Genetically Modified Organisms (GMO)
Public Register Report

Printed on the 02/03/2022 at 09:05:12 - contains 15,326 pages
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Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Trust genetic modification safety committee has been formed as part of the process of initiating the first clinical trial of cancer gene therapy at James Cook University Hospital. The inaugural meeting was held on June 5th 2007, when Terms of Reference were agreed. The remit of the committee is to carry out a risk assessment of all newly emerging therapeutics involving genetically modified organisms within the Trust. A minimum of two committee meetings are to be held, (or more frequently if required) and reporting arrangements are to the Research Governance Committee, with copy to Organisational Safety/Risk Committee for information. Membership of the committee, which is chaired by the Trust's Medical Director, includes the following; Chair of Risk Committee, Chair of Clinical Effectiveness Sub-committee, Clinical Director - Oncology, Chief of Service - Pathology, R&D Director, Research Network representative, Non-executive Director, Patient & Public Representatives, Infection control, Representative from facilities. Staff side representation, Pharmacy representation.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes
Level 2 (GMMs)
Level 3 (GMMs)
Yes
Yes
Yes

Level 4 (GMMs)
Non-microbial

Other (please specify)

Tick if confidential

Bacteriology
Parasitology
Transgenic
Bacteria

Microbiology

Tick if confidential

Virology
Transgenic
Animals

Gene Therapy

Tick if confidential

Mycology
Transgenic
Invertebrates

Other (please specify below)

Transgenic
Plants

For activities involving GMMs, describe the waste management measures which will apply to the activity

Contaminated materials including syringes, vials, swabs and materials used for cleaning will be placed in sharps bins and disposed of by incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

It was agreed that procedures within the Trust would ensure that containment at the required level would be provided. The first version of the risk assessment had stipulated that clinical waste be double bagged, sealed with a red identification tie and identified as GMO waste by indelible pen, however, it was agreed by the committee that waste would instead be discarded into burn bins and sent for incineration as per normal hospital procedures. This was supported by guidance obtained by the representatives from Facilities and Risk Management. The risk assessment was therefore amended to reflect this change.

Project Ref 1/01.1

Date Ackn'd 31/01/2001 02/03/2022
CU2 Project Title FOR INVESTIGATIONS OF MOLECULAR MECHANISMS IN TUMOURIGENESIS
Class 2 1-50 litres
**Project Additional Information**

**Purposes of the contained use**

For investigations of molecular mechanisms in tumourigenesis

**Recipient or parental organism**

The E coli host will be E coli K12 derivatives - HB101, XL1B, DH5α, Y1088, 1089, 1090, C600 and the non-K 12 strain, BLR (rec derivative of BL21)

The source of insect cells will be the SF9 cell line. Mammalian cell lines will include a number of well characterised (immortalised) cell lines of human or murine origin (see appendix in attached risk assessment). For some experiments, primary embryo fibroblasts (rat and mouse) will also be used.

**Host/vector system**

The plasmid backbones are all pUC based and are non-mobilisable. They include: for bacteria; pUC 12, 18, 19, pSP65, pBSBluescript, pCR..., pTOPO-PCR; for mammalian cells; pA64, pT109, pSt5, pCITE, pGY25, pLk-neo, pBABE, pcDNA3; for insect SF9 cells; pVLH6.

Transduction of mammalian and insect cells will be accomplished by DNA-mediated transfection. None of the mammalian vector systems will be used to generate replication defective (or competent) viruses.

**Origin & function**

The inserted sequences (see appendix in attached risk assessment) comprise a number of genes encoding mammalian proteins with either known or strongly suspected oncogenic potential. The perceived hazard arises from manipulation of naked (oncogenic) DNA.

**Evaluation of foreseeable effects**

The oncogenic sequences (see appendix in attached risk assessment) are capable of encoding biologically active proteins which are potentially pathogenic to humans. Some of the prokaryotic and eukaryotic vector systems are optimised for high level expression of protein. The oncogenic sequences are highly unlikely to alter the pathogenicity or properties of the bacterial host cell. Enforced expression of such sequences in mammalian cells will, in some instances, affect the malignant properties of the cells. Sequences encoding biologically active oncogenic proteins are classified as carcinogens for which containment level 2 is appropriate. Neither the DNA, encoded protein, bacterial or mammalian transfectants are likely to have any deleterious effect on the environment. The GM microorganisms are incapable of surviving outside of the laboratory environment. All laboratory procedures will follow good microbiological practice. GM microorganisms will be manipulated in a Class II Microbiological safety cabinet. Written records of staff training will be obtained and checked before commencement of work by individuals. Handling and manipulation of naked DNA will require the wearing of disposable gloves. Mammalian cell cultures for DNA transfection procedures will be handled in a Class 2 cabinet. Where possible, sharps will be avoided in

02/03/2022
all operations. Disposable plasticware (pipettes especially) will be used for mammalian cell culture.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For preparation of plasmic DNA/protein, bacteria will be collected by centrifugation and lysed. Liquid waste material will be rendered non-viable by decontamination overnight with Virkon (1%) and autoclaving prior to sink disposal. Any contaminated solid waste (e.g. contaminated disposable plasticware, agar plates, DNA) will be bagged and autoclaved prior to disposal as normal solid waste in accordance with laboratory practice in the laboratory. Disposal and decontamination of materials used in mammalian cell culture will be done essentially as described above for bacteriological work. The efficacy of autoclaving procedure will be validated as follows:

a) From electronic printout schedule of autoclave cycle parameters to ensure successfully completed autoclave cycle on each occasion.

b) From visual monitoring of internal control "Browne Sterilizer Control Tubes" (Albert Browne Ltd UK) on each occasion.

c) From quarterly monitoring of bacteriocidal performance following a standardised protocol in which a 100ml flask of L-broth innoculated with 10 to the power 7 E coli HB101 (or similar K12 derivative strain) is subjected to a single autoclave cycle. Maintaining sterility of the culture, the flask is then incubated at 37°C on an orbital shaker for 4 days. Under sterile conditions, 0.5ml aliquots of the culture are then plated out in triplicate onto standard L-broth agar plates which are monitored for evidence of bacterial growth after overnight incubation at 37°C.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Copies of the Professor@'s GM project and risk assessment were circulated to members of the University of Essex Genetic Modification Committee for consideration prior to a meeting on the 19th of January 2001. The project was subsequently approved by the committee and signed by the Chair.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2</td>
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</table>
Previous studies, such as microarrays, have identified a number of candidate genes that may be critical regulators of normal haematopoietic cells development. This project seeks to evaluate the role of these genes in murine model systems using genetic knockdown of forced expression experiments. Briefly, murine cell populations will be transduced using lentiviral vector, retroviral vectors or transfected with normal plasmids containing shRNAs or the cDNA of the gene of interest. The cells will then be functionally evaluated using a combination of in vitro and in vivo techniques, the latter involving transplantation of cells in to mice.

Recipient or parental organism
- Lentivirus parental organism- Human Immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3

Host/vector system
- Recombinatt lentiviral vectors pseudotyped with VSV-G envelopes, will be used with 293T cells
- MSCV used with Phoenix A and Phoenix GP cells

Origin & function
Candidate regulator genes for normal haematopoietic cell development will be obtained in the form of cDNA originating from murine genomic or RNA libraries.

**Evaluation of foreseeable effects**

The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal (Details of the origins and deletions within the MSCV and lentiviral constructs are given in appendix 1 of the risk assessment).

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated residual risks.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of Virkon); work carried out in containment 2 laboratories or home office inspected animal facilities; work within class 2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Transduced cells will be transplanted in to mice by intravenous injection. This procedure will be carried out by specifically trained staff only.

The Biological Resource unit (BRU) houses all mice in sealed individually ventilated cages. All handling is within cabinets. The rooms are secured with sealed drains and close sealed doors. There are open ducts within the room. Corridors are sealed with electronically locking flush fitting doors. All corridor risers are sealed. The unit is inspected regularly by Home Office inspectors to ensure that it meets the required standards.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site.

Virkon is routinely used as per the manufacturers recommendations -

- Solid surfaces are disinfected with 1% Virkon solution.
- Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.
- Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation).

**Is an emergency plan required according to regulation 20?**

- [N] No

**If yes, tick to confirm that it is attached to this form**

- [N] No

02/03/2022
A query as to the risk to workers from any free virus whilst administering transduced cells to mice was raised. This is answered in appendix 4 of the risk assessment.

The committee agreed it was a GM Class 2 project.

## Project Containment

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### Large Scale Activities

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## Project Ref 1/99.1

### Date Ackn'd

12/11/1999

### CU2 Project Title

ASSESSING THE STRUCTURE AND FUNCTION OF MICROBIAL COMMUNITIES

### Class

Class 2

### CultureVolClass

Consent Granted

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### Non-GMM

not applicable

### Project notified under transitional arrangements

Y

### Withdrawn

N

### Tick if notifying a connected programme of work

N

### Historical Significant Changes

### Historical Date of Additional Info

### Significant Change ID

### Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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**Name**

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**Comments**

CHANGED FROM IMPERIAL CANCER RESEARCH FUND ON 4/2/2002, CHANGE ON 01/04/2015 FROM CANCER RESEARCH UK

**Date at Which Additional Info Submitted**

| Date             | 28/01/2002 | 19/01/2004 |
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Level 4 (GMMs)

Non-microbial

Other (please specify)  
 tick if confidential

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<th>Parasitology</th>
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<th>Microbiology Research</th>
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<td>Mycology</td>
<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 2/01.1

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<th>Class</th>
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Date Project Ceased: 02/03/2022
Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project notified under transitional arrangements: Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?: N
If yes, tick to confirm that it is attached to this form: N
Tick to confirm that you have attached a risk assessment to this form:
Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<th>Laboratory Activities</th>
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Project Ref 2/01.10

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Date Project Ceased: 02/02/2007

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: TRANSFERRED TO GM CENTRE 973 (2/2/07)

Project notified under transitional arrangements: Y

Project Additional Information

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units  Large Scale Activities  Human Clinical Applications
### Project Additional Information

#### Purposes of the contained use

- [ ] Recipient or parental organism

#### Host/vector system

- [ ] Origin & function

#### Evaluation of foreseeable effects

- [ ] Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
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If yes, tick to confirm that it is attached to this form

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Please enter comments on the GM safety committee on the risk assessment

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Project Ref  2/01.12

Date Ackn'd  15/02/2001
Date Project Ceased  02/02/2007
Withdrawn  N

CU2 Project Title  INTEGRIN SIGNALLING IN MOUSE KERATINOCYTES

Class  Class 2
CultureVolClass2  Non-GMM
Consent Granted  not applicable

Project notified under transitional arrangements  Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Please enter comments on the GM safety committee on the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

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**Project Ref**

2/01.13

**Date Ackn'd**

13/12/2001

**CU2 Project Title**

FUNCTIONAL DISECTION OF VACCINIA GENOME, PARTICULARLY WITH RESPECT TO CELL MOTILITY

**Class**

Class 2

**CultureVolClass2**

< 1 litre

**CultureVolumeClass3-4**

not applicable

**Non-GMM**

Consent Granted

**Withdrawn**

N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
### Purposes of the contained use

To understand the general process of cell motility which plays an important role during development and progression to cancer metastasis.

### Recipient or parental organism

Standard mammalian cell lines, typically HeLa, RK13 and BSC-1.

### Host/vector system

Vaccinia virus, shains Ankara, IHDJ, MVA and WR.

### Origin & function

pBS SKII containing a selectable marker replacing vaccinia gene of interest with upstream and downstream flanking regions to delete the entire gene. Additionally, point mutations, partial deletion or GFP versions of the vaccinia gene of interest will be replaced back into the original gene locus.

### Evaluation of foreseeable effects

In most cases it is likely that pathogenicism of the virus will be reduced and that it would lose the ability to replicate in some host cells. However, some modifications will be made to attempt to restore wild type function to the virus. It is unlikely that any modification made in these experiments would increase the pathogenicity or host range specificity of the GMO over wild type vaccinia virus.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated, for at least 12 hours with Virkon to a final concentration of at least 2%. This will then be discarded to drain. Solid waste will be autoclaved prior to leaving the building to be incinerated as per clinical waste. It is unlikely that any viable GMMs would remain after this treatment, and in any case would require specialist conditions for growth.

Is an emergency plan required according to regulation 20? 

- [ ] Yes
- [x] No

If yes, tick to confirm that it is attached to this form

- [ ] Yes
- [x] No

Tick to confirm that you have attached a risk assessment to this form

- [ ] Yes
- [x] No

Tick if you are claiming exemption from disclosure for section of the risk assessment

- [ ] Yes
- [x] No
The Committee queried whether some vaccinia strains were classed at ACGM level 1. The possibility of vaccinia infection must be controlled by local rules and COSHH assessment.

The Committee confirmed the risk assessment as Containment Level 2 and HSE will need to be notified. There is a possibility of risk to human health and one member of staff with psoriasis will not be working on this project. Some large scale work might require facilities in the 2B1 suite. The risk to the environment was considered to be effectively zero. The project was classified as Class 2.

**Project Containment**

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<td>INDUCIBLE GENE TARGETING IN MOUSE FIBROBLASTS USING RETROVIRAL VECTORS EXPRESSING CRE RECOMBINASE</td>
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Tick if notifying a connected programme of work: N

**Project Additional Information**

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 2/01.3

**Date Ackn’d** 15/02/2001

**CU2 Project Title** PRODUCTION AND EXPRESSION OF TUMOUR SUPPRESSOR GENE CONTAINING ADENOVIRUS VECTORS

**Class** Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM Consent Granted** not applicable

**Project notified under transitional arrangements** Y

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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### Project Ref 2/01.4

Date Ackn'd 15/02/2001

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Consent Granted not applicable

Class 2

CU2 Project Title MANIPULATING IMMUNITY AND TOLERANCE USING REPLICATION DEFECTIVE RETROVIRAL VECTORS EXPRESSING SERRATE AND DELTA

CultureVolClass2 CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Is an emergency plan required according to regulation 20? 

N

If yes, tick to confirm that it is attached to this form

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Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Additional Information**

TRANSFERRED TO GM CENTRE 973 (2/2/07)
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref**: 2/01.6

**Date Ackn'd**: 15/02/2001

**CU2 Project Title**: EXPRESSION OF DOMINANT-NEGATIVE INTEGRIN AND CADHERIN MUTANTS IN HUMAN EPIDERMAL KERATINOCYTES

**Class**: Class 2

**CultureVolClass2**: not applicable

**CultureVolumeClass3-4**: not applicable

**Non-GMM Consent Granted**: not applicable

**Project notified under transitional arrangements**: Y

**Historical Significant Changes**: TRANSFERRED TO GM CENTRE 973 (2/2/07)

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
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If yes, tick to confirm that it is attached to this form  

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Project Ref 2/01.7

Date Ackn'd 15/02/2001

CU2 Project Title ROLE OF FAK IN KERATINOCYTE ADHESION AND DIFFERENTIATION

Date Project Ceased 02/02/2007

Consent Granted

Class 2

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Tick if notifying a connected programme of work

02/03/2022  Page 31 of 15326
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
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**Project Ref** 2/01.8

- **Date Ackn'd**: 15/02/2001
- **CU2 Project Title**: MANIPULATION OF EPIDERMAL STEM CELL FATE BY VERTEBRATE HOMOLOGUES OF DROSOPHILA PATTERNING GENES

**Class** 2

**Non-GMM** Consent Granted: not applicable

**Project notified under transitional arrangements**: Y

**Withdrawn**: N

**Historical Significant Changes**: TRANSFERRED TO GM CENTRE 973 (2/2/07)
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Historical Significant Changes**

TRANSFERRED TO GM CENTRE 973 (2/2/07)

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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- Animal Units
- Large Scale Activities
- Human Clinical Applications

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CU2 Project Title:

ANALYSIS OF INTRACELLULAR TRAFFICKING OF RECOMBINANT POLIOVIRUS TYPE 1 SABIN 1 IN MAMMALIAN MOTOR NEURONS

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work Y

Consent Granted

Project notified under transitional arrangements N

Date Project Ceased

Project notified under transitional arrangements N

Class

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Non-GMM: not applicable
Project Additional Information

**Purposes of the contained use**

This work concerns the analysis of the entry, sorting and retrograde axonal transport of avirulent poliovirus type 1 Sabin 1 strain. This study will aim to clarify the mechanism of internalisation and retrograde transport of poliovirus and its exploitation as a vector for intracellular targeting of spinal cord neurons for gene therapy purposes.

**Recipient or parental organism**

Primary culture of mammalian spinal cord motor neurons. These cells are terminally differentiated and do not divide in culture. They are prepared from rodent (mouse or rat) embryos by isolating the spinal cord and then purifying in vitro the dissociated tissues. These cells are maintained in vitro for 1-2 weeks and are pathogen free.

**Host/vector system**

We will be using a recombinant Sabin 1 strain expressing green fluorescent protein (GFP) as a fusion capsid protein. The Sabin 1 strain has been used in the western world as a vaccine against the virulent poliovirus type 1 (which causes a paralytic poliomyelitis in humans as result from an invasion by circulating poliovirus into the central nervous system). The virus strain is not reported to contain DNA replication sequences.

**Origin & function**

This recombinant Sabin 1 strain will be produced by our collaborators. The virus will be produced in a unique strain of trans-complementing cells, purified in their laboratory and sent frozen to us. No further expansion, purification or titration of the virus will be done at the Lincoln's Inn Fields Laboratories who have a profound experience in producing and manipulating poliovirus will be a visiting Fellow at the Lincoln's Inn Fields Laboratories for all the duration of the project (expected 3 months). We will receive purified, titrated aliquots of virus, which will be stored frozen in a safety freezer in the CONTAINMENT LEVEL 2 at the Lincoln's Inn Fields Laboratories.

**Evaluation of foreseeable effects**

When needed, a virus aliquot will be incubated with living rodent spinal cord motor neurons, microinjected or not with the mammalian expression plasmid encoding the human poliovirus receptor in the CONTAINMENT LEVEL 2 facility. Upon viral entry and removal of free viral particles, uptake and retrograde transport will be monitored in real time by time-lapse fluorescent microscopy. In selected experiments, compartmented cultures of motor neurons will be used to monitor the passage of the virus particle through the cell body.

All the cultures used with the virus are primary and only short-term experiments are programmed (30 min - 6 h). Following incubation and imaging, cells will be disposed following safety rules or fixed with paraformaldehyde/glutaraldehyde and then processed for immunolocalisation studies. The fixing procedure will be different depending upon the technique used to visualise the final specimens. In case of electron microscopy, we will use 4% paraformaldehyde 2% glutaraldehyde overnight at room temperature whereas for light microscopy we will adopt 4% paraformaldehyde 30 min-1 hour at room temperature or 100% methanol at -20 degrees C for 30 min.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The work will be carried out in compliance with Good Laboratory Practice and local safety rules for GM work in a Containment level 2 facility. Protective clothing will be worn. GMM contaminated material and liquid waste is inactivated in 1% Virkon and disposed after 24 hr, as per manufacturer's validated instructions; or autoclaved at 121 degrees C for 30 minutes prior to incineration by validated means through licensed contractor. Accidental spillages of liquid waste will be inactivated with 2% Virkon.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
None at present.

Project Containment

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<th>Growth Rooms</th>
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Project Ref 2/04.2

Date Ackn'd 25/03/2004
CU2 Project Title USING RECOMBINANT RETROVIRUSES TO EXPRESS POTENTIALLY
Class 2
CultureVolClass2 < 1 litre
CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use
To examine the phenotypic effects of specific gene products in primary human cells and to generate immortalised cell strains with defined genetic alterations.

Recipient or parental organism
The intention is to introduce human cDNA sequences into cultured human cells.

Host/vector system
The cDNAs will be transferred into existing retroviral vectors (e.g. pBABE-puro) that are based on the Moloney murine leukaemia virus. The vectors lack the viral pol and env genes and the residual part of the gag gene is non-functional. They will be packaged into replication incompetent particles by transfection of the LinXA packaging cell line (a derivative of 293T). The particles will then be used to infect human fibroblast strains (e.g. W138, Hs68, IMR90) or primary cultures of human epithelial cells (e.g. prostate, mammary glad, skin keratinocytes). The infected cell will be selected in medium containing appropriate antibiotics (either puromycin, neomycin, hygromycin or blasticidin).

Origin & function
The genetic material will be cDNAs encoding a variety of cellular proteins that are already well characterised and in the public domain, including Ras, Myc, hTERT, Bmi1, Cbx7 and the E6 and E7 proteins of human papilloma virus 16. These have the potential to promote tumour formation (i.e. oncogenes). Alternatively, we will use short hairpin sequences that can suppress the expression of tumour suppressor genes such as INK4a, ARF, p53 and pRb. Combinations of these agents should be sufficient to convert a normal cell into one that has the hallmarks of a cancer cell.

Evaluation of foreseeable effects
The expectation is that the human cells expressing appropriate combinations of cDNAs will be capable of indefinite proliferation in culture, and will acquire an ability to form anchorage independent colonies in semi-solid medium. However, they are not expected to represent a hazard to human health as none of the cells used are capable of colonising a healthy individual and the we do not expect the manipulations to alter this. The cells will not be expected to release infectious viral particles. The retroviral vectors are incapable of replication because of removal of the viral pol and env genes and the LinxA packaging cell line has the requisite genes either integrated in the genome or expressed from BPV based episomal vectors that contain no regions of homology with the MMLV based vectors. Three independent recombination events would be required to produce a replication competent virus and the cell system has been tested exhaustively for virus production with consistently negative results.
The amphotropic virus particles are capable of infecting human cells, but will not replicate. In the extremely improbable event that the vector acquires replicative potential by recombining with endogenous retroviral sequences, this single agent is unlikely to cause deleterious consequences in the host. It is estimated that between 4 and 6 separate alterations are necessary to generate a tumorigenic cell. Retroviruses are also inherently unstable and very high titres would be required to produce a persistent infection in an immunologically competent individual. The primary dangers in working practice are expected to be the formation of aerosols and use of sharps. We propose to carry out all manipulations in a Class II microbiological safety cabinet following GLP and any additional local safety guidelines appropriate to this work. There will be no use of sharps and no sonication of solutions, which may contain virus.

None of the cell lines would be expected to be capable of remaining viable outside of the controlled culture conditions used and we do not anticipate that any of the manipulations we are proposing would alter this.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be deactivated in a Virkon trap (1%) and disposed after 24 hr, as per manufacturer's validated instructions. All solid waste will be autoclaved at 121.0°C for 30 minutes prior to incineration by validated means through licensed contractor. All waste form retrovirus work will be handled separately from other tissue culture waste. Accidental spillages of liquid waste will be deactivated with 2% Virkon. We do not expect the scale of low titre viral work to exceed more than 500 ml of amphotropic virus.

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The local GMSC members confirmed the Risk Assessment as ACGM Category 2. The risk to the environment was considered to be low. The project was classified as Class 2.

Project Containment

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</table>

02/03/2022
We will transform C. albicans strain CAI4 (uri-) with different modifications of PACT1 vector that contains the gene for uridine synthesis (uri+).

The transformants will be selected for growth in uridine deficient media and will be used for infecting mice to study both the Ovalbumin-specific immune response and to analyse the immune response against fungal infections.

Pathogenicity of wild type C. albicans: mycosis of superficial layers of skin or mucous membranes (oral thrush, vulvovaginitis, paronychia, onychomycosis, interigo); ulcers or pseudomembranes in esophagus, gastrointestinal tract or bladder; hematogenous dissemination may produce lesions in kidney, spleen, lung, liver, prosthetic cardiac valve, eye, meninges, brain. However, this microorganism is endogenous (part of the normal flora) and can be spread by contact with excretions of mouth, skin, and faeces from patients or carriers.

Origin & function
1) PACT1 - GFP, a yeast encoding yeast enhanced GFP.
2) PACT1 - OVA, the same vector but encoding for chicken ovalbumin.
3) PACT1-GFP-OVA, the same vector but encoding a fusion protein GFP-Ovalbumin.

The transformants will be selected for growth in uridine deficient media and will be used for infecting mice to study both the Ovalbumin-specific immune response and to
Analyse the immune response against fungal infections.

Chicken Ovalbumin and GFP (and other fluorescent variants) are non-pathogenic proteins. Furthermore the gene products would not be expected to cause any hazard to human health.

(Currently we plan to use GFP for tracking fungi within the mice, however, vectors using RFP, YFP or CFP may need to be generated).

**Evaluation of foreseeable effects**

The modification of the fungi by these plasmids would not be expected to change any characteristics such as host range, tropism or pathogenicity.

Non-GM work with wild-type Candida albicans is already in progress at Cancer Research UK at containment level 2. An appropriate COSHH risk assessment has been completed and this will be applicable to the modified Candida too. Please read attached COSHH assessment.

The proposed transgenic modifications will not alter the pathogenicity or otherwise of the Candida, so the fungi present no extra danger than if they were unmodified. If the latter are class 2, so are the modified fungi.

The plasmid construction is class 1 and can be undertaken at containment level 1, but any work with Candida must be undertaken at containment level 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

For only GMMs - application for any derogation from full containment for the class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The work will be carried out in compliance with good laboratory practice and local safety rules for GM work in a containment level 2 facility.

GMM contaminated material and all liquid waste will be deactivated in a Virkon trap (1%) and disposed after 24 hr, as per manufacturers instructions; all solid waste will be autoclaved at 121C for 30 minutes prior to incineration by validated means through licenced contractor. Accidental spillages of liquid waste will be deactivated with 2% Virkon.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Non-GM work with wild-type Candida albicans is already in progress at Cancer Research UK at containment level 2. An appropriate COSHH risk assessment has been completed and this will be applicable to the modified Candida too. All staff working with Candida must read the appropriate COSHH assessment. The proposed transgenic modifications will not alter the pathogenicity or otherwise of the Candida, so the fungi present no extra danger than if they were unmodified. If the latter are class 2, so are the modified fungi.

The plasmid construction is class 1 and can be undertaken at containment level 1, but any work with Candida must be undertaken at containment level 2. The injections will be done at Imperial College Sir Alexander Fleming CBS in containment level 2 facilities following local rules.

Additional address for animal work:
Imperial College, London
CBS
Sir Alexander Fleming Building
Exhibition Rd.
South Kensington, SW7 2AZ.

Project Containment

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Project Ref 2/94.1

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Significant Change ID

02/03/2022
Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
Project Ref 2/96.1

Date Ackn'd 08/10/1996

CU2 Project Title EXPRESSION OF FURIN AND RELATED PROTEINS TARGETED BY SECRETORY GRANULES IN TISSUE CULTURE CELLS USING RECOMINANT VACCINIA VIRUS

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment
### GM Centre Number: 4

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#### Name

UNIVERSITY OF SUSSEX

#### Name 2

Health, Safety and Environment Office

#### Campus Estate or Research Centre

#### Building

#### District

Falmer

#### Town

Brighton

#### County

Sussex

#### Postcode

BN1 9RJ

#### Country

England

#### Tel Number

01273 678446

#### Fax Number

01273 623714

#### Date at Which Additional Info Submitted

02/03/2022

#### Comments

Includes Former GM157

#### HSE Division

East and South East

#### Tel Number

01273 678446

#### Fax Number

01273 623714

#### Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)  
Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 157/01.1

**CU2 Project Title**

ANALYSIS AND EXPRESSION OF HUMAN DNA REPAIR AND CHECKPOINT GENES USING RETROVIRAL VECTORS

**Date Ackn'd** 01/10/2001

**Date Project Ceased**

**Class** 2

**CultureVolClass2** < 1 litre

**Non-GMM Consent Granted** not applicable

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
## Project Additional Information

### Purposes of the contained use
To protect the laboratory workers and the environment from any potential harm.

### Recipient or parental organism
Cultured mammaham cells.
These are characterised cell lines with strict growth requirements and are incapable of growth outside the culture flask.

### Host/vector system
Retroviral vectors or transfection vectors (eg pcDNA3).
The retroviral vectors are defective for replication with no gag, pol or env genes. All vectors are non-mobilisable.

### Origin & function
cDNA libraries derived from human or mouse cells. Hopefully the libraries will be representative of all human cDNAs. Our interest is in those expressing DNA repair and cell cycle checkpoint genes.

### Evaluation of foreseeable effects
The human cDNA library could potentially include harmful genes such as ancogenes. These will however represent a low percentage of the total cDNAs in the library.
The packaging line contain no helper virus and helper functions are encoded by separate segments. The vectors are non-mobilisable and defective for replications. The inserts are however under strong promoters. The insert will not change the host range of the vector.
The mammalian recipient lines have strict growth requirements and are incapable of growth outside the culture flasks.
Taken together the environmental and human risks are minimal.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Liquid waste will be destroyed using Virkon. Disposable plastics destroyed by autoclaving.
- See attached risk assessment for further validation.
- Virkon has been verified to be effective against retroviruses and we consider will inactivate our low litres.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

02/03/2022  Page 50 of 15326
Committee in agreement with risk assessment.

**Project Containment**

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**Project Ref** 157/01.2

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Non-GMM | Consent Granted |
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Withdrawn | Y |

Tick if notifying a connected programme of work | N |

**Project Additional Information**

**Purposes of the contained use**

To protect the laboratory workers and the environment from any potential harm.

Recipient or parental organism
Cultured mammaham cells. These are characterised cell lines with strict growth requirements and are incapable of growth outside the culture flask.

Host/vector system

Retroviral vectors or transfection vectors (eg pcDNA3). The retroviral vectors are defective for replication with no gag, pol or env genes. All vectors are non-mobilisable.

Origin & function

cDNA libraries derived from human or mouse cells. Hopefully the libraries will be representative of all human cDNAs. Our interest is in those expressing DNA repair and cell cycle checkpoint genes.

Evaluation of foreseeable effects

The human cDNA library could potentially include harmful genes such as ancogenes. These however represent a low percentage of the total cDNAs in the library. The packaging line contain no helper virus and helper functions are encoded by separate segments. The vectors are non-mobilisable and defective for replications. The inserts are however under strong promoters. The insert will not change the host range of the vector. The mammalian recepient lines have strict growth requirements and are incapable of growth outside the culture flasks. Taken together the environmental and human risks are minimal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be destroyed using Virkon. Disposable plastics destroyed by autoclaving. See attached risk assessment for further validation. Virkon has been verified to be effective against retroviruses and we consider will inactivate our low litres.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Committee in agreement with risk assessment.

Project Containment
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**Project Ref** 4/07.1

**Date Ackn'd** 09/11/2007

**CU2 Project Title**

Expression of Superantigen genes in E. coli BL21 cells using commercially available expression vector systems pPROEXHT and pET32a-c.

**Class** Class 2

**CultureVolClass2** < 1 Litre

**CultureVolumeClass3-4**

**Non-GMM** Not Applicable

**Consent Granted**

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To protect the operator from transfer of bacterial genetic material and to prevent the transfer of bacterial genetic material to other microorganisms.

**Recipient or parental organism**

Superantigen genes will be expressed in E. coli BL21 cells. These are widely used competent cells which require category I handling once transformed. Toxin genes will be under the control of an IPTG inducible promoter and will only be expressed in the presence of IPTG. IPTG inducible expression results in all metabolic activity being channelled to toxin production, resulting in a biologically unfit microorganism. The GMO would behave in the environment as a SL2I E.coli. It would have no survival advantage over other bacteria.

**Host/vector system**
Commerically available vector systems will be used. Individual risk assessments are attached detailing the particulars of each vector system. See next page for details or foreseeable risks.

**Origin & function**

Superantigens are naturally occurring exotoxins of Streptococcus pyogenes and Staphylococcus aureus. The toxins' biological functions in bacteria are unknown but toxicity in human disease is considered to be due to polyclonal T cell activation. Superantigen genes are widespread and prevalent in wild type Staphylococcus aureus and Streptococcus pyogenes, and therefore the addition of these genes into E. coli cells poses no additional risk to the environment or human health, and in fact due to the IPTG inducible expression described above, risk to human health from these toxins is diminished.

The toxin when expressed forms insoluble granules which would be biologically inert. The toxin is harvested by cell lysis under stringent denaturing conditions (6M urea) and only refolds to an active form under meticulous refolding as denaturing is reversed. Studies have shown that laboratory exposure to these superantigens poses absolutely minimal risk to human health (Rusnak, 2004 Emerging Infectious Diseases Vol10 Issue 9 p1 544-I 549).

We will use the recombinant superantigens as prototypes in experiments aimed at defining novel approaches to identification of superantigens in biological material.

**Evaluation of foreseeable effects**

Hazards associated with expression vector systems:
The plasmid contains an antibiotic (ampicillin) resistance gene. Resistance to ampicillin is already wide-spread amongst strains of E. coli causing human disease. As such, introduction of this gene into E. coli for experimental purposes poses no significant risk.

Although the risk of transfer of genetic material to other species always exists when undertaking this sort of work, risk in this case is in reality very low. The expression of superantigen is tightly controlled by the IPTG inducible promoter. Furthermore the SEK will be expressed as insoluble protein complexes and as such would be biologically inactive without purification.

To further minimise the risk of this work, it will be conducted under category 2 conditions. The sterile nature of the category 2 laboratory conditions with scrupulous disinfection and cleaning protocols will ensure that the risk of transfer of this genetic material to other bacterial species is absolutely minimal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

These measures have been approved by the University of Sussex Genetic Modification Safety Committee from applications:
Expression of recombinant streptococcal superantigens in E. coli BL21 cells
Expression of recombinant streptococcal SEK in E. coli BL2I cells
Expression of recombinant streptococcal SEJ in E. coli BL2I cells
Copies of these are attached.

Once competent E. coli cells have been transformed with complete expression vectors, expression of superantigen genes can be carried out under category I conditions. This is due in part to the IPTG inducible expression of superantigen genes rendering the E. coli cells biologically unfit, as described above, and in part to the findings published by Rusnak et al (Rusnak, 2004 Emerging Infectious Diseases Vol0 Issue 9 p1544-1549) indicating that laboratory exposure to superantigens poses an absolutely minimal risk to human health, in accordance with other category 1 reagents and procedures.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Chemical disinfection of Category 2 biosafety cabinets using 70% IMS. Liquid waste will be neutralised using 10% Virkon solution before autoclaving at 121 degrees C for at least 15 minutes. Autoclave printouts will be checked following each run, and if unsatisfactory the cycle will be repeated. Following autoclaving, liquid waste will be disposed of as standard laboratory waste.

Solid waste such as agar plates will be autoclaved inside plastic bags within a metal container at 121 degrees C for at least 15 minutes. Autoclave printouts will be checked
following each run, and if unsatisfactory the cycle will be repeated. Following successful autoclaving, solid waste will be disposed of as standard laboratory waste. Attempts to grow bacteria from waste disposed of in this manner have proven unsuccessful on numerous occasions, indicating a killing efficiency of >99.999%.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

no comments

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Date Project Ceased

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info
This project aims to define factors that determine virulence in mycobacteria; particularly Mycobacterium tuberculosis, and Mycobacterium bevis. Systems studied include genes involved in metabolism, global gene regulation, stress responses, intracellular survival and in vivo growth. The aim is to produce strains of mycobacteria that have had genes inactivated either by removal or interruption with transposon sequences, over-expressed, or under the control of inducible promoters. The mycobacterial mutants produced will be characterised in vitro and in macrophage models of infection to learn about the biological function of the target genes. The long term aim of this research is to understand mycobacterial virulence, allowing the development of new control measures for tuberculosis, including new drugs and beller vaccines.

The Genus Mycobacteria (gram-positive, rod-shaped bacteria of the Family Actinomycete) consists of more than 40 recognised species, which can be placed into two main groups: i) fast growing non-pathogenic bacteria such as M.smegmatis (commonly used for genetic studies of mycobacteria), M. fortuitum (an environmental mycobacteria found in water sources that may occasionally cause disease in immuno-compromised people ), and M.abscessus (an environmental mycobacteria that may colonise the lungs of cystic fibrosis patients); and ii) the slow growing mycobacteria which consist of the major human pathogenic bacteria which include the TB complex that cause tuberculosis (consisting of M.tuberculosis, M.bovis, M.africanum, M.microti), M.leprae (leprosy) and the M.avium complex (opportunist infections in HIV-infected people). Mycobacteria can infect most species of animals including rodents, birds and fish. M.avium paratuberculosis is also responsible for Johne's disease in cattle and is increasingly thought to be associated with Crohn's disease (a human inflammatory bowel disease). Another slow growing mycobacteria is M.ulcerans, that causes Burul' ulcers in parts of Africa. Other human pathogenic mycobacteria include M.kansasii, M.scrofulaceum and M.gordonae. Mycobacterium tuberculosis (M.tuberculosis) is by far the most important human pathogen in the Genus Mycobacterium in terms of morbidity and mortality and sub-clinically infects a third of the world's population, killing 1.3 million people each year (WHO, Global tuberculosis report 2013). Primary tuberculosis (TB) infection occurs in the lungs resulting in a chronic granulomatous inflammation and eventually cavitating lesions that release fresh bacilli into the lung. Hemoptysis (coughing blood) can occur caused by rupture of the lung blood vessels. The space-occupying granulomatous inflammation eventually overtakes the normal lung tissue and gaseous exchange is increasingly compromised (tuberculous bronchiectasis). Inflammation of the lung parenchyma causes pleural pain. Death is often due to secondary infections and severe loss of lung function. Cough is the most common symptom alongside systemic manifestations including fever, weight loss and malaise. M.tuberculosis can infect most other parts of the body including, the bones (Pott's Disease), the lymph nodes (miliary T8 or of the neck nodes, scrofula), the gut, the skin (lupus vulgaris), genito-urinary tuberculosis, and the meninges (T8 meningitis). The clinical expression of disease is quite varied and depends on factors that include both host and microbe related characteristics. The risk of acquiring T8 in the UK is relatively small, yet possible, and varies as to socia-economic and geographic factors as well as ethnic and cultural factors. Nearly 20% of all T8 in London results from recent transmission of M.tubercuJosis to new cases; the rest is either imported from other countries or is reactivation T8 from prior exposure (often 50 years ago or more) when T8 was more prevalent in the UK. Over 95% of people exposed to M.tuberculosis do not develop
disease, but form a primary lesion in the lung (Gohn’s foci) and the infection is suppressed for years without clinical symptoms (latent tuberculosis). There is a 10% lifetime risk of reactivating this latent infection for immuno-competent people, whereas people with HIV/AIDS have a 5% per annum risk of reactivation. Other risk factors (associated with a compromised immune system) that might increase the likelihood of reactivation of TB include alcoholism, chronic renal failure, diabetes, some cancers, drug abuse, malnutrition, immuno-suppressive drugs. Active pulmonary disease has a 60% mortality over 5 years without treatment, however effective drug therapy is available. A combination of four antimicrobial drugs taken over six months is recommended as treatment, which has a 5% per annum relapse rate due normally to poor patent compliance. Multiple drug-resistant strains of M. tuberculosis have been identified that are most often resistant to two of the first line drugs (Isoniazid and Rifampicin). Treatment requires alternative antimicrobial combinations and prolonged duration (WHO, Global tuberculosis report 2013). There are >20 second line antimicrobial drugs used to treat drug-resistant TB, and a future pipeline of approximately 18 novel compounds at various stages of clinical development.

In addition to effective drug therapy, there is a vaccination against tuberculosis. Mycobacterium bovis BCG (BCG) is a heavily attenuated strain of Mycobacterium bovis, generated through repeated passage by Calmette and Guérin between 1908 and 1921. Since then this strain has been the basis of a live TB vaccine used on the human population throughout the world and has been shown to have multiple (>128) gene deletions compared with virulent M. tuberculosis (Behr, M.A et al. 1999 Science, 284: 1520-1523). There is no harm to humans except in immunocompromised states (such as HIV progression), in which case disseminated mycobacteriosis could occur. BCG is effective as a vaccine to minimise childhood disseminated tuberculosis. Its efficacy to prevent human tuberculosis varies from 0% to 85%, depending on the study and area of the world. Hypersensitivity to M. tuberculosis is usually tested by a skin test (Mantoux or Heaf test). This shows prior exposure to mycobacterial antigens, but it is impossible to distinguish BCG-vaccinated individuals from cases of sub-clinical TB infection. Acute TB often gives a powerful skin test reaction due to enhanced hypersensitivity, but results in negative tests in 25% of cases. Interferon-Gamma Release Assays (IGRA) specific for TB antigens may be used to distinguish BCG-vaccinated from TB-infected individuals and to monitor immune responses to TB antigens over time. BCG vaccination cannot be assumed to produce protective immunity to TB in adults, but because of the safety history of BCG in the past 50 years with billions of doses given without serious consequence, BCG vaccination is still offered in many countries. In some low and middle income countries, where the burden of TB is highest, and in parts of the UK, BGG is given at birth where it provides good protection against childhood disseminating TB and TB meningitis.

M.tuberculosis and M.bovis are classified by ACDP as biohazard group 3 organisms requiring level 3 containment; all work on this organism is conducted within specialised containment facilities with strict guidelines and following rigid protocols in Class I biological safety cabinets to avoid inhalation of M.tuberculosis particles. Transmission is via the respiratory route (droplet nuclei of < 5 microns) with an infectious dose of less than 3 bacteria. This low infectious dose suggests a high risk of laboratory-acquired infection via the inhalation route. M.bovis may also cause disease via the oral route of infection (historically through the ingestion of unpasteurised milk from infected cattle). Direct contact with intact skin is not considered a route of transmission for M. tuberculosis or M.bovis. Trans-cutaneous inoculation is possible but very unlikely to result in infection for M.tuberculosis. It is possible that needle stick injuries could result in disseminated TB. The route of infections for the GMM will be the same as for the parental strain. The infectious dose for M.tuberculosis via the respiratory route is less than 3 bacilli, as this represents the largest cluster of bacilli that can pass the alveoli entrance. For oral or intestinal acquisition, a theoretical infectious dose is considered to be 1 bacillus, but more likely to be thousands or more. The infectious dose for GMMs will be the same as, or greater than (i.e. requires more bacteria to infect, therefore less transmissible), the parental wild-type.

Mycobacterium tuberculosis was included on schedule 5 of the Anti-terrorism, Crimes and Security Act 2001, but removed in 2012.
Gene knockouts and knock-ins in Mycobacteria are generated in three ways: (1) by a system of self-cloning through the introduction of homologous genes with or without selectable markers, (2) using expression vectors with selectable markers which may be used to induce target gene expression, and (3) by the introduction of transposons to deactivate chromosomal genes by interrupting normal gene function.

Homologous recombination
The introduction of altered "self" genes back into the donor to create modified recipient organisms may be regarded as self-cloning. Because the pathogenic mycobacteria are classified as Hazard Group 3 organisms these procedures are included in this risk assessment. Natural genes in wild type mycobacteria will be replaced with the same gene which has been mutated or part-deleted, or a gene from a highly closely related species (i.e from M.bovis into M.tuberculosis, or vice versa) by homologous recombination, in this case DNA sequences already present in mycobacteria will simply be re-introduced. The gene function will thereby be abolished or modified and the resulting GMM will either have A) no change in properties (since the gene may not be necessary for growth in the laboratory model tested, or that the gene has several copies in the genome, or there may be other genes that compensate for the action of the lost gene); B) will have reduced ability to grow or cause disease due to the loss of gene function. In these cases (A and B) the direct hazard of the inserted gene product results in a GMM that is less than or equal to that of the parental wild-type organism. C) There are several hypothetical possibilities which may result in the phenotype of the GMM being altered in favour of increased pathogenicity: (i) By deleting a repressor gene for virulence determinants may result in genes normally only expressed during a particular phase of infection (to cause damage or immune evasion) being switched on permanently. This may at first sight imply an increased hazard, but this is unlikely as the mechanism of pathogenicity in M.tuberculosis is based on the ability to switch multiple sets of genes on and off at specific times and in specific locations. So, expression of, for example, a haemolysin gene (that is normally required for escape from the macrophage killing mechanisms), if expressed prior to arrival at an intracellular location, may make the bacteria more likely to be recognised and killed by the immune system, since such factors are likely to be highly antigenic. (ii) Deletion of a growth repressor; this is highly unlikely as the slow growth rate of mycobacteria is considered to be controlled by the law of mass action and the number of ribosomes as a ratio of the availability of tRNA species and translation initiation factors. Thus multiple genetic manipulations would be required to influence growth rate. There is also no evidence to suggest that increased growth rate would lead to an increase in TS virulence. (iii) Deletion of a dominant antigen involved in immune recognition, thereby allowing the bacteria to avoid recognition and cause more disease. This is again highly unlikely as immune recognition is a multiple-antigen-dependent event and research has shown that TS deletion mutants of dominant antigens (e.g. ESAT6) have reduced or unaltered virulence, not increased.

In consideration of all these possibilities, all M.tuberculosis (and M.bovis) mutants are treated as hazard group 3 organisms, exactly as the donor or wild-type recipient. GMM pathogenicity is unlikely to increase, and infectious route highly unlikely to change, therefore there is no increased exposure hazard risk and no increased disease risk. This is because many of these changes arise by natural mutation in the environment and many uncharacterised clinical strains of M.tuberculosis may well have such altered phenotypes.

(1). Vector systems for homologous recombination
During the cloning strategies to produce GMM by double cross-over homologous recombination, host mycobacteria are made that contain a suicide plasmid (pNIL: a simple plasmid with no mycobacterial origin of replication based on a mycobacterial plasmid pALS000, which contains genes for the maintenance of the plasmid, multiple cloning sites and selectable markers. i.e. simple, non-hazardous vectors of mycobacterial origin). These plasmids are able to replicate in laboratory strains of Escherichia coli (to allow vectors to be generated), however these plasmids cannot replicate in mycobacteria (Parish, T et al. 2003, Infect Immun; 71:1134-40). The plasmid will contain the mutated wild type gene
and will become partially integrated into the host chromosome after a single cross-over event. These single crossover GMM constructs will not have hazards greater than the wild-type. However the Single cross-over recombinants will retain the plasmid with a hygromycin resistance gene selectable marker. Hygromycin is commonly used as a selective marker in mycobacteria; it is not used as a disinfectant nor an antibiotic in clinical settings. The hygromycin resistance gene and the rest of the plasmid DNA is then lost in the second step of GMM generation as a result of counter selection (using sacB and sucrose selection). Selection for a second cross-over event removes plasmid DNA from the mycobacterial chromosome, stably inserting the mutated TB gene. The resulting GMM retains no plasmid DNA and an altered copy of a naturally-expressed TB gene.

(2). Vector systems for complementation and controlling gene expression
Complementation of the GMM will be generated using a plasmid (pAGAN, a simple, non-hazardous mycobacteria plasmid vector related to pNil and pAL5000) containing the wild-type TB gene (Parish, T et al. 2003, Infect Immun; 71:1134-40). The plasmid contains a mycobacterial origin of replication, so it can be sustained in the mycobacterial cell. The vector also has selectable antibiotic resistance marker genes for hygromycin and kanamycin. In a second strategy, plasmids that are able to integrate into the mycobacterial chromosome are used to stably insert a mycobacterial gene (Blokpoel, Me et al. 2005, Nucleic Acids Res; 33:e22). These plasmid vectors also contain inducible promoters to enable the expression of the inserted gene to be controlled or measured using antibiotic or fluorescent markers (Andreu, N et al. 2010, PloS One; 5:e10777), for example, tetracycline, which is not used to treat tuberculosis. These vectors may result in the over-expression of mycobacterial genes, however these genes are naturally expressed by mycobacteria, and since pathogenicity in TS is multifactorial these complemented mutants will have hazards the same as or less than the parent wild-type organism.

(3). Transposon mutagenesis.
A system of transposon mutagenesis will be used where a kanamycin/hygromycin resistant transposon with counterselectable markers, for example sacB (Sassetti, eM et al. 2003; Mol Microbiol; 48:77-84), will be inserted into the mycobacterial genome, inactivating gene function by interrupting the coding strand. Hygromycin is commonly used as a selective marker in mycobacteria, it is not used as a disinfectant in the laboratory or as an antibiotic in a clinical setting for mycobacterial infections. These modified mycobacteria will be treated in the same way and at the same level of containment as the recipient wild type organism.

Origin & function
The origin of genetic material (excluding vector sequences and antibiotic resistance markers discussed in the section above) will be from mycobacteria. Inserting functional or inactivated genes naturally expressed in pathogenic or nonpathogenic mycobacteria. Specific combinations are detailed below:
1. Single or multiple gene inactivation in M.tuberculosis or M.bovis (containment level three) using modified M.luberculosis (or pathogenic or non-pathogenic mycobacteria) gene sequences. GMO containment level three.
2. Single or multiple gene expression (complementation/inducible expression) in M.tuberculosis or M.bovis (containment level three) using modified M.tuberculosis (or pathogenic or non-pathogenic mycobacteria) gene sequences. GMO containment level three.
3. Single or multiple gene inactivation in M.bovis BeG or non-pathogenic mycobacteria (containment level two) using modified M.bovis BeG (or non-pathogenic mycobacteria) gene sequences. GMO containment level two.
4. Single or multiple gene expression (complementation/inducible expression) in M.bovis BeG or non-pathogenic mycobacteria (containment level two) using modified M.bovis BeG (or non-pathogenic mycobacteria) gene sequences. GMO containment level two.
5. Single or multiple gene expression (complementation/inducible expression) in M.bovis BeG or non-pathogenic mycobacteria (containment level two) using modified M.tuberculosis or pathogenic mycobacteria (containment level three) gene sequences. GMO containment level three - note change in containment level. No systematic attempt will
be made to specifically restore M.bovis BeG virulence through multiple genetic manipulations. The function of genetically-modified genes will vary. Virulence in pathogenic mycobacteria is a multifactorial process that does not depend on a single virulence factor (evidenced by greater than 100 gene deletions between the pathogenic M.tuberculosis and vaccine strain M.bovis BeG). Pathogenicity in M.tuberculosis is likely based on the ability to switch multiple sets of genes on and off at specific times and in specific locations during infection. Therefore the manipulation of a single gene is unlikely to increase mycobacterial virulence, unlike some other microbes where expression of a single toxin gene may dramatically affect pathogenicity.

**Evaluation of foreseeable effects**

**Hazards arising from the alteration of existing pathogenic traits**

**Health and Safety Executive**

The pathogenicity of mycobacteria is not fully understood but is widely shown not to be a single gene function; rather mycobacterial virulence is the result of co-ordinated expression of multiple genes. Virulence and pathogenicity of mycobacteria is determined by host-pathogen interactions, with host immunity playing a central role, which is dependent itself on human genetics, nutritional status and other factors. Thus disease, or the potential to harm, is a complex and multi-factorial process, in which single genes play synergistic roles with numerous other genes. The regulation of mycobacterial gene expression and the correct timing of switching genes on and off during infection is therefore most likely to control mycobacterial virulence. This is clearly demonstrated by M.bovis BGG, which is regarded as a regulatory mutant and the abolished virulence is due in part to the inability to regulate genes correctly. Therefore, it is unlikely that the deletion or insertion of a mycobacterial gene will increase the virulence of the recombinant mycobacteria, measured by two or more models of infection. It is remotely possible that mutants in regulatory genes may cause certain virulence genes to be permanently switched on (with the possibility of enhanced virulence in some models of infection), but the current evidence suggests that virulence requires correct temporal and spatial expression of multiple genes within the host making this scenario unlikely. There are also naturally occurring mutants of wild-type M.tuberculosis with only slight differences in virulence, therefore if all mutants are treated as wild-type, no extra hazards will arise. Others researchers have showed that cloning M.tuberculosis genes into non-pathogenic M.smegmatis did not result in a virulent phenotype as tested in mice. This experimental evidence further supports the view that pathogenicity is not a single gene function.

Accidental exposure to GM M.tuberculosis or M.bovis is unlikely to give rise to infection, with disease occurring in 5% of exposure cases (as for the wild type organism). Infection with GM bacilli will be treatable in the same way as the wild type organism with antibiotic treatment for clinical tuberculosis taking 6 months. Drug resistance can occur naturally as a result of random mutation in certain genes, e.g. rpoB, inhA (Gagneux S et al, 2007, Lancet Infect Dis; 7(5):328-37). Such point mutations that lead to drug resistance in M.tuberculosis occur at very low frequency in nature and are only selected for by exposure to antibiotics. If these resistant genes are transferred, the recipient GM bacilli will also be drug resistant and therefore more difficult to treat. Multiple drug-resistant M.tuberculosis strains remain classified as Hazard Group 3 pathogens as they are not more difficult to acquire, nor is disease any more severe. Drug-resistant M.tuberculosis simply requires different combinations of antibiotics to treat infection.

Hazard of sequences within the GMM being transferred to related micro-organisms

Horizontal (lateral) transfer of genetic material in mycobacterial populations is minimal. Mycobacterial plasmids have been identified (Le Dantec, Get al. 2001, Journal of Bacteriology; 183:2157-2164) and conjugal transfer observed in M.smegmatis (Parsons, L.M et al. 1998 Molecular Microbiology; 28:571 · 582). However, the importance of natural horizontal transfer in mycobacteria has not been established and is likely restricted to the environmental-type mycobacteria. Plasmids have not been found naturally in the human pathogenic mycobacteria, M.tuberculosis, M.bovis or M.leprae. Introducing DNA into mycobacteria is a very inefficient process in the laboratory with very low frequencies of recombination. So horizontal transfer would be a very rare event even within the Genus.
Mycobacterium. Transfer of mycobacterial genes from the GMM to other micro-organisms would be highly unlikely, firstly to occur, and secondly if it did, to alter the properties of the recipient bacteria to increase its hazard. Harbouring extra genomic material in bacteria is usually associated with decreased fitness thereby producing a counter selective effect against any recombinant (Gagneux, S et al. 2006, Science; 312:1944-6). Exchange of genes by recombination or complementation between M.tuberculosis and M.bovis is possible and may well be encountered. There are approximately 20 extra genes in M.bovis compared to the lab strain of M.tuberculosis H37Rv in addition to -68 genes that are deleted (Behr, M.A et al. 1999, Science; 284: 1520-1523). However, these genes are also present in clinical isolates of M.tuberculosis. M.bovis also has multiple point mutations compared with M.tuberculosis, as does M.bovis BGG. As M.bovis has a wider host range than M.tuberculosis, but more restricted disease in humans, gene exchange will not result in any increased virulence for either species, and both will remain as hazard group 3 organisms. However, introduction of M.tuberculosis or M.bovis genes back into M.bovis BGG may restore full virulence to BGG by replacing the deleted genes that represent the difference between M.bovis BGG and M.bovis. Such gene replacement will not however overcome the multiple point mutations in BGG that may also contribute to attenuation of BCG. Because the basis for the BeG attenuation is not fully understood, we will assume that it is possible to revert BCG back to a phenotype with increased virulence. As such, any gene introduction into M.bovis BGG from M.tuberculosis or M.bovis that includes one or more of the deleted (putatively attenuation responsible) genes, will have the potential to increase virulence and the resulting GM BGG will be classified as a potential Hazard Group 3 organisms and experiments performed under GL3 conditions (as per wild type M.tuberculosis). Re-introduction of BGG genes back into BeG will not result in any reversion of attenuation and will remain as Hazard Group 2. During laboratory manipulation of recombinant mycobacteria, any mycobacterial cultures found to be contaminated are chemically disinfected (and autoclaved in the CL3 laboratory) and the plastic/glassware autoclaved (see section 12). The risk of accidentally-transformed contaminating micro-organisms not being contained is therefore negligible.

The likelihood that the GMM could cause harm to human health

The likelihood that GM mycobacteria could cause harm to human health is high in the case of the pathogenic mycobacteria such as M.tuberculosis. The pathogenicity of numerous single gene knockouts has been shown to be only partly attenuated in animal models of infection. Complemented mutants of M.tuberculosis re-establish the normal virulence of wild-type organisms. However, gene knockout strains of M.tuberculosis are also being investigated as possible vaccine candidates (Smith, O et al. 2001, Infection and Immunity; 69: 1142-1150) and auxotrophic mutants of M.tuberculosis have been shown to be avirulent even in immuno-compromised mice. Therefore some gene knockouts will be non-pathogenic, but many will remain potential human pathogens. The chance of a knockout mutant being more virulent than the wild type is unlikely (as discussed above and in previous sections). Thus, all GMM will be treated as the parental wild-type bacteria (except in c below).

It is safe to assume that in all cases Genetically Modified mycobacteria pose ‘about the same’ or less hazard as the parental wild type strains. Because the individual effect of every gene in the genome has not been elucidated and because virulence is not the result of the acquisition of a single gene, we will assume that all mycobacterial transformants should be handled in the same way as the recipient wild-type; except in c) below:

a) The ACDP containment level of Category 1 for the non-pathogenic M.smegmatis and its transformants = Class 1 activity;

b) The ACDP Containment Level of Category 2 for M.bovis BCG (and non-pathogenic mycobacteria, such as M.abscessus) and its transformants = Class 2 Activity;

c) EXCEPT where M.bovis BCG is transformed with multiple genes that may complement the deletions responsible for the attenuation of BeG. In this case, where the deliberate attempt to restore some virulence is attempted, the ACDP Containment Level of Category 3 will apply = Class 3 activity;
d) The ACDP Containment Level of Category 3 for M. tuberculosis and M. bovis and their transformants = Class 3 Activity.

The capacity of the GMM to survive and disseminate among other organisms and the environment

The purpose of containment is to prevent exposure of the GMM to laboratory workers and the environment.

Containment Level one protocols will be followed for the non-pathogenic strain M. smegmatis. Containment Level Two protocols will be followed for the vaccine strain M. bovis BCG mutants and non-pathogenic fast growing species (such as M. abscessus). Containment Level Three protocols will be followed for GM-M. tuberculosis/M. bovis strains (and BCG transformants which complement the attenuation-related deletions).

All Containment Level 3 work will be performed in the specialised CL3 laboratories under the ACDP guidance for Hazard Group 3 micro-organisms. Namely, this will involve all work performed with live GMMs to be within a Class I microbiological safety cabinet following identical protocols as for the non-GMM wild type parental bacteria. Transport protocols out of the CL3 suite to another CL3 suite will be followed as for Group 3 organisms. All liquid waste is first chemically sterilised, then autoclaved as standard procedure for all strains in the CL3 laboratory. These methods of sterilisation have been validated and are detailed further in the CL3 code of practice and section 12 below. All waste is sterilised by autoclaving within the CL3 laboratory, followed by incineration.

Contaminated cultures are chemically sterilised and autoclaved as for wild-type M. tuberculosis. GM-M. bovis BCG/M. tuberculosis pose no more threat to the environment than wild type strains. Both wild type and GM strains are disinfected and disposed of according to Level Two Containment regulations (for M. bovis BCG) and Containment Level Three protocols (for M. tuberculosis). Thus, the risk of exposure of live GM mycobacteria to the environment or animal and plants is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMM in the containment level three laboratory (see above sections) will be treated as wild-type M. tuberculosis (ACDP activity class 3). Waste sterilisation and disposal procedures follow validated methods and standard operating procedures detailed in the CL3 Code of Practice. Summarised below:

The disinfectants used in the CL3 laboratory are:

1. 5% Surianios (Laboratoires Anios): N-(3-aminopropyl)-N-dodecylpropan-1,3-diamine 51mg/g, didecylmethy lammonium chloride 25mg/l. Fresh 5% Surianios solution (diluted with water) is made for each new use of the cabinet. Complete decontamination of a high titre liquid culture of M. tuberculosis with 5% Suranios requires a minimum exposure of 3 hours. Complete sterilisation of a 10ml high titre liquid culture of M. tuberculosis (4 x 10^9 colony forming units of bacteria per ml) was achieved after 3h exposure; a kill of greater than 9 logs with 5% Suranios after 3 hours. We treat overnight for simplicity and to ensure a maximal kill.

2. Amphospray 41 (Laboratoires Anios): N-(3-aminopropyl)-N-dodecylpropan-1,3-diamine, didecylmethy lammonium chloride, polyhexamethylene biguanide hydrochloride, 41% v/v ethanol. Amphospray 41 is used as a surface decontaminant (pre-prepared Surianios in 41% alcohol solution). Culture vessels and centrifuge buckets are sprayed when taking them in and out of the cabinet and for use in the cabinet as a surface disinfectant. Amphospray 41 kills M. tuberculosis within seconds; a kill of 8 10^9 units of M. tuberculosis was observed with Amphospray 41 in 5 seconds.
All waste generated in the CL3 laboratory is autoclaved at 121°C for 30 minutes before removal from the CL3 laboratory. Autoclaving at 121°C for 15 minutes is sufficient for >9 logs kill of high titre M.tuberculosis liquid culture (no colonies grew on plates from an autoclaved 7 day M.tuberculosis liquid culture of 2.7x10E9 colony forming units/ml). We have extended this autoclave time from 15 minutes to 30 minutes. Solid autoclaved waste is collected into yellow plastic bags or yellow plastic biosafe bins, sealed and removed from the CL3 laboratory immediately to prevent buildup of waste. Yellow bags and yellow sharps bins are placed into yellow palladins before removal for incineration.

Solid Waste
All solid discard (gloves, paper towels, disposable plastics) is collected into medium sized steam-permeable autoclave bags in the cabinet. Waste is double-bagged (in clear autoclave bags) before removing from the cabinet, and stored in sealable metallin before autoclaving as soon as possible. All tips and pipettes (that could potentially pierce the clinical waste bags on disposal) are placed in separate small steam-permeable autoclave bags (i.e. not with gloves, paper towels, universals etc.) inside the cabinet. This solid waste is double-bagged ‘in clear autoclave bags) before removing from the cabinet and 8uloclaved as soon as possible. After au toclaving, the autoclave bags containing solid discard (gloves, paper towels) are placed into yellow plastic bags for incineration. The autoclave bags containing sharps are disposed of into yellow plastic bins, rather than yellow plastic bags for fear of puncture.

Liquid Waste
Volumes of GMM liquid waste are expected to be small <100ml, since most culture volume is used in subsequent experimental procedures. liquid waste is sterilised chemically before removing from the safety cabinet by adding neat Surfanios to a final concentration of 5% and left overnight (following validated protocols). Reusable culture bottles are soaked in 5% Surfanios and left overnight. The following day the (diSanitised-sterilised) liquid waste is discarded into white polypropylene biosafe jars for autoclave in the CL3 laboratory. After chemical disinfection and autoclave, the liquid waste is disposed of down the sink with lots of water.

Sharps
Sharps are avoided in the CL3 laboratory where possible. All tips and pipettes are placed in a separate small steam permeable autoclave bag (not with gloves, paper towels, universals etc.) in the cabinet, double-bagged and autoclaved as detailed for solid waste above, before placing into yellow plastic sharps bins. If needles are used, they are placed into universal tubes and treated as sharps waste, autoclaving and disposing into yellow plastic bins before removal from the CL3 laboratory.

CL3 laboratory autoclave procedures
The CL3 laboratory is equipped with a L TE K150 front-loading autoclave. In addition, a L TE F150 front-loading autoclave is located in the prep room in case of a failure of the CL3 autoclave. All waste generated in the CL3 lab is autoclaved, at 121°C for 30 minutes, before leaving the laboratory. Autoclave readouts detailing run success and temperature/pressure readings are archived in the CL3 laboratory. The autoclave is connected to the protected power supply that is backed up by generators. The autoclave is serviced by a qualified technician every 6 months. In the event of an autoclave breakdown, CL3 laboratory work will be minimised to avoid generation of large volumes of waste until the CL3 autoclave fault is repaired. The Safety Officer will be informed and the autoclave engineer contacted. Excess waste will be autoclaved in the L TE Prep room autoclave (located in media prep room in the secure basement area situated on the same floor as the CL3 laboratory). Laboratory waste will already be contained in a gas-permeable autoclave bag and a translucent autoclave bag, according to normal waste disposal procedures. This double-bagged waste will be placed inside a steel box and the exterior surface disinfected. These tins will be placed into a wheeled clear plastic waste transporter located inside the CL3 laboratory. The waste transporter will be surface disinfected before transferring from the CL3 laboratory to the Prep room autoclave. After autoclaving, the waste will be disposed of as normal and the wheeled transporter returned to the CL3 laboratory. If both autoclaves breakdown, all CL3 laboratory work will be suspended until at least one of the autoclaves has been repaired. The same disinfection protocols and disinfectants will be used to chemically sterilise ADCP hazard group 1 and group 2 GM-mycobacteria working in containment level two conditions.
### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 L3 L4 L2</td>
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Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

None
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**Name**

UNIVERSITY OF GREENWICH

**Name 2**

FACULTY OF ENGINEERING & SCIENCE

**Campus Estate or Research Centre**

OLD ROYAL NAVAL COLLEGE

**Road Name**

PARK ROW

**District**

GREENWICH

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

SE10 9LS

**Country**

ENGLAND

**Tel Number**

020 8331 8318

**Fax Number**

020 8331 8305

**E-mail**

LONDON

**HSE Division**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Project Ref   5/01.1

Date Ackn'd  19/02/2001
Date Project Ceased  16/06/2017

CU2 Project Title
TRANSFORMATION OF NON-PATHOGENIC STRAINS OF THE BACTERIUM RALSTONIA SOLANACEARUM WITH CLONED PATHOGENICITY GENES ISOLATED FROM R SOLANACEARUM STRAINS PATHOGENIC TO BANANAS

Consent Granted  not applicable

Class  Class 2
CultureVol  CultureVol
Class3-4  Not applicable

Project notified under transitional arrangements  Y
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 5/01.2

- Date Ackn'd: 19/02/2001
- CU2 Project Title: DIFFERENTIAL DISPLAY USING DNA EXTRACTED FROM FOLLICULAR LYMPHOMAS AND DIFFUSE LARGE B CELLS LYMPHOMAS AND CLONING OF POTENTIALLY DIFFERENTIALLY EXPRESSED GENES
- Class: 2
- CultureVolClass2: Class 2
- CultureVolumeClass3-4: Class 2
- Non-GMM: not applicable
- Consent Granted: not applicable
- Project notified under transitional arrangements: Y

- Withdrawn: N
- Tick if notifying a connected programme of work: N

Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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</table>
## Project Additional Information

### Purposes of the contained use

To screen compounds for antileishmanial activity on the promastigote and amastigote stages of the transgenic parasite using fluorescent activated cell sorting analysis.

### Recipient or parental organism

The recipient protozol parasitic strains include *L. aethiopica*, *L. major*, *L. tropica* and *L. mexicana*.

### Host/vector system

1. **Vector systems:**
   - Genomic Vector: *phGFP-S65T*;
   - Episomal vector: *pXG-GFP+

2. **Host:** DH5 alpha E.coli

### Origin & function

The original GFP integration plasmid (*pRib1.2alphaNEOalphaGFP*) intended to create stable expression of GFP in *Leishmania* species following integration into the parasite genome was developed and obtained from Dr. P (Canada). Transient and episomal expression of GFP in *Leishmania* species using vector, *pXG-GFP+*, was obtained from Dr K of the School of Medicine in Washington University in St Louis, USA. The GFP in both vector systems was originally isolated from *Aequorea victoria*. Expression of GFP in *Leishmania* species will enable identification and selection of transgenic parasites, and quantification of intracellular parasite growth following inhibitor screen.
There are no indications that the inclusion of the GFP in Leishmania spp. would increase virulence. There are no past publications revealing increased virulence in GFP transfected Leishmania parasites. These organisms are classified as a Category Level 2 pathogen and will be handled under these conditions at all times.

Evaluation of foreseeable effects

There are no indications that the inclusion of the GFP in Leishmania spp. Would increase virulence. There are no past publications revealing increased virulence in GFP transfected Leishmania parasites. These organisms are classified as a Category Level 2 pathogen and will be handled under these conditions at all times.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Transgenic parasites will always be maintained at the specified class II containment level for the purpose of culturing and drug screening experiments and therefore no derogation from full containment is necessary.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid culture waste will be treated with Virkon or 1% bleach and contained in a designated GMO waste vessel for appropriate disposal in a sink. All solid waste including disposable pipettes, pipette tips, culture flasks/vials and microscopic slides exposed to transgenic Leishmania parasites will be treated with Virkon or 1% bleach, wiped down and the contaminated tissue paper waste will be appropriately discarded as solid waste. Complete level of killing will be achieved with the above processes.

The category II laboratory will be regularly monitored by members of staff and laboratory manager to ensure good housekeeping and hygiene is regularly maintained with particular emphasis on treatment and disposal of infectious biological waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was approved by the genetic modification safety committee and no comments were made.

Project Containment

<table>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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</table>
**Project Additional Information**

**Purposes of the contained use**

The experimental purpose of this work is to assess whether infectious clones of cassava viruses can infect the model plant species Nicotiana benthamiana, Nicotiana tabacum as well as cassava Manihot esculenta to assess the vector ability of cassava begomovirus or Ipomovirus-whitefly populations.

**Recipient or parental organism**

The bacteria Agrobacterium tumefaciens containing the full genome of cassava viruses will be used for infection of plants. Three strain are used as detailed in the risk assessment form attached: EHA105, AGL-1 and LBA1106.

**Host/vector system**

Two binary plasmid vectors are used for the transformation of Agrobacterium: pBINPLUS and pCambia0380.

**Origin & function**

Two types of plant viruses will be inserted as part or full genome inside the vector and transformed into Agrobacterium:

* Sri lanka cassava mosaic virus (SLCMV) This virus possesses a single stranded DNA bipartite genome therefore the two genomic components (DNA-A and DNA-B) have been inserted into two distinct vectors, 
The PBINPLUS DNA-A construct contains 6 overlapping open reading frames (ORFs) (2 virion sense and 4 complementary-sense) encoding the following genes:
- Coat protein (CP) determinant of vector transmission and involved in genome encapsidation
- Movement protein (MP)
- Replication associated protein (Rep)
- Transcriptional activator protein (TrAP)
- Replication enhancer protein (REn)
- Host activation protein

The pBINPLUS-DNA-B construct contains 2 non overlapping ORF encoding 2 proteins involved in the intra- and inter-cellular movement of the virions within the host:
- nuclear shuttle protein
- movement protein

* Ugandan cassava brown streak virus (UCBSV) and cassava brown streak virus are (+)ssRNA virus and their coding region translates into a large polyprotein precursor that is further processed into 10 mature proteins:
  - first protein (P1): serine proteinase
  - third protein (P3) (also encodes a second protein, P3N-PIPO, which is generated by a +2 frameshift)
  - first 6 kilodalton protein (6K1),
  - cylindrical inclusion (CI)
  - second 6 kilodaltons protein (6K2),
  - viral genome linked protein (VPg),
  - nuclear inclusion a (Nia-Pro),
  - nuclear inclusion b (Nib)
  - HAM1h
  - coat protein (CP)

**Evaluation of foreseeable effects**

Agrobacterium tumefaciens is a naturally occurring soil bacterium responsible of crown gall disease in plants. The strains used in this study are disarmed and do not have oncogenic properties however they still possess their gene transfer abilities on the T-DNA supplied by a binary plasmid. In this project on set of Agrobacteria strains were transformed to carry the DNA-a or the DNA-b of the SLCMV and another set to carry parts or the full genome of UCBSV or CBSV.

The objective of this work is to create transient expression of the plant viruses using transformed agrobacterium, not to directly genetically transform the plant. Viral cDNAs are transferred to the plants as T-DNA and the viruses transcribed multiply and spread cell to cell. Three plant species are expected to be infected Nicotiana tabacum, Nicotiana benthamiana and Manihot esculenta using a mechanical inoculation called agroinoculation.

The disarmed Agrobacteria strains are not human or animal pathogens and do not expected to pose an immediate of plant disease upon release. The introduction of the plant viruses genes in the Agrobacterium is not expected to change the virulence of the pathogenicity of the bacteria. The genomes of the viruses inserted in the Agrobacteria have not been altered and are identical to the natural strains they are derived from, therefore their transcription from the T-DNA is not expected to generate a different than from the infection by the natural virus. Due to their ability to transfer the genetic material from their T-DNA into the plant genomes and their wide host range uncontrolled release of transformed agrobacteria could have unknown but potentially adverse effects of the local ecology and plant heat and they should therefore be contained.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable waste (tips, tubes spreaders, gloves and plates etc.) generated from culturing the transformed Agrobacteria will be bagged and autoclaved on a waste cycle before disposal for incineration waste.
All glasswear used for agrobacterium work will be soaked for at least 1 hour in 1 % Virkon before being autoclaved, then processed through washing up.
All working benches used for preparation of Agrobacterium propagation will be decontaminated by wiping the surface with 1 % Virkon after each use.
All plant material agroinfiltrated with the tranformed bacteria, as well as the whiteflies (Bemisia tabaci) feeding on the plants will be kept in a quarantined growth chamber under licence (Defra licence to import, move and keep prohibited invertebrates 24569/210645/9). All waste will added in two plastic bags and kept in a -20C freezer for a minimum of 24 hours (in accordance with the rules of the quarantine facilitie) before being autoclaved and disposed for incineration waste. The bags used have been previously tested as able to sustain a prolonged exposure to -20 degrees without tearing.
The autoclave is on a service contract and is inspected annually to make sure temperature and pressure reach the set parameters.
All the procedures detailed above, the use of quarantined facilities and the inactivation of the waste, will guarantee that no live agrobacteria will be released in the environment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Biological and Genetic modification safety committee of the University of Greenwich has reviewed and approved the risk assessment form, it has requested that an application must be made to the HSE for class II containment

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
GM Centre Number: 6

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Date at Which Additional Info Submitted: 02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory Animal Unit Growth Room Glass House Large Scale
Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  

Tick if confidential

Bacteriology  Parasitology  Transgenic  Microbiology
Birds

Virology  Transgenic  Transgenic  Gene Therapy
Animals  Fish

Mycology  Transgenic  Transgenic  Other (please
Invertebrates  Plants  specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref  101/04.1
We already know that pathogenic E.coli K-1 and pathogenic Pseudomonads lacking active twinarginine transport (Tat) systems are attenuated. We also know that E.coli tat mutants have defects in outer membrane assembly due to problems in targeting cell-wall amidases. We wish to determine whether the lack of active Tat-dependent amidases is the predominant contributing factor which results in the avirulence of tat mutant E.coli strains.

E.coli contains three cell-wall amidases AmiA, AmiB, and AmiC. We have already constructed unmarked deletions of each in E.coli K-12 using the well-established pMAK705 system. We will use our pre-existing pMAK705-based deletion constructs to generate gene knockouts in E.coli K-1. In collaboration with Dr Christoph Tang (Centre for Molecular Microbiology and Infection, Imperial College, London) the modified E.coli K-1 strains will be tested for their ability to infect a mouse model system. (NB. No virulence testing will be done at UEA)

Host: Escherichia coli K-1 is classified as an ACDP category 2 organism. It does not express verocytotoxin. It is a common cause of neonatal meningitis and it invades the bloodstream from the nasopharynx or GI tract. Note that such invasion is rare even in neonates and healthy adults do not suffer from this condition. Mutant strains of E.coli K-1 generated by this project are most likely to have drastically reduced virulence.

Vector: the vector used is pMAK705, chloramphenicol resistant, temperature sensitive for replication) is a derivative of pBR322 and is thus mobilization defective. It is extremely unlikely that pMAK705 will be transferred to other bacteria. The antibiotic resistance gene of pMAK705 is only transiently transferred to the chromosome of the host strain in the early stages of the mutant construction protocol. None of the mutant strains constructed will be chloramphenicol resistant.

Origin & function
This project does not involve the use of 'foreign' DNA in the host organism. All GMO's will lack genes encoding native amidase enzymes.
Evaluation of foreseeable effects

Final GMO: Manipulated strains are most unlikely to be enhanced in terms of their virulence. The available literature suggests strongly that the mutant strains will be severely attenuated and thus probably non-pathogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cultures will be grown in small volumes of liquid medium (10 ml or less, generally 5 ml) and on solid medium in the form of petri-dishes. All solid media will be inactivated by extensive heat and high-pressure treatment in an autoclave. This kill method is 100% effective and will result in no viable organisms remaining. Liquid cultures can also be killed absolutely by this method. Alternatively liquid cultures will be treated with broad-spectrum disinfectants. No viable organisms will survive this treatment. Killed liquid is suitable for disposal trough the general drainage system. Killed solid waste is suitable for land-fill disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All concerns raised by Committee members have been addressed by the current version of the risk assessment.

Project Containment

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Project Ref 197/00.1

02/03/2022
Understanding of the role of P53 in tumour suppression

Class 2

This project has transferred from GM197 on 22/01/2009

Tick if notifying a connected programme of work

Project notified under transitional arrangements

Historical Date of Additional Info

Significant Change ID

Date of Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 197/95.1

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**Historical Significant Changes**

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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**Project Ref** 317/trans A

**Date Ackn’d** 23/02/2007

**CU2 Project Title** MECHANISMS OF VIRAL REPLICATION AND TRANSCRIPTION

**Class**

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM**

**Consent Granted**

Tick if notifying a connected programme of work **N**

**Historical Significant Changes**

**TRANSFERRED FROM GM 317 (23/02/07).**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 317/trans B

Date Ackn'd 23/02/2007
CU2 Project Title PROTEIN DEGRADATION AND TRANSCRIPTIONAL CONTROL

Class CultureVolClass2 CultureVolumeClass3-4
Date Project Ceased

 withdrawn

 Historical Significant Changes

 TRANSFERRED FROM GM 317 (23/2/07).

 Project notified under transitional arrangements

 Project Additional Information

 Purposes of the contained use

 Recipient or parental organism

 Host/vector system

 Origin & function

 Evaluation of foreseeable effects

 Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

 For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

 Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 511/05.1

Date Ackn’d 01/05/2013

CU2 Project Title RNAi knockdown and expression of protein kinases and phosphatases and associated proteins involved in intracellular signalling pathways in mammalian cells using retrovirus vectors

Class 2
CultureVolClass2 1-50 Litres
CultureVolumeClass3-4

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
RNAi knockdown and expression of protein kinases and phosphatases and associated proteins involved in intracellular signalling pathways in mammalian cells will be used to identify the roles of these proteins in relation to the signalling pathways in which they reside. This will be undertaken using retrovirus vectors.

Recipient or parental organism
Commonly used tissue culture cell lines (HEK293, HeLa, COS cells) or embryonic stem cells, hepatocytes, fibroblast and myoblast cells derived from rodents.

Host/vector system
The retroviral vectors to be used for the studies are called pHR-SIN-CSGW, pLK0.1 (Stewart et al., 2003), pMKO.1puro (Masutomi et al 2003) or pRS (commercially available origene vector). Please use full risk assessment for more information on these systems.

Origin & function
The inserts will be either RNAi constructs designed to remove intracellular mRNAs for their target genes or the cDNAs coding for the normal intracellular versions of
kinases, phosphatases, etc. All these proteins are components of signalling pathways and so could affect cell growth. However, none will be known oncogenic versions of the proteins and so should not lead to cellular transformation. It is not known whether any of the proteins are capable of altering the properties of any naturally occurring viruses in any way that would increase their pathogenicity. However, from the known properties of the proteins this would not be predicted. The transfected cultured cell lines will not survive outwith the culture medium and all liquid and solid waste will be autoclaved on site. This rules out the possibility of escape into external environment and survival, establishment or dissemination therein.

Evaluation of foreseeable effects

The RNAi constructs are designed to remove intracellular mRNAs for their target genes and so the predicted direct effect of this will be the removal of the corresponding protein from the cells infected with the virus. This is predicted to interfere with the signalling pathway and this will what will be tested. In the majority of cases it is predicted that the removal of a component from the signalling pathway will lead to the loss of ability for that signalling pathway to function. In some cases it might be that the loss of an inhibitory component could lead to an upregulation of that pathway. Both effects are likely to be deleterious to the cell in the long run and it is not predicted that cell infected with the viruses will beat a selective advantage relative to their parent cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Autoclaving is by validated (internal temperature probe linked to printout) autoclave, in the same building, reserved only for inactivation of waste (effective 100% kill). The autoclave undergoes annual testing to ensure correct operation. Waste will be transferred to the autoclave in covered containers.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee had no major concerns regarding this risk assessment, which was a revised version of the first one submitted and which was revised following comments from the committee regarding the proposed possible use of primary human cells (now not proposed). The work is proposed to take place inside containment two laboratories and these laboratories also are used for work at level 1 containment. The committee did wish to make it quite clear that full level 2 containment practices would need to be employed during use of these retroviral vectors and that other staff working within the facilities must be made aware of the work.

Project Containment

<table>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
Purposes of the contained use

Reactive arthritis is a disease which has close linkage with the MHC Class 1 protein HLA-B27. The disease has been shown to occur post infection with intracellular bacteria such as Salmonella and Yersinia. This project aims to investigate the effect of salmonella infection at the cellular level in relation to the MHC Class 1 protein HLA-B27.

Recipient or parental organism

Both wild type Salmonella enteriditis and the non-virulent strain, Aro A-, will be transformed. The wild type strain is classified as a category 2 pathogen by ACDP. The non-infective strain is equivalent to ACDP category 1. Salmonellosis is a world-wide disease of humans and animals. The most common clinical manifestation of Salmonella infection is self-limiting, uncomplicated gastroenteritis. Mode of transmission is by ingestion of infected food or food contaminated by faeces from an infected animal or person and caecal-oral transmission from person to person. There is no evidence to support aerosol inhalation as a route of infection. Typically, antibiotic therapy is not administered as it appears to increase adverse effects and prolong Salmonella detection in stool.

Host/vector system

The vectors of choice are the non-mobilisable pUC derivatives; pECFP, pEGFP and pEYFP (plasmids supplied by Clontech, Basingstoke) expressing Enhanced Cyan Fluorescent Protein, Green Fluorescent Protein and Yellow Fluorescent protein respectively. The vectors also contain an ampicillin resistance gene for selection purposes.
The expressed proteins are fluorescent and possess no other known biological activity, and are therefore non-harmful.

### Origin & function

Salmonella enteriditis will be transfected with the fluorescent protein plasmids listed in the section above. These transformed bacteria will be used to infect well characterised human cell lines previously transfected with MHC Class 1 molecule proteins. Transformed S. enteriditis expressing fluorescent protein will provide a rapid means of detection in infected cells which will then be fixed and immunochemically counterstained and subjected to microscopy.

### Evaluation of foreseeable effects

Salmonella is capable of surviving for long periods in the environment, has a broad host range in humans and domestic and wild animals and has a large world-wide animal reservoir.

Conferring ampicillin resistance onto a pathogenic bacteria may be perceived to increase the hazard status relative to the host, however antibiotics are not typically used in the treatment of salmonellosis and alternative therapies are available (eg. amoxicillin and chloramphenical).

The ampicillin resistant GM S. enteriditis strain will have no environmental impact due to antibiotic resistance being already present in the wildtype population. This naturally occurring resistance is mainly due to the use of antibiotics in animal feedstuff and in the inappropriate treatment of Salmonella infections in humans. This resistance is transferred by R factors and as the plasmid used is non-mobilisable, should the GMM escape into the external environment, no transfer will occur between the GMM and wildtype Salmonella. Furthermore, when a similar GMM was created at the University of Lund (Sweden), there was a loss of plasmid from the host over time (personal communication from Dr M J Wick). This inability to persist in the genetically modified form further reduces the risk of any environmental impact.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**WASTE DISPOSAL**

Solid waste is collected in an autoclavable, lidded, biohazard labelled, metal bin. After each experiment the bin is taken directly to the autoclave facility for immediate autoclaving. After autoclaving solid waste is disposed of as normal refuse.

Liquid waste is collected in plastic tubes which are placed in an autoclavable, lidded, biohazard labelled, metal bin. After each experiment the bin is taken directly to the autoclave facility for immediate autoclaving. After autoclaving liquid waste is disposed of to drains with copious amounts of cold water.

**MONITORING**

Disinfection: The disinfectant of choice (Virkon) is used in strict accordance with the manufacturer's (Antec's) guidelines.

Autoclaving: To ensure 100% efficacy, testing of the autoclave is carried out annually, by the manufacturer, to demonstrate, using a 12 point test with independent thermocouples, that the correct temperature and pressure have been reached for the required time. On subsequent runs verification that the correct conditions were reached is obtained through use of a temperature probe in the centre of the load. Testing is arranged and test reports are kept by the Health and Safety Co-ordinator.

Inspections: Safety inspections are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health and Safety Co-ordinator.

Microbiological Safety Cabinets: Tested on a six monthly basis with annual KI test. Certificates of conformity are displayed on each cabinet and copies kept by the Health and Safety Information Officer.

Negative Pressure Suites: Tested annually by Estates and Buildings to ensure the suite is at negative pressure relevant to the immediate surroundings. Testing is arranged and test reports are kept by the Health and Safety Information Officer.
The committee agreed that the facilities and procedures are adequate to control level of risk presented to humans and the environment by these genetically modified Hazard Group 2 enteric bacteria.

**Project Containment**

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref 6/02.1**

**Date Ackn’d** 06/03/2002  **CU2 Project Title** INVESTIGATION OF NF-KAPPAB ACTIVATION AND FUNCTION USING ADENOVIRUS EXPRESSED PROTEINS

**Class** Class 2  **Culture Vol Class2** 1-50 litres  **Consent Granted** not applicable

**Non-GMM**  **Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**
The NF-kappaB transcription factor family consists of homo and heterodimers formed from five distinct subunits. NF-kappaB regulates many cellular genes involved in a wide variety of processes. These include immune function, inflammation, cell cycle and apoptosis. In normal cells, NF-kappaB is tightly regulated, being activated only transiently. Aberrant activation of NF-kappaB, when it becomes constitutively nuclear localised, is associated with many human diseases including inflammatory diseases (rheumatoid arthritis, asthma), cancer, atherosclerosis and alzheimer's disease.

Activation of NF-kappaB is controlled by at least three inhibitory proteins, termed IkappaB alpha, beta and epsilon. These proteins keep NF-kappaB in the cytoplasm of the cell until an appropriate signal is received. They then become phosphorylated, which targets them for ubiquitination and degradation, allowing NF-kB to translocate to the nucleus.

The purpose of this project is to assess the function of the different components of the NF-kappaB signalling pathway.

1. Vero/293 cells
These well characterised and authenticated tissue culture lines are used for the propogation of the disabled Adenovirus vector, Ad5-SVR4. In accordance with HSE guidelines this host is especially disabled and considered equivalent to ACDP Hazard Group 1.

2. Other well characterised and authenticated tissue culture cell lines (eg HeLa, U20S, THP-1, MDA-MB-231, MCF7, HT1080 and H1299) equivalent to ACDP Hazard Group 1.
These are principally to study the role of the NF-kappaB pathway in cell survival, cell growth and regulation of gene expression. Protein and RNA extracts will be made from adenivirus infected cells and analysed in various ways in the laboratory. Proliferation and apoptosis assays will also be performed. No new cell lines will be generated within the laboratory during the course of this project.

Recipient or parental organism

Attenuated Adenoviral (Ad5-SVR4) Vector System
The adenoviral vectors to be used are "attenuated" through deletion of the E1 region of the genome (containing E1A and E1B) and through insertions and deletions within the E3 gene that inactivate it. This has several affects on the virus. Firstly deletion of the E1A gene eliminates the potential for viral transformation of cells, since the E1A gene product is absolutely required for this process. It is also required for activation of all other early genes (E1B, E2, E3 and E4). The E1B gene product (also deleted) co-operates with E1A in transformation. The E2 region (E2A and E2B) contains DNA binding (E2A) and DNA polymerase (E2B) activities that are absolutely essential for viral replication. The E3 gene product helps in viral avoidance of the immune system by binding to the major histocompatability complex MHC polypeptides in the endoplasmic reticulum. However, this gene is also inactivated in this strain of adenovirus, resulting in the virus being highly susceptible to immune vurveillance. The E4 gene product is required for formation of an active complex between the E1B gene product and the E4 gene product.

The above characteristics mean that the recombinant virus is totally unable to replicate in E1A-deficient cells of any organism. Under normal circumstances no human or animal cells contain the E1A gene product, resulting in the inability of this virus to replicate in any naturally occurring organism (human or non-human mammalian). Under conditions where an organism (human or non-human mammalian) already was suffering from an adenoviral infection, it is possible that the recombinant virus could be replicated and packaged. Since this is likely to be a relatively rare event, and not one that could sustain a population of recombinant virus, the risks are extremely low. Additionally, any wild-type virus would be at a significant growth advantage with respect to the recombinant virus (for the reasons mentioned above concerning attenuation of the virus), meaning that the wild-type virus would outgrow the recombinant one very quickly.
Also, the attenuated virus cannot recombine with wild-type virus in any way that produces viable virus (Beck T. C et al Methods Cell. Biol. (1994) 43, 161-89). Recombination with a wild-type virus would, by the nature of the recombination event, remove the E1 region from the wild-type virus thereby inactivating its ability to replicate. Taken together, the above means that the recombinant virus is essentially unable to propagate in the external environment.

The wild type Adenovirus serotype 5 causes sub-clinical infections and is categorised by the ACDP as Hazard Group 2. Based on the information given above, the attenuated adenoviral (Ad5-SVR4) vector can be classified as ACDP Hazard Group 1.

Origin & function

The functions of the NF-kappaB pathway components will be elucidated by expressing dominant negative versions of different components (The NF-kappaB subunits, other transcription factors with which they interact, the IkappaBs, the IKKs and other kinases and phosphatases which control their function) in the host cells listed above. Effects on NF-kappaB regulated gene expression and NF-kappaB activation will be assessed, following cellular stimulation with, for example, lipopolysaccharide, ultra violet light or a cytokine such as TNF alpha. Furthermore, effects on cell cycle and apoptosis will be studied. Protein and RNA samples will be harvested from infected cells and analysed. These studies will be performed in a variety of cell lines, both where NF-kappaB regulation is normal and where it is aberrantly active.

Evaluation of foreseeable effects

The constructs to be used in this study, based on the NF-kappaB pathway and associated proteins have not been reported to cause immortalisation or transformation in human cells. The effects of over-expressing some of the various NF-kappaB pathway gene products have not been previously characterised in terms of immortalisation or transformation, however. It is possible, therefore, that changing the expression of genes from this pathway could be harmful. Inhibition of the NF-kappaB pathway could result in activation or inhibition of pathways that regulate the cell cycle and apoptosis, i.e., they could potentially be oncogenic. NF-kappaB also regulates inflammation and immune function. It is also possible that expression of these proteins could therefore produce adverse inflammatory effects and immune suppression. Therefore, adenoviral vectors expressing NK-kappaB pathway components and host cells transfected with these vectors, are considered equivalent to ACDP Hazard Group 2 and require Level 2 Containment.

It should be noted that many, although not all, proteins to be used in this study have been expressed in adenoviral systems in other laboratories, with no known harmful effects on the researchers. For example, adenovirus expressing a dominant negative form of IkappaB alpha, which strongly inhibits activation of the NF-kappaB pathway, is very widely used (see for example Bondeson et al. (1999). "Defining therapeutic targets by using adenovirus: Blocking NF-kappaB inhibits both inflammatory and destructive mechanisms in rheumatoid synovium but spares anti-inflammatory mediators". Proc. Natl. Acad. Sci. USA 96, 5668-5673; Wang et al. (1999). "Control of inducible chemoresistance: Enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kappaB". Nat. Med 5, 412-417). Similarly a dominant negative form of the RelA(p65) subunit has also been expressed using adenovirus (Soares 1998). "Adenovirus-mediated expression of a dominant negative mutant of p65RelA inhibits proinflammatory gene expression in endothelial cells without sensitizing to apoptosis". J. Immunol. 161, 4572-4582).

In the very unlikely event of a release from the Level 2 Containment facility, the virus could survive in the environment for a similar period of time to the wild-type virus. If the virus were to infect a susceptible host, then expression of the recombinant genes would occur in the infected cells. Since the virus cannot propagate (except in exceptional circumstances as detailed above), expression would be limited to the cells initially infected (i.e., once the cell divides, only one daughter cell will contain the adenovirus DNA. Eventually, this would result in loss of the DNA due to natural degradation of the episomal DNA. The effect that expression of the recombinant DNA would have on the cells is unknown and one of the main reasons for undertaking the experiments described in the proposal is to have a better understanding of the functions of these proteins within cells.

It should be noted that many, although not all, proteins to be used in this study have been expressed in adenoviral systems in other laboratories, with no known harmful effects on the researchers. For example, adenovirus expressing a dominant negative form of IkappaB alpha, which strongly inhibits activation of the NF-kappaB pathway, is very widely used (see for example Bondeson et al (1999). "Defining therapeutic targets by using adenovirus: Blocking NF-kappaB inhibits both inflammatory and destructive mechanisms..."

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Disposal
Solid waste is collected in a lined, biohazard labelled, bin. After work the liner is sealed and transported directly to the autoclave facility, in a dedicated wheelie bin, for immediate autoclaving. After autoclaving solid waste is disposed of as normal refuse.
Liquid waste is collected in a plastic, autoclavable flask. After work the sealed flask is transported, in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving liquid waste is disposed of to drains with copious amounts of cold water.

Monitoring
Disinfection: The disinfectant of choice (Virkon) is used in strict accordance with the manufacturer's (Antec's) guidelines.

Autoclaving: To ensure 100% efficacy, testing of the autoclave is carried out annually, by the manufacturer, to demonstrate, using a 12 point test with independent thermocouples, that the correct temperature and pressure have been reached for the required time. On subsequent runs verification that the correct conditions were reached is obtained through use of a temperature probe in the centre of the load. Testing is arranged and test reports are kept by the Health and Safety Co-ordinator.

Inspections: Safety inspections are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health and Safety Co-ordinator.

Microbiological Safety Cabinets: Services and KI tested on an annual basis. Certificates of conformity are displayed on each cabinet and copies kept by the Health and Safety Information Officer.

Negative Pressure Suites: Tested annually by Estates and Buildings to ensure the suite is at negative pressure relevant to the immediate surroundings. Testing is arranged and test reports are kept by the Health and Safety Information Officer.

Committee agreed that facilities and procedures are adequate for level of risk presented by recombinant adenovirus.

Please enter comments on the GM safety committee on the risk assessment

Committee agreed that facilities and procedures are adequate for level of risk presented by recombinant adenovirus.

Project Containment
Purposes of the contained use

This assessment covers experiments using adenovirus constructs in several groups within the Division of Molecular Physiology. Research within the Division concerns the molecular mechanisms involved in the regulation of key cellular processes including energy metabolism, protein turnover, and nutrient transport. These are all important physiological processes, which are interconnected. For example, nutrient availability directly affects energy metabolism and protein synthesis and breakdown. We are investigating the common regulatory pathways that impinge on all three of these systems. Therefore, our research is underpinned by extensive common interests and involves a wide range of collaborative projects. The use of adenovirus constructs is required in several of the cell types we use, in which high level expression of signalling proteins cannot be achieved by standard transfection techniques. The interconnected physiological processes, which are currently under investigation, are described below.

Regulation of protein synthesis. This work focuses on how protein synthesis is regulated in mammalian cells. In particular, the signalling pathways and protein kinases that regulate mRNA translation in response to insulin or nutrients or in disease states such as cardiac hypertrophy. Adenoviral vectors to study signalling in cardiac hypertrophy have been used before. Recently, studies have been initiated to study the role of the AMP-activated protein kinase in the regulation of protein synthesis.

AMP-activated protein kinase. The AMP-activated protein kinase cascade is a signalling mechanism that links cellular energy status to the regulation of energy-producing and consuming processes. It serves to switch such pathways on or off in response to cellular stresses leading to ATP depletion, eg., hypoglycaemia or (in muscle) exercise.
The AMP-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that is activated by cellular stresses that deplete cellular ATP (Hardie & Hawley (2001) Bioessays 23, 1112-1119). Once activated by stress, the kinase phosphorylates numerous targets that switch on catabolic pathways that produce ATP, while switching off anabolic pathways that consume ATP. There are also recent indications that the system may also inhibit other energy-requiring processes, such as translation, protein secretion, and progress through the cell cycle. The broad aim of the project is to gain a detailed understanding of the physiological downstream targets of AMPK by utilising adenovirus vectors to express constitutively active, dominant negative or other mutated forms of AMPK in cultured mammalian cells, thus manipulating the total cellular activity (see Woods et al. (2000) Mol. Cell. Biol. 20, 6704-6711). The adenovirus vectors are particularly used for expression of these proteins in cells where the transfection efficiency is poor, or where high level or prolonged expression is required.

Control of amino acids and glucose transport. The work concerns the mechanisms by which insulin and other stimuli control the transport of glucose and amino acids into mammalian cells. The major thrust of the work is to try and understand the molecular regulation of membrane transport processes (specifically that of glucose and amino acids) in skeletal muscle and fat in response to hormonal, stress and nutrient stimuli. The main aim is defining the intracellular signalling mechanisms by which these stimuli, acutely and chronically, elicit changes in the activity of glucose and amino acid transporters. Understanding such processes is crucial if we are to gain some insight into how defects in glucose and amino acid transport and their regulation by insulin and nutrients may contribute towards the pathogenesis of conditions such as diabetes and complications that are associated with malnutrition. For example, the translocation of GLUT4 is a widely accepted paradigm of membrane trafficking in cell biology. Nevertheless, major gaps still remain in our understanding of how insulin signalling regulates this process.

Collaborative work is undertaken on mechanisms and regulation of amino acid transporters such as System A (SAT2), System N (SN1, -2) and System L (eg Ritchie et al. (2001) Cell. Physiol. Biochem 11, 259-270; Ritchie & Taylor (2001) Biochem. J 356, 719-725; Hyde et al (2001) Biochem. J. 355, 563-568. It was recently discovered that the System L transporter protein (termed IJ12/LAT1) also mediates transport of the thyroid hormones T3 and T4 across vertebrate cell membranes (Ritchie et al. (1999) J. Endocrinol. 163, R5-R9). This discovery provides a previously missing link in the chain by which thyroid hormones (TH) in the blood reach the cell nucleus, where they are generally believed to exert their major biological effects. The identification of TH transporters offers possibilities for the design and development of novel treatments for many types of thyroid disorder, based on the therapeutic regulation of thyroid hormone entry into cells and thus their actions. In order to take this work the next step towards commercial development, we aim to demonstrate that altering TH delivery to cells has significant effects on both nuclear TH appearance and TH-responsive gene transcription.

While activation or inhibition of certain protein kinases might have effects on cell division or other cellular processes that are currently hard to predict, it is important to note that we would only ever use Ad5-based vectors that have E1a and E3 deletions, which can only replicate in cells that provide the E1a function. To this end, an attenuated strain of Adenovirus (Ad5) is used as the base vector. This had had the E1region replaced by a polylinker site. Any recombination event that would restore the self-replication status of the adenovirus will only restore the construct to the wild type Ad5 with the loss of the cloned DNA insert.

The major risk to those involved in the project is in the form of aerosol inhalation during manipulation. This is minimised by the use of class 2 microbiological safety cabinets that are annually serviced and K1 tested to ensure operator protection. Also, during centrifugation steps executed outwith the cabinet, aerosol containment conisters are employed. Furthermore, the tissue culture rooms themselves are classified as Containment Level 2 areas.

Environmental risk comes from the waste produced by the work. All solid and liquid waste is inactivated by autoclaving on site.

Recipient or parental organism

1. Vero/293 cells.
   These well-characterised and authenticated tissue culture cell lines are used for the propogation of the disabled adenovirus vector.
2. We will use a variety of mammalian cell lines and primary cultures (eg muscle, fat and heart cells in primary culture). The mammalian cell-lines we propose to use include human cell lines (HepG2, BelWo, HeLa, CCL13, HEK293A, COS, U2OS) and rodent cell lines (3T3L1, L6, HiI4E, INS1, CHO). These cells have all been well characterised with a history of safe use and are equivalent to ACPD hazard group 1).
   HEK293A cells complement the E1a deletion and will be used only to culture adenoviral stocks. The other mammalian and rodent cell types for experimental studies do not complement the E1a deletion in the adenovirus vector - it remains replication defective. The viral vector does not integrate into the host cell genome, avoiding activation or
inactivation of host genes that could manifest harmful properties not apparent in the recipient. Based upon this information, the GM cells would also be assigned to hazard
group 1.

Host/vector system

Attenuated Adenoviral (Ad5-SVR4) Vector System

The adenoviral vectors to be used are "attenuated" through deletion of the E1 region of the genome (containing E1A and E1B) and through deletions within the E3 gene that
inactivate it. This has several affects on the virus. Firstly deletion of the E1A gene eliminates the potential for viral transformation of the cells, since the E1A gene product is
absolutely required for this process. It is also required for activation of all other early genes (E1B, E2, E3 and E4). The E1B gene product (also deleted) co-operated with
E1A in transformation. The E2 region (E2A and E2B) contains DNA binding (E2A) and DNA polymerase (E2B) activities that are absolutely essential for viral replication.
The E3 gene product helps in viral avoidance of the immune system by binding to the major histocompatibility complex MHC polypeptides in the endoplasmic reticulum.
However, this gene is also inactivated in this strain of adenovirus, resulting in the virus being highly susceptible to immune surveillance. The E4 gene product is required for
formation of an active complex between the E1B gene product and the E4 gene product.

The above characteristics means that the recombinant virus is totally unable to replicate in E1A-deficient cells of any organism. Under normal circumstances no human or
animal cells contain the E1A gene product, resulting in the inability of this virus to replicate in any naturally occurring organism (human or non-human mammalian). Under
conditions where an organism (human or non-human mammalian) already was suffering from an adenoviral infection, it is possible that the recombinant virus could be
replicated and packaged. Since this is likely to be a relatively rare event, and not one that could sustain a population of recombinant virus, the risks are extremely low.
Additionally, any wild-type virus would be at a significant growth advantage with respect to the recombinant virus (for the reasons mentioned above concerning attenuation
of the virus), meaning that the wild-type virus would outgrow the recombinant one very quickly).

Also the attenuated virus cannot recombine with wild-type virus in any way that produces viable virus (Becker T.C., et al Methods Cell. Biol. (1994) 43, 161-89). Recombination with a wild-type virus would, by the nature of the recombination event, remove the E1 region from the wild-type virus thereby inactivating its ability to
replicate. Taken together, the above means that the recombinant virus is essentially unable to propagate in the external environment.

The wildtype adenovirus serotype 5 causes sub-clinical infections and is categorised by the ACDP as Hazard Group 2. Based on the information given above, the
attenuated adenoviral vector can be classified as ACDP Hazard Group 1.

Origin & function

The inserted gene products are described for the three main projects separately. None of the proteins have been reported to be harmful (with the exception of PKB, which
might have oncogenic abilities).

1. Regulation of protein synthesis has been shown to play an important role in cardiac hypertrophy. To study the role of certain signalling pathways in cardiac hypertrophy
we have made use of adenoviral vectors expressing signalling components in the ras-raf-MEK-MAPK pathway (eg. constitutively active MEK (Ser218/222 to Glu), dominant
negative N17 rat (Ser17 to Asn), mouse PKC delta and a dominant negative form (lys376 to Ala), a dominant negative rat PKB (Thr308 and Ser473 to Ala) construct was
used as control) (Wang et al (2001) J. biol. Chem. 276, 32670-32677). These experiments were carried out in the class II facilities of Dr. Sutherland, under his licence. We would like to continue these studies in the facilities on the fourth floor of the MSI.

Recently, in a collaborative study, we have started to study the involvement of the AMP-activated protein kinase (AMPK) in the control of protein synthesis. The proposed
use of adenoviral vectors is described below.

2. Regulation of AMPK. Wild type or mutant (constitutively active T172D or dominant negative D139A) forms of the truncated (312 AA) rat alpha 1 subunit of AMPK.
Constructs will also contain GFP and myc epitope tags for in vitro analysis purposes.

3. Regulation of membrane transport. (The species origin of the genes is indicated between brackets. When two species are indicated this means that the genes from
these species are available).
a. proteins involved in vesicle trafficking: syntaxin 4 (human or rat), VAMP7 (human or rat), VAMP8 (human or rat), tuberin (human or rat), hamartin (human or rat) and
TUB (human or mouse). Overexpression of wild-type and cytosolic domain, binding domain and phosphorylation site mutants.
c. Signalling proteins, eg wild-type PDK1 (rat or human), PKB (rat) (wild-type, constitutively active and kinase-dead), P13KDp85 (rat or human) (dominant negative), wild-type and constitutively active GSK3 (rat), mTOR and wild-type, constitutively active and kinase dead PKC (rat) (Litherland et al (2001). Mol.Membr. Biol. 18: 195-204).

Evaluation of foreseeable effects

The resulting GM virus: Insertion of the constructs will not alter the tissue tropism, or increase the infectivity or pathogenicity of the recipient vector. Scope for recombination with wild type virus is limited and, due to the packaging limits of adenovirus, if any such recombinants did arise they would be unviable. Based upon there being no likelihood of any effect on the phenotypic characteristics of the recipient and the non-harmful nature of the insert, the resulting replication defective recombinant adenovirus/gene vector can be considered equivalent to the recipient vector in terms of hazard status ie hazard group 1. Exception to this is perhaps GM viruses expressing PKB. As mentioned above, overexpression of PKB might have harmful effects, eg promote proliferation and increased cell survival and thereby contribute to cancer progression. However, the safe use of adenoviral constructs expressing PKB has been described before (Becker, et al. (1994) Methods Cell Biol. 43, 161-189; He et al. (1998) Proc. Natl. Acad. Sci. USA 95, 2509-2514) and class 2 containment guarantees the minimised risk associated with the use of these vectors.

In the unlikely event of a release from the Level 2 Containment facility, the virus could survive in the environment for a similar period of time to the wild-type virus. If the virus were to infect a susceptible host, then expression of the recombinant genes would occur in the infected cells. Since the virus cannot propagate (except in exceptional circumstances as detailed above), expression would be limited to the cells initially infected (ie once the cell divides, only ONE daughter cell will contain the adenovirus DNA. Eventually, this would result in loss of the DNA due to natural degradation of the episomal DNA. The effect that expression of the recombinant DNA would have on the cells is unknown and one of the main reasons for undertaking the experiments described in the proposal is to have a better understanding of the functions of these proteins within cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Disposal
Solid waste is collected in a lined, lidded, autoclavable, biohazard bin. Make sure no liquid waste is present in/on the solid waste items.
Single-use plastic, disposable pipettes will be used. Pipettes are fully immersed in 1% Virkon and soaked for at least 5 minutes before draining and disposing of as solid waste.
After work the lidded bin is transported, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving solid waste is disposed of as normal refuse.

Sharps waste are disposed of into autoclavable sharpsafe containers. The use of sharps will be avoided if possible. After work the sealed container is transported, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving the sharp-safe container is disposed of via the clinical waste route.

Liquid waste is collected in a sealable, robust, autoclavable container, stored within the MSC, clearly labelled as biohazard waste and containing enough Terminex to give a final concentration of 1% by volume. Chloros or Virkon are not used - they are not autoclave compatible! Aspirator set-ups will not be used.
After work the container is sealed and transported, in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving liquid waste is disposed of to drains with copious amounts of cold water.

Monitoring
Disinfection: The disinfectant of choice (Virkon) is used in strict accordance with the manufacturer's (Antec's) guidelines. Autoclaving: To ensure 100% efficacy, testing of the autoclave is carried out annually, by the manufacturer, to demonstrate, using a 12 point test with independent thermocouples, that the correct temperature and pressure have been reached for the required time. On subsequent runs verification that the correct conditions were reached is obtained through use of a temperature probe in the centre of the load. Testing is arranged and test reports are kept by the Health and Safety Co-ordinator. Inspectors: Safety inspections are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health and Safety Co-ordinator. Microbiological Safety Cabinets: Services and K1 tested on an annual basis. Certificates of conformity are displayed on each cabinet and copies kept by the Health and Safety Information Officer. Negative Pressure Suites: Tested annually by Estates and Buildings to ensure the suite is at negative pressure relative to the immediate surrounding. Testing is arranged and test reports are kept by the Health and Safety Information Officer.

At meeting of 6 May 2002, the Committee agreed that this work could form a Class 2 connected programme.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
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<td>L3 L4 L2</td>
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### Project Ref 6/02.3

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<th>Non-GMM</th>
<th>Consent Granted</th>
</tr>
</thead>
<tbody>
<tr>
<td>02/03/2022</td>
<td>not applicable</td>
<td></td>
</tr>
</tbody>
</table>
Recent genome projects are leading to identification of the complete gene complement of different organisms. The next big challenge is to discover the functions of all these genes. We will contribute to this by focussing on vertebrate embryonic development, particularly that of the limb. We will test gene function in development of chick and fish embryos because these are good models for vertebrate development and the eggs develop outside of the mother. We will over-express genes that encode for secreted factors by implanting cells transfected with the gene into chick or fish embryos. We will over-express genes in chick embryos using RCAS (Replication Competent Avian Sarcoma virus) or attenuated AdV (Adenoviral Vector) systems. We will inhibit expression of genes in fish embryos by using morpholinos and in chick embryos by developing new antisense strategies based on viral delivery. Some of these approaches have already been used to gain insights into fundamental aspects of embryonic development. In addition, in some cases, these experiments could provide direct links to clinical medicine. Thus it is possible to study, in these model vertebrate embryos, the roles of genes known to be responsible for human congenital defects, such as Holt-Oram syndrome. Furthermore many of the genes expressed in embryos were first identified in tumours and embryos provide an invivo multicellular context in which to find out how products of these genes function. Finally these particular embryo models provide an efficient and cheap way of exploring gene function that does not require killing adult animals.

Recipient or parental organism

1. COS (African Green Monkey) cell lines. These cells will be transfected with genes that encode secreted factors and then used as grafts to deliver the factors to embryos (see below). In addition to transiently transfected cells, stably transfected cell lines will be produced and grafted.
2. QT6 cell lines. These cells will be used for the same purposes as above.
3. Chicken embryonic fibroblasts and chicken DF1 fibroblast cell lines for RCAS production. These cells will be transfected with RCAS vectors containing genes of interest (these genes may be chicken sequences or gene sequences from other vertebrates including mouse, human or fish) and used to produce genetically manipulated virus stocks that can then be used to infect susceptible chicken embryos.
4. HEK/293 cells for adenovirus production. These well-characterised and authenticated tissue culture cell lines will be used for the propagation of the disabled Adenovirus vector.
5. Chick embryos. We will test gene function with RCAS using eggs from SPAFAS (pathogen free) chickens in addition to commercially available eggs because the embryos are more susceptible to viral infection than the embryos from our usual commercial supplier in the UK. Currently we import SPAFAS eggs from Germany. We will infect chick embryos at different stage in development in 3 ways; injection of high titre virus suspensions, grafting fibroblasts producing virus, electroporating the RCAS constructs directly into chick embryos and then letting the infection spread. For the experiments with adenovirus vectors, we will use eggs from our normal supplier and use the same three strategies outlined above to infect embryos.
6. Fish embryos and fry. We will test gene function in early development using morpholinos designed to block the function of specific genes. We will inject the morpholinos into single cells at cleavage stages. We will graft cells expressing secreted factors into embryos and young fry.

Host/vector system

1. Mammalian expression vectors will be used to introduce DNA into COS cells. These vectors only enter isolated mammalian cells under special conditions such as

02/03/2022
integrates in the host genome and the episomal DNA is eventually lost through natural degradation. A virus cannot replicate under normal circumstances, expression would be limited to the infected cell and once this cell divides to one of its daughter cells. The virus never similar period of time to the wild-type virus. If the virus infected a susceptible host, then expression of the recombinant genes would occur in the infected cells. Since the reported harmful effects on researchers. In the very unlikely event of the release of virus from the Level 2 Containment facility, it could survive in the environment for a similar period of time to the wild-type virus. 3. The adenoviral vectors to be used, such as the Ad5-SVR4 system, are attenuated through deletions in both E1 and E3 regions of the genome. The net result of these alterations is that the virus is rendered replication incompetent, the potential for viral transformation is eliminated and the ability to evade the host immune system is abrogated. Unlike the retroviruses above, these viruses do not integrate in to the genome and thus cannot cause insertional mutagenesis. Under circumstances where a host mammal (human or non-human) was already suffering from an adenoviral infection, it is possible that the recombinant virus could be replicated and packaged. However the risks are extremely low because viral recombination will be a very rare event and in any case the attenuated virus cannot recombine with wild-type virus to produce a viable virus that still contains the inserted DNA (Becker et al Methods Cell Biol (1994) 43, 161-189). Harmful effects when cells are infected will depend on the gene being manipulated and the genetically modified chicken embryos will be handled as in (2) above. The wild type Adenovirus serotype 5 causes sub-clinical infections and is categorised by the ACDP as Hazard Group 2. Most human adults are immune to these serotype 5 viruses. Based on the information given above, the attenuated adenoviral (Ad5-SVR4) vector can be classified as ACDP Hazard Group 1. 4. Morpholinos act in a cell autonomous way and must be injected into individual cells. The effect wears off after about 3 days in these genetic modifications are transitory and genetically manipulated fish embryos or fry will not be allowed to reach maturity.

Origin & function

We will obtain sequence information about genes that have already been described or from EST databases that have been assembled from different organisms. We will also design antisense sequences to interfere with function of specific genes. We will test the function of genes in embryonic development by either over-expressing the genes or by trying to inhibit their function as described above. We will assess the phenotype morphologically and also use in situ hyridisation and immunohistochemistry where appropriate to monitor any molecular charges at either mRNA or protein level. The chick system allows local genetic modification and this should enable us to test the functions of genes at different stages and in different regions of developing embryos. We will use this system to examine, in particular, how limb formation is initiated and limb bud outgrowth and patterning is controlled. The experiments with fish embryos should allow rapid screening for the effects of lack of gene function in a vertebrate model system and we will focus our attention on development of paired fins. The vertebrate genes that will be investigated will include genes encoding growth factors, transcription factors, phosphatases, enzymes, receptors, and other genes of so far unknown function.

Evaluation of foreseeable effects

We will be over-expressing products of a range of different genes and these will include both transcription factors and secreted factors. Many of the molecules involved in embryonic development are also found in tumours. However in embryos, the production of these molecules is normally tightly regulated and tumours are not produced. Avian specific retroviral vectors cannot infect humans and strains of chickens differ in their susceptibility or resistance to viruses with different envelope subgroups. Most chicken strains purchased from commercial vendors are typically C/E strains and resistant to viruses with E envelope subgroup, while the viral vectors which we will be using most often will have envelope subtypes A and B (Morgan & Fekete (1996) Methods in Cell Biology 51, 185-214). In the very unlikely event of the release of virus from the Level 2 containment facility, it could survive in the environment for a similar period of time to the wild-type virus and could infect susceptible avian species if they entered the laboratory. This is impossible because the laboratory is behind a locked door. All waste is transported in sealed containers for autoclaving in the same building. Adenoviral vectors and host cells transfected with these vectors are considered equivalent to ACDP Hazard Group 2 and require Level 2 Containment because these viruses can infect humans. These adenoviral systems have been used in many other laboratories eg to over-express both normal and dominant-negative cell adhesion molecules in chicken embryos (Nakagawa & Takeichi (1998) Development 125, 2963-2971; also see Leber et al (1996) in Methods in Cell Biology, 51, 161-181) without any reported harmful effects on researchers. In the very unlikely event of the release of virus from the Level 2 Containment facility, it could survive in the environment for a similar period of time to the wild-type virus. If the virus infected a susceptible host, then expression of the recombinant genes would occur in the infected cells. Since the virus cannot replicate under normal circumstances, expression would be limited to the infected cell and once this cell divides to one of its daughter cells. The virus never integrates in the host genome and the episomal DNA is eventually lost through natural degradation.
### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The chick embryos and embryos infected with genetically modified organisms will be kept in incubators in a room behind a locked door. They will normally only be incubated for a total of 10 days, rarely beyond and will never be allowed to hatch. The fish are kept in aquaria behind a locked door system.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

#### Waste Disposal
Solid waste is collected in a lined, biohazard labelled, bin. After work the liner is sealed and transported directly to the autoclave facility, in a dedicated wheelie bin, for immediate autoclaving. After autoclaving solid waste is disposed of as normal refuse.

Liquid waste is collected in a plastic, autoclavable flask. After work the sealed flask is transported, in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving liquid waste is disposed of to drains with copious amounts of cold water.

#### Monitoring
**Disinfection:** The disinfectant of choice (Virkon) is used in strict accordance with the manufacturer's (Antec's) guidelines.

**Autoclaving:** To ensure 100% efficacy, testing of the autoclave is carried out annually, by the manufacturer, to demonstrate, using a 12 point test with independent thermocouples, that the correct temperature and pressure have been reached for the required time. On subsequent runs verification that the correct conditions were reached is obtained through use of a temperature probe in the centre of the load. Testing is arranged and test reports are kept by the Health & Safety Co-ordinator.

**Inspections:** Safety Inspections are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health and Safety Co-ordinator.

**Microbiological Safety Cabinets:** Serviced and K1 tested on an annual basis. Certificates of conformity are displayed on each cabinet and copies kept by the Health and Safety Information Officer.

**Negative Pressure Suites:** Tested annually by Estates & Buildings to ensure the suite is at negative pressure relevant to the immediate surroundings. Testing is arranged and test reports are kept by the Health and Safety Information Officer.

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**Is an emergency plan required according to regulation 20?**

- [ ] Yes

**If yes, tick to confirm that it is attached to this form**

- [ ] Yes

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Yes

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] Yes

---

**Please enter comments on the GM safety committee on the risk assessment**

On 6th May 2002 the company agreed with the classification

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**Project Containment**
Project Title
Investigation into the proteins regulating chromosome and nuclear function in mammalian cells: regulators of transcription, DNA replication, chromosome dynamics, RNA splicing and nuclear structure

Date Ackn'd
01/03/2005

Class 2
Consent Granted

Tick if notifying a connected programme of work
Y

Historical Significant Changes

Recipient or parental organism

1. Well characterised packaging cell lines, e.g. transformed human embryonic kidney (HEK) cell lines, that provide the genes deleted from the viral vectors in trans either by way of the cells own genome or by transfecting in complementing plasmids. These lines will be used for the propagation of the recombinant viral vectors containing the gene(s) of interest.

2. Other well characterised and authenticated tissue culture cell lines, e.g. HeLa, U2)S, MCF7, HT1080. These lines will be transfected with recombinant viruses then...
analysed in various ways in order to investigate the function of the genes/proteins of interest.

**Host/vector system**

Only well characterised, replication deficient viral vector systems with a history of safe use will be used, for example: adenoviral vector systems such as attenuated Ad5, and retroviral vector systems such as pHR-SIN-CSGW, derived from the human immunodeficiency virus 1 (HIV) genome.

Such viral vectors can only propagate in special packaging cell lines (as identified above). In cells they cannot replicate and, therefore, cannot produce a lytic infection.

**Origin & function**

The work performed in laboratories of the Division of Gene Regulation expression is concerned with different aspects of cell nucleus function. This includes chromosomal processes such as transcription and DNA replication, RNA-splicing and the function of micro RNAs (miRNAs). It also includes investigations of nuclear structure such as the nucleus and cajal bodies. The genetics material inserted into the replication deficient viral vectors will consist of cDNAs encoding for full length or mutant proteins known to regulate these processes and structures. It will also consist of sequences encoding short interfering RNAs (siRNAs), which downregulate the expression of their cellular target miRNAs amd miRNAs, which have a nuclear regulatory role. The specific genetic material to be studied includes:

1. cDNAs coding for proteins that are known or suspected to play a role in transcriptional regulation, for example the NF-kappaB subunits, hypoxia-inducible factors 1, interferon regulated factors and other transcription factors and proteins with which they interact such as the ubiquitin-like proteins, the I kappaBs, the IKKs and other kinases and phosphatases which control their function. The intention is to express these genes in mammalian cells and use various techniques to analyse their effect on gene expression, transcription factor function, the cell cycle and apoptosis. Short interfering RNAs (siRNAs) which downregulate the expression of their cellular target mRNAs will also be expressed. These siRNAs will be used to inhibit the expression of transcriptional regulators and other proteins that interact or regulate them.

2. Primary microRNAs (miRNAs), precursor miRNAs, siRNAs and cDNAs coding proteins ainvolved in mRNA driven transcriptional and translational regulation. The goal of using the retroviral system is to stably express regulatory RNAs and their target proteins in human cell cultures to investigate their role in development, tumour biogenesis and miRNA mediated gene regulation.

3. cDNAs coding for proteins that are known or suspected to play a role in DNA replication and cell cycle regulation, for example the Mcm2-7 proteins, geminin, Ctd1, Cdc6, ORC subunits, Cdc7, Cbf4, cyclins, cyclin-dependent kinases, cyclin-dependent kinase inhibitors and other kinases and phosphatases which control their function. These genes will be expressed in mammalian cells and various techniques will be used to analyse their effects on DNA replication, the cell cycle and apoptosis. Short interfering RNAs (siRNAs) which downregulate the expression of their cellular target mRNAs will also be expressed. These siRNAs will be used to inhibit the expression of DNA replication proteins and other cell cycle proteins that interact or regulate them.

4. Transcription factors, subunits or factors associated with the RNA polymerase I transcription machinery. The intention is to express these proteins in mammalian cells, to study their effect on RNA polymerase I transcription of the rRNA genes in cell growth proliferation. Short interfering RNAs which target the expression of RNA polymerase I transcription machinery components and their regulatory factors will be used to study the cellular effects of down-regulating Pol I transcription in mammalian cells.

5. cDNAs coding for proteins that are known or suspected to play a role in mitotic chromosome dynamics, for example the Aurora B kinase, INCENP, surviving, borealin, topoisomerase II, Aurora A kinase, Eg5, protein phosphatase 1, Sds22 and proteins with which they interact such as the ubiquitin-like proteins, and other kinases ans phosphatases which control their function. The intention is to express these genes in mammalian cells and use various techniques, but especially imaging based assays, often in living cells to analyse their effect on mitotic progression, mitotic chromosome dynamics, and mitotic spindle assembly and function, the cell cycle and apoptosis. Short interfering RNAs (siRNAs) which downregulate the expression of their cellular target miRNAs will also be expressed. These siRNAs will be used to inhibit the expression of the mitotic proteins listed above, and regulators of these proteins.

6. The genetic material involved will consist of cDNAs coding for mammalian nuclear proteins that are either components of the nucleolus and/or components of other classes of subnuclear bodies, including Cajal bodies, PML bodies, Speckles and Paraspeckles. In most cases these will be RNA binding proteins and related factors.
connected directly or indirectly with the biogenesis, maturation and/or transport of nuclear RNAs and RNA-protein complexes. Our intention is to express these cDNAs in mammalian cells and then to study the functions and biochemical properties or the resulting protein factors. For example, we will isolate and characterize their post-translational modifications and their interaction partners. We will also study their localization patterns in the nucleus and determine their dynamic behaviour by expressing the cDNAs as GFP-fusion proteins that can be analysed by time-lapse fluorescence microscopy. Finally, we will use siRNAs to downregulate expression of targeted nuclear proteins, such as spliceosome proteins and nucleolar factors and assess the effect of such reductions in protein levels upon pre-mRNA splicing activity, including alternative splicing patterns, and on nucleolar integrity and function.

**Evaluation of foreseeable effects**

The viral vectors used may infect a wide range of mammalian cell lines and, in the case of the retroviral vectors, they can integrate into the host cell genome and stably express the gene of interest. Some of the proteins being expressed will have the potential to interfere with normal regulation of chromosome function and associated processes such as the cell cycle and apoptosis. An infected individual could, therefore, be at risk of developing a serious disease. However, with the following key controls in place the residual risk is negligible:

1. Replication defective viral vectors are used to ensure proliferation occurs only in special packaging cell lines or in the presence of complimentary plasmids;
2. Containment Level 2 is applied in full to prevent infection of the worker or release into the external environment.

The inserted genetic material is not expected to have any positive effects on the infectivity or replication competency of the viral vectors and it is highly unlikely that there will be any effect on the host range or tropism, since the proteins of interest are not cell receptors or extracellular matrix proteins.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Waste Disposal**
Solid waste is collected in a lined, biohazard, autoclavable bin. After work the liner is loosely sealed and the lidded bin transported directly to the autoclave facility, on a trolley, for immediate autoclaving solid waste is disposed of as controlled waste.

Liquid waste is collected in robust, autoclavable, sealable containers containing enough disinfectant (Terminex) to give a 1% final volume. After work the sealed container is transported, in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving liquid waste is disposed of to the drains with copious amounts of cold water.

Sharps waste is collected in a small, autoclavable sharpsafe. After work the sharpsafe is closed and transported in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving sharpsafes are disposed of as clinical waste and sent for incineration.

**Monitoring**
- Disinfection: Virkon is used to treat spills and wipe down surfaces and equipment after work. Virkon is used in strict accordance with the manufacturer’s (Antec’s) guidelines.

- Autoclaving: During the first four years after installation an annual 12-point validation test, employing independent thermocouples, is used to demonstrate the the autoclave holds the specified temperature and pressure for the required period of time. Thereafter, autoclaves are serviced and calibrated annually, by a reputable service provider, to ensure the validation criteria are met. During normal, daily operation a temperature probe, placed at the centre of the load, is used to ensure the required conditions are achieved. Servicing and testing is arranged and test reports are kept by the SLS Health & Safety Co-ordinator.
Inspections: Safety inspections are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health & Safety Information Officer.

Committee agreed that facilities and procedures are appropriate for these activities. Committee accepted that testing for replication competent retroviruses is not routinely required given that these retroviral vectors have a history of safe use. Committee also endorsed the steps being taken within the division to allay concerns staff not directly involved in the work may have about the use of a HIV based system.

Please enter comments on the GM safety committee on the risk assessment

Committee agreed that facilities and procedures are appropriate for these activities. Committee accepted that testing for replication competent retroviruses is not routinely required given that these retroviral vectors have a history of safe use. Committee also endorsed the steps being taken within the division to allay concerns staff not directly involved in the work may have about the use of a HIV based system.

**Project Containment**

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**Project Ref** 6/07.1

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Historical Significant Changes

Tick if notifying a connected programme of work N
To determine the function of a range of intracellular signalling molecules, including protein and lipid kinases, lipid and protein phosphatases and associated proteins in signal transduction pathways.

Recipient or parental organism

1. Well characterised packaging cell lines (e.g. transformed human embryonic kidney (HEK) cell lines) that provide the genes deleted from the viral vectors in trans either by the way of the cells own genome or by transfecting in complimenting plasmids. These lines will be used for the propagation of the recombinant viral vectors containing the genes of interest.

2. Other well characterised and authenticated tissue culture cell lines, (e.g. HeLa, L6, 3T3-L1, U87MG, 1321N1, MCF10A and MDCK cells) and embryonic stem cells, hepatocytes, fibroblast and myoblast cells derived from rodents. These lines will be transfected with recombinant viruses then analysed in various ways in order to investigate the function of the genes/proteins of interest.

Host/vector system

Only well characterised, replication deficient viral vector systems with a history of safe use will be used, for example:

(1) pHR-SIN-CSGW is derived from human immunodeficiency virus 1 (HIV) genome but with the viral genes deleted. This vector contains the viral long terminal repeat (LTR) but with the U3 region deleted in the 3' LTR to prevent viral enhancer and promoter transfer into target cells (thus rendering it a so-called ‘self inactivated vector’). The vector also contains the Rev Response Element (RRE) to enhance expression, packaging signals, the HIV central polypurine tract (cPPT) to increase viral titre and a WPRE (Woodchuck hepatitis virus post-transcriptional regulatory element) to enhance mRNA stability. Expression of the inserted gene comes from a SFFV (spleen focus forming virus) promoter. Derivatives of this plasmid with altered restriction sites for cDNA insertion will be used. (2) pLenti6/V5-D-TOPO is a similar self inactivating Lentiviral vector for the expression of encoded proteins from a CMV promoter in infected cells. The retroviral vector is pWZL hygro, based upon Murine Moloney sarcoma virus, containing long terminal repeats, the viral gag gene and an inserted hygromycin resistance cassette, within a bacterial plasmid vector. To produce infective virus particles, the virus requires pol and env sequences provided by a packaging cell line. (4) pQCXI vectors from Clontech. These “retro-X” vectors are self-inactivating retroviral vectors designed to express a target gene alongside a selectable or fluorescent marker gene. They are based upon the Murine Maloney Leukemia Virus. These are of similar design to the pWZL vectors, except that they lack the viral gag gene, requiring this function in trans from the packaging cell line. (5) pLKO.1 is a replication incompetent self inactivating lentiviral vector for siRNA expression, encoding a puromycin resistance gene. It is available from Addgene. (6) pMKO.1puuro vector is a modification of the pQCXIN vector (Clontech) in which the puromycin resistance gene was introduced and the human U6 promoter to drive the expression of inserted cDNAs. (7) The Origene retroviral silencing plasmid pRS, contains murine retroviral long terminal repeats (LTR), puromycin resistant gene and a U6 small nuclear RNA gene promoter to effectively express the inserted hairpin DNA and to achieve RNA interference upon introduction into a mammalian cell. Such viral vectors can only propagate in special packaging cell lines (as identified above). In normal cells they cannot replicate and, therefore, cannot produce a lytic infection. Whenever possible, when experiments can be performed using rodent cells, ecotropic viruses will be produced that will not infect human cells.

Origin & function

The inserts to be used will consist of protein and lipid kinases (e.g. AMPK, PI3K, PKB, InsR), protein and lipid phosphatases (e.g. PTEN, SHiP2) and related proteins that interact or are affected by these enzymes (e.g. adaptor, scaffolding proteins, substrates, amino acid transporters). In addition viruses will be used to express short
Interfering RNAs (siRNAs) which down-regulate the expression of their cellular target mRNAs. These siRNAs will be used to target the same proteins listed above. As signalling pathways regulate cell growth and control transformation, it is possible, therefore, that changing the expression of genes from this pathway could be harmful. As signalling pathways also affect the immune system, it is also possible that expression of these proteins could therefore produce adverse inflammatory effects and immune suppression. The inserted genetic material is not expected to have any positive effects on the infectivity or replication competency of the viral vectors and it is highly unlikely that there will be any effect on the host range or tropism, since the proteins of interest are not cell receptors or extracellular matrix proteins.

**Evaluation of foreseeable effects**

It is theoretically possible that recombination between the retroviral vector and the retroviral components (transfected or stably integrated) in the packaging cell line could produce replication competent retroviruses (RCRs). However, the proposed vectors are in wide use and details of the plasmids and the demonstration that they do not give rise to replication competent viruses are available in Zufferey et al. Nat. Biotechnol. 15:871-875, 1997 and Zufferey et al, J. Virol. 72:9873-9880, 1998. There is evidence that such replication defective viruses are not pathogenic when tested in mice and a minimum of 3 recombination events are necessary to produce replication competent, recombinant virus. The effects of expression or knockdown of signalling components are unknown for many signalling molecules. It is possible that cell growth or immune function could be affected. Although retroviral particles and infected cultured cells would not survive outside culture medium, thorough measures must be taken to avoid exposure to workers. It is also possible that accidental exposure to the non-replicative virus would lead to chromosomal insertion of the virus in some cells of the worker. This apparently random insertion could inactivate or activate genes close to its point of insertion, with unknown consequences. However, the chances of such insertion occurring, and at such a pathologically significant site seem very small. Similar risks are associated with many delivery systems. The risk of replicative viral infection with these viruses seem remote. As described, this system has been developed to minimise this risk, which is also indicated by the development of these viruses for gene human therapy (see eg Braybrooke et al, 2005, Clin. Cancer Res. 11, 1512 ; Barquinero et al 2004 Gene Ther. 11 S3-9). The transfected cultured cell lines would not survive outwith the culture medium ruling out any possibility of survival, establishment or dissemination in the external environment. Also, retrovirus particles are relatively fragile, being easily inactivated by 0.1% detergent, chloroform, phenol, 1% bleach, 70% ethanol, 65oC for 30 min, pH <6.5 or >9.0, UV light, or autoclaving.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Waste Disposal | Solid waste is collected in a lined, biohazard labelled, autoclavable bin. After work the liner is loosely sealed and the lidded bin transported directly to the autoclave facility, on a trolley, for immediate autoclaving. After autoclaving solid waste is disposed of as controlled waste. Liquid waste is collected in robust, autoclavable, sealable containers containing enough disinfectant (Terminex) to give a 1% final volume. After work the sealed container is transported, in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving liquid waste is disposed of to drains with copious amounts of cold water. Sharps waste is collected in a small, autoclavable sharpsafe. After work the sharpsafe is closed and transported in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving sharpsafes are disposed of as clinical waste and sent for incineration. Monitoring | Disinfection: Virkon is used to treat spills and wipe down surfaces and equipment after work. Virkon is used in strict accordance with the manufacturer's (Antec's) guidelines. Autoclaving: During the first four years after installation an annual 12-point validation test, employing independent thermocouples, is used to demonstrate that the autoclave holds the specified temperature and pressure for the required period of time. Thereafter, autoclaves are serviced and calibrated annually, by a reputable service provider, to ensure the validation criteria are met. During normal, daily operation a temperature probe, placed at the centre of the load, is used to ensure the required conditions are achieved. Servicing and testing is arranged and test reports are kept by the College of Life Sciences Health & Safety Co-ordinator. Inspections: Safety Inspections are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health & Safety Information Officer. Microbiological Safety Cabinets: Serviced and KI tested on an annual basis. Certificates of conformity are displayed on each cabinet and copies kept by the Health & Safety Information Officer. |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form |

02/03/2022
Committee agreed that facilities and procedures are appropriate for these activities. Committee accepted that testing for replication competent retroviruses is not routinely required given that these retroviral vectors have a history of safe use.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tr>
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<td>L2 L3 L4</td>
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### Project Ref  6/07.2

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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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</thead>
<tbody>
<tr>
<td>15/06/2007</td>
<td>Investigation into the factors involved in the pathogenesis of plant pathogens.</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td></td>
<td>Not Applicable</td>
<td></td>
<td></td>
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<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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<tbody>
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<td>N</td>
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<td>Y</td>
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### Project Additional Information

- Historical Significant Changes
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

Page 112 of 15326
Purposes of the contained use

To identify and investigate the role of genes/proteins involved in determining the pathogenicity and virulence of phytopathogens such as Streptomyces scabies and Pseudomonas syringae. Note: this project does not involve infection of plants - it is confined to the study of the pathogens themselves.

Recipient or parental organism

1. Plant pathogens, for example:
   - Streptomyces scabies: an opportunistic plant pathogen and causative agent of scab on root vegetables. Scab causing Streptomyces sp. are already endemic to the UK and do not cause economically significant damage on crops other than potatoes.
   - Pseudomonas syringae, pathovars maculicola and tomato: coronatine (a chlorosis-inducing phytotoxin) producing plant pathogens, endemic to the UK, causing bacterial leaf spot (or speck) on cruciferous vegetables and tomatoes respectively.

2. Commonly used, non-pathogenic, non-colonising cloning hosts, e.g. Streptomyces lividans, derivatives of E coli K12.

Host/vector system

Commercially available, widely used host/vector systems will be used for recombinant gene expression, including:

- Replication compromised shuttle vectors manipulated in lab adapted strains of E.coli. These recombinant vectors will then be transferred to the recipient via electroporation or by triparental mating via a suicide helper-plasmid.
- Lab adapted strains of E. coli and non-transmissible vectors used to construct cosmid libraries. Clones that contain the gene(s) of interest will be isolated by hybridisation and genes will be expressed in cloning hosts such as S lividans.

Origin & function

Primary focus of the work includes:

- The Tat (twin-arginine translocation) protein export system is found in the cytoplasmic membrane of most prokaryotes and is dedicated to the transport of folded proteins. The Tat system is now known to be essential for many bacterial processes including energy metabolism, cell wall biosynthesis, the nitrogen-fixing symbiosis and bacterial pathogenesis. In this project, Tat system genes in plant pathogens will be deleted/manipulated to identify and investigate the role of potential Tat-dependent pathogenicity determinants.
- Antibiotic-like gene clusters previously identified in certain plant pathogens will be assessed by phenotypic characterisation of transformed hosts. Putative functions will be confirmed by deletion of the antibiotic-like genes from the cosmid clone, and by deletion of the corresponding genes from the chromosome of the pathogen.
- Screening for additional proteins involved in virulence or multicellular behaviour of plant pathogens will be performed by transposon or targeted mutagenesis. Functions of gene products identified by mutagenesis will be ascertained by expressing them in E. coli.
- Phytopathogens may ultimately be modified by the introduction of certain avirulence genes. The purpose of these modifications is to be able to selectively elicit plant disease resistance responses.

Evaluation of foreseeable effects

Deleting/manipulating the tat system, or other genes involved in pathogenesis or secondary metabolite production, is highly unlikely to result in increased pathogenesis. The Tat system has already been deleted from a range of organisms (including plant and animal pathogens) and has universally resulted in strains less fit than the wild-type and, in the case of pathogens, lower pathogenicity than the wild-type. In the unlikely event that the organisms were to escape into the environment, they would almost certainly not persist due to their inability to compete with wild-type organisms. Introducing avirulence genes has the potential to restrict the already narrow host range of the phytopathogens that will be studied.

Therefore, no foreseeable adverse environmental effects are expected and risk to the environment is negligible.
None of the phytopathogens used in this project pose a risk to human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Disposal
Solid waste is collected in a lined, biohazard labelled, autoclavable bin. After work the liner is loosely sealed and the lidded bin transported directly to the autoclave facility, on a trolley, for immediate autoclaving. After autoclaving solid waste is disposed of as controlled waste.

Liquid waste is collected in robust, autoclavable, sealable containers. After work the sealed container is transported, in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving liquid waste is disposed of to drains with copious amounts of cold water.

Sharps waste is collected in a small, autoclavable sharpsafe. After work the sharpsafe is closed and transported in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving sharpsafes are disposed of as clinical waste and sent for incineration.

Autoclaving: During the first four years after installation an annual 12-point validation test, employing independent thermocouples, is used to demonstrate that the autoclave holds the specified temperature and pressure for the required period of time. Thereafter, autoclaves are serviced and calibrated annually, by a reputable service provider, to ensure the validation criteria are met. During normal, daily operation a temperature probe, placed at the centre of the load, is used to ensure the required conditions are achieved. Servicing and testing is arranged and test reports are kept by the College of Life Sciences Health & Safety Co-ordinator.

Disinfection
Virkon, typically as a 1% solution, is used to treat spills and wipe down surfaces and equipment after work. Virkon is used in strict accordance with the manufacturer’s guidelines.

Monitoring
Safety Inspections are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health & Safety Information Officer.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Committee agreed that facilities and procedures are appropriate for these activities.
Project Containment

Laboratory Activities
- L2: Yes
- L3
- L4

Glass Houses
- L2
- L3
- L4

Growth Rooms
- L2
- L3
- L4

Animal Units
- L2
- L3
- L4

Large Scale Activities
- L2
- L3
- L4

Human Clinical Applications
- L2
- L3
- L4

Project Ref 6/09.1

Date Ackn’d 28/01/2009

CU2 Project Title
Investigation of an in vitro and in vivo cellular processes of hazard group 2 bacterial pathogens clinically relevant in the UK

Date Project Ceased

Class
Class 2

Culture Vol
1-50 Litres

Consent Granted
Not Applicable

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID 6/09.1

Date of Significant Change 19/09/2017

Project Additional Information

Purposes of the contained use
To investigate cellular processes at a molecular level that may be of clinical relevance

Recipient or parental organism

Hazard Group 2 micro-organisms listed in The Approved List of Biological Agents, and Hazard Group 1 and 2 micro-organisms as categorised by ACDP guidelines.

Replication defective viruses and associated well characterised packaging cell lines as detailed in Section 2.6 to 2.12 SACGM Compendium of Guidance that can be categorised as ACDP Hazard Group 1 or 2.
Well characterised tissue culture cell lines
Primary cells.

Host/vector system
Well Characterised systems with a history of safe use will be used, for example: commercially available viral vector systems, non-mobilisable plasmid and bacteriophage vectors.

Origin & function
Wild type and mutant forms of genes and genetic material isolated from prokaryotic and eukaryotic organisms or chemically synthesised such as receptors, signalling molecules, transcription factors, enzymes, growth factors, cytokines, non-coding regulatory elements, anti-sense constructs and siRNA, reporter genes and selectable markers.

Evaluation of foreseeable effects
The genetically modified micro-organisms are likely to cause similar effects on human health and the environment as the wild type parent. Therefore, the containment measures for the parental micro-organisms (ie Containment Level 2 and additional measures required by licensing authorities) will be required to reduce the risks to research and support staff and to the broader environment to an acceptable level.

It is extremely unlikely that the genetic modification will alter significantly the fundamental properties of the parental micro-organism such as pathogenicity, infectivity, virulence, survivability, host range and/or response to prophylaxis/treatment. Therefore, additional Containment Level 3 control measures such as sealability for fumigation, HEPA filters on extract air and autoclave within laboratory suite are not merited for the level of risk i.e. not reasonably practicable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Disposal
Solid waste is collected in a lined, biohazard labelled, autoclavable bin. After work the liner is loosely sealed and the lidded bin transported directly to the autoclave facility, on a trolley, for immediate autoclaving. After autoclaving solid waste is disposed of as controlled waste.

Liquid waste is collected in robust, autoclavable, sealable containers. After work the sealed container is transported, in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving liquid waste is disposed of to drains with copious amounts of cold water.

Sharps waste is collected in a small autoclavable sharpsafe. After work the sharpsafe's temporary closure is engaged and it is transported in a plastic tub, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving sharpsafes fully sealed then disposed of as clinical waste and collected by a specialist contractor.

Monitoring
Disinfection: Virkon is used to treat spills and wipe down surfaces and equipment after work. Virkon is used in strict accordance with the manufacturer's guidelines and efficacy data.
Autoclaving: During the first four years after installation an annual 12-point validation test, employing independent thermocouples, is used to demonstrate that the autoclave holds the specified temperature and pressure for the required period of time. Thereafter, autoclaves are serviced every 6 months by a reputable service provider and calibrated annually to ensure the validation criteria are met. During normal, daily operation indicator tape and, in the case of liquid waste a temperature probe placed at the centre of the load, are kept by the CLS Health and Safety Coordinator.

Inspections: Safety inspections are carried out regularly to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health and Safety Information Officer.

Please enter comments on the GM safety committee on the risk assessment

This is a broad connected programme of work supported by five risk assessments:
1. Assessment 1107 involves work with commercially available replication defective retroviral vectors that have a history of safe use to express potentially harmful genes such as those encoding proteins involved in signalling pathways.
2. Assessment 1111 involves work with a replication competent adenovirus to express genes involved in SUMO pathway - these genes could be harmful.
3. Assessment 1125 involves work with replication competent vaccinia virus. The inserted genetic material is unlikely to be harmful, and the insertion is likely to attenuate the virus.
4. Assessment 1124 involves work with Campylobacter jejuni-the most common cause of bacteria gastro-enteritis in the Western world. The genetic modification is likely to reduce its pathogenicity.
5. Assessment 1123 involves work with Salmonella typhimurium that will be modified to express siailated ligands on its surface. This modification could increase the virulence of the pathogen. However, it is unlikely that the modification will alter how these bacteria are transmitted (ie ingestion or injection) or their susceptibility to disinfectants/heat or antibiotics. Therefore, Containment Level 3 measures (ie room fumigation, HEPA extract air, sealable) are not required, nor does the laboratory need to be under negative pressure (not airbourne spread) ie implementation of these measures is not reasonably practicable since they will not significantly reduce the major risk of spread of infection by ingestion or injection.

The committee agreed that the Containment Level 2 facilities and SOP's for this programme of work are adequate for the level of risk. Special attention was given to in vivo work and it was confirmed that this work will be carried out in a dedicated laboratory, IVC and a class 2 change station or Class 2 microbiological safety cabinet will be used.

Therefore, the Committee agreed that this connected programme of work should be notified as Class 2.

Project Containment

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02/03/2022
**Project Ref** 6/12.1

**CU2 Project Title**
Use of genetically modified hazard group 2 viruses indigenous to the UK in molecular studies of innate and adaptive immunity

**Class** Class 2

**Culture Volume** 1-50 Litres

**Project notified under transitional arrangements** N

**Non-GMM Consent Granted**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Project Additional Information**

**Purposes of the contained use**
To investigate at a molecular level the mechanisms of innate and adaptive immunity

**Recipient or parental organism**
Virus species indigenous to the UK and classified by the ACDP into Hazard Group 2, e.g. Influenza A virus, Vaccinia virus and Epstein-Barr virus. Well characterised and authenticated tissue culture cell lines will be infected for propagation of viral stocks. We may also carry out viral propagation in embryonated chicken eggs if this is the only reliable method, e.g. in the case of influenza A. Laboratory mice will be infected for in vivo studies.

**Host/vector system**
HG2 viruses will be received from collaborators ready modified or be genetically modified in-house using well established techniques, e.g. the reverse genetics system established by Enami and colleagues to rescue genes derived from cDNA into influenza A viruses.

**Origin & function**
Existing genes may be mutated or knocked down to investigate the effect this has on the immune response. Foreign sequences may also be inserted into the viral genome.
but only as markers/tags that provide a ‘read out’ and allow us to map and/or measure the immune response, e.g. a foreign epitope which can be detected with a T cell hybridoma following antigen processing.

**Evaluation of foreseeable effects**

Any viruses used in this project will be ACDP Hazard Group 2, i.e. they can cause human disease and may be a hazard to employees but are unlikely to spread to the community and there is usually effective prophylaxis or treatment available.

There is no intention to perform a knock down, mutaton or insertion that will increase the virulence of pathogenicity, or extend the host range, relative to the unmodified parent virus, i.e. any genetic modifications will invariably have a neutral or deleterious effect. As indicated above, inserted genetic material will code for peptide sequences that act as markers/tags and will not be inherently hazardous.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Solid waste will be autoclaved then disposed of to landfill.
- Liquid waste will be autoclaved then put to drain.
- Trace contaminated reusable labware and equipment with will be autoclaved if possible. If this is not possible, it will be disinfected with 1% Virkon overnight before being disposed of to drain.
- Any liquid waste that cannot be autoclaved (e.g. chemical or radiological contamination) will be disinfected with 1% Virkon overnight before being disposed of as special or radiological waste.
- Animal carcasses will be autoclaved then disposed of as clinical waste.

**Is an emergency plan required according to regulation 20?**

- [ ] Yes

**If yes, tick to confirm that it is attached to this form**

- [ ] Yes

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Yes

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] Yes

**Please enter comments on the GM safety committee on the risk assessment**

Risk assessment reviewed and approved by Committee members on 24th Jan 2012

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**Project Containment**
We propose a systematic SILAC-based study of FMDV-induced proteome changes with subcellular resolution and to provide relative evaluations of their significance in FMDV replication and in cells supporting persistent replication. We aim to provide high-depth proteome coverage of 10,000 or more host-cell proteins. Control and FMDV replicon-transfected cells will be pooled at 2, 4, 6 and 8hrs posttransfection then extensively fractionated using ultracentrifugation methods. Fraction proteins will be isolated and further fractionated by 1D SDS-PAGE. Gel lanes will be cut into 12 slices and the proteins from each digested with trypsin, resulting in peptides eluted and analysed by high resolution mass spectrometry (MS). FMDV replicons cannot directly form infectious virus particles as capsid sequences have been deleted preventing the formation of particles to package and transmit the RNA to another susceptible cell.
**Recipient or parental organism**

FMDV replicons will be transfected into HeLa, BHK and a virus free, immortalised bovine cell line*. These host cell lines present no hazard to human health.

*Specific bovine cell line yet to be determined. As cattle is one of the hosts  

8. Containment and control measures for GMOs that are not micro-organisms (e.g. GM animals and plants)  
9. Maximum culture volumes per experiment - for GMMs only for FMDV, we want to make absolutely sure the cell line's genome does not code for FMDV capsid proteins.

**Host/vector system**

FMDV replicon RNA will be transfected directly into the host cells.

**Origin & function**

The FMDV replicon will be supplied by Prof Matrin Ryan, University of St Andrews. It includes sequences derived from foot-and-mouth disease virus genome, however, NO structural protein sequences are included. The replicon is thus is unable to generate virus particles or mediate cell-to-cell spread. It is limited to the cell into which it is introduced by transfection and maintained episomally.

**Evaluation of foreseeable effects**

The FMDV replicon is non-hazardous for the following reasons.

i) It does not encode any structural proteins and therefore lacks the ability for replicon-derived RNA to be packaged. It is therefore impossible to produce FMDV particles from this construct. Naked replicon RNA is highly unstable and cannot survive in the extracellular environment. Furthermore, it can only be transmitted to naïve cells using sophisticated laboratory-based techniques.

ii) Trans-encapsidation with structural proteins from other viruses would be extremely unlikely as this would need to have arisen from a) the presence of both the replicon and a replicating virus within the same cell and b) the ability of the coat proteins produced by the other virus to recognise and package FMDV subgenomic replicon-derived RNA. Even if both of these events were to occur, the resulting particles could not set up an infection as they would lack any genes encoding structural proteins and thus would only be able to undertake cell entry and replication of non-structural proteins. Furthermore, the absence of the structural protein coding sequences would prevent packaging and cell-cell spread.

iii) A recombination event between the replicon and another virus (termed X) could only theoretically occur if these were very closely related (e.g. another picornavirus) and if both were present in the same cell. The lack of any other picornavirus research in the laboratory, together with the containment precautions proposed for this work, mean that this event is extremely unlikely.
to occur. However, even if this highly unlikely event were to occur, recombination involving incorporation of viral structural genes into the replicon would result in the presence of structural proteins from virus X, not from FMDV. Even if the resulting construct was able to make RNA and proteins successfully, the capsid proteins of virus X are highly unlikely to be competent to package the RNA. But even if this was to occur, any particles would have the capsid of virus X (not FMDV) and therefore, due to inability to bind to appropriate receptors, would not duplicate the pathogenic profile of FMDV.

Furthermore, as picornaviruses do not replicate via a DNA intermediate and replicate entirely in the cytoplasm, there could be no host cell mediated homologous DNA recombination. Recombination between host RNA molecules is not a possible route for the provision of structural protein sequences. There is no impact on antibody testing for FMDV due to the absence of FMDV structural proteins.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

FMDV replicon work has been previously notified to HSE, initially by Prof Martin Ryan, University of St Andrews. HSE deemed that Containment Level 2 plus diligent sample and waste tracking were required. Therefore, waste disposal procedures specified in CLS SOP 48, Containment Level 2: Tissue Culture Facilities, as detailed below, will be followed.

*Waste must be disposed of correctly & all waste containers clearly labelled with your name, lab of origin, nature of the waste & the word "BIOHAZARD". Ensure no chemical waste is present in containers destined for the autoclave.*

- True sharps waste & plastic pipette tips must be disposed of into an autoclavable sharpsafe container kept within the MSC. Engage temporary closure before removal for disposal. The use of sharps must be avoided unless essential.
- Do not use large glass pipettes.
- Large plastic pipettes must be filled and drained 3 times using 1% Virkon, kept within the MSC if one is in use, before being drained completely and disposed of as solid waste.
- Solid waste must be collected in a red, lined, biohazard labelled, autoclavable bin (available from Media/Wash-Up). There must be no liquid in the solid waste. Do not use the cardboard biohazard bins.
- Liquid waste must be collected in a sealable, robust, autoclavable container. Do not use the aspirator setups.
- Blood and tissue waste (recognisable as such) must be disposed of as clinical waste (see the CLS H&S web site Waste Disposal section for further details).
- Radioactive or chemically toxic waste must not be autoclaved. Consult your Biological Safety Advisor (BSA) for advice.
At the end of the work session:
Bin liners must be loosely taped around the neck of the bag to prevent spillage during transport to the autoclave. Sharpsafes, with the temporary closure engaged, and liquid waste containers must be placed in the red biohazard bin along side the bags of solid waste and the bin removed to the anteroom. Tape the lid onto the red bin with a strip of autoclave tape and clearly write "WASTE" on the tape. Inform the Wash-Up Technician that there is CL2 waste ready for immediate uplift."
In addition to the above, to ensure a fully auditable trail, the following measures will be implemented:
- A record of all FMDV replicon waste transferred to the autoclaving facility and all autoclave runs will be kept. Note: our standard autoclaving conditions of 124 degrees C for 20 minutes will suffice.
- Once autoclaved, the FMDV replicon waste will be transferred to a large clinical waste bin.
- When full, this bin will be sealed and taken, by Safety Services personnel, the secure clinical waste freezer for final uplift and incineration by an external, specialist contractor.
Virkon, at a final concentration of 1%, will be used for surface disinfection and treatment of spills.

Please enter comments on the GM safety committee on the risk assessment
Members are reassured by the detailed phylogenetic studies carried out by St Andrews University evidencing the likelihood of generating an infectious virus harmful to the environment is extremely low. However, members noted that once inside a cell the Replicon has all the viral sequences required to replicate leading to cell death within a few hours.
Members noted there is no reliable evidence of human illness arising directly, or indirectly, from infection with FMDV-despite it being endemic in several countries and epidemic in others.
Members acknowledged the SACGM decision in 2004 to allow these studies to be carried out at Containment Level 2 with additional measures to keep materials secure at all times. In accord with this decision members agreed CLS Containment Level 2 facilities and Safe Operating Procedures are appropriate for the level of risk with the addition of streaming autoclaved waste as Clinical Waste for incineration.
Members agreed that the risk to the environment from proposed work in the future with extracts from virally infected cells provided by collaborators is not addressed by this risk assessment. This work will have to be risk assessed and approved by this Committee before it commences.

Project Containment

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02/03/2022
Use of genetic tools to study the basic biology/biochemistry of the Cryptosporidium parvum parasite, and to identify and validate potential drug candidates for cryptosporidiosis.

The intracellular parasite Cryptosporidium parvum will be used as a model organism due to the fact that out of 32 identified species Cryptosporidium it is one of only two species that cause disease in humans. Further, this species is amenable to genetic manipulation and is easily maintained in the laboratory. The effects of clinical infection with Cryptosporidium parvum are well known and range from asymptomatic to severe and sometimes fatal illness. However, the latter is only seen in immunocompromised individuals or those with severe underlying health issues. Most people who contract cryptosporidiosis make a full recovery without treatment.

Recipient or parental organism

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Host/vector system

The host is C. parvum Iowa II strain. This is the only strain of Cryptosporidium that will be used. To generate GMO Cryptosporidium, the organism is transfected with (1) A CRISPR/Cas9 plasmid containing a guide RNA sequence that targets the gene of interest and (2) a linear DNA fragment that contains a gene for NanoLuciferase and a selection marker. The plasmid specifically contains Streptococcus pyogenes Cas9 gene under control of Cryptosporidium regulatory elements. This plasmid also contains the Cryptosporidium U6 promoter to produce a guide RNA. The resulting RNA molecule directs the activity of Cas9 to act against a specific sequence of the
Cryptosporidium genome. Plasmids are not maintained in Cryptosporidium, therefore Cas9 and the guide RNA are only transiently expressed by Cryptosporidium and are not maintained from generation to generation. However, the linear DNA molecule containing the gene for NanoLuciferase and a selection marker (neomycin resistance gene) is incorporated into the genome at the site designated by the guide RNA. The guide RNA "guides" Cas9 to make a double stranded break in the chromosomal DNA at a specific sequence and the endogenous homologous repair machinery of Cryptosporidium repair this break by integrating the linear DNA fragment. This linear DNA fragment modifies the chromosomal DNA, for example by deleting a gene of interest, or by inserting an epitope tag at the C-terminus of the encoded protein (to allow for expression and localization studies). To encourage homologous recombination at the site designated by Cas9, the linear DNA fragment is flanked with regions homologous to the chromosomal insertion site. This integrated DNA fragment will be inherited by all progeny organisms and maintained in the chromosomal DNA.

Origin & function

The genetic material is either C. parvum genetic material being used to generate mutant strains or it is marker genes (GFP, YFP, etc) to identify expression patterns, reporter genes (luciferase, etc) to study expression levels, epitope tags (HA, GST, etc) for protein purification or the drug resistance marker (Neomycin resistance) that is needed to select for transgenic organisms. All these materials will be derived synthetically or via production of genetic material in E. coli using standard molecular biology approaches.

Evaluation of foreseeable effects

It is foreseeable that workers could become infected with C. parvum, either the parental strain (Iowa II) or any of the genetically modified strains. It is considered that none of the mutations are likely to increase pathogenicity of the organism and, indeed, many of the mutations will likely be deleterious to the organism. Neomycin Resistance (confers resistance to paromomycin) is introduced into the GM organisms in order to select for the genetic change. Paromomycin is an effective drug for treatment of cryptosporidiosis in immunocompromised mice, but not in human cryptosporidiosis and so will not affect the virulence of the organism.

The inherent homologous recombination machinery of C. parvum will be used to integrate transgenes at specific locations in the genome. All transgenes used to generate transgenic Cryptosporidium strains are chromosome borne. Currently there is only a single drug resistance marker for Cryptosporidium, therefore only a single cassette of transgenes may be introduced into a strain. Transgenic C. parvum are maintained under drug pressure, selecting for growth of only transgenic organisms. Additionally, all transgenic strains are produced from the same parental C. parvum Iowa II strain, so recombination or gene transfer from transgenics to Wild Type does not increase the genetic diversity. Cryptosporidium do not stably maintain plasmids, therefore transgenes are all chromosome borne. Transgenes expressed by Cryptosporidium parvum are not transferred from Cryptosporidium to its host (human cells in co-culture or infected animals - humans included).

Cryptosporidium are water borne, but cannot replicate in the environment. Cryptosporidium parvum can only survive and grow inside the host organism (cow, sheep, humans, immunocompromised mice).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Water bottles and used feed must be autoclaved prior to washing or disposal by placing in taped closed - autoclavable biohazard bag. Autoclave using SLS standard conditions.

Dirty bedding and isolator units must be autoclaved prior to disposal by placing in taped closed, autoclavable biohazard bag - autoclave using SLS standard conditions.

Flasks, pipettes, plates, glassware, spent media, etc will be autoclaved prior to washing or disposal by placing in taped closed, autoclavable biohazard bag - autoclave using SLS standard conditions. Spills/waste (media, feces, tissue, towels, etc) from sample collection will be placed into a biohazard bag in a biohazard bin; other surface spills will be treated with 3-6% H2O2 application for 20 minutes. Any needles/blades used for work or unintentional sharps (from broken glass/plastics) will be placed in a sharps container which is then disposed of via standard SLS clinical sharps disposal route. Biosafety cabinet/work areas - 3-6% H2O2 application for 20 minutes, or use of Diversey Oxivir Disinfectant Spray (or similar) and wipe down following handling of parasites on surfaces or nonporous materials contaminated with parasites. H2O2 made
The GM Safety Committee supported the proposed work. Discussion of the following points took place:

- **Appropriate disinfection protocols for Cryptosporidia, including alternatives to hydrogen peroxide. Neopredisan, a chlorinated phenol derivative was suggested.**
- The risk assessment notes greater risk for the young and immunocompromised. The Committee suggested that there should therefore be extra consideration given to those working with the organism who are immunocompromised or who have young children. Especially, in this regard to pregnant persons and those who have recently given birth/breast feeding. The Committee stated that the decision of how to implement this would be up to the PI, but suggested that the PI may wish to ensure that any such persons are given the option not to work with the organism. This would include anyone else who is significantly immunocompromised (noting, of course, that people must have disclosed this to the PI in order for action to be taken).

- In section 2a - hazards to human health. The Committee approved of a comment about using an attenuated strain if this becomes available. This shows the PI has considered this risk reduction principle (even though at present such a strain is not available). Likewise, the point about in vitro culture (although that is not directly related to human health).

- In section 3 – The Committee noted that the PI made a comment about Cryptosporidia not being able to be aerosolised and asked for more clarification of what is meant by this. The Committee wondered whether the PI meant that desiccation would occur and would inactivate the organism.

- In section 4a – direct effects on the environment of the host. The Committee noted that the organisms is endemic and as such no further comment is required.

- In section 4b - The genetic material. The Committee noted that the PI mentioned the antibiotic resistance cassette used being effective against an antibiotic that is relevant to treatment in immunocompromised mice. The Committee wanted to know whether this has any relevance to wild mice or other organisms in the wider environment.

- In section 5b. The PI answered no to a requirement for work to take place within a microbiological safety cabinet. The Committee wanted more clarification about the aerosol issue noted above in relation to this point.

The Committee wished for more information in relation to spill procedures and whether the PI has a special spill procedure for uncontained spills. For example, if material containing the organism were spilled on the floor of the faecal room, what would the procedure be? Would it be necessary to disinfect the floor with H2O2?

### Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Production of pLGICs (membrane proteins) by the lentiviral transduction of mammalian cell lines

The proposed work will provide a means to study at a molecular and cellular level the functions of proteins involved in a range of fundamental cellular processes such as DNA replication, protein synthesis, inter- and intracellular signalling. This will allow us to investigate the molecular mechanisms that are important for normal cellular functioning and also the causes of a range of serious diseases such as cancer, diabetes and neurological conditions.

A range of eukaryotic cell lines will be the recipient organisms. The cells will generally be well characterised, continuous rodent, primate or human cell lines that have an established history of safe use. However, in some cases finite or less well characterised primary cell lines may be used. In such cases, care will be exercised to minimise the likelihood of adventitious agents being present. This will include careful selection of source material (utilising screening whenever possible). Note that work in E. coli required to produce the viral vectors is covered by an existing class 1 GM risk assessment and so this element of the work is not considered further in this notification.

A range of viral vector systems will be used, including lentiviral, retroviral, adeno-associated and adenoviral vectors. In line with SACGM guidance, vector/insert systems that result in essentially no risk to workers or the environment are covered under a GM class 1 assessment and are not therefore part of this notification. Such systems are:

• 2nd/3rd generation lentivirus or 3rd generation retrovirus
• Replication incompetent and self-inactivating
• Not containing X protein expressing forms of WPRE
• Insert is non-harmful
• Activity does not require sharps

and

• Disabled 2nd or 3rd generation adenovirus vectors
• Likelihood of reversion event must be low
• Stock must be demonstrably free of any replicative virus
• Insert is non-harmful
• Activity does not require sharps

This notification therefore covers vector/insert systems that fall outside of the above classification due to either the nature of the insert (e.g. biologically active, potentially harmful insert such as an active oncogene or cytokine) and/or the vector (e.g. viral vectors containing a WPRE capable of expressing any part of the X protein). Replication competent viruses of any type will NOT be used and viral packaging systems of at least second generation will be used.

Origin & function

The genetic material to be inserted into the viral vector systems will be cloned cDNA sequences derived from a variety of sources (e.g. PCR from cDNA, EST, genomic DNA, synthetic DNA produced by chemical synthesis means). In most cases, the DNA will be of eukaryotic (mainly human or rodent) origin, but some prokaryotic DNA material may be used if this is related to the ability of such organisms to cause disease (e.g. virulence factors that interfere with cellular pathways). In such cases, virulence factors would be expressed individually or, if more than one virulence factor, from separate viral vectors. Where possible, expression of virulence factors would be under inducible control to prevent unintended expression in the event of worker exposure.

Evaluation of foreseeable effects

The cell lines that will result from infection with the viruses are considered to be of low risk. As noted, most cell lines will be continuous well characterised lines, but some may be primary or less well characterised continuous cell lines and this creates a risk (through the potential for adventitious agents to be present) that warrants containment level 2, but is not related to the genetic modification element of the work. However, some elements of the genetic modification could pose a risk to human health or the wider environment. For example, expression of an oncogenic gene or biologically active molecule (such a cytokine) could make the cell line a risk to workers via internalisation (principally via injection). Whilst there will be no work with immortalised cells obtained from workers themselves, it cannot be guaranteed that an immune response would prevent harm occurring in such a situation.

Of more concern though is the potential for infection of a worker by a viral vector via injection or another route (e.g. inhalation). Whilst viral vectors will be replication incompetent, insertional mutagenesis is a risk (although very low, given that there will be no intentional infection of workers with high doses of virus, so exposure dose from an accident is likely to be low). Stable insertion of the viral DNA and expression of the associated transgene(s) is a risk should infection of a worker or someone in the wider environment occur. Expression of an oncogene could cause transformation and expression of other biologically significant proteins (e.g. signalling molecules) could elicit unpredictable responses. Some viral vectors may also contain genetic elements that are of potential concern, such as WPRE X protein sequences in some retroviral vectors, which may have oncogenic properties. Note that whenever possible, viral vectors that do not have potential to express any portion of the X protein will be used. Generally, the intention is to only use commercially available viral vector systems (whether obtained directly from the supplier or from a collaborator). Such systems would be selected on the basis of inherent safety features, one of which would be that they could not express the X protein. However, there is a possibility that a vector system capable of expressing a portion of the X protein (or where it is not clear from available information whether it could express such a portion) may be used.

Whilst the risk posed by all of these issues should be low, they are foreseeable and result in a risk that cannot be said to be insignificant. For this reason, a classification of 2 is appropriate based on these foreseeable effects.
All viral particles generated via the use of packaging helper cell lines will be replication incompetent and once initial infection of target cells has taken place, infective virus should not be present. This will be established by standard means such as PCR of cell culture supernatant.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

No GM animals with a greater potential to cause harm to human health compared to the unmodified organism will be generated and so notification is not required.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste is collected in lined autoclavable lidded boxes. Waste is either solid only or mixed solid and liquid waste. Waste is removed without delay from the site of production to a waste facility located within the same building where it is autoclaved on an appropriate cycle for the type of waste. There are:

- Liquid waste: 134 Degrees Celcius for 5 minutes
- Mixed solid and liquid waste: 121 Degrees Celcius for 20 minutes

These conditions are standard autoclave conditions known to provide 100% degree of kill. Subsequent to autoclaving, solid waste is bagged as “tiger stripe” offensive waste and sent to land fill. Liquid waste is disposed to drain. Each autoclave cycle is monitored by internal sensors and the results recorded (either via computer monitoring or paper printout) to establish that the standard conditions were met. Autoclaves are subject to service and maintenance every six months and an annual calibration, all undertaken by an external contracted service engineer appointed via a University procurement process. Autoclaves are also subject to annual insurance inspection to comply with University insurance requirements and the Pressure Systems Safety Regulations.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
Initial comment from University Biological Safety Advisor (UBSA):

"The risk assessment is generally well written, but more information is needed with regard to the precise nature of the vector system. Currently it states that a modified version of a cited method will be used. The inserts themselves are low risk and so the key to whether this will require notification is the vector system."

Comments from GMSC members subsequent to changes being made in light of above comment:

"VSV-G pseudotyped lentiviral vector containing full length WPRE, i.e. WPRE containing truncated X-protein, therefore Class 2."

"Looking at the list of HSE notifications, titles only, none seem relevant to this project. As previously discussed at UoD BA Committee, ideally, we need a notification to cover all replication defective viral vector work."

"I am happy with the changes made and accept this risk assessment." (UBSA)

### Project Containment

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### Project Ref 6/96.1

- **Date Ackn’d:** 19/11/1996
- **CU2 Project Title:** EVALUATION OF CHEMOTHERAPEUTIC TARGETS IN PARASISTI PROTOZOA

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- **Non-GMM:** Yes
- **Consent Granted:** Yes
- **Project notified under transitional arrangements:** Yes

**Historical Significant Changes:**

- Derogation 15/11/2005, Derogation 24/12/2008

- **Significant Change ID:** 6/96.1b
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment
Project Ref 6/97.1

Date Ackn'd 12/11/1997

CU2 Project Title INTRACELLULAR SIGNALLING PATHWAYS IN CANDIDA ALBICANS

Class Class 2

Consent Granted not applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 6/97.2

Date Ackn'd 21/11/1997

CU2 Project Title CONTROL OF PROLIFERATION AND DIFFERENTIATION IN OCCULAR TISSUES

Class 2

CultureVolClass2

CultureVolumeClass3-4

02/03/2022 Page 133 of 15326
Non-GMM
Consent Granted

Project notified under transitional arrangements

Withdrawn  N  Tick if notifying a connected programme of work  N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Animal Units

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Transgenic expression of human HLA class I major histocompatibility MHC genes in in-vitro mammalian cell lines using vaccinia-HLA constructs

Date Ackn'd: 16/04/1998

Date Project Ceased: 20/02/2006

Class 2

Non-GMM Consent Granted: not applicable

Historical Significant Changes: PROJECT CLOSED AS OF 20/2/06
**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**

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**Tick to confirm that you have attached a risk assessment to this form**

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**Tick if you are claiming exemption from disclosure for section of the risk assessment**

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**Please enter comments on the GM safety committee on the risk assessment**

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Date at Which Additional Info Submitted

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Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 2 (GMMs)</td>
<td>Level 3 (GMMs)</td>
<td>Level 4 (GMMs)</td>
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Non-microbial

Other (please specify)  
Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

- Mycology
- Transgenic Invertebrates
- Transgenic Plants
- Other (please specify below)

Other(s)  
"vaccine"

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
## Project Additional Information

**Purposes of the contained use**  
Increasing basic understanding of the mechanisms underlying bacterial pathogenicity.

**Recipient or parental organism**  
Genes cloned from one pathovar of Pseudomonas syringae (parent) will be transferred into E. coli DH5α and into other pathovars of Pseudomonas syringae.

**Host/vector system**  
Vectors used will be broad host range plasmids such as pLAFR3 and ColEl replicon based pBluescript.

**Origin & function**  
Genes transfer will be used to identify genes for avirulence and virulence by function, using altered pathogenicity as the test. Following detection of activity in cosmid clones (up to 30 Kb) sub-cloning will allow functional open reading frames to be analysed. Proteins will be expressed from vector promoters.
Evaluation of foreseeable effects

The GMOs are not considered a significant risk to human health. Release of the GM Pseudomonas syringae strain with altered virulence is unlikely to result in the infection of plants. The bacteria are spread by seed and through rainsplash onto infected leaves. They are not soil-borne pathogens. Nevertheless the Pseudomonas syringae strains will not be specifically disabled.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Plants infected with GMOs will be maintained in designated growth rooms as specified in the MAFF (now DEFRA), Plant Health Licence, no PHL30A/37554/2001).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Autoclaving at 121°C for 30 mins to destroy broth and petri cultures and infected plant material (leaves and pods).
2. Soaking in a presept bath 140ppm chlorine, overnight to decontaminate glassware and plasticware.
3. Incineration of contaminated soil and pots organised by "Whiterose" No GMOs should survive.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Although the hazards posed by the work were not assessed to require greater than containment level 1 for human health, the use of plant pathogens necessitates notification of Class 2 activity.

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
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<td>Animal Units</td>
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Project Ref 275/03.1
Investigation of cellular immune responses to viruses using vaccinia virus vectors with inserts from (A) human immunodeficiency virus and (B) papilloma virus.

Recipient or parental organism
Vaccinia virus containing one of the following genes: HIV-1, gag, env, pol, nef; influenza; nucleoprotein, matrix protein, pol; papilloma virus (HPV16 and 18) E6 and E7 fusion protein -- the E6 and E7 proteins have oncogenic properties. Transformation requires E7 to bind to retinoblastoma protein. To remove oncogenic potential the E7 protein is mutated at two amino acid residues to abolish binding to retinoblastoma protein. There is no evidence in the literature that modification of the vaccinia increases its pathogenicity.

Host/vector system
Vector: vaccinia virus
Host cell: TK 143, a thymidine kinase negative cell line.

Origin & function
Inserted genes: HIV-1 gag, env, pol, nef,
    influenza matrix, nucleoprotein, pol
    Papilloma virus types 16 and 18 E6 fused to a mutated E7 protein.

The genes will be transfected into human dendritic cells to facilitate antigen presentation to stimulate virus specific cytotoxic T cells.

Evaluation of foreseeable effects
Accidental exposure to the vaccinia modified vector may result in the generation of an immune response to the product of the inserted gene. No other clinical outcome would be expected as a result of the modification.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste (plastics): 2% Virkon overnight. The manufacturer specifies that this will give 100% kill. The decontaminated waste will be then autoclaved prior to disposal via the Chelsea and Westminster Hospital waste disposal services.

Liquid waste: via autoclave, 134 degrees centigrade applied including air extraction, heating and steaming for 30 minutes. The manufacturer specifies that this will give 100% kill. Indicator strips to be used to monitor every autoclave run. Regular six monthly servicing and testing of the autoclave by the manufacturer.

The principal investigator to monitor staff to ensure correct disposal routine is followed.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The committee agreed that a class II classification was appropriate for the work and that the safety precautions outlined were satisfactory.

Project Containment

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<tr>
<th>Laboratory Activities</th>
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Project Ref 275/04.1

Date Ackn'd 02/03/2022  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use
To study cellular immune responses to KSHV in healthy immunocompetent individuals and in individuals infected with HIV.

Recipient or parental organism
KSHV containing a Green Fluorescence protein (GFP) insert. The modified KSHV genome contains an insert of GFP (expressed by the elongation 1-alpha promoter) and the neo gene (expressed by the RSV promoter). This construct was inserted through homologous recombination between ORF 57 and ORF 59 of the KSHV genome (Gao et al, J. Virol 2001.77: 1378-1386). The recombinant KSHV has been designated as rKSHV.152. There is no evidence that the modification of the KSHV increases virulence or pathogenicity.

Host/vector system
Vector: KSHV
Host cells: BCBL-1, a human primary effusion lymphoma cell line containing wild type KSHV.

Origin & function
Inserted gene: GFP derived from Jellyfish.
GFP will be used to detect recombinant KSHV infection in a range of mammalian cell types including endothelial cells, fibroblasts, lymphocytes and myeloid cells.
Infected cells will be used as targets for KSHV specific NK cell and T-cell responses.

Evaluation of foreseeable effects
KSHV infection does not result in pathogenic effects in healthy individuals. This virus can lead to the establishment of tumors in HIV-1 patients.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste (plastics): 2% virkon overnight. The manufacturer specifies that this will give 100% kill. The contaminated waste will then be autoclaved prior to disposal via Chelsea and Westminster Hospital Waste disposal service.

Liquid Waste: via autoclave, 134 degrees centigrade applied including air extraction, heating and steaming for 30 minutes. The manufacturer specifies that this will give 100% kill. Indicator strips to be used to monitor every autoclave run. Regular 6 monthly servicing and testing of the autoclave by the manufacturer.

The principal investigator to monitor the staff to ensure that the correct disposal routine is followed.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The committee agreed that a class II classification was appropriate for the work and that the safety precautions outlines were satisfactory.

Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 Yes | L3 | L2 | L3 | L4 | L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Project Ref 309/01.1

Date Ackn'd | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4
---|---|---|---|---
02/08/2017 | USE OF RECOMBINANT REPLICATION-COMPETENT SENDAI VIRUS (SEV) AS A | Class 2 | < 1 Litre

02/03/2022 Page 147 of 15326
**NEW GENE TRANSFER AGENT FOR GENE THERAPY OF INHERITED AND ACQUIRED DISEASES**

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**Tick if notifying a connected programme of work**

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**Historical Significant Changes**

Transfered to GM8 on closure of GM309

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Recombinant seV will be used to transfer reporter and therapeutic genes to different cell types (in vitro and in vivo) and compared to currently available gene transfer vectors.

**Recipient or parental organism**

Sendai virus (SeV) is an enveloped virus with a nonsegmented negative-strand RNA genome of 15384 nucleotides and is a member of the family Paramyxoviridae. The SeV genome contains six major genes: nucleoprotein (NP), phosphoprotein (P) and large protein (L) form a ribonucleoprotein (RPN) with the SeV genomic RNA. The matrix protein (M) engages in the assembly of viral particles. Two envelope glycoproteins, hemagglutinin-neuraminidase (HN) and fusion protein (F) mediate the attachment of virions and penetration of RNP into infected cells. SeV replication is independent of nuclear functions and does not have a DNA phase. Therefore, it does not transform cells by integrating its genetic information into the cellular genome. SeV has been reported to naturally infect rodents (causing upper respiratory tract infections) and has never been reported to infect humans.

**Host/vector system**

Transgenes are normally inserted immediately before the ORF of the viral 3'-proximan nucleocapsid (NP) protein gene in a full-length SeV CDNA copy. The inserted gene is flanked by "gene start signal" and polyadenilation/stop sequences and recognised by the viral RNA polymerase.

**Origin & function**

The Sendai genome derives from the Z-strain. The recombinant virus has been developed by a Japanese biotech company, DNAVEC. The recombinant SeV will carry different transgenes:

1. bacterial transgenes, such as beta-galactosidase or CAT;
2. Eukaryotic transgenes such as luciferase (from Photinus pyralis) and GFP (from jellyfish);

**Therapeutic genes**

**Evaluation of foreseeable effects**

1. Recombinant SeV does not have an altered tropism compared to wild type virus. However, insertion of a transgene reduces the replication speed as well as the final virus titre in a way, that is proportional to the size of the transgene, carried. In vivo a remarkable attenuated replication and pathogenicity were generally seen (Sakai et al.,

---

02/03/2022
2. Virus replication and transmission to neighbouring cells has been demonstrated in the host species (rodents). SeV has never been reported to infect humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Recombinant Sendai virus is stored at-80°C in 1-2 ml vials separately from the biological samples. All experiments are carried out in class II cabinets. After use, cabinets are UV irradiated for at least 30 minutes, Single-use plasticware is used. The solid waste produced is double-bagged and autoclaved (121°C/1 atm). For the liquid waste, Hypachlor for > 10 min with chlorine (2500 ppm final concentration) is used. Personnel will wear labcoats, gloves, masks and overshoes.

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

The Generic Modification Safety Committee has reviewed the application in great detail. The committee is satisfied that the virus poses no risk to humans and that adequate measures have been taken to ensure that the virus will be wholly contained within the dedicated unit set up specifically for this work.

Project Containment

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Project Ref 309/04.1

Date Ackn'd 02/03/2022
Assessment of 2 Generation Simian Immuno Deficiency virus (SiV) for airway gene transfer.

02/08/2017
Date Project Ceased

Class 2 < 1 Litre

Non-GMM Consent Granted
Not Applicable

Transfer to GM8 on closure of GM309

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Animal Units
- L2 L3 L4 L2 L3 L4 L2 L3 L4

Large Scale Activities
- L2 L3 L4 L2 L3 L4 L2 L3 L4

Human Clinical Applications
- L2 L3 L4 L2 L3 L4 L2 L3 L4

Project Ref 309/16.1

Date Ackn’d 02/08/2017

CU2 Project Title
Lentiviral transfection for overexpression studies to correct defective phagocytosis in human MDM in COPD

Class Culture| Vol Class 2 Culture| Volume Class 3-4
Class 2 | < 1 Litre |
Non-GMM | Consent Granted |

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Transferred to GM8 on closure of GM309
### Project Additional Information

#### Purposes of the contained use

Chronic obstructive pulmonary disease (COPO) is an inflammatory disease that results in poorly reversible airflow limitation, with symptoms including chronic cough, shortness of breath and sputum production. Macrophages are inflammatory cells that are essential in the maintenance of lung homeostasis. These cells are highly phagocytic, and are crucial in the clearance of bacterial and cellular debris. Macrophage phagocytosis is impaired in COPO, resulting in bacterial colonisation and increased risk of infection, and there has been increasing incidences of patients with COPO being infected with Aspergillus. Aspergillus Fumigatus may affect macrophage phenotype and function, as this has also been affiliated with worsening symptoms at exacerbation. We plan to investigate uptake of Aspergillus Fumigatus by macrophages derived from human blood and lung samples.

#### Recipient or parental organism

Aspergillus Fumigatus strain ATCC46645 (ACOP classification 2).

#### Host/vector system

Aspergillus fumigatus has been modified to ubiquitously express GFP so as to be suitable for imaging purposes, FACS and phagocytosis experiments.

1. The eGFP gene (Clontech, Heidelberg, Germany) and the gpdA promoter from A fumigatus were amplified by high-fidelity PCR (Roche, Mannheim, Germany) using oligonucleotides GFP-For, GFP-Rev, gpdA-Prom-For and gpdAProm-Rev. The amplification products were cloned into the pOrive cloning vector (Qiagen, Hilden, Germany) and sequenced using external plasmid-derived primers. The fragments were subsequently cloned into the EcoRV site of pBluescript KS+ (Stratagene, Amsterdam, the Netherlands). The hygromycin resistance cassette containing the hygromycin B phosphotrans-ferase gene of E. coli under the control of the gpdA promoter was isolated from plasmid pUCGH-pyrG and inserted into the KpnI site of pBluescript KS+. Both fragments were cloned into pBluescript KS+ in opposite orientation resulting in plasmid pMAF1, that was transformed into A fumigatus protoplasts using a method described previously (Langfelder et al., 1998).

#### Origin & function

GFP label in Aspergillus is fluorescent on the FITC channel.

#### Evaluation of foreseeable effects

Aspergillus fumigatus has been modified to ubiquitously express GFP so as to be suitable for imaging purposes, FACS and phagocytosis experiments. The addition of the GFP gene allows the microbe to become fluorescent. There are no further functional changes to the microbe, and therefore it poses no greater threat to human health compared to the unmodified strain.

To create this GFP labelled strain, a hygromycin resistance cassette was inserted into the plasmid. The strain would therefore be resistant to this antibiotic. This should be noted in case of accidental infection to a human user.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
A Class II Microbiological safety cabinet (MSC) will be used when handling/processing samples. After use, the safety cabinet will be decontaminated using 2% Distel or 2% Virkon. All buckets used in centrifuges will be fitted with lids when spinning samples, and the centrifuge and buckets will be decontaminated post use using 2% Distel. In case of spillage within the centrifuge, the centrifuge lid will remain closed for 1 hour to allow any aerosol to settle. After 1 hour, the spilled material will be wiped up, area will be sprayed with 2% Distel or 2% Virkon. Distel bottle will be kept near the centrifuge.

Solid Waste: Solid waste will be collected in a biohazard bag inside the Biosafety Cabinet. Pipette tips will be collected in a small Biobin, and the bin closed and deposited into the biohazard bag (in the Biosafety Cabinet) at the end of the work session. Solid waste exposed to A Fumigatus are first disinfected with 2% virkon, prior to disposal in Biobins or biohazard bags. At the end of the work session, the biohazard bag will be closed, sprayed with 70% EtOH, and deposited into a biohazardous waste container. These will be autoclaved, and taken off-site to be incinerated.

Liquid Waste is normally aspirated into a vacuum flask containing 1/10 volume Virkon. The vacuum flask must have a final concentration of at least 10% virkon, for a minimum time of 30 minutes prior to drain disposal. Liquid waste that is not aspirated must be treated with virkon, to a final concentration of 2%, in the hood, allowing a minimum time of 30 minutes to inactivate fungus.
Many thanks for the attached and I note that all changes noted in e-mails below and in the form C have been made to the 8101. However I do have some additional comments for both the CU2 and 8i01. Hope this makes sense. Please send the completed forms back to Biosafety@imperial.ac.uk and we will fill in our bits. Many thanks

CU2 - The must read the same as the BI01 and vice versa. I have the following comments
Section 5 - This should be the Title of the activity from the BI01. - Lentiviral transfection for overexpression studies to correct defective phagocytosis in human MOM in COP. Otherwise if this title no longer fits the activity please change it in the BI01 and the Cu2
Section 6 - The highlighted text does not appear to be in the BI01. Please put this explanation in the BI01. 1.1 is probably the best place for it.
*and there has been increasing incidences of patients with COP being infected with Aspergillus. Aspergillus Fumigatus may affect macrophage phenotype and function, as this has also been affiliated with worsening symptoms at exacerbation. We plan to investigate uptake of Aspergillus Fumigatus by macrophages derived from human blood and lung samples*
Section 7 -
Recipient - This is different to the text for the recipient strain in 2.15 of the BI01. Please make it the same. Le. Aspergillus Fumigatus strain ATCC46645 (ACOP classification 2).
Vector - This is different to the text for the recipient strain in 2.16 of the BI01. Please make it the same. i.e. Aspergillus fumigatus has been modified to ubiquitously express GFP so as to be suitable for imaging purposes, FACS and phagocytosis experiments.
1. The eGFP gene (Clontech, Heidelberg, Germany) and the gpdA promoter from A. fumigatus were amplified by high fidelity PCR (Roche, Mannheim, Germany) using oligonucleotides GFP-For, GFP-Rev, gpdA-Prom-For and gpdAProm-Rev. The amplification products were cloned into the pDrive cloning vector (Qiagen, Hilden, Germany) and sequenced using external plasmid-derived primers. The fragments were subsequence cloned into the EcoRV site of pBluescript KS+ (Stratagene, Amsterdam, the Netherlands). The hygromycin resistance cassette containing the hygromycin B phosphotransferase gene of E. coli under the control of the gpdA promoter was isolated from plasmid pUCGH-pyrG and inserted into the KpnI site of pBluescript KS+. Both fragments were cloned into pBluescript KS+ in opposite orientation resulting in plasmid pMAF1, that was transformed into A. fumigatus protoplasts using a method described previously (Langfelder et al., 1998).
Origins and intended functions of the genetic material - This is different to the text for the recipient strain in 2.17 of the BI01. Please make it the same. Le. GFP labet in Aspergillus is fluorescent on the FITC channel.
7. Please add the following text which is in the CU2 to the 8i01 sections 2.18 and 4.20
*Aspergillus fumigatus has been modified to ubiquitously express GFP so as to be suitable for imaging purposes,
FACS and phagocytosis experiments. The addition of the GFP gene allows the microbe to become fluorescent. There are no further functional changes to the microbe, and therefore it poses no greater threat to human health compared to the unmodified strain.

To create this GFP labelled strain, a hygromycin resistance cassette was inserted into the plasmid. The strain would therefore be resistant to this antibiotic. This should be noted in case of accidental infection to a human user.

Section 9.1 This answer is different to the 8i01 section 4.4 please make sure that the volumes are the same.

Section 12 is different to the waste section 7 in the 8i01. Please take the text from the CU2 which is nicely detailed and add it to the 8i01. Please do not refer to an MSC as a "hood" you can use the phrase Microbiological Safety Cabinet or MSC.

Section 15 I will add this once you have modified the above.

Section 16 this is is usually the PI.

Section 18 we will complete this.

GM Safety Committee 4th Dec 2015

Dear Professor

Following the discussion of your proposal entitled "Lentiviral transfection for overexpression studies to correct defective phagocytosis in human MOM in COP0" (GMIC-4695), the committee request that the following amendments are made to the proposal prior to approval:

Reviewer 1

Section 2.15; This section asks for recipient species and strains with their ACDP classification, add Aspergillus fumigatus here.

Section 2.16 List the vector that has been used for aspergillus here, what resistance cassette is used? What type of GFP?

The added GM Aspergillus fumigatus (GFP labelled) is a class 2 GMO because the host is HG2. This project was originally a Class 1. This will mean that the project will now require notification to the HSE.

Section 2.17 GFP has a functional property, it's fluorescent ... otherwise it would be pointless expressing it

Section 4.16 GFP is not completely non-functional, you use it because it expresses fluorescent protein

Section 4.17, does the GFP vector have a selective resistance cassette? This might have an impact on treatment if somebody gets exposed to Aspergillus

Section 5.5, Who grows the Aspergillus? In which Jab? under which risk assessment? Is it just being donated or are people from RB campus going to SK campus 10 grow this up? Or just to collect?

Section 5.13 Tick the box for using FACS analysis or Sorting and mention SOP

Section 14+15 Aspergillus work is not mentioned here. GM Aspergillus fumigatus (GFP labelled) is Class 2

Reviewer 2

All looks ok in principle. I believe the room that the work will be taking place in is B148 not B146 at Guy Scadding. Also there is a discrepancy between centrifuge spillage procedures, in the form it correctly states that it will be left for 1 hour to allow aerosols to settle, but in all the SOPs it says 15mins - this is needs to be amended.

Could you please ensure that the risk assessment is revised (add GMIC number) and the amendments above addressed. I will then forward the form onto Occupational Health to complete. The completed HSE CU2 form (attached to this email), and copy of the Purchase Order (for BACs Payment) made payable to the HSE should be sent to me, I will then send it all to the HSE. Please ensure the information given in HSE Form relates to that provided in your risk assessment.

For further information, please see http://www3.imperial.ac.uk/safety/subjects/biosafety/gmprocedures

Please note that this work must be notified to the HSE and an acknowledgement receipt received prior to this work commencing.
The aims of this project are twofold. Firstly, to exploit a commercially available E.coli based protein expression system to produce proteins, or parts of proteins, of Salmonella typhimurium. The resultant products will then be used as antigens to stimulate antibody production in rabbits.

Recipient or parental organism

Salmonella typhimurium is ACDP Hazard Group 2, as it is a human and animal pathogen.

Escherichia coli BL21 is also ACDP Hazard Group 2, as it may be pathogenic and is able to colonise humans.
Host/vector system

Salmonella typhimurium is ACDP Hazard Group 2, as it is a human and animal pathogen.

Escherichia coli BL21 is also ACDP Hazard Group 2, as it may be pathogenic and is able to colonise humans.

Origin & function

Source of Host cells: E.coli BL21 commercially available (Stratagene UK), Salmonella typhimurium laboratory maintained strains.

Source of Genetic material (inserts): PCR from S.typhimurium genomic DNA template.

Evaluation of foreseeable effects

Both the GMMs are ACDP Hazard Group 2.

In the case of GM S.typhimurium; the modification takes the form of the addition of an autonomously replicating plasmid which merely serves as a marker for gene expression. The plasmid encodes the green fluorescent protein marker and also confers resistance to ampicillin. As there are a variety of alternative chemotherapeutic agents available for use; this modification is thought to pose no increase risk to human health. The environmental survivability of the GM S.typhimurium is envisaged to be similar to the parental wild type strain.

Likewise in the case of E.coli BL21, the modification takes the form of the addition of a self-replicating plasmid, upon which resides the cloned S.typhimurium gene. Expression is controlled and regulated by the addition of IPTG to growing cultures. In the absence of IPTG expression is minimal. The plasmid also confers resistance to ampicillin. As there are a wide variety of alternative therapies available, this modification is thought to pose no increased risk to human health. The environmental survivability of the GM E.coli BL21 is thought to be the same as the wild type.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Autoclaving of liquid GM waste: Large autoclave; liquid discard cycle 134 celsius for 30 minutes. 100% kill. Machine and cycle validated by thermograph and testing by manufacturer's engineer.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form
The second paper entitled "Investigation of protein expression of Salmonella pathogenicity Island 2 in Salmonella typhimurium using a commercially available expression system (pET) and gene fusions to a plasmid encoded green fluorescent protein" from Professor Holden, this project was approved by the committee, with the following classifications: the Salmonella typhimurium work was classified as Class 2 Containment level 2, work involving E.Coli K12 strains were classified as Class 1, but will be carried out as Containment level 2 due to the level of facilities available, work involving B121 was classified as Class 2 Containment level 2. Again the Class 2 projects must be notified to HSE prior to any work commencing and a new GM Form A completed.

Project reference no. 01/264.
IC reference no. 00083.

### Project Containment

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<thead>
<tr>
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### Project Ref 31/01.6

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<th>CultureVolumeClass3-4</th>
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<td>INSERTIONAL MUTAGENESIS OF THE VIRULENCE GENES OF SALMONELLA TYPHIMURIUM</td>
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Historical Significant Changes: transferred from GM31 on 12/12/2005
Date of Significant Change

Project Additional Information

Purposes of the contained use

The aim of this project is to engineer mutants of Salmonella typhimurium by insertional mutagenesis. The genes to be mutated encode proteins involved in different aspects of Salmonella virulence. The strains will then be tested for phenotypic defects in mammalian cell lines.

Recipient or parental organism

The recipient microorganism is Salmonella typhimurium, which is a human and animal pathogen. It is placed by the ACDP in Hazard Group 2.

Host/vector system

Salmonella typhimurium (Host): pGP704; pFPV25.1; pACYC184 (Vectors).

Origin & function

Source of Host Cells: Laboratory maintained strain. Originally obtained from the PHLS, UK.

Source of Genetic material: inserts PCR from S.typhimurium genomic template.

Source of the material: vectors pACYC184 and pCR2.1 are commercially available; pGP704 and pFP25.1 are gifts from colleagues.

Evaluation of foreseeable effects

The GMM is likely to pose no increased risk to human health in comparison with the wild type parental strain. On the contrary, the GMM is likely to be attenuated in its ability to cause disease, since a putative virulence gene will have been mutated; and so in a functional sense, be disabled.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Autoclaving of liquid GM waste: Large autoclave; liquid discard cycle 134 celsius for 30 minutes. 100% kill. Machine and cycle validated by thermograph and testing by manufacturer's engineer.
For the project entitled "Insertional Mutagenesis of virulence genes of Salmonella typhimurium" from Professor Holden, the project was approved by the committee with the following classifications, the Salmonella typhimurium work was classified as Class 2 containment level 2.

Project Containment

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Animal Units

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Project Ref 31/01.7

Date Ackn'd 12/12/2005

CU2 Project Title

IDENTIFICATION AND FUNCTIONAL CHARACTERISATION OF STREPTOCOCCUS PNEUMONIAE VIRULENCE GENES

Class 2

Consent Granted

Non-GMM Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Transferred from GM 31 on 12/12/2005, transferred to GM553 20/09/2011
Project Additional Information

Purposes of the contained use

The aim of this project is to identify, and characterise the function of Streptococcus pneumoniae genes required for virulence. This necessitates the construction of plasmid vectors containing segments of S. pneumoniae DNA, which are used to transform wild type S. pneumoniae to make GMM strains with specific gene disruptions. The phenotype(s) of these strains will then be assessed using animal and cell culture models of disease.

Recipient or parental organism

The recipient microorganism in Streptococcus pneumoniae, which is a human pathogen. It is placed by the ACDP in Hazard Group 2.

Host/vector system

Host. Streptococcus pneumoniae.

Vectors: pJPC9111, pJPC9112, pLS1GFP, pLS70GFPcat, pucMUT, pDE1, pEVP3, pACH74, p10701

Origin & function

Source of Host Cells - Smithkline Beecham; Prof. James Paton, University of Adelaide
Source of Genetic material: inserts - PCR of Streptococcus pneumoniae DNA
Source of the material: vectors - Prof. James Paton, University of Adelaide; commercial sources.

Evaluation of foreseeable effects

The GMM is likely to pose no increased risk to human health in comparison with the wild type parental strain. On the contrary, since a putative virulence gene will have been mutated the GMM is likely to be attenuated in its ability to cause disease; and therefore be functionally disabled. The insertion mutation protocol utilised in this project renders the GMM resistant to chloramphenicol, spectinomycin, erythromycin or kanamycin. However, there are a wide variety of alternative antimicrobial agents available which are effective against S. pneumoniae infections should infection by the GMM occur. S. pneumoniae is essentially restricted to the human nasopharynx and there is no recognised environmental source. The environmental survivability of the GMM is envisaged to be the same as the wild type, with the caveat that without appropriate selection pressure reversion to the wild type is likely, and there is no increased risk to the environment posed by the GMM compared to the wild type.

All work carried out under this project will take place in appropriate Containment level 2 premises, together with appropriate waste control and management systems.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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manufacturer's engineer.

Autoclaving of liquid GM waste: Large autoclave; liquid discard cycle 134 celsius for 30 minutes. 100% kill. Machine and cycle validated by thermograph and testing by manufacturer's engineer.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project has been approved as a Class 2 project to be carried out under containment level 2 conditions.

Project Containment

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Project Ref   31/97.1

Date Ackn'd 12/12/2005

CU2 Project Title INSERTION DUPLICATION MUTAGENESIS IN STREPTOCOCCUS PNEUMONIAE

Date Project Ceased 20/09/2011

Consent Granted Not Applicable

Project notified under transitional arrangements Y

Withdrawn N

Historical Significant Changes transferred from GM31 on 12/12/2005, transferred to GM553 20/09/2011

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
### Project Additional Information

**Purposes of the contained use**

Lentiviral, adeno-associated viral vectors (AAV) and Sendai viral vectors will be used to deliver reporter or therapeutic genes. Wild-type adenoviruses AD5 will be used for AAV titration. The above vectors, except Sendai, will be manipulated to receive the genes listed below. Viruses will be produced in cell culture under the appropriate conditions and tested for the absence of replication competence. Each gene will be tested for levels of expression and their effects on the recipient organisms. The vectors below will be used to transfer the gene(s) of interest to recipient hosts. The effects of each vector/gene insert will be screened for therapeutic effects or toxicity on the host.

**Recipient or parental organism**
SODKI and LVG-1 cells (HIV-1 based producers, 3rd generation) will be used to generate defective 3rd generation lentiviruses. (HIV-1 based producers, 3rd generation) will be used to generate defective 3rd generation Lentivirus (HIV-1 based) will be produced by transient transfection using a 4 plasmid transfection system which ensures minimal recombination likelihood. The viral genes used to produce these vectors are the gag, pol, rev and VSV-G envelope genes each on discrete plasmids. (1) pMDLg/pRRE induces the rev-dependent expression of gag and pol from the CMV promoter. (2) p tetr expresed rev under tetr control. (3)pVSV-G expresses the VSV-G envelope. (4) plasmid CLL.GFP.CLL contains the viral backbone with the gene inserts detailed below. All other wild-type viral genes have been removed. Homology has been reduced to a few tens of nucleotides. 293T cells will be used to produce these vectors.

Sendai virus will be used as a wild-type recombinant virus. Only the few genes detailed below will be used. No potentially oncogenic genes will be used. Sendai is a respiratory pathogen of rodents which may cause an inflammatory response. The Sendai to be used is derived from the Z-strain. The use of the recombinant virus has significantly less pathogenicity than the wild-type. Animal studies will be short term to determine pathogenic effects. If low pathogenicity is observed then long term studies will be performed on isolated animals. This virus is to be used as an alternative vector which infects the apical surface of lung epithelia unlike the other vectors detailed above which use the baso-lateral surface for infection.

Adeno-associated viruses will be produced from 293 cells. See ris assessment form for vector detail.

Adeno-associated virus with rep and cap genes removed encoding the above gene inserts.
Plasmid pDG will be used encoding the rep, cap and Ad genes E2A, E4 and VA for production of AAV viruses
3rd generation Lentivirus (HIV 1 based) with doxycycline repressible expression of REV/gag/pol and the VSV-G envelope protein encoding the above gene inserts.
Wild-type Sendai vectors will be used containing the inserts lac Z, GFP or CFTR only.

The following genes will be tested for therapeutic genes or as reporter genes. Gene expression will be used to correct the genetic disease associated with the gene of interest below.
Factor IX will be used to correct haemophilia B
GFP is a reporter gene for detection of gene transfer
Lac Z is a reporter gene for detection of gene transfer
HGF is a mitogen used to cause cellular proliferation to allow MLV type retroviruses to infect dividing cells.
Luciferase is a reporter gene for detection of gene transfer
LDLR will be used to lower levels of low density lipoprotein in the host
SODKI (HIV-1 based human embryonic kidney producer cells, 3rd generation) will be used to generate defective 3rd generation lentiviruses after tetracycline induction expressing GFP. This cell line has doxycycline repressible and sodium butyrate induction of expression of REV/gag/pol and the VSV-G envelope protein. LGV-1 (HIV-1 based 293 human embryonic kidney producer cells, 3rd generation) will be used to generate defective 3rd generation lentiviruses after tetracycline induction expressing GFP. This cell line has doxycycline repressible expression of REV/gag/pol and the VSV-G envelope protein. Adeno-associated virus with rep and cap genes removed encoding the gene inserts listed above. AAV will be used to infect HeLa (human fibroblasts), HT1080 (human fibroblasts), NIH3T3 (mouse fibroblasts), HEPG2 (human liver hepatocyte cells) HUH 7 (human liver hepatocyte cells), COS 7, CHO (Chinese hamster ovary cells), V79 (Chinese hamster lung cells).
A plasmid pDG will be used encoding the rep, cap and Ad genes E2A, E4 and VA for production of AAV viruses from 293 cells.
3rd generation Lentivirus (HIV 1 based) with doxycycline repressible expression of REV/gag/pol and the VSV-G envelope protein. Sendai virus will be wild type and only contain lac Z, GFP or CFTR genes to recipient organisms.

The introduction of the above genes and vectors into recipient organisms will not alter the pathogenicity of the genes used.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials used in this work will be disinfected using 1% Virkon and autoclaved as per manufacturer's instructions.
Waste will include solid and liquid.
Degree of kill be be expected to exceed 90%.
Sterilised materials will be tested for kill by tissue culture infectivity assays to ensure viruses are inactive.
The testing procedures will be carried out for each virus preparation and use.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The Imperial College Safety Committee has accepted the containment levels proposed.

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Project Ref 32/05.1

Date Ackn'd 13/02/2006

CU2 Project Title INVESTIGATION OF MEMBRANE TRAFFICKING AND DISEASE

Class 2

CultureVolClass2 < 1 litre

CultureVolumeClass3-4
The lentiviral vectors contain woodchuck hepatitis post-transcriptional regulatory element (WPRE) to enhance transgene expression. This element may be capable of expressing part of the X protein from WHV. A number of publications have suggested that truncated hepadna virus X-proteins may have oncogenic properties and as such WPRE are currently being investigated for oncogenic potential. Appropriate precautions in line with class 2 work therefore need to be used until this has been evaluated.

Recipient or parental organism

Plasmids will be cloned and amplified using competent E. coli cells. Bacteria containing plasmids pose no additional risk to human health.

Lentivirus, AAV and adenovirus will be produced by transient transfection using several plasmids to ensure minimal likelihood of recombination. Vectors will be monitored for replication competence. All viral vectors are designed with low homology to prevent recombination with the host. The multiplasmid transfection system ensures the likelihood/possibility of recombination is effectively zero. Genetic material needed to create a wild-type virus is not carried over with the viral particles and therefore recombination cannot take place.

The GMM cannot survive outside laboratory conditions and so hazard to the environment is minimal.

None of the genes to be inserted encode a pathogen or pathogenic determinant or are known to be oncogenic.

The lentiviral vector contains Woodchuck hepatitis post-transcriptional regulatory element (WPRE) to enhance transgene expression. This element however may be capable of expressing part of the X protein from WHV. A number of publications have suggested that truncated hepadna virus X-proteins may have oncogenic properties and as such WPRE are currently being investigated for oncogenic potential. Appropriate precautions in line with class 2 work therefore need to be used until this has been evaluated.

Adenovirus is deleted in E1 rendering it replication defective and therefore unable to cause illness.

AAV production can involve the use of a helper virus, usually wild-type adenovirus or Herpes Simplex Virus. Although both of these can infect humans, the infections are easily treatable.

The GMM cannot survive outside laboratory conditions and so hazard to the environment is minimal.

In essence no hazards arise from the alteration of existing traits of the host.
Host/vector system

Replication defective HIV-1 based lentivirus will be produced using a 3/4 plasmid transfection system.

Packaging plasmids:
- psPAX2 is a second generation packaging plasmid, deleted in viral auxiliary genes vpr, vif, vpu and nef and without env. The HIV-1 viral genes remaining are gag (virion structural proteins), pol (retrovirus specific enzymes), rev (post transcriptional regulator) and tat (regulatory gene) under a CAG promoter (consisting of a CM V enhancer, chicken b-actin promoter and chicken b-actin intron).
- pMDLg/pRRE and pRSV-Rev are third generation packaging plasmids. pMDLg/pRRE contains genes gag and pol under control of CMV promoter and gene Rev is positioned on plasmid pRSV-Rev separately.
- Envelope: pMD2.G

Expressing the VSV-G envelope using a CMV promoter.

Vectors: This is the only genetic material transferred to target cells and contains the viral backbone and transgene cassette.
- pWPXLd-GFP: a standard vector containing an EGFP reporter gene expressed from an EF-1alfa promoter.
- pWPT-GFP: a standard vector containing an EGFP reporter gene expressed from an intron-less version of EF-1alfa, EF-short.
- pWPI: a bicistronic vector allowing simultaneous expression of a transgene and EGFP marker to facilitate tracking of transduced cells using EF-1alfa promoter.
- pHRsinSFFV: Spleen focus forming virus (sFFV) promoter is driving expression of the transgene, which is fused to the EGFP reporter.

Production of replication defective adenovirus is through homologous recombination with pAd.CMV-Link.1 derived plasmids and Ad5 strain d17001. This strain is deleted in E1 and the majority of the E3 region (3.2kb). The resulting adenovirus will contain a therapeutic or reporter gene under the control of CMV promoter.

AAV vectors are based on the non-pathogenic human papovavirus and are deleted for virally alal virally encoded proteins. The vectors consist of an expression cassette and minimal cis elements necessary for replication and packaging of the recombinant genome. AAV replicative and structural (rep and c ap) genes are supplied in trans on a separate plasmid. Production of AAV is possible by transfecting these plasmids into adenovirus or HSV infected cells. It can also be achieved by co-transfecting with a "helper plasmid" containing the minimal Ad components necessary for helper-function thus avoiding use of helper virus. Replication defective adeno-associated virus of various serotypes will be produced using either a helper plasmid or virus and used to investigate and determine the optimal AAV vector/serotype for the transduction of specific cell types of interest. See ref. JP. Louboutin, L. Wang and J M Wilson J Gene Med2005; 7:442-451. Once this is determined AAV containing therapeutic genes will be produced.

Origin & function

Genes to be inserted:
- EGFP (Enhanced Green Fluorescent Protein) is a reporter gene which will aid in determining efficiency of gene transfer.
- REP-2 is a functional isoform of REP-1 and will be used as a control to demonstrate specific requirements for REP1.
- GDI extract Rabs from the membranes and can recycle them.
- Rab 27a, 27b, 38 and others participate in intracellular transport and targeting of proteins.
- Rab effectors, such as melanophilin and myRIP, determine specificity of Rab function and participate in formation of complexes with other molecules such as motors (myosin V, VII) and cytoskeletal elements (actin).

Evaluation of foreseeable effects

No hazards should arise directly from the inserted gene product or by alteration of existing traits.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Due to the presence of the WPRE in the lentiviral vectors all work involving these vectors will be carried out strictly in line with class 2 work.
Adenovirus and AAV are class 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste such as tissue culture supernatant will be chemically treated with 2% Virkon overnight, validation will be according to manufacturers instructions and disposal will be down the sink.

Solid waste such as pipette tips, culture plates and glass pasteur pipettes will be autoclaved for 20 mins at 120 degrees C 15lb/sw.inch. Validation will be via qualitative monitoring and chart recoder in addition to an annual service maintenance contract. It will then be incinerated and disposed of as clinical waste by an off site contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Lentivirus vectors containing WPRE are potentially oncogenic. At the moment this is one of the most efficient to use, although in future a safer alternative may be found. The researches are proposing to use both lentiviral and adenoviral vectors, if can avoid lentiviral then will. Committee advised the Principal Investigator to incorporate adeno-associated vector (replication deficient Cat 1) to risk assessment. This project is notifiable to the HSE. Agreed: Class 2, containment level 2.

Project Containment

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Project Ref 32/05.2

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
The lentiviral vectors contain woodchuck hepatitis post-transcriptional regulatory element (WPRE) to enhance transgene expression. This element may be capable of expressing part of the X protein from WHV. A number of publications have suggested that truncated hapadna virus X-proteins may have oncogenic properties and as such WPRE are currently being investigated for oncogenic potential. Appropriate precautions in line with class 2 work will therefore need to be used until this has been evaluated.

Plasmids will be cloned and amplified using competent E. Coli. Bacteria containing plasmids pose no additional risk to human health.

Lentivirus and adenovirus will be produced by transient transfection using several plasmids to ensure minimal likelihood of recombination. Vectors will be monitored for replication competence. All viral vectors are designed with low homology to prevent recombination with the host. The multiplasmis transfection system ensures the likelihood/possibility of recombination is effectively zero. Genetic material needed to create a wild-type virus is not carried over with the viral particles and therefore recombination cannot take place.

The GMM cannot survive outside laboratory conditions and so hazard to the environment is minimal.

None of the genes to be inserted encode a pathogen or pathogenic determinant.

The lentiviral vector contains Woodchuck hepatitis post-transcriptional regulatory element (WPRE) to enhance transgene expression. This element however may be capable of expressing part of the X protein from WHV. A number of publications have suggested that truncated hapadna virus X-proteins may have oncogenic properties and as such WPRE are currently being investigated for oncogenic potential. Appropriate precautions in line with class 2 work therefore need to be used until this has been evaluated.

Adenovirus is deleted in E1 rendering it replication defective and therefore unable to cause illness.
The GMM cannot survive outside laboratory conditions and so hazard to the environment is minimal.

In essence no hazards arise from the alteration of existing traits of the host.

Host/vector system

HIV vectors
Replication deficient HIV-1 based lentivirus will be produced using a 4 plasmid transfection system to generate vectors.

Packaging plasmids: pMDLg/pRRE and pRSV-Rev are third generation packaging plasmids. pMDLg/pRRE contains genes gag and pol under control of the CMV promoter and gene Rev is positioned on plasmid pRSV-Rev.
Envelope: pMD2.G expresses the VSV-G envelope from a CMV promoter.
Vector: This is the only genetic material transferred to target cells and contains the viral backbone and transgene cassette. pWPI is a bicistronic vector allowing simultaneous expression of a transgene and EGFP from the EF-1alpha promoter. pWPXl-d-GFP: a standard vector containing an EGFP reporter gene expressed from an EF-1alpha promoter. pWPT-GFP: a standard vector containing an EGFP reporter gene expressed from an intron-less version of EF-1alpha, EF-short.

Adenovirus vectors
Production of replication deficient adenovirus is through homologous recombination between pshuttle (an expression cassette containing the gene of interest and promoter) and pAdEasy-1 (plasmid containing the adeno viral backbone deleted in E1 and E3). The resulting adenovirus will contain a gene under the control of CMV promoter.

Origin & function

Genes to be inserted:
TGFbeta (transforming growth factor) superfamily members and down stream effectors (to include TGFbeta, activin and bone morphogenic protein and the receptor serine threonine kinases (ALK1-6) and Smads). We will initially focus on the Smad proteins: The transforming growth factor-beta superfamily members transduce their signal from the membrane to the nucleus through distinct combinations of transmembrane type I and II serine threonine receptors and their downstream effectors, the Smad proteins, which play a central role in transduction of receptor signals to specific target genes in the nucleus (In the first instance Smad2 will be used).

Cyclin dependent kinase inhibitors. Progression through the cell cycle is regulated by cyclins and cyclin dependent kinases (CDK). The kinase activities of the cyclinD/CDK complexes are negatively regulated by several different molecules including the WAK1/CIP.KIP family which exercise broad acting inhibition of the CDKs and includes p21waf, p57kip2 and p27kip1 and the INK4 family, which specifically inhibit CDK4 and CDK6 and include p15, p16, p18 and p19. (In the first instance p21waf will be used).

EGF (epidermal growth factor) receptor family: The EGF family exert their biological effects by binding to and activating the EGF receptor (c-erbB1), a receptor tyrosine kinase, which occupies a prominent role as a primary regulator of epithelial cell function. (In the first instance c-erbB1 will be used).

FGF (fibroblast growth factor) receptor family: The FGF family contains 23 members in mammals and have been implicated in regulation of many key cellular processes including proliferation, differentiation, migration, apoptosis, angiogenesis and wound healing. They bind to five related, specific cell surface receptors.

Evaluation of foreseeable effects

No hazards should arise directly from the inserted gene product or by alteration of existing traits.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Due to the presence of the WPRE in the lentiviral vectors all work involving these vectors will be carried out strictly in line with class 2 work. Adenovirus are class 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste such as tissue culture supernatant will be chemically treated with 2% Virkon overnight, validation will be according to manufacturers instructions (see www.antechh.com) and disposal will be down the sink.

Solid waste such as pipette tips, culture plates and glass pasteur pipettes will be autoclaved for 20mins at 120°C 15lb/sq.inch. Validation will be via qualitative monitoring and chart recoder in addition to an annual service maintenance contract. It will then be incinerated and disposed of as clinical waste by an off site contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Following the discussion of your proposal entitled "Molecular and cellular pathways underlying development and resolution of airway remodelling" (GMIC -01421.2) at the last GM Safety Committee made the following comments.

Comments: The Chair clarified that the genes to be inserted were not oncogenic. Lentiviral vectors containing WPRE are potentially oncogenic. This project is notifiable to the HSE.

Agreed: Class 2, Containment level 2.

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Project Ref  543/07.1

Date Ackn'd  CU2 Project Title  Class CultureVolClass2 CultureVolumeClass3-4
**Project Additional Information**

**Purposes of the contained use**

Identification and study of the host contribution to bacterial infection and pathogenesis. This is done by infection of the fruitfly *Drosophila melanogaster* with the class 2 pathogen *Mycobacterium marinum*. Both host (*Drosophila*) and pathogen (*M. Marinum*) can be genetically modified.

**Recipient or parental organism**

- *Mycobacterium marinum*
- *Drosophila melanogaster*

**Host/vector system**

- *M. marinum* vectors: pVK173T, pFPV2, and equivalents/derivatives thereof.
- *D melanogaster*: crippled P-element-derived transposable elements (carried in vector pUAST and equivalents/derivatives thereof).

**Origin & function**

- *M. marinum*: transgenes encoding fluorescent proteins such as dsRed and GFP, from corals or jellyfish, used for tracking bacteria cells.
- *D melanogaster*: transgenes encode a variety of molecules (signal-transduction molecules, fluorescent proteins, etc.) of heterologous (human, mouse, jellyfish, coral, bacteria) or homologous origin; used to alter the course of infection and hence to be informative as to the genetic basis of susceptibility to infection or of pathogenesis.

**Evaluation of foreseeable effects**

- **Foreseeable effects**: *M. marinum*: in our hands, fluorescent derivatives typically grow more slowly and are slightly less pathogenic than wild-type strains; they will also be resistant to some aminoglycoside antibiotics (apramycin or kanamycin), the net effect should be a mild decrease in pathogenicity for these strains, since primary treatment for *M. marinum* does not typically depend on aminoglycosides.
D. melanogaster: transgenic files should exhibit reduced fitness relative to other laboratory strains; since lab strains of drosophila are already poorly-adapted for life in the environment, these flies should present no environmental risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

We are very careful to prevent release of any pathogen-infected Drosophila.

1. Individual infected flies are tracked.
2. In most cases flies are infected, placed in culture vials and never again removed. Once the experiment is completed, the entire vial (including contained flies) is frozen to kill flies, then autoclaved to kill bacteria, and finally incinerated.
3. In cases where an experiment requires removal of live infected flies as samples, all flies within the vial are anethetized before the vial is opened, preventing inadvertent escapes.
4. Fly-traps will be placed at frequent intervals in the fly room to attract and contain escaped flies.

Using these techniques, in the course of 6 years doing similar experiments at Stanford University, I observed fewer than 10 escapes of a total of more than 100,000 infected flies. All of these escapes were due to defective vial closures; I have now changed the type of vial-closure to one that is more secure, so fewer escapes are expected.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids: Waste will be double-bagged and autoclaved at 124C for 15 minutes. Appropriate autoclave indicator tape will be used to monitor autoclave function. After autoclaving, waste will be incinerated by White Rose. Waste containing live flies as well as pathogens will be frozen for at least 6 hours at -20C before autoclaving.

Liquid: Addition of disinfectant (chlorine bleach to 5% or 1 haztab/litre) followed by pouring down designated sink.

Both procedures are expected to achieve 100% killing.

Monitoring will be done by attempting to culture M. marinum using standard techniques either after autoclaving or after addition of bleach, as appropriate.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

GMSC approved at meeting of 1 May 2007.

Project Containment
This project is directed at understanding antigenic variation in trypanosomes and how the organism evades the complement system in mammalian hosts.

i) Antigenic variation
The trypanosome wears a dense Variant Surface Glycoprotein (VSG) coat which shields invariant molecules on the trypanosome surface from the immune system. The trypanosome evades the immune system of the mammalian host, by continuously changing its VSG coat. As trypanosomes multiply in the blood of an infected mammal, eventually an antibody response is raised against a given VSG coat, killing all trypanosomes wearing this coat. However, at the population level, trypanosomes are continuously generated that have switched to a new (and temporarily unrecognisable) coat. These switch variants are not recognised by host antibodies, and can form the next wave of infection. Antigenic variation allows trypanosomes to form chronic infections in large mammals which can last for many years.

An individual trypanosome has about 1000 genes encoding different VSG coats. The active VSG gene is located in a VSG expression site, which is invariably located at a
T. brucei brucei 427 strain that we currently work with (and which is sensitive to human serum) grown very well in vitro. In contrast, T. brucei rhodesiense field isolates that are resistant to human serum do not grow in vitro. We would therefore like to create a T. brucei brucei strain that can grow well in vitro in human serum. We would like to genetically modify nonhuman infective T. brucei brucei strains, and insert virulence genes like the Serum Resistance Antigen (SRA) gene into these trypanosomes so that they become resistant to human serum. This will make T. brucei brucei comparable to the T. brucei rhodesiense that we already currently hold. However, these genetically modified trypanosomes will have the advantage of being manipulable in vitro instead of requiring expansion in laboratory rodents. In addition, the virulence genes would be regulatable, greatly increasing the safety of the experiment. We would therefore be able to study the pathogenic aspects of human infectivity in a manipulable in vitro system, and could avoid amplification in laboratory animals.

### E. coli K12 or B derivatives
- **Trypanosoma brucei brucei 427**

### Host/vector system
- **Recipient or parental organism**
  - Bacterial or parasite host with plasmid vectors

### Origin & function

**Antigenic variation**

- Single copy marker genes will be inserted into the repetitive VSG expression sites in order to follow the transcriptional behaviour of single expression sites. Constructs containing fluorescent marker genes like Green Fluorescent Protein (GFP), Yellow Fluorescent Protein (YFP) and DsRed, and drug resistance marker genes like the hygromycin, neomycin, blasticidin, and puromycin resistance genes into various regions of the T. brucei genome including the VSG expression sites. Marker genes introduced into VSG expression sites will allow monitoring of their transcriptional behaviour.

- VSG expression sites include multiple genes of unknown function, in addition to the telomeric VSG gene. RNAi (double stranded RNA inhibition) will be used to inactivate the transcripts from various Expression Site Associated Genes (ESAGs), in addition to transcripts from other candidate genes possibly playing a role in antigenic variation. This technique allows the researcher to investigate the essentiality of different gene families.

**Evasion of the complement system**

- Virulence genes like the Serum Resistance Antigen (SRA) gene will be inserted into nonhuman infective T. brucei brucei strains so that they become resistant to human...
serum. These virulence genes would be inserted behind a tetracyclin inducible promoter, allowing the researcher to turn expression on and off. This will make *T. brucei* comparable to *T. brucei rhodesiense* and facilitate study of the pathogenic aspects of human infectivity in a manipulable in vitro system avoiding amplification in laboratory animals.

**Evaluation of foreseeable effects**

i) Antigenic variation
The genes used here are completely neutral markers encoding fluorescent proteins, or are drug resistance genes for drugs that are not therapeutically relevant for African trypanosomiasis. There is therefore no biochemical mechanism that could explain how these genetic modifications could change the host range or virulence of the genetically modified trypanosome. The genetic modifications proposed would not change *T. brucei* susceptibility to the therapeutic drug suramin and there is no reason to believe that these genetic modifications would change the tissue tropism of the parasite. The double-stranded RNA inhibition (RNA) experiments resulting in the ablation of *T. brucei* RNA transcripts will incapacitate the parasites if the transcripts that are being targeted are essential. Nonessential transcripts being targeted will not result in a change to the trypanosome phenotype. The genetic modifications resulting in the introduction of marker genes into the VSG expression site could result in damage to the active VSG expression site. This would lead to reduced efficiency of antigenic variation. None of these procedures would lead to increased risk to human health and safety.

ii) Evasion of the complement system
These *T. brucei* genetic modifications could make a trypanosome strain that lyases in human serum into one that doesn't lyse in human serum, making it potentially human infective. These genetically modified strains would become similar to the human infective *T. brucei rhodesiense* strains. All published evidence indicates that the SRA gene product is a receptor that interferes with internalisation of the Trypanosome Lytic Factor (TLF) present in human serum. There is no published evidence indicating that expression of the SRA protein could affect the tissue tropism of the trypanosomes. The SRA protein is a receptor for the Trypanosome Lytic Factor, and there is no known biochemical mechanism that could mediate changed drug susceptibilities in an SRA transgenic trypanosome. There is no known biochemical pathway that would confer susceptibility to the drug suramin, currently used in treating human trypanosomiasis, to *T. brucei* SRA gene transgenics. These genetic modifications could increase risk to human health and safety and consequently additional containment and control measures have been assigned. However, even though these genetically modified organisms would be less safe than the original trypanosome, they would be much more safe to work with than human infective *T. brucei rhodesiense* as the tetracyclin inducible nature of virulence gene expression means that the researcher can restrict expression of these genes to the shortest period of time.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

These parasites do not produce spores and are fragile organisms. They are rapidly killed outside the culture vessel or blood by desiccation, osmotic shock, washing with water, detergents, and cannot penetrate unbroken skin. Tsetse flies are the vectors for *T. brucei* and these are endemic only in the disease areas (subSaharan Africa).

The naturally occurring organism does not represent any hazard of infection through aerosol transfer. Aerosols are tiny droplets of liquid. These dry out within minutes under the laboratory conditions. Once dehydrated such material would not be viable.

ACDP guidance on work with *T. brucei* sp.

The Schedule to the certificate of exemption accompanying the Approved List specifies a number of parasites, including *T. brucei* sp. for which full containment level 3 need not be used. ACDP state that work with such parasites does not generally require an inward flow of air to the laboratory or the use of a microbiological safety cabinet as none of these agents if normally infectious by the airborne route. ACDP recommends that for working with these agents in research a separate room should be used or a designated area in a larger laboratory. In ACDP's guidance list is the notation that the laboratory need not be sealable for fumigation.

In relation to the individual control measures to be omitted:
a) Isolated laboratory suite
A dedicated room will be used for working with T. brucei. The room will at all times be operated to derogated Containment Level 3 working practices and have a documented Code of Practice.

b) Laboratory not sealable for fumigation
There is no foreseeable need to fumigate the laboratory. In the event of a major spillage, the bulk of material would be soaked up on absorbent paper towels, then autoclaved, killing any parasites. The area would be wiped down with disinfectant. Any aerosols created that were not cleaned in this initial effort would dry out, killing both host cell and parasite. The area of the laboratory would be left isolated for a period to ensure effective drying. Effective parasite transmission is only possible via direct inoculation of viable parasites.

c) No negative pressure, HEPA filtered extract, microbiological safety cabinet or specified measures to control aerosol dissemination.

The organism presents no hazard of infection by the airborne route. Class II microbiological safety cabinets are in use in the dedicated laboratory, they are necessary for sterile culture of the organism; they are not required for operator safety. Activities involve small scale, standard laboratory techniques with no aerosol generating procedures.

d) Equipment not within the laboratory
Although the laboratory contains most of the necessary equipment for the work, some activities use specialist equipment that cannot reasonably be accommodated within the dedicated facility. In all cases a safe system of transport involving secondary containment is in use, any equipment used is cleaned and disinfected immediately on completion of the work and any contaminated items either returned to the dedicated laboratory or removed for disposal as waste. The following activities are undertaken outside the dedicated laboratory:

i) Storage: transfected parasite stocks are frozen and stored temporarily at -80 degrees C in the adjacent Containment Level 2 laboratory before long term storage in liquid nitrogen within the Containment Level 3 laboratory. The samples will be in Nunc ampules in closed and sealed freezing boxes that are clearly marked as biohazard. The -80 degrees C freezer will be locked during use and accessed only by authorised personnel.

ii) Autoclaving: an autoclave validated for disposal of waste is located within the same building.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (121-125 degrees C for at least 15 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
The aim of this project is to 1) construct strains of these bacteria lacking specific genes, 2) establish whether gene inactivation has affected their ability to cause disease, and 3) further characterise the proteins encoded by these virulence genes.

Escherichia coli, Shigella spp., Neisseria meningitidis, Neisseria gonorrhoeae and Haemophilus influenzae are ACDP Hazard Group 2, as they are human pathogens. The organisms will have the equivalent or less potential to cause human disease as the wild-type bacterium. The insertions will interrupt gene function and are likely to attenuate the host.
Host/vector system

Neisseria meningitidis, Neisseria gonorrhoeae, Haemophilus influenzae (Hosts)/ pMID216 and derivatives (vector)

Shigella and E. coli (Host)/pCR2.1, pUC and pET derivatives, pACYC184, pSTM115, pYH204, pMID216 (vectors)

All these are plasmids which have been used previously with the corresponding microbes. They carry antibiotic resistance markers for agents not usually used to treat human infections with these bacteria.

Origin & function

Source of genetic material (inserts): transposons and antibiotic resistance markers.

Source of genetic material (vectors): pCR 2.1 topo, pACYC184, pUC and pET derivatives: commercially available
pMID216 from collaborator
pYH204, pSTM115: generated in own laboratory

Evaluation of foreseeable effects

All the GMMs are ADCP Hazard Group 2 except the disabled E. coli strain.

The modification of the host bacteria is in the form of insertions into the chromosome that inactivate gene function. The insertions carry antibiotic resistance markers that encode for resistance against antibiotics not used for treatment of infections caused by these micro-organisms. These modifications should not pose any increased risk to human health. The environmental survivability of the GMM will be similar to the parental wild-type strains.

For disabled E. coli, the modifications is in the form of a self replicating plasmid which may contain cloned genes from the pathogenic bacteria. The plasmids confer resistance to ampicillin, kanamycin, and erythromycin. There is a wide variety of alternative agents that can be used to treat successfully infections cause by E. coli. The environmental survivability of the GMM will be similar to the parental strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Chemical disinfection of GMM waste except N. meningitidis:
1% virkon as per manufacturers instructions, at least 1 hour exposure. Plates and plasticare sterilised by autoclaving.
Discard cycle 134 degrees C for 30 min. 100% kill. Machine and cycle validated after each cycle by thermograph; autoclaves thermocouple tested regularly (6 monthly) qualified engineer.

Autoclaving of waste contaminated by Neisseria meningitidis: Discard cycle 134 degrees C for 30 min. 100% kill. Machine and cycle validated after each cycle by thermograph; autoclaves tested regularly qualified engineer.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
It was considered that work with disabled E. coli strains could be conducted in Containment Level 1 facilities. However, this work will be done in a Containment Level 2 laboratory along with the work on Neisseria gonorrhoeae, Haemophilus influenzae, Shigella flexneri, and E. coli K1. The importance of prompt reporting any incidents and episodes of diarrhoeal disease among researchers working with Shigella spp. was highlighted.

People working with N. meningitidis must complete a health questionnaire; any persons with suspected immunodeficiency must be seen by the Occupational Health doctor before working with the bacterium. It was recommended that work with N. meningitidis is carried out in a dedicated (Containment Level 3) room. Only individuals who have undergone a locally approved training programme would be allowed to work with live N. meningitidis. All person must be aware of the signs and symptoms of meningococcal infection, and should seek early medical advice should they become unwell, informing medical staff of their potential exposure to N. meningitidis at the work place. All accidents/spillages to be reported promptly to the Occupational Health Department and Department Safety Officer. A list of individuals allowed to work with the meningococcus, details of the clinical presentation of N. meningitidis infection, and guidelines for safe working is to be kept at the entrance to the designated facility at all times.

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### Project Ref 77/94.3

- **Date Ackn’d**: 16/06/2006
- **CU2 Project Title**: STUDY OF NIESSERIAL BIOLOGY
- **Class**: Class 2
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: Yes
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
<td>L2</td>
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Animal Units

<table>
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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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Project Ref 77/94.4a

Date Ackn'd: 12/12/2005

CU2 Project Title: GENE CLONING IN MYCOBACTERIUM BOVIS BCG

Class: Class 3

CultureVol: Class 2

CultureVolume: Class 3-4

Non-GMM: Consent Granted

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: GM77/95.2, GM77/94.6, GM77/95.4, GM77/97.3, GM77/95.3, GM77/94.5, GM77/97.4, GM77/94.5

Historical Date of Additional Info:
- 01/08/1995
- 04/10/1994
- 30/05/1995
- 04/06/1997
- 04/01/1995
- 08/08/1994
- 21/06/2002
- 12/12/2005

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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### Project Ref 77/94.4c

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<td>Date Project Ceased</td>
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**CU2 Project Title**

**GENE CLONING IN M BOVIS BCG (GENE CLONING IN NON-PATHOGENIC MYCOBACTERIAL STRAINS)**

<table>
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<th>Class</th>
<th>CultureVolClass2</th>
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<tr>
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**Non-GMM Consent Granted**

**Consent Granted**

Not Applicable

**Project notified under transitional arrangements**

Y

**Historical Significant Changes**

transferred from GM77 on 12/12/2005. Project closed as amended to class

**Historical Date of Additional Info**

12/12/2005

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L3 L4 L2 L3</td>
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Project Ref 8/01.1

Date Ackn'd: 05/02/2001
CU2 Project Title: RETINOBLASTOMA PROTEIN PHOSPHORYLATION AND BINDING PROTEINS

Class: Class 2
CultureVolClass2: Non-GMM
Consent Granted: not applicable

Project notified under transitional arrangements [Y]

Withdrawn [N]
Tick if notifying a connected programme of work [N]

02/03/2022
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### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**
- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
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<tr>
<th>Is an emergency plan required according to regulation 20?</th>
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If yes, tick to confirm that it is attached to this form: N
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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<th>Laboratory Activities</th>
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### Project Ref 8/01.10

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Tick if notifying a connected programme of work: N

Historical Significant Changes

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Project Additional Information

02/03/2022

Page 188 of 15326
Purposes of the contained use

*vaccine*

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
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<tr>
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</table>
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref**  8/01.13

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<tbody>
<tr>
<td>06/07/2001</td>
<td>INFECTION OF MAMMALIAN CELLS IN CULTURE AND NEURONS IN VIVO WITH EXOCYTOSIS-INCOMPETENT SNARE CONSTRUCTS VIA A RECOMBINANT REPLICATION-DEFECTIVE ADENOVIRUS</td>
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<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Class</th>
<th>CultureVolClass2</th>
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<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tbody>
<tr>
<td>23/03/2006</td>
<td>Class 2</td>
<td>1-50 litres</td>
<td></td>
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<td></td>
<td>N</td>
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</table>

Withdrawn  

Tick if notifying a connected programme of work

02/03/2022  

Page 191 of 15326
## Purposes of the contained use

Generation of replication deficient and amplification of Adenovirus 5.

## Recipient or parental organism

High efficiency transfection tool for delivering exocytosis competent and incompetent SNAP receptors. These will be capable of affecting exocytosis. The level of protein expression will be regulated either by using a tetracycline inducible promoter or by controlling the level of infection.

## Host/vector system

- E. coli K12 strains and pUC vectors (Clontech).
- Eukaryotic cells (HEK 293) Adeno-X-vectors (Clontech).

## Origin & function

Host cells are from a commercial source (e.g. Invitrogen, Stratagen and Promega).

Mammalian and bacterial plasmid are purchased from Clontech.

## Evaluation of foreseeable effects

These will be safe due to the appropriate procedures and containment. The products are expected to inhibit regulated secretion; should the particular infected cell be important for release of a hormone/transmitter, then the lack of secretion will cause symptoms. However, the use of only small quantities $1\times10^9$ infectious units for animal injection or dilute titres for cell culture experiments (up to $5 \times 10^8$ infectious units/ml) will reduce the risk of harm to health.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Class II microbiological safety cabinet in a containment level 2 laboratory.

## For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for

## Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste will be chemically disinfected by addition 25000 ppm chlorine (Chloros) according to the manufacturers instructions into a designated, downward sink. Excess chloros will be removed by rinsing with water and solid material autoclaved. Liquid waste will be disinfected as above, but liquid will be poured away after disinfection, with copious amount of water.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

From: Minutes of Safety Committee meeting, 21st February 2001, Department of Biochemistry, Imperial College

Dolly-10: 'Infection of mammalian cells in culture and neurons in vivo with exocytosis-incompetent SNARE constructs via a recombinant replication-defective adenovirus'. Various items required clarification; the risk assessment was approved as a Class 2 activity, pending the required revision to the risk assessment. It was also stipulated that the project should be reviewed after 3 months, to check whether some of the assumptions regarding viral toxicity still held. If all was well, the usual 6-month review would be in order. In addition, the HSE would have to be notified and the fee paid.

Project Containment

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<th>Glass Houses</th>
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<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
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<table>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2 L3 L4 L2 L3 L4</td>
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</table>

Project Ref 8/01.14

Date Ackn'd 26/10/2001

CU2 Project Title STUDY OF INTRACELLULAR TRAFFICKING IN MAMMALIAN TISSUE CULTURE CELLS - INFECTION OF MELANOCYTES WITH GFP - CONJUGATED PROTEINS USING A BRCAS VIRUS VECTOR.

Class 2

Culture Vol Class 2 < 1 litre

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

To study protein trafficking and organelle formation in mammalian tissue culture cells by fluorescence and video microscopy. The BRCAS system is to be used to increase infection efficiencies.

Recipient or parental organism

Chick fibroblasts are infected with a disabled Rous Sarcoma Virus. Mouse melanocytes will be transfected with the RSV-A receptor and infected with a disabled Rous Sarcoma Virus. The virus cannot replicate and carries no risk to human or animal health.

Host/vector system

Chick fibroblasts are infected with a disabled Rous Sarcoma Virus. Mouse melanocytes are to be transformed with the RSV-A receptor and infected with a disabled Rous Sarcoma Virus.

Origin & function

BRCAS RNA is derived from Rous Sarcoma Virus. When incorporated into the chick genome, fluorescent proteins from sequences of human DNA are expressed. Fluorescent proteins are to be identified by microscopy.

Evaluation of foreseeable effects

Viral nucleic acid has been engineered so as to make it non-replicative. There are no hazards to human health and all risks are negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Cells infected with BRCAS will be handled in sterile cell culture cabinets fitted with HEPA filters and complying with class II regulations. In the long term the cells will be maintained as frozen stocks.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be chemically disinfected using 1% Virkon for at least 1 hour to give a degree of kill of effectively 100%. The Virkon will be used according to manufacturer's instructions and recommendations, and the manufacturer's protocol will be strictly adhered to. Virkon is effective against retrovirus. Solid waste including petri dishes, plastic pipettes, disposable centrifuge tubes, gloves and paper towels will be autoclaved according to the Biochemistry Department safety instructions and as per available literature. This should give an effective degree of kill of 100% and will be monitored by performing culture tests at regular intervals. All tissue culture plastics in contact with retrovirus will be disposed of in autoclave bags in the tissue culture hood and sealed to prevent aerosol dispersal, prior to autoclaving. Clinical waste is...
removed from the laboratory via yellow bags clearly marked as clinical waste.

It was advised to classify this project as containment level 2 due to the use of a retrovirus which, although disabled, could be potentially hazardous to the environment.

Project Containment

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<tr>
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<th>Glass Houses</th>
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Animal Units
- L2 L3 L4 L2

Large Scale Activities
- L3 L4 L2

Human Clinical Applications
- L3 L4

Project Ref 8/01.2

Date Ackn’d 05/02/2001

CU2 Project Title DISSECTION OF MITOGENIC SIGNALLING PATHWAYS

Class 2

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
Project Ref 8/01.3

Date Ackn'd 05/02/2001

CU2 Project Title IN VITRO CHARACTERISATION OF VIRAL CYCLINS

Date Project Ceased

Class 2

Culture Vol Class 2

Consent Granted

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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<td>Class 2</td>
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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

- **Laboratory Activities**
  - L2
  - L3
  - L4

- **Glass Houses**
  - L2
  - L3
  - L4

- **Growth Rooms**
  - L2
  - L3
  - L4

- **Animal Units**
  - L2
  - L3
  - L4

- **Large Scale Activities**
  - L2
  - L3
  - L4

- **Human Clinical Applications**
  - L2
  - L3
  - L4

**Project Ref** 8/01.6

- **Date Ackn’d** 05/02/2001
- **CU2 Project Title**
  CLONING & EXPRESSION IN E. COLI OF GENES ENCODING CELL WALL PROTEINS FROM THE HUMAN PATHOGEN CLOSTRIDIUM DIFFICILE, A CAUSATIVE AGENT OF ANTIBIOTIC ASSOCIATED DIARRHOEA & PSEUDOMEMBRANOUS COLITIS

- **Class** Class 2
- **Culture**
  - Class Vol Class 2
  - Culture Volume Class 3-4

- **Non-GMM**
  - Consent Granted not applicable

- **Date Project Ceased** 23/03/2006
- **Withdrawn** N
- **Tick if notifying a connected programme of work** N

- **Project notified under transitional arrangements** Y

**Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<th>Laboratory Activities</th>
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02/03/2022
Project Ref 8/01.9

Date Ackn'd 05/02/2001

Date Project Ceased 16/08/2004

CU2 Project Title DEVELOPMENT OF NOVEL MUCOSAL ADJUVANTS

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 8/02.1

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
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<td>13/06/2002</td>
<td>INFECTION OF MAMMALIAN CELLS IN CULTURE WITH ENDOCYTOSIS</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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</tbody>
</table>

02/03/2022
Botulinum neurotoxins (BoNT) act by cleaving specific proteins involved in synaptic vesicle exocytosis resulting in inhibition of neuro-transmission. In order for the various BoNT serotypes to gain access to their specific substrates they have to be taken up by the neuron. This is thought to occur by the binding of the BoNT to a specific receptor on the plasma membrane of the target cell followed by receptor mediated endocytosis of the BoNT. The specific endocytic pathways involved in this have not been defined. One of the best characterised mechanisms of receptor mediated endocytosis involves uptake via clathrin-coated pits. It has been shown that this clathrin dependent endocytosis pathway can be disrupted at a number of specific points by the expression of truncated or mutant forms of the proteins involved, specifically AP180, auxilin (Zhao et al., 2001) and clathrin heavy chain (Liu et al., 1998).

In order to use these tools to access the contribution of clathrin mediated trafficking to the action of BoNT on synaptic vesicle function and hence synaptic transmission it will be necessary to express the above dominant negative forms of the clathrin associated proteins in neuronal cell cultures. Successful high-level introduction of DNA has proved difficult in neurons. Currently the best characterised and most widely used system to introduce DNA into neurons involves the use of replication-deficient adenoviruses. In fact this system has been used successfully for the introduction of constructs expressing other proteins involved in vesicle trafficking into cerebellar granule neurons (Yang et al., 2000).

The aim of this project is to use the replication-deficient adenovirus expression system to express mutant and truncated forms of clathrin associated proteins in neuronal cells in culture, thus disrupting clathrin mediated trafficking pathways. The ability of these cells to take up and traffick BoNT will then be assessed. This will allow us to determine if the routes of entry to the cell and intracellular trafficking of the various BoNT serotypes are mediated by any of the characterised clathrin mediated pathways.


Genes for rat or bovine clathrin associated proteins will be inserted into shuttle vectors and transfected into a commercial human embryonal kidney cell line where they will be packaged into replication-incompetent adenovirus. These will be harvested and used to infect primary cultures of rodent neuronal cells.

Host/vector system

Full length cDNAs for the various clathrin associated proteins will be maintained in non-expressing bacterial vectors (e.g. Bluescript). These will be used as templates for the construction of previously defined truncations (using internal restriction enzyme sites or amplification of specific regions by (PCR) and site directed mutations (using commercial kits eg. Stratagene QuickChange kit). These constructs will be initially cloned into the multi-cloning site of the mammalian expression vector pcDNA1.1 and transfected into cell lines using standard calcium phosphate or lipofectamine reagents. Since transfection of neuronal cells is notoriously difficult with these techniques it is envisaged that in order to achieve sufficiently high transfection efficiencies to allow biochemical studies a commercial adenovirus expression system will have to be used. Therefore the constructs will also be cloned initially into the p-Shuttle vector for testing in the adenovirus expression system and for subsequent studies into the pTRE-Shuttle vector which restricts expression to cells co-transfected with Adeno-X-Tet off virus. Constructs will be amplified in the specified E. coli strains and those in shuttle vectors transfected into the human embryonal kidney 293 cell line were the replication-incompetent adenovirus will be produced and released into the medium. The harvested replication-incompetent adenovirus will be applied to mammalian (non-human) neuronal cells in culture.

Origin & function

All the DNA constructs which will be used have been originally cloned from either rodent or bovine cDNA libraries (Morris et. al 1993; Schroder et. al. 1995; Liu et. al 1995). These will be used to produce truncated and mutated forms of the clathrin associated proteins, AP180, auxilin and clathrin heavy chain. These are expected to have impaired function and to inhibit clathrin mediated endocytosis when expressed in mammalian cells in culture.


Evaluation of foreseeable effects

Truncated genes for clathrin associated proteins will be expressed in wild type (non-human) neuronal cells in culture, using a replication-incompetent adenovirus vector, thus no disabling mutations will be present in the recipient cells. Even if there was a naturally occurring mutation it is unlikely that this could be overcome by expression of proteins which will inhibit clathrin mediated vesicular trafficking pathways. Although, non-regulated expressing adenovirus will be used for pilot studies, the majority of the experiments will use regulatable viruses. This additional level of safety is in built by the necessity to add a second virus, termed the Adeno-X-Tet off expressing the Tet transactivator protein which is necessary for activation of the CMV-TRE promoter in the Adeno-X-TRE virus and allows expression of the gene product contained in the latter. Despite the fact that truncated clathrin associated proteins inhibit receptor mediated endocytosis they have not been reported to cause cell death in non-neuronal cell lines and the relatively small quantities of virus being used would preclude any hazard to the user, particularly when handled under containment. Replication-deficient adenovirus can infect human cells, however risk of infection to the experimenter will be minimised by working in a class II hood. As the adenovirus produced by the packaging cell line is replication-deficient any subsequent infection cannot lead to further propagation. No evidence suggests that the disruption of clathrin mediated trafficking pathways will be either oncogenic nor pathogenic. Transfer of the genetic material to organisms outside the laboratory is highly unlikely since this would require the occurrence of a rare recombination event with wild type adenovirus; wild type virus is not used in the lab.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: 10000 ppm chlorine from chloros is added which will be flushed into designated, downward draw sink. This gives 100% degree of killing as stated by the manufacturer.

Solid waste in compliance with HSE and departmental guidelines complete ‘kill’ of solid GM waste is achieved first by soaking in 1% hypochlorite for at least 1 hour followed by rinsing with water and autoclaving. An automated programmable Rodwell Monach 1534 autoclave is employed and validated by biannual 12 point load tests by departmental approved engineers. The latter test is performed by placing 12 thermocouples at various locations throughout different types of loads during the autoclave cycle and the temperature reading recorded. For the sterilisation of solid GM waste including plastic ware, a program lasting 1 hr 45 mins using a chamber set point of 128 degrees centigrade is performed. To absolutely ensure an efficient autoclave cycle, an additional integral thermocouple in this device is placed in the centre of the load using a 126 degrees centigrade load temperature set point and an audible alarm indicates cycle failure. In addition, hypochlorite (1%) has been validated by others as a suitable method for complete killing of adenovirus. All chloros decontaminated-autoclaved solid waste is placed in sealed biohazard bags tagged with colour coded ‘cable ties’ and sent for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Imperial College genetic modification safety committee considered this proposal to be satisfactory without the requirement for any alterations. They state that all work must be carried out according to the proposal and that no alterations to the proposal should be made without first referring to the GM safety committee.

Project Containment

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Project Ref 8/02.3

Date Ackn’d 02/03/2022
**Project Additional Information**

**Purposes of the contained use**

We wish to over-express foreign proteins of interest in order to study the properties of these proteins. None of the proteins which we would express in this system are hazardous to human health. These recombinant baculoviruses will be occlusion-positive, therefore potentially remain infective for their insect host via the normal oral route.

**Recipient or parental organism**

The parental baculoviruses have individual, narrow host ranges within insects and do not infect humans, other vertebrates, or other non-target invertebrates. Although the GMOs could, theoretically, survive in certain protected environments, the risk to the environment is negligible.

**Host/vector system**

The baculovirus used in these studies are infectious to particular species (mainly agricultural pests) within the Lepidoptera (moths and butterflies). The baculoviruses we will use are: Autographa californica Nucleopolyhedrovirus, Bombyx mori Nucleopolyhedrovirus, and Anticarsia geminitalis Nucleopolyhedrovirus. These viruses will be grown in some of the following cultured insect cells: Spodoptera frugiperda Sf9 and SF21 cells, Trichoplusia ni Tn368 cells and ‘Hi5’ cells, Mamestra brassica cells, Anticarsia geminitalis UFLAG2 cells and Helioverpa zea BCIRL-Hz-AM1 cells. Additionally, these viruses may be reared in T. ni, Spodoptera spp., H. zea and Heliothis virescens larvae.

**Origin & function**

The genes to be inserted into the baculoviruses were obtained from colleagues, commercial sources, or were cloned ourselves. Genes to be individually expressed in these baculoviruses are: UPD-glycosyltransferases from various species, Spodoptera littoralis ecdysone oxidase and 3-dehyroecdysone reductase, Cardiochiles nigriceps polydnavirus genes, Heliothis armigera Entomopoxvirus genes, GFP and derivatives, acetyl CoA synthase, adenylosuccinate synthase, influenza NP, der-PI antigen, LCMV GP, B, mori prothoracicotrophic hormone, H, virescens juvenile hormone esterase, M. sexta eclosion hormone, P. tritici tox34 insecticidal toxin, P. plagiophthalmus luciferase, E. coli beta galactosidase) from occlusion-positive recombinant baculoviruses. In general, we are expressing these proteins using baculoviruses as eukaryotic expression vectors, for the purpose of producing large enough quantities of these proteins for biological and biochemical characterisation of their function. These proteins will be expressed in an occlusion-positive virus background for either of two purposes: 1) To assess how expression of foreign insect proteins affects the oral route of infection and 2) To allow production of proteins as polyhedrin fusion proteins to produce recombinant occlusion bodies for antibody production.
Evaluation of foreseeable effects

For most of these genes, their expression within a recombinant baculovirus will not change the replication properties of the virus in cell culture or in larvae infected via the oral route. A few of the genes (e.g., those encoding products which interact with insect hormone metabolism pathways or the P. tritici tox34 insecticidal toxin) may alter the kinetics of infection within the larval host, but are not envisaged to alter the other parameters of the replication properties of the recombinant baculoviruses compared to parental 'wild' viruses. In the case of baculoviruses which are expressing foreign proteins as fusion constructs, these may decrease the infectivity of the recombinant baculoviruses to the larval host via the normal oral route of infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste containing GMMs will be treated with a solution of 1% Virkon with at least 1 hour contact time, as per manufacturer's instructions. The degree of kill for this method is effectively 100%. Liquid waste and solid waste containing GMMs will be autoclaved using manufacturer's recommendations. Success of each autoclave cycle will be monitored by technical staff with the aid of paper printouts which record equipment performance. Autoclaves are inspected yearly by an independent contractor. Chemically treated and autoclaved liquid waste will ultimately be disposed of down the drain. All autoclaved, solid waste is taken to a central collection point for incineration by an approved contractor. Again, degree of kill for this method is effectively 100%.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The risk assessment proposal was considered satisfactory with no alterations required (GM activity notice dated 01-7-02). However, the work cannot commence until HSE approval has been obtained.

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Large Scale Activities

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Human Clinical Applications

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</table>
**Project Additional Information**

**Purposes of the contained use**
To express small amounts of wild type or mutated a-latrotoxin and a-latroinsectotoxin. Although the micro-organisms used will be unable to survive in the human body or in the environment, the introduced genes encode harmful toxins; so to limit any theoretical risk, the experiments proposed will be carried out under containment. Because the amounts of the active toxin produced will be minimal and the employed procedures will prevent any exposure of the experimenter and the environment, Containment level 2 is sufficient.

**Recipient or parental organism**
Parental organism: the black widow spider (Latrodectus sp.)
Recipient: (1) baculovirus; (2) E.coli K-12 disabled strain; (3) non-human mammalian cell lines (COS7, monkey; NB2A, mouse).

**Host/vector system**
Baculovirus carrying the toxin gene will be used to infect small-scale insect cells cultures (SF9, Hi5). Non viral plasmid vectors carrying the toxin will be introduced into E. coli bacteria and non-human cell lines for small-scale growth and protein expression.

These strains are harmless to humans and will not survive in the human body or the environment.

**Origin & function**
The genes encoding a-latrotoxin and a-latroinsectotoxin have been obtained from Prof. Eugene V Grishin of The Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Russia. The a-latrotoxin gene is also being used by Prof. Thomas C. Sudhof of the University of Texas SW Medical School at Dallas. The isolation and use of these genes has been published previously (Kiyatkin et al., 1993, Eur J Biochem. 213:121-127; Dulubova et al., 1996, J. Biol.Chem. 271:7535-7543;
The latrotoxin genes will be used to introduce point mutations and produce truncated and mosaic mutants of the two toxins, with the intent to express small quantities of the wild type and mutant toxins. The latter will serve as analytical tools to study the mechanisms of neurotransmission in cultured cells or extracted tissues (no in vivo experiments will be conducted).

**Evaluation of foreseeable effects**

The microorganisms used in the study are attenuated and cannot survive in the human body or penetrate into the bloodstream. Only α-latrotoxin may be harmful to humans, and only in its un-mutated form. However, if swallowed, the toxin is unstable in the stomach and intestine and cannot cause any serious damage. The only theoretical and easily avoidable risk is posed if a substantial amount of GMM carrying α-latrotoxin were to enter the bloodstream, in which case the expressed toxin could cause some poisoning. But even in this worst-case scenario, the amount of α-latrotoxin released into the bloodstream would be very small and essentially harmless.

In contrast, α-latroinsectotoxin is not dangerous to mammals but is toxic to insects. However, the GMM carrying the gene for α-latroinsectotoxin will not be released into the environment and cannot survive outside the laboratory conditions.

Importantly, the procedures used will totally nullify the risk of internal exposure or dissemination.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The solid waste produced (bacterial and cell pellets, pipettes, plates, tubes, tips) will be autoclaved and then incinerated. The liquid waste (used media and supernatants cleared of most GMMs) will be autoclaved and discarded after large-volume dilution. The degree of kill is 100% in both cases, and this will be monitored by temperature probes and control plating.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

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02/03/2022
We will introduce genes encoding non-toxic reporter molecules including Green Fluorescent Protein, Luciferase, Chloramphenicol acetyl transferase and beta-galactosidase into different enteric bacteria. These reporter genes will be expressed from either constitutive or regulated bacterial promoters. The aim of the work is to use the reporter gene products to (1) track bacteria to different cells or sub-cellular locations within eucaryotic hosts; (2) to detect the activity of different promoters under different conditions.

The reporter genes will be supplied from existing plasmids as cloned DNA sequences or purchased from commercial suppliers. They will be introduced into any enteric bacteria other than those classified as class III or above. Examples will be Escherichia coli (other than verotoxigenic forms), Shigella spp. Salmonella enterica (other than class III agents such as S. Typhi and S. Paratyphi), Citrobacter spp etc.

The host bacteria will be any enteric bacteria. The genes will either be introduced directly into the chromosome of the bacteria (via suicide vectors or PCR methodologies).
Alternatively the genes will be cloned onto non-transferable plasmids such as those based on ColE1 replicons or pAYC184 (pUC plasmids for example).

**Origin & function**

The genetic material involved is from a variety of sources such as jelly fish (Green Fluorescent Protein), bacteria (beta-galactosidase, luciferase, chloramphenicol acetyl transferase) but will not be cloned from these sources. We will use well-defined existing plasmids or genes from commercial sources. The DNA is simply to be used to report gene expression activity in enteric bacteria under different conditions.

**Evaluation of foreseeable effects**

The genes we have selected are all extremely well characterised and have been used without incident in thousands of research laboratories around the world. We will only use the named reporters associated with this application. If we decide to use any novel reporters we will amend the local risk assessment. To our knowledge there have been no foreseeable effects of these reporters other than high level expression could cause toxicity to the host bacteria (as with over expression of many proteins).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All the experiments will be performed in a well maintained CL2 facility either in The Flowers Building or the CBS facility at Imperial College. A copy of the protocol for handling enteric pathogens in these facilities is enclosed for inspection and has been approved by the local safety committee. Briefly we have detailed policies for handling contaminated liquid and solid waste that involved the contained removal of materials for autoclaving. Hence, all contaminated material is autoclaved or inactivated using chemical disinfection procedures using a variety of accepted and validated disinfectants including phenolics (hycolin etc), Virkon S or chloros. All disinfectants are made up and utilised as described in the enclosed protocol. We routinely validate inactivation using a viable count method based on the killing of control cultures of bacteria within accepted ranges. All waste material is removed using clinical waste disposal methodologies in place at Imperial College.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

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02/03/2022
The aim of the use is to study S. Typhi using a safer attenuated derivative suitable for use at CL2. This will facilitate further examination of (a) the vaccine potential of these derivatives (b) the mechanisms of pathogenesis of S. Typhi.

Recipient or parental organism

Both S. Typhi BRD948/CVD908-htrA (aroC aroD htrA) and ZH9 (aroA aroC ssaV) or similar derivatives have been extensively studied in human volunteers and have been shown so far to be safe and highly attenuated vaccine candidates. ZH9 is through phase II clinical study and is being prepared for an efficacy study in phase III in the field./ Both BRD948 and ZH9 harbour at least three independently, non-reverting attenuating mutations. Both strains fail to colonise the human host and, as S. Typhi, are unable to colonise animals or plants. Both are based on the well characterised S. Typhi strain Ty2 which has been recently fully sequenced. Both strains harbour deletion mutations that are fully sequenced and inactivating. Both strains have been shown to be attenuated in mice as well as humans.

<table>
<thead>
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<th>Host/vector system</th>
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<tbody>
<tr>
<td>Both BRD948 and ZH9 are fully antibiotic sensitive and harbour no foreign DNA.</td>
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<tr>
<td>All DNA is derived from S. Typhi Ty2 with no foreign DNA. The derivatives harbour specific DNA deletions intended to stably attenuate.</td>
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<th>Evaluation of foreseeable effects</th>
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<td>These derivatives have been shown to be highly attenuated in many scores of human volunteers and similar mutations attenuate S. Typhimurium in mice and other species. We see no reason why these organisms should lose their attenuated phenotype as the genetic lesions have been well characterised in multiple in vivo and in vitro systems. Their derivatives have been used by different groups in the UK for many years safely at CL2. For example, BRD948 has originally been recognised by HSE as disabled in 1993.</td>
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<td>The microorganisms will be derogated to CL2 as they are unable to colonise the human host and are highly attenuated but are derived from S. Typhi. The derivatives now have an excellent safety record of being safely handled at CL2 with no evidence of human infection both in the UK and abroad. The microorganism will be handled at CL2 or a higher level of containment at all times.</td>
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<td>We operate a number of comprehensive and proven waste management measures which are conducted according to our local protocol for handling CL2 pathogens. This protocol has been in use for many years and involves autoclaving of clinical waste material, chemical inactivation of liquid waste followed by autoclaving and careful management of waste movement. The GMM will be used within a containment level 2 laboratory in which all bacterial cultures, glassware etc. is decontaminated prior to disposal. Thus drains, sinks etc. do not pose a mode of transmission to the environment. Air movement is also strictly regulated in the laboratory environment. Solid biological waste is autoclaved using temperatures, cycles and conditions appropriate for the inactivation of biological material. Autoclaving is performed by departmental staff using approved conditions. Temperature is 134 C for 3 minutes per cycle. Glassware etc. is decontaminated, as described above, prior to being autoclaved.</td>
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<th>Is an emergency plan required according to regulation 20?</th>
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<td>N</td>
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<tr>
<td>The committee agreed with the classification and containment level assigned to this project.</td>
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02/03/2022
Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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Project Ref 8/03.4

Date Ackn’d: 12/12/2003
CU2 Project Title: MALARIAR RESEARCH - TRANSGENIC PLASMODIUM FALCIPARUM PARASITES: ESTABLISHMENT OF AN INDUCIBLE SYSTEM TO CONDITIONALLY DISRUPT ESSENTIAL GENES AND VALIDATE NOVEL TARGETS FOR INTERVENTION

Date Project Ceased: 20/06/2016

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info: 20/06/2016

Significant Change ID: 8/03.4a

Date of Significant Change: 07/05/2013

Project Additional Information

Purposes of the contained use

The first aim of the project is to establish an inducible gene expression system in the asexual red blood stage stages of Plasmodium falciparum. The strategy will consist in the integration of a plasmid leading to the stable expression of a tetracycline Repressor fused to an activating domain (transactivator) and to the conditional expression of a reporter gene (chloramphenicol acetyltransferase or GFP) controlled by tet-operator dependent promoter, as recently described in Meissner et al 2002, Science). The second aim is to use this inducible system to create conditional knockouts for genes that are essential for the survival of Plasmodium falciparum. The candidate genes to be disrupted include:
- proteases of the rhomboid family potentially required for host cell invasion.
- Calmodulin-dendent kinases potentially involved in a stage differentiaion and organelle secretion
- Surface antigens and secreted adhesins potentially playing a role in parasite differentiation and host cell interaction.

**Recipient or parental organism**

*Plasmodium falciparum* is the causative agent of malaria in human. Its life cycle is split between the human host and an insect vector. Transmission to human occurs via bite by the mosquito vector and only female *Anopheles* mosquitoes are involved as the males do not feed on blood.

*Falciparum* malaria symptoms include: complain of headache, fever and aches and pains all over the body, and diarrhoea and abdominal pain are sometimes present. Spleen and liver are often palpable on clinical examination. This may be misdiagnosed as influenza in non-endemic areas, and, unless treated promptly, the clinical picture can deteriorate rapidly. Can progress to cerebral malaria: confusion, delirium, loss of consciousness. Incubation period: at least 7 days.

Infection could occur through: 1/ Bite from infected mosquito. 2/ Accidental inoculation (needlestick injury) with blood used to culture parasites in the laboratory.

The strain of *P. falciparum* to be used in this study are the two isolates D10 and 3D7, and both of which are sensitive to chloroquine.

**Host/vector system**

The vectors to be employed in the proposed experiments are all derived from the commercially available bluescript from Stratagene. Most of the constructs are designed to express modified proteins or to generate knockout by homologous recombination in *P. falciparum*. These vectors are all described in the literature cited below. The selectable marker genes are the *Toxoplasma gondii* and human dihydrofolate reductase-thymidylate kinase genes. The reporter genes include chloramphenicol acetyltransferase and the green fluorescent protein. The tetracycline repressor originate from *E. coli* and is fused to an activating domain generated in *T. gondii*.

The *P. falciparum* genes to be studied include ROM1, ROM2 and ROM3 (rhomboid-like proteases involved in parasite motility and other undefined functions), cammodulin dependant kinases and surface antigens. Their cDNA will be tagged with epitopes and cloned into the Calmodilin expressing plasmids previously described (Crabb 1997).

The flanking sequences controlling expression of the transgenes are derived from the *P. faciparum* calmodulin genes.


**Origin & function**

The genetic material originates from *P. falciparum*, *T. gondii*, human and E coli.

Rhomboid proteases, kinases, surface antigens.

Gene originating for other organisms: tetracycline repressor (tetR), green fluorescent protein (GFP), chloramphenicol acetyl transferase (CAT). Dihydrofolate reductase (DHFR).
Functions: Selectable marker to integrate and/or disrupt genes by homologous recombination (TgDHFR, human DHFR), reporter genes (GFP) and Creation of chimeric fusion to follow the traffic of protein (GFP), and Creation of chimeric fusion to follow the traffic of protein (GFP), and modulation of gene expression (tetR).

Evaluation of foreseeable effects

1. The genes coding for these proteins will be disrupted in P.falciparum by homologous recombination or by conditional knockout using the inducible tetracycline based gene expression system.

We anticipate an alteration of the ability of parasites to infect cells and to progress into their life cycle.

2. These genes will be expressed as mutants: fused to an epitope tag, insertion of site specific point mutations deletions or GFP fusions of these genes will be used to generate transgenic parasites.

No change increase of virulence is expected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Plasmodium falciparum is a micro-organism

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Plasmodium falciparum is classified as Class 3, but as the pathogen is not transmitted through the airborne route, fumigation of the lab is not required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Propagated of the P. falciparum intraerytotic stages in human blood must take place in the CL3 unit on the 6th floor of the SAF building. The room and equipment fulfills all the safety requirements. The manipulation of live parasites will be done in a safety cabinet. Wearing gloves and laboratory coats are requested and use of no needles or sharps is not allowed. No material or equipment can leave to zone before decontamination or autoclaving.

All the solid wastes will be autoclaved in the Containment 3 zone. Autoclaving: 30 minutes at 121 degrees.C at 15p.s.i. (1.1kg/sq.cm. Monitoring: Autoclave performance is monitored continuously by means of autoclave printout, by the temperature monitoring inserted into the load, and by "thermo-stripes", and checked, serviced and monitored (including a multipoint thermocouple test) annually by an independent company. Work carried out in the Containment level 3 laboratory is autoclaved within the containment room prior to removal to the main autoclave for a second round of autoclaving. The liquid wastes will be decontaminated with 5% of chlorous.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the project involving Plasmodium falciparum can be conducted in a CL3 unit and that the genetic modifications to be introduced according to the project are not anticipated to change the category of containment. In the case of a laboratory exposure to the parasite, the person infected should immediately be treated. For treatment, chloroquine sensitive malaria is controlled by intravenous chloroquine.
### Project Ref  8/04.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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<tbody>
<tr>
<td>02/04/2004</td>
<td>GENERATION OF PLASMODIUMBERGHEI TRANSGENIC PARASITES AND TRANSGENIC ANOPHELES GAMBIAE AND ANOPHELES STEPHENSI MOSQUITOES FOR MALERIA CONTROL...</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
<td>Y</td>
</tr>
</tbody>
</table>

**Project notified under transitional arrangements**  

#### Project Additional Information

**Purposes of the contained use**

To generate transgenic Plasmodium berghei parasites and transgenic Anopheles gambiae and Anopheles stephensi mosquitoes for malaria control

**Recipient or parental organism**

The genes modified on transgenic P.berghei parasites encode for motility proteins or genes involved in a mosquito specific immune reaction. the parasites will have reduced motility and infectivity in mosquitoes.

The transgenic mosquitoes will express marker genes (e.g. luciferase or EGFP) or RNA1 genes against marker genes or mosquito specific genes. The introduction of...
marker genes will not have any effect on the phenotype of the mosquitoes, the RNAi genes against GAGs (glucosaminoglycans) are anticipated to reduce the ability of malaria parasites to invade mosquito salivary glands.

### Host/vector system

In mice and mosquitoes (the GMM hosts), the transgenic parasites are anticipated to have reduced infectivity.

### Origin & function

The genetic material (genes involved in parasite motility, mosquito immunity or marker genes) originates from lab stocks. The intended function of the genetics material is to generate transgenic parasites with knockout or replacements of parasite motility genes, or mosquitoes expressing RNAi genes or marker genes.

### Evaluation of foreseeable effects

The transgenic parasites will have reduced motility and infectivity in mosquitoes.

The introduction of marker genes will not have any effect on the phenotype of the mosquitoes, the RNAi genes against GAGs (glucosaminoglycans) are anticipated to reduce the ability of malaria parasites to invade mosquito salivary glands.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Mosquito cages are designed to effectively prevent escape at all stages. Cages are clearly labeled giving the species, date of collection and relevant modification. Personnel will be wearing lab coats at all times and gloves when necessary. The mosquitoes are contained within the insectary which has a specialised entry system to prevent mosquito escape.

The organisms can not survive at the embryonal or larval stage unless maintained in water, so in the event of a spillage they would not survive. Following a spillage the area will be dried and any solid waste will be autoclaved.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)  
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Mosquitoes: autoclaved (solid cycle) by college staff after killing by starvation and exposing adult mosquitoes to freezing temperatures or by placing larvae in boiling water. The degree of kill is 100% and this practice is standard for mosquito rearing. This is monitored by an autoclave printout and by the use of a thermophile probe inserted into the load, and by thermostrips. The autoclave is serviced and monitored annually by a departmentally approved contractor.

Parasites: Plasmodium parasites are killed by high temperatures (100% effectively), achieved by the autoclave as monitored by a thermograph. This is monitored by an autoclave printout and by the use of a thermophile probe inserted into the load, and by thermostrips. The autoclave is serviced and monitored annually by a departmentally approved contractor.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N
The proposal was considered satisfactory.

**Project Containment**

<table>
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<td>L2</td>
<td>L3</td>
<td>L4</td>
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**Animal Units**

| L2 | L3 | L4 | L2 | L3 | L4 |

**Large Scale Activities**

| L2 | L3 | L4 | L2 | L3 | L4 |

**Human Clinical Applications**

| L2 | L3 | L4 |

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**Project Ref 8/04.2**

**Date Ackn'd**: 07/04/2004

**CU2 Project Title**: THE STUDY OF:- 1) GENOME ORGANISATION IN LEISHMANIA AND TRYPANOSOMA BRUCEI, 2) EXPRESSION OF LEISHMANIA AND TRYPANOSOMA BRUCEI GENES, 3) GENETIC MODIFICATION OF LEISHMANIA AND TRYPANOSOMA .....  

**Class**: Class 3  
**Culture Volume**: 2 litres

**Non-GMM Consent Granted**: Not Applicable

**Project notified under transitional arrangements**: N

**Historical Significant Changes**

| Project GM8/04.2 transferred to GM177 on 07/04/2004 |

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**Project Additional Information**

**Purposes of the contained use**

* to map and sequence target genes in Leishmania species and Trypanosoma brucei, in relation to their chromosomal position and stability between genomes.

* to clone Leishmania and Trypanosoma brucei genes into plasmid vectors engineered for prokaryotic expression: to express and purify the target proteins for further analyses.

* to target specific genes for disruption (by homologous recombination) to generate null, loss-of-function of gain-of-function mutants for phenotypic analyses, by the introduction of antibiotic resistance genes into chromosomal sites in the genomes of non-infective Leishmania or T. brucei stages.
Recipient or parental organism

E. coli K12 strains: eg E. coli BL21 (DE3) pLysS
Leishmania major MHOM/IL/81 Friedlin
Leishmania mexicana MNYC/B762/M379
Leishmania donovani MHOM/SD/82/IS-CL2D
Leishmania infantum MCAN/es/98/LLM-724
Leishmania (Viannia) braziliensis MHOM/BR/75/M<2904
Trypanosoma brucei brucei Lister 427

Host/vector system

For E. coli host:
Plasmid vectors: pUC based such as the pBS, pSK, pGEX or pEt series
Cosmid vectors: pWE15 (Strategene) or similar

For Leishmania/T brucei host:
Plasmid shuttle vectors: pUC based such as the pBS or pSK series.

Origin & function

The sources of parasite material (both Leishmania species and Trypanosoma brucei) are collaborating reference centres, both in the UK and abroad, that supply frozen samples in sealed containers for transport into the containment laboratory. Frozen material is stored in the vapour phase of liquid nitrogen until required for in vitro culture.

The genetic material from these parasites will be used:
* in gene mapping studies
* for expression studies to generate recombinant proteins for structural and functional analysis
* to generate null, loss-of-function or gain-of-function Leishmania or T. brucei mutants for phenotypic analyses

Evaluation of foreseeable effects

Species of Leishmania are causative agents of human Leishmaniasis; the parasite species L. donovani, L. infantum, L. braziliensis are designated hazard group 3f$ (ACDP categorisation). The remaining species covered by this notification, L. major and L. mexicana, are hazard group 2 (ACDP categorisation), as is Trypanosoma brucei, which is non-infective to man. In susceptible individuals, leishmaniasis is treatable (as detailed in the Code of Practice, June 1992).

The target genes for manipulation will be wild type Leishmania or T. brucei sequences that have been fully characterised by DNA sequencing. The products of these genes are not known to have a role in the progression of human disease but this possibility cannot be discounted for those genes coding for proteins of unknown function. The GMMs are unlikely to express proteins at a level significantly higher than wild type parasites that have not been genetically-modified.

The pathogenicity of Leishmania species and the hazard this imposes to experimental work in the Department of Biological Sciences is already covered by Codes of Practice for cultivation in culture (June 1992) and in animals (January 1993). Genetically-modified Leishmania will be subject to the same safety protocols; the environmental risk is therefore no greater than with unmodified parasites. The life cycle of Leishmania involves transmission of extra-cellular parasite stages between hosts by a sandfly vector, followed by intracellular maintenance within macrophages in the mammalian host. No other organism is known to be at risk from infection. There is no sandfly colony on the South Kensington site of Imperial College, so transmission of the parasite, by inoculation of infective parasites into susceptible mammalian hosts, is impossible.

Transgenic Leishmania will be resistant to one or more antibiotics but this is irrelevant to therapy against human leishmaniasis (for which pentavalent antimonials are the current drugs of choice). Transgenic T. brucei will also be resistant to one or more antibiotics but this is irrelevant as T. brucei is non-infective to man.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Table 1a Measure 2 Laboratory: sealable for fumigation. Justification - Parasites not transmissible through the airborne route.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM waste is of 2 types: liquid and solid.
Liquid GMM waste: is treated by chemical disinfection, using Chloros diluted 1:10 (final concentration 10,000ppm available chlorine), with contact time > 12 hours. This gives 100% kill, as validated by testing described in the attached risk assessments.
Solid GMM waste: is autoclaved at 134 degrees C for 15 minutes at 27 psi pressure. This gives 100% kill, as validated by testing described in the attached risk assessments.

All clinical waste is sealed in bags and transported on robust trays from the containment facility to the autoclave, via the interlock hatch. This waste is handled by authorised personnel only. After autoclaving, the material is transferred to yellow bags for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC agreed with the classification and containment measures assigned to this project. Minor modifications were required to be made to the risk assessment form prior to notification to the HSE.

Project Containment

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Animal Units
| L2 | L3 | L4 |

Large Scale Activities
| L2 | L3 | L4 | L2 |

Human Clinical Applications
| L2 | L3 | L4 | L4 |

Project Ref 8/04.3
Genetic modification of Trypanosoma brucei

Purposes of the contained use
Basic research related to coelomate cell biology of eukaryotes and development of therapeutics for protozoan parasites using cell culture and transgenic analysis techniques.

Recipient or parental organism
Trypanosoma brucei brucei Lister 427 and derivatives expressing T7 polymerase and the tetracycline repressor. Lifecycle forms of the parasite both mammalian bloodstream and insect procyclic stages are grown in vitro. The 427 strain of T.brucei has been in vitro cultured for many years and is bloodstream monomorphic. The genetic modifications proposed for this study are on genes of the CCCH zinc finger family that have no "activity" in themselves but may act to regulate gene expression via RNA binding. It is therefore unlikely that either the parental organisms of those generated in this research are hazardous to health or the environment. Plasmids are propagated in standard non-virulent laboratory E. coli strains (E. coli K12-DH5a, XL1Blue or TOP10) and it is unlikely that they represent a hazard to health or the environment.

Host/vector system
Overview:
The aim of this GMO system/study is the expression of mutant and wild type versions of trypanosome proteins and the suppression or ablation of these proteins in trypanosome cells in order to ascertain biological function. The host strains are T. brucei brucei Lister 427 and derivatives 427/SMB, 427/29-13, 427/449. These later forms of the parental strain have been previously engineered to express T7 polymerase and tetracycline repressors (427/SMB and 427/29-13) or the tetracycline repressor alone (427/449). Plasmid vectors are pUC based pGEM or pBluescript series shuttle vectors that are non-mobilisable and contain an ampicillin resistance marker (for cloning in E. coli) and a selectable marker for transfection into T. brucei (Hygromycin, Neomycin or Phleomycin). T. brucei plasmid shuttle vectors for RNAi are p2T7i, for gene knockout are pTNP, pNP, pH and for ectopic expression are pM2C-C/N, pHD451 and pHD430 that are propagated in standard laboratory strains of non-virulent E. coli K12.

Specific notes:
Ectopic expression will be via plasmids (pM2C-C/N, pHD451 or pHD430) engineered for expression in T. brucei that integrate into the trypanosome genome at the tubulin
locus on chromosome 1 or to the rRNA locus on chromosome 9. Due to the fact that these organisms trans-splice their mRNA, and have unique promoter structures, these vectors are incapable of expression in other systems. No attempt is made to optimise expression in these system and there are no reports that expression levels would be greater than 2% of the cellular protein content.

Vector p2T7 is targetted to the ribosomal rRNA locus on chromosome 9. Selection is with phleomycin or hygromycin. This vector expresses RNA that is not capped, and cannot be translated, and hence no protein is expressed. Besides the selectable marker, no protein product is produced. Gene knockout vectors integrate into the 5' and 3' untranslated regions of the targeted gene and result in ablation of the coding sequence and insertion of a selectable marker. Consequently this results in loss of function for the targeted gene and protein product.

Growth of all GM trypanosomes is routinely 10ml in a single culture flask or for large experiments (infrequent) <100ml in a single culture flask. All vectors/GM trypanosomes will carry a selectable marker for hygromycin, phleomycin or neomycin and/or a combination of these drugs and these will be included in culture media. PHD430 and pH451 described in Biebinger S, Wirtz LE, Lorenz P and Clayton C. Vectors for inducible expression of toxic products in bloodstream and procyclic. Trypanosoma brucei. Mol. Biochem Parasitol. 1997 vol 85 p99-112. p2T7 described in LaCount DJ, Bruse S, Hill KL, Donelson JE. Double-stranded RNA interference in Trypanosoma brucei using head-to-head promoters. Mol Biochem Parasitol. 2000 vol 111 p67-76.

For E. col K12 (XL1 Blue, DH5a or TOP10) standard vectors will be used based on pGEM and pBluescript.

Origin & function

CCCH zinc finger proteins are a family of proteins implicated in post-transcriptional control of gene expression, that may be significant in regulation of the Trypanosoma brucei genome. Over the course of this research potentially all known T.brucie CCCH family members will be studied (approximately 40-50 genes). The genetic material will be each of the T.brucie genes encoding CCCH zinc finger proteins that have been amplified from genomic T.brucie DNA and subsequently cloned into T.brucie ectopic expression expression systems (those mentioned above eg: pH451) and T.brucie RNA interference vectors and vectors for gene knockout (those mentioned above eg: p2T7i) for examining the cellular consequence of over-expression from the ectopically expressed gene or gene silencing/ablation once vectors have been stably transfected into T. brucei brucei 427 derivatives (as above). Cloning into the trypanosome vectors (eg: p2T7i and pH451) is performed in E. coli and only the final construct is transfected into T.brucie. The consequences of perturbed expression for individual CCCH proteins can then be phenotypically assessed in order to dissect functionality. Therefore it will be necessary for each vector containing a CCCH insert sequence to be propagated in standard laboratory E. coli hosts (as mentioned above).

E.coli cloning vectors are commercially available and will be transformed into the commercially available XL1-Blue or DH5a host strains or similar. Trypanosoma RNAi and ectopic expression vectors (p2T7i and pH451) are freely available academically generated plasmids that have been widely used for their specific purposes. In these cases E. coli strain XL1-Blue will be used for the propagation of clones prior to transfection of T.brucie.

The CCCH functions in trypanosomes have not been studied in detail (this study addresses these questions), but the suspected mechanism by which function is mediated is through post-transcriptional control of gene expression via RNA binding and subsequent stabilisation or destabilisation. Studies of CCCH proteins in other systems suggest this mode of action. Therefore the CCCH family of proteins has no "activity" in themselves but represent a specific class of regulatory molecules.

Evaluation of foreseeable effects

The genes (and derived GMOs) used in this research are unlikely to be directly part of pathogenic systems. The propogation of T. brucie in the mammalian host is reliant on a cell surface coat comprising a variable surface glycoprotein (VSG). All evidence that is available suggests that this is the primary virulence pathway. Moreover, the parasite does not secrete factors as far as is known that have a role in virulence.

As the CCCH genes have no biological activity in themselves it seems unlikely that they would transfer harm to another organism. CCCH proteins are found in diverse organisms including humans but the specific T. brucie C CCCH genes under study have no identifiable homologues in humans and therefore no identifiable activity or harmful effects.

All experiments that have been previously conducted on T. brucie CCCH proteins report no consequences for VSG expression. All experiments in this research are aimed
at alteration of function; in essentially all cases this results in a less efficient system, and hence the modified organism is less hazardous than the parental strain. It should be noted that the strain selected for study is non-pathogenic to humans as there is a lytic factor in human serum that efficiently kills the organism. None of the intended modifications could be expected to affect this to the extent that resistance will emerge.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid GMM waste will be chemically treated with either.

1. Chloros diluted 1:10 (final concentration 10,000ppm available chlorine), contact time >12 hours or
2. Cultures in flasks or centrifuge bottles, are treated by addition of Virkon powder to a final concentration of 1% or by the addition of an equal volume of a 2% solution of Virkon. Cultures are left in Virkon for a minimum of one hour.

These solutions can then be disposed of down the sink and any glassware sent for washing up. A kill efficiency of 100% is-obtained either procedure.

Viability can be monitored by motility of the organisms and by morphology for

1. Cultures of bloodstream form or procyclic form T. brucei brucei Lister 427 were grown in either HMI-9 or SDM79 media supplemented with fetal calf serum (15%). The cultures were grown to various stages of density. Immediately after the addition of Chloros diluted at 1:10, no live parasites were observed on a slide. After 16hr, >99% of the cells had totally lysed. The results were consistent for all cultures when diluted with Chloros.
2. By Defra standard efficacy suspension testing (manufacturers tests).

Solid GMM waste is collected into dedicated waste bags, sealed and autoclaved (134 degrees C, 15 minutes at 27 psi pressure). A kill efficiency of 100% is achieved by autoclaving and no intact organisms remain following autoclaving. Autoclaving is monitored by temperature indicator paper in each run and this departmental facility is monitored by departmental safety officers.

All waste will be sealed in bags and transported on robust trays from the containment facility to the autoclave. This waste will be handled by authorised personnel only. After autoclaving, the material will be transferred to yellow bags for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC agreed with the classification and containment measures assigned to the proposed project.
**Project Additional Information**

**Purposes of the contained use**

The overall purpose of the project is to evaluate these strains as potential human vaccines.

**Recipient or parental organism**

All strains are derivatives of S. typhi Ty2. All the strains contain deletion mutations in two genes. The first deletion is in the aroC gene. AroC encodes the chorismate synthase enzyme which is critical in the synthesis of aromatic amino acids. Disruption of aromatic amino acid synthesis has been extremely well characterised, and results in severe virulence attenuation in Salmonella (Tacket et al. 1992, O'Callaghan et al. 1988).
The second deletion is in ssaV. This gene encodes a component of the type III secretion system present on the Salmonella pathogenicity island 2 (SP1-2). SP1-2 mutations are severely attenuating and there is evidence that this is because they reduce systemic spread of the organism (Shea et al 1999).

All strains are equally non-hazardous. The parental strain of all the strains to be studies, S. typhi ZH9, has been studies preclinically by Khan et al. (2003) and clinically by Hindle et al (2002). In the latter study, volunteers were given the strain orally. No adverse symptoms were reported.

Host/vector system

All genes are encodes on the chromosome of S. typhi and therefore there is no plasmid vector system involved.

Origin & function

Several genes encoding non-toxic antigens are cloned within the chromosome of the attenuated S. typhi strains.

Evaluation of foreseeable effects

The strains of S. typhi used are designed as potential human vaccines. They are specifically designed to cause a self limiting infection and to induce protective immunity. The parental strain, S. typhi ZH9, has been evaluated both in preclinical and clinical trials and was found to be well tolerated and produced no unforeseen adverse effects.

The antigens to be cloned into these strains are well described in the literature and are non-toxic either by virtue of properties inherent to the molecules or by site directed mutagenesis.

We foresee no risk associated with these strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cultures will inactivated by either chemically or by heat serilisation (autoclaving).

Chemical sterilisation (used of liquid cultures)
Addition of Virkon to a final concentration of 2%; leave overnight; dispose of waste down sink.
Validation - manufacturer's web site.

Autoclaving (used for solid culture waste, agar plates etc)
134 degrees C 3 minutes
Each run is monitored by Departmental staff.
Autoclave is checked periodically by use of a 12 point thermocouple test.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
The Committee approved the risk assessment as Class 2. The experiments use derivatives of Salmonella typhi with two non-reverting attenuating mutations and pose little risk to the workers or to the environment. However as the wild type pathogen is a Hazard Group 3 agent, it was thought prudent to classify this project as Class 2. The control measures in place are adequate for the proposed work.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<tbody>
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<td>L2: Yes</td>
<td>L3</td>
<td>L4</td>
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<td>L4</td>
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<td>L3</td>
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<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

**Project Ref 8/07.1**

- **Date Ackn'd**: 23/02/2007
- **CU2 Project Title**: Molecular Analysis of Fungal Virulence.
- **Class**: Class 2
- **Culture Vol Class 2**: 1-50 Litres
- **Consent Granted**: Not Applicable
- **Non-GMM**: Not Applicable
- **Date Project Ceased**: 15/03/2012
- **Withdrawn**: No
- **Historical Significant Changes**: Project transferred to GM382

**Project Additional Information**
### Purposes of the contained use

The aim of this project is to define, at a molecular level, the virulence attributes of fungi, in particular *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus flavus*, *Candida albicans* and *Candida glabrata*. We also intend to investigate selected attributes in the model fungus *Saccharomyces cerevisiae*.

### Recipient or parental organism

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strains/Attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Strains in common laboratory use e.g. DH 5 alpha, XL-20 will be used as cloning tools. These strains, which are generally derivatives of the K12 strain, have a widespread and long history of safe use. They also contain numerous mutations e.g. thi-1 which render the strains auxotrophic and therefore unlikely to survive outside the laboratory environment.</td>
</tr>
<tr>
<td><em>Aspergillus nidulans</em></td>
<td>There are a huge number of classically produced mutant strains of A. nidulans. These have been constructed in many strain backgrounds and often have been crossed many, many times. These strains have a long history of safe laboratory use and usually contain auxotrophic mutations e.g. argB that would prevent survival outside the laboratory.</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>Af237, D141 and ATCC46645, Af293 and CEA 10 and auxotrophic derivatives thereof eg pyrG or antibiotic selection-mediated gene disruption derivatives thereof eg hygromycin-resistant clones, will be used to construct knock-out, regulatable and reconstituted strains. These backgrounds have a long and safe history of use worldwide. Additionally the pyrG auxotrophies would prevent survival outside the host.</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>SC5314, CA14 and CA110 and auxotrophic derivatives thereof eg his3/his3, ura3 will be used to construct knock out regulatable and reconstituted strains. These strains have a long and safe history of use worldwide.</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>ATCC2001 and auxotrophic derivatives thereof eg his3, ura3, trp1 will be used to construct knock-out, regulatable and reconstituted strains. These strains have a long and safe history of use worldwide.</td>
</tr>
<tr>
<td><em>Pichia pastoris</em></td>
<td>GS115 and SMD 1163 and auxotrophic derivatives thereof eg his4 will be used to express selected fungal proteins. These strains have a long and safe history of use worldwide.</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>The majority of laboratory strains are derived from three major wild type lineages S288C, W303 and Σ1278b. Most contain multiple auxotrophies and would not survive outside the laboratory.</td>
</tr>
</tbody>
</table>

### Host/vector system

We will use standard *E. coli* and *S. cerevisiae* vectors e.g. pUC, pBluescript, YE, YI and YC series. We will use a series of *C. glabrata* episomal vectors (Kitada et al, Gene 175:105, 1996). Mobilisable *Aspergillus* vectors will not be used.

### Origin & function

The majority of inserted genes will be from the same species, eg used to complement a null allele to create a reconstituted strain, and therefore do not constitute the construction of a GMM.

We are investigating fungal virulence and wish to analyse all attributes associated with the ability to cause disease. Current examples of genes under investigation (and the functions that they are known, or predicted to encode) include *C. glabrata* ACE2 (transcription factor) *C. glabrata* CTS1 (endo-chitinase) and *A. fumigatus* PacC (transcription factor). We will also analyse other genes related to virulence in these species.

We will also construct *Aspergillus* and *Candida* DNA libraries in *S. cerevisiae*. We will also add selected tags eg GFP, TAP, HA, etc to selected genes.
We will use heterologous antibiotics genes er ampR, bleR, hygR as markers of transformation.

**Evaluation of foreseeable effects**

The GMM are disabled and are unlikely to be able to survive in the environment and the modifications proposed (gene inactivation) would not confer a selective advantage even if they were to be transferred to a wild type organism. The likelihood of hazard is therefore considered to be no greater than that of the parental strains.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
<thead>
<tr>
<th>Type of Waste</th>
<th>Treatment</th>
<th>Ultimate Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid waste</td>
<td>Addition of Virkon to a final concentration of 1% and then left for at least 120 mins. According to manufacturer's data this treatment results in 100% killing of the GMMs. Treated liquid waste will be then disposed of via a designated sink.</td>
<td>Disposed of by the clinical waste route.</td>
</tr>
<tr>
<td>Solid waste in plastic bags (tissue culture flasks, plastic pipettes, pipette tips and eppendorf tubes)</td>
<td>Autoclaved at 134°C for 30 mins. This will result in 100% killing of the GMMS. A chart recorder will indicate the successful completion of the autoclave cycle and the autoclave is tested by 12 point thermocouple testing. The waste will then be disposed of by the clinical waste route.</td>
<td></td>
</tr>
</tbody>
</table>

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
Q 1.3 not really answered. If none of the proposed GMMs are likely to be any more hazardous than the host without an insert, then this should be stated, and a justification given e.g perhaps there are no known toxins in any of the fungal pathogens being used, or measures will be taken to specifically exclude any toxin genes from being expressed?

Reviewer 2:
2.2.1.1 PI needs to consider the worst case scenario for strains that do not contain auxotrophies.

Reviewer 3:
The hazards need to be identified and then explanations of how the risks are reduced by operational practices mentioned here.
1.21 Paragraph3: give some examples of genes being disrupted and/or replaced. Write explicitly the kind of phenotype assays that will be carried out to test the effects of the genetic modifications.
1.23 Give more details of the plasmids used for transformation of the fungi (Aspergillus sp and Candida sp). Specify the selectable markers used.
2.1.1.2 List the hazards to human health posed by the organisms used. Explain the diseases caused, not just the reasons to think that the risks are mitigated by the auxotrophies.
2.1.1.3 Give examples of the "majority" of genes. List some examples of the remaining "minority". Here is the first mention of complementation of the auxotrophies by the transforming plasmids. This appears to negate some of the mitigating effects described extensively in preceding sections. Therefore clear statements about this must appear in many of the introductory paragraphs.
2.3.2.1 In order to study the function of putative virulence genes, phenotypic assays including infection models require an infectious GMM: this will by definition be a hazard to humans and the environment. Hence this MUST be explicitly stated in the introductory paragraphs to all sections.

### Project Containment

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<td>L4</td>
</tr>
</tbody>
</table>

### Project Ref 8/07.3

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
<th>CultureVolume</th>
</tr>
</thead>
<tbody>
<tr>
<td>26/06/2007</td>
<td>Envelope assembly and gene regulation in Gram-positive bacteria.</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Applicable</td>
<td></td>
<td>N</td>
</tr>
</tbody>
</table>

02/03/2022
Project Additional Information

Purposes of the contained use

Envelope assembly project:
The cell wall envelope of Gram-positive pathogens has important functions as a surface organelle that allows bacteria to interact with their environment, in particular the tissues of the infected host. Major structures found within the cell wall envelope of Gram-positive bacteria include peptidoglycan, carbohydrates, proteins and other wall polymers such as teichoic acid (WTA) and lipoteichoic acid (LTA). The goal of this project is uncover genetic determinants required for the synthesis of these envelope structures, determine how their expression is regulated, biochemically and structurally characterize the function of encoded proteins and assess their contribution to pathogenesis. A more detailed knowledge of these envelope structures will help us design new strategies to prevent their synthesis or function and hence prevent infections.

The staphylococcus aureus research on gene regulation will consist of three research streams.

Stream one will focus on the pleiotropic virulence gene transcription regulator SarA (and homologues therefore) and will explore its interaction with the transcription machinery - the RNA polymerase. Of particular interest is to unravel how SarA influences transcriptional output via modulating the RNA polymerase. Stream two will investigate the genome-wide distribution of SarA during normal growth, infection and in response to antimicrobial compounds. Stream three will be centred on the mechanism of action of bacteriophage-derived small molecular weight proteins that either target or are "re-educated" to target the S. aureus RNAP.

Recipient or parental organism

Listeria strains

EGDe, EGD, 10403, Mack, CLIP11262,F6854,F2365 and derivatives thereof that have defects in cell wall envelope assembly pathways. These strains have a long history of safe use in diverse laboratories around the world.

Escherichia coli:

Wild-type K12 strains and derivatives thereof with required phenotypes for cloning and expressing staphylococcal or bacterial genes of other bacterial origin will be used in these studies. Strains commonly used in the laboratory include strain DH5alpha, XL1blue and CA800 and BL21 (DE3). These strains have a widespread and long history of safe use.

Bacillus anthracis Sterne; This strain is a veterinary vaccine strain.

Bacillus subtilis

Bacillus subtilis strain 168 and derivative thereof, which have defects in the assembly of envelope structures. These strains have a widespread and long history of safe use.

Staphylococcus strains

Newman, SA113, NCTC8325, RN4220, RN450, COL, BB270, N315, NW2, USA300, EMRSA-15, EMSRA-16 and mutants thereof with defects in cell wall envelope pathways and regulatory pathways.

Strain RN4220 and RN 450 are a commonly used laboratory strains. These strains have acquired several mutations that make them less virulent and we will use them for our experiments, whenever possible.

Lower risk strains:

Strains Newman, SA113, NCTC8325, RN4220, RN450 are all methicillin sensitive strains and have been propagated in laboratories for an extended period of time and infections can be treated very effectively with conventional beta lactame antibiotics.
Higher risk strains. Strains COL, BB270, NW2, USA300, EMSRA-15, and EMSRA-16 are higher risk strains, which are methicillin resistant and infections can only be treated with a specific set of antibiotics including vancomycin, trimethoprim-sulfamethoxazole, clindamycin, minocycline, fluoroquinolones or doxycycline and. As outlined in a separate form, additional safety measurements will be taken when working with these higher risk strains.

Host/vector system

We will use standard E.coli vectors i.e. 
- pET vectors and derivatives for protein expression in E.coli
- pGEX - vectors and derivatives for protein expression in E.coli
- pQE30 vectors and derivatives for protein expression in E.coli

We still use standard plasmid vector used in Gram-positive bacteria
- pCL55 and derivatives - single site chromosomal integration vector for S. aureus
- pPL2 and derivatives - single site chromosomal integration vector for Listeria monocytogenes


The list contains the most commonly used vectors but may not be all inclusive. We also seek permission to use additional plasmid vectors with similar characteristics. Again, only plasmid vectors will be used which confer to the target organism antibiotic resistences that naturally occur in these organisms.

Origin & function

The majority of inserted genes will be from the same species or genes from other Gram-positive bacteria that encode for proteins with similar functions.

We are investigating envelope assembly pathways and gene regulation in Gram-positive bacteria and their contribution to cause disease. Current examples of genes under investigation include

- tagO - gene required for the synthesis of the cell wall polymer wall teichoic acid
- femA, B, X - fem factors required for peptidoglycan synthesis
- ypfP - glycosyl transferase - required for glycolipid synthesis
- ItaA - possible glycolipid flipase required for glycolipid anchoring LTA
- srtA - sortase required for anchoring of cell surface proteins
- att - hydrolytic enzyme - required for cell division and part of the bacterial envelope
- sarA and homologues therefore - transcription regulators

We will also construct S. aureus and L. monocytogenes libraries in E. coli
We will also add selected tags i.e. GFP, His, HA etc to selected genes.
We will use antibiotic genes i.e. ermC, tetM, as markers of transformation.

Evaluation of foreseeable effects

The majority of inserted genes will be from the same species or genes from other Gram-positive bacteria encoding proteins with homologous functions. Therefore, the likelihood of hazard is considered to be no greater than that of the parental strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: 1% Virkon final concentration, as per manufacturers instructions. Disposal through designated sink. Solid waste: autoclave: Autoclave cycle is 30 mins to achieve a holding time of 3 mins and monitored by a chartrecorder. Solid waste will be disposed of via clinical waste route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

the contact time required for Virkon disinfection.

Spillages within the centrifuge: Ensure the aerosols are allowed to settle. After disinfection with Virkon rinse either with 70% EtOH or water to remove the Virkon residue.

Project Containment

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<td>Animal Units L2 L3 L4</td>
<td>Large Scale Activities L2 L3 L4</td>
<td>Human Clinical Applications L2 L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 8/07.4

Date Ackn'd 30/08/2007

Date Project Ceased

CU2 Project Title Investigation of the physiological and plant pathogenic properties of Burkholderia cepacia complex organisms.

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 1-50 Litres

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

02/03/2022
A key research focus is aimed at understanding the virulence mechanisms employed by Burkholderia cepacia complex (Bcc) pathogens using plant model systems. A second aim of research in this area is to study aspects of primary and secondary metabolism of Bcc. Both aims will involve random and targeted mutagenesis of selected Bcc strains to identify mutants with altered phenotypes or reduced virulence on a plant host.

Burkholderia cepacia species were originally identified as plant pathogens by Burkholder in the early 1950’s. Subsequently, Bcc strains have been associated with opportunistic infections of, in particular, cystic fibrosis patients. The burkholderia species to be tested include strains of B. cepacia, B multivorans, B graminis, B hospital. All strains are part of the internationally recognized Vandamme LMG stock collection in Ghent, Belgium.

So far, the roles of only a few Bcc virulence factors have been examined in animal infection models. Studying virulence in in vivo animal models is often complex, time-consuming, and expensive. This proposal aims to use the model plants Arabidopsis and Nicotiana benthamiana as hosts for studying virulence mechanisms of selected Bcc strains.

One of the main aims of this project is to generate a collection of random mutants of selected strains of Burkholderia that have been shown to cause disease symptoms on the plant host (Arabidopsis thaliana and/or plasposon technology whereby genes in the recipient organism are randomly inactivated by the insertion of a DNA element. It is important to note at this point that in the case increased virulence mutants of Burkholderia were to be identified in this system, no further work would be carried out on this class of mutants until a new risk assessment would have been prepared and approved. Once mutants of interest have been prepared and approved. Once mutants of interest have been identified, DNA fragments flanking the insertion site will be cloned in standard disarmed E.coli K12 derivatives for sequence analysis to identify the affected gene. It is only at this stage that the identity of the affected gene will become known. The sequence will allow cloning of the wildtype gene for further functional analysis using standard molecular biology approaches (e.g mutation analysis, protein expression, protein interaction studies, etc).

Complementation studies will involve the cloning of wildtype Burkholderia genes in E.coli lab strains and transferring these constructs to the selected Burkholderia mutants to test for complementation.

Apart from the mutant screen approach described above, the following genes will be specifically targeted.

1. Genes encoding respiratory proteins, in particular terminal oxidases
2. Putative HCN biosynthesis genes
3. Two component regulatory genes and sigma factors
4. Putative quorum sensing genes.

It is expected that mutants defective in the targeted pathways be less fit and less pathogenic than the wildtype strains. These mutants will be used in basic biochemical and
regulatory studies on the metabolic pathways and the metabolome of Bcc.

Evaluation of foreseeable effects

As explained above the work described is either aimed at identifying reduced virulence mutants or is expected to result in less fit and less pathogenic mutants. However, in the case increased virulence mutants of Burkholderia were to be identified in this system, no further work would be carried out on this class of mutants until a new risk assessment would have been prepared and approved. Laboratory work with the wild type gene products identified in these screens is very unlikely to lead to increased risk above the use of the wild type organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

As part of the screen for reduce virulence Bcc mutants, plants will be inoculated with randomly generated Bcc mutants in the category 2 lab suite and then transferred to a controlled environment plant growth room (PGR) which is outside the category 2 lab suite, but on the same level in the building and under the same restricted card access control (not open to the public). We will put the following control measures in place:

In a large closed container to the PGRs on the same level. Inside the PGR, plants grown in plastic pots will be placed in sturdy plastic trays to avoid any runoff of water potentially containing Bcc organisms. Infected plant material that category 2 lab suite. This will ensure that all infected plant material, including compost and disposable plastic ware, will be contained and earmarked for autoclaving before leaving the PGR. Plastic trays in growth rooms used for these experiments will be wiped down with both disinfectant and 70% ethanol after each use. Plants infected with Bcc in the plant growth room will be clearly marked and segregated from other plants to avoid any cross-contamination. Only authorized personnel will be allowed to handle infected plants in the PGR and dedicated lab coats and gloves will be supplied within the PGR.

We believe that the above measures will safely contain the use of category 2 Bcc organisms in a category 1 plant growth room. We anticipate that this work will be transferred to a newly commissioned suite of category 2 plant growth rooms at the beginning of 2008.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Culture remnants and preparations originating from the live Bcc cultures will be collected and killed using a chemical disinfectant (Virkon) according to the manufacturers recommendations (essentially by addition of Virkon to a final concentration of 1%, with a minimum contact time of 24 hours) and subsequently discarded in the drain.

Disposable plastic ware that has been in contact with Bcc (including culture plates) will be placed in autoclave buns for disposal, while sharps and any broken glassware are put into autoclave cinbins for disposal as clinical waste after autoclaving. The autoclave cycle to be used is 126 degrees for 10 mins. Autoclave cycles are reported out onto a mounted printer detailing all significant stages of the cycle. The autoclave is on a quarterly preventative maintenance contract, has an annual insurance inspection and cycle 2 (Discard) validated by 12 point thermocouple testing. Equally, a validated kill cycle will be used to autoclave plant and compost waste contaminated with Bcc organisms.

Viable Bcc waste will be transported to the autoclave in sealed autoclave bags inside sturdy white plastic buckets. The material will be taken down on a dedicated metal trolley by the floor technician using the goods lift. Autoclave waste is subsequently incinerated. Chemical disinfection and autoclave treatment will result in 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Deselect containment level 21

2.3.3.1a. The committee recommend the use of plastic containers. Ensure that the risks associated with this project are clearly communicated to those other personnel sharing the laboratory, but not directly involved with this project.

2.3.6: Deselect containment level 1, ensure details provided under containment level 2 are left on form

MSC: Ensure and state that microbiological safety cabinet has the following: 6 monthly maintenance, monthly airflow checks that are recorded by user. Specify which cabinets in the lab are to be used.

3.1.1b. Solid waste – State that sharps will be autoclaved within an autoclavable in a cin bin prior to final disposal.

The committee was informed that the PI had confirmed that a microbiological safety cabinet would be used during the injection process as there is potential for aerosols to be produced.

The laboratories used are containment level 2 while the plant growth room is containment level 1, for this reason the PI has selected both on the risk assessment form. Burkholderia will be handled in containment level 2 facilities, once it is integral to the pant it will be transported to containment level 1 facilities. The plants will be correctly transported between two facilities. The plants will not be allowed to pollinate or flower. All plant waste will be autoclaved before disposal. The committee agreed on the classification, yet advised that risk assessment be sent to another researcher in the department for comment prior to submission to the HSE.

### Project Containment

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<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
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</table>

### Project Ref 8/07.5

- **Date Ackn'd**: 28/11/2007
- **CU2 Project Title**: Study of gene regulation in Pseudomonas that have an effect on infection and persistence within the host.
- **Class**: Class 2
- **Culture Vol**: 1-50 Litres
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

Withdrawn N Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**

*Pseudomonas aeruginosa* is able to infect a wide variety of hosts and tissues and is an excellent model for studying infection by gram-negative bacteria. The project is the coordination of research conducted on two different axes, which are central for the establishment of infection and persistence within the host. One axis concerned the characterization of molecular actors involved in bacterial biofilm formation and dispersion, the second axis is the study of a novel secretion mechanism, called type VI. The type VI secretion system is required for pathogenicity and is proposed to deliver effectors towards the host cell, thus impacting processes such as cytotoxicity or internalization by non-phagocytic cells.

**Biofilm Dispersion.**

One strategy for fighting against a biofilm is to induce its dispersion and thus to make the released bacteria accessible to the immune system and antibiotic treatment. Whereas we have a good knowledge of the biofilm formation mechanisms we have a poor understanding of the dispersion step. This step is required to allow bacteria present into the biofilm to be released and to colonise neighbouring surfaces. The release might be induced upon sensing environmental cues. The focus of the project is to discover the major signalling pathways that result in *P. aeruginosa* biofilm dispersion. Within the signalling cascade we will study the receptors of the environmental cues (chemotactic pathway or two-component regulatory systems). We will analyze the output of the signalling cascade and the intracellular signal molecule cyclic-di-OMP, whose concentration is an important balance in the switch between biofilm and motility. Every single actor of the cascade can be considered as a target for modulating biofilm dispersion. Among the targets whose expression has been shown to be controled by c-di-GMP levels we will particularly study the fimbriae assembled by chaperone usher pathways (Cup).

**Type VI secretion.**

*P. aeruginosa* is known as an extracellular pathogen, but it is capable of transient internalization in non-phagocytic cells. The genetic and molecular basis allowing *P. aeruginosa* internalization are not well-established. Three genetic loci have been identified, that encode a novel type of secretion machinery (called type VI). We have preliminary data that indicate that some of the systems promote internalization of the bacteria whereas others induce cytotoxicity. We suggest that the type VI system transports effector proteins within the host. Some effectors have ACD domain (Actin-cross-linking domains) that may promote cytoskeleton rearrangement and internalisation. The aim will be to understand the molecular mechanisms of the type VI secretion system, to identify and characterize the proteins transported within the host cells and to understand their role in modulating host cell response.

**Recipient or parental organism**

**Escherichia coli**

*E. coli* K12 and derivatives will be used for cloning and as recipient for gene expression and purification. Most commonly used strains will be TG1, I-l.BiOl, C600, DH5alpha, OP5O, CCl8, XLlblue, TOPOF, S17.1. These strains have a widespread and long history of safe use.

**Pseudomonas aeruginosa**

Most well characterized laboratory strains used will be: PA01, PAI4, PAK, CHA, PA103, TB, 5G17.
A collection of Tn mutants (SMlopir/pCM639-ISphoA/hah insertions or SMlOpir/pIT2-ISlacZ/hah insertions) in the PAO1 strain will be used (Jacobs et al., PNAS, 2003). A collection of Tn mutants (MAR2xT7) in the PA14 strain will be used (Liberati et al., PNAS, 2006). Strains isolated from patients will be collected and used in the course of the program. Some isolates will be freshly collected, others have been collected for many years and store at -80°C without any further laboratory manipulations. Pseudomonas aeruginosa is sometimes present as part of the normal flora of humans and the prevalence of colonization of healthy individuals outside the hospital is relatively low.

Pseudomonas syringae
This is a plant-pathogenic organism we will use sequenced strains DC3 000, B728a and 1448a
This work is subject to DEFRA approval and we will not commence work with or hold stocks of this bacterium until we have received such approval
Other Pseudomonas and related species
Pseudomonas fluorescens (Pf-5, SBW25, Pto-I, CHAO), Pseudomonas putida (WCS358, KT2440), Pseudomonas alcaligenes (M-I), Pseudoalteromonas haloplanktis (TAC12S), These are clas organisms with a widespread and long history of safe use
Related Pseudomonas species (class 2)
Ralstonia solanacearum which is a phytopathogen without any known natural antibiotic resistance (Strain GMI1000, Molk2 and 1649). This pathogen is subject to DEFRA approval and we will not commence work with or hold stocks of this bacterium until such approval has been received
Burkholderia cepacia and cenopecia (strain J2315), are opportunistic pathogens which poses little medical risk to healthy people. However, people who have certain health problems like weakened immune systems or chronic lung diseases, particularly cystic fibrosis (CE), may be more susceptible to infections with these organism. Pseudomonas entomophila L48 (strain CT573326) is an entomopathogenic bacterium known to infect Drosophila melanogaster (fruitfly). This pathogen is subject to DEFRA approval and we will not commence work with or hold stock of this bacterium until such approval has been received

Host/vector system

1) We will used standard F. coli vectors:
   NB: These vectors could also be used as suicide vectors for P. aeruginosa mutagenesis.
   b) For gene tagging and expression:
      - pET22b/28c/28a and derivatives, pQE30Xa and derivatives, pEGFP and derivatives, pGEX and derivatives, PAC or PAN and derivatives, pBADmycHisA, B, C and derivatives, pYZ4 and derivatives, GATEWAY plasmids including the entry vector pDONOR and the destination vectors (pDEST derivatives)
   c) For expression of genes under strong promoters:
      - pBAD and derivatives, pT7-1 to pT7-6 and derivatives
   d) For studying protein-protein interaction:
      - pUTI18c and pKT25 and derivatives, pBS1479 and derivatives
2) We will use suicide vectors for the engineering of Pseudomonas mutants:
   - pKNG101 and derivatives, pEX 100 and derivatives
3) We will use broad host range vectors for expression in P. aeruginosa and related organisms (also replicative in E. coli):
   - pLAFR1, pLAFR3 and derivatives, pMMB66, 67, 190, 206 and derivatives, pBBRIMCS and derivatives, pMP220 and derivatives
4) We will use vectors for conjugation in Pseudomonas: pRK2013 and derivatives
5) We will use vectors for transposon mutagenesis in pseudomonas:

This list contains the most commonly used vectors but may not be all inclusive. We also seek permission to use additional plasmid vectors with similar characteristics. Again, only plasmid vectors will be used which confer to the target organism antibiotic resistances that naturally occur in these organisms.
The majority of inserted genes will be from the same species or genes from related organisms and will encode for proteins with similar functions. We are investigating the characterization of molecular actors involved in bacterial biofilm formation and dispersion and a novel secretion mechanism, called type VI. Current examples of genes under investigation include: ladS, retS, xcpA-Z, hpfB-C, vgr, hcp, hsi-I-III, gacA, gacS, psc, icmF, dotU, psi, cupA, cupB, cupC, cupD, cupE, rocA, rocR, rocS, prrA, prrB, tad, pil, lasB, aprA, exoS, exoY, exoU, exoY, etc...

We will also add selected tags i.e. GFP, His, HA etc to selected genes.

**Origin & function**

The majority of inserted genes will be from the same species or genes from related organisms encoding proteins with homologous functions. Therefore, the likelihood of hazard is considered to be no greater than that of the parental strains.

**Evaluation of foreseeable effects**

The majority of inserted genes will be from the same species or genes from related organisms encoding proteins with homologous functions. Therefore, the likelihood of hazard is considered to be no greater than that of the parental strains.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Liquid waste: 1% virkon, as per manufacturers instructions. Disposal through designated sink.
- Solid waste: autoclave: autoclave cycle time is 3 mm - 134°C, 30 minutes monitored by a chart recorder. Solid waste will be disposed of via clinical waste route after autoclaving

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
• 1.21: last sentence, add inactivated by Virkon.
• 2.1.12: Clarify what ‘Class C’ is.
• Pseudomonas syringae needs DEFRA approval, confirm if this host will still be included.
• Clarify where Pseudoalteromonas haloplanktis will be used.
• “the strains include - a full list of all strains would be better.
• Ensure that any transfer of genes from one organism into another is described.
• 2.3.3.4: plastic conical flasks recommended.
• 2.3.3.6: State the animal work will be covered in another Code of Practice and Risk Assessment.
• 2.3.3.7: state risk is yet to be identified, subject to relevant licensing, i.e. DEFRA licence.
• 2.3.4.2: training records will be signed off.
• 2.3.5 OH input is required prior to final approval.
• 3.1.1.a: remove reference to solid waste.
• 3.3: Clarify whether 2L clinical strains will be cultured and whether this will be in glass conical flasks. Plastic conical flasks are recommended.
• 3.5.1: list local P1 as person in control of area.

The GMSC agreed with the classification of this project as Class 2, Containment level 2.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L2</td>
<td>L2</td>
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<td>L3</td>
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</table>

**Animal Units**

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tr>
<td>L2</td>
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<td>L3</td>
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**Project Ref 8/07.6**

<table>
<thead>
<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
<th>Consent Granted</th>
</tr>
</thead>
<tbody>
<tr>
<td>27/12/2007</td>
<td>Functional genomic analysis of Anopheles gambiae innate immunity against and interaction with O'nyong-nyong Virus (ONNV)</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Non-GMM Not Applicable</td>
</tr>
</tbody>
</table>

**Historical Significant Changes**

Withdrawn: N  
Tick if notifying a connected programme of work: N

**Historical Date of Additional Info**

02/03/2022
Project Additional Information

**Purposes of the contained use**

The project aims to identify Anopheles gambiae genes that impact upon ONNV replication and dissemination. To this effect it will use ONNV Alphavirus Transducing Systems (ATS’s) for infection of cell lines and mosquitoes. To facilitate virus detection, viral clones expressing reporter genes, e.g. GFP, EGFP, RFP, EYFP and luciferase, will be used. Furthermore, genes of interest (including but not limited to Toll-9, Dicer-2, Hopskotch) will be silenced using short RNAi cassettes inserted into the ONNV ATS’s (alone or on addition to the reporter genes). Reporter genes and RNAi cassettes will be cloned into a multiple cloning site (MCS) that is under the control of a viral subgenomic promoter. ONNV ATS’s will be first subcloned into E.coli DHSalpha or XLI Blue strains. Transgenic viruses will be produced by in vitro transcription of the modified or non-modified ONNVATS’s, and subsequent transfection of the produced RNA into insect (e.g. Aedes aegypti C6136 and A. gambiae 4a3A) or mammalian (e.g. African green monkey Vero) cells, where the virus is produced and replicates. Cell culture supernatant containing virus will be used to infect further cell lines or mosquitoes.

**Recipient or parental organism**

The SG650 strain of ONNV (isolated from the 1996 outbreak in Uganda) has been used to generate the plasmid (p5'dsONNVic/FOY) containing a cDNA copy of the SG650 strain, a multiple cloning site and a second viral subgenomic promotor (Brault et al 2004). p5dsONNVic/FOY (to be obtained from Colorado State University) will be subject to further genetic modification. In addition a second plasmid (p5'dsONNVic/Foy-GFP) containing a cDNA copy of SG650 and encoding enhanced GFP (Brault et al 2004) will be subject to further genetic modification.

Infectious clones generated from plasmids have been shown to replicate and disseminate at similar, but slightly reduced rates to the SG650 strain (Brault et al 2004). Insertion of reporter genes/RNAi cassettes minimally reduces the ability of the virus to replicate due to difficulties in packaging a larger genome into virion particles.

**Host/vector system**

ONNV ATS’s will be used to infect Anopheles gambiae mosquitoes (03, L35 and Yaounde strains) as well as a variety of insect cell lines (Aedes aegypti C6136, Anopheles gambiae 4a3A, 4a3B, 4a2, SuaEI, SuaIB, Sua4.0, SuaS.1* and L35) and mammalian cell lines (African green monkey Vero’s, Baby hamster kidney BHK’s).

**Origin & function**

1. Reporter genes (e.g. GFP, EGFP, EYFP, luciferase). These are marker genes which emit either fluorescence (GFP, EGFP and EYFP) or luminescence (luciferase) and are valuable tools for tracking and quantifying the replication and dissemination of the virus in the mosquito or mosquito cell lines.

2. RNAi cassettes. These are short (300-600bp) DNA sequence fragments that correspond to genes of interest (e.g. Toll-9, Dicer-2 and Hopskotch), which when transcribed in both directions (through a plasmid vector) or inserted in the alphaviral genome can produce double stranded RNA intermediates which trigger the RNA interference pathways.
Toll-9 belongs to a family of transmembrane proteins involved in signalling during innate immune responses. In Drosophila Toll-9 has been shown to up-regulate anti-microbial peptides via signalling through the Toll pathway. Dicer-2 is an enzyme involved in the RNAi pathway. dsRNA molecules are cleaved into 20-25 nucleotide small interfering RNA’s (siRNAs) by Dicer enzymes. In Drosophila Dicer-2 is believed to be involved in cleavage of dsRNA (including viral RNA), resulting in the targeting of homologous mRNA for degradation. Hopkotch is a Janus kinase (JAK) which in Drosophila has a role in embryo segmentation, and innate immunity by signalling through the JAKISTAT pathway. Hopkotch binds to a receptor, DOME, resulting in the phosphorylation and subsequent activation of a transcription factor, STAT.

ONNV - ONNV is an alphavirus of the family Togaviridae that is transmitted by Anopheleine mosquitoes. The virus has caused several epidemics of human disease in Africa. ONNV causes febrile illness associated with severe joint pain, headache, retroorbital pain and sometime a pruritic maculopapular rash beginning on the neck and face, and spreading to the torso and arms. No deaths have been associated with ONNV infection, with all know patients making a full recovery. In the laboratory risk of infection is highest from infected mosquito bite or needle stick injury. Risk of infection from eye splashes is low. Reporter genes (eg GFP) are non-toxic and non-hazardous and as such do not pose any hazard to human health. RNAi cassettes (e.g. for Anopheles gambiae Toll-9, Dicer-2 and Hopkotch) are short sequences specific to mosquito genes and are not hazardous to human health. Alterations to the traits of the host may include increased ability to infect mosquito cells or adult mosquitoes. Alterations to the host will not result in any increased ability to infect humans and as such should not increase the pathogenicity of the virus in humans. The infectious agent is not air-borne and thus will not escape containment. There are no natural vector mosquito species of this infectious agent in the UK, thus the infectious agent is not an environmental hazard.

Evaluation of foreseeable effects

Containement and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Liquid waste from the culturing of E.coli — Disinfected with 1% virkon for 20-30 minutes followed by drain disposal.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid waste from tissue culture: Autoclave Liquid cycle program (121 degrees centigrade for 15 minutes)</td>
</tr>
<tr>
<td>Solid waste including needles, tissue culture flasks and pipettes, petri dishes and eppendorf tubes: Autoclave solid cycle program (126°C for 10 minutes).</td>
</tr>
<tr>
<td>Floor standing PACS2000 autoclave is monitored by a thermograph. Annual 12-point thermocouple test is carried out by a qualified technition. A back up autoclave is available - GM waste will be stored in yellow Ljltima square clinical waste bins before transport to the back up autoclave.</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]  

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
1.3: State that the SG650 strain will not be used as part of this project.
2.1.1.2: Add the type of rash and where the rash will occur on the individual.
2.3.1.1: Change ‘3$’ to ‘2*’ and expand ‘maximum of 400 mosquitoes’ to ‘pots of 50 mosquitoes’
2.3.2.2.a: Change ‘3$’ to ‘2*’ and the stock of mosquitoes will be stored in room 619.
2.3.2.2b: Replace ‘separate MSC and separate incubators’ with ‘dedicated’ MSCs and incubators.
2.3.3.1.a: reword sentence to specify a dedicated microbiological safety cabinet will be used.
2.3.3.1.a, 2.3.3.2.a, 2.3.3.2.b, 2.3.3.3.d, 2.3.3.4.a and 2.3.3.5: change ‘disabling’ to ‘inactivate’ Specify why 96% EtOFI is used for inactivation rather than 70%.
2.3.3.2.a: State the storage location
2.3.3.2.b: Add that the cytotubes will be quickly transferred into a lidded box following removal from liquid nitrogen, ensure that this procedure is added to the project SOP.
2.3.3.8: answer is ‘No’.
2.3.9.a: Change to State green gowns will be worn in 619 and 630 rooms.
2.3.4.2: Add Induction training will be signed off. This should be added to the project SOP.
2.3.5 01-1 input is required prior to final approval.
2.3.6 Assign the project as Containment level 2, Class 2.
3.1.1.a: Contact time will be 20-30 minutes. Route of disposal is down the drain.
3.1.1.b: Remove E.coli cultures. Route of disposal, autoclaving only.
3.2.a: Change from autoclave to down the drain.
3.2.b: State the box will be ‘leak proof’ and lidded.
3.3: Clarify the volumes of bacteria and cell cultures to be used.
3.5.1: State location of storage here.
Agreed: Class 2, Containment Level 2

**Project Containment**

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<td>L2 L3 L4 L2 L3 L4 L2</td>
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**Project Ref** 8/08.1

<table>
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<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>18/06/2008</td>
<td>Heart muscle can not regenerate after heart attacks, like most other kinds of cells can</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td></td>
</tr>
</tbody>
</table>

02/03/2022
These experiments are aimed at learning with molecules control the formation of heart muscles cells early in life, and what prevents the formation of new heart muscle cells later in life.

The work will use bacterial plasmids and E.coli K12 to generate eDNA which will be put into lentiviral vectors to deliver genes to cells. The use of the lentiviral vector system will enable the efficient infection of cells grown in tissue culture with lentiviruses expressing genes that control cardiac cell growth, differentiation and survival.

E.coli K12 will be used and plasmids will include pQE31, pET28b, pGST. K12 is non-colonising and disabled. Lentivirus particles will be produced by transfection of the 293 human embryonic kidney cell line. Human and mouse cells grown in tissue culture including NIH 3T3, C2C12, primary mouse and rat cardiac myocytes and fibroblasts, muscle, rat and human stem cells and mouse and human ES cells will be the recipients of virus. These cells will express the transgene but will not produce or contain replication-competent virions so are non-infectious.

Recipient or parental organism

E.coli K12 will be used and plasmids will include pQE31, pET28b, pGST. K12 is non-colonising and disabled. Lentivirus particles will be produced by transfection of the 293 human embryonic kidney cell line. Human and mouse cells grown in tissue culture including NIH 3T3, C2C12, primary mouse and rat cardiac myocytes and fibroblasts, muscle, rat and human stem cells and mouse and human ES cells will be the recipients of virus. These cells will express the transgene but will not produce or contain replication-competent virions so are non-infectious.

Host/vector system

1) pLL3.7 - Lentiviral vector. 2) Packaging vector - psPAX2 - lacks both LTRs and has no viral packaging signal. rev is supplied in trans on a different vector, expresses no viral gene products. Envelope (VSV C) is expressed on 3) pMD2.G. 4) pWPI containing the WPRE element. Second generation lentiviral vectors are replication incompetent. No structural genes are present, therefore no replication-competent virions can be produced. E.coli K12 (host), pQE31, pET28b, pGST (vectors).

Origin & function

Ecoli, all strains are K12 derivatives. K-12 derivatives are recognised as non-colonising and disabled. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. Second generation lentiviral vectors which are replication incompetent. No structural genes are present, therefore no replication-competent virions can be produced. For shKnockdowns: pLL3.1 - A Lentiviral vector that expresses shRNA under the mouse U6 promoter. A CMV-EGFP reporter cassette is included in the vector to monitor expression. Packaging vector - psPAX2 - lacks both LTRs and has no viral packaging signal. The following viral genes have been deleted from the packaging vector: env, tat, rev, vpr, vpu, vif and nef. Rev is supplied in trans on a different vector (RSV-Rev). The vector expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion) and expresses no viral gene products. Envelope (VSV C) is expressed on a separate vector - pMD2.G And pWPI containing the WPRE element. For conditional shKnockdowns: This bicistronic vector allows for simultaneous expression of a transgene and EGFP marker to facilitate tracking of transduced cells. The EGFP marker cDNA has been inserted downstream of EMCV IRES. Tet-on systems will also be used. Any
modifications made to the plasmids under this system are biologically neutral and do not change the biosafety of the original second generation lentMral vectors. Clonal cell lines including 293, C2C12, 3T3. Primary cells, rat and mouse cardiac myocytes and fibroblast& Mouse, rat and human stem cells (bone marrow and cardiac). Mouse and human ES cells.

Evaluation of foreseeable effects

K12 is non-colonising and disabled, all plasmids used in K12 will not allow propagation of the strain outside of the laboratory environment Each lentiviral vector produced will be able to infect cells but will not be able to replicate and spread to new cells - The virions produced are replication-incompetent. The foreign proteins that will be expressed in the transfected cell and ultimately injected into mice will not affect the hazard group of the vector or host and will only be expressed by the infected cell. The WPRE element as present in pWPI has been shown to be potentially oncogenic, but as the virus it will be contained within is replication incompetent, an on-going infection in laboratory personnel is not possible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Bacterial and lentiviral cuttures: Liquid culture, spi11s - For metal surfaces that may corrode - Trigene final concentration of 1 :100. For all other surfaces - Viricon final concentration of 2%. Treatment for 30 minutes at room temperature and then disposed of via the drain and paper towels etc will be placed in GM waste bins. Tissue Culture Plastics, pipettes will also be placed in GM waste bins. These will be autoclaved at 121 degrees for 20 minutes and 'disposed of by the Imperial College clinical waste route which ends in incineration off site. autoclaves are monitored by a chart recorder with temperature readout and validated by an annual 12 point thermocouple test.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

The Committee agreed with the classification of Containment level 2, Class 2 assigned to the project, as lentiviral vectors containing WPRE are to be used and injected into animals necessitating the use of sharps. The P1 was advised to separate out the Class 1 aspects of the project, involving the use of attenuated adenoviral and retroviral vectors so that this work could start after College approvals are in place.

Project Containment

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<td>L4</td>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

At the IAVI Core Laboratory in London we have developed and optimised, together with collaborators in Boston and Los Angeles, a functional T-cell based assay to assess the efficacy of HIV vaccine candidates in inhibiting viral replication post-vaccination. As has recently been shown by the HINTNIMerck STEP trial the present methods used to assess vaccine efficacy do not necessarily correlate to in vivo protection, or to a functional in vitro effect.

Our assay is intended for use on clinical trial samples. At present the assay readout is based on the inhibition of a panel of HIV virus strains using a p24 HIV core antigen ELISA for virus detection. While this works very well it is very expensive and labour intensive. Part of our aim is to attempt to limit cost and streamline the assay format, to make it more applicable for trials and eventually for use at trial sites such as those used by IAVI (in developing countries).

We intend to use virus directly labelled with a fluorescent tag. This will enable us to assess viral load in the assay using a high throughput flow cytometer and may enable us to look at an earlier time point in the assay, significantly cutting the assay processing time and cost. This will involve labelling virus isolates for use in our assay.

Our second aim is to assess the ability of vaccinees post vaccination to inhibit viral replication in viruses that directly match the vaccine used in the trial. At present we use a panel of isolates ranging from closely matched isolates to those circulating in the region where the trial has been performed. Our collaborators will generate viruses that contain the exact sequences used within our vaccine candidates. These viruses will more closely match the vaccine insert and allow us to determine if the vaccine is capable of inhibiting the exact sequence used for the vaccine. Using a range of sequences derived from varying primary isolates, in the same backbone, we will be able to assess inhibition directly related to a gene of interest. Informing on what sequences to prioritise in vaccine design. All sequences added into the virus vector will be naturally occurring in the environment and will replace a homologous gene sequence in the vector.
All manipulation and virus generation will be performed by collaborators outside of the UK, and only functional viruses will then be cultured at our lab in London, with no further genetic manipulation.

Recipient or parental organism

All GMOs will be generated by our collaborators, or sourced from the NIH AIDS Research and Reagent program, then shipped to our lab for use. GMOs will be generated by tagging an existing isolate with either a fluorescent tag or the luciferase gene. This tagging is not intended to either increase or decrease pathogenicity, but is often inserted into a gene such as Nef, which is then disrupted and does reduce pathogenicity. The tags themselves are not known to have any pathogenic or detrimental effects. In addition to tagging viruses we will also be generating viruses that have a known backbone but contain a specific gene sequence of interest. This will allow us to directly assess the effect of sequences used in the vaccine and may help to prioritise other sequences for development as vaccine candidates. These inserted sequences will replace a homologue in the virus being used as a vector and will be naturally occurring sequences. This will not result in a virus that is intended to be any more, or less, pathogenic then the original strain. The genes selected for insertion will be based on the sequences used for vaccine trials, and sequences derived from currently circulating primary isolates.

Host/vector system

All modifications will be made to existing viral isolates (primary and lab adapted strains) being used in the Category 3 laboratory. The GMOs will be generated by collaborators and shipped to the IAVI Core Laboratory for use. GMOs will be shipped either as prepared DNA which will be transfected into 293 T-cells to generate virus stocks for experimental purposes, or as virus stocks that have been generated by the collaborator.

Origin & function

Luciferase and fluorescent tags are already at the collaborator laboratories, having been pre-synthesised for use. They are intended simply to act as markers of virus signifying how much virus is present either within the cells in culture or directly secreted into the cell culture supernatant. The various tags will be assessed directly using flow cytometry or a plate reader, while luciferase will be reacted with a substrate (available from Perkin Elmer) and read on a plate reader. Alternative sequences inserted into viral vectors that replace their homologue will either be:

i) synthesised based on vaccine inserts directed against naturally occurring sequences, or

ii) will be taken directly from other isolates. Our collaborators have several methods that allow for sequences from primary isolates to be ‘swapped’ into a known backbone, allowing for an assessment of responses against that specific sequence.

Changing the virus sequences is intended to result in a replicative virus which will allow an assessment of responses against the changed region, relative to the original isolate. This will aid in informing on the efficacy of specific vaccine inserts and help to prioritise vaccine candidates for further trials. Using sequences generated from circulating viruses will allow us to determine other sequences to carry forward into the next generation of vaccines. These changes are not intended to affect the virus pathogenecity, making it more or less harmful, and are intended to reflect naturally occurring sequences already circulating in the environment.

Evaluation of foreseeable effects

The use of a fluorescent or luciferase tag will not have a significant effect on the virus, but may act to limit pathogenecity. This modification is not necessary for the survival of the virus and is likely to be attenuated or lost over several cycles of replication; experiments by our collaborators suggest that the fluorescent tag in their system will be lost after several days in culture. Therefore these modifications are likely to have little or no effect other than making the virus detectable through a variety of assays, such as flow cytometry.

Modifying the sequences within the viral vector will act to replace a gene, or sequence of genes, with direct naturally occurring homologues. This is intended to affect the immune response to the virus in our experimental system, but is unlikely to have a significant affect on other virus strains. The genes selected for insertion into our vector virus will be available in the circulating strains in the environment, acting as a basis for their selection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
All procedures will be performed under existing Category 3 conditions. These conditions are in place for HIV-1 and the proposed GMMs are not expected to require any additional containment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All virus containing media (liquid) will be placed in 2% virkon for a minimum of 30 minutes. This waste will then be discarded down the laboratory sink as detailed in the Category 3 Code of Practice for the IAVI Core Laboratory. This procedure is guaranteed by the manufacturer.

Any solids that come into contact with virus will be placed in 2% virkon for a minimum of 30 minutes. The solid will then be rinsed in water and all liquid discarded down the sink. The solid will then be autoclaved under vacuum air extraction with rapid steam heating to 121C for 25 minutes. Followed by incineration through the Chelsea & Westminster Hospital trust clinical waste system.

The autoclaves are filled with a chart recorder to monitor the autoclave cycle and they undergo an annual 12 point thermocouple testing.

All procedures are performed under GCLP conditions and appropriate for disposal of Category 3 pathogens and are intended to result in 100% death of the GMM.

Is an emergency plan required according to regulation 20? [ ] Y

If yes, tick to confirm that it is attached to this form [ ] Y

Tick to confirm that you have attached a risk assessment to this form [ ] Y

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ] N

Please enter comments on the GM safety committee on the risk assessment
Overall the GMSC were happy with the risk assessment and their comments were as follows:

Dear — Following the discussion of your proposals entitled “Qualification and Development of a T-cell mediated, in vitro, HIV-1 viral suppression assay” (GMIC-01756.1) at the last GM Safety Committee meeting, the committee request that the following amendments are made to the proposals prior to approval:

Comments: PB gave the committee an outline of the project PS stated that they were obtaining the GFP tagged viruses from collaborators and would not be creating them as part of this project US asked for this information to be included in the Form A to clarify that the group themselves were not creating the GMM themselves.

RW asked for further details regarding the pathogenicity of the Nef sequence. SP clarified this by stating that deleted Nef sequences in monkeys resulted in viruses being grown which were not pathogenic.

Comments email by DM: there are a few typos needing correction before it is sent off.

SH asked if the CL3 suite was suitable for this type of work. TN reported that the CL3 suite is operational for this type of work.

The following amendments are required prior to approval:

1. Clarify the work to be conducted by collaborators.
2. Typo ‘Category £’
3. Provide justification to answer
4. Include ‘refer to list...’
5. Specify the volumes of a small or large spillage and that the mom would be fumigated with formaldehyde.
6. OH input required prior to final approval
7. Solid waste, route of disposal: Incinerated
8. ‘robust leak proof container’
9. Include that it is Incinerated
10. Change secondary container to ‘robust leak proof container’
11. Specify the volume of a large spillage.
12. Remove reference to Needlestick injuries as sharps are not to be used in this project.

Agreed: Class 3, Containment Level 3. HSE notification and written consent required prior to commencement.

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**Project Containment**

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**Project Ref** 8/08.3

**Date Ackn’d** 02/03/2022
The overall aim of this research in this area is two fold:
Firstly to study aspects of primary and secondary metabolism of P. aeruginosa. We are interested in:
(i) Discovering global metabolic differences between strains from different sources, and with different genetic backgrounds.
(ii) Studying energy metabolism in P. aeruginosa, regulatory pathways and secondary metabolite production.

Secondly, to understand the survival mechanisms used by P. aeruginosa to survive abiotic stress, and under stationary phase and biofilm growth conditions. We are particularly interested in the roles of universal stress proteins in the survival of P. aeruginosa.

This will involve both random and targeted mutagenesis approaches to disrupt specific metabolic pathways as well as the screening of an ordered, non-redundant library of P. aeruginosa transposon insertion mutants (the PA14NR Set). It will also involve cloning genes, for example those encoding terminal oxidases of P. aeruginosa and this will also involve construction of transcriptional gene fusions of promoter regions of the various genes with lacZ and gfp. Preliminary cloning experiments will be conducted in E. coli strains such as XL1-blue. The genes and gene fusions will be expressed in P. aeruginosa wild type and mutant strains.

Pseudomonas aeruginosa is a ubiquitous environmental organism that can be found at very high population densities in certain environments e.g. in waste water pipes. P. aeruginosa has been associated with opportunistic infections and it is a particular problem in burns patients and patients with Cystic Fibrosis. We will be working with a number of P. aeruginosa strains including the protrophic PAO1, PA14 and PAK strains as well as the auxotroph PAO6049 met-9011 amiE200 strA (PAO1 derivative)

Self cloning in Pseudomonas aeruginosa and in disabled strains of E. coli.
subsequenty cloned for complementation studies. However, proteins that may be identified could include metabolic proteins, transporters and regulators.

Once mutants of interest have been identified, DNA fragments flanking the insertion site will be cloned in standard disarmed E. coli K12 derivatives as a start point to allow cloning of the wldtype gene for further functional analysis using standard molecular biology approaches (e.g. mutation analysis, protein expression, protein interaction studies, etc.).

Complementation studies will involve the cloning of wild-type P. aeruginosa genes in E. coli lab strains and transferring these constructs to the selected P. aeruginosa mutants to test for complementation.

2. For investigation of the energy metabolism of P. aeruginosa, regulatory pathways and secondary metabolite production some of the gene we envisage working with are:
   1. Genes encoding respiratory proteins, in particular terminal oxidases
   2. Putative HCN biodynthesis genes
   3. Two component regulatory genes and sigma factors
   4. Putative quorum sensing genes.
   5. Sigma factor and anti-sigma factor genes involved in the regulation of polysaccharide alginate by P. aeruginosa.

3. In studying the survival mechanisms used by P. aeruginosa to survive abiotic stress and under stationary phase and biofilm growth conditions we will be working with genes encoding universal stress proteins (USPs) of P. aeruginosa, which are thought to play a role in promoting survival under non-growing conditions. These will include the genes encoding the USPs PA3309, PA4352 and PA3017.

Evaluation of foreseeable effects

We will be working with a mutant library with the aim of identifying mutants with interesting metabolic properties, but it would seem most likely that mutants with metabolic defects would have reduced ability to survive in the environment and reduced virulence.

The cloned genes and their gene products we plan to work with specifically are unlikely to alter the pathogenicity of the cloning hosts. The vectors are mobilisable but there are no known environmental or safety concerns relating to the cloned genes.

It is most likely that mutation of the target genes will make the organism less pathogenic, and there is no reason to believe that the GM experiments would increase pathogenicity to organisms in the environment.

The GMM would not pose a risk to the environment. The mutant strains will be less fit than environmental strains; therefore, we do not anticipate an increased hazard to the environment of the mutant P. aeruginosa strains. Proper microbiological practice will minimise risk of environmental exposure but as there would be negligible consequences, even if containment were to be breached, the environmental risk can be judged as effectively zero.

Since the work will involve wildtype P. aeruginosa gene sequences, there is no anticipated hazard with a transfer to related microbes. Containment measures will be in place to avoid such consequences. It is in principle possible that transfer of certain genes, e.g. the terminal oxidase genes, to another bacterium would allow it to colonise a wider range of niche. However, the complex nature of the synthesis and assembly of the prosthetic groups for these enzymes, together with their complex regulation makes this a remote possibility.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Culture remnants and preparations originating from live P. aeruginosa cultures will be collected and killed using a chemical disinfectant (Virkon) according to manufacturer's recommendations essentially by addition of Virkon to a final concentration of 1% with a minimum contact time of 30 mins and subsequently discarded in the drain.
Disposable plastic ware that has been in contact with P. aeruginosa (including culture plates) will be placed in autoclave bins for disposal, while sharps are put into cinbins, autoclaved and then go for disposal as clinical waste. The autoclave cycle to be used is 126°C for 10 min. Autoclave cycles are reported out onto a mounted printer detailing all significant stages of the cycle. The Autoclave is on a quarterly preventative maintenance contract, has an annual insurance inspection and Cycle 2 (Discard) validated by 12 point thermocouple testing. Equally, a validated kill cycle will be used to autoclave plant and compost waste contaminated with P. aeruginosa.

Viable P. aeruginosa waste will be transported to the autoclave in sealed autoclave bags inside sturdy white plastic buckets. The material will be taken down on a dedicated metal trolley by the floor technician using the goods lift. Autoclave waste is subsequently incinerated. Chemical disinfection and autoclave treatment will result in 100% kill.

Comments: The risk assessment was originally submitted prior to 2006 as a class 1 project but not approved by the committee. It has been re-submitted with a new title, scientific changes and as Class 2. An up to date Code of Practice is required before it is sent to the HSE for approval

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE
**Project Additional Information**

**Purposes of the contained use**

The purpose of the project is to gain better understanding of the mechanism used by Legionella to disseminate and cause human disease. Better understanding is essential in order to develop accurate risk assessments and measures to reduce transmission and infection.

**Recipient or parental organism**

We will use the following lab stains:

- Legionella Pneumophila 130b - ATCC BAA-74
- Legionella Pneumophila, Philadelphia-1 - ATCC 33152
- Acanthamoeba castellanii

**Host/vector system**

- **Hosts:**
  - Legionella pneumophila (130b, ATCC BAA-74; Philadelphia-1, ATCC 33151; and derivatives), Escherichia coli (e.g. DH5alpha, BL21, XL1 blue, Top 10), Acanthamoeba castellanii, Dictyostelium discoideum (AX2), Saccharomyces cerevisiae (PJ69-4A, AH109)

- **Vectors:**
  - Commercially available yeast two and three hybrid vectors: pGAD424, pGBT9, pBridge and general cloning vectors.
  - Commercially available E. coli expression vectors including pMAL, pET28(a), pET3, pACYA184, pBR322, pGEX, pKK, pMMB207
  - Commercially available eukaryotic expression vectors including pEGFP-derived and pcDNA3-derived.

**Origin & function**

Origin of Legionella pneumophila - ATCC

Other hosts and vectors - commercial and in-house.

The aim of the project is to investigate the molecular and cellular basis of the interaction of Legionella pneumophila with its eukaryotic host cells.
In particular we will:

1. Site directed mutagenesis of effector genes and phenotypic characterization upon infection of macrophages and amoeba.
2. Cloning of effector genes (with and without tags - HA, GFP, Myc etc..) in bacterial expression vectors for mutant complementation.
3. Cloning the effector genes in eukaryotic expression vectors for transient and stable expression in eukaryotic cells.
4. Cloning the effector genes in yeast vectors for conduction of yeast two hybrid screens (to identify eukaryotic partner proteins).
5. Cloning the effector genes in bacterial expression vectors for protein purification (His, GST, MBP tagged).

**Evaluation of foreseeable effects**

**L. pneumophila** is an opportunistic human pathogen responsible for flu-like or pneumonia Legionnaire's diseases.

Acanthamoeba are free-living amebae belonging to the genus Acanthamoeba. They are the causative agents of granulomatous amebic encephalitis (GAE), a disease of the central nervous system (CNS), and amebic keratitis (AK), a painful sight-threatening disease of the eyes (Clinical Microbiology Reviews, Apr. 2003, p 273-307). Immunocompromised people are most at risk from Acanthamoeba infection.

*Legionella pneumophila* contains over 100 type IV secretion system genes, which conspire to subvert eukaryotic cell functions. In this project we will mutate effector genes (generating single or multiple mutants). The mutants will be complemented by plasmids encoding wild type genes. No foreign DNA will be introduced in *L. pneumophilia*. The effector genes will also be expressed in disabled *E. coli* and yeast hosts. The effector genes will also be expressed, transiently and stable, in eukaryotic cells (e.g. amoeba, macrophages).

The *Legionella* effectors by themselves are unlikely to be harmful (in bacterial or eukaryotic hosts), as their function relies on: 1. The presence of a type IV secretion system (missing form *E. coli*, yeast, eukaryotic cells). 2. Disease is dependent on expression of the whole effector repertoire. As such the most hazardous GMM is presented by over-expressing effector genes in mutant Legionella (for complementation) as this by nature involves somewhat higher level of expression that in theory can increase virulence potential, although we consider that the actual risk is very small (as disease relies on optimal expression levels).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

*L. pneumophila* is acquired by inhalation of contaminated water from showers, air conditioning etc. Building services regularly test for presence of *L. pneumophilia* and take appropriate precautions to ensure water building water systems are not contaminated with *L. pneumophila*.

Everyone working in our lab has to wear a lab coat: the coats are kept at the entry to the lab. Nitrile gloves are used and they are immediately accessible within the lab areas.

We will express *Legionella* effector protein in eukaryotic cells (e.g. Acanthamoeba, epithelial cell, macrophages) ectopically. Any handling of the amoebae will be carried out under aseptic conditions. Any experimental procedure that has the potential to generate aerosols (e.g. sonication) will be carried out within a Class II Biosafety cabinet.

*Legionella* will be handled in class II MSC (e.g. plating, infection, lysis). All our MSC are serviced every 6 months, and they are tested/validated by KI disk test every year (as part of one of the services); an annual insurance inspection is also completed. If the MSC is used for class 2 pathogens or Class 2 work, the users also have to do a monthly airflow test.

02/03/2022
Sharp tweezers might be used to transfer glass coverslips of cells infected with Legionella between dishes. No suitable alternative is available but this use will be avoided as much as possible. After use, the tweezers will be decontaminated in 70% ethanol.

**WASTE MANAGEMENT WITHIN THE LABORATORY**

Within laboratory 1.30, the following procedures are to be followed:

1. **Autoclave waste.** Contaminated plastic flasks, plates, tips and tubes should be placed into sterilin bags mounted in rigid containers for disposal. Objects which are likely to puncture the sterilin bags should be avoided and should be placed in CinBins for disposal (see below). All sterilin bags should be kept close to the work area in the rigid containers provided. Avoid placing objects into the bags that could puncture the sides. Sterilin bags must be fitted to no more than three-quarters full. Prior to disposal the tops of the sterilin bags must be loosely taping to contain contents but allow free circulation of steam. Taped bags should be placed into the blue plastic collection boxes which are specifically designed to contain any spillage. The rigid sterilin containers should be disinfected with Virkon S after each change of bag. Collection bins are strategically placed on the laboratory floor, directly under a sink, in a position which avoids accidental tripping. Each blue box is fitted with a lid held in place using an identity tag that is used to identify the laboratory of origin.

2. **Glass flasks or large plastic flasks for re-use containing contaminated waste must be decontaminated by treatment with one of the approved disinfectants (see Appendix 5). Disinfected material can be dispensed down the sinks provided. Under no circumstances must live microorganisms be dispensed of in this manner.**

3. **Sharps:** all needles, microscope slides and scalpels must be placed in CinBins. These should be sealed when full and always placed in the blue collection boxes for autoclaving.

4. **Broken glass should be placed in the Red Box. If contaminated, no attempt should be made to decontaminate this and advice should be sought from a supervisor or from a Safety Officer (see Appendix 8).**

5. **Clinical waste.** All normal laboratory waste that is not known to be contaminated with biological agents should be placed in the Yellow Bags. This includes tissues, agarose and acrylamide gels, packaging etc. these bags are collected by approved staff, tagged and taken off site for incineration.

6. **Glassware and plasticware for Washing Up must be placed in the collapsible white boxes under the sink. This will not be autoclaved and must not contain contaminated materials. Any container used for hazardous chemicals (see COSHH assessments) should be rinsed with water before placing in box.**

**TRANSPORT OF WASTE TO THE AUTOCLAVE AREAS**

1. All material containing biological waste will be removed from the laboratory for autoclaving in covered blue plastic collection box. These bins will be placed in an approved closed metal trolley and transported by a designated member of staff.

2. The trolley will be taken directly to the autoclave using the service lift and a route approved by the Department. A bottle of Virkon S disinfectant should be placed on the trolley to handle spills in transit.

3. The plastic bins will be unloaded onto the benches next to the autoclave, either directly into the autoclave, or into the designated cold room for temporary storage. Only personnel trained in the use of the autoclave are permitted to load bins into the autoclave.

4. Bins will be autoclaved using the next available cycle.
5. In the unlikely event of a spillage during transport follow the procedures outlined in Section 8C. The Safety support team must be immediately informed and the spillage cleared up as outlined in the protocol for spillage control.

SPILLAGES OF CONTAMINATED MATERIAL

This protocol covers procedures for the containment of spillage of material containing viable microorganisms:

1. Wear full protecting clothing including goggles, gloves and laboratory coat when dealing with spillages. Do not tread in contaminated fluid.

2. The details of approved disinfectants are listed below.

3. Small spillages below two ml should be directly disinfected by use of the squeeze bottles of disinfectant provided. Squeeze the disinfectant solution directly onto the spill. The spillage can then be wiped up using tissue rolls located in the wall racks close to the laboratory exit.

4. Spillages should be cleaned as quickly as safety permits. If you suspect aerosol may be generated allow time for these to settle.

5. For larger spills inform the laboratory supervisor or the Safety Officer. Soak up excess liquid using tissue paper from rolls. Place the contaminated tissue directly into sterilin bags and place in the sterilin bags into the waste collection bins. Decontaminate the contaminated area using direct application of excess disinfectant and repeat the mopping up with tissues.

6. Disinfecting solutions must be available in the laboratory at all times. Prepared and active disinfecting solutions can be found next to the exits from the laboratories. In addition, each worker should maintain a squeeze bottle of 70% ethanol for routine hand disinfection and a bottle of an approved disinfectant for dealing with spillage at the bench.

7. If in doubt consult a senior laboratory member.

List of approved disinfectants

70% ethanol

Ethanol is suitable for disinfecting hands, small spillages and bench surfaces. Ethanol is volatile and, although it does not provide sustained action against microbes, it is particularly effective against enteric bacteria. It must be kept by each worker in small plastic squeezy bottles for personal hand disinfection and for regular swabbing of benches and small spills.

USE ETHANOL FOR HAND WASH AND BENCH SWABBING ONLY

Virkon S

Virkon S is an oxidising agent, which is a powerful disinfectant active against a wide range of bacteria and viruses.

Working stocks of 1% Virkon S must be prepared weekly.

Treatment of spills

The 1% working stock will be used directly to disinfect working surfaces. Spills of contaminated cultures should be mopped up with tissues and then the area treated with 1% Virkon. Larger volumes of spills should be treated directly with Virkon powder and wiped up with tissues. Tissues should be disposed of by the clinical waste route.
Decontamination of cultures

Bacterial cultures e.g. in flasks or centrifuge bottles, are treated by addition of Virkon powder to a final concentration of 1% or by the addition of an equal volume of a 2% solution of Virkon. Cultures should be left in Virkon for a minimum of one hour. The solution can then be disposed of down the sink and the glassware sent for washing up. Culture vessels must be filled to the brim with the Virkon solution.

Dear PF

Following the discussion of your proposal entitled "Host pathogen interaction: Characterisation of Legionella's virulence factors and analysis of their function during infection of eukaryotic cells", GMIC-01790.1 at the last GM Safety Committee meeting, the Committee request that the amendments are made to the proposal prior to approval.

Comments

The Committee questioned if Acanthamoeba castellanii will be genetically modified at a later date, for example, stable versus transient expression of the genes under study. This needs to be clarified at this stage and included in the form in order to prevent any delays to the work as well as further applications should DR F decide to genetically modify Acanthamoeba at a later stage.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 8/08.5

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<td>23/12/2008</td>
<td>Molecular Mechanisms of Attachment, Invasion and Intracellular Survival of Chlamydia</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Chlamydia is an obligate intracellular pathogen that has developed complex strategies to subvert numerous host cell processes, and thus ensures its survival growth and replication inside the cell. The aims of this project are to identify the molecular mechanisms of attachment, invasion, and intracellular survival, and identify any strain- or species-specific differences that may function as determinants of unique pathogenic traits.

Experiments will involve the use of multiple chamydial serovars/species (C. trachomatis serovars L2, B, D, C. pneumoniae, C. muridarum, C. caviae) and different epithelial (HeLa and Hep2) and macrophage-like cell lines (J774, THP-1). Biochemical approaches will be used to identify activation/suppression of signalling components. Confocal microscopy of live and fixed infected cells will be used to study the temporal and spatial controls of these components in the context of chlamydia infection.

The hazardous potential of the GMMs created is considered to be no greater than that of the parental BACTERIAL strain (DH5-alpha, TOP10 (F- mcrA Δ(mrr-hsdRMS-mcrBC) q80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara-leu)7697 galU jaiK rpsL (StrR) endA1 nupG)), which are auxotrophic for some essential amino acids. Genetic modification will not alter the basic genotype, including auxotrophy of the parental strain. Genetic modification of HUMAN CELL LINES (HeLa, Hep2, etc.) will exclusively involve transient transfection of the tagged versions of human genes. Chlamydial genes will also be expressed for studies of the localisation of the chlamydial proteins in transfected cells. No drug selection will be performed for stable transfectants or homologous recombinants, such as in knockouts or transgenics. The cells will either be killed by fixation prior to microscopy, or in the case of live cell imaging, experiments will not last beyond 24 hours.

Recipient or parental organism
The Mammalian and chlamydial genes to be cloned, propagated in E. coli DH5-alpha or TOP10 (see 1.22), and expressed for purification, protein analyses, and microscopy in E. coli or mammalian immortalised cell lines, such as HeLa, Hep2, Cos7, and CHO-K1. Human genes include, though not exclusive to N-WASP, WAVE2, Rac1, Nck, Arp3, Grb2, PI3-kinase, Cdc42, RhoA, Actin, EEA-1 FYVE domain, PI-PLCδ PH domain, BTK-PH domain, N-WASP, WA domain, Rab5a, Rab7, Lamp1, LC3, hGBP1, hGBP2, cPKCs, 14-3-3 isoforms. These genes are typically associated with various modes of actin remodelling relevant to the attachment and invasion portion of the project, and vesicle trafficking, which is related to chlamydia intracellular survival. Chlamydial genes include full-length, and deletion or inactivated derivatives of CT456 (TARP), CT663 (Chaperone), CT043 (Chaperone), CT667 (Chaperone), Cpn0585 (non-annotated, possible modulator of host RAB11 GTPase), CT229 (non-annotated, possible modulator...
of host Rab4 GTPase), engA (bacterial GTPase involved in the stringent response), IncG (inclusion membrane proteins with unknown function), IncA (inclusion membrane protein involved in homotypic fusion of multiple chlamydia incursions), etc. The chlamydia CT456 and chaperone genes are believed to be involved in the triggering of actin remodelling required for invasion, while the others have been implicated in either vesicular traffic or adaptation of chlamydia to different survival cues.

**Evaluation of foreseeable effects**

The hazardous potential of the GMMs created is considered to be no greater than that of the parental bacterial strain (DH5-alpha), TOP10 (F- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZam15 ΔlacX74 recA1 araD139 Δ(ara-leu)7697 galU galK rpsL (StrR) endA1 nupG), which are auxotrophic for some essential amino acids. Genetic modification will not alter the basic genotype, including auxotrophy of the parental strain. Genetic modification of human cell lines (HeLa, Hep2, etc) will exclusively involve transient infection of the tagged versions of human genes. Chlamydia genes will also be expressed for studies of the localisation of the chlamydial proteins in transfected cells. No drug selection will be performed for stable transfectants or homologous recombinants, such as in knockouts or transgenics. The cells will either be killed by fixation prior to microscopy, or in the case of live cell imaging, experiments will not last beyond 24h.

The hazardous potential of the GMMs created is considered to be minimal as they only encode tagged derivatives of normal mammalian genes.

The hosts (E.coli or immortalised mammalian cells) are classified as Level 1 agents, and introduction of gene inserts of interest should pose minimal hazards. If required, experiments will be done within the protective confines of a biosafety cabinet. Recipients (DH5-alpha, TOP10) of inserted genes are non-infectious to humans, animals, plants, and insects. In addition, dessemination to the environment outside the lab will be minimised by restricting use of the GMMs to within the confines of the laboratory and their sterilisation prior to disposal.

Of the known inserts, none have any toxin, allergen, or growth modulatory functions. The presumed function of the CT456 gene is on actin binding. Of the gene inserts with known functions, none have the ability to modulate the expression of crucial metabolic enzymes in the recipient.

The possibility of widespread dissemination of the inserted gene would likely be minimal as GMMs will be contained and used exclusively within the laboratory. The risk is recognised and work procedures have been implemented, such as segregation of the experiments within the shared work area to minimise the risk. In addition, GMMs for disposal will first be sterilised.

Human genes being studied are unlikely to confer altered pathogenicity to the recipient. Chlamydial genes of interest, which are suspected of virulence determinants may endow the recipient E. coli with partial pathogenic traits. Work involving such GMMs will be restricted to containment level 2 within a biocontainment hood, and will use no greater than 10 ml of genetically modified E. coli culture. Handling and disposal procedures will adhere to established level 2 standards.

Dissemination to the environment outside lab will be minimised by restricting use of the GMMs to within the confines of the laboratory, and their sterilisation prior to disposal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Incubation in a shaking incubator will be restricted to small volumes (< 10 ml) contained within Falcon tubes with secured caps. The tubes will be placed in racks that are properly secured to the shaking platform. Spillage will be documented using Virkon and wiped down thoroughly. Incubation in static shelf incubator will be only for cultures grown on solid agar plates.

Routinely, 10 ml cultures, but maximum conceivable is 250ml total.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Liquid Waste**
Derived from culture media; inactivation via 1% Virkon treatment for at least 30 min. From the manufacturer's guide, 30 min treatment is sufficient to kill the bacteria. Waste will be disposed of in the sink.

Solid Waste
**************
Derived from plasticware, agar plates. Inactivation via autoclave (134°C, 3 min). Treatment will be monitored using the chart recorder attached to the autoclave. Inactivated waste will be incinerated.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
COMMENTS
**************

GM work cannot be carried out on Chlamydia, therefore an expression of the Chlamydia genes in E. coli or human cells will be used. Due to unknown effect of expressing potential Chlamydia virulence genes in E. coli it is remembered that the project will be proposed as class 2.

Clarification of whether using cell lines or E-coli is relevant to the entire document and also a general classification of experimental strategy required.

THE FOLLOWING AMENDMENTS ARE REQUIRED PRIOR TO APPROVAL
************************************************************************************
Title: Explained title i.e. 'Molecular mechanisms of attachment, invasion, and intracellular survival of Chlamydia.'
BSO: Stefan Hoyle, CID: 00171599
1.21: Explain on why these genes are involved in the research; what pathways are they associated with? Also it'll be helpful to annotate some of the genes eg engA, IncG, IncA etc.
1.25: Point out the use of human cells and what work will be done with the human genes. List some of the Chlamydia genes and clarify experimental strategy. Include a brief description of methodology.
1.3: Clarify the parental strain is E. coli or other cell line.
Also sentence 'genes are inactivated' is misleading as it implies creation of knockouts.
2.1.1.2: Explain if the parental strain is E. coli or other cell line.
2.1.1.3: The second sentence needs to be moved to section one.
2.1.1.6: State that a potential risk is recognised, but this is minimised. The work area is shared, but segregated which is part of the code of practice.
2.3.3.3c: Specify that samples will be opened in a Microbiological Safety Cabinet and the classification of the cabinet.
2.3.6: Complete as Class 2, Containment Level 2.
3.1.1a: Change 30h to 30 minutes.
3.2b: Remove end bracket and the end of the paragraph.
3.32a: Change this to the maximum conceivable.
3.42: "...pathogens are NOT permitted in my group."
3.5b: Typo: 'Ocupational'
3.6.2.: State there are others working in this area, a Code of Practice is in place for this.
AGREED: Class 2, Containment Level 2, HSE notification and written consent required prior to commencement.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L3</td>
<td>L4</td>
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02/03/2022
The role of the glycostructures on the exterior of the bacterial cell wall in the immune response to Campylobacter jejuni

**Purposes of the contained use**

Campylobacter jejuni is the causative agent of gastroenteritis worldwide. The disease is generally self-limiting, rarely requiring antimicrobial therapy. However, one in 1000 infections caused by C. jejuni may lead to the Guillain-Barre syndrome, a neurological disorder.

The pathogenicity of C. jejuni is not well understood. The aim of the project is to investigate the role the glycostructures of the exterior bacterial cell wall play in the immune response. Several single-gene knockout strains have been produced at the London School of Hygiene and Tropical Medicine which have been shown to have altered glycostructures. These strains, including the wild type strain, will be used to infect mammalian immune cells, like dendritic cells and macrophages, to assess the influence on the immune response.

**Recipient or parental organism**

Campylobacter jejuni causes gastroenteritis which is generally self-limiting and rarely requires treatment with antibiotics. Infection can occur by ingestion and inoculation. Respiratory tract infections are extremely rare.

The wildtype is a hypermotile variant that is thought to resemble the original clinical isolate closely. This strain was first isolated in 1977 and the original isolate has been shown to vary from passaged lab stocks in virulence-associated phenotypes even though no detectable genetic differences could be found.

**Host/vector system**

The GMOs to be used in this activity were constructed at the London School of Hygiene and Tropical Medicine and this activity was notified previously to HSE (HSE reference number GM654/99.2). No new mutants will be constructed in this activity, therefore no host/vector systems will be used.

**Origin & function**

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<td>The role of the glycostructures on the exterior of the bacterial cell wall in the immune response to Campylobacter jejuni</td>
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<tr>
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<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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</table>

**Project notified under transitional arrangements**

N

**Historical Significant Changes**

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**
Campylobacter jejuni has four distinct glycostructures, the capsular polysaccharide, LOS (like LPS, but lacking O-antigen repeats), and 2 glycosylation systems, N- and O-linked. Genetically modified Campylobacter used in this project will include mutations in genes that are responsible in the formation of each of these structures. Examples of some of these genes are given below:

- Knockout of gene cj1439 (glf), a predicted UDP-galactopyranose mutase. This mutant has been shown to be acapsular by HR-MAS NMR. The capsule has been shown to be important for adherence, invasion and protection from phagocytosis.

- Knockout of gene cj1126 (pglB), oligosaccharyl transferase. This mutant does not produce any N-linked glycoproteins.

- Knockout of gene cj1324 (gene function unkown). This mutant lacks legionimic acid on flagella.

- Knockout of gene cj1141 (neuB1), a probable N-acetylneuraminic acid synthetase. This mutant lacks sialic acid residues on LOS.

The knockouts were created at the London School of Hygiene and Tropical Medicine (HSE reference number GM654/99.2) by insertion of a kanamycin resistance cassette derived from Campylobacter species into specific restriction sites. The inserted sequences disrupt genes involved in the formation of C. jejuni glycostructures.

In addition, complement strains may be used where the gene is reintroduced into the mutant strain, within an intergenic region, in addition to a chloramphenicol resistance cassette derived from Campylobacter coli.

### Evaluation of foreseeable effects

All GMOs used have single gene knockouts in the biochemical pathways that are important for the formation of the glycostructures in C. jejuni. The glycostructures are viewed as important for the pathogenicity of C. jejuni. Therefore the mutant strains are likely to be less hazardous than the wild type strain.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

#### Liquid waste

This will be treated with 1% Virkon for a contact time of 30 minutes, as per manufacturer’s instructions, and will then be disposed of via the drains.

#### Solid waste

This will be autoclaved and then sent for incineration with other clinical waste. Autoclave cycle time is 126 C for 10 minutes and is monitored by indicator strips; a record is kept as the chart recorder print-out. Autoclave function is tested by an annual 12-point thermocouple test for validation of treatments.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

02/03/2022
Please enter comments on the GM safety committee on the risk assessment

The following Comments were made by the GM safety committee:

Comments: AR gave a brief summary of the project. YH asked if the London School of Hygiene and Tropical Medicine (LSHTM) had included Imperial College under a connected programme of work in their HSE notification thereby circumventing a further application and hence fees to the HSE. AR clarified that the HSE had approved the LSHTM to use these strains a long time ago and it would be better and more straightforward to apply through Imperial College. IH queried what Guillain-Barre syndrome was. AR explained that it was a neurological disorder. He also asked if the dendritic cells were genetically modified and AR clarified that they were not.

The following amendments are required prior to approval:
Part 1: GM centre number: GM8
Title: Expand title, for example: Role of the glycostructures of the bacterial exterior cell wall in the immune response to Campylobacter jejuni.
1.22: Clarify which genes have been mutated in the strains from LSHTM and the biochemical pathways affected. Also state any hazards associated with 'A hypermotile variant of Campylobacter jejuni strain 11168'.
1.25: State the origins of the mammalian cell cultures; are these primary cultures or cell lines?
1.3: Typo: viewd
2.3.6: Complete section, containment and classification 2.
3.1.1.a: Change the use of Virkon from ‘overnight’ to ‘30 minutes’
3.1.1.b: Solid waste: also include that the autoclave was validated in the last four weeks.
3.2.b: Include that the container will be double contained, robust, and leak proof.

Agreed: Class 2, Containment Level 2. HSE notification and written consent required prior to commencement.

All comments have been addressed and all required changes have been incorporated into the risk assessment attached to this form.

Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 Yes | L3 | L4
L2 | L3 | L4
L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4
L2 | L3 | L4
L2 | L3 | L4

Project Ref 8/09.2

Date Ackn’d 25/06/2009
CU2 Project Title The Genetic Basis Of Anopheles Susceptibility to Plasmodium falciparum
Class CultureVol | Class CultureVolume
Class 3 | 50 ml

02/03/2022  Page 265 of 15326
The goal of this project is to identify the genetic basis of susceptibility to Plasmodium falciparum in the malaria mosquitoes, Anopheles gambiae complex (including gambiae, arabiensis, quadriannulatus, melas and merus), Anopheles stephensi, and Anopheles funestus. The project will use both long-standing lab isolates of Plasmodium falciparum (e.g., NF54, 3D7) and new isolates recently collected in the field by collaborators from Harvard School of Public Health, Makerere University, and Institut de Recherche pour le Développement (IRD). We will investigate susceptibility in both wildtype mosquitoes and mosquitoes that have particular genes knocked down using standard dsRNA techniques. All P. falciparum isolates used will be chloroquine, artemisinin and/or malarone(atovaquone and proguanil) sensitive, and field collected isolates will be tested for this sensitivity before arriving at the lab.

We will create transgenic P. falciparum stocks that express fluorescent proteins in the mosquito stages of the parasite in order to facilitate tracking these life stages of the parasite. We will feed parasites to different colonies of mosquitoes and then compare mosquitoes that are fully resistant to infection with those that are susceptible using various genotyping technologies (e.g., SNP chips). We will also compare mosquito susceptibility in mosquitoes that have certain genes knocked down by RNAi to wild type controls.

Furthermore, we will create knock-outs of genes in the parasite that we think might be important in mosquito infectivity and carry out infections of mosquitoes to test these hypotheses. Similar work has been carried out using Plasmodium berghei but many genes shown to be involved in mosquito susceptibility to P. berghei do not appear to have an effect on P. falciparum susceptibility. Therefore it is important to carry out these experiments using P. falciparum.

Recipient or parental organism

P. falciparum strains 3D7 and NF54. Both are chloroquine sensitive and have been cultured in the lab for decades.

E.coli strains DH5-alpha, TOPTEN, PMC103 and XL10-Gold. TOPTEN, PMC103 and XL10-Gold are genetically modified to tolerate plasmid sequences that are unstable in standard strains such as DH5-alpha. These strains are required due to the high AT content in the extragenic regions of P.falciparum. These strains are available from commercial sources and are CL1.

Host/vector system

P. falciparum expression vector pHH1 with either T. gondii dhfr-ts or human dhfr mutated to encode resistance to WR99210. The plasmid would not reverse the chloroquine sensitivity of the parasite.
Two selection cassettes are mainly used for transgenic Plasmodium falciparum parasite generation. The human dhfr resistance marker is the most widely used, it confers resistance to the folate drug WR99210 (Jacobus pharmaceutical). The other marker is the blasticidin deaminase gene, which confers resistance to blasticidin. We use strains 3D7 and isolate NF54 both of which are chloroquine-sensitive and neither resistance marker confers resistance to chloroquine. However hdhfr selected parasites are resistant to pyrimethamine.

Vectors used for E. coli transformation: The vectors that will be used in bacterial transformation include (a) pLL10 (pBluescript-based plasmid containing two T7 promoters for the purpose of double-stranded RNA production in vitro) (b) pGEM-T easy (for routine AT subcloning of PCR amplicons) (c) various pETM vectors (example , pETM-11, pETM-60, pETM-80 etc.) which originate from pBR322 and are used for protein expression (d) various pGEX vectors (example pGEX-4T-2 and pGEX-4T-3 etc.) which are used to express GST fusion proteins (e) various pQE vectors (example pQE-30 etc.) for the expression of his tagged proteins.

Two types of genetically modified strains of P. falciparum will be generated: reporter strains and knock-out strains. We will create transgenic P. falciparum stocks that express fluorescent or luminescent proteins (such as YFP, GFP, RFP, eGFP, eYFP) in the mosquito stages of the parasite in order to facilitate tracking life stages or tracking proteins of interest in the parasite. We will also create knock-outs of genes in the parasite that we think might be important in mosquito infectivity. Examples include secreted or transmembrane proteins expressed at the gametocyte, gamete and ookinete stages of the parasite.

E. coli will be used as to screen plasmids and amplify them to produce quantities needed for P. falciparum transformation.

Genes of interest in Anopheles species including but not limited to members of the Leucine Rich Repeat family, thioester family, and other immune molecules will be knocked down using RNAi. Both wildtype and knockdown mosquitoes will be infected with GMM falciparum (for example, GFP falciparum to facilitate the observation of the parasite within the mosquito).

Plasmodium falciparum is an infectious parasite and hazardous. If a human becomes infected with P. falciparum, following an incubation period of ~7 days, falciparum malaria can develop. Symptoms include a systemic illness with high fever, rigors, headache, myalgia, prostration and can progress to cerebral malaria: confusion, delirium, loss of consciousness and death.

E. coli strains such as Dh5alpha do not have any significant risks associated with them. Reporter genes (fluorescent and luminescent proteins) are non-toxic and non-hazardous and as such, do not pose any hazard to human health. Knock-outs of genes thought to be important in Plasmodium infectivity to mosquitoes also have no additional hazards to human health apart from the hazards associated with working with falciparum.

Alterations to the traits of P. falciparum might include decreased or increased ability to infect mosquitoes, however they are unlikely to result in any increased ability to infect humans and as such should not increase the pathogenicity of the parasite (which will remain chloroquine sensitive). These alterations are unlikely to affect infectivity to unnatural mosquito hosts, includig those mosquito species that are native to the UK.

The room in which the culturing of Plasmodium falciparum will take place is a Category Level 3 containment facility and separated from all other work and laboratory areas by restricted access corridors. Access is restricted via proximity readers.

Access is granted by the facility manager after an appropriate induction by the Chief Services Technician. All those entering the facility must be on the health surveillance database maintained by the Occupational Health Department.

In vitro culturing of falciparum will be carried out within rooms 627, 630 or 632. All mosquitoes will be in double containment and they will be membrane fed in room 630, and
Transferred to containment room 619 via a hatchway removing the need to transport the mosquitoes in unrestricted areas. The room in which they will be kept has an additional box that allows complete shut-down of the experiment via CO2 gassing should an escape occur. 619 in the insectary is restricted access only.

All people working with P. falciparum and mosquitoes will have Biological agent screening and health surveillance via Occupational Health. When not in culture, Plasmodium falciparum stocks will be kept in liquid nitrogen dewars. These dewars are kept within the CL3 facility and locked. While in culture, the parasite will be stored in a Plasmodium-only incubator in room 627, 630, or 632.

If a release of infectious material were to occur during loading or removing a sample from storage in liquid nitrogen, the area would be wiped down with 70% ethanol. As stated above, aerosolized falciparum is not infectious. Samples will be placed in screw top cryotubes within a microbiological safety cabinet. Tubes will be wiped down with 70% ethanol disabling the parasite before transport to liquid nitrogen. Screwtop lids prevent the risk of lids opening due to sudden changes in temperature. When removing samples from LN2, labcoat, gloves and eye protection will be worn. Cryotubes will be placed immediately into secondary container (in case of ingress of LN2 into cryotube and subsequent expansion at room temperature). These tubes will be taken to MSC, removed from secondary containment within the MSC, and wiped down with disinfectant.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The CL3 has been derogated as the facility cannot be sealed for fumigation purposes. This means the use of hazard group 3 pathogens spread via the inhalation route is prohibited.

The facility is used for working with Plasmodium falciparum (hazard group 3). This is acceptable as P.falciparum (and related organisms) is not spread directly via the inhalation route (infection would only occur via percutaneous injury or mosquito transmission).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste such as E.coli culture waste will be disinfected using 10% Chloros solution as per manufacturer's guidance and disposed of via the sink into the drains. Other liquid waste such as from cell culture waste will be autoclaved. Autoclave cycle time is 121°C for 15 minutes which is monitored by indicator strips; records kept of the chart recorder print-outs. Autoclave function is tested by an annual 12-point thermocouple test for validation of treatments.

Solid waste is collected in dedicated autoclave bags (red colour to distinguish from other waste streams) and autoclaved within the facility. Autoclave cycle time is 126°C for 10 minutes. The autoclave is maintained quarterly, 12 point thermocouple tests and insurance inspection done annually.

Detailed information on waste procedures for the facility can be found in the CoP (page 14 - 16 and SOP 2 & 3 in the appendices) as well as part 3 of the GM risk assessments.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Investigating the genetic basis of Anopheles susceptibility to the human malaria, Plasmodium falciparum. (GMIC-01870.1), Dr G Christophides, Cell and Molecular Biology, Natural Sciences, South Kensington campus.

Comments: This is a new proposal investigating the basis of genetic susceptibility of Anopheles to P. falciparum, the causal agent of malaria. The following amendments are required prior to approval:

1.24: Typo: 'resitance'
1.25: Provide a list of target genes, to make it clearer.
1.4: 'x' mark classification box
2.2.1.1: Correction: 'renders in inviable.' Remove reference to bleach.
2.2.1.4: Correction: add 'P' before falciparum.
2.3.2.2.a: Remove "At the moment" second paragraph. Typo: 'cultring'. Correction: add 'P' before falciparum.
2.3.3.1.a: Room number is incorrect, 632 or 619?
2.3.3.2.b: Clarify last sentence in this paragraph.
2.3.3.6: Typo: 'CO2' (CO2), 'wetable'
2.3.3.8.a: Correction: 'work', should be 'worn'
Part 3: Tick box needs to be ticked
3.1.1.a: Clarify statement '70% ethanol, as per manufacturer's guidance'. Please state clearly your method of disinfection. Your CoP states use of 10% Chlorus. Is this not the case?
3.1.1.b: Typo: 'centigrade'. Change 'technician' to 'Engineer'. Typo: 'clincial'
3.4.a: Clarify statement '70% ethanol, as per manufacturer's guidance'. Please state clearly your method of disinfection. Your CoP states use of 10% Chlorus. Is this not the case?
3.6.1: CID numbers missing.

In the CoP, section 11, page 16, no mention is made of emergency procedures in case of accidental spills. Please rectify.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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Molecular Genetoc analysis of Neisseria meningitidis virulence

Neisseria meningitidis (the meningococcus) is a commensal of the human nasopharynx found asymptptomatically in up to 15% of the population. However, upon crossing of the nasopharyngeal mucosa, which happens rarely, N. meningitidis can cause life-threatening infections mainly in children and young adults. Our scientific goal is to understand the molecular mechanisms that underlie N. meningitidis pathogenesis. The rationale is that a better understanding of meningococcal biology in general, and virulence in particular, could have profound consequences on human health by leading to the rational design of novel therapies. To achieve this goal, we use mainly a molecular genetics approach that relies on the generation and phenotypic analysis of genetically modified N. meningitidis strains. One of the main virulence attributes of Neisseria meningitidis pathogenesis is this bacterium’s ability to adhere to human cells, which relies primarily on surface organelles known as type 4 pili (Tfp). Our primary objective is to understand how these organelles, which are found in numerous bacterial species, are assembled and how they can mediate adhesion and other key virulence functions such as bacterial aggregation, competence for DNA transformation and twitching motility.

We will almost exclusively use a highly adhesive variant of a serogroup C clinical isolate of N. meningitidis, strain 8013 (C:22:NST:L3,7,9), whose genome has been sequenced and which has been used as a lab strain by the Neisseria research community for almost 20 years. This is a variant of a clinical isolate first isolated in 1989 in France that is available from the strain collection of the Institut Pasteur in Paris. Efficient vaccines are available against serogroup C strains and all personnel will be vaccinated. If at any stage of this project the use of other meningococcal strains becomes necessary, we will use one of the other well-characterized sequenced meningococcal clinical isolates belonging to A, C, Y or W135 serogroups for which efficient vaccines exist (e.g. strains FAM18, Z2491, 053442, alpha 14 etc.). We will not use serogroup B strains for which no vaccine is available.
i) For mutagenesis studies, meningococcal genes will be engineered to contain antibiotic resistance cassettes that confer resistance to antibiotics other than those used to treat meningococcal disease:
- genetic modification will be done in E. coli by cloning or by transposition and genes will be subsequently delivered in the meningococcus on pUC- or TOPO-derived cloning vectors,
- genetic modification will be done in vitro by splicing PCR (no cloning involved).
(ii) In order to test competence for DNA transformation, meningococci will be transformed with the pSY6 plasmid which contains a naturally occurring allele of the Neisseria gyrB gene. This vector integrates into the meningococcal chromosome by allelic exchange and confers resistance to nalidixic acid.
(iii) For complementation studies, we will use the pGCC4 vector in which genes of interest can be cloned under an IPTG-inducible promoter. Upon transformation, this vector integrates into the meningococcal chromosome by allelic exchange. We might also clone the genes of interest with their own promoters in the pFP10 vector that can replicate in Neisseria.

Origin & function
To select for mutants in each of the approx. 2,000 N. meningitidis genes, we will use genes encoding resistance to antibiotics. We will use well-established cassettes in the Neisseria field expressing resistance to kanamycin, erythromycin, spectinomycin or chloramphenicol, and/or a mutant allele of Neisseria's gyrB gene conferring resistance to nalidixic acid.
For complementation studies, a key part of molecular genetics, mutants presenting interesting phenotypic defects will be complemented by reintroducing in their genome the corresponding wild-type genes (or orthologs from other species such as Pseudomonas aeruginosa and Neisseria gonorrhoeae) to determine whether this restores wild-type phenotypes. For example, the inserted genes might encode proteins that are suspected to play a role in (i) type 4 pilus biology (our main area of research) or in Neisseria pathogenesis (e.g. proteins involved in resistance to complement-mediated lysis or adhesion to human cells, transcriptional regulators etc.), (ii) which might make good vaccine candidates such as conserved surface-expressed proteins, or (iii) which may be good drug targets such as genes essential for meningococcal life.

Evaluation of foreseeable effects
The hazardous potential of the GM N. meningitidis we will generate is expected to be no greater than that of the recipient strain. Actually, due to the genetic modifications that will be introduced, these GMM will most often be less virulent or even avirulent and hence less hazardous than the recipient N. meningitidis clinical isolate. Moreover, the use of antibiotic resistance cassettes expressing resistance to kanamycin, erythromycin, spectinomycin, chloramphenicol or nalidixic acid, none of which is used to treat meningococcal disease, prevents the risk of generating strains difficult to eradicate in case of accidental infection.

8.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste (cultures). Cultures above 50 are rendered non-viable prior autoclaving by chemical inactivation using 1%
virkon (final concentration). All liquid waste is autoclaved (128°C/60 min). Treatment is monitored by chart recorder attached to autoclave. Waste is finally disposed of by incineration.

Solid Waste (pipette tips, tubes, vials, Petri dishes, plates etc.) is autoclaved (128°C/60 min). Treatment is monitored by chart recorder attached to autoclave. Waste is finally disposed of by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Part 1: GM Centre Number is: GM8
1.21: State what types of species.
1.22: Replace, “We will endeavour” to “we will/will not”.
   - Please be specific about what isolates you intend to use. The risk assessment will need to be amended as additional strains are added.
   - Please clarify whether clinical isolate strain 8013 is a recent isolate or has been in culture for some time. Please clarify whether this isolate has been commercially sourced or recently provided by a patient.
1.25: Include a list of inserts. Please provide some more details about the inserted genes.
   - The committee was under the impression from earlier text that the idea was to interrupt genes of interest. Will you be reinstating the genes as well? Is it likely that any of the inserts increase virulence or host range?
1.3: A sentence in this paragraph does not make much sense and appears either superfluous or there is a word or two missing. Do you mean that the inserted antibiotic genes confer resistance to antibiotics that are not used clinically to treat infection?
   - It is not anticipated that additional hazards are associated directly from the inserted gene products. Genetic modifications in N. meningitidis will most often lead to strains with reduced or even abolished virulence. The major hazard would be to generate strains resistant to antibiotics used to treat meningococcal disease (penicillin G, ceftriaxone and cefotaxime), which will be prevented by using exclusively antibiotic resistance cassettes promoting resistance to other antibiotics. Indeed, to select for transformants and mutants, we will use genes promoting resistance to antibiotics. For the meningococcal work, we will use foreign cassettes encoding resistance to antibiotics such as kanamycin, erythromycin, spectinomycin or chloramphenicol as well as a cloned mutant allele of the Neisseria gyrB gene conferring resistance to nalidixic acid.
2.3.3.1.a: Obviously not “all work” can be carried out in the MSC, there will be storage and incubation to name two work processes outside the MSC. It might be more accurate to state that “all work involving manipulation of live organisms will be carried out in an MSC”.
   - A class 1 and class 2 MSC work in very different ways and offer the operator differing levels of performance in different circumstances. Essentially the class 1 offers more robust protection against aerosols generated by the work activity but does not protect the work against contamination while the class 2 offers protection to both the worker and the subject material but is more sensitive to external air movement interfering with the protection factor. These differences must be understood by the lab worker. Please state what procedures can be carried out in a class 1 MSC and what procedures can be carried out in a class 2 MSC.
   - There is also a statement - “...prevent the potential for aerosol production.” Include a reference to the COP section that is appropriate to explain this, what procedures create an aerosol? How is production prevented and if not possible how is it controlled?
2.3.3.1.c: Remove the highlighted text, as work with live N. meningitidis is always carried out at CL3 at ICL “Not for
work at containment level two but as the work is carried out at level 3, the CL3 suite provides negative pressure”.

2.3.3.3.c: Are the MSC’s tested for KI operator protection with the microfuges in the MSC and running? If not, they should be.

2.3.3.3.d: Please add here that where a leak is suspected inside a centrifuge during operation (e.g. a severe out of balance situation develops) that the centrifuge lid is left closed, the lab evacuated and advice is then sought from the Suite manager, Safety Dept and CSM.

2.3.3.4: How are liquid cultures contained during shaking incubation?

2.3.4.1: Are MSC’s OP tested under operational conditions? i.e. with running centrifuges and other user equipment inside the MSC such as waste containers, tube racks etc? If not they should be.

2.3.5: To be completed by Occupation Health Physician.

3.31a: Provide justification for this answer.

3.5.b: Reconsider the answers to include if the strategies in 3.5.a had failed.

-Who is consulted after an incident to assess the need for fumigation of and MSC and where necessary the room?

-Re centrifuges – Please add here that where a leak is suspected inside a centrifuge during operation (e.g. a severe out of balance situation develops) that the centrifuge lid is left closed, the lab evacuated and advice is then sought from the Suite manager, Safety Dept and CSM.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
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<td>L3</td>
<td>L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
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**Project Ref 8/11.2**

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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
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<tbody>
<tr>
<td>21/12/2011</td>
<td>Bacterial Genome Plasticity - Analysis of integron-mediated acquisition of antibacterial resistance among Pseudomonas aeruginosa and Klebsiella pneumoniae populations</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</tbody>
</table>

- Non-GMM
- Consent Granted

- Project notified under transitional arrangements

| Date Project Ceased | | | |
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<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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Purposes of the contained use

Pseudomonas aeruginosa and Klebsiella pneumoniae are able to infect a wide variety of hosts and tissues and provide excellent models for studying by gram-negative bacteria. Both organisms can cause destructive changes to human lungs including inflammation and hemorrhage with cell death (necrosis) that sometimes produces a thick, bloody, mucoid sputum. Typically these bacteria gain access after a person aspirated colonizing or exogenous oropharyngeal microbes into the lower respiratory tract. Pseudomonas and Klebsiella infections are mostly seen in people with a weakened immune system and/or weakened respiratory host defences.

This project will assess the role of host-imposed stress upon bacterial conjugative plasmid transfer. Specifically we are interested in identifying regulators of integron-mediated acquisition of antibacterial resistance among individuals in host-colonising populations of bacteria. Conjugation consists of DNA transfer, by cell contact, from one bacterium to another. Conjugative circular plasmids have been identified as shuttles and resevoirs for adaptive genes. It is now established that such lateral gene transfer plays an essential role, especially for the antibiotic resistance development and dissemination among bacteria. Moreover, integrons, platforms of mobile gene cassettes, have been instrumental in this phenomenon, through their successful association with conjugative resistance plasmids.

Multi-resistant integrons (RI) have been isolated on mobile elements responsible for their assembly and rapid propagation of multiple antibiotic resistances in Gram-negative bacteria through association with conjugative plasmids. An integron is characterized by an intI gene, coding a site specific recombinase from the tyrosine recombinase family, and an adjacent primary recombination site attI. The intI integrase allows the integration of a circular promoterless gene-cassette carrying a recombination site, attC, by driving recombination between arf and attC. The integrated gene cassettes are expressed from the Pc promoter located upstream of the attI site in the integron platform.

The order of events is: Conjugation > integrase expression> cassette recombination.

Recently it was shown that the conjugative transfer of plasmids triggers a bacterial stress response - the SOS response - in recipient cells which in turn can impact the cassette content of integrons, SOS induction during conjugation is thus most probably able to impact a wide range of genomes. Although the phenomenon has been demonstrated to occur in vitro using laboratory-adapted isolates, it has not yet been demonstrated as a physiologically relevant process in pathogenic isolates. This project will interrogate the relevance of integron-mediated acquisition of antibiotic resistance in Pseudomonas aeruginosa dn Klebsiella pneumoniae.

Our specific objectives will be to 15:04:10

1) To establish the relevance of SOS induction as a general inducer of intI expression during conjugative plasmid transfer in Pseudomonas and Klebsiella pneumoniae.
2) To establish a model for conjugative plasmid transfer between bacterial isolates colonising murine gut and pulmonary niches.
3) To screen for regulators of integrase-mediated gene cassette excision and acquisition using libraries of Pseudomonas and Klebsiella mutants.
4) To screen for regulators of integrase expression using libraries of Pseudomonas and Klebsiella mutants.
5) To assess the epidemiology of integron-bearing plasmids and chromosomes, by PCR screening and sequencing, among local populations of nosocomial Pseudomonas aeruginosa.
Recipient or parental organism

E. coli:
E. coli K12 and derivatives will be used for cloning and as recipient for gene overexpression and purification. Most commonly used strains will be TG1, HB101, C600, DH5α, OP50, CC118, XL1blue, TOP10F, S17.1. These strains have a widespread and long history of safe use.

Pseudomonas aeruginosa (Hazard Group 2, opportunistic pathogen):
Most well characterized laboratory strains used will be: PAO1, PA14, PAK, CHA, PA103, TB, SG17 (Clone C)

Strains isolated from patients will be collected and used in the course of the program. Some isolates will be freshly collected, others have been collected for many years and stored at -80°C without any further laboratory manipulations.

Other Pseudomonas and related species:
Pseudomonas fluorescens (PF-5, SBW25, PFO-1, CHAO), Pseudomonas putida (WCS358, KT2440), Pseudomonas alcaligenes (M-1), Pseudoalteromonas haloplanktis (TAC125).

Related Pseudomonas species:
Ralstonia solanacearum which is a phytopathogen without any known natural antibiotic resistance (Strain Gmi1000, Mok2 and 1649).
Burkholderia cepacia and cenopacia (strain J2315), such as P. aeruginosa is a hazard group 2 opportunistic pathogen which is isolated from cystic fibrosis patients with a poor prognosis.

Pseudomonas entomophilia L48 (strain CT573326) is an entomopathogenic bacterium known to infect Drosophila melanogaster by oral route.

We will also use Klebsiella pneumoniae (Hazard group 2) isolates: LM21, 342 (Fouts et al, 2008, PLoS Genet 4(7) e1000141) and HTUH-K2044 (Wu et al, 2009. J. Bac. 191(14) 4492-4501)

Recipient or parental organism

A collection of Tn mutants (SM10pir/pCM639-ISphoA/hah insertions or SM10pir/pIT2-ISlacZ/hah insertions) in the PAO1 strain will be used (Jacobs et al., PNAS, 2003). A collection of Tn mutants (MAR2xT7) in the PA14 strain will be used (Liberati et al., PNAS, 2006).


Host/vector system

1) We will use standard E. coli vectors:
a) For cloning:
- pBR322 and derivatives: AmpR, TetR.
- pACYC184 and derivatives: ChlR. TetR.

NB: These vectors could also be used as suicide vectors for P. aeruginosa mutagenesis.
B) For gene tagging and expression:
- PET22b/28c/28a and derivatives: ColE1 replicon, AmpR (22) and KanR (28), tag Histidine N or C-terminal
- pQE30Xa and derivatives: Tag histidine (Qiagen), AmpR.
- pEGFP and derivatives: engineering of gfp fusion, AmpR
- pGEX and derivatives: Tag GST (Glutathion S-transferase), AmpR
- PAC or PAN an derivative: AviTag, AmpR
- pBADmycHisA, B, C and derivative: expression from an arabinose promoter, tag histidine and Myc. AmpR
- pYZ4 and derivatives: KanR, Construction of phoA fusion
- GATEWAY plasmids: InVitroGen, System including the entry vector pDONOR and the destination vectors (rpDEST derivatives) for recloning and expressing tagged genes.

c) For expression of genes under strong promoters:
- pBAD an derivatives: arabinose promoter. AmpR.
- pT7-1 to pT7-6 and derivatives: T7 promoter. AmpR. To be used together with pGP1-2 which carries the gene encoding RNA polymerase from T7 phage. KmR.

d) For studying protein-protein interaction:
- pUT18c and pKT25 and derivatives. Two hybrid system from Ladant. pUT18c (pUC19 derivatives), AmpR, contains 3' region of the gene encoding adenylate cyclase (cyaA). pKT25 (pSU40 derivative), KanR, contains 5' region of cyaA.
- pBS1479 and derivatives: AmpR. Tandem Affinity Purification (TAP). The TAP-Tag contains a domain CBP(Calmodium Binding Protein) and a domain Protein A (Affinity for IgG).

2) We will use suicide vectors for the engineering of Pseudomonas mutants:
- pKNG101 and derivatives: StrR. SucR. Only replicate in the E. coli:ambdapir strain CC118.
- pEX100 and derivatives: SucR. Only replicate in E. coli:ambdapir strain CC118.

3) We will use broad host range vectors for expression in P. aeruginosa (also replicative E. coli):
- pLAFR1, pLAFR3 and derivatives: IncP, TetR. Cosmid with low copy number.
- pMMB66, 67, 190, 206 and derivatives: AmpR but pMM206 (ChlR), tac promoter.
- pBRIMCS and derivatives: ChlR (1), TetR (3), KanR (2) AmpR (4), GentR (5).
- pUCP18/19 and derivatives: AmpR. Derivatives of pUC18/19 with a 2.1 kb stabilizing fragment allowing replication in Pseudomonas.

4) We will use vectors for conjugation in Pseudomonas:

5) We will use vectors for transposon mutagenesis in pseudomonas:
- pHP45 and derivatives: Interposons carrying résistances: KanR, TetR, HgR and ChlR.
- pSUP102 and derivatives: Tn5-B10 (KanR), -B11 (GentR), - B13 (TetR), -B20 (KanR), -B21 (TetR), -B22 (GentR), -B30 (TetR), -B40 (KanR et -B41 (TetR).
- pUT-Tn5 and derivatives: TetR.

We will use any or all of the annotated P. aeruginosa genes available from the genome sequence of the PAO1and PA14 strains (http://pseudomonas.com), or Klebsiella pneumoniae isolates: LM21, 342 (Fouts et al, 2008. PLoS Genet 4(7) e1000141) and NTUH-K2044 (Wu et al, 2009. J. Bac. 191(14) 4492-4501).

We will use artificially-constructed minimal type I integron elements designed upon the templates described among naturally-occurring isolates and as curated in the Integrall database (http://integrall.bio.ua.pt/). Genes and gene promoters required and involved in integron-mediated antibiotic resistance will be cloned and/or inactivated. These
genetic elements are required for the shuttling of promoterless antibiotic resistance cassettes between bacterial strains and species. They will include, but not be limited to integrase-type tyrosine recombinase enzymes, and dedicated intergon Pc promoters.

It is not anticipated that additional hazards arise from the alteration of existing traits of the host; only traits similar to traits which naturally exist already in the host will be introduced. Where mutational screens are concerned, we anticipate the vast majority of isolates to exhibit reduced fitness.

**Evaluation of foreseeable effects**

**Pseudomonas aeruginosa: Class 2:**

Pseudomonas aeruginosa as a wild type organism is a Gram-negative, aerobic rod belonging to the bacterial family Pseudomonadaceae. The family includes other genera, which, together with certain other organisms, constitute the bacteria informally known as pseudomonads. These bacteria are common inhabitants of soil and water. They occur regularly on the surface of plants and occasionally on the surface of animals. Pseudomonas aeruginosa and two former Pseudomonas species (now reclassified as Burkholderia) are pathogens of humans. However, Pseudomonas aeruginosa is an opportunistic pathogen, meaning that it exploits some break in the host defences to initiate an infection. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. Pseudomonas aeruginosa infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is 50 percent. Pseudomonas aeruginosa is primarily a nosocomial pathogen. The overall incidence of P. aeruginosa infections in hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital acquired infections. Pseudomonas aeruginosa is sometimes present as part of the normal flora of humans and the prevalence of colonization of healthy individuals outside the hospital is relatively low.

Infection may occur through direct and extensive contact with the organism, however no procedure in the laboratory will require direct contact. In addition, personal protective equipment (PPE) including lab coat, gloves and safety glasses will be worn when working with P. aeruginosa strains to further minimize the risk of direct contact with the organism.

We will use several laboratory P. aeruginosa strains including, PAO1, PA14, PAK, TB, CHA, PA103, SG17 (Class C). We will also work with isolates obtained directly from patients at Hammersmith Hospital and those submitting samples for microbiological testing at HCA Laboratories (Harley Street). The antibiotic susceptibility profile of collected organisms will be known.

**Klebsiella pneumoniae** is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines. Klebsiella pneumoniae is resident in the intestinal tract of approximately 40% of the human population. It is considered to be an opportunistic human pathogen meaning that under certain conditions it may cause disease. For example, nosocomial infections are those that hospitalized patients pick up because they are in a weakened state. Klebsiella pneumoniae is also well known in the environment and can be cultured from soil, water and vegetables. In fact, it is likely that we have K. pneumoniae in our intestine from eating raw foods such as salads. The most common infection caused by Klebsiella bacteria outside the hospital is pneumonia. Mortality in Klebsiella pneumonia is around 50% due to the underlying disease that tends to be present in affected persons. While normal pneumonia frequently resolves without complication, Klebsiella pneumonia more frequently causes lung destruction and pockets of pus in the lung (known as abscessess). The mortality rate for untreated cases is around 90%. Klebsiella can also cause less serious respiratory infections, such as bronchitis, which is usually a hospital-acquired infection. Other common hospital-acquired infections caused by Klebsiella are urinary tract infections, surgical wound infections and infection of the blood.

The hazardous potential of the GMMs created is considered to be no greater than that of the parental strain. In many cases genes are inactivated in the GMM that are required for optimal growth and pathogenesis.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
The disposal of bacterial cultures, culture supernatants, infected cell culture medium and buffers used to wash cells will be initiated by chemical inactivation of GMs by Virkon solution (to 1% final concentration), incubated for 30 minutes at room temperature, and disposed of via designated sinks. Data supplied by the manufacturer indicates that this will result in a validated inactivation of all organisms detailed in this proposal. Contaminated glassware (e.g. flasks) will be decontaminated by the addition of 1% Virkon solution and incubated for 30 minutes at room temperature. Solid waste, including plastics, tubes, pipettes, agar plates etc will be sterilised and disposed of via the university clinical waste service. All contaminated waste is disposed of into dedicated waste bins, which are sealed and subsequently sterilised by autoclaving at 134°C for 30 minutes. Autoclave function is assessed by a chart recorder, 3 monthly servicing and annual 12 point thermocouple validation testing.

Following the discussion of your proposal entitled "Bacterial genome plasticity", GMIC-1338 at the last GM safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

2.3.3.1.a: Specify what work is performed in the Microbiological Safety Cabinet and what is performed on the work bench, for more details of this, make reference to the Standard Operating Procedures.

2.3.3.3.a: Typo: 'liter'

2.3.3.9.a: Include when Safety glasses must be worn.

2.3.3.9.c: Include when Safety glasses must be worn.

2.3.5: To be completed by Occupational Health

3.5.1: Typo 'Elaini'

Comments:

SH queried about how many other Principle Investigators would be working in the same area.

EB confirmed this would be a group of less than 20 people. There are regular floor working progress meetings to discuss any issues.

SH suggested a Local Rules be produced stating the risks shared within the group.

EB also pointed out that there was a list of equipments in the code of practise and a further list of the organism for each of these pieces of equipments.

SH advised to state the volumes you use on the equipment for clarity.

Agreed: Class 2, containment Level 2. This project is notifiable to the HSE

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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Agree: Yes
Staphylococcus aureus is associated with a wide range of infections, many of which can become chronic or recurring. The ability of S. aureus to persist within host tissues in spite of host immune surveillance and antimicrobial therapy is believed to be due, at least in part, to a significant shift of the S. aureus phenotype to what is known as the small-colony variant (SCV). SCVs are able to persist within host cells for significantly greater periods of time than the parental strain and do not trigger an inflammatory response. However, the basis of this enhanced survival is not known. The use of genetic techniques such as transposon mutagenesis will allow us to rapidly screen for S. aureus genes that are important for the formation of SCVs and also intracellular survival. Genes identified by the screen will be further investigated using targeted gene knock-out and complementation studies. In addition to providing important insights into the molecular mechanisms of persistence it is anticipated that this approach will identify novel targets for therapeutic intervention and reveal genes that may form useful biomarkers for the development of persistent infections.

Overview: Initial experiments will involve the creation of a transposon library in S. aureus strain SH1000 using either a bacteriophage mediated system described by Wang et al., 2011 or a plasmid-borne transposon system (Bae et al., 2004). This will result in multiple isolates containing integrated transposon elements encoding erythromycin resistance. It is also likely that targeted mutations will be made to confirm gene function. Mutations giving rise to interesting phenotypes will be complemented by placing genes back on to the chromosome. This project does not involve genetic modification of other pathogens, yeast or mammalian cells.
Recipient or parental organism

E. coli DH5α will be used as a cloning host for shuttle plasmids. Other E.coli strains (K12, M15 or Rosetta) will be used for expression of recombinant proteins. Most genetic manipulations of S. aureus will initially be performed in strain RN4220 before subcloning/transduction into other strains. This is because RN4220 has a defective restriction system allowing the uptake of DNA from E. coli and other sources. SH1000 will be the principle strain modified and used in initial experiments. It is derived from 8325.4 and is methicillin sensitive (the first choice antibiotic for S. aureus infections). Its genome has been sequenced and it is amenable to genetic manipulation.

Detailed list:
- E. coli DH5 alpha, M15, Rosetta, K12
- S. aureus:
  - RN4220 - lab strain capable of accepting DNA from E. coli.
  - 8325.4 - lab strain defective in regulator rsbu. Cured of prophages
  - SH1000 - derived from 8325.4. mutation to rsbu fixed.
  - Col - Lab strain. Isolated from a hospital-acquired infection. (MRSA)
  - USA300 lac - recovered from a patient with community-acquired skin infection. (MRSA).
  - Newman - lab strain defective for fibronectin-binding protein adhesins.
  - MRSA252 - Lab strain. Isolated from a case of hospital-acquired infection (MRSA).
  - MW2 - Lab strain. Recovered from a patient with community-acquired skin infection. (MRSA).

Host/vector system

Initial experiments will involve the creation of a transposon library in S. aureus strain SH1000 using either a bacteriophage mediated system described by Wang et al., 2011 or a plasmid-borne transposon system (Bae et al., 2004). This will result in multiple isolates containing integrated transposon elements encoding erythromycin resistance. It is also likely that targeted mutations will be made to wild-type bacteria to confirm gene function (this will involve the insertion of tetracycline or erythromycin resistance cassettes into bacterial genomes). Mutations giving rise to relevant phenotypes will be complemented by placing genes back on to the chromosome using an integrative vector. Relevant phenotypes include those with the small colony variant phenotype and those with altered adhesion to, invasion of or persistence within cultured human cells.

Relevant mutations (i.e. those that lead to the SCV phenotype or alter intracellular persistence) will be transduced into other S. aureus strains including 8325.4 (lab strain defective in regulator rsbu. Cured of prophages), Col (Lab strain. Isolated from a hospital-acquired infection. (MRSA)), USA300 lac (recovered from a patient with community-acquired skin infection. (MRSA)), Newman (lab strain defective for fibronectin-binding protein adhesins), MRSA252 (Lab strain. Isolated from a case of hospital-acquired infection (MRSA)) and MW2 (Lab strain. Recovered from a patient with community-acquired skin infection. (MRSA)).

S. aureus mutants will be screened for their ability to persist within cultured cell lines. These will not be genetically modified.

Detailed list of vectors to be used:
- pTM401-403
- pTM378
- pBursa and pFA545
- pTS2
- PII39

Controlled expression of S. aureus genes will employ plasmid pRMC2 (Corrigan et al), which confers chloramphenicol and ampicillin resistance.

We will also use standard E. coli vectors:
For cloning:
- pUC18/19-pUCBM20 and derivatives: CoIE1 replicon, AmpR (Boehringer).
- pBR322 and derivatives: AmpR. TetR.

For gene expression:
- pET22b/28c/28a and derivatives: CoIE1 replicon, AmpR (22) and KanR (28). tag Histidine N or C-terminal.
- pQE30Xa and derivatives: Tag histidine (Qiagen). AmpR.
- pEGFP and derivatives: engineering of gfp fusion. AmpR.
- pGEX and derivatives: Tag GST (Glutathion S-transferase). AmpR.

Origin & function

Phage-mediated transposon mutagenesis (doi: 10.1038/nchembio Nature Chemical Biology):
This protocol employs plasmids containing a mariner-based mini transposon system that include promoters with either high or low activity. This system results in gene expression that may be up or down regulated or completely abrogated. The strains employed include: Donor strain RN4220 ΔattBφ11::Orf5 pTM304 TetR, which allows replication of plasmids pTM401-403 (below) but represses phage 11 replication. Plasmids pTM401-403 are maintained in RN4220 ΔattBφ11::Orf5 pTM304 TetR and transduced into either RN4220 or Col containing plasmid pTM378, which encodes the transposase.

The plasmids required are as follows: pTM401-pTM403, which encode the mini-transposon cassette and promoters for capsule, penicillinase and tu respectively. This provides differing promoter strengths at different growth phases. Plasmid pTM378 encodes the transposase and can replicate in RN4220 ΔattBφ11::Orf5 pTM304 TetR but not the recipient strains. This protocol employs staphylococcal phage phi11, which is used to transduce the plasmids from donor to recipient and will also be used to transduce mutated DNA from mutant strains into other strains.

Plasmid-mediated transposon mutagenesis (Bae et al., 2004 doi: 10.1073/pnas.0404728101): This protocol involves 2 plasmids (pBursa and pFA545), which are sequentially transformed into the host strain. Temperature sensitive replication and the acquisition of erythromycin resistance are used to select for transposon mutants. This protocol results in a single integrated transposon, which may or may not disrupt gene expression. Where necessary targeted knockout mutants will be produced using the temperature-sensitive plasmid pTS2, which encodes chloramphenicol resistance (McAleese et al., 2003 doi: 10.1099/mic.0.25842-0).

Mutations will be complemented using a vector that integrates onto the S. aureus chromosome, PII39 that encodes spectinomycin and tetracycline resistance (Luong and Lee, 2007 doi: 10.1016/j.mimet.2007.04.007). Controlled expression of S. aureus genes will employ plasmid pRMC2 (Corrigan et al), which confers chloramphenicol and ampicillin resistance.

We will also use standard E. coli vectors:

For cloning:
- pUC18/19-pUCBM20 and derivatives: CoIE1 replicon, AmpR (Boehringer).
- pBR322 and derivatives: AmpR. TetR.

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- pET22b/28c/28a and derivatives: CoIE1 replicon, AmpR (22) and KanR (28). tag Histidine N or C-terminal.
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- pEGFP and derivatives: engineering of gfp fusion. AmpR.
- pGEX and derivatives: Tag GST (Glutathion S-transferase). AmpR.
Evaluation of foreseeable effects

Whilst all S. aureus strains should be considered infectious, SH1000 is a lab strain and is thus unlikely to be significantly more virulent than clinical strains. However, it is likely that mutations with relevant phenotypes will be transduced into other strains including: 8325-4; Newman; USA300 lac; MW2; MRSA252 and Col to assess the importance of different genetic backgrounds. It is impossible to predict the relative virulence of every transposon mutant since it is theoretically possible that disruption of e.g. a regulator gene might up-regulate expression of certain virulence factors. However, it is anticipated that the vast majority of, if not all, mutations will result in reduced or unaltered virulence. It is relevant to note that previous studies on SCVs in animal models report reduced pathogenesis compared to wild-type (although the organism is able to persist within the host). The intended work will identify mutants with enhanced ability to persist within host cells. Although this may contribute to the persistence of infection, it is associated with reduced damage to host tissues and the down-regulation of cytolytic toxin expression. As such, the screening protocols employed are unlikely to select for mutants with enhanced ability to initiate infection or cause a more severe form of infection.

8.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The disposal of bacterial cultures, culture supernatants, infected cell culture medium and buffers used to wash cells will be disposed of into Virkon solution (to 1% final concentration), incubated for 30 minutes at room temperature and disposed of via designated sinks. Validated data supplied by the manufacturer indicates that this will result in inactivation of all organisms detailed in this proposal. Contaminated glasware (e.g. flasks) will be decontaminated by the addition of 1% Virkon solution and incubated for 30 minutes at room temperature. Solid waste, including plastics, tubes, pipettes, agar plates etc will be sterilised and disposed of via the university clinical waste service. All contaminated waste is disposed of into dedicated waste bins, which are sealed and subsequently sterilised by autoclaving at 134°C for 30 minutes. Autoclave function is assessed by a chart recorder, 3 monthly servicing and annual validation of a typical waste load using 12 point thermocouple testing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Following the discussion of your proposal entitled "Molecular Mechanisms of Staphylococcus Persistence", GMIC - 1387 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

Part 1: GM Centre number: GM8
1.21: Typo: ‘complimented’ change to ‘complemented’ in first and last paragraph.
1.24: Typo: ‘enhanced’

2.3.3.1.a: Specify what work is performed in the Microbiological Safety Cabinet and what is performed on the work bench, for more details of this, make reference to the Standard Operating Procedures.

2.3.4.1: Typo: ‘Quarterly’
2.3.5: To be completed by Occupational Health

Comments:
The Committee suggested adding any further strains that may be possibly used in the research in the future now.
SH queried where the work would be taking place.
AE confirmed this would mostly be bench work and some Microbiological Safety Cabinet work.

RW asked how the agar plates would be sealed?
AE informed the Committee this would be done with a non porous adhesive tape.
IH questioned which parts of this would be done in the MSC and if this could be specified in the form.
IH also queried the volumes used in the centrifuges and how would you know if there was a spill.
RW suggested using lower volumes in the bucket than it can actually handle.
IH recommended adding the centrifuge inspection in the SOP.
RW also recommended using the click-shut eppendorfs for an extra seal.
HK questioned if there would be out of office hours.
AE explained some work may be out of hours.
SH confirmed this would be included in the Local Rules and SOP.
SH then asked about the training methods.
AE pointed out that this was done in conjunction with the Safety managers.
EB also confirmed the training would be signed off by the trainee and trainer.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

### Project Containment

<table>
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<tr>
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<td>L4</td>
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<td>Animal Units</td>
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### Project Ref 8/12.1

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Professor H's group have recently developed a powerful reporter system based on fluorescent dilution that enables direct quantification of the replication dynamics of Salmonella in murine macrophages at both the population and single cell level. Its use has provided the first direct evidence of viable non-replicating Salmonella cells in an infected host. These could represent an important reservoir of persistent bacteria. We now wish to extend the system to other pathogens to study replication dynamics in vivo, and to identify and characterize non-replicating bacteria associated with chronic infections. The group of Norrby-Teglund and others have discovered that Streptococcus pyogenes can survive and replicate in human epithelial cells and macrophages.

In this project we will introduce reporter constructs into wild-type and mutant strains of Streptococcus pyogenes and examine the dynamics of replication in a variety of human cells, including macrophages. Mutant strains will be constructed and analysed to provide insights into mechanisms of survival and growth in host cells. As a result, we expect to gain novel insights into multiplication rates within human cells and to learn more about the nature of persistence in this disease.

Site-directed and transposon mutagenesis will be carried out on recipient strains described below. These vectors will be introduced in S.pyogenes to obtain deletion mutants in conjunction with plasmid based complementation. Various major putative virulence factors will be deleted to investigate the mechanisms that confer intracellular survival in host cells. Any subsequent changes in bacterial fitness will be analysed by confocal microscopy and flow cytometry. Dual fluorescence reporter plasmids will be constructed from pre-existing plasmids obtained from various collaborators. These plasmids will act as a template for our constructs which will contain variations of green and red fluorescent proteins under inducible and/or constitutive promoters. This system will be used for investigating intracellular bacterial replication using fluorescence dilution (Helaine et al, 2010).

Recipient or parental organism

Echerichia coli: Wild type K12 strains and derivatives thereof with required phenotypes for cloning and expressing streptococcal genes or genes of other bacterial origin will be used in these studies. Strains commonly used in the laboratory include DH5alpha, TOP10. These strains have a widespread and long history of safe use.

Streptococcus pyogenes: E.coli shuttle vectors will be introduced into M1T1 5448 strain and M89 H293 strain to generate gene knockout mutants.

The aim of our genetic manipulations will be to knockout major or putative virulence genes and construct fluorescent strains for in vitro visualisation. Therefore, all genetically modified organisms constructed will be no more virulent than the wild type S.pyogenes 5448 or H293 strains to generate gene knockout mutants. The aim of our genetic manipulations will be to knockout major or putative virulence genes and construct fluorescent strains for in vitro visualisation. Therefore, all
Host/vector system


Origin & function

The vector systems mentioned above will be employed to do the following:

1. Suicide vectors for Site directed mutagenesis (pucMUT, pACH74 and pAS1TAG)
2. Transposon mutagenesis (pR412, pJRS233 and pJDM-STM)
3. Cloning of PCR products (pCEM, pCR2, 1TOPO, pLS1ROM, pOri23, pBluescript, pGEM, pPP1/2, pACYC184, pVA838)
4. GFP expressing integrative plasmids (pBaSysBioll, pBSU101, pGFP, YFP, pCFP, pLS1GFP, pMV158GFP, pLS70GFPcat, pMV158GFP)
5. pLS578, pMSP3535, PcEM, PcTet, Janus are used for tagging or replacement of S. pyogenes genes with resistance genes against tetracycline, erythromycin and Kanamycin.
6. pET is an expression vector for protein overproduction
7. pHY304 is temperature-sensitive plasmid for insertional mutagenesis.
8. pSIV and is an inducible shuttle vector system.

All the listed genetic material to carry out cloning and mutagenesis are commercially available. However, for the purpose of GFP expression in the recipient organisms these plasmids will be obtained from various collaborators.

Evaluation of foreseeable effects

The foreseeable effects include the introduction of antibiotic resistance genes which could represent a hazard if released into the environment and transferred to other organisms. All GMOs and GM material is decontaminated and sterilized before disposal and operational protocols within the lab make release unlikely. Therefore transfer of genetic material to other environmental microorganisms remains extremely unlikely.

Human health:

The introduction of additional antibiotic resistance genes into the M1T1 Streptococcus strain poses a greater risk to human health in the instance of infection. However, the organism will remain susceptible to β-lactam antibiotics.

Hazardous GMMs:

One of the major goals of the project is to render the parental strains less invasive and virulent. Thus, it is expected that the GMMs created will not show any increase in virulence or fitness.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: 1% virkon solution for liquid cultures and supernatants for a minimum of 1hr at RT or a final concentration of 1% Biocleanse for 12hr for tissue culture waste. Solid waste and contaminated plasticware sterilised by autolaving. Discard cycle 134 degrees C for 3 min. 100% kill. Machine and cycle checked after each cycle by thermograph; autoclaves are maintained and validated for make safe cycles using a multiple thermocouple test annually by a qualified engineer

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The following amendments are required prior to approval:
1.31: Answer should be 'no'
1.32: Answer should be 'no'
2.2.1.1: Where reference is made to use of a Microbiological Safety Cabinet, the text should refer the reader to the answer in 2.3.3.1.
2.3.2.2a, b and c: Begin with capital "Y".
2.3.3.3.c: Include the buckets are the biocontainment type and have clear lids.
2.3.5: To be completed by Occupational Health
2.3.6: Hatch the containment level 2 and classification 2 box.
3.2.a: Change 'should' to 'will' in answer.
3.2.b: Remove third paragraph.
3.31.a: Provide a justification for the answer.
3.31.d: Provide an answer.

Project Containment

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02/03/2022
Use of new fluorophores to study bacterial infection and heme acquisition in deep tissue

1. Pathogenic bacteria:
Plasmids and transposon systems to implement genetic modifications in pathogenic strains will be propagated in E. coli DH5alpha or JM109 cells. These vectors will then be introduced into the above mentioned pathogens to confer deletions, promoter-reporter fusions, or transposon-generated libraries for various experiments. Libraries will be screened using Fluorescence-Activated Cell Sorting (FCS) to search for iron/heme-specific acquisition determinants. These same strains, and possibly other more virulent versions (stated within this report) will subsequently be used to infect mice and imaged using fluorescent topographic imaging. In the specific case of S. aureus, we will use both plasmid- and bacteriophage-based systems to implement transposon mutagenesis.

2. E. coli:
E. coli GMM strains will also be used to express large amounts of recombinant protein for structural biology and biochemical experiments. BL21 strain derivatives are used for this and expression plasmids confer ampicillin or kanamycin resistance.

3. Yeast:
We will also be using Saccharomyces cerevisiae for yeast-2-hybrid to observe protein-protein interactions (in vivo) of signal transduction components. Vectors to be used are pGBT9 and pGAD425, both of which have ampicillin resistance for E. Coli. Foreign DNA will be introduced into pathogenic bacterial strains. These strains will be cultured and measured for fluorescence intensity. We will also infect animals and monitor fluorescence expression during the infective process with whole mount imaging.

Recipient or parental organism

1. E. coli DH5alpha, JM109, BL21 (class 1)
2. Saccharomyces Cerevisiae (class 1)
3. S pneumoniae R6, TIGR4, D39 (class 2)
4. P aeruginosa PAO1, MPAO1, IA614 (class 2)
5. S. aureus: 8325.4, SH1000, Col, MW2, MRSA252, Newman, USA300 lac, RN4220 (class 2)
6. S. gordonii DL-1 (class 2)
7. S. pyogenes (A40) (class 2)
8. S. agalactiae NEM316, COH1,515, 2603V/R (class 2)
9. M. bovis BCG (class 2)
10. M. smegmatis (class 2)

My current strain inventory only includes numbers 1-4, and number 5- USA300 strain of S. aureus (to be shipped from US)

**Host/vector system**

1. For E. coli pET21b (Novagen Inc), pBAD (Invitorgen Inc.) and pE-SUMO (Lifesensors Inc., http://www.lifesensors.com/) expression systems for protein overexpression, and pUC57 for general propagation of DNA.


3. For P. aeruginosa: a TN7 mini-Tn integration vector (Choi and Schweizer, Nat Protoc. 2006.1: 153-161) to generate deletion mutants or specific chromosome integration, replicating vectors pJN015 and pSB109 for replicating extrachromosomal expression.

4. For S. aureus: phage-assisted mariner transposon system based on doi: 10, 1038/nchembio Nature Chemical Biology). The system uses plasmids pTM401-403 in conjunction with strain RN4220 for maintenance and plasmid pTM304 is also propagated in this strain to introduce the transposase. For S. aureus plasmid-mediated mutagenesis we will use a protocol as per Bae et al., 2004 doi: 10,1073/pnas. 0404728101 that involves plasmids pBursa and pFA545. These plasmids are both transformed to allow a single chromosomal deletion/integration.

Also in S. aureus and where necessary targeted knockout mutants will be produced using the temperature-sensitive plasmid pTS2 (McAleese et al., 2003 doe: 10.1099/mic 0.25842-0). Mutations will be complemented using a vector that integrates onto the S. aureus chromosome, PIIS9 (Luong and Lee, 2007 doi: 10.1016/j.mimet.2007.04.007).

5. For yeast vectors will be transformed by standard chemically competent means.

**Origin & function**

Specific genes to be modified are as follows:

1. For S. pneumoniae: (1) RitR, a transcription factor responsible for iron and oxidative stress regulation (2) Stk, a serine-threonine kinase responsible for cell wall integrity and antibiotic resistance.

2. For P. aeruginosa: (1) HasR, HxuC, PhuR, HO (heme oxygenease) - iron regulatory proteins.

3. For S. aureus: (1) Stk, a serine-threonine kinase responsible for cell wall integrity.

In the case of transposon mutagenesis, this is a relatively ransom procedure intended to create a deletion 'library' that should theoretically cover all genes within the target organism.

4. All systems utilize antibiotic resistance markers for selection. E. coli DH5alpha and JM109 non-infectious strains will be used to propagate DNA for eventual introduction into the above mentions parental species to create GMOs for research purposes.
Evaluation of foreseeable effects

Forseeable effects:

1. Hazards to human health and environment:

General hazards to human health include the propagation of the above mentioned infectious vectors that create an environment to become exposed to levels that might initiate disease.

Hazards to the environment mainly concern the antibiotic resistance determinants in our bacterial strains, and the potential for these genes to enter the environment (e.g. by improper decontamination).

Transfer of genetic information in the form of genes that code for antibiotic resistance determinants is the major concern. The project will use many different strains, and several (previously stated) antibiotic resistance genes as markers of genetic manipulation.

2. Likelihood and consequences of modifications being transferred to other microorganisms:

It is highly unlikely, given the fact that after we propagate samples (cultures) and use them, they are killed and properly disposed of (i.e. autoclaved). In addition, and detailed below, several precautions and cleaning measures are taken to ensure these organisms will not reach area beyond their zones designated for their use.

3. Most hazardous GMMs to be created:

The majority of GMMs will render the strains less efficient at causing disease. It is not anticipated that any GMMs will show increased virulence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste:

1% final concentration virkon solution for a minimum of 1hr at RT for bacterial cultures or 1% Biocleanse for 12 hrs for tissue culture waste. These can then be dispensed down the laboratory sink. Bacterial cultures .5ml are autoclaved prior to disposal. Discard cycle 134 degrees C for 3 min. Machine cycle recorded after each cycle by thermograph; autoclaves thermocoupled validation tested regularly (annually) by a qualified engineer.

Solid Waste:

Bacterial cultures on agar plates and contaminated plasticware are autoclaved prior to disposal. Discard cycle 134 degrees C for 3 min. Machine cycle recorded after each cycle by thermograph; autoclaves thermocouple validation tested regularly (annually) by a qualified engineer.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

Comments: HC gave a brief description of the work.
The following amendments are required prior to approval:
Please complete the Biopersonnel registration form.
1.1: Add all the strains in this section.
2.13: Un-hatch this box.
2.18: State the Class 2 GMO's are the most hazardous, but none are more hazardous than the wild type.
4.2: Add, 'refer to Code of Practise' or other documents.
4.15: Change the order in which this is presented, begin with ‘Mode of Transmission’
4.18: Describe the biological reasons why these vectors cannot be transferred to other organisms in the environment. e.g. Non-mobilisable or, mobilisation defective etc.
5.9: Justify use of the sharps when making blood agar and why a similar gauge blunt needle cannot be used.
5.10: Give explanation for the answer 'no' to shredding of biological agents.
5.12: Answer should be 'no'.
6.5: Make answer clear by stating this depends on the risk assessment associated with which work.
12: To be completed by Occupational Health.
Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

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### Project Ref 8/12.5

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<th>CultureVolumeClass3-4</th>
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<td>21/05/2012</td>
<td>Development of assays to determine neutralizing and binding antibodies in human samples, for application into HIV vaccine trial work</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<tr>
<td>02/03/2022</td>
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This project involves the use of genetically modified Sendai Virus and HIV Pseudoparticles. The use of HIV pseudoparticles has been assessed by the GM8 GM safety committee as falling within the definition of a Class 1 GMO. This application refers only to the use of GM Sendai virus which was assessed as falling within the definition of a Class 2 GMO due to the potential for environmental harm. The descriptions relating to the use of Pseudoparticles are included for information only.

The Human Immunology Laboratory (HIL) based at the Chelsea and Westminster hospital, will conduct and support pre-clinical and clinical studies to assess the safety, immunogenicity and potential efficacy of HIV vaccine candidates. The aim of this project is to measure specific antibody responses against the candidate vaccine vectors and their inserts pre- and post-vaccination. We are particularly interested in the neutralizing and binding antibody responses in samples collected in clinical trials.

The neutralization potential of clinical samples against candidate vaccine vectors such as Sendai Virus (SeV) or against HIV proteins (e.g. envelope, Gag) will be determined in in-vitro cell based assays using immortalized cell lines or PBMCs isolated from human volunteers. Typically, a permissive cell line is infected in a microtiter plate with a low multiplicity of infection of the candidate viral vector or cell culture supernatant of non-replicating HIV pseudoparticles which have been modified to carry a reporter gene (fluorescent proteins, Luciferase or an enzyme). After 1 to 5 days the amount of infected cells is measured using the reporter gene and when applicable after addition of substrate. Addition of the serially diluted volunteer's samples to the virus prior to the in-vitro infection will allow measurement of the presence or absence of specific neutralizing antibodies in the volunteer's samples. Neutralization of viral infection as measured by the decrease of reporter gene activity, will be interpreted as the presence of neutralizing antibodies in the samples. A neutralizing antibody titre will be measured as a function of the sample's dilution.

The presence of binding antibodies in samples will be determined by an Enzyme linked ImmunoSorbent Assay (ELISA). Typically, a preparation of detergent solubilised vaccine vectors, or preparations of purified proteins (or fragments of) from the vectors or inserts (i.e. HIV envelop or gag proteins) is used to coat microtiter plates. After incubation of the coated plates with diluted samples, presence of specific binding antibodies is measured by colorimetry or fluorescence, after the addition of a labelled secondary anti-human antibody (labels include, but are not limited to, Horse radish peroxidase, Alkaline phosphatase or a fluorescent probe).

Recipient or parental organism

All modifications described will be made to an existing Sendai virus (SeV) viral isolate (SeV stain Z). The infectivity of the Z stain is 10,000 times reduced in mice compared to circulating SeV strain. The Z strain described here will not be generated in our laboratory but by collaborators and shipped to the IAVI Core Laboratory for use. No additional modifications will be made in our laboratory.

A replication competent Sendai virus (SeV) (Z strain) expressing a reporter gene (Fluorescence, Luminescence or an enzyme) will be used for the SeV neutralization assay. The reporter gene is located upstream of all SeV genes, and none of the SeV genes have been altered.

For the binding ELISA we will use unaltered SeV as well as SeV expressing HIV-1 proteins or partial protein sequences to coat the ELISA plates. This virus will be identical to the virus used for the immunization of trial participants, or viruses that have been extensively used in the literature, to allow comparisons with other published data. Before coating the ELISA plates the virus will be disrupted with a buffer containing a detergent such as Triton X-100. In addition SeV will be used unmodified as well as SeV expressing HIV-1 proteins or partial protein sequences. SeV will not contain full length HIV at any time. Before use the SeV will be destroyed with a buffer containing a detergent such as Triton X-100.

The pseudoparticles will be generated in our lab, but this work has been classified as containment level 1 by the Imperial College Safety committee and therefore is discussed below for information only.
To produce HIV pseudoparticles two different plasmids will be used: a plasmid expressing the viral envelope sequence of interest and the pNL4.3 Luc+ENV- plasmid which is based on the HIV NL4.3 proviral clone. In this plasmid the luciferase gene is introduced in the nef gene, making it inactive. In addition a deletion at the 5’ end of the envelope gene results in a frameshift which introduces a stop codon thereby preventing the expression of the HIV envelope protein. Transfection of pNL4.3 Luc+ENV-alone can not produce pseudoparticles, because it does not express the HIV envelope protein. To produce pseudoparticles the plasmid is co-transfected with an plasmid expressing the envelope of choice. By cotransfecting both plasmids the transfected cell will produce the pseudoparticles which are secreted into the culture supernatant. The produced particles express the envelope of choice on the outside of the particle and it contains the pNL4.3 Luc+ENV-genome. Because the produced pseudoparticles do not contain a complete genome (they contain the pNL4.3 Luc+ENV-), no new viral particles can be formed after infection of target cells.

For the production of pseudoparticles we will use plasmids expressing HIV envelopes from different clades as well as SIV, human T-lymphotropic virus (HTLV) and Vesicular stomatitis virus (VSV) as control envelopes. We will not use any replication competent constructs of these additional viruses, we will only use their envelope sequences, which are expressed in a plasmid, for the production of pseudoparticles. In addition we will not be inserting these envelope sequences into the HIV genome or the NL4.3 Luc+ENV-plasmid.

To produce HIV pseudoparticles two different plasmids will be used: a plasmid expressing the viral envelope sequence of interest and the pNL4.3 Luc+ENV- plasmid which is based on the HIV NL4.3 proviral clone. In this plasmid the luciferase gene is introduced in the nef gene, making it inactive. In addition a deletion at the 5’ end of the envelope gene results in a frameshift which introduces a stop codon thereby preventing the expression of the HIV envelope protein. Transfection of pNL4.3 Luc+ENV-alone can not produce pseudoparticles, because it does not express the HIV envelope protein. To produce pseudoparticles the plasmid is co-transfected with an plasmid expressing the envelope of choice. By cotransfecting both plasmids the transfected cell will produce the pseudoparticles which are secreted into the culture supernatant. The produced particles express the envelope of choice on the outside of the particle and it contains the pNL4.3 Luc+ENV-genome. Because the produced pseudoparticles do not contain a complete genome (they contain the pNL4.3 Luc+ENV-), no new viral particles can be formed after infection of target cells.

For the production of pseudoparticles we will use plasmids expressing HIV envelopes from different clades as well as SIV, human T-lymphotropic virus (HTLV) and Vesicular stomatitis virus (VSV) as control envelopes. We will not use any replication competent constructs of these additional viruses, we will only use their envelope sequences, which are expressed in a plasmid, for the production of pseudoparticles. In addition we will not be inserting these envelope sequences into the HIV genome or the NL4.3 Luc+ENV-plasmid.

SeV stocks will be generated by our collaborators and sent to our laboratory to be implemented in the antibody assays.

For the ELISA, SeV is the vector system that expresses a variety of HIV-1 proteins or partial proteins. This vector will be used as a source of antigen in the assays, rather than as an expression system

HIV Pseudo particles - For information.

For the production of pseudoparticles we will use plasmids expressing HIV envelopes from different clades as well as SIV, human T-lymphotropic virus (HTLV) and Vesicular stomatitis virus (VSV) as control envelopes. We will not use any replication competent constructs of these additional viruses, we will only use their envelope sequences, which are expressed in a plasmid, for the production of pseudoparticles. In addition we will not be inserting these envelope sequences into the HIV genome or the NL4.3 Luc+ENV-plasmid.

pNL4.3 Luc+ENV- and the different viral envelope expression plasmids will be used to create the pseudoparticles. Co-transfection of both plasmids will result in the formation of non replicating pseudoparticles.

Origin & function

A replication competent Sendai virus (SeV) expressing a reporter gene encoding a fluorescent protein or the luciferase protein will be used to determine SeV antibody neutralization. The fluorescent gene originates from the jellyfish Aequorea victoria, whereas the luciferase gene is either derived from the firefly (Photinus pyralis) or the sea pansy (Renilla reniformis). The reporter gene is located upstream of all SeV genes, and none of the SeV genes have been altered. The reporter gene will be used to quantify infection.

SeV(NP) or SeV expressing HIV-1 proteins or partial protein will be used to measure binding antibodies. The HIV proteins will be derived from different circulating HIV strains. The SeV will never contain the full length HIV-1 sequence or express all HIV-1 proteins in one construct. Before use, SeV will be lysed with a buffer containing a detergent such as Triton X-100.

The use of lysed SeV will enable us to determine specific antibody responses to the proteins present in the vaccine vector. No constructs or viral stocks will be produced in the laboratory, the viral stocks will be produced by collaborators and shipped to our laboratory in accordance with shipment regulations.

The pseudoparticle system has been classified a containment level 1 by the Imperial College safety Committee. These details are included for completeness.

pNL4.3 Luc+ENV -

Nef: luciferase gene is introduced in the nef gene, preventing its expression. Nef is a viral protein that interacts with host cell signal transduction proteins to provide for long survival of infected T cells and for destruction of non-infected T cells (by inducing apoptosis).

Env: The Envelope glycoprotein (Env) is the sole viral protein present on the surface of Human Immunodeficiency Virus-1 (HIV-1) virions. The Env mediates viral entry.

Evaluation of foreseeable effects

The Sendai virus used is a replication competent virus. The insert does not alter the function of the SeV genes. The strain used for the neutralization assay is the SeV Z strain the SeV used for the ELISA is an isolate derived from SeV strain Z. The Z strain is 10e4 less infectious in mice that circulating SeV stains. The loss in infection is cause by prolonged culture of the viral isolate. (K. Kiyotani et al. Arch Virol. 2001)
SeV is a non human pathogen and does not induce disease in humans. SeV is responsible for respiratory tract infection in mice, hamsters, guinea pigs and rats, with infection passing through both air and direct contact routes (Baker, DG. 1998. Clinical Microbiology Reviews. 11:231-266).

The modifications made to the SeV do not impair or alter any of the SeV gene functions and do not increase pathogenicity.

SeV is a rodent pathogen that has never been reported to cause human disease. Symptoms in rodents vary from none at all and no obvious symptoms but lung lesions on post mortem, mild snuffles through to severe pneumonia with breathing difficulties, staring coat, lack of appetite, weight loss, decreased litter size and growth retardation in kittens.

SeV is transmitted by direct contact or by inhalation of aerosols. The virus is easily killed by drying or disinfectants/detergents.

To prevent exposure to the environment all procedures will be performed in a containment level 2 laboratory, equipped with class 2 biosafety cabinet. Sealable flasks will be used for culturing to prevent spillage; in the incubator the flasks will be stored on static shelves and on a plastic tray. Centrifuges are fitted with sealable buckets which will be opened and closed in the safety cabinet. Only trained personnel will work with the above described pathogen and all laboratories are secured by badge readers, accessible by authorized personnel only.

The pseudoparticles are non replicating particles. They are capable of cell entry, however because the pseudoparticle only contains the pNL4.3 Luc+ENV- genome, no infectious particle can be formed in the infected cells. It will produce some HIV proteins (the ones present in the pNL4.3 Luc+ENV-) in the infected cell but because no HIV envelope can be produced (due to the above described deletion) it can not form a whole virion particle. So no viral dissemination will take place.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be decontaminated with 1% Virkon (final concentration) within the microbiological safety cabinet for a minimum of 20 minutes prior to disposal through the laboratory waste sink. The treatment with Virkon completely disrupts the virus thereby eliminating virus activity.

Solid waste will be decontaminated by immersion in 2% virkon solution for at least 20 minutes. The solid waste will then be autoclaved under vacuum air extraction with rapid steam heating to 121°C for 25 minutes. Followed by disposal as clinical waste.

The autoclaves are fitted with a chart recorder to monitor the autoclave cycle and undergo 6 monthly service and an annual 12 points thermocouple test with a mock load.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Comments:
MN gave a brief description of the work.
RW checked if they would be checking the antibody response in humans. MN confirmed this; they would be blocking and binding the antibody to check the response in humans.
SH queried if they would be developing a vaccination to then test. PB explained they would be using the sendai vector, but were unsure if the vaccination would work.
SH then asked if this would involve clinical trials. MN informed the committee that there would not be clinical trial involved in this work.
RW questioned if the sendai virus could replicate in humans. MN stated that it was a non-human pathogen and could not create a disease in humans. Mice population can get infected by the virus, but not humans.
IH queried the impact this would have on the environment, since GFP was being added to the SeV, it would be considered as a pathogen with wild mice and thus under the contained use regs a hazard to the environment. MN informed the group that the minimum Containment Level of the Laboratory was 2.
SH questioned if the pathogen was Class 2, the Containment Level became 2? IH advised if it was a risk to the environment, then yes. BR pointed out to prevent the route of escape, raises the containment level requirement and thus the class.
IH confirmed that the GM Sendai virus was class 2 on the grounds that it presented a risk to the environment.
PB also added that the trial work would be done by International Aids Vaccine Initiative (IAVI).
SH asked if there were any sharps involved in the work; that would lead to a risk of infection. MN explained that the risk of infection was minor. If so, the infected cell would not replicate. A HIV test would come back positive, but further test would confirm a negative result. PB included that all the personnel involved in the work were already working with HIV.
IH asked how the HIV Psuedoparticles were attenuated. MN stated that the HIV pseudoparticles were non replicating and lacked the mechanism for packaging. IH asked if this was a robust attenuation. MN stated that two separate plasmids were required to produce the pseudoparticles, the Nef gene was inactivated and a frame shift induced stop codon prevented expression of the HIV envelope protein. The genome is incomplete and the produced particles are not capable of replication. This is reflected in the risk assessment Activity

Project Containment

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Project Ref 8/12.6

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<th>Date Ackn'd</th>
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<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>24/07/2012</td>
<td>Microglia in Neurodegenerative diseases (Imaging microglia activation in neurodegenerative diseases including full functional assessment of TSPO translator protein on microglia)</td>
<td>Class 2</td>
<td>(&lt; 1 ) Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>

02/03/2022
Purposes of the contained use

Our project is part of a joined European effort (Imaging Neuroinflammation in Neurodegenerative Diseases; INMiND) to better understand neuroinflammation, in particular microglial activation in Neurodegenerative disorders. The project will entail a full functional assessment of TSPO translator protein on microglia. To that purpose we aim to use immortalised murine and human microglial cell lines and astrocytoma cell lines. In addition snap frozen human brain tissue will be used for standard immunohistochemistry techniques.

The work will comprise of placing in culture immortalised microglial cell lines (transformed by recombinant retrovirus) and astrocytoma cell lines, remove the TSPO translocator receptor or by knock-out (siRNA Dharmacon RNAi technology -siGENOME siRNA reagents from Thermo Fisher) or by blocking or activate with agonists. Function of microglia after using various stimuli will be assessed. To that end, RNA, protein and media will be extracted and frozen for relevant molecular and proteomic analysis. Following culture experiments, relevant microglial activation pathway associated with TSPO will be validated on snap frozen brain sections prepared on a cryostat using standard immunohistochemistry techniques.

The J2 retro virus is a recombinant retro virus expressing the oncogenes v-myc and v-raf used to transform the BV2 cell line. Although the cel line is bought in from an external agency, no conclusive evidence can be brought forward to demonstrate that the BV2 cell line is virus free and remains an ectotropic virus, therefore BV-2 cell line has been risk assessed to be a class 2 GMO.

Recipient or parental organism

The BV-2 cloned cells will be purchased. These cells express the nuclear v-myc and the cytoplasmic v-raf oncogene products as well as the env gp70 antigen at the surface level. The BV-2 cell line produces an enveloped recombinant ectropic J2 retrovirus; such virus is known for its in vitro transforming ability and in vivo tumorigenic potential [J Neuroimmunol. 1990 May;27(2-3):229-37]. Similarly the human HMO6 and CHME3 microglial cell lines were infected with the retroviral vector encoding v-myc oncogene (transcribed from mouse leukemia virus LTR plus neomycin-resistant gene transcribed from a SV40 early promotor) inducing propagation of immortalised human microglial cell lines

BV2; mouse (C57BL/6) microglial cells transformed by recombinant retrovirus (v-raf/v-mic); will be purchased at IRCSS
- N9; murine microglia; will be gift from Dr David Dexter, Imperial
- HMO6; human microglia; will be gift from Seung U Kim, Vancouver, Canada
- CHME3; human microglia; will be gift from Professor M Tardieu, Paris, France
- EOC2, EOC13.31, EOC20; murine microglia will be purchased at ATCC
siRNA technology will be used (Dharmacon RNAi technology) - a non viral gene silencing method (for example siGENOME siRNA Reagents from Thermo Fisher). This part of the work does not in our opinion fall under the contained use regs.

However the following Host / vector systems do:
BV2; mouse (C57BL/6) microglial cells transformed by J2 recombinant retrovirus (v-raf/v-mic) purchased at IRCSS Instituto Di Ricovero e Cura a Carattere Scientifico (Italian Research Hospital), Genova, Italy.
Human HMO6 and CHME3 microglial cell lines infected with retroviral vector encoding v-myc oncogene (transcribed from mouse leukemia virus LTR plus neomycin-resistant gene transcribed from a SV40 early promotor) inducing propagation of immortalised human microglial cell lines.

Origin & function

A murine cell line (BV-2) has been generated by infecting primary microglial cell cultures with a v-raf/v-myc oncogene carrying retrovirus. The v-raf-leukemia viral oncogene 1 (RAF1) and v-myc are genes known to play a role in cell cycle progression, apoptosis and cellular transformation.
HMO6 and CHME3 microglial cell lines were infected with the retroviral vector encoding v-myc oncogene (transcribed from mouse leukemia virus LTR plus neomycin-resistant gene transcribed from a SV40 early promotor) inducing propagation of immortalised human microglial cell lines.

Evaluation of foreseeable effects

The cell lines have been transformed with retrovirus in order to produce "immortalised" cell lines.

Knockdown of the TSPO gene will be performed using non viral gene silencing methodologies, which do not fall under the contained use regs.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All handling of cells and media potentially contaminated with the transformed cell lines will be conducted at containment level 2. Activities involving opening of the culture vessels, transferring of cells or media and similar activities where a splash or spill is possible will be conducted in a Class II safety cabinet.

All media and material in contact with cells will be decontaminated before being disposed in designated sink. Liquid will be decontaminated with virkon at a final concentration of 1% for at least 30 minutes and solid waste by autoclaving at 121 for 15 minutes in an autoclave validated for the purpose.

The cells will be double contained for storage and placed in LN2 tank in laboratory accessible only with swipe card.

The lab is shared with other users. Safety cabinet will be decontaminated with 1% Virkon and 70% Ethanol plus with one hour UV lights before other use can use the safety cabinet.

Cell vials will be stored in double containment (cryovial and storage box) in a protected area secure by swipe card entry.

A Microcentrifuge with sealed buckets will be used to contain potential aeroso.

Any spillage will be absorbed and the area treated with 1% Virkon (min contact 15 min). Absorbent material will be disposed of as solid waste via the validated autoclave.

If in direct contact on skin wash abundantly immediately with water. If in contact with mucous membrane seek advice and anti-retroviral or anti-HepC treatment with occupational health immediately. If HO is not available attend local A&E. Include incidents to be reported onto Salus (accident reporting database). Report to Safety Officer and to occupational health immediately.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: Decontaminate with Virkon final concentration 1% min contact time 15 min Max contact time 1 hour.

Solid waste: Decontaminated with 1% Virkon min contact time 15 min Max contact time 1 hour or will be autoclaved (121ºC, 15 min, data logger to confirm successful cycle)

Any material in contact with cells or media will be decontaminated with 1% Virkon min contact time 15 min Max contact time 1 hour or will be autoclaved (121ºC, 15 min, data logger to confirm successful cycle)

Is an emergency plan required according to regulation 20?  

Yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Agreed: Class 2, Containment Level 2.

GMIC Reference Number: GMIC-1412

The J2 retro virus is a recombinant retro virus expressing the oncogenes v-myc and v-raf used to transform the BV2 cell line. This is not work carried out by Dr and the cell line is bought in from an external agency. However it has not been conclusively demonstrated in the risk assessment that the BV2 cell line is virus free, therefore BV-2 cell line has been risk assessed to be a class 2 GMO. This was the view of the committee. They will therefore have to complete a CU2 and pay the HSE the usual admin fee
The specific aim of this work is to identify, sequence, disrupt and analyse the expression of fungal genes involved in virulence. To achieve this genes will be isolated from genomic DNA by PCR, heterologous hybridisation, mutant complementation and related techniques. This will require construction of Aspergillus and Candida libraries in Saccharomyces cerevisiae/Escherichia coli shuttle vectors e.g. YEp24. Genes of interest will be sequenced and characterised further. As part of this further analysis gene disruption and replacement experiments will be performed. In addition these lesions will be complemented using the homologous gene. This will be done by DNA mediated transformation of viable fungal cells using non-mobilisable vectors carrying appropriate nutritional (for complementation of auxotrophy) or antibiotic (eg hygromycin and phleomycin) markers.

Immuno compromised mice will be infected with GM Aspergillus and Candida spp by nasal droplet, or intravenous injection, and after sacrifice tissues excised and
analysed for fungal gene expression.

**Recipient or parental organism**

**Escherichia coli:** strains in common laboratory use eg DH5alpha, XL-10 will be used as cloning tools. These strains, which are generally derivatives of the K12 strain, have a widespread and long history of safe laboratory use. They also contain numerous mutations eg thi-1 which render the strains auxotrophic and therefore unlikely to survive outside the laboratory environment. ACDP HG1

Aspergillus nidulans: there are a huge number of classically produced mutant strains of A. nidulans. These have been constructed in many strain backgrounds and often have been crossed many, many times. These strains have a long history of safe laboratory use and usually contain auxotrophic mutations eg argB that would prevent survival outside the laboratory. ACDP HG1

Aspergillus fumigatus: AF237, D141 and ATCC46645, 293, CEA10 and auxotrophic derivatives thereof eg pyrG will be used to construct knock-out, regulatable and reconstituted strains. These backgrounds have a long and safe history of use worldwide. Additionally the pyrG auxotrophies would prevent survival outside the host. ACDP HG2

Candida albicans: SC5314, CAI4 and CAI10 and auxotrophic derivatives thereof eg his3/ his3, ura3/ura3 will be used to construct knock-out, regulatable and reconstituted strains. ACDP HG2

Candida glabrata: ATCC2001 and auxotrophic derivatives thereof eg his3, ura3, trp1 will be used to construct knock-out, regulatable and reconstituted strains. ACDP HG2

Pichia pastoris: A methylotropic yeast used as a protein expression system, commercially sourced. GS115 and SMD1163 and auxotrophic derivatives thereof eg his4 will be used to express selected fungal proteins. These strains have a long and safe history of laboratory use worldwide. ACDP HG1

Saccharomyces cerevisiae: the majority of laboratory strains are derived from three major wild type lineages S288C,

We also seek permission to use other strains of the spp described above that have essentially the same characteristics as those described.

**Host/vector system**

E. coli and S. cerevisiae vectors including pUC, pBluescript, YE, YI and YC series and similar

We will use a series of C. glabrata episomal vectors (Kitada et al, Gene 175:105, 1996)

Mobilisable Aspergillus vectors will not be used.

**Origin & function**

We will construct Aspergillus and Candida libraries in Saccharomyces cerevisiae/Escherichia coli shuttle vectors e.g. YEp24. Genes of interest will be sequenced and characterised further.

As part of this further analysis gene disruption and replacement experiments will be performed. In addition these lesions will be complemented using the homologous gene. It is not possible to list all of the genes that will be inactivated as we are attempting to characterise complex processes required to maintain fitness en vivo, e.g. nutrient sensing, adherence, metabolic plasticity etc. Disruption of these genes adheres is by definition likely to result in a reduced ability to survive in vivo and therefore to cause disease. Inserted genes will either be from the same species, eg used to complement a null allele to create a reconstituted strain, tags added to selected genes eg GFP, TAP, HA, etc. Or Heterologous antibiotic genes eg ampR, bleR, hygR as markers of transformation.

**Evaluation of foreseeable effects**

The most hazardous GMM will be a reconstituted Hazard group 2 Fungal pathogen from the list of hosts e.g. Aspergillus fumigatus. This will occur where gene disruption and replacement experiments have been performed and lesions complemented using the homologous gene and where DNA mediated transformation of non-mobilisable vectors is used carrying appropriate nutritional genes for complementation of auxotrophy.

Any antibiotic or antifungal selection markers will not be the same as those used for frontline therapy.

The worst case GMO constructed is not anticipated to be any more hazardous than the wild type organisms. It is unlikely that an accidental release would harm the environment. Although classified as Hazard group 2, GM Class 2 the Aspergillus and Candida spp used are opportunistic pathogens that cause disease principally in immuno compromised individuals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste - Treated with virkon at a minimum of 1% concentration after dilution with the waste for a minimum of 30 minutes. Disposal after treatment will be to drains. This has been independently validated as an effective method of inactivasting of the fungal pathogens in use and Aspergillus spores
http://www.therapeuticresource.ca/sporicidal.html

Solid waste - Plastic pipette tips will be disposed of into a 1% virkon solution after use for a minimum of 30 minutes before being drained and subject to autoclave treatment detailed below.

Solid Waste - Solid waste will be placed into autoclave bags and taken in lidded containers to the autoclave for treatment at 134 degrees for up to 30 minutes holding time. This waste will then be placed in orange clinical waste bags for disposal as clinical waste by the authorised Imperial College waste contractor.

The Autoclave is serviced quarterly.

Annually a worst case scenario mock load is prepared and placed in the autoclave. An engineer using a 12pt thermocouple load temperature NAMAS test apparatus ensures that every test point within the load achieves a temperature known to be effective at inactivating pathogenic microorganisms. At the same time the autoclaves load and chamber sensors are calibrated against the test rig.

In all cases a minimum of a $10^5$ reduction in microbiological burden is expected.

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The Committee were satisfied with the risk assessment and had no comments.
Project Ref 8/12.8

Date Ackn'd 03/12/2012

CU2 Project Title Defining the pathogenesis of opportunistic fungal and bacterial infections in transplantation

Date Project Ceased

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Background and Aim:
We are studying the pathogenesis of invasive fungal and bacterial infections in solid organ and haematological transplant patients. I have established a new murine model of transplant immunosuppression in which to define the immunopathogenesis of infection in these patients. Thus far I have investigated the immune responses to Aspergillus fumigatus in these patients, and have appropriate permissions for this work and clearance for Aspergillus fumigatus wild type strains. However I now need to develop murine models of Pseudomonas aeruginosa, Candida albicans/glabrata, and Cryptococcus neoformans and define the host/pathogen-dependent components of virulence in these organisms relevant to solid organ transplantation. I have NREC ethical approval to study peripheral blood immune responses to infection in solid organ transplant patients at Hammersmith Hospital. I am now starting to investigate the immunopathogenesis of invasive fungal and bacterial infections in lung transplant patients from the Harefield Hospital. Appropriate ethical permissions have been obtained from the Royal Brompton and Harefield NHS Trust Biomedical Research Unit Biobank.

Summary of the experimental procedure:
BALB/c/C57Bl/6 or derived knockout mice will be immuno suppressed with standard transplant-relevant immunosuppressants and be infected with Aspergillus fumigatus, Candida spp., Cryptococcus neoformans or Pseudomonas aeruginosa by the intra-nasal or intravenous or oral route and immune responses assayed by organbased RTPCR, ELISA or transcriptomic methods. In addition we will develop florescent/luminescent genetically modified fungal and bacterial strains in order to enable the quantification of pathogen burden either through the use of in vivo florescence imaging or FACS analysis of cellular suspensions. In addition we will breed genetically-modified pathogen gene deletion strains in order to determine the direct effects of transplant immunosuppressants on...
virulence, as many of these drugs have direct anti-fungal or anti-bacterial effects as well as being immunosuppressants (i.e. macrolides, steroids).

We will be undertaking parallel clinical studies of immunity to infection in solid organ transplant patients. This will require the analysis of immune responses in peripheral blood by FACS, RT-PCR and ELISA and similar studies in bronchoalveolar lavage isolates from these patients. All of these patients have regular screening for blood-bourne pathogens, and patients with these blood-bourne pathogens will be excluded from the study. However as part of the study we will culture clinical isolates of Aspergillus spp., Candida spp., Cryptococcus spp., and Pseudomonas spp. for subsequent phylogenetic and virulence analysis.

For in vitro studies we will use plasmid-based siRNA and reporter plasmids in order to knock down specific innate immune effectors in human/mouse primary/cell lines and measure their expression and activation in response to specific PAMP stimulants or infection with wild type pathogens or mutants. In addition we will use specific drug inhibitors and liposomal siRNA transfection.

The primary cell lines in use in the laboratory are RAW24.7, MH-S, U937, A549, HL-60, THP-1, NKL, NK92, YTS

Overview of the GMO's:

For fungal pathogens the key focus will be to develop fluorescent strains suitable for in vivo imaging studies and in addition gene deletion/mutation strains for analysis of the role of the calcineurin pathway in fungal virulence.

For bacterial strains the key focus will be the creation of fluorescent and strains for in vivo imaging analysis. In addition targeted deletions and mutations will be undertaken depending on results of virulence studies.

For mammalian studies we will use adenoviral and lentiviral transduction to either knock down specific components of host immune responses by siRNA, and in addition to enable reporter contracts and fluorescent constructs for in vitro imaging.

For in vivo murine work we will cross CreRecombinase mice with Floxed allele mice for key innate immune components and generate cell-specific inducible gene disruptions for analysis of host immunity.

Recipient or parental organism

Peripheral blood and bronchoalveolar lavage samples and lung transbronchial biopsy samples from patients and healthy controls

Mammalian: RAW24.7, MH-S, U937, THP-1, HL-60, A549, NK92, YTS, NKL from ATCC/HPA

Aspergillus fumigatus Ku80/CEA10/AF293/ATCC46645

Cryptococcus neoformans H99

Candida albicans 5301

Candida glabrata 2001

Pseudomonas aeruginosa PAO1, PA14, PAK, CHA, PA103

E. coli DH5, K12, TG1, HB101

Escherichia coli: strains in common laboratory use eg DH5alpha, XL-10 will be used as cloning tools. These strains, which are generally derivatives of the K12 strain, have a widespread and long history of safe use. They also contain numerous mutations eg thi-1 which render the strains auxotrophic and therefore unlikely to survive outside the laboratory environment.

Aspergillus nidulans: there are a huge number of classically produced mutant strains of A. nidulans. These have been constructed in many strain backgrounds and often have been crossed many, many times. These strains have a long history of safe laboratory use and usually contain auxotrophic mutations eg argB that would prevent survival outside the laboratory.

Aspergillus fumigatus: Af237, D141 and ATCC46645, Af293 and CEA10 and auxotrophic derivatives thereof eg pyrG or antibiotic selection-mediated gene disruption derivatives thereof eg hygromycin-resistant clones, will be used to construct knock-out, regulatable and reconstituted strains. These backgrounds have a long and safe history of use.
worldwide. Additionally the pyrG auxotrophies would prevent survival outside the host.
Candida albicans: SC5314, CAI4 and CAI10 and auxotrophic derivatives thereof eg his3/his3, ura3/ura3 will be used
to construct knock-out, regulatable and reconstituted strains. These strains have a long and safe history of use
worldwide.
Candida glabrata: ATCC2001 and auxotrophic derivatives thereof eg his3, ura3, trp1 will be used to construct knockout,
regulatable and reconstituted strains. These strains have a long and safe history of use worldwide.
Pichia pastoris: GS115 and SMD1163 and auxotrophic derivatives thereof eg his4 will be used to express selected
fungal proteins. These strains have a long and safe history of use worldwide.
Saccharomyces cerevisiae: the majority of laboratory strains are derived from three major wild type lineages S288C,
could not survive outside the laboratory.
The use of Caenorhabditis elegans as a model nematode host for infection modelling. The strain used is: AU37
(genotype; glp-4(bn2) I; sek-1(km4) X. glp-4 was identified in screens for loci that when mutated result in
hermaphrodite sterility; glp-4 was originally defined by a single recessive mutation, bn2, that results in temperatuesensitive
sterility in males and hermaphrodites, with the latter also exhibiting incomplete gonadal morphogenesis;
GLP-4 activity is required during the later larval and adult stages of development, and is necessary for germ cells to
progress efficiently through mitotic prophase and thus continue to proliferate. SEK-1 has MAPKK activity and belongs
to the MAPKK family.
We also seek permission to use other strains that have essentially the same characteristics as those described.
For mammalian studies we will use adenoviral and lentiviral transduction to either knock down specific components of
host immune responses by siRNA, and in addition to enable reporter constructs and fluorescent constructs for in vitro
imaging.
For in vivo murine work we will cross CreRecombinase mice with Floxed allelle mice for key innate immune
components and generate cell-specific inducible gene disruptions for analysis of host immunity. Standard knockout
mice will also be used. GM mice have been assessed seperately.

Host/vector system

For mammalian studies we will use commercially available attenuated adenoviral and 3rd generation lentiviral vector
transduction to either knock down specific components of host immune responses by siRNA, and in addition to enable
reporter constructs and fluorescent constructs for in vitro imaging. e.g. Vira power and Adenoviral Gateway expression
kits from Invitrogen.
For in vivo murine work we will cross CreRecombinase mice with Floxed allelle mice for key innate immune
components and generate cell-specific inducible gene disruptions for analysis of host immunity.
For fungal deletions we will use the plasmids pYes2 and pUChph which are episomal plasmids with selectable
markers. We will use standard E. coli and S. cerevisiae vectors eg pUC, pBluescript, YE, YI and YC series.
We will use a series of C. glabrata episomal vectors (Kitada et al, Gene 175:105, 1996). These carry HIS3, TRP1 or
URA3 markers.
For Cryptococcus neoformans Biolistic deletion and Candida PCR-based deletion will be used.
We will use standard E. coli and S. cerevisiae vectors eg pUC, pBluescript, YE, YI and YC series.
We will use a series of C. glabrata episomal vectors (Kitada et al, Gene 175:105, 1996). These carry HIS3, TRP1 or
URA3 markers. We will also use pSKII for gene disruption in C. glabrata and pCg-ACT14 for reconstitution.
We will used standard E. coli vectors:
a) For cloning:
-pCR2.1 and derivatives: ColE1 replicon, AmpR, KanR ; vecteur TA cloning for PCR products (InVitrogen). Allows
alpha complementation and blue/white cscreening on Xgal containing plates,
- pBR322 and derivatives: AmpR, TetR.
- pACYC184 and derivatives: ChiR, TetR.

NB: These vectors could also be used as suicide vectors for P. aeruginosa mutagenesis.

b) For gene tagging and expression:
- pET22b/28c/28a and derivatives: ColE1 replicon, AmpR (22) and KanR (28). Tag Histidine N or C-terminal.
- pQE30Xa and derivatives: Tag histidine (Qiagen). AmpR.
- pEGFP and derivatives: engineering of gfp fusion. AmpR.
- pGEX and derivatives: Tag GST (Glutathion S-transferase). AmpR.
- PAC or PAN and derivatives: AviTag. AmpR.
- pBADmychHisA, B, C and derivatives: expression from an arabinose promoter, tag histidine and Myc. AmpR.
- pYZ4 and derivatives: KanR. Construction of phoA fusion.
- GATEWAY plasmids: InVitroGen. System including the entry vector pDONOR and the destination vectors (pDEST derivatives) for recloning and expressing tagged genes.

c) For expression of genes under strong promoters:
- pBAD and derivatives: arabinose promoter. AmpR.
- pT7-1 to pT7-6 and derivatives: T7 promoter. AmpR. To be used together with pGP1-2 which carries the gene encoding RNA polymerase from T7 phage. KmR.

d) For studying protein-protein interaction:
- pUT18c and pKT25 and derivatives. Two hybrid system from Ladant, pUT18c (pUC19 derivative), AmpR, contains 3’ region of the gene encoding adenylate cyclase (cyaA). pKT25 (pSU40 derivative), KanR, contains 5’ region of cyaA.
- pBS1479 and derivatives: AmpR. Tandem Affinity Purification (TAP). The TAP-Tag contains a domain CBP (Calmodulin Binding Protein) and a domain Protein A (Affinity for IgG).

2) We will use suicide vectors for the engineering of Pseudomonas mutants:
- pKNG101 and derivatives: StrR. SucR. Only replicate in the E. coli ambdapir strain CC118.
- pEX100 and derivatives: SucR. Only replicate in E. coli ambdapir strain CC118

3) We will use broad host range vectors for expression in P. aeruginosa (also replicative in E. coli):
- pLAFR1, pLAFR3 and derivatives: IncP, TetR. Cosmid with low copy number.
- pUCP18/19 and derivatives: AmpR. Drivatives of pUC18/19 with a 2.1 kb stabilizing fragment allowing replication in Pseudomonas.
- commercially available third generation adenoviral or lentiviral vector systems such as ViraPower™ Adenoviral Gateway™ Expression Kit from Invitrogen

For fungal deletions we will use the E. coli derived plasmids pYes2 and pUChph which are episomal plasmids with selectable markers. We will use standard E. coli and S. cerevisiae vectors eg pUC, pBluescript, YE, YI and YC series.

We will use a series of C. glabrata episomal vectors (Kitada et al, Gene 175:105, 1996). These carry HIS3, TRP1 or URA3 markers. These are available from the Bignell and Haynes labs.

For Cryptococcus neoformans Biolistic deletion and Candida PCR-based deletion will be used. The standard constructs are from Ken Haymes lab, Exeter.

We will use standard E. coli and S. cerevisiae vectors eg pUC, pBluescript, YE, YI and YC series.

We will use a series of C. glabrata episomal vectors (Kitada et al, Gene 175:105, 1996). These carry HIS3, TRP1 or
URA3 markers. We will also use pSKII for gene disruption in C. glabrata and pCg-ACT14 for reconstitution. These are from the Haynes lab.

We will used standard E. coli vectors from the Bignell lab, Imperial College London:

a) For cloning:
   - pBR322 and derivatives: AmpR, TetR.
   - pACYC184 and derivatives: ChlR, TetR.

b) For gene tagging and expression:
   - pET22b/28c/28a and derivatives: ColE1 replicon, AmpR (22) and KanR (28), tag Histidine N or C-terminal.
   - pQE30Xa and derivatives: Tag histidine (Qiagen). AmpR.
   - pEGFP and derivatives: engineering of gfp fusion. AmpR.
   - pGEX and derivatives: Tag GST (Glutathion S-transferase). AmpR.
   - PAC or PAN and derivatives: AviTag. AmpR.
   - pBADmycHisA, B, C and derivatives: expression from an arabinose promoter, tag histidine and Myc. AmpR.
   - pYZ4 and derivatives: KanR. Construction of phoA fusion.
   - GATEWAY plasmids: Invitrogen. System including the entry vector pDONOR and the destination vectors (pDEST derivatives) for recloning and expressing tagged genes.

b) For expression of genes under strong promoters:
   - pBAD and derivatives: arabinose promoter. AmpR.
   - pT7-1 to pT7-6 and derivatives: T7 promoter. AmpR. To be used together with pGP1-2 which carries the gene encoding RNA polymerase from T7 phage. KmR.

c) For studying protein-protein interaction:
   - pUT18c and pKT25 and derivatives. Two hybrid system from Ladant, pUT18c (pUC19 derivative), AmpR, contains 3’ region of the gene encoding adenylate cyclase (cyaA). pKT25 (pSU40 derivative), KanR, contains 5’ region of cyaA.
   - pBS1479 and derivatives: AmpR. Tandem Affinity Purification (TAP). The TAP-Tag contains a domain CBP (Calmodulin Binding Protein) and a domain Protein A (Affinity for IgG).

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   - pBS1479 and derivatives: AmpR. Tandem Affinity Purification (TAP). The TAP-Tag contains a domain CBP (Calmodulin Binding Protein) and a domain Protein A (Affinity for IgG).

2) We will use suicide vectors for the engineering of Pseudomonas mutants:
   - pKNG101 and derivatives: StrR. DucR. Only replicate in the E. coli pambdapir strain CC118.
   - pEX100 and derivatives: SucR. Only replicate in E. coli pambdapir strain CC118

3) We will use broad host range vectors for expression in P. aeruginosa (also replicative in E. coli):
   - pLAFR1, pLAFR3 and derivatives: IncP, TetR. Cosmid with low copy number.
   - pUCP18/19 and derivatives: AmpR. Derivatives of pUC18/19 with a 2.1 kb stabilizing fragment allowing replication in Pseudomonas.
   - commercially available third generation adenoviral or lentiviral vector systems such as ViraPower™ Adenoviral Gateway™ Expression Kit from Invitrogen

For mammalian studies we will use adenoviral and lentiviral transduction to either knock down specific components of
host immune responses by siRNA, and in addition to enable reporter constructs and fluorescent constructs for in vitro imaging. These are commercially available.

For in vivo murine work we will cross CreRecombinase mice with Floxed allele mice for key innate immune components and generate cell-specific inducible gene disruptions for analysis of host immunity. These will be from the Crabtree lab (Stanford), the Gordon lab (Aberdeen) and the Pepy lab (Montpellier).

Calcineurin is required for fungal morphogenesis and calcium signalling. GFP is a fluorescent protein.

As the genomes for these organisms are known, we may wish to knock-out a number of different genes as the work progresses.

**Evaluation of foreseeable effects**

We will be constructing gene deletion strains in the opportunists pathogens Candida albicans, Candida glabrata, Cryptococcus neoformans and Pseudomonas aeruginosa. As we are investing virulence attenuation these strains are likely to be less virulent than wild-type. Of note, all deletions in the calcineurin pathway thus far have severely attenuated virulence. We are also likely to generate fluorescent strains, however again these are unlikely to be no more virulent than wild-type.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All knock-out mice will be housed in secure facilities in the Imperial South Kensington CBS.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All cultures and infected liquids will be sterilised by incubation in 1% Virkon/Distel for 1 hour and disposed of through designated sinks. Validated data supplied by the manufacturer dictates that this will result in inactivation of all organisms detailed in this proposal.

Contaminated glassware will be decontaminated by the addition of 1% Virkon/Distel and incubated at room temperature for 1 hour.

Solid waste, including plastics, tubes, pipettes, agar plates etc will be sterilised and disposed of via the university clinical waste service. All contaminated waste is disposed of into dedicated waste bins, which are sealed and subsequently sterilised by autoclaving at 134°C for 1 hour. Autoclave function is assessed by a chart recorder, 3 monthly servicing and an annual validation of a typical waste load using 12 point thermocouple testing.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N
Dear Dr AJ,

Following the discussion of your proposal entitled "Defining the pathogenesis of opportunistic fungal and bacterial infections in transplantation", GMIC-1438 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

2.16: Include sentence regarding commercially available lentiviral vectors.
4.12: Please answer this question.
5.8: Please include a room number. Check with H C
7.1: Safety Department to confirm alternative to Trigene.
10.2: Explain 'lavage'.
13.1: Include location of IVIS.

Spelling errors throughout the risk assessment.

Comments: S Herbst gave a brief description of the work. IH explained that Dr AJ could not attend the meeting therefore, IH emailed him questions about the risk assessment to present to the Committee. Some of the questions have been addressed by the PI and S H:

Questions from IH:

2.16: Please identify the adeno viral vectors and the lentiviral vectors mentioned in 2.14 and describe the degree to which they are disabled.
Ans: We have a number of these from J C we would like to order, S H has details and will amend the risk assessment.

4.3: Are these T cells from characterised cell lines or from clinical isolates from patients with known histories? If yes please state this, as after 96 hours culture there is an increased risk of propagating adventitious agents and we would need additional assurances that this is unlikely.
Ans: The patients have known histories and are screened (negative) for blood-borne viruses as it standard for transplantation

5.1: You will need to be quite specific about identifying the procedures that potentially generate aerosols. I don't think that the level 5 COP does this. Are these identified in individual SOP's? If so please send them through to the Safety Dept and reference them in the risk assessment.
Ans: I have sent all SOPS as current, these should detail this although I haven't had time to check this.

5.4: Change category 2 agents to infectious substances.
Ans: Agreed, will be done

5.5: Change category 2 agents to infectious substances.
Ans: Agreed, will be done

5.5: Are any of the agents imported from outside the UK in FCS or BSA? If yes please contact the Safety Dept (M B) as you may need an import licence.
Ans: No
5.7: Are rotor buckets opened in an MSC?
Ans: Yes, we will modify this section
5.9: Is there any dissection of animals to remove organs? If so please amend this section
Ans: Agreed, will be done
5.10: Fluorescence imaging of whole animals or organs?
Ans: Both, this will be clarified in the section
5.12: Autoclaving is ok as an inactivation process so long as it is carried out in an autoclave tested and validated for this purpose. Is this the case? Has the microwave inactivation process been validated by yourselves or others and can you provide data to confirm the degree of inactivation?
Ans: The Autoclave is validated for aspergillus, candida and bacteria, crypto is new, SH can ask Jon Perfect
6.5: Please make it clear that normal spectacles do not provide sufficient protection.
Ans: Agreed, will modify this section
7.1: Please specify the final concentration of Trigene e.g. A minimum concentration of 1% after dilution with the liquidwaste.
Ans: Agreed, we will mention that it is 10% for killing fungi.
7.1: change hood to MSC
Ans: Agreed, we will modify the section
8.1: Your local safety Officer should be able to help with the details of the service provider.
Ans: Agreed SH to enquire
14: Please complete this section for the Gmo's that are going to be created.
Ans: Give me a clue about what this box is, I am not sure what project aspect for instance means?
IH: This is fairly straight forwards for example in your case One aspect of the project would be E.coli cloning using non mobilisable vectors such as pUC and pbluescript with non toxic, non pathogenic inserts. Class 1
Second aspect for example Creation of gene deletion mutants in Hazard group 2 fungal pathogens Cryptococcus neoformans, Aspergillus fumigatus in order to study the calcineurin pathway in fungal virulence. Class 2
Third example Creation of gene deletion mutants in hazard group 1 opportunistic fungal pathogens Candida albicans, Candida glabrata in order to study the calcineurin pathway in fungal virulence. Class 1
And so on. I can see two other aspects to this work, the class 2 Pseudomonas and the mammalian cell line studies using adenoviral and lentiviral transduction that need mentioning in the same way. I'm assuming that the latter will be class 1 but this will depend on the level of attenuation of the vectors.
In the Meeting:
RW asked if the animals involved were GMO's.
If there are GM mice involved, please fill in the GM Form B.
HK asked what the samples were screened for.
S Herbst answered the samples were screened for blood borne viruses, such as; Hepatitis and HIV.
HK inquired if the samples were from transplant patients.
S Herbst explained they were, such as lungs, kidneys, etc.
HK checked if this involved clinical trial patients.
S Herbst confirmed, no.
SH queried what they did with samples that came back as positive.
S Herbst explained that positive samples would be disposed of and not used.
HK pointed out that the final concentration of disinfectant after dilution with the liquid waste needed to be stated.
Also, Trigene was no longer available. The Committee discussed alternatives to Trigene and concluded that this would be confirmed once M B (Associate Biological Safety Officer) attended the meeting to discuss this.
SH questioned if the full body imaging facility would be used?
S Herbst confirmed that they would be.
RB suggested including the IVIS and its location in the risk assessment.
S H asked the Committee for advice. They did not currently know what viral vectors if they would be using, should they remove this element form the RA or keep it in?
IH suggested including adding a sentence in Section 2.16 that was broad but confirmed the limitations of what might be using for example using 3rd generation self-inactivating lentiviral vectors that are commercially available.
RW also advised stating 2 or 3 of these suppliers.
IH pointed out that Cryptococcus was coming off the Schedule 5 list soon. Until this is confirmed after the 1st of October, consent for the Cryptococcus cannot be given.
Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.
Could you please ensure that the risk assessment is revised (add GMIC number) and the amendments above addressed. I will then forward the form onto Occupational Health to complete. The completed HSE form (attached to this email), and cheque made payable to the HSE should be sent to me (@imperial.ac.uk), I will then send it all to the HSE. Please ensure the information given in HSE Form relates to that provided in your risk assessment.
For further information, please see http://www3.imperial.ac.uk/safety/subjects/biosafety/geneticmodification.
Please note that this work must be notified to the HSE and an acknowledgement receipt received prior to this work commencing.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L2</td>
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<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L2</td>
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### Project Ref 8/13.1

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<th>CultureVolumeClass3-4</th>
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To characterize the flagellar motor of Campylobacter jejuni. The bacterial flagellar motor is a rotary motor that spins a long tail that acts as a helical propellor, enabling the bacteria to swim. Interestingly, the Campylobacter motor, although related to the "standard" motors of Escherichia coli or Salmonella enterica, is considerably more complex. By studying the Campylobacter motor we can gain comparative insights into how the motor functions, how the motor evolved (as the added complexity of Campylobacter is a relatively recently evolutionary acquisition), and furthermore develop novel, specifically anti-Campylobacter antibiotics (disruption of the motor decreases pathogenicity).

To do this we will be growing wild type and mutants of C. jejuni and imaging them in 3-D in an electron cryo-microscope. Furthermore, the closely related Helicobacter pylori and Helicobacter hepaticus have similar flagellar motors that will also be examined.

Overview of the GMMs to be constructed: Insertion of short peptide tags into pre-existing genomically-encoded flagellar motility proteins in C. jejuni (e.g. FliG, GlgP, MotB); deletion of same genomically-encoded proteins. Use of non-pathogenic lab strains of E. coli (e.g. strain DH5α) for construction of plasmids and for conjugation-mediated plasmid transfer to C. jejuni, H. hepaticus and H. pylori for homologous recombination.

Experimental procedures will include: Genetic manipulation of Campylobacter jejuni, Helicobacter pylori, and Helicobacter hepaticus (electroporation and conjugation to insert genes on plasmids or make chromosomal deletions or insertions), culturing (on agar plates in microaerobic chamber or microaerobic jars), flash-freezing (using pneumatic or gravity-driven plunge freezers to rapidly propel <<1ul of sample on EM grid into liquid ethane/propane cryogen) with subsequent imaging in electron microscope at -180°. Electron microscopy will either in-house in the adjacent Flowers Building, or shipped under liquid nitrogen storage to an off-campus microscope in Manchester, Cambridge or Eindhoven or Leiden (Netherlands).

Recipient or parental organism
All recipient organisms involved are Hazard Group 2 pathogens, and are capable of causing disease in humans. The flagellar motor is important for pathogenesis.

C. jejuni infection causes diarrhea, which may be watery or sticky and can contain blood (usually occult) and fecal leukocytes (white cells). Other symptoms often present are fever, abdominal pain, nausea, headache and muscle pain. The illness usually occurs 2-5 days after ingestion of the contaminated foods or water. Illness generally lasts 7-10 days, but relapses are not uncommon (about 25% of cases). Most infections are self-limiting and are not treated with antibiotics. However, treatment with erythromycin does reduce the length of time that infected individuals shed the bacteria in their faeces.
Helicobacter hepaticus causes chronic hepatitis, liver cancer (hepatocarcinoma) and inflammatory bowel disease in mice. The organism is most likely transmitted by fecal-oral route however the mode of liver invasion is unknown but the production of urease and a soluble hepatotoxic factor may be involved in the pathogenesis of H. hepaticus-induced liver lesions.

The importance of H. hepaticus to humans is not yet completely known.

Over 80% if people infected with H. pylori show no symptoms. Acute H. pylori may appear as an acute gastritis with abdominal pain (stomach ache) or nausea. Where this develops into chronic gastritis, the symptoms, if present, are often those of non-ulcer dyspepsia: stomach pains, nausea, bloating, belching, and sometimes vomiting or black stool.

Host/vector system


Origin & function

Genes involved in C. jejuni flagellar motility as well as homologous genes in H. hepaticus and H. pylori. Key examples include fliG (rotor component of flagellar motor, required for motility), flgP, flgQ, pflA, cjj0413 (unknown function, required but form components of the flagellar motor and are required for motility).

This work will involve genetically modifying proteins naturally encoded by Campylobacter jejuni and related organisms with the intention of adding an additional shape onto the naturally-occurring proteins. We will then be able to directly see where this additional shape appears when we look at the Campylobacter flagellar motor using our electron microscope. This is important, because this will enable us to work out where each protein is found in the motor (not something we are able to do at the moment, as each protein looks very much alike at the resolutions of contemporary electron microscopes). The additional genetic material will derive from known proteins already structurally characterised by international scientists, and is extremely likely to somewhat impair the pathogenic potential of Campylobacter jejuni and relatives because it is likely to interfere with the function of the motor.

Evaluation of foreseeable effects

GMO bacteria will either behave as they usually do, or (more likely), their flagellar motors won't function quite as well after I've added an additional protein onto them. This is directly analogous to welding an additional piece of metal onto an arbitrary part of a motor engine (including moving parts), and expecting it to work normally. Additional proteins that I'll be fusing to existing proteins will be identified according to how well they fit criteria to be grafted onto the existing proteins from an international database (the Protein Data bank, or PDB) that contains the structures of proteins determined by scientists from around the world. If these grafted proteins have an enzymatic function, this will be removed by rational mutation before fusing to existing proteins.

In summary we anticipate that they will all be less harmful than the wild-type C. jejuni, H. pylori and H. hepaticus because some level of motility attenuation which in turn is required for pathogenesis.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated by chemical disinfection prior to disposal (Virkon to 1% final concentration); solid waste placed in heavy-duty plastic autoclave bags, loosely sealed with autoclave tape (to allow ingress of steam) and placed into blue boxes for transport to Biochemistry autoclaves (with affixed lid).
Autoclave is validated for a worst case scenario waste load to achieve a minimum temperature of 121 degrees for 15 minutes across multiple locations within the load itself.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Following the discussion of your proposals entitled "Structural dissection of the flagellar motor from Campylobacter jejuni and related epsilon-proteobacteria", (GMIC -2267) at the last GM Safety Committee meeting, the committee request that the following amendments are made to the proposals to approval:

Comments: MB gave a brief description of the work.
MB informed the Committee that he now has new rooms for his work.
IH pointed out that if MB planned to ship any material to another organisation that he should inform the of the type of material and how it had been treated. Based on the information he provided it would be up to their BioSafety Officer and GM committee to decide how it should be handled at their end and what risk assessments they may need to complete.
HK queried the degree of kill in the freezing experiments.
MB explained when the bacteria was frozen, they were fine, it was during the thawing process when they were killed. The blot, freeze and thaw experiment would be carried out a few times to check this.

The following amendments are required prior to approval:

2.1 and 4.1: Hatch the top box if not using tissue.
10.1: Specify the disinfectant and the concentration required to be achieved after dilution with the liquid spill. E. g. Add an equal volume of 2% virkon.
13: Specify the lab as this will need to be inspected before work can start.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.
Could you please ensure that the risk assessment is revised (add GMIC number) and the amendments above addressed. I will then forward the form onto Occupational Health to complete. The completed HSE form (attached to this email), and cheque made payable to the HSE should be sent to me (address given). I will then send it all to the HSE. Please ensure the information given in the HSE form relates to provided in your risk assessment.
For further information please see http://www3.imperial.ac.uk/safety/subjects/biosafety/geneticmodification.
Please note that this work must be notified to the HSE and an acknowledgement receipt received prior to this work commencing

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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
The primary objective of the research is to examine the asexual and sexual stages of Plasmodium in vitro and in vivo towards dissecting how malaria parasites move and use this understanding to develop new tools for the efficient assessment of potential anti-malarial agents, specifically to assess their ability to impair parasite motility, growth, development and transmission through the lifecycle. This requires the development of transgenic (genetically modified, [GM]) parasites that either: i) express fluorescent proteins across different stages of the asexual or sexual stages of parasite development, allowing for easy visualisation of these lifecycle stages, ii) express heterologous proteins from other organisms that permit complex genetic reorganisation in the parasite cell (e.g. Cre or FLP recombinases) or drug resistance (e.g. dihydrofolate reductase or blasticidin resistance markers) in derived parasite lines to facilitate their selection from non-genetically engineered lines, iii) genetically engineer endogenous proteins to incorporate fluorescent, drug resistant, or antigen tags for their cellular localisation or immunoprecipitation from parasite derived lysates and, iv) have individual genes that are potentially important in motility or development partially or wholly disrupted (knockout [KO] or knockdown [KD]). By examining the derived parasite lines and their resultant phenotypes (in particular for KO or KD), we aim to yield information relating to fundamental parasite cell biology, in particular parasite cell movement and/or development towards understanding growth, development and malaria parasite transmission. Whilst the majority of studies will be entirely via in vitro culture (using human blood from the blood bank in culture dishes), provision is sought to potentially include downstream experiments to explore parasite transmission through mosquitoes (parasites being fed by membrane feeders (as per current Code of Practice [CoP]) to adult Anopheles mosquitoes) or infection models with humanised mice (to explore pre-erythrocytic stages of development).

Most work will be focussed on Plasmodium falciparum with a small comparative set of experiments focussed on another laboratory adapted human parasite species Plasmodium knowlesi. Non-transgenic (non-GM) work with the third major human species Plasmodium vivax, will be undertaken when possible from isolates either available from the malaria reference repository (MR4) or from hospital isolates.
GM mosquitoes and GM mice may be used in the project and GM mosquitoes may be generated through the project (GM mice will only be sourced and will not be directly generated). Where GM mosquito generation is desired, these will be assessed on a Imperial College GM Form B before being used, stored or generated.

Recipient or parental organism

P. falciparum strains 3D7, NF54, D10, CS2 and Dd2 will be used. All are long-standing laboratory strains maintained in continuous culture for decades. P. falciparum is classified as ACDP 3** Annex 1: Biological agents which may be used at less than the minimum containment conditions”. P. knowlesi (strain A1-H) is a widely used laboratory strain classified as human simian malaria ACDP 2.

Host/vector system

pHTK, pCC1, pCC4, pD3HA circular DNA vectors are widely used to transform Plasmodium species and carry either the resistance marker human DHFR mutated to encode resistance to WR99210 or Blasticidin S deaminase gene from Aspergillus terreus to confer resistance to Blasticidin. These do not affect resistance to anti malarials chloroquine, mefloquine, artemisinin or doxycycline which can be used (or their derivatives) to treat malarial infections.

Origin & function

Two types of GMM P. falciparum and P. knowlesi will be generated: reporter strains and knock-out strains. We will create transgenic P. falciparum or knowlesi parasites that express fluorescent or luminescent proteins (for example, but not limited to, YFP, GFP, RFP, PA-GFP) in the asexual stages of the parasite to facilitate tracking life stages, or proteins of interest in the parasite. We will also create knock-outs of genes in the parasite that we think are important in parasite cell motility. Examples include secreted or transmembrane proteins expressed at the merozoite or ookinete stages of the parasite.

Altered and or inserted genes will include:
- Fluorophores, both conventional and photoactivatable
- Epitope tags, including hexa-HIS, GST, HA epitope, FLAG epitope and CLIP epitopes, for localisation or immunoprecipitation based purification
- Plasmodium altered genes may also include those that are knocked out (removed), knocked down (where insertion of domain induces protein instability or gene instability that reduces expression) or flanked by sequences that initiate gene excision (FLP/Cre) for conditional knockout.

Origin & function

The programme of work requires the development of transgenic (genetically modified, [GM]) parasites that either; i) express fluorescent proteins across different stages of the asexual or sexual stages of parasite development, allowing for easy visualisation of these lifecycle stages, ii) express heterologous proteins from other organisms that permit complex genetic reorganisation in the parasite cell (e.g. Cre or FLP recombinases) or drug resistance (e.g. dihydrofolate folate reductase or blasticidin resistance markers) in derived parasite lines to facilitate their selection from non-genetically engineered lines, iii) genetically engineer endogenous proteins to incorporate fluorescent, drug resistant, or antigen tags for their cellular localisation or immunoprecipitation from parasite derived lysates and, iv) have individual genes that are potentially important in motility or development partially or wholly disrupted (knockout [KO] or knockdown [KD]). By examining the derived parasite lines and their resultant phenotypes (in particular for KO or KD), we aim to yield information relating to fundamental parasite cell biology, in particular parasite cell movement and/or development towards understanding growth, development and malaria parasite transmission.

All GMMs to be constructed are expected to be of equal hazard to human health and the environment as parent strains (both Plasmodium species). Insertions, deletions or disruption of genes are not expected to increase the ability of the Plasmodium parasite to infect humans, increase drug resistance (beyond an experimental setting) or pose an increased risk to the environment. At no point will parasites be engineered to increase virulence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Plasmodium falciparum (and derivatives) is a hazard group 3 pathogen requiring CL3 containment. Derogation from full containment is sort in the form of the requirement to decontaminate the laboratory in the event of a spill by fumigation. All other precautions required for full CL3 will be applied.
P.falciparum is not airborne, nor carried by droplet formation it requires direct contact to blood stream. Culture volumes in use are relatively small at 150ml per flask, these agents are extremely easily broken down and cannot survive and/or multiply in the environment, it is not considered necessary by this risk assessment to fumigate the laboratory in the event of a spill or release.
The laboratory proposed for use by this project is currently notified to the HSE as being derogated from full CL3 for fumigation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste - Disinfected in 10% Chloros, final concentration.

Solid waste - the CL3 facility has a dedicated autoclave that is maintained and serviced by the Department. Servicing includes an annual 12 point thermocouple test with dummy loads and regular servicing throughout the year from a competent contractor.

All solid waste is autoclaved before leaving the CL3 facility and waste cycles verified by successful completion and print out.

Details of the autoclave are included in the associated CL3 code of practice for the facility.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Comments: The committee discussed the comments made by IH prior to the meeting. SH and IH have visited the room with the FACs machine and observed the procedure for possible spill incidents. The FACs is an enclosed system except when the sample is drawn into the machine. The lid has to be removed from the sample tube (volume approx. 1.5ML) which is affixed to the machine sample dipper. The sample is taken into the machine by applying a positive pressure to the tube. There is a risk that the tube might be dropped or dislodged from the machine. In practice this is unlikely if proper procedures are followed. Training in these procedures will be key to avoiding incidents. The high throughput sampling system works in a similar manner but uses a multi well plate instead of a single tube.

The following amendments were required prior to approval:

We are going to need specific SOP's and amendments to the existing COP to accommodate the work
1.1: How will the parasites be fed to the mosquitoes? Membrane feeders as per the COP?
1.2: Typo ‘Kensington’
1.3: Specify which Laboratories the work will be carried out in.
2.10: Edit form and add spaces between strains to match up with the names of the agents.
2.15: Remove sentence, “All of these strains are genetically modified to tolerate plasmid…”
4.7: Typo ‘phlebotomist’
4.17: Typo ‘vectorsvectori’
4.18: It is worth stating that "no work known or expected to increase the pathogenicity of the parasite will be carried out" this will help set the limits of the work.
4.21: Typo ‘innocation’
5.2: What is the route to the imaging facility? Does this involve moving parasites along the corridor? If so how are they contained?
5.3: Please describe the procedure including transport and containment. This is likely to be a case for a new SOP.
5.5: Will you import any mosquitoes?
5.9: Current COP states use of sharp needles because the diaphragm of the tipper is quite thick? It would be a great step forwards if blunt needles were used instead.
7.1: Typo "pipet"

Section 12: Should be left blank for the OH physician to complete.
Section 13.1: Please be specific about which Plasmodium species are used at CL 2 and which at CL3.
- Please be specific about which aspects of the protein, DNA work is carried out at CL3 and which at CL2.
- Please state that only fixed materials are removed to CL2. Counting of any live material must be carried out at CL3 although this may be in a derogated facility.

Agreed: Class 2, Containment Level 3. The HSE need to be notified of the significant change to containment.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 Yes</td>
<td>L4 L2</td>
</tr>
<tr>
<td></td>
<td>L3 L4 L2 L3 L4</td>
<td></td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
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### Project Ref 8/14.3

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>01/07/2014</td>
<td>The analysis of Apicomplexan cell motility using model organisms</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
</tr>
</tbody>
</table>

02/03/2022
Here we propose to use the model apicomplexan parasites (as models for human malaria parasites): mouse malaria Plasmodium berghei and the apicomplexan parasite Toxoplasma gondii as model organisms to dissect the molecular basis of host cell interactions during apicomplexan parasite colonisation of the mosquito vector midgut or entry into host cells.

The work requires the development of transgenic (genetically modified, [GM]) parasites that either; i) express fluorescent proteins across different stages of the asexual (P. berghei or T. gondii) or sexual stages of parasite development (P. berghei only), allowing for easy visualisation of these lifecycle stages, ii) express heterologous proteins from other organisms that permit complex genetic reorganisation in the parasite cell (e.g. Cre or FLP recombinases) or drug resistance (e.g. dihydrofolate reductase or blasticidin resistance markers) in derived parasite lines to facilitate their selection from non-genetically engineered lines, iii) genetically engineer endogenous proteins to incorporate fluorescent, drug resistant, or antigen tags for their cellular localisation or immunoprecipitation from parasite derived lysates and, iv) have individual genes that are potentially important in motility or development partially or wholly disrupted (knockout [KO] or knockdown [KD]).

By examining the derived parasite lines and their resultant phenotypes (in particular for KO or KD), we aim to yield information relating to fundamental parasite cell biology, in particular parasite cell movement and/or development towards understanding their growth, development and ultimately to build complete models for extrapolating to human malaria parasite cell biology.

**Recipient or parental organism**

Plasmodium berghei ANKA strain. Toxoplasma gondii RH strain. Both are long-standing strains maintained in continuous passage or culture for decades by groups all over the world. Both are classified as ACDP 2.

**Host/vector system**

Vectors used are derived from various research groups and include: for P. berghei work the PlasmoGEM vectors (Wellcome Trust Sanger Institute) and pL series vectors (Leiden University); and for T. gondii ptet vectors (Glasgow University), pHXGPR and pCT vector series (University of Glasgow and University of Melbourne respectively).
Vectors encode the resistance marker human DHFR mutated to encode resistance to WR99210 or Pyrimethamine or (for T. gondii) the chloramphenicol acetyl transferase (CAT) selectable marker, ble selectable marker conferring phleomycin resistance, mycophenolic acid for HGXPRT.

**Origin & function**

Two types of GMM P. berghei and T. gondii will be generated: reporter strains and knock-out strains. We will create transgenic parasites that express fluorescent or luminescent proteins (for example, but not limited to, YFP, GFP, RFP, PA-GFP) or epitope tags (for example, but not limited to triple HA, 2 x cMyc or Ty tags) in the asexual/sexual stages of the parasites to facilitate tracking life stages, or proteins of interest in the parasite cell. We will also create knockouts of genes in the parasite that we think are important in parasite cell motility. Examples include secreted or transmembrane proteins expressed at the merozoite or ookinete stages of the parasite.

**Evaluation of foreseeable effects**

The programme of work requires the development of transgenic (genetically modified, [GM]) parasites that either; i) express fluorescent proteins across different stages of the asexual or sexual stages of parasite development, allowing for easy visualisation of these lifecycle stages, ii) express heterologous proteins from other organisms that permit complex genetic reorganisation in the parasite cell (e.g. Cre or FLP recombinases) or drug resistance (e.g. dihydrofolate reductase or chloramphenicol resistance markers) in derived parasite lines to facilitate their selection from non-genetically engineered lines, iii) genetically engineer endogenous proteins to incorporate fluorescent, drug resistant, or antigen tags for their cellular localisation or immunoprecipitation from parasite derived lysates and, iv) have individual genes that are potentially important in motility or development partially or wholly disrupted (knockout [KO] or knockdown [KD]). By examining the derived parasite lines and their resultant phenotypes (in particular for KO or KD), we aim to yield information relating to fundamental parasite cell biology, in particular parasite cell movement and/or development towards understanding growth, development and malaria parasite transmission. All GMMs to be constructed are expected to be of equal hazard or (the majority) lesser hazard to human health and the environment as the parent strains. Insertions, deletions or disruption of genes are not expected to increase the ability of the Plasmodium parasite ot Toxoplasma parasite to infect humans, increase drug resistance (beyond an experimental setting) or pose an increased risk to the environment. At no point will parasites be engineered to purposefully increase virulence.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste - Disinfected in 10% Chloros or Virkon and then passed down the drain with water.
Solid waste - All solid waste is autoclaved before leaving the building. The waste cycles have been validated using 12 point thermocouple test and the autoclaves serviced and maintained as per manufacturer’s instructions. All completed sterilisation cycles are checked via a print out form the autoclave. The cycle has been validated for 126°C for at least 10 minutes.
Please enter comments on the GM safety committee on the risk assessment

## Project Containment

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<td>L2 L3 L4</td>
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<tr>
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<td>L2 L3 L4</td>
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## Project Ref 8/14.4

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<th>Date Ackn’d</th>
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<tbody>
<tr>
<td>24/07/2014</td>
<td>Role of inflammasomes during bacterial infections</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
</tr>
</tbody>
</table>

Non-GMM

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

The main focus of the project is to understand the mechanisms of host defence against bacterial infections. Inflammasomes are signalling pathways involved in the activation of inflammatory caspases in cells. Caspases are enzymes that proteolytically process a number of substrates that have various biological functions, including antimicrobial responses. However, apart from a few substrates, most others remain unidentified. The main aim of this project is to identify and characterize novel substrates of caspases in infectious and non-infectious settings. This will involve:

1. Cloning and biochemical characterization of proteins in E. coli, Pichia pastoris and/or Sf9 insect cell lines.
2. Heterologous expression and studies of proteins in immunity against bacterial infections using various cell lines in vitro.
3. Use of fluorescence microscopy to study the cell biology of host immune factors in protecting against bacterial infections.
4. Use of wild-type and gene-knockout strains of mice to study the role of host effectors in antimicrobial defences in vivo.

Most studies will be undertaken using Salmonella and Listera as laboratory ‘work horses’ to dissect the molecular pathways involved in immune activation of caspases. However, after initial experiments are carried out, related studies with other bacteria will be undertaken to compare and contrast mechanisms of host defence against different commensals (e.g. E. coli and Citrobacter), non-pathogenic (E. coli, Bacillus) or pathogenic (as listed in 2.10) microbial agents.

**Recipient or parental organism**

Biohazard group 2:
- *Salmonella enterica* Typhimurium: The following strains will be used - SB136 (InvA::ahpC), SB2199 (delta 0PrgIJ ), SB2176 (delta ssaC), SB762 (flhD::Tn10)
- *Listeria monocytogenes*
- *Streptococcus pyogenes*
- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*
- *Mycobacterium bovis* BCG
- *Mycobacterium avium* subsp. Chester
- *Vaccinia virus*
- *Sendai virus* (HG1 for humans and HG2 for environmental grounds)
- *Adenoviruses* serotype 5

**Host/vector system**

The lambda recombinase system has been used to generate deletion constructs. These include plasmids such as pKD3, pKD4, pKD13, pKD20, pKD46 and related along with helper plasmids for removal of antibiotic markers which are derived from pITNs that expresses the FRT recombinase. In some cases deletion was achieved through phage P22 transductions in Salmonella. Overexpression of proteins such as GFP/RFP use pFPV, pDiGC, pWSK, pRK5 and related plasmids.

**Origin & function**

Genetically modified microorganisms that will be used will be mostly for the generation of fluorescent strains expressing either GFP/YFP/CFP/RFP for use in fluorescence microscopy. Other GMM strains will be obtained commercially or from other scientists.

The following strains of *Salmonella enterica* Typhimurium and *Listeria monocytogenes* have been received - SB136 (InvA::ahpC), SB2199 (delta 0PrgIJ ), SB2176 (delta...
Type I and II secretion systems and flagellin genes will be targeted in Salmonella. GMM organisms of biohazard group 2 will be used for work to assess the role of the host immune response to bacterial infectious agents. This will involve work with cell lines and mice following appropriate operating protocols and rules where applicable. Targeted gene deletions will involve known classes of transcriptional units such as the type-3 secretion systems or flagellins to study their roles in the activation of innate immune signalling pathways. Adenovirus serotype 5 (attenuated, lacking early virulence genes) expressing GFP will be used.

No experiments are planned that involve intentional creation of strains more pathogenic than parental strains. All GMM involves mutation or deletion of virulence genes that should lead to loss of virulence on parental strains. Most gene deletions will be carried out in collaboration with other established laboratories in the MRC CMBI to use their expertise in microbiology and microbial genetics.

Innate immune signalling genes such as capsase-1, caspase-11 and NLR and ALR genes may be expressed in eukaryotic cells using retro or lentiviral transduction to study their role in signal transduction.

Evaluation of foreseeable effects

No experiments are planned that involve intentional creation of strains more pathogenic than parental strains. All GMM involves mutation or deletion of virulence genes that should lead to loss of virulence on parental strains. Most gene deletions will be carried out in collaboration with other established laboratories in the MRC CMBI to use their expertise in microbiology and microbial genetics.

Innate immune signalling genes such as capsase-1, caspase-11 and NLR and ALR genes may be expressed in eukaryotic cells using retro or lentiviral transduction to study their role in signal transduction.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GM animals in use are no more hazardous to human health or the environment than wild type. When infected with Class 2 GMO's the animals will be held in IVC's within the Imperial College Animal facilities at South Kensington.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: Treatment with minimum final concentration after dilution with the waste of 1% Virkon for 60min followed by disposal in the sink. (minimum 1% Surfanios for mycobacteria)
Solid Waste: All other hazardous solid biological waste such as tissue culture plasticware (pipettes, flasks etc) will be autoclaved prior to disposal. Petri dishes with agar will be autoclaved prior to disposal.
Animal carcasses and bedding will be disposed as per BCS guidelines, also described in local rules.

13. * Is an emergency plan required according to regulation 20? Yes No
If "Yes", please check to confirm that it is attached to this form
14. *
Bio1 Form GMIC 2483
Role of inflammasomes during bacterial infections, GMIC-2483. Dr A Shenoy, Microbiology, Department of Medicine, South Kensington Campus.

Comments: HC explained this was a new group, in a new laboratory.
The Committee discussed the difference between a droplet and aerosol and the risk inhalation or of an eye splash. HC confirmed all the tissue culture work would be done in the Microbiological Safety Cabinet.
IH recommended adding an explanation to show human health was being protected while carrying out the work.
IH advised when referencing the Local Rules in the Bio1 form; to specify what section of the local rules to refer to.
The following amendments were required prior to approval: From first committee meeting Jan 2014
2.11: This is not correct about M avium being non-pathogenic. Please remove this statement
2.17: Use of GMO's includes those that have been created elsewhere but you are using in the lab. Please make sure that all imported GMO's are listed.
2.2: Add "and similar" to the cells lines to make it more generic.
4.1:0 Please be specific about the Mycobacterium sp
4.13: What is Vesphene and is there any validation data for this disinfectant?
4.18: Where it says generated; it really means used, so please include all imported GMO's in this statement. We have amended the Bio1.
4.19: Please explain why not?
4.22: Is the 2-3L of E.coli in one flask? Please give approximate numbers and volumes of a typical culture.
For all the following sections: Please provide a more accurate reference to the Local rules to make it easier to find the relevant text.
4.21: Please be specific about the Mycobacterium sp

5.1: Is there any risk of eye splash or inhalation/ingestion during streaking out especially the airborne/inhalation infection route organisms? We have had a few eye splashes during these procedures.
Explain why is Tissue Culture conducted in the MSC and not the work with infectious microorganisms?
5.2: What is meant by a sealed container? The local rules do not specify
5.3: What is being used to double contain the material? The local rules do not specify.
5.7: 30 minutes is a bit short. HSE in the past have suggested at least an hour.
5.8: The local rules do not describe the containment of this material in the shaking incubator. Please provide a description of the sealed containers that you are using.
5.10: There are some blanks in this section that need completing. Where will the work take place? Have you checked with CBS that your training in the US is valid in the UK?
5.12: Is there any plan to run viable material through an FACS?
- Describe that as a result of using Trizol in the process, this will inactivate the materials.
5.13: Where are the protocols for working with LN2 in the local rules? Please provide an accurate reference to the page and section or attach a separate SOP
7.1: Please specify that the concentration of virkon is 1% after dilution with the waste and give the contact time. What is Vesphene and is there any validation data for its effectiveness against M. avium etc
13.4: Please clarify the sentence.
15.1: What about the gene knock outs and deletions?
Agreed: Class 2, Containment Level 2. The HSE need to be notified.

The following comments were required by committee prior to approval following the second meeting in April 2014 to discuss significant changes made to the Bio1 by the PI following the first committee

1.2: Change “tissue culture cabinet” to “microbiological Safety Cabinet”. Please also clarify the ‘fixed samples’.

2.10: The agents listed need to be in the COP also.

2.12: VSV is ordinarily a HG 3 animal pathogen. However you should state that VSV may be worked on at CL2 under the circumstances of this project as the use as part of an assay system and the small quantities proposed allow for reduced containment under a clause in the classification of animal pathogens by DEFRA
A SAPO licence will still be required.

2.15: Sendai virus although Hazard group 1 for humans will be a class 2 GMO on environmental grounds.

2.16: Do any of these plasmids contain the WPRE capable of expressing all or part of the X protein?

2.17: What are the characteristics of the “other GMM strains” obtained commercially or from colleagues? Please clarify.

4.12: Please be specific about the Mycobacterium Sp and list them.

4.13: 10% virkon will not be effective for the Mycobacterium strains listed in 4.12. The Committee recommends surfanios as an alternative.

5.1: Change “cat 2 biosafety cabinet” to Class 2 Microbiological safety cabinet”

5.9: Can you please use blunt needles for cell lysis? They are available from regular suppliers of needles.

5.10: Please complete a GM Form B and submit for approval.

5.12: We will need an SOP for this. It need only be short but this is required to ensure consistency in the process.

5.13: Is there an SOP for the FACS work? This will be needed before this work can proceed.

6.5: The work requiring eye protection needs to be more clearly identified. This can be done in an SOP. It is not clear from this risk assessment when the eye protection is required. 5.1 briefly mentions it but I doubt any member of staff reading this will be clear on exactly when to put on the eye protection. A much clearer statement such as "Eye protection will be worn at all times when working with hazard group 2 microorganisms or class 2 GMO's outside of the MSC" would be better. Even then it should be considered whether this is adequate as eye protection only protects the user not those around them. Where there is a risk of splash then use of an MSC should be given priority.

7.1: Animal carcasses and bedding?

8.1: Autoclave Load validation, and thermocouple calibration requirement is annual. The quarterly testing is for maintenance

Section 9: needs SOP references

Section 10.1: Validation data for Vesphene? Please provide a reference. Also we need SOP references. Where in local rules can this be found?

Section 11: We need the SOP? COP references.

15: Class 2 refers to a GMO, technically the expression of the inserted genes is in a Hazard group 2 organisms which can then lead it to be defined as a Class 2 GMO. This is not always the case Sendai virus is ACDP hazard group 1 but will be a class 2 GMO on environmental grounds.

Comments: : HK will revise the Code of Practice and Local Rules.

IH will confirm if a SAPO licence is required.

Subsequent to the meeting IH confirmed the following. VSV is ordinarily a SAPO HG3 organism. According to DEFRA guidance on SAPO's
Where small quantities of vesicular stomatitis virus are being handled as part of a plaque assay system for human immunodeficiency viruses, Category 2 containment is sufficient. Any procedures likely to cause aerosols must be performed in a microbiological safety cabinet, and any persons having contact with the virus must not have contact with equidae for 48 hours thereafter. In all other circumstances, Category 3 containment is required.

SH pointed out that the list of pathogens needs to be made clear. The committee requests notification of any additional pathogens introduced at a later stage of the research.

SH checked if the cells were commercially available?

IH confirmed they were.

The Committee suggests that 10% virkon will not be effective for the Mycobacterium strains listed in 4.12. BR recommends surfanios.

A GM Form B need to be completed with reference to the information provided in 5.10.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

Could you please ensure that the risk assessment is revised (add GMIC number) and the amendments above addressed. I will then forward the form onto Occupational Health to complete. The completed HSE CU2 form (attached to this email), and copy of the Purchase Order (for BACs Payment) made payable to the HSE should be sent to me (s.joomun@imperial.ac.uk), I will then send it all to the HSE. Please ensure the information given in HSE Form relates to that provided in your risk assessment.

For further information, please see http://www3.imperial.ac.uk/safety/subjects/biosafety/gmprocedures

Please note that this work must be notified to the HSE and an acknowledgement receipt received prior to this work commencing.

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**Project Ref** 8/14.5

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<tbody>
<tr>
<td>04/08/2014</td>
<td>Gene regulation in bacterial pathogens</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

- Withdrawn: N
- Tick if notifying a connected programme of work: N

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Historical Significant Changes

02/03/2022
Project Additional Information

Purposes of the contained use

To understand how bacteria control expression of genes in vitro and in vivo and will involve using strains listed in section 2.1 to:
1. Construct recombinant versions of proteins that make up the transcription machinery
2. Isolate total cellular DNA and RNA
3. Introduce fluorescence reporters to study gene expression processes.
4. Make deletions in selected genes.
Genes of interest will be expressed in the hazard group 2 bacteria listed as hosts below and in the associated Bio1 risk assessment and the phenotype analysed via numerous methods including flow cytometry, mass spectometry, immunoblot, ELISA and several in vitro biochemical analyses etc.
Genes of interest will be deleted from bacteria by site directed mutagenesis and wild type or mutated genes reintroduced into the mutants. Random insertional mutagenesis using mini-Tn5 derivatives will also be used in an untargeted approach to identify new virulence and gene regulatory determinants.
Sorting of bacteria will be conducted using a dedicated FACS situated within a microbiological safety cabinet (to be installed in the Flowers building in May 2014). All other flow cytometry uses fixed samples.

Recipient or parental organism
Staphylococcus aureus (NCTC8325, USA300, Newman) (ACDP HG2)

Host/vector system
pET vector and derivatives, pBAD vector and derivatives

Origin & function
Origin- Genes associated with gene regulation such as genes that encode subunits of the RNA polymerase (the enzyme responsible for RNA synthesis in bacteria) and regulatory factors of the RNA polymerase (e.g. sigma factors, DNA binding transcription factors)
### Evaluation of foreseeable effects

The mutants generated will either be loss of function mutants or carry non-toxic reporter genes. It is not anticipated that bacterial strains produced in the laboratory are more virulent than strains that already exist in the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n.a

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Material used for imaging will be inactivated by fixation using 4% paraformaldehyde for 20min or cold (-20°C) methanol for 3min. Material for isolating RNA/DNA/proteins will be inactivated using the Trizol reagent - which will inactivate biological materials.
- Liquid waste: 1% virkon after dilution with the liquid waste(min exposure time of 30 min) or autoclaved directly.
- Solid Waste: Autoclaved : Solid waste e.g. agar plates Program 7 - Plastic discard 134°C 3 minutes
- Monitor of treatment e.g. chart recorder attached to autoclave: Load temperature probe; a print out of each cycle, retained for 6 months
- Validation of treatment: Annual 12 point thermocouple test; all cycles verified using dummy loads; records of tests kept by Biochemistry lab services
- Route of disposal: After autoclaving, transported in a closed container to the clinical waste compound at the back of the SAF building; removed from site by a specialist waste contractor

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

N
2.10: Staph’s, are these all MRSA please make it more clear which ones are and which are not?
2.10: Xanthomonas oryzae this this may need a FERA license IH will check.
2.12: Xanthomonas oryzae?
2.14: It is not clear that knock out mutants of the pathogens are being reconstituted to full function as described in
1.2. Please make it clear what genetic work is being carried out.
2.15 and 2.16: Are any of the phages going to be used as hosts or vectors?
2.17: Please give some examples to help set the limitations of the work.
4.6 and 4.14: Briefly describe how the phage are isolated from the ultrafuge supernatant of banding. If needles are
used this will need an SOP.
4.16: If this is repeated information reference a section where this info is given, otherwise please complete.
4.21: PA can cause problems through contact with the eyes especially for contact lens wearers.
5.1: There are other organisms not mentioned in this section but listed in 4.21 where you have identified the airways
or eyes as a route of infection and yet work such as plating and streaking out is listed as being carried out on the open
bench. It has been demonstrated in previous accidents that this sort of work can generate splash and aerosol giving
rise to potential for exposure. Why is this work not conducted in an MSC? How do you propose to protect staff both
the worker and those stood next to them from exposure?
I note that ALL tissue culture will be carried out in an MSC. Why is this given priority over handling pathogens?
5.2: Will the tubes have sealed lids?
5.6: ATCSA schedule 5 and Shigella? This has not been mentioned previously. If this is an error please remove
reference to Shigella and work with material on the ATCSA schedule 5 or amend previous sections and speak to the
safety department.
5.7: Applying 70% ethanol or 1% virkon to a puddle of spilled culture is not going to be optimal. You would need to
apply a concentrated disinfectant to the spill such as 5% virkon.
5.8: Any culture of HG 2 or GM class 2 organisms must be affixed in the shaking incubator using clamps and the
culture flask must be sealed sufficiently to prevent a spill if it is dropped or knocked over. Use of sticky mats and
cotton wool or foam bungs is not a sufficiently robust procedure for HG2 and GM class 2 work.
To allow gaseous exchange during culture; screw topped culture flasks with a gas exchange filter top are available
that prevent the loss of liquids even when inverted.
5.9: How are density gradient bands from ultracentrifugation removed from the tubes?
6.5: Safety glasses are not mentioned elsewhere. Where they are used, needs to be made clearer. If necessary
refer to the precise location in the local rules where this is dealt with. Why only use with PA? Other organisms in 4.21
have been identified as infectious though contact with the eyes.
7.1: Specify that the virkon is 1% after dilution with the liquid waste and give a contact time.
10.1: Treat spills with a concentrated disinfectant such as 5% virkon. This is more likely to be effective than 70%
ethanol or 1% virkon, as the ethanol/virkon at this low concentration is diluted to less than its validated concentration by the spill.

Section 14 and 15: Use of radioisotopes was not mentioned in 1.2? Why is it mentioned twice in section 15?

Comments: SW presented an overview of his proposed work.
IH asked if SW could be more specific about the genes that were going to be inserted or altered or at least give some examples.
SW said that he planned to work on at least 250 different genes.
JP asked if the knock outs were then resurrected to full function?
SW confirmed that they were.
JP asked if any of the GMO’s would be more hazardous than the non GM organisms
SW said they were not.
IH asked SW to make it clear in 2.14 that there would be knock out and reconstitution of function associated with the work. This is not clear at the moment
SH asked if Xanthomonas oryzae required a FERA scientific license.
IH said that it was a pathogen of rice and may need a license. IH will confirm this.
SH asked if microorganisms were cultured?
SW said that they were.
IH asked if these were class 2 GMO’s?
SW confirmed that they were.
IH explained that the committee could not approve a project where Class 2 GMO’s were placed on sticky mats in a shaking incubator. They had to be secured in firmly affixed clamps and that the flasks had to be suitably sealed to prevent leakage in the event a flask fell over.
SH stated that it was the PI’s responsibility to ensure that precautions stated in the risk assessment, once approved, were adhered to.
IH reminded all present that it was a contract with ICL, the GM committee and where notifiable, the HSE as well.
SW stated that he would want to culture up to 1L of MRSA USA LAC 300
IH pointed out that the material needed to be properly contained to prevent spills or breakage if dropped, not only in the incubator but during transfer of materials to and from the incubator.
SW stated that he would specify that eye protection was worn.
IH asked SW to specify when in the risk assessment.
IH stated that as he was taking minutes he would send additional comments to the committee secretary by e-mail for consideration by TC.
Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

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**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tr>
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02/03/2022

Page 329 of 15326
### Project Additional Information

**Purposes of the contained use**

Mycoplasmas are the smallest cell wall less, free-living microorganisms. The lack of a cell wall makes them resistant to many of the common antibiotics. Every year, infections caused by Mycoplasmas in poultry, cows, and pigs, result in multimillion euros losses in the USA and Europe. Currently, there are vaccines against \( M \) hyopneumoniae in pigs and \( M \) gallisepticum and \( M \) synoviae in poultry. However, there is no vaccination against many Mycoplasma species infecting pets, humans and farm animals (i.e., Mycoplasma bovis cow infection). Mycoplasma species in many cases are difficult to grow in axenic culture and those that grow need a complex media with animal serum. In large scale production of Mycoplasma species for vaccination aside from the high cost of animal serum, more important is the high irreproducibility in the production process and the possible contamination with animal viruses. All this together highlights what European industry needs: i) a defined cheap reproducible medium that is animal serum free and ii) an universal Mycoplasma chassis that could be used in a pipeline to vaccinate against Mycoplasma species, as well as any pathogen. \( M \) pneumoniae is an ideal starting point for designing such a vaccine chassis. It has a small genome (860 kb) and it is probably the organism with the most comprehensive systems biology data acquired so far. By genome comparison, metabolic modeling and rationally engineering its genome, we will create a vaccine chassis that will be introduced into an industrial pipeline. The process will be guided by the second world largest industry on animal vaccination (MSD), as well as a SME specialized on peptide display and screening. This will ensure the exploitation and commercialization of our work contributing to maintain Europe privileged position in this field. Our ultimate goal is to meet the needs of the livestock industry, taking care of ethical issues, foreseeable risks, and prepare effective dissemination and training material for the public.
Mycoplasma pneumoniae is a human pathogen (hazard group 2) that causes a form of atypical bacterial pneumonia. Symptomatic infections tend to develop over a period of several days and manifestation of pneumonia can be confused with a number of other bacterial pathogens and conditions that cause pneumonia. Common symptoms are mild and include sore throat, wheezing and coughing, fever, headache, coryza, myalgia and feelings of unease, in which symptom intensity and duration can be limited by early treatment with antibiotics (macrolides, tetracycline, ketolides, and fluoroquinolone). 15% of cases, usually adults, remain asymptomatic (Source: http://en.wikipedia.org/wiki/Mycoplasma_pneumoniae).

Host/vector system

GM mycoplasma will be engineered from the M129 laboratory strain (J Bacteriol. 1976 Jan;125(1):332-9). Plasmid based Vector systems used in this project will be customized. Plasmids carrying modifying DNA will be prepared in non pathogenic host E. coli (Top10 strain, GM Class 1), obtained from invitrogen.

Origin & function

The GM aim is to achieve attenuation by deleting/modifying the regulation of key genes on the genome. We will make genomic modifications (deletions and putting essential genes under designed promoters) to attenuate M. pneumoniae, to restrict growth to chemically-modified medium, in vitro only. Thus we will prevent growth in vivo.

Evaluation of foreseeable effects

We will make genetically-modified attenuated M pneumoniae with auxotrophies, "kill-switches" and deleted virulence factors, to allow growth in vitro, but prevent cell division in vivo. The aim is to reduce or abolish pathogenicity, while maintaining potential immunogenicity (vaccine strain). The auxotrophies and kill-switches will be lost in the absence of selection pressure, via cell death. Hence, there will be no increased risk to humans or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: Disinfecting culture will be diluted in a validated disinfectant, virkon to the final concentration of 1% and incubated for at least 30 mins and then only the disinfected cultures will be disposed of correctly (via drains). Solid waste: autoclaved at a minimum of 121 degrees for minimum 15 minutes holding time and disposed of via clinical waste disposal. Autoclave is serviced and subject to a multi point calibration and load validation test annually.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form
Following the discussion of your proposal entitled “MycoSynVac (P51776): Engineering of a Mycoplasma pneumoniae attenuated strain as part of a broad-spectrum animal vaccine”, GMIC-4589, at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

1.3: For clarity please confirm that all the Class 2 work handling and manipulating M pneumoniae is carried out in 443 (COP states that the MSC is in 433? Typing error?) , the lab with the MSC and dedicated incubator, storage is in 439? What happens in 438? General Molecular biology? Section 14 mentions lab 433, what is this lab used for or is this a typing error?

4.22: What is the largest single volume?

5.4: Please add double contained according to IATA regulations and in line with College Policy and guidance on transport of dangerous goods.

5.7: 30 minutes is probably not enough. Please increase this to 1 hour while advice is sought form the lab manager and safety officer.

5.8: Is this a shaking incubator? How are spills from the culture vessels prevented? Are the vessels leak proof? E.g. Ventilated lids rather than cotton wool plug?

6.5: Please answer no if no other PPE used.

7.1: Presumably liquid culture is disposed of via drains, not to the orange bag?

9.0: Is any refresher training carried out for emergency procedures? This could take the form of an annual review of the lab COP with all staff and a review of the emergency procedures.

10.2: Also follow the OH guidance on Laboratory accidents

15: Please mention the organisms in use e.g. Cloning in attenuated K12 E.coli strains Top 10 etc. DNA construct amplification in Attenuated E.coli K12 derived strains

Comments: MI gave a brief description of the work.

JP asked if 1% virkon was effective against mycoplasma?

MI responded that is was susceptible.

HK checked if there were any problems with mycoplasma bacterial contamination from external sources?

MI said they had not had this problem and they strictly kept everything separate for this reason.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

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</tbody>
</table>
### Project Additional Information

**Purposes of the contained use**

Use of *M. tuberculosis* and HG2 mycobacterial strains

My laboratory explores deciphering the environmental adaptation of Mtb within the host. Mainly Metabolomics, and Transcriptomics, Proteomics, and Lipidomics are used as tools for the read-out of the first steps in this adaptation. Effectively, the success of Mtb as a pathogen partially results from its capacity to invade, survive and persist within intracellular phagosomes and extracellular sites in many host tissues. Throughout the cycle of infection, Mtb encounters and survives in a variety of harsh environments in the human body including nutrient-poor, acidic, oxidative, nitrosative and hypoxic niches. Very little is known about the molecular mechanism and kinetics of adaptation of Mtb during the first stages of infection within the host. Deciphering these mechanisms in such defined environments is crucial to understanding the physiology of Mtb within the host and can also inform on us why Mtb is such an efficient intracellular pathogen. The findings will potentially lead to the discovery of new drug targets and have a better understanding on resistant bacteria in context of the host.

**Recipient or parental organism**

*M. tuberculosis* (HG3) use in my laboratory is pathogenic and can cause Human Tuberculosis (TB). TB is a bacterial infection spread through inhaling tiny droplets from the coughs or sneezes of an infected person. It is a serious
condition, but can be cured with proper treatment. TB mainly affects the lungs. However, it can affect any part of the body, including the lymph glands, bones, and nervous system.

M. marinum (HG2) causes opportunistic infections in humans. Typically, clusters of superficial nodules are described at the extremities (hands most of the time) due to its ability to infect cooler part of human body. Its growth is inhibited at 37 degree celsius. M. marinum can causes a rare disease which typically affects individuals who work with fish and keep home aquariums.

M. bovis BCG (HG2) is a vaccine against Human Tuberculosis and is not reported to cause disease in Human as M. smegmatis mc2 155 (HG1).

Non-pathogenic mycobacterial species (M. smegmatis)

Opportunistic pathogenic M. bovis BCG, M. marinum: HG2
M. tuberculosis: HG3

Fully drug sensitive laboratory strains such as M. tuberculosis H37Rv, CDC1551
Transposon mutants of M. tuberculosis from the BEI resources
Severely attenuated mutants of M. tuberculosis for use under Containment 2
Bleupan DleuD DpanCD hygR
mc2 6020 DlysA DpanCD hygR
mc2 6030 DRD1 DpanCD hygR
mc2 7000 DRD1 DpanCD unmarked

Fully drug sensitive field isolates of M. bovis

NB: derivatives mean different background of the strains such as E. coli K12 derived non-toxigenic attenuated lab strains e.g. DH5-alpha, BL21,... for mycobacterial strains, this means generation of genes deletion mutants and complemented strains conducting to a loss or decrease in virulence and pathogenicity. No work will investigate increase in virulence and pathogenicity.

Host/vector system

Plasmids to clone and express recombinant proteins in E. coli; integrative plasmids for mycobacterial mutants and complemented strains
pSMT3 and derivatives: ColE1 and pAL500 origins of replication. Not mobilisable
pYUB854 and derivatives: OriE. non mobilisable
pMV261 and derivatives: OriE. non mobilisable
pHAE159, pHAE87 and derivatives: Derivatives of conditionally replicating mycobacteriophage PH101 (ts; Bardarov et al 97)
pKINTA and derivatives: non mobilisable integrating vectors
pET and derivatives: protein expression system, non mobilisable
pVV16 and derivatives: protein expression system, non mobilisable

Origin & function

Inserted genes may be self cloning of mycobacterial genes, either in the sense orientation to allow over-expression, or in the antisense orientation to allow down-regulation, under the control of either an inducible or constructive promoter. Mycobacteria do not express any toxins and there is little risk of altering pathogenicity by this method.Selectable markers are usually either Hygromycin (not used clinically) or kanamycin (not part of first line therapy), sometimes in combination with the sucrose and the SacB enzyme as a counterselectable marker.

Reporter genes includes a range of fluorescent reporters such as GFP or mCherry, luminescent reporters such as lux operon from Photorhabdus luminescens, or the luciferase genes from Firefly or Gaussia princeps. Reporter enzymes such as beta-galactosidase, or reporter antigens such as the OVA peptide.

Evaluation of foreseeable effects

There is no evidence that any of the recombinant mycobacteria made in this project will be any more hazardous than wild type strains. There is published evidence that...
disrupting some two-components regulators alters virulence in immunocompromised animals, but this does not alter the way in which mycobacteria are handled under containment level 3 conditions. Some samples may have drug resistance so in theory may be more hazardous than wild type that are susceptible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM Class 3 and HG 3 material in CL3 Labs
All materials used in the CL3 facility are to be regarded as potentially contaminated and disposed of accordingly. All waste liquid and solid waste must be autoclaved before leaving the laboratory and disposed of in orange clinical waste bags for clinical waste disposal. Refer to the CL3 COP for details.

Liquid waste is pre treated with Surfanios at 10% final concentration and left overnight in secure vessels.

60L and 150L autoclave. Minimum 121 degrees for 15 minutes holding time. Final disposal into orange bags for clinical waste disposal.

Autoclaves are maintained and calibrated annually and subject to an annual 12pt thermocouple waste load validation test

GM Class 2 Material & Hazard Group 2

Liquid cultures of HG2 and class 2 GM myco bacteria are treated with 10% surfanios overnight before being autoclaved and disposed of as clinical waste.

GM Class 1

Liquid GM class 1 waste is treated with 2% virkon final concentration for a minimum of 30 minutes before disposal to the drains.

All solid waste (contaminated plastics, gloves etc) from CL2 labs that has been in contact with HG 1 and 2 and GM class 1 and 2 material is autoclaved at 121 degrees for a minimum holding time of 15 minutes before final disposal into orange sacs as clinical waste.

Autoclaves are maintained and calibrated annually and subject to an annual 12pt thermocouple waste load validation test

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Dear Dr L,

Following the discussion of your proposal entitled "Chemical biology of Tuberculosis Pathogenesis" GMIC-4885 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

Department: Typo 'Lifes'

Complete all the ticks.

2.2: Bran – should be Brian.

2.3: Please provide an SOP describing how the urine is inactivated and how this is validated.

2.4: Coded - Please clarify that this means the samples are anonymous.

2.10: What is meant by M tb and M marinum derivatives? Please give some examples.

2.10: ACDP classification is HG1, HG2 or HG 3. Class refers to the GM classification not the wild type. Please change class to HG

2.11: Please describe under what circumstances M. marinum can cause disease (it is a HG2 pathogen) and M.bovis BCG is described as a pathogen by the HSE and is also HG2.

2.14: E.coli and derivatives? Please be a bit more specific and give examples such as E.coli K 12 derived non toxigenic attenuated lab strains e.g. DH5Alpha

Please also describe the scope of the GM work, e.g. What sort of modifications you are making and if necessary what you will not do. E.g. Increase virulence or pathogenicity.

2.15: E.coli and derivatives? Please be a bit more specific e.g. E.coli K 12 derived non toxigenic attenuated lab strains e.g. DH5Alpha.

M Bovis BCG and M marinum are described as pathogenic by the HSE, please remove all references to being non-pathogenic.

ACDP classification is HG1, HG2 or HG 3. Class refers to the GM classification not the wild type. Please change class to HG

5.1: Where are you handling the other Mycobacterium sp? Please mention if there are any circumstances where you would handle the HG2 or Class 2 GMO's in an MSC.

In this risk assessment you will need to make it clear where the Class 2 and HG 2 organisms are handled and where the HG3 and class 3 pathogens are handled.

5.7, and 5.8: What about the HG2/class 2 material?

6.4: What about the CL2 labs?

9: Spillage response and emergency procedures. We recommend that staff have refresher training at least annually. This may take the form of a desk top exercise or discussion with staff while reviewing the COP.

10.1: Does virkon work for the M Marinum and M Bovis BCG? Why not use surfanios?

Evacuate the whole lab and seek advice before allowing re-entry. It may take several hours to clear an aerosol at CL2 depending on air changes, titre and volume of the spill.

Comments: GLM gave a brief description of the work.

IH asked if Dr Robertson's (BR) Code of Practise would be used for this work.

GLM confirmed all the techniques were the same therefore; he would be using the same COP.

IH checked if BR would be conducting the training.

GLM said yes.

SH suggested updating the Local Roles for this work.

SH asked GLM to describe the process.

GLM explained TB biomarkers can be traced in 40-60% of human urine samples. The mass spectrometry will be used to analyse it.

SH inquired if the samples from Southampton University would have been decontaminated first and therefore has no bacteria. Do they do random checks for growth?

GLM said there were checks for HIV.

SH suggested getting a protocol from the collaborators regarding decontamination of the samples.

IH suggested including the room number of the mass spectrometry.

MR asked about the use of ethanol.

GLM explained it was very effective.

MR recommended explaining when and how virkon, ethanol, surfanios, etc, was used.

Agreed: Class 3, Containment Level 3. This project is notifiable to the HSE.
In this project, we would like to investigate in detail how the replicative capacity on the transmitted founder virus influences early T cell function, and how this might be associated with disease progression. This will involve amplifying full length HIV genomes from actute HIV patients, sequencing it to identify the transmitted founder virus and creating infectious molecular clones of the founder virus for use in HIV replicative capacity assays, and for a T cell viral inhibition assay. To investigate the relative contribution of each of the HIV proteins to viral replicative capacity, and its role in the viral inhibition assay, we will generate HIV chimeras using a lab adapted HIV backbone, switching each of its genes with the patient derived HIV genes and these use chimeras for replictaive
capacity assays and viral inhibition assays.
Eventually, we hope to identify targets and mechanisms that might be important for T cell inhibition of viral replication that might inform the development of an effective HIV vaccine.

The samples that we are working with are not cultured, but as they are all from acute HIV patients, some of the viral loads are high. Our procedure is to transfer the sealed frozen vial inside a sealed container to a MSC and allow it to thaw, then immediately lyse the virus from the plasma within the MSC, rendering the virus non-infectious.

The Qiagen viral lysis buffer AVL contains 50-100% Guanidinium thiocyanate. Guanidinium thiocyanate is a well-known chaotropic agent that is commonly used to lyse virus before extraction of nucleic acids. The lysis step is described on the highlighted section on page 8 of the attached RNA extraction protocol.

1. Viral RNA extraction from HIV infected plasma, cDNA synthesis and HIV amplification by PCR will be done, and the DNA shipped to Emory university (Atlanta, USA) for full length sequencing.
2. Once the sequences have been analysed and the transmitted founder virus identified, we will generate infectious molecular clones and HIV chimeras.

This will involve:
   a) Cloning of the complete patient-derived HIV genome into a pBluescript vector
   b) Cloning of various HIV genes (Gag, Pol, Nef) onto a lab adapted HIV proviral backbone to generate chimeric HIV that only differs by the inserted gene
   c) Storing the cloned DNA from (a) and (b) above until it is eventually used to generate virus stocks.
   d) Transfecting this DNA onto 293T cells to make virus stocks of the infectious molecular clones and chimeras at the IAVI CL3 lab on the same floor that is routinely used for HIV culture work involving HIV positive sample

Recipient or parental organism

E.coli competent bacteria cells such as JM109 will be used for plasmid manipulation and amplification.
Many derivatives of the E. coli K-12 and B strains have been demonstrated to be non-pathogenic and are well understood.
The host strain bacteria are non-colonising and disabled. They are equivalent to hazard group 1, as they are not pathogenic to humans or animals. They often have auxotrophic requirements which are unlikely to be satisfied outside laboratory cultures. They have very limited survivability in the environment.
These bacteria will be manipulated in a CL2 lab.

Host/vector system

The inserts will be carried on mammalian expression vectors such as pcDNA3, pcDNA3.1, pEF6/V5-His, pENTR-TOPO, pLent6/R4R2/V5-DEST, pBluescript, pUC18, pBR322, pCR-XL-TOPO, pGRN145, pGTU-Luc, pGTU, pEZMO2, pET101, and the DNA vaccine vector pVRC. Several vectors may be tested for efficiency of cloning the HIV insert and the most efficient chosen.
These plasmids also contain bacterial origin of replication and bacterial selection genes such as those encoding Ampicillin, Kanamycin and Chloramphenical resistance. Some plasmids also contain markers of mammalian selection e.g. neomycin, puromycin, hygromycin and blasticidin resistance.
Vectors are considered to be non-mobilisable thus gene transfer is a remote possibility. Neither the vector nor the inserted genes would be expected to confer any selective advantage to the micro-organism or related microorganism. All inserts in the vector will be poorly expressed but the extra burden placed on the micro-organism or on a related micro-organism (in the unlikely event that transfer occurs) will lead to non-transfected micro-organisms having a selective advantage over the GMM.
For the construction of HIV chimeras, a lab adapted HIV proviral backbone will be used. This will be ~12kb, and will be derived from the study cohort and cloned onto pBluescript. Its functional characteristics are expected to be similar to those of HIV proviral DNA.
These vectors will all be handled in a CL2 lab.

Origin & function

We will amplify HIV from plasma obtained from acute HIV infected patients. These plasma samples are from a Kenyan cohort that is predominantly infected with HIV Subtype A, D and A/D recombinants. Once we amplify HIV from the plasma, we will clone it to generate virus stocks of infectious molecular clones and chimeras of the autologous virus.

These infectious molecular clones will be used in viral replicative capacity assays to measure virus fitness and in viral inhibition assays to measure T cell killing of HIV.

1. Full genome HIV will be cloned onto an appropriate vector from 2.16 above - This is expected to have the same characteristics as the patient virus. The purpose of the cloning is not to change any functional property, but rather to study each virus in isolation.

2. Short dsDNA fragments of HIV genome will also be cloned onto a HIV proviral backbone. These will include:
   - GAG (group specific antigen): is one of the core structural protein of HIV and comprises of p24 (makes the viral capsid), p6 & p7 (nucleocapsid) and p17 (protective matrix).
   - POL: encodes the viral enzymes such as RT, Protease and integrase. During viral replication protease cleaves off the Pol polyprotein away from gag to allow viral maturation.
   - NEF(Negative Regulatory Factor): Nef downregulates CD4 (primary viral receptor) and MHC class I molecules. It also increases viral infectivity.
   - TAT (transactivator protein): increases efficiency virus transcription
   - REV: regulates traffic of virus RNA species out of the nucleus,
   - VIF: Counteracts antiviral effect of cellular APOBEC proteins,
   - VPR: Contains nuclear localisation sequence,
   - VPU: Down-regulates CD4, transports Env proteins to cell surface, counteracts antiviral effect of cellular tetherin proteins,
   - ENV: Encodes the viral envelope proteins gp120 and gp41, these proteins mediate virus-entry into target cells.

This manipulation of DNA will all be carried out in a CL2 lab.

Evaluation of foreseeable effects

We are going generate two broad categories of GMMs:

1. E.coli carrying full length HIV genome. HIV will not replicate in E.coli as HIV requires multiple cellular genes to support its life cycle, so no live virus will be in the E.coli culture. E. coli K-12 and B strains have been demonstrated to be non-pathogenic and are well-understood and are non-colonising and disabled. They are equivalent to hazard group 1, as they are not pathogenic to humans or animals. They often have auxotrophic requirements which are unlikely to be satisfied outside laboratory cultures. They have very limited survivability in the environment.

2. HIV infectious molecular clones and chimeras - These cloned viruses are however expected to have the same hazard as the wild type patient viruses - if transmitted can cause AIDS. The purpose of the cloning is not to change any functional property, but rather to study each virus in isolation. The virus stocks will be produced and stored in a CL3 lab.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation. All infectious material will be handled in a CL3 lab.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Containment Level 2</th>
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<tbody>
<tr>
<td><strong>Solid CL2 waste</strong> is disposed in orange bins and autoclaved before disposal to hospital clinical waste.</td>
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</tr>
<tr>
<td><strong>Pipette Tips</strong> are disposed in bio-bins which are sealed and autoclaved before disposal to hospital clinical waste.</td>
<td></td>
</tr>
<tr>
<td>Liquid waste is disinfected in 2% virkon (final virkon concentration 1%) for 30 minutes before disposal to drains. Minimum Log5 reduction in biological agents.</td>
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<table>
<thead>
<tr>
<th>Containment Level 3</th>
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<tbody>
<tr>
<td><strong>Solid CL3 waste</strong> is autoclaved within the CL3 lab before disposal to hospital clinical waste.</td>
<td></td>
</tr>
<tr>
<td>We will not generate large volumes of liquid CL3 waste as all grown virus will be stored. Small volumes of liquid waste (&lt;500ul) will be disposed alongside pipette tips in bio-bins and autoclaved alongside the solid waste within the CL3 lab before disposal to hospital clinical waste.</td>
<td></td>
</tr>
<tr>
<td>Waste Autoclaves are maintained and calibrated annually. The waste load cycle is validated annually using a 12pt thermocouple test on a worst case scenario load. Minimum temperature and holding time is 121 degrees for 15 minutes. This is a sterilization technique.</td>
<td></td>
</tr>
<tr>
<td>In all cases, autoclave tape is used to monitor the process. In addition, a print out detailing the temperatures and duration of each autoclave cycle inspected, signed and retained in a dated diary.</td>
<td></td>
</tr>
</tbody>
</table>

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |
Following the discussion of your proposal entitled "Amplification and cloning of full length HIV genomes to generate patient derived HIV infectious molecular clones, and chimeras", GMIC-5248 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

1.1: Typo in first paragraph ‘relicating’
1.2: Typo in d) ‘riutinely’
1.3: Point 3, the use of ‘infectious’ here would make this section CL3
2.2 and 2.5: Plasma from acute HIV infected patients, with a high likelihood of infection which you have identified as high risk in 2.5. According to COSHH this material should be handled in a CL3 lab. Please amend the risk assessment accordingly.
2.17: Please provide a flowchart and plasmid map with the process of insertion of HIV into a plasmid and at what precise point in the GM process is the viral genome able to express infectious HIV clones. Please also point out at what containment level these parts of the process this will be carried out. The stage at which viral particles can be expressed must be carried out in a CL3 facility.
4.17 and 4.18: You describe transferral across the mucosa as well as by percutaneous inoculation; this differs from what you explain at the meeting. Please clarify.
5.7: The Dangerous occurrence form should be replaced with Salus.
5.8: Generation of infectious clones? Is this double contained and where is it happening?
7.3: High titre HG3 culture waste needs to be autoclaved first. Only larger volumes of low titre culture aspirate can be considered for chemical treatment alone before disposal to drains. Virkon does not sterilize waste it disinfects it, please remove the word sterilized. Disinfection does not kill all microorganisms just reduces their number and virkon is unlikely to reduce the numbers to acceptable levels from high titre culture waste.
Section 10: Change references to an accident form to Salus. Also remove reference to the BioRisk manager and replace by College BioTeam
Why is the volume for a small/large spill different inside and outside the safety cabinet?
Section 14: Handling HIV plasma, change from 2 to 3. Generation of infectious HIV clones also need to be added as a CL3 activity.
Section 15: Needs to be from the point of creation of the HIV infectious clones, not just further manipulation. This point needs some clarification.
Comments: GM gave a brief description of the work.
RW asked if the work would be conducted in new laboratories?
GM confirmed this was correct and routine molecular work would be carried out in Containment Level 2.
MB asked if the high titre serum work would be carried out in the CL3?
DK answered ‘yes’ this would be in the initial phase only, once they have lysed the plasma it will go to CL2.
MB queried at what point would the plasmids become infectious?
DK explained this would be in the end part of the work.
MB And DK had a discussion on whether certain parts needed to be conducted at CL2 and CL3. DK and GM agreed to provide a flowchart explaining the work process and at what containment level that process will take place.
MB checked if splashes were likely.
DK answered this was very unlikely.
JP also pointed out that the initial lyse would render it non-infectious.
The committee needs assurance that the lysis phase reliably and robustly inactivates the virus, reducing viable viral particle levels to an undetectable level. Please forward an SOP to the Safety Department.
Agreed: Class 3, Containment Level 3. This project is notifiable to the HSE.

Project Containment

02/03/2022
Pseudomonas aeruginosa is able to infect a wide variety of hosts and tissues and is an excellent model for studying infection and secretion by gram-negative bacteria. The project is the coordination of research conducted on two different axes, which are central for the establishment of infection and persistence within the host. One axis concerned the characterization of molecular actors involved in bacterial biofilm formation and dispersion, the second axis is the study of a novel secretion mechanism, called type VI.

The type VI secretion system is required by a variety of Gram negative organisms for pathogenicity in a number of plant and animal models. The T6SS is proposed to deliver effectors towards the host cell or bacterial cell. We suggest that this novel secretion mechanism, called type VI, is involved in bacterial competition in a range of environments including plants, plant roots and soil.

We wish to establish the role of Pseudomonas species and their expression levels in lab settings as well as in a plant model of infection. We will also compete pseudomonas species with other plant pathogens and soil organisms to test for a competitive advantage in these conditions.
Biofilm formation may also be a positive characteristic in this environment and or in competition and we wish to investigate if a role is present. We will use Arabidopsis thaliana, Brassica oleracea, Nicotiana benthamiana, Solanum tuberosum and Solanum lycopersicum as hosts. They are all widely-used plant models and none of them will be genetically modified.

We will use a variety of bacteria that are listed in the accompanying risk assessment and in this form. These bacteria are hazard group 1 or 2 organisms however several of them require a FERA licence to be obtained prior to work commencing. Pseudomonas aeruginosa and Burkholderia are potential human infectious agents via broken skin contact. Eye splash and Inhalation. The other organisms are of no concern to animals or humans. However as mentioned several are plant pathogens and good microbiological practice including sterilisation of materials that they come in contact with will be undertaken to prevent any accidental release. All material will be treated in line with ADCP level 2 and local rules.

We are primarily interested in competition between Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae and Agrobacterium tumefaciens. This will be the organisms we mostly work on. Additional strains have been requested as well for infrequent use.

A recent study suggests that the model strain in our work, Pseudomonas syringae pv tomato DC3000, has been largely supplanted over the past decades in the UK and elsewhere, by 11 Pseudomonas syringae pv tomato strains (Cai et al. 2011), reducing risk to the environment.


Over view of the experimentl procedures:

We will study bacteria/host interaction by analysing the effect of P. aeruginosa and other pathogen infection in vivo using Arabidopsis. Arabidopsis will be grown in a plant incubator located in our lab (1.30).

We will study bacteria/host interaction by analysing the effect of P. aeruginosa and other pathogen infection in vivo using Arabidopsis. Arabidopsis will be grown in a plant incubator located in our lab (1.30).

Plant challenges are with Arabidopsis whole plants, and will be via vacuum infiltration. Viable counts will be made on infected leaves. Alternatively plant challenges will be via topical application or inoculation of the roots/surface. Wound models may also be used where a small cut or puncture wound is placed into the plant prior to being inoculated. Inoculations will either be monospecies or co-challenges of two bacteria. We have recently published a paper with our collaborators in Taiwan shown that the T6SS of Agrobacterium Tumefaciens allows it to out compete Pseudomonas aeruginosa but only in the host (Ma, Hachani et al. 2014). Ma, L. S., A. Hachani, J. S. Lin, A. Filloux and E. M. Lai (2014). "Agrobacterium tumefaciens deploys a superfamily of type VI secretion DNase effectors as weapons for interbacterial competition in planta." Cell Host Microbe 16(1): 94-104.

Recipient or parental organism

All plant pathogen strains requiring FERA approval will be handled at containment level 2 and good microbiological practices will be employed to prevent accidental release of these organisms. All FERA licenced strains will be disposed via the internal Biohazard system and any material that comes in contact will be decontaminated with 70% ethanol and/or Virkon (See sections 7 and 10).

Escherichia coli: Hazard Group 1/ GM Class 1

Wild-type K12 strains and derivatives thereof with required phenotypes for cloning and expressing pseudomonas genes or genes of other bacterial origin will be used in these studies. Strains commonly used in the laboratory include strain DH5alpha, XL1 Blue, TG1 TOP10F, CC118, S17.1 and BL21 (DE3). These strains have a widespread and long history of safe use. The BL21 strain carries the gene encoding the T7 ENA polymerase and is used for expressing genes cloned under a T7 promoter.

Pseudomonas aeruginosa: Hazard group 2/ GM Class 2

Pseudomonas aeruginosa is a Gram-negative, aerobic rod belonging to the bacterial family Pseudomonadaceae. The family includes other genera, which, together with certain other organisms, constitute the bacteria informally known as pseudomonads. These bacteria are common inhabitants of soil and water. They occur regularly on the surfaces of plants and occasionally on the surfaces of animals. Pseudomonas aeruginosa and two former Pseudomonas species (now reclassified as Burkholderia) are pathogens of humans. However, Pseudomonas aeruginosa is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. Pseudomonas aeruginosa infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is 50 percent. Pseudomonas aeruginosa is primarily a nosocomial pathogen. The overall incidence of P. aeruginosa infections in hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections. Pseudomonas aeruginosa is sometimes present as part of
the normal flora of humans and the prevalence of colonization of healthy individuals outside the hospital is relatively low. Infection may occur through direct and extensive contact with the organism, however no procedure in the laboratory will require direct contact. In addition, personal protective equipment (PPE) including lab coat and gloves will be worn when working with P. aeruginosa strains to further minimize the risk of direct contact with the organism.

We will use several laboratory P. aeruginosa strains including, PAO1, PA14, PAK, TB, CHA, PA103, SG17, PA7, X13273. We will also work with large collection of isolates that have been issued directly from patients at the hospitals at different stages of their life. The clinical records of the patients are available as well as the antibiotic susceptibility of the collected strains.

Burkholderia cepacia: Hazard group 2/ GM Class 2
B. cepacia is the name for a group or “complex” of bacteria that can be found in soil and water. B. cepacia poses little medical risk to healthy people. However, people who have certain health problems like weakened immune systems or chronic lung diseases, particularly cystic fibrosis (CF), may be more susceptible to infections with B. cepacia. B cepacia is a known cause of infections in hospitalized patients. The effects of B. cepacia on people vary widely, ranging from no symptoms at all, to serious respiratory infections, especially in patients with CF.

We will periodically use several laboratory strains including the sequenced genomovar III CF strain J2315

Pseudomonas putida, Pseudomonas fluorescens and Pseudomonas alcaligenes: Hazard group 1/ GM Class 1
P. putida is a gram-negative rod-shaped saprophytic soil bacterium. It demonstrates very diverse metabolism, including the ability to degrade organic solvents such as toluene. This ability has been put to use in bioremediation, or the use of microorganisms to biodegrade oil. Use of P. putida is a safe strain of bacteria, unlike P. aeruginosa for example, which is an opportunistic human pathogen. Similarly, P. fluorescens has an extremely versatile metabolism, and can be found in the soil and in water. It is an obligate aerobe but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration. Some P. fluorescens strains (CHA0 or Pf-5 for example) present biocontrol properties, protecting the roots of some plant species against parasitic fungi such as Fusarium or Pythium. Finally, Pseudomonas alcaligenes is a Gram-negative aerobic bacterium used as a soil inoculant for bioremediation purposes, as it can degrade polycyclic aromatic hydrocarbons and has no known pathogenic properties.

We will periodically use mostly two strains of Pseudomonas putida, WCS358 and KT2440, knowing that KT2440 genome sequence is available. We will mostly use the P. fluorescens Pf5, SBW25 and PFO-1 strains of which the genome sequence is available, and also the CHAO strain which has commonly been used in laboratories. Finally we will periodically use the P. alcaligenes strains M-1.

Pseudomonas entomophila L48: Hazard group 1/ GM Class 1
Pseudomonas entomophila is an entomopathogenic and metabolically versatile soil bacterium that, upon ingestion, kills Drosophila melanogaster as well as insects from different orders. This strain is not hazardous to human health

We will periodically use the sequenced strain CT573326 to study homologues of the genes in Pseudomonas.

Pseudoalteromonas haloplanktis: Hazard group 1/ GM Class 1
Pseudoalteromonas haloplanktis is a psychrophilic Gram-negative bacterium collected in Antarctic seawater, which produces endoglucanases and other hydrolases. The strain has no known pathogenic properties.

We will periodically use the sequenced strain TAC125 to study homologues of the genes of Pseudomonas.

Pseudomonas syringae: Hazard group 1/ GM Class 2 (FERA approval required)
Pseudomonas syringae is a Gram negative, plant-pathogenic bacterium, strains of which are noted for their diverse and host-specific interactions with different plant species. Specific strains are assigned to one of the over 50 known pathovars based on their ability to infect different plant species. A variety of symptoms are associated with woody plants infected by P. syringae. Symptoms depend on the species of plant infected and the strain of P. syringae. Common P. syringae infection-associated symptoms are: flowers and/or flower buds turn brown to black; dead dormant buds; necrotic leaf spots; discolored and/or blackened leaf veins and petioles resulting from systemic invasion and infection: spots and blisters on fruit. This strain is not hazardous to human health

We will periodically use three strains, all of which have their genome sequenced, i.e, patovar tomato DC3000, patovar syringae B728a and patovar phaseolicola 1448A.

Pseudomonas stutzeri: Hazard group 2/ Class 2 (FERA approval required)
Pseudomonas stutzeri is a nonfluorescent denitrifying bacterium widely distributed in the environment, and it has also been isolated as an opportunistic pathogen from humans. P. stutzeri is ubiquitous in hospital environments and that this species could be considered an opportunistic but rare pathogen.

Agrobacterium tumefaciens: Hazard group 1/ GM Class 2 (FERA approval required)
Agrobacterium tumefaciens is a rod-shaped, Gram-negative soil bacterium and plant pathogen. Agrobacterium tumefaciens is the causal agent of crown gall disease in dicots. Carries the Ti plasmid. This strain is not hazardous to human health.

Agrobacterium vitis: Hazard group 1 / GM Class 2 (FERA approval required)
Agrobacterium vitis is a bacterium species in the genus Agrobacterium. It is gram negative soil bacterium and is responsible for the crown gall disease of grape (plant pathogen). This strain is not hazardous to human health.

Pectobacterium carotovorum: Hazard group 1 / GM Class 2 (FERA approval required)
Pectobacterium carotovorum is a bacterium of the family Enterobacteriaceae; it formerly was a member of the genus Erwinia. The species is a plant pathogen with a diverse host range, including many agriculturally and scientifically important plant species. Two subspecies will be used atrosepticum SCRI 1001 and carotovorum SCRI 194. This strain is not hazardous to human health.

Pectobacterium carotovorum SCRI 1001 (rev 11/08) Page 5 of 15

Ralstonia solanacearum: Hazard group 1 / GM Class 2 (FERA approval required)
Ralstonia solanacearum, previously known as Pseudomonas solanacearum, is a plant pathogenic bacterium commonly found in the soils of tropical and subtropical countries. Certain strains are adapted to milder environmental conditions and have recently been isolated in northern European countries. This organism, responsible for bacterial wilts, can infect over 200 plants species. Major hosts include tobacco, tomato, potato, eggplant, pepper and banana trees. It also causes disease on the model plant Arabidopsis thaliana. This pathogen can lie dormant in water or soil until a host plant grows, then it enters the roots and colonises water-conducting vessels, from where it spreads throughout the plant. This strain is not hazardous to human health.

We will periodically use the sequenced strains GMI1000, together with currently sequenced strains Molk2 and 1649, to study homologues of the genes of Pseudomonas.

Acinetobacter baumannii: Hazard group 1 / GM Class 2
Acinetobacter is a group of bacteria commonly found in soil and water. Outbreaks of Acinetobacter infections typically occur in intensive care units and healthcare settings housing very ill patients. While there are many types or “species” of Acinetobacter and all can cause human disease, Acinetobacter baumannii accounts for about 80% of reported infections. Acinetobacter infections rarely occur outside of healthcare settings.

Acinetobacter baumli ADP1: Hazard group 1 / GM Class 2
Acinetobacter baumli is a strictly aerobic and widely spread soil bacterium with simple growth requirements and wide substrate range. The laboratory strain ADP1 has shown potential for metabolic studies and biotechnological applications, mainly for the compact easily-transformable genome and a unique metabolic network. It is an extremely rare opportunistic human pathogen that has been described once in the literature found in immunosuppressed patients.

Xanthomonas campestris pv campestris: Hazard group 1 / GM Class 2 (FERA not required as European strains)
Xanthomonas campestris pv campestris causes black rot and is considered a important and destructive disease of crucifers, infecting all cultivated varieties of brassicas worldwide. In this project only European strains will be used negating the need for a FERA licence. This strain is not hazardous to human health.

Mesorhizobium loti: Hazard group 1 / GM Class 1
Mesorhizobium loti, formerly known as Rhizobium loti, is a Gram negative species of bacteria found in the root nodules of many plant species where fix nitrogen. M. loti fixes the nitrogen from the atmosphere into ammonium (NH4+) with the enzyme nitrogenase. This is described as a symbiotic relationship because this nitrogen is fixed to a plant usable form; in return, the plant supplies M. loti with carbohydrates, proteins, and oxygen.

Host/vector system

1) We will used standard E. coli vectors:
   a) For cloning:
      - pBR322 and derivatives: AmpR. TetR.
      - pACYC184 and derivatives: ChiR. TetR.
      NB : These vectors could also be used as suicide vectors for P. aeruginosa mutagenesis.
   b) For gene tagging and expression:
      - pET22b/28c/28a and derivatives : ColE1 replicon, AmpR (22) and KanR (28). tag Histidine N or C-terminal.
- pQE30Xa and derivatives: Tag histidine (Qiagen). AmpR.
- pEGFP and derivatives: engineering of gfp fusion. AmpR.
- pGEX and derivatives: Tag GST (Glutathion S-transferase). AmpR.
- PAC or PAN and derivatives: AviTag. AmpR.
- pBADmycHisA, B, C and derivatives: expression from an arabinose promoter, tag histidine and Myc. AmpR.
- GATEWAY plasmids: InVitroGen. System including the entry vector pDONOR and the destination vectors (pDEST derivatives) for recloning and expressing tagged genes.
- pBAD and derivatives: arabinose promoter. AmpR.
- pT7-1 to pT7-6 and derivatives: T7 promoter. AmpR. To be used together with pGP1-2 which carries the gene encoding RNA polymerase from T7 phage. KmR.
- pUT18c and pKT25 and derivatives. Two hybrid system from Ladant. pUT18c (pUC19 derivative), AmpR, contains 3’ region of the gene encoding adenylate cyclase (cyaA). pKT25 (pSU40 derivative), KanR, contains 5’ region of cyaA.
- pBS1479 and derivatives: AmpR. Tandem Affinity Purification (TAP). The TAP-Tag contains a domain CBP (Calmodulin Binding Protein) and a domain Protein A (Affinity for IgG).

2) We will use suicide vectors for the engineering of mutant strains:
- pKNG101 and derivatives: StrR. SucR. Only replicate in
- pEX100 and derivatives: SucR. Only replicate in E. coli lambdapir strain CC118.

3) We will use broad host range vectors for expression in the strains listed in section 2.1 (also replicative in E. coli):
- pLAF1, pLAFR3 and derivatives : IncP, TetR. Cosmid with low copy number.
- pBBRIMCS and derivatives : ChIIR (1), TetR (3), KanR (2) AmpR (4), GentR (5).
- pUC18/19 and derivatives : AmpR. Drivatives of pUC18/19 with a 2.1 kb stabilizing frament allowing replication in Pseudomonas.

4) We will use vectors for conjugation in strains listed in section 2.1:

5) We will use vectors for transposon mutagenesis in these strains:
- pHIP45 and derivatives : Interposons carrying resistances : KanR, TetR, HgR and ChIIR.
- pSUP102 and derivatives : Tn5 –B10 (KanR), -B11 et –B12 (GentR), -B13 (TetR), -B20 (KanR), -B21 (TetR), -B22 (GentR), -B30 (TetR), -B40 (KanR) et –B41 (TetR).
- pUT-Tn5 and derivatives: TetR.
- pBK-miniTn7 and gfp derivatives. pUC19 based vectors with various resistance for delivery of gfp on the chromosom of P. aeruginosa.
- pML1 (GmR) and pLR27 (KmR) and pALMAR3 (TnR) for Tn5 delivery on the chromosom.

6) We will use plasmids to transfect eukaryotic cells:
- pEGFP-C1 and pEGFP-N1.
- pRL-TK or pRK5 vectors and derivatives (Promega).
For Agrobacterium inframe deletions, mutations or insertions will be constructed using pJQ200KS and further cured by a sucrose counter selection procedure. To complement deletion mutants or to over express specific genes in Agrobacterium pRL662, pTrc200, pJN105 or pBBR1 will be utilised.

HOST PLANT SYSTEMS:
We will use Arabidopsis thaliana, Brassica oleracea, Nicotiana benthamiana, Solanum tuberosum and Solanum lycopersicum as hosts. They are all widely-used plant models and none of them will be genetically modified.
The genetic material involved in this project has originated from Prof. Alain Filloux's laboratory. Additional strains will be sourced from approved repositories such as ATCC etc or other scientific lab from around the world (With FERA restrictions on plant pathogens.).

Our project will cover the study of microbial secretion systems and biofilm formation and the regulatory networks govern these two main mechanisms in the listed microorganisms. However, we will primarily focus on four microorganisms: P. aeruginosa, P. syringae, A. tumefaciens and P. putida.

Among the already known genes to be studied, one could name:
- Secretion system genes: structural component of the systems (tss genes, vgrG genes, hcp genes), accessory components (tagF, tagP, ...), effector and immunity genes (tse-tsi genes, ...)
- Biofilm formation genes: lecA, psl operon, pel operon, cup fimbrial clusters, ...
- Regulatory networks: two-component systems (gacA-gacS), quorum sensing system (rel, las, pqs, ...), phosphodiesterase and diguanylate cyclase, ...

**Evaluation of foreseeable effects**

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- Regulatory networks: two-component systems (gacA-gacS), quorum sensing system (rel, las, pqs, ...), phosphodiesterase and diguanylate cyclase, ...

It is not envisaged that the genetically modified organisms will be significantly more fit in the environment than the original. However GMM could alter the expression of some secretion systems and biofilm formation capabilities which may affect virulence. Our data and the literature has shown that increasing these traits tends to decrease acute virulence of strains and indeed we see this with Pseudomonas aeruginosa. GMM targeting the genes we are interested in may increase the minimum inhibitory concentration of certain classes of antibiotics but not all classes. However as most of these are laboratory strains infection in healthy individuals is not a particular concern. GMM with increase/decrease activity of secretion systems (our focus is on the Type 6 Secretion System) may increase/decrease the ability of these strains to kill other bacteria/be killed by other bacteria. Environmental release of these strains could potentially be a problem in specific situations but this is unlikely. As our goal with the plant work is to study the biocontrol properties and potentially increase these properties of several environmental strains in order to help defeat plant pathogens. Therefore the GMM of environmental strains (that don’t cause human or plant disease) is designed to promote interbacterial competition against plant pathogens.

Furthermore the level of containment and procedures should prevent release or infection of these strains. There is a very low likelihood of transfer to related microorganisms as lab benches, equipment and other surface areas used for work with cultures will be decontaminated frequently. As this is the work will be performed in a Class 2 lab this is excellent procedures in place to deal with waste. Furthermore the work will be carried out in very central London further reducing the chance of any worst case scenario release.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

There is not going to be any derogation from full containment

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste
- GM Material, Hazard Group 2 plant pathogens & and plants
  Contaminated plastics and other solid waste eg nutrient agar or small volumes of soil will be placed inside of a biological hazard bag. The bags need to be kept in a bin close to the work area and must be filled to no more than three-quarters full.
  Prior to disposal the tops of the bags must be loosely taped to contain contents but allow free circulation of steam. Taped bags should be placed into the blue plastic collection boxes which will then be fixed with a blue zippy tie. These blue zippy ties have a unique code assigned to our lab.
  Lab bins used to temporarily collect waste destined for autoclaving in the blue boxes are regularly disinfected with 1% Virkon S or another approved disinfectant.
The blue boxes used to transport the collected waste form the lab bins are sterilised as they go through the validated autoclave with the waste (100% kill). Minimum waste load holding temperature 121 degrees C for 15 minutes. Subject to annual calibration of sensors and validation of the waste load cycle. Waste is collected regularly by the Dept. of Life Sciences technical staff and autoclaved in the Sir Ernst Chain Building. Once autoclaved for decontamination, the waste bags are transferred to a clinical waste bag and placed with in a large bin for collection via registered companies for disposal. Liquid waste will be treated with 1% final concentration virkon for a minimum of 1 hour (Minimum viable load reduction = Log5) or small volumes (<5ml) may be autoclaved directly (100% Kill). Liquid waste will then be disposed of down the sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Dear Professor Filloux,

Following the discussion of your proposal entitled "Secretion system mediated competition ex planta", GMIC-5236 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

1.2: 5th paragraph, 1000mL (1 litre) is not small scale, please amend.

2.11: Please can you change ‘class’ to ‘hazard group’ they are two completely different things, class refers to GMO’s only, hazard group to the non GM organisms. Can you also make sure that the hazard group in section 2.10 and 2.11 match up? Whenever you have a hazard group 2 pathogen you cannot say ‘this strain is not hazardous to human health’ if they are plant pathogens (and they have not got a hazard group), can you specify this?

2.12: What about the plant pathogen, Xanthomonas campestris pathovar campestris (Black rot)? Have you confirmed with APHA that no scientific licence is required?

2.17: This is a little vague. Are you implying that genes from any of the listed organisms could be inserted into any of the pathogens? Will any of the deletion, mutations or insertions lead to increased virulence or alterations in host range? Could the down regulation of some genes increase the expression of other genes and increase the virulence of the pathogen? If yes what are the consequences? What are the limits of the work, for example is there anything you won’t do? Please give some examples of the genes you will seek to delete, mutate and insert.

2.18: Please define this better.

4.10: Infection dose is cfu/ml?

4.14: Liquid Culture?

4.18 and 4.20: The list of inserted, deleted or mutated genes is cited in 2.17 as being the annotated genes available from the genome sequence of the PAO1 and PA14 strains (http://pseudomonas.com), as well as other genes found in newly sequenced strains. In addition the genomes of Pseudomonas putida, Agrobacterium tumefaciens, Pseudomonas syringae, Mesorhizobium loti and Acinetobacter baylyi are available. This is a vast array of genes. How can you be certain that your manipulations will not increase pathogenicity or host range? Please explain and define the limits of this work.

5.7: Why only 30 minutes? HSE have previously recommended at least an hour before opening the lid. Remove reference to ‘broken glass’, as it will be double contained.

5.8: Is there any secondary containment in the shaking incubators for the HG2 and class 2 organisms? How are flasks sealed but at the same time allowing gaseous exchange? How are flasks fixed to the shaking platform? Sticky mats are not recommended. Any spill of a class 2 GMO is likely to be reportable.

We discussed this and you need to specify when double containment would be used, especially for environmental risk (plant pathogens). Please amend the risk assessment.

7.1: Is there good efficacy data for 1% virkon against the plant pathogens?

Section 10: Replace references to the accident report form with Salus.

Section 15: The class 2 work needs to be more accurately identified and listed. You may refer to existing information earlier in the form if it clearly identifies the Class 2 work.

Comments: LA gave a brief description of the work.

RW queried if they were planning on using transgenic mutants.

LA explained the core 4 paths will be targeted knock outs and transgenic mutants also.

RW pointed out there was a potential for many of these.

LA confirmed this was correct.

MB suggested defining the limits and putting them in functional groups. Then the rest of the risk assessment could mirror the functional group.

MB asked about the virulence genes.

LA agreed to define and modify.

RB reminded LA to keep in mind what the limits of the work was when completing these sections.

MB asked about secondary containment in the shaking incubators? Possibly putting the flasks in a beaker or using plastic?

AL will put any GM pathogens that are Class 2 or an environmental hazard in secondary containment. AL will specify which pathogens/mutants have to go in secondary containment.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.
The primary aim of my research is to understand how bacteria indigenous to the intestine (the commensal microbiota) influence systemic host defenses to infection by pathogenic microbes. This work will be split into the following phases:

(I) Investigation of what commensal intestinal bacteria, and commensal-derived products, gain access to tissues distal to the intestine.

(II) To understand how the commensal-derived signals described in (I) influence host defenses to bacterial infection by pathogens such as S. pneumoniae and K. pneumoniae.

Phase (I) will include extraction of bacterial products from murine tissue (e.g. spleen, liver and bone marrow) and assaying their concentration and ability to stimulate pattern recognition receptors (PRRs). This will include transfection of eukaryotic cells (e.g. HEK293T cells and macrophages) with PRRs and NFkB expression constructs. Further experiments will be performed to investigate the PRR activating potential of the intestinal microbiota of mice of
different ages and having received different antibiotic treatments. Phase (II) will involve isolation of murine immune cells (eg neutrophils, macrophages and dendritic cells) after treatment of mice with antibiotics to deplete their microbiota. The functions of these cells will be assayed, including the killing of bacterial pathogens, including S. pneumoniae and K. pneumoniae.

**Recipient or parental organism**

S. pneumoniae R6, TIGR4, D39, P1121, 6A (class 2)  
H. influenziae 636 (class 2)  
Klebsiella pneumoniae KP2R1 (class 2)  
Genes will be knocked out to assess their role in the virulence of these organisms

**Host/vector system**

For S. pneumoniae, H. influenziae and K. pneumoniae, vectors will not be used, instead transposon mutagenesis will be used.

**Origin & function**

Genes involved in the biosynthesis of the Streptococcus pneumoniae, H. influenziae and Klebsiella pneumoniae cell wall (for example, but not limited to, Penicillin binding proteins) will be disrupted. This will render all the GM Class 2 organisms more susceptible to killing by immune cells and less able to survive in the environment.

**Evaluation of foreseeable effects**

**S. pneumoniae**

Hazards to human health include transmission to the nasal cavity by hand contact. However, most strains we will use are avirulent. In immunocompromised individuals there is a potential for infection. S. pneumoniae can cause the following diseases: include pneumonia, bacteremia, otitis media, meningitis, sinusitis, sepsis, peritonitis and arthritis. 

Transmission of GM DNA to environmental bacteria is almost zero. GM DNA can be transmitted within the host.

**H. influenziae**

Portal of entry is through the airways, nasal and otherwise where it becomes a commensal in the nasal cavity. This opportunistic pathogen dies rapidly on the bench without being in the host (humans only). Containment will be as above. As we will work with a non-encapsulated version of this organism it is unable to infect or successfully colonise except in cases of severe immunocompromisation (e.g. a patient with cystic fibrosis).

**K. pneumoniae**

Infection can occur with direct contact of mucosal membranes with contaminated surfaces and/or object, and inhalation of infectious airborne secretions, accidental parenteral inoculation and/or ingestion. Klebsiella spp. grow rapidly on surfaces of some vegetation (eg potatoes and lettuce) but do not survive well on the skin. 

The GMMs are disabled and are unlikely to be able to survive in the environment and any modifications proposed (gene inactivation) would not confer a selective advantage even if they were to be transferred to a wild type organism.

The likelihood of hazard is therefore considered to be no greater than that of the parental strains.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste: Treatment with minimum 1% Virkon after dilution with liquid waste for 1h followed by disposal in the sink.
Solid waste: All other hazardous solid biological waste such as tissue culture plasticware (pipettes, flasks etc) will be autoclaved prior to disposal. Petri dishes with agar will be autoclaved prior to disposal.
Sharps: Yellow sharps bins > autoclave sterilisation if known or potentially infected. clinical waste disposal (incineration).
Animal body or recognisable parts: Yellow rigid one way sealed tissue bins. clinical waste disposal (incineration)
Infected or potentially infected lab wastes that have been pretreated before leaving site: Disinfection or sterilisation in the laboratory suite > orange clinical waste bags > clinical waste disposal.

Comments:
2.10: Are all of these Staph strains MRSA? Please clarify which ones are and which ones are not.
2.15 and 2.16: It is unclear as to what is happening with these hosts and inserts. What insert is going into which host? Are all of these vectors only for use in the E.coli? If so please state this more clearly.
2.17: Please list some examples of the genes that you are targeting with the transposon mutagenesis.
2.2 and 4.2: What sort of microorganisms do you expect to be able the culture? Are you interested in anything in particular? If yes these should be added to 2.10 and 4.10
4.22: Please give more details about the volumes of culture in use. E.g. Is this one 4L culture or 40 x 100ml cultures?
5.8: If the culture vessel lids are sealed how is gaseous exchange achieved, please explain?
5.10: Which Form B is this? Please provide a reference number or project title.
Why is there no shedding, please explain?
5.13: Are you Sorting live Class 2 or HG 2 paths? If so where please state this somewhere in the risk assessment?
7.1: Please include the phrase "minimum 1% virkon after dilution with the liquid waste for 1hr" ……… etc
8.1: MSC’s used for operator protection will also have to be airflow tested monthly by the users (or lab manager).
10.1: Make sure it is clear that any spill is treated with concentrated virkon e.g. 5% before removing the mopped up material from the MSC.
Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022
Page 352 of 15326
This project aims to establish standardised conditions for reliable post-transcriptional gene silencing in a parasitic nematode by RNA interference (RNAi), and assay effects on parasite development in vivo. The species selected, Nippostrongylus brasiliensis and Heligmosomoides polygyrus, are ideal for the proposed study as they are phylogenetically closely related to a range of strongylid parasites which cause serious disease in livestock worldwide. Moreover, both species are highly amenable to manipulation in the laboratory. Lentiviral delivery systems enable the transduction of both arrested and dividing cells which allowing increased transduction and knockdown efficiencies. Accomplishing stable, selective gene knock-down could lead to major advances in our fundamental understanding of nematode infections and to radically new methods for the treatment and control of helminthiases. Various recombinant DNA constructs will be used in order to introduce a fluorescent reporter and genes of the RNAi machinery from C. elegans necessary for initial binding and systemic spread of dsRNAs. The constructs will be in the form of bacterial plasmids and will be introduced into cultured cells to generate lentiviral particles that will be in turn used to introduce the relevant transgenes and shRNAmir expression cassettes in parasitic nematodes. Mice or rats will be injected subcutaneously with transgenic parasites expressing a reporter gene and/or miRNAs.
Artificial miRNAs will be designed specifically for the relevant nematode genes and may lead to silencing of gene function which in turn may result in impaired parasite survival in the host and an altered host immune function. The notifiable aspect of the project is the transduced nematodes (H. polygyrus; N. brasiliensis) for assessment of transgene expression and target gene knockdown. They are not infectious to humans but can cause disease in rodents.

Recipient or parental organism

Bacteria: E.coli (Class 1 aspect of the work included for completeness)
E. coli bacteria will be transformed with lentiviral vector plasmids for amplification.
Mammalian cell lines: HEK293T cells, COS7 cells (Class 1 aspect of the work included for completeness)
Mammalian packaging cell line HEK293T will be transfected with lentiviral vector plasmids and several helper plasmids encoding gag-pol-env sequences necessary for production of replication-incompetent virus particles. Lentiviral vector plasmids are transfected into mammalian cell lines (e.g., HEK293T, COS7) either alone or together with plasmids (e.g., pcDNA) encoding the parasite-specific target gene cDNA allowing assessment of transgene expression and to validate different shRNAmir sequences for effective knockdown of target genes, respectively. Transduced cell lines to determine virus titres
Parasites: Heligmosomoides polygyrus, Nippostrongylus brasiliensis. (Class 2 aspect of the work)
Transduced nematodes (H. polygyrus; N. brasiliensis) for assessment of transgene expression and target gene knockdown.
None of the GMO’s will be any more hazardous to human health or the environment than the wild type organisms.

Host/vector system

Packaging vectors:
pCMV-dR8.91 + pCMV-VSVG (lentiviral genome split onto two separate plasmids)
pCMVdR8.74psPAX2 + pMD2.G (lentiviral genome split onto two separate plasmids)
TransLenti packaging mix (lentiviral genome split onto 5 separate plasmids)
Transgene vectors for viral production:
pGIPZ (commercial vector with turboGFP and self-inactivating LTR)
pGIPZ_mCherry (derived from pGIPZ replacing turboGFP with mCherry)
pGIPZ_EV (derived from pGIPZ_mCherry by deletion of IRES/puro)
pGIPZ_SmACT1.1 (derived from pGIPZ_EV replacing CMV with SmACT1.1)
pGIPZ_IPP (derived from pGIPZ_EV replacing CMV with IPP)
pGIPZ_UCOE (derived from pGIPZ_EV encoding UCOE upstream of CMV)
pGIPZ_OVA (derived from pGIPZ_EV replacing mCherry with OVA)
pGIPZ_OVA_GPI (derived from pGIPZ_OVA, GPI-anchor added)
pGIPZ_OVAopt (derived from pGIPZ_OVA replacing OVA with OVA_opt)
pGIPZ_RDE-4 (derived from pGIPZ_mCherry replacing mCherry with RDE-4)
pGIPZ_ERI-5 (derived from pGIPZ_EV replacing mCherry with ERI-5)
pGIPZ_RDE-4_RERI-5 (derived from pGIPZ_ERI-5 and pGIPZ_RDE-4 adding RDE-4/IRES upstream of ERI-5)
Mammalian expression plasmid:
pcDNA3.1 (commercially available plasmid from Invitrogen)

Origin & function

gag - pol - env: lentiviral components allowing the generation of lentiviral vectors;
<table>
<thead>
<tr>
<th>VSV-G: Vesicular stomatitis virus glycoprotein, virus envelope protein allowing fusion of the virus envelope and the host cell membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>turboGFP, mCherry: fluorescent proteins, leading to fluorescent signal produced in transfected/transduced cells;</td>
</tr>
<tr>
<td>OVA, OVA-GPI, OVAopt: Ovalbumin, model antigen expressed in transfected/transduced cells</td>
</tr>
<tr>
<td>CMV, SmACT1.1, IPP: promoter sequences for transgene expression in transfected/transduced cells</td>
</tr>
<tr>
<td>shRNAmir: microRNA-adapted small hairpin RNAs encoded in the 3'UTR of the transgene for specific knockdown of parasite-specific target gene expression.</td>
</tr>
<tr>
<td>RDE-4, ERI-5: C. elegans proteins involved in dsRNA binding and systemic spread in transduced parasites;</td>
</tr>
</tbody>
</table>

**Evaluation of foreseeable effects**

The most hazardous GMM produced will be lentiviruses carrying fluorescent reporters or C. elegans proteins. Lentiviruses are able to infect human cells even when non-dividing, however no harmful constructs will be cloned into such vectors. There is no chance that oncogenes will be packaged into lentiviral particles because these constructs carry packaging signals, which would not lead to enclosure into a lentiviral capsid even in the accidental event of lentiviral packaging cells being transfected with the oncogenic constructs. Lentiviral particles carry a self-inactivating LTR and are replication-incompetent. The risk posed to human health by all GMM produced will be negligible, and further minimised by reducing the use of sharps.  

All GMOs generated, including nematodes, are no more harmful to human or animal health or the environment than the wild type organisms.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Infected rodents are kept in IVCs in a purpose built animal containment unit and carcasses autoclaved and disposed of by CBS staff.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Liquid Waste - Final concentration of not less than 1% Virkon (10g in 1litre) for 30 min |
| Solid Waste - All lentivirus-contaminated plastic are left in contact with Virkon for 30 minutes. Pipettes are then discarded in the dedicated pipette bin to prevent waste bags being pierced. All plastics are then discarded into a transparent autoclave bag into a white bucket bin for autoclaving. |
| Needles and sharps – Placed in a yellow sharps bin for autoclaving and clinical waste disposal |
| Animal Carcasses - carcasses are autoclaved and disposed of by CBS. |

**Is an emergency plan required according to regulation 20?**

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
Dear Professor Selkirk,

Following the discussion of your proposal entitled “Lentiviral delivery of artificial miRNAs to parasitic nematodes” GMIC- 5743 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

SOP: changing the reference from rats to mice in some of the SOPs.

1.2: How are the animals infected? SOP refers to a subcutaneous method of infection, I assume this involves use of a needle?

How are the whole viable nematodes transduced? I didn’t see this in the SOP’s

1.3: Location of the FILM unit?

2.2: Remove mention of the whole viable nematodes form here unless nematode derived cell lines are being cultured.

Remove mention of the whole live mice form this section unless mouse derived cell lines are being cultured.

2.4: Are the mammalian cell lines commercially obtained? If so what guarantees of sterility are provided?

2.13: Add CRISPR gene knock out system here. (It has been decided by the PI that this will not now be incorporated into this experiment)

2.18 and 4.20: Please include a statement about the GMO’s, including the nematodes, being no more harmful to the environment than the wild type organisms.

3.1: Please tick the no box as the GM Nematodes are Class 2 and will need to be handled at CL2

4.15: Add CRISPR gene knock out system here. (It has been decided by the PI that this will not now be incorporated into this experiment)

4.21: Add the infectious route for the nematodes in mice.

5.1: Not all work? I assume this just refers to the genetic manipulation of the lentivirus, cell lines, E.coli and nematodes?

5.3: Please give a typical example of these containers.

5.5: From where are the nematodes obtained?

5.10: I appreciate that there is no shedding of material infectious to humans but what about shedding and dispersal of material

CU 2 2000 (rev 11/08) Page 7 of 12

infectious to rodents, e.g. The nematodes. How is this prevented and controlled. The SOP does not give any more information and this is an important point as the material is GM class 2 in relation to the environment and rodents.

5.12: This section refers to inactivation for further molecular investigation not waste disposal. E.g. Lysis of virus, cells or nematodes for genetic analysis, quantitative PCR etc

7.1: Specify that GM nematode infected rodents will be autoclaved before leaving site for incineration

Section 8: MSC’s used for operator and environmental protection need to be airflow tested by an engineer every 6 months and KI operator protection tested annually. Users must also carry out air flow checks monthly using a 100mm vane anemometer and record the results

Section 9: Any refresher training especially for emergency procedures?

11.5: Please complete this section. Your rooms will have a small coloured circle on the door, probably either yellow or orange. If not please get in touch with Biosafety.

15.1: Whole viable GM virally transduced nematodes expressing molecular markers will be GM Class 2

Comments: MS gave a brief description of the work.

IH pointed out that The Class 2 aspect of this project is the genetic modification of the mouse pathogenic nematodes Heligmosomoides polygyrus, Nippostrongylus brasiliensis. Gene Silencing may not be classed as class 2 GM work if it is transient, however the genetic marker labelling of the whole viable mouse and rat nematode parasites will be GM Class 2.
MS explained that they did know how long it would last, between 10 days and 3 weeks. It was the effects over the period of time they were interested in.

IH queried if the progeny from nematode worms were genetically modified?

MS pointed out that this was difficult to know. It could be passed onto the next generation.

NF suggested adding the CRISPR stage of the project to the application now.

The committee agreed this could be considered a significant change in the future. To save time and costs, it should be added to the form now.

IH asked about shedding?

MS explained there would be none if transduced.

IH wanted to know about the nematode shedding.

MS described that the eggs in the guts moved to the faeces and the bedding would be contained.

JP suggested changing the reference from rats to mice in some of the SOPs.

HK asked about what rooms were used.

MS gave the details of the rooms. The viral GM class 2 work is in the Ernst Chain Building, the transduced parasites in designated rooms, the mice parasites in the CBS (shared room).

NF enquired if the mouse handling would all be in the IVF.

MS confirmed that it was.

Agreed: Class 2, Containment Level 2. This needs to be notified to the HSE.

IC reference number: GMIC-5743

Could you please ensure that the risk assessment is revised (add GMIC number) and the amendments above addressed. I will then forward the form onto Occupational Health to complete. The completed HSE CU2 form (attached to this email), and copy of the Purchase Order (for BACs Payment) made payable to the HSE should be sent to me. I will then send it all to the HSE. Please ensure the information given in HSE Form relates to that provided in your risk assessment.

For further information, please see http://www3.imperial.ac.uk/safety/subjects/biosafety/gmprocedures

Please note that this work must be notified to the HSE and an acknowledgement receipt received prior to this work commencing.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>L3</td>
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<td></td>
<td>L2</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
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<tbody>
<tr>
<td>L2 Yes</td>
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<tr>
<td>L3</td>
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<tr>
<td>L4</td>
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<td>L2</td>
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<tr>
<th>Large Scale Activities</th>
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<tbody>
<tr>
<td>L3</td>
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<tr>
<td>L4</td>
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<td>L2</td>
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</tbody>
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<table>
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<tr>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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<tr>
<td>L3</td>
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<tr>
<td>L4</td>
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<td>L4</td>
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Project Ref 8/16.3

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/07/2016</td>
<td>Screening for novel antibiotics in soil</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Non-GMM</td>
</tr>
</tbody>
</table>

Date Project Ceased 02/03/2022
Aim: To develop the iChip technology (Nichols et al. Applied and Environmental Biology 2010) to now facilitate in situ screening of antibiotic properties of bacteria cultivated in soil. The bacteria strains we seek permission to get registered will be developed to work as the sensors of antibiotic properties. Bioluminescent read outs should reveal whether soil bacteria cultures stress our sensor bacteria and thereby leads to a decrease in light emission. The novel screening device (here called the isCHIP or in-situ screening CHIP) will be buried in the soil outside of a laboratory environment on ICL premises at Silwood Park London. Details of the isChip can be found in the attached documents.

Initial aims are to develop a biosensor in the lab that uses GM Class 1 and 2 sensor organisms and test using soil brought into the lab. Once this phase has been established additional steps will include testing the new isCHIP in the soil in a controlled area.

In the choice of strains we have taken care in selecting strains that are clinically relevant, can survive in soil for extended periods of time, but have limited risks for the researchers and the external environment. An example is E. coli 0157:H7 which is important in several types of E coli infection making it clinically relevant. We have, however, found a strain that has been tested and found to be non-toxigenic. It should therefore not pose a threat to the researcher.

We will be testing the ability of antibiotic producers to inhibit further growth of our reporter strains.

To do this:
We will culture GM strains that have a Lux operon chromosomally integrated.
We will use Lambda red to create our own mutants with chromosomally integrated luminescens.
Finally we will attempt to create more susceptible strains by knocking out genes that protect the bacteria from exposure to antimicrobials. An example could be to knock out tolC.
All knock outs should lead to a decrease in fitness for the organism, and should therefore decrease the organism's chance of survival in the wild.

Procedures therefore include:
Genetic manipulation of a variety of bacteria i.e. electroporation and conjugation to insert genes on plasmids or make chromosomal deletions or insertions and random mutagenesis to optimise expressssson. culturing (on agar plates, some in microaerobic chamber or microaerobic jars), flash-freezing, light microscopy slide preparation with
~ 10ul of dilute cell suspension sealed between glass slides and cover glass using a solidified mixture of vaseline, paraffin wax, and lanolin, coated with a layer of nail polish. Finally cell sorting will be carried out using the ARIA designated for bacteria in the Flowers building. We will be placing the isCHIP in soil outside of a laboratory at Silwood park. The isCHIP is a contained environment that allows biochemicals to diffuse across a membrane and interact with the sensor bacteria but does not allow the escape or ingress of biological agents. Please refer to the attached detailed description of the device.

i) The site will be robustly marked in similar fashion to other projects at Silwood Park.

ii) We have designed the isCHIP to be made of 0.5mm class 2 titanium. There will be two outer layers of titanium that will protect against animals coming in contact with the enclosed bacteria. Each layer of titanium will only allow access to the inner chip through 1536 wells with a radius of 0.4mm (See attached design of prototype)

iii) The isCHIP will be contained in sealed boxes during transport. When buried, it will be buried on an area of 6 x 6 meters. We envisage <50 isCHIPs buried at a time. Each isCHIP will be marked with a flag at the surface of the burial site.

Recipient or parental organism

<table>
<thead>
<tr>
<th>Non-toxigenic strain</th>
<th>E. coli 0157:H7</th>
<th>HG2</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>E.coli O1:K1:H7 (ATCC 11775)</th>
<th>HG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>is an avianpathogen but not a SAPO (See <a href="http://www.ncbi.nlm.nih.gov/pubmed/17293413">http://www.ncbi.nlm.nih.gov/pubmed/17293413</a>)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E. coli O111:K58:H21 (ATCC 29552)</th>
<th>HG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella enterica subsp. enterica serovar Typhimurium HG2 causes acute gastroenteritis with diarrhea, abdominal cramping, fever, and vomiting. Fever will usually subside in 72 hours, with bloody diarrhea lasting between three and seven days. These effects can be more severe or prolonged in children and the elderly. Bacteremia, or the spread of the pathogen into the blood stream, generally occurs in 5-10% of cases and can lead to more severe symptoms such as meningitis and infections of the bones and joints. Treatment involves antibiotics such as chloramphenicol and benzylpenicillin.</td>
<td></td>
</tr>
</tbody>
</table>

Pseudomonas aeruginosa HG2 is a common environmental organism and is often part of the normal microbiome of healthy people. It can act as an opportunistic pathogen, found in wounds (especially burn wounds), urinary tract infections and eye infections (from contaminate contact lens). Rare septicemaia have caused high fever, chills, confusion and shock. Infections are treated by antibiotics.

<table>
<thead>
<tr>
<th>P putida and P flourescens</th>
<th>HG1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterium Smegmatis</td>
<td></td>
</tr>
</tbody>
</table>

Bacillus subtilis HG1. Soil borne organism not known to cause disease in healthy human hosts

Host/vector system

TN5- Transposase (Tnp) TN5 is a member of the RNase superfamily of proteins which includes retroviral integrases. Tn5 can be found in Shewanella and Escherichia bacteria. The transposon codes for antibiotic resistance to kanamycin and therefore allows for positive selection of colonies where Tn5 has been integrated.

Lambda Red - is one of the standard methods for generating insertion, deletion, and point mutations on chromosomal targets in many gram negative bacterial species. Plasmids include PCP20, PKD46, PKD3, and PKD4.

plasmid pMV306hsp. Plasmid pMV306hsp is a modified plasmid that has been shown to have high stability and expression in mycobacteria.

Origin & function

The lux operon consist of a luxAB coding for luciferase and luxCDE coding for the fatty acid reductase complex responsible for synthesizing fatty aldehydes for the
luminescence reaction. They operon derives from V. fischeri.

Firefly luciferase is a euglobulin protein that catalyses the oxygenation of luciferin using ATP and molecular oxygen to yield oxyluciferin. It is derived from Photinus pyralis.

Kanamycin resistance gene.

**Evaluation of foreseeable effects**

Our mutagenesis strategy is to implement targeted mutagenesis. These include the insertion of a) a LuxCDABE operon and b) permutations of knockouts that increases the susceptibility of the reporter strain to antibiotics.

a) the LuxCDABE places a metabolic burden on the host, which will decrease the fitness of the GMO [1.Nunes-halldorson and Duran, 2003]. Hastings and Nealson [2. Hastings and Nealson, 1981] estimated that light emission represents an energy expenditure of approximately six ATP molecules for each photon, assuming 100% efficiency for the reaction.

The effect on fitness has been confirmed experimentally - The result suggested that the incorporation of a lux operon in an E. coli O157:H7 strain had a small negative effect on survival time in ground water (Fig 1) [Ritchie et al, 2003]. Ritchie, J.M., et al., A stable bioluminescent construct of Escherichia coli O157: H7 for hazard assessments of long-term survival in the environment. Applied and environmental microbiology, 2003. 69(6): p. 3359-3367.

b) The targeted deletions will decrease the fitness of the host organism, as the deletions are important functional proteins. An example of a knockout candidate is TolC, which plays a common role in the expulsion of diverse molecules, including protein toxins and antibacterial drugs, from the cell.

Wild type strains of all bacteria will therefore be the most hazardous strain employed; all derivative GMMs will be less pathogenic due to the lack of selective advantage of the genes in question.

We will confirm that wildtype strains have the highest fitness through survival studies before we bury the GM strains in situ.

For in situ experiments:

E. coli:

At present we are targeting three strains of E. coli (W, O1:K1:H7, and O157:H7 with no shigatoxins) for mutagenesis. Different E. coli strains have been shown to be able to survive in soil, although at low concentrations [4]. O157:H7 has specifically been shown to die out over time in soil[3]. The three strains, will, however, have mutations that reduce their fitness. No strain is pathogenic to humans. The E. coli O1:K1:H7 strain causes cystitis in chickens.

B. subtilis, P. putida, P. fluorescens, and M. smegmatis all grow naturally in soil and are non-pathogenic. We will use strains that were isolated in the UK.

These are the only strains we plan to culture for our in situ experiments.


**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

none.
<table>
<thead>
<tr>
<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid waste: Chemical disinfection (Virkon to 1% final concentration for at least 30 minutes) before final disposal to drains.</td>
</tr>
<tr>
<td>Solid waste: Contaminated plastics and other potentially contaminated waste will be placed into clear autoclave bags contained within rigid bins. Objects which are likely to puncture the bags will be placed in a BioBin or similar container. Prior to disposal the tops of the bags will be loosely taped to contain contents but allowed free circulation of steam. Taped bags will be placed into blue boxes for transport to Ernst Chain Building autoclaves (with affixed lid). The autoclaves are validated annually for inactivation of waste loads at a minimum of 121 degrees for 15 minutes measured in the coolest part of the load.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Is an emergency plan required according to regulation 20?</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
<td>N</td>
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<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
</tr>
<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>Y</td>
</tr>
</tbody>
</table>

Please enter comments on the GM safety committee on the risk assessment
Additional note post committee from the BSO
“There might be a problem using the family farm for this work as we might have to register it as a GM centre. I thought you were using Silwood Park? This would be easier as it is already notified to the HSE as a GM centre”.

21.1 Papers for Discussion

21.11 Bio1 Form
Screening for novel antibiotics in soil CL2. Dr H Low, Life Sciences, South Kensington Campus.
Comments: In the absence of the PI and Researcher, SH gave a brief description of the work.
Both IH and SH had discussed prior to the GMSC meeting if this experiment came under the GM Contained Use Regulations or the deliberate release regulations. The structure and robustness of the cell and the environmental locations (a farm as well as the Silwood Park location) were considered. It was decided to put it forwards as a contained use application.

RW asked if small samples of soil were to be bought into the lab environment, would this degrade the sample and alter the experimental outcome?
SH explained that pilot experiments would involve bringing soil into the lab but that the samples were substantial amount e.g. bucket fulls, but that this was just a proof of concept and the main experiments would have to involve environmental sampling.

RW also asked that it was likely that the isChip needed to be grown in a natural environment, so that the soil remained in its natural location with all the external influences that implies e.g. the fall in night temperature, sunlight, pH stability, exposure to rainfall etc to improve the chances of the soil organisms surviving and producing the antibiotics that were being looked for.

RW asked how long the isChip would be incubated in the soil?
SH answered, up to 30 days.
RW wanted to know if the isChip would be buried in the ground.
SH confirmed they would be, however, the depth they were buried at was not clear and this should be stated in the risk assessment and SOP.
SH also added that tests will be conducted in the labs to see if the bacteria would ingress into or leak from the isChip. The paper they cite, shows no ingress or leak.

HK asked what would happen if the isChip was lost or damaged, e.g. by an animal or farm machinery?
SH explained that the Silwood Park grounds and the grounds on the Farm were both controlled areas and the sites would be clearly marked and separated from other activities. This will should be explained in the risk assessment and SOP

RW asked how many devices were they planning on burying?
The Committee agreed this needed to be answered in the risk assessment and SOP

The following amendments were required prior to approval:
SOP: Explain how many isChips were being used, the amount of land to be used, how deep the chips will be buried, and how the chips will be secured to prevent them from going missing or being damaged. A precise location will have to be given for each site. A grid reference should be provided using a GPS device.
Bio1 Form: Typos throughout the whole form, please revise.
1.3: List locations of Silwood Park and external Farm, the field sites should include a GPS location to 8 or 10 figures. The OS app on smart phones can do this.
2.16: Add the functional properties of vectors.
2.17: ‘aldehyde’ should be ‘luciferin’
3.20: Remove ‘melis’. What will be used for decontamination?
4.7: Remove comma, answer ‘yes’
Agreed: Class 2, Containment Level 2. This needs to be notified to the HSE.
IC reference number: GMIC-6023
### Project ref 8/16.4

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<td>Discovering the mechanisms of microbial accommodation inside plant cells</td>
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#### Purposes of the contained use

The aim of our work is to dissect the resistance mechanisms in Solanaceous plants (tomato, potato, and Nicotiana spp.) and understanding the molecular mechanisms underlying plant-microbe interactions. Specific focus will be on perturbations caused by microbial pathogens in the host cell endomembrane transport processes. Mainly, eukaryotic plant pathogens such as the oomycetes Phytophthora infestans and/or Phytophthora capsici, Phytophthora palmivora, Peronospora tabacina will be used to study plant immune responses and processes altered during microbial attack. Plant pathogens target secreted proteins such as cytoplasmic effectors inside host cells where they directly modify or perturb host cellular processes. These pathogens use specialised secretion systems and targeting signals to translocate effector proteins inside host cells. In filamentous pathogenic eukaryotes (fungi and oomycetes) both the nature of the recognition sequences and the focus of any effector’s localisation are largely unknown. In addition, we will also modify plants. Plants attract many parasitic and symbiotic organisms. Some of these parasites and symbionts are accommodated inside invaded plant cells in specialized compartments that are typically separated by host-derived membranes from the plant cytoplasm. This interface is critical in the regulation and development of parasitic infection or symbiotic host colonization for instance by enabling efficient macromolecule exchange. However,
the mechanisms underlining the biogenesis and functions of this intimate host-microbe interface are poorly understood. Specifically, we know little about the reprogramming of cellular and biochemical events that take place inside plant cells that are invaded by microbes. The proposed research aims to characterize the host processes required for accommodation of filamentous plant pathogens inside the plant cells with a specific focus on illustrating the role of plant endomembrane system. To this end we will investigate the mechanisms and perturbations in the plant endomembrane system during accommodation of oomycete pathogens such as Phytophthora infestans (P. infestans) within the host plants. An improved understanding of the molecular basis of the role of host endomembrane system during plant-microbe interactions will provide novel insights in plant pathology and cell biology and improved opportunities for engineering disease resistance in crop plants.

Plants are generated using agrobactrium mediated transformation. Foreign DNA transfer in plants is a mechanism occurring in the wild known as Crown-gall disease and is caused by Agrobacterium tumefaciens (gram negative bacteria). Agrobacterium tumefaciens infects the plant through its tumour inducing (Ti) plasmid which integrates a segment of its DNA (T-DNA) into the chromosomal DNA of its host plant cells. Disarmed Agrobacterium tumefaciens strains, carrying a Ti plasmid lacking tumour genes, are routinely used to insert prokaryotic or eukaryotic DNA fragments into plants of the Solanaceae and many other plants.

Plant DNA fragments are isolated as genomic DNA or cDNA and propagated in plasmids or viral vectors in E.coli. i. Cloned into a range of lambda vectors (eg lambda Flx-II, pRK290 cosmid vectors) and used in disabled E.coli strains (eg P2392, LE392, C600). ii. Cloned into non-mobilisable vectors (eg PUC19) and used in disabled E.coli strains (MC1022, DH5α). iii. Cloned into IncP transferable binary vectors and used in disabled Agrobacterium strains for transient expression in plant tissues and/or in planta as well as stable plant transformation. These clones were used as a source of DNA for manipulation of plant genomes.

The GM plants generated pose no more risk to the environment than the wild type and the bacteria are all GM class 1 and are not part of this notification.

The genetically modified variants of the DEFRA/FERA licensed pathogens (P. infestans, P. capsici, P. palmivora) will be handled under the same conditions as the non modified parental strains. The work will take place in closed containers. contained in small transparent plastic boxes/containers on wet paper towels. Infections will be performed by droplets of zoospore solutions with a pippete (10 ul droplets on the leaf surface). In some cases tissue will be frozen and ground in liquid nitrogen. Phytophthora cultures will be also stored in liquid cultures. Infected leaf samples will be taken to imaging facilities in containers mentioned above.

The eukaryotic plant pathogen; P. infestans is present in the local environment, unmodified recipient strains may be imported from outside of the UK and held under licence from DEFRA.

The premises have been inspected by APHA and the latest scientific license is attached with this notification. A full explanation of the work including the use of class 1 GMO's and GM modified plants not requiring notification can be read in the attached Bio1 risk assessment and Form D.

Recipient or parental organism

Phytophthora infestans
Phytophthora capsici
Phytophthora palmivora

Host/vector system

pTOR (6531 bp) vector with ham34 promoter and terminator from phytophthora infestans will be used. Vector is designed for expression in Phytophthora infestans.

Origin & function
Red Fluorescent Protein (RFP), Green Fluorescent protein and Yellow Fluorescent Protein will be used to achieve fluorescence labeling of Phytophthora species. Depending on the course of the research it is possible that some of the RXLR effectors (Virulence factors induced during infection) could be also mutated/knock out or overexpressed in Phytophthora.

It has been established, for example, that most natural isolates of P. infestans carry a gene for effectors, such as inf1 and Avr3a. It is likely therefore that these genes are required for full virulence and that any suppression of their expression either directly through silencing or indirectly by the expression of other effector genes will lead to loss of virulence.

Fusions to reporter genes (e.g. GFP, GUS) will be made to study the expression and localization of effector genes. All genetically modified strains of eukaryotic plant pathogens so generated will also carry antibiotic resistance genes such as hph encoding hygromycin resistance or nptII encoding G418 resistance in order to select for stably transformed clones.

Evaluation of foreseeable effects

The genetically modified strains of plant pathogens described in this risk assessment will differ from natural isolates in that the expression of effector genes is altered or that they express reporter genes. Any consequent loss of avirulence is unlikely to have an environmental effect in the UK and would not differ from naturally occurring isolates. The available information gives no indication that alteration of the expression of effector genes is likely to have other environmental effects. It has been established, for example, that most natural isolates of P. infestans carry a gene for effectors, such as inf1 and Avr3a. It is likely therefore that these genes are required for full virulence and that any suppression of their expression either directly through silencing or indirectly by the expression of other effector genes will lead to loss of virulence. Taking these factors into account we consider that the risk of harm is effectively low/zero. Therefore, transfer of modifications to related microorganisms in the environment is not likely to occur. The GM plant pathogens created are not predicted to pose any greater risk of harm to human health or the environment than the wild type.

The proposed genetic modifications are predicted to not alter the host range or virulence of the recipient strains which will not be significantly different in their pathogenicity to naturally occurring isolates and are therefore unlikely to pose more of an environmental hazard than wild type strains.

In theory the over-expression of virulence factors might be expected to enhance virulence, however there is no report so far showing this is the case. This is probably because these virulence factors are already expressed highly during infection and efforts of over-expression does not necessarily influence the virulence. Also, there are around 500 different virulence factors that coordinate virulence and changing the expression level of one gene is not likely to have a big difference in virulence.

In addition, sporangiospores and zoospores, the main stages manipulated in the laboratory that could be accidentally released lack vigour and cannot survive for extensive periods as airborne spores in the absence of suitable host plants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid cultures are chemically inactivated using 5% sodium hypochlorite final concentration for 30 minutes, and
autoclaved prior to disposal. Laboratory disposables are autoclaved prior to disposal, sharps are sterilized in sodium hypochlorite before disposal. This waste will then be further autoclaved.

Is an emergency plan required according to regulation 20?  

Y  

If yes, tick to confirm that it is attached to this form  

N  

Tick to confirm that you have attached a risk assessment to this form  

Y  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N  

Please enter comments on the GM safety committee on the risk assessment

Comments from the BSO

Section 2.17 You need to be quite specific about the genes you are altering, knocking out or introducing and what the effects will be on the host organism. There still appears to be genes mentioned in section 1.2 which do not appear in the GM section of the Bio1. See highlighted sections below. I am happy to come across to you and go through the form and the CU2 if it would help. I am free most of tomorrow morning from about 10 – Hope this helps - Ian

"In addition, fusions to reporter genes (e.g. GFP, GUS) will be made to study the expression and localization of effector genes. All genetically modified strains of eukaryotic plant pathogens so generated will also carry antibiotic resistance genes such as hph encoding hygromycin resistance or nptII encoding G418 resistance in order to select for stably transformed clones. Furthermore, eukaryotic plant pathogens will be genetically modified to express reporter genes (e.g. GFP, DsRed) to enable studies of the organisms development in planta.

Comments from GM committee

Dear Dr Bozkurt,

Following the discussion of your proposal entitled “Discovering the mechanisms of microbial accommodation inside plant cells”, GMIC-2549 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

2.11: A tumefaciens. Please confirm that the strains you have named are all disarmed strains well characterised with a safe history of use.

2.15: Where did you get the DEFRA containment levels from?

Section 5 and all other sections onwards from here Please reference the appropriate section of the COP where requested.

7.1: Liquid waste – Time waste is treated for?

Solid waste – Can the autoclave sterilise this amount of soil? Or is soil placed in sealable tissue bins for incineration?

7.4: Please tick the potentially or known infected lab wastes that have been pre treated box.

Section 10: treating a liquid spill with 70% ethanol will not work. All liquid spills should be treated with concentrated disinfectant before being absorbed and disposed of as solid waste. Please check your COP for spills and make sure that the risk assessment and COP agree. Bleach is not a satisfactory disinfectant. You have previously specified Sodium hypochlorite. This must be purchased as a commercial product for inactivation of microorganisms, e.g. Chlorox

Comments: TB presented and overview of his proposed work.

IH asked if all of the Agrobacterium tumafaciens strains were disarmed?

PS and TB confirmed that they were.

IH pointed out that the organisms listed in 2.10 and 2.15 would need to be notified to FERA and a scientific licence obtained.

TB and PS said that they were aware of this.

IH stated that he would forward details of the FERA electronic notifications system to TB.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

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**Project Containment**

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<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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Project Additional Information

Purposes of the contained use

The objective of this project is to study Pseudomonas aeruginosa infection in cystic fibrosis (CF) patients and to develop a diagnosing methodology on excreted fluids. P. aeruginosa and excreted fluids isolated from CF patients will thus be analysed. Genetically modified organisms will also be studied to get a better understanding of the bacterium and the infection mechanisms. Other CF-related pathogens will be analysed in order to identify P. aeruginosa characteristic features.

To achieve our goals, we want to use rapid evaporative ionisation mass spectrometry technology (REIMS) as it has demonstrated promising results on bacteria and is quite simple to implement. During REIMS analysis, an electrical current is applied directly to biological material using forceps. The ions generated become vaporised and are passed through the forceps and channelled into the mass spectrometer.

The Royal Brompton Hospital will provide fluid samples collected from CF patients (e.g. urine, saliva, sputum, skin secretion, exhaled breath condensate) as well as bacterial strains of Pseudomonas aeruginosa and other CF related pathogens (Hazard Group 2) isolated from sputum samples. They will be provided to us as bacterial cultures on petri dishes to be incubated prior to analysis, either at RBH or lab 373C at Sir Alexander Fleming building (SAFB), South Kensington campus (SK). These will not be genetically modified (GM).

Other bacterial cultures will be provided by the microbiology laboratories of SK campus. They will also provide
GM P. aeruginosa isolates for us to analyse in order to study gene metabolic functions. The identity of each bacterial isolates will be known prior to further work and human samples will have been tested at RBH. Antibiotic susceptibilities will be known for some of them. Both samples and bacterial isolates will be transported in a designated transport carrier to SK for REIMS analysis. They will be transported following the Packing Instruction 650, detailed by Imperial College guidelines. Samples and bacterial plates will be stored in a laboratory fridge until analysis (37°C at SAFB); cryovials will be kept in the freezer (373 at SAFB). Samples and bacterial isolates will be discarded according to SOP instructions at SK (SOP Handling Waste). REIMS analysis will be performed at SAFB 373 following SOP. All steps of the procedure will be conducted in a Class II biological safety cabinet.

We will use the complete collection of P. aeruginosa genes which are annotated from the PAO1 genome sequence (http://Pseudomonas.com). This collection comprises over 5500 genes and will be completed by genes identified in newly sequenced P. aeruginosa strains (such as PA14). All the genes are cloned in the pDONOR entry vector (Gateway, invitrogen) and could be moved in the pDEST series of destination vectors that allow overexpression of the genes and production of tagged proteins. These plasmids are maintained in E. coli strains and genes will be overexpressed in E. coli for biochemical and structural applications. For studying the physiological role of the P. aeruginosa genes, the genes could be transferred from E. coli into P. aeruginosa and related strains by re-cloning in broad host range plasmids from the pMMB or pBBRMCS series and conjugated into P. aeruginosa with the help of the pRK2013 plasmid or the S17-1 E. coli strain. Whenever it is required site directed mutagenesis will be performed. The strategy will consist in interrupting the target gene by homologous recombination with an internal DNA fragment cloned into a non-replicative vector for P. aeruginosa or by creating a clean gene deletion using suicide vector which could be further cured by a sucrose selection procedure (pKNG101 or pEX series of plasmids). Moreover random transposon mutagenesis will be performed using delivery systems for Tn5 or Tn7 derivative plasmids. Some of these transposon derivatives will contain a T7 or tac promoter reading outwards for expression of downstream genes, other will carry promoterless lacZ or gfp genes to create chromosomal transcriptional fusion. The antibiotic markers carried by the plasmids and transposon used will essentially be: kanamycin, gentamycin, tetracycline, carbenicillin, ampicillin, chloramphenicol and streptomycin. For complementation studies of the obtained mutants, genes will be cloned on broad host range vectors. In all cases, the desired constructions will be introduced in P. aeruginosa by electroporation or conjugation.

Recipient or parental organism

Class 2
P. aeruginosa (HG2)

Class 1
Attenuated non toxigenis K12 derived E. coli (from standard commercially available clonin kits e.g. DHSalpha) (HG1)

Host/vector system

We will use broad host range vectors for expression in P. aeruginosa (also replicative in E. coli):
- pLAFR1, pLAFR3 and derivatives : IncP, TetR. Cosmid with low copy number.
- Mini-CTX1 and Mini-CTX2 (TetR, pMB1 origin, CTX integrase) for engineering lacZ transcripntional fusion on the chromosome.
We will use suicide vectors for the engineering of Pseudomonas mutants:
- pKNG101 and derivatives : StrR. SucR. Only replicate in the E. coli lambdapi strain CC118.
- pEX100 and derivatives: SucR. Only replicate in E. coli lambdapir strain CC118.
- pUT-Tn5 and derivatives: TetR.
- pBK-miniTn7 and gfp derivatives, pUC19 based vectors with various resistance for delivery of gfp on the chromosom of P. aeruginosa.
- pML1 (GmR) and pLR27 (KmR) and pALMAR3 (TnR) for Tn5 delivery on the chromosom.
- Mini-CTX1 and Mini-CTX2 (TetR, pMB1 origin, CTX integrase) for engineering lacZ transcriptional fusion on the chromosom.
- pFLP2 for excision of CTX backbone from the chromosom (ApR, sacB).

Origin & function

Because of the nature of this project we will use all annotated genes available from the genome sequence of the PAO1 and PA14 strains (http://Pseudomonas.com), as well as other genes which will be found in newly sequenced strains.

Evaluation of foreseeable effects

The hazardous potential of the GMMs created is considered to be no greater than that of the parental strain. In many cases genes are inactivated in the GMM that are required for optimal growth and pathogenesis.

It is not envisaged that the genetically modified organisms will be significantly more fit in the environment than the original. However GMM could alter the expression of some secretion systems and biofilm formation capabilities which may affect virulence. Our data and the literature has shown that increasing these traits tends to decrease acute virulence of strains and indeed we see this with Pseudomonas aeruginosa. GMM targeting the genes we are interested in may increase the minimum inhibitory concentration of certain classes of antibiotics but not all classes. Furthermore the chances of creating a mutant strain with increased virulence is diminishingly small as these mutants should have already arisen in the global population and be highly prevalent. In addition, as most of these are laboratory strains infection in healthy individuals is not a particular concern.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

No liquid waste will be generated in routine use as culture plates will be purchased already filled with solidified growing media. The small quantities of liquid samples will be soaked up with tissue paper to prevent spillage before disposal through the solid waste route (max 200mL/bin)

Solid waste - i.e. bacterial plates, sputum, soaked up liquids - will be sterilised in an autoclave (Waste programmes are run 121C for 20 minutes) before disposal by a specialized company for incineration.

The print outs on the bags are checked to ensure that the cycle has not failed. The autoclaves go through an annual inspection plus a 6-monthly maintenance performed by Ascot Autoclaves. The Imperial staff performs weekly checks in accordance with the manufacturer's instructions.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
Following the discussion of your proposal entitled "Analysis of bacteria-containing samples using Rapid Evaporative Ionization Mass Spectrophotometry (REIMS)" (GMIC-5384), the committee request that the following amendments are made to the proposal prior to approval:

Reviewer 1
1.2 This does not mention the GM work. Please include a brief description and how it fits in with the rest of the REIMS work in this overview.
2.14 Isolation of Burholderia cepacia from patients is mentioned in 1.2 Is this part of the GM process as well?
2.15 Please list all the recipient microorganisms for the GM work i.e. The host organisms. I believe from the description of your work this will include the following:
Pseudomonas aeruginosa HG2
Burkholderia cepaciae HG2
Attenuated non toxigenic K12 derived E.coli (from standard commercially available cloning kits e.g. DH5alpha, ) HG 1
7.1 ‘Liquid waste generated during disinfection’ What is this material, is it liquid culture waste? What happens to the E.coli and Pa liquid cultures? Please describe the use of any chemical disinfection e.g. "Virkon is added to the liquid culture waste to a minimum concentration of 1% for 30 minutes before disposal to the drains”
8.1 Maintenance frequency of the Autoclaves? This is likely to be annual by a qualified engineer and will include a pressure system inspection, probe calibration and waste run validation procedures. This is in addition to users checks in accordance with the manufacturer’s instructions.

Please enter comments on the GM safety committee on the risk assessment

Section 13 Lone working requires completion.
Section 14 Please be more specific about the containment level required for the various processes
The Incubation, plating and storage of Pseudomonas aeruginosa and Burkholderia cepaciae CL2
The incubation, plating and storage of attenuated E.coli CL1 1

Section 15 Please be more specific about the containment level for the various processes
The Genetic modification, incubation, plating and storage of Pseudomonas aeruginosa and Burkholderia cepaciae Class 2
The Genetic modification, incubation, plating and storage of attenuated E.coli Class 1

SOP Spillages – Need to differentiate between spills inside MSC, spills outside and MSC, spills outside a lab. The plates will be transported in double-sealed containers so there is no anticipated scenario that would lead to spillage outside the laboratory.

SOP decontamination – What about the MSC’s? cf SOP for MSC
SOP – Prep of trionic. Eye goggles? Do you mean goggles or glasses.? Goggles are usually reserved for higher risk chemicals. What is it about the chemical or the process that requires goggles? We opted for the glasses, cf SOP Preparation of Trionic Disinfectant
SOP Reims – The SOP does not look as if the forceps are in an MSC and yet the Bio1 says they should be? The picture needs to be updated, I specified in the SOP that this was a demonstration of the operation but that it has to be conducted inside the MSC. This needs to be discussed at committee. Decontamination of the forceps by forcing fluids back through them into a sink may generate infectious aerosols. Can the forceps be soaked in Trionic solution prior to flushing? That's absolutely right, I added a step consisting of sonicating the forceps in IPA before flushing.

SOP – Safety – What sort of eye protection? Goggles and glasses are available, to be chosen according to the chemical risk or as stated in SOP
SOP transport – What about transport of plates within the lab? They will be transported in racks, cf bio1 form.

What about Training and competence? We would need to see training documents detailing what the person has been trained to do with signatures form the trainee, trainer
and PI, as well as periodic refresher training. This is recorded in appendix 5.

Does the Mass Spec require service and are there any protective devices such as filters that need replacement? Is this documented? There’s no need for filter changes, the maintenance of the instruments and pumps is performed by the instrument warden. I added it in “section 8 – Maintenance” of the bio1.

Reviewer 2
4.9 Is it possible to list the organisms sp individually? Are the routes of transmission of infection are different for the different pathogens, if so this should be clearly stated for each pathogen that is being handled.

Reviewer 3
1.1: Little more detail as to why this technique is being developed. It is unclear to me why GMMs need to be created in 2.14.
2.14: What exactly are the biochemical and structural applications that the GMMs over expressing Pseudomonas genes will be used for? Not sure how this fits into the REIMS protocol at all. Relates to my comments on section 1.1
4.21: 30 plates doesn’t give much of an idea of the volume/concentration of the GMMs to be used. E.g. What size plates?

Reviewer 4
2.3: It is not clear to me which samples are being tested. It is stated that patients are regularly screened, then that sputum is not screened but other samples are, then that none of the other samples are screened. I think it should be clarified which samples are tested for what.
5.3 and 5.5: Which samples are sealed with parafilm? I presume these are culture plates or anyway containers which cannot be sealed by screw caps or equivalent.
Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

**Project Containment**

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**Project Ref 8/16.6**

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<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Withdrew N
Tick if notifying a connected programme of work N

Historical Significant Changes
Project Additional Information

The project has 4 clear goals:

1. Evaluate the H2O2-induced Invasion Phenotype (HIP). Host-cell invasion comprises motility, host-cell selection, apical organelle secretion, conoid extension, and active penetration. We will systematically evaluate each to home in on the parasite process/ies stimulated by H2O2.

   Experimental procedures: T. gondii. Continuous culture of T. gondii strains in human cell lines (Human Forskin Fibroblasts or VeroTM cell lines available commercially). Conventional light, immunofluorescence and live cell imaging for cell-based assays of invasion, motility, conoid extension. Western blot will be used as a biochemical readout of organelle exocytosis.

2. Identify molecular machinery necessary for the HIP. We will use a forward chemical genetic screen to identify effector proteins required for its manifestation. This will directly test the hypothesis that T. gondii contains genes that allow tachyzoites to detect and respond to H2O2 signals.

   Experimental procedures: As above, in addition: Chemical mutagenesis of wild-type T. gondii strains to generate mutant parasite libraries. Transfection of replicating forms with plasmid vectors or linear DNA (generated in E. coli via conventional cloning) to create GM parasites. Analysis of chemical mutant and transgenic parasite lines by western blot, southern blot (PCR), immunofluorescence and live cell imaging. Cloning via limiting dilution. Cloning by FACS cell sorting will only be attempted when provision for class II cell sorting is established at the South Kensington Campus. Any other use of FACS will always be using fixed material for counting purposes only. Deep sequencing of parasite genomic material will be undertaken at the Imperial's Genomics Facility.

3. Systematic profiling of H2O2 sensors in T. gondii. We will use a chemoproteomic mass-spectrometry approach to systematically identify cysteine thiol-based H2O2 sensors. We have demonstrated that a novel method we term oxidation-masked isotopic tandem orthogonal proteolysis activity-based protein profiling (OXisoTOP-ABPP) can identify reactive cysteines in tachyzoites, and determine which have the capacity to “sense” exogenously applied H2O2. Is this network of H2O2 sensors strain-specific (and a possible component of virulence differences between types), or hard-wired into T. gondii biology? We will expand our preliminary study of T. gondii type I tachyzoite H2O2 sensors to examine differences between the major parasite genetic backgrounds, and determine the contribution of sensors to parasite biology.

   Experimental procedures: As above, in addition: Proteomic samples will be generated from T. gondii strains and
analysed at the CISBIO Mass Spectrometry Core Facility. All work will be based within the certified laboratory areas of the Child laboratory, level 6 SAF building. T. gondii parasites are grown entirely in vitro. All experimental work which includes parasite purification for staining, imaging, developmental assays, biochemical procedures and genetic modification will be carried in a containment level 2 laboratory, room 639 Level 6 SAF. FACS-based live cell analysis of T. gondii will be performed where required on the 3 laser Fortessa II which is located in room 638 on level 6 of the Sir Alexander Fleming Building, and live sorting on the BD FACs AriaIII enclosed in a hood located in the Flowers Building, room 3.22 Imaging will be undertaken in either the Child laboratory or level 5 rooms associated with FILM. GM mice will not be used. BALB/c mice will be used for the in-house generation of antibodies. This procedure is mild in terms of its severity. This will be necessary as antibodies that recognize T. gondii proteins are not commercially available. Therefore, in order to study the biological function of the H2O2 effectors responsible for the manifestation of the invasion response, and the H2O2 sensing protein networks that this proposal will identify it will be necessary to generate antibody reagents. Before generating any antibodies, research will be done to determine if antibodies raised against proteins of interest are already available through the Toxoplasma research community. If so, we will attempt to obtain these antibodies are a shared community reagent to reduce numbers of animal used in these studies. The future down-stream assessment of the function of specific gene loci identified in goal 2 and 3 might require the assessment of gene function to parasite fitness in vivo using mouse infections. Strains of mice (such as BALB/c) and C57BL/6 will be infected IP with T. gondii parasites. The course of infection will be monitored by twice-daily recording of mouse weight. In order to provide a humane endpoint to the parasite infection model, mice will be sacrificed if they lose 20% of their starting weight. Mouse models of infection remain the only way to assess the contribution of gene function to parasite fitness and virulence in vivo.

Recipient or parental organism

Toxoplasma gondii is an obligate, intracellular, parasitic protozoan that causes the disease toxoplasmosis. Found worldwide, T. gondii is capable of infecting virtually all warm-blooded animals. While the parasite is found throughout the world, more than 50% of people have often been infected. Of those who are infected, very few have symptoms because a healthy person's immune system usually keeps the parasite from causing illness. However, pregnant women and individuals who have compromised immune systems should be cautious for them. Severe toxoplasmosis (found only in immunocompromised), causing damage to the brain, eyes, or other organs, can develop from an acute Toxoplasma infection or one that had occurred earlier in life and is now reactivated. Severe cases are more likely in individuals who have weak immune systems. Suspected infection can also be adequately treated with pyrimethamine and sulfadiazine, or azithromycin, which is given on its own. GMM T. gondii will be generated: reporter strains and knock-out strains. We will create transgenic T. gondii parasites that express fluorescent or luminescent proteins (for example, but not limited to, YFP, GFP, RFP, CFP, PA-GFP) in the asexual stages of the parasite to facilitate tracking life stages, or proteins of interest in the parasite. This will be done by either C-terminal tagging of endogenous loci by singlehomologous site-specific intergration, or using CRISPR-based integration strategies. We will also create knock-outs of genes in the parasite that we think are important in parasite response to H2O2. Examples include secreted or transmembrane proteins expressed at the different lifecycle stages of the T. gondii asexual lifecycle. Genes found to be important via KO will then be genetically complemented to rescue the KO effect. Knockout will be achieved by double homologous crossover with a drug selection cassette. CRISPR-based methods and/or conditional knockout approaches using inducible Cre recombinase activity where standard KO approaches do not work or are not suitable for the loci of interest.
Lab-adapted strains of E. coli will be used for the amplification of DNA plasmid vectors and the heterologous production of recombinant Toxoplasma proteins.

Toxoplasma Gondii strains in use: RH1, Pru, CEP, ME49, GT1. All are long standing well characterised lab strains. E.coli non toxigenic strains used for plasmid amplification will be DH5α or similar, and BL21 or similar for the production of recombinant protein.

Human Foreskin Fibroblast cell lines (HFF) or Vero cell lines (originally isolated from kidney epithelial cells extracted from an African green monkey) both available from ATCC or available commercially.

**Host/vector system**

T. gondii vectors are non-commercially available small circular plasmids. DNA vectors used will be based on those collaboratively available. All contain either: (1) chloramphenicol transferase gene (CAT) that confers resistance to chloramphenicol; (2) the DHFR selectable marker that confers parasite resistance to Pyramethimine; (3) the hypoxanthine-xanthine-guanine phosphoribosyl transferase (HXGPRT) gene that confers resistance to mycophenolic acid and xanthine. No viral vectors or any vectors with pathogenic properties would be used.

All E. coli vectors used are commercially available. Vectors will be pUC19-based for purposes of DNA amplification.

For the recombinant expression of protein, commercially available expression vector systems including pGEX-6P1, pET28a and pET-28b will be used. These vector systems include ampicillin or kanamycin resistance markers for the purposes of plasmid selection.

**Origin & function**

The list of genes that will be altered is currently unknown as it is an aim of the proposed work to discover them, but will likely include: genes encoding proteins with annotated redox functions, an association with invasion, or expression profile that matches other known invasion-associated genes, regulatory (e.g., signaling) machinery genes that mediate transduction of H2O2 signals and could include genes encoding kinases and receptors/channels (based on their predicted coding function).

None when modified would increase virulence (indeed all knockouts or other variants would be expected to be nonviable). Genes encoding fluorescent/luminescent proteins, including but not limited to YFP, GFP, RFP, CFP, luciferase and their derivatives will be introduced into T. gondii to fluorescently/luminescently-tag proteins to facilitate tracking life stages, or proteins of interest in the parasite.

Toxoplasma genes will also be modified with short epitope tags such as HA, Ty, Myc tags to enable biochemical tracking of proteins of interest.

The tagging and knocking out of specific gene loci and selection of tagged strains and KOs will involve the introduction of drug-selectable markers including either: (1) chloramphenicol transferase gene (CAT) that confers resistance to chloramphenicol; (2) the DHFR selectable marker that confers parasite resistance to Pyramethimine; (3) the hypoxanthine-xanthine-guanine phosphoribosyl transferase (HXGPRT) gene that confers resistance to mycophenolic acid and xanthine.

**Evaluation of foreseeable effects**

The knock-out process is targeted. No loci when modified would increase virulence (indeed all knockouts or other variants would be expected to be non-viable). At no stage will any GMM T. gondii line be generated that is expected to have enhanced virulence and therefore enhanced hazard to animals, the environment or human health.

No horizontal gene transfer is known to occur between Toxoplasma and other microorganisms.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The proposed work does not include the generation of GM animals or plants.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| None |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Liquid disinfection will be done using 10% chloros or minimum 1% virkon after dilution with the waste. This will be incubated for 1 hour. This is then followed by disposal to drains. Solid waste will be autoclaved (Minimum 121 C held for 15 minutes) and then disposed of via standard waste routes. |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment
Chemical biology studies of Toxoplasma biology. Dr M Child, Life Sciences, South Kensington Campus.

Review by BSO:

1.3 Where is the main handling / culture / storage of the T. gondii conducted? Where are the animals infected? Please list all the labs in use here.

2.14 How is the gene knock out being achieved?

2.15 Are the T. gondii strains in use those listed in 2.10? Please list the strains. This is important in establishing the limits of the work.

2.16. Do the vectors have commercial names? If so please provide a few examples. Also which vectors are being used in the T. gondii and which are being used in the E. coli. It is not clear from the description currently given. Again this is important in establishing the limitations and boundaries of the work. The HSE will expect to see this specified.

2.17 Please list the genes that you know you will be inserting, deleting or rescuing. At the very least this would be your fluorescence or drug resistance marker genes.

2.18, 4.18 and 4.20 Is the knock out process targeted? For example there might be a concern that if genes controlling the expression of virulence markers are knocked out this might increase the likelihood of harm? Could host range be affected? The committee and HSE are likely to ask how it is known with any certainty that such a wide ranging proposal (list of genes to be altered currently unknown) is unlikely to produce any enhanced GMO’s.

4.6 Have you discussed screening and serotyping with Occupational Health?

5.1 You state that infectious aerosols might be created. You have not indicated that an MSC will be used. COSHH stipulates that at CL2 where there is a risk of exposure to a pathogen by the airborne route that an MSC must be used. Please complete the rest of this section e.g. type of MSC in use and when it will be used?

5.6 Does the LN2 SOP describe how the storage tubes that have been in LN2 liquid phase will be safely removed and thawed, bearing in mind the risk that the occasional tube will burst if defrosted too quickly and any LN2 in the tube expands faster than it can escape. This is a commonly encountered problem with liquid phase storage. Please give the room numbers.

5.7 Main lab is not listed in 1.3. Please give the lab number or location.

5.8 Please give the lab number. It is not mentioned in 1.3

5.9 Are needles or sharps used to infect or dissect animals?

5.12 What percentage paraformaldehyde and is this at room temperature or chilled?

6.5 Are these FFP3 face masks? Are all staff using them face fit tested for these? Why are face masks required? Are the allergens not controlled at source, for example in an MSC or down flow bench? TC hoods are mentioned here. By TC hood do you mean class 2 MSC? If so this was not mentioned in 5.1. Please clarify when and why MSC’s will be
used. Do you use face shields or safety glasses? I am not sure what an eye shield is?
7.1 I assume 70% ethanol is for spraying down bench surfaces after a spill has been absorbed? Please remove reference to it here. This section is for routine liquid culture waste disposal. 70% ethanol is not suitable for this purpose. Is the 10% chloros solution the strength after dilution with the liquid waste?
8.0 MSC’s are mentioned here but not in 5.1 Please add when and why MSC’s are used in 5.1.
11.2 Is the 6th floor designated lab shared with other projects not using T gondii? I thought this was also used by the insectary lab workers for animal holding?
Comments at the meeting: MC gave a brief description of the work.
NF asked what protocols were in place for possible immunocompromised researchers working on the project?
MC stated that he had a discussion with Occupational Health regarding Health Screening. If the researcher was known to be immunocompromised they would have a health screen before work begins. However OH did not routinely screen healthy individuals but would take a base line sample immediately after any potential exposure incident.
NF asked about pregnant workers?
MC would offer them the option not to work with T gondii, and to offer health screening. If immune naïve MC would recommend not working on the project.
NF pointed out the hazards can be easy to forget, what robustness would be in place?
MC explained in previous laboratories he worked in, explanations were given to groups considered at risk when they began on the project. To also operate high standards of cleanliness, label samples and ensure they are secured after use. MC also discussed with IH of possible access restrictions. The lab is on a secure floor and is already dedicated solely to the group, can be locked when not occupied, but is not independently card swipe or PIN secured. IH will look into funding for additional security.
RW checked if all the comments from the BSO had been addressed?
IH confirmed that all the comments had been addressed.
RW questioned if MC covered everything he wanted to work with on the risk assessment?
MC answered that he had also included animal work after IH suggested it.
NF wanted to know if animals could be added at this stage if the HO licence had not yet been obtained?
IH confirmed that animal work could be included with a note that this is for future work.
The following amendments were required prior to approval:
No further comments from the GMSC.
Agreed: Class 2, Containment Level 2. This needs to be notified to the HSE.
IC reference number: GMIC-6856

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<td>L3</td>
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</table>
Legionella pneumophila uses a specialized type IV secretion system (T4SS), the Dot/Icm secretion system, to transport a large number of effector proteins into host cells. Some of the characterized translocated substrates play a role in hijacking host cellular pathways to establish the intracellular niche where the bacteria survive, replicate and ultimately cause disease. The research of my laboratory will focus on understanding the molecular mechanisms of pathogenicity by the intracellular pathogen. We will determine the molecular organization of the large double membrane-spanning Type IV Secretion System by single particle cryo-electron microscopy (cryo-EM) and establish the structural basis of substrate recruitment.

Recipient or parental organism

L. pneumophila subs. Philadelphia strain Lp02: hsdR rpsL thyA
Lp02 is a thymidine auxotroph, hence should not pose any risk to a healthy human health. The Lp02 strain was isolated in 1993 and has been studied in more than 100 publications since then.

E.coli DH5 alpha lambda pir strain. This strain will harbour the pSR47S low copy suicide plasmid. This plasmid contains lambda pir-dependent R6K replication origin; requires lambda pir-containing bacteria strain. This plasmid will carry genetic modifications which are highly specific to L. pneumophila targeted genes, and will not affect any genomic region (or properties) of L. pneumophila Lp02

Host/vector system

pSR47S – KmR; SacB, Low copy suicide plasmid, carries the kanamycin resistance cassette and a SacB (sucrose sensitivity). Contains lambda pir-dependent R6K replication origin; requires lambda pir-containing bacteria strain. Will carry genetic modifications which are highly specific to L. pneumophila targeted genes, and will not affect any genomic region (or properties) of L. pneumophila Lp02

pRK600 plasmid is a natural occurring plasmid that aids the genetic modification carried in the pSR47S plasmid to be transferred into L. pneumophila
L. pneumophila subs. Philadelphia strain (Lp02) will be used as a background strain to isolate the T4SS and will be genetically modified to incorporate different tags which will facilitate the isolation of the multiprotein complex. The introduction of the different tags in the bacterial genome, will be achieved using DNA recombination methods.

**Evaluation of foreseeable effects**

L. pneumophila Lp02 strain, is a thymidine auxotroph. In the unlikely event of the bacteria escaping its growth flask/tube, it does not pose a risk to the environment as it lacks the thymidine necessary for its survival. The strain is non virulent in several infectious models including macrophages and Galleria Mellonella larvae. In the unlikely event of aerosolization, the bacteria is not able to survive, replicate or infect humans since the lung environment lacks the thymidine quantity (100 μg/ml) necessary for its survival.

**Origin & function**

L. pneumophila Lp02 strain, is a thymidine auxotroph. In the unlikely event of the bacteria escaping its growth flask/tube, it does not pose a risk to the environment as it lacks the thymidine necessary for its survival. The strain is non virulent in several infectious models including macrophages and Galleria Mellonella larvae. In the unlikely event of aerosolization, the bacteria is not able to survive, replicate or infect humans since the lung environment lacks the thymidine quantity (100 μg/ml) necessary for its survival.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste such as media is inactivated with Virkon at a final concentration of 1%. Solid waste will be placed into an autoclavable bag for validated autoclave sterilization.

Is an emergency plan required according to regulation 20?

- [N]

If yes, tick to confirm that it is attached to this form

- [N]

Tick to confirm that you have attached a risk assessment to this form

- [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment

- [N]

Please enter comments on the GM safety committee on the risk assessment
Comments at the meeting: TDC gave a brief description of the work.
IH asked if TDC wanted to use attenuated legionella?
TDC explained they needed to use a tiny amount for growth in the agar.
IH questioned the robustness, if it were to escape would it carry growing in the environment.
BR queried what the basis of the mutation was
TDC confirmed; point mutation.
BR asked if it was not partial or complete gene deletion?
TDC explained there were several point mutations and that there were many publications that showed if it was a stable mutation with no evidence of reversion.
IH pointed out that the GM Classification level at which the work for these papers was conducted was not stated in the publication.
TDC added they could monitor for growth on Thymidine deficient media regularly.
RW advised that this may not be wise as it may select for mutants that could overcome the thymidine supplement dependency.
IH suggested if TDC could submit a paper to support multiple deletions which would reduce the risk of spontaneous reversion, this work could be done at containment level 1.
RW enquired if anyone at the College was working with this and at what containment?
IH confirmed Professor Frankel did and at containment level 2.
The Committee agreed that TDC should provide a supporting paper illustrating the extent and robustness of the attenuating mutation to do this at containment level 1.
After the meeting: TDC submitted a paper and his findings, with the following comment: The paper shows that the thymidine mutation in the LP02 strain is indeed a single point mutation in the gene (Table4). Therefore, I believe that a process for a Cat2 project might have to be initiated.
IH also agreed, class 2. The single point mutation seems to suggest that reversion is possible.
Now that this is agreed as a class 2 project the risk assessment needs more details regarding containment. Additional BSO comments are given below:
1.2: Review this wording to include a line that states something along these lines: “the LP02 strain is thymidine dependent and under normal circumstances incapable of establishing an infection or growth outside of the laboratory environment. However the attenuating mutation is only a single point mutation and reversion is not out of the question, therefore this GM work is still considered by the ICL GM committee to be Class 2”
2.14: Is GM E.coli in use? If so please list it as a recipient, and complete the rest of this section with the E.coli work details as well as the Legionella
2.18: Review this section with the finding of the GM committee in mind. See previous statement for 1.2 above.
3.1: change to no
4.16, 4.17, 4.18: Please review with the comments of the GM committee regarding the robustness of the attenuation in mind
5.1: Legionella is infectious via the airborne route. As this is a class 2 GMO activities likely to produce an aerosol will need to be conducted in an MSC. E.g. Are you...
pipetting or handling outside of any primary container liquid culture? If so I would recommend that this is handled in an MSC to control spills, splashes and aerosols generated by pipetting.

5.2: Confirm that this transport process involves double containment and that the plastic box is leak proof and drop resistant.

5.3: Are you not transporting between labs?

5.6: Please add that this material is clearly labelled and easily identifiable
Waste 7.1: Liquid waste. State that the virkon will be used at a final concentration of not less than 1% after dilution with the waste.
Solid waste. Add the words "validated autoclave" into the sentence Solid waste will be placed into an autoclavable bag for "validated autoclave sterilization.

10.1: Spills in the centrifuge should be left for one hour to settle before attempting to open the centrifuge.
A spill in the lab will result in the lab being evacuated for at least an hour to allow any aerosol to settle and be cleared by air handling

10.2: Remove affected clothing, distance yourself and others from the spill, evacuate the lab and wash any contaminated skin with soap and water as you exit.

12: Safety Department will forward this to OH for completion once the final Bio1 is received.

Section 14 and 15: Change to HG2 and class 2 respectively.

Agreed: Class 2, Containment Level 2
IC reference number: GMIC-8608
Reported: This requires HSE consent.

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## Project Containment

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## Project Ref 8/18.2

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<th>CultureVolume</th>
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<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Non-GMM</td>
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**Historical Significant Changes**

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02/03/2022
### Project Additional Information

#### Purposes of the contained use

The aim of this project is to understand the molecular mechanisms that regulate intracellular iron homeostasis in *Trypanosoma brucei*, a parasitic pathogen that infects humans and animals. Three subspecies are endemic in Sub-Saharan Africa: *T. brucei rhodesiense* and *T. brucei gambiense* are human infective while *T. brucei brucei* is only animal infective. All the work proposed here will be done with the non-human infective *T. brucei brucei* species - referred to as hereinafter as *T. brucei*.

The parasites live extracellularly in blood and lymphatic tissues where they interact with their host obtaining essential micronutrients such as iron for survival. Iron is a critical co-factor for numerous important reactions in both eukaryotes and prokaryotes, but in the presence of oxygen iron can be very toxic - creating the need for very tight regulation of free iron levels. In mammalian cells this regulation is achieved by iron regulatory proteins (IRP) containing iron responsive elements (IRE) that control expression of major components of the iron acquisition pathway such as the transferrin receptor (TfR). However, how this task is achieved in African trypanosomes is completely unknown.

Interestingly, African trypanosomes have a surface protein that functions as a transferrin receptor (TbTfR), and expression levels of this protein is also modulated by iron availability. However, TbTfR is structurally distinct from the mammalian TfR, and previous work indicates that *T. brucei* lacks the canonical IRP/IRE iron-based regulatory system. This research will seek to understand if trypanosomes have co-opted and co-adapted alternative eukaryotic mechanisms for iron-sensing and regulation of iron homeostasis.

#### Recipient or parental organism

Organisms and strains:
- E. Coli K12 derivatives, including DH-5 alpha, DH10B, XL-1 Blue and TOP10.
- E. coli BL21 derivative strains.
- T. brucei brucei

#### Host/vector system

Vectors:
- pBluescript, pET and pGEX expression vectors for Histag and GST fusion protein expression in bacteria. These will
be expressed in the E. Coli strains above.
p2T7 RNAi vectors. This is a Bluescript based series of plasmids which has different fragments allowing tetracycline inducible RNA interference (RNAi) inserted into them. Used in T. brucei brucei.

BES targeting vectors. These are Bluescript based plasmids containing BES fragments as target fragments allowing their insertion into T. brucei brucei VSG expression sites.

TIR expression plasmids - these are a series of vectors bearing wild type TIR genes for expression in trypanosomes.

Epitope tagged variants of these vectors will also be used as reporter constructs for iron-stravation experiments.

CRIPR-Cas9 vectors - these are pBlueSript based vectors for expression of Cas9 and sgRNA constructs in trypanosomes.

Origin & function

Selectable marker genes for bacterial plasmid vectors: Kanamycin, ampicillin, chloramphenicol

Fluorescent fusion (eGFP, mCherry, YFP) proteins for localisation studies that bear antibiotic resistance genes

hygromycin, puromycin, phleomycin and blasticidin.

T. brucei Phosphatidic acid phosphatase gene (PAP2), RNA binding protein 5 (RBP5). These proteins are hypothesized to be involved in iron uptake and usage in trypanosome cells.

T. brucei VSG expression sites associated genes (ESAGs). The VSG expression sites are the transcription units where the active VSG gene is expressed. These expression sites contain a variety of gene families (ESAGs) which are thought to be trypanosome surface receptors in addition to the telomeric VSG. These ESAGs appear to be nutrient receptors (for example transferrin receptor encoded by ESAG6 and ESAG7), and do not appear to be involved in trypanosome virulence. They are important for receptor-mediated endocytosis of iron-bound transferrin from the host.

Evaluation of foreseeable effects

E. coli strains are classed by the ACDP as Hazard group 1 and T. brucei is classed as Hazard group 2. Non of the modifications made to these cell lines will change their virulence properties. If anything, we will be making attenuated strains that will be less virulent since we will be deleting or reducing expression of genes by RNA interference.

The genes have no pathogenic determinants by themselves when transfected into host cells will not modify the cell pathogenicity. Both the E. coli and T. brucei strains used are laboratory adapted and do not establish in vivo infections in human.

The E. coli strains and T. brucei strains used are unable to survive outside defined cultured conditions hence are unlikely to pose any specific threats to the environment.

There has never been a documented case of DNA transfer from a trypanosome to another organism. T. brucei will be grown under Containment Level 2 conditions, and all trypanosomes will be destroyed with Virkon or autoclaving before trypanosome infected waste is disposed of. We don't foresee any mechanism for transferral of sequences from GM trypanosomes into the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste: E. coli, T. brucei culturing medium.
chemical inactivation: incubation with 1% Virkon for 30 minutes before disposal down the drains and glassware will be sent for washing
Validation of treatment: all parasites have been shown to lyse in 1% Vrikon 30 seconds after treatment.
Solid waste: Contaminated culture flasks, plates, pipettes.
Treatment: Solid waste will be transferred to autoclave bags and removed for autoclaving at 126°C for 20 mins. Charts are recorded after autoclaving the waste is packed in orange bags before being disposed of via our waste contractor. Autoclaves are serviced each quarter, and validated and insurance inspected once per year in accordance with Imperial College London regulations.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment
Following the discussion of your proposal entitled “Molecular mechanisms of iron sensing in African trypanosomes” GMIC-8841 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

1.1: Begin with full names of the strain, Trypanosoma brucei brucei in the first instance and then shorten for clarity and keep this consistent throughout the form.
2.15: Add CRISPR Cas9 and remove reference to ‘427’ in T. brucei brucei to not restrict your strain list.
Throughout Bio1 form: Remove reference to R1, R2, R3 etc. and change to CoP page number.
Section 7 and Code of Practice: Add how the waste is dealt with. For SAPO licenced work, it has to go into the autoclave by the user.
Section 14 and Code of Practice: Add correct SAPO Licence number, SAPO/77/2017/1
Agreed: Class 2, Containment Level 2. This needs to be notified to the HSE and added to College SAPO licence
Comments:
CT gave a brief description of the work.
RW asked if this work would be based in Sir Ernst Chain Building, Containment Level 2?
CT confirmed it would be and required Access Control measures to be in place.
MB added the CL2 should already have access control in place and key locks on the doors are sufficient.
RW commented that the Bio1 risk assessment form was very complete and if the Code of Practice was based on Professor G R's CoP?
CT confirmed that it was.
RW checked if CT had included strains he thought he would be using in the future?
CT answered he would like to include CRISPR Cas9 in the future and will add this to the list of strains.
MB asked about the FACS machine in the Flowers Building and what was the procedure for disposing of the waste.
CT explained there would be no life cell sorting and the waste would be disposed of by the FACS manager or lab manager.
MB explained this could not be the case for SAPO and CT would need to consider this in his Standard Operating Procedure. Explain you will begin with an empty tank, add virkon and leave for 30 minutes.
JP suggested adding an extra 30 minutes when booking time to use the FACS machine.
MB questioned the reference made on the Bio1 form to the CoP, why R1, R2, R3 etc.
CT thought this would make referencing easier.
MB recommended changing this to referencing the CoP page number instead.
MB pointed out the SAPO licence on the SOP was incorrect and would provide CT with the corrected number.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4 L2 L3 L4</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2</td>
<td>L3 L4</td>
</tr>
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Purposes of the contained use

*M. tuberculosis* is one of about a dozen bacterial species for which some clinical isolates are now resistant to most or all antibiotics (abx) approved for treatment of the infections they cause. Mechanisms of antimicrobial resistance (AMR) in *M. tuberculosis* deserve study for their potential relevance to AMR in other pathogens; because tuberculosis (TB) is now the leading cause of death from infectious disease; and because drug-resistant TB may be the most prevalent of all drug-resistant bacterial infections. Heritable AMR in *M. tuberculosis* emerges with interruption of treatment, and the long duration of TB treatment provides many opportunities for interruption and non-compliance. Prolonged treatment is necessary because of nonheritable resistance, also called phenotypic tolerance or persistence, defined as the transient tolerance of bacteria in an antibiotic-sensitive population to an antibiotic during exposure to an otherwise lethal concentration of that antibiotic. In contrast to “resisters”, whose AMR is genetically encoded, “persisters” are genetically sensitive bacteria whose phenotypic tolerance allows them to survive for prolonged periods during what would otherwise be rapidly curative treatment. The specific aim of this work is to identify genetic determinants that foster phenotypic tolerance in *M. tuberculosis* through the selection and analysis of high-persister (hip) mutants selected in vitro and in vivo; these strains produce a higher proportion of persister cells than a wild-type strain under the same conditions.

Definition of tolerance in this work: Phenotypic tolerance manifests as “the presence of bacteria in an antibiotic-sensitive population that are not killed by that antibiotic but, when allowed to grow in its absence, give rise to populations that can be killed by the antibiotic in the same proportion as before” [C. Nathan, Fresh approaches to anti-infective therapies. Sci Transl Med 4, 140sr142 (2012)]. Of note, in *E. coli*, persisters increase the population surviving antibiotic exposure from which mutations arise that confer heritable antimicrobial resistance (AMR), and the heritably-resistant mutants are then selected during subsequent cycles of exposure to the antibiotic. The same is likely to be the case for *M. tuberculosis*. 
The objective is to identify hip mutants in Mtb that form in vitro or in vivo during infection upon exposure to stresses imposed by a combination of host immunity and chemotherapy. The activities are divided into three parts, namely isolation of hip mutants in vitro, isolation of hip mutants in vivo and follow-up activities that pertain to mutants isolated using both methods.

Experimental procedures will include the use of routine microbiology techniques in Class I Microbiological Safety Cabinets (MSC), infection of cell lines with listed biohazard group 3 bacteria, molecular biology, mouse husbandry, mouse infections, harvesting tissues from mice, imaging of fixed cells using fluorescence microscopy, FACS analysis of fixed biological material, plasmid-based molecular cloning, and various biochemistry methodologies. All procedures are described in the Code of Practice document.

Recipient or parental organism

ACDP 3: Fully drug-sensitive laboratory strains such as M. tuberculosis H37Rv, Erdman or CDC1551 and M. bovis AF2122/97. Please note that the present work does not use M. tuberculosis clinical isolates.

Transposon libraries contructed by himar1 mutagenesis.

A strain of Mtb that bears a plasmid encoding a tetracycline-regulated (tet-on) version of beta-galactosidase. This plasmid encodes an antibiotic resistance cassette.

A strain of Mtb that will be used bears plasmids that encode a selection marker flanked by loxP sites and the Cre recombinase, respectively.

High persistence mutants selected during the research activities described here.

Host/vector system

1. Transposon libraries of Mycobacterium tuberculosis contructed by himar1 mutagenesis
2. A laboratory strain of Mycobacterium tuberculosis that bears a plasmid encoding a tetracycline-regulated (tet-on) version of a reporter such as beta-galactosidase. The plasmid is constructed using the Gateway cloning system technology.
3. A laboratory strain that bears plasmids that encode a selection marker flanked by loxP sites and the Cre recombinase. These plasmids are constructed using the Gateway cloning system technology.
4. High persistence (hip) mutants selected during the research activities. Importantly, these mutants won't be generated but rather selected from a culture of the laboratory strain of Mycobacterium tuberculosis that naturally contains mutations and is not a de facto virulent strain. None of the hip mutants selected will thus be more virulent than wild-type strains listed in 7.1.

5 and 6. Mutations will be further introduced in strains listed in 7.1 to confirm that they confer the hip phenotype. if a gene is essential for growth in vitro, knock-downs will be constructed by transforming Mtb with two plamids: pNitETSacB-kan (https://www.addgene.org/107692), which allows for the replacement of the native gene by a selection marker by recombineering, and a second plasmid that allows for the conditional expression of the gene; this plasmid will be constructed using the Gateway cloning system technology. Alternatively, knock downs will be generated using the CRISPRi technology. Briefly, a dca9 allele is under the control of a Tet-repressor-regulated promoter. Expression of Cas9 is induced in the presence of anhydrotetracycline; a cognate sgRNA (targeting the genomic region of interest) under the control of a tetR-regulated promoter; a tet repressor; a single-copy L5 integrating backbone; a pBR322-derived E. coli replication origin; a kanamycin selection marker. The current optimal backbone for M. tuberculosis is PLJR965. This backbone works as advertised in the Nature Microbiology manuscript "Programmable transcriptional repression in mycobacteria using an orthogonal CRISPR interference platform" [Rock JM et al. Nat Microbiol. 2017 Feb 6;2:16274]. If the gene is not essential, a knock out will be generated using the plasmid pNitET-SacB-kan described above.
Genes identified in the screens described above. Preliminary results in Msm hip mutants obtained in vitro using methodology 1b identified mutations in amino acid biosynthesis pathways, tRNAs and their modifiers, and transcriptional pathways. These are pathways that will be disrupted in M. tuberculosis. Importantly, these mutants won’t be generated but rather selected from a culture of the laboratory strain of Mycobacterium tuberculosis that naturally contains mutations and is not a de facto virulent strain. None of the hip mutants selected will thus be more virulent than the parental strain.

Origin & function

No experiments are planned that involve intentional creation of strains more virulent than parental strains; furthermore, all strains generated will be more tolerant but not resistant to drugs against which they have been selected. If resistance develops, which can be monitored by the presence of growth in the presence of the drug, cultures will be sterilized and discarded according to standard operating procedures so that no work will be conducted on resistant strains. Only second-line antibiotics will be used to select for hip mutants. As such, hip mutants will have increased tolerance, not increased resistance, against second-line antibiotics. Frontline antibiotics will thus remain therapeutic options in the event that an incident occurs. Knock-out or knock-down strains generated in this study using any engineering techniques described above will be as susceptible to frontline antibiotics as wild-type M. tuberculosis.

Evaluation of foreseeable effects

GM animals in use are no more hazardous to human health or the environment than wild type. When infected with Class 3 GMOs, the animals will be held in IVCs within the Imperial College Animal facilities at South Kensington.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: Mycobacterial cultures must be disinfected by the addition of Surfanios to a minimum final concentration of 10% prior to autoclaving and further disposal down drains. Solid waste: All other hazardous solid biological waste such as tissue culture plasticware (pipettes, flasks etc) will be double-bagged and autoclaved prior to disposal via the clinical waste route. Animal carcasses and bedding will be disposed as per CBS guidelines, also described in local rules.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Bio 1 form GMIC-8954 discussed on March 18, 2019. Feedbacks received on March 27, 2019 as follows.

Following the discussion of your proposal entitled “Isolation and study of Mycobacterium tuberculosis cells that are tolerant to antibiotics” (GMIC-8954) at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

2.16. Some of this text looks a bit cut and paste. Please remove any unnecessary text as this will be submitted to the HSE. See example attached.

2.17 Please complete this section with a description of the genetic changes and if practical a list or indicative list of the changes being made, especially any antibiotic resistance genes. This also includes any genes disrupted by CRISPR.

2.18 Please complete this section. For example will any of the mutants show any gain of function, enhanced tolerance or resistance to antibiotics especially important are those used in frontline treatments post exposure. Is there any evidence to support reports that CRISPR might cut outside of the predicted locations and cause unpredictable consequences. Is this actually less likely to occur than risks associated with more established techniques? A quick sentence on this might be useful. If frontline treatments are affected please describe what treatments will remain.

2.19 please complete this section. If no please state no.

3.4 please tick the no box

4.12 What are the consequences of this antibiotic resistance? Will this means extra precautions are needed when selecting for antibiotic tolerant strains of this bacteria? Are other antibiotics available to treat someone in the case of accidental inoculation?

4.14 Please fill this in, possibly centrifugation causing aerosols or the use of sharps or the emergence of resistance during experiments with persisters?

4.18 First mention of disruption of 2 component regulators please give more details on this. For example is this something you intend to do or if this might occur by accident what is the likelihood that this might happen. How is virulence altered? If some of the bacteria are tolerant to antibiotics does this not make them more hazardous than wildtype? Are all of the persisters/resistors still treatable?

4.20 I don’t believe this question has been answered as this asks about the final GMM product not just the vector system. Please include changes such as Drug resistance?

5.1 You will work in room 17 so will need your own CoP (plus please note the copy you sent out of date-latest version of Robertson RA is Jan 17).

5.7 Visual inspection is fine but what if there was a spill what would you do then e.g. how long to wait to open how to clean? Is this in the SoP?

5.10 Additional training required: this does not answer the question and the information here is better suited to the
question about shedding of agents.

5.13 Please supply an SoP for FACS?

7.4. Animal Carcases? Please tick the box.

8. You should also add that equipment is maintained by users in line with the manufacturer's instructions.

10.1 What is Amphospray? Is this a validated method of inactivating Mtb complex? When is this used and when is surfanios used?

10.2 Please include that you will report on Salus as well. All unplanned events with HG3 and class 3 organisms must be reported even if there has been no exposure or no likelihood of exposure.

13.2, 13.3 and 13.4 fill in, lone working is permitted out of hours working is not.

14.1 Mention room 17

Comments:

JV Brief summary of project

Looks at development of tolerance in TB (definition of tolerance given as in Bio1: Definition of tolerance in this work: Phenotypic tolerance manifests as "the presence of bacteria in an antibiotic-sensitive population that are not killed by that antibiotic but, when allowed to grow in its absence, give rise to populations that can be killed by the antibiotic in the same proportion as before"). Persistence precedes resistance

Looking at what mutations lead to the development of persistence

Identify the pathways involved with persistence and target with drugs

Mutations will be selected and their location confirmed with sequencing.

Mutants will then be recreated using Gm to check effects

Dead Cas9 in use which transiently develops knockouts/knockdowns

The link between persisters and resistors has only been shown in ecoli. The development of resistors from persisters cannot be excluded but in ecoli they can only develop under special circumstances with a repeated exposure and growth cycle. It is believed the mutation that allows persistence allows the bacteria the time to then acquire mutations for resistance.

Asked if exposure of persisters can be treated with antibiotics.

JV yes the same as the wild type. However if they become resistors this would become more of an issue if we have been using front line antibiotics in experiments. Therapeutic options would then be limited so this should be avoided. JV suggested to wear masks to prevent exposure to any of these types of resistors. MB stated that the wearing of RPE would not be acceptable as it suggests you are not safe to work at CL3.

There was a discussion about whether the plan was to expose persisters to frontline line antibiotics in a cyclic manner (and potentially lead to resistors) in the next 5 years. Not the immediate plan but could do in the future to study the evolution to resistors. SH said that the project would likely need to return to the committee if JV wanted to extend to
Other option is to use only certain antibiotics so that there are always first line antibiotics available. There was some downside to this scientifically as you want to use clinically relevant antibiotics.

The general consensus was that if mono-exposure to an antibiotic was used this would be acceptable as therapy if usually 3 line and there was agreement to ensure there is always a therapy available.

Discussion about the Gm work: if a gene is non-essential it can be knocked out if essential knocked down. This is inducer dependent.

Gaps need to be filled about which genes are targeted etc. this is tricky as a screen will be used to identify them but some general information to best of knowledge needs to be given.

SH are strains only lab strains, JV yes. SH make sure it’s clear that only lab strains are used not clinical strains, make limitations of strain use and of the project very clear.

MB 4.18 How is virulence altered? BR says this would make it more virulent.

If you’re not aiming to do this (make it more virulent) but it might happen how can you know if it has happened? Lab strains are used often to infect mice so there is a lot of data on this to compare to. Researcher (don’t know her name sorry) they are not creating mutations they are in the starting pool so unlikely to select for increased virulence as it’s unlikely to be in the pool. A tendency for this has not been seen.

IH state in 4.18 that its historical use and an unlikely event.

SH what would you do if you saw increased virulence e.g. mortality in an experiment would you end the experiment?

MB stated a CoP for room 17 is required (RP mentioned the current version of BR CoP is Jan 17)

SH asked if the techniques/work was significantly different to that of Brian Robertson

BR confirmed that it wasn’t significantly different.

The filter technique is already an established technique for other in metabolomics (Gerald) so generic CoP for this can apply. Gerald does use vacuum filtration for metabolomics so will be the same technique.

Amphospray is also used by Gerald for metabolomics and has been validated at the crick. Link to specific protocol is required and RP stated that it should be made clear when amphospray is used and when surfanios is used.

HB made that it would be clear right away if a resister was present as it would grow in the flasks etc.

IH stated that a defined stopping point would need to be added and what action would be taken if a resister had developed so it’s clear.

Action Points:

Check for spelling errors

MB stated a CoP for room 17 is required (RP mentioned the current version of BR CoP is Jan 17)

Add SOPs to RADAR

Agreed: Class 3, Containment level 3. This is notifiable to the HSE.

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Animal Units | Large Scale Activities | Human Clinical Applications
Immune responses are pivotal to immunity and inflammation. Yet, imbalanced immune responses can leave hosts susceptible to infection and/or can cause excessive inflammation and tissue damage. Modulation of immune responses is a promising therapeutic strategy to rebalance immune responses in disease situations. Such approaches could provide strategies to combat infection, including those by antimicrobial resistant (AMR) pathogens, and/or in a range of inflammatory diseases. However, the mechanisms that control the fine-tuning of immune responses are not well characterised. This limits our capacity to understand the full repertoire of mechanisms that could be targeted in immunomodulatory strategies.

The major purpose of this study is to better understand the functions of immunomodulatory immune receptors in the biology of phagocytic immune cells, and to improve our knowledge of how pathogenic microbes exploit these receptors during infection. Phagocytic immune cells express a plethora of immune receptors to recognise, respond to and kill invading microbes. They also express inhibitory immune receptors that act to ensure that phagocytes do not become activated in the wrong place and at the wrong time leading to host damage.

Some receptors belong to families containing activating and inhibitory receptors, and are considered to be immunomodulatory receptors that act to generate balanced immune responses- for example, the CEACAM & LILR families. Though there is improving knowledge of the function of these receptors on adaptive immune cells, there remains a limited understanding of their role in shaping innate immune cell responses in both the infectious and non-infectious setting. This is limited by poor knowledge of native ligands and of cross-talk between receptors. Microbial pathogens are known to directly interact with receptors belonging to these families, most likely to imbalance immune responses and to favour pathogen survival. However, the microbial ligand(s) and/or functional consequences of these host-pathogen interactions remain unidentified. Identification and development of ligands with agonistic and/or antagonistic properties is key to improving knowledge of immune responses, and to recognising mechanisms that could be targeting in future immunomodulatory therapeutics.

The main aim of this project is to identify and characterise native and microbial ligands of immunomodulatory receptors. The work in this project will involve, 1. The use of heterologous expression in cell lines and recombinant forms of receptors to identify native and/or microbial ligands of immune receptors.

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Project Additional Information

Purposes of the contained use

Immune responses are pivotal to immunity and inflammation. Yet, imbalanced immune responses can leave hosts susceptible to infection and/or can cause excessive inflammation and tissue damage. Modulation of immune responses is a promising therapeutic strategy to rebalance immune responses in disease situations. Such approaches could provide strategies to combat infection, including those by antimicrobial resistant (AMR) pathogens, and/or in a range of inflammatory diseases. However, the mechanisms that control the fine-tuning of immune responses are not well characterised. This limits our capacity to understand the full repertoire of mechanisms that could be targeted in immunomodulatory strategies.

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Some receptors belong to families containing activating and inhibitory receptors, and are considered to be immunomodulatory receptors that act to generate balanced immune responses- for example, the CEACAM & LILR families. Though there is improving knowledge of the function of these receptors on adaptive immune cells, there remains a limited understanding of their role in shaping innate immune cell responses in both the infectious and non-infectious setting. This is limited by poor knowledge of native ligands and of cross-talk between receptors. Microbial pathogens are known to directly interact with receptors belonging to these families, most likely to imbalance immune responses and to favour pathogen survival. However, the microbial ligand(s) and/or functional consequences of these host-pathogen interactions remain unidentified. Identification and development of ligands with agonistic and/or antagonistic properties is key to improving knowledge of immune responses, and to recognising mechanisms that could be targeting in future immunomodulatory therapeutics.

The main aim of this project is to identify and characterise native and microbial ligands of immunomodulatory receptors. The work in this project will involve, 1. The use of heterologous expression in cell lines and recombinant forms of receptors to identify native and/or microbial ligands of immune receptors.
2. The use of biochemical and cellular based experiments to study, characterise and define receptor-ligand interactions, including native ligands as well as ligands from bacterial pathogens.

3. The use of cellular based assays to study immune receptor cross-talk, and to study the cell biology of shaping innate immune cell responses.

4. The use of wild-type, gene-knockout and transgenic mice to study the role of immune receptors in modulating immune responses in inflammatory or infectious disease settings.

Experimental procedures include routine cell culture in Class II Microbiological Safety Cabinets (MSC), microbiology, molecular biology, genetic amplification, genetic sequencing, plasmid based cloning, protein expression using routine prokaryotic (e.g. E. coli) or eukaryotic (e.g. EXPI293) expression systems, protein purification, protein biochemistry, retroviral and lentiviral transduction of cells using standard molecular biology procedures, colonisation/infection of cell lines with biohazard groups 1 and 2 bacteria. Analysis and sorting of infected and uninfected live and fixed cells or of bacterial populations by FACS and cell-sorting. Analysis of fluorescence or luminescence of uninfected live and fixed cells or bacterial populations using luminescence or fluorescence based assays. Fluorescent microscopy imaging of infected and uninfected live and fixed cells. Mouse husbandry, mouse infections, harvesting and analysis of tissues from infected and uninfected mice.

GMOs will be used for:-

1) routine cloning in E. coli. This will often involve the generation of vectors for expression of recombinant receptors or bacterial proteins (full or partial) to be expressed in prokaryotic or eukaryotic expression systems). Other work will include construction of vectors to knock-out full/parts of genes in bacterial organisms. Additionally, insertion of genes into shuttle vectors for complementation of mutations in GM bacteria or over-expression in other bacteria. Finally, vectors will be constructed to produce fluorescent bacterial strains for use in FACS or fluorescent microscopy. All of these activities involve biohazard group 1 laboratory strains of E. coli.

2) E. coli to express and purify recombinant proteins cultures using routine methodologies. This will encompass production of recombinant receptors or bacterial proteins (including full proteins, partial proteins or proteins with point or deletion mutations). Vectors in use or to be used are commerically available, or are modifications of commerically available vectors. All of these activities involve biohazard group 1 laboratory strains of E. coli.

3) GMO strains to characterise host-pathogen interactions. Bacterial strains of biohazard group 2 will be generated that have individual genes knocked out, and they will subsequently be used in functional, cellular and in vivo assays. This will include the use of libraries of bacterial mutants, as well as single mutant strains.

4) over-expression of genes in bacteria. GMMMs will be generated by introducing complementation vectors i) into knock-out mutant strains of biohazard group 2 strains, that should re-establish their previous phenotype or ii) into non-pathogenic biohazard group 1 strains, that will provide gain-of-function phenotypes in routinely used Gram positive (e.g. non-pathogenic Class I Lactococcus lactis, Streptococcus mutans) and negative (e.g. non-pathogenic Class I E. coli) species.

5) production of novel cell lines using transduction of 3rd generation lentiviral vectors. Vectors will have receptors inserted and on successful transduction will result in over expression of receptors at the cell surface. All work with lentiviral transduction will be performed in class 2 MSC cabinets.

Phage libraries will then be used to screen and select for phages that bind targets (e.g. cells or recombinant proteins), and genes identified by sequencing phagemid inserted. Currently, phage libraries for S. aureus, S. pyogenes, S. agalactiae, S. uberis, L. monocytogenes, E. coli and H. influenza are developed. Additional libraries for other CL1 and CL2 bacterial pathogens may be acquired, used or developed.

Recipient or parental organism

| Host/Vector system | Host 1: Biohazard group 1: Non-pathogenic organisms  
E. coli (BL21, Rosetta Gam, TOP10, DH5a, K12)  
Lactococcus lactis, Streptococcus mutans, Gemella hemolysans  
Host 2: Biohazard group 2: Streptococcal species  
Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus zooepidemidis, Streptococcus oralis, Streptococcus gordonii, Streptococcus uberis, Streptococcus gallylolicus, Streptococcus sanguinis, Streptococcus aginosus  
Host 3: Biohazard group 2: Staphylococcal species  
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus capitis, Staphylococcus canosnus, Staphylococcus hemolyticus, Staphylococcus lugdensis, Staphylococcus simulans, Staphylococcus saprophyticus  
Host 4: Biohazard group 2: Other Gram positive microbes |

Recipient or parental organism

| Host/Vector system | Host 1: Biohazard group 1: Non-pathogenic organisms  
E. coli (BL21, Rosetta Gam, TOP10, DH5a, K12)  
Lactococcus lactis, Streptococcus mutans, Gemella hemolysans  
Host 2: Biohazard group 2: Streptococcal species  
Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus zooepidemidis, Streptococcus oralis, Streptococcus gordonii, Streptococcus uberis, Streptococcus gallylolicus, Streptococcus sanguinis, Streptococcus aginosus  
Host 3: Biohazard group 2: Staphylococcal species  
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus capitis, Staphylococcus canosnus, Staphylococcus hemolyticus, Staphylococcus lugdensis, Staphylococcus simulans, Staphylococcus saprophyticus  
Host 4: Biohazard group 2: Other Gram positive microbes |
Enterococcus faecalis, Enterococcus faecium, Gardenella vaginalis, Gemella haemolysans
Host 5: Biohazard group 2: Gram negative microbes
Escherichia coli (ExPEC), Klebsiella pneumoniae, Helicobacter pylori, Moraxella catarrhalis, Neisseria gonorrhoeae, Pseudomonas aeruginosa.

Vector 1: Routine cloning in non-pathogenic E. coli
pRSETN, pRSETC, pGEX, pET or similar systems for gene cloning and protein expression in E. coli. All are IPTG inducible and encode ampicillin resistance. pcDNA3.4 or derivatives, pEFGP, pMyc, pFlag, pHA, RP172, RP139, RP137 and similar systems for expression in EXPI293T cells for gene cloning and protein expression in eukaryotic cells. All encode ampicillin resistance. E. coli strains used for routine cloning are susceptible to other antibiotics. To be inserted into Host 1.

Vector 2: 3rd Generation Lentiviral vectors
Lentiviral plasmids being used (RP172, RP139 and RP137, and related) are 3rd generation vectors modified such that they generate replication-defective viruses. These vectors are based on the dual promoter lentiviral vector (no. 2025.pCCLsin.PPT.PA.CTE.4x-scrT.eGFPM.CMV.hPGK.NGFP.PRE) that has been modified to replace the minimal CMV promoter with the human EF1A promoter to facilitate potent expression in immune cells; replace the eGFPM with a cassette containing several unique restriction sites facilitating DNA cloning (Nhel, PacI, Pmel, Afel, SpHl, SpHl and NsiI); and replace the NGFP gene with various combinations of fluorescent proteins (mCherry, mAmetrine) and selection markers (PuroR, BlastR, ZeoR and HygroR), which were fused together by means of the ribosomal skipping peptide T2A. These vectors produce non-replicative viruses, because they lack most virulence genes of these viruses, whilst maintaining only truncated gag, rre and modified long terminal repeats that optimise plasmid packaging and allows gene expression in target cells. Only the post-transcriptional regulatory region of WPRE and not the protein X coding sequence may be present in some lentiviral plasmids.

Packaging will be carried out using separate 2 or 3-plasmid systems that supply Gag + Pol and the VSV-G pseudotyping proteins in trans, to generate viruses that transduce cell lines and lead to the expression of gene of interest. These viruses are incapable of replication within target cells and do not generate infectious ‘daughter’ viruses. To be inserted into Host 1.

Vector 3: Streptococcal / Staphylococcal cloning vectors
pLZ, pDC123, pDC, pDCerm, pMBsacB are routine E. coli/Streptococcal mutagenesis vectors. These vectors encode ampicillin, erythromycin and/or spectinomycin resistance. pCL55, pCN34 are routine E. coli/Staphylococcal mutagenesis vectors that encode chloramphenicol resistance. To be inserted into Host 1, 2, 3 or 4.

Vector 4: Gram-negative cloning vectors
The lambda recombineinase system has been used to generate deletion constructs in Gram negative bacterial organisms. These include plasmids such as pKD3, pKD4, pKD13, pKD20, pKD46 and related along with helper plasmids for removal of antibiotic markers which are derived from plTns that expresses the FRT recombinase. Similar routine cloning vectors will be used in other bacterial systems, using the expertise of other research groups in CMBI. To be inserted into Host 1 or 5.

Vector 5: Transposon mutation vectors
Vectors for construction of random transposon mutants will be used to make libraries of transposon mutants. These systems are well-established in the scientific community and are also commercially available. Vectors typically carry mini-transposons composed of a antibiotic resistance genes flanked by transposon insertion repeats, and a transposase for mobilisation of mini-transposon and insertion into host genome. Streptococcal, staphylococcal and gram-negative vectors available include pGh9:ISS1, mariner system and Tn5 system respectively. To be inserted into Host 2, 3, 4 or 5.

Vector 6: Phagemid vectors for phage display
pDJ01 phagemid vector was created by Jankovic at al. (Massey University, Palmerston North, New Zealand). The vector contains a chloramphenicol resistance cassette and a multiple cloning site and myc-tag sequence in front of the gene encoding the C-terminal domain of the minor coat protein pIII. All phage work to be performed in MSC. To be inserted into Host 1.

Origin & function

Insert1 1: Routine cloning in non-pathogenic E. coli
Genes encoding full, partial or mutated innate immune receptors, bacterial surface proteins, bacterial secreted proteins, or bacterial regulators. This work will involve the generation of various point or deletion mutations or tagging of proteins with appropriate fluorescent or affinity tags for detection. All of these activities involve biohazard group 1 laboratory strains of E. coli. To be inserted into Vector 1 or 2.

Insert 2: Transposons mutant libraries that carry antibiotic resistance cassette
To uncover the molecular basis of other interactions, we will employ a transposon mutant libraries, cell sorting and Tn-Seq. The S. pyogenes mutant libraries (strains, M3 STAB902 & M1 5448) were provided by a collaborator Kevin McIver (Baltimore, USA), and are used widely within the Streptococcal research community. The McIver lab has developed additional libraries in other S. pyogenes strains, in S. agalactiae and other streptococcal species that will be acquired and used if required. The NARSA S.
Staphylococcus aureus mutant library (strain JE2) is available and widely used within the Staphylococcal community, and other libraries have been developed and will be acquired and used if required. Similar transposon mutant libraries for other bacterial species listed in 2.14 may be acquired from commercial sources or scientific collaborations, and used similarly in cell-sorting and/or Tn-Seq experiments. To be inserted into Vector 5.

Insert 3: Targeted mutants in bacterial surface, secreted or regulator proteins

Bacteria interact with immune cells through surface or secreted proteins. Therefore, genes encoding surface proteins, structures, secreted proteins or their regulators will be targeted in Gram-positive and Gram-negative bacterial species, for example those species listed in 2.15, to characterise interactions & infection biology. The molecular basis of CEACAM interaction with S. agalactiae and S. pyogenes has been uncovered, and GMM versions are mutated in genes encoding surface proteins, structures of their regulators (bac, bca, rib, R28, M). No new GMMs will be designed with the intention of enhancing virulence. Most gene deletions will be carried out in collaboration with other established laboratories in the MRC CMBI to use their expertise in microbiology and microbial genetics. In addition, GMMs of non-pathogenic Gram-positive and Gram-negative bacteria from biohazard group I, such as Lactococcus lactis/Streptococcus mutans and non-pathogenic E. coli, respectively, will be generated which have acquired surface or secreted genes to study functions in cellular and in vivo assays. To be inserted into Vector 3 or 4.

Insert 4: Reporter genes

Mutants will be generated or acquired expressing reporter genes, for example (but not limited to) luciferase or fluorescent proteins. To be inserted into Vectors 1, 3 or 4.

Insert 5: Genes encoding innate immune receptors or regulators

Full, partial or mutated forms will be cloned into 3rd generation lentiviral vectors, and will be expressed in eukaryotic cells using lentiviral transduction to study their functions. To be inserted into Vector 2.

Insert 6: Random DNA fragments from bacterial genomes.

Purified genomic DNA is fragmented randomly by sonication, and ligated into pDJ01 phagemid vector. Vectors are electroporated into TG1 E. coli. Subsequently, E. coli are infected with VCSM13 helper phage to generated phage library. To be inserted into Vector 6.

Evaluation of foreseeable effects

No experiments are planned that involve intentional creation of strains that enhance virulence beyond the inherent virulence of parental strains. GMOs are constructed in laboratory strains or well-characterised strains (genomes sequenced) where possible. Primary assessments of virulence phenotypes will be performed compared to parental strains. If the GMO virulence capacity is not enhanced, GMOs may be constructed in clinically relevant strains with known antibiotic susceptibility profiles if required. GMOs will not be constructed in multidrug resistant clinical strains, nor in any strain resistant to last-resort antibiotics. Thus, antibiotics will remain therapeutic options in the event that an incident occurs.

Host Group 1: There is not foreseeable effect of enhanced virulence for GMMs belonging to this group.
- Cloning in E. coli is routine and does not impact virulence properties.
- Over-expression of genes in non-pathogenic organisms such as E. coli, L. lactis or S. mutans does not alter phenotypes beyond the inherent properties of the host organism.
- Insertion of phagemid vectors into E. coli does not impact virulence properties. Phagemids are not capable of infecting human or animal hosts.

Host Group 2, 3, 4 and 5:
- Construction of mutations in CL2 pathogens. Knowledge from ourselves and literature, indicates that mutation of surface proteins, secreted proteins or their regulators does not result in enhanced virulence in comparison to parental strains. Virulence phenotypes are typically multifactorial, in which multiple genes contribute. It is not expected that strains with enhanced virulence potential will be generated. These mutations will first be constructed in well-characterised strains (lab strains, genome sequenced strains or commonly used strains in academic research), prior to construction in clinical strains.
- Transposon mutant libraries of CL2 pathogens. Libraries of transposon mutants are well-characterised and commercially available. There is no evidence to suggest these libraries have enhanced virulence beyond inherent properties on the host organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GM animals to be used in this study are no more hazardous to human health or the environment than their parental wild type. When infected with CL2 GMOs, the animals will be held in IVCs within the Imperial College Animal facilities at South Kensington.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

not applicable
| Solid waste: All hazardous solid biological waste such as tissue culture plasticware (pipettes, flasks etc) will be placed in appropriate waste bins and then securely transported to be autoclaved prior to disposal by trained personnel.  
Liquid waste: Treatment with minimum 2% Virkon after dilution with liquid waste for 1h followed by disposal in the sink.  
Animal carcasses and bedding will be disposed as per CBS guidelines, also described in local rules. |
|---|

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment
2.14 While it's stated that no new GMMs will be designed with the intention of increasing virulence or survival vs wildtype, is this possible with any of the over expressions or re-establishment of functions inserts? If a more virulent/potent strain was created, what action would be taken?

2.16 What generation of lentiviral vectors are these?

2.18 and 4.18. How confident are you that your genetic changes will not have unintended consequences and that the consequences of the changes will have predictable results, for example altering host range?

4.8 Please provide an ethics reference

4.10 Please review the organism you have listed as infectious via the airborne route. For example, you have listed staph spp as infectious via the airborne route but this is not a usual or normal route for infection with these organisms.

4.11 When using vectors in HG2 pathogens, is 50 ml enough to make competent cells?

4.12 I would expect to see in the local rules a good system for identifying the bacterial species to ensure those which should be handled in an MSC or with other additional precautions can be clearly identified. We may need to come back to this once OH have commented on the Bio1.

4.14 This needs to be expanded on? Is this in the local rules? Good lab practice is not a control measure. For example, pipetting and manipulation of HG 2 and class 2 organisms that are infectious via the airborne route.

4.16 Ampicillin as selection for GMO, is there still treatment available when you use this antibiotics?

4.18 How would you mitigate an unexpected more hazardous mutation?

5.1 Eye protection? Is that standard in your labs? If it is and you are working in an area where those not directly involved in your research might be affected, they should also wear eye protection.

2.8 and 2.19. Have you checked with Marian in the safety Dept that none of the animal pathogens require a licence?

2.18 and 4.18. How confident are you that your genetic changes will not have unintended consequences and that the consequences of the changes will have predictable results, for example altering host range?

5.7 This should be 1 hour rather than 30 minutes to agree with 10.1

6.1 Clarification on when gloves are or are not required needs to be confirmed in the local rules

8.1 Worth stating that maintenance of equipment will be conducted according to the manufacturer’s instructions. Most of the routine maintenance can be conducted by trained lab staff.

10.1 Reference to college incident report form should be removed, all incidents should be reported via SALUS

10.5 Virkon? Is it still soluble at this concentration? You will need to add in excess of 20% of the volume of the spill if you use 5% Virkon to achieve a final concentration of 1% after dilution.

As so many bacterial species and strains are involved, it might be good to identify any which could present a risk to a healthy individual or are infectious via the aerosol route clearly.

4.12 With reference to the earlier point about identification of species involved, this would be important in the case of accidental inoculation, so the correct antibiotics were administered so a clear system of identification would be required.

Comments:

AM Role of immune receptors in neutrophils and phagocytes and impact on disease progression. Use of GM for cloning, expression of recombinant proteins, ID of bacterial ligands and use transposon libraries. Knock out genes to validate or make positive expressing strains and to complement them.

Large number of pathogens on project as screen large number then home in.
MB when will you work in an MSC and when on a bench?
AM Inoculation and overnight in a cabinet. Experiments on bench. Mutant strains all work in cabinet
Pathogens max 5-10 mls on bench usually.
Larger volumes when its E.coli
MB/IH discuss what limit to set on volumes to use on the bench and if a limit should be set for pathogens which are airborne or just a general limit as hood availability means its’s not practical to just saw an MSC is used for all work. Or even to state all via airborne route go in MSC.
Agreed its best to set a max volume on bench for all species and strains than differentiate
20mls was agreed as a reasonable limit
MB suggests a trail with water so you have an idea how far 20mls could spread.
4.21 this should be specific meningitides species in use and not spp as Neisseria is excluded.
Can the alteration of surface proteins change virulence? Proteins are related to cell attachment and effect on virulence unlikely as attachment is related to a number of different proteins so unlikely to improve it. This (changes to these proteins) would also happen in nature
IH can you make it clearer that you potentially plan to include additional similar profiles/systems/species. Line is in there but can be more apparent.
Action Points:
Lentivirus in use is 3rd gen-MB put that into Bio1
Agreed: Class 2, Containment level 2. This is notifiable to the HSE.

Project Containment

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**Project Ref 8/21.1**

**Date Ackn'd** 16/07/2021

**CU2 Project Title**
Transfer and gene regulation of different types of mobile genetic elements in Gram-positive and Gram-negative bacteria

**Class** Class 2

**CultureVol** Class2 1 Litre

**CultureVolume** Class3-4

**Class CultureVol**

**Consent Granted**

**Non-GMM**

**Project notified under transitional arrangements** N

**Historical Significant Changes**
**Project Additional Information**

**Purposes of the contained use**

The majority of pathogenic traits in bacteria are encoded in regions of the chromosome that are able to be transferred between bacterial strains. These elements are collectively called mobile genetic elements (MGEs) and include bacteriophages, pathogenicity islands, integrative and conjugative elements (ICEs), plasmids and transposable elements. We aim to characterise these elements present in different Gram-positive and Gram-negative model bacterium organisms. We will study how these elements are transferred between bacteria and how they are regulated, characterising in molecular detail the different mechanisms they use to promote their spread. That would give us an insight as their contribution to the overall bacterial pathogenesis and allow us to design strategies to counteract bacterial infections.

**Recipient or parental organism**

Bacterial strains, either Gram-positive (S. aureus, E. faecalis, B. subtilis) and Gram-negative (E. coli, S. enterica, P. multocida) with different MGEs will be used to generate deletion mutants in the gene(s) of interest involved in the transfer and/or regulation of these elements.

**Host/vector system**

The host used in the project and the vector systems are:

S. aureus and E. faecalis:
- pMAD, pminiMAD, pBT2, pBT2-βgal (low copy number, temperature sensitive)
- pCN51 vector with a Cadmium-inducible promoter and derivatives will be used to complement mutants or overexpress genes in S. aureus
- pCN41 (promotorless) and pCN42 (under the control of a Cadmium-inducible promoter) vectors and derivatives will be used in S. aureus and other Gram-positive bacteria to measure promoter activity by generating a reporter fusion with BlaZ gene.

B. subtilis:
- pDR110 and pDR111 amyE integration vectors and derivatives that contain a multiple cloning site located downstream the IPTG-inducible Pspank promoter, will be used to overexpress genes in B. subtilis.
- pBs3Clux sacA integration vector and derivatives with a promoterless lux-reporter system will be used to measure promoter activity in B. subtilis.
- pDG1663 thrC integration vector and derivatives will be used to generate fusions with a B-galactosidase reporter and measure promoter activity in B. subtilis.
- pDR240a and DR242a vectors with antibiotic resistance cassettes (KanR and ErmR respectively) will be used for construction of single gene deletion mutants in B. subtilis.
A vector carrying the Cre recombinase will be used to promote the recombination between two lox sites. This vector is used to generate scar-deletion mutants in B. subtilis.

E. coli, S. enterica and P. multocida:
- pET-vectors (e.g. pET28a), pGEX-vectors (e.g. pGEX-4T), pProEX-Hta, pLIN, pNIC and derivatives will be used for protein expression and purification of high amounts of proteins in E. coli.
- pKT25, pKNT25, pUT18, pUT18C and derivatives will be used to perform double hybrid assays and determine protein-protein interaction in E. coli and other Gram-negative bacteria.
- pBAD18 vector and derivatives will be used to complement mutants or overexpress genes in E. coli and other Gram-negative bacteria.
- pKD46, pRWG99, pK03 Blue and pCP20 vectors will be used to generate deletion mutants in E. coli and other Gram-negative bacteria.
- pKD3, pKD4 and pWRG717 vectors will be used as templates to amplify different resistance markers to generate deletion mutants in E. coli and other Gram-negative bacteria.

This list contains a general description of the genes of interest present in the different MGEs infecting Gram-positive and Gram-negative bacteria most commonly targeted in our studies, but may not be fully detailed.

Insert 1: S. aureus bacteriophage genes (e.g. phages 11, 80a, 85, among others) to assess functional role in phage biology.
Insert 2: S. aureus pathogenicity islands (SaPIs, e.g. SaPI1, SaPIbov1, SaPI2, among others) to assess functional role in the biology of the MGE.
Insert 3: E. coli bacteriophage and Phage-inducible chromosomal island genes to assess functional role in the biology of the MGEs.
Insert 4: B. subtilis bacteriophage genes (e.g. phages SPBeta, phi3T, phi105) to assess functional role in phage biology.
Insert 5: E. faecalis bacteriophage and Phage-inducible chromosomal island genes to assess functional role in the biology of the MGEs.
Insert 6: P. multocida bacteriophage and Phage-inducible chromosomal island genes to assess functional role in the biology of the MGEs.
Insert 7: S. enterica subsp. enterica serovar Typhimurium LT2 bacteriophage and Phage-inducible chromosomal island genes to assess functional role in the biology of the MGEs.

Origin & function

In some cases, the genes mutated are involved in increasing the pathogenesis of the bacteria. In this sense, the GMMs generated have the same or lower hazardous potential than that of the parental strain. Routinely, strains generated will not have more than two different antibiotic resistance markers and in any case the antibiotics will not be generally used for treatment of infections.

Evaluation of foreseeable effects

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste generated will be treated initially with Chemgen to be inactivated. Treatment of the GMM waste will be done mainly at the SECB autoclaving facility or alternatively using the autoclave in Flowers building, level 6:
- Liquid discard (which includes the agar plates) treated with heat inactivation (temp. 134.5 for 10 min.)
- Dry waste treated with heat inactivation (temp. 134.5 for 5 min.)
- Validation of treatment: Annual 12 point thermocouple test; all cycles verified using dummy loads; records of tests kept by Biochemistry lab services and for the unit on the laboratory (Flowers building, level 6.)
- Route of disposal: After autoclaving, transported in a closed container to the clinical waste compound at the back of the SAF building; removed from site by a specialist
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N

Historical Date of Additional Info

Significant Change ID
The aim of this project is to create genetically engineered bacteria using synthetic biology technology to sense the gut environment and control the gut microbiome. This will include the construction of synthetic DNA constructs and expression (either as plasmids or by integration into the genome) in bacteria strains as appropriate. Further genome engineering (knock-outs, antibiotic resistance gene transfer) may also be made to these bacteria.

Recipient or parental organism

S. typhimurium (LT2, cloning strains, ATCC 14028s, SL1344) B. fragilis (638R, NCTC9343, and biobank isolates from healthy human donors) B. thetaiotaomicron (VPI-5482, and biobank isolates from healthy human donors)

Host/vector system

ColEI derived cloning and protein expression vectors (eg. pBR322, pUC, pGEM, pET, pGEX) for use in all hosts; pGRG36 derived tn7 transposon insertion vectors; RK2 derived expression plasmids EcoFlex cloning vectors P1vir bacteriophage pKD3, pKD4, pKD46 vectors for recombineering p2 bacteriophage for use in Salmonella hosts pNBU1, pNBU2, pKNOCK and similar for cloning, expression , shuttling and genetic insertion in Bacteroides species.

Origin & function

Insert 1: Reporter genes such as avGFP and DsRed derivatives, luciferase variants, beta-lactamase; Insert 2: Bacterial transcriptional repressors and activators, such as lacI, tetR, lambda-cl, lambda-cro.
Insert 3: DNA recombinases/ invertases such as lambda-recombinase, cre, flp, int7,int8, int9, int12.
Insert 4: antibiotic resistance genes and mutations for cloning and bacterial identification purposes to provide resistance to ampicillin, kanamycin, chloramphenicol, streptomycin, spectinomycin, tetracycline.
Insert 5: DNA promoter regions and two-component systems derived from human commensal (eg, E. coli Nissle 1917, MG1655) or pathogenic bacterial species (eg. Salmonella typhimurium, Enterheamorragiic E. coli). Note that no sequences encoding pathogenic protein products will accompany these promoter regions.
Insert 6: Intergenic DNA regions derived from human gut microbiome sequences corresponding to hypothetical small proteins.
Insert 7:Standard synthetic circuit components such as promoters and ribosome binding site variants to control gene expression levels and timing of expression for other insert components (eg. reporter genes, repressors/activators, recombinases/invertases, antibiotic resistance genes, and hypothetical proteins). Transcriptional terminators to insulate expression from adjoining DNA regions.
Insert 8: CRISPR Cas variants and guide RNAs for targetted deletion or insertion, activation and repression of gene
insert 9: synthetic peptides and antibody fragments/nanobodies designed to target specific proteins.

Evaluation of foreseeable effects

GMMs are not expected to be any more hazardous to humans or the environment than their non-modified variants. All modifications involve either non-functional edits or non-pathogenic processes (for example standard reporters or hypothetical genes derived from commensal bacteria found in healthy human guts).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste (e.g., cultures and supernatants from cultures). Virkon will be added to a final concentration of 1% and incubated for at least 30 mins. For bacterial cultures and supernatants <5ml, these may instead be autoclaved prior to disposal.

Solid waste (e.g., agar plates, plastic universal tubes): will be autoclaved before incineration.

All waste management procedures are expected to give 100% kill.

<table>
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<td><strong>Laboratory Activities</strong></td>
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Project Ref 8/93.1

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 8/95.1a

- Date Ackn’d: 15/02/2001
- CU2 Project Title: CONSTRUCTION OF DEFINED TYPHI MUTANTS WITH NULL DELETIONS IN VIRULENCE ASSOCIATED GENES; MUTAGENESIS OF SALMONELLA SPP (EXCLUDING ACDP CAT 3 PATHOGENS)
- Date Project Ceased: 23/03/2006
- Non-GMM Consent Granted: not applicable
- Project notified under transitional arrangements: Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  8/95.3

Date Ackn’d  25/07/1995  CU2 Project Title  CONSTRUCTION OF MUTANT STRAINS OF ENTERPATHOGENIC E. COLI  Class  2  CultureVolClass2  Class  CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref 8/95.4**

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Tick if notifying a connected programme of work [N]

Historical Significant Changes
Historical Date of Additional Info
 Significant Change ID
Date of Significant Change
### Project Additional Information

#### Purposes of the contained use

Mtb is one of about a dozen bacterial species for which some clinical isolates are now resistant to most or all antibiotics (abx) approved for treatment of the infections they cause. Mechanisms of antimicrobial resistance (AMR) in Mtb deserve study for their potential relevance to AMR in other pathogens; because tuberculosis (TB) is now the leading cause of death from infectious disease; and because drug-resistant TB may be the most prevalent of all drugresistant bacterial infections. Heritable AMR in Mtb emerges with interruption of treatment, and the long duration of TB treatment provides many opportunities for interruption and non-compliance. Prolonged treatment is necessary because of nonheritable resistance, also called phenotypic tolerance or persistence, defined as the transient tolerance of bacteria in an antibiotic-sensitive population to an antibiotic during exposure to an otherwise lethal concentration of that antibiotic. In contrast to “resisters”, whose AMR is genetically encoded, “persisters” are genetically sensitive bacteria whose phenotypic tolerance allows them to survive for prolonged periods during what would otherwise be rapidly curative treatment. The specific aim of this work is to identify genetic determinants that foster phenotypic tolerance in Mtb through the selection and analysis of high-persister (hip) mutants selected in vitro and in vivo; these strains produce a higher proportion of persister cells than a wild-type strain under the same conditions.

**Definition of tolerance in this work:**
Phenotypic tolerance manifests as “the presence of bacteria in an antibiotic-sensitive population that are not killed by that antibiotic but, when allowed to grow in its absence, give rise to populations that can be killed by the antibiotic in the same proportion as before” [C. Nathan, Fresh approaches to anti-infective therapies. Sci Transl Med 4, 140sr142 (2012)]. Of note, in E. coli, persisters increase the population surviving antibiotic exposure from which mutations arise that confer heritable antimicrobial resistance (AMR), and the heritably-resistant mutants are then selected during subsequent cycles of exposure to the antibiotic. The same is likely to be the case for Mtb.

The objective is to identify hip mutants in Mtb that form in vitro or in vivo during infection upon exposure to stresses imposed by a combination of host immunity and chemotherapy. The activities are divided into three parts, namely isolation of hip mutants in vitro, isolation of hip mutants in vivo and follow-up activities that pertain to mutants isolated using both methods.

Experimental procedures will include the use of routine microbiology techniques in Class I Microbiological Safety Cabinets (MSC), infection of cell lines with listed biohazard group 3 bacteria, molecular biology, mouse husbandry, mouse infections, harvesting tissues from mice, imaging of fixed cells using fluorescence microscopy, FACS analysis of fixed biological material, plasmid-based molecular cloning, and various biochemistry methodologies. All procedures are described in the Code of Practice document.

#### Recipient or parental organism

ACDP 3: Fully drug-sensitive laboratory strains such as M. tuberculosis H37Rv, Erdman or CDC1551 and M. bovis AF2122/97. Please note that the present work does not use M. tuberculosis clinical isolates.

Transposon libraries constructed by himar1 mutagenesis.

A strain of Mtb that bears a plasmid encoding a tetracycline-regulated (tet-on) version of beta-galactosidase. This plasmid encodes an antibiotic resistance cassette.

A strain of Mtb that will be used bears plasmids that encode a selection marker flanked by loxP sites and the Cre recombinase, respectively.

High persistence mutants selected during the research activities described here.

#### Host/vector system
1. Transposon libraries of Mycobacterium tuberculosis constructed by himar1 mutagenesis
2. A laboratory strain of Mycobacterium tuberculosis that bears a plasmid encoding a tetracycline-regulated (tet-on) version of a reporter such as beta-galactosidase. The plasmid is constructed using the Gateway cloning system technology.
3. A laboratory strain that bears plasmids that encode a selection marker flanked by loxP sites and the Cre recombinase. These plasmids are constructed using the Gateway cloning system technology.
4. High persistence (hip) mutants selected during the research activities. Importantly, these mutants won't be generated but rather selected from a culture of the laboratory strain of Mycobacterium tuberculosis that naturally contains mutations and is not a de facto virulent strain. None of the hip mutants selected will thus be more virulent than wild-type strains listed in 7.1.
5 and 6. Mutations will be further introduced in strains listed in 7.1 to confirm that they confer the hip phenotype. If a gene is essential for growth in vitro, knock-downs will be constructed by transforming Mtb with two plasmids: pNitETSacB-kan (https://www.addgene.org/107692), which allows for the replacement of the native gene by a selection marker by recombineering, and a second plasmid that allows for the conditional expression of the gene; this plasmid will be constructed using the Gateway cloning system technology. Alternatively, knock downs will be generated using the CRISPRi technology. Briefly, a dCas9 allele is under the control of a Tet-repressor-regulated promoter. Expression of Cas9 is induced in the presence of anhydrotetracycline; a cognate sgRNA (targeting the genomic region of interest) under the control of a tetR-regulated promoter; a tet repressor; a single-copy L5 integrating backbone; a pBR322-derived E. coli replication origin; a kanamycin selection marker. The current optimal backbone for M. tuberculosis is PLJR965. This backbone works as advertised in the Nature Microbiology manuscript "Programmable transcriptional repression in mycobacteria using an orthogonal CRISPR interference platform" [Rock JM et al. Nat Microbiol. 2017 Feb 6;2:16274]. If the gene is not essential, a knock out will be generated using the plasmid pNitET-SacB-kan described above.

Origin & function

Genes identified in the screens described above. Preliminary results in Msm hip mutants obtained in vitro using methodology 1b identified mutations in amino acid biosynthesis pathways, tRNAs and their modifiers, and transcriptional pathways. These are pathways that will be disrupted in M. tuberculosis. Importantly, these mutants won't be generated but rather selected from a culture of the laboratory strain of Mycobacterium tuberculosis that naturally contains mutations and is not a de facto virulent strain. None of the hip mutants selected will thus be more virulent than the parental strain.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Animal Units

Large Scale Activities

Human Clinical Applications

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| USE OF YERSINIA ENTEROCOLITICA HABOURING MUTATIONS IN SPECIFIC GENES |

Project Ref 8/trans1

Date Ackn’d

Date Project Ceased

16/08/2004

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

GM8/95

Historical Date of Additional Info

28/11/1995

Significant Change ID

Date of Significant Change

Class

Class 2

Culture

CultureVol

CultureVolume

Class2

Class3-4

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Y

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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**Project Ref** 96/01.1

**Date Ackn'd** 13/02/2006

**CU2 Project Title** Development and use of Adenoviral vectors

**Class** Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM** Not Applicable

**Consent Granted**

**Project notified under transitional arrangements** Y

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes** transferred from GM96 on 13/02/2006

**Historical Date of Additional Info** 13/02/2006

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  96/01.2

Date Ackn'd  13/02/2006

CU2 Project Title  DEVELOPMENT AND USE OF RETROVIRAL VECTORS

Class  Class 2

CultureVol  CultureVolume

Class 2  Class3-4
Date Project Ceased
23/03/2006

Non-GMM
Consent Granted
Not Applicable

Project notified under transitional arrangements
Y

Tick if notifying a connected programme of work
N

Withdrawn
N

Historical Significant Changes
transferred from GM 96 on 13/02/2006. Reclassified to Class 1 on 23/03/2006.

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 96/01.3

- **Date Ackn’d**: 13/02/2006
- **CU2 Project Title**: EXPRESSION OF RECOMBINANT PROTEINS IN MAMMALIAN CELLS
- **Class**: Class 2
- **CultureVolClass2**: Class 2
- **CultureVolumeClass3-4**: Class 2
- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: Y

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: Transferred to GM8 on 13/02/2006. Reclassified to Class 1 on 23/03/2006.
- **Historical Date of Additional Info**: 13/02/2006, 23/03/2006.

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
We aim to define the intracellular signalling events that control differentiation, survival and proliferation of lymphocytes.

Recipient or parental organism

We will transfet E.Coli K12 and derivatives with Blusecript containing mouse cDNAs encoding components of lymphocyte signalling pathways. We will introduce these cDNAs into pBabe retroviral vector and transfect into rodent-specific packaging cells, for infection of mouse cells and tissues with these cDNAs in vitro. Although expression of the genes of interest in the retroviral vector may effect mouse lymphocyte development and differentiation in vitro, the retrovirus cannot infect human cells and so would have no effect on human tissues and will not be oncogenic. Nevertheless, as some of the genes of interest have been shown to be oncogenes in the mouse (eg. Smoothened), we will carry out the work under CLASS II, Containment level II conditions.

Host/vector system

E.Coli K12 type (strains JM101 and derivatives or XL1 and derivatives)

GP+E retroviral packaging line and derivatives (packaging for infection of rodent cells only: Morgenstern and Land, 1990, Nucl. Acids Res. 18, 3587-3596)
Bosc retroviral packaging line and derivatives (Packaging for infection of rodent cells only)
Primary mouse cells and tissue
Mouse cell lines

Vectors:
Blusescript and derivatives
Puc and derivatives
pBabe and derivatives
MIGR1 and derivatives

Origin & function
For E.Coli: Commercial (Strategene) or other labs
For mouse cell lines: other labs
For primary mouse tissues: mice from CBS, Imperial College
For retroviral packaging cell lines: other labs

The genetic material will be PCR products from mouse (geneomic or cDNAs, generated by ourselves or from other labs)
Vectors will be obtained commercially or from other labs.

The function of these genetic manipulations is to study T cell differentiation in vitro.

Evaluation of foreseeable effects
We hope the genes we introduce into mouse T cell cultures will interfer with or alter T cell differentiation in cell cultures, and thus enable us to study T cell differentiation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste will be inactivated using Virkon 1% for at least 3 minutes (according to the manufacturer's recommendations). This will give 100% kill. The material will then be autoclaved, reaching 121 degrees C for 15 minutes. The autoclave will be monitored by autoclave printouts of the status of each run and 6 month checks by external contractors to make sure the correct temperature is reached. The waste will then be removed by a College approved contractor for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The Committee made the following statements:

1. "We are concerned about the potential oncogenic properties of the insert cloned in the vectors. Therefore we recommend that the production and handling of large quantities of DNA are carried out under Class II conditions to minimise exposure to high amounts of DNA with oncogenic potential. The Committee also requests that the packaging cell line used for generating the infectious pBabe does not generate viruses with residual infection for human cells. Could references be provided please".

2. "Please state the required contact time required for Virkon to be effective".

Project Containment

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Project Ref 96/01.5

Date Ackn'd 13/02/2006

CU2 Project Title STUDY OF THE MECHANISM OF HOST CELL INVASION BY TOXOPLASMA GONDI USING REVERSE GENETICS TOOLS

Date Project Ceased 20/06/2016

Class 2

CultureVolClass2 < 1 Litre

Consent Granted Non-GMM Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Transferred from GM96 on 13/02/2006

Historical Date of Additional Info Notification closed 20/06/2016

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

The aim project is to study the mechanism of host cell invasion by the protozoan parasite Toxoplasma gondii by analysing the interactions between three classes of invasion molecules that are expressed in T. gondii. These molecules include molecular motors called myosins, adhesins called microneme proteins and aspartyl proteases. Using stable transfection technique, we propose to disrupt the genes coding for these invasion factors. In parallel, we will express deletion mutant and GFP fusions of these proteins to study their biogenesis. The biochemical analysis of the motor proteins will necessitate the purification of these proteins from toxoplasma gondii.

Recipient or parental organism

Toxoplasma gondii tachyzoites from RH and Prugniaud strains as well as the attenuated strains ts-4 mutant derived from RH strain. Toxoplasma gondii is a mild pathogen infecting human and animals. In human, this parasite is pathogen only under two circumstances: infection by immunosuppressed patients and pregnant women. Toxoplasma gondii acute infection can be treated with antifolates drugs combined to antibiotics of the class of macrolides or atovaquone. The genetic modifications proposed in our project are not anticipated to increase the virulence but rather to impair the ability of recombinant parasites to invade host cells.

Host/vector system

The vectors to be employed in the proposed experiments are all derived from the commercially available bluestrip from Stratagene. Most of the constructs are designed to express modified proteins or to generate knockout by homologous recombination in Toxoplasma gondii. These vectors are all described in the literature and the corresponding publications are listed in the risk assessment protocol.

Origin & function

The genetic material originates from Toxoplasma gondii genes or homologues from other Apicomplexa parasites and E.coli. Genes originating from Toxoplasma gondii and apucomplexa:
- Adhesins proteins secreted by the micronemes at the time of invasion. MIC1 to MIC9
- Molecular motors including myosin light chain and myosin heavy chains and docking proteins: Myosin A to Myosin E, MLC MADP
- Aspartyl proteases. AP1 and AP2
- Functions: Components of the invasion machinery
- Gene originating for other organisms: Beta galactosidase (LacZ), tetracycline repressor (tetR), green fluorescent protein (GFP), chloramphenicol acetyl transferase (CAT)
- Functions: Selectable marker to disrupt genes by homologous recombination (CAT), reporter genes (LacZ) and Creation of chimeric fusion to follow the traffic of protein (GFP) and modulation of gene expression (tetR).

Evaluation of foreseeable effects

1. The genes coding for these proteins will be disrupted in Toxoplasma gondii by homolgous recombination or by conditional knockout using the inducible tetracycline based gene expression system.
   We anticipate an alteration of the ability of parasites to infect cells.
2. These genes will be expressed as mutants: site specific point mutations, deletions or GFP fusions of these genes will be used to generate transgenic parasites.
   No change in virulence is expected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Toxoplasma gondii is a micor-organism.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Propagation of Toxoplasma gondii tachyzoites in tissue culture must take place in a containment level 2. All the proposed experiments will take place in a cell culture.
equipped laboratory located in containment level 3. The room and equipment fulfils all the safety requirements. The room will not be shared with and no containment level 3 activity will take place there. The manipulation of live parasites will be done in a safety cabinet. Wearing gloves and laboratory coats are requested. No material or equipment can leave to zone before decontamination or autoclaving. Both liquids and solids wastes will be autoclaved in the Containment 3 zone. Autoclaving: 30 mins at 121°C at 15p.s.i (1.1kg/sq cm. Monitoring: Autoclave performance is monitored continuously by means of the autoclave printout (including a multipoint thermocouple test) annually by an independent company. Work carried out in the Containment level 3 laboratory is autoclaved within the containment room prior to removal to the main autoclave for a second round of autoclaving.

The safety committee agreed that the project involving Toxoplasma gondii should be conducted in a containment level 2 and that the genetic modifications are not anticipated to change the category of the containment. They agreed that the project will be carried out in a fully equipped cell culture room located in the containment level 3 area since it was the only room available. This room will be exclusively used by the members of the group of D. Soldati. Cell culture will be restricted to Toxoplasma gondii and the host cells to propagate the parasites.

The Committee has suggested to use blunt needles for the rare cases the manipulation of seringes will be necessary. The chemical inactivation of solid wastes before autoclaving has been suggested but since Toxoplasma gondii is not an aerosol borned pathogen, this measure will not be necessary.

Please enter comments on the GM safety committee on the risk assessment

The project involving Toxoplasma gondii should be conducted in a containment level 2 and that the genetic modifications are not anticipated to change the category of the containment. They agreed that the project will be carried out in a fully equipped cell culture room located in the containment level 3 area since it was the only room available. This room will be exclusively used by the members of the group of D. Soldati. Cell culture will be restricted to Toxoplasma gondii and the host cells to propagate the parasites. The Committee has suggested to use blunt needles for the rare cases the manipulation of seringes will be necessary. The chemical inactivation of solid wastes before autoclaving has been suggested but since Toxoplasma gondii is not an aerosol borned pathogen, this measure will not be necessary.

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Animal Units

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Project Ref 96/01.6

Date Ackn’d 13/02/2006

CU2 Project Title THE EFFECT OF GENE KNOCK-OUTS ON GENE EXPRESSION AND

Class 2

Culture Volume Class 2 < 1 Litre
**Project Additional Information**

**Purposes of the contained use**

In this project we wish to establish the role of metabolic and regulatory genes in the stationary phase survival of Mycobacterium bovis BCG. We propose to make gene knock-outs of metabolic and regulatory genes (for example transporter genes, two-component systems) and investigate the effect of these mutations on stationary phase survival and gene expression. We propose to use a single integrating vector to compliment the knock-out mutants. We plan to use macrophage infection studies to establish the effect of the knock-out mutations on virulence/infectivity.

**Recipient or parental organism**

*Mycobacterium bovis BCG* is an ACDP class 2 organism. It is widely used as a vaccination strain.

**Host/vector system**

The vectors that will be used in the project use kanamycin or hygromycin as selectable marker and are non-mobilisable. We will use reporter gene vectors containing XyIE or GFP as reporter genes, single integrating vectors and knockout vectors. All vectors contain an origin of replication for *E. coli* and mycobacteria, except for the knock-out vector, which only has an origin of replication for *E. coli*.

**Origin & function**

All vectors have been constructed and validated in our laboratories. To create the knock-out and complimenting vectors we have used genomic DNA from *M. bovis BCG* and *M. tuberculosis*. The genomic DNA of *M. bovis BCG* originated from our laboratories, while the *M. tuberculosis* genomic DNA was from a collaborating department within Imperial College.

**Evaluation of foreseeable effects**

*M. bovis BCG* is an attenuated vaccination strain, unlikely to survive outside the laboratory. We intend to knock-out genes that are involved in regulatory and metabolic functions. For the genes we propose to knock-out, homologues are described in other organisms and in these organisms the mutation caused the organism to become less virulent. In our laboratories we have made knock-out mutants of genes with metabolic functions in *M. smegmatis* (a non-pathogenic Mycobacterium species), and it was found that these mutants are less viable than wild-type strains. We expect that we will see a similar effect in *M. bovis BCG*.

Mycobacteria are environmental organisms, and gene transfer between mycobacteria and other organisms has been described. However our vectors are non-mobilisable and need antibiotic selection to be sustained in the organism, it is therefore unlikely that the genetic material will establish itself into another population outside the laboratory.
M. bovis BCG is a difficult strain to maintain in the laboratory, with very specific culture methods. Mycobacteria are also sensitive to UV light. If inadvertently genetically modified M. bovis BCG is introduced to the environment, it is unlikely that the organism will survive. In the unlikely event that the genetically modified M. bovis BCG causes infection, the use of kanamycin and hygromycin as selectable markers, will not affect the treatment since the organism will stay full sensitive to Isoniazid and Rifampicin.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GM waste is autoclaved and this should give a 100% kill, as established by Collins, CH (1993), Laboratory Acquired Infections, 3rd edition, pp 146 et seq.. All liquid GM waste is disinfected with a 2% final concentration of Hycolin for at least 16 hours. This should give a 100% kill, as established by Collins, CH (1993), Laboratory Acquired Infections, 3rd edition, pp 146 et seq.. We will monitor this on an ongoing basis by plating out random samples to establish whether any viable cells are present.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GM safety committee approved the project as Class 2, containment level 2. However the committee was concerned about the use of kanamycin as a selectable marker, as this may interfere with susceptibility of the GMO to aminoglycosides such as amikacin. We have submitted evidence, supported by experts in the field of mycobacteria, that anikacin and/or kanamycin are rarely used in the treatment of mycobacterial infection; that M. bovis BCG is fully sensitive to Rifampicin and Isoniazid; and the genetic exchange between mycobacteria is very difficult, therefore making the possibility of transferring resistance from M. bovis BCG to M. tuberculosis very remote. The GM safety committee was very satisfied with our response and granted approval of Class 2, containment level 2.

Project Containment

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02/03/2022
**Project Ref** 96/02.2

**Date Ackn'd** 13/02/2006

**CU2 Project Title** REPLICATION OF PLANT VIRUSES AND VIROIDS

**Date Project Ceased** 16/08/2011

**Class** Class 3

**CultureVolClass2** up to 1 litre

**Consent Granted** Yes

**Non-GMM** Y

Project notified under transitional arrangements N

**Withdrawn** N

**Tick if notifying a connected programme of work** Y

**Historical Significant Changes**

transferred from GM96 on 13/02/2006

**Historical Date of Additional Info** 13/02/2006

**Project Additional Information**

**Purposes of the contained use**

Study of the RNA polymerases and transcription factors involved in the replication of chrysanthemum chlorotic mottle viroid, potato spindle viroid, barley yellow dwarf virus and cereal yellow dwarf virus.

**Recipient or parental organism**

Chrysanthemum chlorotic mottle viroid
Potato spindle tuber viroid
Barley yellow dwarf virus
Cereal yellow dwarf

**Host/vector system**

Escherichia coli K12 derivatives, such as DH5 alpha/ pUC-derived vectors, such as pBluescript, and, for the virus studies, expression vectors, such as the pET and PQE series of vectors.

**Origin & function**

Chrysanthemum chlorotic mottle viroid (infected chrysanthemum plants in Spain, USA)

Potato spindle tuber viroid (infected potato plants in Germany, USA)

Both viroids will be used to study interactions with the cellular RNA polymerases which replicate them.

Barley yellow dwarf virus (Infected barley and other cereal plants in UK, USA)
Cereal yellow dwarf virus (infected barley and other cereal plants in UK, USA)

The RNA of both viruses will be used to study interactions with the cognate virus-encoded RNA polymerases which replicate them.

**Evaluation of foreseeable effects**

Sequences alterations in the viroid or virus RNA which interfere with the ability to bind the cognate RNA polymerase will be likely to reduce the infectivity of the viroid or virus RNA to plants or plant cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Application for derogation of some containment level 3 measures to containment level 2 for the work involving chrysanthemum chlorotic mottle viroid and potato spindle tuber viroid

Table 1a Measure 2 Laboratory: sealable for fumigation

There is no risk to human health as viroids do not infect humans. Any accidental spillages within rooms 418 and 426 can be adequately contained by the disinfectant measures described in the standard operating procedure (attached)

Table 1a Measure 5 Negative pressure relative to the pressure of the immediate surroundings

Viroid-infected plants will not be allowed to flower. Hence there is no possibility of viroid dispersal via pollen or seed. No other parts of the plants are airborne. Plant residues will not be allowed to dry out, so that there will be no possibility of viroid transmission in dust arising from dried infected plant residues. Immediately after use, all viroid-infected plant residues will be placed in sealed containers prior to disposal by autoclaving and incineration. Precautions are in place to prevent the generation of aerosols. For details please see the standard operating procedure (attached). There will be no susceptible plants in the vicinity of rooms 418 and 426.

Table 1a Measure 6 Extract and input air from the laboratory should be HEPA filtered

Viroid-infected plants will not be allowed to flower. Hence there is no possibility of viroid dispersal via pollen or seed. No other parts of the plants are airborne. Plant residues will not be allowed to dry out, so that there will be no possibility of viroid transmission in dust arising from dried infected plant residues. Immediately after use, all viroid-infected plant residues will be placed in sealed containers prior to disposal by autoclaving and incineration. Precautions are in place to prevent the generation of aerosols. For details please see the standard operating procedure (attached). The air intake into the Sir Alexander Fleming Building is filtered to exclude insects and dust.

Table 1a Measure 7 Microbiological cabinet/enclosure

Viroids are not infectious to humans and do not encode any proteins. Hence a microbiological safety cabinet will not be required. Furthermore precautions are in place to prevent the generation of aerosols (see attached standard operating procedure). There will be no plants in rooms 418 and 426 other than those which it is desired to infect as part of the research project.

Table 1a Measure 8 Autoclave required in the lab suite

There is no possible risk of contaminating the environment in transporting infectious viroid-containing material from rooms 418 and 426 to the autoclave. Liquid waste will be absorbed on a clay-based or wood (cellulose)-based absorbent and treated as for solid waste. Solid waste will be placed in sealed containers. The sealed containers will be placed in autoclave bags, the tops of which will be folded over and taped. The autoclave bags will be placed in autoclavable buckets on which lids will be placed. The buckets will be transported to the autoclave in contained trolleys. On reaching the autoclave, the buckets will be removed from the trolley, their lids removed and the tape removed from the autoclave bags. However the containers containing the infectious viroid material will remain sealed. The white buckets will then be placed in the autoclave. During the autoclave cycle, the specially designed sealed containers will melt allowing access of the steam to the viroid waste material. After autoclaving the waste will be placed in clinical waste sacks prior to incineration.

Table 1a Measure 14 Efficient control of disease vectors which could disseminate the GMM
Viroids are not transmitted by aphid vectors when the aphids feed on plants infected by only viroids. However potato spindle tuber viroid (PSTVd) has been reported to be cotransmitted with potato leafroll virus (PLRV) by aphids feeding on potato plants infected with both PSTVd and PLRV. Several precautions are in place to prevent plants becoming infected adventitiously by aphids which may be carrying PLRV, although it is extremely unlikely that there are aphids carrying PLRV in the vicinity of Imperial College because there are no potato-growing areas in the vicinity of the College. (a) No aphids will be used in the project and there are no other projects involving aphids being carried out in the Sir Alexander Fleming Building. The air exchange system in the level 6 glasshouses, where the healthy uninfected plants are grown, is protected by aphid-proof mesh. In the four years that the glasshouse have been in operation, no aphids have been detected. If any aphids were detected, they would be immediately destroyed by application of an aphicide. (b) Plants will be examined for symptoms of virus infection, prior to viroid inoculation. Any putatively virus-infected plants will be destroyed by autoclaving and incineration. (c) Aphids have never been found in rooms 418 and 426 and indeed are unlikely to enter the Sir Alexander Fleming Building because the air intake is filtered and there are no windows opening to the outside of the building. However as an additional precaution, sticky insecticidal tape will be placed in rooms 418 and 426.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 96/99.1

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4
Animal Units

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications

Project Ref 96/99.2

Date Ackn’d 13/02/2006

CU2 Project Title
THE DEVELOPMENT OF DNA-MEDIATED TRANSFORMATION SYSTEMS OF ERYSIPHE SPP

Date Project Ceased

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2

Non-GMM

Consent Granted Not Applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Transferred to GM8 13/02/2006

Historical Date of Additional Info 13/02/2006

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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#### Name

**UNIVERSITY OF LEICESTER**

#### Name 2

**SAFETY SERVICES**

#### Campus Estate or Research Centre

**Building**

#### Road Name

**UNIVERSITY ROAD**

#### Town

**LEICESTER**

#### District

**ENGLAND**

#### Tel Number

0116 252 2020

#### Fax Number

0116 252 2200

#### HSE Division

MIDLANDS

#### Date at Which Additional Info Submitted

02/03/2022
## Significant Change

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**UNIVERSITY OF LEICESTER**

- **MEDICAL RESEARCH COUNCIL**
  - **MRC TOXICOLOGY UNIT**
- **UNIVERSITY OF LEICESTER**
  - **LEICESTER ROYAL INFIRMARY**
- **UNIVERSITY OF LEICESTER**
  - **UNIVERSITY LABORATORIES**
- **UNIVERSITY OF LEICESTER**
  - **DEPARTMENT OF INFECTION, IMMUNITY & INFLAMMATION**
- **UNIVERSITY OF LEICESTER**
  - **GLENFIELD GENERAL HOSPITAL**
- **UNIVERSITY OF LEICESTER**
  - **MAURICE SHOCK BUILDING**
- **UNIVERSITY OF LEICESTER**
  - **HENRY WELLCOME BUILDING**
- **UNIVERSITY OF LEICESTER**
  - **ADRIAN BUILDING**
- **UNIVERSITY OF LEICESTER**
  - **BIOCENRE**
- **UNIVERSITY OF LEICESTER**
  - **CENTRAL RESEARCH FACILITY**
### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes](Y)

Give brief details of the genetic modification safety committee

Chairman, Secretary, Biological Safety Officer, Supervisory Medical Officer, Virologist; GM worker, 4 other GM workers, Technician's Rep, MSF. Rep and Post Graduate Representative.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Non-microbial

Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
- Mycology
- Transgenic Invertebrates
- Transgenic Plants
- Other (please specify below)
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessments are prepared by proposers according to ACGM/HSE/DOE note 7 and are submitted to the central safety office with a local GM Notification/assessment form. The proposals are then circulated to all members of the local GMSC who return their comments to the biological safety officer on the form provided. Comments are forwarded to the proposers for their response and possible amendment of the proposals or risk assessments. The final submissions are discussed at meetings of the local GMSC before final approval is given and the required containment level is agreed.

Project Ref 11/00.1

Date Ackn'd 11/05/2000

CU2 Project Title RESCUE FROM HIV SUB-GENOMIC MOLECULAR CLONES

Class Class 2

CultureVolClass2

CultureVolumeClass3-4

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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## Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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<td>L3 L4 L2 L3</td>
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Project Ref 11/01.1

Date Ackn'd 06/08/2001  
Date Project Ceased 23/12/2010  
Withdrawn N

CU2 Project Title  
THE PRODUCTION OF RECOMBINANT OVINE AND BOVINE PRION PROTEIN (PRP) FOR THE GENERATION OF MONOCLONAL ANTIBODIES SPECIFIC FOR CONFORMATIONAL ISOMERS OF PRP

Class 2  
CultureVol 1-50 litres

Consent Granted not applicable

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

The biological function of the prion protein PrPC is not known, it is thought that the disease isoform of this molecule, PrPSc may be the infectious agent for BSE in cattle and scrapie in sheep. We wish to produce recombinant truncated forms of ovine and bovine PrP. Once purified monomeric truncated PrP will be converted to the -form for use as a target for antibody isolation in order to produce -form PrP conformational specific antibodies. Such antibodies may also be specific for the disease isoform PrPSc.

#### Recipient or parental organism

The proposed E. coli hosts Novablue DE3 and TG1 are routinely used lab strains of E. coli that are inherently safe K12 derivatives. The PrP gene inserts, from bovine or ovine source, are truncated and do not possess the GPI anchor domain or the export signal domain. The truncated versions would therefore be significantly different from the disease and are likely to pose a reduced risk to human or animal health.

#### Host/vector system

The PTrcHis, pRSET, pET, pMal expression vector systems will be used in the K12 derived E. coli host strains Novablue DE3 and TG1.

#### Origin & function

The truncated PrP genes are from bovine or ovine origin. These would be produced in the E. coli host cells using the expression vectors PTrcHis, pRSET, pET, and pMal which produce target proteins fused to either a poly-His tag or maltose binding protein, these tags would then be used to affinity purify the PrP-fusion proteins.

#### Evaluation of foreseeable effects

Due to the truncated nature of the inserts in the constructs proposed here and that the expressed proteins are structurally distinct from the disease isoforms (they need prolonged incubation at low pH in vitro to produce the conformational change thought to be central to the propagation of PrPSc, i.e. from a predominantly α-helical structure to a predominantly β-structure) the hazards to human health are low and containment level 2 is appropriate.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

N/A
Solid waste:
Disposable plastic ware will be used wherever possible, disposable solid waste (including E. coli plates) to be put in biohazard burn bins and sent for incineration (100% kill). Reusable contaminated solids (including E. coli growth flasks) to be soaked in 20,000 ppm available chlorine of sodium hypochlorite for at least 2 hours, except for contaminated metal equipment which will be soaked in 2m sodium hydroxide for at least 2 hours.

Lique waste:
All liquid waste (including E. coli cultures) will be treated with 20,000 ppm available chlorine of sodium hypochlorite for at least 2 hours. Spills would be cleaned up with paper towels which will be treated as solid waste, contaminated spill areas will be soaked in 20,000 ppm available chlorine of sodium hypochlorite for at least 2 hours, except for contaminated metal which will be soaked in 2M sodium hydroxide for at least 2 hours. Where soaking is not possible, contaminated areas will be wiped down with one of the above disinfectants (as appropriate) at least 3 times allowing drying between applications for at least 1 hour. All work using the listed constructs in E. coli will be carried out in the BSE suite (biology Dept., University fo Leicester) and the SOP for this lab followed.

Disinfectant to be used, exposure time and working concentration:
2M sodium hydroxide or 40% (v/v) Haychlor industrial bleach (Chloros hypochlorite bleach). A 40% (v/v) solution of Haychlor industrial bleach contains 20,000 to 64,000 ppm available chlorine (manufacturers data). Decontamination procedures obtained from "transmissible spongiform encephalopathy agents: safe working and the prevention of infection. Advisory Committee on Dangerous Pathogens, Spongiform Encephalopathy Advisory Committee. ISBN 0-11-322166-5.

N.B. The above waste treatment/decontamination procedures are to ensure that contaminating recombinant PrP is destroyed, these procedures will also ensure that viable E. coli are unlikely to remain after treatment. For example University's guidelines from "Genetically Modified Organisms" and "Hazardous Biological Agents" state that solutions containing 1,000 ppm available chlorine (e.g 1% Chloros) are suitable for routine disingection of bench tops and otehr surfaces. 2.5% Chloros or equivalent is suitable for pipette jars and spillages of blood etc. 10% Chloros or equivalent should be used for blood and other materials with a high organic content if the presence of infectious agents is suspected.

Agreed:
1. That a designation of Class 2 is appropriate
2. That the risk assessment is sound and that work should be carried out at Containment Level 2
3. That the proposer should forward the proposal to HSE for prior approval of the project.

Project Containment

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The project aims to characterise the factors that control the generation of genetic variation in a group of related gram negative bacterial organisms that colonise the respiratory tracts of either humans (Neisseria meningitidis, Neisseria lactamica and Haemophilus influenzae) or animals (Actinobacillus pleuropneumoniae, specific for pigs). This project also aims to identify and to characterise the functional consequences of the generation of genetic variation in specific loci.

Recipient or parental organism


Host/vector system

Plasmids: pCR2.1-TOPO, pUC18, pGEM, pACYC, pET, pSU2718, pYHS25, and derivatives of each of these plasmids.

Origin & function
Native genes from each organism. These genes will be inactivated by cloning of the relevant gene, insertion of an antibiotic cassette into the cloned gene and recombination of the mutated gene back into the native organism. All cloning will be performed in E.coli GMM strains. Complementation will also be performed wherein a non-mutated version of the gene re-inserted into another position in the genome. The mutated genes will have functions in various aspects of DNA metabolism, in DNA uptake or will encode surface proteins or enzymes that modify surface structures. Mutations in these genes will reduce infectivity or will mimic naturally-occurring mutations (for example many meningococcal isolates have heightened mutation rates due to mutations in mismatch repair genes) and so construction of strains with these mutations does not constitute any risk to human or animal health.

Antibiotic cassettes. Antibiotic cassettes mediating resistance to kanamycin, tetracycline, erythromycin, chloramphenicol, or streptomycin/spectinomycin will be utilised. These cassettes will be employed in the construction of mutations in specific target genes (see previous paragraph). These cassettes have been derived from other bacteria or plasmids and have been widely utilised for research purposes. These antibiotics are not used for treatment of natural infections by the recipient organisms and so construction of mutants containing these cassettes does not constitute any risk to human or animal health.

Reporter genes. The lacZ reporter gene encodes a beta-galactosidase, an enzyme involved in the metabolism of lactose and of chromogenic substrates such as X-Gal. Fluorescent proteins emit light at particular wavelengths and will include green fluorescent protein or derivatives thereof. The lux genes also emit light. The phoA gene encodes alkaline phosphatase and is involved in the metabolism of phosphate. These genes have benign functions that will not increase the infectivity of GMMs. The reporter genes will be used for detecting production of genetic variants. All these reporter genes have been derived from bacterial sources and widely used for research purposes.

Evaluation of foreseeable effects

Most of the GMM's will not exhibit an increased level of virulence relative to the parental strain. The GMM's with elevated mutation rates may exhibit enhanced virulence in certain tests relative to parental strains but will not be more virulent than wild-type isolates, some of which are known to carry mutations that increase mutation rate. All procedures with these GMM's will be carried out at containment level 2 reducing the chances of spread beyond the laboratory to effectively zero.

The inserted genes (reporter genes/antibiotic cassettes) do not encode functions that will increase the virulence of these organisms whilst the antibiotic cassettes do not mediate resistance to antibiotics that are currently used to control these or related organisms. These genes will present on the chromosome in all but the E. coli GMM's and will not, therefore, be readily transferable to bacterial species or other genera. E. coli GMM's will be in strains/plasmid backgrounds with a limited ability to undergo conjuction such that there will be a limited risk to transfer to other strains of the species. All procedures with these GMMs will be carried out at containment level 2 reducing the chances of spread beyond the laboratory to effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste (paper and plastics): Autoclaving for 30 minutes at 121 degrees centigrade. All material for autoclaving will be transported in appropriate sealed containers (bags or boxes). Autoclaved waste is sent to landfill. Degree of kill: 100%.

Liquid waste: Treatment with 0.5% Prespt or a 1:100 dilution of Trigene for 24 hours prior to disposal down the sink. Degree of kill: 100%.

Small spillages treated with 70% ethanol for 5 minutes and then disposed of as solid waste. Degree of kill: 100%.

Large spillages treated with 0.5% solution of Precept for 60 minutes and then disposed of down the sink. Degree of kill 100%.
The degree of kill will be monitored by plating aliquots of treated samples on appropriate media and incubating overnight under appropriate growth conditions for the relevant organism.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

i. That a designation of Class 2 is appropriate.
ii. The risk assessment is sound and that work should be carried out at Containment Level 2.

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Animal Units

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<td>Generation of cardiovascular cell lines containing excisable telomerase</td>
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<th>Culture Volume Class 3-4</th>
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Non-GMM  
Consent Granted

Project notified under transitional arrangements  
N

Withdrawn  
N

Tick if notifying a connected programme of work  
N

Historical Significant Changes
We have recently shown that shorter telomere length is a causal factor for coronary artery disease. The aim of this project is to produce cell lines with varying telomere lengths from a single genetic background in the absence of confounding factors such as varying stress due to different time periods that the cells are maintained in culture. To achieve this we will employ an established method of introducing an excisable telomerase reverse transcriptase (TERT) using the eRE/Lox system (Salmon P et al 2000, Mol Ther. 2(4):404-14; Stadler et al 2013, Nature Structural and Molecular Biology 20(6) 671-678). Once infected with the pLOX-TERT construct the telomeres will undergo elongation. At different time points TERT will then be excised by the addition of CRE, creating sub clones which will subsequently undergo telomere attrition in the absence of TERT. All sub clones will be grown in parallel, creating clones of varying telomere length at the final time point which have all been maintained in culture for the same period. Although addition of TERT can extend cellular lifespan by preventing senescence that results from telomere loss, other pathways are also involved in cellular senescence, such as the p16-Rb pathway. These can be triggered by cellular stresses that result from maintaining the cells within culture and act independently to telomere induced senescence. Therefore it may not be possible to maintain the cells for the experimental period using only TERT. If necessary we will first generate cell lines which express either CDK4 or a p16-insensitive CDK4 (CDK4 R24C). Expression of CDK4 has been shown to enable growth of the cells for extended periods in culture by preventing nontelomere mediated cell cycle arrest (Stadler et al 2011, Skeletal Muscle 1, 12: Stadler et al 2013, Nature Structural and Molecular Biology 20(6) 671-678). These lines will subsequently be infected with pLOX-TERT.
Human pluripotent stem cells

Host/vector system

DH5-alpha and Stbl3 will be used for maintenance of plasmids
HEK293 will be transfected with the relevant combination of vectors to produce replication deficient retroviral particles (pCMV-VSV-G, pUMVC and either pBabe-hygro-CDK4, pBabe-hygro-CDK4-R24C or pBabe-GW-GFP) or replication deficient lentiviral vectors (pMD2.G, pCMVdR8.74 and either pLOX- TERT-iresTK, pLOX-gfp-iresTK or pLOX-CW-CRE).

Resulting viral particles will be used to infect human cells.

Origin & function

The genes to be expressed are telomerase, CDK4, a CDK4 mutant and green fluorescent protein.
Telomerase is responsible for maintaining telomere length, overexpression will lead to telomere elongation.

Telomerase has been used to immortalise cell lines, and thus needs to be considered potentially oncogenic
CDK4 is cyclin dependant kinase 4 and is expressed during the G1-S phase transition in the cell cycle. In complex with cyclin D and CDK6, it is responsible for phosphorylating pRS. Hypophosphorylation of pRB will drive cells into S phase of the cell cycle. While COK4 is negatively regulated by p161NK4A, the mutated version of CDK4, CDK4 R24C escapes the negative regulation by p161NK4A. As overexpression of both CDK4 and CDK4 R24C lead to dysregulated cell cycle control, both are considered to be potentially oncogenic.

Green fluorescent protein is being used as a marker for transfection.

Evaluation of foreseeable effects

There are three different GMO's being produced during this process
1) Bacteria with the individual components
Bacteria will not contain more than one viral component and therefore are unable to produce viral particles. Strains used are standard K-12 derivative E.Coli, and the presence of the vectors is not expected to alter pathogenicity of the bacteria, nor alter their survivability in the environment.

2) Lentivirus/retrovirus
Individual components for the virus are transfected into HEK293 cells were they are packaged and released. The viral part of the work that involves HTERT and CDK4 is the most hazardous. Both HTERT and CDK4 can be used to immortalise cell because they enable cells to bypass normal growth arrest/cellular senescence regulation. This process has oncogenic potential. Furthermore, as lentiviral systems are capable of infecting non-dividing cells, skin contact or inhalation of aerosols with viral particles may harm human health. Work involving the virus particles will be in a microbiological safety cabinet. Gloves and laboratory coats will be worn and no sharps will be used.

The viral particles produced are non-replicative and self inactivating. The presence of the inserts will not alter this and therefore unlikely to alter survivability in the environment.

3) Infected mammalian cell lines
As the viral particles are non-replicative and self inactivating, once the target cells are infected, the virus will integrate and express the desired proteins, but will not cause any further viral production. Cell lines will have a growth advantage as they will have escaped normal cell cycle regulation, but this process is no different to the myriad of cancer cell lines available. These changes are not expected to alter the pathogenicity of the cell lines. Escaping cell cycle control may indicate a growth advantage. However, given the cell lines requirement to have specialised growth media, transduced cell lines are not expected to have altered survivability.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

02/03/2022
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be autoclaved at 126°C for 20 mins. Autoclaved waste is disposed of as clinical waste via the UHL hospital waste system.

Liquid waste is treated with 10,000ppm free chlorine sodium dichloroisocyanurate (eg presept) for >12 hours.

Small volume spillages will be cleaned up using 1% virkon. Large volume spillages will be cleaned up using 10,000ppm free chlorine sodium dichloroisocyanurate.

Source of validation data. Manufactures data for sodium dichloroisocyanurate and Virkon. Autoclave conditions used are to ensure 121°C for 15 minutes. All runs are recorded and filed. Autoclaves undergo annual service and calibration and statutory inspections. Further checks are made to ensure autoclaves are achieving the correct temperatures.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Agreed:

i. That a designation of Class 2 is appropriate

ii. That the risk assessment is sound and that work should be carried out at Containment Level 2

iii. That the project will be notified to the HSE.

Noted:

i. That the proposer will contact HSE to notify the work as a new project

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In this study we hope to understand the molecular basis of aberrant gene expression observed in people with inflammatory airway diseases such as asthma. The work will involve expression and silencing of various genes in commercially available and primary mammalian cells.

Recipient or parental organism

- Top10
- Stbl3
- DH10B T1R
- Primeplus
- HEK293T
- Hela
- HMC-1
- LAD-2

Primary human cells: T-lymphocytes, innate lymphoid cells, peripheral blood mononuclear cells, airway smooth muscle cells, epithelial cells, mast cells, fibroblasts, fibrocytes

Bacterial expression: All plasmids contain antibiotic resistance for expression in E.coli.
Full length cDNAs obtained from the MGC clone collection are contained within the pCMV-SPORT6 vector and supplied in DH10B T1R bacterial cells. Genes amplified by PCR are placed into the pCR4-TOPO family of vectors for expression in TOP10 bacterial cells to facilitate subcloning into relevant vectors (pCDNA3.1 for mammalian cell expression; lentiviral expression constructs for primary cell work). Amplification of pCDNA3.1 and pCMV-SPORT6 constructs will proceed in TOP10 bacterial cells.
cells, all lentiviral-cDNA constructs will be expressed in Stbl3 bacterial cells.
shRNAs will be supplied in the pGIPZ vector and grown in E.coli Prime plus and Stbl3 bacterial cells.
miRNAs will be supplied in the relevant lentiviral construct (pMIRNA1 and pMIRZ2P) and amplified in Stbl3 cells.
Virus production: Virus particles for transduction of primary human cells will be produced in HEK 293T cells transfected with the expression plasmid containing the insert, the envelope plasmid (pMD2.G), the pRSV-REV plasmid and the required packaging constructs (for pGIPZ constructs psPAX2, all other constructs pMDLg/pRRE).
Gene overexpression and silencing in human primary cells: Tensin1 silencing will be achieved by using a proprietary lentiviral construct from Santa Cruz. All target genes for overexpression in primary human cells will be cloned into the pCDH and pCS2 lentiviral vectors. miRNAs will be expressed in the pMIRNA1 vector and shRNAs in pGIPZ. miRNA silencing will be achieved using pMIRZ2P constructs. These vectors will be used to make lentiviral particles as described above and then the lentiviral particles will be used to transduce human cells.

Mammalian cell transfection: Target genes will be transfected into the various mammalian cell lines using either pCDNA3.1 or pCMV-SPORT6.

### Target genes to be tested

- **Transcription factors:** These transcription factors have been demonstrated to either influence cell phenotype directly or to regulate genes involved in immune cell phenotypic determination and effector cell function. Myb, Maf, Ikaros1, GATA3, TBX21, RORα, RORc, FoxP3, PU1, TCF7, ID2 and IRF4 are involved in cell type specification. The glucocorticoid and Vitamin D3 receptors are nuclear hormone receptors with immunomodulatory functions.

  - **Cell surface receptors:** The target genes (CysLTR1, CysLTR2, KLRG1, IL17RB, IL1RL1, IL15RA, CRTH2) are listed as receptors involved in cell-cell communication, they mainly respond to cytokines and other inflammatory mediators to induce cell chemotaxis or the production of cytokines. The CysLTR1,2, and the CRTH2 receptors are G protein coupled receptors. The KLRG1 receptor is thought to be an inhibitory receptor, whilst the remaining cytokine receptors (IL15RA, IL1RL1, IL17RB) promote cell proliferation and gene transcription in the presence of their respective ligands.

  - **Tensin-1:** This protein is encoded by the TNS1 gene and localizes to focal adhesions, regions of the plasma membrane where the cell attaches to the extracellular matrix. This protein crosslinks actin filaments and contains a Src homology 2 (SH2) domain, which is often found in molecules involved in signal transduction. This protein is a substrate of calpain II and has not been identified as hazardous.

  - **TRP family:** The TRP channel family encodes a large (~30) number of cation channels that, when activated transport either Ca2+ or a mixture of monovalent ions across the cell membrane. Therefore, these channels regulate intracellular calcium concentrations, the most common signal transduction molecule in cells, thereby influencing a wide range of cellular processes. TRP channels are involved in cell differentiation, survival, migration and mediator release.

  - **CEACAM6:** The protein encoded by this gene is a cell adhesion molecule detected on leukocytes and epithelia. It mediates cell-cell adhesion to regulate multiple cellular activities including differentiation, apoptosis, metastasis and modulation of immune responses.

  - **shRNAs:** shRNAs will be employed to decrease amounts of the gene products of target genes, including those listed in this section.

  - **miRNAs:** MicroRNAs (miRNAs) are small RNA molecules that function to silence RNA and regulate gene expression, post-transcriptionally. They play critical roles in the establishment of cellular phenotype. In this project we will deliver both pre- and anti-miRNAs. Evidence on functions of specific miRNAs is sparse and all three listed in this proposal have been associated with cancer. miR-150 has been associated with haematopoietic malignancies, its target genes include myb and myc, therefore silencing miR-150 can lead to increased cell proliferation. miR-31 and miR-155 have both been reported to display highly varied and altered expression profiles across many cancers. miR-31 has many validated targets including, transcription factors, small GTPases and cytokines. miR-155 overexpression reduces mRNA and protein levels of the tumour suppressor gene, TP53INP1.

### Evaluation of foreseeable effects

**Risk from genes to be tested**

- cMaf, cMyb have been shown to have some oncogenic activity, none of the other transcription factors has been reported to be hazardous. There is no evidence that overexpression of any of the cell surface receptors is hazardous, however since they play a role in cell proliferation we will consider them as potentially hazardous. TRP channels are involved in cell differentiation, survival, migration and mediator release. Due to their varied roles and the association of some members of the family with cancer, we will treat all members as potentially hazardous. CEACAM6 has been reported to be overexpressed in several human cancers and has oncogenic properties this gene will be considered hazardous.

**Risk from the use of lentiviral delivery**

02/03/2022
The lentivirus vectors can transduce both dividing and non-dividing human cells and integrate into the genome. There is therefore potential for this to cause insertional mutagenesis. The main hazard to human health following accidental inoculation of viral particles is the potential to cause excessive cell proliferation resulting in tumour or lymphoma formation. However, the likelihood of exposure is low since all staff are specifically trained in cell culture and virus production technique and none of the procedures will include the use of sharps. Therefore the likelihood of exposure is extremely low. Additionally the lentivirus strategies adopted here use three or four plasmids which separates env and gag/pol on to separate plasmids thereby generating replication incompetent virus. Infection is therefore limited to a single round and the likelihood of the GMM causing harm to human health is low. Both second and third generation lentiviral plasmid systems will be used.

Risk to environment

In the event of accidental escape while it is possible for the bacteria to survive for a short time in the environment, the required growth conditions are unlikely to be met, thus it is unlikely that they will be able to persist in the environment. The presence of the inserts is not expected to alter this as none of the targets are being expressed. Mammalian cells require specified growth conditions that will not be met and they are unlikely to survive. Some of the inserts, while potentially oncogenic or may increase proliferation, are not expected to alter this. Due to the use of the split genome lentivirus system there is effectively no chance that genetic material could be transferred to wild type organisms in the environment. The virus is extremely unlikely to revert to wild type and therefore is limited to a single round of infection and could not persist in the environment to cause harm. The vectors used to construct the lentivirus need a specified packaging cell line in order to produce infective particles. Thus it is unlikely if there were co-release of the bacteria containing the lentiviral components that a infectious lentivirus would be produced.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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<tr>
<td>Treated with 10,000 ppm chlorine from dissolved presept tablet 30 min minimum exposure. Presept treated waste will be disposed of down the sink.</td>
</tr>
<tr>
<td>Contaminated Plastics (tips, pipetes, pastettes, etc)</td>
</tr>
<tr>
<td>Treated with 10,000 ppm chlorine from dissolved presept tablet for a minimum of 30 minutes. Presept treated plasticware will be will be placed in an autoclave bag and treated as solid waste.</td>
</tr>
<tr>
<td>Solid Waste</td>
</tr>
<tr>
<td>Autoclaved – 10 min steam followed by 30 min 126oC (10 min stabilisation, 20 min held temperature). Autoclaved waste is disposed of as healthcare waste via University Hospital Leicester.</td>
</tr>
<tr>
<td>Spills</td>
</tr>
<tr>
<td>Spills will be treated with 10% Distel for 30 min before wiping up.</td>
</tr>
</tbody>
</table>

Presept and Distel concentrations have been validated by manufacturer’s for inactivating virus and bacterial particles. Autoclaves undergo annual service, calibration and statutory inspections. Further checks are made to ensure the autoclaves are achieving the correct temperature.

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
Agreed:

i. That a designation of Class 2 is appropriate.
ii. That the risk assessment is sound and that work should be carried out at Containment Level 2.
iii. That the project will be notified to the HSE.

Noted:

i. That the proposer will contact HSE to notify the work as a new project.

### Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
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<td>L2</td>
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<tr>
<td></td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
<td>L2</td>
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<tr>
<td>L4</td>
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<td>L4</td>
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### Project Ref 11/20.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
</tr>
</thead>
<tbody>
<tr>
<td>23/01/2020</td>
<td>Understanding the role of genes linked to eye disorders</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Consent Granted</td>
<td></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

### Project Additional Information

02/03/2022
**Purposes of the contained use**

We are using silica analysis to identify genes involved in eye disorders, in particular uveal melanoma. This project will aim to understand the role of these genes within the disorders that they have been identified from. We shall use lentiviral systems to knock down and overexpress the genes in order to elucidate the function of the genes.

**Recipient or parental organism**

<table>
<thead>
<tr>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top10</td>
</tr>
<tr>
<td>Stbl3</td>
</tr>
<tr>
<td>DH10B</td>
</tr>
<tr>
<td>T1R</td>
</tr>
<tr>
<td>NEB 10-beta</td>
</tr>
<tr>
<td>DH5alpha</td>
</tr>
<tr>
<td>JM109</td>
</tr>
<tr>
<td>BI21</td>
</tr>
<tr>
<td>Prime, Plus</td>
</tr>
<tr>
<td>HEK293T</td>
</tr>
<tr>
<td>Hela</td>
</tr>
<tr>
<td>Primary human cells: UM-T71 OA, UM-T1356A, UM-T1324A</td>
</tr>
</tbody>
</table>

**Host/vector system**

NEB 10-beta and Stbl3 etc will be used for maintenance of all plasmids listed and for routine cloning in pLlV~Gateway

pENTRId-topo, pLV[EXP]-Hygro-EF1A and lentiCRISPRv2

HEK293 will be transfected with the relevant combination of vectors to produce replication deficient lentiviral particles.

Resulting lentivirus will be used to infect the listed human cells

Cell lines may be transfected with pLV[EXP]-Hygro-EF:1A and lentiCRISPRv2

**Hosts**

- E.coli strains: NEB 10-beta, DH5alpha, Top10, Stt?13, DH1 OB T1 R, PrimePlus

- Human cell lines: HEK293T, Hela

- Primary human cells: UM-T710A, UM-T1356A, UM-T1324A

**Vectors**

- To express in E.coli: pLV[EXP]-Hygro-EF1A, pET30, pETM6T1, pGEX, pRSF, pCDF, pBR322 vector, pBR328 vector, pLV-Gateway, pENTRId-TOPO, lentiCRISPRv2

- Transient cell line transfection: pLV[EXP]-Hygro-EF1A, lentiCRISPRv2

- Lentiviral packaging plasmids: pCMVdeltaR8.74

- Lentiviral Envelope plasmid: pMD2G

- Lentiviral vectors for gene expression: pCMVdeltaR8.74, pMD2G, pLV-Gateway, pLV[EXP]-Hygro-EF1A, lentiCRISPRv2

**Origin & function**

Target genes to be tested

**Health and Safety**
We aim to understand the role of genes within the eye disorders that they have been identified from. These genes may influence the processes of growth, proliferation and survival of the cells leading to deregulated expression or function. The exact function of the genes in the disorders is unknown. However, it will not be unreasonable to expect that as a worse case, some of these genes would be oncogenes but could also be tumour suppressor genes or transcription factors.

The first two genes we identified in uveal melanoma are KTN1 and DLK2. These are oncogenes however the exact function of the genes in uveal melanoma is unknown.

KTN1 is involved in cadherin, kinesin and RNA binding as well as in microtubule-based movement, cellular protein metabolic process and post-translational protein modification;

DLK2 is related to canonical and non-canonical notch signalling and the p38 MAPK signalling pathway. DLK2 expression may have a positive and negative effect on the proliferation rate of melanomas.

Evaluation of foreseeable effects

Risk from genes to be tested

Health and Safety

Executive

Contained Use Notification

There are more than 350 hereditary eye diseases; Genetic factors play a key function in many kinds of eye diseases including those diseases that are the leading cause of blindness among infants, children and adults.

We aim to understand the role of genes within the eye disorders that they have been identified from. These genes may influence the processes of growth, proliferation and survival of the cells leading to deregulated expression or function. The exact function of the genes in the disorders is unknown. However, it will not be unreasonable to expect that as a worse case, some of these genes would be oncogenes but could also be tumour suppressor genes or transcription factors.

KTN1 and DLK2 are oncogenes however their exact function in uveal melanoma is unknown.

KTN1 is involved in cadherin, kinesin and RNA binding as well as in microtubule-based movement, cellular protein metabolic process and post-translational protein modification.

DLK2 is related to canonical and non-canonical notch signalling and the p38 MAPK signalling pathway. DLK2 expression may have a positive and negative effect on the proliferation rate of melanomas.

In addition, one of the systems (pLV based vectors) utilises wild type WPRE. This contains a truncated version of protein X that has been demonstrated to be oncogenic under certain circumstances. The other lentivirus system contains a mutant version of WPRE that cannot express protein X.

Unless it has been demonstrated that the identified genes are not harmful to human health, genes identified through this study will be treated as if they could be harmful. All use of pLV based vectors will be treated as if they ‘could be harmful irrespective of the gene being investigated.

Risk from the use of lentiviral delivery

The lentivirus vectors can transduce both dividing and non-dividing human cells and integrate into the genome. There is therefore potential for this to cause insertional mutagenesis. The main hazard to human health following accidental inoculation of viral particles is the potential to cause excessive cell proliferation resulting in tumour or lymphoma formation. However, the likelihood of exposure is low since all staff are specifically trained in cell culture and virus production technique and none of the procedures will include the use of sharps. Therefore the likelihood of exposure is extremely low. 80th second and third generation lentiviral plasmid systems will be used in this study. These systems separate env and gag/pol onto different plasmids and will only produce replication incompetent virus. Infection will therefore be limited to a single round of infection. Thus the likelihood of the GMM causing harm to human health is low.
### Risk to Environment

In the event of accidental escape while it is possible for the bacteria to survive for a short time in the environment, the required growth conditions are unlikely to be met, thus it is unlikely that they will be able to persist in the environment. The presence of the inserts is not expected to alter this as none of the targets are being expressed. Mammalian cells require specified growth conditions that will not be met and they are unlikely to survive. Some of the inserts, while potentially oncogenic or may increase proliferation, are not expected to alter this. Due to the use of the split genome lentivirus system there is effectively no chance that genetic material could be transferred to wild type organisms in the environment. The virus is extremely unlikely to revert to wild type and therefore is limited to a single round of infection and could not persist in the environment to cause harm. The vectors used to construct the lentivirus need a specified packaging cell line in order to produce infective particles. Thus it is unlikely if there were co-release of the bacteria containing the lentiviral components that an infectious lentivirus would be produced.

### Containment and Control Measures for GMOs that are not Microorganisms (e.g. GM Animals & Plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Liquid Waste</th>
<th>Treated with 10,000 ppm chlorine from dissolved presept tablet 30 min minimum exposure. Presept treated waste will be disposed down the sink.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contaminated Plastics (tips, pipettes, pastettes, etc)</td>
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<td>Autoclaved - 10 min steam followed by 30 min 1260°C (10 min stabilisation, 20 min held temperature). Autoclaved waste is disposed of as healthcare waste via University Hospital Leicester</td>
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<td>Spills</td>
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**Is an emergency plan required according to regulation 20?**

- [ ] N

If yes, tick to confirm that it is attached to this form

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] N

Please enter comments on the GM safety committee on the risk assessment
Agreed:
i. That a designation of Class 2 is appropriate
ii. That the risk assessment is sound and that work should be carried out at Containment Level 2
iii. That the project will be notified to the HSE.

Noted:

i. That the proposer will contact HSE to notify the work as a new project.

**Project Containment**

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<th>Laboratory Activities</th>
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<tbody>
<tr>
<td>L2: Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 11/98.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
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<tbody>
<tr>
<td>08/04/1998</td>
<td>ANALYSIS OF RAF-DEFICIENT CELLS</td>
<td>Class 2</td>
<td></td>
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<td>Y</td>
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**Project Additional Information**

- **Historical Significant Changes**
  - **Historical Date of Additional Info**
  - **Significant Change ID**
  - **Date of Significant Change**

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>L3</td>
<td>L4</td>
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<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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</table>
## Project Ref 11/99.1

### Date Ackn'd
10/03/1999

### Date Project Ceased

### CU2 Project Title
GENETIC ANALYSIS OF THE PATHOGENIC MECHANISMS OF CAMPYLOBACTER

### Class
Class 2

### Consent Granted
not applicable

### Non-GMM

### Project notified under transitional arrangements
Y

### Withdrawn
N

### Tick if notifying a connected programme of work
N

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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If yes, tick to confirm that it is attached to this form

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Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<td>Human Clinical Applications</td>
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<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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**Project Ref** 11/99.2

<table>
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<tr>
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<th>Date Project Ceased</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>09/12/1999</td>
<td>STUDIES OF BACTERIAL PHYSIOLOGY AND VIRULENCE</td>
<td></td>
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<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
<td>not applicable</td>
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Withdrawn N  

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
### Project Containment

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</thead>
<tbody>
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<td>L3 L4 L2 L3</td>
<td>L4 L3 L4 L2</td>
</tr>
</tbody>
</table>

- Animal Units
- Large Scale Activities
- Human Clinical Applications

### Project Ref 821/04.2

- **Date Ackn'd**: 17/01/2014
- **CU2 Project Title**: EXPRESSION BY LENTIVIRAL INFECTION OF THE FOLLOWING CLONED GENES - GFP, CEBP FAMILY, BCL11A AND B, MLT, PRDM6 - IN B CELLS.

<table>
<thead>
<tr>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td></td>
</tr>
</tbody>
</table>

- **Non-GMM**: Not Applicable
- **Consent Granted**: Not Applicable

- **Project notified under transitional arrangements**: No

### Project Additional Information

- ** Withdrawn**: No
- **Historical Significant Changes**: Transferred from GM821 on 17/01/2014
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

---

**Tick to confirm that you have attached a risk assessment to this form**: No

**Tick if you are claiming exemption from disclosure for section of the risk assessment**: No
### Purposes of the contained use

To clone and express genes that have been identified as having a possible involvement in haematological malignancies, in primary B cells and B cell lines of human origin. The aim in the first instance is to assess whether the genes of interest have an effect on cell growth and/or apoptosis. Primary B cells cannot easily be made to express cloned genes by any other transfection method.

### Recipient or parental organism

Primary normal human B cells and B cell lines of human origin.

### Host/vector system

Pseudolentiviral particle based on HIV is used to infect the primary B cells (Naldini et al., Science. 1996 Apr 12;272 (5259):263-7). The lentiviral transfer vector pRRLsin.PPT.hCMV.pre will contain the gene of interest under the CMV promoter and between the 5' and 3' LTRs of HIV for integration into the cellular genome. It is transfected into 293T cells together with 3 core packaging plasmids and hybrid viral particles are made with the core proteins and enzymes of a lentivirus and the envelope of vesicular stomatitis virus. All structural, regulatory and accessory genes of HIV are excluded from the lentiviral vector and from the packaging plasmids. Virus particles are made which are replication-defective. The procedure involves transient transfection ie a short time for production of particles. The particles are collected from the culture supernatant, concentrated by centrifugation and can be stored frozen before being used to infect the target cells.

### Origin & function

- **Bcl11A and B** - zinc finger genes, possible transcription factors. Human genes.
- **MLT** - a paracaspase and may be involved in apoptotic pathways. Human gene.
- **PRDM6** - SET domain, may be involved in chromatin remodelling. Human gene.

It is likely that we will use mutated versions of all these genes also. **GFOP** is only used as a test control gene. All the other genes are possible oncogenes.

### Evaluation of foreseeable effects

At the packaging stage the viral particles could infect human cells. Therefore, the production of the virus and infection of host cells with the virus is carried out in containment level 2. Cells infected by the lentiviral particles are not hazardous as the virus cannot replicate. However, they could be overexpressing the gene inserted. The inserted genes are of unknown function and could be oncogenes but it is unlikely they will function on their own without other cellular changes. There is a low possibility of infecting individual cells in vivo. The virus particles cannot replicate and infection would remain localised to the site of entry.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste:

Solid waste will be treated with hypochlorite (10,000ppm) before being autoclaved prior to disposal (100% kill). The sterile waste is then sent for incineration by authorised contractors.
Liquid waste:
Liquid waste will be treated with hypochlorite (concentration 10,000ppm available chlorine overnight and autoclaved before disposal. Any spillages will be cleaned with chlorine disinfectant and the waste paper autoclaved and incinerated.

Autoclave cycle - 121 degrees C for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

It has been agreed by the Genetic Modification Safety Committee that a designation of Class 2 is appropriate for this work and that the risk assessment is sound and should be carried out at Containment level 2.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<td>L4</td>
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### Project Ref 821/05.2

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>17/01/2014</td>
<td>The expression of genes in human and rodent cells using lentiviral infection procedures in order to assess the role of specific genes in toxilogical signalling pathways</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Not Applicable</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work Y
### Project Additional Information

#### Purposes of the contained use

To clone and express genes encoding proteins involved in cellular processes such as apoptosis, the cell cycle, cell signalling and neuronal pathology. Genes will be expressed in cell lines of human and rodent origin.

#### Recipient or parental organism

Human cells including cell lines of human origin and primary cells. Amphotropic packaging cells (for example Phenix 293 cells) will be used to express the core packaging plasmids, therefore the virus produced could infect human cells. However, the packaged virus cannot replicate so infected target cells would not be hazardous, although they would be overexpressing the gene inserted. To minimize risk sharps will not be used and primary human cells will only be obtained from donors outside the building.

Rodent cells will also be used, both primary and cell lines. In this case ecotropic packaging cells will be used to ensure the viruses produced cannot infect human cells.

#### Host/vector system

Inserts will be cloned into the pRRLsin transfer vector. This HIV-derived vector lacks the structural, regulatory and accessory proteins required for making replication competent retrovirus. This vector will be co-transfected into packaging cells alongside the pMDLg/pRRE, pRSV-Rev and pMD2.VSVG vectors in order to create replication-defective lentiviral particles.

#### Origin & function

The genetic material to be expressed will include genes encoding proteins known to regulate cellular processes. This will include genes involved in apoptosis the cell cycle, cell signalling and neuronal pathology. Examples will include ALAS1, CREB, HIF, cJun, CEBP family, BCPN1, MLT, Bcl, m3-muscarinic receptor, PML, DAXX, calpastatin genes and Green fluorescent protein.

Genes will be expressed that are well characterised as well as potential oncogenes or tumour suppressors and also mutated forms of these genes (including the CEBP family, BCNP1, PRDM6, MLT, Bcl11, SNX25 and C2TA). However, it is unlikely these genes would function as potential oncogenes or tumour suppressors on their own in the absence of other additional cellular changes.

#### Evaluation of foreseeable effects

At the packaging stage the virus particles could infect human cells. The virus is, however, replication deficient. Transacting structural genes are excluded from the transfer vector, which only encodes the gene of interest. The essential regulatory genes gag and pol are encoded in a separate plasmid, as is rev gene. These constructs have been altered to contain non-overlapping sequences hence minimising the possibility of recombination. In addition non-essential genes have been eliminated, hence any replication-competent virus generated would lack essential factors for replication and virulence in vivo.
Some inserted genes would be of unknown function and could be oncogenes, however it is unlikely they could function on their own without other cellular changes occurring. It cannot, however, be ruled out that an insert in an environment as yet untested may have a deleterious effect on host cells. For this reason these inserts are considered as potentially harmful.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste
Solid waste will be treated with hypochlorite (10,000ppm) before being autoclaved prior to disposal (100% kill). The sterile waste is then sent for incineration by authorised contractors.

Liquid waste
Liquid waste will be treated with hypochlorite overnight (10,000ppm available chlorine). Any spillages will be cleaned with chlorine disinfectant and the waste paper autoclaved and incinerated.

Autoclave cycle: 121 degrees celsius for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

It has been agreed by the GMSC that a designation of Class 2 is appropriate for this work and that the risk assessment should be carried out at a containment level 2.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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### Name

| UNIVERSITY OF EDINBURGH |

### Name 2

| INSTITUTE OF CELL & MOLECULAR BIOLOGY |

### Campus Estate or Research Centre

| BIOLOGICAL SCIENCES |

### Building

| DARWIN BUILDING |

### Road Name

| MAYFIELD ROAD |

### Town

| EDINBURGH |

### County

| EAST LOTHIAN |

### Postcode

| EH9 3JR |

### Country

| SCOTLAND |

### Tel Number

| 0131 650 1000 |

### Fax Number

| 0131 650 8650 |

### E-mail

| health.safety@ed.ac.uk |

### HSE Division

| SCOTLAND |

### Comments

| GM 12 MERGED WITH GM 207 AS OF 17/02/2004 |

### Date at Which Additional Info Submitted

| 17/02/2004 |
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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### Level 4 (GMMs)

#### Non-microbial

**Other (please specify)**

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<td>Plants</td>
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**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 12/00.1**

**Date Ackn'd** 04/04/2000

**CU2 Project Title** EXPRESSION OF TRANSGENES IN LEISHMANIA MEXICANA & LEISHMANIA MAJOR

**Class** Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM** not applicable

**Consent Granted**

**Date Project Ceased** 02/03/2022
Historical Significant Changes

Historical Date of Additional Info: Merged with GM 207 on 17/02/2004

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**Project Ref**: 12/00.2

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<td>PHYSIOLOGY OF GRAM-NEGATIVE BACTERIA</td>
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Withdrawn: N

Tick if notifying a connected programme of work: N

**Historical Significant Changes**

Merged with GM 207 on 17/02/2004

**Project Additional Information**

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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**Name**

UNIVERSITY OF WARWICK

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

GIBBET HILL ROAD

**District**

COVENTRY

**Town**

COVENTRY

**Country**

WARWICKSHIRE

**County**

CV4 7AL

**Postcode**

ENGLAND

**Tel Number**

024 765 23559

**Fax Number**

024 765 23701

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Project Ref 13/01.1

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<td>15/02/2001</td>
<td>CONSTRUCTION AND USE OF FULL-LENGTH CLONES OF SIMIAN IMMUNO-DEFICIENCY VIRUS (SIV) AND HUMAN IMMUNO-DEFICIENCY VIRUS (HIV) IN ESCHERICHIA COLI.</td>
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02/03/2022

Page 476 of 15326
Project notified under transitional arrangements

Tick if notifying a connected programme of work

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form
### Project Containment

<table>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<td>EXPRESSION OF SEGMENTS AND FULL LENGTH NATIVE AND MODIFIED SYRIAN HAMSTER PRION PROTEIN IN ESCHERICHIA COLI FOR PROTEIN FOLDING STUDIES</td>
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### Project Additional Information

**Purposes of the contained use**

Expression of the full length (residues 23-231) and a segment of the prion (residues 90-231) for structural and folding studies. Expression of mutant forms of the segment.
Recipient or parental organism

Syrian hamster.

Host/vector system

Escherichia coli K12 strain ST11 TIR variant 4 (expression vector carrying the Syrian hamster prion protein segment (residues 90-231). E. coli non-K12 non-B strain BLR (DE3) (BLR is a Rec- version of BL21 and is given an Access factor of 10 -6 - ACGM Compendium of Guidance Part 2A Annex II 12) and a pTrcHis vector.

Origin & function

The strains expressing the full-length prion protein, the segment of the prion protein and mutant forms of both forms of the prion protein were supplied from the Institute of Animal Health, Compton. The strains are only used to express the proteins at Warwick. The purified proteins are then used in structural studies and protein folding analysis.

Evaluation of foreseeable effects

It is not clear that the Syrian hamster prion can cross the species barrier into humans. The GMSC has therefore erred on the side of safety and assumed that it can. We presume in each case because the E. coli can express the prion there is a significant risk to human health if the GMO resides, even for a short time in the gut. This is especially the case as some prion expression is dependent on T7 bacteriophage RNA polymerase, which is itself under the control a lactose inducible promoter. Lactose is a common dietary sugar and so there is high probability the prion would be expressed by bacteria if in the gut. There are, therefore, serious potential health consequences of escape of these GMOs. We also assume that this prion is capable of infecting a wide range of animals and so there is also a very significant risk of Environmental damage if the GMO can escape. Thus the consequence of environmental hazard is severe. As all manipulation of the GMOs occurs under Containment level 3 conditions the likelihood of environmental hazard is negligible.

Evaluation of foreseeable effects

No relevant

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None sought

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the work is carried out in a Containment Laboratory. The Containment Laboratory conforms to all the physical requirements of a Containment Level 4 laboratory (ACGM Compendium of Guidance Part 3A Table 1) but it is run as a Containment Level 3 facility as we do not require complete change of clothing and footwear before entry and exit (ACGM Compendium of Guidance Part 3A Table 1 Section 12). All effluent from hand washing sinks and showers is collected and chemically decontaminated before removal from the room. All GMMs in contaminated material and waste are inactivated by treatment with 20,000ppm chlorine bleach for twelve hours and autoclaving at 134 degrees C. Solid waste is inactivated by treatment with 20,000ppm chlorine bleach for twelve hours and incineration. The detailed protocol for decontamination is given in the letter to HSE dated 10 June 1997.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y
The Brenner scheme for the GMOs bearing the prion gave the following values: BLR (DE3) is given an Access factor of $10^{-6}$; the Damage factor of the prion is $10^{-1}$. As maximal expression is sought, the Expression factor is $10^{-1}$. The overall value is thus $10^{-6}$ and following previous contact with the HSE, this has been ascribed a Containment Level of 3. The fact that the prion is potentially capable of infecting other species means there is a very significant risk of Environmental damage if the GMOs can escape. Thus the consequence of environmental hazard is severe. As all manipulation of the GMOs occurs under Containment level 3 conditions, the likelihood of environmental hazard is negligible.

All of this work has previously been categorised and approved by the GMSC and the HSE. The reason for this resubmission was that following the introduction of the new Genetically Modified Organisms (Contained Use) Regulations 2000, our submission of the continuing project under the Transitional Provisions was rejected as too late.

**Project Containment**

<table>
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<tr>
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<td>L3</td>
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**Project Ref** 13/02.1

- **Date Ackn'd**: 10/05/2002
- **CU2 Project Title**: CONSTRUCTION AND USE OF FULL-LENGTH CLONES OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND SIMIAN IMMUNODEFICIENCY VIRUS (SIV) IN E.COLI, AND GENERATION OF GENETICALLY MODIFIED VIRUS.
- **Class**: Class 3
- **Culture Vol**: 200 ml
- **Consent Granted**: Yes
- **Non-GMM**: Project notified under transitional arrangements

**Historical Significant Changes**

- **Historical Date of Additional Info**: 20/03/2022
### Purposes of the contained use

The aim of this project is to generate virus with specific amino acid changes within certain genes to affect the presentation of B and T cell epitopes to the immune system. The long term aim is to determine which viral epitopes could be used to stimulate a protective response on vaccination.

### Recipient or parental organism

The parent virus used will be full-length molecular clones of HIV or SIV made available to researchers through the NIH AIDS Research and Reference Reagent Program. These generate infectious virus upon transfection into permissive cell lines. Unmodified immunodeficiency viruses are classified in hazard group 3 under the COSHH regulations. HIV-1 and 2 infect humans and there is no effective vaccine, chemotherapy suppresses virus replication but does not eliminate the infection. SIV infects non-human primates.

### Host/vector system

The parent virus is cloned into a non-mobilisable vector pDR8 (a derivative of pUC19) and will be propagated in XL-1 blue cells (E. coli K12). Virus will be generated by transfecting linearised plasmid DNA into human T-cell lines (CEM x 174, H9, C8166, HeLa T4) or human peripheral blood lymphocytes.

### Origin & function

The molecular clones encoding full length HIV and SIV were cloned directly from genomic proviral DNA. These clones produce infectious virus upon transfection into permissive cell lines.

### Evaluation of foreseeable effects

Glycoprotein spikes on the surface of immunodeficiency viruses are responsible for the attachment of the virus to the cell (gp120) and fusion of the virus to the cell membrane (gp41). Both glycoproteins are encoded by the env gene. We intend to modify part of this gene to give rise to specific amino acid substitutions, deletions or insertions. We are not mutating parts of the gene responsible for binding to primary or secondary cell receptors, or the genes responsible for proteins targeted by chemotherapy. HIV causes a lethal human infection and mutations are unlikely to increase its pathogenicity. RNA viruses such as the immunodeficiency viruses have a high level of genetic variability and the changes sought are similar to those which occur in nature.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None sought.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Modification of plasmid DNA is carried out in a containment level 2 laboratory. All solid waste and soiled glassware is autoclaved at 121 degrees C for a minimum of 15 minutes. Paper waste and other porous materials are autoclaved at 134 degrees C for 10 minutes. The autoclave is validated by thermocouple testing once every three months. Liquid cultures are chemically decontaminated by treatment with 2500 ppm chlorine bleach for 12 hours before disposal.

The virus generated will be replication competent and all manipulations will take place within a class 1 safety cabinet within a containment level 3 laboratory which is run under negative pressure and is sealable for fumigation. Access to this laboratory is restricted to authorised personnel only. All waste from this lab is autoclaved before disposal in an autoclave situated within the laboratory suite. The autoclave is validated by thermocouple testing once every three months. All staff are specifically trained in the safety aspects of the work. Written training records are kept. All growth of virus involves standard laboratory protocols; there are no unusual procedures that require additional containment measures.
The committee discussed the extension of the HIV/SIV work to include site-direct modifications of the genomic molecular clones. 

*  The clones are contained in pUC-based, non-mobilisable vectors in disabled host strains.
*  The vectors lack promoter sequences, so no viral proteins are produced.
*  The molecular clones are of half genomes, so no E. coli contains a full complement of viral genes. A restriction site is used to retrieve each half and to religate the DNA in vitro before the transfection of human cells. The half-clones do not overlap - the restriction site is the only homologous sequence, so a full-length viral genome could not be reconstructed by homologous recombination even if the plasmids were present in the same E. coli (which they are not).

Notwithstanding, these GMMs and the DNA in question have always been handled in the category 3 lab using the same precautions as for the live virus. Following new guidelines from HSE, work involving wild-type molecular clones has been classified to containment level 2, and notified to HSE and the work in this department has already been approved by HSE at this level. HSE has indicated that any modified molecular clones should be classified to containment level 3.

The meeting discussed a risk assessment document circulated by NJD and LMcL (attached). The proposed modifications are deletions and point mutations. No foreign DNA will be inserted. The RNA polymerase involved in HIV replication is very error-prone. The viral RNA sequence is highly variable in nature, so the proposed modifications could also arise naturally. Given the large numbers of circulating virus particles, each of the proposed modifications is likely to exist in the wild already. The deletions are known to restrict the host range, and there is no reason to expect that the point mutations would increase the rate of infection, the persistence of virus in the environment or the response to treatment. The disease outcome cannot be worsened by the modifications, because unmodified virus already causes a lethal and incurable disease.

Environmental risks: the receiving environment contains no non-human hosts for HIV or SIV (negligible consequence of release), the likelihood of release from the category 3 lab is negligible, so the overall risk is effectively zero. The committee approved the proposal at containment level 3.

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>Human Clinical Applications</td>
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<td>L3</td>
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Project Additional Information

Purposes of the contained use

The aim of the project is to investigate the control of replication and transcription of pneumoviruses by identifying the role(s) of specific virus proteins in these processes. The ultimate aim of the work is to generate potential vaccines for the human and animal pneumoviruses.

Recipient or parental organism

The cDNA copies of the virus genomes cloned into plasmids cannot be expressed in bacteria or in normal tissue culture, or other, cells unless the bacteriophage T7 RNA polymerase is simultaneously expressed. Consequently, the recombinant viruses can only be generated under controlled laboratory conditions.

With the exception of PVM and APV the viruses are restricted to replication within the respiratory tract of a single host. In the case of PVM there is serological evidence of an antigenically related virus infecting humans, though there is no direct evidence that this is the same organism. There is no evidence that PVM (or its putative relative) causes significant disease in the human population. APVs restricted to birds and has been shown to infect a range of wild and domestic species. RSV is an ACDP group 2 organism. The situation with hMPV is less clear at the moment. It is proposed that the work with wild type hMPV, PVM and APV will be conducted at containment level 2.

It is proposed that specific mutations, expected to produce conditional lethal (primarily temperature sensitive) mutant viruses, will be carried out. Three other types of modification are intended. The first of these involves exchange of specific genes between pneumoviruses. The genes are involved in virus replication and most exchanges are expected to have a detrimental effect on viability. The second is deletion of specific genes, also expected to reduce viability. Both of these categories of experiment and the generation of site-specific mutations will be conducted at containment level 2. The third mutations will involve alteration of genes encoding proteins involved in virus attachment and cell-cell spread and/or cell-cell spread and some involved in eliciting host immune responses. In many cases it is expected that the proposed modifications will result in a reduction in viability of the recombinant virus compared to the parent. It is not anticipated that these mutations will affect the host ranges of the viruses but any modifications involving genes involved in attachment, spread or alteration of the immune system will be carried out at containment level 3. More detail is provided in the attached documents.
The cDNA copy of the virus genome will be cloned into one of several possible plasmid vectors. These will all contain the colE1 origin of replication and be non-mobilisable. The virus genome cannot be expressed in bacterial systems. The bacterial host will be an E. coli K12 strain which is RecA. Commonly used strains will be XL-1Blue and TG2.

Virus will be generated by introducing the plasmid DNA into cells constitutively expressing bacteriophage T7 RNA polymerase (either constitutively or as a result of prior infection with a recombinant poxvirus carrying the polymerase gene). The viruses will be propagated in a range of standard tissue culture cell lines including Hep-2, BHK and HeLa cells. The viruses can only be generated in cells simultaneously expressing the bacteriophage T7 RNA polymerase.

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<tr>
<th>Host/vector system</th>
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<tr>
<td>The molecular clones will be derived from fully sequenced strains of human and bovine respiratory syncytial viruses, pneumonia virus of mice, avian pneumovirus and human metapneumovirus. These clones will provide the genetic backbones for the various alterations to be carried out. They will be used to generate infectious virus following transfection into appropriate permissive tissue culture cell lines expressing the bacteriophage T7 RNA polymerase.</td>
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<tr>
<th>Origin &amp; function</th>
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<tr>
<td>All of the recombinant viruses produced are very unlikely to have altered phenotypes which provide an advantage for survival in the natural environment or to enhance their ability to infect animals or humans. In almost all cases the predicted effect of the alteration(s) to the virus genome(s) will be either to have no effect or to reduce the efficiency of replication and also usually to reduce pathogenicity (with the exception of the PVM experiments identified below). No effect on host range is anticipated for any recombinant viruses.</td>
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<th>Evaluation of foreseeable effects</th>
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<tr>
<td>The initial stage of the work will be to rescue infectious viruses from each of the four pneumovirus cDNAs. These viruses will not contain any mutations but will be identifiable as recombinant viruses because of the novel, non-coding, sequence alterations which result from the cloning process. For purely practical reasons it may be expedient to generate recombinant viruses in which one or more genes from one strain of the same type of virus are inserted into another strain eg humans RS virus genes from one strains into the genome of a different human RS virus to improve the efficiency of the cloning process. These would appear as wild type viruses.</td>
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Subsequently, a range of mutants will be generated. The mutants fall into several classes:

1. Generation of site-specific mutants
   Selected mutations anticipated to induce a conditional lethal (usually temperature sensitive) phenotype, based on characterisation of mutants described in the literature will be selectively introduced into the virus genomes. These mutants will have significantly reduced capacity to replicate.

2. Insertion of genes from homologous viruses
   The genes of a non-pathogenic strain of PVM will each in turn be replaced with the genes of a temperature sensitive pathogenic isolate. The temperature sensitivity of the recombinants will be determined to locate the gene responsible for this phenotype. Any viruses so produced are unlikely to exhibit levels of pathogenicity greater than those found in wild type isolates. The genes of the pathogenic isolate will be replaced with the equivalent gene from the non-pathogenic isolate. This virus will be considerably less pathogenic than wild type isolates. These recombinants will only be grown in tissue culture and will not be used as animal experiments without prior approval of the ACGM at a future date.

3. Generation of deletion mutants of pneumoviruses
   All of the deletion mutants of RSV produced to date are able to replicate both in vitro and in vivo though they are significantly attenuated in their capacity to cause disease. We will investigate the ability of further deletion mutants to replicate to establish the requirements for other virus genes in virus replication for the pneumoviruses. It is anticipated that these viruses will be seriously impaired in their growth characteristics and will almost certainly be significantly attenuated.

4. Insertion of additional genes and non-coding sequences into recombinant pneumoviruses
   We will generate recombinant viruses carrying standard reporter genes such as that for the bacterial chloramphenicaol acetyl transferase, green fluorescent protein luciferase etc. to investigate the limits of the tolerance for carrying additional genes. We will also investigate the possibility of inserting marker genes, such as green...
fluorescent protein, into the coding regions of specific virus genes with the intention of generating molecular tags on on virus proteins. These recombinant viruses will either behave as wild type or be slightly restricted in ability to replicate efficiently.

We will introduce an extra attachment protein gene into the genome of a recombinant virus. This gene will be from a strain shown to be quite distinct from the genetic background of the parental virus. This will establish the level of plasticity of the pneumovirus genome in terms of its ability to support extra virus genes inserted at different locations. We will extend these analyses to generate recombinant viruses carrying additional fusion protein genes to investigate the potential capacity for viruses to carry additional epitopes.

NO RECOMBINANTS CARRYING IMMUNOMODULATORY GENES SUCH AS THOSE FOR CELLULAR CYTOKINES WILL BE PRODUCED IN THIS STUDY.

5. Alteration of the genetic organisation of pneumoviruses

The position of pneumovirus genes relative to the single 'promoter' element is thought to maintain controlled gene expression, and ultimately assembly, of progeny virions. We will disrupt the order of the pneumovirus genes and determine the effects of this. In particular, it will be of interest to investigate the consequence of over expression of the glycoprotein genes and under expression of the N and P protein genes. While it is expected that the permutation of the genome will not destroy infectivity it is likely to result in attenuation of infectivity in vivo.

6. Recombinant viruses carrying genes from other pneumoviruses.

We will construct recombinant pneumovirus genomes in which certain genes have been replaced by their counterparts from other pneumoviruses. Of particular interest will be substitution of the RS virus glycoprotein genes with those of PVM. We would also like to make chimeric viruses in which the genes encoding the major nucleoprotein (N), phosphoprotein (P) M2 and polymerase (L) genes of the pneumoviruses are replaced by their counterparts from other pneumoviruses. We have obtained evidence that these last four genes have different abilities to function in heterologous systems which suggest that recombinant viruses are likely to be impaired in their replication.

Following the analysis above, we would like to generate recombinant N, P, M2 and L genes with specific portions derived from different pneumoviruses to be inserted into recombinant viruses to define the functional domains of the various proteins.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None sought.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For bacterial work all solid waste and glassware is autoclaved at 121 degrees C for a minimum of 15 minutes. The autoclave is validated by thermocouple test annually. Liquid cultures are chemically decontaminated by treatment with 2500 ppm chlorine bleach for a minimum of 2 hours prior to disposal.

Viruses generated and handled under containment level 2 conditions will be handled only in a class 2 microbiological cabinet fitted with two HEPA filters in a designated virus laboratory. The cabinets are tested for microbiological safety annually by an independent company. Solid waste and glassware will be autoclaved as described above. Liquid waste will be chemically decontaminated by treatment with 2500 ppm chlorine bleach for a minimum of 2 hours prior to disposal. Spillages will be immediately treated by flooding the area with detergent (1% Savlon). All pneumoviruses are exquisitely sensitive to detergents and are immediately inactivated. The detergent will be removed with tissue which will be treated as solid waste.

Viruses generated and handled under containment level 3 conditions will be handled only within a class 1 microbiological safety cabinet inside an approved category 3 facility. The containment facility will be run under negative pressure and is sealable for fumigation. Access to the laboratory will be restricted to authorised personnel only. All solid waste from the laboratory will be autoclaved at 134 degrees C for a minimum of 15 minutes, in an autoclave within the containment suite, prior to disposal. The autoclave will be validated by thermocouple test at no less than six monthly intervals. Liquid waste will be decontaminated by treatment with 2500 ppm chlorine bleach for a minimum of 2 hours prior to disposal or by autoclaving.
The committee discussed the extension of the RSV work to include a new human virus, hMPV. The committee has already approved projects to create viruses that recombine genes from human RSV and the related viruses, AVP and PVM.

The committee discussed a risk assessment for extending the project to include the new viruses.

The committee approved the proposal at the same containment levels as for the previous work.

Full minutes of safety committee edited for inclusion on public register.

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### Project Ref 13/03.1

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<td>16/01/2003</td>
<td>GENERATION OF ADENOVIRUS MUTANTS AND VECTORS FOR STUDYING THE ROLE OF AD PROTEINS IN IMMUNE EVASION AND FOR EXPLORING THE APPLICATION IN CANCER THERAPY</td>
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02/03/2022
Project Additional Information

Purposes of the contained use

1. The overall aim of our studies is to investigate the interaction of adenoviruses (Ads) with the host immune system. In particular, we are interested as to how Ads counteract and evade various immune mechanisms. We are concentrating on a set of virus proteins encoded in the early transcription unit E3. Several of these E3 proteins have been shown to function in immune evasion, other E3 functions are still elusive. To investigate the functions of adenovirus E3 proteins, we have used site-directed mutagenesis to introduce (previous work done in Germany) mutations or deletions into the E3 regions of Ad2, Ad3, Ad4, Ad19a to selectively inactivate E3 proteins or to study structure/function relationships. We plan to continue this work and will introduce further mutations or deletions. In some cases, we will also delete or inactivate parts of the E1 region (e.g., E1B 55K and E1B19K) to see the combined effect on apoptosis induction.

2. In addition, we intend to establish new Ad vectors for gene/cancer therapy. We have identified an Ad that efficiently infects professional antigen presenting cells (APCs). With the corresponding vector we should be able to transfer well-characterized tumour antigens (non-oncogenes) that can be recognized by cytotoxic T cells into these APCs. Thereby, we hope that we can stimulate an immune response against tumours carrying the corresponding tumour antigen. These vectors may be utilized in cancer therapy to stimulate an effective immune response to tumor antigens. Moreover, we will test the influence of E3 proteins on such immune responses. We will also test whether or not these new vectors can directly infect and possibly destroy certain tumour cells after replication or upon introduction of an apoptosis-inducing system.

Recipient or parental organism

1. Manipulation of the adenovirus genome is done in E. coli K-12 strains DH10B or DH5.

2. Ads are human viruses that are either endemic or widespread. They cause acute and persistent infections and stimulate lifelong immunity. Some serotypes have been explored as replication-competent or replication-deficient vectors for gene and cancer therapy in humans. Both types of vectors will be generated in our programme. The mutant viruses will contain deletions, specific mutations or may have incorporated a tumour antigen (see below).

3. E3 genes of the various Ads are cotransfected together with an antibiotic resistance gene (neomycin or hygromycin) into 293 cells and other eukaryotic cells to generate permanent cell lines expressing wild-type or mutated E3 proteins (Class 1 work).

Host/vector system

Mutant adenoviruses are constructed by recombination in E. coli K-12 strains. An antibiotic resistance gene is incorporated into the virus genome by in vitro transposition. Subsequently, the virus genome containing the antibiotic resistance gene is recombed with a plasmid containing the left and right end of the relevant Ad genome. The cloned backbone and used to transfect 293 cells to propagate the virus.

Origin & function
1. The constructed Ad mutants only contain specific point mutations or deletions, but no insert. Thus, we are generally not introducing any foreign material except in some cases where short pieces of DNA (restriction sites) or a tag, eg FLAG (artificial octapeptide) or HA (noneptide from flu hemagglutinin) may be inserted for convenient detection.

2. In the Ad vector constructs marker genes are derived from bacteria (B galactosidase), jelly fish (green fluorescent protein) and/or firefly (luciferase). To express these marker genes the expression cassette will contain additional elements (promoter, polyA region) from SV40, cytomegalovirus and/or human tissues (promoter, growth hormone poly A).

3. The tumor antigens inserted will be selected on the basis of their recognition by T cells. To achieve the proof of principle we will concentrate on well-characterized tumor antigens, such as MAGE 1-3 (Melanoma associated tumour antigens) of mouse, human or rat origin.

**Evaluation of foreseeable effects**

Ads are human viruses that are either endemic or widespread. They cause lifelong immunity and some serotypes are used for gene and cancer therapy in humans. Ads do not replicate in animals, except perhaps in cotton rats. We are introducing mutations that should arise in nature. As we are inactivating or deleting potential immune evasion genes, it is expected that these viruses are better recognized by the host immune system. As a consequence, these mutant viruses are presumably less virulent/partially defective in vivo. In summary, the mutant viruses do not pose a greater risk but rather a lower or equal risk to that of wild-type Ads.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None sought

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Modification of plasmid DNA is carried out in a containment level 2 laboratory. All solid waste and soiled glassware is autoclaved at 121 degrees C for a minimum of 15 minutes. Paper waste and other porous materials are autoclaved at 134 degrees C for 5 minutes. The autoclave is validated by thermocouple testing once every three months. Liquid cultures are chemically decontaminated by treatment with 2500 ppm chlorine bleach for 12 hours before disposal.

Virus work will be carried out in a containment level II laboratory within a class II microbiological safety cabinet. All infected waste is autoclaved at 121 degrees C for a minimum of 15 minutes. The autoclave is validated by thermocouple testing once every three months. Liquid waste is chemically decontaminated by treatment with 2500 ppm chlorine bleach for 12 hours before disposal. All growth of virus involves standard laboratory protocols; there are no unusual procedures that require additional containment measures.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N
The project involves the generation of genetically modified adenovirus strains. This virus is a human-specific pathogen that is ubiquitous. None of the modifications were considered to increase the potential risk from the virus. In some cases mutations would mean the virus would be defective and could only propagate in specific tissue culture cells, i.e., 293 cells. Other engineered deletions and point mutations probably already occur in the natural populations and so can be expected not to increase the risk from the virus. Therefore, the constructs were considered to have either identical or reduced risk. In some cases insertions are to be made, i.e., GFP, β-galactosidase, FLAG, HA nonepeptide and tumour antigens (non-oncogenes). The inserts are very well characterised and the GMSC thought it extremely unlikely they could potentiate the pathogenicity of the virus. Therefore, containment at the same level as the unmodified virus (Containment level 2) was considered appropriate. As the virus cannot infect non-humans environmental impact was considered no greater than the unmodified virus.

**Project Containment**

<table>
<thead>
<tr>
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<th>Glass Houses</th>
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**Project Ref 13/07.1**

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<th>Project notified under transitional arrangements</th>
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<tr>
<td>27/02/2007</td>
<td>Generation of recombinant vesiculoviruses.</td>
<td>Class 2</td>
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**Tick if notifying a connected programme of work**

Withdrawn

N

**Project Additional Information**

 Withdrawn

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Purposes of the contained use

The aim of the project is to investigate the interaction of vesiculoviruses with their host cells, at the level of individual virus proteins. The ultimate aim of the work is to extend our knowledge of the ways negative-strand RNA viruses subvert the host cell, and of the mechanisms of persistence of arboviruses in their insect hosts.

Recipient or parental organism

The cDNA copies of virus genomes cloned into plasmids cannot be expressed in bacteria, or in tissue culture cells unless the bacteriophage T7 RNA polymerase is simultaneously expressed. Consequently, recombinant viruses can only be created under controlled laboratory conditions. The parental organisms are the vesiculoviruses, CHPV and ISFV. The viruses cause usually sub-clinical infections in humans, and require an insect vector (not endemic to the UK) for efficient transmission. CHPV and ISFV are both handled at containment level 2. See attached document.

Host/vector system

The cDNA copy of the genome will cloned in a non-mobilisable plasmid vector which cannot be expressed in E.Coli; the host will be a recA-strain such as DH5alpha. Virus will be produced by introducing the plasmid, along with plasmids encoding the viral RNA polymerase complex, into mammalian cells which constitutively express T7 RNA polymerase. Progeny viruses will be propagated in standard cell culture lines including monkey (BS-C-1 and vero), human (Hep-2, 293, A549) and insect (Drosophila S2, C6/36) lines.

Origin & function

Parental strains will be molecular clones of the prototype strains of Chandipura (CHPV) and Isfahan (ISFV) viruses. Three types of recombinant viruses will be made: (1) mutations in the parental viruses; (2) addition of reporter genes; (3) exchange of whole or partial genes between viruses. None of these modifications are expected to increase the ability of the viruses to infect humans or animals, or to survive in the environment, above those of the parental viruses. Most of the alterations are designed, and are likely, to make the viruses less fit. As both parental viruses require an insect vector for efficient onward transmission from infected hosts, it is extremely unlikely that recombinants would lose this vector requirement.

(1) Mutations will be introduced into viral proteins in order to determine the resulting phenotype in terms of virus growth and interaction with host cell proteins. These mutations may be point mutations (some already known to be conditional lethal mutations), or deletions of partial or whole protein coding regions. In some cases short epitope tags (e.g. FLAG, hexaHis) will be added to a viral protein, to facilitate further analysis such as purification of protein interaction partners. All these changes are likely to reduce virus fitness if any effect is seen, and in the case of deletions, to result in defective viruses.

(2) Additional genes will be inserted into the virus which encode a standard laboratory reporter such as chloramphenicol acetyl transferase, luciferase, green fluorescent protein etc. These will enable rapid visualisation and titration of the GMM. These additions are expected to either have no effect on virus fitness, or to reduce it, depending on the site of insertion of the additional gene. The reporter gene products themselves have no known toxicity.

(3) Individual genes will be exchanged between CHPV and ISFV to allow determination of the molecular basis of host-range restriction seen in ISFV. Since parental CHPV already has a very broad host range, recombinants are likely to have a reduced host-range. To enable mapping of functional domains in CHPV proteins, parts of the gene thought to encode functionally relevant domains will be exchanged with the equivalent domain from ISFV. This approach allows the recombinant protein to retain its approximate conformation and can be more useful than a traditional deletion approach. There is no intention to introduce immunomodulatory genes into the viruses.

Evaluation of foreseeable effects

It is very unlikely that the alterations to CHPV or ISFV will make them more pathogenic to humans. Most of the alterations are designed, and are likely, to make the viruses less fit. As both parental viruses require an insect vector for efficient onward transmission from infected hosts, it is extremely unlikely that recombinants would lose this vector requirement. The vector does not occur in the UK (Phlebotomus spp.). There is no evidence for human-to-human or animal-to-animal transmission of these viruses in the absence of the vector.

In some constructs genes will be replaced (in whole or in part) by those from other vesiculoviruses. For genes encoding proteins involved in viral RNA synthesis (replication and transcription; N, P and L proteins) the likely outcome will be to reduce the replicative capacity of the recombinant virus and no hazards are foreseen. For those constructs in which the gene encoding the protein (G) involved in cell attachment/fusion is exchanged between vesiculoviruses, it is unlikely that this will increase the potential host range or pathogenicity of the recombinant viruses. CHPV infects a very broad range of cell types in culture, including human, other mammalian, avian and...
insect cells, suggesting that its receptor is very widely distributed. ISFV is more restricted in that its replication is greatly diminished in certain cell types; this is not simple host-range restriction, as ISFV grows in some human cell lines, but not in others. Replacement of CHPV genes with corresponding ISFV genes is therefore predicted to reduce the host range of the recombinant, if any effect is observed. Furthermore, ISFV and CHPV have essentially the same host ranges in whole organisms. The only animals which show severe consequences of infection are infant mice and hamsters, in which both CHPV and ISFV are lethal. It is very unlikely that recombinant viruses would have any broader host range than this.

The viral M protein is partly responsible for counter-acting the host immune response to infection. Alterations in, or exchange of, the M gene are therefore expected to produce recombinants with reduced ability to resist the immune response, and so which would be attenuated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For bacterial work, all solid waste and glassware is autoclaved at 121 C for at least 15 min. The autoclave is validated by thermocouple test annually. Liquid cultures are chemically decontaminated by treatment with 2500 ppm chlorine bleach for a minimum of 2 hours prior to disposal. Both these treatments give a 100 % kill for standard lab strains of E. coli.

For viruses generated and handled under containment level 2 conditions, all manipulations of the viruses will take place in a class 2 microbiological safety cabinet which contains two HEPA filters on the exhaust. The cabinet is in a designated virology laboratory, and is tested for microbiological safety annually by an independent company. The waste will be autoclaved or chemically treated as described above to ensure that no viable organisms are present prior to disposal. The manipulations will be carried out by trained staff and written records of all experiments will be maintained. Spillages will be immediately treated by flooding the area with detergent-based disinfectant (1% Hibicet). All vesiculoviruses are lipid-enveloped, and so highly sensitive to detergents. The detergent will be absorbed onto tissue which will then be autoclaved as for solid waste. Autoclaving, chlorine treatment and Hibicet treatment all lead to 100 % kill of vesiculoviruses.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Minutes of the Genetic Manipulation safety Committee held on 23rd November 2006 relevant to the notification:

GMSC 688
Recombinant vesiculoviruses expressing reporter genes.
Introduction of reporter gene (GFP, CAT, luciferase and/or SEAP) into the virus genome, either as a separate transcription unit, or as a fusion protein with one of the viral proteins, in order to monitor replication and spread of the virus by detecting the reporter protein. Reporter fusion proteins will be used to monitor distribution of virus proteins within infected cells.
Chandipura virus falls into hazard group 2. Unmodified viruses usually cause sub-clinical infection of humans, sometimes low-grade fever in the case of Chandipura virus. Human-to-human transmission has not been reported, and the insect vector does not occur in the UK. Inserted genes are likely to reduce replicative capacity of the viruses, and reporter proteins have no known toxicity.
Environmental Risks: Consequence: Low; Likelihood: negligible; Overall: effectively zero.
GMSC Decision: Containment Level 2.

GMSC 689
Chimeric recombinant vesiculoviruses.
One or more genes of Chandipura virus will be replaced with the corresponding gene(s) of the related Isfahan virus, or with a chimeric gene composed of parts of the two viruses. Purpose is to study function of the virus proteins in the replication cycle.
Chandipura and Isfahan viruses fall into hazard group 2. Unmodified viruses usually cause subclinical infection of humans, sometimes low-grade fever in the case of Chandipura virus. Human-to-human transmission has not been reported, and the insect vector does not occur in the UK. The modifications are likely to reduce replicative capacity of the viruses.
Environmental Risks: Consequence: Low; Likelihood: negligible; Overall: effectively zero.
GMSC Decision: Containment Level 2

Environmental Risks: Consequence: Low; Likelihood: negligible; Overall: effectively zero.
GMSC Decision: Containment Level 2

Project Containment

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Project Ref 13/07.2

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<td>13/03/2007</td>
<td>Generation and analysis of recombinant hepatitis C virus. This will involve the cloning and modification of cDNA, recovery of virus by transfection of cells in culture and analysis of virus replication.</td>
<td>Class 3</td>
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<td>Non-GMM</td>
<td>Consent Granted</td>
<td>Yes</td>
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Date Project Ceased 02/03/2022
Purposes of the contained use

The broad aim of this project is to better understand the replication of hepatitis C virus. The relatively compact hepatitis C virus genome encodes all the proteins and contains the required genetic elements to replicate in a suitable cell line. We are particularly interested in the structure and function of RNA elements that are involved in the replication process. A better understanding of HCV replication may enable novel therapeutic methods to be developed to limit virus infectivity or spread in the infected host.

Recipient or parental organism

The parental organism is hepatitis C virus, an ACDP level 3 human pathogen. At the time of application this virus only undergoes a full replication cycle in the human hepatoma Huh7 cell line. Therefore virus can only be recovered from cDNA under controlled laboratory conditions and can only be cultivated in a specific cell culture system. The virus cDNA is contained in plasmids is under transcriptional control of a phage T7 promoter. In the absence of exogenously supplied T7 polymerase there can be no transcription and hence subsequent expression of HCV.

Host/vector system

The plasmid vector system is derived from pUC. It is therefore non-mobilisable. It is maintained in standard disabled laboratory strains of E. coli (DH5a, JM101 etc.). Virus production will follow in vitro T7-mediated transcription and transfection of Huh7 cells in culture. Progeny virus will be propagated in Huh7 cells. Huh7 cells are a permanent cell line defective in interferon production. They are the only cell culture system in which hepatitis C replication has been reproducibly demonstrated.

Origin & function

The parental genetic material is hepatitis C virus. Initially studies will be based on the genotype 2a JFH-1 infectious cDNA and virus recovered from this clone (Wakita et al., Nature Medicine 11, 791-796). Future studies will include additional genotypes as they become available in other laboratories. The JFH-1 system reproducibly produces infectious virus in Huh7 cells that can be passaged onto fresh cells. Mutations will be introduced into the cDNA into regions we suspect are involved in controlling virus replication. In particular we are interested in short stem-loop structures that are located in the core protein-encoding and NS5b polymerase-encoding regions. Mutations will be introduced to disrupt these structures and duplications of the structures will be introduced elsewhere in the genome. None of the changes are in regions of the virus implicated in influencing the primary tropism of the virus - they are not in the E1 or E2 envelope-coding regions. Mutations will include deletions, substitutions and short insertions.

A similar range of mutations will be introduced into a sub-genomic replicon system in which the structural proteins of HCV are removed and replaced with a reporter gene (luciferase) or an antibiotic coding gene (conferring G418 resistance). The sub-genomic replicon is transcribed in vitro using bacteriophage T7, the RNA transfected into Huh7 cells, and replication ‘monitored’ by an increase in luciferase activity, or the outgrowth over time of neomycin-resistant colonies in cell culture. The sub-genomic replicon cannot transfer from cell to cell and cannot encode infectious virus.
Some of the substitutions to be introduced will be of analogous regions of other genotypes of HCV. More divergent sequences are not included within the scope of this proposal i.e. the sequences are all derived from hepatitis C virus.

Studies from a number of laboratories have shown that disrupting this type of cis-acting replication elements (that are the targets of this project) is either neutral or deleterious to virus replication. There is no reason to suspect that the mutations to be introduced will enhance replication above that of the parental virus, or that the changes will increase or otherwise influence the tropism or pathogenicity of the recovered virus.

**Evaluation of foreseeable effects**

Hepatitis C virus is exclusively a human pathogen. Transmission is almost exclusively parenteral, with some limited evidence for sexual transmission. There is no evidence for aerosol or faecal-oral transmission of the virus. None of the changes proposed - if they yield viable virus at all - are expected (or designed) to alter the cell, tissue or host tropism of the virus, or influence the route by which the virus can be transmitted. The majority of the changes are expected to be deleterious to virus replication, and to reduce or abrogate virus fitness. There is no reason to expect that the planned changes will reduce the immune response to the virus, which clears infection in about one third of cases. Instead, a less fit virus may be more susceptible to immune clearance.

No vaccine is available to prevent HCV infection. Antivirals - currently ribavirin and interferon, with protease inhibitors shortly - are of limited benefit and lead to viral clearance in a proportion of cases.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For bacterial work, all solid waste and glassware is autoclaved at 121°C for at least 15 min. The autoclave is validated by thermocouple test annually. Liquid cultures are chemically decontaminated by treatment with 2500 ppm chlorine bleach for a minimum of 2 hours prior to disposal. Both these treatments give a 100 % kill for standard laboratory strains of E. coli.

Cell culture work not involving recovery or propagation of viable virus e.g. transfection and analysis of hepatitis C virus sub-genomic replicons, will be conducted in a level 2 tissue culture laboratory. All cells will be handled in a class 2 microbiological safety cabinet with HEPA-filtered exhaust. Waste is autoclaved or chemically inactivated prior to disposal. All staff handling replicon-transfected cells are trained.

Cell culture studies involving the attempted recovery and propagation of infectious hepatitis C virus will be conducted in a level 3 laboratory. Virus and infected cells will be handled in class 1 microbiological safety cabinets by personnel who have been specifically trained and approved to work in a level 3 facility. All biological waste is inactivated by treatment with a quaternary ammonium detergent and waste is autoclaved within the facility prior to disposal. Virus storage is in vapour phase, liquid nitrogen, within the level 3 facility.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Minutes of the Genetic Manipulation Safety Committee meeting held on January 19th 2007 relevant to the notification

GMSC 695 Professor David J. Evans
Cloning and mutagenesis of hepatitis C cDNA
This project aims to dissect the role of structured RNA elements in the replication of hepatitis C virus. The study will involve the manipulation (using standard molecular approaches in E. coli host/vector systems) – by deletion, modification and insertion – of stem-loop structures previously predicted by bioinformatic analysis. The virus cDNA cannot be replicated, or the proteins expressed in E. coli. Expression requires in vitro transcription by bacteriophage T7 polymerase, transfection into a permissive human hepatoma cell line (only one of which has reproducibly been shown to support replication) in which translation occurs. Translation involves cellular factors absent from bacterial cells. The disabled nature of the host, lack of mobilisation or conjugative features of the vector reduce the possibility of environmental risk to effectively zero.

Access 10-6 Expression 10-6 Damage 10-6 Total 10-18 GMSC Decision: Con. Level 1

Environmental Risks: Consequence: negligible; Likelihood: low; Overall: Effectively zero.

GMSC 696 Professor David J. Evans
Analysis of Hepatitis C virus replication using sub-genomic replicons. The aim of this project is to dissect the role of structured RNA elements in the replication of Hepatitis C virus. Functional analysis of the modified stem-loop structures will be undertaken using a hepatitis C virus sub-genomic replicon system. These consist of a virus cDNA from which the coding region for the core, E1 and E2 structural proteins have been deleted and replaced with an antibiotic (or enzymatic) selection marker. Following transfection of susceptible cells with RNA generated in vitro replication can therefore be monitored by an increase in antibiotic resistant colonies, or enzymatic activity. Due to the absence of the structural proteins the sub-genomic replicons is incapable of making virus particles and cannot be transmitted from cell to cell. Replication only occurs in the Huh7 human hepatoma cell line. Protein expression depends absolutely upon replication in one particular human hepatoma cell line. The sub-genomic replicons alone are incapable of being transmitted from cell to cell. Virus pseudotypes can be generated by co-infecting a replicon-containing cell with a suitable helper virus. However, replication would be restricted to a single passage. In the absence of exogenously added helper virus Huh7 cells are known not to contain any viruses capable of pseudotyping the sub-genomic replicons, and are not known to contain any adventitious agents. The requirement for Huh7 cells is due to the exquisite sensitivity of the replicon to the presence of interferon – Huh7 cells are defective in interferon production. Therefore, transfer to the environment is extremely unlikely, and if it did occur then the likelihood of the genome replicating would be very small. Finally, in the absence of any cell-to-cell transmission, damage would be restricted to a single recipient cell. Environmental Risks: Consequence: negligible; Likelihood: negligible; Overall: Effectively zero.

GMSC 697 Professor David J. Evans
Replication of hepatitis C virus – studies using replicating virus system.
The aim of this project is to analyse the role of conserved RNA structures in the replication of hepatitis C virus. Our in vitro and in silico studies have identified a number of stem-loop structures that we believe are implicated in fundamental aspects of virus replication. We propose to modify these structures by mutagenesis, deletion and substitution analysis and determine the effect upon the replication of the virus.

The following points were noted and discussed by the committee:
The project will involve mutagenesis of pre-existing stem-loop structures with the intention of disrupting the structure without altering the encoded protein. The majority of the changes will therefore involve synonymous substitution. It is also proposed to introduce a second copy of the stem-loop structure at an alternative position within the genome – either in a non-coding region, or at a proteolytically cleaved junction of the polyprotein. In these cases the insert will be a 'self' sequence, derived from the same or closely related genotype of HCV.

Changes are expected to have a deleterious effect upon virus replication. All the changes DJE has already constructed in HCV sub-genomic replicons are either detrimental, or have no detectable effect upon replication. None enhance the aspects of replication we are currently able to quantify (i.e. genome copy number per cell).

Furthermore, all the stem-loop structures of interest are located in the region encoding the non-structural proteins of HCV – specifically the NS5b RNA dependent RNA polymerase. There is no evidence that this protein specifically alters the host range of HCV. The virus only replicates efficiently in Huh7 liver cells in culture due to exquisite sensitivity to the presence of interferon – Huh7 cells cannot produce interferon.

Regarding susceptibility to antiviral drugs where treatable, HCV responds to interferon and ribavirin. There is no reason to expect the substitutions to be made to the polymerase will enhance resistance to either of these treatments.

Proposed categorisation: Hepatitis C virus only infects humans. Parenteral transmission to chimpanzees – but not other non-human primates – has been demonstrated, but the virus is cleared and the infected animals only present with transient symptoms (enhanced ALT levels indicative of liver damage). Virus clearance is by the acquired immune response.
Hepatitis C virus is an ACDP level 3 pathogen. GMSC Decision: Con. Level 3.
Environmental Risks: Consequence: severe; Likelihood: negligible; Overall: effectively zero.

**Project Containment**

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**Animal Units**

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**Project Ref 13/13.1**

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<td>01/05/2013</td>
<td>Elucidation and manipulation of antibiotic biosynthetic pathways in Burkholderia species</td>
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02/03/2022
Project Additional Information

The project will use molecular genetic approaches to study antibiotic biosynthetic pathways and to produce antibiotic analogues in Gram-negative opportunist bacterial pathogens belonging to the Burkholderia cepacia complex, and other Burkholderia species classified as ACDP as Hazard Group II biological agents (therefore excluding HG3 Burkholderia including B. mallei and B. pseudomallei).

Prof. Greg Challis at Warwick University and Prof. Esh Mahenthiralingam at Cardiff University have recently identified antibiotics as products of cryptic polyketide biosynthetic gene clusters in members of the Burkholderia cepacia complex. These antibiotics have novel anti-Gram-negative activity against multi-drug resistant strains like: Burkholderia dolosa, Burkholderia multivorans or Acinetobacteria baumannii. The activity against B. dolosa and A. baumannii is particularly significant. B. dolosa is a pathogen that is known to cause chronic infections in cystic fibrosis patients that are associated with accelerated loss of lung function and decreased survival. A. baumannii is resistant to most antibiotics and has emerged as one of the most troublesome pathogens for healthcare institutions globally. It has been implicated in a diverse range of infections, including of the respiratory track, blood stream, skin and soft tissue and kills tens of thousands of U.S. hospital patients each year.

The above findings suggest that such antibiotics may be promising leads for the development of novel drugs to tackle potentially life-threatening infections, e.g. B. dolosa in CF sufferers and A. baumannii infections in hospital patients. However, several aspects of the structures of these compounds present challenges for their development as leads for drug discovery. Additionally, the structural and stereochemical complexity of these metabolite hinder the development of concise and efficient synthetic routes, making the preparation of analogues for structure-activity relationship (SAR), metabolism and toxicity studies via conventional synthetic approaches impractical.

Professors Challis and Mahenthiralingam are developing an alternative approach to the production of novel antibiotic analogues for SAR, metabolism and toxicity studies via engineering of the Burkholderia antibiotic biosynthetic pathways. The intention is to delete genes proposed to be involved in the assembly of intermediates in antibiotic biosynthesis. Generated mutant strains will be fed with chemically-synthesised analogues of the intermediates to produce analogues of the antibiotics. Additionally, it is intended to inactivate genes which are proposed to be involved in late steps of antibiotic biosynthesis to generate analogues of the antibiotics directly.

A system for the construction of targeted unmarked gene deletions in the Burkholderia genus will be adapted from the method described by Flannagan et al. (Flannagan, R. S.et al.; Environmental Microbiology 2008, 10, 1652-1660). The authors reported the development of a system for the generation of targeted unmarked gene deletions in the B. cenocepacia strain K56 2. This method utilises the homing endonuclease I Scel which cleaves at a specific recognition site in DNA and initiates the repair system facilitating recombination of homologous DNA.

Regions flanking the gene(s) of interest have to be amplified and cloned into a suicide plasmid pGPI Scel. This plasmid encodes for a I-Scel recognition site and transmits trimethoprim (Tp) resistance. Triparental mating is carried out with K56-2 and the constructed plasmid, which is incorporated into the K56-2 genome via homologous recombination. The exconjugants are trimethoprim resistant. The plasmid cannot replicate in B. cenocepacia. If, therefore, the exconjugants are trimethoprim resistant, a recombination event must have occurred and the resistance gene is located in the genome, with the cloned flanking regions. A second triparental mating is carried out to introduce a second plasmid pDAI Scel into the K56-2 single cross over insertion strain. This plasmid contains the endonuclease gene I-Scel, expressed constitutively, and transmits tetracycline resistance. I-Scel causes a break at the I-Scel site and a recombination event occurs either restoring the parental DNA, or causing a gene deletion, depending on the site of the crossover. The exconjugants are sensitive to trimethoprim since the recombination event removes the Tp resistance gene, and they are resistant to tetracycline. The correct recombination is screened by PCR and positive clones are grown without antibiotic selection which results in loss of the pDAI-Scel plasmid.

Burkholderia DNA will also be incorporated into broad-host range plasmid vectors (with antibiotic selection markers such as tetracycline, chloramphenicol, kanamycin, trimethoprim) capable of replication in a wide range of Gram-negative bacteria like pUCP vectors, pSCOSBC1/PA1 vectors, pMLBAD, or other from the peer-reviewed...
published literature which all work for various Burkholderia species. After construct preparation in E. coli, these recombinant vectors will then be used for genetic complementation procedures such as the re-introduction of wild-type DNA into a corresponding Burkholderia mutant. Genetic complementation will also be used to examine expression of the cloned DNA. The broad host range vectors used for this procedure can only be introduced by electroporation and are not self-mobilizable between bacterial species.

Recipient or parental organism

- Bacteria belonging to the Burkholderia cepacia complex, e.g. Burkholderia ambifaria AMMD and other ACDP hazard group 2 Burkholderia species.

Host/vector system

Hosts:
- Escherichia coli SY327 strain; ACDP Group 1 microorganisms.
- Escherichia coli HB101 strain; ACDP Group 1 microorganisms.

Vector system:
- pGPI-SceI - suicide donor plasmid incapable of replication outside of E. coli hosts, but capable of transient transfer by conjugal mating procedures; carries I-SceI cut site and trimethoprim (Tp) resistance.
- pRK2013 - encodes genes required for DNA transfer during conjugal mating, carries kanamycin resistance.
- pDAI-SceI - expresses the I-SceI nuclease (yeast homing endonuclease) under the control of the constitutive P1 promoter, carries tetracycling resistance and can replicate in different Gram-negative bacteria.

Origin & function

DNA will derive from Burkholderia cepacia complex, e.g. Burkholderia ambifaria AMMD and other ACDP hazard group II Burkholderia species. In-frame deletions of genes proposed to be involved in antibiotic biosynthetic pathways will be carried out to determine their function. Prepared constructs that will contain fragments of Burkholderia genomic DNA in pGPI-SceI vector will be mobilized from E. coli back into Burkholderia species. Single colonies of generated transformants will be selected to identify correct mutants by genetic analysis. To determine if these disrupted genes play a role in the antibiotic biosynthesis, the wild-type gene will be cloned back into the mutant strain and restoration of the parental phenotype will then be examined.

Evaluation of foreseeable effects

Bacteria belonging to the Burkholderia cepacia complex, e.g. Burkholderia ambifaria AMMD and other ACDP hazard group II Burkholderia species are capable of causing opportunistic human infections. Possibility that a healthy laboratory worker will be infected is low and therapy is available to treat any potential infections. The likelihood of escape of bacteria from the laboratory or transfer to susceptible individuals is low given the level of containment and disinfection procedures employed during handling of these bacteria (including waste handling processes). Escherichia coli cloning strains to be used in the project have no associated hazard. The vector systems to be used carry no associated hazard. The recombinant Burkholderia strains will not present a pathogenicity hazard greater than the original host organism. The GMM is unlikely to disseminate from the research laboratory as it will be handled using ACDP containment level II procedures in a suitable CL2 laboratory. Other organisms will not be infected as no vectors capable of self-transfer to other organisms will be used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste:
- Autoclaving 121 for 75mins (minimum hold time 15 minutes)
Liquid waste:
Autoclaving 121 for 25mins (minimum hold time 15 minutes)
Disinfectant to be used, exposure time and working concentration:
Metal items stand or soak in 1% aqueous solution Virkon (contact time should never exceed 10 minutes).
All contact must be followed by rinsing with water.
Hard surfaces outside of the MSC2
Spray or wipe with 1% aqueous solution Virkon using spray, mop or brush. Rinse/wipe with water if required after 10 minutes. Remove white deposit after Virkon solution has dried with paper towel.
Spillages
Small spills 0.2ml confined to a small area with no splashing can be cleaned with paper towel soaked in disinfectant as for surface disinfection.
Larger spills (up to 10ml) confined to a small area with no splashing can be contained with paper towel and covered with Virkon powder and left for 3 minutes or until all liquid is absorbed. Scrape up waste and place in biohazard bag and then wipe down area with 1% Virkon.
Major spillage Evacuate laboratory and follow Standard Operating Procedure for cat 2 laboratory
Surface disinfection in MSC2
Trigene (Distel) is used as 1% aqueous solution for cleaning of internal surfaces of the safety cabinet and equipment.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The University GMBSC approved the project as AC2, subject to notification to the HSE.

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Project Ref 13/14.1

Date Ackn'd CU2 Project Title

Class CultureVolumeClass2 CultureVolumeClass3-4
A connected programme of work on the characterisation of virulence determinants from pathogenic bacteria of humans and animals on a range of ACDP category 2 pathogens in one laboratory and store room.

Work in the Division focuses on bacterial pathogenesis and genomics, with a particular emphasis on identifying and characterising virulence factors, resistance determinants and potential vaccine and drug targets from bacterial pathogens from ACDP category 2. Our work involves genetic manipulation of pathogenic and commensal strains that colonise or infect humans and other animals (including invertebrates) and the cloning, expression and characterisation of determinants from these strains in other strains or species (usually crippled laboratory strains of Escherichia coli or Bacillus subtilis).

The following is an overview of the GM research activities that form the connected programme of work:

Activity 1. Cloning of unexpressed DNA fragments from Category 2 bacteria into non-mobilisable vectors in laboratory strains of E. coli or B. subtilis. No attempt will be made to express the foreign DNA.

It is often necessary to clone genes or DNA fragments of interest from class 2 pathogens into class 1 disabled laboratory strains of bacteria such as E. coli and B. subtilis to enable subsequent analysis such as DNA sequencing or to enable further constructs to be engineered (Activities 2, 3, 4 and 5). This involves cloning of DNA fragments without any attempt to express them. Procedures within this class would not incur the expression of functional elements. We will exclude from this assessment deliberate cloning of intact soluble toxin genes.

Activity 2. Expression of bacterial genes in a disabled bacterial host.

It is often necessary to clone genes or DNA fragments of interest from class 2 pathogens into class 1 disabled laboratory strains of bacteria such as E. coli and B. subtilis for heterologous expression. This enables gene function to be analysed independently of the other source host phenotypes. Such expression strains may be used for challenges to host models (activity 6), in vitro enzyme assays or protein purification for structure/function studies.

Activity 3. Creation of mutants in hazard group 2 bacterial pathogens.

Allele replacement is a general approach for the creation of mutants in bacteria in which a wild-type gene is replaced by a mutant gene or is disrupted or removed entirely from the bacterial genome. This approach relies on the introduction of mutated non-replicating DNA (e.g. in a suicide plasmid, a low copy unstable plasmid or PCR.
The mutated DNA then integrates into the chromosome by homologous recombination, sometimes augmented with phage-derived recombinase functions (e.g., lambda red) or by group II introns that have been introduced to the cell on a plasmid. If complete ablation of gene function is required, the mutated DNA may contain a copy of the target gene disrupted by an antibiotic resistance gene to provide a selectable marker. However, with modern approach to mutagenesis, in the final GMO, the wild type allele is often replaced with a mutant allele without leaving any antibiotic resistance gene in the cell. In some cases, to monitor gene expression under different environmental conditions, allele replacement will be used to create a transcriptional or translational fusion to a marker gene/protein that is highly unlikely to have any biological effect (e.g., beta-galactosidase, luciferase, green-fluorescent protein). In a similar vein, random mutagenesis is sometimes performed used transposable elements that carry selectable markers or reporter genes to disrupt bacterial genes. Example plasmids that may be used for this activity include pPS856, pSIM5, pFLP2, pRedET, pDS and pLOF series integration plasmids.

Activity 4. Expression of bacterial genes in wild-type strains of bacteria.

The phenotypic effects seen when a gene is disrupted by allele replacement may result from polar effects on transcription downstream, or from other artefacts. In these circumstances it may be necessary to complement the mutation in trans to determine whether the original wild-type phenotype can be restored, and thus any phenotype in the mutant ascribed to the intended mutation rather than to some other cause (this approach is sometimes called “molecular Koch's postulates 2 (Falkow S (1988) "Molecular Koch's postulates applied to microbial pathogenicity." Rev Infect Dis 10(suppl 2):S274-S276). Similarly, to determine the localisation or secretion of a protein, it may be necessary to create and express a fusion to neutral marker or to a domain from another secreted protein. In both cases, this means controlled expression of the gene of interest on a vector that can replicate in the pathogen. The gene is usually cloned first in a crippled strain of E. coli (activity 1 above), and then transferred into the pathogen. This means expressing the gene of interest on a “shuttle vector” that carries origins of replication that work in both E. coli and the pathogen. Although usually the gene under study will have originated from the strain in which it is being expressed, on occasion it may be necessary to express genes from one strain in another strain of the same species. For example, when dissecting function, it may be fruitful to determine whether expression of a homologous but non-identical protein from another strain or species can complement a mutation or elicit a similar phenotype.

Activity 5. Expression of category 2 bacterial genes in yeast cells and in cultured animal and human cells.

Expression of bacterial proteins within eukaryotic cells is necessary for two purposes. Firstly, this approach is useful in identifying and characterising conserved eukaryotic targets of bacterial virulence factors, by identifying the impact of these proteins on cell survival and growth and by determining localisation of fusions to reporters such GFP. Secondly, the yeast two-hybrid system provides a convenient genetic screen for protein-protein interactions.

Activity 6. The use of cultured animal and human cells and invertebrate models of infection.

Well characterised and immortalised animal or human cell lines will be challenged with either wild-type or genetically modified strains of the pathogens described in this assessment. This approach is useful for assessing modes of action and virulence strategies of pathogens. For example we may wish to monitor the ability of a GFP labelled pathogen to invade cultured cells. Such co-culture experiments will all be performed in the class II laboratory and we have a dedicated tissue culture incubator for this. We also plan to use certain invertebrates as model infection hosts. Specifically; Acanthamoeba spp., insects such as Manduca sexta and Galleria mellonella and the nematode Caenorhabditis elegans. Both the amoeba and nematodes are maintained on solid agar plates or in small-scale liquid culture and require no special incubation conditions. Insect host models will be infected by direct injection of low doses of the pathogen using a hypodermic syringe. In these cases training in the procedure will be given before any worker is allowed to perform this. It should be noted that we have previously devised an operating procedure for this that minimises any risk of accidental needle-stick injury. The use of insect hosts in this manner has been previously approved by the UoW GMBSC.

Recipient or parental organism

The recipient bacteria will include crippled laboratory strains of E. coli and B. subtilis. In addition we will also perform various manipulations on the ACDP 2 pathogens themselves.

All the wild-type parent strains that we propose to handle and to potentially genetically modify fall within ACDP category 2. The pathogens can be classified into three overlapping groups according to potential routes of infection:
Respiratory pathogens, where the chief hazard is aerosolisation leading to inhalation. These include:
- Haemophilus influenzae and related species
- Streptococcus pneumoniae and related species
- Staphylococcus aureus and Staphylococcus epidermidis
- Mycobacterium bovis BCG
- Acinetobacter spp (principally A. baumannii)

Gastrointestinal pathogens, where the chief hazard is ingestion. These include:
- Escherichia coli, Salmonell enterica related Enterobacteriaceae (but excluding STEC and typhoidal serovars)
- Bacillus cereus and related species (excluding B. anthracis)
- Campylobacter jejuni
- Helicobacter pylori
- Clostridium difficile and Clostridium perfringens (excluding tetani or botulinum)
- Listeria monocytogenes

Pathogens of soft tissues (including opportunistic pathogens), where the chief hazard is needle-stick injury or infection of wounds or splashes on to mucosal surfaces. These include:
- Staphylococcus spp, including S. aureus and S. epidermidis
- Bacillus cereus and related species (excluding B. anthracis)
- Photorhabdus spp, and related species
- Acinetobacter spp (principally A baumannii)
- Pseudomonas aeruginosa and related species
- Veillonella spp

Host/vector system

Summary of proposed vectors and plasmids described in the six GMO related activities. The bacterial host strains will be ACDP category 1 or 2 as discussed above and the type of vector system will depend on the activity class as detailed above.

Activity 1. Commonly used non-mobilisable plasmids that can allow cloning without expression. Including; pUC-series (in non-lac promoter orientation), pBR322 and derivatives, Large insert vectors, Fosmids, Cosmids and BACs.

Activities 2 and 4. Non-mobilisable vectors that allow expression and reporter fusions (e.g. HIS-, FLAG-, MBP-, CBP-, GST-, GFP-, RFP-, YFP-, LacZ). Including; pUC, pGEX, pET, pBAD and the pCAL series plasmids and pTrcHis2-Topo, pACYC184 and pMTL derivatives.

Activity 4. Unstable or specific host replication dependent integration/suicide vectors Including ; pS856, pS1M5, pFLP2, pRedET, pDS and pLOF series integration plasmids.

Activity 5. Non-mobilisable Eukaryotic host expression plasmids that allow expression and reporter fusions. Including; E. coli -yeast shuttle vectors pYEp13, pYXplac22 and pSEY18 and the yeast two-hybrid vector pDEST22. Mammalian expression plasmids for transient expression (up to 48 hours) in non-replication-permissive cell lines including pRK5myc, pcDNA-DEST47/53 (which contain a conditional SV40-dependent origin of replication).

Origin & function

The origin and intended functions of the genetic material involved will depend upon the activity (1-6): this is set out in detail in the following section.
None of the bacteria we will use represent hazards to the environment. Although most of our work will make use of crippled laboratory strains and non-mobilisable vectors, we will need to use wild type-strains to study the effects of defined chromosomal mutations on virulence and other phenotypes. The 6 activities that we will employ involve standard molecular bacteriology techniques with a track record of substantial safety experience within the research community in the UK and elsewhere. It should be noted that where any cloning vectors or expression plasmids are used in microbial GM work, any specific antibiotic resistance genes employed will be made highlighted to Occupational Health and to researchers should medical attention be required. The lists of bacteria above mainly refer to specific species, although it should be noted we also include the qualifier "and related species" in several cases. We stress that this does NOT include and will not generate any organism that is classified as hazard group 3. Any work with "related species" that have not specifically been named in the above lists will be assessed through UoW procedures prior to work commencing.

Below we provide a detailed description of unexpressed DNA fragments from category 2 bacteria into non-mobilisable vectors in laboratory strains of E.coli or B. subtilis. No attempt will be made to express the foreign DNA.

This work involves commonly used well-characterised disabled laboratory strains from commercial or academic sources (e.g. DH5alpha, JM109, Top10, MG1655 for E. coli, 168, 1012 for B. subtilis, Lactobacillus lactis NZ9800). Cloning vectors would include commonly used non-mobilisable plasmids such as the pUC-series, pBR322 and derivatives, Fosmids, Cosmids and BACs. The bacterial host strains are generally considered incapable of survival in humans or the environment on account of absence of colonisation/virulence factors, auxotrophy etc.

This work involves cloning of DNA fragments without any attempt to express them. In rare cases, genes may be expressed from their own promoters. However, even if potential virulence factors were expressed, there is only a low risk they they will be targeted for secretion or cell-surface expression, particularly as we will exclude any deliberate cloning of intact soluble toxin genes.

We use a number of commonly used well-characterised non-mobilisable or mobilisation-defective vectors from commercial or academic sources. There is negligible risk they they will alter the existing traits of the disabled laboratory host to render it more virulent and a negligible risk they inserted sequences will be transferred to another organism.

ACTIVITY 2: Expression of category 2 bacterial genes in a disabled bacterial host.

It is often necessary to clone genes or DNA fragments of interest from class 2 pathogens into class 1 disabled laboratory strains of bacteria such as E. coli and B. subtilis for heterologous expression.

This work involves commonly used well-characterised disabled laboratory strains form commercial or academic sources (e.g. DH5alpha, JM109, Top10, MG1655 for E. coli, 168, 1012 for B. subtilis, Lactobacillus lactis NZ9800). These strains are generally considered incapable of survival in humans or the environment on account of absence of colonisation/virulence factors, auxotrophy etc.

In this work, we will use non-mobilisable vectors (e.g. pGEX4T3, the pET, pBAD and the pCAL vectors) that express a gene product, either from strong promoters (e.g. P_tac) or from the gene's native promoter in its native form or as part of a HIS-, FLAG-, MBP-tagged, calmodulin-binding peptide-tagged or similar fusion to an innocuous protein (e.g. GST), with a view to purifying and characterising the protein. Even though proteins associated with virulence will be expressed there is only a low risk associated with "damage" because the catalytic subunits of any toxins cannot exert any effect unless delivered in soluble active form to the appropriate compartment of the appropriate cells within the human body. This cannot happen in the context discussed here, as we will ensure that the necessary delivery mechanisms (e.g. a secretion system for getting out of the cell, a binding domain for attaching to cells and mediating entry) are absent from the cloning host for example, we will never express the binding and catalytic domains of a toxin in the same cell.

We use a number of commonly used well-characterised non-mobilisable or mobilisation-defective vectors from commercial or academic sources.

ACTIVITY 3: Creation of mutants in hazard group 2 bacterial pathogens.
This work involves wild-type strains of bacterial pathogens (and their derivatives) that have been assessed as requiring containment level 2 handling.

Example plasmids that may be used for this activity include pPS856, pSIM5, pFLP2, pRedET, pDS and pLOF series integration plasmids.

Antibiotic resistance genes encoded by plasmids and PCR products will be expressed. In most cases, antibiotic resistances will be expressed only transitory during the creation of the GMO, rather than in the final product. However, we will avoid the hazard of creating potential pathogens that are harder to treat than wild type parent strains by avoiding use of resistances to antibiotics that might be used to treat an infection. In particular, we will never manipulate or create pan-resistant strains. In the rare event of infection, the antibiotic resistances and sensitivities of the strain will be communicated to the relevant medical authorities. Any marker genes used will be well-characterised reporters (beta-galactosidase, luciferase, green-fluorescent protein) that have negligible effect on virulence. There is therefore a low risk associated with "expression" and "damage".

Gene deletion or disruption is a naturally occurring process, and so, if a loss of a gene increased the fitness of the pathogen, this would have already been selected for in nature. The disruption of putative virulence genes will thus produce a GMO which will be no more hazardous than the wild-type and which can therefore be handled at the containment level relevant to the wild-type (i.e. containment level 2).

A suicide or other low copy vectors can be propagated in disabled lab strains of E. coli, but will not replicate independently in the target species or strain. When introduced into the target strain a suicide plasmid can survive only by integrating into the chromosome by homologous recombination, while low copy unstable vectors can survive for very few generations after which they have to integrate or are lost. Such vectors will contain a copy of the target gene, disrupted by an antibiotic gene (this construct is created in a crippled laboratory strain of E. coli). Although these vectors are mobilisable, as the genes they carry are disrupted, there is negligible risk that they could cause harm if expressed in another organism. The potential that deletion of genes will enhance the GMM's fitness as a pathogen is considered low to negligible.

Mutagenesis is a naturally occurring process so any disruption or alteration of gene function as a result of deliberate mutagenesis will result in a less fit phenotype, which is likely to be outcompeted by the wild-type if it were to escape into the environment.

Transcriptional fusions will be made only to reporter genes with no conceivable environmental effect (genes for beta-galactosidase, luciferase, or green- or red-fluorescent protein). Any resistance determinants we will use are already abundant in naturally occurring isolates in the environment.

Activity 4. Expression of bacterial genes in wild-type strains of bacteria.

The gene is usually cloned first in a crippled strain of E. coli (activity 1 above), and then transferred into the pathogen. This means expressing the gene of interest on a "shuttle vector" that carries origins of replication that work in both E. coli and pathogen. Although usually the gene under study will have originated from the strain in which it is being expressed, on occasion it may be necessary to express genes from one strain in another strain of the same species.

This work involves wild-type strains of bacterial pathogens (and their derivatives) that have been assessed as requiring containment level 2 handling.

We will use non-mobilisable or mobilisation-defective expression vector including pBR322 derivatives such as pBAD, pTrHis2-Topo,pACYC184, pMTL derivatives. As the goal of this work is to restore wild-type expression genes, potential virulence genes will be expressed from their native or other weak promoters and will be carried on low-copy-number vectors or from a neutral site in the chromosome. The risk associated with "expression" and "damage" is thus identical to that associated with the wild-type parent.

Here we are simply restoring wild-type gene expression and so will produce a GMO that will be no more hazardous than the wild-type gene expression and so will produce a GMO that will be no more hazardous than the wild-type and that can therefore be handled at the containment level relevant to the wild-type (i.e. containment level 2).

Given that we are using non-mobilisable vectors, there is negligible risk that inserted sequences will be transferred to another organisms.
The non-mobilisable vector would not be able to leave the disabled host. Any release of DNA into the environment would be similar to that which occurs during the usual processes of decay. The environmental impact would therefore be effectively zero.

ACTIVITY 5. Expression of category 2 bacterial genes in yeast cells and in cultured animal and human cells.

This work involves expression of bacterial proteins within eukaryotic cells.

Yeast cells present no risks to the experimenter. Well-characterised lines of human and animal cells present negligible risks to the experimenter e.g. HeLa, A549, CACO2 (human colon carcinoma cells), NIH 3T3 (mouse fibroblasts). All of these are standard fully authenticated cell lines that have been safely used in labs for many years or will be purchased from approved tissue culture collections. All therefore count as "especially disabled hosts" according to the SACGM Compendium of Guidance. The use of immortalized cell lines has been approved by the UoW GMBSC committee.

We will study bacterial genes potentially involved in host cell subversion (but which are not oncogenic), fused to biologically irrelevant reporters (short tags, such as the myc tag, or inert proteins such as green fluorescent protein). The genes will have been PCR-amplified and cloned into vectors and propagated in disabled strains of E. coli before introduction into eukaryotic cells (see above for assessment of risks of expressing bacterial genes in E. coli). Bacterial genes may have been subjected to site-directed or truncation mutagenesis so that structure/function relations can be determined. For expression in yeast cells, we will use standard non-mobilisable E. coli-yeast shuttle vectors, e.g. the pYEp13, pYXplac22 and pSEY18 vectors and their derivatives or the yeast two-hybrid vector pDEST22. Constructs will be engineered to express foreign proteins (usually as reporter fusions) under an inducible yeast promoter. For expression in mammalian cells, we will use standard mammalian expression plasmids such as pRK5myc, pcDNA- DEST47/53. Although they contain a conditional SV40-dependent origin of replication, we shall be using them only for transient expression (up to 48 hours) in non-replication-permissive cell lines. They will rapidly be lost upon cell division. It is possible though exceedingly rare, i.e. it is very unlikely, for these vectors to be capable of chromosomal integration in mammalian cells, and they are no transmissible from one cell to another (i.e. "non-mobilisable"). The promoter is the human CMV constitutive promoter. Proteins will, at most, be expressed in nanogram quantities within the mammalian cells. The risk associated with expression and damage is thus effectively zero.

Transfected mammalian cells present no risk to human health, as they are incapable of survival outside of tissue culture media and are unable to colonize the worker. The transfected constructs are not transmissible and cannot replicate within normal human cells.

ACTIVITY 6. The use of cultured animal and human cells and invertebrate models of infection.

Well characterised and immortalised animal or human cell line will be challenged with either wild-type or genetically modified strains of the pathogens. We also plan to use certain invertebrates as model infection hosts. Specifically; Acanthamoeba spp., insects such as Manduca sexta and Galleria mellonella and the nematode Caenorhabditis elegans. Both the amoeba and nematodes are maintained on solid agar plates or in small-scale liquid culture and require no special incubation conditions. Insect host models will be infected by direct injection of low doses of the pathogen using a hypodermic syringe in a previously devised operating procedure that minimises any risk of accidental needle-stick injury. The use of insect hosts in this manner has been previously approved by the UoW GMBSC.

The amoeba will be handled as a class ll pathogen in itself and we already have approval by the Warwick GMBSC to handle this organism. The nematode C. elegans is commonly used for laboratory studies and is not considered hazardous and therefore presents no risk to the experimenter. The insect hosts are larval forms that have limited mobility and in any case will be contained in disposable plastic-ware (no adult forms will be used). They present no hazard to the experimenter. Well-characterised lines of human and animal cells present negligible risks to the experimenter e.g. HeLa, CACO2 (human colon carcinoma cells) and NIH3T3 (mouse fibroblasts). All of these are standard fully authenticated cell lines that have been safely used in labs for many years. All therefore count as "especially disabled hosts according to the SACGM Compendium of Guidance.

Any GM bacterial strain challenged against the tissue culture or invertebrate models will not be able to transfer genetic material to the eukaryotic host. Given that we
are using non-mobilisable vectors, there is negligible risk that inserted sequences will be transferred to another organism.

Infected tissue culture cells present no risk to human health (beyond that of the pathogen itself described in the other activities), as they are incapable of survival outside of tissue culture media and are unable to colonize the worker. Use of immortalized cell lines has been approved by UoW GMBSC. Invertebrate host models will all be contained and have been previously approved by UoW GMBSC.

The non-mobilisable vectors used in any of the GM class 2 pathogens would not be able to leave the eukaryotic hosts. Any release of DNA into the environment would be similar to that which occurs during the usual processes of decay.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste:
Standard sterilisation of contaminated plastics and sharps when discarded into appropriate autoclavable jars, sharps bins or purpose-made autoclave bags before being transported. Items will be sterilised at 121°C for 15 minutes at 15 psi.

Liquid waste: Items will be sterilised at 121°C for 15 minutes at 15 psi.
Contaminated glassware washed in disinfectant prior to cleaning for re-use.

Disinfectant to be used, exposure time and working concentration: Activated chlorine solutions (such as chloros) at 1,000ppm v/v with 12 hour exposure time will be used as it is widely accepted to be lethal for these organisms when used freshly diluted and not inactivated by organic matter. Freshly prepared disinfectant which releases chlorine dioxide (such as Tristel) will be used at 1,000 ppm available in experiments that could produce spores. In addition to this treatment, all potential spore-containing waste will also be autoclaved.

Source of validation data: manufacturer's data and UoW studies

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GMBSC approved the risk assessment of the connected programme of work as Activity Class 2 noting the exclusion of any work with Hazard Group 3 micro-organisms and that the programme was subject to notification to the HSE.

Project Containment
Project Ref 13/15.1

Date Ackn'd 08/01/2015

CU2 Project Title A connected programme of work to understand the physiology, pathogenicity and antibiotic susceptibility of the Mycobacterium tuberculosis complex

Date Project Ceased

Class Class 3

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4 < 1 Litre

Non-GMM Consent Granted Yes

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Mycobacterium tuberculosis is one of the biggest causes of morbidity from a bacterial agent world-wide. We want to investigate the physiology, antibiotic susceptibility and pathogenicity of Mycobacteria. In particular we are interested in the role of nutrient uptake, metabolism and incorporation of nutrients into key biomolecules and signalling networks. The research will involve the use of M.tuberculosis, an ACDP HG3 species, and the related ACDP HG2 Mycobacterium species M.bovis BCG and M. marinum which will mainly be used to determine key differences in metabolism and uptake in non-pathogenic Mycobacterium strains compared with the pathogenic M. tuberculosis. M. tuberculosis is the only member of the Mycobacterium tuberculosis complex that can provide information regarding pathogenicity, virulence, the uptake of nutrients and metabolism with regard to susceptibility to new anti-microbials. Safer strains such as M. bovis BCG and M. marinum and are not such good models due to large differences at the genome level and differences in their pathogenicity to humans.

This project aims to identify new targets which can be developed for new anti-TB therapy and diagnostics. The following is an overview of the GM research activities that form the connected programme of work:
Only pure cultures of M. tuberculosis and other members of the Mtb complex - mainly M. bovis BCG and M. marinum will be used. The physiology and metabolic pathways of M. tuberculosis complex will be studied using a variety of techniques which will include the generation of deletion mutants, conditional gene deletion mutants, complementation of characterized mutants, overexpression of selected ORFs. All Mycobacterium strains will be obtained from ATCC and from the Cole Lab at the Global Health Institute, École Polytechnique Federal de Lausanne, Switzerland.

Recipient or parental organism

Host mycobacteria used are Mycobacterium tuberculosis (both the lab adapted strain H37Rv, and strains Ra, 18b, CDC1551). The work may at some stage include the use of clinical stains of M. tuberculosis but will not include multi-drug resistant strains. Other host mycobacteria will include M. bovis BCG (attenuated vaccine strain) and M. marinum M (can cause infection in fish – optimal growth temperature is 30°C).

Host/vector system

Vector systems that will be used are routinely used in mycobacteria research. These will include pMV261, pMV361, pVV16, p1NIL, p2NIL, phAE87, phAE159, pGEM15, pJG1100, pFRA, cosmid vectors. Mycobacterial phage vectors will also be used (to include phAE180, MycoMarT7).

Origin & function

The genes studied in this work will derive from mycobacterial species: M. tuberculosis, M. bovis BCG, M. marinum along with other pathogenic and non-pathogenic mycobacteria. The genes may be involved in nutrient uptake, metabolism, signaling, intracellular survival, in vitro growth and cell wall biosynthesis and assembly. The aim of this work is to establish the functions of genes involved in these essential processes in M. tuberculosis either through gene deletion, or by introduction of the gene into the host and observing/characterizing the phenotypes of these mutant strains.

Evaluation of foreseeable effects

• Exposure of humans to M. tuberculosis by inhalation or contact with contaminated aerosols could occur in the event of a breach of containment such as the spillage of viable culture. Active tuberculosis or a latent tuberculosis infection could result. It is unlikely that infection would result from any such exposure to M. bovis BCG or M. marinum. M. bovis BCG is the vaccine strain of M. tuberculosis and represents a very low risk to human health. M. marinum will infect fish but is only of risk to severely immunocompromised human individuals.

• All of the vectors are in common usage for TB research. None of the vectors are likely to transfer genetic material to any other environmental microorganisms. The genes which will be transferred or deleted in this investigation belong to specific targeted functional classes the expression or loss of expression of which is highly unlikely to influence the pathogenesis of the hosts. Expression of antibiotic resistance cassettes for selection use resistance to antibiotics (eg kanamycin, gentamicin or streptomycin), which are not used in the clinical treatment of TB.

• The selection strategy will specifically exclude the use of genes likely to be associated with pathogenesis functions such as in the secretion of antigens (eg RD1, ESAT-6, CFP10) or with the production of virulence factors, toxins or known allergens.

• For the reasons outlined above the risk to humans from exposure to the GMMs is highly unlikely to be greater than exposure to the original parental strains. None of the deletion mutants, overexpressing strains or other manipulated strains are likely to be any more or any less pathogenic than the parental strains. The GMM’s are expected to exhibit the same level of pathogenicity as the parental strains and will therefore be handled under the same level of containment (M. tuberculosis at CL3) and (M. bovis BCG & M. marinum at CL2) and with the same stringent handling and disposal procedures as the unaltered parental strains as described in the available associated SOPs and COPs (available on request).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All the project GMO’s are microorganisms.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation from the containment levels specified for this programme.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Disinfectant: Tristel is currently the most efficacious disinfectant against Mycobacterial species. Tristel is a disinfectant based on near instantaneous generation of chlorine dioxide by mixing 2 'safe' components and is the disinfectant recommended by numerous agencies for use in inactivating Mycobacterium tuberculosis and other Mycobacterial species including M.avium-intracellulare, M chelonae and M.terrae. and is compatible with a wide range of laboratory materials and surfaces - plastics, glass and stainless steel. Tristel comes in a variety of different forms for use for different applications of which Tristel One Day Concentrate (T1DC) is the version selected for lab use allowing for many different disinfection applications.

Sources of validation data: The manufacturer’s data and data from T1DC use in a number of mycobacteria labs (Ref: http://www.tristel.com/wp-content/uploads/2012/07/Tristel-for-Labs-Brochure-Jun-2007-Rev011.pdf). Because the disinfectant is versatile we will also validate our methods by in-house testing based on British Standard EN1276 (Ref Anon (1997) "BS EN1276 Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants used in food industrial domestic and institutional areas - test method and requirements", British Standards Institution. Our results will be recorded as the validated methods for disposal of contaminated materials produced in the different procedures in these studies.

Autoclaving: Autoclaving will be carried out in the integral autoclave in the CL3 lab (M126b) using a standard autoclave cycle of 121oC for 20 minutes for simple and liquid loads or 134oC for 5 minutes for dry, complex, loads (disposable gowns, gloves and paper towels). This is the validated treatment to inactivate Mycobacterial strains. All runs are monitored by thermal cycle monitor. All autoclaving will be carried out according to the instructions in the CL3 Code of Safe Working practice for Containment Level 3 Laboratories, document COP_CL004_CL3_2014 (attached).

Autoclaving 121oC for 20 min will be used for waste disposal and culture plates from the CL2 facility according to the instructions in the Code of Safe Working Practice for Containment Level 2 Laboratories, document COP_CL001_CL2_2014 (attached).

Solid waste: All solid waste contaminated with Mtb will be inactivated by autoclaving in the integral CL3 autoclave in M126b. Following successful sterilisation as verified by the run log - the sterile waste is then placed in yellow biohazard bags and disposed of as clinical waste* for incineration. *NB the term 'clinical waste' here is generic for the 'waste stream' for materials to be disposed of by incineration and does not imply any human or animal material content. This will be carried out in accordance with Code of Practice for the CL3 lab. CL2 suite and sterile waste is then bagged as clinical waste* for incineration.

Liquid waste: Liquid waste is inactivated with T1DC – made up according to manufacturer’s guidelines (Ref: http://www.tristel.com/wp-content/uploads/2012/07/Tristel-for-Labs-Brochure-Jun-2007-Rev011.pdf) with a contact time of no less than 5 minutes) OR liquid waste will first be solidified by treatment with Vernagel before being sterilised by autoclaving as described above and the sterile waste then disposed of as clinical waste for incineration.

This will be carried out in accordance with Codes or Practice for the CL3 and CL2 labs.

Laboratory: The floor of the CL3 lab will be cleaned weekly with T1DC or Tristel Fuse, freshly prepared according to the manufacturer's guidelines. Door handles, keyboards and other contact areas are wiped regularly with Tristel Duo Foamer or Contec Prochlor (freshly prepared according to the manufacturer's guidelines). Tristel Products (Tristel Duo, Tristel Fuse and Tristel One day) have been validated by the manufacturer as effective disinfectants for Mycobacterium with exposure times of no less than 5 minutes for Tristel One day and Tristel Fuse, and no less than 30 seconds for Tristel Duo (http://www.tristel.com/wp-content/uploads/2012/07/Tristel-for-Labs-Brochure-Jun-2007-Rev011.pdf).

PPE - Good laboratory practices and rules will be adhered throughout. Hand washing facilities are in place and personnel are trained in hospital hand washing techniques. For the CL3 lab nitrile gloves will be worn at all times and double nitrile gloves when working in the Class I MSC. Back fastening disposable gowns must be worn at all times. All PPE waste will be autoclaved as described above. Safety glasses are to be worn if hazardous chemicals are to be handled. Heat insulated gloves will be worn when handling material from the -80oC freezer and autoclave. Further details of disinfection, autoclave treatments and PPE are provided in the attached Codes of Practice for the CL3 and CL2 labs - documents v. COP_CL004_CL3_2014 and vi.COP_CL001_CL2_2014 (attached).
The project risk assessments, CU2 form, SOPs, initial BSA assessment, were considered by the GMBSC on 25th September 2014. Following the HSE request (letter ref GM13, 28th Jan 2015) revised versions were considered by GMBSC on 25th February 2015. It was confirmed that the project details provided in the revised versions had not changed and the committee considered the project ready for CU2 HSE notification for the CL3 part of the project. The project was confirmed as High Risk Containment level 3 and was approved by the members.

GMBSC Comments: PI's responses to the committee’s comments are appended in blue italicised text.

a) That the Committee required amendments and clarifications to be made to the documentation for Project 136 on the “Connected program of work to understand the physiology pathogenicity and antibiotic susceptibility of the Mycobacterium tuberculosis complex”, noting that the Project required HSE notification and authorisation (via the Health and Safety Department) since the research covered both CL3 and CL2 work.
   1. GMBSC Comment transferred to section 17 (confidential).
   2. That the aims and objectives of the research should clarify how the research will mitigate risk, particularly because the over-expressing or adding of genes generate different risks. The documents have been amended to clarify that no known virulence factor, toxin, allergen will be over expressed in any CL2 or CL3 organisms.
   3. That the SOP for lone working should be revised to clarify exactly what persons can and cannot do in order to remove any ambiguity. Rebecca Allen has provided a copy of the relevant SOP to the Biological Safety Advisor and is included in the supporting documents with this notification form.
   4. That there should be sufficient emergency protocols drafted for handling emergency situations connected to the research. Emergency protocols are provided as part of the general and project-specific SOPs and COPs covering the safe use of the CL2 and CL3 containment facilities.
   5. That the risk assessment and SOPs should be revised and re-submitted to the Biosafety Advisor. Revised versions of these have been provided to the Biological Safety Advisor.
   6. That the preparation of samples could only be conducted under a separate Principal Investigator if there was a project amendment in place which incorporated that element of work. Point noted - any request for changes to the project as described in the RAs will be sent to the GMBSC using the Project Amendment Notification form.
   7. That the Designated Individual for HTA had confirmed that the project did not contain material of human origin. Point noted - any change to the project that might involve any material of human origin or use of human volunteers will be notified to the DI for HTA (Prof Davey).
   8. That all relevant SOPs and revised forms should be returned to the Biosafety Advisor who would help complete the notification to the HSE. All requested SOPs and revised documents have been sent to the Biological Safety Advisor.
   10. That work must not be carried out until both HSE and GMBSC authorisation had been granted. No project work has started pending HSE and GMBSC approval.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L2</td>
<td>L2</td>
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<td>L3 Yes</td>
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Page 510 of 15326
### Project Ref 13/15.2

**Date Ackn’d** 25/03/2015

**CU2 Project Title** Quaternary amine degradation in Proteus mirabilis

**Class** Class 2

**CultureVolClass2** < 1 Litre

**CultureVolumeClass3-4**

**Non-GMM** Consent Granted

**Withdrawn** N

**Tick if notifying a connected programme of work** N

### Historical Significant Changes

### Historical Date of Additional Info

### Significant Change ID

### Date of Significant Change

### Project Additional Information

**Purposes of the contained use**

Investigation of the utilization and role of choline metabolism in growth and swarming motility in Proteus mirabilis. This will be conducted by systematically knocking-out genes involved in trimethylamine production and recomplementation to confirm the function of each gene.

**Recipient or parental organism**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
<td></td>
</tr>
<tr>
<td>E. coli cc118Apr</td>
<td></td>
</tr>
<tr>
<td>E.coli JM109</td>
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</table>

**Host/vector system**

<table>
<thead>
<tr>
<th>System</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
<td>pKNG101 Suicide plasmid, KO genes (see attached list) replaced with kanamycin cassette</td>
</tr>
<tr>
<td>DSMZ 4479 TargeTron</td>
<td>Targeted intron insertion, KO genes replaced with kanamycin cassette.</td>
</tr>
<tr>
<td>pACD4K-C</td>
<td></td>
</tr>
<tr>
<td>pAR1219</td>
<td></td>
</tr>
</tbody>
</table>
pGEM-T Re-complement KO genes with native gene
pBIO1878 lacZ reporter, choline degradation cluster promoter
E. coli cc118pir pKNG101 Plasmid maintenance, stain can replicate plasmid
E. coli JM109 pACD4K-C Plasmid maintenance
pAR1219
pGEM-T
pBIO1878
Plasmid pAR1219 is a pBR322-based vector that expresses T7 RNA Polymerase under control of the IPTG-inducible lac UV5 promoter1 and is intended for use with the commercial TargeTron Gene Knockout System, Catalog Number TA0100 supplied by Sigma-Aldrich. Many TargeTron system plasmids use the T7 promoter for intron expression. By co-transforming plasmid pAR1219 with the TargeTron pACD4 plasmids, the T7 promoter can be used to express the intron and disrupt chromosomal genes in alternative hosts such as Salmonella typhimurium 2 and Shigella flexneri 2. Additionally, chromosomal gene disruptions in non-DE3 strains of E. coli can also be performed using pAR1219 with the pACD4 intron expression plasmids. Ampicillin, at 50 μg/ml, is used to select for pAR1219. To use pAR1219 in conjunction with the pACD4 plasmids, simply co-transform both plasmids and select in a liquid medium containing: 50 μg/ml ampicillin, 25 μg/ml chloramphenicol, and 1% glucose. Glucose is typically included to provide additional suppression of the lac UV5 promoter prior to IPTG-induction.

Origin & function

The aim of this project is to investigate the utilization and role of choline metabolism in the growth and swarming motility in Proteus mirabilis. The investigation will initially centre around the systematic knock-out of Proteus mirabilis genes involved in choline degradation and trimethylamine (TMA) production, followed by the individual complementation of the knocked out genes to confirm the function of each gene. The latter is essentially a 'self cloning' operation since the original Proteus genes will be cloned back into the Proteus KO mutants. The recipient microorganism is an ACDP human hazard group 2 pathogen although as indicated above realistically the risk to human health is only in immunocompromised individuals. The genes for targeted knock-out

The initial operations will produce transgenic Proteus mirabilis strains which are deficient in precisely targeted genes from the following list. In the process of the gene knock out process a functional kanamycin resistance cassette is transferred and used for selection.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene Name</th>
<th>Gene Function</th>
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</thead>
<tbody>
<tr>
<td>PMI2725</td>
<td>tetR</td>
<td>transcription regulator (suppressor)</td>
</tr>
<tr>
<td>PMI2724</td>
<td>transcription regulator: winged-helix DNA-binding HTH domain protein</td>
<td></td>
</tr>
<tr>
<td>PMI2722</td>
<td>microcompartment protein</td>
<td></td>
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<tr>
<td>PMI2721</td>
<td>microcompartment protein</td>
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<td>PMI2720</td>
<td>microcompartment protein</td>
<td></td>
</tr>
<tr>
<td>PMI2719</td>
<td>aldehyde-alcohol dehydrogenase</td>
<td></td>
</tr>
<tr>
<td>PMI2718</td>
<td>microcompartment protein</td>
<td></td>
</tr>
<tr>
<td>PMI2717</td>
<td>cutF alcohol dehydrogenase, class IV</td>
<td></td>
</tr>
<tr>
<td>PMI2716</td>
<td>cutC glycyl radical enzyme - choline degradation</td>
<td></td>
</tr>
<tr>
<td>PMI2715</td>
<td>cutD AE - glycyl radical activating enzyme</td>
<td></td>
</tr>
<tr>
<td>PMI2714</td>
<td>pduJ pduJ propanediol utilisation protein</td>
<td></td>
</tr>
<tr>
<td>PMI2713</td>
<td>pduL pduL propanediol utilisation protein</td>
<td></td>
</tr>
<tr>
<td>PMI2712</td>
<td>transcription regulator</td>
<td></td>
</tr>
<tr>
<td>PMI2710</td>
<td>QacE quaternary ammonium compound resistance protein</td>
<td></td>
</tr>
<tr>
<td>PMI2709</td>
<td>protein-tyrosine phosphatase</td>
<td></td>
</tr>
</tbody>
</table>
PMI3344 pldA Phospholipase, catalyzes hydrolysis of phosphatidylcholine

Evaluation of foreseeable effects

The GMMs will not be any more infectious than the wild type / parental strain. No cases of laboratory-acquired infection from this species has been reported to date. Similarly, no person-to-person transmission has been reported. Infections usually affect surgical sites and the upper urinary tract causing infections such as urolithiasis (stone formation in kidney or bladder cystitis, and acute pyelonephritis). Opportunistic infections can occur in immunocompromised patients, particularly those with indwelling catheters (long-term catheterization) or under frequent antibiotic therapy. The modifications produce transgenic strains deficient in specific targeted genes and these would not be expected to be any more infectious than the starting strain and could be disabled. The knock out strains will have acquired kanamycin resistance from the gene knock-out plasmids. Kanamycin is not an antibiotic commonly used to treat urinary tract infections and there are several effective antibiotics currently used for treating UIs - sulfamethoxazole-trimethoprim; amoxicillin; nitrofurantoin; ampicillin; levofloxacin.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste (agar plates, glassware, plasticware, dry waste): Solid waste collected in biohazard bags supported in metal autoclave containers and then autoclaved at 121°C for 20 min in a validated autoclave before disposal as clinical waste (incineration). Autoclaving under these conditions is adequate to achieve 100% kill of the GMMs described above.

Liquid waste (spent culture media, contaminated solutions and buffers): Inactivated using Presept tablets added freshly to liquid waste to provide 2,500ppm available chlorine (9 x 0.5g tablets (4.5g)/litre) exposed for a minimum of 30 minutes or overnight.

Work surfaces and microbiological safety cabinet surfaces: Wiped down with freshly made up Presept solution (1 tablet in 2 litres water, providing 140ppm available chlorine).

Disinfectant to be used, exposure time and working concentration: at least 30 min at 2500ppm available chlorine. Presept (sodium dichloroisocyanurate) or Clotabs (sodium triclosene), 1% (w/v), at least 30 minutes at room temperature

Source of validation data: (e.g. manufacturer’s data or own studies) risk assessment.

Validation of these data, these details were taken from Safety Data sheets and manufacturers instructions available on-line.


Solids and cultures on agar plates will be autoclaved at 121°C degree for 20min before disposal in ‘tiger’ waste bag as ‘non-infectious waste’ which does not require specialist treatment or disposal.
Project 23 risk assessment is an amendment to an existing non-GM project approved at CL2, to allow the use of gene deletions (gene knockouts), self-cloning and complementation procedures to investigate the role played by specific genes in the degradation of quaternary amines in the ACDP HG2 bacterium Proteus mirabilis.

The project was first considered at a GMBSC* meeting on 25th Sept 2014

Minute (e) That the Committee required further expert clarification whether the amendment to Project 23 on “Quaternary amine degradation” required notification to the HSE as a GM HG2 human pathogen, noting the project involved self-cloning”. (minute 06/14-15 e) of GMBSC meeting of 25th September 2014).

The committee agreed the bacterial host Proteus mirabilis is potentially harmful to human health (HG2) but members felt that the modifications proposed here would be most unlikely to increase the hazard class and the risk to human health. The committee agreed that the GMM was non-hazardous to the environment.

Subsequently Professor Croy contacted HSE and confirmed that notification using the HSE CU2 form was appropriate for this project.

The project GM RA and CU2 form was re-considered at a GMBSC* meeting on the 25th February 2015.

Members considered a need for appropriate validation of the disinfectant method, on the basis that a lower concentration that normal was proposed in the risk assessment for dealing with the disinfection of surfaces. A recommendation was also made to alter the waste disposal from yellow bags to tiger bags following autoclaving, as the waste should no longer require incineration following this process.

The Committee considered the project ready for CU2 HSE notification following review of the validation of the disinfection method (and making any required minor amendment to the risk assessment post this review) and inserting the minor amendment with regards to the waste procedure.

Committee Decision

The project involves genetic manipulation of a HG2 pathogen, is an activity class 2 project requiring CL2 containment facilities and is assigned a University medium hazard level and was approved by members, subject to the following conditions:

1. That the risk assessment is updated to reflect the following conditions:
   • Review of the validation of the disinfection method and revisions being made to the documentation as necessary.
   • Change to the choice of bag for disposal. Post autoclaving the waste material should be classified as offensive waste and placed in a tiger bag, rather than a yellow bag, as this indicates that the material inside is still highly infectious, which is incorrect.

2. The HSE are satisfied with the information provided in the CU2 form.

Professor Andrew Easton
Chair of the University Genetic Modification and Biosafety Committee
Date: 25/02/2015

*GMBSC = Warwick University genetic manipulation and biological safety committee.
Project Additional Information

Purposes of the contained use

Various activities in biological and biomedical research, including but not limited to: expression of known and putative oncogenes, growth factors and cytoskeletal proteins; regulation of gene expression by various methods including RNA interference and the introduction of degrons or repressible promoters; the use of CRISPR/Cas9 systems and single guide RNAs to tag and/or cause breaks in human and animal chromosomes; to induce immortalisation and pluripotency in vertebrate cells; and to administer viral vectors and cells transduced by such vectors to animal models.

Recipient or parental organism

Lentiviral and retroviral vectors based on a range of human and animal viruses, including HIV, EIAV, MMLV, MMTV and MSCV. The split vector systems and packaging
cell lines to be used will conform to designs of second generation or later, and will be demonstrably incapable of replication in humans or the environment. Vectors may have wider tropisms by the use of ecotropic and amphotropic envelope proteins, including VSV-G, and may include either wild-type or mutated forms of the Woodchuck Hepatitis Virus Post-transcriptional Response Element (WPRE).

Host/vector system

Viral vectors will be generated by transfecting all the plasmid components of a split-genome system into packaging cell lines, such as HEK293, or by introducing the backbone vector into commercially-available stable packaging cell lines expressing other components of the viral genome. Viral vectors produced will then be used to transduce a range of human and animal cells. This will include established cell lines from various lineages, mouse embryonic stem cells, and primary human and animal samples, other than those known or suspected to contain agents classified as Hazard Group 3.

Origin & function

As this is a connected programme of work, the genetic material to be introduced will be from a range of origins and have a variety of functions. This will include putative and known oncogenes and tumour-suppressor genes, cytoskeletal proteins, short hairpin RNA (shRNA) molecules intended to inhibit the expression of a range of genes including those or known or suspected to be involved in pathways of oncogenesis, and genes intended to induce immortalisation and the extension of the proliferative lifespan of cells. CRISPR/Cas9 systems may be expressed which are intended to cause double-stranded breaks in DNA, leading to mutation by non-homologous end-joining or insertion of specific mutations or tags with homology-directed repair constructs, but in some circumstances, Cas9 which is disabled for this function will be used to tag or label chromosomes for fluorescence microscopy. This connected programme does not include the use of viral vectors expressing toxin genes or major pathogenesis factors, which will be notified separately.

Evaluation of foreseeable effects

The use of second generation (or later) vector systems in which all accessory genes have been deleted, and where gag, pol, env and rev genes are provided in trans (either on additional accessory plasmids or integrated into the genome of packaging cell lines), means that the risk of production of replication-competent or infectious virus is effectively zero, and there is no documented evidence of replication-competent lentivirus being produced in third-generation vector systems.

However, transduction of a cell with such viral vectors will result in insertion of the viral genome into random sites within the genome of the host cell. While the use of self-inactivating (SIN) viral vector systems, in which promoter and enhancer elements have been removed from the long terminal repeats (LTRs), reduces the risk of transcriptional activation of genes proximal to the insertion site, this could be stimulated by constitutive promoter/enhancer sequences within the inserted genetic material. Insertion of the viral genome could also result in disruption of genes involved in cellular regulation (e.g. tumour-suppressor genes).

The use of WPRE sequences to increase the levels of expressed mRNA is becoming commonplace, especially in lentiviral vectors. Some versions of these elements also express a fragment of the Woodchuck Hepatitis Virus X protein, and there is limited (and controversial) evidence in the literature that this protein is implicated in the formation of hepatocellular carcinomas, albeit when administered systemically at high titres in animal models. It is most common for the intention of the activity to be to take the crude supernatant from a packaging cell line (where the viral titre is unlikely to be higher than 10E+7 particles/ml), and to use that supernatant to transduce a cell culture.

In those circumstances, and in the absence of significant (i.e. >100-fold) concentration of the viral titre and elimination of sharps, it can be argued that the risk of accidental systemic administration, such that the vector is delivered to the liver in sufficient quantity to induce hepatocellular carcinoma, is negligible. Such an activity can therefore be assigned to Class 1 (provided that there is nothing more hazardous in the vector), even in the presence of WPRE expressing protein X.

The inserted genes may also pose specific hazards:
- While oncogenesis is known to be a multi-factorial process, the expression of known or potential oncogenes could act in concert with the insertional effects to contribute to oncogenic pathways.
- The use of systems (shRNA, CRISPR, etc.) to deliberately disrupt tumour-suppressor genes pose a similar level of risk.
- Expression of cytokines and growth factors has the potential to cause inappropriate growth, differentiation or apoptosis of cells, all of which are associated with oncogenesis. Growth factors and cytokines may also be teratogenic, and have other effects on the immune response, so consideration will also be given during the local approval process for risk assessment as to whether expectant mothers and workers with other health issues should be excluded from such work.

All of these events could pose a risk to workers, but only in the event of self-inoculation. This risk will be controlled by eliminating sharps (except where they are required for administration of vectors to animals), and by other measures at Containment Level 2 (documented records of training, laboratories with access restricted to authorised
personnel, and the use of gloves).

All risk assessments for GM activities with retroviral and lentiviral vectors will continue to be subject to scrutiny and local approval by the University Genetic Modification and Biosafety Committee (GMBSC), and the GMBSC will determine if the activity falls within the scope of this Connected Programme. If so, no further notification will be made. The GMBSC will also ensure that the activities are undertaken in approved Containment Level 2 facilities.

The following activities will NOT be permitted within the connected programme of work, and will be the subject of a separate notification:

1. Preparation of high-titre retroviral or lentiviral stocks by methods that require the use of needles to harvest the virus (e.g. density gradient centrifugation)
2. The use of sharps, including hypodermic needles and glass pipettes, OTHER THAN for administration of viral particles to animals
3. Expression of a major pathogenesis factor or a toxin gene.
4. The use of retroviral or lentiviral vectors for which there is evidence for the presence of replication-competent virus.
5. Any other activity which the GMBSC decides constitutes one or more risks that might consign it to a higher Class.

Activities with lentivirus that will be considered as Class 1 genetic modification:

The risks to workers associated with the construction and amplification of the plasmids used to generate the viral vectors are considerably less than with the virus itself, as the passive uptake of circular DNA into human cells is very inefficient, chromosomal integration will not occur, and the plasmids will not be maintained episomally in a dividing cell population. For this reason, work with these plasmid vectors will be assigned as a Class 1 activity, unless the hazardous nature of the inserted gene(s) dictates otherwise.

Furthermore, since the risk to workers from inoculation relies solely on the presence of free viral vector particles, the risk is effectively eliminated by genomic integration step of the life-cycle of the Retroviridae. According to the SACGM Compendium of Guidance, the risks are negligible 'where the cells do not incorporate any helper function and where residual virus titres have been reduced by replacing the potentially infectious cell supernatant medium'. Consequently, cells used after that can therefore be re-assigned to Class 1 GMMs for subsequent use, storage and transport.

Where viral vectors are generated using packaging systems in which the envelope protein is documented as being incapable of infecting human cells (e.g. ecotropic envelope from Moloney murine leukaemia virus), the GMBSC may decide that the activity should be assigned to Class 1, and that notification is not required.

Some aspects of this connected programme will include the administration of lentiviral and retroviral vectors, and cells transduced with such vectors, to GM mice (which represent no increased hazard to human health or the environment compared to the wild-type parental strain).

The animal facility (BSU) has been purpose-built to house rodents and lagomorphs, and was designed in consultation with the Home Office and the HSE. It includes a containment suite capable of operating at CL3, but which is currently run at CL2. Control measures include:

- Rodent barriers to rooms containing mice to prevent escapees exiting the immediate area
- Computerised auditing of the facility rodent population, ensuring absences are quickly detected
- SOPs to monitor for, react to, and record escapes
- The facility and all rooms (including the containment suite) are subject to strict access control
- Staff are trained to handle animal in accordance with Home Office requirements.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste potentially containing lentiviral or retroviral particles will be collected in vacuum traps containing a minimum of 1% Virkon. This will then be disposed of via the sink after a minimum of one hour incubation. Plasticware containing virus packaging cells or transduced cells will be treated with a 1% virkon solution for one hour before disposal OR bagged and sent for autoclaving. All other solid waste will be collected in bags for autoclaving. Autoclaving will be performed for a minimum hold time of 15 minutes at 121°C in autoclaves that
are subject to insurance examination to a written scheme, and are serviced and validated at least annually. Material that has been autoclaved or disinfected is consigned as offensive waste, and sent to land-fill by licensed contractor.

Material that has been autoclaved or disinfected is consigned as offensive waste, and sent to land-fill by licensed contractor.

As this notification relates to a Connected Programme of Work, exemplar risk assessments have been attached.

The risk assessments for Projects 200 and 201 were discussed at the meeting of the GMBSC held on 24th September 2015, having been circulated in advance by email. The following comments were made, which have been incorporated into the attached revised version:
- It is unclear from the information provided whether the Cas9 and the CRISPR guide RNA are being carried on the same vector.
- There should be a greater consideration of the potential for lentiviral vectors to cause mutagenesis by insertion into the human genome, which is theoretically capable of contributing to an oncogenic pathway, although this will not require any higher level of containment than is necessary for work with vectors containing wild-type WPRE sequences.
- The risk to animals in the environment in the event of release of lentiviral vector in Section 6.5 was over-estimated. Taking into account the likelihood of release, the mode of transmission, the replication-defective nature of the viral vector, and the likely survival of the vector in the environment, the Containment Level required to protect human health is adequate to protect the environment.
- There needs to be clarification of how the Class 2 GM materials will be stored safely if they are outside a CL2 lab.
- The waste management and spillage procedures need a little more clarity. Waste that has been treated with Virkon should not be autoclaved subsequently.
- The scientific goals are too far-removed from that of notification 332/07.2 to allow notification of a significant change.

Risk assessments for Project 98 (Amendment) were discussed at the meeting of the GMBSC held on 15th October 2015, having been circulated in advance by email. The following comments were made, which have been incorporated into the attached revised version:
- L2.06 (CL1) is suitable for manipulation and construction of plasmids in E. coli only, due to the reduced likelihood of cellular entry of plasmid DNA, compared to lentiviral vectors.
- The zero sharps policy and necessary PPE and hygiene measures have been identified correctly.
- Waste treatment and spillage processes are appropriate.
- Controls for storage of Class 2 GM materials outside the CL2 lab have been identified.

Please enter comments on the GM safety committee on the risk assessment

As this notification relates to a Connected Programme of Work, exemplar risk assessments have been attached.

The risk assessments for Projects 200 and 201 were discussed at the meeting of the GMBSC held on 24th September 2015, having been circulated in advance by email. The following comments were made, which have been incorporated into the attached revised version:
- It is unclear from the information provided whether the Cas9 and the CRISPR guide RNA are being carried on the same vector.
- There should be a greater consideration of the potential for lentiviral vectors to cause mutagenesis by insertion into the human genome, which is theoretically capable of contributing to an oncogenic pathway, although this will not require any higher level of containment than is necessary for work with vectors containing wild-type WPRE sequences.
- The risk to animals in the environment in the event of release of lentiviral vector in Section 6.5 was over-estimated. Taking into account the likelihood of release, the mode of transmission, the replication-defective nature of the viral vector, and the likely survival of the vector in the environment, the Containment Level required to protect human health is adequate to protect the environment.
- There needs to be clarification of how the Class 2 GM materials will be stored safely if they are outside a CL2 lab.
- The waste management and spillage procedures need a little more clarity. Waste that has been treated with Virkon should not be autoclaved subsequently.
- The scientific goals are too far-removed from that of notification 332/07.2 to allow notification of a significant change.

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- The zero sharps policy and necessary PPE and hygiene measures have been identified correctly.
- Waste treatment and spillage processes are appropriate.
- Controls for storage of Class 2 GM materials outside the CL2 lab have been identified.

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<td>L2 L3 L4 L2</td>
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<td>L2 L3 L4</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>
Project Additional Information

Purposes of the contained use

The majority of work in the laboratory is focused on understanding how plants respond to virulent and avirulent challenges, both in local tissue (leaves) and systemically. We investigate induced defence responses, systemic induced immunity and suppression of plant defence. The majority of the work is done on Arabidopsis thaliana using chemical induced and T-DNA mutants as well as Arabidopsis over-expressing plant or phytopathogen genes either under strong ectopic, specific or conditional promoters. All procedures are standard molecular cloning and transformation. We use Nicotiana benthamiana for transient transformation studies and occasionally tomato, lettuce and banana, but only Arabidopsis is transgenic.

We use a range of pathogens, primarily Pseudomonas syringae pathovars or Xanthomonas campestris pathovars. Pseudomonas syringae pv. tomato DC3000 and pv. maculicola are most frequently used, with DC3000 being the strain we genetically modify, either by deleting effectors or adding effectors in under control of constitutive or inducible promoters. We also routinely use Botrytis cinerea as a necrotrophic pathogen to assess how plants with altered responses to biotrophs respond to this pathogen.

We use information from high temporal resolution microarrays, proteomics and metabolomics to inform on strategies to modify/re-engineer plant defence signaling pathways to prevent pathogen suppression of defence. There is a strong focus on modification of hormone signaling and modification of classical plant disease resistance proteins or pattern recognition receptors, often using bespoke promoters. Golden Gate cloning is used to transform in multiple genes, or biosynthetic pathway components.

A typical experiment would be to use genes identified from microarray studies and other prior knowledge to alter...
the Arabidopsis response to DC3000. We would then investigate how this perturbation occurs using yeast-2 hybrid
to identify interacting partners, over-expression or mutation of the gene of interest, in planta localization and cell
biology and examining the changes in the metabolome. The target is to generate broad spectrum resistance and
this will most likely require crossing lines with modified responses to DC3000 to enhance resistance.

Recipient or parental organism

Pseudomonas syringae is a host pathogen on tomato and a non-host pathogen on Arabidopsis. It is a poor
epiphyte and infection is primarily through wounds or stomata. No strains of P. syringae have been reported to
cause infection or represent any health risk to humans. Transformants would be capable of surviving within infected
plants but not in soil for sufficient time to allow re-infection and transfer to a new host plant. The strains of P.
syringae used are, however, not specifically disabled.
Xanthomonas campestris pathovars have very specific host ranges. There is no risk to humans or animals.

Host/vector system

In P. syringae, plasmid vectors pLAFR3, pVSP61 or pDSK600 in addition to ColE1 will be used. None of the
vectors is self-transmissible but the broad host range vectors are mobilisable via the helper plasmid pRK2013.
Plasmid vectors pDSK600, pVSP61 will be used in X. campestris.

Origin & function

One or more of 30 effector genes from Pseudomonas syringae pathovar DC3000. There will be no expression outside the plant apoplast following infection as these are
tightly controlled by the bacterial Type 3 Secretion System. Gene products are not harmful in isolation.
Effectors identified as differentially present/or polymorphic between virulent and susceptible Xanthomonas isolates. Harm from gene expression is highly unlikely as they
are only expressed in planta in the virulent isolate.

Evaluation of foreseeable effects

No strains of P. syringae have been reported to cause infection or represent any health risk to humans. The possibility that exchange of genes between strains would lead
to significant hazard to human health seems negligible.
The strains of P. syringae used in this work have a very narrow host range (Arabidopsis, Brassica, tomato). Modified P. syringae may in extremely rare cases be
significantly more virulent than the parental strain but coupled with their poor survival rate in the environment and extremely low load in a possible escape and highly
restricted host range, harm to other plants would be expected to be remote.
The likelihood of cloned genes having effects on other microbes in the environment, e.g. soil-inhabiting Pseudomonads becoming pathogenic is unlikely since regions of no
more than 5kb will be cloned and it is known that DC3000 requires type III secretion capabilities before inducing any response in plants.
The likelihood of mobilisation of the plasmid vectors to other Pseudomonas is also remote, and has not been demonstrated in vitro.
Should the transgenic P. syringae have altered pathogenicity this is, therefore, more likely to be manifested only in certain varieties of the plant host. The spread of disease
from heavily infected seed is dependent on the prevalence of rainfall and comparatively high temperatures. Thus in most years plant diseases caused by the bacteria do not
pose a major threat. If the GMM did have enhanced pathogenicity the consequence of release would be at most low and, more accurately, negligible.
Xanthomonas campestris pathovars have very specific host ranges. There is no risk to humans or animals. There is a remote possibility, if this GMM escaped AND came
into contact with on of its very limited hosts, it may infect. In the immediate environment brassica crops would be at risk. However virulence is very varietal specific AND the
GMM is unlikely to enhance this remote possibility.
The work involves examining the function of proteins that are only induced within the plant/host. There is a very specific induction system, so the chances that this GMM
would (i) enhance fitness and (ii) impact the environment is considered negligible. All work with these will never-the-less be undertaken in CL2 facilities.

Some aspects of this programme will include the administration of GM Pseudomonas syringae pathovars to GM strains of Arabidopsis thaliana (which themselves

02/03/2022
represent no increased hazard to human health or the environment compared to the wild-type parental strain). Work with these infected Arabidopsis plants will include transport of GMO material between Growth Facilities. This will be carried out following instructions in relevant SOPs. There is a low risk of seed escape. This risk will be mitigated by use of dedicated lab coats, cleaning all equipment, use of sticky foot mats and filtration of run-off liquid in the PBF (PhotoBiology Facility). The facility is surrounded by gravelled areas, which are regularly treated with herbicides for weed control. Infected plant material will be inactivated by autoclaving at 121°C for a minimum of 30 minutes, ensuring an effective 100% kill.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment in laboratories, growth rooms and glasshouses will be based upon following detailed SOPs. Notably dedicated growth, culturing and inoculation areas will restrict possible pathogen infection.

All solid waste contaminated with GM material [eg. plastic pipettes, plates, small volumes of sealed liquid cultures, gloves, tips etc.] is placed in a rigid container lined with an autoclave bag. These materials are autoclaved on validated cycles ensuring a minimum temperature of 121°C for a minimum hold time of 15 minutes, ensuring an effective 100% kill.

Materials may be initially decontaminated using a validated disinfectant regime prior to autoclaving. Virkon and Jet-5 are effective against both Pseudomonas syringae and Xanthomonas campestris. We have validated the effectiveness of Jet-5 and Virkon in plating experiments carried out at Exeter University in 2008 and 2010. These disinfection methods also work for fungi (Magnaportha oryzae, Botrytis cineria, Alternaria alternata and Trichoderma hamatum). We set a minimum time of 20 minutes soaking in the disinfectant.

Autoclaves are subject to appropriate periodic servicing, validation and insurance inspection against a written scheme of examination. After autoclaving, all waste is placed in Tiger-striped bags, and removed from site by the University’s appointed waste contractor for disposal as offensive waste. All glassware used with plant pathogens is soaked in disinfectant prior to washing. Any spillages will be first taken up with tissue and the contaminated area then cleaned with validated disinfectant (Jet-5).

GM contaminated sharps, including syringes, needles and broken glassware are placed in a sharps bin, autoclaved, then disposed of by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

After extensive consultations between the Principal Investigator and the Technical Assurance Manager and the University Biosafety Adviser, the risk assessment was circulated to the Genetic Modification and Biosafety Committee (GMBSC) for their consideration.

There were no objections to the assignment of the contained use to CLASS 2, with many elements assigned to CLASS 1. Notably, while the GM strains of Arabidopsis thaliana will be infected with Pseudomonas syringae pathovars, these strains are not notifiable in their own right, posing no increased risk compared to the parental unmodified strain.

It is noted that the plant pathogens have been granted a licence by the Plant Health & Seed Inspectorate under the Plant Health Order.

Project Containment
Project Ref 13/16.3

Date Ackn'd 22/09/2016
Date Project Ceased

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

1. To construct models of CF lung biofilm communities using artificial sputum and ex vivo pig lung tissue in order to explore the effects of inter-microbe interactions on infection, virulence, persistence and antimicrobial resistance profile.
2. To use these models to test the clinical potential of existing and novel therapeutics (antibiotics, antivirulence compounds and antibiofilm compounds).
3. To reconstruct anti-infection remedies from pre-modern medical texts and to explore the bactericidal, bacteriostatic and/or antivirulence effects of individual ingredients and whole recipes on a range of soft tissue pathogens in standard lab media, artificial wound models and the ex vivo lung model.
4. To reintroduce unmodified and mutated forms of the deleted genes to the GMMs in trans-complementation assays, followed by analysis in the above models.
### Recipient or parental organism

Parental organisms for the genetic modification are a range of ACDP Hazard Group 2 pathogens which are known or suspected to be involved in the establishment and progression of chronic bacterial infections. The species are transmissible by a number of routes including by aerosol, and come from commercial sources and clinical isolates, with a range of resistances to antibiotics.

GMMs will be applied to ex vivo lung models derived from porcine tissue fit for human consumption, and in in vitro wound models generated from either a) fetal bovine serum, peptone and collagen or b) coagulated blood (not of human origin).

**NB** While these species are listed in the associated risk assessment, amendment to include other HG2 organisms will be considered locally by GMBSC, and will not be considered a significant change provided there is no increase in overall risk.

### Host/vector system

A range of plasmid vectors will be used to deliver the genes of interest, and will include broad-host-range vectors, self-mobilisable vectors, shuttle vectors, suicide vectors, genome insertion vectors, cloning vectors and protession expression vectors with inducible promoters.

### Origin & function

Fluorescent/luminescent reporter constructs, antibiotic resistance markers and transposable elements originating from a variety of species. None known to be inherently harmful. Complementation will also be perfomed by providing unmodified and mutated forms of the deleted genes in trans.

### Evaluation of foreseeable effects

The host species, as Hazard Group 2 agents have the potential to cause disease in humans. In particular, immunocompromised people of any group are at increased risk of infection, most notably those with open wounds, cystic fibrosis, diabetes, or people who are taking broad-spectrum antibiotics or immunosuppressant drugs. *P. aeruginosa* produces toxins (cyanide, exotoxin A) and an allergen (LPS). *S. aureus* produces Toxic Shock Syndrome Toxin-1 and several enterotoxins. Many of the mutants used will be compromised in virulence or persistence, as they will have had virulence factors, biofilm polymers or quorum sensing components knocked out. Apart from the Tn library used for Tnseq, most mutants will be constructed using clean gene deletions or insertion of an antibiotic-resistance marker into the gene of interest. These are unlikely to be overcome, in the latter case because the marked strain will always be cultured in the presence of the selective agent. The genome insertion plasmids insert into the chromosome of a range of bacteria but the conditions for this to occur are very stringent and unlikely to be met outside of the laboratory or by accident, for example the Tn7 derived plasmids require an accessory plasmid to be co-conjugated in order for insertion to occur. The self-mobilisable vectors require a stringent set of conditions that are unlikely to occur outside of the laboratory. Vectors which are required for the expression of a protein will have a regulatory component (wherever possible) so that protein expression is linked to the presence of either IPTG or arabinose, or to species-specific quorum sensing signals, which are unlikely to be found outside the laboratory. The Gram-negative specific transposon mini-Tn5 requires unique conditions for transposition and subsequent mutation to occur. These conditions are likely only to be meet in laboratory environments and are unlikely to occur in the environment, and spread to environmental bacteria is considered highly unlikely.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

SOLID WASTE:-

02/03/2022
Pipettes:
- Plastic pipettes must be discarded into the designated yellow discard. Boxes should be placed in an autoclave bin and removed when full for sterilisation by autoclaving.
- Disposable plastic material:
  - Universals, bijoux, tips and eppendorf tubes etc. must be discarded into a Disposafe jar. Full jars must be transferred into an autoclave tin lined with an autoclave bag and removed for autoclaving.
  - Petri dishes must be placed into an autoclave tin lined with an autoclave bag and removed for autoclaving.
- Contaminated non-disposable items eg flasks, beakers etc
  - Disinfect in 10,000 ppm sodium hypochlorite, dispose of this by the pump/drain system and place items into an autoclave tin without a bag.
- Handtowels and “clean” gloves
  - Handtowels and any gloves that are worn as a second pair underneath outer ones should be placed in the designated autoclave tins lined with an autoclave bag located under the handwash sinks.

The main autoclaves in SLS used for HG group 1 & 2 work are serviced twice a year. Validated loads include porous bagged waste, biobins and petri dishes. They are validated to a cycle of 121°C and 15 psi for 20 mins. Any new waste vessels introduced must be validated before use.

Work surfaces will be cleaned with 1% Chemgene (Medi-Mark Scientific) after each use. Chemgene has been certified by the manufacturer to disinfect Pseudomonas aeruginosa.

LIQUID WASTE:-
- Autoclave as above, or disinfect with sodium hypochlorite to 10,000 ppm chlorine and dispose of via drain.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment was circulated to GMBSC by email on 10th August 2016, and the following specific comments were made:-
- Consideration should be given to listing specific antibiotic resistant strains
- Has sufficient justification been made for the use of sharps?
- Very detailed assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3 L4 L4</td>
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Project Additional Information

Purposes of the contained use

We will induce the expression of exogenous proteins, or alter the expression of endogenous proteins, in the brain in a cell specific manner. These manipulations will be used to:
1) test the role of defined brain cells in specific behaviours and the role of specific proteins in the function of brain cells;
2) functionally dissect neural microcircuits; and
3) manipulate neural control of specific physiologically systems to determine how this affects other systems in the body.

Modifications may arise through:
1) altered excitability of cells;
2) Loss or gain of function; or
3) Cell specific lesions of brain nuclei.

This will be achieved in specific populations of cells in the brain by targeted genetic expression of various ion channels, receptors or toxins. The ion channels and receptors can then be activated in a specific manner (e.g. by light or by specific agonist injection) to achieve either the inhibition or activation of the cell populations. Expression of these proteins may be inducible by expression of other receptors, such as the tamoxifen receptor.

The introduction of these genes may be through transduction with viral vectors, or through breeding of transgenic lines.

Recipient or parental organism

Mice: including GM mice, which present no increased hazard compared to the parental wild-type strain
Rats: wild-type strain
Primary cell culture: including cells from GM rodents, for testing for viral expression in neurons and glia
HEK cells: for testing plasmids and viral constructs
Hela cells: including GM cells, to test the effects of proteins on specific functions
Host/vector system

Adeno-associated viral vectors (all serotypes including modified serotypes such as AAV-DJ)
E1- and E1/E3-deleted adenovirus 5
Lentiviral vector systems (based on HIV)
Plasmids pXcX and p2XcX
AAV plasmids from various sources

Origin & function

Cx26 -ion channel (rodent version; unlikely to result in any harmful effect)
Cre recombinase (bacteriophage enzyme; unlikely to result in any harmful effect)
Flp recombinase (bacteriophage enzyme; unlikely to result in any harmful effect)
Fluorescent proteins (jellyfish; unlikely to result in any harmful effect)
Opsins -ion channel (light-activated; unlikely to result in any harmful effect)
DREADD receptors - (modified receptor only activated by a synthetic molecule, clozapin-N-oxide; unlikely to result in any harmful effect)
Diphtheria toxin receptor (unlikely to result in any harmful effect)
shRNA (inactivating the rodent version of taste receptors; unlikely to result in any harmful effect)
Taste receptors (rodent version; unlikely to result in any harmful effect)
Cell specific promoters such as GFAP, TSH receptor promoter, EF1alpha (Unlikely to result in any harmful effect)
Allatostatin receptor (fruitfly receptor only activated by a non-mamalian ligand; unlikely to result in an harmful effects).
Diphtheria toxin subunit A (Causes apoptosis of transfected cells - may cause harm)
Tetanus toxin light chain (Causes loss of signalling in transfected cells - may cause harm)

Evaluation of foreseeable effects

The expression of toxin subunit genes in adeno-associated viral vectors results in apoptosis, lack of neurotransmitter release or loss of activity of brain cells, and could cause harm in the case of accidental inoculation of workers. However, this risk is minimised by the control measures in place, including the use of tissue-specific promoters of rodent origin, elimination of sharps wherever possible, the use of injection equipment which is distal to the operators' hands, the very low likelihood of the micro-injector pipettes penetrating human skin, and the high levels of operator training.

All viral vectors used in this programme are replication-incompetent, and incapable of forming a productive infection in humans or in the environment.

There is a low risk of third-generation lentiviral vectors inducing mutagenic events as a result of genomic integration.

GM mice will represent no increased risk to human health or the environment, compared to wild-type strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This connected programme will include the administration of viral vectors, and cells transduced with such vectors, to rats and to GM mice (which represent no increased hazard to human health or the environment compared to the wild-type parental strain).

The animal facility (BSU) has been purpose-built to house rodents and lagomorphs, and was designed in consultation with the Home Office and the HSE. It includes a containment suite capable of operating at CL3, but which is currently run at CL2. Control measures include:-
• Animals are housed in sealed IVC systems, minimising risk of escape
• Rodent barriers to rooms containing mice to prevent escapees exiting the immediate area
• Computerised auditing of the facility rodent population, ensuring absences are quickly detected
• SOPs to monitor for, react to, and record escapes
• The facility and all rooms (including the containment suite) are subject to strict access control
• Staff are experienced and trained to handle animals in accordance with Home Office requirements.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
The risk assessment identifies that the injection of virus particles into rodents should be permitted to take place in the BSU outside an MSC. The positioning of, and access to the subject is not achievable in an MSC, volumes of virus involved are very small, and the risks of aerosol are negligible. The overall risk is very low.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid lab waste contaminated with GM material [eg. plastic pipettes, plates, small volumes of sealed liquid cultures, gloves, tips etc.] is placed in a rigid container lined with an autoclave bag. Clear bags are in use at CL1, and red bags at CL2. These materials are autoclaved on validated cycles ensuring a minimum temperature of 121°C for a minimum hold time of 20 minutes, ensuring an effective 100% kill.

At CL2, materials may be initially decontaminated using a validated disinfectant regime (e.g. 5% (v/v) Chemgene solution) prior to autoclaving. Autoclaves are subject to appropriate periodic servicing, validation and insurance inspection against a written scheme of examination.

After autoclaving, all waste is placed in Tiger-striped bags, and removed from site by the University’s appointed waste contractor for disposal as offensive waste. GM contaminated sharps, including syringes, needles and broken glassware are placed in a sharps bin, autoclaved, then disposed of by incineration. Waste in the above streams generated in the BSU is autoclaved within the Unit prior to disposal.

Almost all animals injected with toxins are transcardially perfused with paraformaldehyde post mortem, which inactivates any virus or products generated by the viral inserts, further reducing any risk. As the risk of contaminating GMMs is negligible post mortem, all animal cadavers (in sealed bins) and bedding materials are removed from site for incineration by the University's licensed waste contractor. Post mortem carcases and tissues that have been handled outside the BSU are returned to the BSU for disposal by the same route.

Liquid waste is either autoclaved on a cycle validated to provide an effective 100% kill, or treated with validated disinfectants, prior to disposal via the drains. Residual quantities of viral particle suspensions in tubes, etc, are treated as solid waste. No liquid waste is generated in BSU.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
Y

Please enter comments on the GM safety committee on the risk assessment

This risk assessment was circulated to GMBSC by email on 4th April 2017, and the following specific comments were made:-

1. Clarification requested of appropriate CL2 locations for virus administration in vivo and in cultured human cells.
2. Adenovirus 5 vectors and the HeLa and HEK-293 cell lines to be used should be assigned to HG1/Class 1 not 2 as in the risk assessment, provided they do not contain hazardous gene inserts which may assign them to a higher Class.
3. The Committee agrees with the recommendation to notify this as a connected programme of work.
4. Clarification requested on how separation will be achieved between subject preparation (involving the use of sharps) and the presence of viral vectors which would be harmful to the operator if self-administered.
5. The proposal currently includes shRNA knockdown of unspecified ion channels and receptors. This section should consider the possibility that this might have harmful effects at the individual cell level if an shRNA-expressing vector were to infect a person.
6. It is not accurate to state that ‘All viruses to be used can cause disease in other animals in their wild-type state’. This would actually be exceptional. Both HIV and adenovirus 5 are highly specific for humans, whilst AAV (as stated) doesn’t appear to cause disease in any system.
7. Concern was expressed that the proposal includes the creation and use of viral vectors which express diphtheria toxin A non-specifically that are capable of entering a wide range of human cell types (for lentivirus via the VSV-G pseudotyping, and naturally for AAV and Ad5).

The risk assessment has been amended to address these comments.
## Project Containment

### Laboratory Activities

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### Glass Houses

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### Growth Rooms

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### Animal Units

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### Large Scale Activities

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### Human Clinical Applications

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## Project Ref 13/17.2

### Date Ackn'd

<table>
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<th>Date</th>
<th>09/06/2017</th>
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### CU2 Project Title

Molecular understanding of antibiotic resistance in bacterial cell wall biosynthesis, cell division and tRNA metabolism

### Class Culture Vol Class 2 Culture Volume Class 3-4

<table>
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<th>Class</th>
<th>Culture Vol</th>
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<tbody>
<tr>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>

### Non-GMM Consent

Consent Granted

### Project notified under transitional arrangements

N

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### Purposes of the contained use

Our laboratory studies the mechanism and function of proteins and complexes responsible for the coordination, detection and metabolism of the bacterial cell wall and cell division. These studies include aspects of bacterial metabolism that relate to potential targets for antibiotics in two main areas: bacterial cell wall biosynthesis and aminoacyl-tRNA biosynthesis.

For bacterial cell wall biosynthesis, this includes the proteins directly responsible for its construction and related metabolism, proteins responsible for coordination with bacterial cell division, and signaling proteins related to this area including antibiotic resistance determinants. As part of these studies we recapitulate in-vitro the biosynthetic pathway in order to produce and purify substrate level quantities of the peptidoglycan pathway intermediates.

For tRNA synthetases this includes alanyl, valyl, threonyl and seryl tRNA synthetases from selected bacterial pathogens that require editing mechanisms to prevent...
non-cognate amino acyl-tRNA synthesis. For all of the above areas we may need to create and study the phenotype of in-vivo gene deletions, additions and mutants, in order to more fully interpret in-vitro observations of isolated proteins or protein complexes. For all scientific areas, the translational rational is research that may lead to options for multi-targetting of future and current antimicrobial drugs eg beta-lactams that target multiple penicillin binding proteins.

Recipient or parental organism

Multiple strains of bacterial species classified on the ACDP Approved List at Hazard Group 2, but not those on Schedule 3, Part V of COSHH. All sourced from well-characterised culture collections, and have a range of antibiotic resistance profiles.

Host/vector system

- pET vectors with T7 promoters
- pBAD vectors with arabinose promoters
- pTac and pProEx vectors with tac promotor
- pTet vectors with inducible tetracycline promotors
- High copy number cloning vectors, pUC series, pBluescript
- All vectors are non-mobilisable.

Origin & function

Bacterial housekeeping genes including cell wall biosynthesis genes, Penicillin binding proteins, cell division genes and the wider peptidoglycan biosynthetic pathway that includes protein biosynthetic genes, e.g. tRNA synthetases required in cell wall biosynthesis

Constructs will be made to generate deletions of these genes in HG2 organisms, which will be tested for phenotype.

Genes will also be complemented on plasmids in trans, including related genes from other HG2 and certain HG3 species. None of these will be known or suspected to be toxins or pathogenic determinants. Knockdown will also be achieved using plasmid-based CRISPR/Cas9 technology.

While some of these activities may be defined as self-cloning, they cannot be exempt from the Regulations due to being in HG2 organisms.

Evaluation of foreseeable effects

The expression of these genes is unlikely in humans due to the promoter systems in place, and the likelihood of harm being caused is very low. The resulting GMMs themselves would not be predicted to be any more harmful to human health than the parental wild-type strains, as there are no genes to be expressed that constitute toxins or pathogenic determinants. The most likely effect of gene knockdown, if any, is to reduce viability or pathogenicity. The transfer of genetic material (including antibiotic resistance genes) into related species in the environment is unlikely due to use of non-mobilisable plasmids. None of the genetic modification is likely to confer a survival advantage in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid lab waste contaminated with GM material [eg. plastic pipettes, plates, small volumes of sealed liquid cultures, gloves, tips etc.] is placed in a rigid container lined with a red autoclave bag. These materials are autoclaved on validated cycles ensuring a minimum temperature of 121C for a minimum hold time of 20 minutes, ensuring an effective 100% kill.
At CL2, materials may be initially decontaminated using a validated disinfectant regime (e.g. 5% (v/v) Chemgene solution) prior to autoclaving. Autoclaves are subject to appropriate periodic servicing, validation and insurance inspection against a written scheme of examination. After autoclaving, all waste is placed in Tiger-striped bags, and removed from site by the University’s appointed waste contractor for disposal as offensive waste. Liquid waste is either autoclaved on a cycle validated to provide an effective 100% kill, or treated with validated disinfectants (e.g. 5% (v/v) Chemgene solution, prior to disposal via the drains. Residual quantities of bacterial suspensions in tubes, etc, are treated as solid waste.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment was circulated to GMBSC by email on 17th March 2017, and the following specific comments were made:-
1. There is currently no shaking incubator in that location, but it has been confirmed by the PI that this is not required.
2. The waste practices originally identified are not in accordance with current School practices.

The risk assessment has been amended to address these comments.

Project Containment

<table>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Project Ref 13/17.3

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<td>Construction, growth and experimentation with recombinant adenoviruses and recombinant SV40</td>
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Non-GMM  Consent Granted

Project notified under transitional arrangements N

02/03/2022
### Purposes of the contained use

Adenoviruses and SV40 are amenable to precise genetic manipulation by reverse genetics. This approach is used both to establish the detailed molecular biology of the viruses, typically by targeted disruption of specific viral genome sequences, and to create recombinant viruses capable of expressing a heterologous gene with the purpose of delivering that gene into cells/organisms to achieve its expression - either for experimental studies to dissect virus or mammalian cell biology, or to elicit immune responses (recombinant vaccines), or to alter cell function (gene therapy).

### Recipient or parental organism

- Human adenovirus types 2 and 5 (HAdV-C2, C5)
- Simian virus 40 (SV40)
- E. coli K12 strains
- Human cell lines
- Simian cell lines
- Mouse cell lines
- Hamster cell lines
- Rat cell lines
- Inbred laboratory mouse strains

### Host/vector system

- pAdEasy-1; pShuttleCMV containing insert DNA; pAT153, pML2 or other similar non-mobilizable derivative plasmids containing insert DNA
- pSV4 - clone of SV40 genome in pBR322

### Origin & function

- Reporter genes: luciferase, beta galactosidase, eGFP, RFP and similar derivatives
- HCMV IE promoter/enhancer
- EBV EBNA1 and OriP providing episomal replication in mammalian cells
- Subgenomic or cDNA segments from other adenoviruses, pneumonia virus of mice (PVM)
- HIV-1 rre element and/or Rev gene
- SIVmac p55 Gag or gp120/gp160 Env sequences
- Subgenomic or cDNA segments from mammalian cells, including adenosine deaminase, factor VIII, factor IX, dystrophin plus others but excluding known oncogenes and cell cycle regulators (eg c-myc).

### Evaluation of foreseeable effects

Wild-type Adenovirus types 2 and 5 are classified as HG2, infectious to humans, and transmitted by aerosol. Risk to workers if activities not conducted in MSCs. Many
constructs will be generated which are deleted for combinations of E1a, E1b, E3 and E2/E4 regions, and are likely to be incapable of, or attenuated for replication or infection. The ability of SV40 to cause infection in humans is not well established, although it has an approved classification of HG2, and all constructs will be used at CL2. GM activities will not increase the pathogenicity of the viruses, with many decreasing this activity. In terms of risk to the environment, Adenovirus 2 and 5 are restricted to humans only, and SV40 is unlikely to infect any species that are found in the UK. Inbred laboratory mouse strains pose no greater risk to human health or the environment than the parental wild-type strains, and will only be treated with replication-defective E1-deleted Adenovirus (assigned to Class 1 contained use).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

This programme will include the administration of viral vectors, and cells transduced with such vectors, to GM mice (which represent no increased hazard to human health or the environment compared to the wild-type parental strain). The animal facility (BSU) has been purpose-built to house rodents and lagomorphs, and was designed in consultation with the Home Office and the HSE. It includes a containment suite capable of operating at CL3, but which is currently run at CL2. Control measures include:-

- Animals are housed in sealed IVC systems, minimising risk of escape
- Rodent barriers to rooms containing mice to prevent escapees exiting the immediate area
- Computerised auditing of the facility rodent population, ensuring absences are quickly detected
- SOPs to monitor for, react to, and record escapes
- The facility and all rooms (including the containment suite) are subject to strict access control
- Staff are experienced and trained to handle animals in accordance with Home Office requirements.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None requested

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid lab waste contaminated with GM material [eg. plastic pipettes, plates, small volumes of sealed liquid cultures, gloves, tips etc.] is placed in a rigid container lined with an autoclave bag. Clear bags are in use at CL1, and red bags at CL2. These materials are autoclaved on validated cycles ensuring a minimum temperature of 121C for a minimum hold time of 20 minutes, ensuring an effective 100% kill.

At CL2, materials may be initially decontaminated using a validated disinfectant regime (e.g. 5% (v/v) Chemgene solution) prior to autoclaving. Autoclaves are subject to appropriate periodic servicing, validation and insurance inspection against a written scheme of examination. After autoclaving, all waste is placed in Tiger-striped bags, and removed from site by the University’s appointed waste contractor for disposal as offensive waste.

GM contaminated sharps, including syringes, needles and broken glassware are placed in a sharps bin, autoclaved, then disposed of by incineration. Waste in the above streams generated in the BSU is autoclaved within the Unit prior to disposal. As the risk of contaminating GMMs is negligible post mortem, all animal cadavers (in sealed bins) and bedding materials are removed from site for incineration by the University’s licensed waste contractor. Post mortem carcasses and tissues that have been removed from site for incineration by the University’s licensed waste contractor. No liquid waste is generated in BSU.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
This risk assessment was discussed at the meeting of the Genetic Modification and Biosafety Committee held on 14th July 2016, having previously been circulated by email, and has since been amended in line with the following comments:-

1. Recommend that an active solution is sought to allow administration of these viruses to mice in BSU (and their subsequent holding) to be carried out under suitable containment, rather than CL1 as stated. NB: This has been addressed by clarification that only replication-defective viral vectors assigned to Class 1 will be administered to mice.

2. There is an over-reliance on chemical disinfection of waste materials at CL2, and there should be a greater acknowledgement of the autoclave waste stream for disposable materials. There needs to be a separate paragraph describing the waste procedures within BSU, both for GMMs and infected mice.

3. Spills should not be treated with 70% ethanol other than for small spills.

4. It is unclear from the GMO Public Register whether this activity falls within the scope of an existing notification made under transitional arrangements. NB This has now been clarified and it is not currently covered.

5. New staff/students will require training records to be kept.

**Project Containment**

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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</table>

**Project Ref** 13/17.4

Date Ackn'd 18/08/2017

Date Project Ceased

Investigation of phage therapy of intracellular infection by Staphylococcus aureus and other bacteria in macrophages and human skin fibroblasts

Consent Granted

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Phage therapy is an alternative method to antibiotics for treatment/control of bacterial infections which has nowadays become more important due to the problem of antimicrobial resistance. *Staphylococcus aureus* is a traditional gram-positive extracellular pathogen, responsible for skin and respiratory infections and food poisoning, which has also been shown to be capable of invading host cells, including macrophage cells. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA), which consists a major problem for patients, in combination with the lack of an approved vaccine for *S. aureus*, has made the option of phage therapy very attractive. In this project, we aim to investigate the invasion of *Staphylococcus aureus* in macrophage cells and human skin fibroblasts and the potential of the control of infection using phage targeting *S. aureus*.

While the initial experiments will focus on *S. aureus* and phage K, other HG2 bacterial species and targeting phages may be used within this Connected Programme of Work, without further notification.

**Recipient or parental organism**

Parental organisms for the genetic modification are ACDP Hazard Group 2 pathogens (e.g. *Staphylococcus aureus*) which are known or suspected to be involved in the establishment and progression of bacterial infections. The species are transmissible by a number of routes and come from commercial sources and clinical isolates, with a range of resistances to antibiotics.

GMMs will subsequently be incubated with human cells, including but not limited to macrophages and dermal fibroblasts, to establish the ability of interfering bacteriophages (e.g. phage K) to inhibit the intracellular infection by the bacteria.

NB While the associated risk assessment focusses on initial experiments using *Staphylococcus aureus* and bacteriophage K, amendment to include other HG2 organisms will be considered locally by GMBSC, and will not be considered a significant change provided there is no increase in overall risk.

**Host/vector system**

Bacteria will be transformed with non-mobilisable plasmids

**Origin & function**

Genetic material to be introduced will be fluorescent reporter genes, such as red fluorescent protein from Acropora millepora.

**Evaluation of foreseeable effects**

*Staphylococcus aureus* is an ACDP Hazard Group 2 biological agent, with the ability to cause skin infections and, rarely, invasive infections including pneumonia and sepsis.

The bacterium is prevalent in 30% of the population, but disease is uncommon except in individuals with impaired immunity or skin injury. Infections are treatable with antibiotics.

The genetic modification proposed is unlikely to increase or decrease the level of harm, compared to the unmodified strain.

There is a further low risk posed by the presence of adventitious agents in the primary human cells.

There is a very low/negligible risk to the environment, but the measures to be adopted at Containment Level 2 will ensure that release is prevented.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid lab waste contaminated with GM material [eg. plastic pipettes, plates, small volumes of sealed liquid cultures, gloves, tips etc.] is placed in a rigid container lined with an autoclave bag. Clear bags are in use at CL1, and red bags at CL2. These materials are autoclaved on validated cycles ensuring a minimum temperature of 121°C for a minimum hold time of 20 minutes, ensuring an effective 100% kill.

At CL2, materials may be initially decontaminated using a validated disinfectant regime (e.g. 5% (v/v) Chemgene solution) prior to autoclaving.

Autoclaves are subject to appropriate periodic servicing, validation and insurance inspection against a written scheme of examination.

After autoclaving, all waste is placed in Tiger-striped bags, and removed from site by the University’s appointed waste contractor for disposal as offensive waste.

Liquid waste is either autoclaved on a cycle validated to provide an effective 100% kill, or treated with validated disinfectants, prior to disposal via the drains. Residual quantities bacterial cultures in tubes, etc, are treated as solid waste.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment was discussed at the meeting of the Genetic Modification and Biosafety Committee held on 13th July 2017, having previously been circulated by email.

The Committee were broadly happy with the quality and content of the risk assessment, but there was recognition that the PI would need to identify an appropriate CL2 location for culturing of human cells, which does not generally happen in the location stated.

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
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Project Ref  13/18.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>25/05/2018</td>
<td>Fighting actinomycetoma, a neglected tropical human disease</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>
Actinomycetoma is a neglected tropical disease, and can be caused by the pathogenic species Streptomyces somaliensis (HG2) and Streptomyces sudanensis (HG2). The aim of this project is to genetically modify these two species in order to study the pathogenicity mechanisms that enable them to cause disease. Putative genes involved in pathogenicity will also be cloned and expressed heterologously in alternative hosts. GMMs will be tested in a pathogenicity model using the larvae of the greater wax moth (Galleria mellonella).

Recipient or parental organism

While not classified specifically by ACDP, the recognised ability of Streptomyces somaliensis and S. sudanensis to cause actinomycetoma in humans would indicate a provisional assignment to Hazard Group 2.

These species are transmissible by a number of routes, including by aerosols. Lesions usually occur on the extremities, most often on the feet. They appear as localized swollen nodules that slowly enlarge. Multiple abscesses form, and draining sinuses open to the surface and discharge pus and granules.

Other species of Streptomyces and E. coli K12 to be used are confidently assigned to Hazard Group 1.

NB While the associated risk assessment focuses on two pathogenic species of Streptomyces, amendment to include other closely-related HG2 species will be considered locally by GMBSC, and will not be considered a significant change provided there is no increase in overall risk.

Host/vector system

Bacteria will be transformed with non-mobilisable plasmids.

Origin & function

Cas9 (from Streptococcus pyogenes) will be introduced to HG2 Streptomyces to knock out putative virulence factor genes.

Virulence factor genes from HG2 Streptomyces will be expressed in HG1 species of Streptomyces and E. coli K12 strains.

Other genes from HG2 Streptomyces not involved in virulence (e.g. biosynthesis of non-ribosomal peptides, polyketides, terpenes and other specialised metabolites) will also be expressed in HG1 species of Streptomyces and E. coli K12 strains.
While not classified specifically by ACDP, the recognised ability of Streptomyces somaliensis and S. sudanensis to cause actinomycetoma in humans would indicate a provisional assignment to Hazard Group 2.

Antibiotic treatment is effective for all of the organisms. Vaccines are not available. There is an increased risk of infection in the immunosuppressed.

The proposed use of CRISPR/Cas9 is intended to study the effects of knocking out the expression of virulence factors, so would be very unlikely to increase the level of harm, compared to the unmodified strain.

Expression of virulence factor genes from S. somaliensis and S. sudanensis in E. coli K12 and HG1 strains of Streptomyces has the potential to increase the severity of harm arising from those GMMs, but this may confidently be assigned as a Class 2 contained use.

There is a low risk to the environment, but the measures to be adopted at Containment Level 2 will ensure that release is prevented.

Evaluation of foreseeable effects

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable: the administration of Class 2 GMMs to moth larvae will be in wild-type Galleria mellonella only.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid lab waste contaminated with GM material [eg. plastic pipettes, plates, small volumes of sealed liquid cultures, gloves, tips etc.] is placed in a rigid container lined with an autoclave bag. Clear bags are in use at CL1, and red bags at CL2. These materials are autoclaved on validated cycles ensuring a minimum temperature of 121°C for a minimum hold time of 20 minutes, ensuring an effective 100% kill.

At CL2, materials may be initially decontaminated using a validated disinfectant regime (e.g. 5% (v/v) Chemgene solution) prior to autoclaving. Autoclaves are subject to appropriate periodic servicing, validation and insurance inspection against a written scheme of examination.

After autoclaving, all waste is placed in Tiger-striped bags, and removed from site by the University’s appointed waste contractor for disposal as offensive waste.

Liquid waste is either autoclaved on a cycle validated to provide an effective 100% kill, or treated with validated disinfectants, prior to disposal via the drains. Residual quantities of bacterial cultures in tubes, etc, are treated as solid waste.

Galleria mellonella larvae used in pathogenicity assays are frozen overnight at -20°C, followed by autoclaving as solid waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This risk assessment was circulated to GMBSC by email on 13th September 2017, and the following specific comments were made by members, which have now been addressed:

1. The CL2 lab identified is a small shared facility. Additional facilities will be required if this project increases in scale.
2. Training and SOPs will need to be in place for handling and husbandry of Galleria moths.

**Project Containment**

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<td>Human Clinical Applications L3 L4</td>
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**Project Ref 13/19.1**

- Date Ackn'd: 15/08/2019
- CU2 Project Title: The development of in vitro systems to study Epstein-Barr virus (EBV) - epithelial cell interactions
- Class: Class 2
- CultureVol: < 1 Litre
- Consent Granted
- Project notified under transitional arrangements

**Historical Significant Changes**
- Withdrawn: N

**Project Additional Information**

**Purposes of the contained use**

We plan to interrogate the functional implications of tumour cell interactions with stromal-derived cells (fibroblasts) and immune cells to investigate how these interactions influence the growth, survival and phenotype of EBV infected cell lines in vitro.
Techniques to be used include:-
Infection of established nasopharyngeal and gastric cancer-derived epithelial cell lines with recombinant EBV isolates engineered to express suitable drug resistance genes (G418/Hygromycin B).
Growth and reconstitution of normal epithelial cells and EBV infected epithelial cell lines with normal fibroblasts and/or leukocytes in a 3D organotypic raft or organoid system to develop a model of the tumour and its microenvironment.
The impact of the modified stroma on tumour growth and viral gene expression will be assessed by standard immunohistochemical/immunofluorescence staining and in situ hybridisation (RNAscope).

Recipient or parental organism
HEK293 and B lymphocyte (BL) cells will be infected via a natural process with the GM strains of EBV. The cells pose minimal risk to human health or the environment. Cells will be induced into the lytic phase while in co-culture with a range of mammalian (including human) cell lines, which will subsequently become infected by the GM EBV. These cell lines are all confidently assigned to Hazard Group 1.

Host/vector system
Epstein-Barr virus (EBV) is a hazard group 2 agent which naturally infects a wide range of human cells, but to which approximately 90% of the population are sero-positive. The strains to be used have been genetically modified to include antibiotic-resistance genes to be used in selection techniques. The modification is unlikely to increase the pathogenicity of the EBV compared to unmodified wild-type strains.

Origin & function
The only genetic material involved are selectable marker genes expressing resistance to antibiotics such as neomycin (G-418) and hygromycin B. Other markers of similar function may be introduced during the lifetime of the project.

Evaluation of foreseeable effects
Infection of workers with GM EBV expressing antibiotic resistance markers is unlikely to be any more severe than a normal EBV infection, and the adoption of containment measures at CL2 will be adequate to control the risk from this potentially airborne infection. Antibiotics are not used in the treatment of EBV infection, and there is no vaccine available.

>90% of the population are known to have neutralising antibodies to EBV, although there is a risk of EBV to act as a cofactor in causation of cancers in certain populations and the immunosuppressed.

EBV infection is specific to humans, so the potential impact in the environment is likely to be negligible. Containment measures required for the protection of human health will control any risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid lab waste contaminated with GM material [eg. plastic pipettes, plates, small volumes of sealed liquid cultures, gloves, tips etc.] is placed in a rigid container lined with an autoclave bag. Clear bags are in use at CL1, and red bags at CL2. These materials are autoclaved on validated cycles ensuring a minimum temperature of 121oC for a minimum hold time of 20 minutes, ensuring an effective 100% kill.
At CL2, materials may be initially decontaminated using a validated disinfectant regime (e.g. 5% (v/v) Chemgene solution) prior to autoclaving.
Autoclaves are subject to appropriate periodic servicing, validation and insurance inspection against a written scheme of examination. After autoclaving, all waste is placed in Tiger-striped bags, and removed from site by the University’s appointed waste contractor for disposal as offensive waste.

Liquid waste is either autoclaved on a cycle validated to provide an effective 100% kill, or treated with validated disinfectants, prior to disposal via the drains. Residual quantities of bacterial cultures in tubes, etc, are treated as solid waste.

This risk assessment was discussed by the Genetic Modification & Biosafety Committee at its meeting on 29th April 2019, having been circulated in advance. The following specific comments were made by members, which have now been addressed:

1. Members questioned the suitability of some of the CL2 facilities for the particular activities.
2. It should be emphasised that materials must be fixed and embedded within a CL2 lab BEFORE transfer to the imaging suite.
3. Waste and emergency procedures should be amended to align with current procedures

Project Containment

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Project Ref 13/20.1

Date Ackn’d 24/06/2020

CU2 Project Title Cell biology and pathogenicity of kinetoplastid parasites and mammalian cells

Date Project Ceased

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM

Consent Granted
Trypanosomatids are microscopic vector-borne parasites that cause widespread human and animal diseases. They currently infect ~17 million people, kill 80,000 people annually and are a terrible agricultural and economic burden upon developing countries. There is an urgent need for new therapies because no vaccines are available and existing drugs are often ineffective and toxic.

Central to their ability to spread and cause disease is a protrusion of the surface called the flagellum. The flagellum is essential for parasite movement, sensing and avoiding the human immune system. Furthermore, defects in human flagella cause genetic diseases called 'ciliopathies'. Ciliopathies can affect multiple systems, causing blindness, deafness, chronic respiratory infections, kidney disease, heart disease, infertility, obesity and diabetes.

This project investigates kinetoplastid parasites and mammalian cells, with a special emphasis on understanding flagellum biology. This will give insights into a) fundamental biology, b) pathogenicity of parasites, and c) the molecular mechanisms of human genetic diseases (ciliopathies).

Recipient or parental organism

Leishmania species (including L. major and L. mexicana) - human HG2
Trypanosoma brucei complex (including subspecies T.b. gambiense and T.b. brucei) - human HG2
Trypanosoma congolense, T. evansi, T. equiperdum & T. brucei brucei - SAPO2

(NB An application for a SAPO2 licence has been submitted)

Host/vector system

Non-mobilisable plasmids containing antibiotic resistance selectable markers and fluorescent marker genes.

Origin & function

Fluorescent protein genes (including eGFP, eYFP etc) with a long history of safe use.
Kinetoplastid genes and gene fragments from HG2 and SAPO2 Leishmania and Trypanosoma spp, which are thought to have key roles in parasite motility and lifecycle.

Evaluation of foreseeable effects

None of the genetic modifications that will be made in this project are expected to increase virulence or pathogenicity of the organism. However, both GM and non-GM T.b. gambiense, L. mexicana and L. major are able to infect humans.
Similarly, it is the most likely outcome that the deletion or mutation of individual genes in Trypanosoma brucei, T. congolense, T. equiperdum, T. evansi would either disrupt
or not affect their virulence or pathogenicity in their normal animal hosts. It is likely that expressing the individual kDNA genes, gene fragments or gene fusions in human cells would present negligible risk.

Note that the use of third generation lentiviral vector systems expressing non-hazardous genes has been assigned as a Class 1 activity, and is not part of this notification.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste-handling and spillage procedures will be subject to a standard operating procedure (currently in development) to which all staff will be trained. The procedures to be employed are as follows:

Solid waste that is potentially contaminated with HG1 or HG2 organisms (and Class 1 or 2 GMMs) will be sterilised by autoclaving at 121°C 15psi for a minimum hold time of 20 minutes.

All liquid waste contaminated with HG2 organisms (and Class 2 GMMs) will be sealed securely in autoclavable culture vessels prior to sterilising by autoclaving (121°C at 15psi for 20 minutes).

All waste to be autoclaved will be collected by a dedicated waste team and taken direct from the containment suite to the autoclave room within the same building to be autoclaved immediately, and will not be left unsupervised in an uncontrolled area at any time. All waste autoclaves have independent cycle monitoring recorded on data loggers to ensure that validated cycle conditions have been attained for every run.

All autoclaves used for handling waste are subject to annual validation and calibration by an accredited service provider, and are examined to a written scheme of examination by the University's insurers.

Liquid waste from HG1/Class 1 organisms will be sterilised by autoclaving (121°C at 15psi for 20 minutes) or treatment with 1% Chemgene (final) for 10 minutes.

NB: While autoclaving will always be used as the preferred method of inactivating SAPO parasites and their GM derivatives, the use of 5% Chemgene for one hour is available as a validated contingency (Wang et al, 2008, PMC2556999).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Due to the coronavirus restrictions, the meeting of the Genetic Modification & Biosafety Committee scheduled for 23rd April 2020 was held by email correspondence. The members made the following specific comments when reviewing the risk assessment for this activity, which have now been fully corrected in the attached version:-

1. Elimination of the species infectious to humans will be employed wherever possible, but the assertion that T. brucei brucei is not infectious to humans is contrary to its hazard group.
2. What system will you have in place to ensure that no SAPO or HG2 trypanosomes will be used in the CL1 laboratory you have identified?
3. There is insufficient detail on the lentiviral transduction aspect, to ensure that all the intended transgenes fall within the scope of [the relevant Class 1 GM risk assessment approved by this Committee]. The current description of what will be delivered is open-ended, and needs to be stated to be restricted as per the terms of Generic 008.
4. While it is stated that MSCs will be used when handling organisms posing a hazard to human health, the conditions of the SAPO licence will also require any animal pathogen to be handled in the MSC.
5. Some clarification is required on when CL2 materials will be treated with disinfectant, and when they will be autoclaved (which is the normal procedure at CL2). Is 10 minutes treatment with 1% Chemgene adequate to inactivate the materials?
6. While there is consideration of immunocompromised individuals as a vulnerable group (section 9.2), this should also be the case for expectant mothers. The front-line treatment for early-stage trypanosomiasis is not recommended in pregnancy. Referral of pregnant workers to Occ Health would be recommended.
7. The first aid and primary care protocol applies to Oxford. Please take steps to establish the correct reporting procedure at University Hospital Coventry in the event of a lab exposure.

### Project Containment

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**Project Ref** 13/20.2

**Date Ackn’ed** 25/12/2020

**CU2 Project Title** Virus propagation and gene expression in mammalian and insect cells to study and detect RNA viruses

**Class** Class 2

**Culture Vol Class 2** < 1 Litre

**Culture Volume Class 3-4**

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N

Tick if notifying a connected programme of work N

02/03/2022
The diverse group of RNA viruses includes the pathogens responsible for devastating human diseases such as influenza, Zika, AIDS and Covid-19. These viruses result in substantial mortality as well as having severe economic impacts worldwide. Despite significant progress in the study of these viruses, many fundamental unanswered questions remain. Our laboratory uses a combination of biochemical and biophysical imaging techniques to study the replication processes, morphology and cellular interactions of RNA viruses, as well as develop novel experimental tools for viral diagnostics.

This will be achieved in four ways:

1. Propagation of influenza viruses in eukaryotic cell culture as our primary model system, which will be used for the development of viral diagnostic assays and for imaging experiments to investigate the morphology and cellular interactions of influenza viruses.

2. Propagation of infectious bronchitis virus (IBV), an avian coronavirus, in eukaryotic cell culture, and use as a safe proxy to image and study coronavirus replication.

3. Rescue of recombinant influenza virus strains using plasmid-based transfection systems. We aim to use reverse genetics to generate reassortant influenza viruses with additional reporter gene segments of innocuous and non-viral origin e.g. GFP, or containing tags for virus labelling or purification e.g. histidine, FLASH or SNAP tags, for use in downstream assays such as live cell imaging of fluorescent viruses.

4. Expression of viral polymerase and cofactor proteins from a range of RNA viruses for in vitro studies using transient expression of viral proteins in transfected eukaryotic, bacterial or insect cells. The aim of our work is to study viral replication using single-molecule techniques to measure the structural and functional properties of single replication complexes in real-time. Individual proteins, or subunits, will be expressed and biochemically purified for in vitro experiments.

### Recipient or parental organism

- Low hazard cells of mammalian, insect and avian origin.
- Laboratory-adapted strains of influenza virus, assigned to Hazard Group 2, including vaccine strains A/WSN/33 and A/PR/8/34.
- Seasonal circulating strains of influenza virus, other than those of pandemic potential.
- Avian infectious bronchitis virus (HG2 strains only).
- Respiratory syncitial virus (HG2 strains)
Non-mobilisable plasmids containing antibiotic resistance selectable markers.

**Origin & function**

Genes expressing proteins involved in the expression of viral proteins and viral RNA from influenza, infectious bronchitis virus and SARS-CoV-2. Expression cassettes may include fluorescent and epitope tag fusions.

Note that gene expression factors, etc. from other Hazard Group 2 viruses may be included at a later stage, without further notification, provided there is no increase in risk or requirement for additional control measures. These will be subject to local scrutiny and approval by the GMBSC.

**Evaluation of foreseeable effects**

None of the genetic modifications that will be made in this project are expected to increase virulence, host range or pathogenicity of any viruses or other GMMs.

It is unlikely that expressing individual virus transcription factor and polymerase genes, gene fragments or gene fusions in mammalian cells would result in a GMO of increased risk.

To minimize the risk of human infection and disease, all reassortant viruses will be generated on the background of viruses with low pathogenic potential in humans i.e. the lab-adapted PR8 or WSN strains, found to be non-infectious in humans. Both ‘acceptor’ viruses are classified as Hazard Group 2 organisms, with a long history (> 70 years) of safe use in the laboratory. Furthermore, PR8 forms the backbone of the currently used inactivated influenza virus vaccines. Any insertions into the viral genome are expected to lead to a loss of function, and therefore even further decreased virulence.

The proteins, without the context of a virus particle, pose no toxic or pharmacological hazard, therefore the risk to human health is negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None requested

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste that is potentially contaminated with HG1 or HG2 organisms (and Class 1 or 2 GMMs) will be sterilised by autoclaving at 121°C 15psi for a minimum hold time of 20 minutes.

Liquid waste contaminated with HG2 organisms (and Class 2 GMMs) will be inactivated by treatment with validated disinfectant for the appropriate contact time prior to disposal via the drains. Alternatively, it may be sealed securely in autoclavable culture vessels prior to sterilising by autoclaving (121°C at 15psi for 20 minutes).

All waste autoclaves have independent cycle monitoring recorded on data loggers to ensure that validated cycle conditions have been attained for every run.

All autoclaves used for handling waste are subject to annual validation and calibration by an accredited service provider, and are examined to a written scheme of examination by the University's insurers.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Due to the coronavirus restrictions, the meeting of the Genetic Modification & Biosafety Committee scheduled for 29th October 2020 was conducted by video conference. The members made the following specific comments when reviewing the risk assessment for this activity, which have now been fully corrected in the attached version:

1. You state that you will be expressing (and constructing) the viral genome segments in E. coli, but these are not included in Sections 4 or 6, and need to be added.
2. Polymerase genes from other viruses, fluorescent markers and epitope tags are referred to in Section 3, but not mentioned in 6.4.
3. In Section 6.3, you state that expression from the non-mobilisable plasmid vectors is not likely or intended in humans. This would not appear to be correct, other than for the insect expression.
4. In Section 5.2, members asked for more detail on your direct experience working with respiratory pathogens at Containment Level 2.
5. There is no mention of the increased risk of influenza complications in pregnancy. This ties in with the need for the involvement of occupational health and the provision/offer of seasonal flu vaccine, which the Committee believe should be in place. This will also minimise the potential for generation of novel reassortant viruses, although that risk is admitted quite low.
6. There was concern that the waste team might be at risk from live flu virus in the waste, and that additional controls might be appropriate when disposing of the waste (e.g. ensuring that the bags are folded over before placing the lid on the autoclave tin). It would also be appropriate to offer flu vaccine to the waste team.
7. There is no mention of what infectious bronchitis virus will be used for, but this is infectious in avian species. More detail is needed before the environmental risk can be defined.
8. More detail is required on how ultracentrifugation of infectious material will be conducted safely.

**Project Containment**

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**Project Ref** 13/21.1

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<td>19/02/2021</td>
<td>Structural, functional and activity analysis of bacterial toxins to develop novel therapeutic anti-toxins</td>
<td></td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Our laboratory, with industry partners Bicycle Therapeutics, are looking to develop the next generation of therapeutic anti-toxins. To do this we need to express and purify the target bacterial toxin. This will enable us to screen for toxin inhibitors produced by our partners which we will initially validate by simple (in eppendorf tube) haemolysis assays.

The initial family of ~20 toxins to be considered will be the 'cholesterol dependant' toxins which include pneumolysin, listeriolysin, etc. Pneumolysin is a pore-forming toxin of 53 kDa composed of 471 amino acids. We will express full length wild type toxin and truncated non-toxic versions including domain4 only variants which are demonstrably non-toxic. The study will also investigate anti-toxins to alpha-hemolysin from Staphylococcus aureus which, while not a cholesterol-dependent cytolysin due to its receptor interaction, has a similar structure and mode of action to the CDCs.

### Recipient or parental organism
Multiply-disabled K12 and B lab-derived strains of Escherichia coli

### Host/vector system
- pET vectors with T7 promoters
- pBAD vectors with arabinose promoters
- pTac and pProEx vectors with tac promotor
- High copy number cloning vectors, pUC series, pBluescript
- All vectors are non-mobilisable.

### Origin & function
- Genes from encoding cholesterol-dependent toxins, including the following:-
  - Pneumolysin from Streptococcus pneumoniae
  - Alpha-hemolysin from Staphylococcus aureus

NB: While the initial investigations will focus on these two toxins, it is likely that the work will move on to other members of the CDC family produced by related (HG2) bacterial species. These are unlikely to pose any increased risk or require any additional control measures to be implemented, so will be subject to local approval of the risk assessments by the GMBSC, and will not be subject to a significant change notification.
### Evaluation of foreseeable effects

The genes to be expressed in E. coli constitute secreted toxins which have the potential to display cytotoxic effects in isolation by virtue of pore formation in mammalian cells including macrophages. The resulting risk to human health is low, but cannot be considered nil or negligible, so Class 2 would be appropriate.

The transfer of genetic material (including antibiotic resistance genes) into related species in the environment is unlikely due to use of non-mobilisable plasmids. None of the genetic modification is likely to confer a survival advantage to the E. coli in the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid lab waste contaminated with GM material [eg. plastic pipettes, plates, small volumes of sealed liquid cultures, gloves, tips etc.] is placed in a rigid container lined with a red autoclave bag. These materials are autoclaved on validated cycles ensuring a minimum temperature of 121°C for a minimum hold time of 20 minutes, ensuring an effective 100% kill.

At CL2, materials may be initially decontaminated using a validated disinfectant regime (e.g. 5% (v/v) Chemgene solution) prior to autoclaving. Autoclaves are subject to appropriate periodic servicing, validation and insurance inspection against a written scheme of examination.

After autoclaving, all waste is placed in Tiger-striped bags, and removed from site by the University’s appointed waste contractor for disposal as offensive waste.

Liquid waste is either autoclaved on a cycle validated to provide an effective 100% kill, or treated with validated disinfectants (e.g. 5% (v/v) Chemgene solution, prior to disposal via the drains. Residual quantities of bacterial suspensions in tubes, etc, are treated as solid waste.

### Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
This risk assessment was discussed at a virtual meeting of the University GMBSC held via Teams on 14th January 2021, having been circulated in advance, and the following specific comments were made:

1. There is some detail lacking in Section 6 relating to the components of the genetic modification, which makes it difficult to assess the level of potential risk.
2. There is a little lack of clarity in Section 4 regarding the exact locations to be used for the activities with E. coli expressing toxin genes and with purified toxins.
3. In terms of waste disposal, what is the likelihood that the toxins will survive treatment with the disinfectants named?
4. Chemgene would normally be regarded as effective at 5%, rather than 1% as you have stated.
5. The description of the range of which toxins you intend to study is not detailed enough. Please provide a list of which specific toxins you will be using

The risk assessment has been amended to address these comments.

**Project Containment**

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**Project Ref** 13/97.3

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Project notified under transitional arrangements ✔

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022  Page 550 of 15326
Project Ref 13/99.1

Date Ackn'd 30/11/1999

CU2 Project Title
INVESTIGATION OF THE ANTI-VIRAL ACTIVITY OF DEFECTIVE-INTERFERING SFV Genome

Class 2

CultureVolumeClass3-4

Non-GMM not applicable

Consent Granted

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 13/trans2

Date Ackn'd 02/03/2022  

CU2 Project Title  

USE OF NON-DEFECTIVE VACCINIA VIRUS TO CARRY AND EXPRESS GENES  

Class 2  

Class CultureVolClass2 CultureVolumeClass3-4
FROM BACTERIOPHAGE T7: HUMAN, BOVINE AND PORCINEROTA VIRUS; MURINE AND AVIAN PNEUMOVIRUS; HUMAN AND BOVINE RESPIRATORY... ETC ....

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 13/trans3

Date Ackn'd

Date Project Ceased

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

USE OF NON DEFECTIVE HERPES SIMPLEX VIRUS TO EXPRESS MURINE PNEUMOVIRUS G (ATTACHEMENT) AND F 9 FUSION GENES

Class 2

Consent Granted

Project notified under transitional arrangements

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
USE OF NON DEFECTIVE ADENOVIRUS 5 TO CARRY HUMAN DYSTROPHIN GENE, A BACTERIAL NEOMYCIN RESISTANCE MARKER AND SIV COMPONENTS

Class

Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

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Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
**Project Additional Information**

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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Large Scale Activities

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Human Clinical Applications

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Project Ref 145/01.2

Date Ackn’d: 02/06/2015
CU2 Project Title: VARIOUS STUDIES INVOLVING PSEUDOMONAS SYRINGES AND XANTHOMONAS CAMPESTRIS

Class: Class 2
CultureVolClass2: Non-GMM
Consent Granted: Not Applicable

Date Project Ceased
Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: Transferred to GM13 on closure of GM145

Historical Date of Additional Info
Significant Change ID
Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 145/01.4

Date Ackn'd 02/06/2015

CU2 Project Title PRODUCTION OF INFECTIOUS CDNA CLONES OF TURNIP MOSAIC VIRUS.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work N

Historical Significant Changes Transferred to GM13 on closure of GM145

Historical Date of Additional Info

Project notified under transitional arrangements Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref  145/03.1

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**Project Additional Information**

**Purposes of the contained use**

CMV- 2b-GFP and CMV-GFP
A mutant of cucumber mosaic virus (CMV) that lacks the 2b protein gene and expressing free green fluorescent protein (GFP) and the wild type CMV expressing free GFP obtained in the laboratory of Prof P Palukaitis (SCRI) will be used as a model system to determine whether 2b-less CMV isolates/mutants make better cross-protecting strains and test the hypothesis that cross-protection occurs via virus induced RNA silencing.

Virus movement in the plant will be assayed by detection of GFP under UV light.

Presence of the 2b protein will be determined by Western blotting.

Two well established markers for the silenced state in plants are 25nt oligonucleotides (SiRNA) and increased levels of host RdRP (RNA dependent RNA polymerase) determination of whether levels of these markers are increased will be determined by Northern blotting

TRV-GFP
A tobacco rattle virus (TRV) modified to remove the non-structural 2b and 2c genes and replaced with GFP driven by a second coat protein gene subgenomic promoter derived from a different tobravirus isolate provided by Dr S Macfarlane (SCRI) will be used as a control for CMV-GFP experiments. Virus movement in the plant will be assayed by detection of GFP under UV light.

Two well established markers for the silenced state in plants are 25nt ologonucleotides (SiRNA) and increased levels of host RdRp (RNA dependent RNA polymerase) determination of whether levels of these markers are increased will be determined by Northern blotting.

**Recipient or parental organism**

CMV is one of the most important and widespread viruses of field grown horticultural crops in the world. CMV has a particularly wide host range including Cucumis sativus, Lycopersicon esculentum and Spinacia oleracea. In total it infects over 1,000 species including many weed species. CMV can be transmitted mechanically, and in few cases via seed, but the major mode of infection in vegetable crops is by aphids in a non-persistent manner.

Tobacco rattle virus belongs to one of only two genera of viruses that are transmitted by nematodes, the tobraviruses. Tobraviruses are transmitted only by nematodes belonging to the genera Trichodorus and Paratrichodorus. It can also be transmitted via seed, grafting and by mechanical inoculation. TRV has a wide natural host range including Stellaria media, Voila arvensis. Beta vulgaris. Spinacia oleracea, Capsicum annum Solanum tuberosum Narcissus pseudonarcissus. Tulipa sp. and Hyacinthus sp.
### Host/vector system

**CMV-2b-GFP and CMV-GFP**

To ensure that virus stocks are genetically uniform and satellite-free, 2b-less CMV and the parental wild type CMV will be reconstituted from cDNA clones by in-vitro transcription and the resultant RNA inoculated onto tobacco to bulk up the virus for subsequent experiments.

**TRV-GFP**

Clones of RNA2 of TRV were modified to remove the non-structural 2b and 2c genes and replaced with GFP driven by a second coat protein gene subgenomic promoter, derived from a different tobravirus isolate (pea early-browning virus).

### Origin & function

The CMV-2b-GFP and CMV-GFP will be obtained from the laboratory of Prof P Pulukaitis (SCRI) and the TRV-GFP from Dr S Macfarlane (SCRI). GFP will be used as a reporter gene for virus movement within the plant.

### Evaluation of foreseeable effects

The 2b protein gene in virus interferes with virus induced gene silencing in plants protecting the virus against host defences. It is hypothesised that the properties of the 2b protein may play a role in the induction of cross protection. If the virus lacks a functional 2b protein and is therefore unable to prevent gene silencing it should be less pathogenic. The CMV-2b mutant is less able than the wild type virus to spread systemically and accumulates to lower levels than the wild type in inoculated and uninoculated tissues (Soards A. J. et al 2002, Mol. Plant-Microbe Interact Vol 15, No. 7 pp 647-653). There is therefore no predicted hazard arising from the deleted gene. The inserted gene GFP is well characterized and there is no evidence for it being a hazard.

**TRV-GFP**

The deletion of the non-structural 2b and 2c genes should not alter pathogenicity as infection can occur in the complete absence of RNA 2. The non-structural genes on RNA 2 have been shown to be necessary for transmission of tobraviruses by vector nematodes; thus constructs in which these genes have been deleted should be effectively contained in the plants under test. (Macfarlane and Popvich 2000 Virology 267 29-35).

The inserted gene, GFP is well characterized and there is no evidence for it being a hazard.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Plant pots will be placed on trays or saucers in order to catch run-off water and this water will be allowed to evaporate naturally. All plant material, soil pots and labels will be autoclaved at 121 degrees C before final disposal in the skip. An autoclave is situated in the glasshouse. All trays, saucers, glass, flooring and benching will be thoroughly disinfected with Jet 5 disinfectant (8mls/L)

Plant material used in the laboratory will be autoclaved at 121 degrees C along with bags, tips and any other items used during laboratory procedures. This will then be placed in the skip. An autoclave is present in the same building as the laboratory. Benches in the laboratory are impervious and will be swabbed down with 70% ethanol before and after use.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
The risk-assessment has been discussed and approved by the Genetic Modification and Biological Safety Committee at HRI, Wellesbourne.

### Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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### Project Ref 145/09.1

- **Date Ackn'd**: 02/06/2015
- **CU2 Project Title**: Use of *Pseudoalteromonas syringae* pv *tomato* (DC3000) to deliver oomycete effector genes into plant cells
- **Class**: 2
- **Culture Vol Class 2**: < 1 Litre
- **Project notified under transitional arrangements**: N

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Transferred to GM13 on closure of GM145**

### Project Additional Information

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
Purposes of the contained use

a. Oomycete effector genes (from Hyaloperonospora arabidopsidis and Phytophthora infestans) will be fused to the secretory signal of the Pseudomonas syringae pv Tomato DC300 effector AvrRPS4 and transformed into Pseudomonas syringae pv Tomatoe Dc3000 isolate.

B. Arabidopsis plants will be inoculated with the modified DC300 isolate and tested to determine if the introduced effector triggers a defence response from the plant via recognition by a plant resistance gene (effector triggered immunity) or if the effector increases isolate virulence due to suppression of the plant's basal defence response (effector triggered susceptibility). The following plants may also be inoculated: Potato, Tobacco and Brassica oleracea.

Recipient or parental organism

Pseudomonas syringae pv Tomato is a gram negative, aerobic, rod-shaped bacterium, which is able to survive in soil, in debris form diseased plants and on seeds. Infection is spread by rain splash allowing entry into leaves via natural openings, such as stomata, where it multiplies in the intercellular space resulting in lesions on infected leaves.

Successful infection is dependant upon the translocation of effector molecules into the plant cell to promote disease and suppress/avoid PAMP (pathogen associated molecular patterns) triggered immunity and effector triggered immunity (Ponciano et al 2003, Jones and Dangl 2006)

Pseudomonas syringae pv Tomato is able to cause disease on Solanum Lycopersicum (tomatoe), Capsicum annuum (pepper) and Solanum melongena (aubergine) (Bradbury 1986). In addition, some strains, which include DC3000 are virulent on Arabidopsis thaliana, Brassica oleracea (cabbage, cauliflower, broccoli) and B. campestris (turnip) (Whalen et al 1991). DC3000 is also able to infect Nicotiana benthamiana (tobacco) if the effector hopQ1-1 is removed (Kvitko et al 2009). The isolate DC3000 was isolated in Ontario, Canada by Diane Cuppels and is widely used for moecular studies as it is a naturally occuring pathogen of the model plant Arabidopsis thaliana. In addition, the genomes of both DC3000 and Arabidopsis have been sequenced. Consequently, the technology we wish to use has been developed using this Pseudomonas isolate (Sohn et al 2007).

References:
- Bradbury JF. (1986) Guide to the Plant Pathogenic Bacteria. CAB International Mycological Institute, Kew, UK

Host/vector system

The host strain Pseudomonas syringae pv Tomato DC3000 will be used, which was isolated in Ontario Canada in 1986.

Oomycete effector genes will be cloned into the pEDV6 vector and transformed into DC3000. The pEDV6 vector was developed in Jonathon Jones's lab at the Sainsbury laboratory and contains the N-terminal secretory signal from the DC3000 effector AvrRPS4 and is expressed using the native AvrRPS4 promoter. This allows delivery of the oomycete gene into plants cells via the bacterium's type three secretion delivery mechanism. This vector carries gentimicin to allow antibiotic selection of transformed cells. The pEDV6 vector is a replication compromised plasmid that is unstable and lose in the absence of persistent antibiotic selection.

Origin & function

DC3000 transformants will be grown in liquid culture in volumes of no greater than 30 ml. Cultures will then be adjusted to an aproprite O.D (1 - 2.5) before infiltration of leaves using a 1ml syringe (without needle) or spray inoculation using a hand held atomiser. Infected plants will be analysed after 0-7 days. All plants and soil will be autoclaved at the end of the experiment (see section 12).
All activities will be performed and effectively contained within purpose built laboratories and growth rooms at Warwick HRI, The University of Warwick, Wellesbourne. Pseudomonas syringae bacteria are non-sporulating and can be readily contained using good microbiological practice.

The oomycete effectors may increase, decrease or have no effect on DC3000 virulence when it comes into contact with its host plants. The modified DC3000 isolates may be able to suppress/avoid the host's basal resistance response. Conversely, the introduced effector may act as an avirulence factor and trigger an R gene mediated resistance response in a normally susceptible accession.

Whether the modified DC3000 strains could infect a new host plant has never been tested but this is unlikely to happen, as a single effector, evolved to function in a specific plant-microbe interaction, is unlikely to happen, as a single effector, evolved to function in a specific plant-microbe interaction, is unlikely to overcome the reasons why the plant is a non-host to DC3000 in the first instance. For example, where DC3000 is unable to overcome the plant's passive defence mechanisms, such as a waxy cuticle, rigid cell walls and preformed antimicrobials or effector triggered immunity.

During escape this organism could potentially transfer the oomycete effector gene to other bacteria and possibly cause the undesirable spread of resistance to gentamicin. The likelihood of these hazards occurring is extremely low due to the use of the replication-compromised plasmid that is unstable and lost in the absence of antibiotic selection.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Risk assessment approved by the Genetic Modification Safety Committee at Warwick HRI on 15/06/2009

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

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**Project Ref** 26/99.2

**Date Ackn’d** 18/09/2007  
**CU2 Project Title** MOLECULAR BIOLOGY OF PICORNAVIRUSES

**Date Project Ceased** 06/07/2015

**Class** Class 2  
**CultureVolClass2**  
**CultureVolumeClass3-4**  
**Non-GMM** Not Applicable

**Project notified under transitional arrangements** Y

**Withdrawn** N

**Historical Significant Changes** TRANSFERRED FROM GM 26 (18/9/07) Project transferred to GM317 06

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

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**Project Additional Information**

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Animal Units  
Large Scale Activities  
Human Clinical Applications
Replication deficient lentiviral constructs encoding shRNAs will be used in the study of microtubule-associated proteins.

The production of cells deficient in microtubule-associated proteins or expressing mutant forms in order to study function. Also the production of microtubule-associated proteins with epitope tags or fluorescent proteins for live cell imaging and biochemical experiments.

Cell lines with a history of safe usage within the scientific community. Any primary cells used are isolated from sources held in a controlled environment and should therefore be free of pathogens. Any E.coli variants, cultured cells and replication-deficient lentivirus particles produced cannot survive outside the laboratory. Neither the generated virus particles nor the transduced cells are pathogenic to animals or plants.

Well characterised E.coli K12 derivates, such as TOP10. Lentiviral particles are designed for use in gene therapies and thus have the potential to infect human cells in vivo, however as none of the HIV-1 structural genes are actually present in the packaged viral genome, they are not able to propagate in vivo.

Lentiviral constructs encoding shRNAs targeted against murine microtubule-associated proteins will be transferred, using lentivirus, to cell lines. shRNAs will be used to bring about RNA interference, silencing the targeted genes and enabling studies on the function of microtubule-associated proteins. Other constructs expressing microtubule-associated proteins with epitope tags or fluorescent proteins.
will also be produced in order to allow live cell imaging and biochemical experiments.

**Evaluation of foreseeable effects**

inserts display no potential for a pathogenic phenotype. The likelihood of an environmental hazard is negligible as none of the particles produced can survive outside the laboratory. Neither the generated virus particles nor the transduced cells are pathogenic to animals or plants.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid waste (tissues, gloves, plasticware) is autoclaved at 121 degrees Centigrade for 15 minutes (as measured in load) to give effectively 100% kill. Waste media is inactivated by addition of Virkon tablets to a final concentration of not less than 1% and left overnight. Manufacturer’s validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

Pipettes are decontaminated by complete submersion in 1% Virkon overnight, followed by autoclaving at 121 degrees Centigrade for 15 minutes (as measured in load) to give effectively 100% kill; Virkon manufacturer’s validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

If the use of glassware is unavoidable, this is decontaminated by complete submersion in 1% Virkon overnight. Manufacturer’s validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

**Project Containment**

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02/03/2022
**GM Centre Number: 14**

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**Comments**

GM CENTRES 99,133,134, 147, 521, 596 & 530 CLOSED AND ALL WORK TRANSFERRED TO GM14 24/04/2012

**Date at Which Additional Info Submitted**

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**Significant Change**
- 14/06.2
- GM14/05.3a
- 14/14.8a

**Date of Additional Information (significant change only)**
- 17/03/2006
- 17/08/2009
- 11/01/2016

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Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
**Project Ref** 133/01.2

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<tbody>
<tr>
<td>24/04/2012</td>
<td>THE INVESTIGATION OF P15 INACTIVATION IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)</td>
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<table>
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<tr>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
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</table>

**Non-GMM** Consent Granted  Not Applicable

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

1. To ascertain the extent and significance of inactivation of the tumour suppressor gene p15 in childhood leukaemia.
2. To investigate the relationship between p15 and other known molecular abnormalities in childhood leukaemia.

**Recipient or parental organism**

GMMs generated from this activity have no foreseeable effects to human health or safety. The hosts are all disabled or non-colonising in humans and plasmid and cosmid vectors are non-mobilisable.

**Host/vector system**

<table>
<thead>
<tr>
<th>Vectors</th>
<th>Plasmid vectors pBR327, pGEMT, pBS, PUC, Cosmid and Plasmid artificial chromosome vectors pWE15, pCyPAC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hosts</td>
<td>Disabled K-12 derived E. coli vectors: Sure, JM109, DH5a.</td>
</tr>
</tbody>
</table>

**Origin & function**

| p15 is a known tumour suppressor involved in cell cycle regulation and its loss in human tumours is thought to contribute to the malignant phenotype. Overexpression of |
this gene leads to suppression of cell division and cell death. This activity involves the use of vectors containing several known oncogenes eg c-ABL (J Mol Appl Genet 1983;2(1):57-68) and chimeric gene products that have potential to induce increased cellular proliferation and have been shown to be leukaemogenic in in-vivo systems. Eg MLL/AF9 (EMBO J 1999 Jul 1;18(13):3564-74)

Evaluation of foreseeable effects

The host and vector systems used to generate the constructs present minimum risk to human health and safety and could be handled adequately as a class 1 activity under level 1 containment. However, as this project involves the generation of naked oncogenic DNA it will be subject to the appropriate containment and control measures for a class 2 activity and level 2 containment.

Although there is no direct evidence that contact with naked oncogenic DNA can lead to tumours in humans it is intended to incorporate measures which will limit accidental inoculation or transmission of these DNAs to the laboratory workers.

All experiments will be carried out in ADCP level 2 laboratories with emphasis on the following points in accordance with our local rules and recommendations given in ACGM Compendium of Guidance Part 2A - Annex III and Part 3A - Annex 1.

1. Gloves will be worn at all times and changed when working elsewhere.
2. Steps are in place to limit aerosol production and aerosol contamination (filter tips)
3. Limited use of sharps and glassware to avoid accidental inoculation.
4. Staff are aware of the hazards of handling oncogenic DNA.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste from GMMs will be handled according to our local rules. Bacterial cultures will be inactivated by chemical treatment with fresh Chloros for a minimum of 24 hours. All plastic disposables and gloves will be autoclaved and incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment for this activity has taken into account the oncogenic potential of the DNA sequence to be inserted in the GMO. The activity has therefore been designated as Class 2. Suitable precautions to limit the risk of accidental inoculation of named oncogenic DNA are already practiced in the laboratories designated for Level 2 containment. All other working practices described are in accordance with our local rules.
Primary immunodeficiencies (PID) are congenital disorders that affect the function of the immune system. In recent years, a number of genes causing PID have been cloned, which has made gene replacement therapy for these diseases a realistic alternative for bone marrow transplantation. With increased knowledge of haematopoietic stem cell (HSC) biology and improved retroviral transduction protocols, it is now feasible to use onco-retroviral vectors for efficient corrective gene transfer into HSCs of affected individuals. Since transduced self-renewing HSCs are capable of reconstituting a complete immune system, this approach potentially provides a life-long cure of the disease.
HSCs isolated from cord blood, bone marrow or mobilised peripheral blood will be transduced ex vivo in a closed system with clinical grade retroviral supernatant.

**Host/vector system**

**Vector:** Moloney Murine Leukemia Virus (MoMuLV) based retroviral vectors, containing retroviral 5’ and 3’ LTRs, packaging signal and part of the gag gene, expressing full-length human cDNAs encoding wild type PID causing genes.

**Host:** Human CD34+ haematopoietic cells isolated from cord blood, bone marrow or mobilised peripheral blood.

**Origin & function**

MoMuLV based replication deficient retroviral particles pseudotyped with the envelope of Gibbon Ape Leukemia Virus (GALV), will be used to transduce human CD34+ cells. The GALV envelope has been shown to mediate efficient transduction of HSCs. Replication deficient retroviral particles were generated in packaging cell lines, which provided retroviral gag/pol and env helper functions in trans. The MoMuLV-based retroviral vector was introduced into the packaging cell independently and replication competent retrovirus (RCR) can only be generated after three independent recombination events, which is very unlikely to occur. The production of clinical grade retroviral supernatant, with all the required safety testing, was performed by a licensed commercial organisation.

In the vector, the retroviral genome is replaced by the cDNA encoding the appropriate corrective gene, whereas only 5’ and 3’ viral LTRs, the packaging signal and part of the gag coding sequence (for optimal protein expression) are still present.

Clinical grade retroviral supernatant is provided by a commercial organisation licensed to undertake such a process and has been tested extensively for the presence of RCR. The transduced CD34+ cells will be returned to the patient and the patient will be monitored over time for RCR.

**Evaluation of foreseeable effects**

The patient will be monitored extensively for the possible production of PCR, though as discussed, this possibility has been minimised by the use of packaging cell lines providing the gag, pol and env functions in trans. Expression of the inserted gene will be monitored by PCR, protein and functional assays. The inserted gene is not judged to be an oncogene or growth factor which could cause potentially harmful effects. The gene is unlikely to act alongside existing characteristics of the cell as to endow the cell with altered pathogenic properties.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The work with CD34+ haematopoietic cells constitutes no higher risk than work with fresh blood or blood-related products. Patients will be screened for presence of pathogens prior to donation of cells.

To reduce the risk, all the work is performed in a Class II safety cabinet and cells will be grown in a closed system.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All processes will take place in a closed system inside a class II tissue culture cabinet.

The class II tissue culture cabinets will be swabbed with 70% IMS before and after use, and any spillages will be dealt with immediately using 1% Virkon followed by 70% IMS. In the event of a larger spill, paper towels will be used prior to disinfection.

All solid waste, such as discarded bags, pipettes, tips, towels, etc will be disposed of in autoclave bags. These will be removed when full and autoclaved prior to incineration.

The use of sharps (eg needles, Pasteur pipettes) will be avoided, however when use is unavoidable they will be safely disposed of using sharps bins.
These processes should give 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee was satisfied with the risk assessment and had no further comments.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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Project Ref 133/01.6

Date Ackn’d 24/04/2012

CU2 Project Title INVESTIGATION OF PROTEINS THAT INERACT WITH THE TUMOUR SUPPRESSOR GENE P15

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N
c-Jun Activation domain Binding protein (JAB1) and Geminal centre kinase Like Kinase (GLK) have been shown to interact with the tumour suppressor gene p15 in the yeast two-hybrid system. These nature and significance of these novel interactions will be tested in the mammalian system using both in-vitro and in-vivo techniques. 

In-vitro expression: Full length cDNA and partial constructs of GST-JAP1 and GST-GLK chimeric proteins will be expressed in bacteria and tested for interaction with endogenous p15 protein extracted from established human cell lines. Both proteins will also be transcribed and translated in-vitro using the rabbit reticulocyte system and tested for interaction with in-vitro translated p15. 

In-vivo expression: Full length cDNA and partial constructs of JAP-1 and GLK and p15 will be cloned into mammalian expression vectors and transiently expressed singly or in combination in established human cell lines.

Recipient or parental organism

Bacteria: JM109, SURE, DH5a
All bacterial hosts used in this study are K-12 derivatives and non pathogenic in humans. They are able to survive for short periods in the gut and lung of mammals but are non-colonising. They are able to survive for a similar time in the environment. The plasmid vectors used in this study express antibiotic resistance genes eg ampicillin or neomycin. The vectors are non mobilisable and are defective in one or more functions required to transfer to other hosts. Although transfer of antibiotic resistance to other bacterial hosts is possible, the likelihood is low.

Cell Lines: Hela, MM6, Jurkat, CEM
All human cell lines are established and characterised. Conditions for growth include maintenance at a constant temperature (37 degrees C) in low CO2. They are unable to survive outside these conditions. Both parental and modified cell lines present negligible risk to human health or the environment.

Host/vector system

Vectors  pGex-6p-2, pGEMT, pcDNA3.1, pEGFP-N1, pDsRed1-N1, pGADT7
Hosts  Disabled K-12 derived E.coli vectors: Sure, JM109, DH5a

Origin & function

All cDNAs are human in origin.
p15 full length and partial c-DNA sequences promotor region and exon 1 antisense cDNA. p15 is a known tumour suppressor involved in cell cycle regulation and its loss in human tumours is thought to contribute to the malignant phenotype. Although functional loss of p15 is associated with many human malignancies, there is no evidence to suggest that loss of p15 expression alone is a transforming event. Overexpression in mammalian cells can lead to cell cycle arrest or retardation of proliferation. JAB-1 full length and partial c-DNA sequences. JAB-1 is expressed in a wide range of normal cells and is involved in several signalling pathways involving gene transcription, (JNK), cell cycle control (p27) and integrin signalling. JAB1 is also known to regulate protein levels of the cell cycle regulator p27 by promoting its degradation. Overexpression may lead to cellular proliferation, apoptosis or differentiation.

GLK full length and partial c-DNA sequences. GLK is involved in cellular stress pathways (JNK). It is also expressed in a wide range of tissues. Overexpression may lead to growth retardation or apoptosis.

Evaluation of foreseeable effects

The non-mobilisable GMMs used for this study contain full length and partial cDNA sequences designed for maximum expression in mammalian cells and as GST fusion proteins in bacteria. They are propagated in bacterial hosts, which are disabled or non-colonising in humans. Should the bacteria or GMMs be accidentally released into
the environment their chance of survival is negligible and the potential to cause harm is effectively zero.

HUMAN HEALTH

Inserts into the pGEX vector are designed to be expressed as a fusion protein with glutathione-s-transferase (GST). The expressed proteins are unlikely to change the properties of the parental recipient. Inherent risks and outcomes are the same as the unmodified recipient. i.e. the risk to human health is negligible. Expression of single and multiple proteins from these genes in mammalian cell lines may alter their growth properties. The most likely outcomes are growth arrest and/or cell death. However, overexpression of JAB1 protein may lead to cellular proliferation in some instances. Over expression of either GLK or JAB1 may inhibit the regulatory nature of p15 allowing greater cell proliferation. Expression of antisense p15 is likely to have a similar effect. Accidental insertion of naked cDNA into humans may lead to limited protein expression, however normal immunological response would rapidly eliminate foreign DNA or protein. Modified cell lines present no additional risks or hazards compared with the parental lines.

CONTROL MEASURES

Accidental inoculation of CDNA sequences or gM cell lines into laboratory workers by stick injury or entry via cuts and abrasions may constitute a hazard and therefore use of needles and scalpels will be limited, cuts and abrasions will be covered and gloves will be worn at all times. All cell work is carried out in Class 2 Safety hoods and use of filter tips limits aerosol formation.

All experiments will be carried out in ADCP level 2 laboratories with emphasis on the following points in accordance with our local rules and recommendations given in ACGM Compendium of Guidance Part 2A-Annex III and Part 3A-Annex 1.

1. Gloves will be worn at all times and changed when working elsewhere.
2. Steps are in place to limit aerosol production and aerosol contamination (filter tips)
3. Limited use of sharps and glassware to avoid accidental inoculation.
4. Staff are aware of the hazards of handling potentially damaging cDNA.

GM human cell lines. Accidental inoculation of sequences into laboratory workers by stick injury or entry via cuts and abrasions may constitute a hazard and therefore use of needles and scalpels will be limited, cuts and abrasions will be covered and gloves will be worn at all times. All cell work is carried out in Class 2 Safety hoods and use of filter tips limits aerosol formation.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM human cell lines. Accidental inoculation of sequences into laboratory workers by stick injury or entry via cuts and abrasions may constitute a hazard and therefore use of needles and scalpels will be limited, cuts and abrasions will be covered and gloves will be worn at all times. All cell work is carried out in Class 2 Safety hoods and use of filter tips limits aerosol formation.

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
Please enter comments on the GM safety committee on the risk assessment

No additional comments. Class 2 facilities are available for this activity.

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**Project Containment**

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**Project Ref 133/01.7**

**CU2 Project Title:** MECHANISMS OF MURINE HAEMATOPOIETIC CELL TRANSFORMATION AND APOPTOSIS

**Class:** Class 2

**CultureVol:** < 1 Litre

**Non-GMM Consent Granted:** Not Applicable

**Project notified under transitional arrangements:** N

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### Project Additional Information

**Purposes of the contained use**

Apoptosis regulatory genes and oncogenes will be introduced into established mouse and human cell lines in order to study the effects of these genes on apoptosis and their transforming capacity. Disabled retroviral vectors will be used to transduce the cells with members of the Bcl-2-gene family and with the oncogenes, MLL-AF4, MLL-ENL, MLL-AF9, TEL-AML, and TEL-JAK2.
Recipient or parental organism

E. coli K-12 multiple auxotroph XL1-Blue bacteria will be used. These bacteria are unlikely to survive in the human gut, lung or elsewhere in environment and are non-pathogenic to humans. NIH-3T3, BafF/3 and s49.1 mouse cell lines and primary cells, 293T human cell line. The mouse and human cell lines used are all long-term established cell lines and present no apparent hazard to workers or the environment. Work with primary mouse cells constitutes no higher risk than work with fresh tissue. Animals will be screened for the presence of pathogens prior to the donation of cells. All cells will be grown in a closed system. Despite the possibility that some of the genetic alterations planned may confer growth advantages to the cells, the derivative cell lines would not survive outside the culture conditions (ie. in the environment) used for their propagation. In the extremely unlikely event, that the cells are accidentally introduced into humans the cells would be very unlikely to survive because host allo- and zeno-immune responses would lead to their total destruction.

Host/vector system

pcDNA3.1 and pBluescript plasmid vectors
The plasmid vectors used in this work will not cause expression of inserted genes in XL1-Blue cells and they are non-mobilisable or mobilisation-defective. It is envisaged that neither the original XL1-Blue cells nor their genetically modified derivatives will be harmful to humans, since the risks of the bacteria infecting and persisting in humans and/or transferring genetic information to other micro-organisms is very low.

pMSCV and pRwevTRE retroviral vectors. Both these vectors are disabled retroviral vectors since they do not contain the gag (structural), pol (reverse transcriptase/integrase) and env (coat glycoproteins) genes necessary for particle formation, infection and replication. Replication-incompetent retroviral particles will be made by transfecting the retroviral vectors into the ecotropic Phoenix-eco (ATCC) packaging cell line. This cell line has the gag, pol and env genes stably integrated in its genome. For both the gag-pol and the env constructs non-moloney promoters were used to minimize recombination potential and different promoters for gag-pol and env were used to minimise their inter-recombination potential. After transfection with disabled retroviral vectors, the Phoenix-eco cells produce ecotropic viral particles containing envelope proteins derived from the Moloney Murine Leukaemia virus, which are only capable of infecting murine cells. The Phoenix-eco cell line has been established as being helper-virus free). Recombination would also be limited since retroviral vectors would only be transiently transfected into packaging cell lines and the cells would be disposed of after virus harvest (48 hours after transfection), thus long-term culture of packaging cell lines containing retroviral vectors would be avoided. In the extremely unlikely event of a replication competent virus being made, this would only be ecotropic and thus would not infect humans.

Origin & function

The DNA sequences that will be used in the project belong to the Bcl-2 family of apoptosis regulatory molecules or are known and presumed oncogenes.

Gene sequences: Oncogenes MLL-AF4, MLL-ENL, MLL-AF9, TEL-AML, TEL-JAK2
Apoptosis regulatory molecules Bcl-2, BclXL, BclX, Bid, Bim, Bak, Bax, Bad

Evaluation of foreseeable effects

It is expected that the gene sequences will be active in mammalian cells and will affect their growth and differentiation characteristics. Measures to avoid accidental inoculation and transmission of these sequences to the laboratory workers will be adhered to. For these sequences to be dangerous to humans or other organisms in the environment, they would have to integrate into the genome of the organism in question. Although there is a small risk of this once the DNA sequences are introduced into bacterial and mammalian cells, the use of non-mobilisable and mobilisation defective vectors, disabled retroviral vectors and disabled bacteria will minimise this risk.

Although there is no direct evidence that contact with naked oncogenic DNA can lead to tumours in humans it is intended to incorporate measures which will limit accidental inoculation or transmission of these DNAs to the laboratory workers.

All experiments will be carried out in ADCP level 2 laboratories with emphasis on the following points in accordance with our local rules and recommendations given in AcGM Compendium of Guidance Part 2A - Annex III and Part 3A - Annex 1.

1. Gloves will be worn at all times and changed when working elsewhere.
2. Steps are in place to limit aerosol production and aerosol contamination (filter tips)
3. Limited use of sharps and glassware to avoid accidental inoculation
4. Staff are aware of the hazards of handling oncogenic DNA

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The work with the genetically modified primary cells and established cell lines constitutes no higher risk than work with the parental cells.

Work with these cells is performed in a Class II safety cabinet and cells will be grown in a closed system.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All processes will take place in a closed system inside a class II tissue culture cabinet.

The Class II tissue culture cabinets will be swabbed with 70% IMS before and after use, and any spillages will be dealt with immediately using 1% Virkon followed by 70% IMS. In the event of a larger spill, paper towels will be used prior to disinfection.

All solid waste, such as discarded bags, pipettes, tips, towels, etc will be disposed of in autoclave bags. These will be removed when full and autoclaved prior to incineration.

The use of sharps (e.g. needles, Pasteur pipettes) will be avoided, however when use is unavoidable they will be safely disposed of using sharps bins.

These processes should give 100% kill.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The risk assessment for this activity has taken into account the oncogenic potential of the naked DNA sequences to be inserted in the GMMs and GMOs. Activity has therefore been designated as Class 2. Suitable precautions to limit the risk of accidental inoculation of naked oncogenic DNA are already practiced in the laboratories designated for Level 2 containment. All other working practices described are in accordance with our local rules.

Under these conditions, the risk to the environment or human health from the GMMs is negligible.

Project Containment

<table>
<thead>
<tr>
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</thead>
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02/03/2022
We are conducting a number of projects looking at inflammatory and immunological responses of human dendritic cell, neutrophils, endothelial cells in vitro to the organism Neisseria meningitidis. We will use genetically modified strains lacking a number of known virulence factors (lipopolysaccharide and capsule) to explore the relevance of their presence or absence on these responses.

Recipient or parental organism

Neisseria meningitidis serogroup B will be the recipient organism for this work. This is a gram negative organism which only infects humans. It is carried by up to 25% of the population, and can cause meningitis and/or meningococcal sepsis, particularly in children between 0-5 years, and young adults between 15-19 years old. Meningococcal disease (where there is both meningitis and sepsis) has a case fatality rate of approximately 10%. There is currently no vaccine for this serogroup.

Host/vector system

The strains are already transfected with linearised DNA containing an antibiotic resistance cassette, which results in the disruption of the gene of interest (lpxA and siaD).
These are stable transfectants and do not harbour transmissible plasmids. The kanamycin cassettes released from puc17 and the gene of interest is cloned in proprietary cloning vector pcr2.1. The gene is then excised by restriction digest and the antibiotic resistance cassette inserted. These are subcloned in E.coli, extracted and then linearised by restriction digest.

**Origin & function**

The bacterial strains to be used were generated by insertional inactivation of enzymes involved in the lipopolysaccharide biosynthesis and capsulation. In general all the mutant strains involve loss of virulence compared to the wild type parent strain. For example, one strain, ipxA (1) lacks any LPS, the major virulence factor in N.meningitidis. Other strains to be used include ipxA gene under control of an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible promoter allowing for controlled biosynthesis of LOS by the bacteria (2). In the presence of IPTG, this strain will have similar virulence potential to the wild strain but in the absence of external source of IPTG, bacteria harbouring sequence cannot produce LPS.

Another mutant strain, salD-, lacks the enzyme required for capsule biosynthesis (3). Almost all infection with N.meningitidis are due to capsulated strains therefore this mutant is of no greater virulence than wild type and potentially less.

The mutated strains will not be modified further in any way. These were kindly provided by Dr. Peter van der Ley from the National Vaccine Institute, RIVM, Bilthoven Netherlands. The description of the mutants are available in full in the following references:


They are only intended for in vitro stimulation work and will not be modified in any other way.

**Evaluation of foreseeable effects**

The genetically modified strains should have no more and probably less virulence potential than their wild type derived strains. For the purpose of the projects, they will be handled in the same fashion as the wild type organism.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Culture volumes will not exceed 40mls but in the main are only 5mls.
- Bacteria will be inactivated for use by 20 minute exposure to 2% paraformaldehyde, or heat inactivation at 560°C for 30 minutes. This is accepted practice in laboratories used to dealing with this organism. Testing for killing is not normally performed if equipment is functional; ie thermocouple thermometer in water bath is accurate and reagents are freshly prepared.
- Tissue culture incubated with live bacteria will be inactivated in either 2% formaldehyde or 4% paraformaldehyde. Liquid waste will be disposed of in 5% Hycolin (phenolic surface disinfectant), contained in pot within Class 1 cabinet. This is disposed of by laboratory staff at the end of each working day.
- Dry waste and agar plates are autoclaved.
- Sharps are incinerated.
- Small spills can be inactivated with squeeze bottle of 5% Hycolin allowed to soak for 30 minutes and mopped up with absorbent paper and autoclaved.
- All these waste management procedures are monitored and quality controlled in the Microbiology laboratory which has CPA accreditation.
All laboratory workers should have the necessary training and supervision on any work involving Genetically Modified Neisseria Meningitides and all safety precautions and measures should be placed in the laboratories carrying out this type of work. Furthermore, where possible, culture volumes should be kept to a minimum and all waste disposals should be treated with the appropriate detergent and disposed into designated clinical waste for incineration.

### Project Containment

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### Project Ref 133/10.1

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<td>Identification of genes promoting lethality of MYCN-amplified neuroblastoma cells using genome-wide RNAi screening</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Non-GMM</td>
<td>28/01/2013</td>
<td>N</td>
<td>Project notified under transitional arrangements N</td>
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### Additional Information

- **Historical Significant Changes**
- **Historical Date of Additional Info**
In this research project we aim to identify genes that promote cell lethality in the most aggressive forms of neuroblastomas using shRNAmir library.

Neuroblastomas is a paediatric tumour that derives from primitive sympathetic neural precursors. Amplification of MYCN oncogene is very frequent in a neuroblastoma and is often associated with advanced disease stages with poor prognosis and resistance to therapy. Interestingly, overexpression of MYCN protein in non MYCN-amplified cells can sensitize cells to drug treatment and induce cell death. This could suggest that survival and growth of MYCN-amplified tumour cells relies on expression of genes triggered by MYCN overexpression. When certain gene becomes essential for cell survival in response to MYCN overexpression, this gene can be called "synthetically lethal" partner of MYCN. The mechanism of synthetic lethality is very attractive for the development of drugs that could selectively kill tumour cells, since targeting the synthetic lethal partner of MYCN will cause growth inhibition of MYCN-amplified aggressive tumour cells leaving normal cells relatively intact.

The shRNAmir library consists of over 70,000 pGIPZ individual lentiviral vectors. Each vector contains a unique hairpin sequence designed to target and reduce the expression of most/all genes within the human neuroblastoma cell lines with the shRNAmir library. We will follow the change in relative abundance of individual shRNA over time by microarray hybridization to detect genes essential for cell proliferation since shRNAs important to cell survival will disappear from the population. We will pick the candidate genes by comparing the lethality profile of MYCN-amplified and non MYCN-amplified neuroblastoma cell lines and will validate the candidates after screening using available human neuroblastoma cells. The group of genes common for MYCN-dependent synthetic lethality in several neuroblastoma cell lines will be selected as the potential clinical targets that can selectively kill MYCN-amplified neuroblastoma cells.

Recipient or parental organism

Recipient/Parental organisms:
• Bacterial E.coli strains - HB101, XL1-blue, Top10F, DH5alpha.

Associated risks and likeliness of occurrence:
The bacterial strains used are attenuated non-colonising strains so are incapable of causing human infection and are therefore harmless to humans.
The viral vector to be used will be pseudotyped with the envelope protein VSV-G from Vesticular Stomatis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could enter human cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make a new progeny virus and establish a productive infection, its insertion into cellular DNA could result in a potentially oncogenic mutation. The recipient cells are harmless to humans.
The viral vectors are replication incompetent so therefore can not self-replicate and transfer to another host so are harmless to the environment. The cell that will eventually receive and integrate the DNA cannot survive in the environment as they are only viable under culture conditions and therefore are harmless to the environment. The bacterial strains used are attenuated non-colonising strains that cannot survive outside culture conditions and are therefore harmless to the environment. The viral vectors are replication incompetent so therefore can not self-replicate and transfer to another host so are harmless to the environment. The cell that will eventually receive and integrate the DNA cannot survive in the environment as they are only viable under culture conditions and are therefore harmless to the environment.

Host/vector system

Lentiviral vectors:
Commercial pGIPZ vectors fro Open Biosystems.
**Lentiviral Gag/pol expression vectors p8.9, pPAX2 and derivatives (HIV-1). Viral envelope expression vector pMDG and derivatives (VSV-G).**

**Associated Risks and likelihood of occurrence:**
The viral vector used is a lentiviral vector that contains the CMV (PolI) promoter to drive expression of the hairpin cassette. The lentiviral vector does not contain the appropriate accessory elements to form a viral particle itself and therefore poses no harm to health and the environment. Packaging and envelope proteins are contained on two additional plasmids to the lentiviral expression vector and these plasmids must be introduced into the cell and expressed alongside the lentiviral vector for viable lentiviral particles to be produced.

Woodchuck hepatitis B virus post-transcriptional regulatory element (WPRE) sequence is also incorporated into the pGIPZ vector in order to enhance the stability and translation of the transcripts. This negates the need for rev in the lentiviral vector packaging systems and improves biosafety by eliminating lentiviral genes from the system. However, the WPRE has potential promoter activity and the potential to express part of the X protein from woodchuck hepatitis virus (WHV) which may be oncogenic. There is no direct experimental evidence of oncogenic activities of the truncated fragment of X protein. Moreover, there is low probability that it would be expressed from the X promoter that is present in the WPRE since many studies have suggested that the X promoter is not functional in the absence of a second enhancer, which is not present in the WPRE. There is possibility of expression directed from other promoter/enhancer at chromosomal integration sites as well as the possibility that truncated X protein fragment has oncogenic properties. However, the likelihood of this is very low.

**Origin & function**

The inserted RNAi hairpins are sequences that have been derived de novo and are not from an organism. The sequences contain a 22bp sense and antisense sequence that is homologous to the gene of interest to which the hairpin is designed. These sequences are separated by a small loop sequence and flanked by mir-30 sequences. When transcribed in the cell, the RNA will form a hairpin structure which will initiate a normal cellular in vivo downstream cascade of processing events in response to foreign cellular single-stranded RNA intruder molecules. This process results in the post-transcriptional down regulation of the gene to which the hairpin sequence is designed and targeted by destroying the corresponding mRNA molecules prior to translation. The utilization of this normal endogenous pathway to down-regulate genes of interest is known as RNA interference.

**Evaluation of foreseeable effects**

**Risks and likelihood of risks:**
- The bacterial strains to be used are non colonising and incapable of surviving in the environment or causing human infection. They pose no risk to users or the environment.
- None of the DNA viral vector plasmids are harmful to health or to the environment and do not supply a survival advantage to any microorganisms. The packaging viral components are from the HIV-1 virus. HIV-2 is harmful to humans, however, the packaging vector does not contain full length HIV-2 molecule. Many components of HIV-1 that are critical for HIV-2 infection have been removed from the packaging vector. Only those essential for viral packaging remain.
- The most dangerous GMM is the lentiviral preparations of pGIPZ particles and the most dangerous step is the collecton, concentration and use of this lentivirus, however this risk is minimal. Lentiviral vectors can infect human cells and therefore if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate its genome into cellular DNA. If the inserted sequence were to be expressed in humans after accidental transfer the hairpin could lead to the down-regulation to the gene to which it targets via RNA interference. This could be a gene essential for growth regulation such as a tumour suppressor gene therefore resulting in a potentially oncogenic mutaion. Additionally, physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene, however the likelihood of this is very low. Theoretically the virus can also infect other mammalian cells and knockdown the gene of interest. However this is extremely unlikely as the hairpin is targeted to human genes and many hairpins are not homologous with the genes from other species. The overall risk is very low.
- Woodchuck hepatitis B virus post-transcriptional regulatory element (WPRE) in the pGIPZ vector has potential promoter activity and the potential to express part of the X protein from woodchuck hepatitis virus (WHV) which may be oncogenic. However, there is no direct experimental evidence of oncogenic activities of the truncated fragment of X protein and the likelihood that the expression of this protein would be directed from the promoter/enhancer at chromosomal integration sites in mammalian cells is very low. Any infection would not be severe as the virus is replication incompetent and therefore self-inactivating (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection. Also, it would only be a small number of cells and would not impact on the pathogenic outcome of an exogenous viral infection. Additionally, the viral particles are labile and do not survive on environmental surfaces outside the laboratory making infection unlikely.
- The cell lines used cannot survive outside the laboratory incubators and as such pose no threat to health or environment.
Precautions in place to minimize risks:
The Institute of Child Health is secured by restricted access at all entrances and additionally to all laboratory areas. Additionally, Molecular Haematology and Cancer Biology Unit has level 2 facility. All work involving viruses will be performed in class 2 cabinets in containment level 2 laboratories specified particularly for retroviral and lentiviral GMM work. All DNA plasmids containing viral proteins will be grown separately to avoid opportunity for recombination. To prevent contamination of workers by virus, when handling any GMM or GMM-derived DNA virus, no glass or other sharps will be used in the rooms where the viruses are prepared and used and workers will be protected by a lab coat and nitril gloves. Skin lesions will be covered with a bandage in addition to the protective wear described above. Specific guidelines will be in place to ensure all viral preparations are handled, labelled, stored, transported and cleaned up correctly. A separated clearly labelled compartment in the freezer to store viral supernatant will be used to prevent access to the stored materials for departmental staff and students, who are not involved in the project. All those involved in the project will be made aware of the guidelines and the associated risks as well as anyone else who may use the laboratory. Persons involved on project from other Institutes will work under the supervision of a competent trained worker from this project. No-one visiting the laboratory that is not involved in this work will work on this project. All persons entering the facility will be made aware of the safety precautions upon entering the facility. All equipment used in the level 2 laboratory will be available in other general purpose laboratories for general use so it should not be necessary for others who are not involved in retroviral or lentiviral work to enter the laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Virkon (w/v) will be used to treat liquid waste (18 hours) and also used to decontaminate any spills and for disinfection of any reusable bottles or other equipment and laboratory materials used. Liquid waste is subsequently poured down the sink after treatment with Virkon. Work surfaces will be wiped down with 1% Virkon and 70% IMS after use. Virkon is certified by the PHLS to provide 100% vircidal and GMO kill under these conditions by denaturing and inactivating viral and cellular proteins. Virkon will be used with their lifespan to ensure required kill is achieved. Solid waste will be disposed of into biological waste bags, sealed and autoclaved 132º for 15 mins by trained staff then bagged in yellow biohazard bags and removed by UCL waste services. Autoclave runs are regularly validated by waste management staff.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

A representative group of the UCL Institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees that this work should be classified as an activity class 2 (AC2) notification.

Project Containment

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<th>Growth Rooms</th>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

The aim of this project is to generate induced pluripotent stem (iPS) cells from mouse and human fibroblasts by employing lentiviral vectors coding for 4 reprogramming genes Sox2, Oct4, Klf4 and c-Myc. iPS cell technology has the potential to transform regenerative medicine especially in the areas of stem cell transplantation and patient-specific gene therapy. Briefly, mouse and human fibroblasts will be infected with 4 different lentiviral constructs and reprogrammed to become embryonic stem (ES) cell like cells which are known as iPS cells. Once generated, these cells can be expanded at will maintaining their undifferentiated pluripotent state and can be subjected to differentiation pathways eventually leading to the formation of terminally differentiated somatic cells. As for example, these iPS cells can be induced to become different kinds of blood cells. The efficiency of reprogramming is pretty low (0.01-0.05%) and to obtain sufficient number of iPS cell generation. Upon successful execution, the iPS cells generated from this project will help us to study various genetic defects either from murine disease models or from human patient samples and would be crucial in our therapeutic approaches.

**Recipient or parental organism**

E.coli disabled strains (e.g. DH5alpha), Mammalian cell lines (established, commercially available) and mammalian primary cells. Neither of these are pathogenic to humans, nor capable of survival in the environment.
Host/vector system

Prokaryotic plasmids, eukaryotic expression vectors, replication-deficient self-inactivating (SIN) lentiviral vectors,
The lentiviral vectors used are multi-attenuated meaning it is devoid of all potentially pathogenic HIV-1 encoded functions (1.2). In addition it is replication-defective which means that the vector cannot multiply on its own unless supplied by certain proteins in trans. It is self-inactivating which means that the viral promoter in the U3 region of the 5' LTR has been disabled by genetic manipulation. Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The probability of such an event is extremely low.


Origin & function

Mus musculus, Homo sapiens sapiens

Production of transcription factors that will reprogram the somatic cells to become iPS cells

The vector sequences (sox2, oct4, klf4 and c-myc) code for oncogenic products which if accidentally transferred to a human host might cause tumour formation depending on the size of the inoculums but is most likely expected to be nullified by immuno-competent individual.

Sequences cannot cause harm if transferred to species in the environment; the donor organism is not pathogenic

Evaluation of foreseeable effects

The donor organism does not have apathological or harmful characteristics

Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The probability of such an event is extremely low. The most hazardous GMM are the lentivirus vectors and the most hazardous step is the harvesting of the viral supernatant before infecting the target cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMMs are inactivated for disposal using standard biosafety level 2 procedures. Briefly, liquid and solid wastes are treated with PRESEPT effervescent disinfecting tablets (2.5g). All plastic ware used in tissue culture are autolaved. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern. Proline and IMS are used which have been shown to be 100% effective in tissue-culture related decontamination.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
A representative group of the UCL institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees that this work should be classified as an activity class 2 (AC2) notification.

**Project Containment**

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**Project Ref**  133/10.3

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<td>24/04/2012</td>
<td>Phase I/II Clinical Trial of Haematopoietic Stem Cell Gene Therapy for Wiskott-Aldrich syndrome</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
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**Project Additional Information**
Purposes of the contained use

To undertake gene therapy on autologous HSC from patients with Wiskott Aldrich Syndrome. The study is summarised below.

Phase 1/2 clinical trial of haematopoietic stem cell gene therapy for the Wiskott-Aldrich Syndrome
This is an open labelled, non-randomised, single centre, phase I/II, cohort study involving a single infusion of autologous CD34+ cells transduced with the w1.6_hWASP_WPRE (VSVg) lentiviral vector in up to 5 patients with WAS.

Primary Objectives
To safely administer a lentiviral gene therapy vector encoding the human WAS cDNA to stem cells from WAS patients.
To provide sustained engraftment of WASP-expressing transduced cells, reconstitution of humoral and cell mediated immunity, and correction of microthrombocytopenia

Secondary Objectives
To improve the overall health of the patient, including reduction in frequency of infections, resolution of autoimmunity, and improvement in eczema, reduction in bruising and bleeding episodes.
To evaluate the longitudinal clinical effect in terms of augmented immunity.

Recipient or parental organism

The lentiviral vector (LV) vector used in the proposed clinical trial is a 3rd generation replication-defective hybrid viral particle made by core proteins derived from Human Immunodeficiency virus type 1 (HIV-1) and the envelope of the unrelated Vescicular Stomatitis Virus (VSV). The transfer vector encodes the 12 exons of the human WAS cDNA under control of a 1.6 kb endogenous promoter sequence and leads to the integration of a 5.6 kb sequence.

Replication deficient lentiviral vector is used to transduce hematopoietic cells ex vivo. The GMP grade lentiviral vector will not be directly administered to patient.

Host/vector system

Lentiviral vector (w1.6_hWASP_WPRE (VSVg). There is no recommended International Nonproprietary Name (INN). The vector is pseudotyped with the VSV.G envelope.

The lentiviral vector is a 3rd generation replication-defective, self inactivating, hybrid viral particle made by core proteins derived from HIV-1 and the envelope glycoprotein of the unrelated Vescicular Stomatitis Virus (VSV). The transfer vector encodes the 12 exons of the human WAS cDNA under control of its native promoter. This lentiviral vector leads to the integration of a 5.9 kb proviral sequence into the cellular genome.

The system is based on 4 non-overlapping expression constructs in order to maximize the segregation of cis and transacting functions. The system is engineered in such a way that minimal homology regions are present between packaging and transfer vectors, thus minimizing the likelihood of homologous recombinational events and the generation of replication competent lentiviruses (RCLs).

In addition, the packaging construct is deleted of all HIV accessory proteins (vpu, vpr, nef, vif) and Tat. The conditional packaging system segregates gag/pol and rev genes in two separate plasmids. The Rev responsive element (RRE) maintained in the gag/pol plasmid makes the gag/pol gene expression rev dependant. As the transcripts of gag and pol genes contain cis acting repressor sequences, they are expressed only in the presence of Rev, expressed in trans on separate plasmid, which promotes their nuclear export and expression by binding to RRE.

The transfer vector codes for the therapeutic human WASP gene and for the sequences necessary for expression, incapsidation, reverse transcription and integration of the viral genome. RRE sequences permit the nuclear export and expression of the viral RNA. In order to minimize the risk of RCL generation, the 3' LTR of the transfer vector has been deleted in the U3 region. The introduction of this 400 bp deletion abolishes the production of full-length vector RNA in transduced cells by exploiting the reverse transcriptase mechanism which generates both U3 regions from the 3' of the viral genome, thus transferring the deletion to the 5' LTR of the proviral DNA. The lentiviral particle is therefore conceived with a self-inactivating mechanism that enables the production of infective particle only in the presence of accessory plasmids.

Origin & function

The human WAS protein (WASp) is expressed exclusively in haematopoietic cells where it functions as a regulator of actin cytoskeleton reorganisation by linking various
types of signals to Arp2/3-mediated actin polymerization. Two main molecular activators of WASP are Cdc42 and PIP2. In T cells, WASP is an essential component of the signal transduction cascade initiated by T cell receptor engagement contributing to the establishment of immunological synapse and T cell activation.

Risk of transfer to environmental species is negligible, and would not be expected to cause harm

**Evaluation of foreseeable effects**

The potential for harm is negligible. Work will be at level 2 containment.

All personnel involved in the ex vivo transduction of HSC at ICH/GOSH are trained in GMP cell processing procedures and there competency is assessed every 6 months. Sharps will not be used for the direct manipulation of vector.

A policy is in place for accidental needlestick injuries and can then be assessed via our hospital website (section 2.2.3 http: www.ich.ucl.ac.uk/clinical_information/clinical_guidelines/cpg_guideline 00121. A copy of the document is appended

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

NA

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Autoclave facilities are in an adjoining building. However, all waste will be double bagged and transported by the lab worker in accordance with a written operating procedure to the autoclave facility and this will be recorded in the batch manufacturing record. Sharps will be disposed of in appropriate puncture-proof containers.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

For routine cleaning all surfaces are wiped down with 70% ethanol after use. Liquid GMM waste is first disinfected using Sanichlor (effervescent chlorine tablets) to give a final concentration of 2500ppm chlorine overnight before being disposed of down the sink. This is a standard procedure and viability is below detectable levels. Any solid GMM waste is placed in autoclave bags, labelled with the department's name and autoclaved at the ICH. All sharps are placed in puncture-proof containers and disposed of through the central collection service for contaminated waste. All spillages are cleaned using 1% sodium hypochlorite or 70% ethanol.

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

**Please enter comments on the GM safety committee on the risk assessment**

External reviewer Prof F F, Rayne Institute, Kings College London has advised that the procedures outlined for this study should be undertaken under level 2 containment conditions.

The cell handling and transduction procedures have been approved by the NHRA

The site has been awarded a manufacturing licence for this project.

**Project Containment**
Kidney and urinary tract malformations are the commonest cause of end-stage renal failure in children, whilst progressive destruction of normal kidney tissues by cysts or interstitial fibrosis account for most cases in older patients. Very few of these conditions are monogenic but we have an increasing understanding of the processes involved in maldevelopment, cyst formation and fibrosis, which highlight potential targets for therapies. An example is the multifunctional lectin, galectin-3; this molecule is expressed in normal collecting ducts in the developing kidney and exogenous therapy reduces growth of collecting duct-derived cysts in vitro.

Lentiviruses are excellent tools for gene delivery, due to their relatively large packaging capacity and ability to infect a wide range of cell types when coated with different envelopes. Gene therapy requires a functional version of a gene to be delivered and then expressed over long periods. The aim of this project is to develop lentiviral vectors to modulate galectin-3 in the kidney.

Recipient or parental organism
E. coli disabled strains (e.g. DH5alpha)
Mammalian cell lines (established, commercially available such as human embryonic kidney 293 cells, renal proximal tubular epithelial cells, inner medullary collecting duct cells and Madin-Darby kidney cells) Mammalian primary cells (primarily kidney-derived, such as glomerular, proximal and collecting duct cells, and human fetal renal progenitor cells)
Whole kidneys in organ culture

Mice strains

The bacteria and recipient cells used in this project are not hazardous to human health because they are incapable of causing human infection. Nevertheless, we will still maintain the highest standards to prevent accidental spillage or splashing of workers, and ensure that the equipment is cleaned and disposed of correctly. The infected mice are no greater than normal animals because the virus is not shed; hence standard animal handling precautions will be used. These currently include a gown, protective clothing, gloves and masks (to reduce mouse allergen exposure of the workers). Injection involves use of a needle which would be hazardous in a mobile, mature mouse. However, we use newborn mice which are easily immobilised by hypothermia, and the injection procedure is then carefully controlled by two workers - one places the needle into the vein and holds it in position whilst the other does the injection. These procedures will be done in quiet rooms with limited access, that meet containment level 2 standards, using disposable materials that will be removed at the end of the procedure. The area will then be cleaned and signed off as ready for future work by our personnel.

Recombinant (inactivated non-replicating) lentiviral vectors. Replication deficient lentivirus particles are generated in cell lines following co-transfection of three different plasmids. These supply the self-inactivating transfer vector containing galectin-3 and viral long terminal repeat (LTR) (but lacking any expression of HIV genes; packaging and structural proteins, gag/pol; and envelope (env), vesicular stomatitis virus g (VSV-g). The vector itself is self-inactivating due to a deletion in the promoter enhancer region of the 3LTR. The only expression from the vectors is of the transgene cDNA, controlled by an internal promoter such as SFFV. Galectin-3 mRNA has been cloned into a VSV-G pseudotyped HIV-1-based vector by GeneArt, a commercial biotechnology company. This strategy is similar to the production of Mertk as described in Tschemutter M et al. Gene Ther. 2005 12:694-701.

There is a finite (albeit very small) risk that lentiviral vectors could infect human cells, but we will be using multi-attenuated lentiviruses that are replication incompetent because they lack the gag, pol or env genes. Hence, they are unable to spread even if workers are accidentally exposed by injection, ingestion or through a wound; risk of entry through these routes will be limited by wearing appropriate labwear and gloves, and completely covering open wounds. Although replication incompetent, lentivirus could theoretically insert into cellular DNA and this might result in a potentially oncogenic mutation. The infection with high titre lentiviral vectors encoding growth promoting molecules and selection in vivo for cells with altered growth potential. No adverse affects have ever been described through he accidental exposure to retroviral vectors. The major risks in this project are when the lentivirus is being prepared and then injected but this will be done into immobilised mice by experienced staff, in quiet CL2 standard rooms, wearing a laboratory coat and latex or nitrile gloves.

Origin & function

Gene Sequences: cDNAs encoding human and wild-type and modified galectin-3

Origins and functions of the genetic material involved: Galectin-3 is a lectin that modulates cell adhesion, differentiation and proliferation. It is normally expressed during mammalian development in the kidney, and reactivated in diverse diseases. We predict that increased galectin-3 will reduce the severity of cyst formation and fibrosis in vivo.

Will the sequences cause harm if expressed in humans? Galectin-3 is involved in multiple cellular processes including cancer but the lectin does not have any direct oncological effects by itself, indeed treatment with recombinant galectin-3 protein has been shown to reduce cancer metastases in mice models and the severity of several other renal diseases. Therefore, any accidental increased expression of galectin-3 is unlikely to have any detrimental effects on humans.

Will the sequences cause harm if transferred to the environment? Galectin-3 is a naturally occurring lectin and therefore highly unlikely to cause environmental harm. The potential routes of transmission or escape to the environment of the virus that may occur are known (air or liquids) and full protective measures will be taken to minimise or prevent the access of the virus to other organisms and the environment.

Evaluation of foreseeable effects

The biggest potential harm would be accidental transfer and overexpression of galectin-3 in humans by needlestick injury when the virus is being injected. These risks will
be reduced by carefully controlling the mice, procedure and environment for the injection. We use new born mice which are easily immobilised by hypothermia, and the injection procedure is then done by two workers - one places the needle into the vein and holds it in position whilst the other does the injection. Dr ***, will teach us how to do this procedure and he has already done this over a thousand times without mishap. Even with accidental human injection, the risks are minimal because we are using multi-attenuated lentiviruses which are unable to spread in humans even if they do infect cells.

In addition, we have in place in association with UCL Biological Services various specific control measures for the lentivirus experiments to minimise exposure and have completed a separate risk assessment for all personnel involved in the animal work. The general work will be performed in a containment level 1 environment but preparation work with virus handling will be in level 2 safety cabinets and injection will be in a quiet room that meets CL2 requirements; personnel involved with the virus work will wear gloves, glasses and a mask.

In the case of a laboratory accident, such as defective/leaking plasticware or dropped samples leading to viral spillage, all the necessary procedures will be applied to eliminate any potential hazardous effects of the virus in the environment. These include: wash with virkon, detergents, and disinfectants. Both wild-type and optimised lentiviruses are sensitive to UV, temperature and other physical agents so their chances of survival are low. All of the liquid waste will be treated with virkon and autoclaved; solid waste will be autoclaved and disposed as biohazard materials.

In the case of a needlestick injury, we will contact occupational health immediately or attend an Accident and Emergency Department immediately to assess injury and treatment required for the penetrating wound. Risks from viral inoculation are minimal as described above.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

1. 5% Virkon or equivalent PHLS certified treatment will be used to treat liquid waste (18 hours) and also any spills and for disinfection of equipment and laboratory materials. Work surfaces will be wiped down with this and 70% ethanol after use. Sharps will be placed in sharps bins that will be autoclaved.

2. 199%

3. Virkon is certified by the PHLS to provide 100% viridical kill under these conditions. Additionally, we will ensure that only in-date products are used to maintain optimal efficiency. Autoclave runs are regularly validated by waste management staff.

4. Solid waste will be double-baged in biological waste bags and sharps in sharps bin. Both are autoclaved at 132°C for 15 minutes, then collected for disposal (usually incineration) by the contractors employed by ICH to handle potentially infectious clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
A representative group of the UCL Institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees that this work should be classified as an activity class 2 (AC2) notification.

**Project Containment**

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 133/11.1

- **Date Ackn'd**: 24/04/2012
- **CU2 Project Title**: Lentiviral vector manipulation for shRNA-mediated gene knockdown in mammalian cell lines
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **CultureVolumeClass3-4**: Non-GMM
- **Consent Granted**

**Project notified under transitional arrangements**

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The shRNAmir laboratory is available from Open Biosystems through UCL, and consists of over 70,000 individual E. coli glycerol stocks containing the pGIPZ lentiviral vector. Each stock contains a unique hairpin sequence within the pGIPZ vector designed to target and reduce expression of most/all genes within the human genome via RNAi interference. We will also use other selected shRNA sequences that we will clone into either pGIPZ or another lentiviral vector, pLL3.7, ourselves in case hairpins from the Open Biosystems stocks are ineffective, or if knockdown in non-human cell lines is required.
This project will involve maintenance, storage and use of this shRNAmir library for use in various projects. Specifically, this project will involve lentiviral production from either pGIPZ or pLL3.7 plasmids and the subsequent use of this lentivirus in cell based screening functional assays. Additionally, clones will be pooled at the bacterial, DNA and viral level to make various intermediate pools of clones to be used to transduce cells in screens and subsequently undertake functional assays.

**Recipient or parental organism**

<table>
<thead>
<tr>
<th>Cell lines:</th>
<th>ATDC5, canine-MDCK, DK, D17, C2F; feline-AH927, CRFK, Mya1; Human-HeLa, TE671, HT1080, 293T, HEL, HFFF, BJ1, Jurkat, CEM, supt1, C8166, A549, LECs, tMSCs; Mouse-MDTF, NIH3T3; Avian- QT36; Marsupial- SC300; Bat Tb1lu; non-human primate- FRhK4, LLCMK2, CV1, VERO, Pindak, SMLF; bovine- MDBK, IMR31; Porcine- PK15, SKL, CPK, STIOWA; Mink- mv-1-lu; Rabbit- SIRC, eREP, ratHSN, RAT2, 9L, NRK, TerRPE, human and mouse ES cell lines.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Human cells:</td>
<td>Derived from various human tissues including peripheral blood derived lymphocytes and macrophages.</td>
</tr>
</tbody>
</table>

The bacterial strains used are attenuated non-colonising strains so are incapable of causing human infection and are therefore harmless to humans. The viral vector to be used will be pseudotyped with the envelope protein VSV-G from Vesticular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could enter human cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection, its insertion into cellular DNA could result in a potentially oncogenic mutation. The recipient cells are harmless to humans.

The bacterial strains used are attenuated non-colonising strains that cannot survive outside culture conditions and are therefore harmless to the environment. The viral vector to be used will be pseudotyped with the envelope protein VSV-G from Vesticular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could enter human cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection, its insertion into cellular DNA could result in a potentially oncogenic mutation. The recipient cells are harmless to humans.

The bacterial strains used are attenuated non-colonising strains that cannot survive outside culture conditions and are therefore harmless to the environment. The viral vectors are replication incompetent so therefore cannot self-replicate and transfer to another host so are harmless to the environment. The cell that will eventually receive and integrate the DNA cannot survive in the environment as they are only viable under culture conditions and therefore are harmless to the environment.

**Host/vector system**

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>The viral vector used is a lentiviral vector that contains the CMV (Pol II) promoter to drive expression of the hairpin cassette. The lentiviral vector does not contain the appropriate accessory elements to form a viral particle itself and therefore poses no harm to health and the environment. Packaging and envelope proteins are contained on two additional plasmids to the lentiviral expression vector and these plasmids must be introduced into the cell and expressed alongside the lentiviral vector for viable lentiviral particles to be produced.</td>
<td></td>
</tr>
</tbody>
</table>

**Origin & function**

| The RNAi hairpins are sequences that have been derived de novo and which are not derived from known sequences with both a sense, antisense and loop sequence to form a RNA hairpin when transcribed which will initiate a downstream cascade of events leading to knockdown of the target gene to which the hairpin was designed. |
|---|---|
| The accessory proteins are from the HIV-1 virus. |
The hairpin sequence will be transcribed and the RNA will then form a hairpin structure. The hairpin structure will induce a normal cellular in vivo downstream sequence of processing events in response to foreign cellular single-stranded RNA intruder molecules that will result in the post-transcriptional down regulation of the gene to which the hairpin sequence is targeted.

If the inserted sequence were to be expressed in humans after accidental transfer the hairpin could lead to the down-regulation of the gene to which it targets via RNAi interference. This could be a gene essential for growth regulation such as a tumour suppressor gene therefore resulting in a potentially oncogenic mutation. This, however, would not be severe as the virus is replication incompetent and therefore self-inactivating as none of the structural genes are actually present in the packaged viral genome, such that no new virus can be produced and no other cells can be infected. Physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene, however the likelihood is very low.

VSV-G pseudotyped lentivirus can only infect mammalian cells. Potentially the hairpin could be expressed in another mammalian species and lead to the down-regulation of a growth regulatory gene such as a tumour suppressor gene resulting in a potentially oncogenic mutation. This, however is extremely unlikely as the hairpin is targeted to human genes and many hairpins are not homologous with the genes from other species. If the hairpin were to target and lead to the reduction of a gene via RNA interference it would not be severe as the virus is replication incompetent and therefore self-inactivating as none of the structural genes are actually present in the packaged viral genome so no new virus can be produced and no other cells can be infected.

The envelope protein is VSV-G from the Vesticular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection, its physical insertion into cellular DNA could result in a potentially oncogenic mutation. This would be a very unlikely event and if so only a very small number of cells and would not impact on the pathogenic outcome of an exogenous viral infection. The packaging viral components are from the HIV-1 virus. The full length HIV-1 virus is harmful to humans, however the packaging vector used does not contain the full length HIV 1 molecule. Many components of HIV-1, that are critical for HIV-1 infection but not required for viral packaging, have been removed so the virus is attenuated.

The de novo hairpin donor sequences could potentially be expressed in cells and lead to the knockdown of certain genes leading to the increase of oncogenic potential of the virus. This would, however, only be a very small number of cells and as the virus is replication incompetent and cannot make new viral progeny, it would not impact on the pathogenic outcome of an exogenous viral infection.

Evaluation of foreseeable effects

The envelope protein is VSV-G from the Vesticular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection, its physical insertion into cellular DNA could result in a potentially oncogenic mutation. This would be a very unlikely event and if so only a very small number of cells and would not impact on the pathogenic outcome of an exogenous viral infection. The packaging viral components are from the HIV-1 virus. The full length HIV-1 virus is harmful to humans, however the packaging vector used does not contain the full length HIV 1 molecule. Many components of HIV-1, that are critical for HIV-1 infection but not required for viral packaging, have been removed so the virus is attenuated.

The de novo hairpin donor sequences could potentially be expressed in cells and lead to the knockdown of certain genes leading to the increase of oncogenic potential of the virus. This would, however, only be a very small number of cells and as the virus is replication incompetent and cannot make new viral progeny, it would not impact on the pathogenic outcome of an exogenous viral infection.

The most dangerous GMM, is the lentiviral preparations of pGIPZ particles. The most dangerous step is the collection, concentration and use of the lentivirus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

2.5% Trigene advance or Virkon (v/v) will be used to treat liquid waste (18 hours) and also used to decontaminate any spills and for disinfection of any reusable bottles or other equipment and laboratory materials used. Liquid waste is subsequently poured down the sink after treatment with Virkon. Work surfaces will be wiped down with 5% Trigene advance and 70% ethanol after use. Virkon and Trigene advance are certified to provide 100% vircidal and GMO kill under these conditions by denaturating and inactivating viral and cellular proteins. Trigene advance and Virkon will be used with its lifespan to ensure required kill is achieved. Solid waste will be double bagged in biological waste bags, sealed and autoclaved 132°C for 15 mins by trained staff then bagged in yellow biohazard bags and removed by UCL waste services. Autoclave runs are regularly validated by waste management staff.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

A representative group of UCL institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees that this work should be classified as an activity class 2 (AC2) notification.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<td>Animal Units</td>
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<td>L2 L2 L3 L4 L2 L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
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</table>

Project Ref 133/11.2

Date Ackn'd 24/04/2012

Date Project Ceased

CU2 Project Title Immortalization of human cells

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted
Purposes of the contained use

The aim of this project is to immortalize human cells (e.g. skeletal muscle derived myoblasts and stem cells, blood-derived stem cells and fibroblasts). Cells will be prepared from normal individuals and from patients with neuromuscular diseases (e.g. Duchenne muscular dystrophy). Myogenic cells have a limited life span in culture, which prevents their expansion to sufficient numbers for experimentation and seriously limits any potential use in cell replacement or ex vivo gene therapy. An immortalization protocol for normal human cells (e.g. myoblasts) would allow one to isolate cellular models from various neuromuscular diseases, thus opening the possibility to develop and test novel therapeutic strategies. We intend to use a method developed by M and colleagues (names removed. Aging cell. 2007 Aug; 6(4):515-23. Epub 2007 Jun 8: names removed Biochem Biophys Res Commun. 2009 Oct 16;388(2):333-8. Epub 2009 Aug 6). Cells will be infected with lentiviruses coding for hTERT and cdk-4, selected by antibiotic resistance, expanded in culture and stored in liquid nitrogen. Immortalized cells will be stored and distributed under the auspices of the MRC Centre for Neuromuscular Diseases Biobank.

Recipient or parental organism

HIV.PGK.Puro.CMV.hTERT
HIV.PGK.Neo.CMV.cdk4
Human myoblasts, fibroblasts and stem cells.

The lentiviral vectors used are multi-attenuated, meaning they are devoid of all potentially pathogenic encoded functions. In addition the are replicon-defective which means that the vector cannot multiply on its own unless supplied by certain proteins in trans. It is self-inactivating which means that the viral promoter in U3 region of the 5' LTR has been disabled by genetic manipulation. Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The probability of such an event is extremely low.

The intended recipient organisms are not capable of independent survival in the environment and will not infect or transfer to other hosts.

Host/vector system

HIV-derived lentiviruses, prepared in 293 Cells:
HIV.PGK.CMV.hTERT
HIV.PGK.Neo.CMV.cdk4

The lentiviral vectors used are multi-attenuated, meaning they are devoid of all potentially pathogenic encoded functions. In addition they are replication-defective which means that the vector cannot multiply on its own unless supplied by certain proteins in trans. It is self-inactivating which means that the viral promoter in the U3 region of the 5' LTR has been disabled by genetic manipulation. Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The probability of such an event is extremely low.
WPRE is not present in either lentivirus.

**Origin & function**

Mus musculus, Homo sapiens sapiens

Lentiviruses - can infect human cells.
Puro and Neo - antibiotic resistance genes.

H-TERT - catalytic subunit of telomerase, that lengthens telomeres in DNA strands. Expression of telomerase bipasses telomere-dependent senescence so that cells that would otherwise become postmitotic and undergo apoptosis exceed the Hayflick limit and become potentially immortal.

Cdk4 is the catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression; its expression prevents p16INK4a-associated premature growth arrest.

Cdk4 therefore extends life span and immortalization is induced by hTERT.

There would be a risk of cell immortalization if cdk-4 and h-TERT were introduced into human cells.

The risk of transfer to species in the environment is negligible, but would be a risk of cell immortalization if cdk-4 and h-TERT were introduced into human cells.

The lentiviruses are HIV-derived, but are replication-deficient, therefore represent a low risk.

The donor organism has no pathological or harmful characteristics

**Evaluation of foreseeable effects**

The lentiviruses are HIV-derived, but are replication-deficient, therefore represent a low risk.

The donor organism has no pathological or harmful characteristics.

Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type virus. The probability of such an event is extremely low. The most hazardous GMM are the lentivector viruses and the most hazardous step is dealing with concentrated viral stocks before infecting the target cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All GMMs are inactivated for disposal using standard biosafety level 2 procedures. Briefly, liquid waste is treated with 1% Virkon. Work surfaces are decontaminated on completion of work, or at the end of the day, or after any spill or splash of viable material, with 1% Virkon. Virkon has been shown to achieve 100% GMO kill when used as directed. After decontamination, liquid waste is disposed of via the sink. Solid waste is double-bagged in biological waste bags, sealed, transported to the autoclave,
autoclaved and then removed by waste services

A representative group of the UCL Institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees this work should be classified as an activity class 2 (AC2) notification.

Please enter comments on the GM safety committee on the risk assessment

A representative group of the UCL Institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees this work should be classified as an activity class 2 (AC2) notification.

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</table>

**Project Ref** 133/95.1

**Date Ackn’d** 24/04/2012

**CU2 Project Title**

CONSTRUCTION OF REPLICATION DEFECTIVE RETROVIRAL VECTORS FOR BTK GENE TRANSFER AND EXPRESSION

**Class** Class 2

**Consent Granted** Not Applicable

**Project notified under transitional arrangements** Y

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**
Significant Change ID
Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
Project Ref 133/99.1

Date Ackn'd 24/04/2012

CU2 Project Title TRANSDUCTION EXPERIMENTS WITH MINIMAL HUMAN IMMUNODEFICIENCY VIRUS (HIV) VECTORS

Class 2

Non-GMM Not Applicable

Project notified under transitional arrangements 

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 133/99.2

Date Ackn’d 24/04/2012
CU2 Project Title TRANSDUCTION EXPERIMENTS WITH IMPROVED EQUINE INFECTIOUS VIRUS
Class 2
CultureVolClass2
CultureVolumeClass3-4
**Date Project Ceased**

(EIAV) VECTORS

**Non-GMM**

Consent Granted

Not Applicable

**Project notified under transitional arrangements**

Y

Withdrawn

N

Tick if notifying a connected programme of work

N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 14/01.1

CONSTRUCTION AND PRE-CLINICAL TESTING OF MODIFIED FORMS OF HERPES SIMPLEX VIRUS FOR VACCINATION PURPOSES (PROJECT TRANSFERRED TO GM 785)

Class 2

Non-GMM not applicable

Project notified under transitional arrangements
**Purposes of the contained use**

Construction and pre-clinical testing of modified forms of herpes simplex virus for vaccination purposes.

**Recipient or parental organism**

Herpes simplex virus strains with attenuating mutations but in which replication competence is retained. Attenuating mutations remove HSV genes, which usually inhibit immune responses and also render the virus non-pathogenic. These genes are VHS and/or ICP47 and may additionally include further mutations in ICP34.5, UL43, U35 and/or vmw65. The modified HSV strains also have genes encoding non-HSV antigens inserted (from infectious disease causing agents or which are selectively expressed in tumour cells) and may additionally have inserted genes encoding immune modulatory molecules such as cytokines and chemokines.

The work aims to include immune responses to the delivered antigens and to untoward effects are anticipated. The mutations to the virus have previously been shown to render the virus non-pathogenic and the genes to be delivered are not anticipated to be harmful according to previous work published in the literature.

**Host/vector system**

Herpes simplex virus 1 or 2.

**Origin & function**

Inserted genetic material is of either human or rodent origin and has been cloned by PCR or obtained from collaborators in plasmid form. Genes to be inserted include tumour antigens such as MART-1, MAGE-1, tyrosinase, gp100, Her2neu, Muc-1, PS1, CEA for the development of antitumour vaccines. Antigens from a variety of infectious agents for vaccine development purposes will also be used as will human or rodent forms of various immunomodulatory molecules eg GM-CSF, IL12, CD40L, B7.1, RANTES. The immunomodulatory genes are aimed at the enhancement of the immune response to delivered antigens.

**Evaluation of foreseeable effects**

None of the genes to be inserted are anticipated to result in harmful effects.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste generated during the activity that has been in contact with the GMM will be treated with a Virkon solution. This broad spectrum disinfectant is effective against herpes simplex virus at a 0.5% (w/v) concentration giving terminal disinfection after 10 minutes of contact.

After treatment with the above disinfectant, the waste will then be placed into clinical waste bags and burnt offsite.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
The GMSC opinion is that the viruses to be used are probably Class 2, although as the risks are low, Class 1 could be considered appropriate.

Project Containment

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Project Ref  14/01.3

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<td>DEVELOPMENT OF RETROVIRUS VECTORS AND STUDIES OF RETROVIRUS BIOLOGY USING RECOMBINANT VIRUSES.</td>
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<th>Class</th>
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<td>&lt; 1 litre</td>
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Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: N

Historical Significant Changes

Project Additional Information
Purposes of the contained use

Retrovirus vectors based on an oncovirus, murine leukaemia virus (MLV), or lentiviruses, such as human immunodeficiency virus and equine infectious anemia virus, are promising tools for gene therapy. We aim to develop more efficient and safer vector systems. Better understanding of retrovirus biology, namely how retroviruses interact with host cells and how retrovirus genes function, will lead to such improved vector systems as well as insights into prevention of virus infection, treatment for retrovirus diseases.

Recipient or parental organism

Cells:
Mammalian cell lines: human TE671, HT1080, Hela, Raji, 293, 293T, mink Mv-1-Lu, dog D17, Cf-2-Th, cat CCC, CRFK, mink Mv-1-Lu, mouse NIH3T3, balb/c3T3, MDTF, rat NRK, HSN, pig PK15, PAE, ST-IOWA cells.
Avian cells include quail QT6 and chicken CEF cells.
In addition, primary human, pig, mouse and rat cells may be used, but not of the origin of any worker at Room 302B.

Viruses:
Mammalian type C viruses
which infect human cells in vitro, but not known to infect humans;
porcine endogenous retrovirus, cat endogenous retrovirus RD114, gibbon ape leukemia virus, amphotropic/xenotropic/ polytropic murine leukemia viruses, feline leukemia viruses, baboon endogenous retrovirus Murine type C virus which does not infect human cells: ecotropic murine leukemia virus.

Host/vector system

1. Mammalian cell cultures/Mammalian type C viruses
2. Mammalian cell cultures/Retrovirus vectors based on MLV, HIV and EIAV
3. Culture cells/Cloning plasmids, (lambda) derived disabled phage vectors, eukaryotic expression plasmids, retroviral cDNA expression vectors.
4. Animal retroviruses which are not human pathogen/Cloning plasmids

Origin & function

1. MLV, HIV, EIAV-based vectors with markers: lacZ, green fluorescence proteins (gfp), and neomycin, puromycin, phleomycin resistance genes
2. MLV vectors containing SV40 T-Ag, polyoma virus T-Ag, the catalytic component of human telomerase and marker genes.
3. Cellular genes, unlikely to be oncogenic. Protein tags include green fluorescent proteins, His/myc/flag tags, bacterial lacZ.
4. Genes from animal retroviruses which are not human pathogen: Retrovirus-like sequences endogenous to mammalian cells, including humans. Protein tags including green fluorescent proteins, His/myc/flag tags, bacterial lacZ.

Evaluation of foreseeable effects

The major risk associated with culture cells producing whole, infectious viruses is the possibility of infection of laboratory workers. Pseudotyping is unlikely to cause risk additional to that of RCV, which were classified as non-pathogenic Group 1, by GMAC committee at our former institute, Institute of Cancer Research in 1995. It is proposed to carry out this work at the containment level 2 handling culture cells in Class II safety cabinet with air-circulation through double filters. Although there is no reason why recombinant, replication-competent viruses should be more pathogenic than biological isolates, this possibility cannot be totally excluded. All work with these engineered viruses will therefore be carried out at level 2 containment in Class II safety cabinet with air-circulation through double filters.

The major risk associated with retrovirus vector production and subsequent transduction is occurrence of replication competent, recombinant viruses (RCR). Although a minimum of 3 recombination events are necessary to produce RCE in these systems, this possibility cannot be totally excluded. While RCR derived from HIV and EIAV vectors may cause harm for humans and environment (HIV is ACDP Hazard Group 3 and EIAV is MAFF regulated). It is therefore proposed to carry out this work at the containment level 2 handling culture cells in Class II safety cabinet with air-circulation through double filters and vector preparations and transduced cells will be rigorously tested for the absence of replication competent virus.
Viral T-Ag and the catalytic component of human telomerase are capable of expanding the life span of and/or immortalising certain mammalian somatic cells. Transduction of cells with both viral T-Ag and the telomerase component is likely to immortalise human primary cells in an efficient manner (P.Jat, personal commun.), but the effect in non-human cells is less clearly predicted. As this combination per se results in cell immortalisation, but not cell transformation, accidental transduction of small number of lab workers' cells is unlikely to cause neoplasia, although this possibility cannot be totally excluded. Widely used marker genes are unlikely to be toxic/oncogenic/allergic when expressed in eukaryotic cells.

Retrovirus vectors may undesirably activate cellular genes by insertion mutagenesis. Retroviral cDNA library may contain cDNA for growth factors/oncogenes/allergens. Accidental injection into workers may result in undesirable expression of the above genes.

No gene of the source viruses or protein tags has been shown to be oncogenic or allergic. Replication competent viruses will be produced by transfection of full-length virus genomes or multiple plasmids containing a full set of viral genes. There is no additional risk of such recombinant viruses compared with biological virus isolates. Most of these replication competent viruses have been handled in either Cat 1 or 2 laboratories in our former institute, Institute of Cancer Research. It is highly unlikely that a hybrid or tagged infectious viruses are more harmful than their parent viruses.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

## For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

## Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

### SOLID WASTE DISPOSAL

Laboratory workers are responsible for the follow procedures:

a) the waste is collected in an autoclavable bag which is supported in a flip top white bin.

b) when 2/3rds full the bag is inserted into a 2nd bag and both bags are tied with a blue elastomere band.

c) a piece of autoclave tape is stuck to the outside of the bag.

d) the bag is placed in blue wheely bin.

It is the responsibility of laboratory workers that waste is completely contained before insertion in the blue bin. If bags split or tear a further 2 bags must be used to ensure safe transfer to autoclave.

Wash up staff are responsible for the following steps in the disposal process:

a) the waste is transported in the blue wheely bins to the autoclave room. Waste bags must be transported in a bin - under no circumstances can they be carried by hand to the autoclave room.

b) wearing gloves, the bags are transferred to the autoclave and inserted in the stainless steel bins.

c) remove gloves, discard in yellow waste bag.

d) close the autoclave door and run cycle 3 which is the validated discard cycle.

e) at end of cycle check cycle has operated successfully by checking the display for "finish" light on and no fault lights showing.

f) open autoclave and, wearing insulated gloves, remove waste bags and check autoclave tape has changed (black stripes).

g) transfer waste bags to yellow bags, seal the bags and leave in area for collection by cleaning staff.

h) empty liquid waste from autoclave bins by discarding down the sink with hot water tap running.

### LIQUID WASTE DISPOSAL

Liquid waste is disinfected with DS607 at a final concentration of 5%. Leave overnight before discarding down sink.
These risk assessments were considered at a meeting of the GMSC held on 5 April 2001. The committee, having taken advice from its Advisers, approved the risk assessments as declared by the research group subject to the three amendments indicated and attached to the risk assessments for experiments numbered 603, 604, 606, 607, 609 and 610. The committee noted that the work would be conducted in a containment level 2 laboratory.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### Project Ref 14/01.4

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<td>CLONING AND CHARACTERISATION OF CELLULAR FACTORS INVOLVED IN RETROVIRUSES UNCOATING, NUCLEAR ENTRY AND INFECTION</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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<td>Significant Change ID</td>
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**Project Additional Information**

**Purposes of the contained use**

Cloning cellular factors essential for retrovirus replication will improve the design of retroviral vectors for gene therapy and will help developing anti-retroviral drugs. The interaction between virus and cellular factors may be blocked by small synthetic compounds hence reducing virus spread in humans and animals. On the other hand, cellular factors may be targeted by replication-incompetent viral vectors to improve efficiency of therapeutic gene delivery in gene therapy applications.

**Recipient or parental organism**

For this type of research only replication incompetent retroviral vectors are used. Vectors are based on human immunodeficiency virus type-1 (HIV-1) and Moloney murine leukaemia virus (MoMLV). All viral coding regions have been deleted and are provided in trans by packaging cells. Retroviral vectors are made by 3 separate components: core-enzyme proteins, envelope, vector genome. The core enzyme and envelope plasmids are provided in trans and cannot be packaged in the viral particle. Thus, the viral particle will contain the vector genome only and will be unable to replicate. The use of split core-enzyme and envelope plasmids will reduce the chances of recombination to almost zero. To further reduce the risk of recombinant virus spreading, cells will be screened periodically by polymerase chain reaction (PCR) and reverse transcriptase (RT) assay for the presence of recombinant virus.

Vectors will contain marker genes like GFP or LacZ that cause no harm but may induce some degree of immune response in animals or humans. Viral vectors will be used to infect a variety of cell lines and primary cells from different species including: human, african green monkey, mouse and rat. All infections will be carried out in contained hoods. The presence of pathogens in primary cell donors will be screened beforehand by the appropriate hospital facility. Cell lines are periodically screened for mycoplasma contamination.

**Host/vector system**

**TISSUE CULTURE**

Hosts: mammalian cell lines include TE671, HT1080, AmpliGPE, PA317, TECelB, HeLa, 293, 293T, COS7, NIH373, musdunni, Simian lung fibroblasts, simian kidney epithelial cells (MAFF licence pending for these simian cell lines). Primary cells may include: peripheral blood monocytes, macrophages, lymphocytes, myoblasts, skin fibroblasts.

Vectors: Retroviral vectors: pHR', pHR'cPPT, pCMVd8.2, pCMVd8.9, pMD.G, pLNPOZ and derivatives, pMFG and derivatives. All vectors have been thoroughly tested for the lack of recombination. PMFG has been approved for clinical trials while pHR', pCMVd8.2 and 8.9 are used in pre-clinical studies.

**MOLECULAR BIOLOGY**

Vectors: cloning plasmids: Bluescript, pGEM derivatives, pUC series, Bacteria expression plasmids: pGEX series

Retroviral vectors: pHR', pHR'cPPT, pCMVd8.2, pCMVd8.9, pMD.G, pLNPOZ and derivatives, pMFG and derivatives. All vectors have been thoroughly tested for the lack of recombination, PMFG has been approved for clinical trials while pHR', pCMVd8.2 and 8.9 are used in pre-clinical studies.


**Origin & function**

All viral vectors were cloned by other research groups. TelCeB packaging cell lines were made at the Chester Beatty Laboratories in London by Drs Takeuchi, Cosset and Collins. Cell lines are bought from the American or European tissue culture collections. Primary cells are provided by healthy or diseased human volunteers at appropriate hospitals. Bacterial cloning and expression vectors are bought from specialised companies. Competent bacteria are made in house.
Replication incompetent MoMLV and HIV-1 will be used to infect cells in vitro and prepare intracellular viral reverse transcription complexes. These complexes will be used for electron microscopy analyses. Viral complexes will be labelled and used to study nuclear import in mammalian cells. Cellular factors involved in uncoating and nuclear import of viral complexes will be purified by chromatographic methods and their specific activity tested in tissue culture cells and primary cells. Factors will then be cloned by peptide sequencing and RT-PCR to isolate relevant cDNAs.

Evaluation of foreseeable effects

There is a very low risk of infection by recombinant vectors for humans or animals by accidental contact. In this case, however, the virus will not be able to spread in the infected organism. Moreover, viral vectors used in this study carry marker genes like GFP or LacZ that do not cause harm to humans or animals. To avoid any such risk, all infection procedures will be carried out in Class II MSC hoods with air circulation through double filters. All workers must wear appropriate protecting clothes and gloves and must be trained beforehand on the procedures for handling recombinant virus, dispose of potentially infectious material and waste disposal. In case of accidental contact with recombinant virus, workers are instructed to remove contaminated clothing into an autoclavable bag. Any wound must be let bleeding within reasonable limits, washed with alcide and dressed appropriately. Accidents must be reported immediately to local supervisor or safety officer who will contact the Occupational Health Service if appropriate. All surfaces in working hoods are treated with the appropriate disinfectant to inactivate any vector. Tissue culture media from infected cells will be disposed after appropriate disinfection and infected cells will be autoclaved. Primary cells and cell lines used are not from the persons working in the laboratory and are known to be free of pathogens. These cells will not survive outside of the laboratory without feeding and incubation, nor colonise immunocompetent workers after accidental injection.

Access to the working area is restricted by a code lock. All solid waste is collected in autoclave bags and transferred to a different floor in appropriate sealed bins for autoclaving at 131 degC. Four Class II MSC with air circulation through double filters and 9 CO2 incubators operate in the working area. Biological solid waste from molecular biology work is collected in yellow plastic bags for incineration.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Tissue culture liquids containing recombinant virus are treated with Basol for at least two hours before disposal. Treatment with Basol for 1 hour is sufficient to sterilize cultures. All plasticware, including tips and pipettes, is soaked in Basol and subsequently autoclaved. Surfaces are treated with Alcide at the beginning and at the end of the work. Bacterial cultures are treated with Basol overnight and disposed. Glassware is washed and sterilised by autoclaving. All solid waste from Level 2 labs must be autoclaved before leaving the Windeyer Institute. The following procedure is applied:

a) the waste is collected in an autoclavable bag which is supported in a flip top white bin.
b) when 2/3rds full the bag is inserted into a 2nd bag and both bags are tied with a blue elastomere band.
c) a piece of autoclave tape is stuck to the outside of the bag
d) the bag is placed in a blue wheely bin.
e) the waste is transported in the blue wheely bins to the autoclave room. Waste bags must be transported in a bin - under no circumstances can they be carried by hand to the autoclave room
f) wearing gloves, the bags are transferred to the autoclave and inserted in the stainless steel bins
g) gloves are removed and discarded in yellow waste bag
h) close the autoclave door and run cycle 3 which is the validated discard cycle
i) at end of cycle check cycle has operated successfully by checking the display for “finish” light on and no fault lights showing.
j) open autoclave and, wearing insulated gloves, remove waste bags and check autoclave tape has changed (black stripes).
k) transfer waste bags to yellow bags, seal the bags and leave in area for collection by cleaning staff
l) empty liquid waste from autoclave bins by discarding down the sink with hot water tap running
IF THE AUTOCLAVE CYCLE FAILS OR THE AUTOCLAVE DOES NOT FUNCTION AS NORMAL, WASTE IS NOT REMOVED AUTOCLAVE FAILURE MUST BE IMMEDIATELY REPORTED.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The comments which should be at Section 15 are as below:

"These risk assessments were considered at a meeting of the GMSC held on 5 April 2001. The Committee, having taken advice from the Advisers, approved the risk assessments as declared by the research group. The Committee noted that the work would be conducted in a containment level 2 laboratory."

Project Containment

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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 14/01.5

Date Ackn’d 12/12/2001

CU2 Project Title

CLONING AND FUNCTIONAL ANALYSIS OF HIV ENVELOPE PSEUDOTYPES AND CLONING OF FUNCTIONAL ANALYSIS OF HIV GENES AND HIV SEGMENTS

Class 3

CultureVolumeClass3-4 30ml

Non-GMM yes

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work Y
**Project Additional Information**

**Purposes of the contained use**

Cloning and functional analysis HIV envelope pseudotypes: Molecularly cloned full-length HIV envelopes will be used. HIV envelopes will be amplified from patient material and cloned into mammalian expression vectors. Cotransfection into mammalian cell lines with a full-length HIV backbone defective in the envelope gene results in the production of virus pseudotypes carrying HIV envelopes from patient samples.

The HIV envelope pseudotypes will be used to identify the coreceptors and cell types that are infected by the HIV envelopes derived from patient material.

An adenovirus vector that contains CD4 in the early E1 region of the adenovirus genome will be used to transfact CD4 into primary cells that do not normally express CD4. This will allow for infection of CD4 negative primary cell types by the above HIV envelope pseudotypes.

Cloning and functional analysis of HIV genes and HIV segments: Plasmids containing complete recombinant or chimeric infectious HIV DNA clones of HIV prepared in the containment level 2 laboratory will be transferred into mammalian cell lines to produce infectious virus stocks in the containment level 3 laboratory. Properties of the recombinant HIVs to be tested will include cell tropism, effects on host cells, and neutralisation.

An adenovirus vector that contains CD4 in the early E1 region of the adenovirus genome will be used to transfact CD4 into primary cells that do not normally express CD4. This will allow for infection of CD4 negative primary cell types by the above HIV chimeras.

**Recipient or parental organism**

<table>
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<tr>
<th>HIV envelope pseudotypes:</th>
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<tr>
<td>Recipient organism: HIV-1, HIV-2</td>
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<table>
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<tr>
<th>HIV genes:</th>
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<tr>
<td>Recipient organism: HIV-1, HIV-2</td>
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</table>

**Host/vector system**

HIV envelope pseudotypes and HIV genes:

Vectors: Cloning plasmids (pcDNA3.1, pGEM-Teasy, pGEM13, pBABE, other commercially available ACDP level 1 plasmids).

Host: Disabled bacteria (TopF10', HB101, SCS110, XL1blue), Culture cells (T-cells, fibroblasts, epithelial cells, testicular cells, peripheral blood lymphocytes, and Macrophages).
Expression of CD4 in cells using an Adenovirus vector:
Vector: Recombinant Adenovirus vector that carried the human CD4 gene in the early E1 region of the Adenovirus genome.
Host: primary cell cultures (Leydig cells, astrocytes).

Origin & function

Cloning and functional analysis of HIV pseudotypes: HIV envelope genes amplified from patient material are cloned into mammalian expression vectors. Cotransfection into mammalian cell lines with a full length HIV clone defective in the envelope gene results in the production of virus pseudotypes carrying HIV envelopes from the patient samples.

Cloning and functional analysis of HIV genes: Plasmids containing complete recombinant or chimeric infectious HIV DNA clones of HIV prepared in the level 2 laboratory will be transferred into mammalian cell lines to produce infectious virus stocks in the containing level 3. Various properties of the recombinant HIVs will be tested including cell tropism, and neutralisation. The HIV segments and HIV genes used are cloned by PCR from patient material.

Expression of CD4 in cells using an Adenovirus vector: An adenovirus recombinant vector will be used that carries the human CD4 gene in the early E1 region of the genome. Following infection of cells, CD4 is expressed but late viral genes are not since they are controlled by products encoded by the missing E1 region. The vector however can be produced as virus by infecting HEK293 cells which contain the E1 region (but not other Adenovirus DNA) stably integrated into their chromosomes. We will use the recombinant virus to express CD4 on primary cell cultures before testing infection by HIV. This strategy will reveal primary cells that express functional HIV coreceptors that require CD4 to confer HIV infection.

Evaluation of foreseeable effects

HIV carries the risk of AIDS. The main risk of HIV infection in the laboratory is via cuts or abrasions with contaminated sharp instruments. Aerosols are not likely to be a risk since work will be carried out in class 1 or T-fronted class II microbiological safety cabinets. Work using recombinant HIV carrying mutated or chimeric viral genes are unlikely to be of any higher risk than a typical clinical isolate from an AIDS patient. All work with infectious HIV particles whether using clinical isolates or recombinant strains is therefore carried out in our level three laboratory (as defined in the ACDP level 3 containment) under our local rules. These include a ban on sharp instruments, the use of T-front class II safety cabinets to prevent aerosols in a laboratory with restricted access.

PCR amplification of HIV genes is done under level 2 conditions from HIV cultures that have been treated, in the containment 3 laboratory, with detergents to lyse cells and virus particles in the first nucleic acid purification step. Cloning of amplified HIV genes into disabled bacterial vectors is also done under level 2 conditions. Manipulation of and subcloning into full-length infectious DNA clones of HIV is carried out at level 2. Introduction of infectious HIV DNA into cells by transfection and subsequent production of infectious virus is carried out at containment level 3. Naked viral DNA is manipulated under containment level 2 conditions but the production of infectious virion is carried out in containment level 3 conditions.

The recipient cell cultures are cell lines and primary cells which are not from the persons who work in the same laboratory and are known to be free from human pathogens of ACDP 2-4 groups. These cells will not survive outside of the laboratory, without feeding, nor colonise in immunocompetent workers after accidental injection. There is a small likelihood that the HIV pseudotypes will include some that contain infectious replication competent genomes that result from recombination between the 2 plasmids encoding defective HIVs. These recombinant viruses are no more of a risk than a typical clinical isolate from an AIDS patient. All work after transfection of HIV clones into cell lines is therefore done in containment level 3 and in a class 2 safety cabinet to protect the worker. All pseudotypes are treated as potentially live HIV.

The Adenovirus vector will be propagated in HEK-293 cells which contain the E1 genes that are missing in the recombinant Adenovirus. It is conceivable that the recombinant Adenovirus will recombine with the E1 regions integrated in HEK-293 chromosomes. This would produce a replication competent virus. As far as we are aware, such recombination does not occur. If it did, such a virus would not be any more dangerous than normal Adenovirus. The main danger is exposure to an aerosol containing the recombinant Adenovirus. Such exposure may confer CD4 expression on cells of the respiratory system making them susceptible to HIV infection. The likelihood of significant numbers of cells becoming CD4+ and then being exposed to an HIV aerosol of sufficient concentration to infect them is nil under our level 3 conditions and safety precautions. The recombinant virus will be used in the level 3 containment laboratory in a T-fronted class II microbiological safety cabinet to prevent...
exposure to aerosols. Cells exposed to the vector are then infected with HIV 48 hours later allowing time for CD4 to be expressed. At this time, levels of residual adenovirus will be negligible. The precautions taken will therefore eliminate the envisaged danger.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Containment 3:** All disposable plastic-ware is soaked in Basol detergent overnight before autoclaving. A tray of Basol is kept inside all Safety cabinets during work for the temporary disposal and treatment of micro-pipette tips and small plastic waste. Beside each cabinet there is a tube of Basol for the disinfection of serological pipette tips. Tissue culture flasks and culture tubes are rinsed and filled with Basol and put in a waste bin after use. All pipette tips, disposable plastic-ware, and tissue culture flasks, are treated with Basol after use where they stay until the next day or at least 12 hrs when they are processed in a double-ended autoclave.

All waste is processed through a double-ended autoclave. Waste is loaded on the containment 3 laboratory side and is unloaded on the lobby side of the containment three after the autoclave cycle is finished. The autoclave maintains a temperature of 134 degrees C for 20 minutes that is sufficient to kill all life within the autoclave. A report is printed by the autoclave that confirms the maintenance of temperature during the sterilisation cycle. These reports are kept for a minimum of two months after printing. All waste is labelled with marker autoclave tape before being sterilised in the autoclave. This tape turns black when the appropriate sterilisation temperature has been reached.

Soaking in Basol detergent and subsequent autoclaving is sufficient to kill 100% of all life that is exposed to this treatment.

The efficacy of the Basol has been tested with a blue-spot B-Galactosidase assay for HIV infection. A 2% solution of Basol was added to cell-free HIV supernatant. One hour later, the Basol/HIV solution was plated onto HIV permissive cell lines. After two hours, the inoculum was removed and the cells were washed twice with fresh growth medium. The cells were stained to detect HIV infection three days later. No infection could be detected.

Both solid and liquid wastes are produced in the containment level three laboratory and are treated as containment level three waste and are autoclaved.

**Is an emergency plan required according to regulation 20?** No

**If yes, tick to confirm that it is attached to this form.** No

**Tick to confirm that you have attached a risk assessment to this form.** Yes

**Tick if you are claiming exemption from disclosure for section of the risk assessment.** No

### Please enter comments on the GM safety committee on the risk assessment

The work proposed in this notification was approved by the Genetic Modification Safety Committee at a meeting on 5 April. The Committee agreed with the proposed containment levels and were satisfied that adequate arrangements were in place for safety management.

### Project Containment

02/03/2022
A disabled molecular clone of HXB2 and appropriate complementary domain(s) from patient-derived viruses will be co-transfected into Sup-T1 cells, resulting in the generation of full-length, viable HIV-1 in culture. Using a modified recombinant virus assay (RVA), patient-derived HIV-1 Gag and/or Pol domains will be introduced by intracellular homologous recombination into a constant HIV-1 genetic background (HXB2), and cultured in immortalised human T-cells (Sup-T1 cells - ATCC ref: CRL-1942). The relative fitness of resultant recombinant viruses will be investigated using pairwise competition experiments, also performed in Sup-T1 cells.

Prior to transfection, neither component is thought to present significant risk either to humans or to the environment. The only potential risk would come from complementation of the disabled molecular clone of HIV-1 (HXB2) with endogenous human retroviruses which could in theory generate viable human retroviruses. In view of the specialisation of the Lentivirus genus of Retroviridae, this would seem to be a remote possibility. We intend to use containment level II for the propagation of vector in E. coli.
The risks posed by the experimentally-derived recombinant viruses are equivalent to those of HIV-1 isolates rescued from patient material and which are already present in HIV-infected patients. The requirement for containment of these pathogens, whether experimentally-derived or rescued by conventional culture, is ACDP hazard group III.

References:

Recipient or parental organism
As described above, the recipient for the GMO will be Sup-T1 cells obtained from the AIDS Reagent Project, NIBSC, Patters Bar, Herts, UK (Catalogue Ref. ARP024, ATCC ref: CRL-1942): These have been derived from a patient with non-Hodgkin's Lymphoma not known to bear any know human pathogenic agent.

Host/vector system
A molecular clone of HIV-1 (HXB2) lacking its entire 5’ region will be generated and used as the vector in this work. The 3’ sequences of HXB2 from the integrase domain to the 3’ long terminal repeat will be PCR-amplified and cloned into a commercially-available vector (pUC18, Roche Molecular Biochemicals). Prior to use, this vector will be propagated in E. coli to microgram quantities and linearised using an appropriate restriction enzyme.

Origin & function
Three HIV-1 domains will be rescued from clinical samples - gag, protease, and reverse transcriptase. Either singly and/or in combination, these domains will be amplified by nested RT-PCR directly from nucleic acid extracted from clinical materials. Using unique restriction sites introduced into the termini of the nested RT-PCR products, these sequences will be digested and ligated to previously PCR-amplified flanking HXB2 sequences. This will generate a fragment which comprises a contiguous 5’ coding region. The 3’ end of this resultant fragment will overlap the 5’ end of the 3’ HIV-1 coding region in the vector by approximately 500 bp, facilitating homologous recombination between the two fragments in Sup-T1 cells. This will lead to the generation of a panel of recombinant viruses in which specified sequences from patient-derived viral nucleic acids are expressed in a constant genetic background, enabling phenotypic comparison between gag, protease, and/or reverse transcriptase domains.

Evaluation of foreseeable effects
Linearised vector and recombinant 5’ coding regions will be co-transfected into a human T cell line, whereupon the two fragments will undergo homologous recombination such that contiguous full-length viral nucleic acid is generated. Intracellular expression of this nucleic acid will result in the generation of infectious virus, enabling culture of recombinant viruses.

Prior to transfection, neither component is thought to present significant risk either to humans or to the environment. We consider that the only potential risk would come from complementation of the disabled molecular clone of HIV-1 (HXB2) with endogenous human retroviruses which could in theory generate viable human retroviruses. However, in view of the complex genomic organisation found within primate lentiviruses, any such recombination is extremely unlikely to result in genomes able to express viable viruses. Therefore we intend to use containment level II for the propagation of the vector in E. coli.

The risks posed by the experimentally-derived recombinant viruses are equivalent to those of HIV-1 isolates rescued from patient material by widely used co-culturing techniques, and are present in vivo in HIV-infected patients. The requirement for containment of cultured HIV-1, whether experimentally-derived or rescued by conventional culture, is ACDP hazard group III.

Therefore we intend to use our containment level III laboratory for the handling of these viruses.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard category III containment procedures for the disposal of waste will be adhered to:

Liquid waste, including disposable items used in its manipulation (eg. pipettes) will be sterilised in Virkon solution for a minimum of 16 hours. Both solid waste (eg. gloves and equipment wrappings) and Virkon-treated liquid waste will be autoclaved to destruction (131 degrees C, 2 atmospheres, 30 minutes). Subsequent disposal of autoclaved waste is via the established double-bag system, before finally being incinerated.

Virologically dangerous materials such as cultures will be treated as per liquid waste, and both chemically sterilised and autoclaved before disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The work proposed in this notification has been considered by a representative panel of scrutineers acting for the UCL Genetic Modification Safety Committee. They recommended that approval could be given for this project at the classification proposed by the research group of activity class 3 subject to confirmation at the next full meeting. The laboratory premises where the proposed work is to be undertaken were last inspected on 24th May 2001 and were found to be acceptable for this level of work (containment level 3).

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>Project Ref 14/02.2</td>
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</tbody>
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Date Ackn’d 02/03/2022
GENERATING LENTIVIRAL VECTORS ENCODING FOR ONCOGENIC VIRAL PROTEINS

Date Project Ceased
12/09/2018

Purposes of the contained use
To study the effects of potential cancer causing genes (oncogenes) on cell growth, we need to infect primary cells with these oncogenes. To do this efficiently, we use HIV-1 viruses to carry the oncogene of interest into the primary human cells. These HIV viruses are made incompetent, meaning they can only infect the cells of interest and no new HIV viruses are then produced that are able to infect new cells. The HIV viruses can therefore not replicate.

Recipient or parental organism
The gene delivery system involves 3 plasmids that are transfected into virus producing cells, the 293T cell line. The lentiviral system is secured by gene removal such that no new particles can be produced from infected cells. This system functions as the 293t cells express:
- The viral envelope protein (VSV-G envelope) from a plasmid called p-VSVG.
- The modified HIV-1 genome with the transgene (modified HIV-1 called pHR with deleted envelope and accessory genes like tat, nef and vpr)
- The HIV -1 accessory genes, including the reverse transcriptase and the other viral genes involved in replication (from pCMV8.9 plasmid)
The vector can stably integrate into DNA for sustained long-term expression of the transgene. The vector has the ability to infect primary human cells, with subsequent expression of the foreign gene and GFP in these cells. All progeny cells will also express the foreign genes, but no new HIV-1 virions can be produced from the infected cells (replication defective). Because the HIV-1 envelope, that usually binds CD4 and chemokine receptors, has been replaced by the vesicular stomatitis virus envelope (VSV-G), this vector can infect many different cell types in vitro and possibly in vivo. VSG envelope allows entry of virus into cells by way of binding non-specifically to membrane phospholipid of target cells, rather than relying on specific receptor binding.

Host/vector system
We are testing the functional role of different proteins encoded by Kaposi's sarcoma-associated herpesvirus (KSHV) and by human T-cell leukaemia virus-1 (HTLV-1). These include a viral cyclin homologue (v-cyclin), a latent membrane protein (K15), a latent nuclear antigen (LANA), anti-apoptotic proteins called K7 and a FLIP (FLICE inhibitor protein) homologue (vFLIP) and a membrane protein (K8.1), all encoded by KSHV. The viral cyclin, LANA and K15 are potential oncogenic proteins. We are also testing the mechanisms of transformation by the oncogenic Tax protein encoded by HTLV-1.

Origin & function
To transduce (infect) primary human cells with these different viral genes, we will clone them into a lentiviral vector. The lentiviral vector is based on HIV-1 pseudotyped
with a vesicular stomatitis viral (VSV) envelope, allowing infection into a broad range of cell types. The HIV-1 vector can infect cells but not replicate ("one shot" or "self-inactivating"). In addition, this lentiviral system incorporates a green fluorescent protein (GFP) from a bicistronic transcript allowing the identification of infected cells by immunofluorescence. The transduced cells will include primary human haematopoietic cells, endothelial cells and human stem cells.

Evaluation of foreseeable effects

1. The transduced cells can only survive in tissue culture at 37C.
2. The modified lentivirus cannot survive outside cells (dependent on cells for DNA integration), and is replicative incompetent: thus the lentivirus cannot propagate to infect new cells and pose a negligible risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon (a peroxygen compound) used at a concentration of 1% is known to have a wide range of microbial activity and is bacteriocidal against many human viruses, including HIV. All tissue culture material and disposable plasticware will be soaked for a minimum of 18-24hours in a freshly prepared 1% Virkon solution. The fluid is then disposed of down a laboratory sink with excess water. Solid waste is then double bagged in biohazard bags and autoclaved at 134C for 20 minutes at 3.2 bar pressure. Validation of this method will be by independant thermocouples placed in the centre of the load. Verification that the correct conditions have been reached will be obtained by checking the chart recorder printout. The autoclave undergoes a planned preventative maintenance inspection every 3 months. All inactivated waste is then treated as "clinical waste" and removed from site by a UCL contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

In view of the disabled nature of the HIV, the GM Safety Committee accepted the containment level proposed by the investigator subject to the vigorous application of the risk control measures identified as required by the assessment. The room in which the work will be undertaken was last inspected in June 2001 and was found to be in good order. It is due a revisit in January 2002.

**Project Containment**

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

To investigate the regulation of K+ channels in native tissue by cell signalling pathways.

**Recipient or parental organism**

Existing cDNAs (see Leaney et al PNAS 97:5651 for exact details and source of starting materials) encoding heterotrimeric G-proteins (Galpha i) will be subcloned into an adenoviral shuttle vector ("transfer vector" in pAdEasy system from Qiagen http://www.qbiogene.com/products/adenovirus/). Recombinant adenovirus will be generated according to manufacturer's instructions in HEK293 cells. The virus is replication deficient in any other host. After generation of the virus it will be used to transfect primary neuronal, cardiac and smooth muscle cell lines. If we are not successful with the pAdEasy system we will use an analogous system from Clontech (Adeno-X adenoviral system, http://www.clontech.com/products/families/adeno/index.shtml).

**Host/vector system**

Existing cDNAs (see Leaney et al PNAS 97:5651 for exact details and source of starting materials) encoding heterotrimeric G-proteins (Galpha i) will be subcloned into an...
adenoviral shuttle vector ("transfer vector" in pAdEasy system from QBiogene http://www.qbiogene.com/products/adenovirus/). THESE ARE NOT MEMBERS OF THE SMALL G-PROTEIN FAMILY. A second class of reporter consisting of a small domain of a protein that monitor changes in intracellular lipid concentration by changes in fluorescence may also be used depending on results (PLCd-PH-CFP and C1d-YFP see Botelho et al, 2000 J Cell Biol).

Origin & function

Summary:- Transfection of primary cell lines using recombinant adenoviral expression vectors according to manufacturer's protocols.

Existing cDNAs (see Leaney et al PNAS 97:5651 for exact details and source of starting materials) encoding heterotrimeric G-proteins (Galpha i) will be subcloned into an adenoviral shuttle vector ("transfer vector" in pAdEasy system from QBiogene http://www.qbiogene.com/products/adenovirus/). THESE ARE NOT MEMBERS OF THE SMALL G-PROTEIN FAMILY. A second class of reporter consisting of a small domain of a protein that monitor changes in intracellular lipid concentration by changes in fluorescence may also be used depending on results (PLCd-PH-CFP and C1d-YFP see Botelho et al, 2000 J Cell Biol). Recombinant adenovirus will be generated according to manufacturer's instructions in HEK293 cells. The virus is replication deficient in any other host. After generation of the virus it will be used to transfect primary neuronal, cardiac and smooth muscle cell lines. If we are not successful with the pAdEasy system we will use an analogous system from Clontech (Adeno-X adenoviral system, http://www.clontech.com/products/families/adeno/index.shtml)

Evaluation of foreseeable effects

The virus is replication deficient in any other host other than HEK 293.

There is a risk of self-limiting human infection with the adenovirus when infecting the host cells.

The transferred genes are not likely to be harmful to human health.

The personnel will be asked to report the development of any unusual symptoms especially those of an upper respiratory tract infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To generate the shuttle vector standard molecular cloning techniques will be used. All bacterial strains are attenuated and no risk to human health. Waste is disposed of in designated bins, double-bagged and autoclaved prior to disposal.

The virus will be generated in Class II cell culture facilities in a designated and purpose-built laboratory with designated hoods and incubators. Waste will be disposed of in designated bins, double-bagged and autoclaved prior to disposal. Recombinant virus may be stored prior to use.

Primary cell lines will be isolated or obtained from companies and will be manipulated in Glass II facilities. Infection with the virus will take place in the designated facility above. On completion of the experiment the transfected cell lines will be destroyed.

All reagents will be disposed of on completion of the project (100% kill).
A panel representing the UCL GMSC has given interim approval for this project at Class 2 following the supply of further information, including more detail on the experimental procedures. The project will be discussed at the next meeting of the full GMSC due on 4 July 2002.

Project Containment

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<td>Large Scale Activities</td>
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Project Ref 14/02.5

Date Ackn'd 01/08/2002

CU2 Project Title

BOOSTING OF HIV ENVELOPE EXPRESSION BY A T7 RNA POLYMERASE-CONTAINING VACCINIA VIRUS VECTOR

Date Project Ceased

Class 2

Culture Vol Class 2 < 1 litre

Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

### Purposes of the contained use

HIV-1 envelope expression is boosted by the use of a bacteiophage T7 RNA polymerase recombinant Vaccinia virus VR-2153. The harvested HIV-1 envelopes are then titrated with serum from the envelope donor (autologous serus) or a different donor (heterologous serum) to quantitate the levels of envelope specific antibody present.

### Recipient or parental organism

| VR 2153 Vaccinia virus vector contains Bacteriophage T7 RNA polymerase inserted into the Vaccinia endogenous Thymidine Kinase gene (purchased from American Tissue Culture Collection ACTCC). |

### Host/vector system

| Host: Mammalian culture cells without ACDP 2-4 pathogens (293T, Vero). |
| Vector: VR 2153 Vaccinia virus vector with Bacteriophage T7 RNA polymerase inserted into the Vaccinia endogenous Thymidine Kinase gene. |

### Origin & function

HIV-1 envelope sequences are obtained from patient material. The HIV sequences cloned in this project are envelopes only. Core sequences required for HIV replication will not be amplified or otherwise manipulated. The risk assessment for obtaining HIV-1 envelope sequences from patient material has been submitted and has HSE approval (Cloning and functional analysis of HIV envelopes (GM 14/01.5)).

VR 2153 Vaccinia virus vector with Bacteriophage T7 RNA polymerase inserted into the Vaccinia endogenous Thymidine Kinase gene (purchased from ATCC).

### Evaluation of foreseeable effects

Vaccinia is a poxvirus of the genus Orthopoxviridae. It infects humans and other animals such as cows, horses, rabbits and guinea pigs and is classified as being an ACDP level 2 pathogen. It is infectious through ingestion and contact with open skin and eyes. Infection causes rash and pustules at the site of contact only and recovery is full in the immunocompetent. Vaccinia stably accepts large foreign sequences into its genome so it is a very popular tool in immunology and vaccine research and is a well-established and studied vector.

Vaccinia virus will infect animals other than humans; including rabbits, cows, buffalo and guinea pigs. The containment measures are aimed at protecting both the workers and the surrounding environment.

Vaccinia virus produced from a Vaccinia vector with the T7 polymerase gene insert is not likely to be any more or less infectious than a wild type Vaccinia virus (ATCC) American Tissue Culture Collection product information sheet for VR-2153). Thus, the T7 Vaccinia virus VR-2153 is classified as a USA Biosafety level 2 virus that is equivalent to the United Kingdom AQCDP containment level 2 (http://www.cdc.gov/ohs/biosfty/bmbi4s3.htm).

There is the possibility of recombination between the HIV envelope containing plasmids and the Vaccinia vector genome, however this is unlikely. The resulting pseudo-virus would likely be an HIV enveloped Vaccinia core of reduced fitness when compared to wild type Vqaccinia virus. Once sufficient levels of HIV-1 envelope protein are produced the cultures are lysed with Nonidet P-40 detergent that will inactivate any virion present in culture, whether it is wild type or artificial.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Waste Disposal

Solid Waste - Paper gowns and towels, disposable gloves, filter papers and plasticware must be discarded into an approved autoclavable bag, supported within a labelled biohazardous waste bin. Before the bag is too full, it must be closed with an elastomeric seal before being placed within the autoclave to await a sterilising run. If the autoclave is in use, full bags may be temporarily stored in the bin beside the autoclave. The autoclave discard cycle holds at 134 degrees C for 15 minutes. This is sufficient to kill 100% of life in the autoclave after a cycle has run.

Plastic Ware - Disposable culture dishes, tubes and flasks used for infectious material must not be discarded until after an aliquot of 50% Kleencare DS607 has been added to them (in a MSC). They must then be dealt with as described in 8.1.

Graduated Pipettes - Plastic graduated pipettes must be totally immersed in 1% Kleencare DS607 for at least 2 hours in the grey cylindrical drums. After disinfection the pipettes can be removed from the Kleencare DS607 and allowed to drain. They are discarded into an autoclavable bag and disposed of as for solid waste.

Plastic Straws/pipette tips - Plastic straws and plastic pipette tips must be totally immersed in 1% Kleencare DS607 in a plastic bath within the MSC for at least 1/2 hour before draining and disposal into an autoclavable bag (solid waste). If Gilson tips are used in conjunction with Eppendorf multidosers, the tips must be separated before disposal.

Autoclaving waste - The bags of solid waste are autoclaved in the stainless steel containers in the Priorclave Double Ended Autoclave. On completion of the cycle, the bags are removed in the Prep Room 401E and placed in medibins. When full these bins are sealed and removed from the prep room to await collection for incineration.

Autoclave failure - Should the double ended autoclave be unavailable, bags of solid waste may be autoclaved in the Priorclave EV150 autoclave which is sited in the Prep laboratory, the bags must be sprayed with alcide then placed directly into the autoclave. When the discard cycle is complete, the waste is treated as in 8.7 above.

Liquid Waste - Large volumes of liquid which are, or might be, infectious must be treated with Kleencare DS607 at a final concentration of 5% for at least 12 hours before being discarded.

Solvent waste - Chlorinated and non-chlorinated solvents must be discarded into the appropriately labelled 2.5L plastic containers which are kept in the flammable storage cabinet.

When full, or at least 6-monthly, after having their outer surfaces thoroughly washed with Alcide, they are removed from the lab and disposal is arranged according to Windeyer Institute procedures.

Blood - An equal volume of 50% Kleencare DS607 must be added to all waste blood before autoclaving.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment
A panel representing the UCL GMSC has given interim agreement that this project should be classified at Class 2, prior to consideration by the full Committee at its next meeting.

Although a separate project at a lower classification, it is related to project number GM 14/01.5 and will be carried out in the same laboratories under the precautions described in the risk assessment accompanying this notification. These procedures will adopt the same level of stringency as required for other notified work being undertaken simultaneously in the same laboratories.

### Project Containment

<table>
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<tr>
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<th>Glass Houses</th>
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Animal Units

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<th>Human Clinical Applications</th>
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### Project Ref 14/02.6

**Date Ackn'd**: 14/08/2002

**CU2 Project Title**: USE OF DEFECTIVE HELPER FREE AMPHOTROPIC RETROVIRUSES FOR THE CONDITIONAL IMMORTALISATION OF PRIMARY CULTURES OF HUMAN CELLS.

**Class**: Class 3

**Culture Vol**

<table>
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**Non-GMM**: Yes

**Consent Granted**: Yes

**Project notified under transitional arrangements**: No

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information
### Purposes of the contained use

To generate human continuous cell lines

### Recipient or parental organism

Human cell cultures transduced with one or two viruses

### Host/vector system

- PA317 - pZIPneoSV(X)1 retroviral vector
- TELFLY A and RD cells - pBABEno/hygro, pZIPneoSV(X)1, pLHCX retroviral vectors
- Primary human cell cultures transduced with one or two of these retroviral vectors

### Origin & function

The aim of the project is to use amphotropic viruses to deliver immortalising genes into primary cultures of human cells.

### Evaluation of foreseeable effects

The transduced genes either singly or in combination can immortalise human cells. Therefore the viruses containing these genes could immortalise the operator's cells if they were exposed by inoculation. Safety procedures are in place that make the possibility of self infection remote. In addition, because the viruses are defective and helper free, they should not be able to spread themselves beyond the site of inoculation.

The cultures of human cells transduced with the viruses should not produce infectious virus, because the viruses are defective and helper free. In addition, such cells should be rejected by the immune system following inoculation.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The transduction and selection of the human cell cultures will be carried out in a level 3 containment facility. To test whether the transduced cells are still expressing retrovirus, control cells will be exposed to the culture medium. Once the transduced cells have been proven to be negative for virus production, they will be transferred to a level 2 containment facility for routine culture and subcloning.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- All liquid waste will be treated with chloros for a minimum of 24 hours at a concentration of at least 10% (10,000ppm). This is a validated procedure for 100% inactivation (Parmjit Jat, personal communication). The inactivated liquid waste will be disposed of in dedicated drains leading directly to sewer waste.
- All solid waste will be soaked in chloros for at least 24 hours >10% chloros (10,000ppm), followed by autoclaving for 15 minutes at 120°C, followed by incineration. This is a validated procedure for 100% inactivation (Parmjit Jat, personal communication).
- All waste inactivation procedures will be carried out within the level 3 containment facility.
Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
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L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Project Ref 14/02.7

Date Ackn'd 26/11/2002

Date Project Ceased 28/01/2013

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

To generate high titre pseudotyped lentiviral vector systems containing genes that can be used for the immortalisation of freshly isolated primary human cells. This system will be used for cells that have failed to be immortalised using simple retroviral systems due to low proliferation rates or absence of amphotropic receptors.

Recipient or parental organism

E. coli JS4 - rec A - derived from E. coli MC1061. This is disabled E. coli.

293T kidney cells - produced from human embryonic 293 kidney cells by transformation with SV40T (Studies on in vitro transformation by DNA and DNA fragments of human adenoviruses and simian virus 40, Graham, FL; Abrahams, PJ; Mulder, C; Heijneker, HL; Warnaar, SO; De Vries, FA; Fiers, W; Van Der Eb, AJ. Cold Spring Harb Symp Quant Biol. 1975; 39 pt 1: 637-50) These cells have a history of safe use for the production of lentiviruses.

Cultures of primary human cells and of established human cell lines. The primary human cells will be from colonic epithelial cells and from mammary neoplastic tissue.

Host/vector system


293T will be transduced with the 3 plasmids pVSV-G, p8.91 and pHR into which is inserted hTERT, tsA58-U19 SV40T, U-19 SV40T, bcl-2 to produce the lentivirus.

Primary human cells and cell lines will be transduced with the VSV-G pseudotyped minimal lentiviral vector containing hTERT, tsA58-U19 SV40T, U-19 SV40T, bcl-2.

Origin & function

Minimal HIV1-based lentiviral vectors transducing genes corresponding to a temperature sensitive SV40T-antigen (tsA58-U19), wild type SV40T-antigen (U19), a catalytic protein component of human telomerase (hTERT) and bcl2, a gene that produces anti-apoptotic proteins, will be used to generate immortal cell lines from human primary cell types including colonic epithelial cells and neoplastic mammary epithelial cells.

The immortalising genes are derived from the Papovavirus SV40 and the catalytic subunit of the human telomerase gene.

SV40 large T antigen is an immortalising gene that has the potential to extend the lifespan of human cells. It can inactivate the p53 and RB pathways.

The catalytic subunit of telomerase activity can be used to reconstitute telomerase activity in human somatic cells that are null for telomerase and thus maintain their telomeres. Maintenance of telomeres is a critical step in the immortalisation of primary human somatic cells.

Bcl-2 is a gene sequence that can produce proteins that inhibit the apoptosis pathway. Death of epithelial cells may occur through loss of anchorage to the basement membrane triggering the apoptosis pathway. Therefore introduction of bcl2 has the potential to prolong survival of the cells.

Evaluation of foreseeable effects

The E. coli cells are non-colonising and disabled (recA-) and the inserted sequences are driven by eukaryotic specific promoters (eg CMV). Therefore there is no risk from this part of the work.

The 293T cells will produce a lentivirus containing the inserted genes. The production of a lentivirus increases the risk compared to a simple retrovirus as these can enter non-dividing cells. Also pseudotyping the lentivirus with VSV-G increases the risk as this enables the virus to bind to multiple cells types. However, the virus is replication defective and like all retroviruses these lentiviral particles have a limited survival capacity (half-life of 2-4 hours). The main risk to the worker as for HIV1 is from needlestick
injury and by operating in a sharps-free environment and using a class II hood in a level 3 environment the risk to the worker is very low.

Primary cells once tested for absence of replication competent virus have no risk other than that of being human cells, so possibly contaminated with viral and microbial particles. However, donor tissues will be from cosmetic surgery or cancer surgery in which the likelihood of such contamination, for example, HIV-1 and hepatitis B is less than 0.5%.

The main hazard is from the inserts which are either potentially immortalising oncogenes, telomere stabilising genes or anti-apoptotic genes and therefore each gene either singly or in combination can immortalise, but not transform, human cells and therefore, in principle create a pre-neoplastic clone in vivo in a worker exposed to them. However, the tsA58 is temperature sensitive and has little activity at the non-permissive temperature, which corresponds with near physiological temperatures (38 degrees C). bcl-2 and telomerase are naturally occurring proteins and any risk from them comes from their possible expression at higher than normal levels.

Also, the virus prepared with the inserts is replication defective and therefore cannot be self-propagating, further reducing the risk from these insets, but in any event naturally occurring lentiviruses are not transmitted via aerosols.

The main risk comes from needlestick injury or via an open wound or from an aerosol of these high titre viruses. To minimise these risks the work is carried out in a class II laminar flow cabinet in a level 3 room and working in a sharps-free environment. Any wounds will be covered and gloves and laboratory coats will be worn at all times. Aerosol is minimised by not using a vacuum for aspiration of medium.

Therefore although the consequence of the hazard of transfer of the sequence to the workers could be severe, resulting in the generation of a pre-neoplastic clone(s) in the worker, with the application of the described control measures the risk of the foreseeable effects is extremely low.

The transfection of plasmids to 293T cells and transduction of virus to human primary cells and cell lines will be carried out in a class II hood in a level 3 containment facility. The recipient human cells will be tested to determine whether they are producing lentivirus by taking culture medium from the transduced cells and placing on control cells, EJ and testing for gene transfer. Once they have been shown to be negative for virus production, they will then be transferred to a level 2 containment facility for routine culture and subcloning.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be treated with chloros for a minimum of 24 hours at a concentration of at least 10% (10,000ppm). This is a validated procedure for 100% inactivation. The inactivated liquid waste will be disposed of in dedicated drains leading directly to sewer waste.

All solid waste will be soaked in chloros for at least 24 hours >10% chloros (10,000ppm), followed by autoclaving for 15 minutes at 120 degrees C, followed by incineration. This is a validated procedure for 100% inactivation.

All waste inactivation procedures will be carried out within the level 3 containment facility.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

02/03/2022
This project has been considered by a panel representing the full GMSC. They agreed that Class 3 is appropriate for the proposed work, subject to confirmation at the next meeting of the Committee. The laboratory was inspected most recently on 27 September and found to be free of sharps (including needles, cover slips, sharp-ended forceps). The laboratory is sealable for fumigation.

**Project Containment**

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<thead>
<tr>
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<tr>
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<td>L3 Yes</td>
<td>L4 L2 L3 L4 L2 L3 L4</td>
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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

**Project Ref** 14/03.1

**Date Ackn'd** 10/01/2003

**CU2 Project Title**

THE IN VITRO STUDIES OF HEPATITIS B VIRUS REPLICATION, INVESTIGATING THE EFFECTS OF ANTIVIRAL DRUGS ON VIRAL REPLICATION AND ANTIGEN EXPRESSION

**Class** Class 3

**Culture Vol Class 2** 250 ml maximum

**Non-GMM Consent Granted** yes

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info** project transferred to GM3099
Project Additional Information

Purposes of the contained use

To study the replication and immunobiology of Hepatitis B virus in vitro by:

1. Transfecting plasmids containing HBV-DNA into hepatoma cell lines with transient viral expression.
2. Culturing cell lines that have HBV integrated into their genome and produce full infectious virus in culture.

Recipient or parental organism

Recipient organism: The plasmid containing HBV-DNA will be transfected into 2 human hepatoma cell lines - HepG2 and HUH-7. These cells pose no risk to workers.

Parental organism: A cell line which has the HBV genome integrated into their genomic DNA - HepG2215 cells. These cells produce full infectious Hepatitis B virions in culture. The foreseeable risk in handling these cells is the inoculation of the worker with the cells or the culture supernatant and thereby infecting the worker with HBV.

The most hazardous risk to workers would be of infection by the virus therefore -

1. All workers will be vaccinated against HBV and have proven protective antibody titres prior to commencing the work.
2. All work will be carried out in our containment level 3 (CL3) facility.
3. As the major risk of infection is through needle stick injury there is a NO SHARPS policy in the CL3 laboratory.

If control measure failed the most severe risk would be inoculation of the worker with the virus.

Host/vector system

Hosts: Bacterial - E. coli: Disabled host
Vector: PUC 19 plasmid: non-mobilisable, BOM (-)/NIC (-), MOB (-), TRA (-) and with ampicillin resistance.
There is no foreseeable effects with this host/vector system.

Origin & function

The gene sequence to be inserted into the plasmid is the full HBV-DNA extracted from human patients infected with HBV.

This sequence will be inserted into the PUC19 plasmid vector and transformed into E. coli (DH5-alpha). This DNA will then be extracted and transfected into the HepG2 and HUH-7 cell lines. These cells will be cultured and experiments to monitor viral replication will be carried out.

Evaluation of foreseeable effects

The foreseeable effect of this project, if control measures failed is the infection of the person handling the cell line with HBV.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste generate in the containment level 3 facility will be autoclaved in the double-ended autoclave.

1. Solid waste:
This includes laboratory plastics, laboratory overalls, gloves, paper wastes etc will be collected in double autoclave bags within a sealed container and when 3/4 full loaded into the ‘dirty’end of the autoclave which is within the CL3 laboratory. Once the cycle is complete the worker the waste is collected from the 'clean' end of the autoclave situated outside of the CL3 facility. The receipt from the autoclave is checked to ensure the run has passed (100% kill) and the waste is collected into yellow bags for incineration.

2. Liquid Waste
The liquid waste is decontaminated with Virkon powder - using 1g of powder per 100 ml of culture medium. This has been tested and proven to be 100% effective against Hepatitis B virus.

Preliminary assessment by a panel of expert advisers on behalf of the full GMSC agrees that this is an activity class 3 project.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

If yes, tick to confirm that it is attached to this form

Is an emergency plan required according to regulation 20?

Tick if an emergency plan is required

Project Containment

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Project Ref 14/03.2

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tr>
<td>27/03/2003</td>
<td>LENTIVIRAL EXPRESSION OF RECOMBINANT CDNAS EXPRESSING</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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The aim of this project is to use lentiviruses as an expression system for investigating mechanisms of molecular regulation of a family of ion channels expressed in neuronal cells. A commercially available lentivirus expression kit from Invitrogen will be used. We will make recombinant lentiviruses encoding membrane proteins which we are interested in: e.g., heteromeric G proteins of the Gi/o family (THESE G PROTEINS ARE NOT MEMBERS OF THE SMALL G PROTEIN FAMILY) and fluorescent reporter proteins such as PLCdelta-PH-CFP and PSD95-GFP. The cDNA inserts themselves are very unlikely to have any harmful effects and they are currently used in our research into mechanisms of cellular signalling. The cDNAs encoding these proteins will be subcloned into a viral expression construct (pLenti6, Invitrogen). This will be transfected into HEK293FT cells (producer cells) to generate recombinant virus. The recombinant virus will then be used to infect cultured rat or mouse central neurons in vitro. The neurones will then be used in electrophysiological studies where the effects of the heterologous membrane proteins on the neuronal ion channels will be assessed. These experiments will allow the investigation of specific single proteins (e.g., Gi/o proteins) on the activity of ion channels in their native neuronal environment.

The commercially available kit from Invitrogen is a third generation system based on HIV-1 (see Dull et al., 1998: J. Virol. 72, 8463-71) which allows the construction of a recombinant replication-incompetent, HIV-1-based lentivirus. There are a number of biosafety features which have been introduced to minimise risks. These are as follows: (1) All HIV sequences required for encapsidation and reverse transcription are absent. (2) There is a deletion in the 3' UTR resulting in self-inactivation of virus genome. (3) None of the HIV-1 structural genes contain LTRs and are thus never expressed in the target cell. (4) It contains the minimal number of HIV-1 genes required to generate an efficient vector (only three of the nine are used: gag, pol, and rev). (5) The removal genes are those which are pathogenic therefore no recombinant vector can acquire the pathogenetic features of the parental virus. (6) The tat gene, which is important for replication, has been removed. (7) The packaging genes are contained on 4 separate plasmids and thus the system relies on 4 separate plasmids for the production of transducing particles. (8) These plasmids contain no regions of homology thus preventing any recombination which could occur. (9) The virus is replication incompetent and as a replication-deficient vector it poses significantly reduced risks. Thus these viruses may infect cells and integrate DNA into the target cell but they are incapable of further replication.

Host/vector system
Vectors: HIV-based lentivirus expression vector (pLenti6) into which the gene of interest will be cloned. This vector also contains the elements needed for packaging of the expression construct. Kit also contains viral packaging mix of vectors; this is an optimised mixture of three packaging plasmids which supply the structural and replication proteins required for production of lentivirus.
Hosts: HEK293FT cells will be used to produce the virus. Recombinant proteins will then be expressed in primary neuronal cells.

Origin & function

Recombinant lentivirus will be used to transduce primary central neurones in vitro which have been isolated and cultured from rat or mouse. The intention of this project is to study the effects of the signalling proteins (described in Section 6) on ion channel activity in these neurones by using electrophysiological techniques.

Evaluation of foreseeable effects

Recombinant virus is produced in the HEK293FT producer cells. In these cells the virus can replicate. The potential risk which may arise from this is direct exposure of the viral supernatant produced to the operator. This could arise through the use of sharps and a direct needlestick injury, or if viral supernatant is accidentally splashed onto exposed areas of skin which are cut or scratched. This will be prevented by total avoidance of use of glass and sharps (including needles and forceps) since only sterile, disposable plasticware will be used. The plastic pipettes and pipette tips used are blunt and are also aerosol-resistant and contain a filter thus reducing any aerosol formation. Furthermore labcoats and gloves (and overshoes) are worn at all times. In addition, there is virtually no risk to the environment because retroviruses exhibit poor survival in the general environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All surfaces are swabbed before and after use with 10% Virkon and 70% IMS. Sharps (rarely used) and Pipetman pipettes are placed in specified sharps bins. Waste media, pipette tips and plasticware are doused in Virkon prior to autoclaving in sealed and double-bagged plastic autoclave bags. These procedures will ensure an anticipated 100% kill of virus.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The proposal has been reviewed by a representative panel of the Genetic Modification Safety Committee, which agreed with the proposer’s classification of Class 2. The notification will be submitted to the next meeting of the full Committee on 10 April 2003. The laboratories were last inspected in December 2002 and conditions were found to be satisfactory.

Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
INVOLVEMENT OF RHO GTPASES IN THE REGULATION OF DENDRITIC SPINE MORPHOLOGY AND THE ESTABLISHMENT OF NEURONAL MORPHOLOGY

Primary hippocampal neurons will be transduced by adenoviral or sindbisviral vectors in order to overexpress small GTPases (dominant negative or constitutive versions) or their interacting proteins. Adenoviral vectors are the system of choice for long-lasting experiments due to their low cytotoxicity, while sindbisviral vectors efficiently transduce large number of selectively neurons and are inevitable for batch experiments to ensure only neuronal effects.

Recipient or parental organism

We will work with replication deficient variants of Adenovirus (serotype 5) or a replication deficient, neuron specific variant of the sindbisvirus. In adenovirus this is achieved by a E1 deletion which renders the virus incapable of autonomously reproducing itself, while in the case of sindbisvirus the replication incompetence is achieved by employing a helper virus containing the structural proteins, which cannot be packaged.

Adenovirus will be generated by homologous recombination in 293 cells or be Cre/lox recombination in 293 cells expressing stably Cre recombinase. Sindbisvirus will be generated by cotransfecting in vitro translated RNAs of the helper virus and the transgene containing constructs into BHK (baby hamster kidney) cells.

The replication deficient viral vectors will be used to transduce either neurons or for testing purposes Hela, MDCK and PC12 cells.
Host/Vector System

Host: HEK293 or BHK cells for virus production.
Primary rat and mouse neurons in vitro.
PC12 cells, MDCK, 3T fibroblasts and Hela cells for testing the virus.

Vector: PJM17, pAdlox, PXCHJL1, pAdlox; pAdloxIRESGFP for adenoviral construction
Sinrep5; nsp2S; DH(26S); DH26St for sindbisviral construction.

Origin & Function

Inserts:
- Small GTPases of the Rho family and their interactors (mouse):
- Indicator proteins (GFP, dsRed, β-Galactosidase);
- Cytoskeletal proteins (tubulin and actin from mouse);

Members of the Rho family of small Ras-like GTPases - including RhoA, -B and -C, Rac1 and -2, and Cdc42 - exhibit guanine nucleotide-binding activity and function as molecular switches, cycling between an inactive GDP-bound state and an active GTP-bound state. The Rho family GTPases participate in regulation of the actin cytoskeleton and cell adhesion through specific targets. Identification and characterization of these targets have begun to clarify how the Rho family GTPases act to reulate cytoskeletal structure and cell-cell and cell-substratum contacts in mammalian cells. The Rho family GTPases are also involved in regulation of smooth muscle contraction, cell morphology, cell motility, neurite retraction, and cytokinesis (Kaibuchi et al., 1999).

We want to interfere with the normal polarity establishment of hippocampal neurons using small GTPase mutants and their interactors and image the effect onto hippocampal neurons using fluorescently tagged cytoskeletal proteins.

Since Rho family GTPases are also involved in Ras-mediated cell transformation (Qui et al., 1995) special precautions as described in the next paragraph will be taken when used the active versions of Rho GTPases.

There are no harmful effects caused by the overexpression of cytoskeletal proteins or marker proteins such as GFP or beta Galctosidase.

Evaluation of foreseeable effects

In addition to the described replication incompetence of additional system is build in the used adenovirus system is that DNA of only limited size can be packaged into viral particles. If the virus DNA containing the transgene becomes too large or too small the packaging of it will be prevented. Thus unwanted (even though extremely rare) recombination events will be selected out. Moreover virus is purified by two rounds of plaque isolation. All plaques are tested for the containing of the correct transgene. This safety procedure again will select against the rare event of recombination events may be leading to wildtype viruses. A direct proof of the presence of wt virus in our stocks is the plaque assay on non-host cells, which are transducible by the virus. There should not be a plaque forming activity. Most commonly Hela cells are used for this purpose.

The pSinRep5 alone cannot infect cells and cannot drive the synthesis of Sindbis virions. Together with the Sindbis-helper infectious recombinant viruses can be made, but these are limited to a single round of infection. It is possible that recombination of pSinRep5 and Sindbis-helper may occur in cells transfected with both RNAs. However, this recombination is only likely to occur with very low frequency and will give rise to Sindbis virions.

We will check our stocks for the rare event of recombination by using a plaque assay on BHK cells. The virus should not have plaque forming activity on BHK cells.

Thus the risk of accidental exposure is very low since secondary rounds of infection are avoided. Primary transduction of accidentally exposed tissue could only be harmful using active form of Rho GTPases with potential cell transforming effects.

Thus the risk of accidental exposure will be limited:
- Any spillage will be immediately removed by treatment with 3% Virkon.
- Gloves and coats will be worn all the time and can be autoclaved in the accident of spillage.

02/03/2022
Working in a safety hood will prevent air-mediated exposure.

To avoid any possible transfer of this virus all experiments involving adenoviral and sindbisviral vectors will be conducted in a laboratory suite reserved for virus work. The cultures will be handled within microbiological safety cabinets and appropriate precautions will be adopted throughout. Laboratory workers handling these cultures will have extensive experience in tissue culture and microbiological experiments and will be fully aware of the principals of occupational safety and handling.

By autoclaving cultures and plastic ware, which were in contact with transduced cells and transduced cells the virions will be completely inactivated. Liquid waste will be treated with 3% Virkon for at least 60 mins.

In the viral suite (G17), media containing virus will be removed from cell cultures and disinfected with 3% Virkon for 60 mins.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Before wasting any virion containing media or plastik ware, a 100% killing of viral particles will be sured:

Aerosols may be produced during the handling of supernatants from transduced cultures. Aerosol production will be minimised through the use of microbiological practice and transduced cultures will only be handled within a microbiological safety cabinet.

Contaminated tips, straws, etc. will be placed in the sandwich box filled with 3% Virkon for at least 60 minutes before draining and disposal into an autoclave bag. Bottles of media used for infection should be isolated from all other and immersed in 3% Virkon before being placed into standard glassware containers.

Liquid waste will be treated with 3% Virkon for at least 60 minutes before disposal.

Incubators.

Incubators reserved for transduced cultures (room G17) will be used. Transduced cell culture flasks should be placed in a closed plastic sandwich box with small holes pierced in side in order to kinimise the chance of accidental spillage in the incubator but still allow access of CO2.

Spillage

Spills will be treated with 3% Virkon. If there is a spillage on the laboratory coat, it will be placed into a biohazard bag and autoclaved.

Avoidance of airborne transmission

since adenovirus could be transmitted by air-borne routes, procedures will be adopted to avoid the formation of aerosols. Aerosol production will be minimised through the use of microbiological practice and transduced cultures will only be handled within a microbiological safety cabinet (to BS 5726). Room G17 does not contain windows and is not readily accessible to insects, etc. Windows in adjacent laboratories will be kept closed. The laboratory environment will be monitored for insects and any that are found will be killed.

As indicated above the recombinant virions produced in this sytem do not have the capacity to replicate in host cells or host organisms.

Treatment of Adenovirus and Sinbisvirus before disposal.

By autoclaving cultures and plastic ware which were in contact with transduced cells and transduced cells the virions will be completely inactivated. Liquid waste will be treated with 3% Virkon for at least 60 mins.
Processing of materials for use in locations outside of the viral suite.
In the viral suite (G17), media containing virus will be removed from cell cultures and disinfected with 3% Virkon for 60 min. Cells will then be washed extensively (the wash again disinfected) in order to remove viral particles from the cultures. We will test how often we have to wash to get rid of any viral particles by taking the wash medium to transduce untransduced cell cultures (293 cells, since they are most sensitive by amplification of the virus). Since we assured that the virus does not contain any wt particles (see above) and thus does not contain any infectious particles, there will be no viral particles left with the capacity to infect any other cells, since the virus taken up already lost the competence for further infection. Thus there is no harm connected to work with cultures previously transduced with adenoviral vectors, if the cultures were extensively washed and these cultures can be handled safely. Containers holding the inactivated virus products will be wiped out (3% Virkon) before removal. For subsequent analysis of live cells, culture dishes containing washed cell-cultures will be placed in a tray and analyzed without opening the dish. The dishes will be returned to the viral suite after the experiment and there disinfected with Virkon 3% and autoclaved.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The premises where this work will be carried out were last inspected under the current GMSC programme in February 2003 and found to be satisfactory. This project is notified as class 2 in view of reports that Rho GTPases may be implicated in tumourigenesis.

Project Containment

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<td>L2 L3 L4</td>
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Project Ref 14/03.4

Date Ackn'd CU2 Project Title

22/05/2003 INVESTIGATING THE ROLE OF SMALL GTPASES IN CELL MORPHOLOGY

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 litre
Project Additional Information

Purposes of the contained use

Tissue culture cell lines will be transduced by replication-deficient retrovirus vectors in order to overexpress small GTPases (dominant negative or constitutive versions) or their interacting proteins.

Recipient or parental organism

We will work with replication deficient retroviruses. These viruses are made by transfecting HEK293T cells with: 1) a plasmid expressing the gag and pol genes from murine leukemia virus (MLV) (which encode for viral structural and polymerase proteins, respectively); 2) a gene encoding a viral envelope protein, which will determine the host range of the virus produced; 3) an expression plasmid containing the gene of interest and a packaging signal, allowing it to be incorporated into the virus particles.

Host/vector system

Host: HEK293T cells for virus production.
Immortalized mammalian cell lines in vitro.

Vector: pBabe-puro, pRetroSUPER, pGagPol, pVSV-G

Origin & function

Inserts:
Small GTPases of the Ras superfamily, their upstream regulators, and their downstream effectors (mouse, human)
Small oligos for the production of siRNA in vivo
Indicator proteins (GFP, dsRed, lacZ)
Cytoskeletal proteins (tubulin, actin) (mouse, human)

Intended functions: we would like to interfere with the activity of the Ras superfamily of small GTPases, their regulators, and their effectors, to investigate the involvement of these signalling pathways in cellular behaviours.

Evaluation of foreseeable effects

Primary transduction of accidentally exposed tissue could be harmful using forms of members of the Ras superfamily of GTPases with potential cell transforming effects.
Thus the risk of accidental exposure will be limited:
Any spillage will be immediately removed by treatment with 3% Virkon.

Gloves and coats will be worn all the time and can be autoclaved in the accident of spillage.
Working in a safety hood will prevent air-mediated exposure.

To avoid any possible transfer of this virus all experiments involving retroviral vectors will be conducted in a laboratory suite reserved for virus work. The cultures will be handled within microbiological safety cabinets and appropriate precautions will be adopted throughout. Laboratory workers handling these cultures will have extensive experience tissue culture and microbiological experiments and will be fully aware of the principals of occupational safety and handling.

By autoclaving cultures and plastic ware, which were in contact with transduced cells and transduced cells the virions will be completely inactivated. Liquid waste will be treated with 3% Virkon for at least 60 min.
In the viral suite (G17), media containing virus will be removed from cell cultures and disinfected with 3% Virkon for 60 min.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The local rules for working in the containment level II laboratories will be followed in all locations. These practices include the rules for work in a microbiological safety cabinet.

Gloves and laboratory coats must be worn at all times. All cuts or abrasions will be covered with waterproof dressings.
No sharps of any type (glass Pasteur pipettes, needles, razors) will be present in the vicinity of work when handling viral stocks.
Waste will be bagged for autoclaving, and the bags sealed before removing from the designated rooms.
Eye protection must be worn.
Aerosols may be produced during the handling of supernatants from transduced cultures. Aerosol production will be minimised through the use of microbiological practice and transduced cultures will only be handled within a microbiological safety cabinet (to BS 5726).
Contaminated tips, straws, etc. will be placed in the sandwich box filled with 3% Virkon for at least 60 minutes before draining and disposal into an autoclave bag. Bottles of media used for infection should be isolated from all others and immersed in 3% Virkon before being placed into standard glassware containers.
Liquid waste will be treated with 3% Virkon for at least 60 minutes before disposal.

INCUBATORS
Incubators reserved for transduced cultures (room G17) will be used. Transduced cell culture flasks should be placed in a cloased plastic sandwich box with small holes pierced in side in order to minimise the chance of accidental spillage in the incubator but still allow access of CO2.

SPILLAGE
spills will be treated with 3% Virkon. If there is a spillage on the laboratory coat, it will be placed into a biohazard bag and autoclaved.

AVOIDANCE OF AIRBOURNE TRANSMISSION
Procedures will be adopted to avoid the formation of aerosols. Aerosol production will be minimised through the use of microbiological practice and transduced cultures will only be handled within a microbiological safety cabinet (to BS 5726). Room G17 does not contain windows and is not readily accessible to insects, etc. Windows in adjacent laboratories will be kept closed. The laboratory environment will be monitored for insects and any that are found will be killed.
As indicated above the recombinant virions produced in this system do not have the capacity to replicate in host cells or host organisms.

TREATMENT OF VIRUS BEFORE DISPOSAL
By autoclaving cultures and plastic ware which were in contact with transduced cells and transduced cells the virions will be completely inactivated. Liquid waste will be treated with 3% Virkon for at least 60 mins.

PROCESSING OF MATERIALS FOR USE IN LOCATIONS OUTSIDE OF THE VIRAL SUITE
In the viral suite (G17), media containing virus will be removed from cell cultures and disinfected with 3% Virkon for 60 min. Cells will then be washed extensively (the wash again disinfected) in order to remove viral particles from the cultures. Since the virus used for transduction does not contain a viral genome, and is thus replication deficient, there will be no viral particles produced by the transduced cells. Thus there is no harm connected to work with cultures previously transduced and these vectors, and these cultures can be handled safely. Containers holding the inactivated virus products will be wiped out (3% Virkon) before removal. For subsequent analysis of live cells, culture dishes containing washed cell-cultures will be placed in a tray and analysed without opening the dish. The dishes will be returned to the viral suite after the experiment, disinfected with Virkon 3%, and autoclaved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
A representative group from the GMSC has reviewed the risk assessment and agreed that the work should be ascribed to activity Class 2. The proposed location is a containment level 2 laboratory set aside for this kind of work. The notification will be reviewed at the next meeting of the full committee.

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Project Ref 14/03.5

Date Ackn’d CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
The aim of this project is to investigate the functions of the latent genes expressed by KSHV. This work will involve the cloning and expression of latent viral genes and cellular factors involved in viral transformation and pathogenesis. The viral genes and cellular factors described below will be transfected and expressed in bacterial and mammalian cells by virtue of promoters from expression vector plasmids. In some cases the stable expression of KSHV viral genes in mammalian cell lines will be mediated via lentivirus transduction. Gene modifications will be single mutations, partial deletions and fusion to tags (eg antigen tags, GST fusions). KSHV is an oncogenic virus and we will therefore be working with potential oncogenes. However, this work will focus on the function of individual genes and it is unlikely that any one KSHV latent gene has the capacity to act as an oncogene on its own.

Recipient or parental organism
Human 293T, Jurkat T, NIH 3T3, BJAB, 70Z/3, DG75, Ramos, RPMI, KM3, DEL

Host/vector system
1. Culture cells/Cloning plasmids, eukaryotic expression plasmids
2. Mammalian cell cultures/Lentivirus vectors based on HIV.

Origin & function
1. Cellular genes, unlikely to be oncogenic. Protein tags include His/Myc/FLAG/GST/Xpress will be used to study protein interactions.
2. KSHV latent genes, which may have oncogenic activity. Protein tags include His/Myc/FLAG/GST/Xpress
3. HIV-based vectors with markers: GFP, and neomycin resistance genes.

Evaluation of foreseeable effects
We aim to use lentivirus to transduce cell lines with individual KSHV genes. The risks from accidental infection by lentiviruses are insertional mutagenesis/over expression of cellular genes by random integration. The main risk is to the laboratory personal directly handling the lentivirus. The risk attached to using the replicative defective HIV vectors is minimised because these viruses are extremely susceptible to dessication and will only survive a few hours at room temperature. In addition, the replicative defective nature of the HIV vectors ensures that even in the case of a breach of containment and accidental injection, no virus can spread from individual to individual.
Similar work using lentivirus technology is currently carried out in a category 2 tissue culture laboratory (Rm424) in which the use of sharps and glassware is prohibited to reduce the theoretical risk attached to accidental injection of lentivirus.

The risks inherent to the viral genes to be used are the potential expression of an oncogene or tumour progression factor. Although KSHV is able to transform mammalian cells, it is unlikely that any one gene has the capacity to act as an oncogene on its own. Expressed proteins are not toxic and will not survive more than a few hours at room temperature. They cannot be diffused by aerosol. However, we do identify an increased risk when using these KSHV genes in lentivirus vectors, and extra care will be exercised when handling and expressing these genes and their products. It is possible that in the event of a breach of containment, accidental injection into workers may result in the undesirable expression of the KSHV genes. However, the replication defective vectors will not be transmitted to other cells and all plasmids containing viral genes will be grown separately to avoid any chance of recombination. The cell factors which we intend to express are unlikely to be toxic/oncogenic/allergic as they are expressed in normal cells.

In view of the associated risks of this work, it is proposed to carry out these experiments at the containment level 2 handling culture cells in Class II safety cabinets with air-circulation through double filters.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| Not applicable |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

<table>
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<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
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**Solid waste:**
Uncontaminated waste (eg tissue/wrappings) is disposed of directly into yellow bags. All tissue culture containers exposed to virus must be treated with Kleencare DS607. After disinfection, excess liquid is drained off and the container placed directly into yellow bags. Tips are ejected directly into freshly made up DS607 within the safety cabinet. After draining, tips are placed directly into yellow bags. Pipettes are placed into autoclave bag-lined holders, which are emptied regularly. The double-bagged pipettes are boxed and placed into a yellow bag. It is the responsibility of the laboratory workers to ensure that waste is completely contained before removal by cleaning staff. If bags split or tear, a further two bags must be used.

**Liquid waste:**
All liquid should be removed from tissue culture containers prior to disposal. Liquid waste is disinfected in fresh DS607 at a final concentration of 5%. It is left to soak overnight and then disposed of down the sink.

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<tr>
<th>Is an emergency plan required according to regulation 20?</th>
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<th>Tick if you are claiming exemption from disclosure for section of the risk assessment</th>
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Please enter comments on the GM safety committee on the risk assessment
This project has been considered by a representative panel of scrutineers acting on behalf of the GM Safety Committee pending the next meeting of the Committee. They agreed with the risk assessment of the proposer and the classification of this project at class 2. The premises in which the project will be carried out are suitable for the work.

### Project Containment

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

### Project Ref 14/03.6

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<td>INVESTIGATION OF EMBRYONIC STEM CELL MORTALITY.</td>
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<tr>
<td>Class 2</td>
<td>1-50 litres</td>
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- **Non-GMM**: not applicable
- **Consent Granted**: N

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

To study the effects of potential cancer causing genes (oncogenes) on cell growth, we need to infect primary cells with these oncogenes. To do this efficiently, we use HIV-1 viruses to carry the oncogene of interest into primary human cells. These HIV viruses are made incompetent, meaning they can only infect the cells of interest and no new HIV viruses are then produced that are able to infect new cells. The HIV viruses can therefore not replicate.
All retroviral vectors are based on Moloney Murine Leukaemia Virus. Vectors lack pol and env sequences and only encode a short sequence of ATG negative gag. Third generation LinX (derivative of 293T) packaging cell lines will be used to produce replication-incompetent virus. The gag, pol and env genes are integrated into the genome or expressed from a BPV based episomal vector that contains no regions of homology to the MMLV based vectors. Three separate recombination events would be required to produce replication-competent virus. The LinXE packaging cell line produces ecotropic virus which is incapable of infecting human cells. The cell line has been tested exhaustively for production of replication competent virus; none was detected. The combined choice of the packaging cell lines and the replication defective nature of the vector used will prevent the production of helper/replication competent virus by the transduced cells, reducing the risk to the environment.

The lentiviral system involves three plasmids that are transfected into virus producing cells, the 293T cell line. The system is secured by gene removal such that no new particles can be produced from infected cells. This system functions as the 293T cells express:

a. the viral envelope protein (VSV-G envelope) from a plasmid called pVSVG
b. The modified HIV-1 genome with the transgene (modified HIV-1 called pHIV with deleted envelope and accessory genes like tat, nef and vpr)
c. the HIV-1 accessory genes, including the reverse transcriptase and the other viral genes involved in replication (from pCMV8.9 plasmid).

This vector can stably integrate into DNA for sustained long-term expression of transgene. The vector has the ability to infect primary human cells, with subsequent expression of the foreign gene and GFP in the cells. All progeny cells will also express the foreign genes, but no new HIV-1 virions can be produced from the infected cells (replicative defective). Because the HIV-1 envelope, that usually binds CD4 and chemokine receptors, has been replaced by the vesicular stomatitis virus envelope (VSVG), this vector can infect many different cell types in vitro and possibly in vivo. VSV envelope allows entry of virus into cells by way of binding non-specifically to the membrane phospholipids of target cells, rather than relying on specific receptor binding.

### Host/vector system

**HOSTS:** E. coli strain DH10B, murine Escells, murine embryonic fibroblasts, rat neural precursors, human primary cells.

**VECTORS:** pMARXII, pMARXIV, pWZL, pBabe, attenuated HIV-1 (lentiviral) vector.

### Origin & function

All retroviral vectors are based on Moloney Murine Leukaemia Virus. Vectors lack pol and env sequences and only encode a short sequence of ATG negative gag. Third generation LinX (derivative of 293T) packaging cell lines will be used to produce replication-incompetent virus. The gag, pol and env genes are integrated into the genome or expressed from a BPV based episomal vector that contains no regions of homology to the MMLV based vectors.

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e. the modified HIV-1 genome with the transgene (modified HIV-1 called pHIV with deleted envelope and accessory genes like tat, nef and vpr)
f. the HIV-1 accessory genes, including the reverse transcriptase and the other viral genes involved in replication (from pCMV8.9 plasmid)

### Evaluation of foreseeable effects

1. The transduced cells can only survive in tissue culture at 37 degrees C.
2. The modified lentivirus cannot survive outside cells (dependent on cells for DNA integration), and is replicative incompetent; thus the lentivirus cannot propagate to infect new cells and pose a negligible risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon (a perioxygen compound) used at a concentration of 1% is known to have a wide range of microbial activity and is bacteriocidal against many human viruses, including HIV. All tissue culture material and disposable plastic ware will be soaked for a minimum of 18-24 hours in a freshly prepared 1% Virkon solution. The fluid is then disposed of down a laboratory sink with excess water. Solid waste is then double bagged in biohazard bags and autoclaved at 134 degrees C for 20 mins at 3.2 bar pressure.

The autoclave undergoes a planned preventative maintenance inspection every 3 months together with an annual thermometric testing by the installation company. Chart recorder print-outs are available for checking that the correct temperature and holding time have been achieved.

All inactivated waste is then treated as "clinical waste" and removed from site by a UCL contractor for incineration.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

This project has been considered by a panel representing the Genetic Modification Safety Committee who agreed with the proposer's assessment that this was activity class 2 work. The project will be considered again at the next meeting of the full committee. The laboratories in which the work will be carried have been designed to, and are being operated at the required containment standard.

**Project Containment**

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02/03/2022
### Project Additional Information

**Purposes of the contained use**


HEK293 cells will be used for amplification of the adenovirus, then primary porcine pulmonary endothelial and smooth muscle cells will be infected to express recombinant proteins.

**Recipient or parental organism**

HEK293 cells, of human origin, require handling at containment level 2. Porcine cells are not a hazard to humans and will not survive in the environment. Disabled adenoviral vectors are described below.

**Host/vector system**

Disabled adenoviral vectors due to removal of E1 and E3 elements in genome. Unable to replicate except in an engineered cell line (HEK293). Source: Dr Anne Ridley, Ludwig Institute for Cancer Research, UCL.

Adenoviral vectors have a limited host range, primarily human but they will infect some other animal species, although they are not pathogenic.

**Origin & function**

Disabled adenoviral vectors due to removal of E1 and E3 elements in genome. Unable to replicate except in an engineered cell line (HEK293). Source: Dr Anne Ridley, Ludwig Institute for Cancer Research, UCL.

Adenoviral vectors have a limited host range, primarily human but they will infect some other animal species, although they are not pathogenic.
Insert DNAs:
- constitutively active (V12) RhoA, Rac1 and Cdc42 (source organism: human). Function: Mutant is unable to hydrolyse GTP. Alters actin organization, intercellular junctions and growth regulation.
- B-gal (source organism: E. coli). Function: Hydrolyses lactose to yield galactose and glucose.
- GFP (Source organism: Aequoria victoris). Function: fluoresces green.

Source Laboratory: Dr Anne Ridley, Ludwig Institute for Cancer Research, UCL.

Evaluation of foreseeable effects

The adenoviruses are replication-defective due to removal of E1 and E3 elements in genome and will not multiply if they infect an animal. Although non-replicative, there is a risk that genes encoded by the adenovirus could be potentially harmful to humans. This would be limiting and non-infectious. Dominant negative and constitutively active forms of Rho GTPases: RhoA, Rac1 and Cdc42 have potential growth altering properties. Other inserts, B-gal and GFP can be toxic when expressed at high levels in cultured mammalian cells.

Risk to the environment following accidental release of any of the GMMs is very low. However, the risk of dissemination in the environment will be eliminated by handling in containment level 2 laboratory where all the waste is decontaminated prior to exit from the laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The risk of dissemination in the environment will be eliminated by handling in containment level 2 laboratory where all the waste is decontaminated prior to exit from the laboratory.

Liquid waste: 5,000 ppm hypochlorite (PreSept tablets) for 6-12h, then disposed of down laboratory sinks. This has been shown to kill 100% of infectious organisms and cells.
(Pasteur pipettes, pipette tips, tissue culture dishes): 5,000 ppm hypochlorite for 6-12h, followed by autoclaving at 135 degrees C for 20 minutes, then incinerated.

Solid Waste: 5,000 ppm hypochlorite (PreSept tablets) for 6-12h, then autoclaved at 135 degrees C for 20 mins, then incinerated. This is accepted to kill 100% of infectious organisms and cells.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The project has been reviewed by a panel representing the full GM Safety Committee. It agreed with the proposer's classification (activity class 2) and the containment and risk control measures. The project will be considered at the next full meeting of the GMSC on 7 July 2003.

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Animal Units

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Project Additional Information

Purposes of the contained use

Aims: To identify the molecular basis for the virulence of respiratory pathogens, especially Streptococcus pneumoniae, and their interaction with the host, and to investigate potential new therapies of vaccine candidates.
Methods: Candidate Streptococcus pneumoniae virulence genes will be inactivated by targeted deletion or disruption in the pathogenic bacteria, and the phenotypes of the resulting mutant strains characterised. In order to do this candidate virulence genes will have to be amplified from S. pneumoniae DNA and ligated into shuttle or suicide vectors propagated in laboratory strains of E. coli to construct gene deletion/disruption, complementation, and inducible expression plasmids. In addition S. pneumoniae genes will be expressed in E. coli for purification of the corresponding protein for vaccine experiments and biochemical analysis. Most culture volumes will be small scale (10 to 20 mls), although the occasional large scale protein purification will require culture volumes of up to 2000 mls. As the project is constantly evolving which candidate virulence genes cannot be specified, but will include cell membrane transporters, regulators, cell wall and stress response proteins. In addition, experiments may be performed using other wild-type and genetically modified Gram positive bacteria, including Streptococcus species (S. pyogenes, S. agalactiae and viridans Streptococci) and Staphylococcus species (S. aureus and S. epidermis). The GMM strains of these species will be acquired from other laboratories and not constructed within Dr Brown’s laboratory.

The majority of the work uses capsular serotype 3 (0100993) or 2 (D39) S. pneumoniae strains, and as those are wild type S. pneumoniae clinical isolates capable of causing disease in humans (otitis media, pneumonia, septicaemia and meningitis) they pose the most hazard inherent to the project. However, the genetic modifications to S. pneumoniae will usually either decrease or have no effect on the virulence of the recipient strain. S. pyogenes, S. agalactiae and S. aureus strains will also be clinical isolates capable of causing disease in humans (mainly soft tissue infections and septicaemia). Viridans Streptococci and S. epidermis are only of low pathogenicity in the normal host.

Recipient or parental organism

| Escherichia coli (K12 laboratory strains) |
| Streptococcus pneumoniae (pathogenic and laboratory strains) |
| Streptococcus pyogenes (pathogenic and laboratory strains) |
| Streptococcus agalactiae (pathogenic and laboratory strains) |
| Viridans Streptococci (pathogenic and laboratory strains) |
| Staphylococcus epidermis (pathogenic and laboratory strains) |

Host/vector system

| pEVP3, pID701, pACH74, pUCMUT, pJPC9111, pJPC9112, pLSIGFP, pLS70GFP, pLS70GFPcat, pNE1, puc18/19, pBluescript, Acceptor vectors, TOPO vectors, pACYC184, pQE expression vectors, pET15b, pEMCat, pEMSpc, plus the Magellan2 transposon. |

Origin & function

| Streptococcus pneumoniae genome (to allow construction of vectors for targeted gene disruptions, inducible expression or complementation), antibiotic resistance genes (to allow selection of mutant strains), green fluorescent protein gene and its derivatives, other markers for gene expression (eg lacZ). |

Evaluation of foreseeable effects

| Bacterial species: |
| E. coli - no hazard as laboratory non-pathogenic strain (K12 derivatives, ACDP category 1) |
| Viridans Streptococci and S. epidermis: Both these species are only pathogens in immunocompromised hosts or those with cardiac structural defects. They are present in all humans in very large numbers in the oropharynx (viridans Streptococci) or on the skin (S. epidermis). The only risk of infection is after inadvertent parenteral inoculation of a researcher, and even then this would only have a low risk of causing a significant infection (eg septicaemia) in the absence of severe immunodeficiency. |
| S. pneumonia, S. pyogenes, S. aureus, S. agalactiae - ACDP category 2 organisms. The project will use clinical isolates which could potentially cause invasive disease in humans. Infection will require either accidental parenteral inoculation (all species), transfer to broken epithelial barriers (S. aureus and S. pyogenes) or inhalation of an aerosol (all species). Transfer to damaged epithelial layers of S. pyogenes or S. aureus could cause mild and self-limiting soft tissue infections (eg minor cellulitis or abscesses) but very rarely could cause a more serious illness (erysipelas, necrotising fasciitis, or septicaemia). Inhalation of an aerosol initially could result in asymptomatic nasopharyngeal carriage (all species) followed by invasive disease including pharyngitis (S. pyogenes), pneumonia (S. pneumoniae and S. aureus), |
meningitis (S. pneumoniae) or septicaemia (S. pneumoniae). As nasopharyngeal colonisation by these pathogens is present in many of the population at any one time only a minority of whom develop invasive disease, the rate-limiting step for invasive infection occurs after nasopharyngeal colonisation and limits the potential danger of accidental exposure to an aerosol of these pathogens. Parenteral inoculation is potentially hazardous as it could result in direct introduction of the pathogenic species into the blood. However this can only occur if bacteria are being inoculated using needles which effectively is only during work with animals. Of importance is that any accidental parenteral inoculation is readily identifiable by the recipient. S. agalactiae is not usually a pathogen in adults except after labour and is only likely to cause infection after accidental parenteral inoculation.

S. pneumonia, S. pyogenes, S. aureus, S. agalactiae are all obligate human pathogens and therefore will not be able to spread within the environment. Nosopharyngeal colonisation of a researcher could lead to spread of the bacteria (wild type or GMM strains) to other close contacts. However person-to-person spread of all these pathogens is a continuous process involving a high proportion of the population at any particular time, and the significance of one researcher spreading one of the clinical isolates of the pathogens used for this research would therefore be limited. Most GMM strains will have gene deletions are likely to be less virulent or have a survival disadvantage compared to wild type strains due to disruption of virulence genes and insertion of heterologous DNA. On occasions regulator genes maybe inserted into replicating plasmids under the control of an inducible promoter and transformed into S. pneumoniae, and therefore create a strain which can overexpress a virulence regulator. However the level of expression would be dependent on a specific environmental signal (eg raffinose) which is unlikely to be present at a high concentration within the nasopharynx, and expression would therefore not occur if the strain were to colonise a researcher.

Vectors:
Potentially could spread antibiotic resistance to environmental organisms and hence from there to pathogens, but this is an unlikely train of events in the absence of positive selection. In general genes conferring resistance to antibiotics not routinely used in clinical practice are used as selectable markers (eg kanamycin and chloramphenicol), hence the clinical effect of inadvertent spread of antibiotic resistance to pathogenic species is reduced.

Inserted sequences:
No potential harm likely, as fully functioning virulence determinants usually require interaction with other proteins and only one S. pneumoniae gene will be expressed or carried in E. coli at one time.

GMM:
S. pneumoniae GMM strains will have either a similar or lower pathogenic potential as described for the wild type strains above.

Propagation of plasmids containing S. pneumoniae DNA or expression of S. pneumoniae proteins in E. coli will be very likely to result in increased virulence as (a) only non-pathogenic laboratory strains of E. coli will be used and the expression of a single S. pneumoniae virulence trait will not alter this (b) pneumoniae proteins tend to be detrimental to E. coli replication (c) most S. pneumoniae virulence proteins require co-operation with several other S. pneumoniae structures to achieve virulence.

Potential cross-contamination between GMM bacterial pathogens and one of the other Streptococci or Staphylococci species under investigation is unlikely to occur as usually experiments involving different pathogens will occur at different times and most of the vectors used will not replicate within Gram positive organisms. In addition in general transfer of genetic modifications requires positive selection and specific environmental signals to encourage bacterial uptake of heterologous DNA (eg Competence Stimulating Peptide of S. pneumoniae). Finally, as discussed above most bacterial virulence proteins require co-operation with several other structures to achieve virulence and will be unlikely to substantially affect the virulence of a different bacterial species in isolation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
This project was approved as a class 2 activity by the UCL GM Safety Committee at its meeting on 3 July. The laboratory in which it will be carried out is constructed and managed to containment level 2 standards.

Project Containment

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Project Ref 14/04.1

GENETIC MODIFICATION OF HAEMATOPOIETIC AND VASCULAR CELLS WITH REPLICATION INCOMPETENT VIRAL VECTORS CARRYING THERAPEUTIC GENES.

Class 2

Consent Granted not applicable
We aim to modify haematopoietic and vascular cell progenitors with antioxidant or anti-inflammatory genes and study the effects of these genes on the functional properties of the recipient cells. In future we will examine the therapeutic potential of the genetically modified cells in models of cardiovascular disease. We also aim to modify progenitor and mature vascular cells with genes that alter the activity of the enzyme telomerase in order to elucidate its function in vascular cell physiology.

Recipient or parental organism

The recipient organisms used in this study are human haematopoietic/vascular progenitor cells, or mature endothelial cells and vascular smooth muscle cells isolated from human, rabbit and pig vessels. These are eukaryotic cells which do not survive outside cell culture conditions unless introduced into animals or humans, regardless as to whether they are genetically modified or not. In the case of human cells the work carries an inherent potential risk due to the possible presence of human pathogens (however, cells are derived from donors certified to be free of common pathogens such as HepB and HIV). We have no reason to believe that genetic modification of these cells will trigger additional risks.

Host/vector system

We will use the following retroviral vectors: 1) MFG-IRES-EGFP a vector based on MoMLV, supplied by Queen Mary University of London, containing and IRES sequence and the marker gene EGFP; 2) pBABE-puro, supplied by Harvard Med School, USA, expressing the puromycin resistant gene; 3) pMKO.1-puro which is based on pQCXIN (Clontech), pSUPER.retro.puro which is based on a self-inactivating pMSCV-puro, purchased from OligoEngine, USA. We will also use lentiviral vectors based on HIV-1, AAV based on serotype 2, adenoviral vectors based on serotype 5 and baculovirus, all of which are obtained from University of Kuopio, Finland). All viral vectors are replication incompetent. In the case of retroviral vectors sequences encoding structural genes and envelope proteins essential in replication and virus assembly are mutated or removed totally or partially from the viral backbones. Self-inactivating lentiviral vectors will have deletions in the U3 region. In adenoviral vectors, genes involved in transcriptional regulation, virus replication and virus assembly (ie E1 alone or together with E2A, E2B and E4) are deleted partially or totally. In AAV vector backbones, viral genes are mostly replaced by non-viral DNA and by sequences of oncoretroviral vectors such as the LTR promoter, but not with genes essential for replication and virus assembly. In all cases missing gene functions are provided in trans by packaging cells. Baculoviral vectors are considered safe and nearly all the viral genome is included. The following packaging cells are used to produce viral particles: pHi-NX, PT67 and 293. Transgene expression is driven by commonly used eukaryotic promoters such as CMV, SV40E, U6, PGK and H1-RNA. An IRES sequence is used to obtain a bicistronic mRNA.

Origin & function

All the genes of interest used in this study are based on human sequences and do not alter the characteristics of the vectors carrying them. They correspond to

1) The extracellular superoxide dismutase gene, supplied by Queen Mary University in London. When expressed this gene localize to the extra cellular surface of the cell where it catalyses the conversion of superoxide into hydrogen peroxide. Expression of this gene effects the extracellular redox environment of the cell.

2) A chimeric TNF-alpha receptor antagonist comprising two monomeric forms of the human p75 soluble TNF receptor extracellular domain linked by a 15 amino acid bridge. (Croxford et al. J Immunol 164:2776, 2000). When expressed this gene produces a secreted molecule which binds to TNF-a and neutralises its activity. Expression of this gene will modulate cellular responses where TNF-a is involved, in particular inflammatory processes.

3) A catalytically inactive human telomerase mutant (DN-hTERT) supplied by Harvard Med. School, USA (Hahn et al. Nat. Med. 5:1164, 1999). When expressed this gene will produce a catalytically inactive form of telomerase. Expression of this gene will result in an overall inhibition of intracellular telomerase activity which may interfere with the maintenance of telomere integrity and cellular replicative capacity.

4) A human telomerase catalytic subunit small interfering RNA (hTERT siRNA) comprising hTERT sequences from nt 3114-3134, followed by 9 bp to form a loop and the corresponding anti-sense hTERT nucleotides, followed by 5 uridines, (Masutomi et al. Cell 144:241 2003). When expressed this sequence will activate the destruction of endogenous telomerase mRNA. This will have the same effect as the described for DN-hTERT above.
Evaluation of foreseeable effects

ENVIRONMENTAL EFFECTS: except for adenoviruses which can remain viable for a limited period of time outside laboratory conditions, all other viruses and infected eukaryotic cells do not survive if accidentally released into the environment.

EFFECTS ON HUMAN HEALTH: In case of accidental infection with any of the viruses used in this study there is a theoretical possibility of reversion of the disabling mutations by recombination between the viral vector sequences and endogenous viral sequences already carried by the host. However, the presence of pre-existing viral sequences in the accidentally infected cells is rare. In addition some of the viruses used in this study are self-inactivating, further increasing their safety.

In the case of vectors able to integrate in the host genome (oncoretrovirus, lentivirus and AAV-derived vectors) there is a very low possibility of insertional mutagenesis into the host genome.

In the case of adenoviruses, these are known to cause adverse immunological reactions, but very high exposure is required for this to occur.

The inserted genes used in this study are not oncogenic or known to be harmful by themselves in any other way. However, if a large quantity of viral particles containing anti-oxidant or anti-inflammatory genes used in this study were to gain access to the blood stream or the lung airways accidentally, there is a theoretical possibility that they might temporarily lower the body's natural defences. Rare cases of tuberculosis have been reported in association with anti-TNF-alpha therapy. Where inhibition of telomerase is concerned this is unlikely to have an effect on quiescent cells; it may however confer a growth disadvantage of replicating cells (e.g. blood cells) but the effect may not be manifested before many cell divisions occur, in which case the cells will naturally be replaced by healthy ones.

In case of accidental exposure to the genetically modified cells, these cells are unlikely to survive for a significant period as the host immune system will eventually eliminate them.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cell cultures, liquid waste or any other material coming into contact with viruses will be treated with 10% Virkon (a strong oxidising agent) for more than 12 hours and/or autoclaved. This treatment gives 100% kill. After viral inactivation liquid waste is disposed in designated sinks and solid waste is incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Although this project was considered by the GM Safety Committee to be on the borderline between classes 1 and 2, nevertheless the Committee felt that there was sufficient potential for severe consequences (though low in likelihood) for this project to be classified as an activity class 2 project.

02/03/2022

Page 665 of 1532
### Project Additional Information

**Purposes of the contained use**

A commercially available disabled lentiviral vector (Virapower Lentiviral system (Invitrogen) to be used to transfer genes to human or mouse cells in vitro to determine the effect of their expression on human cells.

**Recipient or parental organism**

The genes will be transferred to human prostate cancer cell lines including DU145 and PC3, normal human prostate epithelial cells including NP1542, mouse fibroblast cell line NIH3T3, dog and human kidney cell lines MDCK and 293T, monkey cell line Cos1 and other related mammalian cells. The vector DNA will be grown up in E. coli.
The genes to be transferred include plexins, semaphorins, Met, Rac, Rho, Rnd, PDZRhoGEF and other related genes.

Host/vector system

A commercially available disabled lentiviral vector (ViraPower Lentiviral system (Invitrogen)) will be used as vector.

Origin & function

The vector system will be obtained from Invitrogen, the cells lines are already available in the lab, previously obtained from the originators. cDNA of the genes to be transferred have been synthesised in the lab or obtained from other labs. The genes will be transferred in order to determine the effect of their expression on human cells.

Evaluation of foreseeable effects

Since the vector to be used is a disabled virus it will be unable to replicate in any host. The virions however could possibly infect human cells in vivo if inadvertently injected. The recipient cells will not survive in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All work to be carried out in a designated laminar flow cabinet. All waste to be disinfected and autoclaved. Sharps will not be used in work involving the virions and gloves and lab coats will be worn.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be disinfected with Virkon and then autoclaved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

A representative panel from the GMSC accept sthat this project should be classified as 2, rather than 1. Oncogenesis may depend on more events than merely the (accidental) insertion of one gene but it is possible that, in a "worst case" scenario, this might happen.

Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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### Project Additional Information

**Purposes of the contained use**

The use of replication deficient lentiviral-based vectors to express cDNAs encoding synaptophysin, synaptotagmin, synaptobrevin, potassium channel Kir2.1, and any of their dominant-negative, and green-fluorescent-protein fusion protein forms, and siRNA against sodium channel subunits, in a number of established rodent and human cell lines, in cultured cells isolated from rodent tissue and in brains of rodents. The aim is delineate the molecular mechanisms of regulating synapse strength and plasticity, and viral vectors are required for efficient delivery of synapse proteins to mark synaptic terminals and/or to perturb their function.

**Recipient or parental organism**

The primary recipient organisms will be cultured cells, including human 293 cells and rodent neurons. In some experiments, we will carry out injections into rat or mouse brain.

**Host/vector system**

The vectors will be based on the latest generation of lentiviral vector design (see Lois et al. 2202 Science 295:868-72), which includes refinements that rended accidental generation of a replication competent virus to be virtually impossible. The viruses, however, contain some HIV sequences and are pseudotyped with VSVg envelope such that human cells can be infected even if they are not dividing. Therefore, production and handling of viruses will require implementation of special safety measures at the containment level "2".

**Origin & function**

The use of replication deficient lentiviral-based vectors to express cDNAs encoding synaptophysin, synaptotagmin, synaptobrevin, potassium channel Kir2.1, and any of their dominant-negative, and green-fluorescent-protein fusion protein forms, and siRNA against sodium channel subunits, in a number of established rodent and human cell lines, in cultured cells isolated from rodent tissue and in brains of rodents. The aim is delineate the molecular mechanisms of regulating synapse strength and plasticity, and viral vectors are required for efficient delivery of synapse proteins to mark synaptic terminals and/or to perturb their function.
The inserted sequences encode brain specific proteins that are expressed in synapses. Our primary purpose of the use of the viral vectors will be to permit visualisation of synapses modification of synaptic efficacy at individual synapses.

**Evaluation of foreseeable effects**

The likelihood of viral vectors causing harm to health and environment is minimal. This is due to the fact that the viruses are defective for replication, and non-oncogenic and the overall non-invasive properties of gene sequences to be inserted.

The inserted sequences encode brain specific proteins and they are not oncogenic. Potential harm, which is expected to be minimal, cannot manifest unless the sequences

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

This derigation applies only to the work in the animal unit, involving the transport and injection of virus particles into rodent brains. Procedures used in the laboratory will be applied to the work in the animal facility. Note that injections require only small amounts of virus particles (less than 1 nl) at a time, and the virus has been modified in such a way that it is virtually impossible to accidentally generate a replicon competent virus. The virus is replication deficient due to (i) separation of packaging genes into two plasmids, each lacking both LTRs and the viral packaging signal (ii) deletion of env, vpr, vpu, vif, and nef viral genes from the packaging vector, (iii) the presence of self-inactivating LTR (TATA box deletion) in the vector expressing the packaged viral genome, and thereby resulting in no expression viral gene products, (iv) the expression of envelope VSVg on a separate vector. A full explanation is provided in the Risk Assessment.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Following virus usage, any material that come into contact with the viruses will be thoroughly decontaminated by soaking in 3% Virkon for at least 60 min, followed by draining and disposal to biohazard waste. Bottles of media used for infection will be isolated from all others and immersed in 3% Virkon before being placed into standard glassware containers. Liquid waste will be treated with 3% Virkon for at least 60 minutes before disposal. Spills will be immediately treated with 3% Virkon. If there is a spillage on the laboratory coat, it will be placed into a plastic bag and autoclaved. Aerosol production will be minimised through the use of microbiological practice, plugged-tips, plugged-pipettes, and infected cultures will only be handled within a microbiological safety cabinet (to BS 5726) in the viral suite (G17). G17 does not contain windows and are not readily accessible to insects, etc. The laboratory environment will be monitored for insects and any that are found will be killed. As indicated above the recombinant virions produced in this system are replication deficient.

**Is an emergency plan required according to regulation 20?**

| N |

**Tick to confirm that it is attached to this form**

| Y |

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

| N |
Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 14/05.2

Investigating the persistence & cellular transformation by Kaposi’s sarcoma associated herpesvirus (KSHV) in cells using bacterial artificial chromosomes (BACs) containing the KSHV genome and retoviral (including lentivirus) vector medicated gene expression and gene ablation

Date Ackn’d 21/07/2005

Date Project Ceased

Class CultureVol

Class 2 < 1 Litre

Consent Granted

Non-GMM Not Applicable

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements N

Project Additional Information

Purposes of the contained use

This connected programme of work aims to investigate how the human herpesvirus, Kaposi's sarcoma associated herpesvirus (KSHV) is able to infect, persist in a latent state, reactivate into a lytic cycle of virus production and in some cases immortalise certain cell types. This connected programme will use a number of standard molecular biology/virology methods to produce recombinant KSHV and monitor KSHV infection, over-express different viral and host genes and ablate through RNAi different virus and host genes. These methods will be used to determine the phenotypic outcomes of KSHV and host interaction. We will use the BAC36 Recombinant KSHV bacterial artificial chromosome to transfect human cells and rescue infectious KHSV virus that has an integrated GFP/puromycin selectable marker expression cassette within the viral genome. This infectious virus will be used to infect eukaryotic cells (including human), both cell lines and primary cells using GFP expression as a marker for virus infection. Further, the BAC36 Recombinant KSHV plasmid will be used to delete additional KSHV
genes by homologous recombination of a Kanamycin resistance, gene disruption cassette with the BAC36 in E.coli DH10B in the presence of the helper plasmid pGETrec. Kanamycin resistant BAC DNA will be isolated from the E.coli and used to transfect human cells to produce gene deletion KHSV mutant viruses for further characterisation. This system will be used to monitor the infection of human cells by KSHV and identify the function of KSHV genes in the appropriate cellular context.

We will use standard eukaryotic gene expression vectors and recombinant retroviral vectors to deliver into eukaryotic cells (including human), both cell lines and primary cells, host and/or KSHV genes. The expression cassettes will direct either the controlled or constitutive expression of said genes to investigate the effect of these genes on host and virus interaction. Similarly, we will use recombinant retroviral vectors to deliver into eukaryotic cells (including human), both cell lines and primary cells, an RNA polymerase III promoter expression cassette that directs the production of shRNA that is converted in the cell to RNAi. These RNAi’s will target either host and/or KSHV genes to ablate their expression at the mRNA level. We will use these methods to investigate the result of the loss of these genes on host and virus interaction.

### Host/vector system

Host cell lines and primary cells including: 293T, HeLa, NIH3T3, B-cell lines from all stages of B-cell including BJAB, Ramos, Raji Tom-1, DEL RPMI-8226, BCP-1, BCBL, HBL-6, BC3, T-cell lines including C8166, CEM, SupT1, monocyctic cell lines including THP1, dendritic cell line MUTZ3, endothelial cells DMVEC, BMVEC. All cell lines and primary cells will NOT be from the workers. The bacterial hosts for expression vectors will be disabled E.coli: XL-1 Blue, TopF10, JM109, SURE. The cloning plasmids will be pGEM derivatives, pUC series, pBLSRIPT series, pcDNA3.1, pCMV/Zeo, and pEGFP-1. The retroviral vectors for either gene expression or shRNA production are retroviral vectors that use the tripatite packaging plasmid pCMVR89.1 (packaging plasmid), pMDG (envelope VSV-G plasmid) and as a transfected cell lines under puromycin selection. The foreseeable effects of transduction of the eukaryotic cells with expression vectors, or the infection of these cells with either recombinant KHSV or recombinant retroviruses would be the production of all cell line with altered properties able to establish a tumour in the laboratory workers.

### Origin & function

The pMHGP36 Bac containing the GFP gene was recombined into the KHSV genome between ORF18 and ORF19 and the resulting BAC propagated in E.coli DH10B. This is supplied to us as a gift from Professor SJ Gao (see J.Virol 2002, 76, 6185-6196 for details). The intended function of the KHSV BAC is to produce infectious KHSV particles containing a genome with an inserted GFP and puromycin expression cassette. These KHSV particles will be used to infect human and animal cells. In addition the defined gene knockouts of KHSV allowing the investigation of KHSV the gene functions. The retrovirus vector systems are standard laboratory reagents within the Division of Infection & Immunity. The vector systems originated from infectious retroviruses of
Evaluation of foreseeable effects

The bacterial strains used for all plasmid propagations within this programme of work are disabled in key bacterial functions (i.e. recombination defective) and are unable to survive in the environment or pass genetic onto host bacterial flora following a breach of containment. The cloning vector/BAC is maintained within the bacteria essentially as an extra bacterial chromosome. Due to its size it is highly unlikely to transmit from the host bacteria to environmental bacteria following a breach of containment. In addition, the GMO (bacteria containing the KSHV BAC) is not a risk to the environment as the bacterial strains used for plasmid propagation are disabled in key bacterial functions (i.e., recombination defective) and are unable to survive in the environment. The bacteria are unable to express the viral genes encoding the complete virus particle and are unable to replicate and package the viral genome contained within the BAC. Therefore there is no risk of KSHV particles being produced from bacterial culture. Similarly, the plasmids used for the production of retroviral vectors split the retroviral genome into three separate plasmids such that virus production is only initiated when all three plasmids are present in a eukaryotic cell. Therefore there is no risk of retroviral particles being produced from bacterial culture. OVERALL THE RISKS OF ADVERSE EVENTS FROM THE HANDLING OF BACTERIA, PLASMIDS (INCLUDING THOSE EXPRESSING KNOWN OR POTENTIAL ONCOGENES) AND THE KSHV BAC ARE LOW.

The risks associated with BAC36. Recombinant KSHV plasmids are through the risk from accidental transfer of the vector to the worker. The main risk is therefore to the laboratory personal directly handling the BAC. If this was to transfer into a permissive host cell there is a small chance of the vector establishing a KSHV infection. This is a very low risk as herpesviruses require viral proteins to initiate the infectious cycle and these would not be present with the BAC DNA as the bacteria are unable to express the viral genes. The BAC36 Recombinant KSHV will only efficiently produce replication competent extracellular KSHV when transfected into human cell lines and induced by the addition of TPA or sodium butyrate. Therefore the control of virus particle production can be contained both physically in a category 2 tissue culture laboratory and by prevention of virus production by the absence of the inducing agent. Recombinant or wild type KSHV are therefore unlikely to be transmitted to, or on from the laboratory workers during routine laboratory experiments or following disposal of laboratory waste.

Wild type KSHV is categorised as a Hazard Group 2 virus similar to Epstein Barr virus. Like Epstein Barr virus, KSHV is none hazardous in a healthy individual but can cause tumours in an immunodeficient setting. BAC36 Recombinant KSHV or any further virus gene deletion mutants will not pose a greater risk to the individual than wild type virus following a breach of containment. KSHV as a Hazard Group 2 pathogen can be handled routinely in a Category 2 tissue culture laboratory and therefore the BAC36 Recombinant KSHV and virus deletion derivatives will also be safe to handle under the same containment. OVERALL THE RISKS OF ADVERSE EVENTS FROM HANDLING VIRUS DERIVED FROM THE KSHV BAC ARE LOW.

For the retroviral gene expression and gene ablation vectors production of infectious viral particles only occurs following transfection of the three plasmid system into eukaryotic cells. Therefore the control of virus particle production can be contained both physically in a category 2 tissue culture laboratory and by prevention of virus production by the absence of all three plasmid vectors. Retroviral vectors are therefore unlikely to be transmitted onto the laboratory workers during routine laboratory experiments involving the retroviral vector plasmids or following disposal of laboratory waste. The main risk is accidental exposure of laboratory workers to recombinant retroviruses. As these retroviral vectors are self-inactivating the risk of onwards transmission to another person is almost zero. The main risk is therefore alteration of the cellular function of the exposed person through insertional inactivation/activation of tumour suppressor genes/oncogenes respectively, or the alteration of cellular function by gene overexpression or ablation from the transferred genetic material within the vector. THESE ARE EXTREMELY UNLIKELY EVENTS THAT HAVE ONLY OCCURRED IN A CLINICAL SETTING FOLLOWING PROLONGED INFECTION WITH HIGH TITRE RETROVIRAL VECTORS AND SELECTION IN VIVO FOR CELLS WITH ALTERED GROWTH POTENTIAL. THEREFORE THE OVERALL RISKS ARE LOW.

Together the risks identified within this integrated programme of work can be minimised to acceptable LOW levels by working to existing laboratory standards and practices in designated Category 2 level containment. This is supported by the accompanying risk assessment.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory workers are responsible for the following procedures:
Method of GMM inactivation: In accordance with Departmental COP Bacterial culture supernatants are treated with Hycolin (1:80 concentration) for at least 16 hours before disposal to the drains. Bacterial plates are double bagged in clear autoclave bags and placed in designated (green) bins for autoclaving.

Method of GMM inactivation: In accordance with Departmental COP cell culture supernatants are treated with Hycolin (1:80 concentration) for at least 16 hours before disposal to the drains. Tissue culture plastics are double bagged in clear autoclave bags and placed in designated (green) bins for autoclaving.

Wash up staff are responsible for the transport of designated green bins to the autoclave room, autoclaving the double bagged contents and transfer of autoclaved waste into yellow bags for collection and disposal by incineration.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

This work has been considered by the UCL GM Safety Committee and approved as an Activity Class 2 project. The containment laboratory is suitable for the work.

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref  14/05.3

02/03/2022  Page 673 of 15326
Our interest is in Schwann cells, the myelinating cells of the peripheral nervous system. These cells are an ideal system in which to study mechanisms controlling cell proliferation, survival and differentiation. Their ability to regenerate after nerve injury may give key insights into increasing the repair process after injury to the central nervous system. By using adenoviral mediated gene transfer, we hope to study the intracellular pathways and transcription factors that control the development and differentiation of this exquisitely specialised cell type.

All adenoviruses used for this work are deleted for the E1 and E3 regions of the adenoviral genome and are therefore replication deficient; these are therefore classified as Category 1. The host cells, Schwann cells, are primary cells which are non-immortalised and non-transformed. The transcription factors such as Pax-3 or molecules that alter intracellular signalling such as the receptor Notch may have effects upon the differentiation and proliferation of the host Schwann cells in vitro.

The preparation of adenoviruses would be performed using the AdEasy adenoviral kit (from Qbiogene Inc), which is a widely used reagent. Adenoviruses used are E1/E3 deleted and are therefore replication deficient. E1 function is complemented in 293 cells for the amplification and production of viral stocks. Adenoviruses would then be used for infection into primary mouse or rat Schwann cells.

The cDNA's encoding the transcription factors and proteins involved in cell signalling would be either of rodent or human origin. Initially for example we would look at the function of Pax-3, a DNA binding transcription factor that regulates gene expression during normal development in cells. The Pax-3 coding sequence that we would express is from the mouse. For the analysis of signalling pathways involved in Schwann cell development and differentiation, we would use either activated or dominant negative forms of the intracellular signalling molecules, for example the p38 MAP kinase pathway, that have been obtained in collaboration with other scientific research groups. These would be used to either switch on or inhibit certain signalling pathways in Schwann cells, and we would then measure the effect of this upon the phenotype of our cells using a number of different analyses.
Expression of molecules such as Pax-3 and Notch may well change the proliferative and differentiation state of the recipient Schwann cells. However, even if there were a complete breakdown of containment, because the adenoviral vectors are replication deficient, the risk to both human health and the environment is low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None applied for

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All adenoviral material is thoroughly decontaminated using Virkon (usually overnight) before disposal for autoclaving, and all surfaces (eg. tissue culture hoods) are decontaminated using Virkon solution after use. All tissue culture plastics are autoclaved after use.

**Is an emergency plan required according to regulation 20?**

N

**Tick if yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

The Committee noted that the applicant classified this project at level 2 on the basis of one report that deregulation of Pax3 can transform mouse cells in culture. The Committee noted further that there is no evidence that it will transform Schwann cells for other cells in culture.

Nevertheless, the Committee agreed with the proposer's assessment that, as a precaution, the work ought to be carried out at containment level 2 and accordingly approved this project as a class 2 activity.

**Project Containment**

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**Large Scale Activities**

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**Human Clinical Applications**

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Project Ref 14/06.1

Investigating the role of cadherin-binding proteins in cell morphology.

Class 2 | < 1 Litre

Non-GMM | Consent Granted
Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Tissue culture cell lines will be transduced by replication-deficient retrovirus vectors in order to overexpress or knock-down cadherin and its binding proteins.

Recipient or parental organism

We will work with replication deficient retroviruses. These viruses are made by transfecting HEK293T cells with: 1) a plasmid expressing the gag and pol genes from murine leukemia virus (MLV) which encode for viral structural and polymerase proteins, respectively, 2) a gene encoding a viral envelope protein (VSV-G) which will determine the host range of the virus produced, 3) an expression plasmid (either pBabe-puro or pRetroSUPER) containing the gene of interest and a packaging signal, allowing it to be incorporated into the virus particles.

Host/vector system

HOSTS: HEK293T for virus production, immortalized mammalian cell lines in vitro.
VECTORS: pRetroSUPER, LZRS-MS-neo, pBabe-puro.

Origin & function

Inserts:
cadherin and its binding proteins
small oligos for the production of siRNA in vivo
indicator proteins (GFP, dsRed, LacZ)

Intended functions:
We would like to interfere with the expression of the cadherin and its binding proteins to investigate the involvement of these molecules in cellular behaviours.

Evaluation of foreseeable effects

Primary transduction, with the recombinant replication defective retroviruses, of accidentally exposed tissue, could be harmful using cell-cell adhesion protein cadherins and their binding proteins with potential cell transforming effects. Thus, the risk of accidental exposure will be limited: Any spillage will be immediately removed by treatment with 3% Virkon.

Gloves and laboratory coats will be worn at all times and can be autoclaved in the case of accidental spillage. Working in a safety hood will prevent air-mediated exposure.

To avoid any possible transfer of this virus all experiments involving retroviral vectors will be conducted in a laboratory suite reserved for virus work. The cultures will be handled within microbiological safety cabinets and appropriate precautions will be adopted throughout. Laboratory workers handling these cultures will have extensive experience tissue culture and microbiological experiments and will be fully aware of the principles of occupational safety and handling.

By autoclaving cultures and plastic ware that were in contact with transduced cells and transduced cells the virions will be completely inactivated. Liquid waste will be treated with 3% Virkon for at least 60 min.

In the viral suite (G17), media containing virus will be removed from cell cultures and disinfected with 3% Virkon for 60 min.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The local rules for working in the containment level II laboratories will be followed in all locations. These practices include the rules for work in a microbiological safety cabinet.

Gloves and laboratory coats will be worn at all times. All cuts or abrasions will be covered with waterproof dressings.

No sharps of any type (glass Pasteur pipettes, needles, razors) will be present in the vicinity of work when handling viral stocks.

Waste will be bagged for autoclaving, and the bags sealed before removing from the designated rooms.

Eye protection will be worn.

Aerosols may be produced during the handling of supernatants from transduced cultures. Aerosol production will be minimised through the use of good microbiological practice and transduced cultures will only be handled within a microbiological safety cabinet (to BS5726).

Contaminated tips, straws, etc. will be placed in a sandwich box filled with 3% Virkon for at least 60 minutes before draining and disposal into an autoclave bag. Bottles of media used for infection should be isolated from all other and immersed in 3% Virkon before being placed into standard glassware containers.

Liquid waste will be treated with 3% Virkon for at least 60 minutes before disposal.

Incubators.

Incubators reserved for transduced cultures (room G17) will be used. Transduced cell culture flasks should be placed in a closed plastic sandwich box with small holes pierced in side in order to minimise the chance of accidental spillage in the incubator but still allow access of CO2.

Spillage

Spills will be treated with 3% Virkon. If there is a spillage on the laboratory coat, it will be placed into a biohazard bag and autoclaved.
Avoidance of airborne transmission.
Procedures will be adopted to avoid the formation of aerosols. Aerosol production will be minimised through the use of microbiological practice and transduced cultures will only be handled within a microbiological safety cabinet (to BS5726). Room G17 does not contain windows and is not readily accessible to insects, etc. Windows in adjacent laboratories will be kept closed. The laboratory environment will be monitored for insects and any that are found will be killed.
As indicated above the recombinant virions produced in this system do not have the capacity to replicate in host cells or host organisms.

Treatment of virus before disposal
By autoclaving cultures and plastic ware which were in contact with transduced cells and transduced cells the virions will be completely inactivated. Liquid waste will be treated with 3% Virkon for at least 60 min.

Processing of materials for use in location outside of the viral suite.
In the viral suite (G17), media containing virus will be removed from cell cultures and disinfected with 3% Virkon for 60 min.
Cells will then be washed extensively (the wash again disinfected) in order to remove viral particles from the cultures. Since the virus used for transduction does not contain a viral genome, and is thus replication deficient, there will be no viral particles produced by the transduced cells. Thus there is no harm connected to work with cultures previously transduced with these vectors, and these cultures can be handled safely. Containers holding the inactivated virus products will be wiped out (3% Virkon) before removal. For subsequent analysis of live cells, culture dishes containing washed cell-cultures will be placed in tray and analysed without opening the dish. The dishes will be returned to the viral suite after the experiment, disinfected with Virkon 3%, and autoclaved.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The cUCL GM Safety Committee approved this project as a class 2 GM activity at its meeting held on 7 February 2006. The details given in the attached full risk assessment are the same as seen by the UCL Committee.

Project Containment

<table>
<thead>
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</tr>
</thead>
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02/03/2022
The objective of this research is to determine the role of HIV-1 infection on human brain and placental cells as models of HIV-1 neuropathogenesis in adults and HIV-1 transmission in utero in children, respectively. Little is known about the direct impact of HIV-1 infection of human neurones and other brain cells and what role this infection may have in the development of HIV-1 associated dementia. In this study we aim to describe this type of infection in terms of neurone subtype infected and the impact this infection has on global gene expression by gene chips. HIV-1 transmission in utero occurs in 14% of newborns with HIV mothers. We know very little about why paediatric neuroAIDS is more aggressive than its adult counterpart. Viral entry into the placenta and brain across the endothelial barrier has not been previously compared. This study will define the protective properties of the placenta and brain barrier to viral infection for the first time and how these are overcome to result in utero transmission of the virus by using cell biology approaches and changes in global gene expression will be assessed by gene chips upon HIV-1 infection.

Human cell lines and primary cells will be transfected with marker sequences that indicate the replication of HIV-1. These cells will be prepared and cultured in containment level 2 laboratory. After a maintenance period, cultures will be transferred to the CL3 laboratory and inoculated with HIV-1 isolates. The cells will then be fixed and assayed for HIV replication.

Recipient or parental organism

Human cells comprising T cell lines, primary human brain and placental endothelial cells are to be grown in culture within a category 2 tissue culture environment. Working with these cells poses some hazard due to the clinical origin of tissue (e.g. hepatitis B, herpesviruses, Mycobacteria (tuberculosis)) to researchers by accidental exposure. However these cells, themselves, do not pose any infectious hazard to researchers, or the environment as the cultures cannot survive in ambient conditions for longer than 30 minutes.

T cell lines transfected with HIV-1 LTR indicator constructs are to be used for measuring viral load in cell supernatants. These modified cells do not contain the full length viral genome and are therefore not infectious. The main hazard to health in this project originates from infection of these human cultures with viruses. HIV lab and primary
Isolates viral stocks are grown in a containment level 3 laboratory environment.

**Host/vector system**

1. Human T and monocyte cell lines/HIV-1 isolates (for viral stocks)
2. Human Indicator T cells/HIV-1 isolates (to quantify viral stocks)
3. Primary human brain cells/HIV-1 isolates (for infection studies)
4. Primary placental endothelial cells/HIV-1 isolates (for infection studies)

**Origin & function**

Indicator T cell lines (MAGI-CCR5 and CVG37), transfected previously with HIV-1 LTR driven indicator marker of β-galactosidase in MAGI cells and green protein GFP in CVG37, are used to quantify viral stocks for infection studies. Other T cells, brain and endothelial cells will be infected with full length HIV-1 isolates that have not been genetically modified and are derived from donated clinical specimens where consent has been given for their use for biomedical research.

**Evaluation of foreseeable effects**

The growth of HIV-1 stocks in human T cell lines by co-cultivation and their concentration by ultracentrifugation pose the greatest risk to the health of researchers by accidental exposure. HIV-1, a blood borne human pathogen is the causative agent of AIDS and as such will be handled and disposed of within a validated containment level 3 environment. Infection of brain cells by HIV-1 results in a persistent infection with limited viral protection. All cell supernatants and lysates are still potentially infectious.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

In the containment level 2 laboratory, cell culture supernatants and excess clinical brain tissue are treated with Hcolin(1:80 concentration) for at least 16 hours before disposal. This approach achieves 100% kill of human cells and their endogenous pathogens. Tissue culture plastics are double bagged and autoclaved. The waste is later incinerated.

In the containment level 3 laboratory, disposal of hazardous and infectious material is tightly regulated, any products resulting from working with HIV-1 infected Human cell lines and Human brain cell cultures: All equipment, surfaces and floors are routinely disinfected with 5% Trigene II; Materials used in research (laboratory clothing, plastic ware) are decontaminated with up to 5% Trigene II for 16 hours prior to being discarded and autoclaved; any products from HIV-1 viral stocks or human cell cultures will be inactivated with 5% Trigene II for 16 hours to ensure 100% kill. Set procedures for waste disposal are in place within the category 3 environment: (1) solid waste (paper towels, laboratory protective clothing, plasticware) are discarded and autoclaved at 134°C for 20 mins and later incinerated; (2) Liquid waste which are, or might be infectious must be treated with 5% Trigene II for at least 12 hours before being discarded; (3) Blood waste is inactivated with 5% Trigene II for a minimum of 12 hours before being autoclaved. 5% Trigene II is a quaternary ammonium compound based detergent sanitiser that has a combined cleaning and disinfection action that is effective in inactivating all known microorganisms (inc. viruses) and ensures, with the combination of autoclaving, 100% kill of viruses in any solid, liquid or blood product that has been deemed potentially bio-hazardous during the course of experimental research in a category 3 environment.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
The UCL GM Safety Committee have approved this project as a class 3 activity involving GMMs. Although the genetically modified cells are not, in themselves, hazard group 3 materials, the experimental procedures that follow their establishment in culture must be carried out under containment level 3 management conditions.

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**Project Ref** 14/06.4

- **CU2 Project Title**: Investigation of the mechanisms regulating virus assembly.
- **Class CultureVolClass2 CultureVolumeClass3-4**
  - Class: Class 2
  - CultureVol: < 1 Litre
  - Consent Granted: Not Applicable

**Project Additional Information**

- **Date Ackn'd**: 27/09/2006
- **Date Project Ceased**: 
- **Historical Significant Changes**: 
- **Historical Date of Additional Info**: 
- **Significant Change ID**: 
- **Date of Significant Change**: 
- **Project notified under transitional arrangements**: N
- **Tick if notifying a connected programme of work**: N
Purposes of the contained use

The aim of this project is to investigate the mechanisms controlling the assembly and cell-to-cell transmission of enveloped viruses, in particular the human immunodeficiency virus (HIV) and the human cytomegalo virus (HCMV). Recent studies have indicated that in many cells these viruses assemble on compartments of the endocytic pathway and that a key cellular machinery, the ESCRT machinery, is essential for HIV budding. Moreover, transfer of viruses from cell-to-cell appears to occur through specialised cell surface domains with similarity to immunological synapses. The aim of these experiments is to use dominant negative forms of proteins that normally control sorting in the endocytic and exocytic pathways, and small interfering RNAs (siRNA), to investigate how the components of these viruses are brought together on late endosomal membranes to allow virus assembly and transmission to occur. Because macrophages will be a main target for analysis, lentiviral vectors will be used rather than non-lenti retrovirus vectors.

Recipient or parental organism

HEK293T for virus production, immortalised mammalian cell lines (e.g. HeLa, Cos, Chinese hamster ovary (CHO), SupT1, Jurkat, CEM, CEMx174), primary macrophages isolated from screened blood obtained from the Blood Transfusion Service, fibroblasts from knock out mice (MEF).

Host/vector system

The vectors will be based on the latest generation of lentiviral vector design (see Lois et al. 2002 Science 295-868-72), which includes refinements that render accidental generation of a replication competent virus to be virtually impossible. The viruses, however, contain some HIV sequences and are pseudotyped with VSVg envelope such that human cells can be infected even if they are not dividing. Therefore, production and handling of viruses will require implementation of special safety measures at the containment level 2.

Origin & function

The inserted sequences encode cellular proteins that are implicated in the assembly and transmission of HIV and HCMV - two major human viral pathogens. Our primary reason for using lentiviral vectors is that they allow transfection and expression of these proteins in non-dividing cells - macrophages - to permit visualisation of assembly and viral transmission.

Evaluation of foreseeable effects

The likelihood of viral vectors causing harm to health and environment is minimal. This is due to the fact that the viruses are defective for replication, and non-oncogenic and the overall non-invasive properties of gene sequences to be inserted.

The inserted sequences encode cellular proteins that are not oncogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All experiments will be conducted in mammalian cell lines kept in ACDP containment level 2 facilities in the MRC-LMCB.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Following virus usage, any materials that come into contact with the viruses will be thoroughly decontaminated by soaking in Trigene (5%) for at least 60 min, followed by draining and disposal to biohazard waste. Bottles of media used for infection will be isolated from all others and immersed in Trigene (5%) before being placed into standard glassware containers. Liquid waste will be treated with Trigene (5%) for at least 60 minutes before disposal. Spills will be treated immediately with Trigene (5%). If there is a spillage on a laboratory coat, it will be placed into a plastic bag and autoclaved. Aerosol production will be minimised through the use of microbiological practice, plugged-tips, plugged-pipettes, and infected cultures will only be handled within a microbiological safety cabinet (to BS 5726) in the viral suite (G17). G17 does not contain windows and are not readily accessible to insects, etc. The laboratory equipment will be monitored for insects and any that are found will be killed. As indicated...
above the recombinant virions produced in this system are replication deficient.

This project has been considered by a representative panel of advisors on behalf of the UCL GM safety committee who agreed with the classification and risk assessment for notification to the HSE. The proposal will additionally be considered in full at the next scheduled GMSC meeting in accordance with the procedures of the committee.

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**Project Ref** 14/06.5

- **Date Ackn'd**: 13/11/2006
- **CU2 Project Title**: Cloning and characterisation of cellular factors involved in the early steps of retroviruses replication.
- **Class**: Class 3
- **CultureVolClass2**: 1-50 Litres
- **Consent Granted**: Yes
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**: 14/06.5 replaces 14/01.4 and the sig. Change to it 14/06.2.
Cloning of cellular factors important for retrovirus replication will foster progress in the development of specifically targeted anti-viral compounds. Mapping the molecular interactions between cellular factors and viral proteins and understanding such interactions at a structural level will increase our chances to find small molecules inhibitors by rational design. Knowledge about the molecular processes involved in retroviruses’ replication will improve the design of both viral and non-viral gene therapy vectors and may reveal unexpected biological phenomena. Ultimately the function of any cloned cellular factor as well as its possible inhibitors will have to be tested with both replication incompetent and replication competent retroviruses, in particular HIV-1 and SIV. HIV-1 is pathogenic in humans and causes AIDS, thus it must be used only in an appropriately contained environment. SIV represent a valid target of research because of its use in primate animal models of AIDS.

1. Recombinant viral vectors. Murine leukaemia virus (MLV), HIV-1 and SIVmac-based retroviral vectors will be used. Theses vectors are made by three separate Componenets: core-enzyme proteins, envelope (wild type or VSV-G) and vector genome. The core enzymes and envelope plasmids are provided in trans and cannot be packaged in the viral particles. Thus viral vectors will be unable to replicate. The use of three plasmids system will reduce the chances of recombination to almost zero. Viral vectors will express marker proteins, specific cDNAs or shRNAs. Viral vectors are infectious and their tropism is increased by pseudotyping with VSV-G envelope. Infection with such vectors may induce an immune response against viral proteins and other proteins expressed by the vectors themselves. Retroviral vectors may activate cellular genes by insertion mutagenesis. Accidental injection into workers may result in unwanted expression of genes carried by the retroviral vector. Expressed proteins are not toxic or dangerous to animals or plants and will survive for more than a few hours at room temperature. They cannot be diffused by aerosol and cannot infect plants. Even in case of total breach of containment, viruses would need to be injected or eaten or drunk to infect humans or animals. The inability of such recombinant viruses to replicate means that even in case of total breach of containment and accidental infection, no virus spread from individual to individual (or animal to animal) is possible.

2. HIV-1 and SIV molecular clones. Replication competent HIV-1 and SIV will be made by transfection of 293T cells with plasmids encoding for such viruses. These viruses are infectious and can replicate in humans (HIV-1) or non-human primates (SIV) and cause AIDS. Tropism is limited to CD4+ cells. Virus produced by transfection 293T cells may be high-titre. SIVmac and HIV cannot infect plants or micro-organisms in the environment. HIV-1 and SIVmac are enveloped viruses, which can survive for a short time in the environment (a few hours). The tropism of these viruses is limited to humans (HIV) or non-human primates (SIVmac). No other mammals can be productively infected.

3. HIV-1 and SIVmac deleted viruses. Mutant viruses, which have a deleted envelope gene or both the envelope and the nef genes, will be used to map viral determinants of the interaction with specific cellular factors. The env-viruses will be pseudotyped in trans with VSV-G envelope, which will broaden their tropism. These viruses are not replication competent because they lack envelope, which is provided in trans. To produce virus, 293T cells will be co-transfected with the mutant virus plasmid and the VSV-G plasmid. There is no significant homology between HIV-1 and SIVmac env genes and VSV-G, thus recombination is very unlikely. These viruses may recombine with wild type HIV-1 or SIV and reconstitute the envelope gene, thus becoming fully infectious. The risk to the environment is similar to that of recombinant viral vectors (see point 1) but viruses pseudotyped with VSV-G have broadened tropism and could infect other mammals in case of total breach of containment. However, viruses will be able to complete one replication cycle only and will not be able to spread within an individual or pass to other animals.

4. Plasmids and recombinant bacteria. Full length molecular clones of HIV-1 and SIV will be grown in competent and disabled E.coli. There is the remote possibility that full-length HIV-1 and SIV plasmid DNA at very high concentration may cause infection and AIDS if infected through the skin or inhaled. Disabled E.coli do not represent a risk to the environment. Plasmid DNA may survive for quite a long time outside the laboratory but it is very unlikely that it will ever generate infectious virus. Even if they do generate infectious virus, the same assessment as for points 2 and 3 applies.

Host/vector system

Vectors: Retroviral vectors: pHR', pHR'cPPT, pCSGW and derivatives, pCMVd8.2, pCMVd8.9,pMD.G, pLNPOZ and derivatives, pMFG and derivatives, pSRVdNBDM and
pSRvdNPurodH. PSIV3+ (SIVmac gag-pol expression vector) and SIVeGFP. All vectors have been thoroughly tested for the lack of recombination. PMFG has been approved for clinical trials while pHR', pCMVd8.2 and 8.9 are used in pre-clinical studies. Cloning plasmids: Blusscript, pGEM derivatives, pUC series, bacteria expression plasmids: pGEX series, pQE60, pET, pQE32 Mammalian expression vector pCMV Tag and derivatives pGEMU6-LINKER.

Recombinant HIV/MLV chimeric vectors (replication incompetent) pME411-delta env, pBRU3-delta env-mMA12, pBRU3-delta env mMA12-luc, pBRU3or-delta env-mMA12CA-luc2. Virus made from these vectors can replicate due to deletion of the envelope sequence and are suitable for single round infection assays only.

Full length HIV-1 molecular clones like NL4.3, SF162, HIIIIB, YU-2, and derivatives, SIVmac full length molecular clones pBRmac239 and pBRmac 239 RT-SHIV and derivatives.

Hosts: disabled E. coli: XL-1 blue, HB101, DH5 alpha, SURE, JM109, BL21, KS1000, DE3.

Hosts: mammalian cell lines include TE671, HT1080, HeLa CD4, 293, 293T, U87, Jurkat, NP-2, SupT1, NIH3T3, mus dunni, Simian lung fibroblasts, simian kidney epithelial cells (OMK), rabbit epithelial cells (SIRC). Primary cells may include: peripheral blood monocytes, macrophages, lymphocytes.

Origin & function

All viral vectors have been cloned by other research groups. Cells are bought from the European or American Tissue Culture Collection. Disabled E. coli are bought from specialised companies. Competent bacteria are prepared in-house. Additional HIV-1 or SIV plasmid DNA may be bought/obtained from the UK AIDS Repository Reagent Programme (NIBSC) or the US AIDS Repository Reagent Programme (NIH). Primary cells will be obtained from anonymous health volunteers through the Blood Bank. Plasmids will be used to generate virus by transfection of 293T cells or other suitable cells. SIVmac and MLV vectors will be used to infect cells in culture and prepare intracellular reverse transcription complexes and to test the effects of specific cellular genes on virus infection and early replication. Viral vectors will also be used to express cDNAs of interest in cultured cells or to knock down expression of specific genes using RNAi. HIV-1 and SIVmac will be used to infect cells in tissue culture to test the effects of specific celluar genes on virus replication and early stages and to test the effects of small molecules inhibitors. HIV-1 and SIVmac deleted viruses and pseudotyped with VSV-G will be used to map viral determinants for interaction with cellular factors in different mammalian cell lines, including mouse, rat and rabbit, which are not normally permissive to these retroviruses. Mapping will be performed by systematically mutating virus genes in the context of an otherwise wild type virus (except for the deletion in envelope).

Evaluation of foreseeable effects

HIV carries the risk of AIDS. The main risk of HIV infection in the laboratory is via cuts or abrasions with contaminated sharp instruments. Aerosols are not likely to be a risk since work will be carried out in class 1 or T-fronted class II microbiological safety cabinets. Work using recombinant HIVs carrying mutated or chimeric viral genes is unlikely to be of any higher risk than a typical clinical isolate from an AIDS patient. All work with infectious HIV particles whether using clinical isolates or recombinant strains is therefore carried out in our level three laboratory under local rules. These include a ban on sharp instruments, the use of T-front class II safety cabinets to prevent aerosols in a laboratory with restricted access. Introduction of infectious HIV DNA into cells by transfection and subsequent production of infectious virus is carried out in level 3 safety laboratory. HIV-1 and SIV plasmid DNA will be grown in disabled E. coli in a level 2 safety cabinet. Sharps and needles will not be allowed while handling HIV-1 or SIV plasmid DNA. Such DNA will be dried in a fume hood to prevent aerosol inhalation and kept diluted in small aliquots. Small DNA stocks will be produced (max 100 micrograms) of replication competent HIV-1 and SIV.

The recipient cell structures are cell lines and primary cells which are not from the persons who work in the same laboratory and known to be free from human pathogens of ACDP 2-4 groups. These cells will not survive outside of the laboratory, without feeding, nor colonise in immuneincompetent workers after accidental injection. There is a small likely-hood that the HIV pseudotypes will reconstitute the envelope gene by recombination with another HIV-1 virus containing the envelope gene. This may happen if the same cells are accidentally infected by two different viruses. However, these recombinant viruses are no more of a risk than a typical clinical isolate from an AIDS patient. Cells used to generate pseudotypes virus will be clearly labelled and kept separated from other infected cells, for example in a dedicated incubator. All work after transfection of HIV clones into cell lines is therefore done in level 3 and all pseudotypes are treated as potentially live HIV.
Accidental infection with retroviral vectors (replication incompetent) may cause an immune response against viral proteins or against other proteins expressed by the vector. It may also cause insertional mutagenesis. Production and handling of these vectors is carried out in a level 2 safety laboratory using class II laminar flow cabinets.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Bacterial cultures are treated with 5% Trigene (final concentration) for 12 hours and disposed. Glassware is washed in 5% Trigene for 12 hours and sterilised by autoclaving. All plasticware is collected in dedicated autoclavable double bags and sterilised by autoclaving. All solid waste is sterilised by autoclaving before leaving the Windeyer Building. The following procedure is applied:

a) The waste is collected in double autoclavable bag, which is supported in a flip top blue bin.
b) When 2/3 rds full the bag is inserted into a 2nd bag and both bags are tied with a blue elastomere band.

Level III safety laboratory

SOLID WASTE

Paper gowns and towels, disposable gloves, filter papers and plasticware must be discarded into an approved autoclavable bag, supported within a labelled "biohazardous waste" bin. Before the bag is too full, it must be closed with an elastomere seal before being placed within the autoclave to await a sterilising run. Double bagging is mandatory to prevent leaks. If the autoclave is in use, full bags may be temporarily stored in the bin beside the autoclave. The autoclave discard cycle holds at 134 C for 15 minutes.

PLASTICWARE

Disposable culture dishes, tubes and flasks used for infectious material must be treated by immersion in 5% Trigene for 12 hours and then autoclaved. Degree of kill: sterilisation.

GRADUATED PIPETTES

Plastic graduated pipettes must be totally immersed in 5% Trigene for 12 hours in the grey cylindrical drums. After disinfection the pipettes can be removed and allowed to drain. They are discarded into an autoclavable bag and disposed of as before. Double bagging is mandatory to prevent leaks. Degree of kill: sterilisation.

PLASTIC STRAWS/PIPETTE TIPS

Plastic straws and plastic pipette tips must be totally immersed in 5% Trigene in a plastic bath within the MSC for 12 hours before draining and disposal into an autoclavable bag. If Gibson tips are used in conjunction with Eppendorf multidosers, the tips must be separated before disposal. Bags are autoclaved as before. Degree of kill: sterilisation.

AUTOCLAVING WASTE

The bags of solid waste are autoclaved in the stainless steel containers in the Priorclave Double Ended Autoclave. On completion of the cycle, the bags are removed in the Prep Room 401E and placed in medibins. When full these bins are sealed and removed from the prep room to await collection for incineration.

Autoclave failure: Should the double ended autoclave be unavailable, bags of solid waste may be autoclaved in the Priorclave EV 150 autoclave sited in the Prep
laboratory, the bags must be sprayed with alcide then placed directly into the autoclave. When the discard cycle is complete, the waste is treated as above.

LIQUID WASTE

Large volumes of liquid, including bacterial cultures, must be treated with Trigene at a final concentration of 5% for at least 12 hours before being discarded in the drain. Degree of kill: sterilisation.

SOLVENT WASTE

Chlorinated and non-chlorinated solvents must be discarded into the appropriately labelled 2.5L plastic containers, which are kept in the flammable storage cabinet. When full, or at least 6-monthly, after having their outer surfaces thoroughly washed with 5% Trigene, they are removed from the lab and disposal is arranged according to Windeyer Institute procedures.

BLOOD

A final volume of 5% Trigene must be added to all waste blood and samples treated for 12 hours before autoclaving.

Queries (below) raised by the GMSC concerning the internal GM notification numbers 934 and 935 were resolved satisfactorily and the committee recommended the approval of this work as a class 3 activity.

"Experiment 934 and 935 should be rolled together into one single overall experiment. If full length HIV DNA is being made and out into mammalian cells this should be carried out at containment level 3".

"Section 1.08 states 2% Trigene for 24 hours, whereas the appendix states 5% Trigene for 12 hours. Which is to be used and has this been validated? If both are validated and both are to be used, this should be stated".

"Sections 2.1: The statement "aerosols are not harmful because naked DNA uptake by the respiratory tract is not documented" is incorrect. The commonly used technique of intra-nasal immunization with naked DNA is very well documented. For a recent example see Hinto et al. J. Imminol. 174, 7423."

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02/03/2022
**Project Ref** 14/07.1

**CU2 Project Title**
Genetic engineering of T-cells for cancer immunotherapy.

**Class**
Class 2

**Culture Volume**
< 1 Litre

**Project Additional Information**

**Purposes of the contained use**
Various novel proteins will be introduced into T-cells with retroviral vectors. These proteins will re-direct T-cells to cancer antigens, direct T-cells to home to appropriate sites of disease, augment and modulate their function. This project involves standard molecular cloning techniques and the generation of replication incompetent retroviral vector by three-plasmid transient transfection. Retroviral vectors will be generated to transduce T-cells or cell lines. These vectors are generated by triple transfection. Replication incompetent. As an additional precaution, these vectors will be handled in level 2 containment.

**Recipient or parental organism**
1. **Bacteria:** Laboratory strain of E. coil transformed with recombinant plasmids providing antibiotic resistance geno to the bacteria.
2. **Retroviral Vectors:** Gagpol and Vector sequence (LTR5, packaging sequence) derived from Moloney Murine Leukemia Virus. The env is derived from RD1 14 — an endogenous Feline retrovirus.
3. **T-cells:** Normal human T-cells from peripheral blood of healthy donors transduced with above retroviral vectors.
1. Bacteria
E. coli will be used to facilitate molecular cloning and to grow up sufficient plasmids for transfection of 293T cells. The competent bacteria used are commercially available NEB 5-alpha Competent E. coli (High Efficiency), New England Biolabs, Catalogue #C2987H. Their genotype is: fhuA2A(argF-lacZ)U 169 phoA glnV44 8O A(lacZ)M15 gyrA96 recA1 relA1 thi-1 hsdR17. Further details can be found in the New England Biolabs catalogue or website.

2. Retroviral Vectors:
The replication deficient oncoretroviral vector is generated by a standard three plasmid transfection process. Plasmid 1 is RDF (generated by Prof. Mary Collins, here at LJCL). This is an expression vector for the RDI14 envelope; Plasmid 2. Peq-PAM-env an expression plasmid for MoMLV gagpol (envelope coding sequence has been deleted). — it was a gift of its creator: Dr Elio Vanin currently working in Children’s Memorial, Chicago. Plasmid 3 is the vector itself which was made in the Mulligan lab. It contains both MoMLV LTRs and the packaging signal. The remaining portion of gagpol (that is not part of the packaging signal) and the env have been deleted. Transgenes are cloned into where the env would have been.

3. Genetically modified T-cells
We will clone many genes and other elements into this retroviral vectors to engineer T-cells. Many will be novel fusions of antibody fragments and signalling molecules. An exhaustive list is not possible as the research will be quite fluid, but a categories of sequences are: chimeric antigen receptors (fusions of antibodies with T-cell signalling molecules), native T-cell receptors (cloned from antigen-specific T-cells), intracellular signalling proteins, homing signalling proteins, suicide genes, cytokines, fluorescent and bioluminescent proteins.

Host/vector system

Origin & function

An exhaustive list is not possible. Genetic material used will be cloned mostly from human cDNA. Some constructs may be made from murine, chicken and bovine DNA. Most of the genetic material will be fusion proteins or modified proteins involved in T-cell biology (e.g. signalling, homing and target killing). Some more exotic proteins (e.g. for jellyfish and insects) will be used for fluorescence and bioluminescent tagging of T-cells.

Chimeric Antigen Receptor Fusions of antibody variable region single chains (scFv) with spacers (usually derived from hinge and CH2CH3 regions of IgGI or CD8) and signalling endodomains from e.g. CD28, CD3-Zeta or 0X404

Native TCR receptors: T-cell receptor alpha and beta chains cloned from antigen-specific T-cells.

Intracellular signalling e.g. calcineurin, Calcineurin mutants, NFAT mutants, LAT (Linker of activated Tcells), Ick, src.

Homing Signals e.g. FLTI

Luciferases e.g. luciferase from Phrixotrix Hirtis, Click Beetle, Firefly and Gaussia.

Suicide Genes: inducible Caspase 9 (iCasp9)

Cytokines e.g. IL-2, IL-15, IL-21

Flourescent proteins e.g. enhanced Green Fluorescent Protein, dsRed, enhanced Blue Fluorescent Protein

Tagging proteins e.g. truncated CD34, truncated Nerve-growth factor receptor, CD20 fragments

miRNA pre-miRNA loops to target various transcripts

expression elements to e.g. NEAT response elements, Chromatin insulators, Scaffold attachment sequences, RNA processing elements.

Evaluation of foreseeable effects

1. Bacteria
Plasmids are engineered such that vector coding genes should not be expressed by E.coli - only antibiotic resistance genes. Even if retroviral proteins were expressed by E. coli they would not be functional in a prokaryote. These are standard molecular biology techniques. The only hazard anticipated is the environmental release of non-pathogenic strain of antibiotic resistant E. coli.

2. Retroviral Vectors:
Triple transfection is very safe since a three plasmid recombination events would need to occur to generate a replication competent vector. Further, the volumes of virus made will be small (maximum batch of 100mL). Retroviral vector is labile. Competent retroviral vector could only infect a researcher by inoculation or exposure to broken skin. Even if a competent retroviral vector were to infect a human host, it would be rapidly cleared by the host immune system. Accidental release of replication incompetent retroviral vector would have no environmental consequence. Accidental release of replication competent vector would result in environmental exposure of a very labile virus that could only infect human hosts by inoculation and even...
that case would likely be rapidly cleared by the host immune system.

3. Genetically modified T-cells

These gene-modified T-cells will not be able to survive out of tissue culture. The only hazard they pose is if they were accidentally injected into a researcher. To minimize even this hazard, researchers will not work with genetically modified T-cells derived from their own blood. Hence, in the very unlikely event of inoculation with these T-cells, an allogeneic immune response will rapidly clear them.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| All waste will be autoclaved. Autoclaved waste will be released to the clinical waste disposal system and destroyed by incineration. |

**Is an emergency plan required according to regulation 20?**

| N |

If yes, tick to confirm that it is attached to this form

| N |

**Tick to confirm that you have attached a risk assessment to this form**

| Y |

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

| N |

### Project Containment

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<tr>
<th>Laboratory Activities</th>
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**Animal Units**

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<th>Human Clinical Applications</th>
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**Project Ref** 14/08.1
The aim is to produce live intact lentivirus based viral vectors. These vectors will be transiently expressed in producer cells (cultured 293T cells) and the viral particles produced from the cells will be concentrated. The concentrated stock of viral particles will be used to infect salamander cells and mammalian cells in culture. The intended sequences are widely used bio markers such as Green and Red fluorescent proteins. In addition to this, newt-specific genes (eg. Prod 1) which is not known to cause any pathogenesis will be expressed in newt and mouse cells. The transfected cells will be used to study cell migration and interaction with other cells.

Recipient or parental organism

The intended recipient organisms are newt cells and mouse cells. Neither should be able to release any infectious agent and no adverse consequences are anticipated. The potential risk is accidental exposure and transfer of inserted genetic material. The intended vector sequences are non-oncogenic in humans and lentiviral vectors so far are not known to cause oncogenesis in humans. Newts represent a negligible hazard in terms of displacing or competing with other species. We do not use poisonous species so there is a negligible hazard in terms of adverse effects on other animals or plants. In terms of the vector employed there is a negligible hazard of transfer of genetic material from the newts to other organisms. If the GM newts were consumed by another animal there is a negligible probability of the products of gene expression causing harm. The possibility that phenotypic or genotypic instability could cause a hazard is negligible. None of the gene products being expressed in the newts could cause a viral disease, and shedding of vector sequences or particles is also of negligible probability given the nature of the constructs.

Host/vector system

Host - HEK293T, XL1 Blue, Newt cells, Mouse cells
Vector system — Trans Lentiviral ORF system consist of the following vectors: pTLA1 -PAK, pTLA-ENZ, pTLAI -ENV, pTLA-REV, pTLAI -TOFF, pLEX-JRED/TURBOGFP, pLEX-MCS
pTLA1-,PAK, pTLA1-FNZ, pTLA-ENV, pTLA-REV and pTLA1-TOFF are he vector and helper constructs contain no significant areas of homology, minimising their chance for recombination. None of the HIV-i genes (gag, pol, rev) will be expressed in transduced cells, instead they are expressed from packaging plasmids lacking packaging signal. The lentiviral vector Long tTerminal Repeats (LTR) do not contain highly active transcriptional enhancers; this feature reduces the possibility of oncogenic genotoxicity resulting from insertional mutagenesis.

pLEX-JREDITURBOGFP vector sequences are marker genes derived from jelly fish and widely used in the laboratory as a biological marker. pLEX-MCS is the vector intended for introducing the newt-specWc gene, Prod-i. The newt-specific sequences are derived from the genome of salamnders which include structural and non-structural gene sequences. These proteins are not known to cause pathogenicity but all are anticipated to play physiological roles.

**Origin & function**

The recipient organism for the vectors a E. coli XL-i -Blue, which is a commercially available E. coli strain derived from E. coli K12 strain and used for routinely in molecular biology studies. This strain has an established safety record in the laboratory with no adverse effects on human health, animal or plant health or the environment. Furthermore, F. coli XL-i-Blue is incapable of survival outside the laboratory.

The inserted sequences are marker genes derived from jelly fish and widely used in the laboratory as a biological marker. The newt-specific sequences are derived from the genome of salamnders which include structural and non-structural gene sequences. These proteins are not known to cause pathogenicity but all are anticipated to play physiological roles. Transient expression of viral construct with packaging plasmids in human 293T cells will produce live viral particles which can infect both dividing and non-dividing cells in a broad host range.

The intended recipient organisms are newt cells and mouse cells. Neither should be able to release any infectious agent and no adverse consequences are anticipated.

**Evaluation of foreseeable effects**

The containment facility is a dedicated animal facility approved by the Home office. The entry to the room is with steel doors and digital lock. Only authorised personnel have access to this facility. GM animals will be held in lidded plastic tanks (20cm x 2 x 1 4 high). Groups of 4 tanks will be enclosed in a custom made Perspex lidded box, which provides a second layer of containment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All disposable plastic ware which come into contact with the liquid during handling within the biological safety cabinet will be disinfected by immersion overnight in peroxygen disinfectant. All other solid waste materials generated in the facility will be collected in autoclavable bags and will be kept in a closed container. These waste materials will be autoclaved within the facility before disposal as clinical waste. The autoclaves are programmed appropriately (124 degrees for 35 minutes) for the degree of kill. Trained personnel are available to perform this work. The incubators have built in decontamination facility for periodical disinfection. All the work surface will be wipe cleaned with peroxygen cleaner after work.

The safety and efficacy of the measures taken will be periodically examined by the Principal Investigator and Departmental Safety Officer. The disinfected materials will be disposed off as clinical waste in sealed bags approved by the university. Facility for daily collection of waste materials is available.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All disposable plastic ware which come into contact with the liquid during handling within the biological safety cabinet will be disinfected by immersion overnight in peroxygen disinfectant. All other solid waste materials generated in the facility will be collected in autoclavable bags and will be kept in a closed container. These waste materials will be autoclaved within the facility before disposal as clinical waste. The autoclaves are programmed appropriately (124 degrees for 35 minutes) for the degree of kill. Trained personnel are available to perform this work. The incubators have built in decontamination facility for periodical disinfection. All the work surface will be wipe cleaned with peroxygen cleaner after work.

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| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |
UCL GM SAFETY ADVISORS HAVE RECOMMENDED TO THE GMSC THAT THIS PROJECT SHOULD BE CLASSIFIED AS A CLASS 2 GM ACTIVITY.

### Project Containment

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### Project Ref  14/08.2

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### Project Additional Information

**Purposes of the contained use**

The aim of this project is to understand the link between single cell mechanical properties and movement, and the mechanics and morphogenesis of multicellular aggregates (such as cysts or cell sheets). To do this, we aim to generate cell lines stably expressing wild type versions of the cytoskeletal and regulatory proteins for observation of protein organisation (or reorganisation) during mechanical perturbation. Mutant versions of the proteins that are involved in resisting mechanical forces will be made, cloned, and stably expressed in cells. The aggregate and cellular mechanical properties will then be reexamined. A similar approach will be taken to determine...
which proteins play a role in simple morphogenetic movements such as invagination and single cell movement. If more acute treatment is needed, purified protein microinjection will be used for rapid perturbation of cell function. Recombinant viruses will be used to create cell lines stably expressing the inserts under the control of the retroviral vector.

Recipient or parental organism

We will utilise commercially available recombinant virus systems such as MMLV retroviruses and lentiviruses for infection of human cell lines. Though commercially available recombinant retro- and lenti-viruses have disabled replication, the risk of accidental recombination with a wild type virus still exists, particularly since a number of cancer cell lines also carry viral loads. The risk in this case is the emergence of a replication competent virus carrying an oncogene under the control of a strong promoter as load. Even in the case where replication deficiency is maintained, recombinant viruses pose a risk through the genes they are carrying. In our experimental work, we wish to express constitutively active mutants of oncogenes. The consequences of upregulation of these oncogenes at the level of a whole organism are difficult to predict and therefore, we believe that these GMQs should be treated as Class 2 risk. Finally, as with all genetic mutations, it is possible that accidental insertion of exogenous DNA sequences within actively transcribed host sequences could have unpredictable and harmful consequences (insertional mutagenesis).

Host/vector system

Hosts; Human and mammalian cell lines.
Retroviruses;
BD clontech retroviral system based on MMLV retroviruses that are replication incompetent. Vectors to be used are pLNCX, pLNCX2, pLXSN, pLXIN, pLPCX, pRetroQ-Dsred,-GFP-C1, GFP-N1, pRevTRE, pRevTetOff, pRevTetOff-IN, pRevTRE-Luc.
Lentiviruses;
Though as of now, we are not using lentiviruses, we do plan to in the future and would be using commercially available kits such as the Virapower expression system commercialised by Invitrogen.

Origin & function

The genes liable to be cloned into viral expression kits can be roughly divided into two categories; coskeletal proteins and regulatory genes (including oncogenes). At present, all of the cDNA in our possession is in purified plasmids. The following genes are being considered at present but any gene playing a role in tissue morphogenesis, cell mechanics, or cell locomotion may be cloned in the future; beta actin, M10277, human tubulin, NM006009, human vimentin, NM003380, human septin 6, SC036240, human anillin, BC070066, human thymosin beta 4, M17733, human myosin regulatory light chain, BC046702, xenopus myosin heavy chain, AF055895, xenopus alpha-actinin, NM001102, human fimbrin, NM005032, human coronin, NM014325, human tropomyosin, BC057705, xenopus PH domain of Phospholipase C delta, U091 17, human Ezrin wild type, T567A, T567D, NM003379, human Moesin, M69066, human keratin 8, BC063513, human keratin 18, BC072017, human daamil, BC038428, human kiaaO861, BC064632, human rho A, NM001664, human rac1, BC107748, human rac2, BC001485, human cdc 42, BC018266, human E-cadherin, U04708, xenopus C-cadherin, U04707, xenopus mdial, BC070412, mouse arp3, AF006083, human net, BC053553, human p115rhogef, human rhoGDI-alpha, NM004309, human p50rhoGAP, NM004308, human cofilin 1, BC012265, human profilin 1, BC002475, human nedd5, AF038404, human tandem dimer red fluorescent protein monomeric red fluorescent protein 1 adducin, BC01393, human annexin 2, NM1002858, human VASP, BC015289, human alpha spectrin, J05243, human integrin beta 1, BC020057, human cor-tactin, BC008799, human gelsolin, BC026033, human myr2, NM023092, rat myr3, NM173101, rat shroom, BC104914, human nhel, AF146439, human protein 41, BC039079, human ankyrin B, BC007930, human Fascin, AF281049, human Rhotein, BC017727, human occiudin, U49185, mouse capZ, BC000144, human tropomodulih, 8C047163, xenopus Proteins not yet cloned but of use in the project:
desmosomal proteins (desmoplakin)
tight junction proteins (alpha-catenin, beta catenin)

**Evaluation of foreseeable effects**

The effects of infection of a human being by a large dose of recombinant viruses are generally unforeseeable. Depending on the nature of the gene, they may range from benign to dangerous. This is especially true since the vectors used are under the control of very strong promoters such as CMV and therefore the quantity of protein expressed may be dangerous for cells rather than the gene itself. When the gene expressed is an oncogene, its overexpression in cells within an organism is unpredictable.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All containment measures will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Risk to the environment with retro- and lenti-viral vectors arise from accidental spillage of solutions containing virus and from improper decontamination of waste products. Spillages will be dealt with immediately, the area will be wiped dry and disinfected as detailed below. The waste material from cleanup will be autoclaved and disposed of in biohazardous waste. Under normal circumstances, risk to the environment should be minimal. Waste products will be in the form of cell culture supernatants, which contain viruses. To disinfect them, we will use commercially available decontamination products such as Virkon S. According to the manufacturer, Virkon S is a powerful disinfectant that has been proven effective against all types of pathogens at dilutions of 1 or 2%. Guidelines from Stoner et al, Journal of Oncological and Pharmacological practices, 2003, 9, 29 recommend using a 2% solution of virkon for disinfection of surfaces potentially contaminated with retroviruses. For cleaning, it needs to be sprayed upon the surface and left to act for two minutes before wiping clean and it kills over 99.99995% of organisms. Waste liquids will then be autoclaved. Final waste is biodegradable and can be released in sewage water.

We will test the infectivity of Virkon containing supernatants and autoclaved waste products by adding them in tissue culture medium of living cells. If the waste liquid still contains virus, we should be able to detect this as the proteins we will express will be tagged with fluorescent reporter constructs such as GFP or mRFP.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

**Please enter comments on the GM safety committee on the risk assessment**

Following minor clarification of the original risk assessment, the UCL GM Safety Committee agreed that this work should be classified as an activity class 2 project at its meeting on 14th November 2007.

**Project Containment**

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02/03/2022
The aim of the project is to identify and characterise mammalian genes that impact on viral infection. We will screen cDNA libraries as well as selecting genes that impact on viral infection. These genes will be expressed, or their expression reduced with RNAi, using non replication competent retroviral vectors, or herpes viruses such as HSV-1 and CMV. Once factors have been identified, their mechanism of action will be characterised by measuring various aspects of viral replication such as DNA synthesis or early protein synthesis. Mutants of the antiviral proteins will be made to inform which parts of the protein are important for their activity. We will also examine the ability of retroviral proteins to influence human gene expression using microarray techniques. This project therefore aims to understand host virus interactions focusing on host virus proteins that effect permissivity to infection and viral proteins that influence gene expression in the host.
the ability of retroviral proteins to influence human gene expression using microarray techniques. This project therefore aims to understand host virus interactions focussing on host virus proteins that effect permissivity to infection and viral proteins that influence gene expression in the host.

Host/vector system

Vectors:
All the vectors (plasmids) will be transformed into E. coli (see above) for cloning and propagation of mammalian and virus sequences. Group 1 vectors (cloning vectors) will only be present in E.coli. Mammalian and virus sequences will also be transferred to expression vectors(2.) and transferred into human cell lines for expression. We will also transfer the sequences to virus expression vectors(3.) to allow long term expression in human cells and infection of otherwise difficult to transfect cell types. Virus expression vectors are packed inside VSV-G pseudotyped virus vectors, made from gag-pol and VSV-G expression vectors transferred into human cells (4.) Details of this retroviral vector system is outlined below. We will also inactivate HIV genes in HIV molecular clones (full-length HIV genomic DNA contained within a bacterial plasmid in E.coli) and other genes in other mammalian retroviruses. (5.)

1. Cloning vectors: pCR-TOPO, pGEM-T, pGEMU61, pCR2.1, p9B-18 and other similar HIV molecular clones,
2. Expression vectors: pcDNA3, pCR3.1, pires family, pTRE2 family expression vectors
4. Gag-pol expression vectors (called packaging constructs) and envelope expression vectors: CMVintron (murine leukaemia virus gag-pol expression vector), p8.9 (HIV-1 env expression vector).
5. HIV molecular clones eg. P9B-18, MLV, FIV and SIV molecular clones.

DETAILS ON RETROVIRAL VECTORS
Retroviral vectors are made by transfecting 3 plasmids, an expression plasmid into which the sequences of interest is cloned, together with a retroviral packing construct, a gag-pol expression vector and an envelope expressing vector (which are not packaged into progeny viruses). All vectors are disabled by separating the packaging sequence, coding sequence and the viral coding sequences from each other on independent plasmids. Retroviral vectors are produced by transfection of all 3 plasmids. These viral particles do not contain genome that encodes virus proteins and so this system cannot produce replication competent virus. VSV-G-enveloped retroviruses are only potentially harmful to human health if introduced into the body by injection, ingestion or through a wound. The potential harm arises from the possibility the virus would enter cells and integrate itself into cellular DNA. Although the virus is not replication competent (does not carry gag, pol or env genes) and so it cannot make new progeny virus and estabilish a productive infection, its insertion into cellular DNA could labile and do not survive on environmental surfaces outside the lab.

MAMMALIAN CELL LINES
Canine MDCK, DK, D17, C2F, feline, AH927, CRFK, Mya 1, human HeLa, TE671, HT1080, 293T, HEL, HFFF, BJ1, Jurkat, CEM, supT1, C8166, U937, THP-1 KG-1, A549, mouse MDTF, NIH3T3, avain QT36; marsupial SC300, bat Tb11u; non human primate FRhK4, LLCMK2, CV1, VERO, Pindak, SMLF; bovine MDBK, IMR31, porcine PK15, SKL, CPK, STIOWA, mink mv-1-lu, rabbit SIRC, eREP, rat HSN, RAT2, 9L, NRK.

Primary human cells derived from peripheral blood including lymphocytes and macrophages.

Origin & function
Mammalian DNA: The aim of the project is to identify and characterize mammalian genes that impact on viral infection. We will screen cDNA libraries as well as selecting genes based on their reported ability to influence permissivity to viral infection. These genes will be expressed, or their expression reduced with RNAi, using replication incompetent retroviral vectors. The modified cells will then be tested to permissivity to retroviruses, tested again using replication incompetent vectors, or herpes viruses, such as HSV-1 and CMV. One factors have been identified their mechanism of action will be characterised by measuring various aspects of viral replication such as protein important for their activity. This project therefore aims to understand how viral permissivity, and species-specificity of viral replication, is controlled by host proteins.

cDNA or cDNA derived clones will be cloned into MLV or HIV based retroviral vectors. Virus genes, identified as having antiviral or interferon modulating properties, will be cloned into mammalian expression vectors. These will be expressed in mammalian cell lines using retroviral vectors. Mammalian cell lines will also be modifieled by transient and stable expression of small interfering RNA. We will also express Bovine diarrhoea virus protein Npro, paramyxovirus SV5 V protein, KSHV vFLIP and EBV
LMP1 in mammalian cell lines and test them for permissivity to viral infection. We will also use expression vectors to express cellular proteins with roles in the immune response such as tripartite motif proteins 1-70, cyclophilins A-H, NFkappaB and interferon regulatory 1-8. Note that all proteins expressed are done so with non-replicating retroviral vectors and not with live virus. Also no live virus related to African swine fever or bovine diarrhoal virus will be used at any point either for expression or testing of permissivity.

VIRUS DNA:
We will also examine the effect of virus proteins on host cell transcription. We will clone individual HIV(Tat, Vpr and Rev), KSHV (LANA, RTA, vIRF, ORF57), EBV (EBNA2, EBNA3, ZTA) or other herpesvirus proteins into cloning vectors and then into expression vectors and retroviral expression vectors. These proteins will be selected based on their ability to regulate human gene expression, as reported in the literature. The expression vectors will also be modified to express HIV proteins from the HIV LTR (promoter element) We will not be introducing virus proteins into viral vectors that can cause the production of replication competent virus. We will tag these proteins with an epitope (HA or FLAG) tag to allow the proteins to be detected. We will also inactivate HIV genes within HIV molecular clones such as p9B-18 by mutating them or deleting them. Once we have identified human genes regulate by virus proteins, we will express these proteins in human T-cells and macrophages from a retroviral vector and knock down their expression levels using siRNAs encoded in retroviral vectors. We will not be introducing full-length HIV or SIV into mammalian cells to produce HIV or SIV virus in these experiments.

None of the vectors are harmful to the environment and do not supply a survival advantage to any microorganisms. The cloning and expression vectors are also not a hazard to human health.

The bacterial strains to be used are non colonising and incapable of surviving in the environment or causing human infection. They pose no risk to users or the environment.

Plasmids encoding full length HIV-1 could theoretically generate infectious HIV if accidently injected directly into the body. These vectors will not be introduced into human cells in tissue culture in these experiments.

Retroviral vectors can infect human cells and therefore, if introduced into the body by injection, ingestion or through a wound, the virus could enter cells and integrate its genome into cellular DNA. Although the virus is not replication competent (the genome does not encode viral gag, pol or env genes) it cannot make new progeny virus and establish a productive infection, its insertion into cellular DNA could result in a potentially oncogenic mutation. The insertion of human genes regulated by HIV into a retroviral vector, or the insertion of siRNAs that knockdown these genes, could theoretically increase the oncogenic potential of the virus. If retroviral vectors encoding genes that affect interferon signalling were injected into a person some cells may become infected and therefore more outcome of an exogenous viral infection. None of the inserted sequences are likely to have oncogenic potential and so retroviral vectors encoding such sequences are extremely unlikely to have pathogenic consequences if accidentally injected. cDNA libraries in retroviral vectors will likely contain oncogenic sequences but the relative infectious titres of these vectors within the population are too low to measure, making the vector preparation very safe.

HSV-1 and CMV GFP viruses are infectious in humans but are of low pathogenicity. The vast majority of individuals have been exposed to environmental infection and will mount an effective immune reaction against it. The insertion of GFP sequences and/or deletion of ICPO in HSV-1 in no way increases pathogenicity or toxicity or hazard to human health or the environment. Exposure of workers to CMV or HSV-1 will be limited, as all work involving infectious virus will be performed in category 2 containment facility in class 2 microbiological safety cabinets. To prevent contamination of workers by virus, no glass or other sharps will be used and workers will be protected by a lab coat, latex or nitrile gloves and eye protection. Skin lesions will be covered with a bandage in addition to the protective wear described above. Pregnant workers will be prevented from working CMV. The risk of harm is therefore extremely low. Both HSV-1 and CMV infection are treatable with drugs (acyclovir and gancyclovir respectively). The modified viruses pose no more threat to the environmental than the wild type virus. None of the virus or vector infected cells cannot survive in the environment.

It is important to note that there are no circumstances where cells will be infected by more than one replication competent virus. There is therefore no chance for any kind of recombination between viruses. No live virus related to African swine fever or bovine diarrhoal virus will be used at any point for expression or testing of permissivity. There are no sharps in the rooms where the viruses are prepared and used. Sharps will also not be used in conjunction with HIV DNA-encoding plasmids. All work with virus will take place inside a microbiological safety cabinet within a category 2 containment lab. Wokers will also be protected by a lab coat. Safety glasses and latex or nitrile gloves when handling any GMM or GMM-derived DNA or virus.
The cell lines used cannot survive outside the lab incubators and as such pose no threat to health or the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**Guidance:**
For micro-organisms and cells in culture nothing need be added here. In addition to the advice in Note 7, it should be noted that some projects may require GMM vectors to be administered to animals (or plants). Appropriate containment measures must be adopted for handling such GMMs during this process even though the intention may not be to create a GM animal (or plant). The best place for these is probably in the risk assessment that is separate from this document.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

5% TriGene (v/v) will be used to treat liquid waste (16 hours) and also any spills and for disinfection of any reusable bottles or other equipment. Solid waste will be double bagged and autoclaved. Work surfaces will be wiped down with 70% ethanol after use. These procedures result in 100% kill of all GMO. Autoclave runs are regularly validated by waste management staff. TriGene is certified by the PHLS to kill viruses. All TriGene will be used within its 3-year life. Solid waste is autoclaved inside two elastic band-sealed clear plastic autoclave bags by trained staff and then these bags transferred to a yellow biohazard waste bag for incineration. Liquid waste is poured down the drain after treatment with TriGene for at least 16 hours. This leads to denaturation and inactivation of viral and cellular proteins.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The GM safety scrutinisers, acting on behalf of the GM Safety Committee agreed that this project was correctly classified at class 2.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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[20/02/2022]
The aim of this project is to investigate the mechanisms controlling the assembly and cell-to-cell transmission of enveloped viruses, in particular the human immunodeficiency virus type 1 (HIV) and the related simian virus (SIVmac). Recent studies have indicated that these viruses assemble on plasma membrane domains that are in some cell types sequestered within the cell, and that cellular proteins including the ESCRT machinery and various adaptor complexes are essential for this process. Moreover transfer of viruses from cell-to-cell appears to occur through specialised cell surface domains with similarity to immunological synapses. The aim of these experiments is to use morphological techniques including electron microscopy and fluorescence microscopy, coupled with molecular biology to investigate how HIV components are bought together within infected cells to allow virus assembly and transmission to occur. These experiments will require the use of replication competent infectious HIV and SIV and will therefore need to be conducted under containment level 3 conditions.

HEK293T for virus production, immortalized mammalian cell lines (eg HEK293, HeLa, SupT1, Jurkat, CEM, CEMx174), primary macrophages and T lymphocytes (isolated from screened blood obtained from the Blood Transfusion Service) will be used to produce viruses or for experimental infections.

Viruses to be used will be X4, R5 and dual tropic HIV and SIVmac239. These will be generated by viral infection and transfection of molecular clones. Infectious virus particles will be stored and handled using protocols and techniques established for the safe handling of HIV.

HIV particles could possibly infect research workers and lead to AIDS. SIVmac239 is not infectious to humans. Mutations introduced into molecular clones to alter the amino acid sequence of specific viral proteins are likely to attenuate infectivity/risk.

To allow reproducible virus production and modification of viral proteins to investigate the role of packaging motifs (eg Gag Late domain motifs) molecular clones will be...
used. We will use molecular clones of HIV-R3A, Ad8, and HxB2 as well as SIV molecular clones from SIVmac239. In some cases viruses will be pseudotyped using cloned envelope proteins from R5 tropic or X4 tropic viruses, or the vesicular stomatitis virus (VSV) G protein.

Viruses will be produced in HEK293T cells by transfecting single or multiple plasmids (for Pseudotyped virus) using lipid based transfection, reagents such as Fugene or Lipofectamine. Virus containing media will be collected 24 and 48 hrs post transfection, clarified of cell debris by low speed centrifugation and stored at -80°C in small volume (~1ml aliquots). All virus preparations will be limited in volume to <60ml.

Mammalian expression vectors, eg pcDNA3, pSV2-neo, pBabe vectors, pUC18/19, SP62, pMD, phage λ EMBL4 (Stratagene).

Origin & function

The genetic material to be used originates from HIV, SIV and VSV viruses originally isolated from infected individuals or animals, molecularly cloned and inserted into mammalian cell expression vectors for production of recombinant, infectious virus particles. Only the VSV G protein will be used and no infectious VSV will be produced.

The use of molecular clones allows us to modify the viruses so that the role of specific viral sequences can be analysed in experimental tissue culture systems. No animal experiments will be undertaken and no virus will be removed from the laboratory unless it has been fixed/inactivated with aldehyde fixatives (as used for light and electron microscopy analysis).

Evaluation of foreseeable effects

The likelihood of the viruses infecting lab personnel is remote. In all cases full bio-containment category three precautions will be taken, including the use of appropriate personal protective clothing (double gloving, etc) no use of glass pipettes or needles, handling of virus only within biological safety cabinets. The personnel involved in the work have previously worked with HIV and SIV under category three conditions as well as with other viruses including Herpes simplex.

In the unlikely event of a research worker becoming exposed to infectious virus particles, he/she will seek immediate medical assistance, and the administration of anti-virals as appropriate, from University College Hospital.

Due to the restricted host range of the constructs, their lack of ability to survive outside laboratory conditions, restrictions on access to the facility and the risk control and disinfection/disposal measures described in this assessment, we conclude that the program of work presents no risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All experiments will be conducted in mammalian cell lines kept in ACDP containment level 3 facilities in the UCL Cancer Centre, Huntley Street. There will be no use of animals.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste: Following virus usage, any materials that have, or may potentially have come into contact with the viruses will be thoroughly decontaminated by soaking in Trigene Advance* (5%) for at least 12 hours, followed by draining and disposal into the autoclave waste stream. The autoclave is a double-ended design situated within the containment facility. The autoclave is validated (12 point thermocouple test) by a competent engineer on an annual basis.

Liquid waste:

Liquid waste will be treated with Trigene Advance (5%) for at least 12 hours before disposal down the laboratory sink.
The genetic modification safety committee has approved this project as a Class 3 activity. It notes that the work is closely similar in risk to notified work currently being undertaken in a different part of this GM Centre.

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**Project Ref** 14/08.5

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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
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<td>IMMORTALISATION OF HUMAN PROSTATE CELLS</td>
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**Historical Significant Changes**

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**Project Additional Information**

**Purposes of the contained use**

**AIM** - The purpose of this project is to produce stable immortalized cell lines derived from human prostate cells, using a construct that will result in minimal genetic changes in the cell lines. This project is an updated version of a similar project aiming to produce immortalized cell lines from prostate cells started in 1997, which produced prostate cell lines, but with extensive genetic changes (Daly-Burns et al., 2006).

We want the prostate cell lines as models for studying the genetic polymorphisms that control susceptibility to prostate cancer. Recently single nucleotide polymorphisms (SNPs) have been identified that are associated with altered susceptibility to prostate cancer (e.g., Gudmundsson et al., 2007). The genotypes of the cell lines we develop will be determined and the cell lines used to provide model systems with which to study the mechanisms underlying altered susceptibility, partly for understanding and partly in the hope that there may be ways of altering susceptibility. One of the potential susceptibility genes that our collaborators have identified is TCF2 (Gudmundsson et al., 2007) and our later studies will involve up and down regulation of selected genes such as TCF2 in the cell lines produced.

**CELL LINES** - The immortalizing vectors extend lifespan allowing transformation to occur either by random spontaneous genetic events (as during crisis following transfection with SV40 T antigen), or with an additional transforming (ie oneogenic) construct containing a gene such as Ras. The sequential use of hTERT and the SV40 large T construct permits both extended lifespan and transformation. The immortalized cell lines we derived using the earlier system rate non-tumorigenic (Daly-Burns et al., 2006), in contrast to about 75% of standard continuous cell lines derived from human carriers. However, immortalization was associated with additional genetic changes and an unstable karyotype. Our collaborators (Professors Jar and O'Hare) have found that a triple SV40 large T mutant (non-DNA binding non-bub1 binding thermolamide U19d189-97tsA58) produces karyotypically stable cells when used in conjunction with reconstitution of telomerase activity (Gjeorup et al., 2007; Hein et al., 2008). We will use the newly developed technology in this study.

In case there is confusion over the use of the terminology "cell lines", it refers in this proposal to the derivatives of the primary cultures. A culture is primary only until the first time it is passaged, at which point it becomes a cell line. Once a cell line has indefinite growth potential it is described as a continuous cell line. We will infect primary cultures or, if greater numbers of cells arc required, cell lines at the first or second passage, with immortalizing vectors to produce continuous cell lines.

**Immortalization**

It is the processes used to grow the vectors and immortalize the primary cultures cell lines using infectious virus that require level 2 containment. The packaging cell system TE-FLY-A is used to package the T and hTERT viruses. Phoenix eco/ampho cells are used for introducing new genes into the immortalised cells.

We are also using this GM proposal to cover a second approach that will allow a lower level of containment. Our collaborator Professor Jat is developing a new system to engineer human cell lines expressing mouse ecotropic receptor. Ecotropic viruses can only infect marine cells and consequently work using them can be carried out at level I, whereas work with amphotropic viruses requires level 2. Human cell lines that express the ecotropic receptor are infectable by ecotropic viruses that normally only infect murine cells.

**Classification**

There has been over a decade of safe usage of these methods. The retroviral vectors have a history of safe use of over 12 years. The construction of the mobilization defective helper free amphotropic retrovirus by Professor Jat at UCL (Institute of Neurology) and their use for the immortalization of primary human cells has HSE Approval at level 2 (NDD reference number NDD/2006/A.016).

**References**


GV, Mayordomo .1f, Catalona WJ, Kiemeney LA, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K.


Recipient or parental organism

Primary human somatic cells and cell lines. The cells are non-mobilizable. The primary cells could be infected with unknown adventitious agents. Infection by such agents is extremely rare and would only take place as a result of poor laboratory practice, ie injury with needles or other sharp instruments. Immortalized cells will be banked under liquid nitrogen and used in culture dictated by the demands of the project. Under good laboratory practice immortalized cells do not constitute any hazard health. The cells can only survive in tissue culture or following implantation into immunosuppressed animals. There is a theoretical risk that as a result of poor practice cells could become implanted in the person using them. However, the cells would have to overcome the immune response and in practice this occurrence has been extremely rare. Anyone who: immunity is compromised for any known reason will not work with transformed cells.

Phoenix ampho cells are a non-mobilizable derivative of HEK293 used for production of high titre amphotropic stocks of virus. They are obtained from ATCC and are free of adventitious agents. History of safe usage.

TEFLYA and RD cells are derivatives of TE671 cells, third generation human packaging cell lines used for the production of stable, high titre defective helper-free amphotropic retroviruses that have a murine or feline envelope. Non-mobilizable. History of safe usage.

PA371 is a second generation mouse amphotropic producer cell lines used to derive the high titre producer of the U19tsA58 T antigen that we used earlier to immortalize prostate cells (Daly-Burns et al, 2006). Non-mobilizable defective.

Host/vector system

pZiPneoSSV(X)1 is derived from a T-antigen construct (U 19tsA58) cloned into a Moloney marine leukaemia virus derivative. The construct is generated in PA317 cells which produce a large T antigen in the form of an amphotropic retrovirus (clone8). Mobilization defective.

TE-FLY-A and RD cells produce pBABEneo/hygro/zeo, pLNCX2, pLi-ICX, pLPCX, pWZLpuro/BlastF. Mobilization defective.

Phoenix ampho cells will be used to generate pBABEneo/hygro/puro, pLNCX2, pL1-ICX, pLPCX, pWZLpuro/BlastF, pTMP, pRetroSuper. Mobilization defective.

Origin & function

Two cell systems will be used to package the LT and hTERT containing viruses for immortalization (TE-FLY-A) and fc introducing new genes (associated with susceptibility to prostate cancer) into the immortalized cells (Phoenix ampho). Bot systems are designed to minimise the risk of inadvertent production of replication competent viruses from endogenous sources.

Both are third generation split function packaging systems, based on human cell lines and capable of generating only replication-defective particles. In each case a separate promoter-driven gag-pol and env plasmid has been separately transduced into the recipient line, and in both cases the plasmids lack both the psi packaging site and have the 3'LTR replaced with SV40polyA sequence. Under these circumstances at least two cross-over events would be required to generate a replication competent virus.
In the case of TE-FLY-A the human cell line used is the rhabdomyosarcoma TE571(TE) transduced with the CEB gag-pol expression vector (FLY) and the AF murine amphotropic (A) envelope vector both driven by an LTR sequence. When transduced with hTERT or LT and an appropriate selectable marker TE-FLY AL will stably produce packaged constructs at a titre of <1E7. For generation of high titre packaging it is generally necessary to clone the transduced lines (hence terminology like clone13 for the hTERT virus) It has been extensively tested by its originators (the Weiss laboratory) and shown to be free of helper virus activity to a level of <1 in 10E7 particles. The Phoenix eco/ampho systems are based on the highly transfectable 293T human cell line and are essentially identical except as the name implies in one case the tropism is governed by an ecotropic env sequence, and in the other by an amphotropic equivalent. The Phoenix systems produce a high level of transient expression from the 293T cells when further transduced with the gene of interest and are therefore not generally subject to further cloning, thus enhancing their safety by reducing risk of cross contamination from a putative helper virus containing line that might be present in the lab. As mentioned above the gag-pol and env sequences were introduced into the packing line separately to minimise possibilities of recombination, which in this system is further reduced by the use of different promoters, CMV for the env and RSV for the gag-pol. Stringent tests by the lab of origin (the Weiss lab) have shown no evidence of the generation of replication competent virus using these systems. In the unlikely event of reactivation of virus, infection could only occur through high-titre safe laboratory practices (needlestick injury).

References
Danos O and Mulligan RC. Safe and efficient generation of recombinant retroviruses with amphotropic and ecotropic host ranges. Proc Natl Acad Sci 1988; 85: 6460-6464, 1988 (Principles)
WS Pear, GP Nolan, ML Scott, and D Baltimore. Production of High-Titer Helper-Free Retroviruses by Transient Transfection. Proc Natl Acad Sci 1993; 90: 8392-8396 (Phoenix, called BOSC23)

Evaluation of foreseeable effects

Human cells could contain unknown adventitious organisms, but infection of laboratory workers by human cells growing in tissue culture is very rare. The vector could cause harm if it infects a laboratory worker and is integrated in DNA in a position that modifies the expression of a cancer-related gene. However, the standard safety and disposal measures make the environmental risk very low. The vector is unlikely to be integrated unless in the absence of deliberate transfection. The inserts are immortalizing genes and therefore have the capacity to extend the lifespan of human cells. Integrated proviruses could be reactivated with helper viruses. The environmental risk is very low due to the short life of the virus and the fact that it needs multiple genetic changes to be reactivated. Infection could only occur through unacceptable practices (needlestick injury).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All work will be strictly confined to 3.17 of Charles Bell House to minimise the possibility of contamination of other laboratory work space. Researchers not involved in this project do not use this laboratory. Cell lines, virus stocks and producer cells will be stored frozen at -20C and used only as needed throughout the project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated with Virkon 4% (w/v) for a minimum period of 24 hours. Treated waste will be disposed of via dedicated drains within the lab. All solid waste will be immersed in 4% (w/v) for minimum of 24 hours followed by autoclaving for 15 minutes at 120 degrees and finally incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The GM safety committee agreed that the application of containment level 2 precautions would be appropriate to manage the risk of this project. The committee therefore approved this project as a class 2 GM activity.

Project Containment

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<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

Project Ref 14/09.1

- **Date Ackn'd**: 01/04/2009
- **CU2 Project Title**: Nuclear Organisation of Immunoglobulin Genes During the Development of Maturation and Activation of B and T Lymphocytes
- **Class**: Class 2
- **CultureVolClass2**: 1-50 Litres
- **Consent Granted**: Not Applicable
- **Non-GMM**: No
- **Project notified under transitional arrangements**: No

**Project Additional Information**

- **Purposes of the contained use**: To understand how nuclear organisation of immunoglobulin genes affects V(D)J recombination and expression
Recipient or parental organism

The following bacteria strains will be used: XL1 BLUE, TOP10F, JM101 and JM105, and other commercially available E.coli strains. Bacteria strains to be used are not pathogenic to humans and incapable of surviving in the environment.

The following viral vectors will be used: modified replication-defective Moloney murine leukaemia virus (MoMLV) vectors under EF1 alpha promoter and blastocytin? puromycin selection, and pMD.G and pMD.OGP vectors or equivalent encoding for modified replication-defective vesicular stomatitis virus G glycoprotein (VSV-G) pseudotyped MoMLV under cytomegalovirus (CMV) or EF1 alpha promoter.

Modified MoMLV viral vectors are not capable of replication but can enter rodent cells, which can lead to virus integration into cellular DNA, potentially disrupting tumour suppressor gene or activating an oncogene, and lead to cancer. Modified MoMLV viral vectors are deficient for replication but can enter insect and mammalian cells including human cells, which can lead to the same effects as described above in rodent cells.

The following cell lines and cells will be used: Adenovirus-transformed and simian virus 40 T large antigen-expressing 293T cell line and derivatives (such as Plat-E cell line that produces modified replication-defective MoMLV under EF1 alpha promoter and blastocytin/puromycin selection), recombination activating gene (RAG) 1 or RAG2-deficient Abelson virus transformed mouse B cell lines, Abelson virus transformed mouse B cell lines previously transduced with retroviral vectors to express RAG1 or RAG2 or truncated or mutated derivative, RAG1 or RAG2-deficient mouse primary lymphoid cells, and primary lymphoid cells derived from mice genetically modified to express truncated or mutated versions of RAG1 or RAG2. Cell lines and cells to be used are not pathogenic to humans and can not survive in the environment.

Host/vector system

The following vectors will be used:
- pBR322 derivatives commercially available, BACS
- Retroviral pMIG, and derivatives that contain RAG1 or RAG2 encoding sequence or truncated or point-mutated derivative, and GFP reporter gene under murine cell virus (MSCV) LTR promoter and IRES.
- Retroviral pRetro, and derivatives that contain RAG1 or RAG2 encoding sequence or truncated or point-mutated derivative, and puromycin selective gene under MoMLV LTR promoter and IRES.
- Commercially available retroviral pCFB-EGSH and pFB-ERV, and derivatives. pCFB-EGSH contains modified EcRE sequence, minimal heat shock promoter (mHSP), MoMLV LTR and CMV promoter, and hygromycin selective gene under herpes simplex virus thymidine kinase (HSV_TK) promoter. RAG sequences from pMIG or pRetro vector derivatives will be inserted into pCFB-EGSH plasmid under modified EcRE sequence, mHSP, MoMLV LTR and CMV promoters. pCFB-ERV contains modified Ecdysone receptors VgEcR and RXR genes and neomycin selective gene under MoMLV LTR and CMV promoters and IRES. pFB-ERV derivative will be obtained by replacing neomycin gene with puromycin selective gene from pRetro vector.

The vectors have low potential for harm to human health or environment, although accidental injection of large amounts of purified plasmids containing RAG expressing sequences could theoretically lead to RAGs-induced recombination of cellular DNA in cells where both RAG1 and RAG2 would be present either physiologically or subsequently to accidental transfer.

Origin & function

The origins of the genetic material involved are: mouse (for RAG sequences), bacteria (for selective genes), or insect (for modified Ecdysone system genes). The donor organisms are not pathogenic.

RAG proteins will induce recombination of Immunoglobulin (Ig) and T-cell receptor (TCR) genes in B and T lymphoid cells, respectively. Expression of RAG1 inserted sequences will induce DNA recombination in RAG1-deficient cells expressing RAG2, and inversely, expression of RAG2 inserted sequences will induce DNA recombination in RAG2-deficient cells expressing RAG1.

Accidental transfer of RAG1 or RAG2 expressing sequences to human or species in the environment could induce recombination of Ig and TCR genes or other endogenous DNA sequences in cells where both RAG1 and RAG2 would be present either physiologically or subsequently to accidental transfer, and could affect immune system or generate lymphoid translocations that could lead to cancer.

Evaluation of foreseeable effects

The most hazardous GMM's are the retroviral vectors when collected, centrifuged and filtered from supernatant or transfected 293T cells or derivatives, transferred to host cells and removed for disposal.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

TriGene will be used to treat liquid waste and any spill. TriGene is certified by the manufacturer to kill all bacteria and viruses to be used, is validated worldwide for its efficiency, and will be used by trained staff within its life-span limitations.

Liquid waste will be poured down the drain only after appropriate treatment with TriGene.

Work surfaces will be wiped down with 70% ethanol after use.

Solid waste will be autoclaved inside two elastic band sealed clear plastic autoclave doubled bags by trained staff before these bags are transferred to a yellow bag for incineration. Autoclave runs are regularly validated by waste management staff and achieve a 100% degree of kill.

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The UCL GM Safety Committee considered that, although the risk of this project is now very high, nevertheless it is appropriate to use containment level 2 conditions to manage the risk. Accordingly it approved the project as a class 2 GM activity.

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Project Ref 14/09.2

Date Ackn'd 02/03/2022  CU2 Project Title

Class CultureVolClass2 CultureVolumeClass3-4
The shRNAmir library consist of over 70,000 individual E.coli glycerol stocks containing the pGIPZ lentiviral vector. Each stock contains a unique hairpin sequence within the human genome via RNAi interference.

This project will involve library maintenance and storage of this shRNAmir library as well as distribution of clones as bacterial stocks, DNA plasmids and/or lentivirus to members within University College London for use in various projects.

Specifically this will involve lentiviral production from the pGIPZ plasmid and subsequent use of this lentivirus in cell based screening functional assays. Additionally, large numbers of clones will be pooled at the bacterial, DNA and viral level to make various intermediate pools of clones to be used to transduce cells in screens and subsequently undertake functional assays. Pooled bacteria/DNA/virus will be distributed to people within the Institute and wider UCL for screening in various other projects.

Recipient or parental organism

- Cell lines: canine- MDCCK, DK, D17, C2F; feline- AH927, CRFK, Mya1; Human-HeLa, Te671, HT1080.293T, HEL, HFFF, BJ1, Jurkat, CEM, supT1, C8166, A549, LECs, tMScs; Mouse- MDTF, NIH323; Avian- QT36; Marsupial- SC300; Bat Tb11u; non-human primate- FRhK4, LLCMK2, CV1, VERO, Pindak, SMLFR; bovine- MDBK, IMR31; Porcine- PK15, SKL, CPK, STIowa; Mink- mV-1-1u; Rabbit- SIRC, eREP, ratHSN, RAT2, 9L, NRK.

- Primary Human cells: Derived from various human tissues including peripheral blood derived lymphocytes and macrophages.


Associated risks and likeliness of occurrence:

- The recipient cells (cell lines and primary cells) that will eventually receive and integrate the DNA are harmless to humans. They are also unable to survive outside conditions and are therefore harmless to the environment.

- The bacterial strains used are attenuated non-colonising strains that are harmless to humans and cannot survive outside culture conditions and are therefore harmless to
Host/vector system

Lentiviral vectors

- Lentiviral expression vectors: Commercial TZV vector from Tranzyme and derivatives (including pGIPZ).

Details on Lentiviral systems and vectors:
Lentiviral vectors are made by transfecting 3 plasmids into a packaging cell line such as HEK293T cells. The 3 plasmids used are contained within or will be transformed into E.coli (see above) for propagation. The plasmids are 1) a packaging plasmid expressing the required accessory proteins for viable viral particle production 2) an envelope plasmid, expressing the envelope protein for the viral particle and 3) the lentiviral vector carrying the cassette with the inserted sequence of interest. Once all 3 vectors are in the cell, each component from the 3 vectors will be expressed. The co-expression of the components from the various vectors will allow the formation of viral particles which will be released from the cells. By seperating the components onto 3 independent plasmids the viral packaging elements are not packaged into the virus themselves making the vector disabled and incapable of self-replication. This system therefore is incapable of producing replication competent virus making it very safe. In this particular project, the shRNA hairpin expression vectors (pGIPZ) are packaged into VSV-G pseudotyped viral vectors made from gag-pol and VSV-G expression vectors transfected into human cells. The lentiviral vector contains the CMV Pol II) promoter to drive expression of the hairpin cassette.

Associated Risks and likelihood of occurrence:
VSV-G enveloped lentiviruses can infect human cells and therefore are potentially harmful to human health if introduced into the body by injection, ingestion or through a wound. The potential harm arises from the possibility the virus would enter and integrate itself into cellular DNA. The physical insertion into cellular DNA could result in a potentially oncogenic mutation. This, however, is unlikely and has only occurred in a clinical setting following prolonged infection with high titre lentiviral vectors encoding growth promoting molecules and selection in vivo for cells with altered growth potential. Some of the hairpin cassettes are potentially oncogenic as some hairpins will target genes with essential cellular function e.g. Tumour suppressors leading to undesirable effects such as cancer. However, the likelihood of infections is very low and no adverse effects have ever been described through accidental exposure to retroviral vectors. VSV-G pseudotyped virus can also infect other mammalian cells but not cells of other origin, however, the virus vectors are of no risk to the environment as viral particles are labile and do not survive on environmental surfaces outside the laboratory.

Origin & function

The inserted RNAi hairpins are sequences that have been derived de novo and are not form an organism. The sequences contain a 22bp sense and antisense sequence that is homologous to the gene of interest to which the hairpin is designed. These sequences are separated by a small loop sequence and flanked by mir-30 sequences. When transcribed in the cell, the RNA will form a hairpin structure which will initiate a normal cellular in vivo downstream cascade of processing events in response to foreign cellular single-stranded RNA intruder molecules. This process results in the post-transcriptional down regulation of the gene to which the hairpin sequence is designed and targeted by destroying the corresponding mRNA molecules prior to translation. The utilization of this is normal endogenous pathway to down-regulate genes of interest is known as RNA interference.

Evaluation of foreseeable effects

Risks and likelihood of risks:
- The bacterial strains to be used are non colonising and incapable of surviving in the environment or causing human infection. They pose no risk to users or the environment.
- None of the DNA viral vector plasmids are harmful to health or to the environment and do not supply a survival advantage to any microorganism. The packaging viral components are from the HIV-1 virus, HIV-1 is harmful to humans, however, the packaging vector does not contain the full length HIV-1 molecule. Many components of HIV-1 that are critical for HIV-1 infection have been removed from the packaging vector. Only those essential for viral packaging remain.
- The most dangerous GMM is the lentiviral preparations of pGIPZ particles and the most dangerous step is the collection, concentration and use of this lentivirus, however this risk is minimal. Lentiviral vectors can infect human cells and therefore if introduced into the body by injection, ingestion or through a wound, the virus could potentially...
enter cells and integrate its genome into cellular DNA. If the inserted sequence were to be expressed into humans after accidental transfer the hairpin could lead to the
down-regulation to the gene to which it targets via RNAi interference. This could be a gene essential for growth regulation such as a tumour suppressor gene therefore
resulting in a potentially oncogenic mutation. Additionally, physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or
oncogene, however likelihood of this is very low. Theoretically the virus can also infect other mammalian cells and knockdown the gene of interest. However this is
extremely unlikely as the hairpin is targeted to human genes and many hairpins are not homologous with the genes from other species. The overall risk is very low. Any
infection would be severe as the virus is replication incompetent and therefore self inactivating (does not carry gag,pol or env genes) so it cannot make new progeny virus
and establish a productive infection. Also, it would only be a small number of cells and would not impact on the othogenic outcome of an exogenous viral infection.
Additionally, the viral particles are labile and do not survive on environmental surfaces outside the laboratory making infection unlikely.
. The cell lines used cannot survive outside the laboratory incubators and as such pose no threat to health or the environment.

Precautions in place to minimise risks
The entire of the Cancer Institute is a containment level 2 facility which is secured by restricted access at all entrances and additionally to all laboratory areas. All work
involving viruses will be performed in class 2 cabinets and in containment level 2 laboratories specified particularly for retroviral GMM work. All DNA plasmids containing
viral proteins will be grown separately to avoid opportunity for recombination. To prevent contamination of workers by virus, when handling any GMM or GMM-derived DNA
or virus, no glass or other sharps will be used in the rooms where the viruses are prepared and used and workers will be protected by a lab coat and nitrile gloves. Skin
lesions will be covered with a bandage in addition to the protective wear described above. Specific guidelines will be in place to ensure all viral preparations are handled,
labelled, stored, transported and cleaned up correctly. All those involved in the project will be made aware of the guidelines and the associated risks as well as anyone else
who may use the laboratory. Persons involved on project from other Institutes will work under the supervision of a competent trained worker from this project. No-one
visiting the laboratory that is not involved in this work will work on this project. All persons entering the facility will be made aware of the safety precautions upon entering the
facility. All equipment used in the retroviral laboratory will be available in other general purpose laboratories for general use so it should not be necessary for others who are
not involved in retroviral work to enter the laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

2-5% Trigene advance or Virkon (v/v) will be used to treat liquid waste (18 hours) and also used to decontaminate any spills and for disinfection of any reusable bottles or
other equipment and laboratory materials used. Liquid waste is subsequently poured down the sink after treatment with Virkon. Work surfaces will be wiped down with 5%
Trigene advance and 70% ethanol after use. Virkon and Trigene advance are certified by the PHLS to provide 100% viricidal and GMO kill under these conditions by
denaturating and inactivating viral and cellular proteins. Trigene advance and Virkon will be used with their lifespan to ensure required kill is achieved. Solid waste will be
double-bagged in biological waste bags, sealed and autoclaved 132°C for 15 mins by trained staff then bagged in yellow biohazard bags and removed by UCL waste
services. Autoclave runs are regularly validated by waste management staff.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
The panel of advisers and the full GM Safety Committee approved this project as a Cass 2 GM activity.

### Project Containment

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<thead>
<tr>
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### Project Ref 14/09.3

- **Date Ackn'd:** 09/11/2009
- **CU2 Project Title:** Gene therapy to the foetus in utero
- **Class:** Class 2
- **CultureVol:** < 1 Litre
- **Consent Granted:** Non-GMM

### Project Additional Information

**Purposes of the contained use**

The aim of the project is to devise safe and effective methods of delivering gene therapy to the fetus or placenta or uterine arteries, with the long term aim of reducing disability and death due to conditions that affect the placenta such as placental insufficiency and fetal growth restriction, and genetic conditions that affect the fetus such as cystic fibrosis, and renal failure.
Recipient or parental organism

The vectors to be used will be adenovirus, retrovirus, lentivirus and adeno-associated virus which contain reporter genes (lacZ beta-galactosidase, Green Fluorescent Protein, luciferase) or therapeutic genes (human factor IX, X or VII, beta-globin, Cystic Fibrosis Transmembrane Regulator).

Only adenovirus vectors will contain Vascular Endothelial Growth Factor, Placental Growth Factor and Insulin Growth Factors.

The vectors will be produced by a number of different sources including Ark Therapeutics Inc, Kuopio, Finland, Department of Haematology (UCL, Dr A N) and Department of Paediatric Immunology (Institute of Child Health, Prof B G).

Host/vector system

Adenovirus: replicative functions are removed such as E1A and placed into packaging cells 293, or removal of E1A and E3, or removal of all early genes (gutless vectors). Supernatant is checked for the presence of wild-type virus.


Lentivirus (second generation) and retrovirus: separated packaging functions transfected into producer cells, theses are gag, pol and env genes. Virus genomes with cDNA will be packaged in vitro.

Origin & function

In this project we will study the use of viral vectors in normal and growth restricted animal fetuses (sheep, rabbit, guinea pig, rat and mouse), their placentae, at various stages of gestation in order to provide basis for gene therapy of fetal animals. Vectors will be delivered to a variety of fetal sites and to the placenta and uterine artery by ultrasound-guided percutaneous needle insertion in the anaesthetised pregnant ewe as well as via chronically implanted fetal catheters in the anaesthetised pregnant ewe, or by injection at laparotomy in anaesthetized animals. Vectors may also be delivered under direct vision by laparotomy and hysterotomy. Some vectors will carry marker genes for beta-galactosidase, Green Fluorescent Protein (GFP) or human factor IX. The former two act as histological markers and allow detection of transgene expression in different tissues while the latter can be detected in blood and will be used to determine the magnitude and timing of gene expression. The remaining vectors will carry therapeutic genes such as for Cystic Fibrosis Transmembrane Regulator, clotting factors VII, IX, and X, beta-globin, Vascular Endothelial Growth Factor, Placental growth Factor and Insulin Growth Factor. Vectors will only be injected into animals held at the Royal Veterinary College, Camden or in the Biological Services Unit, UCL, where post mortem examination will also be carried out. Both the surgery and post mortem examination will be done in level 2 facilities. Animals will be examined at various time points after vector injection. Tissues will be analysed at other laboratory locations including Institute for Womens Health, Rayne Institute and Department of Haematology, UCL. Tissues will be tested using histology, immunohistochemistry, PCR, RT-PCR and ELISA for vector spread, transgene expression and pathology.

Evaluation of foreseeable effects

The transgenes used in these vectors are expected to result in temporary changes in vascular reactivity and blood vessel formation (Vascular Endothelial Growth Factor, Placental Growth Factor and Insulin Growth Factor only present in adenovirus vectors), increase in human clotting factors (VII, IX, and X), or human beta-globin, expression of reporter genes (beta-galactosidase, Green Fluorescent Protein), increased expression of the CFTR gene. None of these effects are likely to be harmful to animals or humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All GM animal material generated by the project will be either stored (fixed in 4% formaldehyde for 24 hours, or snap frozen) or disposed of via incineration arranged by the Biological Services Unit, RVC, Camden.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste such as contaminated needles, tubes and syringes will be treated with 10% chloros solution or incinerated as required. All surgical instruments are cleaned in 10% chloros before autoclaving. Animal post mortems will be performed in a designated post mortem room with category 2 facilities at the royal Veterinary College, Camden or the Biological Services Unit, UCL. All gowns and drapes used during surgery are first sterilized by autoclaving before being washed and then resterilized for use in further...
This project, originally a class 1 activity, was extended to include the administration of integrating viral vectors by injection to animals. This change in activity requires reclassification to a class 2 GM activity as advised in Part 2 of the SACGM Compendium of Guidance.

Please enter comments on the GM safety committee on the risk assessment

This project, originally a class 1 activity, was extended to include the administration of integrating viral vectors by injection to animals. This change in activity requires reclassification to a class 2 GM activity as advised in Part 2 of the SACGM Compendium of Guidance.

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**Project Ref** 14/09.4

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<th>Withdrawn</th>
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**Historical Significant Changes**

**Historical Date of Additional Info**
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to use shRNA, cDNA, RNAi and small molecule libraries, using viral and non-viral vectors, for functional screening of cell-based phenotypes. Cellular processes such as signal transduction, protein trafficking and cytoskeleton organization will be investigated under conditions of cDNA overexpression, small molecule treatment or shRNA/RNAi-mediated knockdown of specific genes. The libraries are envisaged to encompass the complete human genome.

**Recipient or parental organism**

HEK293T for virus production, immortalized mammalian cell lines (e.g. HeLa, Cos, Chinese hamster ovary (CHO)), murine embryonic fibroblasts (MEF), primary rat neurons, primary rat Schwann cells, HUVECs, primary human monocytes and macrophages. Yeast cells.

**Host/vector system**

Non-viral: The cDNA expression vectors are standard expression plasmids (e.g. pEAK12, pCMV) with a CMV, EF1A or beta-chicken actin promoter. A puromycin cassette driven by PGK promoter is also included.

Viral: The viral vectors for shRNA are commercially available from vendors (e.g. SigmaAldrich, Open Biosystems) and will be based on the latest generation of lentiviral vector design that renders generation of a replication competent virus to be virtually impossible. The viruses, however, contain some HIV sequences and are pseudotyped with VSV-G envelope such that cells can be infected even if they are not dividing.

**Origin & function**

The inserted shRNA, cDNA & RNAi sequences encompass the entire human genome. The primary reason for using lentiviral vectors is to allow transfection and expression in non-dividing cells, e.g. neurons and macrophages, to permit functional screening of cellular morphologies in these cells. cDAN expression vectors are to be used to transfect mammalian cell-lines.

**Evaluation of foreseeable effects**

Infection with Lentivirus

The materials and the techniques to be used in this specific work program are well established and routinely used by researchers throughout Europe, North America and Australasia. In all experiments we will use replication defective viruses, derived from molecular clones in which key viral genes have been deleted or replication defective viruses, derived from molecular clones in which key viral genes have been deleted or inactivated, thus rendering the virus particles competent for only a single round of infection and replication.

All work with lentivirus will be performed under containment level 2 conditions and open cultures of virus will always be handled inside a microbiological safety cabinet. Provided the local rules established for labs G17 (LMCB SOP 005) and G17 (LMCB SOP 011) are followed, the risk of infection is negligible. These local rules document arrangements for training and induction, waste disposal, sharps policy, emergency procedures etc.

VSV G pseudo-types

Although VSV G pseudo-types can permit infection of a wide range of cell types and species, there is no evidence that these viruses represent a significant risk when handled under biosafety level 2 conditions. These reagents have been used extensively in research and, to our knowledge, there is no documented evidence of infections of laboratory workers through pseudotyped viruses.
Virus mutations and recombination

Although mutation/recombination could potentially render lentiviral vectors replication-competent, such an event is highly unlikely and, to our knowledge, has not been reported previously in a laboratory setting.

Non viral vectors

There is now potential for harm to human health or the environment using these vectors.

Exposure to infected primary cells

All primary cells for these experiments will be obtained from laboratory animals and serologically tested donors (e.g. HUVECS) and will present no significant risk to human health.

Risk to the environment

Due to the restricted host range of the constructs, their lack of ability to survive outside laboratory conditions, restrictions on access to the facility and the risk control and disinfection/disposal measures described in this assessment, we conclude that the program of work presents no risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated with Trigene Advance* (100% trigene solution added to liquid to make a final concentration of at least 5%) for at least 12 hours before disposal in the laboratory sink.

Solid waste: The LMCB policy is that any solid material containing, or potentially contaminated with, viable biological material must be autoclaved prior to disposal into the clinical waste stream (removed from site by licensed waste carrier and incinerated). Following virus usage, any materials that have, or may potentially have come into contact with virus will be thoroughly decontaminated by soaking in Trigene Advance* (5%) for at least 12 hours prior to draining and placing into an autoclave bag.

Autoclave bags must be doubled (one bag inside the other).

At the end of each session, autoclave bags must be partially** sealed with autoclave tape and left in the blue crate (located in ante-room in G.17 and under lab sink in 1.33) for collection by the autoclave room staff.

Autoclaving is performed in room G.12 (MRC building). The autoclave is validated (12-point thermocouple test) by a competent engineer on a six monthly basis.

*Trigene Advance labs study titled “Virucidal efficacy of a disinfectant for use on inanimate environmental surfaces”, March 2007. This study concluded that TriGene advance (at 1:100) demonstrated complete inactivation of HIV type 1 at 30 min exposure time at RT.

**The autoclaves used pulsed vacuum/stem injection to ensure contact between steam and material. If the bags are completely sealed this process will be compromised.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 14/10.1

**CU2 Project Title**

Maintenance, Distribution and Manipulation of Open Biosystems Human retroviral shRNAmir library, and of retroviral and lentiviral vector systems for gene expression and gene knockdown in mammalian systems

**Date Ackn’d** 09/02/2010

**Date Project Ceased**

**Class** Class 2

**CultureVol** 1-50 Litres

**Class CultureVol** Class 2 Culture Volume

**Consent Granted**

**Non-GMM Consent Granted**

**Project notified under transitional arrangements**

**Tick if notifying a connected programme of work**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

The Open Biosystems shRNAmir library consists of over 70,000 individual E. coli glycerol stocks containing the pSM2 retroviral or the pGIPZ lentiviral vector. Each stock contains a unique hairpin sequence within the pSM2 or pGIPZ vector designed to target and reduce the expression of most/all genes within the human genome via RNA interference (RNAi). Retroviral vectors used for gene overexpression originated from Clonetech. Genes of interest are introduced with standard molecular cloning techniques. Adenoviral vector for expression of recombinant Cre Recombinase is replication deficient due to deletions in the E1 and E3 regions and will be used in mouse embryonic fibroblasts (MEFs) originating from a conditional knock out animal models.

The Project will involve retroviral and lentiviral knockdown and overexpression vector maintenance, storage and use of Open Biosystems shRNA library and adenoviral, retroviral and lentiviral mammalian expression plasmids, as well as distribution of clones as bacterial stocks, DNA plasmids and/or retrovirus/lentivirus to members within University College London for use in various projects. Adenovirus Cre will be used for conditional gene targeting. Cre recombinase is a Type I topoisomerase from bacteriophage P1 that catalyzes the site-specific recombination of DNA between loxP sites. Recombinant adenoviruses will be replication deficient due to deletions in the E1 and E3 regions.

Specifically, this project will involve retroviral/lentiviral production form the pSM2 and pGIPZ plasmid and subsequent use of retrovirus/lentiviral stocks in cell based screening functional assays. Further, adenovirus will be used for conditional gene targeting in cell culture systems.

Recipient or parental organism

Cell lines: HMEC, 293T, 293, canine-MDCK, MCF-7, MCF-10A, DU145, PC3, HELA, Jurkat, BT20, DU4475, HBL100, HCC1007, HCC1008, HCC1143, HCC1187, HCC1395, HCC1419, HCC1428, HCC1500, HCC1569, HCC1599, HCC1739, HCC1806, HCC1937, HCC1954, HCC202, HCC2157, HCC2185, HCC2218, HCC3153, HCC38, HCC70, HCC712, MCF10F, MCF12A, MDAMB134VI, MDAMB157, MDAMB175VI, MDAMB231, MDAMB330, MDAMB361, MDAMB415, MDAMB435, MDAMB435S, MDAMB436, MDAMB453, MDAMB468, SKBR3, SUM102PT, SUM1315MO2, SUM149PT, SUM159PT, SUM185PE, SUM190PT, SUM225CWN, SUM229PE, SUM44PE, SUM52PE, T4, T47D, UACC812, UACC893, ZR751, ZR7530, ZR75B, SF9, OvCA 420, OvCA 429, OvCA432, OvCA433, DOV-13, OvCar 5, SkOV3, OvCar3, OvCa 3, C1, C11, C2, C22, C3, T1, T11, T2, T22, T3

Primary human and mouse cells: derived from various human and mouse tissues including peripheral blood-derived lymphocytes.

Bacterial E.coli strains: HB101, XL1-blue, Top10, PrimePlus, JM109, BL21, DH5alpha, SURE, KS10000, The bacterial strains used are attenuated non-colonising strains so that they are incapable of causing human infection and are therefore harmless to humans.

The viral vectors (lentiviral, retroviral, as well as adenoviral) used will be pseudotyped with replication incompetent envelope protein, so if introduced into the body by injection, ingestion or through a wound, the virus could enter human cells and integrate into cellular DNA. Although the virus is replication incompetent so that it cannot make a new progeny virus and establish a productive infection, its insertion into cellular DNA could result in a potentially oncogenic mutation.

The cancer cell lines are minimal risk to harm human.

The bacterial strains used are attenuated, non-colonizing strains that cannot survive outside culture conditions and are therefore harmless to the environment.

The viral vectors are replication incompetent and therefore cannot self-replicate and transfer to another host and are harmless to the environment.

The cells that will receive and integrate the DNA cannot survive in the environment as they are only viable under culture conditions, and are therefore harmless to the environment.

Host/vector system

Lentiviral vectors: Commercial TZV vector from Tranzyme and derivatives (including pGIPZ).
Retroviral vectors: Commercial retroviral hairpin p2M (pSHAG-MAGIC2) vector from Open Biosystems. Retroviral overexpression vectors, i.e. pLXSN, originate from Clonetech. pBabe.

Adenoviral Vector: Commercial human Adenovirus Type5 (dE1/E3).

The viral vectors used are retroviral, lentiviral and adenoviral vectors. The lentiviral and retroviral vectors contain the CMV (Pol II) promoter to drive expression or the hairpin cassette or gene sequence. The lentiviral and retroviral vector do not contain the appropriate accessory elements to form viral particle itself and therefore pose no harm to health and the environment. Packaging and envelope proteins are contained on two additional plasmids to the viral expression vector and these plasmids must be introduced into the cell and expressed alongside the viral vector for viable viral particles to be produced. There are no differences in risk assessment or technique compared to viral downregulation of genes. The Vectors used for each specific task are specifically designed, meaning that the viral knockdown vectors contain elements, i.e. mir-30 sequence flanking the hairpin containing the shRNA, that enhance gene knockdown efficiency. Otherwise the backbone of viral vectors is very similar.

Concerning the usage of adenovirus, there is no integration with the host system, and therefore no viral replication can take place.

Origin & function

The RNAi hairpins are sequences that have been derived de novo and are not from an organism. They have been designed as small sequences with both a sense, anti-sense and loop sequence to form a RNA hairpin when transcribed which will initiate a downstream cascade of events leading to knockdown of the target gene to which the hairpin was designed. The accessory proteins are from the HIV-1 virus. Sequences from retroviral over-expression plasmids originate from human and mouse genes. The Cre recombinase sequence originates from P1 bacteriophage.

The hairpin sequence will be transcribed and the RNA will then form a hairpin stucture. The hairpin structure will induce a normal cellular in vivo downstream sequence of processing events in response to foreign cellular single stranded RNA intruder molecules that will result in the post-transcriptional down-regulation of the gene to which the hairpin sequence is targeted.

DNA gene sequences in overexpression vectors will express a defined gene sequence. The quantity of gene transcript will depend on the chosen promoter system, which defines the strength of the promoter and subsequently the amount of gene expression.

If the inserted sequence were to be expressed in humans after accidental transfer of the hairpin, this could lead to the down-regulation of the gene to which it targets via RNA interference. This could be a gene essential for growth regulation such as a tumour suppressor gene, therefore, resulting in a potentially oncogenic mutation. Also, over-expression of genes in humans after accidental transfer may result in over-expression of a gene essential for growth regulation, such as an oncogene. This, however, would not be severe as the virus is replication incompetent and therefore self-inactivating as none of the structural genes are actually present in the packaged viral genome, so no new virus can be produced and no other cells can be infected. Physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene, however the likelihood of this is very low.

Retrovirus and VSV-G pseudotyped lentivirus can only infect mammalian cells. Potentially the hairpin could be expressed in another mammalian species and lead to the down-regulation of a growth regulatory gene such as a tumour suppressor gene resulting in a potentially oncogenic mutation. This, however, Is extremely unlikely as the hairpin is targeted to human genes and many hairpins are not homologous with the genes from other species. If the hairpin were to target and lead to the reduction of a gene via RNA interference it would not be severe as the virus is replication incompetent and therefore self-inactivating as none of the structural genes are actually present in the packaged viral genome, so no new virus can be produced and no other cells can be infected. Over-expressed gene sequences could be expressed in another mammalian species and lead to over-expression of growth regulatory genes such as an oncogene resulting in a potential oncogenic mutation. There are no differences in risk assessment or technique compared to viral downregulation of genes. The Vectors used for each specific task are specifically designed, meaning that the viral knockdown vectors contain elements, i.e. mir-30 sequence flanking the hairpin containing the shRNA, that enhance gene knockdown efficiency. Otherwise the backbone of viral vectors is very similar.

If virus is introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate into cellular DNA. Although the virus is replication incompetent (does not cary gag, pol, or env genes) so it cannot make new progeny virus and establish a productive infection, its physical insertion into cellular DNA could result in a potentially oncogenic mutation. This would be a very unlikely event and if so only a very small number of cells would be affected and it would not impact on the pathogenic outcome of an exogenous viral infection. The packaging viral components are from the HIV-1 virus. The full length HIV-1 virus is harmful to the host system, and therefore no viral replication can take place.
humans, however the packaging vector used does not contain the full length HIV-1 molecule. Many components of HIV-1 that are critical for HIV-1 infection but not required for viral packaging, have been removed, so the virus is attenuated. There are no differences in risk assessment or technique compared to viral downregulation of genes. The Vectors used for each specific task are specifically designed, meaning that the viral knockdown vectors contain elements, i.e. mir-30 sequence flanking the hairpin containing the shRNA, that enhance gene knockdown efficiency. Otherwise the backbone of viral vectors is very similar. Recombinant adenoviruses are replication deficient due to deletions in the E1 and E3 regions and considered of ordinary potential harm. In the unlikely event of recombination, wild type, replication competent adenoviruses provoke cold symptoms and strong immune responses in healthy individuals and generally do not cause serious illness.

The de novo hairpin donor sequences or the de novo gene sequences could potentially be expressed in cells and lead to the knockdown or expression of certain genes leading to the increase of oncogenic potential of the virus. This would only be a very small number of cells and as the virus is replication incompetent and cannot make new viral progeny, it would not impact on the pathogenic outcome of an exogenous viral infection. There are no differences in risk assessment or technique compared to viral downregulation of genes. The Vectors used for each specific task are specifically designed, meaning that the viral knockdown vectors contain elements, i.e. mir-30 sequence flanking the hairpin containing the shRNA, that enhance gene knockdown efficiency. Otherwise the backbone of viral vectors is very similar. Recombinant adenoviruses are replication deficient due to deletions in the E1 and E3 regions and considered of ordinary potential harm. In the unlikely event of recombination, wild type, replication competent adenoviruses provoke cold symptoms and strong immune responses in healthy individuals and generally do not cause serious illness.

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Evaluation of foreseeable effects

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The most dangerous GMO is the lentiviral and retroviral preparations of viral particles. The most dangerous step is the collection, concentration and use of the retrovirus/lentivirus.
The UCL GM Safety scrutineers acting on behalf of the GM Safety Committee approved the notification as an activity class 2 activity after their recommended revisions were made.

Please enter comments on the GM safety committee on the risk assessment

The UCL GM Safety scrutineers on behalf of the GM Safety Committee approved the notification as an activity class 2 activity after their recommended revisions were made.

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Project Ref 14/10.4

Date Ackn'd 13/08/2010

CU2 Project Title Determining the mechanism of protein export by the human malaria parasite, Plasmodium falciparum.

Date Project Ceased

Class 3

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4 < 1 Litre

Non-GMM Consent Granted Yes

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**

The malaria parasite lives inside human and red blood cells. It exports proteins into the red blood cell cytoplasm.

**Aim 1:** To find out what determines whether a protein is exported, we will generate parasites that are transfected with GFP (green fluorescent protein) tagged exported proteins. We will introduce mutations into these proteins to see what regions of the protein are required for protein export.

**Aim 2:** Several components of the protein export apparatus have been identified. We will generate malaria parasites that are transfected with these components and then use these parasites for biochemical analysis with the aim of determining what proteins interact with these known components of the export machinery.

**Recipient or parental organism**

GMM/1: lines 1.13, 1.19, 1.20

*Plasmodium falciparum*

*Plasmodium falciparum* causes human malaria. Malaria can be treated effectively with drugs. Parasites used are not resistant to widely used anti-malarial drugs. If untreated, malaria can be fatal.

Recipient organisms are not capable of independent survival in the environment.

**Host/vector system**

GMM/1: lines 1.12, 1.24

Custom made expression vectors will be used (sequences can be provided). These are circular vectors derived from E. coli plasmids but with the following additions that allow them to be propagated in E. coli and plasmodium.

1. A drug selection marker, either human dihydrofolate reductase or Blasticidin deaminase, that will allow selection of transfected parasites using either WR99210 or blasticidin, respectively. Neither of these drugs is used for treatment of malaria and both are widely used in transfection of malaria parasites. Both selection markers will be expressed from a plasmodium promoter, pcDT

2. A plasmodium promoter that allows expression of the proteins in section 1.10 (calmodulin promoter)

3. An AttP site that allows transposase (Bxb1) mediated integration at a specific site into the plasmodium genome, or terminal repeats that allow random insertion of the plasmid into the lasmodium genome using the Piggybac transposase.

4. GFP or strep tag will be fused to the gene to be expressed.

To facilitate plasmid integration into the parasite genome a second plasmid that expresses the transposase Bxb1 or piggybac will also be used. These transposases will be expressed from a plasmodium promoter and the plasmids will not be selected for using a drug selection.

Plasmids also contain an E. coli origin and Ampicillin selection marker to allow propagation of the DNA in E. coli.

**Origin & function**

GMM/1: 1.11, 1.14 - 1.16 - 1.18
DNA is derived from plasmodium falciparum, which causes human malaria

Inserted gene products are expected to have the following activities:

**PFl1755c, pfemp3**: small fragments of these genes, fused to GFP will be used. These fragments will be sufficient to direct the export of GFP into the red cell cytoplasm. They are not expected to have any other biological activity.

**PF11_0175 and Exp2**: proteins are thought to be involved in unfolding of proteins prior to export.

The proteins themselves will not cause harm if expressed in humans after accidental transfer and neither will selectable markers used to select transfected parasites. Parasites will be resistant to drugs WR99210 and Blasticidin but neither drug is used to treat malaria. Parasites will remain sensitive to drugs commonly used to treat malaria such as Artemisinin.

Sequences will not cause harm if transferred to species in the environment

Sequences derived from plasmodium are not implicated in pathological or harmful characteristics of the organism.

### Evaluation of foreseeable effects

**GMM/1: lines 1.17, 1.16, 1.32**

DNA is derived from plasmodium falciparum, which causes human malaria.

Sequences derived from plasmodium are not implicated in pathological or harmful characteristics of the organism.

Potential harm includes infection with malaria or unknown pathogens contained in human blood.

Blood will be obtained from NHS Blood and Transplant (National Blood Service) - product - (NC15-Research Red Cells)

This product is screened, by the NHS, for Syphilis, HIV, Hep B and Hep C. No other blood products will be used.

The most dangerous part of this procedure will involve handling and propagating malaria cultures

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

The laboratory to be used is not sealable to permit fumigation because the malaria parasite cannot survive outside of carefully controlled growth conditions, and so any spillage outside of primary containment (the microbiological safety cabinet) would result in rapid die-off of the infectious agent.

Additionally, the malaria parasite is not transmissible by the airborne route.

No work with other HG3 agents will take place in the laboratory at the same time as work with *P. falciparum* takes place

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**GMM/1: line 1.29 or GMM/2: lines 2.13 (as applicable)**
1. Liquid waste: 10% bleach overnight (liquid waste will be aspirated into a vacuum trap containing sufficient bleach such that even if the trap is full it will contain at least 10% bleach at all times). This is a standard method that results in 100% killing of the malaria parasite.

2. Plasticware: disposed in clearly labelled biohazard bins, double bagged in appropriately labelled bags, and autoclaved. This is a standard method that results in 100% killing of the malaria parasite.

3. Glass microscope slides will be fixed in 100% methanol and autoclaved. This is a standard method that results in 100% killing of the malaria parasite.

This project was reviewed by the University Biological Safety Advisor and one of the UCL GM advisors on behalf of the Committee. Subject to some minor amendments and clarifications, they were content with the risk assessment.

Please enter comments on the GM safety committee on the risk assessment

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Large Scale Activities

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Human Clinical Applications

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Project Ref 14/10.5

Date Ackn'd 24/08/2010

Date Project Ceased

CU2 Project Title Analysis of glial cell function in nerve repair and cancer

Class 2

CultureVolClass2 < 1 Litre

Consent Granted

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to use shRNA and cDNA adenoviral and lentiviral expression vectors to either remove gene function or constitutively express proteins in order to determine the role of specific proteins in glial cell function in relation to nerve repair and cancer. Viral vectors are required to ensure high efficiency of expression. Glial cells interact with other cell types such as neuronal cells and fibroblasts, therefore viral infection of these cells may also be required to determine glial cell function. Many of the genes to be suppressed or expressed will have been identified as a result of genome-wide screens using the facility at the LMCB run by R K.

**Recipient or parental organism**

The hosts will be bacteria used for cloning all E. coli based TopF10, DH5alpha, STBL3). HEK293T for virus production, Rat and mouse Schwann cells and relevant human tumour cell lines (e.g. MPNSTs), immortalized mammalian cell lines (e.g. HeLa, Hek293, Cos, Chinese hamster ovary), fibroblasts from mice (MEFs) and rats (REFs), rodent neuronal cells.

**Host/vector system**

Replication-deficient lentiviral -based vectors for shRNA (available from Open Biosystems or Sigma Aldrich); replicaion-deficient lentiviral-based expression vectors for cDNA expression (FUW or RRL backbone vectors). Replication-deficient adenoviruses for cDNA expression (AdEasy and related vectors (Stratagene and Qbiogene Inc)).

**Origin & function**

Small hairpin RNAs for gene knockdown and cDNA covering a large selection of genes , and indicator proteins (GFP, dsRed, lacZ) or drug selection markers (neo/puro). At the current time it is not possible to be specific about the genes involved as many will be identified in a screen. We will be doing genome-wide lentivirus siRNA screens in collaboration with R K. We would then do a secondary screens and follow-up experiments for the genes which are positive. It is most likely these genes will code for receptors, kinases or phosphatases . Initial experiments will be expressing ligands and receptors of the ephrin signalling pathways and are highly unlikely to be transforming. In any case, all work with infectious virus will be performed in the virus suite which is category 2.

Small hairpin RNAs for gene knockdown cDNA covering a large selection of genes, and indicator proteins (GFP, dsRed, lacZ) or drug selection markers (neo/puro).

All the above are derived from human, mouse or rat genome projects.

shRNA are intended to prevent expression of targeted proteins in recipient cells. cDNA will lead to expression of translated protein in target cells.

**Evaluation of foreseeable effects**

Recipient organisms:
Recipient organism will be E. coli bacteria (Top10, DH5alpha & STBL3) and established & screened cell lines of human & rodent origin. Exposure to these organisms could occur due to ingestion, injection or inhalation of material whilst handling due to poor laboratory practice. The likelihood of exposure is low as workers will handle material in accordance with good laboratory practice (as specified in the LMCB Code of Practice for Laboratory Workers and in the SoPs for lab G.17 (virus lab). None of these organisms are likely to have any harmful effects on human health or the environment.

Viral vectors:

The disabled viral vectors contain some HIV sequences and may be pseudotyped with VSVg envelope such that human cells can be infected even if they are not dividing. Upon infection the virus could conceivably cause harmful effects by disruption to host gene sequences or by constitutive expression of the cDNA due to insertion of viral genome.

Infection with wild-type adenovirus can lead to infection of the upper-respiratory tract resulting on cold/flu like symptoms. Reversion of the replication deficient adenoviral vector to a replication-competent strain is highly unlikely. Moreover, we test for RSV in new virus by testing the supernatant of infected cells for virus. To date we have not been able to detect virus.

Exposure to vectors could occur due to ingestion, injection or inhalation (particularly sub-cutaneous injection in the case of lentivirus and inhalation of adenoviruses) of material whilst handling. Key risks relate to work with adenoviral and lentiviral vectors and are a) spillage during infection b) sub-cutaneous infections during manipulations of plates whilst performing tissue culture and c) generation of aerosols due to spillage or failure of equipment whilst performing tissue culture.

All virus production and cell infections will be performed in the Virus laboratory (G.17), in accordance with the SOP for work in the Virus Laboratory, using class II safety cabinets.

The risk to the environment from the viral vectors is, due to their replication-deficient nature, negligible.

Gene sequences:

Due to their intended effect of shRNAs of supressing targeted proteins it is possible that harmful effects could occur. cDNAs lead to expression of translated protein products which could also have positive harmful effects. It is not possible to predict the severity of the effects but due to the minimal effects of expressing single genes severe effects are unlikely. Exposure to these sequences could occur due to ingestion, injection or inhalation of material whilst handling due to poor laboratory practice. The likelihood of exposure is low as workers will handle material in accordance with good laboratory practice (as specified in the LMCB Code of Practice for Laboratory Workers and in the SoPs for lab G.17 (virus lab).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material containing, or potentially containing adenoviral or lentiviral vectors will be immersed in 5% Trigene solution overnight prior to autoclaving in room G12 (MRC building). Liquid waste will be mixed with Trigene Advance to a 5% solution and left for 12 hours (min) before disposal down a laboratory sink within a Category 2 laboratory (G.17). Green fluorescent reporter constructs for lentivirus and adenoviruses can be assayed for infectivity with & without Trigene treatment. We anticipate that infectivity will be undetectable after Trigene treatment.

Trigene Advance: ATS labs study titled "Virucidal efficacy of a disinfectant for use on inanimate environment surfaces" March 2007. This study concluded that TriGene...
Advance at (1:50) demonstrated complete inactivation of HIV type 1 at 30 min exposure time at RT. Likewise the Abbott Analytical study of Sept 1996 showed that Trigene Advance (at 1:50) completely inactivated adenoviruses following 15 minutes exposure at RT. Consequently, we are confident that Trigene Advance would be equally effective against lentivirus and adenoviruses.

All other GMMs including transfected cells that have been fixed with 4% paraformaldehyde or lysed with Triton x100, will be autoclaved prior to disposal without prior chemical disinfection.

The autoclaves are regularly serviced and a 12-point thermocouple validation is performed every 6-months.

Is an emergency plan required according to regulation 20?  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

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Project Ref 14/11.1

Date Ackn'd 20/01/2011

CU2 Project Title Hepatitis B virus (HBV) genome cloning and phenotypic studies

Date Project Ceased 02/12/2011

Class

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Non-GMM Consent Granted

Project notified under transitional arrangements N
We are conducting studies on the impact of Human Immunodeficiency Virus (HIV) and its treatment among people who are co-infected with the hepatitis B virus (HBV). For this purpose we are collecting blood samples from two cohorts of patients in Ghana and Malawi. In these regions antiretroviral therapy has recently become available to treat HIV infection. The first-line combination includes three drugs active against HIV, typically stavudine, lamivudine (LMV) and nevirapine. Of these drugs, LMV also has activity against HBV. Thus patients who are HIV/HBV co-infected receive three drugs for HIV but only one for HBV. There are two problems associated with the use of LMV as the sole agent against HBV:

1) Control of HBV replication is usually suboptimal, leading to the development of HBV drug resistant variants carrying mutations in the polymerase (pol) gene targeted by LMV. Ongoing HBV replication leads to progressive liver damage and a risk of liver cancer. Thus, as survival increases in these communities due to control of HIV, a potential is emerging for significant HBV-related liver disease to become apparent.

2) As the HBV surface (S) and pol genes are expressed in alternative reading frames from the same region of the viral genome (Figure 1), selective pressure exerted by LMV on pol can cause mutations in both pol and S. Mutations in the HBV surface antigen (HBsAg) can affect key antigenic determinants, creating the potential for immune and vaccine escape. In addition mutations in HBsAg can lead to loss of recognition in diagnostic assays.

We have preliminary data that show high rates of emerging HBV LMV resistance in the two African cohorts and have also identified virus variants that carry highly mutated HBsAg, including some diagnostic escape mutants. We now wish to extend these studies by constructing a system for HBV that will allow us to characterize the viral phenotype, including drug resistance, viral fitness, and HBsAg expression and immune recognition in vitro. We have extensive experience with similar systems applied to the study of HIV phenotypes, where we use both replicative and non-replicate recombinant vectors containing virus gene segments of interest inserted as “cassettes”. The vector may also be modified by site directed mutagenesis (SDM) to study mutations of interest. We plan to follow a similar approach with HBV.

It has long been established that certain circular DNA viruses replicate through a rolling circle mechanism. By mimicking this mechanism, a construct that contains a full length viral genome plus a partial repeat (“full-length plus”) can direct the production of virus particles upon transfection into cell lines in vitro. HBV clones have been traditionally produced in this way. We will amplify the whole HBV genome from one or a few interesting samples and clone it into standard cloning vectors (pUC or pCR based vectors). Full-length plus HBV clones will be generated by adding a partial repeat to the cloned full genome. Individual gene segments will be sub-cloned into generic expression vectors (pCMV or pSV40 expression vectors). In some cases, gene segments derived from certain patient viruses will be exchanged with those of the reference clone to study their effect on phenotype in the context of a common backbone. In addition, mutants of interest will be introduced by SDM. We will then transfect the viral construct in vitro into cell lines with the purpose of studying replication capacity, drug susceptibility and immune recognition of the various viruses.

To facilitate the interpretation of the proposed experiments, we summarise below the main characteristics of the HBV replication cycle (Figure 2). One key feature is that the virus replicates through an RNA intermediate form by reverse transcription, and in this respect it is similar to HIV.

Figure 1. Schematic representation of the structure of the HBV genome with the four opening reading frames (ORFs) P, S, C, and X. The ORFs encode seven different
proteins through use of varying in-frame start codons. ORG P encoded for the polymerase protein; ORF S encodes the three surface proteins; ORF C encodes both the e and core protein; ORF X encodes the X protein. The genome also contains genetic elements which regulate levels of transcription, determine the site of polyadenylation, and mark a specific transcript for encapsidation into the nucleocapsid. The four ORFs transcription are controlled by four promoter elements (preS1, preS2, core, and X).

HBV regions contain a partially double stranded 3.2 kb genome termed the relaxed circle DNA (rcDNA). The negative sense strand is complete while the positive sense strand is only partial. Upon infection of cells, the genome is transported into the nucleus where it is converted into a fully double stranded, covalently closed circular DNA (cccDNA). This persists as an episome in the nucleus, a sort of minichromosome that serves as a template for the transcription of viral RNAs by the cellular RNA polymerase II. Several transcripts are produced, which are classed as genomic and sub-genomic RNAs. Subgenomic transcripts serve as mRNA for the X and surface proteins. The larger genomic transcripts are longer than one genome in length and serve to produce e, core and polymerase proteins. A particular genomic transcript, lacking the ATG start codon for the e protein, is designated as pregenomic RNA (pgRNA). The pgRNA, in addition to serving as mRNA for the core antigen and the viral polymerase, is selectively packaged into immature viral capsids in the cytoplasm, and reverse transcribed by the viral polymerase to produce the rcDNA genome for progeny virions.

UNABLE TO INCLUDE DIAGRAM (Figure 2)
Figure 2. Schematic representation of the HBV life cycle. The virus particle attaches to the hepatocyte through cellular receptor (1). After translocation to the cytoplasm (2), the nucleocapsid releases the viral genome to the nucleus (3) where it is converted into a covalently-closed circular DNA (cccDNA) to serve as template for viral RNA transcription (4). The viral mRNAs are translated into viral proteins in the cytoplasm (5), and the pgRNA (6) is encapsidated together with the viral polymerase into a capsid made of core protein (7). Upon maturation of the virus particle, DNA is synthesized by the viral polymerase using pgRNA as template (8-9). The newly synthesized nucleocapsid can either initiate a new round of cccDNA synthesis in the nucleus (10), or acquire the viral envelope in the ER/Golgi apparatus with release of mature virions by exocytosis (11).
This mode of virus production does not result in cell death

Recipient or parental organism
E. coli strains K12 (for example DH5 alpha, HB101, TOP10 and Mach1)
Cell lines - Epithelial cell lines such a HepG2, HepaRG cells, HepG2.2.15, HeLa; T-cell lines such as Jurkat; Embryonic cell lines such as HEK293.
E. coli K12 (DH5 alpha, HB101, TOP10 and Mach1) used for DNA cloning poses little risk as it is not considered to be pathogenic to humans or animals. It requires biosafety level 1.
A number of cell lines will be required for the proposed experiments.
The human cell line HepaRG poses little risk and requires biosafety level 1. This is a hepatoma cell line which is differentiated before use. The differentiated cells have very similar properties to primary hepatocytes.
The human cell line HepG2 are also poses little risk and requires biosafety level 1. HepG2 cell are adherent, epithelial-like cells derived from the liver tissue of a fifteen year old male with differentiated hepatocellular carcinoma. HepG2 are not tumorigenic even in immunocompromised mice.
HepG2.2.15 cells are HepG2 cells stably transfected with full-length plus HBV genome; they express all viral RNAs and proteins, produce viral genomes, and secrete virus-like particles. HepG2.2.15 cells are a biosafety category 3 cell line that will be handled in containment level 3 laboratory. The cells will be stored in the -80°C freezer in the containment level 3 laboratory in ladled boxes.
The human cell lines HeLa, Jurkat, and HEK293 require as biosafety level 2. The gene that maintains continuous viral growth in vitro may be expressed if accidently transferred into humans and has potential to cause cancers.
E. coli K12 strains have limited survival in the environment. Bacteria that have been transformed by viral constructs do not produce any harmful by-products other than the
viral genome being studied. The inserted plasmids are only maintained with the use of antibiotic selection and are not likely to be maintained within the bacteria in the environment.

Human cell lines HepaRG, HepG2 and HepG2.2.15 cells, HeLa, Jurkat, Hek293 require specialised sterile tissue culture conditions to be maintained and have limited survival in the environment as the serum and nutrient requirements are not likely to be met outside laboratory tissue culture.

**Host/vector system**

Cloning vectors: pUC and pCR vectors.  
Mammalian expression vectors: pCMV and pSV vectors.

The cloning vectors used for this study will be pUC or pCR vectors. They contain a T7 promoter/priming site as well as f1 and co/E1 origin of replication. They also contain genes that confer resistance to the antibiotics Kanamycin and Ampicillin. They are not known to cause harm to humans. If cloning vectors escaped into environment, their antibiotic resistance gene may potentially be taken up by bacteria making them less susceptible to the antibiotics.

The expression vectors used will be pCMV and pSV vectors. They contain promoters/replication origin sites from cytomegalovirus and SV40 respectively, which are needed to maintain plasmids in mammalian cells and transcribe the inserted genes. They also contain genes that confer resistance to Kanamycin and Ampicillin to allow selection in E. coli and to Gentamicin or Puromycin for selection in mammalian cells. They are potentially able to replicate in mammalian cells and cause the cells to become resistant to the specific antibiotics. They may potentially cause drug resistance if they are taken up by and maintained in bacteria.

Two full-length and full-length plus constructs will be produced for each sample in cloning vectors (pUC or pCR) (Figure 3).

The first two products, pF1 and pF2, corresponding to the full-length 3.2kb HBV DNA genome, are produced with two sets of PCR primers targeting different sites of the HBV genome. The third construct, pHB-1, contains full-length HBV DNA plus a partially repeated sequence (“full-length plus”). Only this full-length plus plasmid is capable in vitro of producing all the viral proteins and RNAs required for assembly of HBV virions.

Individual viral genes (i.e. surface antigen, e antigen, core antigen, x protein, polymerase) will be subcloned into expression vectors. The proteins will be expressed in cell lines.

Some of the products will be modified by SDM.

HBV production does not cause major pathogenic effects on the cells. If samples are not handled appropriately, virus proteins may potentially cause immune reactions.

All plasmid production and purification will occur in the containment level 2 molecular laboratory. All bacterial cultures will be grown in plastic flasks and laboratory health and safety procedures will be followed. Plasmids will be stored in clearly labelled boxes at -20°C.

**Origin & function**

**Hepatitis B Virus**

The cloned full-length plus HBV genome will be capable of expressing the proteins and the nucleic acid required for the assembly of HBV particles. The individual gene clones will be able to produce individual proteins, such as HBsAg, e antigen, core antigen, polymerase, x protein. Samples may harbour mutations in certain regions of the genome, either occurring in vivo in the clinical sample under study or introduced SDM. Some of the mutations are expected to modify the susceptibility of HBV to antiviral drugs or immune responses, while others are expected to modify the levels of HBsAg expression and recognition. Some mutations are expected to affect virus fitness i.e. the virus ability to replicate and infect. The mutations that will be studied are those found to occur naturally in infected individuals. In SDM experiments involving the pol gene, it is anticipated that mutations will also indirectly be introduced to the S gene due to overlapping structure of the ORFs.
The likelihood of adverse events after accidental exposure is estimated to be low.

Accidental transfer may occur through percutaneous exposure (e.g., sharp injuries) or membrane exposure (e.g., splash to the eyes) of infectious material. HBV virus particles are quite stable and remain viable for 7 or more days on environmental surfaces at room temperature. The full-length plus HBV sequences and HBV particles are potentially harmful to humans. The outcomes may include acute infection resulting in hepatitis; in a subset of individuals a chronic carrier state may be established. Over several years, a subset of chronically infected individuals will develop complications such as liver cirrhosis and hepatocellular carcinoma.

A number of factors attenuate the risk of adverse outcomes.

Production of infectious HBV by the in vitro system described in this proposal has proved difficult. To date only non-productive infections have been achieved. However, transfection of full-length plus genomes has been shown to produce virions capable of infecting transgenic mice. Thus the possibility of human infection should be taken into account. Clinical samples from HBV infected patients represent a further and more significant potential source of infection.

In the healthcare setting we are very familiar with the management of occupational exposure to HBV and clear protocols are in place. These include minimising the risk of accidental exposure through good laboratory practice (e.g., no sharps, use of personal protective equipment, prompt disinfection of spillages), pre-exposure HBV vaccination, and post-exposure HBV prophylaxis. All people performing the laboratory work have been vaccinated against HBV as per national regulations. Should an exposure occur the laboratory worker has received instructions on how to manage exposed sites by careful washing and first aid kits are available in the laboratory for this purpose. The laboratory worker will then contact the responsible virology consultant immediately (or deputy) for deciding upon post-exposure prophylaxis. This may include further HBV vaccine dose, HBV Immunoglobulin or antiviral therapy as deemed appropriate by the consultant, who will take into account the circumstances of the exposure, the source of the exposure, and the vaccination history of the recipient to guide the decision. Should infection occur despite these pre- and post-exposure interventions, acute hepatitis may result which may require clinical management but very rarely will result in a risk of mortality. Pre-exposure vaccination and post-exposure prophylaxis, even in the unlikely scenario that they are not sufficient to prevent infection, will attenuate the risk of severe acute hepatitis. In immune competent adults, acute hepatitis B rarely (<1-5%) results in a chronic HBV infection, and the risk is further reduced by the use of pre-exposure vaccination and post-exposure prophylaxis.

Thus, the overall risk of exposure to infectious material is considered to be low and the clinical risk associated to an exposure is considered to be minimal.

As a further potential hazard, individual proteins may cause immune reactions and trigger antibody production.

The full-length plus sequences and virus particles are unlikely to cause harm to species in the environment as HBV has a narrow host range which is limited to humans and chimpanzees; in these species it may cause acute and chronic hepatitis. Humans are the main natural host for HBV. As noted above, HBV virus particles are quite stable in the environment and remain viable for 7 or more days on environmental surfaces at room temperature. The individual proteins have the capacity to cause immune reactions if accidently transferred to species in the environment.

These risks will be addressed by HBV vaccination of laboratory workers as per national guidelines, good laboratory practice (e.g., no sharps; use of personal protective equipment including laboratory coats, eye protection and gloved, and prompt disinfection of spillages with either 5% Trigene or 70% ethanol and proper disposal of waste). These procedures are covered in the laboratory Health & safety manual and Standard Operating Procedure (SOP). HBV particles produced in vitro as part of this study will be maintained in the containment level 3 laboratory. It should be noted that the HBV constructs have no or limited ability to establish productive infections (see above).

Evaluation of foreseeable effects

The HBV sequences to be used in this study will be derived from plasma and serum of HBV infected patients. The donor organism is pathogenic if not handled appropriately and may cause infection as described above.

The risk of infection and related adverse clinical events is considered to be low.

The HBV sequences that we aim to amplify and express are the cause of HBV infection and mediate disease pathogenesis.
The full-length plus HBV clone is capable of producing virions when expressed in HepG2 cells; therefore the most hazardous step is transfection of the construct into the cell line and transduction with HBV viral supernatants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste in the Department of Virology is disposed of by autoclaving (Standard autoclave cycle is 135 degrees C, 3150 mBar, 10 minutes) Liquid waste is collected in glass waste bottles and autoclaved. Solid waste is placed in double autoclave bags, autoclaved and then disposed of through approved routes. Details of these procedures can be obtained in the department Safety Manual. Spillages should be absorbed onto paper towels which will be autoclaved and the surface should be decontaminated and cleaned with 70% ethanol.

Waste from the CL3 laboratory (GF478) is additionally treated with the following measures. Virus, infected cells, pipette tips, flasks and all other plastics used will be inactivated in 10% Trigene overnight in disposable jars. Liquid waste, in 105% trigene, is solidified with vernagel prior to removal from the CL3 laboratory. Sealed containers and disinfected bags are placed inside metal tins which will be closed and disinfected prior to transport to the autoclave. The autoclaving conditions are checked with 12 thermocouples. The expected degree of kill is 100%

1. Liquid waste including viruses, infected cells, pipette tips, flasks and all other plastics used will be inactivated by Trigene 5% followed by autoclaving.

Bacterial culture liquid waste and contaminated plastics will be autoclaved. Bacterial cultures will be grown in disposable plastic flasks that will be autoclaved after use.

2. The degree of kill for these procedures is 100%

3. The autoclaving conditions follow those applied to the NHS diagnostic guidelines according to the requirements of Clinical Pathologic Accreditation. Standard autoclave cycle is 135 degrees C, 3150 mBar, 10 minutes. The autoclaving conditions are checked with 12 thermocouples.

4. Sealed and disinfected bags placed inside metal tins which will be closed and disinfected prior to transport to the autoclave.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

In consultation with virology experts at the Royal Free, the GMSC approved this work as an Activity Class 3 project.

Project Containment

02/03/2022
Project Additional Information

One of the barriers to studying neurological diseases is the inaccessibility of diseased tissue for study. One way to circumvent this is to take fibroblasts from patients, and reprogram them to take on an induced pluripotent stem (iPS) cell state, where they behave like embryonic stem cells, and can be directed to differentiate into cells of interest.

We are aiming to use this technique to study inherited neurologic diseases including Parkinson's disease, Alzheimer's disease and frontotemporal dementia amongst others, and we have collections of fibroblast cell lines derived by us from skin biopsies taken from individuals with these diseases.

The next step would be to carry out reprogramming to iPS cells, as described in Takahashi and Yamanak, Cell 2006;131:861-872) which requires the use of retrovirus bearing four transcription factors (Oct4, Sox2, c-Myc and Klf4) to transduce the target fibroblasts. This drives expression of these four genes at a level similar to that seen
in embryonic stem cells, leading to development of an iPS cell phenotype in a small proportion (<1%) of target cells.
This is an essential step before these IPS cells are used to generate cells of interest (neurons and glia) for disease modelling purposes. GMM approval is required for us to carry out the iPS cell generation step of this work.

Recipient or parental organism
The recipient organisms will be replication-deficient amphotrophic retroviruses, platinum A viral packaging cells and human primary fibroblasts cells. The viral particles that will be produced are replication deficient but capable of infecting individual mammalian cells. The viral particles contain genes that will be expressed in the transduced cells including the proto-oncogene c-Myc.

Host/vector system
The vector used will be the PMXs-GW retroviral vector from Cell Biolabs which is based on the Moloney murine leukemia virus (MMLV). The vector backbone contains the psi packaging signal, transcription and processing elements. Replication machinery (e.g. gag, pol and env genes) have been removed. Each of the four genes used in this study have been cloned in to this backbone with the Gateway cloning system (Invitrogen). The pMXs vector backbone with inserted gene is itself not harmful to health or the environment, however the resultant viral particles (formed following transfection of the vectors into Platinum A packaging cells) have the potential to be harmful as described herein.

Origin & function
The genetic material is cloned cDNA from human and mouse tissues. These cDNAs will encode the human and mouse transcription factors c-Myc, Sox2, Klf4 and Oct4. When target cells (patient fibroblasts) are transduced with the combination of four genes (Oct4, Sox2, c-Myc and Klf4), the expectation is that each of these genes will be transcribed at a relatively high level (much higher than endogenous activity in fibroblasts, comparable instead to the level seen in embryonic stem cells) to drive reprogramming to an induced pluripotent stem (iPS) cell state (Takahashi and Yamanaka Cell 2006;131: 861-872). As c-Myc is a protooncogene, prolonged expression has oncogenic potential. Therefore, retroviral particles containing c-Myc sequence could be harmful if accidentally transferred to humans. However, the infected cells (patient fibroblasts) would not themselves be hazardous, because the viral particles are replication-incompetent.

Evaluation of foreseeable effects
The most hazardous step in this procedure is the generation of replication-incompetent retrovirus containing c-Myc, a protooncogene, with the capability of infecting human cells. If accidentally transferred to a worker's tissue through, for example, a needlestick injury, there is oncogenic potential. There is a theoretical risk of recombination of the replication-incompetent vectors with replication-competent retroviruses in the patient samples. This risk is minimal, as the patient cohort recruited into this study is highly unlikely to harbour HIV or HTLV infection due to their age and immune status. Any known HIV or HTLV positive patients would be excluded from recruitment into this study. Further to this, the derived fibroblast cells are CD1 negative, making infection and/or propagation of active HIV or HTLV possible but of low efficiency.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All plasticware, media and disposable waste will be treated with 10% trigene for a minimum of eight hours and then autoclaved prior to disposal as clinical waste. Laboratory surfaces will be treated with 10% trigene and 70% ethanol and cell culture hoods will be UV radiated after use. Trigene has a 100% kill rate against both viral particles and also the recipient cells (from manufacturers instructions). The activity of trigene can be monitored by changes in the colour of cell culture medium associated with a change in pH. Disinfected and autoclaved solid waste will be removed in clinical waste bags for incineration.
The UCL GSMC approved this project without any amendment at its meeting on 17th February 2021.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
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<tr>
<td>L2</td>
<td>Yes</td>
<td>L3</td>
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</table>

Animal Units

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Project Ref 14/11.3

Date Ackn'd 15/06/2011

Date Project Ceased 12/02/2014

Regulation of self-renewal and differentiation in normal and tumorigenic neural stem cells

Class 2

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Transferred to University of Edinburgh 12/02/2014

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

The Open Biosystems library contains over 70000 individual E. Coli glycerol stocks containin the pGIPZ lentiviral vector or pSM2 retroviral vector. Each stock contains a unique hairpin sequence within the vector designed to target and reduce the expression of the majority of genes within the human genomes via RNA interference (RNAi).

The project will involve lentiviral or retroviral knockdown vector maintenance, storage and use of Open Biosystems shRNA library, including lentiviral mammalian expression plasmids, as well as distribution of clones as bacterial stocks DNA plasmids and or lentivirus to members within University College London for use in various projects.

Specifically, these constructs will be used to identify the key functional regulators of normal and tumour-derived neural stem cell lines.

Recipient or parental organism

Cell lines: a panel of mouse and human cell lines derived from mouse or human foetal nervous system and/or adult tissues or tumour biopsies.

Primary cell cultures: derived from mouse or human foetal nervous system and/or adult brain tissues or tumour biopsies.

Established cell lines: 293T (for viral preparations)

Bacterial E.coli strains: HB101, XL1-blue, TOP10, Prime Plus, JM109, BL21, DH5alpha, SURE, KS1000, DE3

Host/vector system

Lentiviral vectors: commercial TZV vector from Tranzyme and derivatives (including pGIPZ).

Retroviral vectors: commercial retroviral hairpin pSM (pSHAG-MAGIC-2) vector from Open Biosystems.

The viral vectors are lentiviral vectors. The vectors contain the CMV (Pol II) promoter to drive expression of the hairpin cassette or gene sequence. The lentiviral vector does not contain the appropriate accessory elements to form a functioning viral particle. Therefore it poses no threat to health or the environment. Packaging and envelope proteins are contained on two additional separate plasmids which are required in combination to produce viral particles. This separation of genetic elements essential for full viral functioning provides an addition safety measure. There shRNA cassettes are designed to target specific genes and will result in their knockdown.

Origin & function

The RNAi hairpins are sequences that have been derived de novo and are not from an organism. They have been designed as short sequences with both a sense and antisense and loop sequence. This results in formation of a RNA hairpin following transcription which in turn will trigger the appropriate RNA interference response (RNAi). The accessory protein derive from the HIV-1 virus.

Evaluation of foreseeable effects

The hairpin sequence will be transcribed following delivery into the host cells. This hairpin structure will trigger a normal cellular response pathway that results in the target sequence being suppressed in its translation or stability - this is known as the RNA interference pathway. The lentivirus and retrovirus can only infect mammalian cells. The target sequence of the shRNA is designed specifically for human sequences. There is a small risk that 'off target' sequences might be hit, including other mammalian species.

As the sequences are designed to target human genes there is the possibility that if the vector was delivered by accidental transfer to human cells it would result in gene...
If the resulting downregulated gene was a gene such as tumour suppressor this might result in aberrant regulation of cell proliferation pathways. However, the virus is replication incompetent and therefore would not spread beyond the initial cell targeted. Genetically normal human cells which received this vector would undergo a process of programmed cell death. Physical insertion of the vector into the host genome might also disrupt critical gene functioning. However, the likelihood of this is extremely low, and similarly cells would trigger an endogenous cell death pathway. Thus, the ability of the virus to be accidentally transferred to human cells is extremely low, there is no possibility for the virus to spread and even if inserted the cells would be removed rapidly as part of their normal cellular response. If virus is introduced into the body by injection, ingestion or through a wound the virus could potentially enter cells and integrate into the genome. Although the virus is replication incompetent (does not carry the packaging protein) its physical insertion into cellular DNA could result in an oncogenic mutation. This would be a very unlikely event and if so only a very small number of cells would be affected. It would not impact on the pathogenic outcome of an exogenous viral infection. The packaging viral components are from HIV-1 virus. The full length HIV-1 virus is harmful to humans, however the packaging vector used does not contain full length HIV-1 molecule. Many components of HIV-1 that are critical for HIV-1 infection but not required for viral packaging have been removed. So the virus is attenuated and replication incompetent and will not make new viral progeny.

The most dangerous GMM is the lentiviral and retroviral preparations of viral particles. The most dangerous step is the collection, concentration and use of retrovirus/lentivirus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- All work will be carried out in level 2 containment facilities within Paul O’Gorman Building. Stably modified human cancer cell lines will ne maintained using level 2 containment facilities.
- All staff involved in the project will be made aware of the associated risks described here and will be required to have appropriate safety training prior to beginning work on the project.
- Workers will be required to wear lab coats as well as latex or nitrile gloves whilst working on all procedures. Any open wounds will be covered with a bandage also.
- All solid waste and any spills will be treated with Virkon and/or trigene advance, both of which are certified to kill 100% of virus, bacteria and cells.
- After treatment, all solid waste will be double-bagged, autoclaved, placed in yellow biohazard safety bags and further treated by UCL waste services.
- All viral supernatant will be stored in double containment and transferred within the laboratory within doubly sealed containers.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- 2-5% Trigene advance or Virkon (v/v) will be used to treat liquid waste (18 hrs) and also used to decontaminate any spills and for disinfection of any reusable bottles or other equipment and laboratory materials used. Liquid waste is subsequently poured down the sink after treatment with Virkon. Work surfaces will be wiped down with 5% Trigene advance and 70% ethanol after use. Virkon and Trigene advance are certified by the PHLS to provide 100% viridal and GMO kill under these conditions be denaturing and inactivating viral and cellular proteins (Degree of kills: 100%). Trigene advance and Virkon will be used with its lifespan to ensure required kill is achieved. Solid waste will be double-bagged in biological waste bags, sealed and autoclaved 132C for 15 min by trained staff then bagged in yellow biohazard bags and removed by UCL waste services. Autoclave runs are regularly validated by waste management staff.

**Is an emergency plan required according to regulation 20?**

- No

**If yes, tick to confirm that it is attached to this form**

- No
The project was approved by the GMSC, subject to minor changes, as an Activity Class 2 project.

Project Containment

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<td>Large Scale Activities</td>
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Project Ref 14/11.4

Date Ackn'd 10/08/2011

Date Project Ceased

Project notified under transitional arrangements N

Development of gene transfer vectors as therapeutics and biosensors: injection of vectors into mice

Class 2 CultureVolClass2 CultureVolumeClass3-4

< 1 Litre Consent Granted

Non-GMM

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Our understanding and application of gene transfer vectors has advanced tremendously in recent years however further optimisations are still required to ensure that this technology can be of maximum benefit to future therapy clinical protocols. In addition, these vectors are also showing great promise for generation of somatic transgenic mice. For development and improvement of gene transfer vectors, the efficacy of these complex entities is poorly modelled by in vitro systems. In contrast, in vivo mouse models provide an excellent platform.

Gene transfer technology has improved dramatically over the past decade and there have now been several clinical trials for gene therapy where therapeutic benefit has been reported. Therefore there is substantial impetus to translate basic gene therapy research into clinical application and preclinical models are an indispensable and valuable step towards achieving this goal.

A variety of disease-specific transgenes will be packaged into various different gene therapy delivery agents (vectors), which will then be tested for their efficacy in either normal outbred mice or the appropriate transgenic mouse model. The diseases studied include defects in the blood clotting cascade (e.g. haemophilia a and B, Factor VII deficiency), metabolic disorders (e.g. Gaucher's disease, Ornithine Transcarbamolyse deficiency) and lung disorders (e.g. Cystic Fibrosis). The gene therapy vectors will include adenoviral, adeno-associated viral (AAV) and lentiviral constructs.

In addition, we have discovered that gene transfer can be used to deliver luciferase under the control of signalling pathway-specific promoters. Therefore, the equivalent of luciferase-reporter mice can be generated easily and quickly without having to purchase and breed entire colonies. This technology is likely to significantly reduce the number of mice used to study disease pathways and we aim to explore the full potential of this technology (“photonic somatotransgenic biosensing”) across numerous disciplines, by using various disease model systems.

This project involves the construction of various viral backbones and so we will be using many combinations of promoter, transgene and viral backbone (e.g. retro/lentivirus, AAV, adenovirus). For example, we may wish to construct an adenoviral backbone containing the human factor X gene controlled by the liver-specific LP1 promoter. Alternatively, we may wish to construct a lentiviral backbone containing the human Protein C gene controlled by the ubiquitous CMV promoter. To keep this application as broad as possible it is not possible to provide exhaustive lists of all constructs. However, as all constructs being dealt with here are non-toxic DNA sequences they all carry the same risks and have the same health and safety considerations.

The production of the viral backbones will be carried out in Lab 103, Chenies Mews and is covered by another GM form (HC66/09; Development of gene delivery vectors for gene therapy). The preparation of all viruses will be stored in Lab 103, Chenies Mews and then taken to the animal unit (Malet Place, Central facility) when required. They will then be administered to mice by various routes of injection and/or instillation. It is the use of these viruses within the Malet Place animal facility that is covered by this application.

Recipient or parental organism

Recombinant adenoviruses:

All adenoviral vectors to be used are E1-deleted first generation adenoviral vectors based on the pJM17 system described by M and co-workers (1) All stocks are routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on cesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the mutation is complemented in trans. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome.

Recombinant adeno-associated viruses (AAV):

All AAV vectors used here are replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a plasmid containing the adenoviral serotype 5 genomic sequence to provide helper
function for AAV replication. Upon a cytopathic effect in the cells, AAV particles are harvested and purified by HPLC.

Recombinant retroviruses (including lenti- and spuma-viruses):

Replication deficient retroviral particles are generated in cell-lines following co-transfection of three different plasmids. These supply the self-inactivating transfer vector containing the insert gene and viral Long Terminal Repeats (LTR) (but lacking any expression of HIV genes); packaging and structural proteins, gag/pol; and envelope (env) in the multi-deleted packaging plasmid pCMVdeltaR8.74 and in the pMD.G2 plasmid (in the case of VSV-G). The env gene is taken from another virus (eg vesicular stomatitis virus G (VSV-G), baculovirus gp-64, filovirus glycoprotein or mouse leukaemia amphotrophic/ecotrophic envelopes) which result in lentiviral particles able to transduce a variety of cell types. The transfer vector also contains a poly-purine tract (cPPT) and the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) for increased efficiency of production. The vector produced is self-inactivating due to a deletion in the promoter/enhancer region of the 3' LTR, that gets copied onto the 5' LTR during transduction of the cell and prevents prouction of viral RNA. The only expression from the vectors is of the transgene cDNA, controlled by an internal promoter.

Recombinant baculoviruses:

Baculoviruses are a family of insect viruses that have recently been developed for use in gene therapy applications. They are produced with a co-transfection protocol in insect cells. As a result of their origin, baculoviruses have the ability to enter mammalian cells but do not have the molecular mechanism for replication i.e. they transduce but do not infect mammalian cells. Additionally, they do not cause any disease or pathogenesis in healthy humans. Their native gp64 envelope gives them a limited mammalian cell tropism but this can be expanded through pseudotyping with the VSVG envelope. The baculovirus used is part of the BacMam Technology range of products commercially available from Invitrogen™

Non-viral DNA constructs:

Mouse, rat, non-human primate and human cells, including ES cells.


The recipient organisms (viral vectors) are not harmful to humans. They are all attenuated versions of the wild-type viruses and so cannot replicate (details can be found in 1.13). If the virus became inserted into cellular DNA, it could result in a potentially oncogenic mutation but the risk for this happening using the amounts and volumes here are very remote.

All viral vectors of all type are replication incompetent so therefore cannot self-replicate and transfer to another host. They are therefore harmless to the environment.

The mice that will eventually receive the recipient organisms would not have any survival advantage in the environment. They could conceivably escape from the holding facility (although this is extremely unlikely) but even then they would not be able to transmit the viral construct to another organism and could also not transmit the DNA in the germ line to an offspring.

Host/vector system

Prokaryotic plasmids, eukaryotic expression vectors, replication-deficient self-inactivating (SIN) lentiviral vectors which are based on third generation lentiviral vectors (ie pCCLSIN) or second generation vectors (ie pHRSIN) with engineered safety features which are resembling third generation vectors in terms of safety. In addition, we are using Moloney murine leukemia virus (MoMuLV) derived vectors in (MSCV, Sfada/W, SRS11) lacking international non-proprietary names (INN), again representing a similar level of safety, when using inserts which are deemed harmless.

Origin & function
All inserted sequences are from either human, mouse, macaque, firefly or bacteria.

All gene products are either enzymes (e.g. Factor VIII, GBA), reporter genes (e.g. GFP), transmembrane proteins (e.g. CFTR) or siRNA hairpin sequences.

None of the sequences fall in to any harmful category such as concogenes and proto-oncogenes, growth factors and GF receptors, any other sequences coding for significant biological activity that might be harmful were these to be expressed in people accidentally exposed to the vector.

In the unlikely event that any of these inserted sequences were to be expressed in humans after accidental transfer, the cDNA sequences or the RNAi hairpins could lead to expression of functional proteins or to the down-regulation of the gene to which the RNAi hairpin it targets via RNAi interference respectively.

Expression of the inserted cDNAs is deemed to hold little risk due to the non-harmful nature of the sequences used and the number of cells that would be affected by accidental exposure. Accidental targeting of RNAi in humans is not deemed to be harmful either due to a combination of the function of the targets, no expected harmful action of other molecules when presence of lower amounts of the target, and the expected relatively low amount of cells involved.

In addition, for both types of sequence, the effect of accidental expression would not be severe due to the fact that all viruses used are replication deficient (see 1.13) and so no new virus particles can be produced so no other cells can be infected. It is possible that physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene, however, the likelihood of this is extremely low.

The risk to the species in the environment is even lower since they would only be exposed to much small amounts of the sequences.

**Evaluation of foreseeable effects**

The mammalian donor organisms are not harmful however some of the donated sequences are from viruses (e.g. the envelope proteins VSV-G from Vesticular Stomatitis virus and gp64 from baculovirus) some of which are infectious to humans.

The sequences used from viral donor organisms are either promoters/enhancers or envelope proteins so none of these are involved in the harmful characteristics of the original donor organism. Even if these viral sequences were introduced into the body by ingestion, through a wound or after spillage onto skin the viral sequences would not enter human cells and would therefore not be expressed. The sequences used from mammalian or bacterial donor organisms are the transgenes or RNAi hairpin sequences, none of which are pathogenic.

The most hazardous GMM is lentivirus. The most hazardous step is injecting the lentiviral constructs into mice due to the use of sharps.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Work surfaces and equipment are decontaminated on completion of work or at the end of the day by spraying with Trigene or 2% Virkon (as per manufacturer's instructions, has been shown to kill 99.9995% of organisms in under 10 minutes). Spills and splashes are mopped up with Wypall and then the area thoroughly sprayed with Trigene or 2% Virkon.

Solid waste, such as tips, eppendorfs or syringes containing lentiviral constructs, will be soaked in 2% Virkon overnight before disposal in a clinical waste sharps bin. Liquid waste will be treated in the same way before disposal down the drain (very small volumes of lentivector are treated in this way, <1 ml). Surgical equipment (e.g. scissors, forceps etc), whilst not in direct contact with any GMM, will also be sprayed with Trigene or 2% Virkon. Once disinfected, the instruments are then cleaned in hot water.
using a fine bristled brush.

The UCL GMSC approved the project as Activity Class 2 with regard to the procedures involving injection of lentivirus into animals with the remainder of the project being Activity Class 1.

Project Containment

<table>
<thead>
<tr>
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Project Ref 14/12.1

Date Ackn'd 07/02/2012
Date Project Ceased

CU2 Project Title Bacterial affects on connexion in chronic wounds

Class 2
CultureVolClass2 CultureVolumeClass3-4
Consent Granted

Tick if notifying a connected programme of work N

Historical Significant Changes

Page 742 of 1532
Chronic wounds are typified by slow, poor healing. All open wounds are colonized with bacteria and it is increasingly being recognized that bacteria can inhibit wound healing without eliciting clinical signs of infection. Biofilms, organized communities of bacteria encased within an extracellular polysaccharide matrix, are receiving increasing interest within wounds, as they are thought to be integral to the resistance bacteria within chronic wounds have to antibiotics and host immunity. Furthermore the biofilm acts as a reservoir, releasing planktonic bacteria into the wound. Research has shown that biofilms of Staphylococcus aureus and epidermidis, two bacteria frequently found in infected wounds, retard re-epithelialization within wounds. Thus due to the inability of the host immune response and current clinical methods to effectively destroy biofilms, local infections can become invasive. The escalating emergence of antibiotic resistant bacterial strains also presents a worrying problem for the healing of chronic wounds and patient morbidity.

Connexin dynamics are increasingly being recognized as being important in the wound healing process. Connexins are hexameric transmembrane proteins that form channels within the plasma membrane, called connexions. These connect with a connexion in an adjacent cell to allow gap junction intercellular communication (GJIC), whereby ions and small molecules can pass freely between the cytoplasms of interacting cells. It is becoming increasingly evident that GJIC is integral in many coordinated cellular activities, and wound healing is no exception. Gap junctions are involved in coordinating keratinocyte proliferation and migration during re-epithelialization, and they are implicated in the immune response in the regulation of leukocyte recruitment and activation. In normal healing wounds, gap junction dynamics are altered within the epidermis in response to wounding, and only return to normal following complete reconnexion which is normally switched off at the epidermal wound edge, showing persistent elevated levels of expression. It is thought that Cx43 expression is required to be turned off in order for keratinocytes to migrate, thus high levels are associated with a lack of re-epithelialization. Preliminary studies within this lab have also shown that bacterial wall components increase Cx43 expression between fibroblasts, which need to migrate into the wound bed in order to rebuild the extracellular matrix. Thus the relationship between bacteria and connexions within wound healing is intriguing, and further understanding may improve our ability to treat chronic wounds.

Through this project we aim to investigate the effects if different bacterial components and biofilms on connexion dynamics and communication, and what affect they have on the healing process. We intend to research the mechanisms behind bacteria induced retarded wound healing and whether this can be reversed or prevented by manipulating connexion dynamics. We will transform specific bacterial strains to contain plasmids encoding fluorescent proteins. These GMOs will make identification of bacterial location and species quick and easy. It will also make it possible to observe the spread of bacteria in situ, within cell culture or an inoculated wound, without necessitating swabbing and sub culturing the bacteria.

Recipient or parental organism

- Staphylococcus aureus ATCC 29213
- Pseudomonas aeroginosa ATCC 27853
- Bacteriodes fragilis ATCC 43935/6
- Escherichia coli ATCC 25922
- Klebsiella pneumonia ATCC 13883

Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli and Bacteriodes fragilis is an obligate anaerobe and is unable to survive in the environment. Escherichia coli can survive in the environment for a few hours to days. Staphylococcus aureus is part of the normal skin flora and is able to survive on a new host. It is also able to survive in the environment for a few days to weeks. Klebsiella pneumonia and Pseudomonas aeruginosa are part of the normal human skin flora but both can also survive in the environment.
The vectors will be plasmids containing antibiotic resistance, an origin of replication and promoter for the host organism. They will actively express functional fluorescent protein in the bacteria. The antibiotic resistant proteins will also be expressed.

No plasmids conveying resistance to an antibiotic currently used to treat individuals infected with these bacteria will be used.

**Origin & function**

The genetic material encodes fluorescent proteins, modified from Aequorea victoria, which is a non-pathogenic organism. The translated protein from the inserted genes will fluoresce when excited by the correct wavelength of light. There will also be an antibiotic resistance gene that conveys antibiotic resistance to any of the bacteria taking up the plasmid. The sequences can cause harm if expressed in humans as the vectors don't contain a mammalian origin of replication. The sequence won't cause harm if transferred to other species in the environment as the species will just fluoresce.

**Evaluation of foreseeable effects**

All GMOs will be essentially the same as the Hazard Group 2 parental agent from which they are derived but will fluoresce and gain antibiotic resistance to one type of antibiotic. The most potentially hazardous GMOs will be the modified Pseudomonas aeruginosa and Klebsiella pneumoniae as they are capable of surviving in the environment. However, these organisms are found naturally in our environment and part of our skin flora, and all precautions will be taken to prevent their release.

The most potentially hazardous step in this procedure is probably transportation of the bacteria between location, but this should be minimal as they will be transported in double sealed containers to prevent any seepage, should, in the unlikely circumstances, a leak occur.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated liquids will be decontaminated in 1% Virkon and autoclaved. Solid waste will be autoclaved. Bacteria within animal wounds will be killed by fixing with 4% formaldehyde. The expected degree of kill is 100%

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The Biological Safety Adviser approved this on behalf of the GMSC, subject to clarification concerning the information that will be given to staff working on the project - although the agents are classified as Hazard Group 2 and do not cause severe disease in healthy humans, they can cause severe disease in those whose immune system is compromised in some way. It was confirmed that it will be made clear to those involved in any aspect of the project, or sharing facilities where the HG2 agents are handled, that the bacteria can cause disease and that immune-compromised individuals are at greater risk.
## Project Additional Information

**Purposes of the contained use**

The main project covered under this application aims to use lentiviral vectors for targeted delivery of lung therapies for both inflammation and cancer.

Our initial approach would be to use lentiviral vectors to transduce bone marrow derived cells (such as macrophages and mesenchymal stem cells) with targeted gene therapies for lung injury and cancer. Genes encoding anti-cancer or anti-inflammatory proteins (for example Keratinocyte growth factor (KGF) and TNF-related apoptosis inducing ligand (TRAIL)) would be used to transduce human bone marrow derived cells to produce modified cells that would be delivered as anti-cancer and anti-fibrotic therapies. Alongside the therapeutic gene controls for tracking and imaging cell behaviour and location (such as fluorescent reporter proteins and luciferase) would also be inserted into the lentiviral vector. Expression of these genes is controlled by the administration of antibiotics (doxycycline).
Recipient or parental organism

GMM/1: Lines 1.13, 1.19, 1.20
Lentivirus: 3rd and 4th generation vector and generation FIV vector with virulence genes deleted, on split plasmids

Cells: standard laboratory cell lines (e.g. 293T, HeLa, A549 and other immortalised cancer cell lines), mouse macrophages cell line IC-21, primary human lung cells and both human and murine bone marrow-derived cells.

The viral vectors themselves are not pathogenic to humans as all virulence genes have been deleted from the original parent viral sequences. However, these vectors have the potential to integrate into human DNA and there is a low risk that infection could damage the health of the laboratory worker directly handling the viruses.

The use of self-inactivating (SIN) vectors to make viral particles has been shown to significantly reduce the risk of insertional mutagenesis. In addition lentiviral vectors have been shown to insert preferentially into coding regions of DNA, rather than the more risky promoter or control regions. Overall, when taking into account these factors the risk to humans are very low and there are no known cases to date where a replication competent virus has been produced.

The vector particles are produced with the Vesicular Stomatitis Virus envelope protein, VSV-G, conferring a broad host specificity. While this may increase the susceptibility of different cells to the virus, it does not increase the risk of insertional mutagenesis.

A WPRE element is included in these vectors to aid translation of the inserted gene. This encodes a putatively oncogenic protein (protein X) which has been mutated in all vectors and is not functional.

These vectors are replication deficient and undergo a single infection of target cells so there is no risk of spread to other recipients. The vectors will only be used as gene delivery systems and transduced cells will not produce viral particles.

Host/vector system

GMM71: lines 1.12, 1.24

Vectors are standard microbiology plasmids and all cloning is performed using commercial disabled bacterial strains which are not harmful to health or the environment. The promoters used for gene expression in lentiviral vectors are not expressed in bacteria.

Origin & function

GMM/1: lines 1.11 - 1.16, 1.17 -1.18
Sequences come from the bacteria DH5α, DH10B and other common disabled E. coli K12 derivatives. All bacterial strains used are disabled.

The inserted sequences may be amplified originally from mouse or human genomic DNA or cDNA before cloning into bacteria.

Keratinocyte growth factor (KGF) is an epithelial mitogen found in many organs, which plays a role in epithelial repair in response to injury. For this project we aim to target KGF expression to the lung parenchyma where it can exert effects on type 2 alveolar epithelial cells. Its expected effects include increased proliferation and reduced apoptosis, increased fluid and electrolyte transport and increased surfactant production.

TRAIL is a transmembrane protein that is bound to circulating immune cells within the body. It has a role in immune surveillance against cancer and virally infected cells by inducing apoptosis or target cells. Whilst inducing apoptosis in cancer cells it has no known effect on healthy cells. We aim to deliver TRAIL to the site of cancer and induce
cancer cell apoptosis.

Luciferase is a naturally occurring oxidative enzyme that catalyses the conversion of luciferin to oxyluciferin and light. It is not known to cause transformation of the target cells and will be used to monitor cell activity and location.

A WPRE element is included in the vector plasmid to aid translation to the inserted gene or sequence. This encodes a putatively oncogenic protein (protein X) which has been mutated in all vectors and is not functional.

**Evaluation of foreseeable effects**

GMM/1: lines 1.17, 1.18, 1.32

The viral vectors themselves are not pathogenic to humans as all virulence genes have been deleted from the original parent viral sequences. However, these vector share the potential to integrate into human DNA and there is a low risk that infection could damage the health of the laboratory worker directly handling the viruses. The use of self-inactivating (SIN) vectors to make viral particles has been shown to significantly reduce the risk of insertional mutagenesis. In addition lentiviral vectors have been shown to insert preferentially into coding regions of DNA, rather than the more risky promoter or control regions. Overall, when taking into account these factors the risk to humans are very low and there are no known cases to date where a replication competent virus has been produced.

The vector particles are produced with the Vesicular Stomatitis Virus envelope protein, VSV-G, conferring a broad host specificity. While this may increase the susceptibility of different cells to the virus, it does not increase the risk of insertional mutagenesis.

A WPRE element is included in these vectors to aid translation of the inserted gene. This encodes a putatively oncogenic protein (protein X) which has been mutated in all vectors and is not functional.

The most hazardous step and the only risk that we identify at the moment is direct delivery of the viral particles into mice.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMM/1: line 1.29 or GMM/2: lines 2.13 (as applicable)

For routine cleaning, all surfaces will be wiped down with 70% alcohol after use which is known to be effective against enveloped viruses. Liquid GMM waste will first be disinfected using 2% Virkon before being disposed of down the sink. This is a standard procedure and viability is below detectable levels. Solid GMM waste will be placed in autoclave bag and autoclaved at 121° for 15 minutes.

It is not possible to estimate a "degree of kill" lentivirus. However, autoclaving is sufficient for a 100% inactivation of enveloped viruses.

For administration of lentivirus to mice pipette tips and needles will be disposed of in dedicated sharps bins (without dissociation from the attached syringe). The sharps bins will be placed in autoclave bags and autoclaved as described above. Mice will be housed in individually ventilated cages.

In the event of spills, 70% ethanol will be used for surface disinfection. This agent is effective and is widely used for inactivating enveloped viruses. Absorbent tissues will be used to mop the affected area and will be disposed of by autoclaving as described above.
This was originally assessed as activity class 1 but after discussion at the UCL GMSC was revised as activity class 2.

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<table>
<thead>
<tr>
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**Project Ref** 14/12.11

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<td>Project notified under transitional arrangements</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
### Project Additional Information

#### Purposes of the contained use

Throughout life, cells follow instructions encoded in our DNA that tell them what type of cell to be, for example brain cells or muscle cells. Cells also follow these instructions in adults when tissues are repaired or when new cells are needed to fight infection. The instructions tell cells when to divide and when to stop and disruptions in how the instructions are read can lead to cancer and other diseases.

We have discovered a new class of RNA molecule that is produced from parts of our DNA that contains these instructions. The molecules appear to be part of the mechanism used to read the instructions because they interact with proteins that control which instructions are read. Evidence suggests that the molecules are also turned off in cancers, which would prevent the instructions from being followed correctly.

We would like to find out more about the new class of molecule and how they work. This will provide information about how cells follow instructions about what type of cell to be and how this goes wrong in cancer. This information might also be useful to generate specific cell types for treating disease and injuries. Generation of GMOs is required for this work in order to test for the function of the RNAs and to produce the proteins that are involved in this process and to test for the activity of such proteins in the cell.

#### Recipient or parental organism

Standard E. coli strains XL1-blue, TOP10, HB101, DH5alpha, SURE, JM109, BL21. Also DH10Bac™, that contains a baculovirus shuttle vector (bacmid bMON14272) and a helper plasmid (pMON7124). All of these strains are disabled and unable to survive in the environment outside the lab. No hazard to human health or the environment.

Virus genome expression vectors: LNCX, CNCG, pSIREN-RetroQ, pMY-family (derived from murine leukaemia virus), pHRSINcSGW/cSRW/cSPW (derived form lentiviruses). See information below.

Insect baculovirus (cannot replicate in mammalian cells). Encoded by bacmid bMON14272.

Human cancer cell lines, eg. HeLa, 293T, HT29, Jurkat, CEM, SupT1, C8166, U937, THP-1, KG-1, Human ES cell lines, eg. H1 and H9. Mouse cancer cell lines eg. NIH3T3. Wild-type mouse ES cell lines, eg E14, JMB, and lines containing various mutations or reporter constructs. Primary human cells derived from peripheral blood including lymphocytes and macrophages. Insect cell lines, eg. SF9. These cells can only survive under carefully controlled laboratory conditions.

#### Host/vector system

Vectors:
All the vectors (plasmids) will be transformed into E. coli (see above) for cloning and propagation of mammalian and virus sequences. Group 1 vectors (cloning vectors) will only be present in E. coli. Mammalian and virus sequences will also be transferred to baculovirus expression vectors (2) or mammalian expression vectors (3) and transfected into cell lines for expression. We will also transfer the sequences to virus expression vectors (4) to allow long term expression in human cells and infection of otherwise difficult to transfect cell types. Virus expression vectors are packed inside VSV-G pseudotyped viruses, made from gag-pol and VSV-G expression vectors transfected into human cells (5) Details of this retroviral vector system is outlined below.

1. Cloning and bacterial expression vectors: pCR-TOPO, pGEM-T, pENTR, pCR-Blunt, pCR2.1. Theses have bacterial promoters driving expression of antibiotic resistance and contains a promoter driving lacZ, into which the transgene is inserted. pGEX bacterial expression vectors - these contain bacterial promoters driving expression of the inserted nucleic acid sequence.

2. Baculovirus (insect) expression vectors: pFastBac and similar baculovirus vectors containing bacterial promoters driving expression of antibiotic resistance and a baculovirus polyhedron promoter driving expression of the inserted sequence, which is only active in the presence of insect baculovirus in insect cells.
3. Mammalian expression vectors: pcDNA3, pCR3.1, pIRES family, pTRE2 family, pYY1Luc, pSICheck-2 mammalian expression vectors. These contain CMV or actin or similar promoters used to drive the expression of transgenes in human cells. In pIRES vectors, the promoter also drives expression of genes encoding antibiotic resistance. In pYY1Luc, the promoter drives expression of luciferase.

4. Virus genome expression vectors: LNCX, CNCG, p SIREN-RetroQ, pMY-family (derived from murine leukaemia virus), pHR'SINcSGW/cSRW/cSPW (derived from lentiviruses). These contain internal promoters used to drive the expression of inserted transgenes in mammalian cells.


Details on retroviral vectors: Retroviral vectors are made by transfecting 3 plasmids; an expression plasmid into which the sequence of interest is cloned, together with a gag-pol expression vector and an envelope expression vector (which are not packaged into progeny viruses). All vectors are disabled by separating the packaging sequence, the envelope coding sequence and the viral coding sequences from each other on independent plasmids. Furthermore, deletion of the U3 of the long-terminal repeat (LTR) ensures that the HIV-derived pHR'SINcSGW vectors are self-inactivating and cannot replicate.

Retroviral vectors are produced by transfection of all 3 plasmids. These viral particles do not contain genome that encodes virus proteins and so this system cannot produce replication competent virus. VSV-G enveloped retroviruses are only potentially harmful to human health if introduced into the body by injection, ingestion or through a wound. The potential harm arises from the possibility the virus would enter cells and integrate itself into cellular DNA. Althouhg the virus is not replication competent (does not carry gag, pol or env genes) and so it cannot make new progeny virus and establish a productive infection, it's insertion into cellular DNA could result in a potentially oncogenic mutation. The virus vectors are of no risk to the environment. Viral particles are labile and do not survive on environmental surfaces outside the lab.

Origin & function

1. Nucelic acid sequences encoding mammalian proteins that act to modify gene transcription and chromatin structure in recipient mammalian cells. Mutant forms of these proteins will be made to identify the parts of the protein important for activity. Sequences encoding epitope tags may be added to facilitate protein identification.

2. Mammalian and viral sequences coding for RNAs that don’t code for proteins (non-coding RNAs). These RNAs may have regulatory functions in mammalian cells.

3. Mammalian and viral regulatory element DNA sequences that don’t code for proteins such as promoters, enhancers, polycomb response elements. These act to recruit mammalian transcription factors and chromatin regulatory proteins and may produce non-coding RNAs.

4 Artificially-designed and natural short hairpin RNAs for knocking down the expression of endogenous transcription factors and chromatin regulators.

None of these sequences are predicted to cause significant harm but it is possible that some proteins encoded in the vectors could be oncogenic, or that they could modulate the expression of oncogenes or tumour suppressor genes. They will have no effect on bacteria or viral vectors and will not cause harm if transferred to species in the environment.

Evaluation of foreseeable effects

None of the vectors are harmful to the environment and do not supply a survival advantage to any microorganisms. The cloning and expression vectors are also not a hazard to human health.

The bacterial strains to be used are non-colonising and incapable of surviving in the environment or causing human infection. They pose no risk to users or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

10% Trigene (Distel) (v/v) will be used to treat liquid waste (16 hours) and also any spills and for disinfection of any reusable bottles or other equipment. Liquid waste is poured down the drain after treatment with TriGene (Distel) for at least 16 hours. This leads to denaturation and inactivation of viral and cellular proteins and results in 100% kill of all GMO. TriGene (Distel) is certified by the PHLS to kill viruses. All TriGene (Distel) will be used within its 3-year life.

Solid waste is double bagged and secured with a cable tie. The waste is autoclaved by trained staff and then these bags transferred to a yellow tiger-stripe biohazard waste bag for off-site removal by UCL waste services.

Work surfaces will be wiped down with 70% ethanol after use. These procedures result in 100% kill of all GMO.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project was approved by the UCL GM Safety Committee following minor changes requested by the advisers

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Project Ref 14/12.12

Date Ackn'd 11/01/2013

CU2 Project Title Epigenetic regulation in the brain and paediatric brain tumours. Role of the histone variant H3.3 and its chaperone DAXX

Date Project 02/03/2022

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 ≤ 1 Litre

Non-GMM Consent Granted
Epigenetic regulation is emerging as a fundamental mechanism by which cells adapt their transcriptional response to developmental and environmental signals. Disruption of this process is believed to contribute to central nervous system pathogenesis, highlighted by the recent realization that most genes associated with mental retardation regulate chromatin structure. In addition to histone modifications and chromatin remodelling, chromatin structure variation is also generated by the deposition of variant histone proteins, with the help of dedicated chaperones. I recently showed that the chaperone DAXX mediates loading of the histone variant H3.3 at promoters and enhancers of selected genes and contributes to the modulation of their transcription. Moreover I showed that DAXX function is regulated by phosphorylation at a specific serine residue.

My experiments aimed at characterising the chromatin binding of DAXX and H3.3 deposition and their transcriptional regulation. I also aim to describe the role of DAXX and H3.3 in key developmental and functional processes of the brain. Overall this research addresses an exciting and yet unexplored area of epigenetics in the central nervous system and will highlight new factors involved in neuropathologies.

Brain tumours are currently the leading cause of cancer-related mortality and morbidity in children. Despite extensive research, complete understanding of the cause and the pathobiology of childhood brain tumours is still lacking. Interestingly the histone variant of H3.3 and its chaperones DAXX and ATRX have recently been found to be mutated in a variety of cancers and particularly in paediatric glioblastoma pathogenesis.

The involvement of DAXX phosphorylation in malignant models has never been studied before. Therefore I propose to study DAXX expression and phosphorylation in paediatric glioblastoma. I aim to define the role of DAXX phosphorylation in the deposition of H3.3 and highlight the pathways regulating DAXX phosphorylation and thus H3.3 loading. Overall this research has the potential to improve our knowledge in the regulation of a pathway newly identified as a driver of paediatric brain tumours and will potentially highlight new therapeutic targets.

Use of genetically, modified organisms will be essential for the completion of this research. Several constructs will be used to express mutant form of DAXX, histone H3.3 and other histone chaperones to understand their role in the central nervous system and in brain tumorigenesis. Specifically, this project will involve lentiviral production from either pGIPZ or TRIP-PGK-ATGm-MCS-WHV plasmids and subsequent use of this lentivirus to infect murine and human cells.

DH5 alpha bacteria, lentivirus, mouse cortical neurons, mouse neural progenitors, Human 293T cells, human glioblastoma cell lines. DH5 alpha bacterial strain cannot survive or attach to human intestinal epithelium.

Lentiviral particles. The envelope protein is VSV-G from the Vesticular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus
could potentially enter cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or VSV-G genes) so it cannot make new progeny virus and establish a productive infection, its physical insertion into cellular DNA could result in a potentially oncogenic mutation. This would be a very unlikely event and if so only a very small number of cells would be infected. Moreover six of the nine genes encoding HIV-1 virulence factors have been deleted from the derived vector system. The resulting multiply-attenuated design of HIV vectors ensures that the parental virus cannot be reconstituted. The recipient organisms for expression of exogenous proteins will be murine or human cells. These cells cannot survive in the environment nor transfer the inserted sequence.

Host/vector system

TRIP-PGK-ATGm-MCS-WHV (from Trono Lab), PGIPZ (from OpenBiosystem) or any second/third generation lentiviral expression vector. pMD.G (From Trono Lab) or any second/third generation packaging system. Viral envelope (VSV-G) expression vector. pCMVdeltaR8.91 (From Trono Lab) or any second/third generation packaging system. Gag and Pol expression vector. pcDNA3, pCMS-GFP, TOPO or any other commercially available backbone vectors commonly used for mammalian protein expression.

Origin & function

Mouse, human jellyfish, drosophila, Bacteriophage P1, HIV-1
DAXX and ATRX are both histone chaperones specific for H3.3.
H3.3 is a histone H3 variant that is expressed throughout the cell cycle.
Calcineurin is a calcium dependent phosphatase implied in cell signalling
PML is a signalling protein
HIPK1/2 are protein kinases
GFP is commonly used in cell biology to label cells.
Cre recombinase is tyrosine recombinase enzyme derived from the P1 Bacteriophage. It catalyses the site specific recombination event between two DNA recognition sites (loxP sites).
shRNA (small hairpin RNA) are small RNA molecules which form a hairpin structure. The hairpin structure will induce a normal cellular in vivo downstream sequence of processing events in response to foreign cellular single-stranded RNA intruder molecules that will result in the post-transcriptional down regulation of the gene to which the hairpin sequence is targeted.

DAXX, ATRX, H3.3, HIPK1/2 and PML have been showed to be differently modulated in human tumours therefore there are potentially harmful effects in the event of accidental transfer to human. Potential harm is oncogenic events. Therefore particular precaution will be used when producing lentiviral particles for expression or downregulation of these factors. The viral vector system used contains numerous safeguards. 1) The sequence encoding the various components are distributed over three independent plasmids to maximize the number of recombination events that would be required to recreate a replication competent virus. Thus far, no replication competent recombinants have been detected among a total of 1.4 x10^10 transducing units. 2) Six of the nine genes encoding HIV-1 virulence factors have been deleted from the derived vector system. The resulting multiply-attenuated design of HIV vectors ensures that the parental virus cannot be reconstituted.

VSV-G pseudotyped lentivirus can only infect mammalian cells. Potentially the lentiviruses could infect other mammalian species and lead to the expression or the down-regulation of the factors. This however, is extremely unlikely as the lentivirus has a very poor survival rate outside the growth conditions. Moreover, the virus is replication incompetent and therefore self-inactivating as none of the structural genes are actually present in the packaged viral genome so no new virus can be produced and no other cells can be infected with new particles.

Evaluation of foreseeable effects

DAXX, ATRX, H3.3, HIPK1/2 and PML have been showed to be differently modulated in human tumours therefore there are potentially harmful effects in the event of accidental transfer to human. However none of these factors have been showed to induced tumour formation when overexpressed or downregulated alone.

The most dangerous GMM, is the lentiviral preparations. The most dangerous steps are the collection, concentration and use of the lentivirus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animal or plant will be used
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| 1% Virkon (v/v) will be used to treat liquid waste (18 hours) and also used to decontaminate any spills and for disinfection of any reusable bottles or other equipment and laboratory materials used. Liquid waste is subsequently poured down the sink after treatment with Virkon. Work surfaces will be wiped down with 5% Trigene advance and 7-% ethanol after use. Virkon and Trigene advance are certified to kill 100% virus and bacteria under these conditions by denaturating and inactivating viral and cellular proteins. Trigene and Virkon will be used with its lifespan to ensure required kill is achieved. Solid waste will be doublebagged in biological waste bags, sealed and autoclaved 132°C for 15 mins by trained staff then bagged in yellow biohazard bags, and stored in a secured, sealed bin for removal by UCL waste services. Autoclave runs are regularly validated by waste management staff. |

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The UCL GMSC approved this project as Activity Class 2 subject to some minor clarifications regarding the nature of the inserted genetic material

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 14/12.2

Date Ackn'd 20/02/2012

CU2 Project Title Gene therapy for the treatment of Adenosine Deaminase Severe Combined

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4
Primary Objectives
To evaluate the safety, feasibility and tolerability of the introduction of autologous cells, transduced with lentiviral vectors encoding relevant transgene, to patients with either HIV-infection, ADA SCID, X-CGD and Netherton Syndrome.

Secondary objectives
A. Gene therapy for ADA SCVID and X-CGD
1. Transduction of CD34+ haematopoietic cells from patients be ex vivo lentivirus-mediated gene transfer
2. Infusion of transduced autologous CD34+ cells into myelosuppressed recipients
3. Evaluation of safety
4. Evaluation of engraftment kinetics and stability
5. Evaluation of biochemical and functional reconstitution in progeny of engrafted cells
6. Longitudinal evaluation of clinical effect in terms of augmented immunity against infections.

B. Gene Therapy for Netherton Syndrome
1. Generation of SPINK5 gene modified keratinocyte stem cells (KSC) from patients with NS
2. Generation of skin sheets for autografting using gene corrected KSC
3. Grafting of gene corrected skin sheets onto patients with Netherton Syndrome
4. Longitudinal evaluation of safety and efficacy clinical effect

C. Gene Therapy for HIV infection
1. Extent of engraftment and survival of gene-containing cells over time, assessed by PCR and flow cytometry
2. Change in plasma HIV viral load;
3. Change in CD4+ T lymphocyte counts;
4. Potential development of CXCR4 or mixed R5/X4 trophic HIV virus

Recipient or parental organism
● The lentiviral (LV) vectors are 3rd generation replication-defective hybrid viral particle made by core proteins derived from Human Immunodeficiency virus type 1 (HIV-1)
and the envelope of the unrelated Vesicular Stomatitis Virus (VSV)

- The transfer vector encodes the human gp91phox/ADA/SPINK 5/C46 gene cDNA under the control of non viral tissue specific promoters.

Replication deficient lentiviral vector is used to transduce autologous haematopoietic or skin cells ex vivo. The GMP grade lentiviral vector will not be directly administered to patients.

The lentiviral vector is not expected to survive in the environment and cause harm. The risk of recombination events between vector and wildtype HIV have not been detected to date. The risk remains theoretical (Cornetta et al. 2011, Molecular Therapy, Vol 19, no. 3, 557-566)

**Host/vector system**

The vector particles are pseudotyped with VSV-g and are produced by transient transfection of four separate plasmids into 293T cells using the calcium phosphate precipitation technique:
- A transfer plasmid encoding the transgenes.
- Three separate accessory plasmids encoding respectively HIV gag pol, VSV-G and HIV Rev which will respectively produce the structural proteins, nucleic-acid polymerases/integrase, reverse transcriptase, surface glycoprotein, and regulatory elements required for packaging a recombinant lentiviral particle.

The system is based on 4 non-overlapping expression constructs in order to maximize the segregation of cis and trans acting functions. The system is engineered in such a way that minimal homology regions are present between packaging and transfer vectors, thus minimizing the likelihood of homologous recombinational events and the generation of replication competent lentiviruses (RCLs).

In addition, the packaging construct is deleted of all HIV accessory proteins (vpu, vpr, nef, vif) and Tat. The conditional packaging system segregates gag/pol and rev genes in two separate plasmids. The Rev responsive element (RRE) maintained in the gag/pol plasmid makes the gag/pol gene expression rev dependent. As the transcripts of gag and pol genes contain cis acting repressor sequences, they are expressed only in the presence of Rev, expressed in trans on a separate plasmid, which promotes their nuclear export and expression by binding to RRE.

The transfer vector encodes the relevant therapeutic transgenes and sequences necessary for expression, incapsidation, reverse transcription and integration of the viral genome. RRE sequences permit the nuclear export and expression of the viral RNA. In order to minimize the risk of RCL generation, the 3’ LTR of the transfer vector has been deleted in the U3 region. The introduction of this 400 bp deletion abolishes the production of full-length vector RNA in transduced cells by exploiting the reverse transcriptase mechanism which generates both U3 regions from the 3’ of the viral genome, thus transferring the deletion to the 5’ LTR of the proviral DNA. The lentiviral particle is therefore conceived with a self-inactivating mechanism that enables the production of infective particle only in the first run of infection.

**Origin & function**

1. **X-CGD gene therapy**
   The gp91phox (phox standing for phagocyte oxidase) protein is large subunit of the NADH oxidase system expressed in phagocytic cells e.g. in neutrophils, monocytes, macrophages and eosinophils. NADPH oxidase is a multicomponent system that includes a membrane bound by flavocytochrome b558 comprised of the large subunit, gp91phox, and a small subunit, p22phox. Phagocytosis of microorganisms leads to translocation of four cytosolic factors (p47, p67, p40phox and Rac2) to the cell membrane to form the activated NADPH oxidase complex, which then binds NADPH and generates the respiratory burst. Defects in the genes that encode any of the NADPH oxidase components may abolish the electron transport from cytoplasmic NADPH to FAD, haeme and onto intraphagosomal molecular oxygen.

2. **ADA SCID gene therapy**
   The ADA transgene encodes the enzyme Adenosine deaminase, expressed in all tissues. During DNA breakdown, ADA catalyses the deamination of deoxyadenosine (dAdo) and adenosine to deoxyinosine and inosine respectively. The lack of the ADA enzyme in ADA patients, results in the accumulation of dAdo in both intracellular and extracellular compartments. Intracellularly, dAdo is then converted by deoxycytidine kinase (dCydK) to deoxyadenosinetriphosphate (dATP) which accumulates within the cell. The buildup of these two metabolites has profound effects on lymphocyte development and function and is the most likely cause of the immunological defects. dATP inhibits enzyme ribonucleotide reductase which is necessary for DNA replication and repair and also induces apoptosis in immature thymocytes. dAdo inactivates the enzyme S-adenosylhomocysteinehydrolase (SAHH): SAHH accumulation inhibits transmethylation reactions and the lack of SAHH activity as a consequence of ADA.
deficiency may also contribute to the immunodeficiency. It has also been speculated that the effects of adenosine acting through G protein receptors on the surface of thymocytes may play a role in the pathogenesis of the disease. Although ADA is expressed ubiquitously, the most profound effects are manifest in the immune system. Introduction of the ADA gene is expected to restore lymphocyte development and function in ADA patients.

3. Gene Therapy for Netherton syndrome

The SPINK5 gene encoded a protein named as LEKTI. It is a serine protease inhibitor expressed in the uppermost compartments of the epidermis, and plays a critical role in the regulation of two serine proteases, kallikrein 5 and 7 which hydrolyze the extracellular corneodesmosomes in the skin. In Netherton Syndrome patients, the control of kallikreins by LEKTI is abolished due to mutations in SPINK5 and as a consequence, a hyper-activated kallikreins can cause premature degradation of corneodesmosomes and result in a defective skin barrier.

4. Gene Therapy for HIV

The C46 component codes for production of a membrane-anchored C-peptide derived from the HIV-1 envelope glycoprotein gp41. The peptide is expressed on the surface of the transduced cell and acts as a fusion inhibitor thereby blocking the entry of HIV into the cell. The sh5gene encodes a short hairpin RNA against the HIV co-receptor CCR5 (sh5RNAi). CCR5 is the major co-receptor for HIV. Studies on a naturally occurring □32 mutant of CCR5 (present in approximately 1% of Caucasians) have shown that these individuals are naturally resistant to HIV infection.

Risk of transfer to environmental species for all of the above is negligible, and would not be expected to cause harm.

**Evaluation of foreseeable effects**

The potential for harm is negligible. Work will be at level 2 containment. All personnel involved in the ex vivo transduction of HSC at ICH/GOSH are trained in GMP cell processing procedures and their competency is assessed every 6 months. Sharps will not be used for the direct manipulation of vector. In the case of needlestick injury, the Cellular Therapies needle stick policy (GTLHS007) and guidelines on UVL website (http://www.ich.ucl.ac.uk/clinical_information/clinical_guidelines/cpg_guideline_00121) are followed.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Autoclave facilities are in an adjoining building. However, all waste will be double bagged and placed in a sealed plastic container. The container is disinfected before being transported using a dedicated trolley by trained staff to the autoclave via service lifts and corridors where possible. This procedure is documented in SOP for the Management of spillage and waste disposal (CT.PR.SOP.012). Sharps will be disposed of in appropriate puncture-proof containers using the above procedure.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

For routine cleaning, all surfaces are wiped down with 70% ethanol after use. Liquid GMM waste is first disinfected using Sanichlor (effervescent chlorine tablets) to give a final concentration of 2500ppm chlorine overnight before being disposed of down the sink. This is a standard procedure and viability is below detectable levels. Any solid GMM waste is placed in autoclave bags, labelled with the department’s name and autoclaved at the ICH as above. All sharps are placed in puncture-proof containers and disposed of through the central collection service for contaminated waste. All spillages are cleaned using 1% sodium hypochlorite or 70% ethanol.

**Is an emergency plan required according to regulation 20?**  
[ ] N

**If yes, tick to confirm that it is attached to this form**  
[ ] N

**Tick to confirm that you have attached a risk assessment to this form**  
[ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
[ ] N
The UCL GMSC approved this as a Class 2 activity and connected programme of work.

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**Project Ref** 14/12.3

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**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The overall aim of the project is to identify and characterise the molecules and processes controlling the self-renewal, lineage commitment and differentiation of haematopoietic stem and progenitor cells. We wish to assess the molecular and cellular consequences of manipulating the expression of normal and selectively modified regulatory factors including microRNAs in a range of murine and human in vitro systems and murine in vivo systems. Some of these factors are already well-characterized and known to play an important role in haematopoiesis while others are candidate regulators identified primarily by their differential expression in different haematopoietic...
stages. Our aim is to deliver regulatory genes such as transcription factors, growth factors, cytokine receptors and signalling molecules to human and murine blood cells and monitor the effects on the survival, growth and differentiation of the recipient cells. The genes may be either wild-type or in the form of the point mutants or chimeric factors generated by chromosomal translocations associated with human leukaemias, or selectively altered variants of any/all of these genes designed to modify and dissect their function.

Recipient or parental organism

Mouse and human ES cells, Human iPS cells, primary human and mouse haematopoietic cells, primary human leukaemia cells, human and mouse cell lines. Primary cells will be a range of haematopoietic cell types including FAC-sorted normal and leukaemic stem/progenitor cells and more committed populations from screened human cord blood, bone marrow, peripheral blood and murine bone marrow, peripheral blood, foetal liver and the aorta-gonad-mesonephros (AGM) region of early embryos.

Human lines: EML, FDCP mix, LHX2, M210B4, Si/Si, BaF3, NIH 3T3, 32D 416B, s17, MEL, BHK-MKL, WEHI3

Bacterial host strains (E.coli): NM522, HB101, XL-1 BLUE, XL-2 BLUE, TOP10 F", JM109, SURE, ABLE, DH5alpha, DH10alpha, DE3.

Both bacteria and cell lines transduced with these GMM are very unlikely to propagate outside the laboratory environment. K12 and B derivatives of Escherichia coli will be used as bacterial cloning hosts. These are disabled strains that cannot colonise the human gut and have a history of safe use. Standard plasmids produced in disabled E. coli present no added risk to human health.

The infected human and murine recipient cells would be unlikely to be pathogenic due to rapid complement-mediated lysis of these cells when recognised by the immune system as of non-self origin. These cells contain only the expression vector, are no longer capable of producing packageable viral genome, so these mice would not carry infective viral particles. Expression of the inserted gene(s) is likely to affect cell growth and differentiation within the target cell but would no cause the animal to be hazardous to the environment.

Host/vector system

All vectors are either third-generation (self-inactivating), replication-defective lentiviral vectors based on human immunodeficiency virus type 1 (HIV-1) virus, or based upon the Moloney Murine Leukaemia Virus (retroviral system)

The lentiviral vectors carry a deletion in the 3'LTR that does not affect generation of the viral geome in producer cells but results in "self-inactivation" of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome. A constitutive Rous sarcoma virus (RSV) promoter has been placed upstream of the 5'LTR in the expression vector to offset the requirement for Tat in the efficient production of viral RNA. Only 3 HIV-1 genes are used in the system (gag, pol and rev). Expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-1 rev-response-element (RRE) in the gag/pol mRNA transcript. The Rev protein itself is expressed from a separate plasmid. Addition of the RRE prevents gag and pol expression in the absence of Rev. The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope protein. Genes encoding components required for packaging are on separate plasmids, which do not contain regions of homology to each other to prevent undesirable recombination events which could contribute to the generation of replication-competent virus. None of these 'packaging plasmids' that allow expression in trans of proteins required to produce viral progeny (i.e. gag, pol, rev, env) in the 293T producer cell line contain LTRs or the psi packaging sequence. Thus none of the HIV-1 structural genes are actually present in the packaged viral genome and, thus, are never expressed in the transduced target cell. The lentiviral vectors are replication incompetent and only carry the gene, microRNA or shRNA of interest.

The viral vectors :


MSCV and derivatives (Hawley et al 1996)

pLentilox 3.7 and derivatives (Rubinson et al Nat Gen 2003)


pGIPZ, pTRIPZ (Open Biosystems)

pLenti6/V5 (Invitrogen)

Non-viral vectors include standard mammalian expression vectors for transient transfection studies. These will be used for transgene overexpression and utilise RPOLII promoters.
**Origin & function**

**Human and Mouse. HIV1. VSV as above.**

**Origins of genetic material:** Vector backbones and fluorescent reporters - commercial sources, inserted genes and microRNA - mouse and human genomic DNA, short hairpins - artificially synthesized.

**Classes of Inserted genetic material:**

Wild-type transcription factors: including but not necessarily restricted to GATA family members, hex, Pax 5. Theses factors exert their function within the nucleus and are expected to alter the balance between self-renewal and lineage differentiation in stem and progenitor cells and in some cases alter the lineage output of committed cells e.g. to elicit erythroid cells from a granulocyte-monocyte progenitor. Forced expression of these molecules is likely to influence the self-renewal and differentiation of haematopoietic cells, based on what is known about their normal function. The factors are not known to be transforming or oncogenic, although point mutations in GATA-1 have recently been reported in Down’s syndrome related acute megakaryoblastic leukaemia and Pax5 expression is deregulated in human medullablastomas.

Other regulatory molecules such as components of the notch pathway, cytokine receptors and growth factors. Although acting primarily outside the nucleus, modulation of the expression of these factors is expected to result in a transcriptional response within the nucleus and to alter the balance between self-renewal and lineage differentiation in stem and progenitor cells and perhaps also to alter the lineage output of apparently committed cells. An example of the type of genes we wish to study is given below.

**Notch pathway:** Human chromosome 9 contains a gene highly homologous to the Drosophila gene ‘Notch’. Transcripts of the human, gene, for which the name TAN1 was proposed for translocation-associated Notch homolog), and its murine counterpart were demonstrated in many normal human fetal and adult mouse tissues but were most abundant in lymphoid tissues. Cytogenetic studies have shown that chromosome 7 band q34-q35, which contains the gene for the beta T-cell receptor, is a common site for translocation in T-cell neoplasms. In t(7;9)(q34;q34.3) translocations from 3 cases of acute T-cell lymphoblastic leukemia, breakpoints occur within 100bp of an intron n TAN1, resulting in truncation of TAN1 play a role in the pathogenesis of some T-cell neoplasms. At least 1 Notch homolog is expressed in human bone marrow CD34+ cells, a population enriched for hematopoietic precursors. Members of the Notch family, including TAN1, may be involved in mediating cell-fate decisions during hematopoiesis.

Other candidate regulatory molecules form differential expression analysis including microRNAs, expected to function in a similar manner to the genes described above. The aim of the study will be to study their function in haematopoietic progenitors and so this study will be to study their function in haematopoietic progenitors and so this may, by definition, be currently unknown. However, the genes of most interest to this study will be those found to have some impact on controlling haematopoietic cell self-renewal, growth and differentiation and are likely to include transcription factors, cytokines and growth factors and their receptors, signalling molecules, cyclins, apoptotic and anti-apoptotic factors, enzymes such as kinases, phosphatases, proteases, acetylases.

**Known products of chromosomal translocations, their normal counterparts, or other naturally occurring mutated versions with deletions, insertions and/or point mutations.** These genes will include, but not necessarily be limited to: ETV6-AML1 (ets variant gene 6-Acute Myelocytic leukaemia 1), PML-RARA (Promyelocytic Leukaemia-Retinoic Acid Receptor alpha), PLZF-RARA (Promyelocytic Leukaemia Zinc Finger-Retinoic Acid Receptor alpha), bcr-abl. These are suspected oncogenes by virtue of their expression as a result of specific translocations associated with different forms of human leukaemia. In particular, experimental expression of bcr-abl appears able to induce leukaemia in mouse models. The bcr-abl p190 oncogene is associated with B-cell acute lymphoblastic leukaemia (B-ALL) in humans. Chimeric mice generated with mutant ES cells carrying a bcr-abl fusion created by homologous recombination systematically develop B-ALL, with the phenotype of the tumours being very close to the phenotype of the leukaemias seen in patients with p190 leukaemias. Similarly, use of tetracycline-responsive expression system has allowed controlled expression of bcr-abl p210 in transgenic mice. Lethal acute B cell leukaemia developed in all mice within 3 months, and complete remission was obtained by suppression of p210 expression. The induction of B-ALL rather than chronic myelogenous leukaemia (CML) - with which p210 is most commonly associated in humans - is probably due to the promoter used, which directed expression to B cells rather than the stem cell compartment.

Selective modifications of any/all of the above: activity of many factors can be rendered inducible by fusion to the ligand-binding domain of estradiol receptor. We have
successfully used this approach with transcription factors GATA-1 and GATA-2 and will continue to use this system where appropriate. In other cases, the transcriptional activation/repression properties of a factor may be modified by fusion to more potent positive and negative trans-activators such as VP16 or engrailed domains. Other modifications will be designed to determine the sequence requirements for oncogenic and other functional features. Mutation of potential sites of post-translational modification such as phosphorylation and acetylation and mutation/deletion of sites of protein-protein interaction will be performed. Altering these sites is likely to alter the regulation of the factor's activity within the cell. Deletion of certain domains to allow further dissection of function will also be performed. Most of the modified versions of the proteins under study would be expected to display essentially the same biological activity in an experimentally inducible manner or to display a subset of the activities of their wild-type counterparts. However, the possibility of inadvertently generating novel biological activities cannot be excluded.

All the technologies described are now standard in biomedical reasearch. The GMM and lent/retroviral vectors cannot survive outside the laboratory culture. In principle however there is a very low risk that a lentivirus may infect an experimenter and thereby pose a risk to human health. The exact effects of such putative viral transfers are unpredictable and would be dependent on the cells infected and the site(s) in the genome where any virus integrated. Nevertheless, these integrations may be oncogenic.

The modified viruses should not pose a risk to the environment. The viruses are unstable, inactivated by detergent, UV light and ethanol, and would not survive outside the laboratroy environment. There is no intention to modify the tissue tropism or host specificity of the viral vectors and it is unlikely that any of the gene inserts proposed would affect these. Similarly no alterations in the interaction of the genetically manipulated retroviral vectors with host defences are anticipated. All of the proposed recombinant viruses are replication defective.

**Evaluation of foreseeable effects**

VSV pseudotyped lentiviruses are highly unstable outside the cell and cannot be passively transferred. VSV is a zoonotic arbovirus, which causes lameness in farm animals. Infection in humans is rare and usually by insect bite. It is often asymptomatic and immunity against VSV has not been extensively studied in humans. In most series the majority of people are seronegative, suggesting that the innate immune system may clear infections, and there is no vaccine. Aerosols are an unlikely source of infection (Schlereth et al J Gen Virol 2003). Gene transfer could possibly occur through accidental exposure to viral particles, via a cut or similar break in the skin. Therefore sharps will not be used in preparations containing viral particles or transfected packaging cells. If an accidental exposure were to occur, such viral particles would conceivably have hazardous effects if they infected a stem cell; reducing the risk to human health. It is standard practice in our group to 'double glove' for lentiviral work. As has been stated already, all vectors are replication defective and so productive or replicative infections are not possible. It should be reiterated that these lentiviruses are extremely unstable even in tissue culture conditions.

The modified viruses should not pose a risk to the environment. The viruses are unstable, inactivated by detergent, UV light and ethanol, and would not survive outside the laboratroy environment. There is no intention to modify the tissue tropism or host specificity of the viral vectors and it is unlikely that any of the gene inserts proposed would affect these. Similarly no alterations in the interaction of the genetically manipulated retroviral vectors with host defences are anticipated. All of the proposed recombinant viruses are replication-defective.

The most dangerous GMM: pantropically packaged lentivirus/retrovirus. The most dangerous step is the harvesting and concentration of packaged virions.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work will be carried out in CL2 containment facilities in the POGB. All staff will be trained prior to starting work and will be aware of the safety hazards. Lab coats and gloves are mandatory. Care will be taken to minimize the production of aerosol.

Solid material will be autoclaved, pipette tips and other small items of plastic-ware will be treated for 18 hrs with 2-5% Virkon (v/v). Treated liquids can either be disposed of in the sink or autoclaved in an autoclavable pastic container. All waste is placed in an autoclave bag and then double bagged. Waste is kept within the lab until it is taken to the autoclave in an enclosed trolley. The waste is then loaded directly into the autoclave for immediate treatment (hold time of 132°C for 15 min) by trained staff then bagged in Biohazard bags for removal by UCL waste services.

The efficacy of this approach has been shown to be 100% elsewhere:

All autoclaves are maintained by the Institute. They have pre-set parameters (132°C for 15min) which if un-met, lead to a 'cycle-fail' warning.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The UCL GMSC approved this activity as Class 2 subject to some minor clarifications

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02/03/2022
Induced pluripotent stem (iPS) cells are a powerful tool for research and provide a new source of cells and tissue for therapies where immune rejection is a common problem. We have previously shown that human embryonic stem cells and iPS cells can be used to generate functional retinal pigment epithelium (RPE). In this project we aim to take cells from informed patients at Moorfields Eye Hospital and generate iPS cells using the vectors described below. We will then differentiate these cells into RPE and retinal cells in order to study the impact of retinal degenerative diseases. We will also use a direct reprogramming approach with the aim of transforming somatic cells directly into RPE cells by transforming somatic cells with candidate genes thought to be involved in RPE cell development. We will also use animal models of retinal degenerative diseases to produce iPS-derived cells for disease study.

**Recipient or parental organism**

Virus: The lentiviral vector systems pSIN, FUW, LL and Fu-tet-o, the retroviral vector systems pMIG, pCL, pCAG system and the episomal vector system pCEP (pEP).

Cells: 293 cell lines, competent bacterial cells, patient and animal derived cells (fibroblast, epithelial and blood cells).

**Host/vector system**

All viral vector constructs will be transformed into competent E. coli cells. There is no potential for harm to health and/or harm to the environment.

Commerically available packaging vectors, such as pMD G, pMD2.G, pVSV-G, psPAX2, pCMV-dR8.2 dvpr, will be used.

All viral vector constructs for somatic programming using iPS technology are available complete from a plasmid repository (Addgene) and already contain the insertion genes (embryonic transcription factors) of interest.
To construct viral vectors for direct RPE reprogramming, intermediate constructs will be made using general lab strain of E. coli. There is no potential for harm to health and/or harm to the environment.

Viral transfer vectors containing inserted genes for direct cell reprogramming (somatic cell to RPE) will be constructed using safe vectors pSIN, FUW, LL and Fu-tet-o and pMIG, pC L, pCAG.

Cells: 293 cell lines, competent bacterial cells, patient and animal derived cells (fibroblasts, epithelial and blood cells).

We will also use commercially available mRNA for direct transfection into the mammalian cell lines.

Origin & function


Functions:
Klf4, Oct4, c-myc, Sox2, Nanog and Lin28 are embryonic transcription factors associated with pluripotency.
Transfection of somatic cells with a combination of these genes (Klf4, Oct4, c-myc and Sox2 or Lin28, Nanog, Oct4 and Sox2) result in the induction of pluripotency.

Direct reprogramming Origin: Genes involved in RPE development, including Otx2, Mitf, Pax6, Rax, Lhx2, Six3, BMPs, Activin, SHH, Wnts, MMPs.

Functions:
Otx2, Mitf, Pax6, Rax, Lhx2 Six3/9 and Hes1 are transcription factors involved in gene regulation during development BMPs and Activin are part of the TGF-b family of proteins, which are important signalling molecules during RPE cell development. SHH and Wnt regulate the patterning of the central nervous system during development. MMPs are thought to play a role in cell proliferation and differentiation

Evaluation of foreseeable effects

The use of replication defective and retroviral vectors to modify mammalian cells is well established and safe. The induction of pluripotency in mammalian cells involves the introduction of potentially oncogenic sequences such as c-Myc and Klf4. The introduction of oncogenic genes is a potential hazard to human health, however working with appropriate containment procedures renders the risk low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The viruses, and cells cannot survive in natural conditions. They will be destroyed and disposed of by standard methods listed below.

E.coli and mammalian cells will be inactivated by treatment with 1% virkon for at least 30 minutes. Liquid waste will be dispensed into a container containing 1% virkon. All plastic ware exposed to virus will be treated with 1% virkon solution for 30 minutes. All waste material will be subjected to UV exposure in the tissue culture hood prior to removal for disposal. All waste will be double-bagged in autoclave sterilisation bags and autoclaved prior to final disposal (by incineration). We expect that 100% of viral particles/cells will be de-activated using the disposal procedure.
Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tr>
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Project Ref 14/12.5

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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Date Project Ceased</th>
<th>Withdrawn</th>
<th>Project notified under transitional arrangements</th>
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<tbody>
<tr>
<td>29/06/2012</td>
<td>Genetic manipulation of neurons to enhance axonal regeneration in the rodent nervous system</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
<td>N</td>
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</table>

Historical Significant Changes

Significant Change ID
The aim of this project is to examine the effects on axonal regeneration of altering candidate gene expression in the rodent nervous system using third generation non-replicating, non-integrating viral vectors. Briefly, viral vectors will be generated in a tissue culture system and delivered into the brain using intracranial injection. The brain and spinal cord tissues will be subsequently collected and the tissues will be examined for the neurotoxic, neurotrophic and regeneration-promoting effects.

Recipient or parental organism

Replication-deficient self-inactivating (SIN) lentiviral vectors with a mutated integrase element so that the vector does not integrate into the host genome (non-integrating lentiviral vectors). The recipient organisms (viral vectors) are not harmful to humans. They are attenuated versions of the wild-type viruses and so cannot replicate or integrate. If the virus became inserted into cellular DNA, it could result in a potential oncogenic mutation, but the risk for this to happen using the amounts and volumes here are very remote. The viral vectors are replication incompetent so therefore cannot self-replicate and transfer to another host. They are therefore harmless to the environment.

The rodents that will eventually receive the recipient organisms would not have any survival advantage in the environment. They could conceivably escape from the holding facility (although this is extremely unlikely) but even then they would not be able to transmit the viral construct to another organism and could also not transmit the DNA in the germ line to an offspring.

Host/vector system

Replication-deficient self-inactivating (SIN) lentiviral vectors with a mutated integrase element so that the vector does not integrate into the host genome (non-integrating). The SIN system relates to the engineered long terminal repeats mutation, which prevents viral genomic RNA transcription from the reverse transcription product residing in the cell after infection. Non-integration, on the other hand results from the mutation in the viral integrase gene, which prevents the integration of the viral genome into the host DNA. In this project, we intend to use the viral integrase mutation D64V. Using this mutation in a population of dividing cells, the expression of transgenes has been shown to become completely extinct within two weeks (Apolonia et al. (2007). Molecular Therapy: the journal of American society gene therapy. 15.11 (1957-54)). Theoretically, a very low level of integration could occur due to endogenous and random DNA recombination. However, we assess that the chance of integration is reduced by the order of $10^3-10^4$ compared to an equivalent integrating vector.

The stability of the D64V mutation is considered equivalent to that of any neutral sequence change resulting from mistakes in DNA polymerisation - it does not alter any survival properties in the self-inactivating system. Ultimately, where the vector persists the risk it poses is definitely not higher than the conventionally used integrating vectors. However, the probability of persistence is very much lower. The lentiviral vectors used are multi-attenuated meaning it is devoid of all potentially pathogenic HIV-1 encoded functions (1.2). In addition it is replication-defective which means that the vector cannot multiply on its own unless supplied by certain proteins in trans. It is self-inactivating which means that the viral promoter in the U3 region of the 5' LTR has been disabled by genetic manipulation. Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The probability of such an event is extremely low.


Origin & function

The inserted sequence comes from Mus musculus, homo sapiens, rattus norvegicus. Inflammatory cytokines will produce perineuronal inflammation. Genes interfering with intracellular signalling (dominant-negative MEK, dominant negative EGFR) will modify the responses of neurons to axonal injury. The inflammatory cytokines could induce a
localised immune reaction. As the vector does not integrate in the genome of the host cells its persistence after the transfer is limited. Further, the reaction is expected to be neutralised by an immune-competent individual. These cytokines are used clinically in humans. DN-MEK and DN-EGFR would be expected to have no harmful effects since they are non-oncogenic and not secreted. The sequence will not cause harm if transferred to species in the environment.

The donor organism is not pathogenic.

Evaluation of foreseeable effects

The donor organism is not harmful, however some of the donated sequences are from viruses e.g. the envelope proteins VSV-G from the Vesicular Stomatitis virus, which may be infectious to humans.

The sequences used from the viral donor organisms are either promoter or envelope proteins so none of these are involved in the harmful characteristics of the original donor organism. Even if sequences were introduced into the body by ingestion, through a wound or after spillage onto skin, the viral sequences would enter the human cells and would therefore not be expressed. The sequences used from mammalian donor organisms are transgenes sequences, non of which are pathogenic.

The most hazardous step is injecting the lentiviral constructs into the mice due to sharps.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMMs are inactivated for disposal using standard biosafety level 2 procedures. Briefly, liquid and solid wastes are treated with PRESEPT effervescent disinfecting tablets (2.5g). All plastic-ware used in tissue culture are autoclaved. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern. Prolin and IMS are used which have been shown to be 100% effective in tissue-culture related decontamination.

Work surfaces are decontaminated on completion of work or at the end of the day by spraying with Prolin and IMS and after any spill or splash of viable material with disinfectants that are effective against the agents of concern. This procedure has been shown to effectively eliminate all possible contamination. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Injected animals will be killed by perfusion with fixative that will remove any risk from the injected tissues.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved at UCL GMSC as Activity Class 2 pending satisfactory replies from the PI to the comments made
### Project Containment

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<td></td>
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**Laboratory Activities**

- Glass Houses
- Growth Rooms

**Animal Units**

- Large Scale Activities
- Human Clinical Applications

**Date Ackn'd**: 10/09/2012

**CU2 Project Title**: DNA modification of selected Gram positive and negative bacteria

**Class**: Class 2

**Culture Volume**: < 1 Litre

**Non-GMM Consent Granted**: Yes

**Project notified under transitional arrangements**: No

**Withdrawn**: No

**Tick if notifying a connected programme of work**: No

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

The ultimate aim of this connected programme of work is to develop novel antimicrobial strategies to combat infections caused by selected Gram positive and negative bacteria. This will be achieved by disrupting genes on the chromosome and determining their role in growth, survival, stress resistance and interactions with host tissues and cells. Complementation of the mutation will follow mutagenesis in an effort to restore wildtype phenotype. Additionally reporter constructs will be made in order to determine the transcriptional and translational effects of mutant genes, environmental stimuli and growth phase on expression of genes involved in virulence and pathogenesis. This will enable us to generate attenuated mutants which will give us clues to genes that are required for...
virulence and fitness in these bacteria.

Recipient or parental organism

1) The firmicutes; (the majority of which are classed as 'group 2 by the ACDP. None are above group 2), Clostridium spp. including C. difficile & C. septicum, Staphylococcal spp. (including S. aureus & S. epidermidis), Streptococci spp. (including S. mutans, S. sanguinis, S. gordonii, S. mitis, S. sobrinus, S. intermedius, S. thermophilus, S. pneumonia & S. pyogenes and other oral streptococci), Enterococcal spp. (including E. faecalis, E. casseliflavus & E. faecium), Bacillus spp. (including B. subtilis & the taiotaomicron), Listeria spp. (including ivanovii, monocytogenes and innocua).
2) Campylobacter jejuni
3) Helicobacter pylori
5) Porphyromonas gingivalis
6) Prevotella intermedia
7) Eschericia coli
8) Pseudomonas aeruginosa
9) Viellonella parvula, V. dispar
10) Propionibacterium aenes
11) Actinomycyes naeslundii
12) Lactobacillus casei
13) Fusobacterium nucleatum
14) Neisseria subflava
15) Pichia pastoris
16) Corynebacterium spp.

The majority of these are classified as human commensal or opportunistic pathogens. However the majority of organisms are not likely to cause disease in healthy humans; e.g. C. difficile primarily causes disease only after treatment with antibiotics therefore the risk of infection to humans with these organisms is low. However, complementation of mutants in potential virulence genes may involve insertion of the wild-type gene into a mutant strain under control of a non-native promoter. Such strains could have increased virulence. Some of the organisms listed above are human pathogens and exposure could result in disease with differing severities of harm (e.g. S. pneumoniae can cause pneumonia, otitis media and meningitis).

The intended recipient organisms are wild type and many are clinical isolates and will therefore be capable of independent survival.

Host/vector system

The vectors have been separated into groups based on the organisms, or groups of organisms in which they will be used (see above)

1) Firmicutes
2) Transposons
   Tn916, (Broad host range conjugative transposon TcR Used for, transposon mutagenesis in a variety of Gram positive bacteria. Recombinant versions used to insert DNA into Gram positive chromosomes).
   Tn917, (Nonconjugative-transposon EmR. Used for transposon mutagenesis in a variety of Gram positive
Self transmissible plasmids

- pMTL9301, Bom + Tra+ (ColE1 origin of replication, ApR & CmR. Suicide vector in B. subtilis. Carries a c. difficile promoter for expression in Gram positives [Minton et al., 1990; Roberts et al., 2003]). pMTL007, (delivery vehicle for re-targeted group II intron from E. coli to Clostridium spp) [Heap et al., 2007].
- pMTL960 (Bom+, Tra+) E. coli-C. difficile shuttle vector [Emerson et al., 2009].
- pG01, (Bom+, Tra+) Used as a mobilisation vector in various Gram positive bacteria.
- pRPF185 (Bom+, Tra+) pMTL960 derivative with fdx transcriptional terminator added after tetR [Fagan and Fairweather, 2011].

Mobilisable defective plasmids

- pAULA, (Bom+, Tra-)
- pG(+)Host9, (Bom+, Tra-)
- pTEX5501 Bom+ Tra-

Non mobilisable plasmids

- pKOR1. (Bom+, Tra-)
- pMAD, (Bom-, Tra-)
- pLTV1, (Bom-, Tra-) contains Tn917
- pD701, (Bom-, Tra-) suicide vector for S. pneumonia derived from pEVP3 CmR
- pACH74, pVA891 derivative containing polylinker from pALTER; EryR CmR. Gram-positive suicide vector.
- pAM120, (pUC18 containing Tn916).
- pGEM-T-Easy (Promega): ColE1 origin of replication; ApR; suicidal in Gram Positive bacteria; classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM.
- pVA838: contains Gram positive and Gram negative replicons, Em-, Mob- and Tra-.
- pFW5: contains Gram negative origin only, suicidal in streptococci, SpR: Bom-, Mob- and Tra-
- pNE1:gfp: contains Gram positive and negative replicons, SpR: Bom-, Mob- and Tra-
- pDL278: contains Gram positive and Gram negative replicons. SpR, Bom-, Mob- and Tra-
- pCR-TOPO: for DNA cloning in E.coli (pBR322 origin, ampicillin resistance, not mobilisable). Suicide vector in these organisms.

PTEX5500 Bom- Tra- shuttle-E.coli/ Enterococcus spp

2) Non-mobilisable Plasmids

- pRRC: (ColE1 origin of replication, CmR, carries C. jejuni 16S-23S rRNA spacer region, suicidal in Campylobacter sp. [Karlyshev and Wren, 2005]; derived from pGEM which is classified as non-mobilisable (Bom-, Mob-, and Tra-minus) by the ACGM).
- pDENNIS: (ColE1 origin of replication, CmR, carries sequences of C. jejuni pseudogene (nucs 205297-207475), suicidal in Campylobacter sp.; derived from pGEM which is classified as non-mobilisable (Bom- Mob- and Tra-minus) by the ACGM).
- pC46: (ColE1 origin of replication, lacking lacZ alpha and AmpR gene, CmR. carries C. jejuni Cj0046 gene sequences amplified from strain NCTC 11168 and fdxA (iron-induced ferredoxin) promoter sequence, suicidal in Campylobacter sp. (van Vliet et al unpublished); derived from pGEM which is
3) Non-mobiliseable Plasmids


plR203C04: (ColE1 origin of replication, CmR, carries H. pylori intergenic region, suicidal in Helicobacter (Langford 2006); derived from pBluescript which is classified as non-mobiliseable (Bom-, Mob-, and Tra-minus) by the ACGM)

plR203K04: (identical to plR203C04 except KmR).

4) Non-mobiliseable Plasmids

pUC18, used as a suicide vector in the Pasteurellaceae. Bom- Mob- Tra- ColE1 replicon ApR.

pGEM-T-Easy (Promega): (ColE1 origin of replication), ApR, suicidal, classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM).

5) Non-mobiliseable Plasmids

pUC18, used as a suicide vector. Bom- Mob- Tra- ColE1 replicon ApR.

pGEM-T-Easy (Promega): (ColE1 origin of replication), ApR, suicidal, classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM).

6) Non-mobiliseable Plasmids

pUC18, used as a suicide vector. Bom- Mob- Tra- ColE1 replicon ApR.

pGEM-T-Easy (Promega): (ColE1 origin of replication), ApR, suicidal, classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM).

7) Non-mobiliseable Plasmids


pET-22b vector for E.coli periplasmic expression (pBR322 origin, ampicillin resistance, not mobilisable)


8) Non-mobiliseable Plasmids

pUC18, used as a suicide vector. Bom- Mob- Tra- ColE1 replicon ApR.

pGEM-T-Easy (Promega): (ColE1 origin of replication), ApR, suicidal, classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM).

9) Non-mobiliseable Plasmids

pUC18, used as a suicide vector. Bom- Mob- Tra- ColE1 replicon ApR.

pGEM-T-Easy (Promega): (ColE1 origin of replication), ApR, suicidal, classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM).

10) Non-mobiliseable Plasmids

pUC18, used as a suicide vector. Bom- Mob- Tra- ColE1 replicon ApR.

pGEM-T-Easy (Promega): (ColE1 origin of replication), ApR, suicidal, classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM).

11) Non-mobiliseable Plasmids

pUC18, used as a suicide vector. Bom- Mob- Tra- ColE1 replicon ApR.

pGEM-T-Easy (Promega): (ColE1 origin of replication), ApR, suicidal, classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM).

12) Non-mobiliseable Plasmids
pUC18, used as a suicide vector. Bom- Mob- Tra- ColE1 replicon ApR.

13) Non-mobiliseable Plasmids

14) Non-mobiliseable Plasmids

15) Non-mobiliseable Plasmids

pPICZA vector: for protein expression in Pichia pastoris (pUC origin. resistance is to zeocin, not mobilisable)

16) Non-mobiliseable Plasmids

As regards health the VECTORS will not have the potential to cause harm (non-mobilisable)

Mobilisable defective have the possibility to transfer if they are complemented in trans by a mobilisable vector. This would require a total breach of containment;

Transposons, self transmissable and mobilisable vectors have the possibility to transfer to organisms in the environment following a total breach of containment

Antibiotic resistance genes will be present on these vectors which may be able to transfer to heterologous species. There will also be promoters cloned into mobilisable vectors (eg) also there will be genes with promoters cloned for complementation studies into integrative conjugative vectors.

The majority of the DNA will come from E. coli (vectors), however gene sequences from the organisms below may be used as substrates for homologous recombination. None of the organisms listed will be above group 2 (classified by the ACDP).

1) The firmicutes; (the majority of which are classed as group 2 by the ACDP. We do not propose to use any organisms which are above group 2). Clostridium spp. (including C. difficile & C. septicum),
2) Campylobacter jejuni
3) Helicobacter pylori
4) The Pasteurellaceae; Actinobacillus actinomycescomilans, Haemophilus innuenzae, Haemophilus ducreyi, Haemophilus somnus, Actinobacillus pleuro pneumoniae, Mannheimia haemolytica, Mannheimia succiniciproducens
5) Porphyromonas gingivalis
6) Prevotella intermedia
7) Escherichia coli
8) Pseudomonas aeruginosa
9) Veillonella parvula, V. dispar
10) Propionibacterium acnes
11) Actinomyces naeslundii
12) Lactobacillus casei
13) Fusobacterium nucleatum ss. polymorphum,
14) Neisseria subflava
15) Pichia pastoris
16) Corynebacterium spp.

The majority of the inserted DNA will encode resistance to an antibiotic and will be used to inactivate
genes in the recipient organisms. Fragments of genes from the donor organisms may be cloned to
facilitate homologous recombination during allelic replacement. It is possible that these fragments may be
expressed. The biological activity of these fragments is likely to be limited but will depend on the gene
being investigated and could include e.g. adhesins, response regulators, transcriptional and translational
repressors and virulence factors. Following knock-out generation complementation studies will be
undertaken which will require the cloning and expression of genes from the parental organism. These will
be recloned into the parental organism potentially with a different promoter and therefore the expression of
the cloned genes may be different to the natural expression.

It is possible that if the gene sequences are expressed accidentally in humans harm would occur
however it is likely that the severity would be low and again will depend on the particular gene under
investigation.

There is always the potential of any given DNA sequence to transfer, by horizontal gene transfer
processes, to other microorganisms in the environment. However by adopting good laboratory practice
and by following the codes of practice for the Division of Microbial Diseases, the chances of a breach of
contamination are effectively zero.

Whilst the majority of the initial cloning will be carried out in E. coli (using disabled lab strains) the majority
of the final experiments/mutations will be carried out in wild-type organisms. The majority of these are
classified as human commensal or opportunistic pathogens. However the majority of organisms are not likely to cause disease in healthy humans; e.g. C. difficile primarily causes disease only after treatment with antibiotics therefore the risk of infection to humans is low. Some of the organisms listed above are human pathogens and exposure could result in disease with differing severities of harm (e.g. S. pneumoniae can cause pneumonia, otitis media and meningitis).

It is envisaged that some of the genes being investigated will be virulence factors, therefore fragments of
these may be cloned to facilitate homologous recombination with the recipient organisms. In the
complementation studies full length genes will be cloned into the parental organisms and expressed.

Evaluation of foreseeable effects

Whilst the majority of the initial cloning will be carried out in E. coli (using disabled lab strains) the majority
of the final experiments/mutations will be carried out in wild-type organisms. The majority of these are
classified as human commensal or opportunistic pathogens. However the majority of organisms are not likely to cause disease in healthy humans; e.g. C. difficile primarily causes disease only after treatment with antibiotics therefore the risk of infection to humans with these organisms is low. Some of the organisms listed above are human pathogens and exposure could result in disease with differing severities of harm (e.g. S. pneumoniae can cause pneumonia, otitis media and meningitis).

It is envisaged that some of the genes being investigated will be virulence factors, therefore fragments of
these may be cloned to facilitate homologous recombination with the recipient organisms. In the
complementation studies full length genes will be cloned into the parental organisms.
organisms and expressed.

Cloning in E. coli should not have pathogenic or ecological disruptive effects. It is not foreseen that DNA insertion onto the chromosomes for the purposes of gene inactivation in the selected bacteria will result in GMMs that are more hazardous than the parental strains. Therefore the most hazardous GMMs are likely to be those that are complemented with the inserted gene under the control of a strong promoter resulting in increased expression. However, we would avoid using strong promoters because we wish to restore the natural phenotype for functional studies.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMM/1 line: 1.29 or GMM/1: line 2.13 (as applicable)

All GMMs will be killed before leaving the laboratories. Liquid and solid waste containing GMMs will be autoclaved on a "kill cycle" at 121°C for 30mins before disposal for incineration. Contaminated glassware will be similarly autoclaved before entering washing-up. Contaminated sharps will be autoclaved in their sharps bins on a kill cycle prior to disposal for incineration. These sharps bins have been tested in-house and stand up to the autoclavage process. Killed liquid waste will be disposed of down the sink. All benches and surfaces will be swabbed down with a 0.1% hypochlorite at the end of each laboratory session.

Degree of kill; 100%

The autoclaves contain internal temperature probes in order to monitor the cycles. In addition, TST strips and autoclave tape are used in every kill cycle.

Killed liquid waste will be disposed of down the sink. All autoclaved solid waste will be bagged into orange bags for incineration and collect from the locked yellow bins by the Eastman's contractors. These bags are labelled with an identifiable cable tie provided by the Safety Officer at the Eastman Dental Institute and are therefore traceable back to the division of Microbial Diseases. Yellow sharps bin. once autoclaved will be deposited in the designated, locked sharps bin container for collection, these are labelled with date, department and researcher. No live GMM will leave the department in the form of waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
Approved as Activity Class 2 after the following alterations/additions were made; more Information on risk to human health; more detail on pathogenic strains; more detail on control measures.

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**Project Ref** 14/12.7

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<td>The generation of integration free human induced pluripotent stem cells</td>
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**Date Project Ceased**

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Project notified under transitional arrangements

**Encapsulation**

Withdrawn

Tick if notifying a connected programme of work

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Human embryonic stem cells (hESCs) are of great interest in the field of regenerative medicine as they are pluripotent and have unlimited proliferative capacity in vitro. Pluripotency describes the potential of the cell to differentiate into any cell type of the body. This would allow us to generate any desired cell type in the lab and replace diseased or absent cells in patients. In our case, we are interested in generating retinal pigment epithelial cells (RPE) in the lab to treat patients with age related macular degeneration. However, the main disadvantage of using hESCs is the possibility of immune rejection given that hESCs are derived from blastocysts.
A landmark technique discovered in 2007 by Takahashi and Yamanaka allowed the generation of hESC-like cells (coined induced pluripotent stem cells or iPS cells) from adult somatic cells without the use of embryonic material. This technique is known as induced pluripotency and allows us to generate pluripotent iPS cells from patient's cells. If RPE cells are differentiated from patient derived iPS cells, they would be genetically identical to the patient and therefore, overcome any potential immune rejection problems. The ability to generate patient specific iPS cells thus has immense potential for cellular therapies.

The original induced pluripotency technique involves the overexpression of key pluripotency genes/factors: Oct4, Sox2, Klf4 and cMyc. This ectopic expression is achieved by retroviral infection of somatic cells with the 4 factors which then integrate into host genome. As the 4 factors are under control of the MMLV (monkey moloney leukemia virus) promoter, once the somatic cell becomes reprogrammed to a pluripotent state, the transgenes will switch off. While this original method of iPS cell generation is useful for in vitro disease modelling and drug discovery, its application to the clinical therapies is limited due to the possibility of insertional mutagenesis and the re-activation of oncogenes, such as cMyc. The objective of this project is to investigate different methods for generating iPS cells and identify the method that would be most translatable to the clinic.

The other methods investigated will include various non genome integrating techniques (episomal plasmid, mRNA and miRNA mimic transfection). Since we do not understand the mechanisms behind reprogramming, we do not know if the method chosen will have an effect on the quality of iPS cells produced. Our aim is to compare the different iPS cell lines generated from the different integration-free methods (episomal plasmid, mRNA and miRNA mimic transfection) with iPS cells generated using the retrovirus gold standard. Comparisons will include the expression of pluripotency markers, genome wide expression and methylation patterns. This comparison will allow us to determine which method is most suitable for the clinical application of iPS cells. Our future studies will include the development of the chosen method to generate GMP (good manufacturing practice) grade iPS cells.

As the mRNA and miRNA mimics methods do not involve use of vectors or generation of viruses and are synthetic oligos/RNA, they will no be discussed in this GMM form as they pose NO harm to humans, the environment and other species as mRNA and miRNA are rapidly degraded when not stored at -80°C in RNA nuclease free conditions.

### Recipient or parental organism

**Mammalian cells**

(Recipient organisms)

- IMR90 human lung foetal fibroblast cell ine (ATCC, USA)
- Patient fibroblast cell lines
  (Support cells)
- Newborn human foetal fibroblasts, Nuffs (Amsbio)

**Bacterial E. coli strains**

- DH5alpha (New England Biolabs)

**Retroviral packaging cell line**

- Platinum A (based on the 293 T cell line encoding gag, pol and env under control of the EF1α promoter from Cell biolabs Inc.)

The likelihood that bacteria transformed with retroviral/episomal vectors and GMM cells will propagate outside the laboratory is extremely low. Its is also highly unlikely that GMM cell lines could persist in the environment and be transferred to other organisms. Both transformed bacteria and GMM cells will not survive outside of the controlled tissue culture environment as these cells are highly dependent on specific in vitro culture conditions (they require low carbon dioxide levels, 37°C, specific growth factors to survive) and thus would not survive outside of the in vitro environment. When handling live recipient organisms, sharps (glass pipettes and needles) will not be used to avoid accidents, hence there is minimal risk associated with working with recipient cells. The minor risk occurs if the recipient cells were directly transferred in vivo to in an immune-compromised individual either by needle stick injury or via the mucosal membranes. However, by following standard safe laboratory practice including local rules for avoiding sharps and using personal protective equipment, their risk associated with working with recipient cells is minimal.
We will follow standard safe laboratory practice for the treatment of waste (vectors and cells) to ensure that the recipient cells is minimal.
We will follow standard safe laboratory practice for the treatment of waste (vectors and cells) to ensure that the recipient organisms are not pathogenic to humans:
- we will treat bacterial cells used to grow vector plasmids and other tissue culture waste (viral supernatant) with Virkon 2-5(v/v)% at least overnight (approximately 18 hours). Virkon powder will be used to decontaminate any spills as the powder absorbs the spill and prevents further spreading of the liquid spill. Liquid waste will subsequently be disposed of down the sink with copious amounts of water. Work surfaces will be wiped down with Trigene Advance (Trigene advance fully meets the criteria of the European Biocidal products directive (ref 98/8/EC) and is bactericidal, fungicidal and virucidal), followed by 70% IMS after use.
- Solid waste will be double bagged in biohazard bags and removed by UCL waste services who autoclave the bags at 132°C with a holding time of 20 mins prior to being incinerated. UCL waste services will validate all the autoclave cycles to ensure no risk to the environment.

Host/vector system

pMXs-hOct4 (Addgene, USA)
pMXs-hSox2 (Addgene, USA)
pMXs-hKlf4 (Addgene, USA)
pMXs-GFP (Cell Biolabs Inc., USA)
pCXLE-hSK (Addgene, USA)
pCXLE-hUL (Addgene, USA)
pCXLE-hOct3/4-shp53 (Addgene, USA)
pCXLE EGFP (Addgene, USA)
pEP4 EO2S ET2K (Addgene, USA)
pEP4 EO2S CK2M EZ2L (Addgene, USA)

The pMXs retroviral vectors used contain an MMLV promoter to drive the expression of the 4 factors and GFP. None of these vectors contain the essential virion replication and packaging elements that are required to form the viral particle itself and so, poses no harm to health and the environment. The packaging and envelope proteins are produced by the packaging cell line, Platinum A(Plat A) cells which expresses the gag, pol and env genes. These packaging and envelope genes are maintained stably in Plat A cells only if placed in medium containing blasticidin and puromycin antibiotics. Therefore, for viable retroviral particles to be produced, the retroviral vector must be transfected using a liposomal reagent into Plat A cells that have been under antibiotic selection. Therefore the viral vectors on their own pose no risk.

The episomal plasmid vectors are not used to make virus and nor does it contain the essential virion replication and packaging elements that are required to form a viral particle itself and so, poses no harm to health and the environment. Furthermore, these episomal plasmids do not contain any sequences that enable integration into the host genome and are maintained in the cytosol of target cells and therefore pose no harm even if directly transferred to humans.

Origin & function

Oct4, Sox2, Klf4, and cMyc sequences were amplified from Homo sapiens sequences and then cloned into the pMXs retroviral vector described by Kitamura et al. (2003).

Oct 4, Sox2, Klf4, L-myc, Lin28, shp53, SVT40 large antigen and Nanog sequences were amplified from Homo sapiens sequences and then cloned into the pCEP4 and pCXLE episomal vectors described by Yu et al, (2009) Okita et al. (2011) respectively.

Overall, the 4 factor (Oct4, Sox2, Klf4 and cMyc) sequences from the pMXs retroviral vectors will be transcribed and the translated transcription factors will act co-operatively to activate the pluripotency network of genes while repressing differentiation pathways. We expect this overexpression to reprogram the target somatic cells and transform them into iPS cells. As the viral vectors are under control of the MMLV promoter, we expect the transgenic sequences to be silenced once the cells are reprogrammed to a pluripotent state. Integration of the MMLV-GFP sequence will have little or no biological consequence aside from labelling cells GFP. The individual known functions and expected activity of each of the 4 factors is described below:

Oct 4 is a key transcription factor in pluripotency and is highly expressed during embryonic development and in embryonic stem cells. It acts co-operatively with Sox2 and Nanog to promote pluripotency and suppress differentiation. Overexpression of Oct 4 in somatic cells is expected to activate the pluripotency network.
Sox2 is a transcription factor that is involved in the regulation of embryonic development and in the determination of cell fate. It acts co-operatively with Oct 4 and Nanog to promote pluripotency and suppress differentiation. Overexpression of Sox2 in somatic cells is expected to activate the pluripotency network.

**Evaluation of foreseeable effects**

Klf4 is a transcription factor that is known to bind putative regulatory elements of Oct 4, Sox2, Nanog and other pluripotency associated genes. Its exact function is not known but is thought to be a key player regulating the pluripotency network. The overexpression of Klf4 in somatic cells is hypothesised to upregulate Nanog while repressing p53.

C-Myc is a transcription factor that plays roles in cell cycle progression, apoptosis and cellular transformation. Mutations, overexpression, rearrangement and translocation of the cMyc gene is associated with various tumours, leukemias and lymphomas. The overexpression of cMYc in somatic cells is thought to cause rapid proliferation of the cells which is expected to promote reprogramming to a pluripotent state.

The genes encoded by the episomal plasmids (pCXLE-hSK, pCXLE-hUL, pCXLE-hOct3/4-shp53, PCXLE EGFP, pEP4 EO2S ET2K and pEP4 EO2S CK2M EN2L) will be transcribed and the translated transcription factors will have similar roles to the ones described above. In summary, these genes will act co-operatively to activate the pluripotency network of genes while repressing differentiation pathways. We expect their expression to reprogram the target somatic cells and transform them into iPS cells. These genes will be expressed by the episomal plasmids alone and will not involve any viral packaging. The episomal plasmids all contain EBNA-1 and OriP sequences for limited maintenance in the host cell without integration into the host genome.

The retroviral vectors used lack viral structural genes and require packaging cells to generate retrovirus, therefore all the retroviruses generated are non-replicating.

The MMLV based GFP is not expected to cause any harm as the sequence only contains a GFP cassette. GFP is a widely used marker of transgene expression following transduction and its expression has not been associated with any adverse effects.

The 4 factor pMXs based retroviruses used in this project are standard practice in iPS cell research and no reports of safety issue have arisen where proper precautions have been taken. If there were an accidental transfer to human iPS cells from target cells is only 0.02% under favourable culture conditions (Takahashi et al., 2007). Furthermore, pMXs retroviruses can only infect actively dividing cells, so is unlikely to be able to infect human cells readily. Furthermore, the recipient organism is not pathogenic.

There is a theoretical possibility of insertional mutagenesis following retroviral transduction of human tissues and uncontrolled cell proliferation if cMyc were to integrate into the genome of human tissues. However, these risks have never been realised in any of the human and non-human primate studies to date using retroviral vectors. The infectivity of retroviral vectors are low as they are labile and do not survive prolonged culture at 37°C. The viruses are not transmissible through unbroken skin or by aerosol. The main risk is by needlestick injury or through the mucous membranes. This would be minimised by safe laboratory practice and restricted access to laboratories where the retroviral procedures are being carried out.

The episomal plasmids (pCXLE-hSK, pCXLE-hUL, pCXLE-hOct3/4-shp53, PCXLE EGFP, pEP4 EO2S ET2K and pEP4 EO2S CK2M EN2L) pose NO harm to humans as they are used as naked DNA plasmids. They will NOT be transfected into viral packaging cells to generate virus. Even if directly transmitted into a human by accidental transfer, the plasmid DNA would be immediately degraded by DNA nucleases on the skin and by other host defences.

It is theoretically possible that the sequences can cause harm to other species in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Autoclave the bags at 132°C with a holding time of 20mins prior to being incinerated. UCL waste services will validate all the autoclave cycles to ensure no risk to the environment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved by the GMSC after review by GM Safety advisors and ratified at the meeting of the GMSC on 3 July.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

- Animal Units
- Large Scale Activities
- Human Clinical Applications

**Project Ref 14/12.8**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>Consent Granted</th>
<th>Non-GMM</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>05/11/2012</td>
<td>Role of hepatitis B virus in regulating immune responses during chronic infections</td>
<td>Class 3</td>
<td>&lt;1 litre</td>
<td>Yes</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

- Date Project Ceased
- Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
# Project Additional Information

## Purposes of the contained use

There are estimated 400 million people worldwide chronically infected with the hepatitis B virus (HBV), leading to deaths resulting from liver diseases as cirrhosis and hepatocellular carcinoma. There is currently no cure for infection and antiviral therapy have limited effect. Successful control of chronic HBV infection depends on mounting a robust T cell response towards infected hepatocytes. A possible therapy to treat chronically infected HBV patients is by redirecting healthy T cells against immunodominant epitopes of HBV antigens. The levels of antigens expressed on the surface of hepatocytes are therefore in focus. Another objective is to investigate immune regulatory surface markers as secondary objective to investigate if redirected HBV specific T cells receive down regulating signals from HBV infected hepatocytes.

We want to investigate if hepatocytes transfected with naturally occurring HBV genotypes have different levels in antigen presentation of immunodominant epitopes. As our research project is about redirecting T cells and antibodies against these HBV epitopes, it is necessary to see if there is a variation in presentation or any cross-specificity of the peptide specific antibodies between HBV genotypes. This will have implications in future therapies and necessity to tailor antiviral therapy according to which HBV genotype the patient is infected with.

## Host/vector system

Naked linearized HBV DNA will be transfected into cells using a liposome reagent (Lipofectamine 2000, Invitrogen). No harm to health or environment according to the product MSDS.

<table>
<thead>
<tr>
<th>GMM/1: lines 1.13, 1.19, 1.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huh7-A2 is a human hepatocellular carcinoma cell line, stable transduced with the human leukocyte antigen A*201. (JCRB No JCRBo403:HuH-7).</td>
</tr>
<tr>
<td>HepG2 is a human hepatocellular carcinoma cell line, natively expressing the human laukocyte antigen A*201. (ATCC No. HB-8065 Hep G2).</td>
</tr>
<tr>
<td>Huh7 A2 and HepG2 are human hepatocellular carcinoma cell lines that do not express any pathogenic substances. These cell lines are categorised as biosafety level 1.</td>
</tr>
<tr>
<td>The human cell lines HepG2 and Huh7 require sterile conditions in specific culture media. They will not survive outside laboratory cell culture conditions.</td>
</tr>
</tbody>
</table>
Plasmid DNA production was done at a biosafety level 2 Laboratory in our collaborator's group in Bordeaux, France. Enzyme digested plasmid DNA is separated with standard electrophoresis methods and linearized HBV is purified from the plasmid backbone. Only the HBV encoding DNA strand is used for transient transfection of Huh7A2 cells. The linearized plasmid backbone containing T7 promoter and ampicillin resistance gene will be trapped in solid agar and disposed as biological waste.

Origin & function

The hepatitis B genome is derived from a patient isolate, described in the article
Genome of hepatitis B virus restriction enzyme cleavage and structure of DNA extracted from Dane particles
PMID: 1060140

The hepatitis B genotype c core sequence is derived from patient isolate, described in the article:
The complete nucleotide sequences of the cloned hepatitis B virus DNA; subtype adr and adw.
PMID: 6300776

Whole HBV genome will be expressing the proteins for the hepatitis B virus and the nucleic acid sequence required for viral assembly.

The hepatitis B virus is non-cytolytic, and will secrete virus particles, HBeAg and HBsAg.

Very little chance of accidental transfer in humans, as the viral DNA is linearized before transfection, making the genomic material unstable and prone to degradation. If infectious HBV viral particles accidentally transfers (sharps injuries), there is a possibility of infection in unvaccinated persons, resulting in an acute liver infection. In a subset of individuals a chronic carrier state may be established. A chronic HBV liver disease may result in cirrhosis and possible hepatocellular carcinoma. Individual proteins may cause immune reactions and trigger antibody production. The HBV genomic DNA will not express properly unless transferred into human liver hepatocytes, as they require human hepatic transcription factors.

HBV infection can cause acute fulminant hepatitis in adults. Estimated 5% of infected adults develop chronic infection with increased risk of developing cirrhosis and HCC. Whole HBV genome is transfected into target cells. The core antigen and the envelope antigen are thought to be the target of immunological response causing hepatic flares and low-level inflammation of the liver.

The HLA-A*0201 gene is derived from human patient isolate

Evaluation of foreseeable effects

HBV infection can cause acute fulminant hepatitis in adults. Estimated 5% of infected adults develop chronic infection with increased risk of developing cirrhosis and HCC. Whole HBV genome is transfected into target cells. The core antigen and the envelope antigen are thought to be the target of immunological response causing hepatic flares and low-level inflammation of the liver.

Potential harm if HBV transfected cells are supernatant from cell culture comes in contact with unvaccinated person with cuts or open sores. The most hazardous GMM are the stable transduced cell lines that constitutively produce HBV at relatively low viral titre: HepG2 117, HepG2 H1.3 and HepG2 2.2.15. Most hazardous step in the procedure is when cells are stimulated to produce viral protein, the cells will go from very low viral titre to producing relatively high viral titres (105-106 genome copies per ml).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No GM animals will be used in this project application
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste is inactivated by 5% (v/v) Distel/Trigene for a minimum period of 12 hours and then discarded to drain. Solid waste is autoclaved. Plasticware is decontaminated by soaking in 5% 9v/v) Distel/Trigene for a minimum of 12 hours and then autoclaved. The degree of kill for these procedures is 100%

The autoclaving is conducted inside the CL3 facility. The autoclaving conditions are 134°C for 30 mins. This is monitored by 12 thermocouples. The records are automatically generated by the autoclave, signed off by the user and retained.

Following autoclave treatment, solid waste bags are placed inside clinical waste containers and sent for incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC approved the risk assessment following minor amendments requested by the advisers

Project Containment

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<tr>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L3 L4 L3 L4</td>
<td>Class 2 ≤ 1 Litre</td>
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Project Ref 14/12.9

Date Ackn’d 16/11/2012

Date Project 02/03/2022

CU2 Project Title Reconstitution of dimethylarginine dimethylaminohydrolase 1 (DDAH-1) in a rodent model of liver disease

Class Non-GMM
CultureVolClass2 ≤ 1 Litre
Consent Granted

Page 782 of 15326
The aim of the study is to demonstrate an attenuation or exaggeration of portal hypertension and liver injury in a rodent model of liver disease with modulation of DDAH-1 (work will be conducted using project license PPL 70/6615, Complications of Liver Disease, PI Dr N D), following transduction of DDAH-1 in diseased liver. The DDAH-1 expressing, or anti-DDAH1 shRNA/miRNA expressing, or control, lentivirus particles will be diluted in DMEM media and injected into anaesthetised (isofluorane) mice. This mode of virus administration for liver transduction has been validated by several investigators[7]. Portal pressure will subsequently be measured 2-6 weeks later.


The lentiviral vectors to be used are: pSIN_Thy1.1_SFFV_DDAH!, pSIN_THY1.1_SFFV_GFP, pSIN_Thy1.1_PGK_GFP, pSIN_Thy1.1_RFP, pGIPZ_shRNA_antiDDAH1.

These vectors are designed to manipulate DDAH1 levels, or be scrambled control vectors expressing GFP or RFP proteins. Lentiviral vectors cannot replicate in humans or animals because complementary packaging and helper proteins are absent and, although insertional mutagenesis is a theoretical possibility, this is not reported for any human or non-human primate studies. Our lentiviral vectors will be used for gene transfer to liver in rodent models. No additional risk is anticipated with any of these procedures; transmission to the germ-line is highly unlikely, while in most cases vectors will have tissue-specific promoters, which further minimizes risk.

The DDAH1 insert is from human cDNA. Other sequences are validated anti-DDAH1 shRNA or anti-miRNA inserts, green or red fluorescent proteins, or scrambled/non-coding control insets. The inserts do not code for oncogenes or other known harmful proteins. The lentivector backbones, and GFP/RFP/scrambled controls are not species-specific.

The transcribed DDAH-1 protein acts to hydrolase ADMA tp :-citrulline. There is no evidence of harm following overexpression of DDAH-1.
Lentiviral vectors are constructed from a three-plasmid system of a transfer vector containing only modified long-terminal repeats (LTRs), the transgene and a suitable promoter. The other plasmids are a gag-pol packaging plasmid and a VSV-G envelope plasmid. This three-plasmid system renders the virus replication deficient since the structural genes are not actually present in the packaged viral genome so no new virus can be produced and no other cells can be infected. Additionally, the 3’ sequence of the U3 element in the transfer plasmid has been modified so that only 18bp remains, this eliminates promoter activity and means that proviruses resulting from this vector cannot transcribe their full genome and are therefore said to be self-inactivating.

The envelope protein is VSV-G from the Vesicular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag. Pol or env genes) so it cannot make new progeny virus and establish a productive infection, its physical insertion into cellular DNA could result in a potential mutation. This would be a very unlikely event and even if it were to occur it would affect only a very small number of cells, and is therefore extremely unlikely to affect the individual. The packaging viral components are from the HIV-1 virus. The full length HIV-1 virus is harmful to humans, however the packaging vector used does not contain the full length HIV-1 molecule. Many components of HIV-1, that are critical for HIV-1 infection but not required for viral packaging, have been removed so the virus is attenuated. This packaging system is routinely utilised in the preparation of lentiviral vectors, with no reported adverse events.

The lentiviral vectors are replication defective and generated by 4 plasmid transfection to minimise potential for recombination and therefore will not cause harm if transferred to other species in the environment.

Evaluation of foreseeable effects

The most hazardous GMM is the lentivirus expressing DDAH1. The most hazardous step is the injection of viral particles into rodents. As previously noted, there is no evidence of toxicity or pathogenicity from DDAH1 overexpression. Good laboratory practice will mitigate the risk of exposure to lentiviral particles. All trained personnel will have specific training in the handling of sharps, and use of gloves/masks, associated with biological vectors, with a dedicated training log. A microbiological safety cabinet will be used for handling low-volume viral culture media. Until they are required for in vivo use, the particles will be stored with restricted access in triple-packed containers.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Since the work will take place in an operating theatre in the BSU, then only trained personnel will have exposure to the vector. Outside of this environment, lentiviral particles will be stored in an area with restricted access, contained in a second sealed, triple-packed box, labelled with description of contents and emergency contact details. The inserts do not pose any health risk upon inadvertent exposure. The antibiotic selection inserts pose a theoretical risk of spreading antibiotic resistance to wild type bacteria. This is extremely unlikely to occur due to good laboratory practice measures that are routinely undertaken. Also, the virus is replication deficient, since the structural genes are not actually present in the packaged viral genome so no new virus can be produced and no other cells can be infected. Additionally, the 3’ sequence of the U3 element in the transfer plasmid has been modified so that only 18bp remains, this eliminates promoter activity and means that proviruses resulting from this vector cannot transcribe their full genome and therefore said to be self-inactivating. Without these processes, the risk to the environment is very low.

Additionally, the following measures are in place at the BSU to prevent rodents escaping to the environment:

* For all rodents the cage represents the primary means of confinement. Rodents are only housed in cageing that is free from damage. The cage type has an outer fitting lid with a spring lock mechanism to ensure tight closure. Placement of the lid securely back onto the box base is most important and staff and user’s are trained to ensure they do this properly. BSU policy is to recapture any escaped rodents.
* All cages are labelled with the number and sex of the rodents.
* Rodents are counted at a minimum frequency of once per week, and discrepancies investigated.
* All of the rodent rooms are fitted with rodent barriers across the doorway, providing a further tier of security to the room.
* Additional passages and doorways off sub corridors or within inner lobbies further protect many of the rodent rooms.
* Storage in animal rooms and in corridors is minimal, allowing few places to hide.
* The main CBU corridors have three sets of double doors in addition to the two main entry/exit points.
* A lobby with two self-return doors protects the main point of entry/exit.
* Vigilance and no attention to detail by BSU staff and user's.
We expect to see less than 1 rodent a year in the BSU main corridors and less than 1% of those making it that far to escape the BSU into the local area. Put another way, 1 rodent in 100 years, could possibly escape beyond the confines of the BSU.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste (including disposable plastics & lab glassware) will be treated with 2% Virkon overnight, and subsequently autoclaved at 134degC for 10 min when the level of GMM inactivation is below detectable level, and then placed in clinical waste bags for disposal and subsequent incineration. Animal carcasses will be disposed of by incineration. The expected degree of kill is 100% - there has been no documented survival of GMMs following incineration.

Bacterial culture medium will be disinfected with 10% Trigene. Other liquid waste will be disinfected overnight with chlorine releasing tablets (Presept) or 2% freshly-made Virkon prior to disposal via a designated laboratory sink.

Solids, such as pipette tips, will be discarded into plastic containers with lids prior to autoclaving, as before.

Surfaces will be routinely cleaned using 70% IMS.

Spillages will be dealt with according to departmental policy, which is available to workers in paper and electronic forms (S:\Hepatology\Safety\spillage\management). Regarding work with E. coli, comply with the safety guidelines as outlined in GM COP in Appendix 1 on S:\CentreForHepatology\Safety\Rik Assessments, Codes of Practice and Standard Operating Procedures\GM Code of Practice.

Disinfection of benches: 70% IMS or Milton solution; Disinfection of centrifuges: 70% IMS only.

Spillages: application of Presept granules for at least 5 min, prior to wiping with a damp cloth and disposal into an autoclave waste bag.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The UCL GMSC approved this as an Activity Class 2 project (July 2012)

Project Containment
The shRNAmir library consists of individual E. coli glycerol stocks containing the pGIPZ lentiviral vector. Each stock contains a unique hairpin sequence within the pGIPZ vector designed to target and reduce the expression of genes within the human genome via RNAi interference. We are particularly, but not exclusively, interested in targetting protein tyrosine phosphatase (PTP) genes.

This project will involve virus library maintenance, viral storage, lentiviral production from pGIPZ plasmids and use of this lentivirus in cell based screening functional assays. Additionally, clones will be pooled at the bacterial, DNA and viral level to make various intermediate pools of clones to be used to transduce cells in screens and subsequently undertake functional assays.

Our specific scientific goals are to reduce the expression of PTP genes, or components of PTP signalling pathways, in neuroblastoma cell lines, in order to identify those PTPs that act as oncogenes in these cancer cells. We will also introduce a limited range of cDNAs into cells using viral delivery, to test further the function of the critical PTP pathways. This will ultimately lead to further development of chemical or RNA/DNA-based inhibitors of PTP pathways that could be used therapeutically to tackle this cancer.
**Recipient or parental organism**

GMM/1: lines 1.13, 1.19, 1.20

(1.13) Cell lines: Human 293T, NIH3T3; human neuroblastoma cell lines including SKNAS, SKNDZ, SKNSH, SHSY5Y, LAN5, LAN1, KELLY, SMS-KCN, SMS-KCNR, IMR32; human glioma cell lines including T98G, KNS242, Res186, Res259, SF188, UW479; simian Cos7.

Primary murine cells: Derived from various mouse adult or embryo tissues and including neurons and adrenal cells.


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(1.19) The bacterial strains used are attenuated, non-colonising strains so are incapable of causing human infection and are therefore harmless to humans.

The viral vector to be used will be pseudotyped with the envelope protein VSV-G from Vesicular Stomatitis Virus. So, if introduced into the body by injection, ingestion or through a wound, the virus could enter human cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes), so it cannot make new progeny virus and establish a productive infection, its insertion into cellular DNA could result in a potentially oncogenic mutation.

The recipient cells are harmless to humans.

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(1.20) The bacterial strains used are attenuated, non-colonising strains that cannot survive outside culture conditions and are therefore harmless to the environment.

The viral vectors and plasmid vectors are replication incompetent so therefore can not self-replicate and transfer to another host so are harmless to the environment.

The cells that will eventually receive and integrate the viral or plasmid DNA cannot survive in the outside environment as they are only viable under culture conditions and therefore are harmless to the environment.

**Host/vector system**

GMM/1: lines 1.12, 1.24

(1.12) Lentiviral vectors: Commercial TZV vector from Tranzyme and derivatives (including pGIPZ).


The plasmid vector to be used are based on well-established, standard laboratory cloning and expression vectors (pCDNA3.1, pClneo, pGEM, pCAβ, pCAβ-IRESGFP). These contain either CMV or beta-actin promoters which are active in mammalian cells. The plasmids are not mobilizable.

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(1.24) The viral vector used is a lentiviral vector that contains the CMV (Pol II) promoter to drive expression of the hairpin cassette. The lentiviral vector does not contain the appropriate accessory elements to form a viral particle itself and therefore poses no harm to health and the environment. Packaging and envelope proteins are contained on two additional plasmids to the lentiviral expression vector and these plasmids must be introduced into the cell and expressed alongside the lentiviral vector for viable lentiviral particles to be produced.

The standard plasmid vectors are not replication competent in mammalian cells. They replicate in host bacteria, but do not express cDNAs in bacteria. Expression of cDNAs occurs only in mammalian cells, under promoters such as CMV, beta-actin or SV40. Plasmids cannot be transmitted from cell to cell and therefore do not represent harmful potential for the environment.
The RNAi hairpins are sequences that have been derived de novo and are not from an organism. They have been designed as small sequences with both a sense, antisense and loop sequence to form RNA hairpin when transcribed which will initiate a downstream cascade of events leading to knockdown of the target gene to which the hairpin was designed.

The cDNAs will be derived from the human or mouse genome and obtained as IMAGE clones or likewise. The accessory proteins are from the HIV-1 virus.

The hairpin sequences will be transcribed and the RNA will then form a hairpin structure. The hairpin structure will induce a normal cellular in vivo downstream sequence of processing events in response to foreign cellular single-stranded RNA intruder molecules that will result in the post-transcriptional down regulation of the gene to which the hairpin sequence is targeted.

The cDNAs will be expressed from viral promoters, or plasmid-derived promoters (e.g. CMV, SV40).

Although we cannot know the exact cDNAs to be used yet, we can expect that they will reflect genes involved in cell signalling and growth control and as such may be growth factors or proto-oncogenes.

If the inserted sequence were to be expressed in humans after accidental transfer the hairpin could lead to the down-regulation of a gene to which it targets cia RNAi interference. This could be a gene essential for growth regulation such as a tumour suppressor gene, therefore resulting in a potentially oncogenic change. This, however would not be severe as the virus is replication incompetent and therefore self-inactivating as none of the structural genes are actually present in the packaged viral genome so no new virus can be produced and no other cells can be infected. Physical insertion of the virus into the genome of the host cell could also potentially disrupt a tumour suppressor or oncogene, however the likelihood of this is very low.

Expression of cDNAs in human cells might induce a growth advantage on those cells. However, this is unlikely to cause significant, harmful effects as very few cells would be affected and the cDNAs used are not expected to have strong oncogenic potential.

VSV-G pseudotyped lentivirus can infect mammalian cells. Potentially the hairpin could be expressed in another mammalian species and lead to the down-regulation of a growth regulatory gene such as a tumour suppressor gene resulting in a potentially oncogenic mutation. This, however, is extremely unlikely as the hairpin is targeted to human genes and many hairpins are not homologous with the genes from other species. If the hairpin were to target and lead to the reduction of a gene via RNA interference it would not be severe as the virus is replication incompetent and therefore self-inactivating as none of the structural genes are actually present in the packaged viral genome so no new virus can be produced and no other cells can be infected.

If the cDNA-containing virus gained access to other animals, it could be expressed in another mammalian species and lead to the down-regulation of a growth regulatory gene such as a tumour suppressor gene resulting in a potentially oncogenic mutation. However, it is very unlikely that the cDNAs would gain access to another species and the viruses are non-replicative.

The envelope protein is VSV-G from the Vesticular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes), so it cannot make new progeny virus and
establish a productive infections, its physical insertion into cellular DNA could result in a potentially oncogenic mutation. This would be a very unlikely event and if so only a very small number of cells and would not impact on the pathogenic outcome of an exogenous viral infection. The packaging viral components are from the HIV-1 virus. The full length HIV-1 virus is harmful to humans, however the packaging vector used does not contain the full length HIV-1 molecule. Many components of HIV-1, that are critical for HIV-1 infection but not required for viral packaging, have been removed so the virus is attenuated.

The de novo hairpin donor sequences could potentially be expressed in cells and lead to the knockdown of certain genes leading to the increase of oncogenic potential of the virus. Similarly the cDNAs may have weak oncogenic potential. This would, however, only affect a very small number of infected cells and as the virus is replication incompetent and cannot make new viral progeny, it would no impact on the pathogenic outcome of an exogenous viral infection.

Evaluation of foreseeable effects

GMM/1: lines 1.17, 1.18, 1.32

(1.17) The envelope protein is VSV-G from the Vesticular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes), so it cannot make new progeny virus and establish a productive infection, its physical insertion into cellular DNA could result in a potentially oncogenic mutation. This would be a very unlikely event and if so only a very small number of cells and would not impact on the pathogenic outcome of an exogenous viral infection. The packaging viral components are from the HIV-1 virus. The full length HIV-1 virus is harmful to humans, however the packaging vector used does not contain the full length HIV-1 molecule. Many components of HIV-1, that are critical for HIV-1 infection but not required for viral packaging, have been removed so the virus is attenuated.

(1.18) The de novo hairpin donor sequences could potentially be expressed in cells and lead to the knockdown of certain genes leading to the increase of oncogenic potential of the virus. Similarly the cDNAs may have weak oncogenic potential. This would, however, only affect a very small number of infected cells and as the virus is replication incompetent and cannot make new viral progeny. It would not impact on the pathogenic outcome of an exogenous viral infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMM/1: line 1.29 or GMM/2: lines 2.13 (as applicable)

2.13 WASTE Management
Describe the waste management measures which you will apply to the activity. Include:
1. The treatment method,
2. Degree of kill,
3. Proposed process testing/monitoring measures to ensure this degree of kill will be achieved,
4. Ultimate form and packaging for removal from the department.

Note that for activity class 2 and 3 GMMs, the organisms must be inactivated by a validated method in the laboratory suite.

The wording is taken from HSE notification form CU2 section 12. This information must be supplied to the HSE when notifying a project.

1. 2.5% Trigene advance or Virkon (v/v) will be used to treat liquid viral waste (at least 18 hours) and also any spills and for disinfection of equipment and laboratory materials used. Work surfaces will be wiped down with 5% Trigene advance and 70% ethanol or IMS after use. Clorox will be used to treat liquid bacterial waste overnight.
2. 100%

3. Virkon and Trigen advance are certified by the PHLS to provide 100% viricidal kill under these conditions. Clorox provides 100% bacterial kill. Additionally Trigene, Clorox and Virkon will be used with their lifespan to ensure effectiveness. Autoclave runs are regularly validated by waste management staff.

4. Solid waste will be double-bagged in biological waste bags and then autoclaved 132°C for 15 mins then bagged in yellow biohazard bags and removed by UCL waste services

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
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<tr>
<td>Animal Units</td>
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<td>Human Clinical Applications</td>
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**Project Ref** 14/13.2

- **Date Ackn'd**: 29/04/2013
- **CU2 Project Title**: Differentiation and function of haematopoietic cells and lymphocytes
- **Class**: Non-GMM
- **Consent Granted**: Consent Granted

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**Date Project Ceased**: 02/03/2022
**Project Additional Information**

**Purposes of the contained use**

In this project we aim to investigate the molecular regulation of T-cell and erythrocyte differentiation. In particular we will focus on the Hedgehog, Bone Morphogenetic Protein and Notch signalling pathways. We will ask how these signalling pathways influence lymphocyte, myeloid and erythroid differentiation, by over-expressing or knocking-down components of the pathway in lymphocytes, erythroblasts, myeloid cells and their progenitors in cell culture, and measuring impact on function, differentiation, survival and cell cycle progression.

**Recipient or parental organism**

Lentivirus eg. HIV-derived lentiviruses, prepared in 293 cells Human and mouse haematopoietic progenitor cells, lymphocytes, erythroblasts, thymic epithelial cells, and cell lines.

The recipient organisms are not harmful to humans and are not capable of surviving in the environment.

**Host/vector system**

HIV-derived lentiviruses, prepared in 293 cells: eg. pLentiLox3.7 (pPLL) encoding eGFP with IRES, packaged in 293 T cells, with packaging proteins on separate plasmids (pMDL, pRev, pENV (vSVG)). This system is described in Rubinson et al, Nat Genet. 2003.

In some instances we may also use commercially available lentiviral supernatants.

The lentiviral vectors used are multi-attenuated, meaning they are devoid of all potentially pathogenic encoded functions. In addition they are replication-defective which means that the vector cannot multiply on its own unless supplied by certain proteins in trans. It is self-inactivating which means that the viral promoter in the U3 region of the 5' LTR has been disabled by genetic manipulation. Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The possibility of such an event is extremely low.

**Origin & function**

Human and mouse sequences

Lentiviruses - can infect human cells.

Puro and Neo - antibiotic resistance genes

The gene products of interest that we insert may modify the differentiation status, signalling capacity or cell cycle status of cells in which they are expressed. The fluorescent markers should have no functional impact on the cells in which they are expressed.
It is unlikely, but possible that knocking-down some of the genes of interest, or over-expressing others, might be oncogenic in human tissue, or contribute to oncogenic transformation if expressed in cells with other mutations.

The risk of transfer to species in the environment is negligible.

The lentiviruses are HIV-derived, but are replication-deficient, therefore represent a low risk.

The inserted genetic material has not been implicated in pathological characteristics of donor organism.

**Evaluation of foreseeable effects**

The lentiviruses are HIV-derived, but are replication-deficient, therefore represent a low risk.

The inserted genetic material has not been implicated in pathological characteristics of donor organism.

Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type virus. The probability of such an event is extremely low. The most hazardous GMM are the lentivector viruses and the most hazardous step is dealing with concentrated viral stocks before infecting the target cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All GMMs are inactivated for disposal using standard biosafety level 2 procedures. Briefly, liquid and solid wastes are treated with either PRESEPT effervescent disinfecting tablets (2.5g) Trigene, or 0.1% Virkon. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern. Virkon and Trigene have been shown to achieve 100% GMO kill when used as directed. Klercide and 70% ethanol are routinely used which have been shown to be 100% effective in tissue-culture related decontamination. Waste will be double-bagged in biological waste bags, sealed, autoclaved and then removed by waste services. Autoclaving will be at 121 degrees centigrade/15 pounds per square inch/for 15 minutes. This range is used to kill all pathogens and spores strips of the Bacillus stearothermophilus are used as indicators of the process. Expected kill: 100%. Tissue culture medium containing viral supernatant will be treated with 0.1% Virkon for at least 10 minutes prior to sealing for autoclaving as described.

Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.

All cultures, stocks, and other regulated wastes are decontaminated with detergent before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be disposed of off-site from the facility are detergent-treated and autoclaved prior to packaging in accordance with applicable local, state, and government regulations, before removal from the facility. Autoclaving will be at 121 degrees centigrade/15 pounds per square inch/for 15 minutes. This range is used to kill all pathogens and spores strips of the Bacillus stearothermophilus are used as indicators of the process. Expected kill: 100%
The UCL Genetic Modification Safety Committee has approved your experiment entitled Differentiation and function of haematopoietic cells and lymphocytes, as an Activity Class 2, Containment Level 2 project.

**Project Containment**

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**Project Ref 14/13.3**

<table>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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</thead>
<tbody>
<tr>
<td>25/07/2013</td>
<td>Identification of viral and host factors associated with the replication of VZV and the attenuated VZV</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
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<td>N</td>
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</tbody>
</table>

**Date Project Ceased**

Withdrawn  N  

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

2022/03/02
Varicella-zoster virus (VZV) infection is manifested as a vesicular rash and its replication in skin is central to the natural history. We aim to:

1. Study in detail the effect of VZV replication on keratinocytes and epidermal differentiation
2. Investigate the effect of epidermal differentiation on the replication of VZV and other alpha herpesviruses e.g. HSV1
3. Identify residues in the live attenuated VZV vaccine responsible for reversion of the vaccine back to a virulent wildtype phenotype.

This will be achieved by

1. Silencing/overexpressing human proteins of interest using lentiviruses
2. Introducing reporter cassettes into a VZV BAC to follow viral replication and cloning individual viral Open Reading Frames (ORFs) into a viral vector system.
3. Introducing single base changes by site directed mutagenesis in genes that have been identified as attenuating by deep sequencing of the live attenuated Oka Varicella-zoster vaccine virus, the wild type virus from which it is derived and other clinically derived samples.

The effect of the above will then be assessed in keratinocyte monolayers and 3-D skin models.

Recipient or parental organism

Top10 F' containing fragments of VZV DNA from a subcloning vector
SW102 containing VZV BAC;
SW102 containing VZV BAC modified with galK or kanSacB
pLNCX2 containing VZV ORFs
Common disabled E Coli K12 or B derivatives

Transfection of the VZV BAC DNA extracted from the recipient organism into permissive cell-lines will result in the production of VZV. VZV and HSV1 infect humans but cause a mild disease and most humans are exposed during childhood. Reactivation of VZV results in the development of herpes-zoster (Shingles) and HSV-1 reactivation results in cold sores. Complications can arise from infection of pregnant women. All DNA work will be carried out using the Oka parental wildtype virus genome bacterial artificial chromosome (BAC) cultivated in non-pathogenic SW102 E.coli. The donated sequences from shuttle vectors pGALK or pKANSACB have a history of safe use.

Introduction of modified VZV sequences into the BAC, by expression of the lamda prophage, within the non-pathogenic SW102 E.coli is not expected to alter the pathogenicity of the SW102 strain.

SW102 and Top10f'strains of E.coli have been modified such that it will only grow under laboratory conditions.

Herpesvirus particles are capable of survival in the environment. However, in environmental tests evidence has been presented that after 30 min at room temperature infectious VZV particles survival ranged from 0.1 to 19% depending on the surface tested (Levin et al Journal of clinical microbiology, Vol. 19, No. 6p. 880-883)

Host/vector system

Derivatives of pBR322
Commercially available vector used to subclone fragments of the VZV genome that will be modified by site directed mutagenesis e.g. Topo vectors
pgalK- available shuttle vector used to direct mutagenesis of BAC
pKanSACB- available shuttle vector used to direct mutagenesis of BAC
Commercially available vectors e.g. pLNCX2 (Clontech) will be used to express individual VZV ORFs.
Lentivectors and gammaretroviral vectors pLNCX2 (Clontech), CMV intron (MLV packaging plasmid from Cosset lab), VSVg
Introduction of point mutations using a site-directed mutagenesis approach will utilise plasmids containing fragments of the VZV genome to be mutated. The fragments of VZV DNA will not be able to be expressed in humans as the vectors used will not contain eukaryotic promoters. These plasmids therefore pose a low risk to workers.

PCR products amplified from pgalK or pKANSCAB contain genes under the expression of prokaryotic promoters. The genes (galK or kanamycin/SACB fusion) in these intermediate products are only capable of being expressed by bacteria and therefore pose low risk to human workers.

Introduction of mutagenic sequences into the VZV genome will be carried out in bacterial strain SW102 This strain is derived from DY380 and contains a lambda prophage recombinering system(http://web.ncifcrf.gov/research/brb/recombineeringInformation.aspx). The SW102 strain has been modified such that the SW102 bacteria will only survive under laboratory growth conditions. These cells therefore pose a low risk to workers.

Varicella Zoster Virus, HIV, MMLV, VSV, HSV1, human

PCR products used in the mutagenesis of the BAC amplified from the shuttle vectors pgalK, pKanSACB or pTopo do not contain eukaryotic promoter sequences and therefore are not expected to have biological activity in human tissue. PCR products amplified from pgalK or pKanSACB and successfully introduced into the BAC are however expected to allow the SW102 bacteria to grow under restricted growth conditions e.g minimal media + galactose (pGalk) or in the presence of kanamycin (pKanSACB). In addition PCR products amplified from pKanSACB are expected to make the bacteria sensitive to the presence of sucrose in growth medium.

VZV recovered from the modified BAC will be expected to maintain biological activity in Mewo cells but it is expected that introduction of the mutations will result in attenuation of the virus in explant culture or keratinocyte cell-lines.

Function of the individual Viral ORFs are varied from viral transactivators to glycoproteins. Human genes cloned will be involved in the process of epidermal differentiation, either as part of the epidermal structure e.g. cytokeratins, signalling pathway e.g. NOTCH, wnt etc or enzymes e.g. kallikreins.

Expression of BAC virus DNA in humans could result in the synthesis of infectious virus particles. Approximately 90% of the population has significant immunity to VZV and the infectious dose required to successfully initiate infection is thought to be high. It is therefore likely that accidental transfer of the virus would be successfully contained by host cell immunity.

The human and individual viral ORF inserts may cause a local immune reaction (skin, draining lymph node), with redness and swelling.

Humans are the only species that VZV can infect.

In susceptible individuals Varicella Zoster virus (VZV) is the causative agent of the acute infection Varicella (Chicken-pox). Reactivation of VZV results in the development of herpes-zoster (Shingles). Complications due to VZV infections are rare but can arise in adults, particularly pregnant women and immunosuppressed individuals. HSV1 is the causative agent of genital herpes and cold sores, complications occur in rare events, can carry risks for pregnant women and foetus.

In this project, regions of VZV DNA will be amplified from the complete VZV genome using PCR and cloned into the commercially available pTOPO vector. These fragments of DNA by themselves will not be pathogenic to humans. The DNA fragments are also not expected to alter the pathogenicity of the Top10F' E.coli harbouring the vector.

The lentivectors and gammaretroviral vectors are deleted for all coding sequences and are replication defective. The other viral sequences encode envelope proteins which are not pathogenic. Top10 F are a non-pathogenic strain of E.coli.
In susceptible individuals Varicella Zoster virus (VZV) is the causative agent of the acute infection Varicella (Chicken-pox). Reactivation of VZV results in the development of herpes-zoster (Shingles). Complications due to VZV infections are rare but can arise in adults, particularly pregnant women and immunosuppressed individuals. HSV1 is the causative agent of genital herpes and cold sores, complications occur in rare events, can carry risks for pregnant women and foetus.

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The lentivectors and gammaretroviral vectors are deleted for all coding sequences and are replication defective. The other viral sequences encode envelope proteins which are not pathogenic. Top10 F are a non-pathogenic strain of E.coli.

The most hazardous step in the procedure is the subsequent production of relatively large quantities of infectious VZV particles following recovery of GM-virus from the BAC by transfection of DNA into the permissive cell-culture line. Human infection via inhalation is considered the most significant hazard to laboratory workers.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Bacterial waste will be disinfected prior to disposal and solid waste autoclaved.

The treatment of waste this way will result in 100% kill of all micro-organisms used in this project.

Class II microbiological safety cabinets will be wiped down with trigene/distel (1:200) or absolute ethanol after work completed.

Liquid and solid waste will be treated with trigene/distel (1:200). Tissue culture plastics will be double bagged and autoclaved according to departmental guidelines, sharps e.g needles will be disposed of within a sharps bin and sent for incineration.

The treatment of waste by disinfection and incineration will be effective at killing 100% of VZV. In environmental tests VZV particles survival ranged from 0.1 to 19%, after 30 min at room temperature, depending on the surface tested (Levin et al Journal of clinical microbiology, Vol. 19, No. 6p. 880-883). VZV will not therefore survive disinfection, which will remove the virus envelope required for infection and incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The project was approved by the GMSC as AC2 following a number of minor amendments/clarifications.
The purpose of this project is to elucidate the precise molecular mechanisms how crosslinking of several members of TNF-R superfamily an IL-1R/TLR superfamily trigger various signalling pathways, i.e. activation of NF-κB, MAPKs or induction of cell death. In order to gain precise knowledge in the molecular mechanism employed by cells, we will use three basic approaches that will employ the use of replication-defective viral vectors. First, we will use retroviral MSCV based vectors to express proteins of interest containing immunoaffinity tag, enabling isolation of these proteins from cells. We will then use mass-spec to analyse the
molecular modification and protein composition of signalling complexes isolated from cells upon TNF-R superfamily or IL-1R/TLR superfamily stimulation. The advantage of using this approach is that we will be able to express our tagged protein at nearly physiological level and thus we will be able to study the physiological composition of signalling complexes triggered upon TNF-R superfamily an IL-1R/TLR superfamily stimulation.

Second, we will isolate mouse embryonic fibroblasts (MEFs) from mice deficient in the proteins of interest. The generation of knockout mice and isolation of MEFs cells does not involve viral vectors and thus is not described in this project risk assessment. The MEFs will be immortalised with large T antigen using lentiviral infection. This approach will enable us to study the signalling in the cells derived from various mouse strains.

Third, we will use lentiviral based vectors to introduce shRNA sequences that will target the mRNA of proteins of interest in cell lines. This will enable us to study the role of the proteins of interest in physiological settings.

This project requires production of viruses that can potentially infect human cells. However, all viruses used in this project will be replication-defective and therefore once the target cells are infected by virus, they will not produce new viral particles. Thus the critical part of this project is viral production and cell infection that must be carefully planned and monitored.

This project was previously performed under the licence from Imperial College London and transferred to UCL in February 2013.

Recipient or parental organism

Retroviral particles will be produced in Phoenix-Ampho (human cell lines) and Phoenix-Eco (mouse cell lines). Phoenix cells are based on the 293T cell line expressing gag-pol and envelope proteins.

Lentiviral particles will be produced in Hek293T.

Recipient human cell lines will be K562, U937, THP1, A549, HepG2 and Jurkat

Recipient mouse cells will be mouse embryonic fibroblasts

The produced viral particles are potentially pathogenic, as they can infect human tissues. However, the produced viral particles are replication defective and thus cannot propagate upon infection. Furthermore, viral particles are highly unstable and are quickly inactivated in cell culture. The most dangerous viral particles produced will be the lentiviral particles containing large-T sequence, that is potentially oncogenic.

The intended target cell lines are not pathogenic and are not able to survive outside the laboratory environment. Upon infection with replication-defective viruses, the target cell lines will not be able to create new viral particles. Some of the recipient cell lines used in this study are immortalized. Accidental infection or inhalation or exposure to wounds could enable these cells to enter the bloodstream or airway and lungs of the operator and this could subsequently lead to potential harmful effect. It is very unlikely that used cell lines will be able to survive in the recipient as these cells will be recognised and disposed of by the immune system.

Host/vector system

For retroviral infections we will use MSCV containing a multiple cloning site followed by IRES and EGFP sequence, enabling selection of cells that have been infected as GFP positive:

MSCV containing IRES-EGFP (Clontech)

For lentiviral infections we will use following constructs:

pMD2G (Tronolab) … Envelope Plasmid for Virus Production in Combination with psPAX2
psPAX2 (Tronolab) … Packaging Plasmid for Virus Production in Combination with pMD2G
pFU-large T vector (kindly provided by John Silke)
… vector containing the sequence of large T that will be transported to the target cell line
For lentiviral expression of shRNA the vector used will be pLKO.1 from Sigma, together with pMD2G and
psPAX2
Neither retroviral (MSCV) nor lentiviral (pFU) vectors containing the sequence of genes of interest
can be transcribed in prokaryotic cells. These vectors do not contain sequences coding accessory
proteins and thus could not form viral particles on their own and do not possess any danger to health or
environment.
In order to produce viral particles, the retroviral vectors must be transfected to Phoenix cell lines that
were previously stably transfected by other two different proteins encoding accessory proteins.
In order to produce lentiviral particles additional two vectors (pMD2G and psPAX2) must be
transfected to HEK293T alongside with pFU-large T or pLKO.1 vectors.

Origin & function

The sequences of genes that will be used in this study are derived from mRNA sequences expressed
in human and mouse tissues.
The sequences of shRNA will be obtained from Sigma together with the expression plasmids.
In this project we will be expressing gene sequences coding several proteins involved in signal
propagation upon TNF-R superfamily an IL-1R/TLR superfamily triggering:
FADD is adaptor linking certain members of TNF-R superfamily an IL-1R/TLR superfamily receptors
to downstream signalling pathway. It functions as an adaptor protein.
Caspase-8 is enzyme involved in initiation of apoptotic cell death upon certain types of stimulation.
RIP1, RIP3 are kinases that mediate activation of certain signalling pathways.
HOIP, HOIL-1, Sharpin are subunits of linear ubiquitin chain assembly complex, that is responsible
TRAIL-R1, TRAIL-R2 and CD95 are members of TNF-R superfamily.
The retroviruses produced in Phoenix-Ampho and lentiviruses are potentially able to infect human
cells. Both lentiviral and retroviral particles are not able to propagate upon infection, thus even after
accidental infection of human cells they will not be able to replicate and infect other cells in the body.
However, there is a risk that upon integration to human cells the viral delivered sequences would be
integrated in genome and this could somehow induce tumorigenic transformation of infected cells. Albeit
unlikely, this danger is common to all retroviruses and lentiviruses used in research and special
measures must be taken so such a situation would not happen.
The intended sequences encode proteins that are not known to be oncogenic, with the exception of
large T antigen that will be used for immortalisation of mouse embryonic fibroblasts.
Since the viruses upon infection are not able to propagate and the genes analysed are derived from
mouse and human genes, it is unlikely that expression of these sequences would cause any harm to
other species in environment. Furthermore, the virus particles are highly unstable outside the laboratory
environment.
The cell lines used for viral production are not pathogenic on their own and they are not able to
survive outside the laboratory environment. However, it should be noted that the cell lines used in this
study are immortalized. Accidental injection or inhalation or exposure to wounds could enable these cells
to enter the bloodstream or airway and lungs of the operator and this could subsequently lead to potential
harmful effect. It is very unlikely that used cell lines will be able to survive in the recipient as these cells
will be recognised and disposed of by the immune system.
Special care should be taken during the production of viral particles. As described in section 1.15 the retroviruses produced in Phoenix-Ampho and lentiviruses are potentially capable of infecting human cells. Thus direct exposure of operator to the viral containing supernatant or to the suspension of virus producing cells can lead to potential infection. This danger is even increased when the virus containing supernatant or virus producing cells would be injected in the bloodstream of operator by sharps or if exposed to open wounds or if inhaled. To minimise this danger, no sharps will be allowed and protective coat and gloves will be worn. All work will be carried in class II cabinet.

Evaluation of foreseeable effects

The cell lines used for viral production are not pathogenic on their own and they are not able to survive outside the laboratory environment. However, it should be noted that the cell lines used in this study are immortalized. Accidental injection or inhalation or exposure to wounds could enable these cells to enter the bloodstream or airway and lungs of the operator and this could subsequently lead to potential harmful effect. It is very unlikely that used cell lines will be able to survive in the recipient as these cells will be recognised and disposed of by the immune system.

Special care should be taken during the production of viral particles. As described in section 1.15 the retroviruses produced in Phoenix-Ampho and lentiviruses are potentially capable of infecting human cells. Thus direct exposure of operator to the viral containing supernatant or to the suspension of virus producing cells can lead to potential infection. This danger is even increased when the virus containing supernatant or virus producing cells would be injected in the bloodstream of operator by sharps or if exposed to open wounds or if inhaled. To minimise this danger, no sharps will be allowed and protective coat and gloves will be worn. All work will be carried in class II cabinet.

The most hazardous step is production of viral particles, that can potentially infect dividing (in case of retroviruses) or both dividing and non-dividing (in case of lentiviruses) human cells. Upon accidental infection of human cells the viruses can no longer divide and thus the cells will not propagate the infection further.

If an accidental infection of human manipulator would occur, two risks are emerging. First, just by introducing the viral sequence in human DNA can promote oncogenic mutation. Second, the expression of target sequences can lead to some modification of cells that can be oncogenic. All sequences used in this study are however non-oncogenic, with the notable exception of mouse large T antigen used for mouse embryonic fibroblast immortalisation.

Thus the most hazardous procedure employed in this project is the production of lentiviruses that will transduce large T antigen to target cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. The treatment method
   All liquid waste will be incubated overnight in the presence of 5% Trigene advance. Any spills will treated with 5% Trigene advance. Work surfaces will be cleaned with 1% Trigene advance followed by 70% ethanol. Laboratory equipment will be decontaminated with 1% Trigene advance followed by 70% ethanol. All solid waste will autoclaved at 134 °C for 30 minutes.
2. Degree of kill
The degree of kill is expected to be 100%. According to our supplier of Trigene Advance (also manufactured under new commercial name Distel) StarLab this compound at 1% concentration is highly active virucidal agent. The killing of several viral strains, including HIV, has been tested by using EPA protocol and complete deactivation was observed.

3. Proposed process testing/monitoring measures to ensure this degree of kill will be achieved:
Trigene advance has been tested to provide complete killing of various viral strains. The functioning of autoclave is validated by waste management staff on regular basis by thermocouples.

4. Ultimate form and packaging for removal from the department:
Upon inactivation with 5% Trigene advance the liquid waste will be disposed in dedicated sink. The solid waste will be disposed in biological waste bags, autoclaved and disposed by UCL waste services.

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>Human Clinical Applications</td>
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Project Ref 14/13.5

- Date Ackn'd 25/07/2013
- CU2 Project Title Understanding the coordination of hepatitis C virus entry and humoral immune evasion
- Class 3
- Culture Vol Class 2 1-50 litres
- Culture Volume Class 3-4 <1 litre

Project Containment

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The UCL GMSC approved this as a Class 2 activity following a minor clarification regarding the use of personal protective equipment.
Previous studies indicate that the mechanism of HCV entry is intrinsically linked to virus particle antibody evasion. This study aims to clarify the interdependence between HCV entry and immune evasion.

HCV establishes a persistent infection in - 80% of cases, leading to hepatitis, cirrhosis and hepatocellular carcinoma. Chronically infected patients raise HCV-specific antibody responses capable of preventing virus interaction with target cells in vitro. However, HCV persists in the face of these apparent neutralising antibodies (nAbs), suggesting that HCV exhibits immune evasion strategies.

The entry of HCV into hepatocytes is a complex process that involves at least four cellular receptors. Like other viruses that use multiple entry factors (e.g. HIV and rotavirus), it is thought that HCV undergoes an ordered sequence of receptor interaction, with each step being accompanied by a conformational change in the virus particle. Such a programme of molecular events allows viruses to shield critical epitopes until the moment that they are required to allow, for instance, co-receptor interaction or membrane fusion. Consequently there is only a narrow window of opportunity in which antibody-mediated neutralisation can occur, allowing the virus to achieve entry to the face of humoral immune assault.

Patient derived HCV is hard to propagate in vitro, therefore studies will be performed with a panel of cell culture proficient infectious clones, referred to here as HCVcc. In this study we will use the HCVcc system to investigate how HCV entry mechanisms inform humoral immune evasion strategies and vice versa. Specifically, we will propagate HCVcc, allowing us to characterise certain properties of virus particles, such as entry mechanisms and sensitivity to neutralising antibodies. We will monitor viral evolution in cell culture and patient quasispecies diversity by sequencing, this will involve extraction of viral RNA, conversion to cDNA and potential onward cloning of these sequences. We will also purify virus particles for biochemical analysis, for instance, by density gradient centrifugation. Viral sequences encoding specific proteins may be sub-cloned to allow production of virus protein in isolation, however these will not pose any risk to human health.

All work with replicative virus will be carried out at containment level 3 (CL3)

Our ultimate goal is to understand the molecular events that allow coordinated HCV entry and nAb evasion. In doing this we hope to design therapies and vaccines to interfere with these mechanisms therefore accessing the untapped potential of patients own humoral immune response.
is possible to cure infected individuals with a combined regime of interferon and ribavirin, furthermore, a range of new anti-virals are dramatically improving treatment efficacy. Notably, the HCVcc infectious clone is a genotype 2 virus, and as such is particularly sensitive to interferon based treatment.

HCV is principally transmitted by percutaneous inoculation. In infected patients the primary routes of infection are needle sharing amongst intravenous drug users and blood transfusions. In a research setting, the primary risk comes from contaminated sharps or, potentially, eye-splash exposure. However, these risk are all but eliminated under CL3.

Host/vector system

HCV is an RNA virus, the HCVcc system comprises the viral genome encoded as DNA in a bacterial cloning vector (pGEM-T Easy) downstream of a T7 RNA polymerase promoter. The DNA clones are generated and maintained in standard disabled bacterial strains. Full-length infectious RNA genomes are transcribed from the promoter and then introduced in to human hepatoma cells by electroporation. The electroporated cells are then cultured under containent level 3 conditions to produce infectious virus particles that can be propagated further.

Origin & function

The original clones were derived from human patients specifically GenBank accession' AB237837.1 and derivatives thereof, originally describe in Lindenbacg, Science, 2005,309,p623-6. We will modify these sequences by introduction of specific mutations that modulate virus entry and immune evasion, and protein tags/fluorescent proteins that allow us to visualise and quantify viral infection. We may also introduce viral sequences derived from patient isolates to study how viruses evolve in their natural host. Viral sequences encoding specific proteins may be sub-cloned into other plasmids to allow production of virus proteins in isolation, however these will pose no risk to human health.

Evaluation of foreseeable effects

Cell culture infectious (HCVcc) is a class three virus capable of causing chronic hepatitis and progressive liver disease. However, it is transmitted exclusily be percutaneous inoculation and is not capable of transmission by air or ingestion. Replicative HCVcc particles and infected cells are handled and stored under containent level three (CL3), this all but eliminates the possibility of accidental inoculation. Notably, although aerosol droplets containing HCVcc may be generated by pipetting and/or vortexing, these will be contained within the microbiology safety cabinet (MSC) and are not a viable route of transmission.

The risks posed by genetically modified HCVcc essentially mirror that of wild type virus; these viruses will have the ability to establish chronic infection and progressive illness in exposed individuals.

We intend to make specific mutations in the viral genome and insert protein tags to visualise virus or monitor infection. We may also introduce sequences mutations in the viral genome and insert protein tags to visualise virus or monitor infection. We may also introduce sequences derived from patients quasispecies. There is a possibility that the specific mutations will increase viral fitness or pathogenesis, whereas it is highly unlikely that the insertion of protein tags will be advantageous to HCV. Importantly, it is extremely unlikely that the proposed modifications will render the virus transmissible by any novel route, for instance by airborne transmission. HCV is highly adapted to its biological niche and multiple. Complex adaptations would be required to alter its route of transmission, it is arguable whether these adaptations would ever be selected for in an experimental setting.

As with wild type virus, CL3 confers a very low risk of exposure.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containent for the Class of activity. (Measures & Justification)

We request that electroporation of human hepatoma cells with HCV RNA be performed at class 2 containent. Once electroporated the cells can be considered infected, however, the virus life cycle dictates that infectious virus particles will not be produced before 12 hours post electroporation (Tellinghuisen et al., 2008, PLoS Pathog 4(3):
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus inactivation will be achieved by overnight incubation in 10% virkon, this will occur in a sealed vessel within a MSC. Contaminated solid waste will be treated in a separate vessel, also inside the MSC. All waste, including inactivated virus, contaminated solid waste and non-contaminated material will be sealed in bags and autoclaved, liquid waste and virkon baths will be solidified using verna gel prior to autoclaving. A standard autoclave destruct run will be used to inactivate waste (30 mins at 135°C). Inactivated and autoclaved materials will be disposed of as clinical waste.

It is impractical to assess the degree of kill regularly, additionally, the concentration of virkon in the inactivation solution will invalidate the standard infectious assay. However, HCV is a relatively instable enveloped virus and extremely sensitive to detergent. The initial virkon treatment will disrupt the virus envelope, thus inactivating the particle, subsequent autoclaving will denature all virus proteins. Based on World Health Organisation guidelines and experimental evidence (Song et al. Virology Journal 2010, 7:40), the above treatment is expected to inactivate all virus.

A record of the autoclave runs will be made, in the event of a failed run the waste will be autoclaved again. Given the level of redundancy in the waste disposal protocol, it is highly unlikely that virus inactivation will fail.

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 14/13.6
The ultimate goal of this project is to develop novel autologous cell therapy strategies for patients affected by different forms of muscular dystrophy. This project will be composed of several different aims, detailed below:

1. Genetic correction of patients' mesoangioblasts by transfer of a Human Artificial Chromosome, expressing dystrophin and several beneficial functions, followed by transplantation into dystrophic/immune deficient mice to test their ability to alleviate the dystrophic phenotype. For other forms where the mutated gene is not large, target selecting lentiviral vectors will be used.

2. Derivation of patients induced pluripotent stem cells (iPSC) from patients affected by muscular dystrophies such as Limb Girdle Muscular Dystrophy 2D, characterized by a strong reduction of pericytes from which mesoangioblasts are derived, or from patients at an advanced stage of disease, when skeletal muscle biopsies no longer yield cells able to proliferate in culture. iPSC cells will be derived from fibroblasts of these patients, expanded, genetically corrected and then differentiated to mesoangioblasts according to a protocol developed in the lab (Tedesco et al., 2012). These cells will be tested for their ability to alleviate the dystrophic phenotype as described in 1.

3. In vivo generation of additional, functional artificial muscles (Fuoco et al., 2012) by combining mesoangioblasts and special biomaterials and implanting the device sub-cutaneously. The artificial muscle receives vessels and nerves from the host and matures almost identically to the underlying normal muscle. This strategy may benefit localized forms of muscular dystrophy (e.g. Oculo-Pharingeal Muscular Dystrophy) or localized lesions of skeletal muscle due to trauma, surgical resections, senile or iatrogen lesion of sphincters.

**Recipient or parental organism**

Organism from which the sequence coe from: Human, Mouse, Dog

Vectors: Lentivirus, Retrovirus

**Host/vector system**
Lentivirus: 2nd and 3rd generation vector with virulence genes deleted

Cells: standard competent cells (XL10-Gold and XL1-Blue Agilent Technologies), Murine cells, Human cells, Canine cells

Origin & function

Origins and Functions of the genetic material involved:

Reprogramming factors (described below); reversible immortalization of cells lines (described below); cDNA of genes encoding for muscle proteins mutated in muscular dystrophies, for transcription factors regulating muscle development and regeneration.

The over expression of the four reprogramming factor (Oct4, Sox2, Klf4 and cMyc) reprogram the target somatic cells. The individual known functions and expected activity of each of the 4 reprogramming factors is described below:

Oct4: is a key transcription factor in pluripotency, its highly expressed during embryonic development and in embryonic stem cells. It acts co-operatively with Sox2 and Nanog to promote pluripotency and suppress differentiation.

Klf4: It’s known to bind putative regulatory elements of Oct4, Sox2, Nanog and other pluripotency associated genes. His overexpression is thought to upregulate Nanog and to repress p53.

C-Myc: is a transcription factor that plays roles in cell cycle progression, apoptosis and cellular transformation. The overexpression of cMyc in somatic cells is expected to promote reprogramin to a pluripotent state.

Regarding the immortalization factors, the simultaneous expression of Bmi-1 and hTert is expected to allow the cells to not enter into senescence.

Bmi-1: the expression of Bmi-1 promotes the cell cycle, it’s a negative regulator of p16 (inhibitor of the cell cycle). His co-expression with hTERT is expected to immortalize the target cells (human and canine mesangioblasts).

hTERT: it’s a catalytic subunit of the enzyme telomeras (RNA-dependent polymerase). Telomerase is a ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG. The enzyme consists of a protein component with reverse transcriptase activity, encoded by this gene, and an RNA component that serves as a template for the telomere repeat. Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells, resulting in progressive shortening of telomeres. Specifically TERT is normally repressed in postnatal somatic cells, resulting in progressive shortening of telomeres. Specifically TERT is responsible for catalyzing the addition of nucleotides in TTAGGG sequence to the ends of a chromosome's telomeres. This addition of repetitive DNA sequences prevents degradation of the chromosomal ends following multiple rounds of replication. hTERT is often up-regulated in cells that divide rapidly, including both embryonic stem cells and adult stem cells. It elongates the telomeres of stem cells which consequently increases the lifespan of the stem cells by allowing for indefinite division without shortening of telomeres. Therefore, it is responsible for the self-renewal properties of stem cells. High expression of hTERT is also often used as a landmark for pluripotency and multipotency state of embryonic and adult stem cells. Over-expression of hTERT was found to immortalize certain cell types as well as impart different interesting properties to different stem cells.

Evaluation of foreseeable effects

All our viruses are replication defective. The retroviral and lentiviral vectors used lack viral structural genes and require [ackaging cells to generate retro/lentivirus, therefore they are non-replicating.

The four reprogramming factors are standard practice in IPS cell research and no reports of safety issue have arisen where proper precautions have been taken. If there were an accidental transfer to humans the likelihood of the person developing IPS cells is negligible given that the efficiency of generating human IPS cells from target cells is only 0.02% under favourable culture conditions. Moreover, these retroviruses can only infect actively dividing cells, so is unlikely to be able to infect human cells readily.
The episomal plasmids cause no harm to humans as they are used as naked DNA plasmids. They will no be transfected into viral packaging cells to generate virus. Even if directly transmitted into a human by accidental transfer the plasmid DNA would be immediately degraded by DNA nucleases on the skin and by other host defences.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

2.5% (v/v) Virkon will be used to treat liquid waste (left overnight) and also used to decontaminate any spills and for disinfection of any reusable equipment and laboratory materials used. Liquid waste will subsequently be disposed in a tank for the biological liquid waste. Work surfaces will be wiped down with 70% ethanol. Virkon was certified by the PHLS to provide 100% GMO kill under these conditions by denaturing and inactivating cells proteins. Solid waste will be double bagged in biohazard bags and removed by UCL waste services validate all the autoclave cycles to ensure no risk to the environment.

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Animal Units

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Project Ref 14/14.1
The research that we have been carrying out is focused on skin diseases including rare genetic skin diseases Netherton syndrome (NS), recessive dystrophic Epidermolysis Bullosa (RDEB), keratitis-ichthyosis-deafness syndrome (KID), and a far more common disease, atopic dermatitis (eczema). We want, through the research, to understand the patho-mechanisms underlying these conditions and develop more specific treatments for these diseases.

For genetic skin diseases, we want to genetically modify patients' stem cells by delivering either wild type gene or siRNA to cells. Lentiviral vectors have been and will be used for the delivery tool as other methods such as non-viral delivery are not efficient enough, especially in primary skin cells (keratinocytes) and some other cell lines. We are also carrying out the research on eczema. Eczema is a multifactorial disease with the feature of impaired skin barrier function. The pathogenesis is complex and remains unclear. We want to use approaches of gain- and loss- of function of candidate genes to demonstrate the patho-mechanism of dysfunctional skin barrier in eczema. Lentiviral vectors will be used to deliver and express candidate genes or siRNAs into skin cells, and the biological interference of these genes involved in skin barrier formation and function will be studied.

As primary skin cells have a limit lifespan under in vitro culture condition, we need to immortalise primary cells with E6 and E7 genes from human papilloma virus type 16 (HPV16) to expand cells' lifespan. Like all papilloma viruses, HPV 16 establishes productive infections only in keratinocytes of the skin or mucous membranes. While the majority of the known types of HPV cause no symptoms in most people, HPV16 can - in a minority of cases - lead to cancers. Therefore, E6/E7 proteins are oncogenic. Vectors used in the project are non-replicating and the most of genes to be expressed by these vectors (listed in section 1.07) are non-pathogenic, non-oncogenic and non-hazardous, except from E6/E7.

The cDNAs listed below have been or will be cloned. These viruses are intended to be used for tissue culture studies in mammalian cell, in invitro organotypic culture and in vivo mouse-human chimaeric skin grafting model. These animals are normally studied for a period of up to six months.

The maximum culture volumes of any of the above handled on one occasion will be less than 210mls for lentivirus production (with each tissue culture flask containing no more than 20 ml of medium for virus production). The inactivation of lentivirus will be by PRESEPT tablets, Distel (formally Trigene) and 70% IMS for liquids and by autoclaving for solids and plastics.

Recipient or parental organism

E. coli disabled strains (e.g. DH5alpha, JM109, Stbl 3);
Mammalian cell lines (established or commercially available), mammalian primary cells including keratinocytes, fibroblasts and bone marrow stem cells;

Replication-deficient self-inactivating (SIN) lentiviral vectors which are based on third generation lentiviral vectors (i.e. pCCL SIN) or second generation vectors (i.e. pHIREN) with engineered safety features which are resembling third generation vectors in terms of safety.

Mooney murine leukemia virus (MoMuLV)- derived vectors (MSCV, Sfada/W, SRS11) lacking international non-proprietary names (INN), again representing a similar level of safety, when using inserts which are deemed harmless.

The following promoters will be used in the lentiviral vectors: Spleen focus forming virus (SFFV), CMV, EF1 alpha, PGK; tissue specific promoters including human involucin promoter, human actin promoter and human SPINK5 promoter.

Commercial TZV vector derivative pGIPZ (from UCL siRNA library) with various hairpin sequences.

The bacterial strains used are attenuated non-colonising strains, so they are incapable of causing human infection and are therefore harmless to humans.

The viral vector to be used will be pseudotyped with the envelope protein VSV-G from Vesticular Stomatitis Virus. If introduced into the body by injection, ingestion or through a wound, the virus could enter human cells and integrate into cellular DNA. Although the virus is replication incompetent so they cannot self-replicate and transfer to another host so are harmless to the environment.

The cell which will eventually receive and integrate the DNA cannot survive in the environment as they are only viable under culture conditions and therefore are harmless to the environment. The mice to be reconstituted with transduced bone-marrow or keratinocytes (skin cells) could conceivably escape into the environment but would be unable to transmit the viral construct to another organism and also not able to transmit in the germ line to an offspring.

Lentiviral Gag/pol expression vectors p8.9 and derivatives (HIV-1). Viral expression vector pMDG and derivatives (VSV-G)

ORIGINS AND FUNCTIONS OF THE GENETIC MATERIAL INVOLVED

Apart from HPV16 E6/E7 which are oncogenes (detailed description see 1.10), all other insert sequences listed above are human cDNA sequences, NONE of which fall into any harmful category, such as:
- oncogenes and proto-oncogenes,
- growth factors and GF receptors
- any other sequences coding for significant biological activity that might be harmful were these to be expressed in people accidentally exposed to the vector.

Sleeping beauty transposase (salmonoid) in present form deemed harmless.

siRNA hairpin sequences in either the commercial TZV vector from Tranzyme and derivatives (including pGIPZ), or in above SIN lentiviral vectors. These siRNA sequences are intended to target and down-regulate expression of the following human mRNAs only: SPINK5, KLKs, GAPDH, EGFP, PAR2. All of these siRNA target down-regulations, even if accidentally occurring after user exposure are not expected to cause any harm, due to a combination of the function of the targets, no expected harmful action of other molecules when presence of lower amounts of the target, and the expected relatively low amount of cells involved.

Host/vector system

Lentiviral Gag/pol expression vectors p8.9 and derivatives (HIV-1). Viral expression vector pMDG and derivatives (VSV-G)

ORIGINS:

Homo sapiens

ORIGIN AND FUNCTIONS OF THE GENETIC MATERIAL INVOLVED

Apart from HPV16 E6/E7 which are oncogenes (detailed description see 1.10), all other insert sequences listed above are human cDNA sequences, NONE of which fall into any harmful category, such as:
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WILL THE SEQUENCE CAUSE HARM IF EXPRESSED IN HUMANS AFTER ACCIDENTAL TRANSFER

If the inserted sequence were to be expressed in humans after accidental transfer cDNA sequences or the RNAi hairpin (described in section 1.10), they could lead to expression of de novo functional proteins or to the down-regulation of the gene to which it targets via RNAi interference, respectively. Such expression of the inserted cDNAs are, however, all deemed to be of little significance due to the non-harmful nature of the sequences used and the expected accidental exposure in cell numbers. Accidental targeting of RNAi in humans is not deemed to be of a harmful nature due to a combination of the function of the targets, no expected harmful action of other molecules when presence of lower amounts of the target, and the expected relatively low amount of cells involved. In addition, for both cDNA inserts and siRNA, the effect would also not be severe due to the fact that the virus is replication incompetent and self-inactivating as none of the structural genes are actually present in the packaged cell genome, so no new virus can be produced and no other cells can be infected. Physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene, however the likelihood of this is low and is drastically reduced by the lack of use of sharps when handling virus. Although E6 and E7 are oncogenes, accidentially occurring of inserted nucleic acid after user exposure are not expected to cause severe harm. The reasons are

i. The oncogenic activities of HPV E6/E7 genes have been documented extensively (Munger et al. Journal of Virology, 2004, p11451-11460). The expression of E6/E7 in primary human keratinocytes effectively facilitates their immortalization. However, HPV immortalized cells are nontumorigenic. They can undergo malignant progression only after extended growth in tissue culture or when additional oncogenes such as ras or fos are expressed. The development of cervical cancers in a transgenic mouse model in which E6/E7 is expressed in basal epithelial cells is also dependendt on long-term exposure to low doses of estrogen. Similarly, progression of HPPV-positive cervical lesions in human occurs at a low frequency and requires the acquisition of host cellular mutations. Therefore, it is unlikely that one will develop skin cancers after exposure E7/E7 to the skin in the lab. In fact, E6/E7 genes have been used to immortalise keratinocytes for more than 20 years in skin research, but there was no report that tumorous cells were grown in people carrying out the experiments.

ii. If the oncogenes were to be expressed in human after accidental transfer, E7/E6 could lead to a potentially oncogenic mutation. This, however, would not be severe as vectors used for our study is replication incompetent and can be produced and no other cells can be infected.

iii. Physical insertion of the genes into the genome of the host cells could potentially disrupt the tumour suppressors. However, as E6/E7 is only partial genes of HPV16, they won't be assembled /matured into virions without the expression of HPV16 late genes L1 and L2 in the transduced cells. So again, the infection would be limited and no new cells can be infected.

WILL THE SEQUENCES CAUSE HARM IF TRANSFERRED TO SPECIES IN THE ENVIRONMENT

No, risk of transfer to environmental species is negligible, and would not be expected to cause harm. VSV-G pseudotyped lentivirus can only infect mammalian cells. Potentially genes or the siRNA hairpin constructed in viruses could be expressed in another mammalian species and lead to gene over expression or the down-regulation of target genes. This is, however, extremely unlikely as the both transgene and hairpin is expressed/targeted to mammalian genome. If happened, it would not be severe as the virus is replication incompetent and self-inactivation. None of the structural genes are actually present in the packaged viral genome, so no new virus can be produced and no other cells can be infected.
IS THE DONOR ORGANISM PATHOGENIC?

The envelope proteins in viral vectors are VSV-G from the Vesticular Stomatitis virus. If introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes), so it cannot make new progeny virus and establish a productive infection, its physical insertion into cellular DNA could result in a potentially oncogenic mutation. However, this would be a very unlikely event and if so only a very small number of cells and would not impact on the pathogenic outcome of an exogenous viral infection. The packaging viral components are from the HIV-1 virus. The full length HIV-1 molecule. Many components of HIV-1 that are critical for HIV-1 infection but not required for viral packaging have been removed, so the virus is attenuated.

IF THE DONOR ORGANISM HAS PATHOLOGICAL OR HARMFUL CHARACTERISTICS, ARE THE DONATED SEQUENCES INPLICATED IN THEM?

The de novo cDNA inserts or hairpin donor sequences could potentially be expressed in cells and lead to the expression of a novel gene or knockdown of the expression of certain genes. However, the inserts/siRNA are all deemed harmless or of very low risk. In addition, this would only involve a very small number of cells and as the virus is replication incompetent and cannot make new viral progeny, it would not impact on the pathogenic outcome of an exogenous viral infection.

SUMMARISE THE POTENTIAL FOR HARM IN THIS PROJECT

Only a very unlikely multiple recombinations during the process of generation of the viral vector can expose the person to wild type HIV-1. The probability of such an event is extremely low. The most hazardous GMM are the lenti-vector viruses and the most hazardous step is the harvesting of the viral supernatant before infecting target cells. However, the most of the inserts used throughout this work is deemed to have harmful effects, except from the oncogenes E6 and E7.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

I) The treatment method: Liquid and solid waste are treated with PRESEPT effervescent disinfectant tablets (2.5g/tablet in 2-5 litres). All plastic-ware used in tissue culture are autoclaved. Work surfaces are decontaminated on completion of work and/or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern. Distel (formerly Trigene) and then 70% ethanol or IMS are used during tissue culture and virus preparation procedures to ensure high effective in tissue-culture related decontamination

II) Degree of kill: 100%

III) Proposed process testing/monitoring measures to ensure this degree of kill will be achieved: Both PRESEPT effervescent disinfecting tablets and Distel are certified by Public Health England (PHE) to provide 100% vircidal kill under these conditions. Additionally, PRESEPT and Distel will be used with its lifespan to ensure effectiveness. Autoclave runs are regularly validated by waste management staff twice a year.

IV) Ultimate form and packaging for removal from the department: Solid waste will be double -bagged in biological waste bags and then autoclaved at 132C for 15 mins, then bagged in yellow biohazard bags and removed by UCL waste services.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Biological Safety Adviser approved this as activity class 2 after the minor concerns of the GMSC advisers were satisfactorily addressed

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 14/14.10

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022  Page 812 of 15326
Use of lentivirus vectors to deliver cDNAs and shRNAs to canine, mouse, human cell lines, mouse primary cells (MEFs) and whole mouse embryos (in vitro culture)

This project is concerned with determining the processes that control the development of neural tube. Defects in neural tube development can give rise to neural tube disorders accounting for 1 to 1000 birth defects. The overall aims of this project are to understand the molecular mechanisms underlying the developmental process of neural tube formation. We are particularly, but not exclusively, interested in targeting the genes, Grhl2 and Grhl3, encoding grainyhead-like protein transcription factors. The purpose of the project is to over-express and knock-down expression of these genes, both of which cause neural tube defects in mouse models. These aims will require lentiviral production to introduce cDNAs or shRNAs into the cells using viral delivery to test the function of grainyhead-like proteins in neural tube closure. Our specific goals are to reduce the expression of grainyhead-like proteins in cell lines in order to identify the downstream target genes and to overexpress these proteins to examine the functional effect on cell behaviour. This could lead to further development of chemical or RNA/DNA-based regulators of grainyhead-like proteins that could be used therapeutically to tackle neural tube disorders.

Recipient or parental organism

Cell lines: Human 293T cells, canine MDCK cells, murine IMCD3 cells, murine NIH-3T3s and other epithelial and endothelial lines Whole mouse embryo in vitro culture (Embryonic days 8.5 and 9.5) Primary murine cells: MEFs

Host/vector system

Lentiviral vectors: The vector is a replication-deficient self-inactivating (SIN) lentiviral vector based on second generation vectors (i.e. pHR'SIN) with engineered safety features resembling third generation vectors in terms of safety using the spleen focus forming virus (SFFV) promoter. The 3rd generation vectors express gag and pol from one packaging vector and rev from another. 3rd generation packaging systems do not express tat. pGIPZ, lentiviral Gag/pol expression vectors, viral envelope expression vector.
Grainyhead-like proteins 2 and 3 are transcription factors essential for neural tube development. cDNA encoding Grainyhead like proteins will be amplified by RT-PCR using RNA derived from tissue of a wild-type mouse.

The RNAi hairpins are sequences that have been derived de novo and are not from an organism. They have been designed as small sequences with both a sense, anti-sense and loop sequence to form a RNA hairpin when transcribed which will initiate a downstream cascade of events leading to knockdown of the target gene to which the hairpin was designed.

In order to fully characterise the function of grainyhead-like proteins we will also introduce cDNAs or shRNAs targeting grainyhead-like protein target genes (transcriptionally regulated by Grhl proteins) into cells. The genes to be knocked down/over-expressed will be decided on the basis of transcriptomic data from ongoing experiments in mice and from RNA sequencing profiling of Grhl expressing cell lines.

If the inserted sequence were to be expressed in humans after accidental transfer, the cDNA sequences or the RNAi hairpin could lead to the expression of de novo functional proteins or to the down-regulation of a gene which is targeted via RNAi interference (provided it is raised against human sequences or conserved murine sequences).

Grainyhead like 2 protein has been described to have oncogenic properties and could lead therefore to a potentially oncogenic change. However, the virus is replication incompetent and therefore self-inactivating. None of the structural genes are actually present in the packaged viral genome so no new virus can be produced and no other cells can be infected. Therefore, risk is considered minimal.

Physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene, however the likelihood of this is very low. Expression of cDNAs in human cells might induce a growth advantage on those cells. However, this is unlikely, to cause significant, harmful effects as very few cells would be affected and the cDNAs used are not expected to have strong oncogenic potential.

The bacterial strains used are attenuated, non-colonising strains so are incapable of causing human infection and therefore harmless to humans. The viral vector to be used will be pseudotyped with the envelope protein VSV-G from Vesicular Stomatitis Virus. If introduced into the body by injection or through a wound, the virus could enter human cells and integrate into cellular DNA. Although, the virus is replication incompetent (does not carry gag, pol or env genes) and cannot make new progeny virus or establish a productive infection, its insertion into cellular DNA could result in a potentially oncogenic mutation. The recipient cells are harmless to humans.

Possible effects in the environment: Potentially the hairpin could be expressed in another mammalian species and lead to down-regulation of a growth regulatory gene such as a tumour suppressor gene resulting in a potentially oncogenic mutation. This, however, is extremely unlikely as the hairpin is targeted to human or mouse genes and many hairpins are not homologous with the genes of other species. If the hairpin were to target and lead to the reduction of a gene via RNA interference it would not be severe as the virus is replication incompetent and therefore self-inactivating as none of the structural genes are actually resent in the packaged viral genome so no new virus can be produced and no other cells can be infected. If the cDNA-containing virus gain access to other animals, it could be expressed in another mammalian species and lead to the down-regulation of a growth regulatory gene such as a tumour suppressor gene resulting in a potentially oncogenic mutation. However, it is very unlikely that the cDNAs would gain access to another species and the viruses are non-replicative.

The bacterial strains used are attenuated non-colonising strains that cannot survive outside culture conditions and are therefore harmless to the environment. The viral vectors plasmid vectors are replication incompetent so therefore cannot self-replicate and transfer to another host so are harmless to the environment. The cells that will eventually receive and integrate the viral DNA cannot survive in the outside environment as they are only viable under culture condition and therefore are harmless to the environment.

The virus will be used in designated level 2 laboratories. Also, in order to prevent contamination of workers by virus no
glass or other sharps will be used with live virus (The lentivirus will be added to the rat serum which is the normal culturing medium for mouse embryos and the expression of the genes of interest will be evaluated after 24h or 48h of culture). Staff will be protected by a lab coat and gloves. Also, the safety cabinet in tissue culture room 203 level 2 ICH will be used as containment. No adverse effects have been described through the accidental exposure to lentiviral vectors. The risk of harm or exposure and harm human health is therefore very low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be double-bagged in biological waste bags, sealed with a biohazard tape and autoclaved at 132°C for 15min by trained staff (Ground floor at ICH room G17) then bagged in biohazard bags and removed by UCL waste services. 10% Distel or 2-5% Virkon (v/v) will be used to treat liquid waste (18 hours) and also used to decontaminate any spills and for disinfection of any reusable equipment and laboratory materials used. Liquid waste will subsequently poured down the sink after the treatment with Virkon. Virkon and Distel are certified by the PHLS to provide 100% virucidal and GMO kill under these conditions by denaturing and inactivating viral, bacterial and cellular proteins. Virkon and Distel will be used within their lifespan to ensure the required kill is achieved.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The UCL GMSC agreed the classification of this work as Activity Class 2 pending some minor clarifications regarding disinfection and disposal of waste.

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<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
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02/03/2022
The proposed project aims to investigate inflammatory and immune responses in mycobacterial infection using the zebrafish Mycobacterium marinum infection model. Mycobacteria are a group of pathogens, several of which are important causes of human disease, including M. tuberculosis: the causative agent of tuberculosis. Model systems for the study of tuberculosis include the zebrafish, and several important advances in understanding mycobacterial disease have now come from this model (Adams and several important advances in understanding mycobacterial disease have now come from this model (Adams 2011, Clay 2007, Davis 2009, Tobin 2010, Volkman 2010)). Infection will be established by microinjection of zebrafish embryos via previously validated routes including yolk sac injection at early stages or injection into the caudal vein at 24-36 hours post fertilisation (hpf) (Carvalho 2011, Benard 2012) of either fluorescently labelled or wild type M. marium. Up to a maximum of five days post fertilisation, zebrafish larvae will be harvested in RNA later, Qiazol or RLT buffer for gene expression studies, SDS reducing buffer for Western blotting or mounted for microscopy studies to acquire qualitative and quantitative information on bacterial load, and interaction with immune cells.

References


M. marinum is a non-tuberculous mycobacterium, and in humans is an opportunistic pathogen which occurs commonly in the environment, with no evidence of person to person spread. M. marinum has been described to cause tenosynovitis in the hand and wrist, associated with penetrating injuries in the presence of contaminated water, occasionally progressing to osteomyelitis. More commonly, M. marinum may cause skin and soft tissue infection following trauma in the presence of contaminated water, most commonly from fish fins, bites or unrelated trauma accompanied by exposure to contaminated water. In temperate climates, infection is commonly related to fishing, fish tank cleaning or in aquarium workers. Due to the lower temperature to which M. marinum is adapted, infection is usually confined to the skin and extremities. Infection begins as one or more nodules at the site of trauma. Spread to deeper tissues can occur. Treatment is with multiple antibiotics, as with other non-tuberculous mycobacteria (Piersimoni, 2008). Infections can be more severe and disseminated in the immunosuppressed, including those on anti-TNF therapies (Ramos, 2010). As noted above M. marinum does have the potential to cause disease in humans. The GMM would be less likely to cause disease given the burden of carrying the insert without any gain to pathogenicity. Any infection could be readily treated with antibiotics as mentioned above.

M. marinum is commonly found in environmental water, and so has the capacity to survive in the environment following inadvertent release. Currently, no environmental hazards have been associated with the organism. Mycobacteria will be separated from research aquaria. It has previously been shown that M. marinum does not grow in standard mammalian tissue culture media at 37 degrees celsius (Ramakrishnan, 1994).

References


origin & function

Mycobacterium marinum is a non-tuberculous mycobacterium, and in humans is an opportunistic pathogen which occurs commonly in the environment, with no evidence of person to person spread. M. marinum has been described to cause tenosynovitis in the hand and wrist, associated with penetrating injuries in the presence of contaminated water, occasionally progressing to osteomyelitis. More commonly, M. marinum may cause skin and soft tissue infection following trauma in the presence of contaminated water, most commonly from fish fins, bites or unrelated trauma accompanied by exposure to contaminated water. In temperate climates, infection is commonly related to fishing, fish tank cleaning or in aquarium workers. Due to the lower temperature to which M. marinum is adapted, infection is usually confined to the skin and extremities. Infection begins as one or more nodules at the site of trauma. Spread to deeper tissues can occur. Treatment is with multiple antibiotics, as with other non-tuberculous mycobacteria (Piersimoni, 2008). Infections can be more severe and disseminated in the immunosuppressed, including those on anti-TNF therapies (Ramos, 2010). As noted above M. marinum does have the potential to cause disease in humans. The GMM would be less likely to cause disease given the burden of carrying the insert without any gain to pathogenicity. Any infection could be readily treated with antibiotics as mentioned above.

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References


Recipient or parental organism

Mycobacterium marinum M-Strain, a clinical isolate, including variants expressing crimson, mCherry and GFP fluorescent reporters. M. marinum is a non-tuberculous mycobacterium, and in humans is an opportunistic pathogen which occurs commonly in the environment, with no evidence of person to person spread. M. marinum has been described to cause tenosynovitis in the hand and wrist, associated with penetrating injuries in the presence of contaminated water, occasionally progressing to osteomyelitis. More commonly, M. marinum may cause skin and soft tissue infection following trauma in the presence of contaminated water, most commonly from fish fins, bites or unrelated trauma accompanied by exposure to contaminated water. In temperate climates, infection is commonly related to fishing, fish tank cleaning or in aquarium workers. Due to the lower temperature to which M. marinum is adapted, infection is usually confined to the skin and extremities. Infection begins as one or more nodules at the site of trauma. Spread to deeper tissues can occur. Treatment is with multiple antibiotics, as with other non-tuberculous mycobacteria (Piersimoni, 2008). Infections can be more severe and disseminated in the immunosuppressed, including those on anti-TNF therapies (Ramos, 2010). As noted above M. marinum does have the potential to cause disease in humans. The GMM would be less likely to cause disease given the burden of carrying the insert without any gain to pathogenicity. Any infection could be readily treated with antibiotics as mentioned above.

M. marinum is commonly found in environmental water, and so has the capacity to survive in the environment following inadvertent release. Currently, no environmental hazards have been associated with the organism. Mycobacteria will be separated from research aquaria. It has previously been shown that M. marinum does not grow in standard mammalian tissue culture media at 37 degrees celsius (Ramakrishnan, 1994).

References


evaluation of foreseeable effects

None of the fluorescent reporter expressing mycobacterium marinum are hazardous. The GMM would be less likely to cause disease in humans, given the burden of carrying the insert without any gain to pathogenicity. There are no environmental risks associated with the inserted genetic material.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All potentially infected solid waste, including M. marinum infected zebrafish larvae, will be chemically disinfected by using 5% Distel (final concentration) before being discarded into an approved autoclave bag. Before the bag is too full it will be closed with a cable tie and then placed in a second autoclave bag, also secured with a cable tie. Bags will be placed in a blue bin to await collection and transport to be autoclaved (The Sterilising Equipment Company). Waste will be autoclaved at 134 degrees Celsius, 20 minutes hold time, 3 bars pressure. Autoclaved waste is categorised as offensive and is double yellow tiger bagged and locked away until collected and incinerated by a UCL contractor (Grundons). Glass microinjection needles will be disinfected using 5% Distel (final concentration) before being disposed of in a sharps bin and then incinerated by a UCL contractor (Grundons). Waste autoclaves alarm and will fail the cycle if any of the above parameters are not met. The staff either re-run the cycle or transfer waste to a different autoclave to ensure decontamination. Each autoclave has a printer attached which records all cycle parameters at set intervals throughout the ~2 hour run. All potentially infected liquid waste will be chemically disinfected using 5% Distel (final concentration) for at least 12 hours before being discarded to drain. Distel disinfectants employ chlorine dioxide chemistry to provide highly effective and safe biocidal cover. Distel has been found to be biocidal with 30 seconds to 5 minutes against a range of mycobacterial species, including, M tuberculosis (Tristel Produc Brochure 2007).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The project was approved by the UCL GMSC (at their meeting on 11/12/2013) as AC2 pending clarification of a number of minor issues relating to the handling of infected fish larvae and risks to immunosuppressed staff

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<th>Human Clinical Applications</th>
</tr>
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<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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To test the hypothesis that nerve growth factor receptor trkA activation via the tumour suppressor p53-dependent repression of the trkA phosphatase SHP-1 expression, promotes differentiation, cell cycle arrest or apoptosis of neuroblastoma cells. Such an understanding of the functional consequences for the tumour cell of the interactions between these proteins will in the longer term, facilitate the design of novel therapies, that would promote targeted cell-cycle arrest, differentiation or cell death of neuroblastoma cells.

We will use known neuroblastoma cell lines to test the outcome of p53-dependent repression of SHP-1, in terms of trkA-phosphorylation and tumour cell proliferation, apoptosis, survival and differentiation.

To determine their role, SY5Y, IMR32, SK-NAS, SK-NBE, Lan-1 and Lan-5 cell lines will be used together with mouse-NIH3T3 and p53-/- murine fibroblasts cell lines. Tranfections with mammalian expression vectors encoding wild type or mutant trkA, p53 or SHP-1 as well as infections with wild type or mutant trkA, p53 and SHP-1 recombinant retroviruses will be undertaken. The role of SHP-1 in trkA-activation will be further assessed by knocking-down SHP-1 expression with mammalian vectors or retroviruses encoding SHP-1-siRNA.

Recipient or parental organism

A, cell lines:
SY5Y, IMR32, SK-NAS, SK-NBE, Lan-1, Lan-5, mouse-NIH3T3, p53-/- murine fibroblasts cell line, Phoenix cells, Platinum cells lines, GP2-293 cells and LinXE cells.

B, Bacterial E coli strains:
HB101, JM109, DH5alpha.

Associated risks and likeliness of occurrence

A, The recipient cells (cell lines) that will receive and integrate the DNA are harmless to humans. These cells cannot survive in the environment as they are only viable
under culture conditions and therefore are harmless to the environment.

B. The bacterial strains used are attenuated non-colonising strains so are incapable of causing human infection and are, therefore, harmless to humans. Also, these strains cannot survive outside culture conditions and are therefore harmless to the environment.

Host/vector system

A. Retroviral vectors

pBABE retroviral vectors contains sequence/elements derived from Moloney murine leukemia virus (MMLV) and IRES-marker genes. pBABE is described by Morgenstern J.P. and Land H. (Nuc. Acid Res. VOL. 18 NO.12 (1990) 3587 and is commercially available from Cell Biolabs Inc.

pLNCX retroviral vector contains sequences/elements from Moloney murine leukemia virus (MMLV) LTR and IRES-marker genes. pLNCX is described by Miller A.D. and Rosman G.J. (Biotechniques VOL.7 (1989) 980) and is commercially available from Clontech.

B. Transfection vectors

pLTR was obtained by cloning proviral Harvey murine sarcoma virus 5' LTR (enhancer, promoter and cap sites) and the SV40 polyadenylation site into a pBR322 backbone containing a EcoRI, XhoI, BglII multiple cloning site, pLTR has been described by Michalovitz d. ET AL., (Cell VOL 62 (1990)671).

pCMV was derived from BCNGneo - miL2 (containing the CMV promoter, splicing and polyadenylation sequences derived from the beta globin gene) of which the human beta globin sequences and the il2 sequences were excised. Also the HSV thymidine kinase gene was introduced to drive expression of the neomycin gene. pCMV has been described by Baker S.J. et al., (Science. VOL 249 (1990) 912).

pMEX was constructed by introducing the Moloney murine sarcoma virus LTR, the SV40 polyadenylation site and the multiple cloning sites of pBluescript into a pBR322 backbone. To this construct the SV40 promoter driving the expression of the neomycin resistance gene was also added. pMEX has been described by Oskam R. et al. (PNAS Vol 85 (1988) 2964).

C. Cloning vectors

pCR2.1 TOPO is a cloning vector with T overhangs, kanamycin and ampicillin resistance, and no mammalian promoter. pCR2.1TOPO is commercially available from Stratagene.

Associated risks and likeliness of occurrence

Modifications of the retrovirus vector (pBABE and pLNCX - retrovirus) have resulted in removal of gag, pol, and env genes from the virus. Thus, the retrovirus is replication incompetent and therefore self-inactivating as none of the structural genes are present in the packaged viral genome. Packaging and envelop proteins are contained in packaging cell lines (Phoenix cells, Platinum cell lines, GP2-293 cells and LinXE cells) and not the virus. The retrovirus is, therefore, attenuated and cannot replicate and form new viable virus.

Also, although the retrovirus is replication incompetent, if introduced into the body by injection, ingestion or through a wound could enter human cells and integrate into cellular DNA. The physical insertion into cellular DNA could result in a potentially oncogenic mutation. However, the likelihood of infection is very low and no adverse effects have ever been described through accidental exposure to retroviral vectors and if so in only a very small number of cells.

The retroviral vectors have been modified to be replication-defective vectors, thus, they have not yet known pathogenicity to humans and cannot transfer to another host and so are harmless. Also, the virus vectors are of no risk to the environment as viral particles are labile and do not survive on environmental surfaces outside the laboratory.
The transfection and cloning vectors do not cause harm to humans or the environment.

**Origin & function**

A, trkA sequences derived from human. These are sequences coding a receptor tyrosine kinase that when stimulated is involved in giving rise to cell signals that promote cell proliferation, differentiation or apoptosis.

B, p53 sequences derived from human and mouse. These sequences code for a tumour supressor gene that is involved maintaining the coding integrity of the cellular DNA and the regulation of the cell cycle.

C, SHP-1 derived sequences from human. These sequences code for the trkA tyrosine phosphatase that regulates trkA tyrosine phosphorylation and function.

**Associated risks and likelihood of occurrence.**

The donor sequences could potentially increase the oncogenic potential of the retrovirus, specifically, some vectors are likely to contain p53 sequences that could have oncogenic potential. However, it is unlikely that these vectors or retroviruses could infect humans. If infection was to occur, it would not be severe as it would only include a very small number of cells. No adverse effects have ever been described through accidental exposure to retroviral vectors and if so only very small number of cells would be infected, thus the overall risk is low. The retroviral vectors that do not encode such sequences with oncogenic potential are extremely unlikely to have pathogenic potential if accidentally injected.

Pooled viral preparations are likely to contain clones with oncogenic potential, however the relative infectious titres of these vectors within the population are too low to measure, making the vector preparation very safe. If a person were infected by infectious retroviral particles they are replication incompetent and therefore self-inactivating, thus there is no chance of transmission to another cell or person. It is extremely unlikely that an infection would occur and no adverse effects have ever been described through accidental exposure to retroviral vectors thus the overall risks are low.

**Evaluation of foreseeable effects**

The intended recipient organisms prior to modification

The bacterial strains to be used are non colonising and incapable of surviving in the environment or causing human infection. They pose no risk to users or the environment.

The cell lines used cannot survive outside the laboratory incubators and as such do not pose a threat to health or the environment.

None of the DNA retroviral vectors are harmful to health or the environment and do not supply a survival advantage to any microorganisms. The packaging viral components of the retroviruses are expressed by the packaging cell lines and have been removed from the retroviral vector.

None of the DNA transfection or cloning vector plasmids are harmful to health or to the environment and do not supply a survival advantage to any microorganisms.

The most hazardous GMM are the retroviral preparations. These could theoretically be hazardous as the retrovirus is amphotropic. If the virus enters the body by injection, ingestion or through a wound, it could enter cells and integrate itself into cellular DNA. However the risk is minimal and likelihood is very low. The virus is replication incompetent and a productive infection. The retroviral vectors have been modified to be replication-defective vectors as the viral particles do not contain all the necessary components. This is because the required components are expressed by the packaging cells (Phoenix cells, Platinum cells lines, GP2-293 cells and LinXE cells) and not the retroviral vector, thus, they have not known pathogenicity to humans and cannot transfer to another host and so are harmless. Also, infection would only be in a small number of cells and would not impact on the pathogenic outcome of an exogenous viral infection. Some of the inserted sequences are likely to have oncogenic potential as their insertion into cellular DNA could result in a potentially oncogenic mutation. However, the likelihood of this occuring is very low as an infection will not be severe and
would only happen in a small number of cells. Therefore the overall risks of the experiment are low. Also, pooled viral preparations are likely to contain clones with oncogenic potential but the relative infectious titres of these vectors within the population are too low to measure, making the pooled vector preparation very safe. Moreover, the viral particles are labile and do not survive on environmental surfaces outside the laboratory making infection unlikely.

Precautions in place to minimize risks

Access to the Institute of Child Health, UCL, is secured by restricted access at all entrances and additionally to all laboratory areas. All work involving viruses will be performed in class 2 cabinets in containment level 2 laboratories specified particularly for retroviral GMM work. All DNA vectors containing viral proteins will be grown separately to avoid opportunity for recombination. To prevent contamination of workers by virus, when handling any GMM or GMM-derived DNA or virus, no glass or other sharps will be used in the rooms where the viruses are prepared and used and workers will be protected by a lab coat and nitrile gloves. Skin lesions will be covered with a bandage in addition to the protective wear. Specific guidelines will be in place to ensure all viral preparations are handled, labelled, stroed, transported and cleaned correctly. All those involved in the project will be made aware of the guidelines and the associated risks as well as anyone else who may use the laboratory. Persons involved on project from other Institutes will work under the supervision of a competent trained worker from this project. No-one visiting the laboratory that is not involved in this work will work on this project. All persons entering the facility will be made aware of the safety precautions upon entering the facility. All equipment used in the retroviral laboratory will be available in other general purpose laboratories for general use so it should not be necessary for others who are not involved in retroviral work to enter the laboratory.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
<thead>
<tr>
<th>All work will be carried out in level 2 containment facilities.</th>
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<tbody>
<tr>
<td>A. All staff and students involved in the project will be made aware of the associated risks and will be required to have appropriate safety training prior to beginning work on the project.</td>
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<tr>
<td>B. Retroviral preparations will only be handled whilst exposed in class 2 cabinets.</td>
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<tr>
<td>C. Workers will be required to wear laboratory coats, that cover the arms, and nitrile gloves and goggles whilst working on all procedures. Any open wounds will be covered with a bandage also.</td>
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<tr>
<td>D. All spills will be treated with 2-5% Virkon which is certified to kill 100% of virus, bacteria and cells.</td>
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<tr>
<td>E. After treatment, all solid waste will be double-bagged, autoclaved, placed in yellow biohazard safety bags and furhter treated by UCL waste services.</td>
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<tr>
<td>F. All retroviral supernatant will be stored in double containment and transferred within the laboratory within doubly sealed containers.</td>
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</table>

We expect 100% kill (routine laboratory practice for viral vectors). 2-5% Virkon (v/v) will be used to treat liquid waste (18 hours) and also used to decontaminate any spills and for disinfection of any reusable bottles or other equipment and laboratory materials used. Liquid waste will be subsequently poured down the sink after treatment with Virkon.

Work surfaces will be wiped down with 2-5% Virkon and 70% ethanol or IMS after use. Virkon is certified by the PHLS to provide 100% viricidal and GMO kill under these conditions by denaturating and inactivating viral and cellular proteins. Virkon will be used within its lifespan of 7 to 10 days to ensure required kill is achieved. Solid waste will be double bagged in biological waste bags, sealed and autoclaved at 132°C for 15 mins by trained staff then bagged in yellow biohazard bags and removed by UCL waste services. Autoclave runs are regularly validated by waste managemet staff.
The University Biological Safety Adviser, on behalf of the UCL GSMC agreed the classification of this project as AC2 following a number of minor clarifications/amendments from 3 UCL GM advisers.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L4 L2 L3 L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
<td>L4 L2 L3 L4</td>
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Project Ref 14/14.4

Date Ackn'd 19/03/2014

CU2 Project Title Cell biology of Poxvirus Infection

Class 2 CultureVolClass2 < 1 Litre

Consent Granted

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N
### Project Additional Information

#### Purposes of the contained use

My Laboratory focuses on determining the mechanisms by which Vaccinia virus subjugates host cells proteins and cellular functions. This work relies heavily on the use of recombinant viruses for cell, molecular, biochemical and virology based experimentation.

#### Recipient or parental organism

Wild type and recombinant vaccinia viruses [strains western reserve (WR) and International health department strain J (IHDJ)] expressing EGFP, mCherry or containing viral structural proteins modified by EGFP, mCherry, HA, FLAG sequences, or a combination thereof.

#### Host/vector system

N/A

#### Origin & function

The GFP sequence was originally derived from Aequorae victoria, and modified to produce mCherry. HA is derived from influenza virus and FLAG is an artificially derived sequence.

#### Evaluation of foreseeable effects

The wild type viruses are the most virulent. The recombinant viruses will either express fluorescent proteins to mark stages of gene expression or package fluorescent structural proteins for visualization of virus particles. All of these insertions either have no impact on virus pathogenicity or limit the pathogenicity of the virus.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid materials (plastic dishes) potentially containing virus will be collected in autoclave bags contained within metal housing containers and autoclaved. Smaller plastic objects (tips used to handle virus or virus containing liquid) will be subjected to UV irradiation up to 12 hours for virus inactivation prior to autoclaving.

Liquid waste will be collected in aspiration bottles containing Trigene Advanced Virucidal solution prior to autoclaving.

For routine infection work pasture pipettes will be utilized for aspirating virus containing media into proper disposal containers. All aspiration will be followed by disinfection of pasture pipettes through aspiration of 70% ETOH which results in a degree of killing of 90% (Block S.S 2001; Disinfection, Sterilization and Preservation).

Vaccinia virus can be 100% inactivated upon autoclaving occurs within 15 min (Espy M.J. et al. 2002; Detection of vaccinia virus by LightCycler by PCR after autoclaving: implications for biosafety of bioterrorism agents).

Trigene: ATS labs study ‘Virucidal efficacy of a disinfectant for use on inanimate environmental surfaces’ March 2007. In addition we have routinely used trigene for vaccinia virus in activation for the last 8 years, prior testing indicated a EC50 of 1% Trigene in vaccinia stock solutions containing 1x10E9 units of infectious virus/milliliter.
The UCL Biological Safety Adviser, on behalf of the UCL GMSC agreed the project classification of AC2, following a number of minor amendments and clarifications.

**Project Containment**

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<th>Laboratory Activities</th>
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**Project Ref** 14/14.5

- **Date Ackn'd**: 24/04/2014
- **CU2 Project Title**: Directed evolution of oxa(thia)zole-containing peptides
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Consent Granted**: Non-GMM Consent Granted
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
Project Additional Information

Purposes of the contained use

Oxazol(in)e and thiazol(in)e heterocycles are present in a wide range of bioactive compounds, including antimicrobials, anti-malarial and anti-tumour agents. Oxazole/Thiazole-containing peptides are naturally produced by a wide range of bacterial and archael organisms through the post-translational modification of ribosomally-synthesised peptides.

Chemical synthesis of polyheterocycles is challenging, making the use of synthetic biology approaches a viable route towards the discovery and synthesis of bioactive compounds. The research program is currently focused on characterising the natural enzymes and the range of viable polyheterocycle products that can be synthesised. That will also include synthesis optimisation of known polyheterocycles such as plantazolicin (an antimicrobial) and telomestatin (a potent telomerase inhibitor), and potentially synthesis scaling up to provide alternative synthetic routes for this class of compounds.

In addition, starting from a library of poly heterocyclic compounds, the program will use directed evolution methodologies to identify novel antimicrobials, novel antitumour agents and novel metamaterials (e.g. UV absorbing peptide aptamers). Those directed evolution methodologies will include in vitro selection strategies as well as bacterial cell display strategies.

Because of the strong correlation between oxathiazoles and bioactive molecules and given the varied nature of activities reported, it is likely that the peptide library will contain a range of biological activities. In a worst case scenario, it is possible that some of the GMMS generated could be capable of synthesising (exporting or displaying) low concentrations of oxathiazole peptides toxic to humans.

Recipient or parental organism

Auxotrophic lab-adapted standard E. coli strains - NEB beta-10, NEB T7 express, DH5alpha.

Host/vector system

pBAD30, pMAL-c2, pET, pTWIN (NEB IMPACT system) - all are well-characterised inducible expression systems

Origin & function

We are focusing on oxathiazole synthase complexes from Bacillus amyloliquefaciens and Pyrococcus furiosus. The synthetase genes being used were commercially synthesised and codon-optimised for E. coli expression. The heterotrimeric synthetase complex catalyses the cyclodehydration of serines, threonines and cysteines in peptides containing specific leader sequences, followed by their dehydrogeneration. Oxathiazole synthetases (and their natural peptide substrates) have not been linked to bacterial pathogenesis in humans.

Some oxathiazole compounds, such as telomestatin and other anti-tumour agents, can affect human health. Because peptide precursor libraries will be synthesised it is possible that compounds that can affect human health will be generated.

The bacterial display systems being considered are already characterised gene fragments from E. coli (EspP) and Pseudomonas syringae (INP) that provide a suitable platform for protein expression on the bacterial surface. The proteins involved in the bacterial display system have not been implicated in pathogenesis. Potential toxicity from fused oxathiazole compounds may be limited by the fusion partner - compounds cannot diffuse into the environment and fusion partner may interfere with toxic function.

Evaluation of foreseeable effects

Synthetase genes will be overexpressed in the bacterial host and purified for in vitro assays. This is of negligible risk.
Peptide precursors will be overexpressed for selection and characterisation. In selection experiments, peptide precursors (alone or as fusion partners) will be co-expressed (using inducible expression) with synthetases to generate the polyheterocycle in vivo. This will result in GMMs expressing potentially bioactive molecules in mixed cultures. Selection experiments will not use more than $10^9$ bacterial cells at any one time.

For characterisation, precursor peptides are going to be expressed and purified prior to in vitro modification by purified synthetases (unless in vivo heterocycle synthesis is significantly more efficient).

Forseeable effects:
Because of the fiversity of the precursor peptide library, it is possible that variants hazardous to human health will be generated. The risk derives from the direct effect of the modified peptides and potentially by altering how the bacterial cell expressing the polyheterocycle variant interacts with human cells. The risks are minimised by using inducible systems and through the small experimental scale.

Effect of gene transfer:
Gene transfer would require concomitant transfer of the three synthetase genes and the peptide precursor (or its cell display version), which will be on separate plasmids.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste, including bacterial agar and plasticware (used in bacterial growth, selection, screening or purification), will be autoclaved before disposal by incineration. Autoclaving will be carried out at UCL, facilities following autoclave's manufacturer recommendations to obtain effectively 100% kill.

Liquid waste, including bacterial cultures and cleared cell lysates, will be chemically inactivated with Virkon (with concentrations above manufacturer's advice to obtain 100% kill) prior to being disposed down the sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The UCL GMSC agreed the classification of AC2 on a precautionary basis subject to some minor clarifications on the risk assessment.

Project Containment
Project Ref 14/14.6

Date Ackn'd 21/05/2014
CU2 Project Title The construction and use of adenoviral vectors for expression or knockdown of cancer related genes, to assess their biological role
Date Project Ceased

Class 2
Culture Volume

Consent Granted

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Proposed activity for a new lab carrying out PI3-kinase research, to generate, introduce, express or knock-down in mammalian cell systems, genes which have a potential for cancer therapy.

Recipient or parental organism

Standard disabled bacteria strains derived from E. coli such as DH5alpha, DH10B, XL-1 and BJ183 for the 'AdEasy' system.

Animals: C57-B6, BALB/c, ICRF nude, and other murine strains as appropriate (see attached animal GM risk assessment).

Host/vector system

Varous well characterised non-pathogenic mammalian cell lines including HEK293, and various human and murine primary and carcinoma cell lines available from CRUK cell services, ATCC and Clonetics which are well characterised, non-pathogenic; pAdtrack and pAdEasy adenovirus expression systems: Ad5 and Ad2 wild type and deletion mutants, containing inserted transgenes;
pAd/BLOCK-iT Adenoviral RNAi Expression System, E1 region is deleted. The pAd/BLOCK-iT Adenoviral RNAi Expression System is a second generation system. It includes a number of safety features designed to enhance its biosafety. The entire E1 region is deleted. Viral replication only occurs in cells that Express E1. Adenovirus produced from the pAd/BLOCK-iT-DEST expression vector is replication-incompetent in any mammalian cells that do not express the E1a and E1b proteins. Adenovirus does not integrate into the host genome upon transduction. Because the virus is replication-incompetent, the presence of the viral genome is transient and will eventually be diluted out as cell division occurs;
Please refer to attached risk assessment for more details.

**Origin & function**

Replication defective viruses will contain the following types of genes:
Genes that can induce differential tumour cell suicide including tumour suppressor and apoptosis-inducing genes including PTEN, p53, p16 and members of the bcl-2/bax family; Dominant-negative mutants including mutants of signal transduction molecules such as PI3-kinase, RAS, AKT, MAPK or molecules that regulate their functions (such as the APPL1 adaptor protein); These would produce molecules that would promote tumour cell death but have no effect on normal cells.

Marker genes such as GFP, beta-Galactosidase and Luciferase will also be used as control vectors.

In all viruses used only one transgene will be expressed in one construct at any one time.

Constructs will be targeted to mammalian cells and models of disease.

**Evaluation of foreseeable effects**

Most gene products as described previously act within tumour cells to promote cell death. Replication defective viruses (E1A deleted) cannot replicate or produce virus from recombination events in normal cells as the genes would not be present to complement.

None of the genes expressed can complement for the defective nature of the viruses: inclusion of a gene such as p53 would actually restore wild type function of the cancer cell (rather than the virus) and make the virus unable to replicate.

Work with replication incompetent adenoviruses will take place within a Cat 2 environment where protected clothing is worn, and will be manipulated within a biological safety cabinet. In case of accidental spillage spills would be immediately inactivated with Distel disinfect and a breathing mask used to avoid inhalation. This will reduce the likelihood of exposure to an extremely low level.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Work with GM animals for this project will be carried out in the BSU facilities at UCL: Within these facilities, the mice will be housed in individually ventilated cages within a strictly contained environment, within a specialised unit staffed by experienced personnel. There is no opportunity for experimental mice to escape to the local environment; rooms where GM animals are housed are fitted with rodent barriers and also all external doors leading to the unit. Drains are fitted with mesh to prevent release of GM animals to the environment. The survival of GM animals in the environment is considered negligible as they are specifically bred laboratory strains with low fecundity.

Staff working within this facility will be given full training on handling GM animals and will work using the appropriate protective clothing. Adenoviral constructs will be administered to the mice via intra-tumoural injection and tail vein injection.

It is highly unlikely that any virus will be shed by the animals, and risk to human health is negligible - work within a Ct 2 facility should minimise exposure. Material exposed to GM animals ie:bedding, carcasses, cages (and anything else that lends itself to autoclaving) will be autoclaved to render it "no longer live GM" before being disposed of as clinical waste

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The likelihood of release of GM organisms from the laboratory is low as all GM waste is decontaminated before incineration through UCL waste contractors. No viable GMOs are discarded.

1. Solid waste:
Contaminated glassware is disinfected first with 1% Distel (Trigene), before autoclaving (134°C, 20min, effectively 100% kill). Distel is a high level disinfectant. Plasticware that has come in contact with the organisms mentioned above is either autoclaved (134°C, 20 min, effectively 100% kill) then incinerated as clinical waste, or put into 1% Distel overnight, washed and then incinerated as clinical waste.

2. Liquid waste:
Tissue culture liquids: Liquid waste is diluted overnight in 1% Distel, after which the solution is disposed of down the sink.

Distel is used following manufacturer's instructions and will provide effective kill from overnight treatment;
Distel: Broad spectrum efficacy within short contact times - 30 seconds to 5 minutes;
Sporicidal, Mycobacterial, virucidal, fungicidal and bactericidal efficacy;
Performance validated within a wide range of peer-reviewed scientific publications;
Acknowledged and recommended by professional societies, public and private sector healthcare institutions


**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

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**Project Containment**

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# Project Additional Information

## Purposes of the contained use

The development of gene delivery technology is a vital component as both a biological research tool and as a means of evaluating gene therapy protocols for future clinical use. Although there has been tremendous progress in developing viral vectors to deliver therapeutic genes into mammalian cells, more research is required to achieve the efficiency required for clinical use. Furthermore, the delivery of reporter genes to cells is a vital component of furthering our understanding of diseases in mouse models. Unfortunately, the use of cultured cells in vitro is not sufficient to model the complex issues at hand and so in vivo rodent models are required. The purpose of our work is to continue developing viral vectors for their eventual use in the clinic and to also use them to further understand disease.

A variety of disease-specific transgenes will be packaged into various different gene therapy delivery agents (vectors) which will then be tested for their efficacy in either normal outbred mice or the appropriate transgenic mouse model. The diseases studied include defects of the nervous system (e.g. Gaucher disease, Parkinson's disease, Infantile Neuroaxonal Dystrophy and Batten's disease). The gene therapy vectors will include adeno-associated viral (AAV) and lentiviral constructs.

In addition, gene transfer can be used to deliver luciferase under the control of signalling pathway-specific promoters. Therefore, the equivalent of luciferase-reporter mice can be generated easily and quickly without having to purchase and breed entire colonies. This technology is likely to significantly reduce the number of mice used to study disease pathways and we aim to explore the full potential of this technology ("photonic somatotransgenic biosensing") across numerous disciplines, by using various disease model systems.

This project involves the construction of various viral backbones and so we will be using many combination of promoter, transgene and viral backbone (e.g. retro/lentivirus, AAV). For example we may wish to construct an AAV backbone containing the human phospholipase A gene controlled by the neuron-specific synapsin promoter. Alternatively, we may wish to construct a lentiviral backbone containing the CLN3 gene controlled by the ubiquitous CMV promoter. To keep this application as broad as possible it is not possible to provide exhaustive lists of all constructs. However, as all constructs being dealt with here are non-toxic DNA sequences they all carry the same risks and have the same health and safety considerations.
The developed viral vectors carrying either therapeutic or reporter genes require to be administered to mice housed at the Biological Services Unit, UCL School of Pharmacy, University College London. These vectors will be injected into the mice by various routes of administration. It is the administration of these vectors to mice within the BSU at the UCL School of Pharmacy that is covered by this application.

Recipient or parental organism

Recombinant adeno-associated viruses (AAV):
All AAV vectors used here are replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a plasmid containing the adenoviral serotype 5 genomic sequence to provide helper function for AAV replication. Upon a cytopathic effect in the cells, AAV particles are harvested and purified by HPLC.

Recombinant retroviruses (including lenti- and spuma-viruses):
Replication deficient retroviral particles are generated in cell lines following co-transfection of three different plasmids. These supply the self-inactivating transfer vector containing the insert gene and viral Long Terminal Repeats (LTR) (but lacking any expression of HIV genes); packaging and structural proteins, gag/pol; and envelope (env) in the multi-deleted packaging plasmid pCMVdeltaR8.74 and in the pMD.G2 plasmid (in the case of VSV-G). The env gene is taken from another virus (eg vesicular stomatitis virus G (VSV-G), baculovirus gp-64, filovirus glycoprotein or mouse leukaemia amphotropic/ecotropic envelopes) which result in lentiviral particles able to transduce a variety of cell types. The transfer vector also contains a poly-purine tract (cPPT) and the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) for increased efficiency of production. The vector produced is self-inactivating due to a deletion in the promoter/enhancer region of the 3' LTR, that gets copied onto the 5' LTR during transduction of the cell and prevents production of viral RNA. The only expression from the vectors is of the transgene cDNA, controlled by an internal promoter.

Host/vector system

Prokaryotic plasmids, eukaryotic expression vectors, replication-deficient self-inactivating (SIN) lentiviral vectors which are based on third generation lentiviral vectors (i.e. pCCLSIN) or second generation vectors (i.e. pHR'SIN) with engineered safety features which are resembling third generation vectors in terms of safety.

Origin & function

All inserted sequences are from either human, mouse, firefly or bacteria.

All gene products are either enzymes (e.g. glucocerebrosidase and phospholipase A), reporter genes (e.g. GFP and luciferase), transmembrane proteins e.g. CLN3 or NPC1) or siRNA hairpin sequences.

None of the sequences fall into any harmful category such as oncogenes and proto-oncogenes, growth factors and GF receptors, any other sequences coding for significant biological activity that might be harmful were these to be expressed in people accidentally exposed to the vector.

In the unlikely event that any of these inserted sequences were to be expressed in humans after accidental transfer, the cDNA sequences or the RNAi hairpins could lead to expression of functional proteins or to the down-regulation of the gene to which the RNAi hairpin targets via RNAi interference, respectively. Expression of the inserted cDNAs is deemed to hold little risk due to the non-harmful nature of the sequences used and the number of cells that would be affected by accidental exposure. Accidental targeting of RNAi in humans is not deemed to be harmful either due to a combination of the function of the targets, no expected harmful action of other molecules when presence of lower amounts of the target, and the expected relatively low amount of cells involved. In addition, for both types of sequence, the effect of accidental expression would not be severe due to the fact that all viruses used are replication deficient and so no new virus particles can be produced so no other cells can be infected. It is possible that physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene, however, the likelihood of this is extremely low.

The risk to the species in the environment is even lower since they would only be exposed to much smaller amounts of the sequences.
Evaluation of foreseeable effects

The mammalian donor organisms are not harmful however some of the donated sequences are from viruses (e.g. the envelope proteins VSV-G from Vesicular Stomatitis virus and gp 64 from baculovirus) some of which are infectious to humans.

The sequences used from viral donor organisms are either promoters/enhancers or envelope proteins so none of these are involved in the harmful characteristics of the original donor organism. Even if these viral sequences were introduced into the body by ingestion, through a wound or after spillage onto skin, the viral sequences would not enter human cells and would therefore not be expressed. The sequences used from mammalian or bacterial donor organisms are the transgenes or RNAi hairpin sequences, none of which are pathogenic.

The most hazardous GMM is lentivirus. The most hazardous step is injecting the lentiviral constructs into mice due to the use of sharps.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Good Laboratory practice will be used and all material that has been exposed to viral vector will be disposed of by trained service staff. All solid waste (e.g. tubes, syringes, plastics, gloves) will be placed into disposable bags and sharps (e.g. needles) will be placed into sharps bins for immediate autoclaving at 131 degree celsius for 30 mins. This will achieve 100% kill and destruction of any viral vector. Any liquid waste (very small volumes of viral vector, <1 ml) will also be autoclaved at 131 degrees celsius for 30 mins also achieving 100% kill and destruction of viral vector. All animal carcasses that have received viral vectors will be destroyed by incineration that will achieve 100% kill and destruction of vectors.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The UCL GMSC agreed the classification of Activity Class 2

Project Containment

<table>
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Examining the progression of neonatal bacterial meningitis (NBM) caused by Escherichia coli K1 using bioluminescent imaging techniques in a neonatal rat model

E. coli O18:K1 strain A192PP (rat passaged strain - Mushtaq et al., 2005) is a human pathogen. The parenteral strain (A192/DSM.No. 10719) was originally isolated from a sepsicaemia patient (Achtman et al., 1983). A192PP may therefore be able to colonise the human adult and neonatal gastrointestinal tract, although rat-passage makes this less likely. Neonates are at higher risk. Additionally, the E. coli A192PP GM strain will have acquired kanamycin resistance. Due to the risk presented to human health contained use is required.


pUTmini-Tn5.

The plasmid can only be maintained in lambda pir+ donor strains (donor strain to be used: E. coli S17-1 lambda pir). Plasmid can be transferred by conjugation from donor to recipient (A192PP) (pir negative strain) and acts as a suicide vector. The mini transposon is disarmed and so forms stable integrations within recipient
chromosome and cannot re-mobilise (Winson et al., 1998).
E. coli S17-1 lambda pir (donor): lambda-pir lysogen of S17-1 (thi pro hsdR3 hsdM. recA RP42-Tc: :Mu-Km: :Tn7(TpR SmR)) (Simon et al., 1983) - enables the donor strain to maintain the pUTmini-Tn5 plasmid.

**Origin & function**

**luxCDABE Km2.**
The entire lux operon (luxCDABE) from the nematode symbiont Photorhabdus luminescens ATCC29999 (Hb strain) (Winson et al., 1998) will be inserted into recipient (E. coli O18:K1 A192PP) chromosome. Along with a kanamycin resistance cassette (Km2).
The operon will provide the mechanics for bioluminescence emission from the recipient. Recipient strains will also become resistant to kanamycin (kanR) due to acquisition of mini-Tn5 luxCDABE Km2 (which will be used for selection purposes).

**Evaluation of foreseeable effects**

- As previously described, the E. coli A192PP strain will acquire bioluminescence (luxCDABE) and kanamycin resistance (Km2).
- **Hazards to human health**
  - E. coli A192PP GM strain (like the parental A192PP strain) may be able to colonise the human adult gastrointestinal (GI) tract if ingested, however rat passage of the parent strain (A192) makes this less likely.
  - E. coli A192PP GM strain (again like the parental strain) may be able to colonise the human neonatal GI tract, however risk of exposure of such individuals is considered low. However, if exposure occurred, the most hazardous outcome could be sepsis and meningitis. Again, as detailed above, rat passage of the parent strain (A192) makes this less likely. However, any individuals at risk/working with this GMM will be informed of possible danger to neonates before entering facilities. No vaccine is available, however antibiotics, including but not limited to betalactams, aminoglycosides, tetracyclines and chloramphenicol can be used, should prophylaxis be required.
  - E. coli A192PP GM strain will acquire kanamycin resistance. However other antibiotics, as stated above, can be used for prophylaxis.
  - The acquisition of foreign genetic material may increase, decrease or have no effect on the virulence of the strain. This will depend on where transposon insertion occurs (random event). However, due to the expected metabolic burden on the strain (caused by the presence of exogenous genetic material), a reduction or no change in virulence is expected to be observed.
  - The acquisition of bioluminescence is not expected to present any hazard to human health.
- **Hazards to the environment**
  - If colonisation of a host with the E. coli A192PP GM strain did occur, the organism could be shed into the environment in faeces/urine.
  - E. coli A192PP GM strain is unlikely to transfer the transposon to other bacterial strains in vitro, in vivo or in the environment, as the mini-Tn5 to be used is disarmed (transposase is present on the suicide vector, but lost during integration into host chromosome). This enables stable integration into the recipient chromosome (i.e. transposon is not self-transposable).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

UCL School of Pharmacy
- Solid waste (contaminated agar plates, rat carcasses, peri-anal swabs, plastics and sharps): autoclavable waste will be placed in two autoclave bags (double-bagging) and placed in blue boxes for collection by UCL School of Pharmacy support staff on a daily basis and be sterilised by autoclaving for a cycle of 120°C for 15 mins. Autoclaved waste will then be disposed of by support staff. Rat carcasses will be macerated/incinerated and disposed of by Biological Services Unit (BSU) support staff. Contaminated sharps (e.g. surgical scissors) will be sterilised by autoclaving. Other sharps (e.g. syringe needles) will be placed in supplied yellow sharps bins and disposed of by UCL support staff for incineration.
- Liquid waste (liquid bacterial cultures, blood, organs (homogenised): autoclavable waste will be placed in two autoclave bags (double-bagging) and placed in blue boxes or placed in buckets for collection by UCL School of Pharmacy support staff and treated as above.
- Personal protection equipment/clothing will be disposed of in accordance with UCL regulations.

Imperial College London
- Waste at Imperial College will be disposed of in accordance with local rules and regulations.
- During imaging procedures, urine/faeces are expected to be shed by neonatal rats - disposable animal beds will be used (with an additional layer of clingfilm will be placed underneath the imaging platform). This waste will be removed after imaging, bags sealed, returned to containment, autoclaved and disposed of in accordance with local Imperial College rules and regulations.
- CBS personal protection equipment/clothing will also be disposed of in accordance with Imperial College regulations.

Is an emergency plan required according to regulation 20?  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The UCL GMSC agreed to the classification of this activity pending clarification of the arrangements for the management of this work at Imperial College.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications
**Project Ref** 14/15.1

**Date Ackn’d** 25/03/2015

**CU2 Project Title** Ranavirus host range and virulence - identifying the genetic mechanisms facilitating host range and virulence of viral pathogen that threatens European amphibian biodiversity

**Class** Class 2

**Culture Vol** < 1 Litre

**Consent Granted**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Non-GMM**

**Historical Significant Changes**

**Recipient or parental organism**

Ranaviruses are an important amphibian pathogen; outbreaks in Europe have involved two lineages of virus (FV3-like and CMTV-like; Teacher et al. 2010, Price et al. 2014) which are both highly virulent but appear to have differing host ranges. We have identified a list of variable loci between the two lineages, which we consider non-essential since they represent genes where presence/absence varies across the virus phylogeny. The aim of knock outs is to explore this variation among ranaviruses at the genome level (novel and truncated genes) and how this may contribute to patterns of virulence and host range that we have observed in the wild. We will be replacing genes of interest with a marker cassette by recombination and will be following an established protocol (Chen et al., 2011).

Wild-type ranavirus strains - No members of this virus genus (or family) vector are known to infect mammals and they cannot grow in cell culture at temperatures above approx. 30C. Neither the wild type viruses nor the GMMs are therefore considered a threat to human health. Virus is cultured in a fish cell line (EPC). Wild type ranaviruses may pose an important risk to amphibian hosts and may also affect reptiles and fish. Amphibian ranavirus infections are notifiable to the OIE. We expect all/most KOs to generate viable GMMs due to our rationale for selecting genes for KO (mentioned above), and consider that standard aseptic procedures used routinely in culturing these viruses will ensure the likelihood of revertants is very low. Revertants have not been observed previously when the same protocols were followed elsewhere.
Host/vector system

Origin & function

Puromycin (antibiotic) resistance marker forms part of the inserted cassette in addition to green fluorescent protein (GFP, not considered hazardous to health). The inserted material will serve to purify and identify the GMM. There are no known direct hazards associated with the inserted material. Puromycin is an antibiotic used in laboratory cell culture and does not have medicinal uses meaning that any theoretical impact of release into the environment should be small. There is a possibility that the inserted cassette could be disseminated and maintained in the outside environment however the inserted genetic material is innocuous and the risk of dissemination is very small and can be limited further through standard containment protocols. Ranaviruses may persist for weeks or months in water and soil (Nazir et al., 2012) but they are obligate parasites of poikilothermic vertebrates that elicit infections in a dose-dependent fashion. Virus inactivation with routinely used disinfectants (bleach, virkon) is highly effective (Bryan et al., 2009) and autoclaving of waste provides a second layer of containment. The importance of adhering thoroughly to aseptic procedures to prevent contamination of virus cultures with xenobiotics and/or other ranavirus isolates being cultured simultaneously serves as a prerequisite barrier to dissemination of virus to the outside environment.

Evaluation of foreseeable effects

Neither the GMMs nor the process of generating them are considered a risk to human health. Ranaviruses can pose a severe risk to poikilothermic vertebrates (especially amphibians) but the innocuous nature of the inserted genetic material, the routine containment and disinfection protocols and the nature of the pathogen as an obligate parasite which is inactivated in the environment through time together identify this GM procedure as low risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Gloves and plastic consumables will be subjected to autoclaving (two 20min cycles at 120 celcius) prior to incineration, animal waste will be bagged securely and incinerated. Unused cultures will be treated with 5% Virkon solution and autoclaving (Two cycles of 20 mins at 120C).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The UCL BSA, on behalf of the GMSC, agreed classification as Class 2 subject to minor clarifications.

**Project Containment**

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
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</table>

**Project Ref** 14/15.2

- **Date Ackn'd**: 01/04/2015
- **CU2 Project Title**: Growth and transformation of Mycobacterium tuberculosis, Mycobacterium bovis (HG3 agents), Mycobacterium marinum, Mycobacterium bovis BCG
- **Class**: Class 3
- **Culture Volume Class 2**: < 1 Litre
- **Class Culture Volume Class 3-4**: < 1 Litre
- **Non-GMM**: Yes
- **Consent Granted**: Yes
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Investigating the expression, turnover and role of regulatory RNA in Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium marinum, Mycobacterium bovis BCG. Infection of cell lines or primary cells with either of the pathogens listed to investigate the effects that the modifications may have on pathogenesis. In addition we will investigate the possibility of using C. elegans as a host for M. marinum.

**Recipient or parental organism**
The most likely consequence of the planned modifications in all the species is a loss of bacterial fitness due to the imbalance in gene regulation. The vector inserts drive the expression of various mycobacterial RNAs or proteins as well as anti-bacterial resistance markers against hygromycin or kanamycin, commonly used laboratory antibiotics which would not be used as a treatment and so no hazard arises from this. Regulatory RNA and cell wall remodelling enzymes could in theory modify the host so it became slightly more tolerant to stress, but it is more likely to results in decreased fitness of the host cell and reduced tolerance to stress and antibiotics. GFP, beta-gal and the myc-tag have not been reported to have any effect on human health.

Host/vector system
Shuttle vectors able to replicate in Escherichia coli for cloning purposes and for expression or deletion of mycobacterial genes. Vectors are derivatives of pMV261, pMV361, pGOAL17. These vectors do not carry any risk by themselves.

Origin & function
The inserted genetic material is all derived from mycobacterial genes mostly those involved in regulatory RNA expression and genes involved in RNA metabolism in addition to reporter genes such as GFP and lacZ. The insertions are made to investigate the expression patterns and functions of regulatory RNAs.

Evaluation of foreseeable effects
The GMMs would be less likely to cause disease in humans or animals than their parental strains, given the burden of carrying the insert or gene deletion as there is no gain to pathogenicity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste is sterilised in 5% Distel for at least 15 minutes after which it is autoclaved as well as incinerated. Agar plates are autoclaved and incinerated. Liquid waste is inactivated by adding Distel to a final concentration of 10% for at least 15 minutes before disposal.
Distel (formerly Trigene Advance): Contact time to kill Mycobacterium tuberculosis (TB) 30 seconds mixed at 1:20; 5 minutes mixed at 1:50. Distel has been found to be biocidal with 30 seconds to 5 minutes against a range of mycobacterial species, including M. tuberculosis.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The UCL GMSC agreed the classification of the work as Activity Class 2 and 3, subject to additional detail being provided, in particular on environmental risks. They agreed notification as a connected programme of work.

## Project Containment

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### Animal Units
- L2
- L3
- L4

### Large Scale Activities
- L2
- L3
- L4

### Human Clinical Applications
- L2
- L3
- L4

### Project Ref 14/15.3

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<th>CU2 Project Title</th>
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<tbody>
<tr>
<td>21/05/2015</td>
<td>Cancer Genome Engineering (CAGE) Facility genome modifications</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Project notified under transitional arrangements</td>
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**Withdrawn**

### Project Additional Information

**Purposes of the contained use**

This project will involve the production, storage, use and distribution of lentiviral and non-lentiviral vector based genome engineering tools, including CRISPR and TALE technologies. Vectors will be distributed as either bacteria, DNA and/or lentivirus to members within the UCL Cancer Institute at University College London for use in various...
projects. This will include lentiviral production from lentiviral vectors expressing genome engineering tools including CRISPR and TALEs, and subsequent use of this lentivirus in cell based screening functional assays. Additionally, large numbers of lentiviruses containing CRISPR guide sequences will be pooled in bacterial, DNA and viral formats to transduce cells in screens to subsequently undertake functional assays. Pooled DNA/virus will be distributed to people within the Institute and wider UCL for screening in various other projects.

Recipient or parental organism

| Cell lines: canine-MDCK, DK, D17, C2F; feline- AH927, CRFK, Mya1; Human-HeLa, TE671 , HT1080, 293T, HEL, HFFF, BJ1, Jurkat, CEM, supT1, C8166, A549, LECs, tMSCs; Mouse- MDTF, NIH3T3; Avian- QT36; MarsupialSC300; Bat Tb1lu; non-human primate- FRhK4, LLCMK2, CV1, VERO, Pindak, SMLF; bovine- MDBK, IMR31; Porcine- PK15, SKL, CPK, STIOWA; Mink- mv-1-lu; Rabbit- SIRC,eREP, ratHSN, RAT2, 9L, NRK. Primary Human and Mouse cells: Derived from various human and mouse tissues including embryonic stem cells, peripheral blood derived lymphocytes and macrophages. Bacterial E coli strains- HB101, XL 1-blue, Top10, PrimePlus, JM109, BL21, DH5alpha, SURE, KS1000, DE3, Lucigen Endura cells. Associated risks and likeliness of occurrence: The recipient cells (cell lines and primary cells) that will eventually receive and integrate the DNA are harmless to humans. They are also unable to survive outside culture conditions and are therefore harmless to the environment. The bacterial strains used are attenuated non-colonising strains that are harmless to humans and cannot survive outside culture conditions and are therefore harmless to the environment. |

Host/vector system

Lentiviral vectors:
Various 2nd and 3rd generation lentiviral expression plasmids including pLKO.1 and derivatives.
Lentiviral packaging vectors:
2nd and 3rd Lentiviral accessory and packaging vectors including Gag/pol expression vectors p8.9, psPAX2 and derivatives (accessory packaging vectors derived from HIV-1) and viral envelope expression vectors including pMDG and derivatives (expresses VSV-G from Vesticular Stomatitis Virus).
Non-viral expression plasmid vectors:
Various non-viral DNA plasmid vectors containing various mutations/genes either to expressed from the cell without integration or regions inserted into the recipient genome by homology dependent repair pathways. This includes PiggyBac vectors from Transposagen which are non-viral/non integrating expression vectors. PiggyBac vectors include two components expressed together in a cell 1) vector containing a cassettes of various genes that will be inserted into the genome at transposon sites 2) Vector expressing transposase that will mediate the integration of the expression cassette in 1 at transposon sites.
Details on Lentiviral system and vectors:
Lentiviral vectors are made by transfecting 3 plasmids into a packaging cell line such as HEK293T cells. The 3 plasmids used are contained within or will be transformed into E.Coli (see above) for propagation. The 3 plasmids are 1) a packaging plasmid expressing the required accessory proteins for viable viral particle production 2) an envelope plasmid, expressing the envelope protein for the viral particle and 3) the lentiviral vector carrying the cassette with the inserted sequence of interest. Once all 3 vectors are in the cell, each component from the 3 vectors will be expressed. The co-expression of the components from the various vectors will allow the formation of viral particles which will be released from the cells. By separating the components onto 3 independent plasmids, that do not have enough
homology to recombine, the viral packaging elements are not packaged into the virus themselves making the vector disabled and incapable of self-replication. This system therefore is incapable of producing replication competent virus making it very safe. In this particular project, the lentiviral expression vectors are packaged into VSV-G pseudotyped viral vectors made from gag-pol and VSV-G expression vectors that have been transfected into human cells.

Associated Risks and likelihood of occurrence:
Non-viral expression vectors including PiggyBac vectors many randomly integrate into the host cell genome but are non-viral and cannot establish an infection. They will not be able to enter cells of non-laboratory cells without specialised cell entry reagents and are therefore very safe.
The lentiviral vectors can infect human cells or any other mammalian cell as they are pseudotyped with the envelope protein VSV-G from the vesicular stomatitis virus and therefore if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate its genome into cellular DNA. Physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene leading to an oncogenic mutation, however the likelihood of this is very low and has only occurred in a clinical setting following prolonged infection with high titre lentiviral vectors encoding growth promoting molecules and selection in vivo for cells with altered growth potential. Additionally, it would not be as severe as the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection. Also, it would only be a small number of cells and therefore would not impact on the pathogenic outcome of an exogenous viral infection. Additionally, the likelihood of infection is very low. No adverse effects have ever been described through accidental exposure to retroviral vectors. The viral particles are labile and do not survive on environmental surfaces outside the laboratory.
The packaging viral components are from the HIV-1. The full length HIV-1 virus is harmful to humans, however the packaging vectors used do not contain the full length HIV-1 molecule. Many components of this virus that are critical for HIV-1 infection have been removed with only those required for viral packaging remain. It is therefore attenuated and cannot replicate and form new viable virus.

Origin & function

The sequences contained within the lentiviral or non-viral expression vectors include:

Health and Safety

Guide RNAs: A component in the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Type II system - a RNA-Guided Endonuclease technology used for genome engineering. The guide RNA is a combination of the bacterial crRNA and tracrRNA molecules. The guideRNA encompasses various small de novo derived 17-20bp sense and antisense sequences that are homologous with a genomic DNA region of interest, which are designed to target anywhere within the Human or Mouse genome. This sequence defines the targeting specificity of the guide RNA where it will bind the DNA, triggering the (CRISPR associated) Cas9 endonuclease to make a double stranded break in the genomic DNA at this site. This triggers a DNA repair mechanism response which in turn results in genome modifications.

CRISPR associated 9 (Cas9) endonuclease: A component in the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Type II system- a RNA-Guided Endonuclease technology used for genome engineering. Cas9 is recruited to the genomic location by the bound guideRNA and cuts the DNA at this location. We will also use mutant versions of Cas9 that cannot cut the DNA but will be linked to various other effector molecules such as transcriptional activators/repressors which result in epigenetic modulation of DNA expression at this location.

Transcription activator-like effectors (TALEs): TALEs are modular proteins that can be designed to specifically bind to a desired region of genomic DNA. These TALE molecules can be linked to nucleases which will result in cutting of the DNA at the genomic location where the TALE binds, trigerring a DNA repair mechanism response and genome
editing, or linked to various other effector molecules such as transcriptional activators/repressors which result in epigenetic modulation of DNA expression at this location.

Other donor sequences:
Contain regions of homology with the genomic DNA of the system of interest alongside additional sequence with desired mutations, additional genes, fluorescence markers or antibiotic resistance genes that will be incorporated into the genomic DNA by DNA repair mechanisms when the TALE or Cas9 molecules have cut the DNA. The sequences contained within the PiggyBac expression vectors include:
Additional genes, fluorescence markers or antibiotic resistance genes, that will be incorporated into the genomic DNA at transposon sites when expressed alongside transposase from a separate non-viral/non-integrating plasmid.

Evaluation of foreseeable effects

Risks and likelihood of risks:
Health and Safety
Executive
The bacterial strains to be used are non colonising and incapable of surviving in the environment or causing human infection. They pose no risk to users or the environment.

None of the DNA viral vector plasmids are harmful to health or to the environment and do not supply a survival advantage to any microorganisms. The packaging viral components are from the HIV-1 virus. HIV-1 is harmful to humans, however, the packaging vector does not contain the full length HIV-1 molecule. Many components of HIV-1 that are critical for HIV-1 infection have been removed from the packaging vector. Only those essential for viral packaging remain.
The most dangerous GMM is the lentiviral preparations and the most dangerous step is the collection, concentration and use of this lentivirus, however this risk is minimal. Lentiviral vectors can infect human cells and therefore if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate its genome into cellular DNA. If the inserted sequence were to be expressed in humans after accidental transfer, Cas9rr AL could cut and induce genome engineering changes in the host genome that may result in mutations that have oncogenic potential or mutations that have a severe effect on cell survival or normal function. Pooled viral libraries will also be used that contain many different guideRNAssrrALEs in the one pool, and some of the guideRNAssrr ALEs in these pools will result in mutations that have oncogenic potential or mutations that have a severe effect on cell survival or normal function. Additionally, physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene. However, the likelihood of infection very low as . all work will be performed in containment level 2 facilities. If a person were infected by infectious lentiviral particles they are replication incompetent (does not carry gag, pol or env genes) so they cannot make new progeny virus and establish a productive infection, so there is no chance of transmission to another cell or person. Also, it would only be a small number of cells and so would not impact on the pathogenic outcome of an exogenous viral infection. It is extremely unlikely that an infection would occur and no adverse effects have ever been described through accidental exposure to retroviral vectors therefore the overall risks of our experiment are low. Additionally the viral particles are labile and do not survive on environmental surfaces outside the laboratory making infection unlikely. Non-viral expression vectors including PiggyBac vectors many randomly integrate into the host cell genome but are non-viral and cannot establish an infection. They will not be able to enter cells of non-laboratory cells without specialised cell entry reagents and are therefore very safe. The cell lines used cannot survive outside the laboratory incubators and as such pose no threat to health or the environment.
Precautions in place to minimize risks
The entire of the Cancer Institute is a containment level 2 facility which is secured by restricted access at all entrances and additionally to all laboratory areas. All work involving viruses will be performed in class 2 cabinets in containment level 2 laboratories specified particularly for retroviral GMM work. All DNA
plasmids containing viral proteins will be grown separately to avoid opportunity for recombination. To prevent contamination of workers by virus, when handling any GMM or GMM-derived DNA or virus, no glass or other sharps will be used in the rooms where the viruses are prepared and used and workers will be protected by a lab coat and nitrile gloves. Skin lesions will be covered with a bandage in addition to the protective wear described above. Specific guidelines will be in place to ensure all viral preparations are handled, labelled, stored, transported and cleaned up correctly. All those involved in the project will be made aware of the guidelines and the associated risks as well as anyone else who may use the laboratory. Persons involved on project from other Institutes will work under the supervision of a competent trained worker from this project. No-one visiting the laboratory that is not involved in this work will work on this project. All persons entering the facility will be made aware of the safety precautions upon entering the facility. All equipment used in the retroviral laboratory will be available in other general purpose laboratories for general use so it should not be necessary for others who are not involved in retroviral work to enter the laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

10% Distel or Virkon (v/v) will be used to disinfection any solid waste including plastic flasks and tubes or other equipment and laboratory and liquid waste (16 hours) and also used to decontaminate any spills. Liquid waste is subseously poured down the sink after treatment. Work surfaces will be wiped down with 10% Distel and 70% ethanol after use.Virkon and Distel are certified by the PHLS to provide 100% viricidal and GMO kill under these conditions by denaturing and inactivating viral and cellular proteins. Distel and Virkon will be used with their lifespan to ensure required kill is achieved. Solid waste will be double-bagged in biological waste bags, sealed and autoclaved 132c for 15mins by trained staff then bagged in yellow biohazard bags and removed by UCL waste services. Autoclave runs are regularly validated by waste management staff

* Work with non-viral expression vectors including PiggyBac vectors will not require the treatment outlined above due to the safety of these reagents (non-viral). Solid waste will not be disinfected and will be double bagged in yellow biohazard bags and removed by UCL waste services for incineration only.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The UCL GMSC approved this project as a Class 2 activity at their meeting on 12/12/14.
### Project Additional Information

**Purposes of the contained use**
Generate and use human and rat olfactory ensheathing cell lines conditionally immortalised using viral vectors.

**Recipient or parental organism**
Primary rat and human primary cells from the nervous system transduced with viral vectors.

**Host/vector system**
JS4, a recA-derivative of E.coli MC1 061, a disabled K12 strain. Non-mobilisable.

PA371, a second generation mouse amphotropic producer cell line that was used to derive the high titre producer for the U19tsA58 T antigen. Non-mobilisable.

TEFL YA and RD cells - both are derivatives of TE671 cells, third generation human packaging cell lines for production of stable, high titre defective helper-free amphotropic retroviruses that have a murine or feline envelope. Nonmobilisable.

Phoenix amphi cells are non-mobilisable derivatives of HEK293, used for production of high titre transient amphotropic virus stocks. Phoenix amphi were originally developed by Gary Nolan but obtained from ATCC. Free of adventitious agents.

pZipNeoSV(X)1, pBabeNeo/Hygro/Puromycin, pBabeNeo/HygroZeo, pBabe-puro-MyCER, pLNCX, pLNCX2, pLHCX, pLPcX, pWZLPuro, pWZLBlastF, pTMP, pUC57, pWZLPuro/BlastF, pRetroSuper

Origin & function

The proteins produced from the immortalizing genes are biologically active in humans and have the potential to extend the proliferative life span. SV40 T antigen, the catalytic subunit of human telomerase, HPV E7 and E6 proteins, Polyoma virus T antigens, Adenovirus E1 A, p53GSE, c-MycER are all immortalizing genes and have the potential to extend the proliferative life span of normal somatic cells. However, The c-MycER and ras-ER are oncogenes only active in the presence of tamoxifen, and tsSV40 U19tsA58 is biologically active only whilst cells grow in permissive conditions (33°C), and when growth in non-permissive conditions, there is an irreversible lost of immortality. VEGF-A 121, VEGF-A 165, VEGF-A 165/PIGF-2123-144, bFGF/PIGF-2123-144, PDGF-AA/PIGF-2123-144, IGF-1/PIGF-2123-144, GST-PIGF-2, GST-PIGF-2123-144 and NT3/PIGF-2123-144 encode for growth factors with or without a promiscuous domain (PIGF-2123-144) that binds with high affinity to extracellular matrix.

SV40 large T antigen and mutants there of obtained from Y. Gluzman, P. Sharp, P. Tegtmeyer, A. Smith, J. Pipas, T. Roberts and constructed by P. Jat.

Human catalytic subunit of telomerase from R. Weinberg.

c-MycER from Littlewood.

Human Papilloma virus 16/18 E7 and E6 gene from D. Shalloway.

Polyoma virus T antigens and mutants there of from B. Griffin, W. Eckhart and M. Fried.

Adenovirus E1 A and mutants there of from S. Lowe. P53 GSE fragment from A. Gudkov.

Full length ORF for cellular genes from ATCC and Gene Service.

Activated ras fused to estrogen receptor from J. Gil.

pmiR-Vec expressing micro RNAs from Gene Service.

VEGF-A121, VEGF-A165, VEGF-A165/PIGF-2123-144, bFGF/PIGF-2123-144, PDGF-AA/PIGF-2123-144, IGF-1/PIGF-2123-144, GST-PIGF-2, GST-PIGF-2123-144 and NT3/PIGF-2123-144 from M. Martino.

Evaluation of foreseeable effects

The transduced genes either singly or in combination can immortalise human cells. Therefore the viruses containing these genes could immortalise the operator's cells if they were exposed by inoculation.

Safety procedures ae in place that make the possibility of self infection remote. In addition, because the viruses are defective and helper free, they should not be able to spread themselves beyond the site of inoculation. The cultures of human cells transduced with the viruses should not produce infectious virus, because the viruses are defective and helper free. In addition, such fells should be rejected by the immune system following inoculation.

Most of the genes alone are unable to cause tumours but can cause tumours in conjunction with other genes. SV40 T antigen is an exception because it can act as an oncogene but at a low frequency and most likely in concert with cellular mutations. Ras does not cause tumours alone- other mutational events are required.

The vector itself could cause harm if it is integrated in the vicinity of an oncogene and induces its expression or if it is
inserted within a tumour suppressor so that its expression was lost. Integrated proviruses are capable of being mobilized by super-infection with helper viruses. The likelihood is low since the human cells that we will use are not producing helper viruses and we will segregate the transduced cells from other primary cultures, in particular mouse lymphoma cells or hybridomas that frequently produce helper viruses. Transduced cell lines for production of retroviruses will be monitored to ensure that the integrated proviruses have not been mobilized.

The most likely route for accidental release of the GMMs to the environment is through the air either by spillage or aerosolisation of high titre viral stocks- this will be avoided. Transmission is also possible through spillage of virus into drains. No virus contaminated liquid waste will be discarded into drains unless it has been decontaminated using sufficient chloros for >10,000 ppm and left for >24 hours. Neither the E. coli, the packaging cells, the amphotropic viruses or the transduced human cells will survive alone within the environment. The most likely route for accidental release of the GMMs to the environment is through the air either by spillage or aerosolisation of high titre viral stocksthis will be avoided.

The recombinant genes do not cause harm but the vectors encode antibiotic resistance markers that could be transferred if the insert was mobilized. The retroviral vectors encode resistance to G418, hygromycin, puromycin, ampicillin, blasticidin and zeocin and thus the GMMs will be resistant to the appropriate drug.

The cell lines PA317 (derivative of NIH3T3), TEFLYA and RD (derivative of TE671), and Phoenix ampho (derivative of HEK293) cells have a history of safe usage within the laboratory and are free of adventitious agents.

Primary rat and human somatic cells have the potential to be infected with adventitious agents.

The retroviral vectors have a history of safe use of over 15 years.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste such as dishes and flasks will be decontaminated with chloros (>10,000 ppm), or by autoclaving and then incineration. E. coli solid waste (agar plates, tips and pipettes) are autoclaved followed by incineration. Solid waste from tissue culture such as tubes, dishes, flasks and pipettes are autoclaved followed by incineration. After autoclaving or chemical disinfection, waste is put into yellow clinical waste bags or containers, collected centrally and transported by an approved waste route for incineration.

E. coli liquid waste is inactivated by treatment with 1 % Virkon for > 10 minutes. Liquid tissue culture medium is collected into containers containing sufficient chloros for > 10,000 ppm and left for >24 hours. After autoclaving or chemical disinfection, waste is put into yellow clinical waste bags or containers, collected centrally and transported by an approved waste route for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The University Biological Safety Adviser, on behalf of the UCL GMSC agreed this as a Class 2 activity following minor amendments to the assessment

Project Containment

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<tr>
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Project Ref 14/15.5

Date Ackn'd: 27/05/2015
CU2 Project Title: Recombinant vaccinia virus purification by monolith chromatography

Class: Class 2
CultureVol: 1-50 Litres

Date Project Ceased: 
Non-GMM: Consent Granted

Tick if notifying a connected programme of work: N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Recombinant vaccinia virus (VVL15RFP) is used as feed material for monolith chromatography studies. We want to
characterise the virus binding capacity to ion exchange monolith column and the elution by various salt to find out the virus recovery and ury levels. Cell culture and virus production, cell harvest and disruption, virus recovery are needed to generate the feed material.

Recipient or parental organism

vaccinia virus lister strain

Host/vector system

Vaccinia Virus Lister strain with thymidine kinase (TK) gene deletion and red fluorescent protein (RFP) gene insertion, VVL15 RFP

Origin & function

The origin of VVL15RFP is the wild type Lister strain vaccinia virus. It was designed so VVL15RFP is incapable of normal replication and need helper functions provided in trans in the packaging cell line to generate the virus particles. Wild-type vaccinia viruses have a natural tropism for tumours. Given the remarkable efficiency of vaccinia virus in inducing tissue destruction, genetic modifications of the virus (VVL15RFP) have been designed to create oncolytic vectors that specifically infect and replicate in tumour cells and attenuate in normal cells/tissues. The construct is a deletion mutant with regions of the viral genes that are critical for efficient viral replication in normal cells but dispensable in tumour cells. VVL15RFP with the thymidine kinase gene deletion further diminish virus replication in normal cells.

The red fluorescent protein gene sequence is from the choral Discosoma striata and has been inserted for assay purposes.

VVL15RFP was constructed by Dr. Yaohe Wang's research group in Institute of Cancer, Barts and The London School of Medicine and Dentistry, Queen Mary University of London. We will not perform any further modification of VVL15 RFP.

Evaluation of foreseeable effects

Attenuated VVL15TK-RFP is unlikely to cause an infection in humans. The wide type virus is only known to cause mild flu like symptoms. The TK-gene deletion in VVL15 RFP demonstrably reduces the virus' ability to infect nonimmortal cells and as such it can be regarded as conditionally replicative. Therefore the VVL15RFP virus attenuated by deletion of Thymidine kinase gene diminishes virus replication in normal cells. Even in the event of a complete breakdown of all containment procedures in the laboratory, the risk to human health would be extremely low.

The use of biological safety cabinets, lab coats and gloves, disinfection and autoclaving of waste will reduce the likelihood of exposure to an extremely low level. The laboratory will have restricted access to only a small number of well trained and experienced staff and postgraduates.

Vaccinia virus is unable to survive outside of a highly controlled laboratory environment, and is not able to pass on genetic information to a host in the event of an infection. This is because it can only selectively replicate in the cytoplasm of a host cell, and so doesn't incorporate it's DNA into the nucleus during infection.

In the event of accidental spillage in the laboratory, which is a containment level 2 facility, it would be disinfected with Virkon leading to the rapid destruction of live material thus eliminating the risk of genetic transfer to the environment. Even in the event of a complete breakdown of all containment procedures in the laboratory, the risk to the environment would be extremely low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Liquid waste and cellular material will be treated with Virkon. 1% Virkon is shown by the manufacturer to provide effective disinfection of 100% kill within 10 minutes. https://www.anachem.co.uk/catalogue/product/itemNo/290012

Waste Disposal of contaminated Solid Waste:
Place all plasticware that comes in contact with biological material in a flask containing 5% Virkon for 60 minutes then drain Virkon liquid down sink (with tap running). The plasticware or equipment will be autoclaved. The disposable plasticware will be transferred to a red bin for incineration.

Waste Disposal of contaminated Liquid Waste:
Pour all liquids containing biological material into a vessel containing 5% Virkon for 60 minutes then drain Virkon liquid down sink (with tap running) and transfer plasticware to a red bin for incineration.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

The UCL Biological Safety Adviser, on behalf of the UCL GMSC approved this project as a Class 2 activity, subject to clarification regarding the risks posed by the FPLC.

Project Containment

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Project Ref 14/16.1
The purpose of this work is to identify cellular components important for infection by different viruses. These cellular components can also be used as molecular targets for the development of broad-spectrum antiviral drugs.

The only GMO is a GFP expressing Semliki Forest virus. The other viruses used (Semliki Forest virus, Sindbis virus, Vaccinia virus, Herpes Simplex 1 virus, and Yellow Fever Vaccine strain 17D) are wild type or attenuated viruses.

The GFP sequence was originally derived from Aequora victoria.

The recombinant virus expresses GFP for visualisation and quantification of infectivity. This modification either has no impact on virus infectivity, or it is likely to limit the infectivity of the virus.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work will be conducted in compliance with the local rules for work in the containment level 2 Laboratory. Waste will be sealed in autoclavable bags inside a metal container before removing from the virus lab for autoclaving in Room G12 (LMCB). Liquid waste will be treated with Distel (5%) for at least 12 hours before disposal in the laboratory sink. Distel, formerly Trigene Advance: ATS labs study titled "Virucidal efficacy of a disinfectant for use on inanimate environmental surfaces", March 2007. This study concluded that TriGene advance (at 1:100) demonstrated complete inactivation of HIV type 1 at 30 min exposure time at RT.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The UCL BSO approved this as a Class 2 activity on behalf of the UCL GMSC (after a number of minor amendments had been made - detial on attached risk assessment).

Project Containment

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Project Ref 14/16.2

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<td>14/04/2016</td>
<td>C. elegans infection models with Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella oxytoca and Enterobacter cloacae</td>
<td>Class 2</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Date Project 02/03/2022
### Purposes of the contained use

Investigating the effect on the life traits (lifespan, development, reproduction) of the model organism *C. elegans* when grown in the presence of the human gut microbes *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Enterobacter cloacae*.

### Recipient or parental organism

All the bacteria to be used are opportunistic pathogens. Although they have been mainly associated with severe disease in the immunocompromised, they can cause infection in healthy humans, albeit with less serious symptoms/consequences. The most likely consequence of the planned modifications in all the species is a loss of bacterial fitness due to the imbalance in gene regulation. The inserts drive the expression of anti-bacterial resistance genes against Spectinomycin. Spectinomycin is only used in the treatment of Gonorrhoea so very little hazard results from this. The inserts are not directly hazardous nor the genes they express. The GMM would be less likely to cause disease as a consequence of the cost of maintaining and replicating the insert. In addition, the insert does not add to the pathogenicity of the bacterial strain. Infections could be treated with a cocktail of antibiotics.

### Host/vector system

Shuttle vectors able to replicate in *Escherichia coli* for cloning purposes and for expression or deletion of *E. faecalis* genes. These vectors do not carry any risk by themselves. Transfer of genes is very unlikely to occur as these strains are not able to mate and therefore not capable of transmitting directly this genetic insert. If it were to occur, antibiotic treatment of the recipient strain would still be efficacious as for the wild-type strain.

### Origin & function

The inserted genetic material is all derived from *E. faecalis* genes mostly those involved in nucleotide metabolism.

### Evaluation of foreseeable effects

The GMMs would be less likely to cause disease in humans or animals than their parental strains, given the burden of carrying the insert or gene deletion as there is no gain to pathogenicity

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All potentially infected solid waste will be discarded in an approved autoclave bag. Before the bag is too full it will be closed with a cable tie and then placed in a second autoclave bag, also secured with a cable tie. Bags will be autoclaved in the autoclave in room SB23 and removed from the autoclave in the anteroom. Waste will be autoclaved at 134°C for 20 minutes, 3 bars pressure. Autoclaved material will be put in the appropriate bins. Autoclaved waste is categorised as offensive and is double yellow tiger bagged and locked away until collected and incinerated by a UCL contractor (Grundons). All infected liquid will be chemically disinfected with Virkon 1% for a minimum period of 24 hours before being discarded in the drain.

Virkon has a wide spectrum of activity against viruses, some fungi, and bacteria. Efficacy against Enterococcus faecium (a very close species to E. faecalis), Enterobacter, Klebsiella pneumoniae (a very close species to K. oxytoca) and P. aeruginosa has been demonstrated (http://virkon.nl/images/VirkonS_effectiviteit_bacteriën_dec2012.PDF) at concentrations of 1% or lower.

Please enter comments on the GM safety committee on the risk assessment

The UCL GMSC agreed the classification of the work as Activity Class 2, subject to additional detail being provided regarding the infectious nature of the agents.

Project Containment

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Project Ref 14/16.3
Expression of non-toxic and non-pathogenic, but potentially oncogenic sequences, as well as non-oncogenic sequences, in animal cells in culture, using plasmids/retroviral/lentiviral expression vector to include the creation of amphotropic pseudotyped virus able to infect mammalian cells (human fibroblasts and/or human iPS cells). Plasmids/retroviral/lentiviral expression vector and cells infected with plasmids/retroviral/lentiviral expression vector may be injected into mouse/rat.

Replication-defective virus that expresses 4 transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) in 293T cells and then infect human fibroblasts to generate human iPS cells will be produced.

Recipient or parental organism

Mouse/Rat work:
The bacterial strains used to produce the plasmid vectors are attenuated non-colonising strains that are harmless to humans and cannot survive outside culture conditions, and are therefore harmless to the environment.
The vectors packaged in Phoenix-ECO are replication-defective, non primate, non infectious and non-pathogenic to humans. There should be no risk to humans from it carrying transgenes, including potential oncogenes.

Human work:
HEK293 is a well characterised cell line derived from human embryonic kidney cells grown in tissue culture.
The recipient cells that will eventually receive and integrate the DNA are harmless to humans. They are also unable to survive outside culture conditions, and are therefore harmless to the environment.
The lentiviral vectors used will all be well characterised, with a history of safe use, and are inherently safe. The lentiviral particles could in theory be a risk to human health as they can infect human cells. However, all vectors will be self-inactivating & replication-incompetent, that is, each lentiviral vector particle infects and gives genomic modification of one cell, and that cell will not (and indeed cannot) subsequently produce and release further viral particles.
However in the unlikely event that the virus enters the body by injection, ingestion or through a wound, it could enter cells and integrate into cellular DNA. Its integration could result in a potentially oncogenic alteration, especially if the inserted sequence codes for an oncogene, activates oncogenes, or represses a tumour suppressor gene.

**Host/vector system**

| pCDNA,    |
| pBird,    |
| FUW,      |
| pEFGFP,   |
| pTdTom,   |
| pLKO.1    |

**Origin & function**

Moloney murine leukemia virus; HIV lentivirus

**Evaluation of foreseeable effects**

The lentiviral particles cannot survive outside of closed controlled cell culture conditions and are rapidly inactivated by dehydration or other environmental insults. They require close contact with contaminated fluids or percutaneous inoculation for transmission. The virus could, in principle, in the unlikely event of it being introduced to the cells of a worker, give genomic modification of cells of the worker.  
Some of the inserted sequences would produce transcription factors that could be oncogenic.  
The survival or retroviral vectors in the environment is poor, due to their fragile envelope. None of the cell lines or primary cells would be expected to remain viable outside the controlled culture conditions.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Lentiviral supernatants will only be handled whilst exposed in class 2 cabinets. They will be clearly labelled and stored in double containment and transferred within the laboratory in doubly sealed containers.  
Sharps will be used for mouse/rats injections. The vector or the cells transduced with the vector will be injected into the mouse/rats tissue. Safer sharps will be used if possible; all sharps will be disposed of after use into an appropriate sharps bin. In addition, all staff carrying the procedure will be training (with competency records kept) which should further limit the chances of sharp injuries.  
However no-sharps policy will be in place for all the human cells work.  
For the transport, the vials containing virus will be placed in a rack which will be in a “Tupperware-type” container with snap lock lid or a screw cap bottle sealed with parafilm, with some absorbent tissue at the bottom of the rack (and a made-up solution of Virkon and spare tissue would have to be brought with during transport in order to clean up a possible spillage promptly)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid Biohazard and GM waste at the WIBR is double bagged in autoclave bags, autoclaved on site at a minimum of 134deg C with a hold time of 20 minutes followed by offsite incineration. The autoclave runs are monitored for temperature and hold time automatically, with a print-out confirming a successful cycle. Sharps are incinerated off
Culture medium: 1% final concentration of Virkon treatment for 24h. Liquid waste will be inactivated with a final concentration of 1% Virkon for 24 hours. (Dupont specification says it has been independently proven that 1% solution kills bacteria within 5 minutes and parvovirus within 10 minutes.) Surfaces are cleaned with a solution of 1% Virkon or Trigene Advance 1:10 spray. Chlorasept tablets are also used on some occasions at a minimum of one 2.5gm tablet per litre of solution, again left for a minimum of 20 minutes.

The University Biological Safety Adviser approved this on behalf of the UCL GMSC subject to some minor amendments.
### Purposes of the contained use

Our model of infection employs adult pregnant mice to study ascending vaginal infection caused by *Escherichia coli* K1. We aim to use this model of infection combined with a genetically modified bioluminescent strain of *E. coli* O18:K1 (A192PP) to track the course of the infection using an IVIS bioluminescence imager. Strain A192 (DSM. No. 10719) (Achtman, et al., 1983) was used to generate this strain. A bioluminescent derivative of *E. coli* A192PP will be generated by introduction of the luxCDABE operon from the nematode symbiont *Photorhabdus luminescens*. The luxCDABE operon will be supplied through mini-Tn5 mutagenesis using the construct pUTm.

### Recipient or parental organism


ACDP hazard group: 2

### Host/vector system

**pUTmini-Tn5**
- Plasmid can only be maintained in lambda pir+ donor strains (e.g. *E. coli* S17-1 lambda pir).
- Plasmid can be transferred by conjugation to other bacterial strains (pir-) and acts as a suicide vector.
- Transposon is disarmed, so forms stable integrations within recipient chromosome and cannot re-mobilise. (Winson, et al., 1998) The mini-Tn5 is also transcriptionally isolated: a in frame stop codon (TAG) was introduced 18 codons upstream of the luxC ATG.

### Origin & function

The inserted transposon is the luxCDABE: lux operon from the nematode symbiont *Photorhabdus luminescens* ATCC29999 (Hb strain) (Winson, et al., 1998). With kanamycin resistance cassette (Km2). The operon will provide the mechanics for bioluminescence emission from the recipient strain. Recipient strains will become resistant to kanamycin (kanR) by acquisition of mini-Tn5 luxCDABE Km2.

### Evaluation of foreseeable effects

*E. coli* A192PP GM strain (like the parental A192PP strain) may be able to colonise the human gastrointestinal (GI) tract if ingested, however rat passage of the parent strain (A192) makes this less likely. As a strain of a bacterium becomes more adapted to a different species, that strain will become less adapted to the original host, thus decreasing in virulence with respect to the original host. (Koskiniemi S., Gibbons H.S., Sandegren L., Anwar N., Ouellette G., Broomall S., Karavis M., McGregor P., Liem A., Fochler E., McNew L., Rosenzweig C.N., Rhen M.,
E.coli A192PP GM strain may be able to colonise human neonates, however risk of exposure of such individuals is considered extremely low. However, if exposure occurred, the most hazardous outcome could be sepsis and meningitis. Again, as detailed above, rat passage of the parent strain (A192) makes this less likely.

E. coli A192PP GM strain will acquire kanamycin resistance. However other antibiotics, including but not limited to beta-lactams, aminoglycosides, tetracyclines and chloramphenicol can be used, should prophylaxis be required.

E. coli A192PP GM strain is unlikely to transfer the transposon to other bacterial strains in vitro, in vivo or in the environment, as the mini-Tn5 to be used is disarmed (loss of transposase during insertion - is present on the suicide vector). This enables stable integration into the recipient chromosome.

The acquisition of foreign genetic material may increase, decrease, or have no effect on virulence of the strain. This may depend on where the transposon insertion occurs (random event). However, due to the expected metabolic burden on the strain (caused by the presence of exogenous genetic material), a reduction or no change in virulence is expected to be observed, rather than an increase.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- Animal housing at Central BSU Animal Facility has individually ventilated cages (IVCs).
- Appropriate personal protective equipment (PPE) will be worn at all times for work with animals; including P2 face masks, disposable gloves, overalls, shoe covers and mob caps.
- Infection of pregnant mice will be done on the bench top in B08 to enable mice to be anaesthised during bacterial instillation. However, only the trained operator will be in room B08 when any work with this bacteria is undertaken.
- Infected mice will be housed in individual IVC cages and will not be touched by BSU staff after infection with this bacteria.
- Imaging of mice colonised with E. coli A192PP (GM strain) will take place at Central BSU, UCL. Imaging procedures will then be done using the IVIS, located in room B08 within the CBS. B08 is a restricted access area (card access only, within the facility). Mice will remain in their IVCs for as long as possible before imaging occurs. During imaging, shedding of urine/faeces from colonised mice will be controlled using a disposable black card placed on the imaging platform (to act as a barrier). This card, as well as eppendorfs containing bacteria, will be disposed of by doublebagging prior to removal from BSU and transported for autoclaving.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We wish to seek derogation in respect of having an autoclave in one of the locations of the work; 86-96 Chenes Mews. Currently only Class 1 GM activities are carried out in this location with liquid waste being disinfected before disposal and solid waste sent direct for incineration to an incinerator notified to HSE as being able to handle waste from Class 1 and Class 2 activities.

Any liquid waste form the proposed Class 2 activity will also be treated with disinfectant overnight (see below). Given that the amount of solid waste anticipated is minimal (no more than 2 autoclave bags/week), and the short term nature of the proposed activity (~6 months), it is proposed that solid Class 2 waste will transported to an adjacent building for autoclaving. Full details are given RA001530 (sent also with this notification) but, in summary, waste will be double bagged (2 autoclave bags closed with coloured cable ties to identify source of waste) and then placed in a small domestic size lidded wheelie bin and the lid fastened. The risk of spillage is therefore negligible during transport.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The imaging platform will then be treated with 70% ethanol at the end of each procedure. Personal protective equipment/clothing will be worn at all times during imaging. Other facilities within the room include hand washing facilities, sharps disposal bins and general waste bins (orange bins).

General microbiology and processing of infected tissues within G13 at Chenies Mews will be done within a Class 2 safety cabinets.

- Bacterial culturing in Lab 103 will be done on a single use basis and the shaking incubator will be clearly labelled with a warning sign for other users of the lab.
- Sharps will be treated before use using 70 % (v/v) ethanol and disposed of after use in supplied sharps-bins for incineration. Surgical scissors and other surgical instruments (e.g. tweezers) will be autoclaved after use (a designated set of surgical instruments will be used for these experiments). All staff receive training in use of sharps as part of their HO PLH training.
- Solid (eg agar place and contaminated plastics) waste will be autoclaved, waste will be collected by UCL IFWH staff and will be sterilised by autoclaving for a cycle of 131oC for 30 mins. Transport of waste to the autoclave is covered by a separate risk assessment (RA001530) - also included with this notification.
- Liquid waste (eg bacterial cultures, blood from mice) will be treated with Presept (4 tabs/litre; final concentration of 2g/l or 4000ppm/l) overnight before disposal to drain.

Mice carcasses will be incinerated and disposed of by BSU support staff.

- Contaminated sharps will be placed in sharps bins and will be incinerated. Surgical scissors and other surgical instruments will be autoclaved after use.

---

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The University Biological Safety Adviser approved this as a Class 2 activity (after minor amendments/clarifications regarding waste handling) on behalf of the UCL GMSC.

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### Project Containment

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Assessment of ingestion of GFP Pseudomonas by drinking water on the development of arthritis

Purposes of the contained use
To be able to track which parts of the gut that ingested bacteria migrate to.

Recipient or parental organism
Pseudomonas aeruginosa is classified as HG2 agent. Pseudomonas lives in soil and water and is normally only harmful in immunodeficient individuals.
The bacterium is a normal skin commensal however it can cause infection in severely immunodeficient individuals, and this is majorly the cases seen (linked to hospitalization). In hospitals, the bacteria can spread through medical equipment, cleaning solutions, and other equipment. For example, pseudomonas is one of the main causes of pneumonia in patients who are on breathing machines. Burn victims and people with puncture wounds may get dangerous pseudomonas infections of the blood, bone, or urinary tract. The bacteria can also get into the body through IV needles or catheters.
Healthy people can develop mild illnesses with Pseudomonas aeruginosa, especially after exposure to water. Ear infections, especially in children, and more generalized skin rashes may occur after exposure to inadequately chlorinated hot tubs or swimming pools. Eye infections have occasionally been reported in persons using extended-wear contact lenses.

Host/vector system
pUCP18?MCSgfpmut3:
Vector size: 5.492kb
Vector type: plasmid
Marker: bla (ampR)
Features:
GFPmut3: fluorescent maker under the control of Plac promoter
Origin of replication: ori from pRO1600
Unique restriction sites: BamHI, EcoRI, EcoRV, SmaI, XhoI
Double restriction sites: HindIII, PstI, SalI, XbaI

Origin & function

Green fluorescent protein GFPmut3: this gene is expressed under the control of the Plac promoter. This construct has been designed for Gram-negative bacteria fluorescence labelling.
GFPmut3.1 is a variant of the Aequorea victoria green fluorescent protein (GFP). This variant gives very bright green fluorescence when expressed in bacteria because it contains the GFPmut3b mutations (Ser-65 to Gly and Ser-72 to Ala) that increase the efficiency of protein folding and chromophore formation at 37°C (1). The GFPmut3.1 fluorophore has an excitation maximum at 501 nm, an emission maximum at 511 nm, and is minimally excited by UV light. The GFPmut3.1 gene also contains a Ser-2 to Arg substitution which creates an Sph I site at the initiating ATG codon.

Evaluation of foreseeable effects

This clone was derived from ATCC 10145TM and contains a multicopy vector encoding the green fluorescent protein GFPmut3. This gene is expressed under the control of the Plac promoter. This construct has been designed for Gram-negative bacteria fluorescence labelling. Ampicillin resistance gene (bla) encoded on a plasmid. Confers resistance to 300 mg/mL ampicillin. NB: this is not the antibiotic of choice for treatment of infection - most infections are susceptible to third-generation cephalosporins (ceftazidime), carbapenems (imipenem and meropenem), aminoglycosides (gentamicin and tobramycin) and colistin.
The wild type is a HG2 agent and there is no evidence to suggest the GFP modification has made the organism less fit/less pathogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste produced in the Rayne Building will be autoclaved. Autoclave cycle is 121°C for 30mins, to kill any residual bacteria; after autoclaving, waste is disposed as offensive waste (in an off-site domestic waste incinerator). For disposal of liquid waste, Virkon will be added to a concentration of at least 1% and left overnight before disposal down the sink. Mice will be incinerated as infectious healthcare waste. Virkon has a wide spectrum of activity against viruses, some fungi, and bacteria. Efficacy against P. aeruginosa has been demonstrated (http://virkon.nl/images/VirkonS_effectiviteit_bacterien_dec2012.PDF) at concentrations of 1% or lower.
This project was approved as a Class 2 activity by the UCL UBSA on behalf of the GMSC after clarification and additional detail given in relation to the controls used for in vivo work.

Please enter comments on the GM safety committee on the risk assessment

This project was approved as a Class 2 activity by the UCL UBSA on behalf of the GMSC after clarification and additional detail given in relation to the controls used for in vivo work.

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Project Ref 14/17.1

Date Ackn'd 23/08/2017

CU2 Project Title Muscle cell Immortalization

Date Project Ceased

Class CultureVol

Class 2 1-50 Litres

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Purposes of the contained use

The aim is to immortalize muscle cells isolated from human muscle biopsies. Indeed, limitation of the proliferative capacity of satellite cells by cellular senescence restricts the development of in vitro models to study disease processes and to develop therapeutic strategies. This project aims to use a retroviral construct carrying the telomerase catalytic unit hTERT and the cyclin-dependent kinase 4 Cdk4 to overcome these limitations.

### Recipient or parental organism

A retroviral vector will be used to transduce human myoblasts.

### Host/vector system

A retroviral construct carrying both the human telomerase catalytic unit hTERT and the murine cyclin-dependent kinase 4 Cdk4. A resistance gene (hygromycin, neocyn, blasticidin etc) is also present.

### Origin & function

- murine Cdk4 overcomes the p16-mediated stress response
- hTERT maintains telomere ends by addition of the telomere repeat TTAGGG
- a resistance gene (puromycin, hygromycin etc) will be also used to select the transduced cells.

### Evaluation of foreseeable effects

hTERT is normally repressed in postnatal somatic cells. Deregulation of telomerase expression in somatic cells may be involved in oncogenesis.

We are using an amphotrophic envelop which can target a broad range of mammalian cells. The virus we produce is a non replicative retrovirus because gag-pol are cloned on a different plasmid. In theory, we cannot exclude any human infection. However, it is probably not likely to occur because all the experiments will be carried out in a Class II safety cabinet, with the appropriate equipments and without any sharp instruments.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All experiments will be carried out in Class II safety cabinets, viral supernatants will be centrifuged in sealed buckets in order to avoid/reduce aerosol., with the appropriate equipments and without any sharp instruments.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- Solid waste is double-bagged in biological waste bags, sealed and removed to be autoclaved by waste services.
- Any material that contain or have come into contact with viral particles will be decontaminated by soaking in the virkon 1% for 1 hour.
- Liquid waste is treated with 1% Virkon. After decontamination, liquid waste is disposed of via the sink.
Virkon has been shown to achieve 100% GMO kill when used as directed. Journal of Hospital Infection 46 (3): 203-209

This project was approved as a Class 2 activity by the UCL UBSA on behalf of the GMSC after clarification and additional detail given regarding control measures.

Please enter comments on the GM safety committee on the risk assessment

This project was approved as a Class 2 activity by the UCL UBSA on behalf of the GMSC after clarification and additional detail given regarding control measures.

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Project Ref 14/18.1

Date Ackn'd 17/01/2018

Date Project Ceased

Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
Genome-editing technologies will be used to manipulate the sequence of the cell genome with the aim to achieve a therapeutic effect.

Commercial E.coli disabled strains commonly used for the propagation of recombinant DNA. These will not express any of the CRISPR-Cas9 system proteins and are therefore unaffected.

The lentiviral vectors used are multi-attenuated meaning they are devoid of all potentially pathogenic virus encoded functions. In addition, they are replication-defective which means that the vector cannot multiply on its own unless supplied with certain proteins in trans. CRISPR-Cas9 system does not encode any protein that could promote replication.

The recipient mammalian cell lines are not considered harmful to immuno-competent humans.

Various 2nd and 3rd generation HIV based lentiviral expression plasmids.

The lentiviral vectors carry a deletion in the 3'LTR that does not affect generation of the viral genome in producer cells but results in "self-inactivation" of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome. A constitutive Rous sarcoma virus (RSV) promoter has been placed upstream of the 5'LTR in the expression vector to offset the requirement for Tat in the efficient production of viral RNA. Only 3 HIV-1 genes are used in the system (gag, pol and rev). Expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-1 revresponse-element (RRE) in the gag/pol mRNA transcript. The Rev protein itself is expressed from a separate plasmid. Addition of the RRE prevents gag and pol expression in the absence of Rev. The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope protein. Genes encoding components required for packaging are on separate plasmids, which do not contain regions of homology to each other to prevent undesirable recombination events, which could contribute to the generation of replication-competent virus. None of these 'packaging plasmids' that allow expression in trans of proteins required to produce viral progeny (i.e. gag, pol, rev,
env) in the 293T producer cell line contain LTRs or the psi packaging sequence. Thus none of the HIV-1 structural genes are actually present in the packaged viral genome and, thus, are never expressed in the transduced target cell. The lentiviral particles are replication incompetent and only carry the sequences of interest.

Non-viral expression plasmid vectors.

Origin & function

The sequences contained within the lentiviral or non-viral expression vectors include:
- artificially synthesized short guide RNAs targeting various genes within human genome,
- Streptococcus pyogenes Cas9 encoding a nuclease that introduces double stranded breaks in the targeted DNA sequence,
- commercially available antibiotic (e.g. puromycin) resistance gene or a fluorescence tag (e.g. GFP) to allow for monitoring of transduction/transfection of the cells
- The lentiviral plasmids also contain WPRE.

Evaluation of foreseeable effects

In mammalian cells, spCas9 has nuclease activity which can lead to loss-of-function mutation of the gene targeted by the short guide RNA. There is a risk that some of these sequences target oncogenes or tumor suppressors or genes with general effect on the host immune system. None of the inserted sequences is expected to confer changes that will confer pathogenicity, immunogenicity, different tissue tropism nor ability to transmit to other hosts.

Gene transfer could possibly occur through accidental exposure to viral particles, via a cut or similar break in the skin. Therefore sharps will not be used in preparations containing viral particles or transfected packaging cells. If an accidental exposure were to occur, such viral particles would conceivably have hazardous effects if they infected a stem cell; reducing the chances of such exposure by not using sharps for viral particle preparations will significantly reduce the risk to human health. As has been stated already, all vectors are replication defective and so productive or replicative infections are not possible. It should be reiterated that these lentiviruses are extremely unstable even in tissue culture conditions.

The modified viruses should not pose a risk to the environment. The viruses are unstable, inactivated by detergent, UV light and ethanol, and would not survive outside the laboratory environment. The recipient cells cannot survive in the environment as they are only viable under culture conditions and as such pose no threat to health or environment.

Gene editing is not expected to provide mammalian cells with the capability to survive in the environment as they are only viable under culture conditions and the inserted sequence will not provide this ability.

The most dangerous GMM: packaged lentivirus. The most dangerous step is the harvesting and concentration of packaged virions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste (culture plates, pipette tips, glass conical flasks) are sprayed with 5% Distel, autoclaved and then disposed of through the clinical waste management system. Autoclave cycles are validated using thermocouple; automatic fail if cycle is not completed.

Distel:
Broad spectrum efficacy within short contact times - 30 seconds to 5 minutes; Sporicidal, mycobactericidal, virucidal, fungicidal and bactericidal efficacy; Performance validated within a wide range of peer-reviewed scientific publications; Acknowledged and recommended by professional societies, public and private sector healthcare institutions http://medi-mark.co.uk/media-centre/article/users-of-trigene-advance Media waste is disposed down the sink with excess water after overnight treatment with 1:10 volume of 100% bleach. Bleach: WT CHLOR from CHELA - Sodium hypochlorite solution. http://www.chela.co.uk/shop/food/wt-chlor/ Using 12.5% hypochlorite (industrial strength bleach) in a 1:10 dilution (one part industrial strength bleach and nine parts water) yields 12,500 ppm or a 1.25% hypochlorite solution, for use within 30 days. One study reported that 25 different viruses were inactivated in 10 minutes with 200 ppm available chlorine. Klein M, DeForest A. The inactivation of viruses by germicides. Chem. Specialists Manuf. Assoc. Proc. 1963;49:116-8. Several studies have demonstrated the effectiveness of diluted hypochlorite and other disinfectants to inactivate HIV. Sattar SA, Springthorpe VS. Survival and disinfectant inactivation of the human immunodeficiency virus: a critical review. Rev. Infect. Dis. 1991;13:430-47.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC approved this as Class 2 subject to clarification of minor queries raised by the Committee.

Project Containment

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Project Ref 14/18.2
The purpose of this work is to generate induced pluripotent stem (iPS) cells from mouse and human cells by employing viral and non-viral vectors coding for reprogramming genes. iPS cell technology has the potential to transform regenerative medicine especially in the areas of stem cell transplantation and patient-specific gene therapy. Briefly, mammalian cells will be treated with reprogramming factors to become embryonic stem (ES) cell like cells which are known as iPS cells. Once generated, these cells can be expanded at will maintaining their undifferentiated pluripotent state and can be subjected to differentiation pathways eventually leading to the formation of terminally differentiated somatic cells. To the best of our knowledge based on currently available scientific literature there is no suitable alternative to the use of the reprogramming vectors for the purpose of iPS cell generation. Upon successful execution, the iPS cells generated from this project will help us to study various genetic defects either from murine disease models or from human patient samples and would be crucial in our therapeutic approaches.

### Host/vector system

1. Lentiviral Gag/pol expression vectors p 8.9 and derivatives (HIV-1).
2. Viral envelope expression vector pMDG and derivatives (VSV-G).
3. A modified, non-transmissible form of Sendai virus (SeV) (ready made and commercially available)
4. A non-viral, non-integrating, self-replicating RNA-based reprogramming vector (ready made and commercially available)
Origin & function

The sequences contained within the lentiviral or non-viral expression vectors include:
1. cDNA sequences of reprogramming transcription factors: Oct-4, Klf4, Sox2, Glis1 and c-Myc
2. Additional sequences that have been added include eGFP reporter gene, luciferase gene, the mutated Woodchuck Posttranscriptional Regulatory Element (WPRE - truncated and with mutated X-protein non-functional start codon in lenti-viral vectors), Internal Ribosomal Entry Sites (IRES elements), F2A self cleaving peptide sequences, puromycin resistance gene

Activity

Evaluation of foreseeable effects

There is a risk that some of these sequences target oncogenes or tumor suppressors or genes with general effect on the host immune system. None of the inserted sequences is expected to confer changes that will confer pathogenicity, immunogenicity, different tissue tropism nor ability to transmit to other hosts.

Gene transfer could possibly occur through accidental exposure to viral particles, via a cut or similar break in the skin. Therefore sharps will not be used in preparations containing viral particles or transfected packaging cells. If an accidental exposure were to occur, such viral particles would conceivably have hazardous effects if they infected a stem cell; reducing the chances of such exposure by not using sharps for viral particle preparations will significantly reduce the risk to human health. All vectors used are replication defective and so productive or replicative infections are not possible.

Sendai virus is not pathogenic to humans. It is non-transmissible and has the fusion protein (F) deleted, rendering the virus incapable of producing infectious particles from infected cells. RNA-based vectors are non-viral, non-integrating and are extremely unstable at ambient temperatures and, therefore, do not present any risk to humans.

The most hazardous GMM are the lentivector viruses and the most hazardous step is the harvesting and concentrating the viral supernatant. Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The probability of such an event is extremely low.

The modified viruses should not pose a risk to the environment. The viruses are unstable, inactivated by detergent, UV light and ethanol, and would not survive outside the laboratory environment. The recipient cells cannot survive in the environment as they are only viable under culture conditions and as such pose no threat to health or environment.

Reprogramming is not expected to provide mammalian cells with the capability to survive in the environment as they are only viable under culture conditions and the inserted sequence will not provide this ability. Accidental exposure of the lentiviral vector itself is not expected to result in transduction of cells in vivo. Successful transduction of cells requires specific culture condition in vitro to achieve gene modification. The target cells for transduction are keratinocytes, fibroblasts and other human cell lines which require specific culture conditions for survival. Therefore, the risk of engraftment of transduced cells is considered negligible. Even if an accidental exposure to the genetically modified cells was to occur, healthy immunocompetent individuals would be expected to promptly reject the allogeneic cells. The risk of transfer to environmental species is negligible, and would not be expected to cause harm as VSV-G pseudotyped lentivirus can only infect mammalian cells. Furthermore, the virus is replication incompetent and self-inactivating with none of the structural genes present in the packaged viral genome, so no new virus can be produced and no other cells can be infected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Solid waste (culture plates, pipette tips, glass conical flasks) are sprayed with 5% Distel, autoclaved and then disposed of through the clinical waste management system. Autoclave cycles are validated using thermocouple; automatic fail if cycle is not completed.

**Distel:**
- Broad spectrum efficacy within short contact times - 30 seconds to 5 minutes;
- Sporicidal, mycobactericidal, virucidal, fungicidal and bactericidal efficacy;
- Performance validated within a wide range of peer-reviewed scientific publications;
- Acknowledged and recommended by professional societies, public and private sector healthcare institutions
  - [http://medi-mark.co.uk/media-centre/article/users-of-trigene-advance](http://medi-mark.co.uk/media-centre/article/users-of-trigene-advance)

Media waste is disposed down the sink with excess water after overnight treatment with 1:10 volume of 100% bleach.

**Bleach:**
- WT CHLOR from CHELA - Sodium hypochlorite solution. [http://www.chela.co.uk/shop/food/wt-chlor/](http://www.chela.co.uk/shop/food/wt-chlor/)
- Using 12.5% hypochlorite (industrial strength bleach) in a 1:10 dilution (one part industrial strength bleach and nine parts water) yields 12,500 ppm or a 1.25% hypochlorite solution, for use within 30 days. One study reported that 25 different viruses were inactivated in 10 minutes with 200 ppm available chlorine.
  - Several studies have demonstrated the effectiveness of diluted hypochlorite and other disinfectants to inactivate HIV.

Is an emergency plan required according to regulation 20? [No]

If yes, tick to confirm that it is attached to this form [No]

Tick to confirm that you have attached a risk assessment to this form [Yes]

Tick if you are claiming exemption from disclosure for section of the risk assessment [No]

Please enter comments on the GM safety committee on the risk assessment

The UCL GMSC agreed this as a Class 2 activity subject to some minor amendments/clarifications

Activity

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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>

02/03/2022
**Project Ref** 14/18.3

**Date Ackn'd** 18/04/2018

**CU2 Project Title**

Lentiviral Vectors Production and transduction of cell lines and primary cells using Class 2 lentiviral vectors

**Class** Class 2

**Culture Volume** < 1 Litre

**Non-GMM Consent Granted**

---

**Historical Significant Changes**

**Historical Date of Additional Info**

**Withdrawn** N

**Tick if notifying a connected programme of work** N

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**Project Additional Information**

**Purposes of the contained use**

Lentiviral vectors encoding for human oncogenes related to hematopoietic malignancies (e.g. BCR-ABL fusion gene, IDHR132K) will be applied to model malignant transformation in cell lines and primary human and murine cells.

**Recipient or parental organism**

- List recipient organism(s) to be used:
  - Cell lines: including Human-TF-1, K562, HEK293T, Jurkat, HEL 1.
  - Primary Human cells: Derived from human Bone Marrow Mononuclear cells isolated from Normal Donors (provided by Lonza) or Leukemia Patients obtained through the Cancer Institute (UCLH).
  - Primary Murine Cells: Obtained from Bone Marrow of wild type mice.

**Host/vector system**

The bacterial strains used are attenuated non-colonising strains so are incapable of causing human infection and therefore are harmless to humans.

Lentiviral vectors: Various 3rd generation lentiviral expression plasmids including pLKO.1 and derivatives. Lentiviral packaging vectors: 3rd Lentiviral accessory and packaging vectors including Gag/pol expression vectors p8.9, psPAX2 and derivatives (accessory packaging vectors derived from HIV-1) and viral envelope expression vectors including pMDG and derivatives (expresses VSV-G from Vesticular Stomatitis Virus).
The lentivirus is pseudotyped with VSV-G envelope so can infect human cells, however the viral vectors are not capable of self-replication as the viral particles do not contain all the necessary components and required components have been split over independent plasmids that do not have enough homology to recombine.

Origin & function

The integrating constructs will include the expression of the following genes:
- Markers of transduction: GFP, OFP, Venus, truncated NGFR.

Health and Safety

Executive
- Activators/repressors of gene expression belonging to the Tet OFF or Tet ON inducible system, ITta lrtTA
- Oncogenes: BCR-ABL (p210), IDH1 mutated - Various short Hairpin RNAs to knock down gene functions,

The lentiviral vectors can infect human cells or any other mammalian cell as they are pseudotyped with the envelope protein VSV-G from the vesicular stomatitis virus and therefore if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate its genome into cellular DNA. Physical insertion of the virus into the genome of the host cell could potentially integrate a oncogene leading to an oncogenic hit, however the likelihood of this is very low and has never occurred in a research setting. Additionally, it would not be severe as the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection. Also, it would only be a small number of cells and therefore would not impact on the pathogenic outcome of an exogenous viral infection. Additionally, the likelihood of infection is very low. The viral particles are labile and do not survive on environmental surfaces outside the laboratory. The packaging viral components are from the HIV-1. The full length HIV-1 virus is harmful to humans, however the packaging vector used does not contain the full length HIV-1 molecule. Many components of this virus that are critical for HIV-1 infection have been removed with only those required for viral packaging remain. It is therefore attenuated and cannot replicate and form new viable virus. The cell that will eventually receive and integrate the DNA cannot survive in the environment as they are only viable under culture conditions and therefore are harmless to human health.

Evaluation of foreseeable effects

Lentiviral particles: The lentivirus is pseudotyped with VSV-G envelope so can infect human cells, however the viral vectors are not capable of self-replication as the viral particles do not contain all the necessary components and required components have been split over independent plasmids that do not have enough homology to recombine. Lentiviral Vector Production: LVs will be produced by transient co-transfection of a permissive cell line (HEK293T) with a combination of packaging and transfer vector constructs. The supernatant from this cells will be collected and subjected to concentration (through ultracentrifugation). After concentration the expected titers of the vector prep will be of 10^7-10^8 Transducing unit/ml. All work involving infectious virus will be performed in containment level 2 facilities in class 2 cabinets. To prevent contamination of workers by virus, no glass or other sharps will be used. Skin lesions will be covered with a bandage in addition to the protective wear described above. The risk of harm is therefore extremely low.

Some of the constructs that will be generated will contain genes that may result in oncogenic potential (e.g. BCR-ABL fusion gene or the mutated form of IDH (IDH1 R132, IDH2 R172). As described above, however, the virus cannot recombine and the likelihood of infection is extremely low as all work will be performed in containment level 2 facilities with all the personal protection described above. Additionally, no adverse affects have ever been described through the accidental exposure to retroviral vectors. Therefore the overall risks of our experiment are low. BCR-ABL fusion gene gives rise to a constitutively activated Tyrosine Kinase, which is able to drive uncontrolled proliferation and cell survival, resulting in leukemic transformation. Importantly, since it has been first characterized, BCR-ABL fusion...
protein has been extensively studied. Experimental work with mouse models and human cells have proved that BCRABL fusion gene can only act as a leukemia driver if the mutation is acquired in rare Hematopoietic Stem Cells residing in the bone marrow (HSCs), but not in more committed progenitors or differentiated cells. IDH1/fDH2 genes encode for isocitrate dehydrogenase. Mutations in these genes have been associated to oncogenic potential in gliomas and in hematologic malignancies. Mutated IDH acquire the ability to convert alpha-KG to 2-hydroxyglutarate (2-HG), which is now considered as an "oncometabolite". IDH1 R132H knock-in mice have a normal life span, similarly do not develop any malignancy.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Work involving viral supernatant will be performed in class 2 safety cabinets and grown within a closed incubator. Centrifugation of samples will be carried out in sealed rotors. After multiple passages and at least 14 days in culture, stably transduced cells may be moved to a standard culture room. Use of sharps is not permitted. If viral supernatent is transported outside of the laboratory, leak proof inner container surrounded by enough absorbent material to absorb the contents if broken and then contained within a second sealed contained.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

NA

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable tissue culture plasticware & cells used for viral production at the end of the procedure: The solid waste will be double-bagged in autoclave waste bags and autoclaved at 121 c for 15mins by trained staff then bagged in tiger bags.

Excess viral supernatant. Media recovered from GMM cells: 10% Distel or Virkon (v/v) will be used to treat liquid waste (16 hours) and also used to decontaminate any spills. Liquid waste is subsequently poured down the sink after treatment. Work surfaces will be wiped down with 10% Distel and 70% ethanol after use. Virkon and Distel are certified by the PHLS to provide 100% viricidal and GMO kill under these conditions by denaturating and inactivating viral and cellular proteins. Distel and Virkon will be used with their lifespan to ensure required kill is achieved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
This project aims to use replication defective lentiviruses to generate stable cell lines in which gene expression has been silenced or genes have been ectopically expressed. We use replication defective lentiviruses to generate cell lines from human and rodent cells which are poorly infected by amphotropic and ecotropic retroviruses. Lentiviruses are much more efficient at infecting non-dividing or poorly dividing cells.

The lentiviral silencing constructs are from the Open Biosystems human and mouse lentiviral shRNAmir libraries maintained and distributed by the UCL RNAi consortium. Both shRNAmir libraries consists of over 100,000 constructs in the pGIPZ lentiviral vector. Each construct, contains 22bp sense and antisense sequences that are homologous to a gene of interest to which the hairpin has been designed to target embedded, within a mir-30 sequence. When transcribed in vivo, these sequences give rise to short hairpin shRNAs which result in posttranscriptional down-regulation of gene expression by RNA interference. Lentiviral DNA constructs are packaged as lentiviruses using HIV accessory proteins for production of viral particles and the VSV-G envelope protein from
### Vesicular Stomatitis Virus

Lentiviral expression constructs encoding cellular full length or mutant genes are prepared and packaged as above. Lentiviral vectors and full length ORFs are obtained from DNA repositories and/or commercial suppliers. All ORFs are sequence verified.

### Recipient or parental organism

<table>
<thead>
<tr>
<th>Organism</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>JS4</td>
<td>A recA- derivative of E. coli MC1061, XL-Blue, DH5a, PrimePlus and other commercially available disabled K12 strains of E. coli; non-mobilisable.</td>
</tr>
<tr>
<td>Human HEK293T cells</td>
<td>Production of lentivirus stocks; non-mobilisable and free of adventitious agents. History of safe usage &gt;30 years within the laboratory.</td>
</tr>
<tr>
<td>Mouse N2a cells</td>
<td>ATCC; non-mobilisable and free of adventitious agents.</td>
</tr>
<tr>
<td>Mouse cell lines including PK1 and CAD5 cells</td>
<td>Non-mobilisable and free of adventitious agents.</td>
</tr>
<tr>
<td>Primary mouse cells</td>
<td>Although these cells are non-mobilisable, primary cells have the potential to be infected with unknown adventitious agents.</td>
</tr>
<tr>
<td>Primary human somatic cells and cell lines</td>
<td>Although these cells are non-mobilisable, primary cells have the potential to be infected with unknown adventitious agents.</td>
</tr>
<tr>
<td>Human cancer cell lines</td>
<td>Non-mobilisable and free of adventitious agents. History of long term safe usage in culture.</td>
</tr>
<tr>
<td>COS cells</td>
<td>Derived from monkey kidney CV1 cells. They are an established cell line with a history of safe usage &gt;30 years in the laboratory.</td>
</tr>
</tbody>
</table>

### Host/vector system

- Lentiviral pGIPZ vector, lenti miRE silencing vectors and other commercially available lentiviral silencing vectors.
- Lentiviral expression vectors pReceiver, pLEX301, pLEX304, pLEX-MCS and pLenti6.2/V5-DEST and other commercially available lentiviral expression vectors. These vectors are all non-mobilisable or mobilisation defective.
- Most lentiviral vectors have deletions in the 3' LTR (SIN-LTR) such that deleted LTRs are generated upon viral replication so that they cannot be mobilised by exogenous virus. Moreover, no replication competent endogenous retroviruses have been detected in the human genome.

### Origin & function

- Genes whose expression will be manipulated are: PRNP, Tau, APP, TDP43, a-synuclein, STX6, GAL3ST1, PDIA4, CXC4R, CHODL, AIM2, FKBP5, ANK1, SLC26A4, PAPSS2, CHGA, IQGAP2, ITGA8, FN1, FST, RGS4, LIN52, BMYB, FOXM1, RELA, CEBP, LARP6, NR3C2, ZNF511, CEBPG, CBF, PRMT3, HMGB1, TFAM, DEK, HMGB2, SMD11, NUF1P1, PRRX1, TEAD4, YEATS4.
- RNAi hairpins for silencing gene expression are sequences that have been synthesized de novo and are not from an organism. They have been designed to target specific genes and comprise both sense and anti-sense as well as a loop sequence.
- Full length ORFs for ectopic expression of cellular genes: are from ATCC, Gene Service or other DNA repositories.
- Full length ORFs for transcription factors: obtained from DNASU plasmid repository, In vitrogen (Thermo Fisher) and Japanese ORF collection.
- The accessory proteins for lentiviral packaging are derived from HIV-1 virus and the VSV-G envelope protein is from Vesicular Stomatitis Virus. Both were provided by UCL Cancer Institute and originate from the laboratory of R. Weiss and G. Towers.
DIRECT EFFECTS
Both the gene silencing and ectopic expression are aimed at investigating the role of the various genes in either growth promotion or neurodegeneration. The viral vectors also encode resistance to a variety of antibiotics to allow for selection of stably transduced cells. None of the shRNAs or the cellular genes are pathogenic but are likely to be biologically active and alter the behaviour of the cells in which they are expressed; this is the aim. They are unlikely to be toxic because if they are, we will not obtain any stably expressing cells. Also unlikely to be allergens or act as hormones/ cytokines.

INDIRECT EFFECTS
The viral vectors have the potential to cause harm by insertional mutagenesis if the virus was to inadvertently infect a cell and integrate in a location that leads to activation of genes or suppression of genes involved in tumour formation or lead to production of a biologically active substance eg. hormone, cytokine.

EFFECT OF GENE TRANSFER
The major effect of silencing and/or overexpression of these genes will either be in promoting growth (tumour formation) or activating neurodegeneration pathways. Lentiviruses are capable of infecting non-dividing cells.

SUMMARY OF RISK TO HUMAN HEALTH
There are two types of risk to human health from these viruses: the shRNAs and genes that are involved in growth promotion have the potential to infect human somatic cells and promote growth, one of the prerequisites for tumour formation. However tumour formation is a multifactorial event. The shRNAs and genes involved in neurodegeneration could potentially infect and promote neurodegeneration, if these viruses were able to infect the appropriate human cell type. The other risk is from insertional mutagenesis, which could promote tumour formation or expression of a biologically active substance eg. hormone, cytokine.

Inadvertent infection by needle stick injury is the most likely route of infection. They will be minimized by not using sharps and needles.

SUMMARY OF RISK TO THE ENVIRONMENT
In the unlikely event of a containment failure, leading to an accidental release of the lentivirus into an environment, these viruses could lead to promotion of tumour formation and activation of neurodegeneration pathways if an animal was accidentally infected. Inadvertent infection by needle stick injury is the most likely route of infection. They will be minimized by not using sharps and needles.

Neither bacteria, human or mouse cells, lentiviruses or transduced cells will survive alone within the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

SOLID WASTE
E. coli solid waste (agar plates, tips and pipettes) are autoclaved followed by incineration. Solid waste from tissue culture such as dishes, tubes and flasks are chemically disinfected followed by incineration. Tissue culture pipettes are autoclaved followed by incineration.

Chemical disinfection is by 1% virkon or > 10,000ppm sodium hypochlorite for >1 hour, preferably overnight followed...
by incineration.

**LIQUID WASTE**

E. coli liquid waste is inactivated by treatment with 1% Virkon for >10 minutes.

Liquid tissue culture medium is collected into containers containing sufficient sodium hypochlorite for >10,000ppm and left for 24 hours.

Lentivirus supernatants are inactivated by treatment with sufficient sodium hypochlorite for >10,000ppm and left for 24 hours.

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
<td>L4</td>
</tr>
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<td>Animal Units</td>
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<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
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**Project Ref** 14/19.1

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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<tbody>
<tr>
<td>07/03/2019</td>
<td>Cryopreserved formulation of autologous CD34+ haematopoietic stem cells transduced ex vivo with CD11 b lentiviral vector encoding for human SGSH gene in subjects with mucopolysaccharidosis type IIIA (MPS IIIA, Sanfilippo syndrome type A)</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
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</table>

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

This is a new research project. The ATIMP is a cryopreserved formulation of autologous CD34+ haematopoietic stem cells transduced ex vivo with the SIN lentiviral vector pCCL_CD11 b-coSGSH-WPRE vector encoding for the codon optimised human N-sulphoglucoasamine sulphohydrolase (SGSH) gene. To create the aforementioned ATIMP product, autologous CD34+ cells HSCs from MPSIIIA patients will be genetically modified using the CLCD11 b.co.SGSG vector; a self inactivating (SIV) lentiviral vector expressing the SGSH gene codon optimised for human use and regulated by hCD11 b. These transduced cells will then be cryopreserved until such time of infusion back to the patients. The cryopreserved formulation will be thawed at the patient's bedside and infused directly without further manipulation.

#### Recipient or parental organism

Autologous Hematopoietics Stem Cells (HSCs) from MPSIIIA patients or healthy donors. No producer cell line will be handled for this project as the vector is manufactured by an external company.

#### Host/vector system

The LV is packaged using four separate plasmids, a plasmid expressing the required viral proteins (gag and pol), a plasmid expressing Rev and a plasmid expressing the glycoprotein VSV-G envelope, combined with a genome plasmid comprising a SIN transfer vector carrying a major deletion in the 3’ L TR region. The system is engineered in such a way that minimal homology regions are present between packaging and transfer vectors, thus minimizing the likelihood of homologous recombination events and the generation of replication competent lentiviruses (RCL). The packaging construct is deleted of all HIV accessory proteins (vpu, vpr, nef, vif) and Tat. The packaging plasmids used are: pMDLg (6.6 ug per 75 cm2), pRSV/Rev (3.3 ug per 75 cm2) and VSV-G glycoprotein expression plasmid pMD.G (4.62 ug per 75 cm2). The transfer vector encodes a codon optimised human SGSH gene cDNA and sequences necessary for expression, encapsidation, reverse transcription and integration of the viral genome. In order to minimize the risk of RCL generation, the 3’ L TR of the transfer vector has been deleted in the U3 region. A WPRE sequence with mutations eliminating expression of the WPRE X protein is incorporated in the vector to enhance transgene expression. The vector is produced as cell-free LV supernatant from the 293T cell line and has an envelope pseudotype from VSV.

#### Origin & function

SGSH cDNA Wpre* (mutated)
The host organism (HSCs) do not represent any risk for the human health and safety as prior to collection, the donors are tested for an extended microbiology and virology panel. Moreover, cells are manipulated at all times inside the isolator and direct contact between the operators and the cells is not expected. Regarding the CD11 b.co.SGSG lentiviral vector, the risk of pathogenicity or toxicity in human cells is negligible. Cases of insertional mutagenesis have been reported in a small number of patients treated with gene therapy for immunodeficient diseases utilising retroviral vectors. However, the risk of accidental inoculation with lentiviral vector and the development of insertional mutagenesis is considered negligible for the following reasons: 1. The risk of insertional mutagenesis is considered to be lower with lentiviral vectors than retroviral vectors. 2. The current vector has been designed with the view to reducing the risks of insertional mutagenesis. 3. Only HIV negative patients will be used as donors for this project so the possibility of recombination with the Wild-type virus is negligible. For all the above reasons, the risks to human health and safety is considered negligible.

The inserted genetic material is not known to have any harmful effects. Its expression will be restricted to cells of the CD11 b lineage. The inserted genetic material is a physiological human. Therefore, it is not expected to alter the properties of the host organism apart from restoring the function of this gene when used to transduce autologous cells from MPSIIIA patients who have a faulty copy of this gene. The insert cannot be disseminated in the outer environment as the vector is non-replicative.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste disposed of following organisation's waste disposal policy. It is placed into dedicated plastic bins with lids, collected by central collection service and incinerated. All sharps are placed in puncture-proof containers and disposed of through the central collection service for infectious waste. Liquid waste is disinfected using Haz-tab (effervescent chlorine tablets) overnight before being disposed of down the sink.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

Approved by the GM safety committee as a class 2 activity, due to the use of sharps in the experimental procedure.
This is a new research project intended to manufacture an advance therapy medicinal product (ATMP) for the treatment of X-linked severe combined immunodeficiency (X-SCID). The ATMP is a cryopreserved formulation of autologous CD34+ haematopoietic stem cells transduce ex vivo with the SIN lentiviral vector pCCL_EF1_IL2RGco_WPRE4 vector (G2SCID) encoding for the codon optimised human IL2RG gene. To create the aforementioned IMP product, autologous CD34+ cells HSCs from X-SCID patients will be genetically modified using a G2SCID vector; a self-inactivating (SIV) lentiviral vector expressing the IL2RG gene codon optimised for human use and regulated by EF1a. These transduced cells will then be cryopreserved until such time of infusion back to the
patients. The cryopreserved formulation will be thawed at the patient's bedside and infused directly without any further manipulation.

Recipient or parental organism

Autologous Hematopoietics Stem Cells (HSCs) from X-SCID patients or healthy donors. No producer cell line will be handled for this project as the vector is manufactured by an external company.

Host/vector system

G2SCID lentiviral vector: The G2SCID lentiviral vector is an integrative, 3rd generation replication-defective, self-inactivating (SIN) human immunodeficiency virus (HIV)-derived LV vector, with a mutated Woodchuck hepatitis virus Posttranscriptional Regulatory Element (WPRE) sequence. It directs IL-2 receptor gamma expression from a codon-optimized form of the IL2RG gene to all hematopoietic and lymphoid lineages. A LV vector derived from HIV-1 has been chosen because it is genetically stable, permanently integrates into the genome of transduced cells, and provides long-term gene expression in vitro and in vivo. The transduction of hematopoietic stem cells (HSC) with such LV can be achieved after limited pre-activation of the cells in short-term cultures with cytokines, in conditions that are compatible with the preservation of the self-renewing capacities of these cells.

The modifications introduced into the organisation of the parental HIV1 genome are described below:

-- Replication defective: The HIV-1 genome is split into 4 constructs consisting of 3 packaging plasmids and one transfer plasmid, which are co-transfected for the production of an infectious particle. The packaging of the particle is therefore conditional on the Rev and gag-pol genes, which are expressed in trans on separate plasmids. The minimal homology between the various cassettes for packaging and gene transfer minimizes the likelihood of homologous recombination events and the generation of replication-competent HIV particles (RCL).

-- SIN: A 400 base pair deletion was introduced in the 3'L TR to remove major transcription factor binding sites. Reverse transcription, which generates both U3 regions from the 3' of the viral genome, transfers the deletion to the 5'L TR of the pro-viral DNA. The integrated provirus therefore contains the therapeutic transgene flanked by two transcriptionally inactive L TRs with U3 deletions.

-- Mutated WPRE: This element is used to enhance the transgene expression in vivo and to increase viral titers during the manufacturing process. It has however been incriminated as the cause of hepatocellular carcinomas observed in mice treated with an equine LV vector. Although such concern has not been evidenced in mice treated with HIV-derived lentiviral vectors with the same WPRE, a mutated WPRE, so-called PRE4, is used in G2SCID in order to prevent any issues.

-- VSV-G envelope: VSV-G has been chosen because it has a broad tropism, and in particular the ability to target HSC.

Origin & function

IL2RG cDNA Wpre* (mutated). The intended function of the genetic material is to correct the genetic mutation present in the SCID-X1 patient HSCs and restore immune system function.

Evaluation of foreseeable effects

The host organism (HSCs) do not represent any risk for the human health and safety as prior to collection, the donors are tested for an extended microbiology and virology panel. Moreover, cells are manipulated at all times inside the isolator and not direct contact between the operators and the cells is expected.

Regarding the G2SCID lentiviral vector, the risk of pathogenicity or toxicity in human cells is negligible. Cases of insertional mutagenesis have been reported in a small number of patients treated with gene therapy for immunodeficient...
diseases utilising retroviral vectors. However, the risk of accidental inoculation with lentiviral vector and the
development of insertional mutagenesis is considered negligible for the following reasons:
1. The risk of insertional mutagenesis is considered to be lower with lentiviral vectors than retroviral vectors.
2. The current vector has been designed with the view to reducing the risks of insertional mutagenesis
3. Only HIV negative patients will be used as donors for this project so the possibility of recombination with the wild-
type virus is negligible.
For all the above reason, the risks to human health and safety is considered negligible.
The inserted genetic material is not known to have any known harmful effects. Although the protein is part of cytokine
receptors, its expression in the membrane of the cells will be regulated by the endogenous expression of the other
proteins that form part of this receptors:

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste disposed of according to the GOSH Pathology waste disposal policy. It is placed into dedicated plastic
bins with lids and collected by the GOSH central collection service for infectious waste and incinerated. All sharps are
placed in puncture-proof containers and disposed of through the central collection service for contaminated/infectious
waste.
Liquid waste is disinfected using Haz-tab (effervescent chlorine tablets) overnight before being disposed of down the
sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Approved by the GM safety committee as a class 2 activity, due to the use of sharps in the experimental procedure.

Project Containment

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</table>

02/03/2022
Use of adeno-associated viruses (AAV) or lentiviruses to study neurodegenerative diseases.

This risk assessment is in preparation for studies that will address the role of axonal transport in animal models of different neurological disorders. Axonal transport is frequently impaired in different neurological diseases, both directly by mutations in genes encoding motor proteins or indirectly by abnormal activity of regulatory pathways. However, the precise mechanisms underlying these defects in neurological diseases remain to be clarified.

Our studies aim to elucidate these gaps in our understanding of axonal transport and will allow the development of novel, targeted approaches to restore axonal transport during the onset and progression of disease in specific animal models of neurodegeneration.

This Risk Assessment covers the administration of recombinant adeno-associated virus (rAAV) or lentiviral vectors expressing non-oncogenic and non-pathogenic constructs to wild-type mice or rats and to animal models of neurological diseases to understand disease mechanisms and assess potential therapeutic efficacy of treatments.

Recipient or parental organism

Recipient organisms are mice or rats. Viral vectors will be injected intravenously, intramuscularly,
intraperitoneally, intra-cranially or intra-thecally.

**Host/vector system**

Non-replicating viral vectors:
1. Recombinant adeno-associated viruses (rAAV) or
2. Lentiviral vectors (2nd or 3rd generation).

Vectors will be obtained from collaborators, from commercial sources, or produced by our laboratory.

**Genetic & function**

Genetic material will include:

1) full-length or engineered versions of genes encoding proteins that are transported in axons (e.g. Mito-YFR to monitor mitochondrial transport) or neurotrophic factors to test effects on disease progression (e.g. brain-derived neurotrophic factor (BDNF));
2) shRNA or siRNA expression constructs to decrease expression of genes to test their involvement in disease progression;
3) reporter genes, (e.g. GFP) to be used as controls to monitor distribution of the rAAV vectors or to identify specific populations of cells;
4) genetically encoded antibodies for detection of specific proteins in vivo.

All the genetic material used is non-oncogenic, non-pathogenic and does not encode toxic proteins. Different promoters will be used to drive expression of transgenes including constitutively expressed promoters (e.g. CMV), inducible promoters (e.g. tetON or tetOFF systems), and tissue-specific promoters (e.g. driving expression in muscles (e.g. MCK) or neurons (e.g. CamKII)).

**Origin & function**

All vectors used in this proposal have been rendered replication-deficient.

The genome of engineered rAAV contains no pathogenic genes. Inverted terminal repeats (ITR) are present making viral self-replication impossible. rAAV are not permanently integrated into the host genome, rather they form stable episomes for transgene expression. Likewise, 2nd and 3rd generation lentiviral vectors are engineered not to self-replicate and the genome does not contain pathogenic genes. 2nd and 3rd generation lentiviral vectors are safe and not pathogenic for humans.

None of the genetic material inserted is a known oncogene or encodes for toxins. Insertion of the genetic sequences listed above will not affect the pathogenicity or transmission ability of the rAAVs or lentiviruses.

Since none of the vectors used are replication-competent, infected animals cannot transmit the virus or transgene. Both rAAV and lentiviruses are not able to replicate in the absence of helper viruses (which are not used here) or other helper functions. Treated animals will be kept in Individually Ventilated Cages.

**Evaluation of foreseeable effects**

All vectors used in this proposal have been rendered replication-deficient. The genome of engineered rAAV contains no pathogenic genes. Inverted terminal repeats (ITR) are present making viral self-replication impossible. rAAV are not permanently integrated into the host genome, rather they form stable episomes for transgene expression. Likewise, 2nd and 3rd generation lentiviral vectors are engineered not to self-replicate and the genome does not contain pathogenic genes. 2nd and 3rd generation lentiviral vectors are safe and not pathogenic for humans.

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Since none of the vectors used are replication-competent, infected animals cannot transmit the virus or transgene. Both rAAV and lentiviruses are not able to replicate in the absence of helper viruses (which are not used here) or other helper functions. Treated animals will be kept in Individually Ventilated Cages.

Only personnel highly trained in the use of ‘sharps’ under aseptic conditions will perform these procedures. Treated animals will be kept in Individually Ventilated Cages. The only risk arises from the use of sharps during the procedure of injection of the viral vectors into the animals. Only personnel highly trained in the use of ‘sharps’ under aseptic conditions will perform these procedures in specific surgery units provided with Laminar Flow Cabinets.

When possible, injections will be performed in anesthetized animals to facilitate handling. When possible, remotely-operated injectors will be used.
GM animals produced by injection of viral vectors will be housed in Individually Ventilated Cages in a dedicated unit of the Cruciform Building or of the Institute of Neurology. In case of necessity of transport, the animals will be maintained in double containment vessels. A separate risk assessment (RA024493/1) covers this activity.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste which has been in contact with the viral vector such as virus containing tubes, disposable equipment for surgery will be disposed of by soaking in a 2% Virkon solution for 1-12 hour and then subjected to autoclaving. Autoclaving (132 degrees celsius, 15 minutes holding time) will give 100% kill at this temperature and time.

Solid sharp waste will be disposed of in the appropriate sharp bin container and ultimately autoclaved.

Liquid waste which has been in contact with the viral vector will be disposed of by adding a 2% Virkon solution for 1-12 hour and then subjected to autoclaving. Autoclaving (132 degrees celsius, 15 minutes holding time) will give 100% kill at this temperature and time.

Carcasses of GM animals will be disposed of via incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GM safety committee were satisfied with the identified hazards, control measures and overall risk assessment.

Tile activity was approved as activity class 2.

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
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This research project intends to manufacture an advance therapy medicinal product (ATMP) for the treatment of Adenosine deaminase (ADA) deficient severe combined immunodeficiency (ADA-SCID). The ATMP is a cryopreserved formulation of autologous CD34+ hematopoietic stem cells transduced ex vivo with EFS lentiviral vector encoding for the human ADA gene (OTL-101). To create the aforementioned product, autologous CD34+ HSCs from ADA-SCID patients will be genetically modified using an OTL-101 vector. These transduced cells will then be cryopreserved until such time of infusion back to the patients. The cryopreserved formulation will be thawed at the patient's bedside and infused directly without any further manipulation.

Recipient or parental organism

Autologous Hematopoietic Stem Cells (HSCs) from ADA-SCID patients or healthy donors. No producer cell line will be handled for this project as the vector is manufactured by an external company.

Host/vector system

The EFS-ADA Lentivirus (LV) used in the manufacture of OTL-101 is an ADA cDNA encoding LV containing the enhance'ri'promoter from the human EF1 a gene. The vector lacks viral coding sequences that may give rise to the formation of replication-competent lentivirus or immunogenic peptides and is also devoid of all LV enhancer-promoter sequences that are known to be involved in insertional mutagenesis by retroviruses and derived vectors. The EF1 a enhancer/promoter used is shortened by deletion of the first intron of EF1 a, which possesses strong enhancer activity and has potential to trans-activate adjacent cellular genes upon insertion. The EF1 a as promoter has
been shown to directly, high level transcription of reporter genes in murine hematopoietic cells and to have significantly reduced trans-activation potential compared with retroviral LTR (Zychlinski et al., 2008). In its plasmid DNA configuration, the EFS-ADA LV contains the following vector modules:

The pCCL SIN LV is derived from HIV-1 (Dull et al., 1998). All open-reading frames of HIV-1 genes have been deleted from the vector, leaving only 2007 bp (20.65% of HIV-1 genome) consisting of: the SIN long terminal repeats (LTRs), the packaging sequences, the revresponsive element (rRE) and the central polypurine tract (cPPT).

The 400 bp SIN deletion from the LTRs removes the promoter and enhancer (from -418 to -18 relative to the U3/R border), leaving only 53 bp with the attachment sequences for chromosomal integration and the polyadenylation signal.

A short promoter fragment (239 bp) from the human EF1 α gene, lacking intronic or enhancer sequences, drives transcription of q normal human ADA cDNA.

The transcriptionally disabled WPRE is downstream. The WPRE sequence used here is devoid of the hepadnaviral X-protein open reading frame and contains a point mutation that destroys the largest residual open reading frame of this element (Schambach et al., 2006). The same WPRE sequence has previously been used without evidence of side effects in a Pha-e I clinical trial in HIV-infected patients receiving autologous T cells transduced with an anti-HIV gamma-retroviral LTR vector containing this element (van Lunzen et al., 2007).

EFS-ADA LV → pCCL-c-EFS→hADA-WPRE. The intended function of the genetic material is to correct the genetic mutation present in the ADA-SCID patient HSCs and restore immune system function.

Evaluation of foreseeable effects

The host organism (HSCs). Do not represent any risk for the human health and safety as prior to collection, the donors’ informed consent was obtained. The genetic material is inserted into the donor cells by recombinant lentivirus particles. The packaging system used in the production of recombinant virus particles is a retroviral packaging system based on the HIV-1 packaging system (Dull et al., 1998). The vector is produced in human cell lines and the virus particles are harvested from the supernatant of the infected cells. The vector is non-replicative and does not integrate into the human genome.

A short promoter fragment (239 bp) from the human EF1 α gene, lacking intronic or enhancer sequences, drives transcription of the normal human ADA cDNA.

The transcriptionally disabled WPRE is downstream. The WPRE sequence used here is devoid of the hepadnaviral X-protein open reading frame and contains a point mutation that destroys the largest residual open reading frame of this element (Schambach et al., 2006). The same WPRE sequence has previously been used without evidence of side effects in a Phase I clinical trial in HIV-infected patients receiving autologous T cells transduced with an anti-HIV gamma-retroviral LTR vector containing this element (van Lunzen et al., 2007).

EFS-ADA LV → pCCL-c-EFS→hADA-WPRE. The intended function of the genetic material is to correct the genetic mutation present in the ADA-SCID patient HSCs and restore immune system function.

Origin & function

EFS-ADA LV ~ pCCL-c-EFS→hADA-WPRE. The intended function of the genetic material is to correct the genetic mutation present in the ADA-SCID patient HSCs and restore immune system function.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste disposed of according to the GOSH Pathology waste disposal policy. It is placed into dedicated plastic bins with lids and collected by the GOSH central collection service for infectious waste and incinerated. All sharps are placed in puncture-proof containers and disposed of through the central collection service for contaminated/infectious waste. Liquid waste is disinfected using Haz-tab (effective chlorine tablets) overnight before being disposed of down the sink.

If the intended waste disposal methods above are disrupted, there is an autoclave located in the building which can be used as a backup.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Approved by the GM safety committee as a class 2 activity due to the use of sharps in the experimental procedure.

Project Containment

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Project Ref 14/19.5

Date Ackn’d 31/05/2019

CU2 Project Title Investigation of streptococcal virulence factors using molecular microbiology and cell

Class 2

Culture Vol Class 2 < 1 Litre

Culture Volume Class 3-4
We seek to elucidate the role(s) of virulence factors in streptococcal pathogenesis using molecular microbiology and cell biology techniques, applied to infection models. This includes using molecular cloning techniques to generate bacterial mutants. We primarily seek to inactivate genes that encode putative virulence factors to induce a loss of gene function and loss of phenotype. Genetic complementation will also be performed to ensure that loss of phenotypes is not due to off-site mutations elsewhere in the chromosome. Streptococcus pneumoniae (pneumococcus), Streptococcus mitis and Streptococcus agalactiae (GBS) are most commonly found as colonizers of the human nasopharynx, mouth, and gut/vaginal tract, respectively, without causing disease. S. pneumoniae and S. agalactiae are also recognized as pathogens that can cause disease such as pneumonia, bacteraemia and meningitis in vulnerable populations such as the very young, the very old and immunocompromised persons. S. mitis is rarely pathogenic but may cause endocarditis and disease in the immunocompromised. S. mitis may also act as a reservoir for antimicrobial resistance and virulence genes for S. pneumoniae.

Given their pathogenic potential, we seek to identify and/or elucidate the mechanism of virulence factors encoded by streptococcal species. Our primary focus is on S. pneumoniae and S. agalactiae. To start, we plan to investigate the role of streptococcal cell surface proteins, toxins and known virulence factors (Ply and LytA for pneumococcus, HvgA for GBS) in establishing association with and transmigration through epithelial and endothelial barriers in vitro. The specific virulence factors that we study will evolve with time based on experimental results and new information in the literature.

Recipient or parental organism

1) Streptococcus pneumoniae is a hazard group 2 microorganism that is most commonly found as a nonpathogenic colonizer of the human nasopharynx, but may cause invasive and non-invasive disease in vulnerable populations such as young children and the elderly.
2) Streptococcus agalactiae is also a hazard group 2 microorganism that is most commonly found as a nonpathogenic colonizer of the human gastrointestinal tract and vaginal tract. It may also cause non-invasive and invasive diseases in vulnerable populations such as infants, elderly and pregnant people.
3) Streptococcus mitis is a nonpathogenic colonizer of the human oral cavity and only rarely causes disease under...
exceptional situations (pre-existing conditions, trauma).

4) Lactococcus lactis is a nonpathogenic species (hazard group 1) commonly used in the production of cheese and other dairy products, which in very rare and exceptional situations can cause endocarditis. L. lactis may be used to express streptococcal proteins to study the function of a gene product in isolation.

5) Escherichia coli is typically a colonizer of the human gastrointestinal tract that does not cause disease; however some strains are particularly virulent and causes enteric disease or urinary tract infections. The particular strains of E. coli we plan to use are lab-adapted, commercially available nonpathogenic strains used for molecular cloning and protein expression. E. coli strains will be used to replicate and store plasmids, and may be used to express proteins for protein purification.

Exposure to all the recipient microorganisms described above, whether WT or GMM, is not known to consistently result in carriage or disease even in vulnerable populations. With the exception of L. lactis, all other bacterial species are most commonly found as non-pathogenic symbionts of humans.

Host/vector system

Multiple suicide and replicative plasmids for molecular cloning, and expression of bacterial gene products. These plasmids are not inherently hazardous and do not alter the pathogenesis of the recipient cells. The plasmids usually contain an origin of replication, one or more antibiotic resistance marker for screening and selection, a multiple cloning site, and in a subset of plasmids a regulatory gene and promoter/operator sequence.

Origin & function

1) Genes from streptococcal species, in particular from S. pneumoniae and S. aga/aetiae, in their native, chimeric or inactivated forms to study the consequences of inactivating, altering or re-introducing the gene and gene product to the fitness and pathogenesis of the bacteria.

2) Commonly used antibiotic resistance markers (aph3, cat, tetM) for selection/screening of plasmids and to inactivate streptococcal genes by allelic replacement or gene insertion.

3) Reporter genes (gfp, mCherry, /aeZ, etc) for downstream functional assays, such as quantification of gene expression via beta-galactosidase assay or determination of protein localization through microscopy and/or subcellular fractionation.

4) Short peptide tags (His6, FLAG3, SUMO, HA etc) for tagging gene products and downstream tracking and functional assays, such as quantification of protein expression via Western blot or determination of protein localization through microscopy and/or subcellular fractionation.

Evaluation of foreseeable effects

Constructed GMMs are unlikely to be more hazardous than wild type (WT) strains. In most cases, we seek to attenuate the recipient micro-organism by mutating one or more genes, leading to a loss-of-function mutation, and to restore WT phenotype by complementation of the mutated gene in the GMM. Such mutations, or reversion to WT genotype, are highly unlikely to make the GMM more hazardous than WT bacteria. Tagging the genes with small peptide tag or placing a reporter gene, under the control of a native streptococcal gene are also highly unlikely to increase the hazardousness of the GMV/l. There may be a small fitness defect for the bacterium with gene and protein mgs. Whenever possible, mutations will be introduced by stable, double crossover homologous recombination into the chromosome to prevent unintended reversion to wild type. Antibiotic selection will be maintained to prevent loss of plasmids by the GMMs.

There is a very small chance that overexpression of certain virulence factors, or heterologous expression of the virulence factors in a non-pathogenic species will increase the virulence of the GMM. However, such virulence factors are also typically surface exposed and may be recognized by the immune response, and thus there is also a small
chance that the same mutation will reduce the pathogenesis of the GMM by stimulating more efficient immune
I clearance in an infected host. To isolate a single gene product to study, or to make large scale bacterial
culturing safer, we may heterologously express gene products in a less pathogenic species (L. /aetis or lab adapted
strains of E. co/i). This may have a small risk at making the recipient organism more pathogenic.(i.e. better able to
bind to epithelial tissue, more resistant to immune killing). Nevertheless, this is a common molecular biology
technique. As a precaution all GMMs will be handled according to containment level 2 conditions to prevent exposure
and contamination.
It is hypothetically possible that any escaped GMM could encounter other isolates of its same species in the
environment or host organism (such as humans) and share antibiotic resistance genes. To minimize risks, proper
containment and handling procedure of WT and GMMs will be carried out in accordance to containment level 2
guidelines. Additionally, we will not introduce any antibiotic resistance cassettes that confer resistance against
antibiotics most commonly used in therapy (ceftriaxone for pneumococcus, penicillin/amoxicillin for G8S).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

We do not plan to construct or use aflY GM animals and plants.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be placed in double-bagged clear autoclave bags. These bags will be sealed when 3/4 full
and autoclaved prior to other forms of disposal. Autoclaving will be done at 121°C, for 20 minutes or 126°C for 15
minutes at 100kfa, by specialized autoclave facility staff according to institutional guidelines. Printout report from the
autoclave's in-built temperature and pressure monitoring system as well as colour change on autoclave tape will be
used to validate the autoclaving process. Microbiology contaminated solid waste dispose in specialized yellow
"biological hazard" cardboard boxes will be incinerated. Liquid waste will be incubated with Virkon at a minimum final "
cQncentration of 1% overnight (>18 hoUrs) to kill the bacteria, followed by disposal in the sink with excess flowing "
water. . . .
Virkon is a commercially available disinfecting agents. Virkon is used according to manufacturer's instruction, which is
a minimum concentration of 1 % to disinfect bacterial cultures and surfaces .. Virkon is effective for killing streptococcus
~nd E. coli when used at the recommended concentratioD, amount and time
(http://wwW.fishersci.co.ukwebfiles/ukiweb-docs/SLSGD05.PDF).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee had no amendments to make and approved the activity as class 2.
Project Additional Information

Purposes of the contained use

The lab is focused on understanding the molecular mechanisms underlying neural stem cell function in normal and disease states. In particular the lab investigates the role of micro environmental niches which impinge on the function of neural stem cells. Using genetically modified mouse lines we can specifically label and follow the activation of neural stem cells in the sub-ventricular zone (SVZ), one of the two neurogenic niches, in the adult brain. We can induce injury in these mice and investigate the activation of astrocytes and neural stem cells mimicking the response of neural cells to a brain insult. We are also focused on understanding the progression of glioblastoma in the mouse brain, recapitulating the human disease in a model system in order to dissect out the molecular basis of induction,
migration and progression of the disease. By introducing lentiviral particles or piggybac plasmids encoding cell specific promoters driving known mutations associated with glioblastoma and tagged with fluorescent proteins directly into mouse brains we can mimic tumour progression in vivo. Thus, using a combination of genetically modified mice and introduction of tumor-inducing viral or plasmid particles we can identify crucial pathways associated with the different stages of the disease which may be drug-targetable in the future. Using the same mouse models eg p53fl/fl and Cdkn2a fl/fl we acutely isolate mouse neural stem cells and astrocytes and, in vitro methods determine the interactions of neural stem cells with their associated niches. Use of lentiviral produced cre recombinase enables deletion of specific tumour suppressor genes which have previously been associated with the progression of glioblastomas. We also isolate cells from our in vivo viral-induced tumours and use single cell sequencing techniques to determine differences in bulk tumour cells and those at the invading margins at the level of the transcriptome.

Recipient or parental organism

Especially disabled E. Coli K12 or B derivatives (e.g. DH1, DH5, HB1 01, JM1 01, JM 105, JM1 09, TG2, SUREtm, XL 1-Blue, Stbl3, BMH 71-18 mutS and ES1301 mutS or derivatives)
Mammalian tissue culture cells: 293T cells will be used to produce replication-incompetent virus. The auxiliary proteins and the VSV-g envelope protein are provided by three separate helper plasmids that contain no regions of homology to the HIV-1-based vectors. Three separate recombination events would be required to produce replication competent virus. The combined choice of the multiple packaging plasmids and the replication defective nature of the vector used will prevent the production of helper/replication competent virus by the transduced cells, reducing the risk to the environment.

Virus particles will be used to infect primary neural stem cells prepared from post-natal mice (PO-P1 0). They will also be used to infect cells from patient-derived glioma stem cells (prepared at the MRC Centre for Regenerative Medicine, Edinburgh). These patient derived cells have been obtained following local ethical board approval and are part of a bank resource of glioma stem cells generated in the MRC Centre for Regenerative Medicine, Edinburgh. Virus particles will also be injected directly into the brains of mice to either delete genes, induce activation of fluorescent proteins and ultimately generate gliomas in mouse brains.

DNA constructs will be electroporated into primary mouse neural stem cells prepared from pos-natal mice (PO-P1 0). They will also be used to infect cells from patient-derived glioma stem cells (prepared at the MRC Centre for Regenerative Medicine, Edinburgh). DNA constructs will also be injected directly into the brains of mice and then electroporated to either delete genes, induce activation of fluorescent proteins and ultimately generate gliomas in mouse brains. No oncogenes (EGRFvlll/PDGFRA) will be integrated via the lentiviral system.

The PiggyBac transposon system will be used to allow insertion of larger pieces of genetically modified DNA into the recipient genome. The system consists of a PiggyBac Vector and the a PiggyBac Transposase which recognizes transposon-specific inverted terminal repeats (ITRs) and efficiently integrates the ITRs find intervening DNA into the genome. The PiggyBac Transposase is delivered to the cell via the PiggyBac Transposase Expression Vector, which is co-transfected with one or more PiggyBac Vectors. Full integration of the plasmid into the genome is only possible when both PiggyBac and Piggybase are expressed concomitantly.

The mouse lines which will be used are commercially available (Jax laboratories) of have been received from the laboratories where they were generated under an MT A. Other non-GM mice will be acquired from commercial breeders (C57Blackl6, CDI NuNu, NSGs).

Host/vector system

PGEM series pcDNA3.1, pUC series, pAlter, pcDNA3, pTOPO, pCineo, pBUDCE4, pYac4 and their derivatives.
Helper-vectors:
Lentiviral vectors: pRSREV, pMD2G, pMDL, RRE, D8.9, VSVg

Lentiviruses will be used that lack viral auxiliary genes together with second generation packaging helper vectors such as pRSREV, pMD2G, pMDL, D8.9, VSVg or RRE. Second generation helper packaging plasmids will be used to ensure that the viruses produced are replication-incompetent.

Origin & function

The mouse lines which will be used are commercially available (Jax laboratories) or have been received from the laboratories where they were generated under an MTA. Other non-GM mice will be acquired from commercial breeders (C57Black/6, CDI NuNu, NSGs).

Evaluation of foreseeable effects

Lentiviral Vectors used in this project are unable to replicate and three separate plasmids are required to produce infectious particle and it is very unlikely to occur in the laboratory. All viral work will be carried out in dedicated level II containment facility. No sharps or needles will be used in any viral manipulations. These measures will ensure minimal or no infection to the user or the environment.

The Piggybac transposon system used in this project requires the simultaneous expression of PiggyBase and PiggyBac in order to effect integration of the plasmid. This will not occur outside of the host system and has no associated hazards to human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The mice used under this application are either from commercial suppliers, or recognised breeding establishments. These are laboratory strains of mice that have had no exposure to the threats of the natural environment and would therefore be badly adapted to survive in the wild or compete with wild strains. These laboratory animals would be extremely unlikely to be able to mate with other animals so would be unable to transfer the inserted genetic material to another animal. Therefore there is negligible risk of disseminating or displacing other animals in the environment.

The mice are kept and maintained in the UCL Central BSU in IVC racks under strict guidelines from the UCL Biological Safety Unit. Standard operating procedures are in place for working with the animals. Everyone working with animals is required to undergo an induction for work within the unit and an assessment by occupational health for any allergies or breathing issues which may be exacerbated by exposure to animal allergens. Everyone working with animals is fully trained and assessed prior to working in the BSU.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For waste produced as a result of, working with mice all PPE are disposed of after use and incinerated. Carcasses are incinerated or macerated after storage at -20°C. Sharps and neeples are disposed of in yellow burn bins and incinerated.

Solid waste generated through production of viral particles will be disposed of in double transparent autoclavable bags and will be destined for autoclaving in the Paul O’Gorman Building. There are 2 autoclaves in the Paul O’Gorman.
building, so a back-up if one breaks down. If both fail, waste is held in designated clinical/lab waste room which is access to staff only.

Contaminated glassware is disinfected first with 50/0 Distel (Trigene) for at least 1 hour, before heat sterilizing on site. Distel is a high level disinfectant. Plasticware that has come into contact with organisms is autoclaved on site (126°C 20 min, effective kill 1000/0) and then put into tiger stripe yellow bags by the building services team for treatment on site by the UCL waste contractors as offensive waste.

Liquid waste will be deactivated by a minimum of 12 hour exposure to Distel (100/0) disinfection. Disinfected liquids will be disposed of via tissue culture specific drains with excess water. The degree of kill is expected to be 1000/0. According to our supplier of Distel, StarLab, this compound at 10/0 concentration is highly active virucidal agent. The killing of several viral strains, including HIV, has been tested by using EPA protocol and complete deactivation was observed. Acknowledged and recommended by professional societies, public and private sector healthcare institutions http://www.medi-mark.co.uk/images/uploads/lTriGene_2p_animal.pdf

The GMSC approved the risk assessment as activity class 2 after minor modifications were made.

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Project Ref 14/20.1

Date Ackn'd | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4 |
-------------|-------------------|-------|------------------|----------------------|
29/01/2020   | GMP Manufacture of autologous CD34+ haematopoietic stem cells transduced ex vivo | Class 2 | < 1 Litre |
with the lentiviral vector

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Manufacture of advance therapy medicinal product (ATMP) for the treatment of patients with:
• Severe combined immunodeficiency due to adenosine deaminase deficiency: ADA
• X-linked severe combined immunodeficiency: IL2RG
• Mucopolysaccharidosis type IIa: SGSH
• Autosomal recessive chronic granulomatous disease: NCF1
• Familial hemophagocytic lymphohistiocytosis type 2: PRF1

The ATMP is a formulation of autologous CD34+ haematopoietic stem cells transduced ex vivo with the SIN lentiviral vector. To create the aforementioned IMP product, autologous CD34+ cells HSCs from patients with the above disorders will be genetically modified using a self-inactivating (SIN) lentiviral vector. These transduced cells will then be cryopreserved until such time of infusion back to the patients. The cryopreserved formulation will be thawed at the patient's bedside and infused directly without any further manipulation. The cells may also be given back to the patients in a fresh formulation which will be dependent on the protocol of the trial.

Recipient or parental organism

Autologous hematopoietic stem and progenitor cells (HSPCs) from patients or healthy donors. No producer cell line will be handled for this project as the LVVs are manufactured by external companies.

Host/vector system

All vectors covered by this RA are integrative, 3rd generation replication-defective, self-inactivating (SIN) HIV-1 derived LVVs, with a mutated Woodchuck hepatitis virus Post-transcriptional Regulatory Element (WPRE) sequence. They direct the expression of different therapeutic transgenes (the transgene used varies according to the disease to be treated in each clinical trial). There are all human physiological genes that are provided within the vector as an extra copy of the same gene that is mutated in the treated patients. The expression of the transgene is controlled in all cases by an internal physiological human promoter.

HIV-1 derived LVVs have been chosen because they are genetically stable, permanently integrate into the genome of transduced cells, and provide long-term gene expression in vitro and in vivo. The transduction HSPCs with such LVVs...
can be achieved after limited pre-stimulation of the cells in short-term cultures with cytokines, in conditions that are compatible with the preservation of the self-renewing capacities of these cells. Gammaretroviral vectors were used in the first gene therapy clinical trials, leading to disease restoration but in some case to malignant transformation (leukemia and myelodysplastic syndromes). This malignant transformation is known as insertional mutagenesis and it is produced when these vectors integrate close to regulatory regions of proto-oncongenes. The full viral L TRs present in these vectors were able to transactivate those regulatory regions, leading to the over expression of the protooncogene and to the subsequent malignant transformation. SIN LVVs represent a much safer approach for human application as: 1) the L TRs have been modified to lack promoter/enhancer activity and 2) they do no integrate in regulatory regions.

The modifications introduced into the organisation of the parental HIV1 genome are described below:
- Replication defective: The HIV-1 genome is split into 4 constructs consisting of 3 packaging plasmids and one (transfer plasmid, which are co-transfected for the production of an infectious particle. The packaging of the particle is therefore conditional on the Rev and gag-pol genes, which are expressed in trans on separate plasmids. The minimal homology between the various cassettes for packaging and gene transfer minimizes the likelihood of homologous recombination events and the generation of replication-competent HIV particles (RCI).
- SIN design: A 400 base pair deletion was introduced in the 3'L TR to remove major transcription factor binding sites. Reverse transcription, which generates both U3 regions from the 3' of the viral genome, transfers the deletion to the 5' L TR of the proviral DNA. The integrated provirus therefore contains the therapeutic transgene flanked by two transcriptionally inactive L TRs with U3 deletions.
- Mutated WPRE: This element is used to enhance the transgene expression in vivo and to increase viral titers during the manufacturing process. It has however been incriminated as the cause of hepatocellular carcinomas observed in mice treated with an equine LVV. Although such concern has not been evidenced in mice treated with HIV1 derived LVVs with the same WPRE, a mutated WPRE is used in order to prevent any issues.
- VSV-G envelope: VSV-G has been chosen because it has a broad tropism, and in particular the ability to target HSPCs.

The intended function of the genetic material is to correct the genetic mutation present in patient HSCs according to the disease being treated.
Therapeutic transgenes: this is different for each of the diseases to be treated
- Severe combined immunodeficiency due to adenosine deaminase deficiency: ADA
- X-linked severe combined immunodeficiency: IL2RG
- Mucopolysaccharidosis type IIIA: SGSH
- Autosomal recessive chronic granulomatous disease: NCF1
- Familial hemophagocytic lymphohistiocytosis type 2: PRF1
- Wpre* (mutated)

Evaluation of foreseeable effects

The host organism (HSPCs) do not represent any risk for the human health and safety as prior to collection, the donors are tested for an extended microbiology and virology panel. Moreover, cells area manipulated at all times inside an isolator (used primarily for product protection) and no direct contact between the operators and the cells is expected. Regarding the LLVs used, the risk of pathogenicity or toxicity in human cells is negligible. Cases of insertional mutagenesis have been reported in a small number of patients treated with gene therapy for immunodeficient diseases utilising retroviral vectors. However, the risk of accidental inoculation with lentiviral vector and the
development of insertional mutagenesis is considered negligible for the following reasons:
1. The risk of insertional mutagenesis is considered to be lower with lentiviral vectors than gammaretroviral vectors.
2. The current vector has been designed with the view to reducing the risks of insertional mutagenesis
3. Only HIV negative patients will be used as donors for this project so the possibility of recombination with the wildtype virus is negligible.
For all the above reason, the risks to human health and safety is considered negligible
The inserted genetic material is not known to have any known harmful effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Disposed of solid waste is conducted following the GOSH Pathology waste disposal policy. A double autoclave bag is used to contain the solid waste. It is placed into dedicated plastic bins with lids and collected by the GOSH central collection service for infectious waste and incinerated. All sharps are placed in puncture-proof containers and disposed of through the central collection service for contaminated/infectious waste, as above.
Liquid waste is disinfected using Haz-tab (effervescent chlorine tablets) overnight before being disposed of down the sink.
Although not used as the principle means of inactivation, autoclaves are available for use in the building; on the ground floor of UCL GOS Institute of Child Health and in the 4th floor laboratory area.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment
Approved by the GM safety committee as a class 2 activity, due to the use of sharps in the experimental procedure.
Corrections were requested by the committee on review and these have been made prior to submission.

Project Containment

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<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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The use of lentiviral vectors as pseudovirus to assess susceptibility of cells to infection by SARS-CoV2

We will purchase commercially available pseudotyped lentiviral particles which use GFP as a reporter to determine if human thymus cells and lymphocytes are susceptible to infection by SARS-CoV-2. The SARS-CoV-2 Spike Pseudotyped Lentivirus was produced with SARS-CoV-2 Spike (Genbank Accession #QHD43416.1) as the envelope glycoproteins instead of the commonly used VSV-G. These pseudovirions also contain enhanced GFP gene driven by a CMV promoter, therefore, spike-mediated cell entry can be conveniently determined via eGFP activity.

The SARS-CoV-2 Spike pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 in a containment level 2 facility. This is a second generation lentiviral vector containing WPRE and so will be a class 2 activity.

Recipient or parental organism

Mammalian cells (human thymus cells and lymphocytes). These are primary human cells that are not immortalised.

Host/vector system

Pseudotyped lentivirus in which the SARS-CoV-2 spike protein (Genbank Accession #QHD43416.1) is the envelope glycoprotein instead of the commonly used VSV-G. As control, Bald Lentiviral Pseudovirion (eGFP reporter) will be used (the same construct lacking the spike protein). The pseudotyped lentiviruses are replication-incompetent. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal. This is a
second generation vector which contains a WPRE.

Origin & function
Enhanced GFP (as a reporter for viral uptake).

Evaluation of foreseeable effects
Risk of pathogenicity or toxicity in human cells by this gene modification is negligible, and would not be expected to cause harm. The only genetic material we will introduce is enhanced GFP. We will not construct lentiviral constructs ourselves, but use commercial viral particles.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste will consist of plastic-ware used in tissue culture and viral preparation including pipettes, tips, culture dishes and flasks. This waste will be double-bagged and then autoclaved at 132°C for 15mins, then removed for off-site incineration. Liquid waste will consist of cells, cell culture supernatant and liquid containing viral vectors. Liquid waste will be treated for 30 minutes with PRESEPT effervescent disinfecting tablets (2.5g/tablet in 2-5 litres) or Distel (at dilution 1:10, according to manufacturer's instructions). Both PRESEPT effervescent disinfecting tablets and Distel are certified by Public Health England (PHE) to provide 100% virucidal kill under these conditions. Following chemical treatment, liquid waste will be placed in sealed containers and disposed of for off-site incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved by the GM safety committee as a class 2 activity.

Project Containment

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<td>Human Clinical Applications</td>
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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Pseudotyped HIV virus particles will be produced using a two-vector system, consisting of a packaging plasmid (encoding different HIV Env proteins or VSV-G) and a plasmid containing the defective HIV genome (env-depleted). Co-transfection of these plasmids results in production of self-inactivating virus, which can undergo only one round of replication. The possibility of recombination of the plasmids is very low but potentially possible, therefore the experiments will be carried out in a MSC class I or MSC class II cabinet in a CL3 laboratory.

**Recipient or parental organism**

Human cell lines HEK293T and TZM-bl.

**Host/vector system**

Plasmids encoding different HIV env proteins will be co-transfected with a plasmid containing the env-depleted HIV genome with minimal sequence-overlap. This two-plasmid system to produce HIV Env-pseudotyped viral particles ensures that the replication is self-limiting to one round of replication and the possibility of recombination to wild-type HIV is minimal.

**Origin & function**

Different HIV Env sequences will be used in neutralisation assays using patient plasma to determine the amount of neutralising antibodies.
Transfection of 293T cells and transduction of TZM-bl cells will result in stable integration of defective HIV genome onto the recipient cells. This will result in expression of some HIV proteins and cell-toxicity, but not the expression of full-length HIV genome. The risk of recombination and therefore production of replication-competent HIV is low.

Evaluation of foreseeable effects

Not applicable

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposal of solid and liquid waste will be carried out according to the detail in the Risk Assessment. Solid waste will be double bagged and sealed inside the safety cabinet and subsequently autoclaved. Autoclaved waste will then be incinerated off-site. Liquid waste will be inactivated with 10% Distel or 3% Virkon over night before disposal to drain in the facility.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The proposed project was reviewed in December 2019, brought as a class 2 assessment. The principal recommendation at that time was to split into two activities, one at class 1 and another at class 3. The assessment was edited and has now been submitted as a class 3 project, with the class 1 activities assessed separately and already approved. Following second review, the activity was approved by the committee as a class 3 activity.

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Additional Information

Purposes of the contained use
To investigate the 4D structure of the CAR-T immune synapse.

Recipient or parental organism
Human T-cells from healthy donor blood.

Host/vector system
No host / vector system in use in this activity.
The GM T-cells proposed in this activity are prepared using self-inactivating lentivirus vectors to create chimaeric antigen receptors against CD19. This activity is carried out at Great Ormond Street Institute for Child Health under a separate Class 2 GM notification. Only the GM T-cells are being used for the microscopy.

Origin & function
All material is human. The GM component is a T-Cell surface protein (anti-CD19)

Evaluation of foreseeable effects
Any exposure to the GM material by workers can be expected to result in prompt rejection of the allogeneic cells. Therefore there are no foreseeable effects to humans.
GM cells will be unable to survive outside of specialised cell culture conditions. There are therefore no foreseeable effects to the environment.
However, as the original source of T-cells is healthy human donors, the presence of (currently unknown) adventitious viruses cannot be excluded. Although very unlikely, the foreseeable effects of these potential hazards cannot be quantified, hence the Class 2.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No derogations applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid Class 2 waste shall be autoclaved at the standard 121°C / 15 minute standard. As the waste may potentially contain chemical waste, the waste will then be removed from the building as clinical waste for incineration.
All liquid class 2 waste shall be deactivated using bleach.

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Approved by the GM safety committee as a class 2 activity.

Project Containment

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Project Ref 14/94.6

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<td>06/07/1994</td>
<td>SEMLIK1 FOREST VIRUS VECTORS IN THE STUDY OF MEMBRANE TRAFFIC</td>
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Date Project Ceased

02/03/2022
Withdrawn: N
Tick if notifying a connected programme of work: N
Project notified under transitional arrangements: Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref:** 147/07.1

**Date Ackn’d:** 24/04/2012

**CU2 Project Title:** Functional characterisation of Mutations in Ion Channels.

**Class:** Class 2
**Culture Vol:** < 1 Litre
**Culture Volume Class:** Not Applicable

**Non-GMM Consent Granted:** Not Applicable

**Project notified under transitional arrangements:** N

**Withdrawn:** N

**Tick if notifying a connected programme of work:** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

Purposes of the contained use

We wish to use third generation lentiviral vectors (PCDH family; Systems Biosciences) to express ion channel genes in neurons and neuronal cell lines. While these lentiviral vectors are deactivated using several approaches and are regarded as a very low risk for recombination (details at www.systembio.com) there remains a
theoretical risk. For this reason, and to protect the target mammalian cells from contamination, as is usual for tissue culture experiments, we will conduct work in containment level 2 facilities. All live animal work will be done in containment level 2 as well.

Recipient or parental organism

The GMOs will be lentiviral particles containing sequences encoding ion channel genes. These viruses will be H
terecombination deficient, but will be capable of transducing individual mammalian cells (including handlers, if skin is L
broken). Any inoculation with these GMOs should be restricted to the subset of cells that come immediately in H
contact, but will not spread. The genes carried by the GMOs will be expressed in inoculated cells, but these genes H
/ion channels/ are not dangerous to neighboring cells.

Host/vector system

The vector system used will be the Systems biosciences PCDH lentiviral kit. These lentiviral vectors are third H
generation and represent a low risk of recombination or of infection. They are self-inactivated, Tat deleted, have the H
packaging genes split on to three separate packing vectors, and mature virus particles (the GMOs) will contain no H
original packaging genes. However as developments in lentiviral research provide safer alternatives we will continue
upgrading our choice of viral system, always in the direction of reduced ability to recombine and increased safety.

Origin & function

The genetic material will be cloned cDNA from human tissues. These cDNAs will encode ion channels that are H
associated with inherited diseases in humans. We have a large collection of mutations in different ion channel genes I
(an expanding group including: SCNs, CACNA1A, KCNA1, and accessory subunits), that have been identified with H
patients presenting with various neurological disorders (including epilepsy, ataxia, and migraine). None of the ion H
channel genes are thought to be oncogenic or dangerous on their own or in combination with other genes. They are L
not secreted but remain in the membranes and cytosol of the cells expressing them. The genes encode ion H
channels and their accessory subunits that allow cells, generally neurons or muscles, to respond to stimuli by H
passing ions across their membranes. We will analyse how the ability to pass ions across the membranes is altered H
when mutations associated with human diseases are expressed in the ion channels. All work with patient DNA is H
done under appropriate ethical approval and consent. H

The neurological nature of the diseases that we are studying, and the specialized nature of the ion channels we are H
studying requires that we explore the behavior of these genes in neurons and muscles. While much of our work can H
be done in vitro, the actual functioning of neurons and muscles in vivo, when expressing the target genes (wild-type H
vs. mutant) will be key to understanding how different ion channels are capable of causing different neurological H
disorders. We hope to investigate the ability of cells inoculated with viruses in vivo (in rodents only) to survive, H
propagate action potentials and to communicate with other neurons, especially when expressing ion channel H
mutants that in humans have been shown to disrupt these processes.

Evaluation of foreseeable effects

We anticipate that the primary effects will be the limited expression of our genes of interest in the desired H
mammalian cells, In general this will have modest effects on the infected cells behaviour, but should not lead to H
changes in neighboring cells. It is possible that the virus particles could infect any human cells including those of H
workers, if they contacted broken skin (i.e. with a sharp glass pipette or syringe needle). Such an infection with an H
inactivated non-replicable virus is likely to present a very small risk to the worker. Individual cells infected by the H
virus would express only the ion channel genes encoded in them and the effects of these genes would be limited to H
the infected cells. However it has been shown in some circumstances where large amounts of virus are used to H
infect embryonic or stem cells that tumors may be produced, although the mechanism has not been clearly linked to the viruses. For these reasons, as well as to maintain the sterility of our tissue culture stocks, and the health of animals during surgery, we will conduct all experiments with viral particles in containment level 2 cabinets. In addition all plasticware, sharps and media that comes in contact with the virus particles will be disinfected with bleach. Innoculated animals will be maintained in containment level 2 conditions and animal remains will be disposed of in sealed containment level 2 clinical waste bags. No animals inoculated with viral particles will be allowed to breed, and no GM animals will be generated.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a Although we hope to use viruses to express human genes in selected animal tissues, we will not breed GM animals for this project (only non-breeding animals will be used).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Both liquid and solid waste will be treated with bleach solutions (degree of kill effectively 100% for both virus particles and transformed mammalian cells). The effectiveness of these solutions is monitored routinely by the p1-1 indicating properties of the media, and bleach is stored in solid form to prevent inactivation. After disinfection with bleach solid waste is disposed as clinical waste. Surfaces in contact with media and virus will be sterilized with ethanol, and will be routinely (usually daily or, exceptionally, weekly) UV irradiation. Animal remains will be disposed of in sealed class 2 clinical waste bags. Live animals will be maintained in Class 2 containment facilities, and used cages will be autoclaved before cleaning. |

**Is an emergency plan required according to regulation 20?**  
N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

In view of current understanding of the risk associated with this vector system the committee argued that containment level 2 precautions are appropriate for this purpose.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

Large Scale Activities

Human Clinical Applications

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**Project Ref 521/01.1**

Date Ackn'd: 24/04/2012

Date Project Ceased

Withdrawn N

CU2 Project Title

TRANSFECTION AND EXPRESSION OF HUMAN ONCOGENES IN MAMMALIAN CELLS

Class 2

Consent Granted: Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 521/01.2

Date Ackn'd: 24/04/2012

CU2 Project Title: PRODUCTION AND USE OF RECOMBINANT HUMAN IMMUNO - DEFICIENCY VIRUS (RHIV) VECTORS

Class: Class 2

CultureVolClass2

Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 521/01.3

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<tbody>
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<td>24/04/2012</td>
<td>TRANSFECTION AND EXPRESSION OF POTENTIAL HUMAN ONCOGENES IN EUKARYOTIC AND PROKARYOTIC CELLS</td>
</tr>
</tbody>
</table>

**Class** 2  
**CultureVolClass2** 1-50 Litres

**Non-GMM**  
Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Withdrawn: N

Tick if notifying a connected programme of work: N

**Historical Significant Changes**
-

**Historical Date of Additional Info**
- 03/07/2013

**Significant Change ID**
- 03/07/2013

**Date of Significant Change**
- 03/07/2013

**Project Additional Information**

**Purposes of the contained use**

To prevent the exposure of humans to risks associated with the Eukaryotic expression of human oncogenes. It is not likely that DNA or cells will infect humans and therefore there is no foreseeable effect. All activity will be Class 2 with special provisions of "no shards".

**Recipient or parental organism**

Disabled strain of E.Coli and tissue culture cell lines as detailed below.

**Host/vector system**

pBl-EEFP, pIIIRES type bicostronic vectors, pTREX toruse in Eukaryotic cells.

In cell lines, Hela, MCF7, HRIE7, ARPE19, CAL02, A431, MRC5, HopI2.cos-1, cos 7. MDCK, CHU, CHUKI, NIH3T3, SWISS 3T3, EPNT.

For prokaryotes pBluescript, pCRII, pBLUNJ, pRSET, TrCHISA, pGEX, pUBEX in TOP10F, E.Coli XL-Blue, E.Coli JM83, BL21, DHSx

**Origin & function**

Human oncogenes have originally been cloned from human tissues, the precise function of which are largely unknown. They will be studied during this project.
Evaluation of foreseeable effects

So long as DNA or transfected cells are not introduced directly to humans there are no foreseeable effects. Class 2 activity is determined due to the expression of human (potential) oncogenes. We will not use any vectors which will directly infect human or animals such as viruses. We will in all instances adhere to precautions and procedures described in ‘Guidance and Construction of Recombinants containing potentially oncogenic nucleic acid servences.’ Para 13-15 GMAC Category 2 containment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All end waste from this project will be autoclaved. Autoclave indicator tape will be used to determine that effective autoclaving has taken place. We have previously determined that autoclave is 100% effective in killing all tissue culture cell lines and all strains of E.Coli used.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Similar work to that described in this notification and in the accompanying risk assessment is currently being undertaken by the Institute. See GM 521/01.1 and is happy that the work can be carried out safely.

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Project Ref  521/02.1
# Project Additional Information

**Purposes of the contained use**

To prevent the exposure of humans to risks associated with the eukaryotic expression of human oncogenes. It is not likely that DNA or Cells will infect humans and therefore there is no foreseeable effect. All activity will be Class II with specific provisions of "no sharps".

**Recipient or parental organism**

Disabled strains of E.coli and tissue culture cell lines as detailed below

**Host/vector system**

HOST: pCDNA3  Tet on/off E1A-deleted (replication deficient) adenovirus expressing GFP pGST, pMBP, pBS, pUC, pSP, pGL2, pCAT, pCH110  

**Origin & function**

Human oncogenes have originally been cloned from human tissues. The precise function of which are largely unknown

**Evaluation of foreseeable effects**

So long as DNA or transfected cells are not introduced directly to humans there is no foreseeable effects. Class II activity is determined due to the expression of human (Potential) oncogenes. We will not use any vectors which will directly affect human or animals such as viruses. We will in all instances adhere to precautions and procedures. Described in "Guidance and construction of recombinants contains potentially oncogenic nucleic acid sequences" Para 13 - 15 GMAC Category II Containment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

none
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste from this project will be autoclaved. Autoclave indicator tape will be used to determine that effective autoclaving has taken place. We have previously determined that autoclaving is 100% effective in killing all tissue culture cell lines and all strains of E.coli used.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment

Similar work to that described in this notification and in the accompanying risk assessment is currently being undertaken at the institute. See GM 521/01.1 and is happy that the work is being carried out safely.

Project Containment

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Project Ref 521/02.2

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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<tr>
<td>24/04/2012</td>
<td>USE OF RECOMBINANT LENTIVIRAL VECTORS TO EXPRESS A RANGE OF NON-ONCOGENIC SIGNALLING PROTEINS AND ADHESION MOLECULES IN EUKARYOTIC CELLS</td>
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<thead>
<tr>
<th>Class</th>
<th>CultureVolClass2</th>
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<tbody>
<tr>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Consent Granted: Not Applicable

Date Project Ceased: 02/03/2022

Project notified under transitional arrangements: N
### Project Additional Information

#### Purposes of the contained use

Purpose of contained use is to minimise/prevent the exposure of humans to risks associated with the eukaryotic expression of a variety of signalling proteins expressed through a human lentiviral gene transfer system. It is not likely that transduced cell lines will produce viable progeny and therefore only handling of the producer cell line with expression vectors and isolated viral particles can be considered a risk. All this activity will be carried out in class II biological safety cabinets and in category containment laboratory facilities.

#### Recipient or parental organism

Strains of E. coli bacteria and a range of human and tissue culture cell lines as detailed below.

#### Host/vector system

<table>
<thead>
<tr>
<th>Hosts</th>
<th>E. coli DH5 alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli DB3.1</td>
<td></td>
</tr>
<tr>
<td>293FT kidney epithelial cells (producer cell line) supplied from invitrogen</td>
<td></td>
</tr>
<tr>
<td>Target cell lines</td>
<td></td>
</tr>
<tr>
<td>Primary cultures of rat brain and retinal endothelial. Primary aortic and lung rat endothelial cells. Cells. GP8/3.9, RBE4 and GPNT cells (rat endothelial cell lines). LD7.4 rat RPE cell lines. ARPE19, hRPE7 human RPE cell lines. CHO cells. COS cells.</td>
<td></td>
</tr>
<tr>
<td>Vectors</td>
<td>pENTR4 (invitrogen)</td>
</tr>
<tr>
<td></td>
<td>pLENT16/V5 (invitrogen)</td>
</tr>
</tbody>
</table>

#### Origin & function

A range of cell adhesion molecules and signalling proteins have originally been cloned from human/rat tissues. The functions of all the proteins are generally known. No human oncogenes will be used.

#### Evaluation of foreseeable effects

As long as the DNA or transfected cell are not directly introduced to humans there is no foreseeable effects. Class II activity is determined to minimise exposure to virus-producing cell lines.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste from this project will be autoclaved immediately. Autoclave indicator tape will be used to determine that autoclaving has reached correct temperature and pressure. We have previously determined that autoclaving results in 100% kill of the GMO used in this project.

Similar work using recombinant lentiviral vectors is currently ongoing at this centre. The local GMAC is happy that the work proposed in this application can proceed safely.

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Project Ref 521/03.1

Date Ackn'd 24/04/2012

CU2 Project Title TRANSFECTION AND EXPRESSION OF POTENTIAL HUMAN ONCOGENES IN EUKARYOTIC CELLS

Class 2

CultureVolClass2 1-50 Litres

Consent Granted Not Applicable

Project notified under transitional arrangements N
**Purposes of the contained use**

The purpose of the contained use is to prevent the exposure of humans to risk associated with the eukaryotic expression of c-myc. It is not likely the DNA or cells will infect humans and therefore there is no foreseeable effects, all activity will be class II with specific provisions of "no sharps".

**Recipient or parental organism**

Tissue culture cell lines as detailed below.

**Host/vector system**

The vector is the pLNC-cmyc ER virus. The virus will be used to transduce primary epithelial cells.

**Origin & function**

Human c-myc was originally cloned from human cells, but its precise functions are largely unknown. The virus will be destroyed during the project.

**Evaluation of foreseeable effects**

So long as the DNA or transfected cells are not introduced directly to humans there are no foreseeable effects. Class II activity is determined due to the expression of human (potential) oncogenes. We will not use any vectors which will directly infect humans or animals. We will in all instances adhere to precautions and procedures described in "evidence and construction of recombinants containing potentially oncogenic nucleic acid sequences" PARA 13-15 GMAC category II containment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

None

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid cultures of viruses, or cells infected with viruses including all tissue culture plasticware will be autoclaved for 30 mins at 121 degrees C under 115lb/sq in pressure. This is effective in producing 100% kill of viruses as assessed by replating 10(6) CFU of autoclaved viruses on indicator cell lines and observing the appearance of no transduced cells. Each autoclaved sample will be marked with autoclave indicator tape. Each load will not be regarded as safe unless indicator tape shows that correct autoclaved parameters have been achieved. In addition we will also include thermosticks with each load to ensure the centre of the load has reached the correct working temperature and pressure. Autoclaves are routinely serviced to ensure correct working temperatures and pressures. Autoclaved material will be marked "inactivated GM waste" discarded to clinical waste and removed from the site by an authorised contractor.
Please enter comments on the GM safety committee on the risk assessment

None

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Project Ref 521/04.1

Date Ackn'd 24/04/2012
CU2 Project Title
Production of recombinant adenoviral (rAd5) vectors and their use to express signal transduction molecules in eukaryotic cells.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 Litre

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
The identification of novel targets for treating neovascular eye disorders

Purposes of the contained use

To work with the transforming oncogene polyomavirus middle T antigen and the growth factors VEGF and Notch ligands in a disabled lentiviral expression system

Recipient or parental organism

In most cases, mammalian gene sequences will be used. Polyomavirus represents the only pathogenic donor organism, the middle T antigen being its transforming oncogene.

Cultured mammalian cells will be transfected with bacterial plasmids containing mammalian gene sequences or transduced with viral vectors containing mammalian gene sequences

Host/vector system

pPICZ yeast expression vectors and bacterial cloning vectors will be used for cloning/expression of VEGF120/121, VEGF164/165 and mutant variants, VEGF 188/189, PIGF and mutant variants, VEGFR-1/2, neuropilin of the pUC series as well as mammalian expression vectors will be used for cloning/expression of Moesin and mutant
variants, PV-1 and mutant variants, Ezrin, Radixin, Caveolin, C5A, Integrins, Notch ligands and receptors, PI GF and PDGF and mutant variants, Hey-1 and -2 and mutant variants, polyoma middle T antigen, and micro RNA sequences for RNA interference of target genes.

ViraPower Lentiviral expression systems (Invitrogen) will be used for cloning/expression of VEGF120/121, VEGF164/165 and mutant variants, VEGF188/189, VEGFR-1/2, neuropilin-1/2, notch ligands and notch receptors and their mutant variants, PV-1 and mutant variants, moesin and mutant variants, polyoma middle T antigen and mutant variants.

Vectors will be introduced into bacteria by heat transformation or by electroporation using a BioRad GenePulser Xcell electroporactor. Vectors will be introduced into yeast cells by electroporation. Vectors will be introduced into mammalian cells in a class 2 safety cabinet either by electroporation using an Amaxa Nucleofector II, or by chemical transfection using lipid based transfection reagents.

Of the inserts to be used, VEGF, PI GF and Notch ligands are growth factors, and VEGFR1/2, neuropilin-1/2 and notch are growth factor receptors; Hey-1 and Hey-2 are nuclear proteins and downstream genes of the notch pathway; moesin, PV-1, radixin and caveolin are structural proteins, linked to the actin cytoskeleton, and it is thought that certain mutant variants of moesin and PV-1 may disrupt cytoskeletal arrangements and affect cell structure; integrins are involved in cell-cell and cell-matrix interactions; C5a, LFA1, CD46, CD55 and Mac-1 are involved in the immune system, C5a Mac-1, CD46 and CD55 in the complement cascade, LFA1 in immune cell recruitment RBP4 is a specific retinol-binding protein; polyoma middle T antigen is a transforming oncogene, and Src, PI3K and Ras are components of the polyoma middle T signalling pathway, and it is thought that mutant variants of polyoma middle T, Src, PI3K and Ras may disable aspects of their activity and therefore affect cell transformation.

MicroRNA sequences are designed with the aim of causing reduced expression of a target gene.

Origin & function

VEGF is a pro-angiogenic and pro-inflammatory growth factor which may exert harmful effects if over-expressed at high levels in the tissue, but with low severity because of the short half-life of the protein. VEGFR1/2 and neuropilin-1/2 are receptors and co-receptors, respectively, which mediate VEGF signalling. Notch ligands and Notch receptors are growth factors and receptors, respectively, and unregulated expression of their mutants variants has been linked to tumorigenesis, but with low severity and a self-limited localisation. C5a, LFA1, CD46, CD55 and Mac-1 may cause transient local inflammation if expressed at high levels locally, but with low severity. Polyoma middle T-antigen is a transforming oncogene with the potential to induce tumour growth, although the severity would be likely to be low and limited to immune-deficient individuals. Src, PI3K and Ras are involved in the Polyoma middle T-antigen signal transduction pathway and may be involved in its transforming ability, indicating a potential for harm, but effects would be limited, localised and transient with low severity. The use of low volumes of culture medium and replication-deficient lentivirus reduce the risk to humans. Sharps will not be used when handling potentially harmful sequences; this will significantly reduce the risk of DNA transfer through needlestick or other puncture injury. Other proteins including the structural proteins such as PV-1 and nuclear proteins such as Hey-2 have no documented risk of causing diseases in humans.

Evaluation of foreseeable effects

VEGF is a pro-angiogenic and pro-inflammatory growth factor which may exert harmful effects if over-expressed at high levels in the tissue, but with low severity because of the short half-life of the protein. VEGFR1/2 and neuropilin-1/2 are receptors and co-receptors, respectively, which mediate VEGF signalling. Notch ligands and Notch receptors are growth factors and receptors, respectively, and unregulated expression of their mutants variants has been linked to tumorigenesis, but with low severity and a self-limited localisation. C5a, LFA1, CD46, CD55 and Mac-1 may cause transient local inflammation if expressed at high levels locally, but with low severity. Polyoma middle T-antigen is a transforming oncogene with the potential to induce tumour growth, although the severity would be likely to be low and limited to immune-deficient individuals. Src, PI3K and Ras are involved in the Polyoma middle T-antigen signal transduction pathway and may be involved in its transforming ability, indicating a potential for harm, but effects would be limited, localised and transient with low severity. The use of low volumes of culture medium and replication-deficient lentivirus reduce the risk to humans. Sharps will not be used when handling potentially harmful sequences; this will significantly reduce the risk of DNA transfer through needlestick or other puncture injury. Other proteins including the structural proteins such as PV-1 and nuclear proteins such as Hey-2 have no documented risk of causing diseases in humans.

pPICZ yeast expression vectors contain the TEF1 and EM7 promoters, which drive the expression of the gene coding for resistance to the antibiotic Zeocin in yeast and prokaryotes, respectively. Insert expression in pPICZ vectors is controlled by the AOX1 promoter, which is induced by methanol. Gene expression is therefore suppressed in the absence of methanol. Bacterial plasmid vectors contain the prokaryotic lac, EM7, T7 and SP6 promoters, and the cytomegalovirus (CMV) immediate-early promoter and the simian virus (SV) 40 promoter for expression in mammalian cells. All bacterial plasmid vectors used are non-mobile, and are therefore unable to transfer into wild-type bacteria in the environment.

The ViraPower Lentiviral expression systems which will be used are derived from HIV-1 but have a number of inbuilt safety features. There are no longer terminal repeats (LTR) in the packaging plasmids, so that HIV-1 structural genes are only expressed in the producer cell and never packaged into virions. Secondly, viral particles are replication incompetent, and they only carry the gene of interest, so that no other viral proteins are produced. Additionally, these viruses have a deletion in the 3’ LTR which makes the virus “self-inactivating”; transduced and integrated lentiviral vectors are no longer capable of producing a packagable viral genome. The insertion of genes would be unlikely to affect the behaviour of the viral vectors.

Cultured mammalian cells transfected with bacterial plasmids containing mammalian gene sequences or transduced with viral vectors containing mammalian gene
sequences may display altered signalling, growth or viability but due to the fact that these cell lines rely on specific culture conditions and would be unlikely to survive outside the laboratory culture conditions, this would not pose a risk to humans. The insertion of genes would be unlikely to alter the recipient organisms' ability to survive in the environment, compete with other organisms or transfer to them the inserted sequences.

The majority of materials used in this project are not considered hazardous to handlers. The transforming oncogene polyoma middle T antigen and the VEGF isoforms and receptors have a potential to cause harmful effects. In the case of the vectors used for VEGF, the risk of natural gene transfer is very low. The most hazardous GMM is disabled lentivirus containing the polyoma middle T sequence, and any procedure in which this construct is handled, particularly with a view to transducing cells, is likely to be the most hazardous.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The inactivation measures which will be used, as appropriate, are chemical disinfection with a 1% solution of Virkon disinfectant for 10 minutes or a 105 bleach (sodium hypochlorite) solution, treatment with UV light, and autoclaving. Virkon disinfectant has been proven to kill 99.999% of microorganisms in less than 10 minutes, and is the disinfectant of choice for inactivating viruses. Sodium hypochlorite is a commonly-used wide-spectrum disinfectant. Autoclaving involves treatment with high pressure steam at 121°C for 15-20 minutes, which kills all bacteria and viruses. The inactivation of bacteria and yeast should be confirmed by spreading treated liquid waste onto the appropriate solid growth medium, incubating overnight at the optimum temperature and checking for micro-organism growth. The inactivation of virus should be confirmed by attempting to infect host cells and testing for infectivity or expression of proteins coded by the viral vector. Inactivated contaminated solid waste should be disposed of in Biohazard bags, and inactivated contaminated sharps in Biohazard sharps bins. Inactivated contaminated liquid waste may be disposed of in normal waste water drains.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

All points were addressed before the risk assessment was finalised

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Additional Information**

**Purposes of the contained use**

Our group aims to contribute to the development of novel methodologies of human disease treatment through identification of novel targets of diseases and their characterisation using in vitro and in vivo systems. We are particularly interested in cancer and ocular disorders. Recently our studies have identified that the small leucine rich proteoglycan family members may inhibit tumourigenesis. To examine this possibility, it is essential to analyse the effect of their activation in in vitro and in vivo cancer models. To this end, the third generation lentivirus system is ideal because it is very safe and can easily produce stable expression. So far, there is no effective treatment of many types of cancer. If the activation is effective, this may provide a novel strategy of cancer treatment.

**Recipient or parental organism**

**Virus:**

The third generation lentiviruses such as Lenti-X system (Clontech), SIN third generation (Gentarget, OpenBiosystem). 

**Cells:**

Non-transformed cells 293, NHU-hTERT
ATCC supplied established human cancer cell lines such as Hela, EJ28, J82, RT112, 253 JBV, and MCF7.

Corneal cells: epithelium, endothelium, and fibroblast

Animals: mouse

Host/vector system

To construct viral vectors, the following intermediate constructs will be made using general lab strain of E. coli. There is no potential for harm to health and/or harm to the environment.

Commercially available packaging vectors are used such as pMDL, prev, and pVSVG.

Lentiviral transfer vectors containing inserted genes of interest will be constructed using commonly used safe vectors such as pLVX-Tight-Puro (Clontech), LT-Exp1 (Gentarget), plenti vectors (Invitrogen), and Express-In™ (Openbiosystems).

Origin & function

Origin: human, mouse, Xenopus (OMD, PRELP), P1 bacteriophage (Cre recombinase), E.coli (Tet-on)

Functions:
OMD and PRELP are secreted proteins in the small elucine rich proteoglycan family. These proteins regulate cellular signalling from extracellular space. We expect that overexpression of these genes show negative effect on tumour cell growth.

Cre recombinase has the activity to induce recombination through the Cre-lox system.

Tet-on (for inducible system) is used for suppression of spontaneous expression of introduced genes.

Evaluation of foreseeable effects

The third generation lentivirus system has been recognised as a very safe system. The inserted DNA and virus are unlikely to be pathogenic. However, the most hazardous GMO is recombinant lentivirus. There is the possibility of mutagenesis caused by random insertion of viral sequences, but the risk of foreseeable adverse effects is very low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Infection of mice will be performed in the animal facility. The protocols are set out in the Home Office Animal Project Licence (PPL70/6750). The mice will be always kept in the animal facility.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The viruses, cells and animals cannot survive in natural conditions. They will be killed by standard methods.

E. coli and eukaryotic cells are inactivated by treatment with Virkon. Also, all waste and used glassware are autoclaved. The autoclaved waste will be discarded using plastic bags. We expect to kill 100% of cells and mice. The conditions are approved to kill E. coli, mammalian cell lines, and mice. Mammalian cells are confirmed by lysis of structure. Death of mice is confirmed by eye. The dead mice will be packed in plastic bags. The bags are temporarily kept in designated -20 freezer in the animal facility.
Then, the dead animals are incinerated by the designated special facility.

Prof O has submitted a new Class 2 risk assessment using third generation lentiviruses. The risk with this proposal lies in the possibility of random positional integration into the genome and the unpredictable effects this could have. The committee reviewed the risk assessment forms and the following points were raised.

GMM1 & 2
- Section 1.11 mentions xenopus but not section 1.13
- Section 2.06 states there is no risk. Clearly there is a risk but this is controlled.
- Section 2.09 - The risk should be low not zero

HSE Risk assessment
- As the form will be submitted to the HSE it must be accurate.
- Section 2.13 needs to be more specific. It is not acceptable to state "such as Virkon" or "suitable bags".
- Section 2.07 again states there is no risk. This needs to be amended.
- We need to make it clear in the risk assessment why we believe the proposal is Class 2, i.e. because of the level of virus titre being used and the injection into animals.

CU2 form
- The sentences relating to the Home Office need to be removed. The HSE are interested in the disposal of the carcasses not how the animals will be killed

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 530/01.1
## Project Additional Information

### Purposes of the contained use

- **Recipient or parental organism**

### Host/vector system

- **Origin & function**

### Evaluation of foreseeable effects

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 530/01.2

Date Ackn'd 24/04/2012

CU2 Project Title

INDUCTION AND EXPRESSION OF PROTEINS IN CULTURES OF COS7 CELLS FOR FUNCTIONAL ANALYSIS AND X-RAY CRYSTALLOGRAPHY

Class

Class 2

Culture Vol

Consent Granted

Not Applicable

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref: 530/01.3

Date Ackn'd: 24/04/2012

CU2 Project Title: USE OF NON-MAMMALIAN PHAGE PARTICLES FOR IDENTIFICATION OF LIGANDS

Class: 2

Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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### Project Ref 530/08.1

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<th>CultureVolumeClass3-4</th>
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| 24/04/2012  | Propagation and use of tumour replicating viral vectors for use in cancer gene therapy. | Class 2 | < 1 Litre

24/04/2012
The purpose of this project is to develop strategies to improve the in vitro and in vivo function of viral vectors for potential use in cancer gene therapy.

Recipient or parental organism

Well characterised, ACDP Class 1 mammalian cell lines (e.g. A549, CaLu 6, CT26, B16-F10, HeLa) well characterised, ACDP Class 2 mammalian cell lines (e.g. HEK293, COS-7, SVEC 4-10, 2F2B) Murine embryonic stem cell line (E4Tg2a) and murine primary cell C57B16, Oalb/c and CD1 immunocompetent mice Athymic (nude) immunodeficient mice

Host/vector system

1. Wild-type Reovirus is replication competent. However, its replication is selective to cells that have an activated Ras signalling pathway (Curr Opin Mol Ther. 2006 Jun; 8(3):249-60)
2. Tumour-replicating Adenovirus. ICOVIR-5 (Ad-DM-E2F-K-Delta24RGD) is an optimised oncolytic adenovirus that combines Ela transcriptional control by an insulated form of the E2F promoter with Delta24 mutation of Ela to improve the therapeutic index of AdDelta24RGD (Mo! Ther. 2007 Sep; 15(9): 1607-15)

Origin & function

Reovirus will be provided by Oncolytics Siotech Inc. Calgary, Canada (Reolysin). This product is currently being used in clinical trials and we wish to investigate its oncolytic potential in cancer gene therapy. The virus is not genetically modified.
ICOVIR has an Ela gene deletion in the retinoblastoma (Rb) protein-binding region, substitution of the Ela promoter for E2F-responsive elements and an RGD-4C peptide motif inserted into the adenoviral fibre to enhance adenoviral tropism.

Evaluation of foreseeable effects

Reovirus will infect human cells however, it is only replication competent in cells with an activated ras signalling pathway therefore, the risk to human cells is minimised. Ras mutations and overexpression of the ras signalling pathway are most frequently associated with a transformed phenotype and the development of malignancy. However, there is a theoretical risk that ras signalling may be activated in growth factor-stimulated cells, cells that proliferate (wound healing) or in people with cellular proliferative
disorders. Therefore we cannot exclude the possibility that Reovirus (Reolysin) might infect and replicate in apparently normal cells, causing lysis. Furthermore, the release of tumour associated antigens following lysis may modify the host immune system to infected cells. However, this is unlikely to result in adverse disease. Reovirus will also be readily transmitted via aerosols. Therefore, to protect people from the potential risk of infection all work associated with the virus will be carried out at Class 2.

ICOVIR is replication defective in normal, healthy cells, However, if ICOVIR were to recombine resulting in replication competence it would be comparable to wild type adenovirus since it contains no additional genetic material. All strains of adenovirus 5 are non-oncogenic. Wild-type Ad5 may cause mild respiratory diseases in children. Primary infection of adenovirus is thought to generate life-long immunity and it is thought that the majority of the adult population are likely to have antibodies to the wild-type virus. Although replication has been shown to occur in the lungs of experimentally infected cotton rats administered with high doses of virus, there is no evidence that adenoviruses can naturally colonise non-human hosts and therefore pose no significant harm to animals, plants or ecosystems.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Wild type Reovirus or Adenovirus-infected animals may excrete (shed) adenovirus (especially in the first 72 hours after infection). Precautions must be taken not to create aerosols when emptying animal waste material, washing cages or cleaning the room. Handling is carried out in Class 2 microbiological cabinet.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated with a 1% Yukon solution for at least 3 hrs. It can then safely be disposed of in the laboratory sink. All surfaces coming in contact with liquid waste will be washed with 1% Virkon and/or 70% isopropanol and/or a mild detergent (e.g. SDS). Plastic tips, syringes, etc or glassware that was used to hold or dispense virus will similarly be soaked in:

1% Virkon solution for at least 3 hrs, but overnight in most cases. The liquid will be disposed of in the laboratory sink, and the remaining solid waste will be collected in the laboratory solids bins.

1% Virkon has activity equivalent to 1000 p.p.m. chlorine without hazards, corrosion potential and loss of activity due to organic challenge.

(Antiviral Res. Inactivation of adenovirus types 5 and 6 by Virkon S. 2004 Oct;64(1):27-33.)

All other solid waste will be inactivated by autoclaving. The autoclave will be validated by external contractors at least once per year and the day to day operation monitored using the instrument readouts of temperature, pressure and time. The autoclaves used for waste also indicate a failed cycle if the required parameters are not met.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The committee feel that the proposed research using replication deficient viruses and tumour replicative competent viruses that are dependent upon ras signaling for adenoviral infection are safe. This research is a continuation of work currently being conducted within other UK institutions, using similar protocols. In these proposed experiments reporter genes are used as markers for transfection efficiency and translation. All cell lines used in these experiments are well characterized and if handled correctly, according to well developed protocols, will not pose any difficulties concerns, even for those cell line assigned at class 2 containment. The proposed experiments are very timely and need to be conducted without undue delay. There are no additional unknown risks associated with using nude mice in these proposed studies as similar studies have been carried out using the very similar experimental protocols as outlined in this proposal.

What happens if humans with cancer (in early stages people might not know that they have cancer) would work with Tumouronly replicative viruses? The worst case scenario would be that of the wild-type viruses. Is it really likely that all of these cell lines will be used? Yes, cell lines held by this laboratory have been acquired specifically for their different properties/species specificity.

The School requires double HEPA filters for Class II Microbiological Safety Cabinets used for GM work. Other points of clarification were dealt with at the draft stage for the Risk Assessment.

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**Project Ref** 530/10.1

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<td>24/04/2012</td>
<td>Cloning and sub-cloning of Clostridium difficile to identify mode of action for small molecules</td>
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**Historical Significant Changes**

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## Project Additional Information

### Purposes of the contained use

We aim to identify the molecular targets for our small antibacterial molecules using a multicopy suppression strategy. This requires the creation of a shot-gun library of Clostridium difficile DNA in specific vectors. These will then be transferred back into Clostridium difficile with the aim of identifying clones that are resistant to our molecules. Further to this our potential targets will be cloned to confirm their role in resistance. An alternative approach to this will be by creating a transposon library of random mutants and testing these for sensitivity against our compounds.

### Recipient or parental organism

Clostridium difficile strains are ACDP hazard category 2. Clostridium difficile R20291 is regarded as the PCR ribotype 027 reference strains. Strains produced in this study are not likely to be more virulent than this. Clostridium difficile 630 and 630 delta erm will also be used in this study but are known to be less virulent than strain R20291. Initial cloning will take place in E.coli and plasmids will be screened to ensure those containing toxin genes will not be placed in C.difficile as the additive effect is unknown. C. difficile only presents a hazard when the normal bacterial flora is compromised and is carried by 25% of the population with no effect to health.

### Host/vector system

The disabled E.coli strains TOP10, JM109 and CA434 will be used as the plasmid host and are not pathogenic. Due to the differences in codon usage between E.coli and C.difficile, it is very unlikely that expression of C.difficile genes will occur in E.coli. Also due to the random fragmentation of the genomic DNA the required elements for expression may not be cloned with the genomic DNA. The pMTL vectors based on the CoIE1 replicon will be used as shuttle vectors in E.coli and C.difficile. These plasmids are nic mob minus and are derived from Gram positive and negative components, many of which (except the Gram positive replicon) do not function in clostridia. These plasmids will also contain antibiotic resistance markers which are regularly used in the laboratory environment and E.coli. The plasmids are not self-transmissible.

### Origin & function

Genomic DNA will be extracted from the Clostridium difficile sequence strains CD630 and R20291. Fragmented segments of this DNA will then be cloned into pMTL80000 series vectors for propagation in E.coli and conjugation into Clostridium difficile. The approach of creating mutants for testing, either by deletion of a gene or insertion of a transposon will make the GMM less virulent. The majority of the genes to be expressed are innocuous bacterial enzymes used for basic metabolic functions and they are unlikely to function in isolation or in E.coli.

### Evaluation of foreseeable effects

**C.difficile:**
The duplication of metabolic genes in the multicopy suppression studies are highly unlikely to increase virulence or survival in the environment.

The effect of gene dosage on the only known virulence determinants of C. difficile, toxins A and B is unknown. Expression levels of the genes encoding toxin A and B vary widely in clinical strains and the experiments here are unlikely to exceed clinical levels even in the worst case scenario.

Random mutagenesis or directed gene deletion may increase or decrease virulence factor expression. None of the antibiotic markers used correspond to antibiotics that are routinely used to treat C. difficile infection.

**E.coli:**
The strains used are disabled, non-pathogenic laboratory strains which are unlikely to cause disease even with the addition of fragmented clostridial DNA or genes. As such infection with recombinant E.coli or survival in the environment is deemed highly unlikely.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be autoclaved to achieve 100% kill and will then be sent to landfill after processing by approved waste disposal contractors.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The GMSC has considered the proposal for work with C. difficile, and after initial discussion to clarify the text of the risk assessment we have no concerns about the genetic modification as planned, and note that it is highly unlikely that a GMO more hazardous than the host will be generated. The main concerns expressed were regarding the safe transport, handling and disposal of the pathogenic host organism. Acceptable safe working practices have been cited in the risk assessment, and reference has been included to indicate the expertise and experience of the key post-doc who has safely and successfully worked with C. difficile, and with other Level 2 and 3 pathogens for 11 years. One committee member has extensive experience of pathogen work including MRSA and has no problem with this straightforward application using category 2 bacteria. The GMSC has approved this proposal.

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Project Ref 596/00.1

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CU2 Project Title IDENTIFICATION AND EXPRESSION OF PROTEINS, FROM ORAL AND  
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CultureVolumeClass3-4
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| Project notified under transitional arrangements | N |

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**Historical Significant Changes**

- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L3 L4 L2 L3 L4</td>
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</table>

Project Ref 596/02.1

Date Ackn’d          CU2 Project Title
24/04/2012          AN INVESTIGATION OF THE ROLE PLAYED BY INTEGRINS AND CELL SURFACE RECEPTORS IN TISSUE REPAIR AND PATHOLOGY.

Class | Culture Vol | Class Culture Vol |
Class 2 | < 1 Litre |

Non-GMM Consent Granted
Not Applicable

Project notified under transitional arrangements
N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
## Project Additional Information

### Purposes of the contained use

The purpose of this programme of work is to examine the role of integrins and cell surface receptors in tissue repair and pathology. The genes for integrins/cell receptors will be cloned and expressed or knocked out in human cell lines and primary cultures of human cells.

### Recipient or parental organism

The recipient cells will either be human cell lines of mesenchymal origin (e.g., epithelial/keratinocyte cell lines) or primary cultures of human mesenchymal cells (e.g., muscle myoblasts). The human cell lines that will be used in this programme of work are cells which have been in safe use for over ten years in a number of laboratories. The primary human cells on the other hand represent a greater inherent risk as they may contain adventitious agents.

### Host/vector system

The hosts will be either human primary mesenchymal cells or human cell lines with a safe record of use such as H357. A number of viral vector systems will be used which lack the structural genes for particle formation and replication. In particular PRcCMV, PBAbePuro, pLXIN, and pLXSN will be used in conjunction with the packaging cell lines AM12, E86, and PT67.

### Origin & function

The genetic material will be obtained from human cells and will code for integrins/cell surface receptors. These genes will be inserted into host cells which lack the proteins they encode so as to determine the role played by these molecules in cell-cell and cell-extracellular matrix interaction.

### Evaluation of foreseeable effects

Parental and recipient cells: The cell lines to be used in this programme of work have been in safe use for over ten years and are unlikely to cause harm to humans or the environment. The human primary cell could contain adventitious agents which may cause harm to humans.

Viral vector systems: The viral vector systems to be used are disabled and as such to not represent a risk to humans or the environment.

GMMs: The GMMs constructed in this programme of work will express integrin/cell surface receptors that may alter their tissue tropism. Thus while these GMMs represent no risk to the environment it is possible that they may cause harm to humans.

### Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Conditioned media from GMMs and contaminated cell culture plasticware will be treated with a 1% solution of Virkon (final concentration) prior to disposal as clinical waste for incineration. Virkon treatment has been shown to rapidly produce 99.9999% virucidal, bactericidal and fungicidal activity. Contaminated serological pipettes and pipette tips will be autoclaved at 121 degrees C for 20 minutes prior to disposal as clinical waste for incineration. The autoclave to be used is regularly validated and serviced.
The work proposed in this programme of work was viewed as principally activity class 1. However it was felt that altering the expression of integrins on the cell surface may alter tissue tropism and thus may conceivably result in the GMO posing a risk to human health.

Project Containment

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<td>L2 L3 L4</td>
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</table>

Project Ref  596/06.1

Date Ackn'd  24/04/2012

CU2 Project Title

The molecular basis of resuscitation of Campylobacter jejuni from a viable but not-culturable (VBNC) state.

Class  2

CulturVolClass2  < 1 Litre

CulturVolumeClass3-4

Non-GMM Consent Granted

Not Applicable

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Withdrawn  N

Tick if notifying a connected programme of work  N
Purposes of the contained use

Construction and complementation of defined Campylobacter jejuni mutants to investigate the function of genes potentially involved in resuscitation from the viable but non-culturable state.

Recipient or parental organism

Campylobacter jejuni NCTC 11168
Campylobacter jejuni 81-176

Host/vector system

Genes encoding putative resuscitation factors will be cloned in E. coli using the plasmid pGEM-T-Easy (Promega UK Ltd., classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM). These genes will be inactivated by insertion of a gene encoding kanamycin resistance (aphA3) and re-introduced into C. jejuni NCTC 11168 and 81-176. As pGEM-T-Easy is not able to replicate in C. jejuni, double cross-over homologous recombination events will direct allelic replacement of the chromosomal wild-type copy of the gene with the mutated allele present in the vector. pGEM-T-Easy carries an ampicillin resistance gene.

Genetic complementation will be done using an E. coli vector (pRRC) that delivers, by homologous recombination, cloned genes (under the control of a Campylobacter-derived promoter) into the 16S-28S rRNA spacer regions on the C. jejuni chromosome (Karlyshev and Wren 2005. Appl. Environ. Microbiol. 71:4004). This vector is not able to replicate in C. jejuni, is classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM and carries a chloramphenicol resistance gene.

Origin & function

C. jejuni genes encoding potential resuscitation factors (Genome sequence Genbank NC_002163; gene ID 904972, possible secreted transglycosylase; gene ID 904469, unknown function; gene ID 905140, possible secreted transflycosylase) will be amplified by PCR from genomic DNA of strain 11168.

Evaluation of foreseeable effects

Although the genetically modified organisms produced in this project will be resistant to kanamycin or to both kanamycin and chloramphenicol, neither are antibiotics of choice for treatment of Campylobacter infections. Therefore the potential risks associated with the genetically modified organisms are the same as those of the parental strains and the likelihood of these risks being realised is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The project will generate the following waste: broth- (of 100 ml maximum volume) and agar plate cultures of the GMOs, contaminated pipettes, pipette tips and glassware. All contaminated waste, will be autoclaved on a "kill cycle" ensuring that the waste is held at a holding temperature of 121 degrees C for at least 15 minutes. This will kill all GMOs. Autoclaves contain temperature probes to check the heat penetration and sterilisation indicator strips are also included in each run. Autoclaved liquid waste will be disposed of by autoclaving on a kill cycle.
disposed down sinks. Autoclaved solid waste will be sent for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Part 1
The committee noted that this project was similar in nature to a programme of work previously approved by the HSE (GM596/99.1) but had the safety advantage over that programme of work in that the vector systems to be used could not replicate in the host organisms under any conditions. The committee also recommended that the inexperienced research on the grant be sent on a training course in good microbiological practice which has now been done.

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Animal Units

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Project Ref 596/98.2

Date Ackn'd 24/04/2012

CU2 Project Title CLONING OF STAPHYLOCOCCAL & STREPTOCOCCAL SECRETED PROTEINS

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 Non-GMM Consent Granted Not Applicable

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

02/03/2022 Page 946 of 15326
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
### Project Containment

#### Laboratory Activities
- L2
- L3
- L4

#### Glass Houses
- L2
- L3
- L4

#### Growth Rooms
- L2
- L3
- L4

#### Animal Units
- L2
- L3
- L4

#### Large Scale Activities
- L2
- L3
- L4

#### Human Clinical Applications
- L2
- L3
- L4

### Project Ref 596/99.1

**Date Ackn'd**: 24/04/2012

**CU2 Project Title**: DNA INSERTION ONTO THE CHROMOSOME OR SELECTED GRAM POSITIVE BACTERIA

**Class**: Class 2

**CultureVolClass2**: Non-GMM

**Consent Granted**: Not Applicable

**Project notified under transitional arrangements**: N

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**
### Origin & function

#### Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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### Project Ref 669/05.1

<table>
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<td>02/03/2022</td>
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Production of a knock-out model of PINK-1 in human cell lines.

**Date Project Ceased**

**Class 1** 1-50 Litres

**Non-GMM**

** Consent Granted** Not Applicable

**Historical Significant Changes** Project transferred to GM14 on closure of GM669

**Historical Date of Additional Info**

**Tick if notifying a connected programme of work**

**Project notified under transitional arrangements**

**Withdrawn**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Generation of PINK-1 gene knockout human cell lines in order to create a model for the study of Parkinson's Disease.

**Recipient or parental organism**

Human cell lines.

**Host/vector system**

Human cell lines such as HEK293, SOAS2, human neuroblastoma cell lines and 197VM human neural stem cells (ReNeuron). JS4, a recA-derivative of E. coli MC1061, a disabled K12 strain. Phoenix ampho cells, a non-mobilisable derivative of HEK293, free of adventitious agents. Vectors: PSUPERretro-puro, PWZLneo EcoR(non-mobilisable), PWZL BlastF(non-mobilisable) and PWZL BlastF EcoR

**Origin & function**

64i nucleotide RNAi constructs designed to knockout PINK-1 expression(Sigma Genesis Ltd). EcoRI fragment of the murine cationic transporter from Julian Downward as pWZLneoEcoR, originally made by D. Conklin.

**Evaluation of foreseeable effects**

Human cell lines will have ability to be infected by murine ecotropic viruses. The gene function of PINK-1 could be knocked out if the PINK-1 RNAi sequences were transferred to a human organism. The consequences of this are not clearly known, although loss of PINK-1 function is thought to be the underlying mechanism in autosomal recisive Parkinson's disease and is therefore associated with neurodegeneration in humans.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No GM animals or plants will be produced.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No derogation required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste and plastic disposable pipettes are chemically decontaminated with either 10,000ppm Chloros or 1% Virkon, followed by incineration (solid material).

E. coli contaminated solid waste (agar plates, tips, pipettes is autoclaved at 121 C for 20 mins, followed by incineration. Solid waste from tissue culture such as dishes, tubes and flasks are also autoclaved before incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been approved by the local GM Safety Committee and accepted at Class 2.

Project Containment

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Animal Units

| L2 | L3 | L4 |

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<td>Date Ackn'd</td>
<td>31/10/2017</td>
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<tr>
<td>CU2 Project Title</td>
<td>Construction and use of defective helper free amphotropic retroviruses for immortalisation of primary human cells.</td>
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<td>Class</td>
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<tr>
<td>Not Applicable</td>
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Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use
To generate and use stable producer cell lines that produce high titre replication defective amphotropic retroviruses to be used for the immortalisation of freshly isolated primary human cells.

Recipient or parental organism
JS4, a recA-derivative of E.coli MC1061 (a disabled K12 strain). PA371, a non-mobilisable second generation mouse amphotropic producer cell line; TEFLYA and RD cells, both derivatived from TE671 cells, non-mobilisable. Phoenix ampho cells, non-mobilisable derivatives of HEK293, and primary human somatic cells.

Host/vector system
SV40 large T antigen and mutants.
Human catalytic subunit of telomerase and human papilloma virus 16/18.
E7 and E6 gene; Polyoma virus T antigens and mutants; Adenovirus E1A and mutants.
P53 GSE fragment and full length ORF for cellular genes.

Origin & function
SV40 T antigen, the catalytic of human telomerase, HPV E7 and E6 proteins, Polyoma virus T antigens, Adenovirus E1A and p53GSE are all immortalising genes that have the potential to extend the proliferative life span of normal somatic cells.

Evaluation of foreseeable effects
No GM animals or plants will be produced.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
No derogation required.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No derogation required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste such as dishes and flasks will be immersed in 1% Virkon for >10 minutes, followed by autoclaving and then incineration.
E.coli liquid waste is inactivated by treatment with 1% Virkon for >10 minutes.
Liquid tissue culture medium is collected into containers containing sufficient Virkon for final concentration of not less than 1% and left for >10 minutes.
E.coli solid waste (agar plates, tips and pipettes) is autoclaved followed by incineration.
Solid waste from tissue culture (tubes, dishes, flasks and pipettes) is autoclaved followed by incineration.

After autoclaving or chemical disinfection, waste is put into yellow clinical waste bags or containers, collected centrally and transported by an approved waste route for incineration.

Established inactivation procedures in molecular biology laboratories, giving effectively 100% kill.

Records of each autoclave run are made and held by the Safety Officer.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The risk assessment has been approved by the local GM Safety Committee and accepted as Class 2.

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Large Scale Activities

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Human Clinical Applications

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Project Ref 669/11.1

Date Ackn’d 31/10/2017

CU2 Project Title Full-length HTT gene expression in human cells using amphotropic retroviral constructs

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

02/03/2022 Page 953 of 15326
Human neurons expressing either full-length normal or mutant Htt will be generated using STROC05 and 197VM neural stem cell lines transduced with pMSCV-based retroviral constructs. They will then be differentiated into neurons by the removal of key growth factors, which can then be used to set up co-cultures of primary human monocytes and macrophages isolated from Huntington's disease patients and control subjects to investigate neuronal-myeloid cell interactions.

The retroviral vectors to be used contain the full-length sequence of the HTT with either a normal (15Q) or mutant (138Q) CAG repeat length encoding the wild-type and mutant Htt proteins respectively. DNA prepared from E. coli bacterial cultures is packaged as a retrovirus by co-transfection into either HEK293 or BOSC23 cells with separate gag/pol and env packaging co-factor plasmids.

E.coli K12 strains (eg DH5α, TOP10)

HEK293T cells (human cell line for retroviral packaging)

BOSC23 cells (human cell line for retroviral packaging)

U937 cells (human lymphoma cell line)

STROC05 cells (human striatal neural stem cell line)

ReNcell 197VM cells (human neural stem cell line)

pMSCVpuro-10366-Htt15Q

pMSCVhyg-10366-Htt138Q

gag/pol+ & env plasmids

The inserted genetic material will encode the full-length human HTT (huntingtin) sequence (with either 15 or 138 glutamine repeats in exon 1 of the gene). Expression of Htt with an expansion in the number of glutamine residues in its exon 1 (>36Q; mHtt) is thought to be the key pathogenic determinant of Huntington’s disease, an inherited neurodegenerative disorder in humans. Htt is widely expressed in human tissue, but its function is unclear. The protein has no sequence homology to other proteins, but it is known to regulate gene expression and may have a functional role in cytoskeletal anchoring or transport of mitochondria or vesicle trafficking. We intend to study the cellular effects of expression of htt in human neural stem cells.
mHTt expression in neurons is thought to be the underlying cause of Huntington’s disease in humans; the inserted gene product could be harmful to health in the event of its expression in the CNS. Viruses packaged from the pMSCV vector are capable of infecting human cells itself, but are unable to replicate because the viral structural genes are absent. Integrated proviruses are capable of being mobilized by superinfection with helper virus, but the likelihood is low because the cells to be used are not known to produce helper viruses and transduced cells will be cultured in isolation from other cell cultures. The GMOs themselves are not able to establish infection and maintain propagation in humans or other species. Cultured mammalian cells, transduced or otherwise, are unable to survive outside of the laboratory environment and are not infectious. The E. coli bacterial strains are disabled, lack the necessary pathogenic mechanisms required to cause infection and will only have limited survival in the environment. Retroviral particles could conceivably infect cells of other species, resulting in the transfer of genetic material. Effects due to expression of target genes would be unlikely, but they could result in disruption of host genes depending on the site of insertion. Such effects would be limited by the inability of the virus to subsequently replicate. The absence of any potential of direct contact and the lack of survival of any of the proposed GMOs limits the potential effects on other species or the environment.

Contents and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be decontaminated with 1% Virkon for a minimum of two hours, prior to disposal with plenty of running water down a suitable designated sink. The degree of kill using 1% Virkon is 100%, as per supplier’s information, and this procedure is well established in molecular/cell biology laboratories. Solid waste such as culture dishes and flasks will be decontaminated with 1% Virkon overnight. Solid waste from mammalian cell culture is autoclaved prior to incineration; this is a standard Departmental procedure for those areas in which Containment level 2 work is carried out. Following chemical disinfection (and autoclaving), all solid waste waste is put into yellow clinical waste bags or containers for collection in designated yellow bins and transport by an approved waste disposal route for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The project was approved by the GM 669 committee. This work poses no risks to personnel or the environment when local rules and decontamination procedures are practised in conjunction with the engineering and administrative control measures for work at containment level 2.

**Project Containment**

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**Project Ref 669/17.1**

<table>
<thead>
<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>Culture Vol Class 2</th>
<th>Culture Volume Class 3-4</th>
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<tbody>
<tr>
<td>31/10/2017</td>
<td>Expression of mouse prion protein in eukaryotic systems</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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<td>N</td>
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**Project notified under transitional arrangements**

**Historical Significant Changes**

Project transferred to GM14 on closure of GM669

**Project Additional Information**

**Purposes of the contained use**

To employ eukaryotic expression systems to produce the normal cellular form of the murine prion protein. The advantage of using such systems is they enable proper protein folding, disulphide bond formation, and posttranslational modifications such as signal cleavage, proteolytic cleavage, or-and glycosylation that we cannot
obtain when using prokaryotic systems.

**Recipient or parental organism**

- Insect cells: Spodoptera frugiperda 9 (Sf9)
- Mammalian cells: Chinese hamster ovary (CHO), human embryonic kidney cells (HEK.293)

**Host/vector system**

- pTriEx, pFastBac HT and other commercially available vectors to be used as necessary for baculovirus expression
- PTriEx, pSV and pCMV series of vectors and other commercially available vectors to be used for expression in mammalian cells.

**Origin & function**

The vectors used are standard commercially available ones and are designed to allow the expression of the gene of interest. The mouse prion gene may be cloned from mouse cDNA or synthesised by commercial gene synthesis.

**Evaluation of foreseeable effects**

None of the strains of GMMs used are able to survive outside of a laboratory. The baculoviruses used are polyhedrin negative and have a much reduced survival time owing to increased susceptibility to dessication and ultraviolet light. The vectors used will confer antibiotic resistance to antibiotics commonly used in the laboratory, namely ampicillin, blasticidin, zeocin or hygromycin.

Although it is theoretically possible that the prion protein could misfold from the cellular form to the disease associated form that could be harmful to health, even were this highly unlikely event to occur, the very low infectious titre of any material in combination with the transmission barrier for prions between mouse and human makes detrimental impact on human health extremely unlikely. The prion protein has been expressed by other laboratories with no reports of changes to the pathogenicity of the host.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be treated with 20,000 ppm sodium hypochlorite for one hour, or where this is not suitable then sodium hydroxide to a final concentration of 1 M will be used. GMM material will then be autoclaved prior to disposal by incineration.

Liquid waste will be treated with 20,000 ppm sodium hypochlorite for one hour, or where this is not suitable then sodium hydroxide to a final concentration of 1 M will be used prior to disposal down the drain.

These represent established protocols for the inactivation of prions and should achieve a 100% "kill rate"

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The project was approved by the GM committee. This work poses no risks to personnel or the environment when local rules and decontamination procedures are practiced in conjunction with the engineering and administrative control measures for work at containment level 2.

### Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2</td>
<td>L3 L4</td>
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**Project Ref** 669/98.1

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<tr>
<td>31/10/2017</td>
<td>STUDIES OF TRANSGENIC MICE EXPRESSING WILD TYPE, MUTANT OF VARIENT HUMAN OR ANIMAL PROTEINS</td>
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<th>Class</th>
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<th>CultureVolumeClass3-4</th>
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<td>Class 3</td>
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</table>

**Non-GMM Consent Granted** Yes

**Project notified under transitional arrangements** Y

**Historical Significant Changes**

GM669/03.1, Project Transferred to GM14 on closure of GM669

**Historical Date of Additional Info** 29/04/2003

**Significant Change ID**

**Date of Significant Change**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022

Page 959 of 15326
This programme of work will use lentiviruses to deliver cDNA encoding specific genes or RNAi to knockout specific genes into various rodent and human cell lines. Established model systems will then assess the effect of these upregulated or downregulated genes in vitro or in cells.

Recipient or parental organism

Replication-defective 2nd and 3rd generation HIV lentiviral vectors and modified vectors constructed in house will be packaged by transient transfection of plasmids into HEK 293T cells. Rodent and human cells will be infected with the packaged lentiviruses. Cell lines are free of adventitious agents, and have a history of safe usage in the laboratory, equivalent to containment level 1.

Host/vector system

Replication-defective lentiviruses used for gene transfer in this study are 2nd and 3rd generation HIV lentiviral
These lentiviruses have a wider tropism than wild-type HIV because the VSV-G Env permits entry into all cells. The virus produced is replication-defective, and all chances of recombination leading to a replicative virus is considered negligible.

### Origin & function

We wish to use lentiviruses to express:

1. P13K isoforms and P13K signalling pathway kinases, phosphatases and other pathway components (bovine, human or mouse) and mutant oncogenic forms of these, identified in various cancers. Of the 8 catalytic subunits of the P13K family, only p11 Oalpha has so far been found to be mutated in cancer. These mutations increase the enzymatic activity of p11 Oalpha. p11 Oalpha mutations can be concurrent with mutations in other genes encoding proteins such as Ras, p53, PTEN, etc. Overexpression of p11 Oalpha in cells has recently been shown to induce p53-mediated growth arrest and mutational inactivation of p53 is required in order for cells to become transformed (Kim JS et al. 2007, MCB, 27(2):662). Ectopic overexpression of wild-type forms of the catalytic subunits p11 Obeta, p11 Odelta and p11 Ogamma can transform avian fibroblasts (Kang et al., 2006, PNAS, 103(5):1289), but there is no evidence for a similar effect in mammalian cells.

2. We also wish to use RNA1 to target the same classes of molecules described in 1. above.


4. Resistance Genes: Neomycin, hygromycin, zeocin resistance genes used as positive selection markers. No potential hazard, have a well-established record of safe use.

5. Gene Regulation: Proteins required for tetracycline regulated expression of genes from tetracycline responsive promoters. These include TetA, TetA2S-M2 and TetR-KRAB; these molecules bind DNA through variants of the tetracycline repressor and do not bind specific eukaryotic DNA sequences. Gre and FLP recombinase. Used to catalyse the site-specific recombination of DNA between loxP sites (Gre) or between FRT sites (FLP). Gre or FLP-coding lentiviruses will be used for infections of cells derived from mice bearing conditional mutations of the genes described above.

### Evaluation of foreseeable effects

2nd generation lentiviral vectors have the genes and sequences for lentivirus formation split between 3 vectors. The backbone of the lentiviral vector only retains the packaging signal and the LTRs between which transgenes of interest are inserted (pHR or similar). Other genes required for synthesis of lentiviruses are supplied in trans on plasmid vectors and are encoded from CMV promoters.

From one plasmid the VSV-G Env protein is expressed (pVSV-G or similar) and from a second plasmid Gag, Pol, Tat and Rev are encoded (pCMV 8.91 or similar). In 3rd generation lentivirus vectors, the LTRs are also self-inactivating, such that upon reverse transcription they are also deleted. Most experiments will use the 3rd generation lentiviral vectors. A favourable system is the ViraPower Lentiviral Expression System (Invitrogen) which combines a mixture of 3 plasmids pLP1, pLP2 and pLPIVSVG encoding gag/pol and Rev response element, Rev, VSV-G env respectively. These are co-transfected with the packaging vector (four are available eg pLenti4N5-DEST) containing the insert of interest.

For the introduction of RNAi to knockdown specific genes, systems such as the pGIPZ from Open Biosystems will be used (http://www.openbiosystems.com/RNAi/shRNAmirLibraries/c3lpzLentiviralshRNAmir).

Lentiviruses have the capacity to infect human cells so infection of normal and HIV infected cells must be considered. Infection of normal cells will result in promoter/transgene incorporation into the genome. Harmful effects could be caused by transgene expression or due to insertional mutagenesis. The lentivirus vectors are replication defective, but there is a theoretical risk of recombination with wild-type HIV-1 or HIV-2. However, there is no report of this ever happening. In any case, recombination restoring all HIV genes — which is necessary to produce a replication - competent virus - would delete the transgene, converting the recombinant virus to wild-type. In order to reduce the risk further, the use of lentiviruses will be at ACGM Containment Level 2.

The recombinant viruses can only be packaged by cotransfecting the plasmid components of the vector system into 293T cells. Transfected cells producing the recombinant virus must be maintained in tissue culture and cannot survive outside the laboratory. Recombinant lentiviruses, are also unstable and unlikely to survive for long with survival time dependent on factors such as temperature and pH. Although theoretically the lentiviruses would be able to infect other animal species such as rodents, the likelihood of this occurring accidentally is negligible. In the event that this did occur, the recombinant virus would be unable to propagate. This programme of work does not include deliberate infection of animal species with lentivirus vectors. No harm to the environment is envisaged.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All basic molecular biology manipulations that do not result in production of virions will be conducted on the bench at Containment Level 1 using GLP. All steps involved in production of infectious virions will be Containment level 2.

Experienced staff used to work in a containment level 2 environment will conduct the experiments. Strict local rules describing protective wear, handling and disposing of
infectious materials will be adhered to. All liquid waste is decontaminated with 1% Virkon overnight. Any potentially contaminated material eg plastic is also soaked in 1% Virkon overnight, double-bagged, transported in leak proof containers to an autoclave in the same building and autoclaved. All equipment that has come into contact with lentivirus will be autoclaved before cleaning or disposal. Aerosols may be produced during the preparation of lentivirus and infection of HEK 293 cells. To prevent the dispersal of aerosols, preparation and infection of the virus will be carried out in a biological safety cabinet class II. All work surfaces will be thoroughly cleaned with disinfectant solution followed by 70% ethanol. Sharps will be used with special care and disposed of into sharps bins. Needles will not be re-sheated before disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be decontaminated by soaking in 1% virkon, then disposed in sealed double-bags which will be autoclaved and incinerated in accordance with local and country regulations for the disposal of biohazardous waste. Inactivation of lentivirus by autoclaving at 121°C for 15 minutes, will give effectively 100% kill. Using a higher temperature, or a longer time, is permissible. Waste will be transported in leak proof containers to autoclaves by trained staff and immediately placed in the autoclave for discard.

If live cells infected with lentiviruses are to be taken out of the Containment Level 2 lab and cultivated elsewhere, they must be tested for replication-competent virus first. In addition it should be noted that live cells infected with lentivirus that are to be used for experiments outside of the isolation room must be transported inside SEALED containers that are wiped over with microsol prior to leaving the hood and then only opened in areas designated as category 2 and for use with lentiviral infected cells. Otherwise cells should be fixed or lysed prior to removal from the hood in the isolation room.

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

"Amendments required by the GMSC have been addressed in the final version of the risk assessment. The oncogenic nature of P13K genes have been assessed, competency and training of workers has been assessed."

Project Containment

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THE ROLE OF THE PROMYEOCYTIC LEUKAEMIA GENE (PML) DAXX AND C-JUN/C-JUN-N-TERMINAL KINASE 1/2 IN THE RESPONSE TO VARIOUS CELLULAR STRESSES

Purposes of the contained use

To study the effect of overexpression or down regulation of PML and DAXX on apoptosis and the regulation of the response to irradiation. The c-Jun and June-N-terminal kinase 1/2 pathway regulates apoptosis induced by UV radiation. We wish to test the effect of the siRNA-based downregulation or functional inactivation of various components of this pathway on the response to UV.

Recipient or parental organism

Mouse and human primary keratinocytes, mouse embryo fibroblasts, mouse bone marrow cells, mouse foetal liver cells, 293 cells (epithelial cell line), primary mouse cerebellum granule cells, neuroblastoma cell lines and adrenal chromaffine cells.

Origin & function

PML and DAXX cDNAs are from human and mouse origin. siRNA oligos for PML, DAXX, c-JUN and JNK1/2 will be designed based on mouse and human sequences.
the genes we intend to study are involved in the regulation of apoptosis.

Evaluation of foreseeable effects

There is a potential risk of infection at the stage of packaging and infection while using the lentiviral vectors, however the inserts we plan to transduce are not a hazard to human health. The host cells and plasmids generated by the parental virus are not harmful. The virus can infect the cells with which it comes into contact but cannot replicate, therefore the hazards to human health are low and containment level 2 is appropriate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste:
Solid waste will be treated with hypochlorite (10,000ppm) before being autoclaved prior to disposal (100% kill). The sterile waste is then sent for incineration by authorised contractors.

Liquid waste:
Liquid waste will be treated with hypochlorite (concentration 10,000ppm available chlorine) overnight and autoclaved before disposal. Any spillages will be cleaned with chlorine disinfectant and the waste paper autoclaved and incinerated.

Autoclave cycle - 121 degrees C for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

It has been agreed by the Genetic Modification Safety Committee that a designation of class 2 is appropriate for this work and that the risk assessment is sound and should be carried out at Containment level 2.

Project Containment

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<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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GENE ADDITION AND GENE CORRECTION STRATEGIES FOR DYSLIPOPROTEINAEMIA

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

| L2 L3 L4 L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 |

Project Ref 99/03.2

Date Ackn'd 24/04/2012

CU2 Project Title

INVESTIGATION OF THE ONCOLYTIC ACTIVITY OF ATTENUATED MEASLES VIRUS (MV) EXPRESSING GROWTH FACTORS.

Class>>

Non-GMM

Consent Granted

Not Applicable

Project notified under transitional arrangements [N]

Withdrawn [N]

Tick if notifying a connected programme of work [N]
## Project Additional Information

### Purposes of the contained use

| Propagation and in-vitro characterisation of attenuated MVs expressing murine and human GM-CSF and IL-12 in-vivo characterisation in mice transgenic for the human MV receptor, CD46. Determination of in-vivo oncolytic effects in relevant tumour models. |

### Recipient or parental organism

| Attenuated (vaccine) strain of MV |

### Host/vector system

| Vector: cDNA encoding murine and human growth factors expressed as additional transcription units of attenuated MV. |
| Hosts: |
| 1. For DNA preparation, K12 strains of E. Coli will be used, typically TOP10 strain. |
| 2. Attenuated (vaccine) strains of MV will express the growth factors. |
| 3. Established mammalian cell lines will be infected by the modified viruses: 293 (human embryonal kidney) Vero (African green monkey kidney), Raji, DOHH2, (human B cell lymphoma). 293-3-46 (modified 293 cells expressing attenuated MV proteins, intended for the rescue of infectious MV from the full-length anti-genomic plasmid). |
| 4. Mice transgenic for the human MV receptor, CD46 and SCId mice bearing humaning tumour xenografts will be intentionally infected by these viruses by various route of infection. |

### Origin & function

| Origins: cDNA encoding GM-CSF cDNA originally purchased from commercial sources (In-vivogen (murine) and ATCC (human)). |
| Full-length MV plasmid obtained from Dr Roberto Cattaneo, Mayo Clinic, Rochester, MN, USA. This plasmid and its use to rescue infectious MV owas originally described in Radecke F, et al Rescue of measles viruses from cloned DNA. EMBO J. 1995, 14:5773. |
| MV GM-CSF constructed in the laboratory of the applicant from starting materials above (Grote et al Cancer Res 2003; 63 6463) |
MV-IL-12 obtained from Dr Roberto Cattaneo (Singh M, Billeter MA. A recombinant measles virus expressing biologically active human interleukin-12. J Gen Virol. 1999, 8:101)

Intended functions:
Infectious MV expressing murine GM-CSF has been used by the applicants laboratory as an oncolytic agent in murine models (Grote et al Cancer Res 2003;63 6463) and is shows greater efficacy than the unmodified parental MV by virtue of stimulating a neutrophil infiltration of the tumors. We are currently further assessing its activity and mode of action. MV expressing human GM-CSF will be used to evaluate the effect on various human cell lines in-vitro. MV expressing murine and human IL-12 will be used similarly.

Evaluation of foreseeable effects

We and others have previously worked with attenuated MVs expressing growth factors and have had the opportunity to evaluate their effects in 2 different in-vivo models. A transgenic murine model expressing human CD46 (which has been accepted by the USA Food and Drug Administration as a suitable model in which to evaluate the toxicity of genetically modified MVs about to enter the clinic in phase 1 human studies) has been used to study the in-vivo effects of attenuated MV expressing murine GM-CSF. Second, a non-human primate model has been used to evaluate an MV expressing human IL-12. In this application, we are thus able to consider the theoretical effects of accidental infection with a growth factor expressing, replicating attenuated MV in the light of in-vivo data. Naive mice were infected by various routes using MV expressing murine GM-CSF and suffered no ill effects. A low level of GM-CSF was detected in the blood at day 3 post-infection, which returned to background levels after that time. No effect was observed on the blood counts. Infection was terminated by an immune response. (Grote et al Cancer Res 2003-63 6463). MV expressing human IL-12 has been administered to non-human primates without ill effects. The virus was eliminated by an immune response (R Cattaneo, personal communication).

Potential Effects on Human Health
The vaccine strains of MV themselves are not pathogens in immune competent individuals, but can cause fever and sometimes a mild rash in approximately 10% of vaccinees. Should humans become accidentally infected by attenuated MVs expressing a growth factor, it is considered that the virus would propagate to a limited extent, allowing some local production of growth factor before being limited by an immune response. By extrapolation from the animal data, it would not be expected that sufficient quantities of growth factor would be produced to cause systemic effects. All workers will be ensured to be immune to MV. Additionally, should all containment be breached, most adult individuals in the Western world, either by prior infection of vaccination. In the very remote case of an unimmunised individual being infected unintentionally, an immune response would be expected to limit infection albeit slightly slower than in a previously immune individual.

Growth factors such as GM-CSF and IL-12 are known to have pleotropic effects on human cells and their functions. Both of these growth factors have been used clinically and their range of effects are well characterised in humans in a variety of clinical scenarios. GM-CSF is a licensed product in fairly common clinical use. IL-12 has also been evaluated in clinical study. At very high doses, however, both GM-CSF and IL-12 can have harmful effects. Our animal data suggested that such toxic dose levels are not achievable when the growth factors are expressed from an attenuated replicating MV.

Bacterial vectors:
MV is a negative strand RNA virus, cDNA encoding the anti-genomic MV plasmid is not infectious, nor can it be used alone to generate infectious virus. Thus there is no foreseeable harm to human health from propagating standard laboratory strain of E coli harbouring MV plasmids since no expression will occur.

Cell lines:
When mammalian cell lines are infected by MVs expressing growth factors, infectious virus is released from the cells, although most of the virus remains cell-associated. Growth factor is secreted into the medium. Concentrations of GM-CSF in the medium are in the ng/ml range. MV-infected cells are the source of stocks of virus to be generated by freeze-thawing of infected cells. Virus concentration in stocks is usually about a million to ten million plaque forming units per millilitre. Average volumes harvested are around 1 to 3 ml. Sharps are not used when working with the cells, accidental inoculation with virus-infected cells is unlikely.

Mice:
Mice do not excrete MV in appreciable quantities. In the event of a worker being bitten by a mouse infected with MV expressing murine GM-CSF, it is unlikely that any
harm would ensue, since the worker would be immune to MV, as discussed above. Additionally, where murine experiments take place, the murine growth factor is expressed. In the case of GM-CSF, there is little or no activity of the murine factor at the human receptor.

Potential Effects on the Environment

Bacterial Vectors
There is no foreseeable risk to the environment in the event of release of bacterial vectors.

Cell Lines
Would not survive outside the controlled environment of the laboratory and thus present no risk to the environment.

Mice
The only natural host for MV is humans. Normal mice are not infectable by MV, hence there is no risk to other mice in the facility nor to animals outside the facility, should a total breach of containment occur. Should MV-infected SCID mice escape into the environment, they would not survive beyond the confines of the laboratory and rapidly perish. Should the MV-infected SCID mice escape into the environment, they would not survive beyond the confines of the laboratory and rapidly perish. They would not be able to infect normal mice with MV. If by chance, these mice bred with local mice, and lost homozygosity for CD46, they would no longer be infectable by MV. It should also be noted that CD46 transgenic mice CANNOT BE INFECTED BY WILD TYPE MV, which uses SLAM and not CD46 as a receptor. Finally, the mouse escape policy from the CBU at the RFH, which demonstrates the very unlikely nature of such an escape, is appended.

Spillage of virus stocks
In the very unlikely event of a spill outside the laboratory, where decontamination procedures for some reason were not able to be applied, which is extraordinarily unlikely, survival outside a host is brief. MV is inactivated by heat and light. Aerosols remain infectious for approximately 30 minutes and survival on surfaces is less than 2 hours. The virus is very unlikely to encounter wild-type strains, for the same reason, and also because in general such strains are usually not circulating in the community. However, considering this very remote possibility, MV is extremely genetically stable and recombination is very unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste generated will fall into the categories below:

Contaminated tissue culture plastics.
Solid waste, for example plastics will be decontaminated within the hood with 1% Virkon before disposal within autoclave bags to be autoclaved before eventual disposal according to the institutional system.

Plastic pipettes and pipette tips.
Will be placed into a rigid container within the hood and eventually, the capped container will be autoclaved before disposal, as above.

Liquid waste
Will be decontaminated with 1% Virkon or 1% sodium hypochlorite before disposal in the drains.
Animal carcasses
Animal carcasses will be disposed of within autoclave bags and autoclaved before disposal according to the institutional system.

Sharps (used during inoculation of animals)
Will be disposed of within an approved sharps container.

Virkon or sodium hypochlorite can destroy the virus so that no detectable virus remains after contact with the agents. No special monitoring will be necessary to monitor decontamination.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment
All non-procedural comments are included (comments such as 'what room will this work be carried out in' are, for example, excluded). Replies are also included.

Comment: Will any primary cells be used?
Reply: There are no immediate plans to use primary cells.

Comment: Consider any risk from various MV-infected mice to humans or the environment.
Reply: possible risks to staff working with MV-infected mice.
Work carried out over the past 4 years at the Mayo Clinic did not require veterinary technical staff performing general housekeeping duties with mice used for similar projects be subjected to the same Occupational Health concerns as staff employed specifically for the projects. Mice infected with vaccine strains of MV do not excrete the virus to any quantifiable extent. In fact, it is rarely even possible to re-isolate the virus directly from MV-infected tissues (for example, lung, spleen) of CD46 transgenic mice by co-culture of tissue with highly permissive cells. Thus, the risk of workers handling the mice and cages for husbandry purposes actually coming into any contact with the virus can be supposed to be extremely low. The case of a person being bitten by a GM MV-infected animal has been considered in this assessment. This situation was discussed at length during the establishment of the policies at the applicant's previous institution. It was felt that no specific action would be needed in the case of an appropriately vaccinated worker, other than general measures, appropriately recording the incident and monitoring the health of the worker. In fact, this incident did occur, approximately 2 years ago, during the applicant's previous post. No harm ensued.

Comment: If new workers join the project, will these issues be addressed through the occupational Health Dept?
Reply: Yes, when new workers join the project, the issue of MV immunity will be addressed through Occupational Health.

Comments:
Pipettes, tips, culture plates etc. do not require disinfection prior to autoclaving.

Dry discard containers with screw caps should be used for tips rather than discard pots.
Reply: Comments are duly noted and will be acted upon.

Comment: Can project staff be named?
Reply: A technician has now been appointed and named.

Comment: Does vaccine mediated immunity protect equally well against the proposed genetically modified strains?
Reply: Murine data from our lab suggest that immunity to the GM-CSF-expressing genetically modified strain of MV is the same in magnitude and timing as to the unmodified MV.

? has sent these comments in after closure regarding your projects.
Comment: It will be important to monitor levels of anti MV immunity in people on the project and in people closely associated with it and to have a program of vaccination in place to boost levels of low response.

Reply: Titres will be checked at starting and if inadequate (<25iu/ml), project workers will be immunised. Regarding further boosting: immunity to MV after natural infection or adequate vaccination is generally accepted to be lifelong. It should not be necessary to boost. There are no data on which to base a scheme of re-testing and possible re-vaccination, this would have to be empirically determined. If it is deemed necessary, I would consult with virology and vaccine experts to try and devise an appropriate scheme.

Project Containment

| Laboratory Activities | Glass Houses | Growth Rooms |

02/03/2022
## Project Ref 99/04.1

**Project Additional Information**

**Purposes of the contained use**

Primary human cells will be isolated from patients: either liver taken at resection or time of transplant, placenta, amnion, bone marrow or peripheral blood, will be immortalised and transformed either by retroviral vector mediated, lentiviral mediated, lipofection, or by electroporation introduction of genes associated with cell cycle and senescence; some of these will be oncogenes. The resulting cells will be grown in vitro, to establish their proliferative and differentiative properties. Suitable cell lines may be chosen as the biological component of a bio-artificial liver machine (should this project be successful their use in a bioartificial liver would be the subject of a future application). A subsequent addendum to this application will be made if and when cell lines have been generated to test the proliferative capacity of these cells in vivo, by injection into nude mice; this will be in conjunction with a home office licence application to use nude mice.

**Recipient or parental organism**

Retroviral vectorial vectors based on murine moloney leukaemia virus: Vectors lack pol and env sequences and only encode a short sequence of ATG negative gag. Third generation Lin X (derivative of 293T) packaging cell lines will be used to produce replication-incompetent virus. The gag, pol and env genes are integrated into the genome or expressed from a Bovine Papiloma Virus based episomal vector that contains no regions of homology to the MMLV based vectors. Three separate recombination events would be required to produce replication-competent virus. The cell line has been tested exhaustively for production of replication competent virus; none was
detected. The combined choice of the packaging cell lines and the replication defective nature of the vector used will prevent the production of helper/replication competent virus by the transduced cells, reducing the risk to the environment. Lentivirus vectors: cPPT-CMV and pCMV 8.91 plasmid) vector is an HIV based vector described in full by Zennou et al (2000). The replication defective virus is produced in TRANS with structural components supplied by the pCMVr8.2 helper plasmid carrying gal, pol, tat, and rev. The vector is pseudo-typed with VSV-G, supplied in trans by the plasmid pMDG. At least 2 independent recombination events with a helper free genome would be required to recreate a viable pathogenic genome.

The second lentiviral gene delivery system involves three plasmids that are transfected into virus producing cell line, the 293t cell line. The lentiviral system is secured by gene removal such that no new particles can be produced from infected cells. This system functions as the host 293t cell express.

1. The viral envelope protein (CVSV-G envelope) from pVSVg.
2. The modified HIV-1 genome with the transgene (modified HIV-1 with deleted envelope and accessory genes)
3. The HIV-1 genome (from the pCMV plasmid 8.91)

The vector can stably integrate into DNA for sustained long-term expression of the transgene. The vector has the ability to infect human primary and stem cells, with subsequent expression of the foreign gene in these cells and their progeny. No new HIV-1 virions can be synthesised by the infected cells due to the deletions in the genome - and they are termed self-inactivating. The VSV-G envelop allows a broader cell target range than HIV1 and is included for this reason.

Host/vector system

Hosts: E.coli strains DH5alpha, DH10B, mammalian cells; primary epithelial and mesenchymal cells derived from human liver, peripheral blood, human placenta and amnion, human stem cells Hepatocyte cell lines Hep G2, human fibroblasts and lines ImR90, packaging cells LinX A and LinX E, 293T cells.

Vectors: pBabe, pWZL, MARX vectors (Genetica Inc, Cambridge, MA USA); LN series (LN, LNSX, LNCX, Miller et al 1989); cPPT-CMV-(eGFP); cPPT-CMV-(hTERT); pCMV 8.91

Origin & function

hTERT (telemersase), myc oncogene E7 oncoprotein, p53 tumour suppressor, E1A (early region 1A adenoviral sequence), mdm2oncogene, cyclin D1, p16 and p189 cyclin dependent kinase inhibitors, SV40 large t antigen, GreenFluorescentPortein, B-galactosidase.
All oncogene-virus constructs will be obtained from Wolfson Institute for Biomedical Research, UCL.

Evaluation of foreseeable effects

Primary human cells may contain adventitious agents and should be handled in containment level 2 according to COSHH. The other human cell lines are established long term lines anad can be regarded as belonging to ACGM activity class 1. The linX amphotropic virus is incapable of replication outside of its packaging cell for following reasons. Third generation LinX (derivative of 293T) packaging cell lines will be used to produce replication-incompetent virus. The gag, pol and env genes are integrated into the genome or expressed from a BPV based episomal vector that contains no regions of homology to the MMLV based vectors. Three separate recombination events would be required to produce replication-competent virus. The cell line has been tested exhaustively for production of replication competent virus; none was detected. The combined choice of the packaging cell lines and the replication defective nature of the vector used will prevent the production of helper/replication competent virus by the transduced cells, reducing the risk to the environment.

Lentivirus vectors: cPPT-CMV and pCMV 8.91 plasmid) vector is an HIV based vector described in full by Zennou et al (2000). The replication defective virus is produced in TRANS with structural components supplied by the pCMVr8.2 helper plasmid carrying gal, pol, tat, and rev. The vector is pseudo-typed with VSV-G, supplied in trans by the plasmid pMDG. At least 2 independent recombination events with a helper free genome would be required to recreate a viable pathogenic genome.

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The vector can stably integrate into DNA for sustained long-term expression of the transgene. The vector has the ability to infect human primary and stem cells, with subsequent expression of the foreign gene in these cells and their progeny. No new HIV-1 virions can be synthesised by the infected cells due to the deletions in the genome - and they are termed self-inactivating. The VSV-G envelop allows a broader cell target range than HIV1 and is included for this reason.

The HIV-1 vectors being used are attenuated and cannot replicate once cells have been infected. The transgene has the potential to be expressed in infected human cells. Because some of the transgenes are potential oncogenes they could, when expressed in cells, give the cells the ability to proliferate. The vector does not replicate, reducing the risk of harm significantly. Aerosol contamination is not possible - the virus can only infect when normal barriers (skin/mucosal membranes) have been penetrated. All work will be carried out in a containment level 2 laboratory, with protective clothing and no sharps inside the safety cabinets. The vector has a small and insignificant potential to become replication competent if combined with wild-type vector. We prevent this by having no live HIV-1 experimentally in the laboratory. All workers on the project will be required to attend Occupational Health department to discuss the risk potential. The overall risk of infection and harm is therefore low.

The project involves the generation of cells with a prolonged cellular lifespan; it is possible that one or several oncogenes could be enriched in some of the cell lines. Genes could belong to any of the categories above but only genes involved with senescence, cell cycle and differentiation will be selected for.

Several oncogenes will be used as controls for the experiments. The effect of an oncogene or tumour suppressor on different cell types is very difficult to predict. For example, myc immortalises prostate epithelial cells but causes terminal differentiation in keratinocytes. Introduction of a single oncogene, however rarily results in malignant transformation. The hazards involved in naked oncogenic DNA will be addressed according to COSHH. Specifically naked oncogenic DNA will be handled in accordance with the "Advisory Committee on Genetic Modification Compendium, Part 3A - Annex 1. Containment level 2 is appropriate. The risk from the viral vectors themselves are minimal.

The main risk is introduction of an amphotropic virus containing an oncogenic sequence into the cells of a worker via a needlestick injury. This may result in the infection of the workers' cells and expression of the oncogene. The number of cells is likely to be small. Entry would have to take place through broken skin or mucosal tissue. The virus is incapable of replication once in the cells and the introduction of a single oncogene into a small number of cells is unlikely to result in tumour formation. This is before we consider the protective measures afforded by level 2 containment required and the class II cabinet that work must be conducted in, without the use of shpms in the hoods. The other risk is that transformed human cells may be introduced via needlestick or mucosal spillage. Because the cells are not derived from the worker they are unlikely to survive for long after entry.

There is no foreseeable risk to the environment in the event of the release of the bacterial vectors or GMMs. The transformed cell lines would not be able to survive outside of the laboratory and also present no risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Containment level 2: negative pressure relative to the pressure of the immediate surrounds: "required where and to the extent the risk assessment shows it is required". The tissue culture laboratories are constructed to Medicines Control Agency specifications and are therefore at a slight positive pressure with respect to their immediate surroundings, the anteroom. The anteroom is negative pressure with respect to the corridor (ie with respect to outside the tissue culture suite). Cell cultures will be manipulated only in Class II microbiological safety cabinets which are at negative pressure. In the event of a breach of containment outside the safety cabinet, GMM cell lines would not survive and would not pose a risk by the respiratory route.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cell culture: All work with mammalian cells will be manipulated in Class II safety cbinets in designated tissue culture laboratories. All laboratory areas are designated
containment level 2. Level 2 procedures will be observed.

Liquid waste: Liquid waste is treated with 1% Virkon or 1% Presept overnight and autoclaved before being discarded down the sink.

Solid waste: non-sharps plastics will be decontaminated within the class II cabinet, before autoclaving at 131 degrees C for 30 min and subsequent discarding as clinical waste.

Plastic pipettes and tips are decontaminated in a rigid but autoclavable container before disposal as per other solid waste.

Sharps: Sharps put into a sharps bin for off-site incineration.

<table>
<thead>
<tr>
<th>Name of disinfectant</th>
<th>concentration</th>
<th>Contact time (approx)</th>
<th>Level of kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virkon</td>
<td>3%</td>
<td>60 mins</td>
<td>below detectable level</td>
</tr>
<tr>
<td>Virkon</td>
<td>1%</td>
<td>overnight</td>
<td>below detectable level</td>
</tr>
<tr>
<td>Presept</td>
<td>1%</td>
<td>overnight</td>
<td>below detectable level</td>
</tr>
</tbody>
</table>

The expected level of kill should be included. This need not be precise eg "approximately 4 logs reduction in viability" or "below detectable level" is acceptable.

#Contact time should be at least one hour.

Autoclave: Length of sterilisation cycle (min): 30

Temperature of sterilisation cycle: 131 C

This is checked using autoclave tape and by inspection of the printout of each autoclave cycle.

Note: If no validation for a disinfectant exists (ie the degree of kill for a particular organism is unknown) then laboratory testing could be required to establish its efficacy.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered
- Containment level is sufficient
- that sharps injury may be considered serious when handling immortalised cells
- the consideration of using temperature sensitive antigens instead of wild type
- the handling of one oncogene containing cell line at one time
- the production of transient cultures rather than stable cell lines for vector production
- the storage of vectors and cells
- risk to health of accidental insertion of the gene sequence into humans
- checks in place to ensure that staff are immunocompetent
- the laboratory facilities and procedures

FOR FULL DETAILS - PLEASE SEE THE WRITTEN NOTIFICATION
Purposes of the contained use

Recombinant vaccinia viruses (NYCBH strain) expressing two HSV-2 genes (UL-7 and UL-46) will be used to infect BSC-40 cells and the cell lysates used in immunological assays. These HSV genes encode viral tegument proteins which are present in the virus particle as well as infected cells and have recently been shown to be targets of the cellular immune response. The viruses have already been prepared by University of Washington, USA. The ability of the vector to replicate is not affected by the insertion of either of the two HSV-2 genes.

Recipient or parental organism

The two HSV-2 genes encoded by the recombinant vaccinia viruses are highly unlikely to alter the tropism of vaccinia or to enhance (or diminish) its inherent pathogenic
potential. The tropism of vaccinia virus will not be affected by the expression of these two HSV proteins, which although present in HSV-2 virus particles, are internal in that structure and not known to play a role in the attachment and receptor-mediated entry of HSV-2 into target cells.

**Host/vector system**

Homologous recombination is a recognised natural event in the replication of poxviruses and although we may grow wild type vaccinia virus the possibility of erroneously co-infecting cells resulting in the acquisition of the HSV-2 genes is extremely low. The two HSV-2 genes would not be predicted to alter the tropism and replication properties of the vaccinia vector alone. Vaccinia virus is not present in the human population and therefore in the case of an individual becoming infected with recombinant vaccinia, there would be no opportunity for wild-type virus to acquire the inserted genetic sequences. Vaccinia virus can infect a number of animal species and any risk of release of virus into the environment by an animal source will be avoided by the physical containment of the work within a restricted access (keycode entry) category 2 containment laboratory. Acquisition of the HSV-2 sequences could not occur in a related micro-organism in the environment as wild-type vaccinia virus is not present in any animal species in the UK. No genetic modification work will be carried out, as the recombinant construct will only be propagated in BSC-40 cells.

**Origin & function**

Recombinant vaccinia expressing two HSV proteins obtained from our collaborator will be grown in BSC-40 cells and the lysates will be used for immunological assays.

**Evaluation of foreseeable effects**

Vaccinia virus, classified as a hazard group II pathogen, may cause severe disease in people with active skin disorders such as eczema or psoriasis or in immunocomprised individuals such as those with HIV. The addition of an HSV regiment protein gene in the recombinant vaccinia would not be predicted to enhance or diminish its ability to cause disease in comparison to the vaccinia virus vector alone (NYCBH strain). All the work will be carried out at containment level 2, in a single laboratory with restricted access (keycode entry, laboratory personnel only, the Department of Virology has swipe card entry system) and appropriate signs will be posted to indicate vaccinia work is being conducted, outlining persons who are potentially at risk. All members of the department will be informed. Vaccinia virus is not present in the human population and therefore in the case of an individual becoming infected with recombinant vaccinia, there would be no opportunity for wild-type virus to acquire the inserted genetic sequences.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

General disinfectant will be 70% ethanol (contact time, 5 min). Bench disinfectant to be used will be Chloros at 1000 parts per million. All liquid and solid/plasticware waste will be double-bagged in autoclave bags and transported in metal tins to the autoclave facility housed within the Department of Virology. For plasticware, autoclave conditions are 10 min sterilisation cycle at 134 degrees C and for fluids, 25 min at 123 degrees C. Each run on the autoclave is validated (pass/fail) and written records kept and the autoclave is services every 6 months by specialist engineers. Spillage procedures are displayed in the laboratories where the work will be carried out (titled ‘Disinfection procedures for GMO spillages’). No sharps will be used, and aerosols will be kept to a minimum as all handling of the viral cultures will be in a class II microbiology safety (serviced every 6 months).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

See attached.

Project Containment

Laboratory Activities Glass Houses Growth Rooms

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Animal Units Large Scale Activities Human Clinical Applications

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Project Ref 99/07.1

Date Ackn’d CU2 Project Title

24/04/2012 Construction of recombinant herpesviruses (herpes simplex virus (HSV) and human cytomegalovirus (HCMV) and their use in studies of herpes virus entry and replication.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 1 < 1 Litre

Non-GMM Consent Granted

Not Applicable

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To gain an understanding of the entry pathways of herpesviruses and the roles played by individual viral proteins in mediating virus entry.
Recipient or parental organism

Recipient/parental viruses:
HSV and HCMV are category 2 human pathogens. They can be propagated and handed under category 2 laboratory conditions. They are routinely cultured in this laboratory under category 2 conditions.
Both viruses are common in the general population and are clinically benign in immunocompetent individuals.
Close contact is generally required for transmission of HSV and HCMV.
Laboratory associated infection with HSV might occur if virus is introduced to the eyes (e.g. by splashing). This risk is minimised by wearing eye protection and working in a class 2 hood. Laboratory associated infection with HCMV is considered to be highly unlikely.
Environmentally, these viruses pose a very low risk. Herpesviruses are enveloped and consequently are labile. Infectivity is completely destroyed by dessication, which is likely to occur in the event of a small laboratory spill. Neither virus has any natural host other than humans, so persistence in the environment, such as in rodents or insects, is considered to be very unlikely.
All the viruses covered by this application retain the necessary genes to confer sensitivity to the licensed anti-viral drugs.

Host/vector system

Characteristics of GMO:
The recombinant viruses fall into two groups;
i) Those in which a gene (or genes) has been disrupted by addition of a stop codon or deleted and replaced with beta-galactosidase, green fluorescent protein (GFP), or derivative, or other marker gene; these viruses are used to probe the function of the deleted gene product.
ii) Those in which GFP (or derivatives) is fused to one or more viral proteins; Incorporation of GFP into these viruses means that they are fluorescent. These viruses are used to track the pathways used for entry into the cell.

Forseeable effects

A: Health
i) Deletion mutants: There is no reason to suppose that the pathogenicity of these viruses is, or will be, increased. Some of the viruses are attenuated in animal models, others are equivalent to wild type. None is known to have enhanced pathogenicity. ii) Fluorescent viruses; In all cases, these viruses are indistinguishable from wild type (other than the presence of the transgenes), indeed they would be of no use experimentally if they did not mimic wild type virus.
Summary; The viruses are considered to be either equivalent to wild type virus or less pathogenic than wild type virus. HSV and HCMV are category II pathogens, thus handling of the recombinant viruses under category II laboratory conditions is considered to be sufficient for containing the risk to health.
In general, close contact is required to infect an individual with HSV or HCMV. The risk of this is minimised by handling the viruses under category II laboratory conditions. Wild type HSV and HCMV are handled routinely and safely in the laboratory in this way.

B: Environment
Herpesviruses are encased in a lipid envelope and thus are labile. They are rapidly inactivated by dehydration or mild detergent. They are very unlikely to persist in the environment.
Neither HSV nor HCMV is known to have any natural non-human hosts, (although HSV can infect laboratory rodents). This lack of natural hosts combined with the environmental instability of the virus particle makes inadvertent transmission to rodents or other wild animals very unlikely.

Origin & function

Origin of viral genomic DNA:
Mutant viruses are constructed by homologous recombination. For HCMV the starting material is a bacterial artificial chromosome (BAC) clone of the HCMV genome. For HSV, purified wild type viral DNA is used. In each case, virus is reconstituted by transfection into a producer cell. All procedures in the protocol prior to the transfection are carried out in E.coli and as such present no infection risk.
Herpesviral genomic DNA can initiate virus production when introduced into a target cell. However, this is very unlikely to happen to a laboratory worker during laboratory manipulation.
Sharps are not used and good laboratory practice means the risk is negligible.
The viruses fall into two categories:
i) Deletion mutants: in which a viral gene is deleted and replaced by marker gene expression cassette encoding beta-galactosidase, GFP (or derivatives), or another
fluorescent protein. In these viruses the function of the inserted genetic material is a) to facilitate selection of recombinant viruses and b) to use as a marker of infection.

ii) Fluorescent viruses: in which one more viral genes is fused in frame with GFP (or derivatives), or other fluorescent protein-encoding genes. These viruses are intrinsically fluorescent and are in all other respects comparable to wild type.

In these viruses the function of the inserted genetic material is to make the virus particles fluorescent, while retaining other wild type characteristics, so that the viruses can be used in fluorescence-microscopic studies of viral entry pathways.

Origins of the inserted genetic material:

Beta-galactosidase: this gene encodes an enzyme that digests lactose or other related substrates. It is widely used in molecular biology. The gene originates from E.coli.

GFP (and related fluorescent proteins): these genes are obtained from jelly fish (Aequoria victoria). They form distinct globular domains that can be fused to other proteins retaining function and rendering those proteins fluorescent, They are widely used in molecular biology.

Evaluation of foreseeable effects

These are detailed in the above sections.

Summary: HSV and HCMV are category II pathogens and so can be handled safely in the laboratory under category II working practice. Close contact is required for infection to occur. Laboratory associated infection is a small risk and this is reduced by the use of appropriate protective clothing.

None of the recombinant viruses is anticipated to be more pathogenic than the parental virus.

HSV and HCMV are rapidly inactivated by dessication and neither virus has a natural non-human host so the risk to the environment in the event of a spill is judged to be minimal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Herpesviruses are 100% inactivated by autoclaving.

All GMM waste, solid (eg flasks, tubes, tips, plates) and liquid (eg culture medium, used virus aliquots) is autoclaved in the department. (Standard discard cycle is 135 degrees C, 3150 mBar, 10 minutes)

Temperature and pressure parameters of each autoclave cycle are recorded and reviewed. Additionally, Browns tubes and autoclave tape are used to assess each run. The autoclave is maintained to a high standard, sufficient for HPA accreditation, by regular servicing and testing with a 12 point thermocouple. Records are kept in the Department.

All autoclaved waste is placed in yellow biohazard bags and taken for incineration.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
This project (local (GM99) reference: F1C36/07) received approval from a panel of advisers representing the Centre GM Safety Committee at a meeting held on Thursday 8 November 2007. The panel agreed with the classification of the activity and further agreed that the precautionary measures stated in the risk assessment were suitable for these laboratory procedures.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 L3 L4 L2 L3 L4</td>
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**Project Ref** 99/10.1

- **Date Ackn'd**: 24/04/2012
- **CU2 Project Title**: Use of lentiviral vectors as gene delivery systems for immunotherapy
- **Class**: Class 2
- **Culture Vol Class**: 1-50 Litres
- **Consent Granted**: Non-GMM Consent Granted

**Historical Significant Changes**
- **Historical Date of Additional Info**: Withdrawn
- **Significant Change ID**: N
- **Date of Significant Change**: N

**Project Additional Information**

**Purposes of the contained use**

The projects covered under this application aim to exploit lentiviral vectors as gene delivery systems for immunotherapy. To this end we have 2 complementary approaches. The first involves the use of lentiviral vectors to deliver immune-modulating (for example, T cell receptors, co stimulatory molecules or cytokines) into immune cells. Genes encoding immune modulating genes will be cloned into lentiviral vectors, and lentiviral particles will be used to transduce mouse or human immune cells or...
haematopoietic cells for the production of modified immune cells which can be used for immunotherapy of cancer. The second approach involves using lentiviral vectors for vaccination strategies. In this case lentiviral vectors encoding model or tumour-associated antigens are used to immunise mice. It has previously been shown that lentiviral vectors are very potent immunising agents and we will be investigating how the immune responses are activated, and whether they would be appropriate immune responses to control an emerging tumour, for example in an in vivo model of leukaemia.

Recipient or parental organism

GMO is the lentivector: 3rd generation HIV vector with virulence genes deleted, on split plasmids

Lentiviral particles will be used to transduce a range of cells in the laboratory: standard laboratory cell lines (e.g. 293T, HeLa, NIH3T3 etc.). Human and mouse haematopoietic cells (in particular T cells and Dendritic cells as well as other immune cells).

The viral vectors themselves are not pathogenic to humans, all virulence genes have been deleted from the original parent viral sequences. However, since these vectors have the potential to integrate into human DNA there is a low risk that infection could damage the health of the laboratory worker directly handling the viruses. We are planning to use self-inactivating (SIN) vectors to make viral particles that have been shown to significantly reduce the risk of insertional mutagenesis. In addition lentiviral vectors have been demonstrated to insert preferentially into coding regions of DNA, rather than the more risky promoter or control regions. Therefore, when considering all these factors the risk to humans is very low.

Because the lentiviral vectors are replication deficient and undergo a single infection of initial target cells, there is no risk of spread to other recipients. The vectors will only be used as an antigen delivery system to immunise, once the viral vector is injected into mice the immunised animal is not considered genetically modified and the viral vector no longer exists. Thus, there is no risk from this point.

A WPRE element is included in these vectors to aid translation of the inserted gene. This encodes a putatively oncogenic protein (protein X) which has been mutated in all vectors. The X protein could potentially be a risk factor, but the use of vectors with non-functional X proteins negates this risk.

The vector particles are produced with a broad host specificity by using the Vesicular Stomatitis virus (VSV) G protein or other coat proteins. This may increase the susceptibility of different cells to the virus, but will not increase the risk of insertional mutagenesis.

Host/vector system

Vectors are standard microbiology plasmids. All cloning is performed using commercial disabled bacterial strains that are not harmful to health or the environment. The promoters used for gene expression in the lentivector are not generally expressed in bacteria.

pUC derivatives or standard molecular biology cloning vectors such as the following, or related vectors: pCMVR8.91 (packaging plasmid), pMD-G (VSV-G envelope plasmid) & pHR'SIN (vector plasmid Dermaison et al (2002) Human Gene Therapy 13:630-640.).

Some vector plasmids obtained from Prof. M C's laboratory at UCL may contain sequences to enhance expression in transduced cells (vFLIP latent gene expressed by KSHV) such as dendritic cells or T cells. Human and mouse genes of the NF-kB pathway. MAPK activators: MKK6EE kinase (p38 activator), MEK1 N3 ED kinase (ERK activator) and MKK7-JNK1 (JNK1 activator).

Origin & function

The genetic material will be of bacterial origin for cloning as follows:

HB101, DH5alpha & other common disabled E. coli K-12 or B derivatives. Only disabled bacterial strains will be used.

The inserted sequences may be amplified originally from mouse or human genomic DNA or cDNA before cloning into bacteria.

For work investigating lentivectors as immunisation agents, the majority of the inserted nucleic acid encodes model antigens. These are inert gene products that do not have a biological function in the transduced cells, and will not effect growth and survival of cells.
receiving these vectors. For the delivery of immunomodulating genes there are genes encoding immune modulating proteins which may alter the immune function of the cells they are expressed in, but it is highly unlikely that this would lead to transformation of the cells in question.

Genes such as the vFLIP latent gene from KSHV or MAPK activators may occasionally be incorporated into lentiviral vectors to enhance immune responses to the inserted gene. It is not expected that these genes will alter the biological activity of inserts encoded in the same vector. However these sequences do have the potential to effect expression of an oncogene or tumour progression factor depending on the site of integration of the vector.

A WPRE element is also included in the vector plasmid to aid translation of the inserted gene or sequence. This encodes a putatively oncogenic protein (protein X) which has been mutated in all vectors and is not functional.

The model antigens, genes encoding immune modulating genes and mutated WPRE element are unlikely to cause harm if accidentally transferred into humans. We do identify an increased risk when using KSHV or MAPK activating genes. It is possible that in the unlikely event of a breach of containment, accidental injection into workers may result in the undesirable insertion of these genes next to an oncogene. However, this risk on insertion at a site such as this is extremely low and it is unlikely that any one gene has the capacity to act as an oncogene on its own.

**Evaluation of foreseeable effects**

For generation of lentiviral vectors only disabled bacterial strains will be used and we do not foresee any effects.

The most hazardous GMMs are the HIV-based vectors. The most hazardous step and the only risk that we identify at the moment is injection of the transducing particles into mice because this involves the use of needles and the risk of personal injury.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All GMOs in this assessment are micro-organisms.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

For immunisation of mice with lentiviral particles it is requested that the animals are injected in a level 2 designated room, but outside a laminar flow hood. Lentiviral particles are extremely sensitive to desiccation and no risk associated with infection due to air-borne particles has been identified. Injection of mice while in a laminar flow hood restricts the movement and in fact increases the risk of needle stick injuries due to the difficulty of manouvering in the confined space. The site of injection is sprayed with ethanol to inactivate any lentiviral particles leaking from the injection site (though this volume is typically less than 10-20 μl).

Once the lentiviral particles are injected into mice, the inserts will be permanently integrated into the genomes of host cells, and no new viral particles will be produced. Therefore, subsequent to immunisation the mice are not considered genetically modified organisms and no risk is associated with their handling and housing. All waste from the housing of mice is disposed of by incineration and this therefore remains the appropriate method of disposal for waste from animals immunised with lentiviral vectors. Likewise cadavers will also be disposed of by incineration according to standard UCL procedure.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

GMM/1: line 1.29 or GMM/2: lines 2.13 (as applicable)

For routine cleaning, all surfaces will be wiped down with 70% alcohol after use which is known to be effective against enveloped viruses. Liquid GMM waste will first be disinfected using Presept overnight (effervescent disinfection tablet containing Troclosene Sodium) to give a final concentration of 2500ppm available chlorine (1x2.5g tablet/500ml waste) before being disposed of down the sink. This is a standard procedure and viability is below detectable levels. Solid GMM waste will be placed in autoclave bag, and autoclaved via the central collection service for GM waste.

The autoclave sterilizing cycle is carried out at 135°C for 5 minutes. All sharp objects, such as pipette tips are placed in a rigid plastic container prior to disposal in autoclave bags. Needles (without dissociation from the attached syringes) are placed in sharps bins which are sealed before being added to the autoclave bag.
It is not possible to estimate a degree of kill for a non-replicative virus. However, autoclaving and 2500ppm available chlorine is sufficient for a 100% inactivation of enveloped viruses.

For immunisation of mice all needles will be disposed of in dedicated sharps bins (without dissociation from the attached syringe). The sharps bins are then placed in autoclave bags and autoclaved via the central collection service for GM waste.

Note that in the event of spills, 70% ethanol will be used for surface disinfection. This agent is effective and widely used for inactivating enveloped viruses. Absorbent tissues will be used to mop the affected area and will then be disposed of by autoclaving as described above.

Temperature and pressure parameters for each autoclave run are recorded on a printout and reviewed. The autoclave is checked weekly and a 12 point thermocouple test is performed annually. The autoclave validation records are audited annually and a certificate is issued.

All solid waste (including sharps) are placed in autoclave bags and collected from the department to be taken to the central autoclave. The bags are transported to the autoclave in a rigid, lidded container.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
1.10 Immuno-modulating genes such as T cell receptors, co-stimulatory molecules, cytokines/chemokines and the receptors for cytokines/chemokines will be studied for their function in the engineered cells. MicroRNA or siRNA sequences to control expression of inserted sequences in specific cell types. For example, the microRNA mir142 restricts expression of upstream genes to non-haematopoietic cells only (Brown et al (2006) Nat Med 12:585). Alternative micro RNA sequences may be used to allow expression only in immune cells, especially dendritic cells. SiRNA would be used to specifically down-modulate protein levels of a gene of interest in transduced cells, for example, a co-stimulatory molecule or cytokine or the endogenous T cell receptor genes.

1.12 Some vector plasmids obtained from Prof. M Cs’ laboratory may contain sequences to enhance expression in transduced cells such as dendritic cells or T cells.

1.13 Cells: standard laboratory cell lines (e.g. 293T, HeLa, NIH3T3 etc.). Human and mouse haematopoietic cells (in particular T cells and Dendritic cells as well as other immune cells).

1.25 They indicate the autoclave waste will be "autoclaved later" is there any issue with waste hanging around to be autoclaved and how long "later" actually would be?. All GM waste is placed in Autoclave bags which are changed on a regular basis. All autoclave bags are kept in rigid containers, All sharps are placed in lidded containers. They are then collected by the porter, who autoclaves them on the service floor. This is what was meant by autoclaved later.

GMM2

2.16 Question: Does your laboratory operating procedure permit the use of sharps (including glass pipettes)?
Answer: No. But they intend to use sharps to inject the lentiviral vectors into mice or does the question relate only to the portion of the work which will be done in the lab rather than in CBU.
Yes, we meant that no sharps are used in the laboratory setting, but of course they are used in the animal house.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
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#### Name

**UNIVERSITY OF BRISTOL**

#### Name 2

##### Campus Estate or Research Centre

**BEACON HOUSE**

#### Road Name

**QUEENS ROAD**

#### Town

**BRISTOL**

#### Country

**BS8 1QU**

#### Tel Number

0117 928 9000

#### Fax Number

0117 925 1424

#### HSE Division

**WALES AND SOUTH WEST**

#### Date at Which Additional Info Submitted

17/09/2002  08/05/2003  07/08/2003
### Accident ID
26.00

### Date Accident Notified
29/05/2013

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity.
Please enter comments of the GM safety committee on the risk assessment

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**Project Ref** 11/97.2

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Withdrawn: N

Tick if notifying a connected programme of work: N

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**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 18/01.10

Date Ackn'd CU2 Project Title  Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
**Purposes of the contained use**

a) clone genes involved in the development of human malignancies.
b) study the normal biological functions of these genes and how these are altered in cancer.

**Recipient or parental organism**

- E. coli K12 and derivatives.
- Saccharomyces cervisiae.
- Cultured mamalian cells.
- Cultured insect cells.

**Host/vector system**

- E. coli: Plasmids, Cosmids, Bacterial artificial chromosomes, (upside down y) vectors, Expression vectors.
- Saccharomyces cervisiae: Yeast artificial chromosomes, Expression vectors.
- Mammalian cells: Mammalian expression vectors.
- Insect cells: Baculovirus expression vectors.

**Origin & function**

Known human oncogenes and tumour suppressor genes, and uncharacterised genes involved in human cancer.

**Evaluation of foreseeable effects**

Organisms will be modified E. coli, S. cervisiae, and mamalian and insect cells expressing potentially oncogenic sequences.

Possible hazards to human health arise from E. coli expressing high levels of potentially oncogenic proteins, and from the use of poorly characterised mamalian cells.

The only viral vectors used (Baculovirus) are disabled vectors that produce unstable, poorly infectious virus that should pose no environmental risk to insects with the
standard containment procedures used (ACBM Annex III).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Some procedures within the overall project only require level 1 containment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste:
Plastics and paper: autoclaved, then into normal refuse.
Animal waste: incinerated or autoclaved as above.
Glass: autoclaved or soaked in disinfectant, then into normal refuse.

Liquid waste:
Autoclaved, the into drains.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Discussed and approved by local Biological and Genetic Modification Safety Committee on 3 July 2000.

Project Containment

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</table>
**Project Additional Information**

**Purposes of the contained use**
Experiments will be carried out in order to evaluate the avidity of the CD8+ CTL response by InsHA mice to pancreatic Islet beta cell-expressed HA. InsHA mice will be immunized with recombinant Vaccinia-Virus expressing either the whole HA protein, or the dominant MHC Class 1 K HA epitope in order to generate K HA-specific CTL. Several murine cell types will be inoculated with the same recombinant vaccinia virus for use as target cells or stimulator cells for the HA-specific CTL.

**Recipient or parental organism**
Both Vaccinia virus Vacc-HA and Vacc-KdHA were kind gifts from Laboratory of Jack Bennink and Johnathan Yewdell at the NIH, Bethesda, USA. Briefly the recombinants were prepared using an isolate of Vaccinia Virus WR. Vaccinia virus HA was produced following in vivo recombination using S variant Vaccinia Virus VTK 79 as rescuing virus and a donor plasmid pDP122B (Panicali et al., 1983 PNAS 80 5364-68).

Vaccinia virus K HA contains the minigene K HA(518-526)-VAC construct, composed of a signal sequence from the adenovirus E3/19K glycoprotein expressing the 9-amino-acid HA natural MHC class 1 determinant 1YSTVASSL.

**Host/vector system**
The parental Vaccinia virus S-variant VTK 79 contains a deletions in the open reading frames of the Thymidine Kinase (TK), gene involved in nucleotide metabolism.

**Origin & function**
The influenza virus HA whole protein and the K HA-epitope in recombinant Vaccinia Virus are for use in studying K HA-specific CTL responses for use in initiating auto-immune responses to tissues expressing HA as well as in vaccination strategies that generate immunity to HA.
Evaluation of foreseeable effects

As a result of the TK deletions both recombinants are highly attenuated and are unable to replicate effectively in TK negative cell types. Replication in TK positive cell types results in the production of non-recombinant wild type Vaccinia Virus having lost the inserted gene.

With regard to the inserted genes, both are derived from mouse-adapted influenza Virus A/PR/8 H1N1. Any accidental release of either the HA gene or the K HA gene would not pose any threat to the environment, or be of hazard to humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste, both from the level 2 containment laboratories and rooms within the vivarium, will be autoclaved. Transfer vessels ie pipettes tubes, will be soaked in 10% bleach before autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

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The Biological and Genetic Modification Committee Meeting of 11/07/01 discussed the fact that the thymidine negative mutant of the WR Vaccinia virus was no longer considered to be apathogenic to humans following the hospitalisation of a laboratory worker. Part 2B Annex III para 71 of the Compendium states “a TK minus phenotype is believed to reduce the virulence of the virus in mice but it is debatable whether this should be taken to imply lower virulence in man or whether this should allow a down-grading of categorisation. *Therefore all work with TK minus Vaccinia virus to be carried out at level 2 containment.*
Further understanding of processing in sensory neurons

The parental strain of the virus to be used is an attenuated form of herpes simplex virus-1 (HSV-1). The wild type human herpes simplex (HSV) type 1 virus is a common human pathogen that causes mucocutaneous lesions usually limited to the oropharyngeal area. Following primary infection, the virus remains dormant in the sensory neurons and may reactivate when virus seeds to epithelium and replicates to infect many epithelial cells. Wild-type HSV1 is listed in ACPD Hazard Group 2. There are attenuated strains of HSV1 that we propose to use, the KOS strain, the ICP4-negative and the ICP34.5-negative strains have deletions of genes essential for viral replication in neurones. These strains of HSV1 are extremely labile. Virus is easily inactivated by drying, lipid solvents such as 50% ethanol, mild detergents and 5% chlorine-based bleach, or by autoclaving.

The cyclooxygenase isoforms are expressed endogenously in the nervous system. COX-2 is dramatically upregulated in many different tumours, and treatment of tumours with selective COX-2 inhibitors can reduce metastasis formation, and cause tumour regression. Although it is not yet clear whether COX-2 upregulation is the
causal event in tumourigenesis, it may play a role, as COX-2 inhibition can result in apoptosis. There is now some evidence that COX-1 may have an oncogenic role in gastrointestinal tumours, and it has also been implicated in some of the pathological features of Alzheimer's disease. There is some evidence that voltage gates potassium channels may be involved in mitogenesis, and that increased voltage gated sodium channel activity may decrease cell proliferation. In addition, voltage gated sodium channels are extremely important in the generation and conduction of action potentials in nerve fibres. As the majority of the studies planned are to introduce antisense constructs against the voltage-gated sodium channels, this may result in altered neuronal properties by inhibition of action potential generation.

As the cDNAs to be expressed may have potential harmful effects on other organisms, and the cloning of these cDNAs is designed to lead to expression in mammalian systems (albeit unlikely that accidental infection could occur in humans), the viral host/vector system requires containment at ACGM level 2. (see also attached risk assessment for further detail).

Origin & function

Cyclooxygenase enzymes and neuropeptides:
Origin: All cDNAs are full open reading frames cloned from rat.
Intended function:
1. to express antisense cDNA (either full length or partial cDNAs) to inhibit expression of a specific gene product in sensory neurons.
2. to express sense cDNA (full coding region) in either mice null for the gene product of interest, or in normal mice or rats.
Sodium and potassium ion channel subunits.
Origin: Rat cDNAs encoding either full open reading frame, or partial fragment.
1. to express antisense cDNA (either full length or partial cDNAs) to inhibit expression of a specific ion channel subunit in sensory neurons.

Evaluation of foreseeable effects

Foreseeable effects relevant to human health and safety.
Direct infection in humans on contact with virus is unlikely, as these strains of HSV-1 cannot infect intact skin. The only site where they may be a risk of infection is the cornea. HSV strains are not infective through an aerosol route but will be handled in a Class II safety cabinet nonetheless. In addition eye protection is mandatory when using these viruses in case of accidental splashes. Barrier methods such as use of appropriate gloves and laboratory clothing will be routine, and, in addition to the above methods of disinfection should be adequate to prevent accidental infection of humans. Recombination of either parental strain with wild type virus after infection in a human is extremely unlikely, as this would require direct contact between an active infection in a human and the laboratory strain. Lab members with such active herpes infections (for example cold sores) would be excluded from working with the recombinant viruses.

TK-negative HSV strains such as recombinant KOS are resistant to acyclovir, which is a potential concern with respect to treatment of an accidental infection. Tis may preclude the use of this vector for gene therapy applications, but with suitable containment in the laboratory this should not present a significant problem for the proposed use. In the unlikely event that an accidental infection does occur, these viruses are still susceptible to "Foscarnet". ICP34.5- and ribonucleotide reductase-negative HSV-1 strains are not resistant to acyclovir.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste likely to be generated is of the following forms and types:
1. Liquid waste from culture sin generation of viral vectors and recombinant virus.
   Liquid waste from all stages of the project will be disinfected immediately by direct drainage into 5% chlorine bleach, followed by further treatment with 1% chlorine bleach prior to disposal.
2. Disposable dry waste eg. contaminated plastics and sharps.
All disposable plastics contaminated with GMMs in this study will be chemically disinfected and autoclaved prior to disposal. Use of sharps with recombinant virus is avoided where possible. Where use of sharps is unavoidable, such as an injection of animals, sharps will be placed in a non-piercable container (Cin-Bin), sealed and incinerated.

3. Non-disposable dry waste eg contaminated glassware.

Laboratory glassware will be chemically disinfected with 5% then 1% chlorine based bleach, will be washed in mild detergent and then autoclaved prior to re-use. All of these procedures will inactivate all wild type and recombinant HSV-1 present, as the HSV1 strains are extremely labile.

Virus is easily inactivated by desiccation, lipid solvents such as 50% ethanol, mild detergents and 5% chlorine-based bleach, or by autoclaving.

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**Project Ref** 18/01.14

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<td>12/12/2001</td>
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At the meeting on 11/07/01 the committee felt that the strains of HSV were not sufficiently attenuated for this project to be level 1 containment, hence HSE notification.
In the proposed clinical trial programme, angiogenic gene therapy product, consisting of a recombinant adenovirus (human serotype 5) containing the human gene for the fibroblast growth factor 4, and referred to as Ad5FGF-4, will be investigated as a treatment for patients with chronic stable angina due to coronary artery disease (CAD). Generation of new blood supply in the diseased heart by intracoronary administration of angiogenic gene therapy product represents a potential new therapeutic approach to relieve this condition.

Recipient or parental organism

The Ad5FGF-4 gene therapy product consists of a recombinant adenovirus vector (human serotype 5, Ad5) with a deletion in the E1 region; from map unit 1.3 to 8.7 of wild-type virus (entire E1A and most of E1B are eliminated). The FGF-4 transgene is inserted, driven by CMV promoter.

Origin & function

The FGF-4 gene was originally isolated from a cDNA library which was constructed from mRNA of Kaposi's sarcoma DNA transformed NIH3T3 cells. The intended function is angiogenesis, the formation of new blood vessels.

Evaluation of foreseeable effects

The probability of adverse consequences resulting from deliberate or accidental release of the gene therapy product Ad5FGF-4 are minimal to nonexistent. Hazards resulting from environmental release (viral shedding from treated persons, inadvertent contamination of the product prior to administration) are negligible or nonexistent for the following reason; infection requires large numbers of infectious vectors, and transfection (expression of the inserted gene) requires a multitude of infectious particles.

Hazards associated with the adenoviral vector can be described as having low potential of adverse environmental consequences in humans or animals. The theoretical consequences to humans of several of the hazards associated with ectopic transgene expression, if they actually occurred, could be considered moderately severe (e.g. promotion of existent malignancy, unknown risk to foetus). However, since any unintended or accidental exposure would most likely be a fraction of the total dose being administered to patients for therapeutic purposes, the relative risk of the occurrence of these types of adverse effects should be very low.

The possible risks to the environment could be assessed as low to effectively zero. This is based on the low probability of infectious adenoviral particles escaping into the environment either therough viral shedding by patients that have received the product, or by incidental exposure during administration procedures. Even if viruses were shed or product spillage occurred the number of infectious viral particles would be too small to result in infection of exposed tissues. The risk to the non-human environment is extremely low to effectively zero because of the species specificity of adenovirus 5, which by natural exposure is only known to infect humans.
The above indicates that the product could be classified as Class 1. However, as a precautionary measure, due to the limited experience available, the product is currently being classified as Class 2. Reclassification into Class 1 may be considered if based on increased data and experience.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Inactivation by autoclaving and effectively 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Discussed at meetings of 11.07.01 and 14.11.01, Committee agreed that project should be notified to HSE due to biological activity of insert. Risk assessment requested 11.07.01.

Project Containment

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Animal Units

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Project Ref 18/01.3

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## Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Project Containment

Laboratory Activities  Glass Houses  Growth Rooms
L2  L3  L4  L2  L3  L4  L2  L3  L4
Animal Units  Large Scale Activities  Human Clinical Applications
L2  L3  L4  L2  L3  L4  L2  L3  L4

Project Ref  18/01.4

Date Ackn'd  CU2 Project Title
23/01/2001  PRODUCTION AND HANDLING OF GENETICALLY MODIFIED FUNGAL PATHOGENS OF PLANTS, INVERTEBRATES AND OTHER FUNGI

Date Project Ceased

Class  CultureVolClass2  CultureVolumeClass3-4
Class 2  

Non-GMM
Consent Granted
not applicable

Project notified under transitional arrangements  Y

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
**Project Ref**  18/01.6

**CU2 Project Title**
VIRAL MEDIATED EXPRESSION OF SIGNALLING PROTEINS AND METABOLIC ENZYMES

**Class**  Class 2

**Consent Granted**
not applicable

**Project notified under transitional arrangements**  Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 18/01.7

Date Ackn'd 23/01/2001
CU2 Project Title ADENOVIRAL-MEDIATED EXPRESSION OF SIGNALLING PROTEINS, STRESS
Class 2
CultureVolClass2 CultureVolumeClass3-4

02/03/2022
Page 1005 of 15326
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 18/01.8

Date Ackn’ed: 23/01/2001

CU2 Project Title: RECOMBINANT ADENO-ASSOCIATED VIRAL AND EQUINE INFECTIOUS ANAEMIA LENTIVIRAL VECTORS (EIAV) GENE THERAPY VECTORS AND AS TOOLS TO STUDY SOME NEURODEGENERATIVE PROCESSES.

Class: Not applicable

Consent Granted: Project notified under transitional arrangements

Historical Significant Changes

Significant Change ID

Date of Significant Change

02/03/2022
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022
Project Additional Information

Purposes of the contained use

Storage, transformation and culture of recombinant trypanosomes with bacterial genes for drug resistance, heterologous reporter genes and trypanosome genes in order to study trypanosome genetics and gene expression for research purposes only.

Recipient or parental organism

African trypanosomes are protozoan parasites of mammals, with an invertebrate vector, the tsetse fly. Neither these trypanosomes nor the insect vector is found naturally occurring in the UK, and the requirement of the insect vector for tropical climatic conditions mean that the natural transmission cycle could not establish itself. The trypanosomes are obligate parasites and required specialized culture conditions. They are not transmissible by aerosol. Genetic modification is not envisaged to render the trypanosomes more hazardous than wildtype. The antibiotic resistance genes used are unrelated to any drugs currently used for treatment of either humans or livestock.

Wildtype trypanosome species are subject to ACPD category 2 and 3 containment and also to handling conditions attached to our MAFF Special Animal Pathogens Order licence. We have a set of Standard Operating Procedures covering all experimental procedures used.
Level 2: Trypanosoma brucei brucei, T. b. gambiense, T. congolense are handled under level 2 containment.
Level 3: Trypanosoma brucei rhodesiense is a derogated ACDP 3 pathogen (exemption certificate No: COSHH/HD/1998/1) and is also handled under derogated level 3 containment.

Host/vector system

Host: Trypanosoma brucei ssp., Trypanosoma congolense

Vector: Plasmid (pGEM) expression vectors for trypanosomes with trypanosome promoter and processing sequences.

Origin & function

1. Bacterial genes for drug resistance, eg hyg, neo, ble.
2. Reporter genes, eg Green fluorescent protein from Aequoria victoria.
3. Trypanosome genes for functional analysis, eg surface coat protein gens, serum resistance antigen genes.

Evaluation of foreseeable effects

1. Trypanosomes are tropical pathogens of humans and livestock. Genetically modified organisms are not envisaged to be more hazardous than unmodified organisms and will be handled under the same stringent safety conditions appropriate for ACDP 2 and derogated ACDP 3 pathogens and MAFF Specified Animal Pathogens Order. These stringent containment conditions ensure negligible environmental risk.
2. The bacterial drug resistance genes confer resistance to antibiotics, which are not related to the drugs currently used for treatment of trypanosomiasis.
3. The heterologous genes are introduced by homologous recombination into the trypanosome genome. The risk of transfer to other organisms is therefore negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Trypanosoma brucei rhodesiense is classified as a derogated ACDP 3 pathogen (Certificate of exemption No: COSHH/HD/1998/1). It is not transmissible by aerosol. We therefore request permission to handle it under derogated level 3 containment, ie laboratory does not need to be sealable for fumigation and does not need negative air pressure; extract and input air from the laboratory do not need to be HEPA filtered; aerosol dissemination needs to be minimized, but not prevented.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The GMO's are simple eukaryote cells without any protective cell wall; therefore they are easily destroyed by hypotonic lysis, heat, dessication or freezing. We already have stringent waste disposal measures in place, since all waste is treated and disposed of according to ACDP 2 guidelines and Standard Operating Procedures attached to our licence issued under the MAFF Special Animals Pathogens Order as follows:

1. Plastic and glassware should be sterilised by complete immersion in 1% Chloros for at least 10 minutes. The liquid waste is then safe to dispose of via the drains and the plastic/glassware autoclaved before disposal.
2. Liquid waste, including unwanted cultures, infected blood or stabilates, is rendered safe by addition of Chloros to 1%, holding for 10 minutes minimum and autoclaving before disposal.
3. Litter from mouse cages is sealed in plastic bags and incinerated. Dead mice are sealed in double plastic bags and frozen before subsequent incineration.
4. Insect (Tsetse fly) dissection waste should be sterilised by complete immersion in 1% Chloros for at least 10 minutes and autoclaved before disposal.
Project Containment

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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 18/02.2

<table>
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<td>THE ROLE OF SURFACE STRUCTURE AND GLOBAL REGULATORY GENES IN CAMPYLOBACTER INFECTIONS OF CHICKENS, PIGS, SHEEP AND MICE AND THEIR INFLUENCE ON ENVIRONMENTAL STRESS SURVIVAL</td>
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<tr>
<td>not applicable</td>
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<tr>
<td>Project notified under transitional arrangements</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
# Project Additional Information

## Purposes of the contained use

The GMMs that will be used in these studies are all knock-out mutants created by insertional mutagenesis. These GMM strains will be compared to the parental strains to ascertain the roles of the surface proteins and regulatory genes. Studies will be carried out during infection of animals to determine the pathogenicity of the GMM relative to the wild type parental strains. Further work will introduce environmental stress factors, such as dessication, heat, low pH and biocides, to monitor the mutated organism's ability to survive these pressures. These studies should allow more effective means of eliminating Campylobacter spp. from the food chain and provide valuable information about the pathogenesis of Campylobacter spp.

## Recipient or parental organism

- **Campylobacter fetus**
- **Campylobacter jejuni**

## Host/vector system

### Deletion mutagenesis in:

**C.fetus:**

Previous generated mutants (Dworkin and Blaser 1997) will be used in addition to novel mutants which will be generated using the same methods. The vector used in pLL570 (Labigne et al 1991), a suicide shuttle vector which only replicates in E. coli strains harbouring the helper plasmid pK212.1 (Figurski et al. 1976). Manipulation of pLL570 is followed by transformation into E. coli:HB 101 harbouring pK212.1. Conjugation facilitates transfer of the plasmid into C.fetus. Within C.fetus the plasmid is unable to replicate and recombination with the host element is only possible with DNA that is homologous with both plasmid and host. Using this method there is a negligible chance of transfer of the DNA to any organisms other than the vector hosts. Transformed hosts have genes insertionally mutated, with antibiotic resistant gene cassettes, by a double cross over recombination event.

**C.jejuni:**

The parent strains are modified by insertional mutagenesis into genes encoding surface structures or global regulators (Linton et al 2000a, 2000b). The inserted gene encodes resistance to an antibiotic eg kanamycin.


## Origin & function

All genetic material originates from Campylobacter sp. The kanamycin resistance cassettes are from C.jejuni, chloramphenicol resistance from C.coli and tetracycline resistance from C.jejuni. Plasmids are derived from E. coli (pLL570, pK212.1) and C.fetus (pLL570) strains.

02/03/2022
Evaluation of foreseeable effects

The GMOs in this study are all knockout mutants. Therefore any manipulated organism is likely to be less viable than the well characterised parental strains. It is very unlikely that these deletions will cause a more virulent organism. The resistance cassettes are introduced into the host cells but only at the expense of the important pathogenic factors under study.

Any release into the environment is unlikely to cause damaging sequelae. The GMMs are all expected to be less viable and highly unlikely to be more dangerous than the parental strains. Appropriate containment level II procedures should ensure that environmental release is highly unlikely.

The organisms under study are responsible for a range of clinical diseases in humans, the majority of being cases of bacterial enteritis. As the GMMs are expected to be no more virulent than parent strains this would not pose a major hazard and the risks of infection would be low due to containment level II controls.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Strict adherence to containment level II control measures including disinfection of all laboratory surfaces after use with validated disinfectant. All bacterial cultures and waste containing GMO's will be autoclaved including agar plates, sharps and glass ware. All animal waste, bedding etc. incinerated on site. Formaldehyde fumigation of all animal rooms after use.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

Discussed at meeting of 20 February 2002 - requires HSE notification.

Project Containment

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</table>

Animal Units  Large Scale Activities  Human Clinical Applications
### Project Ref
18/02.3

**Date Ackn’d**
02/04/2002

**CU2 Project Title**
INVESTIGATION OF THE ROLE OF SURFACE STRUCTURES AND GLOBAL REGULATORS (INC RPOs) FOR SURVIVAL AND VIRULENCE OF SALMONELLA ENTERICA SEROVARS EXCEPT THOSE LISTED IN ACDP HAZARD GROUP 3 AND ......

**Class**
Class 2

**CultureVolClass2**
< 1 litre

**CultureVolumeClass3-4**

**Non-GMM**

**Consent Granted**
not applicable

**Tick if notifying a connected programme of work**
N

**Project notified under transitional arrangements**
N

**Withdrawn**
N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**
It is important to determine what genetic factors determine survival of Salmonella under stress commonly encountered in both natural and man-made habitats in order to assess the effectiveness of different measures to prevent Salmonella from contaminating food. It is even more pertinent to assess if some genetic factors have a possible dual role in promoting survival and virulence in different animal models.

**Recipient or parental organism**
The wild type Salmonella serovars in this project are considered to be analogous to biological agents listed in ACDP Hazard Group 2. The Salmonella strains may be able to colonise humans and animals and cause gastrointestinal disease.

**Host/vector system**
Mutants constructed may be able to survive as well as the wild type Salmonella parent strain in the environment. Salmonella mutants, in which genes related to surface structures eg fimD, pepC, IpfC, flIC, agfA, sefA, motAB, sefA and cheA, global regulatory systems eg rpoS, rpoH, rpoE and bipA or carbon metabolism eg gig genes have been inactivated by the insertion of a gene cassette encoding resistance to an antibiotic eg kanamycin or ampicillin, are unlikely to pose a greater health risk than the parent strain itself. In order to measure the expression of the RpoS protein the following vectors will be used: pSB367 (pUC 18 backbone) and pTopo (pCR2. 1-TOPO backbone); these are non-mobilisable thus reducing the possible hazards from gene transfer. The pSB367 vector contains the spvR gene, the spvA promoterregion and the luxCDABE genes. Most wild type Salmonella strains already contain the spvR gene, the product of this gene has no known toxic function and the expressed protein is therefore not considered to increase pathogenicity. The luxCDABE genes encode the luciferase enzyme and the aldehyde substrate and are not considered to increase the level of risk. The pTopo vector also contains the spvR gene in addition to a gene encoding a green fluorescent protein. The produce of this latter gene is also not considered to
increase pathogenicity.
The role of the genes described above in animals will be investigated using Balb/C mice, chicks and laying hens.

Origin & function

The genetic material involved is normally present in Salmonella strains except for the luxACDABE and gfp genes which originate from Photobacterium fischeri and Actinomyces victoria, respectively.
The genes encoding resistance to different antibiotics originate from a variety of bacteria including E. coli. These genes are used to express a phenotype (bioluminescent, fluorescent or ability to grow in the presence of a certain antibiotic) which will reflect expression of a particular protein (RpoS) or ensure that an intended gene has been disrupted.

Evaluation of foreseeable effects

It is unlikely that wild type strains containing either the antibiotic cassettes or the expression plasmids will be more harmful than the original (wild type) strain itself. There are no particular environmental factors that might affect the likelihood that harm may occur. Given the level 2 containment and control measures it is unlikely that the GMMs will reach the environment and cause harm to humans or animals outside the laboratory. Transmission to people within the laboratory is most likely by ingestion route (medium likelihood). The likelihood of hand to mouth transmission will be reduced to a minimum by observing good laboratory practise appropriate for containment level 2. Access to the facility will be restricted, and appropriate disinfection and waste management procedures are in place. These measures will reduce the likelihood of all hazards to negligible. The overall risk is therefore effectively minimal with the proposed containment and control measures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All bacterial cultures and waste containing GMOs will be autoclaved including agar plates, sharps and glass ware. All potential formites (eg gloves) will be autoclaved.
All animals in contact with bacteria will be killed, double-bagged, and incinerated.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment  

Discussed at meeting of 20.02.02 - notification required.

Project Containment

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**
To create a novel form of recombinant human adenovirus vector that is an improvement on the ones currently widely used.

**Recipient or parental organism**
Class 2 activities;
The E. coli K-12 derivative DH5a, which is recognised as non-colonising and disabled, and may be considered to be equivalent ACDP biological agents hazard group 1.
The recombinant virus vector, adenovirus that is placed in ACDP biological agents hazard group 2. The monkey cell line CV-1 and the human cell line 293 (Human embryonic kidney cell line containing the adenovirus E1 region) which are recognised as non-colonising and disabled.

**Host/vector system**
Class 2 activities:
Host: Escherichia coli K-12 strain DH5a, Monkey Kidney cell line CV-1 HEK cell line 293. A novel CV-1 cell line will be made which will constitutively express the Cre recombinase.
Vectors: Cloning of sequences into recombinant plasmids designed to generate recombinant E1 deleted and E3 deleted adenoviruses based on the human adenovirus serotype 5. The vectors to be used will all be derived from the commercial company Microbix (www.microbix.com) and the details of their plasmids including the sequences are available for them. All the plasmids used are based on pBR322 or pUC. For the generation of the CV-1 based cell line the Cre recombinase will be PCR amplified from the Microbix plasmids and expressed using a pcDNA3.1 based plasmid (available from invitrogen) which is derived from the pUC series of plasmids. The hygromycin marker gene will be introduced from the plasmid pTKHyg available from Clontech and is also based on the pUC series.

Origin & function

The genes are derived from human adenovirus type 5 or from other commercially available sources (eg Hygromycin, EGFP etc). In addition we will generate clones of the E1 region from laboratory isolated wt adenovirus DNA.

Evaluation of foreseeable effects

Will result in the generation of recombinant adenovirus. Some of these will be replication competent in human cells. However all the viruses generated will be less fit than the wild type from which they were derived.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

There is no case for seeking derogation on any of the work.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Class 2 activities.

All biological waste containing GMMs will be inactivated before disposal in accordance with the University Biosafety regulations. Liquid waste (used cultures, culture media, supernatants, and any other solutions which have come into contact with live cells) will be autoclaved at 121 degrees C for 30 minutes before disposal to ensure 100% kill rate of the GMM. Contaminated glassware, re-usable plasticware (eg centrifuge bottles and tubes) and solid waste will be similarly autoclaved before disposal, washing or re-use. The autoclave to be used is housed in the building and is maintained regularly. Each cycle will be monitored using an approved thermosensitive testing strip. Contaminated pipettes will be autoclaved.

Project Containment

Project discussed at the meeting of 7 July 2002 and agreed that it is an activity that must be notified to the HSE.
### Project Additional Information

**Purposes of the contained use**

Investigate the mechanism of action of CTLA3-Ig by comparing its ability to prevent transplant rejection with that of molecules carrying mutations of known functional significance. If delivered systemically CTLA4-Ig prevents transplant rejection and autoimmune disease and has entered phase one clinical trials. This work will further investigate the mechanism of action of this molecule, facilitating its optimal use.

**Recipient or parental organism**

An E1/E3 deleted replication deficient Ad5 vector (AdEasy) will be used to deliver molecules capable of preventing transplant rejection (GTLA4-Ig) to pancreatic islets of Langerhans. Virus will be generated in human kidney 293 cells. Purified murine islets will then be infected with virus by overnight culture and then transplanted into recipient animals (mice) and transplant survival monitored. Generation of virus and infection of islets will take place in a category II facility. Transplants will be performed and animals will be housed in a category II facility in the University of Bristol Medical School animal house.
The likelihood of infection from the mouse is extremely low. No more than 1000 islets are transplanted into each mouse, each islet containing 500-1000 cells. Transfection efficiency is low (approximately 10%). As the virus is non-replicative the number of virus particles in the mouse is thus very low. Although the adenovirus is replication defective, it could be mobilised by co-infection with wild-type adenovirus. Human adenoviruses do not infect mice and the likelihood of this happening with transmission to a second individual is therefore negligible. All waste, including animal bedding, is autoclaved then incinerated.

Host/vector system

The vector to be used is a replication defective E1/E3 deleted Ad5 vector using a CMV promoter. Adenovirus 5 are associated with mild respiratory infections and are non-oncogenic. Replication defective adenoviral vectors have no mechanism for long term maintenance in cells and are unlikely to cause disease. Plasmids expressing CTLA4-ig and mutant molecules will be constructed by cloning into a transfer vector using E. coli. This transfer vector does not contain viral sequences. The inserted gene is under control of a eukaryotic expression vector which does not function in E. coli. Thus, organisms of pathogenic phenotype will not be generated. Recombinant adenovirus vectors will be constructed by homologous recombination in E. Coli of strain BJ5183, which is K12 derived and therefore not pathogenic in man. The vector is non-mobilisable and contains an active resistance gene (kanamycin) for selection. Virus will be generated by transfection of human kidney 293 cells with this recombinant adenoviral vector. Bacteria transformed with plasmids will be stored as glycerol stocks at - 80 degrees C in volumes <5ml.

Origin & function

The inserted gene encodes a fusion protein consisting of murine CTLA4 and murine IgG2a constant regions. This construct was generated by Dr A Gelman, Boston having cloned the respective genes from murine lymphocytes. CTLA4-ig will prevent the immune response to the transplant prolonging transplant survival. Similar work to that proposed here, using vectors containing this insert, has been published (Feng et al Transplantation 1999 67:1607). Plasmids encoding loss of function mutant CTLA4-ig molecules will be generated using site directed mutagenesis. The mutations encode single amino acid substitutions in the mutant molecules resulting in them being defective in binding to selected cell surface ligands. It is predicted that this will inhibit the ability of these molecules to prevent transplant rejection allowing the importance of these interactions to be studied by comparison of the mutant molecule with native CTLA4-ig.

Evaluation of foreseeable effects

Adenoviral vector construction. The inserted gene is under control of a eukaryotic expression vector which does not function in E. coli. All fectors are non-mobilisable and contain an active resistance gene (kanamycin) for selection. Thus, organisms of pathogenic phenotype will not be generated. Freezers containing glycerol stocks will be labelled to indicate that GMOs are stored there. In the event of freezer breakdown or sample spillage storage areas will be disinfected and waste autoclaved before disposal.

Adenovirus will be generated by transfection of human kidney 293 cells. This cell line has been tested for the presence of potentially harmful adventitious agents. The parental organism is a non-pathogenic adenovirus. The insert codes for a molecule capable of inhibiting immune responses. All work will therefore be carried out to ACGM level 2 in a facility which has a class II microbiological safety cabinet for virus handling, designated incubator and equipment necessary for virus isolation and purification. Islets will be infected in this facility. Transplants will take place and animals will be housed in an ACGM level 2 animal facility in the University of Bristol Medical School animal house. Islets will be transferred between these facilities in sealed tissue culture flasks in a sealed box, islets will be washed and resuspended in medium containing no free virus prior to transport. The likelihood of infection from animals receiving transplants is extremely low. No more than 1000 islets are transplanted into each mouse, each islet containing 500-1000 cells. Transfection efficiency is low (approximately 10%). As the virus is non-replicative the number of virus particles in the mouse is very low. All waste, including animal bedding, is autoclaved then incinerated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All animals receiving islet transplants infected with adenovirus will be contained with the ACGM level 2 animal facility in the University of Bristol Medical School animal house.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All liquid waste will be treated with 10% bleach (10,000 ppm sodium hypochlorite) then autoclaved. All solid waste, including animal bedding, will be autoclaved then incinerated. Animal carcasses will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Discussed at meeting of 10.7.02, E. coli BJ5185 from commercially available kit. Agreed that project must be notified to HSE.

Project Containment

<table>
<thead>
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<th>Laboratory Activities</th>
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Project Ref 18/02.8

Date Ackn’d 06/11/2002

CU2 Project Title CONDITIONAL IMMORTALISATION OF HUMAN PODOCYTE AND ENDOTHELIAL CELLS.

Class 2

Consent Granted not applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info
### Project Additional Information

#### Purposes of the contained use
Stable transfection of primary cultured cells with an immortalising gene construct, using amphotrophic retrovirus as the vector.

#### Recipient or parental organism
Cultured mammalian cells.

#### Host/vector system
Amphotrophic retrovirus in cultured PA317 mouse fibroblast cells.

#### Origin & function
- SV40 T antigen - immortalising gene construct
- Human telomerase gene - cell survival gene.

#### Evaluation of foreseeable effects
The retrovirus is replication deficient, and is capable of infecting human cells. There is therefore a theoretical risk to the operator of introducing the described oncogene.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
- Plastics and paper: into yellow bag refuse for incineration
- Glass: autoclaved or soaked in disinfectant, then into normal refuse
- Liquid waste: disinfected with Virkon, then into drains.

All these measures will be expected to result in 100% kill of cultured cells.

---

**Is an emergency plan required according to regulation 20?**

- N

**If yes, tick to confirm that it is attached to this form**

- N

**Tick to confirm that you have attached a risk assessment to this form**

- Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- N

---

02/03/2022
Originally approved at level 1, however at meeting 10.7.02 project reviewed. Compendium 2B Annex III Para 30 "Amphotrophic vectors containing functional oncogenes should be contained at level 2". Therefore project to be notified to HSE, however work actually carried out at level 2 containment.

**Project Containment**

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**Project Ref** 18/03.1

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<td>STUDIES ON THE INTRACELLULAR SIGNALLING PATHWAYS IN BRAINSTEM AND HYPOTHALAMUS USING LENTIVIRAL AND ADENOVIRAL GENE EXPRESSION SYSTEMS.</td>
<td>Class 2 1-50 litres</td>
<td>not applicable</td>
<td>Project notified under transitional arrangements N</td>
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Withdrawn N

Tick if notifying a connected programme of work N

**Project Additional Information**

**Purposes of the contained use**

Essential hypertension remains a highly widespread and damaging disease leading to high morbidity and mortality rates in the industrial countries, such as UK. We have currently obtained financial support to investigate molecular mechanisms of pathological hypertension. Changes in biochemical and biophysical properties of brain neurones which could underlay human hypertension and related disturbances of homeostasis will be studies in animal models of hypertension. A variety of genetic
constructs will be expressed in brain cells in order to a) visualise various stages of intracellular signalling using fluorescently tagged molecules; b) study physiological consequences of suppression of an intracellular signalling cascade.

Examples of "a" are: fusion proteins incorporating mutants of the green fluorescent protein (ie eNOS-EGFP or angiotensin AT1 - receptor-EYPF or ARNO-ECFP), Examples of "b" are dominant negative forms of G-proteins nitric oxide synthases and protein kinase B.

Most of our constructs will utilise cell specific promoters to restrict the expression to selected subpopulations of brain neurones. This research will help to clarify the biochemical pathways responsible for pathological hypertension anre reveal novel targets for drug therapies with a prospect of genetic therapy.

Recipient or parental organism

Viral vectors will be either delivered into the brain of rats (normotensive strains, such as Wistar, or spontaneously hypertensive rat, SHR) or applied to slice cultures made from the brain of these animals or to the acutey prepared brain slices of the rat. As a preliminary step required for construct screening cell lines such as (C12, Neuro2A, CHO, HEK293 will be used.

Rats injected with the viral vectors will be housed within the transgenic facilities of the UOB. Slice cultures will be prepared under sterile conditions and kept in designated incubators for > 7 days before use. It has been demonstrated that once injected of have been applied to cultured cells the viral particles cannot re-infect any other tissue. This can be demonstrated by application of tissue homogenates to a receptive cell line, such as HEK293 showing that no infection will occur.

Host/vector system

Self-inactivating lentivirus (T. lwakuma, Y. Cui, L.-J Chang. Virology 261:120-132, 1999) is a self-inactivating vector with extensive deletions and modifications in both long terminal repeats. Originally derived from HIV, these vectors contain a substitution in the 5’ end promoter, the 3’ end deletion and a number of further modifications which theoretically make formation of replication-competent virus impossible. These and further deletions introduced University of Florida, USA are also thought to also have abolished the potential oncogenic capacity of the native lentiviruses. The self-inactivating lentivirua we intend to use is unable to replicate in any known system and is very unstable in the normal environment.

Replication - deficient adenovirus derived from human adenovirus type 5 with E1a - or combined E1a&E3 deletions have been used extensively around the world for several years. It can replicate only in special cell lines (like HEK 293) and should be unable to recombine to form a replication-competent virus: this has been confirmed by years of its world-wide use.

Origin & function

The self-inactivating lentivirus has been originally developed by the group of Fr. L-J Chang (University of Florida, USA and further modified in the laboratory of Prof M Raizada (University of Florida) to delete vpr, vif and vpu genes for additional safety and we have already gained experience with these vectors through our collaboration with Prof Raizada’s group (see Coleman, J. E. et al, 2003 Physiological Genomics 12, 221-228). The systems for creation of the replication-deficient adenoviral vectors are available commercially from Microbix and Clonetech. We will use the Microbix system as we already have experience with it and have used it for the last three years under supervision of Prof D Murphy (UOB Centre for Neuroendocrinology). This is reflected in a number of publications from our laboratories (ie Kasperov, S. et al., 2002 Experimental Physiology 87, 715-724; Paton, J. F. R. 2001, Acta Physiologica Scandinavica 173, 127-137; Paton J. F. R. et al, 2001, Journal of Physiology 531, 445-458; Waki, H. et al., 2003, Journal of Physiology 546, 233-242; Wong, L. F. et al, 2002 FASEB Journal 16, 1595-1601).

Genes to be cloned into the vectors will come either through third parties (collaborators) or by cloning from cDNA libraries using standard methods. Some of the genes (fluorescent proteins) will be purchased from companies, such as Clonetech. We will concentrate primarily on vectors, which will be targeted to specific populations of brain neurones using specific promoter sequences (ie PRSx8 promoter selective for noradrenergic neurones (Hwang et al, 2001, Human Gene Therapy 12, 1731-1740) supplied by our collaborator K. -S Kim, Harvard University, USA). These constructs therefore will not express transgenes in peripheral tissues. Oncogenes or genes involved in cell division will not be used.
The genes to be expressed
- The main family of transgenes we want to place into the viral vectors will express fluorescent proteins or their fusions (EGFP, HcRed, eNOS-EGFP or angiotensin AT1-receptor-EYPF, PHD-EGFP, synaptophysin-ECFP or ARNO-ECFP). Fluorescent proteins such as EGFP and HcRed will be placed under control of cell-specific promoters (ie PRSx8 promoter selective for noradrenergic cells or neuron-specific NSE promoter which leads to expression in neurones but not any other cell type). They will used to visualise the fine details of connectivity in living neurones using live cells confocal imaging (Kasparov, S. et al, 2002, Experimental Physiology 87, 715-724). The fluorescent fusions will either have no biological activity at all (PHD-EGFP) or be based on signalling molecules, which according do not trigger cell division (ie eNOS-EGFP, AT1 receptor-EYPF, synaptophysin-ECP, ARNO-ECFP). These molecules can be used as tracers for various types of cellular biochemical activity as they are thought to translocate between cellular compartments upon activation of certain signalling cascades. For example, PHD-EGFP contains a sequence which enables it to move towards the plasma membrane in response to PIP3 accumulation. Hence it can be used to visualise PIP3 signalling in living cells such as brain neurones. We will need to use both, cell-specific promoters and HCMV for neuronal types for which no specific promoters are available.

- The other family of vectors will contain dominant negative proteins, which again do not have any known oncogenic properties. We need to generate dominant negative mutants for nitric oxide synthase (NOS), protein kinase B, PIP3-kinase or G-proteins. Such constructs (using HCMV promoter) have been made previously by others and no stimulatory effects on cell division have been reported. Some of these vectors were used in our recent studies (ie Wong L.-F. et al, 2002) but we will need to place them under control of cell-specific promoters in order to identify their site of action. As stated above, most of our constructs will use cell-specific promoters (ie neuron-specific NSE promoter or PRS8 noradrenergic promoter). Viruses with these promoters only express transgenes in neurones or selected neuronal subpopulations. In the event of an accident there is no feasible route by which these viruses may reach susceptible brain cells. Cells outside of brain-blood barrier transfected with a denoviral vectors get rapidly eliminated and as further infection/viral multiplication may not occur the transgene from the peripheral tissues will be eliminated naturally. Fluorescent proteins such as EGFP are not toxic to cells and there are many transgenic animals where EGFP is continuously expressed in some cells.

### Evaluation of foreseeable effects

**Viral vectors potentially can be harmful for humans. This will require that these vectors could a) infect humans; b) multiply in human body; c) bare genes of harmful nature.**

- **a)** both lentivirus and adenovirus can infect humans if delivered to the receptive tissue. As known from HIV, lentivirus has extremely low infectivity unless injected directly into the tissues. Adenovirus can infect human respiratory tract via aerosol route. However, given the use of class II biological safety cabinet, an ACPD category II facility and high degree of worker training and awareness, the risk is greatly minimised. Furthermore, the immune cells in the respiratory epithelium will immediately phagocyte and destroy the viral particles. Given that the volumes used for injection into brain are <2ul and in slice culture/acute slice are ~ 20 ul, the amount of the viral particles which could escape into aerosol should be extremely small (for comparison >10 (to the power of 12) viral particles have been injected into blood during clinical trials). After application to brain slices or cultures or injection into animals, viruses capable of infection may NOT be recovered.

- **b)** neither vector can replicate in a human body. Adenovirus can proliferate in specific cell lines which supply the E1a product in trans, while the lentiviral vector cannot replicate in any system because it lacks most of the original genome and further self-inactivates upon integration into the host genome.

- **c)** in the lentivirus vector all active promoter-like elements have been removed and the only active promoter is the one which is chosen to drive the expression of the transgene. This vector has been modified in such a way that after integration it has no transcriptionally active long terminal repeats anymore. This minimises the risk of detrimental effects of the lentiviral genome itself. The genome of the replication-deficient adenoviral vectors has no known cytotoxic or oncogenic genes. However the transgenes placed in any vector may be harmful, if they have toxic of oncogenic potential, for example, apoptotic genes or constitutively active forms of protein kinases involved in cell division. We will NOT use any such genes and sequences encoding for genes of known harmful potention.

- In addition, the expression of a transgene is dependent on the promoter use. Some promoters, such as the HCMV (human cytomegalovirus promoter) are active in a wide variety of cells while others are selectively active only is some cell types. In this study we intend to use both, cell specific promoters to target selected neuronal populations in the brain and H?CMV for the neuronal populations for which no specific promoters are available. Importantly the cell specific promoters will restrict expression of any transgene to central neurones to which any viral vectors generated in this study may have access only if directly delivered into the brain or put on the neuronal cells of that phenotype in vitro.
Experimentators may potentially have contact with the vectors at two stages: during viral preparation and during their application to the brain cells in vivo or in vitro. During the preparation all required safety measures will be observed and most of the work with recombinant viral particles will be carried out in the class II safety cabinets. The only stage where live viral stock will need to be handled outside of the hoods is the ultracentrifugation but during this step the virus will be hermetically sealed in special tubes. The ultracentrifuge also will be treated with 10% Vircon (antiviral and antiseptic reagent). The same chemical will be also used to neutralise any other contaminated materials, which then will be autoclaved. Transfected animals will be incinerated.

Application of viruses to brain slices (acutely prepared or in organotypic cultures) will also be done in the class II safety cabinets. Microinjection into the brain of experimental animals (rodents) will be carried out in the Cat II designated facility. Adenovirus can infect human respiratory tract via aerosol route while the lentivirus apparently cannot. Considering the use of a class II safety cabinet, an ACDP category II facility, the high degree of worker awareness and qualification and the fact that the viruses are replication-deficient, the risk to the worker is greatly diminished. The volumes used in transfection experiments are very small (2-10-20 ul, containing approximately 2 * 10^7 viral particles). The maximum volume injected into an animal is 2ul and the glass needles will be filled in a category II safety cabinet and buffered by paraffin oil. These considerations make formation of any substantial amounts of aerosol highly unfeasible. Once injected into animals the viral particles cannot re-infect any other tissues and the injected animals are not infectious to the others. Moreover there is no known way for these constructs to re-appear as infection-capable viral particles.

Animals injected with the lentiviral and adenoviral vectors are not infectious and represent no threat to the environment or personal. They will need to be maintained for up to 2 months in the animal facility of the UOB (<20 animals at a time).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All general laboratory waste used for cloning will be collected into the special bags, autoclaved and disposed according to the University regulations. All materials which were in contact with viral suspensions will be neutralised with 10% Vircon and Hycolin and then autoclaved at 121 degrees C for 45 minutes to ensure 100% destruction of any GMM. Pasteur pipettes and similar glass sharps will be avoided to prevent the risk of skin puncture. Scalpel blades will be put into the designated lockable containers and incinerated. Waste materials used for cloning (ie plastic tips etc.) will be collected into designated containers and incinerated. Transfected animals will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Discussed at meeting of 20 November 2002 and Committee agreed that insufficient information had been supplied. Project re submitted to Committee at meeting of 19 February 2003 and agreed that the content was not satisfactory. To be submitted to HSE.
**Project Additional Information**

**Purposes of the contained use**

Fundamental research aimed at understanding the molecular and cellular mechanisms of intracellular survival using bacterial models. Our principal model organisms are *Listeria monocytogenes* and *Rhodococcus equi*. Our experimental approach involves the inactivation of selected genes of these bacteria by standard laboratory protocols and the analysis of the effect of the gene-disabling mutations on the bacterial intracellular proliferation capacity in vitro, on virulence in vivo, and on the response of the whole bacterial genome as assessed by DNA chip and proteomic technologies. The ultimate goal of this research is to identify targets for new vaccines, antimicrobial therapies and diagnostic tools to combat infections due to intracellular parasites.

**Recipient or parental organism**

The research will involve the generation of four types of GMMs.

1. Derivatives of *L. monocytogenes* and *R. equi* in which selected genes have been “surgically” inactivated to deduce their function by phenotypic comparison with the wild-type parental strain. Gene inactivation will usually be carried out by in-frame deletion, a technique that does not leave any trace of foreign DNA in the bacterial
genome. With this technique, GMMs only differ from their parental organism in that they lack a DNA fragment corresponding to the deleted gene(s).

2. Gene-inactivated derivatives of L. monocytogenes and R. equi in which a wild-type copy of the gene under study has been reintroduced to confirm that the observed phenotype is actually due to this gene(s) and not to a secondary, spontaneous mutation affecting other unrelated loci.

3. Non-pathogenic cloning hosts carrying sequences of our model organisms (L. monocytogenes or R. equi), required for the construction of the gene-deletion derivatives described in (1) or for gene function studies. As recipient organisms we will use the following non-pathogenic bacteria:
   - Escherichia coli K-12 or B disabled derivatives (strains DH5α, HB101, C600, HB101, XL1-Blue, etc)
   - Bacillus subtilis
   - Lactococcus lactis
   - Listeria innocua, a non-pathogenic Listeria sp which lacks all known listerial virulence genes
   - A well-characterised derivative of L. monocytogenes (prfA, picA, hly, plcB, actA) which was irreversibly rendered totally non-pathogenic by deletion of multiple essential virulence genes

4. Miscellaneous GMMs. The following GM bacteria may be incorporated as controls in some of our experiments:
   - Salmonella typhimurium SL1344sifA, a derivative of the laboratory strain SL1344 lacking SifA, a protein required for the maintenance of the vacuole in which Salmonella replicates intracellularly
   - Shigella flexneri laboratory strain SC560, rendered non-pathogenic due to an icsA-disabling mutation which impedes host tissue colonisation
   - Yersinia enterocolitica WA-314 attenuated derivatives due to disabling mutations in the sodA (superoxide dismutase) and irp (invasion associated protein), virulence genes.

Host/vector system

For routine gene cloning we will use non-mobilisable or mobilisation-defective plasmid vectors, such as pUC series-based vectors, pBR322 or pBluescript II for E. coli, and pE194 derivatives for gram-positive bacteria (B. subtilis, L. lactis or Listeria spp). To produce gene-disabling mutations we will use thermosensitive derivatives of pE194 in Listeria and pUC derivatives in R. equi. For inactivation of the uhpT gene in Salmonella typhimurium SL1344sifA and Shigella flexneri SC560 (see below) we may use the "suicide" vector pGP704. This vector can only replicate in, and be mobilised from, strain SM10Lpir, a host E. coli C600 derivative engineered to contain the RP4 mobilisation and R6K replication functions integrated in the chromosome. Once conjugally transferred from this host strain, plasmid pGP704 cannot replicate and be mobilised further.

Origin & function

From Listeria monocytogenes.
In the context of a major EU-funded collaborative initiative in functional genomics of Listeria spp., our group will be allocated a number of L. monocytogenes genes to inactivate them by in-frame deletion. A complete description of the 2,853 genes of the L. monocytogenes genome and their predicted products is publicly available at http://genolist.pasteur.fr/ListiList. All the gene-disabled derivatives contributed by the collaborating European partners will then be sytematically analysed in the different laboratories by a number of approaches, including DNA chip technology and proteomics. Our research will particularly focus on the following Listeria genes.

- prfA, encoding a regulatory factory homologous to CAP/Crp from E. coli
- actA, encoding a surface protein involved in actin-based motility
- plcA, plcB and smcL, encoding phospholipases active on phosphatidylcholine and sphingomyelin, respectively
- hpt, encoding a sugar phosphate transporter homologous to the E. coli Uhp T permease
- ptsH, encoding a putative glucose-specific enzyme II permease component of a phosphoenolpyruvate sugar phosphotransferase system (PTS)
- bvrABC, encoding a PTS permease system specific for beta-glucoside sugars
- agl, encoding a two-component regulatory system
- luxS, encoding a putative quorum-sensing signalling system.
From R. equi
Our work will focus on choE and choD genes, encoding cholesterol oxidases that are widespread among saprophyte soil dwelling actinomycetes (eg Streptomyces spp). We believe that these enzymes may contribute to the capacity of R. equi to survive within eukaryotic host cells, whether they are mammalian microphages or soil bacteriovorous protists.

From S. typhimurium and S. flexneri
Our work will focus on the uhpT gene, encoding a glucose phosphate transporter identical to that present in E. coli K-12 and widely distributed among bacteria. We believe that this transporter may play a role in the uptake of sugar phosphates in vivo when bacteria are within host cells.

### Evaluation of foreseeable effects

#### Hazards to human health

L. monocytogenes and R. equi, in which we are going to produce gene-disabling mutations, live as saprophytes in the soil and are widespread in nature. Occasionally, they can cause opportunistic infections in humans and animals and are thus classified in hazard group 2 in the Approved List of Biological Agents. Clinical infections occur only rarely, require a predisposing underlying condition and, presumably also, a high exposure. Both L. monocytogenes and R. equi are considered to have low infectivity and virulence. Under the designation L. monocytogenes we also include the closely related species L. ivanovii (formerly known as L. monocytogenes serovar 5), which is less virulent and has a narrower host spectrum than L. monocytogenes (L. ivanovii is not recognised as human pathogen). The gene "knock-out" derivatives of these bacteria will have the same low virulence as the parent strain if the deleted loci do not play a role in infection, or a lower virulence if the deleted genes play a role in infection. The "reconstituted" mutants, in which the deleted gene is reintroduced back for confirmation that the target gene is responsible for the observed phenotype, will have at most the same virulence as the parent strain. All these GMMs will therefore be handled under containment level 2.

The heterologous bacterial hosts that will be used as recipients of intermediate constructs for deletion mutant construction or to study gene function by complementation (E. coli K-12 or B disabled derivatives, B subtilis, L. lactis or L. innocua) are not considered pathogenic for humans or animals (hazard group 1). The attenuated L. monocytogenes (prfA, plcA, hly, plcB, actA) derivative lacks several critical virulence genes and consequently is entirely avirulent and non-pathogenic. The multiple gene deletions in this L. monocytogenes derivative guarantee that it is stably attenuated, there is no real possibility of reversion, and thus is a totally safe host strain.

Thanks to our participation in the Listeria sequencing projects we have precise information on the putative activities of the products encoded by L. monocytogenes. None of the L. monocytogenes genes code for known dangerous toxins, highly aggressive virulence factors or harmful products which can act directly to cause damage, allergy, oncogenesis or growth modulation. In the case where they do contribute to virulence, their role in infection is always subtle and they need to act alongside a number of other virulence and regulatory factors with which they have co-evolved in the source organisms. Therefore, the risk for one of the above heterologous non-pathogenic host organisms gaining virulence by introduction of L. monocytogenes sequences is negligible. Although in principle they might be handled under containment level 1, as a precaution and because they will be handled in parallel with the other bacteria, they will effectively be worked under containment level 2.

The miscellaneous bacteria that we may incorporate in our experiments all have disabling mutations that attenuate their virulence. S. typhimurium SL1344sifA, lacking the protein SifA required for the maintenance of the vacuole in which Salmonella replicates intracellularly, shows impaired replication in macrophages. The S. flexneri icsA derivative SC560 is virtually non-pathogenic (in fact one of its derivatives, strain SC602, is used at the institut Pasteur as the basis for the development of a vaccine against Shigella infection in humans). Yersinia enterocolitica WA-314, with disabling mutations in the virulence-associated sodA and irp genes, has impaired capacity to survive in vivo in animal tissues and is also used as the basis for a live oral vaccine. Nevertheless, these bacteria can have some residual virulence and therefore will be handled under the containment level required by their corresponding wild-type parental strains, which are classified in hazard group 2 (ie level 2).

The cholesterol oxidase genes choE and choD from R. equi, which are widespread among non-pathogenic actinomycetes (eg Streptomyces spp., Brevibacterium sterolicum), and the uhpT gene from S. typhimurium, encoding a sugar phosphate transporter also widely distributed among bacteria, are not expected to confer any virulence property to a non-pathogenic heterologous host.

The resistance genes used as selection markers in the cloning vectors have been approved for use at containment level 1 and confer resistance to "old" antibiotics for which resistance in clinical isolates is already widespread (and therefore are no longer used in clinical practice).
Hazards to the environment
None of the bacteria we will work with are exotic to the UK, are pathogenic to plants, or cause notifiable diseases under specific surveillance by DEFRA. They are already ubiquitous in the environment and are frequently carried by healthy animals. In some cases they may cause opportunistic infections in these animals.

The gene-disabling mutations introduced in the bacteria (in most cases by in-frame deletion, which does not leave any trace of foreign DNA) should normally lead to attenuation in vivo. Although these mutants may retain some of the original virulence of the parent strains, they will complete less well within an animal host and will be cleared from the infected tissues sooner than the wild type. The risk of pathogenicity is therefore reduced and less important than with the corresponding parent strain. The loss of a trait or genetic sequence that has been selected for through evolution is likely to reduce the fitness of the gene-deleted derivative to establish and survive in nature. Consequently, the gene-disabled derivatives, if accidentally released to the environment, are not expected to displace any indigenous population.

Listeria or R. equi bacteria in which the original phenotype has been restored by introduction of the wild-type allele in a plasmid vector always tend to lose these constructs because the vector is not naturally adapted to these host bacteria and represents a physiological burden (being thus rapidly lost in the absence of selective marker pressure). The non-pathogenic cloning hosts containing sequences from Listeria or R. equi are not expected to become established or be hazardous to the environment because the plasmid vectors will not be mobilised and the foreign genes, which are derived from ubiquitous soil dwelling bacteria, are already present in nature as a result of natural decay processes.

The likelihood of accidental environmental release of the GMMs is very low or negligible given the measures that will be applied to control any risk to human health and safety (level 2 containment). If release was to occur, the consequences can also be graded as very low or negligible. Therefore, the environmental risk can be judged as effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The GMM-contaminated waste will consist of broth/agar cultures, plastic labware and glassware. All this material will be inactivated in the building by autoclaving at 121 degrees C for 20 min (100% kill) prior to disposal or recycling. Carcasses of experimentally contaminated animals will be incinerated on site (100% kill). Non-contaminated general waste generated in the containment level 2 facilities and thus potentially exposed to the GMMs will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Discussed at Committee meeting of 19 February 2003 - extra information required re target genes and comment re pregnant workers suggested.
The research project aims to define the role of alternative pathways in the mitochondrial respiration of filamentous plant pathogenic fungi. It involves cloning genes coding for alternative oxidase and alternative NAD(P)H dehydrogenase, and exploring function through inactivating either gene and comparison with the wild-type in biochemical and infectivity assays. The coding sequence will be disrupted by insertion within each gene of non-coding plasmid DNA. The plasmid construct will also contain a hygromycin resistance gene from E.coli, allowing direct selection of fungal transformants. Sequence identity between the fungal gene and flanking coding sequences of the disrupted gene will allow the targeted replacement of the wild-type gene with the inactivated one. In separate experiments linked reporter genes such as GFP will be cloned into the 3' -end of these fungal genes, in order to define the spatial relationship of alternative components and the core respiratory chain.
Botrytis cinerea (grey mould) laboratory haploid strain Bo5.10
Mycosphaerella graminicola (wheat leaf blotch)

**Host/vector system**

The fungi will be transformed by integrating vectors based on pUC, using protoplast based procedures.

**Origin & function**

The selectable marker for fungal transformation driven from a variety of fungal promoters will be hygromycin B phosphotransferase from E. coli. Green Fluorescent Protein (GFP) from the jellyfish Aequorea victoria will be used as a reporter gene for visualisation of hyphae. The plasmids for construction of gene replacement vectors will carry the E. coli hygromycin resistance gene. This will be under the control of either the promoter and terminator for B. cinerea B-tubulin, or the oliC promoter and trpC terminator from Aspergillus nidulans. Constructs will be based on standard E. coli plasmids, namely pUC and pBluescript.

**Evaluation of foreseeable effects**

Neither of the fungi under study is a pathogen of man or animals. There is no reason to suppose that any of the modifications to be performed will lead to human or animal pathogenicity. Thus there is no risk to health from this work and the containment measures are being used solely for environmental protection. Transformation of the fungi per se is likely to be neutral in effect. The transformation markers do not involve resistance to commercial fungicides, so the resulting transformants are unlikely to have an advantage over untransformed strains. Introduction of reporter genes is unlikely to give the transformant any advantage over wild-type strains. Inactivation of genes by targeted disruption or insertional mutagenesis is likely to be detrimental to the fungus and will certainly not provide a long term advantage compared to wild-type strains.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated by this work will be autoclaved prior to disposal. Any spillages of cultures will be decontaminated by treatment with hypochlorite (3-5 %), while all swabbing materials will be autoclaved prior to disposal. Any potential contaminated surfaces will be sterilised by swabbing with 70% ethanol.

**Is an emergency plan required according to regulation 20?**  
**Y**

If yes, tick to confirm that it is attached to this form

**Tick to confirm that you have attached a risk assessment to this form**  
**Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment

**N**
Project originally agreed at level 1 - picked up by HSE Inspector and review asked for plant pathogen therefore class 2.
Notification and risk assessment discussed at committee meeting 19/2/2003.

## Project Containment

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### Project Ref 18/03.4

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Project notified under transitional arrangements N

### Purposes of the contained use

The research project involves analysis of the organisation of intracellular membranes and the study of the trafficking of molecules between these membranes along cytoskeletal filaments. It involves cloning of markers of intracellular structures and reporters to monitor transport between these structures. Coding sequences will be inserted into replication defective adenoviruses and baculoviruses for the production of proteins in mammalian and insect cells. Markers and reporters will be cloned as fusions with autofluorescent protein coding genes such as that for GFP from the jellyfish Aequoria victoria. This will facilitate imaging of the interrelationships between
these structures and the mechanisms by which transport between compartments occurs.

Recipient or parental organism

Adenovirus (replication defective)
Baculovirus (replication defective)
E. coli
Cultured mammalian cell lines.

Host/vector system

AdEasy adenovirus expression system (Stratagene); viruses generated will be used to infect mammalian cells.
Bac-to-Bac baculovirus expression system (Invitrogen); viruses generated will be used to infect Spodoptera frugiperda cells (Sf9 and Sf21).
Constructs are based on standard E. coli cloning vectors namely pUC, pBR and pBluescript.
Expressed genes will be under the control of polyhedrin and/or p10 promoters in baculovirus and CMV or SV40 promoters in Adenovirus constructs.

Origin & function

Genes encoding markers of membranes and transport pathways will be cloned from mammalian cDNA libraries or obtained from collaborating labs. Where possible, human cDNAs will be obtained (eg COPI and COPII, cytoskeletal markers, Golgi markers;)
GFP from Aequoria victoria, HcRed from Heteractis crispa and DsRed from Discosoma sp. And spectral variants thereof will be used as fluorescent reporters.

Evaluation of foreseeable effects

Adenoviral vectors are capable of infecting human cells. However, the virus used is replication defective. It has been generally accepted that baculovirus cannot infect animal or plant cells however, some recent studies do show a capability for expression of genes from mammalian promoters from baculovirus vectors. Mammalian promoters will not be used in these baculovirus studies. Baculoviruses used are polyhedrin negative and therefore susceptible to insect larval gut conditions, desiccation and environmental conditions such as UV light. The majority of inserted genes are not known to be pathogenic and are unlikely to modify the properties of the host.
Overexpression of inserted genes could result in general disruption of intracellular membrane and cytoskeleton organisation, signalling and ion transport.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated by these procedures will be autoclaved prior to disposal.
Any spillages of liquid will be decontaminated with 5% HiSpec. solution and all swabbing material autoclaved before disposal.
Any potentially contaminated surfaces will be sterilised by swabbing with 70% ethanol.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Project discussed at meeting 19 February 2003, agreed project should be notified because baculoviruses can infect mammalian cells and even though this is a polyhedrin minus strain it contains full length sequences that are biologically active ie could be a hazard to the worker.

**Project Containment**

<table>
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<tr>
<th>Laboratory Activities</th>
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</thead>
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<td>L2 L3 L4</td>
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</tbody>
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**Animal Units**

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<tr>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

**Project Ref** 18/03.5

**Project Additional Information**

The aim of this project is to develop feline foamy virus (FFV) as a gene delivery system in cats for therapeutic purposes. This will involve manipulation of wild type (wt) FFV.

Non-GMM Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Tick if you are claiming exemption from disclosure for section of the risk assessment

**Historical Significant Changes**

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
strain F17 and production of viral proteins through a baculovirus system.

Recipient or parental organism

**FFV strain F17**

E. coli DH5α, E.coli Stbl-2, E.coli XL-1, E.coli M15, E.coli XL10-Gold

E.coli BL21-SI, DH10Bac (pMON7124)

CRFK and FKCU cells

CRFK/pltr1 (FFV)-B-gal (reporter cell for FFV infection)

Baculovirus

SF9 cells

Host/vector system

<table>
<thead>
<tr>
<th>PLASMIDS/VECTORS</th>
<th>PROMOTER</th>
<th>SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR2.1</td>
<td>T7</td>
<td>Commercially available plasmid (Invitrogen)</td>
</tr>
<tr>
<td>Psport 1</td>
<td>SP6, T7</td>
<td>Commercially available plasmid (Invitrogen)</td>
</tr>
<tr>
<td>PQE30/31</td>
<td>T5 and Lac</td>
<td>Commercially available plasmid (Invitrogen)</td>
</tr>
<tr>
<td>PcdNA3.1/eGFP</td>
<td>CMV, SV40 ori/Promoter</td>
<td>eGFP gene (derived from pEGFP1, Clontech ) inserted into commercially available plasmid (Invitrogen)</td>
</tr>
<tr>
<td>PDonr 201</td>
<td>None</td>
<td>Commercially available plasmid (Invitrogen)</td>
</tr>
<tr>
<td>PDest 17</td>
<td>T7</td>
<td>Commercially available plasmid (Invitrogen)</td>
</tr>
<tr>
<td>PHook 3</td>
<td>SV40 Ori/Promoter</td>
<td>Commercially available plasmid (Invitrogen)</td>
</tr>
<tr>
<td>PDest10</td>
<td>Polyhedrin</td>
<td>Commercially available plasmid (Invitrogen)</td>
</tr>
<tr>
<td>Pltr1 (FFV)-B-gal</td>
<td>FFV-LTR (U3)</td>
<td>8.77Kb plasmid, Zoecin and Amp resistance, Lac Z gene</td>
</tr>
<tr>
<td>PMON7124 (in DH10Bac)</td>
<td>SV40 Ori/Promoter</td>
<td>Derived from pClacZ</td>
</tr>
</tbody>
</table>

Origin & function

Genetic material will be derived from FFV strain F17, obtained from the American Type Culture Collection (ATCC VR889). The virus will be used in infection studies and manipulated by standard molecular techniques to create replication competent and replication incompetent viral vectors.

1. The entire genome of feline spumavirus (FFV, strain F17, a wild type strain) will be cloned into pCR2.1 and pSport1 (Invitrogen) and transformed into DH5alpha and Stbl-2 E. coli (Invitrogen). The plasmid will be isolated from small scale culture (10ml) and checked by transfection into CRFK and FKCU feline cell lines. Production of wild type virus produces CPE in these cells.

2. Removal of the Bel 1 (transactivator) gene from pCR2.1/F17. This will be undertaken using conventional restriction enzyme digestion or site directed mutagenesis to insert multiple stop codons into the sequence.

3. Insertion of a CMVpromotor-eGFP-polyA signal expression cassette into the region vacated by the Bel 1 gene and transformation of Stbl-2 E.coli and small scale culture and plasmid purification.

4. Cloning and expression of the Bel 1 gene (transactivator) from FFV/F17 in pQE30 and pQE31 (Qiagen) with expression in XL1-blue and M15 (prep4) E. coli (Stratagene and Qiagen). Also cloning and expression of the Bel 1 gene using the Gateway cloning system (Invitrogen) and pDonr 201 and pDest 17 with expression in BL21-SI E.coli.
5. Construction of a helper cell line for the propagation of replication deficient FFV. The Bel 1 gene of FFV will be cloned into pHook3 (Invitrogen) and transfected into CRFK and FKCU feline cells. Cells containing the plasmid will be selected by Zeocin resistance and cloned by limiting dilution. We will also transfet CRFK and FKCU cells with pHook3/beta gal (a control plasmid in the kit) and stain for beta gal expression.

6. Stably transfected CRFK and FKCU cells thought to be expressing Bel 1 (see 5) will be transfected with the reporter plasmid pLTRI(FFV)B-gal (a plasmid containing the LTR of FFV controlling the B-galactosidase gene. A gift from Prof A Rethwilm, University of Dresden, Germany). pLTR1(FFV)B-gal will be grown in DH5alpha E.coli CRFK or FKCU cells expressing Bel 1 will switch on the LTR and cause expression of B-gal which can be detected by staining.

7. Stably transfected helper CRFK and FKCU cells shown to be expressing Bel 1 (from 6) will be transfected with pCR2.1/F17eGFP (from 3). These cells should produce viable viral particles able to infect other feline cells. Viral CPE will be monitored and virus harvested and transferred to fresh CRFK or FKCU cells where the eGFP gene should be delivered, as monitored by eGFP fluorescence. There should be no viral replication in these cells since they lack the Bel 1 gene and this will be monitored by serial passage into fresh cells.

8. Insertion of a V5 epitope (gly-lys-pro-ile-pro-asn-pro-leu-gly-leu-asp-ser-thr) into the ENV and GAG genes of the parental FFV F17 virus (step 1 above), growth in stbl-e cells (10-50mL) and transfection into CRFK and FKCU cells. Work to date using the plasmid generated in step 3 has not shown the production of any viral particles carrying eGFP. Hence it is proposed to insert the small V5 epitope into the ENV and GAG genes of the parental viral clone generated in step 1 and monitor viral production by immunofluorescent microscopy. It is hoped that this change will be less "invasive" and allow production of viable viral particles. The V5 epitope is a tag and will allow detection of the modified virus, it has no biological activity and would be unlikely to change the viral pathogenicity. Recent work on the insertion of the V5 epitope into the ENV gene, which could alter the viral host range, has proved unsuccessful with no viral particles being produced invitro. Further work is in progress to insert the V5 epitope into the GAG gene, it is not expected that this will alter the host range or pathogenicity. Due to the very limited amount of time remaining on this project it is not anticipated that any other sequences or genes will be cloned into the parental FFV plasmid. Should it become possible to use other genes we will notify the change to the HSE.

9. Expression of FFV GAG, POL and ENV proteins in baculovirus. The GAG, POL and ENV genes from FFV F17 will be amplified by PCR and cloned into the Gateway cloning system (Invitrogen) using pDonr 201 and growth in DH5a E.coli. The resulting plasmids will be used to generate baculovirus expression vectors using the pDest 10 vector and DH10Bac E.coli (Invitrogen). The resulting baculovirus will be grown in SF9 insect cells.

10. Depending on the results of the invitro work to generate modified spumavirus (all of which have failed to date) future work will aim to use these modified vectors as gene delivery systems in the cat. However, due to the limited time left on the project it is unlikely to progress to the cat model system. If it were to become possible to use the cat model system we would notify any change to the HSE.

**Evaluation of foreseeable effects**

Feline foamy virus (FFV) has not been well classified and has hence been assigned to containment level 2. There is no evidence of feline spumavirus infection in humans (Butera et al., 2000, Winkler et al., 1997) and this hazard is considered negligible (Rethwilm, 1996), with infection of humans by FFV not having been reported, despite serological investigations of high-risk groups (Butera et al., 2000).

There is no conclusive evidence that feline spumavirus is a pathogen of cats (Alke et al., 2000, Pedersen et al., 1980, Winkler et al., 1999). FFVs are endemic in the cat population with no evidence of disease causality (Alke et al., 2000 Liniel, 2000, Meiering & Liniel, 2001). The greatest risk of spread would be to the feral cat population. However, FFV is cell associated and spread by contact or droplet, not by air. Cell free virus does not survive in the environment. Hence it is unlikely to spread outside the laboratory, especially when handled under containment level 2 conditions. FFV with a V5 modification would be replication competent, but again would be handled under containment level 2 and no hazards are associated with the V5 epitope.

FFV has not been shown to be pathogenic in cats and hence is unlikely to be pathogenic in other species. FVs experimentally infected into alternative hosts (HFV into rabbits) has been reported as non-pathogenic (Saib et al., 1997), with virus persisting in a defective form.

It is possible for baculovirus to express proteins in mammalian cells, but only when mammalian promotors are use. Hence there is a possibility of gene transfer (FFV GAG, POL and Env) to cats and humans. However, the GAG POL and ENV genes are not modified and the polyhedrin promoter will be used, so expression of these gene
products in humans or cats is unlikely. Recombinant baculovirus lacks the polyhedrin gene making it very susceptible to UV light, desiccation and digestion by insects. Its infectivity is also much lower than the parental virus.

The Bel1 gene is a transactivator. There is very little identify with the transactivator of human FVs, hence it is unlikely that Bel1 could control HFV. The eGFP, GAG, POL and ENV genes and V5 epitope pose no hazards. Alterations to the FFV envelope gene (insertion of a V5 epitope) may change the host range of the virus. Expression of FFV GAG, POL and ENV in baculovirus are unlikely to alter its host range.

REFERENCES


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be disposed of by autoclaving. Tissue waste will be bagged and incinerated. Liquid waste will be treated with fresh 1% Virkon (for FFV and baculovirus) or autoclaved directly (for bacterial work). Glass pasteur pipettes will be immediately discarded into a glass sharps bin for incineration. 70% ethanol will be applied to surfaces for use with E. coli and 1% Virkon for use with spumavirus and baculovirus. Also 10% Microsol (Anachem) will be used for culture surfaces (Incubator and laminar flow hood). Both Virkon and Microsol disinfectants will be used in accordance with manufacturer's recommended instructions to ensure 100% kill. Cultures of E. coli will be handled in a tray to contain spillages. Local rules for handling spills will be followed. Areas will be decontaminated with the appropriate disinfectant (see above). Contaminated lab coats will be placed in autoclave bags and autoclaved prior to washing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
This project was initially approved at containment level 1 in July 1994. Following the GM inspection in November 2002 the Committee were asked to review the risk assessment. As a result of the meeting of 19/02/03 it was decided that all work with viruses would be Class 2 unless they were sufficiently disabled and could therefore be downgraded to Class 1. Discussion re spumaviruses decided that the virus was not well classified and should therefore be a class 2 project and notified to the HSE.

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Project Ref 18/04.1

Date Ackn'd 15/03/2004

CU2 Project Title MODIFICATION AND USE OF VIRAL VECTORS FOR THE STUDY OF CANCER

Class 2

CultureVolClass2 < 1 litre

CultureVolumeClass3-4

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID 18/04.6

Date of Significant Change 07/09/2004
Purposes of the contained use

The aim of this project is to modify the Invitrogen Lentiviral "Gateway" vectors pLenti6/V5-DEST and pLenti4/V5-DEST (encoding blasticidin and zeocin resistance respectively), to create vectors that will allow the control of cloned gene expression (via LacO/LacR and IPTG) in cell lines and transgenic mice stably expressing the lac repressor (characterised in GMMs by Amann et al., 1983).

This will be achieved by the removal of the indigenous CMV promoter from the vectors and its replacement with the SV40 LacO promoter (SV40 promoter containing a lac repressor binding site between the TATA box and start of transcription - created by Hu and Davidson, 1987). This promoter is completely inactive within mammalian cells expressing lac repressor from the SyunLacR construct (lac repressor gene flanked by non-coding mouse B-globin sequences - created by Cronin et al, 2001), and active when IPTG (non-toxic lactose homologue) is added to remove lac repression. This promoter is also active within cells that do not express Lac repressor. The promoters UbC (Ubiquitous), Pax, Hox, H19, Oct4 and Nanog (stem cell-specific) are also to be modified with the lac repressor binding site and inserted into the pLenti vectors.

These regulatable vectors will be further modified to include an internal ribosome entry site (IRES) and yellow fluorescent protein gene (EYFP) downstream of the vector's multible-cloning "att" sites. The IRES-EYFP-poly A will removed from Clontech's pIRES-EYFP vector to achieve this. This feature will allow monitoring of the effectiveness of the inducible system via plasmid cotransfection experiments, and will add an important safety feature - if a worker were to contaminate their skin with lentivirus, the infected area would glow bright yellow under 513nm light (as produced by a standard fluorescence microscopy bulb). The EYFP protein is thought to be harmless as healthy transgenic mice that express it are readily producible (Hadjantonakis et al., 2002).

Initial cloning procedures will be performed in non-pathogenic, competent DH5a E.coli (Invitrogen).

Subsequent introduction of genes for expression into the pLenti vector "att" sites will be performed using pENTR plasmids and the Invitrogen Gateway recombinase-based cloning system. Non-pathogenic competent DB3.1 E.coli (Invitrogen) will be used for these latter cloning procedures. Human insulin-like growth factor 2 (IGF2 - over-expressed in a variety of cancers), IGF2R (Potentially anti-cancerous) or oncogene-silencing siRNA genes (Cancer Research UK), Luciferase, B-galactosidase, viral thymidine kinase are the genes that are to be introduced into these modified pLenti vectors.

The engineered pLenti vectors will finally be used to create replication-deficient lentiviruses using the Invitrogen VirPower kit. These lentiviruses will be used to infect and stably transduce mouse stem cell lines expressing lac repressor. Transduced mouse stem cells may be used to create transgenic mice following bastocyst injection, or to generate teratocarcoma tumours, when expanded cell populations are injected under the kidney capsule.

Recipient or parental organism

The bacteria to be used for cloning pose no hazard. The lentiviral vector does. It is non-replicative and non-pathogenic, but could trigger cancer or disease by random insertional mutation of human chromosomes. Once stably transduced into a cell, the lentiviral vector is present permanently, and the infected cell may not be detected by the immune system. If transduced into a germline cell or gamete it could become heritable. High titre viruses used in human gene therapy have resulted in insertional mutagenesis and leukaemia (Hacein-Bey-Abina 2003). The number of separate integration sites varies, but can be up to 20 depending on number of competent infectious particles. One way of limiting the number of viral integrations, and consequently the risk of insertional mutagenesis, is to adopt low viral titres for experimental testing and to adopt a split-component strategy with self-inactivating LTRs as described (Connolly 2002, Hacein-Bey-Abina 2003). Further, the presence of an insert means there is a possibility that lentivirus particles with altered physical properties could be generated, eg due to the presence of a transgene product on the viral envelope. To present this, only cells stably-expressing the SynLacR repressor will be used for lentiviral work.

One cells have been stably infected by viral integration, growth in culture will facilitate testing of cell phenotype in vivo. Infection and selection of mouse embryonic stem
Host/vector system

The LTR promoters in the pLenti vectors are disabled and the vector can only be packaged into infectious lentivirus particles inside SV40 large T antigen-expressing 293FT cells cotransfected with plasmids pLP1, pLP2 and pLP/VSVG (Invitrogen). The virus particles obtained can infect and stably transduce any mammalian cell, yet are completely replication deficient and lack any viral pathogenicity. The virus genomes also lack any substantial regions of homology to any other viruses, and self-disable their 3LTR upon chromosomal insertion, making recombination with active viruses very unlikely. Whilst these modifications improve the safety of these vectors, they are also more efficient and should retain the same containment approach as retroviral vectors.

The E. coli strains that will be used are all non-pathogenic K12 derivatives.

The plasmid vectors all have a safe history.

The inserts may code for proteins that are harmful to humans if over-expressed.

The lentiviruses, although non-replicative and made safe in many ways, are still potentially hazardous to humans.

In a breach of containment the lentivirus is a risk to human health, although not to the environment. The lentivirus is non-mobilisable and self-inactivating.

Origin & function

The most hazardous part of this project will be the creation of a lentivirus that expresses the human IGF2 gene (Obtained from ATTC or similar scientific sources). This is both a growth factor and oncogene (Hassan and Howell, 2000; Burns and Hassan, 2001). The creation of lentiviruses that express IGF2R or anti-cancer siRNA genes are potentially less hazardous, but still a danger. During these stages of the project (where infectious viral particles are present), stringent containment level 2 safety procedures will be followed. The genetic modifications will neither broaden the host range of the lentivirus nor alter pathogenic traits and tropism.

Use of an inducible system with lac repressor, lac operator modified promoters and IPTG with fluorescent reporter genes means the hazards are minimised. The functions of cancer promoters such as IGF2, and promoters of cancer regression (siGF2R) will be tested in mouse primary cells (fibroblasts and embryonic stem cells), embryos and transplanted tumours. The ultimate aim is to establish novel therapeutic proteins for the treatment of human cancer, delivered by protein infusion and not by these types of vectors.

Evaluation of foreseeable effects

One cells have been stably infected by viral integration, growth in culture will facilitate testing of cell phenotype in vivo. Infection and selection of mouse embryonic stem cells will facilitate the use of this technology to the generation of germline transgenesis. This will be achieved by injection of embryonic stem cells into the blastocyst to generate chimeras. No risk of furthe viral particle production is expected, as the vectors are all self-inactivating (Pfiefer et al 2002). The gene IGF2 should promote cell growth in all systems, with increased survival and proliferation of cells, increased in the size of mouse embryos. The antagonist, IGF2R, should prevent this occurring. Therefore, the considered inserts are potentially harmful to mammals. IGF2 is a growth factor and a potential oncogene. IGF2R is a membrane receptor for IGF2. siRNA genes that silence oncogenes may have undesirable effects in mammalian stem cells. IRES-EYFP is thought to be harmless. The presence of an insert also raises the possibility of creating recombinant lentivirus with altered physical properties (eg through expression of a transgene product on the envelope). To prevent this, only cells stably-transfected with SynLacR will be used for lentiviral work. Mutagenesis secondary to viral integration may occur, but the risk of this will be lessened by using low viral titres to infect. Careful monitoring of tumours and embryos will be performed to identify such events.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Work involving tissue culture cells and lentiviral particles has been provisionally assigned to containment level 2, but as potentially oncogenic viruses may be generated, additional physical barriers will be adopted to further minimise accidental exposure.

(1) GMM lentivirus production will occur in a lockable, HSE-specified containment level 2 tissue culture laboratory (F55, Pathology and Microbiology). There will be dedicated and labelled incubator, fridge and freezer space that will only be used for lentiviral work. There will be appropriate biohazard labelling, fly screens, a rodent control program, eye wash (along with other first aid provisions) and a notice of emergency contact telephone numbers and procedures. All workers will have appropriate training.
HSE biological containment level 2 guidelines are to be strictly adhered to.

Work involving open containers of lentivirus is to be carried out inside a services, labelled level 2 containment safety cabinet, that will not be used for any other work until first cleaned with 1% Virkon, then 70% alcohol, then internally treated for at least 1 hour with UVC light. The cabinet will be treated with formaldehyde fumes (outside working hours) prior to servicing.

A notice will be placed on the lab door prior to work in progress.

There will be no glassware, blades, needles or sharps used in work involving lentivirus particles.

Only 5 experienced molecular biologists may work with the lentivirus particles: Bibe are unnybicinorinuses,

Great care will be taken not to contaminate the mouth, skin or eyes with lentivirus.

There will be a surgical washbasin in the lab with handwash containing alcohol. Hands must be washed and dried before exiting the lab.

1% Virkon and 70% alcohol sprays will be kept to hand at all times for cleaning purposes and for decontamination of spillages. 70% alcohol is not to be sprayed near flames.

Workers must wear clean lab coats, goggles, surgical masks, and two layers of disposable blue vinyl gloves, which must be carefully removed (one layer at a time) for autoclaving after use. These protective items are not to be worn outside the tissue culture lab. Gloves must not be used to handle "clean" surfaces (eg telephone)

Splashes and the creation of aerosols will be avoided.

Screw-on caps are to be used to cover centrifuge rotor heads when in use.

Spills and accidents will be reported to the laboratory director.

Animals not involved in the work are not permitted in the lab.

ADDITIONAL MEASURES

A Bunsen is to be lit near any work involving unopened containers of lentivirus on the bench (eg study of lentivirus-producing tissue cultures under the microscope). This is to help protect workers from any aerosol resulting from accidental spillage.

Production of lentiviral transduced cells will be performed in bulk to minimise the number of times people are exposed to risk.

Containers (eg freezing vials) of lentivirus are not to be more than half-filled and always stored upright, in order to avoid contamination of lids (and subsequently of fingers).

Lentivirus work will always be stored on a bottommost shelf, so as to prevent any spillage contaminating work below.

Stable-transductants are not to be removed from the containment level 2 lab until they have been pelleted and washed at least ten times (in ten volumes of medium).
This should greatly reduce the number of infectious viral particles potentially present, but their presence can never be completely ruled out (by any test). As all constructs will have reporter genes, these will be used to determine the potency of viral integration.

(xx) Matrigel will be used to contain stably transduced cells for transplant into mice.

(xxii) If a worker suspects they have contaminated their skin with lentivirus, they should spray the affected area thoroughly with 70% alcohol, rinse thoroughly with water, then wash with soap. The skin should be subsequently monitored with 513nm light to see if there is any yellow fluorescence. If there is, this area could ater (if oncogenicity is suspected) be treated by laser surgery. Because lentivirus is an enveloped virus, alcohol or soap should be sufficient to inactivate it. Virkon is not suitable for use on human skin.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Liquid waste containing cellular debris and viral particles will be treated with 1% Virkon; plastic pipette tips and disposables will be completely submerged in 1% Virkon in a container in the safety cabinet for at least 1 hour prior to autoclaving and disposal. This applies to primary cell culture media, and post-cell wash supernatants which contain viral particles. All disposal will be performed within the dedicated class II tissue culture facility.

2. There will be a labelled, leak-proof stainless steel autoclave bin (with sealable lid) exclusively for the autoclaving of waste from the lentiviral work.

3. Any equipment used to work with stable transductants must be thoroughly cleaned after use (e.g. a flow cytometer should be flushed-through with paraformaldehyde, then extran, then water. The waste tank should be treated with Virkon prior to disposal of run-off).

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Discussed at committee meeting of 18 Feb 2004 and agreed that it was a class 2 activity and therefore should be notified to the HSE.

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Project Additional Information

Purposes of the contained use

The aim of the project is to enhance the quality of tissue engineered cartilage using gene transfer technology. Sense and antisense sequences of genes of interest will be transferred to mammalian chondrocytes or bone marrow stromal cells. These cells will be seeded onto scaffolds and incubated to generate a new engineered cartilage. Transient expression of sequences will be achieved using commercially available plasmid-based vectors while for long-term expression a commercially available pantropic retroviral vector system will be used. These gene-modified cells will be used for in vitro studies only.

Recipient or parental organism

A Pantropic Retroviral Expression System (Clontech UK) will be used to stably introduce recombinant DNA into dividing cells. This system produces high-titer virus capable of infecting the broadest possible range of mammalian and nonmammalian cells. The Pantropic System uses VSV-G, an envelope glycoprotein from the vesicular stomatitis virus that is not dependent on a cell surface receptor; instead, it mediates viral entry through lipid binding and plasma membrane fusion. The cell line included with the system is GP2-293, and HEK 293 based cell line. The cell line stably expresses the viral gag and pol proteins, but since the VSV-G envelope protein is somewhat toxic to cells, it is not integrated into the genome of the cell line. GP2-293 must be cotransfected with pVSV-G and an expression vector to produce high-titer, replication-incompetent virus. Virus produced can infect target cells and transmit target genes; however, it cannot replicate within target cells because the viral structural genes are absent. Studies of the transient expression will use standard disabled strains of E.coli for propagation of expression vectors. Target cells will include primary cells of musculoskeletal origin such as chondrocytes, synoviocytes and bone marrow stromal cells. These cells pose no significant risk to health although strict ACGM level 2 guidelines will be adhered to during cell isolation from tissue.

Host/vector system

02/03/2022
Laboratory facilities

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

If infection of humans were allowed to occur the outcome could be insertional mutagenesis of host cells.

ii) Exposure to open wounds: Individuals with open wounds will not be allowed access to the tissue culture facility.

i) Needle stick injuries: This project however does not require inoculation using needles and therefore there is no risk of needle stick injury.

The pantropic viruses produced are defective and helper-free. Although not infectious from one human to another they do not have the potential to infect humans through:

- experimental procedures involving the use of human tissue will be carried out in an ACGM category II facility.
- VSV-G envelope protein is toxic, this protein is expressed transiently from pVSV-G. Although the virus can infect target cell lines and transmit a target gene, it cannot replicate because the target cell lines lack the viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation. By using the minimal viral sequences and separately introducing the structural genes into the packaging cell line, the chance of producing replication-competent virus due to recombination events is minimized.

Evaluation of foreseeable effects

The vector to be used transient expression analysis is a pUC series plasmid that is non-mobilisable and propagates in standard disabled strains of E.coli. The vector to be used for long term-expression is pLXRN. It does not contain the structural genes necessary for viral particle formation and replication. The genes encoding the viral gag and pol proteins are stably integrated into GP-293. This cell line has a safe history of use and would be rejected by human hosts if accidentally inoculated. Because the VSV-G envelope protein is toxic, this protein is expressed transiently from pVSV-G. Although the virus can infect target cell lines and transmit a target gene, it cannot replicate because the target cell lines lack the viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation. By using the minimal viral sequences and separately introducing the structural genes into the packaging cell line, the chance of producing replication-competent virus due to recombination events is minimized.

The inserts to be used will include one of the following:

1) TGF-beta and IGF-1 - they drive cell growth and differentiation in a wide variety of cells. TGF-beta was shown to inhibit the initiation stages of oncogenesis but may promote tumour growth later. IGF - 1 is not known to have oncogenic effect on cells. The potential risk therefore of these genes incidentally transferring and expressing to human subjects is minimum.

2) Fibromodulins - these are structural proteins thought to control fibril formation during matrix synthesis by chondrocytes. Their gene product is not known to contribute or have an oncogenic activity.

3) Promoter sequences for genes such as telomerase or nucleostemin. The sequences will be cloned in a plasmid vector for transient expression to drive GFP or firefly luciferase genes. Both are established, harmless reporter genes.

The recipient cells will be primary mammalian musculoskeletal cells such as chondrocytes and bone marrow stromal cells. These cells are non-mobilisable and are therefore effectively safe. they do however carry a risk to human health that is inherent in all primary cells - they may be infected with an unknown infectious agent. All experimental procedures involving the use of human tissue will be carried out in an ACGM category II facility.

The pantropic viruses produced are defective and helper-free. Although not infectious from one human to another they do not have the potential to infect humans through:

- Needle stick injuries: This project however does not require inoculation using needles and therefore there is no risk of needle stick injury.
- Exposure to open wounds: Individuals with open wounds will not be allowed access to this culture facility.

If infection of humans were allowed to occur the outcome could be insertional mutagenesis of host cells.

Origin & function

A variety of genes will be inserted in either vector. The genes of their antisense can be divided into 3 categories: 1) Growth and differentiation genes: this will include TGF-beta and IGF-1. Both are established growth factors that enhance matrix synthesis by chondrocytes and drive the differentiation of bone marrow stromal cells to chondrocytes. Such growth factors may be involved in promoting tumours activity, however their role in initiating tumours is unknown. 2) Structural genes: these include fibromodulins such as decorin, lumican and fibromodulin. these are small leucine rich proteoglycans believed to control collagen fibril size during fibrillogenesis. 3) Promoter sequences: this includes telomerase gene promoter and nucleostemin gene promoter. The study will involve detecting telomerase activity or nucleostemin expression in transfected cells using vectors encoding green fluorescence protein (GFP) or firefly luciferase protein under the control of each gene-specific promoter. The promoter sequences should not have adverse effects on transfected cells since they do not drive the endogenous genes.

For long-term expression studies, the vector to be used is pLXRN from Clontech (Catalogue K1063-1). It contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV), and is designed for retroviral gene delivery and expression. The vector contains the extended retroviral packaging signal, w+, which promotes high-titer virus production and long terminal repeat (LTR) promoter for driving high level expression of target gene. pLXRN can be cotransfected with pVSV-G into the GP-293 Packaging Cell Line (Burns et al 1993) to produce infectious, replication-competent pantropic retrovirus. Dr Jane Burns, the creator of the system, has provided us with written approval for transducing human cells using this system. Indeed, the system has recently been used to infect huan bone marrow stromal cells in vitro (Transfiguracion et al 2003). For transient expression studies, pUC series plasmid vectors, such as pcDNA6 from Invitrogen (Catalogue V22220), will be used. These vectors are designed for overproduction of recombinant proteins in mammalian cells under the control of promoters such as the cytomegalovirus (CMG) promoter or the human elongation factor -1 (EF-1) promoter.
The Academic Rheumatology Group is based on the ground floor of the Avon Orthopaedic Centre at Southmead Hospital, Bristol which is accessible to University staff only. All experimental procedures using the retroviral vectors to transfect human cells, as describe in the attached risk assessment, will be carried out in a recently refurbished ACGM category II facility that is accessible only to trained scientific personnel. As well as negative air pressure generated by the Class II cabinets within the facility, this lab has been designed with and anti-room to prevent the air within the laboratory mixing with the air outside.

Personal exposure
To prevent exposure of skin to naked DNA personal protective clothing (fully-fastened Howie coats, gloves and safety spectacles) will be worn during the experimental procedure. Sharps and needles are not required for this procedure and therefore will not pose a risk. Tissue culture flasks will only be opened inside a class II safety cabinet that undergoes a routine safety check and KI inspection every 6 months.

RISK
When high class II containment levels and controls are in place the risk will be LOW-EFFECTIVELY ZERO.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Means of Inactivation
All solid waste used in this project (plastic pipettes and tissue culture flasks) will be soaked in a 1:100 dilution of Virkon S, and anti-viral agent commercially available from Antecint International. Details of virucidal properties against retroviruses are available on http://www.antecint.co.uk/. Briefly, testing of Virkon against retroviral vectors was carried out at the Central Veterinary Laboratory in November 1997 and a 1:100 dilution was shown to be effective against retrovirus. After soaking in 1:100 dilution of Virkon S for 24 hours the solid waste will be autoclaved at 120 degrees for 15 mins prior to incineration. The autoclave is housed in a small room adjacent to the tissue culture facility. All liquid waste will be treated with Virkon at the appropriate dilution then autoclaved. These measures will be expected to result in 100% kill of retroviruses or their host cells.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

<table>
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</tr>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

The purpose of this project is to understand how regulatory proteins control the formation and turnover of cytoskeletal structures and how these structures contribute to cellular processes such as migration. Lentiviral vectors will be used to infect mammalian immune cells with the cDNAs of cytoskeletal regulators (CRs) to assess their function in cells. Lentiviruses are very efficient vectors for this type of cell which are otherwise very hard to transfect.

**Recipient or parental organism**

The recipient cells will primarily be obtained from wild type and transgenic mice engineered to lack specific cytoskeletal regulators (for example the Wiskott Aldrich-Syndrome Protein, WASP). Immune cells will be purified from bone marrow and spleen and grown in culture. Other cell types may also be purified in a similar fashion. All murine cells/dead mice will be obtained from external sources (not from animals kept within the University). Primary human cells may also be harvested, passaged and transfected in a similar fashion.

**Host/vector system**

The vector used for transfection of cells is an HIV based lentiviral vector. This has been VSV-G pseudotyped giving it a wide cell type target range. It has a heterologous spleen focus forming virus (SFFV) LTR. It contains a U3 deletion in its 3' LTR and is self-inactivating. It is derived from the HIV provirus and has been engineered such that the virulence factors - vpr, vif, vpu and nef genes have been deleted. Pol, rev and tat gene3s are also deleted. Gag and env genes have been truncated and inactivated. Therefore, the viral RNA encompasses, from the 5’ to 3’ end, the HIV 5’ LTR, the leader sequence and the 5’ splice donor site, truncated gag, the rev response...
element (RRE) and a splice acceptor site, an SFFV promoter, cytoskeletal regulator cDNA, and the HIV 3' LTR with U3 deletion.

Origin & function

The cDNAs delivered by the lentiviral vector will be of human, bovine or rodent origin. They may be tagged at their 5' end with enhanced green fluorescent protein (EGFP). WASP/WAVE are proteins that can induce actin polymerisation within cells and are therefore capable of changing cellular morphology. They have no reported oncogenic properties. Wild type and constitutively active and inactive WASP mutants will be studied with respect to their ability to alter the actin based cell morphology of mammalian immune cells and related functions such as endocytosis. These aspects will be monitored using fluorescent microscopy. cDNAs of other regulatory proteins such as protein kinases and Rho family GTPases that impinge on cytoskeletal function may also be used. Examples of kinases used include wild type dominant negative forms of Src family kinases such as Hck. While v-src has oncogenic properties, wild type Src kinases are not known to be oncogenic. RhoGTPases are required for Ras transformation of cells but are not known to have oncogenic properties on their own. To reduce the risk of any oncogenic activity, activated mutants of these molecules will not be used.

Evaluation of foreseeable effects

The host range of the recombinant virus includes human, rat and mouse cells. The virus is self inactivating ensuring that non-specific promoter activity is eliminated. This greatly reduces the risk of non-specific activation of oncogenes following viral insertion. The virus is replication deficient ensuring that the virus will not spread should containment be breached. The insert of cDNAs do not encode active forms of any known oncogenes, significantly lowering the probability that the insert of cDNA will induce oncogenic transformation of infected cells. The risk to humans or the environment should escape/infection occur is extremely low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be autoclaved.
All liquid waste will be treated with 10% chloros followed by autoclaving.
Surfaces will be cleaned with 10% chloros/virkon as appropriate.
All waste will be kept separate from other material.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project discussed by Committee 23/04/2004, confirmed that ACGM Class 2 appropriate and to be notified to HSE.

Project Containment
Project Ref 18/04.5

Date Ackn'd 31/08/2004
Date Project Ceased

CU2 Project Title Genetic manipulation of coronavirus genomes to elucidate the functions of the viral RNAs and proteins

Class 2
Consent Granted Non-GMM

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

The purpose of the contained use is to produce recombinant coronaviruses and autonomously replicating coronavirus RNAs (replicons) that contain defined mutations in amino acid and nucleic acid sequences. This will allow us to investigate the role of cis-acting RNA elements and individual viral proteins in the replication and pathogenesis of coronaviruses. By studying the biological and biochemical properties of recombinant viruses and replicons, our understanding of coronavirus replication and novel antiviral control agents in the future.

Experimental details

In order to produce recombinant coronaviruses and replicons, we shall use a system that is based on vaccinia virus cloning vectors (Thiel and Siddell, 2004). The basic strategy for the generation of recombinant coronaviruses can be divided into three phases.

The assembly of a full-length coronavirus genomic cDNA. This normally involves the generation of numerous subgenomic cDNA fragments that are either amplified as
bacterial plasmid DNA or prepared in large amounts by preparative reverse-transcriptase polymerase-chain reaction (RT-PCR). The cDNAs are then ligated sequentially, in vitro, to produce a small number of cDNAs that encompass the entire genome. The specific ligation strategy is dictated by the sequence of the coronavirus in question but a common feature is the use of convenient, naturally occurring or engineered restriction sites. It is also necessary to modify the cDNAs that represent the 5' and 3' ends of the coronavirus genome. Normally, a transcription promoter sequence for the bacteriophage T7 RNA polymerase is positioned upstream of the coronavirus genome and a (unique) restriction site, followed by the hepatitis ribozyme is placed downstream of the poly-A tail of the coronavirus genome. The terminal cDNA constructs must also have appropriate restriction sites to facilitate cloning into a unique NotI restriction site present in the genomic DNA of vaccinia virus, strain vNotI/tk.

The cloning and propagation of the coronavirus genomic cDNA in vaccinia virus vectors. The next stage is to ligate, in vitro, the coronavirus cDNA fragments and the long and short arms of NotI-cleaved vMptI/tk genomic DNA. This ligation is done in the presence of NotI to prevent religation of the vaccinia virus DNA. Subsequently, the ligation reaction is transfected into mammalian cells that have been previously infected with fowlpox virus. Recombinant vaccinia virus, the genome of which includes a full-length copy of the coronavirus genome, is rescued.

Rescue of recombinant coronaviruses. Essentially, recombinant coronaviruses are rescued by generating genomic-length RNA transcripts from the coronavirus component of the recombinant vaccinia virus DNA template. These transcripts are then transfected into permissive cells. The transcription reaction is normally done in vitro but it is also possible to rescue recombinant coronaviruses via the transcription of template DNA in the permissive cell itself. Also, we have found that the ability to rescue recombinant coronaviruses is significantly enhanced (but not dependent upon) the directed expression of the coronavirus nucleocapsid protein in the transfected cells.

Rescue of recombinant coronavirus replicons. Coronavirus replicons are comprised of the cis-acting elements and viral genes (the non-structural or replicase protein genes) necessary for replication and transcription of the viral RNA. They are devoid of one or more of the viral structural protein genes and, therefore cannot give rise to infectious virus particles. They are constructed as described above for recombinant coronavirus genomes and are rescued by generating replicon-length RNA transcripts from the coronavirus component of the recombinant vaccinia virus DNA template. These transcripts are generated in vitro using bacteriophage T7 RNA polymerase and are then transfected into permissive cells. They may be transiently replicated or, if a gene encoding a selectable marker is incorporated into the replicon, they may be propagated in a stably transfected cell line.

Vaccinia virus-mediated homologous recombination to introduce specific mutations into the coronavirus genomic cDNA. One major advantage of using vaccinia virus as a cloning vector is that the cloned coronavirus cDNA is amenable to site-directed mutagenesis using vaccinia virus-mediated homologous recombination. Essentially, the procedure is based on using the E. coli guanine-phosphoribosyl transferase gene (gpt) as both a positive and negative selection marker. First, a specific region of the coronavirus genome is replaced by the E. coli gpt gene. To do this, we transfect recombinant vaccinia virus-infected CV-1 cells with a plasmid DNA containing the E. coli gpt gene located downstream of a vaccinia virus promoter and flanked by coronavirus sequences that facilitate recombination. Two days post-infection/ transfection, a vaccinia virus stock is prepared and gpt-containing vaccinia viruses are isolated by plaque purification under gpt-positive selection in the presence of mycophenolic acid, xanthine and hypoxanthine. In a second step, we replace the E. coli gpt gene by the mutated coronavirus gene. CV-1 cells are infected with the gpt-containing vaccinia virus and transfected with a plasmid DNA encoding the mutated coronavirus gene. Again, after two days, a vaccinia virus stock is collected from the infected/transfected cells and recombinant viruses are isolated on HeLa-D980R cells under conditions that allow for the selection of vaccinia viruses that have lost the expression of gpt.

Recipient or parental organism

**Donor organisms - non-recombinant coronaviruses**
**Parental organisms - non-recombinant vaccinia virus (strain WR), non-recombinant fowlpoxvirus.**

**Recipient organisms - recombinant coronaviruses (GMO), Recombinant coronavirus replicons (GMO).**

In addition, subgenomic coronavirus cDNA fragments will be cloned in E. coli K-12 derivative DH5a using plasmids of the pUC, pBluescript, pGEM, pBR322 and prSET plasmid lineages. This work is registered as Project 618 at the University of Bristol and has been approved at ACGM containment Level.
Non-recombinant coronaviruses
Coronaviruses are enveloped, positive strand RNA viruses that are associated with respiratory and enteric disease in animals and man. In livestock and companion animals, coronaviruses are responsible for diseases of economic importance and they represent a significant burden on animal health and welfare. Coronaviruses are generally considered to be species specific, although the possibility of zoonotic transmission to humans clearly exist. PLEASE NOTE: THIS APPLICATION DOES NOT INCLUDE THE SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS (SARSCoV).

Vectro organisms - recombinant vaccinia virus (GMO) recombinant N-protein expressing BHK cells (GMO)
Host organisms - non-recombinant BHK cells, human MRC-5 cells, human HRT-18 cells, monkey CV1 cells, murine 17 clone 1 cells, avian CK cells, freline FCWF cells, porcine ST cells, canine A72 cells.

Coronaviruses are pleomorphic but roughly spherical enveloped particles, 120-160 nm in diameter with a characteristic 'fringe' of surface projections 20 nm long. The genome of coronaviruses is a positive-strand RNA molecule of c. 27 000-31 500 nucleotides. The coronavirus genomic RNA has a 5' cap structure and is polyadenylated at their 3' end. The genomes of coronaviruses contain between 6 and 11 functional ORFs. The easiest to define are those located toward the 3' end of the genome, encoding the structural proteins: the surface (S), membrane (M), envelope (E) and nucleocapsid (N) proteins. The genomes of a subset of coronaviruses (eg HCoV OC43, MHV, BCoV) also contain an additional structural protein gene, the haemagglutinin esterase (HE) ORF. In ORFs that encode non-structural proteins, the coronavirus genome is dominated by the replicase (rep) gene (also referred to as the RNA polymerase gene or RNA polymerase locus). The replicase gene consists of 2 large ORFs encompassing 20 000 nt toward the 5'-end of the genome. In contrast to the replicase gene, the pattern and arrangement of the remaining accessory protein ORFs in the genomes of coronaviruses depend on the virus species. The 3' NTR of coronaviruses comprises 200-500 nt following the last functional ORF and preceding the polyadenylate tract.

Poxviruses
Non-recombinant vaccinia virus (strain WR)
Vaccinia virus is the prototype of the genus. Orthopoxvirus. It is a double-stranded DNA virus that has a broad host range. Multiple strains of vaccinia virus exist that have different levels of virulence for humans and animals. The WR strain, which is able to replicate in human cells, is a mouse neuroadapted derivative of the NYCBOH strain. Vaccinia virus can be genetically engineered to contain and express foreign DNA with or without impairing the ability of the virus to replicate. The outcome of accidental laboratory infection with vaccinia virus is varied. In immune persons the infection is usually mild and self-limited. In non-immune, non-immunosuppressed persons, the development of disease is largely dependent upon the site and dose of inoculation and may be mild or moderately severe. In immunosuppressed persons the infection may be systemic and severe.

Non-recombinant fowlpoxvirus (strain HP1.441)
Wild-type fowlpox viruses (FPV) cause proliferative skin lesions that are occasionally lethal in birds. The attenuated FPV strain HP1.441 was obtained from wild-type strain HP-1 by 441 passages in chick embryo fibroblasts.

Non-recombinant cell lines
A variety of continuous cell lines will be used as host organisms for coronavirus replicons. All lines have been screened for bacterial contamination, mycoplasma contamination and the production of virus particles as described by the ATCC.

Vector/recipient organisms
Recombinant vaccinia virus (GMO)
The vaccinia virus vector, vNotI/tk, has been derived from parental vaccinia virus (strain WR) by elimination of a unique NotI site in the Hind/III genomic DNA fragment F and the introduction of a new NotI site immediately after the translation initiation codeon of the thymidine kinase (tk) gene.

Full-length cDNA copies of the coronavirus genome will be inserted at this unique NotI site. The coronavirus sequences are flanked at the 5' end by a bacteriophage T7 promoter and at the 3' end by unique restriction enzyme cleavage sites (which differ depending upon the coronavirus cDNA in question) and a hepatitis virus ribozyme.
Recombinant nucleocapsid protein expressing BHK cells (GMO)

The rescue of recombinant coronaviruses is facilitated by the co-expression of nucleocapsid protein in BHK cells that are transfected with genomic RNA. In order to provide directed expression of the coronavirus N protein, we have produced stable BHK-21-derived cell lines that express coronavirus N proteins. These cell lines are based on the Tet/ON expression system and thus allow the controlled expression of the respective N proteins upon induction and doxycyclin.

Known hazards (e.g., toxicity, or oncogenicity) have not been associated with any of these proteins. Using the strategy described, we will produce a collection of mutated, recombinant coronaviruses. The mutations will be of several types. First, we will produce mutants that fail to express one or more viral genes as a functional protein. This will be achieved by deletion of the coding sequences from the genome, ablation of the cis-active elements necessary for transcription of specific mRNAs or mutation of the initiation codons for the translation of specific ORFs. Second, mutants will be produced by the alteration of catalytic site and/or structural residues when the enzymatic or structural determinants(s) of a specific protein have been identified or predicted by bioinformatics analysis. Third, we will introduce mutations that affect the structural integrity of cis-acting regulatory elements. The consequences of these mutations will be monitored in a number of different assays. In the context of recombinant coronaviruses, we will measure the kinetics and patterns of viral replication in cell culture. In collaboration with colleagues in the USA and Europe, we will also monitor the replication and pathogenesis of mutant viruses in appropriate animal models. These experiments will NOT be done in the UK. ALSO, PLEASE NOTE: WE WILL NOT PRODUCE INTERSPECIES, CHIMERIC RECOMBINANT CORONAVIRUSES. This is because it has been shown that, for example, the exchange of the murine and feline coronavirus surface protein ectodomains can alter the species specificity of coronaviruses in cell culture. We believe it is impossible to predict the biological properties of chimeric coronaviruses at the present time. In the context of the recombinant coronavirus replicons, we will monitor both replication and transcription by reporter gene assays in cell cultures.

Evaluation of foreseeable effects

Working with vaccinia virus

- The following people will be excluded from working with vaccinia virus:
  - People who have, or once had, skin conditions, (especially eczema or atopic dermatitis) or have temporary skin conditions such as burns, herpes or severe acne.
  - People with weakened immune systems, such as those are receiving steroid treatment
  - Pregnant women or women who are breastfeeding
  - People with known heart disease

- People who are allowed to work with vaccinia virus will be:
  - Regular health surveillance by the Occupational Health Nurse and referral to Occupational Health Physician if required
  - Consenting senior scientific or technical staff with training in good microbiological practice at ACGM containment level 2
  - People who are aware of the unusual stability of vaccinia virus and the most common routes of infection
  - People who are aware of the symptoms (especially skin lesions) of vaccinia virus infection and the possibility of transmission to close contacts (including pregnant women or immuno-compromised persons)
  - People wearing protective clothing, including disposable gloves and eye protection

In addition to the standard ACGM level 2 containment procedures, the following local rules will apply:

- Sharps will not be used in any procedure involving vaccinia virus.
- All manipulations with open vessels containing vaccinia virus will be done in Class II biological safety cabinets.
- Plugged pipettes and appendor tips will be used
- Transport of vaccinia virus cultures will be in sealed vessels
- Centrifugation of vaccinia virus cultures will be in sealed bottles
- Centrifuge rotors will be routinely decontamination with either 70% ethanol or 10% SDS
- Any spillages will be immediately decontaminated with 70% ethyl alcohol in water or a 2% sodium hypochlorite solution

In accordance with current practise in the UK, persons working with vaccinia virus will not be routinely vaccinated. Access to areas in which vaccinia virus is handled will be
restricted to authorised workers. All personnel with access to the ACGM level 2 laboratory will be made aware of the symptoms of vaccinia virus infection.

Please note that we have also considered using highly attenuated strains of vaccinia virus (such as MVA) as virus vectors for the coronavirus reverse genetic system. However, this is not possible because MVA has become extensively host range restricted during multiple passage in chick embryo fibroblast cells (essentially the only cell in which it will now replicate efficiently). As, in our system, the recovery of recombinant vaccinia virus from DNA is dependent upon using fowlpox as a helper virus, the use of CEF cells as a host is excluded.

The insertion of coronavirus genomic cDNA in the vaccinia virus genome

Insertion of non-expressed foreign sequences at the vaccinia virus tk locus generally attenuates the pathogenicity of the recombinant vaccinia virus compared to the parental strain. In our strategy, coronavirus genomic cDNAs will be inserted in to the tk locus, downstream from the weak, early tk promoter. Thus, vaccinia virus RNA polymerase activity could, potentially, result in transcripts that include the complete coronavirus genome and, at least theoretically, might give rise to a productive coronavirus infection. However, for a number of reasons, we consider this extremely unlikely. First, the coronavirus cDNA sequences will be flanked at their 5’ end by heterologous bacteriophage and vaccinia virus elements, which should prevent the addition of authentic “cap” structures that facilitate the infectivity of the coronavirus genomic RNA. Second, the vaccinia virus early RNA polymerase termination signal (US5NU) occurs many times in all coronavirus genomic sequences (for example 18 times in the MHV sequence). Thus, it is extremely unlikely that vaccinia virus early RNA transcription will be able to produce a full-length copy of the coronavirus template. Finally, if, against all expectations, an infectious coronavirus genomic RNA was produced in a cell that had been infected with recombinant vaccinia virus, we also think it would be extremely unlikely that a productive coronavirus infection would be established. Vaccinia virus replicates much faster than any coronavirus and essentially destroys the integrity of cellular membranes which are the sites of coronavirus replication. Field trials with recombinant vaccinia virus that express rabies virus antigens have shown that there is little evidence of animal to animal spread of vaccinia virus in the environment.

For these reasons, we are confident that even in the event of a breach of containment, recombinant vaccinia virus vectors would be unlikely to cause disease or illness in humans, animals or plants and they are unlikely to cause adverse effects in the environment.

Recombinant coronaviruses

The available evidence suggests that the ablation or mutation of coronavirus protein genes leads to attenuation of the recombinant virus. For example, de Haan and colleagues have shown that deletion of the MH V accessory protein nspX4 and nspX5a has no effect upon virus replication in tissue culture but severely attenuates virulence in vivo. Similarly, Rottier and his colleagues have shown that deletion of the feline infectious peritonitis nspX3 gene cluster, or the nspX7a and nspX7b genes attenuates virus replication in kittens but not in cell culture. Also, Lavi and colleagues have identified mutations in the surface protein gene of the murine hepatitis virus that abolish demyelination or interfere with virulence and hepatitis. Thus we have no reason to expect that deletion or alteration of coronavirus genes will increase the virulence, pathogenicity or species tropism of the virus.

Furthermore, it is well known that recombination is an important component in the evolution of coronaviruses. Therefore, it is likely that deletion mutations of the type we intend to introduce, occur at high frequency in nature. However, as they are at a selective disadvantage, these mutations will not evolve as a major component of the viral quasispecies.

For these reasons, we are confident that even in the event of a breach of containment, recombinant coronaviruses would be unlikely to cause more severe disease or illness in humans, animals or plants than the parental viruses and they are unlikely to cause adverse effects in the environment.

Recombinant coronavirus replicons

Per definition, coronavirus replicons will not produce infectious virus particles and will not have the ability to spread in the extracellular environment. Also, the replicons we produce will only be maintained within the cell under selection with antibiotic analogues. Once the selection medium has been removed, the replicons will be lost from the cell. Moreover, coronavirus replicons are non-cytopathogenic and, as far as is known, do not exert or confer any deleterious or pathogenic properties on the cell. Also, we will not introduce any replicons or sets of replicons to the cell that contain genetic elements that could, theoretically, recombine to produce infectious virus particles.

For these reasons, we are confident that even in the event of a breach of containment, recombinant coronavirus replicons would be unlikely to cause disease or illness in humans, animals or plants and they are unlikely to cause adverse effects in the environment.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All biological waste containing GMOs will be inactivated before disposal in accordance with the University Biosafety regulations. Liquid waste (used cultures, culture media, supernatants, and any other solutions which have come into contact with infectious material or live cells will be inactivated by spraying with 2% Virkon and autoclaved at 121 degrees C for 30 minutes before disposal to ensure 100% kill rate of the GMO. Contaminated glassware, re-usable plasticware (eg centrifuge bottles and tubes) and solid waste will be similarly disinfected by spraying with 2% Virkon before being autoclaved before disposal, washing or reuse. The autoclave to be used is housed in the building and is maintained regularly. Each cycle will be monitored using an approved thermosensitive testing strip. Pipettes will be disinfected before being washed in the normal manner by total immersion for a minimum of 16 hours in a pipette jar containing a freshly prepared 5% solution of hypochlorite which is made up daily. The pipette disinfection procedure will be tested before use and then periodically to ensure the disinfection procedure maintains a 100% kill rate. Laboratory coats will be autoclaved before being sent for laundry.

Materials that contain or have come into contact with parental or recombinant vaccinia virus will be double-bagged before autoclaving and, after sterilisation, will be disposed of by burning. Any spillages will be decontaminated by wiping with 70% ethyl alcohol in water or a 2% solution of sodium hypochlorite. Accidents, incidents and infections will be reported to the University Safety Officer and Competent Authority.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Agreed by Committee that use of coronavirus would require a Class II designation and thus to be notified to HSE.

Project Containment

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Viral-mediated expression of signalling and regulatory proteins associated with G-protein coupled receptors.

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Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

The proposed work aims to study mechanisms underlying G protein-coupled receptor (GPCR) desensitisation. This will be achieved by expression of GPCRs and other related signalling molecules in mammalian cells in culture or in slice preparations of mature CNS neurons infected with recombinant viruses either directly or by prior injection into the brain of live rodents. Expression will be mediated by recombinant adenovirus when transient expression is adequate. Recombinant lentivirus will be used when prolonged expression is required and Sindbis virus for short term experiments on brain slices where very high expression levels are desirable.

**Recipient or parental organism**

Adenovirus serotype 5 with deletion of E1 gene to render it replication incompetent.

Lentivirus. The virus used is based on HIV, pseudotyped with alternative envelope proteins eg. VSV-G. The virus used is defective, requiring packing genes and the envelope gene to be provided by helper plasmids, and is engineered to be self-inactivating following integration.

Sindbis virus in which the genes encoding structural proteins have been deleted and must be supplied on a helper construct.

Rats, primary cultures of rat neurons and established mammalian cell lines will be infected with virus.

**Host/vector system**

Recombinant adenovirus and lentivirus will be injected directly into rat brain.

Recombinant sindbis virus will be injected into slices of rat brain in culture.

All three virus types will be used to infect mammalian cells in culture, including established cell lines and primary cultures of rat locus coeruleus and other neurons.

**Origin & function**

The genetic material to be expressed encodes G-protein coupled receptors, in the first instance splice variants of the rat u-opioid receptor (MOR1) and other signalling proteins.
molecules, including wild type and mutant forms of G protein-coupled receptor kinases (GRKs), protein kinase C (PKC) isoforms, arrestins, dynamin and EPS-15. These are from a variety of mammalian species, including rat, human and bovine cDNAs. Their intended function is to modify the response of MOR1 in order to elucidate the mechanism of action of morphine and other agonists of this receptor.

Evaluation of foreseeable effects

The inserted DNAs do not encode known oncogenes, however the mutant versions of these signalling molecules could potentially interfere with the function of the endogenous proteins. However, all vectors used are disabled and are highly unlikely to survive long enough in the environment for gene transfer to occur.

Adenovirus can infect humans but the use of a replication-deficient virus, a class II biological safety cabinet, an ACDP category 2 facility, the high degree of worker training and awareness and methods of waste disposal and disinfection (see below) will greatly minimise the risk to the worker. The vector used cannot replicate in host cells unless the E1 sequence is provided in trans (eg in HEK 293 cells). The risk of recombination to give replication competent virus is extremely low.

The lentiviral vectors are derived from HIV. The system proposed here uses a 2nd generation packaging system which lacks auxiliary viral genes. Furthermore, the transfer vector contains a self-inactivating sequence so that the transcripional capacity of the viral long terminal repeat (LTR) is lost after transfer to target cells. This minimizes the risk of emergence of replication competent recombinants (RCR) and avoids problems linked to promoter interference. ACDP category facilities are appropriate for these disabled viruses.

Sindbis virus has a low level of pathogenicity in humans and handling in a class II biological safety cabinet in an ACDP category 2 facility is appropriate. The recombinant particles produced by co-transfection of the replicon RNA and helper RNA can infect cells but have little or no plaque forming capability. The helper RNAs lack a packaging signal so replicon RNA is the predominant RNA packaged. However, helper RNA can be packaged at very low levels and hence pseudovirion preparations may have a very low capacity for replication.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Infected rats will be housed in class 2 facilities until the first feed post-infection and all waste autoclaved before disposal. Subsequently, it is extremely unlikely that any infectious virus remains and no further special precautions are necessary.

Rats are housed in cages in a secure facility.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All virally infected solid and liquid waste will be collected into closed containers. It will be autoclaved by the Department of Pathology and Microbiology's waste disposal service, to give 100% kill before final disposal by incineration. These facilities are already used to dispose of viral waste from other projects in the School of Medical Sciences. Syringes used for injecting rat brains will be disinfected with 1% virkon and work surfaces swabbed with 1% Virkon after use. Virkon solution will be replaced weekly of when colour fades, if earlier, in accordance with manufacturer's recommendations.
This proposal was considered in detail and approved by the University of Bristol's Biological and Genetic Modification Safety Committee (BGMSC) at its meeting of 7th July 2005. No significant issues were raised by members of that Committee.

**Project Containment**

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**Project Ref** 18/06.1

- **Date Ackn'd**: 01/12/2006
- **CU2 Project Title**: Functional analysis of genes involved in childhood cancers.
- **Class**: Class 2
- **CultureVolumeClass2**: < 1 Litre
- **Consent Granted**: Not Applicable
- **Non-GMM**: N

- **Tick if notifying a connected programme of work**: N
- **Project notified under transitional arrangements**: N

**Project Additional Information**

**Purposes of the contained use**

The overall aim of this project is to modulate the expression of genes involved in the development of human cancers (specifically childhood cancers), in order to study their function in normal development and in carcinogenesis. Gene expression will be modulated in cultured cells.
The work will involve 2 complimentary strategies:
1) Knockout the genomic sequences controlling the expression of cancer-associated genes using recombinant adeno-associated virus (AAV).
2) Knock-down of the expression of cancer-associated genes, using RNA silencing (siRNA) via the introduction of lentiviral vectors.

Recipient or parental organism

The initial cloning of recombinant plasmids required for the production of the final viruses will be carried out in K12 derivatives of E.coli and therefore non-hazardous.

Human cultured cells used to produce virus (293 cells) and the cells that will be the target of viral infection are well-established cell lines and therefore non-hazardous.

Ad-CMV-cre (recombinant adenovirus expressing are recombinase for the activation of pSico/pSicoR in lentiviral transduced cells) is replication deficient and therefore non-pathogenic. The nature of the insert (cre) makes it of low risk to human health.

Host/vector system

1) Recombinant adeno-associated virus (AAV) used to knockout genomic sequences controlling the expression of cancer-associated genes.

The system to be used is a gene knockout system based on adeno-associated virus (AVV), a small, ubiquitous single-stranded DNA virus of the Paroviridae subfamily. Kohi et al (2004) Facile methods for generating human somatic cell gene knockouts using recombinant adeno-associated viruses. Nucleic Acids Res. 32 (1):e3. Genomic DNA sequences flanking the region to be knocked out are amplified by PCR, using tagged primers. PCR-based recombination is then used to produce a fusion of these sequences with an antibiotic resistance cassette. The fusion product is then cloned into the AVV vector using standard E.Coli K12 systems. This plasmid is transfected into 293 cells with 2 other helper plasmids and recombinant AVV produced. The recombinant AAV is used to infect cultured human cells (in this case established human cancer cell lines) and hopefully the insert undergoes homologous recombination with the targeted sequence and the antibiotic cassette replaces the sequence, allowing selection of resistant clones. These clones are then grown up, characterized to determine whether the knock-out has been successful; and used in vitro experiments.

This is a "gutless" vector in which all Rep and Cap genes have been deleted, leaving the cloned insert flanked by ITRs. Recombinant virus is thus replication-deficient. Recombinant virus will be produced by co-transfecting the AAV recombinant plasmid into 293 cells, together with pAAV-RC plasmid and pHelper (both commercially available from Stratagene). 293 cells contain adenoviral E1 genes, which together with E2A, E4 and VA genes on proteins needed for AAV replication and packaging are provided by the pAAV-RC.

The advantage of the AVV system is its ability to infect a wide range of human cells and in the specific example here, to produce high efficiency gene knockout. There is a lot of data showing safe use of AVV vectors as both laboratory vectors and gene therapy vectors in humans. AVV vectors used in humans have elicited minimal immune response and no acute toxicity. In vivo experiments have failed to rescue recombinant AAV by superinfection with helper virus. In AVV vectors, site-specific integration is lost, so insertional mutagenesis is a theoretical possibility. In a single report, the use of an AVV vector in mice caused liver tumours but without evidence of insertional mutagenesis and it is unclear what caused these tumours.

2) Lentiviral vectors used to knock-down the expression of cancer-associated genes, using RNA silencing.

The system to be used delivers short hairpin RNAs (targeted to the genes of interest) into cultured cells using a lentiviral vector, in order to down-regulate target gene expression in cultured cells via RNA interference. Oligos containing shRNAs will be ligated into the lentiviral vector, cloned in standard E.Coli K12 and the recombinant lentiviral vector will then be used to infect established human tissue culture cells. The vector system is a cre-regulation with a cre-expressing recombinant adenovirus.

The vectors to be used are third generation lentiviral vectors, which use a split-genome conditional packaging system and a self-inactivating terminal repeats in the final lentivirus, to ensure that the virus is replication-deficient. The packaging system (ViraPower; Invitrogen) uses 3 separate vectors to provide essential viral genes:plLP1 deficient. The plasmids have all been engineered to remove regions of homology, to prevent recombination events that could lead to the production of replication competent virus. The plasmids are co-transfected into 293 cells to produce viral particles. The viral particles, although replication-deficient, are capable of infecting human
cells and the pSico and pSicoR vectors contain the WPRE sequence, which has been suggested to be potentially oncogenic (SACGM information note, 19th November 2004).

The lentiviral vector is HIV-based and there the theoretical possibility of recombination with endogenous retrovirus (as is true for any retroviral delivery system). However, there are no HIV accessory genes in the lentiviral system, so there should be no chance of generating wild-type virus.

There is also a risk to insertional mutagenesis with lentiviral vectors, although this has been minimised by the use of self-inactivating LTRs, which should prevent activation of adjacent host genes at viral insertion sites.

**Origin & function**

The primary focus of this study will initially be the WT1 tumour suppressor gene, which is inactivated in a subset of Wilms' tumours (a common childhood kidney cancer) and acute myeloid leukaemias [Little & Wells (1997) Hum.Mutation 9:209-225]. We have shown that sequences within intron 1 of WT1 control the expression of imprinted transcripts and that expression of these transcripts is deregulated in Wilms' tumour [Malik et al (1995) Oncogene 11:1589-1595; Malik et al (2000) Cancer Res. 60:2356-2360; Dallosso et al (2004) Hum.Mol.Genet.13:405-415]. The purpose of knocking out these sequences is to test whether their removal has any physiological effect on cultured cells by altering WT1 expression.

Future work may carry out similar experiments on cancer-associated genes identified by genomic microarray screening procedures e.g. to identify novel tumour suppressor genes inactivated by epigenetic silencing.

All the targets of this work are thus potentially oncogenic.

**Evaluation of foreseeable effects**

For human, the major hazard is of unintentional infection of a laboratory worker with recombinant virus, but the restrictions on workers with open wounds etc, elimination of sharps and other containment measures will make this very unlikely. Recombination events leading to the production of replication-competent virus are highly unlikely, because neither the AVV or lentiviral vectors carry any viral genes, so any infection would be limited in its consequences to the injured person. The oncogenic sequences cloned into the recombinant viral vectors (WT1 initially) are not thought to be capable of directly initiating cancer development in a single event ie they would require additional genetic alterations. However, infection with one if the recombinant viruses should be considered as potentially oncogenic. In addition, there is a risk of insertional mutagenesis after viral integration, although there is little direct evidence for this in AVV vectors, whilst the lentiviral vectors have self-inactivating LTRs designed to avoid this risk.

The recombinant adenovirus expressing cre-recombinase for activation of pSico/pSioR is replication deficient and therefore non-pathogenic. The nature of the insert (cre) makes it of low risk to human health.

AAV and amphotropic lentiviruses can infect a wide range of mammals, so escape of virus into the environment could harm other animal species. However, the containment procedures used should effectively eliminate any risk to animals. Hazards caused by the viruses to other animals would presumably be the same as to humans ie possibility of oncogenic effects.

The cells used as targets for viral infection are well-characterised, established human cell lines that should not pose any hazard. The genetic modification of these cells will not lead to the production of any adventitious agents (all recombinant viruses are replication deficient) and therefore should not be hazardous after the initial viral infection.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated with 1% Virkon overnight, then disposed of to the drains. Manufacturers data sheets indicate that Virkon is virucidal for lentivirus and paravirus (>99.99% kill). Solid waste (plastic pipettes, tips etc.) will be autoclaved before disposal. Autoclaves are tested monthly with a thermocouple.

Is an emergency plan required according to regulation 20?  N  
If yes, tick to confirm that it is attached to this form  N  
Tick to confirm that you have attached a risk assessment to this form  Y  
Tick if you are claiming exemption from disclosure for section of the risk assessment  N  

Please enter comments on the GM safety committee on the risk assessment

The committee discussed the appropriate containment measures for handling tumour cell lines and recommended that these be handled at Containment Level 2 as a control measure for the purposes of COSHH. Notification of activities involving these cell lines following modification of WT-1 gene expression using lentiviral or AAV vectors was judged appropriate due to a perceived increase risk from the genetic modification. Concern was expressed regarding the broad statement defining future work. While this sets the scope of work the committee will require that the PI submits more information on potential candidate "cancer-associated" genes as it becomes available from microarray studies. However, the committee were satisfied that the risk assessment was adequate and that the control measures described and in place were sufficient to protect human health and the environment for the project as presently proposed. Activities involving plasmid manipulation in E. coli, handling unmodified cell lines and cell lines infected with recombinant adenovirus were approved as Class 1 activities and generation of AAV and lentiviral vectors and work with cell lines infected with these were approved as notifiable Class 2 activities at the Bristol University GMSC Meeting 10th November, 2006.

Project Containment

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Project Ref  18/07.1

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Date Ackn’d  31/05/2007
CU2 Project Title  Infection and immunity of the bordetellae.

Date Project Ceased  02/03/2022

Non-GMM  Consent Granted  Not Applicable
This project is an investigation into the molecular basis for Bordetella infection and immunity. It attempts to elucidate the processes by which these bacteria colonise the host tissues, multiply within these tissues, cause pathology, transmit to other hosts and how the host immune response reacts to the bacteria. In particular, it studies the genetic and cellular basis for these processes. Data arising from this project will obviously expand understanding of the infection biology of these bacteria. However the Bordetella are models for numerous processes that are of general interest to the infection and immunity field, including control of gene expression, interactions with ciliated respiratory epithelia, mucosal immunity in the respiratory tract, host-adaptation and the evolution of virulence.

This project involves studies at the cellular level using whole bacteria and at the molecular level using purified bacterial components (proteins, nucleic acids, carbohydrates, lipids and glycolipids.

Recipient or parental organism

Laboratory strains of Escherichia coli. These strains are used for plasmid maintenance and plasmid amplification and recombinant protein expression. They are non-infectious due to stable mutations. These strains are well characterised, and used extensively world wide for these purposes.

Species of Bordetella B.pertussis and B.parapertussis are pathogens of the human respiratory tract and the causative agents of whooping cough. Infants who have yet to receive the full schedule of pertussis vaccinations are susceptible to whooping cough. Adolescents and adults for whom vaccine or infection induced immunity has waned are susceptible to chronic cough and are sources of infection of susceptible infants. Pertussis is naturally endemic in the human population. Little is known about the contribution of B.parapertussis to the burden of whooping cough disease. Thus, laboratory workers are at risk from infection but the morbidity from such infection is very low. Appropriate control measures to prevent exposure should be used (see below).

B.bronchiseptica infects a very wide range of mammals but is described as a pathogen of just a few (cats, dogs, pigs), causing a relatively mild respiratory tract infection. Human infections are rare and almost always associated with immuno-compromised people or close contact with infected animals. It is not considered a risk to healthy humans. It is a DEFRA controlled organism. B.avium infects mainly turkeys and wild fowl to cause rhinotracheitis. There are no reports of avium infection in humans or mammals.

The other bordetellae cause sporadic disease in humans including respiratory disease and specticaemia. Risk factors for infection by these organisms are unknown, as is their true pathogenic potential. Although infections by these bacteria are rare, appropriate control measures are sensible.

Workers may spread the bacteria, particularly B.avium and B.bronchiseptica, to other susceptible animals if they are carried out of the lab on clothing or person. All workers will be made explicitly aware of the need to maintain rigourous standards of occupational hygiene to minimize the risk of this. All workers will be required to sign that they understand this risk and the control measures required to prevent it on personal safety training/risk assessment records.

Importantly, horizontal gene transfer among the bordetellae is rare. Infact, B.pertussis and B.parapertussis evolved through genome reduction. Thus, they are low risk in terms of transfer of genetic material to other bacteria.
Host/vector system

- pUC based plasmids, pBluescript plasmids, pEX100T, pSS2141, pET vectors, pBBR1 series vectors, pprobe series vectors.

Origin & function

Bordetella DNA will be cloned, maintained and manipulated in lab strains of E.coli. The main purpose for this is to introduce defined mutations into the DNA and then to move the mutate loci back into Bordetella to generate allelic replacement mutants. Mostly, mutations will be constructed by inserting an antibiotic resistance cassette into the Bordetella locus.

The creation of defined bacterial mutants via allelic exchange mutagenesis is a powerful technique for elucidating gene function, is a standard technique in my laboratory and forms the majority of genetic manipulation in this project. The phenotype of the wild type parental stain is compared to that of the isogenic mutants. Changes in phenotype are correlated to the genotype. This approach is a powerful technique for ascribing gene function, for elucidating the role of that gene in the biology of the host organism and is used in studies of bacterial pathogenicity.

Some Bordetella mutants will be used to infect mice to test their phenotype in an in vivo model of infection and immunity. The mouse model of Bordetella infection is well characterised and has been instrumental in understanding the infection biology of these bacteria.

In other cases, Bordetella genes will be cloned in order to express them from the recombinant plasmids ans is also used to study gene function. Complementation of mutations is used to confirm that a mutant phenotype is due to the experimentally constructed mutation and not a secondary effect or artifact. Expression of genes in heterologous hosts is used to identify possible functions of the gene by analysing the phenotypes of the recombinant, purifiable protein for in vitro studies of protein function.

Evaluation of foreseeable effects

It is considered very unlikely that using lab strains of E.coli in this way will result in a pathogenic phenotype. A vast majority of DNA constructs used will not encode functional proteins. Bordetella promoters are not well recognised in E.coli. In the very rare instances where a Bordetella encoded protein is expressed in these E.coli hosts, it is highly unlikely to convert them to an infectious phenotype. Deliberately expressed recombinant proteins will be metabolic enzymes involved in polysaccharide synthesis and not virulence factors.

Deleterious effects from mutating Bordetella are unlikely. Most mutations are of lower virulence/fitness than the parental wild type strains (for example see: Pilione, M.R., Pishko, E.J., Preston, A., Maskell, DJ and Harvill, E.T. 2004. PagP is required for resistance to antibody-mediated complement lysis during Bordetella bronchiseptica respiratory infection. Infect. Immun. 72:2837-42; Burns V.C., Pishko E.J., Preston, A., Maskell, DJ and Harvill, E.T. 2003. The role of Bordetella O-antigen in respiratory tract infection. Infect. Immun. 71:86-94; Cotter, P.A., Allen, A.G., Maskell, D.J and Miller, J.F. 2000. Multiple roles for Bordetella lipopolysaccharide molecules during respiratory tract infection. Infect. Immun. 68:6720-8). To my knowledge, there is no report of a Bordetella mutant showing increased infectivity or causing greater pathology compared to its wild type. There is clear scientific evidence that the host ranges of these bacteria is determined by multiple factors (multiple different adhesions are used, multiple toxins interact with different components of the host immune system, defective metabolic pathways (eg glycolysis, cysteine synthesis) contain multiple genetic deficiencies and thus would not be activated by gain of a single gene.

There are no foreseeable adverse effects from performing in vivo work with Bordetella mutants. The bacteria will be handled in containment level two facilities and infected animals housed in isolator facilities that will not permit release of bacteria in to the environment or to other animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid and liquid waste and contaminated glassware will be inactivated by autoclaving. Autoclaves are validated by temperature probe. Contaminated surfaces will be disinfected using 70% ethonal. 70% ethanol validated by

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 18/08.2

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<td>Evaluation and Optimisation of the Intracranial Administration of Non-viral and Viral Vectors by Convection-Enhanced Delivery (CED).</td>
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Date Project Ceased

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info
The work aims to study and optimise the intracranial administration of therapeutic agents by a novel technique termed convection-enhanced delivery (CED). This work is focusing on the delivery of both non-viral and viral vectors expressing marker genes or fluorescent tags. The project may progress to the delivery of therapeutic transgenes such as neurotrophic factors, depending on the results of our initial studies. The normal blood-brain barrier profoundly limits the access of potentially therapeutic agents to the brain, when administered systemically. Convection-enhanced delivery (CED) circumvents the blood-brain barrier by utilising extremely fine intracranial catheters and very low infusion rates, to deliver agents directly into the brain extracellular space. Whereas the blood-brain barrier can be a significant obstacle to systemically administered drugs, CED can utilise this to compartmentalise agents that would not normally cross this barrier, such as viruses and proteins, within the brain, potentially limiting systemic toxicity.

We have access to a number of viral and non-viral vectors that are suitable for the delivery of therapeutic genes into the brain. We are primarily interested in the delivery of non-viral, adenovirus and HSV-mediated gene therapy for brain tumours and adeno-associated virus (MV) and lentivirus-mediated gene therapy for neurodegenerative diseases.

The primary objectives of the project are:

• Understand the important factors in achieving convection-enhanced delivery.
• Evaluate and optimise the administration of viral and non-viral vectors within disease-specific neuroanatomical structures.
• Evaluate and minimise the immune response to various therapeutic agents.
• Evaluate different MR acquisitions in visualising drug distribution.
• Develop mathematical models of drug distribution by convection-enhanced delivery.
• Evaluate and optimise the delivery of other therapeutic agents including monoclonal antibodies, neurotrophic proteins and conventional chemotherapeutic agents.

At present viral vector production is carried out externally to this GMAG application, therefore it use replication- incompetent viruses expressing EGFP and 3-Gal vectors to be utilised in this study will be:

- Adenoviral vectors
- Adeno associated viral vectors
- Lentiviral vectors
- Herpes Simplex viral vectors

The nature of the viral systems to be used:

At present viral vector production is carried out externally to this HSE/GMAG application, as we are working in collaboration with both academic and Bio scientific companies. Vectors to be utilised in this study will be:

- Adenoviral vectors
- Adeno associated viral vectors
- Lentiviral vectors
- Herpes Simplex vectors

The project will at a later stage, will progress to produce viral vectors - We intend to use two commercially available viral systems for the expression of the sequences detailed later; the ViraPower Lentiviral System from Invitrogen and the adenoviral systems Adenovirus (Tet-on system developed locally and AdEasy from Stratagene as
well as vectorbiolabs. All of these systems produce replication-competent, self-inactivating pseudoviral particles that can be used to transduce almost any cell including post-mitotic and non-proliferating cells such as neurons.

### Origin & function

**Nature of the genetic material involved:**
Initially all vectors will be obtained from collaborators and will only include marker sequences:
- **EGFP**
- **13-Gal**

In the future the following gene inserts may be used:
- neurotrophic factors, such as glial cell line derived neurotrophic factor (GDNF) within the Adenoviral and lentiviral vectors.
- human and mouse 3-hexosaminidase (a and b subunits) within the AAV vectors.
- Gibbon Ape Leukaemia Virus (GALV) fusogenic membrane protein (FMP) GALV contain a 16 amino acid truncation in the transmembrane R-peptide of the wild type protein. This 16 amino acid sequence normally serves to restrict fusion of the envelope until it has been cleaved during viral infection. The alteration in the glycoproteins renders the proteins constitutively highly fusogenic, and therefore cytotoxic to human tumour cells (2000) Diaz Gene Ther.7(19):1656-63 (in the presence of the prodrug), will be incorporated in HSV vectors supplied by biovex.

### Evaluation of foreseeable effects

**Genetic Material:**

**Viral Production.**
All the cloning vectors used to construct the viruses (E.coli K-12 strains and 8L21) are laboratory adapted and are not considered hazardous to humans. The cell lines used as targets for viral infection (HEK 293) are well characterised, established human cell lines that should not pose any hazard. The genetic modification of these cells will not lead to the production of any adventitious agents (all recombinant viruses are replication deficient) and therefore should not be hazardous after the initial viral infection.

Risks for humans: the major hazard is of unintentional infection of a laboratory worker with recombinant virus where it is appropriate work will be carried out in a biological safety cabinet, which will minimise the risk to workers.

If all containment measures are followed this is very unlikely.

All Recombination events leading to the production of replication-competent virus are highly unlikely, because the vectors do not carry any viral genes. In order to decrease the risk of insertional mutagenesis after viral integration, lentiviral vectors have self-inactivating LTRs designed to avoid this risk. However, infection with one of the recombinant viruses should be considered as potentially oncogenic.

**Risks for Animals:**
The containment procedures used should effectively eliminate any risk to animals. Hazards caused by the viruses to other animals would presumably be the same as to humans i.e. possibility of oncogenic effects.

**Gene Inserts**
The control inserts are deemed not to exert any hazardous effects on the recipient organism. The proposed inserts are also not deemed to be hazardous to the recipient organism.

EGFP and B gal are markers and are therefore not considered to be hazardous to humans or the environment. GDNF is a cell survival factor and human and mouse cx-hexosaminidase are also not considered hazardous. Gibbon Ape Leukaemia Virus (GALV) fusogenic protein consists of a 16 amino acid sequence that normally serves to restrict fusion of the envelope until it has been cleaved during viral infection. It is thought very unlikely that any of the products of this gene, particularly at the very low levels likely to be generated following accidental exposure, could cause untoward effects. This is only active in the presence of the prodrug (5-FC).

Since all of these inserts will be in non replication competent viruses, it is thought that the severity and likelihood of hazard to both humans and the environment is low.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Derogation to animal containment level 1 for animal handling and holding facilities. Once injected the risk to human health from these animals is considered to be low. In most cases the GMO will be restricted to the brain and in animals that have been injected intravenously the risk to humans from bites and scratches during animal handling is considered to be minimal due to the nature of the inserts and the amount of non-replicative GMO in the circulation. The extra precautions described in the risk assessment will be taken to reduce the risk from any virus that is shed from any animals that may be injected intravenously in the future. Should an animal escape containment (which is unlikely due to the design of the animal facility) we consider the risk to the environment from these animals to be low due to the nature of the genes that would be expressed.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The Functional Neurosurgery Research Group (FNRG) AOC waste policy will be followed. Specifically:
- Solid waste coming into contact with GMMs will be stored in ‘yellow bins’ All plastic consumables and solids will be deposited into “Biohazard” marked bags and either autoclaved or incinerated via the University hazardous waste stream.
- Liquid Waste (e.g. samples, culture supernatants, tissue culture media) will be made safe by 1% Virkon used (with at least 10 mm contact time) and discharged to drains.
- Viral samples will be treated with a 1% Virkon disinfectant solution or equivalent (at least 10mm contact time — but usually hours) and discharged to drains.
- Sharps waste. Needles will be disposed of directly into sharps bins and incinerated via the University hazardous sharps waste system.
- Spillages In the case of any spillages, the spilled materials will be soaked up using a spill kit located in the containment level 2 facility and disposed of as solid, contaminated waste. Chemical (Virkon) disinfection: used at 1%, mm contact time 10 mins.
- General Disinfectant procedure 1% Virkon disinfectant solution should be used to decontaminate surfaces (at least 10mm contact time).
- Work within ASU theatres. All the above will be done to inactivate the waste created whilst surgical procedures are carried out.
- The disposal of carcasses will be performed by the ASU staff. Carcases will ultimately be disposed of by incineration (University licensed incinerator).

Validation.

Virkon, made fresh, effectively inactivates 100% of all viral vectors in use, under the above conditions (Manufacturers efficacy data: http://www.antecint.co.uk/mainfftpfiles.htm)

Incineration effectively inactivates 100% of all viral vectors in use (licensed incinerator).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was discussed at the meeting of Bristol GMSC on 21 January 2008. The researchers were asked to clarify the expected effects of GALV expression. The committee were also concerned that insertion of a gene encoding a potentially fusogenic protein into a lentiviral vector may have profound effects upon the vector phenotype and consequently the risks from that vector. The researchers were also advised to provide information on risks and precautions for animal care workers as necessary and to ensure that such information had been communicated and discussed with these workers or their line managers. The research team have confirmed that only the short 16 amino acid sequence of the GALV fusogenic membrane glycoprotein will be expressed and that insertion will be restricted to the HSV vector.

Project Containment

Laboratory Activities

Glass Houses

Growth Rooms

02/03/2022
The overall aim of this project is to modulate the expression of genes involved in the development of murine regulatory T cells (Tregs), in order to study their function in normal differentiation of Tregs within a transgenic TCR mouse model. Gene expression will be modulated in primary cells. The work will involve two complementary strategies:
1. Over expression of cDNA sequences of genes that have been associated with Treg development
2. Knock-down of the expression of Treg associated genes, using RNA silencing (51RNA)

The introduction of genetic material into primary lymphocytes for research purposes is associated with a number of practical problems, primarily the extremely low rate of transfection achieved by standard plasmid transfection methods. An alternative method for introducing genes into a number of cells types is to use replication deficient retrovirus as a transfer tool. Retroviruses efficiently infect dividing cells and integrate into the host cells genome, thus creating stably transfected cells that can be used for further studies. Because such vectors are replication deficient, once applied to cultured cells, they cannot proliferate or re infect other cells types or organisms. This work aims to develop a method of generating replication deficient MSCV-based retroviral constructs (Clontech) and introducing them into primary murine cells. MSCV vectors will be used as they have been shown to infect primary lymphocytes at a higher efficiency that MuLV-based vectors.

The work also aims to create bone marrow chimeras using donor cells that have been infected in vitro with commercially available retroviral vectors which include...
pSuper.Retro (OligoEngine Inc), pMSCV (Clontech) derivation retroviral vectors which have all been designed specifically for biosafety. These vectors generate replication incompetent viruses through separation of the packaging function, addition of self inactivation, deletions and the use of packaging cell lines which restrict viral tropism. The packaging cell line tropism will be modified to generate ecotropic viruses which will recognise only mouse and rat cells. The viral vectors will be used to infect and stably transduce mouse cells derived from existing University colonies.

Bone marrow has the ability to differentiate into a wide range of haemopoietic cells types and provides a way to circumvent many of the difficulties arising from the use of fully differentiated T lymphocytes, and offers the potential to generate adult mice that cannot be generated from modifying pre-implantation embryos (ie pronuclei injection and creation of ES chimeras). The use of syngeneic strains will also circumvent any rejection issues arising from cell transfer.

### Recipient or parental organism

Primary murine cells i.e. bone marrow and lymphocyte cultures

### Host/vector system

Viral vector particles based on pMSVC will be generated by a two-plasmid co-transfection system (of human 293T cells) in which plasmids containing the genome (pMSCV, or pSUPER.retro) or gag/polfenv proteins (pCL.Eco) are used as a mixed DNA preparation. This significantly reduces the probability of the production of replication competent virus.

- **pMSCV**: Clontech
- **pCL.Eco**: Retromax Retroviral system, Imgenex Corporation
- **pSUPER.retro RNAi System**: OligoEngine, Inc.

### Origin & function

Retroviral and helper plasmids purchased from commercial sources (Addgene, Imgenex, OligoEngine)

All cDNAs generated from cDNA libraries using cloning standard techniques or to be purchased from IMAGE collection (MRC) where possible

All siRNA oligos to be purchased from OligoEngine

### Evaluation of foreseeable effects

Wsx I and irf-1 have not been identified as potentially oncogenic. However, both molecules are required for the development and function of Th1 cells. Murine over-expression and knock-out studies of both wsx 1 and irf-1 have resulted in impaired cytokine signalling and immune dysregulation, but have not shown an increase in survival or tropism for cells expressing these genes. Conversely, both conventional knock-out studies of wsx 1 and irf 1 mice have shown some immune dysregulation, but no increase in either survival, tropism or infectivity in these animals.

EGFP has no known toxic or oncogenic potential

None of the gene products form secreted proteins

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All animal work will be carried out within local designated facilities following their rules regarding animal containment and welfare. At no time will live animals be removed from this facility. By following the procedures for washing and preparation of infected cells for transfer, no free virus should be introduced into the animal facility. The cells carrying the modification will carry some viral sequences, but will only express the transgenes of interest, as is the case when making stable transfectants using non-viral vectors. No increase in infectivity or tropism is expected in the infected cells, nor are the protein products of the modification excreted in vivo or into the environment. Based on this risk assessment, the work with GM animals is considered to be equivalent to a class 1 activity and not notifiable. Animals will be housed within the designated LAF cabinets under a minimum of animal containment level 1 and in accordance with local rules. Analysis of tissue from chimeras will be undertaken post-sacrifice. Animal waste will be incinerated post-sacrifice.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Because of their lipid-derived membrane, recombinant retroviruses are extremely labile and are inactivated by ethanol, detergents, or bleach. Liquid waste will be inactivated by exposure to 1% yukon for a minimum of 25 minutes (a procedure shown to completely inactivate retrovirus particles by the manufacturer). Solid waste will be inactivated in the same way, all within a microbiological safety cabinet. The solid waste will than be placed in autoclavable bags, sealed and disposed of by autoclaving in accordance with current departmental procedures regarding solid biological waste.

Before removing cells that have been infected with recombinant viruses from the tissue culture laboratory for further experimentation, they will be rendered non-infectious by thorough washing with ice cold PBS.

MSCV particles are extremely heat labile and washing in this way removes free virus particle from cultures and dramatically reduces the infective half-life of any particles that may remain.

Autoclave — effectively 100% kill. Autoclaves undergo a 12-point thermocouple validation every 3 months and all runs are monitored and recorded.

The committee asked that the group demonstrate either experimentally or by reference to published data that the washing procedure proposed effectively removes free virus from cell cultures or reduces infectivity before the first transfer of cultures to animal and FACS analysis facilities for downstream applications. The committee must also be kept informed of any significant changes to the risk assessment as a result of new inserts identified by microarray analysis.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref** 18/08.4

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Project Additional Information

Purposes of the contained use

To investigate the role of red blood cell adhesion molecules! red cell membrane Band 3 complex proteins, apoptotic pathway proteins, signalling molecules! transcription factors and the role of protein trafficking and internalisation and autophagy of these molecules in human erythropoiesis (red blood cell production).

Recipient or parental organism

1. Lentivirus-based plasmids will be propagated in laboratory strains of the bacterium E.coli. There are no foreseeable effects except that the GMM will be transformed to antibiotic(ampicillin) resistance to facilitate selection. The GMM will not express the target genetic material and, like the parent bacterial strain, will be unable to survive outside the laboratory nor colonise mammalian gut.
2. Lentivirus-based transduction particles will be produced in a cultured, human, embryonic kidney cell line. There are no foreseeable effects - the GMM will not express the target genetic material, will be cultured transiently and will be unable to survive outside the laboratory.
3. Cultured, human, haematopoietic, progenitor cells and cultured, human, haematopoietic cell lines will be transduced using these particles and thus rendered resistant to an antibiotic (puromycin) to facilitate selection. The GMM will express the target genetic material but will be unable to survive outside the laboratory.

Host/vector system

1. Lentivirus based plasmid vectors:
   - Transfer vectors - pLKO. 1, Sigma, St Louis, USA or pTRIP transfer vector, Chameau et al., Gene Ther. 2001, 8(3):190-8, or pTRIPZ vector purchased from Open Biosystems;
   - Compatible packaging vectors - Sigma Mission ShRNA or psPAX2, Chameau et al., Gene Ther. 2001, 8(3): 190-8, now sold by Addgene;
   - Compatible envelope vector - pCMV-VSV-G, Sigma, St Louis, USA or pMD2-VSV-G; Steward et al., RNA 2003, 493-501, now sold by Addgene.
   These vectors will be propagated in XL-1 blue E. coli cells.
2. Lentivirus-based transduction particles will be produced in HEK293T, a human embryonic kidney epithelial cell line.
3. The ultimate host will be cultured, human, haematopoietic, progenitor cells or cultured, human, haematopoietic cell lines. These cells will be transduced using lentivirus-based transduction particles produced as above.

Origin & function
Genetic materials are small, inhibitory RNA sequences (short hairpin RNA or shRNA) or cDNA sequences for the human genes of the proteins listed below:

1. Green fluorescent protein — a control protein used to determine multiplicity of infection values and to demonstrate effectiveness of transduction or knockdown protocols.

2. Cell adhesion molecules — found on many normal human cells and expressed during erythroid development (e.g. ICAM-4 [CD242]; Lutheran blood group protein [CD239]). Tetraspannins — cell adhesion accessory molecules found on many normal human cells and expressed during erythroid development (e.g. CD81;CD82; CD51).

3. Band 3 (CD233 the chloride/bicarbonate exchange transport protein) macromolecular complexes. Includes some blood cell restricted molecules such as the blood group active proteins glycoporphin A, ICAM-4 and RhD/CE; Rh associated glycoprotein and other, more widely expressed, proteins such as CD47. Includes associated structural/cytoskeletal proteins such as ankyrin and its isoforms and protein band 4.2 and also cellular proteins involved in the efficient trafficking of Band 3 and associated proteins. The overall function of the complex is gas-exchange between the red cell and tissues.

4. Other well characterised mammalian membrane transport proteins found on red blood cells (including Kidd blood group active protein [urea transporter]: glucose transporter Glut-1 and CD98 an amino acid transporter).

5. Red cell membrane proteins that form structural complexes involved in the maintenance of red cell integrity (including glycoporphin C and associated cytoskeletal proteins such as protein band 4.1, actin and spectrin).

6. Transcription factors - these control gene expression in a tissue dependent manner and thus direct cell differentiation and function. Examples are well characterised human haematopoietic transcription factors GATA-1 KLF1 and Spi-1. Haematopoietic transcription factors will be targeted. These molecules are normally expressed during erythroid differentiation but inappropriate or mis-timed expression (such as in this case where expression will be driven from a viral promoter) or knockdown of expression by siRNA may result in arrest of normal differentiation and therefore transformation of the target cell to immortality. For this reason, certain transcription factors such as Spi-1 may be considered as oncogenic or leukaemogenic and their encoding sequences as oncogenes.

7. Intracellular signalling molecules with a role in erythropoiesis. Target molecules include kinases and phosphatases of the Src and MAP pathways.

8. Proteins involved in apoptotic pathways in erythroid cells. Includes pro-apoptotic proteins such as Bak/Bax, BH3-only proteins, caspases, Fas & FasL and associated signalling molecules such as GSK-3.

9. Proteins functioning in the autophagy pathway and required for packaging/elimination of erythroid organelles. To include regulatory molecules such as Beclin-1 and components of the autophagosome biogenesis pathway such as Atg4 (mammalian orthologues A, B, C, D), Atg5, Atg7, Atg8 (mammalian orthologues include LC3, GATE-16, GABARAP L1-3).

The intended use is to enable the study of loss of function or rescue of function of the targeted proteins within a tissue culture, laboratory model of human red blood cell maturation (erythropoiesis). Ongoing screens might identify novel proteins that fall into one of these categories. The risk assessment will be reviewed to take account of such circumstances.

**Evaluation of foreseeable effects**

It is predicted that the GMOs produced in this study will have very similar characteristics to the host cells or organism. E.coli cells used to propagate plasmid vectors will be transformed to antibiotic (ampicillin) resistance but will not express proteins or shRNA encoded by the inserted genes within the vectors. Human embryonic kidney packaging cells will produce lentivirus-based transduction particles. Transduced, target, haematopoietic cells are predicted to be silenced for expression of the gene target sequence or to over-express the sequence but other characteristics are predicted to remain the same as the host cells. None of the GMOs will be capable of survival outside the exacting conditions of culture used in the laboratory. The system is designed such that virus that is capable of replicating within host cells is not produced.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No plants or animals will be used in this study.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

1. Contaminated bacterial cultures, petri dishes, tips, etc. will be collected in an autoclave bag, sealed with an indicator tape and autoclaved for 30 minutes at 125°C. Degree of kill 100%. The bag will be inspected and if the indicator strip has changed colour, thus verifying the temperature and duration of heat treatment, the bag will be disposed of as non-hazardous waste. The autoclave is serviced annually.

2. Contaminated tissue culture plastic (dishes, tubes, pipette tips, pipettes etc.) will be transferred to a sealed, non-porous plastic ‘sweetie jar’ kept inside the class II
microbiological safety cabinet. Once full, the “sweetie jar” will be decontaminated on the outside with 1% w/v virkon and sent for autoclaving.
3. In the Class II microbiological safety cabinet, spent tissue culture medium, including those contaminated with lentivirus particles, will be transferred to discard containers containing 2% w/v Virkon. These will be filled until a 1% w/v virkon solution of Virkon has been reached and after a minimum of 10 minutes contact (usually much longer) contents discarded to drains as non-hazardous waste.

Inactivation methods:
Autoclaving, effectively 100% kill (annual validation) Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection with Virkon (dipotassium peroxodisulphate, Antec International, Sudbury), used according to manufacturers instructions under standard conditions, manufacturers validation (e.g. 99.998 % kill).
1% w/v Virkon disinfectant has been validated by the manufacturer to inactivate lentiviruses even in the presence of high concentrations of protein (such as inactivation of HIV in blood). To inactivate contaminated liquid, add an equal volume of 2% w/v Virkon (to obtain 1% w/v virkon final concentration) and leave for at least 10 minutes before disposal down the sink.

The risk assessment was discussed at the Bristol GMSC meeting of 5 June 2008. The committee generally agreed that the risk assessment was very detailed and clearly identified the risks and suitable control measures. It discussed the large number of inserts being manipulated in an activity being undertaken by one group. However, as the risk assessment now makes more clear! the work is being undertaken by two collaborating groups in the department and some of the work is also being undertaken by these groups at the National Blood Transfusion Service (GM176,for which a separate notification has been made) where honorary contracts are held. The risk assessment is intended to cover all anticipated future work that may be undertaken on University premises. The committee have asked the person responsible to seek further advice and review their risk assessment as on-going screening activities identify further proteins for manipulation.
Investigation into the proteins regulating transcription and nuclear function in mammalian cells: regulators of transcription, DNA replication, the cell cycle, RNA splicing and nuclear structure
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 18/08.6

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<td>Regulation of cell behaviour and gene expression during wound repair</td>
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<tr>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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- **Non-GMM**
- **Consent Granted**
  - Being Processed

Tick if notifying a connected programme of work

Historical Significant Changes

Withdrawn

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
### Purposes of the contained use

Wound healing is a dramatic regenerative episode which triggers cells that would otherwise be dormant to proliferate and migrate, and this in turn requires induction of numerous genes including those that encode for cell cycle regulators, matrix molecules, integrins, proteases, etc (reviewed in Martin, 1997, Science). We have recently shown that the expression of epigenetic regulators, including polycomb transcriptional repressors, DNA methyltransferases, and DNA demethylases, changes during mammalian wound repair. The objective of the proposed lentiviral experiments is to test whether the polycomb family of transcriptional repressors and/or DNA demethylases play key roles in regulating some of the genes that are activated at sites of wound repair.

### Recipient or parental organism

The microorganism is a lentivirus that will be used to infect primary and cultured cell lines as well as mice.

### Host/vector system

Virus production will be performed using a second generation 3 plasmid system. The first ‘transfer’ plasmid is either pLG3 or pLKO.1 and contains viral and insert DNA outlined in point 1 above. The second ‘packaging’ plasmid (pCMVdeltaR8.91) contains wild type gag, pol, rev and tat genes driven by a CMV promoter in a puci 8-derived vector. The third ‘envelope’ plasmid contains the VSV-G envelope protein driven by a CMV promoter. These are introduced into HEK 293 cells and virus harvested.

### Origin & function

Genes (Bmil, Eed, Ezh2, GADD45a) will be obtained either through GeneService (IMAGE clones), collaborators, or standard cloning techniques; short hairpin sequences for knock-down will be obtained from collaborators (pLKO.1-Scrambled from Addgene), or ordered from Invitrogen. Lentiviral vectors will be used to infect cells in culture (1. MOCK; 2. mIMCD3; 3. Swiss 3T3; 4. mouse embryonic fibroblasts; or 5. primary mouse keratinocytes) or in vivo mouse wounds with the cDNA5 of, or short hairpin sequences against, polycomb proteins Bmil, Eed, and Ezh2, as well as the demethylase GADD45.

### Evaluation of foreseeable effects

Based on the literature, it is thought that if a human were to be infected with the GMM thus altering the expression levels of the named genes, this could result in broad changes in gene expression, and consequently changes in the rate of cell proliferation, migration, and survival. Because of this ability to affect cell proliferation and survival, one could speculate that the absolute worst-case hazard for an infected human could be tumour formation; however, the instability of the virus together with proper handling of the lentivirus and infected animals and cells reduces this risk to virtually zero. There are no foreseeable effects on the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Mice will be housed individually and wounds covered with Tegaderm or OpSite (surgical bandages impermeable to virus). In unlikely event that surgical bandages fail and wounds become exposed then research staff will be informed. Such exposure should only present a hazard for a short time after application. In such cases disinfection protocols will followed to decontaminate the cages and bedding (prior to incineration) to prevent spread of contamination. Handling procedures will minimise the risk to human health. However, as an extra precaution against transfer of virus animals will be handled while wearing gloves and cages will be disinfected after animals are removed. Bedding and animal carcasses will be incinerated. Infected animals will be well marked and animal facility staff will be trained on appropriate procedures for handling and husbandry. The animal facility is an isolated unit with restricted access. The genetically modified animal is, itself, considered no greater risk than the non-modified animal.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Infected solid material from tissue culture experiments will be kept separate from all other waste material and will be destroyed by autoclaving. Liquid material will be similarly kept separate from all other waste, and will be neutralised by using Virkon prepared as per manufacturer's instructions. Animal bedding will be incinerated with animal carcass waste.

Is an emergency plan required according to regulation 20?  
Y

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The University GMSC agreed with the evaluation of risks and selection of control measures at its meeting January 21 2008

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms
L2  Yes  L3  L4  L2  L3  L4  L2  Yes  L3  L4
L2  L3  L4  L2  L3  L4  L2  L3  L4

Animal Units  Large Scale Activities  Human Clinical Applications

Project Ref  18/09.1

Date Ackn'd  10/11/2009  CU2 Project Title  Use of bioluminescence to quantify bacteria in infection models

Date Project Ceased

Withdrawn  N  Tick if notifying a connected programme of work  N

Project notified under transitional arrangements  N
Our laboratory performs a number of murine bacterial infection models, which are used in the investigation of novel antimicrobial agents. The ability of the novel compounds to inhibit growth of bacteria is tested. The test will be a determination of the numbers of bacteria present and the hope is that treatment will reduce these numbers.

For some of our studies, we aim to quantify the numbers of bacteria at serial timepoints through the course of infection, by using non-invasive imaging methods during the in-life phase. In order to do this it is necessary to use bacteria which have been genetically modified to produce light (bioluminescence). These light-producing bacterial strains are commercially available and the light production is due to the introduction of a gene such as luciferase (exploiting the same principle as the production of light in many species in nature, for example the firefly). By quantifying the amount of light, the number of bacteria can be determined at several different timepoints during the course of infection.

This work encompasses the following bacterial strains: Staphylococcus aureus, Streptococci pneumoniae, Escherichia coli, Pseudomonas aeruginosa, and Acinetobacter baumannii.

These recipient (host) micro-organisms are bacteria which are ACDP hazard group 2 and are naturally present in the environment or in human hosts.

Acinetobacter is readily found throughout the environment including drinking and surface waters, soil, sewage and various types of foods. Escherichia coli is commonly found in the intestines & faeces of humans and animals. P. aeruginosa is commonly in soil and water. Staphylococcus aureus is a common coloniser of human skin and can be found on environmental surfaces around infected individuals. Streptococcus pneumoniae is part of the normal human upper respiratory tract flora & has been isolated from aquatic environments such as seawater.

These bacteria do not tend to cause disease in immunocompetent individuals, but can be problematic in the immunosupressed, neonates or the elderly.

The risk assessment outlines primary containment and PPE measures which have been assessed for the handling & containment of these organisms.

The host organisms covered by this risk assessment are bacteria which are ACDP hazard group 2 and are genetically modified to render them bioluminescent (in the
presence of luciferin substrate).

The genetically modified bacteria will be obtained from a commercial source (Caliper Life Sciences) or from a scientific collaborator. This risk assessment covers the use of genetically modified bacteria and their wild type equivalents (not the generation of genetically modified bacteria within this institution).

The genetic material is stably inserted into the bacterial genome. It does not alter the pathogenicity of the bacteria in vivo or in vitro. No host genes are inactivated or disturbed at the site of integration. The bacteria can be selectively grown on kanamycin.

The vector for bioluminescent transformation was the gram-positive lux transposon cassette, Tn4001 luxABCDE Kmr.

Examples of the transformation strategy are further detailed in K.P. Francis, et al, Infection & Immunity 2001 (69(5)3350-3358) and J.L. Kadurugamuwa et al Infection & Immunity, 203 (71(2) 882-890)

**Origin & function**

Insertion of genetic material from the luciferase gene (photorhabdus luminescens lux operon) renders the bacteria bioluminescent (in the presence of oxygen and the enzymatic substrate, luciferin), hence allow their detection using non-invasive in vivo imaging using a Caliper-Xenogen IVIS system.

**Evaluation of foreseeable effects**

The main risks of these products used relate to the risk to human health by these ACDP hazard group 2 organisms.

The bacteria used are each commonly found either in the environment or in human hosts. Furthermore, the genetically modified bioluminescent strains are no more virulent than the parental strains. This project should not contribute any additional risk to the environment even if there were to be an accidental release.

The containment & disposal measures detailed will minimise any chance of release of the viable bacteria into the environment

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Virkon** is a bacterial agent, which is used at a concentration of 1%. It has been shown to cause a 5-log reduction in bacterial counts (Hernandez, A 2000. Journal of Hospital Infection. 46: 203-209).

In vitro GMM liquid waste will be disposed of into 1% Virkon. After 24 hours, this will be discarded to drains. Bacterial plates will be sealed and autoclaved. Contaminated tips will be soaked in 1% Virkon prior to autoclaving. Any contaminated glassware (kept to minimum) will be soaked in Virkon for 24 hours, then rinsed prior to collection by media facility collection service.

Other solid waste (plasticware eg homogenizing tubes, pipettes, pipette tips, flasks, tubes) will be disposed of as biohazard waste (clear plastic bags) and autoclaved. Disposable plastic waste will be disposed of via the biological waste route. Biohazard waste will be removed from the laboratory through a central twice daily collection service which removes all biohazard waste to autoclaves for on site destruction.

Most solid (plasticware & pipettes) will be generated during the in vitro phase, with a bare minimum of plastic waste generated in ASU facilities (only gavage tube/needle
used for bacterial inoculation). Any such plastic waste created in ASU (eg gavage tubes) will be autoclaved then disposed of via the non hazardous waste route.

In vivo waste: cages and bedding will be autoclaved.

Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-135°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

The risk assessment was reviewed at meetings of the Biological and Genetic Modification Safety Committee on 15th July 2009 and, following updated information requested regarding animal containment measures, on 14th October 2009. The committee agreed that there was no increase in risk to human health or the environment from the genetic modification and that measures selected were suitable for containment of both the wild-type and genetically modified Hazard Group 2 bacteria and the deliberately infected animals involved in the activity.

Project Containment

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Project Ref 18/09.2

Date Ackn’d: 16/11/2009

CU2 Project Title: Containment of Influenza A viruses, for use in studies of vaccination or treatment of influenza infection

Class: Class 2

Culture Vol Class 2: < 1 Litre

Non-GMM Consent Granted

Date Project Ceased: 02/03/2022
Experiments will be carried out to establish the effectiveness of influenza vaccines & treatments in murine and ferret models. This work also encompasses propagation of established viral strains in eggs.

Immunologists studying influenza vaccines have realised that by combining wild type human influenza A strains with attenuated strains, they are able to achieve reassortant viruses with the properties of each, namely: a virus with the relative safety of an attenuated virus (PR8) yet possessing the ability to stimulate immune responses to surface viral proteins from wild type human influenza viruses (eg H3 & N2 or H5 & N1).

We wish to use an established ACDP hazard group 2 viruses available from the Health Protection Agency. For example, NIBRG-14 is a reassortant virus consisting of 6 genome segments from A/PR/8/34 (PR8)(H1N1 virus) and 2 genome segments A/Vietnam/1194/2004 (H5N1 virus). This was derived by a process of reverse genetics and the resulting virus is avirulent (or "de-pathogenised") compared to the parent H5N1 virus. This process involved deletion of sequences of the H5N1 haemagglutinin responsible for its virulence - hence the ACDP hazard group 2 designation. Other than the two surface haemagglutinin & neuraminidase genome encoded segments, the remainder of the genome is PR8. This virus has the ability to grow in eggs (a PR8 property) & is attenuated for human infection, but possesses the surface haemagglutinin & neuraminidase necessary to stimulate and investigate immune responses to H5 and N1.

PR8 has over 100 passages in mice, ferrets & embryonated chicken eggs, which has reulted in the complete attenuation of its ability to replicate man.

Reassortant influenza virus strains have been generated by recombining viral genome RNAs from established viral laboratory influenza strain PR8 (H1N1) with RNAs of other human infectious strains (eg H5N1) by reverse genetics, resulting in attenuation of pathogeneity of the latter.

As described above, the haemagglutinin & neuraminidase gene segments are derived from human influenza A viral strain. These are combined with established laboratory strain PR8, which is unable to replicate in man.

The inserted genetic material can be used to stimulate immune responses to influenza.

We will be using established ACDP hazard group 2 viruses available from the Health Protection Agency. For example, NIBRG-14 is a reassortant virus consisting of 6 genome segments from A/PR/8/34 (PR8)(H1N1 virus) and 2 genome segments of A/Vietnam/1194/2004 (H5N1 virus). This was derived by a process of reverse genetics.
and the resulting virus is avirulent (or "de-pathogenised") compared to the parent H5N1 virus. This process involved deletion of sequences of the H5N1 haemagglutinin responsible for its virulence - hence the ACDP hazard group 2 designation. Other than the two surface haemagglutinin and neuraminidase genome encoded segments the remainder of the genome is PR8. The resulting virus has the ability to grow in eggs (a PR8 property) & is attenuated for human infection (compared to the wild type virus), but possesses the surface haemagglutinin & neuraminidase necessary to stimulate and investigate immune responses to H5 and N1.

PR8 has over 100 passages in mice, ferrets & embryonated chicken eggs, which has resulted in the complete attenuation of its ability to replicate in man.

The attenuated viruses (eg NIBRG-14) do not pose a significant threat in terms of their replication in humans. However, if the virus were to enter a human who is already infected a human influenza virus (circulating in the population) then there is a theoretical risk of secondary reassortant to generate a new infectious human viral strain. The containment, PPE & other measures outlined in the accompanying risk assessment acknowledge this possibility and serve to minimise this risk.

The naturally occurring influenza virus in the human population evolves over time, with new variants emerging constantly (a phenomenon known as genetic or antigenic drift). In an attempt to keep pace with this, each year the composition of the influenza vaccine is designed to protect against the strains currently in circulation & most likely to be encountered the coming winter. Annual, seasonal influenza vaccines are available and routinely offered to at risk individuals, as defined by underlying chronic medical conditions.

Workers involved in this project (including animal husbandry) will be offered the annual, seasonal influenza vaccine. They will also be required not to work on the project if displaying flu-like symptoms. These measures, combined with relevant PPE constitute preventative measures to minimise any opportunity for viral strain reassortants.

Adherence to primary containment & PPE procedures will reduce the risk of any viral transmission outside of the designated rooms/units.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In the case of waste generated from infection of eggs, this material will be put into pots containing chloros solution, then placed in an outer, sealed plastic bag, promptly removed to be autoclaved & sent then for incineration.

For in vivo elements of the study, bedding will be bagged for incineration. The cages are then washed and autoclaved. Animal carcasses should be double bagged prior to incineration.

Laboratory waste: Solid waste will be disposed of as biohazard waste (clear plastic bags) and autoclaved. Disposable plastic waste will be disposed of via the biological waste route. Biohazard waste will be removed from the laboratory through a central twice daily collection service which removes all biohazard waste to autoclaves for on site destruction.

Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg tissue culture media) - treat overnight at room temperature using 10% sodium hypochlorite, always made fresh, then send for autoclaving. A 1% solution of sodium hypochlorite can be used for wiping down surfaces.
The Biological and Genetic Modification Safety Committee reviewed this revised risk assessment at its meeting on 14th October 2009. The committee had originally reviewed and approved the assessment under COSHH and University Policy for work with Hazard Group 2 biological agents. The revised assessment included work with an influenza virus produced by reverse genetics to be obtained from the Health Protection Agency and therefore requires notification under GMO(CU). The committee agreed (after further advice from HSE regarding hazard group classification) that containment level 2 measures with extra respiratory protection would be appropriate for this activity. RPE (FFP3-type masks) was advocated at all times when handling infected animals outside of primary containment. The committee had further concerns regarding the risk of viral reassortment should mixing occur between a virus carried by a worker naturally infected with seasonal (or other) influenza and the experimental viruses or should the different virus subtypes in used be accidentally inoculated into the same eggs for propagation. The committee therefore recommended additional measures to reduce the risk assessment. These measures included better management controls when propagating viruses (either purchasing sufficient virus or propagating one virus at any one time) and decontamination and disposal of contaminated egg material), a moratorium on workers (including animal husbandry staff) on handling differing animal models within the same 72 hour period (different species models are also located in different buildings), reinforcing the instructions given regarding signs of human infection and not undertaking this work if these become apparent; and recommending that workers are offered the seasonal influenza immunisation as an added measure while emphasising to staff that this is not for health protection. The practicability of immunisation of staff with a swine influenza vaccine will be kept under review.
The principal aim of the research activity is to understand the interplay between bacteria and their human host during colonisation and progression of disease. By understanding key mechanisms used by bacteria to interact with human cells and tissues candidates for intervention therapies can be identified. My research focuses on bacterial species residing within the respiratory tract and oral mucosa from these sites a number of bacterial species under investigation are able to disseminate and/or infection other regions of their human host.

I am currently involved in a number of projects which would benefit from genetic manipulation of bacteria in order to further elucidate mechanisms of pathogenesis. These projects principally include interactions of commonly isolated Gram negative and Gram positive bacterial species with mucosal associated tissue of the human respiratory tract and oral mucosa. In addition, other projects examine the association of bacterial species (principally Gram positive also commonly isolated from the respiratory tract) with cells associated with normal bone development, structure and function.

Collectively these projects examine bacterial species including Neisseria meningitidis (and related Neisserial species), Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Group A streptococcal species, Group B Streptococci, Pseudomonas aeruginosa, Staphylococcus aureus and commensal Staphylococcal species, Bordetella species and Fusobacterium species (all organisms listed fall under hazard groups 1-2). In addition, strains of E. coli will be used as vectors for plasmids containing genes from the bacterial species listed above and transient controlled expression of bacterial proteins.

Organisms to be used within these studies include Neisseria meningitidis (and related Neisserial species), Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Group A streptococcal species, Group B Streptococci, Pseudomonas aeruginosa, Staphylococcus aureus and commensal Staphylococcal species, Bordetella species and Fusobacterium species (all organisms listed fall under hazard groups 1-2). In addition, strains of E. coli will be used as vectors for plasmids containing genes from the bacterial species listed above and transient controlled expression of bacterial proteins.

Genes of interest will be PCR products from the appropriate bacterial species and vectors used as above. Firstly, the inserted DNA disrupts the function of known/putative virulence proteins within strains of the above mentioned bacteria invariably making the less or certainly no more virulent than the parent strain. In addition, the DNA will encode an antibiotic resistance cassette (e.g. kanamycin) allowing specific laboratory selection of the phenotype. Despite resistance to these antibiotics the strains will be

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<th>Host/vector system</th>
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<td>Briefly, plasmids will be derivatives of pUC/pBR322 e.g. pCR, pQE and pET plasmids. Genes of interest will be PCR products from the appropriate bacterial species. The antibiotic resistance cassettes will be obtained ere obtained from restriction digests/PCR of pE194 (erythromycin) and pCW59/pACYC184 (tetracycline) pUC4K (kanamycin) and pACYC184 (Chloramphenicol).</td>
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<th>Origin &amp; function</th>
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sensitive to the antibiotics used in the clinic e.g. penicillin, methicillin and vancomycin. Thus should a worst case scenario arise resulting in laboratory acquired infection this could be treated with antibiotics commonly used in the clinic. Note where clinical isolates of S. aureus are used strains will be resistotyped by the HPA prior to collection and high risk organisms e.g. MRSA/VRSA will not be used for GMO work.

Secondly, swapping existing putative virulence genes between strains of the same species is a process which occurs naturally in the wild leading to development of families of so called mosaic genes. This work will only be done in species where such events occur naturally, hence such work only mimic events in the real world. Such gene swapping will not lead to a strain that is anymore virulent than the host organisms form which the single gene is derived.

Both of the above rely on the genes of interest replacing an exiting region of DNA by homologous recombination from linear DNA hence no plasmid derived DNA will remain following outgrowth and selection of desired mutants.

Thirdly, expression of single putative virulence genes in E.coli using IPTG expression vectors presents a low risk the protein of interest will invariably not fold correctly under over expression conditions and bacteria will be quickly lysed to release the protein of interest for subsequent purification. Further no/low level protein expression will occur until the appropriate induction agent is added.

**Evaluation of foreseeable effects**

- **Evaluation of foreseeable effects**
  
  Whilst the majority of bacterial species are common colonisers of human skin and mucosa. The majority have the capacity to cause disease, particularly if there is an opportunity for the bacteria to enter the body. Illnesses such as skin and wound infections, urinary tract infections, pneumonia and bacteraemia (blood stream infection) may then develop if the bacteria enter the body. Toxins from certain strains of S. aureus can also cause food poisoning. Most strains of these bacteria are sensitive to many antibiotics, and infections can be effectively treated. The most common spread is still by human to human contact. Generally survival in the environment has not been widely studied but none of the organisms form endspores so survival should be limited. One exception is Pseudomonas aeruginosa which is commonly resident in soil and water naturally. It should be noted that risks associated with the GMMs are as those for parental bacterial species and as such will be treated like the wild-type hazard group 2 microorganism. Infections associated with the above species are wide ranging and include mild spots and boils to septicaemia and associated meningitis. In addition, these organisms are capable of causing infections of the respiratory tract and oral cavity and so have the potential to be spread via aerosol. Regarding swapping genes between strains of the same species is a process which occurs naturally in the wild leading to development of families of so called mosaic genes. This work will only be done in species where such events occur naturally, hence such work only mimic events in the real world. Such gene swapping will not lead to a strain that is anymore virulent than the host organisms form which the single gene is derived. Overall pathogenicity change from the parental strain will be minimal and in some cases diminished. The majority of bacterial species studies are naturally carried by high numbers of humans any way and this poses the greatest route of spread. Whilst some GMM’s will have antibiotic resistance genes introduced, the antibiotics in question are not generally used in the clinic to treat such infections.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All plastics coming in contact with GMM’s will be decontaminated by immersion in 1% Virkon for a minimum of 30 minutes sufficient to kill species from all genera listed in the work to be done (see efficacy pdf). All plastics will be subsequently autoclaved prior to disposal at landfill sites. Liquids/media containing GMM will be decontaminated by mixing with Virkon to a final concentration of 1% to ensure killing and incubated overnight prior to disposal down the sink. Following decontamination in Virkon any glassware will be washed and autoclaved for reuse.
The committee requested qualification of the types of genes that will be manipulated and "swapped" between bacterial species so that a more informed assessment could be made of the likely effects. This is reflected in the attached risk assessment. The activity was discussed at its meeting on January 27th 2010.

### Project Containment

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### Project Ref 18/12.1

- **Date Ackn’d**: 22/05/2012
- **Date Project Ceased**: 
- **Local and systemic immune responses to sub-retinal gene delivery using lentiviral vectors**
- **Class**: Class 2
- **Consent Granted**: Consent Granted
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**: 

**Significant Change ID**: 

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22/05/2012
Immune-mediated inflammatory eye disease (Uveitis) and retinal degenerative disorders including age related macular degeneration (AMD) account for the majority of visual handicap in the working age population and the ever increasing ageing population. Our research focuses on how one of the essential immune cells, the macrophage mediates damage and angiogenesis to the retina in models of uveitis, (experimental autoimmune uveoretinitis; EAU) and of age-related macular degeneration (laser-induced choroidal neovascular membranes (CNV)). Both EAU and CNV are established preclinical models that are widely used for the assessment of immunotherapeutic efficacy towards the translation of therapy, allowing us to see how we can inhibit damage and restore tissue function.

The recent advances in gene transfer techniques has lead to the use of retroviral and lentiviral based vectors to transduce tissues of the eye including the retina and retinal pigment epithelium, to achieve effective, stable long-term gene expression (Yanez-Munoz et al., 2006 Mar;12(3):348-53 Nat Medicine; Balaggan KS & Ali RR. Ocular gene delivery using lentiviral vectors. Gene Therapy 2012 19: 145-153). Lentiviral vectors can be used both to introduce a gene product into in vitro systems or animal models, but are also effective tools to block or "knock-down" expression of a specific gene using RNA interference technology.

The project seeks to establish whether lentiviral vectors that express anti-inflammatory cytokines or pro- or anti-angiogenic proteins, or "knock-down" vectors that express short-hairpin RNA (shRNA) to block expression of such proteins, when administered to the eye can directly modulate macrophage phenotype and thus influence severity in both EAU and CNV disease models. In another experimental approach, we will generate mouse chimeras following standard protocols, in which donor bone-marrow that is transfected with the "knock-down" gene expressing viral vectors is transferred, into irradiated recipients. To this end, mice will be injected, and animals sacrificed in the majority within 2 months and occasionally experiments extending up to 1 year post-injection where in all animals, tissues will be removed for histological assessment. To determine whether the expression of vectors influences the kinetics and degree of cellular infiltrate observed in the eye during disease, some eyes will be dissected and infiltrating immune cells isolated for full phenotypic characterisation by flow cytometry (FACS).

Cloning, generation and in vitro assessment of recombinant Lenti-viral vectors has already been conducted by our collaborators form the Molecular Therapy Division based at the Institute of Ophthalmology, University College London.

**Recipient or parental organism**

Human Immunodeficiency Virus (HIV) is in group 3 according to the ACDP definition of biological hazard and although HIV causes a fatal disease, the virus presents a low risk of spreading since retroviruses require close contact for their transmission, and survival in the environment is poor. The minimal HIV vectors have been developed to be replication defective, non-pathogenic and non-immunogenic. It is expected that both would have similar survival characteristics, and do not pose a risk to the environment. Since these vectors can not produce HIV in biological system, he risk of developing AIDS upon accidental injection of an individual is extremely low. Furthermore, the vectors have been designed to minimise the chance of any recombination events resulting in replication competent virus (RCV). Testing of the system for RCV has shown absence of detectable helper virus. Although the minimal HIV vector pseudotyped with the various envelopes can access all human cells, dissemination of the viral vector should not occur, which is in contrast to the wild type virus.

**Host/vector system**

HIV retroviral vector particles are produced by a standard three plasmid co-transfection procedure which has been designed to minimise the risk of generating replication competent virus. The vector genome pH4 is based on the HIV genome but has large deletions resulting in the absence of tat, vpr, vpu, vif and nef. The posttranscriptional transactivator rev and its responsive element (RRE) remain in the vector to increase transgene expression. The second component is an HIV gag/pol expression vector. The vectors have been designed to minimise the chance of any recombination events occurring where replication competent retroviruses (RCR) may result. The minimal HIV vector systems can access human cells by virtue of the amphotropic Moloney murine leukemia virus (MoMuLV), primate retroviral Gibbon ape leukemia virus (GALV) or vesicular stomatitis virus G-protein (VSV-G) envelope. VSV is a Biological Hazard Group 2 agent and should therefore be used at Containment level 2. The minimal HIV vector system, lacking tat, pseudotyped with one of these envelopes should be handled at a minimum of ACGM Containment level 2. The system has been tested for
RCR's and has been shown to be free of helper virus

Origin & function

All cDNA generated from cDNA libraries using standard cloning techniques.
sh RNA targeting sequences were designed using RNAi sequence design software and commercially synthesized.


The biological action of the proposed inserts are (1) immuno-modulatory, and are known to reduce inflammation and therefore regarded as immune- suppressive, including cytokines II-1Ra, II-4, IL-10 and other molecules CD200; or (2) anti-angiogenic, and inhibitors of vascular endothelium growth factor (VEGF) function and retinal neovascularisation, such as soluble Flt-1.

Evaluation of foreseeable effects

If rHIV were to infect another mammalian host, the risks would be minimal as the vector cannot recombine, and the genes expressed are not plasmid borne. The encoded genes could be expressed if they were transferred to another organism following a breach of containment, but the encoded products are not toxins or hormones and are unlikely to be expressed at levels high enough to have any systemic effect. The cDNA and shRNA inserts are mouse-specific so could have an effect in rodents however the vector is unlikely to survive in the environment long enough or be in a form that could readily affect an animal if containment were breached.

The cDNA or shRNA sequences are not associated with any risk such as an increase in pathogenicity and are not oncogenic so pose no risk to humans or animals. Recombiantion events leading to the production of recombinant viruses are unlikely, so an infection would be very limited in its consequence to exposed animals. It is unlikely that rHIV will survive outside of laboratory conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Handling of mice that have been infected with rHIV poses no extra risk to Animal Unit Staff, as following injection of virus particles or transfected bone marrow cells, mice will only contain stably transfected cells and no free virus will be present. Therefore the use of standard personal protective equipment (gown, face-mask and gloves) as stated in the Animal Services Unit (ASU) standard operating procedures, will provide sufficient protection during all animal handling. There are potential hazards associated with injection of rHIV particles into wild-type or Genetically Altered (GA) mice, such as accidental needle-stick injury. To prevent this occuring only experienced and licensed staff will undertake any regulated procedures required for this part of the project.

The ASU animal house is a high security facility with many measures in place to prevent escape of mice into the environment. Mice are housed in secure cages in rooms designated to one individual research group per room. Rodent barriers exist to prevent mice from escaping and travelling between rooms and exits. Animal bait traps are placed throughout the facility and regular checks are made to ensure that if any cracks/holes in walls, doors etc appear they are repaired immediately. However, in the unlikely event that any experimental mice do escape, they would represent no more of a risk to the general population than conventional wild-type mice.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The departmental waste policy will be followed. Spesifically:

rHIV are extremely labile and are inactivated by ethanol, detergents or bleach.

All solid waste will initially be inactivated by exposure to 2% Virkon for at least 30 minutes. All solid waste (animal tissues, tissue culture plates) will be placed in
autoclavable bags, and (plastic tips, pipettes or slides with sections) in an appropriate BioBin or CinBin, sealed and disposed of by autoclaving (121-125°C for 15 mins) and discharged to drains.

Animal waste - carcasses will be disposed of by incineration following the Animal Services Unit rules.

For any minor spills of viral preparations, and all bench tops on which procedures involving virus has been used, 2% Virkon disinfectant solution will be used to decontaminate surfaces (10 min contact time), followed by 70% ethanol. Laboratory tissue waste will be disposed as potentially infectious solid waste via the autoclaving and incineration route: Autolaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection with Virkon, used according to manufacturer's instructions under standard conditions, manufacturers validation (e.g. 99.998% kill).

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The Bristol Biological and Genetic Modification Safety Committee reviewed this risk assessment at its meeting on 27th April 2012. The committee agreed that the work described involving lentivirus/inserts with extensive use of sharps in the work procedures would require containment level 2 measures specifically access restrictions and assessment of staff training and experience (recorded). They also agreed that following infection of animals and bone marrow for transplant and removal of free virus that the risk would be reduced to a level commensurate with containment level 1.

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Animal Units

| L2  | L3  | L4  | L2  | L3  | L4  | L2  | L3  | L4  |

Large Scale Activities

| L2  | L3  | L4  | L2  | L3  | L4  | L2  | L3  | L4  |

Human Clinical Applications

| L2  | L3  | L4  | L2  | L3  | L4  | L2  | L3  | L4  |

Project Ref 18/16.1

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<th>CultureVolumeClass3-4</th>
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<td>19/02/2016</td>
<td>Expression of immune receptors and ligands at the surface of mammalian cells using</td>
<td>Class 2</td>
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Our ultimate aim will be to use lentiviral vectors as tools for the generation of stable cell lines expressing immune molecules of interest, in order to advance our understanding of their functions and involvement in various disease settings.

Recipient mammalian cells to be transduced using lentiviral delivery will include either immortalised cell lines (e.g. Jurkats, HEK293T, C1Rs) purchased from commercial sources (e.g. ATCC) or peripheral blood mononuclear cells (PBMCs) obtained with appropriate ethical approval and informed consent (in the case of samples of human origin). This work involving lentiviral vector is considered to be a class 2 activity to be undertaken at containment level 2 (until cells are virus free).

The lentiviral vector being used in this study would be considered to be a hazard group 1 biological agent possessing the safety features of a 3rd generation system as discussed in the risk assessment (multiple plasmids, self-inactivating, lack of WPRE sequences). That said, some of the inserts in the modified virus, could under a worse case scenario if accidental exposure occurred, lead to inappropriate immune responses (e.g. autoimmunity). Although the virus is replication defective, there could be enhanced effects due to evasion of immune tolerance mechanisms by inadvertent modification of immune cells following any accidental exposure (e.g. leading to clonal expansion).

We consider that the likelihood of exposure in this study is low because:
• No sharps will be used
• Small volumes will be used
• Cell cultures require additional treatment for efficient transduction with lentivirus in vitro and so the likelihood of expression in vivo is likely to be lower even if exposure occurred.

However, exposure remains possible under certain circumstances e.g. due to skin conditions or mucosal contamination through splashing which would be reduced by the wearing of gloves and use of a microbiological safety cabinet (which are used anyway for work protection). SACGM guidance also recommends that a class 1 designation is not appropriate where inserts are considered to be “biologically active” as we feel is the case in this study and has been outlined in the first paragraph.

Once the mammalian cells have been transduced, washed free of virus and passaged, the cells would present the same hazards as the original unmodified cells. Cells, unless fixed chemically, are handled under containment level 2 conditions to protect from any native contaminants or other hazardous properties as considered in COSHH risk assessments that are already in place.

8. Containment and control measures for larger GMOs (e.g. GM animals and plants) - Public Register
9. Maximum culture volumes per experiment - for GMMs only - Public Register
10. For GMMs only - the level of containment that will be applied - Public Register

Level 2 - Laboratory contained use
11. For GMMs only - Application for any derogation from full containment for the class of contained use (measures and justification) - Public Register

I would not like to request derogation from full containment measures
12. Description of waste management measures - Public Register
13. Emergency Plan - Public Register
14. Risk Assessment - Public Register

I confirm that I have attached a risk assessment to this form.
I am not claiming exemption from disclosure for sections of the risk assessment.

Summary of files attached to this notification
Attachments Uploaded
This notification therefore relates to the work with the genetically modified lentivirus vector at containment level 2 (GM class 2).

Origin & function

The inserts will fall into the following categories:
1) Immunoregulatory molecules (e.g. T-cell receptors, CD4/CD8 coreceptors, MHC-I/MHC-II molecules, coinhibitory/costimulatory and cytokine/chemokine receptors).
2) Reporter proteins (e.g. GFP, tCD34, rCD2).
3) Transgene expression enhancers (e.g. 2A peptide).
4) Mammalian target gene repressors (e.g siRNA).

The cDNA insert sequences are synthesised de novo and purchased from commercial suppliers (e.g. GeneArt) and are not sourced from any organism.
Evaluation of foreseeable effects

The lentiviral vector being used in this study would be considered to be a hazard group 1 biological agent possessing the safety features of a 3rd generation system as discussed in the risk assessment (multiple plasmids, self-inactivating, lack of WPRE sequences). That said, some of the inserts in the modified virus, could under a worse case scenario if accidental exposure occurred, lead to inappropriate immune responses (e.g. autoimmunity). Although the virus is replication defective, there could be enhanced effects due to evasion of immune tolerance mechanisms by inadvertent modification of immune cells following any accidental exposure (e.g. leading to clonal expansion).

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However, exposure remains possible under certain circumstances e.g. due to skin conditions or mucosal contamination through splashing which would be reduced by the wearing of gloves and use of a microbiological safety cabinet (which are used anyway for work protection). SACGM guidance also recommends that a class 1 designation is not appropriate where inserts are considered to be “biologically active” as we feel is the case in this study and has been outlined in the first paragraph.

Once the mammalian cells have been transduced, washed free of virus and passaged, the cells would present the same hazards as the original unmodified cells. Cells, unless fixed chemically, are handled under containment level 2 conditions to protect from any native contaminants or other hazardous properties as considered in COSHH risk assessments that are already in place.

This notification therefore relates to the work with the genetically modified lentivirus vector at containment level 2 (GM class 2).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid culture wastes are treated for 12 hours with sodium dichloroisocyanurate (Haz-tab granules; 10,000 ppm) and disposed to drain with a copious amount of running water. Disinfection validated for lentivirus in manufacturer’s data - referenced at http://guest-medical.co.uk/productspage/disinfectants/haz-tab-tablets/)
Solid waste such as pipette tips and plasticware, that has been in contact with lentivirus, is similarly decontaminated with sodium dichloroisocyanurate in situ prior to removal for autoclave sterilisation. Autoclaves are routinely maintained and validated and individual runs monitored. Autoclave sterilisation is considered to give 100% final kill.

The University Biological and Genetic Modification Safety Committee (BGMSC) reviewed this risk assessment and approved it for notification as a class 2 activity at its meeting on 5th February 2016. The committee discussed at length the arguments for and against a class 1 classification in relation to previous discussions with and presentations given by HSE. The arguments for a class 1 designation were based upon the safety features of the lentivirus vector, the small scale nature of the work and the elimination of sharps. However, the committee concluded that the PI had made a valid argument about the potential severity of any effects taking into account the inserts and although the likelihood of this being realised is not high, a class 2 designation would be consistent with previous activities reviewed by this committee and the guidance contained in the SACGM compendium for work with lentiviruses. The committee also agreed that a class 1 designation would be appropriate for virus-free modified cell cultures but that due to the inherent nature of these cells they should be handled under CL-2 conditions under COSHH until they had undergone chemical fixation.

Please enter comments on the GM safety committee on the risk assessment

The University Biological and Genetic Modification Safety Committee (BGMSC) reviewed this risk assessment and approved it for notification as a class 2 activity at its meeting on 5th February 2016. The committee discussed at length the arguments for and against a class 1 classification in relation to previous discussions with and presentations given by HSE. The arguments for a class 1 designation were based upon the safety features of the lentivirus vector, the small scale nature of the work and the elimination of sharps. However, the committee concluded that the PI had made a valid argument about the potential severity of any effects taking into account the inserts and although the likelihood of this being realised is not high, a class 2 designation would be consistent with previous activities reviewed by this committee and the guidance contained in the SACGM compendium for work with lentiviruses. The committee also agreed that a class 1 designation would be appropriate for virus-free modified cell cultures but that due to the inherent nature of these cells they should be handled under CL-2 conditions under COSHH until they had undergone chemical fixation.

Project Containment

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<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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<tr>
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<td>Human Clinical Applications</td>
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<tr>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L3 L4 L2 L3 L4</td>
<td>L3 L4</td>
</tr>
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</table>

Project Ref 18/18.1

Date Ackn'd   CU2 Project Title

02/03/2022
The study of influenza A virus-host interactions using genetically modified strains and cell lines

UTS People's placeholder

Class 2

< 1 Litre

Non-GMM

Consent Granted

Not Applicable

Date Project Ceased

Tick if notifying a connected programme of work

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The purpose of the contained use is the deeper understanding of influenza virus and its dependence on host cell proteins. Reporter cells that have been genetically modified are used to facilitate the detection of virus infection and virus-host interactions.

Recipient or parental organism

CELL LINES
Cell lines used are already genetically modified A549GFP1-10, A549GFP1-9 (both human cell lines), and MDCK GFP1-10 (canine cell lines), all of which are for combinatorial use with a GMO influenza strain harbouring the GFP11 (11th beta strand of GFP) for complementation GFP fluorescence analyses. The genetically modified cell lines are considered no more harmful to human health than the non-modified cells. They will be handled at containment level 2 under COSHH and while lentivirus is present or until they have been chemically fixed.

BACTERIA (E. coli)
Used for plasmid manipulation and are non-pathogenic strains. This work is considered class 1/containment level 1.

INFLUENZA A VIRUS
The influenza viruses to be used are mouse-adapted WSN strains (H1N1) and considered hazard group 2. The work does not involve use of influenza viruses of pandemic potential or of avian origin.

Host/vector system

LENTIVIRUS
The lentiviral vector being used in this study would be considered a hazard group 2 biological agent due to it being 2nd generation in formation and used with biologically active inserts. Lentiviruses do not have the capacity to infect as airborne pathogens and no sharps are required for this work. The virus is replication defective but there could be enhanced effects due to evasion of immune tolerance mechanisms by inadvertent modification of immune cells following accidental exposure (e.g. leading to clonal expansion).

**INFLUENZA VIRUS AND INSERTED MATERIAL**

(A). WSN PA-GFP has been genetically modified to express GFP as a fusion protein at the C-terminal part of the Influenza viral polymerase subunit PA protein. The maximum yield of this genetically modified strain is $5 \times 10^6$ PFU/mL whereas the wild-type can reach over $1 \times 10^9$, thus the strain is 200-fold attenuated compared to wild type. The strain was provided by Seema Lakdawala (University of Pittsburgh).

(B). WSN PB2-GFP has been genetically modified to express a split GFP11 (16 amino acids the 11th beta-strand of GFP) as a fusion protein at the C-terminal part of the Influenza viral polymerase subunit PB2. Its yield can reach $1 \times 10^8$ PFU/mL and attenuated compared to wild-type. The strain was provided by Nadia Naffakh (Pasteur Institute).

**LENTIVIRUS / CELL LINES AND INSERTED MATERIAL**

Cell lines will be transduced with genetically modified lentivirus vectors to produce stable cell lines expressing genes of interest for interaction with influenza virus proteins. These are detailed in the risk assessment with examples and include organelle marker genes, cytoskeletal marker genes, camelid nanobodies and binder proteins and cell/nuclear transport molecules. These are all generated as oligonucleotides.

**Evaluation of foreseeable effects**

**CELL LINES**

The same hazards exist as for the GM lentivirus during cell culture with the virus present. Once cells have been passaged and washed free of virus the GM cell line isn’t any more hazardous than the original cell.

Many in vitro cell lines (including the ones used for lentiviral transduction) already have defects in tumour suppressor genes and are handled under containment level 2 conditions without additional precaution. Once fixed chemically, the inherent hazards with the cells due to potential contamination would also be reduced and as the likelihood of exposure is low the risk would therefore be much lower.

**LENTIVIRUS**

The vector being used in this study would be considered a hazard group 2 biological agent due to it being 2nd generation with biologically active inserts. The virus is replication defective but there could be enhanced effects due to
evasion of immune tolerance mechanisms by inadvertent modification of immune cells following accidental exposure (e.g. leading to clonal expansion). Some of the inserts in the modified virus could under a worstcase scenario if accidental exposure occurred lead to inappropriate immune responses (e.g. autoimmunity). In the most extreme outcome, viral vector/inserts could inactivate tumour suppressors in the accidentally infected person, triggering cellular transformation and oncogenesis.

Lentiviruses do not have the capacity to infect as airborne pathogens and no sharps are required for this work thus minimising the likelihood of exposure.

**INFLUENZA VIRUS**

In the most extreme case, a GMO influenza strain may revert to wild-type after several passages but will still be a minimal threat to the workers. Any other recombinant viruses (such as by accidental co-infection with another influenza strain) are unlikely to arise in the short length (under 12 hours) of our typical experiments. Long infection assays (up to 3 days) are performed only for the purpose of virus stock propagation which is always performed in a flask with a filtered lid thus minimising any risk of cross contamination of viral strains. It is highly unlikely that any higher pathogenic strains will arise as a result of the use of these GMO strains.

Mouse models of IAV are not suitable for transmission assays, since they do not display flu-like symptoms (sneezing, coughing) that are essential for animal-to-animal transmission. Therefore, even if an infected rodent escaped out of captivity it is unlikely it can be a source of transmission and contaminate wild life/other rodents. Furthermore, there are no rodent experiments planned in this IAV risk assessment using.

At the relatively low doses of viruses used in this assessment, it is highly unlikely that an escaped virus is capable of infecting animals outside of containment or contaminating plants and wild life in any way. Furthermore, removal of lab coats and hand washing following virus experiments substantially reduces the likelihood of viruses escaping outside containment.

The wild-type IAV may survive a few hours or days if released from containment in a micro droplet (e.g. aerosols, typically caused by sneezing or coughing of an infected human patient). If a viral particle escaped containment attached to clothing, for instance, they will still be susceptible to extermination without the protective environment of an aerosol within a short period of time.

Handling the genetically modified viruses has been assessed as a class 2 activity.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste e.g. contaminated plasticware will ultimately be autoclaved to sterilise. Waste will be removed from the laboratory through a central twice daily collection service which removes biohazard waste to autoclaves for on-site destruction prior to disposal. Autoclave runs are monitored and autoclaves validated for waste treatment. Liquid waste (e.g. cell culture media containing IAV and lentiviral particles) will be disinfected for 12 hours using Virkon which has been validated as effective at the final concentrations to be used by the manufacturer. Disinfected liquid waste is disposed to drain with copious amounts of water.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The University GMSC discussed this work at its meeting on 14 February 2018. The committee agreed with the classification of the activity due to the work with influenza virus but it required clarification from the proposer regarding the generation of lentivirus system to be used, which wasn't clear in the original risk assessment, and consideration of environmental risks. Following receipt of further information and a revised risk assessment the committee approved the work for notification.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
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<tbody>
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<td>L2  Yes</td>
<td>L3  L4  L2  L3  L4  L2  L3  L4  L2  L3  L4</td>
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<td>L2  L3  L4  L2  L3  L4  L2  L3  L4</td>
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**Animal Units**

| L2  L3  L4  L2  L3  L4  L2  L3  L4 |

**Large Scale Activities**

| L2  L3  L4  L2  L3  L4  L2  L3  L4 |

**Human Clinical Applications**

| L2  L3  L4  L2  L3  L4  L2  L3  L4 |

**Project Ref** 18/18.2

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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<td>20/09/2018</td>
<td>Studies of mammalian cell growth pathways</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>
The aim of this programme is to determine how the fundamental function of eukaryotic trafficking and signalling pathways controls cell growth and division and how their dysregulation leads to health conditions such as cancer, chronic inflammation and cardiovascular disease. Cell growth and proliferation are dependent on an interplay of proteins regulating cell-cycle progression, DNA damage repair, signal transduction, cytoskeletal organisation and metabolism. Furthermore, proteins controlling cell growth also regulate homeostatic functions such as metabolism and immunity. Indeed, many noncanonical functions have recently been discovered for cell cycle genes in post-mitotic cells. Ultimately our research should identify new targets for the design of therapies to treat patients with chronic health conditions.

This programme involves use of viral vectors to express or modulate expression of cell cycle and DNA repair genes. Accidental infection of the researcher could potentially affect endogenous tumour suppressors and oncogenes. In the worst-case scenario, accidental infection may predispose to cellular transformation and tumorigenesis.

Recipient or parental organism
- Non-pathogenic strains of E. coli e.g. DH5α strains (class 1 activity)
- Plasmodium chabaudi (rodent pathogen, class 1 activity)
- Established mammalian cell lines e.g. HeLa, RPE-1, BJ5Ta, HEK-293
- Primary non-human cells e.g. mouse and rat fibroblasts
- Primary human cells e.g. hematopoietic, endothelial, keratinocytes
- Insect cell lines e.g. Sf9, SF2, Hi5

Host/vector system
- Non mobilisable plasmids including antibiotic resistance genes (e.g. Amp, Kan)
- Mammalian expression plasmids including antibiotic resistance genes (e.g.
Pur, Neo) and non-specific tissue promotors such as CMV, SV40 and tissue
specific promotors such as EF1-a
- CRISPR and shRNA plasmid vectors
- Adeno-associated virus AAV (disabled human vectors from helper virus free
systems lacking genes required for replication)
- Adenovirus (attenuated E1/E3 deleted Ad5 strains)
- Lentivirus (attenuated 3rd and 4th generation vector systems with SIN
sequences by preference. 2nd generation systems will only be used where
the insert/vector combinations already exist in these vectors as gifts or as
purchased or where absolutely required by the experimental design)
- Baculovirus (attenuated AcNPV)

Origin & function

All inserts will be amplified from mammalian cDNA by rtPCR or synthesised
oligos. The following categories of genes will be silenced, deleted or inserted
and expressed in the final recipient using the vector delivery systems stated.

Cell cycle genes
e.g. CDK6, p21cip
Regulating DNA replication, mitosis, microtubule dynamics, spindle
assembly checkpoints.

DNA repair genes
e.g. ATM, ATR, CHK1
Genes regulating DNA damage detection, checkpoint initiation, DNA repair,
apoptosis.

Cytoskeleton proteins, including motor proteins
e.g. Eg5, NEK2, dyneins, kinesins
Regulators of cell division, motility, intracellular trafficking.

Metabolism regulators
e.g. pentose phosphate genes, tafazzin
Regulators of energy production, mitochondrial function, biosynthetic
pathways.

Signal transduction genes
e.g. PKC, Rac, T-Cell Receptor
Cell surface receptors, kinases, phosphatases, enzymes catalysing posttranslational
modifications, proteases, cell-cell communication.

Regulators of cellular homeostasis
e.g. ATM, neutrophil elastase
e.g. ATM, neutrophil elastase
Proteases, genes involved with degradative pathways, such as autophagy,
immune defence, cellular stress response.

Structural proteins
e.g. Lamin A Histone H1
Nuclear proteins (eg. lamins), plasma membrane proteins, proteins involved
structural integrity, organisation and function of subcellular components.

Cytokines
**Evaluation of foreseeable effects**

**HUMAN HEALTH**
The vectors involved in this programme are generally considered to be crippled laboratory organisms, attenuated strains, or restricted to rodents. However, accidental infection with viral vectors remains a possibility and there is a risk of biological effects being realised when biologically active inserts are used, or genes are being targeted. The processes being targeted include human cell cycle proteins and their modification in experimenter’s cells after accidental infection could potentially modulate endogenous tumour suppressors or oncogenes. However, oncogenesis requires the concerted action of several oncogenes and modulation of a single gene does not lead to tumorigenesis. Nevertheless, in the worst-case scenario, accidental infection with any of the genetically modified viruses targeting these sequences may predispose to cellular transformation.

Regulatory sequences used will include promoters designed to drive high level expression in a broad range of tissue types and therefore the risk of effects from accidental infection is likely to be increased in these cases.

**ENVIRONMENTAL SAFETY**
We consider that all work in this programme is likely to be of more risk to human health and safety than to environmental safety because:

- E. coli strains that will be used are attenuated for laboratory use and cannot survive in the environment.
- The viral vectors to be used will also be defective such that they are unlikely to survive in the environment.
- Eukaryotic cell lines and primary cultures can only survive in specialised cell culture medium with optimised nutrients, pH and temperature.
- Animals infected with the P. chabaudi will not excrete the genetically modified parasite and the only way to transmit to other animals (rodents) in the environment would be via a mosquito bite which is extremely unlikely to happen in the animal containment facilities. The GMO is effectively contained in the animal until it is incinerated.
- We do not consider any planned modifications, including gene editing, to confer an advantage to the GMOs; indeed, the converse is most likely to be true, that we will in fact limit the ability of cells to divide.
### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable (animal infection studies only)

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Liquid waste (e.g. cell culture media containing lentiviral particles) will be disinfected based on individual risk assessments. Sodium dichloroisocyanurate will be used at 10,000 ppm for 12 hours contact time (manufacturers testing) or Virkon at 1% final concentration for at least 10 minutes, usually 12 hours, (manufacturers testing). Disinfected waste rendered safe to handle will be discarded to drains.</th>
</tr>
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<tr>
<td>Solid waste will normally be autoclaved in the building using validated autoclaves (considered 100% deactivation) prior to disposal by land-fill or incineration (based on University waste contractor arrangements). Autoclave runs are monitored and logged. Solid class 1 waste and some class 2 waste may be deactivated and disposed of by incineration where an individual risk assessment has fully considered this method.</td>
</tr>
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<td>Sharps waste and infected animal carcases and tissues will be incinerated by the University's hazardous waste contractor. University hazardous waste collection and disposal contractors are fully audited by the University’s Hazardous Waste Manager to ensure compliance with requirements for the storage and transport of infectious goods. Audits also include GM premises registration checks.</td>
</tr>
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</table>

### Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The University GMSC has reviewed 2 risk assessments covering activities that are proposed under this CPW to date. The risk assessment example which is enclosed with this notification was reviewed at its meeting on 14th February 2018 but has not yet been notified. The committee agreed with the classification and made several recommendations that the investigator incorporated into the final risk classification and made several recommendations that the investigator incorporated into the final risk assessment.

The proposed CPW (and the second class 2 risk assessment) was discussed at its meeting on 2nd May 2018 and the committee agreed that the scope of work covered by these two risk assessments that is ready to take place and the anticipated work in this building was suitable for a CPW. The final CPW scoping document (also enclosed with this notification) was reviewed by the committee at its meeting on 5th September 2018 and notification approved. The committee concluded that this document and the limits set out in it can be used by the committee when considering any further risk assessments for activities to be covered by this CPW.

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### Project Ref 18/21.1

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<td>Studies on viral vaccine development and cure</td>
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- Project notified under transitional arrangements: N

<table>
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<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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<tr>
<td>N</td>
<td>Y</td>
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</table>
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The aim of this connected programme of work is to develop innovative and efficacious prophylactic and therapeutic vaccines against infectious agents such as HIV and Influenza. Through this work we hope to develop the next generation of vaccine, and elucidate novel information pertaining to the basic biology of infection and how the immune system can be harnessed to prevent infection. We will use a variety of different vaccine technologies such as DNA, recombinant protein and nanoparticle-based immunogens to elicit protective immune responses. Additionally, our research aims to identify and develop novel adjuvants which can significantly augment anti-pathogen immune responses in the systemic circulation and at mucosal portals of entry.

**Recipient or parental organism**

- E. coli (non-pathogenic strains such as K-12 derivatives)
- Saccharomyces cerevisiae
- Mammalian cell lines
- Human Immunodeficiency Virus-1 (HIV-1)
- Influenza virus (no pandemic strains)

**Host/vector system**

- Lentivirus - modified vectors only. No full length (5'LTR-3'LTR inclusive), replication competent virus, will be inserted into pREC vectors.
- Standard non-mobilizable plasmids for routine cloning with antibiotic resistance genes e.g., ampicillin, kanamycin.
- Mammalian expression plasmids including antibiotic resistance genes e.g., puromycin, neomycin, blasticidin, hygromycin; promoter regions e.g., CMV, SV40, EF1-α, tetracycline-repressible promoters.

**Origin & function**

**Class 3 activity**

Near full length HIV genome (gag-3'LTR) or individual genes.

Source: PCR amplified from proviral DNA, RNA isolated from blood, or from DNA expression vectors.

Function: HIV protein expression for recombinant protein or nanoparticle self-assembly. Particle expressing plasmids do not have a 5'LTR, contain viral RNA packaging defects and a nonfunctional integrase. VLPs are therefore non-infectious particles, if transfecting with a complementing vector (pREC_Sbf1). Gene editing of HIV proteins (e.g. incorporating stabilizing disulphide bonds, amino acid sequence changes or glycan modification etc.) is expected to render the proteins more immunogenic while reducing the fitness of viruses that contain them.

**Class 2 activity**

Genes encoding Influenza HA, NA, M1 and M2.

Source: Purchased oligo or PCR amplicon from purchased plasmid vector.

Function: Produce Influenza proteins or nanoparticles using proteins expressed from genes isolated from lab adapted strains (X31, PR9 and WSN), and generally circulating influenza viruses such as H1N1 and H3N2. Pandemic and HPAI (e.g. H5, H7 and H9) are not being used. The HIV gag gene may be included into these constructs for studies involving RNA adjuvant packaging. Gene editing of HA and NA surface proteins (e.g. incorporating stabilizing disulphide bonds, amino acid sequence changes or glycan modification) is only expected to render the protein more immunogenic.

**All activities**

Immuno-stimulatory RNA motifs

Source: PCR amplicon from purchased plasmid vector
Function: Generates immunostimulatory RNA transcripts that can be used as exogenous adjuvants for vaccines or for selective packaging into particles. The RNA will trigger pattern recognition receptors (PRR) within cells.

Reporter genes e.g., GFP and luciferase
Source: Purchased oligo or PCR amplicon
Function: Reporter genes for quantifying cellular proliferation and subcellular localization of proteins

Evaluation of foreseeable effects

Full or partial Envelope (Env) genes cloned into the common HIV-1 backbone within our pREC vectors are not expected to have any alteration of existing traits beyond that observed with other characterized HIV-1 viruses as they are derived from individuals naturally infected with circulating viruses or lab adapted strains. The Env genes are expected to bind human CD4 and chemokine receptors just like those of known HIV strains. Env-deleted viruses pseudotyped with VSV-G would be capable of by-passing normal entry requirements and entering target cells by endocytosis. The VSV-G would only be used in conjunction with Env deleted vectors, making this a single round infection only.

Genetic modifications to the hemagglutinin and neuraminidase proteins of the influenza virus are expected to have either no effect, or will impair the viruses ability to replicate or increase its susceptibility to immune mediated clearance. In the unlikely event that the genetically modified virus recombines with another influenza virus, we would expect the resulting virus to either replicate as normal, be more susceptible to host mediated immunity, become attenuated or even inactivated.

We do not consider any of the planned modifications, including gene editing, to confer an advantage to the GMOs affecting environmental safety; indeed, the converse is most likely to be true, that any modification will be deleterious to the virus.

The virus like particles generated in these activities are devoid of viral genomes and due to the engineered modifications cannot replicate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We seek 4 derogations for work involving HIV at CL3. Specifically:
1. Derogation from implementing an air pressure within the CL3 laboratory that is negative to atmospheric pressure.
2. Derogation from HEPA filtration of extracted air from the CL3 laboratory.
3. Derogation from using a laboratory that is sealable for fumigation.

Justified as HIV-1 cannot survive outside a host cell and outside optimal tissue culture settings degrades very rapidly. We have also minimized and compartmentalized the volumes of virus being grown and processed, have stringent disinfection procedures in place, and ensured multiple layers of containment during both transportation of virus between the MSC and incubator as well as within the centrifuge. There is no evidence for airborne transmission, none of the work will affect this and measures being taken will minimise the likelihood of aerosol release.

4. Derogation from the requirement to have an autoclave in the laboratory suite.

This can be justified using the same arguments as above regarding transmission route, limited survivability, compartmentalization of the volumes of virus being grown and processed and the stringent disinfection procedures in place. All waste will be disinfected before leaving the CL3 and/or will be in sealed containers and always handled by CL3 trained workers for immediate autoclaving. No waste will be stored outside the CL3. There is an autoclave in the same building as the laboratory.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Low titre liquid class 3 waste and all class 2 liquid waste – disinfection with 2% Distel or Virkon for 30 minutes minimum (in line with manufacturers microbiological testing
validation). This will render the liquid sufficiently safe for discard to drain.

High titre class 3 liquid waste – will be placed directly into a leakproof Dispo safe jar containing virucidal solution (final solution will not be less than 2% Virkon or Distel). Containers are then incubated for 30min, sealed, placed within autoclave basket lined with autoclavable Biohazard bag. Waste is then sent for autoclaving. Effectively non-hazardous waste discarded to drain.

All solid waste (e.g., plasticware) – disinfectant soak with 2% Distel or Virkon for 30 minutes minimum. Then autoclave sterilisation. Non-hazardous waste route for disposal.

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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02/03/2022
Project Ref: 18/93.1

Date Ackn'd: 29/03/1993

CU2 Project Title: STUDY OF GENE EXPRESSION OF HERPES SIMPLEX VIRUS BY USE OF RECOMBINANTS CONTAINING REPORTER GENES

Class: Class 2

Culture Class: Class 2

Culture Volume Class: Class 3-4

Non-GMM Consent Granted: not applicable

Withdrawn: N

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Animal Units**

| L2 | L3 | L4 |

**Large Scale Activities**

| L2 | L3 | L4 |

**Human Clinical Applications**

| L2 | L3 | L4 |

**Project Ref** 18/93.2

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Date Project Ceased

Tick if notifying a connected programme of work  N

Historical Significant Changes
GM18/01.12

Historical Date of Additional Info
13/09/2001

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Animal Units**

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**Project Ref** 18/93.3

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Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Y

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 18/94.1a

Date Ackn'd 24/10/1994

Date Project Ceased

CU2 Project Title CLONING OF GENES FROM AND THEIR RE-INTRODUCTION INTO ACDP HAZARD GROUP 2 BACTERIA

Class

Culture

Culture Vol

Class 2

Consent Granted not applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Project Ref 18/94.1b

Date Ackn'd 24/10/1994

CU2 Project Title CLONING OF GENES FROM THEIR RE - INTRODUCTION INTO ACDP HAZARD

Class 2

Class CultureVolClass2 CultureVolumeClass3-4
GROUP 2 BACTERIA: IDENTIFICATION AND ANALYSIS OF ENTEROPATHOGENIC ESCHERICHIA COLI (EPEC) VIRULENCE DETERMINANTS

Date Project Ceased
17/10/2004

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Animal Units | Large Scale Activities | Human Clinical Applications

| L2 L3 L4 | L2 L3 L4 | L2 L3 L4 |

Project Ref 18/94.1c

Date Ackn’d 24/10/1994

CU2 Project Title CLONING OF GENES FROM THEIR RE - INTRODUCTION INTO A:CDP HAZARD GROUP 2 BACTERIA: CLONING EXPRESSION AND MUTATION OF GENES FROM ORAL BACTERIA

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Project Ref 18/96.1

Date Ackn’d 12/02/1997
Date Project Ceased 25/02/2003
Non-GMM Consent Granted not applicable

Tick if notifying a connected programme of work N

Use of BHV-2 as a Mammalian Expression Vector

Class CultureVolClass2 CultureVolumeClass3-4
Class 2

Project notified under transitional arrangements Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Project Ref 18/99.1

Date Ackn'd 06/04/1999  CU2 Project Title REGULATION OF PROTEIN PHOSPHORYLATION AND GENE EXPRESSION BY  Class CultureVolClass2 Class 2  CultureVolumeClass3-4
Date Project Ceased

INSULIN-STIMULATED PROTEIN KINASE

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrew

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 18/99.2

Date Ackn’d 19/12/1996

CU2 Project Title ION CHANNEL EXPRESSION IN MAMMALIAN CELL LINES USING RECOMBINANT VACCINIA VIRUS

Class 2

Consent Granted not applicable

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022

Page 1118 of 15326
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**Project Additional Information**

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  85/08.1

Date Ackn'd  23/11/2017  
CU2 Project Title  Studying the function of putative virulence genes of S.aureus.

Class  Class 2  CultureVolClass2  1-50 Litres  CultureVolumeClass3-4
### Project Additional Information

#### Purposes of the contained use

Staphylococcus aureus is a major human pathogen and the purpose of our contained use will be to study the role specific genes play in diseases caused by the bacterium.

#### Recipient or parental organism

Wild type Staphylococcus aureus (ACDP category 2) will be used (e.g. strain 8325-4). E. coli strain DH5α (ACDP category 1) will be used (genotype: F- cp8OdlacZAM15 A(lacZYA-argF)U169 deoR, recA1 endA1 hsdRI7(rk- mk+ phoA supE44 A- thi-1 gyrA96 relA)). Wild type Lactococcus lactis strain MG1363 (ACDP category 1) will be used.

#### Host/vector system

pUC based plasmids e.g. the pLL plasmids (Luong et al. 2007. J. Micro. Methods. 70:186-190) and the p5K plasmids (Grkovic et al. 2003. Microbiology. 149:785-794) will be used to manipulate S. aureus. For expression in L. lactis the pKS8O plasmids will be used (Massey et al. 2001. Cell. Micro. 3:839-851). For purification the pQE vectors provided by Qiagen will be used. These plasmid contain a multiple cloning site into which the gene in question can be cloned. It results in the fusion of this gene to a poly-histidine tag which facilitates easy purification of the gene product.

#### Origin & function

This project will involve functional studies of genes believed to contribute to S. aureus' pathogenicity. Specifically, it will involve the cloning of pieces of DNA amplified from the chromosome of S. aureus by PCR into plasmid vectors in E. coli. In E. coli the genes may be further manipulated for example by performing site-directed mutagenesis (Strategene kit) to identify important amino acids. Proteins may be purified at the stage using a His-tag system. Where we need to inactivate genes in S. aureus a shuttle vector will be used. These are typically pUC based plasmid into which a S. aureus origin of replication has been cloned, which allows the plasmid to replicate in both host organisms. Gene inactivation will be performed as follow: an antibiotic resistance cassette will be cloned into the middle of the gene in E. coli. The resulting plasmid will be electroporated into the restriction minus, modification plus S. aureus lab strains RN4220. Here the temperature sensitive plasmid will be forced to jump into the chromosome by shifting between temperatures. From here the inactivated gene can be transduced using Phage 11 into the final recipient wild type S. aureus strain. S. aureus genes may also be cloned and expressed in the bacterium Lactococcus lactis. This bacterium is used as a heterologous expression system to study bacterial proteins in a non-invasive or non-pathogenic bacterium. Here the virulence genes will be cloned into the pKS8O plasmid.

The types of genes to be studied will be those involved in host-pathogen interactions. For example the fibronectin binding proteins, the Eap proteins. These are both involved in adherence of the bacterium to human tissues.
With good microbiological practice the host organisms pose minimal risk to human health. Expression of individual S. aureus proteins in either E. coli or L. lactis may make them behave more like this ACDP cat 2 organism but is unlikely to make them more pathogenic than S. aureus itself. There are no foreseeable hazards associated with inactivating these genes in S. aureus above what is already associated with working with wild-type S. aureus. If anything we will be making them less pathogenic. With good waste kill management and microbiological practice environmental contamination with these organisms is unlikely, however should a breach in procedure occur the genetic manipulations will make them less fit than wild type environmental organisms and they will therefore not be competitive. The plasmid vectors used in this study are not naturally mobilisable. They contain origins of replication specific to the host strains and antibiotic resistance cassettes, but not ability to form conjugative pili. Under laboratory conditions (e.g. electroporation of high concentrations of purified plasmid into a strain) they can be made to move between E. coli and S. aureus. But as neither of these organisms (nor L. lactis) are naturally transformable and neither have the ability to form conjugative pili, the plasmids are considered non-mobilisable from this point. Poor microbiological techniques are used it is possible that the GMMs could escape into the environment on the hands/clothes of laboratory workers. All workers will be trained in this and will not be allowed to work with the GMM5 until their techniques are considered adequate. If any of our decontaminating procedures could fail, for example the departmental autoclave could malfunction, resulting in the release of contaminated plasticware. Or an inactive batch of Virkon could be purchased, However control measure are in place to prevent this, such as regular testing of the efficacy of our Virkon, and routine servicing and testing of the departmental autoclave.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
comments on first draft of risk assessment:
Further detail on the following issues is needed as requested by the HSE advisors:
1. mobilisation of the vector
2. decontamination procedures
3. genetic characteristics of strains used (ie DH5a genotype)
These suggested amendments were all incorporated into this application and to the attached risk assessment. The final draft was then reassessed and considered to meet local requirements.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Project Ref 85/13.1

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<td>28/01/2019</td>
<td>Hsp90 and kinase-mediated regulation of the fungal stress response, development and drug resistance</td>
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Tick if notifying a connected programme of work N

Historical Significant Changes

Transferred from GM85 28/01/2019

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

The study of fungal virulence factors

Recipient or parental organism

Class 2 fungal species: Candida albicans, Candida spec., Sporothrix schenckii, and Cryptococcus neoformans var. neoformans C. neoformans var gattii.

Host/vector system

Vectors used to transfer fungal DNA onclude pBluescript derivatives, pSP72 derivatives, pUC19 and pUC derivatives

Origin & function

Genetic material involved originated from fungi, including the class 1 species Saccharomyces cerevisiae and Ashbya gossypii

Evaluation of foreseeable effects

We will delete or replace genes that are involved in diverse aspects of fungal virulence. Thus, we assume, that most strains created here will be less virulent due to reduced gene function

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid cultures will be treated with Dettol for 5 minutes. This is expected to kill >99% of microbes. Validation could be achieved by test plating of a de-contaminated culture prior to disposal. Solid cultures and contaminated solid waste will be collected in appropriate bins and autoclaved (126C, 30 minutes). Color-changing autoclave tape is used as the standard for a successful autoclave run. Autoclaving should eradicate all fungi completely.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Genetic Safety Committee approved the risk assessment without further comments.
<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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Animal Units | Large Scale Activities | Human Clinical Applications
| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |
GM Centre Number: 20

Data Premises Notified (Originally): 01/07/1979

Transferred from 1992 Regs?: Y

Transitional Premises Class: 3

Data Premises Closed: N

Emergency Plan Required?:

Non-GMMs: Y

Withdrawn: N

Name:

MRC LABORATORY OF MOLECULAR BIOLOGY

Name 2:

Department:

Campus Estate or Research Centre:

CAMBRIDGE BIOMEDICAL CAMPUS

Road Name:

FRANCIS CRICK AVENUE

Building:

District:

Town:

CAMBRIDGE

County:

CAMBRIDGESHIRE

Postcode:

CB2 2QH

Country:

ENGLAND

Tel Number:

01223 248011

Fax Number:

01223 213556

E-mail:

HSE Division:

EAST AND SOUTH EAST

Comments:

Date at Which Additional Info Submitted:

05/08/2002 24/05/2005
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</table>
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

**Y**

Give brief details of the genetic modification safety committee

Chairman, Deputy Chairman, Alternate Chairperson/Deputy Chairperson, Nominees of staff side of local & joint committee, Representing Users, Supervisory Medical Officer from Addenbrookes Hospital & Representative of the Hospital, Representative of Hutchison & MRC research centre, MRC Centre Safety Advisor, Biological Safety Officer & Deputy Biological Safety Officer.

The quorum for a meeting consists of 2 from the Chairman, the Deputy Chairman (or the alternate), the 2 Nominees of the Staff side (or named substitutes) plus the Biological Safety Officer or Deputy Biological Safety Officer.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Bacteriology | Parasitology | Transgenic Birds | Microbiology Research |

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

The quorum for a meeting consists of 2 from the Chairman, the Deputy Chairman (or the alternate), the 2 Nominees of the Staff side (or named substitutes) plus the Biological Safety Officer or Deputy Biological Safety Officer.

---

**Project Ref**  20/00.1

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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Withdrawn  N  Tick if notifying a connected programme of work  N
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
Project Ref 20/00.2

Date Ackn'd 28/11/2000

Date Project Ceased

CU2 Project Title HIV GENE EXPRESSION

Class 2

CultureVolClass2

Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2   L3   L4   L2   L3   L4   L2   L3   L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2   L3   L4   L2   L3   L4   L2   L3   L4</td>
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Project Ref  20/00.3

Date Ackn’d            CU2 Project Title                     Class       CultureVolClass2       CultureVolumeClass3-4
01/12/2000             STRUCTURE OF V-TYPE ATPASE IN ENTEROCOCCUS HIRAE   Class 2       1-50 litres
Project Additional Information

Purposes of the contained use

Overproduction of the V-ATPase of *E. hirae* into which the V-ATPase operon has been cloned. (This work is self-cloning and the GMM has already been constructed and characterised elsewhere.)

The overproduction is wanted to allow purification of the V-ATPase for structural studies.

Recipient or parental organism

*Enterococcus hirae* (formerly *Streptococcus faecalis*), ATCC9790 and 25D.

Host/vector system

*E. hirae* (as above) as host; pHY300LK vector.

This is a commercially available, mobilisation defective vector from Yakuruto Co. (Japan). It is one of the smallest hybrids of a series of chimeric plasmids using the parental plasmids pACYC177 of *E. coli* and pAM-a of *Streptococcus faecalis*. This shuttle vector for *E. coli* and *B. subtilis* contains an RNA primer gene for ori-177; the R-Tc gene; the R-Ap gene; Rep-alpha-1 gene, which is the plasmid replication gene; two replication origins, one for *E. coli* and the other for *B. subtilis*; and a polylinker.

Origin & function

The genetic material cloned is derived from *E. hirae* and is cloned back into the micro-organism as host, i.e. the work is self-cloning. The resultant construct has already been characterised elsewhere (in Japan) and the work within the UK will involve growth, cell lysis and purification of the cloned protein.

Evaluation of foreseeable effects

*Enterococcus hirae* is a human commensal, which occurs in the intestine of normal healthy people and only occasionally causes disease due to getting into the wrong environment (e.g. the bloodstream). Since it can cause disease, it is classified as a Hazard Group 2 pathogen - in the same way as *Eschericia coli*.

The genetic modification involves self-cloning of an operon from *E. hirae*, in order to obtain increased yields of the protein product from this operon. The protein is membrane bound and will not increase the pathogenicity of the host (which has it naturally).

The only foreseeable affect is the disabling of the *E. hirae*, due to overproduction of one protein rather than the appropriate amount being made as in the wild-type cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms involved in this work.
Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

After growth, the micro-organisms will be centrifuged and collected. The culture supernatant will be disinfected with a clear phenolic disinfectant. During the initial experiments the optimum disinfection conditions will be determined, to obtain <1 viable cell/ml, and these conditions then be applied to all supernatants before discharge down the drain into the public sewer. The cell pellet will be resuspended and the cells lysed to prepare the protein. Any substantially solid residues will be inactivated by autoclaving at 135 for 15min, which is expected to give - 100% killing.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

It was noted that E. hirae is the new name for Streptococcus faecalis, which is a normal component of the gut microflora in healthy individuals. However, since it does occasionally cause illness (mostly by infection of wounds) it is classified as Hazard Group 2 under ACDP guidelines.

Clones have already been constructed elsewhere, which have the V-ATPase operon self-cloned for high level expression in E. hirae. The vector is mobilisation defective.

It was agreed that neither the insert nor the vector increases the pathogenicity of the host, so this requires Containment Level 2 and is therefore Class 2.

Project Containment

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<tr>
<th>Laboratory Activities</th>
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Project Ref  20/02.1

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<td>17/04/2002</td>
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**Project Additional Information**

### Purposes of the contained use

To study the control functions of the HIV LTR when inserted into the host cell genome by the viral integrase.

### Recipient or parental organism

Human tissue culture cell lines.

### Host/vector system

Laboratory strains of E. coli K12.

Tissue culture cells (human).

3rd generation HIV-based packaging constructs which give pseudo-typing with the envelope protein of vesicular stomatis virus, as described by Vigna & Naldini (J Gene Med, 2, 308-316 (2000)) and Dull et al. (J Virol, 72, 8463-8471 (1998)). This system involves co-transfection of the viral producer cells with separate plasmids expressing HIV gag-pol under the control of the CMV promoter, a Rev gene under the control of the RSV LTR, and a VSV envelope gene under the control of CMV.

### Origin & function

The inserts are a series or reporter genes, including luciferase genes, green fluorescent proteins (and their variants) and drug resistant genes, including neomycin, hygromycin and blastocidin resistance genes.

These inserts will be inserted under the control of the HIV LTR, in order to help in studying the control of transcription from this.

### Evaluation of foreseeable effects

The major potential hazard arises from the production of defective retrovirions, which are pseudo-typed with the VSV coat protein, following transfection of the t.c. cells. These will have a wide host range for infection, including humans. The inserted genes are all harmless reporter genes, which will not cause any hazard even if expressed in an unintended context.

The vectors being employed were developed for gene therapy and, despite being HIV-based, have a very low risk of any hazard other than that potentially arising from a single cycle infection with the inserted genes.

The classification of work with retroviral vectors is considered in ACGM Guidance Notes, Part 2B, Annex III paras. 14-34. The vector system for the eukaryotic work is based on HIV, which is a hazard group 3 pathogen. However, the individual plasmids do not contain a complete HIV genome between them, in particular lacking the tat.
env and nef genes. Moreover, in this 3rd generation packaging system the gag/pol and rev genes are on separate plasmids with no common sequences to allow homologous recombination and neither of these vectors has the packaging signals. This results in a particularly low recombination probability and no packaging of HIV genes. The only vector with packaging signals will contain only the inserted genes, which are of extremely low hazard and will not increase the pathogenicity of the cells in any way, although their expression might lead to cell death of individual cells.

Although the VSV-pseudo-typing of the defective retroviruses, produced from the packaging step in the t.c. cells, will broaden the host range, this is not considered to have any significant effect on the overall hazard, due to the very low hazard of the inserted genes in this work. It is therefore considered that Class 2 is appropriate for a provisional classification of the work in t.c. cells.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| Full containment level 2 will be applied. |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing. Containment level 2 waste will be autoclaved (136C for 10min), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing. |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

### Please enter comments on the GM safety committee on the risk assessment

This work involves the use of "third generation" HIV-based vectors to generate a retrovirus pseudo-typed with the vesicular stomatitis virus surface protein, which is capable of a single infective cycle in human cells. The intention is to obtain cell lines containing the gene for a reporter protein, under the control of the HIV LTR, which has been inserted into the cell genome by the normal function of the HIV integrase. The BSO pointed out that the ACGM Compendium of Guidance, Part 2B, Annex III, para. 26 says that work employing vectors based on primate lentiviruses should generally be Class 3, unless there is a clear justification for using a lower level of containment. The committee considered the particular vector system proposed for this work and concluded that it would require too many specific, non-homologous recombination steps for the generation of a normally infectious retrovirus to have anything more than a negligible likelihood. Moreover, the inserted genes are all for reporter proteins which do not pose any hazard to either humans or the environment. It was therefore agreed that there was a clear justification, in this case, for a lower level of containment and that the steps involving the production of the viral vector, infection of the host tissue culture cells and their subsequent propagation should be Class 2. The committee considered that any stable cell lines should be checked to ensure that they are not generating virions, by assaying the supernatants from cell cultures for reverse transcriptase, before they are brought out of CL2. The initial cloning steps, working with laboratory strains of E. coli K12, were considered to be class 1.
**Project Ref** 20/02.2

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>14/05/2002</td>
<td>REGULATION OF EPIDERMAL STEM CELL FATE.</td>
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<table>
<thead>
<tr>
<th>Class</th>
<th>Culture Vol Class 2</th>
<th>Culture Volume Class 3-4</th>
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<tbody>
<tr>
<td>Class 2</td>
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<table>
<thead>
<tr>
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<th>Consent Granted</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>not applicable</td>
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Tick if notifying a connected programme of work: **N**

<table>
<thead>
<tr>
<th>Historical Significant Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Transferred from GM20 to GM921 on 02/08/2005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Significant Change ID</th>
<th>Date of Significant Change</th>
</tr>
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</table>

**Project Additional Information**

**Purposes of the contained use**

To study the pathways regulating differentiation of human cells, in particular how stem cell fate is regulated.

**Recipient or parental organism**

Human and mouse tissue culture cell lines.

**Host/vector system**

- Laboratory strains of E. coli K12.
- Phoenix-ecotropic and amphotropic retroviral producer cells.

**Origin & function**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L4 L3 L2 L4</td>
<td>L3 L4 L2 L3 L4</td>
<td>L3 L4 L2 L3</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
<td></td>
</tr>
</tbody>
</table>

Historical Date of Additional Info

02/08/2005

Withdrawn: **N**
Inserts used are all derived from mouse, human and Xenopus cDNA libraries and comprise wild type and mutated forms of Notch ligands, Notch receptors, suppressor of hairless homologues, mastermind homologues, and Notch target genes including the hairy/enhancer of split family of transcription factors. In addition mutant Xenopus beta catenin and mutants of MAP kinase will be expressed to determine how the Notch pathway interacts the wnt and Map kinase signalling pathways. Cell cycle inhibitory proteins such as p21Cip1 and Geminin will also be used.

In addition reporter constructs derived from the pGL3 series of vectors will be used; these consist of Notch and wnt responsive promoter elements, driving expression of the firefly and renilla luciferase genes. In some experiments green fluorescent protein will be expressed as a reporter.

The intended use is to study how these genetic elements control the cell development.

**Evaluation of foreseeable effects**

The main hazard arises directly from the recombinant retroviruses produced from the packaging cells. These yield both ecotropic and amphotropic retrovirions, depending upon the envelope protein supplied by the cell.

The packaging cell lines used to generate the retrovirus have either ecotropic or amphotropic envelope proteins, and the retrovirus therefore can either infect only mouse cells, or have a wider host range including human cells. Their infectivity is unstable and infection is only obtained by co-cultivation of the packaging cell line with ther recipient cells. The retrovirus is self-inactivating and there is thus no possibility of further transfer. With the ecotropic packaging cell line, there is therefore negligible risk to the worker or the environment.

With the amphotropic packaging cell line, the risk of infection of a worker during the packaging/infection step is small, but still not negligible. Although the inserted genes are not known to be oncogenic, they have this potential and it is considered that this part of the work is Class 2. In view of the, at worst, low-oncogenic nature of the inserted genes and also the self-inactivating nature of the retroviruses, there likelihood of any hazardous replication competent virus being generated is negligible and is no need to test for such RCV.

The single non-negligible hazard arises from the potentially oncogenic or cytotoxic nature of some of the inserts. This risk can be controlled if DNA grown up from such clones is handled appropriately; ie gloves should be worn, sharps avoided and allwastes be rendered harmless before disposal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 mins), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N
This work involved the cloning of some potentially oncogenic genes into disabled retrovirus vectors, with the use of packaging cell lines which have an amphotropic envelope protein.

From the ACGM Compendium of Guidance, Part 2B, Annexe III, particularly para. 30, this requires Class 2 (as already concluded in the risk assessment).

The Committee noted that the risk of the potentially oncogenic DNA had already been considered in the assessment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
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<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
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<tr>
<td>L3</td>
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<tr>
<td>L4</td>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
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<table>
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<tr>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L2</td>
<td>L3 L4 L2 L3 L4</td>
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**Project Ref 20/02.4**

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<td>FUNCTIONAL SILENCING OF GENES INVOLVED IN DNA REPAIR AND CELL CYCLE: 2</td>
<td>Class 2</td>
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<td>not applicable</td>
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<table>
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<tr>
<th>Date Project Ceased</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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**Historical Significant Changes**

Transferred to GM921 from GM20 on 02/08/2005

**Historical Date of Additional Info**

02/08/2005
### Project Additional Information

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<th>Date of Significant Change</th>
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#### Purposes of the contained use
To functionally silence genes whose inactivation in cancers gives rise to genetic instability, using a recently developed stable RNA interference approach, to ascertain the molecular basis for the abnormal division of cancer cells and their increased genetic instability.

<table>
<thead>
<tr>
<th>Recipient or parental organism</th>
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</thead>
<tbody>
<tr>
<td>Tissue culture cells of human origin.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory strains of E. coli K12 derivatives; tissue culture cells of human origin.</td>
</tr>
<tr>
<td>pUC-based plasmid vectors with RNA polymerase III promoter (e.g. pSUPER);</td>
</tr>
<tr>
<td>Phoenix-amphotropic retroviral producer cells.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>The plan is to use vectors based on pSUPER in which an RNA polymerase III promoter drives expression of a short (~25 mer) interfering RNA from a synthetic DNA insert. Details of the categories of interfering RNAs to be inserted are as follows:</td>
</tr>
</tbody>
</table>

A) RNAs which target DNA double-strand break repair pathways: The proteins encoded by BRCA1, BRCA2, RAD51 and its paralogs, Nbs1, ATM, ATR, Ku70, Ku80 and DNA-PK are nuclear molecules which participate in the sensing and repair of DNA double-strand breaks. In mice, homozygosity for null mutations in these genes leads to cell death, embryonal lethality and a DNA repair defect accompanied by genetic instability.

B) RNA which target cell cycle regulators: The proteins encoded by the mammalian cyclin/CDK genes, the mitotic kinases Pik1, nek1, Aurora-A/B/C, Bub1/BubR1 and the chromatid cohesion molecules Scc1, SMC1/3 and Eco1, are required for cell cycle progression and mitosis.

C) RNAs which target regulators of the G1, S and G2/M checkpoints: These inserts will include proteins encoded by checkpoint regulators such as p53, chk1/chk2 and chfr, or proteins involved in the downstream enforcement of these checkpoints (including cdc14, ranGAP, cyclin G, cdc25 or PP1).

<table>
<thead>
<tr>
<th>Evaluation of foreseeable effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>The inserts encode short interfering RNAs which are only active within the cell, and cannot encode an exogenously expressed protein. Expression of these RNAs will not enhance the pathogenicity of the host cells. The effect of expression of the insert RNAs will be to functionally silence the target genes. In most cases, functional silencing is known to impair cellular function resulting in defective cell cycle progression or in genetic instability. In no instance is functional silencing of the target genes known to work directly to transform primary cell cultures. A non-negligible hazard arises from the potentially oncogenic or cytotoxic nature of the cellular effects of some of the interfering RNA inserts. It is considered that DNA from such clones can be appropriately handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras 8-10, in which the potential hazard is specifically considered.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only micro-organisms (including tissue culture cells) are involved in this work.</td>
</tr>
</tbody>
</table>

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This work involves the cloning of short DNA inserts under a eukaryotic promoter, in order to generate ~25-mer interfering RNA. The genes targeted in this work are those for sensing and repair of DNA double-stranded breaks, cell cycle progress and mitosis, and checkpoint regulators or downstream enforcement proteins. The cloned DNA is therefore potentially cytotoxic or oncogenic.

Initial cloning is into non-mobilisable vectors grown in laboratory E. coli K12 strains. The vectors are pUC-based and are disabled retroviral vectors, which require a helper t.c. line to produce virus, which in turn is only capable of a single infectious cycle. This stage is therefore Class 1.

Subsequently the retroviral vectors will be transfected into mouse emphotropic cell lines, with the packaging construct in more than one component, to produce the disabled retrovirus with an amphotropic envelope. Following consideration of the advice in the ACGM Compendium of Guidance, Part 2B, Annex III, paras 21-30, it was agreed that Class 2 is appropriate for this work. If cell lines are being maintained for a long time, consideration should be given to testing them for the presence of replication competent virus.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Animal Units</td>
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<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>
### Project Additional Information

**Purposes of the contained use**

The project aims to determine the molecular basis of the differentiation of human and mouse epithelial cells. Pathways regulating differentiation include Notch, wnt and the MAP kinase signalling cascade. In addition we wish to explore the interaction of Notch with cell cycle regulators such as p21 Cip1 and Geminin.

**Recipient or parental organism**

Tissue culture cells of human or murine origin.

**Host/vector system**

Laboratory strains of E. coli K12 derivatives; tissue culture cells of human origin.

pUC-based plasmid vectors with RNA polymerase III promoter; Phoenix-amphotropic retroviral producer cells.

**Origin & function**

The expression of inserts encoding siRNAs, consisting of sense and antisense 21-29 base sequences derived from the coding region of the target mRNA with a spacer to generate a hairpin RNA, in human primary epidermal keratinocytes, mouse primary keratinocytes and mouse and human cell lines, including squamous carcinoma cells and telomerase immortalised keratinocytes. Once expressed the siRNA oligonucleotides are expected to decrease levels of RNA encoding the target protein by over 70%.

Target genes are those of the pathways regulating epithelial stem cell fate, including Notch receptors, suppressor of hairless homologues, mastermind homologues, and...
the hairy/enhancer of split family of transcription factors. In addition components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins such as p21 Cip1 and Geminin will also be targeted.

Control siRNAs directed against enhanced green fluorescent protein and luciferase will be required for some experiments.

**Evaluation of foreseeable effects**

The inserts encode short interfering RNAs which are only active within the cell, and cannot encode an exogenously expressed protein. Expression of these RNAs will not enhance the pathogenicity of the host cells.

The effect of expression of the insert RNAs will be to functionally silence the target genes. In most cases, functional silencing is known to impair cellular function resulting in defective cell cycle progression or in genetic instability. In no instance is functional silencing of the target genes known to work directly to transform primary cell cultures.

A non-negligible hazard arises from the potentially oncogenic or cytotoxic nature of the cellular effects of some of the interfering RNA inserts. It is considered that DNA from such clones can be appropriately handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras. 8-10 in which the potential hazard is specifically considered.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

**Is an emergency plan required according to regulation 20?**

- [ ] N

**If yes, tick to confirm that it is attached to this form**

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] N
This work involves the cloning of short DNA inserts under a eukaryotic promoter, in order to generate ~25-mer interfering RNA. The genes targeted in this work are those for pathways controlling epithelial stem cell fate, components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins. The cloned DNA is therefore potentially cytotoxic or oncogenic.

Initial cloning is into non-mobilisable vectors grown in laboratory E. coli K12 strains. The vectors are pUC-based and are disabled retroviral vectors, which require a helper t.c. line to produce virus, which in turn is only capable of a single infectious cycle. This stage is therefore Class 1.

Subsequently the retroviral vectors will be transfected into mouse cell lines, with the Phoenix packaging constructs, which give either ecotropic or amphotropic envelopes, to produce the disabled retrovirus with either type of envelope. Following consideration of the advice in the ACGM Compendium of Guidance, Part 2B, Annex III, paras 21-30, it was agreed that Class 1 is appropriate with the ecotropic envelope and Class 2 with the amphotropic. If cell lines from the amphotropic constructs are being maintained for a long time, consideration should be given to testing them for the presence of replication competent virus.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>L3 L4</td>
<td>L2 L2 L4</td>
</tr>
</tbody>
</table>

**Project Ref 20/03.2**

- **Date Ackn'd**: 26/02/2003
- **CU2 Project Title**: GENETIC ANALYSIS OF MAMMALIAN SIGNAL TRANSDUCTION
- **Class**: Class 2
- **Culture Vol**: ≤ 1 litre
- **Non-GMM**: not applicable
- **Consent Granted**: Project notified under transitional arrangements

<table>
<thead>
<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
**Purposes of the contained use**

A program for the discovery of gene function related to mammalian signal transduction.

Mammalian reporter cell lines will be constructed, with reporter genes or selectable markers cloned under the control of pathway specific promoters. These cell lines will be used to screen mammalian cDNA libraries, to identify genes acting in a particular pathway. Individual cDNAs will be identified, based on the phenotypic changes that they cause in the reporter cell line, and then studied further by truncation, point mutation etc.

**Recipient or parental organism**

Tissue culture cells of human or murine origin.

**Host/vector system**

- Laboratory strains of E. coli K12 derivatives;
- Tissue culture cells of human or murine origin.
- Retroviral producer cells, such as the Phoenix-ecotropic and amphotropic and VSV-G expressing retroviral producer cells.
- PUC-based plasmid vectors; Moloney Leukaemia virus based vectors.

**Origin & function**

- Reporter or selectable resistance genes, cloned under pathway specific promoters, to study changes in the control of the particular pathway.
- Mammalian cDNA libraries, to probe to select genes involved in the control of these specific pathways.
- Genes identified from this screen/selection, and altered forms of these genes, to study their role in controlling these specific pathways.

**Evaluation of foreseeable effects**

The initial inserts code for either marker proteins or resistance proteins which will allow quantitation and/or sorting or selection of the tissue culture cells which express them at sufficient levels. These are cloned under the control of pathway specific promoters, to allow study of the control of these pathways.

Subsequent cloning into these cells will be to screen cDNA libraries for genes which do control these pathways. Genes obtained in this way will then be modified (by truncation, point mutation or fusion to other genes), in order to gain an understanding of how they function in the cell.

These steps will be expected to alter the expression levels of the previously cloned marker or resistance proteins, with this alteration acting as the marker for the function of the controlling genes.

A non-negligible hazard arises from the risk of insertional mutagenesis and the potentially oncogenic or cytotoxic nature of some of the inserts. DNA grown up from such clones should be handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras. 8-10, ie gloves should be worn, sharps avoided and all wastes be rendered harmless before disposal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
For the part of the work classified as Class 2, full containment level 2 will be applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 1 waste, generated during the initial steps, will be inactivated by either treatment with 1% v/v Clearsol, before discharge down the drain into the public sewer, or by incineration (in a registered facility). Data have been obtained from the manufacturer to show that the disinfection will achieve >10 (to the power of 5) loss of infectivity in the times used, while incineration will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This is a project to study mammalian signal transduction, by establishing stable cell lines which have reporter genes under the control of various pathway specific promoters. These cells will then be challenged with cloned cDNAs which are thought to have a role in controlling these promoters and either up-or-down regulation be checked using the reporter protein. In some cases the reporter gene will code for a selectable marker, so that cells in which the promoter activity has been changed can be selected, rather than just quantitated.

The host cells are either standard E. coli K12 derivatives, used for generation of the cloned DNA, or standard mammalian t.c. cell lines. In addition various retroviral packaging cell lines will be used for the generation of replication-defective virions with ecotropic or amphotropic envelopes, or with VSV-G envelope protein. These retroviral packaging lines are based on the Phoenix system, with the helper functions in 3 separate parts of the genome, thus reducing the likelihood of recombination to produce replication competent virus to a negligible level. The host cells are all highly disabled. The vectors are either standard plasmids (with eukaryotic promoters were appropriate) (eg pUC-derivatives, wityout a promoter, or pCDNA), or disabled Moloney Murine Leukaemia virus (MMLV) based plasmids which can be used to generate disabled virions in the helper, packaging cell lines.

These vectors are all non-mobilisable in E. coli and also normal t.c. cells and neither they, nor the inserted transgenes, will increase the pathogenicity of the host cells.

The MMLV constructs are, by design, mobilised from the packaging cell lines, to generate defective virions, capable only of a single infective cycle, and even these have a limited efficiency of infection and special steps are usually taken to obtain a good infection rate. Depending upon the envelope protein, these virions may have a restricted or a wider host range.

It is considered that the precautions needed for safety of the workers will reduce the likelihood of hazard to the environment to a negligible level, with the resulting risk being effectively zero.

Having considered the potentially cytotoxic or oncogenic nature of some of the transgenes and the advice in the ACGM Compendium of Guidance, Part 2B, Annex III, paras, 14-34, it was agreed that the work is correctly classified as Class 1 for all the E. coli work and the work with t.c. cells and ecotropic retrovirus, and as Class 2 for the work with t.c. cells and amphotropic or VSV-G protein pseudo-typed retrovirus.

It is noted that the potential hazard of the naked DNA has already been considered in the risk assessment and that appropriate precautions are proposed.
Project Containment

Laboratory Activities

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<th>L4</th>
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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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<th>L4</th>
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Human Clinical Applications

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<th>L3</th>
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Project Ref 20/03.3

Date Ackn'd 07/07/2003

CU2 Project Title THE GENETIC REQUIREMENTS FOR RETROTRANSPOSITION

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2 L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
<td></td>
</tr>
</tbody>
</table>

Project Ref 20/03.4

Date Ackn'd CU2 Project Title

Class CultureVolClass2 CultureVolumeClass3-4
The project aims to determine the molecular basis of the differentiation of human and mouse epithelial cells. Pathways regulating differentiation include Notch, wnt and the MAP kinase signalling cascade. In addition we wish to explore the interaction of Notch with cell cycle regulators such as p21 Cip1 and Geminin.

Tissue culture cells of human or murine origin.

Laboratory strains of E. coli K12 derivatives; tissue culture cells of human or mouse origin, including murine cells expressing helper functions for defective retrovirus production.

pUC-based plasmid vectors with RNA polymerase III promoter; Plasmids containing lentiviral helper proteins - see diagrams attached to risk assessment.

The expression of inserts encoding siRNAs, consisting of sense and antisense 21-29 base sequences derived from the coding region of the target mRNA with a spacer to generate a hairpin RNA, in human primary epidermal keratinocytes, mouse primary keratinocytes and mouse and human cell lines, including squamous carcinoma cells and telomerase immortalised keratinocytes. These cells will include those infected with lentiviruses or retroviruses engineered to include a LoxP sequence flanked cDNA sequence.

Also expression of non-oncogenic genes involved in these pathways.

Target genes are those of the pathways regulating epithelial stem cell fate, including Notch receptors, suppressor or hairless homologues, mastermind homologues, and the hairy/enhancer of split family of transcription factors. In addition components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins such as p21 Cip1 and Geminin will also be targeted.
Control siRNAs directed against enhanced green fluorescent protein and luciferase will be required for some experiments.

**Evaluation of foreseeable effects**

The inserts encode either non-oncogenic proteins or short interfering RNAs which are only active within the cell, and cannot encode an exogenously expressed protein. Expression of these RNAs will not enhance the pathogenicity of the host cells. The effect of expression of the insert RNAs will be to functionally silence the target genes. In most cases, functional silencing is known to impair cellular function resulting in defective cell cycle progression or in genetic instability. In no instance is functional silencing of the target genes known to work directly to transform primary cell cultures.

The expression of the Cre recombinase will recombine the LoxP sites. Among other resulting deletions will be the gene for Cre itself.

Anon-negligible hazard arises from the potentially oncogenic or cytotoxic nature of the cellular effects of some of the interfering RNA inserts. It is considered that DNA from such clones can be appropriately handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras. 8-10 in which the potential hazard is specifically considered.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before eit[e subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

**Is an emergency plan required according to regulation 20?**

- [ ] N

**If yes, tick to confirm that it is attached to this form**

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] N
This work involves the cloning and expression of non-oncogenic proteins involved in pathways controlling epithelial cell fate. It also involves the cloning of short DNA inserts under a eukaryotic promoter, in order to generate ~25-mer interfering RNAs. These genes targeted in this work are those for pathways controlling epithelial stem cell fate, components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins. The cloned DNA is therefore potentially cytotoxic or oncogenic.

Initial cloning is into non-mobilisable vectors grown in laboratory E. coli K12 strains. The vectors are pUC-based and are disabled lentiviral or retroviral vectors, which require a helper t.c. line (or helper plasmids in a t.c. cell line) to produce virus, which in turn is only capable of a single infectious cycle. This stage is therefore Class 1.

Subsequently the retroviral vectors will be transfected into mouse cell lines, together with plasmids supplying helper functions, which give either ecotropic or amphotropic envelopes, to produce the disabled lentivirus with either type of envelope. Following consideration of the advice in the ACGM Compendium of Guidance, Part 2B, Annex III, paras. 21-30, it was agreed that Class 1 is appropriate with the ecotropic envelope and Class 2 with the amphotropic.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
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</table>

### Project Ref 20/04.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>17/03/2004</td>
<td>GENETIC ANALYSIS OF THE HUMAN FANCD2 GENE</td>
<td>Class 2</td>
<td>≤ 1 litre</td>
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</table>

<table>
<thead>
<tr>
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</thead>
<tbody>
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</table>

Project notified under transitional arrangements N

<table>
<thead>
<tr>
<th>Withdrawn</th>
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</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

### Historical

Significant Changes

Date of Additional Info

Significant Change ID
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

A study of the human FANCD2 gene, which is a downstream effector molecule in the Fanconi anaemia pathway. The cloned FANCD2 gene (and point mutants which are thought to be inactive for DNA repair) will be used to establish infection into human fibroblast cell lines that are mutant for FANCD2. Expression will be monitored and cells studied for complementation of the FA defect. Tagging of the gene will also be carried out to facilitate detection in cells. To establish complementation, cells will be studied for survival after exposure to DNA damaging agents, using FACS analysis, chromosome breakage studies and cell viability assays.

**Recipient or parental organism**

Tissue culture cells of human origin.

**Host/vector system**

Laboratory strains of E.coli K12 derivatives; Tissue culture cells of human or murine origin. Retroviral producer cells, such as the Phoenix-ecotropic and amphotropic and VSV-G expressing retroviral producer cells. pUC-based plasmid vectors; Moloney Leukaemia virus based vectors.

**Origin & function**

The human FANCD2 gene (and mutants thereof), to study their effect in complementing natural mutations.

**Evaluation of foreseeable effects**

The expected effect of expression of the transgene is a possible repair of the defective DNA repair of the defective DNA repair pathway in the original cell lines in which FANCD2 is mutant, if the inserted gene is either wild-type or contains a mutation which still allows it to complement the mutant already existing in the host cell line.

A non-negligible hazard arises from the risk of insertional mutagenesis and the potentially oncogenic or cytotoxic nature of some of the inserts. DNA grown up from such clones should be handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex I, paras.8-10; i.e. gloves should be worn, sharps avoided and all wastes be rendered harmless before disposal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 2 waste will be autoclaved (136 degrees for 10min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

02/03/2022
This work involves cloning the human FANCD2 gene (and point mutants thereof) into a defective Moloney murine leukaemia vector, using the Phoenix eco-and amphotropic packaging systems. The initial cloning is correctly assessed as Class 1. Since the mutant transgenes are considered as potentially oncogenic (in the absence of sufficient information to decide otherwise), the retroviral cloning was assessed in this light. With the ecotropic envelope, Class 1 complies with the ACGM Compendium of Guidance, Part 2B, Annex III, para.29 while with the amphotropic envelope this becomes Class 2, according to the ACGM Compendium of Guidance, Part 2b, Annex III, para.30.

It was also noted that the risk from the potentially oncogenic DNA has been considered and appropriate precautions proposed.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tbody>
<tr>
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<td>L3</td>
<td>L2</td>
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<tr>
<td>L3</td>
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**Animal Units**

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<th>L4</th>
</tr>
</thead>
</table>

**Large Scale Activities**

<table>
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<tr>
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<th>L4</th>
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**Human Clinical Applications**

<table>
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<tr>
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<th>L4</th>
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</table>

**Project Ref** 20/04.2

Date Ackn'd 17/03/2004

Date Project Ceased

Date notified under transitional arrangements N

Tick if notifying a connected programme of work N

Tick if you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This work involves cloning the human FANCD2 gene (and point mutants thereof) into a defective Moloney murine leukaemia vector, using the Phoenix eco-and amphotropic packaging systems. The initial cloning is correctly assessed as Class 1. Since the mutant transgenes are considered as potentially oncogenic (in the absence of sufficient information to decide otherwise), the retroviral cloning was assessed in this light. With the ecotropic envelope, Class 1 complies with the ACGM Compendium of Guidance, Part 2B, Annex III, para.29 while with the amphotropic envelope this becomes Class 2, according to the ACGM Compendium of Guidance, Part 2b, Annex III, para.30.

It was also noted that the risk from the potentially oncogenic DNA has been considered and appropriate precautions proposed.
**Project Additional Information**

**Purposes of the contained use**

A program for the discovery of gene function related to mammalian signal transduction. Mammalian reporter cell lines will be constructed, with reporter genes or selectable markers cloned under the control of pathway specific promoters. These cell lines will be used to screen mammalian cDNA libraries, to identify genes acting in a particular pathway. Individual cDNAs will be identified, based on the phenotypic changes that they cause in the reporter cell line, and then studied further by truncation, point mutation etc.

**Recipient or parental organism**

Tissue culture cells of human or murine origin.

**Host/vector system**

Laboratory strains of E.coli K12 derivatives; tissue culture cells of human or mouse origin. pUC-based plasmid vectors, including plasmids containing retroviral helper proteins.

**Origin & function**

A program for the discovery of gene function related to mammalian signal transduction. Mammalian reporter cell lines will be constructed, with reporter genes or selectable markers cloned under the control of pathway specific promoters. These cell lines will be used to screen mammalian cDNA libraries, to identify genes acting in a particular pathway. Individual cDNAs will be identified, based on the phenotypic changes that they cause in the reporter cell line, and then studied further by truncation, point mutation etc.

**Evaluation of foreseeable effects**

The initial inserts code for either marker proteins or resistance proteins which will allow quantitation and/or sorting or selection of the tissue culture cells which express them at sufficient levels. These are cloned under the control of pathway specific promoters, to allow study of the control of these pathways.

Subsequent cloning into these cells will be to screen cDNA libraries for genes which do control these pathways. Genes obtained in this way will then be modified (by truncation, point mutation or fusion to other genes), in order to gain an understanding of how they function in the cell. These steps will be expected to alter the expression levels of the previously cloned marker or resistance proteins, with this alteration acting as the marker for the function of the controlling gene.

A non-negligible hazard arises from the risk of insertional mutagenesis and the potentially oncogenic or cytotoxic nature of some of the inserts. DNA grown up from such clones should be handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex I, paras. 8-10; i.e. gloves should be worn, sharps avoided and all wastes be rendered harmless before disposal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

For the part of the work classified as Class 2, full containment level 2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give 100% killing.

This project is basically very similar to others already considered and approved (2003/004, HSE Ref. GM20/03.2; 2003/023), involving the same transgenes in defective retroviruses with eco- and amphotropic envelope proteins. The difference is that the current work, rather than employing a helper cell line which provides the necessary proteins for packaging and envelopment of the retroviral RNA, these functions will be provided by co-transfection of the packaging cell line with a helper plasmid, coding for these functions, as well as the plasmid producing the defective retroviral RNA with the transgene. The helper plasmid provides functions from the mink cell focus forming virus, with either ecotropic or amphotropic envelope protein, and is commercially marketed by Imegemex (Naviaux et al). It was agreed that this modification of how the helper functions are supplied does not alter the risk and that the work remains Class 1 for the initial cloning and ecotropic work, and Class 2 for the amphotropic work. It was noted that appropriate precautions are to be taken for handling the potentially oncogenic DNA.

Please enter comments on the GM safety committee on the risk assessment:

This project is basically very similar to others already considered and approved (2003/004, HSE Ref. GM20/03.2; 2003/023), involving the same transgenes in defective retroviruses with eco- and amphotropic envelope proteins. The difference is that the current work, rather than employing a helper cell line which provides the necessary proteins for packaging and envelopment of the retroviral RNA, these functions will be provided by co-transfection of the packaging cell line with a helper plasmid, coding for these functions, as well as the plasmid producing the defective retroviral RNA with the transgene. The helper plasmid provides functions from the mink cell focus forming virus, with either ecotropic or amphotropic envelope protein, and is commercially marketed by Imegemex (Naviaux et al). It was agreed that this modification of how the helper functions are supplied does not alter the risk and that the work remains Class 1 for the initial cloning and ecotropic work, and Class 2 for the amphotropic work. It was noted that appropriate precautions are to be taken for handling the potentially oncogenic DNA.

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<tr>
<td>L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
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Animal Units

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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 20/04.3

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<th>CU2 Project Title</th>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>17/06/2004</td>
<td>Using Somatic Knockouts for novel compound screening and target/pathway validation</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
</tr>
<tr>
<td>Date Project Ceased</td>
<td></td>
<td></td>
<td></td>
<td>Not Applicable</td>
</tr>
</tbody>
</table>
Project Additional Information

Purposes of the contained use

To use somatic cell line knockout technology to generate assay reagents for combinatorial libraries of potential therapeutic compounds and for pathway analysis of potential new therapeutic targets.

This project requires gene disruption through homologous recombination in human epithelial cells in vitro; rAAV (adeno-associated virus) vectors permit such knockouts in human cells.

Inserts will encode LoxP sites, human genomic targets and positive selectable marker genes (e.g., neomycin or hygromycin). Target cells are human epithelial cell lines, including HCT-116, DLD1, A549, CAL51 and MT3 cell lines. These cells will be infected with rAAV (e.g., AAV-293).

Target genes are those of the pathways regulating DNA repair, apoptosis, histone acetylation, including BRCA2, HDCA1, SIRT1 and ADA3.

Recipient or parental organism

Tissue culture cells of human origin.

Host/vector system

Laboratory strains of E. coli K12 derivatives; Tissue culture cells of human origin.

Human t.c. lines both for recombinant virus production (e.g., AAV-293 cells derived from HEK 293 cells will be used as producer cells) and for subsequent infection to produce recombinant t.c. cell lines.

pUC-based plasmid vectors; Moloney Leukaemia virus based vectors.

AAV Helper-Free system (Stratagene) (pCMV-MCS for initial cloning, pAAV-LacZ to produce the ssDNA for encapsidation, pAAV-RC and pHelper to provide the adenoviral proteins required for encapsidation); the resulting recombinant adeno-associated viral vectors will be used for transduction of human cells.

Origin & function

Target genes are those of the pathways regulating DNA repair, apoptosis, histone acetylation, including BRCA2, HDCA1, SIRT1 and ADA3.

Evaluation of foreseeable effects

The planned knockout of the target genes in human t.c. cell lines is not foreseen as increasing their pathogenicity from its negligible level for the cell lines.

The one significant hazard arises from the rAAV produced from the packaging cells. This is capable of infecting human cells and contains inserts designed to knockout target genes in the pathways regulating DNA repair, apoptosis, histone acetylation, including BRCA2, HDCA1, SIRT1 and ADA3. Their effect is therefore considered as...
potentially oncogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? (Y/N) N

Tick to confirm that it is attached to this form (Y/N) N

Tick if you are claiming exemption from disclosure for section of the risk assessment (Y/N) N

Please enter comments on the GM safety committee on the risk assessment

The work involves the cloning of inserts to knockout various genes, their incorporation into recombinant adeno-associated virus (rAAV) and the subsequent infection of human epithelial cell lines, in order to establish stable cell lines with the appropriate genes knocked out. The target genes are those in the pathways regulating DNA repair, apoptosis and histone acetylation, including BRCA2, HDCA1, SITR1 and ADA3. The host cells are all highly disabled and the modifications will not increase their pathogenicity. With the exception of the rAAV, the vectors are non-mobilisable and present no risk. The exception is the AAV, which can infect human cells.

Knocking out the target genes is potentially oncogenic and, while this is highly unlikely to increase the pathogenicity of the intended host cells, it does present a risk to workers who might become infected with the rAAV.

For this reason the packaging and infection steps are considered as Class 2, while the preliminary cloning steps and subsequent propagation of any modified cells are Class 1.

(Note from BSO: I have taken advice from HSE and the Inspector, to whom I was referred, agreed with this assessment.)

Project Containment

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<tr>
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</table>

Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
Genetic analysis of the human Fanconi anaemia genes

Our studies will focus on the human genes that function in the Fanconi anaemia DNA repair pathway. The human Fanconi genes will be cloned into the vectors outlined. In addition to the known and established Fanconi genes, we wish to test other known DNA repair genes for their role in the FA pathway. In these instances, we plan to introduce these candidate genes into cells that harbour the Fanconi defect, but in which the mutated genes are have yet to be identified. These will then be used to establish infection into human immortalised fibroblasts or lymphoblasts that are either mutant for the relevant FA gene, or candidate FA gene. Expression of the recombinant FA gene will be monitored by western blot and the cells will be studied for complementation of FA defect.

To establish complementation, the cells will be studied for survival after exposure to DNA damaging agents. This will be done by FACS analysis, chromosome breakage studies and cell viability assays.

Recipient or parental organism

Tissue culture cells of human origin, from patients with Fanconi anaemia.

Host/vector system

E. coli K12 derivatives (for example DH5a, MC1061); human cell lines.
Retroviral producer cells: HEK293 derived lines, such as Phoenix-ecotropic, Phoenix-amphotropic, or VSV-G expressing retroviral producer cells.
Mammalian tissue culture cells, including human cell lines.
Target genes are those of the Fanconi anaemia pathway (usually involved in DNA repair) and candidate genes to test for presently unknown members of the pathway.

Evaluation of foreseeable effects

The work is to compare known and established Fanconi genes and also candidate genes, which are other known DNA repair genes, for their role in the FA pathway. These candidate genes will be introduced into cells that harbour the Fanconi defect, but in which the mutated genes are have yet to be identified.

To establish complementation, the cells will be studied for survival after exposure to DNA damaging agents. (This will be done by FACS analysis, chromosome breakage studies and cell viability assays). It is expected that the correct complementing gene will increase the survivability of the cells, while other genes will have minimal effect.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136 degrees C for 10 minutes), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

This work involves cloning of genes on the Fanconi anaemia pathway, and also candidate genes, and their transfer into human primary fibroblasts which are mutant for the relevant FA gene. Candidate genes are known to be involved in DNA repair, but have not been definitely assigned to the Fanconi pathway and could be involved in some other pathways.

Initial cloning will be into pUC-based vectors and then the constructs transferred into disabled Moloney Murine Leukaemia-based plasmids, all of this work using laboratory E. coli K12 strains as host. Subsequently the plasmids will be transfected into t.c. cells of the Phoenix packaging lines (HEK293-derived), for production of disabled, single-cycle retroviruses, with ecotropic and amphotropic envelope proteins and also VSV-G protein pseudotyped.

Although wild type FA genes are tumour suppressing, there is a small, but not negligible, possibility that some of the candidate genes might be potentially oncogenic. With the ecotropic envelope, the work is correctly classified as Class 1 under ACGM guidance. However, with an amphotropic envelope (or VSV-G protein pseudotyping), the potential oncogenic nature of some inserts means that it becomes Class 2 during the packaging and infection stages. Once stable cell lines have been established free from residual virus, they can again be brought down to Class 1.

In practice, for in house convenience, the work will all be carried out in a Containment Level 2 facility, but this is only a safety requirement for the specific stages considered above.

It is noted that LREC clearance may well be required for obtaining and using the human tissues involved in the cell lines.
Project Containment

Laboratory Activities
- L2: Yes
- L3
- L4

Glass Houses
- L3
- L4

Growth Rooms
- L2
- L3
- L4

Animal Units
- L2
- L3
- L4

Large Scale Activities
- L2
- L3
- L4

Human Clinical Applications
- L2
- L3
- L4

Project Ref 20/05.1

Date Ackn’d 31/01/2005

CU2 Project Title
Use of lentiviral vectors in cloning and expression of genes involved in DNA repair and the cell cycle.

Date Project Ceased 02/08/2005

Class
- Class 2

Culture Volume
- Class 2
- < 1 Litre

Non-GMM
- Not Applicable

Consent Granted
- Project notified under transitional arrangements

Withdrawn
- N

Tick if notifying a connected programme of work
- N

Historical Significant Changes
Project Transferred from GM20 to GM921 on 02/08/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
Cancer cells exhibit abnormalities in cell cycle progression, chromosome structure and segregation and in the capacity to repair DNA damage that are central to transformation. These abnormalities are often the result of mutations in tumour suppressor genes, such as the breast cancer gene BRCA2. The overall goal of our studies is to ascertain the molecular basis for the abnormal division of cancer cells, and their increased genetic instability.

Recipient or parental organism
Murine-embryo-fibroblasts and HeLa and other cancer cell lines.
Host/vector system

E.coli laboratory K12 derivatives/pUC based vectors;
Vertebrate tissue culture cells including 293T cells, murine-embryo-fibroblasts and HeLa and other cancer cell lines/Lentiviral vectors, with polytropism through viral pseudo-typing using VSV-G protein.
Lentiviral system: The viral system to be used employs a well-characterised lentiviral vector and packaging cell lines(Refs below). A number of safety features are incorporated to eliminate or very greatly reduce the possibility of generating productive recombinants. The vector lacks gag, pol, env, tat, rev, vpr, vpu, vif and nef functions. A chimeric LTR offsets the requirement for Tat in expressing genes transcribed from the LTR. The Rev function is provided in trans from a separate plasmid in the packaging cells, as are the Gag/Pol functions. The viral vectors are self-inactivating due to deletions in both the LTRs, and thus incapable of replication after one round of infection of target cells. The packaging cell lines are effectively helper-free, since the rev, gag/pol and env (in this case, pseudo-typed with VSV-G protein) genes are expressed from separate plasmids. Rubinson et al Nat Genetics 34:231 (2003); Dull et al., J Virol 72:8463 (1998); Myoshi et al J. Virol 72:8150 (1998).

Origin & function

Specific aims with details of the categories of inserts to be used are as follows:

A) BRCA2-deficient cells exhibit defects in DNA repair (1). The defect is likely related to the inability of mutants BRCA2 to form functional complexes with RecA homologs in the Rad51 family (eg Rad51, xrcc2 & 3, Rad51B-D). The molecular basis for this phenotype will be studied by functional and biochemical analyses in transfected cells. Wild-type or mutant forms (including point mutations, deletions and fragments) will be studied.

B) Abnormalities of chromosome structure and number are abundant in BRCA2-deficient cells (2-4). To investigate the molecular basis for these phenotypes, the function of genes controlling chromatin structure (eg histones, histone-modifying-enzymes, HP1), the cell cycle (eg cyclins A, B, D, E; cdk1, 2 & 4, polo-like kinases), chromosome number and structure (eg SMCS1 & 3, sccl, Ecol), cytokinesis (eg chromokinesins, INCENP, surviving) and the centrosome cycle (eg nek1, nap, g-tubulin, aurora kinases) will be investigated by functional & biochemical analyses in transfected cells. Wild-type or mutant forms (including point mutations, deletions and fragments) will be studied.

C) We have shown that mutations inactivating mitotic checkpoint genes including Bub1 or Mad3L (BubR1), are necessary for the neoplastic transformation of cells lacking the BRCA2 tumour suppressor (5). Besides these, we will test the possibility that genes (eg chk1/chk2, cdc14A/cdc14B, PP1delta, cdc25C, m TERT or h TERT, ranGAP & cdh1) whose normal function is to regulate cell cycle progression and chromosome stability, particularly during the S phase and in mitosis, will be targets for inactivation by secondary genetic changes during tumour evolution in cells that lack genes such as BRCA2, using studies of transfected cells. Wild-type or mutant forms (including point mutations, deletions and fragments) will be studied.

D) To identify novel genes that regulate these processes, cells will need to be transfected with pools (libraries) of vertebrate cDNAs or short-hairpin RNA molecules that interfere with the expression of vertebrate genes, for phenotypic screens. Here, the precise nature of the insert cannot be specified in advance.

In A-C above, fusions of wild-type & mutant genes to GFP or other fluorescent tags will be used to determine intracellular localisation.

3) Yu et al., Genes Dev 14:1400 (2000)
5) Lee et al., Molecular Cell 4:1-10 (1999)

Evaluation of foreseeable effects

Possible changes in the behaviour of cells in tissue culture, helping to elucidate the abnormal division of cancer cells, and their increased genetic instability.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before subsequent discharge doen the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

None

Project Containment

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Project Ref 20/05.2

Date Ackn'd 24/03/2005

CU2 Project Title Evolution of antibodies inhibiting gp120-mediated cell entry

Date Project Ceased

Class 2 CultureVolClass2 < 1 Litre

Class CultureVolumeClass3-4

Non-GMM Consent Granted

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info
**Purposes of the contained use**

It is planned to evolve antibodies and chemokines which inhibit HIV gp120-mediated cell entry of retroviral particles using compartmentalisation techniques.

**Recipient or parental organism**

CD4+human cell lines.

**Host/vector system**

Hosts: laboratory E.coli K12-derived strains (e.g. Top F10, Electro Ten blue); HEK293T cells, CD4 positive T-cells

Vectors: pUC-based vectors encoding MLV gag/pol and amphotropic envelope proteins (GaLVEnv, aMLV Env, VSV-G) but without any other retroviral sequences; further vectors generating packable RNA encoding either HIV env, vpu and nef (but lacking gag/pol and any HIV packaging signal), or antibody and chemokine libraries. These vectors contain MLV LTRs outside a packaging signal and the cloning site for the transfers.

**Origin & function**

In a first step, a CD4+ line expressing an antibody library will be generated by transduction with MLV vector particles pseudo-typed with amphotropic envelope proteins (e.g. GaLV Env, aMLV Env, VSV-G). To obtain these pseudo-type particles, HEK293T cells will be transfected with plasmids encoding MLV gag/pol and amphotropic envelope proteins but lacking any viral LTR and packaging signals. Furthermore, an MLV-packagable vector encoding membrane-anchored antibodies, which might have been pre-selected in other systems (phage-orribosme-display) for binding of HIV gp120, has to be generated and co-transfected. In particular, this vector will encode antibodies which have been fused to the N-terminus of the platelet-derived growth factor receptor transmembrane domain (PDGFR-TM) via a protease cleavage site. Furthermore, an antibiotic resistance marker will be encoded on the applied vector (inside the LTRs). In an alternative approach, diversified variants of RANTES (CCL5), also fused to the N-terminus of PDGFR-TM, will be used instead of antibodies. This library will be encoded on the same vector as described above.

In parallel, MLV(HIV Env) particles that have packaged a vector encoding the HIV genes env, vpu and nef will be generated. For this purpose HEK293T cells will be transfected with a plasmid encoding MLV gag/pol, an MLV-packagable vector encoding the HIV genes described above and a plasmid encoding a C terminally truncated variant of gp120 (exhibiting a cytoplasmic tail of only seven amino acids). Two days post-transfection the particles will be harvested and applied to the selection procedure.

**Evaluation of foreseeable effects**

Possible selection cells expressing antibodies or mutant chemokines which inhibit infection of CD4+cells by retroviruses with HIV envelope protein.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full CL2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

CL2 waste will be autoclaved (136 degrees for 10min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.
This work involves various aspects of compartmentalisation of cells and their secretion products, but from the GM viewpoint it consists of essentially two main steps: the construction of human CD4+ cells expressing either antibodies, or variants of RANTES, as cell surface proteins; and the construction of retroviral particles, containing HIV env, vpu and nef genes under the control of murine leukaemia viral LTRs, with HIV envelope protein to test the ability of the antibodies or RANTES variants to block infectivity.

None of the single proteins, if released into the medium, poses any hazard and the sole concern is the HIV genes, particularly vpu and nef whose full functions are still rather obscure. It was therefore agreed that the preparatory work, using laboratory E.coli K12 derivatives as host and non-mobilisable vectors is correctly classified as Class 1.

The replication-defective pseudo-retroviruses will have HIV envelope protein, and therefore be capable of infecting human cells. With the non-hazardous inserted genes, they will be Class 1, but with HIV vpu and nef, and particularly with env, vpu and nef together, it was agreed that they are correctly assessed as Class 2.

It was noted that appropriate precautions are given for working with the naked DNA encoding the HIV env, vpu and nef genes.

**Project Containment**

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**Project Ref** 20/06.1

- **Date Ackn'd**: 04/04/2006
- **CU2 Project Title**: Interfering with A-I RNA editing in vivo.
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Non-GMM**: Not Applicable
- **Consent Granted**: Not Applicable
- **Date Project Ceased**: 02/03/2022
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Please enter comments on the GM safety committee on the risk assessment**

This work involves various aspects of compartmentalisation of cells and their secretion products, but from the GM viewpoint it consists of essentially two main steps: the construction of human CD4+ cells expressing either antibodies, or variants of RANTES, as cell surface proteins; and the construction of retroviral particles, containing HIV env, vpu and nef genes under the control of murine leukaemia viral LTRs, with HIV envelope protein to test the ability of the antibodies or RANTES variants to block infectivity.

None of the single proteins, if released into the medium, poses any hazard and the sole concern is the HIV genes, particularly vpu and nef whose full functions are still rather obscure. It was therefore agreed that the preparatory work, using laboratory E.coli K12 derivatives as host and non-mobilisable vectors is correctly classified as Class 1.

The replication-defective pseudo-retroviruses will have HIV envelope protein, and therefore be capable of infecting human cells. With the non-hazardous inserted genes, they will be Class 1, but with HIV vpu and nef, and particularly with env, vpu and nef together, it was agreed that they are correctly assessed as Class 2.

It was noted that appropriate precautions are given for working with the naked DNA encoding the HIV env, vpu and nef genes.

**Is an emergency plan required according to regulation 20?** N

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To understand whether/how RNA editing of glutamate receptors contributes to the formation and synaptic targeting of these receptors, and the potential role of this process in sporadic ALS (amyolateral sclerosis).

Recipient or parental organism

Primary neuronal cultures (cortical, spinal motor neurons);
organotypic brain slice cultures (derived from cortex, brainstem, spinal cord).

Host/vector system

Hosts:
E. coli (XL1 blue, DH5alpha);
Human embryonic kidney (Hek) 293 cells;
Primary neuronal cultures (cortical, spinal motor neurons); organotypic brain slice cultures (derived from cortex, brainstem, spinal cord).

Vector(s)
pUC-based lentiviral (HIC1) vectors, allowing for constitutive or conditional transgene expression, will be used for initial cloning and vector construction and then for production of pseudo-virions in Hek293 cells. These vectors contain the Woodchuck Hepatitis B virus WPRE.
The pseudo-virions will be used to infect the t.c. cells to study the effects of the transgenes.

Origin & function

Non-oncogenic cDNA variants of the GluR2 AMPA receptor subunit, under its native and under neuronal-specific promoters. To allow for RNA processing (ie alt. Splicing, A-1 editing) of the insert it will cloned as a cDNA : genomic DNA hybrid. Moreover, the insert will be tagged with peptide epitopes and, will be inserted upstream of a fluorescent marker, in a bi-cistronic fashion, to allow for optical identification of successfully targeted neurons. This design may be used for other glutamate receptor subunits. In some instances, the vector will also contain shRNAi modules, driven by a PolIII promoter, to specifically knock-down endogenous GluR2 message.
In addition antisense RNA molecules (such as sno [small nucleolar] RNAs) may be expressed under neuronal-specific promoters to interfere with RNA processing (splicing, editing) of iGluR receptor mRNAs in neurons.

Evaluation of foreseeable effects

The only effects foreseeable are on the formation and synaptic targeting of glutamate receptors, in both the primary neuronal cells and the cells in the organotypic brain slices due to the effects of RNA editing of the mRNA.
These will only be expressed in culture, as the cells and slices are cultures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
No GM plants or animals are involved in this work. While the brain slices are not strictly micro-organisms, they are cultured in dishes in a very similar fashion and the containment is exactly the same as for micro-organisms of the same GM class.

For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Full containment level will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

The work involves the cloning of cDNA variants of the GlutamateR2 AMPA receptor subunit under its native and other neuronal-specific promoters, both alone and with peptide epitopes and as a fusion with fluorescent marker proteins (eg GFP).

Cloning will be into pUC-based lentiviral vectors, which contain the Woodchuck Hepatitis viral WPRE. Initial cloning will be in standard laboratory E. coli K12 derivative, then standard Phoenix HEK helper cells will be used to supply the helper functions for pseudo-virion production, using eco- and amphotropic envelope proteins and also pseudo-typing with the VSV-G protein. The replication-defective pseudo-viruses will be used to infect cells in primary neuronal cultures and also organotypic brain slice cultures.

The preliminary stages of the work are correctly assessed as Class 1, while the viral work is also correctly assessed as Class 2 due to the presence of the WPRE (otherwise it would also be class 1).

The potentially oncogenic nature of the isolated DNA, due to the WPREm is noted and appropriate precautions suggested.

The committee notes the training requirements of, at least one of, the workers and strongly recommends that he is trained, in the virological methods, by other workers who are already using this host/vector system in the laboratory.

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02/03/2022
Rhomboid proteases play a critical role in regulation of the epidermal growth factor (EGF) receptor signalling in the fruit fly Drosophila melanogaster. Notably, deregulated EGF receptor activity contributes to cancer in humans; however, the role of Rhomboids in this process has not yet been investigated. Therefore, we propose to investigate the role played by mammalian rhomboid proteases and rhomboid-like proteins in human disease, including cancer.
cDNAs for mammalian rhomboids and their homologues will be expressed either in mammalian tissue culture cells or primary cells derived from transgenic mice. Co-transfection with fluorescent marker proteins (GEP or luciferase) will be used to determine successfully transfected cells. The substrates for rhomboids, such as epidermal growth factor, will be co-expressed with the expressing mammalian cDNAs for e.g. EGE rhomboids by
In addition, shRNAs will be expressed to specifically knockdown expression of the rhomboids

Evaluation of foreseeable effects

The rhomboids, their substrates and the shRNAs will not be expressed in E. coli, because the vectors do not contain suitable bacterial promoter or translational initiation sequence for these inserts. Expression of rhomboids and/or their substrates (e.g. EGF), or shRNAs designed to knockdown rhomboid expression, will not have any effect on the pathogenicity of the mammalian cell lines or primary murine cells.

Origin & function

cDNAs for mammalian rhomboids and their homologues will be expressed either in mammalian tissue culture cells or primary cells derived from transgenic mice. Co-transfection with fluorescent marker proteins (GEP or luciferase) will be used to determine successfully transfected cells. The substrates for rhomboids, such as epidermal growth factor, will be co-expressed with the expressing mammalian cDNAs for e.g. EGE rhomboids by
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Evaluation of foreseeable effects

The rhomboids, their substrates and the shRNAs will not be expressed in E. coli, because the vectors do not contain suitable bacterial promoter or translational initiation sequence for these inserts. Expression of rhomboids and/or their substrates (e.g. EGF), or shRNAs designed to knockdown rhomboid expression, will not have any effect on the pathogenicity of the mammalian cell lines or primary murine cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

FuW containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contamination level 2 waste will be autoclaved (136°C for 10 mm), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Rhomboids are integral membrane proteases that cleave substrates to produce signalling molecules such as Epidermal Growth Factor (EGF). This project will express human rhomboids, EGFs, shRNAs to depress rhomboid expression and marker proteins (GFP, luciferase) using amphotrophic retroviruses, ecotropic retroviruses and lentiviruses in mouse primary cell cultures and standard tissue culture cells. The host cells for plasmid production (E. coli K12 laboratory strains) and the tissue culture cells are highly disabled and can be considered to be ACDP hazard group 1. All production of the pUC-based plasmid vectors in E. coli is correctly assessed as class 1. Ecotropic retroviruses are incapable of infection of human cells, so presenting negligible risk to the worker, and can also be considered to be HG1; expression of the reporter genes, rhomboids, shRNAs or EGFs will not alter the pathogenicity of the host therefore the work is correctly classified as class 1. In effect, for local convenience work of this nature is performed in the CL2 labs. All work using the amphotropic retroviruses containing the potentially oncogenic rhomboids and EGFs is correctly classified as class 2. It is noted that appropriate use of gloves, absence of sharps and appropriate disposal routes are recommended for handling potentially oncogenic naked DNA. All work using the lentiviruses, regardless of insert type, is class 2 due to the presence of the WPRE with the downstream X protein gene, despite a point mutation at the initiator Met.

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**Project Ref 20/08.2**

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Enzymatic modification of DNA leading to mutation is a molecular event essential to both innate and adaptive immunity. Both these processes are triggered by targeted deamination of deoxycytidine (dC) to deoxyuridine (dU) residues within the immunoglobulin locus - a process catalysed by the AID enzyme. The aim of the project is to further understand the AID pathway and how it is modulated.

**Recipient or parental organism**

Hosts: E.coli K12 derivatives (e.g. DH5a, MC1061);
Mammalian tissue cell lines (e.g. DT40, DG75, RAMOS, 293T).
Retroviral producer cells: HEK193, derived lines, such as Phoenix-ecotropic cells.
Primary mouse cells (for example B cells).

**Host/vector system**

Vectors:
Second generation HIV-based lentiviral system (plasmid pHRSinUBEm) containing WPRE and X gene (single inactivating mutation at initiator Met)

Helper plasmids pCMVR8.91 and pMD-G

**Origin & function**

cDNA inserts generally correspond to individual, well characterized ORFs (for example reporter genes such as GFP, transcription factors, AID and its relatives, AID-associated proteins, proteins implicated in nuclear trafficking) or their derivatives (for example, truncated, point mutated, or fused to other genes).

Work also includes the creation of lentiviral supernatants from mammalian cDNA libraries which may contain hazardous genes such as oncogenes.

**Evaluation of foreseeable effects**

cDNAs will not be expressed in E.coli because the vectors do not contain a suitable bacterial promoter or translation initiation sequence for these inserts. There is a small risk that some inserts (e.g. those encoding transcriptional factors or AID (or its interactors/homologues)) might increase the pathogenicity of the transduced cells. Whereas expression of reporter genes and those encoding nuclear trafficking proteins will have no effect. The expression of cDNA libraries from recombinant lentiviruses could result in the expression of oncogenes that could potentially transform mammalian cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

only GM micro-organisms will be produced in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 2 waste will be autoclaved (136 degrees Centigrade for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.
Enzymatic modification of DNA leading to mutation is a molecular event essential to both innate and adaptive immunity. Both these processes are triggered by targeted deamination of deoxyxytidine (dC) to deoxyuridine (dU) residues within the immunoglobulin locus - a process catalysed by the AID enzyme. This project will express cDNAs encoding AID, proteins involved in the AID pathway and standard protein markers (GFP, luciferase etc) using lentiviruses in mouse primary cell cultures and standard tissue culture strains. The host cells for plasmid production (E.coli K12 strains) and the tissue culture cells are all highly disabled ans can be considered to be ACDP hazard group 1. All production of the pUC-based plasmid vectors in E.coli is correctly assessed as class 1.

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Project Ref 20/08.3

Date Ackn'd 03/12/2008

Role of innate immunity for the restriction of facultatively intracellular bacteria.

Class 2

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Tick if notifying a connected programme of work
### Project Additional Information

**Purposes of the contained use**

Facultatively intracellular bacteria are able to proliferate in phagosomes of host cells, from where they occasionally escape into the cytosol. It is poorly understood how bacterial proliferation in the eukaryotic cytosol is restricted but the induction of inducible transcription factors of the IRF family is thought to be involved. The purpose of the current project is to investigate the role of the IRF pathway in the intracellular restriction of bacterial pathogens. To this end we will test how Salmonella typhimurium and Shigella flexneri behave if the IRF pathway is modulated.

**Recipient or parental organism**

- **Salmonella typhimurium**
  - S typhimurium strain 1344

S typhimurium strain 1344 contains GFP gene integrated into the genome; there are no plasmids involved and there are no antibiotic resistance genes integrated.

**Host/vector system**

- **Vectors:**
  - pDsRed2; pUC-based plasmid, ampicillin resistant, expressing a red fluorescent protein. This will be transformed into S typhimurium, wild-type.

**Origin & function**

- **GFP:** Green fluorescent protein from Aequorea victoria
- **DsRed2:** red fluorescent protein from Discosoma sp. red coral

**Evaluation of foreseeable effects**

The two strains of S typhimurium will be expressing fluorescent marker proteins, either dsRed or GFP, that will not alter the pathogenicity of the strains. Whilst no other proteins will be heterologously expressed in S typhimurium 1344, S typhimurium (pDsRed2) will also express the antibiotic resistance gene for ampicillin, but it will still be sensitive to quinolone antibiotics if treatment of an infection is ever necessary. The plasmid is based upon pUC and is non-mobilisable.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- Only GM micro-organisms will be produced in this work.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

---

Two hazard group 2 pathogens, Salmonella typhimurium and Shigella flexneri, will be grown to investigate the role of the IRF pathway in the intracellular restriction of bacterial pathogens. All experiments involving these bacteria are therefore class 2. The experiments require the growth of small volumes (1-3ml) of the bacteria, which can be accommodated in the bacterial CL2 lab B17. Two strains of genetically modified S.typhimurium will be used, each expressing either GFP or DsRed, neither of which will alter the pathogenicity of the bacteria.

The bacteria will be used to infect HeLa cells that have altered expression of IRF components, already assessed by the BSC in projects 2008/03 and 2008/04. Project 2008/03 was assessed as Class 2 and involves the expression of cDNAs encoding the IRF components in mammalian cells. Project 2008/04 was expressing the siRNAs in mammalian cells and was assessed as Class 1. Transfected mammalian cells will be brought to the bacterial CL2 lab in a sealed boxed and all further steps involving incubation with S.typhimurium and S.flexneri will be performed there. A class 1 Microbiological Safety Cabinet will be used for all manipulations with S.typhimurium and S.flexneri, the bacteria will be grown within CL2 lab and all wastes will be inactivated by autoclaving before incineration.

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Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L2</td>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref**: 20/08.4

**Date Ackn'd**: 02/03/2022

CU2 Project Title

Class

CultureVolClass2

CultureVolumeClass3-4
**Project Additional Information**

**Purposes of the contained use**

We wish to use replication deficient lentiviruses for the delivery of certain oncogenes and SiRNAs (in the form of shRNAs or microRNAs) to examine how oncogenic lesions perturb the processes of ribosome biogenesis and translation.

**Recipient or parental organism**

Hosts: E.coli K12 derivatives (eg DH5a, XL-10 gold);
Mammalian tissue cell lines (eg normal diploid fibroblasts and fibroblasts derived from patients with Schwachman-Diamond Syndrome (SDS) or Diamond Blackfan anaemia (DBA);
Primary mouse cells (mouse embryonic fibroblasts, bone marrow stem cells, neutrophils' and lymphocytes).

**Host/vector system**

Vectors:
Second generation HIV-based lentiviral system (plasmid pLKO1) and the retroviral vector pBABE
Helper plasmids pCMVR8.91 and pMD-G

**Origin & function**

cDNAs correspond to oncogenes and the siRNAs will be used to knock-down known tumour suppressor genes. These will be expressed in the mouse and human cell lines and the effects of their expression upon ribosome biosynthesis determined.

**Evaluation of foreseeable effects**

The cDNAs correspond to known oncogenes and the siRNAs are to known tumour suppressors. These will be expressed from amphotropic retroviruses and lentiviruses, both of which can infect human cells. It is well recognised that multiple oncogenic lesions are required before tumourigenesis, but expression of either the cDNAs or siRNAs will increase the probability of cell transformation and therefore there is a non-negligible risk to oncogenesis.
Three types of expression systems will be used. Ecotropic retroviruses will be used in studies on mouse primary cell lines; these can enter human cells and are of negligible risk and are not part of this Notification. Both amphotropic retroviruses and lentiviruses will be able to infect human cells and will be used to express oncogenic cDNAs or siRNAs to tumour suppressors. Even though oncogenesis requires multiple lesions, the recombinant amphotropic retroviruses and lentiviruses pose a small risk of oncogenesis if introduced into a worker. None of the inserts will alter the pathogenicity or tissue tropism of the retroviral or lentiviral vectors.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 10 mins), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20?  No

Tick to confirm that you have attached a risk assessment to this form  Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment  No

Please enter comments on the GM safety committee on the risk assessment

Project 2008_08 describes work towards the study of ribosome biogenesis in mammalian cells. The work entails the expression of known oncogenes and the suppression with siRNAs of known tumour suppressor genes, using both retroviruses and lentiviruses in human cell lines. Plasmid construction will use non-mobilisable plasmids in laboratory strains of E.coli K12 where none of the inserts will be expressed; this aspect of the work is correctly assessed as Class 1. There are two aspects to the retroviral work. Ecotropic retroviruses will be used to express oncogenes and/or siRNAs in primary mouse cell lines. Ecotropic retroviruses cannot infect human cells, the inserts will not alter the pathogenicity or tissue tropism of the retroviruses and the cell lines are equivalent to ACDP HG1; this aspect of the work is correctly classified as class 1. Amphotropic retroviruses and lentiviruses will be used to infect primary human cell lines derived from donors with Scwachman-Diamond Syndrome (SDS) or Diamond Blackfan anaemia (DBA) or from healthy volunteers. The inserts will not affect the pathogenicity or tropism of the viruses, but both cDNA and siRNA inserts are potentially oncogenic. Although it is accepted that multiple events are required for tumourgenesis, there is a small but non-negligible risk to the operator. This aspect of the work is therefore class 2. The primary human cell lines have undergone multiple passage over at least two weeks and have been assessed as Class 1 in the associated risk assessment (HTA/2008_01); appropriate ethical consent has been obtained for both making the cell lines and for their usage.

Project Containment

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<tr>
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**Project Ref** 20/09.1

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<td>RETROVIRAL AND LENTIVIRAL EXPRESSION OF TRIM PROTEINS TO STUDY THEIR FUNCTION AND MOLECULAR MECHANISM.</td>
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**Date Project Ceased**

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<tr>
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**Non-GMM Consent Granted** Not Applicable

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Project Additional Information**

**Purposes of the contained use**

Human cells contain a variety of factors that prevent unrestricted proliferation of bacteria and viruses that have entered the cytoplasm, which forms part of the innate immune system. This intracellular restriction can be caused by a large family of proteins called TRIM (Triparite Motif proteins). The aim of the project is study the molecular mechanisms of how they prevent the proliferation of intracellular pathogens.

**Recipient or parental organism**

Hosts: E.coli K12 derivatives (e.g. DH5a, XL10 gold);
Mammalian tissue cell lines (e.g. HEK293, HeLa, CRFK, C2C12).
Retroviral producer cells: HEK293 derived lines, such as Phoenix-ecotropic cells.

**Host/vector system**

pCNCG &pEXN=CMV, MLV based lentiviral vector expressing Red fluorescent protein or Neo, respectively.
pMDG=CMV-VSVG expresses VSV-G for pseudotyping of viral particles
pCMV1=MLV-Gag-Pol, helper plasmid expressing MLV gag,pol, tat and rev.
pKLO1 =for the expression of shRNAs commercially available from OpenBioSystems.
pBabe = for the expression of cDNAs, commercially available from AddGene.
Mammalian cDNAs for the following proteins will be used:
(i) TRIM21, a cytosolic IgG receptor
(ii) TRIM5 and TRIM-Cyp, cytoplasmic restriction factors
(iii) TRIM7, a putative regulator of glycogenin function
(iv) TRIM 20, a gene linked to susceptibility to FMF
(v) TRIM 1&18, genes in which defects lead to the developmental defect Optiz syndrome
(vi) TRIM 19, so-called nuclear-body protein involved in viral restriction and cell cycle.
(vii) TRIM 25, putative restriction factor via interaction with RIG-1.

In addition, siRNAs that selectively repress the expression of each of these proteins will be constructed and expressed.

Evaluation of foreseeable effects

cDNAs and siRNAs will not be expressed in E.coli, because the vectors do not contain a suitable bacterial promoter or translational initiation sequence for these inserts. None of these proteins are known to be toxic or oncogenic, and neither are they likely to change the tissue tropism of the viruses or alter the pathogenicity of the host cells. The siRNAs for repressing the expression of the proteins are also not known to be toxic or oncogenic, and neither will they alter the tissue tropism of the viruses or the pathogenicity of the host cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The TRIM family of proteins are found in the cytoplasm where they are of an innate mechanism to prevent the proliferation of viruses and/or bacteria; they are not known to be either toxic or oncogenic and neither will they alter the pathogenicity of the recombinant viruses or the host cells. Cloning will be performed in standard E.coli K12 strains using non-mobilisable plasmids and this aspect of the work is correctly classified as Class 1. Expression of the TRIM proteins and siRNAs will be either from amphotropic retroviruses or lentiviruses; due to the potentially oncogenic nature of the viruses and their ability to infect human cells, gloves must be worn and hence the work is Class 2. Expression may also be performed in mouse cells from ecotropic retroviruses that are incapable of infecting human cells; this work is correctly classified as Class 1.
Project Containment

Laboratory Activities
L2 L3 L4 L2 L3 L4
Animal Units
L2 L3 L4 L2

Glass Houses
L3 L4 L2 L3
Large Scale Activities
L2 L3 L4

Growth Rooms
L2 L3 L4
Human Clinical Applications

Project Ref 20/09.2

Expression of endocytic and exocytic proteins using VSV-G pseudotyped lentiviruses

Date Ackn’d 22/09/2009
CU2 Project Title Expression of endocytic and exocytic proteins using VSV-G pseudotyped lentiviruses

Date Project Ceased

Class 2
CultureVolClass2 < 1 Litre
Consent Granted

Historical Significant Changes
Historical Date of Additional Info

Project notified under transitional arrangements N

Withdrawn N
Tick if notifying a connected programme of work N

Project Additional Information

Purposes of the contained use
Mammalian cells regulate the composition of their surface membranes by the processes of exocytosis and endocytosis. Pathogens and viruses gain entry into cell via these pathways. The purpose of this project is to study the molecular mechanisms of these pathways.

Recipient or parental organism
Hosts: E.coli K12 derivatives (e.g. DH5α, MC1061);
Mammalian tissue cell lines (e.g. HEK293, HeLa, Co115).
Retroviral producer cells: HEK293 derived lines, such as Phoenix-ecotropic cells.
Host/vector system

FUW for expression of cDNAs; includes WPRE and geneX
Packaging plasmids pMD2.G, pMDLg/pRRE and pRSV-Rev

Origin & function

Mammalian cDNAs that express proteins involved in endocytosis and exocytosis will be used, including proteins such as intersectin, GRAF, FCHO, synaptotagmins; and receptors and ligands like ephrin receptor and ephrins.

Evaluation of foreseeable effects

cDNAs will not be expressed in E. coli, because the vectors do not contain a suitable bacterial promoter or translational initiation sequence for these inserts. None of these proteins are known to be toxic or oncogenic, and neither are they likely to change the tissue tropism of the viruses or alter the pathogenicity of the host cells.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project 2009/05 will express proteins involved in endocytosis and exocytosis using VSV-G pseudo-typed lentiviruses in mammalian cells. The mammalian host cells are highly disabled and equivalent to hazard group 1. The E.coli K12 strains used for plasmid production are hazard group 1. The plasmids to be used are all non-mobilisation and will not alter the pathogenicity of the host cells; this aspect of the work is therefore correctly classified as Class 1. The third generation lentiviral system being used will produce non-replicative lentiviruses that are capable of infecting human cells and will express proteins involved in the trafficking of endocytic and exocytotic vesicles. None of the proteins are toxic or oncogenic, and neither will they change the host range of pathogenicity of the lentiviruses. However, the lentiviruses all have a WPRE and a downstream gene X that is potentially oncogenic. Therefore the production and use of recombinant lentiviruses is classified as Class 2.

Project Containment

Laboratory Activities

Glass Houses

Growth Rooms
The aim of this project is to improve our understanding of meiotic prophase in mouse oocytes using high resolution live cell confocal microscopy. Homologous pairing, recombination and chiasmata formation occur in oocytes during embryo development. We will use lentiviral and retroviral systems to overexpress meiotic proteins fused to well characterized marker proteins to study their changing distributions during meiosis.

**Hosts:** E. coli K12 derivatives (e.g. XL, DH5α);
Mammalian tissue cell lines (e.g. HEK293, He La, Cos 7, Pnt1A).
Retroviral producer cells: PT67 and HEK293 derived lines, such as Phoenix-ecotropic cells.
Mouse oocytes

**Host/vector system**
Lentiviral system: pLL3.7 for expression of cDNAs; includes WPRE and gene x and the packaging plasmid pCMV delta8.91, with env from pMD.G
Retroviral systems: MMLV-based vectors and the MSCV retroviral expression system

Origin & function

Mammalian cDNAs encoding meiotic proteins will be fused to well characterized marker proteins such as mEGFP, mCherry or PAGFP. The proteins that will be overexpressed are the commonly used markers of different components and structures of a meiotic cell: components of the synaptonemal complex (such as SCP1, SCP2, SCP3), cytoskeletal reporters (such as tubulin, actin and proteins binding to microtubules and F-actin), motor proteins (such as myosins, kinesins), histones (such as TRF1, TRF2, Pot1, Rap1), centrosome components (such as pericentrin, gamma-tubulin), components of the meiotic cohesion complex (such as SMC1/3, SCC1, Rec8, STAG1), nuclear pore complex proteins and other nuclear membrane components including SUN and KASH domain proteins, as well as meiotic proteins involved in chiasma formation and resolution (such as Rad 51, DMC1, MLH1).

Evaluation of foreseeable effects

cDNA's will not be expressed in E.coli, because the vectors do not contain a suitable bacterial promoter or translational initiation sequence for these inserts. None of these proteins are known to be toxic or oncogenic, and neither are they likely to to change the tissue tropism of the viruses or alter the pathogenicity of the host cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment
Project 2009/09 aims to study meiotic prophase in mouse oocytes. A variety of proteins that are components of cytoskeleton, nuclear membrane, pore core complex, centromere and telomeres will be over-expressed as GFP fusion proteins and overexpressed in mammalian cells or mouse oocytes by transfection with replication defective lentiviruses and retroviruses. All the proteins to be expressed are non-hazardous and none of them will change the pathogenicity of any of the host cells. Host cells include E.coli K12 strains for the initial cloning and plasmid preparation and the mammalian cells to be transfected; all these host cells are highly disabled and are equivalent to hazard group 1.

Three different forms of recombinant viruses will be used.
1. Recombinant ecotropic retroviruses cannot infect human cells. The proteins to be used will not change the host range or pathogenicity of the virus. This aspect of the work is therefore correctly classified as Class 1.
2. Recombinant amphotropic retroviruses can infect human cells, but cannot replicate due to the segregation of essential genes onto three separate plasmids. The proteins to be used will not change the host range or pathogenicity of the virus and none of the proteins is oncogenic. There is a small but non-negligible risk of insertional mutagenesis, but the risk can be reduced to effectively zero by the wearing of gloves and not using sharps. This aspect of the work is therefore correctly classified as Class 1 (Section 2.11 of the SACGM compendium of guidance).
3. Recombinant lentiviruses are not capable of replication but can infect human cells. The proteins to be used will not change the host range of pathogenicity of the virus and none of the proteins is oncogenic. However, the lentiviral vectors contain the WPRE and downstram X gene, which has the potential to be oncogenic. This aspect of the work is correctly classified as Class 2; gloves will be worn and sharps will not be used.

Project Containment

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<tr>
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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 20/10.2

Date Ack’n’d 09/03/2010
CU2 Project Title Rhomboid inhibitors studied in Providencia stuartii

Class 2

Culture Vol

Class 2 1 Litre

Project notified under transitional arrangements

Consent Granted

Historical Significant Changes

Tick if notifying a connected programme of work

N

Historical Date of Additional Info
### Project Additional Information

#### Purposes of the contained use

The aim of the research is to study the biological function and biochemistry of the intramembrane protease rhomboid. From a wide screen of homologous proteins, rhomboid from Providencia stuartii was found most amenable for analysis due to its high expression level and high rate of substrate cleavage. The native substrate is unknown and therefore the function of rhomboid in P. stuartii is to be studied.

#### Host/vector system

| Hosts: E.coli K12 derivatives (e.g. DH5α, XL1); | Providencia stuartii; either wild-type or rhomboid-knockout strain (aarA) |
| Plasmids: pET vectors e.g. pET21a, pET25b+ and the pBR322-based vectors pMAL and pMPM |

#### Origin & function

Plasmids-encoded rhomboid substrates are fusions of the transmembrane domains from Drosophila Gurken and Spitz, E.coli LacY TMD2 or Providencia stuartii TatA fused to maltose-binding protein and thioredoxin. The proposed substrates will be expressed from plasmids in Providencia stuartii and their cleavage monitored by western blotting.

#### Evaluation of foreseeable effects

None of the reporter proteins are known to be toxic or oncogenic, and neither are they likely to alter the pathogenicity of the host cells or their ability to survive in the environment.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.
Containment level 2 waste will be autoclaved (136º for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Project 2010/03 from M F Is to study the rhomboids (intramembrane proteases) and their potential inhibition in the bacterium Providencia stuartii, which is an HG2 organism as it has been implicated in urinary tract infection and perhaps travellers diarrhoea. The bacteria will be transformed with plasmids encoding putative rhomboid substrates whose cleavage can be monitored, and the effects of various rhomboid-inhibitors on this process will be studied. Two strains of P. stuartii will be used, a wild-type strain and an aarA strain that has its native rhomboid knocked out. The expression of rhomboid substrates or the knocking out of the rhomboid gene will neither affect the pathogenicity or the ability of the bacteria to survive in the environment. This work is therefore correctly classified as Class 2 and the work will be performed in the Class 2 pathogen lab in the Containment Suite Note that it is policy in all the Class 2 laboratories for double-gloves to be worn for all work and sharps to be avoided.

Please enter comments on the GM safety committee on the risk assessment

Project 2010/03 from M F Is to study the rhomboids (intramembrane proteases) and their potential inhibition in the bacterium Providencia stuartii, which is an HG2 organism as it has been implicated in urinary tract infection and perhaps travellers diarrhoea. The bacteria will be transformed with plasmids encoding putative rhomboid substrates whose cleavage can be monitored, and the effects of various rhomboid-inhibitors on this process will be studied. Two strains of P. stuartii will be used, a wild-type strain and an aarA strain that has its native rhomboid knocked out. The expression of rhomboid substrates or the knocking out of the rhomboid gene will neither affect the pathogenicity or the ability of the bacteria to survive in the environment. This work is therefore correctly classified as Class 2 and the work will be performed in the Class 2 pathogen lab in the Containment Suite Note that it is policy in all the Class 2 laboratories for double-gloves to be worn for all work and sharps to be avoided.

Project Containment

Laboratory Activities

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Glass Houses

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Large Scale Activities

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Human Clinical Applications

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Project Ref 20/10.3

Date Ackn'd 18/03/2010

CU2 Project Title Intracellular immunity in mammalian cells to pathogenic bacteria and viruses

Class CultureVol Class 2 CultureVolume Class 3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Date Project Ceased

02/03/2022

Page 1184 of 15326
Despite extracellular immune responses, viral and bacterial pathogens are able to infect cells. Infected cells must therefore have mechanisms to neutralise or at least limit the replication of pathogens intracellularly. We would like to investigate how this is accomplished. We aim to identify and characterise restriction factors to determine how they target pathogens and the mechanism by which they neutralise them. This will be performed by infecting mammalian cell lines with human pathogens, either with or without siRNA expression to down regulate intracellular restriction.

**Recipient or parental organism**

**Hosts:** E.coli K12 derivatives (e.g. DH5α, XL1) and mammalian cells e.g. HeLa & HEK293  
Shigella flexneri, wild-type  
Salmonella typhimurium, wild-type and strain 1344  
Cocksackie virus (clone CVB3-GFP)  
Recombinant Sindbis virus and recombinant Adenovirus; both expressing GFP (both CL1, but part of project)

**Host/vector system**

pDsRed2; pUC-based plasmid, ampicillin resistant, expressing a red fluorescent protein. This will be transformed into S. typhimurium, wild-type. S. typhimurium contains GFP integrated into its genome with no associated antibiotic resistance marker. The recombinant sindbis virus and adenovirus are replication defective and both express GFP (assessed as Class 1). The cosackie virus is replication competent and expresses GFP.

**Origin & function**

GFP: Green fluorescent protein from Aequorea victoria  
DsRed2, red fluorescent protein from Discosoma sp. Reef coral  
siRNAs directed towards the repression of genes such as TRIM21, Atg8 (and other autophagy genes), encoding E2 enzymes, TRIM5, TRIM19, TRIM25

**Evaluation of foreseeable effects**

The two strains of S. typhimurium will be expressing fluorescent marker proteins, either dsRed or GFP, that will not alter the pathogenicity of the strains. Whilst no other proteins will be heterologously expressed in S. typhimurium 1344, S.typhimurium (pDsRed2) will also express the antibiotic resistance gene for ampicillin, but it will still be sensitive to quinolone antibiotics if treatment of an infection is ever necessary. The plasmid is based upon pUC and is non-mobilisable. Neither of the reporter proteins are known to be toxic or oncogenic, and neither are they likely to alter the pathogenicity or host range of the non-replicative recombinant sindbis virus, the non-replicative recombinant adenovirus or the replicative cosackie virus.
Expression of siRNAs in mammalian cells will not alter the cells' ability to survive in the environment or their pathogenicity. In addition, during co-infection of mammalian cells with the siRNAs and with either S. flexneri, S. typhimurium, adenovirus, sindbis virus or coxsackie virus, the sRNAs will not alter the pathogenicity, host range or ability to survive in the environment of any of the pathogens.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied to all work using S. typhimurium, S. flexneri and cosackie virus

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136º for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20?  

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Mammalian cells will be infected with viruses or bacteria (recombinant Sindbis virus, cosackie virus, recombinant adenovirus, Shigella flexneri, Salmonella typhimurium); studies will then be performed on the effect of knocking-down expression of interferon-upregulated genes by siRNA. pUC-based non-mobilisable plasmids expressing siRNAs will be produced in standard laboratory E.coli K12 strains; the plasmids will neither affect the pathogenicity or survivability of the hosts and Class 1 is appropriate. All mammalian cells are highly disabled and Class 1 is also appropriate. Recombinant Sindbis viruses are replication defective, although they are still able to infect human cells. The viruses will express GFP, which is non-toxic and non-oncogenic and, at worst, upon infection of a worker's cells would result in transient expression of GFP. This aspect of the work is therefore correctly classified as class 1. Similarly, recombinant adenovirus is also replication incompetent and GFP will not affect the virus’ host range or pathogenicity; Class 1 is therefore appropriate. The recombinant cosackie virus expressing GFP is fully replication competent. Cosackie virus is a HG2 pathogen with high-infectivity via aerosols or by oral transmission, producing symptoms such as fever, headache, sore throat, gastroenteritis and sometimes chest and muscle pains. GFP will not alter its pathogenicity or host range and therefore Class 2 is appropriate. Given the high-infectivity via aerosols, all work is to be performed in a Class 1 or 2 Microbiological Safety Cabinet and procedures likely to cause aerosols (particularly centrifugation in a microfuge) must be further risk assessed. Shigella flexneri and Salmonella typhimurium will be resistant to beta-lactams, but will still be sensitive to quinolones in the case of a worker infected. Work with recombinant S. typhimurium and S. flexneri is therefore appropriately classified as Class 2.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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</table>

02/03/2022
The aim is to study the effect of mutations on the propensity of aggregation for aggregation-prone proteins, such as huntingtin derivatives, superoxide dismutase 1, APP derivatives, synuclein derivatives. In addition, factors that affect the aggregation will be studied. The research will involve the expression of aggregation-prone proteins from replication-incompetent lentiviruses and the modulation of the proteins’ aggregation will be studied by co-expressing shRNAs and a mammalian cDNA library.

Recipient or parental organism

Hosts: E.coli K12 derivatives (e.g. DH5α, XL1, MC1061, Stbl3) and mammalian cells e.g. AR42J, Min6 & HEK293 and primary mouse cell lines (embryonic fibroblasts and neurons)

Host/vector system

The pLenti system from Invitrogen will be used to produce replication-incompetent lentiviruses; this uses the four plasmids pLenti derivatives, pLP1 (expresses HIV-1 gag/pol and contains the HIV-1 RRE), pLP2 (expresses HIV-1 Rev) and pLP/VSIG (expresses the VSV G glycoprotein)

Origin & function

Mammalian (e.g. mouse, human) huntingtin derivatives, insulin mutants, superoxide dismutase 1, APP derivatives, synuclein derivatives. GFP fusions will be made to these proteins as well.
shRNAs directed towards the repression of genes such as mammalian huntingtin derivatives, superoxide dismutase 1, APP derivatives, synuclein derivatives etc.

A mammalian cDNA library

Evaluation of foreseeable effects

All the aggregation proteins and GFP fusions to be expressed are neither toxic nor oncogenic. Note that prions are specifically excluded from this work. The proteins will neither alter the pathogenicity or the host range or the recombinant lentiviruses. In addition, the recombinant lentiviruses will not alter the host cells pathogenicity or their ability to survive in the environment.

Expression of shRNAs from recombinant lentiviruses that are directed towards the down-regulation of the aggregation proteins in mammalin cells will not alter the pathogenicity or host range of the recombinant lentiviruses.

Expression of a mammalian cDNA library from a nonreplicative lentivirus is unlikely to affect the pathogenicity of the host cells or the recombinant virus, or their ability to survive in the environment.

The plasmids used to produce the recombinant viruses will not have any affect on the pathogenicity or ability to survive in the environment of the E. coli host clls used for their production.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied to all work using the nonreplicative recombinant lentiviruses

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The aim of project 2010/6 is to study protein aggregation in standard human cell lines and isolated primary mouse cells. Proteins that are known to aggregate, such as mutants of insulin, huntingtin and superoxide dismutase, will be expressed using recombinant lentiviruses. All of the proteins to be expressed are neither toxic nor oncogenic. Tissue culture cells and mouse explants are highly disabled and are therefore considered to be equivalent to ACDP hazard group 1. Plasmids will be produced in laboratory E. coli K12 strains that are non-colonising and are HG1. The plasmids used the pLenti expression system are pUC-based non-mobilisable plasmids that will neither alter the pathogenicity or ability of the E. coli host cells to survive in the environment, and therefore plasmid production is correctly classified as Class 1. VSV-G pseudotyped lentiviruses have a broad host range and can infect human cells. The recombinant lentiviruses are replication-incompetent and are made by co-transfection of HEK293 cells with 3 helper plasmids. The lack of homology between the plasmids reduces the risk of any recombination event and therefore the likelihood of regenerating a replication incompetent virus is negligible and the testing for such RCVs is considered unnecessary. The one non-negligible hazard associated with the expression systems it contains the WPRE and downstream X gene fragment that is considered to have oncogenic potential. Thus the production and use of the recombinant lentiviruses are correctly assessed as Class 2. Oncogenic DNA will be handled and disposed of appropriately. Obtaining the mouse tissues for this work is covered by project license PPL 80/2035. The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pairs of gloves.
### Project Additional Information

<table>
<thead>
<tr>
<th>Purposes of the contained use</th>
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</thead>
<tbody>
<tr>
<td>The aim of the research is to study the biology of circadian rhythms. Replication deficient lentiviruses and adeno-associated viruses (AAVs) will be used for the delivery of mammalian cDNA with known or suspected roles in signal transduction or circadian timing, such as period proteins, adenylyl cyclase, calcium regulated kinases etc., and their effects on circadian rhythms in primary mouse neuron cultures will be studied.</td>
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<th>Host/vector system</th>
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<td>Non-replicative lentiviruses will be constructed using co-infection of HEK293 cells with the plasmids pCCLsin.PPT.hPGK.Wpre, pMDLg/pRRE (gag/pol expression), pMD2-G (VSV-G expression), pRSV_Rev (Rev expression). Non-replicative AAV production will be performed by co-infection of HEK293T cells with plasmids pAAV-6P-SEWB, and helper plasmids pDP1 &amp; pDP2 (rep and cap proteins, adenovirus E2A, E3, E4 proteins and VA RNA).</td>
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<th>Origin &amp; function</th>
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<tr>
<td>All the proteins that will be used which have known or suspected roles in signal transduction or circadian timing, such as period proteins, adenylyl cyclase, calcium regulated kinases, are mouse sequences. Recombinant optogenetic factors will be used to monitor intracellular changes. GFP; Green fluorescent protein from Aequorea victoria. Channel rhodopsin: green algae. Luciferases: fire-fly. Light regulated adenylyl cyclase: mouse.</td>
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<th>Evaluation of foreseeable effects</th>
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<td>The reporter proteins, the proteins involved in circadian rhythms and the proteins involved in downstream-signalling are not toxic or oncogenic, and neither are they likely to alter the pathogenicity of the host cells or their stability to survive in the environment. In addition, the proteins are also unlikely to alter the host range of the recombinant no-replicative viruses or their ability to survive in the environment.</td>
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</table>

02/03/2022 | Page 1190 of 15326
The aim of project 2010/05 is to study circadian rhythms in isolated primary mouse neurons and brain slices. Standard reporter proteins and proteins that are likely to affect circadian rhythms, such as period proteins, will be expressed using recombinant lentiviruses and recombinant adeno-associated viruses (AAVs). The proteins to be expressed are neither toxic nor oncogenic. Tissue culture cells and mouse explants are highly disabled and are therefore considered to be equivalent to ACDP hazard group 1. Plasmids will be produced in laboratory E. coli K12 non-mobilisable plasmids that will neither alter the pathogenicity nor the ability of the E. coli host cells to survive in the environment, and therefore plasmid production is correctly classified as Class 1. Both lentiviruses and AAVs have a broad host range and can infect human cells. The recombinant systems both produce replication-incompetent viruses by co-transfection of HEK293 cells with either 3 helper plasmids for the lentiviral system or 2 helper plasmids with the AAV system. The lack of homology between the plasmids reduces the risk of any recombination event and therefore the likelihood of regenerating a replication competent virus is negligible and the testing for such RCVs is considered unnecessary. The one non-negligible hazard associated with the expression systems is that they both contain the WPRE and downstream X gene fragment that is considered to have oncogenic potential. Thus the production and use of the recombinant lentiviruses and AAVs are both correctly assessed as Class 2. Oncogenic DNA will be handled and disposed of appropriately. Obtaining the mouse tissues for this work is covered by project license PPL 80/2310. The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pair of gloves.
### Purposes of the contained use

SOX2 has been identified as the most likely candidate oncogene within the distal segment of chromosome 3q that is amplified in lung cancer. There are other non-coding RNAs (ncRNAs) (e.g. SOX2OT) and genes (PIK3CA) that may well play a synergistic role. We propose to investigate the role of these and other loci known to be dysregulated or mutated in epithelial carcinogenesis by using mammalian cell lines and primary cells as in vitro model systems.

### Host/vector system

Non-replicative lentiviruses will be constructed using a co-infection of HEK293 cells with the plasmids pLL3.7 or pLVTHM (contains inserts and WPRE), pCMV Δ8.91, psPAX2 or pCMV-R8.74 (gag/pol expression), pMD.G or pMD.2G (VSV-g expression). Non-replicative amphotropic retrovirus production will be performed by co-infection of HEK293T cells with plasmids pSuper retro, and plasmids pCMV Δ8.91 (gag/pol expression) and pMD.G (VSV-G expression).

### Origin & function

Inserts carried by the retroviruses and lentiviruses will express shRNA or mir30-based shRNA-mirs targeted for SOX2, SOX2OT and related genes, non-coding RNA e.g. SOX2OT and cDNA versions of SOX2 and related proteins and their putative substrates controlling, for example, the TGFbeta and SMAD pathways.

### Evaluation of foreseeable effects

SOX2 overexpression is potentially oncogenic and there is a likelihood that overexpression of other components within the pathway could be oncogenic. Similarly, there is a possibility that some of these genes are tumour suppressors, so down regulation by shRNA expression could be potentially oncogenic. The inserts to be expressed are not toxic and neither will they alter the pathogenicity of host range of the lentiviruses or retroviruses.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing

Project 2010/11 aims to study the role of SOX2 amplification and associated genes in squamous epithelial carcinogenesis. Proteins and RNAs will be expressed in recombinant lentiviruses and amphotropic retroviruses. Proteins to be expressed include SOX2 and related proteins and components of the SOX2-controlled pathways. shRNA or mir30-based shRNA-mirs targeted for SOX2 will also be expressed. SOX2 overexpression is potentially oncogenic and there is a reasonable likelihood that overexpression of other components may also be oncogenic. Host cells include E. coli K12 strains for the initial cloning and plasmid preparation and the mammalian cells to be transfected; all these host cells are highly disabled and are equivalent to hazard group 1. Two different forms of recombinant viruses will be generated.  
1. Recombinant amphotropic retroviruses can infect human cells. The proteins to be used will not change the host range or pathogenicity of the virus and the proteins/RNAs to be expressed are potentially oncogenic. This aspect of the work is therefore correctly classified as Class 2.
2. Recombinant lentiviruses are not capable of replication but can infect human cells. The proteins to be used will not change the host range or pathogenicity of the virus and the proteins/RNAs are potentially oncogenic. The lentiviral vectors also contain the WPRE and downstream X gene, which also have the potential to be oncogenic. This aspect of the work is therefore correctly classified as Class 2.

The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pair of gloves

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2</td>
<td>L3</td>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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**Project Ref** 20/12.1

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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tr>
<td>02/04/2012</td>
<td>Development of an amber suppressor system in mouse neurons</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</tbody>
</table>
## Project Additional Information

### Purposes of the contained use

We propose to develop a new system for the expression of proteins in neurons that will allow the labelling of the proteins at specific sites with unnatural amino acids using amber suppression. This will be used to study the role of kinases in signal transduction and in the control of circadian rhythms.

### Recipient or parental organism

Hosts: E. coli K12 derivatives (for example DH5a, MC1061, XI1blue, XL10 gold, TOP10); mammalian cell lines (for example HeLa, HEK293T); primary mouse cells.

### Host/vector system

Non-replicative lentiviruses will be constructed using co-infection of HEK293 cells with the plasmids pCCLsin.PPT.hPGK.EGFP.Wpre (contains inserts and WPRE), pRSV.Rev, pMDLg/pRRE (gag/pol expression), pMD2-G (VSV-G expression)

### Origin & function

Inserts carried by lentivirus will express tRNA synthetase and the corresponding tRNA, a reporter gene (eGFP), and a variety of mammalian kinases

### Evaluation of foreseeable effects

Overexpression of mammalian kinases is potentially oncogenic. The tRNAs, tRNA synthetases and GFP homologues to be expressed are not toxic and neither will they alter the pathogenicity of host range of the lentiviruses. All the lentiviruses contain a WPRE which is potentially oncogenic; it is acknowledged that the start codon is mutated, but this is considered insufficient to prevent the possibility of mutation and Gene X expression.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Project 2012/02 wishes to develop an amber suppressor system in mouse neurons. This will initially involve the expression of tRNA synthetases and tRNAs in conjunction with a fluorescent reporter gene such as GFP. Once the system has been developed, the aim will be to use it for the study of mammalian kinases. All the proteins and tRNAs are non-toxic, although the kinases have oncogenic potential or, at worst, are known to be oncogenic. E. coli and mammalin cells are all ACDP hazard group 1. Plasmids are all mobilisation defective or, at worst, mobilisation deficient. Amplification in E.coli of plasmids containing the target proteins/tRNAs will not alter their pathogenicity or their ability to survive in the environment, and this aspect of the work is therefore correctly classified as Class 1. The non-replicative VSV-G pseudo-typed recombinant lentiviruses expressing the target proteins and tRNAs will be able to infect human cells, but the viral tropism and their ability to survive in the environment is unchanged by their inserts. Due to the presence of a WPRE in the lentiviral vectors and its potential oncogenic nature, coupled to the ability of the lentiviruses to potentially infect human cells, all work in lentivirus production and their usage is Class 2, regardless of the insert used.

The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pairs of gloves.

**Project Containment**

<table>
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<tr>
<th>Laboratory Activities</th>
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<th>Animal Units</th>
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**Project Ref** 20/12.3

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<tbody>
<tr>
<td>06/09/2012</td>
<td>Synaptic transmission in visual systems</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>

| Date Project | 02/03/2022 | 02/03/2022 | Page 1195 of 15326 |
**Project Additional Information**

**Purposes of the contained use**

Our goal is to understand the molecular basis for synaptic transmission, using the paradigm of the visual systems in mammals and fish. This research requires the use of recombinant lentiviruses and adeno-associated viruses for the expression or down-regulation of specific proteins involved in synaptic transmission and also a series of reporter proteins that either report on intracellular levels of calcium ions, sodium ions or pH, or directly alter their levels through light-activated channels (optogenetics).

**Recipient or parental organism**

Hosts: E.coli K12 derivatives (for example DH5a, MC1061, X11blue, Top10); mammalian cell lines (for example HeLa, HEK293T); primary mouse cells, mouse brain slices, isolated retinas or zebra fish.

**Host/vector system**

Non-replicative lentiviruses will be constructed using co-infection of HEK293 cells with plasmids pCCLsin.PPT.hPGK.EGFP.Wpre (contains inserts and WPRE), pRSV.Rev, pMDLg/pRRE (gag/pol expression), pMD2-G (VSV-G expression).

Non-replicative adeno-associated viruses (AAVs) will be constructed in HEK293 cells by co-infection of plasmid pAAV-6P-SEWB (included WPRE) and the helper plasmids pDP1 and pDP2 (encode rep, cap, E2A, E3, E4 and VA).

**Origin & function**

Inserts carried by the recombinant lentiviruses and AAVs will include proteins expressed at the synapse (such as synaptophysin, ribeye and PSD95, and mutants of them), shRNAi molecules to knockdown the expression of endogenous synaptic proteins (such as ribeye, clathrin, synaptophysin etc), cDNAs encoding fluorescent reporters of cellular activity (such as GFP derivatives that sense calcium ions, sodium ions or pH), fluorescent reporters (gfp or luciferase fusions of synaptic proteins) and optogenetic factors (such as channel rhodopsin and light-regulated adenyl cyclase).

**Evaluation of foreseeable effects**

All the synaptic proteins, their mutants and gene fusions, shRNAi and reporter proteins and optogenetic factors to be expressed are not toxic and neither will they alter the pathogenicity or host range of the lentiviruses or AAVs. All the lentiviruses and AAVs contain a WPRE which is potentially oncogenic; it is acknowledged that the start codon is mutated, but this is considered insufficient to prevent the possibility of mutation and gene X expression.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only GM micro-organisms will be produced in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

02/03/2022
Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The project aims to study synaptic transmission and signal transduction in primary cells, brain slices or in vivo in zebrafish (Danio). Standard reporter proteins and proteins and siRNAs that are likely to affect synaptic transmission, such as synaptophysin, ribeye, PSD95 etc, will be expressed using recombinant lentiviruses and recombinant adeno-associated viruses (AAVs). All of the proteins and siRNAs to be expressed are neither toxic nor oncogenic. Tissue culture cells, mouse explants and Danio are highly disabled and are therefore considered to be equivalent to ACDP hazard group 1. Plasmids will be produced in laboratory E. coli K12 strains that are non-colonising and are HG1. The plasmids used in both expression systems are pUC-based non-mobilisable plasmids that will neither alter the pathogenicity or ability of the E. coli host cells to survive in the environment, and therefore plasmid production is correctly classified as Class 1. Both lentiviruses and AAVs have a broad host range and can infect human cells. The recombinant systems both produce replication-incompetent viruses by co-transfection of HEK293 cells with either 3 helper plasmids for the lentiviral system and 2 helper plasmids with the AAV system. The lack of homology between the plasmids reduces the risk of any recombination event and therefore the likelihood of regenerating a replication competent virus is negligible and the testing for such RCVs is considered unnecessary. The one non-negligible hazard associated with the expression systems is that they both contain the WPRE and downstream X gene fragment that is considered to have oncogenic potential. Thus the production and use of the recombinant lentiviruses and AAVs are both correctly assessed as Class 2. Oncogenic DNA will be handled and disposed of appropriately.

The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pairs of gloves.

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Project Ref 20/13.1
To fully understand the mechanism of T-cell activation, we need to elucidate its underlying principles at the molecular level. To achieve this, the minimal set of components required to recapitulate the initial triggering steps of the pathway have to be reconstituted. This requires using lentiviral transduction technology to stably express multiple gene products in mammalian cell lines.

Hosts: E. coli K12 derivatives (for example DH5a, MC1061, Xl1blue, Top10); mammalian cell lines (for example NIH3T3, HEK293T)

Recipient or parental organism

Non-replicative lentiviruses will be constructed using co-infection of HEK293 cells with the plasmids pHRSIN-CSGW (contains inserts and WPRE), pCMVd8.91 (gag/pol; expression), pMD2.G (VSV-G expression).

Origin & function

Human cDNAs encoding the following will be used: membrane receptors such as the whole T-cell receptor complex, CD4, CD8 and CD28, as well as mutants and chimeras of them; tyrosine kinases such as Lck, Fyn, ZAP70 and CSK, and mutants and truncations of them; phosphatases such as CD45 (ptprc), shp1, cd148; Adapters such as CBP?PAG, LAT, SLP76, GRb2, GADS; Dimerisation domains such as FKBP/FRB and LOV2 or PhyB. In addition fluorescent reporters such as GFP and its analogous will be used.

Evaluation of foreseeable effects

None of the inserts will affect the pathogenicity or survivability of the host cells (E. coli or mammalian cells). None of the inserts will affect the host range, pathogenicity or ability to survive in the environment of the recombinant lentiviruses. None of the inserts are toxic and none of them are oncogenic, except for Lck and Syk. All the lentiviruses contain a WPRE which is potentially oncogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136° for 20 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Project Containment

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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Despite extracellular immune responses, viral and bacterial pathogens are able to infect cells. Infected cells must therefore have mechanisms to neutralise or at least limit the replication of pathogens intracellularly. We would like to investigate how this is accomplished when cells are infected with respiratory syncytial virus. We aim to identify and characterise restriction factors that target respiratory syncytial virus and the mechanism by which the virus is neutralised.

**Hosts:** mammalian cell lines (for example HeLa, HEK293T)

**Recipient or parental organism**

**Replication-vector system**

Recombinant respiratory syncytial will express fluorescent reporter proteins, either GFP (rgRSV-RW30) or DsRed (rrRSV-BN1). The genes are cloned into the L-gene terminus (3' end of the positive sense transcript) and described in Hallak et al. (J Virol 74:10508, 2000). A control recombinant RSV (rRSV) with no insert will also be used.

**Origin & function**

Recombinant respiratory syncytial will express fluorescent reporter proteins. GFP and DsRed fluorescent reporter proteins are not toxic and neither will they alter the pathogenicity or host range of the RSVs.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only GM micro-organisms will be produced in this work.
Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 20 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project 2012/12 is to study the role of Trim21 in the intracellular immunity to Respiratory Syncytial Virus (RSV). RSV is a (-)ssRNA virus belonging to the Paramyxoviridae family that causes respiratory tract infections. In young children (less than 2 years old) infection can lead to hospitalisation, but in healthy adults the symptoms are like a mild cold, but immunosuppressed adults could be affected more severely. There is near ubiquitous seropositivity for RSV in adults. The virus is highly infectious from aerosols and is a robust virus probably capable of surviving for hours on a contaminated surface. RSV is classified as a HAZARD Group 2 pathogen. Recombinant virus expressing either GFP (rgESV-RW30), dsRed (rrRSV-BN1) or no colour protein (rRSV) will be obtained from Prof. M Peeples (Hallak et al. J Virol 74:10508, 2000). The recombinant viruses are all replication competent. Expression of GFP or dsRed will not alter the pathogenicity, host range or survivability of the virus in the environment. Class 2 is therefore appropriate for this work. Given the ease of infection by aerosols, all work must be performed in a Class ll MSC and processes that generate aerosols e.g. sonication will need further risk assessment if required. Centrifugation of viral supernatants must be performed in screw-capped tubes, not snap-capped eppendorfs. The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pair of gloves.

Project Containment

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Project Ref 20/13.3

Date Ackn’d CU2 Project Title | Class CultureVolClass2 CultureVolumeClass3-4
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Expression of mammalian proteins, siRNAs and reporters in eukaryotic cells using viral delivery systems

A fundamental tenet of understanding biological systems is the ability to formulate and test hypotheses on the function of proteins within a cellular context. This entails altering the expression levels of proteins in vivo, either through the overexpression of the native protein or decreasing expression through RNA silencing. All these manipulations are facilitated through the use of recombinant non-replicative viruses.

Hosts: E. coli K12 derivatives (for example DH5a, MC1061, Xl1blue, Top10); mammalian cell lines (for example HeLa, CHO, HEK293T); primary cell lines from e.g. mouse

Host/vector system

(1) HIV-based non-replicative recombinant lentiviruses e.g. based on pCCLsin.PPT.hPGK.EGFP.Wpre or pLKO1;
(2) MLV-based non-replicative recombinant retroviruses e.g. based on pCL-10A1 and VSV-G pseudo-typed
(3) AAV-based non-replicative recombinant adeno-associated virus e.g. based on pAAV-6P-SEWB
(4) deltaGp-RV non-replicative recombinant Glycoprotein-deleted rabies virus e.g. based on pSADdeltaG-F2

All the above require helper plasmids for the generation of viral particles upon cotransfection in mammalian cells.

Origin & function

Mammalian proteins to be expressed fall into all major classes of proteins found in the cell and secreted from the cell and fall into the following classes: (A) Intracellular proteins such as: DNA modifying and repair proteins, post-translational modifying systems (such as phosphorylation, ubiquitination, SUMOylation) metabolic enzymes, proteases, cytoskeletal proteins, motor proteins, nucleoproteins, membrane-binding proteins. (B) Secreted proteins (c) Integral membrane proteins such as transporters, ion channels, receptors, enzymes, structural proteins, molecular chaperones, apoptosis regulators. Specific exclusions include prion agents known or suspected to cause neurological disorders and any biological toxin.

RNAs to be expressed fall into all major classes of RNA found in the cell and also RNAs specifically designed for the modulation of expression levels of native RNAs: (A) Anti-sense RNAs, siRNAs or shRNAs designed to decrease levels of endogenous mRNAs encoding proteins described above. (B) NON-coding RNAs that modulate cellular activities. (c) Structural RNAs

Specific exclusions include any agent that may include the expression of prion agents.
Reported proteins to be expressed include a variety of natural and engineered proteins from bacteria, insects and jellyfish such as GFP derivatives, luciferases, channelrhodopsins, light regulated adenyl cyclase, GFP based calcium sensors such as GCaMP5/6, R-GECo1, pH-sensors or sodium sensors.

Evaluation of foreseeable effects

None of the inserts will affect the pathogenicity or survivability of the host cells (E. coli or mammalian cells). None of the inserts will affect the host range, pathogenicity or ability to survive in the environment of the recombinant lentiviruses. None of the inserts are toxic. Some of the proteins are oncogenic and some of the lentiviral vectors and AAV vectors contain a WPRE which is also potentially oncogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied where the risk assessment identifies Class 2 as the appropriate classification.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated level 2 waste will be autoclaved (136°C for 20 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The comments are too long to insert in the space given, so please see the attached Risk Assessment for the full comments. In brief:

Lentiviral vectors containing WPRE, regardless of insert: Class 2
pLKO1 lentiviral vectors for RNA expression, Class 1, unless the insert is oncogenic, then Class 2 AAV vectors containing a WPRE, regardless of insert: Class 2
DeltaGp-rabies virus vectors, class 1, unless the insert is oncogenic, then Class 2

Note that inserts that encode prions or biological toxins are specifically excluded from this risk assessment.

Oncogenic DNA will be handled and disposed of appropriately.

The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pair of gloves.

Waste generated in this project will be effectively inactivated by using the approved disinfectants in the LMB as described in the Safety Handbook. The environmental impact will therefore be negligible.

Project Containment
### Project Additional Information

**Purposes of the contained use**

We wish to produce virus-like particles and replication-incompetent viruses in order to study the dynamics and physiochemical requirements of the membrane fusion event that delivers the viral genome into the cytoplasm during infection. We plan to determine the structures of these viruses or virus-like particles, and their membrane fusion intermediates, by electron microscopy. We also plan to image virus-like particles and replication incompetent viruses by light microscopy as they fuse with synthetic lipid membranes of live cells in culture.

**Recipient or parental organism**

Hosts: E. coli K12 derivatives (for example DH5α, X11blue, Top10); mammalian cell lines (for example HeLa, Vero, CHO, HEK293, BHK)

**Host/vector system**

Production of non-replicative virus like particles for (i) closely related flaviviruses (such as dengue virus, West Nile virus, Japanese encephalitis virus), (ii) closely related phleboviruses such as Rift Valley fever virus, (iii) ΔG vesicular stomatitis virus (VSV) for pseudotyping with viral envelope proteins from other viruses, (iv) Putative endogenous retroviruses from C. elegans and other nematodes.
Genes encoding structural proteins of the relevant viruses will be used to make recombinant non-replicative viruses for dengue virus, West Nile virus and Japanese encephalitis virus and other closely related viruses from the flavivirus genus; West Nile virus; Rift Valley fever virus and other closely related viruses from the phlebovirus genus; yellow fever virus; Hepatitis C virus; HIV-1 ΔG vesicular stomatitis virus; endogenous retroviruses from C. elegans and other nematodes.

Proteins to be expressed in the lentivirus system also include (i) envelope glycoproteins from flaviviruses (E), hepaciviruses (E1/E2) or bunyaviruses (Gn/Gn), (ii) Eukaryotic protein sequences bearing sequence homology to the ectodomains of viral envelope proteins (e.g. from C. elegans and Ancylostoma ceylanicum), (iii) Toll-like receptors 7, 8 and 9 from mammals e.g. mouse, human etc.

Evaluation of foreseeable effects

None of the inserts will affect the pathogenicity of survivability of the host cells (E. coli or mammalian cells). None of the inserts will affect the host range, pathogenicity or ability to survive in the environment of the recombinant lentiviruses. None of the inserts are toxic and none of them are oncogenic. All the lentiviruses contain a WPRE which is potentially oncogenic and therefore Class 2 is appropriate.

All the virus-like particles cannot replicate and therefore Class 1 is appropriate for all the work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied where the risk assessment classes the activities as Class 2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 20 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Lentiviral expression will also be used for the expression of a number of receptors for structural studies, such as RIG-I like receptors and Toll-like receptors, and because this lentiviral system contains a WPRE and X gene, this is correctly classified as Class 2. To facilitate pseudo typing with viral envelope proteins from hepaciviruses, deltaG VSV will be generated using vaccinia strain vB18R-T7 (an attenuated strain) and co-transfection of 5 plasmids. The HCVpp-pseudotyped delta G VSV will only be able to infect hepatic cells, but since the vaccinia strain is not fully attenuated, Class 2 is appropriate.

The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pair of gloves.
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
We seek to understand the host & pathogen interactions that occur during tuberculosis (TB), by using a model system where zebrafish (Dania rerio) are infected with Mycobacterium marinum. In addition, we will use the zebrafish model to study the mechanism of infection of other pathogenic bacteria, viruses and helminthes.

Recipient or parental organism
Escherichia coli K12, listeria monocytogenes, Mycobacterium abscessus, Mycobacterium avium complex, Mycobacterium bovis (BCG strain), Mycobacterium smegmatis, Mycobacterium marinum, Mycobacterium ulcerans,
| Pseudomonas aeruginosa, Salmonella arizonae, Salmonella typhimurium, Staphylococcus aureus, Streptococcus iniae, and Streptococcus pneumonia, Candida albicans, Candida glabrata, and Cryptococcus neoformans |

### Host/vector system

Expression of fluorescent markers such as GFP will be from pUC-based plasmids which have Ampicilln ChloramPhenicol, Spectinomycin, Kanamycin, and/or Hygromycin resistance markers, not exceeding 2 antibiotic resistance markers per plasmid. Gene products from the host bacteria/yeast that have been deleted, will be expressed from plasmid pCEB3 to check that complementation does occur. Potentially any gene would want to be expressed back into its host cell.

### Origin & function

Antibiotic resistance genes for ampicillin, chloramphenicol, spectinomycin, kanamycin, and/or hygromycin will be used for plasmid selection. Fluorescent markers such as green fluorescent protein are from Aequorea victoria. Other genes identified in bacteria/yeast upon deletion that lead to interesting phenotypes will be re-expressed in the deleted mutant to check the deletion phenotype is reversible. Potentially any gene in the bacterial yeast could be deleted and then re-expressed from a plasmid.

### Evaluation of foreseeable effects

None of the inserts will affect the pathogenicity or survivability of the host cells (bacteria or yeast). None of the inserts will affect the host range, pathogenicity or ability to survive in the environment of the recombinant GMMs. None of the inserts are toxic and none of them are oncogenic.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied where the risk assessment classes the activities as Class 2.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 20 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

N
The aim of project 2014110 is to understand host and pathogen interactions that affect the infectivity of a wide range of pathogenic bacteria and yeasts, specifically the ACDP hazard group 2 pathogens Listeria monocytogenes, Mycobacterium abscessus, Mycobacterium avium complex, Mycobacterium bovis (BGG strain), Mycobacterium smegmatis, Mycobacterium marinum, Mycobacterium ulcerans, Pseudomonas aeruginosa, Salmonella arizonae, Salmonella typhimurium, Staphylococcus aureus, Streptococcus iniae, and Streptococcus pneumonia, Candida albicans, Candida glabrata, and Cryptococcus neoformans. Genes will be knocked-out and, if an interesting phenotype is observed, then the gene will be re-introduced to ensure the wild-type phenotype is recoverable. Knocking-out genes from these pathogens is highly unlikely to increase pathogenicity or their ability to survive in the environment. Similarly, re-introduction of these knocked-out genes or expression of fluorescent reporter proteins will neither alter pathogenicity or the survivability of any of the above pathogenic micro-organisms. Therefore all aspects of work involving the above bacteria and yeasts are correctly classified as Class 2. Plasmids will be produced in laboratory E. coli K12 strains that are non-colonising and are HG 1. The plasmids are pUC-based non-mobilisable plasmids that will neither alter the pathogenicity or ability of the E. coli host cells to survive in the environment, and therefore plasmid production is correctly classified as Class 1.

The Code of Practice for the CL2 lab where this work will be performed dictates the avoidance of all sharps and the wearing of two pair of gloves.

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**Project Ref** 20/14.3

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Project notified under transitional arrangements [N]
**Project Additional Information**

**Purposes of the contained use**

We seek to understand the host & pathogen interactions that occur during tuberculosis (TB), by using a model system where zebrafish (Danio rerio) are infected with Mycobacterium marinum. Validation of the zebrafish biochemical pathways that have been implicated in affecting the infectivity of bacterial or viral pathogens will be tested in human cell lines by either making knock outs or overexpressing the relevant proteins.

**Recipient or parental organism**

Escherichia coli K12, and a range of mammalian cell lines such as HEK293, A549, J774, THP-1, Mono-mac6, U87MG, human bronchial epithelial cell lines, L929, RAVV264.7, J774A.1, COS7, CHO, and fish cell lines (e.g. ZF4, CLC)

**Host/vector system**

The Trans-Ienliviral packaging system from Dharmacon I GE life sciences will be used, which comprises plasmids pTLA1-PAK (gag pro), pTLA1~ENZ (pol), pT~1--NV. (env - VSV-G), pTLA1-TOFF (tet-off), pTLA1-TAT/REV (TAT/REV) and pGIPZ (contains 5' Ilr, 3' self-Inactivating Ilr, and shRNA, WPRE, and marker genes (GFP and puromycin) between the I TRs).

**Origin & function**

The lentiviral expression system will be used to express or knock-out a wide variety of proteins in the human cell lines such as (a) Cytokines, chemokines, and mediators of the immune response and their receptors, scaffold proteoids and effector proteins during signal transduction (TNFa, IL-1b, CCL2, CCR2, TNFRs, RIPKs, MAPKs, aSMase, antimicrobial peptides, etc) (b) Enzymes and enzyme associated proteins related with metabolism of mediators implicated in immune responses (LTA4H, LTD4DH, Alox etc); (c) Effectors and mediators of cell death pathways (Caspases, pro-apoptic factors - BAX, BAK, BID, PUMA-, pro- survival factors -BCL2, MCL etc); (d) Effectors and mediators of cell migration pathways (SAM68, SFK etc); (e) Effectors, mediators and regulators of immune cell functions (efferocytosis, phagocytosis, endocytosis, lysosome function etc); (f) Genes implicated in gene transcription, RNA metabolism and RNA translation (transcription factors, RNA binding protein etc) (g) Effectors related with protein post-translational modifications (kinases, methylases, acetylase etc); (h) Genes linked to epigenetic modifications

Note that there are explicit exclusions from this list, which include any proteins known to act as infectious prions or toxins
None of the inserts will affect the pathogenicity or survivability of the host cells (bacteria or yeast) or the lentiviruses.
None of the inserts will affect the host range, pathogenicity or ability to survive in the environment of the recombinant
GMMs. None of the inserts are toxic or function as prions. Some of the proteins are potentially oncogenic, but this will
not affect the properties of the host virus/cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only GM micro-organisms will be produced in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied where the risk assessment classes the activities as Class 2.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 2 waste will be autoclaved (136°C for 20 min) before subsequent discharge down the drain into
the public sewer or incineration. It is expected that this will give effectively 100% killing.

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**Evaluation of foreseeable effects**

The aim of project 2014/08 is to study the role of proteins identified as being important in zebrafish for their ability to
affect infectivity of bacterial pathogens in mammalian cell lines. A wide variety of mammalian proteins will be
expressed. some of which may be oncogenic. Expression will be performed using non-replicative recombinant
viruses using HIV-based lentiviruses. All of the proteins and RNAs to be expressed are neither biological toxins nor
prions. Tissue culture cells are highly disabled and are therefore considered to be equivalent to ACDP hazard group
1. Plasmids will be produced in laboratory E. coli K12 strains that are non-colonising and are HG 1. The plasmids are
pUC-based non-mobilisable plasmids that will neither alter the pathogenicity or ability of the E. coli host cells to
survive in the environment, and therefore plasmid production is correctly classified as Class 1. Lentiviruses have a
broad host range and can infect human cells . The recombinant systems produces replication-incompetent viruses by co-transfection of HEK293 cells with between 5
helper plasmids that have been engineered to ensure that there are
no identical regions within the plasmids that would facilitate recombination and therefore the lik.elihood or regenerating a replication competent virus is negligible and the
testing for such RCVs is considered unnecessary. &
The one non-negligible hazard associated with the expression systems is that the lentiviral expression system for
protein expression contains the WPRE and downstream X gene fragment that is considered to have oncogenic
i potential. Thus the production and use of the recombinant lentiviruses is correctly assessed as Class 2, regardless of
the insert to be expressed (provided it is not a biological toxin or a prion). Oncogenic DNA will be handled and disposed of appropriately.
The Code of Practice for the CL2 lab where this work. will be performed dictates the avoidance of all sharps and the
wearing of two pair of gloves.
The aim is to define the role of factors such as cytokines, receptors, signalling molecules and transcription factors in the development of the immune system and in haematopoesis. We will study this in a variety of model systems such as primary cell lines and established well-characterised eukaryotic cell lines. The methodologies to be used will be CRISPR/Cas9 system, retroviral and lentiviral transductions, and the creation of novel stable cell lines using these technologies.
Hosts: E. coli K12 derivatives (for example DH5a, MC1061, Xl1blue, Top10) and E. coli B cell strains such as BL21 (DE3), C41, Origami); mammalian cell lines (such as HEK293).

### Host/vector system

Production of non-replicative virus like particles for (i) MSCV retroviruses (ecotropic and amphotropic); (ii) pLent6-based lentiviruses; pLKO.1 lentiviruses expressing RNA; lentiviruses expressing human cell directed CRISPR/Cas9 libraries

### Origin & function

The origins of the cDNAs/RNAs to be used will be either murine or human. Strategies will include overexpression of specific cDNAs (e.g. Bcl11 b, GATA3, c-Myb, Nfil3, plzf, Id2, Ets1 etc) and regulatory RNAs (e.g. shRNA to tumour suppressors such as Tcf?, Eomes etc) in mammalian cells. In addition, any gene in the murine or human genome may be knocked-out or knocked-in. In addition, the CRISPR/Cas9 system will be used in either murine retroviruses or lentiviruses to screen knock-outs or knock-ins of cDNA/RNA libraries; there is no intention to make gene drives.

### Evaluation of foreseeable effects

None of the inserts will affect the pathogenicity or survivability of the host cells (E. coli or mammalian cells). None of the inserts will affect the host range, pathogenicity or ability to survive in the environment of the recombinant lentiviruses. None of the inserts are toxic, but many of the cDNAs/RNAs are potentially oncogenic. Class 2 is appropriate for lentiviruses containing a WPRE (expressing any insert) and also for amphotropic murine retroviruses expressing oncogenic cDNA/RNA. Class 1 is only appropriate for lentiviruses and amphotropic murine retroviruses NOT containing a WPRE and NOT expressing an oncogenic cDNA/RNA.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied where the risk assessment classes the activities as Class 2.

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Please enter comments on the GM safety committee on the risk assessment

02/03/2022
There is insufficient room for all the comments from the SSC, so please see the attached risk assessment. Verbatim comments for CL2 activities are below, and all other activities were classified as Class 1.

*Lentiviruses and VSV-G pseudo-typed MSCV have a broad host range and can infect human cells. The recombinant systems both produce replication-incompetent viruses by co-transfection of HEK293 cells with between 2-4 helper plasmids that have been engineered to ensure that there are no identical regions within the plasmids that would facilitate recombination and therefore the likelihood or regenerating a replication competent virus is negligible and the testing for such RCVs is considered unnecessary. The one non-negligible hazard associated with the expression systems is that the lentiviral expression system for protein expression sometimes contains a WPRE and downstream X gene fragment that is considered to have oncogenic potential. Production and use of the recombinant lentiviruses containing a WPRE is correctly assessed as Class 2, regardless of the insert to be expressed (provided it is not a biological toxin or a prion). Expression of siRNA from the pLK01 lentiviral expression system, expression of proteins from pLent6 and expression using amphotropic retrovirals do not require a WPRE and therefore class 1 is appropriate for most inserts, unless the inserts' are potentially oncogenic, when Class 2 is the correct classification. Ecotropic retroviruses may be regarded as Class 1, regardless of insert, as they cannot infect human cells. The CRISPR/Cas9 work will be used to make knock-outs or knock-ins in cell lines in tissue culture and there is no intention to make gene drives. Where human lentiviral CRISPR/Cas9 RNA or cDNA libraries are used, this aspect of the work is considered to be Class 2 due to the potential presence of cDNAs that could be oncogenic or iRNAs that could target tumour suppressor genes. The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pair of gloves."

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<td>L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
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<table>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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**Project Ref 20/17.2**

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<th>CU2 Project Title</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<tbody>
<tr>
<td>21/04/2017</td>
<td>Lentivirus mediated expression and knockouts of genes involved in host responses to bacterial infection</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
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</table>

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**Project notified under transitional arrangements**

02/03/2022
### Project Additional Information

**Purposes of the contained use**

The aim of the project is to produce cell lines that are stable knockouts, knock-down or overexpressed proteins of interest involved in the cellular responses to bacterial infection. The cell lines will be made using either CRISPR-Cas9 libraries, specific gene alterations using CRISPR-Cas9 or insertion of the lentiviral genome for protein overexpression.

**Recipient or parental organism**

Hosts: E. coli K12 derivatives (for example Stbl3, DH5a, MC1061, Xl1blue); mammalian cell lines (such as HEK293T), murine primary cell lines.

**Host/vector system**

Production of non-replicative virus-like particles for lentiviruses expressing proteins and RNAs and human cell directed CRISPR-Cas9 libraries.

**Origin & function**

The human proteins/RNAs to be used in the context of the project fall into a number of major categories including autophagy, phagosome maturation, inflammasome components, toll-like receptors etc, but any gene in the genome may be targeted, including tumour-suppressor genes and potential oncogenes.

Specific exclusions include the expression of any agent that may be a prion agent or toxin.

**Evaluation of foreseeable effects**

The lentiviral expression systems used are non-replicative in the absence of specific factors required for virus propagation. The major risk to the user is therefore the expression of elements encoded by the non-replicative virus and the DNA encoding the protein/RNA of interest. Some lentiviruses contain the WPRE that is potentially oncogenic and therefore this is regarded as Class 2. The greatest risk is posed by any of the non-replicative viruses expressing siRNAs that are potentially tumour suppressors, such as critical cell cycle checkpoint regulators (e.g. p53, Rb etc) or DNA repair genes (e.g. MSH2, BRCA1 etc), and proteins that potentially induce oncogenesis upon overexpression, such as tyrosine kinases (e.g. src), transcriptional activators (e.g. myc) or small G protein mutants (e.g. ras). In all these cases, the GMMs are classified as Class 2 and will be handled only in a Class 2 facility in a Class II microbiological safety cabinet. The SOP for this work dictates the use of gloves and the avoidance of sharps to reduce risk of accidental inoculation via a puncture wound to essentially zero.

Specific exclusions include the expression of any agent that may be a prion agent or toxin.

All the expression systems rely on expression of component plasmids in E. coli; none of the inserts will affect the,
pathogenicity or survivability of the host cells. Similarly, none of the inserts will affect the host range, pathogenicity or ability to survive in the environment of the recombinant lentiviruses. CRISPR/Cas9 work explicitly excludes the creation of gene drives, with components either encoded on separate plasmids or purified gRNAs/Cas9 being used.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only GM micro-organisms will be produced in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied where the risk assessment classes the activities as Class 2.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 2 waste will be autoclaved (136°C for 20 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

There is insufficient room for all the comments from the SSC, so please see the attached risk assessment. Verbatim comments for CL2 activities are paraphrased below, and all other activities were classified as Class 1. *All genes encoding proteins or RNAs in the mammalian cells will be targeted, some of which may be oncogenic or tumour-suppressors. Gene targeting will be performed using non-replicative recombinant viruses, i.e HIV-based lentiviruses. All of the proteins and siRNAs to be expressed/targeted are neither biological toxins nor prions. Non-replicative lentiviruses have a broad host range and can infect human cells. The one non-negligible hazard associated with the expression systems is that the lentiviral expression system for protein expression sometimes contains a WPRE and downstream X gene fragment that is considered to have oncogenic potential. Production and use of the recombinant lentiviruses containing a WPRE is correctly assessed as Class 2, regardless of the insert to be expressed (provided it is not a biological toxin or a prion). Expression of siRNA from the pLK01 lentiviral expression system do not require a WPRE and therefore class 1 is appropriate for most inserts, unless the inserts are potentially oncogenic, when Class 2 is the correct classification. The CRISPR/Cas9 work will be used to make knock-outs, knock-downs or knock-ins in cell lines in tissue culture and there is no intention to make gene drives or use whole animals. Where human lentiviral CRISPR/Cas9 RNA or cDNA libraries are used, this aspect of the work is considered to be Class 2 due to the potential presence of cDNAs that could be oncogenic or iRNAs that could target tumour suppressor genes. The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pair of gloves.*
**Project Additional Information**

**Purposes of the contained use**

The goal of the project is to identify and characterize proteins involved in initiation of immune responses by dendritic cells (DC). This includes proteins involved in sensing of pathogen and cancer associated signals, antigen processing and presentation, as well as protein trafficking pathways and transcriptional programmes that lead to DC activation and maturation.

**Recipient or parental organism**

Hosts: E. coli K12 derivatives (for example DH5α, MC1 061, XL1 blue, Top10); mammalian cell lines (such as HeLa).
CHO, HEK293, murine primary cell lines.

**Host/vector system**

Production of non-replicative virus-like particles for lentiviruses expressing proteins and RNAs; adeno-associated viruses expressing proteins and RNAs; lentiviruses expressing human cell directed CRISPRiCas9 libraries

**Origin & function**

Cellular proteins to be studied fall into three main classes according to activities: (A) Intracellular proteins such as: DNA modifying and repair proteins, post-translational modifying systems (such as phosphorylation, ubiquitination, SUMOylation) metabolic enzymes, proteases, cytoskeletal proteins, motor proteins, nucleoproteins, membrane-binding proteins. (B) Secreted proteins such as antibodies, hormones, extracellular proteases, molecular chaperones, lipid/metabolite binding proteins. (C) Integral membrane proteins such as transporters, ion channels, receptors, enzymes, structural proteins, molecular chaperones, apoptosis regulators. Specific exclusions include prion agents known or suspected to cause neurological disorders and any biological toxin.

RNAs to be expressed fall into all major classes of RNA found in the cell and also RNAs specifically designed for the modulation of expression levels of native RNAs: (A) Anti-sense RNAs, siRNAs or shRNAs designed to decrease levels of endogenous mRNAs encoding proteins described above. (B) Non-coding RNAs that modulate cellular activities. (C) Structural RNAs

Specific exclusions include any agent that may induce the expression of prion agents.

Reporter proteins to be expressed include a variety of natural and engineered proteins from bacteria, insects and jellyfish such as GFP derivatives, luciferases, channelrhodopsins, light regulated adenylyl cyclase, GFP based calcium sensors such as GCaMP5/6, R-GEC01, pH-sensors or sodium sensors.

In addition, any gene in the murine or human genome may be knocked-out or knocked-in. The CRISPRiCas9 system will be used in either murine retroviruses or lentivirus9s to screen knock-outs or knock-ins of eDNA/RNA libraries; there is no intention to make gene drives.

**Evaluation of foreseeable effects**

Two viral expression systems will be utilised, both of which have the properties of being non-replicative in the absence of specific factors required for virus propagation. The major risk to the user is therefore the expression of elements encoded by the non-replicative virus and the DNA encoding the protein/RNA of interest. Some lentiviruses and AA Vs contain the WPRE that is potentially oncogenic and therefore this is regarded as Class 2. The greatest risk is posed by any of the non-replicative viruses expressing siRNAs that are potentially tumour suppressors, such as critical cell cycle checkpoint regulators (e.g. p53, Rb etc) or DNA repair genes (e.g. MSH2, BRCA1 etc), and proteins that potentially induce oncogenesis upon overexpression, such as tyrosine kinases (e.g. src), transcriptional activators (e.g. myc) or small G protein mutants (e.g. ras). In all the aforementioned cases, the GMMs are classified as Class 2 and will be handled only in a Class 2 facility in a Class II microbiological safety cabinet. The SOP for this work dictates the use of gloves and the avoidance of sharps to reduce risk of accidental inoculation via a puncture wound to essentially zero.

A number of genetic modifications are specifically outside the boundaries of this work. In particular, the following modifications are forbidden: expression of any protein mutant that is suspected of being a prion e.g. PrP(+CJD mutations); expression of biological toxin that are highly toxic to humans e.g. ricin or co-expression of both A and B chains of botulinum toxin.

All the expression systems rely on expression of component plasmids in E. coli; none of the inserts will affect the pathogenicity or survivability of the host cells. Similarly, none of the inserts will affect the host range, pathogenicity or ability to survive in the environment of the recombinant lentiviruses or recombinant AA Vs.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied where the risk assessment classes the activities as Class 2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 20 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

There is insufficient room for all the comments from the BSC, so please see the attached risk assessment. Verbatim comments for CL2 activities are below, and all other activities were classified as Class 1.

"A wide variety of proteins will be expressed including standard reporter proteins, mammalian proteins and siRNAs, some of which may be oncogenic. Expression will be performed using non-replicative recombinant viruses, i.e HIV-based lentiviruses and AAVs. All of the proteins and siRNAs to be expressed are neither biological toxins nor prions. Lentiviruses and AAVs have a broad host range and can infect human cells. The recombinant systems both produce replication-incompetent viruses by co-transfection of HEK293 cells with between 2-4 helper plasmids that have been engineered to ensure that there are no identical regions within the plasmids that would facilitate recombination and therefore the likelihood or regenerating a replication competent virus is negligible and the testing for such RCVs is considered unnecessary. The one non-negligible hazard associated with the expression systems is that the lentiviral expression system for protein expression sometimes contains a WPRE and downstream X gene fragment that is considered to have oncogenic potential. Production and use of the recombinant lentiviruses containing a WPRE is correctly assessed as Class 2, regardless of the insert to be expressed (provided it is not a biological toxin or a prion). The CRISPR/Cas9 work will be used to make knock-outs or knock-ins in cell lines in tissue culture and there is no intention to make gene drives. Where human lentiviral CRISPR/Cas9 RNA or cDNA libraries are used, this aspect of the work is considered to be Class 2 due to the potential presence of cDNAs that could be oncogenic or iRNAs that could target tumour suppressor genes. The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pair of gloves."

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
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02/03/2022
The aim of the project is understand the cellular mechanisms of circadian rhythms. A variety of different report I proteins will be expressed downstream from promoters of genes involved in circadian rhythms ('clock reporters') to monitor effects in different cells and the presence or absence of viruses.

Recipient or parental organism

Hosts: E. coli K12 derivatives (for example DH5alpha, XL 1, Top10); mammalian cell lines (such as human pluripotent stem cell lines, HEK293), murine primary cell lines.

Host/vector system

Production of non-replicative virus-like particles for lentiviruses and Sendai viruses expressing reporter proteins; replicative Herpes Simplex virus (HSV1) expressing reporter proteins and replicative influenza virus PR8 expressing luciferase.
Production of non-replicative virus-like particles for lentiviruses and Sendai viruses expressing reporter proteins; replicative Herpes Simplex virus (HSV1) expressing reporter proteins and replicative influenza virus PR8 expressing luciferase.

**Evaluation of foreseeable effects**

The major risk to the user of the non-replicative lentiviruses is the expression of elements encoded by the lentivirus as they contain a WPRE that is potentially oncogenic and, therefore this is regarded as Class 2. None of the inserts will affect the host range, pathogenicity or ability to survive in the environment of the recombinant lentiviruses. Thus the GMMs are classified as Class 2 and will be handled only in a Class 2 facility in a Class II microbiological safety cabinet. The SOP for this work dictates the use of gloves and the avoidance of sharps to reduce risk of accidental inoculation via a puncture wound to essentially zero.

Influenza strain PR8 is a highly attenuated strain that is unable to infect human cells and the expression of the reporter proteins will not affect its host range, pathogenicity or ability to survive in the environment; Class 1 is therefore appropriate for this work.

Sendai viruses can infect human cells but are non-pathogenic to humans. The recombinant viruses are non-replicative and the expression of the reporter proteins will not affect its host range, pathogenicity or ability to survive in the environment; Class 1 is therefore appropriate for this work.

Herpes simplex virus HSV1 is an ACDP HG2 pathogen that can replicate in humans and cause a variety of diseases. Expression of the reporter proteins will not affect its host range, pathogenicity or ability to survive in the environment; Class 2 is therefore appropriate for this work.

All the expression systems rely on expression of component plasmids in E. coli; none of the inserts will affect the pathogenicity or survivability of the host cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only GM micro-organisms will be produced in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied where the risk assessment classes the activities as Class 2.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 2 waste will be autoclaved (136°C for 20 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
There is insufficient room for all the comments from the SSC, so please see the attached risk assessment. Verbatim comments for CL2 activities are below, and all other activities were classified as Class 1.

"Non-replicative lentiviruses have a broad host range and can infect human cells. The recombinant system produce replication-incompetent viruses by co-transfection of HEK293 cells with between 2-4 helper plasmids that have been engineered to ensure that there are no identical regions within the plasmids that would facilitate recombination and therefore the likelihood or regenerating a replication competent virus is negligible, and the testing for such RCVs is considered unnecessary. The one non-negligible hazard associated with the expression systems is that the lentiviral expression system for protein expression sometimes contains a WPRE and downstream X gene fragment that is considered to have oncogenic potential. Production and use of the recombinant lentiviruses containing a WPRE is correctly assessed as Class 2, regardless of the insert to be expressed. Oncogenic DNA will be handled and disposed of appropriately.

HSV1 is a HG2 pathogen and the non-hazardous proteins to be expressed will not alter the pathogenicity or survivability of the viruses. Therefore this work is correctly classified as Activity Class 2. Due to complications in pregnancy, pregnant women should not work with HSV1.

All the Activity Class 2 work will be performed in a Class 2 laboratory following the COP for the lab, which includes wearing of gloves and lab coat."

Project Containment

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Animal Units

<table>
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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
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Project Ref 20/17.5

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Historical Significant Changes
Project Additional Information

Purposes of the contained use

The goal of the project is to define in high-resolution the architecture of neurotransmitter receptors, their supra-molecular assemblies and, eventually, whole synapses. We aim to link structural work with neuronal physiology, in order to provide fundamental mechanistic insights into the basic biology of neurotransmission.

Recipient or parental organism

Hosts: E. coli K12 derivatives (for example DH5alpha, XL 1 blue, Top10); mammalian cell lines (such as HeLa, CHO, I COS Jurkat, HEK293), murine primary cell lines. II

Host/vector system

Production of non-replicative virus-like particles for lentiviruses and adeno-associated viruses (AAVs) expressing I proteins and RNAs and human cell directed CRISPRCas9 libraries; recombinant replicative baculoviruses expressing oncogenic proteins from a CMV promoter in mammalian cells (BacMam system).

Origin & function

Reporter proteins to be expressed include a variety of natural and engineered proteins from insects and jellyfish such as GFP derivatives and luCiferases, respectively.

The human proteins/RNAs to be used in the context of the project fall into a number of major categories such as structural proteins at the synapse, receptors, G proteins etc, but any gene in the genome may be targeted, including tumour-suppressor genes and potential oncogenes. Specific exclusions include the expression of any agent that may be a prion agent or toxin.

Evaluation of foreseeable effects

The lentiviral and MV expression systems used are non-replicative in the absence of specific factors required for virus propagation. The major risk to the user is therefore the expression of elements encoded by the non-replicative viruses and the DNA encoding the protein/RNA of interest. Some lentiviruses and all the MVs contain the WPRE
that is potentially oncogenic and therefore this is regarded as Class 2. The greatest risk is posed by any of the
non-replicative viruses expressing siRNAs that are potentially tumour suppressors, such as critical cell cycle
checkpoint regulators (e.g. p53, Rb etc) or DNA repair genes (e.g. MSH2, BRCA1 etc), and proteins that potentially
induce oncogenesis upon overexpression, such as tyrosine kinases (e.g. src), transcriptional activators (e.g. myc) or
small G protein mutants (e.g. ras). In all these cases, the GMMs are classified as Class 2 and will be handled only in
a Class 2 facility in a Class II microbiological safety cabinet. Similarly, any expression of potentially oncogenic
proteins using the BacMam baculovirus system in mammalian cells is Class 2. All CRISPR/Cas9 work using human
libraries expressed from lentiviruses also classified s Class 2, due to the high proportion of potential oncogenes and
tumour-suppressors in the human genome. The SOP for all Class 2 work dictates the use of gloves and the
avoidance of sharps to reduce risk of accidental inoculation via a puncture wound to essentially zero.
Specific exclusions include the expression of any agent that may be a prion agent or toxin.
All the expression systems rely on expression of component plasmids in E. coli; none of the inserts will affect the
pathogenicity or survivability of the host cells. Similarly, none of the inserts will affect the host range, pathogenicity or
ability to survive in the environment of the recombinant lentiviruses.
CRISPR/Cas9 work explicitly excludes the creation of gene drives, with components either encoded on separate
plasmids or purified gRNAs/Cas9 being used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied where the risk assessment classes the activities as Class 2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 20 min), before subsequent discharge down the drain into
the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
Lentiviruses and AAVs have a broad host range and can infect human cells. The recombinant systems both produce replication-incompetent viruses by co-transfection of HEK293 cells with between 2-4 helper plasmids that have been engineered to ensure that there are no identical regions within the plasmids that would facilitate recombination and therefore the likelihood or regenerating a replication competent virus is negligible and the testing for such RCVs is considered unnecessary. The one non-negligible hazard associated with the expression systems is that AAVs and the lentiviral expression system for protein expression sometimes contains a WPRE and downstream X gene fragment that is considered to have oncogenic potential. Production and use of the recombinant AAVs and lentiviruses containing a WPRE is correctly assessed as Class 2, regardless of the insert to be expressed (provided it is not a biological toxin or a prion). Expression of siRNA from the pLK01 lentiviral expression system, expression of proteins from pLenti6 do not require a WPRE and therefore class 1 is appropriate for most inserts, unless the inserts are potentially oncogenic, when Class 2 is the correct classification. The CRISPR/Cas9 work will be used to make knock-outs or knock-ins in cell lines in tissue culture and there is no intention to make gene drives. Where human lentiviral CRISPR/Cas9 RNA or cDNA libraries are used, this aspect of the work is considered to be Class 2 due to the potential presence of cDNAs that could be oncogenic or iRNAs that could target tumour suppressor genes. The SacMam system has a broad host range and can infect human cells and will be used to expressed oncogenic proteins, and therefore this correctly classified as Class 2.

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**Project Containment**

<table>
<thead>
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<td>L3 L4 L2 L3</td>
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**Animal Units**

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<th>Large Scale Activities</th>
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**Project Ref** 20/19.1

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<td>Using fluorescently-labelled UPEC to investigate the role of immune sentinels during infection of the urinary tract</td>
<td>Class 2 ≤ 1 Litre</td>
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Non-GMM Consent Granted
The aim is to better understand the pathogenesis of urinary tract infection and chronic pyelonephritis and how this contributes to renal failure by investigating the role of different immune sentinels in the defence of the urinary tract.

Hosts: uropathogenic Escherichia coli (UPEC)

Stable integration of fluorescent reporter proteins into the genome of UPEC

Fluorescent proteins such as GFP, mCherry and all their derivatives

Expression of fluorescent reporters such as GFP or mCherry (and any of their derivatives) will not affect the pathogenicity or the ability of the UPEC to survive in the environment. The GM UPEC will therefore have the same properties as the wild type UPEC and therefore will zero environmental impact and no effect on human health.

Only GM micro-organisms will be produced in this work.

Full containment level 2 will be applied where the risk assessment classes the activities as Class 2.

Containment level 2 waste will be autoclaved (136°C for 20 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.
Project 2018/08 is study the process of infection of uropathogenic E. coli (UPEC) using fluorescently-tagged bacteria. GFP, mCherry or their derivatives will be expressed in UPEC to make them fluorescent. The bacteria will be introduced into mice via a catheter and the organs then analysed for the present of bacteria. UPEC are categorised as HG2. The fluorescent reporters will not alter the pathogenicity of the bacteria and will not make them more effective at surviving in the environment. Introduction of GM UPEC into mice will be via a catheter and thus avoids the use of a sharp needle. Dissection of potentially UPEC-contaminated tissues using sharps will be performed in a Class III MSC and will use forceps to hold tissues during excision and dicing, reducing risks to the workers of needle stick injuries. The project is therefore correctly classified as Activity Class 2.

Project Containment

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<td>L2</td>
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</table>

Animal Units
Large Scale Activities
Human Clinical Applications

Project Ref  20/19.2

Date Ackn'd  30/10/2019
Date Project Ceased

Expression of mammalian proteins, siRNAs and reporters in eukaryotic cells using viral delivery systems

Class | CultureVolClass2 | CultureVolumeClass3-4
Class 2 | < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
### Project Additional Information

#### Purposes of the contained use

The overarching purpose of the work is to understand signal transduction and transport processes in normal and abnormal (diseased) vertebrate cells during cellular activities such as neurotransmission, host pathogen interactions, cell division and cellular repair mechanisms. These integrated mechanisms will be studied at the cellular and molecular levels through either the disruption/activation of pathways by siRNA expression or through the expression of proteins and protein mutants with defined activities.

#### Recipient or parental organism

**Hosts:** E. coli K12 derivatives (for example DH5a, MC1 061, X11 blue, Top10); mammalian cell lines (for example HeLa, CHO, HEK293T); primary cell lines from e.g. mouse and human; tissue explants for e.g. mouse; in vivo work in mice.

#### Host/vector system

1. HIV-based non-replicative recombinant lentiviruses e.g. based on pCCLsin.PPT.hPGK.EGFP.wpre or pLK01;
2. MLV-based non-replicative recombinant retroviruses e.g. based on pCL-10A1 and VSV-G pseudo-typed
3. AAV-based non-replicative recombinant adeno-associated virus e.g. based on pAAV-6P-SEW8
4. deltaGp-RV non-replicative recombinant Glycoprotein-deleted rabies virus e.g. based on pSADdeltaG-F2
5. BacMam replicative baculovirus

#### Origin & function

Cellular proteins involved in signal transduction and transport processes fall into three main classes according to activities: (A) Intracellular proteins such as: DNA modifying and repair proteins, post-translational modifying systems (such as phosphorylation, ubiquitination, SUMOylation) metabolic enzymes, proteases, cytoskeletal proteins, motor proteins, nucleoproteins, membrane-binding proteins. (B) Secreted proteins such as antibodies, hormones, extracellular proteases, molecular chaperones, lipid/metabolite binding proteins. (C) Integral membrane proteins such as transporters, ion channels, receptors, enzymes, structural proteins, molecular chaperones, apoptosis regulators. Specific exclusions include prion agents known or suspected to cause neurological disorders and any biological toxin.

RNAs to be expressed fall into all major classes of RNA found in the cell and also RNAs specifically designed for the modulation of expression levels of native RNAs: (A) Anti-sense RNAs, siRNAs or shRNAs designed to decrease
levels of endogenous mRNAs encoding proteins described above. (8) Non-coding RNAs that modulate cellular activities. (C) Structural RNAs
Specific exclusions include any agent that may result in the expression of TSEs or biological toxins.
Reporter proteins to be expressed include a variety of natural and engineered proteins from bacteria, insects and jellyfish such as GFP derivatives, luciferases, channelrhodopsins, light regulated adenyl cyclase, GFP based calcium sensors such as GCaMP5/6, R-GECo1, pH-sensors or sodium sensors.
The vectors will also be used to screen human CRISPR/Cas9 libraries for either gene knock-out, knock-down or knock-up.

Evaluation of foreseeable effects

LFour mammalian viral expression systems will be utilised, all of them have the properties of being non-replicative in "the absence of specific factors required for virus propagation. The major risk to the user is therefore the expression of "elements encoded by the non-replicative virus and the DNA encoding the protein/RNA of interest. Some lentiviruses and AAVs contain the wild type WPRE that is potentially oncogenic and therefore this is regarded as Activity Class 2. In contrast recombinant MLV, delta Gp rabies virus and some lentiviruses do not contain any potentially oncogenic elements and therefore Activity Class 1 is appropriate. The greatest risk is posed by any of the non-replicative viruses expressing siRNAs that are potentially tumour suppressors, such as critical cell cycle checkpoint regulators (e.g. p53, Rb etc) or DNA repair genes (e.g. MSH2, BRCA1 etc), and proteins that potentially induce oncogenesis upon overexpression, such as tyrosine kinases (e.g. src), transcriptional activators (e.g. myc) or small G protein mutants (e.g. ras). In all the aforementioned cases, the GMMs are classified as Activity Class 2 and will be handled only in a Class 2 facility. The SOP for this work dictates the use of gloves and the avoidance of sharps to reduce the risk of accidental inoculation via a puncture wound to essentially zero. The BacMam expression will also be used, which is based on recombinant replicative baculovirus, that cannot replicate in human cells, but can express proteins under the control of the CMV promoter. Where the insert to be expressed is oncogenic or a tumour suppressor, then this is classified as Activity Class 2 and will be used only in a Class 2 facility wearing gloves and avoiding sharps.

A number of genetic modifications are specifically outside the boundaries of this work. In particular, the following modifications are forbidden: expression of any protein mutant that is suspected of being a prion (e.g. PrP+CJD mutations); expression of biological toxin that are highly toxic to humans (e.g. ricin or co-expression of both A and B chains of botulinum toxin).

All the expression systems rely on expression of component plasmids in E. coli; none of the inserts will affect the pathogenicity or survivability of the host cells. Similarly, none of the inserts will affect the host range, pathogenicity or ability to survive in the environment of the recombinant lentiviruses or recombinant AAVs.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GM micro-organisms will be produced in this work.
Future work may include the introduction of the AC2 GM vectors (lentiviruses and AAVs) into mice. All mouse work is approved by appropriate Home Office licenses. Where possible, mice will be injected with the use of a stereotactic frame or using suitable restraints. Minimal quantities of the AC2 vectors will be used, typically 10 ul. The work will be performed only by highly experienced personnel and gloves will be worn. After injection, mice will be held in a post-operative recovery cabinet (Hepa filtered air supply) for 24 hours and not handled. Bedding will be treated as CL2 waste (autoclaved followed by incineration) and will be removed after 24 hours within a Class II MSC. After 24 hours the viral load in the mouse will be significantly depleted and so the mice will be transferred to CL 1.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Full containment level 2 will be applied where the risk assessment identifies Class 2 as the appropriate classification.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solids from the Containment level 2 lab (e.g. plasticware) will be autoclaved (136°C for 20 min), before disposal by incineration. It is expected that this will give effectively 100% killing.

All liquids from the Containment Level 2 lab will be inactivated by treatment with RBS50 (minimum final concentration of 1 %) for a minimum of 1 hour before subsequent discharge down the drain into the public sewer.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The comments are too long to insert in the space given, so please see the attached Risk Assessment for the full comments. In brief:
Lentiviral vectors containing a wild type WPRE, regardless of insert: Class 2
Lentiviral vectors with mutated WPRE (no expression of X gene): Class 1, unless insert is oncogenic, then Class 2
pLK01 lentiviral vectors for RNA expression, Class 1, unless the insert is oncogenic, then Class 2
MV vectors containing a wild type WPRE, regardless of insert: Class 2
MV vectors with a mutated WPRE (no expression of X gene): Class 1, unless insert is oncogenic, then Class 2
DeltaGp-rabies virus vectors, class 1, unless the insert is oncogenic, then Class 2
BacMam expression system, class 1, unless the insert is oncogenic, then Class 2
All human CRISPR/Cas9 libraries in lentiviruses (CasH and gRNAs on same vector): Class 2
Note that inserts that encode prions or potent biological toxins are specifically excluded from this risk assessment. Potentially oncogenic DNA will be handled and disposed of appropriately.
The Code of Practice for the Containment Suite where this work will be performed dictates the avoidance of all sharps and the wearing of two pair of gloves.
Waste generated in this project will be effectively inactivated by using the approved disinfectants in the LMB as described in the Safety Handbook. The environmental impact will therefore be negligible.
This project is an update of previously notified CL2 activities; the list of previously notified activities is in Additional Comments. All of these projects were previously approved by the BSC (comments can be found with Notifications).

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
Project Additional Information

Purposes of the contained use

The investigation of the cellular genetic requirements for efficient retrotransposition using the technically tractable chicken cell line DT40 and Moloney murine leukaemia virus based vector with amphotropic envelope.

Transgenes will be fluorescent marker proteins and also genes to complement the genetic defects engineered into the host DT40 eg genes involved in DNA damage avoidance and repair.

Recipient or parental organism

Avian cell lines (eg DT 40).
Retroviral producer cells: HEK293 derived lines, such as Phoenix-amphotropic, or VSV-G expressing retroviral producer cells.

Host/vector system

MD

Origin & function

Transgenes will be fluorescent marker proteins and also genes to complement the genetic defects engineered into the host DT40 eg genes involved in DNA damage avoidance and repair.
### Evaluation of foreseeable effects

Inserts carried by retroviruses generally correspond to individual, well characterised cDNAs (e.g. reporter genes such as GFP, genes involved in DNA damage avoidance and repair e.g. RAD18, signalling molecules such as NF-kB) or their derivatives (e.g. truncations, point mutations, or fusions to marker proteins, e.g. GFP). Work also includes the creation of retroviral supernatants which may contain hazardous genes such as proto-oncogenes. All inserts are unlikely to increase the pathogenicity of the tc target cell lines.

A non-negligible hazard arises from the risk of insertional mutagenesis and the potentially oncogenic or cytotoxic nature of some of the inserts. DNA grown up from such clones should be handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1m, paras. 8-10, i.e. gloves should be worn, sharps avoided and all wastes be rendered harmless before disposal.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

For the part of the work classified as class 2, full containment level 2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 1 waste, generated during the initial steps, will be inactivated by either treatment with 1% v/v Clearsol, before discharge down the drain into the public sewer, or by incineration (in a registered facility). Data have been obtained from the manufacturer to show that the disinfection will achieve >10 (to the power of 5) loss of infectivity in the times used, while incineration will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

**Is an emergency plan required according to regulation 20?**

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**Tick to confirm that you have attached a risk assessment to this form**

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**Tick if you are claiming exemption from disclosure for section of the risk assessment**

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Please enter comments on the GM safety committee on the risk assessment
This work involves the cloning of fluorescent marker proteins and also genes involved in DNA damage avoidance and repair. The ultimate host in both cases is the chicken cell line DT 40, but initial cloning will be into standard laboratory E. coli K12 strains and also, as the vectors include Moloney murine leukaemia virus, appropriate retroviral packaging cell lines.

The transgenes are already covered for work in E. coli under existing projects, and the new vectors to be included in this project are standard mammalian expression plasmids (eg pCDNA) and also the MMLV-based disabled retroviral vectors. These will be packaged using the Phoenix-amphotropic packaging line, or with VSV-G protein pseudo-typing, to produce disabled retroviral particles, capable of only a single infective passage, but with envelope proteins allowing promiscuous infectivity.

The E. coli work and also that with the pCDNA (or similar vectors is correctly classified as Class 1. With the retroviral vectors, there is no hazard with the marker transgenes, and therefore Class 1 is appropriate. With the genes involved in DNA damage avoidance and repair, which are potentially oncogenic, Class 2 is appropriate, as assessed.

It is noted that the potential hazard from the naked DNA for proto-oncogenes has been considered.

### Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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### Project Ref 553/17.9

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<td>Use of bacteria to investigate mechanisms of biofilm formation and implications for infection</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Notification transferred from GM553

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**

The aims of the project are to understand how adhesion of bacteria to surfaces, followed by defined cell-cell interactions between bacteria, lead to the formation of macroscopic three-dimensional bacterial communities called biofilms.

**Recipient or parental organism**

| Hazard Group 2 bacteria such as Pseudomonas aeruginosa |
| Hazard group 1 bacteria such as Pseudomonas fluorescens and disabled strains of E. coli (K-12 and B derivatives) |

**Host/vector system**

| E. coli K-12 or B derivatives and non-mobilisable vectors |

**Origin & function**

| Standard reporter genes such as GFP and Luciferase |
| Standard antibiotic resistance genes such as Amp and Kan |
| Over expression or knockdown of genes proposed to be involved in the bacterial adhesion process, such as fimbriae genes, Ag43, Pel and Psl. |
| Over expression or knockdown of genes identified from bacterial samples from Cystic fibrosis patients to be involved in biofilm formation. |

**Evaluation of foreseeable effects**

Addition of standard reporter genes has a history of safe use and is not expected to alter the pathogenicity of the host. Standard antibiotic resistance genes are not used to treat the disease and therefore will not alter the ability to treat infections. Over expression of proteins in disabled E. coli K-12 or B derivatives to enable production of proteins for study is not expected to overcome the disabled nature of the strains. Overexpression of genes involved in biofilm formation in P. aeruginosa is not expected to increase virulence. Where there may be potential for affecting virulence the work will be done in strains which are Type IV pili negative. Deletion mutants are likely to have a reduced ability to form biofilms and consequently are less likely to cause infection. Parallel work in P. fluorescens and E. coli will be done to the same standards as P. aeruginosa to ensure consistency.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - sharps are not required for this project.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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<tr>
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Animal Units

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Project Ref 678/09.1
Use of luminescently-tagged and fluorescently-tagged bacteria to study pathogen-phagocyte interactions

<table>
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<td>24/02/2016</td>
<td>Use of luminescently-tagged and fluorescently-tagged bacteria to study pathogen-phagocyte interactions</td>
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- **Project notified under transitional arrangements**: No

**Withdrawn**: No

**Historical Significant Changes**: Transfer from GM678 24/02/2016

**Date of Significant Change**: 09/03/2015

**Historical Date of Additional Info**: 24/02/2016

### Project Additional Information

#### Purposes of the contained use

The project aims to understand how non-tuberculous mycobacteria (NTM), specific types of gram negative bacteria (E. coli (non-verotoxigenic strains isolated from sputum), P. aeruginosa) and gram positive bacteria (S. pneumoniae, S. aureus) interact with macrophages to avoid intracellular killing and how pharmacological manipulation of cellular processes (such as the phagosomal ionic environment and macro-autophagy) might enhance intracellular bacterial killing and/or modulate release of inflammatory mediators.

#### Recipient or parental organism

NTM (excepting M. bovis BCG) are ubiquitous organisms occurring naturally in the environment (in domestic water, soil and houses). While they can cause lung infections in people with pre-existing structural lung disease (such as Cystic Fibrosis and Chronic Obstructive Pulmonary Disease), they are not pathogenic in healthy people and are not transmissible from person to person. Untransfected, it is usually handled in CL2 areas.

M. bovis BCG is still in current world-wide use as a live vaccine against M. tuberculosis and is not pathogenic in healthy people.

#### Host/vector system

pSMT1 plasmid carrying the luxAB genes from Vibrio harveyi and green fluorescent protein (GFP), Cyan FP, Yellow FP or Red FP; under the control of the constitutively active mycobacterial GroEL (hsp60) promoter; mycobacterial and E. coli origins of replication and a hygromycin resistance cassette. The vectors will not be translated by eukaryotic cells.

#### Origin & function

pSMT1 plasmid carrying the luxAB genes from Vibrio harveyi and green fluorescent protein (GFP), Cyan FP, Yellow FP or Red FP; under the control of the constitutively active mycobacterial GroEL (hsp60) promoter; mycobacterial and E. coli origins of replication and a hygromycin resistance cassette.

LuxAB genes from Vibrio harveyi and green fluorescent protein (GFP) purchased from Clontech

Episomal insertion of plasmid DNA will result in generation of cytoplasmic green fluorescent protein (GFP) and luciferase (lux) as previously utilised in M. bovis and M. tuberculosis (Kampmann et al 2000). No selection advantage is anticipated for LUX-tagged or GFP tagged mycobacteria or bacteria.

**Evaluation of foreseeable effects**

NTM (excepting M. bovis BCG) are ubiquitous organisms occurring naturally in the environment (in domestic water, soil and houses). While they can cause lung infections in people with pre-existing structural lung disease (such as Cystic Fibrosis and Chronic Obstructive Pulmonary Disease), they are not pathogenic in healthy people and are not transmissible from person to person. Untransfected, it is usually handled in CL2 areas.

M. bovis BCG is still in current world-wide use as a live vaccine against M. tuberculosis and is not pathogenic in healthy people. Untransfected and luminescently tagged M. bovis BCG are currently handled in CL2 facilities.

Untransfected and luminescently tagged S. pneumoniae, S aureus, P aeruginosa and E. coli are currently handled in CL2 facilities. While all can cause infections in man, the risk to healthy laboratory staff of these bacteria is extremely low. They will be handled in the tissue culture hoods and no procedures have the potential to generate aerosolised bacteria. The risk of inhalation therefore is extremely low.

**ii) the inserted genetic material**

The inserted plasmid has no eukaryotic promoter, encodes a luciferase gene and green fluorescent protein, which, in all mycobacteria examined to date, is retained intracellularly. The encoded proteins, lux and fluorescent proteins, have benign metabolic type activities (light production, fluorescence) which would have no significant effect on bacterial or cellular gene expression, though products may be toxic to cells expressing high levels. Extracellularly they have no signalling effects and are of very low toxicity. There is no effective way to deliver genetic material to human cells in these conditions.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Bench tops and spills will be wiped with a disinfectant (1% sodium hypochlorite, or 70% alcohol). Spills will be covered with disinfectant soaked towels for at least 15 min and then wiped. GMM will be killed by addition to 1% sodium hypochlorite for at least 15 min.

(As recommended in ‘Standard safety practices in the microbiology laboratory: www.cdc.gov’).

Liquid and solid waste will be double bagged, autoclaved and then incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
# Project Containment

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<th>Laboratory Activities</th>
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Comments

Date at Which Additional Info Submitted

02/03/2022
Significant Change: 22/12.2a  Date of Additional Information: 21/03/2014

## Premises Addresses

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<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial
- Other (please specify)

Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 14/02.2

Date Ackn'd 12/09/2018

CU2 Project Title GENERATING LENTIVIRAL VECTORS ENCODING FOR ONCOGENIC VIRAL PROTEINS

Class 2

Culture Vol

Class 2 1-50 Litres

Consent Granted Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes GM14/02.3, Transferred from GM14 ON 12/09/2018
## Project Additional Information

### Purposes of the contained use

To study the effects of potential cancer causing genes (oncogenes) on cell growth, we need to infect primary cells with these oncogenes. To do this efficiently, we use HIV-1 viruses to carry the oncogene of interest into the primary human cells. These HIV viruses are made incompetent, meaning they can only infect the cells of interest and no new HIV viruses are then produced that are able to infect new cells. The HIV viruses can therefore not replicate.

### Recipient or parental organism

The gene delivery system involves 3 plasmids that are transfected into virus producing cells, the 293T cell line. The lentiviral system is secured by gene removal such that no new particles can be produced from infected cells. This system functions as the 293t cells express:

- a. The viral envelope protein (VSV-G envelope) from a plasmid called p-VSVG.
- b. The modified HIV-1 genome with the transgene (modified HIV-1 called pHR with deleted envelope and accessory genes like tat, nef and vpr)
- c. The HIV-1 accessory genes, including the reverse transcriptase and the other viral genes involved in replication (from pCMV8.9 plasmid)

The vector can stably integrate into DNA for sustained long-term expression of the transgene. The vector has the ability to infect primary human cells, with subsequent expression of the foreign gene and GFP in these cells. All progeny cells will also express the foreign genes, but no new HIV-1 virions can be produced from the infected cells (replication defective). Because the HIV-1 envelope, that usually binds CD4 and chemokine receptors, has been replaced by the vesicular stomatitis virus envelope (VSV-G), this vector can infect many different cell types in vitro and possibly in vivo. VSG envelope allows entry of virus into cells by way of binding non-specifically to membrane phospholipid of target cells, rather than relying on specific receptor binding.

### Host/vector system

We are testing the functional role of different proteins encoded by Kaposi's sarcoma-associated herpesvirus (KSHV) and by human T-cell leukaemia virus-1 (HTLV-1). These include a viral cyclin homologue (v-cyclin), a latent membrane protein (K15), a latent nuclear antigen (LANA), anti-apoptotic proteins called K7 and a FLIP (FLICE inhibitor protein) homologue (vFLIP) and a membrane protein (K8.1), all encoded by KSHV. The viral cyclin, LANA and K15 are potential oncogenic proteins. We are also testing the mechanisms of transformation by the oncogenic Tax protein encoded by HTLV-1.

### Origin & function

To transduce (infect) primary human cells with these different viral genes, we will clone them into a lentiviral vector. The lentiviral vector is based on HIV-1 pseudotyped with a vesicular stomatitis viral (VSV) envelope, allowing infection into a broad range of cell types. The HIV-1 vector can infect cells but not replicate ("one shot" or "self-inactivating"). In addition, this lentiviral system incorporates a green fluorescent protein (GFP) from a bicistronic transcript allowing the identification of infected cells by immunofluorescence. The transduced cells will include primary human haematopoietic cells, endothelial cells and human stem cells.

### Evaluation of foreseeable effects

1. The transduced cells can only survive in tissue culture at 37°C.
2. The modified lentivirus cannot survive outside cells (dependent on cells for DNA integration), and is replicative incompetent: thus the lentivirus cannot propagate to infect new cells and pose a negligible risk to the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon (a peroxygen compound) used at a concentration of 1% is known to have a wide range of microbial activity and is bacteriocidal against many human viruses, including HIV. All tissue culture material and disposable plasticware will be soaked for a minimum of 18-24 hours in a freshly prepared 1% Virkon solution. The fluid is then disposed of down a laboratory sink with excess water. Solid waste is then double bagged in biohazard bags and autoclaved at 134C for 20 minutes at 3.2 bar pressure. Validation of this method will be by independant thermocouples placed in the centre of the load. Verification that the correct conditions have been reached will be obtained by checking the chart recorder printout. The autoclave undergoes a planned preventative maintenance inspection every 3 months. All inactivated waste is then treated as "clinical waste" and removed from site by a UCL contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

In view of the disabled nature of the HIV, the GM Safety Committee accepted the containment level proposed by the investigator subject to the vigorous application of the risk control measures identified as required by the assessment. The room in which the work will be undertaken was last inspected in June 2001 and was found to be in good order. It is due a revisit in January 2002.

Project Containment

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Project Ref 22/01.1

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Date Project Ceased

Notification reclassified to class 1 on 14/02/2012

Historical Significant Changes

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 22/01.10

Date Ackn’d 23/02/2001

Date Project Ceased 14/02/2012

CU2 Project Title BIOGENESIS OF E.COLI HEAT-LABILE TOXIN

Class Consents

Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref** 22/01.2

**Date Ackn’d** 23/02/2001

**CU2 Project Title** HIGH-LEVEL EXPRESSION OF NOVEL PROTEINS AND PROTEIN DOMAINS IN E.COLI (BL21(DE3)) FOR IN VITRO FUNCTIONAL AND STRUCTURAL STUDIES

**Class** Class 1

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM** not applicable

**Consent Granted**

**Project notified under transitional arrangements** Y

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes** Notification reclassified to class 1 on 14/02/2012

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref  22/01.3

Date Ackn'd 23/02/2001  CU2 Project Title CLONING, EXPRESSION AND MODIFICATION OF GENES IN CANDIDA  Class 2  CultureVolClass2  CultureVolumeClass3-4

02/03/2022  Page 1247 of 1532
ALBICANS

Date Project Ceased
14/02/2012

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements

Historical Significant Changes

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? ☐

If yes, tick to confirm that it is attached to this form ☐

Tick to confirm that you have attached a risk assessment to this form ☐

Tick if you are claiming exemption from disclosure for section of the risk assessment ☐

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 22/01.5

Date Ackn'd 23/02/2001

CU2 Project Title INVESTIGATION OF COOPERATIVITY AND STRUCTURE/FUNCTION RELATIONSHIPS IN MUSCLE THIN FILAMENTS

Date Project Ceased

Class 1

CultureVolClass2

CultureVolumeClass3-4

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Notification reclassified to class 1 on 14/02/2012

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 22/01.6

Date Ackn’d 23/02/2001

CU2 Project Title REGULATION OF THE FIM SWITCH IN E.COLI

Class 1

CultureVolClass2 CultureVolumeClass3-4

Class 1
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### Historical Significant Changes
- Notification reclassified to class 1 on 14/02/2012

### Project Additional Information

#### Purposes of the contained use

#### Recipient or parental organism

#### Host/vector system

#### Origin & function

#### Evaluation of foreseeable effects

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
**Project Containment**

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**Project Ref** 22/01.7

- **Date Ackn’d**: 23/02/2001
- **Project Title**: LUX MARKED BACTERIAL BIOSENSOR
- **Class**: Class 2
- **Non-GMM Consent Granted**: Not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ] N

If yes, tick to confirm that it is attached to this form [ ] N

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ] N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref  22/01.8

CLONING, EXPRESSION AND MANIPULATION OF DEFINED GENE SEQUENCES
IN ESCHERICHIA COLI

Date Ackn'd  23/02/2001
Date Project Ceased

Class  Class 1
CultureVolClass2  Non-GMM  Consent Granted  not applicable
CultureVolumeClass3-4

Tick if notifying a connected programme of work  N

Notification reclassified to class 1 on 14/02/2012

Historical Significant Changes

Historical Significant Changes

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  22/01.9

Date Ackn'd  23/02/2001  
CU2 Project Title  NATIVE DISULPHIDE BOND FORMATION IN THE ER  
Class  Class 2  
CultureVolClass2  
CultureVolumeClass3-4  

02/03/2022  
Page 1257 of 15326
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
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Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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<tr>
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Project Ref 22/03.1

<table>
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<tr>
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<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<tbody>
<tr>
<td>06/11/2003</td>
<td>ANALYSIS OF DIMORPHISM IN CANDIDA ALBICANS AND CANDIDA DUBLINIENSIS</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td>not applicable</td>
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Historical Significant Changes

Date Project Ceased

<table>
<thead>
<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>
### Project Additional Information

#### Purposes of the contained use

The purpose of the activity is to understand which genes control polymorphism in Candida albicans.

#### Recipient or parental organism

Candida albicans is a yeast. It is a commensal residing mostly in the anal cavity as well as the gut of individuals. In humans with severe immunosuppression it can cause anal "thrush". In hospital settings such as intensive care units invasive infections have been reported. Candida albicans is a typical "opportunistic" pathogen.

#### Host/vector system

Candida albicans and Escherichia coli.

#### Origin & function

The genetic material will origin from C. albicans on C dubliniensis.

It is intended to use the material, propagate it in E. coli in specific (standard vectors such as pBlusknip) and construct "disruption cassettes" to delete genes involved in the regulation of polymorphism.

See the detailed project description.

#### Evaluation of foreseeable effects

In this study we intend to identify genes of Candida albicans and Candida dubliniensis that are involved in the control of dimorphism. We intend to delete these genes in C. albicans and C. dubliniensis. It is expected that the resulting deletion mutants will be less capable to filament/replicate and thus be severely compromised in their ability to cause disease. It is not expected that amplification of these genes in E. coli will confer novel properties on the latter making it more virulent. Indeed E. coli will only be used as a host to amplify genetic material from C albicans or C dubliniensis. We do not foresee risks to human health on the environments involved in this study. Specifically we do not intend to release any of our studies into the environment.

See risk assessment form and project description.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All of the waste involving class 2 organisms is collected in dedicated biohazard bags as well as containers. All of the waste containers are sealed and then autoclaved. The autoclaving process is monitored with each lot.

A dedicated servicing contract assures proper monitoring and functioning of our autoclave facilities.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
There have been no comments when discussing this application with our Health and Safety Committee.

Project Containment

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<td>L2 Yes L3 L4</td>
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<td></td>
<td>L2 L3 L4</td>
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Animal Units

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<tbody>
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Project Ref 22/12.1

<table>
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<tr>
<td>07/03/2012</td>
<td>Mechanisms of Influenza virus assembly and budding</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Class Culture Vol</th>
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</thead>
<tbody>
<tr>
<td>09/01/2020</td>
<td>Class 2 &lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
# Purposes of the contained use

The research described in this proposal is designed to elucidate the molecular mechanisms of influenza virus assembly and budding as mediated by the M2 protein. To better elucidate the functions of the M2 cytoplasmic tail, mutations will be made throughout the region and incorporated into recombinant viruses which will then be tested for activity.

## Recipient or parental organism

- **Influenza A virus**
- Mammalian and insect tissue culture cell lines.

## Host/vector system

- E. coli and baculovirus

## Origin & function

Individual influenza virus genes will be incorporated into plasmids for growth in E. coli for ultimate expression in mammalian tissue culture cells. Individual influenza genes will also be incorporated into plasmids for growth in E. coli where they will be mutated for subsequent reintroduction into recombinant influenza viruses. Additionally, influenza genes will be incorporated into baculovirus vectors for introduction into insect tissue culture cell lines to allow for influenza virus protein expression and purification. The function of these experiments is to determine the role of influenza virus proteins in the assembly and budding of the virus.

## Evaluation of foreseeable effects

The construction of the GMOs should inform as to the functions of influenza virus proteins in viral budding. The construction of the tissue culture and vector GMOs will not have any adverse effects and has no foreseeable adverse effects. The construction of the GMO influenza viruses will not add any measures of virulence or pathogenicity and will likely attenuate virulence. Thus, there are not foreseeable adverse effects.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

## For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

## Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This project will generate liquid waste from tissue culture media and virus growth and solid (typically plasticware) waste. Liquid waste will be sterilized via treatment with 30% NaClO for 30 min to give 10% kill. 100% kill will be assessed via testing for viral growth on fresh tissue culture cells every 6 months. After sterilization, waste will be diluted with water and disposed of as non-toxic liquid waste. All solid waste will be subjected to heat inactivation (via autoclave) to give 100% kill. Sterilization will be confirmed via autoclave indicator tape that will be processed with all waste. Sterilized waste will then be disposed of via university services.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
It is the opinion of the GMSC that, as the project will not introduce new genes into the organism, but rather mutate native genes, it is highly likely that there will be either no change or an attenuation of the virulence of the organism. Moreover, the committee felt that performing the work at containment level 2 would result in the GMMs posing low risk of infection. The committee agrees with and supports the categorisation of this project as class 2.

Project Containment

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<td></td>
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</tbody>
</table>

Project Ref 22/12.2

Date Ackn'd 07/03/2012
CU2 Project Title Nitric oxide tolerance in bacteria

Class Culture Vol Class 2
Class 2 < 1 Litre
Culture Volume Class 3-4
Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID 22/12.2a
Date of Significant Change 21/03/2014

Project Additional Information

Purposes of the contained use

The main goal is to provide a greater understanding of how Uropathogenic E. coli strains are adapted for survival during infection. In particular, the importance of mechanisms involved in nitric oxide tolerance and the export of glutathione will be investigated. The aim is to measure the contribution of specific genes to the tolerance of...
stresses encountered during infection. This work will be extended to clinical bacteraemia-causing E. coli isolates from collaborative work with Prof. F M (East Kent Hospitals University NHS Foundation)

Recipient or parental organism

Uropathogenic E. coli strains
Clinical bacteraemia-causing E. coli isolates

Host/vector system

Mutations will be introduced via transformation of linear DNA fragments. Complementation vectors are conventional expression plasmids with a safe history of use:
pWSK29 (Gene 100, 195-199)
pSU2718(Gene 68, 159-162)

Origin & function

E. coli will be constructed with deletions in genes involved in NO-detoxification, respiratory components, ABC transporters etc. The native genes will be re-introduced on a complementation vector with a safe history of use. Genetic manipulation of E. coli strains involves insertion of antibiotic resistance cassettes. However, these are standard antibiotics for lab use (e.g. kanamycin, chloramphenicol, ampicillin, gentamycin) that are not used in clinical treatments of infections caused by pathogenic E. coli.

Evaluation of foreseeable effects

The proposed modifications to the E. coli strains are predicted to make the recipient bacterium less viable.

There are naturally-occurring strains of Uropathogenic E. coli and bacteraemia-causing E. coli that possess resistance to all the antibiotics intended for use, so there is no danger of engineering E. coli strains with unprecedented combinations of antibiotic resistance. The introduced antibiotic cassettes are standard (ampicillin, kanamycin, chloramphenicol, gentamycin), and are introduced to the GMM via linear transformation and lambda-red mediated recombination, so there is a low risk of the genetic manipulation technique contributing to gene spread.

Other gastrointestinal bacteria, such as Shigella, are potential targets for gene transfer events with E. coli, although this would require an unlikely breach to take place. Furthermore, the antibiotic resistance genes intended for use are already widely disseminated, so such a breach would pose only a small contribution to the spread of antibiotic resistance throughout gastrointestinal flora. The likelihood of worker infection is very low, given that the work will be performed in a level 2 facility by trained individuals using correct technique.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMM waste will be autoclaved (plates) or treated with Bleach/Virkon (liquid cultures). Bleach.Virkon is the industry standard for killing E.coli.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The committee noted that the proposed genetic modifications would result in the mutation of native genes that are well characterised as involved in virulence and would therefore be expected to decrease virulence. One concern was that antibiotic resistance could arise. However, the antibiotics to be used are not used clinically and in any case are widely used on class 1 E. coli strains. Uropathogenic E. coli (UPEC) strains are common in the wild and likely to be encountered naturally by the general population. However, as they are capable of causing human disease it was considered that class 2 was the most appropriate designation for this work.

Project Containment

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<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 22/12.3

Date Ackn’d 16/07/2012
Date Project Ceased

CU2 Project Title Bioengineering and elucidation of modified tetrapyrroles metabolic pathways

Class 2
CultureVol 1-50 Litres
Non-GMM Consent Granted

Project notified under transitional arrangements N
### Purposes of the contained use

This project is aimed at increasing our understanding of how biochemical pathways operate, how they are controlled and how they can be engineered to enhance the metabolic ability of the host cell:

- by studying in molecular details the step-by-step synthesis of hemes along a novel pathway going via siroheme.
- by investigating unstable metabolite intermediate channelling along the complex vitamin B12 pathway.
- by transferring the genetic software that allows bacteria to make vitamin B12, into E. coli, Lactobacillus, yeast and a higher plant that are unable to make it, and therefore conferring upon these host organisms the ability to make this essential nutrient

### Recipient or parental organism

The recipient microorganisms used for these studies are mainly Escherichia coli K12 and E.coli B (BL21 (DE3) strains). It can also be Bacillus megaterium. Lactococcus lactus, Lactobacillus reteri, Salmonella enterica serovar Typhimurium, Saccharomyces cerevisiae.

### Host/vector system

All vectors used were originally commercial vectors, known to have a safe history of use and are non-mobilised. For example: pET vectors (Novagen), pKK223.3, pGEM (Promega), pACYC vectors, pMal Vectors, pGEX vectors, pRS yeast vectors, pNZ vectors (NICE).

### Origin & function

The inserted genes used from Brucella melitensis, Staphylococcus aureus and Pseudomonas aeruginosa are only orthologous genes of known vitamin B12 biosynthetic genes. The human aminolevulinic acid synthase was subcloned from a plasmid harbouring its cDNA. The source of genes used for this project can also be from Bacillus megaterium, Rhodobacter capsulatus, Rhodobacter sphaeroides, Escherichia coli K12, Desulfovibrio vulgaris, Desulfovibrio desulfuricans, Geobacillus thermoglucosidasius, Anoxybacillus flavithermus, Synechocystis sp., Paracoccus pantotrophus, Synechococcus elongatus, Thermosynechococcus elongatus, Methanosarcins barkeri, Methanobacterium thermoautotrophicum, and ortholog genes from other Archaea will also be used. Also B12 genes from Mycobacterium tuberculosis, B12 biosynthetic genes from different species are cloned in plasmids harbouring standard antibiotic resistances (ampicillin, chloramphenicol and tetracycline) and used in cross-species complementation studies.

### Evaluation of foreseeable effects

All genetic modifications are not expected to alter the pathogenicity of the host organisms. All vectors used in this study have a safe history of use and are non-mobilised.

The work involves a class 2 recipient micro-organism, Salmonella enterica serovar Typhimurium. Salmonella enterica serovar Typhimurium is a pathogenic Gram-negative bacterium predominantly found in the intestinal lumen and can cause gastroenteritis in humans. Salmonella B12 mutant strains are only used in cross-species complementation studies to restore the B12 phenotype. These GMO strains will not gain novel or harmful properties. Only B12 biosynthetic genes form different species that are cloned in plasmids harbouring standard antibiotic resistances (ampicillin, chloramphenicol and tetracycline) and used to transform Salmonella. No gene with a potentially harmful biological activity will be introduced. There is a negligible chance of transfer to other micro-organisms. GMO work will not increase the pathogenicity of this strain.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMM waste will be autoclaved (plates) in a validated facility or treated with Bleach/Virkon (liquid cultures).
The committee noted that the proposed genetic modifications would simply restore vitamin B12 synthesis in the Salmonella strain. As B12 is a significant factor in the host-pathogen relationship this will restore the pathogenicity of Salmonella B12 mutant strains and could, if higher than normal production of B12 is achieved, potentially increase virulence. However, treatment for Salmonella infections is readily available and combined with the minimal likelihood of increased virulence occurring, means that class 2 containment is suitable and sufficient for this work.

**Project Containment**

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</thead>
<tbody>
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Animal Units

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

**Project Ref** 22/13.1

<table>
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<th>CultureVolumeClass3-4</th>
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<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>19/09/2013</td>
<td>Understanding the mitochondrial functions of diverse microbial eukaryotic parasites</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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</table>

<table>
<thead>
<tr>
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<th>Date Project Ceased</th>
<th>Tick if notifying a connected programme of work</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
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</tbody>
</table>
Purposes of the contained use

The main goal is to understand and unravel patterns and evolutionary mechanisms that shaped the genomic, proteomic, cellular and biochemical adaptations of the many independent lineages of eukaryotic parasites. Specifically the protein composition of their mitochondrial-related organelles will be investigated using proteomic and transcriptomic analyses under different environmental conditions. In addition, knock-down and/or knock out parasite strains will be developed to investigate the requirement for individual mitochondrial proteins and pathways for the lifestyle of the different organisms. As part of this approach homologous genes will be transfected into knockdown strains in order to examine the conservation of protein function across parasitic eukaryotes.

Recipient or parental organism

- Acanthamoeba castellanii
- Blastocystis hominis
- Cryptosporidium hominis
- Cryptosporidium parvum
- Entamoeba histolytica
- Giardia lamblia
- Plasmodium yoelii
- Trichomonas vaginalis

Host/vector system

Cloning and evaluation of the corresponding genes will take place using the pGEM-T Escherichia coli system

Origin & function

Individual genes encoding components of different mitochondrial pathways - Fe-S cluster biosynthesis, pyruvate decarboxylation, protein translocation, folding and degradation, metabolic exchange, oxidative phosphorylation, mitochondrial morphology and apoptosis - from all the eukaryotic parasites listed above will be incorporated into plasmids for developing knock-down strains and/or consequently for heterologous expression of the corresponding gene. For complement experiments the T7/TetR system will be employed using p2T7-177 vector for knocking down corresponding gene and pABPURO, PCF4 (for Acanthamoeba, Blastocystis, Cryptosporidium), tag Vag 2 Trichomonas vaginalis), pEi-ck-Myc (for Entamoeba), pL0016 (for Plasmodium) and pRANneo or 5'Δ5N-Pac (for Giardia) vectors for the expression of corresponding proteins.

Evaluation of foreseeable effects

The GMOs constructed during this work are either knock downs of mitochondrial genes or knock downs complemented by homologous genes from the host itself or other species or microbial eukaryotic parasite. The knock downs will be attenuated in comparison to the native organism and this is also likely to be true for the complementation strains. As the genes targetted for knock down and replacement are house-keeping genes, even in the most effective cases the complementation strains are only likely to be as infectious as the original native organism. The most construction of the GMO strains will not add any measure of virulence or pathogenicity and no foreseeable adverse effects are envisioned.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This project will generate liquid waste from culture media and solid (typically plasticware) waste. Liquid waste will be sterilized via treatment with bleach Virkon and subsequently autoclaved. All solid waste will be sterilised by autoclaving. Spills or potentially contaminated work surfaces will be disinfected as outlined below.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Treatment Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthamoeba castellanii</td>
<td>10% Hydrogen peroxide</td>
</tr>
<tr>
<td>Blastocystis hominis</td>
<td>10% Bleach solition, 70% Ethanol</td>
</tr>
<tr>
<td>Cryptosporidium hominis</td>
<td>6% Hydrogen peroxide, 13 min exposure</td>
</tr>
<tr>
<td>Cryptosporidium muris</td>
<td>6% Hydrogen peroxide, 13 min exposure</td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>6% Hydrogen peroxide, 13 min exposure</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>Chlorine</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>Chlorine 8mg, 10 min exposure</td>
</tr>
<tr>
<td>Plasmodium yoelii</td>
<td>10% Bleach, 10% Hydrogen peroxide, or 70% Ethanol</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>10% Bleach solution, 70% Ethanol</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

It is the opinion of the GMSC that, as the project will not introduce new genes into the organism, but simply mutate native genes and replace them with homologous genes from other eukaryotic parasites, it is highly likely that there will be an attenuation of the virulence of the organisms in question and at the most native virulence will be regained.

Moreover, the committee felt that strict adherence to standards of laboratory practice applicable to work at containment level 2 would result in the GMM's posing a low risk of infection. The committee agrees with and supports the categorisation of this project as class 2

Project Containment

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**Project Additional Information**

**Purposes of the contained use**

The overall goal is to understand the mechanisms of chromatin regulation in C. albicans. Specific goals are to characterise:

a) The pathways responsible for centromere establishment and maintenance.
b) Regulation of gene expression by the RNAi machinery.
c) The Biology of DNA repeats

**Recipient or parental organism**

C. albicans strains: CA14, ura3: CA18, ura3, ade2: RM1000, his1, ura3; BWP17, his1, ura3, arg4 and derivatives of these strains carrying further disabling markers

**Host/vector system**

Vector system:
1. Non-mobilisable E. coli vectors (including pUC18/19, pBluescript, pGEM-T; lac-based expression plasmids such as pET vectors).
2. Specific non-mobilisable C. albicans vectors (low copy replicating and integrating vectors [YPB1, Clp10, Clp20, Clp30]: low copy replicating and integrating vectors for ectopic expression [YPB-ADHpt, pACT1]; integrating expression vectors regulated by methionine [MET3 promoter], maltose [MAL2 promoter], glucose [PCK1 promoter], or doxycycline [tet ON promoter and tet OFF promote].

These vectors will be prepared from E. coli cultures. We will always use non-pathogenic E. coli strains such as the multiple disabled K12 derivatives, and the rec deficient BLR strain. The E. coli strain BLR is a disabled (recombination deficient) derivative of the strain BL21

**Origin & function**

We are planning to generate null mutants in genes involved in:
1 Centromere assembly and propagation. Null mutants will be expected to compromise centromere function resulting in lethality.
2. RNAi Null mutant will be expected to impair siRNA production of specific chromosomal loci.
3. Histone modifications. Null Mutants will be expected to alter the chromatin structures of specific chromosomal loci. In some experiments, we will express specific genes in C. albicans ectopically using a MET3, MAL or tet promoter

**Evaluation of foreseeable effects**

Transformations will be performed in multiple disabled C. albicans strains (ura3, ade2, arg4, or his1) to ensure that all transformants are avirulent. In none of the cases this is likely to increase virulence; instead transformants will always carry a disabling mutation, thereby rendering them completely avirulent. No null mutations have been described that increase the virulence of C. albicans.

In some experiments, the CA14 strain will be transformed with URA3 plasmids. Insertion of the URA3 marker gene will partially restore the virulence of CA14, but this restoration is not complete because the genes neighbouring URA3 remain inactivated. This is enough to attenuate virulence partially.

In some experiments, we will express specific genes in C. albicans ectopically using a MET3, MAL or tet promoter. In these experiments, the expression level is not maximised. Their ectopic expression levels will be <1% of total cell protein. The ectopic expression of most specific genes is unlikely to affect virulence at all, and in some cases it will be expected to reduce C. albicans virulence by adversely affecting growth. In experiments where we over-express genes, expression levels will be carefully regulated by addition of doxycycline; without doxycycline protein levels will be at or below wild type levels. The key point is that the probability of increasing virulence by ectopic expression of a single gene is very low, because pathogenicity is complex and polygenic trait requiring a high level of fitness of the C. albicans cell (Odds [1994] ASM News, 60, 313). Indeed there is evidence that ectopic expression of factors involved in virulence traits does not increase virulence (Bailey [1997] PhD thesis, University of Aberdeen; Leng [1999] PhD thesis, University of Aberdeen; Rodaki et al. [2006]).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All yeasts will be killed by either heat treatment or addition of appropriate levels of bleach (10% for 30 min) in accordance with standard lab procedures post experimentation

Is an emergency plan required according to regulation 20?  

<table>
<thead>
<tr>
<th>Y</th>
<th>N</th>
</tr>
</thead>
</table>

If yes, tick to confirm that it is attached to this form

| N |

Tick to confirm that you have attached a risk assessment to this form

| Y |

Tick if you are claiming exemption from disclosure for section of the risk assessment

| N |

The applicant proposes that the work be subject to class 2 restrictions. The mutations that the applicant proposes to engineer can in no way be expected to increase either the pathogenicity or augment any potential environmental effect.

The committee is therefore satisfied that class 2 is an appropriate level of containment using the measures proposed.

02/03/2022
The research described in this proposal is designed to elucidate the molecular mechanisms of species-specific Ebola virus replication, assembly and budding. At present, the published VLP system expresses proteins from the Zaire species of Ebola virus. To better elucidate the functions of each Ebola virus protein, we will engineer the VLP system to express the proteins from other (lower pathogenicity) virus species (Reston, Sudan, Bundibugyo and TaJ Forest). This will create a variant VLP system reflecting the other Ebola virus species, whilst still retaining the non-infectious nature of the published system. With these systems in place, the activity of the different species proteins can be compared during VLP assembly and budding. Outside of changing the Ebola virus species, no change in the system...
Recipient or parental organism

Plasmids transfected into mammalian tissue culture cell lines

Host/vector system

Mammalian tissue culture cell lines (human, mouse and non-human primate) and E. coli for plasmid growth.

Origin & function

Individual VLP plasmids expressing the Zaire VLP system genes as published will be obtained from Heinz Feldmann at the US National Institutes of Health. Using standard restriction-digest cloning, the Zaire genes in the system will be exchanged for the genes for other Ebola virus species and will then be amplified in E. coli for ultimate expression in mammalian tissue culture cells. Genes to be exchanged include: single protein expression plasmids for the viral proteins NP, L, VP30 and VP35; and a mini-genome expression plasmid containing the genes GP, VP40 and VP24. This exchange will create non-infectious VLP systems for comparative analysis of the assembly and budding of the other Ebola virus species. At no point will the full infectious genome be present for any Ebola virus species and new individual genes will be provided as gene-expression plasmids.

Evaluation of foreseeable effects

The construction of the GMOs (non-infectious plasmid transfected mammalian tissue culture cells) should inform as to the species-specific functions of Ebola virus proteins during viral assembly and budding. The construction of the tissue culture and vector GMOs will not have any adverse effects and has no foreseeable adverse effects. The VLP system has been previously published and widely used worldwide at CL2 containment level. The system is noninfectious and is not capable of producing infectious, replication-competent viruses. The VLP system expresses the viral NP, L, VP30 and VP35 proteins from individual DNA expression plasmids in transfected cells only. When combined with transfection of the negative-sense RNA producing mini-genome expression plasmid, virus-like particles are produced. These VLPs only contain the mini-genome (expressing the genes for GP, VP40 and VP24), thus without the individual DNA expression plasmids, the VLPs are non-infectious, non-replicating and non-hazardous. Thus, the system remains biologically-contained and there is no possibility of producing any infectious replication-competent viruses or particles and as a result there are not foreseeable adverse effects. In addition, because the NP, L, VP30 and VP35 genes are present only as individual DNA gene expression plasmids, the full Ebolavirus genome will never be present, as only the mini-genome is capable of producing the necessary negative sense RNA and this is only for the genes GP, VP40 and VP24, which are not sufficient to produce an infectious virus particle. For added precaution, all work will be performed in a certified category II biological safety tissue culture hood with a users properly trained and wearing laboratory coats and gloves. No sharps will be used during these protocols.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

No infectious replication-competent organisms will be generated or used in this project. This project will generate
liquid waste from tissue culture media and solid (typically plasticware) waste. Liquid waste will be sterilized via treatment with 30% NaClO for 30min (this has been proven to give 100% kill of enveloped viruses such as influenza virus). After sterilization, waste will be diluted with water and disposed of as non-toxic liquid waste. All solid waste will be subjected to heat inactivation (via autoclave) to give 100% kill. Autoclave tape will be utilized on all autoclave sterilization procedures, however, the sterilization will be validated once a month while GMO contained use procedures are being performed. Validation will be though the use of 3M Comply™ (Thermalog™) Steam Chemical Integrators, used as per the manufacturer's directions. Sterilized waste will then be disposed of via University services.

This project utilises a transcription and replication competent virus-like particle (trVLP) system that essentially splits the Ebola viral genome into two: the 4 genes required for viral genome replication and transcription are supplied by a corresponding number of DNA expression plasmids, the 3 structural genes (together with a reporter gene) are provided by a monocistronic negative sense RNA minigenome. When all components are co-expressed, virus-like particles containing the minigenome are produced. These trVLPs have the ability to enter all cells but minigenome replication and transcription and formation of a new generation of trVLPs can only occur in specific target cells that have been pre-transfected with the 4 replication / transcription gene expression plasmids. For all intents and purposes the trVLPs can be considered non-infectious to anything other than these target cells.

In addition, the provision of the two components of this system in different nucleic acids systems, together with the fact that the expression plasmids used do not share any homologous sequence with the minigenome means that there is no potential for recombination to occur. Supporting this, the authors of the paper describing this system state that no recombination events has ever been observed in filoviruses.

Finally, it is important to note that all other viral genes that will be used in this study are being provided as individual clones and no complete viral genomes will be present on site at any time. For these reasons the committee supports the designation of this project as containment level 2.
Project Ref 22/18.1

Date Ackn’d 27/12/2018

CU2 Project Title Expression of proteins in human cancer and other cell lines using Adenoviral vectors

Class Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Withdrawn

Tick if notifying a connected programme of work

Project Additional Information

Purposes of the contained use

The aim of this project is to express proteins in human cancer and other standard laboratory cell lines using adenoviral vectors. This includes, but it not limited to, proteins involved in the regulation of growth factor signalling such as proteins of the MAPK (Mitogen Activated Protein Kinase) pathway. Human cancer cell lines that have been genetically modified (GM) with Adenoviral viral vectors will be assayed to identify changes in several cancer related phenotypes such as the response to anti-cancer drugs.

Recipient or parental organism

Human cancer cells lines such as the ovarian cell lines A2780, PEO1, PEO4, EFO-27 and OVCAR3, breast cancer cell lines HCC38, MDA-MB468 and T47D, and colon cancer cell lines HT29, SW620, HCT116. In addition standard laboratory culture cell lines such as CHO may be used.

Host/vector system

Human Adenovirus serotype 5. The Adenoviral viral vectors in this project have the E1 and E3 regions of their genome deleted rendering them replication deficient, minimizing the hazards posed to human health and the environment.

Origin & function

Human genes encoding proteins involved in the regulation of growth factor signalling such as proteins of the MAPK (Mitogen Activated Protein Kinase) pathway. Most of the proteins expressed within this project will also be tagged to validate their expression (e.g. Myc-tag) or visualise their localisation within the cell (e.g. Green Fluorescent Protein).
Human Adenovirus serotype 5 is not a recognised animal pathogen. The inserted genetic material will be of human origin and is therefore unlikely to be able to impact the fitness/infectivity of the Adenovirus or cause alterations in its existing pathogenic traits. Horizontal gene transfer is not known to take place between Adenoviruses, other members of the Adenoviridae family or any other related micro-organisms. In addition, any transfer of genetic material would require the infection and subsequent viral replication inside host cells. The Adenoviral viral vectors being used are missing the E1 region of their genome rendering them replication deficient.

It is possible that loss of containment could result in exposure to human populations. However, any work that carries the risk of generating aerosols will be undertaken in a biological safety cabinet. In addition, wild-type Adenovirus causes self-limiting infections of the upper respiratory tract and the common cold, which usually do not require any treatment. GM Adenovirus may mimic some the characteristics of wild-type Adenovirus but is far less pathogenic due to the E1 and E3 deletions which render the virus replication deficient. Upon culture in a helper cell line, such as HEK293 cells, a replication competent virus has the potential to be produced. However, this is very unlikely as the E1 sequences in HEK293 cells do not overlap with the deleted E1 region. Therefore, two non-homologous recombination events would have to occur to produce a replication competent Adenovirus. Any replication competent virus generated would still be lacking the E3 gene (HEK293 cells do not contain E3) and would therefore have reduced fitness as an infective agent (E3 counteracts host defence mechanisms). In addition, human adenoviral infection is very common; most adults have been infected and have immunity. Taken together the risk from infection by an Adenoviral viral vector is low.

Some GM Adenoviruses generated as part of this project could contain inserted genes that are oncogenes, and therefore could pose a hazard to human health. The risk is mitigated by using a replication deficient Adenovirus (as discussed previously), and that during the Adenoviral life cycle its genome does not insert into the host genome. Expression of an inserted oncogene would therefore be transient and pose little hazard to human health.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated liquid waste will be treated with a 1% w/v Virkon® S solution for ≥5 minutes. McCormick and Maheshwari (Inactivation of adenovirus types 5 and 6 by Virkon S. Antiviral Res. 64(1):27-33. 2004) showed that a 5 min exposure to ≥0.9% w/v Virkon® S is sufficient to inactivate the crude virus (in the supernatant/cell lysate of HEK293 cells) of Adenovirus type 5. This comparable to the conditions that will be present in our experiments.

Treated waste will subsequently be autoclaved in-house at 126°C for 10 mins, 2.5 bar pressure, prior to disposal down the sink.

Contaminated solid waste, such as tissue culture plastics, is to be double-bagged and autoclaved in-house at 126°C for 10 mins, 2.5 bar pressure, prior to disposal by the waste contractor Viridor. Any work using sharps will be disposed of in the same manner.

Autoclaves are serviced every quarter and undergo an annual thermocouple test by BMM Western Ltd, Kent, UK (http://bmmweston.com/infection-control/).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
This risk assessment clearly outlines the low risk of hazards to human health and the environment. Due consideration has been given to the contingencies that are built in that preclude serious disease or persistence in the environment in the unlikely event of a breach. The viral pathogen has a safe history of use and the suggested laboratory containment level (2) is appropriate. Sufficient control measures are in place for nature of work to be undertaken and the outlined procedures for waste management are appropriate for such a project.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 L3 L4 L2 L3 L4 L3</td>
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</table>

- **Project Ref**: 22/19.1

**Date Ackn'd**: 13/12/2019

**CU2 Project Title**: Synthetic biology approaches to compartmentalisation in bacteria and the construction of novel bioreactors

**Class**: Class 2

**Culture Vol**: < 1 Litre

**Non-GMM**: Consent Granted

- **Project notified under transitional arrangements**: N

- **Historical Significant Changes**: Withdrawn

- **Historical Date of Additional Info**: Tick if notifying a connected programme of work

**Project Additional Information**
Purposes of the contained use

This project investigates how proteins are targeted and incorporated into bacterial microcompartments. The aim is to direct specific enzymes to the bacterial microcompartments in order to make bespoke bioreactors. The development of these bioreactors in compartments within the cell would allow for greater control and regulation of the process, detaching the encased process from any negative influences within cellular metabolism.

Recipient or parental organism

Pseudomonas aeruginosa and Listeria monocytogenes

Host/vector system

All vectors used were originally commercial vectors, known to have a safe history of use and are non-mobilised. For example: pET vectors (Novagen), pGEM (Promega), pACYC vectors

Origin & function

Pseudomonas aeruginosa will be transformed with plasmids harbouring only Pseudomonas genes in order to overexpress proteins. The genes of interest, NCBI-GeneID: 878574 and 881385, are predicted to be part of the LemA family of membrane restructuring proteins that when overexpressed in E.coli produce intracellular membranous structures with bioengineering applications. These experiments are aimed at recreating these structures in Pseudomonas.

Listeria monocytogenes strains in which genes had been deleted from the ethanolamine (eut) operon (eutB and eutM) will be transformed with stable chromosomal integrative plasmids harbouring the original genes in order to restore the original function (vitamin B12 dependent).

Evaluation of foreseeable effects

This GMO work will not increase the pathogenicity of recipient organismsthis strain. Pseudomonas aeruginosa is known to cause infections in both humans and animals. However, severe infections in humans usually occur in a nosocomial setting in immunocompromised patients. In animals, Pseudomonas can cause otitis and urinary tract infections in dogs, mastitis in dairy cows and endometritis in horses. Overexpression of the native LemA protein family genes in this organism is not expected to affect the pathogenicity or fitness of Pseudomonas in the host or the wider environment.

Listeria monocytogenes is a causative agent of listeriosis in animals and humans. The deletion of eut genes from Listeria monocytogenes are not expected to confer any advantageous effect on its fitness neither in the host nor the wider environment. In fact, by deleting genes that belong to a pathway that is suspected to help the bacteria grow in specific conditions, the modifications are expected to make these Listeria strains less likely to cause infection and compete in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
GMM solid waste (plates) will either be autoclaved and disposed in nonmal waste or will be handed to a registered waste disposal company for incineration.
GMM liquid waste will be treated with BleachNirkon. Virkon will be used at 1% solution as recommended by manufacturer. After an overnight treatment in 1% virkon, the liquid waste will be disposed of via the laboratory sink. Virkon is commonly used as a laboratory disinfectant to kill a wide range of micro-organisms from E. coli to MRSA.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This seems a pretty straightforward Class II project given that they are only planning to express native genes in Listeria and Pseudomonas with no change in potential risk. Moreover the antibiotics utilised for selection are commonly used laboratory antibiotics not used in the treatment of infections by these organisms, there is no known mechanisms of sequence transfer within these orgnaism or between them and other species, and the work in Listeria uses vectors that result in highly stable genome integrations.
Overall we support the classification of this project as Class II.

Project Containment

<table>
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<td>L3</td>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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GM Centre Number: 25

Data Premises Notified (Originally) 06/11/1978

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed N

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

CR UK THE BEATSON INSTITUTE FOR CANCER RESEARCH

Name 2

CANCER RESEARCH UK

Department

Campus Estate or Research Centre

GARSCUBE ESTATE

Building

Road Name

SWITCHBACK RD

District

BEARSDEN

Town

GLASGOW

County

EAST RENFREWSHIRE

Postcode

G61 1BD

Country

SCOTLAND

Tel Number

0141 330 3953

Fax Number

0141 942 6521

E-mail

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

<table>
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<tr>
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<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>County</th>
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<td>CRC BEATSON LABORATORIES</td>
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<td>SWITCHBACK ROAD</td>
<td>BEARSDEN</td>
<td>GLASGOW</td>
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<td>SCOTLAND</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

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<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment.

Tick if you are claiming exemption from disclosure for sections of the risk assessment.

Please enter comments of the GM safety committee on the risk assessment.

---

**Project Ref** 125/03.2

**Date Ackn'd** 03/08/2007

**CU2 Project Title** CYTOSKELETAL GENE ANALYSIS IN ENTAMOEBA AND DICTYOSTELIUM

**Class** Class 2

**CultureVolClass2** 1-50 Litres

**Non-GMM Consent Granted** Not Applicable

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** Y

**Historical Significant Changes** TRANSFERRED FROM GM CENTRE 116 - 3/8/06.

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**
## Purposes of the contained use

To express cDNAs encoding Entamoeba cytoskeletal and signalling proteins in both Entamoeba and Dictyostelium cells.

## Recipient or parental organism

<table>
<thead>
<tr>
<th>Hosts</th>
<th>E. coli XL1-blue - disabled and slow growing;</th>
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<tbody>
<tr>
<td></td>
<td>DH5 - as XL-blue.</td>
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</tbody>
</table>

## Host/vector system

| Vector | pUC18 - standard molecular biological plasmid vector. |

## Origin & function

### Origin of genetic material

- **Dictyostelium strain AX3** - a free living non-pathogenic soil amoeba which grows at 22 degrees centigrade and is harmless to crops and agriculture.

- **Antamoeba histolytica strain HM-1:IMSS** -- standard laboratory strain. Cysts are the only infectious stage of this parasite and these cannot develop or form in standard lab cultures, hence the parasite is not transmissible to humans in this setting. The cultured form, ie the trophozoite, is very fragile and susceptible to desiccation and detergents. As they are unable to persist in the environment the chance of environmental damage is remote.

### Genetic material

- Full length cDNAs encoding Entamoeba Arp2 and Arp3 - major skeletal proteins involved in movement and cell shape. Selectable markers conferring G418 resistance.

## Evaluation of foreseeable effects

### Effects on human health:

- **Dictyostelium** - None. It is a non-pathogenic soil amoeba, unable to grow at human body temperature and is harmless to crops and agriculture.

- **Antamoeba histolytica** - No likely risks. It is a parasitic enteric protozoan that is responsible for amoebiasis in humans. The disease is characterised by diarrhoea/dystentery, intestinal tissue invasion and potential liver access formation. Epidemiology, the organism is ubiquitous and has a worldwide distribution, although prevalence is much higher in developing, disadvantaged countries and tropical regions. In contrast, rates of infection are low in the developed world, with the majority of these infections being found in specific groups (eg travellers from developing or high incidence areas). Symptomatic invasive amoebiasis only develops in 10% of individuals harbouring the parasite. The organism can exist as both cysts and trophozoites. Transmission is feco-oral and results from ingestion of viable cysts in contaminated food or water. Cysts can remain viable for a long time depending on environmental conditions but are extremely sensitive to desiccation. Trophozoites on the other hand are more fragile and degenerate rapidly in the external environment as well as being easily destroyed by acidic pH (eg stomach acid).

As the only infectious stage of the parasite, the cyst, does not form in standard laboratory cultures the parasite is not transmissible to humans in this setting. Accidental infections have never been reported, but effective drug treatments are available should this happen.

### Environmental hazards:

- **Dictyostelium** - negligible hazard. The strains used are very weak due to multiple mutations and would not compete with local Dictyostelium species.

- **Entamoeba** - The organisms are very sensitive to desiccation and detergents and are not free living. As they are unable to persist in the environment the chances of environmental damage are remote.

The work will be carried out at CL2 and the likelihood of accidental release is very low.
E. coli XL-blue/DH5 - non-colonising, disabled lab strains, no risk to human health or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation applied for CL2 applied. The laboratory set aside for this work is a relatively new facility and is currently being fitted with a hand wash basin.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Dictyostelium - to kill spent cultures: 1% chlorine bleach.
Entamoeba - Tubes containing liquid cultures and contaminated disposables will be placed in autoclave bags and autoclaved at 121 degrees for 50 minutes and discarded as "clinical waste" (incineration).
Agar plates, loops, tips etc will all be autoclaved. 100% kill.

All methods are more than sufficient to kill Dictyostelium which are fragile, as well as Entamoeba which are sensitive to desiccation and detergents and are not free-living.

Bacterial cultures will be killed with Virkon at the recommended dilution (1:100)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
I feel information on human health should be transferred to the relevant section; the nature of the "normal laboratory clothing and protective equipment" should be defined; the autoclave used for disposal of cat 2 waste should be monitored (it is anyway, why not say so for completion?); and the disinfection regimen should be defined, i.e., is 1% chlorine bleach 1% available chlorine, and what is the actual dilution of Virkon used?

**POINTS ADDRESSED IN ASSESSMENT**
- There is no mention of any possible alteration in phenotype resulting from the introduction of cytoskeleton and signalling proteins in Entamoeba and Dictyostelium. I assume they will not become (more) pathogenic but perhaps this could be addressed.

- No increase in virulence/pathogenic capacity is expected. As mentioned in the application, Dictyostelium are free-living non-pathogenic soil amoebae which grow at 22 degrees and therefore do not pose a threat to humans. Likewise Entamoeba does not pose a risk to humans in this/our setting because the cyst, which is the only infectious stage of the parasite, can/do not develop or form in standard laboratory cultures, hence the parasite is not transmissible to humans.

The GMSC also asked whether the Class 1 and Class 2 work should be split into two separate proposals. The investigator felt this was not necessary and not desirable because the work is one experiment, in which cDNAs encoding Entamoeba cytoskeletal and signalling proteins will be expressed in both Entamoeba and Dictyostelium cells.

The GMSC has approved this assessment.

### Project Containment

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### Project Ref 25/01.1

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<td>TRANSDUCTION OF HUMAN FIBROBLASTS AND KERATINOCYTES WITH AMPHOTROPIC RETROVIRUSES ENCODING ONCOGENES INVOLVED IN GROWTH FACTOR SIGNALLING</td>
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<td>&lt; 1 litre</td>
<td></td>
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Withdrawn N  
Tick if notifying a connected programme of work N
The aim of this project is to develop human cell lines expressing oncogenic components of the growth factor-Ras signal transduction pathway. The modified cells will be used to investigate the changes in gene expression which underlie growth factor regulation of invasion and cell cycle control. The long term aim is to use this system to identify new targets for the diagnosis and treatment of cancer.

The cells to be used will be immortalised human foreskin fibroblasts (iHFFs) and human epidermal keratinocytes (iHEKs). DNA transfection is inefficient in these cells, therefore replication defective murine retroviruses will be used for gene transfer. Human cells in culture are not considered to pose any inherent hazard to human health, nor do they have any capacity to survive autonomously outside of the laboratory. It is not anticipated that the proposed modification will alter these properties. The final GMO cell lines will therefore not exert any foreseeable effects on either human health or the environment.

Replication defective murine retroviruses will be generated and used to transfer oncogenes into the iHFFs and iHEKs cell lines. Such viruses have the potential to infect but not to replicate in human cells. It is unlikely that replication defective retroviruses encoding single oncogenes would be effective carcinogens in humans in the short term. However, retroviral infection and oncogene expression in human stem cells could reduce the number of additional genetic changes required for malignant conversion and thus could conceivably increase the probability of cancer occurring in an infected individual in the long term. ACGM level 2 containment is therefore indicated to prevent exposure to infectious amphotropic virus. This, combined with rigorous waste inactivation procedure and the inherent fragility of retroviruses mean that the risk to the environment is negligible.

The genetic material to be introduced into the iHFF and iHEK cell lines consists of genes which are frequently mutated in human cancers and which are known to comprise components of the growth factor-Ras signal transduction pathway. It is anticipated that studies of the modified cell lines will lead to a better understanding of how these oncogenes cause cancer.

The final GMO human cell lines will contain stably integrated DNA copies of the retroviral vectors encoding the transferred genes but will be free of infectious retrovirus. As a result, they will pose no hazard to human health or to the environment, since they are unable to colonise humans or survive outside of laboratory culture. It will however be important to prevent human exposure or environmental release of the replication defective retroviruses which are used transiently to effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation procedures.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk to human health it will be essential to prevent exposure to infectious amphotropic virus. To this end the retroviral regeneration and target cell infection manipulations will carried out in Class II biological safety cabinets in the Class 2 containment laboratory. All viral supernatants and spent medium inactivated using precept tablets before removal from the Class 2 laboratory for autoclaving and final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class 2 laboratory and autoclaved prior to final disposal by incineration. These procedures will result in 100% inactivation of infectious retrovirus.

Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by both infection of appropriate indicator cells and assays of reverse transcriptase activity. Once these criteria have been met the infected target cells will be removed from Class 2 containment and handled using standard tissue culture procedures. All cell cultures and spent medium will be autoclaved prior to disposal by incineration. This will achieve 100% killing of GMO cell lines finally used in experimental procedures.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The Risk Assessment pertaining to this notification was discussed in detail at a meeting of the CRC Beatson Laboratories Safety Committee held on Mon 11 December and approved with minor modification. The Safety Committee requested to be informed when the final GMO cell lines were shown to be free of replicating retrovirus and released from Class 2 containment.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
INTRODUCTION OF Dyrk Family Genes INTO PC12 AND OTHER NEURONAL DERIVED CELL LINES USING AN ADENOVIRUS TYPE 5 DERIVED SHUTTLE VECTOR

One of the fundamental goals of developmental biology is to understand how neuronal stem cell populations are instructed to proliferate in order to produce the correct number and kind of neuronal progeny, and subsequently directed to withdraw from the cell cycle, terminally differentiate and selectively express neuronal cell-specific genes. Rat pheochromocytoma (PC12) cells provide one of the few available tissue culture-based model systems with which to study neuronal growth, differentiation and survival. In PC12 cells, treatment with epidermal growth factor (EGF) induces a mitogenic response while treatment with nerve growth factor (NGF) results in cessation of cell proliferation and terminal differentiation. Therefore, like neuronal stem cells, PC12 cells can exit the cell cycle and terminally differentiate or continue to divide in an undifferentiated state.

We have identified Dyrk2 as a gene which is expressed in a differentiation specific manner in PC12 cells and wish to understand its role. Analysis of Dyrk-family members has the potential to address such issues as developmental neurogenesis, neuronal-based cancers, and neuronal regeneration.

Recipient or parental organism

The cell lines used to propagate the adenoviral GMM (HEK293 or GH329) and the cells to be infected with the GMM (rat pheochromocytoma [PC12] cells and oligodendrocyte-type2 astrocyte progenitor [0-2A] cells) do not pose any inherent risk to human health and cannot survive outside laboratory tissue culture conditions. As DNA transfection is inefficient in these neuronal cells, Dyrk-related genes will be introduced into PC12 and other neuronal derived cell lines using the Adenovator vector system. The system is based on the wild type Ad5 virus which is a normal human pathogen. Approximately 80% of the population express antibodies to this virus indicating prior exposure. Ad5 has been extensively studied in numerous laboratories for well over 30 years with no indication of serious harm to humans. AdenoVator was constructed by deleting sequences from the viral genome making it replication-deficient, preventing the GMM from propagating itself.

Host/vector system

The AdenoVator vectors have E1 deleted (Ad5 nucleotides 1-3533) to remove potential oncogenic sequences and to block viral gene expression, and the E3 region deleted...
(Ad5 nucleotides 28130-30820), which encodes proteins that modulate the host's immune response and are not essential for growth in tissue culture. Such vectors must be grown in E1 expressing cell-lines, such as HEK293 cells or GH329, and consequently the spread of virus outside these cell-lines is prevented because the viruses produced are non-replicative. Deletion of E3 is expected to result in greater presentation of viral antigens to the immune system than in a normal adenoviral infection, resulting in a greater inflammatory response (which might, however, accelerate the clearance of the infected cells). Virus stocks will be stored in aliquots at -20°C, with secondary containment and clearly labelled.

**Origin & function**

Apart from the Dyrk-related genes, no new sequences are present for transfer to related micro-organisms. Dyrk-related sequences have not been reported in any micro-organism and should these sequences be incorporated they are highly unlikely to confer any advantage to the organism or to pose a threat to human health. In vertebrates, Dyrk genes encode a serine/threonine-tyrosine dual-specificity kinase and are associated with brain development. In yeast, the Dyrk homolog has been characterised as an antagonist of the RAS/protein kinase-A pathway and a negative regulator of growth. There is no indication that any Dyrk family member is either oncogenic or toxic.

**Evaluation of foreseeable effects**

Human exposure to either the GMM replication-deficient vector virus or the Dyrk-containing version of the virus will be prevented by appropriate physical containment rigorous inactivation procedures. Furthermore it is highly unlikely that a human would encounter levels of these adenoviruses to trigger a harmful immune response. In addition, the replication deficiencies introduced into the virus make it highly unlikely that exposure would result in a productive infection. Addition of the Dyrk gene should not increase the threat of harm to humans.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

none

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All stocks of virus, whether during production or infection of cell lines, will be handled safely in Class II biological safety cabinets, avoiding the production of aerosols and the use of sharps. Recombinant plasmids containing Dyrk family genes and derived from pAdenoVatorCMV5-IRE5-GFP/pAdEasy-1 will be transferred into HEK293 or GH329 cells by lipofection. Transfection and viral production will be monitored by GFP expression. Viral supernatant will be recovered by freeze-thawing HEK293 or GH329 cells. (Most virus remains cell-associated, only being released into the culture medium if the cell is disrupted.) These supernatants will be amplified by further rounds of infection of HEK293 or GH329 cells. Supernatants will be applied to neuronal cell lines PC12 or 0-2A. Before removal from the Class II facilities, all infectious cells or viral supernatents will be inactivated with precept tablets prior to being double bagged, autoclaved and incinerated. Solid waste materials will also be double bagged autoclaved and incinerated. Hence 100% killing of GM virus and cell lines will be achieved.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
This activity notification's risk assessment was discussed in detail by the CRC Beatson Laboratories' Safety Committee on Monday 11 December. The proposal was approved with minor modifications to the section (i)/b on "Hazards arising directly from the inserted gene product".

### Project Containment

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### Project Additional Information

**Purposes of the contained use**

The aim of this project is to generate replication-defective adenoviruses that express the cell death inducing molecules p53, E2F and Ask1 including mutations, truncations and fusion protein thereof. This will enable the expression of these in whole cell populations and tumours which will aid investigations in to the way in which these proteins cause tumour cell death. The ultimate goal of the project is to identify factors regulating cell death that may well prove to be novel targets for therapeutic intervention.
The cells to be used will be a range of human and mouse cultures including established and primary cells. In addition, experimental tumours generated in nude mice will also be used. The analysis of cell death in whole cell populations is important, but doing this using standard transfection methods is impossible due to the death of cells before a uniform population is established. As a result, an adenoviral expression system will be employed enabling widespread expression both in vitro and in vivo. The target cells in culture or mice are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory. The adenoviral infection is only transient and the target cells or mice will not be able to produce infective virus. The cells or mice will therefore not exert any foreseeable effects on either human health or the environment.

Host/vector system

The system to be used to transfer genes known to cause cell death involves disabled replication-defective adenoviruses. These viruses only have the potential to infect human cells when they have been packaged in appropriately engineered packaging cells. Once packaged these infective viruses are extremely labile and do not have the capability to replicate or produce infective virus in other non-engineered cells or mice. In addition to lacking components required for replication, the adenoviruses also lack factors found in wild-type adenoviruses which are involved in evading an immune response. As a result of these safeguards, these viruses cannot exist outside of the laboratory and are incapable of widespread infection. As a result they do not pose a risk to human health or the environment. A risk of minor localised infection does exist when handling the packaged infectious virus, but this is prevented by using the appropriate level of containment and inactivation procedures - hazard containment 2 is therefore recommended and all work will be carried out in Class II biological safety cabinets.

Origin & function

The genetic material to be introduced via the adenoviral system consists of genes (p53, E2F and Ask1) which are known to cause programmed cell death. The activity of these genes is often perturbed during the formation of many cancers causing the tumours to become resistant to the effects of many chemotherapeutic drugs. It is anticipated that by studying the way these factors induce programmed cell death will lead to a better understanding of chemosensitivity and hopefully lead to the identification of novel targets of therapeutic intervention.

Evaluation of foreseeable effects

Disabled replication-defective adenoviruses are labile at 37. In addition, the final GMO cell lines or mice do not contain the necessary components to compliment the disabled virus in order to make an infectious viral particle. Therefore, the final GMO cells or mice will be free of infectious adenovirus. In addition, since the cells or mice are unable to survive outside of the laboratory, it is not foreseeable they will pose any hazard to human health or to the environment. It will, however, be important to prevent human exposure or environmental release when dealing with the infective viral cultures when being transferred from the viral packaging cells to the final target cells or mice. Although unlikely, it is possible that these agents at this stage may cause localised cell killing upon exposure to an infectable organism. The risk though will be nullified by the use of the appropriate level of physical containment and inactivation procedures. Hazard containment 2 is therefore recommended and all work will be carried out in Class II biological safety cabinets.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk to human health it will be essential to prevent exposure to infectious adenoviral particles. All work will therefore be carried out under Class 2 containment conditions. Following procedures, all viral supernatants and spent medium will be inactivated using precept tablets before removal from the Class II laboratory and autoclaved prior to final disposal by incineration. Solid waste will be double-bagged in biohazard bags prior to removal and autoclaved prior to final disposal by incineration. These procedures will result in 100% inactivation of infectious adenovirus. Target cells will be infected with adenovirus by incubation with medium containing the virus. After three hours this medium will be removed and replaced by non-infective
medium. Being as the replication-defective virus is labile at 37, it is considered that after 24 hour, the culture dish containing the target cells will now be non-infectious. Even though, all cells and medium from these manipulations will be treated with precept tablets before removal from the Class II laboratory and autoclaved prior to final disposal by incineration. Should it be required that target cells are removed from the Class II laboratory then their infectivity will be tested by testing cell supernatant from these for its potential to infect new target cells. Only when the cells have been shown to be non-infective will they be removed from the Class II containment laboratory. Gene transfer using the adenoviral vectors in mice will be by intra-tumoural injection. Procedures will be carried out in a Class II biological safety cabinet in a designated containment laboratory. Animals will be maintained in this laboratory, separate from other animals and any potentially contaminated material including laboratory materials, bedding and killed animals which have previously been injected will be autoclaved together with the cage in the facility prior to incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was discussed in detail at the Cancer Research UK Beatson Laboratories Safety Committee held on Monday 7th April 2003 and approved with minor modification.

Project Containment

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Project Ref 25/03.2

Date Ackn'd 31/07/2003

Date Project Ceased

CU2 Project Title GENE TRANSFER USING DISABLED REPLICATION-DEFECTIVE ADENOVIRUS VECTORS TO STUDY PROGRAMMED CELL DEATH

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

The aim of this project is to generate replication-defective adenoviruses that express the cell death modulating molecules p53, p63, p73, p73 na, p73 nB, p73TAa, mdm2, ARF, L11, Puma, Java, ASPP, iASPP, ASPP1, ASPP2, and cyclin G1 including mutations, truncations and fusion protein thereof. This will enable the expression of these in whole cell populations which will aid investigations into the way in which these proteins cause tumour cell death. The ultimate goal of the project is to identify factors regulating cell death that may regulate novel targets for therapeutic intervention.

#### Recipient or parental organism

The cells to be used will be a range of human and mouse cultures including established and primary cells. The analysis of cell death in whole cell populations is important, but doing this using standard transfection methods is impossible due to the death of cells before a uniform population can be established. As a result, an adenoviral expression system will be employed enabling widespread expression both in vitro and in vivo. The target cells in culture are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory. The adenoviral infection is only transient and the target cells will not be able to produce infective virus. The cells will therefore not exert any foreseeable effects on either human health or the environment.

#### Host/vector system

The system to be used to transfer genes known to modulate cell death involves disabled replication-defective adenoviruses. These viruses only have the potential to infect human cells when they have been packaged in appropriately engineered packaging cells. Once packaged these infective viruses are extremel labile and do not have the capability to replicate or produce infective virus in other non-engineered cells or mice. In addition to lacking components required for replication, the adenoviruses also lack factors found in wild-type adenoviruses which are involved in evading an immune response. As a result of these safeguards, these viruses cannot exist outside of the laboratory and are incapable of widespread infection. As a result they do not pose a risk to human health or the environment. A risk of minor localised infection does exist when handling the packaged infection virus, but this is prevented by using the appropriate level of containment and inactivation procedures - hazard containment 2 is therefore recommended and all work will be carried out in Class II biological safety cabinets.

#### Origin & function

The genetic material to be introduced via the adenoviral system consists of genes (p53, p63, p73, p73 na, p73 nB, p73TAa, mdm2, ARF, L11, Puma, Java, ASPP, iASPP, ASPP1, ASPP2, and cyclin G1) which are known to modulate programmed cell death. The activity of these genes is often perturbed during the formation of many cancers causing the tumours to become resistant to the effects of many chemotherapeutic drugs. It is anticipated that by studying the way these factors induce programmed cell death will lead to a better understanding of chemosensitivity and hopefully lead to the identification of novel targets for therapeutic intervention.

#### Evaluation of foreseeable effects

Disabled replication-defective adenoviruses are labile at 37 degrees. In addition, the final GMO cells lines or mice do not contain the necessary components to compliment the disabled virus in order to make an infectious viral particle. Therefore, the final GMO cells will be free of infectious adenovirus. In addition, since the cells are unable to survive outside of the laboratory, it is not foreseeable they will pose any hazard to human health or the environment. It will, however, be important to prevent human exposure or environmental release when dealing with the infective viral cultures when being transferred from the viral packaging cells to the final target cells. Although
unlikely, it is possible that these agents at this stage may cause localised cell killing upon exposure to an infectable organism.

The risk though will be nullified by the use of the appropriate level of physical containment and inactivation procedures. Hazard containment 2 is therefore recommended and all work will be carried out in Class II biological safety cabinets.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimize the risk to human health it will be essential to prevent exposure to infectious adenoviral particles. All work will therefore be carried out under Class 2 containment conditions. Following procedures, all viral supernatants and spent medium will be inactivated using precept tablets before removal from the Class II laboratory and autoclaved prior to final disposal by incineration. Solid waste will be double-bagged in biohazard bags prior to removal and autoclaved prior to final disposal by incineration. These procedures will result in 100% inactivation of infectious adenovirus.

Target cells will be infected with adenovirus by incubation with medium containing the virus. After three hours this medium will be removed and replaced by non-infective medium. Because the replication-defective virus is labile at 37 degrees, it is considered that after 24 hr, the culture dish containing the target cells will now be non-infectious. As an extra precaution, all cells and medium from these manipulations will be treated with precept tablets before removal from the Class II laboratory and autoclaved prior to final disposal by incineration. Should it be required that target cells are removed from the Class II laboratory then their infectivity will be tested by testing cell supernatant from these for its potential to infect new target cells. Only when the cells have been shown to be non-infective will they be removed from the Class II containment laboratory.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was discussed in detail at the Cancer Research UK Beatson Laboratories Safety Committee held on Thursday 17th July 2003 and approved with minor modifications.

Project Containment

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The knockdown of tumour suppressor and Wnt family gene expression by RNA interference using amphotropic retroviral and lentiviral vectors.

### Project Additional Information

#### Purposes of the contained use

The aim of the project is to generate replication defective amphotropic retroviruses and lentiviruses that express shRNAs against negative regulators of the oncogenic canonical Wnt signalling pathway (Dickkopf genes: DKK-1, -2, -3 and -4, frazzled protein: FZB) and the tumour suppressor gene Wnt-5A.

This will allow the knockdown in expression of these genes using short hairpin RNAs that generate the stable expression of small interfering RNAs (RNAis) within the target cell. This will enable evaluation of the role of the above genes in human keratinocyte senescence.

#### Recipient or parental organism

The cells used will be primary cultures of human epidermal and oral keratinocytes. The target cells in culture are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory.

Retroviral or lentiviral infection is only transient in the target cell and it will not be able to produce the virus. The cells will therefore not exert any foreseeable effects on either human health or the environment.

#### Host/vector system

The system used to allow expression of small interfering RNAs against genes mediating senescence in human keratinocytes involves replication-defective amphotropic...
The retroviral vector (p RETROSUPER) and lentiviral vector (pLentLox3.7) will be used to transfer the shRNAs into human keratinocytes. The lentiviral vectors have the advantage of being able to infect non-dividing keratinocytes.

These viruses have the potential to infect human cells only when they have been packaged in appropriately engineered packaging cells. Once packaged these infective viruses are extremely labile and do not have the capability to replicate or produce infective virus in other non-engineered cells.

In more detail:
The p RETROSUPER vector is derived from the Murine Embryonic Stem Cell Virus (pMSCV). The vector has a specifically designed 3'LTR that has a deletion in the LTR promoter elements. This deletion results in inactivation of the LTR mediated transcription upon retroviral integration. The p SUPER constructs will be grown in the excision repair-deficient E.coli strain GT116 to avoid removal of the shRNA sequences. The resulting plasmids will then be transfected in Phoenix-E cells to produce ecotropic retrovirus and then into PT67 cells to generate high titre amphotropic retroviruses. Each of these cell lines contains gag, pol and env genes encoded by 2 separate plasmid expression vectors both of which lack the retroviral cis-acting packaging signal to minimise the likelihood of replication competent virus arising through recombination.

The lentiviral vector used is a third generation system (self inactivating vectors) derived from HIV-1 incorporating many safety features. These include deletion in the enhancer region of the 3'U3 of the long terminal repeat which results in self inactivation after transduction of the target cell. Genes encoding the structural and other components are split into four plasmids which are engineered not to contain any regions of homology to prevent any recombination and none contain LTRs or psi packaging sequence. Thus none of the HIV-1 structural genes are present on the packaged viral genome and thus are never expressed in the transduced target cell. Short hairpin RNAs (shRNAs) will be cloned into the pLentLox3.7 (pLL3.7) lentiviral transfer vector and delivered to the cells in the form of an amphotropic lentivirus. The resulting infected cells will then generate a continuous supply of RNAi in the target cells. Infected cells can be identified by the co-expression of the GFP FLAG with the shRNA. The pLL3.7 vector was engineered by introducing the mouse U6 promoter upstream of a CMV-EGFP expression cassette to create a vector that simultaneously produces shRNAs and a reporter gene. To facilitate the introduction of RNAi stem cell loops, a multiple cloning site was placed immediately after the U6 promoter. The pLL3.7 constructs will be grown in either DHs5 alpha or the excision repair-deficient E.coli strain to avoid removal of the shRNA sequences. The resulting plasmids will then be transfected into 293FT cells along with the packaging vectors pMDLg/pRRE (gag/pol elements), pRSV-REV and pMD.G (VSVG envelope glycoprotein vector) to produce high titre amphotropic lentiviruses. These are available commercially from Invitrogen as pLP1, pLP2 and pLP/VSVG vectors. The lentiviruses will then be recovered by ultracentrifugation and resuspended in phosphate-buffered saline before titration on HeLa cells. Concentrated viruses 10 million infectious units per ml will then be used to infect the normal human keratinocytes.

The genetic material to be introduced via the retroviral and lentiviral systems consists of short hairpin RNAs that target a number of genes involved in mediating senescence in human keratinocytes. These include the four Dickopf genes (DKK-1, -2, -3, -4), frazzled related protein (FZB) and the tumour suppressor gene WNT-5A. These genes have been shown to act as negative regulators of the oncogenic canonical Wnt signalling pathway. Wnt-5A is frequently lost in immortal human keratinocytes and the other genes have been found to be transcriptionally up regulated in senescent human keratinocytes.

It is anticipated that by knocking down the expression of these genes their role in keratinocyte senescence can be further understood.

In addition, the lentiviral vector has been engineered co-express GFP FLAG with the shRNA constructs to act as markers of infected cells.

The final GMO human cells will contain stably integrated DNA copies of the retroviral and lentiviral vectors encoding the transferred shRNAs against the genes listed above. The cells will be free of retrovirus or lentivirus. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside of laboratory culture.
It will however, be important to prevent human exposure or environmental release of the replication defective retroviruses or lentiviruses which are used to transiently effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimize the risk to human health it will be essential to prevent exposure to infectious retroviral or lentiviral particles. All work will therefore be carried out under Class II containment conditions. Following experimental work, all viral supernatants will be autoclaved and spent medium will be inactivated using precept tables (10,000 ppm chlorine) before removal from the Class II laboratory for disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class II laboratory and then autoclaved before final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by both infection of appropriate indicator cells and assays of reverse transcriptase activity. Once these criteria have been met the infected target cells will be removed from Class II containment and handled using standard tissue culture procedures.

Separate lab coats are worn within the Class II laboratory, gloves will be worn, plastic ware will be used and all use of sharps will be avoided.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

The risk assessment pertaining to this notification was discussed at the CRUK Safety Committee of the 12th May 2004 and was approved with minor modifications.

**Project Containment**

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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
The aim of this project is to develop replication-defective lentiviral vectors for the transfer of siRNA sequences into human tumor cell lines. Short hairpin RNAs (shRNAs) will be cloned into lentiviral vectors and delivered to the cells in the form of replication incompetent lentivirus. The resulting infected cells will then generate a continuous supply of siRNAs in the target cells. We plan to examine the effect of down regulation of both cell-matrix and cell-cell adhesions on the behaviour of human tumor cells. Cell-cell adhesion proteins (E-cadherin, p120ctn) and cell matrix adhesion proteins (beta 1 integrin, FAK) and other signalling proteins involved in regulation of these adhesions (Src family kinases, Fer and Abl tyrosine kinases, phosphodiesterase type 4 isoforms) will be targeted and effects on tumor cell motility and invasion determined.

The vectors will be supplied by Genscript (pRNAT-U6.1/Lenti and pRNAT in-H1.2/Lenti) and virus will be generated using the invitrogen ViraPower Lentiviral expression system. The virus based on human HIV-1 which has a deletion in the 3'LTR that results in the self-inactivation of the lentivirus after transduction of the target cell.

The recipient cells are human carcinoma derived cell lines: MCF-7 (breast), HT29, H630, KM12C, KM12L4A (colon), A2780 (ovarian), A431 (cervical). These are widely used by research laboratories. Their use has been classified as containment level 1. The target cells in culture are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory.

The ViasPower Lentiviral expression system is a third generation lentiviral system based on HIV-1 and consists of an expression vector and packaging genes contained on 3 additional plasmids. The expression vector contains the gene of interest. In this case Genescript Vectors pRNAT - U6.1/Lenti and pRNATin-H1.2/Lenti will be used. These commercially available vectors are compatible with the invitrogen ViraPower system. The vectors contains a neomycin resistance gene for establishing a stable cell line.
line and a coral GFP gene for tracking transfection efficiency, which are driven by a CMV promoter and SV40 promoter respectively. H1.2 and U6 promoters are used to drive siRNA expression. The HIV-1 genes env, vif, vpr, vpu and nef have been deleted from the vectors and thus recombination to produce replication competent virus is not possible. The three packaging plasmids pLP1, pLP2 and pLP/VSVG supply the helper function as well as structural and replication proteins in trans for production of Lentivirus. The plasmids have been engineered not to contain any regions of homology with each other to prevent recombination. None of them contains LTRs or the packaging sequence which means none of the HIV-1 structural genes are present in the packaged viral genome.

The 293T producer cell line stably expresses SV40 large T antigen under the control of the CMV promotor. When the expression vector and packaging vectors are co-transfected into 293FT cells, replication incompetent lentivirus is produced which will be used to infect the recipient cells.

These viruses have the potential to infect human cells only when they have been packaged in appropriately engineered packaging cells. Once packaged these infective viruses are extremely labile and do not have the capability to replicate or produce infective virus in other non-engineered cells. They would be unable to spread beyond the infected cell. Mobilisation through interaction of lentivirus with endogenous human retroviruses in the target cells is highly unlikely since the cells are of known origin and clear of HIV.

Origin & function

The genetic material to be introduced via the lentiviral systems consists of short hairpin RNAs that target a number of genes involved in cell-matrix and cell-cell adhesions. Targets include cell-cell adhesion proteins (E-cadherin, p120ctn) and cell matrix adhesion proteins (beta1 integrin, FAK) and other signalling proteins involved in regulation of these adhesions (Src family kinases, Fer and Abl tyrosine Kinases, phosphodiesterase type 4 isoforms) will be targeted and effects on tumor cell motility and invasion determined. Loss of E-cadherin has been casually linked to tumour progression however, there is no evidence that it is a tumour suppressor (Perl et al, Nature 392:190-3, 1998). It is unlikely therefore that its down regulation in human cells would lead to enhanced susceptibility to malignancy. Beta1 integrin and FAK protein levels regulate cell survival during matrix detachment (anoikis) (Xu et al Cell Growth Diff.7:413-418, 1996) and down regulation of these proteins may render the recipient cells more susceptible to anoikis. It is not known what the effect on normal cells would be.

Evaluation of foreseeable effects

The replication defective lentiviral vectors containing the siRNAs described above are unlikely to be hazardous to humans. RNAi sequences that target tumour suppressor genes could act in a similar way to oncogenes however, it is unlikely that replication defective lentiviruses would be effective carcinogens in humans in the short term. It will however, be important to prevent human exposure or environmental release of the replication defective lentiviruses which are used to transiently effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes.

The possibility of exposure to recombinant viruses generated by recombination with wild type virus needs to be considered and may, although unlikely, cause pathological effects in humans. Careful attention will, therefore, be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation.

The final GMO human cells will contain stably integrated DNA copies of the lentiviral vectors encoding the transferred siRNAs against the genes listed above. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside of laboratory culture.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
To minimise the risk of human health it will essential to prevent exposure to lentivirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Class II containment laboratories, with access restricted to authorised staff. The amount of lentivirus handled will be limited to no more than 25ml of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Prespekt tablets (10,000 ppm chlorine) before removal from the Class II laboratories for autoclaving and final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated bottle of medium that will not be used to passge other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area. To assess infection efficiencies, the cultures will be sealed and carried in a secondary sealed box and GFP-positive clones identified using the fluorescent microscope situated in the main tissue culture facility. Positive clones will be expanded and checked for virus production before removal from the Category II laboratory. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells. This will be done by treating the parental cell line with culture medium from the derivatised cells and monitoring for GFP production which would indicate the presence of replication competent virus.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment pertaining to this notification was discussed at the CR UK Safety Committee of the 19th January 2005 and was approved with minor modification.

Project Containment

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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 | L2 | L3 | L4 |
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to test the role of Raf kinase inhibitor protein (RKIP) depletion in a variety of human tumour cell lines (origin of eg, breast, skin, prostate, colon). Down regulation of RKIP will be achieved using DNA vector based siRNA technology. Small DNA inserts encoding short hairpin RNA (shRNA) targeting the gene of interest are cloned into a vector and expressed in the target cell. The shRNA expressed is rapidly processed into siRNA which destroys the cognate RNA. However, in contrast to continuous down regulation, here a tetracycline-regulated expression system is used. This allows for a much more defined approach as the same cell can be examined both in presence and absence of shRNA expression.

**Recipient or parental organism**

The recipient cells would be:
- telomerase immortalised normal human cells (fibroblasts hTERT-BJ1, breast epithelial cells hTERT-HME1)
- human breast tumour cell lines MCF-7, MDA-MB 231, MDA-MB 453, MDA-MB 468, T47D
- human colon tumour cell lines DLD-1, HCT116, HT129, LoVo, SW480
- human melanoma cell lines Mel Im, Mel Ho, Mel Ei, Mel Wei, Mel Ju, Mel Juso
- human prostate tumour cell lines C42, C42B, DU145, LNCaP, PC3
- monkey cell line COS-1 human embryonic kidney cell line HEK293

These are widely used by research laboratories. The target cells in culture are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory.

**Host/vector system**

The ViraPower™ T-Rex™ Lentiviral Technology allows tetracycline-regulated, in vitro or in vivo delivery of a target gene or RNA to dividing and non-dividing mammalian cells using a replication-incompetent lentivirus. It is based on the lentikat™ system developed by Cell Genesys (Dull et al., J. Virol., 72, 8463-8471, 1998). The lentiviral and packaging vectors supplied in the Block-iT™ Inducible H1 lentiviral RNAi system are third-generation vectors based on lentiviral vectors developed by Dull et al., 1998.

Although the lentiviral vector does have the potential to infect non-dividing human cells, by its design it is unable to spread beyond the infected cell. The likelihood of mobilisation through recombination is highly unlikely. Mobilisation through interaction of lentivirus with endogenous human retroviruses in the target cells is highly unlikely.
since the cells are of known origin and clear of HIV.

The third-generation HIV-1-based lentiviral system includes a significant number of safety features designed to enhance its biosafety and to minimize its relation to the wild-type, human HIV-1 virus. The pLenti4/BLOCK-iT™-DEST and pLent6/TR vectors contain a deletion in the 3' LTR (U3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.

* The number of genes from HIV-1 that are used in the system has been reduced to three (ie gag, pol, and rev)
* The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns et al., 1993; Emi et al., 1991; Yee et al., 1994).
* Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids (ie three packaging plasmid and pLenti4/BLOCK-iT™-DEST or pLent6/TR). All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).
* Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (eg gal, pol, rev, eng) in the 293FT producer cell line, none of them contain LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
* The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.
* Expression of the gag and pol genes from pLP1 has been rendered Rev dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al., 1998).
* A constitutive promoter (RSV promoter) has been placed upstream of the 5' LTR in the pLenti4/BLOCK-iT™-DEST and pLent6/TR vectors to offset the requirement for Tat in the efficient production of viral RNA (Dull et al., 1998).

Origin & function

RKIP is a putative tumour suppressor gene and its loss renders cells metastatic (Chatterjee et al., 2004; Keller, 2004). In contrast, there is no evidence that RKIP plays a direct role in tumourigenesis. However, it cannot be ruled out that amphotropic viruses encoding shRNAs against RKIP might also raise the possibility of tumour formation in addition to their contribution to metastasis.

Literature:


Evaluation of foreseeable effects

The replication defective lentiviral vectors containing the siRNAs described above are unlikely to be hazardous to humans. RNAi sequences that target tumour suppressor genes could act in a similar way to oncogenes however, it is unlikely that replication defective lentiviruses would be effective carcinogens in humans in the short term. The lentiviruses are disabled and cannot replicate unless packaging proteins are supplied, and thus would be unable to spread beyond the infected cell. It will however, be important to prevent human exposure or environmental release of the replication defective lentiviruses which are used to transiently to effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes.

The possibility of exposure to recombinant viruses generated by recombination with wild type virus neeeds to be considered and may, although unlikely, cause pathological effects in humans. Careful attention will, therefore, be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation.

The final GMO human cells will contain stably integrated DNA copies of the lentiviral vectors encoding the transferred siRNAs against the genes listed above. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside of laboratory culture.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

To minimise the risk to human health it will be essential to prevent exposure to lentivirus. Virus generated and infection of target cells will be carried out in Class II biological safety cabinets in the Class II containment laboratories, with access restricted to authorised staff. The amount of lentivirus handled will be limited to no more than 25ml of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Class II laboratories for autoclaving and final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area.

Positive clones will be expanded and checked for virus production before removal from the Category II laboratory. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells.

Is an emergency plan required according to regulation 20?  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The risk assessment pertaining to this notification was discussed at the CR UK Safety Committee of the 19th January 2005 and was approved with minor modification.

Project Containment

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>

02/03/2022

Page 1303 of 15326
### Project Ref 25/05.3

**Date Ackn’ed**: 01/07/2005  
**CU2 Project Title**: Retroviral infection of rat or human fibroblasts with genes found to be differentially expressed between Ras or Fos transformed fibroblasts cell lines.

**Class**: Class 2  
**Culture Vol Class**: < 1 Litre  
**Consent Granted**: Not Applicable  
**Project notified under transitional arrangements**: N

**Withdrawn**: N  
**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**:  
**Significant Change ID**:  
**Date of Significant Change**:  

**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to test the role of a number of genes found to be differentially expressed (or which physically interact with differentially expressed gene products) between Ras or Fos transformed fibroblast cell lines by retroviral infection of the cells with the genes in order to improve the understanding of invasion mechanisms in cancer.

These genes are targets of Ap-1 (AP-1 activity is required by oncogenes that function in the growth factor ras signal transduction pathway) and are involved in cellular invasion; up-regulated genes function as effectors of invasion and down-regulated genes function as suppressors of invasion.

**Recipient or parental organism**

The recipient cells would be:
- Telomerase immortalised (hTERT) human foetal fibroblasts (Tiff cells)
- Tiff cells stably expressing the v-FosFBJ/R gene
- Tiff cells stably expressing the v-Ha-Ras gene
- Rat fibroblasts (208F) cells
- Rat fibroblasts (208F) cells expressing the v-FosFBR gene

The target cells in culture are not considered to pose any inherent hazard to humana health, nor do they have the capacity to survive outside the laboratory. All the mammalian cells to be used have been tested and shown to be free of replicating retrovirus, either in house or others (Curran T, Verma, IM (1984) Virology, 135 p229).

The probability that harmful sequences may be transferred to related viruses is therefore very low.

**Host/vector system**
The retroviral vectors pLNCX, pLHCX and pLPCX (available from Clontech) are based on the Moloney Murine Leukaemia Virus (MOMuLV) and are replication defective since they do not encode the necessary gag, pol and env genes. Transfection into the packaging cell line, Phoenix ampho (Nolan Labs) or RetroPak-PT67 (Clontech), provides these gene products, permitting production of infective virus. Each of these cell lines contains gag, pol and env genes encoded by 2 separate plasmid expression vectors both of which lack the retroviral cis-acting packaging signal to minimise the likelihood of replication competent virus arising through recombination.

Virus produced by the packaging cell line is capable of infecting human cells, however, further production of virus from an infected cell is extremely unlikely, since the virus lacks the gag-pol and env genes. In the packaging cell line these genes are carried in two separate constructs and under the control of non-Moloney promoters, therefore, removing the risk that a single recombination event could result in the virus acquiring the genes necessary to become replication competent. In addition, once packaged these infective viruses are extremely labile. According to the ACGM compendium of guidance part 2B note 15 the appropriate level of containment to control the inherent risk of amphotrophic, replication defective viruses is AGCM Level 1. A risk of minor localised infection does exist when handling the packaged infection virus, but this is prevented by using the appropriate level of containment and inactivation procedures - hazard containment 2 is therefore recommended and all work will be carried out in Class II biological safety cabinets.

The gene sequences are of human origin (with the exception of Qsulf-1 which is of avian origin) and are involved in suppressors of invasion.

- Krp1 - kelch-related protein 1 (Krp1) is necessary for pseudopod elongation.
- Lasp-1 is necessary for cell pseudopod extension and invasion.
- Prex1 - phosphatidylinositol 3, 4, 5-triphosphate-dependent exchanger acts as a guanine nucleotide exchange factor for the RHO family of small GTP-binding proteins (RACs)
- Tiam-1 is a guanine nucleotide exchange factor that activates the RHO family of small GTP binding proteins (RACs) and is known to have oncogenic properties.
- Sulf-1 (human and quail) is a heparan sulfate (HS) 6-0 endosulfatase with preference, in particular, toward trisulfated IdoA2S-GlcNS6S disaccharide units within HS chains. It can function cell autonomously to remodel the sulfation of cell surface HS and promote Wnt signaling when localised either on the cell surface or in the Golgi apparatus.
- Integrin-linked kinase (ILK) is a focal adhesion protein activated by both extracellular matrix and growth factors, is required for the activation of Rac and Cdc42 in epithelial cell. It has known oncogenic activity.
- Integrin linked kinase associated phosphatase (ILKAP) regulates ILK signaling and inhibits anchorage-independent growth. It has known oncogenic activity.
- PINCH1 is a focal adhesion protein that complexes with ILK and is involved in cell spreading and motility. It is proposed to express the above genes and mutated versions which are inactivated or constitutively active. Three of the genes are known oncogenes the others are involved in invasion processes. It is unlikely that amphotropic retroviruses encoding the genes would represent a significant hazard as a localised infection in normal cells. It is proposed to express the above genes and mutated versions which are inactivated or constitutively active.

Evaluation of foreseeable effects

The replication defective retroviral vectors containing the genes described above are unlikely to be hazardous to humans. Some of the gene sequences are oncogenic however, it is unlikely that replication defective retroviruses would be effective carcinogens in humans in the short term. Single oncogenes are not generally considered sufficient to convert primary human cells to full malignancy, and replication defective viruses are not capable of dissemination even in a permissive host, however there is potential for harm to human health in the event of infection. It will however, be important to prevent human exposure or environmental release of the replication defective retroviruses which are used to transiently to effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes.

The possibility of exposure to recombinant viruses generated by recombination with wild type virus needs to be considered and may, although unlikely, cause pathological effects in humans. Careful attention will, therefore, be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation.

The final GMO human cells will contain stably integrated DNA copies of the retroviral vectors encoding the transferred genes listed above. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside of laboratory culture.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

To minimise the risk to human health it will be essential to prevent exposure to retrovirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Class II containment laboratories, with access restricted to authorised staff. The amount of retrovirus handled will be limited to no more than 25 ml of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Class II laboratories for final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area.

Positive clones will be expanded and checked for virus production before removal from the Category II laboratory. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells or assay or reverse transcriptase activity. Once these criteria are met the infected target cells will be removed from Class II containment and handled using standard tissue culture processes.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

The risk assessment pertaining to this notification was discussed at the CR UK Safety Committee on 24 June 2005 and was approved with minor modification.

**Project Containment**

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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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02/03/2022
Use of retroviral and lentiviral human siRNA libraries to knock down expression of proteins that interact with known target genes by measuring GFP activity, in order to investigate the events that are required for a tumorigenic phenotype.

The aim of this project is to generate replication-defective retroviruses and lentiviruses that express shRNAs from RNA interference libraries. This will enable the expression of siRNAs in a target cell population which will allow for their selection and identification based on a target gene/reporter construct. The ultimate goal of the project to identify factors regulating cellular processes that may offer novel targets for therapeutic intervention.

The cells to be used in the first instance, will be the human RPE-tert cell line (telomerase-expressing human retinal pigment epithelial cells) These have been modified by transfecting and selecting for a target gene. PUMA, a propapoptotic gene (Nakano, K and Vousden, K.Mol.Cell.7:683-694,2001) linked to a GFP reporter. The RPE-tert cells are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory. The cells will therefore not exert any foreseeable effects on either human health or the environment.

Other common laboratory cell lines that will be used to complement the GFP reporter construct and subsequent siRNA screening include:
telomerase immortalised normal human cells (fibroblasts hTERT-BJ1, breast epithelial cells hTERT-HME1)
human breast tumour cell lines MCF-7, MDA-MB 231, MDA-MB 453, MDA-MB 468, T47D
human colon tumour cell lines DLD-1, HCT116, HT29, LoVo, SW480
human melanoma cell lines Mel Im, Mel Ho, Mel Ei, Mel Wei, Mel Ju, Mel Juso
human prostate tumour cell lines C42, C42B, DU145, LNCaP, PC3
Burkitt lymphoma EBV negative cell lines CA46, Ramos, BL40, BL41, Bl2
monkey cell line COS-1
Human embryonic kidney HEK293

These are widely used by research laboratories. They are not considered to pose any inherent hazard to human health nor do they have the capacity to survive outside the laboratory and do not harbor any adventitious agents.

### Host/vector system

The system to be used to transfer the retroviral shRNA library involves disabled replication-defective retrovirus pRetroSuper (Brummelkamp, T et al. Cancer Cell 2, 243-7 (2002) or a similar retroviral vector pSM2c (Paddison et al., Nature methods 1:2, 163-7 (2004) available from Open Biosystems Inc. Both these vectors contain the retroviral packaging signal which will only allow them to be packaged when they have been placed in appropriately engineered packaging cells. Once packaged the infective viruses are extremely labile and do not have the capability to replicate or produce infective virus in the other non-engineered cells.

In more detail:

The pRETROSUPER vector and pSM2c vectors are derived from the Murine Embryonic Stem Cell virus (pMSCV). The vectors have a specifically designed 3'LTR that has a deletion in the LTR promoter elements. This deletion results in inactivation of the LTR mediated transcription upon retroviral integration.

The pSM2c cloning vector is roughly equivalent to pSM1 (Paddison et al., methods Mol.Biol.265, 85-100 (2004) with a few notable exceptions. First, the cloning strategy has been changed. Previously "PCR-SHAG" was used to clone hairpins by adding the entire hairpin onto the end of a PCR primer. Now a single oligo is used, containing the hairpin and common 5' and 3' ends, as a PCR template. That is, the oligo is PCR amplified using universal primers that contain XhoI (5' primer) and EcoRI (3' primer). These PCR fragments are then cloned into the hairpin cloning site of pSM2c. The mir30-styled hairpins are expressed from the human U6 promoter. The 5' and 3' flanks are derived from 125 bases surrounding the Human mir30 microRNA.

The pSUPER constructs will be grown in the excision repair-deficient E.coli strain GT116 to avoid removal of the shRNA sequences. The pSM2c constructs will be g rown in E. coli DH10Bpir116 and DH10Bpir116 and DH10BF DOT competent cells. The shRNA plasmids use the pir-dependent R6Ky replication origin. The PirPlus E. coli express the protein (pir gene product), which is essential for cloning, and propagation of the shRNA vectors. The resulting plasmids will then be transfected into the packaging cell lines Phoenix-Ampho (Orbigen Inc) based on 293T (a human embryonic kidney cell line transformed with adenovirus E1a and carrying a temperature sensitive T antigen) or RetroPak-PT67 (BD Biosciences) based on NIH 3T3 to produce amphotropic retrovirus. These helper free cell lines contain gag, pol and env genes encoded by 21 separate plasmid expression vectors both of which lack the retroviral cis-acting packaging signal to minimise the likelihood of replication competent virus arising through a single recombination event.

Transfection into the packaging cell line, Phoenix ampho or RetroPak-PT67, provides these gene products, permitting production of infective virus. At this stage the virus is capable of infecting human cells, however, further production of virus from an infected cell is extremely unlikely, since the virus lacks the gag-pol and env genes. In the packaging cell line these genes are carried in two separate constructs and under the control of non-Moloney promoters, therefore, removing the risk that a single recombination event could result in the virus acquiring the genes necessary to become replication competent.

The vector for the lentiviral shRNA library is a derivative of pLentihair called pLKO.1 (Stewart et al, RNA [2003], 9:493-501) (http://www.addgene.org/pgvec1?f=c&identifier=8453&cmd=findpl). This vector system is based upon HIV-1 lentivirus, although is lacking all HIV-1 genes except gag-pol and env, which are provided in trans by a transient transfection strategy. Also, the virus produced is to be pseudotyped with VSV-G envelope protein rather than HIV-1. The vector requires cotransfection into 293T cells with two other plasmids providing packaging (gag-pol) and envelope (env) functions in trans in order to produce retroviral particles. 293T cells are not employed in this capacity as a packaging cell line, merely as a host for this entirely exogenously introduced viral system. In combination with a self-inactivating LTR on pLKO.1 the transient transfection of a three separate plasmid system effectively eliminates the possibility of recombination to form replication-competent retrovirus. As such the vector system is highly unlikely to cause harm to humans.

### Origin & function
shRNA libraries produce double-stranded RNA identical in sequence to that of the gene of interest that can interfere with its function. Depending on the library to be used the resulting siRNAs can be 21 to 30 nucleotides long.


The lentiviral Expression Arrest-TRC human shRNA library is available commercially from Open Biosystems. This library was designed and developed by the RNAi Consortium (TRC) at the Broad Institute of MIT and Harvard. Currently the human shRNA library (TRC-Hs 1.0) contains over 34,000 shRNA constructs, cloned into lentiviral expression vectors, covering 7300 human genes.

It is anticipated that by knocking down the expression of these genes in relation to a target gene/GFP reporter a link can be made in the target gene pathway/phenotype.

The knock down of genes will involve the tumour supressor gene p53 plus others including, p3000, CBP, retinoblastoma, P16, P27. It is possible that amphotropic viruses encoding shRNAs against tumour suppressor genes might raise the probability of transformation in infected cells.

Other targets are BIK and BAX genes which are pro-apoptotic proteins that when expressed at sufficient levels and in the correct context can induce cell death. Knockdown of these genes in cell lines is predicted to protect them from stimulation induced cell death. BIK has so far been found to be mutated in a few glioma and peripheral B-cell lymphomas and its loss may therefore contribute to oncogenesis. Bax mutations have also been found in colorectal tumours with a microsatellite instability phenotype. Loss of these genes may enable cells to escape specific stimulation induced apoptosis and may therefore contribute to oncogenesis. The frequency of these events in human cancer is low.

Evaluation of foreseeable effects

The final genetically modified cells will contain stably integrated DNA copies of the retroviral or lentiviral vectors encoding the transferred shRNAs. The cells will be free of retrovirus or lentivirus. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside the laboratory culture. It will however, be important to prevent human exposure or environmental release of the replication defective retro- or lentiviruses which are used to transiently effect gene transfer. The worse case scenario in case of infection is the selection of an shRNA that knocks down expression of a gene necessary for tumour suppression. Risk against this will be reduced through the use of appropriate physical containment and inactivation processes. Hazard containment 2 is therefore recommended and all work will be carried out in Class II biological safety cabinets.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk to human health it will be essential to prevent exposure to retrovirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Class II containment laboratories, with access restricted to authorised staff. The amount of retrovirus or lentivirus handled will be limited to no more than 25ml of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during
experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Class II laboratories for final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated vials of medium that will not be used to passae other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area.

Positive clones will be expanded and checked for virus production before removal from the Category II laboratory. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells or assay of reverse transcriptase activity. Once these criteria are met the infected target cells will be removed from Class II containment and handled using standard tissue culture processes.

The risk assessment pertaining to this notification was discussed at the CR UK Safety Committee of the 21st September 2005 and was approved with minor modification.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</table>

The risk assessment pertaining to this notification was discussed at the CR UK Safety Committee of the 21st September 2005 and was approved with minor modification.
**Project Additional Information**

**Purposes of the contained use**

Primary human cells from normal or tumorigenic tissue are to be transfected using amphotropic retroviruses containing human telomerase catalytic component (hTERT) and then analysed for RNA and protein expression, proliferation and other cell culture assays. The function of the expressed hTERT, cDNAs is to immortalise human cells whilst retaining a normal non-neoplastic phenotype. The resulting cells will allow the study of normal and dysplastic human cells in vitro where previously only neoplastic or rodent in vivo models have been available.

**Recipient or parental organism**

The recipient cells would be primary cells that senesce in culture after 3-62 population doublings and will be of the following origins: fibroblasts, keratinocytes, glial, epithelial, striated muscle, kidney etc.

The target cells in culture are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory. They are derived from patients with no known retroviral disease or Hepatitis.

**Host/vector system**

The retroviral vectors such as pBabe, pRetroSuper, pLNCX, pLHCX and pLPCX are based on the Moloney Murine Leukaemia virus (MMLV) and are replication defective since they do not encode the necessary gag, pol and env proteins. They are available commercially from companies such as BD Biosciences, Invitrogen and Stratagene etc. Phoenix packaging cell lines (Nolan Lab) are second-generation retrovirus producer lines for the generation of helper free retroviruses. The lines are based on the 293T cell line (a human embryonic kidney line transformed with adenovirus Ela and carrying a temperature sensitive T antigen co-selected with neomycin). Transfection of the phoenix E packing cells will produce an ecotropic retrovirus. When this retrovirus is used to infect the Phoenix Amphi packaging cell line, an amphotropic retrovirus is produced which is capable of infecting human cells. However, further production of retrovirus from the infected human cells is extremely unlikely since the virus lacks the gag-pol and env genes.

An alternative packaging cell line is RetroPak-PT67 (BD Biosciences) a mouse fibroblast NIH 3T3-derived cell line designed for the production of infectious, replication-incompetent virus. PT67 contains the Moloney murine leukemia virus (M0MuLV) gag, pol, and env (IOA1-derived) genes. Transfection with a retroviral vector containing the retroviral packaging signal and a target gene allows production of replication-incompetent virus. In the PT67 packaging cell line these genes are carried in two separate constructs and under the control of non-Moloney virus promoters, therefore removing the risk that a single recombination event could result in the virus acquiring the genes necessary to become replication competent.

Virus produced by the packaging cell line is capable of infecting human cells, however, further production of virus from an infected cell is extremely unlikely, since the virus lacks the gag-pol and env genes. In the packaging cell line these genes are carried in two separate constructs and under the control of non-Moloney promoters, therefore,
removing the risk that a single recombination event could result in the virus acquiring the genes necessary to become replication competent. In addition, once packaged these infective viruses are extremely labile. A risk of minor localised infection does exist when handling the packaged infection virus, but this is prevented by using the appropriate level of containment and inactivation procedures — hazard containment 2 is therefore recommended and all work will be carried out in Class II biological safety cabinets.

**Origin & function**

The gene sequences are of human origin: hTERT (Human telomerase reverse transcriptase). It is the catalytic component of telomerase, with similarity to reverse transcriptases; may represent universal subunit for telomerases. Bodnar et al (Science 279:349, 1998) reported bypassing cellular senescence when they transfected human somatic cells lacking telomerase activity with the hTERT gene. Counter et al. (PNAS 95:14723, 1998) reported alteration of the C-terminus of hTERT did not affect enzymatic activity but impeded telomere maintenance, and cells with this mutant telomerase would not immortalize. hTERT has been shown to prolong the lifespan of human cells and has been implicated in the process of epithelial cell immortalisation (Muntoni et al. Oncogene 22, 7804-08 (2003)). It is difficult to envision how the proposed manipulation of the levels of this gene in our cultures could pose a greater risk than the use of existing immortal cancer cell lines in the laboratory. It is therefore unlikely that amphotropic retroviruses encoding these genes would represent a significant hazard as a localised infection of the cells under investigation.

**Evaluation of foreseeable effects**

The replication defective retroviral vectors containing the genes described above are unlikely to be hazardous to humans. hTERT has been shown to prolong the lifespan of human cells and has been implicated in the process of epithelial cell immortalisation. However, it is unlikely that replication defective retroviruses would be effective carcinogens in humans in the short term. Single oncogenes are not generally considered sufficient to convert primary human cells to full malignancy, and replication defective viruses are not capable of dissemination even in a permissive host, however there is potential for harm to human health in the event of infection. It will however, be important to prevent human exposure or environmental release of the replication defective retroviruses which are used to transiently to effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes. The possibility of exposure to recombinant viruses generated by recombination with wild type virus needs to be considered and may, although unlikely, cause pathological effects in humans. Careful attention will, therefore, be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation.

The final GM human cells will contain stably integrated DNA copies of the retroviral vectors encoding the transferred genes listed above. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside of laboratory culture.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

To minimise the risk to human health it will be essential to prevent exposure to retrovirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Class II containment laboratories, with access restricted to authorised staff. The amount of retrovirus handled will be limited to no more than 25ml of virus stock. The most likely routes through which individuals could be exposed to virus are needlestick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Class II laboratories for final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.
Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area. Positive clones will be expanded and checked for virus production before removal from the Category II laboratory. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells or assay of reverse transcriptase activity. Once these criteria are met the infected target cells will be removed from Class II containment and handled using standard tissue culture processes.

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**Project Ref** 25/07.2

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**Non-GMM**

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**Project notified under transitional arrangements**

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Project Additional Information

Purposes of the contained use

The aim of this project is to generate replication-defective lentiviruses that express shRNAs against proteins that are important in cancer metastasis and maintenance of cell morphology and signalling. The goal of the project is to test the requirements for specific proteins in various cellular processes such as migration, survival and growth.

Recipient or parental organism

The cells to be used in the first instance, will be the primary and immortalised mouse embryo fibroblast cells (MEF and iMEF). The MEF and iMEF are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory. The cells will therefore not exert any foreseeable effects on either human health or the environment. These cells are not competent to produce replication defective virus either. Other common laboratory cell lines that will be used include:

- Telomerase immortalised normal human cells (fibroblasts hTERT-BJI, breast epithelial cells hTERT-HMEI)
- Human breast tumour cell lines MCF-7, MDA-MB 231, MDA-MB 453, MDA-MB 468, T47D
- Human colon tumour cell lines DLD-1, HCTI 16, HT29, LoVo, SW480
- Human melanoma cell lines Mel Im, Mel Ho, Mel Ei, Mel Wei, Mel Ju, Mel Juso, A375
- Human prostate tumour cell lines C42, C428, DU145, LNCaP, PC3
- Human squamous cell carcinoma SCC9
- Mouse fibroblast cell lines- NIH3T3, Swiss 3T3
- Mouse myoblast cell line C2C12
- Burkitt lymphoma EBV negative cell lines CA46, Ramos, 8L40, BL41, BL2
- Monkey cell line COS-1
- Human embryonic kidney HEK293

These are widely used by research laboratories. They are not considered to pose any inherent hazard to human health nor do they have the capacity to survive outside the laboratory and do not harbor any adventitious agents.

Host/vector system

Host bacteria for growth of the plasmids are standard disabled laboratory strains of E. Coli such as DH5α and JM109.

The vector systems will be a third generation lentiviral system where all six regulatory/accessory proteins (Rev, Tat, Vif, Vpr, VPu and Nef) are removed, except Rev. Rev acts at the post transcriptional level and is necessary for HIV gag/pol expression. Rev binds to an RNA motif (Rev responsive element RRE) and facilitates the cytoplasmic export of gag/pol messenger. As an extra safety measure Rev is placed on a separate vector (RSV-Rev) from the transfer vector. Only 700bp of the HIV envelope protein are present in the transfer vector, plus RRE and the packaging signal. The packaging construct contains the minimal RRE of 374 bp and the gag/pol genes. Through deletions in both LTR's and the absence of the accessory proteins, including the replication essential Tat, it is highly unlikely that replication of competent virus is produced. However the WPRE could express part of the X protein from the woodchuck hepatitis B virus which may have oncogenic properties. The ACOM advises that vectors containing these sequences are handled at category II or higher.

The vectors to be used in this study are available from Addgene or OpenBiosystems.

The lentiviral packaging cells will be 293T which will be used for the initial rescue of the virus from transfected DNA and for general virus propagation and will also express...
The transfected protein or shRNA of interest.

pWPI from Addgene- vector information available at www.addgene.org

This is the HIV-derived viral transfer vector. The description of the vector is copied from the Trono laboratory website, where we obtained the vector from: “As an analogue of the “self inactivating (sin)” retroviral vectors a sin lentiviral vector was constructed. The standard vector contains the post transcriptional regulatory element of woodchuck hepatitis virus (WPRE, abbreviated in plasmid names) has been inserted to enhance transgene expression as well as a central polypurine tract (cPPT), a cisacting element that improves the efficiency of gene transfer by a few-fold in many targets. All contain a transgene expressed from an elongation factor 1-alfa promoter (or its intron-less version EF1-a short), a robust transcriptional element in most cell types. Unique restriction sites at key positions will allow you to change promoter and transgene. The bicistronic vector allows for simultaneous expression of a transgene and CFP marker to facilitate tracking of transduced cells. Recently, we have developed a series of lentivectors that allows for constitutive or conditional RNA interference. In order to insert your own shRNA a pLVTH vector was designed in a way that Hi Pol III promoter can be easily replaced with Hi-siRNA cassette from pSUPER using EcoRi - Clal (or BamHl - Clai to obtain pLVH version). Alternatively, Hi promoter or tetO-Hi cassette can be substituted by any other siRNA expression cassette (e.g. U6-siRNA etc.).” In third generation lentiviral vectors derived from HIV in general, the total U3 region of the 5’ LTR is replaced by a heterologous Rous Sarcoma Virus (RSV) promoter. The U3 region of the 3’ LTR has a 400 bp deletion encompassing the TATA box. This means that the viral transfer vector has no complete LTR and the LTR’s of the provirus will not lead to transcription after integration into the genome, because of the removal of the U3 promoter. In the other packaging constructs no HIV LTR elements are present, so that it is impossible to get a complete LTR by recombination. The vector contains HIV elements that are necessary for the packaging of the viral RNA, the leader sequence, 360 bp gag, and 700 bp env. The total remaining HIV sequence is 1700 bp, which is 18% of the original viral genome.

pLVTHM from Addgene- vector information available at www.addgene.org

This is a modified version of the pWPI vector, described above, in which the Hi promoter drives expression of shRNA.

pMDLg/pRRE from Addgene- vector information available at www.addgene.org

This is the viral packaging construct. This vector contains 4700 bp HIV sequence: the gag/pol genes, 374 bp of env and the rev responsive element (RRL). Transcription is under control of the CMV promoter for GFP and the U6 promoter for the short oligonucleotides.

pRSV-rev from Addgene- vector information available at www.addgene.org

This vector contains the HIV-rev gene which is under control of the RSV promoter. There will be no translation of HIV gag/pol without expression of Rev, because Rev is necessary for the export of gag/pol messenger from the nucleus to the cytoplasm. This is an additional safety measurement. It is highly unlikely that replication competent virus will be formed from the pMDLg/pRRE vector because this vector will not express gag/pol in the absence of Rev. In the unlikely event that expression were to occur then only very low or undetectable levels are anticipated.

pMD2.g from Addgene- vector information available at www.addgene.org

This is the viral envelope expression vector. Vesicular stomatitis virus glycoprotein expression is under control of the CMV promoter.

pLVTHM- from Addgene- vector information available at www.addgene.org

This is a mammalian expression vector used for cloning the insert into prior to subcloning into pLVCT-TR-KRAB. It is similar to the previous Trono lab pLVTH but it contains a 3bp substitution that generates a unique MluI site for direct cloning of shRNA into MluI-Clal.

pLVCT-TR-KRAB- from Addgene- vector information available at www.addgene.org

This is a mammalian lentiviral expression vector which allows for drug controllable RNA interference using Tet-on shRNA. A vector map is available at the Addgene website.

pMV-CI N-ZEO from http://www.openbiosystems.com

This is a third generation lentiviral vector for the expression of shRNA that may be used as an alternative to pLVTHM, described above, because its microRNA-adapted shRNAmir design may increase levels of gene knockdown and its neomycin selection cassette allows for stable expression. The pCMV-GIN-ZEO characteristics are as follows: CMV promoter driving expression of a GFP marker and an IRES-neomycin selection cassette; a cPPT to help translocation to the nucleus; a WRE to enhance transgene expression ampicillin and zeocin bacterial selectable markers; a 5’ LTR; a pUC origin of replication to promote high copy maintenance in E. coli; a self-inactivating 3’ LTR; a Rev response element; and an RNA polymerase II promoter to drive shRNAmir expression.

Origin & function

Green fluorescent protein

Sequence encodes Green Fluorescent Protein (GFP) which functions as a reporter molecule that fluoresces bright green upon exposure to UV light.

Mouse and human proteins

The cytoskeletal proteins cortactin, HS-1, Scar-i, Scar-2 and Arp are implicated in the regulation of changes in morphology in platelets following stimulation by collagen.
Mutant mouse and human proteins
Point mutants or deletions of N-WASP, Arp2, Scar, Rac, Cdc42, mDia or related cytoskeletal proteins
Short hairpin RNAs corresponding to mouse and human proteins of the actin cytoskeleton
Short oligonucleotide sequences are designed to form short hairpin RNAs which will knock down expression of the above mouse and human genes.
No known hazard could arise from expression of these cDNAs or the short hairpin sequences designed to reduce their expression. All have been widely studied in a similar capacity before (e.g. overexpression by transient or stable transfection or knockdown) by our lab and other labs around the world.

**Evaluation of foreseeable effects**

The final genetically modified cells will contain stably integrated DNA copies of the lentiviral vectors encoding the transferred shRNAs. The cells will be free of lentivirus. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside the laboratory culture, It will however, be important to prevent human exposure or environmental release of the replication defective retro- or lentiviruses which are used to transiently effect gene transfer. The worse case scenario in case of infection is the selection of an shRNA that knocks down expression of a gene necessary for tumour suppression. Risk against this will be reduced through the use of appropriate physical containment and inactivation processes. Hazard containment 2 is therefore recommended and all work will be carried out in Class II biological safety cabinets.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

To minimise the risk to human health it will be essential to prevent exposure to retrovirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Class II containment laboratories, with access restricted to authorised staff. The amount of retrovirus or lentivirus handled will be limited to no more than 25m1 of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Class II laboratories for final disposal. Solid waste will be double- bagged in biohazard bags prior to removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area.

Positive clones will be expanded and checked for virus production before removal from the Category II laboratory. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells or assay of reverse transcriptase activity. Once these criteria are met the infected target cells will be removed from Class II containment and handled using standard tissue culture processes.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
The proposal was reviewed and approved as Class II at the Safety Committee Meeting on the 20th of November 2007.

Please enter comments on the GM safety committee on the risk assessment:

the proposal was reviewed and approved as Class II at the Safety Committee Meeting on the 20th of November 2007.

Project Containment

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Project Ref 25/08.1

Date Ackn’d: 11/07/2008

CU2 Project Title: Retroviral infection of mouse or human tissue culture cells to express or knockdown proteins involved in cell signalling, tumourgenesis, cell motility, invasion, metastasis and cell death and survival.

Class: Class 2

Culture Vol: < 1 Litre

Non-GMM: Not Applicable

Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

The ultimate goal of these projects is to identify factors regulating cellular processes that may offer novel targets for therapeutic intervention in human disease. The retroviral vectors, pLNCX or pLPCX or pLHCX, pRetro-X Tet-On Advanced, pRetro-X-Tight-Hyg, pRetro-Tight-Pur (Clontech) and the pSM2 based vectors (LMP, TMP, and derivatives, Open Biosystems and Scott Lowe laboratory) will be introduced, by standard transfection techniques into the viral packaging cell lines, Phoenix-ampho, Phoenix-eco (Orbigen Inc, Nolan Labs), RetroPak PT-67, GP2-293 (Clontech) which will generate amphotrophic or ecotrophic retroviruses. The retroviral vectors will carry either siRNA or microRNA sequences to knock down target protein expression or sequences coding for target proteins that we want to overexpress. We will also express mutated forms of these proteins which are inactivated or constitutively active.

Recipient or parental organism

Host bacteria for growth of the plasmids are standard disabled laboratory strains of E. Coli such as DH5a and JM1 09. The use of E.coli as the recipient micro-organism, has been subject to a risk assessment and designated a Class 1 procedure.

The virus produced by the Phoenix-ampho, RetroPak PT-67, OP2-293 Phoenix-Eco cells will be used to infect human or mouse tissue culture cell lines: Human melanoma: A375, CHL-1, Me1505, Colo-829, A375M2, wm266.4, WM35, Skmel 119, Skmel2, Skmel 147, Skmel 173, RPM18332, Skmel23, BFTC905

Human Breast cancer: TMX2-28, MDA-MB231 and derivatives, MCF1O and derivatives, MCF-7, Hs578t, MDA-MB157, MDA-MB435s, MDA-MB468, MDA-MB8361, BT-549, T47D

Neuroblastoma: SHSY5Y,

Human Osteosarcoma: SAOS-2, U2OS

Human Cervical carcinoma: Hela

Human Keratinocyte cell line: HaCaT

Human head and neck squamous cell carcinoma: SCC9, FaDu, Detroit512, HN5, HN30, H413

Human Vulval 3CC: A431, UMSCV1A, UMSCV1B, LJMSCV6A, UMSCV2, UMSV7

Human T cell lymphoma: Jurkat

Human Burkitts Lymphoma: Ramos, CA46, BL41, BL2, 6L30, L3055, 8L40

Human Ovarian Carcinoma: A2780, JAMA2, 1847, OVCA433, OVCAR3, SKOV3, TR175, 1847, IGROV, QAW42, PA-i

Human Colon carcinoma: HT29, RKO, Colo205, SW620, SW48, 5W480, DLD1, Lovo, Ls174t, SKCO-1, 5W948, HCTI16

Human Prostate tumour: C42, C42B, DU145, LNCaP, PC3 Human Bladder tumour: SW1670, 5637, T24, VMcubl

Human Lung tumour: KNS-62, A549

Human Pancreatic tumour: panc-1, BxPC3, aspc-1, MIA-Paca-2

Human Retinal pigment epithelial cells: RPE

Human telomerase immortalised cell lines: hTERT-BJ1, hTERT-HME1, RPE-tert, Tif-puro

Mouse cell lines: 67NR, 4To7, 4T1, NIH3T3, Swiss 3T3, primary MEFs, immortalised MEFs, MEFs derived from knockout mice, primary mouse T cells, primary mouse melanocytes.

All the mammaliam cell lines are in routine use in many laboratories and have been provided by ATCC. CR UK cell bank, ICR cell bank or from other laboratories and have been tested either in house or at source to be free from replicating retroviruses. The probability that harmful sequences may be transferred from endogenous related viruses is therefore low.

Primary Human Cells: mammary epithelial cells, melanocytes, umbilical vein endothelial cells (HUVECs), fibroblasts, Peripheral blood lymphocytes, tonsillar lymphocytes. These are not considered to pose any inherent hazard to human health. They are derived from patients with no known retrovirial disease or Hepatitis. Derivatives of these cell lines engineered to express the ecotropic retrovirus receptor, the tetracycline transactivator ITA and reporter genes (Luciferase or florescent protein reporters) will also be used. The relevant drug (puromycin, hygromycin or neomycin) will then be used to select cells which have stable expression/knockdown of our gene of interest or appropriate controls.
**Host/vector system**

Host bacteria for growth of the plasmids are standard disabled laboratory strains of E. Coli such as DH5a and JM109. The use of E.coli as the recipient micro-organism, has been subject to a risk assessment and designated a Class 1 procedure. The packaging cells will be Phoenix-ampho, RetroPak PT-67, GP2-293 or Phoenix-Eco cells which will be used for the initial rescue of the virus from transfected DNA and for general virus propagation and will also express the transfected protein or shRNA of interest.

The retroviral vectors pLNCX, pLHCX and pLPCX are based on the Moloney Murine Leukaemia Virus (M0MuLV) and is replication defective since it does not encode the necessary gag, pol and env genes. The pRetroX-Tet-on Advanced and pRetroX-Tight-pur and pRetroX-Tight-hyg vectors are based on mouse sarcoma virus (MSV) sequences and are replication defective. pSM2 is based on the murine stem cell virus and is also replication defective. Transfection into the packaging cell line, Phoenix amphi, Phoenix eco, GP2-293 or RetroPak-PT67, provide these gene products, permitting production of infective virus. At this stage the virus from amphotropic packaging cell lines is capable of infecting human cells, however, further production of virus from an infected cell is extremely unlikely, since the virus lacks the gag-pol and env genes. In the packaging cell line these genes are carried in two separate constructs and under the control of non-Moloney promoters, therefore, removing the risk that a single recombination event could result in the virus acquiring the genes necessary to become replication competent.

**Origin & function**

Some of the genes we wish to express will include known oncogenes (for example Vl2HaRas, V600EBRaf, c-myc) and likewise some of the genes we will knockdown will include known/suspected tumour suppressor genes (for example p53, Dab2). Current knowledge indicates that the de-novo transformation process of human cells requires the peturbation of six-pathways (Rangarajan et al, Cancer Cell, 2004 (2):171-83) and therefore manipulation of individual pathways is not expected to confer a serious cancer risk to normal human cells. Similarly individual expression of activated oncogenes alone results in cellular senescence and/or apoptosis (Wajapeyee et al, Cell 2008 1 32(3):363-74). None of the genes we wish to manipulate encode toxins. Other examples include expression, knockdown and reexpression or mutant constructs for the following: Arp2/3 complex, WASP, N-WASP, Scarl-3, WASH. Cortactin,dynamin, TOCA-1, Rac1, RhoB, RhoF, RhoBTB1,2, Cdc42, IRSp53, MTSS1, ABBA1, Fascin.

**Evaluation of foreseeable effects**

Some of the genes we wish to express will include known oncogenes and likewise some of the genes we will knockdown will include known tumour suppressor genes. Current knowledge indicates that the denovo transformation process of human cells requires the peturbation of six-pathways (Rangarajan et al, Cancer Cell, 2004 (2):171-83) and therefore manipulation of individual pathways is not expected to confer a serious cancer risk to normal human cells. None of the genes we wish to manipulate encode toxins.

Hence, it is unlikely that replication defective retroviruses would be effective carcinogens in humans in the short term. Single oncogenes are not generally considered sufficient to convert primary human cells to full malignancy, and replication defective viruses are not capable of dissemination even in a permissive host, however there is potential for harm to human health in the event of infection. It will however, be important to prevent human exposure or environmental release of the replication defective retroviruses which are used to transiently to effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes. The possibility of exposure to recombinant viruses generated by recombination with wild type virus needs to be considered and may, although unlikely, cause pathological effects in humans. Careful attention will, therefore, be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation.

The final GM human cells will contain stably integrated DNA copies of the retroviral vectors encoding the transferred genes listed above. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside of laboratory culture.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

n/a
To minimise the risk to human health it will be essential to prevent exposure to retrovirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level 2 laboratories, with access restricted to authorised staff. The amount of retrovirus handled will be limited to no more than 1 OmI of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Class II laboratories for final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus. Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area. Positive clones will be expanded and checked for virus production before removal from the Category II laboratory. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells or assay of reverse transcriptase activity. Once these criteria are met the infected target cells will be removed from Class II containment and handled using standard tissue culture processes.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The project was approved as Class II by the Safety Committee in May 2008 and amendments reviewed in June 2008

Project Containment

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Project Ref 25/08.2

Date Ackn'd 26/11/2008  CU2 Project Title Gene transfer using replication defective adenovirus vectors to study the regulation of

Class 2  CultureVolClass2 < 1 Litre  CultureVolumeClass3-4

02/03/2022  Page 1320 of 15326
The aim of the project is to use replication-defective disabled adenovirus vectors expressing GFP and/or full length and mutated forms of transcription factors and their known putative regulators to study transcription and cell proliferation. For example Brf1 will be used for gene transfer into cells such as human diploid fibroblast (IMR90) and mouse embryonic fibroblast (MEF) cell lines, and full length id2 and E47 for gene transfer into cells such as the HeLa cell line. The purpose of the experiments is to study the role of id2, E47 and Brf1 in the regulation of transcription of RNA polymerase III.

The cell lines to be used will be a range of established human and mouse cell lines, for example MEF, IMR90 and HeLa, and pose no significant hazard to human health when used under appropriate containment.

The vectors were originally generated using the commercially available replication-defective disabled adenovirus vector system termed AdEasy, (Quantum Biotechnologies, Montreal, Canada). The vector is based on adenovirus serotype 5 (Ad5). Wild type Ad5 is a human pathogen and classified in hazard group 2. The Adeasy adenovirus vector is deleted for E1 and E3 virus sequences rendering the virus replication defective. The virus s unable to replicate except in a complementing cell lines such as 293 cells (a human embryonic kidney cell line which expresses the left 11% of the Ad5 genome). These viruses have the potential to infect human cells when they have been packaged but are extremely labile and do not have the capability to replicate or produce infective virus. The adenoviruses also lack factors found in wild type adenoviruses which are involved in evading immune response. The replication defective vector can be considered unlikely to cause disease and designated as Class I.

Origin & function

It is not anticipated that the inserted sequences will alter the host range or tissue tropism of the virus. Some of the genes we wish to express may encode oncogenic proteins. Current knowledge indicates that the de-novo transformation process of human cells requires the perturbation of six pathways (Rangarajan et al, Cancer Cell, 2004 (2):171-83) and therefore manipulation of individual pathways is not expected to confer a serious cancer risk to normal human cells. None of the genes we wish to manipulate encode toxins. Hence, it is unlikely that replication defective retroviruses would be effective carcinogens in humans in the short term. Single oncogenes are not generally considered...
sufficient to convert primary human cells to full malignancy, and replication defective viruses are not capable of dissemination even in a permissive host, however there is potential for harm to human health in the event of infection. It will however, be important to prevent human exposure or environmental release of the replication defective adenoviruses which are used to transiently to effect the transfer. This will be achieved through the use of appropriate physical containment and inactivation processes.

The possibility of exposure to recombinant viruses generated by recombination with wild type virus need to be considered and may, although unlikely, cause pathological effects in humans. Careful attention will, therefore, be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation.

The final GM human cells will contain stably integrated DNA copies of the adenoviral vectors encoding the transferred genes listed above. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside of laboratory culture.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk to human health it will be essential to prevent exposure to adenovirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level 2 laboratories, with access restricted to authorised staff. The amount of adenovirus handled will be limited to no more than 25+ - ml of virus stock.

The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Containment Level II laboratories for final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Containment Level II laboratories and autoclaved prior to final consignment to waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area.

Positive clones will be expanded and checked for virus production before removal from the Containment Level II laboratory. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells or assay of reverse transcriptase activity. Once these criteria are met the infected target cells will be removed from Containment Level II containment and handled using standard tissue culture processes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The project was approved as Class II by the Safety Committee in October 2008.

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**Project Ref** 25/09.1

**Date Ackn'd** 19/03/2009

**CU2 Project Title**

REPORTER ANALYSIS OF CANCER- AND SENESCENCE-ASSOCIATED PROMOTER ACTIVITIES IN CANCER CELL LINES AND PRIMARY CELLS USING LENTIVIRUS CONSTRUCTS.

**Class** Class 2

**Culture Vol Class 2** ≤ 1 L

**Consent Granted** Yes

**Non-GMM**

**Historical Significant Changes**

Withdrawn **N**

**Historical Date of Additional Info**

This project has transferred to GM383

**Date of Significant Change**

02/03/2022

**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to generate replication-defective lentiviruses that express marker genes including firefly or renilla luciferases and/or destabilised variants, GFP and related fluorescent proteins or bacterial nitroreductase under the transcriptional control of various human, viral, or synthetic gene regulatory elements including normal or sitemutated variants of the human telomerase hTR and hTERT promoters, the human p16, p21 and CDK4 promoters and commonly used "constitutive" viral promoters. The constructs will be used to assess basal activity and, through the use of small molecules, siRNA, or transient overexpression of cell signalling genes, regulatory mechanisms of cancer and...
senescence associated gene promoter by reporter assay or cell ablation studies in a variety of cancer and normal cell types including primary haematopoietic and stem or progenitor cells.

**Recipient or parenteral organism**

Recipient cells will include both commonly used normal (untransformed) cell strains such as W138 and MRC5 and common cell lines that are widely used by research laboratories and are not considered to pose any inherent hazard to human health nor have the capacity to survive outside laboratory and do not harbor any adventitious agents.

Examples of cell lines to be used include:

- Additional recipient cells include sub-clones of common cell lines such as 293T, A2780-CP70 and HCT116-p53/- and stably transfected variants such as BJ-hTERT wherein the genetic and epigenetic traits associated with the sub-clone or transgene are not considered to increase the overall inherent hazard to human health of the cell line itself or following transduction with lentivirus.
- Additional recipients are to include normal or transformed primary human cells obtained from donors with appropriate consent such as T-lymphocytes, CD34+ haematopoietic progenitors, mesenchymal stem cells and cells derived from ascitic fluid of patients displaying advanced tumours of the peritoneal cavity. Additional recipients are to include primary cells from animals obtained under appropriate license including rat primary neural cells. All such primary cells will be cultured exclusively under category II containment.

**Host/vector system**

Host Bacteria for growth of the plasmids are standard disabled laboratory strains of E.Coli such as DH5α and JM109 or the Invitrogen proprietary strains Stbl£ (F-mcrB mrr hsdS20(rB-, mB-) recA13 sup E44 ara-14 galK2lacY1 proA2 rpsL20(StrR) xyl-5 - leu mtl-1) or TOP10 (F-mcrA *(mrr-hsdRMS-mcrBC) *80lacZ*M15 *lacX74 recA1 araD139 *(araaleu) 7697 galU galK rpsL (StrR) end A1 napG).

The system to be used for lentivirus construction is the Invitrogen ViraPowere Promoterless Lentiviral Gateway Expression System (www.invitrogen.co, catalogue number K5910-00). The system combines gateway cloning and lentiviral cloning technologies for construction of a 3rd generation lentivirus pseudotyped with the G Glycoprotein gene from vesicular stomatitis virus. The system allows incorporation of any transgene and gene regulatory element in the lentiviral vector. The system comprises:

1. The pENTR 5'-TOPO® TA Cloning Kit for production of a gateway entry clone containing a gene/promoter of interest
2. The vector contains no sequences necessary for virus production.
3. A promoterless pLenti6/R4R2/V5-DEST destination vector into which the promoter and gene of interest are transferred. This expression plasmid contains elements that allow packaging of the construct into virions.
4. Additional packaging plasmids pLP1, pLP2, and pLP/VSVG. And 293FT packaging cells. The packaging cells will be 293FT which will be used for the initial rescue of the virus from transfected DNA and for general virus propagation and will also express the marker genes of interest.

Vector features necessary for viral production:

- pLenti6/R4R2/V5-DEST:
  - Rous Sarcoma Virus (RSV) enhancer/promoter for Tat-independent production of viral mRNA in the producer cell line.
  - Modified HIV-1 5' and 3' Long Terminal Repeats (LTR) for viral packaging and reverse transcription of the viral mRNA (Note: The U3 region of the 3’ LTR is deleted (*U3) and facilitates self inactivation of the 5’ LTR after transduction to enhance the biosafety of the vector.
- HIV-1 psi (*) packaging sequence for viral packaging.
- HIV Rev response element (RRE) for Rev-dependent nuclear export of unspliced viral mRNA.
- pLP1:
  - HIV gag/pol coding sequences encoding genes required for structure and replication of the virus.
- pLP2:
  - HIV Rev OTF under transcriptional control of the RSV promoter encodes the Rev protein, which interacts with the RRE on pLP1 to induce gag and pol expression, and on the pLenti6/V5 expression vector to promote the nuclear export of the unspliced viral RNA for packaging.
- pLP/VSVG:
  - VSVG coding sequence under control of the CMV promoter encodes the envelope G glycoprotein from Vesicular Stomatitis Virus to allow production of a pseudotyped
The system comprises numerous biosafety features designed to produce a self-inactivating, replication defective lentivirus with a negligible risk of homologous recombination between the different plasmid elements. Risk of mobilisation is therefore negligible.

- A deletion in the 3' LTR (*U3) results in "self-inactivation" of the lentivirus after transduction of the target cell.
- The number of genes from HIV-1 that are used in the system has been reduced to three (gag, pol, and rev).
- The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope.
- Genes encoding structural and other components required for packaging are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent recombination events.
- None of the packaging plasmids contain LTRs of the * packaging sequence. Thus, none of the HIV-1 structural genes are present in the packaged viral genome.
- The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.
- Expression of the gag and pol genes is prevented in the absence of Rev.
- A constitutive promoter (RSV promoter) has been placed upstream of the 5' LTR in the pLenti6/R4R2/V5-DEST vector to offset the requirement for Tat.

* replaces a symbol as symbols can't be shown

**Origin & function**

No protein is expected to be expressed in bacterial strains used for plasmid propagation. The marker gene products will be expressed in the transfected cells used for virus propagation and in the target cells. All of the protein coding gene sequences to be used in this study have previously been widely used in similar studies and none are considered to present a significant hazard to human health.

Renilla and firefly luciferases derived from commercially available pGL3 or pGL4 (firefly) or phRG orpRL vector families (Promega) encode reporter proteins which catalyse luminescent conversion of the substrates luciferin and coelenterazine.

Nitroreductase (NTR) is an E.Coli derived flavoprotein which catalyses the reduction of a variety of nitroaromatic small molecules such as the nontoxic substrate CB1954, which is converted to a toxic bifunctional alkylating agent in NTR expressing cells. NTR has been used by several laboratories for enzyme/pro-drug gene therapy applications and cell ablation studies and has been safely expressed in human tissues in clinical trials.

Fluorescent proteins such as GFP (green fluorescent protein), or related proteins CFP, RFP, and YFP are used widely as reporter proteins to mark cells in which transgene expression is induced by exposure of transfected cells to appropriate wavelengths of light.

The hTR and hTERT promoter sequences direct expression of the encoded transgenes exclusively in cells with telomerase activity such as cancer cells or some stem cells. Senescence associated gene promoters p16 and p21 are expected to limit transgene expression to cells undergoing senescence. Viral promoters such as SV40 or CMV will be used as positive controls and are expected to direct expression of the encoded transgenes in all cells

**Evaluation of foreseeable effects**

The final genetically modified cells will contain stably integrated DNA copies of the lentiviral vectors encoding the transgene expression cassettes. The cells will be free of lentivirus. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside the laboratory culture. Nevertheless, it will be important to prevent human exposure or environmental release of the replication defective lentiviruses which are used to transiently effect gene transfer. The worse case scenario in case of infection is integration at a site that disrupts expression of a gene necessary for tumour suppression. Risk against this will be reduced through the use of appropriate physical containment and inactivation processes. Hazard containment II is therefore recommended and all work will be carried out in Class II biological safety cabinets.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
To minimise the risk to human health it will be essential to prevent exposure to retrovirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Class II containment laboratories, with access restricted to authorised staff. The amount of retrovirus or lentivirus handled will be limited to no more than 25ml of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area.

Positive clones will be expanded and checked for virus production before removal from the category II laboratory. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells or assay of reverse transcriptase activity. Once these criteria are met the infected target cells will be removed from Class II containment and handled using standard tissue culture processes.

The proposal was reviewed and approved as Class II at the Safety Committee Meeting on the 13th May 2008.

Project Containment

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Project Ref 25/12.1

Retroviral infection with tRNA genes to assess their effect on cancer progression and...

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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
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<td>13/03/2012</td>
<td>Retroviral infection with tRNA genes to assess their effect on cancer progression and</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Replication deficient retroviral vectors (pLHCX) will be used to deliver tRNA genes to normal and cancer cell lines. This strategy will then be used to analyse the effects of overexpressing tRNA genes on cancer progression and metastasis. Commercially available recipient cell lines will host a helper cell aided retroviral vector (pLHCX) containing an insert with a putative cell proliferation capability.

Recipient or parental organism

Commercially available, authenticated cell lines which are widely used within the international research community and cannot survive outside laboratory culture such as, Telomerase- immortalised fibroblast, Mouse epithelial fibroblasts, Prostate (PC3) and Melanoma-epithelial (WM266.4) cell lines will be used. As a result they are not considered to pose any inherent risk to human health or the environment.

Host/vector system

Replication-incompetent retroviral particles will be produced by transfection of pLHCX (containing our gene of interest) into a commercially available amphotropic ‘Phoenix-ampho’ (helper) cell line.

pLHCX is a replication-incompetent retroviral vector (Clontech used to overexpress genes of interest. It allows transient and stable expression of the gene of interest in selected cell lines, and the production of retroviral particles in retroviral packaging cell lines. pLHCX does not contain the structural gene Gag, Pol and Env necessary for viral particle production. The introduced viral particles cannot therefore replicate within the recipient (e.g. PC3 or WM266.4) cell lines, as the particles do not contain the viral structural genes, gag, pol and env - which remain in the ‘Phoenix’ (helper) cells and prevents the production of replication-competent virus due to recombination events during cell proliferation. As a result they are not considered to pose any inherent risk to human health or the environment.

Origin & function

Overexpression of tRNA imet has been shown to increase cell proliferation in mouse cell lines (MEFs): Cell: 2008 Apr 4;133(1):78-89. This has not been demonstrated in human cell lines.

Evaluation of foreseeable effects

It is not anticipated that the inserted sequence will alter the host-range or tissue tropism of the virus.
Current knowledge indicates that the de-novo transformation process of human cells requires the perturbation of six-pathways (Rangarajan et al, Cancer Cell, 2004 (2):171-83) and therefore manipulation of individual pathways is not expected to confer a serious cancer risk to normal human cells. None of the genes we wish to manipulate encode toxins.

Hence, it is unlikely that replication defective retroviruses would be effective carcinogens in humans in the short term. Single oncogenes are not generally considered sufficient to convert primary human cells to full malignancy, and replication defective viruses are not capable of dissemination even in a permissive host, however there is potential for harm to human health in the event of infection. It will however, be important to prevent human exposure or environmental release of the replication defective retroviral particles which are used transiently to effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes.

The highly unlikely possibility of a recombination event occurring between the created recombinant viral particles with wild type virus needs to be considered and may cause pathological effects in humans. Careful attention will therefore, be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation.

The final GM human cells will contain stably integrated DNA copies of the viral vectors encoding the transferred genes listed above. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside of laboratory culture.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus generation and infection of target cells will be carried out in Class II Biological safety cabinets in the Containment Level II laboratories, with access restricted to authorised staff.

All viral supernatants and spent medium will be inactivated using 10,000 ppm free chlorine before removal from the Containment Level II laboratories for final disposal. Solid waste will be double bagged in biohazard bags prior to removal from the Containment Level II laboratory and then autoclaved before final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells and assays of reverse transcriptase activity. Only once these criteria have been met will the infected target cells be removed from Containment Level II laboratory and handled using standard tissue culture procedures.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

The project submitted to the BSO for assessment, modification and amendment prior to its presentation and approval as Class II at a meeting of the Safety Committee in February 2012.

Project Containment

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<tr>
<td>Animal Units</td>
<td>L2 L3 L4 L2</td>
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| Project Ref 25/13.1 |

Date Ackn'd: 25/06/2013
CU2 Project Title: The construction and evaluation of adenovirus vectors as therapy for cancer
Class: Class 2
CultureVolClass2: < 1 Litre
Consent Granted

Date Project Ceased: 29/04/2014

Withdrawn: N
Tick if notifying a connected programme of work: N

Historical Significant Changes
Transferred to GM383 29/04/2014

Project Additional Information

Purposes of the contained use

The aim of this project is to amplify wild-type and deletion mutant oncolytic adenovirus vectors already produced in the applicant’s previous lab; to evaluate their anti-tumour efficacy in in vitro and in vivo models of human malignancy, particularly ovarian cancer.

Adenovirus serotypes Ad5, Ad11 and Ad35 will be used as reference standards for work performed with Ad5 (mainly E1A) deletion mutants.
Replication competent and replication defective mutants will be constructed using the 'Ad-Easy' system to infect permissive human cell lines. These new mutants may encode transgenes that could induce tumour cell killing alone or enhance the anti-tumour properties of replication-competent deletion mutants. Evaluation of viral efficacy of mutants will be performed on Human and Murine cell lines by a variety of methods. Viral manufacture of replication defective vectors will be performed in HEK293 cell line to provide the E1 'in-trans'.

Concentration of adenoviral constructs from 750ml final cultures will be via Caesium Chloride centrifugation.

Recipient or parental organism

The recipient cell lines HEK293 or A549 will be used. Both are commercially available cell lines which require specific laboratory culture and as such pose negligible risk to humans. HEK 293 is rated Biosafety level 2 because of an E1 fragment of adenovirus embedded in it. It is this fragment that is essential to this series of experiments with E1 deletion adenoviral mutants. Cell line A549 is rated Biosafety level 1.

Host/vector system

One vector system will be oncolytic deletion mutants of Adenovirus. Replication of oncolytic deletion mutants of Adenovirus is attenuated in normal cells/tissue. Multiple mechanisms for this attenuation exist, depending upon the nature of the deletion. Publications attest to the selectivity of replication –between normal human cells compared to a panel of human malignant cells – see Heise et al Nature Med (2000) 6:1134. In addition, multiple phase I and II trials of oncolytic adenovirus have shown no replication in normal tissues.

Wild-type adenoviral vectors, used as reference standards, are replication competent and could infect humans. However, wild-type adenovirus infection in humans with an intact immune system leads to a mild and self-limiting 'flu-like illness.

Origin & function

Mammalian genes are to be inserted into the adenovirus genome and have been chosen to perform their intended function; either to increase tumour-specific killing by the virus (e.g. tumour suppressor or apoptosis-inducing genes), to inhibit the activity of oncogenic pathways or to increase the immunogenicity of infected tumour cells. Documentation exists to indicate that this can be achieved safely. No wild-type oncogenes or direct toxins will be inserted.

The virus will be applied to various established human and murine ovarian carcinoma cell lines; obtained from ATCC and other reputable sources and have consequently been screened for bloodborne viruses. In addition, the human cell lines have also all been previously STR verified and are known to be mycoplasma-free.

Evaluation of foreseeable effects

The deletions induced in adenovirus vectors are designed to narrow the host range, so that there is reduced replication and killing within normal cells, thus increasing the therapeutic index in ovarian cancer cells. Mechanisms to achieve this will include introduction of specific deletions to restrict replication and the use of tumour and tissue-specific promoters.

During process development for clinical grade manufacture of one of the mutants being used, no recombination between vector and HEK293 sequences was detected (limit of detection 1 in 109 genomes) – thus it is believed that the risk of reversion to wild-type virus following amplification in HEK293 cells is extremely low.

Replication-deficient viruses are incapable of replication in cells lacking adenovirus E1 function. Infection of normal human cells as well as malignant cells results in no infectious virion production.

The replication competent adenoviruses used will contain mutations within the viral genome that attenuates replication in normal cells and tissues. The deletions occur in regions of the viral genome that are necessary for replication in normal cells but are complemented by the altered gene expression in cancer cells. Mutants used can only replicate efficiently in cells with deregulated p53 and pRB pathways respectively.
The tumour selective mutants can infect normal cells but the ability to replicate is highly attenuated.

Wild-type adenoviral vectors are replication competent and could infect humans. Wild-type virus infection in humans with an intact immune system leads to a mild and self-limiting 'flu-like illness.

The normal route of adenovirus infection is via aerosol. The use of correctly trained and supervised staff, Class II biological safety cabinets, lab coats and gloves, disinfection and autoclaving of waste will reduce the likelihood of exposure to an extremely low level.

The virus will be applied to various established human and murine ovarian carcinoma cell lines. Because ovarian cancer cells do not contain any adenovirus genomic DNA, there is no risk of recombination of deletion mutants to generate wild-type revertant virus. Thus, generation of adenoviruses and infection of ovarian cancer cells is not considered to pose any inherent hazard to human health. The GM viruses do not have the capacity for survival outside of the laboratory. The recipient cells will therefore not have any foreseeable effect on human health.

E.coli strains are highly attenuated laboratory strains and do not present any hazards to humans.

Human adenoviruses do not replicate in murine tissue - this is true of wild-type, replication-restricted oncolytic viruses and replication-defective E1-deleted vectors.

The only replication following injection into in vivo tissue will be in malignant tumour tissue.

All injections of virus into vivo tissue will be undertaken in class II hoods within the specialist unit, thus minimising the risk of an aerosol-mediated infection during the injection process.

Multiple human studies demonstrate that virus shedding after intraperitoneal, intravenous or intratumoural injection is undetectable. Thus, the risk to humans from murine cell line tissue is negligible as the viruses are replication-deficient or tumour specific and the gene products are not considered harmful to health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Full containment level 2 will be used, as set out in the Regulations, including appropriate treatments for bulk, contaminated solid waste.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be used, as set out in the Regulations; i.e. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level II laboratories, with access restricted to authorised staff. Including appropriate treatments for bulk, contaminated solid waste.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All viral supernatants and spent medium will be inactivated using 10,000 ppm free chlorine before removal from the Containment Level II laboratories for final disposal. This process will result in 100% inactivation of infectious virus.

Solid waste will be bagged in biohazard bags prior to removal from the Containment level II lab and then placed in boxes which are sealed prior to removal from the CL2 suites. Boxes remain sealed and are autoclaved before the contents is removed for collection by accredited waste contractors. Autoclaves produce a digital record of load temperature achieved and are validated by annual thermocouple mapping. This process will result in 100% inactivation of infectious virus.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N
Questions were raised and answered regarding management of containment during centrifugation of virus.

**Project Containment**

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**Project Ref** 25/14.1

- **Use of a Lentiviral based system to remove genes of relevance to cancer progression**
- **Class Culture Vol Class 2 Culture Volume Class 3-4**
  - Class 2
  - < 1 Litre
- **Non-GMM Consent Granted**
- **Project notified under transitional arrangements**
- **Withdrawn**

**Project Additional Information**
Purposes of the contained use

To generate cell lines which have had specific genes "knocked out" using LentiCRISPR / CAS9 technology (Clustered Regularly Interspaced Short Palindrome Repeats), by using specific unique guide sequences obtained from the Genome - scale CRISPR Knock - Out (GeCKO) library; which targets 18,080 genes using 64,751 of these guide sequences.

The need for contained use stems from the use of a lentiviral based delivery system of the components which remove target genes. Target genes include those involved in actin binding and regulation, apoptosis, autophagy and lipid metabolism. It is possible, but not an immediate priority that P53 and P53-like genes may be targeted.

The lentiviral based system designed to deliver CAS9 and target guide RNA to recipient cells. The lentiviral vector is LentiCRISPR (Shalem et al Science 343, 84-87 (2014)) and contains a safety modified WPRE component (has been truncated in such a way as to remove the promoter region and any open reading frame for the X protein). The LentiCRISPR used here is based on an pLKO vector backbone. This will be transfected into 293T cells along with PSPAX2 and VSVG packaging vectors to produce lentiviral particles. The guide sequences that will be cloned into LentiCRISPR will target murine and/or human cell lines.

Host/vector system

The "LentiCRISPR" vector systems utilise a replication-incompetent lentiviral vector chosen for expression of CAS9 and guide RNAs (Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells Shalem et al, Science 343, 84-87 2014). Different versions exist employing different lentiviral backbones.

This lentiviral system allows for the production of viral particles using lentiviral packaging cell lines and includes a number of safety features designed to enhance its biosafety and to minimize its relation to the wild-type, human HIV-1 virus. In doing so, this system, like may others, prevents the generation of recombinant viral particles that possess the required functional Gag-Pol structure for DNA mobilization and the emergence of replication competent lentivirus.

LentiCRISPR contains 2 antibiotic resistance markers, ampicillin as bacterial selection marker and puromycin for mammalian cells.

Although the packaging and envelope plasmids psPAX2 and pVSVG allow expression in trans of genes required to produce (lenti)viral progeny (e.g. gag, pol, rev, tat, env) in the TLA-HEK293T producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell.

The replication-defective viruses are extremely labile and only infective when produced in the appropriate packaging cell line together with the required packaging vectors.

As noted in section 6 (above) the WPRE component has been modified to remove effects from "X protein".

Origin & function

Parental organisms (cell lines) are losing targeted genetic material through these procedures. The targeting of genes (of interest) is accomplished using a technique employing a Genome - scale CRISPR Knock - Out (GeCKO) library which targets 18,080 genes using 64,751 unique guide (RNA) sequences.

The genes removed relate to metabolic pathways associated with cancer (Actin binding and regulation, Apoptosis, Autophagy and lipid metabolism) and could not be foreseen to increase risk. Tumour suppressors such as P53 or P53-like genes (repressors of oncogenic behaviour) may cause oncogenic behaviour limited to the cell lines which are treated.
Evaluation of foreseeable effects

The removal of the genes of interest are not foreseen to increase the risk of an unfavourable event. Removing P53 or P53-like genes (repressors of oncogenic behaviour) may cause oncogenic behaviour in the cell lines which are treated but that will be limited to those cell lines alone.

Hazards arising directly from the inserted gene product:
Guide RNAs will be expressed, and in conjunction with CAS9 will result in deletion of the target genes listed. These target genes have been proposed to play a role in cancer metabolism. Based on current knowledge, deletion of any of these genes is not expected to impart significant hazard.

Hazards arising from the alteration of existing pathogenic traits (e.g. alteration of host range or tissue tropism):
It is not expected that the inserted guide sequences would alter either the host-range or target tropism of the virus.

The possibility of potentially hazardous sequences within the GMM being transferred to related micro-organisms is considered extremely low and would require additional viruses to be present. Both virus-producing and recipient cells are certified virusfree from the supplier.

The genetically modified cells will contain stably integrated DNA copies of the lentiviral vectors encoding the inserts. These cells will not produce lentivirus. Consequently, the cells pose no hazard to human health, since they cannot grow outside of a laboratory environment.

The possibility of plasmid inserts recombining with native lentiviruses could theoretically lead to widespread infection. However, this remains a remote possibility, and even if it were to occur, would be expected to have no detrimental effects due to the nature of the proteins encoded by these inserts, as described above.

To minimise the risk to human health it will be essential to prevent exposure to lentivirus. Careful attention will be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation.

Accidental injection, inhalation or ingestion of genetically modified (GM) cells such as these is not considered (in the wider GM community) to pose a threat of actual harm.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level II suite, with access restricted to authorized and trained staff.

The amount of virus-containing medium handled will be limited to no more than 25ml.

The most likely routes through which individuals could be exposed to virus are needle-stick injures or aerosols generated during experimental procedures. Plastic ware will be used throughout and no needles or sharps will be used during virus preparation.

The use of the Class II biological safety cabinet will guard against exposure to aerosols.

All cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm free chlorine) before removal from the Containment Level II suite for final disposal.

Solid waste will be double bagged in biohazard bags prior to removal from the Containment Level II suite and then autoclaved before final consignment of waste to the approved contractor.

Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100%
inactivation of infectious virus. Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Containment Level II suite that are not worn in the general laboratory area. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells and assays of reverse transcriptase activity. Only once these criteria have been met will the infected target cells be removed from Containment Level II suite and handled using standard tissue culture procedures.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Discussed and approved.

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Project Ref 25/15.1

Date Ackn'd  
05/01/2015

Date Project Ceased  
01/01/2018

CU2 Project Title  
Evaluating the anti-tumour efficacy of oncolytic adenovirus and oncolytic Herpes Simplex Virus

Class  
Class 2

Culture Vol Class 2  
< 1 Litre

Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work

02/03/2022  
Page 1335 of 15326
**Project Additional Information**

**Purposes of the contained use**

The purpose of the project is to evaluate the anti-tumour efficacy of oncolytic adenovirus and oncolytic Herpes Simplex Virus, in in-vivo models of human malignancy, particularly ovarian cancer. This will be achieved using Wild-type Ad5, Ad11, Ad35 adenoviral vectors as well as a number of Ad5 deletion mutants. Herpes Simplex Virus vectors are based upon HSV-1 single gene “vector 1716” mutants (neuro-virulence gene ICP34.5) as well as derivatives of this single mutant encoding fluorescent markers. Double deletion mutants (e.g. ICP34.5 and ICP47), with increased selectivity for malignant cells may also be evaluated. Novel derivatives of the adenovirus and HSV vectors (encoding transgenes that could induce tumour cell mortality alone or enhance the anti-tumour properties of replication-competent deletion mutants) will be evaluated.

In some viral treatments, tumour and tissue specific promoters (e.g. CEA, PSA) may be used to drive virus replication (via transcriptional control of E1A transcription) or to drive transgene expression.

**Recipient or parental organism**

Viruses are to be evaluated in various human and murine established carcinoma cell lines growing within subjects (either immunodeficient for human cell lines, or immunocompetent for murine cell lines). These cell lines have been obtained from reputable sources and have consequently been screened for HIV, HepB, HepC and EBV.

Because these tumour cells do not contain any adenovirus or HSV genomic DNA, there is no risk of recombination of deletion mutants to generate wild-type revertant virus. ICP34.5-deleted HSV vectors have been injected into over 200 human patients with advanced malignancy - there are no records of any wild-type reversion in any patient. The recipient cells are not therefore, considered to pose any inherent hazard or foreseeable effect to human health.

**Host/vector system**

Replication of oncolytic deletion mutants is attenuated in normal cells/tissue, depending upon the nature of the deletion and the nature of the vector. Multiple publications from the proposer's lab and others attest to the selectivity of replication – see Heise et al Nature Med (2000) 6:1134. In addition, multiple phase I and II trials of oncolytic adenovirus as well as E1-deleted non-replicating vectors have shown no replication in normal tissues.

Wild-type adenoviral vectors are replication competent and could infect humans. However, wild-type adenovirus infection in humans with an intact immune system leads to a mild and self-limiting 'flu-like' illness.

ICP34.5-deleted HSV is dramatically attenuated in normal non-malignant tissue including neuronal cells, both murine and human. Direct intra-cranial inoculation of 1716 has been demonstrated to be safe in subjects. No wild-type HSV-1 will be used in any experiment.

**Origin & function**

Genes that will be inserted into the adenovirus or HSV genome are designed either to increase tumour-specific mortality by the virus (e.g. tumour suppressor or apoptosis-inducing genes), to inhibit the activity of oncogenic pathways or to increase the immunogenicity of infected tumour cells. There are multiple examples in the
literature where this can be achieved safely. No wild-type oncogenes or direct toxins will be included.

The deletions induced in adenovirus and HSV vectors are designed to narrow the host range, so that there is reduced replication and mortality within normal cells, thus increasing the therapeutic index in ovarian cancer cells. Mechanisms to achieve this will include introduction of specific deletions to restrict replication and the use of tumour and tissue-specific promoters.

During process development for clinical grade manufacture of one of the mutants, no recombination between vector and HEK293 sequences was detected (limit of detection 1 in 109 genomes) – thus it is believed that the risk of reversion to wild-type virus following amplification in HEK293 cells is extremely low. For HSV, there is no evidence from any previous pre-clinical or clinical study that HSV vector 1716 can transfer genetic material to other organisms.

**Evaluation of foreseeable effects**

Replication competent adenoviruses contain mutations within the viral genome that attenuates replication in normal cells and tissues. The deletions occur in regions of the viral genome that are necessary for replication in normal cells but are complemented by the altered gene expression in cancer cells. These mutants can only replicate efficiently in cells with deregulated p53 and pRB pathways. Tumour selective mutants can infect normal cells but the ability to replicate is highly attenuated. Human adenoviruses do not replicate in murine tissue - this is true of wild-type, replication-restricted oncolytic viruses as well as replication-defective E1-deleted vectors. The only replication following injection into tumour-bearing subjects will be in malignant tissue. The normal route of adenovirus infection is via aerosol. 1716 and other ICP34.5-deleted HSV vectors have been administered to over 200 patients with cancer with no significant toxicity. All vectors proposed contain an intact thymidine kinase gene, and thus HSV-infected cells are sensitive to treatment with the anti-viral drugs aciclovir and ganciclovir. All injections of virus will be undertaken in class II hoods within the unit, thus minimising the risk of aerosol-mediated infection during the injection process. Multiple human studies demonstrate that virus shedding after intraperitoneal, intravenous or intratumoural injection is undetectable. Thus, the risk to humans from subjects is negligible as the viruses are replication-deficient or tumour specific and the gene products are not considered harmful to health. In addition, laboratory standard operating procedure in relation to biological agent handling and waste management are such that the likelihood of exposure to a biological agent is reduced to an extremely low level. The risk to human health is low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste to be double bagged in biohazard bags prior to removal from the Containment Level 2 laboratory and then autoclaved (15 mins @ 134.C) [verified by thermocouple] before collection by licensed professional waste contractors.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

Considered and accepted by GMSC

02/03/2022
The aim of this project is to test the differential effect of certain proteins on cell survival, growth and metabolism in relation to the p53 status of cell lines. This will be done by altering the cells using engineered replication-defective lentiviruses that express proteins that are important in the pathways underlying mutant p53 ‘gain-of-function’ in tumours; such as amino acid transporters (such as Slc7a11, Slc3a2), enzymes involved in amino acid metabolism (such as PRODH), and enzymes involved in regulation of bioenergetic pathways.
Recipient or parental organism

The cell line to be used in the first instance will be: MDA-MB-231 a Human breast tumour cell line. MDA-MB-231 is not considered to pose any inherent hazard to human health, nor does the line have the capacity to survive outside the laboratory. The cells will therefore not exert any foreseeable effects on either human health or the environment.

The other cell lines which may be used are of a similar biosafety risk (1) and include:

- Human breast tumour cell lines (MCF-7, MCF10A), Human colon tumour cell lines (HCT116, HT29, SW480, RKO), human ovarian cancer cell lines (A431, PEO1, PEO4, OVCAR3, OVCAR4, CAOV3, COV318), RPE-normal retinal pigment epithelial cells immortalized with h-TERT, Osteosarcoma cell lines (U-2 OS, Saos-2), and a non-small cell lung carcinoma (H1299). All of these cell lines are incapable of producing the replication defective virus used as the vector.

Host/vector system

The ViraPower Lentiviral Expression System (Invitrogen) used is a third generation vector system based upon a HIV-1 lentivirus. It uses a pLenti expression vector that contains many features which prevent the possibility of new replication-competent virus being produced. This includes a non-homologous four plasmid system that allow expression in 'trans' of proteins required to produce viral progeny (e.g. gal, pol, rev, and env) in the 293FT producer cell line. None of the plasmids contain LTRs or the \( \Psi \) packaging sequence, which means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell.

The virus produced uses VSV-G envelope protein rather than that of HIV-1. A deletion in 3’ LTR results in “self-inactivation” of the lentivirus after transduction of the target cells. As a result, once integrated, the lentiviral genome is no longer capable of producing a packageable viral genome.

Origin & function

No protein is expected to be expressed in bacterial strains used for plasmid propagation. The proteins will be expressed in the transfected cells used for virus propagation and in the target cells. Sequences encoding fluorescent proteins (such as enhanced Green Fluorescent Protein (eGFP) and mCherry) may also be inserted. They will function purely as reporter molecules, fluorescing upon exposure to UV light. Human proteins: No known hazard has arisen from expression of the cDNA’s of the tumour suppressor protein p53, the point mutants of p53, the metabolic
genes identified as potential targets of p53 and its mutants [including amino acid transporters (such as Slc7a11, Slc3a2)], enzymes involved in amino acid metabolism (such as PRODH) and enzymes involved in regulation of bioenergetic pathways.

Mutant p53 proteins have been shown to contribute to transformation and should be treated as being as oncogenic as myc and activated Ras proteins. These proteins have been widely studied in a similar capacity (e.g. overexpression by transient or stable transfection) by our lab and others without any reportable hazard. However, to maintain safe working practices, these experiments will be carried out under Containment Level II conditions with appropriate precautions.

**Evaluation of foreseeable effects**

Correct use of the replication defective lentiviral vectors carrying the inserts described above is unlikely to be hazardous to human health. The possibility of widespread infection is very small due to the requirement for 'helper' sequences. There is a minor risk that the virus may integrate at a locus which could be detrimental to health or that the virus could recombine to generate an agent that could cause pathological effects in humans. Careful attention will, therefore, be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation. Level 2 containment will be implemented using biological safety cabinets and appropriate procedures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk to human health it will be essential to prevent exposure to lentivirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level II tissue culture laboratory, with access restricted to authorised staff. The amount of lentivirus handled will be limited to no more than 25ml of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injures or aerosols generated during experimental procedures. Plastic pipettes will be used throughout and no needles or sharps will be used during virus preparation, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosols. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Containment Level II laboratory for final disposal. Solid waste will be double bagged in biohazard bags prior to removal from the Containment Level II laboratory and then autoclaved before final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious
virus. Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells and assays of reverse transcriptase activity. Once these criteria have been met the infected target cells will be removed from Containment Level II tissue culture and handled using standard tissue culture procedures. Authorised staff will wear dedicated lab coats in the Containment Level II laboratory that are not worn in the general laboratory area.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Amended version of RA passed by GMSC

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3</td>
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<td>L2 L3 L4</td>
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Project Ref 25/16.1

Date Ackn'd 06/09/2016

Date Project Ceased

CU2 Project Title Lentivirus mediated overexpression of oncogenes in mouse or human cells to study proteins involved in cell signalling, tumorigenesis, invasion, metastasis, cell death and survival.

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Consent Granted

Project notified under transitional arrangements N
Replication deficient lentiviral vectors will be used to over express oncogenic moieties such as small GTPases of the RAS superfamily, their regulators, and their interacting and co-operating proteins, and may therefore produce a potentially more hazardous product. Members of the RAS example are known oncogenes and interfering with RAS signalling may offer a potential therapeutic strategy in colorectal cancer.
This over expression will be used to study the survival, apoptosis and invasion of infected cells with the intention of mapping these moieties in these cells.
Viral supernatant may be made in the laboratory or may be purchased as pre made viral supernatants such as those supplied by Genetarget Inc. San Diego. CA

Recipient or parental organism

The lentiviral packaging cells will be HEK293 and derived subtypes which will be used for the initial rescue of the virus from transfected DNA and for general virus propagation and will also express the transfected protein of interest.
Recipient of parental organism

The mouse and human tissue culture cell lines to be used will be well characterised and authenticated cell lines, sourced from reputable suppliers and will pose no inherent risks themselves or risks from adventitious agents.
Human cell lines such as Hela, U20S, MDA-MB 231, MDA-MB 453, HCT116, LNCAP, PC3, MCF-10A, DU145, Calu-3 and the [ATCC CRL-11609] strain of (HPV18- free) RWPE-1.
Mouse cell lines such as 3T3 SA, NIH 3T3, C2C12.
In addition mouse primary cells will be used which will pose no inherent risks themselves or risks from adventitious agents. The cells will therefore not present a hazard to human health.

Host/vector system

Host bacteria for growth of the plasmids are standard disabled laboratory strains of E. Coli such as DH5a, JM109 or Stbl3.
Third generation lentiviral vector systems such as those available from Genetarget Inc will predominantly be used. These contain a reporter gene (GFP or RFP) linked to a target, proprietary CMV promotor (suCMV) and an optional tetracycline inducible CMV promotor. Other third generation lentiviral vector systems to be used include the ViraPower™ Lentiviral Expression System (Invitrogen) which is based on the lentikit™ system developed by Cell Genesys (Dull et al., 1998 J Virol. 1998 Nov. 72(11):8463-71).

A number of features are embedded within these vectors to enhance their biosafety:

• All pathogenic genes have been removed from the wild type HIV-1 genome
• The HIV-1 envelope has been completely removed and replaced (pseudotyped) with the VSV-G gene from Vesicular Stomatitis Virus.
• The packaging genes have been separated from the transfer vector so that the packaged viral genome does not contain any packaging components.
• Sequence homology between the packaging plasmid and transfer plasmid has been reduced to the maximum extent possible to further minimize the risk of plasmid recombination during co-transfection. As a result, packaging genes of these “third generation” lentiviral vectors are non-functional outside of the virus- producing cells.
• A self-inactivating (SIN) transfer vector has been created with a deletion in the 3′-LTR region to ensure it produces only replication-incompetent virus. (Zufferey, R., et al., Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. J Virol. 72, 9873-80 (1998).)

In some instances it may be necessary to use plKO.1 derivatives vector systems; e.g. pLex and pLix. These systems include similar safety features designed to enhance its biosafety and to minimize its relation to the wild-type, human HIV-1 virus.

• The reverse transcriptase (RT) and integrase (IN) proteins are split from the native Gag-Pol polyprotein and are provided ‘in trans’ on separate plasmids.
Removing the RT & IN components from the packaging construct prevents the lentiviral replication machinery from functioning, thereby rendering these plasmids as inherently replication-deficient. This system prevents the generation of recombinant viral particles that possess the required functional Gag-Pol constructs for DNA mobilisation and the emergence of replicationcompetent lentiviral particles.

• The packaging and envelope plasmids, psPAX2 and pVSV-G, allow expression in trans of genes required to produce viral progeny (e.g. gag, pol, rev, tat, env) in the ‘packaging cells’ (293-FT), none of these contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced cells.
• The parental pLKO plasmid contains the ampicillin-resistance gene for bacterial selection, and the puromycin-resistance gene replaced by alternate mammalian selection markers, e.g. Zeocin, Neomycin, Hygromycin B, or mammalian selection markers, e.g. Zeocin, Neomycin, Hygromycin B, or Blasticidin.
• Other derivatives replace the puromycin gene with a membrane- or nucleus-targeted fluorescent protein.
• Note that these are changes that only affect the selection method in mammalian cells and do not affect any other structural feature of the viral particles, and as such do not exert any foreseeable effects on human health or the environment.
• Details of the parental plasmids:
  o pLEX_301, 302, 303, 304, 305, 306, 307,
  o pLIX_401, 402,
  o pLKO.1 can be found on the Addgene website.

Expression of genes, such as those involved in RAS signalling, are associated with cancer progression and growth, promoting cell survival, growth, and the invasive behaviour of tumour cells. It may prove harmful if such genes were inserted into normal human cells via accidental injury or inhalation.
However, current knowledge indicates that the de-novo transformation process of human cells requires the perturbation of six-pathways (Rangarajan et al, Cancer Cell, 2004 (2):171-83) and therefore manipulation of individual pathways is not expected to confer a serious cancer risk to normal human cells.
Similarly individual expression of activated oncogenes alone results in cellular senescence and / or apoptosis (Wajapeyee et al, Cell 2008 132(3):363-74).

Origin & function

Evaluation of foreseeable effects

Because of the physical segregation of components in 3rd or 4th generation lentiviral constructs, the likelihood of disease propagation beyond the culture dish is very low / negligible. However, lentiviral particles with a VSV-G envelope constitute a minor risk as they are able to infect human cells.
Exposure to most human cells from lentivirus particles is unlikely to cause infection. The likely routes of infection are through aerosol formation and needle sticks where the protective barriers of the body are bypassed.
Therefore there is a small risk to the experimenter of exposure to viral particles, during lentiviral particle delivery phase, via those two routes:
1) Inhalation of aerosol and
2) Inadvertent or accidental needle-stick injury.
There is negligible risk of aerosol inhalation as the amounts of lentiviral particle-containing solutions used during the experiment are tiny and no
In aerosol-producing manipulations (e.g. centrifugation) will be used without suitable containment.

Infection via inhalation or injection of virus would be limited to mucosal cells or cells at the site of injection. (In vivo experiments have shown (Lerchner et al. Gene Therapy (2014) 21 233-241) diffusion of infectious particles no more than millimetres from the injection site. No sharps will be used in the Containment Level II suite.

Assuming a worst case scenario: Even if the infected worker had a preexisting HIV infection, the chances of recombination are extremely small due to the design of the vector.

There is no available evidence for transfer of infectious viral particles to other vulnerable cells via the bloodstream or lymphatic system - except in cases where the intention was to transduce cells of the immune system in the bloodstream / bone marrow.

Viral particles that do not infect cells are estimated to become inactive within hours and no viable virus should remain after 48-72 hours.

It is not anticipated that the inserted sequences will alter the host-range or tissue tropism of the virus.

The probability of harmful sequences being transferred to related viruses is extremely low and would require suitable adventitious agents to be present in the cells being handled. The cells to be used should not pose any inherent risk from adventitious agents.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

To minimize the risk to human health the following safety precautions would be taken.

1. Prevent exposure to lentivirus. Careful attention will be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation. Virus infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level II laboratories, with access restricted to authorised staff.

2. Minimize volumes of virus handled. The amount of lentivirus handled will be limited to no more than 25ml of virus stock, and aliquots will be clearly marked.

3. Prevent infection routes. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. Plastic ware will be used throughout and no needles or sharps will be used during the procedure, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosols. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium.

4. Proper handling of waste.

All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm free...
chlorine) before removal from the Containment Level II laboratories for final disposal. Solid waste will be double bagged in biohazard bags prior to removal from the Containment Level II laboratory and then autoclaved, before final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

5. Usage of designated safety equipment. Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Containment Level II laboratories that are not worn in the general laboratory area.

6. Follow up on the infected cells. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells and assays of reverse transcriptase activity. Only once these criteria have been met and it has been cleared by the BSO will the infected target cells be permitted to be removed from the Containment Level II laboratory and handled using standard tissue culture procedures.

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**Project Containment**

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**Project Ref** 25/19.1

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The purpose of these experiments is to allow us to efficiently and specifically over-express R-spondin 1 in a temporal manner, to investigate the mechanisms of intestinal function in homeostasis and in preclinical cancer models.

Recipient or parental organism

C57Bl6 and mixed background subjects that may be genetically modified are to be used.

Host/vector system

Adeno-associated virus (AAV) vectors using a CMV IE promoter and dimeric hRSP1-hFc fusion will be obtained from overseas collaborators. The virus will be ready to use when arriving at the Institute. The AAV vectors are based on AAV viruses. Our collaborators have experience of using this virus and similar experiments have previously been published (Yan et al., Nature 2017. Non-equivalence of Wnt and R-spondin ligands during Lgr5+ intestinal stemcell self-renewal).

The vector can infect human cells (AAV9 is associated with infection in the intestine as well as CNS, heart, liver, lung and skeletal muscle) but it cannot replicate in them, and it cannot be mobilised. The vectors are regarded as non-pathogenic in humans and not currently known to cause any diseases in humans or animals. The vector capsid can induce a mild localized inflammatory response in humans (Mingozzi et al. Blood 2013).

Origin & function

RSPO-1 is known to modulate Wnt signalling.

The replication defective adenovirus expressing RSPO1 or a control vector
will be used for infection. RSPO-1 is documented as having an ambiguous role as either a mitogen or tumour suppressor. De Cain et al, Oncogene 2017. The purpose of these experiments is to allow us to efficiently and specifically over-express Rspondin 1 in a temporal manner. The AAV vectors contain recombinant transgene sequences flanked by the AAV inverted terminal repeats (ITRs). In dividing cells, AAV DNA is lost through cell division, since the episomal DNA is not replicated along with the host cell DNA this makes it highly unlikely that the AAV could infect a neighbouring cells. The probability of sequences being transferred to other related or unrelated species is extremely low since the virus is replication deficient. It is not anticipated that the inserted sequence will alter the host-range or tissue tropism of the virus. The possibility of transmission of the virus is extremely low since the virus is replication deficient.transfer genetic material to other organisms.

**Evaluation of foreseeable effects**

In the event of exposure it is extremely unlikely that this virus could cause harm to human health. The integration rate of AAV vectors is extremely low if it occurs at all. The risk of human exposure is low, and all appropriate care will be taken to prevent needle stick injury at the time of injection. The method of delivery through intraperitoneal injection as opposed to intravenous injection has been chosen specifically to minimise the risk of needle-stick injury. The protein produced as a result of infection will be an R-Spondin1 protein fused to an Fc domain. It has been widely reported that role of the R-spondin proteins is to potentiate Wnt-signalling in a Wnt-ligand dependent manner, which results in expansion of the Lgr5+ stem cell pool. We (and others) have demonstrated that this expansion of the stem cell pool is a tumour suppressive mechanism in vivo (Huels et al, Nature Communications, 2017; Vermeulen et al, Science, 2013).

Gene fusion events incorporating the RSPO2 or RSPO3 gene loci are found in a proportion of colorectal cancers (Seshagiri et al, Nature, 2012) where it has been postulated that they may potentiate Wnt-signalling in an APC independent fashion. Critically, these fusion proteins are not seen to exist in isolation, and are found associated with strong oncogenic driver mutations such as BRAF or KRAS and are Wnt-ligand dependent. Indeed it has been reported that the RSPO1-Lgr5 signalling axis suppresses tumour progression in colorectal cancer (Zhou et al, Cancer Research 2017). In the unlikely event of user exposure, and of a proliferative response driven by the fusion protein, the use of a non-incorporating AAV delivery system will result in the event being self-limiting.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste to be double bagged in biohazard bags prior to removal from the Containment Level 2 laboratory and then autoclaved (15 mins @ 134°C) [verified by thermocouple] before collection by licensed professional waste contractors.

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Lentiviral or retroviral infection of activated oncogenes to induce senescence

<table>
<thead>
<tr>
<th>Class CultureVolClass2 CultureVolumeClass3-4</th>
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<tr>
<td>Class 2 &lt; 1 Litre Non-GMM Consent Granted</td>
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</table>

Considered and accepted by GMSC.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Project Containment

Project Ref 383/09.1

Date Ackn'd 05/07/2012

Date Project 02/03/2022
Replication-deficient lentiviral vectors (pFG12 vector) will be used to deliver the activated oncogenes RasV12 and BRAFV600E to primary human cell lines to induce senescence. This strategy will be used to further analyze the senescence pathway.

A replication-deficient retroviral vector (pBABE-puro) will also be used to deliver the activated oncogene RasV12 to the human fibroblast cell line IMR-90 (ATCC Number CCL-186) in order to induce senescence. This strategy will be utilized in order to investigate mechanisms of the senescence pathway.

The GMM will be transfected into 293FT cells in order to produce virus and the virus will then be applied to primary human cell lines, such as fibroblasts, melanocytes and keratinocytes, which are widely employed in laboratories and have all been screened for HIV, Hep B, Hep C and EBV. There is no risk from the cells infected. Cells are typically obtained from ATCC.

The GMM (pBABE-puroRasV12) will be transiently transfected into Phoenix cells, a 293T-derived packaging cell line that contains the Gag, Pol and Env viral proteins required for making active virus. Subsequently, viral supernatants produced by Phoenix cells will be used to infect human fibroblasts (IMR-90). Both the Phoenix packaging cell line and IMR-90 fibroblasts are used in extensively in laboratories and have been screened and found to be negative for HIV, Hep B, Hep C and EBV. There is no risk from the infected cells. Cells are obtained from ATCC and have been rigorously characterized.

The ViraPower Lentiviral Expression System (Invitrogen) uses a pLenti expression vector that contains a deletion in the 3’ LTR that results in “self-inactivation” of the lentivirus after transduction of the target cells. When integrated into the target cell the lentiviral genome is therefore no longer capable of producing packagable viral genome. This third generation vector system is based upon a HIV-1 lentivirus, although it is lacking all HIV-1 genes except gag-pol and env, which are provided in trans by a transient transfection strategy. Also, the virus produced is to be pseudotyped with VSV-G envelope protein rather than HIV-1. The vector requires three other packaging plasmids that allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line. These packaging plasmids separately allow for the expression of gag and pol, a RRE-containing sequence, and the rev protein sequences from HIV-1. None of the packaging plasmids contain LTRs or the Ψ packaging sequence, and therefore, none of the HIV-1 structural genes are actually present in the packaged viral genome so they are never expressed in the transduced target cell. No new replication-competent virus can be produced. (Dull, et al., J. Virology, 8463-8471, 1998).

The pBABE vector system is a retroviral vector construct that has been derived from Moloney murine leukemia virus (MMLV). As with lentiviral constructs, the pBABE retroviral vector also requires three additional packaging plasmids that drive expression in trans of proteins required to produce viral progeny (i.e. gag, pol, rev and env) upon transfection into a producer cell line (Phoenix). The pBABEpuro vector has been designed to avoid homology between the vector and packaging constructs, in order
To decrease the change of homologous recombination in the Phoenix packaging cells.

Origin & function

The Ras and BRAF genes belong to the MAP kinase pathway, which mediates cellular responses to growth signals. When constitutively activated through mutation (RasV12 and BRAFV600E) senescence is conferred through the upregulation of the tumor suppressors p53 and p16INK4a. Activating BRAF mutations are present in up to 80% of melanomas while mutations in the ras gene are very common in adenocarcinomas of the pancreas (90%), the colon (50%), and in myeloid leukemia (30%). Although these gene products are very common in human cancers, additional mutations are required to drive a malignancy. Also, the hazards of the activated oncogene are very minimal due to the inability of the 293FT and Phoenix cells to create a replication-competent virus. In addition to the inserted oncogenes, the carrier plasmid (FG12) contains the woodchuck hepatitis virus posttranscriptional regulatory element (WRE) to increase the level of transcription. Although controversial, WRE may have oncogenic activity but should not pose a hazard due to the safety precautions that are already built in for the activated oncogenes.

Evaluation of foreseeable effects

It is not anticipated that the inserted sequences will alter the host-range or tissue tropism of the virus. Some of the genes we wish to express will oncogenic proteins. Current knowledge indicates that the de-novo transformation process of human cells requires the perturbation of six-pathways (Rangarajan et al, Cancer Cell, 2004 (2):171-83) and therefore manipulation of individual pathways is not expected to confer a serious cancer risk to normal human cells. None of the genes we wish to manipulate encode toxins.

Hence, it is unlikely that replication defective viruses would be effective carcinogens in humans in the short term. Single oncogenes are not generally considered sufficient to convert primary human cells to full malignancy, and replication defective viruses are not capable of dissemination even in a permissive host, however there is potential for harm to human health in the event of infection. It will however, be important to prevent human exposure or environmental release of the replication defective adenoviruses which are used to transiently effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes.

The possibility of exposure to recombinant viruses generated by recombination with wild type virus needs to be considered and may, although unlikely, cause pathological effects in humans. Careful attention will, therefore, be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk to human health it will be essential to prevent exposure to virus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level 2 laboratories, with access restricted to authorised staff. The amount of virus handled will be limited to no more than 40ml of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cutures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Class II laboratories for final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area.
The project was approved as Class II by the Safety Committee in December 2008

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L3 L4 L2</td>
<td>L2 L3 L4</td>
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</table>

**Project Ref 383/12.1**

**Investigating the role of apoptosis, mitochondria and autophagy in cancer through the retroviral generation of stably expressing cell lines**

**Class** Class 2  **Consent Granted**

**Culture Volume** < 1 Litre

**Date Ackn’d** 13/08/2012  **Date Project Ceased** 13/08/2012

**Project notified under transitional arrangements** N

**Withdrawn** N

**Historical Significant Changes** Project transferred to GM 25

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
Project Additional Information

Purposes of the contained use

Retroviral vectors will be used to produce retrovirus in order to generate a variety of human and murine, normal and cancer cell lines stably expressing the below proteins. These will be used to study apoptosis, autophagy and mitochondria in relation to cancer development and treatment response.

Recipient or parental organism

Producer cells: Phoenix Ecotropic and Phoenix Amphotropic packaging cells These were obtained from Dr. Gary Nolan, University of Stanford and are extensively used for retroviral generation.

Target cells: Hela, MCF-7, SVEC 4-10, NIH3T3, L929, NIH SA, SV40 transformed murine embryonic fibroblasts, U2OS, HT90, Caco-2, Jurkat. These cell lines are widely used by research laboratories, are well characterized, and have been obtained from either ECACC or ATCC and subsequently been screened for human pathogens including HIV, HBV, HCV and EBV. They do not harbour adventitious agents. They are not considered to pose any inherent hazard to human health nor do they have the capability to survive outside the culturing environment of the laboratory.

Host/vector system

Retroviral vectors: pBabe puro, pBabe hygro, pLZRS ires zeo, PMX ires GFP, pSuper retro

The vectors are again widely used by research laboratories and have been developed to provide the viral package signal, transcription and processing elements, and a target gene. The Packaging cells, into which the vectors are inserted, provide the envelope components.

Origin & function

Inserts:

Apoptosis related: Bcl-XL, Mcl-1, tBid, PUMA, Bax, Bad, Bak, Bfl-1, Mcl-1, Bcl-2, Caspase-3, DD Caspase 3, Caspase-9, Caspase-8, RIP3K, RIP1K, FADD, TNFR-1, Trail, Fas, PGAM5, Drp-1, Cytochrome-c GFP, Smac mCherry, Omi mCherry, caspase activity FRET reporter probe, Parkin, PINK1

Autophagy: mCherry LC3, eGFP LC3, eGFP mCherry LC3, eGFP p62, mCherry p62, Atg7, Atg5, Atg12, DD Atg5

Mitochondrial: mito matrix dsRed, mito matrix YFP, mitochondrial outer membrane targeted GFP, mitochondrial intermembrane space targeted Cre recombinase

Evaluation of foreseeable effects

Hazards associated with target/recipient cell lines

Phoenix Ecotropic or Phoenix Amphotropic cells are extensively used for retroviral generation. The target cells have been sourced from either ECACC or ATCC and subsequently been screened for human pathogens including HIV, HBV, HCV and EBV. They are widely used within and outside the Institute and are unable to survive outside a laboratory environment. Consequently, the cells should not have any foreseeable effects on human health or the environment.

Hazards associated with the vector system and inserted gene products

pBabe puro/pBabe hygro (Addgene), pLZRS zeo (Addgene), PMX ires GFP (Addgene), pSuper Retro (Addgene) are all replication incompetent retroviral vectors that allow production of retroviruses encoding proteins or shRNA targeting proteins of interest. Stable cell lines will be selected with puromycin (pBabe puro/pSuper retro), zeocin (pLZRS zeo), hygromycin (pBabe hygro) or sorted for GFP expression (PMX ires GFP).

Using the Phoenix cells and the above plasmids, viral progeny are only produced following transfection of the plasmid encoding the gene of interest (because this provides
the viral packaging signal in trans). Produced virus are replication incompetent, since they lack various genes essential for viral replication. The genes, including Gag-Pol and Env are provided in trans by the Phoenix packaging cell line.

None of the gene inserts to be used are known oncogenes in themselves. Pro-apoptotic proteins such as Bax and Bak can only kill cells upon massive overexpression, it is not anticipated that retroviral transduction will attain the required levels. Many of the fluorescent fusion proteins are used solely as reporters, with no known toxic or oncogenic properties. None of the gene inserts to be used are expected to alter viral tropism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level II laboratories, with access restricted to authorized and trained staff. The amount of retrovirus handled will be limited to no more than 5ml of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. Plastic ware will be used throughout and no needles or sharps will be used during virus preparation. The use of the Class II biological safety cabinet will guard against exposure to aerosols.

All cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm free chlorine) before removal from the Containment Level II laboratories for final disposal.

Solid waste will be double bagged in biohazard bags prior to removal from the Containment Level II laboratory and then autoclaved before final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Containment Level II laboratories that are not worn in the general laboratory area.

Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells and assays of reverse transcriptase activity. Only once these criteria have been met will the infected target cells be removed from Containment Level II laboratory and handled using standard tissue culture procedures.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project was approved as ClassII by the Safety Committee on 12 June 2012.
### Project Containment

<table>
<thead>
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<th>Laboratory Activities</th>
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#### Name

UNIVERSITY OF GLASGOW

#### Name 2

**Department**

DIVISION OF VIROLOGY

#### Campus Estate or Research Centre

**Road Name**

CHURCH STREET

#### District

**Town**

GLASGOW

**County**

EAST RENFREWSHIRE

**Postcode**

G11 5JR

**Country**

SCOTLAND

**Tel Number**

0141 330 4029

**Fax Number**

0141 337 2236

**E-mail**

**HSE Division**

SCOTLAND

#### Comments

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 3 (GMMs)</td>
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Tick if confidential

Bacteriology
Parasitology
Transgenic
Birds
Microbiology
Research
Virology
Transgenic
Animals
Transgenic
Fish
Gene Therapy
Mycology
Transgenic
Invertebrates
Transgenic
Plants
Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
## Project Additional Information

### Purposes of the contained use

The aim of the activities is to utilise SFV for expressing a variety of foreign proteins that could be of either viral or cellular origin. The foreign proteins could be derived from viruses that are human pathogens such as hepatitis C virus (HCV) or respiratory syncitial virus (RSV). The purpose is to use SFV to express high levels of the proteins in mammalian cells. This permits the study of the properties and functions of individual components of a particular virus in the absence of all other viral components.

### Recipient or parental organism

The vectors used for introduction of foreign sequences into SFV are derived from the SFV viral genome. SFV is a well studied virus which does have a wide natural host range (including birds, rodents and anthropod vectors) but this does not include man.

It is intended eventually to use Sindbis virus also.

### Host/vector system

- **Host system(s):**
  - Bacterial: Disabled E. coli K12-derived strains such as DH5.
Mammalian: a range of tissue culture cell lines of mammalian origin.
Vector system(s):
Mobilisation-defective plasmids pGEM1, pSFV1 and its derivatives. SFV-based expression in animal cells requires in vitro synthesis of recombinant RNA from pSFV1-derived and pSFV3 plasmids.

Origin & function
Hepatitis C virus (HCV), GBV-B and respiratory syncytial virus (RSV)
The system will be used for studying proteins encoded by these viruses.

Evaluation of foreseeable effects
Semliki Forest Virus is being used to study proteins from other viruses in this programme and evaluation of effects has to take into account both it and the other virus species involved. HCV is a natural human pathogen that causes chronic infection. The only documented route of infection is by contact with infected blood. The natural host for GBV-B is unknown, although there are literature reports that it is infectious in tamarins. It is not known whether humans are susceptible to infection. From studies elsewhere on infection of chimpanzees by HCV and GBV-B, infection is only successful using the entire genome. Generation of full-length genomic RNA of HCV in the SFV system is not possible. Furthermore, in the SFV system, there will be no combination of vectors introduced into cells that permits generation of full-length HCV and GBV-B genomes by any recombination mechanism. Hence, infectious HCV cannot be produced. It is not expected that HCV and GBV-B proteins will be toxic to man.

Although wild-type SFV can infect animals, the system produces disabled virus that will give only a single round of infection. The most significant consequence of infection by direct inoculation may be an immune response to SFV proteins and those inserted into the vector. There is a possible risk of infection by either aerosols or direct inoculation. Therefore, any SFV particles produced by the vector system will be handled in a safety cabinet and no sharps will be used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

DISINFECTION POLICY
The three methods for disinfection are as follows:
1. Chloros - 1% v/v
2. Virkon - 1% w/v
3. Autoclaving
All of the above methods can be used for herpes, adeno, vaccinia, bunya, respiratory syncitial, measles, chandipura and baculo viruses. Material derived from other viruses must be disinfected by autoclaving and possibly incineration as described in the relevant risk assessment form.
The methods are also suitable for E.coli.
Chloros (outside the laboratory)
For bulk overnight steeping of glassware
Laboratories will be supplied daily with a trolley on which are large round vats for heavy glassware and smaller rectangular vats for light glassware. The appropriate volumes of stock Chloros is pre-dispersed in accompanying plastic bottles.
N.B. With the exception of empty pipette cans and chloros containers, no other items are permitted on this trolley.
Virkon (within the laboratory)
For bulk liquids (e.g. discarded tissue culture media)
Virkon powder* should be added to give a 1% w/v final concentration and the container mixed thoroughly.
For large containers (e.g. burrers, large and medium tissue culture flasks)
Treat with 1% w/v Virkon, either by adding powder and filling with water as above, or by dilution of a fresh concentrate. Stock solutions of Virkon must be dated: they retain efficacy for 1 week. Care should be taken to resuspend any pelleted or clumped material and to completely fill the container, swabbing the threads and then inverting closed flasks and burrers to ensure the neck is treated.

With both of the above methods the treated containers must be soaked for a minimum of 1 hour before disposing liquid down the sink, glassware to normal wash-up and plasticware to normal solid waste (black bags) - laboratory personnel must remove any large bulk waste to cleaning department bins. It is recommended that materials being disinfected are clearly marked. For large glass conical flasks (greater than 1 litre capacity) These should be filled with 1% w/v Virkon and placed on the designated trolleys located on the first and second floors. The washroom staff will empty these trolleys first thing in the morning. These trolleys are for the disposal of large flasks only - other material must not be left on them. For surfaces, equipment and instruments Surfaces should be swabbed with 1% w/v Virkon (less than one week old).

Equipment and instruments can be similarly disinfected but extreme caution should be exercised with electrical equipment. If in doubt see the Technical Services Manager. While Virkon is regarded as an extremely safe compound, care should be taken when dispensing dry powder in bulk. A protective face mask could be used to prevent inhalation.

Autoclaving For small vessels (e.g. petri dishes and small tissue culture flasks) These should be placed in the biological waste discard boxes (lined with clear bags) in each lab. For higher risk organisms (e.g. clinical isolates, hepatitis, HIV) These should be placed in the biological waste discard boxes (lined with biohazard bags) which will be autoclaved prior to incineration of the contents.

For contaminated material (e.g. burrers with fungi or bacteria) These should not be opened but transported and loaded, at the allotted time, into the "dirty" autoclave in the washroom for inclusion in liquid discard cycle. All lids must be loosened at the autoclave when the material is loaded. In the event that the "dirty" autoclave is still running the previous cycle, being serviced or out of order, all discard boxes or other contaminated materials must be returned to their lab of origin. They MUST NOT be left at the autoclave or given to the washroom staff. Other methods - Any other method or disinfecting agent (e.g. sensitivity reactions) must be treated as a separate experimental procedure with its own evaluation of efficacy and risk assessment form. General Aerosols Benchworkers should be wary of all techniques and manipulations which give rise to aerosols when using hazardous materials e.g. filling used burrers contaminated with viruses. Where practicable these procedures should be performed in an appropriate safety cabinet. Biological materials - It is recommended that all vessels used with biological materials be disinfected e.g. burrers used for non infected cell growth be treated with Virkon before discarding to normal waste. WASTE DISPOSAL The disposal of waste chemicals and solvent can often cost more than the original material. It is important, therefore, when orders are placed for chemicals that only sufficient is ordered for your immediate use. Waste disposal must be considered as integral to all laboratory procedures. These notes summarise the principles of waste disposal organisation and describe the approved System of Work for discarding labware. The guidance principle is that scrupulous care must be taken to protect the interest of cleaners, other staff, the public and the environment.

Waste-bag colour code and disposal routes BLACK = Non-hazardous; cleaners may remove RED = These are for material requiring the attention of specialist waste disposal companies and are for compounds (e.g. ethidium bromide) identified in risk assessment forms. See "Waste Chemicals" below. YELLOW = These will not be available to laboratory workers. They are for incineration of autoclaved clinical waste. TRANSPARENT/BIOHAZARD = Microbiological waste for autoclaving. These must be transported in the metal disposal boxes and placed in the "dirty" autoclave in the washroom at the appropriate times. General Waste General waste in the laboratory may be put into the normal disposal containers. However, it is important that broken glass and other hazardous waste is kept separate. Waste solvents are collected in metal drums at the back of the spirit store. Potential hazards exist in mixing different solvents and there are separate drums for each of 3 categories of waste: 1) Flammable, e.g. ether, methanol, acetone. 2) Non-flammable, e.g. DMSO, phenol, chloroform. 3) Oils, e.g. waste from centrifuges, pumps, etc.

The material and amount disposed of in each category must be entered into the appropriate log book provided stating your name and date. Consult The Technical Services Manager if you are in any doubt as to which category your waste belongs. Make sure that there is adequate room left in the drum before starting. There should always be an empty drum below the one in use. If you finish a drum, inform The Technical Services Manager. Absorption granules for soaking up any solvent spillage are available in the solvent store.
From Divisional Safety Committee minute of 22/09/2000

5 Genetic modification proposal: Analysis of GBV-B structural proteins
In discussion there emerged more concern with the introduction of a new virus to the institute than with the proposed GM aspects. It was not known whether the virus is human or tamarin originally. The chief interest was as a model for HCV infection and, in this case, its structural proteins.

5.1 RME noted that there was one report in the literature of VSV glycoproteins being expressed in Semliki Forest Virus.
5.2 There was some concern with the statement "not envisage..... will produce infectious virus" However, part 10 did address concerns.
5.3 It was important to check with the ACDP on the classification GBV. (see 4.2 above)
5.4 The question has to be answered whether it extended previously agreed work, and hence whether a new notification to the HSE was needed.
5.5 It was recommended that the reference to "Experience of expression with HCV...." be moved from part 10 to part 7. Subject to these constraints the proposal was accepted. From the Virology Safety Committee minutes of 07/02/2001

24 Use of Semliki Forest Virus to study RSV proteins
It was noted that the original use of Semliki Forest virus has not constituted genetic modification. A further proposal was now considered to introduce GM of this virus and Notification of a new Activity was indicated. The present proposal represented an additional GM use of SFV.
24.1 The autoclaving of all waste was queried: the normal regime should be adequate (AW to check). Also, the ACGM Guidance Notes provided a model for the environmental risks. (RT to discuss with RS).
24.2 Subject to these changes the proposal was accepted at class 2.

Project Containment

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Project Ref 26/01.2

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Non-GMM Consent Granted

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use
- Study of the structure and function of Norwalk-like viruses, and related viruses of public health significance.

#### Recipient or parental organism
- Human small round structured virus (SRSV)
- Feline calicivirus (FCV)

#### Host/vector system

**Hos system(s)**
1. **Prokaryotic**
   - Plasmid propagation: E.coli TG2, JM101, DH5α, MC1061
   - Expression in E.coli: E. coli BL21, BL21 (DE3)

2. **Eukaryotic**
   - Yeast protein expression system: Pichia pastoris
   - Insect cell lines: Spodoptera frugiperda
   - Mammalian cell lines: MRC5, HeLa, Ohio HeLa, 293, BSC-1, VERO, RD, CHO, BHK

**Vector system(s)**
1. **Prokaryotic**
   - Plasmid propagation: pBR322, pUC, PGM, pJM1, pEMBL, PACYC184, pBLUESCRIPT
   - Protein expression: pGEMEX, pET, pUC119

2. **Eukaryotic**
   - Protein expression in yeast: pPIC9, pPIC3
   - Protein expression in insect cells: Autographa californica baculovirus
   - Protein expression in mammalian cells: pcDNA3, pREP, pTM1 & pTM3 (vaccinia expression), vTF7-3 (vaccinia T7 system), MVA-T7, fowlpox T7, non-cytopathic Sindbis virus replicons
   - Virus expression in eukaryotic cells
FCV full genome and cloned segments of SRSV will be used to study the structure and function of individual proteins and the replication of the virus.

**Origin & function**

The predicted characteristics of the proteins indicate that there will be no toxic effect on humans or the environment. (Those produced in other laboratories have been used to raise antibodies and had no toxic effect).

**Evaluation of foreseeable effects**

Origin & function

The predicted characteristics of the proteins indicate that there will be no toxic effect on humans or the environment. (Those produced in other laboratories have been used to raise antibodies and had no toxic effect).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

none

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**DISINFECTION POLICY**

The three methods for disinfection are as follows:-

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3. Autoclaving

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The methods are also suitable for E.coli

Chloros (outwith the laboratory)

For bulk overnight steeping of glassware

Laboratories will be supplied daily with a trolley on which are large round vats for heavy glassware and smaller rectangular vats for light glassware. The appropriate volumes of stock Chloros is pre-dispensed in accompanying plastic bottles.

NB  With the exception of empty pipette cans and chloros containers, no other items are permitted on this trolley.

Virkon (within the laboratory)

For bulk liquids (eg. discharged tissue culture media)

Virkon powder *should be added to give a 1% w/v final concentration and the container mixed thoroughly.

For large containers (eg. burrlers, large and medium tissue culture flasks)

Treat with 1% w/v Virkon, either by adding powder and filling with water as above, or by dilution of a fresh concentrate. Stock solutions of Virkon must be dated: they retain
efficacy for 1 week. Care should be taken to resuspend any pelleted or clumped material and to completely fill the container, swabbing the threads and then inverting closed flasks and burrlers to ensure the neck is treated.

With both of the above methods the treated containers must be soaked for a minimum of 1 hour before disposing liquid down the sink, glassware to normal wash-up and plasticware to normal solid waste (black bags) - laboratory personnel must remove any large bulk waste to cleansing department bins. It is recommended that materials being disinfected are clearly marked.

For large glass conical flasks (greater than 1 litre capacity)

These should be filled with 1% w/v Virkon and placed on the designated trolleys located on the first and second floors. The washroom staff will empty these trolleys first thing in the morning. These trolleys are for the disposal of large flasks only - other material must not be left on them.

For surfaces, equipment and instruments

Surfaces should be swabbed with 1% Virkon (less than one week old). Equipment and instruments can be similarly disinfected but extreme caution should be exercised with electrical equipment. If in doubt see the Technical Services Manager.

*While Virkon is regarded as an extremely safe compound, care should be taken when dispensing dry powder in bulk. A protective face mask could be used to prevent inhalation.

Autoclaving

For small vessels (eg petri dishes and small tissue culture flasks)

These should be placed in the biological waste discard boxes (lined with clear bags) in each lab.

For higher risk organisms (eg. clinical isolates, hepatitis, HIV)

These should be placed in the biological waste discard boxes (lined with biohazard bags) which will be autoclaved prior to incineration of the contents.

For contaminated material (eg burrlers with fungi or bacteria)

These should not be opened but transported and loaded, at the allotted time, into the "dirty" autoclave in the washroom for inclusion in liquid discard cycle. All lids must be loosened at the autoclave when the material is loaded.

In the event that the "dirty" autoclave is still running the previous cycle, being serviced or out of order, all discard boxes or other contaminated materials must be returned to their lab of origin. They MUST NOT be left at the autoclave or given to the washroom staff.

Other methods

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General

Aerosols

Benchworkers should be wary of all techniques and manipulations which give rise to aerosols when using hazardous materials eg. filling used burrers contaminated with viruses. Where practicable these procedures should be performed in an appropriate safety cabinet.

Biological materials

It is recommended that all vessels used with biological materials be disinfected eg. burrers used for non infected cell growth be treated with Virkon before discarding to normal waste.

WASTE DISPOSAL

The disposal of waste chemicals and solvent can often cost more than the original material. It is important, therefore, when orders are placed for chemicals that only sufficient is ordered for your immediate use. Waste disposal must be considered as integral to all laboratory procedures. These notes summarise the principles of waste disposal organization and describe the approved System of Work for discarding labware.

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Waste-bag colour code and disposal routes

BLACK = Non-hazardous; cleaners may remove
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TRANSPARENT/BIOHAZARD = Microbiological waste for autoclaving. These must be transported in the metal disposal boxes and placed in the "dirty" autoclave in the washroom at the appropriate times.

General waste

General waste in the laboratory may be put into the normal disposal containers. However, it is important that broken glass and other hazardous waste is kept separate.

Waste solvents

Solvent waste is collected in metal drums at the back of the spirit store. Potential hazards exist in mixing different solvents and there are separate drums for each of three categories of waste:

1. Flammable, eg ether, methanol, acetone.
2. Non-flammable, eg DMSO, phenol, chloroform.
3. Oils, eg waste from centrifuges, pumps, etc.

The material and amount disposed of in each category must be entered into the appropriate log book provided stating your name and date. Consult The Technical Service Manager if you are in any doubt as to which category your waste belongs. Make sure that there is adequate room left in the drum before starting. There should always be an empty drum below the one in use. If you finish a drum, inform The Technical Services Manager. Absorption granules for soaking up any solvent spillage are available in the solvent store.
From the Divisional Safety Committee minute of 03/05/2000


RME spoke to the proposal which, if accepted, would represent a new programme of work requiring notification to the HSE.

9.1 Caliciviruses caused human gastrointestinal disease typified by an acute but usually short-lived episode of severe diarrhoea and vomiting. Currently they were classified at ACDP level 2.

9.2 Growth in tissue culture could not yet be achieved and there was no testing of Virkon effectiveness. It was noted that were a tissue culture system to become available this would materially alter the safety implications (though not a genetic modification issue in itself).

9.3 The project would involve recovery of unmodified virus from cDNA clones. For future modification of clones a further proposal would be brought to the Committee.

9.4 The Committee accepted the proposal. A submission to the ACGM would follow.

Please enter comments on the GM safety committee on the risk assessment

From the Divisional Safety Committee minute of 03/05/2000


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Project Ref 26/01.3

Date Ackn'd: 26/02/2001

CU2 Project Title: GENETIC MODIFICATION OF PARAMYXOVIRUSES

Class: Class 2

CultureVolClass2: < 1 litre

CultureVolumeClass3-4:
Project Additional Information

**Purposes of the contained use**
To study respiratory syncytial virus and related viruses of public health significance.

**Recipient or parental organism**
Respiratory Syncytial Virus (RSV)

**Host/vector system**

1) Propagation of viral cDNA. Standard laboratory hosts.
   - JM109
   - DH5
   - Late6 (a derivative of SURE, developed for maintenance of large, unstable plasmids such as the RSV antigenome).
2) Expression in mammalian cell-lines ie production of infectious virus particles. Established cell lines.
   - HepC
   - BHK
   - Cos-7

**Origin & function**
Isolates of RSV
**RSV is relatively common in the human population. The experiments are designed to study the functions and interactions of the proteins encoded by the RSV genome. It is expected that engineered virus will be no more hazardous than the wild type. Eventually it is hoped to identify viral proteins which are potential targets for antiviral drugs.**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| n/a |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| none |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**DISINFECTION POLICY**

The three methods for disinfection are as follows:

1. Chloros - 1% v/v
2. Virkon - 1? w/v
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All of the above methods can be used for herpes, adeno, vaccinia, bunya, respiratory syncitial, measles, chandipura and baculo viruses. Material derived from other viruses must be disinfected by autoclaving and possibly incineration as described in the relevant risk assessment form.

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From Divisional Safety Committee minutes 13/09/2000

10. Genetic modification proposal: Genetic manipulation of respiratory syncytial virus

It was intended to use a commercially available system (from Aviron) which did not need M2. Although the proposer would start using vaccinia strain T7 he would move away from this to a fowlpox or sindbis virus system.

10.01 Fundamentally it would be an RSV rescue system and as such represented a new programme of work. On this basis it was agreed by the Committee at level 1 or (vaccinia) level 2.

10.02 This would be submitted to the HSE as a paramyxovirus programme.

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Project Ref 26/01.4

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<td>not applicable</td>
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Date Project Ceased

02/03/2022
Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form
Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

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Project Ref  26/01.5

Date Ackn’d  15/02/2001
CU2 Project Title  GENETIC MODIFICATION OF BACULOVIRUSES

Date Project Ceased  26/08/2021

Class  Class 2
CultureVolClass2  Non-GMM  Consent Granted  not applicable
CultureVolumeClass3-4  Project notified under transitional arrangements  Y

Withdrawn  N
Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
### Project Additional Information

**Purposes of the contained use**

To study the proteins of hepatitis C virus.

**Recipient or parental organism**

BVDV

**Host/vector system**

- **Host system(s):** Disabled E. coli K12-derived strains such as DH5
- **Mammalian:** Human hepatocyte derived cell lines (eg Huh-7, HepG2 etc), BHK, MDBK

**Origin & function**

HCV genomic cDNA sequences expressing viral proteins will be used for structure and function analysis.
If successful, the system will generate chimeric pseudotype BVDV particles carrying HCV structural proteins which may change its host and tissue tropism. Furthermore, the pseudotype particles are likely to encapsidate replication competent chimeric cDNA genome carrying HCV structural genes. Therefore, these particles could infect cells and replicate if injected into humans. It is virtually impossible to predict the outcome of accidental infection of humans with the chimeric particles and hence the categorization of the proposed work as class 3 activities.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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DISINFECTION POLICY
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2. Non-flammable, eg DMSO, phenol, chloroform.
3. Oils, eg waste from centrifuges, pumps, etc.

The material and amount disposed of in each category must be entered into the appropriate log book provided stating your name and date. Consult the Technical Services Manager if you are in any doubt as to which category your waste belongs. Make sure that there is adequate room left in the drum before starting. There should always be an empty drum below the one in use. If you finish a drum, inform the Technical Services Manager. Absorption granules for soaking up any solvent spillage are available in the solvent store.
From the local Committee minute of 01/05/2002:


It was noted that all relevant staff were CL3 trained.

6.1 In discussion no major problems were identified but it was noted that, with BVDV being modified, HSE approval of a new GM Activity would be required.

6.2 It was noted that RME and others had used a similar HCV protease-dependent system.

6.3 Some textual revisions were required, to strengthen the proposal. The introduction should state that BVDV is not a Specified Animal Pathogen. Also, a few more papers on existing recombinants should be cited since there are more recent papers on recombination in vitro (RME to advise). Section 6 should mention that some recombinants will have both BVDV glycoproteins and HCV proteins.

6.4 Subject to these changes, the proposal was agreed for presentation to the HSE as a new class 3 notification.

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Project Ref 26/04.1

Date Ackn’d 07/04/2004
CU2 Project Title USE OF DISABLED ADENOVIRUS VECTORS DESIGNED FOR EXPRESSION OF
Class 2
Culture Vol
Class 2 ≤ 1 litre

02/03/2022
Page 1380 of 15326
This project covers the broad area of the use of disabled adenovirus vectors for the expression of cloned genes of interest in mammalian cells. Several such vector systems are now available, some commercially, and while they vary in detail, they have in common the lack of the essential E1A gene, and therefore they can be propagated only in complementing cell lines. This genetic modification proposal is intended to register the use of adenovirus vectors for gene expression in the Division of Virology. The risk assessment for each such project will be considered on an individual basis as such proposals are made, with specific reference to the vector system and cloned inserts being used. These future proposals will be considered as an amendment to this parent proposal.

Recipient or parental organism

Complementing cell lines 293 and 911, which express the adenovirus E1A protein, will be used to propagate stocks of the recombinant adenoviruses.

Host/vector system

The viruses will be used for experimentation in standard mammalian cultured cell lines such as Hep2, human foetal fibroblasts and Vero cells. The virus vectors are those commercially supplied by BD Biosciences (for full information see http://www.bdbiosciences.com/clontech/techninfo/manuals/index.shtml). These are rountinely used in many laboratories around the world and are accepted as Category 2 pathogens.

The adenovirus vectors to be used are of two types. Firstly those in which constitutive expression of the cloned genes is under the control of the human cytomegalovirus immediate-early promoter region. Secondly, a tri-partite inducible expression system, in which expression of the cloned genes is controlled by an inducible tetracycline responsive promoter, and the tetR-VP16 fusion activator protein provided in trans by a second adenovirus vector (Ad.CMV-rtTA). Thus the recombinant proteins will be expressed only in cells treated with tetracycline derivatives and co-infected with both adenoviruses.

Origin & function

The initial list of the viruses to be utilised is:
- Ad.CMV-null: Adenovirus vector with HCMV IE promoter and no insert.
- Ad.TRE-null: Adenovirus vector with Tet-responsive promoter and no insert.
- Ad.CMV-GFP: Adenovirus vector with HCMV promoter and EGFP insert.
- Ad.TRE-GFP: Adenovirus vector with Tet-responsive promoter and EGFP insert.
- Ad.CMV-PMLwt: Adenovirus vector with HCMV promoter and wt PML insert.
Ad.CMV-rtTA: Adenovirus vector expressing the doxycycline-inducible transactivator of the TRE promoter.
Ad.TRE-PMLwt: Adenovirus vector with Tet-responsive promoter and wt PML insert.
Ad.TRE-ICPO: Adenovirus vector with Tet-responsive promoter and ICPO insert.
Ad.TRE-n212: Adenovirus vector with Tet-responsive promoter and ICPO insert truncated at residues 212.

Abbreviations:
HCMV  Human cytomegalovirus.
TRE  Tetracycline responsive element.
GFP  enhanced green fluorescent protein
PML  Promyelocytic leukaemia protein.
ICPO  Infected cell protein O of HSV-1
HSV-1  herpes simplex virus type 1

Evaluation of foreseeable effects

The adenoviruses to be used are defective for the essential E1A gene products and they are also deleted for the E3 region, so they are unable to replicate and spread in normal human cells. However, the inserted genes are designed to be expressed in mammalian cells, and therefore the project is designated as Level 2. Increased expression of the PML proteins is very unlikely to be harmful since PML is expressed in a wide range of cell types, and high level expression of PML is a normal consequence of interferon treatment of cells. ICPO is very unlikely to be more harmful to man when expressed in the tet inducible vector virus pair than when expressed during the course of a normal HSV-1 infection. Detailed safety guidance concerning these adenovirus vectors, and the protocols approved for their use in the National Institutes of Health, Bethesda USA, is available at: http://www.bdbiosciences.com/clontech/techinfo/manuals/index.shtml

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Extract from the Divisional Safety Handbook.

DISINFECTION POLICY

The three methods for disinfection are as follows:
1. Chloros - 1% v/v
2. Virkon - 1% w/v
3. Autoclaving

All of the above methods can be used for herpes, adeno, vaccinia, bunya, respiratory syncitial, measles, chandipura and bacula viruses. Material derived from other viruses must be disinfected by autoclaving and possibly incineration as described in the relevant risk assessment form.

The methods are also suitable for E. coli.

Chloros (outwith the laboratory)
For bulk overnight steeping of glassware
Laboratories will be supplied daily with a trolley on which are large round vats for heavy glassware and smaller rectangular vats for light glassware. The appropriate volumes of stock Chloros is pre-dispensed in accompanying plastic bottles.

NB With the exception of empty pipette cans and chloros containers, no other items are permitted on this trolley.

Virkon (within the laboratory)

For bulk liquids (eg discarded tissue culture media)
Virkon powder *should be added to give a 1% w/v final concentration and the container mixed thoroughly.

For large containers (eg burriers, large and medium tissue culture flasks)
Treat with 1% w/v Virkon, either by adding powder and filling with water as above, or by dilution of a fresh concentrate. Stock solutions of Virkon must be dated: they retain efficacy for 1 week. Care should be taken to resuspend and pelleted or clumped material and to completely fill the container, swabbing the threads and then inverting closed flasks and burriers to ensure the neck is treated.

With both of the above methods the treated containers must be soaked for a minimum of 1 hour before disposing liquid down the sink, glassware to normal wash-up and plasticware to normal solid waste (black bags) - laboratory personnel must remove any large bulk waste to cleansing department bins. It is recommended that materials being disinfected are clearly marked.

For large glass conical flasks (greater than 1 litre capacity)
These should be filled with 1% w/v Virkon and placed on the designated trolleys located on the first and second floors. The washroom staff will empty these trolleys first thing in the morning. These trolleys are for the disposal of large flasks only - other material must not be left on them.

For surfaces, equipment and instruments
Surfaces should be swabbed with 1% w/v Virkon (less than one week old). Equipment and instruments can be similarly disinfected but extreme caution should be exercised with electrical equipment. If in doubt see the Technical Services Manager.

* While Virkon is regarded as an extremely safe compound, care should be taken when dispensing dry powder in bulk. A protective face mask could be used to prevent inhalation.

Autoclaving
For small vessels (eg petri dishes and small tissue culture flasks)
These should be placed in the biological waste discard boxes (lined with clear bags) in each lab.

For higher risk organisms (eg clinical isolates, hepatitis, HIV)
These should be placed in the biological waste discard boxes (lined with biohazard bags) which will be autoclaved prior to incineration of the contents.

For contaminated material (eg burriers with fungi or bacteria)
These should not be opened but transported and loaded, at the allotted time, into the ‘dirty’ autoclave in the washroom for inclusion in liquid discard cycle. All lids must be loosened at the autoclave when the material is loaded.

In the event that the ‘dirty’ autoclave is still running the previous cycle, being services or out of order, all discard boxes or other contaminated materials must be returned to
their lab of origin. They must not be left at the autoclave or given to the washroom staff.

Other methods
Any other method or disinfecting agent (e.g., stericol, gluteraldehyde, SDS) required for scientific or personal reasons (e.g., sensitivity reactions) must be treated as a separate experimental procedure with its own evaluation of efficacy and risk assessment form.

General

Aerosols
Benchworkers should be wary of all techniques and manipulations which give rise to aerosols when using hazardous materials e.g., filling used burriers contaminated with viruses. Where practicable these procedures should be performed in an appropriate safety cabinet.

Biological materials.
It is recommended that all vessels used with biological materials be disinfected e.g., burriers used for non-infected cell growth be treated with Virkon before discarding to normal waste.

WASTE DISPOSAL
The disposal of waste chemicals and solvent can often cost more than the original material. It is important, therefore, when orders are placed for chemicals that only sufficient is ordered for your immediate use. Waste disposal must be considered as integral to all laboratory procedures. These notes summarise the principles of waste disposal organisation and describe the approved System of Work for discarding labware.

The guiding principle is that scrupulous care must be taken to protect the interests of cleaners, other staff, the public and the environment.

Waste-bag colour code and disposal routes.

BLACK = Non-hazardous; cleaners may remove.
RED = These are for material requiring the attention of specialist waste disposal companies and are for compounds (e.g., ethidium bromide) identified in risk assessment forms. See "Waste Chemicals" below.
YELLOW = These will not be available to laboratory workers. They are for incineration of autoclaved clinical waste.
TRANSPARENT/BIOHAZARD = Microbiological waste for autoclaving. These must be transported in the metal disposal boxes and placed in the "dirty" autoclave in the washroom at the appropriate times.

General waste
General waste in the laboratory must be put into the normal disposal containers. However, it is important that broken glass and other hazardous waste is kept separate.

Waste solvents
Solvent waste is collected in metal drums at the back of the spirit store. Potential hazards exist in mixing different solvents and there are separate drums for each of three categories of waste.

1. Flammable, e.g., ether, methanol, acetone.
2. Non-flammable, e.g., DMSO, phenol, chloroform.
3. Oils, e.g., waste from centrifuges, pumps etc.
The material and amount disposed of in each category must be entered into the appropriate log book provided stating your name and date. Consult the Technical Services Manager if you are in any doubt as to which category your waste belongs. Make sure that there is adequate room left in the drum before starting. There should always be an empty drum below the one in use. If you finish a drum, inform the Technical Services Manager. Absorption granules for soaking up any solvent spillage are available in the solvent store.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Extract from the Minutes of the Divisional Safety Committee meeting of 17.2.2004


The intended work was described. It was noted that the adenovirus technology was a very useful and widely used technique. However on winding up previous GM work involving adenovirus at the time the current Regulations came into force, the Division had formally discontinued GM of adenovirus. Formal Notification of the proposed work was required as a new Activity. The capability to do this work was important. The technique was expected to be useful to a number of teams in the Division.

3.1 A disabled strain of adenovirus would be used. The vectors listed in the proposal would not be the only ones and were specific to the immediate project, so Notification in that sense would be generic. The proposal could appropriately be made more general (Action: RDE).

3.2 No reservations were expressed on safety issues. The project was approved for work at level 2. AW to progress Notification (Action: AW). (The Notification fee was likely to rise from the current 576 as from 1.4.2004, so it should be done then).

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Project Ref 26/11.1
Arboviruses are transmitted to susceptible vertebrates by arthropod vectors such as mosquitoes or ticks. Most arboviruses belong the Flaviviridae, Bunyaviridae and Togaviridae families of RNA viruses. While arboviruses can cause disease in vertebrates - ranging from febrile illness to more severe complications, depending on virus and/or host - as well as cell death in infected cultured cells, this appears not to be the case in the arthropods that transmit them. In fact, arbovirus-infected arthropods display few or no signs of disease (Fragkoudis et al., 2009), and this effect can be mimicked in arthropod cell culture.

Infection of mosquito or tick cells usually begins with an acute phase with high titre virus production followed by a persistent infection which, once established can last the life time of the arthropod cell culture. The biological reasons behind persistence are poorly understood and are believed to be linked to innate immune responses of the arthropod host. Research on antiviral immune responses in vector arthropods has only in recent times seen some progress yet this area of research is crucial in understanding this virus/host interaction and develop novel antiviral control strategies targeting the vector. It is now clear, mainly from research on arbovirus-infected mosquitoes, that vectors do not passively replicate arboviruses but that immune responses are activated. These can result in activation of immune signalling pathways (STAT, TOLL, IMD etc.), humoral responses in the arthropod hemocoel and most crucially RNA interference (RNAi) responses. It is not known how immune signalling pathways are activated following infection or how exactly they would mediate antiviral activities, and extracellular responses such as phenoloxidase activities also appear to be involved. The RNAi responses are better understood and form a major antiviral defence. Viral replication induces production of double stranded RNA which serves as a substrate for dicer-type nuclease activities to produce virus-induced small interfering RNAs (also called viRNAs in the context of viral infection) which are integrated into the RNA-induced silencing complex (RISC). The RISC presumably mediates antiviral activities by finding viral RNAs through complementarity with viRNAs and then mediating nucleolytic cleavage of those viral RNAs. Although some steps in this antiviral pathway are known, mechanisms, triggers etc. are poorly understood. In addition, arthropod cells might differentially regulate subsets of their own small RNA repertoire following infection by arboviruses.

In this project, we aim to further analyze:

- The induction, regulation and mechanisms of antiviral signalling pathways such as STAT, TOLL, IMD etc. in arthropod cells such as mosquito and tick cells, as well as mosquitoes and tick organ cultures. This project will look at protein functions and signalling regulation within these pathways.
- The induction, regulation and mechanisms of humoral, extracellular antiviral responses. We will analyse viral triggers and key components that lead to activation of extracellular responses. This project will be carried out in arthropod cells such as those derived from mosquitoes and ticks, but also live mosquitoes, and tick organ culture.
- The induction, regulation and mechanisms of small RNA pathways including antiviral RNAi in arthropod cells such as mosquito and tick cells, as well as live mosquitoes and tick organ cultures. We will analyse the nature and characteristics of small RNAs including those derived from cells as well as the regulation of the RNAi machinery (including that of proteins involved in RNAi).
- The relationship between virus/cell interactions and virus replication and induction of immune responses. Virus replication rates as well as interactions with host proteins are likely key determinants in the outcome of infection and those will be investigated.

To infect arthropod cells, mosquitoes or tick organ cultures, we will use arboviruses that are well understood and for which molecular tools are available. In addition to strains of the viruses, we will use genetically modified viruses as well as replicons derived from these viruses. Replicons are replicating viral RNA elements which lack the components to form virions (packaging signals, structural proteins) but contain RNA elements and proteins sufficient to replicate the replicon RNA. Replicons can either be introduced into cells by transfection, or packaged by co-expression of structural proteins into so-called virus-replicon particles.

Virus models to be studied are viruses of both the Togaviridae (genus Alphavirus) and Flaviviridae (genus Flavivirus) families, and/or derived replicons:

1. As a model for tick-borne arboviruses, we will study infections by Langat virus (LGTv). LGTV is a tick-borne flavivirus of the Flaviviridae family. It was first isolated from Ixodes granulatus ticks in 1956 in Malaysia (Smith, 1956). The natural transmission cycle of the virus is believed to involve rats in areas such as Thailand, Malaysia and Russia. LGTV can cause encephalitis in experimentally infected mice (Webb et al., 1968) but is not very neuroinvasive compared to other more virulent members of the tick-borne flavivirus group (Thind & Price, 1966) and is considered the non-pathogenic member of the Tick-borne encephalitis complex. The naturally attenuated Malaysian strain TP21 (the most common laboratory strain), is not associated with human disease under natural conditions (Rumyantsev et al., 2006) and LGTV has been used as a live attenuated vaccine in humans (Dubov et al., 1972).

A cDNA copy of the LGTV genome (a positive-stranded RNA of approx. 11 kb encoding a polyprotein with structural sequences followed by non-structural sequences) is available, allowing manipulation of the genome (Rumantsyev et al., 2006). This plasmid has been or will be engineered to delete to targets sequences (for example for replicon production), mutate specific sequences or insert genes such as reporter genes (for example fluorescent molecules such YFP, eGFP or various types of luciferase), insect immune response inhibitors or drug resistance genes (to facilitate selection of cells harbouring replicons). Genetic changes that will be introduced into the virus sequences (point mutations, deletions, insertions) will be targeted at disrupting specific functions of virus RNA or virus proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virulence. The viral envelope glycoprotein and capsid protein sequences which are the major determinants of host range and cell tropism will not be changed beyond the addition of marker genes such as GFP (as fusion or cleavable inserts into this coding region); host range and cell tropism are therefore unlikely to be altered. Changes to viral sequences will target disruption of viral protein functions or sequences.

LGTv can be directly used to infect cells. cDNA plasmids with LGTV sequences will be amplified using standard disabled laboratory strains of bacteria (usually strain BD1528 which is routinely used for flavivirus cDNAs). Plasmids or transcribed RNA will be transfected into eukaryotic cells. Depending upon the construct, in eukaryotic cells this may result in replication of virus RNA and production of new virions. Virus or virus replicon particles generated from these plasmids will be used to infect eukaryotic cell and tissue cultures, to investigate virus replication in arthropod cells such as tick cells or tick organ cultures (derived from ticks). The use of LGTV expressing a reporter gene such as GFP or luciferase will allow accurate, rapid, efficient, localised and repetitive monitoring of infection of cells and tissues which is important for immunity studies.

In addition to the use of LGTV, we plan to carry out comparative studies between LGTV replicons and replicons of other arthropod-borne flaviviruses such as tick-borne encephalitis or dengue virus; those replicons are not covered by Schedule 5 and no sequences for complete schedule 5 virus genomes (ie sequences encoding missing structural genes that are required to complement the full set of viral proteins to produce infectious virion) are stored in-house. Those replicon RNAs will not be packaged; only RNA will be introduced into cells to study replication; replicons will not be mixed in the same cells (either by co-transfection/infection or potential superinfection by spreading virus) with sequences of related viruses from within the same genus to avoid recombination events during co-infection (recombination can only occur when viruses replicate in the same cell). Like LGTV-derived replicons, other flavivirus replicons pose no risk to safety to human and animal health as they cannot spread and transfected cells will be eliminated by cell death or the immune system. Experiments with these replicons will be carried out to study host innate immune responses (RNAi, immune signalling) and/or virus-host cell interactions (protein interactions, replication complex localization in cells etc.). These experiments will complement studies carried
out with LGTV and allow us to verify how broadly relevant some findings (those that do not rely on formation of infectious virus or/and virus spread) obtained with a model virus are to other members of the family.

2. As a model for Togaviridae (genus Alphavirus) infection of arthropod cells, we will study infections by Semliki Forest virus (SFV). SFV is naturally found in central and Southern Africa and is transmitted between animals and birds by mosquitoes (mainly Ae. aegypti and africanus). SFV can infect man and is classified as a hazard group 2 pathogen by the ACDP. The alphavirus genome consists of a single strand of positive sense RNA of around 12 kb. This RNA codes for two open reading frames. The first codes for the non-structural viral replicase proteins (nsP1, 2, 3 & 4), the second for the viral structural proteins (C, E1, E2 & E3). Expression of the replicase proteins is under the control of a promoter (P) in the 5’ non-coding region of the virus. Expression of the structural proteins is under the control of a subgenomic promoter (SP) which is activated later in infection and leads to a high level of expression from this second open reading frame.

There are several strains of SFV which have been derived from natural isolates, these include prototype SFV4, L10, A7 and A7(74). Each of these strains has been cloned and sequenced and is available as a series of plasmids which can be amplified in standard bacterial systems. In vitro transcription of the plasmid cDNA can give rise to an RNA which when transfected into cells can give rise to infectious virus; alternatively RNA is transcribed from transfected plasmid under control of eukaryotic promoters such as the CMV promoter. The SFV cDNAs can be engineered to give rise to virus mutants or engineered viruses. The prototype strain SFV4 forms the basis of the SFV vector system which is widely used for eukaryotic gene expression (Smerdou and Liljestrom, 1999). SFV natural isolates, cDNA derived viruses and mutant viruses derived from these cDNA clones have been used extensively by us and others. In mice, different strains show different pathogenicity and these are likely to be due to sequence differences. We will produce and use genetically engineered SFV viruses mutated in viral proteins or sequences expressing additional genes (reporter genes such as luciferase or fluorescent proteins, insect immune response antagonists etc.) as part of the non-structural open reading frame, the structural or from duplicated subgenomic promoters (Siu et al., 2011; Attarzadeh-Yazdi et al., 2009). If the coding sequence for a protein such as a reporter gene would be inserted into the viral structural polyprotein-coding sequence, in the resultant hybrid polyprotein the added protein would be separated from the structural proteins by protease cleavage sites. No changes to the sequence of the structural proteins would ensue so that there would be no changes to virus tropism resulting from changes to the structural proteins. Introduction of additional sequences into SFV is likely to provide no change or to decrease virulence. Changes to viral sequences will target disruption of viral protein functions or sequences.

In addition to infection with SFV, it is possible to use virus-derived replicons. In replicons, the structural genes are replaced by a polylinker which allows insertion of foreign sequences. The system exploits the wide cell tropism of SFV, but has no potential for virulence since genes for the viral structural proteins are deleted. A helper system is also available whereby recombinant RNA can be packaged into infectious virus-replicon particles (VRP); this system is deemed extremely safe (Smerdou and Liljestrom, 1999). Packaging of genomic RNA into new virions is absolutely dependent on a packaging sequence in the replicase gene. Packaged VRPs are made by using cells which also express the viral structural genes (usually by cotransfection of structural gene-encoding mRNA transcripts). In the absence of genes encoding the viral structural proteins in replicons, new infectious virus particles capable of initiating a propagating infection cannot be regenerated in VRP-infected cells; VRPs are thus also referred to as ‘suicide particles’. Replicons can be used to express reporter genes or other genes of interest such as insect RNAi inhibitors.

SFV and engineered variants will be used to infect eukaryotic cell and tissue cultures, as well as arthropods such as mosquitoes. The use of genetically modified marker viruses will allow monitoring of infected cells and tissues, determination of the spread of infection and dissection of specific tissues / areas or even specific cells for further analysis. Again these properties are very useful to study immune responses in arthropod cells and tissues.

In addition to the use of SFV we plan to carry out comparative studies with other arthropod-borne alphavirus replicons such as those derived from chikungunya virus; those replicons are not covered by schedule 5 and no sequences for the complete genome (ie sequences encoding structural genes required to complement the viral protein set to give infectious virions) will be stored in house. Those replicons will not be packaged into VRPs, only RNA will be introduced into cells to study replication; replicons will not be mixed with related viruses from within the same genus in the same cell (either by co-infection/transfection, or potential superinfection by spreading virus) to avoid recombination events during co-infection. Like SFV-derived replicons, other alphavirus-derived replicons pose no risk to safety or human health as they cannot spread and transfected cells will be eliminated by cell death or the immune system. Experiments with these replicons will be carried out to study host innate immune responses (RNAi, immune signalling) and/or virus-host cell interactions (protein interactions, replication complex localization in cells etc.). These experiments will complement studies carried
out with SFV and allow us to verify how broadly relevant some findings (those that do not rely on formation of infectious virus or virus spread) obtained with a model virus are to other members of the family.

References:


Recipient or parental organism

Arboviruses and derived replicons used will be of the Flaviviridae and Togaviridae (genus Alphavirus) virus families.

Togaviridae:
Semliki Forest virus prototype (SFV4), and strains such as A7 and A7(74).
Semliki Forest virus-derived replicons and virus-replicon particles.
Other arthropod-borne alphavirus replicons (for example derived chikungunya virus).

Flaviviridae:
Langat virus (strain TP21, the most commonly used lab strain, or others).
Langat virus-derived replicons.
Other arthropod-borne flavivirus replicons (for example derived from tick-borne encephalitis virus and dengue virus).

Host/vector system

All organisms will be used at containment level 2, which is routine practice.

02/03/2022 Page 1389 of 1532
Plasmid vectors:
Viral sequences (including full length clones or partial sequences of SFV or LGTV) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. RNAs can be transcribed through bacterial promoters (ie. T7 or SP6) or eukaryotic promoters (CMV, SV40 etc.) Plasmids contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin) and sometimes a selectable eukaryotic drug resistance marker such as neomycin or puromycin resistance. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

Hosts:

Prokaryotic hosts:
Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste should prevent release of viable organisms. The risk to the environment is therefore effectively zero.

Eukaryotic hosts:
Vertebrate cell lines of various origins (typically mouse, hamster, etc.) and invertebrate cell lines of various origins (mosquito, tick etc.). Mosquitoes, mainly strains of Ae. aegypti (such as the laboratory strain Liverpool red eye). Tick organ cultures from dissected ticks.

In prokaryotic cells, only selectable (eg antibiotic resistance) genes will be translated.

Virus sequences will be under the control of a promoter that will only generate transcripts in eukaryotic cells or in vitro transcription reactions.

In eukaryotic cells, RNA will be translated into the following gene products:

- Arboviral proteins and RNAs (SFV and LGTV, or replicons of related arboviruses): Proteins involved in replication and transcription of viral genetic material and virus structural proteins. Some of these virus proteins will interact with host-cell components and may affect host cell responses to infection; these interactions are not currently known. If all virus sequences are translated new virions may be generated; replicons are non-propagative viral RNAs (capable of replication). Partial RNAs do not give rise to propagating infectious material but can display biological activities (ie. RNAi inhibitory, expression of some viral proteins for example for packaging of replicons).

- Non-arboviral genes: Genes of prokaryotic (eg CAT) or eukaryotic (eg. reporter genes such as luciferases or fluorescent proteins such as GFP; inhibitors of insect immunity ie. RNAI inhibitors, signalling inhibitors, melanization inhibitors etc.) origin. No proteins known to interfere with vertebrate immune responses will be cloned into full length SFV or LGTV. The recombinant foreign proteins to be expressed provide no significant increase in the hazard to human health; none are toxic or likely to produce disease in the quantities that could be produced by accidental exposure to these systems. Non translated RNA sequences have no known toxic effects and pose no risk to human health.

Techniques used to introduce insert or vectors into cells:

Prokaryotic cells: Introduction of DNA into E. coli will be by heat shock/chemical transformation. These techniques have been extensively described and are widely used; they rely on getting DNA very close to the bacterial membrane and introducing the genetic material through pores or membrane fusion.

Eukaryotic cells: Mammalian cells will be transfected using reagents such a lipofectamine or by electroporation. Virus genomes will be introduced into cultured cells or tick organ cultures by infection with virus or virus replicon particles (VRP), using transfection reagents or by electroporation.
Note - location and nature of where experiments are to be carried out:

GM 26 (CVR Church Street):
- Preparation, characterisation and genetic manipulation of viruses and replicons.
- Infection of cell lines and organ cultures, preparation of lysates, extracts and fixed tissues for downstream analysis.

GM223 (CVR Garscube Estate):
- Infection of Ae. aegypti mosquitoes with Semliki Forest virus (SFV) (wild type or genetically modified) or VRPs by bloodmeal.
- Isolation of SFV, or nucleic acids or proteins from Ae. aegypti mosquitoes for downstream analysis (for example real time PCR for quantification of gene expression, reporter gene assays etc.).
- Fixation of mosquito tissues for downstream analysis (immunofluorescence, fluorescent reporter gene expression).

Evaluation of foreseeable effects

Foreseeable effects/risk assessment for human health and safety (also see Risk Assessments):

Plasmid vectors:
Viral sequences (including full length clones or partial sequences of SFV or LGTV) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Transcription of even complete viral cDNA (SFV or LGTV) will result in no infectious RNA since the promoters are not active in prokaryotic hosts. Systems for prokaryotic gene expression are under control of an inducible prokaryotic promoter. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV or SV40), eukaryotic (eg actin promoter, pathway inducible promoter such as STAT) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human cells. None of the genes used in these studies are oncogenes. The hazard of expressing 'foreign' genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the foreign gene product and is discussed in more detail below.

SFV:
In Africa, natural infections have been associated with mild febrile illness (Mathiot et al., 1990). Various laboratory strains of SFV including prototype-SFV4, L10, A7 and A7(74) have been used extensively in many laboratories and even (in the past) for student practical classes. There is one report of laboratory based human disease. Death of a laboratory worker in Germany was associated with infection by the Osterrieth strain of SFV (Willems et al., 1979). This (Osterrieth) strain of SFV is no longer used experimentally. Whilst the above case report is noted, it dates back over 30 years and is difficult to reconcile with the extensive use of other strains of SFV with no reported adverse effects over a 50 year period. This case report makes the unpublished observation that antibodies against SFV can be demonstrated in the serum of many laboratory personnel working with it. It is generally considered that the individual who died was probably immunosuppressed and therefore highly susceptible to infection. Anecdotal reports and reported laboratory incidents indicate no human clinical signs after accidental inoculation with various laboratory strains of SFV. The classification into ACDP hazard group 2 indicates current expert opinion that containment level 2 precautions are considered adequate for controlling the risks associated with working with SFV. The viral envelope glycoproteins and capsid protein sequences are the major determinants of host range and cell tropism and will not be changed beyond the addition of marker genes such as GFP (as fusion or cleavable inserts into this coding region) into the structural open reading frame; host range and cell tropism are therefore unlikely to be altered. SFV is classified as ACDP hazard group 2. Personnel working with this virus are expected to inform occupational health should they become pregnant or immunosuppressed.

LGTV:
Under normal conditions, LGTV is only very rarely pathogenic to humans; live virus has been used in vaccine trials in humans (Dubov et al., 1962). Development of this vaccine was stopped in the 1980s because of rare (1 in 20,000) occurrences of post-vaccinal neurological disease (Rumyantsev et al., 2006). Genetic changes that will be introduced into the virus sequences will be targeted at disrupting specific functions of virus RNA or virus proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virus virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences such as...
reporter genes which confer no advantage to virus replication. As for SFV, the viral envelope glycoproteins and capsid protein sequences which are the major determinants of host range and cell tropism will not be changed beyond the addition of marker genes as fusion or cleavable inserts into the region coding the structural proteins; host range and cell tropism are therefore unlikely to be altered. LGTV is not listed on the ACDP list but should be classified as hazard group 2 (current use in the UK and internationally, see http://www.healthsafe.uab.edu/pages/biosafety/biosafetymanual.pdf).

Personnel virus working with this virus are expected to inform occupational health should they become pregnant or immunosuppressed.

The host/recipient organism:
Prokaryotic organisms: All strains will be disabled, commercially available E. coli derivatives classified as ‘especially disabled hosts’ by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic cells and tick organ cultures:
Cell lines to be used would not survive inside the human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA will confer no growth or survival advantage in or outside the laboratory to cell lines of vertebrate or arthropod origin. The tick organ cultures are derived from dissected ticks and are immobile culture systems. As with the cell lines they could not survive in humans. Organ cultures are generally derived from ticks sourced from ‘pathogen-free’ colonies; ticks from other sources (for example ‘field ticks’) could carry other pathogens and will not be used in these studies.

Mosquitoes (also see paragraph 8, below): These will be sourced only from ‘pathogen-free’ laboratory colonies (provided by Prof. Eileen Devaney, University of Glasgow). Precautions will be taken to minimise the risk of bites or escapes in strict compliance with standard operating procedures (attached separately). Work with virus replicons and VRPs will be done under the same procedures and containment as work with infectious virus (wild type or genetically modified). Measures to prevent human infection include storage of infected arthropods in sealed containers; work in designated rooms with restricted access; wearing of gloves and cooling of arthropods to slow their movement and facilitate handling. Samples (dead arthropods or material from these) will be transported to our CL2 laboratory according to standard regulations.

The inserted/donated genetic material:
Viral RNA and proteins, cellular proteins: Most individually expressed viral or cellular proteins (for example arthropod immune response inhibitors) are unlikely to have harmful effects in eukaryotic cells, however some could perturb normal arthropod cellular metabolism, predispose or protect against cell death or render cells more or less susceptible to other infections. It is very unlikely that SFV or LGTV (and other alphavirus, flavivirus or insect virus) RNA or proteins would have any harmful toxin-like effect outside cells. RNA containing the complete SFV or LGTV sequence can give rise to infectious virus in eukaryotic cells; alphavirus and flavivirus replicon RNAs are non-propagative and pose no harm (individual infected cells are likely to die or be eliminated by the immune system). Viral non-coding RNA sequences are likely to have minimal effects on virus replication, especially outside arthropod cell systems, and do not encode biologically active molecules that are likely to induce physiological effects in humans. As described under (9), no proteins or sequence which could interfere with immune responses in vertebrates will be cloned into viral genomes.

Reporter genes: Reporter genes of prokaryotic or eukaryotic origin (eg luciferase, fluorescent proteins etc.). No harmful properties have been attributed to these proteins. There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Phontinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function.

Other non-coding RNA sequences: Any effects these elements have on gene expression are likely to be minimal. They may affect gene function or virus replication in individual cells but are unlikely to lead to whole body physiological effects as they do not code for the production of secreted bioactive molecules, and are unlikely to have any biological effects outside arthropod cell systems.

Sequence changes in viral sequences:
Mutation or deletion will target disruption of non-structural protein functions or non-coding viral sequences and are likely to have no or deleterious effects on viral replication, thus not increasing risk or hazards to human health associated by viruses genetically modified in such a way.

Control measures – assign provisional containment level:
GM bacteria transfected with plasmids containing viral sequences - Containment Level 1.
Eukaryotic cells (including tick organ cultures) containing alphavirus or flavivirus sequences unable to form complete infectious virus – Containment Level 1.
Eukaryotic cells (including tick organ cultures) containing full length SFV/LGTV sequences (capable of forming infectious virus, or capable of undergoing recombination or minor mutations to form infectious virus) – Containment Level 2.
Arthropods containing viral sequences unable to form complete infectious virus – Arthropod Containment Level 2.
Arthropods containing full length viral sequences (capable of forming infectious virus, or capable of undergoing recombination or minor mutations to form infectious virus) – Arthropod Containment Level 2
All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. For work with mosquitoes, wear a white laboratory overall with elastic cuffs, gloves and a mosquito net that covers the head. A microbiological safety cabinet will be used where appropriate.

Foreseeable effect/risk assessment for the environment:

SFV: This virus is not endemic in the UK It is not on the SAPO list. It is not clear whether suitable vertebrate hosts and insect vectors are present to establish an infection in this country. SFV can infect vertebrates (such as rodents) and certain biting insects. It is unclear whether suitable hosts are present. Transmission to vertebrates is by inoculation and to insect is by feeding on blood of an infected vertebrate. SFV is naturally transmitted by mosquitoes such as Aedes aegypti and Aedes africanus. Given the absence of these specific mosquitoes in Scotland and low pathogenicity of strains, the chance of infected laboratory mosquitoes (unlikely to survive for any extended length of time in the local environment) initiating a transmission cycle sustained by local insects is negligible. The modified viruses or replicons or replicons of other arthropod-borne alphaviruses will not have any inserted foreign genes other than non-harmful genes or sequences, or disruptive changes in non-structural genes or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. In the absence of a suitable host or vector these viruses would not survive in the environment and the risk is effectively zero.

LGTV: This virus is not endemic to the UK. It is not on the SAPO list. LGTV is naturally transmitted by ticks; it was originally isolated from Ixodes granulatus ticks in SE Asia. The competence of UK ticks for this virus is unknown. A related virus, Louping-III virus, is found in ticks throughout the British Isles. Given the location of our laboratories, tick populations are unlikely to be very low in the immediate vicinity. Ticks generally acquire infection from a blood meal on vertebrates and as ticks are excluded from our laboratories, and viruses stored in closed containers the chance of accidental tick infection is negligible. Modified viruses or replicons or replicons of other arthropod-borne flaviruses do not contain anything other than non-harmful genes or sequences, or disruptive changes in non-structural genes or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. The likelihood of virus released from our laboratory infecting a local tick is effectively zero (see disinfection and waste disposal procedures).

Hosts:

Prokaryotic organisms: Disabled, commercially available E. coli strains. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. Acquiring antibiotic resistance (from the plasmid vector) or additional sequences (virus or marker gene sequences) will not give these strains any survival advantage in the environment. The risk to the environment is therefore effectively zero.

Eukaryotic cells: Cell lines (and tick organ cultures) to be used are not viable outside the laboratory and thus pose no threat to the environment. Addition of plasmid vectors or virus will confer no growth or survival advantage in the environment.

Mosquitoes: Arthropods will be contained as described above under ‘risk to human health’ (and as detailed below, section 8). If arthropods carrying infectious material were to escape from our facilities they would have no survival advantage in the environment but could transmit the infection by bite. Whether this would initiate a natural sustainable cycle of SFV infection in Scotland is unlikely given the generally low mosquito activity. Given that escaped uninfected arthropods and natural arthropods will be
rare and that virus samples are in sealed containers and generally used in microbiological safety cabinets, the chances of arthropods becoming infected in the laboratory outside containment are virtually zero.

In summary

i) consequence/severity of effects: Low

ii) likelihood of effects being realised: Negligible

iii) overall risk: Effectively zero

Final classification: GM bacteria transfected with plasmids containing viral or other eukaryotic sequences (unable to cause disease, minimal hazard to health and environment) - Containment Level 1.

Eukaryotic cells (including tick organ cultures) containing viral sequences unable to form complete infectious virus – Containment Level 1.

Eukaryotic cells (including tick organ cultures) containing full length SFV or LGTV sequences (capable of forming infectious virus, or capable undergoing recombination or mutation to form infectious virus) – Containment Level 2.

Arthropods containing viral sequences (full length, partial) – Arthropod Containment Level 2.

All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. For work with mosquitoes, wear a white laboratory overall with elastic cuffs, gloves and a mosquito net that covers the head. As a routine measure all work will be carried out Containment level 2. A microbiological safety cabinet will be used where appropriate.

References:


Infection of mosquitoes will take place in a dedicated insectary at arthropod containment level 2 (AcCL2). A standard operating procedure (SOP) has been added separately.

Whole mosquitoes will be injected or fed (membrane feeding or capillary tube) with liquid (including blood) containing virus derived material, infectious virus, or a mixture of these. Viruses, RNA and proteins will be isolated from crushed mosquitoes. Virus infected live mosquitoes may be monitored by microscopy. Arthropods will be studied inside closed containers. In addition wingless mosquitoes may be used (wings will be removed from cold immobilised mosquitoes inside containment – glove box; see below.

ArCL2 is suitable for arthropods infected with hazard group 2 pathogens (human or animal) or Class 2 GMOs eg Semliki Forest virus, Langat virus etc.

Containment:

- The ArCL2 room will be arthropod-proof. Entrance door into insectary separated from surrounding facilities by additional door.
• door to facility will have clear signage indicating ‘arthropod containment level 2, no unauthorized entry’;
• any ventilation inlets and outlets of room and incubators will be screened with gauze or similar of pore size small enough to trap the smallest arthropod life cycle stage;
• windows (if present) and doors will be sealed;
• sinks (if present) will have an adequate arthropod-proof trap or be sealed;
• measures will be taken to enable escapees to be easily detected and recaptured or destroyed (white/clear walls);
• solid and liquid waste will be autoclaved (some invertebrates are not killed by chemical disinfectants or fumigants);
• an insecticidal spray will be available for use in an emergency (ONLY) (use of insecticides may render the room unfit for invertebrates for a long period, if not permanently; non-residual type insecticides should be chosen; insecticides do not have an immediate “knock-down” effect on ticks, but could cause long-term damage);
• infected flying insects will be kept in primary and secondary containment (glove box, incubator);
• primary containers for mosquitoes will be either (i) solid material sealed with a tight-fitting (tape secured with an elastic band) net lid or, (ii) a sleeved netting cage;
• secondary containment for mosquitoes will be either: (i) ‘arthropod-proof’ incubator, transparent or with transparent inner door, (ii) large and robust transparent sealed plastic bag with pin-prick air holes, (iii) transparent glove box, (iv) transparent Perspex box sealed (tape or elastic band) with a net cover;
• experimental containers (both primary and secondary) will be labelled to indicate the date, workers initials, arthropod species, number of arthropods and the nature of any infectious agent; the number of arthropods in each standard primary container will be kept to a minimum and for flying arthropods will not exceed numbers that can be easily counted (ie. 20)
• numbers will be adjusted as arthropods are removed (or to record escapees); each arthropod will be accounted for as the work proceeds through to final fixation or disposal;
• all experiments will also be recorded in a log book (species, worker initial, date, number of arthropods, nature of infectious agent;
• flying or crawling arthropods will be handled on white trays to detect escapees;
• infected arthropods will be killed before they are taken out of the containment facility;
• operating protocols designed to minimise escape and bites will be adhered to.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM26 (CVR Church Street)

Solids from cell culture (eg plasticware such as flasks, tubes, pipettes tips etc.) - soak in 1% Virkon (w/v) for a minimum of 12 hours. Dispose of as normal solid waste (black bag).

Other solids (agar plates, gloves etc.) placed in a metal disposal box (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 1210C for 30 minutes.

Arthropod-derived material such as the remains of mosquitoes or tick organ cultures will be placed in a metal disposal box (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 1210C for 30 minutes.

Liquids (eg samples, culture supernatants, tissue culture media) – add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Liquids (E. coli culture medium)- add Virkon to final concentration of 1% (w/v) for a minimum of 12 h, then discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave by using a make safe cycle of 1210C for minutes.

Degree of kill:
Chemical Sterilization by Virkon- effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).

GM223 (CVR Garscube Estate):

Solids (pipettes tips, gloves etc.)- place in container (lined with sealed bag) and dispose of by autoclaving using a make safe cycle of 121-123°C for 30 minutes.

Arthropod-derived material such as the remains of mosquitoes will be placed in a sealed container (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 121-123°C for 30 minutes.

Liquids (eg. samples) – dispose of by autoclaving using a make safe cycle of either 121-123°C for 30 minutes.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
1. GM 26 committee comments (approval date 25/08/11; see below):

The CVR Church Street GM subcommittee met on 22/08/11 to consider the proposal:

"Arthropod host cell/arbovirus interactions and immune responses induced by virus replication."

Subcommittee members:
R E
J M
A P
F R

The subcommittee members agreed recommendations for modifications to the following topics:

In Section 12 (Describe the waste management measures etc) of the HSE Notification and Section 16 (Proposed experiments etc, sub-heading Waste Disposal) of the Risk Assessment :-

Some of the autoclaving cycles specified were not available on the autoclave at the CVR Church Street site.

Procedures appropriate for clinical waste, incineration and microwaving were either not in use at the CVR Church Street site or would be expensive to implement. They were considered to be unnecessary for this proposal.

The procedure specified placing arthropod derived material into a sealed container for autoclaving. However, autoclaving requires containers be open to allow entry of steam.

No procedures for disinfection of contaminated material were described. All material is to be rendered safe by autoclaving, which would entail contaminated material being stored and transported to the autoclaves. This was considered an unacceptable risk, especially for liquid waste, and requires an effective disinfection procedure to be put in place.

The subcommittee decided that the relevant entries under section 12 in the HSE Notification and section 16 of the Risk Assessment should be replaced with the existing waste disposal and disinfection policies employed at the CVR Church Street site. These are available on the local intranet. Before this can be approved, validation must be obtained regarding the effectiveness of Virkon for inactivating LGTV and SFV.

In Section 6 (Purpose of the contained use), reference is made to subgenomic replicons of other arboviruses (eg chikungunya and dengue) that are not otherwise included in this proposal. A full description of the work to be carried out with these agents, the nature of the hazards and the precautions required should be included in both the HSE Notification and Risk Assessment.

Clarification is required for section 8 (Containment and control measures for GMOs that are not micro-organisms; page 11) of the HSE Notification and for section 11, sub-heading Hosts (page 8) of the Risk Assessment concerning the transport of arthropods. The latter states that; "Samples (dead or non-mobile arthropods (our italics) or material from these) will be transported to our CL2 laboratory according to standard regulations." The subcommittee agreed that no living infected arthropods would be allowed in the CVR Church Street site and that they should only be handled in an insectary.
The reference to; “the city centre location of our laboratories” on page 10 of the Risk Assessment is not appropriate for the CVR Garscube site and should be removed.

Under section 7, sub-heading Control measures (page 8) of the HSE Notification, the use of appropriate PPE (not just gloves) should be specified.

An estimate of virus quantities (eg pfu/ml) should be provided in addition to the culture volumes in the HSE Notification, section 9 (Maximum culture volumes per experiment; page 11) and the RA section 16 (Culture volumes; page 14).

The requested changes were made and on 25/08/11 the revised documents were circulated to and approved by the subcommittee members.

2. GM223 committee comments (approval date 20.10.2011, see below):

"The risk assessment was clear but clarification on local mosquito activity was required; more detail on any full length viruses being held, and careful consideration of any possible recombination events was deemed essential. Risks to immune-suppressed personnel needed to be mentioned.

These points have been addressed in form CU2 and Risk Assessment, and on the 20.10.2011 the revised documents were approved.

Dr. G on behalf of the committee."

**Project Containment**

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 26/12.1

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<td>Bunyavirus molecular biology and virus/host interactions</td>
<td>Class 2</td>
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The Bunyaviridae is one of the largest taxonomic arbovirus groups (over 300 named isolates). Viruses within the Bunyaviridae are classified into five genera: Orthobunyavirus, Hantavirus, Nairovirus, Phlebovirus and Tospovirus following molecular organization and serotyping [1]. Within a genus, viruses show similar patterns in the sizes of their genome segments and structural proteins, and whether or not non structural proteins are encoded. Bunyaviruses are important examples of emerging viruses, including pathogens of humans and/or animals [2,3]. The bunyavirus genome is a tri-segmented single-stranded RNA genome of negative (or ambi-sense) polarity that encodes four structural proteins. The genome segments are called L (large), M (medium) and S (small). The L RNA encodes the L protein, the M RNA segment Gn and Gc, and the S RNA the N protein. In addition, some viruses encode non-structural proteins called NSm (M segment) and NSs (S segment). The segments are encapsidated by the nucleocapsid (N) protein and interact with the viral RNA dependent RNA polymerase, the L protein, to form ribonucleoprotein complexes (RNP) known as nucleocapsids. During replication, a positive-stranded antigenome is produced which serves as the template for the de novo production of negative-stranded genomes. The polymerase L also transcribes shorter mRNAs which direct synthesis of bunyavirus proteins; interestingly, caps are “snatched” from cellular mRNAs. The bunyavirus terminal 3’ and 5’ sequences are largely complementary and interact to give the genome a characteristic pan-handle structure; these terminal sequences (and other sequence elements adjacent to the protein-coding regions) contain elements regulating transcription and replication of the bunyavirus genome. The RNPs are contained within a lipid envelope into which the two viral glycoproteins Gn and Gc are inserted. Replication occurs in the cytoplasm of infected cells, and viruses mature by budding at Golgi membranes (reviewed in [4]).

Most bunyaviruses are transmitted by arthropod vectors such as mosquitoes or ticks. Infection of arthropod cells is usually not cytopathic while vertebrate cells die following infection. Understanding the molecular basis for the different outcomes of infection in these cells of different origin is of fundamental importance, and may help in the development of new control measures for arboviral diseases. This is likely to imply immune responses (which differ in both hosts) and virus host interactions [5,6]. Research on antiviral immune responses in arthropod vectors has focused on positive-strand RNA viruses. Arthropod vectors activate immune responses upon infection, i.e. immune signalling pathways, humoral responses and most crucially RNA interference (RNAi) responses. Viral replication induces production of double stranded RNA, the RNAi-inducing substrate cleaved by dicer-type nuclease activities to produce virus-induced small interfering RNAs (also called viRNAs in the context of viral infection) which are integrated into the RNA-induced silencing complex (RISC). The RISC mediates nucleolytic cleavage of complementary viral RNAs. In addition, arthropod cells might differentially regulate subsets of their own small RNA repertoire following infection by arboviruses. In vertebrates, it is clear the interferon response is a key factor in limiting bunyavirus replication and this process, as well as bunyavirus antagonism of the interferon (as well as other host antiviral responses such as PKR) is an area which has seen important progress although important questions remain [6].

This project will investigate bunyavirus molecular biology and virus/host interactions, including structure-function analyses of viral proteins and the role of signals within viral RNAs, the interactions of bunyaviruses with host cell components, as well as the role of immune responses in controlling bunyavirus replication.
Two orthobunyaviruses will be at the core of this project. Bunyamwera virus (BUNV) is the prototype virus of the family [7]. Originally isolated in Uganda from infected mosquitoes, most of our understanding of bunyavirus biology is due to studies on this model bunyavirus. We will also study Schmallenberg virus (SBV), which was recently isolated from cattle in Germany and leads to disease (fever, diarrhoea, malformations, abortion) in cattle and sheep [8,9]. Non-hazardous minigenome systems (see below) of bunyaviruses of higher containment levels such as Rift Valley fever virus (RVFV) [10] may also be studied.

A reverse genetics systems that allows manipulation of the BUNV genome is available [11,12]. Cultured cells constitutively expressing bacteriophage T7 RNA polymerase are transfected with a mixture of 3 plasmids, each containing a full-length cDNA copy of one of the bunyavirus genome segments under control of T7 promoter and terminator sequences, and a hepatitis delta ribozyme sequence. Intracellular transcription produces exact copy RNA transcripts that act as viral mRNAs and templates for viral RNA synthesis that result in production of infectious virus. The system was initially established with BUNV and was subsequently adopted by other bunyaviruses such as the orthobunyavirus LaCrosse [13,14]. A reverse genetics system allows us to introduce mutations into the orthobunyavirus genome, as well as to introduce new sequences (such as reporter genes) or change or delete sequences [15,16,17]. Similarly, minigenome systems can be constructed in which only the coding sequences between the terminal orthobunyavirus sequences (either genomic or antigenomic) are replaced with a reporter gene such as Renilla luciferase (RLuc) or eGFP. A minigenome will be transcribed if N and L proteins are co-expressed from expression plasmids, which is useful to study the role of sequences and proteins in replication and in packaging as minigenome RNPs can be introduced into virions following superinfection with BUNV for example [18,19] and potentially also virus replicon particles (VRPs) by co-expression of glycoproteins. Minigenomes are on their own non-propagating and are used to mimik virus replication, and thus are a safe alternative to study for example replication of high-containment bunyaviruses.

In this project, we aim to work on the following topics as listed below. Eukaryotic host systems will be cells of vertebrate or invertebrate origin.

1) Development and use of reverse genetics systems for BUNV and SBV. Such a system is already in use for BUNV, and the SBV system will be designed along the same principles as the BUNV system. Similar systems for other bunyaviruses of the same hazardgroup may be considered for comparative studies.

Reverse genetics systems will be used to:
- Introduce mutations into the bunyavirus sequences. These can target coding or non-coding sequences although amino acid sequences of the glycoproteins will not be changed, to avoid alterations of host cell tropism.
- Introduce deletions and insertions into bunyavirus sequences, targeting coding or non-coding sequences. Deletions can for example target parts of non-coding regions, insertions can be reporter genes, bunyavirus genes may be completely replaced with other sequences.
- Exchange sequences between bunyavirus segments and within segments. Sequences from within bunyavirus segments will be transferred to other segments or transferred to other regions of the same segment.

Recombinant and wild type bunyaviruses will be used to:
- Study viral growth, gene expression, replication and packaging mechanisms in host cells.
- Study interactions with the host cell (replication, interaction with host cell factors, immunity).

2) Development and use of bunyavirus minigenome systems
- A BUNV minigenome is already in use and similar system will be developed for SBV. Minigenome systems will be used (as read-outs) in studies on protein functions (cellular, viral), sequence function, segment packaging (packaging by glycoproteins co-expression, or following co-infection) and complementation studies (ie. BUNV N in SBV minigenome).
- Minigenome systems of others bunyaviruses will be developed or obtained for studies on replication and packaging (Rift Valley fever virus etc).

3) Functional studies and expression of bunyavirus proteins.
- Manipulation of protein sequences by introduction of point mutations, deletions, insertions, fusion to others sequences such as reporter genes, tags etc.
- Expression of bunyavirus protein to study interactions with host cell components and cellular localisation.
- Expression of bunyavirus proteins by prokaryotic expression systems, for example for protein purification.
- Expression of bunyavirus proteins by eukaryotic expression systems (plasmid, retroviral expression), for example to produce packaging cell lines, cell lines expressing virus-like particles, or cell lines to study protein function.
- Expression of bunyavirus proteins by eukaryotic expression systems (plasmid, retroviral expression), to produce packaging cell lines for bunyavirus sequences (segments or minigenomes; production of single round infectious particles) and cell lines expressing virus-like particles.
- Silencing of bunyavirus gene expression or host genes involved in bunyavirus replication by RNA interference.

4) Studies on host immunity.

- Interactions of bunyaviruses (wild type, recombinant, minigenome) with vertebrate host responses, and counteraction of host responses (for example by NSs proteins) will be investigated. Immune signalling/antiviral responses (including activation mechanisms), and viral interference therewith, will be analysed. This will involve studies of extra- and intra-cellular host molecules. Role of immunity in host cell tropism (infections of cells from different species) will also be investigated. These studies will also use reporter genes inducible upon activation of a given pathway. Vertebrate-origin cell culture models are available.
- Interactions of bunyaviruses (wild type, recombinant, minigenome) with arthropod host responses, and counteraction of host responses will be analysed. Studies of the RNA interference response (host proteins regulating this mechanisms, identification and role of cellular and viral small RNAs, role of bunyavirus sequences in countering host responses) as well the role of immune signalling pathways and antiviral responses (including activation mechanisms) will be investigated. This will involve extra- and intra-cellular pathways (for example extracellular melanisation pathways). These studies will also use reporter genes inducible upon activation of a given pathway. Arthropod-origin cell culture models are available.
- The roles of bunyavirus RNAs in activitating or inhibiting vertebrate or arthropod immune responses will be investigated (expressed in viral systems such as Semliki Forest virus or plasmid-based expression).

References:


Recipient or parental organism

Viruses and derived replicons to be used will be of the Bunyaviridae family:
- Schmallenberg virus (SBV) (Orthobunyavirus).
- Bunyamwera virus (BUNV) (Orthobunyavirus).
- Recombinant viruses derived from BUNV and SBV.
- Minigenome systems derived from BUNV, SBV, and other bunyaviruses (Rift Valley fever virus etc.).

Other containment level 2 bunyaviruses (Guaroa virus, Maguari virus, Toscana and Sandfly fever phleboviruses etc.) may be considered as required.

No infectious containment level 3 or 4 viruses will be used.

Host/vector system

All organisms will be used at containment level 2, which is routine practice.

Plasmid vectors:
Viral sequences (full length clones of BUNV or SBV; partial sequences of these and other bunyaviruses) and other genes (cellular, viral, reporter) are available in plasmid-encoded cDNA form. RNAs can be transcribed from bacterial promoters (ie. T7 or SP6 by in vitro transcription or plasmid-expressed RNA polymerase in cells) or eukaryotic promoters (CMV, SV40, cellular promoter, baculovirus promoters etc.). Plasmids for expression of bunyavirus sequences (virus rescue or minigenome) contain a T7 RNA polymerase promoter, T7 terminator and a hepatitis delta-derived ribozyme sequence. Plasmids contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin) and sometimes a selectable eukaryotic drug resistance marker such as neomycin or puromycin resistance. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

Lentiviral expression vectors: only at GM Centre 26; use is covered in CVR GM form 41 and amendments.

Hosts:

Prokaryotic hosts:
Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste should prevent release of viable organisms. The risk to the environment is therefore effectively zero.
Eukaryotic hosts:
Vertebrate cell lines of various origins (typically human, mouse, hamster, bovine etc.) and invertebrate cell lines of various origins (mosquito, culicoides, tick etc.). The vertebrate BSR-T7/5 cell line (derived from BSR, a clone of BHK) (Buchholz et al., 1999) expresses T7 RNA polymerase and will be used for minigenome assays and virus rescue, as may similar cell line expressing T7 RNA polymerase.

In prokaryotic cells, only selectable (eg antibiotic resistance) genes will be translated; prokaryotic cells may also be used for protein expression and subsequent purification.

In eukaryotic cells, the following RNAs will be produced which may be translated into the following gene products:

- Bunyavirus proteins and RNAs (BUNV and SBV, or minigenomes): Proteins involved in replication and transcription of viral genetic material and virus structural proteins. Some of these virus proteins will interact with host-cell components and may affect host cell responses to infection; few of these interactions are currently known. If all virus sequences are translated new virions may be generated; minigenomes are non-propagative viral RNAs (capable of replication). Partial RNAs do not give rise to propagating infectious material but may display biological activities (ie. RNAi inhibitory).

- Non-bunyavirus genes or sequences: Genes of prokaryotic (bacteriophage polymerases) or eukaryotic (eg. reporter genes such as luciferases or fluorescent proteins such as GFP; inhibitors of arthropod innate immunity, vertebrate or arthropod host genes etc.) origin. Reporter gene sequences will also be present in bunyavirus minigenome RNA. The recombinant foreign proteins to be expressed provide no significant increase in the hazard to human health; none are toxic or likely to produce disease in the quantities that could be produced by accidental exposure to these systems. Non translated RNA sequences (including T7 terminator and hepatitis delta ribozyme in plasmids used for virus rescue or minigenomes) have no known toxic effects and pose no risk to human health.

Techniques used to introduce insert or vectors into cells:

Prokaryotic cells: Introduction of DNA into E. coli will be by heat shock/chemical transformation. These techniques have been extensively described and are widely used; they rely on getting DNA very close to the bacterial membrane and introducing the genetic material through pores or membrane fusion.

Eukaryotic cells: Cells will be transfected using transfection reagents such a lipofectamine or by electroporation. Virus genomes or sequences will be introduced into cultured cells by infection with virus or virus replicon particles (VRP). Lentiviruses will be packaged and used to infect target cells.

Note- Nature of experiments are to be carried out at Church Street and Garscube Estate:

GM 26 (CVR Church Street):
- Preparation, characterisation and genetic manipulation of bunyaviruses and bunyavirus minigenomes.
- Protein expression and purification (prokaryotic cells), protein expression and purification (eukaryotic cells including lentiviral expression), protein interaction and modification studies.
- Infection/transfection of cell lines, preparation of lysates for downstream applications (including sequencing).
- Reporter gene assays, immunofluorescence studies.

GM223 (CVR Garscube Estate):
- As above; exception: separate GM notification for lentiviral/retroviral expression required.
Foreseeable effects/risk assessment for human health and safety (also see Risk Assessments):

Plasmid vectors:
Viral sequences (including full length clones or partial sequences of SBV or BUNV or other bunyaviruses) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Transcription of even complete viral cDNA (SBV or BUNV) will result in no infectious RNA since the promoters are not active in prokaryotic hosts. Systems for prokaryotic gene expression are under control of an inducible prokaryotic promoter. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV or SV40), eukaryotic (eg actin promoter, pathway inducible promoter such as STAT, interferon etc.) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human cells. None of the genes used in these studies are oncogenes. The hazard of expressing ‘foreign’ genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the foreign gene product and is discussed in more detail below.

BUNV:
Infection with this virus can cause a febrile illness with headache, arthralgias, rash and infrequent central nervous system involvement (Gonzalez and Georges, 1988). The classification into ACDP hazard group 2 indicates current expert opinion that containment level 2 precautions are considered adequate for controlling the risks associated with working with BUNV. The viral glycoprotein amino acid sequences are the major determinants of host range and cell tropism and will not be changed beyond the addition of marker genes as fusion or cleavable markers into the structural open reading frame. Genetic changes that will be introduced into the virus sequences will be targeted at disrupting or changing specific functions of virus RNA or virus proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication.

SBV:
This an animal pathogen, and not likely to be a risk to human health by current guidelines (http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=795). Genetic changes that will be introduced into the virus sequences will be targeted at disrupting or changing specific functions of virus RNA or virus proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virus virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication. As for BUNV, the viral glycoprotein amino acid sequences which are the major determinants of host range and cell tropism will not be changed beyond the addition of marker genes as fusion or cleavable markers. SBV is not listed on the ACDP or SAPO list but is recommended to be classified as hazard group animal pathogen 2 (see attached recommendation). We propose to treat SBV like BUNV in terms of risk for human infections and health unless other regulation is in place for a particular pathogen. In addition, personnel working with SBV should not be in contact with susceptible animals or visit farms/zoos where they could potentially come into contact with susceptible species for a period of 48 hours. A form outlining that they have understood their responsibility with regards to minimising the risks to the environment associated with this virus will be signed by personnel working on SBV.

Personnel working with these viruses are expected to inform occupational health should they become pregnant or immunosuppressed, and if planning pregnancy inform the PI so that precautionary measures can be introduced following advice from occupational health.

Bunyavirus minigenomes:
Minigenomes pose no risk to human health, including those derived from pathogenic bunyaviruses such as Rift Valley fever virus. They cannot propagate on their own, replication requires co-expression of L and N proteins. Even if packaged by co-expression of glycoproteins and thus used to infect cells, their own transcriptional activity would be minimal. If packaged into virions, the associated risk is that of the virus itself as reporter genes in minigenomes are not reported to be toxic.

Note 1: Should material such as minigenomes or other sequences be obtained for bunyaviruses listed under schedule 5 regulations, no additional sequences that could potentially be used to assemble the full length genome of a schedule 5 pathogen will be stored in locations that do not have required licenses.
Note 2: No reassortment experiments between BUNV, SBV or closely related orthobunyaviruses will be carried out as class 2 activity, to eliminate the possibility of creating viruses with enhanced pathogenicity such as Ngari (Gerrard et al., 2004). No work with two or more viruses of different bunyavirus species will be carried out in parallel in the same microbiological safety cabinet. Microbiological safety cabinets are decontaminated between experiments (UV and/or alcohol). The risk of accidental co-infection is effectively zero.

The host/recipient organism:
Prokaryotic organisms: All strains will be disabled, commercially available E. coli derivatives classified as ‘especially disabled hosts’ by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic cells:
Cell lines to be used would not survive inside the human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA or infection will confer no growth or survival advantage in or outside the laboratory to cell lines of vertebrate or arthropod origin.

The inserted/donated genetic material:
Viral RNA and proteins, cellular proteins: Most such bunyavirus-expressed viral (such as inhibitors of insect immunity; where tested in vivo this has led to more rapid death of arthropod vectors) or cellular proteins are unlikely to have harmful effects in eukaryotic cells, however some could perturb normal cellular metabolism, predispose or protect against cell death or render cells more or less susceptible to other infections if overexpressed or silenced. It is very unlikely that BUNV or SBV (and hazard group 2 bunyaviruses) RNA or proteins would have any harmful toxin-like effect outside cells. Only antigenomic RNA containing a bunyavirus sequence can be translated or give rise to infectious virus (if all necessary genomic information is provided) in eukaryotic cells; minigenome RNAs are non propagative and pose no harm (individual cells replicating minigenome are likely to die or be eliminated by the immune system). Viral non-coding RNA sequences are important in bunyavirus replication but do not encode biologically active molecules that are likely to induce physiological effects in humans on their own.

No proteins that are known to interfere with immune or host responses in vertebrates will be cloned into bunyavirus genomes for the purpose of virus rescue under containment level 2 conditions, to avoid producing potentially highly pathogenic bunyaviruses by adding a more potent host response antagonist that those already present in bunyavirus genomes.

Reporter genes: Reporter genes of prokaryotic or eukaryotic origin (eg luciferase, fluorescent proteins etc.). No harmful properties have been attributed to these proteins. There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Photinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function. Introduction of these reporter genes is unlikely to increase the risk associated with these viruses.

Other non coding RNA sequences (siRNAs, dsRNAs, other RNAs): Any effects these elements have on overall gene expression are likely to be minimal. They may affect gene function or virus replication in individual cells but are unlikely to lead to whole body physiological effects as they do not code for the production of secreted bioactive molecules.

Other sequence changes in viral sequences:
Mutation or deletion will target disruption of non-structural protein functions or non-coding viral sequences and are likely to have no or deleterious effects on viral replication, thus not increasing risk or hazards to human health associated by viruses genetically modified in such a way. No changes will be introduced that will change the amino acid sequence of the glycoproteins Gn and Gc (with the exception of in-frame fusion to reporter genes) so viral tropism is not affected.
Summary:
Genetic changes that will be introduced into the BUNV or SBV sequences as described above can be expected to attenuate virus fitness (with the exception of insect immunity inhibitors, but this does not concern human health); such changes are highly unlikely to increase virus virulence as they modify virus sequences (which are optimized for replication), or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication or have no effects outside the infected cell. Therefore we do not expect increased risk or hazards associated with viruses modified in such a way.

Control measures – assign provisional containment level:
GM bacteria transformed with plasmids containing viral sequences, reporter genes, other eukaryotic/prokaryotic genes or non-coding sequences - Containment Level 1.
Eukaryotic cells containing bunyaviral sequences unable to form complete infectious virus, or plasmids expressing reporter genes, other eukaryotic/prokaryotic genes or non-coding sequences – Containment Level 1.
Eukaryotic cells containing hazard group 2 bunyavirus sequences (capable of forming infectious virus, or capable of undergoing recombination or minor mutations to form infectious virus) – Containment Level 2.

All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. A microbiological safety cabinet will be used where appropriate (see below). Work with infectious bunyaviruses should take place in a microbiological safety cabinet. Spray gloves with alcohol when working outside the hood or before disposing.

Foreseeable effect/risk assessment for the environment:
BUNV: This virus is not endemic in the UK, and it is not on the SAPO list. It is not clear whether suitable vertebrate hosts and insect vectors are present to establish an infection in this country. BUNV can infect vertebrates (such as rodents) and certain biting insects. Transmission to vertebrates is by inoculation and to insect is by feeding on blood of an infected vertebrate. BUNV was isolated from mosquitoes in Uganda. Given the absence of these specific mosquitoes in Scotland and low pathogenicity of virus strains, the chance of accidentally infected arthropods initiating a transmission cycle sustained by local insects is negligible. The modified viruses will not have any inserted foreign genes other than non-harmful genes or sequences (with exception of inserted insect immunity inhibitors, which may lead to rapid death of infected arthropods), or (usually disruptive) changes in viral genes (with the exception of glycoproteins, whose amino acid sequences will not be changed) or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. In the absence of a suitable host or vector these viruses would not survive in the environment and the risk is effectively zero. Arthropods are kept out of labs and the risk of accidental infection of insects by ingestion of contaminated material is also effectively zero for BUNV.

SBV: This virus has now been detected in the UK, and infections are most likely the result of midges carried from mainland Europe to the UK (Veterinary Record, News & Reports- see reference below). However other blood-feeding arthropods cannot be ruled out and vector competence studies are required. It is not yet on SAPO or ACDP lists and the recommendation (including for infection of insects) from Scottish Government (Rural and Environmental directorate) is to handle this pathogen as a category 2 animal pathogen (see attached recommendation). We propose, until further notification, to work with SBV at containment level 2 similarly to BUNV. The modified viruses do not contain anything other than non-harmful genes or sequences (with the exception of inserted insect immunity inhibitors, which may lead to rapid death of infected arthropods), or disruptive changes in genes (with the exception of glycoproteins, whose amino acid sequences will not be changed) or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. If SBV was to escape the lab and infect local midges (or possibly other arthropods), there is a significant risk it could initiate a transmission cycle although this virus might arrive here by natural means (competence of local midges for SBV is not known but there is a significant risk they may be able to act as vectors). However arthropods are kept out of labs and the risk of infection of arthropods by ingestion of contaminated material is low and the likelihood of virus released from our laboratory infecting local vectors is effectively zero (see disinfection and waste disposal procedures). In addition, advice will be given to lab personnel that they should not be in contact with susceptible animals or visit farms/zoo where they could potentially come into contact with susceptible species for a period of 48 hours following work with SBV. A form outlining that they have understood their responsibility with regards to minimising the risks to the environment associated with this virus will be signed by personnel working on SBV.
Note: No proteins which could interfere with immune or host responses in vertebrates will be cloned into bunyavirus genomes for the purpose of virus rescue under containment level 2 conditions, to avoid producing potentially highly pathogenic bunyaviruses by adding a more potent host response antagonist that those already present in bunyavirus genomes.

We propose to use containment group 2 bunyaviruses (see point 7) similar to BUNV unless other regulations are in place for a particular pathogen.

Minigenomes: These systems derived from BUNV, SBV or other bunyaviruses are non-propagative and even if packaged, pose no or no additional hazard.

Hosts:

Prokaryotic organisms: Disabled, commercially available E. coli strains. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. Acquiring antibiotic resistance (from the plasmid vector) or additional sequences (virus or marker gene sequences) will not give these strains any survival advantage in the environment. The risk to the environment is therefore effectively zero.

Eukaryotic cells: Cell lines to be used are not viable outside the laboratory and thus pose no threat to the environment. Addition of plasmid vectors or virus or minigenomes will confer no growth or survival advantage in the environment.

In summary

i) consequence/severity of effects: Severe

ii) likelihood of effects being realised: Low

iii) overall risk: Low

Final classification: GM bacteria transformed with plasmids containing viral sequences, reporter genes, other eukaryotic/prokaryotic genes or non-coding sequences (unable to cause disease, minimal hazard to health and environment) - Containment Level 1.

Eukaryotic cells containing bunyaviral sequences unable to form complete infectious virus, or plasmids expressing reporter genes, other eukaryotic/prokaryotic genes or non-coding sequences – Containment Level 1.

Eukaryotic cells containing bunyavirus sequences capable of forming infectious virus, or capable undergoing recombination or mutation to form infectious virus – Containment Level 2.

All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. As a routine measure all work will be carried out at Containment level 2. A microbiological safety cabinet will be used where appropriate. Work with infectious bunyaviruses should take place in a microbiological safety cabinet. This additional layer of security also is to prevent accidental contamination of any arthropods (thus reducing risk to the environment). Spray gloves with alcohol when working outside the hood or before disposing. Traps to catch arthropods will be present in labs were work with live infectious bunyaviruses is carried out.

References:


<table>
<thead>
<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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<tbody>
<tr>
<td>Not applicable.</td>
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<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
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<tr>
<td>Not applicable.</td>
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<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
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<tr>
<td>Disinfect bunyavirus-contaminated material immediately.</td>
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**GM26 (CVR Church Street):**

Solids from cell culture (eg plasticware such as flasks, tubes, pipette tips etc.) - soak in 1% Virkon (w/v) for a minimum of 12 hours. Dispose of as normal solid waste (black bag).

Other solids (agar plates, gloves etc. )- placed in a metal disposal box (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 121°C for 30 minutes. Gloves used for bunyavirus work should be disinfected with alcohol before putting in containers.

Liquids (eg samples, culture supernatants, tissue culture media) – add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Liquids (E. coli culture medium)- add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave by using a make safe cycle of 121°C for 30 minutes.

**Degree of kill:**
- Chemical Sterilization by Virkon- effectively 100% kill.
- Autoclaving - effectively 100% kill (annual validation).

**GM223 (CVR Garscube Estate):**

Disposable solids (eg plasticware such as flasks, tubes, pipette tips etc.): soak in 1% Virkon (w/v) for a minimum of 12 hours. Transfer solid contents to clear autoclave bags and autoclave at 121°C for a minimum of 20 minutes prior to final disposal by district council to land fill, remaining liquid to be discharged to drain.

Other solids (agar plates, gloves etc.): placed in a marked box lined with a clear autoclave bag, and disposed of by autoclaving using a make safe cycle of 121°C for at least 20 minutes. Seal contaminated plates (with for example bacterial GMOs) before placing in bag, to avoid lid falling off. Gloves used for bunyavirus work should be disinfected with alcohol before putting in containers.

Liquids (eg. samples, bacterial culture media, tissue culture media) - add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave by using a make safe cycle of 121°C for at least 20 minutes, then incinerated.

**Degree of kill:**
- Chemical Sterilization by Virkon- effectively 100% kill.
- Autoclaving - effectively 100% kill (annual validation).
1. GM 26 committee comments:

The CVR Church Street GM subcommittee met on 22/08/11 to consider the proposal:
"Bunyavirus molecular biology and virus/host interactions."

Present:
*********** personal details removed

The following topics were discussed:

The committee members had no concerns regarding the overall aims of the proposed experiments. Overall the work with Bunyamwera virus (BUNV) was approved for handling under normal class 2 conditions. Questions were raised over some aspects of the work with Schmallenberg virus (SBV). Additional questions on risk to the environment and containment were also discussed.

This points have been addressed in form CU2 and the Risk assessment, and on the 15.03.2012 the documents were approved.
On behalf of the CVR Church Street subcommittee, Dr. P"

2. GM223 committee comments:

"The risk assessment was clear and comprehensive but a number of clarifications in particular on risk to the environment by SBV (vectors, accidental contamination of local arthropods) were required.

These points have been addressed in form CU2 and Risk Assessment, and on the 15.03.2012 the revised documents were approved.

Dr. G on behalf of the committee."

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Drosophila C virus (DCV) belongs to the genus Cripavirus now classed within the Dicistroviridae family (previously Picornaviridae) within the order Picornavirales. It is a single stranded, positive-stranded RNA virus with a genome length of approximately 9300 bases. Unlike picornaviruses, the genome encodes two open reading frames (ORF) which encode (towards the 5’ end) an open ORF coding for non-structural proteins and (towards the 3’ end) an ORF coding structural proteins. The non-structural ORF encodes proteins involved in virus replication (including the RNA interference inhibitor 1A), while the structural ORF encodes viral envelope proteins. Both open reading frames are translated via separate internal ribosome entry sites in the genome. A Vpg protein is attached to the 5’ end the 3’ end contains a poly A sequence. These viruses are non-enveloped (1).

DCV has a worldwide distribution (including UK) and is also found in laboratory strains of Drosophila melanogaster and cultured cells (D. Obbard, University of Edinburgh, personal communication); it can also infect a variety of drosophila species (6). Infection leads to more rapid development, females are heavier and lay more eggs. Infection occurs by ingestion and infection is not pathogenic, although this may vary between flies. Interestingly, infection by injection leads to rapid death of flies (4). DCV is not a SAPO or ACDP pathogen; it does not infect vertebrate cells.

The genetic and molecular tools available for D. melanogaster make it a very useful model to study virus-host interactions and virus pathogenesis in an easily grown and genetically manipulated host. DCV has been particularly used in studies on antiviral immune signalling and RNA interference responses in drosophila (7); this research has led the insect virology field for many years. The DCV 1A protein acts as a viral suppressor of RNA interference (10), yet the virology of these virus/drosophila interactions is overall poorly understood in contrast to the extensive knowledge we have of drosophila genes and physiology. DCV is also inhibited by endosymbiotic Wolbachia bacteria in drosophila (9). This is similar other RNA viruses such as arboviruses in mosquitoes (which may be a useful control measure in public health strategies) yet it is not known...
how inhibition by the endosymbiont is mediated and powerful drosophila genetics may be required to solve this question and improve the uses of Wolbachia endosymbionts (5).

Despite the strength of D. melanogaster genetics, a major drawback of drosophila immunity research is that no reverse genetics system is available to manipulate and study the genome of DCV. This project aims to develop an infectious DCV clone to apply reverse genetics technology to this virus. A dicistrovirus infectious clone has so far only been described for Rhopalosiphum padi dicistrovirus (RhPV) (2, 8). This clone is not very efficient and one of the major drawbacks is that the length of the 3’ poly A tail is not known for these viruses; it is likely to be similar to picornaviruses and of 20-60 nucleotides in length. However the RhPV infectious clone has no such sequence, bar a very short internal poly A stretch. This may be one of the reasons why the RhPV infectious clone is relatively weak, and possibly efforts to make a DCV infectious clone so far may have failed due to missing 3’ poly A tails.

The prototype DCV strain is isolate EB, but sequences of other isolates are available.

We propose to:
- Propagate the cloned DCV sequence. The DCV genome will be cloned into a plasmid backbone such as pUC-type or other vector. Transcription of the viral cDNA into RNA will be under control of a bacteriophage RNA polymerase promoter (T7, SP6 or similar) or eukaryotic promoter, and RNA introduced into insect cells or infectious clone cDNA transfected into insect cells for virus rescue. Where required, ribozyme-encoding sequences (hammerhead ribozyme, hepatitis delta ribozyme or similar) will be added to the genome ends (5’ and/or 3’ ends) to generate correct termini in the transcribed RNA as has been described previously (3).

- Manipulate the cloned DCV genome by molecular biological methods. This will be achieved by insertion of sequences (reporter genes such as luciferase or fluorescent proteins, drug resistance genes, other eukaryotic genes, non-coding sequences), deletion of sequences (point deletions, deletion of larger sequences) or point mutations within the DCV sequence. No changes will be made to sequences encoding the structural proteins, to avoid changes in viral tropism. Alterations to viral sequences such as those described above usually result in a fitness decrease. Modifications of the viral genome sequence will also include adding of poly A tails either by in vitro poly-adenylation or adding a cloned poly A sequence to the viral cDNA. Chimeras between strains of DCV (exchange of protein-coding sequences or non-coding sequences) will be created to study the role of strain-specific differences on replication, immunity and pathogenesis.

- If infectious DCV can be successfully rescued, we will delete the structural open reading frame 2 (or parts of it) to create non-propagating replicons and a packaging system (co-expression of DCV structural proteins in replicon-transfected cells) to obtain virus replicon particles.

- Individual DCV sequences (coding or non-coding) will be cloned into eukaryotic or prokaryotic vectors (plasmid or Semliki Forest virus or derived replicon) for molecular biological manipulation and/or expression purposes. Manipulated sequences may be re-introduced into the DCV infectious clone.

Studies to be undertaken with a DCV infectious clone and/or derived replicons:
- Immune responses of insect cells against DCV infection. We will study RNA interference and other mechanisms (immune signalling) that control DCV replication and viral interference therewith.
- Replication and polyprotein processing in insect cells. We will analyse sequences and mechanisms involved in these processes, as well as cellular factors involved in promoting or inhibiting DCV replication and spread.
- Virus entry, replication compartment formation and exit from insect cells. The tools for DCV manipulation (in particular viruses encoding reporter genes and replicons/virus replicon particles) developed in this project will allow to track these processes more efficiently (timing of events, determination of virus production etc.).
- Above studies will also be carried out in Wolbachia-infected insect cells to further study the mechanisms by which this micro-organism inhibits RNA virus replication.
- No experiments in vertebrate cells are planned.


Recipient or parental organism

Drosophila C virus. The infectious clone will be based on the sequence of strain EB; other strains or sequences from other strains may also be cloned.

Host/vector system

All organisms will be used at containment level 2, which is routine practice throughout the Church Street CVR site.

Plasmid vectors:

Viral sequences (including full length clones or partial sequences of DCV) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. RNAs can be transcribed through bacterial promoters (ie. T7 or SP6) or eukaryotic promoters. Plasmids contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin) and sometimes a selectable eukaryotic drug resistance marker such as neomycin or puromycin resistance. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

Hosts:

Prokaryotic hosts:

Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste should prevent release of viable organisms. The risk to the environment is therefore effectively zero.
Eukaryotic hosts: Invertebrate cell lines of various origins (drosophila, mosquito, tick etc.). These will be used to grow virus or express/silence individual proteins or sequences. Cells grown in standard media such as L-15 or Schneiders and are maintained in cooled cell culture incubators.

Origin & function

In prokaryotic cells, only selectable (eg antibiotic resistance) genes will be translated.

Virus sequences will be under the control of a promoter that will only generate transcripts in eukaryotic cells or in vitro transcription reactions.

In eukaryotic cells, RNA will be translated into the following gene products:

- Viral proteins and RNAs: Proteins involved in replication and transcription of viral genetic material and virus structural proteins. Some of these virus proteins will interact with host-cell components and may affect host cell responses to infection (for example 1A RNAi suppressor). If all virus sequences are translated new virions may be generated; replicons are non-propagative viral RNAs (capable of replication). Partial RNAs do not give rise to propagating infectious material but can display biological activities (ie. RNAi inhibitory, expression of some viral proteins for example for packaging of replicons).

- Non-viral genes: Genes of prokaryotic (eg CAT) or eukaryotic (eg. reporter genes such as luciferases or fluorescent proteins such as GFP; inhibitors of insect immunity ie. RNAi inhibitors, signalling inhibitors, melanization inhibitors etc.) origin. The recombinant foreign proteins to be expressed provide no significant increase in the hazard to human health; none are toxic or likely to produce disease in the quantities that could be produced by accidental exposure to these systems. Non-translated RNA sequences have no known toxic effects and pose no risk to human health.

Techniques used to introduce insert or vectors into cells:

Prokaryotic cells: Introduction of DNA into E. coli will be by heat shock/chemical transformation. These techniques have been extensively described and are widely used; they rely on getting DNA very close to the bacterial membrane and introducing the genetic material through pores or membrane fusion.

Eukaryotic cells: Cells will be transfected using reagents such a lipofectamine or by electroporation. Virus genomes will be introduced into cultured cells by infection with virus or virus replicon particles (VRP), using transfection reagents or by electroporation.

Evaluation of foreseeable effects

Foreseeable effects/risk assessment for human health and safety (also see Risk Assessments):

Plasmid vectors:
Viral sequences (including full length clones or partial sequences of DCV) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Transcription of even complete viral cDNA will result in no infectious RNA since viral elements are not active in prokaryotic hosts. Systems for prokaryotic gene expression are under control of an inducible prokaryotic promoter. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV, baculovirus-derived IE promoter), eukaryotic (eg actin promoter, pathway inducible promoter such as STAT) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human cells. None of the genes used in these studies are oncogenes. The hazard of expressing ‘foreign’ genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the foreign gene product and is discussed in more detail below.

DCV:
This virus is not known to infect or replicate in human cells or other cells of vertebrate origin. It is not a ACDP pathogen. Work with this virus or recombinant DCV does not pose a risk to human health. Replicons are non-propagating even within virus-replicon particles and pose no risk.
The host/recipient organism:
Prokaryotic organisms: All strains will be disabled, commercially available E. coli derivatives classified as 'especially disabled hosts' by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic cells:
Insect cell lines to be used would not survive inside the human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA will confer no growth or survival advantage in or outside the laboratory to these cell lines.

The inserted/donated genetic material:
Viral RNA and proteins, cellular proteins: Most individually expressed viral or cellular proteins (for example arthropod immune response inhibitors) are unlikely to have harmful effects in eukaryotic cells, however some could perturb normal arthropod cellular metabolism, predispose or protect against cell death or render cells more or less susceptible to other infections. It is very unlikely that DCV RNA or proteins would have any harmful toxin-like effect outside cells. RNA containing the complete DCV sequence can give rise to infectious virus in insect cells; replicon RNAs are non-propagating and pose no risk. Viral non-coding RNA sequences are likely to have minimal effects outside arthropod cell systems, and do not encode biologically active molecules that are likely to induce physiological effects in humans.

Reporter genes: Reporter genes of prokaryotic or eukaryotic origin (eg luciferase, fluorescent proteins etc.). No harmful properties have been attributed to these proteins. There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Photinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function.

Other non-coding RNA sequences: Any effects these elements have on gene expression are likely to be minimal. They may affect gene function or virus replication in individual cells but are unlikely to lead to whole body physiological effects as they do not code for the production of secreted bioactive molecules, and are unlikely to have any biological effects outside arthropod cell systems.

Sequence changes in viral sequences:
Mutation or deletion will target disruption of non-structural protein functions or non-coding viral sequences and are likely to have no or deleterious effects on viral replication, thus not increasing risk or hazards to human health associated by viruses genetically modified in such a way. Deletions in the structural genes to produce replicons will result in non-propagating virus-derived RNAs that pose no risk, even if packaged into virus replicon particles. Expression of sequences by Semliki Forest virus and derived replicons is covered under GM26/CVR_AK_1.

Control measures – assign provisional containment level:
GM bacteria transfected with plasmids containing viral or other eukaryotic sequences - Containment Level 1.
Eukaryotic cells containing DCV sequences unable to form complete infectious virus – Containment Level 1.
Eukaryotic cells containing full length DCV sequences (capable of forming infectious virus, or capable of undergoing recombination or minor mutations to form infectious virus) – Containment Level 2.

All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. A microbiological safety cabinet will be used where appropriate.

Foreseeable effect/risk assessment for the environment:
DCV: This virus is not on the SAPO/ACDP lists. It can infect drosophila by ingestion; recombinant virus is likely to be less fit than wild type but may be able to infect
drosophilid species and other insects. Virus is kept within containment and no accidental infections of arthropods can take place. Replicons are non-propagating even within
virus replicon particles and pose no risk. Arthropods and food are kept away from the laboratories and waste sterilized before disposal (see below) and it is therefore
unlikely that any lab-acquired infection and/or escape can take place.

Hosts:

Prokaryotic organisms: Disabled, commercially available E. coli strains. These strains can only survive in a controlled laboratory environment and will be used for
propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. Acquiring antibiotic resistance
(from the plasmid vector) or additional sequences (virus or marker gene sequences) will not give these strains any survival advantage in the environment. The risk to the
environment is therefore effectively zero.

Eukaryotic cells: Cell lines to be used are not viable outside the laboratory and thus pose no threat to the environment. Addition of plasmid vectors or virus will confer no
growth or survival advantage in the environment.

In summary

i) consequence/severity of effects: Low

ii) likelihood of effects being realised: Low

iii) overall risk: Low

Final classification: GM bacteria transfected with plasmids containing viral or other eukaryotic sequences (unable to cause disease, minimal hazard to health and
environment) - Containment Level 1.
Eukaryotic cells containing DCV sequences unable to form complete infectious virus – Containment Level 1.
Eukaryotic cells containing DCV sequences (capable of forming infectious virus, or capable undergoing recombination or mutation to form infectious virus) – Containment
Level 2.

All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. As a routine measure all work will be carried out
Containment level 2. A microbiological safety cabinet will be used where appropriate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids from cell culture (eg plasticware such as flasks, tubes, pipettes tips etc.) - soak in 1% Virkon (w/v) for a minimum of 12 hours. Dispose of as normal solid waste
Other solids (agar plates, gloves etc.) placed in a metal disposal box (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 121°C for 30 minutes.

Liquids (eg samples, culture supernatants, tissue culture media) – add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Liquids (E. coli culture medium)- add Virkon to final concentration of 1% (w/v) for a minimum of 12 h, then discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave by using a make safe cycle of 121°C for minutes.

Degree of kill:
Chemical Sterilization by Virkon- effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
1. GM 26 committee comments (approval date 22/02/12; see below):

The CVR Church Street GM subcommittee met on 31/01/12 to consider the proposal:
"Establishment of a reverse genetics system for drosophila C virus"

Subcommittee members:
R E
J M
J M
AP
F R (convener)

As Drosophila C virus only infects arthropods, it is not considered hazardous. Therefore, no objections were raised against the proposed work. However, clarification was requested for the following points:

How will the virus be propagated? The culture conditions should be described.

The Risk Assessment contains the statement “DCV: This virus is not a ACDP pathogen. It is not known to infect or replicate in human cells, and there is no risk associated with using this virus.” (page 7, Vectors and GMOs). In the GM application the same section (page 5, point 7) states “DCV: This virus is not able to infect or replicate in human cells or other cells of vertebrate origin.” These statements should be made to be consistent and accurate.

More clarity is required over the potential for infection of, and expression in, mammalian cells. This is particularly relevant in light of the subsequent discussion over the need for Notification (see below), which would only be required if there is a threat to human health or the environment. For example, it is proposed to clone genes under the CMV (and possibly other mammalian) promoter. Is this promoter active in insect cells and if not, why is it being used?

Also, SFV can infect mammalian cells so its use as a vector is potentially harmful. In the Notification (page 3) it is stated that “-Individual DCV sequences (coding or non-coding) will be cloned into eukaryotic or prokaryotic vectors (plasmid or Semliki Forest virus or derived replicon) for molecular biological manipulation and/or expression purposes.” In the Risk Assessment the sentence reads: (plasmid or Semliki Forest virus replicon or derived replicon). These statements should be made to be consistent and accurate.

Although only experiments in insect cells are listed under “Detailed descriptions and description/use of genetic modification:” (page 2-4 of Notification) a positive statement that no experiments in mammalian cells are planned should be inserted.

On page 2 of the Notification it is proposed to clone the DCV genome into a plasmid backbone. However, the section on Host/vector system (page 4) contains the statement that “Viral sequences (including full length clones or partial sequences of DCV) and other genes (cellular, viral or reporter) are available in plasmid-encoded..."
The discussion on this proposal raised the question of whether a Notification is required in this case. DCV is an insect pathogen that appears to pose no risk to human health and would have low or negligible environmental consequences if it escaped from the laboratory. It therefore resembles baculovirus, which is classified at containment level 1 except when specifically intended as a vector for gene expression in mammalian cells or when encoding viral functions that could themselves transmit to mammalian cells. Page 9-10 of the Risk Assessment Form includes the statement: “If the answer to questions 11 and 12 is negligible, you may believe that you have sufficient information at this stage to classify the project to class 1, as defined in the Contained Use Regulations 2000. In order to do this you should be confident that in the event of a total breach of containment the GMO would be of no or negligible risk to human health or the environment.” The committee therefore requested that the reason for suggesting that work involving DCV is classified as containment level 2 should be further evaluated and the results of such an evaluation be conveyed back to the subcommittee. If a Notification is deemed unnecessary a Risk Assessment would still be required for GM work.

As part of the same discussion it was suggested that to save effort and expense, planned future work on Arboviruses should be assessed in terms of the likely need for further applications for Notification. If more potential applications are identified consideration should be given to the possibility of combining the proposals into a single Notification covering as many viruses as possible.

Response from A K to GM Committee comments (06.02.2012):

I would like to thank the committee members for their helpful suggestions. My response to several comments is outlined below:

- Culture conditions for DCV have now been indicated under “Eukaryotic hosts”, GM notification page 4 and RA page 2.
- It is now stated that “... is not known to infect or replicate in human cells”, to be consistent. Infection of vertebrate cells has to my knowledge never been tried. Although highly unlikely, I do not believe this is a risk as no true insect virus has ever been described as an emerging pathogen, or is known to move between insects and vertebrates for example. However even baculoviruses are known to enter vertebrate cells and initiate transcription. Therefore some precaution is advisable. The CMV promoter is active in some insect cell lines and we use pRL-CMV for example to express luciferase, as well as other eukaryotic reporter plasmids.
- Comment on SFV now reads “plasmid or Semliki Forest virus or derived replicon”.
- Statement “-No experiments in vertebrate cells are planned” has been added (RA page 3, GM form page 3).
- The DCV genome of strain EB is currently being synthesized; we do not have the plasmid in the lab yet but it should be delivered over the coming weeks. I inserted this comment as by the time HSE receive the form, we should be about to receive the plasmid.

I suggest to class this virus as class 2 activity. This is mainly based on the observation that DCV can in fact infect far more species of drosophila than previously thought, and although it is rapidly cleared from mosquitoes it can potentially infect these insects too. There is therefore a theoretical possibility of infecting other species beyond the
drosophilids. With current debates around pollinators such as bees, which are highly susceptible at least to viruses closely related to DCV, it may be advisable to take into account the risk of introducing GM forms of DCV into the environment. In the risk assessment parts of the two forms I have now made it clear that we are aware of the fact that recombinant DCV could potentially infect drosophila “and other insects”. I do not foresee any risk to human health.

The revised documents and explanations were circulated to and approved by the subcommittee members

F. R(convener) 24/02/12.

1. GM 26 committee comments (approval date 22/02/12; see below):

The CVR Church Street GM subcommittee met on 31/01/12 to consider the proposal:
"Establishment of a reverse genetics system for drosophila C virus"

Subcommittee members:
Roger Everett
John McLauchlan
Joyce Mitchell
Arvind Patel
Frazer Rixon (convener)

As Drosophila C virus only infects arthropods, it is not considered hazardous. Therefore, no objections were raised against the proposed work. However, clarification was requested for the following points:

How will the virus be propagated? The culture conditions should be described.

The Risk Assessment contains the statement “DCV: This virus is not a ACDP pathogen. It is not known to infect or replicate in human cells, and there is no risk associated with using this virus.” (page 7, Vectors and GMOs). In the GM application the same section (page 5, point 7) states “DCV: This virus is not able to infect or replicate in human cells or other cells of vertebrate origin.” These statements should be made to be consistent and accurate.
More clarity is required over the potential for infection of, and expression in, mammalian cells. This is particularly relevant in light of the subsequent discussion over the need for Notification (see below), which would only be required if there is a threat to human health or the environment. For example, it is proposed to clone genes under the CMV (and possibly other mammalian) promoter. Is this promoter active in insect cells and if not, why is it being used?

Also, SFV can infect mammalian cells so its use as a vector is potentially harmful. In the Notification (page 3) it is stated that “Individual DCV sequences (coding or non-coding) will be cloned into eukaryotic or prokaryotic vectors (plasmid or Semliki Forest virus or derived replicon) for molecular biological manipulation and/or expression purposes.” In the Risk Assessment the sentence reads: (plasmid or Semliki Forest virus replicon or derived replicon). These statements should be made to be consistent and accurate.

Although only experiments in insect cells are listed under “Detailed descriptions and description/use of genetic modification:” (page 2-4 of Notification) a positive statement that no experiments in mammalian cells are planned should be inserted.

On page 2 of the Notification it is proposed to clone the DCV genome into a plasmid backbone. However, the section on Host/vector system (page 4) contains the statement that “Viral sequences (including full length clones or partial sequences of DCV) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form.” The source and nature of the already available DCV clones should be described.

The discussion on this proposal raised the question of whether a Notification is required in this case. DCV is an insect pathogen that appears to pose no risk to human health and would have low or negligible environmental consequences if it escaped from the laboratory. It therefore resembles baculovirus, which is classified at containment level 1 except when specifically intended as a vector for gene expression in mammalian cells or when encoding viral functions that could themselves transmit to mammalian cells. Page 9-10 of the Risk Assessment Form includes the statement: “If the answer to questions 11 and 12 is negligible, you may believe that you have sufficient information at this stage to classify the project to class 1, as defined in the Contained Use Regulations 2000. In order to do this you should be confident that in the event of a total breach of containment the GMO would be of no or negligible risk to human health or the environment.” The committee therefore requested that the reason for suggesting that work involving DCV is classified as containment level 2 should be further evaluated and the results of such an evaluation be conveyed back to the subcommittee. If a Notification is deemed unnecessary a Risk Assessment would still be required for GM work.

As part of the same discussion it was suggested that to save effort and expense, planned future work on Arboviruses should be assessed in terms of the likely need for further applications for Notification. If more potential applications are identified consideration should be given to the possibility of combining the proposals into a single Notification covering as many viruses as possible.

Response from Alain Kohl to GM Committee comments (06.02.2012):

I would like to thank the committee members for their helpful suggestions. My response to several comments is outlined below:
Culture conditions for DCV have now been indicated under “Eukaryotic hosts”, GM notification page 4 and RA page 2.

It is now stated that “... is not known to infect or replicate in human cells”, to be consistent. Infection of vertebrate cells has to my knowledge never been tried. Although highly unlikely, I do not believe this is a risk as no true insect virus has ever been described as an emerging pathogen, or is known to move between insects and vertebrates for example. However even baculoviruses are known to enter vertebrate cells and initiate transcription. Therefore some precaution is advisable. The CMV promoter is active in some insect cell lines and we use pRL-CMV for example to express luciferase, as well as other eukaryotic reporter plasmids.

Comment on SFV now reads “plasmid or Semliki Forest virus or derived replicon”.

- No experiments in vertebrate cells are planned (RA page 3, GM form page 3).

The DCV genome of strain EB is currently being synthesized; we do not have the plasmid in the lab yet but it should be delivered over the coming weeks. I inserted this comment as by the time HSE receive the form, we should be about to receive the plasmid.

I suggest to class this virus as class 2 activity. This is mainly based on the observation that DCV can in fact infect far more species of drosophila than previously thought, and although it is rapidly cleared from mosquitoes it can potentially infect these insects too. There is therefore a theoretical possibility of infecting other species beyond the drosophilids. With current debates around pollinators such as bees, which are highly susceptible at least to viruses closely related to DCV, it may be advisable to take into account the risk of introducing GM forms of DCV into the environment. In the risk assessment parts of the two forms I have now made it clear that we are aware of the fact that recombinant DCV could potentially infect drosophila “and other insects”. I do not foresee any risk to human health.

The revised documents and explanations were circulated to and approved by the subcommittee members

F. Rixon (convener) 24/02/12.

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**Project Containment**

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**Project Ref** 26/13.1

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02/03/2022
The Orthomyxoviridae is defined by viruses that have a negative-sense, single stranded and segmented RNA genome. Viruses within the Orthomyxoviridae are classified into five genera: Influenza viruses A, B, C, Thogotovirus and Isavirus. Within a genus, viruses show similar patterns in the sizes of their genome segments as well as structural and non-structural proteins. Influenza A viruses are typical examples of emerging viruses, including pathogens of humans and/or animals (5). Influenza B virus appears predominantly restricted to humans but it has also been isolated from seals; and influenza C virus infects humans and pigs. Types A and B are the cause of seasonal annual epidemics of acute respiratory disease among humans, and type A viruses have caused occasional pandemics – worldwide epidemics caused by a virus having new antigenic surface proteins among an antigenically naive population. Influenza A virus infections can cause a severe and debilitating febrile illness that can lead to fatal pneumonia and increase the risks associated with secondary bacterial chest infections, particularly in the very young, chronically ill, immunocompromised or elderly.

The influenza A virus genome is composed of eight segments of single-stranded RNA of negative polarity that encodes for up to 14 proteins. The genome segments are called PB2 (polymerase basic), PB1, PA (polymerase acidic), HA (haemagglutinin), NP (nucleoprotein), NA (neuraminidase), M (matrix) and NS (non-structural). Influenza A viruses are sub-classified according to the antigenic characteristics of the surface glycoproteins, the haemagglutinin (H) and neuraminidase (N). There are 17 H and 10 N subtypes known to date. The four major pandemics of the last hundred years were caused by H1N1 (1918 [Spanish flu], and 2009 [swine flu]), H2N2 (1957, Asian flu) and H3N2 (1968, Hong Kong flu) viruses. Currently, influenza A virus subtypes H1N1 and H3N2, and influenza B virus, are co-circulating in the human population. Avian viruses (such as H5N1, H7N7, and H7N9 viruses) have also caused human infections, and epizootics of highly pathogenic H5N1 and H7N9 avian viruses in several Asian countries since 1997 have raised the spectre of a new pandemic.

During replication, a positive-stranded antigenome is produced which serves as the template for de novo production of negative-stranded genomes. The influenza A virus terminal 3’ and 5’ sequences are highly conserved and largely complementary, and interact to give the genome a characteristic pan-handle structure. The RNPs are contained within a lipid envelope into which the viral glycoproteins (HA, NA and M2) are inserted. Replication occurs in the nucleus of infected cells.

Most influenza viruses are transmitted by direct and indirect contact. Some influenza viruses are transmitted through aerosol droplets. Cytopathic effect (CPE) depends on both viruses and host cells. For example, pandemic H1N1 virus causes CPE in Madin-Darby canine cells whereas most equine influenza viruses do not. At the same time, some equine influenza viruses cause CPE in dog tracheal explants whereas others do not. Understanding the molecular basis for the different outcomes of infection in these cells and tissues of different origin is of fundamental importance, and may help in the development of new control measures for influenza emergence. This is likely to imply immune responses (which differ in both hosts) and virus-host interactions.

Although much is known about the function(s) of individual influenza virus proteins, much more remains to be learned about the factors involved in host–pathogen
interactions that affect host range and disease outcome. The overall aim of our research is to elucidate the molecular and evolutionary determinants of host range, replication and pathogenicity of influenza viruses.

The work to be undertaken forms part of a comprehensive programme of viral emergence and molecular pathogenesis, involving researchers from different groups within the Centre for Virus Research (CVR). It involves work on several areas including, epidemiology, mathematical modelling of virus evolution, structural biology, and studies on virus-host interactions and molecular pathogenesis. It is the last aspect i.e. virus-host interactions and molecular pathogenesis with which this GM risk assessment is concerned. Within the CVR, the groups of Pablo Murcia and Benjamin Hale will generate the tools to produce recombinant influenza viruses and these viruses will form the basis of the work covered in this risk assessment. The work proposed will involve both in vitro, ex vivo, and in vivo infections. Embryonated chicken eggs will also be used as a system for growing some virus stocks.

Many Hazard Group 2 (HG2) influenza viruses will be at the centre of this project: equine influenza virus (EIV), canine influenza virus (CIV), low pathogenic avian influenza viruses (AIV), and human influenza viruses (H1N1, H3N2). Other viruses which will be used will include laboratory-adapted and mouse-adapted strains, as well as currently circulating influenza viruses and influenza viruses that infect other animal species (e.g. swine). We will also generate viruses that contain segments from highly-pathogenic avian influenza A viruses in the background of an attenuated laboratory strain. These viruses will lack a major virulence determinant from highly-pathogenic viruses and will also be engineered such that they do not possess the antigenic novelty associated with pandemic-potential of these strains. Non-hazardous minigenome systems (see below) of influenza viruses will also be studied.

Reverse genetics systems that allow manipulation of the influenza virus genome are readily available (2). Cultured cells are transfected with a mixture of 8 to 12 plasmids, each containing a full-length cDNA copy of one of the influenza virus genome segments under control of a Pol I promoter and terminator sequences, and a hepatitis delta ribozyme sequence. Intracellular transcription produces exact copy RNA transcripts that act as viral mRNAs and templates for viral RNA synthesis that result in production of infectious virus. Similarly, minigenome systems can be constructed in which only the coding sequences between the terminal influenza A virus sequences (either genomic or antigenomic) are replaced with a reporter gene such as Renilla luciferase (RLuc) or eGFP. A minigenome will be transcribed if the polymerase and nucleoprotein genes are co-expressed from expression plasmids, which is useful to study the role of sequences and proteins in replication and also in packaging as minigenome. Minigenomes are on their own non-propagating and are used to mimic virus replication.

1- In vitro and ex vivo infections to study influenza virus replication, host range and pathogenesis.
We will establish and characterise influenza virus infection in cell lines of various origins (human, canine, equine, etc), primary cultures derived mainly from humans, horses and dogs, as well as canine and equine tracheal explants. Primary cell cultures and explants from other animal species might also be employed.

For the canine/equine project, we will mainly be using H3N8 viruses derived from canine and equine strains (for example A/canine/New York/2008 and A/equine/Ohio/2003). Other equine and canine viruses of the same lineage will also be cloned and rescued by reverse genetics. All equine and canine influenza viruses belong to the ACDP hazard group 2. It should be noted that no equine or canine influenza virus has been associated with human disease.

We will also generate by reverse genetics the following human influenza viruses: mouse-adapted A/WSN/33 (H1N1), A/PR/8/34 (H1N1) and the non-mouse adapted 2009 H1N1 pandemic strains (e.g. A/England/195/2009, or A/California/04/2009) as well as other human seasonal strains (e.g. A/New York/312/2001 (H1N1) and A/Victoria/3/75 (H3N2)) as appropriate. All human influenza viruses used will belong to ACDP hazard group 2 and will be predicted to be antigenically similar to components of the seasonal trivalent or quadrivalent vaccine. The viruses will therefore be used at CL2.

Reverse genetics will also be used to construct recombinant viruses with mixed gene segments enabling identification of virulence determinants or identification of host factors that impact specific strains. The ability to perform site directed mutagenesis to produce viruses containing targeted mutations in specific genes will allow further characterisation of the role of specific proteins. Readouts will include the capacity for virus replication in tissue culture cells, or explants, as well as ability of the viruses to induce histological changes and the susceptibility of infected cells to apoptosis. In addition, we will examine a number of changes in functional attributes following virus infection, such as ciliary beating if appropriate.
2- Viral genetics of host restriction, adaptation, and interactions with host-cell functions. The contribution that individual virus genes (focusing on the 'internal' or non-glycoprotein genes) make to defining host range and pathogenicity will be probed by creating reassortants between the strains of influenza described above in section (1). For the highly-pathogenic avian influenza A viruses, we will not generate nor seek to possess complete sets of reverse genetics clones, and will only use a selection of individual internal segments to generate viruses in the background of attenuated laboratory-adapted influenza viruses (such as WSN or PR8 strains). In this regard, it is important to note that we do not possess reverse genetics clones for the haemagglutinin (HA) or neuraminidase (NA) genes of highly pathogenic avian influenza viruses, and will not attempt to generate viruses expressing such antigens to which the human population does not have herd immunity. We will only generate recombinant reassortment viruses that contain the internal segments of these avian viruses.

We will ensure that reassortants between CIV strains, EIV strains, and between CIV and EIV viruses will not affect any immunological protection as they are all phylogenetically very similar (CIV is a direct descendant of EIV). Reassortants between the H3N8 avian-like strains and PR8 is unlikely to result in any increased risk as use of PR8 internal genes together with novel surface glycoproteins is standardised in traditional influenza virus vaccine production worldwide (even with highly virulent influenza viruses such as H5N1), and has been approved by the World Health Organization (6).

Virus reassortments will be generated by reverse genetics that contain select internal segments from highly pathogenic viruses with the surface glycoproteins (HA and NA) from lab-adapted strains (e.g. PR8 or WSN). This will ensure that the viruses are likely to be antigenically similar to the currently used human vaccine, and sensitive to clinically approved NA inhibitors such as oseltamivir. Reassortants will be limited to the 'internal' genes of the highly pathogenic virus and are therefore expected to retain the basic tropism of the parental strain, as well as any immunological protection. In the case of using the PR8 and WSN backgrounds, this also means that attenuated characteristics are likely to be maintained. The HA gene of H5N1 viruses is a primary pathogenicity determinant and deletion of its polybasic cleavage site severely attenuates the virus in mammalian and avian models of infection. In addition, the classification of HPAI influenza involves the nature of the haemagglutinin – H5 or H7 subtypes with a polybasic cleavage site expected to produce systemic spread in infected poultry. Current ACDP guidelines state that H5 or H7 viruses modified such that they do not possess a polybasic cleavage site in HA can be handled under CL2 conditions. By only working with the 'internal' genes of these reassortants, we will further reduce any risk as not only will the polybasic cleavage site be removed, but there will be no novel HA (such as H5 or H7) to which humans have never been exposed to previously. The reassortants are unlikely to fall under SAPO regulations as no H5 or H7 HA with a polybasic cleavage site will be used. This will be clarified, and appropriate SAPO licences sought, prior to any work commencing. Use of lab-adapted HA and NA genes (from WSN and PR8) will accentuate any attenuation in humans (e.g. Clements et al., J Clin Micro, 1992, 30 655-62, and Clements et al., J Clin Micro, 1989, 27, 219-22) and is likely to provide cross-protection from H1N1-containing vaccines such as the current seasonal vaccine (the 2009 H1N1 pandemic vaccine (still used in current trivalent vaccines) provides protection against "old" H1N1 viruses between the years 1918 and 1947 (Medina et al., Nature Communications, 2010, Manicassamy et al., PLoSPathogens 2010). PR8 was isolated in 1934, whilst WSN was isolated in 1933.

We will use viruses generated by ourselves and also plan to use recombinant viruses based on A/equine/Ohio/2003, A/canine/New York/2008 (both H3N8), A/WSN/33 or A/PR/8/34 (both H1N1) and A/California/04/2009. In some cases we will add fluorescent tags for labelling and live tracking of viral particles. One possible approach will be to use a FIAshH currently being developed at Institut Pasteur. The FIAshH tag (Invitrogen) is a short (6 residues) peptide tag that has been fused to influenza gene NP and used in a reverse genetics plasmid-based system to produce infectious viral particles carrying the tagged NP protein. Cell-permeable FIAshH reagent, binding with high affinity to the FIAshH tag, is used to stain the cells, and can be excited at 508 nm wavelength. Emission at 528 nm can be detected using standard fluorescence microscopy settings, allowing identification of infected cells. Alternatively, we will use the similar technology SNAP tag, or the autofluorescent GFP/RFP protein domains. Luciferase genes may also be incorporated into recombinant viruses.

In order to compare host range determinants of equine and canine viruses, we will use reverse genetics to construct mutant versions of A/canine/New York/2008 and A/equine/Ohio/2003. The dose of the inoculum will vary, although for most of our experiments we infect explants with a very small dose (200 pfu).

As a reverse genetics system is not currently available for some canine and equine viruses, such a system will be constructed. We will clone various CIV and EIV sequences into an 8-plasmid system widely used for influenza reverse genetics. Genomic segments corresponding to genes coding for proteins HA, NA, M, NS, PB1, PB2, PA and NP including the 3’ and 5’ non-coding regions will be PCR-amplified and cloned into pHW2000 vector in which the cloning site is flanked by the Poll promoter and
hepatitis delta ribozyme sequences. If necessary, sequences corresponding to PB1, PB2, PA and NP genomic segments will be cloned into pHMG, where protein expression will be under the control of a mouse hydroxymethylglutaryl-coenzyme A reductase (HMG) promoter.

The method to be used for the production of recombinant EIV and CIV by reverse genetics is adapted from previously described procedures (3). The 8-12 plasmids will be co-transfected into a sub-confluent monolayer of co-cultivated HEK293T and MDCK (Madin-Darby canine kidney) cells. Subsequent amplifications of the virus will be performed on MDCK cells or embryonated chicken eggs.

Reassortant and wild type influenza viruses will be used to:
- Study viral growth, gene expression, replication and pathogenesis in host cells and tissues.
- Study interactions with the host cell (replication, interaction with host cell factors, immunity).

3- Viral genetics using influenza virus minigenome systems
- Minigenome systems for a broad range of influenza A viruses are already in use (1, 4). We will use minigenome systems that have been generated by others. If required, we will derive existing minigenome systems by cloning the segments of interest of the viruses under study (e.g. EIV, CIV, etc). Minigenome systems will be used (as read-outs) in studies on protein functions (cellular, viral), sequence function, segment packaging (packaging by glycoproteins co-expression, or following co-infection) and complementation studies (ie. CIV PB2 in EIV viral polymerase complex).

4- Functional studies and expression of influenza virus proteins.
- Manipulation of protein sequences by introduction of point mutations, deletions, insertions, fusion to others sequences such as reporter genes, tags etc.
- Expression of influenza virus proteins to study interactions with host cell components and cellular localisation.
- Expression of influenza virus proteins by prokaryotic expression systems, for example for protein purification.
- Expression of influenza virus proteins by eukaryotic expression systems (plasmid, retroviral expression), for example to produce packaging cell lines, cell lines expressing virus-like particles, or cell lines to study protein function.
- Expression of influenza virus proteins by eukaryotic expression systems (plasmid, retroviral expression), to produce packaging cell lines for influenza virus sequences (segments or minigenomes; production of single round infectious particles) and cell lines expressing virus-like particles.

5- Studies on host immunity.
- Interactions of influenza viruses (wild type, reassortants, minigenome) with host responses, and counteraction of host responses will be investigated. Immune signalling/antiviral responses (including activation mechanisms), and viral interference therewith, will be analysed. This will involve transcriptomic studies as well as studies of extra- and intra-cellular host molecules (e.g. proteins). Role of immunity in host cell tropism (infections of cells from different species) will also be investigated. These studies will also use reporter genes inducible upon activation of a given pathway. Cell culture models are available.

References:
### Parental organisms (non-GMOs from which GMOs would be derived)

<table>
<thead>
<tr>
<th>Parental Organism</th>
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<tbody>
<tr>
<td>A/California/04/09 (pdmH1N1)</td>
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<tr>
<td>A/Netherlands/602/09 (pdmH1N1)</td>
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<tr>
<td>A/Mexico/4108/09 (pdmH1N1)</td>
</tr>
<tr>
<td>A/Brisbane/59/07 (H1N1)</td>
</tr>
<tr>
<td>A/Brisbane/10/07 (H3N2)</td>
</tr>
<tr>
<td>A/Wyoming/3/03 (H3N2)</td>
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<tr>
<td>A/Moscow/10/99 (H3N2)</td>
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<tr>
<td>A/Panama/2007/99 (H3N2)</td>
</tr>
<tr>
<td>A/Texas/36/91 (H1N1)</td>
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<tr>
<td>A/Puerto Rico/8/34 (H1N1) [PR8]</td>
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<tr>
<td>A/WSN/33 (H1N1) [WSN]</td>
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<td>A/equine/Miami/1963 (H3N8)</td>
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<td>A/equine/Fontainebleau/1979 (H3N8)</td>
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<td>A/equine/Argentina/1995 (H3N8)</td>
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<td>A/equine/Newmarket/1993 (H3N8)</td>
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<td>A/equine/South Africa/2003 (H3N8)</td>
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<td>A/equine/Ohio/2003 (H3N8)</td>
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<td>A/canine/New York/2008 (H3N8)</td>
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<td>A/equine/Mongolia/2009 (H3N8)</td>
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<td>A/wild bird/Mongolia/2009 (H3N8)</td>
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<td>A/wild bird/Mongolia/2010 (H3N8)</td>
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<td>A/wild bird/Mongolia/2011 (H3N8)</td>
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<tr>
<td>A/swine/England/495/2006 (H1N1)</td>
</tr>
<tr>
<td>B/Yamagata/88</td>
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</tbody>
</table>

Note: that all of the above viruses are HG2, and will therefore be handled under CL2 conditions.

A/Vietnam/1203/2004 (HPAI H5N1), or related [only the internal segments of this virus will be used]
A/Shanghai/1/2013 (H7N9), or related [only the internal segments of this virus will be used]

The above 2 virus types are HG3, but we will only use genetically modified versions (as detailed below in section 11) which are modified to remove major pathogenicity determinants and the antigenic novelty associated with strains of pandemic-potential. The justifications are reasoned in section 11, and such modified viruses have been assessed locally to be HG2 pathogens, which will be handled under CL2 conditions.

### Recipient organisms:

- Equine influenza virus H3N8 (genus Influenzavirus A), and derived recombinant viruses.
- Canine influenza virus H3N8 (genus Influenzavirus A), and derived recombinant viruses.
- Human influenza virus H3N2 and H1N1 (genus Influenzavirus A), and derived recombinant viruses (e.g. see above).
- Other containment level 2 influenza viruses (e.g. avian viruses previously characterised as exhibiting low pathogenicity, or swine viruses, etc.) may be considered as long...
as they are unrelated to those capable of causing infection of humans.

At no point will viruses be produced that have surface antigens with a multibasic cleavage site in H5 or other livestock influenza virus HA proteins. It should also be noted that the equine/canine project and the human/avian projects will be performed by different investigators at different sites. We will therefore physically separate work to minimise the likelihood of reassortment between different viruses. We do not exclude possible future work on viruses expressing H5N1 antigens at CL2 as per current ACDP guidelines (e.g. low-pathogenic H5N1 viruses engineered to lack the polybasic cleavage site: “HALo”). However, such work will only be done after a separate notification to the local safety committee and relevant addendum to our local Risk Assessment.

Human influenza B virus (e.g. B/Yamagata/16/88), and derived recombinant viruses.

Reporter systems mimicking Influenza virus replication:
Minigenomes of CIV, EIV, and other influenza A and B viruses (for example swine influenza virus).

**Host/vector system**

**Plasmid vectors:**
Viral sequences (full length clones of Influenza viruses; partial sequences of these and other influenza viruses) and other genes (cellular, viral, reporter) are available in plasmid-encoded cDNA form. RNAs can be transcribed from bacterial promoters (i.e. T7 or SP6 by in vitro transcription or plasmid-expressed RNA polymerase in cells) or eukaryotic promoters (CMV, SV40, cellular promoters, baculovirus promoters etc.). Plasmids for expression of influenza virus sequences (virus rescue or minigenome) typically contain an RNA polymerase I promoter and a hepatitis delta virus ribozyme or a mouse RNA polymerase I terminator. Plasmids contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin) and sometimes a selectable eukaryotic drug resistance marker such as neomycin or puromycin resistance. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

**Hosts:**

**Prokaryotic hosts:**
Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste should prevent release of viable organisms. The risk to the environment is therefore effectively zero.

**Eukaryotic hosts:**
Vertebrate cell lines of various origins (typically human, mouse, avian, hamster, bovine, canine, equine, etc.), primary cells and explants of various origins (typically human, swine, equine and canine).

In prokaryotic cells, only selectable (e.g. antibiotic resistance) genes will be translated; prokaryotic cells may also be used for protein expression and subsequent purification. Virus sequences will be under the control of a promoter that will only generate transcripts in eukaryotic cells or in vitro transcription reactions.

In eukaryotic cells, the following RNAs will be produced which may be translated into the following gene products:

1-**Influenza viruses and RNAs (or minigenomes):** Proteins involved in replication and transcription of viral genetic material and virus structural proteins. Some of these virus proteins will interact with host-cell components and may affect host cell responses to infection; few of these interactions are currently known. If all virus sequences are translated new virions may be generated; minigenomes are non-propagative viral RNAs (capable of replication). Partial RNAs do not give rise to propagating infectious material.

2-**Non-influenza virus genes or sequences:** Genes of prokaryotic (bacteriophage polymerases) or eukaryotic (e.g. reporter genes such as luciferases or fluorescent proteins such as GFP; plant or arthropod immunity modulators) origin. Reporter gene sequences will also be present in influenza minigenome RNA. The recombinant foreign
proteins to be expressed provide no significant increase in the hazard to human health; none are toxic or likely to produce disease in the quantities that could be produced by accidental exposure to these systems. Non translated RNA sequences (including T7 terminator and hepatitis delta ribozyme in plasmids used for virus rescue or minigenomes) have no known toxic effects and pose no risk to human health.

Techniques used to introduce insert or vectors into cells:

1-Prokaryotic cells: Introduction of DNA into E. coli will be by heat shock/chemical transformation/electroporation. These techniques have been extensively described and are widely used; they rely on getting DNA very close to the bacterial wall and introducing the genetic material through pores or membrane fusion.

2-Eukaryotic cells: Cells will be transfected using transfection reagents such a lipofectamine or by electroporation. Virus genomes or sequences will be introduced into cultured cells by infection with virus or virus replicon particles (VRP).

**Origin & function**

The genetic material will originate from the parental organisms:

A/California/04/09 (pdmH1N1)
A/Netherlands/602/09 (pdmH1N1)
A/Mexico/4108/09 (pdmH1N1)
A/Brisbane/59/07 (H1N1)
A/Brisbane/10/07 (H3N2)
A/Wyoming/3/03 (H3N2)
A/Moscow/10/99 (H3N2)
A/Panama/2007/99 (H3N2)
A/Texas/36/91 (H1N1)
A/Puerto Rico/8/34 (H1N1) [PR8]
A/WSN/33 (H1N1) [WSN]
A/equine/Miami/1963 (H3N8)
A/equine/Fontainebleau/1979 (H3N8)
A/equine/Argentina/1995 (H3N8)
A/equine/Newmarket/1993 (H3N8)
A/equine/South Africa/2003 (H3N8)
A/equine/Ohio/2003 (H3N8)
A/canine/New York/2008 (H3N8)
A/equine/Mongolia/2009 (H3N8)
A/wild bird/Mongolia/2009 (H3N8)
A/wild bird/Mongolia/2010 (H3N8)
A/wild bird/Mongolia/2011 (H3N8)
A/swine/England/495/2006 (H1N1)
B/Yamagata/88
A/Vietnam/1203/2004 (HPAI H5N1), or related [we will only use the internal segments from these viruses]
A/Shanghai/1/2013 (H7N9), or related [we will only use the internal segments from these viruses]

Alternatively, viral sequences of these and related viruses (similar strains and same ADCP classification) can be synthesised. We do not exclude possible future work on viruses expressing H5N1 surface antigens at CL2 as per current ACDP guidelines (e.g. low-pathogenic H5N1 viruses engineered to lack the polybasic cleavage site:...
The intended functions of the genetic material involved is the production, purification and characterisation of influenza viruses, as well as the production and use of minigenomes. In turn, the viruses produced will be used to infect human or animal cell tissues and cells to determine the cellular processes involved in pathogenesis and replication through virological and molecular biological methods.

**Evaluation of foreseeable effects**

**Plasmid vectors:** Viral sequences (including full length clones or partial sequences of influenza A virus or other influenza viruses) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Systems for prokaryotic gene expression are under control of an inducible prokaryotic promoter. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV or SV40), eukaryotic (eg actin promoter, pathway inducible promoter such as STAT, interferon etc.) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human cells. None of the genes used in these studies are oncogenes. The hazard of expressing 'foreign' genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the foreign gene product and is discussed in more detail below.

**Viruses:** Influenza is typically a mild and self-limiting infection, acquired generally by the respiratory route. Severity depends on the strain of virus and host but in general tends to fall in a spectrum between asymptomatic to ~ 1 week of fever and malaise in otherwise healthy people. Severe illness can occur but is rare, occurring in less than 1% of cases (see below). This level of risk applies to most wild type human H1N1 and H3N2 viruses and the porcine viruses. Equine H7N7, H3N8 and canine H3N8 viruses have not been associated with significant illness in humans. In addition, the laboratory adapted PR8 and WSN strains of H1N1 human influenza are known to be avirulent in man from many volunteer challenge studies as well as a long (> 70 years) history of safe use in the lab. The GM viruses containing internal segments from H5N1 or H7N9, but with the surface glycoproteins from WSN or PR8 would retain antigenic properties of the lab-adapted PR8/WSN viruses, which are likely covered by the current human influenza vaccine, and which have not been associated with disease despite their wide use worldwide for decades.

Even seasonal influenza can have potentially serious consequences for anyone who is pregnant, immunosuppressed, asthmatic, or has other respiratory/underlying chronic diseases. Vaccines are available for the circulating human H1N1 and H3N2 strains and the majority of the population has at least some prior immunity to these viruses. All laboratory workers and staff within the department are recommended to receive the annual human influenza vaccine, and all workers using shared resources (even if not directly involved in the work) are made aware of the risk assessment for influenza virus work. Those at risk of severe consequences of influenza (e.g. pregnant women) will be separately notified of the risks of these pathogens immediately after they identify themselves to their PI and occupational health. Individual risk assessments will be carried out governing these specific cases.

The antivirals oseltamivir, zanamivir and amantidine are also available for treatment or prophylaxis if needed. We have procedures for staff to seek medical advice and prophylactic treatment following any possible significant accidental exposure events. Note, we will not handle any known antiviral-resistant strains of influenza virus in the same location as other viruses with different antigenic profiles in order to limit the possibility of accidental reassortment generating an anti-viral resistant virus with new antigenic properties.

Vaccines are also available for horses/dogs against EIV and CIV. Workers with these viruses will be advised to not have contact with either horses or dogs for 48h after last handling the viruses.

Personnel working with these viruses are expected to inform occupational health should they become pregnant or immunosuppressed, and inform the PI so that precautionary measures can be introduced following advice from occupational health. We also have a policy whereby those with flu-like symptoms do not come to work, do not handle influenza viruses experimentally, and inform their PI immediately. This policy will further limit any possible accidental reassortment between laboratory viruses and circulating human viruses, should a worker accidentally expose themselves to a laboratory virus whilst simultaneously infected with a circulating virus. The local PI (Pablo Murcia or Benjamin Hale) will take responsibility for enforcing this, and will similarly assume responsibility for the safety and compliance of any visiting workers.
Influenza A virus minigenomes: Minigenomes pose no risk to human health, including those derived from pathogenic influenza A viruses. They cannot propagate on their own. Even if packaged by co-expression of glycoproteins and thus used to infect cells, their own transcriptional activity would be minimal. If packaged into virions, the associated risk is that of the virus itself as reporter genes in minigenomes are not reported to be toxic.

Note: No reassortment experiments involving the glycoproteins of human and animal influenza A viruses (for example an influenza A virus carrying a human H1 and an equine N8) will be performed, to eliminate the possibility of creating viruses for which humans or animals have no prior immunity. Furthermore, we aim to physically separate work on different influenza viruses such that accidental reassortment to generate a more pathogenic strain, or an antigenically novel strain with new properties, cannot occur from our work (see details in Risk Assessment).

Hosts

The host/recipient organism: Prokaryotic organisms: All strains will be disabled, commercially available E. coli derivatives classified as ‘especially disabled hosts’ by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic cells: Cell lines and tissues to be used would not survive inside the human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA or infection will confer no growth or survival advantage in or outside the laboratory to cell lines and tissues.

What hazards do the inserted genetic material or other genetic modification pose?

The inserted/donated genetic material:

Viral RNA and proteins, cellular proteins: Most individually expressed viral or cellular proteins are unlikely to have harmful effects in eukaryotic cells, however some could perturb normal cellular metabolism, predispose or protect against cell death or render cells more or less susceptible to other infections if overexpressed or silenced. It is very unlikely that Influenza A virus RNA or proteins would have any harmful toxin-like effect outside cells. Only antigenomic RNA containing an influenza A virus sequence can be translated or give rise to infectious virus (if all necessary genomic information is provided) in eukaryotic cells; minigenome RNAs are non-propagative and pose no harm (individual cells replicating minigenome are likely to die or be eliminated by the immune system). Viral non-coding RNA sequences are important in influenza virus replication but do not encode biologically active molecules that are likely to induce physiological effects in humans on their own. None of the influenza virus gene products are known to be secreted cytotoxins.

Reporter genes: Reporter genes of prokaryotic or eukaryotic origin (eg luciferase, fluorescent proteins etc.). No harmful properties have been attributed to these proteins. There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Photinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function.

Sequence changes in viral sequences:

Mutation or deletion will target disruption of structural and non-structural protein functions or non-coding viral sequences and are likely to have no or deleterious effects on viral replication, thus not increasing risk or hazards to human health associated by viruses genetically modified in such a way.

Foreseeable effects to the environment:

Canine influenza virus: This virus is not thought to be endemic in the UK, and it is not on the SAPO list. In fact, it is not highly transmissible in the only country in which is endemic (USA). Transmission is common only in highly dense dog populations such as those found in animal shelters. CIV was first isolated from greyhounds in USA.
Experimental infection results in mild respiratory disease. Serological evidence of infection in foxhounds in the UK has been shown, although no outbreaks have been associated with it. Given the low transmissibility and low pathogenicity of CIV, the chance of accidentally infecting dogs initiating a transmission cycle sustained by local dogs is negligible. Reassortants carrying gene segments from EIV will likely display lower fitness and will pose minimal risks to dogs, if any. The modified viruses will not have any inserted foreign genes other than non-harmful genes or sequences, or (usually disruptive) changes in viral genes or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. In the absence of a suitable host these viruses would not survive in the environment and the risk is effectively zero. Dogs are kept out of labs and the risk of accidental infection of dogs by contact of contaminated material is minimal for CIV. However, as some members of staff bring their dogs to work there is a potential risk of infection of those animals if they access the laboratories.

Equine influenza virus: This virus has been endemic in the UK for over 40 years and infections are most likely the result of horse movement and inadequate vaccination schedules. EIV is not on SAPO or ACDP lists. There is a vaccine available for EIV, although most horses have natural immunity due to vaccination or natural infections. The modified viruses do not contain anything other than non-harmful genes or sequences, or disruptive changes in viral genes or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. If EIV was to escape the lab and infect local horses, there is minimal risk it could initiate a transmission cycle. Horses are kept out of labs and the risk of accidental infection of horses by contact of contaminated material is minimal. The closest horses to the lab are kept at the Weipers Centre and up to date vaccination against EIV is a requirement for admission.

Avian-like equine influenza virus (H3N8): This virus is highly pathogenic in horses (20% mortality) and there is no cross reactivity between classical EIV and avian-like EIV. Reassortants of this virus will be done using PR8 as a backbone (HA and NA of avian like EIV + the six internal genes of PR8), so the resultant viruses will be highly attenuated. No other reassortant viruses including the surface glycoproteins (HA and NA) of avian like EIV will be generated. Reassortants carrying the internal genes of avian-like EIV and the external genes of classical EIV will have a minimum risk to initiate a transmission cycle as most horses in the UK have immunity against the surface glycoproteins of classical EIV. It is important to note that PR8 has been used as a backbone to generate vaccines against highly pathogenic H5N1 viruses and thus is very safe to use. If these reassortants were to escape they would have very low fitness due to the attenuation conferred by the PR8 genetic background.

Human influenza viruses: Currently circulating human influenza A viruses (pdm H1N1, seasonal H1N1, and H3N2) are globally distributed, there are available vaccines, and the majority of the population has at least some prior immunity to these viruses. The antivirals oseltamivir and zanamivir are also available for treatment or prophylaxis if needed.

Viruses constructed will be based on the widely used laboratory strains A/WSN/33 (H1N1), A/PR/8/34 (H1N1) (all mouse adapted and known [WSN, PR8] to be attenuated in man) or more recent low pathogenicity human strains to which most people will have substantial amounts of pre-existing immunological protection: A/Udorn/72 or A/Victoria/3/75 (H3N2) viruses, the current human influenza strains A/England/195/2009, A/California/04/2009 (both 2009 H1N1 pandemic viruses) and the A/New York/312/2001 strain (‘seasonal’ H1N1). Such antigenic and attenuated properties will be maintained in viruses bearing the surface glycoproteins of WSN/PR8 and the internal segments of H5N1 or H7N9 viruses.

The experiments proposed involve gene deletion, mutation or replacement of specific genes with the corresponding gene from one of the other strains listed. For reassortants between the human-derived or laboratory adapted viruses, this is not predicted to create viruses with novel tropism or pathogenicity as they either already share similar characteristics (the recent human isolates) or are highly attenuated in humans (PR8, WSN). In the case of reassortants incorporating genes from animal strains of influenza, we also do not expect any change in tropism because the human or laboratory adapted HA and NA genes will be retained. In the case of reassortants incorporating genes of the avian-like equine influenza virus, the six internal genes will be derived from PR8, thus attenuating the virus significantly.

We propose to use containment level 2 for all other HG2 influenza A viruses (as specified on page 2) similar to CIV and EIV unless other regulations are in place for a particular pathogen.

Minigenomes: These systems derived from other influenza A viruses are non-propagative and even if packaged, pose no or no additional hazard.
Hosts:
Prokaryotic organisms: Disabled, commercially available E. coli strains. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. Acquiring antibiotic resistance (from the plasmid vector) or additional sequences (virus or marker gene sequences) will not give these strains any survival advantage in the environment. The risk to the environment is therefore effectively zero.

Eukaryotic cells: Cell lines to be used are not viable outside the laboratory and thus pose no threat to the environment. Addition of plasmid vectors or virus or minigenomes will confer no growth or survival advantage in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We propose to generate influenza viruses bearing the 6 internal gene segments from H5N1 or H7N9, with the 2 surface glycoproteins of laboratory-adapted H1N1 viruses WSN or PR8. Currently, both H5N1 and H7N9 viruses should be handled at CL3 according to ACDP guidelines.

ACDP guidelines stipulate mitigating circumstances whereby some CL3 viruses can be used at CL2. This includes genetically modifying the HA molecule of HPAI viruses to remove the polybasic cleavage site (a major pathogenicity determinant). This renders the virus apathogenic in chickens, and highly reduces the pathogenicity in mammalian models. We propose to go further, by replacing the entire HA and NA segments of H5N1 virus with the HA and NA segments of the laboratory-adapted strains PR8 or WSN. This will not only remove the pathogenicity-associated polybasic cleavage site, but will also render the virus antigenically similar to existing human vaccines, and susceptible to prophylactic antivirals. The virus will also be expected to be antigenically similar to viruses currently circulating in humans meaning there will be a degree of herd immunity in the general population. Furthermore, such a virus is likely to retain attenuated characteristics of WSN/PR8 in humans due to their extensive laboratory passage over 70 years. WSN/PR8 have not caused any known serious laboratory infections despite their use world-wide in decades.

We will apply the same strategy to generate viruses bearing the internal segments of human H7N9 viruses and the surface glycoproteins of the laboratory-adapted WSN/PR8 strains. Such viruses will thereby lose any possible antigenic novelty, minimising any risk to either worker or the broader human population.

WE DO NOT POSESS THE HA OR NA GENE SEGMENTS OF EITHER H5N1 or H7N9. We do not exclude possible future work on viruses expressing H5N1 antigens at CL2 as per current ACDP guidelines (e.g. low-pathogenic H5N1 viruses engineered to lack the polybasic cleavage site: “HALo”). However, such work will only be done after a separate notification to the local safety committee and relevant addendum to this Risk Assessment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfect influenza virus-contaminated material immediately.

GM26 (CVR Church Street)

Solids from cell culture (eg plasticware such as flasks, tubes, pipettes tips etc.) - submerged soak in a minimum of 1% Virkon (w/v) for at least 12 hours. Rinsed solids then go to landfill. Virkon is added to the plasticware prior to removal from the MSCII.

Other solids (agar plates, gloves etc. ) placed in a sealed container (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 121oC for 30 minutes. Gloves used for influenza virus work should be disinfected with alcohol before putting in containers prior to autoclaving.

Liquids (eg. samples, culture supernatants, tissue culture media) – add Virkon to final minimum concentration of 1% (w/v) for at least 12 hours, then discharge to drains.
Virkon is added to the liquid waste prior to removal from the MSCII.

Liquids (E. coli culture medium)- add Virkon to final minimum concentration of 1% (w/v) for at least 12 hours, then discharge to drains.

Sharps (e.g. needles/syringes, scalpels) - decontaminate in 1% Virkon overnight, then place in sharps bin for incineration.

Spills of infectious liquid - immediately inactivated with powdered Virkon (rather than liquid spray, to minimise aerosol generation - large spill), or absorbed into paper towels soaked in Virkon (minimum 1% - small spill). Soaked paper towels then autoclaved. Surface wiped down with Virkon soaked paper towels and then disinfected with alcohol prior to drying.

Degree of kill:
Chemical Sterilization by Virkon- effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).

GM223 (CVR Garscube Estate):

Disposable solids (eg plasticware such as flasks, tubes, pipette tips etc.)- soak in 1% Virkon (w/v) for a minimum of 12 hours. Transfer solid contents to clear autoclave bags and autoclave at 121oC for a minimum of 20 minutes prior to final disposal by district council to land fill, remaining liquid to be discharged to drain.

Other solids (agar plates, gloves etc.)- placed in a marked box lined with a clear autoclave bag, and disposed of by autoclaving using a make safe cycle of 121°C for at least 20 minutes. Seal contaminated plates (with for example bacterial GMOs) before placing in bag, to avoid lid falling off. Gloves used for influenza virus work should be disinfected with alcohol before putting in containers.

Liquids (eg. samples, E. coli culture media, tissue culture media)- add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Sharps (eg needles, syringes, scalpels) - decontaminate in 1% Virkon overnight then place in sharps bin for incineration.

Degree of kill:
Chemical Sterilization by Virkon- effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
1. GM 26 committee comments:

The CVR Church Street GM subcommittee met on 29/04/2013 to consider the proposal:
"Influenza virus pathogenesis, host range and virus/host interactions"

Present:
Roger Everett
John McLauchlan
Joyce Mitchell
Arvind Patel
Emma Thomsen
Frazer Rixon (convener)

The subcommittee agreed the following recommendations.

Vaccination
The existing recommendation is that anyone working with influenza in Church Street should be vaccinated. This requirement for vaccination is not clearly stated in the RA. For example, on pages 16, 20 and 22 of the RA it is stated that vaccines are available against the human influenza but there is no mention that vaccination is advised. In addition, the potential seriousness of infection is downplayed. Thus, on pages 11, 13, 17 and 22 infection is described as mild, or typically mild and self-limiting. This takes no account of the potentially serious consequences for anyone who is pregnant, immunosuppressed, asthmatic, or has other respiratory diseases. We suggest that text is inserted into the forms at appropriate places stating that people working with influenza should be vaccinated and that anyone with a flu-like illness should not report to work or go to A&E, but should inform Occupational Health. In addition, vulnerable groups should be identified in the documents and warned of the potential risk.

Risk
The subcommittee considered that the greatest risk of generating unforeseen viruses through reassortment was posed by workers who were already infected with a circulating strain. Therefore, it is particularly important that the guidelines should make it clear that people suspected to be infected should not work on this project and these guidelines must be enforced. As the proposed work covers two separate sites (Church Street and Garscube), a local PI must assume responsibility for the safety and compliance of any visiting workers at Church Street and this must be clearly stated.

Scope
Although the proposal states (p11 of RA) that “equine, canine, and low pathogenicity avian viruses are unlikely to be used at this (Church Street) site”, the subcommittee did not wish to rule out such use in future. Therefore, the above statement should be removed and the text modified so as not to preclude use of these viruses at Church Street.

Disinfection
Under Waste Disposal (RA page 20) it should be made clear that Virkon is added to infectious liquid waste before it is removed from the safety cabinet.

Text
A number of matters were identified relating to the layout and wording of the RA:

The Church Street site (GM26) is designated as both a primary location (RA page 9) and a secondary location (page 11) for proposed laboratory work. This is ambiguous
and needs to be clarified.
Change BSL 2 laboratory to containment level 2 laboratory (RA page 9).
Church Street policy at present is that oseltamivir-resistant flu can only be handled in room 310. This should be specified.
References to Virkon treatment should make it clear that the final concentration must be ≥1%.
Spills of infectious or contaminated liquids should be inactivated with Virkon before being cleaned up (RA page 15).
Remove the reference to visual inspection of the safety cabinet by the PI (RA page 17).
List COSHH 237 under point 15 (RA page 19).

Following discussion between the co-applicant Ben Hale and Frazer Rixon, a second meeting of the CVR Church Street GM subcommittee was called for 27/05/2013, to
discuss further the handling of reassortants containing genome segments from high pathogenicity avian influenza strains.
Present:
John McLauchlan
Joyce Mitchell
Arvind Patel
Emma Thomsen
Frazer Rixon convener.

Invited: Ben Hale

Apologies: Roger Everet

The subcommittee agreed the following additional recommendations:

Background information on the polybasic site and its importance for pathogenicity should be included in the proposal and it should contain a clear statement that viruses containing a polybasic site would not be generated or used.

The proposal could include mention of low-pathogenic H5N1 viruses engineered to lack the polybasic cleavage site: "HALo" but should contain a clear statement that work with such viruses would require prior consideration and approval by the local safety committee.

Methods to prevent accidental reassortment of genome segments resulting in viruses with potentially novel antigenic properties should be specified.
The requested changes were made and on 23/06/13 the revised documents were circulated to and approved by the subcommittee members on 25/06/13.

2. GM223 committee comments 13/05/13

The comments of GM 223 were that this was a detailed risk assessment with a lot of evidence, justification of arguments and references. The recommendations were primarily in agreement with those of GM 26 as detailed above and the PIs were notified accordingly. Clarification on any SAPO licence requirements was requested. Careful consideration should be given to the potential for reassortment of genome segments and this should be reflected in the laboratory practices. All workers using the designated facilities should be made aware of all pathogens and GMOs being handled in the areas. All solid waste generated from this project will be autoclaved and it is recommended that Virkon treated liquid waste also be autoclaved. Where this is not possible then references/documentation should be provided to support Virkon disinfection as a validated means alone for inactivation of the liquid waste generated.

GM 223 approved the revised documents on 24/06/13

Project Containment

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Project Ref 26/18.1

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Historical Significant Changes

Project transferred to GM223

02/03/2022
The objective of this project is to investigate the interaction of hepatitis B virus (HBV) and hepatitis D virus (HDV) with host cells with a view to understanding the roles of viral and host factors in virus infection and replication, and their contribution to virus-associated pathogenesis. We will first establish in vitro cell culture systems to generate infectious virus using well-characterized prototype and patient-derived replication-competent viral genomic DNA. Viruses generated thus will be then used to infect different target cell lines to study, at the mechanistic level, the functions of the relevant viral and host components (e.g., proteins and/or nucleic acids) and the impact of their interactions on virus pathogenesis. It is hoped that the results obtained will inform development of novel anti-viral therapeutic avenues and may lead to bats as a new animal model for HDV.

Description of activities:

HBV is the prototypic member of the family Hepadnaviridae, which includes avian, primate and rodent hepatitis viruses. All hepadnaviruses share the property of restricted host range, a strong, but not exclusive, tropism for hepatocytes, and the ability to cause liver damage. HBV is a major human pathogen causing a chronic inflammatory liver disease often leading to the development of hepatocellular carcinoma (HCC). The clinical significance of HBV is easily demonstrated by the fact that more than 250 million people worldwide are chronically infected. HBV has a relaxed circular partially double stranded DNA genome of approximately 3.2 kb. Following infection, the HBV genome is transported to the nucleus and converted to covalently closed circular (ccc) DNA which serves as a template for transcription of viral RNAs. Following transport of the pre-genomic RNA and translation in the cytoplasm, selective encapsidation of the RNA into the nucleocapsid ensues, along with encapsidation of RNA polymerase. In the immature capsid, the 3.5 kb mRNA is reverse-transcribed, to yield minus strand DNA. The RNA is degraded and the DNA strand is replicated, producing the second, shorter DNA strand. Nucleocapsids containing DNA genomes then acquire their outer envelope, probably by budding into the endoplasmic reticulum (ER) in areas where viral surface antigens (HBsAg) are transported. The resulting particles are then transported from the cell by normal pathways of vesicular transport. There are 3 forms of HBsAg (called large, medium and small or L-HBsAg, M-HBsAg and S-HBsAg, respectively) which all share the same C-terminus (i.e. S-HBsAg), with the M-HBsAg being extended at its N-terminus by 55 amino acid (aa) preS2 segment and L-HBsAg additionally containing an N-terminal 108 to 119 aa preS1 domain. HDV belonging to the genus Deltavirus causes chronic hepatitis D. The virion carries a circular single-stranded negative-sense RNA genome of ~1.7 kb in length. The viral replicative intermediate of positive-sense polarity encodes the delta antigen (HDAg) that is not packaged into the virion. HDV requires HBV as a helper virus in the form of its envelope proteins (i.e. HBsAg) to form infectious virus particles. Co-infection with HDV is associated with more severe pathological complications (e.g. increased risk of liver failure in acute infections and development of liver cancer in chronic infections) compared to HBV alone. HDV RNA replication occurs in the nucleus and the viral ribonucleoprotein is transported to the Golgi where it is enveloped by HBsAg. The secretion of the assembled virion is thought to occur via Golgi. Co-expression of HDV genome with HBsAg results in secretion of HDV particles into cell medium. This medium can be used as a source of HDV to infect susceptible naïve cells in which the virus initiates a single-cycle infection; i.e. the virus delivers its replication-competent genome, but is unable to produce progeny virions as the cells lack HBsAg. While HDV has historically been considered a virus of humans, recent metagenomic sequence data obtained here at the MRC – University of Glasgow Centre for Virus Research (CVR) has discovered RNA which appears to represent an analogous virus (HDVb) circulating among wild bats (Desmodus rotundus) in South America (Bergner et al., unpublished data). Little is known of HDVb, but genomic similarities to HDV suggest it is likely to have a similar biology (i.e., narrow host range and requirement for a helper virus such as bat hepadnavirus). Ongoing sequencing studies here at the CVR aim to identify the analogous bat-associated HBV using metagenomic sequencing and PCR. On account of the presence of HBsAg on HBV and HDV virions, both viruses share the same cell entry pathway although their genomes replicate differently. Cell entry is mediated by initial attachment of the virus to the cell surface heparin sulfate proteoglycans (HSPGs) followed by a specific interaction with the hepatocyte-specific sodium taurocholate co-transporting polypeptide (NTCP) (Yan et al., 2012 eLife1,e00049). The virus interaction with HSPG is mediated by the N-terminal preS1 domain of L-HBsAg and the antigenic loop of the S-HBsAg, while the interaction with NTCP is solely mediated by preS1. It is likely that additional as yet unidentified host factors are also required for virus entry into target cells.

The proposed project consists of 6 major components:

1. Generation of infectious viruses: We will use the well-characterized tetracycline-inducible HBV expressing cell line, Hep38.7-Tet (Ogura et al., Biochemical and Biophysical Research Communications 452 (2014), 315–321), to generate HBV for use in work described here. Additionally, we will also transfet mammalian cells (including hepatocytes) with replication-competent HBV DNA derived from well-characterised isolates or patients infected with different viral genotypes and isolates. This is expected to initiate a fully productive virus life cycle in transfected cells. Evidence of virus infection and replication will be monitored by the detection of viral RNA (by
3. Molecular epidemiology of HBV infection: We are interested in studying the molecular epidemiology of HBV infection in different population groups and identifying mutations that confer resistance to current antiviral treatments and vaccines in circulating strains. We are using next-generation sequencing technologies to sequence full-length viral genomes from patient samples. We will use the previously described (Pollicino et al., 2006, Gastroenterology 130, 823-37) HBV cccDNA-dependent transfection system to investigate antiviral resistant variant fitness and assess the evolution of HBV antiviral resistant strains. Briefly, single genome-length linear monomeric HBV DNA will be produced from a subset of the patient sequencing data and introduced into the human hepatocytes cells. The introduced monomers are expected to circularise into cccDNA and act as templates for transcription of HBV genomes, thus initiating and then recapitulating the entire viral replication cycle. We will perform these experiments in the presence or absence of clinically approved antivirals and experimental drugs, and measure the levels of cccDNA, the associated transcripts (e.g. the pre-genomic RNA), the intracellular encapsidated DNA, and the virion-associated extracellular DNA using established methods. We will assess the ability of the extracellular virions produced in this system to infect the human hepatocyte cell lines that stably express the virus entry receptor NTCP. The specificity and the mechanisms of viral entry will be explored further using a panel of in-house generated antibodies to preS1, the N-terminal domain of the L-HBsAg that plays a role in viral entry via its interaction with NTCP. Together, these experiments will allow us to measure and study the mechanics of virus entry and resistance to drugs, and to evaluate vaccine escape.

4. Infection of cells with clinical HBV isolates: Sera collected from HBV-infected individuals will be used as an inoculum for infection of appropriate cell lines. Initially all patients will be recruited in UK and only samples tested negative for cytomegalovirus and HIV will be used. Samples from overseas and/or from patients screened positive for HIV might be used at a later stage of the project. In this case, samples will be screened for the infectious agents most likely to be present in the sera (depending on their origin). Samples containing adventitious agents will be excluded and any import into the CVR will be subjected to the local Safety group approval. Evidence of infection will be monitored as described in step 1 above.

5. Investigating the effects of interferon-stimulated genes (ISGs) in HBV and HDV infection: Type I interferons (IFNs) suppress the replication of HBV in vitro and in vivo. However, the success of IFN therapy is variable and influenced by the HBV genotype. Despite this, relatively little is known about which host ISGs inhibit HBV and why some genotypes respond better to IFN therapy. We have developed libraries of ISGs from humans, rhesus macaques and cows as well as diverse ISGs from diverse vertebrate species (Schoggins et al., 2011, Nature 472(7344):481-485; Kane et al., 2016 Cell Host Microbe 20(3):392-405). These libraries are arrayed so that in a single screen the ability of hundreds of individual ISGs to inhibit viral replication can be considered. Briefly, hepatocytes, including Hep38,7-Tet (or other permissive target cells) will be transduced with lentiviral vectors encoding each ISG prior to infection with HBV representing different genotypes and isolates. Alternatively, ISG-encoding vectors will be co-transfected together with replication-competent HBV DNA and the yield of HBV will be quantified. In all instances, the methods described in step 1 above will be used to quantify the level of virus infection/gene expression and/or replication in order to identify inhibitory ISGs. Similarly, we are interested in which ISGs inhibit the replication of HDV and whether such inhibition is direct or a consequence of HBV inhibition (indirect). Thus, we will examine the ability of different ISGs to inhibit HDV replication in cultured hepatocytes or other permissive target cells (vertebrate origin).

6. In vitro evolution of HBV and HDV: To reveal viral determinants of sensitivity/resistance to antiviral factors and reveal potential resistance pathways to antiviral molecules or antiviral candidates we will propagate HBV (and HBV and HDV) in the presence of antiviral factors (genes from various species), candidate antiviral molecules, approved antivirals, antiviral cytokines or experimental drugs. The methods described in step 1 and sequencing approaches will be used to monitor changes in the viral genome(s) selected during the passage experiments.
We will use a genetically engineered stable cell line, Hep38.7-Tet, conditionally replicating and secreting HBV particles as source of virus in our studies. Additionally, we will generate HBV and HDV by reverse genetics following transfection with patient-derived infectious genomic DNA into hepatocyte cell lines. Where necessary, appropriate genetic modifications will be introduced into the relevant part of the viral genomic cDNA to generate genetically modified (GM) viruses of interest. These modifications may include specific mutation of amino acids that are functionally and structurally important in virus infection/replication, for reactivity to antibodies of interest, and for conferring antiviral resistance (the latter will be based on those observed in circulating strains, or those identified in our studies on ‘In vitro evolution of HBV and HDV’ as described in point 6, of Section 6). Additionally, we will also attempt to generate viruses expressing reporter genes or epitope tags (including but not limited to GFP, RFP and Luciferase). The modifications are not expected to alter host tropism.

**Host/vector system**

**Viruses:** Different WT and GM derivatives of HBV and HDV, including a bat-associated subtype of HDV (HDVb).

**Bacterial:** Disabled E. coli K12-derived strains such as DH5α. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids.

**Mammalian:** Various vertebrate hepatocyte and non-hepatocyte stable cell lines.

**Vector systems:** Non-mobilisable pUC-derived plasmids, pBlueScript, pCDNA and lentiviral vectors

**Origin & function**

Virus will mainly be obtained from a HBV producer cell line, Hep38.7-Tet, which will be obtained from collaborators in Japan. This cell line is well-characterised by them (Ogura et al., Biochemical and Biophysical Research Communications 452 (2014), 315-321) and is widely used by different groups. It will be imported from Japan as ‘UN2814 Category A infectious Substance affecting humans’ and will be handled in our Containment Level 3 (CL3) facilities. Additionally, we will produce both HBV and HDV by reverse genetics using patient-derived viral genomic nucleic acids for HBV and HDV and bat-derived viral genomic nucleic acids for HDVb. We also plan to attempt to directly propagate viruses derived from clinical samples (serum/plasma) following inoculation into cultured cells.

GM cell lines that ectopically express host proteins of interest (e.g. the virus receptor NTCP, viral dependency or restriction factors) will be generated. In some cases cell lines lacking host factor(s) identified as of interest in the study will be generated either through transient knock-down or permanent gene knock-out technologies. These GM cell lines will allow assessment of the functions of the relevant viral and/or host factors on virus infection, replication and pathogenesis.

**Evaluation of foreseeable effects**

**Risk associated with HBV and HDV generated in this project:** Viruses will be rescued by reverse genetics. There are no plans to carry out extensive mutational analyses of the viral genomes - mutations introduced will be mainly based on sequences circulating in patients including those conferring resistance to drugs. Additionally, in order to understand and delineate at molecular level aspects of viral life cycle (e.g. entry, replication, assembly) we may create specific amino acid changes in viral or host proteins of interest, or insert reporter genes or epitope tags (including but not limited to GFP, RFP and Luciferase). However, we expect these modifications to not adversely alter the replication, transmission or tropism of the viruses. If anything, the modifications would result in attenuated viruses.

**Risks associated with GM cell lines:** As mentioned above, the HBV producer cell line, Hep38.7-Tet, will be used as one source of virus. This cell line will always be handled under our CL3 facilities: the Richard M. Elliott Biosafety Laboratories (REBL) and/or MacRobert Blood-Borne Virus (BBV) laboratory (see section 11 below). All work will be strictly performed as per the criteria laid out in the REBL and BBV CL3 laboratories Codes of Practice and Risk Assessment associated with this Notification (see disinfection and waste disposal procedures). Risk of accidental infection to personnel working in the CL3 laboratory is very low, and the likelihood of virus being released from the laboratory is effectively nil. GM cell lines modified to express or not host genes of interest are not expected pose any risk to laboratory personnel or the environment. These cell lines would not survive inside the immunocompetent human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA or infection will confer no growth or survival advantage in or outside the laboratory to these cell lines.

**Risks to the environment:** Both HBV and HDV are Hazard Group 3 pathogens. In accordance with this classification all work involving the generation and handling of these viruses proposed will be performed in our REBL or MacRobert BBV CL3 facilities as described above (see section 11 below). HDVb is currently unclassified with respect to ADCP Hazard Group, and has never been found or suspected to infect humans or species other than bats. Nevertheless, as a precaution, potentially infectious HDVb will be handled only in our CL3 facilities. All work is strictly performed as per the criteria laid out in the REBL and BBV CL3 laboratories Codes of Practice and Risk Assessment associated with this Notification (see disinfection and waste disposal procedures). Risk of accidental infection to personnel working in the CL3 laboratory is very low, and the likelihood of virus being released from the laboratory is effectively nil. Therefore, the risk to the environment is effectively zero.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We have recently been granted derogation under Regulation 19 of the Genetically Modified (Contained Use) Regulations 2014 to dispense with sealability for the MacRobert BBV CL3 laboratory in relation to work with wild-type and genetically modified Hepatitis C virus (HCV) and Human Immunodeficiency virus (HIV) - notified activities GM26/99.3a and GM223/13.1b, respectively.

Similarly to HCV and HIV, HBV and HDV are blood-borne viruses, they are not infectious through the aerosolised/airborne route. Although aerosolised infection by highly concentrated HBV or HDV stocks remains a theoretical transmissible route, no case has been documented. Moreover, both viruses are sensitive to common chemical disinfectants such as 70% Industrial Methylated Spirit (IMS), Virkon and Distel. Little is known of HDVb, but genomic similarities to HDV suggest it is likely to have a similar biology (i.e., narrow host range and a requirement for a helper virus such as bat hepadnavirus). Considering the transmission routes of HBV and HDV and the scope of work we intend to do (as detailed in this form and the attached risk assessment), we consider that the risk of using highly toxic fumigants (such as formaldehyde or hydrogen peroxide) surpasses the risk of contamination by or release of the viruses and we propose to dispense with fumigation for room disinfection. Instead, we propose to use chemical surface disinfectants for regular and emergency disinfection procedures, as detailed in the attached risk assessment.

Hence, we are seeking derogation from full containment level 3 measures and propose to handle HBV and HDV/HDVb in the MacRobert BBV derogated CL3 laboratory in the same way as already consented by HSE for HCV and HIV: uncontained infectious material will only be handled in microbiological safety cabinets (MSC); no concentration of virus by ultracentrifugation will be permitted and propagation of virus will be limited to no more than 10 to the 6 genome copies/ml. No laboratory-acquired infection of HBV or HDV via aerosols have ever been reported in this concentration range. Any work requiring higher titres of HBV or HDV will necessitate concentration of the virus by ultracentrifugation or other means and will be carried out in the REBL CL3 Suite, which operates under more stringent safety procedures and is suitable for room fumigation for regular and emergency disinfection. See associated risk assessment for fully detailed containment measures, inactivation and waste disposal procedures.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment Level 2 areas:
Liquid waste: add Virkon to final concentration of 1% (w/v) overnight, then discharge to drains.
Disposable solids (eg plasticware such as flasks, tubes, pipette tips etc.): soak in 1% Virkon (w/v) overnight. Transfer solid contents to autoclave bags and autoclave at 121°C for a minimum of 15 minutes prior to final disposal by district council to landfill, remaining liquid to be discharged to drain.
Other solids (agar plates, gloves etc.): place in a marked box lined with a clear autoclave bag, and autoclave at 121°C for at least 15 minutes. Seal contaminated plates before placing in bag, to avoid lid falling off. Gloves should be disinfected with alcohol before putting in containers.
Degree of kill:
Chemical sterilisation by Virkon - effectively 100% kill
Autoclaving - effectively 100% kill (annual validation)

MacRobert BBV derogated CL3 laboratory:
Liquid waste: decontaminate in Virkon 1% w/v (final concentration) overnight. When handling protein rich liquid, Virkon powder should be added to give a final concentration of no less than 1% w/v. After inactivation, discharge liquids to drain.
Solid waste from within the MSC: discard paper waste within the MSC into a metal bin lined with autoclave bag for autoclaving. Cell culture plastics which can be submerged must be soaked overnight in 1% w/v Virkon prior to autoclaving. Flasks, once liquid has been removed, can be rinsed with 1% w/v Virkon, capped, surface-decontaminated with 70% IMS and disposed of into the metal bin outside the MSC for autoclaving. Disposable plastic pipettes must be submerged overnight in 1% w/v Virkon prior to autoclaving. Gloves must be sprayed with 70% IMS prior to being discarded into an autoclave bin.
Sharps which arise inadvertently or glass slides/cover slips must be placed into clearly labelled sharps bins containing enough 1% w/v Virkon solution to cover all material and autoclaved.
Solid waste from outside the MSC: other waste generated within the laboratory, including soiled lab coats/gowns must be placed directly into disposable bags for autoclaving.
All solid waste is to be autoclaved at 121°C for 15 minutes prior to being sent for incineration.
**Degree of kill:**
Chemical sterilisation by Virkon - effectively 100% kill
Chemical sterilisation by 70% IMS - effectively 100% kill
Autoclaving - effectively 100% kill (annual validation)

**The Richard M. Elliott Biosafety Laboratories (REBL) CL3 Suite:**
Liquid waste: decontaminate in Virkon 1% w/v (final concentration) overnight. When handling protein rich liquid, Virkon powder should be added to give a final concentration of no less than 1% w/v. After chemical inactivation, liquid waste must be either autoclaved at 121°C for 15 min or disposed through ACTINI effluent treatment plant (135°C for 2 minutes).

Solid waste from within the MSC: spray paper waste with 1% w/v Virkon within the MSC, then with 70% IMS outside the MSC prior to autoclaving. Solid which can be submerged must be soaked overnight in 1% Virkon w/v (final concentration) prior to autoclaving. Solids which cannot be submerged must be filled with or soaked in Virkon 1% w/v (final concentration) for 10 minutes, wiped down with 1% w/v Virkon within the MSC then with 70% IMS outside the MSC prior to autoclaving. Sharps which arise inadvertently or glass slides/cover slips must be placed into clearly labelled sharps bins containing enough Virkon solution to cover all material and autoclaved.

Solid waste from outside the MSC: other waste generated within the laboratory, including soiled protective clothing, must be sterilised by autoclaving. Solid waste is to be autoclaved at 134°C for 5 minutes, fabrics are to be autoclaved at 134°C for 3.5 minutes.

Following ACTINI treatment or autoclaving, liquid waste is disposed to drain. After autoclaving solid waste is sent for incineration.

**Degree of kill:**
Chemical sterilisation by Virkon - effectively 100% kill
Autoclaving - effectively 100% (annual validation)

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### Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2 Yes</td>
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<td>L4 L2</td>
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Project Ref: 26/94.1

Date Ackn'd: 07/11/1994
CU2 Project Title: PRODUCTION OF BUNYAVIRUSES CONTAINING GENOME SEGMENTS DERIVED FROM CLONED CDNA

Date Project Ceased: 26/08/2021

Class: Class 2
CultureVolClass2: Class 2
CultureVolumeClass3-4: Class 3-4

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: N

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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<tr>
<td>Animal Units</td>
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<td>Human Clinical Applications</td>
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Project Ref 26/99.2

Date Ackn'd 08/04/1999

Date Project Ceased 18/09/2007

Withdrawn N

Tick if notifying a connected programme of work N

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements Y
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
<thead>
<tr>
<th>Is an emergency plan required according to regulation 20?</th>
<th>N</th>
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</thead>
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<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
<td>N</td>
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</tbody>
</table>
Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Project Ref 26/99.3

Date Ackn'd 08/04/1999

CU2 Project Title GENETIC MODIFICATION OF HEPATITUS C VIRUS

Class 3

Date Project Ceased 26/08/2021

Non-GMM Consent Granted yes

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Project transferred to GM223

Historical Date of Additional Info

Significant Change ID 26/99.3a

Date of Significant Change 26/07/2017

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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## Project Ref 26/trans1

<table>
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<th>CU2 Project Title</th>
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<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>15/12/1993</td>
<td>GENETIC MODIFICATION OF HERPESVIRUS</td>
<td>Class 2</td>
<td></td>
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</tbody>
</table>

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: yes

### Historical Significant Changes

- Project transferred to GM223

### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
1. GM 26 committee comments (approval date 25/08/11; see below):

The CVR Church Street GM subcommittee met on 22/08/11 to consider the proposal:
“Arthropod host cell/arbovirus interactions and immune responses induced by virus replication.”

Subcommittee members:
R E
J M
A P
F R (convener)

The subcommittee members agreed recommendations for modifications to the following topics:

In Section 12 (Describe the waste management measures etc) of the HSE Notification and Section 16 (Proposed experiments etc, sub-heading Waste Disposal) of the Risk Assessment:

Some of the autoclaving cycles specified were not available on the autoclave at the CVR Church Street site.
Procedures appropriate for clinical waste, incineration and microwaving were either not in use at the CVR Church Street site or would be expensive to implement. They were considered to be unnecessary for this proposal.

The procedure specified placing arthropod derived material into a sealed container for autoclaving. However, autoclaving requires containers be open to allow entry of steam.

No procedures for disinfection of contaminated material were described. All material is to be rendered safe by autoclaving, which would entail contaminated material being stored and transported to the autoclaves. This was considered an unacceptable risk, especially for liquid waste, and requires an effective disinfection procedure to be put in place.

The subcommittee decided that the relevant entries under section 12 in the HSE Notification and section 16 of the Risk Assessment should be replaced with the existing waste disposal and disinfection policies employed at the CVR Church Street site. These are available on the local intranet. Before this can be approved, validation must be obtained regarding the effectiveness of Virkon for inactivating LGTV and SFV.

In Section 6 (Purpose of the contained use), reference is made to subgenomic replicons of other arboviruses (eg chikungunya and dengue) that are not otherwise included in this proposal. A full description of the work to be carried out with these agents, the nature of the hazards and the precautions required should be included in both the HSE Notification and Risk Assessment.

Clarification is required for section 8 (Containment and control measures for GMOs that are not micro-organisms; page 11) of the HSE Notification and for section 11, sub-heading Hosts (page 8) of the Risk Assessment concerning the transport of arthropods. The latter states that; “Samples (dead or non-mobile arthropods (our italics) or material from these) will be transported to our CL2 laboratory according to standard regulations.” The subcommittee agreed that no living infected arthropods would be
allowed in the CVR Church Street site and that they should only be handled in an insectary.

The reference to; “the city centre location of our laboratories” on page 10 of the Risk Assessment is not appropriate for the CVR Garscube site and should be removed.

Under section 7, sub-heading Control measures (page 8) of the HSE Notification, the use of appropriate PPE (not just gloves) should be specified.

An estimate of virus quantities (eg pfu/ml) should be provided in addition to the culture volumes in the HSE Notification, section 9 (Maximum culture volumes per experiment; page 11) and the RA section 16 (Culture volumes; page 14).

The requested changes were made and on 25/08/11 the revised documents were circulated to and approved by the subcommittee members.

2. GM223 committee comments (approval date 20.10.2011, see below):

“The risk assessment was clear but clarification on local mosquito activity was required; more detail on any full length viruses being held, and careful consideration of any possible recombination events was deemed essential. Risks to immune-suppressed personnel needed to be mentioned.

These points have been addressed in form CU2 and Risk Assessment, and on the 20.10.2011 the revised documents were approved.

Dr. A G on behalf of the committee.”

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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<td>L4</td>
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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 26/trans2

<table>
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<td>GENETIC MODIFICATION OF HUMAN PAPILLOMAVIRUS</td>
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Date Project Ceased

01/11/2007

Withdrawn       N

Tick if notifying a connected programme of work       N

Historical Significant Changes

TRANSFERRED TO GM 223 (1/11/07)

Project notified under transitional arrangements    Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 26/trans3

Date Ackn’d 15/12/1993
CU2 Project Title GENETIC MODIFICATION OF VACCINIA VIRUS AND OTHER CLASS 2 POXVIRUSES

Class 2
Non-GMM Consent Granted not applicable
Project notified under transitional arrangements Y

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<p>| Laboratory Activities | Glass Houses | Growth Rooms |</p>
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Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

| Date Premises Closed | Name                  | Department | Name 2          | Campus Estate or Research Centre | Building        | Road Name    | District | Town              | County         | Post-code | Country       | With-drawn |  |
|----------------------|-----------------------|------------|-----------------|----------------------------------|----------------|--------------|----------|-------------------|----------------|-----------|---------------|------------|
| 14/10/2013           | MRC HUMAN GENETICS UNIT |            |                 | WESTERN GENERAL HOSPITAL         | EVANS BUILDING | CREWE ROAD   |          | EDINBURGH          | EAST RENFREWSHIRE | EH4 2XU   | SCOTLAND      | N          |

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 28/03.1**

**Date Ackn'd** 26/11/2003

**CU2 Project Title** USE OF HIV-DERIVED LENTIVIRUS VECTORS TO EXPRESS GENES IN CULTURED CELLS, ORGAN CULTURE AND MOUSE TRANSGENESIS.

**Date Project Ceased** 14/10/2013

**Class 2 Culture Volume** < 1 litre

**Non-GMM Consent Granted** not applicable

**Project notified under transitional arrangements** N

**Historical Significant Changes** TRANSFERRED TO GM 207 ON CLOSURE OF GM28

---

**Project Additional Information**
Purposes of the contained use

Studies in cancer genetics and developmental genetics, that are an integral component of the concerted research programme on cancer and development genetics that is underway here at the Medical Research Council Human Genetics Unit.

Recipient or parental organism

These lentivirus vectors are infective to non-dividing human cells and are integrative to the genome therein, but are non-replicative, ie, self-inactivating, and so infected cells would not produce viral particles and hence pose no risk.

There is a risk of oncogene activation in infected cells due to the insertion, but the risk is low, and the risk of consequent tumourigenesis very low, as changes to several genes are required for these events to occur.

Host/vector system

A third generation packaging system after Dull et al (1998) J. Virol 72 8463-8471 will be used, which requires genes from three different vectors for packaging into infectious particles. These vectors designed so as not to be packaged into viral particles and not recombine with each other or with the lentivirus.

These vectors transiently provide at high titre the viral functions required for packaging of the lentiviral construct.

The lentivirus vector to be used initially is LentiLox 3.7. This infects non-dividing human cells under the above circumstances, is stably integrative, but is non-replicative, ie, is self-inactivating. Vector expresses no viral functions once transduced. Thus, target cells can be infected with viral particles made in the packaging line but to infectious particles can then be made by the infected target cells. Wildtype virus does not infect mice and is rare in humans, so recombination with wildtype virus very unlikely. Airborne particles, if produced, would be at very low concentration and unstable.

We wish to include other similar lentivirus vectors to be developed in the future which have the same properties as outlined above (or properties giving lower risk) within the scope of this application.

Origin & function

We intend to use the lentiviral system to express the following classes of gene. Construct-specific risks will be covered in (future) GM Risk Assessments, and experiments only permitted if the GM Safety Committee here categorises the proposed work as Hazard Group 2 (or 1).

i) Expression of siRNAs against genes involved in development and tumorigenesis. siRNAs (short inhibitory RNAs) are short dsRNA molecules which can give sequence-specific knockdown of the expression of endogenous target genes, a process also referred to as RNAi (RNA interference). siRNAs will be expressed, if possible, as short hairpin RNA molecules, or otherwise as pDECAp constructs which express long dsRNA hairpins which are endogenously converted to functional siRNAs. Target genes will mainly be murine, so whenever possible the target sequences will be murine-specific. If this is not possible, such as when the target is human cells in culture, if practicable then the expression of the siRNA will be regulated by an inducible system (ie tetracyclin inducible) to further limit the potential risk of siRNA expression in humans. Examples of genes we intend to study are genes involved in testis and kidney development and tumorigenesis, such as WT1 and its downstream target genes. Lentiviral siRNA vectors will be used to infect cell culture (murine or human), organ culture (murine) and in the generation of transgenic mice.

ii) Reporter genes, like lacZ, GFP, or its derivates. Promoters responding to endogenous cellular signals involved in tumorigenesis or development, or tissue-specific promoters, will be cloned in front of these reporter genes and used to monitor the signalling pathways involved. These inserts pose no risk to human health. Reporter constructs will be used in cell culture (murine or human), organ culture (murine) or transgenesis (murine).

After surveying the promoter activity with reporter genes we will use these promoters to drive expression of developmental genes. These genes are predicted to affect early developmental stages and we propose to drive both the active genes and siRNAs. Genes that will be studied for example are Gli3, dHAND, Shh, and members of the Tbx, Nkx, Bmp and Fgf families.
iii) Expression of Cre or FLP recombinases. Cre and FLP are used in combination with conditional mouse models in which part of a gene is placed between loxP or FRT sites. Expression of Cre/FLP will result in deletion of the sequence between the loxP/FRT site and inactivation of the gene. By expressing the recombinases in an inducible or tissue-specific manner full control of inactivation of the target gene is possible. Expression of Cre or FOP poses no risk for human health. Lentiviral expression systems will be used in cell culture, organ culture and transgenesis, all murine.

iv) Expression of accessory genes involved in different tetracyclin responsive expression systems, like rtTA, tTA and Tet-R. These genes regulate the expression of genes the promoters of which have been extended with tet-binding elements, and the expression of the genes will be activated by the presence or the absence (depending on the system) of tetracyclin or its analogue doxycyclin. Expression of rtTA, tTA or Tet-R poses no risk to human health. Lentiviral expression of these genes will be used in cell culture, organ culture and transgenesis.

Evaluation of foreseeable effects

RISK TO HUMAN HEALTH ARISES DUE TO:
Lentivirus vector infective to non-dividing human cells
Lentivirus vector integrative
Lentivirus vector at high titre
thence potential for insertional mutagenesis of body surface cells by the lentiviral vector and thence potential for oncogene activation in infected cells.

RISK TO HUMAN HEALTH AMELIORATED AS:
Third generation packaging system to be used - requires three different vectors which cannot recombine with each other or the lentivirus.
Integration is stable.
Lentivirus vector is non-replicative, ie, self-inactivating - no viral function expressed once transduced
Tumourigenesis very unlikely
Wildtype virus does not infect mice and is rare in humans - recombination thus very unlikely
Airborne particles, if produced, would be at very low concentration and unstable
siRNA whenever possible will be highly specific to murine genes
Knockdown of the siRNA targeted genes in an adult human would not cause transformation
Targeted murine genes under developmental-specific and tissue-specific enhancer and although it is a very unlikely possibility, tests will nonetheless be done to ensure that the lentivirus does not become replicative by recombination

APPROXIMATE EVALUATION OF ABOVE RISK (APPLYING BS8800:1996) TO HUMAN HEALTH:
Estimated likelihood = highly unlikely
Potential severity of harm = extremely harmful
Risk = moderate and thus control = Containment level 2 or rationalising.

According to the ACGM Compendium of Guidance (Part 2B, Annex III, paragraph 26) HIV-derived lentivirus (such as this) should be Hazard Group 3, unless there is justification for a lower classification. The vector here requires three different vectors for packaging (ie a 'third generation system') and is self-inactivating/non-replicative. Further, the probability for oncogene activation in infected cells is anyway low, and for consequent tumourigenesis is very low. We thus consider there to be sufficient justification for classification of this proposed work as Hazard Group 2, rather than 3.

AND RISK TO THE ENVIRONMENT:
Negligible - transduced cells/animals not infective (see above), all changes somatic (see Section 8), and all virus, cells and tissues/animals destroyed (see Section 12).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Any live-birth transduced mice will be entirely reared, housed and killed within our Transgenic Mouse Facility.
This is a secure 'barrier' unit, and as such escape of mice or entry of exogenous rodents is almost impossible. Such animals anyway pose negligible risk to human health or to the environment, as they could not be infective with the lentivirus vector (is self-inactivating/non-replicative).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Complete immersion in 1% Virkon for at least two hours will be used to destroy all cultures and sterilise all items. Virkon is a potent peroxygen disinfectant that will give 100% kill of all agents used, the lentivirus in particular. Virkon is known to give 100% kill of the closely related HIV (US Environmental Protection Agency). Liquid waste will then be disposed to drains. Solid waste is then routinely autoclaved and then sent for commercial incineration. Larger tissue samples/whole animals (ie mice) are routinely disposed by commercial incineration (there is no viral risk from such items anyway).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Four GM Risk Assessments attached. Relevant comments include the following.

Committee noted this proposed use at high titre of an integrative retrovirus that could infect non-dividing human cells. According to the ACGM Compendium of Guidance (Part 2N, Annex III, paragraph 26) this should be HG3, unless there is justification for a lower classification. It was further noted that the virus requires three different vectors for packaging (ie a 'third generation system') and is self-inactivating/non-replicative. However, the former must be considered in the context of its infectivity once packaged. Committee were mindful of the potential, albeit very low probability, for oncogene activation consequent upon infection of and integration into the genome of a worker's cells. Nonetheless, the non-replicative nature of the construct was agreed as sufficient justification for classification of this proposed work as HG2 rather than HG3. The proposed (section 15) HG1 is not sufficient.

Committee requires, as a condition of this approval, that packaging lines are assayed by plaque formation colony assay or equivalent to ensure that the viral vector has not become replicative by recombination (although it is accepted that this is unlikely, it was considered that the high titres used might possibly increase the overall likelihood). This assay must be performed initially, and then repeated after three months of actual project work, and then after every six months of actual project work thereafter. And the acceptable result is to be zero replicative viral particles.

Project Containment

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

N

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Name**

QUEEN MARY & WESTFIELD COLLEGE

**Name 2**

UNIVERSITY OF LONDON

**Department**

SCHOOL OF BIOLOGICAL SCIENCES

**Campus Estate or Research Centre**

**Building**

**Road Name**

MILE END ROAD

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

E1 4NS

**Country**

ENGLAND

**Tel Number**

020 7882 3200

**Fax Number**

020 8983 0973

**E-mail**

biology@qmw.ac.uk

**HSE Division**

LONDON

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Animal Unit</th>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

02/03/2022
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For activities involving GMMs, describe the waste management measures which will apply to the activity

All contaminated waste, including microbiological cultures, culture medium and plastic ware is collected into 20 litre biohazard bags which are sealed and autoclaved within the building prior to being collected on a weekly basis by a commercial firm for incineration. The autoclave provides a printout of the temperature and pressure throughout the cycle and additional monitoring is provided by autoclave tape attached to the bags. No more than about 10 bags of waste (200 litres) are produced from the site per week.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Data Premises Notified (Originally) | 24/04/1978 |
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Transferred from 1992 Regs? | Y |
Transitional Premises Class | 2 |
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Transitional Premises | N |
Emergency Plan Required? | |
Non-GMMs | Y |
Withdrawn | N |

**Name**

**IMPERIAL COLLEGE SCHOOL OF MEDICINE AT HAMMERSMITH CAMPUS**

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**Comments**

**Date at Which Additional Info Submitted**

| 14/08/2001 | 09/10/2001 |
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

- **Mycology**
- **Transgenic Invertebrates**
- **Transgenic Plants**
- **Other (please specify below)**

- **Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment
Project Ref 25/13.1

**Date Ackn’d:** 01/01/2018  
**CU2 Project Title:** The construction and evaluation of adenovirus vectors as therapy for cancer  
**Class:** Class 2  
**CultureVol:** < 1 Litre  
**Consent Granted:** Non-GMM  
**Date Project Ceased:** 27/02/2018  
**Historical Significant Changes:** Transferred from GM383 01/01/2018  
**Historical Date of Additional Info:**  
**Significant Change ID:**  
**Date of Significant Change:**  

**Project notified under transitional arrangements:** N

**Tick if notifying a connected programme of work:** N

**Tick if you are claiming exemption from disclosure for sections of the risk assessment:**

---

**Purposes of the contained use**

The aim of this project is to amplify wild-type and deletion mutant oncolytic adenovirus vectors already produced in the applicant's previous lab; to evaluate their anti-tumour efficacy in in vitro and in vivo models of human malignancy, particularly ovarian cancer. Adenovirus serotypes Ad5, Ad11 and Ad35 will be used as reference standards for work performed with Ad5 (mainly E1A) deletion mutants. Replication competent and replication defective mutants will be constructed using the 'Ad-Easy' system to infect permissive human cell lines. These new mutants may encode transgenics that could induce tumour cell killing alone or enhance the anti-tumour properties of replication-competent deletion mutants. Evaluation of viral efficacy of mutants will be performed on Human and Murine cell lines by a variety of methods. Viral manufacture of replication defective vectors will be performed in HEK293 cell line to provide the E1 'in-trans'. Concentration of adenoviral constructs from 750ml final cultures will be via Caesium Chloride centrifugation.
### Recipient or parental organism

The recipient cell lines HEK293 or A549 will be used. Both are commercially available cell lines which require specific laboratory culture and as such pose negligible risk to humans. 

HEK 293 is rated Biosafety level 2 because of an E1 fragment of adenovirus embedded in it. It is this fragment that is essential to this series of experiments with E1 deletion adenoviral mutants. 

Cell line A549 is rated Biosafety level 1.

### Host/vector system

One vector system will be oncolytic deletion mutants of Adenovirus. Replication of oncolytic deletion mutants of Adenovirus is attenuated in normal cells/tissue. Multiple mechanisms for this attenuation exist, depending upon the nature of the deletion. Publications attest to the selectivity of replication – between normal human cells compared to a panel of human malignant cells – see Heise et al Nature Med (2000) 6:1134. In addition, multiple phase I and II trials of oncolytic adenovirus have shown no replication in normal tissues.

Wild-type adenoviral vectors, used as reference standards, are replication competent and could infect humans. However, wild-type adenovirus infection in humans with an intact immune system leads to a mild and self-limiting 'flu-like illness.

### Origin & function

Mammalian genes are to be inserted into the adenovirus genome and have been chosen to perform their intended function; either to increase tumour-specific killing by the virus (e.g. tumour suppressor or apoptosis-inducing genes), to inhibit the activity of oncogenic pathways or to increase the immunogenicity of infected tumour cells. Documentation exists to indicate that this can be achieved safely. No wild-type oncogenes or direct toxins will be inserted.

The virus will be applied to various established human and murine ovarian carcinoma cell lines; obtained from ATCC and other reputable sources and have consequently been screened for bloodborne viruses. In addition, the human cell lines have also all been previously STR verified and are known to be mycoplasma-free.

### Evaluation of foreseeable effects

The deletions induced in adenovirus vectors are designed to narrow the host range, so that there is reduced replication and killing within normal cells, thus increasing the therapeutic index in ovarian cancer cells. Mechanisms to achieve this will include introduction of specific deletions to restrict replication and the use of tumour and tissue-specific promoters.

During process development for clinical grade manufacture of one of the mutants being used, no recombination between vector and HEK293 sequences was detected (limit of detection 1 in 109 genomes) – thus it is believed that the risk of reversion to wild-type virus following amplification in HEK293 cells is extremely low.

Replication-deficient viruses are incapable of replication in cells lacking adenovirus E1 function. Infection of normal human cells as well as malignant cells results in no infectious virion production.

The replication competent adenoviruses used will contain mutations within the viral genome that attenuates replication in normal cells and tissues. The deletions occur in regions of the viral genome that are necessary for replication in normal cells but are complemented by the altered gene expression in cancer cells. Mutants used can only replicate efficiently in cells with deregulated p53 and pRB pathways respectively.

The tumour selective mutants can infect normal cells but the ability to replicate is highly attenuated.

Wild-type adenoviral vectors are replication competent and could infect humans. Wild-type virus infection in humans with an intact immune system leads to a mild and self-limiting 'flu-like illness.

The normal route of adenovirus infection is via aerosol. The use of correctly trained and supervised staff, Class II biological safety cabinets, lab coats and gloves.
disinfection and autoclaving of waste will reduce the likelihood of exposure to an extremely low level.

The virus will be applied to various established human and murine ovarian carcinoma cell lines. Because ovarian cancer cells do not contain any adenovirus genomic DNA, there is no risk of recombination of deletion mutants to generate wild-type revertant virus. Thus, generation of adenoviruses and infection of ovarian cancer cells is not considered to pose any inherent hazard to human health. The GM viruses do not have the capacity for survival outside of the laboratory. The recipient cells will therefore not have any foreseeable effect on human health.

E.coli strains are highly attenuated laboratory strains and do not present any hazards to humans.

Human adenoviruses do not replicate in murine tissue - this is true of wild-type, replication-restricted oncolytic viruses and replication-defective E1-deleted vectors.

The only replication following injection into in vivo tissue will be in malignant tumour tissue.

All injections of virus into vivo tissue will be undertaken in class II hoods within the specialist unit, thus minimising the risk of an aerosol-mediated infection during the injection process.

Multiple human studies demonstrate that virus shedding after intraperitoneal, intravenous or intratumoural injection is undetectable. Thus, the risk to humans from murine cell line tissue is negligible as the viruses are replication-deficient or tumour specific and the gene products are not considered harmful to health.

Full containment level 2 will be used, as set out in the Regulations, including appropriate treatments for bulk, contaminated solid waste.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All viral supernatants and spent medium will be inactivated using 10,000 ppm free chlorine before removal from the Containment Level II laboratories for final disposal. This process will result in 100% inactivation of infectious virus.

Solid waste will be bagged in biohazard bags prior to removal from the Containment level II lab and then placed in boxes which are sealed prior to removal from the CL2 suites. Boxes remain sealed and are autoclaved before the contents is removed for collection by accredited waste contractors. Autoclaves produce a digital record of load temperature achieved and are validated by annual thermocouple mapping. This process will result in 100% inactivation of infectious virus.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
The purpose of the project is to evaluate the anti-tumour efficacy of oncolytic adenovirus and oncolytic Herpes Simplex Virus, in in-vivo models of human malignancy, particularly ovarian cancer. This will be achieved using Wild-type Ad5, Ad11, Ad35 adenoviral vectors as well as a number of Ad5 deletion mutants. Herpes Simplex Virus vectors are based upon HSV-1 single gene "vector 1716" mutants (neuro-virulence gene ICP34.5) as well as derivatives of this single mutant encoding fluorescent markers. Double deletion mutants (e.g. ICP34.5 and ICP47), with increased selectivity for malignant cells may also be evaluated.

Novel derivatives of the adenovirus and HSV vectors (encoding transgenes that could induce tumour cell mortality alone or enhance the anti-tumour properties of...
replication-competent deletion mutants) will be evaluated. In some viral treatments, tumour and tissue specific promoters (e.g. CEA, PSA) may be used to drive virus replication (via transcriptional control of E1A transcription) or to drive transgene expression.

Recipient or parental organism

Viruses are to be evaluated in various human and murine established carcinoma cell lines growing within subjects (either immunodeficient for human cell lines, or immunocompetent for murine cell lines). These cell lines have been obtained from reputable sources and have consequently been screened for HIV, HepB, HepC and EBV. Because these tumour cells do not contain any adenovirus or HSV genomic DNA, there is no risk of recombination of deletion mutants to generate wild-type revertant virus. ICP34.5-deleted HSV vectors have been injected into over 200 human patients with advanced malignancy - there are no records of any wild-type reversion in any patient. The recipient cells are not therefore, considered to pose any inherent hazard or foreseeable effect to human health.

Host/vector system

Replication of oncolytic deletion mutants is attenuated in normal cells/tissue, depending upon the nature of the deletion and the nature of the vector. Multiple publications from the proposer’s lab and others attest to the selectivity of replication – see Heise et al Nature Med (2000) 6:1134. In addition, multiple phase I and II trials of oncolytic adenovirus as well as E1-deleted non-replicating vectors have shown no replication in normal tissues. Wild-type adenoviral vectors are replication competent and could infect humans. However, wild-type adenovirus infection in humans with an intact immune system leads to a mild and self-limiting 'flu-like' illness. ICP34.5-deleted HSV is dramatically attenuated in normal non-malignant tissue including neuronal cells, both murine and human. Direct intra-cranial inoculation of 1716 has been demonstrated to be safe in subjects. No wild-type HSV-1 will be used in any experiment.

Origin & function

Genes that will be inserted into the adenovirus or HSV genome are designed either to increase tumour-specific mortality by the virus (e.g. tumour suppressor or apoptosis-inducing genes), to inhibit the activity of oncogenic pathways or to increase the immunogenicity of infected tumour cells. There are multiple examples in the literature where this can be achieved safely. No wild-type oncogenes or direct toxins will be included.

The deletions induced in adenovirus and HSV vectors are designed to narrow the host range, so that there is reduced replication and mortality within normal cells, thus increasing the therapeutic index in ovarian cancer cells. Mechanisms to achieve this will include introduction of specific deletions to restrict replication and the use of tumour and tissue-specific promoters.

During process development for clinical grade manufacture of one of the mutants, no recombination between vector and HEK293 sequences was detected (limit of detection 1 in 109 genomes) – thus it is believed that the risk of reversion to wild-type virus following amplification in HEK293 cells is extremely low. For HSV, there is no evidence from any previous pre-clinical or clinical study that HSV vector 1716 can transfer genetic material to other organisms.

Evaluation of foreseeable effects

Replication competent adenoviruses contain mutations within the viral genome that attenuates replication in normal cells and tissues. The deletions occur in regions of the viral genome that are necessary for replication in normal cells but are complemented by the altered gene expression in cancer cells. These mutants can only replicate efficiently in cells with deregulated p53 and pRB pathways. Tumour selective mutants can infect normal cells but the ability to replicate is highly attenuated. Human adenoviruses do not replicate in murine tissue - this is true of wild-type, replication-restricted oncolytic viruses as well as replication-defective E1-deleted vectors.

The only replication following injection into tumour-bearing subjects will be in malignant tissue. The normal route of adenovirus infection is via aerosol. 1716 and other ICP34.5-deleted HSV vectors have been administered to over 200 patients with cancer with no significant toxicity. All vectors proposed contain an intact thymidine kinase gene, and thus HSV-infected cells are sensitive to treatment with the anti-viral drugs aciclovir and ganciclovir.

All injections of virus will be undertaken in class II hoods within the unit, thus minimising the risk of aerosol-mediated infection during the injection process. Multiple human studies demonstrate that virus shedding after intraperitoneal, intravenous or intratumoural injection is undetectable. Thus, the risk to humans from subjects is negligible as the viruses are replication-deficient or tumour specific and the gene products are not considered harmful to health.
In addition, laboratory standard operating procedure in relation to biological agent handling and waste management are such that the likelihood of exposure to a biological agent is reduced to an extremely low level. The risk to human health is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste to be double bagged in biohazard bags prior to removal from the Containment Level 2 laboratory and then autoclaved (15 mins @ 134.0C) [verified by thermocouple] before collection by licensed professional waste contractors.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Considered and accepted by GMSC

Project Containment

Laboratory Activities

Glass Houses

Growth Rooms

Animal Units

Large Scale Activities

Human Clinical Applications

L2 L3 L4 L2 L3 L4 L2 L3 L4

L2 Yes L3 L4 L2 L3 L4 L2 L3 L4

Project Ref 31/01.1

Date Ackn'd 09/02/2001

CU2 Project Title CLONING AND EXPRESSION OF BACTERIAL ENTEROTOXINS

Class 2

Culture Vol Class 2 < 1 litre

Culture Vol Class 3-4
### Project Additional Information

**Purposes of the contained use**

The aim of the project is to clone, express and purify bacterial enterotoxins from Staphylococcus aureus, specifically the SEG and SEI enterotoxins. The genes for these proteins will be obtained by PCR amplification from DNA isolated from a particular isolate of S. aureus obtained from a patient at St Mary's Hospital. The PCR products will be cloned into E. coli expression vectors capable of expressing the clones genes from the bacteriophage T7 promotor in case the gene products are toxic to E. coli.

**Recipient or parental organism**

The genes of SEG and SEI will be obtained by PCR amplification from DNA isolated from a patient at St Mary’s Hospital. The DNA will be cloned into plasmid vectors for propagation in lab adapted disabled strains of E. coli.

**Host/vector system**

Bacterial Host cell lines: E. coli DH5a, E. coli BLR (DE) pLysS, E. coli HMS174 (DE) pLysS, E. coli HMS174(DE) pLysE
Cloning Vector: pCRT7/NT-TOPO

**Origin & function**

Staphylococcus aureus is the origin of the DNA. The genes encode enterotoxins, and the genes will be expressed with the aim of producing recombinant protein.

**Evaluation of foreseeable effects**

The E. coli strains are disabled and there is very little likelihood of the establishment of an infection in humans and/or animals. The encoded gene product may cause symptoms of food poisoning. There is very little possibility of the GMO surviving in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste is chemically treated with freshly made 2% hycolin for a minimum of 15 minutes, in practice overnight treatment is usual. This effectively yields a 100% kill.

Solid waste is autoclaved at 134 degrees C for 30 minutes, and effectively yields a 100% kill.

Periodic testing of a test culture to determine survival after the above treatments is used for monitoring the effectiveness of the procedures.

Solid waste is subsequently transported in sealed bins for incinerating at an off site facility.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project concerns the cloning of enterotoxin genes from S. aureus. Due to the nature of the encoded gene products, it was considered that the proposal is assessed as a Class 2 project, even though the project is for the production of the proteins in E. coli cells only.

IC Occupational Health did not consider it necessary for Health Surveillance.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
<tr>
<td>L2</td>
<td>L3</td>
</tr>
</tbody>
</table>

Project Ref 31/01.2

Date Ackn'd 19/02/2001

CU2 Project Title

USE OF ATTENUATED SALMONELLA STRAINS FOR VACCINATION AGAINST TUMOUR-ASSOCIATED ANTIGENS

Date Project Ceased 02/03/2022

Class 2

Consent Granted not applicable
Withdrawn  N  

Tick if notifying a connected programme of work  N  

Project notified under transitional arrangements  Y  

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**  N  

**If yes, tick to confirm that it is attached to this form**  N  

**Tick to confirm that you have attached a risk assessment to this form**  

02/03/2022
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L3 L4 L2 L3 L4</td>
<td>L3 L4 L2 L3 L4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
</tr>
</tbody>
</table>

**Project Ref** 31/01.3

**Date Ackn’d** 29/03/2001

**CU2 Project Title** PRODUCTION OF E.COLI AND S. TYPHIMURIUM CONSTITUTIVELY EXPRESSING GREEN FLUORESCENT PROTEIN

**Class** Class 2  **CultureVol** < 1 litre

**Non-GMM** not applicable

**Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N  **Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to transform bacteria used in in-vivo models of infection, with a plasmid vector carrying the gene for green fluorescent protein, in order to develop
Recipient or parental organism

E. coli 0111:B4, E. coli J5 and S. typhimurium LT2 are routinely used in mouse models of E. coli and Salmonella infections. The vector pFPV25.1 is mob+, colE1+ and contains an ampicillin resistance gene (bla). Constitutive GFP expression is under the control of the promoter for the S. typhimurium rpsM gene, which encodes for the S13 ribosomal protein. It has been previously used to transform E. coli and S. typhimurium for similar applications.

Host/vector system

Bacterial Host cell lines: E. coli 0111:B4, E. coli J5 and S. typhimurium LT2. Cloning Vector: pFPV25.1 (mob+, colE1+)

Origin & function

Aequoria victoria is the origin of the DNA encoding the GFP gene. The plasmid construct will express GFP enabling bacterial cells to be visualised fluorescence microscopy and flow cytometry.

Evaluation of foreseeable effects

The E. coli and S. typhimurium strains have been used for studies of salmonella infections. Thus, there is a possibility of the strains causing symptoms of food poisoning. There is very little possibility of the GMO surviving in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste is chemically treated with freshly made 2% hycolin for a minimum of 15 minutes, in practice overnight treatment is usual. This effectively yields a 100% 'kill'. Periodic testing of a test culture to determine survival after the above treatments is used for monitoring the effectiveness of the procedures. Solid waste is subsequently transported in sealed bins for incinerating at an off site facility.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The project concerns the use of a plasmid vector expressing GFP in E. coli and S. typhimurium to enable visualisation of bacterial cells when used to infect mammalian cells. It was considered that the proposal be assessed as a Class 2 project, due to the strains of bacteria that were been used to harbour the plasmid vector. IC Occupational Health did not consider it necessary for Health Surveillance.
Project Containment

Laboratory Activities
- L2 Yes
- L3
- L4

Glass Houses
- L2
- L3
- L4

Growth Rooms
- L2
- L3
- L4

Animal Units
- L2
- L3
- L4

Large Scale Activities
- L2
- L3
- L4

Human Clinical Applications
- L2
- L3
- L4

Project Ref 31/01.4

Date Ackn'd 07/06/2001
CU2 Project Title ATTENUATION OF VIRULENCE IN SALMONELLA TYPHIMURIUM FOR USE IN CANCER THERAPY

Date Project Ceased 19/11/2002

Class
- Class 2

CultureVol
- Class2 1-50 litres

Consent Granted
- not applicable

Non-GMM

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Withdrawn N

Tick if notifying a connected programme of work N

Project Additional Information

Purposes of the contained use

The purpose of this work is to exploit the ability of salmonella typhimurium to accumulate and preferentially replicate within solid tumours. A variety of bacterial genes associated with virulence will be knocked out by homologous recombination and their effects on bacterial virulence and anti-tumour ability will be assessed. In addition, vectors carrying the reporter genes GFP and luciferase will be used to follow the location of the bacteria within host cells.

Recipient or parental organism

- S.typhimurium 12023
- S.typhimurium LT2
S. typhimurium SL1344
All three strains are commercially available and have been widely used in several laboratories for many years. They are classified as containment level 2 by the ACDP.

Host/vector system

<table>
<thead>
<tr>
<th>Host Cells</th>
<th>Vectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium</td>
<td>pGP704</td>
</tr>
<tr>
<td></td>
<td>pFPV25.1</td>
</tr>
<tr>
<td></td>
<td>pSP-luc+</td>
</tr>
<tr>
<td></td>
<td>pGP704</td>
</tr>
<tr>
<td>E. coli DH5a</td>
<td></td>
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</tbody>
</table>

The E. coli strain is a widely used, high attenuated commercial strain.

Origin & function

The vector pGP704 is a simple suicide vector, carrying an oriR for E. coli only. DNA from the parental organism will be amplified by PCR and inserted into the plasmid, using E. coli as a host organism. This will then be transformed into the parental organism and successful recombination events selected for (by antibiotic resistance marker). Genes to be targeted will be involved with LPS biosynthesis, auxotrophy or the type III secretion system. Inserted DNA will be heavily disrupted and so unlikely to be active. The disruption of the targeted genes is likely to reduce the pathogenicity of the parent organism. It will also make the host strain resistant to ampicillin, but suitable alternative therapies are available if necessary.

The vectors pFPV25.1 and pSP-luc+ are expression vectors for the genes encoding GFP and luciferase respectively. All vectors are defective for mobilisation so genetic transfer is unlikely.

Evaluation of foreseeable effects

The results of genetic modification will reduce the pathogenicity of the parental organism. No adverse effects are anticipated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is deactivated using 'Virkon' according to the manufacturer's instructions, it has been validated as being effective at this concentration within 5 minutes, see www.antech.com/virindex.html. All solid waste is first autoclaved at 134 degrees for 30 minutes in an autoclave on the 6th floor, MRC Cyclotron building before being removed for incineration. The autoclave is under a service contract and is serviced quarterly by arrangement with ICSM estates. Thermalogue strips will be included with every run. Steris verify kits used every 6 months. Records to be kept.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The committee recommended that this project be classified as class 2 due to the ACDP classification of the wild type S.typhimurium strains as containment level 2, despite the fact that created GMMs will be attenuated for virulence.

### Project Containment

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</table>

### Project Ref 31/01.5

- **Date Ackn'd:** 19/07/2001
- **Date Project Ceased:** 12/12/2005
- **Consent Granted:** not applicable

INVESTIGATION OF PROTEIN EXPRESSION OF SALMONELLA PATHOGENICITY ISLAND 2 IN SALMONELLA TYPHIMURIUM, USING A COMMERCIAL EXPRESSION SYSTEM (PET) AND GENE FUSIONS TO A PLASMID ENCODING GFP

- **Class:** Class 2
- **Culture Volume Class 2:** < 1 litre

- **Non-GMM Consent Granted:** not applicable
- **Project notified under transitional arrangements:** N

#### Purposes of the contained use

The aims of this project are twofold. Firstly, to exploit a commercially available E.coli based protein expression system to produce proteins, or parts of proteins, of Salmonella typhimurium. The resultant products will then be used as antigens to stimulate antibody production in rabbits.
Secondly, we wish to employ a strategy of engineering fusion genes to elucidate in which cell lines or stage of infection certain Salmonella typhimurium genes are expressed.

**Recipient or parental organism**

Salmonella typhimurium is ACDP Hazard Group 2, as it is a human and animal pathogen. Escherichia coli BL21 is also ACDP Hazard Group 2, as it may be pathogenic and is able to colonise humans.

**Host/vector system**

Salmonella typhimurium is ACDP Hazard Group 2, as it is a human and animal pathogen. Escherichia coli BL21 is also ACDP Hazard Group 2, as it may be pathogenic and is able to colonise humans.

**Origin & function**

Source of Host cells: E.coli BL21 commercially available (Stratagene UK), Salmonella typhimurium laboratory maintained strains.

Source of Genetic material (inserts): PCR from S.typhimurium genomic DNA template.

Source of Genetic material (vectors): pET 15b from Stratagene. pFPV25 is a gift from collaborator.

**Evaluation of foreseeable effects**

Both the GMMs are ACDP Hazard Group 2. In the case of GM S.typhimurium; the modification takes the form of the addition of an autonomously replicating plasmid which merely serves as a marker for gene expression. The plasmid encodes the green fluorescent protein marker and also confers resistance to ampicillin. As there are a variety of alternative chemotherapeutic agents available for use; this modification is thought to pose no increase risk to human health. The environmental survivability of the GM S.typhimurium is envisaged to be similar to the parental wild type strain. Likewise in the case of E.coli BL21, the modification takes the form of the addition of a self-replicating plasmid, upon which resides the cloned S.typhimurium gene. Expression is controlled and regulated by the addition of IPTG to growing cultures. In the absence of IPTG expression is minimal. The plasmid also confers resistance to ampicillin. As there are a wide variety of alternative therapies available, this modification is thought to pose no increased risk to human health. The environmental survivability of the GM E.coli BL21 is thought to be the same as the wild type.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Chemical Disinfection of liquid GM waste: 2% hycolin solution, as per manufacturer's recommendations, overnight exposure. 100% kill validated by periodic plating out of cultures after exposure to hycolin disinfectant.

Autoclaving of solid GM waste: Large autoclave; solid discard cycle 134 celsius for 30 minutes. 100% kill. Machine and cycle validated by thermograph and testing by manufacturer's engineer.
Autoclaving of liquid GM waste: Large autoclave; liquid discard cycle 134 celsius for 30 minutes. 100% kill. Machine and cycle validated by thermograph and testing by manufacturer's engineer.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The second paper entitled "Investigation of protein expression of Salmonella pathogenicity Island 2 in Salmonella typhimurium using a commercially available expression system (pET) and gene fusions to a plasmid encoded green fluorescent protein" from Professor Holden, this project was approved by the committee, with the following classifications: the Salmonella typhimurium work was classified as Class 2 Containment level 2, work involving E.Coli K12 strains were classified as Class 1, but will be carried out as Containment level 2 due to the level of facilities available, work involving B121 was classified as Class 2 Containment level 2. Again the Class 2 projects must be notified to HSE prior to any work commencing and a new GM Form A completed.

Project reference no. 01/264.

IC reference no. 00083.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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</thead>
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<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
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**Project Ref** 31/01.6

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<tr>
<td>19/07/2001</td>
<td>INSERTIONAL MUTAGENESIS OF THE VIRULENCE GENES OF SALMONELLA</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td>Class 3-4</td>
</tr>
</tbody>
</table>

02/03/2022 Page 1485 of 15326
# Project Additional Information

## Purposes of the contained use

The aim of this project is to engineer mutants of Salmonella typhimurium by insertional mutagenesis. The genes to be mutated encode proteins involved in different aspects of Salmonella virulence. The strains will then be tested for phenotypic defects in mammalian cell lines.

## Recipient or parental organism

The recipient microorganism is Salmonella typhimurium, which is a human and animal pathogen. It is placed by the ACDP in Hazard Group 2.

## Host/vector system

**Salmonella typhimurium (Host):** pGP704; pFPV25.1; pACYC184 (Vectors).

## Origin & function

- **Source of Host Cells:** Laboratory maintained strain. Originally obtained from the PHLS, UK.
- **Source of Genetic material:** inserts PCR from S.typhimurium genomic template.
- **Source of the material:** vectors pACYC184 and pCR2.1 are commercially available; pGP704 and pFP25.1 are gifts from colleagues.

## Evaluation of foreseeable effects

The GMM is likely to pose no increased risk to human health in comparison with the wild type parental strain. On the contrary, the GMM is likely to be attenuated in its ability to cause disease, since a putative virulence gene will have been mutated; and so in a functional sense, be disabled.

However, the insertional mutagensis protocol utilised in this project renders the GMM resistant to ampicillin. But there are a wide variety of alternative chemotherapeutic agents available should infection by the GMM occur.

Similarly, there is no increased risk to the environment posed by the GMM in comparison to the wild type. The latter is widespread in the environment. The environment survivability of the GMM is envisaged to be the same as the wild type, with the caviat that without appropriate selection pressure reversion to the wild type is likely. All work carried out under this project will take place in appropriate Containment level 2 premises, together with appropriate waste control and management systems.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

For the project entitled "Insertional Mutagenesis of virulence genes of Salmonella typhimurium" from Professor Holden, the project was approved by the committee with the following classifications, the Salmonella typhimurium work was classified as Class 2 containment level 2.

Project Containment

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</table>

Project Ref 31/01.7
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to identify, and characterise the function of Streptococcus pneumoniae genes required for virulence. This necessitates the construction of plasmid vectors containing segments of S. pneumoniae DNA, which are used to transform wild type S. pneumoniae to make GMM strains with specific gene disruptions. The phenotype(s) of these strains will then be assessed using animal and cell culture models of disease.

**Recipient or parental organism**

The recipient microorganism in Streptococcus pneumoniae, which is a human pathogen. It is placed by the ACDP in Hazard Group 2.

**Host/vector system**

**Host.** Streptococcus pneumoniae.

**Vectors:** pJPC9111, pJPC9112, pLS1GFP, pLS70GFPcat, pucMUT, pDE1, pEVP3, pACH74, p10701

**Origin & function**

**Source of Host Cells** - Smithkline Beecham; Prof. James Paton, University of Adelaide

**Source of Genetic material:** inserts - PCR of Streptococcus pneumoniae DNA

**Source of the material:** vectors - Prof. James Paton, University of Adelaide; commercial sources.

**Evaluation of foreseeable effects**

The GMM is likely to pose no increased risk to human health in comparison with the wild type parental strain. On the contrary, since a putative virulence gene will have been mutated the GMM is likely to be attenuated in its ability to cause disease; and therefore be functionally disabled. The insertional mutagenesis protocol utilised in this project renders the GMM resistant to chloramphenicol, spectinomycin, erythromycin or kanamycin. However, there are a wide variety of alternative antimicrobial agents available which are effective against S. pneumoniae infections should infection by the GMM occur. S. pneumoniae is essentially restricted to the human nasopharynx and there is no recognised environmental source. The environmental survivability of the GMM is envisaged to be the same as the wild type, with the caviat that without...
appropriate selection pressure reversion to the wild type is likely, and there is no increased risk to the environment posed by the GMM compared to the wild type.

All work carried out under this project will take place in appropriate Containment level 2 premises, together with appropriate waste control and management systems.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Chemical disinfection of liquid GM waste: 2% hycolin solution, as per manufacturer's recommendations, overnight exposure. 100% kill validated by periodic plating out of cultures after exposure to hycolin disinfectant.

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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

This project has been approved as a Class 2 project to be carried out under containment level 2 conditions.

**Project Containment**

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02/03/2022
The purpose of this work is to exploit the ability of Vaccinia Virus (VV) to accumulate and preferentially replicate within solid tumours. A variety of viral genes associated with virulence or evasion of the host immune system will be knocked out and their effects on viral tissue specificity and anti-tumour ability will be assessed. In addition, vectors carrying the reporter genes GRP and luciferase will be used to follow the location of the virus within host systems.

Vaccinia virus strains Western Reserve, Lister and Wyeth
All three strains are commercially available and have been widely used in several laboratories for many years. In addition, strains Lister and Wyeth have been used as Smallpox vaccines in millions of people worldwide. They are classified as containment level 2 by the ACDP.

Host/vector system
Host cells - E.coli DH5a, K12
Vectors - pUC18, pUC4K, pSJH7
The E.coli strains are widely used, highly attenuated commercial strains.

Origin & function
DNA from wild type VV strains will be amplified by PCR and cloned first into standard, commercially available plasmids (pUC18, pUC4K) where deletion mutants will be constructed. The modified DNA will then be cloned into the specialist plasmids (pSJH7) for transient dominant selection. These DNA manipulations will take place in commercially available E.coli strains, acting as a cloning vector. The plasmids will then be co-transfected into the cell lines TK143 or D98 along with a second vector containing an attenuated version of the wild type VV genome, such that viral replication can only occur following recombination of the two plasmids. Virus can then be
picked from plaques and successful recombination checked by PCR and Southern blotting. Genes to be targeted will be involved with the evasion of the host immune system (IFN binding proteins, anti-apoptotic proteins). Inserted DNA will be heavily disrupted and so unlikely to be active and the disruption of these genes is likely to reduce the pathogenicity of the host organism.

In addition, this same technique will be used to insert the reporter genes GFP and luciferase into the viral genome.

Evaluation of foreseeable effects
The results of genetic modification will reduce the pathogenicity of the parental organism. No adverse effects are anticipated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All liquid waste is deactivated using 'Virkon' according to the manufacturer's instructions, it has been validated as being effective at this concentration within 5 minutes, see www.antech.com/virindex.html. All solid waste is first autoclaved at 134 degrees for 30 minutes in an autoclave on the 6th floor, MRC Cyclotron building before being removed for incineration. The autoclave is under a service contract and is serviced quarterly by arrangement with ICSM estates. Thermalogue strips will be included with every run. Steris verify kits used every 6 months. Records to be kept.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The Committee recommended that this project be classified as class 2 due to the ACDP classification of the wild type VV strains as containment level 2, despite the fact that created GMMs will be attenuated for virulence.

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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
The study has been set up to assess the biological activity of different strains of attenuated HSV in different types of human tumours in the ex-vivo setting. The study involves injecting vectors directly into either discarded tumour blocks or purified tumour cells extracted from discarded tumour specimens. The purpose of the contained use is to prevent the vectors which are Class 2 GMOs being a hazard to workers.

HSV1 vectors deleted for the neurovirulence factor ICP34.5 and ICP47 with insertion of a human granulocyte macrophage colony stimulating factory (hGM-CSF) or green fluorescent protein (GFP) will be used. These vectors will be produced offsite. ICP34.5 deletion vectors are designed to replicate in tumours but not surrounding tissue (considerable literature demonstrates such a phenotype for HSV1 and 2 ICP34.5 deleted viruses). As ICP47 is an HSV gene that minimise immune responses to HSV infection, it's deletion results in enhanced immune response. ICP34.5 and ICP47 deleted vectors have been shown individually to generate a non-pathogenic, though still replication competent virus. The insertion of the hGM-CSF gene is intended to further stimulate the immune system in the clinical setting. The insertion of the GFP gene is for assessment of the biological activity.

There are two copies of ICP34.5 which are in identical portions of the genome, therefore the virus cannot exist in a stable form with 1 copy different to the other (ie only one copy disabled). This is because recombination with the region around the other copy would occur rapidly, this would either delete the inserted gene to give two copies of ICP34.5 or result in a virus with two insert genes in ICP34.5 ie a disabled virus. Therefore it is not possible for a virus not disabled but with a single copy of the transgene to have a stable existence in the environment.
Herpes Simplex Virus 1.

Origin & function

hCM-CSF gene was cloned from an IMAGE clone 2340997/5808-K14 (UK HGMP Resource Centre). GM-CSF is a potent cytokine responsible for the differentiation and proliferation of dendritic cell precursors and therefore is a potent immune stimulator. Thus if it were to enter a human it would be anticipated to give an improved immune response against HSV.

GFP was purchased from Contech which is not biological active but is used as an aid to assess the activity of the vector in the ex-vivo setting.

Evaluation of foreseeable effects

The gene to be inserted is not anticipated to result in harmful effects.

The aim of this experiment is to evaluate the biological activity of herpes simplex vectors in different types of tumours and will provide invaluable information towards the setting up of clinical trials in the future.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste generated during the activity that has been in contact with the GMM will be treated with 2% freshly prepared bleach for a minimum of 2 hours. This broad-spectrum disinfectant is effective against herpes simplex virus at a 0.5% (w/v) concentration giving terminal disinfection after 10 minutes of contact. The waste is then double bagged, autoclaved and put in incineration bins which will be taken by contractors for incineration or microwaved.

All liquid waste will be treated with 2% freshly prepared bleach for a minimum of 2 hours and then discarded.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Teh GMSC opinion is that the viruses to be used are probably low risk Class 2.

Project Containment

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02/03/2022
The aim of the project is to define the role of a number of exotoxins of the pathogen Pseudomonas aeruginosa in invasion and penetration of the respiratory epithelium. We will construct mutants of this organism lacking specific toxins and investigate the effects of these mutations on invasion of the pulmonary epithelium. To confirm that the effects of a mutation are specific, we will also complement mutated strains with the wild type toxin gene.

Different strains of Pseudomonas aeruginosa will be modified to delete one or more of the genes encoding toxins secreted by the type 1 secretion system of this organism. Some of these strains will then be complemented with the wild type gene for one or more of these toxins. The changes to these microorganisms will be used to define the role of these exotoxins in causing human disease. No material will be introduced into the microbe that does not already exist in wild type strains of this microorganism. Selectable markers to be used will confer antibiotic resistance to the microorganism but this resistance already exists in the wild type organism and other antibiotics are available to treat these infections. The organism can survive in the environment but no enhanced danger is produced by the modification to be carried out in the project.
Host/vector system

Bacterial strains
E.coli K12 derivatives
E.coli SM10
Pseudomonas aeruginosa PAO1 and clinical strains

Vectors:
TOPO series vectors (Invitrogen)
pUCPneo pKS Km
pEX100T pJQ200SK
pUCP18
pSMC2

Origin & function

Genes to be deleted will be replaced by kanamycin resistance cassette from a Gram negative transposon (mTn5). Complementation of deleted genes will be with wild type toxin amplified from clinical strains of Pseudomonas aeruginosa. The genes to be deleted will be for exoenzymes S, T, U and Y. The toxins encoded by these genes are introduced into mammalian cells by the specialised type 111 secretion system. The toxins can only exert their effect when introduced into cells by this means; they are not toxic outside the cell.

Evaluation of foreseeable effects

The deletion of the exotoxin genes will remove known virulence factors of Pseudomonas aeruginosa, reducing its ability to cause disease. Restoration of these genes will increase the virulence of a particular strain that does not express these toxins, but only to the level of naturally occurring Pseudomonal strains. P. aeruginosa does not cause disease in healthy humans. It can cause pneumonia in patients with cystic fibrosis, those who are ventilated and in those with extensive burns. The modifications will introduce resistance to the antibiotic kanamycin into these organisms. Such resistance already exists in naturally occurring strains and other antibiotics can be used to treat infection. Although modified organisms could potentially survive in the environment, they would present no greater risk than naturally occurring P aeruginosa, which is widely spread in the environment eg freely growing in soil and water.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid GM waste will be treated with Hycolin 3% for 15 minutes which produces effectively 100% kill. This is monitored by quantitative plating of treated bacteria. In addition, both solid and liquid GM waste will be inactivated by autoclaving at 121 degrees C for 15 minutes. This provides 100% kill as evidenced by quantitative plating of treated bacteria.

Following autoclave treatment waste is then removed from the site by a contracted service, to be disposed of as clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The risk assessment was considered by the local genetic modification safety committee and approved.

Project Containment

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Project Ref  31/03.2

Date Ackn’d: 24/11/2003
CU2 Project Title: INVESTIGATION OF EVENTS INVOLVED IN THE DIFFERENTIATION OF CD8+ T CELLS DURING A PRIMARY IMMUNE RESPONSE.

Class: Class 2
CultureVolClass2: < 1 litre
Consent Granted: not applicable
Project notified under transitional arrangements: N

Withdrawn: N
Tick if notifying a connected programme of work: N

Historical Significant Changes: Transferred to GM77 on 03/02/2005

Project Additional Information

Purposes of the contained use:
Recombinant vaccinia virus will be grown and used in containment level 2 facilities with class II cabinets.
Recipient or parental organism

GMO: Recombinant vaccinia virus WR strain with disrupted TK gene and insertion of either influenza nucleoprotein or a peptide (366-374) from the influenza nucleoprotein.

TK disruption will attenuate pathogenicity of the virus

Insertion of influenza nucleoprotein or peptide will not have any foreseeable effect.

Host/vector system

Recombinant vaccinia will be cultured in vitro in TK143 cells.

Origin & function

Influenza nucleoprotein is an RNA binding protein that encapsidates the viral genome. It comes from influenza virus.

The influenza nucleoprotein peptide comprises amino acids 366-374 of the nucleoprotein and has no intended function. It serves as a peptide epitope presented by the MHC class 1 molecule Db. It comes from influenza virus nucleoprotein.

The F5 T cell receptor is a T cell receptor that recognises the influenza nucleoprotein 366-374 peptide presented by the MHC class 1 molecule Db.

Evaluation of foreseeable effects

Disruption of the vaccinia virus TK gene will be attenuate pathogenicity.

Insertion of the coding sequence for influenza nucleoprotein or the nucleoprotein peptide 366-374 into the vaccinia virus will allow for expression of the protein/peptide within cells of infected mice. This will stimulate a T cell response to the nucleoprotein.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory.

Solid waste: Treatment with 1% Virkon for minimum of 10 minutes. 100% kill. This has been validated on the Antec (Virkon) website. The product has been tested to be effective at 1% solution and 10 minutes contact time. The web site will be monitored for any changes in specification.

Solid waste will be placed in sterilin bags and put in specially designated grey boxes within the laboratory or cold room. When these are full they will be tied using a specially designated yellow tag and sent for autoclaving.

Solid waste will be autoclaved at 134 degrees centigrade for a minimum of 30 minutes. A 12 point thermocouple test is conducted every 6 months on a simulated worst case worst load to establish that an effective and validated sterilisation temperature and time is achieved in all areas of the load. An internal thermocouple monitors the chamber temperature on every run. The current autoclave program sets temperature of 134 degrees C for 30 minutes and this ensures that all parts of a worst case load will always achieve a validated lethal temperature for the microorganisms mentioned in this assessment.

Liquid waste: Treatment with 1% Virkon for minimum of 1 hour. 100% kill. This has been validated on the Antec (Virkon) website. The product has been tested to be effective at 1% solution and 10 minutes contact time. The web site will be monitored for any changes in specification.
Mouse bedding and cages: These will be removed for cleaning and decontamination by CBS staff. Bedding will be autoclaved at 134 degrees C for 30 minutes and disposed of via the clinical waste route. Cages will be autoclaved at 121 degrees for 15 minutes and then wasted for re-use.

Is an emergency plan required according to regulation 20? Y
If yes, tick to confirm that it is attached to this form Y
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
GM safety committee have approved the risk assessment.

Project Containment

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Project Ref 31/04.1

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Historical Significant Changes

02/03/2022
### Project Additional Information

**Purposes of the contained use**

Induce downregulation of selected genes in various Mammalian cells/Cell lines. Approach could potentially be applied to any gene of interest. In the first instance targets will be hypoxia regulated genes/Genes regulated by HIF (hypoxia inducible factor).

**Recipient or parental organism**

Various Mammalian cells and/or cell lines, primarily of human or mouse origin.

**Host/vector system**

Modified Lentivirus, produced with PLL3.7 vector containing ShRNA inserts. 
Inserts will be various small RNA stretches tailored to the gene to be downregulated. Lentivirus is a 3rd generation multiple vector system with multiple modifications to increase safety and ease of handling, ie

1. Packaging vector lacks both LTRs and has no viral packaging signal ( )
2. The following viral genes have been deleted from the packaging vector: env, tat, rev, vpr, vpu, vif and ref.
3. Rev is supplied in trans on a different vector (RSV-Rev).
4. The vector expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion) and expresses no viral gene products.
5. Envelope, in this case VSVG, is expressed on a separate vector.

**Origin & function**

Intention is to use Modified lentivirus to introduce interfering RNA into cells, leading to downregulation of Targeted Genes. No new genetic material will be introduced.

**Evaluation of foreseeable effects**

Up to 95% downregulation of targeted genes expressed by Mammalian Cells. Gene downregulation is specific dependant on the original ShRNA sequence inserted into the modified Lentivirus. No other effect are anticipated. Modified Mammalian cells are Non micro-organism GMOs. Cells are cultured only in a Class 2 suitable lab, with all relevant control procedures. No viable cells will enter the environment as all cells and Tissue culture waste will be decontaminated by an approved method. Decontamination of liquid and solid (ie flasks, pipettes etc) will be with a 1% virkon solution approved for chemical inactivation.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Chemical inactivation of all liquid and solid (flasks, pipettes etc) waste associated with culture of modified mammalian cells. Agent of choice is Virkon in a 1% solution overnight, giving an effective kill of 100% (independent tests by manufacturer). Monitored with continued reference to manufacturer guidelines and test against culture every six months.

Solid waste is disposed of after chemical treatment in yellow clinical waste bags, tied, labelled and disposed of by the clinical waste route.

Liquid waste is disposed of via the drains after chemical inactivation.

Autoclaving may also be used for solid waste. Autoclaving gives a temperature of at least 120 degrees for 30 minutes monitored by an internal thermocouple, giving an effective kill of 100%. Autoclave function is validated with a 12 point thermocouple every six months.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The classification of this GM project was queried by the GMSC and a virologist independent of the GMSC was consulted for further advice. An extract of the advice received is provided here: "the inhibition of gene expression using these vectors is very broad. This is usually not a problem but if inhibition is for say tumour suppressor genes then these vectors, which can infect human cells when Pseudotyped with a VSV0G envelope, could potentially be hazardous and be at a level higher than 1."

Project Containment

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<tr>
<td>L2</td>
<td>L3 L4 L2</td>
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Project Ref  31/04.2

Date Ackn'd  28/09/2004  Project Title  Induction and study of Coxsackievirus B3 (CVB3) myocarditis.

Class  Class 2  CultureVol  < 1 litre  CultureVolumeClass

02/03/2022  Page 1500 of 1532
Date Project
Ceased

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
The aim of the project is to induce and study inflammation in the heart using models of CVB3 infection. Live CVB3 will be generated from cDNA clones which contain the entire viral genome of the wild-type or mutated forms.

Recipient or parental organism
CVB3 is an ubiquitous picornavirus, a small single positive strand RNA virus. Infection is usually asymptomatic, transmission is mainly faecal-oral.
E coli is a gram negative bacillus that forms part of the normal gut flora.
Vero cell is derived from African Green Monkey kidney.
HeLa cell is derived from human cervix epitheloid carcinoma.

Host/vector system
Hosts:
E coli TG1
XL1
JM109

Eukaryotic cell line
HeLa cells
Vero cells

Vectors:
Bluescript
pGEM
pUC
m13
Lorist6
pSVN (previously generated from pSVA13 by insertion of a Not I linker in the blunter EcoRI site)
p2732B (a derivative of pBR322 containing the ampicillin-resistance gene, the plasmid origin of replication, and a cloning site array between two EcoRI site)

Origin & function

Origins:
Plasmids vectors are obtained commercially and from Enterovirus Research Laboratory, Department of Pathology and Microbiology, University of Nebraska Medical Centre, 986495 Nebraska Medical Centre, Omaha NE68 198-6495, USA and Imperial College London.

CVB3 genome cDNA derived originally from CVB3 isolated from infected human tissues eg Nancy strain are obtained Enterovirus Research Laboratory, Department of Pathology and Microbiology, University of Nebraska Medical Centre, 986495 Nebraska Medical Centre, Omaha NE68 198-6495, USA and Imperial College London.

Intended functions:
These genetic materials are used to generate live infectious CVB3 with genomic sequences identical to that of the original wild-type isolate or modified in a controlled manner. Briefly entire RNA genomes of CVB3 are cloned as infectious cDNA copies in plasmids. Disabled E. coli host is used for cloning and amplification of the recombinant plasmids. Transfection of eukaryotic cell lines are used to generate live infectious CVB3 with genomic sequences dictated by the cDNA insert in the recombinant parental plasmids. CVB3 recovered from the supernatant medium and cell lysates can be further propagated by CVB3 infection of eukaryotic cells, purified and kept as stocks. Wild-type and genetically modified CVB3 thus generated will be tested in established models of enteroviral myocarditis to assess phenotype, including changes due to particular sequence modification of the viral genome. Strategies for genetic modification will include site-directed mutagenesis and substitution of homologous regions of the genome (resultant cDNA genomic constructs verified by restriction endonuclease mapping and sequence analysis).

Evaluation of foreseeable effects

General human health and environmental hazards of CVB3
CVB3 is an ubiquitous picornavirus (commonly present in the environment), of which man is the natural host. It has been estimated that at least 70% of the human population has come into contact with the endemic CVB3. Infection is usually asymptomatic. Uncommonly, CVB3 infection may manifest clinically as aseptic meningitis, meningocerebralitis, paralysis (infrequent), pleurodynia, myo- or peri-carditis, upper respiratory illness and pneumonia, rash, hepatitis, conjunctivitis, undifferentiated febrile illness, severe systemic infection in infants and fatal neonatal disease. The disease is likely influenced by host and viral genetic/non-genetic determinants. Currently there is no specific therapy for CVB3 infection. There is no vaccine.

In addition to viral and host determinants (eg virulence of the strain, host immune status), animal data suggests that the higher the infecting dose the greater the risk of infection/severity of the disease. In man, CVB3 infects via the mouth or oropharynx. Incubation period is 7-14 days (range 2-35 days). The virus infects the mucosal tissues of the pharynx. Gut or both, finally entering the blood stream and gaining access to cells of the reticuloendothelial system and specific target organs such as the meninges, myocardium and skin. The virus can generally be recovered from the pharynx during the first week of illness and from the faeces for 1-4 weeks after onset of illness; they have been isolated from CSF, spinal cord, brain, heart, conjunctivae and skin lesions.

To date, laboratory acquired CVB3 infection in man has not been reported in the literature. Although the risk of transmission to the laboratory worker varies throughout the natural history of CVB3 infection in mice, at all times the risk of transmission to the laboratory worker is small, and reduced to negligible levels with routine laboratory practice eg. wearing personal protective equipments such as laboratory gown/coat, mask, gloves in the containment areas. Changing animal cages at least 3 days post inoculation to remove bedding contaminated with infectious animal excrements (+/- blood from fights) may further reduce the risk of transmission to the laboratory worker. Possible routes of transmission of CVB3 infection to the laboratory worker are: (i) needle-stick injury or splashes/aerosol of CVB3 inoculum; (ii) faecal-oral or accidental ingestion of infectious matter; and possibly (iii) bites from infected animals.

Mice are rarely susceptible to natural CVB3 infection (ie through the faecal-oral route). Thus CVB3 infection in mice in the laboratory is achieved by direct inoculation of the virus ip. Housing of CVB3 infected adult mice in the same case as non-infected adult mice showed no evidence of cross-infection by virus isolation, serology or tissue histology.

CVB3 is a small single positive strand RNA virus. It does not have a lipid envelope and is therefore resistant to ether and other water-immiscible solvents. CVB3 may
CVB3 is a small single positive strand RNA virus. It does not have a lipid envelope and is therefore resistant to ether and other water-immiscible solvents. CVB3 may survive at room temperature for days, this is prolonged to weeks when in faeces. However, it is rapidly inactivated by ultraviolet light, drying temperature (esp > 50C), Virkon, Cidex OPA High Level Disinfectant Solution and formalin. All infectious animal tissues retained will be rendered non-infectious with formalin before transported out of Hammersmith BSU. Movement of wastes containing viable CVB3 will be as per existing local protocol and outlined below:

Non-radioactive wastes containing viable CVB3/plasmids/E. coli
Sharps waste will be place in sharps bin. At the end of the experiment, the lid will be closed, the outside of the bin sprayed with 1% Virkon* and the bin disposed of via the local autoclave route at routine times. Tissues, paper towels, gloves and other non-sharps will be placed in autoclavable waste bags, sealed, the outside sprayed with 1% Virkon* and the bag disposed of via the local autoclave route at routine times and incinerated by waste contractor. Formalin fixed tissues are non-infectious and can be disposed as non-infectious biological wastes. Liquid wastes will be rendered non-infectious by mixing with Virkon, aiming for a final concentration of 2% Virkon in the mixture, allowed to stand for at least 10 min, and disposed down the laboratory sink with copious amounts of water.

Radioactive wastes containing viable CVB3/plasmids/E. coli
CVB3 biologically contaminated radioactive material should not be autoclaved as this will create radioactive aerosols. Soak CVB3 biologically contaminated radioactive waste in 1-2% Virkon for at least 10 min then dispose the liquid down the designated radiation sink, recording an estimate of the level of radioactivity disposed. Then double bag the well drained waste in Radiation disposal bags. Spray the outside of the bag with 1% Virkon,* and remove immediately to an appropriate radiation bin for solid waste disposal. Record the radioactivity levels of waste disposed and monitor all areas that have been in contact with the waste for radiation contamination. Clean as appropriate. Existing college safety rules will be observed.

Likely effects due to genetic modification of CVB3
The virulence of genetically modified CVB3 will be attenuated or no more severe than wild-type/parental strain because candidate sequences (eg determined previously by comparative nucleotide sequencing of wild-type, attenuated and revertant viruses) will be introduced into the wild-type sequence. Wild-type CVB3 (eg Nancy strain - originally isolated from a patient with febrile illness in Connecticut USA) is both pancreovirulent and cardiovirulent in mice. Genetically modified CVB3 can be non-cardiovirulent +/- non-pancreovirulent in mice. This is similar to known CVB3 strain isolates from human tissues. Virulence is assessed by dose-effect comparisons between wild-type/parental and modified strains, as well as changes in phenotype ie revertant or disappearance of cardiovirulence or pancreovirulence. Currently the virulence determinant region(s) of the viral genome is not well understood. The human health and environmental hazards of genetically modified CVB3 are not expected to differ significantly from natural CVB3.

E. coli obtained commercially are disabled strains. Plasmids do not naturally infect humans or bacteria.
The degree of kill which should be achieved by Virkon, Cidex OPA High Level Disinfectant Solution, formalin, or autoclaving is 100%. Process testing and monitoring measures will include regular renewal and dating of disinfectants, monitoring of autoclave print outs as well as maintenance and testing of safety cabinets and autoclaves. Routine decontamination of other laboratory equipment will be undertaken. Regular inspections and internal reviews will take place. Workers will be under appropriate health surveillance. Information, instruction and training will be in the form of attendance at College Safety courses, local induction and procedures training, and the use of Good Microbiological Practice. Procedures for containment work on ACDP Group 2 pathogens will be followed. This work will only be undertaken by trained workers or whilst supervised by competent persons.

*The outside of the waste bag/sharps bin is sprayed with 1% Virkon to remove any glove contamination during the process.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The GM Safety Committee asked for several minor points on the Risk Assessment to be clarified. All points have now been addressed.

**Project Containment**

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**Project Ref**  
31/05.1

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02/03/2022
### Project Additional Information

#### Purposes of the contained use

To study cellular immune responses. In their recombinant form vaccinia virus and adenovirus can be used in vitro as a vector for protein preparations in mammalian cells, studying protein functions or assessing protein cytotoxic T cell antigenicities. This will form the basis for the development of control strategies such as vaccines. The recombinant viruses are also useful vectors for delivery of genes in vivo for animal model studies.

#### Recipient or parental organism

K12 and B derivatives of Escherichia coli will be used as bacterial cloning hosts. These are disabled strains that cannot colonise the human gut and have a history of safe use. These hosts may be considered equivalent to ACDP hazard group 1.

293T, TK143. The mammalian cell lines used all can be regarded as low hazard for GM activities and, as hosts, are suitable for containment level 1 precautions. However for the purpose of COSHH risk assessment, any human or primate cells or lines that are not well characterised and authenticated (eg unscreened or primary material or from sources that cannot verify status) will be handled at Containment Level 2 with a microbiological safety cabinet.

#### Host/vector system

The vaccinia virus vector is based on the Western Reserve (WR) strain. This is a replication competent strain that is able to infect humans and cause disease. Genes are inserted into the thymidine kinase (TK) locus. The TK minus phenotype reduces the virulence of the virus in mice but no comparable data are available for humans.

The adenovirus Ad5 vector has a deletion removing most of the E1 region and as such is unable to replicate except in complementing cell lines such as 293 (a human embryonic kidney cell line that expresses the left 11% of the Ad5 genome). Gene inserts are put into the E1 site. Deletion of the E3 region allows insertion of larger sequences and also makes the virus less fit. E1 deleted hosts are regarded as unlikely to cause disease and assigned to ACDP hazard group 1.

Genes to be inserted into the Vaccinia viral genome will first be cloned into transfer vectors such as pSC11 and their derivatives under a synthetic, vaccinia derived early or early/late promoters. Followed by in vitro homologous recombination between a transfer plasmid and the wild type vaccinia in TK143 cells. Recombinant E1 deleted Ad5 adenoviruses will be produced by cotransfection of plasmids into 293T cells (E1 helper cell lines).

#### Origin & function

**Details of the inserted genes and gene sequences:**

1. From viral sources
   - I) Influenza A virus genes: fragments and full length proteins. They are coding for constitutive viral genes:
     - M1 matrix protein, major structural protein lies under lipid bilayer
     - M2 matrix protein acts as proton channel
NS1  non-structural protein, unknown function
NS2  non-structural protein involved in export of vRNA
NP  nucleoprotein, associates with RNA, aids replication and transcription
PB1  subunit of RNA polymerase which copies viral genome
PB2  subunit of RNA polymerase which copies viral genome
PA  subunit of RNA polymerase which copies viral genome
HA  haemaglutinin, surface glycoprotein
NA  neuraminidase, surface glycoprotein

ii) Dengue virus genes: fragments and full length proteins:

C    nucleocapsid protein

preM/M precursor membrane glycoprotein
E    envelope glycoprotein
NS1 non-structural protein 1, role in early RNA replication
NS2A non-structural protein 2A, binds strongly to the 3' untranslated region, NS3 and NS5
NS2B non-structural protein 2B, cofactor of NS3
NS3 non-structural protein 3, serine protease and NTPase-helicase
NS4A non-structural protein 4A, unknown function but binds strongly to most of the non-structural protein
NS4B non-structural protein 4B, unknown function
NS5 non-structural protein 5, RNA-dependent RNA polymerase

iii) HIV & SIV genes:

a) individual whole HIV or SIV proteins and their truncations:

gag group specific antigens - structural capsid proteins, RNA nuclear transport
pol enzymes - protease, reverse transcriptase
env envelope (gp41+gp120=gp160) - external viral glycoprotein
nef many functions - e.g. downregulation or upregulation of some specific cellular proteins
tat viral transcriptional transactivator
rev RNA transport and stability
vif promotes virion maturation and infectivity
vpr promotes nuclear localisation, inhibits cell division
vpu promotes release of virions, degrades some specific cell proteins
vpx SIV homologue of vpr

2. From various sources

i) CTL epitopes: CTL recognize and kill cells displaying on their surface 8- to 10- amino acid-long peptides derived from microbial proteins. To study T cell responses it is sometimes necessary to express target antigens by transfection into recipient cell lines. Another approach to minimize the amount of protein administered during vaccination is to engineer artificial proteins consisting of CTL epitopes. The sequences coding for short peptide epitope are derived from HIV, SIV, influenza virus or
expression of other proteins in vaccinia virus would also not be expected to affect host range or tissue tropism.  Similarly, the virus envelope during morphogenesis because of the unusual way in which vaccinia virus acquires its outer envelope from the trans-Golgi network.  Recombinant vaccinia virus has been widely used and there is no evidence that its tissue tropism or host range has been altered such that the expressed sequences will not be biologically active because, for example, they are synthesised as modified or disrupted coding sequences or the expression products will be rapidly degrade.

The insertion of gene sequences from mammalian sources and many of the genes from viral sources is not expected to affect the pathogenicity of the viral vectors.  However, some of the viral genes that will be expressed are known to modulate immune responses.  In the majority of cases the effect will be to upregulate the immune response resulting in a less virulent virus.  Some of the inserted sequences may downregulate the immune response.  Particular attention will be paid to ensure no gene sequences are expressed in vaccinia virus whereby it is reasonably foreseeable this would increase the virulence of the virus by downregulating the immune response.  For recombinant adenovirus, since this is a replication defective adenovirus, down regulation of the immune response does not present significant additional risk.  Adenoviruses have no mechanisms for cytolysis so viral particles remain cell associated and any effect would remain localized.

For the mammalian cell line and bacterial hosts no significant hazards have been identified.  Insertion of the foreign sequences into these is not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms.  Gene transfer is possible but unlikely to be hazardous.  The resulting GMOs are not expected to carry any additional risks compared to that of the un-modified recipients.

No hazards to the environment were identified for genetically modified bacteria and cell lines.  The E. coli recipient strains K12 and B derivatives are disabled and neither these nor cell lines can survive in the environment.  Expression of inserted genes does not present a hazard.  Since these genetically modified micro-organisms are non-colonising, and none of the inserted sequences would affect the level of risk, they would not be harmful to animals, plant or humans in the environment.

Adenoviruses are non-enveloped viruses that are relatively resistant to desiccation and can survive in aerosols.  Consequently the adenovirus may survive in the environment in the event of accidental release.  Recombination between modified and wild type adenoviruses could possibly occur, but because the foreign genes are inserted into the E1 deleted site it is highly unlikely that a replication competent virus carrying foreign sequences would result.  Further, there is no evidence that human serotypes can naturally infect animals and the recombinant viruses are not expected to have altered host specificity.  If the recombinant adenoviruses were to survive for a short time in the environment it is unlikely that these would cause harm.  Recombination between modified vaccinia and other poxviruses resulting in the transfer of foreign sequences is extremely low because there is no natural reservoir within the UK.

Vaccinia virus is able to survive in the environment for some time.  The natural host of vaccinia is unknown but it is able to infect a wide range of mammalian species.  Of particular note regards environmental risks is that there was no colonisation of domestic or wild animals in the UK following the widespread administration of vaccinia virus to humans in the smallpox eradication campaign, nor has the recombinant vaccinia virus that has been used to control rabies in parts of Belgium or France led to colonisation of wild species.  There is no reason to believe that the recombinant vaccinia viruses produced in this work are any more likely to survive and become established in the environment than the wild type virus.

There is no intention to modify the tissue tropism or host specificity of the viral vectors and it is thought unlikely that any of the modifications would affect these.  In any case the recombinant adenoviruses are replication defective.  Recombinant vaccinia virus has been widely used and there is no evidence that its tissue tropism or host range has been altered by expression of any membrane protein from any other virus.  This is probably because foreign membrane proteins do not get incorporated into the vaccinia virus envelope during morphogenesis because of the unusual way in which vaccinia virus acquires its outer envelope from the trans-Golgi network.  Similarly, the expression of other proteins in vaccinia virus would also not be expected to affect host range or tissue tropism.
The insertion of gene sequences from mammalian sources and many of the genes from viral sources is not expected to affect the pathogenicity of the viral vectors. However, some of the viral genes that will be expressed are known to modulate immune responses. In the majority of cases, the effect will be to upregulate the immune response resulting in a less virulent virus. Some of the inserted sequences may downregulate the immune response, this is discussed further in the risk assessment. For recombinant adenovirus, since this is a replication defective adenovirus, down regulation of the immune response does not present significant additional risk. Adenoviruses have no mechanisms for cytolysis so viral particles remain cell associated and any effect would remain localised.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste, non-disposable plastics and culture vessels - chemical inactivation in 1% Virkon (final concentration) for a minimum contact time of 30 minutes following manufacturers validation data against the organism in use. After 100% inactivation liquid waste can be disposed of to the drains.

Solid phase GM waste, disposable plastics, pipettes and pipette tips, animal bedding - double bagged in autoclave bags, autoclaved (134 degrees C for 30 minutes). Autoclaving is validated by using indicator strips and chart data from the autoclave. Autoclaves are validated by annual 12 point thermocouple testing. After autoclaving all solid waste is incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The GM safety committee agreed with the containment measures and classification assigned to the project.

Project Containment

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**Project Ref 31/06.1**

**Use of a lentivirus-based vectors for the transfer of complementary DNA into human and murine cells.**

**Date Ackn'd**: 01/02/2006  
**CU2 Project Title**: Use of a lentivirus-based vectors for the transfer of complementary DNA into human and murine cells.  
**Date Project Ceased**:  
**Class**: Class 2  
**Culture Vol**: < 1 Litre  
**Non-GMM**: Not Applicable  
**Consent Granted**: Not Applicable  
**Project notified under transitional arrangements**: No

### Historical Significant Changes

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

The proposed lentiviral vector is pHR-SIN-CSGWdlNotl that contains the WPRE sequence which has been implicated in the development of liver tumours in mice in a pre-clinical study using lentiviral vectors (SACGM Information Note, November 2004). Therefore the pHR-SIN-CSGWdlNotl vector will be used under conditions of Class 2 containment, as recommended by the SACGM Secretariat.

**Recipient or parental organism**

pHR-SIN-CSGWdlNotl will be expanded in the bacterial strain E. coli-derived Top 10F: F’(lacI q Tn10 (tetR)) mcrA. (mrr-hsdRMS-mcrBC) O80lacZ.M15.lacX74 deoR recA1 araD139.(ara-leu)7697 galU galK rpsL endA1 nupG. This is a highly disabled laboratory variant of E. coli. The presence of pHR-SIN-CSGWdlNotl is not expected to modify the properties of the host and thus pose increased risk to humans or environment. In addition, bacteria carrying pHR-SIN-CSGWdlNotl cannot survive outside the conditions offered by the optimum culture medium.

**Host/vector system**

The vector system pHR-SIN-CSGWdlNotl is a lentivirus-based plasmid that when co-transfected with gag-pol and pMDG VSC-G expressing plasmids in packaging cell lines such as 293T generates VSV-G pseudotyped virions released in the supernatants that are capable of infecting human as well murine cells. Primary human and murine cells of haemopoietic and epithelial origin as well as a variety of cells lines will be subjected to infection.

**Origin & function**

HR-SIN-CSGWdlNotl, gag-pol and pMDG VSC-G plasmids were from UCL, London. cDNAs amplified from human and murine cells will be inserted into pHR-SIN-CSGWdlNotl and expressed in human and murine cells respectively. The functions of these cDNAs include transcription regulation, promotion of haemopoiesis, presentation of antigens to immune cells.
Evaluation of foreseeable effects

In some cases, lentivirus-infected human or murine cells will be transferred to murine recipients by parenteral route. However, direct injection of the virus or the vector will not be undertaken. Mice treated with lentivirus-infected human or murine cells will be maintained according to the procedures and rules of the local Biosafety Unit.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All the procedures with regards the handling of GMM are Containment level 2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated with Virkon 5% overnight and then discarded in the sink. Solid waste: immersion of all solid materials used (tips, pipettes, glassware) into Virkon 5% overnight before disposal of solid waste into GM designated bins and autoclaved at 134 degrees Celsius for 30 min. Overnight treatment with Virkon 5% is expected to kill 100% of GMM (manufacturer's warranty).

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The proposal was considered satisfactory with no alterations required.

Project Containment

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<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Project Ref 31/06.2

Date Ackn'd 02/03/2022  CU2 Project Title

Class CultureVolClass2 CultureVolumeClass3-4
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to prepare recombinant retroviruses and lentiviruses to transduce human or mouse muscle precursor cells with high titre, helper-free recombinant lentivirus or retrovirus coding for nuclear or cytoplasmic GFP, or for nuclear or cytoplasmic B-gal, or proteins such as Pax3, Pax 7, or for therapeutic proteins, such as dystrophin, or for U7 constructs designed to skip specific exons of the dystrophin gene. These transfected cells will either be used for in vitro studies and/or be implanted into mouse muscle. The contribution of marked cells to the host muscle will be analysed on sections of injected muscles, or in cells cultured from injected muscles. The virus may be used to mark and track cell clones using the sequence and/or integration sites of the virus as well as the GFP or B-gal reporter.

Other experiments, with vector/host systems of similar or lesser risk, may also be performed, eg. Retrovirus or lentiviruses coding for a growth factor, or viruses coding for genes such as CD34, c-met, or microRNA's involved in myogenesis, eg. miR181, miR1, miR-133 (and others, as they become available).

**Recipient or parental organism**

1. The lentiviral or retroviral vector plasmids will be grown up in bacteria from glycerol stocks and may be purified by caesium choride/ethidium bromide gradient.
2. The vector plasmid, packaging plasmid, and envelope plasmid will be transfected into a packaging cell line, eg. Human kidney cell line. The cell line that we will most commonly use is 293T, a human renal epithelial cell line which is transformed by adenovirus E1A gene product, which also expresses SV40 large T antigen, allowing episomal replication of plasmids containing the SV40 origin and early promoter region. Such producer cells are capable of producing helper-free ecotropic and amphotrophic non-replicating retroviruses or lentiviruses.
3. Alternatively, retroviral or lentiviral producer cells will be grown up in tissue culture and supernatent medium containing helper-free recombinant virus will be collected.
4. Viruses may be concentrated by centrifugation and may either be used immediately, or frozen in aliquots for later use.
5. Retroviruses or lentiviruses will be used to infect mouse or human cells, stem cells of blood, bone marrow or other origin, in tissue culture.
6. In some experiments, we will obtain lentiviral or retroviral vectors from collaborators, eg. The lentivirus coding for U7 and antisense oligonucleotides designed to keep dystrophin exons. These will be used to infect muscle or stem cells in tissue culture.

**Host/vector system**

Human 293 T cells
Phoenix 293 T cells
Bosc 23 cells
Mouse muscle cells
Human muscle cells
Human or mouse stem cells, e.g. Bone marrow, mesenchymal stem cells, AC 133+ cells

pMSCVpuro (modified to add IRES-eGFP and Pax7)
pRRL.ppt.hPGK.GFP.pre.sin-18 lentivirus
pMFG nls LacZ
AmpliGPE LNPOZ
pSUB201 containing IGF1 driven by the MLC1/3 promoter
pMCSext-IRES2-eGFP
pMSCVpuro (modified to add IRES-eGFP and Pax7)
pMDLg/pRRE.REV 3rd generation core packaging
pRR1-SIN-18PPTpgk.GFPW self-inactivating (SIN) transfer
pMD2VSV.G envelope plasmid
pCMVdR8.74
pRSV-Rev
pCCL.hPGK.nlsLacZ
pRRL.CMV.LacZ

Origin & function
Plasmid constructs, and producer cells have been obtained either from collaborators or from commercial suppliers.
The expressed genes include marker genes, proteins such as Pax3, Pax7, therapeutic proteins, such as dystrophin, U7 constructs designed to skip specific exons of the dystrophin gene, growth factors, CD34, c-met, microRNA's involved in myogenesis.

Evaluation of foreseeable effects
Lentiviral or retroviral vectors coding for marker genes (eg. GFP, b-gal), or proteins such as Pax3, Pax7, or for therapeutic proteins such as dystrophin, or for U7 constructs designed to skip specific exons of the dystrophin gene, or coding for a growth factor, or viruses coding for genes such as, CD34, c-met, or microRNA's involved in myogenesis, eg. miR-181, miR-1, miR-133.
These vectors will be used to infect mouse or human cells in tissue culture, so that the gene of interest is expressed in the transduced cells and their progeny.
Retrovirally and lentivirally transduced cells and their progeny, will express the gene of interest. In the case of a marker gene, this may be used to follow the fate of cells following their transplantation in vivo. As these viruses are integrated into the host genome, the insertion site may be used to determine the fate of individual clones of cells.
When a potentially therapeutic gene has been introduced into muscle or other stem cells, these will contribute to regenerated muscle fibres following their transplantation into host mouse muscles. We will then determine whether the therapeutic gene is expressed within muscle fibres and whether it has a beneficial effect.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Culture media - Virkon, presept or Klercide 5 will be applied via spray or direct mixing/aspiration. Contact time is of 5 minutes minimum. Dilutions are made following manufacturer's directions (Virkon 1%, presept 10,000ppm, Kercide neat). 100% kill achieved, no viable cells present when disinfected cultures returned to incubator. Disposal via laboratory sink.

Solid waste - cell pellets, tissue culture plastics, scalpels, syringes - Autoclave 134 C for minimum 30 minutes. Internal temperature continuously monitored and displayed on chart recorder annual 12-point termocouple test. Disposal via clinical waste after autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

This activity was considered by the GMSC on 29 March 2006 and they are content with the proposal and classification of the activity - please refer to the attached approval form from the GMSC.

Project Containment

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<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 31/06.3

Date Ackn'd 22/12/2006

CU2 Project Title Use of Listeria DPL-1942 (ova) to examine the CD8 T cell response to a bacterial

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

22/12/2006
Listeria monocytogenes DPL-1942 (ova) was constructed and described by Pope, C., et al. in 2001: A rLM (rLM-ova) was constructed that expresses a secreted form of OVA and an erythromycin-resistance marker. An Ag expression cassette was constructed that consists of the entire coding sequence of OVA fused to the signal sequence and promoter of the hly gene and an erythromycin resistance gene for selection. The Ag expression cassette was introduced into the Listeria, and double-crossed into the Listeria chromosome by homologous recombination. Integration of the Ag cassette into the LM genome and secretion of the OVA fusion protein by rLM-ova were confirmed by PCR and Western blot analyses, respectively. "-- Pope, C., et al. 2001. "Organ-specific regulation of the CD8 T cell response to Listeria monocytogenes infection." J. Immunol. 166, 3402-9.

Origin & function

Chicken ovalbumin is a major component of the chicken egg and is widely used in many experimental systems. The antigenic epitopes of ovalbumin have been very well characterized and many experimental reagents are available for its study.

Evaluation of foreseeable effects

This DPL-1942 strain is attenuated (ActA-deficient). The addition of the chicken ovalbumin gene does not increase its virulence or pose any additional risks.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid and solid waste will be disinfected with a minimum of 2% Virkon for at least 30 mins. Pipettes, tissue culture flasks, gloves and paper towels will be autoclaved at 134°C for 20 min. at a full cycle time of 1hr 44 min.

Is an emergency plan required according to regulation 20? \( \text{N} \)

If yes, tick to confirm that it is attached to this form \( \text{N} \)

Tick to confirm that you have attached a risk assessment to this form \( \text{Y} \)

Tick if you are claiming exemption from disclosure for section of the risk assessment \( \text{Y} \)

Please enter comments on the GM safety committee on the risk assessment
The GMSC agreed with the classification of this project as Class 2 Containment Level 2.

Project Containment

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Project Ref 31/09.1

<table>
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<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Class CultureVolClass2 CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>08/07/2009</td>
<td>Generation of bioluminescent and/or fluorescent Staphylococci in order to study strain transmissibility, colonisation and characteristics of pathogenicity.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Date Project Ceased

Withdrawn \( \text{N} \)

Tick if notifying a connected programme of work \( \text{N} \)

Historical Significant Changes
The aim of the work is to better understand the transmission and colonisation plus pathogenicity of pathogenic Staphylococci using new models of infection (both in vitro and in vivo). This project aims to generate bioluminescent and/or fluorescent derivatives of laboratory and clinical isolates of Staphylococci e.g. S. aureus. The reporter genes (including native and synthetic codon-optimised luciferases derived from bacteria, insects and marine crustaceans and fluorescent proteins) will be carried either on replicative plasmids or integrated into defined regions of the Staphylococcal genome. The purpose of the genetic modification is simply to create strains that can be more easily tracked in these models.

The following are laboratory strains used in the initial genetic manipulations: Escherichia coli (Novoblue, DH5alpha, and similar).
S. aureus RN440 (reference/lab strain)
S. aureus CYL316 (RN440 strain harbouring the pYL112619 vector). CmR
S. aureus CYL557 (RN440 strain harbouring pLL2787 vector). CmR
Clinical isolates of S. aureus are then transduced with the bioluminescence enzymes/fluorescent protein containing plasmids. The majority of these clinical isolates will be methicillin sensitive (MSSA) strains (<1% will be methicillin resistant/MRSA). GMMs derived from the most virulent parental strain will be the most virulent e.g. clinical strains from patients with bacteremia. The inserted reporter genes encode for enzymes that catalyse bioluminescent reactions (such as from the firefly or luminescent bacteria), or for fluorescent proteins (such as from sea pansies and jellyfish). Therefore no GMM is expected to be more virulent than the parent strain. Genetically-modified strains of S. aureus obtained from collaborating laboratories will not be any more virulent than the naturally-occurring parent strains thus there is no extra perceived hazard from use of these GMMs. Furthermore, the antimicrobial resistance patterns of these previously-modified host strains are limited to the same range antimicrobial selection markers as the GMMs described in this current proposal.

S. aureus isolates are as indicated above. The vectors being used (with their resistance markers indicated) are:
pCL55 – AmpR, CmR. S. aureus integrative vector; possesses the Staphylococcus bacteriophage L54a int gene and attP attachment site.
pCL25 – TetR, SpecR. S. aureus integrative vector; possesses the L54a attP site.
pLL29 – TetR, SpecR. S. aureus integrative vector; possesses the L54a and φ11 attP sites.
pMK4 – AmpR, CmR. S. aureus replicative plasmid.
pYL112619 – CmR. Possesses the L54a int (integrase) gene.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All mouse experiments are conducted in the facilities of the central biomedical services (CBS) at the Hammersmith or South Kensington campuses of Imperial college. There is already a separate risk assessment for use of bacterial pathogens in CBS Hammersmith that encompasses work with S. aureus. Transport of GMOs between laboratory and CBS- secondary containment to be conducted. Bacteria transported in sealed tubes, not in syringes.

Theoretical risk of aerosolisation when drawing up or administering bacterial suspension in CBS (though this risk is also encountered in the normal laboratory environment). Needles are not attached until after syringe is filled. The CBS experimental mouse area has been approved for administration of bacterial GMOs im, ip, iv as the risk to other personnel sharing the procedure room is minimal, especially as all personnel are wearing FFP2 or FFP3 masks and PPE. Needles are not attached until after syringe is filled. The CBS experimental mouse area has been approved for administration of bacterial GMOs im, ip, iv as the risk to other personnel sharing the procedure room is minimal, especially as all personnel are wearing FFP2 or FFP3 masks and PPE.

Intranasal infections will not use sharps and will be conducted in the containment area of the CBS to facilitate autoclaving of cages, as there is a theoretical risk that cage bedding is contaminated. Infections will be initiated in a safety cabinet. Mice infected experimentally with S. aureus either clear the bacterium completely or die. One exception is the case of nasal colonisation, where staphylococci may be carried in the nasopharynx for up to 42 days without illness. If such a mouse escaped, there is a theoretical risk that the GMO could spread into the environment. However, this risk is considered to be extremely small, as (i) indefinite carriage does not occur and (ii) the bacterium is a human pathogen and does not spread naturally among other animal species (iii) escape is a remote possibility. Rarely, infections in bacteria may be inoculated into the skin via a bite from a bacteremic animal, potentially causing human skin infection needing antibiotics or via respiratory secretions (in the case of nasal carriage experiments), causing human nasal carriage. The risk of mouse to human respiratory transmission is considered to be significantly less than the usual risk of human to human transmission of wildtype S. aureus. All handlers will be notified of the infected status of the animals. Any bite or scratch will prompt an accident report and must be discussed with occupational health with regard to antibiotic prophylaxis; resistance patterns of strains will be notified to Occupational Health and kept on record. Masks will be worn to protect staff from the theoretical risk of respiratory transmission in the case of pharyngeal carriage experiments, and mice with pharyngeal infection will be housed in filter cages, as advised by Occupational Health/Safety Office.

There are new cage washing facilities in the west wing of the CBS. BD disposes of bedding into orange bags for alternative technology disposal. All incineration is conducted off-site at Hammersmith. Infected cadavers from the CBS are disposed of in labelled orange bags for immediate incineration via the designated fridge in containment.

For nematode (Caenorhabditis elegans) killing assays this work will be carried out in the designated class II laboratories (BS4+5 of the Commonwealth building, Hammersmith campus) in class II safety cabinets. All petri dishes containing GMM infected C. elegans are wrapped in parafilm to prevent accidental opening of dishes outside containment. Infected C. elegans are disposed of by autoclaving as per for solid bacterial waste.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid bacterial waste (e.g. agar plates, pipette tips, falcon tubes) are placed in clear autoclavable bags. These are then loosely folded when 1/2 full and placed in a grey box used for autoclaving. A blue tag is used to temporarily lock the box, which is transported down a corridor to the room housing the primary autoclave. Autoclaving is performed at 134°C, 30 minutes (waste disposal cycle). All runs are recorded and kept in log books. There is an annual validation of the waste cycle by 12 point thermocouple testing as part of the maintenance schedule. Autoclaved waste is disposed via biological waste route on campus.

Liquid bacterial waste (e.g. centrifugation supernatant, culture media) is treated with Virkon or Trigene/Microsol at 1% for 30 minutes. Efficacy data sheets are obtained from suppliers. All inactivated liquid waste is poured down the drain.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y
The GM Safety Committee made the following comments:
- Old version of form has been completed. Please fill in new version of form.
  1.21: Transfer information provided in 2.3.2.1 to fully answer this question.
  1.22: Add ‘with known antibiotics used...’ to the strain Clinical isolates of S. aureus. Also add more information from 2.3.2.1 such as details for pathogenicity of main isolates of interest i.e. in comparison to the wild type and lab strains.
  2.1.1.4: Answer: n/a
  2.1.1.5: Typo in line 5: Italy not Ialy
  2.3.2.1: Remove information about in vitro and in vivo and transfer to Part 1 of the form.
  2.3.2.2b: What other Class 2 organisms are being worked on in the vicinity?
  2.3.3.1a: The centrifuge tubes should really not be opened outside of a MSC.
  2.3.3.3.a: Add in brackets: Total 200mL.
  2.3.3.3b: As this is a class 2 project, the centrifuge should be opened in the MSC if a sealed rotor is not being used.
  2.3.3.6: CBS only use orange bags at present. New cage washing facilities in West Wing, BD disposes of bedding into orange bags for alternative technology disposal. All incineration is conducted off-site at Hammersmith.
  2.3.3.7: State source and type of human cells and purpose of the experiment.
  2.3.3.9.c: Delete answer and replace with: ‘None’
  2.3.4.2: ‘Local CoP is prepared...’
  3.1.1.b: Confirm that all solid waste from CBS (including cadavers and cage bedding) is being transported back to 8N1 for autoclaving as stated on form.
  3.2.b: GMM’s are being transported to CBS as stated in part 2. Please detail containment measures for this. Also cadavers and bedding being transported to 8N1 (see above), please detail containment measures for this. Also transport from TC labs to autoclave.
  3.3: Total is 200 mL
  3.6.1: Add the CID numbers of personnel.
  3.6.2: Cleaners, maintenance staff for labs (EVS etc).
  3.7: PI to check box.

Comments: C. Bateman gave a brief summary of the project. CR emailed his comments to be included. YH confirmed that this proposal will have to be notified to the HSE.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

All of the comments have been addressed and the required changes incorporated into the risk assessment attached to this form.

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**Project Ref**  31/11.1
### Project Additional Information

#### Purposes of the contained use

The research group is interested in studying how the immune system responds to infection with different pathogens. This is of particular importance as many patients with chronic lung damage are susceptible to infection and as such have a worse prognosis. Lung associated pathogens (Streptococcus pneumoniae and pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa and Haemophilus influenzae, Aspergillus fumigatus) will be transformed with a vector containing the operon for a luciferase or fluorescent protein. This will label the pathogen to allow for imaging. Other vectors and/or imaging luciferases / fluorescent proteins may be used.

#### Recipient or parental organism

- **Streptococcus pneumoniae and pyogenes**
- **Staphylococcus aureus**
- **Pseudomonas aeruginosa**
- **Haemophilus influenzae**
- **Aspergillus fumigatus**

#### Host/vector system

- pAUL-A Tn4001 transposon encoding bacterial luxCDABE

Alternative vector systems and/or luciferases and/or fluorescent proteins and/or labelling techniques may be used.

#### Origin & function

Lung associated pathogens (Streptococcus pneumoniae & pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenzae, Aspergillus fumigatus) will be genetically modified to express a single copy of bacterial luciferase (luxCDABE) from Photorhabdus luminescens. This will lead to the pathogens to bioluminescence.
or "glow" to allow for imaging. Alternative vector systems and/or luciferases and/or fluorescent proteins and/or labelling techniques may be used.

### Evaluation of foreseeable effects

The pathogens used in this project can potentially infect people. However, insertion of bacterial lux operon into these pathogens is not linked with increased virulence and should not pose any additional hazard to the user. Precautions must be taken when culturing the microorganisms. These issues have been addressed with risk assessments (GMIC-1375) to minimise potential contamination and/or hazards to the user.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| None |

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid cultures for GMMs will be decontaminated with virkon or a similar agent overnight before disposal down the drains. Plating out decontaminated liquid will confirm the GMMs have been killed. All solid waste (bacterial plates, tips, stripettes, sharps that have come into contact with GMMs) will be autoclaved within the department before disposal by incineration. Autoclaves are maintained and monitored routinely. All waste management has been covered by risk assessment (GMIC-1375).

13. * Is an emergency plan required according to regulation 20?  
   Yes No

Public Public Register Public Register  
Register

Please enter comments on the GM safety committee on the risk assessment

Y  N  
Tick if you are claiming exemption from disclosure for section of the risk assessment

N
This project has been reviewed by and approved by the Imperial College genetic modification safety committee. Following the discussion of your proposal entitled "Infection with bioluminescent pathogens", GMIC-1375 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

**Part 1: GM Centre Number is GM31**
1.23: Change answer to ‘Yes’ and the reference number to the Form B is GMIC-01266.1
1.4: Containment level 2

**Part 2: GM Reference number required**
2.3.3.1.a: Change answer to ‘Yes’
2.3.3.a: Include all lab location/rooms
2.3.3.3.c: Room number should be 8S5, not 9S5.
2.3.3.3.d: Include final concentration in this paragraph.
2.3.3.5: Please state that the biological hood is a Class 2 Microbiological Safety Cabinet
2.3.3.8.c: Answer question.
2.3.4.2: Local Code of Practice has to be submitted to be sent to the HSE. Answer “Yes”.
2.3.5: To be completed by Occupational Health.
2.3.6: Complete this section.
3.1.1.a: Typo, ‘virkin’. Reduce 24 hours.
3.31.a: Remove hatch in ‘Low risk’ box
3.31.c: Remove ‘yes’ by inhalation
3.32.b: Explain that the 500ml culture volume will be in smaller volumes of .XXml. Please specify volume.
3.42: Typo ‘infec’
3.5.b: Typo ‘wiht’
3.6.1: Include all labs and 4S1
3.7.1: Move the names from 3.7.2 to this section.
3.7.2: Add the names of the CBS Staff from Professor Sriskannd in this section.
3.8: PI to complete this section.

Comments: DC gave a brief description of the project.

MJ queried if they were using normal human pathogens.

AS confirmed they were original wild type pathogens that were already modified and from a company.

AC asked about where the imaging would take place.

DC stated that she would like the IVIS to be located in a specialist lab. A room number will be provided.

MJ requested a flowchart outlining the experimental process from beginning to end. This should make it clear which parts of the work are conducted in different labs. It should show techniques such as imaging, transport between locations and culture work.

The Committee also suggested that they add other strains and organisms that may be used in future such as Aspergillus fumigatus to the risk assessment before it is submitted to the HSE to avoid charges associated with significant changes.

Please provide SOP’s and COP for work in the 8th floor culture labs

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE

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<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022
The aim of the project is to identify DNA damage and resulting genomic lesions that are associated with successful human malignancy.

Recipient or parental organism

a) Non-invasive/non-pathogenic TOP10/DH10B bacteria cells (E.coli, Invitrogen) will be transfected with expression vectors during vector design and cloning (non-hazardous GMMs).

b) Expression vectors will be purified from cells generated in a), and BOSC/293T and GP2-293 cells (human) will be transfected with these expression vectors together with helper plasmids to produce mouse-specific retrovirus. Mouse bone marrow cells and spleenocytes will be then infected with viral supernatant from these cultures to induce expression of selected genes.
Retroviral mammalian expression vectors (MuLV related or MSCV related backbones) deficient for GAG and POL genes (replication-incompetent, infection-incompetent). These vectors will be used together with ecothrophic pECO vectors, which allow infection of mouse cells but NOT human cells. To produce virus, these will be introduced into a packaging cell line (293T cell derivative) expressing GAG and POL. Resulting virus produced by these cells is infectious to mouse cells but NOT to human cells and replication incompetent.

a) pRetroX-TRE3G (Clontech/Takara) system: the pRetroX-TRE3G Tetracycline-inducible expression vector and the pEco helper plasmid for packaging into mouse-specific retroviral particles. These vectors will be used to induce oncogene expression in mouse bone marrow B cells and to further induce mouse leukemia in vitro. These are derived from MuLV.

Please note: Produced mouse leukemia cells are not infectious and do not represent a potential harm for human workers. Furthermore, oncogene expression in this system requires the further addition of the tetracycline derivate TetEXPRESS and is therefore tightly controllable by the investigator.

b) pSingle-tTS-shRNA vector (Clontech/Takara) system: pSingle-tTS-shRNA vector and the pEco helper plasmid as described above. These vectors will be used to induce shRNA expression in bone marrow B cells, which leads to a downregulation of a yet undefined target gene.

Please note: Also here, the artificial expression of the shRNA in target cells is dependent on the presence of the tetracycline derivate Doxycycline and is therefore tightly controllable by the investigator.

c) pMX-IRES-GFP expression vector (DF Robbiani, Nussenzweig Laboratory), including pEco Vector helper plasmid (DFR) for packaging into mouse-specific retroviral particles. These vectors will be used to induce oncogene expression in mouse bone marrow cells, which are already infected with the expression vectors described in (b). Its backbone is derived from murine stem cell virus (MSCV).

d) pRRL.PPT.T6-GFP-PGK-M2-pre, pRRL.PPT.T11-GFP-PGK-M2-pre are inducible expression vectors that might be used for oncogene expression as (a) and that are a kind gift from the laboratory of Dr A. Karadimitris.

e) TRMPV is an inducible shRNA expression vector that is from Addgene and a kind gift from the laboratory of Dr A. Karadimitris. It might be used as in (b). Its backbone is derived from murine stem cell virus (MSCV).

Please note: As the viral particles are mouse specific and replication incompetent, these infections do not bear any harm for human workers. Similarly, the mouse leukemia cells produced in vitro are not infectious and do not represent any harm to human workers.

**Origin & function**

The vectors described will be used to induce the expression of BCR-ABL1, v-Abl, RAS and C-MYC (and potentially E2A-PBX1 and TEL-AML1) in in vitro cultured mouse bone marrow cells.

The Vectors described will also be used to induce expression of shRNAs targeting mouse candidate genes.

**Evaluation of foreseeable effects**

Introduction of oncogene-encoding expression vectors in recipient cells will result in malignant transformation of the recipient cell resulting in tumour clones in cell culture media. If injected into immunocompromised mice these cell will induce development of leukaemia in thses chimeras.

GMM bacteria produced are non-invasive/non-pathogenic. Viral particles are mouse cell specific and NOT infectious to human cells and replication-inactive. Mouse lines used are non-pathogenic. Infected mouse cells are nonpathogenic.

In the unlikely case of a release of the virus to the wild mouse population, this could result in the development of leukemia in the infected mice. As the virus is replication incompetent, these mice would die by leukemia but would not infect any further mice. The release would thereby terminate itself.

Therefore, there are no GMM's produced in this project that are hazardous to human health, but there is a low risk of harm to the environment that requires us to work at containment level 2.
GM animals are not part of this notification.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste containing GMOs or GMMs will be incubated with Virkon (1% final concentration, 10min contact time) to neutralise any potential capacity to affect environment of health, solid waste including sharps will be autoclaved (121C, 20min).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Reviewer 1
The big question with this project relates to the Mouse specific Lenti virus. From the description in 1.2 it appears to me that this is a GMO. If it is a GMO according to the GM contained use regulations this is likely to be a class 2 virus unless evidence can be produced that it is heavily attenuated and unlikely to spread to the environment. Please see below for further comments.

2.9 Section 2.14 describes the mouse specific retro virus as "no GMM" If that is true it should be listed in section 2.9
2.14 In this paragraph it is stated that the mouse retro virus is "no GMM" However section 2.16 describes several vectors for packaging into a mouse specific lenti virus, this seems to indicate that the lentivirus is genetically modified. Please explain the process more fully and clarify whether the mouse specific lenti virus is a GMO. Section 1.2 paragraph a seems to indicate that the lentiviral particles are GMO's

2.18 If the mouse specific lentivirus is a GMO then this may represent a hazard to the environment. Please clarify in the GM section whether the virus is GM or wild type and also whether it is attenuated.
3.1 If the mouse lentivirus is GM then this may be a class 2 project on environmental grounds unless the virus can be demonstrated to be highly attenuated. More explanation is required as requested in the comments above.
4.15 What about the environment? If the GM virus is infectious to mice then this should be mentioned as it is a potential environmental concern as defined by the GM contained use regulations.
4.16 This is subject to clarification on the GM status of the mouse specific lenti virus.
4.17 If the Mouse specific Lenti virus is a GMO then this must be considered in this section.
4.18 If the Mouse specific Lenti virus is a GMO then this must be considered in this section.
4.19 If the Mouse specific Lenti virus is a GMO then this must be considered in this section.
4.20 If the Mouse specific Lenti virus is a GMO then this must be considered in this section.

Section 5. If the mouse retro virus is a class 2 GMO (see comments above) then we will need to see a COP for this work.
5.1 If the Mouse specific lentivirus is a GMO then this must be considered in this section and the MSC may be required to protect the environment from contamination.
5.2 What about the risk to the environment?
5.4 Will this material be shipped to other campuses or outside of the University?
5.6 Section 4.21 states that no GMO will be stored but 5.6 states that the retro virus will be stored if the virus is a GMO please explain this apparent contradiction.
5.7 If the retro virus turns out to be a class 2 GMO then you will need to use sealed rotors to spin these plates.
5.9 What are these processes and is there an SOP?
Section 6. If the mouse retro virus is a class 2 GMO (see comments above) then we will need to see a COP for this work.
Section 7. If the mouse retro virus is a class 2 GMO (see comments above) then we will need to see a COP for this work.
Section 8. 9 10 and 11 will need to be completed if the mouse retrovirus is a class 2 GMO
Section 13. If the mouse retro virus work is class 2 then this section will need to be altered to reflect this
Section 14 If the mouse retro virus work is class 2 then this section will need to be altered to reflect this
Reviewer 2
Section 6.6 Remove reference to restroom handwash facilities as hands must be washed before leaving the lab areas.
A Form B needs to be completed.
Section 2.18: Please consider the environmental impact on virus release to the wild mouse population.
Section 4.15: Please consider the environmental impact on virus release.
Section 4.16: The question is asking about the hazards posed by the vector systems being used. Please modify your response accordingly.
Section 4.20: E. coli can infect humans and therefore the question does require to be answered fully.
Section 4.21: You have stated that no GMM will be stored. However in section 5.6 you have stated storage in -80C freezer. Please correct this discrepancy.
Section 5.13: LN2 is indicated for cell storage but this is not listed in section 5.6. Please amend as appropriate.
Section 7.4: Packing material is highlighted for disposal but section 5.5 states no receipt of material. Please correct discrepancy.
Reviewer 4
Section 2.2(a) – luciferase not luciferase
2.4 NR only needs checking and not the other boxes
2.8 No
Reviewer 5
Section 2.18: need to identify the most hazardous GMM (the answer does not reflect this, plus the environment need to be considered).
Section 4.15: Need to consider not only risk to human health but the environment.
Section 4.16: As above
Section 4.18: Need to consider the consequences (not explained what would happen, only that it is unlikely / prevented from happening)
Section 4.21: States no GMM will be stored. This in not consistent with Section 5.6 and 5.13 (storage in LN2)
Section 5.3: Secondary containment recommended while transporting cells
Section 5.7 & 7.1 : Contact time for virkon only 10 mins.

Project Containment

<table>
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</tr>
</thead>
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<tr>
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<td>L3 L4</td>
<td>L2 L3 L4</td>
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</table>
### Project Additional Information

**Purposes of the contained use**

A number of associated projects are being undertaken to analyze the roles of different host innate immune receptors and adaptor molecules in infection and inflammation. TLRs, NLRs, MyD88, ASC, Casp-1, NLRP3, NLRs, NPC, SLCs are all mammalian proteins that are directly involved in these processes, and are being investigated in the lab. The aims of these projects are to study the individual roles of these proteins in cells and mice lacking these proteins. Additionally, these proteins will be over-expressed in mammalian cells to study their precise functions. From a microbial perspective, we are interested in deciphering the roles of different microbial antigens, including cytolysins, in the modulation of innate immunity. Thus, we will employ microbial strains either wild-type or lacking individual antigens to evaluate their ability to modulate the above-mentioned host signaling molecules/pathways. Cells (or mice) lacking different host innate molecules will be exposed to microbial antigens or microbes. In addition, they will be exposed to pathogens lacking distinct antigens or cytolysins to decipher their roles in innate immunity. Different plasmids expressing wild-type or mutant forms of the above genes will also be employed to over-express the DNA/proteins in E. coli and mammalian cells.

**Recipient or parental organism**

Hazard group 2 recipients (leading to Class 2 GMO’s)

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**Project Ref** 31/17.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>Consent Granted</th>
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<tbody>
<tr>
<td>06/09/2017</td>
<td>Innate Immune Responses to Pathogens</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Not Applicable</td>
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</tbody>
</table>

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

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**Purposes of the contained use**

A number of associated projects are being undertaken to analyze the roles of different host innate immune receptors and adaptor molecules in infection and inflammation. TLRs, NLRs, MyD88, ASC, Casp-1, NLRP3, NLRs, NPC, SLCs are all mammalian proteins that are directly involved in these processes, and are being investigated in the lab. The aims of these projects are to study the individual roles of these proteins in cells and mice lacking these proteins. Additionally, these proteins will be over-expressed in mammalian cells to study their precise functions. From a microbial perspective, we are interested in deciphering the roles of different microbial antigens, including cytolysins, in the modulation of innate immunity. Thus, we will employ microbial strains either wild-type or lacking individual antigens to evaluate their ability to modulate the above-mentioned host signaling molecules/pathways. Cells (or mice) lacking different host innate molecules will be exposed to microbial antigens or microbes. In addition, they will be exposed to pathogens lacking distinct antigens or cytolysins to decipher their roles in innate immunity. Different plasmids expressing wild-type or mutant forms of the above genes will also be employed to over-express the DNA/proteins in E. coli and mammalian cells.
Listeria monocytogenes (GFP/RFP-labelled, or lacking hly, LLO, ActA, ActB) - HG2
Salmonella typhimurium (GFP/RFP labelled, or lacking SPI1, SPI2, flagellin, SipB - HG2
Citrobacter rodentium, (GFP/RFP labelled) - HG1 (Class 2 GMO)
Hazard group 1 recipients (leading to Class 1 GMOs) Included for information as they are relevant to the project as a whole.
(E. coli K12-derived lab strain (GFP/RFP-labelled) - HG1
Francisella novicida (GFP labelled, or lacking mglA, iglA, iglC) - HG1
M. smegmatis (lacking ESX-1) - HG1
Cell lines - J774, U937, Hep2, HEK293, RAW, mouse primary macrophages or derived cell lines, THP1, 16HBEs

Host/vector system
Used to create the Class 2 GMO's
pLOFKmgfp
pML523 derived
pET derived
pLSV1 derived
pBSK derived
pMP590 derived
pKK derived
Used principally in the Class 1 GMO's
pUC derived
Public Register Public Register Public Register Public Register
Health and Safety
Executive
CU 2 2015 (rev 11/15) Page 3 of 11
pBR322 derived
px458 derived
px459 derived
pCMV derived
psPAX2 derived
pcDNA derived
J2-cre derived
pLenti-derived

Origin & function
NLRs 1-22, involved in host defense
NPC 1-2, involved in cholesterol trafficking
ASC, adaptor molecular in the inflammasome complex
Casp-1, a protease
TLRs 1-11, involved in host-defense
RLRs, involved in viral sensing
ALRs, involved in DNA sensing
GFP, Green fluorescent protein
RFP, Red fluorescent protein
LLO - codes for Listeriolysin O
ActA,B - genes involved in actin-dependent movement
Flagellin - flagellum,
SPI - Salmonella pathogenicity island
ESX-1 - codes for bacterial secretion system

Evaluation of foreseeable effects

The GMO's listed above are no more hazardous than the non GM recipient organism. Additionally, since the GMMs will lack important factors required for their virulence, both mice and likely humans are expected to be more resistant and in the event of infection the symptoms are likely to be mild until the infection is cleared by the immune system.

Listeria monocytogenes: potential, but low, infection risk from accidental inoculation or ingestion, ranging from subclinical to severe systemic illness. Specific risk of harm to fetus through transplacental infection in 3rd trimester.

Higher risk if significantly immunocompromised.

Salmonella typhimurium: Infection risk [gastro-enteritis] from accidental inoculation or ingestion of culture. Likelihood low.

E. Coli: K12 derived non pathogenic strains

Citrobacter rodentium: not pathogenic in humans, may cause mild infection in rodents depending upon the dose and route of infection.

Franciscella novicida: rare opportunistic human pathogen. Risk of infection even if exposed will be low. Main symptoms include muscle pain, pneumonia, and fever.

Mycobacterium smegmatis: Considered non-pathogenic in humans and rodents.

The listed vector system hazards are the same or less. These are common, non infectious systems and with appropriate handling and containment processes (mainly Hazard Group I), they do not constitute a hazard. The transfer plasmids are all replication incompetent and thus there is no risk of transfer to other organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste - Microbial cultures- Inactivated by adding Distel (a commercially available chemical disinfectant classified as a biocide under the EU Biocides Regulation) used at a final concentration of no less than 1:100 for a contact time of at least 30 minutes. At least a log5 reduction in viable organisms.

Solid Waste - Laboratory plastic disposables and glass - Sterilized by validated autoclave (compliant to British and European Standards). Minimum 121 degrees for a holding time of 15 minutes. At least a Log 5 reduction in viable organisms.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Following the discussion of your proposal entitled "Innate immune receptors in infection and inflammation" (GMIC-7900), the committee request that the following amendments are made to the proposal prior to approval:

Reviewer 1
2.3 “most of these…” Please be more specific. I agree that Igose obtained commercially are unlikely to be infected with pathogens, but what about the PBMC's from patients mentioned in 2.2? what are these screened for?
2.15 Please indicate the ACDP or the DEFRA classification. FYI Listeria monocytogenes and Salmonella Typhimurium are HG2 hosts. Citrobacter rodentium, is ACDP HG 1 (but will be a class 2 GMO as it causes disease in an environmental organisms i.e. mice) Francisella novicida HG1 M. smegmatis HG1 You also mention E.coli without further description. E.coli is a pathogen and may be HG2 or HG3 unless it can be demonstrated that it is suitably attenuated – Please provide a more detailed description E.g. K12 derived non toxigenic auxotrophic lab strain or something more hazardous such as E. coli O157:H7. 2.15 Regarding this statement “The above listed GM infectious agents have been obtained from collaborating labs. They will not be modified any further and thus there are no further vectors listed for these in section 2.16.” Unfortunately it is still classed as your GM work whether or not they were obtained from colleagues. If you can obtain more information please include the vectors that were used to create the GMO in 2.16.
2.17 Please add the inserts mentioned in the added organisms in 2.15
2.18 Please complete this section. If you are certain then you can state that none of the GMO’s are expected to be any more hazardous to human health or the environment than the non GM host. Please identify the most hazardous GMO’s regarding human health and the environment. 4.16 The recipients are not all HG2 organisms. Please identify the disease and harm that they can cause to human health and the environment. You need to consider each HG2 organism in turn and describe the disease and symptoms
8.1 Centrifuges will need to be inspected and maintained if they are used to handle and contain pathogens. Elements of the inspection e.g. condition of bio seals on rotors or buckets, can be carried out by the users and recorded monthly. Please specify what these arrangements are.
MSC’s will need to be airflow checked monthly by the users and this recorded if used to handle pathogens as stated in 5.1 of this Bio1
Section 15. Not all the work is Class 2. Please separate and identify what is Class 1 and what is class 2.
Reviewer 2
Section 2.18: Please complete
Section 4.5: Centrifugation (see 4.6)
Section 4.17: Please answer the question asked. E.g. can the vector system transfer material to other organisms?
Section 4.18: Please answer the question asked. Some of the GMO hosts are pathogens
Section 4.20: Please answer the question asked. Some of the GMOs are pathogens
Section 5.10: Discrepancy in routes of infection listed in sections 5.10 and 4.21 also M. bovis and M. avium missing from section 4.21. Also discrepancy between organisms listed in section 5.10 and 2.15.
Reviewer 3
The comments concerning about Q 2.3, 2.4, 2.5 and 4.8, it was mentioned 4.2 that PBMC from patient and health individuals will be used therefore ethic approval has to be provided. It was not clear from where PBMC from healthy individuals will be obtained? And will they be screened for infectious agents? Please make this clear in section 2.2
Need answers for the following questions
Page 2 Q2.4 last question " If yes, and for human material, how will the information be disseminated in the course of the project"
Page 5 Q2.18
Page 6 Q4.14

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</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4 L2 L3 L4</td>
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Project Ref 31/18.1

Date Ackn'd 10/08/2018
Date Project Ceased
CU2 Project Title
Generation of a genetic tool in stable and transient passaged primary cells, cell lines and mice to analyse cancer heterogeneity in 3D models
Class 2
Culture Vol Class 2
≤ 1 Litre
Consent Granted

Historical Significant Changes
Historical Date of Additional Info

02/03/2022
The aim of the project is to establish heterogeneity in cell lines, primary cells and in murine mammary glands and monitor tumour development over time. Plasmids under the control of an inducible promoter will either overexpress, downregulate or knock out genes involved in breast cancer evolution, which will be delivered to the targeted cells via viral systems. The multigene construct will be designed in several steps. Where first, genes involved in breast cancer evolution will be tagged with fluorophores in lab-adapted bacterial strains. Secondly, these constructs will be cloned into functional lentiviral vectors to validate the functionality of the tagged genes. Once validated the multigene constructs will be integrated into a baculovirus delivery system to infect host organisms.

For the lentivirus system generation, two or three plasmids will be used for overexpression, knock down and knock out. Both generations of lentiviruses are encoding for a replication-defective system, which will be produced in HEK293 and will enclose a plasmid with multigene constructs.

For the baculovirus-mediated gene delivery, multigene shuttle plasmid will be designed using E.coli Rec-strains. Afterward a recombinant virus DNA is produced by a site-specific integration in to a variation of a DH10Bac E.coli. Isolated viral DNA will be transfected into Sf9 or Sf21 insect cell and will be either purified for murine experiment or used to directly transduce mammalian cells.

Both transduction methods will be used to produce stable expression and promote an integration of the multigene construct into the host genome. The induction of the multigene construct will promote the modification of genes that are involved in breast cancer evolution and will allow spatio-temporal analysis of tumour development in a heterogeneous background.

Recipient or parental organism
- E.coli lab-adapted strains (hazard group1) - NEB stable, STBL3, DH5a, Top10, CCdB
- Panel of breast cancer cell lines including MCF10A, MCF7, MDAMB-231, MDAMB468, BT474, T47D, SKBR3
- murine mammary cells
- passaged primary epithelial/luminal cells
- HEK293
- Insect cells Sf21 or Sf9

Host/vector system
To design the multigene construct the MXS vector system will be used. MXS_MCS1, MXS_MCS2, MXS_MCS3, MXS_MCS4, SMX DEST vector, MXS_TagBFP, MXS_Cerulean, MXS_LSS-mKate2, MXS_mEGFP, MXS_mAzamiGreen, MXS_Citrine, MXS_mKO2, MXS_tdTomato, MXS_mCherry, MXS_mPlum, MXS_E2-Crimson, MXS_IRFP670, MXS_PA-GFP, MXS_Kaede, MXS_PGK, MXS_minPGK, MXS_PGKenhancer, MXS_CMV, MXS_minCMV, MXS_CMVenhancer, MXS_CAG, MXS_EF1a, MXS_Tet, MXS_BiTet, MXS_bGpA, MXS_bGhpA, MXS_SV40pA, MXS_CreERT2, MXS_rTA2, MXS_tTA2, MXS_IoxP, MXS_linker, MXS_H2B, MXS_P2A, MXS_PEST2D, MXS_CMV::HygroR-bGhpA, MXS_PGK::HygroR-bGhpA, MXS_CMV::NeoR-bGhpA, MXS_PGK::NeoR-bGhpA, MXS_CMV::PuroR-bGhpA, MXS_PGK::PuroR-bGhpA, MXS_CMV::ZeoR-bGhpA, MXS_PGK::ZeoR-bGhpA, MXS_CMV::CreERT2-bGhpA, MXS_PGK::CreERT2-bGhpA, MXS_CMV::rTA3-bGhpA.
Additionally, the breast cancer evolution genes of interest will be subcloned into the pDONR223 to perform a Gateway cloning to get an overexpression construct, which will be tested in the pINDUCER21 (Addgene 46948) containing the mutated (non-oncogenic) WPRE. For knock down or knock out gene silencing constructs will be cloned into the 3rd generation plasmid TET-pLKO-puro (mutated WPRE; Addgene 21915) or 3rd generation CROPseq-Guide-puro (Addgene 86708) or similar plasmids respectively. Envelope and packaging plasmids will be pMDLg/pRRE, pRSVRev, pCMV pCMVR and pMD2.G or similar. Other plasmids used for subcloning will be pcDNA3.1, pUC18, pUC19, Pjet, pTOPO, pM5, pBABE-puro, pLenti6/V5-TOPO and similar.

For the baculovirus system a shuttle plasmid based on the pFastBac1 plasmid will be used to introduce a mammalian multigene construct, consisting of an inducible promoter and a SV40 promoter controlling a resistance gene, into the baculovirus genome via a transposition between a mini-att Tn7 target site.

Genes characterised as being involved in breast cancer evolution, like TP53, PTEN, Her2, MP3KCA and similar genes. Those genes will be manipulated via overexpression, shRNA or CRISPR.

To mark the cells for lineage tracing fluorescent proteins line GFP, dsRed, Citrine, Azurite, Plum, mCherry and similar will be introduced.

Chosen virus systems are not able to propagate due to lack of structure proteins and/or not able to replicate in mammalian cells. At the same time the risk posed by the genetic insertions is minimised by using inducible promoters. All vectors will be engineered using only an inducible promoter for expression of the genes potentially involved in cancer evolution.

Low risk nature of most baculoviral systems to both humans and environment indicates that minimal containment measures will be required. Nevertheless, the lentiviral particles will contain a tropism for expended host range, which could lead to insertion of the vector into hosts/human genome, this could in rare cases lead to a risk. The best methods to prevent exposure are proper technique, training, and personal protection equipment. This will include carefully reviewing the use of sharps in laboratory procedures and eliminating them when possible: for example, plastic substitutes for glass Pasteur pipettes are commercially available. Needle/syringes should only be used if no alternative is possible.

Indirect aerosol exposure with droplets containing lentiviral particles may occur in the laboratory. It can be prevented if working in MSC and centrifuge in sealed containers.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Virus injection into mouse mammary tissue will be undertaken to induce heterogeneous tumours in CL2 facilities.

Human lentivirus or insect baculovirus does not replicate in murine tissue. Therefore the risk of shedding is negligible.

Needles will be used to infect mice with baculovirus via intraductal, injection. Staff will be trained in the correct use and disposal of sharps and gloves will be worn at all times.

Sharps may be occasionally used to prepare cell lysates or to dissect tissue samples.

All tissue dissection will take place in a class 2 safety cabinet. Sharps will be disposed in yellow sharps bin after use.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Liquid Waste: | 10% final concentration distel solution for a minimum of 1 hour at room temperature for bacterial cell cultures or 10% final concentration distel overnight for tissue culture waste. These can then be dispensed down the laboratory sink. Bacterial cultures <5ml are autoclaved prior to disposal. |
| Solid Waste: | All solid waste including bacterial cultures on agar plates and contaminated tissue culture plates and other disposable plastics, cell pellets and other material in contact with GM material will be collected inside a clear autoclave bag and transferred into another autoclave bag after completion of experiment or at the end of the day. The double-bagged GM waste is then labelled and left in the GM waste bin located in the cold room. The GM autoclave waste run is carried out twice a week where the waste is autoclaved at 121 degrees C for 15 minutes. A chart recorder attached to autoclave and thermolog strips in autoclave bags will be used to validate treatment. Solid waste is autoclaved and then disposed of in orange clinical waste bags into EUROCARTS for Alternative Treatment. Tips and stripettes are collected within a designated clearly labelled sharps box for autoclaving with the other GM waste prior to disposal. |

**Is an emergency plan required according to regulation 20?**

- [ ] N

**If yes, tick to confirm that it is attached to this form**

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] N

Please enter comments on the GM safety committee on the risk assessment
Following the discussion of your proposal entitled “Generation of a genetic tool in stable and transient passaged primary cells, cell lines and mice to analyse cancer heterogeneity in 3D models” GMIC-8832 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

Please describe in the form or a flowchart what vectors are proposed to be built. The Committee is aware that this is not exactly known yet and likely change during the project, therefore provide a broad description of potential vector/plasmid outcomes and in which organisms these are planned to be used in. Set some boundaries of what will and will not be done for this project. Add vector maps for the backbones.

2.9 and 4.9: Remove the details from the Biological Agents section.

2.14: Make sure the boundaries of the project are described here.

2.15: The appropriate ACDP classification would be ‘hazard group’ and not ‘level’ for bacteria/virus. Remove the level/classifications of the cell lines and hazards in the pink sections. For E.coli; describe these as ‘lab-adapted strains’ with some of the examples, i.e. what is in the green section. Lentivirus and baculovirus are not really hosts, these are vector systems.

2.16: Name the vectors used and describe the WPRE gene in the 2nd generation lenti virus.

2.17: Name the genes being proposed to investigate, make it broader by saying ‘gene x and related genes’. A description is required here to understand the boundaries of the work.

2.18: Replication incompetent lenti-virus, with human tropism are able to infect human cells and integrate their genome into the host cells, whether or not they are induced. This increases the risk of accidental exposure. Think carefully about this section and identify the real risks.

4.3: Typo ‘underwent’

4.4: Increase volumes to either 100ml or 250ml to avoid restriction of 25ml and 4 flasks.

4.16: Remove ‘present no harm’ and ‘impose no risk’ when producing lentiviral particles with the WPRE gene, as this will upregulate potential oncogenes. Re-write this section carefully addressing the potential risk/harm.

4.18: See 2.18

4.20: Typo ‘specific’

4.22: Give a best estimate on how many virus particles might be produced/used.

5.6: Give an estimate what high or low titre might be?

5.7: Clarify that everything will be in the MSC and remove “…leave for 30 minutes to allow aerosol to settle.”

5.9: Change ‘category’ to ‘class’

5.12 and 5.13: Please provide a Standard Operating Procedure for the FACS and Sorting. This is not the same as the FACS facility Code of Practice. In this SOP identify what the operators of the Sorter must use to disinfect the machine after your sort.

10.1: Clarify that everything will be in the MSC and remove “…leave for 30 minutes to allow aerosol to settle.”

10.1: ‘category II waste’ change to either ‘GM waste’ or ‘containment level 2 waste’

11.2 ‘category II’ change to ‘containment level 2’

14 and 15: Change to classification and containment level 2.

Agreed: Class 2, Containment level 2.

IC reference number: GMIC-8832

Project Containment
Patients with cystic fibrosis and bronchiectasis are susceptible to infection by a number of different pathogens. Pathogens that are problematic for this patient group are **Burkholderia cenocepacia**, **Burkholderia multivorans** and **Pseudomonas aeruginosa**. Our research group is interested in understanding the underlying disease pathology in the lung.

**Recipient or parental organism**

**Burkholderia Multivorans**, *Burkholderia cenocepacia* and other similar virulence strains, *Pseudomonas aeruginosa*. All ACDP classification hazard group 2

**Host/vector system**

pAKN69 plasmid carrying YFP gene (or similar fluorescent markers), pBluescript or similar commercially available phagemid cloning plasmids

**Origin & function**
Genetically modified Burkholderia multivorans strains are less hazardous than their wild-type counterparts due to the antibiotic sensitivity conferred by their mutation. Genetically modified Pseudomonas aeruginosa in this work are less hazardous than their wild-type counterparts. Introducing YFP or similar fluorescent markers into a bacterium has no impact on pathogenicity.

<table>
<thead>
<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not applicable</td>
</tr>
</tbody>
</table>

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| Not applicable |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Liquid waste: Placed into Distel (2%, final concentration) for a minimum contact time of 30 minutes in line with the manufacturers recommendations. A minimum of a log5 reduction in viable microorganism numbers is anticipated. Solid Waste: Placed into an autoclave waste bag and autoclaved before disposal. The autoclave is validated annually for this waste type by a competent service engineer complying with the relevant BS and using a calibrated 12 pt thermocouple monitoring device. Minimum temperature achieved in the waste load is 121 degrees celcius for at least 15 minutes. Complete sterilisation of the waste load is anticipated. |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | Y |

Please enter comments on the GM safety committee on the risk assessment
Request for partial non disclosure - see section 17.

Following the discussion of your proposal entitled “Infection with Burkholderia cenocepacia and Pseudomonas aeruginosa.” (GMIC-8925) at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

1.3. Add the location of the FACS and Sorter

5.1 Add that MSC's will be used for all work involving manipulations of the HG 2 and Class 2 micro-organisms and this includes the FACS and Cell sorter

5.7 Amend the lid closed time from 30 mins to 60 mins

8.1 Add that Centrifuge maintenance will be carried out in accordance with the user manual.

10.1 State that you will use at least an “equal” volume of 2% virkon / Distel to treat the spill.

Comments:

gave brief description of the work is aware of the need for occupational health screening in case anyone would be at increased risk due to their health status.

asked are they all class 2?

responds yes

1.3. Where is the FACS machine located?

responds the FACS machine is in a Class 2 cabinet on the 8th floor.

Added that a sorter is also located on the 8th floor and is enclosed in a class 2 MSC

Commented on the phrase “A class 2 Microbiological safety cabinet will be used for all procedures involving this bacterium” asked Which bacterium? Or should this be “These Bacterium”

responded All hazard group and GM class 2 bacteria

commented that it was unlikely that there would be no hazards.

Responded that they meant that the GMO would be easier to treat than the wild type. They would remove the word none and leave the explanation about the GMMs sensitivity to antibiotics.

5.7 I would recommend that the lid of the centrifuge is left closed for 1 hour rather than 30 minutes. This is based on previous correspondence with the HSE. This also matches your ERPs described in 10.1 asked about the answer to 5.9 as it states no sharps but 7.4 states that there is sharps waste

8.1. Centrifuge maintenance should be carried out at the frequency indicated in the user manual. Some will be daily, weekly, monthly and annual, for example visual o ring inspections and visual checks of the rotor integrity should be carried out every time the centrifuge is used, swing out bucket trunnions may only require cleaning and greasing monthly.

recommended stating ‘at the frequency indicated in the user manual’ to answer the question regarding maintenance frequency for centrifuges.

Please specify that you will use at least an “equal” volume of 2% virkon / Distel to treat the spill.

stated that this would be amended

Asked for the SOPs to be uploaded to RADAR

On RADAR you mention use of PA and Burkholderia multivorans as GM hosts, which agrees with the Bio1. and pointed out that work with GMOs included any use and therefore we need to record the GM characteristics of all GMOS in use on the Bio1

There was a discussion about how to broaden the scope of the assessment to include possible further expansions to the work so a re-submission would not be required.

confirmed that he would send and some guidance.

Agreed: Class 2, Containment level 2. This is notifiable to the HSE.

Could you please ensure that the risk assessment is revised and the amendments above addressed. I will then forward the form onto Occupational Health to complete
# Project Additional Information

**Purposes of the contained use**

The enteric nervous system is an important pathway in the regulation of glucose homeostasis and is made up of both neural and endocrine components. The neurotensin receptor 1 (NtsR1) has been identified in the enteric nervous system and is implicated in controlling a profound glucose lowering effect following glucose challenge. This project aims to investigate the expression of the NtsR1 on neural projections extending between the gut and pancreas by injecting an attenuated transsynaptic, retrograde pseudorabies virus (PRV-Bartha) that expresses fluorescent proteins (e.g. PRV152 or PRV614) into the pancreas. This virus will then trace neural projections between the gut and pancreas allowing for specific analysis of NtsR1-expression on distinct enteric neuronal populations.

Mice will be injected with PRV-Bartha into the head, body and tail of the pancreas. This should lead to transsynaptic, retrograde labelling of neurons from the pancreas.
back to the wall of the gut. 3 days following surgery, animals will be culled and tissues collected for immunofluorescent analysis. This will be visualised on a microscope.

Recipient or parental organism

Pseudorabies virus

Host/vector system

No new GMOs will be created as part of this project.

PRV-Bartha is a pseudorabies virus created by the Viral Neuroengineering Laboratory, Princeton Neuroscience Institute, Princeton University. It is an attenuated vaccine strain of the wild-type PRV-Becker that contains the CMV-mFP reporter gene cassette inserted into the gG locus of the viral genome that allows for transsynaptic retrograde tracing of neuronal circuits.

Origin & function

PRV-Bartha infection is initiated by binding to cellular receptors to allow penetration into the cell. After reaching the nucleus, the viral genome directs a regulated gene expression cascade that culminates with viral DNA replication and production of new virion constituents. Progeny virions then self-assemble and exit host cells. This allows for retrograde transsynaptic tracing of enteric neurons that originate in the myenteric plexus of the gut wall and terminate in the pancreas.

Evaluation of foreseeable effects

PRRV-Bartha is a pseudorabies virus created by the Viral Neuroengineering Laboratory, Princeton Neuroscience Institute, Princeton University. It is an attenuated vaccine strain of the wild-type PRV-Becker that contains the CMV-mFP reporter gene cassette inserted into the gG locus of the viral genome that allows for transsynaptic retrograde tracing of neuronal circuits. PRV-Bartha is less virulent than PRV-Becker due to the fact it only travels via efferent routes in the PNS en route to the brain, unlike PRV-Becker that transits via both afferent and efferent routes. Due to the fact that PRV-Bartha is an attenuated virus, its cytotoxicity is reduced meaning that animals live several days longer after infection without significant symptoms. Unlike the virulent PRV strain (PRV Becker) that causes self-mutilation of mouse flank skin in response to virally induced pruitus and death due to infection of the CNS, PRV-Bartha-infected animals have no skin lesions and do not appear pruirtic at any time during infection. Infection of the CNS occurs following infection with PRV-Bartha. However, PRV-Bartha infected animals survive approximately three times longer than PRV-Becker infected animals. For this reason, animals will be culled 3 days after injection.

The virus replicates and is shed via aerosols. However, PRV-Bartha can only be transferred to other animal by injection of the virus, not by aerosol or inadvertent or casual contact. Studies have shown that leaving an uninfected animal in a cage with an infected animal resulted in no cross-infection. PRV-Bartha causes no risk to human health.

The largest risk when using PRV-Bartha is the causation of Aujezky's Disease. This is a viral disease that occurs in swines. No pigs are kept at the Hammersmith Campus at ICL and the risk of this occurring is therefore very low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All labware that comes into contact with the material will be either autoclaved or treated with 10% sodium hypochlorite for 5 minutes to inactivate the material. Waste will be double bagged to minimise risk of spillage if a bag bursts. Liquid waste will be collected and autoclaved to inactivate the material. This can then be safely put down the sink.
Infected animals will be culled 3 days after injection with PRV-Bartha. All animals will be immediately transcardially perfused with phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA). Tissues will then be extracted and placed in 4% PFA overnight. Tissues will be further processed by paraffin-embedding or set in 1% agarose. Carcasses will be placed in yellow clinical waste bags to be collected by CBS staff for transfer to CBS yellow Euro Carts and disposal by contracted clinical waste service managed by the Trust.

Infected animals will be kept in individually ventilated cages in Containment, H1. These cages are washed according to specific Cat2 procedures. This includes the spraying, bagging and autoclaving of cage waste and high temperature wash followed by acid-clean of the cages themselves.

Following the discussion of your proposal entitled “Neural Tracing” (GMIC-9493) the committee request that the following amendments are made to the proposal prior to approval:
- Section 4.7: does not need to be completed (as far as I can tell, PRV-Bartha is a GM virus) this will make the form consistent as section 2.11 was not completed.
- Section 5.11: How will post-perfused PBS (potentially containing live virus be disposed of (as no liquid waste treatment in section 7.1)?
- Points 2.27 and 4.17: “Studies have shown that leaving an uninfected animal in a cage with an infected animal resulted in no cross-infection” please provide a reference.
- I am not aware of the cleaning system of mouse cages. If they are washed (with anti-viral agent) and sterilised then it is fine. If not, should it not be done?
- I suggest we add some comments to the Bio1 regarding the survivability and stability of the Pseudorabies in the environment in the event of a breach of containment, as this is a requirement under SACGM (reference https://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/)

Class 2 agreed for this project.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Project Ref 31/21.2
Single molecule imaging of herpes simplex virus type 1 DNA replication

Herpes simplex virus type 1 (HSV-1) is a widespread pathogen, which has been studied extensively. Nevertheless, we still lack a complete understanding of the mechanism of genome replication. Although a model for replication exists, this model fails to explain certain observations made. We propose to revisit long-standing questions of HSV-1 replication using cutting-edge single molecule imaging techniques. Single molecule assays enable the detection of transient structures and heterogeneous behaviour, revealing crucial insights into the fundamental process of genome replication. The current model for HSV-1 genome replication comprises an origin-dependent followed by an origin-independent stage. Whereas we have evidence and a good understanding of the origin-independent phase, we completely lack evidence for the proposed structures of the origin-dependent phase. We want to study aspects of the origin-dependent replication phase. Specifically, we want to reveal the role of the HSV-1 origin binding protein UL9 during replication since UL9 was proposed to act as a switch from the first to the second stage. It has been shown that UL9 is an essential protein during the origin-dependent stage but dispensable and even inhibitory during the second, origin-independent stage. We propose to use single-molecule imaging techniques to reveal the inhibitory effect of UL9 on HSV-1 mediated replication, characterise the specific conditions of UL9 origin binding and assess its role during the assembly of the HSV-1 replication machinery.

Recipient or parental organism

Herpes simplex virus type 1 (HSV-1) strains F, 17 and KOS.
Most HSV-1 infections are asymptomatic, the most common symptom are cold sores (blisters on mucosal skin), in rare cases HSV-1 infections lead to keratitis, encephalitis or meningitis. HSV-1 infections can be life-threatening for newborn babies. HSV-1 infections are irreversible, the virus will establish latency in sensory neurons and might lead to recurring cold sores.

Common E.coli strains will be used for cloning.

HSV-1 will be passaged in and harvested from common cell lines (Vero and HEK293 cells)

Host/vector system

HSV-1 (strains F, 17 and KOS)
Origin & function

Wildtype HSV-1 DNA will be used because the aim of the project is to clone individual viral proteins involved in HSV-1 genome replication and will be tagged for example to enable a visual readout at the microscope (e.g. fluorescence) or to purify the specific protein (e.g. with a streptavidin tag).

BAC plasmids containing the entire HSV-1 genome (e.g. YE102)

Established cloning and protein expression vectors such as pCM, pUC18, pGEX, pET30, pRF or similar and derivatives thereof (in which the MCS has been modified in-house or commercially for different fusion proteins, different cleavage sites etc)

Evaluation of foreseeable effects

HSV-1 strains will be modified to include fluorescent or non-fluorescent (e.g. GST-tag) tags

HSV-1 deletion mutants where specific genes are missing will be used

HSV-1 GMMs used have the same biohazard classification as the wild type strain, as the genetic modifications do not enhance any virulence factors. The HSV-1 GMMs used in this project generally show lower infectivity and virulence when compared to the wildtype strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

no derogation from full containment

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

HSV-1 containing liquid waste will be treated with disinfectant (e.g. Distel (1%), Microsol (10%) or 2% Virkon, hypochlorite bleach (1% 10,000ppm)) (decontamination for 10 min) before being flushed with running water down the drain. Small volumes of liquid waste (<5ml) in eg. culture tubes may be disposed of with tube as solid autoclave waste

HSV-1 contaminated solid waste will be treated with disinfectant (e.g. Distel (1%), Microsol (10%) or 2% Virkon, hypochloric bleach (1% 10,000ppm) and then autoclaved before disposal as clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Reviewer 1: I'm happy that this is GM Class 2.

Reviewer 2: I am happy with the class 2 classification.

Section 5.2: Why so many different decontamination chemicals used – would it not be easier to standardise on 1 protocol?

Section 5.7: Why not using sealed buckets/rotors?

Section 6.1: How does wearing gloves impact laser safety?

Section 7.2: Why use bleach for liquid waste but other chemicals for solid waste? Why use both chemical disinfection and autoclaving (Just autoclaving would reduce handling of contaminated plasticware which would save time and be safer for the staff).

Section 7.6: Storing hazardous waste if fume cupboards is not recommended.

Section 8.3: Please uncheck N/R box

Section 9.5: Please uncheck N/R box

Section 10.1: As centrifuge will only be opened in MSC (Section 5.7), waiting for 1 hour for aerosols to settle is not required.

Reviewer 3: I agree that this project is Class 2.

Reviewer 4: The PI has checked low risk of infection since the virus is most likely rendered inactive by the extraction method (but to be verified) in 2.6 and High risk in 3.1 but HSV1 is not a Schedule 5 pathogen and toxins. I would suggest 3.1 to be changed to low risk. I think it is a CL2 project.

Reviewer 5: I am in agreement with the classification of Class 2, since the wildtype strain is Hazard Group 2.

Reviewer 6: I’m happy with the proposal.

## Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
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</thead>
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<tr>
<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
</tr>
</tbody>
</table>

**Animal Units**

**Large Scale Activities**

**Human Clinical Applications**

<table>
<thead>
<tr>
<th>Project Ref</th>
<th>31/22.1</th>
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</thead>
<tbody>
<tr>
<td>Date Ackn'd</td>
<td>06/01/2022</td>
</tr>
<tr>
<td>CU2 Project Title</td>
<td>Immunometabolic analysis of infected glial and myeloid cells</td>
</tr>
<tr>
<td>Class</td>
<td>Class 2</td>
</tr>
<tr>
<td>CultureVolClass2</td>
<td>≤ 1 Litre</td>
</tr>
<tr>
<td>CultureVolumeClass3-4</td>
<td></td>
</tr>
<tr>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

02/03/2022
Fluorescent tagged bacteria (M. bovis BCG, S. pneumoniae and S. pyogenes) will be used for microscopy and/or flow cytometry purposes to identify percentage of infected cells and their cellular location and potential interaction with other molecules. S. pneumoniae and S. pyogenes without bacterial capsule may also be used to study the effect of the bacterial polysaccharide component in modulating the host immunometabolic responses.

Recipient or parental organism

No effect is foreseen as fluorescent tag is not known to be transferable across species or to increase virulence. Bacterial strains without the capsule are known to be more readily killed by the host cells as they cannot easily escape from phagocytosis. The non-capsule strains are highly unlikely to acquire genes for the capsule through the environment as horizontal transfer of such large genetic regions is rare.

Host/vector system

Fluorescent tagged strains are expected to have the same effect as parental strains to the host. For bacterial strains without the capsule, they are expected to have the same or less impact to the host than the parental strains due to loss of major virulence factor.

Origin & function

For the fluorescent tag, green fluorescence protein (GFP) originates from jellyfish, while red fluorescence protein (RFP) originates from Discosoma sea anemones. These proteins emit visible colour when exposed to light and have long been used in microscopy. For the uncapsulated bacteria (Streptococci species), some of the strains are naturally occurring, while others are genetically modified by removing genes that encode for the polysaccharide capsule (conserved genetic regions between dexB and aliA genes). Capsules are major virulence factor of Streptococci species that allow the bacteria to escape host immune response such as phagocytosis. It is important to use the GM strain to allow a direct comparison to the parental strain.

Evaluation of foreseeable effects

The fluorescently tagged strains are expected to have identical properties as to their unmodified parental strains counterparts. Genetic removal of capsule from Streptococci bacteria is anticipated to have either no effect or to weaken the virulence of the bacteria as the capsule is a major virulence factor.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
All GM waste will be disinfected and disposed in accordance to COP. Plastic containers used to culture GM microbes or GM-infected cells will be disinfected for at least 30mins with approved disinfectants (e.g. Virkon, Distel, or Microsol) before being discarded in biohazard bags for sterilisation (autoclaving). Other work surface such as biosafety cabinet will be sprayed with 70% Ethanol for disinfection.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

All members of the committee agreed that the project is GM class 2.

### Project Containment

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### Project Ref 31/22.2

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<th>CU2 Project Title</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<tbody>
<tr>
<td>25/02/2022</td>
<td>Immortalization of human and mammalian primary cells</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Consent Granted</td>
<td></td>
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</tbody>
</table>

Project notified under transitional arrangements N
**Project Additional Information**

### Purposes of the contained use
To immortalize primary human and mammalian cells to produce cell line models for in vitro investigation.

### Recipient or parental organism
**Human cell line HEK-293T** - for virus particle production.

Primary human and mammalian cells (for example stromal and endothelial cells). Human and mouse embryonic stem cells and adult stem cells either obtained commercially or from collaborators and repositories or from biopsy material (for example; mesenchymal stem cells).

### Host/vector system
**Lentivirus (or retrovirus) cultured in HEK-293T cells for oncogene induction (e.g. hTERT, HPV, BMI-1, SV-40)** in recipient (primary) cells for the purpose of their immortalization.

**Human cell line HEK-293T** - for virus particle production.

### Origin & function
The genes to be expressed for immortalization are potential oncogenes such as:
- **human telomerase reverse transcriptase (hTERT)**. hTERT is a ribonucleoprotein polymerase that maintains the length of telomeres. Telomerase expression plays a role in cellular senescence and is normally suppressed in postnatal somatic cells.

- **human papilloma virus 16 E7 (HPV16-E7)**. HPVs have been found in 90% of cervical cancers as well as other carcinomas. Viral early proteins E6 and E7 induce immortalization and transformation in a variety of rodent and human cell types.

- **SV40 Large T-antigen (from the DNA tumour virus simian virus 40)**. The SV40 large T-antigen is found in early region of the virus together with the small T-antigen which together are sufficient for transformation of cell lines.

- **B lymphoma Mo-MLV insertion region 1 homolog (BMI-1)**. Overexpression of Bmi1 role in several types of cancer, such as bladder, skin, prostate, breast, ovarian, colorectal as well as hematological malignancies. Bmi-1 is required for the maintenance of some adult stem cells partly because it represses genes that induce cellular senescence and cell death.

### Evaluation of foreseeable effects
The use of lentiviral second generation (or later) vector systems, in which all accessory genes have been deleted, and where gag, pol, env and rev genes are provided in...
trans (either on additional accessory plasmids or integrated into the genome of packaging cell lines), means that the risk of production of replication-competent or infectious virus is effectively zero.

Most commonly the crude viral supernatant from a packaging cell line (where the max viral titre is unlikely to be higher than 10E+9 particles per ml), will be used to transduce a primary cell culture. In those circumstances, and in the absence of significant concentration of the viral titre and elimination of sharps, it can be argued that the risk of accidental systemic administration, such that the vector is delivered to the in sufficient quantity to induce a significant health hazard (e.g. hepatocellular carcinoma linked to WPRE protein X), is negligible. Thus the use of lentivirus would be assigned to Class 1 (provided that there is nothing more hazardous in the vector - see below), even in the presence of WPRE expressing protein X.

However, given that inserted immortalization genes can pose a specific oncogenic hazard, and the expression of these known or potential oncogenes could act in concert with the insertional effects (lentiviral vector system) to contribute to oncogenic pathways (but only in the event of self-inoculation), this work could pose an increased risk to workers and thus is required to be assigned as Class 2. This risk will be controlled by eliminating sharps, and by other measures at Containment Level 2.

The following activities will NOT be permitted within programme of work:-
1. Preparation of high-titre retroviral or lentiviral stocks by methods that require the use of needles to harvest the virus
2. The use of sharps, including hypodermic needles and glass pipettes
3. Expression of a major pathogenesis factor or a toxin gene.
4. The use of retroviral or lentiviral vectors for which there is evidence for the presence of replication-competent virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste:
Tissue culture medium/liquid waste will be treated with Virkon for 24 hours, the final concentration of Virkon must be 2% or higher. Virkon treated liquid waste (minimal contact time of 24 hours) an then be disposed off down drain.

Solid waste (plasticware, tips, cell pellets etc.):
All solid waste (pipettes, tips, plasticware etc.) will be treated by immersion in 2% virkon overnight (24 hours), then autoclaved at 121°C for 30 min in a validated autoclave before being disposed in clinical waste route.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
In the risk assessment Bio 1 form, immortalisation of primary cells using lentiviral vectors is considered by the Imperial Biosafety team as containment level 2 because of the cumulative risks. Other activities on this Bio 1 form are considered containment level 1.

Following an overview of the project by WS, the committee discussed various aspects of the work with particular reference to what classification the work required. The work involves using replication incompetent 2nd and 3rd generation of adeno, lenti and retro viruses to transfect and immortalise primary adipocytes. It was confirmed that the viruses would be pseudotyped with VSV-g and that some of the work would involve using viral vectors containing transformative hTERT, HPVE6/7 or BMI-1 oncogenes. However, the target cells would initially only be exposed to a single vector (vector constructs would always contain only one of the oncogenes). If necessary, and following a period of 2-3 weeks, selected cells may undergo a second transfection with a vector containing an alternative oncogene. It was confirmed that doing this would not significantly increase any risk to the researchers.

The use of VSV-g pseudotyped viruses was deemed the most appropriate technique to create the desired immortalised cells. Buying in pre-immortalised cells would be prohibitively expensive and use of alternatives such as liposomes is ineffective in these cells.

It was deemed that not all the work described in the bio 1 necessarily falls under a class 2 classification. However, HSE guidance indicates that in a situation as described in this assessment where there is a combination of a pseudotyped virus containing oncogenes then this would require containment level 2 control measures and, as such, this work needs to be classified as class 2. Once cells have been transfected with the lentivirus and after there has been a period of washes and passaging, those cells can be taken out and worked with at containment level 1 as the replication deficient lentivirus will have been cleared from the cultures.

The following information is required prior to approval:

1. Amend the overall project classification to GM class 2
2. Section 4.5: tick box answer should be changed to yes
3. The distinction between the different gene work and their classification needs to be made clear on the bio1 – oncogenes in VSV-g pseudotyped viruses for immortalisation is class 2 but other genes for screening metabolic functions in similar pseudotyped viruses is class 1.

**Project Containment**

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Project Ref** 31/97.1

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02/03/2022 Page 1550 of 15326
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid cultures for GMMs will be decontaminated with virkon or a similar agent overnight before disposal down the drains. Plating out decontaminated liquid will confirm the GMMs have been killed. All solid waste (bacterial plates, tips, stripettes, sharps that have come into contact with GMMs) will be autoclaved within the department before disposal by incineration. Autoclaves are maintained and monitored routinely. All waste management has been covered by risk assessment (GMIC-1375).

13. * Is an emergency plan required according to regulation 20?

Yes No
Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  N
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment
Project Ref 32/05.3

Date Ackn'd 22/12/2005

CU2 Project Title Genetic Modification of Endothelial, Dendritic and Mesenchymal Stem Cells.

Date Project Ceased 08/02/2022

Class  Class 2

Culture Volume Class 2  1 L

Consent Granted Not Applicable

Non-GMM

Tick if notifying a connected programme of work  Y

Historical Significant Changes This should have been notified originally under GM31 but was put under GM32 - has now transferred back to GM31 as of 22/12/2005

Project notified under transitional arrangements N

Recipient or parental organism

E. coli DH5α cells will be transformed with pONY3.1 pRV67 and pSMART2G (or a genetically modified form of pSMART2G - see below). 293T cells (human embryonic kidney cell line) will then be co-transfected with 3 vector plasmids to generate single cycle infective pseudotyped EIAV viral stocks. D17 cells (canine osteosarcoma cell line) will be transduced for routine titration of lentiviral vector stocks. Dendritic, endothelial and mesenchymal stem cells will be transduced with genetically modified EIAV lentivirus for in vitro and in vivo studies.

02/03/2022 Page 1554 of 1532602/03/2022
Host/vector system

Vectors for third-generation, self-inactivating (SIN) EIAV lentivirus will be used (provided by Oxford Biomedica). Functional, non-replicating/self-inactivating EIAV lentiviruses generated by modifying the transcriptional enhancer and promoter regions in the 3' U3 long terminal repeats (LTR). The following lentiviral plasmids will be used:

- pSMART2G - Oxford Biomedica (GFP expression construct)
- pONY 3.1 - Oxford Biomedica (EIAV gag/pol expression construct - also expressing Tat, REV,S2 and part of ENV)
- pRV 67-Oxford Biomedica (VSV-G expression construct - ENV expression)
- pESynRev - Oxford biomedica
- pONY 8.7 NCG Si - Oxford Biomedica (GFP expression construct)

Origin & function

The immunomodulatory genes used encode intracellular fusion proteins such as CTLA4-KDEL and ICOS-KDEL, and extracellular fusion proteins such as CTLA4-Ig, ICOS-Ig, PDL-1/L-2 Ig, and CD83-Ig. CTLA-4, ICOS and PDL-1/L-2 are molecules involved in T cell costimulation, and KDEL is an endoplasmic reticulum retention sequence. IDO (indoleamine 2,3-dioxygenase) is a tryptophan-degrading enzyme that has been demonstrated to have immunoregulatory properties. The enhanced green fluorescent protein (eGFP) is a protein obtained by adding an enhancer sequence to a naturally fluorescent protein (GFP) found in the jellyfish Aequorea victoria.

The aim of the scientific project is to genetically modify dendritic and endothelial cells to express novel intracellular fusion proteins and an immunomodulatory enzyme such that there is a substantial downregulation in T cell alloreactivity and subsequent induction of transplantation tolerance. This would be achieved by inhibition of dendritic cell/T cell co-stimulation in the former case (due to the retention of key costimulatory ligands within the endoplasmic reticulum of dendritic cells), and T cells tryptophan starvation in the latter case (due to upregulation of IDO activity in dendritic cells). EIAV lentiviruses will be used to genetically modify dendritic and endothelial cells.

Mice will be injected with dendritic cells that have been transduced with EIAV lentiviral vectors to express the intracellular fusion proteins stated above. Corneal and heart transplantation will have to be carried out on mice for the latter/in vivo stages of the project. Histopathological analysis will have to be carried out on mice injected with transduced cells for the in vivo stages of the project.

Mice will be injected with Mesenchymal Stem cells that have been transduced with EIAV lentiviral vectors to express the eGFP reporter protein stated above.

The aim of the scientific project is to genetically modify dendritic and endothelial cells to express novel intracellular fusion proteins and an immunomodulatory enzyme such that there is a substantial downregulation in T cell alloreactivity and subsequent induction of transplantation tolerance. This would be achieved by inhibition of dendritic cell/T cell co-stimulation in the former case (due to the retention of key costimulatory ligands within the endoplasmic reticulum of dendritic cells), and T cells tryptophan starvation in the latter case (due to upregulation of IDO activity in dendritic cells). EIAV lentiviruses will be used to genetically modify dendritic and endothelial cells.

Evaluation of foreseeable effects

There is no human pathogenicity associated with the lentiviral vector to be used in this activity (Equine infectious Anaemia Virus). There is no possibility of in vivo infection and subsequent in vivo propagation. E.Coli DH5a cells (which will be transformed to produce the 3 vectors pSMART2G/pSMART-CTLA-KDEL/pSMART-IDO, pRV67 and pONY 3.1) are non-colonising and disabled, and we are not pathogenic to humans or animals.

Although the gene product expressed will be biologically active, there are no hazards or pathogenicity associated with the proposed intracellular and extracellular fusion proteins. The fusion proteins will only express the extracellular porteins of the T cell co-stimulatory markers. The enhanced green fluorescent protein (EGFP) is a protein obtained adding an enhancer sequence to a naturally fluorescent protein (GFP). No specific hazard can be identified.

The host strains are not human pathogenic and the inserted gene does not subsequently alter/increase the pathogenicity of the host. A3 plasmid technique will be used for transduction of endothelial and dendritic cells - EIAV is not human pathogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Cellular work will be undertaken within a designated containment 2 laboratory which is adequately separated from other areas. This containment 2 laboratory has restricted access only to workers who will be involved in using intracellular and extracellular fusion proteins for the T cell immunomodulatory effects stated in section 7. A microbiological safety cabinet will be required for routine cell culture of 293T cells, D17 cells, and dendritic and endothelial cells that will be transduced with the lentiviral vector stocks. Aerosols or splashes generated during the activity will not pose a risk of infection to the workers. No other form of local exhaust ventilation will be required. There are no particular requirements for room ventilation.

Static shelf incubators will be used for the culture of 293T cells post co-transfection with three vectors to generate single-cycle infective pseudotyped viral stocks. Shelf incubators will also be used to culture dendritic and endothelial cells transduced with EIAV lentiviral vectors.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Lentiviral vector supernatant stocks will be stored in liquid nitrogen within protective cryotubes with screw-top lids. This will prevent any release of material until cryotubes are opened within microbiological safety cabinets for subsequent transduction of cells of interest. Security measures are in place for liquid nitrogen storage—routine catalogues are maintained. Any missing samples from liquid nitrogen compartments would be immediately identified. Ultracentrifugation (~17,000 RPM) will be used to concentrate lentiviral supernatant harvested from transfected 293T cells, and centrifugation will be used to pellet lentivirus-transduced cells. Buckets will be used with screw-top lids for this activity. In the case of a leak or spillage, immediate containment, extensive use of Virkon and spillage granules will be used if necessary.

Protective laboratory coats are always available, laundered weekly, and stored in the department wash room. Powder-free nitrile gloves will be worn during any cell culture activity associated with this project. Face shields will be used for liquid nitrogen usage. All staff to be involved with project have experience with genetic modification and have attended safety courses.

Vaccination is not required as EIAV is not human pathogenic. The GMM does not have the ability to establish in vivo infection in humans and subsequent in vivo propagation. Health Surveillance is not required. Hepatitis B immunisation would be required (related to isolation of peripheral blood mononuclear cells (PBMCs) not related to GMM product).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For liquid GMO cultures and non-disposable material, 2% Virkon will be used for a maximum of 12 hours. Virkon has been tested by independent laboratories and been proven to be effective against a total of 20 virus families (including HIV/AIDS), 43 bacterial genera and 27 fungal genera Disposables that come into contact with liquid GMO cultures will be autoclaved at a 134°C, a sterilisation time of 20 mins, and full cycle time of 1 hour 44 mins. This treatment will be monitored using test strips and validated using quarterly multi point thermocouple tests of the autoclave.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Risk Assessment Designated Class 2, Containment 2 Activity.

Project Containment
Project Additional Information

Purposes of the contained use

The aim of this project is to evaluate the therapeutic potential of selectively replicating ('oncolytic') Herpes simplex virus 1 vectors in human cancers, especially ovarian cancer. Specifically, we aim to evaluate the pathways of cell death activated by oncolytic HSV in ovarian cancer and the role of innate and adaptive immune responses in modulating therapeutic efficacy of oncolytic HSV1 vectors.

Recipient or parental organism

Human ovarian cancer cells; murine ovarian cancer cells; African green monkey kidney cells

Host/vector system
Origin & function

All vectors will be based upon the vector 1716, which is HSV-1 deleted in the neurovirulence gene ICP34.5. 1716 itself has no inserted viral gene products. There are also derivatives of 1716 encoding GFP, Firefly luciferase and RFP. Further double deletion mutants (eg ICP34.5 and ICP47) with increased selectivity for malignant cells may also be evaluated.

Evaluation of foreseeable effects

Deletion of ICP34.5 dramatically reduces neurovirulence. Although 1716 remains capable of replicating within malignant cells, replication is dramatically attenuated in normal neuronal tissue, as confirmed in clinical trials of 1716 and other ICP34.5-deleted vectors. Risk to human health is therefore effectively zero. Parental Wild-type HSV1 causes fatal encephalitis in mice. However, previous data show that 1716 is avirulent in mice, including by direct intracranial injection. There is no reason to suspect that 1716 has enhanced environmental survival factors or altered host range compared with HSV-1, which is already present in the environment. Overall risk to organisms other than humans and to the environment is therefore effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

To minimise the risk to the laboratory staff all work will be undertaken within dedicated MSC class 2 cabinets within class 2 tissue culture facilities. All materials will be decontaminated in 10,000 ppm chlorine (e.g. Virkon, Chlorsept) before removal from MSC class 2 cabinets and autoclave-mediated destruction. Virus is stored at -80°C in cryovials. Following defrosting on ice, cryovials are not opened until placed within MSC class two cabinets, thus minimising the risk of spillage within the lab. In the case of accidental spillage either within or without the MSC class two cabinet, all materials will be decontaminated with 10,000 ppm chlorine.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection
All materials potentially contaminated with 1716 will be decontaminated in 10,000 ppm chlorine (Chlorsept) before removal from MSC class 2 cabinets.

Autoclaving
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving, using the following regime; gradual pulsing of temperature and pressure to final pressure of 3040mBar and temp of 134°C - 39 mins, sterilisation cycle @ 3040mBar, 134°C - 20min, Pressure venting - 1min drying and pressure vent – 30s, air wash, pressure vent and cooling - 3mins, air Break - 1min, cooling - 11mins (100% kill), prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each...
run will be monitored by continuous chart (or digital) recording of the temperature/time profile. Following disinfection and autoclaving, all waste will be incinerated.

The project was assessed as Class 2 by the GM383 Safety Committee in February 2014.

Please enter comments on the GM safety committee on the risk assessment

The project was assessed as Class 2 by the GM383 Safety Committee in February 2014.

**Project Containment**

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<th>Laboratory Activities</th>
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**Project Ref** 8/08.1

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### Project Additional Information

#### Purposes of the contained use

The work will use bacterial plasmids and E.coli K12 to generate eDNA which will be put into lentiviral vectors to deliver genes to cells. The use of the lentiviral vector system will enable the efficient infection of cells grown in tissue culture with lentiviruses expressing genes that control cardiac cell growth, differentiation, and survival.

#### Recipient or parental organism

E.coli K12 will be used and plasmids will include pQE3I, pET28b, pGST. K12 is non-colonising and disabled. Lentivirus particles will be produced by transfection of the 293 human embryonic kidney cell line. Human and mouse cells grown in tissue culture including NIH 3T3, C2C12, primary mouse and rat cardiac myocytes and fibroblasts, miRuse, rat and human stem cells and mouse and human ES cells will be the recipients of virus. These cells will express the transgene but not produce or contain transferral virions so are nongenous.

#### Host/vector system

1) pLL3.7 - Lentiviral vector. 2) Packaging vector - psPAX2 - lacks both LTRs and has no viral packaging signal. rev is supplied in trans on a different vector, expresses no viral gene products. Envelope (VSV C) is expressed on 3) pMD2.G 4) pWPl containing the WPRE element. Second generation lentiviral vectors are replication incompetent. No structural genes are present, therefore no replication-competent virions can be produced.

#### Origin & function

E.coli, all strains are K12 derivatives. K-12 derivatives are recognised as non-colonising and disabled. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. Second generation lentiviral vectors which are replication incompetent. No structural genes are present, therefore no replication-competent virions can be produced.

For shKnockdowns: pLL3J - A Lentiviral vector that expresses shRNA under the mouse U6 promoter. A CMV-EGFP reporter cassette is included in the vector to monitor expression. Packaging vector - psPAX2 - lacks both LTRs and has no viral packaging signal. The following viral genes have been deleted from the packaging vector: env, tat, rev, vpr, vpu, vif and nef. Rev is supplied in trans on a different vector (RSV-Rev). The vector expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion) and expresses no viral gene products. Envelope (VSV C) is expressed on a separate vector - pMD2.G And pWPl containing the WPRE element.

For conditional shKnockdowns: This bicistronic vector allows for simultaneous expression of a transgene and EGFP marker to facilitate tracking of transduced cells. The EGFP marker cDNA has been inserted downstream of EMCV IRES. Tet-on systems will also be used. Any modifications made to the plasmids under this system are biologically neutral and do not change the biosafety of the original second generation lentiviral vectors.

Clonal cell lines including 293, C2C12, 3T3. Primary cells, rat and mouse cardiac myocytes and fibroblasts, Mouse, rat and human stem cells (bone marrow and cardiac). Mouse and human ES cells.

#### Evaluation of foreseeable effects

K12 is non-colonising and disabled, all plasmids used in K12 will not allow propagation of the strain outside of the laboratory environment. Each lentiviral vector produced will be able to infect cells but will not be able to replicate and spread to new cells. The virions produced are replication-incompetent. The foreign proteins that will be expressed in the transfected cell and ultimately injected into mice will not affect the hazard group of the vector or host and will only be expressed by the infected cell. The WPRE element as present in pWPl has been shown to be potentially oncogenic, but as the virus it will be contained within is replication incompetent, an on-going infection in laboratory personnel is not possible.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Bacterial and lentiviral cultures: Liquid culture, sp11s - For metal surfaces that may corrode - Trigene final concentration of 1 :100. For all other surfaces - Viricon final concentration of 2%. Treatment for 30 minutes at room temperature and then disposed of via the drain and paper towels etc will be placed in GM waste bins. Tissue Culture Plastics, pipettes will also be placed in GM waste bins. These will be autoclaved at 121 degrees for 20 minutes and disposed of by the Imperial College clinical waste route which ends in incineration off site. Autoclaves are monitored by a chart recorder with temperature readout and validated by an annual 12 point thermocouple test.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee agreed with the classification of Containment level 2, Class 2 assigned to the project, as lentiviral vectors containing WPRE are to be used and injected into animals necessitating the use of sharps. The P1 was advised to separate out the Class 1 aspects of the project, involving the use of attenuated adenoviral and retroviral vectors so that this work could start after College approvals are in place.

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GM Centre Number: 32

Data Premises Notified (Originally) 01/12/1994

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Name

IMPERIAL COLLEGE SCHOOL OF MEDICINE

Name 2

Department

DIVISION OF BIOMEDICAL SCIENCES

Building

SIR ALEXANDER FLEMING BUILDING

District

Road Name

EXHIBITION RD

Town

LONDON

County

GREATER LONDON

Postcode

SW7 2AZ

Country

ENGLAND

Tel Number 020 7594 3011

Fax Number 020 7594 3100

E-mail

HSE Division LONDON

Comments

Date at Which Additional Info Submitted

23/04/2001
### Premises Addresses

<table>
<thead>
<tr>
<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
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<td>IMPERIAL COLLEGE SCHOOL OF MEDICINE</td>
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<td>SW7 2AZ</td>
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<td>N</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Level 2 (GMMs)</td>
<td>Level 3 (GMMs)</td>
<td>Level 4 (GMMs)</td>
<td>Non-microbial</td>
</tr>
<tr>
<td>Other (please specify)</td>
<td>Tick if confidential</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research |
Virology | Transgenic Animals | Transgenic Fish | Gene Therapy
---|---|---|---
Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

### Project Ref 32/01.1

<table>
<thead>
<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>02/04/2001</td>
<td>DEVELOPMENT OF VIRAL VECTORS FOR GENE THERAPY</td>
<td>Class 2</td>
<td>1-50 litres</td>
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Date Project Ceased: 13/02/2006

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: transferred to GM8 on 13/02/2006

Historical Date of Additional Info: 13/02/2006

Consent Granted: not applicable

Project notified under transitional arrangements: N
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Lentiviral, adeno-associated viral vectors (AAV) and Sendai viral vectors will be used to deliver reporter or therapeutic genes. Wild-type adenoviruses AD5 will be used for AAV titration. The above vectors, except Sendai, be manipulated to receive the genes listed below. Viruses will be produced in cell culture under the appropriate conditions and tested for the absence of replication competence. Each gene will be tested for levels of expression and their effects on the recipient organisms. The vectors below will be used to transfer the gene(s) of interest to recipient hosts. The effects of each vector/gene insert will be screened for therapeutic effects or toxicity on the host.

**Recipient or parental organism**

<table>
<thead>
<tr>
<th>Organism/Vector</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SODKI and LVG-1 cells</td>
<td>(HIV-1 based producers, 3rd generation) will be used to generate defective 3rd generation lentiviruses. (HIV-1 based producers, 3rd generation) will be used to generate defective 3rd generation Lentivirus (HIV-1 based) will be produced by transient transfection using a 4 plasmid transfection system which ensures minimal recombination likelihood. The viral genes used to produce these vectors are the gag, pol, rev and VSV-G envelope genes each on discrete plasmids. (1). pMDLg/pRRE induces the rev-dependent expression of gag and pol from the CMV promoter. (2)p tet rev expressed rev under tet control. (3)pVSV-G expresses the VSV-G envelope. (4). plasmid CLL.GFP.CLL contains the viral backbone with the gene inserts detailed below. All other wild-type viral genes have been removed. Homology has been reduced to a few tens of nucleotides. 293T cells will be used to produce these vectors.</td>
</tr>
<tr>
<td>Sendai virus</td>
<td>will be used as a wild-type recombinant virus. Only the few genes detailed below will be used. No potentially oncogenic genes will be used. Sendai is a respiratory pathogen of rodents which may cause an inflammatory response. The Sendai to be used is derived from the Z-strain. The use of the recombinant virus has significantly less pathogenicity than the wild-type. Animal studies will be short term to determine pathogenic effects. If low pathogenicity is observed then long term studies will be performed on isolated animals. This virus is to be used as an alternative vector which infects the apical surface of lung epithelia unlike the other vectors detailed above which use the baso-lateral surface for infection.</td>
</tr>
<tr>
<td>Adenoassociated viruses</td>
<td>will be produced from 293 cells. See ris assessment form for vector detail.</td>
</tr>
</tbody>
</table>

**Host/vector system**

<table>
<thead>
<tr>
<th>System</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>See above</td>
<td>Adeno-associated virus with rep and cap genes removed encoding the above gene inserts. Plasmid pDG will be used encoding the rep, cap and Ad genes E2A, E4 and VA for production of AAV viruses. 3rd generation Lentivirus (HIV 1 based) with doxycycline repressible expression of REV/gag/pol and the VSV-G envelope protein encoding the above gene inserts. Wild-type Sendai vectors will be used containing the inserts lac Z, GFP or CFTR only.</td>
</tr>
</tbody>
</table>

**Origin & function**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor IX</td>
<td>will be used to correct haemophilia B</td>
</tr>
<tr>
<td>GFP</td>
<td>is a reporter gene for detection of gene transfer</td>
</tr>
<tr>
<td>Lac Z</td>
<td>is a reporter gene for detection of ten transfer</td>
</tr>
<tr>
<td>HGF</td>
<td>is a mitogen used to cause cellular proliferation to allow MLV type retroviruses to infect dividing cells.</td>
</tr>
<tr>
<td>Luciferase</td>
<td>is a reporter gene for detection of gene transfer</td>
</tr>
<tr>
<td>LDLR</td>
<td>will be used to lower levels of low density lipoprotein in the host</td>
</tr>
<tr>
<td>SODKI (HIV-1 based human embryonic kidney producer producer cells, 3rd generation)</td>
<td>will be used to generate defective 3rd generation lentiviruses after tetracycline</td>
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</tbody>
</table>
induction expressing GFP. This cell line has doxycycline repressible and sodium butyrate induction of expression of REV/gag/pol and the VSV-G envelope protein. LVG-1 (HIV-1 based 293 human embryonic kidney producer cells, 3rd generation) will be used to generate defective 3rd generation lentiviruses after tetracycline induction expressing GFP. This cell line has doxycycline repressible expression of REV/gag/pol and the VSV-G envelope protein.

Adenoassociated virus with rep and cap genes removed encoding the gene inserts listed above. AAV will be used to infect Hela (human fibroblasts), HT1080 (human fibroblasts), NIH3T3 (mouse fibroblasts), HEPG2 (human liver hepatocyte cells) HUH 7 (human liver hepatocyte cells), COS 7, CHO (Chinese hamster ovary cells), V79 (Chinese hamster lung cells).

A plasmid pDG will be used encoding the rep, cap and Ad genes E2A, E4 and VA for production of AAV viruses from 293 cells. 3rd generation Lentivirus (HIV 1 based) with doxycycline repressible expression of REV/gag/pol and the VSV-G envelope protein. Sendai virus will be wild type and only contain lac Z, GFP or CFTR genes to recipient organisms.

Evaluation of foreseeable effects

The introduction of the above genes and vectors into recipient organisms will not alter the pathogenicity of the genes used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials used in this work will be disinfected using 1% Virkon and autoclaved as per manufacturer's instructions. Waste will include solid and liquid. Degree of kill be expected to exceed 90%. Sterilised materials will be tested for kill by tissue culture infectivity assays to ensure viruses are inactive. The testing procedures will be carried out for each virus preparation and use.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Imperial College Safety Committee has accepted the containment levels proposed.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<tbody>
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</tbody>
</table>
Purposes of the contained use

The lentiviral vectors contain wood chuck hepatitis posttranscriptional regulatory element (WPRE) to enhance transgene expression. This element may be capable of expressing part of the X protein from WHV. A number of publications have suggested that truncated hepadna virus X-proteins may have oncogenic properties and as such WPRE are currently being investigated for oncogenic potential. Appropriate precautions in line with class 2 work therefore need to be used until this has been evaluated.

Recipient or parental organism

Plasmids will be cloned and amplified using competent E. coli cells. Bacteria containing plasmids pose no additional risk to human health.

Lentivirus, AAV and adenovirus will be produced by transient transfection using several plasmids to ensure minimal likelihood of recombination. Vectors will be monitored for replication competence. All viral vectors are designed with low homology to prevent recombination with the host. The multiplasmid transfection system ensures the likelihood/possibility of recombination is effectively zero. Genetic material needed to create a wild-type virus is not carried over with the viral particles and therefore recombination cannot take place.

The GMM cannot survive outside laboratory conditions and so hazard to the environment is minimal. None of the genes to be inserted encode a pathogen or pathogenic determinant or are known to be oncogenic.
The lentiviral vector contains Woodchuck hepatitis post-transcriptional regulatory element (WPRE) to enhance transgene expression. This element however may be capable of expressing part of the X protein from WHV. A number of publications have suggested that truncated hepadna virus X-proteins may have oncogenic properties and as such WPRE are currently being investigated for oncogenic potential. Appropriate precautions in line with class 2 work therefore need to be used until this has been evaluated.

Adenovirus is deleted in E1 rendering it replication defective and therefore unable to cause illness.

AAV production can involve the use of a helper virus, usually wild-type adenovirus or Herpes Simplex Virus. Although both of these can infect humans, the infections are easily treatable.

The GMM cannot survive outside laboratory conditions and so hazard to the environment is minimal. In essence no hazards arise from the alteration of existing traits of the host.

Host/vector system

Replication defective HIV-1 based lentivirus will be produced using a 3/4 plasmid transfection system.

Packaging plasmids:
- pSPAX2 is a second generation packaging plasmid, deleted in viral auxiliary genes vpr, vif, vpu and nef and without env. The HIV-1 viral genes remaining are gag (virion structural proteins), pol (retrovirus specific enzymes), rev (post transcriptional regulator) and tat (regulatory gene) under a CAG promoter (consisting of a CMV enhancer, chicken b-actin promoter and chicken b-actin intron).
- pMDLg/pRRE and pRSV-Rev are third generation packaging plasmids. pMDLg/pRRE contains genes gag and pol under control of CMV promoter and gene Rev is positioned on plasmid pRSV-Rev separately.
- Envelope: pMD2.G

Expressing the VSV-G envelope using a CMV promoter.

Vectors: This is the only genetic material transferred to target cells and contains the viral backbone and transgene cassette.
- pWPXLd-GFP: a standard vector containing an EGFP reporter gene expressed from an EF-1alfa promoter.
- pWPT-GFP: a standard vector containing an EGFP reporter gene expressed from an intron-less version of EF-1alfa, EF-short.
- pWPI: a bicistronic vector allowing simultaneous expression of a transgene and EGFP marker to facilitate tracking of transduced cells using EF-1alfa promoter.
- pHRsinSFFV: Spleen focus forming virus (sFFV) promoter is driving expression of the transgene, which is fused to the EGFP reporter.

Production of replication defective adenovirus is through homologous recombination with pAd.CMV-Link.1 derived plasmids and Ad5 strain d17001. This strain is deleted in E1 and the majority of the E3 region (3.2kb). The resulting adenovirus will contain a therapeutic or reporter gene under the control of CMV promoter.

AAV vectors are based on the non-pathogenic human papovavirus and are deleted for virally alal virally encoded proteins. The vectors consist of an expression cassette and minimal cis elements necessary for replication and packaging of the recombinant genome. AAV replicative and structural (rep and c ap) genes are supplied in trans on a separate plasmid. Production of AAV is possible by transfecting these plasmids into adenovirus or HSV infected cells. It can also be achieved by co-transfecting with a "helper plasmid" containing the minimal Ad components necessary for helper-function thus avoiding use of helper virus. Replication defective adeno-associated virus of various serotypes will be produced using either a helper plasmid or virus and used to investigate and determine the optimal AAV vector/serotype for the transduction of specific cell types of interest. See ref. JP. Louboutin, L. Wang and J M Wilson J Gene Med2005; 7:442-451. Once this is determined AAV containing therapeutic genes will be produced.

Origin & function

Genes to be inserted:
- EGFP (Enhanced Green Fluorescent Protein) is a reporter gene which will aid in determining efficiency of gene transfer.
- REP-2 is a functional isoform of REP-1 and will be used as a control to demonstrate specific requirements for REP1.
GDI extract Rabs from the membranes and can recycle them. Rab 27a, 27b, 38 and others participate in intracellular transport and targeting of proteins. Rab effectors, such as melanophilin and myRIP, determine specificity of Rab function and participate in formation of complexes with other molecules such as motors (myosin V, VII) and cytoskeletal elements (actin).

**Evaluation of foreseeable effects**

No hazards should arise directly from the inserted gene product or by alteration of existing traits.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Due to the presence of the WPRE in the lentiviral vectors all work involving these vectors will be carried out strictly in line with class 2 work.

Adenovirus and AAV are class 1.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste such as tissue culture supernatant will be chemically treated with 2% Virkon overnight, validation will be according to manufacturers instructions and disposal will be down the sink.

Solid waste such as pipette tips, culture plates and glass pasteur pipettes will be autoclaved for 20 mins at 120 degrees C 15lb/sw.inch. Validation will be via qualitative monitoring and chart recoder in addition to an annual service maintenance contract. It will then be incinerated and disposed of as clinical waste by an off site contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

Lentivirus vectors containing WPRE are potentially oncogenic. At the moment this is one of the most efficient to use, although in future a safer alternative may be found. The researches are proposing to use both lentiviral and adenoviral vectors, if can avoid lentiviral then will.

Committee advised the Principal Investigator to incorporate adeno-associated vector (replication deficient Cat 1) to risk assessment.

This project is notifiable to the HSE.

Agreed: Class 2, containment level 2.
**Project Additional Information**

**Purposes of the contained use**

The lentival vectors contain wood chuck hepatitis posttranscriptional regulatory element (WRPE) to enhance transgene expression. This element may be capable of expressing part of the X protein from WHV. A number of publications have suggested that truncated hapapdna virus X-proteins may have oncogenic properties and as such WPRE are currently being investigated for oncogenic potential. Appropriate precautions in line with class 2 work will therefore need to be used until this has been evaluated.

**Recipient or parental organism**

Plasmids will be cloned and amplified using competent E. Coli. Bacteria containing plasmids pose no additional risk to human health.

Lentivirus and adenovirus will be produced by transient transfection using several plasmids to ensure minimal likelihood of recombination. Vectors will be monitored for replication competence. All viral vectors are designed with low homology to prevent recombination with the host. The multiplosasms transfection system ensures the likelihood/possibility of recombination is effectively zero. Genetic material needed to create a wild-type virus is not carried over with the viral particles and therefore recombination cannot take place.
The GMM cannot survive outside laboratory conditions and so hazard to the environment is minimal.

None of the genes to be inserted encode a pathogen or pathogenic determinant.

The lentiviral vector contains Woodchuck hepatitis post-transcriptional regulatory element (WPRE) to enhance transgene expression. This element however may be capable of expressing part of the X protein from WHV. A number of publications have suggested that truncated hepatitis virus X-proteins may have oncogenic properties and as such WPRE are currently being investigated for oncogenic potential. Appropriate precautions in line with class 2 work therefore need to be used until this has been evaluated.

Adenovirus is deleted in E1 rendering it replication defective and therefore unable to cause illness.

The GMM cannot survive outside laboratory conditions and so hazard to the environment is minimal.

In essence no hazards arise from the alteration of existing traits of the host.

Host/vector system

HIV vectors
Replication deficient HIV-1 based lentivirus will be produced using a 4 plasmid transfection system to generate vectors.

Packaging plasmids: pMDLg/pRRE and pRSV-Rev are third generation packaging plasmids. pMDLg/pRRE contains genes gag and pol under control of the CMV promoter and gene Rev is positioned on plasmid pRSV-Rev.
Envelope: pMD2.G expresses the VSV-G envelope from a CMV promoter.
Vector: This is the only genetic material transferred to target cells and contains the viral backbone and transgene cassette.

pWPI is a bicistronic vector allowing simultaneous expression of a transgene and EGFP from the EF-1alpha promoter.
pWPXl-d-GFP: a standard vector containing an EGFP reporter gene expressed from an EF-1alpha promoter.
pWPX-t-GFP: a standard vector containing an EGFP reporter gene expressed from an intron-less version of EF-1alpha, EF-short.

Adenovirus vectors
Production of replication deficient adenovirus is through homologous recombination between pshuttle (an expression cassette containing the gene of interest and promoter) and pAdEasy-1 (plasmid containing the adenoviral backbone deleted in E1 and E3). The resulting adenovirus will contain a gene under the control of CMV promoter.

Origin & function

Genes to be inserted:
TGFbeta (transforming growth factor) superfamily members and downstream effectors (to include TGFbeta, activin and bone morphogenic protein and the receptor serine threonine kinases (ALK1-6) and Smads). We will initially focus on the Smad proteins: The transforming growth factor-beta superfamily members transduce their signal from the membrane to the nucleus through distinct combinations of transmembrane type I and II serine threonine receptors and their downstream effectors, the Smad proteins, which play a central role in transduction of receptor signals to specific target genes in the nucleus (In the first instance Smad2 will be used).

Cyclin dependent kinase inhibitors. Progression through the cell cycle is regulated by cyclins and cyclin dependent kinases (CDK). The kinase activities of the cyclinD/CDK complexes are negatively regulated by several different molecules including the WAK1/CIP.KIP family which exercise broad acting inhibition of the CDKs and includes p21waf, p57kip2 and p27kip1 and the INK4 family, which specifically inhibit CDK4 and CDK6 and include p15, p16, p18 and p19. (In the first instance p21waf will be used).

EGF (epidermal growth factor) receptor family: The EGF family exert their biological effects by binding to and activating the EGF receptor (c-erbB1), a receptor tyrosine kinase.

02/03/2022
kinase, which occupies a prominent role as a primary regulator of epithelial cell function. (In the first instance c-erbB1 will be used).

FGF (fibroblast growth factor) receptor family: The FGF family contains 23 members in mammals and have been implicated in regulation of many key cellular processes including proliferation, differentiation, migration, apoptosis, angiogenesis and wound healing. They bind to five related, specific cell surface receptors.

Evaluation of foreseeable effects

No hazards should arise directly from the inserted gene product or by alteration of existing traits.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Due to the presence of the WPRE in the lentiviral vectors all work involving these vectors will be carried out strictly in line with class 2 work. Adenovirus are class 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste such as tissue culture supernatant will be chemically treated with 2% Virkon overnight, validation will be according to manufacturers instructions (see www.antechh.com) and disposal will be down the sink.

Solid waste such as pipette tips, culture plates and glass pasteur pipettes will be autoclaved for 20mins at 120°C 15lb/sq.inch. Validation will be via qualitative monitoring and chart recoder in addition to an annual service maintenance contract. It will then be incinerated and disposed of as clinical waste by an off site contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Following the discussion of your proposal entitled "Molecular and cellular pathways underlying development and resolution of airway remodelling" (GMIC -01421.2) at the last GM Safety Committee made the following comments.

Comments: The Chair clarified that the genes to be inserted were not oncogenic. Lentiviral vectors containing WPRE are potentially oncogenic. This project is notifiable to the HSE.

Agreed: Class 2, Containment level 2.

Project Containment
Project Additional Information

Purposes of the contained use

The induction of transplantation tolerance by genetically modifying denticritic and endothelial cells to express novel intracellular molecules such that there is substantial downregulation of alloresponses. This will be achieved using genetically modified lentiviral vectors (EIAV). The mechanism of homing and engraftment of mesenchymal stem cells will also be evaluated by genetically modifying these cells with a lentiviral vector to express a long-term fluorescent marker.

Recipient or parental organism

E. coli DH5α cells will be transformed with pONY3.1 pRV67 and pSMART2G (or a genetically modified form of pSMART2G - see below). 293T cells (human embryonic kidney cell line) will then be co-transfected with 3 vector plasmids to generate single cycle infective pseudotyped EIAV viral stocks. D17 cells (canine osteosarcoma cell line) will be transduced for routine titration of lentiviral vector stocks. Dendritic, endothelial and mesenchymal stem cells will be transduced with genetically modified EIAV lentivirus for in vitro and in vivo studies.

Host/vector system

Vectors for third-generation, self-inactivating (SIN) EIAV lentivirus will be used (provided by Oxford Biomedica). Functional, non-replicating/self-inactivating EIAV
lentiviruses generated by modifying the transcriptional enhancer and promoter regions in the 3' U3 long terminal repeats (LTR).

The following lentiviral plasmids will be used:

- pSMART2G - Oxford Biomedica (GFP expression construct)
- pONY 3.1 - Oxford Biomedica (EIAV gag/pol expression construct - also expressing Tat, REV,S2 and part of ENV)
- pRV 67-Oxford Biomedica (VSV-G expression construct - ENV expression)
- pESynRev - Oxford biomedica
- pONY 8.7 NCG Si - Oxford Biomedica (GFP expression construct)

**Origin & function**

The immunomodulatory genes used encode intracellular fusion proteins such as CTLA4-KDEL and ICOS-KDEL, and extracellular fusion proteins such as CTLA4-lg, ICOS-lg, PDL-1/l-2lg, and CD83-lg. CTLA-4, ICOS and PDL-1/L-2 are molecules involved in T cell costimulation, and KDEL is an endoplasmic reticulum retention sequence. IDO (indoleamine 2,3-dioxygenase) is a tryptophan-degrading enzyme that has been demonstrated to have immunoregulatory properties. The enhanced green fluorescent protein (eGFP) is a protein obtained by adding an enhancer sequence to a naturally fluorescent protein (GFP) found in the jellyfish Aequorea victoria.

The aim of the scientific project is to genetically modify dendritic and endothelial cells to express novel intracellular fusion proteins and an immunomodulatory enzyme such that there is a substantial downregulation in T cell alloreactivity and subsequent induction of transplantation tolerance. This would be achieved by inhibition of dentritic cell/T cell co-stimulation in the former case (due to the retention of key costimulatory ligands within the endoplasmic reticulum of dentritic cells), and T cells tryptohan starvation in the latter case (due to upregulation of IDO activity in dentritic cells). EIAV lentiviruses will be used to genetically modify dentritic and endothelial cells.

Mice will be injected with dentritic cells that have been transduced with EIAV lentiviral vectors to express the intracellular fusion proteins stated above. Corneal and heart transplantation will have to be carried out on mice for the latter/in vivo stages of the project. Histopathological analysis will have to be carried out on mice injected with transduced cells for the in vivo stages of the project.

Mice will be injected with Mesenchymal Stem cells that have been transduced with EIAV lentiviral vectors to express the eGFP reporter protein stated above.

**Evaluation of foreseeable effects**

There is no human pathogenicity associated with the lentiviral vector to be used in this activity (Equine infectious Anaemia Virus). There is no possibility of in vivo infection and subsequent in vivo progation. E.Coli DH5a cells (which will be transformed to produce the 3 vectors pSMART2G/pSMART-CTLA-KDEL/pSMART-IDO, pRV67 and pONY 3.1) are non-colonising and disabled, and we are not pathogenic to humans or animals.

Although the gene product expressed will be biologically active, there are no hazards or pathogenicity associated with the proposed intracellular and extracellular fusion proteins. The fusion proteins will only express the extracellular portiane of the T cell co-stimulatory markers. The enhanced green flourescent protein (eGFP) is a protein obtained adding an enhancer sequence to a naturally flourescent protein (GFP). No specific hazard can be identified.

The host strains are not human pathogenic and the inserted gene does not subsequently alter/increase the pathogenicity of the host. A3 plasmid technique will be used for transduction of enothelial and dentritic cells - EIAV is not human pathogenic.

**Cellular work will be undertaken within a designated containment 2 laboratory which is adequately separated from other areas. This containment 2 laboratory has restricted access only to workers who will be involved in using intracellular and extracellular fusion proteins for the T cell immunomodulatory effects stated in section 7.**
A microbiological safety cabinet will be required for routine cell culture of 293T cells, D17 cells, and dendritic and endothelial cells that will be transduced with the lentiviral vector stocks. Aerosols or splashes generated during the activity will not pose a risk of infection to the workers. No other form of local exhaust ventilation will be required. There are no particular requirements for room ventilation.

Static shelf incubators will be used for the culture of 293T cells post co-transfection with three vectors to generate single-cycle infective pseudotyped viral stocks. Shelf incubators will also be used to culture dendritic and endothelial cells transduced with EIAV lentiviral vectors.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Lentiviral vector supernatent stocks will be stored in liquid nitrogen within protective cryotubes with screw-top lids. This will prevent any release of material until cryotubes are opened within microbiological safety cabinets for subsequent transduction of cells of interest. Security measures are in place for liquid nitrogen storage-routine catalogues are maintained. Any missing samples from liquid nitrogen compartments would be immediately identified. Ultracentrifugation (~17,000 RPM) will be used to concentrate lentiviral supernatent harvested from transfected 293T cells, and centrifugation will be used to pellet lentivirus-transduced cells. Buckets will be used with screw-top lids for this activity. In the case of a leak or spillage, immediate containment, extensive use of Virkon and spillage granules will be used if necessary.

Protective laboratory coats are always available, laundered weekly, and stored in the department wash room. Powder-free nitrile gloves will be worn during any cell culture activity associated with this project. Face shields will be used for liquid nitrogen usage. All staff to be involved with project have experience with genetic modification and have attended safety courses.

Vaccination is not required as EIAV is not human pathogenic. The GMM does not have the ability to establish in vivo infection in humans and subsequent in vivo propagation. Health Surveillance is not required. Hepatitis B immunisation would be required (related to isolation of peripheral blood mononuclear cells (PBMCs) not related to GMM product).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For liquid GMO cultures and non-disposable material, 2% Virkon will be used for a maximum of 12 hours. Virkon has been tested by independent laboratories and been proven to be effective against a total of 20 virus families (including HIV/AIDS), 43 bacterial genera and 27 fungal genera. Disposables that come into contact with liquid GMO cultures will be autoclaved at a 134°C, a sterilisation time of 20 mins, and full cycle time of 1 hour 44 mins. This treatment will be monitored using test strips and validated using quarterly multi point thermocouple tests of the autoclave.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Risk Assessment Designated Class 2, Containment 2 Activity.

Project Containment
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GM Centre Number: 36

Data Premises Notified (Originally)  
Transferred from 1992 Regs? Y  
Transitional Premises Class 3

Data Premises Closed  
Transitional Premises N

Emergency Plan Required? Y  
Non-GMMs Y  
Withdrawn N

Name

UK HEALTH SECURITY AGENCY (UKHSA)

Name 2

CENTRE FOR EMERGENCY PREPAREDNESS & RESPONSE

Department

DIVISION OF BIOTECHNOLOGY

Campus Estate or Research Centre

Building

PORTON DOWN

Road Name

District

Town

Country

SALISBURY

WILTSHIRE

SP4 OJG

Tel Number 01980 612100  
Fax Number 01980 611310

E-mail

HSE Division WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
Significant Change
36/05.5
36/05.8
36/05.7
36/05.6
36/05.9
36/05.10
36/05.11
Date of Additional Information (significant change only)
28/11/2005
28/11/2005
28/11/2005
28/12/2005
19/12/2005
19/12/2005
19/12/2005

Premises Addresses

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Project Ref 36/00.2A

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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<td>21/02/2001</td>
<td>THE DEVELOPMENT OF VACCINES AGAINST AIDS USING THE SIV MAQUE MODEL</td>
<td>Class 2</td>
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Tick if notifying a connected programme of work N

Withdrawn N

Project notified under transitional arrangements Y
Historical Significant Changes
GM36/03.2, GM36/05.9
Historical Date of Additional Info
03/10/2003, 30/12/2005
Significant Change ID
36/05.9
Date of Significant Change
19/12/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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#### Project Ref 36/00.3

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<td>24/05/2000</td>
<td>DEVELOPMENT OF VACCINES AGAINST AIDS USING SIV CONTAINING DELETIONS WITHIN REGIONS WHICH DO NOT CODE STRUCTURAL PROTEINS</td>
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#### Withdrawn

| N |

#### Historical Significant Changes

| GM36/der, |

#### Historical Date of Additional Info

| 17/07/2001, |

#### Significant Change ID

| 36/05.7 |

#### Date of Significant Change

| 28/11/2005 |

#### Project notified under transitional arrangements

| Y |
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Project Ref: 36/00.4

Date Ackn’d: 03/08/2000

CU2 Project Title: PATHOGENICITY OF CANDIDA ALBICANS PMT-1 MUTANT IN NEUTROPAENIC MICE

Class: Class 2

Non-GMM: not applicable

Consent Granted: 

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: GM36/03.2

Historical Date of Additional Info: 03/10/2003

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 36/01.1

Date Ackn’d 25/01/2001

Date Project Ceased

Withdrawn N

CU2 Project Title THE DEVELOPMENT OF VACCINES AGAINST TB USING THE MOUSE AND GUINEA PIG MODEL

Class 2 CultureVolClass2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N
### Project Additional Information

**Purposes of the contained use**

Recombinant vaccinia virus expressing non-toxic proteins will be used in Mycobacterium tuberculosis (MTB) immunogenicity studies using the mouse and guinea pig models. These recombinant viruses have been constructed elsewhere. No genetic modification will take place at CAMR. The recombinant vaccinia viruses will be propagated in, or used for in vitro assays in, 143B, CV1, EL4 and CEF cells. The viruses will be delivered to animals via several routes for example; intra-nasally or by intra-muscular injection.

**Recipient or parental organism**

Vaccinia virus (VV) or modified vaccinia Ankara (MVA)

**Host/vector system**

Vaccinia virus (VV) or modified vaccinia Ankara (MVA) 143B, CV1, EL4 and CEF cells.

**Origin & function**

The proteins to be expressed individually, are the 38kDa and 19kDa non-toxic proteins of M.tuberculosis. The 38kDa protein is a binding protein in phosphate transport, it is associated with complex-specific antibody response in smear-positive patients and proliferative T-cell responses in infected individuals and after BCG vaccination. The 19kDa protein is a lipoprotein and evokes an antibody response and a proliferative T-cell response in humans. They are intended only to function as antigens after expression in VV or MVA infected cells.

**Evaluation of foreseeable effects**

The functions/properties of the MTB proteins suggest that the tissue tropisms, host range, infectivity and pathogenicity potential of the virus vector will be unaltered by the modification. The efficacy of the vaccinia virus as a protective vaccine will also be unaffected. No disabling feature of the vector will be altered. However, acyclovir and gancyclovir will no longer be effective as thymidine kinase has been disabled. However these are not normally used as therapy against these viruses.

**Effects on Humans:** Vaccinia virus is capable of infecting humans but does not normally cause disease except in immunocompromised individuals. Handling vaccinia virus in Class 2 containment laboratories should prevent release of the virus into the population. When the intra-nasal inoculation of experimental animals is performed this will be performed in a Class 1 safety cabinet, to control increased risk from aerosolisation.

**Effects on Animals:** Vaccinia virus can infect cows, pigs, buffaloes and rabbits. The risk of exposure however is low/negligible when using the appropriate containment levels.

**Effects on the environment:** Vaccinia virus is capable of surviving in the environment for extended periods of time. When the appropriate containment levels are used however the releases of virus into the environment is unlikely.
### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| n/a |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| n/a |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Virus suspension: fluid containing live virus will be inactivated autoclaving (121°C for 10 min) to achieve sterilisation (100% kill). |
| Contaminated glassware: will be autoclaved (121°C for 10 min) to achieve sterilisation (100% kill). |
| Contaminated waste: will be incinerated or autoclaved (121°C for 10 min) to achieve destruction (100% kill). |
| Surfaces: will be decontaminated with 10% Chloros (approx 100% kill) |
| Animal wastes: will be autoclaved to destruction or incinerated. (100% kill) |

### Is an emergency plan required according to regulation 20?

| N |

### If yes, tick to confirm that it is attached to this form

| N |

### Tick to confirm that you have attached a risk assessment to this form

| Y |

### Tick if you are claiming exemption from disclosure for section of the risk assessment

| N |

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## Project Containment

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<th>Laboratory Activities</th>
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<th>Human Clinical Applications</th>
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## Project Ref 36/01.10

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 36/01.11

Date Ackn’d 21/02/2001

CU2 Project Title RECOMBINANTS OF MODIFIED VACCINIA VIRUS ANKARA EXPRESSING 5T4 ONCO-FOETAL ANTIGEN

Class 2

Non-GMM Consent Granted not applicable

Tick if notifying a connected programme of work N

Historical Significant Changes GM36/03.2

Historical Date of Additional Info 03/10/2003

Significant Change ID

Date of Significant Change

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project notified under transitional arrangements Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Individual domains (the enzymatic domain (DipA) and the cell targeting domain (DipB)) of diphtheria toxin will be cloned and expressed in E. coli. The isolated protein domains will be characterised and used in cell targeting experiments.

Recipient or parental organism

E. coli K12 is a laboratory adapted strain of E. coli unable to colonise or survive outside the laboratory.

Host/vector system

E. coli K12

Plasmid vectors are based on pUC and defective for the functions required for transfer to other hosts. The vectors can be considered to be non-mobilisable.
The diphtheria toxin gene was supplied by Dr Cesare Montecucco, University of Padova as a plasmid clone of the non-toxic variant CRM197. The point mutation in CRM197 will be corrected to allow the expression of an isolated active DipA domain.

The dipA and dipB genes will be expressed and the proteins purified. These proteins will be used to define the regions involved in the interaction between the two domains and in cell targeting studies.

Evaluation of foreseeable effects

Individually DipA and DipB are non-toxic.

The host is unable to persist in the environment. The vector system is non-mobilisable and cannot be transferred to other organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be incinerated. Culture waste will be disposed of in 10% hypochlorite solution and autoclaved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The CAMR GMSC had no objections to the risk assessment undertaken.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
**Project Ref**  36/01.5

**Date Ackn'd**  21/02/2001

**CU2 Project Title**

USE OF RECOMBINANT MUTANTS OF SIMIAN DEFICIENCY VIRUS (SIV), MODIFIED WITH THE REPORTER GENE GREEN FLOURESCENT PROTEIN FOR STUDYING THE VACCINE POTENTIAL OF A VESICULAR STOMATITIS VIRUS PSEUDOTYPED SIV

**Class**  Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM**  not applicable

**Consent Granted**

**Project notified under transitional arrangements**  Y

**Withdrawn**  N

**Tick if notifying a connected programme of work**  N

**Historical Significant Changes**  GM36/03.2

**Historical Date of Additional Info**  03/10/2003

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 36/01.6

Date Ackn’d 21/02/2001

CU2 Project Title

USE OF RECOMBINANT MUTANTS OF SIMIAN IMMUNODEFICIENCY VIRUS, MODIFIED WITH THE REPORTER GENE GREEN FLUORESCENT PROTEIN FOR STUDYING INFECTION AND EXPRESSION IN VITRO

Date Project Ceased

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes GM36/03.2

Historical Date of Additional Info 03/10/2003
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
Project Ref  36/01.7

Date Ackn'd  21/02/2001

CU2 Project Title  SUBCLONING AND EXPRESSION OF RECOMBINANT HUMAN TRANSFERRIN (RHTF)

Class  Class 2

Consent Granted  not applicable

Non-GMM  N

Project notified under transitional arrangements  Y

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes  GM36/03.2

Historical Date of Additional Info  03/10/2003

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 36/01.8

Date Ackn'd 21/02/2001  CU2 Project Title SUBCLONING AND EXPRESSION OF DISCREET COMPONENTS OF THE  Class 3  CultureVolClass2  CultureVolumeClass3-4  500ml
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 36/01.9

- **Date Ackn’ed**: 21/02/2001
- **CU2 Project Title**: EXPRESSION OF HETEROLOGOUS GENES IN CLOSTRIDIUM BEIJERINKII NCIB
- **Class**: Class 2
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**

- **GM36/03.2**: 03/10/2003
- **Historical Date of Additional Info**: 03/10/2003
- **Date of Significant Change**

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**Date Project Ceased**

---

**Withdrawn**

---

Tick if notifying a connected programme of work

---

Tick if you are claiming exemption from disclosure for section of the risk assessment

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Tick to confirm that it is attached to this form

---

Tick to confirm that you have attached a risk assessment to this form

---

Tick if an emergency plan is required according to regulation 20?
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref** 36/02.1

**Date Ackn'd** 09/05/2002

**CU2 Project Title** EXPRESSION OF ENZYMATICALLY INACTIVATED TOXINS

**Class** Class 2

**CultureVol** 1-50 litres

**Non-GMM** not applicable

**Consent Granted**

**Date Project Ceased** 

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes** GM36/03.2

**Historical Date of Additional Info** 03/10/2003

**Project notified under transitional arrangements** N

### Project Additional Information

**Purposes of the contained use**

To express and purify inactivated clostridial neurotoxins in E. coli for characterisation.

**Recipient or parental organism**

E. coli K12 is a laboratory adapted strain of E. coli unable to colonise or survive outside the laboratory.

**Host/vector system**

E. coli K12

Plasmid vectors are based on either

(a) pUC based; Defective for the functions required for transfer to other hosts. The vectors can be considered to be non-mobilisable.

(b) pMAL, pET, pGEX based; Defective in mobilisation functions. Considered mobilisation defective.

**Origin & function**
Neurotoxin fragments from native and synthetic genes prepared at CAMR. The toxins have multiple mutations in the active site and are non-toxic. The purified protein will be used for cell binding and delivery studies.

Evaluation of foreseeable effects
The neurotoxin fragments to be expressed are non-toxic. The host is unable to persist in the environment. The vector system is either non-mobilisable or mobilisation defective, minimising the risk of transfer to other organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid waste will be incinerated. Culture waste will be disposed of in 10% hypochlorite solution and autoclaved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

The Committee was broadly happy with this submission. There was some discussion of the likelihood of the mutant form of the L-chain reverting to wild type, and the advantages of employing a deletion mutant. However, 2 separate independent point mutations would be required to produce a protein with activity. The likelihood was therefore very low. However, the Committee did recommend that details were included in the proposal relating to the absolute activity/toxicity of the mutant L-chain.

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Animal Units

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Project Ref 36/02.2
**Project Additional Information**

**Purposes of the contained use**
The work aims to produce novel therapeutic agents for the treatment of disease.

**Recipient or parental organism**
E.coli K12 is a laboratory adapted strain of E.coli unable to colonise or survive outside the laboratory.

**Host/vector system**
E.coli K12
Plasmid vectors are based on the vector pUC or equivalent. Defective for the functions required for transfer to other hosts. The vectors can be considered to be non-mobilisable.

**Origin & function**
Gene fragments from native and synthetic genes prepared at CAMR. The proteins to be expressed are prototype therapeutic agents for treatment of human disease.

**Evaluation of foreseeable effects**
The proteins as expressed are inactive.
The host is unable to persist in the environment. The vector system is non-mobilisable minimising the risk of transfer to other organisms.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be incinerated. Culture waste will be disposed of in 10% hypochlorite solution and autoclaved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

ok

Project Containment

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Project Ref 36/03.1

Date Ackn'd 15/05/2003

CU2 Project Title EXPRESSION OF HUMAN, BOVINE AND MOUSE PRION PROTEIN

Class 2

CultureVolClass2 1-50 litres

CultureVolumeClass3-4

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Historical Significant Changes GM36/03.2
**Project Additional Information**

**Purposes of the contained use**
To use the product in analysis of therapeutic agents.

**Recipient or parental organism**
An especially disabled derivative of E. coli K12 - a laboratory adapted strain of E. coli incapable of surviving outside the laboratory.

**Host/vector system**
X1776, MRC1
pGEX-4T-2, pQE, or pMAL. Plasmids considered to be mobilisation defective.

**Origin & function**
Gene fragments from native and synthetic genes prepared at Health Protection Agency, Porton Down. The proteins to be expressed will be used in the design of therapeutic agents.

**Evaluation of foreseeable effects**
The system described is expected to produce the PrP in the cellular non-pathogenic isoform. The host is especially disabled and therefore incapable of existing either within the human gut or in the environment. The vectors selected are mobilisation defective, minimising any possibility of gene transfer.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Full containment would be level 3, however, as the PrP expressed is not expected to cause the conversion of human PrP to the pathogenic isoform, the HSE guidelines (Part 2A - AnnexIII) indicate a containment level of 2 is sufficient.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Disposal of liquid waste in sodium hypochlorite solution for 12 hours followed by autoclaving. Solid waste by incineration.

---

**Is an emergency plan required according to regulation 20?**

- N

If yes, tick to confirm that it is attached to this form

- N

Tick to confirm that you have attached a risk assessment to this form

- Y
None.

**Project Containment**

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**Project Ref** 36/04.1

- **Date Ackn’d**: 15/07/2004
- **CU2 Project Title**: The Creation of Master and Working Cell Banks for non-replication competent retroviral (Lentiviruses) packaging and producer cell lines
- **Class**: Class 2
- **Culture Volume**: < 1 litre
- **Non-GMM Consent Granted**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Project Additional Information**

- **Purposes of the contained use**: This notification describes our intent to create Master and Working cell banks for retroviral packaging cell lines and non-replication competent retroviral producer cell lines. The viral potency of infection produced by conditioned - medium from producer lines will be assessed using a standard viral titre assay in recipient cells.
In this particular instance we propose to create cell banks from expanded cultures of lentivirus packaging or producer cell lines to the extent where we will be able to cryopreserve stock ampoules of these cell lines, typically 2 x 10^6 (to the power of 6) cells per ampoule. For each cell line we will establish a Master bank of 10 ampoules and a Working cell bank of 25 ampoules. These ampoules of frozen cells will stored in liquid nitrogen vapour-phase and will be eventually distributed to the research community.

Non-replicative competent retroviruses have the ability to infect cells but do not carry the genetic material necessary to reproduce themselves within a cell. They irreversibly integrate into the host DNA and are not capable of producing complete viruses. The safety aspect is achieved by the viral packaging genes being cloned from the wild type virus into a simple plasmids which are then transfected separately into a candidate cell line to produce a packaging cell line. Packaging cell lines containing these cloned genes express the proteins required for the production and packaging of the non-replicative virus.

The viral vector carrying, the gene to be expressed, as an engineered expression vector carrying the minimum number of viral gene sequences, typically just the viral promoter region, required for the expression of that gene. This vector is transfected into the packing cell line where the transcript of the candidate gene is packaged into a non-replicative virus by the expressed proteins from the genes previously transfected into the cell line.

Although recombination is still a theoretical possibility it would require all three packaging genes and the viral vector to combine to produce a replication competent virus. In this unlikely event such a recombinant virus would still lack all the genes required for the production of a complete intact virus. So in this instance no intact HIV virus will be produced.

In this particular instance we propose to create Master and Working cell banks for eight lentivirus packaging cell lines and three non-replication competent lentivirus producer cell lines. To test the viral potency of infection produced by conditioned - medium from the three producer lines using a standard viral titre assay in recipient cells.

### Recipient or parental organism

**Eight packaging cell lines.**

1. **STAR-G.** Human kidney epithelial cell line 293T transfected with HIV synthetic GAG-POL expressed from Murine Leukemia virus (MLV) vector; HIV REV gene expressed from a MLV vector; GALV envelope (with MLV c-tail) expressed from a plasmid.
2. **STAR-RD-pro.** Human kidney epithelial cell line 293T transfected with HIV synthetic GAG-POL expressed from Murine Leukemia virus (MLV) vector; HIV TAT gene expressed from MLV vector; HIV REV gene expressed from a MLV vector; RD114 envelope (with HIV protease cleavage site) expressed from a plasmid.
3. **STAR-A.** Human kidney epithelial cell line 293T transfected with HIV synthetic GAG-POL expressed from Murine Leukemia virus (MLV) vector; HIV TAT gene expressed from MLV vector; HIV REV gene expressed from a MLV vector; amphotropic envelope expressed from a plasmid.
4. **293T-GPRT1+R1.** Human kidney epithelial cell line 293T transfected with wild type HIV-GAG-POL; -TAT and -REV genes expressed from a MLV vector and amphotropic envelope expressed from a MLV vector.
5. **293T-GPRT1 +R1-A.** Human kidney epithelial cell line 293T transfected with wild type HIV-GAG-POL; -TAT and REV genes expressed from a MLV vector and wild type HIV-REV expressed from a MLV vector.
6. **STAR.** Human kidney epithelial cell line 293T transfected with HIV synthetic GAG-POL expressed from Murine Leukemia virus (MLV) vector; HIV TAT gene expressed from MLV vector; HIV REV gene expressed from a MLV vector.
7. **HT-STAR-A.** Human fibrosarcoma (HT1080) transfected with HIV synthetic GAG-POL expressed from Murine Leukemia virus (MLV) vector; HIV TAT gene expressed from MLV vector; HIV REV gene expressed from a MLV vector and amphotropic expressed from a MLV vector.
8. **HT-STAR.** Human fibrosarcoma (HT1080) transfected with HIV synthetic GAG-POL expressed from Murine Leukemia virus (MLV) vector; HIV TAT gene expressed from MLV vector; HIV REV gene expressed from a MLV vector.

**Three producer cell lines.**

1. **STAR-G-HV#2.** Human kidney epithelial cell line 293T transfected with HIV synthetic GAG-POL expressed from Murine Leukemia virus (MLV) vector; HIV TAT gene expressed from MLV vector; GALV envelope (with MLV c-rail) expressed from a plasmid and infected with an HIV vector.
expressing green fluorescent protein (GFP).
2. STAR-Rdpro-HV#1. Human kidney epithelial cell line 293T transfected with HIV synthetic GAG-POL expressed from Murine Leukemia virus (MLV) vector; HIV TAT gene expressed from MLV vector; HIV REV gene expressed from a MLV vector; RD114 envelope protein with HIV protease cleavage site expressed from a plasmid and infected with an HIV vector expressing green fluorescent protein (GFP).
3. STAR-A-HV#2. Human kidney epithelial cell line 293T transfected with HIV synthetic GAG-POL expressed from Murine Leukemia virus (MLV) vector; HIV TAT gene expressed from MLV vector; HIV REV gene expressed from a MLV vector; amphotrophic envelope protein expressed from a plasmid and infected with an HIV vector expressing green fluorescent protein (GFP).

Recipient cell lines: Human kidney epithelial cell line (293). Used in titering the supernatant from producer cell lines.

Host/vector system

The Moloney leukemia virus based vectors served as basic constructs for the generation of plasmids containing HIV gag-pol, Tat or Rev sequences. In particular the construct pCNC-MCS was used to generate the constructs pCNC-GPRT; pCNC-SYNGP; Pnc-Tat and pCNC-Rev.

To construct the producer lines 293T cells were transduced with pH7G; pHRSIN-CSGW and pHV. As described in Ikeda Y et al Nat. Biotechnol 2003 May 21(5): 569-572. UK patent application 0220467.5

Origin & function

The cell lines; STAR-G-HV#2; STAR-Rdpro-HV#1; STAR-A-HV#2; STAR-G; STAR-RDpro; STAR-A; 293TGPRT1+R1-A; 293TGPRT1+r1; star; ht-star-a AND ht-star; were provided by Department of Immunology and Molecular Pathology, Windeyer Institute of Medical Sciences, Windeyer Building, 46 Cleveland Street, London, W1T 4JF. Tel 020-7679-9343; Fax 020-7679-9652.

The packaging cell lines (STAR-G; STAR-RDpro; STAR-A; 293TGPRT1+R1-A; 293TGPRT1+r1; STAR; HT-STAR-A and HT-STAR) are intended as research tools in the production of non-replication competent lentiviruses. The producer cell lines (STAR-G-HV#2; STAR-Rdpro-HV#1; STAR-A-HV#2;) are meant to serve as sources of non-replication competent lentiviruses encoding the marker protein GFP for research studies involving cell tracking and identification.

Evaluation of foreseeable effects

As both cell type described here are intended for research purposes the foreseeable effects cannot be evaluated at this time. Details of the MLV constructs and demonstration that they do not give rise to replication competent MLV viruses are available in the publication; Soneoka Y. et al. Nucleic Acid Res. 23-628-633, 1995.

There is further evidence that such replication defective retroviruses are not pathogenic when tested in monkeys are presented in; Besnier C et al. Proc. Natl. Acad. Sci USA 99: 11920-11920 (2002).

Safety. Supernatants from the stable packaging cell lines and producer cell lines will be for the absence of replication competent virus using assays fro HIV-1 gag sequences and reverse transcriptase activity. Both of these assays are described in the publication Ikeda Y. et al. Nat Biotechnol. 2003 May 21(5): 569-572.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The packaging cell lines and producer clones are classified as ACDP Hazard Group 2. They are genetically modified cell lines developed for the production of non-replication competent viruses and are classified for use at ACGM level 2 and are worked with in Level 2 containment and conditions.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation from level 2 containment and use is expected. All activities with these cell lines will be performed in Lab 237 at the National Pathogenic Virus Collection located in the main building at CAMR.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste generated in class-2 safety cabinet in Room 237:

1. Prior to work taking place, each cabinet is checked to see that it contains a ‘new’ or ‘in use’ liquid waste container. A ‘new’ liquid waste container comprises a 1L polypropylene bottle containing a single formulated tablet containing a chlorine-releasing agent sodium dichloroisocyanurate (NaDCC).

2. Add all liquid waste generated in the cabinet goes into this container, until a final volume of approximately 500 ml is reached (equivalent to a final concentration of 2,500ppm available Chlorine).

3. At the end of each working period, all surfaces of the open bottle are sprayed and wiped down with 0.25% v/v of NaDCC solution, equivalent to a final concentration of 2,500ppm available Chlorine.

4. After all work has finished the capped ‘full’ liquid waste bottle is removed from the cabinet (a ‘new’ waste bottle should be placed in the cabinet immediately).

5. The removed ‘full’ liquid waste bottle is allowed to stand for 24 hours.

6. The inactivated liquid waste can then be disposed of by laboratory personnel into a designated sink with running water.

Solid waste generated in class-2 safety cabinet:

1. All solid waste generated in the cabinet is placed in portex plastic bags.
2. The bagged waste is placed into an autoclave tin.
3. Once the autoclave tin is full. Spray and wipe the outside of the tin with 2,500ppm chlorine-based disinfectant.
4. Transfer the tin to the lobby area; notify Central Autoclaving Services of its presence and await collection.
5. The tin is autoclaved by Central Autoclaving Services.
6. After successful autoclaving, Central Autoclaving Services will return the tin to laboratory 237. Laboratory personnel will transfer the contents to yellow clinical waste sacks for incineration.
7. The waste in yellow sacks is then transferred to an on-site incineration plant for incineration. All steps in the disposal of waste from laboratory 237 must be recorded and signed off on the form shown below. A copy of the form must accompany the waste tin and be returned to the laboratory supervisor.

The use of chlorine-release tablets to make up disinfectant solutions is now well established. Formulated tablets using Sodium Dichloroisocyanurate (NaDCC) a potent chlorinating agent has been shown to be more effective than sodium hypochlorite solution or liquid bleaches where organic material is present.

Bloomfield S F. The bacterial capacity of Sodium dichloroisocyanurate formulations; the sterilisation of infant feeding bottles and teats. Lab Practice 1973. 22. 617-623.


Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
**Project Containment**

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**Project Ref** 36/05.1

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<tr>
<td>See Derogation GM36/15.3a</td>
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</table>

**Project Additional Information**

**Purposes of the contained use**

The European Collection of Animal Cell Cultures (ECACC) offers a service of safe storage, under appropriate conditions, for animal cell lines, viruses and bacteria as a back-up contingency to customers with their own storage facilities. A number of these deposits will be classified as genetically modified organism ACDP category 2 or 3 (GMO 2 or 3) by the depositor and as such will have already received HSE approval. ECACC requires submission of a biohazard risk assessment form with such deposits and evidence of HSE notification and approval prior to shipment from the owner to ECACC. These documents will be reviewed by the GMSC at HPA Porton Down before a deposit is accepted. The safe deposits are stored under appropriate conditions (either liquid nitrogen vapour or -80 degrees C freezer) in the secure ultra-low temperature.
cryostore facility at HPA Porton Down. Samples are always returned to the customer for disposal at the end of the storage period. For depositors outside the UK a biohazard risk assessment is required and, where available, a notification and approval by a national regulatory body with equivalent jurisdiction to the HSE will be sought.

ECACC also provides storage for patent deposits under the terms of the Budapest Treaty. The same requirements are made for these as for the safe deposits (see above). However, under the conditions of the Budapest Treaty, the contents of a representative ampoule has to be tested and shown to demonstrate viability and number of the contents of a representative ampoule thereby conforming with patent requirements. The purpose of this submission is to notify and obtain permission to store deposits (GMO class 2 & 3) at ECACC where the depositor has already demonstrated approval of a regulatory authority equivalent to the HSE for the work at their institution where the GMO was generated.

Further to notify the HSE that ECACC proposes to determine the viability and count the contents of a representative ampoule of a patent deposit under appropriate containment conditions by fully trained personnel. No additional genetic modification will be performed with the deposit at ECACC, HPA Porton Down in connection with the safes and patent storage facility.

Each customer will already have previously demonstrated notification of an appropriate regulatory body (eg HSE) and approval for the work that has generated the GMO class 2 or 3 patent deposit. Each customer will also provide ECACC with a detailed risk assessment for each type of patent deposit. These will be reviewed by the GMSC prior to any patent deposit being allowed on site. Any testing beyond the technical scope of the trained staff at ECACC will be contracted out to other department within the HPA or accredited external contractors.

Manipulation of patent deposits will be kept to a minimum and involve the determination of viability and number of content of a representative ampoule. This type of activity will result in no extensive cell culture, less than 72 hours; less than 100mls of culture medium or other liquid reagent.

ECACC expects the majority of the safes and patent deposits it will store and/or test will be mammalian or non-mammalian cell lines requiring bio-safety level containment up to and including ACDP containment levels 2 and 3. ECACC intends to test cell lines at ACDP 2, in room 237 located at the National Collection of Pathogenic Viruses (NCPV). ACDP 3 cell lines will be tested in the ACDP 3 laboratory (Room 237a) in the NCPV. In addition, room 21 in the ECACC main building is also equipped for ACDP level 2 work. Working with cell lines requiring ACDP2 containment ECACC would expect the most extreme hazard from such cell lines would be that they may produce mobile, self-replicating, transforming viruses or toxic agents. An extreme example of this would be the production of replication competent retrovirus with a broad tropism and containing a transforming gene, such as HPV16E6 and E7 or SV40 large T-antigen. The possibility of such an occurrence will be evaluated and assessed from the biohazard risk assessment and its review by the genetic-modification safety committee (GMSC).

The containment and waste management procedures described in sections 8 and 12 below are designed with this possibility in mind.

**Recipient or parental organism**

ECACC expects the majority of the safes and patent deposits it will store and/or test will be mammalian or non-mammalian cell lines requiring bio-safety level containment up to and including ACDP containment levels 2 and 3. ECACC intends to test cell lines at ACDP 2, in room 237 located at the National Collection of Pathogenic Viruses (NCPV). ACDP 3 cell lines will be tested in the ACDP 3 laboratory (Room 237a) in the NCPV. In addition, room 21 in the ECACC main building is also equipped for ACDP level 2 work. Working with cell lines requiring ACDP2 containment ECACC would expect the most extreme hazard from such cell lines would be that they may produce mobile, self-replicating, transforming viruses or toxic agents. An extreme example of this would be the production of replication competent retrovirus with a broad tropism and containing a transforming gene, such as HPV16E6 and E7 or SV40 large T-antigen. The possibility of such an occurrence will be evaluated and assessed from the biohazard risk assessment and its review by the genetic-modification safety committee (GMSC).

The containment and waste management procedures described in sections 8 and 12 below are designed with this possibility in mind.

**Host/vector system**

The possible cell lines that are anticipated to require storage or patent testing would include but not be limited to the following: animal stem cells, both embryonic and adult, (with current exception of human embryonic stem cells and related tissues); primary cultures derived from various tissues of animal and insect species; established/permanent cell lines (normal or spontaneously transformed; hybridomas or other cell hybrids derived from fusions; cell lines immortalised by introduction of transforming genes chemical mutagens or physical carcinogens (radiation); continuous cell lines produced by insertional mutagenesis; or animal and insect cell lines producing recombinant proteins, disabled or attenuated viruses, growth factors, antibodies, hormones, structural proteins or metabolites.

Animal cell lines described above further genetically modified by transfection or viral transduction to express non-endogenous genes or inactivate endogenous genes by homologous recombination.

**Origin & function**
In the instance where genetically modified cell lines are to be stored as a Safe or Patent deposit the origins and functions of the material introduced into the cell line will be diverse. It is anticipated that they will include but not be limited to: bacterial plasmid constructs containing homologous and heterologous regulatory sequences driving the expression of exogenous genes of bacterial, viral or animal origin. Retroviruses and adenoviruses containing similar genetic sequences as previously described may also be used to modify animal cell lines.

A full listing of most of the plasmid and viral systems commonly used to carry exogenous genes and genetically modified cell lines is given in the Advisory Committee on Genetic Modification Compendium of Guidance, Issue March 2000, Part 2A-Annex II.

Evaluation of foreseeable effects

These will have been evaluated by the depositor and communicated to ECACC in the form of the biohazard risk assessment. Additional guidance on cell lines that have undergone specific types of genetic modification can be sought in the Advisory Committee on Genetic Modification Compendium of Guidance, Issue March 2000, Part 2A-Annex II.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Containment and control measures are to include, but not be limited to, a separate laboratory with an appropriate air handling and monitoring systems for the level of containment. This laboratory will include a microbiological safety cabinet suitable for handling ACDP2 materials (or ACDP3 materials as appropriate), an incubator, if required, hand washing facilities, sink for disposal of disinfected liquid waste and a documented waste management scheme for the disposal of solid waste. Personal protection will consist of appropriate attire (gowns, gloves, hats and masks) which will be worn at all times thereby minimising risk. Further, operatives will be disqualified from working in the Level 2 or 3 containment facilities if they have any pre-existing condition that may render them susceptible to disease following any potential exposure to the biological being tested.

Safe and patent deposits will be supplied by laboratories and institutions outside HPA Porton Down, accordingly European and International regulations govern the packaging and shipment of such biological materials.

The supplier will be responsible for the shipment and will strictly follow the principle of triple packaging systems. This is absolutely necessary in the case of infectious and non-infectious biological substances. It is the obligation of the supplier to comply with the regulations relating to the biological material being transported.

Basic triple packaging system.

The system consists of three layers as follows:

1. Primary receptacle. A labelled primary watertight, leak-proof receptacle will be used to contain the cell lines or biological agents. The receptacle will be wrapped in enough absorbent material to absorb all fluid in case of breakage.
2. Secondary receptacle. A second durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle(s) is to be used. Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material will be used to cushion multiple primary receptacles.
3. Outer shipping package. The second receptacle will be placed in an outer shipping package, which will protect it and its contents from outside influences such as physical damage and water while in transit.

Specimen data forms, letters and other types of information that identify or describe the specimen and also identify the shipper and receiver will be taped to the outside. Accurate and informative signage on the outer shipping package is to be used.

2. Receiving broken or damaged specimen packages

If packages are received from the shipper and the contents are not intact, then the package and contents will be destroyed by disinfection and autoclaving. The shipper (and) courier will be notified and if necessary amendments made to the packaging. Records of destroyed specimens are to be kept.

Storage

Storage of cell lines (or viral isolates) in a state of cryopreservation will be in ampoules stored in liquid nitrogen vapour-phase in ACDP2 and ACDP3 designated tanks with appropriate inventory control. Cell lines up to and including ACDP2 and products derived from such are to be stored in liquid nitrogen vapour in warehouse at ECACC. The
warehouse at ECACC, located at the Health Protection Agency, Porton Down and is a secure site monitored on a 24 hour basis. Cell lines of ACDP2 and higher together with any products derived from such are to be stored in liquid nitrogen vapour in designated tanks at NCPV, room 237.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All activities with these cell lines will be performed in Lab 237/237A at the National Pathogenic Virus Collection located in the main building at HPA Porton Down in accordance with the Bio-hazard risk assessments. Alternatively Lab 21 located in the ECACC building sited next to the main building at HA Porton Down can be used for bio-safety level 2 only. No derogation from bio-safety level 2 or 3 containment and use is expected.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be decontaminated under the appropriate conditions in operation in that particular area of ECACC or the National Collection of Pathogenic Viruses. Currently all procedures requiring bio-safety level 2 containment are performed at Porton Down. Currently all procedures requiring bio-safety level 3 containment are performed in Porton Down. In addition, Lab 21 in the ECACC building is available for bio-safety level 2 work.

FOR BIO-SAFETY LEVEL 2 CONTAINMENT THE WASTE MANAGEMENT MEASURES ARE AS FOLLOWS:

Liquid waste generated in class-2 safety cabinet in Room 237:

1. Prior to work taking place, each cabinet is checked to see that it contains a 'new' or 'in use' liquid waste container. A 'new' liquid waste container comprises a 1L polypropylene bottle containing a formulated tablet containing a chlorine-releasing agent sodium dichloroisocyanurate (NaDCC) in water.
2. All liquid waste generated in the cabinet goes into this container, until a final volume of approximately 500ml is reached (equivalent to a final concentration of 10,000ppm available Chlorine). Four tablets per litre.
3. At the end of each working period, all surfaces of the open bottle are sprayed and wiped down with 0.25% v/v of NaDCC solution, equivalent to a final concentration of 2,500ppm available Chlorine. One tablet per litre of water.
4. After all work has finished the capped ‘full’ liquid waste bottle is removed from the cabinet (a ‘new’ waste bottle should be placed in the cabinet immediately).
5. The ‘full’ liquid waste bottle is allowed to stand for 24 hours.
6. Inactivated liquid waste can then be disposed of by laboratory personnel into a designated sink with running water.

Solid waste generated in class-2 safety cabinet:

1. Solid waste such as serological pipettes and pipette tips generated in the cabinet are disinfected by being immersed in an approximate 1 litre solution chlorine-releasing reagent at a final concentration of 2, 500ppm in container (1 litre polypropylene bottle).
2. All solid waste generated in the cabinet is placed in portex plastic bags.
3. The bagged waste is placed into an autoclave tin.
4. Once the autoclave tin is full. Spray and wipe the outside of the tin with 2,500ppm chlorine-based disinfectant.
5. Transfer the tin to the lobby area; notify Central Autoclaving Services of its presence and await collection.
6. The tin is autoclaved by Central Autoclaving Services.
7. After successful autoclaving, Central Autoclaving Services will return the tin to laboratory room 237.
8. Laboratory personnel will transfer the contents to yellow clinical waste sacks for incineration.
9. The waste in yellow sacks is then transferred to an on-site incineration plant for incineration.
10. All steps in the disposal of waste from laboratory 237 must be recorded and signed off on the form shown below.
11. A copy of the form must accompany the waste tin and be returned to the laboratory supervisor.

The use of chlorine-release tablets to make up disinfectant solutions is now well established. Formulated tablets using Sodium Dichloroisocyanurate (NaDCC) a potent chlorinating agent has been shown to be more effective than sodium hypochlorite solution or liquid bleaches where organic material is present.

Bloomfield S.F. The bacterial capacity of Sodium dichloroisocyanurate formulations; the sterilisation of infant feeding bottle and teats. Lab Practice 1973. 22. 617-623.
FOR BIO-SAFETY LEVEL 3 CONTAINMENT THE WASTE MANAGEMENT MEASURES ARE AS FOLLOWS:

It is a requirement of DEFRA that an audit trail of the waste generated in 237A is kept. (See Code of Safety Practice 1998 - Chapter 11; and COP 237A Addendum 1)

Liquid waste
All aqueous liquid waste generated within the ACDP3 containment laboratory, must be treated with Eegodor to a final concentration of not less than 3%. Prior to work taking place, each cabinet must be checked to see that it contains a 'new' or 'in use' liquid waste container. A 'new' liquid waste container comprises a 1L polypropylene bottle containing 50ml neat Tegador disinfectant.

Add all liquid waste generated in the cabinet to this container, until a final volume of approximately 500ml is reached (ie a final Tegador concentration of approximately 10% v/v)

At the end of each working period, all surfaces of the open bottle are sprayed and wiped down with 3% v/v Tegador, and the cabinet is fumigated as normal. Only after fumigation can the capped 'full' liquid waste bottle be removed from the cabinet (a 'new' waste bottle should be placed in the cabinet immediately).

After venting the fumigated cabinet, remove the 'full' liquid waste bottle and place into a rack within an autoclave tin.

Once the autoclave tin is full; unscrew each of the waste bottle caps by half a turn; place the lid on the autoclave tin; spray and wipe the outside of the tin with 2,500ppm chlorine-based disinfectant.

Transfer the tin to the lobby area; notify Central Autoclaving Services of its presence and await collection. This tin is autoclaved by Central Autoclaving Services on liquid cycle.

After successful autoclaving, Central Autoclaving Services will return the tin now that now contains the disinfected/fumigated/autoclaved liquid waste bottles. The inactivated liquid waste can be disposed of by laboratory personnel into designated Centre for Emergency Preparedness and Response (CEPR) effluent treatment plant for heat treatment prior to release.

Solid waste
All solid waste generated in the cabinet is placed in portex plastic bags and fumigated in the cabinet as normal.

Only after fumigation can the waste be removed from the cabinet after venting. The bagged waste is placed into an autoclave tin.

Once the autoclave tin is full; wipe the outside of the tin with 2,500ppm chlorine-based disinfectant. Transfer the tin to the lobby area; notify Central Autoclaving Services of its presence and await collection.

The tin is autoclaved by Central Autoclaving Services.
After successful autoclaving, CAS will return the tin to laboratory. Laboratory personnel will transfer the contents to yellow clinical waste sacks for incineration.

The waste in the yellow sacks are transferred to on-site incineration plant for incineration.

DOCUMENTATION
All steps in the disposal of waste from laboratory must be recorded and signed off on the form in appendix 1. A copy of the form must accompany the waste tin and be returned to the laboratory supervisor at step 17.2.9 or 17.3.6.
ORGANIC WASTE
All organic solvent liquid waste generated in the ACDP3 containment laboratory, must be treated with 40% formaldehyde (aqueous formalin) solution to a final concentration of not less than 4% (1:10 dilution). Solvent waste generated in the MSCs should be placed into 1L polypropylene bottles and fumigated before removal from the cabinet. Solvent liquid waste must be transferred to a specialist contractor via a SA.

GLASS SLIDES
Glass slides should be disposed of as ‘sharps’; laid out in CAMR Code of Safety Practice 1998 - Chapter 7, Section 5.3

DISINFECTANT
Chlorine-based disinfectants must not be used on metal surfaces as they will cause corrosion. Chlorine-based disinfectant, made up to 2,500 parts per million using Haz-tabs, is to be used for general disinfection of laboratory benches at the end of each work session and at the end of the day. It can be used, in conjunction with Haz-tab absorption granules, to deal with spillage and potentially infectious fluids eg serum etc.

Tegodor is a non-corrosive disinfectant. Tegor is a gluteraldehyde/formaldehyde based disinfectant with quaternary ammonium compounds plus a wetting agent. The concentrate is diluted with distilled water to give a working 3% or 10% solution. 3% Tegodor must be used to decontaminate laboratory equipment that may be attacked by chlorine based disinfectants, eg centrifuges or other apparatus containing metal, prior to servicing or as part of a regular cleaning program. 10% Regodor is used on heavily contaminated areas (eg a spill of virally infected liquid) within the MSC III. Tegador must only be used on a well-ventilated area in accordance with COSHH assessments NCPV/000.

Committee satisfied.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if an emergency plan is required according to regulation 20? N

Tick to confirm that it is attached to this form N

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L2 L3 L4 L2</td>
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**Project Ref** 36/05.3

**Date Ackn'd** 27/09/2005

**CU2 Project Title** Production of Avian influenza vaccine.

**Date Project Ceased**

**Class** Class 3

**CultureVolClass2**

**CultureVolumeClass3-4** 500 cultures

**Non-GMM** Yes

**Consent Granted**

**Project notified under transitional arrangements** N

**Withdrew** N

**Tick if notifying a connected programme of work** N

**GM36/05.11**

**Historical Significant Changes**

**Historical Date of Additional Info** 30/12/2005

**Significant Change ID** 36/05.11

**Date of Significant Change** 19/12/2005

**Historical Significant Changes** GM36/05.11

**Historical Date of Additional Info**

**Significant Change ID** 36/05.11

**Date of Significant Change** 19/12/2005

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**Project Additional Information**

**Purposes of the contained use**

We are making a vaccine from a safe strain of influenza virus. The virus is inactivated with a chemical after production and before release to third parties. We are not doing any of the genetic manipulation at HPA-Porton Down. All safety checks will have been undertaken and validated before transfer from NIBSC to HPA-Porton Down under DEFRA licence.

**Recipient or parental organism**

The strain of influenza virus to be used is comprised of 6 genomic segments from the well-characterised PR8 strain (H1N1) and two segments derived from an avian pandemic strain (e.g. H5N1, H7N1, H7N7 or other currently circulating avian viruses). These 2 segments encode the haemagglutinin (HA) and neuraminidase (NA) genes. The HA gene has been genetically truncated which further reduces its pathogenicity. A reassorted virus has been generated based on the PR8 backbone with the modified HA and NA segments.

**Host/vector system**

See recipient or parental organism section above for full description.

**Origin & function**

Production of GMP seed-stock for manufacture of influenza vaccine.

**Evaluation of foreseeable effects**

Accidental release of the organism: This virus is incapable of entry into and replication in human cells, thus the risk is very low. Virus will be chemically inactivated before removal from the laboratory suite. Recombination with a Wild type Virus: there is a theoretical possibility of this, however the likelihood is extremely low as virus will only be
removed from the suite as killed suspension or in suitable containment. Staff who contract a WT virus will not be exposed to the PR8 reassorted virus as all manipulations are carried out in contained facilities.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation is made.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be decontaminated using 3% (v/v) Kleencare prior to removal from the Class III Microbiological Safety Cabinet. Following disinfection, waste will be autoclaved using a validated destruction cycle and the autoclave waste finally incinerated. These procedures ensure that there can be no release of live virus from the laboratory facility.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

The committee had no objections to the risk assessment that had been undertaken.

Project Containment

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Animal Units

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Project Ref 36/05.4

Date Ackn'd 03/10/2005

CU2 Project Title Assessment of the viricidal potential of non-nucleoside reverse transcriptase inhibitors

Class 3

CultureVolClass2 25ml

CultureVolumeClass3-4
### Project Additional Information

**Purposes of the contained use**

The purpose of this work is to use a recombinant Simian Immunodeficiency Virus (SIV) containing a Human Immunodeficiency Virus (HIV) type 1 reverse transcriptase (RT/HSIV) in studies designed to investigate the efficacy of non-nucleoside reverse transcription inhibitors (NNRTI). The recombinant SHIV is non-infectious for humans, but susceptible to NNRTI and is therefore safer to work with.

**Recipient or parental organism**

Simian Immunodeficiency Virus

**Host/vector system**

No new constructs will be made, no new manipulations will be performed at the Centre for Emergency Preparedness and Response. GMO is a chimeric virus, SHIV which is a recombinant SIV expressing an HIV type 1 reverse transcriptase.

**Origin & function**

The recombinant Simian Immunodeficiency Virus containing a Human Immunodeficiency Virus (HIV) type 1 reverse transcriptase has been prepared by Dr L Alexander at Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut, USA. No genetic modification will take place at The Centre of Emergency Preparedness and Response. The reverse transcriptase from HIV, unlike that of SIV, is sensitive to the NNRTI's, otherwise both reverse Transcriptases perform the same role and are equivalent. The recombinant SHIV is thus essential for these studies.

**Evaluation of foreseeable effects**

A breach of containment with release into the environment of the self-replicating recombinant:

The risk of this event occurring is negligible as all work will be carried out at containment level 3. The recombinant SHIV cannot survive outside the primate host. The SHIV is less fit than the parental strain of SIV, is unlikely to be pathogenic to humans and is susceptible to treatment with anti-retroviral drugs.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A
The Centre of Emergency Preparedness and Response (formally CAMR) applied for derogation from the requirement for autoclave destruction of CL3 laboratory waste to be carried out in the laboratory. The documents accompanying this notification should be consulted for further details of measures to be implemented. This derogation is in place for a number of projects and this application should be considered to be included in the derogation request.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Chemical disinfection and autoclaving procedures used with hazard group 3 work at CEPR are validated to give 100% kill efficiencies. Disposable plasticware will be used for all manipulation involving GMO's; these materials will be disposed of by autoclaving. Culture media and other liquids potentially containing GMOs will be pre-treated chemically (10% tegodor final conc.) for 24 h prior to autoclaving.

As indicated in detail in the derogation request accompanying this notification, GMO-containing waste will be transported in UN-approved purpose-designed sealed containers from the laboratory to the destruction autoclave, which is located in the CEPR main building, along with the laboratory in use for this project.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC were satisfied with the RA that had been undertaken.

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### Project Ref 36/06.2

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<th>CultureVolumeClass3-4</th>
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<td>Expression of fragments of the virulence factors of Clostridium difficile</td>
<td>Class 2</td>
<td>1-50 Litres</td>
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</table>
Purposes of the contained use

The aim of this work is to generate a panel of non-toxic fragments derived from the toxins and other virulence factors of C. difficile and use these as tools for the study of pathogenesis of the disease and investigate therapy options for C. difficile-associated disease.

Recipient or parental organism

Derivatives of E.coli K12 or B (for example JM109, HMS 174, RV308, TOP10, ER2566, DH5a, BL21, Novablue) for the generation of plasmid stocks, general DNA manipulation, and protein expression. E.coli K12 strains are E.coli K12 derivatives that are recognised as non-colonising and disabled, and may be considered to be equivalent ACDP hazard group 1. They are not considered pathogenic to humans or animals, but are thought capable of surviving for up to seven days in the gut. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. BL 21 and derivatives have been demonstrated to be non-pathogenic according to Chart et al. 2000. An investigation into the pathogenic properties of Escherichia coli strains BLR, BL21, Dha, EQ1.J. Appl. Microbiol. 89, 1048-1058.

Host/vector system

The pMTL expression vectors contain a mutant form of the CoIE1 origin of replication and are Bom/Nic in addition to Mob and Tra, making them non-mobilisable.

The pMAL expression vector is derived from pBR322 and has been manipulated in house to ensure Bom/Nic in addition to Mob and Tra, making it non-mobilisable.

The pGEX expression vector is derived from pBR322 and is Bom/Nic in addition to Mob and Tra, making it mobilisation-defective.

The pET expression vector is derived from pBR322 and is Bom/Nic in addition to Mob and Tra, making it mobilisation-defective.

Origin & function

All protein sequences are in the public domain and genes will be produced by contract synthesis using these published protein sequences. The intended use of the proposed expression products is to investigate the pathogenesis and therapy of Clostridium difficile-related diseases.

Evaluation of foreseeable effects

The chosen hosts are non-infectious E.coli K12 and B derivatives which are incapable of surviving outside the laboratory environment for a prolonged period. Because of
these properties they can be considered non-hazardous.

The expressed proteins represent fragments of toxins which lack an essential domain(s) required for the expression of toxicity. All of the C. difficile toxins so far identified require entry into the mammalian cell to exert their toxic effects. The expressed constructs in the present proposal fall into two categories:

1. Protein domains which represent the enzymically active part of toxins but lack the domains which are essential for delivery of this effector into the mammalian cell
2. Protein domains which are the delivery vehicles for cellular effectors but which are expressed with the effector domain removed from the toxin structure.

Both these construct types are non-hazardous because they completely lack one or more protein domains essential for biological activity.

The above constructs will be inserted into hosts which are either mobilisation defective or non-mobilisable making the risk of gene transfer very low. It must also be stressed that genes encoding the complete toxins are already widespread in nature. In summary, the risk of harmful effects to either man or the environment is exceedingly small.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/a.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Describe the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste is treated with 10% (v/v) sodium hypochlorite for 12 hours and autoclaved by centralised validated autoclave cycle. Solid waste is decontaminated by centralised validated autoclave cycle or incineration.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

Please enter comments on the GM safety committee on the risk assessment

The committee had no objections to the risk assessment that had been undertaken.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>L3</td>
</tr>
</tbody>
</table>

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

This proposal covers the generation by PCR, cloning and expression of non-toxic chimeric Botulinum type A toxins. The binding and translocation/catalytic (Hcand LHn) domains of BoNT/A toxin subtypes A1, A2, and A3 will be synthesised using E.coli codon bias to incorporate 2 separate mutations that remove function in each of the binding and catalytic domains. The purpose is to investigate the vaccine efficacy potential of chimeric toxins incorporating domains from different toxin subtypes. Custom gene synthesis and PCR using synthetic oligonucleotides will be used to construct clones of the various domains which will be cloned into disabled E.coli and sequenced. These genes will then be suncloned into non-mobilisable expression vectors (pMTL series etc.) for expression in disabled E.coli strains and purification. Fusion constructs between domains from different toxin subtypes will be constructed by PCR and the hybrid genes subcloned for sequencing and expression as described above.

**Recipient or parental organism**

Derivatives of E.coli K12 or B (JM109, HMS174, RV308, TOP10, ER2566, DH5a, BL21, Novablue) for the generation of plasmid stocks, general DNA manipulation and protein expression. These E.coli strains are recognised as non-colonising and disabled, and may be considered to be equivalent ACDP hazard group 1. They are not recognised pathogenic to humans or animals, but are thought capable of surviving in the gut for up to seven days. They have limited survivability in the environment and have auxotrophic requirements which are unlikely to be satisfied outside the laboratory.

**Host/vector system**

The initial synthesised genes will be cloned in the pCR series of vectors supplied by Invitrogen. These carry an E.coli origin of replication, multiple cloning site and
ampicillin and kanamycin resistance genes. The vectors are Bom, Nic, Mob and Tra, and therefore are non-mobilisable. These genes will be subcloned into expression vectors either as fusions with a variety of 5’ and 3’ affinity tags or without fusion partners to identify systems to give optimal yields of protein. Examples of affinity tags would be GST, (His)6, maltose binding protein (MBP) and GFP. Therefore, initially the expression vectors used will be pMAL-c2 (modified in house to be non-mobilisable) and the pMTL series (HPA-CEPR in house) and derivatives. These vectors are Bom, Nic, Mob, and Tra and therefore non-mobilisable. pET series expression vectors will also be used. These are Mob and Tra, but Bom and are therefore considered mobilisation defective.

Origin & function
The sequences of botulinum toxin genes are available in the public domain, as are the mutations required to render them non-toxic.

Evaluation of foreseeable effects
Since the chosen hosts are non-infectious E.coli K12 and B derivatives they are unable to survive outside the laboratory environment and are therefore regarded as non-hazardous. The DNA fragments inserted into the hosts encode non-toxic but nevertheless potentially biologically active proteins which have very low levels of toxicity. These sequences neither encode a pathogenicity determinant nor compensate for any host attenuation and therefore will not lead to an increase in the infectivity or pathogenicity of the host. The DNA fragments will be inserted into a range of non-mobilisable or mobilisation defective vectors such that the risk of gene transfer is negligible. The organism is neither pathogenic to man, animals nor plant, therefore the risk to the environment is effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste is treated with 10% Sodium Hypochlorite for 12 hours and autoclaved via centralised validated autoclave cycle. Solid waste is decontaminated by centralised autoclave cycle and incineration

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The GM Committee had no objections to the risk assessment that had been undertaken.

Project Containment

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<td>Yes</td>
<td>L3</td>
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</table>
A number of Tn5 transposon knock out mutants of Bordetella pertussis were produced at CAMR in the 1980s. These are currently stored as freeze dried organisms in a Bordetella pertussis culture collection at CEPR. They have not been cultered since the late 1980s or early 1990s. The strains are knock outs of the virulence components fimbriae (Fim), filamentous haemagglutinin (FHA), pertactin and adenylate cyclase. There is also a mutant with a disabled vir regulon that is unable to determine if the strains retain viability by culture on charcoal agar containing 25 ug/ml kanamycin and then in 100ml shake flask cultures containing the same concentration of Kan. The bacteria will then be stained with a fluorescent stain, killed and used in immunoassays. No further genetic manipulation is proposed.

Bordetella pertussis is a CL2 pathogen that requires containment where there is a potential risk of aerosol infection. The Tn5 mutants are likely to be attenuated in virulence but the Tn5 knockout strains will be handled in the same manner as wild type B pertussis using CL3 safety cabinet for all subculture procedures.

**Recipient or parental organism**

Bordetella pertussis strains

Background – ACDP hazard group 2. Humans can be infected and pertussis (whooping cough) develop. Transmission – B. pertussis is spread by inhalation of large aerosol droplets from infected persons.
Symptoms
- Incubation period approximately 10 days
- Catarrhal stage approximately 1-2 weeks Flu-like symptoms
- Paroxysmal stage approximately 1-6 weeks. Symptoms are forceful hacking successive coughs with paroxysms
- Complications include pneumonia and asthma

Treatment – Antimicrobials are only effective during incubation and catarrhal stage i.e before cough.

Vaccination – Effective pertussis vaccines are available and are part of the UK vaccination schedule.

Host/vector system
Previous produced Tn5 knock out mutants will be grown. No further genetic manipulation is proposed.

Origin & function
The Tn5 knock out strains were produced at Centre for Applied Microbiology and Research in the 1980s. Strains are described in:

A kanamycin resistance gene is included in the Tn5 transposon. This does not affect treatment options as other antibiotics such as azithromycin, clarithromycin or erythromycin are used.

We intend to determine if the strains retain viability by culture on charcoal agar containing 25ug/ml kanamycin and then in 100ml shake flask cultures. The bacteria will then be strained with a fluorescent stain. Killed and used in immunoassays to determine the role of the deleted antigens in the immune responses to current and new vaccines.

Evaluation of foreseeable effects
Accidental release: The genetically modified B. pertussis strains are unlikely to survive any better than wild type B pertussis which survives poorly outside the human host or on specific culture media. Available pertussis vaccines have shown efficacies exceeding 80% in clinical trials and the strains will be handled in a CL3 cabinet. Thus risks are no different from current wild type strains used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No application for derogation required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste will be decontaminated using 1% SuperQ disinfectant solution prior to removal from the Class 3 Microbiological Safety Cabinet. Following disinfection, waste will be autoclaved and the autoclaved waste finally incinerated. Waste will be tracked through the destruction process according to standard procedures. These ensure that there can be no release of live organisms from the laboratory.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
Please enter comments on the GM safety committee on the risk assessment

The committee had no objections to the risk assessment that had been undertaken

Project Containment

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<td>L2 L3 L4</td>
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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 36/08.3

- **Date Ackn’d**: 15/05/2008
- **CU2 Project Title**: Generation of recombinant Baculovirus for the expression of specific Filoviral proteins (GP, NP, VP40 and VP24) and virus-like particles (VP40 + GP) from transfected insect cells for the generation of antibody against these purified, recombinant proteins.

- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Consent Granted**: Not Applicable

- **Non-GMM**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

This project is part aimed at generating reagents and assays to efficiently and accurately detect and differentiate between alternative types of Filoviral infection. Essential to these goals are the generation of the purified structural proteins GP, NP, VP24 & VP40 and Virus like particles with analogous structure to mature filovirus virions. Due to their complex post-translational modification processes, these proteins will need to be expressed in a eukaryotic system to ensure they have the correct
modifications to allow relevant antibody generation downstream. As such, the purpose of this project is to create a panel of Baculoviruses, each expressing either GP, NP, VP24 or VP4O from a specific subtype of filovirus. In addition we will also create Virus Like Particles (i.e. VP4O and OP proteins) for each subtype by co-infection.

Recipient or parental organism

Sf9 insect cells from S. puclopterafrugiperda (cell culture adapted): Cell culture transformed insect cell line. Addition of Genetically modified material (BaculoDirect construct) will result in expression and assembly of recombinant Baculovirus. Not deleterious effects on environment or human health.

Infection of this cell line with the previously described recombinant Baculovirus will result in over expression of the corresponding structural protein, and eventual cell death. Of the over-expressed proteins the only foreseeable effects, from accidental exposure, would arise from the immunogenic nature of the OP gene product and from the VP4O/GP VLPs. Although these products have been previously shown to be immunogenic (resulting in protective antibody response and dendritic cell activation/endothelial inflammation) they will not have any other deleterious effects and are not classed as toxic or pathogenic in any way.

Host/vector system

BaculoDirect baculovirus from Autogrepha california nuclear polyhedrosis virus (AcNPV Baculovirus): This vector, and the recombinant vectors created by insertion of the previously described filoviral structural genes, will only replicate/express viral proteins in insect cells due to the specific promoter/enhancer usage. They therefore pose no risk to the human health of the user or general public. Two of the products (OP and VLPs) resulting from infection with this vector are immunogenic as described above.

Origin & function

- **Glycoprotein (GP) and the (edited/full length) extracellular domain of GP:** The OP protein is responsible for Filoviruses binding to their specific cell surface receptors (e.g. DC-SIGN) allowing viral entry into target cells (1, 2, 3, 4).
- **VP24 and the extracellular domain of VP24:** The Filoviral VP24 protein or minor matrix protein is membrane associated and predicted to have a structural role in the formation of mature filovirus particles. (3,5).
- **VP4O Matrix protein:** The filoviral matrix protein is sufficient to drive the budding of VLP5 from the cell membrane and is responsible for the incorporation of Nucleocapsid/RNP into Virions. As such VP4O is the main driver for virion assembly and budding. (3,6).
- **Nucleoprotein (NP):** Filoviral Nucleoprotein is essential for RNA binding and incorporation into assembling virions. NP is the main constituent of filoviral nucleocapsid — implying an important structural role within mature virions. It is thought to be packaged into virions/VLPs via interactions with VP24/VP4O and VP35 (3,4,7) References for previous section (for gene function):
  4. Klenk, H. - D. 'Marburg & Ebola Viruses’1999; Springer publishing

Evaluation of foreseeable effects

The inserted gene products are non-hazardous structural proteins with no toxic, endogenic, or oncogenic properties. Any Minor risk associated with each of the resultant recombinant proteins, once over expressed by the vector-infected host cell line and purified, are described below:
• Glycoprotein (GP) and the (edited or full length) extracellular domain of QP. The GP protein is responsible for Filoviruses binding to their specific receptors allowing viral entry into cells. Although there are cytotoxic effects if over-expressed in cell lines, the purified protein has not been shown to have any pathogenic effects and the DNA constructs made in this project do not contain promoters that would all over-express in any mammalian cells (1, 2). No other pathological effects have been reported for OP. Although immunogenic (triggering antibody and inflammatory responses), it has no deleterious effects (3), however, the truncated ‘form of GP, sOP, has been shown to be immunosuppressive (4); it is not going to be expressed during this project (due to mutagenesis of the corresponding region in the OP ORF).

• 1P24 and the extracellular domain of VP24: The Filoviral VP24 protein predicted to have a structural role in the formation of mature filovirus particles (4). To date no Pathogenicity has been demonstrated for this protein when expressed recombinantly/purified. (4)

• VP40 Matrix protein: VP40 is the main driver for virion assembly and budding. To date no Pathogenicity has been demonstrated for this protein when expressed recombinantly/purified. (4)

• Nucleoprotein (NP): Filoviral Nucleoprotein is essential for RNA incorporation into virions and is the main constituent of filoviral. To date no Pathogenicity has been demonstrated for this protein when expressed recombinantly/purified. (4)

References for above:
4.Klenk, I-IL- 0. ‘Marburg & Ebola Viruses’ 1999; Springer publishing

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
The only GMO's Generated are micro-organisms (Baculovirus & Transformed Cell line) - no transgenic animal or plant work is associated with this project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No Derogation from Full ACGM level 2 and ACDP class 2 containment will be needed for this work. All work with the resultant GMMs will be conducted under level 2 conditions and in a class 2 cabinet. Any work that does derogate from full class 2 containment will only be carried out after appropriate de-activation/lysis of any GMM that may be in that sample.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Hypochlorite — Haztabs
For liquid waste with in the Class II safety cabinet, a solution of sodium hypochlorite is made by adding 1 HazTab tablet to 1 litre of water/liquid waste (2,500ppm available chlorine) in a waste pot. For non-infectious tissue culture this is transferred to drain with copious amounts of water. If virus-infected waste is produced, this is left in hypochlorite overnight, then transferred to drain with copious amounts of water.
70% Isopropanol
Isopropanol stock is 70% in purified water. It can be used to disinfect surfaces that are not heavily contaminated. All items of equipment taken out of the laboratory should be first sprayed with 70% Isopropanol. Its use should be avoided where bacterial spores, mycobacteria or non-enveloped viruses maybe present.
Formaldehyde
The Class II safety cabinet should be fumigated before servicing (or when bacterial/fungal contamination is observed) using 50% formalin (20% formaldehyde) diluted in deionised water. 30ml of 50% formalin is added to a boiling pot and allowed to evaporate in an enclosed cabinet. This is left for at least 6 hours, or preferably overnight before cabinet is switched on for reuse.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
The committee has no objections to the risk assessment that has been undertaken.

Please enter comments on the GM safety committee on the risk assessment

The committee has no objections to the risk assessment that has been undertaken.

**Project Containment**

<table>
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<tr>
<th>Laboratory Activities</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L2 L3 L4</td>
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<td></td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4</td>
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**Project Ref** 36/08.4

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<td>Using the ClosTron Knockout system to disable virulent genes in Clostridium Difficile.</td>
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<tr>
<th>Date of Significant Change</th>
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</table>

**Projects Additional Information**

02/03/2022
Purposes of the contained use

Generation of specific gene knock out mutants of C. difficile 63 OAERM

Recipient or parental organism

Derivatives of C. difficile 63OAERM with specific virulence genes “knocked out”. Genes to be specifically targeted in this study are detailed in the table below and are those of known function whose attenuation will not cause known virulence (Sebaihia et al. 2006).

<table>
<thead>
<tr>
<th>Gene</th>
<th>General category</th>
<th>Specific function or location</th>
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<td>tcdA</td>
<td>Virulence factor</td>
<td>Toxin A</td>
</tr>
<tr>
<td>tcdB</td>
<td>Virulence factor</td>
<td>Toxin B</td>
</tr>
<tr>
<td>tcdE</td>
<td>Virulence factor</td>
<td>Holin protein — associated with toxin export</td>
</tr>
<tr>
<td>tcdD</td>
<td>Virulence factor</td>
<td>Positive regulator of toxins A and B</td>
</tr>
<tr>
<td>CID 1228</td>
<td>Virulence actor</td>
<td>Collagen protease</td>
</tr>
<tr>
<td>CD0065</td>
<td>Adaptation and survival in GI tract</td>
<td>bile-acid inducible NADP+- dependent 7-alpha-hydroxysteroid dehydrogenase</td>
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<tr>
<td>CD32 15</td>
<td>Adaptation and survival in GI tract</td>
<td>Ortholog of the bilEA gene from L. monocytogenes which act as a bile exclusion system and confer bile acid tolerance.</td>
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<tr>
<td>CD3216</td>
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<td>Ortholog of the bilEB genes from L. monocytogenes which act as a bile exclusion system and confer bile acid tolerance.</td>
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<td>s1pA</td>
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<td>S-layer precursor protein (1-1MW and LMW)</td>
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<td>Glucosidic hydrolase catalytic core</td>
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<td>CD2794</td>
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<td>CD2797</td>
<td>Surface protein</td>
<td>Fibronectin binding</td>
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<tr>
<td>SpoOA</td>
<td>Sporulation</td>
<td>Sporulation protein A</td>
</tr>
</tbody>
</table>


Host/vector system

The knock out delivery system (‘ClosTron’) is based on the shuttle vector pMTLO07C-E2 (depicted below) as described

Heap et al. (2007).


Origin & function

The DNA sequences for the C. difficile virulence factor genes are in the public domain (Sebaihia et al., 2006). The intended functions are to use these sequences to engineer knock out mutant derivatives of C. difficile 630SERM (Hussain et al., 2005).

Hussain et al. (2005) Generation of an erythromycin-sensitive derivative of Clostridium difficile strain 630 (630Deltaerm) and demonstration that the conjugative transposon Tn91 6DeltaE enters the genome of this strain at multiple sites. J. Med. Microbiol. 54, 137-141.


Evaluation of foreseeable effects

Foreseeable effects are expected to be the generation of C. difficile mutants with attenuated virulence profiles. The target genes
have been selected on the basis of known function and it is not anticipated that there would be any enhanced virulence of the wild type strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste is treated with 10% sodium hypochlorite for 2 hours and autoclaved via centralised validated autoclave cycle. Solid waste is decontaminated by centralised validated autoclave cycle and incineration.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The GM Safety Committee had no objection to the risk assessment that has been undertaken.

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<td>Large Scale Activities L3 L4 L2</td>
<td>Human Clinical Applications L3 L4</td>
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Project Ref  36/09.1

Date Ackn'd  19/03/2009  
CU2 Project Title The aim of this work is to evaluate a combined TB and malaria vaccine  
Class 2  Culture volume: Class 2 ≤ 1 Litre
Project Additional Information

Purposes of the contained use

In the work covered in this proposal, CEPR will carry out testing of a novel recombinant BCG vaccine expressing malarial antigens, followed by an adenovirus-malarial antigen boost, that may offer protection against both tuberculosis and malaria disease. The role of CEPR will be to provide vaccine evaluation of rBCG (+ malaria antigens) and Adenovirus (+ malaria antigens) constructs in the guinea pig model of tuberculosis to ensure that insertion of the malaria antigens into BCG has not diminished the potency of the BCG to protect against TB infection. The recombinant BCG and viruses have been prepared externally as a 'ready to test' formulation. No genetic modification will take place at HPA.

Recipient or parental organism

Mycobacterium Bovis BCG vaccine strain has multiple mutations rendering them unable to survive outside the laboratory.

Host/vector system

Mycobacterial shuttle vector PMV261 (derivative of pAL5000)

Origin & function

Mycobacterium bovis BCG vaccine strain has a listeriolysin gene (Hly) from L. monocytogenes inserted into its genome knocking out the BCG urease subunit C gene (ureC). The insertion increases the immunogenicity of BCG to the host without increasing virulence. In addition, up to three antigens from Plasmodium falciparum will be cloned into the rBCG:ureC:Hly BCG known to be immunogenic in humans.

Evaluation of foreseeable effects

Expression is performed in a recombinant M. bovis BCG vaccine strain. The addition of listeriolysin gene, from L. monocytogenes, into BCG, has proven not to increase its virulence and is unlikely to survive outside of the laboratory. Plasmodium falciparum do not possess the classical virulence factors such as toxins, capsules and fimbriae. Therefore, expression of a single component in BCG is unlikely to be toxic. The shuttle vector pMV261, a derivative of pAL5000, used to express the malaria antigens is non mobilisable. It is also unlikely that individual non-mycobacterial gene products are hazardous to the environment, being proteins they will be rapidly degraded outside stable laboratory storage.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Any potential shedding of the GMM by animals is controlled by the husbandry practices. Animals vaccinated with Class 2 GMM are housed in the containment facility.
section of animal wing and all material (bedding, waste food etc) is disposed via the facility autoclaves. Waste bags are autoclaved within the containment facility and then incinerated.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable - appropriate containment level being applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste will be treated according to the site waste management plan. For Class 2 material this would compromise autoclaving and disinfection of liquid waste with 2,500ppm available chlorine followed by autoclaving.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

**The committee had no objection to the risk assessment that had been undertaken**

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<tr>
<td>L2</td>
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<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tbody>
<tr>
<td>L2 L3 L4 L2</td>
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**Project Ref** 36/09.2

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>30/11/2009</td>
<td>Generation of recombinant Baculovirus for the expression of structural viral proteins and virus like particles</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>

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02/03/2022
Essential to several ongoing virology projects is the ability to express antigenic viral proteins in a protein expression system that allows effective expression and post-translational modification. Expressing these viral antigens allows us to purify large amounts of protein that can be used in diagnostics (e.g. Antibody ELISAs/serology). Such proteins can then be used to raise antibodies and generate specific antibody reagents for the detection and differentiation of specific viral species/strains.

In order to obtain high yields of easily recoverable protein, we intend to express specific viral structural proteins, namely Envelope Glycoprotein (GP/env), Nucleoproteins (NP) and Matrix proteins (e.g. filovirus VP40) in individual (i.e. one per protein) Baculovirus vectors, to allow infection and subsequent over expression of both epitope tagged and native forms of the above proteins in insect (Sf9 or Sf21) cells. We also intend to express envelope proteins and matrix proteins in the same cells (infected with two baculoviral vectors simultaneously - one expressing each protein), to allow the production of virus-like particles (VLPs) that will be structurally analogous to whole virions but remain non-infectious, containing no genomic material or viral enzymes.

All this work will be sub-genomic; cloning only a single ORF from viral RNA (from previously infected cells) into Baculoviral transfer plasmids. At no time will full viral genomes be reconstructed.

Recipient or parental organism

Sf9, Tni.Hi5 and Sf21 insect cells from Spodoptera frugiperda (cell culture adapted)

Cell culture transformed insect cell lin. Addition of Genetically modified material will result in expression and assembly of recombinant Baculovirus. Not deleterious effects on environment or human health.

Infection of this cell line with the previously described recombinant Baculovirus will result in over expression of the corresponding structural protein, and eventual cell death. Of the over-expressed proteins the only foreseeable effects, from accidental exposure, would arise from the immunogenic nature of the Glycoprotein/Envelope gene product and from their corresponding VLPs. Although these products have been previously shown to be immunogenic (resulting inprotective antibody response and dendritic cell activation/endothelial inflammation) they will not have any other deleterious effects and are not classed as toxic or pathogenic in any way.

Host/vector system

Baculoviruses, modified from Autographa california nuclear polyhedrosis virus (AcNPV Baculovirus):

- Baculovirus entry vectors: are all simple cloning vectors allowing cloning, modification and recombination (into Baculoviral DNA) of gene of interest - all are non-mobilisable and contain modified pUC origins or replication. Namely, vectors used will include:
Origin & function

This scheme of work involves the insertion of three different genes into separate Baculoviral vectors, but from a range of viral pathogens. The functions of the genes is as detailed below, across the range of viral pathogens listed:

1. Envelope Glycoprotein: responsible for binding to specific cell surface proteins/receptors, allowing viral entry into target cells. As such these genes are responsible for viral tropism. Their exposed conformation on the viral envelope means they are heavily glycosylated but still are the main drivers of the host immune response to the virus.
2. Nucleoprotein: Viral Nucleoproteins are essential for ensuring viral genomes are packaged into progeny virions as the virus assembles. As such their main functions are binding to viral genomes and forming the main structural component of the viral core.
3. Matrix proteins: Viral Matrix proteins are important structural elements of enveloped viruses, bridging interactions between nucleocapsids and envelope glycoproteins. They are responsible for the incorporation of nucleoproteins into assembling viral particles. In many cases (e.g. filoviruses) they are responsible for driving viral budding.

Previous expression studies on such genes were performed at BSL/CL1-2

The above genes will be cloned into baculoviral vectors from the panel of viruses listed below:

- Filovirus (Strains of Ebola and Marburg viruses)
- Flaviviruses (including Dengue, Yellow fever, Japanese Encephalitis)
- Influenza viruses (included pandemic H1N1 and H5N1 influenza)
- Nairoviruses (e.g. Crimean-Congo Hemorrhagic Fever-CCHF)
- Arenaviruses (including strains of Lassa Fever virus and Lujo)
- Phlebovirus (e.g. Rift Valley fever)

Evaluation of foreseeable effects

Minimal hazards to human health: The inserted gene products are non-hazardous structural proteins with no toxic, endogenic, or oncogenic properties. Any Minor risk associated with each of the resultant recombinant proteins are described below:

- Matrix proteins and nucleoproteins: No pathogenicity or hazardous effects have been reported from the recombinant expression of viral Matrix or nucleoproteins, and previous expression studies on such proteins have been performed at BSL 1-2.
Envelope Glycoprotein: Some envelope glycoproteins give cytotoxic effects if over-expressed in cell lines, these proteins have not been shown to have any pathogenic effects, furthermore the DNA constructs made in this project do not contain promoters that would allow over-expression in any mammalian cells. No other pathological effects have been reported for viral glycoproteins. Glycoproteins are, by their nature and function, immunogenic (triggering antibody and inflammatory responses), previous expression studies on such proteins have been performed at BSL 1-2/CL1-2.

Recombinant baculovirus will not be able to establish an infection in human cells, they will not have altered host range or tissue tropism.

None of the gene products (described above) have any directly hazardous effects, baculovirus derived Virus Like Particles and heterologous protein from a range of these viruses (e.g. filoviruses) have been previously shown to illicit an immune response. Exposure to these proteins and VLPs, like many proteinaceous materials could have immuno-regulatory effects such as the generation of antibodies via Dendritic and B-cell activation and pro-inflammatory responses such as Endothelial cell activation. VLPs are not infectious and the expressed proteins are neither pathogenic nor toxic.

To minimise any risk, all handling of recombinant baculovirus, supernatants containing VLP's purified VLP's, expressed protein and co-infected cells will be carried out under level 2 containment conditions in class 2 cabinets, so affording suitable protection to the user. The fact that such VLP's are replication deficient ensures there is no risk of infection occurring and no wider risk to co-workers, etc.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The only GMO's Generated are micro-organisms (Baculovirus & Transformed Cell line) - no transgenic animal or plant work is associated with this project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No Derogation from Full ACGM level 2 and ACDP class 2 containment will be needed for this work. All work with the resultant GMMs will be conducted under level 2 conditions and in a class 2 cabinet. All GMM's will be inactivated before removal from ACGM 2 licensed areas.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Hypochlorite - Haztabs
For liquid waste with in the Class II safety cabinet, a solution of sodium hypochlorite is made by adding 1 HazTab tablet to 1 litre of water/liquid waste (2,500ppm available chlorine) in a waste pot. For non-infectious tissue culture this is transferred to drain with copious amounts of water.

70% Isopropanol
Isopropanol stock is 70% in purified water. It can be used to disinfect surfaces that are not heavily contaminated. All items of equipment taken out of the laboratory should be first sprayed with 70% Isopropanol. Its use should be avoided where bacterial spores, mycobacteria or non-enveloped virus maybe present.

Formaldehyde
The Class II safety cabinet should be fumigated before servicing (or when bacterial/fungal contamination is observed) using 50% formalin (20% formaldehyde) diluted in deionised water. 30 ml of 50% formalin is added to a boiling pot and allowed to evaporate in an enclosed cabinet. This is left for at least 6 hours, or preferably overnight before cabinet is switched on for re-use.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

02/03/2022
The committee has no objections to the risk assessment that has been undertaken.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<tr>
<td></td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
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<td>L3 L4</td>
<td>L2 L3 L4</td>
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### Project Ref 36/10.1

<table>
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<tr>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>30/06/2010</td>
<td>Growth of Yersinia pseudotuberculosis Yp111 pTrc99A-LcrV and use in cell based assays</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

### Project Additional Information

**Purposes of the contained use**

The Yersinia strain will be grown and used as a reagent in cell based assays to determine the ability of a novel recombinant vaccine to generate functional immune responses in immunised subjects.

**Recipient or parental organism**
Yersinia pseudotuberculosis YpIII pIB19:

**Host/vector system**

pTrc99A containing the lcrV gene of Y. pestis

**Origin & function**

The recombinant lcrV is from Yersinia pestis. It has been introduced into a Y. pseudotuberculosis strain which has had its own lcrV gene deleted to enable the Y. pseudotuberculosis strain to act as a surrogate organism for Y. pestis in the cell based assay.

**Evaluation of foreseeable effects**

Insertion of the lcrV gene into a lcrV mutant of Y pseudotuberculosis will return the organism to the wt phenotype.

The inserted gene product, the V antigen of Y. pestis is unlikely to have a hazardous effect. It has been used in phase 1 clinical trials with no adverse effects (Williamson et al., Inf. Lmm., 73:3598).

Yersinia species harbouring the recombinant plasmid will gain resistance to ampicillin and kanamycin, however they remain sensitive to aminoglycosides, tetracycline, chloramphenicol and cephalosporins.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

NA

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be placed in clearly marked discard tins for autoclaving. After autoclaving, waste will be incinerated. Liquid waste will be decontaminated by treatment with a 10,000ppm available chlorine solution and left overnight before autoclaving. Decontaminated, autoclaved waste will be sent to drain before the containers are sent to wash up.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

Please enter comments on the GM safety committee on the risk assessment

The HPA CEPR GMSC has no objections to the Risk Assessment that has been undertaken
Project Containment

Laboratory Activities

- L2
- L3
- L4

Glass Houses

- L2
- L3
- L4

Growth Rooms

- L2
- L3
- L4

Animal Units

- L2
- L3
- L4

Large Scale Activities

- L2
- L3
- L4

Human Clinical Applications

- L2
- L3
- L4

Project Ref 36/10.2

Cloning and expression of a type VI secretion system subunit Acinetobacter baumannii in E Coli

Class 2

< 1 Litre

Non-GMM

Consent Granted

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Generation of recombinant material to enable the purification and characterisation of the components of the Type VI secretion system.

Recipient or parental organism

Disabled strains of E. coli K12 derivatives (TOP10, DH5α and Novablue) will be used for the purposes of plasmid propagation with no foreseeable effects envisaged. For expression of the gene products, disabled E. coli K12 or B - derived host strains (JM109, RV308, HMS174±DE3, Novablue±DE3, ER2566±DE3 and BL21±DE3) will be employed. These E. coli strains are recognised as non-colonising and may be considered to be equivalent to ACDP hazard group 1. They are not considered pathogenic to humans or animals, but are thought capable of surviving in the gut for up to seven days. They have limited survivability in the environment and have auxotrophic...
requirements which are unlikely to be satisfied outside of the laboratory.

The genes to be cloned and expressed are the hep gene, AIS_1296 and the vgrI, vgrII & vgrIII genes AIS_0550, AIS_3364 & AIS_1288 respectively.

Host/vector system

The synthesised gene will be cloned in the pCR series of vectors supplied by Invitrogen. These carry an E. coli ColE1 origin of replication, multiple cloning site and ampicillin and kanamycin resistance genes. The vectors are Bom\(^{-}\), Nic\(^{-}\), Mob\(^{-}\) and Tra\(^{-}\), and therefore non-mobilisable.

The genes will be subcloned into expression vectors either as fusions with a variety of 5’ and 3’ affinity tags or without fusion partners to identify systems that give optimal yields of protein. Expression vectors that will be used include both non-mobilisable vectors, eg pMAL-c2 (modified in-house to be non-mobilisable), pMTL series (HPA-CEPR in-house), and mobilisable defective, eg the pET series, pMAL.

Origin & function

The DNA sequences for the A. baumannii genes are in the public domain (Pubmed). The intended functions are to use these sequences to maximise expression of the gene products in disabled E. coli host strains.

Evaluation of foreseeable effects

Forseeable effects are expected to be the generation of disabled E.coli variants with antibiotic resistance to either Ampicillin, or Kanamycin.

It is highly unlikely that the pathogenicity of the host will be attenuated by the expression of the above gene products as the individual subunits only form a small part of the type VI system and therefore are unable to form an active secretion system.

It can not be completely excluded, however, that the individual proteins may have some biological activity of their own although the potential to cause harm in isolation is very low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The only GMO's generated are micro-organisms - no animal or plant work is associated with this project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste is treated with 10% sodium hyperchlorite for 12 hours and autoclaved via centralised validated autoclave cycle. Solid waste is decontaminated by centralised validated autoclave cycle and incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The committee has no objections to the risk assessment that has been undertaken

### Project Containment

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<td>L3  L4</td>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2</td>
<td>L3  L4</td>
<td>L2  L3  L4</td>
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</table>

### Project Ref 36/12.1

- **Date Ackn’d**: 05/01/2012
- **Date Project Ceased**: 05/01/2012
- **CU2 Project Title**: Cloning and expression of secretion system subunits from gram negative bacteria - RD/1011-428
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Consent Granted**: Non-GMM

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**: 05/01/2012

**Significant Change ID**: N

**Date of Significant Change**: 05/01/2012

### Project Additional Information

**Purposes of the contained use**

Generation of recombinant material to enable the purification and characterisation of the components of the Type VI secretion system

**Recipient or parental organism**

Disabled strains of E.coli K12 derivatives (TOP10, DH5α and Novablue) will be used for the purpose of plasmid propagation with no foreseeable effects envisaged. For expression of the gene products, disabled E. coli K12 or B-derived host strains (JM109, RV308, HMS174ΔDE3 and BL21ΔDE3 will be employed. These E. coli strains are
recognised as non-colonising and may be considered to be equivalent to ACDP hazard group 1. They are not considered pathogenic to humans or animals, but are thought capable of surviving in the gut for up to seven days. They have limited survivability in the environment and have auxotrophic requirements which are unlikely to be satisfied outside of the laboratory.

The gene to be cloned and expressed include:

E.coli (CFT0173); hcp, AE016765_238; vgrl, AE016765_240; vgrll, AE016760_202; vgrlll, AE016760_207; lcmF, AE016765_245; ClpV, AE016765_239

K. pneumonia (MGH 78578); hcp, KPN_01327; vgrl, KPN_01329; vgrll, KPN_02273; vgrlll, KPN_03170; lcmF, KPN_03142; ClpV, KPN_01328.

**Host/vector system**

The synthesised gene will be cloned in the pCR series of vectors supplied by Invitrogen. These carry an E. coli ColE1 origin of replication, multiple cloning site and ampicillin and kanamycin resistance genes. The vectors are Bom-, Nic-, Mob- and Tra-, and therefore non-mobilisable. The genes will be subcloned into expression vectors either as fusions with a variety of 5’ and 3’ affinity tags or without fusion partners to identify systems that give optimal yields of protein. Expression vectors that will be used include both non-mobilisable vectors, eg pMAL-c2 (modified in-house to be non-mobilisable), pMTL series (HPA-CEPR in-house), and mobilisable defective, eg the pET series, pMal.

**Origin & function**

The DNA sequences for the E. coli and K. pneumoniae genes are in the public domain (Pubmed). The intended functions are to use these sequences to maximise expression of the gene products in disabled E. coli host strains.

**Evaluation of foreseeable effects**

Foreseeable effects are expected to be the generation of disabled E. coli variants with antibiotic resistance to either Ampicillin, or Kanamycin.

It is highly unlikely that the pathogenicity of the host will be attenuated by the expression of the above gene products as the individual subunits only form a small part of the type VI system and therefore are unable to form an active secretion system.

It can not be completely excluded, however, that the individual proteins may have some biological activity of their own, although the potential to cause harm in isolation is very low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The only GMO’s generated are micro-organisms - no animal or plant work is associated with this project.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste is treated with 10% sodium hyperchlorite for 12 hours and autoclaved via centralised validated autoclave cycle. Solid waste is decontaminated by centralised validated autoclave cycle and incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The committee has no objections to the risk assessment that has been undertaken.

### Project Containment

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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

**Animal Units**

- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

### Project Ref 36/12.2

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<tbody>
<tr>
<td>08/06/2012</td>
<td>Use of Gene Knock-out Plasmids to Disable Genes in Bacteria from the Enterobacteriaceae family - RD/0412-438</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Class</th>
<th>CultureVol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
</tr>
</tbody>
</table>

**Non-GMM Consent Granted**

Project notified under transitional arrangements

### Project Additional Information

**Purposes of the contained use**

- Generation of knockout mutants to investigate the role of the Type VI secretion system in pathogenesis.
Recipient or parental organism

The recipient strain is K. pneumonia subsp. MGH 78578 (ATCC 700721). The organism was isolated from the sputum of a 66-year old ICU patient in 1994. The strain is resistant to ampicillin, ticarcillin, trimethoprim-sulfamethoxazole, and gentamicin, but I susceptible to amikacin, ciprofloxacin, and imipenem.

The genes to be knocked include:

Hcp1, Hcp2, vgr!, vgr2, vgr3, lcmF1, lcmF2, lcmF3, ClpV1, ClpV2

Host/vector system

Synthetic DNA sequences corresponding to subgene fragments (c. 300bp) of the knockout gene targets are cloned either side of a kanamycin resistance cassette and subcloned into the delivery plasmid, pJTOOL-3. This plasmid can only be propagated in specific E. coli K12 derived strains (egS17-1pi). The plasmid is introduced into the recipient strain by conjugation. The synthetic target DNA sequences introduced into the recipient are not genes but short (c.300bp) fragments that are partially homologous to regions of the genes being targeted in the chromosome.

Origin & function

The DNA sequence for the targeted genes are in the public domain (Pubmed). The intended functions are to use these gene fragments to knockout the corresponding genes in the recipients chromosome.

Evaluation of foreseeable effects

It is highly unlikely that the pathogenicity of the host will be attenuated as the knock out mutants are more likely to be rendered less virulent by preventing expression of key components of the Type VI secretion system (the Type VI system has been shown to be involved in pathogenesis in other Gram negative bacteria). The introduction og the synthesised genes into the recipients chromosome is highly unlikely to result in a harmful biological activity as they are subgene fragments that are homologous to existing regions in the chromosome.

Knockout mutants will be kanamycin resistant but this antibiotic is not the drug of choice for this organism

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The only GMO's generated are micro-organisms, however, Galleria mellonella insect larvae will be infected with the genetically modified bacteria. These will be handled and disposed of in the laboratory at containment level 2.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste is treated with 10% sodium hyperchlorite fo 12 hours and autoclaved via centralised validated autoclave cycle. Solid waste is incinerated.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
The committee has no objections to the risk assessment that has been undertaken.

**Project Containment**

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<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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</table>

- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

<table>
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</table>

- **Project Ref 36/12.3**

- **Date Ackn'd**: 08/06/2012
- **CU2 Project Title**: Cloning and expression of outer membrane Proteins from Acinetobacter and bacteria of the Enterobacteriaceae family - RD/0412-443
- **Class**: Class 2
- **Culture Vol Class 2**: < 1 Litre
- **Consent Granted**: Project notified under transitional arrangements

- **Significant Change ID**: 
- **Date of Significant Change**: 

- **Project Additional Information**

- **Purposes of the contained use**: Generation of recombinant material to enable the purification and characterisation of the OmpA protein
- **Recipient or parental organism**: 

02/03/2022
Disabled strains of E. coli K12 derivatives (TOP10, DH5α and Novablue) will be used for the purpose of plasmid propagation with no foreseeable effects envisaged. For expression of the gene products, disabled E. coli K12 or B-derived host strains (JM109, RV308, HMS174Δ3, NovablueΔDE3, ER2566ΔDE3 and BL21ΔDE3) will be employed. These E. coli strains are recognised as non-colonising and may be considered to be equivalent to ACDP hazard group 1. They are not considered pathogenic to humans or animals, but are thought capable of surviving in the gut for up to seven days. They have limited survivability in the environment and have auxotrophic requirements which are unlikely to be satisfied outside of the laboratory.

Host/vector system

The synthesised gene will be cloned in the pCR series of vectors supplied by Invitrogen. These carry an E. coli ColE1 origin of replication, multiple cloning site and ampicillin and kanamycin resistance genes. The vectors are Bom-, Nic-, Mob- and Tra-, and therefore non-mobilisble. The genes will be subcloned into expression vectors either as fusions with a variety of 5’ and 3’ affinity tags or without fusion partners to identify systems that give optimal yields of protein. Expression vectors that will be used will be non-mobilisble eg pMAL-c2 (modified in-house to be non-mobilisble) or the pMTL series (HPA-PORTON in-house).

Origin & function

The OmpA genes to be cloned and expressed will be sourced from strains of A. calcoaceticus and A. Iwoffi which are opportunistic pathogens of low virulence.

Evaluation of foreseeable effects

Forseeable effects are expected to be the generation of disabled E. coli variants with antibiotic resistance to either Ampicillin or Kanamycin.

The OmpA protein from A. baumannii has been shown to possess a relatively low toxicity against mammalian cell lines. It is therefore proposed to carry out the work at containment level 2 to provide adequate protection from disabled E. coli strains that have acquired a biologically active protein that shows a low level of cytotoxicity against mammalian cell lines.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The only GMO’s generated are micro-organisms - no animal or plant work is associated with this project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste is treated with 10% sodium hyperchlorite for 12 hours and autoclaved via centralised validated autoclave cycle. Solid waste is incinerated.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Committee has no objections to the risk assessment that has been undertaken.
**Project Additional Information**

**Purposes of the contained use**

The purpose of this work is to facilitate robust characterisation of previously constructed Clostridium difficile gene knockout mutants by re-introducing wild type copies of the inactivated genes. These mutants will be used to investigate colonisation factors which may be targets for Clostridium difficile infection.

**Recipient or parental organism**

Clostridium difficile 630 knockout strains, in which the chromosomal copy of the gene of interest has ben previously inactivated by targeted insertion of a group II intron.

**Host/vector system**
Wild type genes will be amplified from C. difficile genomic DNA and cloned into a mobilisation defective E. coli/C. difficile shuttle vector. Genes will be introduced into C. difficile knockout strains by conjugation from E. coli CA343, a K-12 derivative carrying a plasmid which provides mob+ functionality in trans.

**Origin & function**

**Evaluation of foreseeable effects**

The complemented C. difficile mutants are expected to have an identical phenotype to the wild type organism, as the inserted plasmid-borne gene will simply replace the inactivated chromosomal copy. The complemented mutants are therefore not expected to be any more hazardous than the parental wild type strain.

These are four genes we specifically exclude from this work - genes encoding the classical C. difficile toxins tcdA, tcdB, and those encoding the binary toxin ctdA and ctdB. There is therefore no risk of creating strains more toxigenic than the wild type.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

In accordance with the HPA code of safety practice liquid waste is treated with 10% sodium hypochlorite for 12 hours or autoclaved via centralised validated autoclave cycle. Solid waste is decontaminated be centralised validated autoclave cycle and incineration.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

The proposal was reviewed by the local GM safety committee. They were confident that the risk assessment was appropriate and comprehensive and approved it with no concerns and where happy for the work to proceed.

**Project Containment**

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<td>L3 L4 L2 L3 L4 L2</td>
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</tbody>
</table>

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Burkholderia thailandensis is a non-pathogenic relative of Burkholderia pseudomallei and Burkholderia mallei which has significant genetic homology to both pathogenic species (B. pseudomallei and B. mallei). We propose to use genetically modified strains supplied by Dstl to allow expression of putative vaccine candidates (polysaccharides). Dstl will provide HPA with genetically modified B. thailandensis strains. In these strains one of the genes involved in the biosynthesis of the O-antigen of lipopolysaccharide (LPS) is inactivated, either through insertion of mutation or in-frame gene deletion. This results in abrogation of O-antigen biosynthesis. These strains will be grown at HPA to enable production and ultimately purification of B. thailandensis capsular polysaccharide which is not contaminated with LPS. The purified material will be shipped to Dstl for further treatment to develop vaccines.

**Recipient or parental organism**

No GMMs will be constructed at HPA. Strains will be received from dstl and handled in an ACDP/ACGM II laboratory. The strains to be used all lack O-antigen biosynthesis, either through insertion of inactivation of selected genes or in-frame deletion of selected genes. The wild type host is B. thailandensis E555. It was isolated from the environment and has not been modified beyond the modification reported here.

**Host/vector system**

The only vector DNA present in the GM organisms is integrated into the chromosome. It contains the DNA sequences for kanamycin resistance, the R6K origin or replication and the RP4 origin of transfer. The functions of the latter two markers are rendered redundant by their integration into the host chromosome.
The recombinant DNA in these organisms, beyond those found in the vector detailed above, lead to gene inactivation. These all encode metabolic proteins involved in polysaccharide biosynthesis. Please note that gene numbering is based on strain E264 (ATCC700388) since E555 genes have not been assigned numbers. The genes in E555 are all more than 90% identical to the E264 genes.

Evaluation of foreseeable effects

The genetically modified B. thailandensis strains in this protocol will be provided by dstl; none are deemed more hazardous than the other and no further modifications are being undertaken. All of the GM organisms contain inactivating mutations resulting in abrogation of O-antigen biosynthesis. Thus, the strains are likely to be phenotypically indistinguishable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid and liquid waste will be removed from the laboratory according to the local GM procedure for waste removal; 10% sodium hypochlorite solution

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Minor additional information was required to be inserted into the assessment; insertion of the ATCC reference for strain E264 reference text was added to questions 8 and 9 the route of delivery was included in section 10

Project Containment

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02/03/2022
Project Ref 36/12.6

Date Ackn'd 22/10/2012
CU2 Project Title Development of a novel delivery system for Chlamydial Vaccines - RD/0512-445

Class 2
CultureVol
Class 2 Volume
Consent Granted

Non-GMM

Historical Significant Changes
Historical Date of Additional Info

Withdrawn
Tick if notifying a connected programme of work

N

Project notified under transitional arrangements

N

Project Additional Information

Purposes of the contained use
Transformation of Neisseria with chlamydial genes. Preparation of outer membrane vesicles containing chlamydial proteins.

Recipient or parental organism
For conjugation experiments the donor strain will be E. coli disabled laboratory strains derived from K12:
E. coli S17-I carries the transfer genes of the broadhost-range INcP-type plasmid RP4 integrated into the chromosome (Simon, R et al. 1983).
E. coli S18 is a hemA deletion mutant E. coli S17-I. This strain requires 5-aminolevulinic acid (ALA) for growth. (Thoma, S et al. 2009).

Commensal Neisseria recipient strains; N. lactamica, N. cinerea, N. flavescens, N. flava, and N.subflava. These are not pathogenic to humans or animals and have limited survivability in the environment (the neisserial comensals are only able to colonise humans).

Host/vector system
The E. coli to Neisseria shuttle vector, pMIDG, will be used for transfer of chlamydial constructs into commensal Neisseria. pMIDG was initially developed from the fusion of a 4.2 kb gonococcal cryptic plasmid and a 7.0 kb beta-lactamase plasmid. pMIDG contains a kanamycin resistance cassette as a selectable marker.
pMIDG is not self-mobilising. Although it contains an origin of transfer it does not carry the additional genes (mating-pair formation & DNA processing and transport genes) for self-mobilisation.

Chlamydial genes will be cloned into pMIDG via the use of subcloning vectors that have been developed from pUC vectors, are non-mobilizable, and can only be...
Origin & function

The DNA sequences for the chlamydial genes are in the public domain (Pubmed/JCVI). MOMP: (JCVI annotated locus name: NT01CT0727)
The chlamydial MOMP is a porin that is both structurally and immunologically the dominant protein in the chlamydial outer membrane complex. It is a well defined chlamydial antigen with both T and B cell epitopes identified. It does not appear to have any virulence capabilities.

Polymorphic membrane proteins (Pmps); (JCVI annotated locus names: NT01CT0435, NT01CT0436, NT01CT0437, NT01CT0868, NT01CT0929, NT01CT0930, NT01CT0931, NT01CT0932, NT01CT0935)
The Pmps are a family of 9 genes (A-I) encoding outer membrane proteins. These proteins contain auto-transporter domain allowing secretion via a type V pathway. Pmps represent 13.6% of the coding capacity of the C. trachomatis genome, suggesting they have a critical role in chlamydial biology. However, the role of Pmps in chlamydial virulence is not well understood.

Evaluation of foreseeable effects

The family of chlamydial polymorphic membrane proteins (Pmps) have been shown to be immuno-dominant with certain members demonstrating an ability to induce protective immunity against genital tract infection (Eko FO, Okenu DN et al. 2011) (Eko FO et al. 2011)(Yu H et al. 2010)(Patent No. US 6,642,023). All nine Pmps are expressed on the surface of chlamydial elementary bodies (EB) and C. trachomatis-infected patients can produce antibodies to each Pmp subtype. However, the role of Pmps in chlamydial virulence is not completely understood. Recently it has been found that PmpD may act as an adhesion (Swanson KA et al. 2009) (Crane, D. D et al. 2006). Therefore, it is possible a Neiseria GMM could be created expressing a possible Chlamydia adhesin factor.

Although there is a potential role for Pmp proteins in chlamydial pathogenesis, it is currently thought that the full complement of Pmp genes would be required for any virulence traits to be achieved (Carrasco JA et al. 2011). There is also no evidence that these genes are directly toxic and by ensuring that only one gene is transformed per host cell we will be removing any possibility of any combined virulence being transferred to the host. In addition, these genes are unique to members of the order Chlamydiales. Due to lack of evidence for horizontal gene transfer in the Chlamydiales the Pmps are likely to have evolved specifically for the unique biphasic life cycle of the chlamydial organisms, and their unique intracellular niche of the inclusion body. Thus, it is highly unlikely that they will be capable of making the recipient commensal Neisseria strains pathogenic.

The use of a mobilizable plasmid for the transfer of the chlamydial genes into commensal Neisseria may appear as though it could be possible for this plasmid to undergo further mobilisation, and transfer chlamydial genes to other bacteria. However this is deemed highly unlikely due to pMIDG not being self mobilizing, unlikely to be able to persist outside of laboratory cultures due to a lack of selection pressure. Kanamycin resistance will not allow for stabilisation of the transferred plasmid in natural populations of the nasopharynx as this antibiotic is not used in a clinical setting for neisserial disease, thus there will be no selection pressure.

Finally, there is a possibility that the E. coli donor may be able to mobilise pMIDG to other bacteria. Again, this is considered to be highly unlikely as the E. coli donor strains S17 and S18 are K12 derivatives that have limited survivability in the environment (Isaza LA et al, 2011) (Simon R et al. 1983) (Thoma S et al. s009). More specifically the strain S18 has an auxotrophic requirement for 5-aminolevulic acid that will not be satisfied outside laboratory culture (Thoma S et al. 2009). Furthermore, any additional concerns regarding expression of chlamydial proteins in the E. coli donor strains can also be considered insignificant as the chlamydial genes will be driven by a neisserial promoter, and contain a neisserial signal peptide sequence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The only GMOs generated are micro-organisms

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid cultures will be disposed of via the site incineration service. Liquid waste will be disposed via the site autoclaving service.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment  

The committee has no objections to the risk assessment that has been undertaken

Project Containment

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Project Ref 36/12.7

Date Ackn'd 19/10/2012

CU2 Project Title

The growth and use, in assay development, of modified strains of non-typeable Haemophilus influenzae that will be generated by external collaborators and supplied to the HPA

Class CultureVol

Class 2 CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

02/03/2022  

Page 1651 of 15326
**Purposes of the contained use**

With a view to developing improved vaccines and or immunological assays, the GMMs will be used to investigate the role of functional antibodies to the conserved NTHi outer membrane proteins PD, LicD, PilA and PE using target strains that are deleted in these antigens.

**Recipient or parental organism**

The strains of non-typeable Haemophilus influenzae (NTHi) to be modified are those of MPJ003, 3224A and 3655. All strains used are ACDP 2 organisms specific to humans. Associated disease includes sinusitis, otis media, chronic bronchitis, and in very rare cases meningitis and septicaemia.

Modifications to strains were made by allelic exchange, using antibiotic resistance cassettes to replace the knocked out NTHi genes. The knockout phenotypes are stable in the absence of antibiotic selection demonstrating that allelic exchange has gone to completion so as to leave behind no plasmid relics.

Antibiotic resistance genes have been integrated for kanamycin, spectinomycin or both.

The knocked out genes include:

hpd, licD, pe and pilA

**Host/vector system**

Modifications to strains are made by allelic exchange, using antibiotic resistance cassettes to replace the knockout NTHi genes. No plasmid remains and the knockout phenotypes are stable in the absence of antibiotic selection as the sequences have been integrated into the host chromosome.

**Origin & function**

This genetic material involved will be used in immunological assays that will have been developed (using the wild type organisms) as part of this study to investigate the role of functional antibodies to the conserved NTHi outer membrane proteins PD, LicD, PilA and PE using target strains that are deleted in these antigens. The strains deficient in the proteins of interest will be produced by our collaborator on this study, therefore this proposal is strictly directed at growth and manipulation of the mutant strains for use in assays.

**Evaluation of foreseeable effects**

The modifications that will be made to the host strain involve the deletion of NTHi surface proteins. Proteins PD, PilA and PE have either been shown or suspected of playing a pathogenic role in adhesion and entry into upper respiratory epithelial cells. LicD is another conserved protein phosphorylchlorine (PCho) to LOS. The presence of the PCho epitope as part of the outer membrane LOS results in the promotion of NYHi infection and persistence by reducing the host inflammatory response. Deletion of the genes expressing these proteins will therefore not result in any increased hazard to human health.

The use of kanamycin or spectinomycin resistance as a selection marker will not have any impact in a clinical setting owing to these antibiotics no being used in the treatment of NTHi disease.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid cultures will be disposed of via the site incineration service (yellow clinical waste sacks). Liquid waste will be treated using 2.5% sodium hypochlorite and disposed of via the site autoclaving service (autoclave tins). Waste disposal procedures do not need to be amended to accommodate the genetic modifications (ACGM 2 activity).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee has no objections to the risk assessment that has been undertaken.

Project Containment

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Project Ref 36/13.1

Date Ackn'd 28/01/2013

Date Project Ceased

CU2 Project Title Recombinant influenza strains based on attenuated influenza A virus PR8 (H1N1) vectoring vaccine antigens

Class CultureVol

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**
- Evaluation of novel vaccine candidates

**Recipient or parental organism**
- Attenuated Influenza A virus PR8 (H1N1)

**Host/vector system**
- N/A No vectors are used; recombinant strain(s) are received ready to evaluate

**Origin & function**
- Well characterised bacterial antigens serving as vaccine candidates

**Evaluation of foreseeable effects**

The host strain is attenuated and there is no reason to believe that the inserts will increase the hazards to human health or the environment: Attenuated Influenza A virus PR8 (H1N1)

This virus was passaged over 100 times in mice, ferrets and embryonated chicken eggs. As a result of this passage history a complete attenuated virus was generated unable to replicate in humans (WHO Technical Report Series No 941, 2007 Annex 5, WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines).

The infectivity absence in humans was attributed to the surface glycoproteins of the PR8 virus; the Haemagglutinin (HA) and Neuraminidase (NA) (Beare AS, Schild GC, Craig JW (1975) Trials in man with live recombinants made from A/PR/8/34 (H0 N1) and wild H3 N2 influenza viruses. Lancet 2: 729-732).

The inserted gene products (vaccine antigens) are not harmful. This has been shown in preclinical and clinical studies. There is also preclinical and clinical evidence for their not being harmful when delivered in a viral vector.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
- N/A (No GM animals or plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
- N/A (All measures specified as relevant for the containment level will be applied)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste will be treated according to the site waste management plan. Solid waste will be incinerated. Liquid waste will be treated with sodium hypochlorite 10,000 ppm and...
then autoclaved

The committee had no objection to the risk assessment that had been undertaken

Please enter comments on the GM safety committee on the risk assessment

The committee had no objection to the risk assessment that had been undertaken

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### Project Containment

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### Project Ref 36/13.2

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**
HIV-1-based plasmids will be adapted to allow the cloning and study of viral genes from HIV-1. These genes can be sequences derived from lab adapted viruses, molecular clones, partial clones or clinical HIV material. The plasmids contain all the necessary HIV-1 genes required for HIV-1 replication in a single vector. Therefore the transfection the plasmids into cells produces viruses that are replication competent and capable of multiple rounds of replication in susceptible host cells. The viruses will then be used in cell-based drug susceptibility and replication fitness and drug selection studies.

**Recipient or parental organism**
Bacterial or eukaryotic transformed or primary human or animal cell lines.

**Host/vector system**
The plasmids have a standard antibiotic resistance backbone and therefore do not constitute any more hazard than any other cloning or expression plasmids containing genes of HIV origin. The plasmids are maintained in disabled E. coli strains such as HB101 and DH5α. The plasmids promoter are eukaryotic hence not specific for expression within the bacterial cells. The plasmids are transfected into eukaryotic transformed human or animal cell lines such as HEK293T cells. Upon transfection, viral particles will be produced and secreted into the tissue culture medium.

**Origin & function**
The genetic material to be used will be derived from laboratory adapted viruses, full or partial molecular clones or clinical HIV material.

**Evaluation of foreseeable effects**
The recombinant viruses produced are capable of multiple rounds of infection in HIV-1 susceptible cells and will be used in replication fitness and drug selection assays where viral production will be quantified using indicator cell lines or by ELISA. Exposure of a lab worker could lead to infection and integration of the packaged HIV-1 genome and expression of HIV-1 genes. Thus hazards associated with the recombinant viruses are through the risk from accidental infection resulting in HIV-1 infection and/or insertional mutagenesis causing over-expression of cellular genes by random integration. The main risk is to the laboratory personnel directly handling the recombinant viruses and the risk is equivalent to that of a laboratory worker handling clinical specimens from HIV-infected individuals. The final GMM is not a risk to the environment as the recombinant viruses are extremely susceptible to desiccation and will only survive a few hours at room temperature.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Autoclave sterilisation of all solid waste and residual fluid waste. Autoclave waste programmed to 134°C for 40 minutes, with 100% kill., Autoclave validation by thermocouples in every load.
Some liquid culture waste disinfected with proprietary disinfectant containing 2500ppm available chlorine over 2 hours or more. Disinfection validated by demonstrating presence of available chlorine after disinfection time.
Two comments were made:
1. If the volume and concentration handled is to be greater than that expected in clinical samples, an additional risk assessment would be required to address this. GMSC has been informed that this will not be the case, and that the risk assessment is therefore sufficient.

2. The risk assessment submitted to the GMSC should include, in the environmental section, information about storage of the material and shipment arrangements. This additional information has been added to the risk assessment and returned to the GMSC (see attached copy).

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 36/14.1

Date Ackn'd 22/01/2014  CU2 Project Title Production of pseudotype viruses in mammalian cell lines for identification of therapeutic antibodies

Class 2  CultureVolClass2 < 1 Litre  Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**

This project is aimed at generating reagents and assays to efficiently and accurately detect antibodies with neutralising properties to Hazard Group (HG) 3 and 4 virus pathogens.

Essential to these goals are the generation of the Human Immunodeficiency Virus 1 (HIV-1) replication deficient pseudoviruses (PV) that display HG3 and 4 surfaces proteins (glycoproteins, GP). Due to their complex post-translational modification processes, these proteins will need to be expressed in a eukaryotic system to ensure they have the correct folding and modifications to determine cellular tropism for downstream applications (virus neutralisation assay).

As such, the purpose of this project is to create a panel of PV, each expressing a GP from a range of HG3 and 4 viruses, which can be used to assess antibodies for virus neutralising properties.

**Recipient or parental organism**

Mammalian cell lines (culture adapted):

- Vero cells;
- Vero E6 cells;
- Human Embryonic Kidney 293 and derivatives;
- SW13 (adrenal cortex from human);
- Multicystic dysplastic kidney (MCDK) cells;
- Chinese hamster ovary (CHO) cells and derivatives;
- COS cells (CV-1 (simian) in Origin, and carrying the SV40 genetic material);
- HeLa cells.

The cell lines will be used for two applications:

1. Transfection with plasmids (DNA) for production of PV. Depending on the cell line chosen, transfected cell lines will either amplify plasmid DNA and over-express the heterologous genes, or over-express the heterologous genes from the transfected plasmids. The over-expressed gene products include, HG3 or 4 surface GP, HIV-1 gag
[capsid protein [p24], nucleocapsid protein [p9], matrix protein [p17], p6), pol (reverse transcriptase, protease, Rnase H, Integrase), tat, rev (only the regions needed for the tat and rev genes are maintained) and a reporter gene (green fluorescent protein (GFP) or luciferase). The production of these proteins in combination with HIV-1 regulatory and packaging material will self-assemble to form replication deficient HIV-1 pseudoviruses, which display a heterologous HG3 or 4GP.

2. Infection with PV to screen for virus neutralisation. Cells are infected with a pseudovirus, if the virus enters the cell it cannot replicate at it's replication deficient; however, once in the cell and unpackaged the modified viral genome can integrate into the hosts genome and over-express a reporter gene.

The forseeable effects from accidental exposure to a laboratory worker would arise from over-expression of the immunogenic protein GP, which is present in and on the host cell, and the surface of PVs. The GP also determines cellular tropism, enabling PVs to infect humans and animal cells, although the likelihood is extremely low and considered to be non-hazardous.

Virus GPs have been shown to be immunogenic (resulting in protective antibody response and dendritic cell activation/endothelial inflammation), however, they are considered to have no deleterious effects and are not classed as toxic or pathogenic to humans, or hazardous to the environment.

The PV system described here is well established in the US and the UK for producing high titre retroviral vectors. There is a very low likelihood that exposure to any components of this project will cause harm. The risk of accidental transfer of reporter gene to the host is extremely low but in the event of such a transmission the potential for harm would be by insertional mutagenesis/over-expression of a reporter gene by random integration acting to deregulate normal cell function. Again the likelihood that this will cause harm is low as the vector is replication defective and therefore will not spread within or between individuals; also more than one genetic lesion is required for most cell transforming and altering events. We will use the retroviral vector system to transduce cell lines. The main risk is to the laboratory personal directly handling the retroviral vectors. The risk attached to using replication defective HIV vectors is minimised because these viruses are extremely susceptible to desiccation and will only survive a few hours at room temperature. In addition, the replication defective nature of the HIV vectors ensures that even in the case of a breach of containment, no virus can spread within the individual or from individual to individual.

The PV intended for experimental use will be produced using an adapted version of the HIV-1 gene therapy vector system; this system comprises three plasmids:

Plasmid 1 (p8.91 or p8.9NSX) - packaging construct, supplies the structural (gag), transcriptional (pol) and regulatory (tat and rev) proteins to form a HIV-1 core particle.

Plasmid 2 (pMDG, pcDNA3.1, pCAGGS, pCI or pSI containing a HG3 or 4 GP) - envelope construct, produces a foreign surface envelope protein (e.g. VSV-G), provides virus identity for cell recognition.

Plasmid 3 (pHIR-SIN-CSHW or pCSFLW) - expression construct, this supplies the HIV-1 RNA packaging signals, regulatory elements and reporter gene; this is the genetic material that will be carried by the PV for cell entry studies.

To produce PVs, all three plasmids must be delivered into the host (mammalian cells); plasmids are delivered into the host by transfection. Once in the cell the packaging plasmid produces structural proteins required to make virions; however, it also produces viral transcription and regulatory proteins that are required by the PV for infectivity studies. The expression construct produces a modified RNA HIV-1 defective genome, which is packaged into the virions; no viral structural genes are packaged into the virions. The envelope plasmid produces a foreign GP, which is incorporated into the surface of the virions; the GP subsequently determines the type of cell the PV can enter.

The PVs produced can be used to infect a broad-spectrum or specific cell line depending on the GP it carries, once cells are infected with a PV the genetic material (RNA defective reporter genome) is released and the transcription protein (reverse transcriptase) processes the RNA genome into a DNA form, which is then integrated into the host cells genome. Once intergraded, regulatory elements transcribe the reporter gene into its protein constituen (e.g. GFP), providing a quantifiable readout for analysis.
Plasmids:

p8.91 or p8.9NSX (and derivatives of) encoding HIV-1 gag-pol, tat and rev but with virulence genes vif, vpu and nef deleted as well as deletion of env (only the regions needed for the tat and rev genes are maintained) - Naldini, L. et al (1996) Science 272, 263-7.


pHR-SIN-CSGW encoding GFP, and derivatives of that encode alternative reporter genes, including pCSGW-YFP encoding Yellow Fluorescent Protein and pCSFLW encoding firefly luciferase. These all have the HIV-1 packaging sequence and the SFFV promoter leading to the expression of the reporter gene, flanked by self inactivating HIV-1 Long Terminal Repeats.

Envelope glycoprotein (GP) is responsible for binding to specific cell surface proteins/receptors, allowing viral entry into target cells. As such these genes are responsible for viral tropism. Their exposed conformation on the viral envelope means they are heavily glycosylated but still are the main drivers of the host immune response to the virus.

Gag contains around 1500 nucleotides, and encodes four separate proteins which form the building blocks for the HIV viral core:

- Capsid protein, CA, p24
- Nucleocapsid protein, NC, p9

Viral capsid and nucleoproteins are essential for ensuring viral genomes are packaged into progeny virions as the virus assembles. As such their main functions are binding to viral genomes and forming the main structural component of the viral core.

- Matrix protein, MA, p17 (this protein isn’t actually part of the viral core but the “matrix” which anchors the core to the viral envelope).
- p6 - The protein p6, one of the products of the gag gene, helps vpr to be incorporated into newly-made virus particles.

The most significant role of the gag gene is therefore to encode important proteins which will make up the viral core.

Pol is one of the main retroviral genes. It encodes four proteins, of which the most important is Reverse Transcriptase. Reverse Transcriptase performs a job which is unique to retroviruses, in that it copies the virus’ RNA genome into DNA. Since most organisms and viruses keep their genes in DNA form in the first place, they have no need to perform this task. The copying of the HIV genome into DNA form is one of the key stages of the HIV life-cycle. The other three products of pol are:

- Protease - which processes proteins made from HIV’s genome so that they can become part of new fully-functioning HIV particles.
- RNAse H - which breaks down the retroviral genome following infection of a cell
- Integrase - which integrates the DNA copy of HIV’s genome into the host DAN

"tat" is short for “transactivator” - its’ a regulatory gene which accelerates the production of more HIV virus. Infact, it's crucial to HIV, because HIV completely fails to replicate itself without it.

HIV regulator gene "rev" stimulates the production of HIV proteins, but suppresses the expression of other HIV's regulatory genes, rev helps intact mRNA to be exported from the cell nucleus. It binds to the mRNA at a specific point (the Rev-Responsive Element or RRE), and this complex of RNA and rev is sent out of the nucleus. A molecule of rev can "shuttle" in and out of the nucleus, potentially taking a new set of RNA out each time it leaves the nucleus. The RRE is not present in completely-spliced HIV mRNA - it will have been chopped out.
5' and delta 3' Long Terminal Repeats (LTR) and self-inactivating (SIN) - the LTRs function in packaging to create lentiviral particles, but are self-inactivating after integration into the target cell genome and thus render the integrated lentiviral construct incapable of being mobilised into infectious particles even in the presence of active endogenous or exogenous retrovirus.

Central polypurine tract (cPPT) serves to enhance the efficiency of integration and insert gene expression by facilitating nuclear translocation of pre-integration complexes.

Spleen focus-forming virus promoter (SFFV) - drives expression of reporter genes, upon host integration of the defective modified HIV-1 genome.

Woodchuck Hepatitis Virus Post-transcriptional Regulatory Element (WPRE) - enhance expression of the genes harboured in the expression construct. Its mechanism of action is unclear, but may involve the facilitation of nuclear export and/or stability of viral mRNA, or enhanced transcriptional termination and avoidance of read-through beyond the construct. This element was first described in Donello et al., 1998 and in Zuffrey et al., 1999 and there has been a good deal of work on it since. Kingsman et al., 2005 wrote a summary highlighting the concerns about the presence of a promoter and partial (60 amino acids) coding sequence of the WHV X protein within the first generation WPRE element. Protein X has been reported to be a major factor in the increased incidence of liver cancer after infection by natural routes with Hepatitis B-type hepadnaviruses. It is unclear whether there is any real evidence that this 60 amino acid N-terminal fragment of protein X is directly oncogenic, although it may act as a cofactor for oncolycygenicity with weak effect. It also seems that partial-Protein X cannot be expressed under the control of the promoter in the WPRE element, even in lentiviral constructs, except in the presence of a second enhancer, examples of which are not present in the WPRE construct. It could potentially be expressed as a result of read-through from endogenous genes through effects would be reduced by the deliberate inclusion of insulator elements flanking the expressed portions of the integrated construct. It is also possible to abrogate the potential expression from the included protein X promoter using newer generation WPRE elements lacking all or part of this promoter, and/or by site-directed mutagenesis to using newer generation WPRE elements lacking all or part of this promoter, and/or by site-directed mutagenesis to remove the start codon or other portions of the coding sequence. These modifications have been shown not to affect the efficiency of the WPRE element in enhancing expression of genes contained within the lentiviral construct.

The green fluorescent protein (GFP) is a protein composed of 238 amino acid residues (26.9kDa) that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. Although many other marine organisms have similar green fluorescent proteins, GFP traditionally refers to the protein first isolated from the jellyfish Aequorea victoria.

Luciferase is a generic term for the class of oxidative enzymes used in bioluminescence and is distinct from a laboratory reagent often refers to P. pyralis luciferase although recombinant luciferases from several other species of fireflies are also commercially available.

An application of GFP and luciferase for virology, cell and molecular biology includes reporters of expression.

- Bainbridge, J. et al. (2001) Gene Therapy 8, 1665-8

Evaluation of foreseeable effects

Minimal hazards: the inserted gene products are non-hazardous structural proteins with no toxic or oncogenic properties. Any minor risk associated with each of the resultant recombinant protein or PV are described below.

Envelope Glycoprotein: Some envelope glycoproteins give cytotoxic effects if over-expressed in cell lines, these proteins have not been shown to have any pathogenic effects. No other pathological effects have been reported for glycoproteins. Glycoproteins are, by their nature and function, immunogenic (triggering antibody and inflammatory responses), previous expression studies on such proteins have been performed at CL2 (GM36/09.2).

pP8.9NSX (and derivatives of) encoding HIV-1 Gag-Pol, Tat and Rev but with virulence genes vif, vpu and nef deleted as well as deletion of env (only the regions needed for the tat and rev genes are maintained). The combination of these genes will produce empty virions; however, the virions will not possess a surface GP and are not considered hazardous.
pHR-SIN-CSGW encoding GFP (and derivatives of that encode alternative reporter genes, including pCSFLW encoding firefly luciferase). These all have the HIV-1 packaging sequence and the SFFV promoter leading to the expression of the reporter gene, flanked by self inactivating HIV-1 LTR. The products from this construct is not considered hazardous.

Combination of gene products from p8.9NSX, pHR-SIN-CSGW and pMDG-GP (GP of interest) will produce a replication deficient PV capable of entering human and animal organs specific to the tropism of the GP displayed. This is considered highly unlikely, but if in the event this was to happen then no pathogenic affects are expected; the modified genetic material carried by the PV has the potential to be integrated into host cells and over-express the inserted reporter gene. The nature of this insertion and expression is also considered non-hazardous as the inserted genetic information cannot be mobilised due to the presence of the SIN and partially deleted LTR. Production and handling of PVs should be performed at Containment Level 2 (CL2).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The only GMO's generated are micro-organisms - no transgenic animal or plant work is associated with this project

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No Derogation from Full ACGM level 2 and ACDP class 2 containment will be needed for this work. All work with the resultant GMMs will be conducted under level 2 conditions and in a class 2 cabinet. Any work that does derogate from full class 2 containment will only be carried out after appropriate de-activation/lysis of any GMM that may be in that sample.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Hypochlorite - Haztabs
For liquid waste with in the Class II safety cabinet, a solution of sodium hypochlorite is made by adding 1 HazTab tablet to 1 litre of water/liquid waste (2,500ppm available chlorine) in a waste pot. For non-infectious tissue culture this is transferred to drain with copious amounts of water. If virus-infected waste is produced, this is left in hypochlorite overnight, then transferred to drain with copious amounts of water.

70% Isopropanol
Isopropanol stock is 70% in purified water. It can be used to disinfect surfaces that are not heavily contaminated. All items of equipment taken out of the laboratory should be first sprayed with 70% Isopropanol. Its use should be avoided where bacterial spores, mycobacteria or non-enveloped viruses maybe present.

Formaldehyde
The Class II safety cabinet should be fumigated before servicing (or when bacteria/fungal contamination is observed) using 50% formalin (20% formaldehyde) diluted in deionised water. 30ml of 50% formalin is added to a boiling pot and allowed to evaporate in an enclosed cabinet. This is left for at least 6hours, or preferably overnight before cabinet is switched on for re-use.

Autoclave sterilisation and incinderation of all solid waste.

Is an emergency plan required according to regulation 20?  
No

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
The committee has no objections to the risk assessment that has been undertaken.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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### Project Ref 36/14.2

#### Date Ackn’d
17/06/2014

#### CU2 Project Title
Genetic manipulation of Gram-negative ACDP2 bacteria

#### Date Project Ceased

#### Class
Class 2

#### CultureVol
< 1 Litre

#### Class CultureVol
Class 2 Culture Vol

#### Class 3-4
Consent Granted

#### Non-GMM

#### Project notified under transitional arrangements

#### Withdrawn
N

#### Tick if notifying a connected programme of work
N

#### Historical Significant Changes

#### Significant Change ID

#### Date of Significant Change

### Project Additional Information

#### Purposes of the contained use

The work proposed covers the genetic manipulation of ACDP2 Gram-negative bacteria, specifically targeted gene knock-outs and knock-ins in Enterobacteriaceae (e.g. Klebsiella pneumoniae, ACDP2 Escherichia coli, Enterobacter spp, Serratia, Citrobacter) and Pseudomonads (e.g. Pseudomonas aeruginosa, Stenorophomonas maltophilia). The main goal of the project is to generate site specific gene knock out derivatives in order to assess their susceptibility to common clinical disinfectants and to define the function of specific genes within the related stress response. The project will also generate fluorescent and bioluminescent derivative strains which can be used to develop more rapid measurements of microbial growth.
**Recipient or parental organism**

Klebsiella pneumoniae:
Clinical strains (e.g. TW3, M3, M6; Wand et al 2013; Complex interactions of Klebsiella pneumoniae with the host immune system in a Galleria mellonella infection model. J MedMicrobiol, 62:1790-8) and library isolates (e.g. NCTC13368, MGH78578)
Murray strains; isolated from the 1920s to the 1950s and available from PHE Culture collections.

Pseudomonas aeruginosa
PA01, NCTC 13359 and 372261 (resistant to chloramphenicol and the penicillin class of antibiotics).

Clinical and type isolates of Escherichia coli (acdp-2), Enterobacter spp, Serratia, Citrobacter and Stenotrophomoas maltophilia ). Details of specific strains or additional ACDP-2 bacteria will not be notified to the local GMSC committee.

**Host/vector system**

Vectors:
Suicide plasmids:
- pKOV suicide plasmid (Link et al, 1997) temperature sensitive Psc101 replication origin; CmR, SacB counter selection marker.
- pK0V has a temperature sensitive Psc101 replication origin. Strains harboring the plasmid must be grown at 30°C under chloramphenicol selection.
- pDM4 suicide plasmid, mobRP4; oriR6K; CmR; sacB counter selection marker;

The origin of replication (oriR6K) requires for its function the pi protein. This gene product is not present on the vector. It is supplied by specific E. coli strains that have had the prophage (lambda pir) introduced. It is therefore unable to replicate in strains that do not express the pi protein.

For plasmid complementation:
- pET-28a(+) expression plasmid (mobilisation defective). (Novagen) T7 promoter and terminator, pBR322 origin, f1 origin, iacI coding sequence, KanR
- pMF230 pTrc vector pKK233-2, oriVSF oriT Aor GFP mut2 (Nivens et al 2001; J Bac 183p1047) (mobilisation defective)
- pACYC184 broad host range plasmid (mobilisation defective; Change and Cohen, Construction and characterisation of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid, J Bacteriology 1978)

**Origin & function**

The functions of many of the genes to be targeted for knock-out and/or introduced for complementation are in the stress or stringent response. The upstream and downstream fragments are to allow insertion of the knock-out construct and are for the purpose of targeting only. They are extremely unlikely to encode peptides with any deleterious biological activity, and no attempt will be made to construct these fragments in a way that would allow high level expression.

Full length genes used to complement knock-out mutants will be inserted under the control of their own native promoter either back into their original chromosomal position or onto an expression plasmid. Doing this will not generate any deleterious biological activity beyond normal wild-type phenotype.

Genes expressing fluorescent proteins such as the gfp gene and its derivative e.g. egfp, bfp, rfp, cfp, etc will also be inserted either into the chromosome or onto an expression plasmid. Again there is no evidence to suggest that expression of these genes leads to any deleterious biological activity. The Photorhabdus luminescens lux genes encoding for the luciferin-luciferase system will be used to generate bioluminescent strains.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Putative function</th>
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<tbody>
<tr>
<td>mazG</td>
<td>nuceloside triphosphate pyrophosphohydrolase</td>
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<tr>
<td>katG</td>
<td>catalase-peroxidase</td>
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<tr>
<td>qacE, ΔE</td>
<td>putative efflux pumps</td>
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<td>relA</td>
<td>Stringent response, production of alarmone</td>
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<tr>
<td>ompA</td>
<td>Outer membrane protein, function in stress survival</td>
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<tr>
<td>cepA</td>
<td>putative efflux pump</td>
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<td>spoT</td>
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<td>Superoxide dismutase</td>
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<td>pmrA/pmrB</td>
<td>Bacterial two component regulator</td>
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</table>

**Evaluation of foreseeable effects**

The expectation is that the majority of the knock-out mutants generated in the study would be attenuated to some degree, compared to their parental strains. There is no expectation that the traits being modified would generate clones that were significantly more virulent than their parental strains. The aim of the project is to understand the role of specific genes in the ability of the organisms to adapt to biocide or other environmental stresses. As such it would be expected that the knock-outs would either increase the susceptibility to specific biocides and/or prevent the organisms from acquiring resistance. The introduction of wild type genes to complement the knock-out strains, even where this is achieved using an intact plasmid copy of the gene of interest on a multicopy plasmid, would not be expected to have a significant effect on the range of biocides that are effective against the organism, although it might affect the relative level of susceptibility compared to parental strains.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The only GMO's generated are micro-organisms - no transgenic animal or plant work is associated with this project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Autoclave sterilisation and incineration of all solid waste.

Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment  

The committee has no objections to the risk assessment that has been undertaken  

Project Containment

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Project Ref 36/14.3

Date Ackn’d 14/10/2014  
Date Project Ceased  
Withdrawn  
Historical Significant Changes  

CU2 Project Title  
Allelic exchange/transposon knockout strains of B. pertussis and plasmid-borne copies of endogenous B. pertussis genes

Class  
CultureVol  
Consent Granted  
Non-GMM  
Project notified under transitional arrangements  

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<tr>
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<td>1-50 litres</td>
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</table>

Tick if notifying a connected programme of work  

See Derogation GM36/15.3a
Current whooping cough vaccines are either whole cell formulations (wP) containing killed/detoxified Bordetella pertussis cells, or acellular formulations (aP) containing up to five purified B. pertussis proteins. Acellular pertussis vaccines were introduced in response to concerns about the reactogenicity of wP, but it is now apparent that aP vaccination has accelerated the emergence of new alleles of the vaccine antigens. There is also evidence that the immunity elicited by aP is shorter-lasting and less effective at blocking transmission compared to that elicited by wP. The allelic exchange and knockout strains described here are of value in distinguishing the contribution made by each of the proteins encoding by the deleted and/or allelic-exchanged genes to host immunity or to immune evasion. Their purpose is thus to aid the development of improved vaccines that overcome the shortcoming of existing wP and aP.

Bordetella pertussis. All available strains were isolated from whooping cough patients and are handled as virulent strains. B. pertussis is a respiratory pathogen transmitted from person to person via aerosol drops. There is evidence that asymptomatic (or mildly symptomatic) carriage may occur in persons who have received current acellular vaccines. Containment measures must therefore be sufficient to prevent un-noticed dissemination via vaccinated laboratory workers.

E. coli ST18 is used as a conjugation donor for construction of new GM B. pertussis strains. E. coli ST18 is a derivative of widely used non-colonising K12 strains. E. coli ST18 has chromosomally integrated tra genes from plasmid RP4, and a markerless deletion of hemA (these two modifications fall within the exemption in Schedule 2 Part lll of the Contained Use Regulations 2000). The hemA deletion prevents this strain from growing without supplementary 5-aminolevulinic acid (required at concentrations exceeding those in the human respiratory tract), and thus effects additional attenuation.

Recipient or parental organism

Bordetella pertussis. All available strains were isolated from whooping cough patients and are handled as virulent strains. B. pertussis is a respiratory pathogen transmitted from person to person via aerosol drops. There is evidence that asymptomatic (or mildly symptomatic) carriage may occur in persons who have received current acellular vaccines. Containment measures must therefore be sufficient to prevent un-noticed dissemination via vaccinated laboratory workers.

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Host/vector system

Allelic exchange vectors pSS4940 and PSS4894:

These were constructed elsewhere from a pUC replication origin, an RP4 origin of transfer, a selectable marker (gentamycin resistance in pSS4940, streptomycin resistance in pSS4894), and a counterselectable marker (the coding sequence for restriction enzyme I-SceI under the control of a Bordetella promoter). These vectors replicate episomally in E. coli, but after conjugation into B. pertussis, can persist only as chromosomal cointegrates via recombination between chromosomal sequences and homologous insert sequences.

Episomal vector pBBR1GW-Tet:

pBBRGWTet is a GATEWAY®-adapted derivative of the natural Bordetella bronchiseptica S87 plasmid pBBR1, with tetracyclin resistance added as a selectable marker.
The original pBBR1 is a 2.6kb plasmid that can replicate episomally in the genera Bordetella, Escherichia, Pseudomonas, Rhizobium and Vibrio, with 30-40 copies per cell in B. pertussis and E. coli. It can co-exist with incompatibility groups IncP, IncQ and IncW, and it can be mobilised by any host that provides RP4 tra function in trans (including E. coli ST18).

Origin & function

Transposon knockouts: The heterologous genetic material (Tn5) has no function other than to disrupt endogenous B. pertussis genes. The sole phenotypic side-effect is kanamycin resistance.

Cointegrates (transient intermediates in allelic exchange process); the heterologous genetic material is the backbone of pSS4940 or pSS4984 (see above).

Markerless knockouts: No heterologous genetic material present. B. pertussis genes cleanly deleted.

Allelic exchange mutants: No heterologous material present. Alleles with >80% amino acid similarly have been shuffled between different isolates of the same species.

Strains with upregulated antigen production: Limited to endogenous gene relocation within the B. pertussis chromosome or in to pBBRGWTet, i.e. no heterologous material other than the vector pBBRGWTet (described above)

Evaluation of foreseeable effects

Transposon knockout and markerless knockouts may be attenuated relative to the wild-type organism, but the cautious assumption is that they are still able to cause disease. The possibility of upregulated toxin expression arising from altered transcriptional context has been examined and discounted for each knockout.

Allelic exchange mutants are unlikely to pose new hazards because diversification of these genes into alleles is driven by immunological pressure, and the encoded products of different alleles are functionally equivalent. Similarly, strains with upregulated antigen expression are unlikely to have gained virulence since natural selection ensures that these antigens are already expressed at optimum levels (for the organism) in disease isolates. Upregulation of toxin genes has not been attempted and is not proposed.

Horizontal transfer risks: The risk of horizontal transfer of pBBRGWTet constructs is low because hosts strain of B. pertussis do not have RP4 tra function (checked from genome sequences). For cointegrates of pSS4940 or pSS4894, this risk is minimised by the transient nature of these constructs (~5 days agar plate culture before cointegrate resolution).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is sought

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Microbiologically contaminated liquids are treated with sodium hypochlorite or Super Q™, to produce a final concentration of no less than 2.5% (v/v) and left for 30 minutes before removing from the flexible film isolator or microbiological safety cabinet. These liquids are then autoclaved by the site autoclaving service prior to disposal to drain.

Solid waste is discarded into a nylon autoclave bag, sealed with a swan-neck bend, secured with a cable tie and sprayed with 2.5% sodium hypochlorite or Super Q™ (v/v) prior to being bagged into a second nylon autoclave bag sealed using a swan-neck bend secured with cable-tie. This is then sprayed with 2.5 sodium hypochlorite or Super Q™ (v/v) and left to vent in the flexible film isolator pass box or microbiological safety cabinet for 5 minutes prior to removal. The double-bagged waste is then placed in waxed paper-lined autoclave tins, autoclaved by the site autoclaving service and finally incinerated. Autoclave tins are surface-decontaminated prior to removal from the laboratory.
Waste is tracked throughout the destruction process according to standard site-wide procedures.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
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**Project Ref**  36/15.2

- **Date Ackn'd**: 18/11/2015
- **CU2 Project Title**: A rescue system for Hazara virus
- **Class**: Non-GMM
- **Consent Granted**: Consent Granted

**Project notified under transitional arrangements**  N

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

02/03/2022
The development of a rescue system for HAZV will enable the replication cycle of HAZV to be studied in much greater detail. The HAZV rescue system will enable us to make (i) precise mutations, (ii) deletions and (iii) gene fusions in the support proteins and or genome of HAZV. These will permit a molecular understanding of Hazara virus biology through subsequent experiments in comparison to wild type virus. We aim to use site directed mutagenesis to modify amino acid residues in the L-protein, N-protein, and the viral glycoproteins to determine the roles of these residues within the function of the protein and the replication cycle of the virus. We also aim to modify the viral glycoproteins and/or nucleocapsid protein through the addition of an EGFP tag to enable better visualisation of viral replication. This system will also allow the modification of the viral 3' and 5' UTRs to determine the minimum cis-acting factors required for viral replication, and the testing of anti-viral compounds. Initially wild-type (unmodified) infectious HAZV will be recovered from BSRT7 cells following transfection of plasmids encoding cDNA corresponding to the viral L-, M-, and S-segments. We will then generate plasmids encoding the following specific point mutations:

1) In the L segment plasmid, D685A, G710A and K726A These amino acid mutations will eliminate the putative endonuclease site responsible for cap-snatching during the replication cycle of HAZV, and should result in no or significantly decreased virus replication. Mutation of the equivalent amino acids (0693,0718 and K734) in the CCHFV virus-like particle system (which uses the CCHFV Nand L- proteins in trans to support transcription and replication of a CCHFV minigenome, and co-expression of the viral glycoproteins to create virus-like particles) abrogates minigenome transcription (0693A and K734A) or significantly reduces it (D718A). Oevignot S, Bergeron E, Nichol S, Mirazimi A, Weber F. 2015. A Virus-Like particle system identifies the endonuclease domain of CrimeanCongo hemorrhagic fever virus. J Virol. 89(11):5957-67.


An EGFP and other fluorescent reporter tags will be inserted into the viral glycoprotein precursor and N protein to determine whether the protein can tolerate the insert, and to better visualise HAZV replication. This insertion will either not affect, or will detrimentally affect HAZV replication. Shi X, van Mierlo JT, French A, Elliot RM. 2010. Visualising the replication cycle of bunyamwera orthobunyavirus expressing fluorescent protein-tagged Gc glycoprotein. J Virol. 84(17):8460-9.

The genetic changes proposed in this work will be detrimental to the WT version of HAZV and they will result in no or negligible risk to human health and the environment.
Recipient or parental organism

The recipient organism is Hazara virus. HAZV is listed by the ACDP in hazard group 2 and is used in a containment level 2 environment. HAZV is not known to cause any disease in humans or animals (other than severely debilitated transgenic mice which lack an interferon response) and does not pose a risk to the environment or community. HAZV has been isolated from Ixodes redikorzevi licks in Pakistan, and antibodies against Hazara virus have been detected in wild rodent sera. Transmission of HAZV is therefore thought to be through the bite of an infected tick.

Host/vector system

The vectors for the rescue system are all simple cloning vectors that allow the site directed mutagenesis of their inserts, but are non mobilisable and contain a Cot E1 origin of replication along with an antibiotic resistance gene. The vectors for the plasmids are:
- pOK (HAZV L-segment and M-segment rescue)
- pMK-RQ (HAZV S-segment rescue)
- pMK-RQ (HAZV L segment support and N support)
The plasmids will be propagated in and purified from the E.coli strain DH5a. The plasmids will be transfected into BSRT7 cells, which are modified BHK21 cells (an immortalised cell line originally derived from baby hamster kidney cells) that have been stably transfected with a plasmid encoding the T7 polymerase, which is constitutively expressed in BSRT7 cells. The rescue system plasmids contain a T7 promoter, which will allow the initiation of transcription of the HAZV genomic segments and support plasmid open reading frames in the cytoplasm of transfected cells by the T7 polymerase.

Origin & function

The genetic material (rescue plasmids encoding cDNA corresponding to the Hazara virus genomic segments, as well as support plasmids encoding the open reading frames of the HAZV L- and N- proteins) was originally synthesised by GeneArt (Thermo Fisher). The functions of the proteins encoded by these plasmids are detailed below.
The L segment rescue and the L segment support plasmid encode the HAZV RNA dependent RNA polymerase. This enzyme is responsible for transcription and replication of all three segments of the HAZV RNA genome. The M segment rescue plasmid encodes the viral glycoproteins Gn and Gc. Gn and Gc are the viral envelope glycoproteins that form a heterodimer on the virion surface. The function of Gn and Gc is to bind to mammalian cell surface receptors and facilitate entry of virions into cells. The S segment rescue and S segment support plasmid encode the viral nucleocapsid protein (N protein). The N protein binds to and encapsidates the viral RNA segments, protecting them from degradation, and forming ribonucleoprotein particles which are essential for both transcription and replication of the viral RNA genome.

Evaluation of foreseeable effects

The proposed HAZV rescue system poses minimal hazards to human health. All of the planned mutations have been previously generated in equivalent amino acid residues in related viruses, and have been shown to be detrimental to replication either of the rescued virus itself, or in a minireplicon system. Therefore we predict that the planned mutations will be detrimental to HAZV replication, and will result in a virus that is replication-comprised in comparison to wild type. These mutations will not increase the hazard of HAZV, they will not result in HAZV being turned into a pathogen. The planned mutations are not in the M segment which encodes the viral glycoproteins and therefore will not affect the tropism of HAZV or the immunogenic properties of the virions. None of the proposed mutations in the Nand L protein alter known virulence determinants or immunogenic properties of HAZV, and there are no hazardous properties associated with the proposed gene insert (EGFP). Also, none of the gene products
(described above) have been shown to have any directly hazardous effects. In accordance with wild type HAZV being classified as a hazard group 2 pathogen by the ACDP, all handling of supernatents from transfected cells, supernatent containing rescued virus and purified rescued virus will be carried out under containment level 2 conditions in MSC class 2 cabinets. This provides suitable protection to the user and minimises the risk of co-workers and the wider community being exposed to any GMOs.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A - no transgenic animal or plant work is associated with this project. |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A - no derogation from full containment level 2 procedures applicable to work with any ACDP classified hazard group 2 organisms will be required for this project. All work with the resultant GMMs will be conducted under containment level 2 conditions in an MSC class 2 cabinet and all GMMs will be inactivated before removal from ACGM 2 licensed areas. |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Liquid laboratory waste will be disinfected with 1% virkon solution overnight and diluted to drain with copious water (or other approved disinfectants such as sodium hypochlorite, or sodium dichloroisocyanurate can be used at a concentration of 2,500 ppm available chlorine). Solid laboratory waste will be discarded in yellow sacks for incineration. Virkon has been validated as an effective disinfectant for other members of the Bunyavirus family by the manufacturers (DuPont Rely+on virkon efficacy data) and by other groups (Evaluation of the efficacy of disinfectants against Puumala hantavirus by real time RT-PCR. Journal of virological methods. Maes, Li et al. December 2006) and is active in the presence of organic material. Sodium Dichloroisocyanurate (Haz-Tabs) have also been validated as an effective virus disinfectant. 70% isopropanol (100% isopropanol diluted in purified water) will be used as a surface and equipment disinfectant. when these are not heavily contaminated. 70% isopropanol will also be used to spray all items of equipment taken out of the laboratory. The MSC class 2 cabinet will be fumigated before servicing (or when fungal or bacterial contamination is observed) using 50% formalin (20% formaldehyde) diluted in deionised water to minimise the risk of exposure of GMMs to co-workers. |

### Is an emergency plan required according to regulation 20?  

| N |

If yes, tick to confirm that it is attached to this form  

| N |

Tick to confirm that you have attached a risk assessment to this form  

| Y |

Tick if you are claiming exemption from disclosure for section of the risk assessment  

| N |

Please enter comments on the GM safety committee on the risk assessment  

Project Containment
Project Ref 36/15.3a

Date Ackn'd 23/12/2015

Request for derogations concerning use of autoclaves

Consent Granted Yes

Derogation applies to GM36/05.1, GM36/12.8 & GM36/14.3

Historical Significant Changes

Project notified under transitional arrangements N

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref**  36/18.1

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<td>18/05/2018</td>
<td>Expression of Clostridium difficile virulence factors in Bacillus Megaterium</td>
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We propose to express DNA encoding whole or parts of virulence factors from C. difficile in Bacillus megaterium, as part of a commercial expression kit from MobiTec using standard techniques. This will involve inserting the relevant genets into a plasmid which can be propagated in E. coli cloning strain(s). This plasmid will then be harvested, and transferred to B. megaterium via transformation of protoplasts. Following this the genes will be expressed in B. megaterium under the control of a xylose inducible promoter.

Recipient or parental organism
B. megaterium - Host strains
MoBiTec™ offers three different B. megaterium strains (WH320, MS941, and YYBm1) for protein production. All strains are supplied as protoplasts, ready-to-use for transformation.
1. The strain WH320 is a chemical mutant of strain DSM319, which is deficient in the production of betagalactosidase (β-lacZ). It was described by Rygus and Hillen (1991).
2. The strain YYBm1 carries the nprM deletion and an additional deletion of the xylose isomerase gene xylA. It is thus unable to metabolize xylose, which is used as inducer for gene activation (Yang et al. 2006). (Genotype: Defined deletion mutant of wild type strain DSM319: ΔnprM, ΔxylA)
3. The strain MS941 was generated from the Wild-type strain DSM319 by deletion of major extracellular protease gene nprM (Wittchen and Meinhardt 1995). Because of reduced extracellular protease activity, this strain is well suited for extracellular protein production.

DSM319 been a workhorse in food and pharmaceutical production processes for decades (i.e., a and β-amylases used for starch modification in the baking industry and penicillin acylases essential for the synthesis of novel 3-lactam antibiotics, among others).
MoBiTec host strains are asporogenic on common media (vegetative cells die on plates kept at 4°C within two weeks) and are therefore not expected to persist in the environment.
B. megaterium is ubiquitous in the environment. These asporogenic mutants have a long history of safe use.

E. coli - Host strains
Derivatives of E. coli K12 or B (for example JM109, TOP10, ER2566, DH5alpha/10-beta, Novablu) will be used for the generation of plasmid stocks, general DNA manipulation. E. coli K12 strains are E. coli K-12 derivatives that are
recognised as non-colonising and disabled, and may be considered to be equivalent ACDP hazard group 1. They are not considered pathogenic to humans or animals, but are thought capable of surviving for up to seven days in the gut. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture.

### Host/vector system

**Expression vectors**

**Health and Safety**

Executive

With exception of pMGBm19, all shuttle vectors are derived from the original pWH1520 vector (Rygus and Hillen, 1991) with following features:

- Xylose-inducible expression system: strong PxylA promoter and repressor gene xylR; after xylose addition, the repressor is released from the PxylA that activates transcription initiation
- Ribosomal binding site (RBS) and start codon (ATG) upstream of the multiple cloning site (MCS)
- The MCS is located within the xylA' reading frame and allows easy cloning due to identical restriction sites in all vectors (from BglII to Nael, except for the pWH1520)
- Replication origins: CoIE1 ori (E. coli) and pBC16 ori (Bacillus)
- repU gene encoding for the replication protein RepU of Staphylococcus aureus, necessary for replication in Bacillus
- Resistance genes for selection: J3-1actamase (ampicillin resistance for E. coli), tetl encoding a tetracycline efflux pump (tetracycline resistance for Bacillus)

There are two kinds of vector series available:

- vectors of the 1520 series: with a) far-off located stop codon (> 120 bp downstream of start codon or b) stop codon directly downstream of an existing C-terminal tag
- vectors of the 1622 series: size-reduced vector variants with a) closely located stop codon directly downstream of the MCS or b) stop codon directly downstream of an existing C-terminal tag (Malten et al., 2006)

- pWH1520
- pMM1522
- pMM1525
- pHIS1522
- pHIS1525
- pSTREP1525
- pSTREPHTS 1525
- pSTOP1622
- pC-His1622
- pC-Strep1622
- pN-His-TEV1622
- pN-Strep-TEV1622
- pN-Strep-Xa1622

Vectors for special requirements

- pMMEc4

### Origin & function

The genes to be cloned and expressed will be sourced from either directly from C.difficile or synthetically synthesised based on genes available in the public domain.
The proposed inserted genes will be C. difficile toxins A (TcdA) and B (TcdB), and C. difficile transferase toxin (COT). The single-chain toxins TcdA and TcdB are the main virulence factors. They bind to cell membrane receptors and are internalized. The N-terminal gluCoQsyltransferase and autoprotease domains of the toxins translocate from low-pH endosomes into the cytosol. After activation by inositol hexakisphosphate (InsP6), the autoprotease cleaves and releases the glucosyltransferase domain into the cytosol, where GTP-binding proteins of the Rho/Ras family are mono-O-glucosylated and, thereby, inactivated. Inactivation of Rho proteins disturbs the organization of the cytoskeleton and affects multiple Rho-dependent cellular processes, including loss of epithelial barrier functions, induction of apoptosis, and inflammation. COT, the third C. difficile toxin, is a binary actin-ADP-ribosylating toxin that causes depolymerization of actin, thereby inducing formation of the microtubule-based protrusions.

Evaluation of foreseeable effects

The most hazardous GMM constructed will express active proteins which have the functions defined in Section 6 iii. The primary potential hazard is therefore towards human health. Toxins (TcdA and TcdB) are the main virulence factors of C. difficile (though there is no real difference in the virulence of these two). The most hazardous GMO produced here will therefore be Bacillus megaterium expressing high levels of one of these toxins. Although Knoblock et al (2011) reports expression of genes under the xyiA promotor in E.coli. The GMM produced here (E.coli and B. megaterium) are producing active toxin, as such will be handled in the same way as the native pathogen to mitigate any risks associated with the expression of biologically active virulence factors.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste is treated with 10% sodium hyperchlorite for 12 hours and autoclaved via centralised validated autoclave cycle. Solid waste is incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee has no objections to the risk assessment that has been undertaken
Project Additional Information

**Purposes of the contained use**

The particular micro-organisms for this Notification are genetically-modified derivatives of Aspergillus fumigatus. They are null/knockout mutants for transcription factors were generated by means of homologous recombination. The genetically-modified derivatives 01 Aspergillus fumigatus will be delivered to CC-PHE in preformatted and packaged multi-well plates frozen and in double-barrier containment packaging. They will be stored at -BOC in the ultra-low temperature storage facility Building 17, Culture Collections-Public Health England at Porton Down. These genetically-modified derivatives of Aspergillus fumigatus will be stored and shipped from Building 17, Culture Collections-Public Health England and Porton Down.

We will only store and distribute these strains. No laboratory work will be performed.

Risk Assessments to be used: BA100419. These GM strains have been developed and deposited with Culture Collections-PHE by COFUN 2.30D Core Technology Facility, Grafton Street, School of Biological Science* Faculty of Biology, Medicine and Health, University of Manchester, M13 9NT* UK.
Recipient or parental organism

The strain used to create the library of null mutants strains in a genetically modified strain with the designation A1160P+ (MF1G001). This strain has virulence levels indistinguishable from the isogenic wild-type clinical isolate CEAIO.

Host/vector system

Vectors containing hygromycin cassettes were constructed using the Gateway™ pDONR221 Vector (Thermo Fisher) and the hygroR cassette from pCBI003 Knock out vectors pDONR HPH A and pDONR HPH B vectors (plasmid A and plasmid B), representing two orientations of the hygroR cassette were constructed

These vectors were used to construct the knockout vectors

The generation of Knockout cassettes in A. fumigatus was performed using a generic methodology for the generation of gene KO cassettes using PCR fusion and these constructs.

PCR fusion and Aspergillus transformation has been described in detail by Berl Oakley's group (Nat. Protoc. 2006;1 (6):3111-20. Szewczyk E).

Origin & function

The selectable marker hph, which confers hygromycin resistance. The genetically-modified derivatives of Aspergillus fumigatus that have been constructed are null/knockout mutants for transcription factors generated by means of homologous recombination. The genetic KO constructs comprise the selectable marker hph, which confers hygromycin resistance, flanked by 1 kb fragments upstream and downstream of the gene to be removed. The hygromycin cassette was delivered into A. fumigatus and transforming colonies were selected using hygromycin at 200mg/L. Transformants have been validated by PCR to confirm insertion of the cassette into the specified locus.

Evaluation of foreseeable effects

Consequences to Risk Assessment for Human Health:

Aspergillus fumigatus is an opportunist pathogen. Individuals with a normal immune system are not at risk from systemic aspergillosis. Severe immunological defects are required for individuals to become infected [Singh & Paterson, Clin. Microbiol. Rev. (2005) 1 B: 44-69].

Aspergillus fumigatus is found as a normal component of the microflora of the atmosphere and no systemic disease is seen in the healthy population. It is therefore very unlikely that Aspergillus fumigatus poses a significant risk to immune-competent laboratory workers or other support staff.

Although genetically-modified Aspergillus strains produced pose no significant threat to healthy immunocompetent individuals. Immunocompromised individuals or women of childbearing age who are expecting a child or planning a pregnancy will be excluded from handling these pre-packaged Aspergillus strains.

Risk: Low

The stock held at CC-PHE will consist of preformatted and packaged multi-well plates containing the genetically modified strains of A. fumagatus will be delivered to Culture Collection-PHE frozen and in double-barrier containment packaging. They will be stored at -BOC in the ultra-low temperature storage facility Building 17, Culture Collections Porton Down. The stock held will consist of ten batches of five x 96 multiwell plates (containing a total of 400 individual strains of GM Aspergillus).

We will not propose to perform any laboratory work with these strains. Storage and distribution only

Shipping: Aspergillus Fumigatus is a Hg2 fungus whether wild type or genetically modified these will be shipped as UN3373 - Biological substance Category B and would be packaged according to Packing Instruction 650.
We have consulted the Mycology Reference Laboratory, Public Health England, Bristol. Their recommendation for handling the Aspergillus strains are: Aspergillus fumigatus is a HG2 pathogen and is manipulated on the bench at CL2. A 0.5% Virusolve + solution is used with a contact time of more than 1 minute for local disinfection where a reduction of spore numbers is adequate to limit contamination.

Total elimination of genetically-modified A. fumigatus spores, for example, a spill in a MSC, would be fumigation with formaldehyde. This is the routine procedure at the Mycology Reference Laboratory. Spillage outside of the MSC would probably require fumigation of entire the laboratory.

As no culture work is proposed we need only consider the release of fungal strains in the event of breach of the packaging.

The disinfection procedures in the event of a breach of the packaging will be as follows:

Spillage: allow aerosols of dry spores or spore suspensions to settle; wearing protective clothing, gently cover spill with paper towels and apply 0.5% Virusolve +. After 30 minutes clean entire area with 0.5% Virusolve + and dispose of all materials by steam sterilization.

Liquid waste to be inactivated with 0.5% Virusolve +.

All waste materials (solid and liquid) will be initially autoclaved followed by incineration.

Contaminated equipment, e.g., freezer, would be cleaned using 5% Virusolve + solution.

(Virusolve + is supplied by Amity International). This reagent is 1509001 &13485 compliant, Certificate Numbers: GB06/69741 & GB0669740. Basic fungicidal and anti-yeast activity has been tested in accordance with BS EN 1275 (European Suspension Test) and demonstrates a > log.5 reduction in counts at 20°C after 1,2, and 5 minute contact times.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We will not propose to Rerorm any laboratory work with these strains. Storage and distribution only. No derogation is requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Control and Waste Management procedures

Health and Safety Executive

Shipping: Aspergillus Fumigatus is a HG2 fungus whether wild type or genetically modified these will be shipped as UN3373 - Biological substance Category B and would be packaged according to Packing Instruction 650.

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The disinfection procedures in the event of a breach of the packaging will be as follows:
Spillage: allow aerosols of dry spores or spore suspensions to settle; wearing protective clothing, gently cover spill with paper towels and apply 0.5% Virusolve +. After 30 minutes clean entire area with 0.5% Virusolve + and dispose of all materials by steam sterilization. Liquid waste to be inactivated with 0.5% Virusolve +. All waste materials (solid and liquid) will be initially autoclaved followed by incineration. Contaminated equipment, e.g., freezer, would be cleaned using 5% Virusolve + solution. (Virusolve + is supplied by Amity International). This reagent is ISO9001 & 13485 compliant. Certificate Numbers: GB06/69741 & GB0669740. Basic fungicidal and anti-yeast activity has been tested in accordance with BS EN 1275 (European Suspension Test) and demonstrates a > log.5 reduction in counts at 20C after 1, 2, and 5 minute contact times

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick if you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

None

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Non-GMM Consent Granted

02/03/2022
The work proposed covers the genetic manipulation of Candida species to enable studies on new antifungal agents. Much of the genetic engineering will be carried out by our collaborators at King's College London (KCL) but 1 strains will be transferred to the organisation for use in drug screening, mechanisms of action studies and for virulence studies in Galleria mellonella infection models. Studies focus particularly on Candida albicans, as the most common cause of candidiasis, and the recently emerged species C.aurantis. Additional species, such as C.glabrata, C.tropicalis, may be used to answer specific questions about drug resistance mechanisms in these species. A specific objective of the work is to generate strains with single and multiple deletions in the efflux pump genes, associated with resistance to antifungal agents and genes affecting cell wall permeability. These mutants will help in defining how specific efflux pumps contribute to resistance and to improve initial drug screening. These strains also help validate these targets for the development of new antifungals, acting either directly on the cell wall/membrane or as “resistance breaking” adjuvants targeting efflux pump.

C. auris strains TIDG1912, NCPF8985, NCPF8971, and NCPF8977 from Public Health England (now UKHSA) and NCTC. Strains are resistant to fluconazole and voriconazole, variably resistant to echinocandins, but sensitive to caspofungin and amphotericin B (according to broth dilution and presumptive EUCAST breakpoints). The strains is also sensitive to nourseothricin used as a selectable marker in these studies. C. albicans strain SC5314 (the wild-type strain sequenced by the Candida genome sequencing project), was provided by King's College London (Odds et al 2004). The strains is susceptible to all antifungal agents tested. The strains is also sensitive to nourseothricin.

Genetic modification of Candida strains is achieved using CreLoxP systems based in intergration cassettes carrying a selectable marker, which can subsequently be excised from the genome. In the GMOs covered here there are no vectors used.

Efflux pump genes and regulators.
Genes that appeared to be significantly upregulated in fluconazole-resistant isolates of C. albicans or C. auris were CDR1 and CDR2 (multidrug transporter of ABC superfamily), MDR1 and MD2 (MFS family multidrug efflux pump) and MLT1 (vacuolar membrane transporter of ABC family) will be deleted.

Metabolic genes.
Metabolic genes will be deleted to demonstrate utility of the knockout process and to generate auxotrophic variants for some applications. Selected genes will include:

- URA3 encodes orotidine-5'-phosphate (OMP) decarboxylase, an enzyme required for the biosynthesis of uracil.
- ADE2 encodes phosphoribosylaminomimidazole carboxylase, an enzyme involved in the catalysis of 'de novo' purine nucleotide biosynthesis.

Cell wall / membrane biosynthetic genes.

- ss-(1,3)-glucan synthase (GS): involved in biosynthesis of ss-(1,3)-glucan, the major component of the fungal cell wall. Molecular targets of echinocandins
- Cell wall kinase Yck2
- Trehalose synthases: involved in synthesis of trehalose
- Gwt1, a key component of GPI anchor protein biosynthesis
- Chitin synthase involved in synthesis of chitin, one of the key cell wall components in fungi.

Putative virulence genes.

- Hog1-related stress-activated protein kinase (SAPK), is a known stress-response gene linked to virulence in Candida and other fungal pathogens; Day et al 2018.

Peptide tags.

- C-myc, 6-HA and 6-His tags are short peptides recognised by antibodies and have no biological function in their own right.
- HiBiT is a short 11 amino acid peptide which combines with LgBiT to form a functional luciferase for ultrasensitive bioluminescent detection.

Evaluation of foreseeable effects

The expectation is that the majority of the knock-out mutants generated in the study would be attenuated to some degree, compared to their parental strains, either in terms of reduced fitness/virulence and/or by reducing their resistance to antifungal agents. There is no expectation that the traits being modified would generate clones that were more virulent than their parental strains. The aim of the project is to understand the role of specific genes in the antifungal resistance of the organisms and to generate strains to improve initial stages of antifungal screening. As such it would be expected that the knock-outs would either increase the susceptibility to specific antifungals and/or prevent the organism from acquiring resistance. Specific knockouts might also have reduced virulence traits, such as the ability to infect Galleria mellonella as a model host, or to form biofilms as an important facet of infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The only GMMs generated are micro-organisms - no transgenic animal or plant work is associated with this project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No Derogation from Full ACGM level 2 and ACDP class 2 containment will be needed for this work. All work with the resultant GMMs will be conducted under level 2 conditions and in a class 2 cabinet.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Hypochlorite — Haztabs

For liquid waste with in the Class II safety cabinet, a solution of sodium hypochlorite is made by adding 1 HazTab tablet to 1 litre of water/liquid waste (2,500ppm available chlorine) in a waste pot. For non-infectious tissue culture this is transferred to drain with copious amounts of water. If virus-infected waste is produced, this is left in hypochlorite overnight, then transferred to drain with copious amounts of water.

70% Ethanol

isopropanol stock is 70% in purified water. It can be used to disinfect surfaces that are not heavily contaminated. All items of equipment taken out of the laboratory should be first sprayed with 70% Isopropanol. Its use should be avoided where bacterial spores, mycobacteria or non-enveloped viruses maybe present.

Formaldehyde

The Class II safety cabinet should be fumigated before servicing (or when bacterial/fungal contamination is observed) using 50% formalin (20% formaldehyde) diluted in deionised water. 30ml of 50% formalin is added to a boiling pot and allowed to evaporate in an enclosed cabinet. This is left for at least 6 hours, or preferably overnight before cabinet is switched on for re-use.

Autoclave sterilisation and incineration of all solid waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The committee has no objections to the risk assessment that has been undertaken.

Project Containment

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<th>Growth Rooms</th>
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Project Ref 36/94.2

Date Ackn’d CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
Gene Transfer in Clostridium Botulinum

26/05/1994

Date Project Ceased

Class 2

Non-GMM

Consent Granted

Not Applicable

Tick if notifying a connected programme of work

N

Withdrawn

Y

Historical Significant Changes

GM36/03.2

Historical Date of Additional Info

03/10/2003

Project notified under transitional arrangements

Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Name

INSTITUTE OF BIOMEDICAL & LIFE SCIENCES UNIVERSITY OF GLASGOW

Name 2

Department

BIOCHEM & MOL. BIO., INFECT & IMMUNIY IMMUNOLOGY

Campus Estate or Research Centre

Building

BOWER BUILDING

Road Name

District

Town

GLASGOW

County

EAST RENFREWSHIRE

Postcode

G12 8QQ

Country

SCOTLAND

Tel Number

0141 339 8855

Fax Number

0141 330 4447

E-mail

HSE Division

SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee
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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**Project Additional Information**

**Purposes of the contained use**

P. falciparum is a serious human pathogen classified by ACDP as Group III* (derogation applies such that they can be handled in category II facilities, with no requirement for positive pressure of HEPA filtration, since there is no risk of airborne contamination.).

No significant additional hazards have been identified above and so it is appropriate to assign the same containment level as that required for the non-GM organism. This is categorised by ACDP as Class III, but derogation is permitted to allow work to proceed under containment level 2. A COSHH form pertaining to P. falciparum cultures has been signed by all relevant personnel.

**Recipient or parental organism**

The function of the inserted parasite gene product will frequently be unknown, or can only be implied through homology with other organisms. Gene knockout experiments targeting genes linked to invasion should result in parasites with lowered invasion potential. Knockouts of potential drug targets (e.g., protein kinases) are aimed at reducing the fitness of the knockout parasites, preferably to produce non-viable or severely functionally-impaired parasites. Allelic replacement of protein kinase genes with those exhibiting hypersensitivity to inhibitors should result in parasites with increased sensitivity to these inhibitors. Growth rates are not expected to be affected, other than negatively, in any of these experiments.

**Host/vector system**

Growth of *Plasmodium falciparum* in human red blood cells.

**Origin & function**

Stable transfection systems for the human malaria parasites have only recently become available, and provide a means to investigate gene function using gene...
modification or ablation ("knockout") 2. Transfection is achieved through electroporation of either red blood cells prior to infection by the parasite, or ring-stage parasites (asexual development cycle in the blood), using in vitro cultured parasites grown in human erythrocytes, with specific plasmid vectors such as pHRPCAT. These vectors contain a drug-selectable marker, usually the dihydrofolate resistance gene (DHFR) from pyrimethamine-resistant strains of either P. falciparum or Toxoplasma gondii, for selection with pyrimethamine, or the human DHFR for selection with the antifolate drug WR99210 3. We will also use plasmids based on the pHHT-TK vector 4, which allows negative selection using gancyclovir.

Transient transfection (without permanent modification of the parasite;"s genome) will be performed using plasmids encoding reporter genes such as GFP, luciferase or CAT. The mRNA levels of specific genes will be targeted by RNAi.

Plasmodium genes considered for possible GM work:

* Protein kinases (a preliminary examination of the PlasmoDB database indicated the presence of approximately 60 genes of this family in the parasite's genome, any of which can be of interest in the context of our work).
* Cyclins (pfcyc-1, -2, -3, -4)
* Apical complex proteins (esp. pFRH1, -2A, -2B, -3, and -4)

Evaluation of foreseeable effects

Any environmental risk is more likely due to the organism itself rather than the genetic modification. Because we will not be using mosquitoes, the release of the parasite in to the environment is exceedingly unlikely.

The function of the inserted parasite gene product will frequently be unknown, or can only be implied through homology with other organisms. Gene knockout experiments targeting genes linked to invasion should result in parasites with lowered invasion potential. Knockouts of potential drug targets (eg protein kinases) are aimed at reducing the fitness of the knockout parasites, preferably to produce non-viable or severely functionally-impaired parasites. Allelic replacement of protein kinase genes with those exhibiting hypersensitivity to inhibitors should result in parasites with increased sensitivity to these inhibitors. Growth rates are not expected to be affected, other than negatively, in any of these experiments. For all new transgenic strains, growth rates will be checked as part of phenotypic characterisation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Similar containment requirements are for unmodified P. falciparum. As for unmodified P. falciparum, derogation from various aspects of Level 3 containment is appropriate. The reasons for derogation relate to the fact that infection by aerosol or ingestion does not occur, and spread of the disease requires the appropriate vector species of mosquitoes, which are absent in the area where the work will occur.

We request derogation from standard level three containment on the three points detailed below. Plasmodium cannot be transmitted by aerosol. The normal route of transmission is by mosquito bite. In laboratory conditions, the only means of infection is by an accidental puncture wound, for example with a contaminated syringe needle. Also, plasmodium is exceptionally fragile. To survive outside its host species (humans and mosquitoes), it must be kept in complex media. Plasmodium dies instantly if placed in water and is also killed by drying.

1. Negative pressure relative to the pressure of immediate surrounds. Derogation requested. Plasmodium transmission by aerosol does not occur.
2. Extract and input air from the laboratory should be HEPA filtered. Derogation requested. Plasmodium transmission by aerosol does not occur.
3. Protective footwear. Derogation requested for footwear. The risk of live/infectious parasites escaping to the environment in this way is nil as they cannot survive outside the host in the environment - not necessary as this requirement is is only where and to the extent the risk assessment shows it to be required.
4. Autoclave required in the laboratory suite.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste is inactivated using HibiTane prior to disposal. Solid waste for autoclaving will be double-bagged and transported to the autoclave room in a plastic

02/03/2022
The culture room is kept locked and access is restricted to trained personnel. Written training and culture records are maintained. The sensitivity of new transgenic strains to killing by hibitane will be tested.

We feel this is a good and well thought out risk assessment. From an original discussion of the proposal, the committee raised a variety of questions, all of which have been answered to our satisfaction, by the principle investigators. In particular, we feel that the areas of derogation from level 3 are appropriate because of the extremely low risk of spread of plasmodium into the wider community and environment. We believe the only significant risk is to the actual individuals involved in the project where infection as a result of a puncture wound could theoretically occur. The risk of a puncture wound occurring is minimised by avoiding the use of sharps in handling transgenic plasmodium.

**Project Containment**

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**Project Ref** 37/00.3

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Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 37/01.1

Date Ackn'd: 15/01/2001

CU2 Project Title: OVEREXPRESSION OF EUKARYOTIC MEMBRANE PROTEINS

Class: Class 2

CultureVolClass2: 1-50 litres

Consent Granted: not applicable

Historical Significant Changes

Withdrawn: N

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: N

**Project Additional Information**
### Purposes of the contained use

To overexpress membrane proteins of interest in sufficient quantities as to provide the necessary material for subsequent purification and crystallisation experiments.

### Recipient or parental organism

CHO, BHK cells

### Host/vector system

Semliki Forest Virus (SFV)

### Origin & function

Receptors (in particular G-protein coupled receptors (GPCR), ion channels (both voltage - and ligand-gated) and transporters from the human, rat, mouse Drosophila, C. elegans, yeast and higher plant genomes.

### Evaluation of foreseeable effects

The consequence of escape to the operator is low: If the worker is exposed to virus or virus infected cells, the likelihood of disease is very low. Any gene expression that does occur would be restricted in its duration and limited to those cells that have been infected. The only potential hazard associated with this possibility depends upon whether or not the transgene is toxic. The genes that we intend to express, even though some maybe from organisms that are non-human are non-toxic. As 'live' virus can, theoretically, be produced by RNA recombination events, there exists a very small possibility that a user could become infected with replication-competent virus. This would result in mild disease symptoms before the immune system restores the person to full health. Given that the major carrier of SFV is the mosquito, infections with SFV arising from natural sources in the UK are infrequent. This means that the possibility of generating replication-competent virus within an SFV-infected human through RNA recombination events, between wild-type and recombinant RNAs, are next to zero. The possibility of an infected operator infecting other people is also very low.

The consequence of escape to the environment is low: If virus was to be removed from the lab (e.g. for shipment or transfer to another lab), it would be the inactive form of the virus that would be sent. Activation of the virus with a-chymotrypsin will always occur in a class II hood, prior to infection of the mammalian cells. Even if the virus escaped by accidental damage to its container during transit, it would remain non-infectious.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Cell suspensions infected with recombinant SFV will be grown in a purpose built incubator for up to 48 hours, during which time expression of the transgene will occur. This is an absolute requirement as optimal gene expression usually occurs at temperatures between 30-40C. The bio-reactors will be autoclaved after they have been used. Following the period of gene expression, the cells will be harvested by centrifugation in a stand-alone centrifuge. The cell suspension will be poured into sealed containers, which will be autoclaved after use.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated tips and tubes will be placed in autoclave bags situated within a metal bin. The bin has a metal lid, and the whole bin will be replaced into the autoclave without removing the bag from it. Centrifuge pots and bioreactors will be autoclaved directly after use.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
This application proposes to use an attenuated Semliki Forest Virus Vector to overexpress a variety of proteins. Since SFV is a category 2 pathogen a full RA and notification to HSE is required. The vector system is an off-the-shelf one that utilises defective strains of the virus. Live virus should not be produced. In the unlikely event of live virus being generated by RNA recombination, a mild and short lived human infection might be produced. The committee were satisfied that the probability of this occurring was low and that the consequences unlikely to be serious. The recommendation of the committee was that the application be approved and forwarded to HSE.

### Project Containment

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### Project Ref  37/02.1

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<td>18/02/2002</td>
<td>USE OF ADENOVIRUSES (SEROTYPE 5 REPLICATION-DEFICIENT/E1-DELETED ADENOVIRUSES) TO DELIVER FOREIGN GENES INTO CELLS</td>
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<td>&lt; 1 litre</td>
<td>Consent Granted: not applicable</td>
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Date Project Ceased

- Withdrawn: N

- Historical Significant Changes

- Historical Date of Additional Info

- Significant Change ID

- Project notified under transitional arrangements: N
### Project Additional Information

#### Purposes of the contained use

The purpose of these experiments is to drive high level expression in cellular populations to determine the physiological role of these proteins in either insulin signalling or regulated membrane trafficking. These are the two main areas of research activity in this lab.

We seek to use Adenovirus as it is an established method for high efficiency infection of adipocytes in culture (which cannot be achieved by other means), and because such high levels of expression can be reached, it offers advantages for other cellular systems over more standard transfection approaches.

#### Recipient or parental organism

- Murine 3T3-L1 adipocytes, Chinese Hamster Ovary cells, Rat pheochromocytoma cells (PC12 cells), HeLa cells.
- Rat L6 muscle cells, Human Endothelial Artery cells (commercial - not primary isolated cells) and similar cells.

All cells will be purchased from commercial suppliers.

#### Host/vector system

Serotype 5 replication-deficient/E1-deleted adenoviruses

The adenoviral vectors will be purchased from commercial suppliers of these (Invitrogen).

Intended function is to use these viruses to delivery foreign genes into cells. These genes include components of membrane trafficking pathways (syntaxins, components of the so-called exocyst complex (sec6, sec8, sec10 and sec15), cysteine string proteins, domains of trafficking proteins (such as Hrs domains, ear domains of adaptins) and Arf protein effectors. In addition, we will express some components of signalling cascades which impinge on membrane traffic, specifically IKK kinase isoforms alpha and beta, protein kinase B and dominant negative mutants of protein kinase B.

#### Origin & function

The viral vectors are replication deficient, and cannot replicate in the cell lines to be used. Infection of humans is highly unlikely to result in replication as the viruses are E1 deficient.

The consequences of escape to the operator are mild. If the worker is exposed to virus or viral infected cells, the likelihood of disease is very low. Any gene expression which may occur will be restricted to the infected cells and its duration of expression would be short. The only potential hazard could be if the transgene is toxic.

The consequences of escape to the environment are minimal, as the proteins being expressed using these viral vectors are non-toxic or pathogenic and there is no potential for any of the defective isolates recombining with wild type adenovirus present in the human population.

#### Evaluation of foreseeable effects

The consequences of escape to the operator are mild. If the worker is exposed to virus or viral infected cells, the likelihood of disease is very low. Any gene expression which may occur will be restricted to the infected cells and its duration of expression would be short. The only potential hazard could be if the transgene is toxic.

The consequences of escape to the environment are minimal, as the proteins being expressed using these viral vectors are non-toxic or pathogenic and there is no potential for any of the defective isolates recombining with wild type adenovirus present in the human population.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
All replication of viruses will be performed under level 2 containment. All infections of cells for experimental analysis will be performed in level 2 facilities. Once infection is completed, media containing viruses will be removed from cells, the cells will be washed and at that stage manipulations will involve washing in different buffers, stimulation of cells with insulin or other agonists, treatments with inhibitors etc. These require incubations in defined buffers on a hot-plate or on ice, depending on the experiment, and cannot be performed in level 2 hoods. We will therefore carry them out with the derogations from level 2 detailed below. Subsequent disruption (eg homogenisation of lysis) will, however, be performed in level 2 hoods to minimise any risk from aerosols. Subsequent fractionation of homogenates or lysates will be carried out with the derogations detailed below.

It must be emphasised that all cell lines proposed for these experiments do not support replication of the infectious virus.

Derogations from level 2 for manipulation of cells and subsequent fractionation of homogenates or lysates: Numbers refer to Part II, Table 1a, pp 80-82.

5. Negative pressure relative to surroundings: Not required by RA.
7. Microbiological Safety Cabinet. Not required by RA for these procedures.
9. Access to authorised personnel. Work to be carried out, and stocks to be kept in room with restricted access to authorised personnel. No public access to labs within the building.
10. Specific measures to control aerosols. Not required for these handling procedures. Required for cell disruption (to be carried out in level 2 hood).
14. Efficient control of disease vectors. Not required as adenovirus is not spread by vectors. Defective virus used for GMM not infectious to rodents.
15. Specific disinfection procedures in place. Specific procedures not required by RA.

Written records of Staff Training. All workers covered by COSSH and must be adequately trained and complete COSSH records, all GM workers registered locally with GMSC. Specific records not required.

Media derived from cells (HEK293 cells) producing virus will be immediately mixed with strong bleaching agents (such as chloros) prior to disposal. Media from cells after infection (test cell lines which do not support viral replication) will likewise be treated with bleach then disposed of in designated sink/drain area.

All contaminated plasticware will be rinsed in chloros prior to autoclaving and disposal.
All contaminated pipettes will be autoclaved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Following prior consultation the GM Safety Committee (Assessment Subcommittee) met on 06/12/01 to consider this proposal. The subcommittee was satisfied that the proposal meets the safety criteria for a class 2 project.

Project Containment
Project Ref 37/02.2

Date Ackn'd 20/05/2002
CU2 Project Title MOLECULAR AND CELL BIOLOGY OF BACTERIAL RESPIRATORY PATHOGENS

Date Project Ceased 11/01/2013
Class 2
CultureVolClass2 < 1 litre
CultureVolumeClass3-4
Non-GMM not applicable
Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes Project Transferred to GM116

Recipient or parental organism
Streptococcus pneumoniae
Neisseria meningitidis
Staphylococcus aureus

Host/vector system
Introduction of linear DNA generated by polymerase chain reaction (PCR)
Transposon Tn917 for gene inactivation
Plasmid pMTL23 for gene inactivation in Neisseria

Purposes of the contained use
Construction of gene detection mutants or gene replacement mutants. Analysis of phenotype of altered mutants in invitro and in vivo (animal) systems.
Modified genes from the pathogens including virulence factors such as toxins, surface proteins and signalling molecules.

**Origin & function**

All the organisms used are carried as commensals in the normal human population. All genetic alterations involve the removal of genes or protein function such that the GMO will be no more harmful to people or the environment than the parental organism. In most cases the risk will decrease in the GMO due to the genetic modification partially disabling the organism. Antibiotic resistance markers will only be used if they are associated with the normally occurring population.

**Evaluation of foreseeable effects**

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

none

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMO Liquid cultures (volume up to 100ml) will be autoclaved. Following autoclaving cultures will be sterile. Other material (equipment etc) will be sterilized by disinfection. Animals infected with GMOs are incinerated. No GMO will be released into the environment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The GMSC assessed this proposal on 21/2/2002. The committee was satisfied that this work could be carried out under the containment indicated and that the proposal could be classified class 2.

**Project Containment**

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02/03/2022
USE OF ADENOVIRUSES (SEROTYPE 5 REPLICATION-DEFICIENT/E1-DELETED ADENOVIRUSES) TO DELIVER FOREIGN GENES INTO CELLS

The purpose of these experiments is to drive high level expression in cellular populations to determine the physiological role of these proteins in cell signalling. This is the main area of research activity in this lab.

We seek to use Adenovirus as it is an established method for high efficiency infection of various cells in culture, in particular, cardiac myocytes, human vascular endothelial cells, and vascular smooth muscle cells which cannot be achieved by other means. Because such high levels of expression can be reached, this offers many advantages for analysing these various cellular systems over more standard transfection approaches.

Recipient or parental organism

Murine 3T3-L1 adipocytes, murine F442A adipocytes, Chinese Hamster Ovary cells, rat cardiac myocytes (primary isolates), human arterial endothelial cells (commercial - not primary isolated cells), monkey kidney cells (COS1 and COS7), Rat1 fibroblasts, human umbilical vein endothelial cells, human pulmonary artery smooth muscle cells (commercial).

All cells apart from the rat cardiac myocytes will be purchased from commercial suppliers.

Host/vector system

Serotype 5 replication-deficient/E1-deleted adenoviruses - see risk assessment.

Origin & function

The adenoviral vectors will be purchased from commercial suppliers of these (Invitrogen)
Intended function is to use these viruses to deliver foreign genes into cells. These genes include the following G-protein-coupled receptors:

Human: EDG1, EDG2 and EDG3 lipid receptors. A1, A2A and A3 adenosine receptors.

AND catalytically inactive forms of all the above AND other forms engineered to have disruptions in intracellular targeting and phosphorylation.

Also Green Fluorescent Protein (GFP) and receptor-GFP chimeras.

Evaluation of foreseeable effects

The viral vectors are replication deficient, and cannot replicate in the cell lines to be used. Infection of humans is highly unlikely to result in replication as the viruses are E1-deficient.

The consequences of escape to the operator are mild. If the worker is exposed to virus or viral infection cells, the likelihood of disease is very low. Any gene expression which may occur will be restricted to the infected cells and its duration of expression would be short. The only potential hazard could be if the transgene is toxic. Further details are in the enclosed risk assessment.

The consequences of escape to the environment are minimal, as the proteins being expressed using these viral vectors are non-toxic or pathogenic and there is no potential for any of the defective isolates recombining the wild type adenovirus present in the human population.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All replication of viruses will be performed under level 2 containment. All infections of cells for experimental analysis will be performed in level 2 facilities. Once infection is completed, media containing viruses will be removed from cells, the cells will be washed and at that stage manipulations will involve washing in different buffers, stimulation of cells with insulin or other agonists, treatments with inhibitors etc. These require incubations in defined buffers on a hot-plate or on ice, depending on the experiment, and cannot be performed in level 2 hoods. We will therefore carry them out with the derogations from level 2 detailed below. Subsequent disruption (eg homogenisation of lysis) will, however, be performed in level 2 hoods to minimise any risk from aerosols. Subsequent fractionation of homogenates or lysates will be carried out with the derogations detailed below.

It must be emphasised that all cell lines proposed for these experiments do not support replication of the infectious virus.

Derogations from level 2 for manipulation of cells and subsequent fractionation of homogenates or lysates: Numbers refer to Part II, Table 1a, pp 80-82.

5. Negative pressure relative to surroundings: Not required by RA.
7. Microbiological Safety Cabinet. Not required by RA for these procedures.
9. Access to authorised personnel. Work to be carried out, and stocks to be kept in room with restricted access to authorised personnel. No public access to labs within the building.
10. Specific measures to control aerosols. Not required for these handling procedures. Required for cell disruption (to be carried out in level 2 hood).
14. Efficient control of disease vectors. Not required as adenovirus is not spread by vectors. Defective virus used for GMM not infectious to rodents.
15. Specific disinfection procedures in place. Specific procedures not required by RA.

Written records of Staff Training. All workers covered by COSSH and must be adequately trained and complete COSSH records, all GM workers registered locally with GMSC. Specific records not required.
Media derived from cells (HEK293 cells) producing virus will be immediately mixed with strong bleaching agents (such as chloros) prior to disposal. Media from cells after infection (test cell lines which do not support viral replication) will likewise be treated with bleach then disposed of in designated sink/drain area.

All contaminated plasticware will be rinsed in chloros prior to autoclaving and disposal.

All contaminated pipettes will be autoclaved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The proposal was considered the members of the GM Safety Committee Applications Subcommittee. This proposal involves the use of commercially available vectors and is almost identical to a previous proposal (GM 37/02.1) that has already been approved by HSE.

The GMSC therefore approved this proposal for notification to HSE under class 2.

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Project Ref 37/02.4

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<td>01/08/2002</td>
<td>USE OF ADENOVIRUSES (SEROTYPE 5 REPLICATION-DEFICIENT/E1-DELETED ADENOVIRUSES) TO DELIVER FOREIGN GENES INTO CELLS</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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Date Project Ceased: 02/03/2022
The purpose of these experiments is to drive high level expression in cellular populations to determine the physiological role of these proteins in cell signalling. This is the main area of research activity in this lab.

We seek to use Adenovirus as it is an established method for high efficiency infection of various cells in culture, in particular, cardiac myocytes, human vascular endothelial cells, and vascular smooth muscle cells which cannot be achieved by other means. Because such high levels of expression can be reached, this offers many advantages for analysing these various cellular systems over more standard transfection approaches.

Recipient or parental organism

Murine 3T3-L1 adipocytes, murine F442A adipocytes, Chinese Hamster Ovary cells, Rat pheochromocytoma cells (PC12 cells), HeLa cells, rat cardiac myocytes (primary isolates).

Rat L6 muscle cells, Human Endothelial Artery cells (commercial - not primary isolated cells) and similar cells. Monkey kidney cells (COS1 and COS7), Rat1 fibroblasts, SK-N-SH neuroblastoma (human), HUVEC human umbilical cord endothelial cells, human pulmonary artery smooth muscle cells (commercial)

All cells apart from the rat cardiac myocytes will be purchased from commercial suppliers.

Host/vector system

Serotype 5 replication-deficient/E1-deleted adenoviruses - see risk assessment.

Origin & function

The adenoviral vectors will be purchased from commercial suppliers of these (Invitrogen). Intended function is to use these viruses to delivery foreign genes into cells.

These genes include the following PDE4s

Human: 4A1, 4A4, 4A10, TM3, 2el, 4B1, 4B3, 4B4, 4C1, 4C2, 4C3, 4D1, 4D2, 4D3, 4D4, 4D5.

Rat: 4A8, 4A5.

Human: EDG1, EDG3, and A2A adenosine receptors.

AND catalytically inactive forms of all the above AND other forms engineered to have disruptions in intracellular targeting and phosphorylation.
Also green fluorescent protein (GFP) PDE4-GFP and receptor-GFP chimeras.

Evaluation of foreseeable effects

The viral vectors are replication deficient and cannot replicate in the cell lines to be used. Infection of humans is highly unlikely to result in replication as the viruses are E1-deficient.

The consequences of escape to the operator are mild. If the worker is exposed to virus or viral infected cells, the likelihood of disease is very low. Any gene expression which may occur will be restricted to the infected cells and its duration of expression would be short. The only potential hazard could be if the transgene is toxic. Further details are in the enclosed risk assessment.

The consequences of escape to the environment are minimal, as the proteins being expressed using these viral vectors are non-toxic or pathogenic and there is no potential for any of the defective isolates recombining with wild type adenovirus present in the human population.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All replication of viruses will be performed under level 2 containment. All infections of cells for experimental analysis will be performed in level 2 facilities. Once infection is completed, media containing viruses will be removed from cells, the cells will be washed and at that stage manipulations will involve washing in different buffers, stimulation of cells with insulin or other agonists, treatments with inhibitors etc. These require incubations in defined buffers on a hot-plate or on ice, depending on the experiment, and cannot be performed in level 2 hoods. We will therefore carry them out with the derogations from level 2 detailed below. Subsequent disruption (eg homogenisation of lysis) will, however, be performed in level 2 hoods to minimise any risk from aerosols. Subsequent fractionation of homogenates or lysates will be carried out with the derogations detailed below.

It must be emphasised that all cell lines proposed for these experiments do not support replication of the infectious virus.

Derogations from level 2 for manipulation of cells and subsequent fractionation of homogenates or lysates: Numbers refer to Part II, Table 1a, pp 80-82.

5. Negative pressure relative to surroundings: Not required by RA.
7. Microbiological Safety Cabinet. Not required by RA for these procedures.
9. Access to authorised personnel. Work to be carried out, and stocks to be kept in room with restricted access to authorised personnel. No public access to labs within the building.
10. Specific measures to control aerosols. Not required for these handling procedures. Required for cell disruption (to be carried out in level 2 hood).
14. Efficient control of disease vectors. Not required as adenovirus is not spread by vectors. Defective virus used for GMM not infectious to rodents.
15. Specific disinfection procedures in place. Specific procedures not required by RA.

Written records of Staff Training. All workers covered by COSHH and must be adequately trained and complete COSHH records, all GM workers registered locally with GMSC. Specific records not required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Media derived from cells (HEK293 cells) producing virus will be immediately mixed with strong bleaching agents (such as chloros) prior to disposal. Media from cells after infection (test cell lines which do not support viral replication) will likewise be treated with bleach then disposed of in designated sink/drain area.

All contaminated plasticware will be rinsed in chloros prior to autoclaving and disposal.

All contaminated pipettes will be autoclaved.

02/03/2022
The proposal was considered by the members of the GM Safety Committee Application Subcommittee. This proposal involves the use of commercially available vectors and is in fact very similar to a previous proposal that was approved by the GMSC for notification to HSE. The GMSC approved this proposal for notification to HSE under class 2.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2 Yes</td>
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<td>L3</td>
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<td>L3 L4</td>
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<td>L4</td>
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<td>L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4 L2</td>
<td>L3 L4</td>
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<td>L3</td>
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<td>L3 L4</td>
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<td>L4</td>
<td></td>
<td>L3 L4</td>
</tr>
</tbody>
</table>

**Project Ref** 37/02.5

**Date Ackn'd** 07/08/2002

**CU2 Project Title** GENETIC MODIFICATION OF HUMAN MALARIA PARASITES FOR FUNCTIONAL GENETIC ANALYSES

**Class** Class 3

**CultureVolClass2** 200ml

**Consent Granted** yes

**Non-GMM**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N
**Project Additional Information**

**Purposes of the contained use**


**Recipient or parental organism**

Plasmodium falciparum - ACDP Class 111 (derogated to Class 11)
Plasmodium vivax - ACDP Class 11

**Host/vector system**

Plasmid pHRPCAT is modified pBluescript (Strategene) with DHFR-TS gene as selectable marker flanked 5’ and 3’ by regions of the gene of interest. This allows recombination of the selectable marker gene into the gene of interest, thereby disrupting it and ablating the function of the protein encoded.

E. coli XL1-Blue MRF’ is host for pBluescript

Human malaria parasites grow in human erythrocytes in vitro and infect mosquitoes of genus Anopheles

**Origin & function**

Gene disruption (knockout) of parasite genes thought to be important in mosquito infection processes. Genes are those known to be expressed in mosquito life-cycle stages, or in transmission stages (gametocytes), or which have been identified by linkage analysis studies. Allelic replacement of these genes from different cloned lines of P. falciparum or from P. vivax.

Investigation of novel potential anti-material drug targets, including protein kinases, cyclins, DNA polymerases, by gene disruption and replacement of wild-type with alternative alleles (naturally occurring).

Localisation of parasites or proteins using green fluorescent protein-tags or purification of epitope-tagged parasite proteins.

Inserted material

DHFR-TS selectable marker is from P. falciparum or Toxoplasma gondii (for selection with pyrimethamine)

DHFR selectable marker is from Homo sapiens (for selection with WR99210).

Green Fluorescent Protein gene is from Aequorea victoria (jellyfish).

Epitope tags are synthetic.

**Evaluation of foreseeable effects**

Genetically transformed parasites will be resistant to the antifolate drug pyrimethamine or to the drug WR99210.

GMM is unlikely to be more harmful to humans or the environment than the parental organism.

It is more likely that the GMM will be of reduced fitness (lowered transmission success to mosquitoes, increased susceptibility to inhibitory compounds, slower growth rates, reduced or non-viability) than of increased fitness relative to the parental parasite.

Transmission of antifolate resistance genes to other species is unlikely due to the strict containment measures applied for this pathogen.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The GM parasites will be used to infect mosquitoes. These are not GM mosquitoes. Infected mosquitoes are maintained in a secure insectary at a containment level appropriate to the pathogenic nature of the parasites. Access to the insectary is through a sealed anteroom and all doors have rubber seals and brushes to prevent
mosquitoes escaping from the insectary area. Within the inner insectary, mosquitoes are kept inside secure escape-proof containers. A copy of the mosquito protocols (for COSHH purposes) is attached which gives details of safety measures in place to ensure no mosquito leaves the secure insectary alive.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Plasmodium falciparum has been specifically exempted from full ACDP Class 11 containment. This formal derogation has been applied because the risk of airborne transmission is deemed to be low.

For in vitro culture of GM P. falciparum, the following derogation from full Class 3 containment are requested.
1. The laboratory is not sealable for fumigation
2. Entry to lab via airlock is not required
3. There is no negative pressure relative to the immediate surroundings
4. Extract and input air are not HEPA filtered
5. Autoclave is not required in the laboratory suite
6. Inactivation of GMMs in effluent from handwashing sinks and showers and similar effluents is not required

Justification: Infection of human or vector is not via aerosol or skin contact but requires direct blood stream (human) or stomach (ingestion by mosquito) contact. Parasites cannot survive desiccation and there is no air-borne infection route. Parasites are inactivated by bleach, detergent or disinfectant.

For work with GM P. falciparum parasite-infected mosquitoes the following derogation from full Class 3 containment are requested (the insect facility is classified as an animal facility)
1. The laboratory is not sealable for fumigation
2. Entry to lab via airlock is not required.
3. There is no negative pressure relative to the immediate surroundings
4. Extract and input air are not HEPA filtered
5. Autoclave is not required in the laboratory suite
6. Inactivation of GMMs in effluent from handwashing sinks and showers and similar effluents is not required
7. Incinerator for disposal of animal carcasses is not required (mosquitoes)

Justification: A 14-day developmental period is required within the mosquito post-infection before parasites are capable of infecting humans. At this point direct inoculation of the parasites into the human blood is required, through the bite of the insect. There is no air-borne route of infection. There is no aerosol formation. Parasites are inactivated by bleach, detergent, disinfectant, or desiccation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management suitable for ACDP Class 111 (derogated) will be applied.
Solid waste, e.g. contaminated paper towels, plastic tissue culture flasks and pipettes, microscope slides, infected mosquitoes:
Autoclaved prior to disposal.

Liquid waste e.g. spent culture medium, waste culture: Inactivated by bleach or an appropriate detergent or disinfectant.
Parasites are lysed (killed) by water or mild detergent. Infected mosquitoes are killed by deep freezing at -20 degree C for 24 hours, or by exposure to chloroform vapour and ethanol immersion. The former process kills both parasite and mosquito. The latter kills only the mosquito, allowing viable parasites to be examined. The parasites are subsequently killed by detergent lysis.

All mosquito waste contaminated with GM parasite material is autoclaved prior to disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
This proposal has been carefully evaluated. We sought advice from HSE as to the class under which the proposal would be submitted and were advised that although many of the activities could be derogated to Cat 2 containment, the nature of the GMO indicated that the proposal should be submitted as Class 3. The GMSC is satisfied that the containment proposed can be achieved with the facilities available.

Project Containment

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<td>L2 L3 L4 L2 L3 L4 L2</td>
<td>L4 L2 L3 L4 L2</td>
</tr>
</tbody>
</table>

Project Ref 37/02.6

Date Ackn'd 24/09/2002

CU2 Project Title USE OF ADENOVIRUSES (SEROTYPE 5 REPPLICATION-DEFICIENT/E1-DELETED ADENOVIRUSES) TO DELIVER FOREIGN GENES INTO CELLS

Class 2

Consent Granted not applicable

Date Project Ceased

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
## Purposes of the contained use

The purpose of these experiments is to drive high level expression in cellular population to determine the physiological role of these proteins in either cell signalling cascades or either cell cycle and the subsequent effect on protein synthesis and transcription by RNA polymerase (pol) and pol III. These are the main areas of research activity in the lab.

We seek to use adenovirus as it is an established method for high efficiency infection of fibroblast cells and cardiomyocytes in culture (which cannot be achieved by other means). Because such high levels of expression can be reached, it offers advantages for cellular systems over more standard transfection approaches.

## Recipient or parental organism

Murine Balb/c 3T3 fibroblasts, Chinese Hamster Ovary cells, Human embryonic kidney 293 cells, HeLa cells and similar cells.

All of these cells will be purchased from commercial suppliers.

In addition, primary cultures of neonatal rat cardiac myocytes will also be utilised for our studies.

## Host/vector system

Serotype 5 replication-deficient/E1-deleted adenoviruses - see risk assessment.

## Origin & function

The adenoviral vectors will be purchased from commercial suppliers of these (Invitrogen)

Intended function is to use these viruses to deliver foreign genes into cells. These genes include components of signalling cascades which impinge on RNA polymerase III transcription such as CKII, mitogen-activated protein kinase (ERK) and dominant negative mutants of this as well as protein kinase B and dominant negative mutants of protein kinase B. In addition, RNA pol III is regulated during the cell cycle and genes that encode components of the cell cycle machinery will also be delivered into host cells including retinoblastoma protein RB, as well as cell cycle inhibitory proteins (p27kip1 and p16). In addition, we will express some components of the basal RNA pol I and pol III transcriptional machineries such as the transcription factors UBF, TFIIIB and TFIIIC. In addition, p53 has been found to regulate pol I and pol III and wild-type and inactive constructs of p53 can also be delivered to host cells.

## Evaluation of foreseeable effects

The viral vectors are replication deficient, and cannot replicate in the cell lines to be used. Infection of humans is highly unlikely to result in replication as the viruses are E1-deficient.

The consequences of escape to the operator are mild. If the worker is exposed to virus or viral infected cells, the likelihood of disease is very low. Any gene expression which may occur will be restricted to the infected cells and its duration of expression would be short. The only potential hazard could be if the transgene is toxic. Further details are in the enclosed risk assessment.

The consequences of escape to the environment are minimal, as the proteins being expressed using these viral vectors are neither non-toxic nor pathogenic and there is no potential for any of the defective isolates recombining with wild type adenovirus present in the human population.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.
will, however, be performed in level 2 hoods to minimise any risk from aerosols. Subsequent fractionation of homogenates or lysates will be carried out with the derogations detailed below. However, it must be emphasised that apart from the HEK293 cells that will be used for amplification of adenovirus preparations, none of the other cell lines proposed for these experiments support replication of the infectious virus.

Derogations from level 2 for manipulation of cells and subsequent fractionation of homogenates or lysates: numbers refer to Part II, Table 1a, pp80-82.

5. Negative pressure relative to surroundings. Not required by RA.
7. Microbiological safety cabinet. Not required by RA for these procedures.
10. Specific measures to control aerosols. Not required for these handling procedures. Required for cell disruption (to be carried out in level 2 hood).
14. Efficient control of disease vectors. Not required as adenovirus is not spread by vectors. Defective virus used for GMM not infectious to rodents.
15. Specific disinfection procedures in place. Specific procedures not required by Ra.
21. Written records of staff training. All workers covered by COSHH and must be adequately trained and complete COSSH records. All GM workers registered locally with GMSC. Specific records not required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Media derived from cells (HEK293 cells) producing virus will be immediately mixed with strong bleaching agents (such as chloros) prior to disposal. Media from cells after infection (test cell lines which do not support viral replication) will likewise be treated with bleach then disposed of in designated sink/drain area.

All contaminated plasticware will be rinsed in chloros prior to autoclaving and disposal.
All contaminated pipettes will be autoclaved.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

This project is very similar to three previous projects, 37/02.1, 37/02.3 and 37/02.5 all of which involve the use of the same defective adenovirus vector. The GMSC did not consider that the proposed sequences to be clones offered any greater risk that the previous three projects, all of which were approved for submission to HSE.

**Project Containment**

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<td>L3</td>
<td>L4</td>
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</table>

**Animal Units**

**Large Scale Activities**

**Human Clinical Applications**
Genetic and phenotypic studies of enteropathogenic (EPEC) enterohaemorrhagic Escherichia coli EHEC), Salmonella enterica, Citrobacter rodentium and other enteropathogenic bacteria. Genetic and phenotypic studies of Clostridium.

The work involves dissecting the molecular basis of virulence of the listed enteric pathogens by targeted mutation of bacterial genes and analysis of wild-type, mutant and trans-complemented strains in vitro and in vivo. The cellular localisation and expression of proteins is also determined using green fluorescent-tagged and epitope-tagged constructs.

The GMOs are likely to be less virulent or of comparable virulence compared with the parent strain. Deletion of genes involved with virulence or the regulation of virulence generally leads to a reduction in pathogenicity. The complementation of genes on plasmids or by chromosomal exchange using alternative promoters may lead to higher copy number of compared with the parental strain but, in isolation, this step is unlikely to increase pathogenicity. Furthermore, the stringent disposal and handling of all material should effectively limit any chance of infection.

For EHEC, pACYC184 and 177: medium copy vectors used for promoter::reporter (GFP/lacZ) fusions to determine gene expression. Also used to generate complements of specific deletions. pUC1S: used to carry/express gene::GFP translational fusion constructs. p18307: low copy number, temperature sensitive vector used to create specific gene deletions using allelic exchange. pKD201: expresses lambda red recombinase enzyme, used to create specific gene disruptions For C.difficile, ClosTron or alternative transconjugative plasmid will be used.
Inserted material: the majority of material cloned and expressed on the plasmids is derived from the host (pathogen) strain itself. Typically, these are promoter regions of genes or regulators that have been deleted in order to study their function. The fact that the genes are from sequenced strains allows excellent bioinformatic based analyses before any cloning takes place which informs the researcher of the likely function of the target gene. Promoter fusions drive known and well characterised reporter genes.

Some exogeneous material is used: green fluorescent protein is from Aequorea victoria Ocell's fish) and epitope tags are synthetic in origin.

<table>
<thead>
<tr>
<th>Evaluation of foreseeable effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains transformed with any of the reporter plasmids are extremely unlikely to be more harmful to humans or the environment than the parental organism. The plasmids themselves are unlikely to be disseminated as all material is disposed of by autoclaving.</td>
</tr>
<tr>
<td>Strains that carry cloned genes for complementation work should be equivalent to parental strains or less harmful.</td>
</tr>
<tr>
<td>Strains with deletions in specific genes are most likely to be less harmful than the parental strain. A small chance that a regulator is deleted leading to an upregulation of virulence is possible but it will only affect existing virulence mechanisms and is unlikely to change the disease process or severity of the disease.</td>
</tr>
</tbody>
</table>

<table>
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<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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<tbody>
<tr>
<td>The procedures in place are suitable for ACDP class II material</td>
</tr>
<tr>
<td>(1) Exposure of liquids containing the biological agent to an appropriate disinfectant at a known effective concentration.</td>
</tr>
<tr>
<td>(2) All liquids containing the agent will be autoclaved.</td>
</tr>
<tr>
<td>(3) Disposable items of equipment/material will be autoclaved</td>
</tr>
<tr>
<td>(4) Collection of inoculated petri-dishes, closed with clear tape to prevent the lid from falling off, and culture flasks for autoclaving</td>
</tr>
<tr>
<td>(5) To reduce the potential contamination of the environment with C. difficile spores, where possible work will be performed in the restricted environment of an anaerobic chamber (which will be regularly treated with agents with known activity to spores). Other environmental contamination will be limited by treatment of benches and equipment with such reagents. (Bench swabs will be used regularly to monitor for failures in such controls)</td>
</tr>
<tr>
<td>Infection of animals</td>
</tr>
<tr>
<td>(1) Appropriate risk assessments have been written and are in place with respect to infection and decontamination of infected animals and cages in the C.R.F, Biological Services.</td>
</tr>
<tr>
<td>(2) Appropriate measures are also in place to control against escape of infected animals.</td>
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</table>

<table>
<thead>
<tr>
<th>Is an emergency plan required according to regulation 20?</th>
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<tr>
<td>N</td>
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<tr>
<td>N</td>
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<table>
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<tr>
<th>Tick to confirm that you have attached a risk assessment to this form</th>
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<tr>
<td>N</td>
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</tbody>
</table>
This application was considered at the committee meeting of 7.11.07 and the addition of project work by considered at the committee of 24.1.08. The application was passed by the committee subject to minor amendments.

**Project Containment**

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<td>L3 L4</td>
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<tr>
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**Project Ref** 37/08.2

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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</thead>
<tbody>
<tr>
<td>07/05/2008</td>
<td>Genetic manipulation of the protozoan Trichomonas vaginalis.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>Class 2</td>
<td>1-50 Litres</td>
<td></td>
</tr>
</tbody>
</table>

**Non-GMM**

Consent Granted: Not Applicable

Withdrawn: N

Tick if notifying a connected programme of work: N

**Project Additional Information**

Purposes of the contained use

The work involves the investigation of metabolism of T. vaginalis in order to identify novel biochemical pathways that might be suitable for therapeutic intervention. The essentiality of potential drug targets will be analysed by gene knockout (either by gene deletion or RNAi). The cellular localisation and expression of proteins will also be determined using epitope-tagged constructs.
Recipient or parental organism
The parental organism is Trichomonas vaginalis, which is listed in ACDP hazard group 2. The parasite infects the human urogenital tract and is sexually transmitted — there are no other known routes of transmission.

Host/vector system
Reticulation of T. vaginalis will be derived from the non-mobilisable E. coli vectors. They will contain well characterized T. vaginalis promoters such as the ap65-1 or SCS promoters to direct expression of homologous and heterologous genes. Vectors for gene deletion will contain a selectable marker gene flanked by sequences homologous to sequences of the target gene. RNAi methods will use a tetracycline inducible system.

Origin & function
Inserted material: the majority of material cloned and expressed on the plasmids is derived from T. vaginalis. Typically, these are genes that will have been deleted in order to study their function. The fact that the genes are from the genome strain of the parasite (for which a draft genome is available) allows excellent bioinformatic based analyses before any cloning takes place, which informs the researcher of the likely function of the target gene. Promoter fusions drive known and well characterised reporter genes (such as Green Fluorescent Protein and its derivatives) and epitope tags of synthetic origin.

Evaluation of foreseeable effects
Trichomonas vaginalis is listed in ACDP hazard group 2. The parasite infects the human urogenital tract and is sexually transmitted — there are no other known routes of transmission. Infections are asymptomatic to mild (vaginitis or urethritis) and are successfully treated with the nitroimidazole drugs, metronidazole and tinidazole. The parasite has no cyst stage and the organism has no capacity for survival in the environment. Humans are its only natural host. The genetic manipulations in this study are not envisioned to lead to over-expression of proteins or other metabolites that are harmful to humans. None of the genes in this study are thought to encode proteins that are sufficient to alter the pathogenic properties of T. vaginalis with respect to host range or tissue tropism. The genetic manipulations planned in this study are intended to lead to either no change in virulence or a loss of virulence. The antibiotic resistance genes used to select genetically modified parasites (such as puromycin acetyl transferase (PAC) in combination with puromycin) do not confer resistance to drugs used in the treatment of T. vaginalis infections (metronidazole or tinidazole). Genetically modified T. vaginalis strains will be propagated in-vitro as trophozoites in complex defined medium. They are not able to survive in the environment. Sexual exchange of genetic information has not been reported for this organism. Genetic exchange between T. vaginalis and other microorganisms has not been reported. It is envisioned that the genetically modified T. vaginalis strains will be either less hazardous or about the same as the parental organism (ACDP Hazard group 2). The containment level required is ACDP laboratory containment level 2. No additional measures above ACDP laboratory containment level 2 are required.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The procedures in place are suitable for ACDP class II material
(1) Exposure of liquids containing the biological agent to an appropriate disinfectant at a known effective concentration.
(2) Disposable items of equipment/material will be autoclaved
(3) Disposable culture flasks will be autoclaved
The committee assessed the proposal as falling into class 2. The Committee examined the Risk Assessment and were content for the proposal to be sent to HSE.

Please enter comments on the GM safety committee on the risk assessment

The committee assessed the proposal as falling into class 2. The Committee examined the Risk Assessment and were content for the proposal to be sent to HSE.

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</table>

Project Ref 37/10.1

Date Ackn'd 22/02/2010

Date Project Ceased

CU2 Project Title

Molecular biology of Toxoplasma gondii. Systematic analysis of essential parasite genes linked to invasion of the host cell by Toxoplasma gondii

Class 2

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

22/02/2010
**Project Additional Information**

**Purposes of the contained use**

Toxoplasma gondii is an obligate intracellular parasite that can invade basically every warm blooded, nucleated cell. The aim of this project is to study the formation, maintenance and regulation of the complex machinery the parasite has evolved in order to actively invade its host cell. To do so, forward and reverse genetic approaches will be combined to identify and characterise factors believed to play a critical role in host cell invasion. We have previously demonstrated that parasites mutated in some of these factors are unable to invade the host and can be used as experimental live vaccines. Furthermore this study will allow the validation of potential drug targets.

**Recipient or parental organism**

Toxoplasma gondii is capable of infecting a wide range of warm blooded animals, including man, cattle and rodents. Transmission is through ingestion. Symptoms are normally mild to asymptomatic although congenital transmission can be serious. Infection with the RH strain (virulent) may be serious if left untreated.

Toxoplasma gondii is classed as ACDP hazard group 2. Both non-genetically modified parasites and genetically modified parasites are handled in accordance with ACDP guidelines. It is considered unlikely that genetically modified parasites T. gondii will cause harm to humans or the environment above that inherent in the pathogenicity of the wild type parasites themselves.

**Host/vector system**

The host for genetic manipulation will be T. gondii. Genetic manipulation of the parasite will exclusively be performed in cell culture, propagating the parasite in a variety of mammalian cell lines. Drug resistance genes that will be used to generate stable transfected parasites include:

- DHFR (confers resistance to pyrimethamine) (see: Donal and Roos (1993), PNAS 15;90(24):11703-7)
- HXGPRT (positive selection under mycophenolic acid and negative selection under 6-thioxanthine) (Donald and Roos (1993), Molec.Biochem.Parasitol. 91, 295-305) This system has the benefit of not introducing any resistance genes into the T. gondii.
- Green fluorescent protein and resistance genes of prokaryotic origin (Ble, CAT)

**Origin & function**

Selection methods for gene knockout studies and stable transfection and gene expression in T. gondii require plasmid vectors that can be amplified in bacteria (Escherichia coli) and are based on pBluescript. In addition vectors contain selectable markers as described above for stable transfection of the parasite. The intended applications are to either remove or overexpress a gene of interest (including mutants of the respective gene) in T. gondii.

DNA encoding genes of the parasites (and mutated versions) and in some instances heterologous genes from other organisms (e.g. Plasmodium may be expressed in the parasites in order to characterise the function of the respective gene during the asexual life cycle of the parasite.

It is the aim to identify genes required for host cell invasion. To do so, libraries of parasite mutants will be established by two complementary strategies:

1) Transfection of an inducible overexpression library (normalised cDNA fused to the ddFKBP-regulation system (see Herm-Gotz et al., 2007). In case parasites will be transfected in a medium throughput format and subsequently standard screening procedures will be applied to identify loss and gain of function mutants.

2) Random integration mutagenesis. It is possible to generate parasite mutants by transfection with linear DNA fragments, containing a selectable marker (see above). These fragments will integrate into the genome of the parasite at random positions at high frequency. Thus it is possible to generate random parasite mutants that can subsequently be screened for certain phenotypes (for and example see: Meissner et al., 2002)

For the reverse genetic screen, the focus will be on homologous of factors involved in vesicular traffic, like RabGTPases, Dynamins, Motor proteins and Syntaxins. All these candidates will be expressed under control of an inducible system as trans-dominant mutants (see above for an example of the respective plasmids employed). (see: Agop-Nersesian et al., 2009 or Breinich et al., 2009)

**Evaluation of foreseeable effects**

The overall aim of this project is to perform a systematic, functional analysis of important parasite genes linked to host cell invasion. Therefore a combination of
bioinformatics, forward and reverse genetics will be employed to:
- Generate and analyse parasite mutants unable to invade the host cell
- Establish and open access database of characterised genes of interest (GOIs) as a community resource
- Screen for chemical inhibitors (drug development) for the most promising GOIs

This should facilitate drug target identification, validation and future design.

The main interest of this project is the mechanisms involved in host cell invasion by Toxoplasma gondii, with a special focus on the biogenesis and maintenance of specialised organelles linked to invasion. In the past we isolated and characterised several mutants that were deficient in host cell invasion (Meissner et al., 2002; Kessler et al., 2008, Agop-Nersesian et al., 2009, Breinich et al., Current Biology 2009). All of these mutants have reduced virulence and are unable to grow, when the respective protein is depleted or expressed as a trans-dominant mutant. Genes to be analysed in Toxoplasma include:
- mutants obtained from forward genetic screens
- trafficking factors, such as Rab-proteins, dynamins, motor proteins, kinases and syntaxins.

Foreseeable effects of gene disruption or overexpression of a GOI

(i) Considerations relating to whether the product of the inserted gene has a biological activity

Three types of genetic modification are envisaged.

(a) Gene disruption.
Generation of mutant parasites by removing a GOI via homologous recombination (exchange of the GOI for a selectable marker by homologous recombination). It is considered likely that gene disruptions will be detrimental to parasite survival or will have no phenotypic difference from wild type cells. The selectable markers mentioned above will be used (DHFR, HXGPRT; CAT, Ble).

(b) Homologous and heterologous gene expression.
The expression of genes in T. gondii will be achieved after integration of a expression vector into the genome. These experiments are designed to test for cross species complementation (eg. Homologous genes from Plasmodium) and for generation of overexpression phenotypes (to identify genes required for host cell invasion, etc.). Other heterologous genes include reporter genes such as green fluorescent protein (GFP) from the jelly fish Aequorea victoria (including mutants of this protein engineered to give greater fluorescent or a different colour of light emission) and luciferase from the firefly Photinus pyralis (or mutant versions of this gene from this or other organisms).
The heterologous DNAs to be used for transfection of the parasited do not encode a toxin, oncogenic protein, allergen or other protein with a potentially harmful biological activity.
Risk assessment. As access will be restricted to contained handling in a containment level 2 laboratory and expressed genes will be non-toxic, risk levels are kept to a minimum.

(ii) Considerations relating to whether the inserted gene encoded a product that might act alongside the existing characteristics of the recipient micro-organism, so as to endow the GMM with altered pathogenic properties.
It is possible that disruption of genes will alter the pathogenicity of the parasited. It is envisaged that genes modified in this project will lead to either no change in virulence or a loss of virulence. Drug resistance genes of prokaryotic origin, such as Ble or CAT will be used. These drug resistance genes do not confer resistance to drugs commonly used to treat T.gondii, in man or animals. DHFR selection confers resistance to pyrimethamine one of the current drugs used for treatment of T. gondii. Alternatives are available (eg. Sulphadiazine)

(iii) Consideration of the most hazardous GMM to be created
The most hazardous GMM to be generated are pyrimethamine resistant T. gondii (see above). With alternatives to pyrimethamine available (Sulphadiazine) and the unlikeliness of environmental release (see below), the hazard is very low.
Most of the proposed genetic modifications are expected to produce attenuated organisms lacking one or more genes or that express trans-dominant versions of an
essential protein.

(iv) Considerations relating to wether an inserted sequence, that does no give rise to a harmful phenotype in the recipient micro-organism, could give rise to harm as a result of natural gene transfer to another, possibly related, organism. T.gondii can reproduce sexually but this only happens in the domestic cat. Genetic exchange with other organisms has not been reported for T. gondii.

The genetically modified parasited will be contained with ACDP containment level 2 facilities. The chance of escape into the environment is negligible. The details of the appropriate systems of work are held within the appropriate COSHH risk assements and standard operating procedures.

Part 2 Animal work
Genetically modified T. gondii will be grown in mice. The University of G;asgow has an animal facility with ACDP containment level 2 classification. It is not envisaged that genetically modified T. gondii will present any additional environmental hazard risk envisaged in using the genetically modified micro-organisms rather than the parental micro-organisms in genetically modified animals.

GM modified T. gondii may be inoculated into wild type or KO mice (of various backgrounds eg. BALB/c) to analyse their virulence. These animals will be housed in a security monitored home office licensed facility in appropriate surroundings at containment level 2. The risk of these reaching the external environment is negligible. All animal tissue will be incinerated (by the Universiyt appointed contractors) and spillage of body fluids and surfaces that have been in contact with tissue will be treated with Virkon. All animal handling will involve the use of gloves. All workers should have tetanus vaccination up to date. Bites and scratches should be squeezed to allow blood flow, treated with 70% ethanol, dried and covered with an antiseptic plaster. Toxoplasma cannot be transmitted by bites or scratches.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All parasite manipulation will be conducted in accordance with ACDP guidelines. T. gondii will be handled under containment level 2.

1) Are any of the work procedures likely to generate aerosols?
Aerosols are unlikely since most work with the parasite will be performed in a class II hood. Aerosols could be generated if, for example, a tube of T. gondii was dropped during FACS analysis of live cells. However, the volumes of material would be small (1-2ml) and T. gondii infection cannot be spread by aerosols.

2) How will you dispose of waste materials?
Animal waste will be disposed of through an authorised waste disposal company, provided through the Clinical Waste Service. Solid waste is stored in small waste bins within the laboratory. The bins have lids and hold inner liners, which are autoclave bags. When the liners are no more than 3/4 full, they are removed from the bin, loosely sealed with autoclave tape (to allow steam penetration during autoclaving ), labelled with lab number, operator and date and then transported to the autoclave room on a trolley. The bags are logged into the room and autoclaved on the same day that they are delivered. The bags and the logging procedure designates them 'GM', which results in their inclusion on a 'run' on the validated autoclave with recorder. The record is inspected to ensure that the correct temperature has been reached for sufficient time. The record is kept on file by the autoclave room staff. The autoclaved material is disposed of in solid waste by an authorised waste disposal company, provided through the Clinical Waste Service. The routine autoclave regime will be 126°C for 30 min, which has previously been established to produce a 100% kill (British Pharmacopeia 2005, Vol IV, Appendix XVIII A385-A388).

Liquid waste (e.g cultures in flasks or 'spent broth' wherein the cells have been seperated from the culture broth by centrifugation) will be sterilised in the culture flasks the same day by addition of Virkon® to a final concentration of 1% w/v, and left overnight. Virkon-disinfected solutions will be disposed of into the waste water supply. Virkon solutions are stable for up to 7 days (manufacturer's data), and thereafter disinfectant solutions will be discarded (the pink colouration fades). Virkon is a peroxygen compound and disinfection requires a contact period of at least 10 min for 1% w/v solution: see http://www.antechh.com/frameset.html.
Contaminated glassware and centrifugation buckets from cultures will be disinfected by complete submersion in 1% w/v Virkon® overnight (polypropylene and polystyrene centrifugation buckets, steel and glass are not corroded by 1% w/v Virkon). Glassware and buckets will then be rinsed in tap-water and dried.

Small volume spills (up to 100ml) on hard surfaces (floors, work benches, trolleys) will be disinfected by mopping up the spill with absorbent tissue paper and spraying the surface with a 1% w/v Virkon solution or sprinkling the area with powder Virkon and leaving for at least 20 minutes and drying with paper towel to remove any remaining white deposit. Paper towels will then be bagged for disposal as solid waste (above).

Large volume spills (100ml to 0.5l) on hard surfaces (floors, work benches, trolleys): Absorbent granules will be used to cover the spill, if necessary, contained using absorbent booms. Granules and booms will be bagged for disposal as solid waste (above). The contaminated area will then be disinfected by wiping or spraying the surface with a 1% w/v Virkon solution or sprinkling the area with powder Virkon and leaving for at least 20 minutes and drying with paper towel to remove any remaining white deposit. Paper towels will then be bagged for disposal as solid waste.

3) Does your laboratory avoid sharps?
Yes, where possible. However, where it is necessary to use sharps, the laboratory provides Sharps containers and has guidelines for the use and safe disposal after use.

4) Has the disinfectant been validated?
Yes, Virkon kill has been validated in house to kill 100% of the Toxoplasma when used as per manufacturer's instructions (1% weight/volume). When genetically modified organisms are created these will also be tested. This is done by incubation in Virkon at different concentrations for different lengths of time and examination by microscopy. The autoclave has also been validated.

5) Does the nature of the work preclude it being undertaken by any workers who have a serious skin condition?
No. All workers must wear adequate protective clothing, including laboratory coats and disposable gloves, safety glasses,

6) Will workers receive any vaccination?
No vaccinations are currently available for toxoplasmosis

7) Is the recipient micro-organism controlled by DEFRA?
No

ASSIGNMENT OF CONTROL MEASURES
It is believed that the genetically modified T. gondii provide no significant additional risk over and above the wild type parasites. Non-genetically modified T. gondii are handled at Containment level 2. All genetically modified T. gondii, will also be handled in Containment 2 facilities only.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The GM safety Committee deemed this risk assessment satisfactory as per minute of meeting 03/09/09 and back-up documentation.
Project Containment

Laboratory Activities
- L2 Yes
- L3 L4 L2
- L3 L4 L2
- L3 L4 L2
- L3 L4 L2
- L3 L4 L2
- L3 L4 L2
- L3 L4 L2

Glass Houses
- L2
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Growth Rooms
- L2
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Animal Units
- L2
- L3
- L4
- L2
- L3
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- L2
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Large Scale Activities
- L2
- L3
- L4
- L2
- L3
- L4
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- L3

Human Clinical Applications
- L2
- L3
- L4
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- L3

Project Title
Investigation of regeneration, repair and plasticity in rodent models of central and peripheral nervous system injury & Functional restoration of neuronal phenotype using exogenous Mecp2 in mouse models of Rett syndrome

Date Ackn'd
22/02/2010

Date Project Ceased

Class
Class 2

CultureVol
Non-GMM

ClassVolume
Consent Granted

Project notified under transitional arrangements

Withdrawn
N

Tick if notifying a connected programme of work
N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
Sub-project 1 - Dr R is investigating how neurons and nerves can be repaired after injury and will use lenti constructs to express marker protein in cells around the injury site and in cells transplanted into the injury site. Proteins potentially involved in regulating nerve regeneration, differentiation and plasticity would also be expressed. Sub-project 2 - Drs C and B are investigating therapeutic avenues for Rett syndrome, a neurodevelopmental disorder and will use lentiviral constructs to deliver wild-type Mecp2 to mice deficient in this gene or cre to reactivate the gene in targeted mice to look for restoration of normal neuronal functioning in a regulated manner.

Recipient or parental organism
Recipients - Mouse, Rat
Parental organisms - transgenes with biological activity will be rodent or human-specific cDNAs

Host/vector system

4th generation lentiviral vector systems based on pLenti and pLentiLox4.0 (pLL4.0) and pLentiLox4.2 (pLL4.2). Three plasmid packaging system (pLP from Invitrogen or pMD series). VSV-G envelope. Can infect all mammalian cells.

Origin & function

The inserted transgenes will compromise full length cDNAs encoding plasticity-related proteins (including Mecp2-e1), neurotrophins (e.g. Ngf, Bdnf, Cntf, Gdnf), and chemorepulsive factors (e.g. semaphorins, ephrins, Slit, Neureilin). Constructs containing cre recombinase will be used in some experiments to reactivate Lox-STOP-inactivated targeted genes. Some constructs will also employ fusions with eGFP and RFP, FLAG and MYC tags, HS4 insulators (chicken-derived), WPRE (encoding non-expressible portion of N-term fragment of WHV-X protein). A variety of nervous system-specific and ubiquitous promoters will be employed to express the transgenes/fusions/markers, including CMV, UbiC, U6, CamKII, PGK, synapsin 1, Tau. All are intended to influence neurite-extension, maturation and mature functioning/plasticity properties of neurons in the central and peripheral nervous system.

Evaluation of foreseeable effects

Risk to experimenter and other co-workers involved in the delivery of lentiviral particles to the experimental animals - there is a small risk that the experimenter and associated lab workers present in the room during the surgery and lentiviral particle delivery phase will be exposed to viral particles via inadvertent or accidental needlestick injury. The viruses cannot survive for more than a few hours in the environment and are easily inactivated by use of standard antiviral reagents and cleaning regimes. If needlestick injury does occur, it could lead to two consequences. Firstly, lentiviral particles will infect cells local to the injury and may provoke inflammatory responses or immune responses to the infected cells. Such responses are likely to be mild and not prolonged. Exposure volumes would be tiny, and infections are unlikely to become established locally or by spreading away from the needlestick injury site. Secondly, integration of the construct may cause mutagenic effects on the genome in infected cells local to the delivery site. The chances of insertional inactivation of a tumour suppressor gene are very low, but finite. If the construct inserts adjacent to cellular oncogene, there is a very low but finite risk of disruption of the normal regulation of the Tumourigenesis, if it did occur, could be of 'mild' consequence, if localised, benign, or succesfully treated, or 'sever' consequence if malignant and untreated. Recombination between natural lentiviruses and constructs in an exposed worker is of negligible likelihood. Virus particles will become inactive within a few hours and no viable virus should remain by 48 hours. No virus shedding is expected from the GM rodents created. There will be no germline integration of lentiviral construct. We have no reason to believe that the GM animals produced by lentiviral infection and/or transplantation of infected exogenous cells will be any more aggressive or likely to bite handlers, or more anxious and prone to escape, than mice and rats not undergoing the infection procedure.

Risk of altering or affecting other conspecifics and other species - the mice and rats used in vivo work are either wild type inbred (or partially outbred) lines with poor ability to compete in the environment, or genetically modified (knockout) mice with low or reduced fitness relative to WT. None of the constructs or their use increases the risk that GMOs or GMMs will escape into the environment or come into contact with non-GM organisms, and there is no reason to believe that escape of GMOs/GMMs would affect the environment in deleterious ways.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Containment of infected animals will be as per the standard GM rodent containment risk assessment used by Biological Services. Essentially, all GM rodents are identified by methods appropriate for the species and records of disposal and experimental use are kept electronically in the unit. All animals are kept in secured, labeled cages with a record card containing the number of animals kept in each cage. If escape from the cages occurs, this can be readily identified and dealt with accordingly. A count of the animals held within the animal unit is carried out routinely on a weekly basis. During animal handling the holding room door is kept closed. A knee high rodent barrier is also fitted to each animal holding room door to contain any animals that may have escaped during husbandry or procedural activities. This barrier can be removed for cleaning but is kept in place at all other times. The layout of the animals unit is such that there are multiple doors separating the animal/procedure rooms from the outside (emergency exits excpeted). Live rodent traps are placed in rooms when working with GM rodents to assist in the recapture of escaped animals. All these measures are more than the minimum appropriate for the planned activities and the nature of the GMOs.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
In survival experiments, viral particles are not expected to survive in infectious form by the time the recipient animals are sacrificed. Where animals die unexpectedly within 48 hours, or in acute application experiments where recipients animals are sacrificed within 48 hours, carcasses will be autoclaved and bedding and absorbent towels treated with Virkon and autoclaved, as is routine for all GM animals. Cages and incubators used to house treated animals within 48 hours of infection will be sprayed with Virkon and/or fumigated after removal and disposal of bedding. Unused aliquots of packaged viral particles will be inactivated using Virkon and autoclaved. Kill is expected to be 100% complete in all these circumstances, so no special monitoring is planned.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This is a well constructed proposal using the most up-to-date and safest lentiviral vector system. The committee was satisfied that had the project been confined to cell lines, it would clearly have fallen into category 1. The project falls into category 2 solely because of the intention to use live animals and the consequental risk to the experimenter in carrying out injection procedures as defined under SACGM discussion paper 20/11/08

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

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Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 37/15.1

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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tr>
<td>23/12/2015</td>
<td>Creation, complementation and characterisation of gene deletion mutants of</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
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Pectobacterium spp. are phytopathogenic bacteria that cause soft rot, stem rot, bacterial wilt and black leg disease in angiosperm species. The aim of this work is to identify genes that may be involved in iron uptake and susceptibility to protein antibiotics. This work could ultimately lead to better control strategies against this important plant pathogen.

Recipient or parental organism
Pectobacterium spp. are major pathogens of potatoes and other field vegetables causing blackleg, aerial stem rot, and tuber soft rot. P. carotovorum is very common and has an extensive host range, including most fleshy vegetables, whereas P. atrosepticum is associated mostly with potatoes. Tuber soft rot can be caused by either of these bacteria and blackleg is usually caused by P. atrosepticum carried on contaminated seed tubers. Most lots of seed tubers are contaminated to some degree. In contrast, aerial stem rot is usually caused by P. carotovorum contained in infested soil or introduced to the crop by irrigation water. Other sources of bacteria include infected plant debris and contaminated potting media, tools or soil. However, Pectobacterium spp. do not cause disease unless environmental conditions are favourable and amount of bacterial disease that actually develops in a crop is strongly influenced by factors such as soil moisture and temperature.

Host/vector system
The suicide plasmid pMRS101 will be used for the creation of deletion mutants in Pectobacterium spp. This vector is derived from pKNG101 with the addition of the high copy number CoIE1 origin of replication on a NotI fragment to increase plasmid yield and to allow initial manipulations in E. coli DH5α. pKNG101 has a conditional origin of replication requiring the replication factor *pir for propagation and thus is unable to replicate in most Gram-negative bacteria. For complementation we will clone relevant iron uptake related genes into a standard expression vector (pUC, pET or pJexpress) and transform this vector into Pectobacterium strains by electroporation with ampicillin selection.
The origin of the genetic material to be transferred is the host Pectobacterium species. Thus genes or fragments of genes encoding putative proteins involved in iron uptake will be transferred. Typically the encoded protein will be TonB-dependent outer membrane receptors, inner membrane ABC transporters, periplasmic binding proteins or homologues of TonB. The intended function of the transferred material in the deletion mutants is to enable the construction of the deletion mutant by homologous recombination. For plasmid based complementation, the purpose of the genetic material is to allow production of the putative iron uptake protein to complement the loss of function of the deletion mutant.

Evaluation of foreseeable effects

Since the recipient organisms are UK field isolates the capacity of the GMM to cause disease, given favourable environmental conditions is high. However, this would not be expected to be increased over and above the parent strain as virulence would not be expected to be increased. Indeed, since the acquisition of iron is a critical factor in the bacterial infection of plants the capacity of some of the deletion mutants to cause disease may be reduced, although possible redundancy in iron acquisition genes does not make this a certainty. In the case of the complemented deletion mutants, which may show higher expression levels of complemented gene products, it is again unlikely that this will lead to increased virulence since they will be only one component of a multi-component iron uptake system. There is also no reason to suspect that the proposed genetic modification would have an impact on host range since Pectobacterium spp. are necrotrophic pathogens with an already broad host range. Therefore accidental release would not be expected to impact on other environmentally sensitive plant species. In addition, since Pectobacterium spp. are already present in high numbers in the environment, the very low bacterial numbers that might be released would be expected to have a negligible impact on the environment. Since most crop losses are due to contamination of seed tubers or existing soil contamination where host plants have been grown, there is also a very low probability that any accidently released GMM would find a route to infect crop plants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The GMSC has agreed that work need not be undertaken in a microbiological safety cabinet, since the proposed experiments (which do not include infection of live plants) are physically easy to contain. The maximum culture volume will be 50 ml and aerosols will be kept to a minimum. A designated area will be assigned for this GMM work when in progress and this are will be thoroughly disinfected after use. Specified measures to control aerosol dissemination will include avoidance of vigorous mixing or shaking of cultures and the avoidance of vigorous pipetting. The GMM will be stored in a secure area. Good microbiological practice will be followed at all times.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile. After inactivation as described above, waste will undergo conventional disposal. These procedures are appropriate for the disposal of Class 2 containment material.
This application was submitted to the GM safety committee on 21/08/15. The application was discussed at the committee meeting held on 09/09/15 and some minor clarifications sought. The amended version was circulated to the committee who were satisfied that these met the requests for clarification. The application was approved for submission to HSE and signed by the GMBSO on 19/11/15.

Please enter comments on the GM safety committee on the risk assessment:

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Animal Units:
- L2
- L3
- L4

Large Scale Activities:
- L2
- L3
- L4

Human Clinical Applications:
- L2
- L3
- L4

Project Ref: 37/17.1

Date Ackn’ed: 02/08/2017

CU2 Project Title:
- Investigating virulence and transmission in malaria parasites using wild type and genetically modified Class 2/Hazard Group 2 Plasmodium species

Class: Class 2

CultureVolClass2: < 1 Litre

Consent Granted

Non-GMM: Consent Granted

Project notified under transitional arrangements: N
### Purposes of the contained use

Overall the goal is to understand the biology of Plasmodium blood stages using molecular, biochemical, cellular and omics tools. In vitro and in vivo (rodents) experiments with Plasmodium blood stages will be performed to study parasite host interactions, gene and protein expression, and subcellular localization of proteins. Only Class 2/Hazard Group 2 Plasmodium species will be used in this work, for example P. berghei, P. knowlesi, P. cynomolgi. Wild type and genetically modified strains will be used.

### Recipient or parental organism

Class 2 and Hazard Group 2 Plasmodium species will be used in this work, for example P. berghei, P. knowlesi, P. cynomolgi. Wild type and genetically modified strains will be used.

### Host/vector system

Shuttle vectors include those for genetic knock-outs (Maier et al, Cell 2008), epitope tagging by 3’ replacement (Dvorin et al, Science 2009) and overexpression (Buchholz et al, JID 2011), and for conditional knockdown (Coleman et al, CHM 2014). The vectors may also contain promoter DNA to drive expression of the genes of interest, DNA to confer resistance to specific drugs for selection purposes, and DNA to express specific markers for protein identification. Vectors developed for other Plasmodium species, eg P. falciparum, P. berghei, and P. vivax, may be used, as evidence to date suggests that Plasmodium is a universal acceptor for DNA from other Plasmodium species.

### Origin & function

Plasmodium is generally the source of the DNA for integration, eg P. berghei, P. falciparum, P. vivax. Additionally genetic elements that find general use in genetic transformation systems (e.g. Crispr/CASS, Cre-recombinase, AID degron, fluorescent/bioluminescent protein encoding genes) will be employed (see below). There are a number of specific elements to this work:

1. The cloning of promoter regions and gene fragments to create transcriptional and translational reporter fusions. This will be achieved using standard molecular techniques to analyse the expression and regulation of genes. The promoter regions of genes will be PCR amplified from the target genome and cloned into reporter vectors (pDEFhDHFR or pDEFSSUhDHFR(RV) derivatives, published Franke Fayard et al (2004) Mol.Biochem. Parasitol 137. 23-33) to produce promoter-reporter gene fusions for expression studies. These plasmids would be then be transformed into host strains to allow expression of the gene to be monitored by measuring reporter activity. The protein product of individual genes may be analysed through the creation of fusion genes where the open reading frame (ORF) of the gene of study (GoS) may be fused to a reporter gene, such as the gene encoding green fluorescent protein, a non-toxic and well studied reporter gene. Additional reporters include red fluorescent protein, luciferase (both firefly and Renilla), CFP, YFP, mCherry (from the mFruit series), and beta-galactosidase. All these products have been used extensively in numerous studies and are non-toxic. These foreign (eg human, toxoplasma gondii) DNA sequences have been used extensively in malaria parasites without negative consequences.

2. Specific gene deletions. These will be achieved using standard molecular techniques to analyse the role of specific genes in pathogenesis.

3. Complementation of specific gene deletions. This will be achieved using the appropriate standard techniques.

Plasmid vectors have been obtained from commercial sources - pET (Stratagene), pcAL-N-FLAG (Stratagene), peGFP (Clontech), or from a former laboratory in Leiden (http://www.lumc.nl/1040/research/malaria/malaria.html) where a database of vectors with sequence and maps is available.
available and can be supplied if required. The database is password protected (User: Waters. Password: mal-2008) All vectors will be engineered and amplified inside of E.coli plasmids. Plasmogem vectors from a publicly available resource (http://plasmogem.sanger.ac.uk/) will also be used.

**Evaluation of foreseeable effects**

*P. berghei* is a rodent malaria pathogen and does not infect humans. During the proposed experiments with *P. berghei*, exposure of humans to the infectious stage of the parasite would require either a bite by an infected escapee mosquito or an injection of the infected rodent blood. Both events can be easily prevented by appropriate laboratory practice. Moreover, even in the event of such exposure *P. berghei* would be extremely unlikely to invade and replicate in human cells. Successful invasion of human erythrocytes by *Plasmodium* was studied extensively and was shown to require multiple species-specific ligand-receptor interactions, which are present in human parasites but not in *P. berghei*. Additionally, despite presumably multiple accidental exposures of humans to *P. berghei* in its natural environment, no human *P. berghei* infection has ever been reported in the literature and a phylogenetic tree of *Plasmodium* parasites shows no evidence of frequent crossing of the host-species barrier.

Simian *Plasmodium* Hazard Group 2 species e.g. *P. knowlesi*, *P. cynomolgi*, predominantly infect simians but can infect humans and cause malaria. Human *Plasmodium* Hazard Group 2 species e.g. *P. vivax* also cause malaria. Exposure to humans of the infectious stage of the parasite during our proposed experiments would require either a bite by an infected escapee mosquito or an injection of the infected human blood. Appropriate laboratory practice should reduce the likelihood of these events from happening, such as by avoiding the use of any sharps whenever possible. However, in the event of such an occurrence the individual will be treated with anti-malarials (Singh et al, Lancet 2004; Ta et al, Malar J. 2014). There are no known instances of human-mosquito-human transmission of simian *Plasmodium* species. Only monkey-mosquito-human. All species to be employed in the laboratory are sensitive to common anti-malarials e.g. chloroquine. The sources of inserted gene products are as described above. The resultant genetically modified organisms, including those overexpressing target genes, are not expected to be any more virulent than the unmodified host organism. DNA when introduced into *Plasmodium* overwhelmingly interacts with the target genome and integrates through mechanisms of homologous recombination. Random integration into the genome is not observed and therefore unexpected mutant parasites are generally not generated unless through specific experimental design and the use of integrases of low target site specificity (e.g. piggyBac).

The risk that insertion of foreign DNA, especially that from *P. falciparum* or *P. vivax* DNA into rodent or simian *Plasmodium* species will confer pathogenicity to humans is very low as the genes that we are interested in are not known to play any part in the infectivity of the organism. Invasion of human erythrocytes by *Plasmodium* has been studied extensively and it is known that species-specific ligand-receptor interactions are required. We are not working on any of these proteins and therefore should not confer an increase in infectivity to humans. The gene products we are investigating are not known toxins or allergens.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Parasites are completely inactivated by desiccation, or by the addition of detergent, bleach, or even water to culture material. All *P. berghei* liquid waste is inactivated by the addition of detergent or 5% Chemgene for a minimum of 16 hours (overnight) and then flushed down the sink with copious amounts of water. All *P. berghei* solid waste is either autoclaved or incinerated. Since the cultured material for any of the simian *Plasmodium* species, eg *P. knowlesi* and *P. cynomolgi*, also contains human blood and serum, an autoclaving step is necessary to prevent any infection by viruses etc, which are not inactivated by detergent, bleach or desiccation. It would also have the effect, in GM parasites, of denaturing the genetic material. All solid waste used with simian *Plasmodium* species is soaked in detergent or bleach for the
same minimal period before autoclaving. GMO infected mice carcasses are autoclaved and then frozen until removal for incineration by an appropriate waste disposal company following procedures described in the Biological Services risk assessment 334. The above procedures are common practice in Plasmodium laboratories as a reliable method for 100% kill.

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L2 Yes L3 L4</td>
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<tr>
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<td>L3 L4 L2</td>
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Animal Units

| L2 Yes | L3 L4 | L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 | L2 L3 L4 | L2 L3 L4 |

Human Clinical Applications

Project Containment

Project Ref 37/20.1

Date Ackn’d 29/04/2020

CU2 Project Title Genotypic and Phenotypic studies of Mycobacterium species

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N
### Project Additional Information

**Purposes of the contained use**

We intend to use type strains of *Mycobacterium smegmatis* (Class 1), *Mycobacterium haemophilum* (Class 2), *Mycobacterium abscessus* (Class 2), *Mycobacterium avium* (Class 2), *Mycobacterium bovis BCG* (Class 2), and *Mycobacterium Aurum* (Class 2) to carry out genotypic and phenotypic studies of identified proteins involved in growth and virulence by targeted mutation of bacterial genes and analysis of wild-type, mutant, and complemented strains in vitro.

**Recipient or parental organism**

The GMOs are likely to be less virulent or of comparable virulence with the parent strain; deletion of genes involved with virulence or the regulation of virulence generally leads to a reduction in pathogenicity. The complementation of genes on plasmids using alternative promoters may lead to higher copy number of proteins compared with the parental strain but, in isolation, this is unlikely to increase pathogenicity. Furthermore, stringent disposal and handling of all materials should effectively limit any chance of infection.

**Host/vector system**

- *Mycobacterium smegmatis* (Class 1) is an acid-fast bacillus commonly found in the environmental habitats, mainly soil. It is a fast-growing non-pathogenic bacterium commonly used in the laboratory for genetic studies of mycobacteria.
- *Mycobacterium haemophilum* (Class 2) is an acid-fast bacillus commonly found in the environmental habitats, mainly water. It is a slow-growing opportunistic pathogenic C bacterium that could colonize immunocompromised hosts (humans and animals), but rarely in immunologically competent individuals.
- *Mycobacterium abscessus* (Class 2) is an acid-fast bacillus commonly found in the environmental habitats, mainly soil and water. It is a slow-growing opportunistic pathogenic C bacterium that could colonize immunocompromised hosts (mammals and animals), but rarely in immunologically competent individuals.
- *Mycobacterium avium* (Class 2) is an acid-fast bacillus found in the environmental habitats, such as soil and water. It is a slow-growing opportunistic pathogenic bacterium that could colonize immunocompromised hosts and cause Mycobacterium intracellulare, Mycobacterium Avium Complex (MAC) infection, but rarely in immunologically competent individuals.
- *Mycobacterium bovis BCG* (Class 2) is the attenuated form of the *Mycobacterium bovis* strain Calmette-Guerin (BCG), an acid-fast bacillus, that is currently used as a vaccine for *Mycobacterium tuberculosis* (causative agent of tuberculosis).
Mycobacterium aurum (Class 2) is an acid-fast bacillus commonly found in the environmental habitats, mainly soil and water. It is a slow-growing opportunistic pathogenic bacterium that could colonize immunocompromised hosts (humans) resulting in bacteremia, but rarely in immunologically competent individuals.

pML1335-GFP or pML 1357 was a gift from Michael Niederweis (Addgene plasmid # 32378; http://n2t.net/addgene:32378; RRID:Addgene_32378). The intended function is to introduce GFP gene into the to re-introduce gene back to a mutant organism.

pKM444 was a gift from Kenan Murphy (Addgene plasmid # 108319 ; http://n2t.net/addgene:108319; RRID:Addgene_1 08319). The intended function is for creating knockouts of target genes by oligonucleotide-mediated recombineering of bacterial chromosomes. This shuttle vector expresses the Che9c phage RecT annealase and the Bxb1 phage integrase (Int) from an anhydrotetracycline (ATc)-inducible Ptet promoter.

pKM464 was a gift from Kenan Murphy (Addgene plasmid # 108322; http://n2t.net/addgene:108322; RRID: Addgene_108322). The intended function is for creating knockouts of target genes by oligonucleotide-mediated recombineering of bacterial chromosomes. This is a payload plasmid that does not replicate in mycobacteria and includes a hyg resistance marker for selection in mycobacteria and a Bxb1 attB site.

pKM468-EGFP was a gift from Kenan Murphy (Addgene plasmid # 108434; http://n2t.net/addgene:108434; RRID: Addgene_108434). The intended function is for creating EGFP-His fusions to target genes by oligonucleotide-mediated recombineering of bacterial chromosomes. C-terminal tag: EGFP-4xGly-TEV-Flag-6xHis.

pKM491-Flag-His was a gift from Kenan Murphy (Addgene plasmid # 109282 ; http://n2t.net/addgene:109282; RRID: Addgene_1 09282). The intended function is for creating Flag-His fusions to target genes by oligonucleotide-mediated recombineering of bacterial chromosomes. C-terminal tag: 4xGly-TEV-Flag-6xHis.

pKM493. The plasmid is not yet available, but will be used in the future. The intended function is for creating FlagEGFP fusions to target genes by oligonucleotide-mediated recombinering of bacterial chromosomes. C-terminal tag: TEV-Flag-4xGly-EGFP.

Origin & function

Materials are obtained from Group Leader of the Mycobacterial Metabolism and Antibiotic Research Laboratory and Group Leader of the Host-Pathogen Interactions in Tuberculosis Laboratory, at The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK. Inserted gene products are all from mycobacterial origin. In most cases, the inserted gene will be the same previously deleted, to demonstrate rescue of the phenotype.

Evaluation of foreseeable effects

Deletion, mutation or introduction of genes, is unlikely to alter its ability to infect humans and cause disease as usually these are dependent on several genes acting in concert. Alteration would likely reduce the pathogenic traits. Most mutants generated will contain gene deletions or mutations which are associated with a fitness cost. Therefore, these strains will be further attenuated and less fit to survive in the environment. Strains that carry cloned genes for complementation work should be equivalent to parental strains or less harmful.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Disinfection and autoclaving will be carried out where required. All waste materials will be disposed of into the autoclave bags, yellow clinical waste bags or sharps bins as required. All used sharps will be placed immediately after use into a sharps bin. Sharps bins will be located on the bench where the sharps are used so that they can be disposed of directly after use.

All disposable consumables that come into contact with mycobacteria will be chemically inactivated by soaking in 10% Surfanios for at least 15 minutes. All other surfaces will also be chemically inactivated by spraying with Amphospray and left to air dry for at least 5 minutes. This include the external surface of rubbish bags used to collect the waste.

The chemically-inactivated waste will then be heat-inactivated by autoclaving at 121°C for 20 minutes.

Disinfectant:

10% surfanios in a tub inside the microbiological safety cabinets (MBSC) will be used for decontamination of plastics.

Stripettes will be decontaminated by flushing with 10% surfanios;

Soaked for a minimum of 15 minutes and placed in a bag to prevent piercing of the autoclave bag.

• The liquid waste from the tub should be poured down a sink;

Pouring this through a sieve device (colander) to collect the decontaminated items which can then be put in the autoclave bags.

For mycobacterial cultures, surfanios is added to obtain a 10% concentration. A minimum of 15 minutes contact time should be allowed for decontamination.

Amphospray 41 can be used for surface decontamination.

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile or chemical indicators (eg Browne TST indicator test strips). This is carried out within the Institute of Infection, Immunity and Inflammation general waste protocol.

(1) Solid waste: where practicable, solid waste should be chemically disinfected prior to disposal in autoclave bag for autoclaving.

(2) Liquid waste: all liquid waste must be thoroughly treated with the disinfectant appropriate for killing the specified organism.

(3) Glassware: the use of glassware should be avoided if at all possible. If the use of glassware is unavoidable then glassware used as a container for infectious materials must be;

a. Thoroughly disinfected inside and out using the approved disinfectant, Amphospray or Surfanios.

b. Glassware is to be rinsed with water once after disinfection.

c. If the glassware has not been in contact with infectious materials then all outer surfaces must be disinfected using an appropriated approved diSInfectant, Amphospray or Surfanios.

d. Disinfected glassware should be placed in the glassware collection tray situated in the lab for Glass wash & Media Preparation staff to collect.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The mycobacterial species are normally found in the environmental habitats, and our mutants will not likely to affect other organisms. No potential hazards to the environment and other organisms associated with the recipient mycobacteria can be predicted. Again, most mutants generated will contain gene deletions or mutations which are associated with a fitness cost. Therefore, the mutant strains will be further attenuated and less fit to survive in the environment.

### Project Containment

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### Project Ref 37/98.1

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### Project Additional Information

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

The project is notified under transitional arrangements.
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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#### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Date Ackn'd | 01/03/1999
Date Project Ceased
Withdrawn | N

CU2 Project Title
MOLECULAR GENETICS OF TRYPANOSOMES AND LEISHMANIA

Class | CultureVolClass2 | CultureVolumeClass3-4 |
Class 2 | Non-GMM |

Consent Granted | not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work | N

02/03/2022
GM37/00.2, **** PROJECT TRANSFERRED BACK TO GM 37 (26/10/07)*

02/06/2000

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 37/99.2

Date Ackn'd 27/02/1999
CU2 Project Title GENETIC MANIPULATION OF TRYPANOSOMAS AND LEISHMANIA

Class 2
Non-GMM Consent Granted not applicable
Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
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#### Project Additional Information

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

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Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 73/trans1

Date Ackn'd 07/03/1988

CU2 Project Title EXPRESSION OF CLONED BORDETELLA PERTUSSIS ANTIGENS IN BORDETELLA HOSTS

Date Project Ceased

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref  821/15.1

Investigation of the signalling properties of protein kinases in Plasmodium falciparum

Date Ackn'd  24/08/2016

Date Project Ceased

Consent Granted  Yes

Project notified under transitional arrangements  N

Historical Date of Additional Info

transferred from GM821 24/08/2016

Historical Significant Changes

Tick if notifying a connected programme of work  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Project Additional Information
Purposes of the contained use

Aims:

Contained Use Notification
Plasmodium falciparum is one of the parasites responsible for human malaria. The aim of this study is to establish the role of protein phosphorylation in maintaining the blood stage of the parasite and the essential processes involved in progressing the parasite through the blood stage. For this we will be generating and importing mutant parasite strains where certain protein kinase genes have been mutated as well as other genes involved in invasion and gametogenesis.

General overview of the project:
It is estimated that 300-500 million people worldwide suffer from malaria with over 2 million deaths per year, mostly in children under the age of 5 (Breman, 2001). The most severe cases of malaria are caused by the parasite Plasmodium falciparum. This parasite is transmitted to humans in the saliva of an infected mosquito. Once in the bloodstream, the parasite makes its way to the liver where it invades liver cells. Inside liver cells the parasite divides and changes into a form that is able to infect red blood cells. The infected liver cells eventually release parasites into the bloodstream where they quickly invade red blood cells. Once inside the red blood cells the parasite once again divides filling the red blood cells with new parasites which are released when the cell membrane eventually ruptures. These released parasites invade other red blood cells and the cycle is repeated.

Many studies aimed at developing novel treatments for malaria focus on the blood stage form of P. falciparum (Pleass and Holder, 2005). Hence a detailed understanding of this stage in the life cycle will provide robust targets for further drug discovery.

We will study the biochemical processes of invasion of P. falciparum into human red blood cells, progression of the parasite through the red blood cell cycle and gametogenesis with particular focus on the role of protein phosphorylation. The following will be undertaken:

1. Investigation of novel potential drug targets including protein kinases and signalling molecules involved in parasite development and gametogenesis. Gene knockout experiments have demonstrated that certain proteins are essential for viability of malaria parasites. Among these genes are those that encode for protein kinases. Therefore, replacement of wild-type alleles of protein kinases with alleles carrying a mutation conferring hypersensitivity to inhibitors will allow validation of these proteins as potential drug targets.

2. Localisation and purification of tagged proteins/parasites: Subcellular localisation of Plasmodium proteins is important for determining how they may be pharmacologically targeted. Localisation of potential drug targets to different parts of the cells will be achieved through GFP or epitope-tag gene constructs. The addition of tags (such as 6-His, HA) to proteins such as kinases, cyclins and metabolic enzymes will allow direct purification of these proteins complexed with their natural protein partners, which can then be analysed further. Such purified proteins can also be used to directly screen against potential drug compounds in luminescent assays.

3. Investigation of erythrocyte invasion by malaria parasites. Gene knock-out of Plasmodium genes of the EBA family presumed to be involved in invasion will determine the role of individual proteins in this process.

4. Determination of the phospho-proteome of plasmodium falciparum using wild-type and genetically-modified parasitoids in comparative studies.

We will be looking at mutants of various protein kinases and signalling proteins including PfCPDK1, PfCLK1, PfCLK3, PfGSK3, PfPK6 and the gale keeper mutant of PfPKG and NEK family (PfNEK1-4) kinase mutants as well as mutants in invasion-related proteins (such as the EBAs) and regulatory proteins including K13-propeller and PfRh protein.

Recipient or parental organism
Host/vector system

DNA vector to be used will be pHH1
This vector will confer ampicillin resistance

Origin & function

The genetic material used has its origin in an eDNA library obtained from the reverse transcription of mRNA derived from the schizont stage of P. falciparum.

The specific genetic material is discussed below:

Dominant negative mutants of:

- PfGSK3 (+GTP/HA/His tagged versions)
- PfPK6 (+GTP/HA/His tagged versions)

We will inhibit the action of specific protein kinases by over expression of dominant negative mutants. These will include dominant negative mutants to GSK3 and PK6.

PfGSK3 is a protein kinase. The normal function of this kinase in malaria is unknown other than it is an essential kinase to maintain the blood stage of the parasite.

PfPK6 is a protein kinase. This kinase is homologous to the cyclin-dependent kinase and therefore likely to be involved in the cell cycle.

Gate keeper mutant of:

PfPKG (+GTP/HA/His tagged versions)

Mutants of the essential protein kinase PKG that render the parasite insensitive to inhibitors of PKG will be used to dissect the role of this kinase in the survival of the parasite.

PfPKG is a protein kinase. It is an essential kinase for parasite survival and is involved in parasite development in the red blood cell, egress and invasion into red blood cells. This kinase is also involved in formation of gametocytes.

Protein kinases:

- CDPK1 (HA-tagged)
- NEK kinase family (NEK1 -4)(HA and GFP tagged)
- CLK1 and CLK3 (+GTP/HA/His tagged versions)

Wild type and mutants of a number of protein kinases that are essential for the progression of the parasite through the erythrocytic cycle will be investigated to establish the role played by these kinases in maintaining the viability of the parasite. The mutations to be introduced either involve epitope tags or removal of regulatory phosphorylation sites or introducing mutations that change sensitivity to protein kinase inhibitors.

PfCLK1/3 are protein kinases. They are involved in RNA processing in the parasite

Invasion proteins

- EBA family (e.g. EBA-175)
- K13-propeller. This is a transcriptional regulator of unknown function.

Drug resistance is a major problem in malaria. It is becoming clear that a number of proteins including K13-propeller proteins are mutated in drug resistant parasites. We will use mutants of these proteins to establish what mechanisms are in play during drug resistance.
The PfRH-family are a group of rhoptry proteins which are released from the parasite during invasion and are essential for the invasion process.

**Evaluation of foreseeable effects**

P. falciparum is a serious human pathogen but the parasite is unable to infect any vertebrate species apart from man and certain primates, and can only infect certain species of Anopheles mosquitoes. Natural infection of humans occurs through the bite of an infected mosquito vector of the genus Anopheles. The research programmes that will be implemented in the Cat3 facilities do not involve mosquito vectors, so the risk of insect-borne inoculation is very low. The only way that the GM could enter the environment and survive is if a mosquito able to transmit malaria was to enter the lab, feed on the culture and pick up from the culture gametocytes. This is almost impossible for the following reasons: Firstly there are only a few species of mosquito in the UK able to act as the vector for P. falciparum, of those that do exist they are in very low numbers in Leicestershire. • The mosquito would then have to enter the closed environment of the malaria lab and gain access to the class 2 hood, or the incubator, which is extremely unlikely. The mosquito would have to feed on the culture (which it is unlikely since it is only 10% human blood) In the meal the mosquito would need to pick up gametocytes which are in extremely low numbers in Pf in vitro cultures (less the 0.5% of the infected red blood cells) After all this it is almost impossible for a single infected mosquito to successful propagate malaria. For this to happen a large number of mosquitoes would have to go through the above process. In the laboratory environment, infection of humans may take place by direct inoculation of infected blood (cultured material) into the bloodstream of the laboratory worker, e.g. through contaminated hypodermic needle. This risk is minimal since no needles or any glass are used in the dedicated culture room. Since the parasite may enter through a cut in the skin then any skin abrasions should be cover in a plaster and the operator where gloves and appropriate PPE. This should reduce any risk of infection to close to zero. The only parasite lines which will be used in this programme (307, NF54) are all susceptible to chloroquine and, importantly, the effectiveness of commonly used antimalarial drugs, e.g. chloroquine, is unaffected by the genetic modification. The function of the inserted parasite gene product will frequently be unknown, or can only be implied through homology with other organisms. Knockouts of potential drug targets (e.g. protein kinases) are aimed at reducing the fitness of the knockout parasites, preferably to produce non-viable or severely functionally-impaired parasites. Allelic replacement of protein kinase genes with those exhibiting hypersensitivity to inhibitors should result in parasites with increased sensitivity to these inhibitors. Growth rates are not expected to be affected, other than negatively, in any of these experiments. The GMM is very unlikely to be more harmful to humans than the parental organism. Pathogenicity in Plasmodium does not depend on any known toxin or clonable agent. Malaria parasites are extremely host-specific and there have been no reports of malaria parasites crossing species barriers, with the exception of some non-human primate malarias that are able to establish very mild infection in humans, and experimental infection of certain non-human primates with human malarias. It is not possible for the human malarias to establish infection in any other vertebrates.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

P. falciparum is a parasite responsible for human malaria. As such this organism is categorised by ACOP as a Hazard
group 3 parasite but handling without negative pressure/HEPA filler is permitted (appendix 3.2 Biological agents managing the risks in laboratories and healthcare premise). This is justified on the following grounds:

- The only way to contract malaria from an in vitro erythrocytic culture of P. falciparum is through a puncture wound of the skin. It is not possible, for example, to gain infection via spillage on the skin, touching contaminated cloths or by touching or rubbing eyes with contaminated hands or tissues.
- It is not possible to be infected by inhalation of parasites contained in aerosols, for example caused by centrifugation or by pipetting, or from evaporation from culture media.
- It is not possible to be infected by contaminated clothing.
- It is not possible to become infected by spillages unless some how the spillage was able to enter the body through punctured skin.

Since the parasite may enter through a cut in the skin then any skin abrasions should be cover in a plaster and the operator where gloves and appropriate PPE. This should reduce any risk of infection to close to zero.

For these reasons it is not necessary to implement full cat 3 containment. Specifically the following key elements of cat 3 contained are not required for P. falciparum in vitro culturing for the following reasons:

- **The laboratory is sealable of fumigation:**
  - This is not necessary as there is no inherent risk of infection from P. falciparum through inhalation. Hence fumigation does not present any inherent risk of parasite infection. Thus, it is not necessary to conduct fumigation in a sealed room. It would seem that this would in fact seriously increase the risk associated with the fumigation procedure with no benefit in decreasing the risk of parasite infection.

- **There is an autoclave available within the laboratory suite:**
  - It is not possible to become infected by the parasite culture other than through a puncture wound. Thus, the solid waste is currently double bagged and autoclaved in the building autoclave facility because it is not possible to contract an infection through accidental contact of any residual waste culture material with the skin, by inhalation or ingestion. There is no justification therefore for autoclaving in the malaria lab. In fact having an additional autoclave would increase risk of injury through autoclaving.

- **Note that all liquid waste is decontaminated in the malaria lab and disposed of in the malaria lab therefore is not autoclaved.**

- **Entry to the lab is via an airlock:**
  - This is unnecessary since it is not possible to be infected by the malaria parasite from inhalation or from aerosols or vapour or any airborne source. Hence an airlock is not necessary.

- **The lab is at negative pressure relative to the pressure of the immediate surroundings:**
  - This is not necessary for the same reasons as described above for the airlock.

- **Extract and input air from the laboratory must be HEPA filtered:**
  - This is not necessary for the same reasons as described above for the airlock/negative pressure.

Controls to mitigate risk of infection from in vitro P. falciparum cultures.

To mitigate the risk of infection from a puncture wound the following standard operating procedure for P. falciparum in vitro culture has been in operation for some time in line with the recommendations from the University and MRC safety office and as approved by the HSE.

- **a) Only the culture of P. falciparum is permitted in this laboratory.**
- **b) Each culture batch will be up to 30ml**
- **c) Only staff that have gone through the appropriate safety training and that have been approved by the University**
and MRC safety officers will be allowed to work in lab 201.
d) Access to lab 201 will be limited to personnel who have received training and approval by the supervisor/group leader.
e) Only trained personnel will be given the access code to enter the lab that will be locked at all times with a swipe card entry lock.
f) All the equipment required for the culture of P. falciparum will be contained in lab 201. This includes the class 2 microbiological cabinet, incubator, centrifuge and microscope.
g) Liquid tissue culture medium is stored in lab 202/209.
h) The use of sharps is strictly forbidden. This includes in particular glass pipettes and glass bollies. Glass slides will, however, be permitted for the staining of parasites in culture.
i) Liquid waste will be inactivated with presept (1,000ppm equivalent to 4 x 2.5g tablet per 500ml) and disposed of down the designated sink.
j) All solid waste will be double bagged and autoclaved.
k) Gloves will be worn at all times.
l) Safety glasses to be worn at all times.
m) Dedicated blue lab coats will be worn at all times.
n) Buckets in the centrifuge must be capped when spinning tubes containing the parasite cultures.
o) In the event of spillage, liquid waste will be absorbed onto CLAN Unisafe absorbent granules and placed in solid waste and autoclaved. Area will then be washed with presept (10,000ppm equivalent to 4 x 2.5g tablet per 500ml) for disinfecting.
p) In the event of centrifuge disruption or if a spill is suspected then the following procedure must be adhered to:
1. Leave 20 minutes before opening
2. If the integrity of the buckets is not comprised, these (with caps) will be moved into the class II microbiological cabinet and opened.
3. Liquid will be disposed of in liquid waste container, treated with presept (1,000ppm equivalent to 4 x 2.5g tablet per 500ml) and disposed of in the designated sink. Solid waste will be disposed of in the solid waste disposal bin and autoclaved.
4. If the integrity of the buckets is comprised, then liquid waste will be absorbed onto CLAN Unisafe absorbent granules and placed in solid waste and autoclaved.
5. The centrifuge should then be thoroughly cleaned with presept (10,000ppm equivalent to 4 x 2.59 tablet per 500ml) and all waste disposed of as solid waste and autoclaved.
q) Transport of potentially infected material from laboratory 201 (e.g. cell lines, frozen ampoules, serum etc.) will be done in an approved sealed vessel clearly labeled and a trolley will be used.
r) All solid waste is autoclaved.
s) Eating, chewing, drinking, smoking, taking medication, storing food and applying cosmetics is forbidden in the laboratory.
t) Mouth pipetting is forbidden.
u) In case of a spillage of P. falciparum on broken skin or a puncture wound (from for example a contaminated slide) the wound should be washed thoroughly under running water and Occupational Health consulted.
v) When leaving the malaria lab (room 201) with malaria samples gloves and lab coats need to be changed.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be inactivated by addition of Presept (Johnson & Johnson Medical) - 4x2.5g tablet per 500ml (equivalent to 10,000ppm*) - overnight and disposed of down the designated sink.
In the event of a large spillage, liquid waste will be absorbed onto CLAN Unisafe absorbent granules and placed in
solid waste and autoclaved. Area will then be washed with Presept (10,000ppm equivalent to 2.59 tablet in 500ml). In the event of small spillages or splashes, the surface should be cleaned with 70% IMS. All solid waste will be placed in an appropriate autoclave bag, taped closed and autoclaved at 133°C for 12 mins. It is handled as detailed in the appropriate standard operating procedure for the Hodgkin Building. All pipettes will be placed in a sealed orange topped 7L sharps bin and autoclaved at 133°C for 12 mins. All waste will then be incinerated by an approved contractor. *Note: Presept data - a solution of presept was made up in water where the presept concentration was 10,000 ppm. 1ml of this solution was added per 10ml 307 or NF54 P. falciparum culture for 24 hours (final concentration was 1,000 ppm). In the control culture parasitemia was 1% and in the presept treated culture, no live parasites were observed. Therefore a final concentration of 10,000 ppm is deemed far higher than necessary to kill the parasites.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee have agreed the following:

i. That a designation of Class 2 is appropriate
ii. That the risk assessment is sound and that work should be carried out at Containment Level 3 but can be handled without negative pressure/HEPA filter
iii. That the project will be notified to the HSE, and, further, they have noted:
   i. That the proposer will contact HSE to notify the work as a new project.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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### Name

| Name                  | John Innes Centre            |

| Name 2 | Sainsbury Laboratory          |

| Campus Estate or Research Centre | Norwich Research Park       |

| Road Name | Colney Lane          |

| Town      | Norwich             |

| County   | East Anglia         | Postcode | NR4 7UH | Country | England |

| Tel Number | 01603 450000 | Fax Number | 01603 456844 |

| E-mail | HSE Division | East and South East |

| Comments |

| Date at Which Additional Info Submitted | 21/10/2003 | 06/11/2003 |

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Project Ref  38/01.1

Date Ackn'd  07/03/2001
Date Project Ceased

CU2 Project Title

TRANSFORMATION OF THE POWDERY MILDEW FUNGUS Erysiphe graminis WITH A GREEN FLUORESCENT PROTEIN (GFP) CONSTRUCT TARGETED TO THE MITOCHONDRIA.

Class  Class 2
CultureVolClass2 < 1 litre
CultureVolumeClass3-4

Non-GMM  Consent Granted
not applicable
Purposes of the contained use

To investigate the sexual reproduction of the fungus Erysiphe graminis with particular reference to the inheritance of nuclei and mitochondria. The mitochondria of the two mating fungi will be differentially recognised using two forms of green fluorescent protein (GFP). The mitochondria cannot be transformed directly, so we will transform nuclear DNA using a GFP construct with a mitochondrial-targeting signal. Positive selection of transformants will use bialophos (BASTA), which since E. graminis is an obligate biotroph will also require a susceptible wheat line to be bialophos resistant. A bialophos resistant wheat variety (Bobwhite) has already been generated at the John Innes Centre. This procedure will enable us to establish the mechanism by which mitochondria, but not the nucleus, from the male parent are excluded from the developing progeny.

Recipient or parental organism

The recipients will be two isolates (Fe109 and J1W2) of the powdery mildew fungus Erysiphe graminis f. sp. tritici.

Host/vector system

The plasmids used will be derivatives of the pBT-BAR plasmid. This plasmid contains the bialophos resistance gene (bar) (for positive selection of fungal transformants) bordered by fungal promoter and terminator sequences. The rest of the vector consists of the plasmid pBLUESCRIPT KS(-) available from Stratagene. pBLUESCRIPT contains a bacterial promoter plus ampicillin resistance genes to enable positive selection of bacterial transformants during plasmid propagation. These will be disabled Escherichia coli strains such as DH5-a.

A GFP construct with a mitochondrial-targeting signal will be engineered into the above background.

Origin & function

The different components of the above vector have the following origins:

i) The bar gene is isolated from the bialophos-producing Streptomyces hygroscopicus. The bar gene encodes a phosphinothricin acetyl transferase which inactivates the antibiotic bialophos.

ii) The fungal promoter and terminator sequences are derived from the B-tubulin gene from E. graminis.

iii) pBLUESCRIPT is a commercially available plasmid from Stratagene.

iv) The GFP gene originates from the jellyfish Aequoria victoria and is commercially available.

v) The mitochondrial-targeting signal will be the 5' end of the citrate synthase gene from either Aspergillus niger or E. graminis.

The modified pBT-BAR plasmid will be transformed into nuclear DNA of Erysiphe graminis f.sp. tritici using biolistic transformation. The mitochondrial-targeting signal will then enable GFP to be specifically expressed in the fungal mitochondria, where under appropriate conditions it will cause the mitochondria to fluoresce. These isolates will
then be studied microscopically. The bialophos resistance genes will be expressed conferring bialophos resistance upon the host organism for selection purposes only. All transformants are for experimental purposes only.

**Environmental Risk**

Plant, fungal and bacterial colonies will be killed by autoclaving prior to disposal, eliminating risk of environmental contamination by this route. Likewise all compost and residual plant material generated in containment glasshouses is autoclaved prior to disposal. Infected plants growing in containment glasshouses will be contained in plastic tubes, sealed at the bottom to the plant pots, and at the top to hoods prepared from a double layer of tea bag paper. This system has been routinely tested for escape of contaminating spores and has proved highly effective. In addition, containment glasshouses are MAFF-approved and have an air filter system, which prevents accidental release of airborne spores into the environment. The air filter system is tested at regular intervals (every 4 months) using rust fungal spores and the filters changed regularly. The soiled filters are bagged and autoclaved prior to disposal. In the highly unlikely event of accidental release no adverse effects are likely, as E. graminis is an obligate biotroph and will not survive long in the absence of a susceptible wheat host. Laboratory manipulations will be performed in sterilised airflow cabinets and isolates stored in sealed plastic boxes. Transformants will be resistant to the herbicide bialophos, which non-transformed isolates are susceptible to; however, bialophos is not used to control fungi, nor indeed is it used as a herbicide in the UK.

**Human Health Risk**

The plasmids used are geared for expression in bacteria and fungi and should have no expression potential in humans. The transformed organisms are for experimental purposes only and are not intended for human consumption. The bacterial hosts are disabled laboratory strains with negligible risk to human health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Containment level 2 is appropriate for activities involving plant manipulations.

The susceptible wheat line (Bobwhite) which has been previously transformed with the bialophos resistant gene at the John Innes Centre will be grown in MAFF-approved containment level 2 glasshouses. The plants will be grown to seedling stage (10 day old) and inch long leaf segments removed for infection with the transformed E. graminis isolates in the laboratory (G31 Bateson Building). Transfer of the transformed isolates between the laboratory and glasshouses will be in sealed containers to prevent dissemination. Plant material infected by E. graminis will be contained in plastic tubes, sealed at the bottom to the plant pots, and at the top to hoods prepared from a double layer of tea bag paper. This system has been routinely tested for escape of contaminating spores and has proved highly effective. This infected plant material will be grown in containment glasshouses and transported to the laboratory in sealed plastic bags. Laboratory manipulations will be completed in airflow cabinets, which are sterilised before and after use. Material stored in the laboratory will be in sealed plastic boxes. All residual plant material (both infected and uninfected) will be disposed of by autoclaving. Autoclaving facilities are present within the containment glasshouse facilities and within the laboratory (G68) adjoining G31.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation from containment level 2 required.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste material generated will be in different forms:

i) Residual plant material and soil will be disposed of by autoclaving.

ii) Plant material infected with transformed E. graminis isolates will be stored in sealed boxes which will be disposed of by autoclaving.
iii) Liquid cultures generated by bacterial propagation will be autoclaved prior to disposal. Plasmid manipulations of bacterial cultures will be completed in disposable plastic vessels, which will be autoclaved prior to disposal.

All autoclaving results in 100% kill. Autoclave procedures are as follows:
1. Soil is autoclaved in stainless steel containers for 1.5 hours (15 min vent, 30 min sterilisation at 7 vacuums). Resultant waste is transported in trailers to a farm site where it stands for a 3 year period prior to disposal.
2. Plant waste generated in the glasshouses is autoclaved for 1.33 hours (15 min vent, 45 min sterilisation at 2 vacuums).
3. Laboratory material will be autoclaved in room G68 (adjoining room G31) at 121 degrees C for 18 minutes.
Routine monitoring of autoclave efficiency is completed using Thermolog S strips.

Run-off from containment glasshouses is channelled to a filtration plant with a 5000 L holding tank. 1500 L aliquots are removed and treated with sodium hypochlorite and circulated in the tank for a 24 hour period, following this treatment is with sodium thiosulfate. The water is then filtered and disposed of.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

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<tr>
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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref  38/01.2

Date Ackn’d  11/05/2001  CU2 Project Title  INFECTION OF ARABIDOPSIS WITH PSEUDOMONAS SYRINGAE SPP  Class  2  CultureVolClass2  < 1 litre  CultureVolumeClass3-4

02/03/2022  Page 1758 of 1532
EXPRESSING RECOMBINANT AVIRULENCE PROTEINS.

Purposes of the contained use

To investigate the plant resistance response to bacterial pathogens expressing or lacking specific avirulence determinants. Pseudomonas syringae episomally expressing recombinant avirulence determinants derived from related Pseudomonads will be infiltrated directly into Arabidopsis leaves. Leaves will be subsequently harvested and analysed for bacterial growth, defence gene induction, and physiological changes.

Bacterial tagged with the lux operon will be used in viability studies.

Recipient or parental organism

Recipients will be Pseudomonas syringae pv maculicola, pv.tomato, pv.lachrymans, pv.pisi, pv.phaseolicola, pv.glycinea, and pv.apii.

Host/vector system

The plasmids used will be derivatives of pRK2 and pRSF1010. In both cases modified derivatives of these plasmids will be used that are defective in mobilisation (mob). The plasmids are also replication defective and are rapidly lost in the absence of antibiotic selection in vitro. These plasmids contain genes encoding resistance to tetracycline (RK2) and kanamycin (RSF1010) for selection in vitro. These plasmids contain lac promoter to drive expression of introduced avirulence genes. Manipulation of these plasmids and avirulence genes will be performed in non-colonising rec derivatives of E.coli K12.

The lux operon of Photohabdus luminescens will be introduced by Transposition insertion with a disabled mini-Tn5 construct.

Origin & function

1. The avirulence genes to be used are derived from Pseudomonas pathovars as follows: avrA, B, C (pv glycinea), avrRpt2 (pv.tomato), avrRpm1 (pv maculicola), avrRps4 (pv.pisi), avrPphB (phaseolicola). Additionally, the lux operon from Photohabdus luminescens will be used in some viability studies.

2. The tetracycline resistance gene is native to the parental RK2 plasmid.

3. The kanamyycin resistance gene is native to the parental RSF1010 plasmid.

4. pBluescript is commercially available from Stratagene.
All constructs will be introduced into the respective Pseudomonas pathovars via electroporation and maintained episomally in plasmids under antibiotic selection with the exception of the lux operon which will be maintained chromosomally after plasmid integration. The lux operon will enable the direct determination of viability to be made by scoring for relative luminescence.

Evaluation of foreseeable effects

The transformed Pseudomonads will be essentially identical to their non-transformed parents except for the expression of avirulence proteins. These avirulence proteins are well characterised molecules that are known to elicit specific plant defence responses. These Pseudomonads are expected to be equal or less virulent than their parental counterparts as they will be expressing specific molecules that enable a plant response.

Environment risk:

The bacteria and infected plant material will be killed by autoclaving prior to disposal. Likewise all compost and residual plant material generated in the containment glasshouses is autoclaved prior to disposal. Containment glasshouses are MAFF approved and have an air filter system (the filters are changed regularly and autoclaved prior to disposal) which prevents accidental release of aerosols. These are non-spore forming bacterial and hence such risks are negligible.

In the event of release:

In the event of an accidental release no adverse effects are expected. The recombinant avirulence proteins used in these experiments are common in their wild-type counterparts and are being expressed on unstable plasmids that are rapidly lost in the absence of persistent antibiotic selection in vitro.

Human health risk:

The plasmids and constructs being used are directed for bacterial expression only. The E.coli hosts used are disabled non-colonising rec derivatives of E.coli K12. The Pseudomonads in use are unable to colonise humans as they cannot replicate at 37 deg. No adverse human health effects are foreseen.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Level 1

Level 2 where infected with GMM

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Class 2 GMM (plant pathogen) (containment level 2). All GMM material handled using good microbial practice. All GMM material autoclaved prior to disposal.

Residual lant material will be stored in sealed containers and disposed of by autoclaving.

Liquid bacterial cultures will be chemically inactivated using Hycolin or autoclaved prior to disposal.

Autoclave procedures are as follows: Soil is autoclaved in stainless steel containers for 1.5 hours (15 min vent, 30 min sterilisation at 15 psi). Resultant waste is transported in trailers to a farm site where it stands for a 3 year prior prior to disposal.

Plant waste generated in the glasshouses and growth rooms is autoclaved for 1.33 hours (15 min vent, 45 min sterilisation at 15 psi).
Routine monitoring of autoclave efficiency is completed using Thermolog S strips.

Run-off from containment glasshouses is channelled to a filtration plant with a 5000 L holding tank. 1500 L aliquots are removed and treated with sodium hypochlorite and circulated in the tank for a 24 hour period followed by a treatment with sodium thiosulfate. The water is then filtered and disposed. Plants in the growth rooms will be watered by sub-irrigation in leak proof trays such that no run-off is generated.

The RAs indicate that level 2 containment is required for GMM work. The BSC agree that this is appropriate and that the facilities are available to ensure containment is effective.

Please enter comments on the GM safety committee on the risk assessment

The RAs indicate that level 2 containment is required for GMM work. The BSC agree that this is appropriate and that the facilities are available to ensure containment is effective.

**Project Containment**

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref  38/01.3**

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**Project Additional Information**

**Purposes of the contained use**

To investigate plant-virus interactions - in particular, the plant genes involved in virus infection and changes in plant gene expression in response to virus infection. We will introduce transgene or virus constructs into non-transformed or transformed plants (Arabidopsis or tobacco) using Agro-inoculation, rub-inoculation, particle bombardment or stable transformation using Agrobacterium. The virus constructs will be wild-type or recombinant ie tagged with reporter genes or parts of plant genes.

**Recipient or parental organism**

Plant viruses: eg potato virus X (PVX); tobacco rattle virus (TRV); tobacco etch virus (TEV); tobacco mosaic virus (TMV); tomato golden mosaic virus (TGMV). These viruses will be tagged with reporter genes eg beta-glucuronidase; green fluorescent protein. TGMV, PVX and TRV will have parts of plant sequences inserted into their genomes. These sequences will not encode plant proteins.

**Host/vector system**

**Plant Hosts**: Arabidopsis thaliana and Nicotiana spps.

The plasmids used will be derivatives of the binary vectors pBIN19, pSLJ729/2, pSLJ75515/6, pGreen or derivatives of pUC18/19, pBLUESCRIPT KS, pGEM-T.

**Bacteria hosts**: Escherichia coli DH10B (Disabled); Agrobacterium tumefaciens GV3101, LBA4404, C58C1 (pCH32) - all disarmed.

**Origin & function**

The reporter genes will be commonly used markers such as the uidA gene from E. coli, that encodes beta-glucuronidase (GUS); the green fluorescent protein (GFP) from the jellyfish Aequoria victoria. The plant sequences will be from A. thaliana or Nicotiana spps. and will not encode proteins. The transgenes will be GFP or GUS reporter genes or viral genes expressed from generally used promoters eg the cauliflower mosaic virus 35S promoter, the nopaline synthase promoter.

**Evaluation of foreseeable effects**

**Environmental risks**: None expected.

There is no evidence that the host range of the recombinant viruses can be increased by insertion of a reporter gene or by insertion of part of a plant gene sequence. Symptoms of the recombinant viruses are very similar to those produced by the non-recombinant (wild-type) virus. The inserted sequences are rapidly lost through recombination through duplicated viral sequences. The agrobacterium is disarmed and therefore not oncogenic. Agrobacterium may survive in the environment as a free-living soil microbe but the binary vector would be lost in nature very quickly in the absence of antibiotic selection. Transfer and expression of the constructs requires the presence of a chemical that induces T-DNA transfer. Agro-inoculation will only be used as a delivery system for what are already mechanically transmitted viruses. The plant hosts are not wind pollinated. All wild-type and recombinant viruses will be propagated in Class 2 containment glasshouses. All transgenic plants will be grown in Class 2 containment glasshouses or a Weiss controlled environment room (CER).

**Human health risks**: None expected.
The marker genes have no known toxicity and have a history of safe usage over the previous 5-10 years. The plant sequences used in the viruses will not encode proteins. The plant viruses do not cause infection in humans. The bacteria are disabled and do not cause infection in humans.

All manipulations of GMOs will be performed in laboratories which comply with HSE level 2 containment facilities. All agro-inoculations will be performed in Class 2 containment glasshouses. All virus inoculations will be performed in Class 2 containment glasshouses or a Weiss CER.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Containment level 2 is appropriate for activities involving plant manipulations.

All GM plants will be grown in Class 2 containment glasshouses or a Weiss CER.

Glasshouse: All run-off water is sterilised before discharge into the sewerage system. Access is restricted to named personnel and monitored. Entry is via a sterilisation foot mat. The glasshouses are physically protected against insect ingress and a solid floor prevents insect or nematode vectors. Unrelated experiments are segregated.

Weiss CER: All run-off water is collected in trays below the benching and evaporates. The chamber is sterilised at the end of each experiment. Access is limited to named personnel only and the chamber is locked. Entry is via a sterilisation foot mat. Entry to the area housing the CER is via several closed doors. Flowering is prevented because the CER is run at short day length and plants are removed at about 3 weeks post pricking out. Non-GMO work is not performed in the chamber.

All pots, soil, plant material are autoclaved after each experiment.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All manipulations of GMMs will be performed in the laboratory with GMP. The laboratories comply with HSE level 2 containment facilities. All materials coming in contact with the GMO and all biological waste is inactivated by autoclaving (121C for 18 min) or verified chemical means before leaving the building.

All experiments using GMMs will be performed in Class 2 containment glasshouse or Weiss CER. The glasshouse run-off water is sterilised before discharge into the sewerage system (using hypochlorite solution circulated for 24h, followed by sodium thiosulfate treatment and filtration). The CER water is collected in trays and evaporates. The trays are sterilised at the end of each experiment. All plant material and soil is autoclaved after use (Soil: 15 min vent, 30 min. sterilisation at 7 facuums; plant material: 15 min. vent, 45 min sterilisation at 2 vacuums). All autoclaving results in 100% kill. Routine monitoring of autoclave efficiency is completed using Thermolog S strips.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

This work has been reviewed by the local JIC Biological Safety Committee. The modified organisms should be no fitter than the wild-type and are probably less fit. Given the containment levels, environmental hazards are minimal.
**Project Additional Information**

**Purposes of the contained use**

To transform avirulent isolates of powdery mildew to virulence, and hence identify avirulence genes which are specifically recognised by the corresponding resistance gene in plants.

**Recipient or parental organism**

The recipients will be isolates of powdery mildew fungi, (*Blumeria graminis*).

**Host/vector system**
The plasmids used will be derivatives of pCAMBIA and they will be grown in Agrobacterium for transformation. Initial experiments to establish transformation efficiency will require the resistance gene (bar) for the antibiotic bialophos, and will require susceptible barley or wheat line to be bialophos resistant. However, the experiment to isolate avirulence genes will not require bialophos resistance since selection of transformants will be directly on cereal plants containing the corresponding resistance gene. The plasmids contain a kanamycin resistance gene to ensure that the bacterium can be grown without contamination.

Origin & function

The pCAMBIA series of plasmids are available from the Centre for the Application of Molecular Biology to International Agriculture (CAMBIA), Canberra, Australia. The bar gene is isolated from the bialophos-producing Streptomyces hygroscopicus. The bar gene encodes a phosphinothricin acetyl transferase which inactivates the antibiotic bialophos.

A pCAMBIA vector will be delivered to the nuclear DNA of Blumeria graminis by incubation of growing colonies of mildew with the Agrobacterium strain AGCI 563 containing the vector. In the initial experiment to establish efficiency of transformation, the mildew colonies will be inoculated onto a bialophos-resistant line of barley or wheat and sprayed with bialophos. In the experiment to isolate avirulence genes, a derivative of the pCAMBIA vector with the bar gene removed will be used. Selection will be directly onto commercially available cultivars of wheat or barley with the corresponding resistance gene. Any colonies that grow can be expected to disrupted in their ability to produce the avirulence gene specifically recognised by the resistance gene, enabling the avirulence gene to be isolated. All transformants are for experimental purposes only.

Evaluation of foreseeable effects

The purpose of the experiment is not to introduce foreign genes into powdery mildew, but to disrupt the expression of a functional avirulence gene by random insertion of the Agrobacterium T-DNA into the genome. This make the fungus become virulent on a wheat or barley plant carrying the corresponding resistance gene, and will enable us to establish the genetic location of the avirulence gene. Selection is highly specific for disruption in the target avirulence gene, and transformants with altered expression in non-target genes will be recognised by the plant and will die. Mutation to virulence in cereal powdery mildew occurs frequently in nature, and we will use R genes which have already ‘broken down’ in the field, ie for which matching virulence is common in European populations of the fungus. Thus the transformed fungus will not be any more pathogenic than naturally-occurring isolates.

Even though transformed mildew is not expected to be any more pathogenic than naturally-occurring isolates, procedures to minimise risk of escape into the environment will be implemented. Plant, fungal and bacterial colonies will be killed prior to disposal in an autoclave located in the laboratory where the transformants are generated. Laboratory manipulations will be performed in sterilised airflow cabinets and isolates stored in sealed plastic boxes. During the initial experiment to investigate transformation efficiency, transformants will be resistant to the herbicide bialophos. However, bialophos is not used to control fungi, nor indeed is it used as a herbicide in the UK.

The plasmids are geared for expression in bacteria and fungi, and will have no expression potential in humans. The transformed mildew will be used for experimental purposes only and are not intended for human consumption.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Susceptible wheat and barley lines (Bobwhite and Golden Promise respectively) which have been transformed with the bialophos resistance gene will be used during the initial experiment to assess transformation efficiency. These will be grown in DEFRA-approved containment level 2 glasshouses. The plants will be grown to seedling stage (10 day-old) and inch long sections removed and taken to the laboratory (G31 Bateson building) in sealed plastic containers for infection with the transformed mildew isolates. The experiments to isolate avirulence genes will not require any genetically-modified plants.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation from containment level 2 required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste material generated will be in different forms:

1. Residual plant material and soil will be disposed of by autoclaving.
2. Plant material infected with transformed Blumeria graminis isolates will be stored in sealed plastic boxes which will be disposed of by autoclaving.
3. Liquid cultures generated by bacterial propagation will be autoclaved prior to disposal. Plasmid manipulations of bacterial cultures will be completed in glass or disposable plastic vessels, which will be autoclaved prior to disposal.

All autoclaving results in 100% kill. Autoclave procedures are as follows.

1. Soil is autoclaved in stainless steel containers for 1.5 hours (15 min. vent, 30 min. sterilisation at 7 vacuums). Resultant waste is transported in trailers to a farm site where it stands for a 3 year period prior to disposal.
2. Plant waste generated in the glasshouses is autoclaved for 1.33 hours (15 min. vent, 45 min. sterilisation at 2 vacuums).
3. Laboratory material will be autoclaved in Room G68 (adjoining room G31) at 121 degrees C for 18 minutes.

Routine monitoring of autoclave efficiency is completed using thermolog S strips.

Run-off from containment glasshouses is channelled to a filtration plant with a 5000 litre holding tank. 1500 litre aliquots are removed and treated with sodium hypochlorite and circulated in the tank for a 24 hour period, after which they are treated with sodium thiosulphate. The water is then filtered and disposed of.

Details of how the facilities at the John Innes Centre reduce the risk to effectively zero have been supplied, and more details of the experience of the researchers has been provided. Although there is no environmental of human risk, this procedure has been classified as a category 2 activity because it involves work with the powdery mildew fungus, which is a plant pathogen. Under DEFRA guidelines, all work involving plant pathogens is a category 2 activity. Experiments to identify multiple tDNA insertions and insertion of vector backbone have been described in accordance with the recommendations of the safety committee.

Please enter comments on the GM safety committee on the risk assessment

Details of how the facilities at the John Innes Centre reduce the risk to effectively zero have been supplied, and more details of the experience of the researchers has been provided. Although there is no environmental of human risk, this procedure has been classified as a category 2 activity because it involves work with the powdery mildew fungus, which is a plant pathogen. Under DEFRA guidelines, all work involving plant pathogens is a category 2 activity. Experiments to identify multiple tDNA insertions and insertion of vector backbone have been described in accordance with the recommendations of the safety committee.

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Animal Units  Large Scale Activities  Human Clinical Applications
Project Additional Information

Purposes of the contained use

To characterise signal transduction events in the interactions between plants and their pathogens, particularly disease resistance conferred by the tomato Pto gene in response to strains of Pseudomonas carrying the avrPto gene. The GM organisms described here have been constructed to 1) provide identical strains of Pseudomonas syringae differing only for the presence of avrPto, and 2) to allow transgenic expression of Pto or avrPto in the tomato relative Nicotiana benthamiana for experimental purposes. The GM organisms will be imported from a laboratory in the USA under DEFRA Plant Health License PHL 161/3914.

Recipient or parental organism

1) Pseudomonas syringae pathovar tomato strains DC3000 (unmodified) and T1 (modified).
2) Nicotiana benthamiana (wild tobacco).

Host/vector system

1) Pseudomonas strains have been modified with the derivatives of the broad host-range vector (pDSK519 (Gene 1988) 70, 191-197). Some of the constructs express the avrPto gene from Pseudomonas syringae pv tomato strain TO, from its genomic promoter.
2) N. benthamiana has been modified by Agrobacterium-mediated transformation to express 1) Pto from the 35S promoter, or 2) avrPto from a dexamethasone-inducible promoter.

Origin & function

1) pDSK519 is a derivative of the broad host-range vector RSF1010 (Gene (1983) 24, 299-308). The vector carries the Kan gene for kanamycin resistance, the lacZ gene
and multiple cloning site from pUC18, and where indicated, the avrPto gene with 0.5 Kb of genomic promoter sequence. The broad host range origin allows propagation of
the vector in both E. coli and Pseudomonas. The lacZ<CS facilitates cloning of genes to be expressed, and avrPto confers avirulence to Pseudomonas on hosts carrying
the Pto gene.

2. The Pto gene was cloned into the standard binary vector pB1121 and is expressed from the strong, constitutive 35S promoter. The vector also carries the Kan gene on
the T-DNA. Transgenic plants thus constitutively express Pto from the 35S promoter and are resistant to kanamycin.

3. The avrPto gene was cloned into the binary vector pTA7002 (Curr Opin Biotechnol (2000) 11(2): 146-51) and is expressed conditionally in the presence of
dexamethasone. pTA7002 contains the mammalian GVG gene under control of the constitutive 35S promoter. The T-DNA also contains the HPT gene for resistance to
hygromycin. Thus transgenic plants express GVG and hygromycin resistance constitutively. avrPto expression is silent until specifically induced with the glucocorticoid
dexamethasone.

All GM organisms described here are for experimental procedures only.

**Evaluation of foreseeable effects**

1. Pseudomonas strains carrying pDSK519 will be resistant to the antibiotic kanamycin. Kanamycin is not used to control the bacteria in the field and so this will not add
fitness to the genetically modified organism. Genetically modified Pseudomonas carrying pDSK519 are expected to be slightly less fit than the unmodified bacteria because
of the metabolic cost in replicating the vector. Pseudomonas carrying pDSK519 modified to express the avrPto gene will be avirulent (non-infectious) on hosts expressing
the Pto gene. Therefore, Pseudomonas strains expressing avrPto are expected to be considerably less fit than the unmodified organism. The protein encoded by AvrPto is
only known to be active within the plant cell, and is not known ro have any biological effects on humans. The sequence of the avrPto gene does not suggest any
relationship with known toxins. The Pseudomonas strains used here are not known to be pathogenic on humans. AvrPto is not known to increase the virulence of
Pseudomonas, and in fact should decrease the host range of the modified bacteria. Therefore, Pseudomonas carrying pDSK519 expressing the avrPto gene should be
less fit than the unmodified organism.

2. Transgenic Nicotiana benthamiana 38-12 expressing 35S:pto will be resistant to Kanamycin. Kanamycin is not used to control plants in the field and this is not expected
to make any difference to the fitness of the organism. Plants expressing 35S:Pto will be resistant to bacteria that contain the avrPto gene. The resistance is specific to
micro-organisms and will not cause the plant to become more toxic to humans or animals. The resistance will not be selected against in the wild so the transgenic plants
will not be fitter than unmodified plants. Therefore I foresee no potential for these plants to pose a hazard as weeds.

3. Transgenic Nicotiana benthamiana containing the T-DNA derived from vector pTA7002, or this vector containing the avrPto gene, will be resistant to hygromycin, a
herbicide. Thus the plants will no longer be susceptible to this herbicide, but can still be contained by application of unrelated herbicides (multiple herbicide resistance will
not be a result of the modification). The modified plants are not expected to be fitter than unmodified plants. Transgenic N. benthamiana containing the PTA7002 T-DNA
containing the avrPto gene will express AvrPto protein only when induced with the specific chemical inducer, dexamethasone. AvrPto protein will not be expressed under
any other conditions so the chance of expression of AvrPto after escape to the wild is negligible. AvrPto is not knonw to be toxic to humans or animals, and is only known
to be active when expressed within the plant cell. Expression of AvrPto will activate plant defences only in the presence of the host resistance gene, Pto. Plants modified
with the capability to express AvrPto upon induction with dexamethasone are not expected to be fitter than unmodified plants, and do not possess greater potential to be
weeds than the unmodified plant.

**ENVIRONMENTAL RISK**

Plant and bacterial material will be killed by autoclaving prior to disposal, eliminating risk of environmental contamination by this route. Laboratory surfaces exposed to
genetically modified micro-organisms will be decontaminated by treatment with bleach or 70% ethanol. All compost and residual plant material generated in containment
glasshouses is also autoclaved prior to disposal. Containment glasshouses are DEFRA-approved and have air filter and waste-water treatment systems. The GMMs
described here are expected to be less fit than unmodified bacteria.

**HUMAN HEALTH RISK**

The plasmids used are designed for bacterial use and should have no expression potential in humans. The transformed organisms are for experimental purposes only and
are not intended for human consumption or environmental release. The bacterial hosts are not pathogenic to humans.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Containment level 2 is appropriate for GM plants that are imported under a DEFRA Plant Health License (PHL 161/3914). All GM material will be imported as seed in sealed vials.

Transformed plants will be grown in DEFRA-approved containment level 2 glasshouses. Transfer of the GM material between the laboratory and glasshouses will be in sealed plastic bags to prevent dissemination of the material. Plants will be grown to 4-6 weeks old prior to infection or sampling for biochemical analyses. Where infection of plants with GMMs is necessary, infected plants growing in containment glasshouse will be maintained free from invertebrates. Infected plants will be grown on saucers to prevent spillage of soil or drainage water, and inflorescences will be bagged to contain pollen and seed, which will be destroyed by autoclaving or stored within the glasshouse in a suitably labelled, secure container. Plant material infected with GM bacteria may be transported from containment facilities to the laboratory in sealed plastic bags, however most material will not leave the glasshouse but be destroyed by autoclaving of plants, soil, and contaminated material, in the dedicated autoclave within the glasshouse.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste material generate will be in different forms:

(i) Residual plant material and soil will be disposed of by autoclaving.
(ii) Plant material infected with transformed P. syringae strains will be stored in sealed boxes which will be disposed of by autoclaving.
(iii) Liquid cultures generated by bacterial propagation will be autoclaved prior to disposal.

All autoclaving results in 100% kill. Autoclave procedures are as follows:

1. Soil is autoclaved in stainless steel containers for 1.5 hours (15 min vent, 30 min sterilisation at 7 vacuums). Resultant waste is transported in trailers to a farm site where it stands for a 3 year period prior to disposal.
2. Plant waste generated in the glasshouses is autoclaved for 1.33 hours (15 min vent, 45 min sterilisation at 2 vacuums).
3. Laboratory material will be sealed in plastic bags and boxed, before being autoclaved in room G68 (adjoining room G31) at 121 degrees C for 18 minutes.

Routine monitoring of autoclave efficiency is completed using Thermolog S strips.

Run-off from containment glasshouses is channelled to filtration plant with a 5000L holding tank. 1500 L aliquots are removed and treated with sodium hypochlorite and circulated in the tank for a 24 hour period, following this treatment is with sodium thiosulfate. The water is then filtered and disposed of.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
Please enter comments on the GM safety committee on the risk assessment

Acceptable - no further comments.

Project Containment

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Project Ref 38/04.1

Date Ackn'd 03/02/2004

CU2 Project Title
TO DETERMINE HOW THE HRT GENE IN ARABIDOPSIS CONFERS RESISTANCE AGAINST TURNIP CRINKLE VIRUS.

Date Project Ceased

Class 2 CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 litre

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements N

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Turnip crinkle virus cDNA clones were obtained from Dr Dan Klessig, Boyce Thompson Institute, Ithaca, NY, USA under DEFRA license PHL 161A/4391 (01/2003). The cDNA will be inoculated either as cDNA or as in vitro transcripts to arabidopsis plants. The level of accumulation will indicate whether the virus is able to accumulate either in the presence or absence of HRT.
The experiments will generate TCV that are as virulent or less virulent than the wild type constructs. The most virulent constructs would have the ability to survive, establish and disseminate in the environment. However, given the use of containment there is only a low hazard from these experiments.

Turnip crinkle virus is not known as a hazard to human health and nor is there any reason to suppose that it could be unless specifically modified to express toxic proteins or proteins that produce toxic products.

Host/vector system

Origin & function

Turnip crinkle virus cDNA clones were obtained from Dr Dan Klessig, Boyce Thompson Institute, Ithaca, NY, USA under DEFRA license PHL161A/4391 (01/2003). The cDNA will be inoculated either as cDNA or as in vitro transcripts to Arabidopsis plants. The level of accumulation will indicate whether the virus is able to accumulate either in the presence or absence of HRT.

Evaluation of foreseeable effects

Turnip crinkle virus is not known as a hazard to human health and nor is there any reason to suppose that it could be unless specifically modified to express toxic proteins or proteins that produce toxic products. To eliminate this hazard we shall not express toxic proteins or proteins known to produce toxic products. The host plants used in the experimental work include Arabidopsis, potato, tomato, Nicotiana species, Chenopodium species, Gomphrena globosa. None of these experimental plants is a human health hazard under the conditions used in our experimentation. There is no reason known to me why infection with wild type or modified forms of turnip crinkle virus should cause these plants to be a human health hazard under the conditions used in our experimentation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Bacterial cultures containing the TCV clones will be propagated in the laboratory in axenic culture and will be disposed of by autoclaving. All materials in contact with these cultures will also be sterilized to give 100% kill before disposal. Infected plants will be maintained in the containment glasshouse at the John Innes Centre. Standard operating procedures for the glasshouse and for disposal of glasshouse waste will be strictly adhered to. These procedures provide physical containment, biological containment by minimising opportunities for unwanted spread of the virus infection and for disposal of waste after sterilization by autoclaving.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Autoclaving of all materials to give 100% kill. Autoclaves are regularly monitored with Thermalogues. All inactivated waste is sent to landfill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The background to the proposed work is a project in which the inf1 extracellular protein of P. infestans was identified as an avirulence determinant on N. benthamiana (Kamoun et al., 1998). P. infestans strains that produce this protein do not exhibit extensive growth or sporulation whereas strains in which the inf1 protein are not produced exhibit extensive hyphal growth and do sporulate whereas strains in which the inf1 protein are not produced exhibit extensive hyphal growth and do sporulate. The role of inf1 as an avirulence determinant is associated with its ability to elicit a hypersensitive necrotic response in N. benthamiana (Kamoun et al., 1997). This hypersensitive response is manifested in a simple assay in which a solution of the inf1 protein is infiltrated into leaves of N. benthamiana. After 2-3 days the infiltrated patch becomes...
necrotic and eventually dies. We shall be using the genetically modified isolate of P. infestans that does not produce inf1 as a control in tests of N. benthamiana genes that may be involved in the resistance pathway.

Recipient or parental organism

The modified strains of P. infestans will carry antisense transgenes to suppress expression of inf1. These strains also carry a kanamycin resistance gene that was used as a selectable marker in the production of the strains. We intend to use these strains as a negative control in the experiments described above. If we have completed ablated the resistance against P. infestans in N. benthamiana the wild type strains will grow as well and will sporulate at the same level as the inf1 strains. There is no reason to suppose that there is a human health risk since P infestans is not known as a human pathogen and the genetic modification will have resulted in loss of gene expression. The genetic modification in P. infestans transformants isolates 88069 Y15, PY23 and PY37 results in the ability of the strain to overcome natural resistance in N. benthamiana. However this modification does not affect the pathogen on potato, tomato or tobacco. (Kamoun, S., vanWest, P., Vleeshouwers, V., deGroot, K.E. and Govers, F (1998). Resistance of Nicotiana benthamiana to Phytophthora infestans is mediated by the recognition of the elicitor protein INF1. Plant Cell 10, 1413-1425.). Since N. benthamiana is not a UK species (it is from Australia) there is no reason to think that the gm strains would pose an environmental hazard.

Host/vector system

Not applicable.

Origin & function

The P. infestans isolates were imported under DEFRA licence from Wageningen, The Netherlands

Evaluation of foreseeable effects

The most severe consequences that could be envisaged would be a novel pathogen of N. benthamiana species that are used for experiments in the JIC. However as no experiments will be carried out in glasshouses this is an unlikely scenario. All proposed experiments will be carried out on detached leaves of plants maintained in propagators that are kept in incubators with illumination.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All proposed experiments with infected plants will be carried out on detached leaves of plants maintained in incubators in the laboratory. The level 2 containment measures will include:

* the use of microbiological safety cabinets when handling cultures of the isolates
* autoclaving to be carried out in the laboratory building
* all handling of liquid cultures and samples in microbiological safety cabinets and using procedures that minimise aerosol production.
* cultures to be handled only by named personnel
* laboratory coats to be worn at all times when handling cultures and samples
* disinfectant procedures as specified in standard operating procedures
* inactivation of contaminated material and waste by autoclaving prior to disposal
* cultures will be maintained in designated freezers and storage areas.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. SECURITY
   a) Areas containing quarantine material: all bioassays should be carried out in the laboratory under the following conditions: inoculations of agar plates and plant tissue with licensed isolates of P. infestans should be performed in microbiological safety cabinets designed for experiments dealing with fungi. For long-term storage, stocks of P
infestans zoospores in 15% DMSO are stored in cryotubes in a -80 degrees C freezer. Regarding the bioassays, mycelium should be grown on appropriate agar medium in petri dishes sealed with parafilm. Bioassays will be carried out on plant detached leaves that will be placed in screw-capped plastic containers or sealed petri dishes. Plates and plants should be kept in sealed containers at 18 degrees C in a growth chamber designated for P infestans bioassay experiments located in the laboratory.

b) Access to quarantine areas: the growth chamber is used exclusively for experiments using licensed isolates of P infestans, therefore only those persons working with these isolates will be authorised to use this facility. One person will be in charge of monitoring whether standard operating procedures are followed.

c) Quarantine areas should be labelled clearly with the following information: this facility is to be used for P infestans work only, with a description of the licensed material, the name of the persons authorised to use the growth chamber and the name of the person in charge of the project. Information on the type of work that is being carried out at any one time should also be listed.

d) Authorised personnel will have to read and sign the operating procedures before they can work on the licensed material.

2. ADMINISTRATION AND RECORDING:

a) Dated records of all introductions of licensed material and description of the work will be kept in a log book.

b) Licensed material should be labelled as such together with the name of the corresponding P infestans isolate, the date and the name of the user, through all stages of experimentation.

c) Inventories of stocks will indicate that the material is held under licence and that users should be authorised.

3. TRANSPORT BETWEEN AND WITHIN LICENSED AREAS

a) Infected material should be kept at all times in sealed containers and transported in these containers between the microbiological safety cabinet and the growth chamber or -80 degrees C freezer so that escape of the pathogen is prevented.

b) The licensed material should be sent as rye-agar slants of pure P. infestans mycelium covered with mineral oil, in screw-capped tubes placed in a metal container to avoid the possibility of damage. To eliminate any fungal or bacterial contaminants, P. infestans isolates should initially be grown on clean-up media containing a cocktail of antibacterial and antifungal agents prior to starting any other manipulation.

4. CONTAINMENT FACILITIES

a) All experiments should be carried out in the laboratory using three types of facilities: a -80 degrees C freezer, a growth chamber and a microbiological safety cabinet. It should be noted that the latter will not be used for storage of material but for inoculation experiments only.

b) The -80degree C freezer and the growth chamber (Sanyo versatile environmental test chamber) are located on the first floor of the Sainsbury Laboratory in room SL1-40. The microbiological safety cabinet is located on the second floor of the Sainsbury Laboratory in room 241.

c) Licensed facilities can be used by authorised staff as many times as required provided that they follow strictly the procedures described here.

d) The growth chamber should not be overcrowded, no more than 200 leaf samples and no more than 20 mycelium plates should be kept at any one time. Up to 100 cryotubes containing stocks P. infestans will be stored in the -80 degrees C freezer. The microbiological safety cabinet will only be used for inoculation experiments.
typically inoculations of 50 samples can be done at any one time.

e) Sharing of the facilities: the growth chamber will only be used for growing licensed material whereas the -80 degrees C freezer and the microbiological safety cabinet will be shared between licensed and non-licensed material.

f) Type of work to take place in each area:
- microbiological safety cabinet: inoculations of agar plates and plant tissue with P. infestans. Preparation of infected leaf samples for microscopy and extraction of nucleic acids.
- growth chamber: incubation of P. infestans and infected leaves in sealed containers at 18 degrees C.
- -80 degrees C freezer: storage of pathogen material.

g) The licensed material will not include plants for planting.
h) There are no traps to detect any escapes of the licensed organism.

5. EXPERIMENTAL PROCEDURES

a) Protocol: Inoculations of agar plates and plant tissue with P. infestans are performed in a microbiological safety cabinet designed for experiments dealing with fungi. Stocks of P. infestans zoospores will be stored in cryo-containers at -80 degrees C. To carry out bioassays, mycelium will be grown from single zoospores taken from the cryotubes in petri dishes containing the appropriate growth medium (typically rye-agar or pea-agar). Plates will be sealed with parafilm and incubated at 18 degrees C in the growth chamber for us to three weeks. Isolation of P infestans zoospores will be done by adding cold water on the surface of the mycelium that is growing on the agar plate and incubating the plate at 4-8 degrees C in an ice box. After 1-2h, water suspension containing zoospores is collected and filtered through cheesecloth to separate the zoospores from the mycelium. Bioassays are carried out on detached leaves from Nicotiana benthamiana, potato and tomato plants. Aliquots of zoospores from the water suspension are taken with a Gilson pipette fitted with filtered tips and placed on the surface of the leaves. Typically, each leaf will is inoculated with 20ul droplets containing between 100 and 1000 zoospores. Leaves are placed in sealed containers and incubated at 18 degrees C in the growth chamber for up to 8 days. To assess biotrophic growth of the pathogen, pieces of leaf are cut with a scalpel at different days and placed immediately in screw-capped tubes containing a fixing solution (1 volume of chloroform, 3 volumes of acetic acid, 6 volumes of ethanol), such a treatment will neutralise the pathogen and bleach the leaves so that they can be used for microscopy analyses. For DNA analyses, plant tissue is placed directly in a lysis solution that will also neutralise the pathogen. The scalpel will be sterilised by dipping into ethanol and flaming, it will be kept in the microbiological safety cabinet in ethanol at all times.

b) Staff should wear lab coats and gloves at all times. Gloves will be disposed of immediately after use and autoclaved.

c) Infected material should be kept at all times in sealed containers and transported in these containers between the microbiological safety cabinet and the growth chamber or -80 degrees C freezer so that escape of the pathogen is minimised.

6. HYGIENE AND DISPOSAL

a) Infected areas will be disinfected with 70% ethanol.

b) Disposal of waste: waste will consist of sealed petri dishes containing mycelium, screw-capped tubes containing zoospores/sporangia in water, gloves, screw-capped plastic containers containing infected plant tissue, pipette tips used for inoculation of plant tissue. All contaminated material will be placed in autoclave bags in closed containers and autoclaved immediately after use using autoclaves that are located on the first floor of the Sainsbury Laboratory or other locations that can be reached through indoor corridors and passageways.
Approved November 7th 2003.

Please enter comments on the GM safety committee on the risk assessment

Approved November 7th 2003.

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Project Ref 38/04.3

Date Ackn'd 04/02/2004
CU2 Project Title THE AIM OF THE WORK IS TO INVESTIGATE VIRUS ENCODED PROTEINS AS SUPPRESSORS OF GENE SILENCING.

Class 2 Culture Vol Class 2 Culture Volume Class 3-4
Class 2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

The experiments will involve expression of these viral proteins in plants in a transient expression system in transgenic plants. The findings will be informative about the mechanisms of silencing and, through the identification of host proteins that interact with the suppressors, about the host proteins involved. In addition by coexpression of viral suppressors and non viral transgenes encoding foreign proteins, it will be possible to enhance expression of these foreign proteins in plants.

The coexpression experiments will be carried out using agrobacterium transient expression in leaves of Nicotiana species. These experiments will not generate transformed plants - the agrobacterium strains are unfiltrated into leaves and expression of the suppressor and the foreign protein is assayed within 14d.

Transgenic Arabidopsis and solanaceous plants will be developed to express these suppressor proteins as part of our work to investigate the mechanism of silencing.

**Recipient or parental organism**

The viral suppressors will be derived from beet western yellows virus, cotton leaf curl virus, African cassava mosaic virus, tomato bushy stunt virus, cucumber mosaic virus. None of these viruses is known as a hazard to human health and nor is there any reason to suppose that they could be in the proposed experiments. The experiments will not involve expression of these viruses as infectious agents - the isolated genes for the suppressor proteins will be used.

PO from beet western yellows virus (Pfeffer et al., 2002)
beta C1 protein from cotton leaf curl virus (Briddon et al., 2001)
p19 from tomato busy stunt virus (Silhavy et al., 2002)
2b from cucumber mosaic virus (Ji and Ding, 2001)
AC2 from african cassava mosaic virus (Voinnet et al., 1999)

**Host/vector system**

The cDNA clones of the suppressor proteins will be maintained in bacterial plasmids maintained either in E. coli or A. tumefaciens. The E. coli strains will be standard laboratory strains and the A. tumefaciens will be standard non oncogenic isolates used for plant transformation.

**Origin & function**

The cDNA clones were obtained under DEFRA license PHL161A/4391(01/2003)

**Evaluation of foreseeable effects**

It is unlikely that accidental release of transgenic plants expressing these suppressor proteins would have a harmful effect because the proteins are not known to be toxic.
and because the plants are normally impaired in growth and development and because they are hypersusceptible to virus disease.

The effects of the potyvirus suppressors on development is described (Anandalakshmi et al., 2000; Kasschau et al., 2003) and it likely to be a guide for the effects of other suppressors in transgenic plants. The effect of p189 on arabidopsis development has been described by Dunoyer et al (2004) and is described in a paper in press. The hypersusceptibility phenotypes to virus disease are described for a potyvirus suppressor of silencing (Pruss et al., 1997) and for the 2b protein of cucumber mosaic virus (Li et al., 1999).


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Bacterial cultures containing the suppressor clones will be propagated in the laboratory in axenic culture and will be disposed of by autoclaving. All materials in contact with these cultures will also be sterilized to give 100% kill before disposal. Plant tissues that are transiently expressing these proteins or that are stably transformed to express these proteins will be maintained in the containment glasshouse at the John Innes Centre. Standard operating procedures for the glasshouse and for disposal of glasshouse waste will be strictly adhered to. These procedures provide physical containment, biological containment by minimising opportunities for disposal of waste after sterilization by autoclaving.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Autoclaving of all materials to give 100% kill. Autoclaves are regularly monitored with Thermalogues. All inactivated waste is sent to landfill.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment
Approved November 7th 2003 and revised after request from HSE for more information and decision by investigator to remove PVX expression experiments. The BSC made the following comments on the revised risk assessment.

Two points:
Section B(ii) of the GMRA - the same paragraph appears twice.
Section 12 of the Notifications suggest the following:
Autoclaving of all materials to give 100% kill. Autoclaves are regularly monitored with Thermalogues. All inactivated laboratory waste is sent to landfill and inactivated soil waste is composted on site for 2 years prior to use on JIC field plots.

I've had a look at bogh assessments and they look fine. The previous comments concerned the problems which might arise if suppressors of silencing were expressed from a viral vector (PVX) and resulted in increased virulence. The PVX expression work is no longer requested and the new versions appear to have assessed the potential hazards correctly.

I found one typographical error in GMRA 154 Revised: The paragraph starting: "The Agrobacterium strains and Arabidopsis and Nicotiana species would survive in the environment but neither are likely to cause damage…" In Part B (ii) is repeated twice.

This revised version appears to be OK. Only one minor point: Section (ii) of the GMRA refers to African cassava mosaic virus but, unlike other viruses mentioned, there is no reference (Voinnet et al., 1999) provided. This also applies to section 7 of the HSE Notification.

Everything appears to be in order.
The appropriate minor corrections were made to the GMRA and HSE notification form in response to these comments.

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**Project Ref**  38/07.1

**Date Ackn'd**  19/01/2007

**Date Project Ceased**  02/03/2022

**CU2 Project Title**  Expression of candidate oomycete and fungal effector proteins in planta, in Agrobacterium sp., Xanthomonas spp., Erwinia sp. And in Pseudomonas syringae.

**Class**  Class 2

**CultureVolClass2**  < 1 Litre

**CultureVolumeClass3-4**

**Non-GMM**  Not Applicable

**Consent Granted**
**Project Additional Information**

**Purposes of the contained use**

Please see page 10 for section 6. (additional comments page).

**Recipient or parental organism**

Recipients will be standard laboratory strains of Escherichia coli, disarmed or non-oncogenic isolates of Agrobacterium tumefaciens, Pseudomonas syringae (all pathovars), Xanthomonas campestris pv. vesicatoria (=Xanthomonas axonopodis pv. vesicatoria), Xanthomonas translucens pv. hordei (Xanthomonas translucens pv. Translucens) and Xanthomonas campestris pv. campestris and Erwinia carotovora. These experiments may generate transgenic plant pathogenic bacteria with altered host ranges or pathogenicity.

**Host/vector system**

Plasmids used will be recombinant pBBR, pVS1, pVSP61 (a derivative of pVS1), pGreen, pRK290 derived binary vector plasmids, pBIN19 and pTA7002 (a derivative of pBIN19). These plasmids are either non-mobilisable or non self-mobilisable and encode genes for resistance to gentamycin, spectinomycin, kanamycin and tetracycline for selection in vitro. Recombinant plasmids may carry effector fusions that could deliver oomycete or fungal effector proteins into plant cells.

**Origin & function**

Candidate plant pathogen effector molecules will be identified and genes cloned from the oomycete pathogens; Hyaloperonospora parasitica (Hp), Albugo candida (Ac), Phytophthora infestans (Pi), Phytophthora capsici, phytophthora sojae and from the fungal pathogens; Golovinomyces spp., Erysiphe spp., Blumeria spp. and Colletotrichum spp.

Many of these candidate effectors from Hp.Ac.Pi, Phytophthora capsici, Phytophthora sojae, Golovinomyces spp. Erysiphe spp., Blumeria spp. Or Collectotrichum spp. Will be fused to the N terminal TTSS secretion signal of a Pseudomonas,Erwinia or Xanthomonas TTSS- secreted protein, introduced into Pseudomonas, Erwinia, Agrobacterium or Xanthomonas, and then tested for their phenotypes on various species and strains of Arbidopsis, Medicago, Tricitcum, Brassica, Solanum, Lycopersicon, Horedonium, Brachypodium, Oriya and Nicotiana whole plants grown in Glasshouses and CERS.

**Evaluation of foreseeable effects**

Human Health and Environment

The E.coli to be used in this study are rec-non-colonising derivatives of K-12. The Pseudomonas, Erwinia and Xanthomonas phytopathogens in this study are endemic in the UK and have specific host-ranges as indicated by their pathovar designations. The genetic manipulation of these bacteria will be performed using replication-compromised plasmids that are unstable and lost in the absence of persistent antibiotic selection. The transformedPseudomonas, Agrobacterium, Erwinia and Xanthornonas spp. Will be essentially identical to their non-transformed parents except for the expression of pathogens effector molecules. These bacteria are
non-sporulating water-borne organisms that are readily contained using standard microbiological practices. The genetic manipulations in this study may alter the pathogenic properties of the bacteria, but these changes are as likely to result in reduced host range as in increased host range, because bacteria regularly exchange plasmids that encode effector proteins that plants have evolved the capacity to recognize, and plants have resistance proteins that recognize effectors from diverse microbes. Because of the narrow host range of these bacteria and the genetic instability of these vectors outside the laboratory, no adverse environmental effects are expected.

The Pseudomonas, Xanthomonas and Agrobacterium strains to be used are unable to colonise humans, as they are incapable of growth at 37°C. The Erwinia species used are not human pathogens. No adverse human health effects are foreseen. It is not foreseen that plant infected with any of these transgenic plant pathogenic bacteria would constitute a human health hazard.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For plant material infected with GMM, containment level 2 is appropriate. Infected plants will be maintained in containment level 2 growth facilities at the John Innes Centre. Standard operating procedures for the use of plant growth facilities and for the disposal of waste will be strictly adhered to.

The bacteria infected plant material and compost will be killed by autoclaving prior to disposal. The containment glasshouses have an approved air filter system to minimise the accidental release of aerosols, and as these are non-spore forming bacteria, such as risks are negligible.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Class 2 GMM (plant pathogen) (containment level 2). All GMM material will be handled using good microbiological practice (GMP). All GMM material will be autoclaved prior to disposal.

Residual plant material will be stored in sealed containers and disposed of by autoclaving. Liquid bacterial cultures will be autoclaved prior to disposal.

Autoclave procedures are as follows: Soil and plant waste generated in glasshouses and growth rooms is autoclaved in stainless steel containers for 1.5 hours (15 minutes vent, 30 minutes sterilisation at 15 p.s.i) Resultant waste is transported to a farm site where it is left to stand for three years prior to disposal. The compost banks are inspected twice yearly and sprayed with a general purpose herbicide.

Routine monitoring of autoclave efficiency is completed using Thermolog S strips.

Run-off from containment glasshouses is collected into 5000L tank. 1500L aliquots are removed and treated with sodium hypochlorite and circulated in the tank 24 hours followed by a treatment with sodium thiosulphate. The water is then filtered and disposed. Plants in growth rooms will be sub-irrigated in teak proof trays such that minimal run-off is generated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The John Innes Centre GM Safety Committee have reviewed the risk assessment and the HSE notification both electronically via e-mail and also at a general meeting on the 6th November 2006. A series of minor improvements and text additions were recommended and incorporated by the group leader. The classification for the containment were agreed.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<td>L3</td>
<td>L4</td>
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<tr>
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### Project Ref 38/08.1

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<td>USE OF PLANT VIRUS-BASED VECTORS FOR GENE SILENCING STUDIES IN MONOCOTYLEDONOUS HOSTS.</td>
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<th>Project notified under transitional arrangements</th>
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<td>Not Applicable</td>
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### Project Additional Information

**Purposes of the contained use**

The purpose of this project is the use of plant virus-based vectors for analysis of monocotyledonous plant gene function through silencing. Plant viral cDNAs, under the control of bacteriophage promoters for in vitro transcription, will be genetically modified through the insertion of cereal derived genes to determine the effects of silencing on...
Recipient or parental organism

Barley stripe mosaic virus (BSMV) gene silencing vector
As tested by SCRI, Dundee (HSE centre number GM 250)

Host/vector system

Virus induced gene silencing (VIGS) using the BSMV-based gene silencing vector will be performed in different cereal species (intially with Hordeum and Triticum aestivum). BSMV vector viral cDNAs will be contained in plasmids (such as pUC-based plasmids) under the control of bacteriophage or plant promoters. Plasmids will be cultured in disabled Escherichia coli strains (such as XL1-Blue and DH5a).

Origin & function

VIGS of cereal genes will be performed using partial cDNAs of candidate genes isolated from different cereal species (such as Hordeum vulgare and Triticum aestivum) inserted into the BSMV vector. Also reported genes such as Green fluorescent protein (GFP) from Aequorea and ß-glucuronidase (GUS) from Escherichia coli will be used.

Evaluation of foreseeable effects

The viral proteins produced by the recombinant viruses occur in nature and have no known risks to human health. The reporter proteins GFP and GUS have been expressed in a wide range of experimental systems and do not present a risk to human health. BSMV is non-indigenous plant pathogen and thus subject to legislative control and is a cause of disease in wheat and barley. In order to provide robust biological containment, portions of the coat protein genes of BSMV have been deleted to render them non-transmissible by mechanical routes (system developed by Holzberg et al 2002). Previous insertion of reporter protein genes into plant viral genomes has resulted in the creation of attenuated viruses and no mechanism for enhanced pathogenicity can be foreseen. Overall the coat-protein disabled BSMV can be considered to present less risk to the environment than the natural progenitor viruses.

The modified BSMV strain has been used successfully in several labs (Lacomme et al 2003, Hein et al 2005, Scofield et al 2005 and Cloutier et al 2007) to silence targeted genes, both reporter genes and partial cDNAs from cereals. The vector to be used for this study will be obtained from the Scottish Crop Research Centres (SCRI) in Dundee where the BSMV based VIGS system is used under CL2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All materials in contact with viral material will be sterilized to give 100% kill before disposal. Sterilization will be carried out when plants are four weeks old. Plant tissues that are transiently silenced will be maintained in the containment glasshouse at the John Innes Centre. Standard operating procedures for the glasshouse and for disposal of glasshouse waste will be strictly adhered to. These procedures provide physical containment, biological containment by minimising opportunities for disposal of waste after sterilization by autoclaving.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Infected plants and associated materials (soil, plants, gloves) will be autoclaved using a protocol validated to give 100% kill. Plant extracts prepared in the laboratory and material derived from them will be autoclaved using a protocol validated to give 100% kill. Containment surfaces will be disinfected with a proprietary validated viricide.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
John Innes Centre Biological (GM) Safety Committee agrees with the containment level 2 classification. Main Comment:

Although BSMV can infect many different species of plants from a range of plant families, it may be that this virus and vectors derived from it would not necessarily be able to trigger gene silencing in all of them. This is the case for PVX induced plant gene silencing. One could say that the native virus poses a greater risk to the environment than the proposed genetic modification. It is necessary to address the risk of silencing genes in plants from the same or other plant family, which may include plants in the neighbouring environment. In the absence of pollen transfer this virus would be contained absolutely, since it can no longer be mechanically transmitted.

The risk assessment states that silencing of endogenous genes could result in more severe infection symptoms (if host defence genes are silenced. The notification to the HSE states that no mechanism for enhanced pathogenicity can be foreseen with reference to viral genomes with inserted reporter protein genes. If silencing of endogenous genes could result in greater disease symptoms, then include it in the HSE notification (the logic being that a pathogen will be more pathogenic on a plant that is less fit). It could be that no known host defence genes will be targeted for silencing and say so if that is indeed the case. It would probably be useful to indicate the nature of the genes to be silenced in the Project Description Overview.

**Project Containment**

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**Project Ref** 38/11.1

**Date Ackn'd** 03/05/2011

**CU2 Project Title**
Virus induced gene silencing (VIGS) and virus-mediated heterologous gene overexpression in the Graminae: Aegilops spp., Triticum spp., Hordeum spp. and Brachypodium distachyon and virus induced gene silencing (VIGS) in the Fabaceae

**Date Project Ceased**

**Class** Class 2
**CultureVolClass2** < 1 Litre

**Non-GMM Consent Granted**

**Project notified under transitional arrangements** N
Project A;
Virus induced gene silencing (VIGS) and virus-mediated heterologous gene overexpression in the Poaceae; Aegilops spp., Triticum spp., Hordeum spp., Avena spp., and Brachypodium distachyon.

This project aims to establish protocols for: (1) virus-induced gene silencing (VIGS) and (2) virus-mediated gene overexpression in Aegilops sharonensis, (Sharon goatgrass), Ae. Longissima, Ae. Bicornis, Ae. Searssii and Ae. Speltoides, Triticum species, especially Triticum aestivum (bread wheat), T. durum (pasta wheat), T. boeoticum (domesticated Einkorn wheat), T. monococccum (wild Einkorn wheat), T. diococum (domesticated Emmer wheat) and T. dicoccoides (wild Emmer wheat), Hordeum vulgare (Barley) and Hordeu, valgare subsp. Spontaneum (wild Barley), Avena sativa (oat) and Brachypodium distachyon. VIGS, if achieved, would be a useful tool to test the function of candidate wheat stem rust (Puccinia gramiis f.sp tritici) and wheat yellow rust/stripe rust (Puccinia striiformis f. sp. Tritic) resistance genes. We will test cloned viruses with a proven potential above all in monocots, including brome mosaic virus (BMV), barley stripe mosaic virus (BSMV), tomato yellow leaf curl virus: (TYLCV), and soil-borne wheat mosaic virus (SBWMV).

The same viruses identified in the screen above will also be tested for their ability to over express candidate effector genes from wheat stem rust and wheat stripe rust.

Project B;
Virus induced gene silencing (VIGS) in the Fabaceae.
Bean pod mottle virus (BPMV) and tomato yellow leaf curl virus; (TYLCV)

This project aims to establish protocols for and identify whether either of these viruses are suitable vectors in order to achieve virus induced gene silencing (VIGS) and/or virus-mediated heterologous overexpression in the Fabaceae.

Virus osolates we will use are infectious modified clones. They are full length clones that have had certain restriction enzyme sites modified or introduced (to facilitate cloning) and also in some cases have been engineered to carry foreign "marker" gene(s), including the green fluorescent protein (GFP), b-glucoronidase (GUS), the bar gene from Streptomyces hygrosporius coding for phosphimothricin (PPT) acetyltransferase, and a fragment of wheat and or barley pds (phytoene desaturase) for gene silencing. In the case of BSMVb we also plan to employ a widely used clone, BSMVbDa, that has the ba gene (coat protein) deleted (Holzberg et al., 2002). The modified tri-partite virus (longhand, BSMV a::bDba::g) spreads systemically better within barley (Holzberg et al., 2002) but considering that seed transmission determinants map to RNAa and RNAg (Edwards, 1995) it is conceivable that such a mutant would display reduced seed transmission.

The identified hazards would be the escape of GM virus that could silence wheat stem rust and/or stripe rust resistance genes in plants in the local environment, thereby rendering infected plants more susceptible to these fungal pathogens or the GM virus could cause plants in the local environment to die, probably not as a result of the pathogenicity of the virus, but as a result of the host plant's recognition of fungal effector proteins. The GM virus will likely be more pathogenic on plants with compromised...
host defence mechanism. Viral vectors expressing genes such as fluorescent or antibiotic resistance markers will have a neutral effect on their host plant in that the genetic modification will not affect viral pathogenicity. Recombinant viruses could survive in the environment, but the genetic modification is unlikely to persist due to the instability of recombinant viral vector genomes.

Host/vector system

The genetically modified viruses will be the vectors for the delivery of gene constructs that would direct either the overexpression or silencing of wheat stem/stripe rust genes and/or host plant resistance genes. We may consider cloning the virus in the T-DNA of a standard laboratory binary vector, such as pBIN19 but not exclusive to, and delivered from a disarmed Agrobacterium strain.

Origin & function

We may engineer BSMV (or other viral) infectious clones expressing putative fungal stem rust and/or stripe rust effector(s) (some of which will have been identified in the lab of Fumiaki Katagiri, J G and L S, University of Minnesota, USA; and in the lab of Sophien Kamoun and Cristobal Uauy, The Sainsbury Laboratory, The John Innes Centre, Norwich, UK)

The identification of novel eukaryotic plant pathogen effector molecules from wheat stem and stripe rust and the identification of host plant resistance genes will lead to novel strategies for the control of these and other emerging crop pathogens.

Evaluation of foreseeable effects

Human Health;

None of the recipient organisms used in these projects is a human pathogen and does not, therefore, constitute a hazard to human health. The proposed genetic modifications of plant viruses here described will not cause the modified viral vectors to become human or animal pathogens and are therefore of no risk to human or animal health. None of the host plants used in the experimental work, is nor is likely to become, a human health hazard under the conditions used in our experimentation.

Environmental Safety;

Virus-Induced Gene Silencing (VIGS)
The silencing of endogenous plant gene(s) may compromise host defence, enabling the virus to be more virulent than the corresponding wild type isolate. The GM-virus infected plants will be more susceptible to wheat stem rust and stripe rust. The identified hazard would be the escape of GM virus that could silence wheat stem rust and/or stripe rust resistance genes in plants in the local environment, thereby rendering infected plants more susceptible to these fungal pathogens.

Virus-Mediated Gene Over expression (VMGO)
The virus mediated overexpression of plant fungal pathogen effector proteins may cause the infected plants to grow more slowly or even lead to plant death. The identified hazard would be the escape of GM virus that could cause plants in the local environment to die, not as a result of the pathogenicity of the virus, but as a result of the host plant's recognition of fungal effector proteins. The GM virus will likely be more pathogenic on plants with compromised host defence mechanisms.

The endogenes that will be silenced or the transgenes that will be overexpressed with virus vectors will be candidates for either wheat stem rust/stripe rust resistance or wheat stem rust/stripe rust avirulence determinants.

It is unlikely that either the overexpression of avirulence determinants from these plant fungal pathogens or the silencing or wheat stem rust/stripe rust resistance genes will pose a threat to UK farming or the natural environment. Wheat stem rust has only been found in the south coast of the UK well after normal harvesting time for cereals, it was last identified in 1981 and has never been observed spreading into the UK in the last century (R B, personal communication) and wheat stripe rust is routinely controlled with fungicides

Survival of GM virus in the local environment

Since all the viruses that will be genetically modified have broad host ranges, there is a high likelihood that GM virus could find a suitable host in the local environment. The
insertion of genes into the viral genome is extremely unlikely to affect the virus host range, viral pathogenicity or means of transmission. Only BSMV is pollen transmissible and such virus infected plants will not be allowed to flower. BMV, TYLCV and BPMV are primarily transmitted by insects and therefore plants infected with these viruses will be kept in the containment glasshouse block under positive pressure (B41). SBWMV is transmitted by zoospores of the soil borne fungus, Polymyxa graminis. All wastewater run off is treated within B41. Virus infected plants contained in B35 room 505 produce no waste water run-off.

It is generally the case that when foreign transgenes are inserted into viral vector genomes, these modified forms tend to be much less stable than wild type virus. Continued passage of the virus through successive host plants will select for deletion variants. There is therefore a degree of biological containment inherent with working with such types of modified virus. Genetic stability of the virus will cause loss of the insert and of the corresponding hazard. This has been demonstrated for PVX (Avesani et al., 2007) and BSMV (Bruun-Rasmussen M. et al., 2007).

The plant viral pathogens; BSMV, BMV, TYLCV and SBWMV are widely distributed and very likely to be present in the local environment, even if the unmodified recipient strains here used may be imported from outside of the UK and held under licence from DEFRA. Therefore the risk posed by their potential accidental escape is not related to the introduction of a new plant disease, but rather to factors that relate to their genetic modification(s) BPMV is from the USA and widespread in soya bean growing areas. This virus vector will be used to infect members of the FABaceae with transgenes to silence marker proteins/genes only.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
<thead>
<tr>
<th>For plant material infected with GMMs, containment level 2 is appropriate. Infected plants will be maintained in containment level 2 growth facilities at the John Innes Centre. Standard operating procedures for the use of plant growth facilities and for the disposal of waste will be strictly adhered to.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant pathogens, infected plant material and compost will be killed by autoclaving prior to disposal.</td>
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### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Class 2 GMM (plant pathogen) (containment level 2). All GMM material will be handled using good microbiological practice (GMP). All GMM material will be autoclaved prior to disposal.</th>
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</thead>
<tbody>
<tr>
<td>Residual plant material will be stored in sealed containers and disposed of by autoclaving. Viral transcripts and viral infected plant material will be autoclaved prior to disposal.</td>
</tr>
<tr>
<td>Autoclave procedures are as follows; Soil and plant waste generated in glasshouses and growth rooms is autoclaved in stainless steel containers for 1.5 hours (15 minutes vent, 30 minutes sterilisation at 15 p.s.i.). Residual waste is transported to a farm site where it is left to stand for three years prior to disposal. The compost banks are inspected twice yearly and sprayed with a general purpose herbicide.</td>
</tr>
<tr>
<td>Routine monitoring of autoclave efficiency is completed using Thermalog S strips.</td>
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<td>Run-off from containment glasshouses is collected into a 5000L tank. 1500 L aliquots are removed and treated with sodium hypochlorite and circulated in the tank for 24 hours, followed by a treatment with sodium thiosulphate. The water is then filtered and disposed. Plants in growth rooms will be sub-irrigated in leak proof trays such that minimal run-off is generated.</td>
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**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N
Members of the Biological Safety Committee reviewed the risk assessment along with the HSE Notification form and were generally very satisfied with the standard and contents of the documents. These were considered to be very comprehensive. Two typographical errors were pointed out and corrected.

It was stressed that it is imperative to ensure that good procedures are in place to kill BSMV plants before they are allowed to flower including a reasonable margin of safety.

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<td>25/07/2011</td>
<td>The expression of GM marker genes and/or candidate oomycete and fungal effector genes in eukaryotic plant pathogens. The silencing of eukaryotic plant pathogens. Also the expression of genes thought to induce fungicide insensitivity in eukaryotic plant pathogens.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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**Historical Significant Changes**

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<th>Significant Change ID</th>
<th>Date of Significant Change</th>
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</thead>
</table>
## Purposes of the contained use

The aim of our work is to dissect the resistance mechanisms in Solanaceous plants (tomato, potato, and Nicotiana spp.), sweet potato (Ipomoea batatas), cereals (wheat and barley), Brassicas including Arabidopsis thaliana that operate against eukaryotic plant pathogens such as the oomycetes Phytophthora infestans and/or Phytophthora brassicae, Phytophthora capsici, Phytophthora ipomoeae, Phytophthora palmivora, Albugo candida and Albugo laibachii, as well as ascomycete fungi such as Blumeria spp., Mycosphaerella graminicola, M. brassicicola, Leptosphaeria maculans, Botrytis cinerea, Sclerotinia sclerotiorum, Fusarium spp., Pyrenophora spp., Phaeosphaeria nodorum, Oculimacula spp. and Ramularia colo-cygni.

## Recipients or parental organism

Recipients will be wild type strains of eukaryotic plant pathogens (oomycetes and fungi), some of which will be native to the UK environment, some of which will have been imported and held under Plant Health Licence from FERA/DEFRA. These experiments may generate transgenic eukaryotic plant pathogens with altered host ranges, pathogenicity and/or insensitivity to fungicides.

## Host/vector system

Plasmids used will be recombinant pTOR and its derivatives, including the pTOR derivative pNC2. It is identical to pTOR apart from the MCS which is replaced by a Gateway MCS. Also, pAN7, pAN5 or pCamb, pCAMDsRed, pCAMBgf, and pCamb-CYP51-2. These plasmids encode genes for resistance to kanamycin/geneticin G418 or hygromycin. Recombinant plasmids may carry plant pathogen effectors and/or effector fusions as well as effector hairpin constructs and fungicide insensitivity genes that will be delivered and integrated into eukaryotic plant pathogen genomes.

## Origin & function

Candidate eukaryotic plant pathogen effector molecules and genes that encode insensitivity to fungicides will be identified and cloned from the oomycete pathogens; Phytophthora infestans, Ohytophthora brassicae, Phytophthora capsici, Phytophthora ipomoeae, Phytophthora palmivora, Albugo candida and Albugo laibachii and the ascomyte fungi such as Blumeria spp., Mycosphaerella graminicola, M. brassicicola, Leptosphaeria maculans, Botrytis cinerea Sclerotinia sclerotiorum, Fusarium spp., Pyrenophora spp., Phaeosphaeria nodorum, Oculimacula spp. and Ramularia colo-cygni.

Many of these genes will be transferred back into various recipient strains which may be of the same or different species of plant pathogen. Stably transformed genetically modified eukaryotic plant pathogens will be inoculated onto host plants and tested for pathogenicity and/or insensitivity to fungicides. Host plants include tomato, potato, and Nicotiana spp., sweet potato (Ipomoea batatas), cereals (wheat and barley), Brassicas including Arabidopsis thaliana. Other species of plants may be tested for resistance.

## Evaluation of foreseeable effects

### Human Health;

None of the recipient organisms used in these projects is a human pathogen and does not, therefore constitute a hazard to human health. The proposed genetic modifications of eukaryotic plant pathogens will not cause the modified strains to become human or animal pathogens and are therefore of no risk to human or animal health.

### Environmental Safety;

The eukaryotic plant pathogens; P infestans, P. brassicae, Albugo candida, Albugo laibachii, Blumeria sp., Mycosphaerella graminicola, M. brassicicola, Leptosphaeria maculans, Botrytis cinerea, Sclerotinia sclerotiorum Fusarium spp., Pyrenophora spp., Phaeosphaeria nodorum, Oculimacula spp. and Ramularia colo-cygni are all present.
in the local environment, even if the unmodified recipient strains here used may be imported from outside of the UK and held under licence from DEFRA. Therefore the risk posed by their potential accidental escape is not related to the introduction of a new plant disease, but rather to factors that relate to their genetic modification. The graminicola, M. brassicicola, Leptosphaeria maculans, Botrytis cinerea, Sclerotinia sclerotiorum Fusarium spp., Pyrenophora spp., Phaeosphaeria nodorum, Oculimacula spp and Ramularia collo-cygni will result in GM plant pathogens that could survive in the local environment. Some of the genetic modifications will result in pathogens that are more virulent than the recipient strain, but are nonetheless unlikely to be significantly more virulent than naturally occurring isolates.

The eukaryotic plant pathogens; P. capsici, P ipomoeae and P. palmivora are unlikely to be present in the local environment since they are pathogens of subtropical/tropical plants and are unable to survive in the local environment, due both to the absence of suitable hosts and environmental adversity. P. capsici will be used as a model organism, receiving candidate effector genes from different donor oomycete plant pathogens. The hazard identified is that GM-P. capsici may have an altered host range and be able to infect plants that may grow in the local environment. However, it is extremely unlikely that the genetically modified P. capsici will differ from wild type strains in its ability to survive in the UK environment. Moreover, P. capsici is not likely to be present in the UK and therefore not able to reproduce sexually with the GM strain.

The silencing of candidate plant pathogen effector genes could lead to growth of the GM-pathogen on an otherwise resistant plant host, especially if the silencing resulted in a loss of avirulence determinants. However, potato varieties harbouring genes conferring P. infestans resistance remain susceptible to other diverse naturally occurring virulent races of the pathogen.

It has been demonstrated that the Phytophthora infestans a virulence protein Avr3a is required for full virulence of the pathogen. The silencing of effector genes is not likely to alter the GM-pathogen’s host range, but is likely to have a negative effect on virulence.

Where eukaryotic plant pathogen genomes receive genes that are suspected to be involved in reducing sensitivity to fungicides, the hazard identified is the production of genetically modified plant pathogens that are more able to establish in the UK environment. The choice of fungicides insensitivity genes will be made to ensure that there is no additional risk from fungi transformed with these genes. For example, fungicide insensitivity genes used to transform B. graminis will only be those that have already been shown to be present in the UK population of this fungus. There would therefore be no risk of releasing alien genes into the UK environment.

For genetically modified plant pathogens that could survive in the UK environment, it is envisaged that none of the modifications will lead to races off pathogen that are more virulent than naturally occurring races; the recipient strains are not the most virulent and the most virulent naturally occurring strains will not be modified, except to receive GM marker genes. Moreover since the primary objective of these investigations is to monitor the virulence of eukaryotic plant pathogens, any pathogens where the genetic modifications lead to a significant increase in virulence will be killed by autoclaving.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For plant material infected with GMMs, containment level 2 is appropriate. Infected plants will be maintained in containment level 2 growth facilities at the John Innes Centre. Standard operating procedures for the use of plant growth facilities and for the disposal of waste will be strictly adhered to.

Plant pathogens, infected plant material and compost will be killed by autoclaving prior to disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

A derogation is requested for the use of an autoclave in a building other than the building where the work is conducted. The transport distance between the building where the work is conducted and the building housing the autoclave is short, both buildings are located on the same site. The waste materials would be transported from the building where the work is conducted to the autoclave in bags inside sealed plastic boxes, the boxes have locking lids to prevent the accidental release of waste materials during transportation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Class 2 GMM (plant pathogen) (containment level 2). All GMM material will be handled using good microbiological practice (GMP). All GMM material will be stored in sealed containers and disposed of by autoclaving.
Oomycete and fungal cultures and culture plates will be autoclaved prior to disposal. The compost banks are inspected twice yearly and sprayed with a general purpose herbicide.

Routine monitoring of autoclave efficiency is completed using Thermalog S strips.

Run-off from containment glasshouses is collected into a 5000L tank. 1500 L aliquots are removed and treated with sodium hypochlorite and circulated in the tank for 24 hour, followed by a treatment with sodium thiosulphate. The water is then filtered and disposed. Plants in growth rooms will be sub-irrigated in leak proof trays such that minimal run-off is generated.

Autoclave procedures are as follows; Soil and plant waste generated in glasshouses and growth rooms is autoclaved in stainless steel containers for 1.5 hours (15 minutes vent, 30 minutes sterilisation at 15 p.s.i). Resultant waste is transported to a farm site where it is left to stand for three years prior to disposal.

Members of the Biological Safety Committee reviewed the risk assessments along with the HSE Notification form and were generally very satisfied with the standard and contents of the documents. These were considered to be well written, covering the necessary points.

It was suggested that the original HSE Notification title be changed from "The expression of fluorescent marker...” to "The expression of GM marker genes...”, to cover GUS, hph, nptII etc., used in experiments, and this suggestion was accepted.

In Section C of the GMRAs (Provisional Assessment), in both cases it was stated that the experiments are "excluded from Class 1" but did not actually propose which class they should be in. Although it was stated later that Class 2 is appropriate, it was suggested this should be stated in the provisional assessment as well. The GMRAs were amended accordingly.

Please enter comments on the GM safety committee on the risk assessment

Members of the Biological Safety Committee reviewed the risk assessments along with the HSE Notification form and were generally very satisfied with the standard and contents of the documents. These were considered to be well written, covering the necessary points.

It was suggested that the original HSE Notification title be changed from "The expression of fluorescent marker...” to "The expression of GM marker genes...”, to cover GUS, hph, nptII etc., used in experiments, and this suggestion was accepted.

In Section C of the GMRAs (Provisional Assessment), in both cases it was stated that the experiments are "excluded from Class 1" but did not actually propose which class they should be in. Although it was stated later that Class 2 is appropriate, it was suggested this should be stated in the provisional assessment as well. The GMRAs were amended accordingly.

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The aim of our work is to dissect the resistance mechanisms in monocotyledonous plants such as wheat, barley, Brachypodium and Aegilops spp. that operate against, among others, the fungal plant pathogens Puccinia striiformis and P. graminis. This will be done by expressing candidate plant pathogen effector genes in B. glumae.

The characterisation of capsular polysaccharide prepared from genetically modified B. thailandensis will be used to further research the production of a vaccine for B. pseudomallei.

Exopolysaccharides purified from genetically modified P. aeruginosa will be used to probe the PAMP (pathogen associated molecular patterns)-triggered immune response in Nicotiana benthamiana and Arabidopsis thaliana.

Deletion mutants of P. aeruginosa will be constructed by allelic exchange and examined for attenuated/enhanced PAMP triggered immune responses in N. benthamiana and A. thaliana.

The recipient strain of B. glumae, the bacterial panicle blight pathogen of rice, was isolated from a rice paddy field from outside the UK and, as a non-native plant pathogen, will be held under Plant Health Licence from FERA/DEFRA. The genetic modification of B. glumae will allow it to operate as an effector delivery system capable of delivering filamentous phytopathogen effectors into monocotyledonous plants under controlled laboratory conditions.

B. thailandensis, strain E555 occurs naturally in the soils as a soil saprophyte and was isolated from the soil in Cambodia. It is an opportunistic human pathogen affecting only immuno-compromised individuals. The characteristics of the genetically modified strain will likely render it between one tenth and one hundreth of the virulence of wild type and it is essentially avirulent in mammals.
P. aeruginosa PAO1 used in this study was isolated from an infected human patient. PAO1 is a commonly used strain for research purposes and is an opportunistic human pathogen affecting immuno-compromised individuals. Any mutants used in this study will likely have a reduced fitness.

P. aeruginosa strains have been reported as an opportunistic pathogen in Arabidopsis and lettuce, however, as in humans this would be as a secondary opportunistic infection.

**Host/vector system**

Plasmids used to genetically modify B. glumae will be largely derived form the broad-host range cloning vector pBGR1MCS. Recombinant plasmids will harbour genes encoding for putative plant phytopathogen effectors. The genetically modified B. thailandensis carries the pKock plasmid inserted in to the wbl gene, abrogating LPS 0-antigen synthesis and conferring kanamycin resistance.

**Origin & function**

Candidate filamentous phytopathogen effector molecules will be identified and cloned from, in the first instance, Puccinia striiformis and P. graminis. These genes will then be fused to the N terminal type III secretion signal of a Pseudomomas type III secreted protein, introduced into Burkholderia glumae and used in plant infections assays.

Burkholderia thailandensis strain E555 wbl (knock-KmR) is a kanamycin resistant derivative of E555, isolated from the soil in Cambodia. Characterisation of capsular polysaccharide produced by this strain of bacteria will be used to advance research in to the production of a carbohydrate vaccine against Burkholderia pseudomallei.

Pseudomonas aeruginosa strain PAO1 is a domesticated laboratory strain originally isolated from the wound of a patient in Australia. Lipo/exopolysaccharides produced from mutant strains of this bacterium will be used to investigate PAMP-triggered immune responses in Nicotiana benthamiana and Arabidopsis thaliana.

**Evaluation of foreseeable effects**

**Human Health:**

The bacterial genus Burkholderia comprises in excess of 40 species most of which are typically non-pathogenic for humans. B. glumae is a plant pathogen of rice (Oryza sativa) and considered to be an organism with extremely low human virulence. Only one case of human infection has been reported in an infant with chronic granulomatous disease (Weinberg et al., 2006). As this case was associated with a pre-existing complication this suggests that immune-competent humans are not at risk of infection.

B. glumae strain 106619 was isolated from wild paddy field rice (Oryza sativa). The genetic modification(s) of this strain, here described will likely have no effect on its virulence in humans.

B. thailandensis is a soil-saprophyte and is considered to be an organism with extremely low virulence. Only four human infections with B. thailandensis have been recorded, two in Southeast Asia and two in the United States, suggesting that it is much less virulent than B. pseudomallei (Dharakul et al. 1999; Lertpatanasuwan et al. 1999). Indeed, these cases have been in immuno-compromised individuals. The characteristics of the genetically modified strain will likely render it between one tenth and one hundredth of the virulence of wild type and it is essentially avirulent in mammals.

P. aeruginosa rarely causes disease in healthy humans; however it is an opportunistic nosocomial pathogen of immuno-compromised individuals. Diseases caused by P. aeruginosa include skin and soft tissue infections in burns victims and patients with wound infections, outer ear and urinary tract infections, colonisation of medical devices (e.g. catheters), and diffuse bronchopneumonia in cystic fibrosis patients (Lowbury, 1975).

Strain PAO1 is a domesticated laboratory strain originally isolated from the wound of a patient in Australia (Holloway, 1955). It is perhaps the most widely studied laboratory strain of P. aeruginosa at present. PAO1 is known to be less virulent than PA14, the other extensively studied laboratory strain (Mikkelsen et al., 2011).

The genetically modified strains of P. aeruginosa here described will likely be unaffected with respect to their virulence in humans.
Environmental Safety:

Bacterial panicle blight of rice, which is caused by B. glumae, has been reported in Central and South America and Asia. B. glumae strain 106619 will be imported from outside of the UK and held under licence from DEFRA.

B. glumae is unlikely to survive in the local environment, due both to the absence of suitable hosts and environmental adversity.

B. glumae will be used as a system for the delivery of filamentous phytopathogen effectors to monototyledonous plants. It is not envisaged that there would be any expansion in B. glumae host range outside controlled laboratory conditions. The recipient strains are not the most virulent and the most virulent naturally occurring strains will not be modified. This strain will be used in pathogenicity assays and any alterations in pathogenicity will be closely monitored.

The genetically modified P. aeruginosa strains used to infect plants will not be more virulent than the wild type strain and as they are opportunistic plant pathogens they do not present a hazard to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For plant material infected with GMMs. Containment level 2 is appropriate. Infected plants will be maintained in containment level 2 growth facilities at the John Innes Centre. Standard operating procedures for the use of plant growth facilities and for the disposal of waste will be strictly adhered to.

Genetically modified micro-organisms, infected plant material and compost will be inactivated by autoclaving prior to disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMM material will be handled using good microbiological practice (GMP). All GMM material will be inactivated by autoclaving prior to disposal.

All residual plant material will be stored in sealed containers and disposed of by autoclaving.

Autoclave procedures are as follows; Soil and plant waste generated in glasshouses and growth rooms is sterilised by autoclaving at 121 degrees C for a minimum of 30 minutes at 15p.s.i.

Routine monitoring of autoclave efficiency is completed using Thermalog S strips. Annual 12 point validation of autoclaves is performed.

Run-off from containment glasshouses is collected into a 5000L tank. 15000L aliquots are removed and treated with sodium hypochlorite and circulated in the tank for 24 hours, followed by a treatment with sodium thiosulphate. The water is then filtered and disposed. Plants in growth rooms will be sub-irrigated in leak proof trays such that minimal run-off is generated.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]
Two risk assessments attached (GM RA 175 and GM RA 176):

Caps/LPS gene-deletion mutants. (Reference GM RA 175) and Genetic modification of Burkholderia glumae (Reference GM RA 176).

The risk assessments were reviewed by members of the Biological Safety Committee.

For GM RA 175, the following observations were made during the review process:

It looks informative, clear and accurate in its risk assessment. It would be good to define IN (by the IN route, page 3) for clarity, or write in full. The GM RA was amended accordingly.

On considering the potential hazards to the environment for the HSE notification, I noticed that in Part B; Project Description (i) Overview, the modified P. aeruginosa will be tested in plants for PAMP-triggered immune responses. I understand that P. aeruginosa/plant infection(s) can be used as a model to understand animal infections. However, since P. aeruginosa can be pathogenic on Arabidopsis in nature. I think that there should be a statement in (ii) Hazard identification in respect of human health and environmental safety that relates to the effect of the genetic modification(s) on the virulence in plants. One sentence should suffice to demonstrate that the risk has been assessed-something along the lines; The modified P. aeruginosa used to infect plants will not be significantly more virulent than the wild type strain and does not present a hazard to the environment. The GM RA was amended.

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Project Ref 38/15.1

Date Ackn'd 05/11/2015

CU2 Project Title Examining the contribution of various Pseudomonas syringae and Streptomyces loci to infection and colonisation in model plant systems

Class 2

Consent Granted
The work will include the development of genetic tools including vectors and gene replacement systems and the subsequent expression, deletion and modification of bacterial genes and pathways in P. syringae and plant pathogenic Streptomyces strains, plant infection assays in model systems including Arabidopsis thaliana, tomato and potato, comparative genomics and other whole-cell techniques, biochemical analysis of purified proteins, and the study of signalling pathways, secondary metabolism, and bacterial behaviour in different plant-associated environments (including plants and soil).

P. syringae is an ubiquitous and widespread phytopathogen that is responsible for a range of important plant diseases including tomato speck, bleeding canker and halo blight. It is an aggressive plant pathogen that uses a combination of secreted phytotoxins and effectors to subvert plant defences and kill host cells. There are over 50 known pathovars of P. syringae, which specifically infect a wide range of different plant species depending on their particular encoded effector proteins. The most commonly employed pathovar in this project will be Pto DC3000, which infects A. thaliana and tomato, and as such has become a model organism for plant-pathogen interaction studies.

The genetically modified P. syringae strains used in this work will be no more virulent (and are highly likely to be less virulent) than the wild type strain. Furthermore, as these variants will be compromised for important signalling and phenotypic output operons they are unlikely to thrive outside of a protective and defined laboratory environment. As such, it is unlikely that they will present a hazard to the wider environment.

Streptomyces plant pathogens are prevalent all over the world and infect tuber and root crops. The most well characterised is S. scabies, which is one of the causative agents of potato scab, where it forms lesions on the potato surface, which decreases the quality of the crop but is not destructive. Other closely related Streptomyces strains include S. reticuliscabiei, S. turgidiscabies, S. acidiscabies and S. europaeiscabiei. These all form similar lesions on tubers and roots. The most commonly used strains for this project will be S. scabies DSM 41658, which is the type strain, and S. scabies 87-22, which is the best characterised S. scabies strain and has become the model organism for the study of potato scab.

Mutations will be made to Streptomyces strains to inactivate biosynthetic pathways that make natural products. It is therefore highly likely that the strains will be less virulent than the wild type strains, as natural products have been shown to be important determinants of S. scabies pathogenicity.
Various plasmid-based systems will be used to delete or complement P. syringae genes. These will include the broad host-range vectors pME6032, pBBR-MCS2-5, pME3087, pUC18-miniTn7-Gm, and derivatives of these. These plasmids will confer resistance to gentamycin, kanamycin, tetracycline and other antibiotics in common laboratory use. Genes in Streptomyces strains will be mutated or complemented using standard vectors and methodology developed for the genetic modification of Streptomyces strains. These will include pYH7, pKC1132, pSET152, p1J10257, pIB139 and derivatives of these, which confer resistance to apramycin, hygromycin or kanamycin. These will be introduced into Streptomyces strains by intergeneric conjugation from E. coli ET12567 harbouring a plasmid required for the conjugalltransfer of DNA, such as pUZ8002.

**Origin & function**

P. syringae are a widespread species group of phytopathogenic, Gram negative bacteria. Pathogenic Streptomyces strains are a closely related widespread group of Gram-positive bacteria. They have been isolated from around the world, and both the DSM 41658 and 87-22 strains were originally isolated in the USA. All genes manipulated in these experiments will either be derived from the species under observation, or will be wellcharacterised genes that will not affect the pathogenicity or virulence of the species under investigation (e.g. GFP, antibiotic cassettes used in cloning).

**Evaluation of foreseeable effects**

**Environmental Safety:**
Pseudomonas syringae is already ubiquitous in the environment, including in the soil and on plant surfaces. The genetically modified P. syringae strains used in this work will be no more virulent (and are highly likely to be less virulent) than the wild type strain. Furthermore, as these variants will be compromised for important signalling and phenotypic output operons they are unlikely to thrive outside of a protective and defined laboratory environment. As such, it is unlikely that they will present a hazard to the wider environment.

Streptomyces are one of the most common bacterial genera found in the soil, and pathogenic variants are prevalent in all fields that contain root or tuber crops. They mainly affect the appearance of potatoes rather than the yield and the genetic modifications made in this work will not increase the virulence of these strains. It is therefore unlikely that they would present a hazard to the wider environment.

**Human Health:**
Neither organism is able to infect humans, except under very exceptional circumstances where susceptible individuals would be severely immuno-suppressed. Such individuals would not be present in the workplace. There is consequently no appreciable risk to human health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For plant material infected with GMMs, containment level 2 is appropriate. Infected plants will be maintained in containment level 2 growth facilities at the John Innes Centre. Standard operating procedures for the use of plant growth facilities and for the disposal of waste will be strictly adhered to.

Genetically modified micro-organisms, infected plant material and compost will be inactivated by autoclaving prior to disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMM material will be handled using good microbiological practice (GMP). All GMM material will be inactivated by
autoclaving prior to disposal. All residual plant material will be stored in sealed containers and disposed of by autoclaving. Autoclave procedures are as follows; Soil and plant waste generated in glasshouses and growth rooms is sterilised by autoclaving at 121 degreesC for a minimum of 30 minutes at 15p.s.i. Routine monitoring of autoclave efficiency is completed using Thermalog S strips. Annual 12 point validation of autoclaves is performed. Run off water from watering plants in the containment glasshouse is collected and treated using a thermal treatment system at 1210C, 15 psi for 15 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 38/18.1

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The work will include the development of genetic tools (vectors and gene replacement systems) for the deletion and modification of bacterial genes and pathways in *S. aureus*, for microscopy experiments, and for biochemical analysis of purified proteins, with an overall aim of understanding the mechanism of chromosome segregation and cell division in *S. aureus*.

**Recipient or parental organism**

*S. aureus* is the most common pathogenic staphylococcus, which is often part of the normal human microflora and linked to opportunistic infections. It is a pathogen of man and other mammals. Around one third of healthy individuals carry this bacteria in their noses, pharynx and on their skin. In normal healthy and immunocompetent person, *S. aureus* colonization of the skin, intestinal tract, or nasopharynx does not lead to any symptoms or disease. However, in immunocompromised people, *S. aureus* may cause: minor skin infections, such as pimples, impetigo etc.; boils (furuncles), cellulitis folliculitis, carbuncles; scalded skin syndrome and abscesses. Moreover, it may lead to lung infections or pneumonia; brain infections or meningitis; bone infections or osteomyelitis; heart infections or endocarditis; generalized life threatening blood infections or Toxic shock syndrome (TSS), bacteremia and septicaemia.

The genetically modified *S. aures* strains used in this work will be no more virulent (and are highly likely to be less virulent) than the wild type strain. Furthermore, as these mutants will be compromised for important mechanisms necessary for cell division and chromosome segregation they are unlikely to thrive outside of a protective and defined laboratory environment. As such, it is unlikely that they will present a hazard to the wider environment.

**Host/vector system**

Various plasmid-based systems will be used to delete or complement *S. aureus* genes. These will include the broad host-range vectors such as pIMAY, pRAB11, and derivatives of these. These plasmids will confer resistance to cloramphenicol, ampicillin and other antibiotics in common laboratory use. Plasmids will be constructed in JM110 *E. coli* strain and introduced to *S. aureus* strains by electroporation or conjugation. The *S. aureus* bacteriophage 11 will be used for DNA transduction as well.

**Origin & function**

*S. aureus* is a Gram-positive spherical bacteria, member of the Firmicutes of important clinical and biotechnological relevance. It is frequently found in the nose, respiratory tract and on the skin. *Staphylococcus* was first identified in 1880 in Aberdeen, Scotland, by surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint. It has been estimated that 20% to 30% of the human population are long-term carriers of *S. aureus*. Pathogenic *S. aureus* strains have been isolated from around the world.

*S. aureus* RN6390 and RN4220 strains are laboratory strains derivatives of NCTC8325-4 (RN450), a modified *S. aureus* NCTC8325 strain originally isolated in 1960 from a sepsis patient and primarily utilized in the laboratory for the study of *S. aureus* genetics. While RN6390 was generated via sequential phage transduction (Peng et al., 1988), RN4220 was mutagenized via nitroguanoside to enrich for mutants that would efficiently accept plasmid DNA from *E. coli* (Kreiswirth et al., 1983; Herbert et al., 2010).

All genes manipulated in these experiments will either be derived from the species under observation, or will be well-characterised genes that will not affect the
pathogenicity or virulence of the species under investigation e.g. GFP, antibiotic cassettes used in cloning, or genes that encode proteins that are important for chromosome segregation such as parB, smc, scpA and scpB. Other related genes may be used, these would present no greater risk to the environment, humans or animals.

Evaluation of foreseeable effects

Environmental Safety:
Staphylococcus aureus is already ubiquitous in the environment, it is part of the human microbiota and it colonises about the 30% of human population. The genetically modified S. aureus strains used in this work will be no more virulent (and are highly likely to be less virulent) than the wild type strain. Furthermore, as these mutants will be compromised for important phenotypic output operons they are unlikely to thrive outside of a protective and defined laboratory environment. As such, it is unlikely that they will present a hazard to the wider environment.

Human Health:
S. aureus is a human pathogen, however it doesn't lead any symptoms or diseases when it colonizes healthy and immuno-competent people. Only under very exceptional circumstances, where susceptible individuals would be severely immuno-suppressed it could represent a risk. Such individuals would not be present in the workplace. There is consequently no appreciable risk to human health in the workplace.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Genetically-modified S. aureus will be maintained in containment level 2 growth facilities at the John Innes Centre. Standard operating procedures for the use of the growth facilities and for the disposal of waste will be strictly adhered to.

Genetically modified micro-organisms will be inactivated by autoclaving prior to disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All GMM material will be handled using good microbiological practice (GMP). All GMM material will be inactivated by autoclaving prior to disposal.
All residual microbiological material will be stored in sealed containers and disposed of by autoclaving
Autoclave procedures are as follows: Microbiological solid waste generated in growth rooms is sterilised by autoclaving at 121 degrees C for a minimum of 30 minutes at 15p.s.i.
Routine monitoring of autoclave efficiency is completed using Thermalog S strips. Annual 12 point validation of autoclaves is performed.

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
The Genetic Modification Safety Committee requested that a list of genes the investigator intends to delete or express in the target strain is included in the GMRA and the HSE CU2 form.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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**Animal Units**

- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

---

**Project Ref** 38/21.1

- **Date Ackn'd**: 02/09/2021
- **CU2 Project Title**: Genetic modification of Enterococcus faecalis and other related hazard group 2 Enterococcus species using plasmids encoding antibiotic resistance and fluorescent markers
- **Class**: Class 2
- **Culture Volume Class 2**: ≤ 1 Litre

- **Non-GMM**: Consent Granted

- **Historical Significant Changes**: Withdrawn

**Project Additional Information**

**Purposes of the contained use**

The study will visualise how GM Enterococcus faecalis, and other related hazard group 2 Enterococcus species, colonise the larvae of the moth Galleria mellonella. This will be done through feeding and injection studies. The plasmid enables easier visualisation and differentiation between the E. faecalis colonising the larvae and the native bacteria of the larvae, which are also enterococci.

**Recipient or parental organism**

02/03/2022
Larvae of the moth Galleria mellonella will be colonised by GM Enterococcus carrying antibiotic resistance and fluorescent marker genes. Visualisation studies will be performed to study colonisation and to allow differentiation between the native enterococci and the GM E. faecalis and other related species. There are no harmful effects foreseen.

Host/vector system

E. faecalis is classified as being a Hazard Group 2 organism. It is a commensal bacterium inhabiting the gastrointestinal tract of healthy humans. They can be used as a probiotic. They are opportunistic human pathogens that would not present a problem to healthy individuals but could cause infection in immuno-compromised people. Treatment of infections caused by these organisms is well documented and effective antibiotic treatments are known.

The plasmid carried by E. faecalis confers antibiotic resistance and fluorescent markers for visualisation studies. The plasmid contains a spectinomycin resistance cassette; a commonly used antibiotic marker in research laboratories. The plasmid contains no other antibiotic resistance genes and is not considered to pose an additional hazard.

Origin & function

The GM Enterococcus faecalis will colonise Galleria mellonella larvae and the fluorescence marker carried on the plasmid will enable visualisation and differentiation between the E. faecalis colonising the larvae and the native bacteria of the larvae, which are also enterococci.

There is a theoretical possibility that the plasmid could be passed to the native bacteria in Galleria, these are generally E. gallinarum or E. casseliflavus which also belong to hazard group 2. They are less pathogenic than E. faecalis so this would not pose an additional hazard. Effective containment measure will prevent risk of un-authorised escape to the environment.

Infected Galleria larvae will maintained in a dedicated incubator in the lab, in Petri dishes closed with tape, and these will only be opened within biological safety cabinets. Galleria larvae will be destroyed by 24 hour freeze treatment, followed by autoclaving.

Evaluation of foreseeable effects

The GM Enterococcus faecalis will colonise Galleria mellonella larvae and the fluorescence marker carried on the plasmid will enable visualisation and differentiation between the E. faecalis colonising the larvae and the native bacteria of the larvae, which are also enterococci.

There is a theoretical possibility that the plasmid could be passed to the native bacteria in Galleria, these are generally E. gallinarum or E. casseliflavus which also belong to hazard group 2. They are less pathogenic than E. faecalis so this would not pose an additional hazard. Effective containment measure will prevent risk of un-authorised escape to the environment.

Infected Galleria larvae will maintained in a dedicated incubator in the lab, in Petri dishes closed with tape, and these will only be opened within biological safety cabinets. Galleria larvae will be destroyed by 24 hour freeze treatment, followed by autoclaving.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All routine studies involving the GM E. faecalis and the contained larvae of Galleria mellonella will be conducted within a Containment Level 2 laboratory.

Occasionally longitudinal multigeneration studies may be performed to see if the Galleria mellonella moths can transfer the bacterium E. faecalis to their offspring. These will be conducted in the designated Entomology Dept with appropriate containment facilities as required by the Genetically Modified Organisms (Contained Use) Regulations. Moths will also be destroyed by 24 hour freeze treatment, followed by autoclaving.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Infected Galleria larvae and moths will be treated by 24 hour freeze treatment, followed by autoclaving.

All solid biological waste will be sterilised by autoclaving at 121°C for 20 minutes, or 30 minutes for dense loads.

Liquid biological waste will be sterilised by autoclaving at 121°C for 20 minutes.

Routine monitoring of autoclave efficiency is completed using Thermalog strips and annual 12 point validation of autoclaves is performed.
Project Containment

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Animal Units

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Project Ref 38/95.1

Date Ackn'd 12/06/1995

CU2 Project Title

CLONING AND MANIPULATION OF SEPTORIA GENES IN BACTERIA AND FUNGI

Class 2

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref**  38/98.1

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- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref  38/98.10

Date Ackn'd  09/02/1998  CU2 Project Title  MOLECULAR GENETICS ANALYSIS OF PATHOGENICITY OF GAEMANNOMYCES  Class  Class 2  CultureVolClass2  Class CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 38/98.11

Date Ackn'd 09/02/1998

Date Project Ceased

CU2 Project Title MOLECULAR GENETICS OF PATHOGENICITY OF XANTHOMONAS

Class 2

CultureVolClass2

CultureVolumeClass3-4

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

#### Is an emergency plan required according to regulation 20? [N]

- If yes, tick to confirm that it is attached to this form [N]
- Tick to confirm that you have attached a risk assessment to this form [N]
- Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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#### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 38/98.3

Date Ackn'd 09/02/1998  CU2 Project Title AGROINOCULATION OF NON-INDIGINOUS DNA VIRUSES THAT HAVE NO

Class CultureVolClass2 CultureVolumeClass3-4

Class 2
FORESEEN POTENTIAL FOR RECOMBINATION WITH INDIGENOUS VIRUSES

---

Non-GMM

Consent Granted

not applicable

---

Project notified under transitional arrangements  

---

Withdrawn  

Tick if notifying a connected programme of work  

---

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

---

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  
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Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Animal Units**

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Tick if notifying a connected programme of work  N

**Historical Significant Changes**

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02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 38/98.4a

Date Ackn'd 09/02/1998

Date Project Ceased 24/02/2004

CU2 Project Title ORGANISATION AND EXPRESSION OF THE GENOMES OF PLANT POTYVIRUSES

Class 2

Consent Granted not applicable

Non-GMM

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes GM38/03.1

Historical Date of Additional Info 06/11/2003

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 38/98.4b

Date Ackn'd 09/02/1998
CU2 Project Title ORGANISATION AND EXPRESSION OF THE GENOMES OF PLANT
Class 2
CultureVolClass2
CultureVolumeClass3-4

Page 1816 of 1532
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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</table>

Project Ref 38/98.5

Date Ackn’d 09/02/1998

CU2 Project Title
GENETIC MANIPULATION OF PEA EARLY BROWNING VIRUS

Class 2

Consent Granted not applicable

Tick if notifying a connected programme of work N

Withdrawn N

Non-GMM

Project notified under transitional arrangements Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022
<table>
<thead>
<tr>
<th>Date Ackn'ed</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<tr>
<td>09/02/1998</td>
<td>GENETIC MANIPULATION OF CAULIFLOWER MOSAIC VIRUS AND ITS USE AS A GENOME</td>
<td>Class 2</td>
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**Withdrawn** N

Tick if notifying a connected programme of work N

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form N
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
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Project Ref 38/98.7

Date Ackn'd 09/02/1998
CU2 Project Title EXPRESSION OF THE CLADOSPORIUM FULVUM AVR4 AND AVR9 AVIRULENCE
Class 2
Class CultureVolClass2 CultureVolumeClass3-4
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 38/98.8

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<tr>
<td>09/02/1998</td>
<td>IDENTIFICATION OF TOMATO- CLADOSPORIUM FULVUM INTERACTION USING RECOMBINANT C FULVUM RACES</td>
<td>Class 2</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 38/99.1

Date Ackn’d 28/02/2000

Date Project Ceased

CU2 Project Title
AGROBACTERIUM INFILTRATION OF LEAF PANELS FOR TRANSIENT
EXPRESSION OF FOREIGN GENES & PROMOTERS IN PLANTS. ISOLATION OF
PLANT GENES INVOLVED IN GENE SILENCING

Class 2

Non-GMM Consent Granted
not applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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02/03/2022  Page 1826 of 15326
GM Centre Number: 40

Data Premises Notified (Originally) 18/12/1979

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

UNIVERSITY OF DURHAM

Name 2

Department

SCHOOL OF BIOLOGICAL AND BIOMEDICAL SCIENCES

Building

SCIENCE LABORATORIES

District

Road Name

SOUTH ROAD

Campus Estate or Research Centre

Town

DURHAM

County

DURHAM

Postcode

DH1 3LE

Country

ENGLAND

Tel Number 0191 334 2667

Fax Number 0191 334 2661

E-mail

HSE Division YORKSHIRE AND NORTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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<th>Date Premises Closed</th>
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<th>Department</th>
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<th>Building</th>
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<td>UNIVERSITY OF DURHAM</td>
<td>SCHOOL OF BIOLOGICAL AND BIOMEDICAL SCIENCES</td>
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<td></td>
<td>UNIVERSITY OF DURHAM</td>
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<td></td>
<td>QUEENS CAMPUS</td>
<td>WOLFSON RESEARCH INSTITUTE</td>
<td></td>
<td>STOCKTON-ON-TEES</td>
<td>CLEVELAND</td>
<td></td>
<td>TS17 6BH</td>
<td>ENGLAND</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<tr>
<td>Level 4 (GMMs)</td>
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</tbody>
</table>
All activities so far assessed and carried out at GM40 are class 1: Activities of no or negligible risk, for which containment level 1 is appropriate to protect human health and the environment. The scale of work carried out at present is small with GMM cultures of up to a maximum of 10L; GMO work with animals is on a much smaller scale and is limited to cell culture; GMO work with plant materials is on a slightly larger scale with several projects producing transgenic lines of up to a hundred plants grown in containment growth rooms or glasshouse facilities. Waste management for GMM and GMO cell culture materials is similar - microbial and cell cultures are pretreated with an effective disinfectant (usually a hypochlorite-type) prior to autoclaving and disposal as normal biological waste. Waste GM plant materials including all tissue culture media and compost are autoclaved on an extended cycle which has been checked to achieve and maintain the temperature/pressure necessary for deactivation of any microorganisms/plant pollen or seeds. Autoclaves used for waste processing are tested annually for maintenance of temperature and pressure cycles. Routine checks for waste deactivation include 'load-activated' temperature sensors which control the autoclaves as well as indicator tape or tabs (temptabs etc.).

For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
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<th>Non-microbial</th>
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<td>Bacteriology</td>
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<td>Invertebrates</td>
<td>Plants</td>
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<td>Other (please specify below)</td>
<td>Other (please specify below)</td>
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</table>

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 40/05.1
Structure/function analysis of Mononegavirales proteins with relation to their role in viral replication, assembly, virion morphology and host-pathogen interactions.

---

**Project Additional Information**

**Purposes of the contained use**

Academic research and training

**Recipient or parental organism**

Standard eukaryotic cell-lines (human and primate) such as A549s, HeLa, and 293Ts and CV-1 for the propagation of recombinant viruses.

The genes for insertion are derived from Human Respiratory Syncytial virus or Measles virus grown in standard mammalian cell-lines.

The recipient viruses are vaccinia, baculovirus, and lentivirus (HIV-1 derived) and mouse (MMLV) retroviruses.

**Host/vector system**

Vaccinia will use plasmids pTM-1, pTM-3 or similar for initial cloning systems, propagated in E.coli before use in targeted recombinant transference in mammalian cell-lines. Recombinant Respiratory syncytial virus generated using reverse genetics, the viruses will be generated and propagated in mammalian cells such as CV-1, A549s and other mammalian lines. The system was a gift from Hong Jin, Aviron Inc (USA)

Baculoviruses created in E.coli using the Bac-to Bac system (Invitrogen) using transfer vector based on pFastbac-1

A third generation Lentivirus system using plasmids pWPI and pMDL g/p RRE from Trono's lab in Geneva

A MMLV based system will use the shuttle vectors pNCLX and pNCLX2 or other related plasmids.

**Origin & function**

Vaccinia virus, a poxivirus, is used routinely to express or drive the expression of recombinant proteins in mammalian cells. The viruses, created using homologous recombination, will either contain the gene for the protein of for a polymerase, such as T7 RNA polymerase. The polymerase then drives the expression froma transfected plasmid containing the gene of interest downstream of a T7 polymerase promotor. The virus was the basis for the smallpox vaccine but infection can be problematical. Adverse effects were noticed in 1/50,000 vaccinations. Most of the viruses in the lab are based on the Copenhagen strain however the use of more attenuated strains.
(MVA) will be employed were possible. The Lentivirus system is a third generation expression system that has been developed to remove the possibility of recombination generating viable HIV. The virus particles produced from this system will not contain a viable genome as the L3 domain has been mutated prevent formation of the LTR at the 5' domain. The system will be used to express proteins in non-dividing cells, for example human primary cells.
The MMLV-derived retroviral system will be used to routinely express proteins in tissue culture.
Baculoviruses will be used for the large scale production of recombinant proteins, suitable for structural studies e.g. crystallography.
Recombinant RSV will be generated by cloning genes of interest into a plasmid containing the full length RSV genome. The recombinant plasmids will be used to transfect mammalian cells together with helper plasmids and vaccinia supplying T7 polymerase. The resultant viruses will be used in experimental procedures to determine the effect of mutating or deleting structural proteins that are important in replication and/or assembly.

Evaluation of foreseeable effects

The MMLV and Baculovirus systems have been used in many labs for a number of years and have demonstrated excellent biosafety. Baculovirus can infect mammalian cells but fail to replicate, if a mammalian promotor is present a transgene can be expressed, however there is practically no chance of propagation and spread of the vector. Each construct will therefore be assessed individually on the impact of the expression of the recombinant protein on a cell.

The Lentivirus system, whilst based on HIV-1 (HSE CAT 3) has been designed to maximise its biosafety, particularly with regard to the likelihood of producing infectious HIV-1 particles. The proposed system is a third generation series developed by the Trono lab (http://www.tronolab.unique.ch/x_hime2.htm) and the inherent design reduces the likelihood of this to near zero. The system comprises three main components, a transfer vector which the transgene is inserted. This is the component that will eventually be packaged into the virion and contains viral sequences such as the packaging signal for viral assembly and the LTRs need for integration of the proviral DNA. The 3' LTR has been modified such that the ability of the provirus to reconstitute active viral promoters is destroyed. Expression of the transgene is driven by an upstream mammalian promotor. The structural and enzymatic components of the viral particle are provided by a second plasmid containing the viral gag-pol genes. This plasmid cannot be packaged into the virion thus the virus does not contain the necessary resources to replicate upon subsequent infection of a cell. Finally the viral glycoprotein, gp169 has been replaced by vesicular stomatitis virus (VSV) G protein, provided in trans from a third plasmid which again cannot be packaged into nascent virions. Whilst there is a minimal chance of infecting a lab worker the ability to replicate, or recombine with endogenous retroviruses has been reduced to insignificant levels.

Vaccinia virus (HSE CAT 2), a poxvirus, is used routinely to express or drive the expression of recombinant proteins in mammalian cells. Recombinant viruses, created using homologous recombination, will either contain the gene for the protein or for a polymerase, such as T7 RNA polymerase. The polymerase then drives expression from a transfected plasmid containing the gene of interest downstream of a T7 polymerase promotor. The virus was the basis for the smallpox vaccine but infection can be problematical. Adverse effects were noticed in 1/50,000 vaccinations. Infections by vaccinia is still a possibility. Possible routes of infection for workers are ingestion, droplet spread and exposure to aerosols. The virus may cause a particularly severe skin disease in those suffering from eczema or other skin conditions. Most of the viruses in the lab are based on the Copenhagen strain however the use of more attenuated strains (MVA) will be employed when possible.

The risk of changing the glycoproteins of RSV using the rescue system may result in a change of cell tropism. However no work will be undertaken were both viral glycoproteins are changed, thus in the case of accidental exposure the worker is likely to be able to mount an effective immune response preventing any disease. As RSV is endemic to the general population no foreseeable risks are envisaged.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Individual schemes for dealing with the waste disposal from each expression system is described in the attached risk assessments. Also code of practice for the labs has been drawn up detailing the waste streams available in the CAT 2 labs in the Wolfson. Basically, all work is carried out in class II MSC's that are decontaminated before
and after use with either 70% ethanol or 1% Virkon. Before servicing the hood, or if there is a major spill, the hood can be sterilised by fumigation with Formaldehyde. Liquid waste will be treated with Virkon overnight before disposal as normal waste. Solid waste will be neutralised by autoclaving before disposal via the normal waste stream and removed by council/contractors to approved waste sites. Plasticware will be soaked overnight in 1% Chloros before disposal in the normal waste stream. In the advent of a large, unattended spill, solid Virkon will be added directly to the liquid and mopped up using absorbent tissue. The contaminated tissue will be autoclaved before disposal to ensure complete neutralisation of any biological material. At all times personnel will wear suitable safety gear, lab coats, gloves, eye protection etc. The autoclave is tested regularly to ensure it is functioning efficiently.

The above schemes represent procedures similar to that used in many labs. The efficiency of the above has been tested in the author's previous place of employment, the Medical Research Council's Virology Unit in Glasgow. The effect to reduce titre/infectivity was demonstrated to be suitable assays.

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Project Ref 40/05.2

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<th>CultureVolumeClass3-4</th>
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<td>20/04/2005</td>
<td>The study of protozoan lipid biosynthesis and trafficking in host pathogen interactions</td>
<td>Class 2</td>
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<td>Non-GMM</td>
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This proposal has been reviewed by five members of the GMSC and altered according to comments made. The safety committee are satisfied that HSE notification can now take place and the work can proceed once acknowledgement is received.
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
ACADEMIC RESEARCH AND TRAINING

Recipient or parental organism
LEISHMANIA MAJOR, LEISHMANIA MEXICANA, TRYPANOSOMA BRUCEI BRUCEI, AND TOXOPLASMA GONDII GMO POSE NO ENHANCED THREAT TO HUMAN HEALTH AND THE RISKS OF ENVIRONMENTAL CONTAMINATION ARE INSIGNIFICANT.

Host/vector system
LEISHMANIA SPECIES AND TRYPANOSOMA BRUCEI. Px, Ptex, Pxs, P2t7 AND DERIVATIVES. TOXOPLASMA GONDII. Psag, Pmincatbap, Pc3m2m3 AND DERIVATIVES.

Origin & function
GENETIC MATERIAL FROM LEISHMANIA SPECIES, TRYPANOSOMA BRUCEI, TOXOPLASMA GONDII SPECIES, GENES ANALYSED TO ASCERTAIN FUNCTION IN GROWTH AND INFECTIVITY.

Evaluation of foreseeable effects
N/A

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
NONE

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
LEISHMANIA, TRYPANOSOMA AND TOXOPLASMA GONDII: SPILLAGES AND INACTIVATION OF SPENT CULTURES REQUIRE FRESHLY DILUTED CHLORS AT 1:10V/V (FINAL CONC. 10,000PPM). ROUTINE DISINFECTION OF PLASTICWARE REQUIRES FRESHLY DILUTED CHLORS AT 1:40V/V (FINAL CONC. 2500PPM). THIS HAS BEEN VERIFIED AS GIVING 100% KILLING (SEE LOCAL CODE OF PRACTICE). CHLORS WASTE IS SUBSEQUENTLY DISPOSED OF DOWN THE DRAIN.

02/03/2022
Page 1833 of 15326
This proposal has been reviewed by five members of the GM safety committee and altered according to comments made. The safety committee are satisfied that HSE notification can now take place and the work can proceed once acknowledgement is received.

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Project Ref 40/05.3

Date Ackn'd 09/08/2005

CU2 Project Title

The use of viral expression systems to analyse structure-function relationships involving the cytoskeleton, cell junction, protein chaperone and cell protection mechanisms in multicellular tissues.

Date Project Ceased

Class 2

Culture Class 2

1-50 Litres

Non-GMM

Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

02/03/2022  Page 1834 of 15326
**Purpose of the contained use**

To prevent the operator infection by the viral systems used. Class 2 safety cabinet ensures appropriate protection and reduces to effectively zero the environmental risk for this project.

The project boundaries are set by the effect of disease causing mutations that cause the loss of function in neurones, astrocytes, muscle, the eye, fibroblasts and keratinocytes and other epithelia. We need to establish tissue culture systems to studying normal cell function and then use transduction methods to alter protein expression and cell function. One part of this project will be the immortalisation of lens epithelial cells taken with informed consent from human patients and donors. This project requires containment level 2 as immortalisation will be achieved by attenuated virus Ad5SVR4 containing SV40 large antigen. Of all the constructs that we plan to use, this one is unique as it is the only one that clearly has the potential to immortalise primary cells. All our other constructs do not have this ability. It is the possibility of opportunistic infection, particularly in the case of the primary human cells when using either Ad5 based transduction methods that require a level 2 containment. Other parts of the project involves the transduction of a wide range of primary cells with the similar constructs for which viral-based vectors offer the most consistency. These cells include primary astrocytes, neurones, fibroblasts, epithelial and muscle cells. These constructs will cause changes in the cytoskeleton, chaperone, cell adhesion, cell architecture or protection properties of the cell. Some constructs will induce protein aggregation or protect cells against stress and other insults. Others will express control proteins such as GFP or beta-galactosidase. We also have access to a wide range of transgenic animals and others expressing mutant proteins and we will be using our transduction strategies to try and reverse the functional deficits in primary cells derived from these animals. Conventional transfection strategies are notoriously inefficient for primary cell cultures, which is the main reason for using these viral transduction approaches. Primary cells are an excellent cell culture system because it is most similar to the tissue situation. These experiments range from clear containment level 1, where structural genes encoding normal proteins will be introduced into primary rodent cells, to the situation where primary CNS, epithelial, muscle and fibroblast cells will be transduced with RNAi and other constructs to modify function and cell responses to the presence of mutant proteins or external insults. Lentiviral systems are the preferred choice and commercial, published and current clinical trials are based on HIV-1 because of the extensive knowledge base that exists for this virus, the unique control methodologies and the existence of drugs to treat infections. We are also planning to transduce some constructs that affect signalling pathways, cell survival, cell polarity and neuroprotection. This project outline is necessarily broad due to the biological questions themselves, but it is also a unique strength as we seek to discover the common pathways that link protein aggregation, cell protection and cell misfunction to disease. By necessity, the project will also use constructs to disrupt normal cell processes in order to test hypotheses concerning cytoskeletal function, epithelial barrier and cell protection mechanisms. The neuroprotection studies will include tracing multi-synaptic neural pathways in rodents with the neurotropic virus, herpes simplex virus type 1 (HSV-1). When injected into a peripheral tissue or brain region, the controlled infection spreads in a sequential manner through the neural network making it possible then to trace how specific genes affect neuroprotective pathways. The HSV-1 utilised for trans-neuronal tracing retains the ability to replicate and as such falls into containment category 2 (Compendium of Guidance 2000, Part 2B, annex 1 & II herpes simplex virus 51-66). The HSV-1 is tagged and these constructs will be introduced into animals in the animal facility by injection using established protocols developed at the University of Birmingham.

Such studies will be very unlikely to alter the tropic properties of the transduced cells or cause immortalisation or transformation of these target cells.

**Recipient or parental organism**

Replication defective Adenoviral, Lentiviral and Baculoviral systems for transduction of animals and human cells in culture. These unmodified GMOs pose no significant health or environmental risk. Tissue culture cells are severely compromised and need highly specialised growth conditions. Exposure to any conditions other than the tissue culture environment will result in their death.

Adenovirus: Replication defective Ad5 will be used. The Ad5 E1E3 or Ad5 E1E3E4 deleted regions deleted so as to prevent replication in human cells and genes will be inserted into the site of the disabling deletion (He et al., 1998, Becker et al, 1994) ensuring that any recombination event that restores replication will remove the expressed gene. The replication deficient adenovirus is infectious to humans by aerosol/droplet but it has no mechanism for long term maintenance in cells. This may be considered to
occurs the resultant virus will not be harmful as the animals are considered to be dead end hosts (Compendium of Guidance 2000, Part 2 Annex 1 & III part 66). There is

or tropism and therefore create a novel virus. In studies where two viruses expressing different coloured markers are introduced into the animal, even if recombination

infects. If left long enough it can kill the infected neurone, but this is not the intended experimental outcome. The individual virus cannot produce variations in pathogenicity

Injection of the virus into the host animal causes a controlled infection to spread through the neural circuit of interest. The virus does not alter the genome of the neurones it

Birmingham and will be provided by them.

2001). For lacZ (strain SC17) expression driven by IE 110 promotor, lacZ is inserted into US5 gene. These constructs are those authorised for use at the University of

mammalian cell lines and primary cell lines. The vector has also been designed to express GFP independent of the shRNAs, enabling facile determination.

access to a fully sequenced genome. Such advantages have resulted in BV being increasingly explored as a possible alternative to more traditional types of viral vectors as

promotors, but the insect genes will not be active in mammalian cells. In addition, no alteration to their genome is envisioned that could lead to a risk to human health. They

They contain deletions that make them susceptible to ultraviolet light and dessication, therefore they cannot survive an an external environment. The deletions also prevent

regard to human health. Although it infects human cells it actually requires a very high titre for efficient infection. The inserted genes will be driven by mammalian

Baculovirus (Details of commercial vector given http://www.invitrogen.com/content/sfs/manuals/baculodirect_man.pdf) The insect virus to be used is considered safe with

baculovirus to a range of cultured mammalian cells, in particular liver-derived cells such as primary hepatocytes (Boyce and butcher, 1996). This list of primary cells, hepatic

promotors, but the insect genes will not be active in mammalian cells. In addition, no alteration to their genome is envisioned that could lead to a risk to human health. They

infection (m.o.i.). Consequently, Baculovirus is often given Class 1 status. There are also a range of commercial systems designed to generate recombinant virus, and

Gene expression of RNAi constructs (Nicholson et al., 2005). There are important safety features that are attractive to the system. Baculovirus is inherently incapable of

replicating within non-natural host cells because it needs host transcription factors and the transduced cells exhibit no apparent cytotoxicity even at a high multiplicity of

infection (m.o.i.). Consequently, Baculovirus is often given Class 1 status. There are also a range of commercial systems designed to generate recombinant baculovirus, and

Horsep Simplex virus type 1: The HSV-1 (clinical isolate HSV 17) retains the ability to replicate and will be used to produce a controlled infection that spreads in a

Herpes Simplex virus type 1. This eliminates the possibility that pathogenic, replication competent strains arise (Dull et al 1998, Zufferey et al 1998). Retroviruses, including

Herpes Simplex virus type 1. This eliminates the possibility that pathogenic, replication competent strains arise (Dull et al 1998, Zufferey et al 1998). Retroviruses, including

Para A of the Lentinus virus vector reduced the likelihood of activation of endogenous oncogenes upon infection, as has sometimes been observed with Moloney-based

are found in a commercial system produced by Genesys and sold by Invitrogen. Replication-deficient retroviruses have been developed by mutating these genes and inserting complimentary picking into packaging cell lines (Danos and Mulligan 1988). Therefore the disabled recombinant viral vector containing an inserted therapeutic gene can now only replicated in the packaging cell line, but the resulting viruses produced by the packaging cells are still capable of infecting other cells. This limits infection to the initial target population. Elimination of the U3 region in the 3 LTR

region of the Lentivirus vector reduced the likelihood of activation of endogenous oncogenes upon infection, as has sometimes been observed with Moloney-based retrovirus systems. In essence the system comprises the self-inactivating transfer vector (SIN -self-inactivating LTR by a TATA box deletion, expressing no viral gene products (Miyoshi et al 1998) and containing the woodchuck hepatitis virus post regulatory element (W) (Deglon et al 2000). Mouse PGK and CMV are used as internal promotors in this construct. The viral particles are produced by transient clacium phosphate transfection of HEK 293T cells with pCMVDR-8.92 (packaging construct providing Gag and Pol, but lacking both LTRs and has no viral packaging signal, y, as well as Env, Vif, Vpr, Vpu and Nef), pMD.G (Producing VSVG as the envelope component), pRSV-Rev (encoding Rev), and SIN-W-PGK or SIN-W-CMV transfer vectors as previously described (Hottinger et al 2000). The invitrogen system provides a modified cell line 293 (293FT) containing the packaging construct under tetracyline regulation. These systems comply with the HSE recommendation in Part 2B Annex III para 19. Baculovirus (Details of commercial vector given http://www.invitrogen.com/content/sfs/manuals/baculodirect_man.pdf) The insect virus to be used is considered safe with regard to human health. Although it infects human cells it actually requires a very high titre for efficient infection. The inserted genes will be driven by mammalian

Promoter/envelope constructs, pCMVDR-8.92 (packaging construct providing Gag and Pol, but lacking both LTRs and has no viral packaging signal, y, as well as Env, Vif, Vpr, Vpu and Nef), pMD.G (Producing VSVG as the envelope component), pRSV-Rev (encoding Rev), and SIN-W-PGK or SIN-W-CMV transfer vectors as previously described (Hottinger et al 2000). The invitrogen system provides a modified cell line 293 (293FT) containing the packaging construct under tetracycline regulation. These systems comply with the HSE recommendation in Part 2B Annex III para 19. Baculovirus (Details of commercial vector given http://www.invitrogen.com/content/sfs/manuals/baculodirect_man.pdf) The insect virus to be used is considered safe with regard to human health. Although it infects human cells it actually requires a very high titre for efficient infection. The inserted genes will be driven by mammalian promotors, but the insect genes will not be active in mammalian cells. In addition, no alteration to their genome is envisioned that could lead to a risk to human health. They contain deletions that make them susceptible to ultraviolet light and dessication, therefore they cannot survive an an external environment. The deletions also prevent

regard to human health. Although it infects human cells it actually requires a very high titre for efficient infection. The inserted genes will be driven by mammalian promotors, but the insect genes will not be active in mammalian cells. In addition, no alteration to their genome is envisioned that could lead to a risk to human health. They contain deletions that make them susceptible to ultraviolet light and dessication, therefore they cannot survive an an external environment. The deletions also prevent

regard to human health. Although it infects human cells it actually requires a very high titre for efficient infection. The inserted genes will be driven by mammalian promotors, but the insect genes will not be active in mammalian cells. In addition, no alteration to their genome is envisioned that could lead to a risk to human health. They contain deletions that make them susceptible to ultraviolet light and dessication, therefore they cannot survive an an external environment. The deletions also prevent
The HSV-1 utilised for trans-neuronal tracing retains the ability to replicate and as such falls into containment cat 2 (Compendium of Guidance 2000, Part 2B annex 1). Keratinocytes; rat and mouse hepocytes; myoblasts; myotubes; fibroblasts; neurons and astrocytes. Because they are not of neural origin they pose only a low risk. The recombinant, replication defective viruses will be used to transduce established mammalian cell cultures from such donors. Cells from such donors will be uncharacterised, but will have a very low risk of contamination by blood-borne viral infections (eye lens is avascular). Human cell cultures will be established from donated material taken from the anterior chamber of the eye. (Compendium of guidance 2000, Part A, annex IV). Using table 1, month animals not suspected of carrying BSE and taken from beef cattle will be used for these studies and again pose no significant threat to human health. Primary cultured as unmodified cells. Sometimes bovine cells from the anterior chamber of the eye will be cultivated. Following Compendium guidelines (2000 Part 2A, Annex III), 6 month animals not suspected of carrying BSE and taken from beef cattle will be used for these studies and again pose no significant threat to human health. Primary cells are therefore group 1 when propagated under barrier conditions and health screening already monitors rodent viral infection as a key health status check. These primary cells are therefore group 1 when used for these applications. Vector systems based on the well-established and well-characterised adenovirus, Baculavirus, Lentivirus transduction systems. HSV-1 is a tried and tested virus system used and developed at the University of Birmingham to be used in our neuroprotection studies. The Adenoviral, Baculaviral and Lentiviral vector systems are severely compromised and are replication defective. The inclusion of the genes indicated in this application, which interfere with cytoskeletal, cell adhesion or protection function, will not change infectivity or enable replication of these compromised virus systems. Any recombination events that occur via unintended recombination events or via adventitious viral contamination of cell cultures, will result in simultaneous loss of the gene of interest and therefore remove the risk of generating virus particles with altered host or growth characteristics. The use of a specific cell line for the propagation of the virus particles restricts the potential for altered infection essentially to this stage as both systems involve severely compromised systems where essential components have to be supplied in trans to create infective particles. These particles will infect, but not be able to propagate in their hosts. Unintended adventitious infections can increase the risk as when using primary cell cultures. As a general principle, these primary cells from both animal and human sources will die within a few passages and have no ability to survive outside the laboratory or cell culture dish. Cells from knockout and mutant mouse models will be transduced to either modify gene expression or produce immortalised cell lines. The primary cells pose no significant threat as the animals are not of neural origin they pose only a low risk. The recombinant, replication defective viruses will be used to transduce established mammalian cell lines with a long track record of safe use in which both the adenovirus, baculovirus, lentivirus systems are unable to replicate such as: rat hepatoma cells; mouse keratinocytes; rat and mouse hepatocytes; myoblasts; myotubes; fibroblasts; neurons and astrocytes. The HSV-1 utilised for trans-neuronal tracing retains the ability to replicate and as such falls into containment cat 2 (Compendium of Guidance 2000, Part 2B annex 1). Herpes Simplex Virus 51-66). However, the virus sequence is known (Elliott & O'Hare, 1999; Donnelly & Elliott, 2001). The virus has been used under license to researchers.
at the University of Birmingham since 2000 (HSE ref GM166/99.3). The rodents to be used in these experiments are considered dead end hosts, as they are unable to infect sentinel animals kept in the same cage and therefore pose no additional health or environmental risk than uninfected animals.

**Origin & function**

Origins: The DNA will be derived from human, mouse, rat, bovine, chicken, zebra fish, Arabidopsis, Castania, Jellyfish and e.coli. For cell immortalisation, the large T antigen of SV40 virus will be used in an Ad5-based vector, as a tried and tested method for extending primary cell lifespan.

Intended function: This project aims to use viral vectors to introduce DNA into cells that will result in the expression of proteins that will aid in the identification of the molecular mechanisms by which the cytoskeleton, cell junction, protein chaperone function and cell protection mechanisms operate. Identification of such pathways that are required for sensory cell function will allow the analysis of these pathways and their constituent proteins in specific disease states. Molecules identified will then become potential points for therapeutic intervention. Thus we must express wild type and mutant forms of these proteins. The systems we study are differentiated or isolated cells, as well as ex vivo tissue samples. These are notoriously difficult to transiently transfect, therefore we use an adenoviral or lentiviral vector system to introduce the recombinant DNA into cells. Replication defective adenovirus and lentivirus vector systems offer a number of key advantages over plasmid transfection based systems. The host-vector systems to be used do not generate replication enabled viral particles. The replication deficient viruses are infectious to humans by aerosol/droplet but have no mechanism for long term maintenance of cells. It is the introduction of the SV40 large T antigen that changes the classification of the recombinant Ad5 virus from class I to class III due to its affect on potentially oncogenic signalling pathways. There are no reports, as such, to link any of the other constructs proposed in this study to cell immortalisation or cell transformation, but the pathways concerned do involve cell signalling events that will change cell adhesion, cell motility, intercellular communication and cell survival.

**Evaluation of foreseeable effects**

The research strategies are designed to disrupt cell function by the introduction of mutants with either dominant negative, continuously active or simply inactivate. These reagents are anticipated to disrupt the normal function and process of cell signalling events that will change cell adhesion, cell motility, intercellular communication and cell survival in the sensory cells under investigation. In some situations this could cause the cell lines to die; in others the cells may be more resistant to specific stresses. Alternatively, signal transduction pathways will be disrupted by the expression of these constructs and an output could be altered cell shape, cell motility and increased sensitivity to chemical and physical stresses. The established cell culture cells to be used can be considered as especially disabled hosts and are unlikely to infect humans. The transduction experiments are very unlikely to confer a growth advantage or alter the host tropism as none of the sequences to be transduced except the SV40 large T antigen can be considered oncogenic (Part 2A annex IV).

Human adenoviruses do not naturally infect animals and have only been shown to infect animals (cotton rats) when deliberately administered at high doses of viruses under experimental conditions (see Part 2B - Annex III of the HSE guidelines) and of course the system is highly attenuated as are the lentivirus to be used. Baculavirus only infect mammalian cells if very high titres are used and the system used takes advantaged of an attenuated virus.

The HSV-1 will be used to infect animals in laboratory in a controlled manners and in a manner that positively prevents neuronal cell death as the objective is to map living neural networks. We therefore do not expect any detrimental effects on the experimental animals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None. Although a class 2 containment activity, the risk of an emergency is effectively zero. In the highly unlikely situation of a release of a virus stock from the facility, the virus cannot replicate therefore propagate outside the helper cell lines. A standard Emergency Procedure code of practice has been prepared for the facility.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Viruses will be applied to target cells via cell culture medium for 2-4 hr, the medium then removed and disposed of, leaving no unctact, inafective particles in the cells being studied. Operations do not involve sharps such as glass pipettes or needles and therefore the major risk of operator contamination is by aerosols.
All solid waste will be autoclaved before disposal. Autoclaves are tested annually for Hse compliance. Annual testing includes thermometric test for small and normal load and safety check, vacuum leak test of automatic controls.

Liquid waste will be either treated by autoclaving or by the addition of the viral and cell killing reagent eg VIRKON to a final concentration of 1% w/v as an accepted and thoroughly tested route for 100% kill.

Treatment with 70% ethanol has been shown to be an effective disinfectant for adenovirus (Rutula 1996), but we will use the Virkon product to disinfect and decontaminate.

Decontaminated solutions will be disposed of to the drain with copious volumes of water.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

What is the source of the HSV-1 material to be used in the project?

This is the same as those used by the group who trained Dr S Pyner at the University of Birmingham.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref** 40/09.1

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<td>&lt; 1 Litre</td>
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Non-GMM

Date Project Ceased
Notification is provided here as recommended in communications with HSE since the work will involve use of GM-modified plant pathogen (Pseudomonas syringae) strains.

The objective of this project is to further characterise the molecular, biochemical and cellular events occurring in plant cells infected with virulent and avirulent strains of the pathogen(12,10),(988,977) described in the attached document “Signalling and cell biology of plants responding to psedodmonas infection” and risk assessment.

Recipient or parental organism

Recipient of parental organism: Pseudomonas syringae pv. tomato DC3000 - virulent plant pathogen.

GMM's

* P. syringae pv. tomato DC300 (avr8) - avirulent plant pathogen.
* P. syringae pv. tomato DC300 (avrRPM1) - avirulent plant pathogen.
* P. syringae pv. tomato DC300 (AvrRpt2) - avirulent plant pathogen.
* P. syringae pv. tomato DC300 (luxABCDE) - virulent plant pathogen expressing bacterial luciferase for imaging.
* P. syringae pv. tomato DC300 (AvrRpt2) (luxABCDE) - a virulent plant pathogen expressing bacterial luciferase for imaging.
* P. syringae pv. tomato DC300 (hrpA mutant) - has reduced virulence due to impaired TTSS
* P. syringae pv. Tabaci (hrcV mutant) - has reduced virulence due to impaired TTSS

Relevant genes are described in more detail in the accompanying risk assessment (Appendix 1), Table 1 below and in the accompanying document (Appendix 2) and pathogen profile (Appendix 3).

Host/vector system

* P. syringae strains are plant pathogens as described in the accompanying documents. Some of the strains contain mutations which render them avirulent on the host plant species. Most of the strains to be used are based on P. syringae pv. tomato DC3000. (See attached paper Appendix 4)
* pDSK600 (see diagram opposite) is a broad-host-range cloning and expression vector, (Spr/SMr, IncQ replicon, 3x lac UV5 promoter).

[See attached paper, Appendix 5].

[NB: Appendices / attached papers referred to are available for reference in hardcopy].
[It is on the hardcopy if required for reference].

Origin & function
*avrB, avrRPM1, avrRpt2 are all proteins which are recognised by host plant with appropriate resistance gene, to allow resistance mechanism to occur i.e. makes the pathogen avirulent. See also Appendices 1-3. *luxABCDE encode subunits of the bacterial luciferase enzyme, producing light, used as a reporter gene.

**Evaluation of foreseeable effects**

The consequences of the hazards being manifest are considered to be very low and the likelihood of their manifestation is negligible when the containment proposed above is taken into account. P. syringae strains are plant pathogens that have never been isolated from human hosts; they are unable to grow at 37 deg celsius. Vectors contained in some of the strains harbour antibiotic resistance genes, giving marginal risk of transfer to other strains, however this is highly unlikely and should not affect the risk assessment nor the level of containment required. Taking into account the control measures assigned above, the overall risks to the environment from the genetically modified micro-organisms produced or used in this work are low. Therefore few additional containment or control measures are considered necessary to protect the environment other than those described to protect human health and safety. These include containment of infected plants in growth chambers and measures to prevent insect transmissions from infected plants. Two of the strains we will use contain mutations (P. syringae pv. tomato DC3000 hrpA mutant and P. syringae pv. Tabaci hrcV mutant) which make these bacterial strains contain plasmids which express proteins e.g. AVR proteins which also make these strains more vulnerable i.e. easier for the plant to detect, and hence less pathogenic. Hence if the plasmids were lost, or the mutations reversed, the bacterium would, in a 'worst case scenario', revert to the natural law of pathogenicity of the wild type (DC3000). The assessment presented here and in the risk assessed document (Appendix 1) is thus based on a 'worst case scenario' of the full virulence of the wild type (DC3000) being realised in a genetically modified strain. The only strain to which this applies directly is P. syringae pv. tomato DC3000 (luxABCDE), which expresses harmless bacterial luciferase. Loss of this plasmid or mutation would only lead to loss of luminescence from the luciferase, and hence no change to the risk to the environment or to human health.

Overall it is inconceivable that any of the GM strains to be produced or used could become more pathogenic to plants since there is no intention to manipulate any of the virulence genes - we only intend to work on strains which have already been manipulated and there is a history of safe use of the GM strains to be used.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No GMO's are associated with this project.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Small samples of infected plant materials for sectioning or biochemical assessment will be taken from CL2 containment to CL1 facilities for processing. Surplus materials will be treated as described in the accompanying schedule prior to disposal. Samples will be leaf explants or tissues taken from treated plants growing under CL2 conditions and mounted on slides for microscopy or placed in sealed eppendorf tubes for extraction and analysis in CL1 laboratories. Residual materials will be processed as described by autoclaving and/or incineration.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**WASTE MANAGEMENT MEASURES**

All autoclaving is carried out in autoclaves dedicated for GM and CL2 waste processing and equipped with thermal logging system.

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclaved using a 'make safe cycle' as specified in BS 2646, Part 3, 1993 (either 121-125 deg Celsius for at least 15 minutes or 126-130 deg Celsius for at least 10 minutes), any excess liquid discharged to drains, disposal of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream or landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - EITHER autoclaved using a 'make safe cycle' as specified in BS 2646, Part 3, 1993 (either 121-125 deg Celsius for at least 15 minutes or 126-130 deg Celsius for at least 10 minutes), and discharging to drains OR treated with sodium hypochlorite (at 1% available chlorine) OR virkon (at 1% w/v) for at least an hour or overnight and then discharged to drains.

Agar plates - autoclaved using a 'make safe cycle' as specified in BS 2646, Part 3, 1993 (either 121-125 deg Celsius for at least 15 minutes or 126-130 deg Celsius for at least 10 minutes), any excess liquids discharged to drains, solid disposedof via the industrial (black bag) waste stream for landfill.
Sharps (e.g. needles, syringes, scalpels) - disposal as biohazardous sharps waste via clinical waste stream for incineration.

Plants, plant material and soil - autoclaved as specified in DEFRA Plant Health Licence (this in practice involves 50 mins at 121 deg Celsius in the autoclave, and such material is dealt with through a University-approved contractor), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste system for landfill.

DEGREE OF KILL
Autoclaving, effectively 100% kill (annual validation, cycle thermal logger)
Incineration, effectively 100% kill (incinerator by licensed contractor)
Chemical disinfection with Virkon, used according to manufacturers instructions under standard conditions. Hypochlorite used at 1% available chlorine.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
No further questions / suggestions were raised on the Pseudomonas risk assessment so the local risk assessment is approved.

Project Containment

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Project Ref 40/12.1

Date Ackn'd  
14/11/2012

Date Project  
02/03/2022

CU2 Project Title  
Virus-induced gene silencing (VIGS) A functional genomics approach to identify plant genes required for growth and development, and those involved in resistance or required for diseases caused by plant pathogens

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<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
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</table>
The project aims to understand how plants cope with invading pathogens. By reducing the expression of candidate genes by a disarmed virus based gene-silencing system we can ascertain the role of the candidate genes in plant response to infection. The virus based gene silencing system will not be used outside the containment facility nor will the plants be allowed to flower or release pollen to the outside.

Recipient or parental organism

* BSMV (barley stripe mosaic virus). Jacksons et al., 2009, Annu. Rev. Phytopathol. 47: 385-422,
* TRV (tobacco rattle virus), PVX potato virus X. All these are disarmed viruses and are cloned in pGreen based cloning vector (Bachan et al., 2012, Methods Mol Biol. 894:83-92).
* Wheat barley, rice, maize, N. benthamiana
* Agrobacterium tumifaciens GV3101

Host/vector system

* pTRV (kanamycin resistance)
* pPVX (kanamycin resistance)
* These are pGreen based gene silencing vectors which contain the disarmed viruses (TRV and PVX see references above) fore generating VIGs based siRNA for gene silencing. Plant sequencing will be cloned between viral sequences in these vectors for gene silencing. For details of how silencing works please refer to (Bachan et al., 2012, Methods Mol Biol. 894:83-92).
* pBSMV (kanamycin resistance), see vector details described in attached risk assessment

Origin & function

* Genes affecting the ubiquitin pathway
* Plant cytoskeleton
* Genes involved in general signal transduction
Short portions (22-500bp) of a transcribed sequence from various plants including wheat, barley and related cereal species (the host species identified under "recipient or parental organism" in section 7 above). The intention is to silence endogenous genes rather than express additional ones

Evaluation of foreseeable effects

The basic strategy is to create infectious BSMV in N. benthamiana leaves, which is then used to produce an infectious extract able to secondarily infect host cereals by rub infection with carborundum powder.
After sub-culturing in liquid medium A. tumefaciens strains carrying BSMV alpha, beta and gamma genome components will be combined in 1:1:1 ratio and infiltrated using a needless syringe into leaves of young Nicotiana benthamiana plants. The bacteria transfer Ti plasmids to the plant cells, and the double CaMV 35S constitutive promoter present in the binary plasmids drives synthesis of infectious BSMV transcripts within the cells of the host plant. These transcripts initiate the formation of virus particles that infect the tissue surrounding the inoculation site, and subsequently move systemically to invade upper uninoculated leaves. Infectious BSM viruses are thus only created by a combination of individually non-infectious components within a host Nicotian plant. The viral vector is therefore not transmissible apart from by experimental means.

The cereal hosts include species that are native to the UK and will survive outside containment facility but cannot compete with native forms of the plant as these are not elite cultivars. We are only silencing endogenous genes and not adding new genes so it is unlikely that hazards may arise from this form of research. In the event that the GMO is inadvertently released into the environment via seed or pollen, it is likely that germination will occur. Also, cross-pollination with wild-type relatives may take place. The consequences are unlikely to be severe as the genes being silenced are endogenous to cereals and the virus cannot replicate outside the Agrobacterium transient assays system. There are unlikely to be hazards to human health as cereal leaves are not consumed by humans. The inserted gene is not expected to result in an increase in the hazards posed by handling plant material. Even if the plants were to be accidentally consumed, no harmful effects are known or anticipated. Therefore, the consequences are considered to be negligible.

The basic strategy is to create infectious BSMV in N. benthamiana leaves, which is then used to produce an infectious extract able to secondarily infect host cereals by rubbing infection with carborundum powder. This then allows BSMV based gene silencing in cereals.

TRV is a single stranded RNA virus which is transmitted by a nematode vector. Virus particles are rod shaped and comprise of long (180-197nm) and short rods (46-114nm). Both are required for the production of intact virus particles. The genome is bipartite with RNA1 encoding putative replicase and cell-to-cell movement proteins. The shorter RNA2 encodes the coat protein and an additional protein is required for nematode transmission. The cloned TRV gene silencing vector does not carry the additional protein required for nematode infection in RNA 2 but is replaced by the multiple cloning site for cloning plant genes of interest. The viral vector is therefore not transmissible apart from by experimental means.

Similarly PVX has very wide natural and experimental host ranges. It is reported to occur naturally in at least 62 plant species of 27 families and to be transmissible experimentally to another 348 species in 33 families (Edwardson and Christie, 1997). Transmission of the virus is mechanical e.g. by contact. Both TRV and PVX will be only used to silence or overexpress genes in N. benthamiana. These vectors will be Agroinfiltrated into N. Benthamiana plants for silencing genes of interest. Control measures for BSMV will be similarly employed for TRV and PVX systems.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All the work will be done in a CL2 room and plants will be kept in an approved containment glasshouse and growth room arrangements to contain the micro-organisms (growth room 1 and 2). To eliminate the spread of the virus, infected plants will be discarded before they reach the flowering stage and no other flowering plants would be present in the compartment where the experiment was in progress.

There are 5 people involved in the project. A rota will be drawn up that ensures in any one time at least 1 person will be primarily responsible for the VIGs experiments including plants and viral cultures. While a second person will act as a back up in case the key contact is not available in an emergency. The persons responsible will monitor plant status, i.e. ensure plants do not reach flowering stage, viral cultures are monitored and that standard operating procedures are followed (see comments below).

Standard good practices by adhering to Code of Practice for transgenic glasshouse and growth rooms will be ensured by the PI and the two duty personnel on the rota.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To eliminate the spread of the virus, infected plants will be discarded before they reach the flowering stage and no other flowering plants would be present in the compartment where the experiment was in progress. Both liquid and solid laboratory waste, waste from glass houses and waste from experiments with infected plants will be double bagged and autoclaved. All viral cultures will be disinfected with bleach. In previous cases these measures have proved to be effective in containing the viral
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

A key aspect of maintaining containment for this project is that GM plants will not be propagated long enough to flower. The committee requested details about systems to ensure that a single individual is not solely responsible for ensuring that plants do not flower. These are described in section 8.

Project Containment

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Project Ref 40/12.2

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Historical Significant Changes

Historical Date of Additional Info
Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The project will investigate the underlying mechanisms of resistance to penicillin and meticillin in laboratory-generated strains of S. aureus and determine whether these mutations are important in clinical isolates.

Recipient or parental organism

Staphylococcus aureus Oxford and its derivatives are the main strains to be used in this programme. Expression of penicillin- and meticillin - resistance determinants in this strain would alter the antibiotic resistance profile of the organism, but is very unlikely to alter its ability to invade and establish itself in humans. Any recombinant strains would remain sensitive to a number of other antibiotics (vancomycin, linezolid, trimethoprim-sulfamethoxazole, clindamycin, minocycline).

S. aureus strains Newman, SH1000, NCTC8325, RN4220, RN450 are meticillin-sensitive strains widely used as cloning intermediates (RN4220) and as typical genetic backgrounds in which to study gene expression.

Other strains including COL, BB270, NW2, USA300, EMSRA-15, and EMSRA-16) are meticillin resistant, which means that infections can not be treated with beta-lactam antibiotics can be treated with a number of other antibiotics including vancomycin, linezolid, trimethoprim-sulfamethoxazole, clindamycin, minocycline,

Host/vector system

pUC and derivative vectors for cloning and analysis in E. coli.

pMAD (or similar) is a temperature-sensitive plasmid used for allele replacement in S. aureus (Memmi et al., 2008. Antimicrob. Agents Chemother. 52:955-66).

pLOW (or similar). Stable low copy number E. coli-S. aureus shuttle vector with IPTG-inducible Pspac promoter. (Liew et al., Microbiology-SGM 157:666-676)

pCL%% and derivatives - single site chromosomal integration vector for S. aureus (Lee et al., 1991 Gene 103:101-105)

pGL485 is a E. coli-S. aureus multi-copy shuttle vector carrying the chloramphenicol acetyl transferase gene (cat). (Cooper et al., 2009 BMC Microbiol 9:266)

Transducing phage, such as 80 alpha

Origin & function

The genes involved are resident in S. aureus Oxford, which is a strain that has a long history of use in hospital pathology laboratories and is sensitive to penicillin and meticillin. The genes of interest are those that have changed in laboratory selection of strains that are resistant to these antibiotics, but are presently unknown. Inactivation of these genes is likely to lead to strains that are less fit than the parental strain. Addition of these altered genes to other genetic backgrounds may increase resistance to penicillin/meticillin, but will not alter levels of resistance to other clinically useful antibiotics.

One family of genes identified as targets are the penicillin binding proteins (pbp 1,2,3 and 4). These genes are involved in peptidoglycan biosynthesis and are the target of penicillin and meticillin antibiotics

Evaluation of foreseeable effects

It is likely that one or more genes that we identify will contribute to decreasing the sensitivity of the host organism to penicillin/meticillin. It is unlikely that any (other) gene in isolation will have a dramatic effect on antibiotic resistance or virulence.

S. aureus can infect animals which can act as a reservoir for human infection.

Primary containment measures should be directed towards protecting laboratory workers, but also ensure that these organisms cannot enter the environment.

As the genes that we are pursuing are involved in antibiotic resistance, if they were transferred to related microorganisms, they may have an impact on levels of resistance to penicillin and metacillin. However, it is unlikely that this would cause and added concern to the health of humans or animals as many strains in hospitals and in the
community carry high-level resistance to these antibiotics already.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The disposal of bacterial cultures and other contaminated liquids, e.g. culture supernatants, will be carried out by adding Virkon solution, to 1% final concentration, and incubation for 30 minutes at room temperature prior to disposal via designated sinks. Validated data supplied by the manufacturer indicates that this will result in inactivation of S.aureus strains. Contaminated glassware, centrifuge tubes and work surfaces will be decontaminated by the addition of 1% Virkon solution and incubation for 30 minutes and room temperature.

Solid waste, including plastics and agar plates will be placed in clear autoclave bags for sterilization by autoclaving at 134C for 30 minutes. A printed record of each disposal cycle is checked for time and temperature achieved during the run before disposal of the sterilized waste and annual validation of the waste cycle is carried out using 12 point thermocouple testing.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The committee noted that the proposal involved a single research worker at times would be working in a restricted access facility. It was noted that there was a departmental policy for lone working that would be adhered to in this situation

Project Containment

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Animal Units

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Project Ref 40/13.1
The aims of the project are two-fold: Firstly, to exploit a commercially available E. coli based protein expression system to produce proteins and mutant variants of Salmonella enterica serovar Typhimurium. Secondly, to generate Salmonella enterica serovar typhimurium mutants lacking metal homeostasis genes and expression such proteins extra chromosomally, and monitoring gene expression.

Recipient or parental organism

1. Escherichia coli BL21 (DE3), JM109, DH5α and BW25141
2. Salmonella enterica serovar Typhimurium strains (ACDP Hazard Group 2 as they may be pathogenic and able to colonise humans):
   - LB5010a - a restriction deficient, modification proficient strain that is known to be attenuated due to a specific mutation.
   - SL1344 - wildtype that is a derivative of a strain isolated in 1977 which is auxotrophic for histidine and has been safely used in research since this date.

Origin & function

Source of host cells:

E. coli BL21 (DE3) is already a laboratory maintained strain at Durham University.

E. coli JM109 and BW25141 will be supplied by a long standing collaborator, Dr J C from the University of Manchester.

S. Typhimurium LB5010a and SL1344, and bacteriophage P22HT105/1-int will also be supplied by DR J C from the University of Manchester and were originally sourced.
from the Salmonella genetic stock centre.

Source of genetic material
S. Typhimurium genes or genetic regions (e.g. operator-promoter region) will be amplified by PCR using specific primers (no random DNA) from S. Typhimurium cells or purified genomic DNA.

Plasmid vectors - pGEM-T (Promega) and the pET vector system (Novagen) are already laboratory maintained; pUC series, pACYC184, pBR322 series, pKD3, pKD4, pKD46, and pCP20 plasmid vectors will be supplied by Dr J C from the University of Manchester.

Intended use
S. Typhimurium genes encoding metal homeostatic proteins will be cloned into pGEM-T prior to sub-cloning into the pET vector system for protein over-expression, purification and in vitro characterisation. Mutant derivatives will be generated by site-directed mutagenesis in pGEM-T, followed by sub-cloning into the pET vector system.

The lambda red gene disruption method will be used to generate S. Typhimurium strains lacking metal homeostatic genes. Briefly, antibiotic resistant cassettes will be amplified from pKD3 or pKD4 (maintained in E. coli BW25141) with specific primers to allow homologous recombination into the chromosome of S. Typhimurium LB5010a (expressing the lambda Red system from PKD46) and subsequent gene disruption. The gene deletion will be moved into S. Typhimurium SL1344 by bacteriophage P22HT105/1-int and antibiotic resistance removed using the FLP-recombinase encoded by pCP20. Mutations will be complemented using expression of the gene on pACYC184.

Operator-promoter regions of S. Typhimurium metal-responsive genes will be cloned into the pBR322 vector series using E. coli JM109 or DH5a and transformed into S. Typhimurium SL1344 (via LB5010a) to monitor gene expression using the B-galactosidase assay.

S. Typhimurium SL1344 is a derivative of a strain that was isolated in 1977 which has been modified (1981) to be auxotrophic for histidine. SL1344 has been safely used in research since this date without the requirement for microbiology safety cabinets. Laboratory passage means this strain is likely to be more attenuated than the original isolate. LB5010a is a derivative of a strain (S. Typhimurium LT2) that is known to be attenuated due to a specific mutation and therefore the risks associated with this strain are considered to be low. This strain is required to facilitate movement of DNA from E. coli to strain SL1344, as this cannot be done directly.

Evaluation of foreseeable effects
S. Typhimurium SL1344 is a derivative of a strain that was isolated in 1977 which has been modified (1981) to be auxotrophic for histidine. SL1344 has been safely used in research since this date without the requirement for microbiology safety cabinets. Laboratory passage means this strain is likely to be more attenuated than the original isolate. LB5010a is a derivative of a strain (S. Typhimurium LT2) that is known to be attenuated due to a specific mutation and therefore the risks associated with this strain are considered to be low. This strain is required to facilitate movement of DNA from E. coli to strain SL1344, as this cannot be done directly.

The genetic manipulations will be of genes encoding non-toxic products and use well characterised harmless vectors. They will not increase the pathogenicity of the organism. The likelihood of harm to human health in the event of exposure would be no greater than that with non-GM S. Typhimurium and can be considered negligible. The chance of transfer of the vectors to other organisms in the environment can be considered low. It is difficult to envisage how any of the genetic manipulations proposed in this project could confer a harmful phenotype or competitive advantage to the host strains in the environment. The environmental hazards associated with the project are therefore considered no greater than those associated with handling non-GM S. Typhimurium. The project involves small-medium scale work for research purposes and all contaminated material will be completely inactivated by autoclaving prior to disposal. In the highly unlikely event of release into the environment, no risks are envisaged.

Genes subject to characterisation are either characterised or novel homologues of metal homeostasis genes (including metal-responsive transcriptional regulators, chaperones and transporters). Although the inducing metal ions for some of these are unknown, all are predicted to have no deleterious side effects. The gene disruptions of S. Typhimurium will not increase the hazard of the organism. The most likely outcome following removal of metal homeostasis genes will be a hazard no greater than the non-GM S. Typhimurium or even a decrease in pathogenicity towards humans.

No combination of host, vector and insert is considered more hazardous than non-GM S. Typhimurium.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The GMM-contaminated waste will consist of broth/agar cultures, plastic labware and glassware. The bacteria will be viable but can be successfully and rapidly inactivated by a number of disinfectants (for example 70% ethanol, 1% Virkon, Virkon powder and 10% Microsol). Following experimentation, E. coli and S. Typhimurium strains will be decontaminated before disposal either by steam sterilisation or chemical disinfection. They are susceptible (100% kill) to moist heat (121°C for at least 15 minutes). A suitable autoclave will be used which is validated by annual thermocouple mapping (including a probe within a dummy waste run) and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile. All agents are efficiently killed with a 1% Virkon solution (Du Pont™ Rely+On™ Virkon®) which will be used according to manufacturer's instruction. In addition, Gram-negative bacteria are sensitive to 70% ethanol. Any delicate equipment will be cleaned with Microsol 3+ which has a proven efficacy against bacteria (EN1276 for gram negative and gram positive bacteria) yet is non-corrosive. Benches used for S. Typhimurium work will be routinely swabbed with 70% ethanol. All contaminated waste will be completely inactivated (100% kill) before disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 40/16.1

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<td>Class 2</td>
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Date Project

Page 1850 of 1532
The aim of the project is to characterise the function of genes encoded by Mycobacterium tuberculosis. In order to achieve this in as closely related organism as possible to the virulent M. tuberculosis we will utilise a highly attenuated and genetically validated M. tuberculosis generated by Prof W Jacobs (Albert Einstein College of Medicine, New York). The M. tuberculosis mc(2)7000 strain is an unmarked derivative of the mc(2)6306 M. tuberculosis strain harbouring disabling deletion mutations in RD1 region (associated with the attenuation of bacilli Calmette-Gurin (BCG) vaccine) and panCD (associated with pantothenate synthesis). RD1 mutations limit the ability of M. tuberculosis to grow within animal tissue and have been validated in humans through the use of the BCG vaccine, panCD mutations limit growth in vivo by restricting the availability of essential nutrients to the bacteria. Evidence supporting these statements is to be found in the supporting documents. Additional parallel studies will be performed in Mycobacterium bovis BCG, an avirulent strain of M. bovis, and similar HG2/1 mycobacteria (e.g. Mycobacterium smegmatis, Mycobacterium marinum, Mycobacterium thermoresistibile).

We will exploit these background strains in phenotypic screens. Nulling deletion mutations will introduced in protein coding genes within M. tuberculosis and additionally complement with a mycobacterium specific inducible expression vector. The mutants will enable us to identify the function of these genes using basic phenotypic characterisation studies. Potential foreign genes, which are exploited already within the field as validation markers and reporters, will be cloned into mycobacterium specific vectors to create viable test strains (e.g. luxAB genes from Vibrio harveyi). Antimicrobial testing and mutant generation using these strains will assist our efforts in mode of action studies of novel anti-tuberculosis compounds.

Additionally genes of interest will be expressed at high levels in disabled host bacterium (E. coli) to enable further biochemical and biophysical analysis of the target protein.

**Recipient or parental organism**

The parental Mycobacterium tuberculosis H37Rv strain is a fully sequenced laboratory strain of TB classified as a HG3 organism. The route of transmission is via aerosol but can also infect via oral routes. This strain will not be used in the notified activities. The highly attenuated M. tuberculosis mc(2)7000 strain and its derivatives, classified as hazard group 2, will be used. The GM activities in this notification are grow1h, complementation, and storage of GM mc(2)7000 strains.

Other Mycobacterium related strains with potential opportunistic activities will also be used; Mycobacterium marinum (HG2)
Rhodococcus equi (HG2)
The vaccine strain;  
Mycobacterium bovis BCG  
Non-pathogenic;  
Mycobacterium smegmatis  
Mycobacterium thermoresistibile 
(HG2)  
(HG1)  
Disabled laboratory strains of E. coli including K12/MC1061 or similar/derivatives thereof will be utilised in construction of genetic elements using standard protocols. TOP10, XL-10 Gold (host for routine cloning); BL21, C41, BL21 star (for protein expression).

Host/vector system

The pJV53 and pAL70 series of vectors for generating marked and unmarked mutants in Mycobacterium spp. including M. tuberculosis mc(2)7000 will be used (van Kessel JC, Hatfull GF Nat Methods. 2007. 4(2):147-52, Cascioferro et al., Appl Environ Microbiol. 2010; 76(15): 5312-5316). These vectors carry a hygromycin resistance gene for selection which can be removed by successive passaging of the organism in the absence of the selective marker.

pHAE181 phage is a recombinant phage that contains a derivative of the mariner-based Tn5371. The recombinant phage pHAE181 is derived from a temperature sensitive mutant of the lytic Mycobacteriophage TM4, which can infect many slow and fast growing Mycobacterium species, including M. tuberculosis, M. bovis, M. smegmatis and M. marinum. The phage can replicate inside a mycobacterial host (with a productive lytic cycle) at 30°C, but not at the non-permissive temperature of 37°C. Tn5371, which contains a hygromycin resistance gene (hyg), can thus be delivered by infecting the target Mycobacterium host at 30°C, followed by selection of Tn-mutants on hygromycin-containing plates at 37°C (Kriakov et al., 2003 J Bacteriol 185(16):4983-91).

DNA sequences will be introduced into Mycobacterium spp. including M. tuberculosis mc(2)7000 using well characterised and harmless non-mobilisable (Parsons et al., 1998 Mol. Microbiol. 28: 571-582) shuttle vectors that can replicate in E. coli and mycobacteria (e.g. pJEM15, pMV261, pMV306, pW16, pPR27 and their derivatives). These vectors contain an E. coli origin of replication (from the pUC series of cloning vectors), a mycobacterial origin of replication from plasmid pAL5000 and antibiotic resistance markers (e.g. kanamycin and hygromycin). Additionally pMIND and pMEND derivatives will be used in complementation and overexpression studies (Blokpoel et al. Nucleic Acids Res. 2005 . 33(2):e22. 10.1093/nar/gni023). pNV18 and pNV19 are constructed by inserting a 1.8 kb DNA fragment carrying the pAL5000 origin of replication (Snapper et al. 1990 Mol. Microbiol. 4:1911-1919; Stolt 1997 Nucl. Acids Res.25:3840-3846) into the unique Nhel site of pk18 or pk19 (Pridmore 1987 Gene 56:309-312) for expression in Rhodococcus equi.

E. coli recombinant protein expression for crystallographic and biochemical studies will utilise the non-mobilisable pET expression systems.

Origin & function

Source: M. tuberculosis H37Rv
Genetic material: DNA fragments encoding various well defined genes involved in cell wall metabolism and function, e.g. transferases, fatty acid biosynthesis, polyketide biosynthesis, siderophore biosynthesis, p450 enzymes, core biosynthetic genes, regulatory enzymes and shock response genes. The intention is not to work with any sequences where insertion or deletion could be reasonably foreseen to increase the hazardous characteristics of the organism.
Source: Depending on marker - E. coli for β-galactosidase, luminescent vibrios for luciferase, jellyfish for GFP, Streptomyces for agarases.

The genetic material that are to be expressed are marker protein which are highly unlikely to have any biological effect, eg GFP, luciferase, B-galactosidase, agarase.

Antibiotic resistance genes expressed from their native promoters.

Evaluation of foreseeable effects

1. Hazards to human health:
The parental strain M. tuberculosis H37Rv is capable of causing TB, the deletions of RD1 and panCD are welldocumented in peer-reviewed papers as attenuating the ability of these strains to colonise and cause infection in mammals (see accompanying summary of relevant literature). Furthermore, there is no reduction in the susceptibility of the GMMs to frontline treatments for TB. The likelihood of the 10 disabling gene deletions being overcome is extremely unlikely. The possibility of generating a fully infectious TB strain is therefore effectively zero. Subsequent genetic modifications by over-expression of potential target gene candidates by plasmid expression or through further gene deletions are unlikely to modify the bacteria's virulence or infective route as long as the mutations are not rescuing or reverting the original RD1 and panCD deletion genotype. The majority of GMMs will render the strains less efficient at causing disease. It is not anticipated that any GMMs will show increased virulence.

2. Possible impact on the environment:
The disabling mutations were introduced by multiple gene replacement events in the H37Rv chromosome. The likelihood of mobilisation of the genetic modification from the mc(2)7000 derivatives, or transfer into other species in the environment in the event of a release is diminutive. This is reduced further still when the waste control measures are taken into consideration. All potentially infected material is autoclaved prior to disposal. 100% killing of GMMs is required. Disinfectants such as 2% Trigene and 10% Chloros are available for spills/disinfection of contaminated surfaces. Potential modes of transmission to the environment: Drains, aerosols, faulty autoclaves, and carried on individuals. These risks are reduced to moderated/unlikely, firstly by the disabling mutations within the bacteria, which reduce the likelihood of survival outside the laboratory (panCD) and the control measures used in the contained environment (see BioCOSHH and risk assessment).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The parental Mycobacterium tuberculosis H37Rv strain is a fully sequences laboratory strain of TB classified as a HG3 organism. This strain will not be used in any part of this notification. The HG2 classified M. tuberculosis mc(2)7000 strain and its derivatives will be used at our premises. The M. tuberculosis mc(2)7000 strain is an unmarked derivative of the mc(2)6306 harbouring disabling deletion mutations in RD1 region (associated with the attenuation of bacilli Calmette-Gurin (BCG) vaccine) and panCD (associated with pantothenate synthesis). RD1 mutations limit the ability of M. tuberculosis to grow within animal tissue and have been validated in humans through the use of the BCG vaccine, panCD mutations limit growth in vivo by restricting the availability of essential nutrients to the bacteria. Therefore we seek a derogation to allow the reclassification of the M. tuberculosis mc(2)7000 strain to HG2. Similar derogations for work with mc(2)7000 and its parental strain have been granted to existing projects in the GM public register, including project Ref 8/15.2 & Ref 552/13.2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials used in the CL2 facility are to be regarded as potentially contaminated and disposed of accordingly to
ensure 100% killing. All solid waste will be autoclaved in a validated autoclave before leaving the laboratory and disposed of in clinical waste bags for clinical waste disposal. Liquid waste is pre-treated with Surfanios at 5% final concentration and left overnight before autoclaving and disposal of as clinical waste. Minimum 126°C degrees for 45 minutes, 10 minutes free-steam. Activities will be separated from those which use Virkon for chemical disinfection, as Virkon is documented as being ineffective against Mycobacteria.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

Please enter comments on the GM safety committee on the risk assessment

The mc27000 strain is attenuated by two independent means, and letters presented from the safety boards of US institutions indicated that in their opinion this organism could be considered as hazard group 2. One of the attenuating mutations, requiring the organism to scavenge pantothenate, would still permit it to grow in mammalian cells. This is because the organism would be able to obtain some pantothenate from the environment it was infecting. It was agreed that a case for derogation of this organism to hazard group 2 should be made to HSE.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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**Project Ref** 40/17.1

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<td>24/08/2017</td>
<td>The roles of trace nutrient metal ions in bacterial physiology and pathogenesis.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Date Project Ceased

02/03/2022
This project will examine the ability of medically significant pathogens to manage nutrient and toxic metals (copper, zinc, iron, manganese). These metals are essential for the function of nearly half of all bacterial proteins but they are toxic if present in excess or if found in the wrong location. The battle to control metal location and availability is a key component of host-pathogen interactions. Key host immune strategies include starving pathogens of iron and zinc, and harnessing metals (mainly copper) to poison invading pathogens. Consequently, bacterial mechanisms that acquire iron and zinc, and those that expel excess copper are important for pathogenesis. The results from this study will be used to guide development of metal-based strategies for managing infections in a clinical setting.

The activities described herein (activities 1-7 below) form a connected programme of work that involve four mucosal pathogens:

a. uropathogenic Escherichia coli (UPEC)
b. Neisseria gonorrhoeae (GC)
c. Streptococcus pneumoniae (pneumococcus)
d. Streptococcus pyogenes (GAS)

UPEC and GC cause genitourinary infections while the pneumococcus and GAS cause respiratory infections. All four have all been identified as key threats to public health as a result of increased resistance to antibiotics (CDC 2013, WHO 2017).

Specific activities in this programme are:

1. Overexpression and purification of proteins (including artificial site-directed mutants) from the pathogens in an attenuated E. coli host to study protein function and activity ex vivo.
2. Subcloning of DNA fragments from the pathogens in attenuated E. coli cloning strains to aid activities 3-7 below.
3. Generation of pathogen mutants by allelic exchange using DNA fragments or shuttle vectors that carry the intended mutations and antibiotic markers.
4. Restoration of gene function in pathogen mutants by allelic exchange using DNA fragments or shuttle vectors that carry the wild type gene.
5. Restoration of gene function in pathogen mutants by expression of the wild type gene off the native or an inducible promoter (e.g. IPTG) on a shuttle vector.
6. Expression of an artificial reporter gene off native promoters on a shuttle vector to monitor gene expression.

Organisms for Activities 1-2 are attenuated (non-colonising) E. coli K-12 strains and are considered ACDP Hazard
Group 1 organisms. They have a long history of safe use in commercial and research settings.

Activity 1.
Cloning hosts (JM109, DH5a) and overexpression hosts (BL21, BL21(DE3), BL21(DE3) pLySS, BL21(DE3) Rosetta).

Activity 2.
Cloning hosts (JM109, DH5a).

Activities 3-6.
Virulent/pathogenic bacteria a-d as listed in Box 6 above (more details below). All are used widely in research worldwide to understand pathogenicity and antibiotic resistance traits.

a. UPEC (strains UTI89 and EC958).
UPEC causes urinary tract infections (UTI). UPEC can also exist within the human intestinal tract as part of the normal microbiota. Both clinical isolates used here have been sequenced. EC958 is the best characterised ST131 strain that has emerged rapidly and disseminated globally in recent years. It produces Extended Spectrum Beta Lactamases (ESBLs) and causes multidrug-resistant infections.

b. GC (strains 1291, FA19, FA1090).
The gonococcus causes the sexually transmitted infection gonorrhoea. Both of these clinical isolates are likely to be attenuated from decades of laboratory passage but we will assume that they remain virulent. Both have been sequenced.

c. Pneumococcus (strain D39).
The pneumococcus is normally found as a harmless commensal of the human upper respiratory tract but it may lead to invasive infections like pneumonia when the immune system weakens. The D39 strain is likely attenuated in the human host following decades of laboratory passage but this strain remains virulent in mouse models of infection and will be assumed as pathogenic to humans. This strain has been sequenced.

d. GAS (strain SF370, MGAS 5448).
GAS is a common causative agent for scarlet fever and strep throat. MGAS 5448 is an isolate of the hyperinvasive M1T1 strain that has been globally disseminated and is associated with life-threatening conditions.

Host/vector system

Activities 1-2.
Standard commercial non-mobilisable vectors that can be propagated in E. coli:

Activity 1.
cloning vectors (pUC, pT7, pGEM, and pBAD series) and overexpression vectors (pET series).

Activity 2.
cloning vectors (pUC, pT7, pGEM, and pBAD series) and shuttle/helper/complementation vectors described below for Activities 3-6.

Activities 3-6.
All vectors well established in the literature. They are non-mobilisable, ie. they can be propagated in E. coli but they are not stable in non-E. coli host pathogen.

Activity 3.
a. UPEC - helper plasmids encoding components of the lambda-red recombinase system (pKD46, pKD3, pKD4, pCP20).
b. GC - cloning vectors listed in Activity 2 above.
c. Pneumococcus - cloning vectors listed in Activity 2 above.
d. GAS - temperature-sensitive shuttle vector pHY304.

Activity 4.
Origin & function

SOURCES:
All recipient organisms and vectors, including those already carrying genetic modifications described here, will be supplied by Dr Karrera Djoko from the University of Queensland (Australia). Additional genetic modifications will be generated at Durham.

ORIGINS:
Activity 1.
Partial and full-length genes from the pathogens.
Activity 2.
Partial and full-length genes from the pathogens.
Activity 3.
Partial and full-length genes from the pathogens.
Activity 4.
Full-length genes from the pathogens.
Activity 5.
Full-length genes from the pathogens.
Activity 6.
Common reporter genes that are unlikely to have any biological effect in the recipient organism (eg. betagalactosidase gene, green fluorescent protein gene)

FUNCTION:
Genes of interest in this programme are those involved in metal homeostasis, namely the import, sequestration, and export of iron, copper, zinc, or manganese. These genes encode:
- Transmembrane transporters that allow metal ions to enter or exit the bacterial cytoplasm or periplasm.
- Metal-responsive transcription factors that control the expression of metal homeostasis genes.
- Soluble and membrane-anchored metal carriers that capture metal ions from the solvent and deliver them to target enzymes or transporters.
- Soluble and membrane-anchored proteins that store metal ions.
- Enzymes that require metal ions for activity.
**HUMAN HEALTH:**

For all activities, no combination of vector, host, and insert is considered more hazardous than non-GM host.

Activities 1-2.

No risk to human health. These activities fall into hazard/containment level 1.

Activities 3-6.

The pathogens used in these activities fall into hazard/containment level 2. The activities will not produce a GMO that is any more hazardous than the wild type. The likelihood of harm to human health in the unlikely event of exposure to GM strains will be no worse than exposure to non-GM strains.

Activity 3.

The intended mutations are likely to decrease the fitness of the pathogen and not increase it. Studies have shown that metal homeostasis genes are important for the pathogenesis of all four organisms described here. Disruption of the genes usually leads to attenuation of the pathogen in human or animal model hosts of infections. So far, there is no indication that deletions of metal homeostasis genes will lead to increased fitness of the pathogen in animal model hosts.

The intended genetic modifications will be introduced in the chromosome and not on mobile genetic elements. It is expected that the mutations will be confined in the GMO and not be transferred to other organism.

In the case of an accidental exposure, antibiotic resistance markers will not affect the clinical management of infections caused by the pathogens. Antibiotics used as selection markers (ampicillin, kanamycin, tetracycline, chloramphenicol, erythromycin, spectinomycin, gentamicin) are widely used in research. They are already widely disseminated in the environment, are not clinically relevant to the pathogens, or are obsolete and are no longer in clinical use. Previous studies have not found that the presence of these antibiotic markers in metal homeostasis mutants confer any advantage in the fitness of pathogens in animal model hosts of infections.

Activities 4-5.

These activities will simply reintroduce a gene that was originally present in the wild type pathogen but had been removed by mutagenesis in Activity 3. The resulting GMO will not be any more hazardous than the wild type.

Activity 6.

Insertion of the reporter gene (β-galactosidase, green fluorescent protein) into the recipient organisms described here is common and has not been reported to increase fitness or pathogenicity. The resulting GMO will not be any more hazardous than the wild type.

**ENVIRONMENTAL HEALTH:**

For all activities, no combination of vector, host, and insert is considered more hazardous than non-GM host.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The activities will generate a mixture of solid and liquid waste, including live bacterial cultures, spent media, and contaminated paper towels, gloves, plasticware, and glassware.

All waste will be decontaminated (100% kill) using steam sterilization (autoclaving) at 120°C for at least 30 min. The autoclave will be checked monthly with an accepted biological indicator (e.g., *Geobacillus* or *Bacillus* spores).
commercially available) or more frequently during periods of heavy use. The autoclave will be serviced annually.
Each run will be monitored by a continuous chart or digital recording of the temperature and time. After autoclaving,
solid will be disposed in the clinical waste stream for incineration and landfill using a licenced contractor approved
by the university. Autoclaved liquid waste will be discharged to drains.
Protective clothing (lab coats or gowns) will be autoclaved monthly before laundering using a licenced contractor
approved by the university.
All working surfaces (benches, equipment, tools) will be decontaminated by chemical disinfection using 2% Virkon
(100% kill) or 70% ethanol (100% kill). Accidental spills will be first mopped up with a paper towel and the surface
will be decontaminated with 2% Virkon. The paper towel will be autoclaved before disposal in the clinical waste
stream.

Please enter comments on the GM safety committee on the risk assessment

The Durham GM safety committee noted comments made by the University of Queensland safety committee on
this work regarding the provision of information sheets detailing the antibiotic susceptibility and resistance
characteristics of the organisms involved in this application.
The Durham GM committee commented that clarification was needed regarding one of the proposed methods of
preparing material for autoclaving, as there was a risk that the method described would prevent steam penetration
and hence adequate sterilisation of all parts of the load. A revised procedure that addressed these concerns was
produced.

Project Containment

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Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

BREWING RESEARCH INTERNATIONAL

Name 2

BREWING RESEARCH FOUNDATION

Campus Estate or Research Centre

Building

LYTTEL HALL

District

NUTFIELD

Town

REDHILL

County

SURREY

Postcode

RH1 4HY

Country

ENGLAND

Tel Number 01737 822 272

Fax Number 01737 822 747

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

UNIVERSITY OF CAMBRIDGE

**Name 2**

GENETICS

**Campus Estate or Research Centre**

DOWNING STREET

**Road Name**

CAMBRIDGE

**Town**

CAMBRIDGESHIRE

**County**

CB2 3EH

**Postcode**

ENGLAND

**Country**

**Tel Number**

01223 333999

**Fax Number**

01223 333992

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Tick if confidential

02/03/2022
All waste is autoclaved. 15lbs 15 mins. Autoclaves routinely tested with meters and live organisms.

For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste is autoclaved. 15lbs 15 mins. Autoclaves routinely tested with meters and live organisms.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 42/16.1

Date Ackn'd
20/05/2016

Date Project Ceased

CU2 Project Title
Expression of oncogenes and cell signalling genes in cultured human keratinocytes by retroviral transduction

Class
Class 2

CultureVol
< 1 Litre

Class CultureVol
Class 2 < 1 Litre

Non-GMM
Consent Granted

Historical Significant Changes

Tick if notifying a connected programme of work
N
**Project Additional Information**

### Purposes of the contained use

Project MF02/16: The goal of this research is to isolate, propagate and characterise stem cells from adult human tissues and tumour tissues in order to generate in vitro-ex vivo model systems to study self-renewal differentiation and tumorigenesis.

Project FMW03/2016: The goal of this project is dissect the mechanisms involved in the differentiation of stem cells in the human epidermis.

### Recipient or parental organism

Projects MF02/16 and FMW02/2016:
- E. coli (e.g. DH10B, Stabl2, 4) or derivatives.
- Helper-free retrovirus producer cell line Phoenix-eco.
- Mouse cell line GP + envAm12.

Project MF02/16:
- Human primary cells and primary tumour cells.

Project FMW02/2016:
- Mouse embryonic stem lines (established over 25 years).
- Epithelial tumour cell lines (commercial available with a history of safe usage).
- Human keratinocyte cell lines derived from normal infant skin from screened healthy individuals having a negligible risk of contamination with adventitious human pathogens.
- Primary human keratinocytes purchased from established sources (for instance Lonza; http://www.lonza.com).

### Host/vector system

- E. coli / standard mammalian expression vectors such as pGL, pcDNA or commercially available derivatives
- Human Cell Lines / non-mobilisable viral plasmid vector pBabe (puro) and derivatives, based on Moloney Murine Leukaemia Virus or commercially available lentivirus vectors (such as pRRLSIN_cPPT.PGK-GFP.WPRE).
- Human Cell Lines / pSuperRetro or lentiviral vectors (such as pLKO, pLentiCRISPRv2 and their commercially available derivatives)
Projects MF02/16 and FMW03/2016:
- Genes with autocrine pathogenic effect, some of which are classified as oncogenes or tumour suppressor genes:
  - Dominant negative and activated forms of Myc, β-catenin, Tef/Lef, MEK1, Ras, Raf, Rac, Rho, cdc42, PI3K, Klf4,
  - Oct4, Sox-2, p53, histone methyltransferases, and splice factors.
- Genes with paracrine pathogenic effect, some of which are classified as oncogenes or tumour suppressor genes:
  - Wild-type and mutant constructs of alpha - and [I, integrin subunits, Ephrins, and E-cadherin, components of the
  - Hedgehog signalling pathway (Serrate, Delta, Notch, Sonic Hedgehog, Indian Hedgehog, Patched), TGF-signalling
  - Pathway (Smads), EGF-signalling pathway (LRIG).
- Genes or non-coding RNA genes with poorly characterised pathogenic effects: NSun2, NSun3, NSun6, SRSF2,
  - 7SK, and Vault RNAs.
- Genes with no growth-promoting capacity e.g. GFP, dsRED, tdTomato, confetti, structural genes.
All DNA will be propagated in E coli (e.g. DH10B or derivatives).
The plasmid will first be transfected into an ecotropic packaging cell line, Phoenix-eco, and the retrovirus produced
will be harvested over 1-2 days.
The helper-free retrovirus producer cell line Phoenix-eco, was developed by Dr. G. Nolan at Stanford University,
(http://www.stanford.edu/group/nolan/index.html) and has been used by over 2500 labs. Phoenix-eco cells express
the gag-pol and envelope genes using different non-Moloney promoters to minimize both recombination and intertypic
recombination potential. This cell line has a history of safe usage.
This ecotropic retrovirus will then be used to stably infect a second packaging cell line, GP + envAm12, which
produces disabled amphotropic retrovirus. The mouse cell line GP + envAm12 has been widely used for the
production of amphotropic virus. Tests for the safety of the GP + envAm12 packaging line showed no evidence for the
generation of wild type virus and it has a history of safe usage. Markowitz, Goff and Bank. Virology 167,400-406
(1988). Defective amphotropic virus would then be used for the infection of human keratinocytes. Transduced cells
would be selected and maintained in medium containing an appropriate antibiotic before further use in experiments.
All genes will be inserted downstream of the viral LTR with the intention of achieving a moderate level of expression in
recipient cells. Infection of keratinocytes will be by transfer of supernatant culture fluids without any concentration of
the virus particles.
Lentiviral vectors will be used to deliver transgenes directly into a variety of dividing or nondividing cell types. These
vectors are commercially available and have a history of safe use. The components necessary for virus production
are split across multiple plasmids (3 for 2nd-generation systems, 4 for 3rd-generation systems)
(http://www.addgene.org/viral-vectors/lentivirus/lenti-guide/).
Recipient human cell lines expressing the individual genes under study will be created which are likely to have some
growth advantage or induce increased proliferation in an autocrine or paracrine manner.

Evaluation of foreseeable effects
Recipient human primary cells, human primary tumour cells, human keratinocytes and established cell line
expressing the individual genes under study will be created which are likely to have some growth advantage or
induce increased proliferation in an autocrine or paracrine manner. Alternatively, modified human primary cells,
primary tumour cells and human keratinocytes could be induced to terminally differentiation.
Bacterial strains of E coli such as DH10B and its derivatives are not capable of colonising a healthy individual.
The amphotropic retrovirus is able to infect cells from a broad host range, including humans but can not replicate
unless it recombines with endogenous retrovirus. Exposure of an individual to amphotropic retrovirus, by possibly a
break in the skin, could result in a localised transfer of the pathogenic properties of the particular gene. None of the
cell lines would be expected to remain viable outside the controlled culture conditions and we do not anticipate that
any of the genetic manipulations we are proposing would alter this. It is predicted that over-expression of oncogenes and signalling molecules could lead to an increased rate of cell division although tumour progression and disease in the whole organism is unlikely. The major possible source of hazard to the environment would be accidental escape of high titre virus solutions expressing cDNAs, which could then infect rodents. Retroviruses require close contact for their transmission and their survival in the general environment is poor due to their fragile envelope. The vector is non-mobilisable and the successful infection of a cell would include stable integration of the viral genes into host DNA. Although the amphotropic virus would be able to infect humans it could not replicate unless it recombined with endogenous retrovirus. Even then it is unlikely that expression of a single oncogene or inactivation of a tumour suppressor would cause tumours, as multiple genetic alterations (between 4 and 6) are required for inducing tumours, and safeguard mechanisms exist impeding the accumulation of these alterations in the organisms (i.e. single events like Ras or myc expression instead of unrestrained proliferation, induce growth arrest or apoptosis) (Hanahan&Weinberg, Cell 100, 57-70; 2000).

Lentivirus can infect human dividing and non-dividing cells. The potential for generation of replication-competent lentivirus is minimized by the design of the vectors and by safe laboratory practice. The transfer vector encodes the gene of interest and contains the sequences that will incorporate into the host cell genome, but cannot produce functional viral particles without the genes encoded in the envelope and packaging vectors. Unless recombination occurs between the packaging, envelope, and transfer vectors, and the resulting construct is packaged into a viral particle, it is not possible for viruses normally produced from these systems to replicate and produce more virus after the initial infection. Many of the lentiviral transfer vectors are self-inactivating (SIN) vectors. These vectors have a deletion in the 3'LTR of the viral genome that is transferred into the 5'LTR after one round of reverse transcription. This deletion abolishes transcription of the full-length virus after it has incorporated into a host cell.

The use of sharps will be strictly controlled and avoided wherever possible. Needles and scalpels will not be permitted. Plastic Pasteur pipettes will be used for aspirating culture medium.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable. Generation of larger GMOs (animals/plants) is not proposed.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be sealed in double autoclave bags and transported to the Departmental autoclave where it will be exposed to steam at 1270°C for 25 minutes. The Departmental autoclave has effectively 100% kill as validated by the annual testing certificates which are held by the Department. Printed readouts from each run will be retained to ensure temperatures within the autoclave were maintained during the cycle. All liquid waste will be inactivated by treatment with Anigene at a final concentration of 10% before disposal via the drains.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Risk Assessments were reviewed by the Department of Genetics Biological Safety subcommittee including Mark Elsdon (Biological Sciences School Safety Officer) as well as Keff Tibbles (Clinical School Safety Officer) who has a background in virology and a special interest in Lentiviral Vector safety. Comments were returned to Michaela Frye's group who modified the Risk Assessments appropriately. Similar project work has been undertaken with HSE approval by Michaela Frye and her research group at the Wellcome Trust-MRC Cambridge Stem Cell Institute since 2007.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
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- **Animal Units**
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  - L3
  - L4

- **Large Scale Activities**
  - L2
  - L3
  - L4

- **Human Clinical Applications**
  - L2
  - L3
  - L4
### UNIVERSITY OF CAMBRIDGE

#### Name

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### Comments

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
- Virology
- Transgenic
- Animals
- Transgenic
- Fish
- Gene Therapy
- Mycology
- Transgenic
- Invertebrates
- Transgenic
- Plants
- Other (please specify below)
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

<table>
<thead>
<tr>
<th>Project Ref</th>
<th>159/15.1</th>
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<td>CU2 Project Title</td>
<td>Transformation of Chlamydia trachomatis</td>
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**Project Additional Information**

**Purposes of the contained use**

The obligate intracellular bacterium Chlamydia trachomatis has been considered genetically intractable. However,
there have been multiple recent reports of transformation from different laboratories using recombinant plasmids based on hybridisation of E.coli and the endogenous Chlamydia trachomatis plasmids. We intend to use these vectors to express epitope-tagged or fluorescent fusion proteins in Chlamydia trachomatis to investigate the localization of virulence effectors and components of the type III secretion system biochemically and using imaging techniques.

Recipient or parental organism

The following aspects of this project are assigned to Class 1.
Cloning and manipulation of the vectors and their propagation in E.coli strains
The following aspects of this project are assigned to Class 2.
Transformation and propagation of transformed C.trachomatis within cultured mammalian cells.

Host/vector system

Wild-type Chlamydia trachomatis (this is a Biosafety level 2 organism and not disabled)
E.coli DH5α and XL-1 Blue will be used for plasmid propagation and sub-cloning procedures. These are E.coli K12 derivatives and are classified as ‘disabled and non-colonising’ by the ACGM.
X11-Blue recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F ’ proAB lacIqZΔM15 Tn10 (TetR)]
DHSalpha F- phi80lacZ::M15 delta(lacZYA-argF)U169 deoR recA1 endA1 hsdR17 (r- m+) phoA supE44 thi-1
 gyrA96 relA1 lamdA pASKGFPL2
 pTRL2-GFP
 pCDSSKO
 placZ-CD55KO

Origin & function

We plan to study the localisation and function of C.trachomatis proteins encoding components of the type III secretion system (inner and outer membrane protein, ATPase, translocon and needle) and the substrates, which are delivered into the boundary membrane of the replicative vacuole (hydrophobic inclusion proteins), host cytosol or nucleus.
These genes will be amplified by PCR from chromosomal DNA of C-trachomatis held in our laboratory or by gene synthesis (as some genes are exceptionally difficult to amplify by PCR). Epitope tags will be added to the sequence as part of the PCR primer or synthesised product (e.g. FLAG, Myc) when required. In the case of fusion proteins to MBP, GFP, mKate2 these will be amplified from existing commercial vectors (e.g. pEGFP-C2; Clontech) or obtained from collaborating laboratories where necessary.

Evaluation of foreseeable effects

E.coli. will be used to make the expression constructs. The Chlamydia trachomatis will be used to express the various components of the epitope-tagged or fluorescent fusion proteins to investigate the localization of virulence effectors and components of the type III secretion system biochemically and using imaging techniques. The GMOs themselves will not, in our assessment, cause any additional harm to human health above the virulence of the wild-type organism. Chlamydia elementary bodies survive for only a very short time outside their host cells (30 minutes to 1 hour), so risks from environmental release do not significantly exceed those of the wild-type organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
The control systems in place are designed to ensure that any contaminated waste is efficiently sterilised and any GMOs killed. There are two main routes for the sterilisation of laboratory waste to be used in this notification. All liquid and solid laboratory waste will either be incubated with 1% Virkon for at least 30 minutes or will be autoclaved on the same day using validated conditions. Each autoclave cycle is validated with the inclusion of a thermolog S strip to monitor that the target temperature for killing the GMOs has been successfully achieved. The sterile, solid autoclaved material can then be disposed of via our normal waste route. The 1% Virkon treated sterile liquid waste would go down the sink route.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The risk assessment entitled "Transformation of Chlamydia trachomatis" was considered at the College GM safety committee on Wednesday 29th October 2014. The committee approved the risk assessment level at GM category 2 and agreed that it should be forwarded to the HSE for notification and consideration with the appropriate fee.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment entitled "Transformation of Chlamydia trachomatis" was considered at the College GM safety committee on Wednesday 29th October 2014. The committee approved the risk assessment level at GM category 2 and agreed that it should be forwarded to the HSE for notification and consideration with the appropriate fee.

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Project Ref 43/01.1

Date Ackn'd 05/01/2001  CU2 Project Title IDENTIFICATION OF NATURAL KILLER CELL ACTIVATING LIGANDS  Class: Class 2  CultureVolClass2: < 1 litre  CultureVolumeClass3-4:
Natural killer cells are a population of peripheral blood lymphocytes able to lyse certain virus-infected and tumor cells in a non-MHC restricted fashion. Normal human fibroblasts are not lysed by human NK cells, but the same fibroblasts infected with e.g. HSV are attacked and killed by NK cells. The aim of this project is to directly test the hypothesis that the expression of incorrectly folded MHC Class I molecules at the cell surface is one of the mechanisms by which a given target cell can become susceptible to attack and lysis by human Natural Killer cells.

Recipient or parental organism

The recombinant organism to be used in the course of this work include mutants of Herpes Simplex Virus (HSV)-1 (all of which are at least gH and thus unable to produce infectious virions) and some recombinant vaccina viruses (derived from strain WR, but thymidine Kinase deficient).

Host/vector system

The mutant and recombinant viruses will be used to infect in vitro maintained cultures of human foreskin fibroblasts and the human B cell line 721.221 and various Class I MHC transfectants of the parental cell line.

Origin & function

The mutant HSV strains were provided by Dept. of Pathology, University of Cambridge and MRC Virology Unit, Glasgow. The recombinant vaccina viruses were gifts of NIH and University of Cambridge Vet School.

The aim of this work is to directly test the hypothesis that the expression of incorrectly folded MHC Class I molecules at the cell surface is one of the mechanisms by which a given target cell can become susceptible to attack and lysis by human Natural Killer cells. Expression of incorrectly folded MHC Class I molecules at the cell surface is one of the effects of the protein product of the HSV gene ICP-47, thus the hypothesis can be tested by examining:

1. Does infection with mutant HSV strains lacking ICP-47 still result in enhanced susceptibility to NK cell lysis?
2. Is the introduction of solely the ICP-47 gene, via the recombinant vaccina, sufficient to cause enhanced susceptibility to NK cell lysis?
The work proposed would not include the use of experimental animals and would be carried out in the ACGM category 2 tissue culture suite in the department under the guidelines for good practice laid out in the ACGM compendium of guidance. Accidental ingestion, inoculation or aerosol exposure represent possible routes of laboratory worker contamination and thus these viruses will only be used in category 2 (operator safety) microbiological cabinets by experienced workers wearing gloves and labcoats. No sharps should need to be used during these experiments.

The HSVs to be used in these experiments can be considered replication defective, by virtue of lacking the gH protein and the probability of this mutation being complimented in vivo by a wild-type virus can be estimated as being very low. The experiments with HSV are extremely likely to pose any risk to the environment. Humans are the only natural host for HSV and the virus is extremely labile thus the overall risk can be considered to be effectively zero.

The recombinant vaccina viruses have been attenuated by loss of the TK locus and the likelihood of recombination in vivo is low. Moreover although the recombinant vaccina expressing ICP-47 might be considered to have an increased ability to evade recognition by cytotoxic T cells (CTL), it is worth pointing out that even when ICP47 isexpressed as an immediate early gene product in HSV infection a substantial CTL response to HSV can still be generated. Further, a neutralising antibody response, which ICP47 expression would not be expected to affect, is a significant component of the immune response to vaccina virus. Finally, all evidence that is isolated down-regulation of MHC Class I glycoproteins increases immune rejection of tumor cells through the action of NK cells. The other recombinant vaccina expresses the p25 gag gene of maedi-visna virus, a virus infection of sheep. The protein product of this gene forms the core of the visna-virus particle. Expression of this gene product would not be expected to alter any of the pathogenic characteristics of the vaccina.

Vaccina has been extremely widely used as a vaccine vector for both man and animals and the accumulated experience indicates that although the virus can survive long periods for example in dried scabs, the virus is unlikely to become established in the wild. However to minimise the risk of escape the virus will only be used in the ACGM category 2 tissue suite in the department under the guidelines for good practice laid out in the ACGM compendium of guidance. This should minimise any risk to the environment and thus the overall risk is low.

### Evaluation of foreseeable effects

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| None |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid and solid waste will be autoclaved, this should result in effectively 100% kill of all microorganisms. As a precautionary measure before autoclaving, liquid waste will be treated with 1% vircon. Validation of kill via autoclaving will depend on regular testing of the autoclave installed in the ACGM category 2 tissue culture suite in the department.

**Is an emergency plan required according to regulation 20?**

| N |

**If yes, tick to confirm that it is attached to this form**

| N |

**Tick to confirm that you have attached a risk assessment to this form**

| Y |

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

| N |
Project Additional Information

Purposes of the contained use

There are two objectives:

1. to design cellular immune assays using recombinant adenoviruses to determine cytotoxic T cell responses to papillomavirus antigens.
2. to investigate the effect of papillomavirus proteins on the eukaryotic cell cycle in vitro.

Recipient or parental organism

The parental organisms are the canine oral papillomavirus COPV and human papillomavirus types 6, 16, 18. The recipient organism is a replication defective adenovirus.
**Host/vector system**

Vector amE1 deleted replicator defective adenovirus (Ad-5).

**Origin & function**

COPV early genes. - The E6, E7, E2, E1 and E4 genes of COPV are cloned from COPV DNA. The COPV isolate was derived from a clinical case of oral warts in a retriever. The virus has not been passaged in vitro, generates warts in beagle dogs, does not transform cells in culture. The early genes will be expressed via a replication defective adenovirus to generate peptide fragments for recognition by T cells.

HPV 16, 6, 18 - The E2, E1, E6 and E7 genes are cloned from reference DNAs originally isolated from clinical samples. These genes will be expressed in replication defective adenoviruses. The viruses will be used to infect cells in tissue culture to determine the effects of the HPV genes on the eukaryotic cell cycle.

**Evaluation of foreseeable effects**

Vector - an E1 deleted replication defective adenovirus (Ad-5). This modified virus cannot initiate a full replication cycle and there is no survival value. Recombinant viruses expressing COPV proteins - COPV is not an oncogenic papillomavirus, there is no evidence that any COPV protein delivered via an oxogenous vector can immortalise human cells. COPV does not induce carcinoma in the natural host. Recombinant viruses expressing HPV proteins - HPV 16 and 18 are oncogenic viruses, encoding the oncogenes E6 and E7. Co-expression of both proteins is required for immortalisation of human cells in vitro. Expression of E6 or E7 alone in primary cells results in apoptosis. Experiments will involve the use of adenoviruses expressing either E6 or E7 or E1 or E2. The recombinant adenoviruses cannot initiate a full replication cycle and there is no survival value. All work is small scale using standard laboratory procedures. Replication defective adenoviruses are Class I GMOs. The insertion of the HPV genes with the properties detailed above raises these GMOs to Class 2. Containment is therefore at level 2. The identifiable risk is infection of the laboratory worker by aerosol; therefore all work is done in a laminar flow class II hood. Full containment level 2 requirements will be applied and the risk to human health and the environment is minimal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The identifiable risk is infection of the laboratory worker by aerosol, therefore all manipulations with recombinant viruses are conducted in a Class II laminar flow hood in a restricted culture facility under negative pressure. Air is exhausted to the exterior through HEPA filters. The restricted culture facility is for work at Class 2 level containment only.

Gloves, face masks and protective clothing are worn within the facility. Soiled and used protective clothing is double bagged and autoclaved within the facility before disposal. Glassware is avoided wherever possible and disposable pipettes, tubes, culture flasks etc are employed. Used disposables are double bagged in autoclave bags and autoclaved within the facility before disposal. Sharps are disposed in sharps bins that are autoclaved within the facility before disposal.

Infected media is collected in aspirator traps containing available chlorine at 2000 ppm [100ml chloros/litre or 5 klorsept 7 (Jencons) tablets/litre]. Any glassware is immersed fully in a solution containing available chlorine at 2000 ppm for 2 hours.

Spillages are disinfected with Klorsept granules which contain and absorb the spill.
All the work involves standard laboratory procedures on a small scale (maximum culture volume/flask 150 ml). The level 2 containment and control measures in place make it improbable that the GMOs will reach the environment and cause harm to humans or animals outside the laboratory.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Our Genetic Modification Safety Committee have agreed it is Class 2 containment.

Project Containment

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Project Ref 43/01.3

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<th>Class</th>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info
### Project Additional Information

**Purposes of the contained use**

To investigate the functions of influenza virus gene products in virus replication and pathogenesis.

**Recipient or parental organism**

Vaccina virus - coding sequences derived from influenza viruses.
Influenza virus - 'reporter genes' (e.g. LacZ, firefly luciferase, green fluorescent protein) coding sequences derived from influenza virus genes

**Host/vector system**

Eukaryotic viruses (ACDP group 2)/mammalian and avian (established, commercially available).

**Origin & function**

**Origins:** Sequences will be sub-cloned from pre-existing plasmid clones. In some instances influenza virus sequences will be isolated from purified viral RNA.

**Functions:** The biological properties of influenza virus genes are considered in the detailed risk assessment. These genes will be expressed in eukaryotic tissue culture cell lines (via eukaryotic vectors) to examine the location, trafficking and function of influenza virus proteins, or to provide helper functions for disabled influenza viruses.

'Reporter genes' will be inserted into influenza virus genomes to enable tracking of the tagged viruses.

**Evaluation of foreseeable effects**

Influenza virus genes act in concert to achieve virus replication. Some of the gene products are known to be cytotoxic but these are not secreted and only the expressing cell is expected to be affected. Influenza viruses are not oncogenic and transforming genes have not been identified in these viruses. Influenza virus gene function is, however not fully understood and some influenza virus genes are known to modify host immune responses. The eukaryotic vectors used are either heavily attenuated or replication incompetent in mammalian hosts. No change in tissue tropism or host range of the vectors expressing influenza virus genes is foreseen (see detailed risk assessment). The assessment to human health indicated no greater hazard from those of the wild type unmodified virus.

The insertion of 'reporter genes' into the influenza virus genome is likely to either reduce the replication capacity and virulence of the recipient virus or to leave it unchanged. No increase in virulence, alteration of tissue tropism or modification of host range is predicated.

The creation of replication competent reassortant influenza viruses might conceivably result in increased virulence or host-range properties. However, the recipient and donor viruses are all of low pathogenicity for humans. In addition, reassortant influenza viruses arise continually in nature and there is also a long history of their safe creation in the laboratory. As discussed in the detailed risk assessment (attached), the risk of creating a virus with increased pathogenicity is extremely low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

02/03/2022
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard validated methods will be used:

Liquids: made 2000ppm available chlorine (5% chloros) and disposed via drains after a minimum of 12 hours.

Mixed disposable waste: Validated autoclave, 121 degrees/30 min, then disposed as domestic waste.

Disposable pipettes: Either a) as for mixed disposable waste or b) immerse in solution of 2000ppm available chlorine (5% chloros) for minimum 12 hours, followed by incineration.

Re-usable materials: Autoclave, 121 degrees/30 min.

I had forgotten to inform you that the local ACGM committee had reviewed and approved the 6 applications.

Project Containment

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Animal Units

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Project Ref 43/01.4

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**Project Additional Information**

**Purposes of the contained use**

To investigate the functions of herpesvirus gene products in virus replication and pathogenesis.

**Recipient or parental organism**

- Adenovirus 5, Amphotropic retrovirus, Vaccinia virus: coding sequences derived from mammalian alpha or beta herpesviruses.
- Herpes simplex virus, Human cytomegalovirus: 'reporter genes' (eg LacZ, firefly luciferase, green fluorescent protein).

**Host/vector system**

Eukaryotic viruses (ACDP group 2)/mammalian cell lines (established, commercially available).

**Origin & function**

- Origins: Sequences will be sub-cloned from pre-existing plasmid clones. In some instances herpes virus sequences will be isolated from purified viral DNA.
- Functions: The biological properties of herpes virus genes are considered in the detailed risk assessment. These genes will be expressed in mammalian cell lines (via eukaryotic vectors) to examine the location and trafficking of herpes virus proteins, or to provide helper functions for disabled herpesviruses. 'Reporter genes' will be inserted into herpesvirus genomes to inactivate specific virus genes and to identify the resulting disabled or attenuated viruses.

**Evaluation of foreseeable effects**

The insertion of 'reporter genes' into viral genomes is expected to reduce the replication capacity of the recipient virus or to reduce its virulence. No increase in virulence, alteration of tissue tropism or modification of host range is predicted.

Herpesvirus genes act in concert to achieve virus replication. Some of the gene products are known to be cytotoxic but these are not secreted and only the expressing cell is expected to be affected. Mammalian alpha and beta herpes viruses are not oncogenic and transforming genes have not been identified in these viruses. Herpesvirus gene function is, however not fully understood and some herpesvirus genes are known to evade or modify host immune responses. The eukaryotic vectors used are either fully disabled or heavily attenuated. No change in tissue tropism or host range of the vectors expressing herpesvirus genes is foreseen (see detailed risk assessment). The assessment to human health indicates no greater hazard than those of the wild type unmodified virus.
**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Standard validated methods will be used:

- **Liquids:** made 2000ppm available chlorine (5% chloros) and disposed via drains after a minimum of 12 hours.

- **Mixed disposable waste:** Validated autoclave, 121 degrees/30 min, then disposed as domestic waste.

- **Disposable pipettes:** Either a) as for mixed disposable waste or b) immerse in solution of 2000ppm available chlorine (5% chloros) for minimum 12 hours, followed by incineration.

- **Re-usable materials:** Autoclave, 121 degrees/30 min.

**Is an emergency plan required according to regulation 20?**

- **Yes**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- **No**

**Tick to confirm that it is attached to this form**

- **No**

**Tick to confirm that you have attached a risk assessment to this form**

- **Yes**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- **No**

**Please enter comments on the GM safety committee on the risk assessment**

I had forgotten to inform you that the local ACGM committee had reviewed and approved the 6 applications.

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</table>
**Project Additional Information**

**Purposes of the contained use**

To investigate the functions of herpesvirus gene products in virus replication and pathogenesis.

**Recipient or parental organism**

- Adenovirus 5, Amphotropic retrovirus, Vaccinia virus: coding sequences derived from mammalian alpha, beta or gamma herpesviruses, murine cellular genes.
- Murine gamma-herpesvirus 68 (MHV-68): 'reporter genes' (e.g., LacZ, firefly luciferase, green fluorescent protein) coding sequences derived from MHV-68.

**Host/vector system**

Eukaryotic viruses (ACDP group 2)/mammalian cell lines (established, commercially available).

**Origin & function**

**Origins**: Sequences will be sub-cloned from pre-existing plasmid clones. In some instances, herpes virus sequences will be isolated from purified viral DNA or amplified from genomic DNA/cDNA by PCR.

**Functions**: The biological properties of herpes virus genes are considered in the detailed risk assessment. These genes will be expressed in mammalian cell lines (via eukaryotic vectors) to examine the localisation and function of herpesvirus proteins, or to provide helper functions for disabled herpesviruses. 'Reporter genes' will be inserted into herpesvirus genomes to inactivate specific virus genes and to identify the resulting disabled or attenuated viruses. MHV-68 coding sequences will be re-inserted back into the MHV-68 genome in order to rescue disabled knockout viruses.
The insertion of 'reporter genes' into viral genomes is expected to reduce the replication capacity of the recipient virus or to reduce its virulence. No increase in virulence, alteration of tissue tropism or modification of host range is predicted.

Herpesvirus genes act in concert to achieve virus replication. Some of the gene products are known to be cytotoxic but these are not secreted and only the expressing cell is expected to be affected. We have not found MHV-68 to be an oncogenic virus in vivo or in vitro, and the equivalents of other gamma-herpesvirus transforming genes have not been reliably identified. Herpesvirus gene function is not fully understood and some herpesvirus genes are known to evade or modify host immune responses. The eukaryotic vectors used are either fully disabled or heavily attenuated. No change in tissue tropism or host range of the vectors expressing herpesvirus genes is foreseen (see detailed risk assessment). The assessment to human health indicates no greater hazard than those of the wild type unmodified virus.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard validated methods will be used:

- **Liquids**: made 2000ppm available chlorine (5% chloros) and disposed via drains after a minimum of 12 hours.
- **Mixed disposable waste**: Validated autoclave, 121 degrees/30 min, then disposed as domestic waste.
- **Disposable pipettes**: Either a) as for mixed disposable waste or b) immerse in solution of 2000ppm available chlorine (5% chloros) for minimum 12 hours, followed by incineration.
- **Re-usable materials**: Autoclave, 121 degrees/30 min.

### Is an emergency plan required according to regulation 20?  

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

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### Project Containment

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02/03/2022
CLONING AND EXPRESSION OF GENES OF VIRAL, HUMAN, MOUSE OR PARASITE ORIGIN AND OTHER RELATED ACTIVITIES (TRANSFERRED TO GM353)

Purposes of the contained use

1. Identification of viral proteins that block various aspects of the host immune response.
2. Characterisation of the activity of viral and parasite proteins in vitro, in binding assays to several ligands and functional analysis, and comparison with the properties of the human or mouse counterparts.
3. Understanding the role of viral proteins and their cellular counterparts in viral pathogenesis and immune modulation in a mouse model of infection.

Recipient or parental organism

Vaccinia virus and amphotropic retroviruses expressing reporter genes (eg LacZ, firefly lucerase, green florescent protein), selectable genes that confer resistance to specific compounds (EcoGpt, puromicin resistance gene) or the ability to grow in a rabbit cell line (vaccinia virus K1L gene), or coding sequences derived from viruses (poxviruses, herpesviruses), parasites (leishmania, schistosoma), humans or mice.

Ectromelia viruses expressing (i) reporter genes (eg LacZ, firefly lucerase, green florescent protein), (ii) selectable genes that confer resistance to specific compounds (EcoGpt, puromicin resistance gene), (iii) gene from proxviruses or herpesviruses or (iv) mouse or human genes.
Host/vector system

Eukaryotic viruses (ACDP group 2).

Ectromelia virus (ACDP group 1).

Mammalian cell lines (established, commercially available) (ACDP group 1).

Origin & function

Origins: Sequences will be amplified by polymerase chain reaction using viral, parasitic or cellular DNA as a template, or will be subcloned from pre-existing plasmid clones.

Function: The biological properties of the genes are considered in the detailed risk assessment. These genes will be expressed in mammalian cell lines (via eukaryotic vectors) to examine the location and function of proteins. Reporter and selectable genes will be inserted into viral genomes to inactivate specific viral genes and to identify the resulting recombinant virus.

The biological effects derived from inactivation of genes in proxviruses (vaccinia or ectromelia viruses) or from the replacement of ectromelia virus genes by homologous genes will be determined in vivo in a mouse model of infection.

Evaluation of foreseeable effects

The insertion or reporter or selectable genes into viral genomes is expected to reduce the replication capacity of the recipient virus or to reduce its virulence. No increase in virulence, alteration of tissue tropism or modification of host range is predicted.

The eukaryotic vectors used (vaccinia, retrovirus) are either fully disabled or heavily attenuated. No change in tissue tropism or host range of the vectors expressing foreign genes is foreseen (see detailed risk assessment). The assessment to human health indicated no greater hazard than those of the wild type unmodified virus.

Whereas vaccinia virus and cowpox virus can replicate in a number of mammalian species, ectromelia virus is restricted to mice. Recombinant poxviruses lacking specific genes are expected to be attenuated and to transmit poorly between individuals. No change in tissue tropism or host range of the vectors expressing foreign genes is foreseen (see detailed risk assessment). The assessment to human health indicates no greater hazard than those of the wild type unmodified virus.

The ectromelia virus mutants lacking immunomodulatory genes are expected to be attenuated. Insertion into ectromelia virus mutants of related genes from other proxviruses or herpesviruses, or mouse/human genes, with similar functions, is unlikely to increase ectromelia virus virulence to levels higher than wild type. However, if an increased virulence is observed, it is unlikely to change the tissue and host tropism of ectromelia virus (highly restricted to mice), and therefore to represent a greater hazard to human health than wild type ectromelia virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard validated methods will be used:

Liquids: made 2000ppm available chlorine (5% chloros) and disposed via drains after a minimum of 12 hours.
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Disposable pipettes: Either a) as for mixed disposable waste or b) immerse in solution of 2000ppm available chlorine (5% chloros) for minimum 12 hours, followed by incineration.

Re-usable materials: Autoclave, 121 degrees/30 min.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref 43/01.7**

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**CU2 Project Title**

EXPRESSION OF HERPES SIMPLEX VIRUS GENES USING REPLICATION DEFECTIVE VIRUS VECTORS

**Class**

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**Non-GMM Consent Granted**

not applicable

Project notified under transitional arrangements [N]
### Purposes of the contained use

To investigate the functions of herpesvirus gene products in virus replication and pathogenesis.

### Recipient or parental organism

- **Adenovirus 5, coding sequences derived from mammalian alpha or gamma herpesviruses.**
- **Adeno associated virus**
- **Amphotropic retrovirus**
- **Herpes simplex virus, 'reporter genes' (eg LacZ, firefly luciferase, green fluorescent protein)**
- **Murine gammaherpes virus 68, coding sequences derived from Gammaherpesviruses.**

### Host/vector system

- **Eukaryotic viruses (ACDP group 2)/mammalian cell lines (established, commercially available).**

### Origin & function

**Origins:** Sequences will be sub-cloned from pre-existing plasmid clones. In some instances herpes virus sequences will be isolated from purified viral DNA.

**Functions:** The biological properties of herpesvirus genes are considered in the detailed risk assessment. These genes will be expressed in mammalian cell lines (via eukaryotic vectors) to examine the location and trafficking of herpesvirus proteins, or to provide helper functions for disabled herpesviruses. 'Reporter genes' will be inserted into herpesvirus genomes to inactivate specific virus genes and to identify the resulting disabled or attenuated viruses.

### Evaluation of foreseeable effects

The insertion of 'reporter genes' into viral genomes is expected to reduce the replication capacity of the recipient virus or to reduce its virulence. No increase in virulence, alteration of tissue tropism or modification of host range is predicted.

Herpes simplex virus genes act in concert to achieve virus replication. Some of the gene products are known to be cytotoxic but these are not secreted and only the expressing cell is expected to be affected. Mammalian alpha herpes viruses are not oncogenic and transforming genes have not been identified in these viruses. Herpes simplex virus gene function is, however not fully understood and some herpesvirus genes are known to evade or modify host immune responses. The eukaryotic vectors used are either fully disabled or heavily attenuated. No change in tissue tropism or host range of the vectors expressing herpes simplex virus genes is foreseen (see detailed risk assessment). The assessment to human health indicates no greater hazard than those of the wild type unmodified virus.

Murine gammaherpes virus 68 is a naturally occurring herpesvirus of rodents and has been isolated from a number of rodent species in Europe. There is no direct evidence that this virus infects man although the virus is known to replicate in human cells. Inactivation of virus gene products and the insertion of reporter genes into viral genomes
is expected to reduce the replication capacity of the recipient virus or to reduce its virulence. In common with other well characterised gammaherpesviruses MHV-68 is likely to encode gene products with transformation potential thus expression of virus genes with unknown function or known transformation potential in will be restricted to fully disabled vectors. No change in tissue tropism or host range of the vectors expressing MHV-68 gene is expected (see detailed risk assessment). The assessment to human health indicates no greater hazard than those of the wild type unmodified virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard validated methods will be used:

Liquids: made 2000ppm available chlorine (5% chloros) and disposed via drains after a minimum of 12 hours.

Mixed disposable waste: Validated autoclave, 121 degrees/30 min, then disposed as domestic waste.

Disposable pipettes: Either a) as for mixed disposable waste or b) immerse in solution of 2000ppm available chlorine (5% chloros) for minimum 12 hours, followed by incineration.

Re-usable materials: Autoclave, 121 degrees/30 min.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

I had forgotten to inform you that the local ACFM committee had reviewed and approved the 6 applications.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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</table>

Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
### Project Additional Information

**Purposes of the contained use**

To analyse papillomavirus gene functions in eukaryotic cells and innate and adaptive immune responses to these proteins.

**Recipient or parental organism**

Recipient/Vector: Inserted Material.

Adenovirus 5 Vaccinia virus: coding sequences from human papillomaviruses and canine oral papillomavirus.

**Host/vector system**

Eukaryotic viruses (ACDP group 2).

Eukaryotic cells.

**Origin & function**

Origins

papillomavirus genes - sequences will be sub cloned from pre-existing plasmid clones (HPV and COPV). The COPV isolate was derived from a clinical case of oral warts
In a retriever. The virus has not been passenged in vitro, generates oral warts in beagle dogs and does not transform human or canine cells in future.

Functions

The biological functions are considered in the detailed risk assessment. The genes are expressed in tissue culture cells via recombinant viruses to:

1. general peptide fragments for recognition by immune cells
2. generate viral proteins
3. analyse biological functions of viral proteins in eukaryotic cells in vitro.

Evaluation of foreseeable effects

Vectors

An E1 deleted replication defective adenovirus (Ad-5). This modified virus cannot initiate a full replication cycle and there is no survival value.

Vaccina virus. Insertion of papilloma sequences in the thymidine kinase (TK) site resulting in a TK negative Phenotype. The recombinant virus is attenuated by several orders of magnitude compared to the wild type. The inserted genes are expressed under the control of promoters operational only in the vaccinia lytic cycle, the expression of the inserted gene is therefore always accompanied by cell lysis.

Recombinant genes expressing COPV proteins.

COPV is not an oncogenic virus and there is no evidence that expression of COPV protein delivered via an exogenous vector can immortalise human cells. COPV does not induce carcinoma in the natural host.

Recombinant viruses expressing HPV proteins

The HPV E6 and E7 proteins must be expressed co-ordinately for immortalisation of human cells in vitro, expression of these genes singly results in cell death by apoptosis.

All work is small scale using standard laboratory procedures. The identifiable risks to workers are by aerosol and therefore all work is done in a laminar Class II hood and full containment level 2 requirements are applied.

The risk to human health and the environment is minimal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Cloning and expression in prokaryotic transfer vectors is a Class I activity and is covered under "Papillomavirus protein expression" Project Number 21 ACGM Centre 43.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The identifiable risk is infection of the laboratory worker by aerosol, therefore all manipulations with recombinant viruses are conducted in a Class II laminar flow hood in a restricted culture facility under negative pressure. Air is exhausted to the exterior through HEPA filters. The restricted culture facility is for work at Class II level containment only.
Gloves, face masks and protective clothing are worn within the facility. Soiled and used protective clothing is double bagged and autoclaved within the facility before disposal. Glassware is avoided wherever possible and disposable pipettes, tubes, culture flasks etc are employed. Used disposables are double bagged in autoclave bags and autoclaved within the facility before disposal. Sharps are disposed in sharps bins that are autoclaved within the facility before disposal. Mixed disposable waste: validated autoclave 121 degrees/30 mins then disposed as disposable waste.

Infected media is collected in aspirator traps containing available chlorine at 2000 ppm [100ml chloros/litre 5 Klorsept 7 (Jencons) tablets/litre]. After a minimum chlorine treatment of 12 hours liquids are disposed via drains. Any glassware is immersed fully in a solution containing available chlorine at 2000ppm for 2 hours, rinsed within the facility and autoclaved. Validated autoclave 121 degrees/30 mins.

Please enter comments on the GM safety committee on the risk assessment

I had forgotten to inform you that the local ACGM committee had reviewed and approved the 6 applications.

Project Containment

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Project Ref 43/05.1

<table>
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<th>Class</th>
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<th>Date Project Ceased</th>
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<tr>
<td>13/05/2005</td>
<td>Expression of mammalian apoptosis-regulating genes in mouse mammary epithelial cells by recombinant adenovirus.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Not Applicable</td>
<td>17/03/2014</td>
<td>Non-GMM</td>
<td>N</td>
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</table>
Project Additional Information

Purposes of the contained use

We aim to express mammalian genes that putatively regulate apoptosis pathways into mouse mammary epithelial cells. Changes in the rate of cell death, cell cycle, cell motility and/or cell adhesions will be monitored in these cells. cDNA will be cloned into a commercially available prokaryotic vector (pAdTrack or pShuttle), recombined with a replication defective disabled adenoviral backbone (pAdEasy) in a bacterial host, BJ5183, then linearised and transfected into cultured 293 cells to produce replication defective Ad. 293 cells will be passaged through 1-5 rounds of infection and high titre virus then purified on a CsCl gradient. Established mouse mammary epithelial cells lines will be infected with diluted purified virus and subsequently monitored for cellular changes in situ and by harvesting cell lysates for molecular analysis.

Recipient or parental organism

Attenuated E. coli strain BJ5183 (recipient micro-organism), if accidentally ingested in large quantities may produce mild temporary gastrointestinal symptoms. Wild-type Ad5 (not used here) is common in the population (>50% children carry antibodies to Ad5) and manifests a mild respiratory disease on initial infection or in immunocompromised hosts. Persistence of infection is dependent on replication competence and infected epithelial cells are eliminated and replaced by the host apoptosis mechanisms. Replication deficient adenovirus (used here) can only be propagated in 293 cells, due to the absence of the essential E1 gene in the viral genome. In addition the replication deficient vector has a deletion of the E3 immune evasion gene which, although non-essential in vitro, is likely to be an important virulence determinant. Adenovirus has a short lifetime outside the host and is inactivated immediately on contact with virkon. Adenovirus can be transmitted by aerosol.

Host/vector system

Hosts: E. coli BJ5183
Transformed and semi-transformed mouse mammary epithelial cells lines (KIM-2, NMuMG, EP-H4).
Transformed human embryonic kidney cells (AD-293) carrying E1 gene.

Vector system: Replication defective human adenovirus (serotype 5) derivative: pAdEasy, pAdTrack-CMV, pShuttle (Commercially available as AdEasy Adenoviral Vector System, Stratagene)

Origin & function

Apoptosis regulators may suppress or promote cell survival. However, as apoptosis is a cell autonomous process, this will not affect the survival of neighbouring cells and will not be propagated to cell progeny. Promotion of survival may prolong the life of damaged, excess or unwanted cells and therefore may increase the risk of cells accumulating genetic damage. However, apoptosis pathways in the cell are redundant and the regulators proposed to be used here are extremely unlikely to block all apoptosis pathways.

Evaluation of foreseeable effects

Cells infected by recombinant virus will express high levels of inserted gene within 24 hours (in culture), which may persist for the life of the cell, however transduced genes will not integrate into the host genome and therefore will not be propagated by the infected cell. Only cells that come into direct contact with the packaged virus will become infected. The routes of transmission to deliver the virus or its products to tissues are airways and eyes. So, the identifiable risk is infection of the laboratory worker by...
aerosol, or through needle stick injury during the purification procedure. The training of staff on the standard operating procedure for CsCl purification will be given particular attention in order to minimise the risk of needle stick injury. Adenoviruses used in this set of experiment are deficient in the E1 gene. Viral vector and recombinant vectors are stable in propagating bacterial and mammalian cells. The probability of reversion to the wild type is low as recombination in absence of E1 is performed in bacterial host prior to propagation in 293s. Consecutive rounds of amplification in 293 cells have the potential to increase titres of recombined replication-competent adenovirus, so amplification passages will be restricted to a maximum of 4 rounds. Dependent on the regulatory gene expressed, infected cells may either become susceptible to or resistant to cell death by specific apoptosis pathways but alternative mechanisms of cell death (eg. Other apoptosis pathways, necrosis, immune mediated lysis, senescence) will be unaffected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All media/supernatants will be pipetted into Virkon. All cultures performed in sealed flasks and handled in Class II laminar flow hood in a restricted culture facility under negative pressure. Air is exhausted to the exterior through HEPA filters. Gloves, facemasks and laboratory coat are worn within the facility. Any disposable flasks, pipettes, filtered tips, plastic tubes etc. are fully immersed into Virkon for 24h and disposed by an approved decontamination method following autoclaving. Work surfaces and spillages are decontaminated by spreading Virkon. Liquids: transfer by filtered pipettes to Virkon. Soak for 24 hrs and disposed by sink. All the work involves standard laboratory procedures on a small scale. The level 2 containment and control measures in place make it highly improbable that the GMOs will reach the environment and cause harm to humans or animals outside the laboratory.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Department of Pathology GM safety committee identified two issues:
1. The generation of replication competent adenovirus containing the genes involved in apoptosis.
2. The handling of highly concentrated replication incompetent viruses.

The Committee is now satisfied that both issues were specifically addressed by:
1. Screening for replication competent viruses and limiting passage.
2. Identification of needlestick injury during CsCl virus purification with the implementation of specific training to minimise the risk.

Project Containment
The use of lentiviral and retroviral expression vectors to influence gene expression and cell signalling in myelinating oligodendrocytes.

**Purposes of the contained use**

The study of oligodendrocyte differentiation, and the oligo-axonal interactions involved in myelination, so as to develop new strategies to enhance repair in Multiple Sclerosis. This project aims to induce the expression of adhesion molecules involved in multiple phases of oligodendrocyte development in myelinating oligodendrocytes. A second objective is to inhibit the expression of candidate genes hypothesized to regulate axo-glial adhesion and myelination using short hairpin RNA’s. The use of viral expression is necessary for these investigations, because myelination occurs over multiple weeks, and the duration of gene expression or repression required is greater than what can be attained using conventional transfection methods.

**Recipient or parental organism**

DH5alpha E.coli (disabled) and other common disabled E.coli K12 strains. LinX or equivalent packaging cell line for retrovirus, and HEK293 FT or equivalent for lentivirus. Cells to be infected are rodent oligodendrocyte and other precursors, neurons, neurospheres or oligospheres.

**Host/vector system**

D7alpha E.coli (disabled) and other common disabled E.coli K12 strains. LinX or equivalent packaging cell line for retrovirus, and HEK293 FT or equivalent for lentivirus. Cells to be infected are rodent oligodendrocyte and other precursors, neurons, neurospheres or oligospheres.
Bacterial cloning vectors (e.g. pUC based vectors). Retroviruses including pSM2 and LMP (Open Biosystems), lentiviruses including pLenti6.2v5 and ViralPower Packaging mix (a mixture of pLP1, pLP2 and pLP/VSVG plasmids; Invitrogen).

**Origin & function**

The objective of our studies is the knock-down of gene expression by the use of virally expressed small interfering RNA's, and/or disruption of protein function by virally expressed dominant negative isoforms, resulting in the inhibition of adhesion signalling pathways implicated in myelination, leading to alterations of myelin-forming capacity or the generation of myelin-forming cells.

DNA clones for expression (encoding wild-type or dominant negative forms of adhesion and related signalling molecules) will be obtained from collaborators, or generated by standard molecular biology techniques from DNA libraries held within the laboratory. Short-hairpin RNA sequences will be obtained from Open Biosystems Inc. Or will be designed using software such as "shRNA Retriever" from RNAi Central and then synthesised commercially and inserted into the vectors described above.

**Evaluation of foreseeable effects**

It will cause serious harm to the researcher is very low as the decrease in gene expression could not spread beyond the small number of cells directly exposed to the virus, either within the individual or to other individuals. Similarly, the ectopic expression of wild-type or inactive forms of oligodendrocyte proteins such as integrins may likewise alter the function of cells that are infected. The risk this poses will be minimised in two ways. First, the great majority of proteins expressed or knock-down to study myelination will have no effect on cell proliferation - an entirely different cellular process. In those cases were proteins implicated in myelination have been shown to have secondary effects on proliferation, the constructs used will be designed to express proteins that inhibit or prevent cell proliferation. Second, the very small number of cells affected by any contact of a researcher with the (replication-incompetent) viruses would limit the consequences of such an accident.

c) The vector (LMP) is derived from a mouse stem cell retrovirus (MSCV) vector, and while replication competent, cannot be packaged without viral gag, pol, and env genes, which it does not contain. pLenti contains a deletion in the 3’ long terminal repeat (LTR) that results in self-inactivation of lentivirus after transduction of the target cell, resulting in the virus being replication-incompetent. In both cases, once integrated into the transduced cell, the virus can no longer be packaged. The potentially harmful effects to the environment are effectively zero because

a) Genetic material will not have any outside target mammalian cells, and in the case of the small interfering RNA's will be species-specific.

b) The donor micro-organisms are disabled and will therefore not cause environmental harm by transfer to other organisms.

c) The vectors are replication-incompetence that will prevent dissemination or transfer to other organisms.

d) The resulting genetically modified micro-organism are extremely susceptible to dessication and will only survive a few hours at room temperature. Taken with the non-oncogenic nature of the genetic sequences to be used, the specificity of the vectors for mammalian hosts and their replication incompetence the resulting GMM poses no effective risk to the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste will be decontaminated by chlorine bleach and solid waste by autoclaving.

**Is an emergency plan required according to regulation 20?**

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y
Email received, Dept. of Pathology ACGM dated May 16th 2007.
"The Dept. of Pathology ACGM has approved your risk assessment entitled: “The use of lentiviral and retroviral expression vectors to influence gene expression and cell signalling in myeliating oligodendrocytes” as a class 2 project. Moreover the committee had no substantive comments so the risk assessment can be used as is".

Project Containment

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Animal Units

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</tbody>
</table>

Project Ref 43/07.2

1. Cloning and expression of herpesvirus genes
2. Manipulation of herpesvirus genomes
3. Cloning and expression of known and putative cellular and viral oncogenes

Date Ackn’d 13/07/2007

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Purposes of the contained use

To investigate the function of herpesvirus and cellular gene products in viral oncogenes.

### Recipient or parental organism

<table>
<thead>
<tr>
<th>Recipient/vector</th>
<th>Inserted Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) KSHV</td>
<td>&quot;reporter gene&quot; e.g. LacZ, green fluorescent protein, firefly luciferase</td>
</tr>
<tr>
<td>2) Retroviruses</td>
<td>coding sequences from gamma herpesviruses and mammalian cellular genes and &quot;reporter gene&quot; LacZ, green fluorescent protein, firefly luciferase</td>
</tr>
<tr>
<td>3) Mammalian expression plasmids</td>
<td>coding sequences from gamma herpesviruses and mammalian cellular genes</td>
</tr>
<tr>
<td>4) bacterial expression plasmids</td>
<td>coding sequences from gamma herpesviruses and mammalian cellular genes</td>
</tr>
</tbody>
</table>

### Host/vector system

**Eukaryotic viruses (ACDP group 2)/ mammalian cell lines (established, commercially available) mammalian primary cells**

### Origin & function

**Origins:** sequences will be subcloned from existing plasmid clones. In some instances, herpesvirus sequences will be amplified from cDNA or genomic DNA by PCR.

**Functions:** the biological properties of herpesvirus and mammalian genes are considered in the detailed risk assessment (appended). These genes will be expressed in mammalian primary and cultured cell lines (via eukaryotic vectors) to examine their function.

### Evaluation of foreseeable effects

The insertion of reporter genes into viral genomes is not expected to alter the tropism of the virus/

Pseudo-typed, replication defective retroviruses will be capable of infecting a wide range of cell types. The modified lentivirus is replication-defective, i.e., is capable of only single round of virus production, and viral particles are labile. Infected primary cells can only survive in tissue culture.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard validated methods will be used.

- Liquids will be made 2000ppm available chlorine (5% chloros) and disposed of via cdrains after a minimum of 12 hours.
- Mixed disposable water: validated autoclave, 121°C for 30 minutes, then disposed of as domestic waste.
- Disposable pipettes: either (a) as for mixed disposable waster or (b) immerse in solution of 2000ppm available chlorine (5% chloros) for a minimum 12 hours; followed by incineration.

**Is an emergency plan required according to regulation 20?**

| N |

**If yes, tick to confirm that it is attached to this form**

| N |
The dept. of pathology GM committee reviewed the risk assessment and approved it without further modification or comments.

**Project Containment**

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**Project Ref** 43/07.3

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<tr>
<td>12/09/2007</td>
<td>An investigation into mechanisms of Tumorigenesis</td>
<td></td>
<td>Class 2</td>
<td>1-50 Litres</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

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<td>11/01/2017</td>
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Project Additional Information
### Purposes of the contained use
To investigate the role of protein products generated as a result of genomic abnormalities in tumour development.

### Recipient or parental organism
The ultimate recipients for the inserts will be well characterised mouse and human cell lines.

### Host/vector system
The third generation retroviral vector systems employed will ensure that replication-competent viruses are not produced and thus that replication cannot occur in workers in the unlikely event that they are exposed to infectious virus.

The mouse ecotropic viruses will not infect humans. Amphotropic viruses could infect individual cells in exposed individuals, but without subsequent spread; infection is most likely via percutaneous introduction therefore the use of sharps will be minimised. The effects of operator infection with viruses generated without expressed inserts would likely be due to insertional mutagenesis. This risk is real, but of low probability. The infection risk itself is minimised by physical containment coupled with relatively low stability and ease of inactivation. Biologically the probability of an untoward event must be proportional to the number of infected cells and their ability to proliferate following infection.

The experience from the French SCID-X1 gene therapy is not fully informative in this context but it is clear that insertional mutagenesis leading to premalignant proliferation only occurred in two of nine patients and that the efficient ex vivo infection of a stem cell population must have been crucial to the outcome. It is highly unlikely that any conceivable laboratory accident would fulfil these preconditions.

### Origin & function
The genetic material will be derived and isolated from human and mouse genomic DNA generated from primary human tissue samples, bacterial artificial chromosomes and/or well characterised cell lines.

The inserted genetic material will encode oncogenes with potentially deleterious effects if inserted into, amongst others, lymphoid cells. The candidate oncogenes are from human tumours with therefore some expectation that their introduction into appropriate cells will result in transformation/oncogenesis. This is indeed the basis for the proposed assays. The in vitro assays will use cell lines that have already accumulated a background of mutations such that the further addition of a candidate oncogene will produce an altered growth phenotype. It is likely that oncogenic effects in humans or experimental animals would depend on the sequential accumulation of multiple changes in proto-oncogenes against the background of high level expression of the candidate oncogene. The time taken for tumours to develop in rodents infected with high doses of oncogene-expressing retroviruses reflects the need for this accumulation of additional genetic changes. It is thus unlikely that any single oncogene candidate would be able to "transform" a normal cell in the unlikely event of exposure to DNA by inoculation or inhalation. The exposure risk to the oncogene is presented by high concentrations of plasmid DNA containing oncogene inserts as well as the viral vectors; therefore care must be taken when handling these DNA preparations. Sharps will not be used and aerosol exposure prevented by handling concentrated solutions of oncogene containing DNA in a class 2 cabinet. Any gene-specific adverse effect of insertion will probably be dependent on transcription. While retroviral insertion occurs preferentially in transcriptionally active regions the majority of insertions will not lead to transcription and subsequent translation.

### Evaluation of foreseeable effects
The recombinant retroviruses capable of expressing a human oncogene combine the risks associated with inappropriate expression of the inserted gene and the insertional mutagenesis risk associated with the vector alone. The risk of infection even following a puncture wound is likely to be low. These risks are only associated to any significant extent with the amphotropic, not the ecotropic, constructs. Again the control of exposure coupled with inability to replicate in the unlikely event of operator infection are crucial.
exposure provide the major controls on risk. The possibility that any given insertion event might lead to adverse effects is itself of low probability. These adverse effects could however be severe, but are further mitigated by a requirement for the accumulation of multiple genetic changes in the target cell (or its progeny) before tumour formation. These changes would only occur following sustained proliferation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GM animals are housed in a barrier facility. All staff entering the facility change into clean clothes inside the unit and remove this clothing on exiting. All staff wear the necessary safety equipment including gloves, hats, overshoes and safety spectacles as required. The GM animals are kept in isolator cages (IVCs) and do not leave the facility. Containment measures in place include physical barriers and deposition of rodent poisons on the floor around the facility.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Producing retroviruses will be housed in a category II facility in a marked incubator. Waste liquid will be inactivated following overnight incubation in a 1% solution of Virkon. Solid waste will be autoclaved and incinerated before disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee has considered the risk assessment for the project entitled “An investigation into mechanisms of tumorigenesis” and believes that it is consistent with the guidance provided by the SACGM compendium and with our knowledge of the facilities available in the laboratory in which the work would be carried out. We therefore approve the risk assessment subject to comment from the HSE.

Project Containment

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<td>L2 L3 L4 L2 L3 L4</td>
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</table>
Investigation into the mechanisms of tumorigenesis in human tumours

To investigate the biological functions of the candidate oncogenes and tumour suppressor genes as well as their associated mechanisms in tumorigenesis

Recipient or parental organism
Human and murine established cell lines, primary human cancer-derived cells

Host/vector system
The use of third-generation retroviral systems will ensure that replication-competent viruses are not produced in the laboratory environment and thus replication cannot occur in the unlikely event that a laboratory worker was exposed to infectious virus. The same replication-deficient criteria also apply to the AAV system and the recombinant adenovirus. The mouse ecotropic viruses will not infect humans. Amphotropic viruses could infect cells in exposed individuals, but without subsequent spread. The most likely route of infection is through percutaneous introduction which can be avoided by banning the use of sharps. The outcome of an infection with viral vectors devoid of any inserts would likely be insertional mutagenesis. Although real, this risk is of low probability. The infection risk itself is minimised by physical containment coupled with relatively low stability and ease of inactivation. Biologically the probability of an untoward event must be proportional to the number of infected cells and their ability to proliferate following infection. An insertional mutagenesis leading to premalignant proliferation occurs very rarely. It is highly unlikely that any conceivable laboratory accident would fulfil these preconditions.

The adeno-associated virus AAV-2 is a naturally defective virus not known to cause any human disease. Production of nild-type recombinant virus is prevented by the lack of homology between the required individual plasmids. Furthermore the helper-free system serves to eliminate the requirement for wild-type adenovirus co-infection. The AAV-2 virus replicates epichromosomally, viral integration into the genome can occur but is a rare event, limited to chromosome 19 reducing the probability of intact viruses to disrupt an endogenous gene still further.

No host genome integration for recombinant adenoviruses
the genetic material will be derived and isolated from human genomic DNA extracted from human tumours and corresponding normal tissues and also well-characterized cell lines currently used for in vitro experiments. The genetic material we wish to insert into eukaryotic vectors represents a number of novel candidate oncogenes or TSGs initially identified by genome-wide CGH array screening in our laboratory. In order to investigate the role of these genes in tumourigenesis in vitro, we need to produce tagged molecules and transflect them into an appropriate cell line. The tagged molecule will aid in the detection and purification of the corresponding recombinant proteins for further functional-protein interaction studies. The low efficiency of transient transfections in some cell lines represents a major barrier to observe cell behavioural phenotypes. For example, a routine cell proliferation assay requires a high proportion of transfected cells representing a homogenous population expressing a particular oncogene or TSG to clearly identify a growth promoting/reducing effect as compared to untransfected cells. In order to circumvent this problem, we aim to produce recombinant stable cell lines derived from single cell clones expressing the transgene after integration into the host genome hence the need for retroviral vectors. Finally tagging of the endogenous gene will bring yet another level of control in those studies by keeping the gene under tight physiological regulation. This approach requires the use of the AAV system which allows for targeted homologous recombination. In summary, insertion of the genetic material under investigation into a wide range of vectors will allow for a greater understanding the biological function of these important genes and the multiple roles that they play in tumourigenesis.

The inserted genetic material may encode an oncogene with potentially deleterious effects if incorporated into, amongst others, neuronal cells. Our candidate genes have been initially cloned from human tumour DNA. Their introduction into appropriate cells could therefore lead to transformation or tumorigenesis. Our current understanding the transformation process requires a well-ordered series of genetic changes. Ourestablished cell lines have already accumulated a background of mutations (some of them identified by our own studies) in which the addition of a candidate oncogene/TSG will produce an altered growth phenotype detectable through standard in vitro assays. It is thus unlikely that overexpression of any single gene would be able to replicate the effect of the accumulation of multiple changes in specific genes to “transform” a normal cell in the unlikely event of exposure to DNA by inoculation.

Inhalation. The exposure risk inherent in the use of an oncogene is presented by high concentrations of plasmid DNA as well as the viral vectors. These DNA preparations will be handled carefully avoiding the use of sharp instruments and aerosol exposure by working in a class II safety cabinet. Any gene-specific adverse effect of gene insertion will also probably be highly dependent on active transcription. While retroviral insertion occurs preferentially transcriptionally active regions the majority of insertions will not lead to active transcription and subsequent protein translation. For epitope tagging of endogenous proteins, the inserted genetic material will be a combination of an epitope tag such as Flag or HA and an antibiotic resistance gene, neither of which are likely to possess a deleterious effect in host cells.

### Evaluation of foreseeable effects

The recombinant retroviruses expressing a suspected human oncogene combine the risks associated with the expression of the inserted genetic material and the inherent mutagenesis risk of the inserted vector alone. The risk of percutaneous infection is likely to be low and is only associated to any significant extent with the amphotropic, not the ecotropic, constructs. The minimal exposure to the genetic material coupled with the replicative deficiency of the recombinant viruses constitute strong safeguards in the unlikely event of operator exposure. Again as above, the possibility that any given insertional event might lead to adverse effects is itself of low probability. These adverse effects could however be severe, but are further mitigated by a requirement for the accumulation of multiple genetic changes in the target cell (or its progeny) before tumour formation. These changes would only occur following sustained proliferation. The AAV-2 recombinant vector will only carry a small carboxy-terminal tagged sequence of the target gene together with an antibiotics-resistance gene and therefore present the same risks as the constructed retroviral recombinant vectors but at a reduced probability of adverse effects. The recombinant adenovirus will only be transiently expressed in host cells.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Host cells producing viruses will be housed in a category II facility in a marked incubator. Liquid waste will be inactivated through overnight incubation in a freshly made
solution of 1% Virkon. Solid waste will be autoclaved and incinerated before disposal. The degree of kill is 100% and no viable GM organisms remain after treatment.

The committee has considered the risk assessment for the project entitled “Investigation into mechanisms of tumorigenesis in human tumours” and believes that it is consistent with the guidance provided by the SACGM compendium and with our knowledge of the facilities available in the laboratory in which the work would be carried out. We therefore approve the risk assessment subject to comment from the HSE.

**Project Containment**

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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**Project Ref** 43/11.1

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Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes N
**Project Additional Information**

**Purposes of the contained use**

2. Introduction of mouse and human cellular genes, including oncogenes, into mammary cells and transplantation of these cells into mouse mammary fat pads

**Recipient or parental organism**

1) Retroviruses-lentiviral (3rd generation) vectors - All vector systems have been described in the literature and are self inactivating. The lentiviral packaging and envelope components (gag,pol, rev) are separated from the vector containing the DNA sequences of interest that will be incorporated into target cells. The packaging plasmids share no significant homology to any of the expression lentiviral vectors, expression vector or any other vector to prevent generation of a recombinant replication-competent virus. The packaging plasmids lack any packaging signals so are not present in the packaged viral genome and therefore the lentiviral particles are replication incompetent. A deletion in the enhancer of the U3 region of 3' LTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic-DNA of target cells. Therefore no new HIV-1 virons can be produced from infected cells. Retroviruses are rapidly inactivated outside the host therefore spread into the environment is unlikely. There is very small theoretical risk that the attenuated HIV-1 vector can recombine with wild type (thus replicative) HIV-1 virus. To prevent this we shall not use tissue known to be infected with the wild type HIV-1 virus. Because the HIV-1 envelope, which usually binds CD4 and chemokine receptors, has been replaced by the vesicular stomatitis virus envelope (VSV-G), this virus can infect many different cell types in vitro and possibly in vivo. This introduces a potential aerosol/contact transmission threat in addition to percutaneous hazard. Particles are more physically stable but are sensitive to complement. The virus will carry the WPRE cassette, increasing vector titre and transgene expression and therefore risk. The vector integrates into host cell's DNA so there is some risk of long term expression or of insertional mutagenesis. Vector is considered ACDP HG1.

2) All mammalian cells used (primary or cell lines, mouse or human) are especially disabled and cannot survive outside laboratory culture. They are unable to colonize humans and survive in the environment. Any human cell lines used will be thoroughly characterised with a history of safe use. In such cells adventitious pathogens are unlikely to be present, therefore the risk of colonization is low. In primary cells there is a slightly elevated risk owing to the increased risk of adventitious pathogens being present, although the likelihood of colonization remains low. Human cells will not be used from patients known to be HIV+ or hepatitis+. Mouse cells give negligible risk because of the phylogenetic difference means adventitious pathogens and colonization both become very low probability.

3) Bacteria are disabled E. coli K12-derived strains and cannot survive outside of laboratory culture. They are unable to colonise humans or survive in the environment.
**Origin & function**

Origins: Sequences will be sub-cloned from existing plasmid clones or from cellular genes. In some instances, cellular sequences will be amplified from cDNA or genomic DNA by PCR.

Functions:
1) LacZ, green fluorescent protein, mCherry fluorescent protein, tdTomato fluorescent protein, EY fluorescent protein or some other fluorescent protein or firefly luciferase will be inserted into mammalian cells and expressed as reporter molecules.

2) Coding sequences for mammalian cellular genes and reporter genes (e.g. LacZ, green fluorescent protein, mCherry fluorescent protein, tdTomato fluorescent protein, EY fluorescent protein or some other fluorescent protein or firefly luciferase) will be inserted into retroviral or lentiviral vectors for long-term expression in mammalian cells. These cells may be introduced into animals by injection at various sites or by transplantation into mammary fat pads.

3) Coding sequences of mammalian cellular genes and reporter genes will be introduced into mammalian cells using the viral vectors for long term expression in mammalian cells to facilitate the study of their functions in cell death, growth and differentiation processes. These cells may be injected into mouse mammary fat pad and allowed to grow to assess the functions of specific genes in regulating stem cell activity and progenitor potential or in controlling cell death.

4) Coding sequences from mammalian cellular genes will be inserted into lentiviral vectors for expression in mammalian cells. These cells will then be transplanted at various sites or injected into mammary fat pads. We will study their role in directing the phenotype of cancer after targeting to various cell types. The oncogenes we will use include, but are not restricted to, ErbB2, Ras, Wnt, Notch and Jak2V617F.

6) Sequences designed to interfere with specific endogenous mammalian cellular genes (for example shRNA) will also be inserted into lentiviral vectors in order to study the role of the cellular gene. The target genes will be associated with normal mammary cell growth, differentiation and death and may also be tumour suppressors.

**Evaluation of foreseeable effects**

1) The insertion of reporter genes into vector genomes is not expected to alter their potentially harmful properties.

2) Lentiviral vectors bearing reporter genes and/or cellular genes of interest are capable of introducing these genes into cells of the body. While the number of cells affected will be small and limited to the initial round of infection, the expression may be long term and involve potentially transforming events. However, the genes are unlikely to enable full oncogenic transformation in isolation and long term expression has been difficult to achieve in practice even in instances of deliberate optimisation for gene therapy trials. Cells expressing viral protein will be targeted by the immune system.

3) The properties of mammalian cells with respect to hazards to human health and the environment will remain unaffected by any of the non-oncogenic genes introduced. Should a vector with an oncogene be inoculated into human cells in situ, the oncogene could potentially stimulate division of the cell, but will not be able to propagate or infect other cells. The risk of inoculation will be minimal as work will be carried out in a tissue culture hood with no glass or sharps present. Also should the mouse cells be inoculated directly into a human they should be recognised by the immune surveillance system and removed. No toxic proteins will be produced.

4) The genes mainly encode eukaryotic functions that are unlikely to affect the properties of bacteria. The proteins produced are not known to be toxic.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Animals infected with retroviruses and lentiviruses will be killed within the animal facility, tissues fixed in formaldehyde or frozen, and carcasses disposed of by incineration.
Virkon (a peroxygen compound) used at a concentration of 1% is known to have a wide range of anti-microbial activity and is bacteriocidal against many human viruses, including HIV. All tissue culture material and disposable plastic ware will be soaked for a minimum of 18-24hrs in a freshly prepared 1% Virkon solution. The fluid is then disposed of down a laboratory sink with excess water. Solid waste is then double bagged in biohazard bags and autoclaved at 134°C for 20 minutes at 3.2 bar pressure.

Validation of this method will be by independent thermocouples placed in the centre of the load. Verification that the correct conditions have been reached will be obtained by checking the chart recorder printout. The autoclave undergoes a planned preventive maintenance inspection every 3 months. All inactivated waste is then treated as "clinical waste" and removed from site by a contractor.

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Approved on 28/03/2011

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Date Project Ceased 02/03/2022
Withdrawn: N

Tick if notifying a connected programme of work: N

## Project notified under transitional arrangements

### Historical Significant Changes

### Historical Date of Additional Info

**Significant Change ID** 43/11.2a

**Date of Significant Change** 02/06/2016

## Project Additional Information

### Purposes of the contained use

Work in our lab involves comparative computational analysis of virus sequence data in order to identify new functional elements, followed by experimental analysis to verify computational predictions and to characterize the function of the elements identified. Recent examples include the discovery of an essential overlapping 'hidden' gene in the Potyviridae (the largest and most economically important family of plant viruses), short overlapping coding sequences in the alphaviruses and the Japanese encephalitis serogroup of flaviviruses, both of which are translated via ribosomal framshifting, and a short overlapping gene in equine arteritis virus and other arteriviruses. Experimental work for these projects took place outside of the UK. However, we continue to identify new functional elements in new viruses and the purpose of this GMO form is to cover the GM work required to investigate such cases. Work typically involves the generation of mutant viruses in which the predicted functional element is mutated, and comparison of wild-type and mutant virus phenotypes via standard virological assays. It is expected that mutant viruses will most likely be either similar to wild-type virus or attenuated.

Currently we intend to start work on a project involving cardioviruses but we envisage the possibility of amending this form at a later date, if necessary, to include other viruses.

### Recipient or parental organism

We will use infectious cDNA clones of two cardioviruses (family Picornaviridae, genus Cardiovirus): the mengovirus strain of Encephalomyocarditis virus (EMCV), and Theiler's murine encephalomyelitis virus (TMEV).

Infection with wildtype EMCV is associated with sporadic cases and outbreaks of myocarditis and encephalitis in domestic pigs, in numerous species of nonhuman primates, and in other mammalian species. Transmission is generally via ingestion of contaminated water or feed or dead animals, but transplacental, contamination of wounds and other close-contact mechanisms of transmission may also occur. In susceptible animals disease is often fatal. Virus isolation aand serologic studies indicate EMCV is distributed worldwide. Serologic studies indicate that humans have been infected by EMCV or immunologically related viruses but clinical disease in humans is relatively infrequent and, when observed, generally occurs as a self-limiting febrile illness. However, our EMCV work will be limited to the mengovirus construct vMCO - a mutant that grows like wild-type virus in cell culture but is severely attenuated in mice (10^6 to 10^9) and other mammals via removal of a 50-115 nucleotide poly-C tract in the 5'UTR (Martin et al, 1996, J Virol 70:2027-2031, PMID 8627731).

MEV is not known to be a human pathogen despite being used extensively in the scientific community. TMEV infects mice and is transmitted faecal-orally and transplacentally. Most infections are subclinical; however susceptible mice may develop severe neurological disease. We will use strain GDVII.

Both EMCV and vMCO and TMEV may be considered to be ACDP hazard group1. Handling of these virus under BSL2 conditions is sufficient to control any slight risk of infection.

### Host/vector system

02/03/2022
Plasmids will be grown in disabled strains of E. coli which have a long history of use without harm to human health or the environment and lack reasonably conceivable means to cause such harm.

Origin & function

RNA viruses have very compact genomes that are subject to intense selection pressure. As a result, the identity of most nucleotides have been highly optimized and mutation most often either have no effect or attenuate the virus. Using computational analyses of virus sequences data we have identified a number of highly conserved elements. Such high evolutionary conservation suggest functional importance and we now wish to verify and explore the functionality of these elements via targeted mutations and comparison of the phenotypes of mutant viruses with wildtype viruses in cell culture. Mutations are most likely to have either no affect or attenuate the virus.

Evaluation of foreseeable effects

Genetically modified disabled E. coli would be no more hazardous than the unmodified host. Little or no expression of virus sequence should occur in E. coli and this is unlikely to provide a suitable environment for virus production. No adverse effects are expected for low level expression of any of the viral genes in E. coli.

Both EMCV vMCO and TMEV may be considerd to be ACDP hazard group 1. TMEV is not known to be a human pathogen despite being extensively in the scientific community. EMCV vMCO is very highly attenuated and not observed to revert to wild-type. Handling of these virus under BSL2 conditions is sufficient to control any sight risk of infection. In the wild, TMEV (strain GDVII) causes acute encephalomyelitis in susceptible mice (most infections are subclinical) but there is very low risk of the virus coming into contact with potential hosts.

It is unlikely that the genetically modified cardioviruses would be any more hazardous than the unmodified host: mutations in RNA viruses most often either have no effect or attenuate the virus. None of the mutations involve the introduction of any known toxins or oncogenic sequences. No changes are proposed that would be predicted to alter the host range or tissue tropism of the virus, or alter its susceptibility to host defence mechanisms except in so far as the virus may be attenuated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste- autoclaved (run shown to reach and hold temperature ).

Liquid waste - autoclaved as above or treated with appropriate disinfectant, Lifeguard (2.5%) for bacterial cultures, Chloros for virus-containing fluids.

Surface decontamination - Lifeguard for bacterial work, chloros for virus work (2% virkon for metal surfaces such as the surfaces of class II cabinets or incubators but work should be performed in plastic trays to avoid the need for this).

Pipettes soaked overnight in 2% virkon (bacterial work at level 1), plastic pipettes soaked overnight in chlorine-based disinfectant, >2500 ppm available chlorine (virus work at level 2).

All procedures expected to give 100% kill.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

02/03/2022
The GM committee has approved your project "Analysis of Functional elements of RNA virus genomes" as a CL2 project. The project involves the mutagenesis of two cardioviruses endemic in mice (TMEV and ECMV) and although the risk to human health is low, in the case of ECMV human infections have been documented. For this reason it was considered that the work should be carried out at CL2.

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**Project Ref 43/14.1**

- **Date Ackn'd**: 20/03/2014
- **CU2 Project Title**: Studying Gene Expression in Toxoplasma gondii
- **Date Project Ceased**: 
- **Class**: Class 2
- **Culture Volume Class 2**: < 1 Litre
- **Non-GMM Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
### Purposes of the contained use

To study gene expression and proteomics in *Toxoplasma gondii* and in mammalian host cells infected with *Toxoplasma gondii*.

### Recipient or parental organism

| A. E. coli | Attenuated K-12 derivatives of *E. coli* will be used. The standard intermediate recipients *E. coli* (XL-1 Blue, DH-10 etc) are highly disabled strains. |
| B. *T. gondii* | *Toxoplasma gondii* is an obligate intracellular parasite and neither the tachyzoite nor bradyzoite can survive outside the host. *Toxoplasma gondii* is an ACDP Hazard Group 2 organism and thus requires Level 2 Containment. Wild type, haploid, tachyzoite forms of *T. gondii* (eg Types I, II and III strains), as well as their mutant derivatives: hxgprt gene knockout mutants, ku80 gene knockout mutants (lack nonhomologous end-joining DNA repair mechanisms), lac Z+, TATi-1 strain (contains a functional tetracycline transactivator to regulate reversible gene expression). See Appendix to the Risk Assessment for further technical details. |
| C. Mammalian Cells | Well-characterised mammalian cells in culture, eg NIH/3T3, HeLa, BHK, 293T, HFF, RAW264. |

### Host/vector system

| A. E. coli | Standard cloning plasmids including puC series, BACs, cosmids and Lambda ZAP II. |
| B. *T. gondii* expression vectors | Expression vectors, engineered with *T. gondii* protein promoters, loxP flanking regions, and selectable markers such as CAT or HXGPRT, and fluorescent protein genes. See Appendix to the Risk Assessment for further technical details. |
| C. Mammalian expression vectors used are pcDNA 5/FRT/TO plasmids (and similar variants; Invitrogen) that can be used in both transient assays and integration into host cells modified with a chromosomal FRT site. For transient assays, tetracycline induction of the hybrid CMV/TetO2 promoter drives gene expression. Hygromycin resistance is selected upon integration into the FRT site via co-transfection with the pOG44 plasmid that expresses the FLP recombinase. Hygromycin can only be expressed upon FRT site integration as the recombination event is required to place the hygromycin gene next to the SV40 promoter. |

### Origin & function

**Origins of genetic material involved:**
The origin of the genetic material involved is from *Toxoplasma gondii* and related apicomplexan species, cloned in *E. coli*. DNA encoding *Toxoplasma gondii* genes (and mutant versions) and in some instances orthologous genes from other relevant apicomplexans may be expressed in the parasite.

**Expected biological action of genetic material involved:**
(i) knock out endogenous gene products,  
(ii) express genes from conditional promoters,  
(iii) generate mutant gene replacement products (eg inter-strain).
(iv) label *T. gondii* proteins with standard fluorescent proteins and allow localisation and monitoring of expression,
(v) express *T. gondii* proteins directly in host cells.

**Evaluation of foreseeable effects**

While these manipulations may alter parasite and host cell transcriptional and proteomic processes, they are not expected to increase parasite virulence nor are they likely to expand the host cell range.

(i) The presence of fluorescent proteins will not affect the parasite's virulence
(ii) Construction of knockouts will either not change or might even decrease the parasite’s virulence.
(iii) In cases where gene constructs will be interchanged between parasite strains, no resultant parasite will be more virulent than the wild type Type I, which itself is only virulent in mice.
(iv) Variant/altered gene products would not be expected to change either the host range or host cell type, which already extends to all nucleated cells in warm-blooded animals.

None of the vectors used for these manipulations is able to persist in mammalian host cells. Thus, the possibility of adverse effects due to accidental exposure is severely reduced. Moreover, mammalian cells expressing *T. gondii* genes are not able to infect humans or other animals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All experiments will be performed in a Containment Level 2 suite. In accordance with the waste management procedures of the suite, no material or equipment can be removed without first having been decontaminated or autoclaved.

Liquid waste (cell culture medium containing live GMOs) is treated overnight with Distel (v/v 5%) as recommended by the manufacturer's validated instructions.

Solid waste which may have come into contact with live GMOs (such as empty culture vessels) is autoclaved in the Containment Level 2 Suite, before being incinerated as clinical waste.

Each autoclave run is logged with the operator's name, the date and contents of the run, as well as the conditions of the run (1 hour at 125°C in a Boxer Petite). At the end of each autoclave run, the name of the operator emptying the load is also recorded.

The use of sharps is limited to those operations where it is essential. Sharps that may have come into contact with live GMOs are disposed of in a dedicated labelled sharps bin which, once full, is sealed, autoclaved and then incinerated (as with other solid waste above).

Is an emergency plan required according to regulation 20?  
**N**

If yes, tick to confirm that it is attached to this form  
**N**

Tick to confirm that you have attached a risk assessment to this form  
**Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment  
**N**

02/03/2022  Page 1914 of 15326
The project was approved by the Departmental Biological Safety Committee in February 2014. The Committee was satisfied, after discussion, that for this work the use of sharps could not be entirely avoided and that controls were sufficient to minimise the risk of infection.

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
</tr>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### Project Ref 43/14.2

- **Class**: Class 2
- **CultureVolClass2 CultureVolumeClass3-4**: < 1 Litre
- **Non-GMM Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

### Project Additional Information

#### Purposes of the contained use

The work in this project has been devised by and will be carried out under the supervision of Professor Ian Goodfellow.

The project aims to understand the basic mechanism of RNA virus biology, including the identification of cellular and viral proteins involved in the life cycle of a number of
RNA viruses.

The work can be broken down into various subsections as detailed below:

1) Expression of virus products for functional, structural and immunological studies:
Viral genes from members of the Caliciviridae, Astroviridae and Hepeviridae will be expressed in E.coli and mammalian cells to study their function. Plasmid expression systems and retroviruses will be used as delivery systems to examine the effect of viral gene expression on host cell pathways and virus replication.

2) Expression and inhibition of cellular pathways involved in RNA virus life cycles:
Cellular proteins or inhibitors of cellular proteins involved in various aspects of the virus life cycle will be expressed in cells by either plasmid transfection or retrovirus-mediated delivery and their effect on virus replication monitored. Examples include translation initiation factors, transcription factors, RNA binding proteins and proteins involved in the innate immune response.

3) Immortalization of mammalian and avian cells:
In order to develop new cell lines for the study of RNA virus replication we aim to generate a number of lentiviral vectors expressing proteins known to prolong cell survival and facilitate immortalization. These include: SV40 Large T antigen, telomerase, vRel and vRas.

4) Generation of modified viruses for studies of virus replication/attenuation:

(a) Caliciviridae
The overall aim of the project is to further our understanding of the molecular mechanisms caliciviruses use for replication and translation. Specifically we aim to address the role various RNA structures, viral proteins and host cell proteins play in the calicivirus life cycle as well as their relative contribution to viral virulence. The project will involve the use of human caliciviruses, including human norovirus and sapovirus as well as cellular factors thought to play a role in the viral life cycle (e.g. RNA binding proteins, translation initiation factors).

Development of a cell culture system for human caliciviruses. In order to fully understand the roles that RNA structures as well as viral and cellular proteins play in the various aspects of the virus life cycle we need to introduce mutations into the viral genome and study their effects on virus replication. A reverse genetics system already exists for feline calicivirus, however the efficiency of this system is poor. No systems are currently available for human noro and sapoviruses. We will attempt to develop such systems.

In addition, we wish to produce genetically tagged viruses containing innocuous biochemical markers such as green fluorescent protein (GFP) and luciferase to monitor virus replication.

(b) Hepeviridae
The aims of this project are to identify viral and cellular components required for efficient virus replication in cell culture. Initially the project will exclusively use non-infectious hepatitis E virus replicons that lack the capsid sequences and are therefore unable to produce infectious virus. We will use proteomics to identify host factors that interact with viral RNA structures and viral proteins. We then aim to use viral replicons to characterise the role of these interactions on virus replication in cell culture. In some instances we may wish to express the viral capsid proteins in trans to examine the ability of capsid proteins to encapsidate viral RNA. The particles produced by such an approach will remain unable to go through muli-cycle replication and are considered defective. Due to the replicon construction, co-transfection of cells with replicon and plasmids expressing viral proteins cannot result in recombination that would restore the capsid open reading frame. The replicon carries only the first 3 nucleotides of the capsid coding region therefore homologous recombination cannot occur.

(c) Astrovirus
The aims of this project are to identify and characterise viral and cellular factors required for viral replication. Human astrovirus infectious cDNA clones will be used to generate mutant and WT viruses as well as viruses carrying reporter proteins such as luciferase, GFP etc. Human astrovirus infects >90% of individuals prior to the age of 5 and results in acute gastroenteritis only. Other astroviruses (e.g. murine) have yet to be replicated in cell culture but this is one of the aims of this project.
Recipient or parental organism

1) Human Caliciviridae members - all human caliciviruses are ACDP hazard group 2 pathogens and cause acute gastroenteritis. These are common pathogens with >80% of the UK population being seropositive. Their genomes are positive sense single stranded RNA. We foresee that all the modifications to the viral genome will have little to no appreciable affect on viral virulence and in most cases is likely to debilitae replication.

2) Hepatitis E virus (non-infectious replicons only) - This work is currently limited to non-infectious replicons that lack the capsid sequences only. Hepatitis E virus causes acute hepatitis and is currently classified as ACDP hazard group 3 pathogen, however as replicons do not possess capsid sequences they are unable to form infectious particles and infect cells. Their genomes are positive sense single stranded RNA. We foresee that all the modifications to the viral genome will have little to no appreciable affect on viral virulence and in most cases is likely to debilitae replication.

3) Astroviruses, including animal and human members. Human members are classified as hazard group 2 pathogens and cause acute gastroenteritis. As with caliciviruses, these are common with >90% seropositivity reported in some instances. Their genomes are positive sense single stranded RNA. We foresee that all the modifications to the viral genome will have little to no appreciable affect on viral virulence and in most cases is likely to debilitae replication.

4) E.coli (lab strains only). These are lab adapted and highly attenuated strains of E.coli, widely used around the world. No pathogenic potential. We do not foresee any of the modifications to increase pathogenic potential.

5) Mammalian and avian cell lines. We will use numerous widely available immortalised human and avian cell lines e.g. (293, HeLa, Caco, DF1) as well as cell lines that will be generated in house from primary tissues. Part of the aim of this project will be to produce novel immortalised cell lines from primary tissue.

6) Disabled/replication defective retroviruses. These are non-replicating and require the transfection of cells with components that are expressed on separate plasmids. Self inactivating LTRs are employed. In a small number of cases, we intend to generate retroviruses carrying known oncogenes (telomerase, SV40 large T antigen, vRAS and vREL). These will possess oncogenic potential but will not be used with sharps and strictly used under BSL containment level 2 conditions.

Host/vector system

Plasmids will be grown in disabled strains of E. coli which have a long history of use without harm to human health or the environment. pUC-based vectors have a long history of use without harm to human health or the environment and lack reasonably conceivable means to cause such harm.

Mammalian and avian cells. Include currently available immortalised human and avian cell lines. In addition a number of novel cell lines will be produced during the course of the project.

Retrovirus vectors. These are non-replicating and require the transfection of cells with components that are expressed on separate plasmids. Self inactivating LTRs are employed. In a small number of cases, we intend to generate retroviruses carrying known oncogenes (telomerase, SV40 large T antigen, vRAS and vREL). These will possess oncogenic potential but will not be used with sharps and strictly used under BSL containment level 2 conditions.

Origin & function

1) Plasmid vectors in E. coli:
   a) Positive strand virus sequences even if expressed will have no toxic or oncogenic potential. These will include viral gene products encompassing both structural and non-structural genes of Hepeviridae, Caliciviridae and astroviridae members.
   b) Cellular proteins involved in the RNA virus life cycle even if expressed will have no toxic or oncogenic potential. These include cellular RNA binding proteins, translation initiation factors, molecular chaperones etc.

2. Retrovirus vectors: All vectors will be replication defective
a) Viral gene products will again not have toxic or oncogenic potential.
b) Cellular factors involved in the life cycle of RNA viruses: examples include RNA binding proteins, translation initiation factors, transcription factors. No toxic or oncogenic potential.
c) Cellular cytokine or interferon stimulated genes – expression may result in increased local inflammation.
d) Oncogenes and proteins required for cellular transformation: These are limited to the following: telomerase, SV40 large T antigen, vRAS and vREL. They function in various ways to enable cell immortalization including the inactivation of cellular tumour suppressors including p53 and pRb.

Evaluation of foreseeable effects

1) Plasmid vectors in E. coli:
   a) Positive strand virus sequences even if expressed will have no toxic or oncogenic potential. These will include viral gene products encompassing both structural and non-structural genes of Hepeviridae, Caliciviridae and astroviridae members.
   b) Cellular proteins involved in the RNA virus life cycle even if expressed will have no toxic or oncogenic potential. These include cellular RNA binding proteins, translation initiation factors, molecular chaperones etc.

2. Retrovirus vectors: All vectors will be replication defective
   a) Viral gene products will again not have toxic or oncogenic potential.
   b) Cellular factors involved in the life cycle of RNA viruses: examples include RNA binding proteins, translation initiation factors, transcription factors. No toxic or oncogenic potential.
   c) Cellular cytokine or interferon stimulated genes – expression may result in increased local inflammation.
   d) Oncogenes and proteins required for cellular transformation: These are limited to the following: telomerase, SV40 large T antigen, vRAS and vREL. They function in various ways to enable cell immortalization including the inactivation of cellular tumour suppressors including p53 and pRb.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste - autoclaved (run shown to reach and hold temperature).
Liquid waste - autoclaved as above or treated with appropriate disinfectant, Lifeguard (2.5%) for bacterial cultures, Chloros, Virkon or Perasafe for virus-containing fluids.
Surface decontamination - Lifeguard for bacterial work, chloros for virus work (2% virkon or Perasafe for metal surfaces such as the surfaces of class II cabinets or incubators).
Pipettes soaked overnight in 2% virkon, plastic pipettes soaked overnight in chlorine-based disinfectant, >2500 ppm available chlorine (virus work at level 2).
All procedures known to give 100% kill.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
**Project Containment**

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**Project Ref** 43/15.1

- **Date Ackn'd**: 26/01/2015
- **CU2 Project Title**: Use of recombinant Lentivirus, Retrovirus, Adenovirus and Vaccinia virus expression vectors to study the life cycle and pathogenesis of human papillomaviruses and their gene products
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **CultureVolumeClass3-4**: Consent Granted

**Project notified under transitional arrangements** N

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**

**Project Additional Information**

**Purposes of the contained use**

Production and/or use of replication-defective lentivirus, adenovirus, retrovirus and vaccinia virus particles to enable:

1. The expression of individual human papillomavirus proteins in human keratinocytes and other cell types.
2. The expression of mediators of signaling pathways (cellular proteins) relevant to HPV-mediated disease.
Recipient Organism

Viruses:
1. Defective retroviruses and lentivirus – viral vector systems in which all the viral genes have been removed and which contain self-inactivating mutations/deletions.
2. Defective Adenoviruses deleted in E1 and E3.
3. Thymidine Kinase negative Vaccinia Viruses.

Bacteria:
1. Disabled E.Coli strains, including DH5a, DH10b, Top10, 2 T1R

Cells and cell lines:
1. Hela, W12, S12, SiHa, CaSki, C33A, which are derived from human cervix.
2. U2OS, Saps-2, which are derived from human bone.
3. FSK, NEK, HaCat, NIKS, EF1F, which are derived from human skin.
4. COS, CV-1, which are derived from monkey epithelium.
5. CHO, HEK-293, which are derived from hamster epithelium.
6. 293, 293T, 293TT, phoenix cells, which are derived from human epithelium.
7. J2 3T3, NIH 3T3, TK143, which are of mouse fibroblast origin.
8. Primary keratinocytes and other cell types derived from normal human or mouse tissue (skin, tonsil).

Host/vector system

Genetic Material and Vectors

Plasmids containing Viral and Cellular Sequences

Genetic material for use in these experiments will be derived from papillomavirus or cellular DNA sequences cloned into standard bacterial vectors. The laboratory cloning vectors used in this study will generally consist of pUC series plasmids (or related plasmids) containing human or mouse genes, or viral genes encoded by mouse or human papillomaviruses. Included amongst these are commercially-available bacterial plasmids such as pENTR, pShuttle and pDONR (all non-mobilisable).

In addition, we will also use standard mammalian expression vectors containing papillomavirus or cellular DNA sequences. Amongst these will be pBabeNeo, pBabepuro, pBabehygro, pLXSN, pQCXlH, pQCXlPuro, pQCXZeo, pQCXIbsd, CSII-CMV, CSII-TRE-Tight, pCAG-HIVgp, pCMV-VSV-G-RSV-Rev, pCL-Gag-pol, pHCMV-VSV-G, pAdEasy-1.

Plasmids containing Complete Papillomavirus Genomes.

The complete double-stranded DNA genomes of HPV and MusPV have been cloned into bacterial vectors. These genomes contain endogenous viral promoters, and in some instances additional mammalian promoters (e.g. CMV, EF1F or TRE-Tight) positioned to drive the expression of reporter genes (such as GFP), or drug-resistance markers (such as blasticidin). There is no expected biological action of papillomavirus genes in bacteria and no modifications will be made to increase the level of expression of viral gene products or reporter genes in bacterial hosts.
Retrovirus and Lentivirus vector systems.

These are defective, unable to propagate themselves after infection, and contain self-inactivating deletions. Lentivirus vectors (CSII) contain a deletion in the 3' long terminal repeat (LTR) that results in self-inactivation, and lack the pol, tat, rev, env and gag genes. They cannot produce packaged virions unless co-transfected with pCAG-HIVgp and pCMV-VSV-G-RSV-Rev. Retrovirus vectors lack gag, pol and env cannot be packaged without pCL-Gag-pol, and pHCMV-VSV-G. In both cases, once integrated into the transduced target cells, the virus can no longer be packaged. Cell lines used for packaging contain genes required for virus replication on a separate cassette in order to prevent autonomous virus production. The cell lines used to receive genes of interest are not hazardous and are unable to infect people or animal. Genes will be expressed in mammalian cells from CMV, TRE-tight, EF1F, CAG or TGK promoters, or from the retro/lenti virus promoter contained in the viral LTR. While the cell lines that receive the genes of interest are not expected to be hazardous, some of the genes under investigation have well described oncogenic effects (e.g. HPV 16 E6 and E7). The standard code of practice for virus-handling will be followed and work will be carried out under category 2 containment.

Adenovirus and Vaccinia virus vector systems.

Recombinant Adenoviruses will be prepared using the pAdEasy (Ad 5) backbone, which is deleted in the E1 and E3 genes. The E1 deletion renders the viruses defective for replication and incapable of producing infectious viral particles in target cells. The E3 region encodes proteins involved in evading host immunity and is dispensable. The deletion of both genes creates room for up to 7.5 kb of foreign DNA that can be inserted into the Ad5 genome. The E1 gene, which is necessary for the production of viral particles, is provided in trans by AD-293 cells. pAdEasy-1 carries the ampicillin resistance gene, which is lost after recombination with a shuttle vector. Recombinant vaccinia viruses expressing papillomavirus proteins have a thymidine kinase –ve phenotype (TK-). TK- vaccinia are typically 1000 to 10,000 fold less virulent than parental TK+ viruses. Recombinant Adeno and Vaccinia viruses expressing HPV proteins will be used at category 2 containment level. The standard code of practice for virus handling will be followed.

Origin & function

Inserted Genetic Material

The human papillomavirus type 16 E6 and E7 proteins are expected to provide cells with an increased ability to progress through the cell cycle and to proliferate, when compared to parental cells. The HPV E4 protein functions to inhibit cell cycle progression, and mediates cell cycle arrest in the G2 phase of the cell cycle. The other viral genes that may be expressed in these systems include E1, E2 and E5, which will be subcloned from the full length papillomavirus genome. E1 is a replication protein with helicase activity. E2 is a transcription factor and is involved in viral replication. E5 is a membrane protein that can affect cell receptor density and responsiveness to growth factors, and which can increase cell growth rate.

In the first instance, the cellular genes studied will include components of the Hippo, Wingless (Wnt), NF-kb and Notch pathways. Our analysis will focus on the Yap and Taz transcriptional co-activators, which control cell proliferation (Hippo pathway), β-catenin and for the Notch and NF-kb pathways we will focus on the Notch Intracellular Domain (NICD) and on IkB. All are transcriptional regulators or co-activators. The following constructs will be typical of those used in this study …

Retroviral and Lentiviral Vectors.

i) pQCXIH-YAP 5SA (encodes a constitutively active form of human YAP carrying 5 serine to alanine mutations [Addgene Plasmid #33093])
ii) pQCXIH-YAP ΔCT (Dominant negative form of human YAP lacking the C-terminal trans-activation domain [Addgene Plasmid #33095])
iii) pQCXIH-TAZ (Wild type human TAZ [Addgene Plasmid #32841])
iv) pQCXIH-TAZ 5SA (Constitutively active form of human TAZ carrying 5 serine to alanine mutations [obtained by site-directed mutagenesis of the wild type protein])
v) pQCXII-TAZ ΔCT (Dominant negative form of human TAZ lacking the C-terminal trans-activation domain [obtained by site-directed mutagenesis of the wild type protein])
vi) pLXSN-β-catenin ΔN90 (Constitutively active form of human β-catenin lacking the N-terminal phosphorylation sites for GSK3-3β [Obtained through the recombination of pENTR-N90-beta-catenin [Addgene Plasmid #31787] with the empty pLXSN vector using the Gateway Technology])
vii) pLXSN-β-catenin ΔN90 ΔCT (Dominant negative mutant of human β-catenin lacking the C-terminal trans-activation domain [obtained by site-directed mutagenesis of the wild type protein ΔN90 β-catenin protein])
viii) pBabe-ixBalpa-mut (super-repressor) (mutant form of IκBα with two serine to alanine mutations which increase its repressive activity to NF-κB [Addgene Plasmid #15291])
ix) pLXSN-HPV-16 E6 (Encoding for the wild type E6 protein of HPV-16 [the sequence was subcloned from pre-existing plasmid clones])
x) pLXSN-HPV-16 E7 (Encoding for the wild type E7 protein of HPV-16 [the sequence was subcloned from pre-existing plasmid clones])
xii) pLXSN-HPV-16 E6 SAT (Encoding for a HPV-16 E6 mutant form defective in the p53 binding activity [obtained by mutagenesis of the wild type HPV-16 E6 coding sequence])
xiii) pCSII-HPV-16 E1 (Encoding for the human papillomavirus E1 wild type protein)
xiv) pCSII-HPV-16 E1mut (Dominant negative form of E1 obtained by site-directed mutagenesis of the wild type protein)

Adenovirus and Vaccinia virus vectors.

pAd-HPV-16 E4 (which encodes the human papillomavirus type 16 E4 proteins), pAd-LacZ (which encodes the E.coli β-galactosidase protein) and rVacc-E4 (recombinant Vaccinia virus encoding the papillomavirus E4 protein) have been described previously (Davy,C.E. et al 2002 J Virol 76:9806-9818. ; Doorbar,J. et al (1991) Nature 352:824-7). The papillomavirus E4 protein expressed from these vector systems was obtained from cloned HPV DNA. The E4 protein is an inhibitor of cell cycle progression.

Evaluation of foreseeable effects

Retrovirus and Lentivirus for the generation of stable cell lines.

Although recombinant Retro and Lentiviruses can infect humans, both are defective and unable to be propagated after infection because of the presence of self-inactivating deletions. Cell lines used for lenti/retrovirus packaging require the expression of replication genes from a separate cassette, which eliminates the possibility of autonomous virus production. The cell lines used as recipients of foreign genes are not hazardous, as they are unable to infect people or animals. Foreign genes will be expressed in mammalian cells from either the CMV, TRE-tight, EF1F, CAG or TGK promoters, or from the Retro/Lenti virus promoter contained within the viral LTR. While the cell lines that receive the genes of interest are not expected to be hazardous, some of the genes under investigation have well described oncogenic effects (e.g. HPV 16 E6 and E7). Retro/Lentivirus production and infection will be carried out under category 2 containment, and we will follow the standard code of practice for virus-handling.

Recombinant Adenovirus and Vaccinia virus vector systems.

Recombinant Adenoviruses will be prepared using the pAdEasy (Ad 5) backbone, which is deleted in genes E1 and E3. The E1 deletion renders the viruses defective for replication and incapable of producing infectious viral particles in target cells. The E3 region encodes proteins involved in evading host immunity and is dispensable. The deletion of both genes creates room for up to 7.5 kb of foreign DNA that can be inserted into the Ad5 genome. The E1 gene, which is necessary for the production of viral particles, is provided in trans by AD-293 cells. pAdEasy-1 carries the ampicillin resistance gene, which is lost after recombination with a shuttle vector. The viral and cellular sequences expressed from the recombinant Adenovirus vectors will not affect the vector characteristics. As the HPV E6 and E7 genes have well described oncogenic function, the work will be carried out under category 2 containment as described for Lentil/Retroviruses above.

We do not anticipate a need to produce new recombinant Vaccinia viruses able to express foreign genes, but will use recombinant Vaccinia viruses that we have prepared previously. Recombinant vaccinia viruses expressing papillomavirus proteins have a thymidine kinase –ve phenotype (TK-). TK- vaccinia viruses are typically 1000 to
10,000 fold less virulent than parental TK+ viruses. Recombinant Adeno and Vaccinia viruses will be used at category 2 containment level with adherence to the standard code of practice for virus handling.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The primary identifiable risk is infection of the laboratory worker by aerosol. As a result, all manipulations with recombinant viruses will be conducted in a class II laminar flow hood in a restricted cell culture facility under negative pressure (Class 2 level containment). Gloves, protective masks and protective clothing will be worn within the facility. Soiled and used protective clothing will be sealed in bags and autoclaved before disposal. Glassware is avoided wherever possible and disposable pipettes, tubes, culture flasks etc will be employed. Used disposables will be sealed in bags and autoclaved before disposal. The use of sharps is discouraged when working with recombinant viral vectors because of the risk of contamination through needle-stick injury. When required, sharps will be disposed of in sharps bins that will autoclave prior to final disposal disposal. Liquid waste will be decontaminated using Virkon Tablets (Du Pont), and solid waste will be treated by autoclaving according to standard protocols for the category 2 containment facility.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

N

### Project Containment

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Please enter comments on the GM safety committee on the risk assessment

A requirement to attach the Code of Practice for working with recombinant viral vectors was made. This is now attached. A standard operating procedure to the disposal of recombinant viral vectors was also made. Again, this is now attached.
The project aims to understand the role of viral proteins in the papillomavirus life cycle using an organotypic raft culture approach. Part of this work will look at the process by which HPV causes new lesions, which will be carried out using infectious papillomavirus particles isolated from organotypic rafts. Differences in infectivity between HPV virions prepared from wild type and mutant papillomavirus genomes (e.g. papillomavirus gene knock-outs and point mutants) will allow us to establish the role of individual viral proteins during infection and during the full productive life-cycle. An initial interest centres around our working hypothesis that the viral E4 protein can associate with virus particles to influence infectivity, virulence and virion survival outside the cell. In the first instance, we will focus on the assembly and release of infectious virions, and on the role of viral proteins such as E4 that may have a putative role in virus survival and resistance to desiccation in the environment.

A second aim of our work is to establish how viral genomes from high and low-risk groups are maintained in the infected cell, and to understand how virus infected cells respond to the presence of viral episomes. This work is expected to provide new insight into the initial stages of disease development, and will involve the use of papillomavirus reporter genomes in which a region from the L1 and L2 genes has been replaced with the coding sequence for Green Fluorescent Protein. Such mutant genomes will be made in the context of the wild type papillomavirus background, or in the context of specific mutants that may affect genome replication success. It is hoped that such studies will provide key insight into the different mechanisms that high and low-risk HPV types use to cause disease in humans.

Work on polyomaviruses will investigate the replication of these viruses, with a primary focus on virus host interactions. Recombinant viruses will be constructed that lack the entire genes or certain domains of viral proteins that are thought to be important for interaction with host cell processes. These recombinant viruses will be used to investigate the role of viral protein – host protein interactions for virus replication. In addition, reporter viruses expressing markers such as GFP or luciferase, either as soluble proteins or as fusions with viral proteins, will be constructed. These will be used to aid the identification of infected cells and the expression and localisation of specific viral proteins.
Recipient Organism

Human Papillomaviruses and Polyomavirus genomes of different type, including:

1. HPV 1 (V01116), HPV 2 (X55964), HPV 4 (X70827), HPV 5 (M17463), HPV 6 (X00203), HPV 7 (X74463), HPV 8 (M17463), HPV 11 (M14119), HPV 16 (K02718),
   HPV 18 (X05015), HPV 31 (J04353), HPV 33 (M12732), HPV 45 (X74479), HPV 52 (X74481), HPV 57 (X55965), HPV 58 (D90400), HPV 60 (U31792), HPV 65 (X70829),
   HPV 88 (NC_010329), HPV 95 (AJ620210), HPV 126 (AB646346), Mouse Papillomavirus type 1 genome (GU808564), BKPyV (NC_001538.1), JCPyV (NC_001699.1),
   Murine PyV (NC_001515.1)
Genbank Accession numbers are indicated in brackets

Bacteria:

1. Disabled E.coli strains, including DH5a, DH10b, Top10, 2 T1R

Cells and cell lines:

1. Hela, W12, S12, SiHa, CaSki, C33A, which are derived from human cervix.
2. U2OS, Saos-2, which are derived from human bone.
3. FSK, NEK, HaCaT, NIKS, EF-1F, which are derived from human skin.
4. COS, CV-1, Vero, which are derived from monkey epithelium.
6. 293, 293T, 293TT, phoenix cells, which are derived from human epithelium.
7. RPTEC, which are a commercially available primary human renal epithelial cells
8. U87-MG, which are derived from human glioblastoma
9. J2 3T3, NIH 3T3, TK143, which are of mouse fibroblast origin.
10. Primary keratinocytes and other cell types derived from normal human or mouse tissue (skin, tonsil), or from mice with different genetic backgrounds

Host/vector system

Genetic Material and Vectors:

1. Plasmids containing Viral and Cellular Sequences.

Genetic material for use in these experiments will be derived from papillomavirus or cellular DNA sequences cloned into standard bacterial vectors. The laboratory cloning vectors used in this study will generally consist of pUC series plasmids (or related plasmids) containing human or mouse genes, or viral genes encoded by mouse or human papillomaviruses.

2. Plasmids containing Complete Papillomavirus or Polyomavirus Genomes:

The complete double-stranded DNA genomes of various HPV types and of MusPV and human polyomaviruses have been or will be cloned into bacterial vectors. These genomes contain endogenous viral promoters, and in some instances additional mammalian promoters (e.g. CMV, EF1F or TRE-Tight) positioned to drive the expression of reporter genes (such as GFP), or drug-resistance markers (such as blasticidin) will be inserted. In some instances, mutant viral genomes will be used that are defective
in normal virus protein function. There is no expected biological action of papillomavirus genes in bacteria and no modifications will be made to increase the level of expression of viral gene products or reporter genes in bacterial hosts.

3. Cells and Cell lines:

Papillomavirus or polyomavirus genomes will be excised from their parental vector, before being re-ligated and transfected into the cell systems outlined above. Propagation of whole papillomaviruses genomes will generally be carried out in the NIKS keratinocyte cell line or in primary keratinocytes. Some level of viral gene expression will occur in monolayer cell culture, with life-cycle completion and virus synthesis being limited to cells stimulated to differentiate. Assembled virus particles will be harvested from differentiating cells, either in organotypic raft culture or following differentiation with high-calcium or methyl cellulose. For propagation of polyomaviruses RPTE cells (for BKPyV) or U87-MG cells (for JCPyV) will be used.

Origin & function

Inserted sequences will be either reporter genes such as GFP or Luciferase, or components of recombination systems that allow expression.

1. Cre recombinase (P1-phage); A Type I topoisomerase that catalyzes the site-specific recombination of DNA between loxP sites.
2. LoxP sequence (P1-phage); Target sequence for Cre recombinase which mediates the site-specific recombination of DNA.
3. FRT sequence (Saccharomyces cerevisiae); Target sequence for Flp (flippase) catalyzes the site-specific recombination of DNA between FRT sites.
4. CreERT2; Cre recombinase - estrogen receptor T2 fusion gene. Tamoxifen- inducible Cre recombinase.
5. GFP and its derivatives (Aequorea victoria); Green fluorescent proteins.
6. Gaussia Luciferase (Gaussia princeps); A secreted reporter luciferase.
7. Blastcidin resistance (Bacillus cereus); Blasticidin-S deaminase.
8. LacZ (Escherichia coli); β-galactosidase.

The expression of reporter genes will be directed by several promoter sequences including

1. Human cytomegalovirus promoter (human cytomegalovirus)
2. P tight promoter (synthetic Tetracycline responsible promoter, Clontech, USA)
3. EF-1α and HTLV fusion promoter (rat and HTLV-1, respectively)

Evaluation of foreseeable effects

Wild-type human papillomaviruses typically cause benign epithelial tumors in humans. The HPV types used in this study are typical of those found in the general population, and have the same virulence and epithelial tropisms. In some individuals, HPV-induced lesions can persist and can cause neoplasia. HPV-types (e.g. HPV 11) that are not associated with neoplasia comprise the majority of HPV types, and these types are classified as low-risk. HPV16 and 18 are high-risk HPV types are considered as high-risk, and can be associated with the development of neoplasias and cancers at particular epithelial sites.

The mutated human papillomaviruses produced in this study include knock-out mutants that cannot express particular virus proteins, as well as less severe mutations that express modified viral proteins. Some of the modified viral genomes will have their late genes replaced with a reporter gene such as GFP or luciferase. Such mutant genomes are expected to decrease genome virulence when compared to their wild type counterparts. Gene substitutions that lead to the loss of the virus late genes necessary for virion production will not be able to complete their productive cycle.

MusPV, like all papillomavirus is host and tissue specific, does not infect humans. MusPV is prevalent in wild European mice but is associated only with inapparent infections that are controlled by the animals immune system. Mutations in MusPV are not expected to increase genome virulence, or to change its host range.

Polyomaviruses are small non-enveloped DNA viruses that are ubiquitous pathogens of humans. These viruses establish long-term persistent infections in humans and the majority of the population are infected by these viruses early in childhood. Polyomaviruses cause relatively benign or asymptomatic infections in immunocompetent hosts and appear to cause pathology only in immunocompromised patients. Given that the majority of the population is seropositive for these viruses and they do not appear to
cause disease in healthy individuals, handling these viruses would only conceivably pose a risk to immunocompromised, seronegative people. Polyomaviruses have theoretical oncogenic potential; they all express large-T and small-t antigens and these proteins from some polyomaviruses have the capacity to transform cells in culture. However only one human polyomavirus (MCPyV) has a well-documented association with human cancer, a rare form of skin cancer known as Merkel Cell Carcinoma, in immuno-compromised patients. However the other human polyomaviruses have not been associated with any neoplastic conditions, despite long-term or even life-long persistent infection by these viruses. The recombinant viruses that will be constructed will either contain reporter genes (such as GFP or luciferase) and/or mutations that are expected to be deleterious (such as gene deletions and point mutations designed to inhibit function). No alterations that could be expected to alter virus tropism will be introduced into polyomavirus genomes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The primary identifiable risk is contamination of the laboratory worker either by aerosol or needle stick inoculation. As a result, all manipulations with recombinant viruses will be conducted in a class II laminar flow hood in a restricted cell culture facility under negative pressure (Class2 level containment). Gloves, protective masks and protective clothing will be worn within the facility. Soiled and used protective clothing will be sealed in bags and autoclaved before disposal. Glass ware will be avoided wherever possible and disposable pipettes, tubes, culture flasks etc will be employed. Used disposables will be sealed in bags and autoclaved before disposal. The use of sharps will be discouraged when working with recombinant viral vectors because of the risk of contamination through needle-stick injury. When required, sharps will be disposed of in sharps bins that will be autoclaved prior to final disposal. Liquid waste will be decontaminated using Virkon Tablets (Du Pont) or hypochlorite (e.g. Chloros at 10% v/v), and solid waste will treated by autoclaving according to standard protocols for the category 2 containment facility. Work areas will be disinfected with 1% Virkon (or equivalent) treatment after use.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

A requirement to attach the Code of Practice for working with recombinant viral vectors was made. This is now attached. A standard operating procedure to the disposal of recombinant viral vectors was also made. Again, this is now attached.

Project Containment

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

We study host-parasite interactions within the context of blood-stage malaria infection and Trichinella spiralis muscle stage infection. This involves looking at the host cellular responses to the parasite as well as studying parasite pathways important to maintaining homeostasis. Trichinella parasites will not be maintained on site, we will be working with specific parasite proteins that we will individually express in mammalian myoblasts to study their effects on the host. For the malaria work we will be genetically modifying parasite lines either by knock-in or knock-out techniques to study the function of individual genes in the parasite itself and in its interaction with host antigen presenting cells (either cell lines or primary cells).

The aim of this work is to gain insights into mammalian cell biology by first understanding how parasites manipulate host pathways to their advantage.

**Recipient or parental organism**

Organisms that will be genetically modified as part of this project:

- A. Mammalian Cells
- Well-characterized mammalian tissue culture cell lines including THP-1, RAW264.7, DC2.4, HeLa, 293T, C2C12 as well as primary human macrophages
B. E. coli
Standard laboratory strains such as XL-10, XL-1 Blue, DH-10, BL21, Rosetta2 which are all highly disabled strains.

C. Plasmodium falciparum parasites
Parasite lines to be modified include laboratory-conditioned strains 3D7, NF54, Dd2.

Host/vector system

A. pGEMT, pcDNA3.1+, pLVX, pLPCX, pPRETO, lentiviral and retroviral accessory plasmids gag/pol, vsvg, tat, rev.
Viral vectors are under the CMV or the UBC promoters. Retroviral and lentiviral vectors are 3rd and 4th generation, commercially available, and replication defective, therefore unable to propagate themselves after infection.

B. pGEMT, pet28, pet29, pGEX, pUC series plasmids

C. pUML, pCAM-BSD, pCC1, pDC2-AttP, Plasmodium expression vectors containing a drug selection marker to either WR 33063 or blasticidin antimicrobials and the hsp86 promoter.

Origin & function

Plasmid vectors in E. coli: DNA plasmids will be propagated in E. coli.
Protein expression in E. coli: E. coli will be transformed to express recombinant proteins.
Lentiviral and retroviral production/infection in mammalian cells: we will be using viral transduction to create transgenic primary cells and cell lines. We will be focusing mammalian proteins involved in antigen capture, processing and presentation. These include cell-surface receptors on antigen presenting cells and components of the endosomal pathway. We plan to fluorescently or biochemically tag these proteins and to express them in relevant mammalian cells. In addition, we plan to express genes of unknown function of Trichinella spiralis in mammalian cells to understand what they do.
Knock-out and knock-in mutants in Plasmodium falciparum: We will be knocking out specific genes and observing parasite viability to assess whether they are essential to Plasmodium survival. For knock-in mutants, we will be replacing endogenous genes with tagged version to allow for biochemical and imaging analyses. The resulting parasite lines will be used to query gene product behaviour and function.

Evaluation of foreseeable effects

Plasmid vectors in E. coli: This approach will not have any toxic or oncogenic effects.
Protein expression in E. coli: None of the proteins to be expressed are toxic or oncogenic.
Lentiviral and retroviral production/infection in mammalian cells: We will be using the commercially available 3rd generation lentiviral systems from Clontech where all essential replication genes have been removed. Lentivector and packaging functions (gag/pol, tat, rev, vsv-g) have been separated into 4 vectors and the envelope protein has been replaced with the G protein of vesicular stomatitis virus. Viral plasmids will be co-transfected into mammalian packing cell lines (293T cells) with accessory plasmids gag/pol, tat, rev and vsv-g. Cell supernatants will contain live viruses that are replication defective and are, therefore, unable to propagate themselves after infection.
Recombination events giving rise to viruses competent of reproduction have not been observed and are highly unlikely given that at least 4 recombinations in non-homologous regions would need to occur. The cell lines that will be subsequently transduced by these viruses are not infectious or hazardous to humans. The specific genes of interest that will be expressed in cells are also non-hazardous and have no known deleterious effects. We will not be using any known oncogenes or genes with high oncogenic potential, but we will rather be focusing on mammalian proteins involved in antigen capture, processing and presentation. These include cell-surface receptors on antigen presenting cells and components of the endosomal pathway.
We plan to characterize the function of individual Trichinella spiralis proteins by expressing them in mammalian cell lines and assessing changes in phenotype. Most of these proteins have known orthologs in other organisms and
others are unknown and T. spiralis-specific. The constitutive expression of a parasite protein in a mammalian cell is a very artificial system and aims to specifically characterize the function of the individual proteins. Therefore, the risk of creating a more infectious organism is entirely non-existent. The minimal clinical symptoms associated with Trichinellosis on a whole organism level underscore the implausibility of T. spiralis proteins posing a higher risk on an individual level, particularly when expressed in well-defined, commercially available cell lines. The standard code of practice for virus-handling will be adhered to for lentivirus production and infection.

Knock-out and knock-in mutants in Plasmodium falciparum: Although these manipulations may alter parasite transcriptional and proteomic processes, they are not expected to alter parasite virulence. Plasmodium falciparum is a very well-studied organism and there is no evidence, either published, anecdotal or in our own experience, that eliminating a gene results in a parasite with increased infectivity or virulence. Tagged genes will be targeted to the endogenous locus via homologous recombination and will effectively replace the wild-type version of the gene. Therefore, the resulting parasite line will be phenotypically no different than wild-type.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

DEROGATED CONTAINMENT LEVEL 3 FOR PLASMODIUM FALCIPARUM:
Blood-stage infection is not transmitted by aerosols. However, the wild type strains themselves are infective to humans at the erythrocytic stage with which we will be working. To avoid infection, parasites will be handled within a microbiological safety cabinet, using a labcoat gloves and safety glasses (standard laboratory PPE) and use of sharps will be avoided. In case of suspected exposure to infected erythrocytes by needlestick, standard protocols for Plasmodium-exposure that are in place will be followed, including treatment with chloroquine. Plasmodium falciparum is a human pathogen that is treatable but that can cause significant symptoms. It is, therefore, classed as a Hazard Group 3 pathogen and needs to be handled with care to minimize the risk of self-inflicted infection. Given that it does not aerosolize, needs a mosquito vector to be naturally transmitted, and exposure to it can be minimized with standard laboratory PPE, it can be handled in a derogated Containment Level 3 setting. Specifically:
- the laboratory does not need to be maintained at an air pressure negative to atmosphere because the agents are not transmissible by the airborne route
- the laboratory does not need to have exhaust air extracted using HEPA filtration, although any work that could give rise to an aerosol of infectious material will be carried out in a microbiological safety cabinet,
- the laboratory does not need to be sealable to permit fumigation because these agents are extremely easily broken down and cannot survive and/or multiply in the environment

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste pertaining to bacterial work including liquid cultures, contaminated glassware and all solid waste will be autoclaved using validated temperatures and recorded temperature cycles. Likewise, Any equipment used during viral production and cellular transduction will be decontaminated with Virkon, Perasafe or Chloros (hypochlorite) under manufacturers’ validated conditions and all solid waste will be autoclaved using validated and recorded temperature cycles.
Plasmodium falciparum work and handling of human red blood cells will be done under derogated CL3 conditions and all waste will be autoclaved before exiting the CL3 area.
Autoclaving is expected to kill all organisms.
The project was reviewed by our internal Biological Safety Committee, and amended as follows:

- More detail added regarding the genes to be propagated and expressed in E. coli
- More detail added regarding the genes to be modified in Plasmodium falciparum; specifying that gene knock-ins are repairs of knock-outs
- A statement that all staff working with P. falciparum were to be notified to Occupational Health.

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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 43/16.2

Date Ackn’d 29/07/2016

CU2 Project Title Use of Lentiviral vectors for the discovery and investigation of cancer pathways, including pathways directly influenced by oncogenic viruses, and development of clinically significant marker genes

Class Class 2

Culture Volume Class 2 ≤ 1 Litre

Class 3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info 02/03/2022
Project Additional Information

Purposes of the contained use

The Aim of this project is to identify new cancer pathways and to discover clinically significant marker or target genes. We have and continue to identify genes which are amplified or deleted as well as genes which transcription is deregulated in cancers of interest (in particular cervical cancer or other HPV induced cancers, SCC in general as well as childhood cancers, including malignant germ cell tumours). We also have and will continue to identify novel fusion genes which are the results of translocation events in cancers of interest. Depending on the characteristics of the genes/fusion genes identified, a variety of experimental work is carried out. Identified genes as well as other genes associated in relevant pathways are investigated to study the effects of neoplastic changes and evaluate their potential as clinical marker or target genes. When appropriate, the effects of overexpression of genes of interest or of genes involved in the regulation of the genes of interest are studied in details in appropriate cell models through the establishment of stable cell lines. Conversely, we may want to study the effects of temporary or permanent suppression of genes associated in relevant pathways on neoplastic changes and evaluate their potential as clinical marker or target genes. This will be achieved by stably expressing shRNAs or by genome editing of the genes of interest.

We are also interested in identifying specific effects that LR and HR-HPV may have on cellular gene functions including expression of genes modulating innate immune responses, cell growth, neoplastic changes, as well as in regulating the expression of their own genes. We already use cell lines containing all of the listed genes either as episomal viral genomes, or as integrated virus, but these studies will help dissecting out the contribution of each individual gene to effects we have already identified in cell lines containing the whole viral genome. In some cases we will modify by genome editing genes or regulatory regions in the already existing cell lines to study the effects of these changes, in particular with reference to the regulation in cis or trans of specific regions due to chromatin remodelling following viral integration.

Recipient or parental organism

Replication defective Lentiviruses/retroviruses (generally considered ACDP1 if not containing exogenous material) are produced following co-transfection of 293T or similar cells with two or three plasmids independently providing packaging and replication. Although they are replication-incompetent, they may have a broad tissue tropism due to
the presence of a VSV-G envelope. The ultimate goal is to use the GM viruses to transduce cells so that we can alter expression of genes of interest. The obtained cell lines will then be used for further investigations.

**Host/vector system**

Viral vectors: The second/third generation of lentivirus has been significantly modified for biosafety, including some or all of the following: 1. Packaging vector lacks both LTRs and has no viral packaging signal (y); 2. The viral genes (env, tat, vpr, vpu, vif and nef) have been deleted from the packaging vector; 3. Rev is supplied in trans on a different vector (3rd generation only); 4. The vector expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion) and expresses no viral gene products; 5. Envelope is expressed from a separate vector. In some cases, mutations to the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) sequence may be also present. The presence of WPRE significantly increases transgene expression in target cells: however the WPRE sequence contains a truncated form of the WHV X gene, which has been implicated in animal liver cancer. Similarly retroviruses vectors lack gag, pol and env cannot be packaged without provision in trans. Although they are replication-incompetent, they may have a broad tissue tropism due to the presence of a VSV-G envelope.

**Origin & function**

Lentivirus/Retrovirus for the generation of stable cell lines: We will produce viruses to ultimately produce cell lines of interest. Viruses will be produced using second generation or later packaging systems which have improved safety. As a minimum, the cell lines that are used for virus packaging contain, either transiently or stably, the genes required for virus replication on separate cassettes in order to eliminate the possibility of autonomous virus production and no viral accessory proteins (Vif, Vpu, Vpr or Nef). Additionally, the viruses contain self-inactivation deletions, such as deletion in the U3 region of the 3’ –LTR. Moreover, mutations to the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) sequence may be also present. The presence of WPRE significantly increases transgene expression in target cells: however the WPRE sequence contains a truncated form of the WHV X gene, which has been implicated in animal liver cancer. The GM viruses will contain genes that will be expressed from promoters that are active in mammalian cells such as CMV, TRE3GV, TRE-tight, EF1F, CAG or PGK promoters, or from the retro/lenti virus promoter contained in the viral LTR. We may use constitutive or induced expression systems. The use of inducible system of expression will allow the study of particular pathways without the added effects that long-term expression of these genes may have on cellular processes. The use of inducible systems provides an extra layer of safety allowing the expression of the genes of interest in a controlled fashion. In some cases the inserted genes will have well described oncogenic effects (e.g. HPV 16 E6 and E7). We will also generate viruses expressing sh-RNAs to silence genes of interest. Finally, crRNA, tracrRNA, sgRNA and/or Cas9 expressing viruses will be produced to carry out gene editing of genes of interest or other genomic areas of interest using the CRISPR/Cas9 system. Although these viruses can infect humans, they are defective and unable to propagate themselves after infection.

**Evaluation of foreseeable effects**

Genes/miRNA/molecules of interest:

There are many genes/molecules of interest we have identified in the course of our studies. Current work primarily focusses on the ones listed below, but new ones are identified continuously. We study the genes/molecules of interest themselves as well as other genes/molecules of interest that may be part of downstream or upstream pathways e.g. the signalling pathways that the genes/molecules of interest activate. This is not an exclusive list, but gives an idea of the direction of our studies. The genes described are typical of the type of genes that will be genetically manipulated and the phenotypes of genes not explicitly listed here are not expected to be more severe than for the listed genes.

1. Cytokines, cytokine receptors and their downstream pathways.

We are interested in how cytokines influence cancer cells and in particular we focus on cytokines which receptor is
overexpressed in cervical and other cancers. In particular we are currently interested in: OSMR and its ligand OSM: OSMR is a member of the type I cytokine receptor family. OSMR hetero-dimerizes with interleukin 6 signal transducer to form the type II oncostatin M receptor and with interleukin 31 receptor A to form the interleukin 31 receptor, and thus transduces oncostatin M and interleukin 31 induced signalling events. Oncostatin M is a member of a cytokine family that includes leukemia-inhibitory factor, granulocyte colony-stimulating factor, and interleukin 6. This gene encodes a growth regulator which inhibits the proliferation of a number of tumour cell lines. It regulates cytokine production, including IL-6, G-CSF and GM-CSF from endothelial cells.

STATs: In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. Various cytokines and growth factors including IFNs, EGF, IL5, IL6, HGF, LIF and BMP2 induce such phosphorylation. STAT family members mediate the expression of a variety of genes in response to cell stimuli, and thus play a key role in many cellular processes such as cell growth and apoptosis.

HIFs: These genes encode transcription factors involved in the induction of genes regulated by oxygen, which is induced as oxygen levels fall. They are regulated by and in turn regulate STATs.

2. microRNAs and the genes/pathways they regulate:
There are numerous microRNAs expressed in human cells. We are particularly interested in a subset of microRNAs which are either overexpressed or under-expressed in Germ Cell Tumours. These include miR-371–373, and the clusters miR-302/367, as well as miR-9, the miR-30 family, miR-125 and miR-181. Not all functions of microRNA have been elucidated to date, some of the identified roles include regulation of cell proliferation (miR371–373, miR125, miR-9), alternative splicing (miR-30), metastasis (miR371–373, miR-9) and cell metabolism (miR-181).

DROSHA: A members of the ribonuclease III superfamily of double-stranded (ds) RNA-specific endoribonucleases which participate in diverse RNA maturation and decay pathways in eukaryotic and prokaryotic cells. The RNase III Drosha is the core nuclease that executes the initiation step of microRNA (miRNA) processing in the nucleus.

3. Chromatin modellers and Transcription regulators:
Changes in chromatin structure and regulation of gene expression by epigenetic mechanisms, such as histone posttranslational modification, are involved in most cellular biological processes. Abnormal regulation of epigenetics is implicated in the occurrence of various diseases, including cancer. We are interested in modulating complexes which have a role in chromatin structure, as we have found that chromatin epiregulation at HPV16 integration site has a role in determining the levels of viral genes transcription. We would like to study the effects of silencing by shRNA and/or genome editing of cellular genes involved in these processes on the transcription levels of the viral genes. In some cases genome editing will be used to constitutively upregulate the expression of some genes of interest. We will also want to target for genome editing binding sites for the genes of interest, including on the HPV genome integrated in cells. Genes we will be targeting include for example CDK9, HATs such as TIP60 and p300, HDACs, lysine methylases and generally enzymes involved in regulating the presence of epigenetic marks on histones. We are also interested in dissecting the roles of transcription regulators such as transcription factors and hormone receptors on the level of viral gene transcription, as the HPV16 URR contains numerous binding sites for a variety of transcription factors (AP-1, YY1, GR, etc). We may also want to target other structural regulatory proteins such as CTCF and their binding sites, which also have a role in regulating gene transcription. Finally we are also interested in genes adjacent or distal to the viral integrations sites which are deregulated by novel chromatin interactions following HPV integration, to assess if the viral induced modification of chromatin structure has an effect on transcription of genes that may promote carcinogenesis.

4. Human Papilloma Virus genes:
There are two classes of HPV: high risk and low risk – the classification reflecting their capacity to promote cancer. Most of our work will use high risk HPV16 type but we may use low risk viral genes as well for comparison. Although
the functions of the viral proteins are somewhat similar in high and low risk viruses, high risk viruses genes tend to have more functions and potentially have a higher risk associated with them. We have used plasmid to make stable cell lines, but this has not always been successful. We are proposing the use of retroviruses to increase the efficiency of delivery. Three HPV genes have known oncogenic effects.

E7 protein of HPV16 has both transforming and trans-activating activities. Disrupts the function of host retinoblastoma protein RB1/pRb, which is a key regulator of the cell cycle. Induces the disassembly of the E2F1 transcription factors from RB1, with subsequent transcriptional activation of E2F1-regulated S-phase genes. Inactivation of the ability of RB1 to arrest the cell cycle is critical for cellular transformation, uncontrolled cellular growth and proliferation induced by viral infection. Stimulation of progression from G1 to S phase allows the virus to efficiently use the cellular DNA replicating machinery to achieve viral genome replication. Interferes with histone deacetylation mediated by HDAC1 and HDAC2, leading to activation of transcription. More biological actions are expected in the transfected cells as E7 has also roles in immune modulation and new interactions are still being described for this protein.

E6 protein of HPV16 plays a major role in the induction and maintenance of cellular transformation. Acts mainly as an oncoprotein by stimulating the destruction of many host cell key regulatory proteins. E6 associates with host E6-AP ubiquitin-protein ligase, and inactivates tumour suppressors TP53 and TP73 by targeting them to the 26S proteasome for degradation. In turn, DNA damage and chromosomal instabilities increase and lead to cell proliferation and cancer development. The complex E6/E6P targets several other substrates to degradation via the proteasome including host NFX1-91, a repressor of human telomerase reverse transcriptase (hTERT). The resulting increased expression of hTERT prevents the shortening of telomere length leading to cell immortalization. Other cellular targets including Bak, Fas-associated death domain-containing protein (FADD) and procaspase 8, are degraded by E6/E6AP causing inhibition of apoptosis. E6 also inhibits immune response by interacting with host IRF3 and TYK2. These interactions prevent IRF3 transcriptional activities and inhibit TYK2-mediated JAK-STAT activation by interferon alpha resulting in inhibition of the interferon signalling pathway. More biological actions are expected as the list of proteins E6 interacts with is continuously increasing.

E5 of HPV16 acts to keep host cells in a proliferation-competent state upon differentiation. Enhances host epidermal growth factor receptor (EGFR) activation after stimulation by EGF by inhibiting EGFR internalization. Induces a redistribution of host caveolin-1 and glycosphingolipid (ganglioside GM1) components of lipid rafts to the plasma membrane. Since GM1s inhibit cytotoxic T-lymphocytes, block immune synapse formation, and enhance proliferative signalling by the EGFR, E5 may enhance immune evasion and cell proliferation via a common mechanism. E5 also alters endosomal pH by interacting with the vacuolar H+-ATPase, which is a proton pump responsible for acidifying cellular organelles. Additionally, E5 prevents transport of the major histocompatibility class I to the cell surface and retains the complex in the Golgi apparatus. More biological actions are expected as not all E5 interactions are fully understood.

No significant hazard is anticipated with regard to the expression of the other viral genes and their mutants in mammalian cells.

The E1 protein is a replication protein with helicase activity.

The E2 protein is a transcription factor and is involved in viral replication and viral genome segregation during cell division.

The E4 protein inhibits cell proliferation and interferes with the normal structure of the cytoskeleton.

5. CRISPR/Cas9 system:

We will use the CRISPR/Cas9 system for genome editing using lentiviruses to deliver Cas9 and crRNA/tracrRNA/sgRNA. CRISPR/Cas9 systems use the ability of Cas9 to be recruited by crRNA/tracrRNA to specific genomic sites and in turn create DNA double strand breaks which can be then repaired in an altered manner. In genome editing systems, Cas9 is directed to the site of interest via a synthetic guide RNA (sgRNA), which can be
provided in cis or trans. Catalytically inactive or "dead" Cas9 can also be recruited by gRNAs to specific target DNA sites and used as a platform for recruitment of heterologous effector domains to specific genomic loci. For example, dCas9 fusions to a transcriptional activation domain (VP64 or the p65 subunit of nuclear factor kappa B; NF-kB) or a transcriptional repression domain (the Krüppel-associated box (KRAB) domain) have been shown to regulate the expression of endogenous genes in human and mouse cells. This technology has become widely used but is still being further developed. Currently there are two main systems in use. One delivers Cas9 separately from the crRNA/tracrRNA/sgRNA and when this system is used Cas9 expression is generally (but not exclusively) inducible under the control of the TET repressor, and only after treatment of transduced cell with doxycycline or similar compounds there is expression of Cas9. The alternative system will deliver Cas9 and crRNA/tracrRNA/sgRNA via the same lentivirus, and in this case Cas9 expression is always constitutive. Constitutive expression of Cas9 can result in non-specific ds breaks, which in turn could lead to mis-repairing and potential mutagenesis. The chosen system has implications for the safety of the GM lentivirus with inducible Cas9 expression introducing a further level of safety. Both systems will be used in this project, although preference will be given whenever possible to Cas9 inducible systems.

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<td>Transduced cell lines: Lentiviral transduction will be used to make new stable cell lines expressing naive, as well as mutated active or inactive forms of genes of interest. This will include both human, mouse and viral (i.e. Human Papillomavirus) genes. In other cases, we will make cell lines in which a gene or set of genes will have been silenced by either the use of shRNA or by CRISP-R/Cas9 based genome editing. In some case genome editing will be used to express constitutively genes of interest. All of the established cell lines utilised are well characterised or authenticated with a low risk of endogenous infection with a biological agent and which have been tested for the most serious pathogens. As such they will pose minimal risk to both human health and the environment. In primary cells there is a slightly elevated risk owing to the increased risk of adventitious pathogens being present, in particular for human primary cells, although the likelihood of colonization remains low. Mouse cells give negligible risk because of the phylogenetic difference means adventitious pathogens and colonization both become very low probability. This risk is not going to be greatly increased by the addition of the genes of interest. The expectation is that there will be an effect from the transduction of these cells, which will be the subject of investigation; we are expecting a variety of effects which will vary depending on the gene of choice. Some of the genes under investigation have well described oncogenic effects (e.g. HPV 16 E6 and E7), or are tumour suppressor genes, however, the cell lines that receive the genes of interest are not expected to be more hazardous as a result of the genetic manipulation, as they do not produce infectious material and are unable to infect people or animals.</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
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<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
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<tbody>
<tr>
<td>Virkon (a peroxygen compound) used at a concentration of 1% is known to have a wide range of microbial activity and can inactivate many human viruses, including HIV. Tissue culture will be carried out using disposable material. Disposable pipettes will be soaked in a freshly prepared 1% Virkon solution (or other Peroxygen Compound at equivalent efficacy) for a minimum of 18-24hrs after which they will be placed in an autoclave bag and autoclaved at 134° C for 20 minutes at 3.2 bar pressure. All other disposable plastic ware if containing minimum liquid contamination, will be placed in a biological waste bag while work is carried out. At the end of the day work (i.e. on the same day) the bag will be sealed, bagged again and autoclaved at 134° C for 20 minutes at 3.2 bar pressure.</td>
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02/03/2022
Liquid waste will be inactivated with 1% Virkon solution (or other Peroxygen Compound at equivalent efficacy) for a minimum of 18-24hrs after which the fluid will be disposed down a laboratory sink with excess water. Validation of this method will be by independent thermocouples placed in the centre of the load. Verification that the correct conditions have been reached will be obtained by checking the chart recorder printout. The autoclave undergoes a planned preventive maintenance inspection.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project was passed by our internal Biological Safety Committee after the addition of addition information on the vector systems used and more specificity regarding the molecules of interest.

Project Containment

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<td>L2 L3 L4 L2</td>
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Animal Units

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Project Ref 43/17.1

Date Ackn’d 05/01/2017

CU2 Project Title Studying the consequences of deficiency in Fbxo7 expression in humans

Class 2 Culture Vol Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

02/03/2022

Page 1937 of 1532
A patient has a germline mutation in Fbxo7. Our aim is to study the effects of the loss of Fbxo7 expression for this patient, and to determine the cellular pathways that are defective in his cells. To do this our aim is to complement his mutation, introducing back in WT expression and also to use his cells to create fibroblasts that contain mutations in Fbxo7 that have been associated with cases of PD.

Recipient or parental organism
Cultured patient fibroblasts

Host/vector system
Retroviruses-lentiviruses (3rd generation)

Origin & function
Cells are obtained from a skin biopsy from the patient, and they will be cultured in vitro for biochemical and cellular assays.

Evaluation of foreseeable effects
Patient fibroblasts have a limited lifespan in culture, typically between 1-3 months before undergoing senescence. The introduction of a wild-type copy of the gene may allow the cells to grow for longer in culture. Fbxo7/PARK15 gene expression can transform already immortalized cells. The recipient patient cells are primary fibroblasts, and will likely undergo senescence as normal fibroblasts do. It is extremely unlikely that introducing this single gene back into the patient's fibroblasts will do more than complement the known mutation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Standard validated methods will be used:
- Liquids will disposed of in Virkon (1% solution) and disposed of via drains after a minimum of 12 hours. Effectively 100% kill.
- Mixed disposable plastic waste: validated autoclave, 121°C for 30 minutes, then disposed of to incinerator. 100% kill.
- Disposable pipettes: either (a) as for mixed disposable waste or (b) immersion in solution of Virkon (1% solution) for a minimum of 12 hours; the Virkon drained off, followed by autoclaving and incineration. 100% kill.

The Department's Biological Safety Committee reviewed the attached risk assessment and approved it once the waste disposal policy had been made consistent, and the requirement for appropriate vaccination of workers had been added.

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Animal Units
| L2 | L3 | L4 |

Large Scale Activities
| L2 | L3 | L4 |

Human Clinical Applications
| L2 | L3 | L4 |

Project Ref 43/19.1

Date Ackn'd 21/11/2019

CU2 Project Title The p53 protein family: Tp53, Tp63 and Tp73 gene silencing or over-expression to investigate their biological functions

Date Project Ceased

Class 2 CultureVolClass2 < 1 Litre

Consent Granted
During the initial experimental phase, we will generate knock-out cell lines for p53, p63 or p73 by different strategies. On the counterpart, over-expression of the proteins will be obtained through simple plasmid DNA transfection for episomal transient expression. Knock-out of either of the three genes will be obtained through small interfering RNA transfection or through the CRISPR/Cas9 technology.

CRISPR/Cas9 is a gene editing technique that exploits a DNA sequence-specific guide RNA (gRNA) to target a bacterial nuclease Cas9 on a selected nucleotide sequence. In our case, the designed gRNAs, specific to either of the p53 family genes, recognise its target being partially complementary to its nucleotide sequence, hybridise with it and recruit the Cas9 enzyme. Cas9 cleaves DNA inducing a double strand-break shortly downstream of the gRNA recognition sequence. The DNA double strand break is then repaired by the DNA repair machinery, usually resulting in frameshift mutation of the target gene and therefore its knock-out.

Concurrent expression of the gRNA and the Cas9 nuclease, essential for gene knock-out, could be obtained through simple transfection methods, using standard transfection agents, or by lentiviral or retroviral infection of the target cells.

Cells will be screened for the gene deletion through Sanger sequencing of the gene locus to prove the efficiency of the knock-out.

Finally, genetically modified cells will be used in various cellular biology assay such as proliferation, citotoxicity, migration assays, etc.

The host micro-organism used during the cloning phase of the gRNA or the gene of interest in each vector, is the bacterial E. coli TOP10 strain (which is a K12 derivative strain). This is not a mammalian expression strain. Expression can happen but there is a very low risk potential.

Defective lentivirus or retrovirus produced from packaging mammalian cell lines, in which the helper genes are located in two separate plasm ids, thus eliminating the possibility of a reversion to replication competence by a single recombination event.

Immortalised human cell lines:
- H1155 (lung cancer)
- H23 (lung cancer)
- H1299 (lung cancer)
A549 (lung cancer)
HEK293FT (embryonic kidney)
293T (embryonic kidney)
U251 (glioblastoma)
SH-SY5Y (neuroblastoma)
MCF7 (breast carcinoma)
U20S (bone osteosarcoma)
Immortalised mouse cell lines:
KC (pancreatic cancer harbouring k-ras mutation)
KPC1 270 (pancreatic cancer harbouring k-ras mutation, and mutated p53)
KPCB 172 (pancreatic cancer harbouring k-ras mutation, and mutated p53)
The host micro-organism used during the cloning phase of the gRNA or the gene of interest in each vector, is the bacterial E. coli TOP10 strain (which is a K12 derivative strain). This is not a mammalian expression strain. Expression can happen but there is a very low risk potential. Defective lentivirus or retrovirus produced from packaging mammalian cell lines, in which the helper genes are located in two separate plasmids, thus eliminating the possibility of a reversion to replication competence by a single recombination event.

Immortalised human cell lines:
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SH-SY5Y (neuroblastoma)
MCF7 (breast carcinoma)
U20S (bone osteosarcoma)
Immortalised mouse cell lines:
KC (pancreatic cancer harbouring k-ras mutation)
KPC1 270 (pancreatic cancer harbouring k-ras mutation, and mutated p53)
KPCB 172 (pancreatic cancer harbouring k-ras mutation, and mutated p53)
KPiC (pancreatic cancer harbouring k-ras mutation, and knocked-out p53)

• Primary cell lines:
MEF (Mouse embryonic fibroblast)
Human keratinocytes

Host/vector system

Vector(s)
Lentiviral vectors:
pMD2.G (Addgene)
pMDLg/pRRE (Addgene)
pRSV-Rev (Addgene)
pLP1 (Invitrogen)
pLP2 (Invitrogen)
<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Description</th>
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<tbody>
<tr>
<td>pLIVSVG (invitrogen)</td>
<td>Health and Safety Executive</td>
</tr>
<tr>
<td>LZRSpBMN GFP (HK2 expressing - not produced by our laboratory, but received and ready to use)</td>
<td>Cas9 and or gRNA delivering plasmids:</td>
</tr>
<tr>
<td>FUCas9Cherry (Addgene)</td>
<td>FgH1tUTG (Addgene)</td>
</tr>
<tr>
<td>ipVSEPR</td>
<td>pSpCas9(BB)-2A-GFP PX458 (Addgene)</td>
</tr>
<tr>
<td>pSpCas9(BB)-2A-Puro PX459 (Addgene)</td>
<td>U6-gRNNCMV-Cas9-GFP (Sigma)</td>
</tr>
<tr>
<td>Vectors used for plasmid DNA transfection:</td>
<td>pcDNA</td>
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<tr>
<td>pGL3</td>
<td>pCMV6</td>
</tr>
<tr>
<td>pEZX-PG02 (Gencop)</td>
<td>pLKO,1 puro</td>
</tr>
<tr>
<td>pLKO-Tet-On</td>
<td>pRSV-RW</td>
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</tbody>
</table>

**Origin & function**
The host micro-organism used during the cloning phase of the gRNA or the gene of interest in each vector, is the bacterial E. coli TOP10 strain (which is a K12 derivative strain).

**Evaluation of foreseeable effects**
The knock-out of each of the mentioned genes might have oncogenic potential. Lentivirus are able to infect human cells. However, GM viruses are replication deficient. Any exposure to the virus may carry some hazard, but the separation between the Cas9 and the gRNA delivering viral particles will minimise the possibility of an accidental gene knock-out in the case of exposure of a worker. In case the knock-out might be obtained through classical transfection techniques, instead, the risk for exposure is effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
No GM animals or plants will be used, but only micro-organisms

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
GMMs will be stored in a dedicated -80°C freezer located in the containment level 2 designated tissue culture room. The containers will be clearly labelled with the name of the operator, the date, and a concise description of its content. In case GMMs will need to be stored outside the CL2 rooms, the freezer will be clearly labelled to specify that it contains hazard group 2 organisms.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Solid waste: solid waste will be autoclaved before disposal. Plasticware will be pre-treated with Prespekt overnight before autoclaving. Prespekt (which contains sodium dychloroisocyanurate) will be used at a final free chlorine.

02/03/2022
concentration of 10,000 ppm. Before autoclaving, the excess detergent will be drained off. Tissues and gloves should be also placed in the autoclave bin. Liquid waste: liquid waste will be treated with Presept for 24 hours and then discarded. In case of spillages surfaces and instruments can be treated with 10% Chloros (10,000 ppm available chlorine).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Biological Safety Committee assessed the risk assessment and gave its approval after the author had provided clarification of the appropriate level of PPE and disinfectants to be used, as well as further detail on the levels of risk attributed to the procedures involved.

Project Containment

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Project Ref 43/19.2

Date Ackn'd 04/12/2019

CU2 Project Title

Investigating the effects of evolutionary divergent intracellular pathogens on host cell gene expression and immune response.

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N
To investigate gene expression of intracellular pathogens and host cells during infection. In particular, this study addresses how the pathogens manipulate host cell immune response and gene expression.

Recipient or parental organism

- Listeria monocytogenes

Wild-type Listeria monocytogenes (strains 10403S and EGO, both of which are serotype 1/2a) and derivatives where genes of interest are deleted through means of homologous recombination. For example, genes encoding the protein virulence factors listeriolysin 0 (LLO), internalin A (InIA), InIB and actin assembly-inducing protein (ActA). Knock-in L. monocytogenes mutants, expressing reporter proteins (e.g. YFP or GFP) will also be utilised. 10403S is a streptomycin-resistant derivative of the clinical isolate 10403 and is the predominant laboratory strain of L. monocytogenes used for research in the United States of America (USA). It was originally isolated from a human skin lesion in 1968. Infected individual by streptomycin-resistant strains will declare they have been exposed to a streptomycin-resistant strain when presented at the hospital.

EGO is a strain derived from the original L. monocytogenes strain that was isolated from rabbits in 1926. It's original supplier was the Trudeau Institute, who have since distributed the strain to laboratory's across the globe. Deletions in each of the virulence factors are expected to reduce the virulence of L. monocytogenes due to the role of these genes in the infection and colonisation process. Virulence for reporter knock-in mutants are expected to be reduced compared to wild-type.

A recent overview of these strains can be found at 001: 10.1128/mBio.00969-14.

- Salmonella typhimurium

Wild-type Salmonella typhimurium (SL 1344 and SJW11 03, both derivatives of the original lab adopted parental strain L T2) and derivatives where genes of interest are deleted through means of homologous recombination. The parental strains SL 1344 and SJW11 03 are auxotrophic for histidine with reduced fitness, and has been used safely in research labs since 1977. Both strains are able to cause localised gastroenteritis in humans if ingested but resolves itself with 3-7 days or can be managed with antibiotics. Although laboratory passage means both SL 1344 and SJW11 03 are likely attenuated.
- **Listeria monocytogene**
  pMTL20, pMTL23, pE194ts for allelic exchange to generate in-frame deletion of virulence factors.

- **Salmonella typhimurium**
  Well characterised vector systems with a history of safe use will be used, for example, commercially available nonmobilisable/mobilisation defective plasmids, pUC series, pACYC184, pWSK29, pBR322 series vectors, and pKD3, pKD4, pKD46, pCP20, pET, pGEX, pTrc99, pBAD, pLG339, pOU71, pMRS101, pWRG730 plasmid vectors.
  Mutations of Salmonella Typhimurium will be created by means of homologous recombination by using lambda red recombinase expression plasmids (e.g. pWRG730, pKD46) to facilitate introduction/removal of genes of interest.

**Origin & function**

- **Salmonella typhimurium**
  Source of genetic material- pACYC184 (NEB), and pUC series (e.g. NEB), pBR322 series (e.g. NEB) vectors, and pKD3, pKD4, pKD46, pCP20 (Datsenko and Wanner 2000), pET, pGEX, pTrc99, pBAD, pLG339 pOU71, pMRS1 01, pWRG730, all plasmid vectors are already laboratory maintained. These vectors carry antibiotic resistance markers of no or limited clinical value such as ampicillin, kanamycin or chloramphenicol. Bacterial virulence genes will by amplified by PCR from parent.

  Intended function
  High-copy pUC vectors will be used to express GFP or RFP that will act as a reporter to allow direct visualisation of the pathogen during cell infection.
  Low-copy pBR322/pACYC184 vectors encoding tagged virulence effectors (e.g. GFP-tagged prgJ) will be used to complement parental null mutant strains and investigate virulence factors inside the target host cell.
  Mutations of Salmonella Typhimurium will be created by means of homologous recombination by using lambda red recombinase expression plasmids (e.g. pWRG730, pKD46) to facilitate introduction/removal of genes of interest.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment protocols that minimise aerosols and spillages during bacteria culture via use of airtight sealed tubes, i.e. falcon tubes, in as small a volume as possible, typically 1 ml.

Autoclaving of waste material at 121°C for 60 minutes provides 100% inactivation. The autoclave used for waste is located in the building; all waste to be transported is double bagged in autoclave bags and then sealed in a plastic container. Annual validation of the autoclave by independent means will be performed.

For spillages, ethanol is an effective surface disinfectant in an aqueous solution of 70% (v/v), and ready-to-use proprietary disinfectant, such as Distel spray (formerly known as Trigene) which is proven to kill bacteria. Virkon
powder can be used directly for larger spillages.
- Liquid waste will be aspirated into liquid vessels containing proprietary Distel or Virkon proven to disable bacteria/viruses, incubated for at least 30 minutes before disposal of the deactivated waste down the sink.

The Biological Safety Committee was happy to give approval for the risk assessments once a number of details had been clarified regarding disinfection procedures. Clarification was also sought on safe working procedures, both for the laboratory and animal facility staff.

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### Project Ref 43/20.1

- **Date Ackn’d**: 03/04/2020
- **CU2 Project Title**: Using CRISPR/Cas9 genome editing on 2D and 3D cultures of primary cells from the human placenta and uterus to study placental development and the influence of the uterine microenvironment
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Non-GMM**: Consent Granted
- **CultureVolumeClass3-4**: 
- **Project notified under transitional arrangements**: N

02/03/2022
How does the human placenta develop and how is this influenced by the maternal uterine microenvironment? This is the central question we are trying to address in lab. Normal growth and development of the fetus depends on the placenta, the extra-embryonic organ derived from trophectoderm and the establishment of interactions between the placenta and the decidua, the pregnant lining of the uterus. Defective placental development and interactions at the maternal/fetal interface results in miscarriage, pre-eclampsia, fetal growth restriction and stillbirth.

Understanding the molecular and cellular mechanisms underlying these maternal/fetal interactions has been challenging due both to practical and ethical limitations and lack of reliable in vitro models. I have recently derived 3D culture systems (organoids) from human decidua and placenta that will provide the essential tools. We will use these organoids combined with co-culture methods with other cell types, single cell genomics and Crispr/Cas9 genome editing to study: (i) the molecular mechanisms that drive the development of the placenta and (ii) the role of the signalling from the maternal uterus in regulating placental development.

Primary human cells from the placenta and uterus grown as 2D and 3D cultures (organoids) will be used for gene targeting using CRISPR/Cas9 gene editing.

No vectors will be used. Synthetic crRNA/tracrRNA molecules will be combined with recombinant purified Cas9 protein to generate a ribonucleoprotein (RNP) complex. The crRNA will be homologous to the targeting region of interest, so its sequence will be specific to the gene we will target. The RNP complex will be directly transduced into cells by electroporation.

Genes of interest in placental and uterine cells will be edited by inducing double-stranded breaks in target sequence, to achieve knockout/knockin at the locus of interest. The expected outcome of the gene targeting is disruption/correction in these cellular processes. We will also introduce fluorescent tags to create placental and uterine cell lines that will be fluorescently labelled to allow co-culture studies with other cell types.

The RNP complex presents very low risk of activity in humans if exposed. For RNP delivery, sgRNA and Cas9 protein will be mixed only immediately prior to delivery into the cells and are inactive beforehand. In the unlikely event of direct contact, RNP delivery in the absence of electroporation is extremely inefficient. In addition, the RNP has a short half-life in the cell. It is only active for up to 12 hours before being degraded. Furthermore, no sharps will be used in the process of preparing the RNP complex. Thus the process of gene targeting of human cells will not have any additional risks to the research staff. The cultures will also bear no
additional harmful properties compared to the starting biological material. As cells are isolated from unscreened human tissue is used, thus the potential harmful properties regard pathogens. However, these samples are obtained from women from the Cambridge area thus are considered low risk. In the event that a potentially hazardous pathogen is later revealed in any samples, the procedure will be to remove the sample from the study and destroy all stocks by incineration. We will notify relevant workers of the finding and to have them followed up with Occupational Health. The possibility that this issue might arise will need to be explained to workers at the outset so they can make an informed choice about whether they are comfortable with handling the samples. Also, the workers will be following risk assessments and SOPs, and handling specimens as if they DO harbour potentially hazardous pathogens therefore the overall risk is very 'low'.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All genetically-modified human cells and any other liquid reagents used in experiments, will be inactivated using 1% Virkon solution (which is virucidal/bactericidal/fungicidal), and will be incubated with solution for 24 hrs after which is safe to discard down the sink. Any plastic waste produced will be collected as biological hazard waste for autoclave and then send to landfill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project was given approval by the Biological Safety Committee on 12/12/2019.

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Project Ref 43/21.1

Date Ackn'd 05/02/2021
CU2 Project Title Determination of the response of placental cells to human cytomegalovirus

Date Project Ceased

Class 2
CultureVolClass2 < 1 Litre
CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To help us understand how cytomegalovirus crosses the placental barrier

Recipient or parental organism
Pathogenic clinical strain (TB40E) of HCMV.
Health and Safety
Executive
Activity Notification
Organism is already genetically modified and provided by Prof. Joh Sinclair, Department of Medicine, University of Cambridge.
In vitro assays will be used to infect human placental cells and blood monocyte derived macrophages with HCMV.

Host/vector system
Plasmid encoding yellow flourescent protein, fused with the viral protein IE-2

Origin & function
Gene product is expressed during early stages of viral infection of cells, and acts as a flourescent marker, localised to the nucleus. This will allow us to identify cells in which the virus is replicating. In particular, we plan to use in vitro systems to develop our understanding of the interactions of CMV with human placental cells. Lab procedures involved include infecting human placental macrophages and other blood monocyte derived cells with CMV (TB40E). Viral
spread is monitored by light and UV microscopy. Assays are harvested by treating virus infected cells with trypsin to detach cells, these are inactivated with 2% paraformaldehyde prior to analysis on a flow cytometer. Supernatants are harvested to determine productive viral infection. Supernatants are added to human fibroblast cultures and infection is quantified. Supernatants are harvested to test for cellular cytokine production upon infection.

Viral spread assays are also carried with human fibroblasts that are infected with HCMV. Viral spread is monitored as stated above. Human fibroblasts will be seeded with infected human Hofbauer cells or blood monocyte-derived cells, to determine the extent of cell-to-cell spread of virus. Viral spread is monitored as stated above.

Immunofluorescence of HCMV infected cells: HCMV infected cells in 96 well plates, on slides or in slide chambers are fixed in 70% EtOH at -20C. Fixed cells are washed in PBS and stained with antibodies. Slides are sealed with a coverslip and analysed on the confocal microscope.

Flow cytometry sorting of live HMCV infected cells: Cells are infected as stated above. Cells will be transported from the containment level 2 laboratory to the containment level 2 Department of Pathology flow facility. Tubes will be sealed and within a secondary box lined with absorbent material.

**Evaluation of foreseeable effects**

This Gene product is expressed during early stages of viral infection of cells, and acts as a flourescent marker, localised to the nucleus.

HCMV is in the ACDP human pathogen hazard group 2.

Primary infection can cause an acute infectious mononucleosis like illness with high temperatures; this is self-limiting and very rarely requires medical attention. Infection of HMCV seronegative women during pregnancy can lead to infection of the developing foetus and serious morbidity.

The gene product expressed by CMV does not alter the morbidity or risk associated with CMV infection.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Access to containment level 2 laboratory is limited to laboratory personnel and other specified persons.

All work takes place in a class II microbiological safety cabinet (MSC) with a UV light source.

All solid and liquid waste is inactivated by immersion in 5% virkon for 1 hour prior to disposal. Virkon kills 99.999% of organisms, that is it kills organisms to an extent where they no longer pose a threat to health. All waste is then placed in doubled sealed bags before autoclaving followed by incineration.

Remaining inactivated liquid waste is poured down the appropriate lab sink and water is then run down the sink for 5 minutes.

Work surfaces are cleaned down with disinfectant 5% viron, then 70% ethanol. The safety cabinet is the irradiated with UV for 15minutes to deactivate any potential aerosols.

All items that were in MSC with the virus and need to be reused, such as pipettes will be irradiated with UV light for 15 minutes and sprayed with 70% ethanol before removal from the MSC.

Any accidents and spillages that occur will be dealt with immediately. Minor spills in the MSC will be cleaned up with 5% virkon and paper towels. These towels will be inactivated in 5% virkon for 1 hour and double bagged in autoclave bags, and autoclaved prior to incineration. If spills occurs in sealed centrifuge buckets and sealed containers, the buckets and containers will only be opened once placed in MSC. Again 5% virkon and 70% ethanol and paper towels.
will be used to clear spillage. If spills occur outside of MSC, work will be stopped. Other people in the area will be informed and asked to evaluate the immediate until the spill is cleared. The spillage will be isolated and immediately cleaned up. The spillage will be cleaned up with 5% virkon and 70% ethanol and paper towels. The papers towels will be inactivated as described above.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The Biological Safety Committee approved this risk assessment after a few small amendments were made as requested.

Project Containment

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Project Ref 43/21.2

Date Ackn’d 14/05/2021
Date Project Ceased

Characterisation of MHC class I and class II antigen presentation pathway components controlling peptide selection for immune recognition

Class 2
Culture Vol Class 2 < 1 Litre
Consent Granted

Project notified under transitional arrangements N
Withdrawn N
Tick if notifying a connected programme of work Y
Peptide presentation on major histocompatibility complex (MHC) molecules is central to mounting effective antiviral and antitumoural immune responses. Our overreaching aim is to identify and characterise components of the antigen presentation pathway, including the peptide editor TAPBPR, MHC-I and MHC-II and their interaction partners. Several aspects of this work require the use of class 2 GM material:

1. Generation of mammalian cell lines with modified expression of antigen presentation pathway components
   Through lentivirus transduction, components of the MHC antigen presentation pathway (e.g., MHC-I, MHC-II, TAPBPR, tapasin) and their potential ligands/interaction partners will be knocked out or introduced and overexpressed. This may include natural variants and mutated versions of these proteins. Furthermore, the expression of proteins that are identified as antigen in either T cell responses or recognised by specific antibodies will be modified using knockout techniques or overexpressed. This may include the expression of variants and mutated forms of these proteins.

2. Characterisation of the immunopeptidome in hepatitis B and hepatocellular carcinoma
   Potential hepatitis B virus (HBV) peptides presented on MHC molecules will be defined. This work requires the use of a panel of mammalian cell lines expressing selected MHC class I and class II molecules and stably transduced with individual HBV proteins or all HBV proteins except the viral polymerase (HBpol). These cell lines are generated using lentiviral vectors.

3. Functional characterisation of TAPBPR, and other known or novel antigen presentation pathway components, in the context of virus infections
   Virus-mediated manipulation of the antigen presentation components will be assessed. This project requires the use of hazard group 2 biological agents such as human cytomegalovirus (HCMV), vaccinia virus (VACV), herpes simplex virus (HSV)-1, Zika virus, influenza A virus and human respiratory syncytial virus (RSV). These viruses may be modified to knockout gene function or to express a tag (e.g., HA-tag or green fluorescent protein [GFP]). Additionally, mammalian cell lines transduced with viral proteins will be generated. Phenotypes of the mutant viruses and cell lines expressing viral proteins will be assessed using standard virological and immunological assays.

E. coli disabled strains (ACDP hazard group 1) have a long history of use without observation of harmful effects.

All mammalian cells that will be used are widely available (e.g. ATCC, ECACC), well established and have a long history of safe use, e.g. HeLa, HEK293T, A549, Jurkats, HFFF-TERTs, Hep G2.

Lentiviruses (produced following co-transfection of 293T or similar cells with two or three plasmids independently providing packaging and replication) are replication-incompetent but have a broad tissue tropism due to the presence of a VSV-G envelope. The third generation of lentivirus has been significantly modified for biosafety, including 1) Packaging vector lacks both LTRs and has no viral packaging signal; 2) The viral genes (env, tat, rev, vpr, vpu, vif and nef) have been deleted from the packaging vector; 3) Rev is supplied in trans on a different vector; 4) The vector expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion) and expresses no viral gene products; 5) Envelope is expressed from a separate vector.

Other viruses used are categorized by ACDP as hazard group 2 biological agents and can cause disease in healthy adults:

- Vaccinia virus (VACV) has been widely used as a vaccine in the smallpox eradication campaigns. The virus may cause particularly severe disease during pregnancy, in
people with active skin disorders such as eczema or psoriasis, or in immunocompromised individuals such as those infected with HIV.

- Human Cytomegalovirus (HCMV) causes a life-long infection and has a prevalence of 55-100% within the human population. Primary HCMV infection is generally asymptomatic in healthy hosts but it can cause severe and sometimes fatal disease in immunocompromised individuals and neonates. HCMV infection is often controlled by early treatment with antivirals.

- Herpes Simplex Virus (HSV)-1 is a highly contagious infection, that is common and endemic throughout the world. Most HSV-1 infections are acquired during childhood, and infection is lifelong.

- Influenza A virus. Naturally occurring and/or vaccine-induced immunity exists against the PR8, WSN, and Eng glycoproteins, as well as their variants, and should provide a large measure of protection against infection and spread of the proposed mutant viruses.

- Zika viruses are transmitted via a vector (mosquito) and thus pose an extremely low risk for contamination through the described work.

- Human respiratory syncytial virus (RSV). Natural immunity against RSV exists. RSV causes a mild-respiratory disease in adults and children, but it can be severe in infants who are at increased risk of acute lower respiratory tract infection.

**Host/vector system**

Lentiviral expression vectors are used to generate second or third generation self-inactivating and replication disabled lentiviruses. This system is used to knockout (KO) or introduce components of the MHC class I and class II antigen presentation pathway in mammalian cells or to transduce mammalian cells to stably express a viral protein. Furthermore, it may be used to KO or introduce a protein which is the target of a T cell or antibody response. Promoters used will be either constitutively expressed or under the control of a repressor (inducible systems).

Small expression plasmids (standard commercially available cloning plasmids) and in vitro transcribed RNA are used for reverse genetics to generate virus particles (incl. lentivirus), as well as bacterial artificial chromosome (BAC) and BAC helper plasmids required to produce virus particles. Overall, these vectors are of no human or environmental risk.

**Origin & function**

Lentiviral vectors

Lentiviral expression vectors are used to modify expression of known or novel components of the MHC class I and class II antigen presentation pathway including their ligands/interaction partners, to modify expression of identified or potential targets of T cells and antibodies, and/or to introduce a viral protein of interest into mammalian cell lines. Lentivirus particles will be produced using second generation or later packaging systems which show improved safety. As a minimum, the cell lines that are used for virus packaging contain, either transiently or stably, the genes required for virus replication on separate cassettes in order to eliminate the possibility of autonomous virus production. Additionally, the viruses may contain self-inactivation deletions. The GM lentiviruses will contain genes that will be expressed from promoters that are active in mammalian cells (e.g. CMV promoter) or from the lentivirus promoter contained in the viral LTR. We may use constitutive or induced expression systems.

Mammalian cell lines

Resultant modified cell lines are not expected to be more hazardous as a result of the genetic manipulation, as they do not produce infectious material and are unable to infect people or animals. CL2 will be necessary for the transduction of the cell lines with the lentivirus vectors. All established cell lines will be utilised in CL1 laboratories. Although expression of certain viral genes may be expected to be toxic to the expressing cells, there is no expectation that neighbouring cells or the environment will be affected. Of the viral proteins of interest, only HBV protein x (HBx) has been postulated to have oncogenic effects in human hepatocytes. Overexpression of HBx in non-dividing cells does not have deleterious effects, which is consistent with the lack of a cellular stress response to HBV-infected hepatocytes. Introduction of any of the other viral proteins of interest in mammalian cells is not expected to result in proliferation of the cell. Some of the mammalian cell proteins that are recognised by specific T cell and antibodies whose expression will be manipulated through knockout techniques or via overexpression may potentially be oncogenic. Although there is a risk if accidental gene transfer occurs, the absence of a promotor region mean there would be a negligible impact on human health. The use of inducible systems provides an extra layer of safety allowing the expression of the genes of interest in a controlled fashion.

Viruses

Hazard group 2 viruses will be grown in mammalian cells. GM viruses are expected to behave the same as WT virus. KO of viral genes or inserting tags poses no risk to human health and is highly unlikely to increase virulence or fitness of the virus above that of the parental wild-type virus. These viruses are by no means expected to display enhanced replication as gene products of genes altered/deleted from viruses are not expected to be involved in replication.
Evaluation of foreseeable effects

The packaged lentiviruses with the gene of interest inserted will be able to potentially infect humans. Depending on the gene inserted, the effects will be different. Accidental exposure to this material without a promoter region will have negligible impact on human health. Accidental exposure when already cloned in a plasmid, with a constitutive promoter before it, may potentially have a localised effect. However, there will be no spread to other cells as the virus cannot replicate or infect other cells. The risk of inoculation will be minimised, as work will be carried out under CL2 conditions.

Virus will be produced in mammalian cells when deliberately introduced in genomic form, but there is a negligible chance of this being introduced to susceptible hosts. It is unlikely that the GM viruses with tagged/modified/deleted genes would be any more hazardous than the unmodified viruses as our experiments are not designed to enhance function. None of these manipulations can increase virulence or stability and CL2 is therefore appropriate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All of the cell lines to be utilised are well characterised or authenticated with a low risk of endogenous infection with a biological agent presenting no apparent harm to laboratory workers and which have been tested for the most serious pathogens and as such will pose minimal risk to both human health and the environment. This risk is not going to be greatly increased by the addition of the genes of interest. Although expression of certain genes may be expected to be toxic to the expressing cells, there is no expectation that neighbouring cells or the environment will be affected or that proliferation of the cell will result. The use of inducible systems provides an extra layer of safety allowing the expression of the genes of interest in a controlled fashion.

Activity

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

E. coli strains are especially disabled and have a negligible chance of surviving in the environment as well as a negligible risk of such release occurring. Disinfection using solutions of phenolic compounds (e.g. Virkon) will achieve 100% kill, preventing these GMOs from reaching the environment beyond the lab. Solid contaminated waste is disposed of through autoclaving and landfill disposal. There are multiple autoclaves available in the department, thus there are alternatives available if the regular autoclave is out of service. If disinfection/autoclaving were to fail for any reason, the E. coli strains used are highly attenuated and will not survive in the environment. The inserts contained within the plasmids pose no hazard to the environment.

Mammalian cells used are well characterised or authenticated and as such will pose minimal risk to both human health and the environment. Liquid waste is inactivated using solutions of phenolic compounds (e.g., Virkon), which will achieve 100% kill, preventing these GM cells from reaching the environment beyond the lab. Solid contaminated waste is disposed of through autoclaving and landfill disposal.

Viruses used in this study (lentivirus vector and other viruses) do not pose a hazard to the environment. Handling of these viruses under CL2 conditions as described above is sufficient to control any risk of infection.

All liquid waste is inactivated using either chemical treatment Virkon, Perasafe or Chloros (hypochlorite) under the manufacturers’ validated conditions. Liquid waste will be treated for at least 30min before discarding. - Effectively 100% kill.

Solid wased is autoclaved using validated and recorded temperature cycles. Disposable plasticware is double bagged and stored in a container in a CL2 location until autoclaved. The department has multiple autoclaves available, thus there are alternative available if the regular autoclave is out of service. As a contingency, solid waste will be soaked in Virkon if the autoclaves have broken down. - Effectively 100% kill.

Surface contamination in safety cabinets is cleaned using 70% ethanol & UV irradiation. - Effectively 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The Department Biological Safety Committee approved the related risk assessments for this work with corrections from the research group.

**Project Containment**

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**Project Ref** 43/21.3

- **Regulation of innate immune responses by host cell intrinsic signalling**

- **Class**: 2
- **Culture Volume**: < 1 L
- **Non-GMM Consent Granted**: Yes

**Historical Significant Changes**

- **Historical Date of Additional Info**: 23/05/2021

**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to understand how pathogens interact with pattern recognition receptors (PRRs) of the innate immune system. PRRs initiate immune responses by binding to conserved pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs). These ligation events drive the production of...
interferons, cytokines and chemokines from the PRR-expressing cells to initiate the immune response to pathogens or damaged tissues. We are specifically interested in the detection of foreign and self nucleic acids by intracellular PRRs. The sensing of DNA and RNA by these receptors is essential for the type-I interferon response to virus infection and is implicated in the pathogenesis of multiple auto-inflammatory and auto-immune diseases. To study the interactions between host cells and pathogens we will use mammalian and avian cell lines and infect them with DNA or RNA viruses. These viruses will activate DNA or RNA sensing PRRs and initiate the downstream signalling pathways that we are studying. PRR signalling, downstream of the receptors, relies on a combination of adaptor proteins and kinases that propagate the signal, resulting in the activation of specific transcription factors that drive the expression of interferons and pro-inflammatory mediators. Examples of specific pathways we are studying include, but are not limited to, the sensing of DNA viruses by the cytoplasmic DNA PRRs DNA-PK and cGAS, which operate via the adaptor protein STING, leading directly to activation of TBK1 kinase and the type-1 interferon-inducing transcription factors IFR-3/7 and NF-kB. We are also studying the RNA sensing PRRs RIG-I and MDA5 and how they activate the same transcription factors via the adaptor protein MAVS. These signalling pathways are regulated by several fine tuning mechanisms, particularly using the ubiquitin machinery to positively and negatively regulate PRR signalling outputs. By studying the PRRs themselves and the proteins that propagate the downstream signalling pathways we can understand how our cells and bodies sense the presence of infection. In turn, viruses make proteins that inhibit these signalling pathways in order to suppress the innate immune response and promote their survival in tissues. To study these pathways in the host and the viral inhibitors we use genetically modified viruses in which one or more of these immunomodulatory genes has been deleted or modified by genetic recombination. These techniques allow for the deletion, mutation or epitope tagging viral genes to generate viruses to be used in experiments that will explore the mechanisms and functions of these genes in inhibiting immune responses.

**Recipient or parental organism**

1. Vaccinia virus (VACV, strains WR, Copenhagen, MVA, SKV, Lister)
2. Fowlpox virus (FPV, strain FP9)
3. Herpes simplex virus 1 (HSV-1, strains 16 and 17)
4. Marek’s disease virus (MDV, low virulence strains)
5. Influenza A virus (IAV, strain A/PR/8/1934)

**Host/vector system**

pUC, pCDNA based plasmid vectors

**Origin & function**

We will use sections of genetic material (not containing open reading frames) cloned from the viruses into the plasmid transfer vectors. These vectors will be used to generate modified viruses by homologous recombination in mammalian cells that are infected with the target WT viruses.

**Evaluation of foreseeable effects**

We will generate attenuated viruses by deleting or mutating viral genes that interact (or are predicted to interact) with the host innate immune system. The deletion or mutation of such viral genes will most likely result in pathogens with equal or lower virulence than the wild-type viruses and will not alter the tropism or transmissibility.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Disposing of the viral supernatant will be done within a tissue culture hood while wearing appropriate PPE (lab coat and nitrile gloves). Virus will be inactivated in 1% Virkon before being removed from the hood. Expected 100% kill efficiency for all viruses. Solid waste is double-bagged, sealed and autoclaved. Surface contamination in safety cabinets – Virkon 1% solution will be used for 10 minutes (100% kill for all viruses), followed by wiping with 70% ethanol. Non-proteinaceous virus-containing liquid waste –
Virkon added to 1%, left >30 minutes and then disposed of - effectively 100% kill for all viruses. For virus-containing tissue culture supernatants (or other proteinaceous solutions) – Virkon 5% solution – effectively 100% kill for all viruses. Disposable plastic ware is double bagged and autoclaved - effectively 100% kill for all viruses.

The Biological Safety Committee approved these risk assessments.

**Project Containment**

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**Project Ref 43/92trans**

- **Date Ackn’d**: 03/03/1992
- **CU2 Project Title**: EXPRESSION OF PRP GENES USING HERPES SIMPLEX VECTOR
- **Class**: Class 3
- **CultureVolClass2**: Class 3
- **CultureVolumeClass3-4**: Class 3
- **Non-GMM**: yes
- **Consent Granted**: Yes
- **Date Project Ceased**: 17/03/2014
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: GM43/98.1
- **Historical Date of Additional Info**: 12/05/1998

**Project notified under transitional arrangements**: Y
**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Is an emergency plan required according to regulation 20? [N]
- If yes, tick to confirm that it is attached to this form [N]
- Tick to confirm that you have attached a risk assessment to this form
- Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

**Project Containment**
Project Ref 678/12.1

Date Ackn'd 16/05/2013

CU2 Project Title T-cell signalling mechanisms. Over-expression or down-regulation of the target genes in cell lines and primary cells using transfection

Class 2

Culture Volume < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes transferred from GM678 16/05/2013

Project Additional Information

Purposes of the contained use

The nature of T-cell surface receptors (TcR, costimulators CD28, CTLA-4, ICOS) signalling pathways responsible for regulation of T-cell activation and adhesion and motility. Our objective is to study the role of costimulators CD28, CTLA-4, ICOS, PD-1 as well as adaptor proteins SLP-76, ADAP and SKAP-55 and the kinases including ZAP70, LCK FYN and GSK3. Over-expression or down regulation of the target genes in cell lines and primary cells using transfection. T-cell function in vitro including activation, proliferation, and adhesion will be examined

Recipient or parental organism

Mammalian cells: Mammalian primary cells or cell lines are especially disabled and unable to survive or propagate outside of laboratory culture.

Host/vector system
Host Human or murine cell lines, such as Jurkat DC27, T8.1, A20, 3A9 or primary cells and other cell lines such as 3T3, 293T, phoenix cells.

Retroviral vectors (pinco, MigR1, pMX, pMDG, pMLV-GP, p8.91, pGIPZ)
Lenti-viral vector (HIV-1 self-inactivating vector SIN, pFIP)
pLKO.1 vector (lentiviral (HIV)-based plasmid)
plasmid encoding the envelope plasmid derived from vesicular stomatitis virus (VSV) G protein

Cells will be transfected with retroviral or lentiviral plasmids and viral supernatant harvested (max vol 30ml/vector/transfection) and used to infect target T-cell lines or primary T-cells. After infection twice target cells are harvested and used for functional assessments. Max culture vol of target cells in 100ml/cell type, if viral supernatant is frozen for stock, viral supernatant is aliquot in small vial (~2ml/vials) and stored in allocated areas or boxes in -80 degree freezer. 200 µl will contain approximately 1 x 10^6 TU/ml

For example, shRNAs will be cloned into the pLKO.1 transfer vector which is compatible with standard two plasmid (packaging vector with rev gene and envelope vector) or a three plasmid (packaging vector without rev gene, envelope vector, and rev expression vector) packaging systems. In the case of MISSION shRNA, the viral particles cannot be propagated and are replication incompetent.

A three plasmid transient transfection system will be used to generate recombinant replication-defective retroviruses 293T cells, a human cell line carrying SV40 T cell antigen will be transfected with (a) An HIV-1 self inactivating vector (SIN) cDNAs encoding the genes of interest will be cloned into the HIV-1 derived SIN vectors, which have a deletion in the U3 region of the 3’ long terminal repeat (LTR) of the DNA used to produce the vector RNA. During reverse transcription this deletion is transferred to the 5’ LTR and abolishes the transcriptional activity of the LTR and as a result the production of full-length vector RNA in transduced cells is abolished (b) An HIV-1 derived retroviral sequence encoding viral gag/pol tat and rev genes, from which the virulence genes vif, vpr, vpu and nef have been deleted and (c) a plasmid encoding the envelope plasmid derived from vesicular stomatitis virus (VSV) G protein. This is a well defined lentivirus system that is in use under our GM centre No 678.

Origin & function
The genes targeted are involved in aspects of cell signalling, activation and proliferation and are not expected to be toxic.
None of the proteins are known oncogenes and are therefore highly unlikely to foster tumour progression.

Evaluation of foreseeable effects
Mammalian primary cells or cell lines are unable to survive or propagate outside of laboratory culture.

The genes targeted are involved in aspects of cell signalling, activation and proliferation and are not expected to be toxic
None of the proteins are known oncogenes and are therefore highly unlikely to foster tumour progression.

The vectors used will be self inactivating (SIN). A deletion inactivates transcription from the proviral LTR, so reducing the potential for transcriptional activation of cellular genes and also prevents mobilization of any RCL. However as the virus integrates into the host cell DNA there is still a potential for insertional mutagenesis to occur.

A plasmid encoding the envelope protein from vesicular stomatitis virus envelope protein G (VSV-G) increases the host cell range/tissue tropism and makes it permissive for human cells, including quiescent cells. This feature means that there is a risk of infection through contact/aerosol transmission in addition to the normal HIV percutaneous route. Such particles are also physically more stable.

The genes or other sequences of interest will be delivered by an integrative process leading to potential long-term expression for the life of the target cell. The results of such expression for most of the sequences will not be severe and will be limited to the cells targeted and no further.

VSV-G enables the vector virus to infect a wide variety of mammalian cells. There may also be enhanced environmental stability. However, the viruses are replication disabled, they cannot produce progeny virus and so cannot spread to the wider human population or other animals. The possibility of RCL being generated and released is negligible.
The MISSION TRC lentiviral particles are replication incompetent. Non of the modifications will alter the broad properties of the vectors. They will remain replication disabled and so are biologically contained.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable tissue culture plastics will be autoclaved and incinerated. Culture medium will be treated using vernagel and disposed of via Containment Levek II disposal route.

Benches are cleaned with 10% trigene and 70% ethanol.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

This project has been reviewed by the Institutes Biological & Genetic Modification Safety Committee. The Committee is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>L2</td>
<td>L3</td>
</tr>
</tbody>
</table>

Project Ref 77/01.2
GENETIC MANIPULATION OF VACCINIA VIRUS AND OTHER CLASS 2 POXVIRUSES

Fundamental research in virology and vaccine development.

Recipient or parental organism

The genetically modified viruses will have characteristics either similar to the parent virus, or of reduced virulence.

The expression of foreign genes by vaccinia virus will not increase the hazard posed by the virus and often the insertion of these recombinant viruses is associated with a reduction in virulence because the gene is inserted into a site in the virus genome that affects virulence. The foreign proteins pose no toxic hazard and are designed to promote appropriate immune responses that will be beneficial in preventing or reducing disease caused by infectious disease and cancer.

The deletion of existing vaccinia virus genes will either be neutral or, more often, decrease virus virulence. Removal or genes affecting the ability of the virus to be released from infected cells and spread to other cells is accompanied by a dramatic reduction in virulence. Removal of genes encoding proteins that aid virus escape from the immune system also reduces virus virulence since the virus infection is better recognised and cleared by the host immune response.

Other poxviruses to be used include the orthopoxviruses cowpox and camelpox virus and the yata poxviruses tanapox and yaba-like disease virus. Cowpox virus is similar to, and often confused with, vaccinia virus and poses a similar hazard. Camelpox virus is a less well characterised orthopoxvirus that causes a severe disease in camels but does not cause disease in man. Tanapox virus and yaba-like disease virus are simian viruses that have caused rare zoonoses in man. Such zoonoses usually produce a single lesion that heals without adverse consequence.

Host/vector system

Vaccinia virus is the live vaccine used to eradicate smallpox and has been used widely as an expression vector since 1982. For cowpox virus, yaba-like disease virus and tanapox virus see comments above under section 7. All these viruses will be treated as for vaccinia virus and pose similar or reduced risk to man. All viruses will be housed in our category 2 containment laboratory and will not be released from that laboratory. The genetic manipulation of all these viruses requires construction of plasmids in bacterial vectors (level 1 GM work).
### Origin & function

Each genes to be deleted from or inserted into vaccinia virus or other class 2 poxviruses are described in detail in the accompanying document.

In summary, the genes to be deleted from these viruses encode proteins that either enable the virus to be released better from infected cells, or affect interactions with the host immune system. The latter group of proteins interfere with the host response to infection, and the deletion of these genes therefore may reduce virulence and enhance immunogenicity.

Foreign genes to be expressed in these viruses are either marker proteins that enable the virus to be detected in cell culture, such as β-galactosidase or green fluorescent protein, or antigens from other micro-organisms against which an immune response is sought. These latter proteins are not toxic but are important targets for immune responses that help prevent or reduce disease.

### Evaluation of foreseeable effects

The foreseeable effects of deleting genes from vaccinia virus and other class 2 poxviruses are to make the virus less fit, less virulent and less able to cause disease and survive in nature. These virus genes selected encode proteins that aid virus dissemination from infected cells or to aid virus escape from the immune system. It follows that deletion of such genes, will reduce virus virulence and fitness. The foreseeable effects of expressing foreign genes in vaccinia virus are to make the virus a candidate vaccine for different infectious diseases or cancers. The foreign proteins to be expressed pose no toxic hazard and the immune responses to them are likely to be beneficial.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material from our virus containment room will be autoclaved, or incubated in 1% (weight/volume) virkon overnight to ensure no infectious virus remains.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

N

---

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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The local safety committee reviewed and approved the proposed work at category 2 containment in October 2000.
This programme of work will use lentivirus vectors to aid analyses of the functions of viral proteins involved in poxvirus entry, morphogenesis and immune modulation as well as human orthologues of these proteins. The lentivirus vector system will enable the efficient infection of cells grown in tissue culture with lentiviruses expressing cellular and viral proteins.

Recipient or parental organism

Lentiviruses will be produced by transfection of the SV40 transformed human embryonic kidney cell line HEK 293T. Human and rodent cells grown in tissue culture will be the recipients of the lentivirus GMOs. These cells will include (but are not restricted to) BSC-i, HeLa, and TK-143 cells and other human or rodent (e.g. NIH 3T3) cell lines supplied by ATCC. In addition, some primary cells will be used including rat embryo fibroblasts. After trypsinisation these cells will express the transgene but will not produce or contain any lentiviruses and so are non-infectious.

Host/vector system

The vectors are based on mutated lentivirus genomes, pseudotyped with a vesicular stomatitis virus (VSV) envelope glycoprotein (VSV-G) allowing infection of a broad
range of cell types and containing a gene for puromycin resistance to allow the selection of expressing cells. The vectors are pCMVR8.91 (encodes the lentivirus genes), pMD (encodes VSV-G), pdlNotI’MCS’R’Pk and pdlNotI’nPk’MCSR, (each contain a multiple cloning site to allow cloning of gene of interest fused to a N- or C-terminal V5 epitope tag respectively, and a puromycin resistance gene).

Origin & function

1. Poxvirus proteins involved in entry or morphogenesis
A33, A34, A36, A56, A56, B, D, E2 and K2 each have a role in the entry or exit of vaccinia virus. Each will be cloned into the lentiviral vector.

2. Poxvirus immunomodulators
K7, B14, Ni, A46, A52, A49, C6, A41, A44, B15, B18, and v-GAAP are all non-essential for virus replication in vitro but have roles in altering the host response to infection in vivo. Each of these will be cloned separately into the lentivirus vector. In addition, uncharacterised poxvirus proteins, which have a putative immunomodulatory role, may be expressed in this way in the future.

3. Cellular orthologues of poxvirus proteins
Many poxvirus proteins have cellular orthologues, the function of some of which is not yet fully understood, and each of these may be cloned into the lentivirus vector. This includes the newly characterised human Golgi anti-apoptotic protein (h-GAAP), which shows 73% amino acid identity to the vaccinia virus v-GAAP (Gubser et al., 2007. PLoS Pathogens 3:ei7).

Evaluation of foreseeable effects

Each lentivirus vector produced will be able to infect cells, but is not able to replicate in those cells and so cannot spread to new cells. These vectors are therefore classified as replication-defective. The nature of the foreign proteins expressed by these vectors is not likely to affect the hazard group of the parent vector and will only be expressed from the infected cell. Lentivirus vectors provide an efficient means to transduce genes into human cells. In theory, these genes can be transduced into laboratory personnel working with the packaged viruses, however, the virus is replication-defective, and so an ongoing infection with these viruses is not possible. The ability of h-GAAP to block apoptosis means that any cells transduced with h-GAP? would be resistant to cell death, which would in theory increase the chance of those cells becoming cancerous. Because the lentivirus is replication-defective, there is a very low probability that infection will occur in stem cells with the potential to become cancerous. This programme of work will not include viral oncogenes which have multiple oncogenic activities such as the SV40 T antigen, nor will it include clusters of genes such as HPV16 E6-E7. Genes encoding toxins will not be included in this work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste
Treated with 1% final concentration of Virkon disinfectant for a minimum of 30 minutes at room temperature. 100% effective kill rate as specified by manufacturer (http://www.antechn.com/virkongd.html) Treated waste will then be disposed of via the sink.

Solid Waste
Waste will be autoclaved using a cycle of 121 deg C, 20 minutes. Treatment is monitored via a chart recorder attached to the autoclave and validated by an annual 12 point thermocouple test. Once autoclaved the waste will join the hospital clinical waste.
Following the discussion of your proposal the following amendments are requested prior to approval:

1-25: Remove the sentence; "Any other viral gene subsequently implicated in morphogenesis may also be included.” The Committee recommend that further genes be considered on a case by case basis in new (Form C, if appropriate) submissions.

1.3: The sentence; The ability of h-GMP to block apoptosis indicates that it may be a proto-oncogene and contribute to cancer” is too broad and needs clarification.

3.8: Tick declaration box.

Agreed: Class 2, Containment Level I IC reference number: GMIC-01698.1

Please enter comments on the GM safety committee on the risk assessment

The use of lentiviral vectors to study poxvirus proteins and their cellular orthologues, Virology, Investigative Sciences, St Mary's.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Name**

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**Name 2**

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**E-mail**

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**Comments**

**Date at Which Additional Info Submitted**

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee
<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Invertebrates</td>
<td>Plants</td>
<td>specify below)</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The aims of the study are to investigate the mechanisms of replication of RNA viruses and how they subvert the cellular machinery to achieve this goal. Recombinant viruses will allow us to analyse this process in greater detail and is fundamental to this research strategy and its aims. The long term aims are the improvement of fundamental knowledge in the area of RNA virus replication and the future development of vaccines and antivirals.

The GMOs are viruses derived from DNA plasmid copies of RNA viruses, specifically non-segmented RNA viruses from the Mononegavirales order such as Measles virus. The target viruses are all classed as ACDP2 or lower and will not include ACDP3+ or SAPO restricted pathogens from this Order. The GMOs will also include pseudotyped replication defective lentiviruses that are capable of transducing mammalian cells to overexpress viral or host genes e.g. to over-express genes relevant in studying pathogenesis. The recipient hosts for these GMOs are mammalian cells (primary derived or immortalised cell lines) and/or mice (lab-strains or transgenic derivatives). Bacteria (commercial non-pathogenic strains) will also be used to replicate the DNA clones prior to manipulation.

The vector system for these studies is based on the use of full length DNA clones of RNA virus genomes. The non-segmented RNA virus genomes of ACDP2 (and lower) pathogens are cloned downstream of promoters that can drive transcription of a genome like RNA. This RNA, in the correct context, and with the correct support, is capable of producing infectious virus. The plasmid backbones used for this system are based on standard commercially available versions. The lentiviral system for over-expression of chosen genes is based on the commercially available ViraPower based system. A replication defective and truncated HIV1 genome is used as a vector to generate pseudotyped particles capable of infecting (non-productively) target cells. Integration and selection is anti-biotic mediated however, no nascent virus can be produced.

Origin & function

Recipient or parental organism

The GMOs are viruses derived from DNA plasmid copies of RNA viruses, specifically non-segmented RNA viruses from the Mononegavirales order such as Measles virus. The target viruses are all classed as ACDP2 or lower and will not include ACDP3+ or SAPO restricted pathogens from this Order. The GMOs will also include pseudotyped replication defective lentiviruses that are capable of transducing mammalian cells to overexpress viral or host genes e.g. to over-express genes relevant in studying pathogenesis. The recipient hosts for these GMOs are mammalian cells (primary derived or immortalised cell lines) and/or mice (lab-strains or transgenic derivatives). Bacteria (commercial non-pathogenic strains) will also be used to replicate the DNA clones prior to manipulation.

Host/vector system

The vector system for these studies is based on the use of full length DNA clones of RNA virus genomes. The non-segmented RNA virus genomes of ACDP2 (and lower) pathogens are cloned downstream of promoters that can drive transcription of a genome like RNA. This RNA, in the correct context, and with the correct support, is capable of producing infectious virus. The plasmid backbones used for this system are based on standard commercially available versions. The lentiviral system for over-expression of chosen genes is based on the commercially available ViraPower based system. A replication defective and truncated HIV1 genome is used as a vector to generate pseudotyped particles capable of infecting (non-productively) target cells. Integration and selection is anti-biotic mediated however, no nascent virus can be produced.
The genetic material (viral or cellular) will be sourced from biological isolates of these RNA viruses (Mononegavirales of ACDP2 and lower classification only), from mammalian cell-lines, from primary cell cultures or through in vitro commercial genetic synthesis. All ethical approval for the receipt of the relevant clinically associated samples will be in place prior to the commencement of work. Importantly, the genetic material will be used to generate recombinant RNA viruses with equivalent or reduced pathogenesis relative to the parental strain. Also, the pseudotyped lentiviruses are incapable of producing productive progeny virus. Recombinant tagged/mutant viruses will be used to determine the molecular basis for pathogenesis both in vitro and in vivo (in mice only).

Evaluation of foreseeable effects

Recombinant viruses: Only ACDP2 class 1 and 2 pathogens from the Mononegavirales order will be 'rescued'. Work with ACDP3+ and SAPO restricted pathogens will not be carried out. The viruses rescued will be designed in such a way as to not increase pathogenesis, relative to the parental strain. This will obviously be confirmed using in vitro tests under ACDP2 conditions in the lab. The foreseeable effects to the organism of such mutations and/or addition of tags (fluorescent etc) are therefore to either maintain its virulence or attenuate it. The risks to the environment and public are therefore effectively zero. This bears true for the recombinant lentiviruses as well as these are replication competent and incapable of producing nascent virus after the primary infection step.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Transgenic mice will be used for some of the proposed work, where virus is used to infect mice or transgenic mice. These will be held in a secure Animal facility, with the use of isolators to house the infected mice. Agreed procedures will be in place for the removal of carcasses.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - full CL2 applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM MeV in waste will be inactivated with 1% Distel (formerly Trigene) or 1% Virkon, which has been shown to be an effective agent for inactivation of a wide range of enveloped viruses including Measles. Measles is also susceptible to 70% Ethanol.

All biological waste generated in the IBR is then autoclaved at 135°C for 15 mins under pressure before removal as clinical waste by licensed contractors. Nothing is released to the environment. All autoclave runs are monitored and recorded by trained staff - any failed runs are treated as infectious and re run.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

Please enter comments on the GM safety committee on the risk assessment

Generation of recombinant Measles virus from cloned cDNA: The assessment stated that "At no-point" will gain of function viruses be intentionally generated i.e. those with increased scope for pathogenesis, replication, broader host-range*. A reviewer queried this, and asked how would the researcher identify what changes might potentially increase the scope for pathogenesis: "Although in general, certainly, one would generally expect mutations to reduce 'fitness' of the virus, might there be instances where this is not the case? I would suggest you include a bit more discussion around this issue; presumably, faster replication/spread, greater infectious yield of a novel GM derivative would become apparent in your initial in vitro experiments with the virus - so you could get an early indication of whether 'fitness' were unexpectedly increased."

This was addressed within the assessment.

02/03/2022
The objective of this project is to enhance fundamental understanding of the neuropathogenesis of RNA viruses by determining genetic loci in viral genomes which determine phenotypic properties of neurotropic viruses.

Recipient or parental organism:
Semliki Forest Virus, Bunyamwera Virus and Theiler's Virus.

Host/vector system:

cDNA derived, Semliki Forest Virus, Bunyamwera Virus, Theiler's Virus.

Origin & function

Specific gene loci will be targeted in Semliki Forest Virus, Bunyamwera Virus, Theiler's Virus. These sequences may be swapped between different natural isolates that have varying phenotypes or may be mutated or deleted. Any transfer of genetic material will only be between different strains of each virus, there will be no gene transfer between the different species.

Evaluation of foreseeable effects

Most of the genetic changes that will be introduced into the viruses can be expected to attenuate fitness. However in some cases changes in the sequences of avirulence virus cDNAs which change the sequence to that of a virulent virus cDNA would be expected to increase virulence but the changes are such that it would be only to a level seen in wild type isolates. These engineered recombinant viruses would only have sequences found in natural virus isolates, they are therefore considered highly unlikely to have increased virulence compared to that of wild type isolates. The viral envelope glycoproteins and capsid proteins which are the major determinants of host range and cell tropism will not be changed in these studies; host range and cell tropism are therefore unlikely to be altered. The changes are not expected to significantly alter susceptibility to host defence mechanisms. There are no vaccines or treatments available for these viruses and so effects of the modifications on these aspects are not applicable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
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<th>Solids (eg plasticware such as pipettes, flasks, tubes etc) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.</th>
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<tbody>
<tr>
<td>Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge to drains.</td>
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</tr>
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<td>Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), dispose via clinical waste stream for microwaving or via the industrial (black bag) waste stream for landfill.</td>
</tr>
<tr>
<td>Degree of kill:</td>
</tr>
<tr>
<td>Autoclaving - effectively 100% kill (annual validation)</td>
</tr>
<tr>
<td>Incineration and microwaving - not applicable, all waste is autoclaved prior to disposal by incineration or microwaving.</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3</td>
<td>L4 L2 L3 L4</td>
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Animal Units

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<tr>
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Large Scale Activities

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Human Clinical Applications

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<tbody>
<tr>
<td>L2 L3 L4 L2 L3</td>
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</table>

**Project Ref** 207/07.4

**Date Ackn'd** 27/05/2016

**CU2 Project Title** Investigations into the pathogenesis of poxviruses.

**Class** Class 2

**Culture Vol Class** 1-50 Litres

**Non-GMM Consent Granted** Not Applicable

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info** Transferred from GM207 27/05/2016

**Project Additional Information**
Purposes of the contained use

To investigate how Vaccinia virus (VACV) and Cowpox virus (CPXV) replicate and interact with the host’s immune system, leading to a better understanding of VACV genes that affect virulence. This work requires genetic manipulation of VACV and CPXV including the deletion and modification of genes to investigate their role in viral virulence.

Recipient or parental organism

Vaccinia virus
Cowpox virus

Host/vector system

Viral host with plasmid vector

Origin & function

Genes likely to affect the host’s response to viral infection will be deleted or mutated. In particular, genes that may be involved in cytoskeletal rearrangement or the modulation of the ubiquitin / proteasome system. Mutations involve minor alterations to amino acid sequence to identify functional domains. Standard non-harmful marker genes (such as E. coli gpt, β-gal, luc or enhanced fluorescent proteins) and epitope tags (such as Flag, c-myc, His or HA) will also be inserted.

Evaluation of foreseeable effects

The genetically modified viruses produced in this work are not expected to have increased virulence, pathogenicity, extended tissue tropism or host range or altered susceptibility to host defence mechanisms when compared to wild type strains and in many cases viruses will be attenuated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via clinical waste stream for heat treatment.

Animal bedding and carcasses – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose of carcasses via clinical waste stream for incineration and bedding via clinical waste stream for heat treatment or via the industrial (black bag) waste stream for landfill.
Degree of kill:
Autoclaving - effectively 100% kill (annual validation using 12 point thermocouple of worst case loads)
Heat treatment or Incineration – not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

Project Containment

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</tr>
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<td>L4</td>
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Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
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Project Ref 207/10.4

<table>
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<tr>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>25/09/2012</td>
<td>Response of ticks and tick cells to infection</td>
<td>Class 2</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Date Project Ceased

Withdrawn  

Tick if notifying a connected programme of work  

Project notified under transitional arrangements  

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Ticks carry a number of infections of importance in medicine and veterinary medicine. Little is known about how tick cells and ticks respond to microbial infections, how microbes persist in ticks for long periods of time and whether responses to one microbe affect responses or the outcome of infection to another microbe. This project will use a genetically modified tick-borne virus, Langat virus (LGTV) and a genetically modified tick-borne bacterium, Borrelia burgdorferi to investigate the responses of tick cells and ticks to infection, how one infection affects another and how and where these infections persist within ticks.

**Recipient or parental organism**

For LGTV: Disabled, commercially available E. coli strains and eukaryotic cell lines.

**Host/vector system**

LGTV sequence with changes in the replicase genes or insertion of reporter gene sequences in standard bacterial plasmids containing antibiotic resistance genes.

Non-human eukaryotic cells infected with LGTV, LGTV mutants or LGTV containing inserted reporter genes.

Plasmid JAH2 derived from the pBLS590 B. burgdorferi - E. coli shuttle vector. pJAH2 contains the gene for kanamycin resistance and a reporter gene driven by a bacterial ‘erp’ promoter.

**Origin & function**

LGTV containing a reporter gene and B. burgdorferi containing a reporter gene will be used to monitor infection or co-infection of eukaryotic cells, arthropods and rodents.

**Evaluation of foreseeable effects**

The LGTV structural proteins will not be changed; there should thus be no change to the tropism or host range of this virus. LGTV is not associated with human disease and has been used as a vaccine for other tick-borne encephalitis viruses. LGTV is not known to be present in the UK environment. It is not known whether it could be naturally sustained here if released, however release from our laboratories would be very unlikely to result in infection of ticks (naturally infected by blood meal) or vertebrates (naturally infected by bite). LGTV is not on the SAPO list. Borrelia causes borreliosis (Lyme disease) in humans. Laboratory acquired human infections have not been reported.

Infection can be treated with antibiotics. This bacterium is present in the UK environment. The GM bacterium would have no selective advantage for survival and establishment in the environment and if released from our laboratories would be very unlikely to infect ticks or vertebrates (natural route of infection is by bite). No harmful properties have been attributed to the inserted reporter genes.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste disposal procedures and protocols for biological material are in place in the Virology Towerblock, Summerhall Square. All biological material is inactivated by autoclaving or incubation with chlorine (as appropriate) before being disposed of through established waste disposal channels.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned

Project Containment

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<td>L2  L3  L4  L2</td>
<td>L3  L4  L2  L3  L4</td>
<td>L2  L3  L4</td>
</tr>
</tbody>
</table>

Animal Units  Large Scale Activities  Human Clinical Applications

| L2  L3  L4  L2 | L3  L4  L2  L3  L4 | L2  L3  L4  |

Project Ref  53/00.1

Date Ackn'd  03/08/2000

CU2 Project Title  ASSESSMENT OF RECOMBINANT ADENOVIRUS VACCINES AGAINST FOOT-AND-MOUTH DISEASE

Date Project Ceased  02/03/2022

Class  CultureVolClass2  CultureVolumeClass3-4

Class  2  

Non-GMM  Consent Granted  not applicable
Withdrawn: N

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?: N

If yes, tick to confirm that it is attached to this form: N

Tick to confirm that you have attached a risk assessment to this form: 

02/03/2022
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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</tbody>
</table>

**Laboratory Activities**
- L2
- L3
- L4

**Glass Houses**
- L2
- L3
- L4

**Growth Rooms**
- L2
- L3
- L4

**Animal Units**
- L2
- L3
- L4

**Large Scale Activities**
- L2
- L3
- L4

**Human Clinical Applications**
- L2
- L3
- L4

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**Project Ref 53/01.4**

<table>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Consent Granted</th>
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</thead>
<tbody>
<tr>
<td>02/10/2001</td>
<td>EXPRESSION OF AN INHIBITOR OF THE TRANSCRIPTION FACTOR NF-KB ENCODED BY AFRICAN SWINE FEVER VIRUS (ASFV) GENES USING A SINDBIS VIRUS VECTOR</td>
<td>Class 2</td>
<td>≤ 1 litre</td>
<td>not applicable</td>
<td></td>
</tr>
</tbody>
</table>

**Non-GMM**
- not applicable

**Withdrawn**
- N

**Project notified under transitional arrangements**
- N

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**Project Additional Information**

**Purposes of the contained use**

The aim of the experiment is to analyse the effects of an inhibitor of the transcription factor NF-kB encoded by an African swine fever virus (ORF A238L) on Sindbis.
virus-induced apoptosis. The inhibitor is encoded by ORF A238L from BA71V and ORF 5EL from Malawi isolates of ASFV. It is cloned into a replicon of Sindbis virus designated SinRep5 (Invitrogen). The replicon encodes Sindbis non-structural proteins and an SP6 promoter that allows transcription of the Sindbis genes and the gene of interest. Recombinant RNA produced from the replicon is transfected into BHK cells for high level expression of protein. The RNA from this replicon can be transfected together with a defective helper, DH26S or DHBB, which provides the structural proteins in trans. The transfected cells produce pseudovirions, which can be used to infect a second cell type, such as primary vascular endothelial cells. The pseudovirions only undergo one round of infection, because they should not contain RNA for the helper virus encoding the structural proteins. Infection with pseudovirions enables primary cells which are difficult or impossible to transfect to produce high levels of protein from the gene of interest. This is useful for investigating co-associating proteins, biological activity and localisation of the protein in the cell and to study mutated protein. Vascular endothelial cells are easily infected by both Sindbis virus and ASFV, and undergo NF-κB activation and apoptosis. Phenotypic characterisation of these highly differentiated cells will determine the mechanism of action of the IkB homologue without the influence of other ASFV genes.

Recipient or parental organism

Sindbis virus naturally infects mosquitos and other animal species such as birds. The risk to humans is low if the virus is handled in appropriate safety cabinet. The risk of spread is minimal in the absence of available competent mosquito vector. There should be no risk of altered infectivity with our recombinant vector.

There is a risk that fully infectious virus will be generated by recombination events during RNA replication. Recently, it has been shown that there is a low level of infectious virus produced where both structural and non-structural RNAs are packaged. The titre of this type of virus increases with subsequent passaging (Lu and Silver 2001) Transmission of replication-defective sindbis helper vectors encoding caspid and envelope proteins J. Virol. Methods 91: 59-65) Moreover, a report has appeared in the literature that recombination of the two RNAs can take place resulting in a replicon coding for the non-structural and structural proteins as well as the gene of interest (Herrmann et al, 1998 BiocheM Biophys Res Comm 253: 524-531). The risk of a recombinant replication competent Sindbis virus containing the ASFV IkB homologue is still considered to be of low risk to humans and the environment.

Host/vector system

RNA from SinRep5 plasmid (Invitrogen) or the recombinant SinRepA238L plasmid are cotransfected in BHK cells or primary cultures of porcine aortic endothelial cells (PAECs). The plasmid SinRep5 contains the SP6 promoter, non-structural proteins 1-4 and a subgenomic promoter. The DH26S helper virus is derived from a naturally occurring defective RNA and the sequence upstream of the subgenomic promoter is rearranged with no open reading frame. DHBB is a helper that gets packaged very, very poorly.

Origin & function

The ORF A238L is from the BA71V isolate of African swine fever virus (ASFV) and the ORF 5EL is from the Malawi isolate of ASFV. The intended fuction of the gene is unknown, but it may delay apoptosis and prevent inflammation in host cells. It has been shown to bind to the transcription factor NF-κB and to protein phosphatase 2B, also called calcineurin. It inhibits expression of genes controlled by NF-kB and by the transcription factor NF-AT.

Evaluation of foreseeable effects

The effects of A238L/5EL expression by Sindbis virus in vascular endothelial cells will be assessed by FACs analysis, RT-PCR, Western blotting and co-immunoprecipitation. It is expected that A238L will bind to NF-κBp65 and prevent expression of NF-kB dependent genes. This will result in inhibition of secretion of cytokines, surface expression of adhesion molecules and alter expression of apoptotic factors.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals or plans will be used. Animal cells in culture will be destroyed after the experiment with Stericol and autoclaving.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None for Class 2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste plastics and glassware is autoclaved. The plastics are then incinerated and glassware washed and sterilised. Waste liquids are treated with Stericol before sterilisation with alkali in our sewage treatment.

The safety committee considered that a Class 2 risk assessment was appropriate.

Project Containment

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<td>Animal Units</td>
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<td>L3 L4 L2 L3</td>
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<td>L2 L3 L4 L2</td>
<td>L2 L3 L4 L3</td>
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<tr>
<td>Human Clinical Applications</td>
<td>L2 L3 L4 L2</td>
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Project Ref 53/06.1

<table>
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<tr>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>05/06/2006</td>
<td>Infection-Interference Strategy for the First Defence against FMD</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
</tr>
</tbody>
</table>

Non-GMM Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info
### Purposes of the contained use

The objective of the study is to induce early immunity for the protection against FMDV through the enhancement of innate and passive immunity using defective (E1 and E3 sites deleted) human recombinant adenoviruses type 5 expressing various interferons, heavy and light chains of FMDV neutralising Mabs, FMDV structural or non-structural proteins or Sh RNAs (short hairpin RNAs) directed against the highly conserved sequences of structural and non-structural proteins of FMDV virus. A) Interferons are required for production of innate immunity, b) Mabs are required for humoral antibody, c) Structural and non-structural proteins are required to generate adaptive immunity and d) Sh RNA will provide the anti virals for the protection against FMDV infection.

### Recipient or parental organism

- The recipient is human adenovirus 5. It produced from the pAd/CMV/V5/ΔE1/ΔE3-DEST or pAd/PL/ΔE1/ΔE3-DEST expression vectors, is replication-incompetent in any mammalian cells that do not express the E1a and E1b proteins. The recombinant viruses could grow only in 293 A human embryonic kidney cells in tissue culture where E1 has been supplied.

### Host/vector system

- Adenovirus vectors produced from a) the pAd/CMV/V5-DEST and Block iT™ Adenoviral expression system, Invitrogen, USE, b) pAd/PL-DEST is obtained from Adeno Quest system, Q Biogen, Canada.

### Origin & function

- The genetic material involved is the recombinant adenovirus, interferon genes, heavy and light chains of FMDV neutralising Mabs, FMDV structural or non-structural proteins or Sh RNAs (short hairpin RNAs) directed against the highly conserved sequences of structural and non-structural proteins of FMDV virus.

- Interferons are required for production of innate immunity, b) Mabs are required for humoral antibody, c) Structural and non-structural proteins are required to generate adaptive immunity and d) Sh RNA directed against the highly conserved sequences of structural and non-structural proteins of FMDV virus will provide the anti virals for the protection against FMDV infection.

The recombinant adenoviruses will be amplified in 293 A HEK cells allowing (by supplement of E1) for the propagation of these recombinant constructs and titrated. Titrated viruses will be transduced in to the (bovine thyroid) BTY/IBRS-2 cells (no viral replication) and then challenged with various serotypes of FMDV 24 hours later. These recombinant adenoviruses will also be used to immunise cattle by an appropriate route (parenteral/nasal) and challenged with one of the seven sterotypes virulent FMDV 24 hours later. Different clinical samples like blood, nasal and ocular secretions will be collected prior to immunisation and at a regular interval during the period of study. The anti-viral activity of the various constructs will be studied by different virological and immunological assays.

### Evaluation of foreseeable effects

- The ability of the GMMs to extend the tissue tropism or host range of the AD5 vector is not expected to be increased by manipulation(s) to be carried out in this study. No toxicity is expected to recipient. There is no risk at all in case of this replication deficient adenovirus containing different FMS, FMD Mab constructs and ShRNAs and may be considered as class 1 containment level. However, the vector expressing the interferon (cytokines) may represent a risk to human health. In the extremely unlikely event of accidental inoculation there is possibility of accumulation of cytokines locally, mainly in the upper respiratory tract and the eye where they might have inflammatory or other immunological consequences. Therefore, this may be considered as class 2 containment levels.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Decontamination of all liquid and solid waste in the class II hood should be done in 2% FAM. Persons handling the interferon - expressing viruses to wear gloves; eye protection to be worn when administering virus to animals.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The local ACGM safety committee agreed that the only potentially hazardous constructs were the interferon-expressing recombinant viruses, and the potentially harmful nature of the expressed proteins from these constructs are the reason for this project being raised from the normal class 1 for helper cell-dependent human adenovirus 5 to class 2.

Project Containment

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<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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Project Ref 53/08.1

Date Ackn’d CU2 Project Title

10/09/2008 Use of Lentivirus-based vectors to study the function of proteins from animal virus

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2 < 1 Litre
For all the animal pathogens that are the focus of research at the institute, there is a common need to be able to study the roles of viral protein in isolation, to identify the host proteins with which the viral proteins interact, if any, and to characterise the role that interaction plays in the viral life cycle. This program of work involves the production of lentivirus-based pseudovirions to define the functions of proteins expressed by African swine fever virus, rinderpest virus, peste des petits ruminants virus, classical swine fever virus or other virus causing disease in livestock. The work proposed will use these vectors in several different approaches. These are: firstly to use the vectors to transduce cells and thereby construct cell lines expressing the virus gene of interest. These cell lines will then be used in various functional assays as appropriate, including the affinity purification of viral proteins and the characterisation of co-purifying host or viral proteins by proteomic systems. Secondly, the vectors will be used to express interfering RNAs targeted against either specific virus or host cell genes in cell lines. These cell lines will be used to study the effect of knocking down expression of either virus or host genes on the replication of the respective virus or the modulation of host cell functions caused by virus infection. Thirdly, the vectors will be used to construct cell lines overexpressing individual host genes which are identified as being targeted by virus infection.

Recipient or parental organism

The recipient vector is a replication deficient lentivirus-based vector. This is comprised of a transfer vector consisting of proviral cDNA from which virus coding sequences have been deleted or replaced with a genetic insert. The packaging system consists of three helper constructs that express viral genes needed to generate infectious virus particles (two plasmids for the retroviral genes and a third to express vesicular stomatitis virus surface glycoprotein G (VSV-G); VSV-G is required to replace the viral env protein and enables the resulting pseudovirions to enter most mammalian cells including non-dividing cells. The generation of replication competent virus (RCV) and insertional mutagenesis as a result of provirus integration pose the major safety issues when handling retrovirus vectors. Although transformation with lentivirus-based vectors has not been seen when using lentivirus-based systems in a broad range of in vitro and animal studies, some reports indicate that tumours can arise following administration of some lentivirus-based vectors. The woodchuck hepatitis post-transcriptional regulatory element included in some vectors is thought to be associated with these tumours. The lentivirus-based vectors are replication incompetent. Generation of replication competent lentiviruses through recombination is a very unlikely event since it would require simultaneous recombination between three plasmids during the intial transfection. Further safety is provided by the modification of the insertional elements (deletion of most of U3 region of 3’ LTR) to prevent reversal of the process, creating a so-called self-inactivating vector.

The animal virus coding sequences to be inserted in the lentivirus-based vectors include those for proteins involved in replication and in modulating host functions, including immunomodulatory genes. If individuals were accidentally infected by such constructs, and inserted genes integrated into stem cells, there is a possibility that the function of these cells may be affected. For example, the host immune system may be suppressed in the affected cells. A more likely event is the local entry of the GMOs into cells in exposed tissue (e.g. eyes, subdermal tissues exposed by cuts or abrasions). As discussed above, integration of DNA from some lentivirus-based vectors into
host genomes may cause tumours. These risks are managed appropriately by preventing contact of personnel with large amounts of the GMOs through the work being
carried out at ACGM
level 2. The interfering RNAs expressed will be specific for individual virus genes or non-human mammalian host genes. The
sequence specificity of interfering RNAs makes it very unlikely that these would be active against human genes.

Host/vector system
The lentivirus-based vector systems that will be used include those marketed under the name ‘ViraPower’ by Invitrogen, or similar commercial and non-commercial
systems (e.g. the Stanford University Helix system or the derivatives ofpHR-SIN
CSGW(Demaison C et al. (2002) 13, 803) based on HIV-I LTR sequences that have been modified to prevent reforming of live
virus, and also to prevent deintegration of DNA sequences after integration into the target cell genome. The interfering RNAs
will be expressed using the BLOCK-IT lentiviral RNAi system from Invitrogen, or similar. This expresses sbRNAs under
control of a pol II dependent promoter.
These constructs will be used to establish a range of different mammalian cell lines.

Origin & function
The genetic material involved is the lentivirus-based vector, protein coding sequences from animal virus pathogens and individual mammalian host genes. The function of
the animal virus pathogen coding sequences is either in replication of the virus or in modulation of host cell function. In particular, animal virus pathogen genes involved in
interfering with host defence systems will be investigated. Host genes from non-human mammalian sources will also be used. These will include genes from host pathways
targeted by virus infections. shRNAs will be targeted against individual animal virus genes or non-human mammalian genes. The genes to be used do not have known oncogenic
properties. Separate risk assessments will be prepared for each category of use of lentiviruses that is for each animal virus pathogen as well as for different types of host
gene or interfering RNA.

Evaluation of foreseeable effects
The individual animal virus pathogen genes to be expressed have a variety of functions in replication and in manipulation of host cell functions, particularly in inhibition of
host defence pathways such as the interferon pathway. Some of the genes to be expressed have no known function. The genes are from animal virus pathogens which are
not oncogenic and non of the genes to be studied have known oncogenic activity. They are therefore considered not to pose a risk significantly above that of the
lentivirus-based vector alone. The interfering RNAs to be expressed are specific for individual animal virus pathogen genes or non-human host genes and there is therefore
a negligible risk that they would pose a risk to human health. There is therefore a negligible risk of an adverse effect on human health associated with the described work
when handled at class 2 containment level.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Decontamination of all liquid and solid waste will be carried out in Class II MBSC using FAM at 1 in 200 dilution.
Individuals will wear protective clothing including a back fastening gown and gloves when handling non-sealed vessels
containing lentivirus-based vectors.
There were no additional comments from the committee.

**Project Containment**

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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- **Animal Units**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Large Scale Activities**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Human Clinical Applications**: L2 L3 L4 L2 L3 L4 L2 L3 L4

**Project Ref** 53/10.1

**Class** 2  
**CultureVolClass2** < 1 Litre  
**CultureVolumeClass3-4** Non-GMM Consent Granted

- **Date Ackn'd**: 25/03/2010  
- **CU2 Project Title**: The use of vaccinia virus to investigate viral protein function in eukaryotic cells

- **Date Project Ceased**:  
- **Withdrawn**: N  
- **Tick if notifying a connected programme of work**: Y  
- **Historical Significant Changes**:  
- **Historical Date of Additional Info**:  
- **Significant Change ID**:  
- **Date of Significant Change**:  

**Is an emergency plan required according to regulation 20?** N  
**If yes, tick to confirm that it is attached to this form**: N  
**Tick to confirm that you have attached a risk assessment to this form**: Y  
**Tick if you are claiming exemption from disclosure for section of the risk assessment**: N
Project Additional Information

Purposes of the contained use

The vaccinia virus (VV) Copenhagen strain, VC-2, will be used as the recipient virus. Using homologous recombination, open reading frames encoding selected viral proteins will be inserted into non essential loci of the vaccinia genome. Target viruses include dsRNA viruses and other ssRNA viruses, particularly flaviviruses, alphaviruses and phleboviruses. The recombinant organism would retain its interferon resistant phenotype. In the case of dsRNA viruses, some of the proteins are supposedly acting as anti-interferon (anti-PKR) proteins and therefore will be used to rescue the replication a VC-2 E3L defective strain (vp1080, that is sensitive to interferon). Therefore, some of the proteins that will be tested would not alter the interferon resistant phenotype of VC-2, while other proteins would rescue the interferon resistant phenotype of a VC-2 E3L defective strain: hence the contained activity is regarded as class 2.

Recipient or parental organism

The vaccinia virus that would be used is the VC-2 copenhagen strain. A VC-2 derivative vp1080, which is an E3L defective virus will be used to test the anti-PKR anti-interferon activity of some of the dsRNA binding proteins of dsRNA viruses. The wild type VC-2 strain would replicate in a wide range of cell lines and can infect humans. A vaccinia virus infection is very mild and is typically asymptomatic in healthy individuals, but it may cause a mild rash and fever. The characteristics of the vp1080 strain were published earlier by Beattie et al., Journal of Virology, 1995,69: 499-505. The deletion of the E3L was found to block the replication in Vero (Monkey Kidney), HeLa (Human), and L929 (Murine) cells, but not in chick embryo fibroblasts. The blocking of the replication is attributed to the loss of the anti-PKR/interferon phenotype.

Host/vector system

The virus will be used to study protein functions in cell culture models and there would be no involvment of any animal models.

Origin & function

A Vaccinia virus infection is very mild and is typically asymptomatic in healthy individuals, but it may cause a mild rash and fever. However, skin lesions and lesions to the eyes could occur in some individuals. Infection could occur if there is exposure of mucous membranes (eye conjunctiva, lips, mouth) to virus aerosols or exposure of skin cuts or by inhalation of large quantities of aerosolized virus. This is unlikely as material will be used within a class II microbiological safety cabinet.

Evaluation of foreseeable effects

A Vaccinia virus replication is inhibited by an authorised antiviral, cidofovir (an acyclic nucleoside phosphonate). Infection can be controlled through the administration of antivaccinal immunoglobulin.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Contaminated plasticware will be soaked in a disinfectant such as Activanios® with an appropriate dilution (0.2% glutaraldehyde + quaternary ammonium salt) for 45 minutes inside the safety cabinet. The material is then transferred into an autoclave where it will be further decontaminated at 134°C for 30 minutes. The surface of the container is decontaminated with a Virkon (1%) spray. Any work surfaces will be contaminated with Virkon spray and wiped with paper towels, which will be autoclaved at 134°C for 30 minutes. All autoclaved solid waste will be placed in heavy duty waste bags and incinerated.

Any liquid waste will be decontaminated with an appropriate disinfectant (0.2% glutaraldehyde + quaternary ammonium salt) contained in autoclavable polypropylene jars that can be closed hermetically. The jars are kept for 45 minutes under the hood, surface decontaminated then autoclaved at 134°C for 30 minutes. The liquid waste can then be disposed down the drains.

The dilution of Activanios that are used, kill the virus within 30 minutes of contact. Testing virus viability after treatment is done by removal of the disinfectant using ultrafiltration and washing any residual material with cell culture media. The recovery of any residual material and inoculation of permissive cell cultures such as Vero, HeLa or L-929 cells indicate that the virus has been inactivated irreversibly.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This is a connected programme of work for which a first risk assessment was provided and authorisation is granted for this particular activity. The risk assessment covers the usage of the E3L defective vaccinia virus (vp1080 to identify dsRNA binding proteins with an anti-PKR/interferon activity. Further risk assessments will be submitted in due course to the BAGMSC before a different activity is to be started, particularly before using the non modified VC-2 strain.

The risk assessment 07/08 was originally approved by the ACGM in 2007. Due to the delay in starting this work the RA was reviewed again at the local BAGSMC and approved on 24/03/2010

Project Containment

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### Project Additional Information

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 53/16.2

Date Ackn'd 26/05/2016

CU2 Project Title Impact of genetic reassortment and mutations on infectivity, replication and potential pathobiology of avian influenza viruses circulating in poultry

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 3 1-50 Litres

Non-GMM Consent Granted Yes

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

02/03/2022
Project Additional Information

**Purposes of the contained use**

**Research Question:**
What are the basic virological properties of emerging H7N9 viruses infecting poultry and what is their zoonotic transmission potential?

**Objectives**
1) To generate viable recombinant influenza viruses based on H7N9 with variable combinations of internal genes derived from prevalent H7N9 and H9N2 viruses,
2) To determine the virological properties of the recombinant viruses in vitro using cultured avian and mammalian cells and ex vivo organ cultures.
3) To determine the virological properties of the recombinant viruses in embryonated eggs

**Recipient or parental organism**

This project focuses on LPAI strains of H7N9 and H9N2 viruses. The novel H7N9 virus emerged through complex genetic reassortment events involving HA and NA genes from H7N3 and H7N9 viruses, which are prevalent in duck and wild aquatic birds respectively, and the six internal gene segments from H9N2 viruses, which are enzootic in chickens in China (1). Infection of chickens with H7N9 produces no clinical disease or mortality in infected chickens (2), therefore they are not HPAI. However, due to its ability to cause zoonotic infections and in some cases severe and fatal disease in humans with case fatality rate of 39% (3), it must be handled as a ACDP HG 3 agent (4). All reported human infections have been linked to direct contact with live poultry or potentially contaminated environments, particularly markets trading live birds. The virus does not appear able to transmit from human-to-human (5).

Recent evidence suggests that as H7N9 spreads in China it is undergoing further genetic reassortment and acquiring internal genes from distinct strains of H9N2 endemic in local regions (6-7). The H9N2 viruses are the most prevalent subtype in bird population worldwide and are also undergoing rapid genetic diversification by acquiring internal genes from other AlVs subtypes infecting poultry, including HPAI H7 and H5 viruses (8-9). Epidemiological and phylogenetic models suggest that highly diversified and distinct clades of H9N2 are enzootic in poultry in different countries (10). Modelling studies also predict that there is high likelihood that the H7N9 virus will potentially spread to other countries where distant clades of H9N2 are endemic (11). The likely co-circulation of both H7N9 and novel genotypes of H9N2 viruses containing diversified internal genes therefore could result in the emergence of novel reassortant H7N9 virus.
strains with unknown pathobiological features. This raises a number of important basic scientific and control policy-related questions that we aim to tackle through this research. Part of this project was recently funded by the BBSRC.

To determine the virological properties and in vitro host tropism of H7N9 Influenza virus strains infecting poultry and humans, we will use influenza virus Reverse Genetics (RG) approach to generate Genetically modified microorganisms (GMMs) H7N9 RG viruses (12). These GMMs will mimic the potential natural evolution of H7N9 and contain internal genes from diversified strains of H9N2 or H7N9 viruses currently prevalent in poultry in different regions of the world (6-7).

The GMMs will contain HA and NA genes of currently circulating H7N9 viruses and single or multiple internal genes from different strains of H7N9 or H9N2 viruses isolated from birds. These GMMs will be produced from known sequences in public databases of influenza viruses. The GMMs will have single or multiple internal genes identical to natural H7N9 or H9N2 virus isolates derived from infected birds or humans. Internal genes sequences of representative H7N9 or H9N2 strains (prevalent at different geographical regions) will be used for generation of H7N9 PG viruses. The representative internal genes of H7N9 or H9N2 viruses will be produced from known sequences using either synthetic gene synthesis approach (produced commercially) or through site-directed mutagenesis approach. All gene products to be used for generation of H7N9 AG viruses will be subjected to sequence analysis and integrity of gene sequences of rescued PG viruses will also be verified by complete sequencing of virus genome.

The activities that will be undertaken with these viruses are detailed below:
- Generation by reverse genetics methodology in cell culture.
- Propagation in embryonated hen eggs.
- Characterisation of growth and fitness in cells and ex-vivo organ cultures.
- Inactivation and extraction of PNA for sequencing.

**Host/vector system**

**Bacteria**

cDNA clones will be generated in E.coli and plasmids containing the influenza genes will be transfected into cells of eukaryotic origin (avian or mammalian) to rescue infectious virus.

**Origin & function**

DNA plasmids containing influenza virus gene segments (HA, NA) of H7N9 and internal genes (PB2, PB1 and PA, NP, M and NS) of H7N9 or H9N2 viruses will be generated from sequence data on the NCBI database by de novo synthesis by a commercial gene synthesis company. All sequences will be analysed by Sanger sequencing prior to use in viral rescue.

Point mutations in the nucleotide sequences representative of natural field isolates will be generated by us through site-directed mutagenesis and confirmed by sequencing the genetic segment prior to virus generation.

Once generated these viruses will be used to test their replication fitness such as infectivity and plaque forming morphology in permissive cell line such as MDCK cells, chicken embryo fibroblast (CEF), or replication in ex vivo chicken or ferret tracheal organ cultures or replication in embryonated eggs.

**Evaluation of foreseeable effects**

**Bacteria:**

No potential hazards to human health are envisaged with the manipulation of MV derived sequences in bacteria. The standard laboratory strains, disabled E. coli K12, will be used in this project. These strains pose no harm to human health. The inserted influenza genes are under the control of viral or eukaryotic promoters, so viral genes will not be expressed in the bacteria.

**H7N9 RG viruses:**
All the H7N9 RD viruses detailed in this assessment should be considered as capable of infecting humans, but the control measures in place in the Containment Level 3 laboratory (AWW-COP-i rev 2) are such that the virus will be contained appropriately.

Human infection with H7N9 viruses can cause severe and, on occasion, fatal disease (13). The widespread circulation of these viruses in birds and infection in humans is resulting in novel strains of R7N9 (14). Currently, limited data is available describing the impact of natural evolutionary changes in the internal genes on virus pathogenicity or host tropism compared to the wild-type H7N9 strains isolated from birds and humans. The proposed changes in internal genes of H7N9 that will contain single or multiple internal genes from diverse range of circulating H7N9 or H9N2 viruses may alter (increase or decrease) virus infectivity and replication characteristics. Any of these anticipated phenotypic characteristics will be monitored through our routine in vitro characterisation of novel influenza viruses. The routine in vitro characterisation of all isolates engineered in this study will provide evidence as to whether they possess any changes in virus infectivity or replication or plaque forming morphology in permissive cells such as MDCK and chicken embryo fibroblast (CEF) cells, ex vivo chicken or ferret tracheal organ cultures or replication in embryonated eggs. Work on viable recombinant viruses that show marked in vitro phenotypic changes (such as significant increase in virus titres and for plaque formation) compared with the wild type virus will be analysed by performing full genomic sequence analysis. This will allow identification of any molecular changes that the viruses might acquire during generation and replication in cells or eggs. Particular emphasis will be to identify unintended changes that potentially alter virulence such as insertion of polybasic residues at the HA cleavage site [conversion of low pathogenicity to high pathogenicity phenotype] or any known changes in NA (such as amino acid substitution H274Y, N294S, R292K and Eli 9V) that potentially increase resistance to antiviral drugs. Any viruses with identified mutations in HA or NA will be destroyed, the Pirbright Institute biosafety team will be informed.

All viruses generated by RD will be handled at Containment Level 3 in a dedicated suite of laboratories for avian influenza viruses. No changes in the HA gene will be made that may alter virus Phenotype from low pathogenicity to high pathogenicity. No change in the NA gene will be made therefore all GMM’s will remain sensitive to existing antiviral drugs.

In any potential cases of viral exposure diagnostics and antiviral treatment procedures will be implemented as detailed in The Pirbright Institute Q-Pulse document (H&S-GUIDE-29).

H7N9 viruses have been circulating in China for 3 years and there is no evidence of human-to-human transmission (15). Therefore, in the event of human infection any spread in the community is highly unlikely. All the RG viruses produced in this assessment will be handled separately in a class III microbiological safety cabinets or a flexible-film isolator with dedicated or clean (via a validated means including chemical disinfection or fumigation) equipment (pipettes etc). This procedural control will help to prevent potential mixing of influenza genetic segments via reassortment in vitro.

Reference:
4. HSE Advice on Experimental working with Influenza Viruses of Pandemic Potential
7. Cui et al. (2014). Dynamic reassortments and genetic heterogeneity of the human-infecting influenza A (H7N9)
8. Iqbal et al. (2009). Novel genotypes of H9N2 influenza a viruses isolated from poultry in Pakistan containing ns genes similar to highly pathogenic h7n3 and h5n1 viruses. PLoS ONE 4(6): e5788.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| No derogations required, these GMMs will be worked with under full ACGM conditions. |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| All work with these GMMs will be undertaken in a suite of high containment laboratories for working on avian viral disease pathogens up to and including Advisory Committee on Dangerous Pathogens (ACDP) hazard group (HG) 3 pathogens and pathogens up to and including group 4 of the Specified Animal Pathogen Order (SAPO). The Avian suite (Avian west wing, AWW), is a self-contained suite of laboratories within The BBSRC National Virology Centre: The Plowright Building at The Pirbright Institute, herein alter called The Plowright Building. All work involving these GMMs will be undertaken within primary containment, microbiological safety cabinets (MBSC) class I/III in class III mode or flexible film biosisolators within the AWW. Disposable equipment will be used where possible. All waste will be decontaminated by disinfection with one of the validated methods below; Virkon 1% 10 minutes Distel 10% 10 minutes Formaldehyde 40% 12 hours FAM 30 1:100 dilution 30 minutes Validation: Tests have been carried out by the Harbin Veterinary research Institute in China confirming the efficacy of Distel and Virkon against H5N1 virus. See Distel Medical test summary for validated data against influenza viruses. (DISTEL-1) FAM 30 is a DEFRA approved disinfectant again notifiable avian influenza viruses. http://evansvanodine.co.uk/asset/2-FAM-30-PROFILE-ISSUE-7.pdf Following disinfection the waste will be placed within a autoclave bag and removed from the primary containment before being sealed within an autoclave container. These containers have a PTFE seal to prevent the transfer of |
unfiltered air and a space for a plastic tag to secure the lid and indicate that the container is full. The containers are made from aluminium or specialised plastic containers to reduce weight. These are then transported to the dedicated within suite autoclave, where validated autoclave cycles for various types of waste including, solid, liquid and eggs are used by trained individuals. Autoclaved waste is removed from the clean side of the autoclave outside of the ADCP level 3 SAPO 4 suite and taken for incineration via The Pirbright Insitute clinical waste stream or if non-disposable taken to wash-up for reuse.

All users of the AWW suite will be trained in the correct disposal and operation of the within suite autoclave prior to becoming an authorised user of the suite. All training is documented by The Pirbright Institute training management systems. A risk assessment by the biosecurity team will be undertaken should the autoclave fail and robust procedures are in place to manage such an event. The autoclave within the suite is serviced and validated every six months as part of the planned preventative maintenance schedule and after any unscheduled maintenance work.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Reviewed by BAGMSC on 08/03/16 and approved at ACGM3.

Project Containment

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref  53/16.3

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Date Project

02/03/2022
To study the phenotypic effects and biological functions of defined host cellular and viral proteins in mammalian and avian cells; to silence expression of target genes; and to genetically program primary cells. For example:

1. We will use a 3rd generation replication deficient recombinant lentivirus vector to express GFP-fused to Npro from classical swine fever virus in order to study Npro’s localisation in relation to subcellular organelles/compartments and apoptotic proteins in porcine cell lines and primary cells.
2. We will use a 2nd generation replication deficient recombinant retrovirus vector expressing Bcl-6 and Bcl-xL to prevent the terminal differentiation of porcine memory B cells and enable their continuous proliferation with secretion of monoclonal antibodies.

The generation of recombinant retroviruses and lentiviruses will involve initial amplification of transfer, packaging and envelope plasmids using bacteria and commercial plasmid purification kits. All bacteria used for such amplification will be standard commercially-available ACGM class 1 strains of Escherichia coli (E.coli) such as JM109 and TOP10. Genes encoded by the plasmids are unlikely to be expressed in such strains of E. coli and pose no risk to the environment. Persistence is unlikely as all transformed E. coli will be lysed during plasmid preparation and all waste discarded into 1% Virkon (Gasparini, 1995). In addition, bacteria will never be transformed with more than one type of plasmid.

Only standard packaging cells, such as the human HEK 293T cell line, will be used to generate retrovirus stocks. Retroviral mediated expression of the host and viral proteins or silencing RNAs will be carried out in transduced cell lines or primary cells of mammalian or avian origin.

No infection of animals with recombinant retroviruses and lentiviruses will be conducted.

This project will utilise the retrovirus delivery and expression system, comprised of either the Moloney murine leukemia retrovirus or lentiviral (e.g. HIV-1-based) transfer, packaging and envelop plasmids. Recombinant retrovirus encoding mammalian, avian or viral genes will be prepared and transduced into mammalian or avian cells. These vectors may be pseudotyped with other envelope proteins such as gibbon ape leukemia virus envelope (GALV-env) or Vesicular stomatitis (VSV)-G protein to produce amphotropic viral particles.

Only 2nd or 3rd generation retrovirus systems designed to be replication incompetent will be used. The 2nd
generation system requires co-transfection of 3 plasmids encoding transfer, packaging and envelop proteins to generate replication incompetent retrovirus. The 3rd generation system requires an additional plasmid because the packaging proteins are split between two plasmids; in addition the 3rd generation system does not express the Tat protein.

Only retroviral transfer vectors such as pLJM1, that do not contain the post-transcriptional regulatory element from woodchuck hepatitis virus (WPRE), will be used.

**Origin & function**

The genetic materials involved will be cDNA encoding host cellular proteins involved in a range of processes including immune responses, growth factors (such as cytokines) and oncogenes (such as Bcl-6 and Bcl-xL) and viral proteins (such as classical swine fever virus Npro), which are known or suspected to interact with host cellular processes/pathways.

Genetic materials may encode interfering RNA molecules designed to silence specific genes of interest. In addition, the genetic material may contain or be solely cDNA encoding epitope tags (such as Myc, His, Strep, V5) or marker proteins (such as GFP, GST and luciferase) for the purposes of purification and detection of the above mentioned mammalian and viral proteins.

Genetic material may also contain a selectable drug marker e.g. the pac gene encoding a puromycin N-acetyl transferase, which confers puromycin resistance and enables selection of transduced cells.

**Evaluation of foreseeable effects**

The expectation is that cells transduced with recombinant retro and lentiviral vectors will have the proviral DNA inserted into the genome resulting in constitutive expression of the transgene(s). There is the potential for this to disrupt the normal pattern of gene expression within the infected cell, including the possibility of cellular transformation. Since the vectors to be used are replication incompetent no infectious progeny would be produced and disseminated.

Only second and third generation packaging systems will be used to propagate pseudotyped viral particles and thus the risk of generating replication competent virus is negligible.

The amphotropic virus particles are capable of infecting cells of both human and non-human animal species, but will not replicate. In the extremely improbable event that the vector acquires replicative potential by recombining with endogenous retroviral sequences, this single agent is unlikely to cause deleterious consequences in an accidental host. It is estimated that between 4 and 6 separate alterations are necessary to generate a tumorigenic cell.

Retroviruses are also inherently unstable and very high titres would be required to produce a persistent infection in an immunologically competent individual. The primary dangers in working practice are expected to be the formation of aerosols and use of sharps. We propose to carry out all manipulations in a Class II microbiological safety cabinet and any additional local safety guidelines appropriate to this work. Work involving glassware and sharps will be avoided where possible to mitigate the risk of exposure through non-intact skin. Where essential, work involving glassware and sharps will only be conducted by fully trained personnel and after risk assessment. The sonication of solutions, which may contain virus, will only be conducted by indirect sonication which prevents aerosols or cross-contamination.

None of the cell lines would be expected to be capable of remaining viable outside of the controlled culture conditions used and we do not anticipate that any of the manipulations we are proposing would alter this.

The risk of exposure to retro and lentiviral vectors in transduced cells subsequently used for other activities e.g. non-

CU 22015 (rev 11/15) Page 3 of 10
HSE Health and Safety
Executive

fixed flow cytometric cell sorting is negligible since the cells do not incorporate any helper function and residual
infectious virus particles are reduced by replacing the potentially infectious cell supernatant medium. In these circumstances, no additional containment measures beyond those needed for the non-transduced cells are required. Please refer to the overarching risk assessment CM 16/08, which users may work under as part of this connected program, although they must also complete a separate, project specific risk assessment, which will be assessed and approved by BAGMSC (see attached examples CM 16/05 and CM 16/07) prior to work commencing, however, should BAGMSC feel that there is an increased risk or the work falls outwith the scope of this notification, the work will be notified to HSE. Initially, this connected program will commence using the attached risk assessment GM16/07, although similar work will follow.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The proposed work consists of small-scale in vitro experiments to produce recombinant pseudotyped retroviral and lentiviral vectors and to transduce cells with these vectors. The following measures will be implemented to safely manage waste and safeguard human health and the environment:

1. A lab coat and gloves will protect the operator from skin contact with the pseudotyped viral particles.
2. A validated disinfectant e.g. 1% Virkon S or 70% ethanol will be used to disinfect any exposed surfaces. Both are effective at inactivating retroviruses and lentiviruses.
3. Accidental spillages of liquid waste will be deactivated with a validated disinfectant e.g. 2% Virkon S; the use of double strength disinfectant will ensure that it is not diluted past the effective concentration.
4. All liquid waste will be inactivated by a validated disinfectant for the contact time (e.g. 1% Virkon S for 10 minutes) and then disposed.
5. All solid waste will be autoclaved at 121 oC or 134oC dependent on the autoclave for 30 minutes prior to incineration by validated means.
6. Work involving glassware and sharps will be avoided where possible to mitigate the risk of exposure though non intact skin. Where essential, work involving glassware and sharps will only be conducted by fully trained personnel and after risk assessment.
7. All ACGM Class 2 work will be carried out in a class II microbiological safety cabinet (MBSC).
8. Centrifugation must be carried out in closed containers with sealed rotors or safety cups. Safety cups are to be loaded and unloaded inside the MBSC. After use safety rotors/cups are disinfected using a validated disinfectant.
9. Work will only be conducted in laboratories with restricted access to unauthorised personnel.
10. Flow cytometric analysis of transduced cells will be conducted following fixation of cells e.g. 4% paraformaldehyde for 20 minutes at 4oC.
11. Flow cytometric sorting of transduced cells will be conducted in a cell sorter fitted with an aerosol evacuation system and housed within a MBSC unless the risk assessment deems the risk of residual infectious virus to be negligible, whereby, no additional containment measures beyond those needed for the non-transduced cells are required.
Reviewed by BAG MSC on the 12/07/16 and approved at ACGM2 with the following amendments included:
Expand the scope of the risk assessment to include expression of interfering RNAs and selectable drug markers.
Include flow cytometric cell sorting of live cells on the bench provided that the risk of residual infectious virus had been reduced to negligible by washing the cells to replace the potentially infectious cell supernatant medium.

Please enter comments on the GM safety committee on the risk assessment
Reviewed by BAG MSC on the 12/07/16 and approved at ACGM2 with the following amendments included:
Expand the scope of the risk assessment to include expression of interfering RNAs and selectable drug markers.
Include flow cytometric cell sorting of live cells on the bench provided that the risk of residual infectious virus had been reduced to negligible by washing the cells to replace the potentially infectious cell supernatant medium.

Project Containment

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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 53/17.1

Date Ackn'd: 08/05/2017
CU2 Project Title: In vitro and in vivo studies with recombinant porcine reproductive and respiratory syndrome viruses

Consent Granted

Non-GMM

Consent Granted: Yes

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info
Porcine reproductive and respiratory syndrome (PRRS) is one of the most important pig diseases worldwide with a huge economic impact, estimated in the USA and Europe to exceed US$600 million and 1.5 billion Euros p.a., respectively. The PRRS virus (PRRSV) is an arterivirus, which exists in two distinct genotypes: PRRSV-l (formerly European) and -2 (formerly North American). Both genotypes are widely distributed although PRRSV-l remains the predominant genotype in Europe and PRRSV-2 predominates in North America and Asia. PRRSV-l is endemic in the UK whilst PRRSV-2 has never been reported. Common to most RNA viruses, the PRRSV replication cycle is prone to mutation and recombination events resulting in rapid evolution. This characteristic is most dramatically illustrated by the emergence of highly pathogenic variants in Southeast Asia and Eastern Europe. Vaccination is considered a key element to PRRS control but existing vaccines are evidently failing to control the PRRS panzootic. An improved understanding of PRRSV and its interactions with the porcine immune system is urgently required to support the development of improved vaccines and disease control intervention strategies. The purpose of the contained use is to create and manipulate cDNA copies of the genomes of PRRS viruses in plasmids and use these to recover live viruses with specific modifications. Viruses will be modified for example to:

1. Prevent expression of specific viral proteins to examine their effect on viral replication and on the host cell response.
2. Introduce specific modifications/mutations to individual viral proteins to study the effects of those mutations on viral replication and/or interaction with host proteins, or to create epitope tags on the viral proteins allowing these proteins to be recognised by specific antibodies.
3. Exchanging genomic regions, untranslated regions or open-reading frames with equivalent genetic material from other strains of PRRSV with different pathogenicity, to identify virulence factors.
4. Introduction of extra genes to express heterologous proteins which act as infection markers (e.g. GFP) or to express non-PRRSV proteins so that the virus may act as a polyvalent vaccine.

Recombinant PRRS viruses will be studied both in vitro in primary cells and cell lines, and, in vivo in the natural porcine host.

The generation of recombinant PRRS viruses will involve initial amplification and modification of plasmids carrying cDNA copies of the PRRSV genomes using bacteria and commercial plasmid purification kits. All bacteria used for such amplification will be standard commercially-available ACGM class 1 strains of Escherichia coli (E.coli) such as JM109 and TOP10. Genes encoded by the plasmids are unlikely to be expressed in such strains of E. coli and pose no risk to the environment. Persistence is unlikely as all transformed E. coli will be lysed during plasmid preparation and all waste discarded into 1% Virkon (Gasparini, 1995). In addition, bacteria will never be transformed with more than one type of plasmid.

Recombinant PRRSV will be rescued following transient transfection of cell lines, e.g. BHK-21 or MARC-145 cells, or primary porcine cells, e.g. porCine alveolar macrophages with infectious copy plasmids or RNA derived from in vitro transcription of infectious copy plasmids. Recombinant PRRSV will be propagated in vitro using MARC-145 cells or porcine alveolar macrophages. The phenotype of recombinant PRRSV will be studied in vitro using MARC-145 cells or primary porcine cells, and, in vivo, in experimentally infected pigs. Recombinant PRRSV are expected to retain the
phenotype of the parental wild type strain from which the infectious cDNA clone was derived. Genetically modified PRRSV may display an attenuated phenotype due to the introduction of modifications/mutations affecting the expression or function of individual viral proteins. Introduction of a specific viral gene or genomic region from a highly virulent strain to a low virulence strain may result in an increase in the virulence but this is not expected to exceed that of the virulent parental wild type strain.

Host/vector system

This project will utilise mammalian expression plasmid vectors e.g. pCMV to manipulate and express full-length infectious cDNA clones of the PRRSV genome. Cell lines e.g. BHK-21 cells will be used for initial transfection of plasmids or RNA derived from in vitro transcription of infectious copy plasmids. MARC-145 cells and porcine macrophages and related myeloid cells will be used for virus rescue and subsequent in vitro experiments. In vivo experiments will be conducted in pigs, the only species susceptible to PRRSV infection.

Origin & function

The genetic materials involved will be cDNA encoding genotype 1 and 2 PRRSV. These may be derived from viral strains of defined virulence (e.g. attenuated, low virulence, high virulence). Alternatively, these materials may be chemically synthesised based on PRRSV genome sequence data. Genetic materials may have specific modifications/mutations introduced into individual PRRSV open-reading frames (ORFs) or 5' and/or 3' untranslated regions (UTR). Alternatively genomic regions or ORFs may be replaced with those from heterologous PRRSV strains. In addition, the genetic material may contain cDNA encoding epitope tags (such as Myc, His, Strep, V5) or marker proteins (such as GFP, GST and luciferase) for the purposes of purification and detection of the recombinant PRRSV or defined PRRSV proteins. These materials will allow the production of recombinant PRRS viruses which will be used to study basic aspects of viral biology, to study the interaction of the virus and individual viral proteins with the innate and adaptive immune systems and to develop novel vaccines.

Evaluation of foreseeable effects

Recombinant PRRS viruses derived from infectious cDNA clones are expected to display the same phenotype as the parental wild type strain from which they are derived. None of the novel genes which might be inserted will encode toxic or immunomodulatory proteins, nor will any oncogenes be added. No gene will be inserted which would alter the host cell tropism or extend the host-range of the recombinant virus. The majority of the manipulations to the PRRSV genome including the introduction of mutations to disrupt the interactions of viral proteins with host ligands or the disruption of the expression of specific viral proteins are expected to reduce the virulence and fitness of the virus. An increase in the virulence and fitness of the virus may be achieved by the introduction of genetic material from a virulent strain into the genome of an attenuated/low virulence strain, however, the resultant virulence of the chimeric strain is not expected to exceed that of the virulent parental strain. Therefore, all proposed mutations/modifications will leave the GMM with the same or lessened hazard than the parental virus. There is no evidence that recombinant (or wild-type) PRRSV could infect humans or cause harm to human health. However, recombinant (and wild-type) PRRSV will infect domestic and wild pigs and thus they pose an environmental hazard which would be controlled by the control measures detailed below.

Please refer to the overarching Connected Programme of Work (CPW 17IXX), which provides further details of the context and scope of the proposed work. Users will complete project specific risk assessments, which will be assessed and approved by The Pirbright Institute Biological Agents and Genetic Modification Safety Committee (TPI...
BAGMSC) (see attached example GM 17/ZZ) prior to work commencing, however, should BAGMSC feel that there is
an increased risk or the work falls outwith the scope of this notification, the work will be notified to HSE. Initially, this
connected program will commence using the attached risk assessment GM17IXX, although similar work will foNow.
Since TPI carries out work on highly contagious pathogens of livestock, all animals on-site have to be housed in high
containment (SAP04) facilities. In vivo work with recombinant PRRSV-1 (ACGM class 2 GMM) may be conducted in
lower containment using CL2 animal facilities of the Animal and Plant Health Agency (APHA), Weybridge. Project
specific risk assessments covering such in vivo work will be assessed and approved by both the TPI and APHA
BAGMSCs.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogations required, these GMMs will be worked with under full ACGM conditions.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The proposed work consists of small-scale in vitro experiments to produce recombinant PRRSV-1 and -2 viruses.
These viruses will subsequently be used in in vitro and in vivo studies. In vitro and in vitro work with recombinant
PRRSV-1 (ACGM class 2) will be conducted in CL-2 laboratories and animal facilities whereas work with recombinant
PRRSV-2 (SAP03 ACGM class 3) will be conducted in laboratories and animal facilities under SAPO level 4
containment. The following measures will be implemented to safely manage waste and safeguard human health and the
environment:

In vitro ACGM class 2 work with PRRSV-1 (handled within CL-2 Jenner Laboratory, The Pirbright Institute (TPI»
1. A lab coat and gloves will protect the operator from skin contact with GMM.
2. A validated disinfectant e.g. 1 % Virkon S or 0.5% FAM 30 will be used to disinfect any exposed surfaces. Both are
effective at inactivating PRRSV.
3. Accidental spillages of liquid waste will be deactivated with a validated disinfectant e.g. 2% Virkon S or 1 % FAM
30; the use of double strength disinfectant will ensure that it is not diluted past the effective concentration.
4. All liquid waste will be inactivated by a validated disinfectant for the required contact time (e.g. 1 % Virkon S for 10
minutes) and then disposed to drains.
5. All solid waste will be autoclaved at 121 oC or 134oC dependent on the autoclave for 30 minutes prior to
incineration by validated means.
6. All work with live virus will be carried out in a class II MBSC.
7. Centrifugation must be carried out in closed containers with sealed rotors or safety cups. Safety cups are to be
loaded and unloaded inside the MBSC. After use safety rotors/cups are disinfected using a validated disinfectant.
8. In vitro work with PRRSV-1 will only be conducted in CL-2 virus handling laboratories with restricted access to
unauthorised personnel.

In vitro ACGM class 3 work with PRRSV-2 (handled within SAP04 Plowright Laboratory. TPI)
1. A lab coat and gloves will protect the operator from skin contact with GMM.
2. A validated disinfectant e.g. 1 % Virkon S or 0.5% FAM 30 will be used to disinfect any exposed surfaces. Both are
effective at inactivating PRRSV.
3. Accidental spillages of liquid waste will be deactivated with a validated disinfectant e.g. 2% Virkon S or 1% FAM
20; the use of double strength disinfectant will ensure that it is not diluted past the effective concentration.
4. All liquid waste will be inactivated by a validated disinfectant for the required contact time (e.g. 1 % Virkon S for 10
minutes) and then disposed to the heat effluent treatment plant.
5. All solid waste will be autoclaved at 121°C or 134°C dependent on the autoclave for 30 minutes prior to
incineration by validated means.
6. All work with live virus will be carried out in a class II MBSC, in sealable rooms, with room air having a single
supply and double extract HEPA filter, with the room operating under negative pressure.
7. Centrifugation must be carried out in closed containers with sealed rotors or safety cups. Safety cups are to be
loaded and unloaded inside the MBSC. After use safety rotors/cups are disinfected using a validated disinfectant.
8. In vitro work with PRRSV-2 will only be conducted in SAP04 virus handling laboratories with restricted access to
unauthorised personnel.

In vivo ACGM class 2 work with PRRSV-1 (handled within Animal and Plant Health Agency (APHA) CL2 Animal Units.
samples processed in CL2 Jenner Laboratory. TPI)
1. In vivo work with recombinant PRRSV-1 may be conducted in a CL-2 animal facility at APHA. Weybridge.
2. Recombinant PRRSV-1 will be prepared for inoculation within CL2 virus handling laboratories at TPI and
packaged within a secure secondary container for transport to APHA.
3. PPE (disposable overall, boots, gloves) will be worn whilst working with infected animals. Gloves and overalls to
be disposed after use, bagged up. bags disinfected with 2% Virkon S or 1% FAM 30. left for 1 h and incinerated.
Boots will be cleaned with 2% Virkon S or 1 % FAM 30.
4. All solid waste including bedding will be bagged and incinerated. No liquid or solid waste will be allowed to enter
the normal drainage system and all drains in the relevant area will be blocked. In effect liquid waste will soak into
bedding and be disposed of as solid waste.
5. Samples will be packaged in the animal facility with the outer packaging disinfected with a validated disinfectant
e.g. 1% Virkon S or 0.5% FAM 30, and then packaged within a secure secondary container for transport to CL2 virus
handling rooms in the Jenner Laboratory. TPI. Unpacking of samples is carried out in a class II MBSC.

In vivo ACGM class 3 work with PRRSV-1 and -2, respectively (handled within TPI SAP04 animal isolation
facility, samples processed in SAP04 Plowright Laboratory. TPI)
1. In vivo work with recombinant PRRSV-1 may be conducted in TPI isolation units, which operate under SAPO level 4 containment.
2. In vivo work with recombinant PRRSV-2 will be conducted in TPI isolation units, which operate under SAPO level
4 containment.
3. Staff will remove clothing, access via an airlock and put on scrubs. Staff will don waterproof clothing prior to
entering animal rooms, which are disinfected on exit from animal rooms. Staff remove clothing and shower on exit
from the facility.
4. Liquid waste is disposed to drains and heat-treated to 94°C for 1 hour in the effluent treatment plant. All solid
waste will be autoclaved at 1210°C or 134°C dependent on the autoclave for 30 minutes or incinerated in the barrier
incinerator. Removal of items or equipment that cannot be autoclaved or incinerated are surface disinfected prior to
fumigation with formalin vapour.
5. Animal rooms are sealable and have single HEPA on supply air and double HEPA on extract air, with the room
operating under negative pressure.
6. Samples will be packaged in the animal isolation rooms with the outer packaging disinfected with a validated
disinfectant e.g. 1 % Virkon S or 0.5% FAM 3D, and then packaged within two secure containers resulting in 4 layers of
containment. Samples are then moved directly to virus handling rooms within the SAP04 Plowright Laboratory.
Unpacking of samples will be carried out in a class II MBSC.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

02/03/2022
Please enter comments on the GM safety committee on the risk assessment

Reviewed by BAGMSC on the 14/03/17 and approved at ACGM3 with the following amendments included:
Increased description of the containment and control measures.
Remove mention of specific laboratories/rooms and 'risk spaces'.

### Project Containment

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<tr>
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<td>Large Scale Activities</td>
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### Project Ref 53/18.2

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Tick if notifying a connected programme of work N

Historical Significant Changes

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Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Project Additional Information

Purposes of the contained use

The purpose of our projects is to increase our understanding of the viral determinants for host range, transmission and pathogenesis of influenza A viruses (IAV) and to develop tools that could enhance control of influenza viruses in poultry, humans and swine.

Recipient or parental organism

We intend to generate several different groups of IAVs, all which are assessed as ACDP HG 2. None of the IAV we generate will be categorised as highly pathogenic as defined by HSE (in possession of a multiple basic cleavage site in the HA gene or having an intravenous pathogenicity index score in chickens of over 1.2). We will also not generate viruses with altered characteristics that enhance the replication and transmission in humans or surrogate human animal models (e.g. the ferret).

Group 1: Wild-type IAV strains. Human IAV strain (Pre 2009 H1N1, pandemic 2009 H1N1, H3N2 but not H2). Swine influenza viruses (H1N1, H3N2, H3N1 but not H2 viruses). All subtypes of low pathogenicity avian influenza virus where the natural HA sequence does not contain a multiple basic cleavage site (defined as greater than 2 basic amino acids). Will not generate any highly pathogenic virus (HPAI) as defined in the HSE guidance. We will also not generate viruses known to cause severe disease in humans as these are categorised as ACDP HG3 (e.g. H7N9 and H10N8).

Group 2a: Segment swaps in whole viral strains: Segment swaps between IAVs isolated from the same species. For example any of the genetic segments could be swapped individually or in combination between the chicken LPAI viruses H9N2 and H6N1. Segments from highly pathogenic viruses will not be swapped into LPAI viruses. We will not swap segments between different species for example between the human H1N1 and the chicken H9N2 virus.

Group 2b: Segment swaps between IAVs isolated from different avian species (e.g. ducks and chickens). The only exception to the previous boundary regarding segment swaps between different species is within the avian genus since the ability of all avian species isolated viruses to replicate in mammalian hosts is limited and reassortment between these viruses is not likely to generate strains with enhanced ability to replicate or transmit in mammals. Segments may be swapped individually or in combination. Segments from highly pathogenic viruses will not be swapped into LPAI viruses only swaps between LPAIs will be made.

Group 3a: Sequence mutations in viral proteins: Mutations in IAVs that occur in other IAV strains isolated from the same species in nature. Mutation of single or multiple residues will be made in the genetic material of IAVs in coding or non-coding regions of the genome that are reflective of sequences deposited on the database from viruses isolated in the field from the same species. For example where there is a chicken H9N2 virus sequence on the
NCBI influenza database containing a Leucine residue at position 226 in the
HA protein we may alter the HA of a chicken H6N1 virus or chicken H9N2
virus at position 226 to a Leucine residue. Mutations only observed in
sequences from highly pathogenic viruses will not be engineered into LPAI
viruses. Mutations only observed in isolates from one species will not be
engineered into IAV isolated from different species, (e.g. sequences only
observed in human IAV isolates and not identified in natural chicken isolated
IAVs will not be engineered).

Group 3b: Point mutations in IAVs that occur in other IAV strains isolated
from different avian species in nature. The only exception to the previous
boundary regarding engineering mutations into IAVs only seen in different
species is within the avian genus since the ability of all avian species
isolated viruses to replicate in mammalian hosts is limited and mutational
switches between these groups of viruses are not likely to generate strains
with enhanced ability to replicate or transmit in mammals. Mutations only
observed in sequences from highly pathogenic viruses will not be engineered
into LPAI viruses.

Group 4a: Deletion: The deletion of coding or non-coding portions of the IAV
genome. The deletion may be achieved by generating IAV segments that
have portions removed or by introducing stop codons in protein coding
regions to prevent expression of the region. Genes may be deleted partially,
fully or in combination with others.

Group 4b: Insertion: The insertion of tags or reporters into the genetic coding
capacity of IAVs. Tags such as small epitope tags for the purpose of gene
identification using ubiquitous antibodies may be added to the N or C
terminus of IAV proteins in the viral genome (e.g. V5, FLAG, (His)6).
Reporter genes such as fluorescent proteins (GFP, mCherry etc) or
luminescent proteins (luciferase) may be inserted into the viral genome,
either as fusions with IAV proteins or as standalone proteins released by
proteases (such as self-cleaving proteases like 2A from porcine teschovirus-
1 or endogenous proteases). Insertion of such tags and reporters may occur
in a number of sites individually or in combination (such as in the NS, PB2 or
PA segments (3-9)). Group 5a: PR8 backbone IAVs: IAVs with vaccine
strain (A/PR/8/34) internal segments (M, NS, NP, PA, PB1, PB2) and the HA
and NA of any other HA (H1-H18) or NA (N1-N11) seen in nature. H5 and
H7 HAs that do not possess a multiple basic site only will be produced (i.e.
from low pathogenic H5 and H7 IAV isolates only). If the HA from a highly
pathogenic H5 or H7 is to be used in such a virus then the multiple basic cleavage site must be engineered out of the HA as has been exemplified by
the NIBSC vaccine strain candidates for avian influenza viruses such as
RG14 (HA from HPAI H5N1 A/Vietnam/1194/04 virus with an excised
multiple basic cleavage site) or RG23 (HA from HPAI H5N1
A/turkey/Turkey/1/05 virus with an excised multiple basic cleavage site).

Group 5b: PR8 backbone IAVs that include mutation from the natural
sequence in HA or NA proteins. Similar to the above group 5a PR8 backbone IAVs these viruses will contain the internal genetic segments (M, NS, NP, PB1, PB2, PA) from the vaccine strain A/PR/8/34 and HA from any of the H1-H18 and NA N1-N11 segments. Mutation of the HA segment may occur with such purpose to alter the antigenic profile of the viruses, to remove glycosylation sites, alter the receptor binding preference, HA stability or ability to facilitate membrane fusion. Mutation of the NA segment may occur with such purpose as to modify the NA stalk length, glycosylation, antigenic profile, sialidase activity.

Group 6a: PR8 pseudotyped IAVs: IAVs with the internal genetics segments from any influenza virus (M, NS, NP, PB1, PB2, PA) and the HA and NA (as a minimum) from the A/PR/8/34 (PR8) IAV strain. The internal genetic components may come from any natural IAV isolate including HPAI virus. More PR8 genetic segments other than the minimal HA and NA can be included up to 7 from the vaccine strain PR8.

Group 6b: PR8 pseudotyped IAVs that include mutation from the natural sequence in the internal genetic segments. Mutation of the PR8 HA and NA will not occur when mutation of internal genetic components occurs.

Host/vector system

cDNA clones will be generated in E.coli and plasmids containing the above mentioned influenza genes will be transfected into cells of eukaryotic origin (avian or mammalian) to rescue infectious virus.

Origin & function

cDNA constructs for the rescue of IAVs will be generated by de novo synthesis according to our sequences or obtained from collaborators where they will be fully Sanger sequenced prior to use in IAV rescue. Point mutations may be introduced via site-directed mutagenesis using the guidelines set out above. Once generated these viruses will be used in vitro to assess fitness in avian and mammalian cell lines by measuring replication characteristics, innate response antagonism and the ability to interact with host derived partners. The viruses may be used to inoculated avian species (e.g. chickens, turkeys or ducks) to assess fitness in a whole animal model and look at transmission. Insertion of influenza A virus genes or reporter or tags and the associated gene products to K-12 derived E.coli will not alter the pathogenic traits of the bacterial host since none of these have known harmful characteristics.

Group 1 – Wild type influenza viruses will not be altered in sequence from the natural isolate and the use of reverse genetics will not alter the characteristics of the virus from the parental isolate.

Group 2 & 3 – Segment swapped viruses and sequence mutation viruses in the whole are not likely to alter existing pathogenic traits in humans since segment swaps or point mutations will only occur amongst viruses isolated
from the same species host (e.g. human virus segment swaps or identified point mutations into other human viruses) or between avian species only (e.g. duck virus segment swaps or point mutations into isolates from chickens). The swapping of avian influenza segments into other avian influenza virus isolates may result in the introduction of a mammalian adaptation motif into a strain that previously did not have that motif. For example the swapping of the PB2 (viral polymerase) segment for one that contains a mutation at amino acid position 627 to a Lysine, many avian strains in nature do possess this mutation. Mutation at 627K in PB2 is known to enhance the ability of the influenza viral polymerase complex to replicate genomic RNA in mammalian cells. However since avian virus mammalian adaptation is multi-factorial including receptor preference changes, virion stability enhancement as well as viral polymerase activity an increased ability in one characteristic as a result of a segment swap or cluster of point mutations in a segment will not lead to greatly enhanced replication of an avian virus in mammalian hosts (10-12). We will not generate avian viruses that might be predicted to have more than one of these characteristics enhanced in mammal. Low pathogenicity avian viruses that contain a single mammalian virus motif may infect a human after exposure to a high aerosolised dose and replicate in lung cells but this will not be efficient replication, the virus would be susceptible to anti-viral drugs and no onward transmission would occur.

Group 4 – Insertions and deletions to influenza viruses. The deletion of viral genetic segments or non-coding portions of the viral genome are on the whole likely to reduce pathogenicity of influenza virus strains in humans. Many of the viral proteins are essential for efficient replication in a cellular environment. In addition many of the accessory proteins have also been demonstrated to be important in conferring pathogenicity in vivo (13). The majority of the influenza genomic segments encode for one or two proteins so deletion of proteins does not alter or may only alter the level of protein expression of one other viral protein. There are no known toxic or oncogenic characteristics known for any of the viral proteins. Insertion of tags or reporters into the genome of influenza viruses will not increase the pathogenic traits of the virus, tags and reporters are notoriously unstable in influenza viruses and their fusion to viral proteins will not change the function of viral proteins in an adverse way. If anything the insertion of tags or reporters will disrupt virus replication or protein function leading to an attenuation of virus pathogenesis.

Evaluation of foreseeable effects

Group 5 - PR8 backbone IAVs. These viruses contain the internal genetic segments (M, NS, NP, PA, PB1 and PB2) from the well characterised lab adapted vaccine strain A/PR/8/34 (PR8) and the HA from any low pathogenic HA subtype (H1, H2, H3, H4, H5 (without multi-basic site only), H6, H7 (without multi-basic site only), H8, H9, H10, H11, H12, H13, H14, H15, H16) coupled with any NA subtype (N1, N2, N3, N4, N5, N6, N7, N8, N9). These viruses will be lowered in their pathogenic potential compared to the parental virus from which the HA and/or NA originate from. PR8 is a highly laboratory adapted influenza strain that has been passaged in cell culture, mice and ferrets over 1000
times and as a consequence has become highly attenuated in humans (14). The exact mutations that have led to this attenuation in humans has not been documented but human volunteer trials of recombinant influenza viruses possessing the six internal genes (PB1, PB2, PA, NP, M, NS) of the highly lab adapted vaccine strain A/PR/8/24 and the 2 surface proteins (HA & NA) from other human virulent influenza strains demonstrates that the attenuation is conferred by the presence of the six PR8 genes and that the HA & NA genes of PR8 are not required for attenuation in humans (14, 15). Viruses with the same genetic constellations as described above are generated by many laboratories all over the world and are generated in huge quantities worldwide at ADCP HG 2 during part of human influenza vaccine manufacture, including in the UK at NIBSC. Alteration of the natural HA and/or NA in sequence to change receptor preference, HA stability, antigenicity or NA activity will not increase the pathogenicity of the PR8 backbone IAVs. We will not generate PR8 backbone viruses that are altered in the NA segment to be resistant to neuraminidase inhibitor drugs.

Group 6 – PR8 pseudotyped IAVs. These viruses contain the HA and NA (as a minimum) from the well characterised lab adapted vaccine strain PR8 and the remaining segments from any other IAV, including those categorised as highly pathogenic in their parental form. The pathogenic traits of these viruses will be lower or equivalent to the parental viruses from which the other genetic segments originate. The hazards directly associated with recombinant influenza viruses bearing the surface antigens of the attenuated vaccine strain PR8 virus is low since the PR8 HA has a receptor preference for α2-3 linked sialic acid which is not found abundantly in the upper respiratory tract of mammals and thus are highly unlikely to infect an individual or transmit (Roberts et al 2011). The host range is not predicted to be extended for any of these viruses. In the extremely unlikely scenario these viruses do infect a human the PR8 HA & NA, which have a preference for α2-3 linked sialic acid, may allow replication deeper in the respiratory tract where these sialic acids are found in humans rather than the typical upper respiratory tract infection usually experienced by human influenza infections and this may cause increased respiratory symptoms. These viruses remain susceptible to neuraminidase inhibitors that are routinely used for treatment in humans against influenza infection. Moreover, there is considerable cross-reactivity between the pdmH1N1 2009 human virus and PR8, so people infected in 2009 or subsequently vaccinated will have protection against infection with the PR8 based viruses (16). For highly pathogenic viruses it has been shown that removal of the multiple basic cleavage site is sufficient to reduce the pathogenicity of the virus genetic constellation (17). The multiple basic cleavage site in the HA of highly pathogenic viruses is responsible for viral spread beyond the respiratory tract and the cause for the extreme pathogenicity observed by these viruses. The substitution of highly pathogenic HA and NA genes with those from the restricted PR8 virus will substantially lower the pathogenicity of these viruses in mammals.
in vivo work will be performed in the Poultry BSU both in containment level 2 laboratories on The Pirbright Institute site.

1. A lab coat and gloves will be worn to protect from contamination
2. All work with infectious influenza virus will be performed in primary containment (a class II MBSC, negative pressure poultry isolator or on a down-table with a face-fitted FFP3 face mask). All primary containment is serviced annually by an outside contractor and records maintained.
3. Influenza virus work is carried out in restricted access containment level 2 laboratories.
4. Training is given to all workers prior to unsupervised work on identification of influenza viruses that can be used concurrently, the need for separated equipment for different IAV groups and disposable consumables including filtered tips, and the segregated storage of plasmids carrying IAV genetic information, how to respond to an uncontained spill of IAV (exit the laboratory and inform the duty biological safety officer).
5. All IAVs will be stored appropriately in screw-capped tubes with O-rings and labelled.
6. All contaminated waste will be chemically decontaminated by a validated disinfectant (5% Distel for 5 minutes or 1% Virkon for 10 minutes) before being disposed down the sink or autoclaved before leaving the building. The only exception is infected eggs or avian carcasses which are disposed of in double bags in sealed and labelled burn bins and disposed of via a clinical waste route.
7. Accidental spillages inside primary containment dealt with double concentration disinfectant (e.g. 10% distel for 5 minutes or 2% Virkon).
8. Occupational Health screening and policy in place to identify those at greater risk from influenza virus infection, (immune-suppressed, pregnancy etc) (H&S-GUIDE-29).
9. Vaccination with human seasonal influenza vaccine offered to all workers and confidence of workers to report influenza like illness to line managers to prevent community influenza being brought into the laboratories.
10. All new HA and/or NA segments will be sanger sequenced prior to reverse genetics rescue to determine that the expected sequence is correct following cloning or synthesis of plasmids. This is of particular importance when using plasmids encoding for the influenza sequences of H7 or H5 HAs where the multiple basic cleavage site has been engineered to be single basic and therefore of low pathogenicity.
11. All PR8 backbone viruses will be attenuated for replication in humans due to the six internal genetic segments from the PR8 strain which has been shown be limited in human hosts (7, 8).
12. No known mutations to alter the susceptibility of the NA gene for the anti-influenza neuraminidase inhibitor class of drugs (e.g. Oseltamivir, Zanamivir and derivatives) will be introduced.

Validation of disinfectants
1% Virkon with a contact time of 10 minutes and 5% Distel for 5 minutes have been validated against various strains of influenza virus or are approved by DEFRA as a method of disinfection for avian influenza viruses (a) (b) (c).
(c) Q-Pulse document: AWW-VALID-01- Disinfectant validation report – Avian influenza

Is an emergency plan required according to regulation 20?  [N]
If yes, tick to confirm that it is attached to this form [N]
Biological Agents and Genetically Modified Safety Committee (BAGMSC) authorised risk assessment attached.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

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Project Ref 53/19.1

Date Ackn'd: 13/03/2019

CU2 Project Title: Connected program of work - CPW no. 4.3: Genetic manipulation of herpesviruses

Date Project Ceased:

Consent Granted

Non-GMM

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements

Project Additional Information

02/03/2022
Purposes of the contained use

The purpose of this document is to describe projects involving genetically modified animal herpesviruses at The Pirbright Institute. The projects will involve: 1) use of animal herpes viruses as vaccine vectors to control animal viral disease, and 2) understanding pathogenesis of animal herpesviruses in their natural hosts. A list of the herpesviruses is provided in section 7 of this document.

Recipient or parental organism

Among the members of the subfamily Alphaherpesvirinae of Herpesviridae, at The Pirbright Institute, genetic modification of viruses is being conducted on Marek's disease virus (MDV; also known as gallid herpes virus 2, GaHV-2), MDV-2 (also known as MDV strain 2, MDV strain SB-I, MDV strain HPRS24 or gallid herpesvirus 3, GaHV-3), infectious laryngotracheitis virus (ILTV, also known as gallid herpesvirus 1, GaHV-1), herpesvirus of turkey (HVT, also known as Meleagrid herpesvirus 1), duck enteritis virus (DEV), psittacid herpesvirus 1 (PsHV-1, also known as Pacheco's parrot disease virus), perdicid herpesvirus 1, pheasant herpesvirus, stork herpesvirus, quail herpesvirus, bovine herpesvirus 1 (BHV-1) and equid herpesvirus 1 (EHV-1).

The only Betaherpesvirinae member which will used in GM related projects at the Pirbright Insitute is porcine cytomegalovirus (PCMV).

The only Gammaherpesvirinae member which will be used in GM related projects at the Pirbright Institute is bovine herpesvirus 4 (BHV-4).

The viruses mentioned above will be refered to as the parental organisms throughout this document.

Host/vector system

BHV-1 and BHV-4 mainly affect cattle. PCMV is only known to infect pigs. The host system for EHV-1 is horse. No positive serological results have been shown in samples from humans working with horses infected with EHV-1.

Domestic chicken (Gallus gallus domesticus) is considered as the host organism for GaHV-1, GaHV-2 and GaHV-3. Domesticated turkey (Meleagris gallopavo) is considered the host for MeHV. Anatidae species (ducks) are hosts for DEV. MDV is isolated from species other than domestic chicken such as partridges, european quails, ducks or geese.

There is no confirmed report indicating infection of MDV or other avian herpesviruses listed above in human. Stork herpesvirus was isolated from black storks (Ciconia nigra) and it has shown no serological cross reactivity with other avian herpesviruses. Pheasant herpesviruses affect pheasants, as their name implies.

E.coli strains (mainly strain DH10Beta, its derivatives and in some cases strain DH5alpha or B strain derivates) will be used for cloning and/or genetic manipulation of the parental viruses. These strains are avaliable commercially and are considered as non-pathogenic strains widely. In addition, E.coli Strains EL250, EL350 and SW102 are derivatives of E.coli strain DH1 OB. These strains contain a defective " prophage with recombination proteins exo, bet and gam.

Origin & function

Bacterial artificial chromosome (BAC) technology and/or CRISPR/Cas9 method will be employed to generate genetically modified microorganisms (GMMs) from the parental organisms (listed above). The inserted gene products will be heterologous antigens, such as a surface or internal viral protein. We will also consider insertion of cytokines such as IL 18 that may result in a better immune response to the vaccine. Individual risk assesments will be required in such occasions. Genetic materials for the vaccines will be chosen from a wide range of sources and for different purpouses (explained below):

1- For vaccine studies and expression of heterologous antigens of other viral origins: parental organisms will be used to deliver antigens selected from pathogen of the hosts which are listed in the section 7. For example, GaHV-2 will be
used to deliver antigens from sources such as avian influenza viruses (e.g. HA and/or NA glycoproteins of AIV), infectious bursal disease virus (e.g. VP2 protein), ILTV (e.g. glycoproteins 0, I or B), infectious bronchitis virus (e.g. spike protein), MDV (e.g. glycoprotein B), Newcastle disease virus (e.g. F glycoprotein). Similarly, BHV-4 will be employed as a multispecies vaccine to deliver heterologous antigens from sources such as Nipah virus (NiV). PCMV will be used as a porcine vaccine vector to deliver heterologous antigens from sources such as porcine reproductive and respiratory syndrome viruses.

2- Vaccine studies and expression of immunomodulatory genes to boost the immune response to the vaccines. More specifically, studying the effect of IL18 in enhancing immune response in GaHV-2 or GaHV-3 origin vaccines delivering gO, gI or gB antigens from ILTV. In such cases, individual risk assessments will be performed to ascertain the safety of the vaccines.

3- Mutagenesis techniques or deletions of specific genes (such as gB, Meq’ or pp38 of MDV, or Bc12 of HVT) to study the effect of the genes on the parental viruses entry, replication, egress, pathogenesis and/or oncogenicity.

4- Exchange of genes from two related viruses (such as exchange of HVT origin gE, gI and/or gM with their counterpart from MDV) to study the effect of the glycoproteins in cell free virus production.

Evaluation of foreseeable effects

Evaluation of any foreseeable effects on human health:
MDV, GaHV-2, GaHV-3, DEV, PsHV-1, perdicid herpesvirus 1, pheasant herpesvirus, quail herpesvirus and stork herpesvirus are not pathogenic in human. It is not expected genetic manipulation of the viruses would expand the tropism of the viruses to humans.

There is no evidence that BHV-1, BHV-4 or PCMV can infect humans. Sera from naïve humans contain natural antibodies that efficiently neutralize BHV-4, which may provide an innate immune mechanism preventing crossspecies transmission of BHV-4 to humans. No positive serological results have been shown in samples from humans working with horses infected with EHV-1. It is not expected to see a higher level of pathogenicity in BHV-1, BHV-4, PCMV and EHV in their host or in human.

Any mutation or gene expression in the context of the viruses which may result in a change in viral tropism is outside of the scope of this document. In such cases, a separate risk assessment will be required.

Evaluation of any foreseeable effect on the environment:
Avian herpesviruses such as MDV and HVT do not infect and replicate in mammals, as a result the only potential hazard is to poultry. HVT and GaHV-3 are widely used as vaccines against MDV and as vaccine vectors to deliver antigens from other viral pathogens to chicken safely. It is not expected to see a higher level of pathogenicity in HVT or GaHV-3 delivering a heterologous antigen, as the fitness of the recombinant viruses are usually lower than that of their parental virus. It is also not expect to see a change in tropism of the viruses due to insertion of heterologous antigens. Majority of the heterologous antigens are usually localised across the cytoplasmic membrane and herpesviruses have a nuclear maturation cycle. As a result, the risk of changing the parental virus tropism due to inclusion of the antigens in the mature herpesvirus particles is very minimal. The only concern is with expression of ILTV gO/1 or gB in the context of GaHV-2 or other avian herpesviruses. It is likely that the recombinant GaHV-2 would carry IL TV gO/1 or gB in its viral particle. However, considering that both viruses are viruses of poultry with a tropism to the respiratory system, it is unlikely to see a change in the virus pathogenicity, tropism and/or widening its host range. In addition, gB from ILTV are used safely in a fowlpox system (001: 10.1080/03079450120044542) to vaccinate chickens against ILTV. As it was noted earlier, it has been reported widely that alterations to MOV, HVT or GaHV-3 genome to insert antigens from other viruses usually is associated with a reduction in the fitness 01 the parental virus which results in further attenuation 01 the recombinant virus vectors. Delivery 01 antigens with GaHV-3, HVT or attenuated vaccine strain of MOV is shown to be safe, it is used commercially worldwide. It is expected that delivery of gB or gOlgl using GaHV-3 will be safe too.

The vaccine vectors will not be used to express any oncogenes or toxins. However, usage 01 cytokine genes such as
IL-18 that may result in an enhanced immune response to the vaccines will be considered. IL-18 cytokine has been used safely in fowlpox virus vector vaccines (00110. 1016~ .vaccine .2006.03 .006 and 10.1111/j.1574-695X.2011 .00850.x). Chicken immunised with FPV expressing IU8 and heterologous antigens, have shown a superior immune response to the vaccine compared to the control group. It is also thought the expression of IL-18 helps chicken to overcome the toxico-side effects 01 FPV (10.1 016/j.vaccine.2006.03.006). Although, there is no report indicating expressing IL8 using an avian herpesvirus, there are records reporting safe usage 01 HVT in chicken delivering different cytokines including IFN-gamma. Considering the relation between IU8 and IFN-gamma and based on the previous reports, we are not expecting to see any adverse effects in GaHV-3 vaccines delivering chicken IU8 and ILTV gB or gOli.

Usage 01 OEV, PsHV-l, perdicid herpesvirus 1, pheasant herpesvirus and stork herpesviruses will be limited to deliver heterologous antigens to their natural hosts. Unfortunatley, there has been a lack 01 information regarding the above viruses. However, based on our experience with them, we are not expecting to see a higher level 01 infectivity in their recombinant compared to that 01 their parental strains. Work involving GMO derived MOV, GaHV-2, GaHV-3, MeHV, ILTV, OEV, PsHV-l, perdicid herpesvirus s 1, pheasant herpesvirus, quail herpesvirus and stock herpesvirus will be performed in ACGM containment level 2 facilities.

BHV-l, BHV-4, EHV-I and PCMV:

BHV-I causes infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis in cattle. BHV-I can infect sheep, goats and pigs, but without causing disease. BHV-4, in contrast, causes no illness in cattle. BHV-4 can infect goats, but not sheep or pigs. BHV-I and BHV-4 are found in cattle all over the world and are not mentioned in animal health orders, except in Oenmark and Switzerland that have eradicated BHV-I. Cattle can become latently infected with BHV-I and BHV-4. However, BHV-I gE and gI deletion mutants are not pathogenic in their natural host, cattle, do not appear to establish latent infections and are being considered as live vaccines. The same considerations 01 the effects of foreign viral or cytokine genes on the pathogenicity 01 BHV-I lor animals other than cattle, apply as lor humans. BHV-I is a labile virus and is spread by direct contact rather than airborne transmission. Virus is therefore unlikely to survive lor any length 01 time in the environment. Insertion of foreign viral or cytokine genes into attenuated BHV-I may confer a selective advantage in infected animals in the wild, therefore all infected material, including animals, have to be properly contained and disposed 01. Recombination between modified BHV-I and another bovine herpesvirus is low as the homology between the two viruses would be too low to support a recombination event. Although expression olloiregional viral genes may increase the virulence 01 gE or gl-deleted mutants 01 BHV-I for cattle, the virulence 01 the recombinants is unlikely to be greater than that 01 wild-type BHV-I. Since expression 01 certain bovine cytokine genes may increase the virulence 01 the recombinant virus lor cattle, all animal experiments will be done under high containment so that the potential 01 infection from experimental animals should be negligible. The hazard 01 recombinant BHV-4 vectors to humans and to the environment is extremely low and the risk is no more than that 01 the wild type virus. The safety 01 BHV-4 viral vectors has been demonstrated in a range 01 mammalian species. BHV-4 viral vectors are highly attenuated in species other than cattle. In non-bovine species, BHV-4 does not establish a viraemia or result in virus shedding. Rather BHV-4 vectors show a highly attenuated, transient replication localized to the site 01 inOCUlation and are incapable 01 spreading to other animals. As a member 01 the enveloped Herpesviridae family, BHV-4 and other viruses listed in this document are expected to possess low environmental stability and direct contact between cattle is typically necessary for spread. The hazard of BHV-4 viral vectors to the environment is extremely low and the risk is no more than that 01 the wild type virus.

EHV-I is a pathogen of equine species and BHV-I is known to cause several disease worldwide in cattle including respiratory disease, abortion, genital disease, or occasionally encephalitis. Glycoprotein E and I deleted EHV-I recombinant viruses has been used as vaccine candidates. Oeletion 01 gE in BHV-I was associated with attenuation
01 the virus. Glycoprotein I or glycoproteins II deleted BHV-I was used as candidate vaccines. It can be concluded both gE and/or gl deleted EHV-I and BHV-I are associated with the viruses pathogenicity. Hence, the risk of gE/gl deleted viruses to the environment will be minimal.

PCMV is highly species-specific and is ubiquitous within its pig host, where infection is generally subclinical. PCMV infects the nasopharyngeal region of the upper respiratory tract and similar to all herpesviruses establishes a latent/persistent life-long infection. Recombinant PCMV are expected to possess low environmental stability and direct contact between pigs is typically necessary for spread. The hazard of recombinant PCMV viral vectors to the environment is extremely low and the risk is no more than that of the wild type virus.

Any work related to genetically modified organisms related to BHV-I, BHV-4, PCMV and EHV discussed in this section will be assigned to ACGM containment level 2. No specific containment under SAPO is required.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In vitro and in vivo work with recombinant organisms listed in this document (ACGM class II) will be performed in the containment level II facilities. The following measures will be implemented for safely management of waste:

In vitro ACGM class 2 work with GMs listed in this document will be performed in compliance with the SACGM compendium of guidance:

1. A lab coat and gloves will protect the operator from skin contact with GMMs.
2. All in vitro work with live virus will be carried out in a class II microbiological safety cabinet (MBSC).
3. A validated disinfectant such as 1 % Virkon S [Dupont], Trigene or 0.5% FAM 30 [Evans Vanodine] will be used to disinfect any exposed surfaces. Both are highly effective virucidal disinfectants.
4. Any solid waste, culture vessels or exposed disposable equipment needs to be autoclaved at 120°C for 30 minutes.
5. Any liquid waste will be disinfected appropriately using a validated disinfectant (1 % Virkon for 15-30 minutes).
6. Only authorised personnel will have access to the GMMs.
7. Accidental spillages of liquid waste will be deactivated with 2% Virkon S or 1 % FAM 30; the use of double strength disinfectant will ensure that it is not diluted past the effective concentration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All agreed and accepted by BAGMSC Committee with final approval 08/01/19.
Project Additional Information

Purposes of the contained use

The Pirbright Institute is a research institute focusing on preventing and controlling viral diseases, especially of livestock but also zoonotic and human pathogens. Much of this work can be conducted in various types of cultured cells, in keeping with our commitment to replace, reduce and refine the use of animals in research. Contained use in this respect relates to addition/modification of genetic material to cultured cells of animal origin.

Recipient or parental organism

The cultured cells covered by this application are not viable except in cell culture. In some very special cases, e.g.
mouse embryonic stem cells, the cells could be recovered into a whole-animal in vivo form (mouse); such recovery and its products are not included. Human pluripotent stem cells (human embryonic stem cells (hESC) or human induced pluripotent stem cells (human iPSCs) are also excluded.

**Host/vector system**

The exogenous nucleic acid may be inserted into the cell using chemical or physical means, e.g. chemical transfection agents (calcium phosphate, Lipofectin or other proprietary systems) or physical methods such as electroporation. Viral vectors are not covered by this risk assessment.

**Origin & function**

Genetic inserts/genes - Expressed genes under this RA must not encode known toxins, e.g. Schedule 5 toxins such as ricin. Genetic material inserted into cells must not be infectious (i.e. capable of reproducing a whole pathogen), this is particularly relevant when cloning the genome of positive sense RNA viruses. When transfection of genetic material representing viral genomes is undertaken (including DNA or RNA versions) no more than 80% of the genome can be encoded in aggregate between the DNA/RNA molecules used in a single transfection and care should be taken that no overlap of concurrent work where the whole genome might be in contact occurs (recombination). Examples of nucleic acids considered within this Risk Assessment include protein-coding genes from other organisms, plasmids, non-coding RNAs such as guide RNAs, hairpin RNAs, synthetic DNA, non-coding RNA (all with the exclusions noted above and below, e.g. non-infectious).

Gene editing - it is not entirely clear under current legislation (as of Jan 2019) whether products of gene editing that do not incorporate exogenous nucleic acid are to be considered GM under UK legislation (“mutagenesis” exemption, Part 3(a) of Schedule 2 of The Genetically Modified Organisms (Contained Use) Regulations 2014, Guidance on Regulations, Schedule 2, Part 3(a)) or, for genetic material derived from the same or similar species “self-cloning” exemption at Part 3(b)). Conservatively, animal cells that are such products are included in this risk assessment as their risk profile is considered similar to other animal cells with modified genetic material; this does not imply a position as to whether any such cell or cell line is or is not a GMO under relevant legislation.

**Evaluation of foreseeable effects**

GMOs are cultured cells with additional/modified genetic material. Cultured animal cells are unable to survive or replicate outside an artificially-maintained tissue culture environment and pose negligible risk to the environment. In some cases, e.g. mouse embryonic stem cells, the cells can be recovered into a whole-animal form (mouse); such recovery and its products are not included in this risk assessment. Animal cells, including human cancer-derived cells ex vivo, are not known to pose significant hazards to human health. Cells may harbour animal pathogens or other environmental hazards and may therefore need to be handled at higher containment levels or with cell-type specific mitigations (but cells known or likely to harbour group 3 or group 4 agents are excluded from this Risk Assessment).

Genetic material used under this risk assessment must not encode for known toxins, e.g. Schedule 5 toxins, or infectious material. For genetic material encoding other potentially hazardous molecules, such as immune modulators, growth factors, allergens, hormones or oncogenes, consideration must be given as to whether exposure could be sufficient to cause harm to human health or the environment - this is a priori considered unlikely for the cells but may affect assessment of downstream processes, e.g. purification of such molecules from the cells or tissue culture supernatant.

The genetic inserts will not be known toxins. The genetic inserts will also not be capable of producing an infectious pathogen. The genetic inserts will be unable to be passed to bacteria as the vectors are non-mobile or mobilisation defective. The GM cells/organisms are considered to be no more harmful to human health or the environment than the unmodified form. Exposure to large quantities of cells, tissue culture media or gene products, e.g. by ingestion,
could conceivably cause harm to human health, this is appropriately mitigated by good laboratory practice, e.g. as per applicable building and laboratory codes of practice, appropriate to the unmodified form.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| not applicable (no larger GMOs included) |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| N/A |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The GMOs - cultured animal cells - are not viable outside special culture conditions, e.g. tissue cell culture media, controlled temperature, etc. For disposal, all viable material will be rendered inviable by physical or chemical means, e.g. autoclaving 134°C 30 minutes or using 2% Virkon for the required contact time. Where possible disposable consumables will be used. Contaminated disposables are placed in waste bags which are sealed, placed in labelled metal tins and transferred to Central Services Unit for autoclaving and disposal. Spillages and contaminated surfaces will be treated with a validated disinfectant, e.g. 2% Virkon, and wiped with absorbant tissue until dry; said tissue disposed as disposable consumables. Non-disposable items will be soaked in 1% Virkon for the required contact time and autoclaved.

**Project Containment**

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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Approved by BAGMSc April 2019.
Project Ref 53/trans6

Date Ackn'd

CU2 Project Title

TRANSIENT EXPRESSION OF BLUETONGUE VIRUS RNA AND PROTEINS USING RECOMBINANT VACCINIA VIRUS

Class

Class 2

CultureVolClass2 CultureVolumeClass3-4

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

N

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
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Animal Units

Large Scale Activities

Human Clinical Applications

**Project Ref** 94/trans2

<table>
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<tr>
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<tr>
<td></td>
<td>EXPRESSION OF FOOT AND MOUTH GENES USING RECOMBINANT VACCINA VIRUS VECTORS</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
Significant Change ID
Date of Significant Change

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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<tr>
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<tr>
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<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
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</table>

**Please enter comments on the GM safety committee on the risk assessment**

**Project Containment**

02/03/2022
The purpose of our projects is to increase our understanding of the viral determinants for host range, transmission and pathogenesis of influenza A viruses (IAV) and to develop tools that could enhance control of influenza viruses in poultry, humans and swine.
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? □

If yes, tick to confirm that it is attached to this form □

Tick to confirm that you have attached a risk assessment to this form □

Tick if you are claiming exemption from disclosure for section of the risk assessment □

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
<td>L2</td>
<td>L3 L4 L2 L3</td>
<td>L3 L4 L2 L3</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4 L2</td>
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Project Ref 97/00.4

Date Ackn'd 02/03/2022

CU2 Project Title

Class CultureVolClass2 CultureVolumeClass3-4
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>Characterisations of Human &amp; Bovine Respiratory Sincytial Virus (BSV)</td>
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Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  [N]

If yes, tick to confirm that it is attached to this form  [N]

Tick to confirm that you have attached a risk assessment to this form  [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment  [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref**  97/00.7  

**Date Ackn'd** 01/02/1998  

**CU2 Project Title** LARGE DNA VIRUSES - VACCINIA VIRUS  

**Class**  

**CultureVolClass2**  

**CultureVolumeClass3-4**  

**Non-GMM**  

**Consent Granted** Not Applicable  

**Project notified under transitional arrangements** Y  

**Historical Significant Changes** Previously 97/98.7 Transferred from GM97 28/07/2015  

**Historical Date of Additional Info**  

**Significant Change ID**  

**Date of Significant Change**  

### Project Additional Information

#### Purposes of the contained use

#### Recipient or parental organism

#### Host/vector system

#### Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

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Animal Units

<table>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tr>
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Project Ref 97/01.1

Date Ackn’d 18/01/2001

CU2 Project Title CPN 7E IMMUNOLOGY - CYTOKINES

Class 2

Class CultureVolClass2 CultureVolumeClass3-4

Class 2
Date Project Ceased

Withdrawn  N

Historical Significant Changes
Transferred from GM97 28/07/2015

Project notified under transitional arrangements  Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 97/01.3

- **Date Ackn'd**: 18/01/2001
- **CU2 Project Title**: CPW7A IMMUNOLOGY OF FARM ANIMALS - GENERAL
- **Class**: Class 2
- **Non-GMM**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: Y
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: Transferred from GM97 28/07/2015
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**: 02/03/2022

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref** 97/01.6

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- **Non-GMM** Consent Granted: Not Applicable
- **Project notified under transitional arrangements**: Yes

**Withdrawn**: No

**Tick if notifying a connected programme of work**: No

**Historical Significant Changes**: Transferred from GM97 on 06/01/2016

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? ❌

If yes, tick to confirm that it is attached to this form ❌

Tick to confirm that you have attached a risk assessment to this form ❌

Tick if you are claiming exemption from disclosure for section of the risk assessment ❌

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 97/01.8

Date Ackn'd 06/01/2016

CU2 Project Title CPW4.3 LARGE DNA VIRUSES -HERPES VIRUS

Class 2

CultureVolClass2

CultureVolumeClass3-4
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<td>Purposes of the contained use</td>
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<td>Recipient or parental organism</td>
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<td>Origin &amp; function</td>
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<tr>
<td>Evaluation of foreseeable effects</td>
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<tr>
<td>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</td>
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Project Containment

Laboratory Activities  Glass Houses  Growth Rooms
L2  L3  L4  L2  L3  L4  L2  L3  L4
L2  L3  L4  L2  L3  L4  L2  L3  L4
Animal Units  Large Scale Activities  Human Clinical Applications
L2  L3  L4  L2  L3  L4  L2  L3  L4

Project Ref  97/03.1

Date Ackn’d  26/03/2003
Date Project Ceased

CU2 Project Title  CHARACTERISATION OF CHIMERIC BOVINE RESPIRATORY SYNCYTIAL VIRUS (BRSV) WITH GENE SUBSTITUTIONS FROM HUMAN RESPIRATORY SYNCYTIAL VIRUS (HRSV)

Class CultureVolClass2  CultureVolumeClass3-4
Class 2  < 1 Litre

Non-GMM  Consent Granted
Not Applicable

Withdrawn  N
Tick if notifying a connected programme of work  N

Historical Significant Changes  Transferred from GM97 28/07/2015
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
### Purposes of the contained use

In order to investigate the relative contribution of RSV proteins to the host range phenotype of RS viruses, the replication of recombinant BRSV, in which the surface glycoproteins or the non-structural genes have been replaced either alone or in combination by those from HRSV, will be investigated in different bovine and human primary cell cultures and cell lines. In addition, the replication of these chimeric viruses in cattle and mice will be investigated.

Characterisation of the role of different RSV proteins in determining the host range phenotype of RS viruses may lead to the development of a small animal model of BRSV and/or the development of attenuated RSV vaccines for man and cattle.

### Recipient or parental organism

Bovine respiratory syncytial virus.

### Host/vector system

Bovine RSV in which the F, G, SH, NS1 and/or NS2 genes have been replaced with those from human RSV.

### Origin & function

Bovine RSV in which the F, G, SH, NS1 and/or NS2 genes have been replaced with those from human RSV have been made by collaborators.

The F protein mediates virus attachment and penetration of cells.

The G protein mediates virus attachment and may also have other, as yet unidentified, functions.

The function of the SH protein is not known.

The NS proteins are involved in aspects of virus replication, in resistance to the antiviral effects of type I interferons, in regulating the induction of type I interferons and may also have other, as yet unidentified, functions.

### Evaluation of foreseeable effects

HRSV is a major cause of respiratory disease in young children and BRSV causes a similar disease in young calves. Although closely related, with greater than 80% amino acid identity for 8 of the 11 proteins, BRSV and HRSV display a restricted host range in vivo. However, HRSV and BRSV have an overlapping host range in cell culture, in vitro. Replacement of BRSV genes by genes from HRSV may alter the host range phenotype of the virus. Thus the chimeric BRSV may be able to infect humans, chimpanzees, cotton rats and/or mice and may replicate more efficiently in primate cells than the parental BRSV. It is possible that some of the chimeric BRSV may replicate less efficiently than the parental virus in some cells or animals. If replication is poor the chimeric virus may adapt and evolve giving rise to virus with altered pathogenicity.

HRSV is ubiquitous in the human population, the majority of whom have experienced one or more HRSV infections and therefore have some pre-existing immunity. HRSV infection in adults is usually restricted to the upper respiratory tract. However, HRSV infection in young infants, the elderly or in immunosuppressed individuals can be severe. Similarly infection with BRSV is widespread in the cattle population and reinfection is usually restricted to the upper respiratory tract. If chimeric rBRS viruses were to infect an adult, pre-existing immunity to the F protein of HRSV, which is the major protective antigen of the virus, is likely to limit the replication of the chimeric virus. Thus there is only 19% amino acid difference between the BRSV and HRSV F proteins and neutralising monoclonal antibodies specific for the F protein recognise both the HRSV and the BRSV F proteins. These anti-F mAbs protect against experimental HRSV infection in mice, HRSV in children and BRSV infection in calves. Bovine sera will neutralise HRSV and human convalescent sera neutralise BRSV. However, the neutralisation titres are higher against homologous viruses. Intramuscular injection of cattle with HRSV will protect against BRSV infection and infection of cotton rats with BRSV will protect against HRSV infection. Infection of adults with the GMM is therefore unlikely to produce disease any more severe than a cold. In contrast, infants and immunosuppressed individuals are at risk of developing bronchiolitis and pneumonia if they became infected.

The chimeric viruses may be more hazardous to humans than wild-type BRSV, but are unlikely to be as hazardous to humans as wild-type HRSV.
Since the organisms will be handled as ACDP category 2 pathogens for all experiments, the likelihood of environmental exposure is small. Furthermore, RS viruses do not replicate in the environment outside of the host animal and would not be expected to survive for long in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

RS viruses are labile viruses and are readily inactivated. All contaminated or potentially contaminated category 2 laboratory waste is autoclaved or incinerated (100% kill).

Pipettes and similar objects are disinfected, and surfaces are wiped, with hypochlorite solution or 70% ethanol (100% kill). Waste animal bedding and small animal boxes will be autoclaved (100% kill). Large animal rooms will be fumigated with formaldehyde (100% kill). Animal carcasses will be incinerated (100% kill).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Initially, the proposed genetic modification work was brought to the attention of the Genetic Modification Safety Committee in the form of an amendment to be considered as part of a previously notified CU2 project in 2000 (re-notified under transitional arrangements in 2001) but the Committee felt this was inappropriate and had requested to the project proposer that a new full risk assessment must be carried out and suggested some amendments. This was reviewed by the Committee and is the risk assessment attached here.

Project Containment

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</table>
As part of the current programme on immunity to TB in cattle, we will evaluate immune responses and protective efficacy of recombinant adenovirus expressing antigen (Ag) 85 of Mycobacterium bovis. The objectives of the project are:

1. To evaluate the immune responses elicited in cattle by the recombinant human adenovirus type 5 expressing Ag85A (Ad85A) of M. bovis.

2. To evaluate the use of recombinant the Ad85A for in vitro stimulation of T-cells.

No construction of vector will take place at IAH as the GMO will be supplied by our colleagues at Veterinary Laboratory Agency. E.coli DH5alpha or equivalent.

Recipient or parental organism

Recombinant, replication deficient human type 5 adenovirus.

The vector is pACCMV or equivalent, such as pjW24. This is a widely used shuttle vector for producing recombinant adenoviruses.

Host/vector system


Recombinant DNA will be placed between the cytomegalovirus promoter and the SV40 poly(A) sequence.

Origin & function
Mycobacterial DNA encoding Antigen 85A (Ag85A). The gene encoding Ag85A will be inserted into the adenovirus vector mentioned above for expression in mammalian hosts for expression and immunization.

Evaluation of foreseeable effects

Recombinant Ag85A expressed in adenovirus vectors will induce Ag85A specific immune responses in immunised hosts. This construct has been shown to elicit immune responses in cattle (Vordermeier, H.M. Infect Immun 74:1416-1418).

Due to its attenuation, no new virus particles will be produced in the host. Ag85A has no known toxic effects, it is not a known virulence gene and it is not expected to confer replication competency to the adenovirus vector.

The adenovirus to be used in these experiments is human type 5, which is a double-stranded DNA virus. Wild-type, unattenuated adenovirus most commonly causes self-limited infections in the respiratory tract, causing the "common cold" and the gastrointestinal tract, causing diarrhoea.

Wild type adenovirus have been safely used as oral vaccines to prevent adenoviral infections to more than 10 million people and is also being given to military personnel in the USA and Canada (Rubin B.A. and Rorke, L. B. Adenoviral vaccines. In: Plotkin & Mortimer (Eds). Vaccines. (1988) W.B. Saunders. Philadelphia, pp 492).

The vector to be used in these experiments will have deletions in the E1 and E3 regions of the adenoviral genome and has been described by Wang, J. et al. J Immunol 173:6357-6365. In brief, the Ag85 sequence is in frame downstream of human tissue plasminogen activator signal peptide, which in turn is downstream of the murine cytomegalovirus promoter. 3' of the Ag85 sequence will be the SV40 poly (A) signal. The E1 region is essential for replication and propagation and its removal renders the virus replication deficient. Deletion of the E3 region is not essential for viral growth, but its removal allows the insertion of guest genes in this region. (Trappe, B. Adenoviral vectors for gene transfer. Advance Drug Delivery Rev. (1993). 12:185.; Bramson, J.L. et al. The use of adenoviral vectors for gene therapy and gene transfer in vivo. Curr. Opin. Biotech. (1995). 6:590).

The recombinant adenoviral vector has been evaluated in a number of clinical trials and found to be safe. The safety and efficacy data from both clinical and pre-clinical studies using recombinant adenoviral gene transfer vectors have widely been reported (Zhang, W. W. Development and application of adenoviral vectors for gene therapy of cancer. Cancer Gene Ther. (1999). 6:113).

The main hazard with adenovirus would be infections of humans and other animals, but no detrimental effects are expected on either. Exposure monitoring by eg. Serological testing of animal carers, etc. is not necessary. These tests would be unable to distinguish between wild type viruses and the attenuated virus used in this study. Wild type adenovirus is a common cause of mild upper respiratory infections worldwide and many of us will already have seroconverted. The hazard is also minimal given the replication deficiency of the construct. We intend to reduce even this minimal risk by working with the construct at ACGM level 2.

Given the replication deficiency and the containment level at which we intend to work, the likelihood of release of the recombinant vector to the environment is effectively zero. There is no production of new viral particles in vaccinated hosts.

All work under this proposal will be carried out at HG 2 containment. No additional hazards over those of wild type adenovirus are foreseen. Cattle inoculated with recombinant adenovirus will be housed in appropriate category 2 containment facilities at all times during the experiment.

Accordingly this proposal seeks approval for a confirmed containment level 2 for work to be carried out under this proposal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Waste materials generated by these experiments will be inactivated mainly by autoclaving at 121°C for 30 min and/or by incineration. Where necessary, materials will be soaked with 1% solution of Virkon for a minimum of 20 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
None.

Project Containment

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Project Ref 97/09.2

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02/03/2022
# Project Additional Information

## Purposes of the contained use

The aim is to assess the potential of new vaccination strategies in protection against bovine TB. Prime-boost strategies utilising MVA expressing mycobacterial antigens have been used in human studies and show enhanced immunogenicity compared to conventional vaccines (McShane et al, Nature Med 2004)

Cattle will be vaccinated with BCG (prime) and boosted at defined intervals with MVA expressing immunodominant antigens from Mycobacteria. Immune responses will be assessed in vaccinated cattle to assess vaccine efficacy. This vaccination strategy is expected to induce broad protective immunity.

## Recipient or parental organism

Modified vaccinia virus ankara (MVA) expressing M. tuberculosis antigens will be produced by infection of permissive chick embryo fibroblast cell and transfection with a shuttle vector containing the recombinant genes plus a marker gene (green fluorescent protein). The shuttle vector recombines with the viral genome inside cytoplasm of the cell. The MVA viruses will be manufactured at Jenner Institute Vector Core facility at Oxford University.

## Host/vector system

Modified Virus Ankara (MVA) is an attenuated vaccinia strain that, by extensive passage in chick embryo fibroblasts, has had multiple regions of the genome deleted. This strain has been demonstrated not to replicate in a range of primary human cells and mammalian cells, but has recently been shown to replicate in a baby hamster kidney cells. Genes from M. tuberculosis will be expressed in a shuttle vector under the control of the MVA 7.5 promoter.

## Origin & function

Mycobacterial DNA encoding Ag85A, TB9.8, TB10.4 and Acr2. The genes have been cloned at the Jenner Institute, Oxford.

The proteins to be expressed (Ag85A, TB9.8, TB10.4 and Acr2) are immunodominant proteins produced by Mycobacteria species during in vivo infection, these are major targets for T cell recognition.

The aim is to test the recombinant MVA expressing these proteins as part of vaccination strategies in cattle: by incorporating immunodominant proteins into the vaccine the protective T cell response should be enhanced leading to immunity from infection
Evaluation of foreseeable effects

MVA is highly attenuated and can only form infectious particles in permissive avian cells.

MVA expressing mycobacterial antigen 85A has been used in phase II human vaccine trials and has an excellent safety record (Hawksridge et al, J Infect Dis 2008).

No infectious particles can be generated in human or animal cells and therefore MVA cannot cause infection or disease.

MVA cannot disseminate in the environment due to its replication deficiency.

None of the expressed genes are oncogenic or toxic and are not expected to increase virulence of MVA

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory procedures: blood samples from MVA immunised cattle will be handled at containment level 2 within class II microbiological safety cabinets according to IAH vaccinia virus rules. Liquid waste will be treated overnight with 2% virkon prior to disposal into drains. Solid waste (plasticware) will be autoclaved (121°C, 30 min) prior to incineration. The safety cabinets will be disinfected with 2% Virkon wipe down at the end of any experimental procedure.

Animal housing procedures: animals will be housed at containment level 2 in locked accommodation with restricted access. All personnel handling MVA immunised animals will be required to wear protective accommodation with restricted access. All personnel handling MVA immunised animals will be required to wear protective clothing and gloves, which will be disinfected by wash down with 2% virkon following contact with animals. Walls and floors within the animal accommodation will be scrubbed with 2% virkon solution (minimum 10 minutes); wash down will be into drains with traps which contain 2% virkon (held for minimum of 10 minutes). Solid waste will be covered with 2% virkon for > 30 minutes prior to removal for incineration. All animals will be culled at the end of the experimental period and the carcasses incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This assessment has been reviewed and passed by the IAH Compton BAGMSC

Project Containment
The determinants of replication, transmission and pathogenicity of avian influenza viruses

The purpose of these experiments is to increase our understanding of the viral determinants for host adaptation, increased transmissibility and virulence of influenza viruses (AI) of poultry and mammalian species. This may lead to improved vaccines and better control measures against avian influenza infections in poultry and hence reduction of the viral load in the environment. We will use the reverse genetics system to produce recombinant influenza viruses derived from wild-type animal viruses to define which polypeptides, combination of polypeptides or particular amino acid residues are responsible for increases in either virus-host interactions, increased virus attachment and replication of viruses in different avian or mammalian cells and in animal models.

Recombinant low pathogenicity influenza viruses will be generated by transfecting eukaryotic cells (avian or mammalian) with the plasmids pHW 2000 or pHWScdB containing PB2, PB1, PA, HA, NP, NA, M and NS genes from low pathogenicity influenza A viruses, specifically:
(1) A/chicken/Italy1279/00 (H7N1)
(2) A/chicken/Pakistan/UDL-1/08 (H9N2)
(3) A/turkey/Turkey/1/05 (H5N1). The HA gene derived from this virus will be modified to delete polybasic amino acids at the cleavage site. The GMP construct will have a
mono basic HA cleavage site identical to that present in the low pathogenicity phenotype of H5N1 avian influenza viruses. (4) A/PR/8/34. This virus will be used as recipient of HA, NA and PB2 genes derived from the H7N1 and H9N2 and H5N1 viruses described above (number 1-3).

<table>
<thead>
<tr>
<th>Host/vector system</th>
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<tbody>
<tr>
<td>cDNA clones will be made in E. coli and plasmid DNA containing influenza virus genes will be transfected into cells of eukaryotic origin (avian or mammalian) to rescue influenza virus</td>
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<thead>
<tr>
<th>Origin &amp; function</th>
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<tbody>
<tr>
<td>The recombinant viruses produced will be used in tissue culture and animal models to study the effect of specific changes in their replication capacity, pathogenesis and transmission.</td>
</tr>
<tr>
<td>Alterations in amino acids will be introduced by site directed mutagenesis in the genes that encode polypeptides of haemagglutinin, neuraminidase and polymerase (PB2) genes. These will mimic strains that have emerged during the adaptation of avian influenza viruses found in waterfowl which act as reservoir for viruses affecting poultry.</td>
</tr>
<tr>
<td>As described in the attached risk assessment, the changes in the HA gene will be restricted to those observed in the field isolates. These include the variation in the potential glycosylation sites in the HA and deletion of amino acids in the stalk length of the NA gene.</td>
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<tr>
<th>Evaluation of foreseeable effects</th>
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<tr>
<td>The characteristics that we plan to introduce are those observed in viruses isolated from field outbreaks in poultry and are not foreseen to be any more hazardous than parental strains; thus we propose to handle these viruses in the same way as we handle the parental strains.</td>
</tr>
<tr>
<td>To minimise the risk of infection to workers, the engineered viruses will be worked on in a Class II Microbiological Safety Cabinet. Furthermore additional procedures will be used to ensure minimal risk to the operator during transfer from the cabinet to incubators, freezers and autoclaves. Individual workers will wear disposable gloves and appropriate protective clothing at all times when handling influenza viruses.</td>
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<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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<tr>
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**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Liquid waste containing infectious virus will be treated with 1% Virkon for a minimum of 30 minutes inside the Class II Microbiological Safety Cabinet (Class II-MSU), then a 50-100 ml maximum volume (in plastic flasks) will be placed into double autoclave bags (double autoclave bags will be made by inserting one autoclave bag inside the other one, this will create two protective plastic layers of the autoclave bags which further reduce the chances of any potential leakage of the contaminating material inside the autoclave bags) and into the designated metal tins, transferred to Microbiological Services (within the same building) and autoclaved at 135° C for 30 minutes prior to disposal by incineration. |
| Solid waste: Solid waste contaminated with infectious virus (plastic tips, flasks) will be immersed in 1% virkon for minimum of 30 minutes inside the Class II-MSU, then placed in double autoclave bags, put in a secure metal tin and autoclaved as described above and disposed by incineration. |
| Other solid waste such as eggs will also be disposed of by bagging in double autoclave bags within the ClassII-MSU, put in a secure metal tin and autoclaved as described above and disposed of by incineration. |
The autoclave is serviced quarterly and annually validated; records are kept and examined.

The Class II-MSU are tested and validated every six months during a scheduled maintenance period.

Animal waste in the animal facility (EAH corridor D) will be placed in the autoclave bags and sprayed with 1% virkon before being removed for autoclaving. They will then be autoclaved within EAH corridor D building before disposal by incineration.

The proposal has been extensively reviewed by the local Biological Agents and Genetic Modification Safety Committee and is now considered to be acceptable for notification.

Please enter comments on the GM safety committee on the risk assessment:
The proposal has been extensively reviewed by the local Biological Agents and Genetic Modification Safety Committee and is now considered to be acceptable for notification.

Project Containment

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Animal Units

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Project Ref 97/11.1

Date Ackn’d 17/10/2011

Date Project Ceased

CU2 Project Title Vaccine efficacy of recombinant Newcastle disease virus expressing respiratory syncytial virus fusion (F) glycoprotein

Class 2

CultureVolClass2 < 1 Litre

Consent Granted

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to evaluate immune responses and protective efficacy induced by recombinant Newcastle disease virus (rNDV) expressing either bovine (B) or human (H) respiratory syncytial virus (RSV) fusion (F) glycoprotein (rNDV-F) in calves. The rNDV has been produced by Dr*, Mt Sinai School of Medicine, New York, USA. The RSV F gene has been inserted between the P and M genes of the Hitchner B1 strain of NDV. Calves will be inoculated intranasally (i.n.) or i.n. and intratracheally (i.t.) with rNDV-F and analysed at intervals for excretion of NDV, induction of RSV-specific immune responses, and clinical signs of disease. Vaccinated calves will be challenged with BRSV to determine the effect of vaccination on BRSV replication in the nasopharynx and lungs, and the development of pulmonary pathology.

**Recipient or parental organism**

The Newcastle disease virus (NDV) to be used is the lentogenic, avirulent Hitchner B1 strain, which is a licensed live vaccine virus, available for use in the UK.

**Host/vector system**

NDV is an avian paramyxovirus serotype 1 (APMV-1), and belongs to the Avulavirus genus within the Paramyxoviridae family. NDV is classified as ACDP CL2 and has been reported to cause short-lived conjunctivitis and 'flu-like symptoms in man. Many of the reported instances of NDV infection in humans have been the result of direct inoculation into the eye, either by laboratory workers or those handling vaccines. Reports of 'flu-like illnesses associated with NDV are rare. Both virulent (mesogenic) and avirulent (lentogenic) strains of NDV appear to be equally able to cause conjunctivitis in humans. However, both mesogenic and lentogenic strains of NDV have been tested in man as oncolytic viruses and cancer vaccines with no adverse effects, signs of evolution towards human pathogenicity, recombination or transmission from human to human. Furthermore, virus could not be recovered from either the nose or tracheal lavage of African green monkeys that had been inoculated intranasally and intratracheally with a lentogenic strain of NDV expressing the HN protein of human parainfluenza virus-3 (HPIV3).

Lentogenic strains of NDV are used as live vaccines in poultry. The Hitchner B1 strain is not classified as a specified animal pathogen by Defra (see page 133 of [http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part2.pdf](http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part2.pdf)) and is licensed for use in the U.K. Statutory action to control NDV is taken only for strains which are classified as "virulent" ([http://archive.defra.gov.uk/foodfarm/farmanimal/diseases/vetsurveillance/profiles/documents/sp-newcastle-disease.pdf](http://archive.defra.gov.uk/foodfarm/farmanimal/diseases/vetsurveillance/profiles/documents/sp-newcastle-disease.pdf)). NDV does not appear to infect cattle and vaccine strains of NDV are highly attenuated in experimentally infected calves. Although, NDV has been isolated from pigs with an influenza-like illness, experimental inoculation of piglets with a strain of NDV that resembles the B1 strain did not produce any clinical signs of disease.

**Origin & function**

The RSV F protein mediates fusion of the virus envelope with the cell membrane and fusion of infected cells with adjacent uninfected cells. The F protein is the major protective antigen of RSV and does not contain any potentially toxic or oncogenic sequences. Inoculation of mice or cattle with eukaryotic expression plasmids expressing the RSV F gene under the control of the HCMV promoter does not result in any harmful effects. There is no evidence that the RSV F protein activates TLR4 and induces the production of pro-inflammatory cytokines. However, no adverse effects have been seen following vaccination of small laboratory animals or cattle with recombinant virus vectors expressing the F protein.
RSV and NDV are related viruses of the Paramyxovidae family and cause respiratory disease in their respective host. rNDV-F expresses the native NDV F and HN proteins, which are involved in viral attachment and invasion of cells, and does not incorporate the RSV F protein into the virion. Therefore, expression of the HRSV or BRSV F protein will not be expected to alter the host range or tissue tropism of NDV. Furthermore, a lentogenic strain of NDV expressing the HN protein of HPIV3, which is more closely related to NDV than RSV, was highly attenuated in the nose and lungs of African green monkeys that had been inoculated intranasally and intratracheally with high titres of the recombinant virus.

If accidentally released into the environment, rNDV-F could infect susceptible individuals, but is unlikely to cause disease in livestock or poultry. Thus, following i.n. and i.t. inoculation of calves with high doses of a lentogenic strain of NDV expressing the bovine herpesvirus-1 (BHV-1) gD protein, NDV was not isolated from nasal swabs of any of the animals and none of the animals developed clinical signs of disease, even when the gD protein was incorporated into the NDV virion. Furthermore, there is evidence that insertion of a foreign gene between the P and M genes of lentogenic strains of NDV attenuates the virus further for poultry. Thus, rNDV (Hitchner B1 strain) expressing an influenza virus HA gene is attenuated in embryonated chicken eggs, in contrast to the parent wild-type. Similarly following ocularnasal inoculation of 1-week-old SPF chickens with 10^6 EID50 of a lentogenic vaccine strain of NDV expressing influenza virus H5 HA gene, replication of rNDV-H5 was attenuated in the lungs and oropharynx compared with the control rNDV, rNDV-H5 was not detected in cloacal swabs, and there were no clinical signs of disease.

Recombination between single-stranded, negative-sense RNA (ssRNA(-) viruses such as NDV is rare. The ssRNA(-) genome of NDV is found exclusively in a RNAse-resistant nuclecapsid that also contains the viral polymerase. Based on studies with other ssRNA(-) viruses, the potential for intermolecular recombination during mixed infection with closely related parainfluenzaviruses appears to be very rare. However, analysis of the genome of NDV isolates became infected with the GMM and a closely related virus and recombination occurred, transfer of the RSV F gene is unlikely to alter the tropism or virulence of the related micro-organism as virulence of paramyxoviruses is multigenic with the GMM and a related virus, it may be possible that pseudotype viruses could be produced in which the genome of one may be packaged in a virion which includes the surface glycoproteins expressed by the GMM. Such virus particles are unlikely to have an altered cell tropism as they infect similar pulmonary epithelial cells, and their progeny would be that encoded by the viral genome.
This assessment was reviewed and approved by the Biological Agents and Genetic Modification Committee, who agreed it should not be notified as a stand-alone activity.

**Project Containment**

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**Historical Significant Changes**

- Transferred from GM97 28/07/2015

**Project Additional Information**

**Purposes of the contained use**

The purpose of these experiments is to determine if the PB1-F2 protein which is extensively conserved in avian influenza strains is a virulence factor in the infection of poultry by influenza viruses. This will help understand this protein's interactions with the host and elucidate whether the PB1-F2 protein is a valid target for anti-viral strategies. We will use reverse genetics to generate avian influenza strains that express the PB1-F2 protein and isogenic viruses that do not and assess their fitness and probe their interactions in vitro and in vivo using poultry lines.
We will generate recombinant avian influenza viruses which possess:
- The surface proteins (HA & NA) of the attenuated vaccine strain A/PR/8/34 (H1N1) and the internal genes (M, NS, PB2, PA & NP) of either:
  1) A/Turkey/Turkey/01/05 (H5N1)
  2) A/Turkey/England/50-92/91 (H5N1), or
  3) A/Chicken/Pakistan/UDL-1/08 (H9N2).

This will generate 2:6 recombinant influenza viruses safe to use at containment level 2.

These viruses will then have various mutations made to them in the PB1 segment to truncate or abolish the expression of the PB1-F2 protein which is in a +1 frame on the PB1 gene.

At no time with the HA & NA if H5N1 influenza viruses (either LPAI or HPAI) be rescued in conjunction with these genes. Therefore no complete H5N1 viruses will be generated in this process.

These viruses will be assessed for replication, innate immune response induction, pathogenicity and transmission in vitro in cell lines and ex vivo tissue sections as well as in vivo in poultry species.

Recipient or parental organism

Origin & function

The hazards directly associated with this class of GM influenza virus are small because they bear the surface antigens of the attenuated vaccine strain PR8 virus and thus are highly unlikely to infect a human or transmit. The main hazard would be from inadvertent co-infection of a cell culture with these viruses and other viruses that bear human or avian influenza surface antigens. This will be minimized by either working with these viruses in a separate MSC and incubator than used for other routine work with influenza viruses or fumigation of the MSC prior to use.

Clear signage on the outside of the hood will be used to indicate the last time the hood was used and which virus was used. We eliminate the possibility that workers are 'mixing vessels' by our OH policy of seasonal influenza vaccination. These viruses remain susceptible to both neuramidase inhibitor used routinely as anti-viral treatment for human influenza infection. Mutation of the PB1 gene segment to remove the expression of PB1-F2 or truncate it should reduce any risk from these viruses as PB1-F2 is thought to be a virulence factor.
The hazards to the environment are small from these GM influenza viruses due to the HA & NA from the vaccine strain PR8. This HA & NA combination should be capable of infecting avian species but the control measures in place in both the CL2 in vitro lab (G1B) and EAH are such that the virus will be contained appropriately. This HA & NA combination will render these viruses low pathogenic in nature in avian species. Manipulation in G1B will be carried out exclusively inside a class II MSC, transportaion will be under double containment, liquid waste will be decontaminated via contact with 1% Virkon for at least 10 minutes and all associated waste directed to autoclave. Poultry infected with these viruses inside the EAH will be done so in containment level 2 rooms and housed inside isolators. Every room in EAH has rodent barriers the input and output air is filtered and all waste and drainage liquid is autoclaved. Poultry to be infected will be screened for pre-infection by Influenza virus by looking for the presence of Influenza antigens or antibodies. Experiments will not be performed in animals previously infected with Influenza virus preventing the chance of a natural reassortment event in poultry. It is highly unlikely that the animals would be exposed to Influenza prior to the experiment as all poultry are reared on site at IAH under good biosecurity. Mutation of the PB1 gene segment to remove the expression of PB1-F2 or truncate it should reduce any risk from these viruses as PB1-F2 is thought to be a virulence factor.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Stewart building lab G1B - in vitro assays

In vitro assays with live virus will be carried out exclusively in a class II MSC cabinet. Cabinets are routinely serviced and tested on an annual basis by an outside contractor and service records are maintained. The risk to laboratory workers can be virtually eliminated by carrying out all work with potentially infectious samples, virus or infected material, in a class II microbiological safety cabinet (MSCII).

- All workers will wear appropriate protective clothing.
- All work in the MSCII will be carried out in benchcote.
- All items will also be sprayed with 70% Ethanol before removal from the MSCII. Items which cannot be directly sprayed with ethanol before being removed from the MSCII will be placed in plastic storage containers prior to removal, the containers will be sprayed before removing from the MSCII.
- Disposable equipment should be used where possible and filtered tips should be used on pipettes.
- Contaminated waste from the hoods will either be;
  (a) soaked in 1% Virkon for at least 10 minutes and then disposed of through the incineration route for laboratory waste or
  (b) disposed of directly into a double bagged autoclave bag which will be sealed with tape inside the hood and placed directly into an autoclave tin. The autoclave tin will be marked with G18 and INFLUENZA VIRUS before being taken to microbiological services for autoclave.

Infected material will be transported from the MSCII to the destination within a sealed container. e.g. plastic storage containers to incubator, sealed rotors to centrifuges. All items will be sprayed with 70% Ethanol before removal from the MSCII.

Infected tissue culture will be incubated in a designated CO2 incubator. Cells will be transferred to the incubator in a sealed container, once in the incubator the lid will be unsealed and removed to allow circulation of CO2. This will prevent spills and drips whilst inside the incubator also.

Influenza viruses are routinely grown in 10-day old embryonated hen's eggs

- Allantoic fluid will be harvested within the MSCII by cracking the top of the egg shell (placing a disposable pipette tip in the hole made previously by the egg gun) and removing the disc of shell with blunt forceps. The embryo is pushed to one side with disposable equipment (pipetter or wooden spatula), the allantoic fluid is removed using a jumbo pastette or disposable pipette and placed into 50ml falcon tubes.
- Eggs are discarded into a double autoclave bag within the MSCII which is sealed then placed into a metal tin for autoclaving. The tin should then be marked with contents (EMBRYONATED EGG WASTE) and taken immediately to microbiological services for processing.

02/03/2022
- Any non-disposable instruments (e.g. metal forceps) will be left in 70% Ethanol for 30 minutes and then sent for autoclaving.

All infective material will be autoclaved, incinerated or otherwise rendered sterile, unless being transported to another CL2 facility or being put to storage. Infective material will not be voided through the drain system, but inactivated with either by autoclaving or using 1% Virkon. Where possible disposable consumables will be used. Non-disposable items will be soaked in 1% Virkon for the required contact time, any attached labels removed and transferred to the Microbiological Services Department (in the same building) in labelled lidded metal tins for autoclaving and disposal.

This assessment was reviewed by BAGMSC on 14/02/2012 and the committee approved this RA as a GM2 project

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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Animal Units

L2 Yes | L3 L4 | L2 L3 L4 | L2 L3 |

Large Scale Activities

L2 Yes | L3 L4 | L2 L3 L4 | L2 L3 L4 |

Human Clinical Applications

Class CultureVol Class

Class 2 < 1 litre

Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements

N

Project Ref 97/14.1

Date Ackn'd 06/03/2014

CU2 Project Title Manipulation of Newcastle disease virus vaccine strain (LaSota) genome.

Class CultureVol Class

Class 2 < 1 litre

Non-GMM Consent Granted

Not Applicable

Tick if notifying a connected programme of work

N

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to understand the biology of recombinant Newcastle disease virus (NDV) vaccine strain (LaSota) expressing either fusion 9F) or haemagglutination-neuraminidase (HN) genes, single or jointly, closer to the promoter-proximal region of the genome. Growth kinetics of these construct will be evaluated in the chicken and duck cell culture systems. Having an improved NDV vaccine candidate in hand, genes of viruses will be introduced into this recombinant La Sota strain. The V protein, which is a non-structural protein of NDV, will be knocked out from vaccine strain of NDV to investigate the effect on the pathogenesis of the virus. The NDV infectious clone used in this work is tagged with GFP marker and has been produced by Dr Ben Peeter at central veterinary laboratory, The Netherlands. The modified viruses along with vaccine strain of NDV (La Sota) will be used to infect chicken and duck cell-lines to understand the role of these proteins playing in the replication and expression of the surface glycoproteins. The rescued viruses will be inoculated in 9-days old embryonated eggs to propagate and to make a stock. The same stocks will be used for quantification and titration purposes.

**Recipient or parental organism**

The vaccine strain of NDV to be used in this proposal is the lentogenic, avirulent (non-pathogenic) La Sota strain, which is a licensed live vaccine virus and is being used in Major endemic countries. NDV has extensively been used as vaccine vector for both veterinary and medical viruses with satisfactory results without change in the pathogenicity of the vaccine strain of NDV (Bukreyev, et al. 2005) Moreover, the genomic rearrangement presented in this work has already been made in other paramyxoviruses of medical origin such as vesicular stomatis Virus and Respiratory Syncytial Virus (Krempl At Al 2002). Such genetic rearrangements did not affect the pathogenicity of these medical viruses (Wertz, Et al 1998). It is therefore plausible to expect that such rearrangement in NDV, a similar virus but of veterinary importance, Will not affect its pathogenicity.

**Host/vector system**

The NDV is an avian paramyxovirus serotype 1 (APMV-1), and belongs to the Avulavirus genus within the Paramyxoviridae family. Vaccine strains of NDV are classified as ACDP CL2 and has been reported to cause short-lived conjunctivitis symptoms in man (Capua and alexander, 2004). Many of the reported instances of NDV infection in humans have been the result of direct inoculation into the eye, either by laboratory workers or those handling vaccines. However, reports of illnesses associated with NDV are rare. Both virulent (mesogenic) and avirulent (lentogenic) strains of NDV appear to be equally able to cause conjunctivitis in humans. However, both mesogenic and lentogenic strains of NDV have been tested in man as oncolytic viruses and cancer vaccines with no adverse and effects (Zamarin and Palese, 2012) Signs of evolution towards human pathogenicity , and recombination or transmission from human to human. Furthermore, virus could not be recovered from either the nose or tracheal lavage of African green monkeys that had been inoculated intratracheally with a lentogenic strain of NDV expressing the HN protein of human parainfluenza virus-3 (HPIV3) (Bukreyev, et al 2005)

Lentogenic strains of NDV are used as live vaccines in poultry. The hitchner B1 strain, which is very similar to LaSota strain in terms of pathogenicity and genome characteristics, is not classified as a specified animal pathogen by Defra and is licensed for use in the uk. NDV does not appear to naturally infect cattle, and vaccine strains
of NDV are highly attenuated in experimentally infected calves. Although, NDV has been isolated from pigs with an influenza-like illness, experimental inoculation of piglets with a strain of NDV did not produce any clinical signs of disease. Genomic rearrangements will likely to attenuate the vaccine strain of NDV as it did in medical viruses such as vesicular stomatitis Virus and Respiratory Syncytial Virus (Krempl et al 2002; Wertz et al 1998).

**Origin & function**

Three Genes, Presented in this proposal, will be modified. Briefly (i) the HN protein determines the host tropism by binding to sialic acid receptor of the host. (ii) the F protein mediates fusion of the NDV envelope with the cell membrane and fusion of infected cells with adjacent uninfected cells. Both HN and F protein are the major protective antigens of NDV and do not contain any potentially toxic or oncogenic sequences. (ii) the V protein is a non-structural protein which is known to involve interferon regulations activities.

**Evaluation of foreseeable effects**

Genomic rearrangement may lead to attenuation of the LaSota vaccine strain of NDV as it has been experienced with viruses of same family. Since the same gene of La Sota vaccine strain will be moved to promoter proximal region, it is unlikely that the resultant virus will change any tropism. Moreover, all the modifications, presented in this protocol, will be made only in the vaccine strains of NDV. Based on the reported studies, it is very likely that these recombinant NDV vaccine viruses will have increased virulence (Wertz et al 1998; Krempl et al 2002). Examples are available indicating the expression of any foreign gene in NDV can lead to attenuation in the animals that had been inoculated intranasally and intratracheally with high titres of the recombinant virus (Bukreyev, A et al 2005).

Recombination between single-stranded, negative-sense RNA (ssRNA(-)) viruses such as NDV is rare. The ss RNA9(-) genome of RDV is found exclusively in a RNAse-resistant nucleocapsid that also contains the viral polymerase. Based on studies with other ssRNA(-) viruses, the potential for intermolecular recombination during mixed infections appears to be very rare. However, analysis of the genome of NDV isolates from China suggests that homologous recombination of NDV can occur naturally, which is a rare event.

In the unlikely event that this genomic rearrangement will alter the tropism of the NDV since gene of the same vaccine will be moved to promoter region, virulence is unlikely to increase because pathogenicity in NDV is determined by the cleavage site in the F protein and we are not intended to mutate cleavage site in this proposal. Moreover, such rearrangements in other paramyxoviruses including vesicular stomatitis virus and respiratory syncytia virus have not increased the virulence of the recombinant viruses but has induced the protective immune responses, collectively, indicating least possible hazard associated.

The V protein of NDV is associated with interferon antagonism. Generation of LaSota NDV, Not expressing V Protein, is very much likely to be attenuated due to immune responses produced against NDV in infected cells. Therefore, there appears to be no adverse foreseeable effects with this modification.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Contaminated laboratory plastics will be decontaminated by 1% Virkon for 30 minutes prior to disposal by incineration and contaminated glassware will be autoclaved. Any tips, flasks, or other plastic wear that has been exposed to virus will also be decontaminated prior to disposal by incineration. Spillages and contaminated surfaces will be decontaminated with 2% Virkon for a minimum of 30 minutes.
Project Containment

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Project Ref 97/14.2

The use of recombinant Murine Cytomegaloviruses as vaccine vectors

Class 2

< 1 Litre

Consent Granted

Non-GMM

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Transferred from GM97 28/07/2015

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Reviewed by the BAGMSC and Dr M B (a paromyxovirus research group leader) on 26/11/13 and approved at GM2.
## Project Additional Information

### Purposes of the contained use

To develop the use of MCMV as vaccine vectors against human and livestock diseases.

### Recipient or parental organism

Mice

### Host/vector system

Murine cytomegalovirus

### Origin & function

- **Nucleoprotein (NP) and matrix (M1) proteins of X31 Influenza Virus.**
- The intended function is to induce a durable immune protective immune response against Influenza viruses.

### Evaluation of foreseeable effects

MCMV does not pose any harm to human health. MCMV has been shown to infect human cells in vitro, but infection leads to an abortive replication cycle. Accidental infection could induce immune response to MCMV and inserted transgenes, which would not be harmful.

Release of rMCMV into the environment is very unlikely due to the standard procedures employed in the lab or animal house. However even if such an event occurred, although rMCMV could infect wild mice (prevalence of wild type MCMV infection in wild mice is 65-90%) this is unlikely to occur because rMCMVs are attenuated and naturally infected wild mice should be cross protected against rMCMV. Furthermore even if infection did occur, attenuated rMCMVs are very unlikely to cause any pathology. The risk of infection of any other species is extremely low.

MCMV is an enveloped virus and therefore has poor survival in the environment. MCMV transmission is thought to require direct contact with contaminated excreta/secretions and cross-contamination between cages has so far not been reported.

The inserted genes are internal proteins from Influenza viruses to which humans are frequently exposed and which are known to induce immune responses. As stated above, because the risk of rMCMV infecting humans is minimal the risk of recombination between the transgene and human Influenza Virus is extremely low. Modified vaccinia Ankara or Adenovirus expressing NPM1 has been administered already to mice, pigs, chicken and humans without ill effects and these genes are present in the influenza vaccines, administered to humans.

In the case of the MCMV infected animals being challenged with Influenza virus there is no increased risk of these animals excreting a virus that could be transmissible to people compared to any other mouse strain. We shall use either A/PR/8/34 or PR8 based viruses. These challenge viruses pose very little risk for humans since PR8 is poorly infectious in people. The animals will be handled in isolators so that the risk is minimal.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Contaminated laboratory plastics will be incinerated and contaminated glassware will be autoclaved. Spillages and contaminated surfaces will be decontaminated with 2% Virkon for a minimum of 10 minutes. All waste will be disposed following MSD-113.

All waste and rubbish from the animal house should be placed in an autoclave bag. This should then be double bagged and sprayed with 1% Distel before it is removed from the room. The waste should then be autoclaved before being placed in yellow clinical waste bags. These yellow bags should then be placed in the yellow bins for incineration. Staff handling the mice will shower out on exit of the unit.

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Project Ref 97/15.1

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<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Withdrawn N  

Tick if notifying a connected programme of work N
The purpose of this work is to generate reverse genomics systems for endemic avian viruses (Chicken Astroviruses (including Avian Nephritis virus), and Chicken Anaemia virus) and uses these systems to investigate the mechanisms of genome packaging employed by these viruses. Improving understanding of the mechanisms used by these viruses to encapsidate viral genomic nucleic acids as a key step in virion formation may contribute to efforts to develop new antiviral strategies. Such new strategies are required to provide new control measures to improve and maintain the sustainability of global poultry production and improve animal health and welfare.

Chicken Astrovirus (CAstV including Avian Nephritis virus (ANV)): cDNA genome clones will be generated from strains of virus that are circulating within the UK. Selected strains are considered clinically mild by comparison with Asian strains of this group. Strains used will be: CAstV 11672 and 612, ANV G4260. Astroviruses are +ve sense ssRNA viruses with a genome of 6·8kb, encoding 3 ORFs 1a/1b/2. ORF1a and 1b encode non structural proteins and the RNA dependent RNA polymerase (AdRp) respectively, while ORF2 contains the capsid protein precursor, which is expressed from a subgenomic RNA transcript. Progeny genomes are synthesised via a -ve strand intermediate by viral RdRp.

Chicken Anaemia Virus (CAV): genomic DNA clones will be generated from vaccine strain virus (P4). CAV encodes 3 ORFs, VP1, VP2 and VP3. VP1 is the capsid protein. VP2 a dual specificity phosphatase which may be involved in VP1 maturation and VP3 (also known as apoptin) specifically causes apoptosis of transformed cells. CAV has a closed circular -ve sense ssDNA genome of 3kb.

We propose to alter Packaging signal motifs within the viral genome clones in order to understand their contribution to encapsidation of the viral genome. any mutations will be, as far as practicable, conservative at the amino acid level to retain wild type (WT) function of the encoded gene product where these signals are identified within viral ORFs.

Host/vector system

Genome clones (cDNA or gDNA) will be generated and maintained in laboratory strains of E. coli. Viral genome clones will be transfected into appropriate eukaryotic cells for recovery of virus. For CAstV plasmids will be used as templates for in vitro transcription prior to transfection. CAV genomes will be regenerated by excision of bacterial vector sequences and religated to circularise the genome and establish a replication competent form.
Viral genomic RNA for CAstV (and ANV) will be provided by Dr. Victoria Smyth Agri-Food and Biosciences Institute Northern Ireland from reference virus collections used in diagnostic procedures. Strains to be used are, CAstV 11672 and 612, and ANV-1 strain G4260, which are mild strains circulating in the UK. These genomic ANAs will be used as templates from which to reverse transcribe full length eDNA clones for subsequent propagation and manipulation in E. coli. Genome clones will be flanked 5' with T7 promoter sequences, and 3' with the Hepatitis delta ribozyme and a T7 terminator to allow generation of the WT RNA without exogenous flanking sequence.

Genomic DNA of CAV (strain P4) will be isolated from a commercial vaccine and used as the basis for construction of a plasmid based genome clone.

Putative packaging signals will be identified from a library of RNN/DNA aptamers panned against CAstV/ANV/CAV isolated and purified capsid proteins. Alterations to packaging sequences in viral genome clones will be undertaken following in silico modelling and computational identification of genome nucleotide regions which are either similar by nucleotide composition or secondary structure. These regions will then be mutated, again guided by computational modelling to remove, weaken or strengthen the packaging signals and their interaction with cognate capsid protein. Alterations will be undertaken using standard restriction digest/site directed mutagenesis techniques. Mutations will be designed to maintain the WT amino acid composition of any viral OAF in which the signals fall, to retain WT function and behaviour as far as is practicable. Our proposed alterations to genome packaging signals, designed to maintain NT coding at the amino acid level, are not expected to alter host range or tropism nor alter the pathogenicity of these mutant viruses relative to WT parental strains.

Virus genomes generated in this way will be used in in vitro assays either using purified components (RNA/DNA) and protein) or within avian cells (after transfection of genome). To study the encapsidation of the genome under altered conditions. The resulting virus will be examined to identify any deficiencies in assembly and relate these to mutations introduced into the genome packaging signals to understand the contributions of these signals to the process of genome encapsidation.

**Origin & function**

The WT clones considered here should behave as circulating field strains of the virus and are highly unlikely to show altered pathogenicity or host tropism after rescue from bacterial hosts. CAstV and ANV clones are derived from UK samples and are considered clinically mild, as these are endemic viruses many birds in the population should have immunity to them. CAY clones will be derived from a live vaccine strain used to provide protection to chicks via maternal antibodies against CAV. In both cases there is likely extensive existing immunity in flocks and as such these viruses represent little risk to avian health unless introduced to unprotected young chicks. 80th viruses seem to be host restricted limiting the likelihood of transmission to non avian species.

Packaging signal mutants derived from the WT clones are expected to be highly disabled compared to WT. The distribution and strengths of these sequences in a WT genome is likely to have been selected over the course of virus evolution and could be considered optimised for the efficient encapsidation of viral genome and ultimately for production of mature virions. Likewise these signals may also have been selected to ensure the subsequent release of the genome on infection of a new host. As such it is highly likely that as we alter the strength of the Packaging signal: capsid protein interaction either increasing or decreasing it, the result will be to perturb viral assembly or disassembly in such a way as to result in defective non viable particles incapable of initiating infection. Similarly alteration of signal distribution is likely to drive genome encapsidation down kinetically trapped pathways that form incomplete non viable virions incapable of establishing further infections. In all cases it is highly unlikely that a fitness enhancing mutation would be introduced, and much more likely that mutations would have deleterious effects on genome encapsidation and virion assembly.

There is low foreseeable hazard to human or avian health from the GM CAstV, ANV and CAY proposed here.
Exposure of ideal hosts (young naive or immunocompromised chicks) could result in establishment of an infection with WT virus, or in a situation where modifications were not significantly disabling compared to WT. Handling of these agents under CL2 conditions in the CL2 in vitro lab should ensure that virus is appropriately contained and release avoided. Rescue of virus by transfection will be undertaken in Class II MSCs and all waste rendered noninfectious by treatment with virkon. For AstV exposure to 1.5% virkon for a minimum of 30min is indicated and for CAV 1% virkon for 30 minutes or more in line with the group's COP. Subsequently all solid waste will be directed to the clinical waste stream for incineration in line with site COPs.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

As the most stringent conditions are required for inactivation of AstroVirus these will be used as the minimum level for both CAstV and CAV. AstroVirus or CAV contaminated waste will be dealt with in line with published conditions for inactivation 01 Turkey AstV. For in vitro work (assembly assays/cell culture and transfection) waste will be decontaminated by exposure to 1.5% virkon for a minimum of 30 minutes. Subsequently solid waste (tips/flasks etc) will be processed through the clinical waste stream for incineration as per established site procedures In MS0-113. All cell work will be conducted in Class II MBSGs which are serviced and tested for performance annually by an external contractor. with service records maintained centrally. All contaminated waste will be decontaminated with exposure to 1.5% virkon for a minimum of 30 min. Filtered tips will be used on pipettes when handling CAstV or CAV samples. Aller exposure to virkon solid waste will be directed into the clinical waste stream for incineration. Cells containing CAstV gANA or CAV gONA will be transported within closed containers from the MBSC to the incubator. All transport containers will be surface decontaminated with 70% ethanol before removal from the MBSC. Cells will be incubated in a specific C02 Incubator, after transport tissue culture vessels will remain within the transport container without the lid to permit gas exchange but minimise the chance of drips or spills within the incubator. All contaminated material will be rendered sterile unless it is to be transported to another CI2 laboratory or stored. Disposable consumables/equipment will be used wherever practicable where this cannot be done. sterilisation will be undertaken using 1.5% virkon for 30mins minimum contact time prior to transfer to the Microbiological services department (located in the same building) for autoclaving In labelled containers.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
## Project Containment

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### Animal Units

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- L3
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### Large Scale Activities

- L2
- L3
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- L3
- L4

### Human Clinical Applications

- L2
- L3
- L4

## Project Ref 97/98.1

### Date Ackn'd

12/06/1998

### CU2 Project Title

INFECTIONOUS BOVINE VIRAL DIARRHOEA VIRUS FROM CLONED CDNA

### Class

Class 2

### Culture Volume

Class 2

### Consent Granted

Not Applicable

### Project notified under transitional arrangements

Y

## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<th>Growth Rooms</th>
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**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**

Tick if confidential

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.

---

**Project Ref 55/01.1**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref**: 55/01.3

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**Tick if informing a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 55/01.4

Date Ackn'd 19/02/2001

CU2 Project Title USE OF IMMORTILISED HUMAN CORNEAL CELL LINES (EPIDERMAL, STROMAL)

Class 2

CultureVolClass2 ClassVolumeClass3-4
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**Historical Significant Changes**
**Historical Date of Additional Info**
**Significant Change ID**
**Date of Significant Change**

**Project Additional Information**

* Purposes of the contained use

* Recipient or parental organism

* Host/vector system

* Origin & function

* Evaluation of foreseeable effects

* Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

* For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

* Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 55/01.5

Date Ackn’d

09/05/2001

CU2 Project Title

USE OF A 3D HUMAN CORNEAL EPITHELIAL (HCE) MODEL CONSTRUCTED FROM AN IMMORTALISED HUMAN CORNEAL EPITHELIAL CELL LINE

Class

Class 2

CulureVolClass2

< 1 litre

Consent Granted

not applicable

Project notified under transitional arrangements

N
**Purposes of the contained use**

SkinEthicTM (Nice, France) supply a 3D human corneal epithelial (HSC) model for use in eye irritation testing. This model is constructed from corneal cells originally derived from a cornea removed from a normal human donor. The cells were transformed using a replication defective retrovirus vector LXSN16E6E7, which encodes the human papilloma virus (HPV) type 16 E6 and E7 open reading frames and the neo antibiotic resistance gene. This cell line is used by SkinEthicTM to construct an in vitro 3-D model of the cornea. The 3D model will be purchased ready made from SkinEthicTM and used to assess their suitability as a model for assessing the mechanisms of eye irritation.

**Recipient or parental organism**

The corneal epithelial cell line was derived from primary human corneal cells isolated from a donor cornea from a 35 year old male, by Roger W. Beuerman, LSU Eye Centre, Louisiana State University School of Medicine, New Orleans, Louisiana 70112, USA.

**Host/vector system**

The corneal cells were transformed using a replication defective retrovirus vector LXSN16E6E7 (manuscript in preparation) using methods described in a previous paper (Nguyen et al., 1999). This vector (also described in 16risk99) was produced using the packaging cell line PA317 LXSN 16E6E7 (Halbert et al., 1991), obtained by introduction of retroviral vector pLXSN16E6E7 into the amphotropic packaging cell line PA17 (Miller and Buttimore, 1986; Rhim et al., 1998). The retroviral vector pLXSN16E6E7 was derived from the Moloney murine leukemia virus (MoMLV) and lacks the structural genes (gag, pol, env) required for particle formation and replication. pLXSN16E6E7 also contains

i) the HPV type 16 E6 and E7 open reading frames under the control of the 5' MoMLV LTR promoter

ii) the neo gene, which codes for kanamycin-neomycin antibiotic resistance under control of the SV40 early promoter gene

iii) pBR322 DNA containing the origin of replication (pBR322 ori) and the bacterial B-lactamase gene (ampicillin resistance).

**Origin & function**

No further genetic material was involved.

**Evaluation of foreseeable effects**

The Host: The corneal cells were derived from a normal human cornea. The resulting primary cells were cultured in vitro prior to transformation. Eukaryotic cell culture systems are normally considered as "especially disabled hosts" unless the vector can infect other hosts. In this case, the vector can enter both mouse and human host cells but it is unable to transmit viruses to other host cells. Thus the cell line cannot be termed 'especially disabled'. However, the cells would not survive outside of tissue culture conditions and would not survive in the human body. Also, the cells per se will not be subcultured in the laboratory, as the model is received ready for use and has a limited shelf-life, thus reducing risk of escape. Thus the host is a 'disabled or non-colonising' host and of low hazard.

Retroviral Vector pLXSN16E6E7: This vector is capable of integrating into both mammalian and bacterial cells and therefore the potential hazard from this vector is that of transfer to other cells, in vitro or in vivo. Vector pLXSN16E6E7 is derived from the amphotropic retrovirus MoMLV and has been multiply disabled such that it cannot further replicate and infect other cells. The packaging cell line, PA17, used to produce the vector, also has deletions in its packaging system genes to reduce packaging function transfer to vectors. Vectors produced by the PA317 cell line were shown to produce <1 helper virus/ml. At least two viral recombination events would be required to allow production of infectious virus. The ACGM guidelines state that viral vectors from which no infective virus can be produced should be regarded as minimal risk. While this is considered to be an externally unlikely occurrence, electron microscopy will be carried out initially to check for the presence of virus as well as to investigate cell ultrastructure. Any cells producing virus will be destroyed.

Genetic Inserts: The following are inserted into the vector:
HPV type 16 E6 and E7 open reading frames: These regions are responsible for cell transformation. HPV virus infection is common in humans and infects epithelial basal cells. The E6 and E7 genes are used to transform cells by interfering with cell cycle control mechanisms. Thus it is possible that this insert could be transferred to other cells, either in vitro or in vivo and cause cell transformation. However, a transformed cell is not an immortalised cell and the presence of E6/E7 sequences alone is not sufficient for immortalisation to occur. Several unknown processes are required for immortalisation and not all transformed cells become immortal. It is also considered that the risk of transfer of these genes to other cells or organisms is likely to be low. Other genes required for the production of complete HPV (e.g. L1 viral capsid protein) are not present on the vector.

Neo gene: This gene confers resistance to the kanamycin/neomycin class of antibiotics. The expression of this gene has not been optimised. There is a negligible risk associated with this gene used as a selection marker in that resistance may be transferred to the gut bacteria if ingested.

PBR322 DNA: This contains the pBR322 origin of replication and the ampicillin resistance gene. The pBR322 sequences do not produce a gene product alone and is a non-coding region of DNA. The ampicillin resistance gene expression has not been optimised. There is a negligible risk associated with this gene (used as a selection marker) in that resistance may be transferred to bacteria if ingested.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This cell model is handled in microbiological safety cabinets, maintained on a 6 monthly basis, within an ACGM/ACDP Containment Level 2 suite. The suite is entered via a lobby and only trained personnel will handle the models. The suite also has restricted access. There are two methods of disposal - autoclave or incineration. Autoclaving is the preferred route, performed prior to disposal as clinical waste via a specialist waste contractor, and is considered to provide 100% kill of the GMO. Service of autoclaves takes place quarterly by ACE to meet standards set down in Health and Technical Memorandum 2010 Part 3. Details of temperature profiles for each run are recorded. Clinical incineration is used as a route of disposal if the cells have been treated with chemicals which are very toxic or whose toxicological properties are unknown. In these circumstances, the hazard generated from autoclaving these very toxic chemicals was considered far greater than that from any potential exposure to the GMOs. If the GMOs are intended for incineration, they are usually but not always sealed inside a container (e.g. tissue culture flask or Universal), then always double bagged on sawdust (to absorb liquid) before being placed in another plastic bag (sealed) inside non-returnable, sealed waste bins. Thus accidental release to the environment is considered highly unlikely.

In case of spillages in the laboratory, fresh 1% Virkon is the disinfectant of choice and is always readily available. Spillages are treated with Virkon, mopped up with paper towels and these are autoclaved prior to disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
The committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.

**Project Containment**

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**Animal Units**

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Withdrawn N

Tick if notifying a connected programme of work N

**Project Additional Information**

**Purposes of the contained use**

The use of green fluorescent protein (gfp) has become an important tool in the investigation of gene expression in cells. Numerous bacterial clones are available with gfp-fusions inserted for example into the promoter regions of the pathogenicity islands of Salmonella spp. Such organisms can be investigated in situ using cytometric measurements of their fluorescent under a variety of physiological and non physiological conditions (eg stress) to determine gene activation. A variety of clones and organisms will be used to investigate physiological changes under simulated conditions in the environment, to test a variety of environmental challenges of food treatment...
processes or physiological changes following infection in human or animal tissue using flow and image cytometry.

Recipient or parental organism

Salmonella enterica serovars Typhimurium and Enteritidis currently called S. enterica subsp. enterica serovar Typhimurium and S. enterica subsp. enterica serovar Enteritidis are classified as class 2 pathogens, as determined by the ACDP and are food isolates from the Institute of Food Research in Norwich, UK.

Host/vector system

Mutations or gfp fusions are introduced into the chromosome of the pathogenic strains by transformed linear fragments of DNA into the cells of interest harbouring an oriR101-based temperature-sensitive plasmid (pKD20/pKD46) encoding the Red recombinase under control of the tightly regulated arabinose-inducible ParaB promoter. The fragments of DNA contain the mutated allele/reporter-gene construct and the antibiotic-resistance marker for kanamycin or chloramphenicol. The plasmids also encode ampicillin and/or chloramphenicol resistance and araC. The use of an arabinose inducible recombinase and proteins required to inactivate the host's RecBC exonuclease V system avoid unwanted recombinatorial events under noninducing conditions allowing efficient recombination of the linear DNA fragments into the host chromosome. The plasmid also encodes a temperature-sensitive RepA protein, only allowing replication of the plasmid at temperatures up to 30 degrees C. None of the genes encoded by the plasmid are considered likely to enhance the virulence of the strains harbouring them.

The organisms are cured of the plasmids and transfectants tested for the absence of Amp resistance to exclude the possibility of remaining plasmid or insertion of the resistance gene.

Origin & function

Promoter sequences for pathogenicity islands/stress response genes and the green fluorescent protein (gfp) reporter gene are amplified by PCR and fused by a recombinant PCR approach. Promoter sequences from the isolates were selected based on strength of induction upon stress as determined by the DNA array work at the Institute of Food Research. gfp variants are based on McCormak gfp-mut3 solely to indicate the activation of the promoter sequence.

Evaluation of foreseeable effects

The Host.

Salmonella is pathogenic to animals and humans, although it causes only limited gastro-intestinal infection in healthy individuals. Therefore, the consequence of hazard is restricted.

Mutants contain kanamycin and/or chloramphenical resistance genes; these are not the drugs of choice for the treatment of infections by these infectious agents and thus would present no selective advantage in the event of an accidental release.

The Salmonella strains containing the promoter-gfp fusions are anticipated to have virulence characteristics close to wild-type levels. Introduction of the fusions are not expected to enhance the virulence properties of these strains because they only contain virulence gene promoters, not the entire structural genes. In fact the inserted constructs should lead to a dilution of promoter activity as it is partially used to produce gfp instead of virulence factors.

Control measures are in place to minimise the risk for accidental release of the organism.

Genetic Material:

Plasmid pKD20/46 and its derivatives cannot replicate at 37 degrees C. Strains are cured of the plasmid prior to experimental work at Colworth thus further transfer of genetic material is unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| None |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Bacteria and animal tissue handled in liquid broth, buffer or on agar plates. Waste will be discarded via autoclaving which achieves effectively 100% kill and/or incineration (100% kill) depending on the contact with potentially carcinogenic dyes. Service of autoclaves takes place quarterly by ACE to meet standards set down in Health and Technical Memorandum 2010 Part 3. Details of temperature profiles for each run are recorded.

Cell culture waste for incineration is always sealed inside a container (eg tissue culture flask or Universal). Incineration waste is double bagged inside the yellow UN approved one-way bins containing absorbant in the inner and Virkon inside the outer bag. Burn bins are stored in a refrigerated container vehicle, supplied and collected by a licenced contractor.

Diluted liquid waste from the cytometer will be treated with active chlorine at 2500ppm (10 tablets/2 ltr) overnight, neutralised with 10g sodium thiosulphate. In the absence of a chlorine demand from the sheath liquid the available chlorine concentration and incubation time at room temperature will exceed the 100% kill achieved for most vegetative cells by more than 10,000 fold, for spores by more than 1000 fold.

| Is an emergency plan required according to regulation 20? N |
| If yes, tick to confirm that it is attached to this form N |
| Tick to confirm that you have attached a risk assessment to this form Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment N |

Please enter comments on the GM safety committee on the risk assessment

The Committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.

**Project Containment**

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Project Ref 55/03.1
The aim of the project is to stably express antioxidant genes (Catalase (CAT1), Cu/Zn Superoxide Dismutatse (SOD1), Mn Superoxide Dismutase (SOD2)) in immortalised cultured human cells using retroviral gene transfer. A series of pBABE based vectors have been constructed under a previous risk assessment (not notified) and these will be used to stably express the cDNAs for human CAT1, SOD1 and SOD2 in the keratinocyte cell line, HaCaT. In the appropriate producer cell lines the pBABE vectors produce eotrophic and amphotrophic retroviruses where gene expression is driven by the Moloney murine leukemia virus (Mo MuLV) LRs. All retroviruses are replication defective as many helper functions are supplied by the producer cell lines pCRIP and E. Cells that have stably integrated the retroviral expression cassette will be selected on the basis of drug resistance to puramycin and neomycin.

The packaging cell lines E pBABE (CAT1); E pBABE (SOD1) and E pBABE (SOD2) will be generated through infection of the E packaging cell line with the pBABE vectors. The eotrophic supernatant produced by these cell lines, containing secreted retroviral virions, will be used to transfect C RIP cells to produce the packaging cell lines p CRIP pBABE (CAT1); p CRIP pBABE (SOD1) and p CRIP pBABE (SOD2). In turn, the amphotrophic supernatant produced by these cells will be use to transfect the HaCaT cell line (immortalised human keratinocytes).

The risk assessment addresses the steps involved in the production of antioxidant expressing human cells as follows:

1) E cells harbouring human cDNA for the genes identified above (preparation of stable eotrophic producer cell lines);
2) p CRIP cells harbouring human cDNA for the genes identified above (preparation of stable amphotrophic producer cell lines); and
3) HaCAT immortalised human cells expressing human cDNA for the genes identified above.

The titre of the retroviral virions, secreted from the amphotropic producer cell lines described above, will be assessed through infection of human carcinoma A431 cells.
minimal sequence overlap and decreased sequence homology achieved by ‘codon woddling’.

p CRIP is a high titre amphotropic helper-free packaging cell line derived from NIH 3T3. It is ideal for use with pBABE vectors as the risk of generation of wild type virus is reduced due to extensive changes in the codon usage of the viral proteins. pCRIP were engineered by sequential co-transformation of NIH 3T3 cells with two mutant Moloney murine leukaemia virus-derived proviral genomes carrying complementary mutations in the gagpol or env regions. Each genome contains a deletion of the Psi sequence necessary for the efficient encapsulation of retroviral genomes into virus particles and additional alterations at the 3’ end of the provirus. Viral producers derived from pCRIP do not transfer the packaging function or yield helper virus.

Both the above cells were supplied by University of Wales College of Medicine, Cardiff, UK.

HaCaT cells are spontaneously immortalized human keratinocyte cell line (non-virion producing), originally derived from normal male skin at the distant periphery of a melanoma. The cells were supplied from Heidelberg, Germany.

**Host/vector system**

Each of the above cell lines will be transfected with a pBABE vector. All vectors used are based on the pBABE family of vectors and differ only in respect to the insert DNA they harbour (CAT1, SOD1 or SOD2) and the antibiotic resistance gene carried to allow selection in mammalian cells. The pBABE vectors are capable of integrating into both mammalian and bacterial cells and therefore the potential hazard from these vectors is that of transfer to other cells, in vitro or in vivo. The pBABE series of vectors are derived from the Moloney murine leukaemia virus (MoMuLV) and have been multiply disabled by deletion of the pol and env genes. Therefore, pBABE vectors on their own cannot further replicate and infect other mammalian cells. At least two recombination events would be required in order to get a functional virus. Retroviral vector produced from the packaging cell line, E, can enter mouse host cells but is unable to transmit viruses to other host cells. There is a slight risk that successive viral recombination events could occur to give risk to infectious virus producing cells. This possibility has been minimised significantly through the construction of the E cells, which are regarded by the HSE as third generation packaging cells. These cells harbour the viral structural genes, gagpol and env on separate constructs to limit the risk of recombination.

Retroviral vectors produced from pCRIP can infect the cells of their host and the cells of other species including human cells. However, they are not capable of further cycles of replication. Therefore the risk of subsequent spread of infection is minimal. The HaCaT cell line should not produce live viral vectors and this will be monitored on a 6-monthly basis.

The vectors also contain the bacterial B-lactamase gene, which confers resistance to ampicillin. The neo vectors contain the NeoR gene, which confers resistance to the kanomycin/neomycin class of antibiotics and is expressed from the SV40 early promoter of the pBABE series of vectors. The puro vectors contain the Pac gene, which confers resistance to puromycin and is also expressed from the SV40 early promoter of the pBABE series of vectors.

**Origin & function**

- pBABE neo (CAT1) and pBABE puro (CAT1) carry the human CAT1 cDNA (catalase). Catalase is an anti-oxidant and is ubiquitously expressed in mammalian cells.
- pBABE puro (SOD1) and pBABE neo (SOD1) carry the human SOD1 cDNA (Cu/Zn Superoxide dismutase). SOD1 is an anti-oxidant expressed in the cytosol of mammalian cells.
- pBABE pur (SOD2) and pBABE neo (SOD2) carry the human SOD2 cDNA (Mn Superoxide dismutase). SOD 2 is an anti-oxidant expressed in the mitochondria of mammalian cells.

Antioxidants counteract the harmful effects of reactive oxygen species generated in cells through normal metabolic processes or as a result of exposure to pro-oxidant chemicals.

**Evaluation of foreseeable effects**

THE HOST CELLS
E cells: E cells are regarded by the HSE as a third generation packaging cell line. Eukaryotic cell culture systems are usually considered as 'especially disabled hosts' except where vectors they harbour can infect other hosts. In this case the vector can enter mouse host cells but it is unable to transmit viruses to other host cells. Consequently, the genetically modified cell line cannot be termed 'especially disabled'. However, the cells would not survive outside of tissue culture conditions and would not survive in the human body. Thus the host is a 'disabled' or non-colonising' host and of low hazard.

pCRIP cells: pCRIP is a high titre amphotropic helper-free packaging cell line derived from NIH 3T3. Viral producers derived from pCRIP do not transfer the packaging function or yield helper virus. Accordingly, pCRIP cells are regarded by the HSE as a third generation packaging cell line. Eukaryotic cell culture systems are usually considered as 'especially disabled hosts' except where vectors they harbour can infect other hosts. In this case the host is used to produce amphotropic viruses. These are able to infect the cells of their host and the cells of other species including human cells. Consequently, the cell line cannot be termed 'especially disabled'. However, the cells would not survive outside of tissue culture conditions and would not survive in the human body. Thus the host is a 'disabled or non-colonising' host and of low hazard.

HaCaT cells: HaCaT cells are a spontaneously immortalised human keratinocyte cell line, originally derived from normal male skin at the distant periphery of a melanoma. All eukaryotic cells and tissue culture systems can be considered as especially disabled hosts provided that the cell line is unable to colonise the worker and contains no known adventitious agents that are potentially harmful. In this case the vector can enter both mouse and human host cells but it is unable to transmit viruses to other host cells. Consequently, the cell line cannot be termed 'especially disabled'. However, the cells would not survive outside of tissue culture conditions and would not survive in the human body. Thus the host is a 'disabled or non-colonising' host and of low hazard.

All the above cells have a selective advantage over other non-transformed cells, as they are immortalised and harbour antibiotic resistance genes (ampicillin and puromycin resistance or ampicillin and neomycin resistance). However, it is extremely unlikely that suitable culture conditions could be found outside of the tissue culture environment. There is a slight risk that the antibiotic resistance genes used as selection markers could be transferred to the gut bacteria following accidental ingestion but this is highly unlikely.

RETROVIRAL VECTOR:
All vectors used are based on the pBABE family of vectors and differ only in respect to the insert DNA they harbour (CAT1, SOD1 or SOD2) and the antibiotic resistance gene carried to allow selection in mammalian cells. The possible hazards associated with the use of these vectors will be discussed collectively. The pBABE vectors are capable of integrating into both mammalian and bacterial cells and therefore the potential hazard from these vectors is that of transfer to other cells, in vitro or in vivo. The mobilisation functions between bacteria are unknown. Therefore, it must be assumed to be mobilisable. The pBABE series of vectors are derived from the Moloney murine leukaemia virus (Mo MuLV) and have been multiply disabled by deletion of the pol and env genes. Therefore, pBABE vectors on their own cannot further replicate and infect other mammalian cells.

In addition, the packaging cell line, pCRIP, used to produce the vector, has deletions in its packaging system genes to reduce packaging function transfer to vectors. At least two viral recombination events would be required to allow production of infectious virus. The ACGM guidelines state that viral vectors from which no infective virus can be produced should be regarded as minimal risk. Retroviral vectors produced from pCRIP can infect the cells of their host and the cells of other species including human cells. However, they are not capable of further cycles of replication. Therefore the risk of subsequent spread of infection is minimal. Since the retrovirus is 'defective' for replication, the hazard is therefore limited to handling of the producer cells and their culture medium, which contains the infected virions. The potential harm from accidental infection of the worker derives from the introduction of oncogenic sequences into exposed cells which, although almost certainly incapable of giving rise to a tumour alone, might nevertheless then have an increased chance of subsequently becoming tumourigenic if they acquired further spontaneous mutations. The chance of the above event occurring is greatly reduced by the following considerations:

- Even in optimised experimental conditions (containing fusogens such as polybrene) the efficiency of infection of human cells is still very low (estimated to be less than 1%).
- These viruses are extremely fragile and are, for example, exquisitely sensitive to desiccation.
- No doubt as a result of the above factors, a model human retroviral disease (AIDS) has never been associated with airborne droplet spread. By extrapolation, it is
extremely unlikely that any such route of infection would be significant with the vectors described here. In practice therefore, the only significant route of exposure is likely to be inoculation by 'needle-stick' injury and working practices have been designed to minimise aerosol production and eliminate the risk of penetrating injuries.

As above, there is a slight risk that successive viral recombination events could occur to give rise to infectious virus producing cells. This possibility has been minimised significantly through the construction of the pCRIP cells, which are regarded by the HSE as third generation packaging cell lines. These cells harbour the viral structural genes, gagpol and env on separate constructs to limit the risk of recombination.

The HaCaT cell line should not produce live viral vectors and will be monitored during initial culture for the presence of virus by electron microscopy, and then on a six monthly basis. Any cells producing virus will be destroyed.

GENETIC INSERTS: The following are inserted into the vectors:

Anti-oxidants: pBABE neo (CAT1) and pBABE pur (CAT1) carry the human CAT1 cDNA (catalase). Catalase is an anti-oxidant and is ubiquitously expressed in mammalian cells. pBABE puro (SOD1) and pBABE neo (SOD1) carry the human SOD1 cDNA (Cu/Zn Superoxide dismutase). SOD1 is an anti-oxidant expressed in the cytosol of mammalian cells. pBABE puro (SOD2) and pBABE neo (SOD2) carry the human SOD2 cDNA (Mn Superoxide dismutase). SOD2 is expressed in the mitochondria of mammalian cells. Anti-oxidants as a class function to counteract the harmful effects of reactive oxygen species generated in cells through normal metabolic processes or as a result of chemical exposure. There is no evidence to suggest that the increased expression of any of these anti-oxidants in human cells would constitute a risk to health.

ANTIBIOTIC RESISTANCE GENES

Bacterial B-lactamase gene confers resistance to ampicillin and is harboured by all pBABE plasmids. The expression of the bacterial B-lactamase gene has not been optimised.

The NeoR gene is carried by the pBABEneo vectors and confers resistance to the kanomycin/neomycin class of antibiotics. It is expressed from the SV40 early promoter of the pBABE series of vectors. The expression of this gene has not been optimised.

The Pac gene is carried by the pBABEpuro vectors and confers resistance to puromycin. It is expressed from the SV40 early promoter of the pBABE series of vectors. The expression of this gene has not been optimised.

For all the above antibiotic resistance genes, there is a slight risk that the gene could be transferred to gut bacteria following accidental ingestion but this is highly unlikely. Furthermore antibiotic resistance is lost in the absence of selective pressure. Alternative antibiotic therapies are available which could be used to limit the proliferation of transformed cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work surfaces will be treated with Virkon before and after handling these cultures. These cell lines are handled in microbiological safety cabinets, maintained on a 6 monthly basis, within a Containment Level 2 suite entered via a lobby, by trained personnel. The suite has restricted access and the cells are autoclaved before leaving the laboratory or are disposed of via incineration. Autoclaving is the preferred route and is considered to provide 100% kill of the GMO. Incineration will be used when the cells have been in contact with chemicals which are very toxic or whose toxicological properties are unknown. It was considered that the hazard generated from autoclaving these very toxic chemicals was far greater than that from any potential exposure to the GMOs. Incineration is via the Clinical Waste route at Colworth and is removed from site and incinerated by external contractors. Cultures and associated media are usually but not always sealed inside a container (eg tissue culture flask or Universal), then always double bagged on sawdust (to absorb liquid) before being placed in another plastic bag (sealed) inside sealed yellow one-way bins. Thus making it virtually impossible for the GMOs to escape during disposal. Accidental release to the environment is considered highly unlikely.
Servicing and validation of autoclaves takes place quarterly by specialist contractors to meet standards set down in Health and Technical Memorandum 2010 Part 3. Chart recordings of each run are examined before opening the machine to ensure that correct time and temperature have been reached. Process indicators are used with each load. Regular safety inspections are carried out in the laboratory to ensure high standards of GOSH and GMP are maintained and that the controls detailed in this assessment are in place.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.

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Project Ref 55/03.2

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<td>STABLE EXPRESSION OF ANTIOXIDANT GENES (CATALASE (CAT1), CU/ZN SUPEROXIDE DISMUTATSE (SOD1), MN SUPEROXIDE DISMUTASE (SOD2)) IN THE CULTURED HUMAN CELL LINES, SVPGC2A AND SQCC/Y1.</td>
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Non-GMM Consent Granted

Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N
Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The aim of the project is to stably express antioxidant genes (Catalase (CAT1), Cu/Zn Superoxide Dismutase (SOD1), Mn Superoxide Dismutase (SOD2)) in immortalised cultured human cells using retroviral gene transfer. A series of pBABE based vectors have been constructed under a previous risk assessment (not notified) as these will be used to stably express the cDNAs for human CAT1, SOD1 and SOD2 in the SV40 T-antigen transformed human buccal epithelial cell line, SVpgC2a and the buccal carcinoma cell line, SqCC/Y1. In the appropriate producer cell lines the pBABE vectors produce ecotropic and amphotropic retroviruses where gene expression is driven by the Moloney murine leukaemia virus (Mo MuLV) LTRs. All retroviruses are replication defective as many helper functions are supplied by the producer cell lines (Psi)CRIP and (Omega)E. (Cannot input symbols See form for correct symbols for this previous passage) Cells that have stably integrated the retroviral expression cassette will be selected on the basis of drug resistance to puramycin and neomycin.

The packaging cell lines (Omega)E pBABE (SOD1) and (Omega)E pBABE (SOD2) will be generated through infection of the (Omega)E packaging cell line with the pBABE vectors. The ecotropic supernatant produced by these cell lines, containing secreted retroviral virions, will be used to transfect (Psi)CRIP cells to produce the packaging cell lines (Psi)CRIP pBABE (CAT1); (Psi)CRIP pBABE (SOD1) and (Psi)CRIP pBABE (SOD2). In turn, the amphotropic supernatant produced by these cells will be used to transfect the SV40 T-antigen transformed human buccal epithelial cell line, SVpgC2a and the buccal carcinoma cell line, SqCC/Y1.

The risk assessment addresses the steps involved in the production of antioxidant expressing human cells as follows:
1) (Omega)E cells harbouring human cDNA for the genes identified above (preparation of stable ecotropic producer cell lines);
2) (Psi)CRIP cells harbouring human cDNA for the genes identified above (preparation of stable amphotropic producer cell lines); and
3) Cell lines SVpgC2a and SqCC/Y1 expressing human cDNA for the genes identified above.

The titre of the retroviral virions, secreted from the amphotropic producer cell lines described above, will be assessed through infection of human carcinoma A431 cells.

Recipient or parental organism

(Omega)E is a high titre ecotropic helper-free packaging cell line derived from NIH 3T3 mouse fibroblasts. It is ideal for use with pBABE vectors as the risk of generation wild-type virus is reduced due to extensive changes in the codon usage of the viral proteins. (Omega)E cells have separated gagpol and ecotropic env expression constructs with only minimum sequence overlap and decreased sequence homology achieved by ‘codon wobbling’.

(Psi)CRIP is a high titre amphotropic helper-free packaging cell line derived from NIH 3T3. It is ideal for use with pBABE vectors as the risk of generation of wild type virus is reduced due to the extensive changes in the codon usage of the viral proteins. (Psi)CRIP were engineered by sequential co-transformation of NIH 3T3 cells with two mutant Moloney murine leukaemia virus-derived proviral genomes carrying complementary mutations in the gagpol or env regions. Each genome contains a deletion of the Psi sequence necessary for the efficient encapsulation of retroviral genomes into viru particles and additional alterations at the 3’end of the procvirus. Viral producers derived from (Psi) CRIP do not transfer the packaging function or yield helper virus.

Both the above cells were supplied by Dr Christopher J Jones, University of Wales College of Medicine Cardiff, UK.
SVpgC2a is an immortalised bucal keratinocyte cell line that was derived from human buccal tissue obtained from a non-cancerous female non-smoker patient undergoing maxillo-facial reconstructive surgery. The cells were transformed using a plasmid, pRSV-T, described by Sakamoto et al, (1993). This plasmid consists of pBR322 DNA containing the origin of replication (pBR322 ori) and the bacterial B-lactamase gene (coding for ampicillin resistance), and an SV40 DNA sequence including the early promoter and the T antigen both of which are expressed from the Rous Sarcoma virus 3’ Long Terminal Repeat. The cells were supplied by Professor R C Grafstrom of the Karolinska Institute, Stockholm, Sweden.

SqCC/Y1 is a bucal carinoma cell line that was derived from a primary bucal squamous carcinoma from a human female. The cells were supplied by Professor R C Grafstrom of the Karolinska Institute, Stockholm, Sweden.

Host/vector system

Each of the above cell lines will be transfected with a pBABE vector. All vectors used are based on the pBABE family of vectors and differ only in respect to the insert DNA they harbour (CAT1, SOD1 or SOD2) and the antibiotic resistance gene carried to allow selection in mammalian cells. The pBABE vectors are capable of integrating into both mammalian and bacterial cells and therefore the potential hazard from these vectors is that of transfer to other cells, in vitro or in vivo. The pBABE series of vectors are derived from the Moloney murine leukaemia virus (MoMuLV) and have been multiply disabled by deletion of the pol and env genes. Therefore, pBABE vectors on their own cannot further replicate and infect other mammalian cells. At least two recombination events would be required in order to get a functional virus. Retroviral vector produced from the packaging cell line, (omega)E, can enter mouse host cells but is unable to transmit viruses to other host cells. There is a slight risk that successive viral recombination events could occur to give rise to infectious virus producing cells. This possibility has been minimised significantly through the construction of the (omega) cells, which are regarded by the HSE as third generation packaging cells. These cells harbour the viral structural genes, gagpol and env on separate constructs to limit the risk of recombination.

Retroviral vectors produced from (PSI)CRIP can infect the cells of their host and the cells of other species including human cells. However, they are not capable of further cycles of replication. Therefore the risk of subsequent spread of infection is minimal. Neither SVpgC2a and SqCC/Y1 should produce live viral vectors and this will be monitored on a 6-monthly basis.

The vectors also contain the bacterial B-lactamase gene, which confers resistance to ampicillin. The neo vectors contain the NeoR gene, which confers resistance to the kanomycin/neomycin class of antibiotics and is expressed from the SV40 early promoter of the pBABE series of vectors. The puro vectors contain the Pac gene, which confers resistance to puromycin and is also expressed from the SV40 early promoter of the pBABE series of vectors.

Origin & function

- pBABE neo (CAT1) and pBABE puro (CAT1) carry the human CAT1 cDNA (Catalase). Catalase is an anti-oxidant and is ubiquitously expressed in mammalian cells.
- pBABE puro (SOD1) and pBABE neo (SOD1) carry the human SOD1 cDNA (Cu/Zn Superoxide dismutase). SOD1 is an anti-oxidante expressed in the cytosol of mammalian cells.
- pBABE puro (SOD2) and pBABE neo (SOD2) carry the human SOD2 cDNA (Mn Superoxide dismutase). SOD2 is an anti-oxidant expressed in the mitochondria of mammalian cells.

Antioxidants counteract the harmful effects of reactive oxygen species generated in cells through normal metabolic processes or as a result of exposure to pro-oxidant chemicals.

Evaluation of foreseeable effects

The Host Cells:
(Omega)E cells : (Omega)E cells are regarded by the HSE as a third generation packaging cell line. Eukaryotic cell culture systems are usually considered as ‘especially disabled hosts’ except where vectors they harbour can infect other hosts. In this case the vector can enter mouse host cells but it is unable to transmit viruses to other host cells. Consequently, the genetically modified cell line cannot be termed ‘especially disabled’. However, the cells would not survive outside of tissue culture conditions and would not survive in the human body. Thus the host is a ‘disabled or non-colonising’ host and of low hazard.

(PSI)CRIP cells : (PSI) CRIP is a high titre amphotropic helper-free packaging cell line derived from NIH 3T3. Viral producers derived from (PSI)CRIP do not transfer the packaging function or yield helper virus. Accordingly, (PSI)CRIP cells are regarded by the HSE as a third generation packaging cell line. Eukaryotic cell culture systems
are usually considered as ‘especially disabled hosts’ except where vectors they harbour can infect other hosts. In this case the host is used to produce amphotropic viruses. These are able to infect the cells of their host and the cells of other species including human cells. Consequently, the cell line cannot be termed ‘especially disabled’. However, the cells would not survive outside of tissue culture conditions and would not survive in the human body. Thus the host is a ‘disabled or non-colonising’ host and of low hazard.

The SVpC2a cell line is an SV40 T antigen-immortalised buccal epithelial cell line, originally derived from human buccal tissue obtained from a non-cancerous female non-smoker patient undergoing maxillo-facial reconstructive surgery. Eukaryotic cell lines are usually considered as ‘especially disabled hosts’ except where vectors they harbour can infect other hosts. In this case the retroviral vector can enter both mouse and human cells but it is unable to transmit viruses to other host cells. Consequently, the cell line cannot be termed ‘especially disabled’. In addition, this cell line contains the pRSV-T plasmid, which is based on pBR322 DNA and contains the origin of replication (pBR322ori), the bacterial B-lactamase gene (coding for ampicillin resistance), and an SV40 DNA sequence including the early promoter and the T-antigen. The SV40 virus is a member of the papova group of small nonenveloped DNA viruses that cause lytic infection in monkey cells. Humans are non-permissive hosts for this virus, meaning that the virus cannot replicate in human cells. However, the viral DNA may become integrated into the host genome. When produced in quantity, the SV40 large and small T-antigens interfere with the normal growth regulatory systems, causing transformation. The level of transformation is low in human fibroblasts (Mayne et al, 1986). Southern blotting analysis has shown that the pRSV-T plasmid sequences were stably integrated into the genome for at least 90 passages (Kulkarni et al, 1995). Additionally, the presence of SV40 sequence alone is not sufficient for immortalisation to occur - further undefined process leads from transformation to immortalisation and not all transformed cells will become immortal. The mobilisation properties of the pRSV-T plasmid are unknown, therefore it must be considered as mobilisable but the parent vector pBR322 is considered ‘monilisation defective’ (Southern and Berg, 1982). In addition, these cells would not survive outside of tissue culture conditions and would not survive in the human body. The capacity of the parental line, SVpC2a, to produce tumours when transplanted into experimental animals has been investigated. These cells were non-tumourigenic when injected into athymic nude mice (Kulkarni et al, 1995). Thus the SVpC2a cell line is ‘disabled or non-colonising’ and of low hazard.

The S9CC/Y1 cell line was derived from a primary buccal squamous carcinoma from a human female. As above, this cell contains a vector that can enter both mouse and human host cells but it is unable to transmit viruses to other host cells. Consequently, the cell line cannot be termed ‘especially disabled’. The capacity of the parental line, S9CC/Y1, to produce tumours when transplanted into experimental animals has been investigated. These cells were tumourigenic when injected into athymic nude mice, are highly proliferative and are resistant to terminal differentiation (Kulkarni et al, 1995). Since its establishment in 1983 (Pitman et al, 1983) the S9CC/Y1 cell line has a history of safe use. However, there is a potential hazard that this cell line may be tumourigenic in humans especially for immuno-compromised individuals. Consequently, this host can not be regarded as of low hazard.

Vector DNA:
All the above cells have a selective advantage over other non-transformed cells, as they are immortalised and harbour antibiotic resistance genes (ampicillin and puromycin and neomycin resistance). However, it is extremely unlikely that suitable culture conditions could be found outside of the tissue culture environment. There is a slight risk that the antibiotic resistance genes used as selectin markers could be transferred to the gut bacteria following accidental ingestion but this is highly unlikely.

Retroviral Vector:
All vectors used are based on the pBABE family of vectors and differ only in respect to the insert DNA they harbour (CAT1, SOD1 or SOD2) and the antibiotic resistance gene carried to allow selection in mammalian cells. The possible hazards associated with the use of these vectors will be discussed collectively. The pBABE vectors are capable of integrating into both mammalian and bacterial cells and therefore the potential hazard from these vectors is that of transfer to other cells, in vitro or in vivo. The mobilisation functions between bacteria are unknown. Therefore, it must be assumed to be mobilisable. The pBABE series of vectors are derived from the Moloney murine leukaemia virus (Mo MuLV) and have been multiply disabled by deletion of the pol and env genes. Therefore, pBABE vectors on their own cannot further replicate and...
In addition, the packaging cell line, (PSI)CRIP, used to produce the vector, has deletions in its packaging system genes to reduce packaging function transfer to vectors. At least two viral recombination events would be required to allow production of infectious virus. The ACGM guidelines state that viral vectors from which no infective virus can be produced should be regarded as minimal risk. Retroviral vectors produced from (PSI)CRIP can infect the cells of their host and the cells of other species including human cells. However, they are not capable of further cycles of replication. Therefore the risk of subsequent spread of infection is minimal. Since the retrovirus is ‘defective’ for replication, the hazard is therefore limited to handling of the producer cells and their culture medium, which contains the infected virions. The potential harm from accidental infection of the worker derives from the introduction of oncogenic sequences into exposed cells which, although almost certainly incapable of giving rise to a tumour alone, might nevertheless then have an increased chance of subsequently becoming tumourigenic if they acquired further spontaneous mutations. The chance of the above event occurring is greatly reduced by the following considerations:

- Even in optimised experimental conditions (containing fusogens such as polybrene) the efficiency of infection of human cell is still very low (estimated to be less than 1%).
- These viruses are extremely fragile and are, for example, exquisitely sensitive to desiccation.
- No doubt as a result of the above factors, a model human retroviral disease (AIDS) has never been associated with airborne droplet spread. By extrapolation, it is extremely unlikely that any such route of infection would be significant with the vectors described here.

In practice, therefore, the only significant route of exposure is likely to be inoculation by ‘needle-stick’ injury and working practices have been designed to minimise aerosol production and eliminate the risk of penetrating injuries. As above, there is a slight risk that successive viral recombination events could occur to give rise to infectious virus producing cells. This possibility has been minimised significantly through the construction of the (PSI)CRIP cells, which are regarded by the HSE as third generation packaging cells. These cells harbour the viral structural genes, gagpol and env on separate constructs to limit the risk of recombination. The SVPGC2a and SqCC/Y1 cell lines should not produce live viral vectors and will be monitored during initial culture for the presence of virus by electron microscopy, and then on a six monthly basis. All cells producing virus will be destroyed.

Genetic Inserts: The following are inserted into the retroviral vectors:

- Anti-oxidants: pBABE neo (CAT1) and pBABE puro (CAT1) carry the human CAT1 cDNA (Catalase). Catalase is an anti-oxidant and is ubiquitously expressed in mammalian cells. pBABE puro (SOD1) and pBABE neo (SOD1) carry the human SOD1 cDNA (Cu/Zn Superoxide dismutase). SOD1 is an anti-oxidant expressed in the cytosol of mammalian cells. pBABE puro (SOD2) and pBABE neo (SOD2) carry the human SOD2 cDNA (Mn Superoxide dismutase). SOD2 is expressed in the mitochondria of mammalian cells. Anti-oxidants as a class function to counteract the harmful effects of reactive oxygen species generated in cells through normal metabolic processes or as a result of chemical exposure. There is no evidence to suggest that the increased expression of any of these anti-oxidants in human cells would constitute a risk to health.

Antibiotic Resistance Genes:

- Bacterial B-lactamase gene confers resistance to ampicillin and is harboured by all pBABE plasmids. The expression of the bacterial B-lactamase gene has not been optimised. The NeoR gene is carried by the pBABEneo vectors and confers resistance to the kanamycin/neomycin class of antibiotics. It is expressed from the SV40 early promoter of the pBABE series of vectors. The expression of this gene has not been optimised.
- The Pac gene is carried by the pBABEpuro vectors and confers resistance to puromycin. It is expressed from the SV40 early promoter of the pBABE series of vectors. The expression of this gene has not been optimised. For all the above antibiotic resistance genes, there is a slight risk that the gene could be transferred to gut bacteria following accidental ingestion but this is highly unlikely. Furthermore antibiotic resistance is lost in the absence of selective pressure. Alternative antibiotic therapies are available which could be used to limit the proliferation of transformed cells.
All work surfaces will be treated with Virkon before and after handling these cultures. These cell lines are handled in microbiological safety cabinets, maintained on a 6 monthly basis, within a Containment Level 2 suite entered via a lobby, trained personnel. The suite has restricted access and the cells are autoclaved before leaving the laboratory or are disposed of via incineration. Autoclaving is the preferred route and is considered to provide 100% kill of the GMO. Incineration will be used when the cells have been in contact with chemicals which are very toxic or whose toxicological properties are unknown. It was considered that the hazard generated from autoclaving these very toxic chemicals was far greater than that from any potential exposure to the GMOs. Incineration is via the Clinical Waste route at Colworth and is removed from site and incinerated by external contractors. Cultures and associated media are usually but not always inside a container (e.g. tissue culture flask or Universal), then always double bagged on sawdust to absorb liquid) before being placed in another plastic bag (sealed) inside sealed yellow one-way bins. Thus making it virtually impossible for the GMOs to escape during disposal. Accidental release to the environment is considered highly unlikely.

Servicing and validation of autoclaves takes place quarterly by specialist contractors to meet standards set down in Health and Technical Memorandum 2010 Part 3. Chart recordings of each run are examined before opening the machine to ensure the correct time and temperature have been reached. Process indicators are used with each load. Regular safety inspections are carried out in the laboratory to ensure high standards of GOSH and GMP are maintained and that the controls detailed in this assessment are in place.

The SqCC/Y1 cell line is tumourigenic when injected into athymic nude mice. Consequently there is a potential hazard of tumourigenicity through accidental exposure of the worker, although the extent of this is unknown. This hazard is limited to all use of the SqCC/Y1 cell line as well as the SqCC/Y1 derived cell lines. The potential harm from accidental infection of the worker can be greatly reduced through working practices. In this respect working practices have been designed to minimise aerosol production and eliminate the risk of penetrating injuries. The use of needles, sharps and glassware is forbidden for these experiments. Workers will use two pairs of gloves during all use of the cell lines described above and all cuts will be covered with plasters. If a worker cuts themselves during use of the cell line, the cut must be encouraged to bleed freely and then washed thoroughly with soap and water. The relevant first aid should be sought and the incident should be reported via the Colworth Incident Report system. By use of these measures, the cell line can be designated at ACDP Level 2.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
**Project Additional Information**

**Purposes of the contained use**
Identification of functionally active molecules through the use of stably-transfected HEK293 cells.

**Recipient or parental organism**
Human embryonic kidney cell line (HEK293).

**Host/vector system**
Host cells are HEK293 cells, and are transfected with a pCR3 (invitrogen) vector expressing a human cDNA.

**Origin & function**
Genetic material involved is of human origin and is exogenously expressed in a human cell line (HEK293). This new cell line created will be used to screen for inhibitors of the enzyme expressed from the vector transfected. The vector is used to give high-level expression of the enzyme in a cell line not known to express that protein.

**Evaluation of foreseeable effects**
HEK293 cells are a transformed human cell line which is commercially available. These cells are well characterised and authenticated by ECACC. HEK293 cells are human embryo kidney cells which have been transformed with sheared human Ad5 DNA. Ad5 is considered to be non-oncogenic and the use of sheared DNA to transform the cells further reduces any potential risk as no functional virus can be produced. The Ad insert has been shown to consist of a colinear segment from nts 1 to 4344 integrated into chromosome 19 (19q13.2). These cells are unlikely to survive outwith standard cell culture conditions and so pose only a very slight risk to human health.
The transfected vector contains the following elements (CMV promoter sequence driving expression of the cDNA of interest; T7 promoter; SP6 promoter; TK polyA signal; Kanamycin/neomycin resistance gene; SV40 promoter/origin; Ampicillin resistance gene; F1 replication origin). The vector is non-mobilisable and expresses a protein already present in human cells. Constitutive over-expression of this protein at levels far in excess of those naturally occurring may result in undesirable effects. However, it is very unlikely that this would occur in the human as the vector is non-mobilisable and all handling will be performed in Microbiological Safety Cabinets. Although antibiotic resistance genes are also expressed, without continued selection the vector will be lost.

The transfected cell line poses only a slightly higher risk than the parent cells. The transfected cell line will not survive without antibiotic selection and so possesses a low risk.

Eukaryotic cell culture systems are usually considered as ‘especially disabled hosts’ except where vectors they harbour can infect other hosts. In this case the vector is non-mobilisable and thus this cell line is considered “especially disabled host”.

Both the parent cells and the transfected/genetically modified cells have a history of safe use in other laboratories.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Cells will be handled in microbiological safety cabinets, maintained on a 6 monthly basis, within a containment level 2 lab. Access to room is restricted by means of a keypad lock, the number of which is only known by trained personnel. All waste will be disposed of as "clinical waste" in accordance with local codes of practice. Autoclaving is the preferred route and is considered to result in 100% cell kill. All staff using these cells have been trained in compliance with GM procedures and other workers in these labs will be GM aware. All spillages will be cleaned with 1% Virkon and gloves and bactigowns will be worn at all times when handling these cells.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All cell material and waste which has been in contact with GM materials will be sealed in autoclaveable bags, which in turn will be sealed in autoclaveable boxes. These boxes will then be autoclaved which is considered to result in 100% cell kill.

Service of autoclaves takes place quarterly by ACE to meet standards set down in Health and Technical Memorandum 2010 Part 3. Details of temperature profiles for each run are recorded.

In case of spillages in the laboratory. 1% Virkon is the disinfectant of choice and is always readily available.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

**Please enter comments on the GM safety committee on the risk assessment**

The committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.
### Project Containment

#### Laboratory Activities

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#### Glass Houses

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#### Growth Rooms

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### Laboratory Activities

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<tr>
<th>Animal Units</th>
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<th>Human Clinical Applications</th>
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### Project Ref 55/07.1

#### Date Ackn'd

07/03/2007

#### CU2 Project Title

Use of bioluminescent lux transformed ACDP class 2 bacteria.

#### Class

Class 2

#### CultureVol

< 1 Litre

#### Consent Granted

Not Applicable

#### Project notified under transitional arrangements

N

#### Withdrawn

N

#### Tick if notifying a connected programme of work

N

### Historical Significant Changes

#### Historical Date of Additional Info

07/03/2002

### Project Additional Information

#### Purposes of the contained use

Visualisation and quantification of bioluminescent bacteria attached to surfaces and in liquid suspension.

#### Recipient or parental organism

Staphylococcus aureus Xen36: S.aureus Xen36 was derived from the parental strain Staphylococcus aureus ATCC 49525. The parental strain was initially isolated from a bacteremia patient and its pathogenic. No mutations as such have been made to disable this strain. S aureus Xen36 us a Hazard Group 2 microorganism.

Staphylococcus Aureus29: S.aureus Xen29 was derived from the parental strain S.aureus 12600, a pleural fluid isolate which is also designated as NCTC8532. Xen29 is a Hazard Group 2 organism and a pathogenic strain. No mutations as such have been made to disable this strain.
Psuedomonas aeruginosa Xen41: P. aeruginosa Xen41 was derived from the parental strain P. aeruginosa PAO1, obtained from Pesuedomonas Genetic stock Center (PGSC). PAO strains are derived from the ATCC parent strain 15692. ATCC 15692 was isolated from an infected wound and it is a pathogenic strain. No mutations as such have been made to disable this strain. P. aeruginosa Xen 41 is a Hazard Group 2 microorganism.

Psuedomonas aeruginosa Xen05: P. aeruginosa Xen 5 was derived from the parental strain P. aeruginosa ATCC 19660, a mucoid strain isolated from a clinical case of human septicemia. No mutations as such have been made to disable this strain. P. aeruginosa Xen5 is a Hazard Group 2 microorganism.

Escherichia coli Xen14: E. coli X14 was derived from the parental strain E. coli WS2572, a clinical isolate from Weilhenstephen Culture Collection. No mutations as such have been made to disable this strain. E. coli Xen 14 is a Hazard Group 2 microorganism.

Psuedomonas aeruginosa Xen41: P. aeruginosa Xen 41 possesses a single stable copy of the P. luminescens lux operon on the bacterial chromosome. There are no disabling mutations in this strain. Xen41: Tetracycline (60 ug/mL) resistance gene used as selective marker.

Psuedomonas aeruginosa Xen05: P. aeruginosa Xen5 was engineered through conjunction and transposition of plasmid carrying transposon Tn5 luxCDABE. P. aeruginosa Xen5 possesses a single stable copy of the P. luminescens lux operon on the bacterial chromosome. There are no disabling mutations in this strain. Xen05: Tetracycline (60 ug/mL) resistance gene used as selective marker.

Escherichia coli Xen14: E. coli Xen14 possesses a stable copy of the Photorhabdus luninescens lux operon on bacterial chromosome. Xen14: Kanamycin (30 ug/mL) resistance gene used as selective marker.

Host/vector system

Staphylococcus aureus X36: S. aureus Xen36 possesses a stable copy of the modified Photorhabdus luninescens Kanamycin (200 ug/mL) resistance gene used as selective marker.

Staphylococcus aureus Xen29: S. aureus Xen29 possesses a stable copy of the modified Photorhabdus luninescens lux ABCDE operon at a single integration site on the bacterial chromosome. There are no disabling mutations in this strain. Xen29: Kanamycin (200ug.ml) resistance gene used as selective marker.

Psuedomonas aeruginosa Xen41: P. aeruginosa Xen 41 possesses a single stable copy of the P. luminescens luxCDABE operon on the bacterial chromosome. There are no disabling mutations in this strain. Xen41: Tetracycline (60 ug/mL) resistance gene used as selective marker.

Psuedomonas aeruginosa Xen05: P. aeruginosa Xen5 was engineered through conjunction and transposition of plasmid carrying transposon Tn5 luxCDABE. P. aeruginosa Xen5 possesses a single stable copy of the P. luminescens lux operon on the bacterial chromosome. There are no disabling mutations in this strain. Xen05: Tetracycline (60 ug/mL) resistance gene used as selective marker.

Escherichia coli Xen14: E. coli Xen14 possesses a stable copy of the Photorhabdus luninescens lux operon on bacterial chromosome. Xen14: Kanamycin (30 ug/mL) resistance gene used as selective marker.

Origin & function

The primary objective of the inserted gene is to transform the parental bacterial strain into a bioluminescent one. As a result, growth of such bacteria can be monitored by imaging systems (such as the Caliper-Xenogen IVIS system).

In their natural host, the nematode symbiont and insect pathogen, Photorhabdus luminescens. The lux bioluminescence genes function as quorum sensing system, in which the genes responsible for bioluminescence (lux genes) are expressed in response in increasing cell density.

Five, non-regulatory, lux genes are associated with the expression of bioluminescence. The genes are present on a single operon (the lux operon). These five genes are called luxA-luxE, with luxA and B encoding the alpha and beta subunits of the heterodimeric luciferase and luxCDE encoding proteins for production of the luciferin substrate.

Evaluation of foreseeable effects

Staphylococcus aureus X36, Staphylococcus aureus Xen29, Pseudomonas aeruginosa Xen41, Pseudomonas aeruginosa Xen05 and Escherichia coli Xen14 are all commercially available bacterial strains that have been modified to contain the Lux ABCDE gene operon of Photorhabdus luminescens.

All 5 bacteria strains are classified as Hazard Group 2 and as such represent a potential hazard to human health and will therefore only be used in suitable equipped laboratory facilities.

The Lux ABCDE operon contains the genetic material required for synthesis of the luciferase enzyme and the generation of its substrate luciferin. The reaction of luciferase with luciferin generates light (bioluminescence) and so can be used as an indicative marker of both the presence of bacteria and of their viability.

Although prolonged contact with the enzyme (luciferase) is associated with skin sensitization amongst sensitive individuals, both luciferin and luciferase are commercially available as purified products and are not classified as hazardous materials.
The inclusion of the lux operon in the target bacteria is not associated with any increase in virulence, however, the plasmid vector used to transfer the organism carries an antibiotic resistance gene in order to assist in the selection of transformed strains. Consequently the transformed organisms will have a broader antimicrobial resistance spectra than the parent strains. The details are given above, but in summary, strains Xen36, Xen29 and Xen14 will display enhanced resistance to Kanamycin and the strains Xen41 and Xen05 will show elevated resistance to tetracycline.

Horizontal gene transfer of the bioluminescent lux operon from the transformed organism to the external environment is unlikely due to the contained use protocols governing the proposed experimentation. If, however, any release did occur the capacity to emit bioluminescence is not associated with any evolutionary advantage outside the specific niches of those few organisms that have evolved such a capacity. The lack of any evolutionary advantage thus makes it unlikely that the lux operon would be stably maintained in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cell material and waste which has been in contact with GM materials will be placed in autoclave bags, and then in autoclave boxes. These boxes will then be autoclaved which is considered to result in 100% kill. A waste form will be filled in by the GMO worker to record the type of waste being disposed of and this will accompany the waste to the autoclave. This form will be archived in the GMO autoclave log book. The autoclave is serviced and calibrated four times per year.

In case of spillages in the laboratory either a solution of 1% Virkon or 70% ethanol will be used as the disinfectant of choice depending on the chemical tolerances of the surface being disinfected.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.

**Project Containment**

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02/03/2022
### Project Additional Information

#### Purposes of the contained use

The purpose of the contained use is to investigate the physiological and molecular features of transfected (immortalised) human epithelial cells derived from the skin.

#### Recipient or parental organism

see section 17

#### Host/vector system

The genetic material will be cDNAs encoding cellular proteins that are already well characterised and in the public domain i.e. The E6 and E7 proteins of human papilloma virus. The HPV16/E6/E7 genes probably act by blocking the inhibition of cell cycle progression by inhibiting the activity of such genes as CIP-1/WAF-1/p21, Rb, p53 and p16 thus giving an increased life span, reducing DNA surveillance, and giving an enhanced opportunity for further mutations within the immortalised cells.

#### Origin & function

The expectation is that the human cells expressing th cDNAa will be capable of indefinite proliferation in culture, and will acquire an ability to form anchorage independent colonies in semi-solid medium. However, they are not expected to represent a hazard to human health as none of the cells used are capable of colonising a healthy individual and we do not expect the manipulations to alter this. The cells will not be expected to release infectious viral particles. The retroviral vectors are incapable of
replication because of the viral pol and env genes and the PT67 packaging cell line also has the requisite genes deleted to prevent such an occurrence. Forseeable effects will be the immortalisation of skin derived cell lines.

**Evaluation of foreseeable effects**

The infection of the cultured human epithelial cells with HPV16E6E7 inserts will result in the production of immortalised cell lines. The cells will continue to multiply only when grown in suitable culture conditions. The cells should not survive outside culture conditions or in the human body, and therefore, unlikely to spread or cause damage to the environment. We propose to carry out all manipulations in a Class II microbiological safety cabinet following GLP and any additional local safety guidelines appropriate to this work.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All cell material and waste which has been in contact with GM materials will be placed in autocleavable bags, which in turn will be placed in autocleavable boxes. These boxes will then be autocleaved which is considered to result in 100% cell kill. A waste form will be filled in by the GMO worker to record the type of waste being disposed of and this will accompany the waste to the autoclave. This form will be archived in the GMO autoclave log book. Servicing of autoclaves takes place quarterly to meet standards set down in Health and Technical Memorandum 2010 Part 3. Details of temperature profiles for each run are recorded. Clinical incineration is used as a route of disposal if the cells have been treated with chemicals which are very toxic or whose toxicological properties are unknown. In these circumstances, the hazard generated from autoclaving these very toxic chemicals is considered greater than that from any potential exposure to GMOs. If the GMOs are intended for incineration, they are usually but not always sealed inside a container (eg tissue culture flask), then always double bagged on sawdust (to absorb liquid) before being placed in another plastic bag (sealed) inside non-returnable, sealed waste bins. In case of spillages in the laboratory either a solution of 1% Virkon or 70% ethanol will be used as the disinfectant of choice depending on the chemical tolerances of the surface being disinfected.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

Y

**Please enter comments on the GM safety committee on the risk assessment**

The committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.

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02/03/2022
Purposes of the contained use

The CHO-K1 cell line has been used to generate three different clones that stably express shRNA targeting the hamster antioxidant genes Catalase (CAT), Superoxide Dismutase 1 (SOD1) or Glutathione Synthetase (GSS) and Glutamate Cysteine Ligase (GCLC), with the aim of producing cell lines with gene knockdowns for those antioxidant genes and therefore a greater susceptibility to the DNA damaging activity of pro-oxidant materials compared to normal CHO-K1 cells.

Recipient or parental organism

Chinese Hamster Ovary (CHOK-K1) cell line.

Host/vector system

The host are CHO-K1 cells, they were transfected with the pRS-Puro (Origne Inc.) vector containing a shRNA sequence targeting the hamster antioxidant genes, CAT, SOD1 or GSS or GCLC.
The genetic material involved is of hamster origin.

Pro-oxidant chemicals which generate reactive oxygen species (ROS), are often detected as genotoxic in in vitro mammalian cell based assays. In vivo, the damaging effects of ROS are counteracted by enzymes such as catalase and superoxide dismutase, as well as other antioxidants defence mechanisms, therefore it is widely regarded that pro-oxidant chemicals have a threshold below which no genotoxicity is observed. In vitro cell systems with limited antioxidant defences may be expected to be more susceptible to the DNA damaging activity of ROS and as such the genotoxicity of pro-oxidant chemicals seen in vitro may not be relevant to the in vivo situation. These CHO-K1 cell lines with gene knockdowns for CAT, SOD or GSS & GCLC antioxidant genes should show a greater susceptibility to the DNA damaging activity of pro-oxidant materials compared to normal CHO-K1 cells. These cell lines will be evaluated using in vitro genotoxicity assays such as the in vitro micronucleus assay and/or the Comet assay.

Evaluation of foreseeable effects

Host

CHO-K1 cells are commercially available epithelial cells derived as a subclone from the parental CHO cells. The cell line is not known to harbour an agent known to cause disease in healthy adult humans and is reverse transcriptase negative. The cells would not survive outside culture conditions and they would not survive in the human body. Thus the host is a 'disabled or non-colonising' host and of low hazard. The genetically modified clones described in the risk assessment have been screened for mycoplasma and bacterial contamination with negative results (i.e. no contamination detected).

Vector for CHO-K1 gene knockdowns

The HuSH shRNA cloning plasmid vector, pRS-Ouro (Origene Inc.) was used to generate three CHO-K1 cell lines with hamster gene knockdowns for CAT, SOD1 or GSS and GCLC. The vector allows stable delivery of a shRNA expression cassette into host cells by direct transfection.

The pRS-Puro plasmid vector contains the following functional elements:
- Both 5' and 3' long terminal repeats (LTRs) of the Moloney murine leukaemia virus
- Human U6 polymerase III promoter located for shRNA expression
- SV40 early promoter for regulation of the mammalian cell drug resistance marker, puromycin
- Puromycin-N-acetyl transferase gene, conferring resistance to puromycin
- pBR322 bacterial origin of replication
- Ampicillan resistance gene for bacterial selection

The pRS-Puro vectors are capable of integrating into mammalian cells therefore the potential hazard from these vectors is that of transfer to other cells in vitro or in vivo. Although these vectors could potentially be incorporated into bacterial cells, the promoters driving expression of the mammalian gene would be expected to be low non-functional. The pRS-Puro vectors contain retroviral LTRs and packaging signal but do not contain the retroviral DNA sequences gag, pol or env and therefore require a packaging cell line in order to produce a virus. The 3' LTR of the pRS vector has been mutated (has a deletion) so that the virus is self-inactivating (SIN) and therefore replication deficient. In the unlikely event that a virus is generated from this vector, the virus would not be able to further replicate and therefore could not infect other mammalian cells. The ACGM guidelines state that vectors from which no infective virus can be produced should be regarded as minimal risk.

Insert DNA

The CHO-K1 knockdown (KD) cell lines all contain a shRNA sequence targeting the hamster antioxidant genes, CAT, SOD1 or GSS & GCLC. It is unknown whether the cloned shRNAs targeting the hamster genes have sufficient sequence homology to target the human genes. However the catalase and SOD1 genes in particular are highly conserved between species. It should therefore be assumed the shRNAs have the potential to target the human genes.

Catalase (CAT)

The CHO-K1 CATKD cells contain a shRNA sequence targeting the hamster catalase gene. Catalase is ubiquitously expressed in mammalian cells where its function is to counter the harmful effects of reactive oxygen species, predominantly peroxyl radicals, which are generated in cells through normal metabolic processors or as a result of chemical exposure. Decreases in catalase activity in humans does not show life threatening harmful effects under normal levels of oxidative stress due to the presence of...
other antioxidant enzymes, such as glutathione peroxidase, which also removes hydrogen systems. Detects in the gene encoding for the catalase protein can cause acatalasaiain humans, which is a rare autosomal recessive disorder resulting from the absence of catalase activity. Although it is usually asymptomatic, a syndrome of oral ulcerations can occur.

Superoxide Dismutase 1 (SOD1)
The CHO-K1 cells contain shRNA sequence targeting the hamster SOD1 gene. SOD1 is expressed in the cytosol of mammalian cells where its product counters the harmful effects of reactive oxygen species, specifically superoxide radicals, which are generated in cells through normal metabolic processes or as a result of exposure to pro-oxidant chemicals. In mice, inactivation of SOD1 has been shown to contribute to hyperplasia in the liver and hepatocellular carcinoma through the presence of persistent oxidative damage to proliferating cells. It is therefore possible that a decrease in SOD1 activity in humans could result in an increase in oxidative DNA damage leading to cancer promotion.

Glutathione synthetase (GSS) and glutamate-cysteine ligase (GCLC)
The CHO-K1 GSS&GCLCKD cells contain a shRNA sequence targeting the hamster GSS and GCLC genes. Glutathione synthetase catalyzes the second step of glutathione biosynthesis, which is the ATP-dependent conversion of gamm-L-glutamyl-L-cysteine to glutathione. Glutamate-cysteine ligase, also known as gamma-glutamylcysteine synthetase is the first rate limiting enzyme of glutathione synthesis. It is anticipated that knockdown of both of these genes will result in decreased levels of glutathione. Glutathione is important for a variety of biological functions, including protection of cells from oxidative damage by free radicals, detoxification of xenobiotics, and membrane transport. Glutathione synthetase deficiency is an autosomal recessive disorder that prevents the production of glutathione. Individuals with the disorder often exhibit a tendency to haemolysis and in severe cases acidosis and neurological defects.

Antibiotic resistance genes
The Puromycin-N-acetyl transferase gene confers resistance to puromycin and is expressed from the SV40 early promoter in the pRS-puro vector to allow selection in bacteria. There is a slight risk associated with these genes used as a selection markers in that resistance may be transferred to the gut bacteria if ingested. However, alternative antibiotic therapies are available which could be used to limit the proliferation of transformed cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work surfaces will be treated with Virkon before and after handling these cultures. These cell lines are handled in microbiological safety cabinets, maintained on a 6 monthly basis, within a Containment level 2 suite entered via a lobby, by trained personnel. The suite has restricted access and the cells are autoclaved before leaving the laboratory or are autoclaved or are disposed of via incineration. Autoclaving is the preferred route and is considered to provide 100% kill of the GMO. Incineration will be used when the cells have been in contact with chemicals which are very toxic or whose toxicological properties are unknown. It was considered that the hazard generated from autoclaving these very toxic chemicals was far greater than that from any potential exposure to the GMOs. Incineration is via the Clinical Waste route at Colworth and is removed from site and incinerated by external contractors. Cultures and associated media are usually but not always sealed inside a container (e.g. tissue culture flask or Universal), then always double bagged on sawdust (to absorb liquid) before being placed in another plastic bag (sealed) inside sealed yellow one-way bins. Thus making it virtually impossible for the GMOs to escape during disposal. Accidental release to the environment is considered highly unlikely.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.

Please enter comments on the GM safety committee on the risk assessment

The committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Project Ref 55/12.1

Date Ackn'd 11/10/2012

CU2 Project Title The use of lux transformed ACDP class 2 bacteria

Class 2 CultureVolClass2 CultureVolumeClass3-4
< 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Withdrawn N

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

The use of bacterial bioluminescence to assess the viability of the target organism in preservative challenge testing.

Recipient or parental organism

The 14 recipient bacterial strains (listed below) were originally isolated from industrial sources with the exception of the genome reference strain Burkholderia lata strain 383 which was recovered from soil and the reference Staphylococcus spp. that originated from a clinical source. Engineered genetic control measures (Biological containment) were not employed to attenuate or disable the recipient strains; they are unchanged form their original isolation. All strains are classified as ACDP Hazard Group 2 in accordance with CoSHH.

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<td>ESH749</td>
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<td>ESH750</td>
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<td>BCC0803</td>
<td>Burkholderia lata strain 383</td>
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Host/vector system

The following vector systems will be used to create bioluminescent derivatives of the recipient strain panel described above. All vector systems selected have been widely used in scientific research and have a track record of publication associated with their construction and use.


2. Gram positive broad host range plasmid vector. Plasmid pAL2 is a broad-host range vector that carries the lux-ABCDE operon from P.luminescens and an erythromycin resistance gene as a selective marker for transformants. This vector system was used specifically for the Gram-positive Staphylococcus spp. The plasmid is non-mobilisable and requires electroporation for its introduction into recipient bacteria. The construction and use of this vector has been described by Beard, S. et al. Antimicrob Agents Chemother, 2002 46(2)S38-542.

3. Integrative transposon vector. The mini-Tn5-lux vector contains the inner and outer transposase recognition sequences flanking a promotless luxCDABE operon from
P. luminescens, and a tetracycline resistance gene for transformant selection. Once mobilised and inserted into a new recipient strain, the integrated mini-Tn5 luxCDABE construct is not self transposable and is stably maintained in the absence of antibiotic. The construction and use of this vector has been described by Winson, M.K. et al. FEMS Microbiol Lett. 1998. 163(2) 193-202; Lewwnza, S. et al. Genome Res. 2005. 15(4)538-589.

4. A pseudomonas integrative vector. The ФCTX-based integration-proficient mini-CTX-lux vector is a non-replicative plasmid system that allows the integrase mediated insertion of the lux gene operon from P. luminescens at the attB sequence (phage attachment site) located on the P. aeruginosa chromosome. Following integration, unwanted sequences, including plasmid-associated promoters, integrase, and the tetracycline resistance gene marker can be removed in vivo by yeast Flp recombinase. The construction and use of this vector has been described by Hoanf, T., et al. Plasmid, 2000.43(1)59-72; Schwartz, H.P. Curr Opin Niotech, 2001. 12(5)439-445.

5. Well characterised bacterial lux-vectors from published literature will be used as necessary. The effect of using such vectors on the fitness (i.e. growth characteristics), preservative and antibiotic susceptibility will be investigated using published methodology; this will ensure the GMOs are not higher risk than the wild-type. The lux construct including selective marker, of such vectors will not be self-transmissible in recipient bacteria.

**Origin & function**

The primary role of the inserted lux gene operon is to generate a self-bioluminescent transformant of the parental bacterial strain. As a result, the growth of such bioluminescent bacteria can be monitored using an appropriate imaging system (such as Tecan Infinite M200 PRO). In their natural host, the ACDP Hazard Group 1 soil bacterium P. luminescens, the lux bioluminescence genes produce light exclusivity during the post-exponential phase of growth when the bacteria is infecting insect larval hosts at high cell densities.

Light production in bacteria requires the presence of a give-gene operon luxCDABE. The luxA and luxB genes encode the alpha and beta subunits of heterodimeric luciferase. The product of the luxC, luxD and luxE genes is predicted to form a fatty acid reductase that provides the luciferase enzyme with an aldehyde substrate.

Other than the emission of light, the lux gene system is not associated with any other known function such as antimicrobial resistance, virulence or persistence, that would confer an advantageous phenotype on recipient strains.

**Evaluation of foreseeable effects**

All fourteen bacterial strains have been assigned to ACDP Hazard Group 2 and as such may pose a threat to human health and will therefore only be used in a suitably equipped laboratory facility.

The lux ABCD operon contains the genetic material required for the synthesis of the luciferase enzyme and the generation of its substrate luciferin; the reaction of luciferase with luciferin generates light (bioluminescence). Light output from these bioluminescent bacteria is a highly sensitive reporter of metabolic activity which can be used to monitor the viability of the target organism in preservative challenge testing.

As in the case with many enzymes, prolonged or repeated exposure to luciferase may cause sensitisation in some sensitive individuals; however, both the enzyme luciferase and its substrate luciferin are commercially available as purified chemicals and are not classified as hazardous materials. The level of exposure to this enzyme within the reporter bacteria is below the risk associated with excess exposure to ACDP Hazard Group 2 bacteria.

The insertion of the lux ABCDE operon as a plasmid or into the chromosome of the target bacteria is not associated with an increase in virulence; however, the use of antibiotic resistance markers to aid selection of transformants and retention of genetic material, will confer extended antimicrobial resistance, will confer extended antimicrobial resistance spectra on the transformed bacteria relative to the wild type parental strain. However, all the antibiotic selection markers used (tetracycline, cloramphenicol, gentamycin, trimethoprim and erythromycin) have a long and safe track record of use in the genetic modification of bacteria.

Horizontal gene transfer of the bioluminescent lux operon or antibiotic resistance gene marker from the transformed bacteria to the external environment is highly unlikely due to the use of containment protocols governing the proposed experimentation. In the highly unlikely event of a release the capacity to emit bioluminescence would confer no evolutionary advantage on the organism and so is unlikely to favour the long term bioaccumulation of any released organisms in the environment. Similarly the potential
for the horizontal spread of genes encoding antibiotic resistance would not confer an evolutionary advantage outside an environment where antibiotics are in frequent use, such as a hospital.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All of the GMOs cited under this notification are micro-organisms.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All cell material and waste which has been in contact with GM material will be sealed in autoclavable bags, which in turn will be placed into autoclavable boxes. These boxes will then be autoclaved which is considered to result in 100% kill. A waste form will be filled in by the GMO worker to record the type of waste being disposed of and this will accompany the waste to the autoclave. This form will be archived in the GMO autoclave log book.

The autoclave has an electronic chart recorder/data logger for monitoring load and drain temperatures. Before the autoclave is opened, the paper chart will be checked to confirm the required process has been achieved.

The autoclave is serviced 6 monthly and validated (according to Health and Technical Memorandum 2010 part 3) by specialist contractors quarterly. In case of spillages in the laboratory either a solution of 1% Virkon or 70% ethanol will be used as the disinfectant of choice depending on the chemical tolerances of the surface being disinfected. After treatment with disinfectant the spill will be absorbed using paper towels and all material used in this clean up will be subjected to autoclaving as above to ensure complete destruction of the GMO.

**Is an emergency plan required according to regulation 20?** No

If yes, tick to confirm that it is attached to this form No

Tick to confirm that you have attached a risk assessment to this form Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment No

Please enter comments on the GM safety committee on the risk assessment

The committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.

**Project Containment**

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<th>Laboratory Activities</th>
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**Animal Units**

**Large Scale Activities**

**Human Clinical Applications**
### Project Ref 55/98.1

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#### Withdrawn

| N |

#### Tick if notifying a connected programme of work

| N |

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### Project Additional Information

#### Purposes of the contained use

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Human Clinical Applications

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02/03/2022
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Name

UNIVERSITY OF WALES ABERYSTWYTH

Name 2

Department

INSTITUTE OF BIOLOGICAL SCIENCES

Campus Estate or Research Centre

PENGLAIS CAMPUS

Building

EDWARD LLWYD BUILDING

Road Name

District

Town

ABERYSTWYTH

County

CEREDIGION

Postcode

SY23 3DA

Country

WALES

Tel Number

01970 621515

Fax Number

01970 622350

E-mail

HSE Division

WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Level 4 (GMMs)
Non-microbial

Other (please specify)

Tick if confidential

Bacteriology
Parasitology
Transgenic
Microbiology

Birds
Research

Virology
Transgenic
Animal
Transgenic
Gene Therapy

Animals
Fish

Mycology
Transgenic
Invertebrates
Transgenic
Other (please
Plants
specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

For our waste management all solid and liquid waste is deactivated by autoclaving. On average there are five autoclave runs daily across the different locations in machines up to 150L volume. The autoclave performance is monitored by assigned technical staff using print-outs, direct temperature measurements and bioindicators.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 245/02.1

Date Ackn'd 08/04/2002
CU2 Project Title GENETICALLY MODIFYING THE BIOCHEMICAL AND PHYSIOLOGICAL

Class 2
CultureVolClass2 < 1 Litre
CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use
To improve understanding of plant metabolism, especially in legumes, by altering gene expression.

Recipient or parental organism
Lotus and Trifolium species
Agrobacterium rhizogenes strain C58C1 LBA9402
[Plant pathogen. Class 2. Use requires Plant Health license from DEFRA]

Host/vector system
Disabled E. coli/pUC/pLC/pBluescript/pBin19 derivatives used to transform Agrobacterium rhizogenes
Agrobacterium rhizogenes is used to transform plants genetically eg Lotus and Trifolium.

Origin & function
The Genetically Modified Micro-organism Agrobacterium rhizogenes acts as a plant vector and the modifications do not involve manipulating its plant pathogenicity.

Genes isolated from a range of bacterial and plant sources will be introduced into A. rhizogenes, which will then be used to transform plants. None of the inserted genes will be expressed in A. rhizogenes. The inserted sequences include selectable marker genes, reporter genes and known genes or non-characterised plant sequences inserted in sense and antisense orientations that will potentially alter secondary metabolism and symbiotic interactions of the plants transformed with the sequences concerned. Inserted sequences will include
a) selectable markers for antibiotic resistance - kanamycin and hygromycin (from bacteria) - and herbicide tolerance - phosphinothricin (PPT, bacteria).
b) reporter genes - glucuronidase (bacteria) and green fluorescent protein (jellyfish).
c) secondary metabolism genes from flavonoid pathway - chalcone synthase (plant), dihydroflavonol reductase (plant)
d) symbiotic genes - early and late nodulins, calcium binding protein (plant)
e) sequences isolated from plants, but whose role in the biochemical pathway is unknown, and is being determined.

Evaluation of foreseeable effects
Genetically Modified Microorganisms. A. rhizogenes:
<table>
<thead>
<tr>
<th>Risk to human health</th>
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<tbody>
<tr>
<td>Access</td>
<td>10 (-3) A. rhizogenes equivalent to Streptomyces sp. or Bacillus subtilis</td>
</tr>
<tr>
<td>Expression</td>
<td>10 (-6) Site not facilitating expression</td>
</tr>
<tr>
<td>Damage</td>
<td>10 (-9) Biological effect unlikely</td>
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<tr>
<td>Overall</td>
<td>10 (-18)</td>
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The inserted bacterial and plant genes have no foreseeable effect on human health.

Ris to Environment

Agrobacterium rhizogenes is a soil-borne plant pathogen causing hairy root disease. Current information indicates that its pathogenicity has little economic impact on crop production. A rhizogenes is used as a plant vector and the modifications do not involve manipulating its plant pathogenicity. The inserted sense and antisense genes - antibiotic or reporter genes, known genes or non-characterised sequences related to metabolism in plants - are not expressed in the bacterium and are themselves unlikely to alter the pathogenicity of the bacterium.

While A. rhizogenes has the potential for a minor impact on the environment as a plant pathogen, the inserted genes are unlikely to increase that potential. In the unlikely event that genetically transformed A. rhizogenes were to escape and effect indigenous plants, the resultant hairy root tissue would contain and could express any of the inserted genes. However, any expression would be restricted to the hairy roots themselves and these hairy roots are unlikely to regenerate into new plants. Plant regeneration from roots usually requires very specific, controlled culture conditions, and then only occurs in a limited number of species.

The inserted gene would not be expected to confer any significant advantage on the hairy roots unless other unlikely conditions were also met. For example, hairy root tissue transformed with the PPT herbicide tolerance gene (as the result of accidental escape of A. rhizogenes) might be at an advantage if it came into contact with the herbicide. However, this herbicide is not used routinely in British agriculture and, normally, roots die without a shoot system. In a worse case scenario, these plants might be unusual and be able to regenerare shoots directly from the herbicide tolerant hairy roots.

The containment measures adopted will prevent escape of genetically manipulated A. rhizogenes. All bacterial and plant/bacterial work will be done in Class 2 safety cabinets. The bacterium will be maintained in sealed units, cultures will be contained within the laboratory and kept in growth rooms. A warning notice will be displayed next to bacterial cultures and plant cultures potentially containing A rhizogenes as agreed by DEFRA. Plant material will be rigorously tested for sterility to ensure that A. rhizogenes is removed prior to transfer to soil or equivalent in Plant Containment Level A growth rooms and glasshouses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The resulting GM plants will be grown under established facilities under agreed procedures for Containment Level A, in a locked, insect-proof compartment with no free drainage.
All transgenic material will be free of Agrobacterium species before transfer to soil.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All waste is collected in labelled autoclave bags and autoclaved at 121 degrees C, 40 minutes, 15 psi
Autoclave is tested annually using a thermocouple and whenever autoclave is re-commissioned after maintenance work.
Autoclaved waste collected and disposed of by the local authority.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The local Genetic Modification Safety Committee (GMSC) considers that the risk assessment meets current regulations even though the comments on the original risk assessment were made under the regulations enforced in 1993. The agreed procedures state that all manipulations using A. rhizogenes will be done in a Class 2 hood.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Project Ref** 56/02.1

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<td>02/10/2002</td>
<td>MUTAGENESIS OF WILD TYPE BACTERIAL STRAINS USING MOBILISABLE GENETIC ELEMENTS OR BY HOMOLOGOUS RECOMBINATION</td>
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Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**

Microbial populations mutated with mobile genetic elements will be screened for transcriptional activity in response to environmental stimuli in Chromobacterium violaceum, Pseudomonas aeruginosa, Burkholderia cepacia, Pseudomonas fluorescens and Pseudomonas putida, Pseudomonas aerantiaca and Acinetobacter spp. In many cases the mobile elements will encode transcriptional reporter genes e.g. luciferase and associated genes (luxCDABE) or green fluorescent proteins (gfp/yfp) (see Section 7). Transcription of the reporter genes will be driven by the regulatory sequences ("promoter") of the gene within which the transposon has inserted (assuming the reporter gene is appropriately orientated). Thus, these reporter fusions will be used to monitor gene activity in response to environmental stimuli. Key genes identified through random mutagenesis and screening may suggest further candidates for gene-knockouts or non-disruptive reporter insertion. These could represent homologous genes identified by database searches, functionally linked components of signalling or biosynthetic pathways or adjacent co-regulated genes (part of an operon).

Directed mutagenesis will be carried out using in situ exchange of an endogenous gene with a mutated version by homologous recombination. The mutated gene will be generated by the introduction of a ‘cassette’ containing a selectable marker (an antibiotic resistance gene) and genes (luxCDABE, gfp/yfp) coding for luminescence/fluorescence.

**Recipient or parental organism**

Mutated populations will be generated in the category 2 wild type strains: Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas aurantiaca; and in the following category 2 wild type strains (where strains from the same species have been identified as opportunistic animal/human pathogens): Chromobacterium violaceum, Pseudomonas aeruginosa, Burkholderia cepacia and Acinetobacter spp.

**Host/vector system**

The following genetically mobilisable systems based on transposon Tn5 (de Bruijn and Lupski JR. 1984 Gene27:131-149) will be employed:

(i) Mini - Tn5 transposon (de Lorenzo et al., 1990, J. Bact. 172 172; 6568-6572) derivatives to include a promoterless luxCDABE operon (Winson et al., 1998 FEMS Microbiol Lett. 163:193-202) or gfp (encoding green fluorescent protein (Miller & Ludow 1997 191;149-153)). The mutants generated in this way will include one or a combination of elements containing a gene coding for resistance to kanamycin, streptomycin, chloramphenicol and tetracycline introduced into the bacterial strain on the pUT suicide plasmid (the suicide plasmid will not be maintained within the bacterial cell).

(ii) Genetic constructs for marker exchange will be introduced into recipient strains by conjugation from an auxotrophic E. coli S17-1 pir donor using the following suicide vectors which lack a suitable origin of replication for maintenance in the target strain. (A) pUCMOB = pUC18 origin of mobilisation (oriT) cloned from pSUP5011 (Simon et al., Mol.Gen. Genet. (1984) 196: 413-420; Winson (1991) PhD UW Bangor "Molecular Biological Studies on Catabolic Plasmids").

(B) pMOB variants 1,2,3,4 all containing oriT and kanamycin resistance genes. Variants pMOB2 and 3 encode levansucrase (sacB) whilst pMOB3 and pMOB4 encode chloramphenicol resistance. (Schweizer (1992) Mol. Microbiol. 6, 1195-1204). (C) The REDIRECT (Rapid Efficiently Direct Recombination) construct pK20 which encodes the hygB gene which confers hygromycin resistance. In this system recombination efficiency between a cloned DNA segment and the target DNA is aided by recombinate (Datsenka KA, Wanner BL. 2000) "One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products". Proc Natl Acad Sci USA.;97:6640-5).

**Origin & function**

**MUTAGENESIS USING MOBILE GENETIC ELEMENTS**

Origins: The introduced genetically mobile elements are derived from the Tn5 transposon which was originally isolated from the Gram-negative bacterium Escherichia coli (Herrmann et al., 1978 Mol Gen Genet 159:171-8). However, unlike the original Tn5, the transposase function for these mini-Tn5 elements is placed outside of the
Functions: For the mini-transposon based reporter system two functions are expected 1) disruption of the gene/operon within which the element inserts and; 2) reporting the upstream promoter activity of the disrupted gene/operon. The reporter element consists of either 1) a promoterless luxCDABE operon which encodes both luciferase and enzymes responsible for generating the aldehyde substrate leading to bioluminescence when driven by a promoter outside and upstream of the inserted element or 2) gfp which provides for a fluorescent phenotype when driven by a promoter outside and upstream of the inserted element. 

Introduction of a mini-Tn5 reporter element leads to random mutation of the genome in the target strain and each individual mutant strain isolate is expected to possess a different single transposon-disrupted gene.

MUTAGENESIS USING MOBILE GENETIC ELEMENTS

The use of Mini-Tn5's and plasposons will allow the derivation of mutant populations which are likely to exhibit either no apparent difference or a reduction in "fitness". These will be screened to identify individuals which are altered in their ability to detect and respond to environmental stimuli. It is considered extremely unlikely that mutagenesis will result in strains with either increased general "fitness" or virulence. 

Expression of the luxCDABE operon in mini-Tn5 elements will have no effect on bacterial fitness or virulence. However, insertion of selectable markers possessing their own promoter sequence into mini-Tn5 and plasposons could activate the genes flanking the inserted locus. In a measure to reduced these effects the selectable marker and promoter is directed so that transcription is driven into and not out of the main body of the genetic element.

MUTAGENESIS USING MOBILE GENETIC ELEMENTS. This form of mutagenesis further reduces the chance of unpredictable effects since the target is well defined and the likely effects of selectable marker promoter-mediated activation are known. Thus, in each case the effects of recombination mutagenesis will be to have either no effect or reduced fitness/virulence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All experiments will be carried out in designed Category 2 containment facilities. These are located in F22 of the Cledwyn Building and SF1, SF2 and SF21 of the Edward Llwyd Building Annex. Entry to each laboratory is via keypad-controlled doors; floors are all sealed and only dedicated lab-coats are worn within the laboratory. These
never leave the category 2 laboratories and are periodically autoclaved and washed. All workers leaving the lab must thoroughly wash their hands using bactericidal soap in a hand washbasin located at the lab entrance. All cultures are grown within the category 2 facility and all spent bulk liquid cultures are disinfected and autoclaved prior to disposal. All glassware is autoclaved after use. Petri dishes and contaminated plasticware are collected in autoclave bags. All spills are treated with hycolin and absorbed onto paper towels. These are placed in an autoclave bags. All items which are due to be autoclaved are placed within two autoclave bags (one within the other) and sealed. Bags destined to be autoclaved are not allowed to accumulate. These are transported to the autoclave within a clearly marked metal container. Following autoclaving and cooling all waste is disposed of as domestic waste.

A large capacity autoclave is available in F35 and provides a full read-out which is recorded. Annually, the performance of this autoclave is independently tested using a EBRO EBI-125A logger for which the manufacturers provide a calibration certificate.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved by the GM Safety Committee.

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**Project Ref 56/02.2**

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<td>02/10/2002</td>
<td>MUTAGENESIS OF WILD TYPE PLANT PATHOGENIC BACTERIA USING MOBILSABLE GENETIC ELEMENTS OR BY HOMOLOGOUS RECOMBINATION.</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td></td>
<td></td>
<td>not applicable</td>
</tr>
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</table>

Date Project Ceased | 02/03/2022
Purpose of the contained use

Overall purpose: Populations of bacterial pathogens mutated with mobile genetic elements will be screened for altered interactions with the model plant species - Arabidopsis and tobacco (Nicotinia tabacum).

(A) The introduced mobile "transposable" genetic elements are derived from Tn5. These encode selectable antibiotic resistance genes to kanamycin or tetracycline whose transcription is driven by upstream regulatory sequences ("promoter"). We propose to use these mobile elements to abolish gene function and following screening identification of genes whose mutation leads to reduced virulence. In certain cases the mobile elements will encode transcriptional reporter genes luciferase (luxCDABE) (see Section 7). Transcription of this reporter cassette will be driven by the promoter of the gene within which the transposon has inserted (ie. outside of the introduced genetic element and assuming the reporter is appropriately orientated). Such reporter fusion's will be used identify bacterial genes which respond to plant-derived signals by enabling their activity to be monitored during plant-pathogen interactions.

(B) Key genes identified through random mutagenesis and screening may suggest further candidates for gene-knockout. These new targets could represent (1) homologous genes identified by database searches, (2) functionally linked components of a signalling or biosynthetic pathway or (3) adjacent co-regulated genes (ie. part of the same "operon").

Directed mutagenesis will be carried out by homologous recombination using in situ exchange of the endogenous gene with an introduced mutated version. Within the introduced mutated gene will contain an antibiotic resistance gene so that successful integrations into the genome to be selected for (where integration has not occurred the bacterium will not display the specified antibiotic resistance, See Section 7).

Recipient or parental organism

We propose to manipulate the following species of Pseudomonas syringae - all of which are bacterial plant pathogens.

Pseudomonas syringae pathovar phaseolicola - isolated from French Bean
Pseudomonas syringae pathovar tomato - a wide host range including tomato and arabidopsis.
Pseudomonas syringae pathovar tabaci - isolated from tobacco.
Pseudomonas syringae pathovar syringae - though originally isolated from lilac this species exhibits a wide host range.

Individual species vary in their host range and degree of virulence. Pseudomononas syringae pathovar tabaci cause "wildfire disease" which, as the name suggests, is a highly aggressive infection.

Host/vector system
The following genetically mobilisable systems based on transposon 5-Tn5 (de Bruijn and Lupski JR. 1984 Gene27:131-49) will be employed -

(i)  Mini -Tn5 "transposons" (de Lorenzo et al., 1990, J. Bact. 172, 172; 6568-6572) derivatives to include a promoterless luxCDABE operon.  (Winson et al., 1998 FEMS Microbiol Lett. 163:193-202)  The inserted transposons include tetracycline or kanamycin resistance genes and will be introduced into the bacterial strain on the pUT suicide (ie. this will not be maintained within the bacterial cell) plasmid.

(ii)  Plasposons/TnMod - Integration of a mobile element will result in the introduction of an selectable marker (tetracycline or kanamycin resistance) and bacteriophage CoIE1 origin of replication bounded by Tn5 "inverted repeats" which mark the beginning and end of the mobile genetic element.  TnMod-encoding kanamycin and tetracycline resistance will be introduced into bacterial cells on the suicide plasmid - pTnMod-OTC respectively (Dennis and Zylstra, 1998, App.  And Environmental Microbiol. 64, 2710-2715).  Mutated genes may be easily isolated by extracting genomic DNA from the mutated lines, restriction enzyme digestion, ligation (to form DNA "circles") and transformation of E. coli cells.  Only the circularized mutated gene fragment will possess both an origin of replication and a selectable antibiotic marker, to allow maintenance in E. coli and selection of transformed cells.

Candidates for marker exchange will be introduced into the desired strains on the following suicide vectors.


(ii)  pMOB variants 1, 2, 3, 4 HP all containing oriT and kanamycin resistance genes.  Variants pMOB2 and 3 encodes levansucrase (sacB) whilst pMOB3 and pMOB4 encode chloramphenicol resistance.  (Schweizer [1992] Mol. Microbiol. 6, 1195-1204).

(iii)  The REDIRECT (Rapid Efficiently Direct Recombination) construct PKD20 which encodes the hygB gene and confers hygromycin resistance.  In this system recombination efficiency between a cloned DNA segment and the target DNA is aided by recombinase which is encoded on the same plasmid (Datsenko KA, Wanner BL. [2000] One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products.  Proc Natl Acad Sci USA.;97:6640-5).

Mutagenesis using mobile genetic elements

Origins:  The introduced genetically mobile elements are derived from the Tn5 transposon which was originally isolated from the Gram-negative bacterium Escherichia coli (Herrmann et al., 1978 Mol Gen Genet 159:171-8).  The luxCDABE operon on the Mini-Tn5 was isolated from Photorhabdus luminescens (Winson et al., 1998 FEMS Microbiol Lett. 163:193-202).

Functions:  For Plasposons, it is expected to disrupt genes in Pseudomonas syringae and isolate of the mutated gene in E. coli.  However, for Mini-transposon based systems - two functions are expected -disruption of gene/operon at the site of integration and reporting the mutated gene/operon's promoter activity.  This is carried out using a promoter-less luxCDABE operon which encodes genes making both luciferase and its substrate.  Bioluminescence will only be observed when transcription of the luxCDABE operon is driven by a promoter outside of the inserted element.

Strains will be randomly mutated throughout their genomes; thus individual strains possess different gene "knock-outs".  Over entire population it is expected that most genes encoded by the bacteria will be mutated.

Mutagenesis by homologous recombination

Origins:  All vectors used to introduce genes for mutagenesis will be derived from sequences originating from either E.coli or bacteriophages.

Functions:  The introduced plasmids will allow the homologous recombination between gene sequences encoded by the plasmid and genomic gene sequences.  Successful integration is indicated through the stable acquisition of an antibiotic resistance the transcription of which is driven from its own promoter.  Target genes to be mutated will be to a great extent determined by the outcome of the mutant screens (see Section 6).
Evaluation of foreseeable effects

MUTAGENESIS USING MOBILE GENETIC ELEMENTS
As the nature of the proposed genetic manipulation is a gene- "knock-out", it is considered unlikely that mutagenesis will result in strains with either increased general "fitness" or virulence. Expression of the luxCDABE operon in Mini-Tn5 elements in themselves will have no effect on bacterial fitness or virulence.

Nevertheless, two identifiable risks exist. The selectable marker genes possess their own promoter sequence which could activate the genes around the inserted genetic element by "read-through". Additionally, gene activation could also occur if the mobile element inserts and abolishes the function of the gene(s) that suppresses the activity of other genes or their products. Hence, abolition of such a gene could result in the expression/display of eg. virulence gene(s) or product(s). But as mutation of such a suppressor function would undoubtedly have occurred naturally in the wild type population, it is not expected that this scenario would result in strains which were more virulent than already described for that particular pathogen.

MUTAGENESIS BY HOMOLOGOUS RECOMBINATION. This form of mutagenesis further reduces the change of unpredictable effects since the target is well defined and the likely effects of selectable marker promoter-mediated activation are known. The targets for mutagenesis will be virulence determinants or involved in other aspects of pathogenic interactions with plants (eg detection) and thus genetic manipulation will result in reduced fitness/virulence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

It is envisaged that mutated Pseudomonas syringae strains will be at some stage used to challenge GM plants. The generation of transgenic plants is covered by DEFRA licence PHF123/3011 and the facilities used to generate, maintain, store seed and dispose of transgenic material are subject to twice yearly DEFRA inspection.

Transgenic plants will be germinated and grown in dedicated greenhouses (T4 and T5) and the header-house at the University Garden Penglais Hill or in the Transgenic Growth Rooms (Edward Llwyd Building Annex). The greenhouses have double doors and key pads control entry. Entry is via a positively pressured entrance vestibule or "air-lock". All compartments within the glasshouses are positively pressured within the exception of the central vestibule at "ambient" pressures. These pressures are maintained by "forced air" over-head, or in-wall fans where the input air is passed through 5 um filters. The net effect of these measures is to prevent the entry of insects into the glasshouses or into the plant growing compartments. Blue-coloured lab coats are placed within the entrance vestibule which all users MUST wear to prevent transfer to fungal spores from the general environment into the glasshouses and also transgenic pollen to the outside. The blue-coats are periodically autoclaved.

There are no drains in the glasshouse and the sparse volumes of run-off water are collected using dedicated "liquid" vacuum cleaners and autoclaved prior to disposal with the common liquid waste. Any loose-leaf material will be collected for autoclaving. Any plants (which must be non-flowing) to be transported to the main laboratory (~500 yards distant) are transferred in a van which is dedicated for garden use. All loose soil or leaf material will be collected and autoclaved. After completing an experiment, all plants, soil and plastic pots will be autoclaved.

Transgenic plants are also maintained in Transgenic Growth Rooms (TGR1-8), which are located in the basement of the Edward Llwyd Building Annex. Entry to TGR1-8 is via keypad controlled double doors. TGR1-8 is positively pressured to prevent insect and fungal spore entry. Blue coats are also used in these facilities.

AUTOCLAVING: All GM plant material, pots and soils will be autoclaved. Designated green-coloured bins have been placed in the main laboratories - GF20b, FF1, FF2 and FF16 Edward Llwyd Building and TGR1-8. Large capacity autoclaves have been placed in the immediate vicinity ofGF20b, FF2 and TGR1-8 and also in the Penglais Hill Garden Header-house. These autoclaves provide a paper report slip which is examined and stored. All autoclaves are checked yearly using EBRO EB1-125A logger.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All experiments will be carried out in designed Category 2 containment facilities. These are located in GF20a, GF20b, and FF2 of the Edward Llwyd Building Annex. Entry to each laboratory is via keypad-controlled doors; floors are all sealed and only dedicated lab-coats are worn within the laboratory. These never leave the category 2
laboratories and are periodically autoclaved and washed. All workers leaving the lab must wash their hands using bactericidal soap in a hand washbasin located at the entrance. All cultures are grown within the category 2 facility and spend broth is treated with effective detergents (eg. hycolin) prior to disposal. All glassware is autoclaved after use. Petri dishes and contaminated plastic-wear are collected in autoclave bags. Spills are treated with hycolin, absorbed on to paper towels and then placed in autoclave bags. GF20a is also negativity pressured to prevent pathogen aggress and the designated lab-coats are maroon in colour. All items to be autoclaved are placed within two autoclave bags (one within the other) and sealed. These are moved to the autoclave within a metal container.

A large capacity autoclave is positioned immediately outside GF20a/b and provides a full read-out which is recorded. This autoclave is annually tested using EBRO EB1-125A logger. Bags destined to be autoclaved never allowed to accumulate eg around the autoclave or in the labs. Following autoclaving and cooling all waste is disposed of as domestic waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved by the GM Safety Committee.

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Project Ref 56/11.1

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<td>Characterisation of a novel family of Sperm Coat-like Proteins (SCP) from Schistosoma mansoni, and identification of their protein interactive partners</td>
<td>Class 2</td>
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<td>Non-GMM Consent Granted</td>
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Date Project Ceased 02/03/2022

02/03/2022 Page 2118 of 15326
Schistosomiasis remains a significant cause of morbidity and mortality within countries of the Developing World, with an estimated 200 million active infections and a further 600 million people in the tropics and sub-tropics at risk from infection. Although there is highly effective antihelmintic (praziquantel) to treat the disease, the re-infection of treated individuals often occurs. There is an urgent need for an effective vaccine, as well as novel second-line anthelmintics to deal with emerging praziquantel resistance in the parasitic schistosome worms responsible for this neglected tropical disease.

We have established that many of the 29 S. mansoni SCP protein family members are differentially expressed throughout the various life stages of the parasite, and at least some are recognised by the host immune system. The purpose of this study is the functional characterisation of SCP proteins in S. mansoni to understand their role in the biology of the parasite, and their potential involvement in the human immune response to schistosome infection.

Recipient or parental organism

- Escherichia coli strains K12, TOP 10 and BL21 Star (Invitrogen)
- Saccharomyces cerevisiae strains AH109 and Y187

Host/vector system

- E.coli Top 10/pGEM-t Easy
- E.coli BL21 Star/pET30a C-term 6x His
- S.cerevisiae/pGBKT7 and /pGADT7 Rec

Origin & function

Origin

Some SmSCP/TAPS genes will be amplified from cDNA synthesized from total RNA previously extracted from various life-cycle stages of S. mansoni. Others will be amplified directly from pCR-TOPO 2.1-SCP/TAPS (Invitrogen) constructs already available to us.

No living parasite material, or exposure of workers to live parasites, is necessary for generating any of these source DNAs.

Function

The pGEM-t Easy constructs will be used for the initial cloning and maintenance of the genes from PCR products. The pET30a plasmid constructs containing cloned S. mansoni SCP genes will be made solely for the purpose of overexpression of SCP proteins.

pGBLT7 will be used to express Val proteins as fusions with the GAL 4 DNA binding domain.

pGADT7-Rec will be used to express host proteins of interest as a fusion with the GAL 4 activation domain (AD). These constructs constitute respectively the "bait" and "prey" components of the BD MatchMaker yeast 2-hybrid system.
The SCP/TAPS family of proteins have a very wide distribution throughout the natural world. The biological function of most family members is unknown [1], but they are thought to be involved in many fundamental biological processes, for example sperm/egg recognition and plant stress responses (for a review, see [2] below). Whilst a very limited number of SCP/TAPS genes encode proteins with either pathogenic or allergenic characteristics [3], a large number of SCP/TAPS proteins have already been safely expressed and purified from bacterial or eukaryotic hosts[2].

Our preliminary data [4] suggest that in S. mansoni, some SmSCP/TAPS proteins are recognized by the human immune system, may modulate cellular responses, and are secreted during penetration through the dermis of the infective ( cercarial ) stage of the parasite. However, life cycle expression analysis, coupled with phylogenetic evidence of considerable gene duplication and divergence suggest that no one SmSCP/TAPS protein has an overreaching role in invasion or immune modulation.

As SCP cannot act in isolation from the pathogen (e.g. have no intrinsic toxicity) and limited evidence of allergenicity, we suggest that the SCP family of S. mansoni proteins present a low risk to human health. Thus, SCP expressing E. coli and yeast strains will be inherently safe to work with providing all manipulations of GM material are carried out in adequate Laboratory Containment Level 2 facilities, and best microbiological practice is adhered to throughout. In the event of escape from these containment facilities the E. coli and yeast strains will have no selective advantage in the environment as S. mansoni SCP could only increase fitness within a pathogenic host (and not auxotrophic laboratory strains of E. coli and yeast). Further, the use of non-mobilisable vectors, and microbiological containment of all GM material will effectively eliminate the possibility for transfer of the sequences to other organisms. The widespread distribution of SCP/TAPS family proteins throughout the plant and animal kingdoms suggests that the highly improbable accidental transfer of any SmSCP/TAPS sequence to other organisms would have essentially benign consequences.


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid cultures of GM material, and all disposable plasticware and glassware that has been in contact with GM organisms, will be sterilized (100% kill) by wet heat (121°C/15 mins) in an approved autoclave housed within the CL2 containment facility. The process cycle will be monitored using a single calibrated thermocouple linked to a recorder producing a printout of cycle performance will be monitored according to the standards outlined in HPA national standard QSOP6 (http://www.hpa-standardmethods.org/documents/qsop/pdf/qsop6.pdf)

Autoclaved waste will be disposed of to landfill (solid waste) or to sluice (liquid).

Laboratory coats used within the CL2 facility will be autoclaved prior to being sent for cleaning.

All laboratory work surfaces, and equipment used for the culture or processing of GM material, will be routinely cleaned and disinfected with a peroxygen- based disinfectant (‘Virkon’) at an ‘in use’ dilution of 1% (100% kill). The laboratory floor will also be cleaned and disinfected weekly with 1% Virkon.

Procedures for the containment and disinfection (100% kill) of accidental spillages of GM material within the CL2 facility are the subject of a separate SOP (attached)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The local Genetic Modification Committee considers that the risk assessment meets current regulations. The project seeks to over-express Schistosoma mansoni Sperm Coating Proteins (SCP) in E. coli in volumes of 1-6L. The biological function of most SCP/TAPS family of proteins is unknown and the involvement of a very limited number of SCP/TAPS genes with either pathogenic or allergic characteristics, indicates that this work should be classified as a Class 2 activity. The work will be done in a newly refurbished Containment Level 2 laboratory, containing its own autoclave for destruction of GM materials.

### Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Name**

UNIVERSITY OF SOUTHAMPTON

**Department**

CENTRAL HEALTH & SAFETY GROUP

**Campus Estate or Research Centre**

**Road Name**

UNIVERSITY ROAD

**District**

**Town**

SOUTHAMPTON

**Country**

ENGLAND

**County**

HAMPSHIRE

**Postcode**

SO17 1BJ

**Tel Number**

023 8059 6850

**Fax Number**

02380 594319

**E-mail**

biosafety@soton.ac.uk

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Y

02/03/2022
Genetic Modification and Bio-Safety Committee

Terms of reference

(i) To consider, monitor, provide guidance and make recommendations to the Safety and Occupational Health Committee on matters relating to safety in work involving Hazard Group 3* dangerous pathogen activities under the Control of Substances Hazardous to Health (as amended) Regulations 2002, Regulation 7(10) Schedule 3, "Additional provisions relating to work with biological agents". In exceptional circumstances only will this remit extend to non-GM Hazard Group 2 dangerous pathogen activities.

(ii) To consider, monitor, provide guidance and make recommendations to the Safety and Occupational Health Committee on all matters relating to safety in work involving genetic modification as defined in the Genetically Modified Organisms (Contained Use) (as amended) Regulations 2000.

(iii) To consider, monitor, provide guidance and make recommendations to the Safety and Occupational Health Committee on all matter relating to safety in work involving the deliberate release of any genetically modified organism into the environment as detailed under the Genetically Modified Organisms (Deliberate Release) (as amended) regulations 2002.

(iv) To consider, monitor, provide guidance and make recommendations to the Safety and Occupational Health Committee on all matters relating to safety in work involving animal pathogens under Schedule 1 of the Specified Animal Pathogens Order 2008.

(v) To consider, monitor provide guidance and make recommendations to the Safety and Occupational Health Committee on matters relating to safety in work involving agents under Part 7, Schedule 5 of the Anti-Terrorism, Crime and Security Act 2001.

(vi) To receive for scrutiny, review and notification to the relevant authority, Project Proposals, Risk Assessments, Codes of Practice and Standard Operating Procedures for activities (I), (ii), (iii), (iv), and (v) above.

(vii) To approve items in (vi) above such that work may commence with the exception of Activity Class 1 projects which may be approved for commencement by the Biological Safety Officer prior to formal review by the GMBSC and HSE notification.

(viii) To consider and make recommendations to the Safety and Occupational Health Committee on reports received from the Biological Safety Officer on procedures adopted by Schools on work in the fields specified in (I), (ii), (iii), (iv) and (v) above.

(ix) To conduct safety inspections of work and facilities identified in (I), (ii), (iii), (iv) and (v), to monitor implementation of the regulations, and to review the outcomes of these inspections.

Membership:
- Chair appointed by Council
- The Biological Safety Officer
- A technically competent member (one each) from the various disciplines in Medicine, Life Sciences and Engineering
- Two members of the academic staff (one each from the Highfield campus and Southampton General Hospital campus)
- Two members from the dissolved Biosafety Committee
- A member of the Divisional?School Genetic Modification Liaison Officers
- The Faculty Safety Adviser for Medicine or representative of the Central Health and Safety Group
Two postgraduate students (one from Life Sciences and one from a group based at SGH)

* This group of dangerous pathogens will also include three specified Hazard Group 2 agents, listed in Part V of COSHH Schedule 3: Bordetella pertussis, Corynebacterium diphtheriae, Neisseria meningitidis. Other agents may be considered as judged appropriate or necessary.

+ Appointed after consultation by the Chair of the Committee

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| Other (please specify) |            |             |             |             | Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Transgenic Animals
- Transgenic Fish
- Transgenic Invertebrates
- Transgenic Plants
- Microbiology Research
- Gene Therapy
- Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity
Virkon and Trigene are used widely (final concentrations as appropriate) to inactivate GMMs in liquid cultures. Concentration x Time values vary from species to species and media to media. Inactivated liquids are discharged direct to foul sewer (drain) and flushed with copious amounts of water.

Contaminated non-disposable labware that cannot be autoclaved is soaked as above in appropriate disinfectants with appropriate Ct values.

All other waste (primarily solids) is autoclaved in validated loads, is double bagged, and disposed via landfill. Waste containing clinical items are disposed via the incineration route.

Autoclaves are initially validated according to types of loads and are then serviced annually. Subsequent self-validation using biological indicators is exercised for Activity Class 1-2 waste autoclaves. Autoclaves handling Activity Class 3 waste are validated professionally on an annual basis. All autoclaves handling GMM waste are required to have recording capabilities.

The attached risk assessment is the template used to assess the human and environmental risks for any activity involving GMMs (Form A), Gm Animals (Form B), GM Plants (Form C) or Clinical Trials (Form D).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The attached risk assessment is the template used to assess the human and environmental risks for any activity involving GMMs (Form A), Gm Animals (Form B), GM Plants (Form C) or Clinical Trials (Form D).
### Project Additional Information

#### Purposes of the contained use

The aim of the project is to i) to perform functional screening of cDNA libraries, including the identification of new tumour specific T cell antigens; ii) to investigate the processing and presentation of antigens to T cells.

#### Recipient or parental organism

1) Vaccinia virus (Western Reserve strain which is attenuated, and Modified Virus Ankara which is replication incompetent in normal cells); Moloney Murine Leukaemia virus (which is replication defective)

#### Host/vector system

- Non-mobilisable pUc-based vector containing amp(r), lacZ and the vaccinia 7.5K promoter. CDNA expression library in non-mobilisable, commercial vector (ViraPort retrovirus DNA marketed by Invitrogen).

#### Origin & function

- i) Non-functional proteins and protein fragments which can be processed by infected cells for presentation to T cells.
- ii) cDNA expression libraries from normal and tumour cells.

#### Evaluation of foreseeable effects

The vaccinia virus is the WR strain which was used world-wide for vaccination against smallpox. It has been extensively investigated and attenuated. It has a fairly broad mammalian host range and would be transmitted efficiently by the needlestick route. Recombinant virus is modified by recombination into the thymidine kinase gene which reduces virulence by 10(6) fold (Buller et al Nature 317, 813, 1985). Clinical inoculation with high titre virus carries a 1:10(6) risk of viral encephalitis. This risk is reduced to 1:10 in the GMM. The insert should not increase pathogenicity of the GMM and in isolation does not have toxic, oncogenic or allergenic properties.

- ii) Influenza A strains include H1N1 (A/PR/8?1934), H2N2 (A/JAP/305/1957), H3N2 (A/Aichi/1968) and have been extensively investigated. They have a fairly broad mammalian host range and would be transmitted efficiently by aerosol. These organisms are not genetically modified.

- iii) Moloney Murine Leukaemia virus used are replication defective since production of infectious virus requires functions supplied in cis on three separate plasmids. These have minimal or no sequence overlap and the chance of homologous recombination creating competent virus is extremely low. CDNA from tumour cells is likely to contain some oncogenic sequences. However, oncogenic transformation requires multiple events and we will not develop retroviruses expressing multiple co-operating oncogenes. The viruses are inactivated by human complement. There is a very low environmental risk as viral particles are extremely labile and do not survive on environmental surfaces.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Immunisation of mouse strains with influenza A and GM vaccinia would be carried out under level 2 containment and post-mortem tissue produced in the laboratory also level 2 containment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- none

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Liquid waste containing GMMs and disposable items such as pipette tips will be submerged in 1% virkon disinfectant and sealed in plastic before terminal disinfection by autoclaving. All animal waste such as bedding will be disinfected by autoclaving and carcasses by incineration.
1) Project approved as required level 2 containment.
2) Sharps and sharp-precursors must not be used unless essential. Gloves will be worn and any abrasions covered with surgical tape in all procedures where exposure to virus is possible.
3) Centrifuge buckets and rotors will be disinfected by autoclaving according to manufacturer’s instructions.
4) Requirements for work with non-GMMs must comply with relevant regulations and necessary approvals must be in place before this part of the work is commenced.
5) Staff who work in the level 2 facilities where the viruses are used must be registered with the University occupational health group. Registered workers will be offered vaccination where appropriate.

Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 57/02.2

Date Ackn'd 19/02/2002

Date Project Ceased

Class 2

CultureVolCultureVolume

Class 2 < 1 litre

Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to use the modified viruses to achieve extended focal expression of pro-inflammatory and anti-inflammatory cytokines in models of human disease.

**Recipient or parental organism**

Human adenovirus Group C (Ad5) The vectors are all based on adenovirus serotype 5 (Ad5), which is a double-stranded DNA virus comprising over 40 serotypes all of which are categorised as hazard group 2 biological agents. Ad5 is a respiratory pathogen and in vivo infections are generally limited to the epithelial cells lining the respiratory tract. Unmodified Ad5 is classified in biological agents hazard group 2. Ad5 is generally associated with mild respiratory infections in children, and it is thought that the majority of the population is likely to have antibodies to the wild-type virus. Immunity to adenovirus infections is thought to be life-long following primary infection, although latent infection of tonsil and adenoid tissues is a frequent occurrence following childhood infection by Ad5. The precise mechanism of latency is unknown, but free virus is only rarely detected in lymphoid tissue and is present in <1 in 107 cells. Reactivation of latent Ad5 can lead to serious complications in immuno-compromised individuals and it has been isolated at high frequency from patients with AIDS. Human serotypes do not normally infect other animals and there are few reports of virus replication following inoculation of experimental animals. The cotton rat, a species not indigenous to the UK, provides an animal model of infection. Group C viruses (eg Ad5) are non-oncogenic. However, all human adenoviruses, including Group C, transform rat cells in culture. Despite this, there is no evidence of any association between adenoviruses and human cancer.

**Host/vector system**

Disabled human adenovirus Group C (Ad5) (E1 and E3 deleted)  
The lytic cycle of Ad5 is divided into the early and late phases. The early genes are expressed from four regions of the genome. The two transcription units of the early region 1 (E1a and E1b) are responsible for cell transformation and tumourigenicity. Both alter transcriptional regulation during infection and transform by directly interacting with cell proteins involved in transcription and cell cycle regulation (eg p53 tumour suppressor protein). The vectors used under this application are E1 and E3 deleted. The E1 deletion region prevents the expression of E1a and E1b genes. The viruses are unable to replicate except in complementing cell lines such as 293 (a human embryonic kidney cell line which expresses the left 11% of the Ad5 genome. Thus the replication deficient viruses do not generate infective units following the initial infection of susceptible cells other than 293 cells. The replication defective Ad vectors have no mechanism for long term maintenance in cells; expression in the lining of the respiratory epithelium declines with time and is limited to ~2 months. In the absence of any significant episomal replication of E1a deleted Ad5 in normal human cells, long term maintenance requires integration into the host chromosome. This can occur at a frequency of about 1 per 10e5 pfu in exponentially growing cultures of primary human cells. Deletion of the E3 region (which is non-essential for in vitro growth) allows inserts of up to approximately 8 kb to be cloned. An E3 deletion also reduces the likelihood of a GMM causing harm as a consequence of making the virus less able to establish and maintain an infection within the cells of an infected individual. It should be noted, however, that there is little evidence that the pathogenicity of an E3-deleted virus, in any cells which do become infected, will be reduced.
The genetically modified replication deficient viruses were produced by manipulation of partial viral genomes in bacterial plasmids by the co-transfection of overlapping plasmids into 293 cells to allow the generation of complete genomes by homologous recombination as previously described (1). The following genes were introduced into the E1-E3 deleted region: XCMVhumanIL-1b, XCMVmurineTNF-wt, XCMVmurineTNF-membrane bound, XCMVmurines B7.1, XCMVTFGFb1, XCMVTFGFb1 (active), XCMVmurineIL-10, XCMVmB7.1/IL-2, XCMVmLymphotoactin, XCMVmLymphotoactin/IL-2, XCMVbv-gal, or XCMVd170-30 (control), or similar (no material difference). After 5-7 days, recombinant viral particles were released by freeze-thawing the transfected 293 cells. The vectors were purified by plaque assay and a viral stock was generated on CsCl gradients. The stocks were titrated by plaque formation (3 x 10^13 pfu/ml) and checked for integrity of viral DNA (by HIRT, restriction digest and Southern Blot), expression of the desired cDNA (by Western Blot) and presence of replication - competent virus (by PCR to detect E1 sequences as described (2)). Additionally, presence of endotoxin was monitored by the amebocyte horseeshow crab lysate method (E-toxate assay, Sigma). No E1 sequence or endotoxin could be detected in the stocks. The intended function of the inserted genetic material is to give rise to the focal over expression of cytokines in experimental models. The introduction of the non-adenoviral genetic material is not expected to alter the infectivity profile (only humans) of the replication deficient adenovirus. Indeed, the cytokines are likely to increase the level of the inflammatory response, which will give rise to an increased rate of clearance. Tissue tropism is also not expected to be different in the GM adenoviruses.


Evaluation of foreseeable effects

Wild-type adenoviruses are non-enveloped, relatively resistant to desiccation stress and can survive in aerosols. But there is no evidence that human Ad serotypes can naturally infect animals, and replication is very limited in rat cells, for example. Replication has been shown to occur in the lungs of experimentally infected cotton rats administered a high doses of virus. However, the replication deficient adenoviruses cannot replicate outside 293 cells and the insertion of the cytokine genes will not alter this characteristic. Homologous recombination between E1a- Ad and wild-type virus (or viral sequences in the 293 cells lines) may occur at low frequency, but, were this to happen the packaging limits of Ad would be expected to delete the transgene ir naje sytg a recombinant unviable. It has also been shown that recombination only occurs between serotypes within a subgenus and not between subgenera. Co-replication between E1a deletants and WT could also occur due to transcomplementation. This requires co-infection of both viruses and has so far only been demonstrated at high multiplicities of infection (moi). As no new virus or viral stoc ks are to be generated in Southampton, the likely hood of a recombination event, occurring under this proposal would be highly improbable. Overall, it is not thought that the modified virus would pose a serious risk to the environment. The main environmental risk would be to susceptible children. However, the modified viruses will be employed in very small volumes (<1µl), which are unlikely to enter the atmosphere.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The modified virus is replication deficient and no threat to the environment is likely. The preparation of aliquots from stock virus will be performed in a biological safety cabinet. Staff will be specifically trained in the safety aspects of this work with written training records being kept. Waste materials will be autoclaved with 100% kill. Appropriate, validated disinfectants will be used to decontaminate exposed work areas. These containment and control measures will protect both the workers and the environment.

02/03/2022
The genetic modification safety committee recommended that the project should be assigned to activity class 2. The laboratory facilities will be inspected by members of the committee prior to work commencing.

### Project Containment

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- **Animal Units**: L2 Yes L3 L4 L2
- **Large Scale Activities**: L3 L4 L2
- **Human Clinical Applications**: L3 L4

### Project Ref  57/03.1

**Date Ackn’d**: 24/10/2003  
**CU2 Project Title**: ADENOVIRAL EXPRESSION OF PLEIOTROPHIN IN HUMAN MESENCHYMAL STEM CELLS.

**Class**

- **Consent Granted**
  - Non-GMM: not applicable

- **Project notified under transitional arrangements**: No

**History Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

02/03/2022  
Page 2132 of 15326
### Purposes of the contained use

Pleiotrophin (Ptn) has been suggested to induce differentiation of progenitor cells to an osteoblast phenotype and to recruit osteoblasts to the site of new bone formation. This study aims to investigate the action of Ptn on human stem cells and its role in bone formation. Cells transduced with adenovirus expressing pleiotrophin will be implanted in diffusion chambers in nude mice to study ex vivo bone formation.

### Host/vector system

| Adenovirus, specifically the AdEasy Adenoviral Vector System. Plasmids will be propagated in E. coli DH5α, XL10-Gold and BJ5183. Adenovirus serotype 5 with genes E1 and E3 deleted. Virus is propagated in HEK293 cells. |

### Origin & function

In human pleiotrophin gene will be used in this study. Ptn is oncogenic in certain cell lines and is expressed in various tumours. Ptn also stimulates neurite outgrowth, angiogenic and an osteoblast stimulating agent. Pleiotrophin is associated with lung cancer, may be an early indicator of lung cancer and serum levels correlate with the stage of the disease. In this study, the specific activity that will be examined is the potential for stimulation of osteoblast function.

### Evaluation of foreseeable effects

The most hazardous GMM is adenovirus expressing pleiotrophin. E. coli strains are disabled and non-colonising bacteria, non-pathogenic to humans; all are derived from K12 strains. Adenovirus Ad5 with E1 and E3 deleted is replication deficient. Hazards associated with adenovirus are from inhalation. Airway epithelia are fairly resistant to infection by adenoviral vectors therefore, the risk of infection is low and may cause the symptoms of a mild cold. The persistence of infection with a replication deficient virus would be limited.

Potential exists for the emergence of replication competent adenoviruses (RCA). RCAs can result from double crossover events during amplification in the HEK293 cell line which carries the E1 region. Care is taken to always plaque purify the recombinant virus and to keep the HEK293 cells passage number low, ideally less than 4. This double crossover event results in the loss of the recombinant protein.

The primary human bone marrow cells are not screened for adventitious agents and are treated as potentially pathogenic.

Ptn is oncogenic in certain lines and is expressed in various tumours. Potential exists for expression of Ptn in the respiratory tract before immune clearance of the virus if inhaled and potential for alteration of lung tissues. Pleiotrophin is associated with lung cancer, may be an early indicator of lung cancer and serum levels correlate with the stage of the disease.

The recombinant virus is replication deficient and as such, is actually less pathogenic than the wild-type. Therefore, there is little capacity for colonisation. If exposed to the environment it is unlikely to survive for extended periods.

If the virus were released into the environment, potentially, humans could be infected with consequences the same as for the laboratory worker. There is no evidence to suggest that plants or animals could be infected with adenovirus (with the exception of the cotton rat which is not indigenous to this country).

In the extremely unlikely event of an animal becoming infected with the GMM, Ptn would probably be expressed transiently. If the animals cells could respond to human Ptn
the result may be mitogenic activity, recruitment of osteoblasts to the site of the infection, or angiogenesis.

There is no evidence to suggest that Ptn has any involvement in pathogenicity, nor is it likely to be expressed on the surface of the virus or on any cell surface.

Potentially, the recombinant virus could recombine with a wild-type adenovirus producing a replication competent virus expressing Ptn.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Although animals will be used in this study, they are not GM animals. The GMM will be maintained within the diffusion chamber and since the GMM is replication deficient, virus will not be passed to the animal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All bacterial waste used in the construction and propagation of the plasmid vectors is decontaminated in freshly prepared 1% (v/v) Hycolin for a minimum of 16 hours. The solid waste is then autoclaved, liquid is poured to waste with copious amounts of water. All cell culture waste is disinfected in freshly prepared 1% (w/v) Virkon and solid waste autoclaved, liquid is poured to waste with copious amounts of water. Cell culture waste, both solid and liquid, that includes adenoviral waste is disinfected in 1% (w/v) Virkon, and remains in Virkon until autoclaved. The autoclave is located within the hospital building. Waste is transported in sealed containers avoiding public corridors where possible. The autoclave is validated for disinfection of waste. Both Virkon and Hycolin are used according to the manufacturers recommendations and have been validated for decontamination of bacterial and viral agents.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment for this project was first discussed at the GMSC meeting of 21 January 2003. Members agreed that the project was borderline AC1/AC2 and agreed to seek advice from the HSE. The response from the HSE was carefully considered, as was the guidance in ACGM Newsletter 31, regarding the risk assessment of GMMs expressing cytokines. Following discussions with the project leader, all parties agreed that the project should be classified as AC2, and the notification process initiated. It was advised that the animal work should receive particular attention in the risk assessment, as no MSC or isolator will be used.

The chair of the GMSC and the two University BSOs will carry out an inspection of these laboratory facilities in November 2003.

Project Containment

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02/03/2022
THE USE OF CELL LINES, GENETIC CONSTRUCTS (PLASMIDS), ADENOVIRUSES AND TRANSGENIC MICE TO ASSESS THE MOLECULAR MECHANISMS LEADING TO NEUROPATHOLOGY IN POLYGLUTAMINE DISEASES WITH A FOCUS ON HUNTINGTON'S DISEASE

I will construct and propagate new/already available plasmids and adenoviruses that express proteins with polyglutamine stretches fused or not fused to green/red fluorescent proteins, various neuroprotective heat shock proteins and dominant negative forms of protein kinases in order to express the transgenes in cell lines and primary neuronal cells in vitro to investigate their effects on cellular function (eg mitochondrial function) and neuronal survival. I will also use brain cells for in vitro studies of transgenic mice. This approach will be used to identify and elucidate important cellular signaling pathways that may be altered during pathology induced by expanded glutamine stretches in various polyglutamine diseases with a focus on Huntington's disease. This research will lead to a better understanding of these pathologies and may contribute to the identification of novel therapies for these currently untratable neurological disorders.

I will use various cell lines for over-expression studies (see below for over-expressed transgenes). Routinely used cell lines will be HEK293 and 911 cells (in order to propagate the Adenovirus; these cells are E1 complementing cells); COS-1 and COS-7 cells (monkey origin), an immortalized striatal full-length huntingtin knock-in mouse cell line, NT-2 cells (mouse neuronal precursor cells), SK-N-SH, SH-SY5Y, HeLa, 143B cells that express the Huntington's disease Exon 1 With/without glutamine expansions upon induction with the antibiotic doxycycline (PC12 Tet-On Q23, Q43, Q53, Q74), PC12 naïve (rat origin) and some other not yet specified mouse and human cell lines. I will also use primary neurons of the mouse and/or rat cerebrocortex, hippocampus, striatum, celineblum and peripheral nervous system into which the transgenes (see below) are transfected. Furthermore, I will use primary neurons of huntingtin Exon 1 with/out polyQ expansion or full length mouse huntingtin where a
polyQ expansion has been “knocked-in” at the HD locus (knock-in mice). The transgenic mice are already available.

The recipient micro-organisms are bacterial and adenoviral vectors. All the transgenes in plasmid vectors and Adenovirus are driven by CMV promoters. However, we intend to use a series of Adenovirus vectors expressing huntingtin Exon 1 with/without polyglutamine expansion fused to the red fluorescent protein (mRFP) that are driven by the neuronal specific synapsin-1 promoter in conjunction with the WPRE (woodchuck post-transcriptional regulatory element, from woodchuck hepatitis virus; see reference below, Glover et al., 2002). The use of the WPRE sequence has recently been reported to bear a risk of tumor formation in mice (SACGM information note, see also www.advisorybodies.doh.gov.uk/genetics.gtac/). Therefore the GTAC has called for special attention for the use of the WPRE sequence in an open letter until further notice and recommended containment level 2 for all use with WPRE sequences. I will use WPRE sequences only for in vitro experiments where Adenovirus bearing WPRE sequences are expressed in primary neuronal cells in vitro and we will strictly follow the current recommendation of the GTAC including changes in future recommendations.


In collaboration, I intend to construct and use lentivirus that expresses the same transgenes as the ones expressed by the Adenovirus (htt Exon 1, see above). The WPRE sequences will NOT be used for the construction and use of lentivirus. The lentivirus will be used in an identical way compared to the Adenovirus (infection and expression of transgenes in primary neuronal cell cultures).

Hazards to human health

Hazards associated with the various cell lines
There are no known adverse effects.

Hazards associated with the recipient micro-organism (e.g. bacterial host or viral vector)
Inhalation or ingestion of adenovirus could be harmful. Hence the transmission route could be "oral". Infection is generally limited to the epithelial cells lining the respiratory tract. However, the Adenovirus is modified in that the E1 gene is missing and hence the adenovirus is replication deficient. Unmodified Adenovirus serotype 5 is classified in hazard group 2 under the COSHH regulation. Bacterial strains are classified in hazard group 1.

Identification of any hazards to the environment.

Hazards associated with the recipient microorganism (e.g. bacterial host or viral vector)
It is thought that recombination negative Adenovirus serotype 5 would not pose a risk to animals and plants in the environment because there is no evidence that human adenovirus can infect animals or plants.

The potential hazards of sequences within the GMM being transferred to related microorganisms
Recombination with animal wild type adenovirus or virus fragments would not pose a risk other than above if it occurred.

The recipient micro-organisms are bacterial and adenoviral vectors. All the transgenes in plasmid vectors and Adenovirus are driven by CMV promoters. However, we
intend to use a series of Adenovirus vectors expressing huntingtin Exon 1 with/out polyglutamine expansion fused to the red fluorescent protein (mRFP) that are driven by the neuronal specific synapsin-1 see reference belwo, Glover et al., 2003). The use of the WPRE (woodchuck post-transcriptional regulatory element, from woodchuck hepatitis virus; reported to bear a risk of tumor formation in mice (SACGM information note, see also www.advisorybodies.doh.gov.uk/genetics/gtac/). Therefore the GTAC has called for special attention

Host/vector system

Recipient bacterial strains used:
For the production and propagation of the transgenes and adenoviruses I will use E.coli (disabled, K12) that are either recombinant deficient (Dalpha, TG1, JM101) or recombinant proficient (BJ5183).

List of vectors used:
- Adenovirus 5 (pAdEasy-1/2, the 33.4 kb plasmid containing the Adenovirus serotype 5 genome with E1 deleted, this adenovirus is replication deficient)
- plasmid vectors: pcDNA1, pcDNA3, pCMV5, pEGFP-N1, pEGFP-C1, pShuttle, pShuttleCMV, pAdTrack, pAdTrack-CMV, pECFP, pEYFP-C1, pmRFP, GFPu (destablized GFP)

Origin & function

DNA for the wildtype, constitutively active and/or dominant negative forms (mutant) of the following genes will be used:
1) Kinases/other: JIP-1, MEK1/2, M KK3
2) Bcl-2 family proteins: Bcl-2, E1B19K
3) Huntington's disease genes and fragments fused or not fused to green/red fluorescent protein or other proteins tags: httExon 1 Q25/47/72/103 with/out NLS (nuclear localisation signal), htt aa500Q17/68, full-length httQ15/130, huntingtin Exon1 Q20, Q40 and Q50 GST- fusion DNA
4) Heat shock proteins: Hsp22/25/27, alphaB crystalline, members of the Hsp40, Hsp60, Hsp70 and Hsp90 family
> Please see the appendix on page 7 & 8 for details of each gene and its function/activity with selected references and for information relating to which vector system will be used for each transgene.

Human Health

Hazards arising directly from the inserted gene product Incetracellular expression of heat shock proteins could be cancerpgenic because it could prevent cells from dying. The opposite effect could occur by expressing polyglutamine proteins or dominant negative forms of kinases by decreasing the activity of intracellular survival pathways or activation cell death pathways. No immune response is expected. The WPRE sequence has been recently associated with an oncogenic risk (see above).

Hazards arising from the alteration of existing traits No alterations of existing traits are expected because no viral genes are expressed and no alteration of the capsid or of any receptor is attempted.

Environment

Hazards arising directly from the inserted gene product Expression in animals of the transgenes would produce similar harmful effect as in humans because the cellular pathways targeted by heat shock proteins, dominant negative forms of the kinases and the polyglutamine proteins are expected to be evolutionary conserved.

The potential hazards of sequences within the GMM being transferred to related microoganisms The genes would not represent a hazard if it were to recombine into a wildtype adenovirus other than outlined above. The risk of formation of replication proficient adenovirus (expressing the transgene) is minimal since I will perform a diagnostic polymerase chain reaction for the presence of the E1 gene on each preparation of the E1 gene on each preparation of Adenovirus produced in the E1-positive mammalian cell lines before use. Should there be a positive Adenovirus preparation it will be appropriately destroyed (see below).
Evaluation of foreseeable effects

Given the identification of the potential hazards and detrimental effects on human health and the environment described above it is evaluated that the foreseeable effects are generally of a minor risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

I will breed transgenic mice ubiquitously over-expressing the heat shock protein 27 (Akbar et al., 2003; see belwo) or expressing the first Exon of the human huntingtin gene with a polyglutamine mutation (R6/2 strain, Davies et al., 1997; see below) or full length mouse huntingtin where additional glutamines have been "knocked-in" (Shelbourne et al., 1999, see below). These mice will be held and bred according to standard procedures in the transgenic mouse facility.


For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the work procedures are not likely to produce aerosols. All the waste including bacteria (E.coli) and adenovirus and material/plastics that have been in contact with GMMs described above will be disposed off by VIRKON treatment (disinfectant concentration: 1 per cent; time of treatment: 24 hours). This disinfectant will be made up on a weekly basis (it is stable for up to 6 days). VIRKON is a balanced stabilised blend of peroxygen compounds, surfactant, organic acids and an inorganic buffer system and is recommended disinfectant for the 100 per cent killing -off of a large variety of pathogens including the GMMs proposed to use in this application. Liquid GMM material treated with VIRKON in this way is then disposed off conventionally (sink). All non-liquid material (such as plastic culture dishes and wells) will additionally be autocleved and after that process treated as conventional waste.

Process of testing: To ensure that all GMMs are killed to 100 per cent efficiency, VIRKON treated samples (see above) will be tested in the following way:

1) Bacterial waste-> after VIRKON treatment described above, a whole sample of E.coli will be centrifuged at standard speed in order to pellet potentially living E.coli cells (10,000 rpm) and subsequently plated on LB agar plates and incubated at 37C for 24 hours. No growth of colonies is expected if E.coli cells have been killed off. My previous experiences has shown that this is the case.

2) Adenoviral waste-> E1-expressing mammalian cells such as HEK293 known to complement the E1-deficient Adenovirus will be exposed to the adenovirus containing sloution after VIRKON treatment (see above) for 60-90 minutes. Because 1 per cent VIRKON has a toxic effect on the HEK293 cells, the VIRKON containing sloution will be diluted appropriately (1:50). After 60-90 minutes the medium will be replaced by normal growth medium containing 1 per cent fetal bovine serum. The HEK293 cells will then be observed over a 2-3 week period for the production of a visible CPE (cytopathic effect). A single viable viral particle will be sufficent to induce such a CPE within 48 hours which then will be amplified and easily visible after 2-3 weeks in the culture dish (CPE, positive cells will round-up and detach from the culture dish). As a positive control a non-VIRKON treated sample of a similar viral particle dilution will be used.

These two procedures (1 and 2, see above) have been previously tested successfully. These procedures will be performed once on each purchase of VIRKON before use.
The Biological Safety Officer made the following comments on the draft project proposal/risk assessment that was initially submitted:

"Although replication deficient adenovirus alone would be classified at AC1, I assume that expression of htt, polyQ and/or EGFP in human cells, in vivo, is being considered as potentially harmful to the individual, and therefore AC2 selected. AC2 projects require notification of the HSE, and for this purpose the risk assessment needs to be somewhat more detailed - the rubric in each section of the GMM form is very helpful in this respect. In general, all statement should be justified. The ACGM Compendium of Guidance is useful (http://www.hse.gov.uk/infection/gmo/acgm/acgmcomp/index.htm), in particular the section on adenoviral vectors. The following comments also help: Part 1 (b)(ii) Which cell lines will be used? Cells in culture are considered to be microorganisms, and they are also "recipients". (b)(iv) Are the activities and functions of the individual genes/proteins known? If so, they should listed, either here or in an addendum. References may be helpful. Particular attention should be paid to genes to be expressed in adenovirus. Stress that the adenovirus is replication deficient and not wt. Part 2 (a)(I) Hazard group of wt adenovirus and vector strain? How is it disabled? Transmission route? Cell tropism? (a)(ii) What are the potential effects of polyQ, EGFP and hsp expression? Immune response expected? (a)(iii) No, but justify no alteration of existing traits. Not viral genes. Not incorporated into capsid. Not receptors. (a)(iv) Possible recombination with wt virus or virus fragments - see ACGM Compendium. State risk of formation of replication proficient virus expressing transgene. (c) (I) This is the risk assuming that release to the environment has already occurred. Does the vector infect indigenous species? (c)(ii) Would expression of the transgenes in animals produce the same harmful effects as in humans? Why? 9c)(iv) Recombination with animal wt adenovirus or virus fragments? (d)(ii) Disinfectant concentration, time in contact, made up daily? Validated for adenovirus by manufacturer/externally? (d)(viii) Would workers with known immunological defects be permitted to work with viral vectors? Or with eczema? Route of infection? Would they be referred to occ.health? (d)(x) Training overseen by project leader? What procedures? (e) What will be the max volume of culture? (f)(I) E.coli manipulations. (f)(ii) Adenovirus manipulations. (g) Highest level of containment is CL2 using class II MSCs?"

The project proposal/risk assessment was modified accordingly.

At a meeting of the GM Committee on 20/01/05, members felt that it would be prudent to insert an additional sentence in the notification form to indicate that there is an awareness of the oncogenic potential of some of the constructs (namely, the ones expressing Bcl-2 and E1B19K), and also a familiarity with the relevant HSE guidelines. It was agreed that the work should be classified as AC2, and that the HSE should be notified once the required amendments had been made to both the risk assessment and this notification form.

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02/03/2022
**Project Ref** 57/06.1

**Date Ackn’ed** 20/04/2006

**CU2 Project Title**

The use of genetic constructs to assess pathogen survival and growth in the environment.

**Historical Significant Changes**

**Historical Date of Additional Info**

**Withdrawn** NO

**Tick if notifying a connected programme of work** NO

**Project Additional Information**

**Purposes of the contained use**

The work concerns the use of genetic constructs to:

a) Elucidate the genes involved in biofilm formation by Staphylococcus aureus and Pseudomonas aeruginosa. For S. aureus, this will involve the use of genetically modified sensor strains to identify and quantify which of four different autoinducing peptide (AIP) types the wild type strains produce. For P. aeruginosa, green fluorescent protein (GFP)-based reporters under the control of the inducible tac promoter of pMEKm12 or the mutD gene encoding DNA polymerase III for detecting mutagenesis within biofilms in situ and in real time.

b) To enable microscopy detection on surfaces of HG2 E. coli (wild type NCTC 12900), Salmonella enterica serovar Thompson (RM 2311 and RM 2313) and Campylobacter jejuni (RM1997 and RM2112) containing the green fluorescent protein (GFP) or similar fluorescent protein such as cyan (CFP) or yellow (YFP). Also, that after microbial extraction from environmental samples, the HG2 microorganisms can be detected clearly on membranes or agar media. In this way the efficiency of bacterial extraction procedures can be monitored and novel methods for bacterial detection and decontamination investigated.

c) To enable microscopy detection on surfaces of HG3 enterohaemorrhagic E. coli O157 (RM2323, RM2325 and ATCC 43890) containing GFP or similar fluorescent protein such as CFP or YFP. Also, that after microbial extraction from environmental samples, the HG3 microorganisms can be detected clearly on membranes or agar media. In this way the efficiency of bacterial extraction procedures can be monitored and novel methods for bacterial detection and decontamination investigated.

**Recipient or parental organism**

S. aureus, P. aeruginosa, E. coli, S. enterica and C. jejuni are known human pathogens that are spread by contact or ingestion. They are defined as Hazard Group 2 organisms by the Advisory Committee for Dangerous Pathogens. They can cause skin rashes and septicaemia, or food poisoning if ingested in contaminated food. There is also a risk of infection by parenteral routes. Enterohaemorrhagic E. coli are Hazard Group 3 organisms that can cause food poisoning and kidney failure if ingested in...
contaminated food. The S. aureus mutants have lost AIP production, making them less virulent and able to function as sensors of AIP produced by wild types strains. P. aeruginosa, E. coli, S. enterica and C. jejuni contain the green fluorescent protein (GFP) of Aequorea victoria, encoded by the reporter gene gfp, or the cyan and yellow variants. These fluorescent proteins have been expressed in a wide variety of micro organisms for research purposes and have no known toxicity toward humans. For the purposes of risk assessment the expressed gene product can be considered to be non harmful and do not change the hazard grouping of the organisms.

**Host/vector system**

1. **S. aureus** For the construction of the GMM strains the P3 promoter of the Agr system is linked to the beta-lactamase gene on a plasmid (Lyon et al., 2000, 2002). A shuttle vector, pRN7035, was used that contains plasmid replicons for E. coli and S. aureus along with antibiotic resistance cassettes: ampicillin for E. coli and erythromycin for S. aureus. This plasmid was constructed by cloning a PCR product containing the 180-nt agr P2P3 region into the pUC polylinker site of plasmid, pRN7034, which contains a beta-lactamase reporter gene. In the new construct, the P3 promoter is fused to the beta-lactamase reporter gene. Plasmids pRN7062, pRN7105, pRN 7107 and pRN 7131 were constructed by cloning PCR fragments containing agrC, agrA, and downstream termination signals from the pUC polylinker of pRN7035. These strains are not able to produce AIP themselves; however when exogenous AIP is added it activates the P2 and P3 promoters and therefore the beta-lactamase enzyme is transcribed. In order to be able identify the specific AIP produced by the test strains and to study the effect of AIP on biofilm formation, filtered supernatant taken at various stages from the test strains will be used to activate the bioassay. 2. **P. aeruginosa** Green fluorescent protein (GFP)-based reporters will be used for detecting mutagenesis within biofilms in situ and in real time: i) GFP expression will be placed under the control of the inducible tac promoter of pMEKm12 (Lu et al., 2002); in the absence of the IPTG inducer, GFP expression will be repressed and no fluorescence is observed. If mutations occur in the tac control region, de-repression can occur and allow for GFP expression. A similar GFP reporter system was used to detect mutagenesis in E. coli batch cultures (Cariello et al., 1998). ii) We will generate a GFP reporter containing a +1 DNA frameshift in its coding region - this GFP will be non-fluorescent unless a frameshift mutation causes reversion to the wild-type. iii) The gfp gene will be fused to the regulatory region of the mutD gene of P. aeruginosa. This gene encodes the highly error-prone DNA polymerase III, the expression of which leads to increased mutation frequencies in P. aeruginosa. Using these reporter systems, we will investigate biofilm development in glass flow cell reactors and examine the spatial and temporal distribution of mutagenesis during biofilm development. 3. **E. coli**, S. enterica and C. jejuni Campylobacter jejuni and Salmonella enterica serovar Thompson have already been genetically manipulated to contain plasmids containing either GFP (pWM1001 or pWM1007), YFP (pWM1008 or pWM1011) or CFP (pWM1009 or pWM10012) (Miller et al., 2000; Brandl and Mandrell., 2002). Each plasmid contains a kanamycin resistance gene (kan) and the mob (mob) and repB (rep) genes required for replication. Non-toxigenic E. coli O157 is the NCTC strain 12900 lacking the Shiga toxin genes and will be used to receive the plasmids containing the fluorescent proteins from the S. enterica strains. 4. **Enterohaemorrhagic E. coli**. coli O157:H7 strains RM2323 and RM2325 will be obtained from ATCC, and introduced into strain PAO1 (obtained from Australia). 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Evaluation of foreseeable effects

S. aureus: Pathology of S. aureus includes: Superficial lesions (abscesses wound infections and skin rashes), deep seated and systemic infections (osteomyelitis, endocarditis and pneumonia, and toxemia syndromes, toxic shock syndrome, scarlet fever and food poisoning); mastitis in livestock. In most cases treatment from various antibiotics is readily available, irrespective of the ampicillin and erythromycin resistance markers used in the GMM constructs. The beta-lactamase reporter gene is expressed by a wide variety of microorganisms, and has no known toxicity toward humans. For the purposes of risk assessment the expressed gene product can be considered to be non harmful and does not change the hazard grouping of the organisms. E. coli, S. enterica and C. jejuni: Pathology of Campylobacteriosis includes: diarrhoea, vomiting and has been linked with neurological disorders such as Guillain-Barre syndrome and Miller-Fisher syndrome, which are forms of reactive arthritis. Treatment is readily available for the disease. A person infected with Salmonella usually has fever, abdominal cramps, and diarrhea beginning 12 to 72 hours after consuming a contaminated food or beverage. The illness usually lasts 4 to 7 days, and most persons recover without antibiotic treatment.
do not change the hazard grouping of the organisms. Enterohaemorrhagic E. coli O157 has been shown to cause hemorrhagic colitis and can also cause hemolytic uremic syndrome. These strains produce Shiga toxins that cause the characteristic sequential process of the disease and have been designated as HG3. Following an incubation period of 1-5 days, watery diarrhea develops, often accompanied by abdominal cramping and vomiting. Diarrhoea becomes bloody in 1-2 days in most patients. Fever is present in about a third of cases. Illness duration is typically 4-10 days. Cattle and sheep are the reservoir (EHEC is a common inhabitant of the animal intestine) and humans can serve as a reservoir for person-to-person transmission. Transmission occurs through ingestion of contaminated food, primarily inadequately cooked beef, beef products, or foods that contact bovine-exposed soil (such as alfalfa sprouts). Waterborne transmission has also been associated with outbreaks, such as fecally contaminated drinking water and swimming pools. Non-toxigenic E. coli O157 lack the Shiga toxin genes and are designated HG2 pathogens.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

For laboratory operations the use of standard Containment Level 2 and 3 facilities, and good microbiological practice, will be sufficient to limit contact with humans and the environment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

S. aureus: Waste material containing GM S. aureus will be pretreated by immersion in the microbiocide Virkon 1%, for two hours. Virkon will be made fresh, at the time of disinfection. Validation of the effect of Virkon on S. aureus has been carried out by the manufacturer. The waste will then be followed by autoclaving at 121°C for 30 minutes, on a solids or liquids cycle, as appropriate. The autoclave used for waste is located in the building; all waste to be transported is double bagged in autoclave bags and then sealed in a plastic container. The autoclaves are validated for waste decontamination once per year. P. aeruginosa, non-toxigenic E. coli, S. enterica and C. jejuni: Waste material will be disposed of by autoclaving at 121°C for 30 minutes, on a solids or liquids cycle, as appropriate. The autoclave used for waste is located in the building; all waste to be transported is double bagged in autoclave bags and then sealed in a plastic container. The autoclaves are validated for waste decontamination once per year. Enterohaemorrhagic E. coli: Waste material will be disposed of by autoclaving at 121°C for 30 minutes, on a solids or liquids cycle, as appropriate. The autoclave used for waste is located within the CL3 laboratory itself. Indicator tape will be used on the containers to show that the working temperature as been met. Monthly monitoring will be undertaken using Browns tubes, and an annual validation of the autoclave by independent means will be performed. The final waste bag can then be disposed of as normal Category 1 laboratory waste. Work surfaces in both CL2 and CL3 laboratories will be cleaned with the microbiocide Hycolin (2% final concentration; validated by the manufacturer to completely kill the pathogens described here).

Is an emergency plan required according to regulation 20? [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Comments on the project proposal/risk assessment forms originally submitted. These points were addressed in the final versions. The Committee's members were in agreement with the levels of containment proposed, and therefore the final activity class designation.

Project Containment
Hepatitis C virus (HCV) is a positive strand enveloped virus belonging to the family Flaviviridae. It is also a hepatotrophic blood borne human pathogen that often establishes a life-long chronic infection in infected individuals and is responsible for significant morbidity and mortality throughout the world. Currently there is no therapeutic intervention that is able to eliminate viral infection effectively, and as such the ACDP guidelines require that handling of wild type virus be done under containment level 3. The purpose of the proposed work is to identify host and viral proteins, as well as viral RNA structures that are important in controlling and maintaining viral replication within cells. It is also planned to examine how viral infection of hepatocytes modulates host cell immune responses, the effect of which enables chronic HCV infection to be established. Delivery of HCV genetic material into mammalian cells will either be through DNA transfection, RNA transfection or baculovirus transduction. It should be noted that the use of baculovirus to deliver HCV genetic material into cells means that this GM entity can be considered both as a recipient organism and as a vector (for the purpose of this application it will be referred to as a vector although associated risks will be assessed in both contexts). It is hope that this work will shed light on aspects of HCV infection that may facilitate improved clinical intervention strategies.
Bacterial strains:

(1) DH5α: F- 80lacZ M15 (lacZYA-argF) U169 deoR recA1 endA1 hsdR17(rk-, mk+) phoA supE44 thi-1 gyrA96 relA1
(2) DH10Bac: F- mcrA (mrr-hsdRMS-mcrBC) 80lacZ M15 DlacX74 endA1 recA1 deoR (ara,leu)7697 araD139 galK galU rpsL l- / bMON14272 / pMON7124

Both strains are commonly used laboratory strains derived from E.coli K-12 and are considered to be able to survive in the human gut. DH5α will be used for the cloning and propagation of pUC-based plasmid vectors containing HCV genetic material. DH10Bac will be used for the generation and maintenance of bacmids (a genetically modified attenuated baculovirus genome that maintains itself as a low copy episome within the bacteria (Luckow et al. (1993) J. Virol. 67. 4566-4579). Work with these genetically-modified bacteria will be deemed to be a class one activity unless the plasmid/bacmid they contain has a cDNA copy of a full length infectious HCV genome under the control of a constitutive mammalian promoter. Under such circumstances this work will be deemed a class two activity and will be performed under containment level 2 (This represents a slight amendment to what was originally proposed and relates to comments from GMSC-see section 15). This is in case of the extremely unlikely event that a sharp injury to lab personnel could lead to such constructs being taken up and transcribed to generate an infectious virus in the cells of that individual (the reason why this is considered a low risk is that naked DNA transfects cells poorly and initiation of infection would most likely require uptake by hepatocytes rather than any other cell line).Human cell lines:(1) HepG2 cells: cells derived from human hepatocellular carcinoma cell line (ECACC 85011430),(2) Huh7 cells: human hepatoma cell line (Japanese Cancer Research Resources Bank Number JCRB0403),(3) HeLa cells: human carcinoma cell line (ECACC 93021013),(4) Primary human hepatocytes.No latent viral infection such as EBV is known to exist in the first three transformed cell lines. The intention is to introduce HCV genetic material under the control of either a constitutive or tetracycline-regulable mammalian promoter into these cell lines, either stably or transiently, using plasmid transfection or baculovirus transduction. As with the bacterial work, most cell culture work will be deemed to be a class one activity. However, if the plasmid or baculovirus used contains a cDNA copy of a full length infectious HCV genome, this work will be considered a class 3 activity. If genetic material is introduced into the cells such that, were a recombination event to occur, an infectious viral genome could in theory be reconstituted this work will be considered a class 2 activity. As a result, these latter work activities will be performed under containment level 3 and 2 respectively.

Hepatitis C virus:

The subgenomic replicon: Modifications that will be made to the viral genome will for the most part be profoundly disabling for viral infectivity. A large amount of work will use the HCV sub-genomic replicon (Lohmann et al. (1999) Science), a construct that is already widely used throughout the HCV research community. The sub-genomic replicon is capable of replicating within select tissue culture cell lines but lacks the viral structural proteins necessary for virus particle formation and is therefore non-transmissible. Foreign genetic materials that will be inserted into the replicon are listed below. Most represent reporter genes or selectable markers that allow assessment of sub-genomic replicon replication. Some inserted genetic materials will be derived from other viruses but do not represent pathogenic determinants that would lead to increased virulence or a change host range. As such none of the constructs will represent any greater risk than an 'empty' sub-genomic replicon (a more detailed description of these genetic elements is presented in section 6)Selective markers: neo, bsd, hyg, pac.Reporter genes: lacZ, Luciferase gene from Photinus pyralis, Luciferase gene from Renilla mullerei, secreted alkaline phosphatase gene, green fluorescent protein gene.Host genes: Ubiquitin gene.Non-HCV viral sequence:

Encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES), Foot and Mouth disease virus (FMDV) 2A protease, Hepatitis delta virus (HdV) ribozyme.

All work undertaken with the HCV subgenomic replicon will be considered a class 1 activity unless, as stated earlier, work in these constructs is performed with concomitant expression of HCV structural proteins from a second DNA-based construct (plasmid or baculovirus). Under such circumstances it is possible, however unlikely, that multiple recombinations events could in theory lead to reconstitution of the full length HCV genome. Such work will considered a class two activity and be performed under containment level 2 conditions. It should be emphasised that should such an unlikely event occur, the resulting genome would be expected to represent no more of a risk to human health or the environment as wild type HCV, as no other non-HCV pathogenic determinants will be present in the constructs used.Full length viral constructs: Some work will be undertaken with the purpose of mammalian expression constructs (plasmid and baculovirus) are used for the purpose of transcribing HCV transcripts which contain and express the entire HCV open reading frame but lack either the 3’ or 5’ untranslated region of the viral genome (both these RNA elements are essential for replication of the virus (Freibe et al. (2001) J Virol 75, 12047-12057; Freibe and Bartenschlager (2002) J Virol 76, 5326-5338)). As such these constructs will be profoundly replication deficient and handling of them will be considered a class 1 activity. In contrast, work that involves the introduction of full length wild type or genetically modified HCV transcripts into mammalian cells (either by RNA transfection, poli-based delivery using plasmid transfection or baculovirus transduction) is designated as a class 3 activity and will be performed under containment level 3. All genetic modifications to the full length HCV genome will either be as a result of directed point mutation, or through introduction of the same non-pathogenic foreign genetic sequence that is proposed for use with the subgenomic replicons (see above). Therefore, any HCV-related
material produced will represent no more of a risk to human health or the environment than the parental HCV virus itself.

Host/vector system

Baculovirus: As well as using RNA and plasmid transfection to introduce HCV-related transcripts into mammalian cells, this work will also rely on transduction of cells by the baculovirus Autographa californica nuclear polyhedrosis virus, which has already been shown to be more efficient than DNA transfection (McCormick et al. (2002) J Gen Virol 83, 383-394). Generation of recombinant baculovirus will be achieved using the commercial Bac-to-Bac system supplied by Invitrogen. This system relies on transposition of genetic material from a donor plasmid vector into baculovirus shuttle vector (bacmid) propagated in the E.coli strain DH10Bac (Lucow et al. (1993) J. Virol. 67, 4566-4579). The parental bacmid contains a modified baculovirus genome which is maintained in E.coli as a large (approximately 130kb) low copy number plasmid by virtue of a mini-F replicon cassette with the genome. Transposition into the bacmid is required as the bacmid genome is too large to be modified easily by standard genetic manipulation. The bacmid genome is attenuated through deletion of the polyhedrin gene, the product of which is necessary for survival of the virus under conditions of environmental stress (desiccation, u.v. inactivation, etc.) and is requisite for transmission of the virus to an uninfected host during the natural life cycle of the virus (Rohrmann (1986) J Gen Virol 67, 1499-1513). Recombinant bacmids are constructed by transposing a mini-Tn7 element from a donor plasmid into the mini-attTn7 attachment site on the bacmid, which is brought about by Tn7 transposition functions that are provided in trans by a helper plasmid. Recombinant baculoviruses are then generated through transfection of the bacmids into the insect cell line Sf9, and the viruses used to transduce mammalian cells. Use of recombinant baculovirus to introduce HCV-related material into mammalian cells is not anticipated to represent any additional hazard above and beyond that encountered from transfecting cells with the comparable RNA transcripts. In other words, working with a recombinant baculovirus that expresses an HCV replicon lacking the viral structural genes represents the same risk as working with the stable cell line in which the same HCV replicon is being stably maintained. Justification for this statement is based on a number of arguments. The first is that baculovirus and HCV represent two completely different classes of viruses and possess such divergent mechanisms to facilitate replication in host cells it is unlikely that a virulence factor from one of these two viruses would provide a selective advantage to the other. Moreover the two types of virus infect do not infect related host species (baculovirus host range is limited to insects and crustaceans and HCV is limited in host range to humans and chimpanzees) making it even more unlikely that a change in host tropism could occur through introduction of HCV genetic material into the baculovirus genome. Furthermore, recombinant baculoviruses will be attenuated through lack of the polyhedrin gene, and so are unlikely to survive or propagate should accidental release occur. In addition, previous studies suggest that in the extremely unlikely event that the attenuated virus was able to be propagated outside a laboratory environment, there is selective pressure for foreign DNA to be lost by spontaneously deletion (Pijlman et al. (2003) J Gen Virol 84, 2669-2678). Finally, the virus is extremely susceptible to complement through environmental stress (desiccation, u.v. inactivation, etc.) and is requisite for transmission of the virus to an uninfected host during the natural life cycle of the virus (Rohrmann (1986) J Gen Virol 67, 1499-1513). Recombinant bacmids are constructed by transposing a mini-Tn7 element from a donor plasmid into the mini-attTn7 attachment site on the bacmid, which is brought about by Tn7 transposition functions that are provided in trans by a helper plasmid. Recombinant baculoviruses are then generated through transfection of the bacmids into the insect cell line Sf9, and the viruses used to transduce mammalian cells. Use of recombinant baculovirus to introduce HCV-related material into mammalian cells is not anticipated to represent any additional hazard above and beyond that encountered from transfecting cells with the comparable RNA transcripts. 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Accordingly, generation and propagation of baculovirus constructs containing genes encoding individual HCV proteins, HCV subgenomic replicons or HCV genomes lacking either the 3' or 5' untranslated region will be considered a class 1 activity. In contrast, generation and propagation of baculovirus constructs containing an unmodified HCV genome or a genetically modified full length HCV genome will be considered class 3 activities and performed under containment level 3.

Origin & function

HCV genome

activity. This proteolytic activity has also been shown to suppress host innate immune responses. NS4A: acts as a co-factor for the NS3 protease. NS4B: induces membrane alterations in cells, a process thought to be required for formation of HCV replication complexes where viral RNA synthesis takes place. NS5A: both necessary for viral RNA synthesis and affects a number of cell signaling pathways in the host cell. NS5B: RNA-dependent RNA polymerase. Selectable markers: neo: encodes resistance to the aminoglycosidic antibiotic G418. bsd: encodes resistance to the nucleoside analogue blastcidin. hyg: encodes resistance to aminoglycosidic antibiotic Hygromycin B. pac: encodes resistance to aminonucleoside antibiotic puromycin. Reporter genes: lacZ: encodes for bacterially derived $beta$-galactosidase. Luciferase gene from Photinus pyralis (North American firefly). GFP: green fluorescent protein.

Non-HCV viral genetic material

EMCV internal ribosome entry site (IRES): The IRES represents an alternative way to use a 5’ cap structure to recruit translationally active ribosomes to RNA transcripts. While EMCV is a picornavirus that displays a wide host tropism and is infectious to humans, infection rarely leads to clinical complications. The EMCV IRES itself is not a virulence factor for the virus and it’s use in this work is merely to allow a second open reading frame to be introduced into the replicon transcript. FMDV 2A protease: This is an extremely small protein present within the FMDV genome. The role of it is to facilitate cleavage at the FMDV 2A/2B boundary without the requirement for additional viral or host cell protease activity. Recent data suggest that cleavage occurs not as a result of protease activity but due to a novel ribosome ‘skip’ mechanism. No other functions have been assigned to FMDV 2A and non-viral homologues that also make use of the ribosome ‘skip’ mechanism are known to exist. Use of the 2A protease allows generation of sub-genomic replicons were reporter gene or selectable marker expression can be maintained while also allowing HCV IRES-directed expression of the HCV non-structural proteins. HdV ribozyme: A RNA sequence from hepatitis delta virus capable of cleaving itself away from RNA located at its extreme 5’ boundary. Use of the HdV ribozyme allows precise definition of the 3’ end of RNA transcripts produced from the polII-derived expression systems.

Mammalian promoters

Immediate early CMV promoter: Widely used constitutively active polII promoter derived from cytomegalovirus that functions well in the majority of mammalian cell lines. ‘CAG’ promoter: Widely used constitutively active polII promoter/expression cassette that is a composite between an immediate early CMV enhancer element, a minimal chicken b-actin promoter and human b-globin polyadenylation signal.

Tetracycline responsive promoter: A composite genetic element where multiple copies of a tetracycline response element (which is recognised by the tTA element in the absence of tetracycline; see below) is upstream of a minimal immediate early CMV promoter.

Other genetic elements

tTA element: A widely used artificial protein where the Herpes Simplex virus VP16 trans-activating domain is coupled to the tetracycline repressor (tetR) protein of E. coli, giving a protein that enables transcription from a second separate ‘tetracycline-responsive’ promoter to occur (Gossen & Bujard (1992) PNAS 89, 5547-5551). The tetracycline-responsive promoter is inactive alone, and when the tTA element is expressed, transcription from it is also suppressed in the presence of tetracycline. The tTA element will be expressed from a different DNA construct to the DNA constructs containing the HCV genetic material. Ubiquitin: Small host protein that is attached onto and targets cellular proteins for degradation. This will be used as has been done previously (Blight et al. (2002) J Virol 76, 13001-13014; Friebe et al. (2005) J Virol 79, 380-392) to physically separate selectable markers and reporter genes from the HCV polyprotein as a result of protease cleavage at the extreme carboxy-terminus of the ubiquitin sequence.

Evaluation of foreseeable effects

Hazards to human health

(i) Hazards associated with the recipient micro-organism: Plasmid and RNA based HCV constructs: HCV is an ACDP hazard group 3 blood-borne virus that establishes a chronic infection in humans. In the majority of individuals this leads to chronic infection, which progresses to cirrhosis in about 20% individuals after 20 years. Treatment of chronically infected individuals with current regimens (pegylated interferon-b and ribavirin) leads to long term viral eradication in 55% of cases. However, >95% receiving treatment within the first six months following infection have long-term responses to therapy. Replication of the virus is almost exclusively within hepatocytes of the liver, although it is likely that a very low level of replication also occurs within leukocytes. No vaccines are currently available for HCV. Almost all cloned full length infectious HCV genomes are unable to replicate in tissue culture without the presence of culture-adapted mutations. Interestingly, although inclusion of culture-adapted mutations within the HCV genome results in efficient replication of the viral transcripts within cells, the formation of virus particles is still blocked. Moreover, these culture-adapted mutations are detrimental to viral infectivity and have been seen to revert to wild-type sequence when culture-adapted genomes are introduced into chimpanzees (the only robust animal model for HCV infection). A recently identified full length infectious clone (termed JFH-1) is currently the only HCV isolate that can be propagated in tissue culture and which is able to infect chimpanzees.
Baculovirus-based HCV constructs: Baculovirus will also be used to introduce full length HCV genomic constructs and full length HCV replicons into cells in tissue culture. This is an ACDP hazard group 1 virus whose replication is limited to insects. While baculoviruses can transduce (introduce their genome to the cell nucleus) mammalian cells in tissue culture viral replication does not occur and there is no evidence to date that baculoviral promoters are able to drive viral gene expression in mammalian cells. All baculovirus constructs used lack the polyhedrin gene, required for transmission of the virus under natural conditions. Furthermore, recent reports demonstrate the extreme difficulty in using baculovirus to transduce cells in vivo due to rapid inactivation of the virus by complement (Hoare et al. (2005) J Gene Med 7, 325-333).

(ii) Hazards arising directly from the inserted gene product Plasmid and RNA based HCV constructs: The inserted genes to be introduced into the full length HCV constructs will Hazards to human health(i) Hazards associated with the recipient micro-organismPlasmid and RNA based HCV constructs: HCV is an ACDP hazard group 3 blood-borne virus that establishes a chronic infection in humans. In the majority of individuals this leads to chronic infection, which progresses to cirrhosis in about 20% individuals after 20 years. Treatment of chronically infected individuals with current regimens (pegylated interferon-b and ribavirin) leads to long term viral eradication in 55% of cases. However, >95% receiving treatment within the first six months following infection have long-term responses to therapy. Replication of the virus is almost exclusively within hepatocytes of the liver, although it is likely that a very low level of replication also occurs within leukocytes. No vaccines are currently available for HCV.

Almost all cloned full length infectious HCV genomes are unable to replicate in tissue culture without the presence of culture-adapted mutations. Interestingly, although inclusion of culture-adapted mutations in the viral genome results in efficient replication of the viral transcripts within cells, the formation of virus particles is still blocked. Moreover, these culture-adapted mutations are detrimental to viral infectivity and have been seen to revert to wild-type sequence when culture-adapted genomes are introduced into chimpanzees (the only robust animal model for HCV infection). A recently identified full length infectious clone (termed JFH-1) is currently the only HCV isolate that can be propagated in tissue culture and which is able to infect chimpanzees. Baculovirus-based HCV constructs: Baculovirus will also be used to introduce full length HCV genomic constructs and full length HCV replicons into cells in tissue culture. This is an ACDP hazard group 1 virus whose replication is limited to insects. While baculoviruses can transduce (introduce their genome to the cell nucleus) mammalian cells in tissue culture viral replication does not occur and there is no evidence to date that baculoviral promoters are able to drive viral gene expression in mammalian cells. All baculovirus constructs used lack the polyhedrin gene, required for transmission of the virus under natural conditions. Furthermore, recent reports demonstrate the extreme difficulty in using baculovirus to transduce cells in vivo due to rapid inactivation of the virus by complement (Hoare et al. (2005) J Gene Med 7, 325-333).

(ii) Hazards arising directly from the inserted gene product Plasmid and RNA based HCV constructs: The inserted genes to be introduced into the full length HCV constructs will either be widely used selectable markers to allow for selection of cells carrying the construct (neo, bsd, hyg, pac) or widely used reported gene constructs to assess viral replication (Firefly and Renilla luciferase, b-galactosidase, secreted human placental alkaline phosphatase or GFP). By themselves, none of these genes or gene products are thought to constitute a biological risk, nor is there any reason to consider that they would represent a greater risk when expressed from an HCV construct than if they were absent from the construct. In order for these constructs to function it is also necessary to physically separate the expression of the viral polyprotein from the inserted gene product. This will be done either by using the EMCV IRES (which will drive translation of the viral polypeptide while expression of the inserted gene product will be driven by the HCV IRES) or by use of ubiquitin or FMDV 2A protease. Inclusion of the EMCV IRES is unlikely to represent a hazard as the native HCV genome already has an IRES. Indeed the EMCV IRES already been included in a number of full length HCV constructs and in one case using JFH has been shown to reduce the fitness of the virus (Wakita et al. (2005) Nat Med 11, 791-796). Use of FMDV2A is not thought to constitute an additional risk as the only ascribed function for this protein is facilitation of ribosome stuttering that breaks the peptide link between FMDV2A and the protein sequence downstream from it. Finally the use of ubiquitin is also thought unlikely to enhance virulence, as although insertion of ubiquitin sequence into the related bovine virus diarrhoea virus (BVDV) does lead to the virus developing a cytopathic phenotype (Tautz et al. (1993) 197, 74-85), this is because of enhanced cleavage of the NS2/NS3 boundary which in non-cytopathic BVDV undergoes only minimal cleavage (Agapov et al. (2004) J Virol 78, 2414-2425). In contrast, the boundary between NS2 and NS3 in HCV is already rapidly cleaved in the wild type virus and so alterations to this trait would not be expected to increase virulence.Baculovirus-based HCV constructs: With regard to insertion of HCV genetic material into baculovirus, given that their modes of replication (baculovirus is a large DNA virus that replicates in the insect cell nucleus, HCV is a small RNA virus that replicates in the cytoplasm) and host cell tropisms are so different, there is negligible risk that inclusion of HCV genetic material into a genetically modified baculovirus would alter baculovirus host range or pathogenicity. The other possibility, that HCV could acquire baculovirus genes due to chance recombination events while propagating recombinant baculovirus carrying HCV constructs, is remote. However, if such events took place it is not envisaged that the resultant recombinant HCV virus would pose any greater risk than wild type HCV.

(iii) Hazards arising from the alteration of existing traitsThere is no reason to believe that the full length HCV constructs will have altered pathogenicity or host range tropism as a result of the genetic modification proposed. The same applies for recombinant baculoviruses carrying HCV genetic material.
(iv) The potential hazards of sequences within the GMM being transferred to related microorganisms: Plasmid and RNA based HCV constructs: Because HCV is a positive strand RNA virus with no DNA intermediate, and has replication machinery that is unlikely to be able to process other viral RNAs, there is already little chance that any inserted gene in HCV would be able to be transferred to another pathogen. In addition, as stated before it is likely that an HCV construct carrying the proposed inserted genes would have an attenuated phenotype and so would not persist as effectively as an unmodified HCV virus. Finally, none of the genes proposed to be introduced into HCV would be likely to represent a risk if expressed by another organism. Baculovirus-based HCV constructs: As stated before, replication of baculovirus is limited to insect cells, the baculovirus constructs we are using are unlikely to be transmitted in the natural environment due to lack of the polyhedrin gene, and foreign gene inserts are known to be lost during multiple rounds of viral propagation. For this reason if an accidental release of the recombinant virus was to occur, there is little chance that this would result in transfer of the HCV genetic material to another insect virus. Moreover, if such a transfer did occur, there are at present little or no insect transmissible viruses found within the UK, yet further reducing the opportunity for recombination of the HCV genetic material with other human viral pathogens.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not applicable.**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Literature supplied by the manufacturer (Dupont) of the disinfectant Virkon, detailing the effectiveness of this product in independent tests, indicates that 1% Virkon is effective at destroying HCV after a 10 minute contact time at room temperature. The details of this study are somewhat limited (it appears that inactivation of the virus was probably ascertain through use of RT-PCR to detect viral genome), but the results are consistent with another study looking at the virucidal activity of Virkon on Yellow Fever Virus and West Nile Virus (both in the same virus family as HCV) where a solution of 0.4% Virkon was sufficient to completely inactivate both of these viruses after 10 minutes (and 0.1% Virkon sufficient to inactivate all virus after 30 minutes). The literature available on the effectiveness of Virkon for inactivation of baculovirus is limited, but numerous reports exist demonstrating the effectiveness of a 1% solution of Virkon for the inactivation of Herpes and Pox viruses (both enveloped dsDNA viruses like baculovirus) and in these instances similar contact times of 10 to 20 minutes were employed. We therefore plan to disinfect all liquid waste from both class 2 and class 3 activities by addition of Virkon to a final concentration of 2% (w/v), and allowing the resultant solution to sit overnight at room temperature before being disposed of down the sink. Prior to commencing work, an independent study will also be performed to confirm the effectiveness of Virkon at inactivating baculovirus under the conditions just described. No such study can as yet be performed for HCV, as we are not at a point in time where we can propagate HCV in tissue culture (this is because the institute's containment level 3 facility is still under construction). Once we are at this stage, tests examining the effectiveness of Virkon will be extended to look at inactivation of HCV. Solid waste from class 2 and class 3 activities will be sterilized by autoclaving at 126ºC for 30 minutes in an autoclave that is checked periodically to ensure adequate performance (in the case of class three activities, this will be done within the confines of the containment level 3 facility with a browning tube included in each autoclave run to confirm effective sterilization) and then sent for incineration.

**Is an emergency plan required according to regulation 20?**

**If yes, tick to confirm that it is attached to this form**

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**
The committee's members agreed with your categorisation of certain aspects of the project as Activity Class 2 and others as Activity Class 3. As such, these parts of the project will require HSE acknowledgement or approval, respectively, prior to their commencement. With regard to the elements that you have classified as Activity Class 1, the committee's members were slightly concerned with the handling at containment level 1 (CL1) of DNA or RNA constructs incorporating a full complement of hepatitis C genes. In theory, such genes could be expressed if the nucleic acid were to gain access to the operators cells via, for instance, mucous membranes, abrasions or needlestick. Virus replication may result from this expression. Handling such constructs at CL1 would suggest that there was no risk, or an insignificant risk, to normal healthy individuals. This may be the case, but please explain why this is the case. (NOTE: Modifications were subsequently made so that DNA constructs that contained a full length HCV genome under the control of a constitutive mammalian promoter would be handled under containment level 2. Handling of full length naked RNA transcripts will still be a designated a containment level 1 activity, as this form of nucleic acid is extremely labile and recovery of infectious virus from it is known to require direct intrahepatic inoculation. This was discussed with the biological safety officer and deemed acceptable.) Also, members of the GMSC were interested to know where it is proposed to carry out the AC3 work, as the University does not currently have a working containment level 3 laboratory. The Committee recommended that personnel intending to work on the AC2 or AC3 aspects of the project should be referred to the Occupational Health department for assessment of their baseline hepatitis C status, prior to the commencement of work. This can be dealt with at the OH department here at SGH, but they have asked me to inform them of the staff names prior to referral.

### Project Containment

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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### Project Ref 57/08.1

**Date Ackn'd** 21/02/2008

**Date Project Ceased**

**CU2 Project Title** Use of lentiviral particles and rhinovirus (HRV) to study inflammation, infection, repair and lipid metabolism.

**Class** Class 2

**CultureVolClass2** < 1 Litre

**CultureVolumeClass3-4**

**Non-GMM** Not Applicable

**Consent Granted**

**Project notified under transitional arrangements** Y

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**
**Purposes of the contained use**

The project aims at gaining a deeper understanding of the mechanisms steering the immune response to self, infection, and tumors and the ensuing tissue repair responses. For this purpose, we will make use of a range of mammalian cell lines either overexpressing specific genes, or cell lines in which genes have been silenced via small inhibitory ribonucleic acids (siRNA, e.g. small hairpin ribonucleic acids, shRNA). We will use Lentiviral particles, which are a third generation, self-inactivating retroviral expression system, to stably express either transgenes or siRNAs in mammalian cell lines and primary cells. Our studies will focus on understanding the interaction and signalling of cellular receptors, on trafficking of molecules within the cells, and on the effect of metabolic alterations within the cell. Furthermore, we will define the role of microRNAs in host defence and in modulating the immune response. To complete this goal, we will study viral infection employing human rhinovirus (HRV).

Human Rhinovirus (HRV) is a positive strand, non-enveloped RNA virus belonging to the Picornaviridae family and is one of the major causes of the common cold. It is also implicated in acute exacerbations of asthma in all age groups, which is a major cause of morbidity and mortality. Currently there are no established methods for effective prevention or treatment of rhinovirus infections. In order to elucidate and understand the nature of rhinoviral replication in human lung cell culture models, and the effect of microRNAs on this process, we propose to construct a recombinant form of HRV with a reporter gene. This will involve the generation of full-genome HRV cDNA in which a reporter gene is inserted either between the 5’ UTR and ORF or between structural and non-structural, i.e. P1 and P2, regions of the genome. RNA transcribed in vitro and introduced into cells is predicted to replication and produce virus, based on similar studies with other picomaviruses. Such a virus will be used to re-infect cell lines and replication will be assayed by level of reported gene expression. This system will allow the analysis of primary infection and bystander effects in a human lung explant model. Some of the cell lines will have been previously stably transfected with lentiviral particles, in order to evaluate the role of different microRNAs in the cellular immune response.

1. Tang et.al. (1997) JVirol 71;7841-7850

**Recipient or parental organism**

**Bacterial strains**

1) DH5a: F Ø80lacZAM15 A(lacZYA-argF) U169 deoR recA1 endA1 hsdRI7(rk, mk) phoA supE4 R171 gyrA96
m1A1 This strain is a commonly used laboratory strain derived from the K-12 E. coli strain, derivatives of which are considered to be unable to survive in the human gut
2) XL1-0-Gold cells have the lacZA. lacZ. lacZ M15 gene
3) XL-1 blue has the lacIq gene and overexpresses the lacIq protein, and thus represses the lacZ alpha gene on plasmids like pUC19

**Mammalian cells**

1) Ohio HeLa cells: human carcinoma cell line
2) Primary bronchial epithelial cells
3) Primary bronchial fibroblast cells
4) Peripheral blood mononuclear cells
5) Bronchial tissue explants
6) Primary epithelial cells
7) Primary fibroblast cells

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*Date of Significant Change*

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*Project Additional Information*

**Purposes of the contained use**

The project aims at gaining a deeper understanding of the mechanisms steering the immune response to self, infection, and tumors and the ensuing tissue repair responses. For this purpose, we will make use of a range of mammalian cell lines either overexpressing specific genes, or cell lines in which genes have been silenced via small inhibitory ribonucleic acids (siRNA, e.g. small hairpin ribonucleic acids, shRNA). We will use Lentiviral particles, which are a third generation, self-inactivating retroviral expression system, to stably express either transgenes or siRNAs in mammalian cell lines and primary cells. Our studies will focus on understanding the interaction and signalling of cellular receptors, on trafficking of molecules within the cells, and on the effect of metabolic alterations within the cell. Furthermore, we will define the role of microRNAs in host defence and in modulating the immune response. To complete this goal, we will study viral infection employing human rhinovirus (HRV).

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1) Ohio HeLa cells: human carcinoma cell line
2) Primary bronchial epithelial cells
3) Primary bronchial fibroblast cells
4) Peripheral blood mononuclear cells
5) Bronchial tissue explants
6) Primary epithelial cells
7) Primary fibroblast cells
(8) Tissue explants
(9) HEK293T cells (human embryonic kidney cancer cell line)
(10) T2 lymphoblast line and derivatives (e.g. T2.721.174)
(11) THP1 monocytic line
(12) U937 monocytic line
(13) Hep2G hepatoma line
(14) HL60 human leukemia line
(15) Jurkat cells
(16) Raji Burkitt lymphoma line
(17) Human Melanoma lines
(18) MCF7 human breast cancer line
(19) Human Fibroblast lines
(20) CaCo2 human colon carcinoma line
(21) K562 erythroblast line
(22) Rat basophilic leukemia line
(23) CHO (Chinese hamster ovary) cells
(24) C1R B lymphoblast line

Viral strains:
(1) Human rhinovirus — 16
(2) Human rhinovirus — 1 f3

Host/vector system

Host: We will be using a third generation lentivirus system comprising different plasmid vectors for individual components of the final virus particle. The retroviral genes in these vectors have been modified/truncated to make recombination/replication impossible (i.e. they are self-inactivating). Furthermore, the packaging signal for the virus particles is encoded by a different plasmid vector from the target sequence.

The pathogenic properties of any modified form of the rhinovirus genome are likely to be reduced relative to wild type virus. The proposed wild type picornaviruses are classified by the ACDP as hazard group 2. (J Virol 1998 Jan; 72 (1): 20-31)

Vectors: The genes of interest (GOI) are transferred into a lentiviral transfer vector, such as pSIHl-H1-PURO, pHRsinl8, WPT, pLVTHM, pLV/ITR_KRAB_Red, or pLKO.1-puro. The GOI is now flanked by the non-coding retroviral LTRs, while no retroviral genes are encoded on this plasmid and therefore no retroviral genes transferred into the particles.

In order to obtain the modified rhinovirus, modifications will be made to viral cDNA contained in standard non mobilisable DNA plasmids, grown in laboratory adapted strains of Escherichia coil that are unlikely to survive in the human gut (Eur J Clin Microbiol 1982 Jun; 1(3): 186-92)

Origin & function

In the first part of the project human genes will be identified that affect the immune response to self, infection, and tumors and the ensuing tissue repair responses. For this purpose mammalian cells will be subjected to gene silencing by lentiviral particles, which will be generated from the GeneNet H.50K siRNA lentiviral plasmid library. As a readout we will use functional assays as well as recombinant fluorescent immunoreceptors or antibody based approaches. Promising cell clones will be FACS sorted and the genes responsible for their individual phenotypes will be sequenced.

In the second part of the project, specified individual human genes will be targeted in various human cell lines by gene silencing (using 5hRNA encoding lentiviral particles). This is to confirm the findings of part one of the project. In the third part of the project we will overexpress individual genes in various human cell lines. The rational for overexpressing these genes will be derived from the first two parts of the project.

In a second project, we will identify genes that affect the immune response to self, infection, and tumors and the ensuing tissue repair responses by overexpressing mammalian genes in eukaryotic cells. For this purpose mammalian cells will be subjected to gene overexpression using lentiviral particles, which will be generated from a
human lentiviral open reading frame cDNA libraries. As readouts, we will use functional assays, recombinant fluorescent immunoreceptors or antibody based approaches. Promising cell clones will be FACS sorted and the genes responsible for their individual phenotypes will be sequenced.

Finally, we will overexpress, in different mammalian cell lines and primary cells, microRNAs to study their role in immune responses and tissue repair. One & the main goals will be to evaluate the role of these microRNAs in the cellular host defense against viral infection. This will be tested employing human rhinovirus (HRV).

Generation of full-genome HRV cDNA constructs in which a reporter gene (green fluorescent protein, LacZ or luciferase) is inserted either i) between the 5' un-translated region (UTS) and PRF or ii) between structural and non-structural regions of the genome. RNA transcribed in vitro and introduced into cells is predicted to replicate and produce virus. Such a virus will be used to re-infect cell lines and replication will be assayed by level of reporter gene expression. This system will allow analysis of cell-entry and early events in viral infection in culture.

Well documented 'reporter' genes expressing proteins such as Green Fluorescent Protein, @-Galactosidase or Luciferase, are non-toxic, non-allergenic and are not predicted to alter the nature of the virus.

The genes of interest (GOI) are transferred into a lentiviral transfer vector, such as pSINHi-PURO, pHRsinI8, WPT, pLVTHM, pLViTR_KRAB_Red, or pLKO.1-puro. The GOI is now flanked by the non-coding retroviral LTRs, while no retroviral genes are encoded on this plasmid and therefore no retroviral genes are transferred into the particles. This construct is packaged into particles using a HEK293T cell or HEK293FT cell based packaging system. For the packaging the HEK293T or HEK293FT cells are co-transfected with additional plasmids (such as pLP1 and pLP2, pPAX2, pMD2G, or pFIV-34N/pVSV-G). These plasmids express the envelope protein from VSVg and the non-structural proteins of the virion; none of these genes are transferred into the particles since they lack the packaging signal, which is only present on the lentiviral transfer vector plasmids. After transfecting the producer cells, the particles can be harvested from the supernatant over the next three days.

Hazard to Human Health:

The Lentiviral Expression Systems used include the following key safety features:

• They contain a deletion in the 3' LTR (AU3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
• The number of retroviral genes that are used in the system has been reduced to three (Le. gag, p0!, and rev.
• The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes.
• Genes encoding the structural and other components required for packaging the viral genome are separated onto three to four plasmids. All plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus.
• Although the packaging plasmids used in these system allow expression in trans of proteins required to produce viral progeny (e.g. gal, p01, rev, env) in the HEK293T or HEK293FT producer cell lines, none of them contain LTR5 or the LI) packaging sequence. Therefore, none of the retrovirus structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
• The lentiviral particles produced in these systems are replication-incompetent and only carry the gene of interest. No other viral species are produced.
• Expression of the gag and pol genes from LP1 has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag! pol mRNA transcript. Addition of the RRE prevents gag and p01 expression in the absence of Rev.
• A constitutive promoter (RSV promoter) has been placed upstream of the 5' LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA.

The Modified rhinoviruses present no more human hazard than wild type virus. The infection resulting from such an exposure is predicted to be significantly reduced relative to wild type virus, due to the reduced replication efficiency of the modified virus, based on previous studies with other members of the picomaviridae family of viruses 1,2 The effect of expression of a non toxic reporter gene product in infected cells would be negligible relative to the effect of normal wild-type virus replication which destroys the cell. The growth of replication-defective mutants is likely to be very inefficient. Furthermore, in the context of a natural infection, the genetic instability of the inserted gene combined with normal in vivo selection pressures is predicted to cause rapid reversion to a genotype effectively identical to wild-type1.

Infection of stably transfected cell lines, that were previously transduced with lentiviral particles don’t pose an additional hazard to standard infection experiments. As mentioned above, none of the retrovirus structural genes
are actually present in the packaged viral particles, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced and no cross-recombination can occur. Finally, regarding the hazard that inserted genes could pose, we will exclude from this expression system genes encoding:

a) proteins of retroviral origin
b) toxins
c) oncogenes

In the specific gene expression approaches, genes that will be used for overexpression or silencing are involved in the immune or repair response and metabolism. There are no described adverse effect regarding their overexpression in healthy organisms, tissues or cells. In addition, gene silencing of mammalian genes in cell lines using whole genome libraries (such as the one used in our project) has not been described to be hazardous.

Environmental and Activity considerations:
We are confident that the final lentiviral GMMs pose no hazard to the environment. Lentiviral particles are very inefficient infecting cells (They need very specific experimental conditions, including manipulating the charge of the cell surface by adding the chemical substance Polybrene before transduction of the virus particles to the cells), and are unable to replicate (self-inactivating replication-incompetent viruses are used). Lentiviruses have a short half-life and are unstable at room temperature. In addition, as described above, lentiviral particles are low infective and unable to replicate (self-inactivating). Although VSVG-pseudotyped virus particles can transduce, in theory, all mammalian cells, transduction is also highly dependent on the concentration of the virus particles in a given medium. In addition, primary cells are much more difficult to transduce with lentivirus particles than in vitro generated cell lines. For these reasons, it is very unlikely that the lentivirus particles pose a risk for the environment.

Oral ingestion of lentiviral particles will be prevented by standard laboratory working practices. Aerosol formation resulting from an accident outside of the class II safety cabinet and involving Lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory workers’ skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low risk for humans. Firstly, they are replication-incompetent. Secondly, these particles carry an extremely low infection risk, and transduction of a laboratory worker’s skin or mucosa is virtually impossible. In addition no genes encoding toxins, oncogenes, or pathogen-derived genes are used in our studies. In the unlikely event of accidental transduction of a laboratory workers skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

For work involving HEK293FT cells to produce the lentiviral particles the following procedures will be deployed:
Work with these cells will be conducted within a class 2 microbiological safety cabinet (MSC). The MSC is serviced and tested (inflow/downflow) on a regular basis. The cells will be maintained in closed tissue culture vessels and incubated in a dedicated incubator within the laboratory. Harvesting of the lentiviral particles will be carried out 1 8-72hrs-post transfection. The culture vessels will be transferred to the class 2 cabinet, and only then will the lids be opened. In some cases the lentiviral particle containing media will be used directly to transduce cells in culture. In other cases the virus particle containing media will be decanted into sealable centrifuge containers and the particles pelleted by centrifugation. The supematants will be directly used for cell transduction or decanted directly into a 1% w/v Virkon solution. The culture vessels will then be given fresh media and be returned to the class 2 MSC. The pellets will be re-suspended in PBS and then be transferred to a —80°C freezer.

The particles will then be used to transduce cell lines by adding a small amount (~500ul) of particle suspension (with up to 1 x10⁷ particles per ml) to the host cells. Effective transduction of mammalian cells requires the addition of the chemical Polybrene (5-15 microgramms/ml) to the cell cultures. 24h later transduced cells will be passaged at least once before analysis of gene expression by FACS calibur (which will be flushed with a “clean” solution (provided by Becton Dickinson) containing bleach. After at least 2-3 passages, when no viral particles will be present anymore in
the culture medium, the transduced cells will be sorted using FACS. Before FACS sorting the cell culture medium will be checked for the presence of infectious virus particles by transducing HeLa cell line (in the presence of polybrene) and FACS analysis of this cell line (GFP fluorescence of the HeLa line after “transduction” with culture cell medium). Upon passage of the transduced cells infectious particles are no longer present and special containment measures won’t be necessary in the FACS facility. Subsequent work will then be carried using good microbial practices.

When working with HRV, the following procedures will be contemplated:

The primary route of transmission for HRV is by aerosol droplet inhalation. Oral ingestion will be prevented by standard laboratory working practices. Aerosol formation resulting from an accident outside of the class II microbiological safety cabinet and involving HRV-containing liquid may lead to infection of laboratory workers with modified viruses. The infection resulting from such an exposure is predicted to be very much reduced to that resulting from wild type virus, due to the reduction in viral replication described previously. Infection of the community at large with a modified virus is unlikely for reasons of genetic instability described earlier.

There would be negligible economic or ecological consequences of a major escape. No infection of animals with wild type or recombinant HRV has been reported.

The existing containment (CL2) and normal working practices in use for work with wild type HRV are sufficient to reduce the risk of aerosol infection of laboratory workers to a very low level. These include the use of a class II cabinet for all cell culture manipulations to prevent formation or escape of aerosols from samples taken outside of the cabinet (eg. Samples contained in plastic screw-capped vials, centrifugation carried out in sealed rotors etc.)


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste containing viral particles is inactivated by overnight treatment with Virkon (2% final concentration). Fresh Virkon solution will be made up before each daily experiment. All viral exposed materials, except for ultracentrifuge tubes and work bench surfaces, will be disinfected with Virkon. Ultracentrifuge tubes and work bench surfaces will be disinfected with 70% ethanol. After virus inactivation dry waste goes into the yellow bags (which will be collected by the contractor, macerated, autoclaved and landfilled with the hospital’s clinical waste). Class II cabinets are tested once a year by the KI discus method as recommended by HSE guidelines (NOTE: Lentivirus particles are irreversibly inactivated by 20% - 75% Ethanol; Virkon irreversibly inactivates all enveloped viruses). The manufacturer of Virkon (DuPont/Antec International) was consulted on the effectiveness of this disinfectant against rhinoviruses. Their response was “Unfortunately we have no direct efficacy test data for Virkon against Rhinoviruses. However, Rhino viruses are from the same family of viruses as F M 0 and Polio (picornaviridae) for which we do have data. Therefore we can advise that Virkon will be effective against rhino viruses. Polio virus is considered to be the most resistant to biocides therefore if Virkon is effective by extension it should be effective against Rhinovirus.” Their efficacy data is attached.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

None

Tick to confirm that you have attached a risk assessment to this form

None

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y
Lentivirus project (extract of the minutes of a meeting of the Genetic Modification and Biosafety Committee held on 15 January 2008):

"Use of lentiviral practices automatically required classification at Activity Class 2 and these components of the project would therefore require USE notification. A draft of the notification was currently being prepared. Work on this component of the project could not begin until USE acknowledgement was received. The work would take place in an appropriate CL2 laboratory.

Some concern had been expressed about FACS sorting and the potential for infection — however each cell would be washed to remove infectious particles before any FACS sorting took place. The remaining cells could not infect because they were replication-incompetent.

(A member) raised queries about disposal (waste) particularly as the researchers were new to the University. (It was) commented that he had discussed this with them in detail, and emphasised that the P1 had undertaken similar work in Switzerland, including FACS work. It might however be appropriate to schedule the laboratory for a visit relatively soon.

Resolved That the elements of the project relating to the propagation of plasmid vectors in E Coil be approved as Activity Class 1; and that the remaining elements be classified as Activity Class 2, requiring HSE notification."

Rhinovirus (extract of the minutes of a meeting of the Genetic Modification and Biosafety Committee held on 17 October 2007):

"Members supported the assessment that this project contained both AC1 and AC2 components. USE notification was therefore required for the AC2 elements, and work could not begin until USE agreement was received. It was confirmed that sharps would not be used,

Resolved That the elements of the project proposal relating to propagation of the plasmid and generation of RNA transcripts be approved at Activity Class 1; and that the elements relating to the transfection of RNA transcripts into cells and the infection of recombinant virus into primary cell cultures be approved at Activity Class 2."

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**Project Containment**

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<td>02/12/2008</td>
<td>Contrasting immune adaptation in peripheral organs and the CNS in response to chronic infection in health and disease.</td>
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<th>Project notified under transitional arrangements</th>
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02/03/2022 Page 2156 of 15326
## Project Additional Information

### Purposes of the contained use

Our strategy is to employ a live attenuated bacterial strain to mimic a bacterial infection in otherwise healthy mice or mice with ongoing neurodegeneration (ME7 prion infected mice). We believe that this can only be achieved using live bacteria, rather than heat-killed bacteria or bacterial products such as lippolysaccharide (LPS). While LPS induces inflammation and is widely used in animals to model aspects of bacterial infection, it does not mimic the dynamics of a real live infection, characterised by prolonged, often low grade exposure to replicating bacteria. Attenuated Salmonella strains are ideal for our studies due to its potential to elicit all three forms of immunity: mucosal, systemic and humoral with a low risk to induce human disease. A second advantage is the well characterised innate and acquired immune responses in mice following infection with Salmonella.

### Recipient or parental organism

Wild-type Salmonella enterica serovar Typhimurium is an intestinal pathogen which causes infection in mice and human. In this project we propose to use the attenuated strain SL3261, an AroA mutant. This mutation makes the bacteria unable to grow but will persist in the antigen presenting cells in host tissue for several weeks, but it is not likely to cause disease.

Hoiseth et al were the first to introduce the aroA mutation and measured the level of attenuation between SL3261 and its parent strain SL1344. In this study, 3x10 cfu of the AroA mutant SL3261 did not cause ill effects in mice while the LD50 for SL1344 was only <20 cfu. From this study it can be concluded that the level of attenuation of SL3261 is 6 log reduction in cfu relative to its (grand) parent strain, SL1344. (Hoiseth SK and Stocker BA, Aromatic-dependant Salmonella Typhiumurium are non-virulent and effective as live vaccines. Nature 1981 May 21:291 (5812):238-9)

Salmonella organisms do not multiply significantly in the natural environment (out of digestive tracts), but they can survive several weeks in water and several years in soil if conditions of temperature, humidity and pH are favourable. When working in a laboratory it is unlikely that the SL3261 strain will survive in the environment. Furthermore, laboratory mice that have not been exposed to predators or finding their own food would be unlikely to survive for long in the environment should escape occur. Salmonella may be shed in the faeces of exposed animals and increase the risk of exposing other animals.

Innocation of mice with a high dose of SL3261 strain may cause sickness behaviours in mice, such as pilperection, hunched posture, which may persist for weeks. Overt symptom including fever and diarrhoea may occur when high doses ie 10 (8) 0 10 (9) cfu of SL3261 are used.

Many attenuated Salmonella vaccines have been constructed and used in experimental conditions and some attenuated Salmonella vectors have already been evaluated in humans for clinical use. None of these studies have reported side effects, despite using relative high doses of bacteria ie 10(9) cfu. In our project we will not exceed does of 10 (8) cfu and, therefore if bacteria are accidentally ingested or needle stick accidents occur, there is low risk for human disease. Based on this information we consider the most hazardous GMM to be a ME7 prion infected mouse inoculated with 10 (8) cfu/ml of attenuated S.Typhimurium SL3261.

### Host/vector system

The GMM Salmonella strain SL3261 will be used, but no further genetic modification undertaken.
**Origin & function**

The GMM Salmonella strain SL3261 will be used, but no further genetic modification undertaken.

**Evaluation of foreseeable effects**

The GMM Salmonella strain SL3261 will be used, but no further genetic modification undertaken. The characteristics of this strain are detailed above.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

In this project we will use the ME7 prion agent to induce chronic neurodegeneration. Our animal facility has designated rooms that are level 2 containment. If we use bacteria to infect animals or genetically modified animals, such as mice deficient for cytokines, we will use the same control measures as the ME7 prion infected mice. The measures include inactivation of bacteria in cage and consumable using a 10 minute soak in 1:10 diluted chlorine bleach. Animal tissue will be autoclaved to inactivate bacteria prior to incineration.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

We will use disposable consumables for bacterial cultures and preparation of glycerol stocks. Bacterial cultures that are discarded will be neutralized with freshly prepared chlorine bleach or by 30 minutes treatment at 121°C using an autoclave. The autoclaves we will use are validated for a bacterial waste management cycle ie 30 minutes at 121°C and we will regularly check the inactivation of bacteria (for example S.Typhimurium SL3261) using sterilization indicator ampoules. The autoclaves are a LTE300SH and a Kestrel. It has been shown that steam heated to 121°C for at least 15 minutes will inactivate all fungi, bacteria, viruses and also bacterial spores. Work benches will be wiped down with freshly prepared Chlorine bleach to inactivate any spilled bacteria and we will take regular bacterial swabs to check the inactivation of bacteria on work benches. Chlorine bleach is an accepted liquid sterilizing agent. Household bleach consists of 5.25% sodium hypochlorite. To inactivate S.Typhimurium SL3261, we will use a 1:10 dilution. The dilution factor must take into account the volume of any liquid waste that it is being used to sterilize. Bleach will kill many organisms immediately, but for full sterilization is should be allowed to react for 20 minutes. Bleach will kill many, but not all spores. It is highly corrosive and many corrode even stainless steel surgical instruments. Bleach decomposes over time when exposed to air, so fresh solutions should be made daily.

Use of bacteria in vivo:

In this project we will inject bacteria in otherwise healthy mice and mice infected with ME7 prion agent. We will use disposable needles to inject mice with bacteria. Gloves will have to be worn during the injection procedure and needles will be disposed of using incineration containers. To minimise the risk of needle stick injury, only experienced workers in the facility will be allowed to inject the bacteria. We will assess this by an appropriate animal handling assessment. Bacteria may be shed in the faeces of exposed animals. Infected animals will be housed in such a way that there will be an appropriate barrier to the possible spread of infection. For example, mice will be kept in filter top cages in separate holding room and animal’s handlers must wear gloves and wash their hands after handling infected mice. At the end of the experiment, we will inactivate bacteria that are left in the cage, including bedding, cage lid and animal tissue that will not be used for further analysis, using an autoclave (bacterial waste management cycle 30 minutes 121°C). We will use an autoclave located in the animal facility. This autoclave will be validated for inactivation of bacteria using sterilization indicator ampoules and the inactivation of bacteria will be checked regularly using similar sterilization indicator ampoules. Inactivated animal tissue, in particular those that contain ME7 prion agent, will be incinerated after autoclave treatment. Incineration will burn any organism to ash and is used to sanitise medical and other bio hazardous waste before it is discarded with non-hazardous waste. Work benches will be wiped with 1:10 diluted bleach to neutralise any spilled bacteria and bacterial swaps taken to check the inactivation of bacteria.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

02/03/2022

Page 2158 of 15326
Committee members commented that there appeared to be some conflicting sentences in the original proposal regarding the degree of hazardousness – if a safety cabinet was to be used for the work, did this not imply there was a potential risk to health? It was commented that work involving wt salmonella would usually require level 2 containment, but that in this instance as the S.Thyhmurine strain to be used was highly attenuated, perhaps CL1 would be appropriate? (the prion work as part of the project would of necessity require CL2 – work with this agent had already been approved). It was suggested that a good case had been made regarding the safety of the salmonella strain. Perhaps it would be useful to find out how the collaborators on the project classified their work with this salmonella strain? Alternatively advice might be sought from the HSE.

It was resolved that enquiries would be made from the collaborators as to how they classified this work; and that further advice would be sought from the HSE if necessary. After the meeting, the collaborators were contacted. It was confirmed that work with this salmonella strain had been classified as AC2. Therefore, it was decided to classify the work with this strain at Southampton as AC2.

### Project Containment

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### Project Ref 57/09.1

- **Date Ackn’d**: 08/10/2009
- **CU2 Project Title**: Surfactant Protein D (SP-D) as a potential therapeutic against recombinant avian H5N1 influenza A virus infections
- **Date Project Ceased**: 
- **Class**: Class 2
- **Culture Volume Class 2**: < 1 Litre
- **Culture Volume Class 3-4**: 
- **Non-GMM**: Consent Granted
- **Project notified under transitional arrangements**: No
- **Withdrawn**: No
- **Tick if notifying a connected programme of work**: No
- **Historical Significant Changes**: No
### Project Additional Information

**Purposes of the contained use**

To investigate if surfactant protein D can be used as a therapeutic against influenza A virus infections.

**Recipient or parental organism**

The two recombinant avian H5N1 influenza strains are attenuated viruses and candidate strains for the human vaccine against avian H5N1 influenza. They are made by reverse genetics at the 'National Institute of Biological Standard and Control' (NIBSC). The genes encoding the 'internal' of the virus is from human H3N2 PR/8/34 and the 'external' is avian H5N1 genes in a mix of 6:2. Furthermore, the basic amino acids in the region cleaved in haemagglutinin has been genetically modified to contain neutral amino acids. These precautions ensure that the strains will not replicate well in human beings.

**Host/vector system**

Genes encoding for viral proteins are cloned into plasmids, each gene into an individual plasmid. The plasmids are transfected into a cell line and a recombinant influenza strain is generated according to the genes cloned into the plasmids. The recombinant influenza strain is then amplified in embryonated hen eggs according to established procedures. The specific information can be obtained from NIBSC.

**Origin & function**

The external of the recombinant H5N1 virus strains are originally isolated from human clinical samples. The internal of the recombinant H5N1 strains are from the PR/8/34 influenza strain which is a human attenuated strain and has a track record of being a safe strain to use for human vaccines against influenza viruses. These recombinant virus strains are candidates to be used in the human vaccine against the H5N1 influenza A virus. These two recombinant H5N1 strains will be used as 'model H5N1' strains' when evaluating the potential of surfactant protein D as a therapeutic against influenza infections.
These viruses are made so they replicate well in embroyolated hen eggs (standard amplification procedure) but will have a low pathogenicity to human beings. The risk for humans contracting and being infected with these recombinant H5N1 strains is therefore minimal. The constellation of these recombinant viruses, with an external H5N1 surface and internal genes from H3N2 PR/8/34, together with the removal of basic amino acid residues in the hemagglutinin, is an efficient two-step procedure to ensure the low pathogenicity of the viruses. During several amplification rounds at the NIBSC no mutations were found in the HA of the viruses verifying that these viruses are stable viruses.

Evaluation of foreseeable effects

These viruses are made so they replicate well in embroyolated hen eggs (standard amplification procedure) but will have a low pathogenicity to human beings. The risk for humans contracting and being infected with these recombinant H5N1 strains is therefore minimal. The constellation of these recombinant viruses, with an external H5N1 surface and internal genes from H3N2 PR/8/34, together with the removal of basic amino acid residues in the hemagglutinin, is an efficient two-step procedure to ensure the low pathogenicity of the viruses. During several amplification rounds at the NIBSC no mutations were found in the HA of the viruses verifying that these viruses are stable viruses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Pathogenicity has been assessed by NIBSC using the intravenous chicken pathogenicity test, a ferret pathogenicity test, a test for plaquing in absence of trypsin and a test for lethality in eggs. In the chicken pathogenicity test, both strains showed no pathogenicity with a score of 0.0. In the ferret pathogenicity test, both strains was less pathogenic than the original H5N1 strains (which was only slightly pathogenic) and similar to PR8 which was non-pathogenic. In the plaquing test, both strains produced plaques on MDCK cells in the presence of trypsin but not in the absence of trypsin. On egg passage, bot strains grew to high titre and all embryos survived; in contrast, passage of the original H5N1 strains in eggs resulted in the deaths of all embryos. In addition, gene sequencing of the HA segment in both recombinant strains confirmed the absence of the polybasic amino acids that determine pathogenicity. In conclusion, these strains are considered to be non-pathogenic. Following six additional serial passages in eggs, both NIBRG-14 and NIBRG-23 were assessed for pathogenicity in eggs and stability of HA sequence through the deleted polybasic cleavage site. Both strains grew to high titres in eggs and all embryos survived. Results of sequencing confirm that these recombinant strains do not contain the polybasic cleavage site of the original wildtype virus and has retained the mutations introduced to prevent a new site reforming.

The strains are of low pathogenicity for humans and can be used in a normal CL2 laboratory setting with controlled management. i.e. neutral air pressure, the laboratory can only be entered by appropriately trained staff and all waste is disinfected by virucidal solution (1% Virkon solution) followed by autoclaving before disposal. The work will take place in a class II safety cabinet with HEPA filtration of air prior to exhaust, staff will be offered seasonal influenza vaccination before working with the viruses and antiviral drug, as Tamiflu, will be available as prophylaxis if required.

All work with viable virus will take place in the specified laboratory. If further analysis of virus infected samples are required outside the laboratory, virus will be killed with 4% paraformaldehyde and solid materials will be wiped down with a 1% Virkon solution before leaving the laboratory.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste (both solids and liquids) will be subjected to a double-inactivation procedure to ensure 100% kill of the virus: Initial, all waste will be soaked in 1% Virkon solution for a minimum of 30 minutes before disposal in an autoclave box followed by autoclaving for a minimum of 20 min at 1210C.

Monitoring that viruses are killed 100% can be detected using a plaque assay to detect viable virus from wipe tests from solid waste or liquids, respectively.

02/03/2022
The University GM & Biosafety Committee reviewed this project proposal and associated risk assessment on the 29th September 2009 and approved the recommendation that the work be classified as Activity Class 2 and could be safely conducted in a Containment Level 2 laboratory with the recommended safety precautions.

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**Project Ref** 57/09.2

- **Class CultureVol**
  - Class 2
  - CultureVolume: Class 2

- **Date Ackn'd**
  - 29/10/2009
- **CU2 Project Title**
  - Modulation of Apoptosis by BCL-2 Family Proteins
- **Date Project Ceased**
- **Class CultureVol**
  - Class 2
  - CultureVolume: ≤ 1 Litre
- **Non-GMM Consent Granted**
- **Withdrew Consent**
  - N
- **Tick if notifying a connected programme of work**
  - N
- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
BCL-2 family proteins are key regulators of apoptosis, a form of programmed cell death. The primary aim of this work is to investigate the function of the pro-apoptotic BIM protein in controlling apoptotic cell death. The secondary aim is to compare the function of BIM with other structurally and functionally related BCL-2 family proteins.

Virus: Lentivirus, originally derived from HIV-1. The virus is disabled and transfection of cells with the lentiviral plasmids will not result in the formation of replication competent virus.

Recipient cells: Established cell lines derived from human solid and liquid malignancies. We will also use the viruses to infect malignant and non-malignant primary human cells. Non-malignant cells will be obtained from healthy individuals or from patients with non-malignant disease. Malignant cells will be from clinical samples.

A three plasmid lentiviral system will be used. Lentiviral plasmids:
- pCMVR8.91 (containing gag/pol/rev/tat)
- pMD.G (containing VSV-g)
- pCG-H and pCG-F (containing measles virus glycoproteins hemaglutinin (H), fusion protein (F))
- plasmid encoding the feline immunodeficiency virus (FIV) coat protein RD114
- Backbone vector for expression of target siRNA or protein

Various measures have been taken to ensure transfection of cells with these lentiviral plasmids will not result in the formation of replication competent virus, as mentioned below.

This project will create replication incompetent lentivirus carrying shRNA to reduce expression or RNA to overexpress specific BCL-2 family proteins that normally act to regulate apoptosis.

BCL-2 family proteins are key regulators of apoptosis, a form of programmed cell death. Over 25 individual mammalian BCL-2 family proteins have been identified to date (listed and described in detail at BCL2DB). These proteins share primary sequence and structural homology and act together via protein:protein interactions to coordinately control apoptosis, at least in part, by regulating the release of pro-apoptotic factors from mitochondria. Individual BCL-2 family proteins act to either promote or suppress apoptosis and therefore function as potential tumour suppressors or oncproteins in malignant disease. For example, BCL-2, BCL-XL and MCL-1 suppress apoptosis whereas BIM, BAK and BAK promote apoptosis. Overexpression of certain anti-apoptotic molecules and deletion of certain pro-apoptotic genes has been shown to promote tumour formation in experimental animals.

The goal of our work is understand the functional significance of BCL-2 family proteins in cancer. Our principle focus is the pro-apoptotic protein BIM and we aim to decrease expression of BIM using RNA interference technology (using shRNA to decrease BIM RNA) or overexpress the BIM-encoding RNA to determine to what extent its expression levels modulate apoptosis susceptibility in different cell types and to understand the mechanisms by which it modulates apoptosis.
Since BIM functions as part of a family, it will also be important to consider the role of other related BCL-2 family proteins and a secondary aim will be to compare these results with those obtained by modulation of other functionally and structurally related BCL-2 family proteins. This will also involve shRNA-mediated knock-down or RNA overexpression.

Anti-apoptotic Pro-apoptotic
Bcl-2 Bak Bik Bcl212
Bcl-XL Bax Bim Bid
Bcl-w Bok Bmf Bad
Bfl-1 Bcl7 Noxa Bnip1
Mcl-1 Bcl-rambo Puma Bnip2
Bcl210 Bfk Hrk Bnip3
BAG-1

We will also utilize viruses to overexpress CD38. This is a cell surface protein and will be used to monitor infection efficiency (following antibody staining) and as a marker to select transduced cells.

We will also utilize viruses to overexpress GFPs. These naturally fluorescent proteins will be used to monitor infection efficiency.

Evaluation of foreseeable effects

The lentivirus will be used to transfecet either siRNA or expression constructs into the CLL cells, causing either knockdown or overexpression of the proteins in the CLL cells. Various measures have been taken to ensure transfection of cells with these lentiviral plasmids will not result in the formation of replication competent virus, including the use of three physically separate plasmids and deletion of the packaging sequence on helper plasmids. This prevents helper/packaging sequences from entering fully formed virus, so the virus cannot make structural proteins and hence cannot propagate once inside the target cells. The multiple plasmid system also reduces the chances recombination into replication competent virus. The national gene vector laboratory at the National Institutes of Health has screened over 60 liters of lentiviral products and has not been able to detect any recombinant, replication competent lentivirus (recombinant DNA advisory committee guidance document, NIH 2006). The virus is self inactivating as the U3 sequence, required to prevent loss of DNA during replication, has been deleted. The 5' promoter region is lost upon replication. Additionally no pathogenic HI'V accessory proteins, such as Vif, Vpr, Vpu and Nef, required for viral RNA synthesis and processing and other replicative functions, are present. The use of alternative coat proteins also prevents recombination into replication competent virus. Heterologous envelope proteins such as VSV-g or proteins from feline immunodeficiency virus or measles virus will be used. Therefore the risk to human health is small. The genetic inserts, such as the BIM shRNA, pose a slight risk as work in experimental animals has shown that overexpression of certain anti-apoptotic molecules and deletion of certain pro-apoptotic genes promotes tumour formation. Although expressed highly in poor prognosis groups of leukemia, CD38 has not been shown to act directly as an oncprotein and is therefore considered of lower risk.

The lentivirus produced will have coat proteins compatible with entering human cells. The target cells of the study are from humans so this cannot be avoided. Therefore there is some risk associated with using this lentiviral system, though this is small, and the health and safety procedures will provide sufficient protection to workers.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: decontamination in Trigene Advance from MediChem International (1 in 10 dilution) for 4 hours within the biosafety cabinet. Trigene has been validated for use against lentivirus. After 4 hours the liquid waste will rinsed down the sink with lots of running water.

Solid waste: Decontamination of solids waste will be by autoclaving prior to disposal as clinical waste for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMBSC agreed that this work could safely be considered an Activity Class 2 project and carried out under standard Containment Level 2 conditions. It was agreed, contingent on the adoption of safe systems of work, that the use of 2nd generation lentiviral vectors with inserts from the BCL-2 family of proteins posed minimal risk to human health or the environment.

Project Containment

Laboratory Activities

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Glass Houses

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Human Clinical Applications

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Project Ref  57/10.1

Date Ackn'd  19/07/2010

CU2 Project Title  Genetic transformation of Chlamydiae

Class  Class 2

CultureVolClass2  < 1 Litre

CultureVolumeClass3-4

Non-GMM  Consent Granted
### Purposes of the contained use

Genome sequencing has now provided a significant DNA resource to make the task of searching for chlamydial gene function much easier. Genomic evidence has been accumulating that chlamydia can exchange genetic information by recombination during natural infections. This has now been clearly demonstrated experimentally in the laboratory in the USA and it is now possible to transform Chlamydia using allelic exchange. However, this approach of direct transformation targeting the chromosome imposes some limitations – the unique chlamydial developmental cycle is thought to involve up to 30% of the genome coding capacity and mutations in these genes are likely to cripple the host organism and possibly render it unable to further infect cells. Our overall goals are to use and improve the recently developed transformation techniques (Binet and Maurelli, 2009 PNAS 106, 292-297) to produce simple stable vectors that can be used to study the basic genetics of chlamydial development.

### Host/vector system

We will use C.trachomatis and Chlamydophila strains, these are all classified as Hazard group 2 micro-organisms. We do not plan to genetically modify the host chromosomal DNA but to complement function with homologous genes. Our vector systems will be based on well-characterised endogenous chlamydial extrachromosomal elements: the endogenous chlamydiaphage and the cryptic plasmid.

### Recipient or parental organism

We plan to use only well-characterised, attenuated laboratory strains of Chlamydiae. Adaptation of Chlamydia to growth in cell culture causes mutations in a virulence factor attenuating the strains for mouse infectivity (and thus presumably human infectivity). There are no foreseeable effects from our proposed work that will increase the virulence of the strains in our study. We do not plan to modify the virulence attributes of Chlamydiae.

### Origin & function

In addition to the chlamydial replicon sources described under ‘host/vector’ we will include the following nonchlamydial markers (either singly or in combination): beta-galactosidase, beta lactamase, chloramphenicol acetyl transferase, the green fluorescence protein as well as other non selected markers from the chlamydial genome.

### Evaluation of foreseeable effects

This project is intended to optimise a plasmid transformation protocol for Chlamydia (mainly C.trachomatis). Success in the project will not generate a more hazardous recipient strain than already exists because the selectable markers
we want to use are not used in chlamydial treatment. However, despite its poor infectivity C.trachomatis and C.pneumoniae are exclusively human pathogens and are both categorised as hazard group 2 microorganisms; transformation to express the selectable or indeed the non-selectable markers listed above will not increase virulence of the host GMM. Thus there are no foreseeable effects that will allow the construction of GMO (GMM) that is more virulent than the original host. This risk assessment places the work at level 2 simply because C.trachomatis/C.pneumoniae are HG2 microorganisms and no increase in hazard to human health or the environment is envisaged. Thus containment and handling precautions will be the same as for the control untreated chlamydia.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon is the accepted treatment of choice for inactivating Chlamydiae because it has been demonstrated to destroy both vegetative bacteria and spores. Virkon is a hypochlorite based disinfectant and can be corrosive. Liquid waste will be neutralised with Virkon but then additionally treated by autoclaving and therefore finally destroyed by incineration. We will use only diposable consumables for chlamydial cultures and preparation of stocks. Thus solid waste will take the form of pipettes (1 - 20ml), micro pipette tips and general cell culture plasticware (24 and 6 well trays and flasks from T25 to T175 and tissues. All will be soaked in Virkon before being sent for autoclaving. All work handling/manipulating infected material will take place within the class 1 cabinet in laboratory LC78 and performed on impermeable, white, plastic spill-control trays. This work surface will be wiped down with freshly prepared 70% methanol prior to work commencing. At the end of each day/experimental procedure (whichever is the more frequent) the spill control tray will be wiped down with 5% Virkon. As an alternative we also have Virusolve which is a modern triamine disinfectant that destroys RNA and has been proven effective in killing clostridial spores. Virusolve is used to inactivate C.trachomatis by the HPA in the routine handling of clinical samples containing wild - type chlamydia.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University of Southampton's GMBSC reviewed the risk assessment via electronic circulation and approved this project proposal on 16 July 2010.

Project Containment
Project Additional Information

Purposes of the contained use

The epithelial-specific integrin alphavbeta6 is not expressed on normal epithelium, but is upregulated in numerous cancers, particularly squamos cell carcinomas. We have shown previously that alphavbeta6 promotes tumour cell invasion directly, and also indirectly through modulating normal cells within the tumour stroma. This latter effect is predominantly mediated through integrin-dependent activation of TGF-beta 1. TGF-beta 1 is a pleiotropic cytokine, which is involved in many signalling pathways, and through its activation, alphavbeta6 modulated many processes associated with acquisition of a malignant cell phenotype ("hallmarks of malignancy"), including motility, metastasis, apoptosis, growth, metabolism and immune evasion. These data suggest that alphavbeta6 is an attractive tumour target: it is not expressed on normal cells, it is expressed on the cell surface and it has tumour promoting function. The aim of this study is to use lentiviral and retroviral vectors to overexpress or silence genes that mediate alphavbeta6/TGFbeta signalling, in order to gain further understanding of the biological functions controlled through these pathways.
Producer cell lines
293T Cell Line is derived from the 293 Cell Line and stably expresses the SV40 large T antigen controlled by the human cytomegalo-virus (CMV) promoter. Producer cell lines. The 293 parental cell line was established from primary embryonal human kidney transformed with sheared human adenovirus type 5 DNA.

Phoenix-Ampho HEK293T is a second-generation retrovirus producer line for the generation of helper free amphotropic retroviruses. The lines are based on the 293T cell line. The lines were created by placing into 293T cells two separate constructs capable of producing gag-pol, and envelope protein for ecotropic and amphotropic viruses.

Plasmid production
Plasmids will be propagated in E. coli K-12 strains such as DH5, JM109 and TOP10, One Shot (R) Stbl3™ which have been demonstrated to be non-pathogenic and have well-understood, stable genetic lesions in the bacterial chromosome, which render the microorganism auxotrophic and dependent upon nutrients that must be supplied in the culture media. Furthermore, these strains are often rendered incapable of colonising mammalian hosts, either due to introduced biological restrictions or sensitivity to common agents. Thus these E. coli strains are non-colonising and disabled.

Recipient cell lines
For the study of the gene function we will use panels of well-characterised cancer cell lines from head and neck cancers, colorectal cancers, pancreatic cancers, oesophageal cancers, breast cancers etc as previously.

Primary epithelial cells and fibroblasts from healthy donors will also be used as previously.

Host/vector system

A) Lentiviral (replication-incompetent)
Third generation lentiviral expression vectors contain a deletion in the 3’LTR (∆U3) that does not affect the generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
The number of genes from HIV-1 used in the system has been reduced to three (i.e. gag, pol, and rev). The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope.
Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus. Although the three packaging plasmids allow in trans expression of proteins required to produce viral progeny (e.g., gag, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
The ViraPower Lentiviral System (Invitrogen) contains additional features:
Expression of the gag and pol genes from pLP1 has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev.
A constitutive promoter (RSV promoter) has been placed upstream of the 5’ LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA.

B) Bacterial (replication incompetent)
Second generation retroviral vectors have the genes and sequences required for retroviral production split between 3 vectors. The backbone of the retroviral vector retains only the packaging signal and LTRs between which the transgene of interest is inserted. Other genes required for synthesis of retrovirus are supplied in trans by Phoenix-Ampho HEK 293T packaging cells which have been transduced with gag, pol and env genes (expressed from 2 different inserts).
Vectors:
pBABEpuro based on the Moloney Murine Leukemia Virus (MoMuLV). Inserted genes are expressed from the MMLV Long Terminal Repeat, which has been proven to be more efficient than most internal promoters in a number of cell lines.
pMSCVpuro and pSUPER retro are vectors based on Murine Stem Cell Virus. These vectors contain a specially designed long terminal repeat (LTR) from the murine stem cell PCMV virus. This LTR differs from the MMLV LTR by several point mutations and a deletion that together enhance transcriptional activation and prevent
transcriptional suppression. As a result, the PCMV LTR drives high-level, constitutive expression of the target gene in mammalian cell lines. The pSUPER retro vector uses the polymerase-III H1-RNA gene promoter, as it produces a small RNA transcript lacking a polyadenosine tail and has a well-defined start of transcription and a termination signal, and is therefore suitable for delivery of sHRNA sequences. pSUPERIOR retro is an inducible version of pSUPER retro.

Packaging cells:
The Amphotropic Phoenix-Ampho HEK293T packaging cell line is a second generation and thus the gag, pol and env genes are expressed from 2 different inserts.

Origin & function

Genes to be expressed/silenced relate to alphavbeta6/TGF-beta signalling and related genes: The following list identifies specific genes, and likely function:

Components of the canonical and non-canonical TGF-beta signalling pathway - alphavbeta6, TGF-beta, TGFBRI, Smad, smurf, SCF/Roc, MAP kinase, NOX, nrf2, keap1, ATM, CHK, ATR, BMI1, p53 GLI1, RAS.

TGF-beta-dependent genes-thrombospondin, regulators of EMT, VEGF, Transglutaminase, PKC, caveolin alphavbeta6-dependent genes - regulators of motility, - fyn, Akt, MMPs, serine proteases, COX-2, PI3 kinase.

Regulators of alphavbeta6-dependent cell adhesion/spreading -Rho, Rac, cdc42, Rap

Regulators of alphavbeta6-dependent actin cytoskeletal rearrangement -WASP, Wave, eps8, ARP2/3, abi1, sos myosin light chain.

Regulators of alphavbeta6 function CtBP, EGRF, EP4, Talin, Kindin, Her2, clathrin.

TGF-beta dependent genes regulating the stromal response, SMA, HGF, periostin, p16, -21, FAK, fibronectin, H2AX, cMET.

Reporter genes eg Firefly and Renilla luciferase, Fluorescent proteins (EGFP, YFP, BFP, RFP). These have no molecular/physiological function.

Evaluation of foreseeable effects

The major risk in this project is from transduced (virus-producing) 293T cells (and pertains to human infections).

a) potential for generation of replication-competent lentivirus or retrovirus
b) potential for oncogenesis

a) The risk is mitigated by the safety features of these latest generation vector systems, detailed above, including:

1) Lentiviral vectors
   - separation of vector and packaging functions onto four or more plasmids
   - use of additional safety features (e.g. they do not encode Tat, which is essential for replication of wild-type HIV-1)
   - a deletion in the 3'LTR (ΔU3) that results in "self-inactivation" of the lentivirus after transduction of the target cell

2) Retroviral vectors
   - the genes and sequences required for retroviral production split between 3 vectors. The backbone of the retroviral vector retains only the packaging signal and LTRs between which the transgene of interest is inserted. Other genes required for synthesis of retrovirus are supplied in trans by Phoenix Amphi HEK293T packaging cells which have been transduced with gag, pol and env genes (expressed from 2 different inserts).

Although the virions are replication-incompetent, as an added precaution, target cells are routinely tested for production of replication competent viruses by transferring condition medium onto virgin 3T3 fibroblasts and testing for drug resistance.
b) although no single gene can induce malignant transformation in vivo, the pathways to be investigated in this proposal could be regarded as potentially oncogenic in certain circumstances (eg perhaps if expressed constitutively at high levels). Since lentiviral vectors can infect primary human cells, precaution against inadvertent infection by the user must be taken.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid Waste: Everything that contacts virus-containing solutions or vessels is decontaminated or contained before exiting the containment level 2 room. Solid waste is collected in a biohazard bag inside the tissue culture hood. Pipette tips are collected in a disposable plastic box (e.g., an empty P-1000 box), and the box closed and deposited into the biohazard bag (in the tissue culture hood) at the end of the work session. At the end of the work session, the biohazard bag is closed, sprayed with 70% EtOH, and deposited into a further biohazard bag, sealed and autoclaved, (15 ins at 121°C and ultimately incinerated.

Liquid Waste is aspirated into a vacuum flask containing appropriate disinfectant eg 1/10 volume concentrated bleach (or 1% Virkon or Klosept [10,000ppm of chlorine]). At the end of the work session, 25-50ml of concentrated bleach is aspirated through the vacuum tubing, into the vacuum flask. The vacuum flask has a final concentration of at least 10% bleach, for a minimum time of 30 minutes prior to drain disposal. Liquid waste that is not aspirated is treated with bleach, to a final concentration of at least 10%, in the hood, allowing a minimum time of 30 minutes to activate virus. Liquid thus treated is disposed to drain.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

The University GM & Biosafety Committee reviewed this project proposal and associated risk assessment on the 17th January 2012 and approved the recommendation that the work be classified as Activity Class 2 and could be safely conducted in a Containment Level 2 laboratory with the recommended safety precautions.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
The molecular pathogenesis of myeloproliferative neoplasms and related disorders

This risk assessment covers the production of amphotropic lentivirus for use in the transfection of human cell lines and human primary cells. The genes we wish to transfet will be oncogenes identified as pathogenic in MPNs and genes encoding micro-RNAs and small interfering RNAs (siRNAs). Mammalian gene products that are either wild-type or inactivated/activated by mutation will be transfected by lentiviral particles human cell lines or primary cells.

Cell lines covered by this risk assessment are restricted to those with a history of safe use, offering no known hazard to human health or the environment and therefore regarded as of low risk (ACDP hazard group 1). All will be handled at Containment Level 2.

Producer cell lines
The cell line HEK293T is derived from the 293 cell line and stably expresses the SV40 large T antigen controlled by the human cytomegalo-virus (CMV) promoter.

Plasmid production
Plasmids will be propagated in E. coli K-12 strains such as TOP10, DH5 or One Shot® Stbl3 which have been demonstrated to be nonpathogenic and have well-understood, stable genetic lesions in the bacterial chromosome, which render the microorganism auxotrophic and dependent on nutrients that must be supplied in the culture medium. Furthermore, these strains are often rendered incapable of colonising mammalian hosts, either due to introduced biological restrictions of sensitivity to common agents. Thus these E. coli strains are non-colonising and disabled.
Recipient cell lines
For the study of gene function we will use panels of well-characterised human cancer cell lines derived from leukaemias (UT-7, K562, EOL-1, HL60 and EBV-immortalised B cells), cervical cancer (Hela), fibrosarcomas γ-2A derived from 2C4), and those with a stem cell like phenotype (EML-1). Additionally, leukocytes derived from peripheral blood and bone marrow will also be used as recipients for lentiviral transfection.
Peripheral blood or bone-marrow derived primary cells are not able to survive without growth factors in vitro. However, using patient material does carry risk from undiagnosed infections (e.g. hepatitis, HIV), but the culturing of these cells will be for a minimal time duration using standard techniques widely used at WRGL at containment level 2.

Host/vector system

A) Description of the Lentiviral Expression System:

The gene of interest is cloned into a lentiviral vector, with a typical structure of:

- PRSV/5'LTR - Ψ -RRE-PCMV - Transgene - WPRE - Δ3-3'UTR

More specifically, the destination vectors proposed for use here are the pLenti-DEST vectors that are adapted for use with Invitrogen's ViraPower Lentiviral Expression system. Depending on the antibiotic marker chosen and the promoter strength, the following expression vector will be selected:

- Plenti6/V5-DEST or PLenti6-2/V5-DEST: CMV and blastidicin
- PLenti4/V5-DEST: CMV and Zeocin

Inserts (gene of interest or non-coding RNA) of up to 10kb can be packaged into the Lentiviral expression vector. The insert will originate from PCR from cDNA or DNA and will be mammalian in origin.

The packaging plasmids each carry genes which separately encode the structural and assembly units for packaging a viral genome, and allow the creation of a replication-incompetent, HIV-1-based lentivirus that can deliver the target gene to mammalian cells. The three packaging plasmids comprising the ViraPower Lentiviral Expression system are:

- PLP1: CMV - gag/pol - RRE
  The RRE signal is a HIV-1 Rev response element, and permits Rev - dependent expression of the gag and pol genes.
- PLP2: RSV - rev ORF - HIV-1 LTR
  The Rev ORF encodes the Rev protein that interacts with the RRE on pLP1 to induce gag and pol expression, and on the pLENTI6 expression vector to promote the nuclear export of viral RNA for packaging into viral particles. The HIV-1 LTR allows efficient transcriptional termination and polyadenylation of mRNA.
- PLP/VSVG: CMV - VSV-G
  VSV-G encodes the envelope G glycoprotein from VSV to allow production of a pseudotyped retrovirus with a broad host range.

B) Safety features of the Lentiviral Expression System

- The pLenti expression vector contains a deletion in the 3' LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in 'self-inactivation' of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zuffrey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).
- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK293T producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
- The lentiviral particles produced in this system are replication-competent and only carry the gene of interest. No other viral species are produced.
- Expression of the gag and pol genes from pLP1 Has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al., 1998).
- A constitutive promoter (RSV promoter) has been placed upstream of the 5' LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA (Dull et al., 1998).
Genes to be expressed/silenced relate to the pathogenesis of MPNs. The following list identifies specific genes and likely functions:

- Components of signalling pathways, including signalling intermediates (for example, JAK2, SH2B3), or cell surface receptors, (for example MPL, EPOR or integrins), or extracellularly secreted proteins, (for example EPO, SCF).
- Epigenetic proteins (i.e. histone modifiers, and demethylases, for example EZH2, SUZ12, DNMT3A,)
- Transcription factors (for example, STATs, MYB, MECOM)
- Splicing factors (for example, SH3BP1)
- Non-coding RNAs (for example, microRNAs such as miR-181a, or si RNAs against genes like EZH2)
- Reporter genes (for example, Firefly and Renilla luciferase, fluorescent proteins like GFP, YFP, RFP, all of which have no physiological function)

**Evaluation of foreseeable effects**

The major risk in this project is from transduced (virus-producing) HEK293T cells, and pertains towards personnel conducting the experiment.

a) Potential for generation of replication-competent lentivirus
b) Potential for oncogenesis.

a) The risk is mitigated by the safety features of these latest generation vector systems, detailed above, including:
- Separation of vector and packaging cell line system.
- Use of additional safety features (e.g. the vectors do not encode Tat, which is essential for replication of wild-type HIV-1)

b) Regarding accidental exposure of skin or mucosal tissue to infectious lentivirus, there is a theoretical risk of oncogenesis, caused by insertional mutagenesis or integration and expression of the gene encoded on the vector. However, there is currently no evidence that wild-type or mutant forms of the gene products listed previously are oncogenic in mucosal epithelial cells. This application does propose the transfection of mutated genes that have been shown to contribute to the initiation of MPNs, and it is conceivable that high expression of wild-type as well as mutant proteins could be potentially dangerous in the event of being delivered to the appropriate target tissue. However our current understanding is that oncogenesis would only occur if the proposed genes were integrated into a rare subset of cells within the bone marrow. It is conceivable that these sequences may have other unknown oncogenic effects. Altogether, precaution against inadvertant infection by the user must be taken.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Solid waste:** All items that contact virus-containing solutions or vessels will be decontaminated or contained before exiting the containment level 2 room. Solid waste is first decontaminated with 2% Virkon and left for a minimum time of 30 minutes, sealed and then collected in a biohazard bag inside a metal container beneath the tissue culture hood. Pipette tips are collected in a disposable plastic container (i.e. an empty medium bottle) and the box closed and deposited in the biohazard bag, under the tissue culture hood, at the end of the work session. Serological pipettes will be disinfected prior to removal from biosafety cabinet by placing in 2% Virkon for a minimum of 30 minutes before being transferred to the biohazard bag for disposal. At the end of the work session, the biohazard bag is sealed, sprayed with 70% ethanol, and deposited into a further biohazard bag. This is then sealed and autoclaved (10 minutes at 135 degrees with chamber pressure of 3130Mb, taking an appropriate total cycle time of 28 minutes), and ultimately incinerated.

Liquid waste is placed into a sealable plastic container (an empty medium bottle) containing ~20 ml 2% Virkon, and left for a minimum of 30 minutes, before being disposed of down the drain with plenty of water.
The original risk assessment was recorded as Activity Class 1. Members disagreed with the AC1 classification; some elements were considered to be more akin to AC2. A short critique on why the project was judged to be AC2 was sent to the PI and the researcher(s). This was then agreed by the PI and the BSA and the project was approved as AC2.

**Project Containment**

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**Project Ref**  57/12.3

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**Project Additional Information**

**Purposes of the contained use**

The experiments carried out in the laboratory involve culturing Mtb, measuring mycobacterial growth, infecting cells, harvesting supernatants and mRNA and developing 3-dimensional human granuloma models. In order to carry out these studies, Mycobacterium tuberculosis (Mtb) will be cultured and maintained in the containment level 3 facility. The primary strain used will be H37Rv. Clinical strains known to be fully drug susceptible will also be cultured within the facility, and genetically modified Mtb that are either luminescent or fluorescent will be utilised in specific experiments. No drug resistant Mtb strains will be cultured within the facility, apart from kanamycin or hygromycin resistant GMTB. These antibiotics are not used to treat patients with tuberculosis. Human cell cultures (A549 cells, primary human bronchial epithelial cells, peripheral blood monocytes, alveolar macrophages, fibroblasts and THP-1 cells) will be infected with Mtb. The supernatants of these cells and cells themselves will be analysed at specified time points after infection. For the development of 3-dimensional granuloma models, we will mix cells and live Mtb with collagen/alginate mix (50:50) and we will electrostatically generate beads to form spherules using a bead generating machine housed within the class I MSC in the TB lab. We will study the cell supernatants and cells themselves at the specified time points.

The genetically modified strains are Mtb (H37Rv) or clinically isolated strains which are fully sensitive to existing anti-tuberculosis drugs that contain fluorescent reporters GFP or mCherry and luminescent reporters from Photorhabdus luminescens, or the luciferase genes from Firefly or Gaussia princesses. The GFP strain contains pSMT3LxEGFP which a dual reporter plasmid containing fluorescent enhanced EGFP gene and bacterial luciferase. Positive clones were selected by growing on plates and media supplemented with µg/ml hygromycin. The mCherry strain contains codon optimized mCherry genes inserted into pSMT3, an E.coli - mycobacterium shuttle vector containing hygromycin resistance (pCherry3) and selected similarly as a EGFP positive Mtb (H37Rv). The plasmids in luminescent reporters are pMV306hsp+FFlucWT for Firefly luciferase, pJ201:26462 for Gaussia luciferase and pSMT3+Lux for bacterial luciferase encoding vectors. The Firefly luciferase plasmid is a pMV306hsp encoding the codon optimized for M.tuberculosis and kanamycin resistance. The bacterial luciferase contains vector containing the LuxABCDE operon from P. luminescens modified for expression for M. tuberculosis namely mc2 6030 and mc2 7000 which have deleted RD1 region, which is the main pathogenicity island of Mtb. All strains were previously reported in published articles and for this programme of work we are using the already transformed bacteria so the depth of the work is limited to the introduction of the bacteria to cell culture or monitoring the growth of bacteria following interventions.

**Recipient or parental organism**

Mycobacterium tuberculosis strain H(37)Rv and fully drug sensitive field isolated of M.tuberculosis strains (Beijing, Indo-Oceanic, Eurasian).

**Host/vector system**

- **pSMT3LxEGFP** - A plasmid based on pSMT3 Esherica coli/mycrobacteria shuttle vector and containing the human codon-optimized and fluorescence-enhanced EGFP gene from pEFP cloned under the control of the mycobacterial 19kDa protein promoter, and the luxAB genes from Vibrio harveyi under the control of hsp60 promoter. The shuttle vector has hygromycin resistance (Hygr) originally.
- **pCHERRY3** - It is developed on pSMT3 backbone with hygromycin resistance (Hygr) to give a high copy number using site-directed mutagenesis. After that, a strong promoter Psmyc was introduced to codon optimized Pshe60 and then the mCherry sequence was cloned which are codon optimized for Mtb codon preferences.
- **pMV306hsp+FFluc** - This is vector which is based on Mycobacterial integrating vector (pMV306) which holds kanamycin resistance gene (Kmr) and part of E. coli cloning vector encoding the firefly luciferase (Ffluc) codon optimized for Mtb.
- **pMV306hsp+GlucWT+SS** - This is vector which is based on Mycobacterial integrating vector (pMV306) which holds kanamycin resistance gene (Kmr) and part of cloning vector encoding the Gaussia luciferase gene including the secretion signal (GlucSS) codon optimized for Mtb.
- **pMVhsp+LuxAB+G13+CDE** - This is developed on pSMT3 backbone with hygromycin resistance (Hygr) in which the promoter Pshe60 is replaced with G13 promoter (PG13) and cloned front of luxC of LuxABCDE operon.

**Origin & function**
* **pSMT3LxEGFP** - The plasmid confers the Mtb strains to express green fluorescent proteins (GFP) under control of hygromycin resistance. GFP has excitation of 475 nm and emission of 509 nm in fluorescent microscopy.

* **pCHERRY3** - The plasmid confers the Mtb strains to express red-shifts excitation and emission maxima (558nm and 583nm respectively) protein under control of hygromycin resistance.

* **pMV306hsp+Ffluc** - The plasmid gives ability Mtb strains to express firefly luciferase (Ffluc) and emit light upon the addition of D-luciferin (benzothiazole) which can be detected at 557nm.

* **pMV306hsp+GlucWT+SS** - The plasmid confers Mtb strains to express blue light (480nm) of Gaussia princeps upon addition of coelenterazine.

* **pMVhsp+LuxAB+G13+CDE** - The strains which contain this plasmid produce light in the wavelength 490nm (blue range) without exogenously added substrate

**Evaluation of foreseeable effects**

The parent Mycobacterium tuberculosis or Mycobacterium tuberculosis derivatives which contain inserts incorporating fluorescent reporters such as GFP or mCherry, luminescent reporters such as the lux operon from Photorhabdus luminescens, or the luciferase genes from Firefly or Gaussia princeps have no resistance to clinically used anti-TB drugs. They contain hygromycin or kanamycin resistance (Himar1 and hyg-R, respectively), but these antibiotics are not used for treatment of human disease, which is treated with rifampicin, isoniazid, pyrazinamide and ethambutol. All the genes are well characterized and have been used in many applications and has no hazard arising from alteration of existing traits of the host in terms of alteration of pathogenicity, host range or tissue tropism. Therefore, the genetically modified strains of Mtb have no greater virulence than the recipient of Mtb strains.

Mtb is exclusively a pathogen of man and transfer of the plasmids/part of plasmids to other pathogens has not reported previously. The work is done in Category III laboratory and all waste for disposal will be chemically disinfected then autoclaved prior to release from the lab. The chance of GMO being transferred to other microorganisms is nil.

Despite widespread use in research laboratories, H37Rv has not caused any documented cases of tuberculosis (TB), suggesting it has become attenuated through repeated passage. Typically, Mtb is passed from person to person in a community by the droplet route and the site of infection is the respiratory tract. The creation of aerosols therefore represents the major risk to laboratory workers. The survival of Mtb in aerosols is less than 1% at 1 hour, though surviving mycobacteria can then persist in the environment for long periods. Therefore any dust particles should be regarded as potentially infectious. Live Mtb is susceptible to 70% ethanol or commonly used laboratory disinfectants (10% Surfanios, a disinfectant detergent which gives >9 log kills in 3 hours, 3% Hycolin or 3% Virkon), though secondary autoclaving after chemical disinfection is necessary to ensure sterility. The highest risk procedure here is the culturing and handling of Mtb (or its derivatives).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

* Liquid waste - 10% final concentration Surfanios treatment overnight then autoclaving in Rodwell autoclave within CL3. Surfanios (10%) was demonstrated to be the most effective detergent tested at killing Mtb in studies comparing different detergents, with a >9 log kill withing 3 hours. This may not achieve full sterility so is followed by autoclaving which will kill all Mtb. Liquid waste will include TB cultures and cell cultures.

* Solid waste - Autoclaving in Rodwell autoclave within CL3. Autoclave load temperature of 126 degree C for 30 minutes is significantly greater than the time needed to kill Mtb, so will ensue灭ility. For example, milk Pasteurisaton kills Mycobacteria completely and this is a much shorter, lower temperature treatment. Solid waste includes
culture plates, pipette tips, eppendorfs, absorbent towel, cell scrapers, falcon tubes plus waste from the lobby used for hand drying.

* Autoclavig is done using Rodwell autoclave which is exclusively used for TB work. It will have 6-monthly service visit with annual 12 calibration run, and records will be kept from each run for 6 months.

* The back-up autoclave will be in the adjacent laboratory in the CL3 suite. Therefore, if required, waste will be double contained, taken along the CL3 suite corridor (approx 3 meters) and autoclaved in next room with assistance from the CL3 suite manager.

After chemical treating and autoclaving, the waste will be placed in the hospital yellow bag waste route and will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GM & Biological Safety Committee reviewed this project proposal and associated risk assessment at an extraordinary meeting called on the 3rd September 2012 and approved the recommendation that the work be classified as Activity Class 3 and could be safely conducted in a Containment Level 3 laboratory with the recommended safety precautions. The minutes and recommendations from this meeting will be recorded at the next full GMSC meeting on the 9th October 2012.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 (Yes)</td>
<td>L4</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 57/14.1

Date Ackn’d 30/01/2014

CU2 Project Title Use of retro viral vectors to reprogramme mammal cells cultured in vitro

Class 2

CultureVolClass2 < 1 Litre

Non-GMM Consent Granted

Date Project 02/03/2022
The aim of this research is to use patient-specific stem cell technology (1) to understand the role of genetic defect in the development of retinal degeneration; (2) to screen potential drugs for the personalized treatment; (3) to generate (induced pluripotent stem cells) iPCs using non-oncogenic factors for the treatment of retinal degenerative diseases (proof of principle study).

To do this, we will drive human and mouse somatic cells to iPSCs, using pluripotent transcription factors. Transcription factors (including Sox2, Oct4, cMyc, Klf4) will be over-expressed in mammalian cells using retroviral particles. This is a widely used iPSCs reprogramming protocol developed by Yamanaka, who won the Nobel Prize (Physiology/Medicine) for the ground-breaking discovery of iPSCs generation in 2012. Recently Yamanaka's work has also shown that these factors along with Lin28 can generate iPACs from peripheral blood cells. This low invasiveness cell/tissue collection is very attractive for clinical use. In addition, to promote cell differentiation towards retinal cells in vitro, retinal genes, such as Pax6, Crx, Otx2, Chx10, NeuroD, rhodopsin or their combination are also to be used in this study.

Recipient or parental organism
- Human cells derived from the eye, blood and skin
- Mouse cells derived from the eye and skin

Host/vector system
Moloney Murine leukemia Virus (MoMuLV) expression systems are used as vector system, which include the following features:
- All the genes required for producing full length viral RNA and packaging it into viable viral particles (gag-pol, rev and env) have been removed from the pRRL plasmids. Gag-pol and rev are expressed separately as is the VSV-G coat protein. The plasmids have been engineered so as to have no recombination sequences that would allow these genes to be inserted into the pRRL plasmid. This allows for a viral particle to be produced that can enter dividing cell, but that once inside does not have the relevant genes necessary to re-package itself or to re-generate full length viral RNA following genomic integration.
- This MuMLV retro Expression Systems used include the following key safety features:
  - The number of retroviral genes that are used in the system has been reduced to three (i.e. gag, pol and rev)
  - The VSV-G gene from Vesicular Stomatitis Virus is used in place of other retroviral envelope genes.
  - Genes encoding the structural and other components required for packaging the viral genome are separated onto three plasmids. All plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus.
  - The packaging plasmid encodes for the gag and pol proteins in trans. It also retains the minimal cis acting viral sequences necessary for packing, reverse transcription and integration. The envelope plasmid encodes for the envelope protein in trans. So, when the virus particles are assembled, the accessory genes required for subsequent viral replications are not included, therefore, making the vector replication deficient.
  - The viral particles produced in these systems are replication-incompetent and only carry the gene of interest. No other viral species are produced.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct4</td>
<td>This gene encodes a transcription factor containing a POU homeodomain. It plays a role in embryonic development, especially during early embryogenesis, and it is necessary for embryonic stem cell pluripotency.</td>
</tr>
<tr>
<td>Klf4</td>
<td>Can act both as activator and as repressor. Binds the 5'-CACCC-3' core sequence. Binds to the promoter region of its own gene and can activate its own transcription. Regulates the expression of key transcription factors during embryonic development. It plays an important role in maintaining embryonic stem cells, and in preventing their differentiation. Required for establishing the barrier function of the skin and for postnatal maturation and maintenance of the ocular surface. Involved in the differentiation of epithelial cells and may also function in skeletal and kidney development.</td>
</tr>
<tr>
<td>cMyc</td>
<td>Encodes a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. It functions as a transcription factor that regulates transcription of specific target genes. Mutations, overexpression, rearrangement and translocation of this gene have been associated with a variety of hematopoietic tumors, leukemias and lymphomas, including Burkitt lymphoma.</td>
</tr>
<tr>
<td>Lin28</td>
<td>Acts as a 'translational enhancer', driving specific mRNAs to polysomes and thus increasing the efficiency of protein synthesis. Its association with the translational machinery and target mRBAs results in an increased number of initiation events per molecule mRNA and, indirectly, in stabilizing the mRNAs. Diseases associated with LIN28A include breast cancer susceptibility, and rhabdoid tumor.</td>
</tr>
<tr>
<td>Crx</td>
<td>A mammalian gene that plays a role in the differentiation of photoreceptor cells. We may also use other transcription factors/growth factors important for retinal specific differentiation.</td>
</tr>
<tr>
<td>Homeobox protein (OTX2)</td>
<td>A mammalian gene which acts as a transcription factor and may play a role in brain and sensory organ development.</td>
</tr>
<tr>
<td>NeuroD (Neuronal Differentiation)</td>
<td>A mammalian gene also called Beta2, which is a basic helix loop helix transcription factor expressed in certain parts of brain.</td>
</tr>
<tr>
<td>Chx10</td>
<td>Plays a significant role in the specification and morphogenesis of the sensory retina. May also participate in the development of the cells of the inner nuclear layer, particularly bipolar cells (by similarity).</td>
</tr>
<tr>
<td>Pax6</td>
<td>A transcription factor with important functions in the development of the eye, nose, central nervous system and pancreas.</td>
</tr>
<tr>
<td>Rx (Retina and Anterior Neurol Fold Homeobox)</td>
<td>Plays a critical role in eye formation by regulating the initial specification of retinal cells and/or subsequent proliferation. Binds to the photoreceptor conserved element-1 (PCE-1/Ret 1) in the photoreceptor cell-specific arrestin promoter.</td>
</tr>
<tr>
<td>Rhodopsin</td>
<td>Protein for image-forming vision at low light intensity. Required for photoreceptor cell viability after birth. Light-induced isomerization of 11-cis to all-trans retinal triggers a conformational change leading to G-protein activation and release of all-trans retinal.</td>
</tr>
<tr>
<td>Nrl</td>
<td>Transcription factor which regulates the expression of several rod-specific genes, including RHO and PDE6B.</td>
</tr>
</tbody>
</table>

Above genetic materials are from human.
### Evaluation of foreseeable effects

Approximate 0.01-0% cells will be reprogrammed into iPSCs following infection with four pluripotent markers (such as Sox2, Oct4, cMyc and Klf4). They may exhibit embryonic-like stem cell features. The appearance of iPS colonies will be assessed following infection in the culture plates. These colonies will be subject to purification, subculture and characterisation using immunocytochemistry, RT-PCR, Western Blotting etc. The iPSCs should have the ability to retinal specific markers and exhibit relevant functionality. Cell phenotypes will be examined using immunocytochemistry, RT-PCR, Western Blotting etc. Functionality will be assessed using ion indicator, electrophysiology etc.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Cells infected with retroviral vectors may be subcutaneously or intramuscularly injected into immunodeficient mice or transplanted into the eyes of mice after continuing passaging and subculture. This will be done in a certified animal facility. The transplanted cells will be subjected to long-term culture and washed thoroughly prior to injection, thus no infectious agent will be present in the eyes or under the skin of the animals. Experimental animals will not be subjected to further breeding. No GM animal or plant will be produced in the project.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for any derogation from containment for the class activity

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Liquid waste**, such as spent media and culture supernatant, will be subjected to chemical inactivation using Virkon. This has been validated for retroviral inactivation (antec-biosentry).

- **Solid waste**, such as contaminated laboratory plastics, gloves, will be soaked in Virkon before being autoclaved and sent for incineration.

### Is an emergency plan required according to regulation 20?  N

### If yes, tick to confirm that it is attached to this form  N

### Tick to confirm that you have attached a risk assessment to this form  Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment  N
Received The risk assessment

This project could be AC1 or AC2. Mr Lockey suggested that he seek clarification of some issues; he confirmed that the work would be carried out at Containment Level 2. If the project was confirmed at AC2, it would require notification to the HSE. Professor K proposed that the project be confirmed at AC2. If the researchers accepted this classification, there is no need to re-submit the project. However, if there was any dispute, the researchers would be asked to attend a meeting of the GM&BSC to put their case for a different classification.

Resolved That the project be confirmed at Activity Class 2, subject to acceptance of this by the researchers.

GMBSC meeting 21st January 2014 item 9.3 GMBSC 72 (minute 06.3)

Mr L reminded members that they had recommended approval of this project at Activity Class 2. He confirmed that the researchers had accepted the recommendation and were preparing for the HSE notification.

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Animal Units

<table>
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Project Ref  57/14.2

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
<th>CultureVol</th>
</tr>
</thead>
<tbody>
<tr>
<td>01/04/2014</td>
<td>Targeted mutation of genes in biofilm development in Streptococcus pneumoniae and Pseudomonas aeruginosa by allelic replacement and insertional mutagenesis</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info
### Project Additional Information

#### Purposes of the contained use

Previous studies of biofilm formation in *S. pneumoniae* have shown the emergence of a sub-population of pneumococci with a small colony morphology (SCV) and distinct phenotypic differences compared to the parent strain, namely increased biofilm formation, reduced capsule production and slower growth rate. We have found that all SCV's contain mutations within the DNA-directed RNA polymerase delta subunit (RpoE). To confirm that the phenotype seen in the SCV sub-population is a direct result of the mutations this work aims to generate a ΔpoE) knockout (KO) mutant in *S. pneumoniae*.

Biofilm formation in *Pseudomonas aeruginosa* is controlled by the bacterial second messenger cyclic dimeric guanosine monophosphate, (c-di-GMP). Intracellular levels of c-di-GMP are modulated by diguanylate cyclases (DGCs) and phosphodiesterases (PDEs). Work from the PI has shown that nitric oxide (NO) plays a role in the regulation of bacterial biofilm formation through altering PDE activity. Other groups have independently described the role played by NO in signalling pathways that control the activity of PDEs. We aim to investigate the role of DGCs and PDEs in biofilm formulation, and the mechanism by which their activity is regulated by NO, targeted deletion of these genes in *P. aeruginosa*.

**Recipient or parental organism**

A clinical isolate of *Streptococcus pneumoniae* serotype 22F.

*Pseudomonas aeruginosa* PAO1 (Stover et al., Nature 406, 959-64 (2000))

**Host/vector system**

For streptococcus pneumoniae, the aim of this work is to disrupt the RpoE gene by targeted mutagenesis using a PCR mutagenesis approach. An RpoE KO will be generated via PCR ligation mutagenesis (PLM) by allelic replacement as previously described (Lau et al PCR ligation mutagenesis in transformable streptococci: application and efficiency 2001). This method of mutagenesis requires no vector construct. The KO construct will be generated by PCR amplification and ligation of regions of homology approximately 500 bp 5' and 3' of the RpoE gene. These regions of homology will include approximately 120bp of the RpoE gene to ensure specificity. The 5' and 3' regions of homology will be ligated using Acl and FseI, respectively, either side of a erythromycin cassette. Once generated the cassette will be transformed into a clinical isolate of *S. pneumoniae* serotype 22F isolated during an on-going carriage study at University Hospital Southampton.

For *Pseudomonas aeruginosa* two methods will be used - insertional mutagenesis, and allelic replacement as described below:

1. We plan to make use of a commercially available library of transposon mutants for *P. aeruginosa* (Jacobs MA, et al. Comprehensive transposon mutant library of P.
eurigonsa. Proc Natl Acad Sci USA 100, 14339-44, 2003). This is held at the University of Washington Department of Genome Sciences. The library was created using transposons ISphoA/hah (4.83 Kb) andIslacZ/hah (6.16 Kb), derived from the ISSOL element of transposon Tn5 and generate alkaline phosphatase (phoA0 or beta-galactosidase (lacZ) translational gene fusions where inserted in a target gene in the appropriate orientation and reading frame. The transposons contain the tetracycline resistance gene tet. The mutants are shipped on antibiotic free medium without selection, but on receipt will be maintained on media containing tetracycline.

2) Allelic replacement. The allelic replacement strategies that we will use to make mutations in genes for NbdA, FimX, MucR and BifA, as examples the genes listed in Appendix 1 are described in the supplementary documents. The protocols outlined are derived from previously published descriptions of mutagenesis of these genes as described, and it is anticipated that one or more of the strategies identified will be appropriate to knock out any of the DGC or PDE-coding genes listed in Appendix 2. The knockout constructs will be electroporated into wild-type P. aeruginosa.

Origin & function

We will use vectors and knockout strategies, as previously published for the deletion of the P. aeruginosa DGC and PDE-containing genes: pGEM T-Easy, PEX100T, pEX18, pFLP2, pS858. See addended documentation for further information and source references.

For Streptococcus pneumoniae the erythromycin gene cassette will be obtained from the pCR2-ΔrpoE plasmid as previously described (Xue et al. Lack of the delta subunit of RNA polymerase increases virulence related traits of Streptococcus mutans, PlosOne, vol6, issue 5, 2011)

We will also make use of antibiotic resistance elements for the purpose of i) disruption of the target genes described and ii) to provide a selectable marker to confirm the successful generation of mutant strains.

The function of the genetic material is to disrupt the gene function of the target genes to better understand their role within the organism and to provide a selectable marker.

Evaluation of foreseeable effects

Introduction of an erythromycin cassette into a clinical S. pneumoniae strain introduces a source of antibiotic resistance. Introduction of gentamicin or tetracycline resistance into P. aeruginosa introduces a source of antibiotic resistance.

There are no expected changes to the hazards associated with these organisms other than in acquiring the antibiotic resistance genes. In S. pneumoniae, the delta subunit of RNA polymerase RpoE is thought to play a role in environmental adaptation and stress response regulation, and therefore deletion of RpoE is likely to be deleterious for the survival and pathogenicity of the organism (reduced human hazard). While few studies have examined RpoE function in S. pneumoniae, other work has shown that inactivation of RpoE in the human dental caries pathogen Streptococcus mutans causes impaired growth and loss of important virulence traits, including biofilm formation, resistance to antibiotics, and tolerance to environmental stresses (Xue et al. The delta subunit of RNA polymerase, RpoE, is a global modulator of Streptococcus mutans environmental adaptation, J bacteriol 192, 19, 2010), although can also lead to an increase in some virulence-linked traits including a broader substrate spectrum (Xue et al. Lack of the delta subunit of RNA polymerase increases virulence traits of Streptococcus mutans, PlosOne, vol 6, issue 5, 2011). Further work in other species including Bacillus subtilis and Streptococcus agalactiae have shown RpoE mutants to have increased sensitivity to killing in whole-blood bacteriocidal assays, suggesting attenuated virulence (Jones et al The Delta subunit of RNA polymerase is required for virulence of Streptococcus agalactiae vol 71 issue 7 Infect Immun 2003). However the relevance of these findings to S. pneumoniae has not previously been investigated.

For P. aeruginosa, certain virulence-associated traits are controlled by multiple DGCS and PDEs through alterations in c-di-GMP levels. There are numerous studies from many laboratories worldwide demonstrating that insertions in different DGC and PDE genes result in distinct phenotypes, including alterations to motility and biofilm formation (e.g,Kulasakara et al., 2006. Analysis of Pseudomonas aeruginosa diguanylate cyclases and phosphodiesterases reveals a role for cyclic-di-GMP in virulence. PNAS vol. 103 pp 2839-2844). In the latter study, a set of P. aeruginosa mutants in DGC- and PDE-encoding genes exhibited attenuated virulence in a mouse infection model. In this study, we will examine mutations in the genes previously studied by Kulasakara et al., (2005) for alterations in their ability to respond to NO during the process of biofilm dispersal.

Although there may be a risk of interspecies transfer, the knockout constructs used are thought to be low-risk as described above. All work is undertaken in a containment...
level II laboratory. The knock-out strategies employed are commonly used for other Streptococcus pneumoniae and Pseudomonas aeruginosa GM Studies.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This work will not involve the use of GM animals or plants.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All work with HG2 organisms will be carried out in a containment level II laboratory. No derogation is required.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All GM waste will be discarded in clearly labelled GM DispoSafe jars and autoclaved at 121°C and subsequently incinerated according to the University of Southampton local code of practice:

- **Solid waste** - sterilisation by autoclaving (double-bagged in clear autoclave bags or DispoSafe jars with lid loosely attached and placed in a clear autoclave bag (followed by disposal in yellow bin bags into yellow Eurocart).

- **Liquid waste (small volumes)** - collection onto absorbent material, sterilisation by autoclaving (double-bagged in clear autoclave bags or DispoSafe jars with lid loosely attached and placed in clear autoclave bag) followed by disposal in yellow bin bags into yellow Eurocart.

- **Sharps** - placed in orange or yellow lidded sharps bins, sterilised by autoclaving, tagged and placed in yellow Eurocart.

All uncontaminated waste (paper hand towels, outer packaging, etc) will be placed in black bin bags and taken to purple Eurocart.

Any Bacterial spills will be disinfected with 70% Ethanol and absorbent qirls bluw roll before discarding as GM waste. Bacterial liquid culture or solid medium, will be autoclaved at 121°C and subsequently discarded according to the University of Southampton local code of practice:

If spill is of a large volume (over 100 ml) and involves an aerosol forming agent and is outside of a MSC, vacate the laboratory and prevent other users from entering. Spill can then be cleaned providing a respirator is worn to protect from aerosol risk (this would be done by a specialist contractor). However, large volumes of such agents are not planned and risk assessments detail how aerosol producing agents must be securely contained prior to being removed from a MSC.

All other cases, use absorbent material (such as blue roll) to soak up the spill. This material should then be treated as contaminated solid waste. Surface will be decontaminated with 70% Ethanol.

---

**Is an emergency plan required according to regulation 20?**  

- N

**If yes, tick to confirm that it is attached to this form**  

- N

**Tick to confirm that you have attached a risk assessment to this form**  

- Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  

- N

---

Please enter comments on the GM safety committee on the risk assessment
GM risk assessment GMBSC/0080 was sent to GMBSC members by email for review prior to the next meeting on the 13th May.

The GMBSC had the following question:

"Are the antibiotic resistance genes being introduced ones that can be found in wild isolates or are there the group creating S. pneumoniae and P. aeruginosa strains with new antibiotic resistance profiles nor normally seen? If it is the latter it would be good to see justification for why they have chosen the resistance genes they have."

The response from the researcher was as follows:

For Streptococcus pneumoniae, an erythromycin gene was selected because the strain to be transformed is both phenotypically and genetically sensitive to erythromycin (MIC 0.06 µg/ml) and previous work has used erythromycin as a selectable marker for this organism. Erythromycin resistant clones are found in the wild so we are not creating an new antibiotic profile here for Streptococcus. Likewise, for Pseudomonas aeruginosa, we have selected antibiotics (Gentamicin and Tobramycin) to which our laboratory strain is normally sensitive, which are used widely as slectable markers for genetic studies in P. aeruginosa, and for which P. aeruginosa strains with resistance to both Gm or Tb have previously been reported to occur naturally in a variety of environmental and clinical settings.

The GMBSC approved the project as AC2 by email and this approval will be officially noted on the minutes of the next GMBSC meeting on 13th May.

### Project Containment

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<td>L2</td>
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</tr>
<tr>
<td>L2</td>
<td>L3</td>
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</table>

### Project Ref 57/14.3

**Date Ackn'd** 22/07/2014

**CU2 Project Title** Investigating the pathogenesis of infection with Neisseria species

**Class** Class 2

**CultureVolClass2** ≤ 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

**Withdrawn** N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

02/03/2022
The aim of this programme is to investigate the pathogenesis of neisserial infection by studying bacteria both in culture and interacting with eukaryotic cells. Overall, we aim to identify and characterise bacterial interactions with eukaryotic cells and the concomitant cellular responses made by eukaryotic cells to the presence of infection. We aim to improve our basic scientific understanding of the mechanisms of sepsis-inducing diseases.

**Recipient or parental organism**


**Neisseria gonorrhoeae**: Strain FA1090, Strain MS11; Strain 1291, Strain 35/02, Strain DGI18, Strain FA6140, Strain FA19, Strain PID18, Strain PID1, Strain PID24-1, Strain PID332, Strain SK-92-679, Strain SK-93-1035, Strain DG12, Strain F62 and mutant derivatives thereof. See: [http://www.broadinstitute.org/annotation/genome/neisseria_gonorrhoeae/GenomeDescriptions.html#Ng_1291_V1](http://www.broadinstitute.org/annotation/genome/neisseria_gonorrhoeae/GenomeDescriptions.html#Ng_1291_V1) for derivation information.

**E. coli**: Strains of E. coli to be used are all derivatives of the disabled K12 strain (e.g. Dh5-alpha, JM-series, TG-1, C600, XL1-Blue and K12 strain AW405, a chemotactic, motile variant of K12. The exception is BL21, which will be used for high-level expression of some genes. BL21[DE3] is considered inherently safe, in the sense that it is broadly equivalent to K12, and offers no additional risks to the use of K12 derivatives.

**Salmonella enterica sv. Typhimurium**: Strains of S. enterica sv. Typhimurium to be used include: Strain LT2/SGSC1412/ATCC 700720, SL1344 NCTC 13347, D23580, DT104 NCTC 13348, DT2

None of the GMOs are anticipated to have increased pathogenicity over the wild type strains. All strains of pathogenic Neisseria and any genetically-modified Neisseria lactamica will be handled as a Category II pathogen regardless of resistance phenotype. Non of the antibiotics to which the GMOs are resistant are used clinically to treat Neisseria/Salmonella infection.

pGCC4 and derivatives: A plasmid for use in complementing mutations using the NICS (Neisseria intergenic complementation site) system. Inserts a functional copy of the gene ectopically at an unlinked chromosomal locus between the lctP and aspC genes under the control of lac regulatory sequences, and linked to an Erythromycin (Ery) resistance cassette.

pEN11 and derivatives: A plasmid based on the pFP10 E.coli/Neisseria shuttle vector containing the origin of replication of a naturally occurring gonococcal plasmid, a lac-derived promoter and ribosomal binding site of the highly expressed omp85 gene, plus Ndel and AatII sites for subcloning of any ORF. The shuttle vector has chloramphenicol (Cam) resistance.

Homologous genetic material to be (re) introduced:

   A comprehensive list of putative virulence-associated genes (Table 1 therein) and potentially phase variable genes coding for proteins responsible for the interaction of Neisseria meningitidis with eukaryotic cells (Table 2 therein) are included in this Reference. NB: Gene nomenclature provided refers to MC58 complete genome sequence, though Ng. and NI. Homologues can be accessed online:
   FA 1090: http://microbes.ucsc.edu/lists/neisGono_FA1090_1/refSeq-list.html
   MS11: http://www.broadinstitute.org/annotation/genome/neisseria_gonorrhoeae/GenomesIndex.html
   ST-640: http://www.xbase.ac.uk/genome/neisseria_lactamica-y92-1009
   Where appropriate, homologous genes in Salmonell enterica sv. Typhimurium will be targeted:
   http://bacteria.ensembl.org/salmonella_enterica_subsp_entnerica_serovar_typhimurium_str_ltt2/Info/Index

   Members of the Neisseria meningitidis nssR regulon (Table 2 therein). These genes are de-repressed in response to high concentrations of nitric oxide such as those encountered under conditions of nitrosative stress. They function to promote survival of the bacterium under these conditions. Gene nomenclature provided refers to MC58 complete genome sequence: see online resources above for Ng. and NI. Homologues.

   Members of the Neisseria meningitidis fur modulon (Fig 2 therein). The expression of these genes is modulated by the availability of free iron, a process involving the Ferric Uptake Regulator (Fur protein. Gene nomenclature provided refers to MC58 complete genome sequence: see online resources above for Ng. and NI. Homologues.

Heterologous genetic material to be introduced:

Codon-optimised (Neisseria) enhanced Green Fluorescent Protein (eGFP) from Aequoria victoria and derivatives, such as mCherry, which are designed to fluoresce at different wavelengths.

Luciferase from Firefly and Renilla reniformis
lux gene operon from Vibrio fisheri bacterial species
B-galactosidase from E.coli
Bacteriophage T7 RNA polymerase gene
FLP Recombinase (and derivatives), originally isolated on the 2 micron plasmid from yeast
Bacillus subtilis levansucrase; SacB
Transposon Tn10 tetracycline responsive repressor protein; tetR
Neisseria meningitidis: The sole biological reservoir of Neisseria meningitidis is the human nasopharynx, and between 10-30% of a given population is carrying the organisms as a nasopharyngeal commensal. The transmission of Neisseria meningitidis is through droplet inhalation and via close contact with carriers of the bacterium. Large increases in the number of carriers in a population typically precede an epidemic outbreak. The incidence of cases of invasive meningococcal disease is relatively small, with less than 1000 cases of disease per annum in England and Wales. A majority of these cases are caused by serogroup B strains of Nm, which express a non-immunogenic polysaccharide capsule. The number of cases is predicted to decrease further however, following the advent of multivalent protein vaccines derived from invasive serogroup B strains. Invasive meningococcal disease is a rapid onset, potentially fatal infection characterised by sepsis syndrome: including microcirculatory collapse, multiple organ failure and death in approximately 8% of cases. Symptoms of meningococcal disease involve lethargy, fever and vomiting, aversion to light, sore neck and headache in the early stages, though not all cases present with all symptoms. In cases of meningococcal septicaemia, there is the development of a petechial and purpuric rash and death can occur within as little as 4h. Between 5-30% of survivors of meningococcal disease have significant neurological sequelae. Laboratory workers manipulating high numbers of meningococci are at risk of becoming colonised. If colonised with a disease-causing strain, there is a risk that the organism will cross the nasopharyngeal epithelium and cause invasive meningococcal disease. Humans vary greatly in their susceptibility to invasion and disease, but the risk of disease, given colonisation with a hypervirulent lineage of serogroup B or C ranges from 1:10,000 - 1:100,000. The laboratory serogroup B strain Neisseria meningitidis MC58 was originally isolated from a patient in the Gloucester area in 1991 (Mcguines et al. (1919) Lancet. 1991Mar2;337(8740):514-7). Untyped isolates obtained from participants in either of the two human lactamica carriage studies conducted by the Read group are, by definition, carriage isolates and are no more likely to cause invasive meningococcal disease than any other strain used in these studies. Clinical treatment of meningococcal disease involves injection of cephalosporin antibiotics, to which MC58 and its GMO derivatives remain sensitive.

Neisseria gonorrhoeae: The gonococcus is sexually transmitted and can colonise the genital tract and the oropharyngeal mucosa. Occasionally it may cause a conjunctivitis in babies when the mother is infected. It is exclusively a human pathogen. A laboratory worker could theoretically become infected if they auto-inoculated the oropharynx or genital mucosa. The infection could then be passed on with high efficiency to their sexual contacts. The laboratory strain MS11 was originally isolated by J S in the 1960's from an uncomplicated infection, whilst FA1090 is a serum-resistant proline-requiring strain isolated from a patient with disseminated gonococcal infection.

NB: Neisseria species - The inserted gene products do not carry additional pathogenicity or virulence factors that would increase the overall pathogenicity of GMO strains beyond that of the wild type.

Salmonella enterica sv. Typhimurium: Salmonella enterica sv. Typhimurium is an intracellular pathogen, causing a non-fatal morbidity in infected humans and a typhoid-like illness in mice. Infections with Salmonella begin by ingestion of the bacterium, where different studies have shown a median infective dose in humans ranging from 200-10^6 bacteria, depending on the strains used and the immunological competence of patients. Infection of humans with nontyphoidal Salmonella typically results in a self-limiting gastroenteritis, with symptoms emerging between 6 to 48 h after ingestion and characterised by nausea, vomiting and diarrhoea. Preence of fever is not clinically useful, but may occur - with increased likelihood of fever in immunocompromised individuals, who are also more at risk of developing enteric fever or Salmonellosis. Standard therapy for uncomplicated typhoid consists of 15 mg/kg day of a fluoroquinolone such as ciprofloxacin or ofloxacin, administered orally for 5 to 7 days. As such, introduction of antibiotic resistance genes into Salmonella for the purposes of selection do not pose an increased clinical risk, beyond the further dissemination of these genes to other intestinal commensals.

Echerichia coli: E.coli K12 and its derivatives are multiply disable and are designated as Class 1 organisms. Good microbiological practice will be followed when using these organisms. For protein expression in E.coli we will preferably use BL21[DE3] or B21[DE3]pLysS and its derivatives as expression strain of choice. This strain is essentially equivalent to K12 (Chart et al. (2000) J. Applied Microbiol. 89: 1048-1058). It is believed to be unlikely to colonise and establish a persistent infection in the gut of a healthy individual. Overall, E.coli is considered inherently safe.

NB: Escherichia coli: The vectors proposed for use in cloning neisseria genes are non-mobilisable. Sequences of neisseria DNA inserted into cloning vectors will not increase the pathogenicity of E.coli.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

As meningococci are passed from person to person by the droplet route and the site of colonisation is the human nasopharynx, the creation of aerosols represents the major risk to laboratory workers. Infection with Salmonella enterica sv. Typhimurium requires the bacteria to be ingested, so Good Laboratory Practice should preclude infection. All Neisseria are acutely susceptible to common disinfectants (Kendall, A. I. (1928). The Meningococcus-Gonococcus group. Bacteriolog: general, pathological and intestinal (3rd ed., pp. 292-309)) and are fastidious organisms that do not survive for long outside of their biological niche. Neisseria can be inactivated by exposure to 1% sodium hypochlorite, 70% ethanol, phenotics, 2% glutaraldehyde, formaldehyde, and peracetic acid (Rutala, W. A. (1996). APIC guideline for selection and use of disinfectants. American Journal of Infection Control, 24(4), 313-342), and 1% Virkon, although secondary autoclaving after chemical disinfection is necessary to ensure sterility.

Contaminated plasticware such as tips, spreaders and inoculating loops will be disinfected with 2% Virkon solution overnight before decanting into suitable labelled "Biological Hazard" bins (blue) for movement to the HPS autoclave on Level A, Southampton General Hospital. Contaminated petri dishes will be placed in the same tins with the lids on: colonies of Neisseria on culture plates are not robust and will expire outside of the CO2 incubator overnight.

Decanted, infected supernatants will be chemically disinfected with 2% Virkon overnight. Autoclaving will result in 100% kill of potentially infectious agents.

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<th>Is an emergency plan required according to regulation 20?</th>
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<td>If yes, tick to confirm that it is attached to this form</td>
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<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
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Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment
GMBSC Meeting 15th October 2014 item 10.4 GMBSC 52 - Investigating the pathogenesis of infection with Neisseria species (Medicine)

Received The risk assessment

Mr L explained that the researcher was waiting for approval. The laboratory was already set up for what would be a long-term GM and a non-GM project. The HSE had been notified in respect of work at a previous university, but further notification was required, as this was a change of premises and a new employer. Mr L suggested that this was an AC2 project, and would be notified to the HSE as required for biological agents under Part 1 of schedule 3 of the COSHH regulations. He proposed to audit the project in a year's time.

Members had a query over a statement of keeping a 'door closed' and also the use of both white laboratory coats and blue surgical gowns. The project was approved subject to satisfactory responses to these queries.

Resolved That the project be approved as Activity Class 2 subject to satisfactory responses to the queries (action: Mr L). [Post meeting note: The risk assessment has been changed to reflect that at CL2 the door must remain closed and that the staff will wear oversleaves while working in the MSC instead of using two different lab coats.]

GMBSC Meeting 21st January 2014 item 11 Project amendment: GMBSC 52

Received The amended risk assessment.

Mr L reminded members that this project had received GMBSC approval at AC2 in January 2013 but work had not started as the researchers were waiting to submit an amendment to the original risk assessment before notifying the HSE. The researchers had submitted an amended risk assessment of which the only difference from the original was the addition of a new bacteria; this would be of the same nature as the one in the original risk assessment, the amendment could be notified at the same time if approved today.

GM&BSC was content to approve the amendment at AC2 but asked for a list of the genes contained in the risk assessment to be circulated. Subject to this circulation, the amendment was approved and the project could go forward for HSE notification.

Resolved That the project amendment be approved at Activity Class 2, and a list of the genes contained in the risk assessment to be circulated.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 57/14.4
The use of lentivirus to investigate phosphoinositide metabolism and function

Phosphoinositides are lipids that function as messenger molecules that control various cellular functions such as membrane trafficking and endocytosis and in doing so impact on cell proliferation and apoptosis. These lipids are also present in the nucleus where their function is not completely understood. We will use attenuated lentiviral transduction vectors to knockdown or overexpress genes that modulate the levels of phosphoinositides or that might act as downstream targets. In this manner we expect to understand how signalling through phosphoinositides impacts on cellular functions that are relevant to human diseases such as cancer, neurodegenerative and myopathies.

Well established human and mouse cell lines.

HIV derived lentiviral vectors
PLKO1 and 2, and pRRL backbone
Overexpression vectors based on promoters driven by EF1a

The genetic material has either been chemically synthesised and amplified using PCR and then cloned into suitable vectors. Alternatively the genetic material was amplified from established mouse or human cell lines and then cloned in appropriate vectors.

The genetic material is aimed to
1. Suppress the expression of endogenous genes in murine or human cell lines in order to establish their functional properties.
2. To modify human and mouse cell lines in order to overexpress fragments, mutants or whole proteins in order to establish what their functional role in cells might be.
3. To identify novel targets that might be useful for the development of therapeutic molecules to treat human diseases.
Evaluation of foreseeable effects

We expect that genetically modifying cell lines with the vectors described above will lead to:
1. Increased growth characteristics
2. Suppression of growth and the induction of cell cycle arrest or apoptosis
3. Effect differentiation of cells down different cell lineage pathways
5. Change morphological characteristics.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

We will not modify plants or animals but will modify well established mouse or human cell lines. All studies with these cell lines will be carried out in a containment level 2 facility. Hazards to human workers from the GMO are minimal and are limited to ingestion or percutaneous injection of the cell lines. Even in this unlikely event the immune system and the foreign environment will likely prevent any further growth. Possible local inflammation from percutaneous injection can be effectively monitored and controlled. Genetic transfer from the modified cell line is gag, pol genes as well as VPR, VIF, VPU and NEF (required for immune host evasion), and require co-transfection of other plasmids for the generation of competent virus.

Controls in place.
1. No needles will be used with the genetically modified cells thereby minimising the likelihood of percutaneous injection.
2. All studies will be carried out in a class 2 microbiological safety cabinet in a secure room (access to the laboratory is restricted and training is required to gain card access).
3. Aerosols and splashes will be minimised by using good laboratory practice and minimal use of centrifuges.
4. Spills will be cleaned immediately with 1% VIRKON
5. All tissue culture usage will be decontaminated by VIRKON and by autoclaving.
6. Personal protective equipment is mandatory in this laboratory and includes a change of laboratory coat when entering, and the use of safety glasses and gloves.
7. Hand washing facilities are present.

For only GMMs - application for any derogation from full containment for the class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste from either the GMM or the GMO will be treated with 1% VIRKON disinfected for 2 hours. Solid waste (cell culture lactic) will be treated with 1% VIRKON and then double bagged and autoclaved at 121 degrees for 15 minutes. Tissue waste will be double bagged and autoclaved.

Both the GMM and the GMO are susceptible to killing by either 1% virkon or by the use of 70% ethanol. Accidental splashes will be contained by wiping with tissues and 70% ethanol followed by Virkon (1%) disinfectant. All tissues used for clean up will be immediately placed in biohazard bags for decontamination by autoclaving. After treatment with VIRKON and Autoclaving the final solid waste will be placed in the normal waste disposal. The liquid waste after treatment with VIRKON will be disposed of in the drainage system.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
GMBSC meeting 14/10/14
That the project be approved in principle at Activity Class 2, subject to clarification of containment level of the lab and evidence of a code of practice in place for the lab. Official approval to be suspended until these concerns are resolved.

The laboratory was reviewed confirmed as CL2 by the University BSA on 31/10/14. The local code of practice has been reviewed with regard to the new AC2 project and circulated to all users.

Approval given by the University BSA prior to the next GMBSC meeting on 13/1/15

### Project Containment

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### Project Ref 57/15.1

- **Date Ackn'd**: 06/05/2015
- **CU2 Project Title**: The pathogenesis of bacterial infection and development of vaccines
- **Class**: Class 2
- **Culture Vol Class**: < 1 Litre
- **Consent Granted**: Non-GMM

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

02/03/2022
The generation and use of several GMOs are as follows:

1. We will generate Neisseria meningitidis strains containing individual gene deletions through insertional inactivation with non-clinically utilised antibiotic resistance cassette(s). The genes of interest are those that have been identified as important for pathogen interactions with eukaryotic cells and genes regulating or contributing to neisserial virulence and/or pathogenesis. For example, we will generate knock-out organisms that lack the adhesin complex protein (ACP), macrophage infectivity potentiator (MIP) proteins, ABC transporter proteins amongst others and also the corresponding knock-in strains [Hung et al., 2011, 2014]. It is anticipated that other genes with known function will be deleted in Neisseria spp. These genes can be found in the published genome sequence(s) and are also mentioned in our proteome studies (Williams et al., 2009, 2014).

2. Generation of green fluorescent protein-containing Neisseria species, Pseudomonas spp and E. coli spp (for plasmid maintenance). GFP-Neisseria will be produced according to the paper of Christodoulides et al (2000) and GFP-Pseudomonas will use the plasmid from Alice Prince, Columbia University, New York. We will also acquire several strains of PAO-1 and PA14 that are deficient in expression of pili, flagellae and type-1 III secretion systems. These reporters will be used in human cell infection experiments to investigate bacterial interactions. PA-14 strains will be provided by Prof. George A. OToole of the Geisel Schoof of Medicine at Dartmouth; and PAO-1 GFP strains by Professor Tolker-Nielsen in Copenhagen, Denmark.

3. We will also construct E.coli strains using commercially available cloning and expression vectors for the production of recombinant proteins for vaccine studies [Phillips et al., 2013]. In addition, reporter gene-fusions, e.g. for beta-galactosidase or other defined enzymes will be made in order to quantify the expression of pathogenesis-associated gene(s) during experimental infection or growth.

4. Strains of Streptococcus agalactiae, deficient in several virulence factors will be provided by Or. Kelly Doran, San Francisco, USA. They include mutants that do not express beta-haemolysin/cytolysin, lagA, CiaR, PilB, Srr-1 components. Selective antibiotics for these mutants are chloramphenicol and kanamycin. These strains are all susceptible to drugs used to treat clinical infection and will be used to study host cell-pathogen interactions in vitro.

5. Strains of Haemophilus influenzae and Staphylococcus aureus will be sourced (from ATCC or collaborators) for ex vivo infection studies as future projects are developed in respiratory disease and skin infection.

The experiments to be done involve i) culturing bacterial pathogens, ii) measuring bacterial growth in the presence and absence of additives/anti-microbials iii) infecting human cell cultures and tissue explants, iv) harvesting supernatants and developing appropriate models to investigate bacterial interactions with human cells in vitro v) generating recombinant pure bacterial proteins in heterologous host (E.coli) for investigating vaccine potential and for structure-function studies. For this programme, all bacterial pathogens named will be grown in LC91 containment level 2 laboratory, Molecular Microbiology, Level C, South Laboratory and Pathology Block, Southampton General Hospital.

Selected References:


iv) Hung, M.C, Heckels, J.E., Christodoulides, M. 2013. The Adhesin Complex Protein of Neisseria meningitidis is a


Recipient or parental organism

1. Neisseria meningitidis strains MC58, MC58.6.SiaD (Cap-), Opa+/-, Pil+/-, Opc+/-, ACP+/-, MIP+/- and other derivatives phenotypically selected.
2. Neisseria gonorrhoeae Strain P9, FA1090, MS11 (and phenotypic variants selected).
3. Escherichia coli BL21 (DE3), DH5α, TOP10 and TOP10F, JM109; K1; IH3080
4. Pseudomonas aeruginosa PAO-1, PAO-1 tn7-GFP, flimtn7-GFP, pilAtn7-GFP, flim pilAln7-GFP, PA14-GFP (pUCP19)
6. Streptococcus agalactiae strains A909; H36B; 18RS21 ; NEM 316; 2603V/R; COH-I; COH-1 HY106; NCTC 10/84, strains mutated in CytE, Srr-1, Pi IS; lagA, CiaR.
7. Haemophilus influenzae HiS (Pillman type strain)
8. Staphylococcus aureus ATCC strain(s), e.g. Newman.

Host/vector system

1. pRSET A plasmid (Invitrogen, UK) - maintained in E. coli DH5α (Invitrogen, UK) will be used for cloning a gene encoding MIP (Macrophage Infectivity Potentiator Protein), Adhesin Complex Protein (ACP) and other vaccine antigens; E. coli BL21 (DE3)/pLysS (Invitrogen, UK) will be transformed by recombinant pRSET A for protein expression.
2. pGEM®-T Easy Vector System I (Promega, UK) - E. coli TOP10 (Invitrogen, UK) will be transformed with this vector carrying 6.mip-kanamycin construct, this plasmid will be used to create N.m. Mess .6.mip mutant (with pTOPO kanamycin cassette inserted). The same process will be used for other knock-outs e.g. ACP, and other genes.
3. pGCC4 plasmid (personal communication, UK) - E. coli TOP10 (Invitrogen, UK) will be transformed with this vector, N.m. MC58 .6.mip mutant will be transformed with this plasmid to obtain the complemented strain N.m. MC58 .6.mip(mip). The same process will be used for generating other complemented strains.
4. pTOPO plasmid (Invitrogen, UK) - source of the kanamycin cassette for constructing of the N.m. MC58.6.mip mutant; pACYC177 plasmid (New England Biolabs, UK) - alternative source of the kanamycin cassette.
5. pEN11 and derivatives: a plasmid based on the pF10 E. coli/Neisseria shuttle vector containing the ORF of a naturally occurring gonococcal plasmid, a Lac-derived promoter and ribosomal binding site of the highly expressed OMP85 gene, plus Ndel and AattI sites for subcloning of any ORF and chloramphenicol resistance.
6. pUCP19-GFP; P9-cryptic plasmid-GFP.
7. pET-24a-d(+). The pET-24a-d(+) vectors carry an N-terminal T7’Tag® sequence plus an optional C-terminal His’Tag® sequence. These vectors differ from pET-21a-d(+) only by their selectable marker (kanamycin vs. ampicillin resistance).
8. Pseudomonas aeruginosa complementation: PA14 .6flgk pUC19-f1gK; PA14 fipop8, pUC19-popB; and also the PA14 .6.f1gK pUC19 and the PA14 .6.popB pUC19.

Origin & function

1. MIP (Macrophage Infectivity Potentiator) gene will be inserted into N.m. MC58 .6.mip mutant to obtain a complemented strain reverting to the wild-type N.m. MCS8 strain in functionality; this gene encodes for MIP protein, which is a surface exposed lipoprotein in N.m., a bacterial pathogenicity factor.
2. ACP (adhesin complex protein) gene will be inserted into N.m. MC58 MC7 mutant to obtain a complemented strain reverting to the wild-type N.m. MCS8 strain in functionality; this gene encodes for ACP protein, which is a surface exposed protein in N.m., a bacterial pathogenicity factor. For both MIP & ACP, a kanamycin cassette will be inserted into N.m. MC58 for selection purposes (provides the resistance to kanamycin antibiotic). Also an erythromycin cassette will be inserted into N.m. MC58 for selection purposes (provides the resistance to erythromycin antibiotic).
4a) Aequoria victoria Green fluorescent protein (GFP) and newer derivatives, e.g. mCherry, which are designed to fluoresce at different wavelengths. b) Luciferase from Firefly and Renilla reniform is. c) Lux gene operon from Vibrio fisheri bacterial species. d) E. coli β-galactosidase enzyme. e) Bacteriophage T7 RNA polymerase gene. These will be introduced into bacteria for imaging and selection purposes.
5. For bacteria belonging to genus Streptococcus, Haemophilus, Staphylococcus, Pseudomonas for which mutants will be provided by collaborators, no further genetic manipulation is expected.

Evaluation of foreseeable effects

Due to removal of virulence-associated genes, none of the GMOs will have increased pathogenicity over the wild type strains. The bacteria and strains that carry induced gene-deletions are attenuated, i.e. they show a reduced capacity to interact with human cells. Any inserted gene products (for complemented strains) will not carry additional pathogenicity or virulence factors that would increase the overall pathogenicity of GMO strains beyond that of the wild type organisms. Furthermore, bacteria that carry GFP plasmids will be similar to wild-type organisms; in our experience, these GFP-bacteria show no difference to wild-type organisms with respect to host cell interactions. All strains of pathogenic Neisseria and other Class II organisms are handled as hazard group 2 pathogens, regardless of their resistance phenotype. None of the antibiotics to which the GMOs are resistant are used clinically to treat infections caused by these bacteria. Risk assessments for handling bacteria have been conducted and below are descriptions of the pathogens and potential outcomes of infection. With appropriate risk assessment and good microbiology practice, and where all materials are chemically killed and then autoclaved, the possibility of GMO
bacteria infecting laboratory workers is effectively reduced to nil. The most hazardous procedures are growth and manipulation of high titre bacterial cultures in the Class II microbiological safety cabinets, CO2 incubators and floor standing sealed shaking incubator, which could generate aerosols in the event of significant spillage. Risk assessments for all procedures handling bacterial pathogens are available.

1. Neisseria meningitidis (meningococcus, Nm): is a causative agent of bacterial meningitis and septicaemia in humans. The organism is a strict human pathogen that colonises the mucosal epithelium of the nasopharynx. The organism can be considered as a commensal that is present in 15-25% of the population, but for reasons still unclear can become invasive for a few susceptible individuals, dependent on co-infection, genetic pre-disposition and naïve immunity. Transmission of Nm is through droplet inhalation and close contact with carriers of the bacterium. The organism can cause isolated and sporadic outbreaks in temperate countries to epidemics in Sub-Saharan countries. In the UK, annual incidence of Nm disease is ~1000-1500 cases, with ~10% mortality rate. Invasive meningococcal disease caused by capsulated bacteria is a rapid onset and potentially fatal disease exhibiting as a meningitis and/or sepsis. Sepsis is characterised by disseminated intravascular coagulation, intravascular inflammation, petechial rash, multi-organ failure, ARDS and death, if untreated within 4h. Meningitis is characterised by lethargy, photophobia, vomiting, neck pain and headache. Survivors can be left with permanent neurological and physical sequelae, ranging from deafness to brain damage to limb loss. Vaccines are available for all major serogroups causing disease, except MenB, although new licensed vaccines are now available from 2014. Clinical treatment involves the injection of cephalosporin antibiotics (MC58 and GMO strains are sensitive).

2. Neisseria gonorrhoeae (gonococcus, Ngo): the sister organism to meningococci, is non-capsulated and a strict human pathogen that colonises the lower reproductive mucosal epithelium of men and women. In men, infection causes inflammation, painful urethritis with a purulent discharge, whereas in women, infection can be asymptomatic and ascending into the upper reproductive tract. Untreated infection in women can lead to pelvic inflammatory disease, a syndrome characterised by endometritis, salpingitis, pelvic pain, peritonitis, tubal infertility and ectopic pregnancy. The organism is transmitted sexually and cases are increasing again in the UK and US, often in tandem with Chlamydia. Reports of antibiotic-resistant gonococci are increasingly (including to cefixime, no longer used). Ceftriaxone still has clinical efficacy in combination with one of two oral antibiotics, either azithromycin or doxycycline (CDC recommendation).

3. Streptococcus pneumoniae (pneumococcus, Spn): is a causative organism of invasive (meningitis, septicaemia, pneumonia, empyema) and non-invasive (otitis media, ocular infection) disease. Pneumococcal disease affecting the young and elderly has fallen significantly with introduction of effective capsular polysaccharide conjugate vaccines (Prevenar 7, 13). Antibiotic-resistance has been increasing, but clinical treatment (depending of invasive or noninvasive) involves the use of antibiotics including amoxicillin (otitis), fluoroquinolones (sinusitis), cephalosporin (ceftriaxone or cefotaxime) for pneumonia, macrolide (or doxycycline) for community-acquired pneumonia, vancomycin plus ceftriaxone or cefotaxime at meningcal doses (meningitis).

4. Streptococcus agalactiae and Escherichia coli K1 are intestinal organisms that are the major causative pathogens of neonatal meningitis; transmission from mother to foetus can occur intra-amniotically or during birth. Neonatal infection is particularly devastating, characterised by sepsis and meningitis with high mortality (50%) and high morbidity for survivors (e.g. 50% will suffer permanent sequelae, bra in damage). No vaccines currently exist for immunisation of pregnant mothers and pre-screening for GBS/E.coli carriage prior to birth is not universal. Clinical treatment involves the use of intravenous penicillin, ampicillin, or cefazolin (for exposed individuals and for mothers prior to birth and ampicillin for neonates). Escherichia coli vectors proposed for use in cloning Neisseria and other pathogen genes are non-mobilisable. Sequences of Neisseria and other bacterial pathogen DNA inserted into cloning vectors will not increase the pathogenicity of E. coli. GMO strains of laboratory-grown E. coli used for protein production are extremely unlikely to
demonstrate increased virulence. E.coli K12 and its derivatives are multiple-disabled and designated as Class I organisms (attenuated). Good microbiological and laboratory practice will nevertheless be used when handling these stains. Strains BL21 (OE3) is a K12 derivative used for heterologous protein expression and unlikely to colonise healthy individuals.

5. *Pseudomonas aeruginosa:* is a saprophyte and opportunistic human pathogen, demonstrating a wide range of pathophysiologies. Often found in ocular infection and in cystic fibrosis patients, *Pseudomonas* can form persistent biofilms. No vaccines are available for prophylaxis. Clinical treatment involves the use of fluoroquinolones, ciprofloxacin or ofloxacin (PAO-1, PA14 are sensitive to these antibiotics).

6. *Haemophilus influenzae* was an important pathogen causing sepsis and meningitis; with the HiB-conjugate vaccines, this organism now does not cause significant reported disease. Non-typeable strains are increasingly found in respiratory infections.

7. *Staphylococcus aureus* is a commensal organism and ubiquitous in the population; it is involved in many infections as an opportunist pathogen and antibiotic-resistance is a serious concern.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Prior to describing waste management procedures, some information on CONTROL measures is warranted.

**'CONTROL MEASURES'**

1. **Security, Isolation and Segregation**

Understanding the pathogenesis of infections caused by these organisms requires the use of live, virulent bacteria, especially for investigating the role of specific virulence factors. Any laboratory adaptation does not represent the natural biology of the organisms during host interactions.

Entry to Molecular Microbiology, Level C, South Laboratory and Pathology Block, Southampton General Hospital is restricted behind code-locked and swipe-card locked outer and inner doors. Access by the public is prevented and only members of the Academic Unit of Clinical and Experimental Sciences can gain access through the inner doors with University approved swipe-identity cards. The LC91 containment level 2 laboratory is locked when not in use by authorised users, and is accessible to non-authorised users only by 1) agreement with the PI or the Level C laboratory manageress, in his absence, 2) in the presence of an authorised user or 3) when equipment to be inspected/serviced has been decontaminated and a permit to work has been issued (with prior consultation with the PI). Access to unauthorised users is PROHIBITED, especially when pathogen handling is being done. In addition, the names of authorised workers is listed on the door and updated regularly.

All live bacterial strains will be handled within this room ONLY and in no other laboratory. The PI has several measures in place to limit access: 1) all staff handling pathogens are inducted in safety and handling procedures according to COSHH guidelines 2) staff are given written permission to use this room only after training and competency evaluation 3) cleaning staff and service engineers are allowed access ONLY when no pathogen handling is being done and only after consultation and agreement with the PI, or in his absence, the laboratory manageress and safety officer. Operator numbers in the laboratory are restricted with a booking system for cabinet use.

2. **Controlling exposure**

All operators will wear appropriate PPE - in the containment level 2 laboratory this also means wearing designated
blue gowns, that are not allowed outside the laboratory except for cleaning. Class II microbiological safety cabinets are required for the handling of all pathogens (wild-type and GMO alike). Class I microbiological safety cabinets will be used only when handling bulk volumes of bacteria, e.g. E.coli for recombinant protein expression or other organisms for antigen extraction and purification. Splashes are contained within the cabinets and aerosol generation is not a significant risk. All cabinets are safety-inspected and tested every 6 months. All procedures for pathogen handling have been assessed for risk according to COSHH guidelines and assessments are in place.

3. Transport
For transporting cultures within the laboratory, all primary containers (Bijoux, universals, cell culture flasks, etc.) must be screwed closed during transit. Centrifuge tubes must be transported within sealed centrifuge buckets. Cell culture plates carrying infected cells must be carried with closed lids and in a plastic box. All experimental flasks, samples etc. must be labelled with name, cell type, organism and date. Materials in freezers must be clearly labelled and sealed in secondary containers.

If the need to transport organisms outside of the laboratory arises, we will use a re-usable sealed transport container, Biological Substance-Category B (UN3373). Movement of waste: each Cabinet holds a bucket with a solution of 2% (v/v) virkon into which all contaminated plasticware such as tips, spreaders and inoculating loops is placed. Such plasticware is left overnight as a minimum before decanting and disposal into blue autoclave bins. These bins are moved from Level C to HPA autoclave on Level A in large sealed plastic carriers with "Biological Hazard" markings. Contaminated petri dishes and cell culture flasks are placed in the same blue bins for autoclaving. Decanted, infected supernatants will be chemically disinfected with the addition of 2 % (v/v) Virkon overnight.

Shipment and receipt of biological material - some of these biological agents will be moved within the UK or overseas. Hazardous agents and materials will be packaged by qualified personnel according to the World Health Organisation guidance on regulations for the transport of infectious substances and transportation will use approved couriers and follow the advice of the University DGSA. Bacteria and strains will be received from overseas as part of ongoing research collaborations. These receipts will be frozen stocks on dry ice or cultures on agar slopes or lyophilised samples. Samples sent from overseas collaborators will be subject to strict regulations and packaged according to international regulations and the conditions of carriage applied by the courier(s). Transportation of bacterial cultures and cell cultures by air follows the WHO guidance on regulations for the transport of infectious substances (guidelines from the International Air Transport Association (IATA)( http://www.iata.org/Pages/default.aspx) and the ADR-carriage of dangerous goods, HSE (http://www.hse.gov.uk/cladg/manualadrcarriage.htm).

4. Storage and inventory - Long term storage of GMO bacteria will be in LN2 storage, the dewar kept in the containment level 2 laboratory. E.coli strains will be stored usually at -80C. Operators must refer to the relevant Risk Assessments for handling and growth of bacteria in force for PI and all laboratory users. Material being stored in liquid nitrogen must use cryovials with screw-cap lids to prevent explosive thawing (NUNC cryotubes must only be used). Removal or addition of potentially infectious material from/to LN2 will be performed by authorised users wearing appropriate PPE (face visor, cryo-compatible gloves over nitrile gloves, laboratory coat) and be done with emergency spill kits - including virkon satchets for disinfection - immediately to hand. For biological samples maintained in liquid nitrogen and at -80C , the PI holds a master file that identifies the pOSitions of each sample in the reservoirs. Each vial is identified by content, date of deposition and users initials. Only the PI has access to this information and has the authority to make changes depending on sample removal and/or replacement. Any samples unaccounted for must be reported to the PI immediately. Samples that appear to be 'lost' are invariably a result of not being replaced; the biological hazard of samples left outside the storage facilities is negligible as viability is compromised.
1. Liquid waste - Virkon chemical (2% v/v solution overnight) before disposal. Virkon is sufficient to kill the Class II pathogens (both Gram positive and negative) used. Bactericidal activity is observed within hours of treatment. Liquid waste is disposed down appropriate laboratory sinks.

2. Solid waste - physical (autoclave). Disposable plastic waste (loops, pipettes) will be chemically disinfected overnight (with 2% v/v Virkon solution). On the following day, the disinfected waste will be placed into a blue autoclave bin. Petri dishes, cell culture plates etc will also be placed in blue autoclave bins. These bins are autoclaved in the HPA autoclave, Level A, SGH.121 C for 15-30 minutes, at 2.68 Kg/cm², is sufficient to kill bacterial pathogens and to ensure sterility.

3. Use of centrifuges - for large volumes of medium containing pathogens or bacterial extracts. For cell culture infection experiments, bacterial pathogens need to be centrifuged. Up to 100 ml of potentially-infectious material, with a maximum concentration of 10⁸ cfu/ml (20 ml volume maximum in sealed universal) will be centrifuged. Risk assessments for the handling of bacteria and centrifugation of infectious material are documented and must be consulted before procedures are started. For the preparation of recombinant proteins in E.coli laboratory strains, the maximum culture volume is 21 (4x 21 flasks, each containing 500 ml growth medium). Culture of such volumes is done in a floor-standing shaking incubator and volume handling in a Class I microbiological safety cabinet. Preparation of centrifuge buckets and balancing must be done in the Class I microbiological safety cabinet. All infectious material for centrifugation will be contained in sealed biological safety buckets, which can only be opened in Class 1111 cabinets. In the event of leakage/spillage, for decontamination and waste management, if the leakage was confined to a sealed bucket, open the centrifuge, sterilise the compartment with an alcohol spray (70% methanol) and wipe-down, sterilise the outside of the bucket before removing it to the nearest Class II microbiological safety cabinet. If spillage is within a Cabinet, open the centrifuge bucket and decontaminate by adding 2% (v/v) Virkon solution or 70% (v/v) alcohol, allowing at least overnight contact time before decanting of waste material into the Virkon-containing bucket kept inside the cabinet. For the preparation of bacterial extracts and other components from pathogens and the growth of E.coli for recombinant protein expression, larger volumes may be required. In the unlikely event of breakages within ultracentrifuge rotors, the additional concern of aerosol production needs to be considered. Thus, decontamination of ultracentrifuge(s) and removal of waste should be done only within Class I cabinets using appropriate decontamination solutions (alcohol, Virkon).

4. Use of incubators - infected cultures will usually be cell cultures in flasks (25 ml volume maximum per flask) or in plates (lidded, up to 6 well containing 5 ml) that have been challenged with bacteria for periods of up to 24-48h (depending on human cell viability). Cultureware are kept on static shelves. All cultures/flasks/plates must be labelled with organism, operator and date. Waste samples from these experiments are disposed of as follows: flask supernatants decanted into Virkon (2% v/v) bucket. Culture plate wells emptied by sterile pipette into same solution. All plasticware in contact with pathogens are placed into blue autoclave bins for transport to HPA autoclave on Level A, Southampton General Hospital. In the event of a spillage inside the CO2 incubator, the incubator should be closed, turn off the incubator to allow aerosols (if generated, but unlikely) to settle. Open the incubator after 24h and spray internal compartment with 70% v/v solution of alcohol, wipe down and then clean with dilute Virkon solution to disinfect. All wipes must be disposed of within blue autoclave bins.

5. For bacterial cultures grown in orbital shakers inside a CO2 incubator, these will be done in either 15 ml or 50 ml Bio-Reaction tubes (CellTreat Scientific Products), which contain a 0.22 um filter in the lid for gas-exchange and the prevention of aerosol generation inside the incubator. Tubes will be clamped and shaking of cultures will not exceed 200 rpm. Staff trained in the use of the orbital shaker will also be trained to carry out routine, dynamic risk assessment of the incubator and shaker when they open the outer door of the incubator: any accidental spillage of
bacterial culture can be identified by visual inspection through the inner glass door. In the event of spillage, the orbital shaker(s) will be switched off at the wall socket and an excess period of 6 hours given for any potential aerosols to settle. Evacuation of the laboratory is unnecessary as the aerosols will be contained within the gas-tight incubator. Spills can then be decontaminated and cleaned up using a 2% Virkon solution and at least 15 minutes of contact time. All paper waste will be disposed of as contaminated biological waste for incineration in blue autoclave bins.

For E.coli cultures for recombinant protein production, appropriate glass culture flasks are microbiologically sealed and shaken within a floor-standing shaker (Sony Gallenkamp). Any breakage within the shaker can be observed through the glass lid, which is sealed to prevent potential aerosol escape. In the unlikely event of a breakage, turn off the incubator, DO NOT open the lid and leave the incubator closed for at least 24 hours to allow any aerosol to settle and bacteria to die. Decontamination will involve absorbent paper for spillage, followed by alcohol and Virkon decontamination. Paper waste will be disposed as above.

For spillages that may occur on lab equipment such as incubators or centrifuges, after decontamination, it is advisable to swab the cleaned area and culture on appropriate agar media to demonstrate sterility, prior to subsequent use.

6. PPE contamination - in the containment level 2 laboratory, ONLY side-fastening blue gowns must be worn. These gowns are kept in this lab only and must NOT be worn elsewhere. The gowns must not share same hooks as standard white laboratory gowns. The blue gowns provide cover from the neck to beyond the knee. When appropriate (e.g. every fortnight), the blue gowns will be placed into a designated, labelled bin for washing and will be replaced with fresh gowns by the lab technician. Gowns are taken, in sealed bag/box, to the HPA facility on Level A, Southampton General Hospital for cleaning. In the unlikely event of a spillage onto the gown, operator should remove the garment and decontaminate by spraying initially with alcohol (70% v/v) and then washing with diluted Virkon solution. Place the washed garment immediately into a bag for cleaning.

7. Personal hygiene - disposable nitrile gloves (all sizes XS, S, M, L) are available to operators. These gloves do NOT leave the laboratory, if contaminated they are discarded into autoclave bins. The laboratory has a sink for hand-washing, with a pH neutral soap solution as well as an alcohol gel. Hands MUST be washed before leaving the laboratory. The laboratory also holds an eye-wash bottle solution.

8. Animal work with bacterial pathogens - at present, infection of animals with any of these GMO agents is NOT anticipated. If this becomes necessary in the future, the Biomedical Research Facility has dedicated Class II microbiology containment facilities and cabinets for infection work and holding pens for infected animals, next to decontamination and autoclaving facilities.

Comments from the University GMBSC held on 14/10/14.
This project was clearly Activity Class 2; it did not fulfil the criteria for assignment to Activity Class 3. The project was approved as Activity Class 2 subject to notification to the HSE.

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
### Project Additional Information

**Purposes of the contained use**

This project aims to investigate the use of novel acellular strategies for tissue repair. In this project we will deliver as part of our activities, genetic material to cells for bone regeneration. These strategies will be compared to conventional methods of intracellular delivery, such as lentivirus delivery.

**Recipient or parental organism**

Lentivirus particles produced by 2nd generation packaging system
**Host/vector system**

- psPAX2, pMD2.G, pSIN

**Origin & function**

- Human gene sequences for:
  - VEGF (stimulates angiogenesis)
  - HIF1a (transcription factor used in hypoxic conditions)
  - BMP2 (bone and cartilage development)
  - GFP (Green Fluorescent Protein - control gene providing a fluorescent marker)

**Evaluation of foreseeable effects**

- The cells to be transfected are primary tissue from human samples. All samples are from patients undergoing routine hip replacements. Patients are screened for Hepatitis B prior to surgery. We have consent from all patients to use their samples.
- We only receive cell samples from haemotologically normal patients.
- We are not generating the viral particles.
- The viral particles cannot replicate. Once a cell replicates, the viral particle load is split between daughter cells.
- The more a cell replicates, the lower the cellular viral load. Hence, if the operator becomes inoculated then the affect of the gene will be limited to only a few cellular replications.
- The inserted genes are normally expressed in human bone cells. Over-expression of the genes may make the cells form bone quicker than normal, but without significantly changing there normal traits.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- No derogation

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Waste will be exposed to 1% Virkon solution (final concentration) for minimum of 1 hour prior to autoclaving at adjacent NHS site. 100% kill.
- Liquid waste will be treated with Virkon to 1% final concentration for at least 1 hour according to manufacturer's instructions.
- Solid waste will be immersed in 1% Virkon for at least 1 hour according to manufacturer's instructions.

**Is an emergency plan required according to regulation 20?**

- N

**If yes, tick to confirm that it is attached to this form**

- N

**Tick to confirm that you have attached a risk assessment to this form**

- Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- N
This project has been resubmitted at AC2 following advice from the GMBSC.
Resolved: That the project be approved at Activity Class 2, subject to minor amends.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L3 L4 L2 L3 L4</td>
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<th>Human Clinical Applications</th>
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### Project Ref 57/16.1

<table>
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<tr>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
<th>CultureVolume</th>
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<tbody>
<tr>
<td>07/01/2016</td>
<td>Structure function studies of cell surface proteins</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>

Withdrawn N
Tick if notifying a connected programme of work N

### Project Additional Information

**Purposes of the contained use**

As a retroviral vector system to express protein constructs of interest on human peripheral blood mononuclear cells.
Recipient or parental organism

N/A

Host/vector system

Retroviral vectors pMIGR1 (derived from MSCV- Murine Stem Cell Virus vector) and SFG (derived from Moloney Murine Leukemia Virus vector) and helper plasmids to allow transduction of proliferating human PBMCs

Origin & function

The vector pMIGR1 is derived from MSCV (Murine Stem Cell Virus) vector. The vector SFG is derived from Moloney Murine Leukaemia virus vector. Vectors will be used to introduce cDNA encoding gene constructs for cell surface proteins into PBMCs.

Inserted in the vector are genetic material encoding parts of the following proteins:

Antibody Fragments
Fragments of anti-CD20 antibodies encompassing the antigen binding domains, cloned by PCR from original antibody constructs made in house, allowing recognition of CD20 on cell surfaces.

Signalling domains from the following proteins, generated by GeneArt as oligonucleotides (commercial company):

Human CD28
CD28 is a co-stimulatory molecule expressed on the surface of T lymphocytes, NK cells and plasma cells and is required for T cell activation and signaling. The cytoplasmic region interacts with signaling molecules including to allow cell activation upon antigen recognition. Uniprot ref: P10747.

Human CD137:
CD137, also referred to as 4-1BB in literature, is a member of the tumour necrosis factor receptor (TNFR) superfamily present on lymphocytes and acts as a costimulatory molecule for T cell signaling. It consists of four cysteine rich extracellular domains and a small cytoplasmic tail. A sequence annotation of the translated protein is available in the Uniprot database (reference: Q07011).

Human CD3 ζ:
CD3 ζ is a protein expressed on T lymphocytes as part of the CD3-TCR (T-cell receptor) complex. It comprises a short extracellular domain, helical transmembrane domain and a signaling cytoplasmic tail encompassing three ITAM domains. These ITAMs interact with signaling molecules in the cell and, once phosphorylated at the tyrosine residues, help to initiate T cell activation. The transmembrane regions allow dimerization and interaction with the TCR. Uniprot ref: P20963.

The cellular function of all these signalling domains will be to induce cell activation upon antigen recognition. The antigen recognition will be elicited by the antibody fragments.

Vector constructs will be transfected into producer 293T cells. The viral supernatant produced will be used to infect PBMCs and then removed, neutralised and discarded. The PBMCs will then be washed of all virus supernatent and used in subsequent experiments.

Evaluation of foreseeable effects

Environmental risks:
The viral vectors used pose no environmental risks. They are incapable of replicating and would not be able to survive or be infective outside of laboratory conditions.

Risks to human health:
The viral vectors pose a minimal risk to human health. They are infective but non-replicative so do not multiply inside any host cell. The only potential harmful foreseeable event would be intravenous injection to a human with concentrated viral particle-containing supernatant. However, no sharp instruments are used throughout the protocol, and the volumes handled are kept to a minimum at any one time. There is minimal risk posed by any cells in culture throughout this process when they are incubated within the viral supernatant. They will only be incubated in the viral particle-containing media for a short period of time and then all virus will be neutralised. The resulting cells will then be safe to use.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| All waste that has contact with viral supernatant will be treated with Virkon solution to neutralise retroviral particles. Liquid waste will be treated for 16hr then disposed of down the sink. Solid waste will be treated for 16hr then disposed of by incineration. Virkon is a DEFRA approved disinfectant and has independently been validated as proof against retrovirus: [http://www.sphsupplies.co.uk/categories.php?category=Disinfectants/Virkon](http://www.sphsupplies.co.uk/categories.php?category=Disinfectants/Virkon) |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

### Please enter comments on the GM safety committee on the risk assessment

GMBSC meeting 12/5/15

AC1 project GMBSC-71 Structure function studies of cell surface proteins re-submitted with additional vectors. Amendments were received and the Committee decided that these added vectors had an increased risk and this project should now be classified as AC2.

Comments from the GMBSC

Application to use amphotropic packaging line to transduce human cells, requiring change from CL1/AC1 to CL1/AC2 - this should be CL2/AC2

Amphotropic now means significantly more hazardous (to humans). We will now need a harmless insert, SIN vector, and 3rd generation packaging to remain at AC1. Otherwise it will become AC2.

The Committee requested that the Researcher fill out a new risk assessment form taking into account the additional vectors.

Resolved: Mr Lockey to request that the researcher complete an AC2 RA form.

GMBSC - 71 re-submitted as an AC2 project and approved by committee via SharePoint

Researcher placed project on hold until December 2015
Project Containment

Laboratory Activities  Glass Houses  Growth Rooms
L2  Yes  L3  L4  L2  L3  L4  L2  L3  L4  L2  L3  L4
Animal Units  Large Scale Activities  Human Clinical Applications
L2  L2  L3  L4  L2  L3  L4  L2  L3  L4

Project Ref  57/18.1

Date Ackn'd  09/03/2018
CU2 Project Title
The assay of S. aureus USA300 reporter for anti-virulent drug identification

Date Project Ceased

Class  CultureVolClass2  CultureVolumeClass3-4
Class 2  < 1 Litre
Non-GMM  Consent Granted
Not Applicable

Withdrawn  N
Tick if notifying a connected programme of work  N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
One excellent drug target in S. aureus is the signal transduction system SaeRS two component system TCS, which is conserved in all clinical S. aureus strains; the SaeRS TCS controls production of more than 20 important virulence factors including toxins, coagulates, adhesins and enzymes. We plan to utilise this TCS for the development of antistaphylococcal drugs. Using the USA300 strain we plan to screen organic molecules synthesized in the laboratory to identify the potential inhibitors

Recipient or parental organism
Staphylococcus aureus USA300 FPR3757
Staphylococcus aureus USA300-0114, Yes this strain will be used experimentally

**Host/vector system**

pCL55, contains the ori and the AmpR gene from pBR322, can be selected in E. coli. Plasmid pCL55 also contains a CmR gene for selection in S. aureus.

**Origin & function**

gfp (green fluorescene protein)

**Evaluation of foreseeable effects**

S. aureus can cause sometimes a multitude of diseases via direct infection particularly when skin/mucosal barriers have been breached, or due to production of toxins by the bacteria. However, our reporter strain has one of its gene (i.e. alpha-hemolysin) replaced with benign gfp gene. Since there are many other virulent factors, the virulence seems to remain unchanged.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not relevant

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not necessary

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All the manipulation will be carried out in the class II MSC hence the exposure due to aerosol or splashes will be minimum. Transport within the laboratory is minimal. Cell culture flasks will be transferred between the shaker incubator, Class II MSC and the centrifuge, which are all located within the same laboratory. All the liquid waste generated will be treated with 2% Virkon (contact time 1 hour) before discarding it. All other waste will be autoclaved and only after autoclaving the waste would be transported through the building to the bins located at the rear of the building. Further details are given in the form GM Section 3.2

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Please enter comments on the GM safety committee on the risk assessment
GMBSC meeting January 2017

GMBSC–175 S. aureus USA300 reporter for anti-virulent drug identification

An early draft of this AC2 project had been received and returned to the project leader with initial queries, along with a reminder that the project could not continue without approval. Further follow up with the project leader would take place.

GMBSC meeting May 2017

Members reviewed the revised risk assessment and approved as AC2.

**Project Containment**

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**Project Ref 57/18.2**

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<td>Use of genetic complementation to make comparisons of the different replication mechanisms used by members of Flaviviridae family</td>
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**Historical Significant Changes**

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**Project Additional Information**

02/03/2022
Purposes of the contained use

His project is concerned with understanding the biology of certain members of the Flaviviridae family of viruses and develop novel intervention strategies. Flaviviridae family members are positive strand RNA viruses that encode their structural and non-structural proteins as a single polyprotein that gets processed into individual proteins by host and viral-encoded proteases. Viruses or viral constructs worked on will be derived from hepatitis C virus (HCV), Bovine Viral Diarrhoea Virus (BVDV), Dengue Virus (DV), Yellow Fever Virus (YFV) genomes.

Pre-existing approved GM applications already allow us to undertake certain HCV work at containment level 1, 2 and 3 and BVDV work at containment level 1. This application extends the GM work we will be undertaking for HCV and BVDV as we are now proposing to use a novel Venezuelan Equine Encephalitis Virus (VEEV) replicon to express HCV and BVDV non-structural proteins for genetic complementation studies. For such studies stable VEEV-replicon containing cell lines will be generated where the VEEV replicon not only expresses its own non-structural proteins, but also expresses either HCV or BVDV non-structural proteins. The cell lines will then be transfected with an HCV or BVDV replicon carrying various lethal mutations in order to see whether rescue of these constructs is possible. Genetic complementation using this system has already been shown to be safe and effective for HCV work (Kazakov et al. (2015) Plos Pathogens 11(4):e1004817), and the authors of this report will be collaborating with us to get the system working in Southampton.

We will also use the same VEEV system to express flavivirus non-structural proteins for comparable genetic complementation studies with YFV and DV. As with the above HCV and BVDV work, we will restrict ourselves to transfecting VEEV-containing stable cell lines with subgenomic DV and YFV replicons that do not express viral structural proteins.

Finally, work will focus on critical elements of the full length YFV genome important for replication as well as intervention strategies to either block, or detect YFV replication in vitro and in vivo. For this we will use the full length 17D-204 vaccine strain of YFV. In this instance, viral transcripts will be generated from a 17D-204 cDNA clone by in vitro transcription and transfected into Vero cells to recover virus to ensure we are generating a ‘safe’ low passage defined working stock of virus. Virus will be propagated in BHK-21 cells and Vero cells. Any changes made to the 17D-204 backbone will be ones expected to be neutral or further attenuate the virus. Insertion of foreign genetic material will be restricted to epitope tags, GFP and luciferase coding regions. No changes will be made that reverse any of the nucleotide substitutions which are potentially linked to the 17D-204 attenuated phenotype (Hahn et al. (1987) PNAS 84;2019; Galler et al. (1998) Vaccine 9-10:1024).

The GM work being performed doesn't easily lend itself to being categorized into "Recipient organism" and "host/vector system". For this reason I am listing all viral and subgenomic viral constructs to be made in this category.

Recipient or parental organism

The GM work being performed doesn't easily lend itself to being categorized into "Recipient organism" and "host/vector system". For this reason I am listing all viral and subgenomic viral constructs to be made in this category.

HCV: HCV is a human hepatotropic virus, exposure to which typically results in a life-long chronic infection that in a significant minority of cases can lead to severe morbidity and mortality due to liver cirrhosis, hepatocellular carcinoma and liver failure. Over the last few decades, the advent of new direct acting antiviral drugs means that cure rates for HCV now approach 100%, although treatment costs mean that these new drugs can only be afforded by a small proportion of the global population. Transmission of HCV is almost exclusively by parenteral means, typically as a result of being given contaminated blood products, poor tattooing practices or intravenous drug users sharing needles. Work using full length infectious HCV currently requires containment level 3 facilities. In contrast, work with HCV replicons, RNA transcripts that do not express the viral structural proteins, is typically permitted at containment level one. HCV constructs we will be using will be exclusively subgenomic replicons that do not express any structural proteins and which will only be modified to express non-pathogenic foreign genetic elements that enable detection (epitope tags and luciferases), visualization (GFP) or facilitate expression of polypeptide cassettes in certain contexts (e.g. Internal Ribosome Entry Sites and the "Stop-Go" sequence found in Foot-and-Mouth Disease Virus 2A protein that allows ribosome stuttering and separation of translated products from a single open reading frame).

BVDV: Is a pestivirus responsible for causes self-limiting diarrhoea in adult cattle, but which if it infects calves in utero causes either embryonic death or a persistent infection that limits growth and milk production. Persistently infected cows shed high levels of virus and are susceptible to mucosal disease, a form of BVDV induced pathology that is associated with contracting viral strains carry naturally occurring re-arrangements of the BVDV genome and which is invariably fatal. BVDV is endemic across most of Europe, including the UK. Work with full length infectious BVDV can be carried out at containment level 1. This application is to cover work carried out using BVDV subgenomic replicons lacking structural proteins and expressing the same range of foreign genetic elements described for the HCV replicons (see above).
DV: DV is a mosquito-borne virus that causes an acute fever which ranges in severity, but which at its most extreme results in dengue haemorrhagic fever and dengue shock syndrome, both of which are associated with significant mortality. While infection results in life-long protection against the same serotype of DV, the existence of separate serotypes represents a significant hurdle to vaccine development. Indeed, there is good evidence that the non-neutralizing antibody response generated from a prior DV infection enhances the pathogenicity of subsequent infections with other DV serotypes. DV is endemic in tropical regions of the globe, but is not found in Europe due to the climatic conditions being unable to support vector-based transmission. Containment requirements for work with DV infectious virus and replicons typically mirrors that of HCV. This application is to cover work carried out using DV subgenomic replicons lacking structural proteins and expressing the same range of foreign genetic elements described for the HCV replicons (see above).

YFV: YFV is another mosquito-borne virus that causes acute haemorrhagic disease. Symptoms of infection include fever, headache, jaundice, muscle pain, vomiting and fatigue. A small proportion of those contracting YFV will develop severe symptoms which carry a risk of death 7-10 days after the start of infection. As with DV, the virus is only endemic in tropical countries where the climate allows for productive mosquito borne transmission. Current vaccination for YFV involves the injection of a live attenuated form of the virus (typically referred to as the 17 strain) developed by serial passage of the virulent Asibi strain in chicken embryo cells. The vaccine is considered to be both effective and safe, producing life-long immunity in 99% of vaccinated persons within 30 days of a single injection. Severe complications do occur, but are rare. Yellow fever vaccine-associated neurological disease (YEL-AND) occurs for between 0.19 and 0.8 cases per 100,000 whereas the incidence of yellow fever vaccine-associated viscerotropic disease (YEL-AVD) is between 0.004 and 0.4 per 100,000 doses. For YEL-AVD at least, evidence suggests that these rare events are not due to the virus reverting to a virulent phenotype but is due to some other co-factor.

Regards the pedigree of the 17D virus used for vaccine production, the 17D actual virus stock is no longer available, and it is closely related sub-strains derived from it (17DD, 17D-204) that serve as seed stocks for vaccine production. Sequencing of these vaccine strains and another 17S sub-strain (17D-213) show that there are a total of 67 nucleotide substitutions compared to the virulent Asibi strain, 48 of which are common to all 3 attenuated strains and which are assumed to be responsible for the attenuated phenotype (Galler et al. (1998) Vaccine 9(10):1024). These 48 substitutions result in 22 amino coding changes as well as 4 nucleotide alterations in the 3'UTR of the virus, all of which have the potential to contribute to virus attenuation. Work involving the use of fully virulent YFV needs to be performed at containment level 3, whereas work involving YFV replicons would typically be allowed in a laboratory with containment level 1 facilities. This application is to cover work carried out using 17D-derived YFV replicons, similar to those described for HCV, BVDV and DV. It is also to carry out work with the full length 17D vaccine strain of yellow fever, either using unaltered vaccine strain virus recovered from an infectious cDNA clone (Bredenbeek et al. (2003) JGV 84:1261)) or using the same 17D infectious cDNA clone but modified to express a non-pathogenic insert to enable detection (specifically either an epitope tag, a fluorescent protein such as GFP or a luciferase). It is the work with full length YFV that we consider to be part of the class 2 activity within this project.

VEEV: VEEV is a mosquito-borne alpha virus that can be lethal for equine species and which exists as enzootic and epizootic subtypes. Like the other viruses we are working with it is an enveloped positive strand RNA virus, but derives from a different virus family, being an Alpha Virus. Enzootic subtypes tend to be localized to discrete areas, maintained through a local mosquito-rodent transmission cycle, and while they can cause human illness, do not present a problem to horses. In contrast, epizootic subtypes can spread rapidly and affect both human and horses alike. The virus causes an acute infection in humans, with healthy adults typically displaying mild to moderate flu-like symptoms and suffering no long-term sequelae. In contrast, viral infection of the young and old can result in a life-threatening illness. A vaccine strain, TC-83, has been developed for military personnel and laboratory workers at risk of contracting VEEV, but which can result in side effects and does not fully immunize the patient. Infectious VEEV has to be handled at containment level 3 and is also a SAPO3 agent. However, as with other positive strand RNA viruses, removal of the structural genes to generate a replicon dramatically reduces risk. We have already sought assurances that handling of VEEV replicons does not require a SAPO license. We are seeking approval to use VEEV replicon cDNA carries non-structural coding regions from BVDV, YFV and DV, to make stable replicon-containing cell lines. These stable cell lines will then be transfected with the same HCV, BVDV, DV or YFV replicons described above. We will not transfet full length 17D vaccine strain YFV into the VEEV replicons. While no structural proteins will be expressed/present during this work, this is the second part of our project where we feel there is the potential for it to need to be categorized as a class 2 activity due to the complex nature of the genetic elements being introduced into the same cellular environment.
The various genetic elements to be used in this project are listed below:

Genetic elements foreign to the Flaviviridae and Alpha Virus family: These are all non-hazardous genes with no known pathogenic potential. They are the neomycin resistance gene (NPTII), the puromycin resistance gene (PAC), green fluorescent protein (GFP), Firefly luciferase, Renilla luciferase, Nanoluciferase, the EMCV IRES, the FMDV2A ribosome stutter element, ubiquitin and epitope tags such as FLAG, V5, myc. Their intended use is to allow us to select for stable replicon containing cell lines, to monitor replicon and virus replication, to facilitate gene expression from viral subgenomic RNAs and to visualize/detect replicon and viral proteins.

HCV non-structural proteins: Recoded versions of HCV non-structural proteins NS3, NS4A, NS4B, NS5A and NS5B will be inserted into the VEEV replicon. In some instances these genetic elements may carry additional non-hazardous gene elements, specifically the same as those listed above (i.e. GFP, luciferase, etc). The intention is to study the potential of these genes to complement defects present in an HCV replicon carrying a lethal mutation in the same genes.

BVDV non-structural proteins: Recoded versions of BVDV non-structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B will be inserted into the VEEV replicon. In some instances these genetic elements may carry additional non-hazardous gene elements, specifically the same as those listed above (i.e. GFP, luciferase, etc). The intention is to study the potential of these genes to complement defects present in a BVDV replicon carrying a lethal mutation in the same genes.

DV and YFV non-structural proteins: Recoded versions of DV and YFV non-structural proteins NS2A, NS2B, NS3, NS4A, 2K, NS4B and NS5 will be inserted into the VEEV replicon. In some instances these genetic elements may carry additional non-hazardous gene elements, specifically the same as those listed above (i.e. GFP, luciferase, etc). The intention is to study the potential of these genes to complement defects present in a DV or YFV replicon carrying a lethal mutation in the same genes.

Evaluation of foreseeable effects

All replicon vectors lack any structural proteins and are therefore non-transmissible and non-hazardous. The 17D vaccine strain infectious virus that will be generated from a cDNA clone will possesses the same pathogenic potential as the vaccine strain of YFV used to vaccinate against YFV. As such it only represents a hazard to at risk groups for whom vaccination is advised against (see below). The genetic elements to be introduced into the 17D infectious virus are non-hazardous and therefore expected to have either have no impact on virulence or further attenuate the virus. Any personnel who would normally be excluded from 17D vaccination because of being in a higher risk category will not be allowed to work with the 17D infectious constructs that will be generated.

The guidelines for administration of 17D YFV are that WHO advise is that pregnant women should ideally avoid travelling to areas where there is YFV transmission. However, if travel is necessary then the benefits of vaccination outweigh the risks. Vaccination is contraindicated for infants under 6 months of age and not recommended for those aged 6-8 months. Vaccination is not recommended for those with severely immunocompromising conditions. Conditions and treatments considered to be severely immunocompromising include: primary immunodeficiencies, thymus disorder, symptomatic HIV infection or CD4 T-cell values <200 per mm3, malignant neoplasm treated with chemotherapy, recent haematopoietic stem cell transplantation, drugs with known immunosuppressive or immunomodulatory properties (e.g. high-dose systemic corticosteroids, alkylating drugs, antimetabolites, TNF-α inhibitors, IL-1 blocking agent, or other monoclonal antibodies targeting immune cells), and current or recent radiation therapies targeting immune cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Yellow Fever Virus is currently classified as a HG3 pathogen. We are not wishing to work with virulent YFV but are instead applying to work with the attenuated 17D vaccine strain of YFV only, one of the safest and most effective vaccines there is. Modifications we will make are expected to be neutral or cause further attenuation of virulence. We will not reverse any of the potential nucleotide substitutions that might be associated with loss of virulence. 17D virus titres typically reach levels no more than 10^6 pfu/ml and vaccination involves administration of ~3.5 x 10^4 viruses. When working with unconcentrated viral supernatant we believe it is inconceivable that accidental transmission would involve volumes of more than 35 microliters of viral supernatant being injected into a lab worker as any rare exposure event would likely involve...
splashes and abrasions and hence much smaller volumes entering the body. However, if working with concentrated virus the risk of exceeding the vaccination threshold of 3.5 x 10⁴ viruses increases. For this reason any worker involved in concentrating 17D YFV or handling of concentrated 17D YFV stocks will undergo prior YFV immunization as a precaution.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All biological (human and non-human) fluids will be inactivated in a freshly prepared 1% virkon solution for a minimum of 30 minutes before being disposed of via the sink. The manufacturer of Virkon claim to have tested their against a range of viruses including Flaviviruses. Given that both these viruses are enveloped and thus more susceptible to chemical inactivation than non-enveloped viruses which are also inactivated by 1% Virkon there is reason to think that 1% Virkon is well above the threshold of the chemical needed for complete inactivation of the viruses we will be handling. Indeed our in house testing of Virkon against baculovirus, another enveloped virus, suggests that exposure of virus to 0.1% Virkon for 2 minutes when in a solution of 10%FCS is enough to result in a 10⁵ drop in infectious titres and exposure to 1% Virkon for 2 minutes reduces titres to below a detectable level. Once we have the 17D YFV infection system up and generating sufficient titres to carry out this sort of testing, we will look at the effectiveness of Virkon against this virus as well. Cell lines carrying replicons will be even more susceptible to inactivation by Virkon and would be unable to survive more than a few minutes outside a cell culture environment. Because of this reason and because material from these cell lines is not infectious we do not deem it necessary to test the effectiveness of Virkon on cell killing.

All biological (human and non-human) samples that are non-fluids will be disposed via the blue boxes which when full are placed in sealed metal boxes and transported to the HPA facility one floor below the labs for disposal by autoclaving. The HPA routinely validate their autoclaves to confirm that they are operating at the required specifications to ensure complete microbial sterilization and maintain these records.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
Use of genetic complementation to make comparisons of the different replication mechanisms used by members of Flaviviridae family

Dr CM was the author of the brief and explained the project to the committee and clarified that it was low risk.

Dr CM informed the committee that he had put a GM application in to bring the plasmid across from America, which was approved.

Dr CM felt that the project should be a AC2 activity but that he needed confirmation from committee.

It was noted that the collaborators view was that it is not a higher risk but Dr CM was slightly concerned it was higher risk and so stalled the project.

It was clarified that there was secondary containment around anything that involved plates and dishes.

Professor BK asked Dr CM if it was possible for him to specify that he will not work with any other strains at the same time. Dr CM felt that this was very restrictive for him.

Dr CM informed the committee that it would be vetted by HSE as well.

Professor BK noted that he could see that Dr DC had thoroughly researched the project.

Resolved: That this project be approved for Activity Class 2 subject to HSE notification.

**Project Containment**

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<tr>
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Non-GMM Consent Granted

Project notified under transitional arrangements N
Acute Myeloid Leukemia (AML) remains an unmet medical need with ~20,000 new cases and ~10,000 deaths in US alone each year. Current treatments for AML fail to induce long-term tumour regression, demonstrating the need for new therapies. Recent studies demonstrate a role for leukocyte immunoglobulin-like receptors (LILRs) in regulating leukocyte function, and that inhibitory LILRs (LILRBs) are dysregulated in haematological malignancies, including AML. We have produced and extensively characterised a unique panel of monoclonal antibodies (mAb) specific for human LILRB3. Using the previously generated reagents and established pre-clinical platforms and our expertise in mAb and chimeric antigen receptor (CAR) T cell therapy, we propose to develop LILRB3-specific CAR-T cells and evaluate their capacity against AML in vivo.

This proposal aims to 1) generate and optimise autologous T cells targeting LILRB3, CD33 and other unique antigens highly expressed on AML cells, and 2) evaluate the efficacy and safety of CAR T cells in humanized AML and patient-derived xenograft mouse models.

In order to study cell surface receptors as potential therapeutic targets:

These will comprise single chain variable fragments (scFvs) derived from antibodies (eg, anti-human LILRB3, CD33) linked to various protein domains to allow expression and signaling within the T cell. This will be used to investigate the differences in response when alternative scFvs are included and the roles of protein domains in the signaling pathways of the cell. The chimeric receptors will be expressed using lentiviral vectors, detailed below, in established cell lines and primary human peripheral blood mononuclear cells. Some will be transferred to immunocompromised NSG mice to look at target cell depletion elicited by the CAR-expressing cells.

Recipient or parental organism

Human peripheral blood mononuclear cells from healthy donors and humanised mice.

Second generation replication incompetent lentiviral plasmids will be used, which include delta 8.9, VSV-G and pAdvantage constructs.

Origin & function

Human CD28

CD28 is a co-stimulatory molecule expressed on the surface of T lymphocytes, NK cells and plasma cells and is required for T cell activation and signaling. The molecule consists of 3 domains; an immunoglobulin extracellular domain, a transmembrane helical domains and a cytoplasmic signaling tail. The extracellular domain binds ligands CD80 and CD86 on target cells whilst the cytoplasmic region interacts with signaling molecules including PI3kinase. CD28 is expressed as homodimer, linked by disulphide bonds. Uniprot ref: P10747.

Human CD137:

CD137, also referred to as 4-1BB in literature, is a member of the tumour necrosis factor receptor (TNFR) superfamily present on lymphocytes and acts as a costimulatory molecule for T cell signaling. It consists of four cysteine rich extracellular domains and a small cytoplasmic tail. A sequence annotation of the translated protein is available in the Uniprot database (reference: Q07011).
Human CD3ζ:
CD3ζ is a protein expressed on T lymphocytes as part of the CD3-TCR (T-cell receptor) complex. It comprises a short extracellular domain, helical transmembrane domain and a signaling cytoplasmic tail encompassing three ITAM domains. These ITAMs interact with signaling molecules in the cell and, once phosphorylated at the tyrosine residues, help to initiate T cell activation. The transmembrane regions allow dimerisation and interaction with the TCR. Uniprot ref: P20963.

Other modifications include the use of leader sequences, transmembrane and hinge regions (eg, human CD8) and CD20 receptor. Depending on the project directions, other co-stimulatory molecules and target genes may be included in the future.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The use of microbiological safety cabinets are standard for all cell culture techniques. Spillages are reduced by good aseptic technique. Spillages are only considered a risk in this project when dealing with Amphotrophic viral media as this poses a low risk threat to humans. The work with this media will be carried out in a containment 2 laboratory and the following procedures adhered to:
Use racks and keep samples capped whenever possible to reduce risk of spillage.
Use Virkon solution to clean up any spillages using paper towels, then clean the area again with Trigene 1:50 and 70% ethanol.
At completion of experiment, wipe area with Trigene then 70% IMS.
All plates of infected cells will be sealed prior to removal from safety cabinet

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

4.7 GMBSC-0283 Generating chimeric antigen receptor leukocytes for immunotherapy of cancer

GMBSC felt the project was borderline Activity Class One or Two and needed clarification as to where the project was taking place.

The Chair stated that if the principal investigator was getting the cells in and not handling the virus then the project would be classed as Activity Class One, if handling the virus then this must be AC2.

Clarification was requested and the risk assessment was approved as AC2.
Project Additional Information

Purposes of the contained use

Bronchial epithelial cells (16HBE14o- cell line) will be grown in a sealed microfluidic device for 5 days (to enable barrier formation), cells will then be apically challenged with respiratory bacterial pathogen S. pneumoniae and left for 1 day to analyse the changes in the epithelial barrier through electrical readouts (magnitude of impedance and phase) and analysis of basolateral collections. This will allow us to better understand the disease pathogenesis of S. pneumoniae.

Recipient or parental organism

Streptococcus pneumoniae strain TIGR-4, Serotype 4, Strain P1672
**Host/vector system**

Green Fluorescent Protein (GFP) construct

**Origin & function**

Streptococcus pneumoniae (TIGR-4, Serotype 4, Strain P1672) originally from a clinical isolate, Primarily from Tim Michell (University of Birmingham) provided to Jeremy Brown (University College London) and being given to University of Southampton by Caroline Weight (University College London).

GFP construct was originally obtained from Jan-Willem Veening (University of Groningen, Netherlands). The Green Fluorescent Protein (GFP) construct was then combined with the S. pneumoniae strain by Caroline Weight (University College London) to form GFP-TIGR4 by inserting GFP into an inactive area of the S.pneumoniae genome (into histone like protein A locus). The GFP insertion is not expected to have increased the risk of the modified strain (GFP-TIGR4) so can be treated in the same way as the wild-type strain (TIGR4).

The GFP construct enables tracking of S. pneumoaniae across the epithelial barrier and increases ease of endpoint analysis.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid and solid waste is submerged in chemical decontaminant solution (4% Virkon) for at least an hour. Virkon’s efficiency has been independently proven highly effective against over 100 strains of viruses in 22 viral families, over 400 strains of bacteria and over 100 strains of fungi, which includes streptococci, where a 1:100 dilution provides bactericidal efficacy. ([https://relyondisfection.com/fileadmin/user_upload/RelyOn_Virkon_UK.pdf](https://relyondisfection.com/fileadmin/user_upload/RelyOn_Virkon_UK.pdf) and [virkon.com/product/biosecurity-products/biosecurity-disinfectants/virkontm-s/proven-chemistry-proven-results/](https://virkon.com/product/biosecurity-products/biosecurity-disinfectants/virkontm-s/proven-chemistry-proven-results/)). This can also be validated by trying to grow organisms using agar plates after decontamination. After chemical decontamination solid waste is incinerated with other decontaminated biological waste.

In addition to this the isolate of S. pneumoniae being used is self inactivating in liquid culture (8 hours) or when grown on agar plates (24 hours), so the risk of infection from waste to workers who collect and transfer waste is very low.

**Is an emergency plan required according to regulation 20?**  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
The University of Southampton Genetic Modification and Biological Safety Committee reviewed the risk assessment on line. The following questions were asked by the Committee:

- Bronchial epithelial cell line 16HBE14O is a genetically modified cell line, could you confirm that the use of this is on an approved risk assessment, and if so please add the risk assessment number to section 1.1.
- Is the Lung on Chip system sealed, and only opened within the class II MSC?
- What is the risk of aerosolised S. pneumoniae being produced during incubation?
- Is the incubator and perfusion system entirely within the MSC?

The questions were answered satisfactorily and the Committee approved the risk assessment as AC2.

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GM Centre Number: 66

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Name

UNIVERSITY OF NOTTINGHAM

Name 2

SCHOOL OF CLINICAL LABORATORY SCIENCES

Department

GENETICS

Campus Estate or Research Centre

Building

Road Name

QUEENS MEDICAL CENTRE

District

Town

NOTTINGHAM

County

NOTTINGHAMSHIRE

Postcode

NG7 2UH

Country

ENGLAND

Tel Number

0115 970 9398

Fax Number

0115 970 9906

E-mail

www.nottingham.ac.uk/genetics/

HSE Division

MIDLANDS

Comments

GM66 Merged with GM470 on 18/02/2005 and all projects Transferred.

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

**Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 66/00.1

Date Ackn'd 03/04/2000

Date Project Ceased 18/02/2005

Withdrawn N

Historical Significant Changes

Project Transferred from GM66 to GM470 on 18/02/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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02/03/2022
CONVERSION OF FIBROBLASTS TO MYOBLASTS USING RETROVIRAL DELIVERED MYO D

Historical Significant Changes
GM66/01.1 Transferred to GM470 on 18/02/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

| L2 | L3 | L4 |

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| L2 | L3 | L4 |

<table>
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<th>Human Clinical Applications</th>
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</table>

| L2 | L3 | L4 |

Project notified under transitional arrangements

**Project Ref 66/04.1**

**Date Ackn'd** 02/09/2004

**CU2 Project Title** INVESTIGATION OF MOLECULAR BASIS OF ASEXUALITY/SEXUALITY IN ASPERGILLUS FUMIGATUS

**Class** 2

**Culture Vol** < 1 Litre

**Consent Granted** Not Applicable

**Date Project Ceased** 18/02/2005

**Withdrawn** N

Tick if notifying a connected programme of work N

02/03/2022

Page 2226 of 1532
The project aims to investigate whether the filamentous fungus Aspergillus fumigatus has the potential to undergo sexual reproduction. Recent genome analysis has revealed the presence of a series of genes involved with sexual reproduction, some of which are known to be expressed. However, the fungus is only known to reproduce by asexual means. Resolving this question is of major significance to understanding the population biology of this species in the wild and related management of the fungus. Insights into the sexual potential of the species might also allow exploitation of the sexual cycle in classical genetic studies to study inheritance of traits such as fungicide sensitivity/resistance or pathogenicity determinants.

There are two broad areas in which recombinant gene technologies will be used.

A) Cloning of putative genes involved in sexual development from A. fumigatus into E. coli hosts and vectors. This comprises genes regulating sexual compatibility, genes involved in sexual pathway signalling and genes involved with fruit body development.

B) Transformation of fungal genes involved in sexual development into fungal hosts. This encompasses:

- Transformation of putative sexual genes from A. fumigatus into class 1 ADCP host strains which lack the ability to undergo sexual reproduction as a result of previous gene disruption. This will allow complementation studies to assay the functionality of the A. fumigatus genes.
- Transformation of known functional sexual genes from class 1 ADCP host strains into A. fumigatus. These genes will be either under the control of native, constitutive or inducible promoters. Such studies may result in enhancement/restoration of sexual development in A. fumigatus, allowing possible exploitation of the sexual cycle.

Such studies have already been performed with sexual and asexual relatives of the plant pathogens Cochliobolus and Bipolaris species in the USA.

Fungal strains. Aspergillus fumigatus is a common environmental species, widespread in soil and in decomposing vegetation. It is not normally a risk to healthy persons. However, it occasionally causes aspergillosis, an invasive disease of the lungs and other organs, upon opportunistic infection of immunocompromised patients. It may also cause opportunistic infections of patients with existing medical disorders. The spores may in addition cause allergic reactions in certain patients. Thus the fungus is classified as ACDP class 2 (a potentially pathogenic microbe). More detail available in risk assessment.

Bacterial strains. The E. coli strains to be used are all Rec A-derivatives such as DH5a, XL1-Blue, SURE and TOP10. These strains are disabled and non-colonising and are equivalent of ACDP category 1 organisms (ie non pathogenic to humans or animals). They have limited survivability in the environment as they require specific nutrients supplements not required by wild-type organisms.

Host/vector system

Fungal vectors. These are pUC and pBR322-derived plasmids that will be maintained in E. coli host before transfer to a fungal host organism. Some plasmids will have
fungal promoters that are either constitutive or induced under certain environmental conditions. Vectors are integrated into the fungal genome and considered non-mobilisable or mobilisation defective.

Bacterial vectors. These are pUC derivatives encoding ampicillin, zeomycin or other antibiotic resistance markers. Considered to be non-mobilisable.

**Origin & function**

Inserts are genes involved with sexual development in filamentous fungi. These may be categorised into three main areas:

a) Genes involved with determination of sexual compatibility. Examples include mating-type (MAT) genes determining sexual identity, pheromone precursor genes involved with production of short chain amino-acid pheromones; pheromone processing genes involved with enzymatic modification of pheromone precursor molecules.

B) Genes involved with sexual signalling pathways. Examples include pheromone receptor genes encoding transmembrane surface receptors, a series of MAP kinase elements involved with signal transduction, and final transcription factors activating sexual development.

C) Genes involved with fruit body development. These include genes encoding hormonal factors regulating hyphal morphogenesis, and transcriptional factors altering developmental pathways.

These genes are not virulence determinants so unlikely to alter the pathogenicity of the host organism. The antigenicity of these proteins is not known. Previous work with similar genes involved with sexual development in fungi has not revealed any risk, so the protein products are considered unlikely to pose a toxic hazard.

**Evaluation of foreseeable effects**

*Aspergillus* is classified as an ACDP class 2 organism (a microbe potentially capable of causing human disease). It is a common environmental fungus associated with decomposition of organic matter in soil and vegetation. It is not normally a risk to healthy persons. However, it occasionally causes aspergillosis, an invasive disease of the lungs and other organs, upon opportunistic infection of immunocompromised patients. It may also cause opportunistic infections of patients with existing medical disorders. The spores may in addition cause allergic reactions in certain patients.

The potentially most hazardous GMMs to be constructed in this work are the transformed strains of the ACDP class 2 fungus *Aspergillus fumigatus*. However, it should be stressed that these will be transformed with genes involved in sexual reproduction, which themselves have no known link to virulence or pathogenicity. They are not involved with toxin production, so unlikely to alter the pathogenicity of the host organism. Also the strains to be transformed will be pre-screened to ensure that they are sensitive to the main antifungals (Amphotericin B and Litraconazole) used in clinical treatments.

The genes under study, those involved with sexual reproduction in ascomycete fungi, are widespread in numerous ascomycete fungi in the wild with the same functional gene families present in many harmless fungi. Thus potential transfer of such genes is not considered a risk, as it is stressed again that the genes under study do not related directly to the pathogenic potential of *A. fumigatus*.

There is the potential hazard that resultant GMM strains of *A. fumigatus* may have enhanced sexual fertility, which may be spread into wild-type strains of *A. fumigatus* through sexual recombination, promoting gene flow within the species. This might conceivably include flow of genes linked to virulence. However, there is no known occurrence of a sexual cycle of *A. fumigatus* in the wild and it is thought that *A. fumigatus* reproduces only by asexual means. Thus, if sex does occur in the wild it is at likely to be of very low occurrence if at all. Therefore there is low risk of spread of genes promoting sexual fertility. The hazard of spread of antibiotic resistance genes, used as selective markers, is thus also considered low risk.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Solid waste and plasticware exposed to GM material will be autoclaved prior to disposal.
Liquid waste and glassware exposed to GM material will be autoclaved prior to disposal/wash up.
Class 2 autoclave disposal facility available in same lab suite.

2% trigene will be used to swab benches and disinfect as required. It has been shown by the manufacturer to be effective for control of filamentous fungus and is used in accordance with their instructions.
Standard procedure for spillage/breach of containment involves absorbing onto paper towels followed by cleaning of area with disinfectant (2% Trigene). Paper towels then sent for autoclave.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<td>L3 L4</td>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Name

UNIVERSITY OF BIRMINGHAM

Name 2

CRC INSTITUTE FOR CANCER STUDIES

Department

CANCER STUDIES

Campus Estate or Research Centre

THE MEDICAL SCHOOL

Building

Road Name

District

Town

BIRMINGHAM

County

MIDLANDS

Postcode

B15 2TT

Country

ENGLAND

Tel Number

0121 414 5251

Fax Number

0121 414 3309

E-mail

Comments

THIS CENTRE MERGED INTO CENTRE 116 - 4/4/06.

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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<td>B15 2TT</td>
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Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

  - Laboratory
  - Animal Unit
  - Growth Room
  - Glass House
  - Large Scale
  - Level 1 (GMMs)
  - Level 2 (GMMs)
  - Level 3 (GMMs)
  - Level 4 (GMMs)
  - Non-microbial

- Other (please specify)  
  - Tick if confidential

02/03/2022
### Bacteriology
- Parasitology
- Transgenic Birds

### Virology
- Transgenic Animals
- Transgenic Fish

### Mycology
- Transgenic Invertebrates
- Transgenic Plants

### Other (please specify below)

---

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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**Project Ref 67/00.3**

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**Date Project Ceased**
- 04/04/2006

**Withdrawn**
- N

**Historical Significant Changes**
- TRANSFERRED TO GM CENTRE 116 - 4/4/06.
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment
Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Large Scale Activities

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Human Clinical Applications

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Project Ref 67/01.1

Date Ackn'd

| 29/05/2001 |

CU2 Project Title

| REPLICATION DEFECTIVE ADENOVIRUS RETARGETED VIA INTEGRINS |

Date Project Ceased

| 04/04/2006 |

Class

| Class 2 |

CultureVol

| < 1 litre |

Class CultureVol

| Class 2 < 1 litre |

Class 2

| not applicable |

Consent Granted

| not applicable |

Project notified under transitional arrangements

| N |

Withdrawn

| N |

Tick if notifying a connected programme of work

| Y |

Historical Significant Changes

| TRANSFERRED TO GM CENTRE 116 - 4/4/06. |

Historical Date of Additional Info

|  |

Significant Change ID

|  |

Date of Significant Change

|  |

Project Additional Information

Purposes of the contained use

| Laboratory - based, preclinical studies developing gene therapy for cancer |

Recipient or parental organism

| Adenovirus; cell lines and primary tumour material |

Host/vector system
E1-deleted, replication-defective adenovirus vectors; probably E3-deleted. The wild-type fibre gene will be substituted with genetically modified derivatives intended to permit binding to integrins (e.g. by insertion of peptide motifs into the HI loop). Binding to the normal receptor (CAR) will initially not be modified, but may be ablated in later vectors. The viruses will be generated from transfected plasmid DNA constructs, and grown, in cells that complement the E1 deficiency, e.g. HEK293 cells.

Origin & function

The vector sequences are derived via plasmid cloning from adenovirus type 5; integrin-binding motifs designed based on known binding motifs. Some cancers, e.g. chronic lymphocytic leukaemia (CLL) have only low levels of the coxsackie and adenovirus receptor CAR, and so adenoviral gene transfer to these is very inefficient. Retargeting the virus by insertion of integrin-binding peptide motifs into the capsid fibre protein is expected to allow more efficient delivery of therapeutic genes to the cancer cells and, if the natural CAR-binding ability is simultaneously removed, may confer some selectivity of gene delivery to the cancer cells. A range of transgenes may be inserted into the E1 region of the viral vectors; the initial risk assessment covers reporter genes (e.g. green fluorescent protein); and the potentially therapeutic genes CD80, 4-1BB ligand, CD40 ligand, and GM-CSF (secreted or membrane-anchored).

Other potentially therapeutic genes may also be inserted under this connected programme of work including e.g. other immunomodulatory genes; prodrug activating enzymes; tumor-suppressor genes; we understand these would not require further notification to HSE if the GMSC agrees on the basis of risk assessments that there is no significant increase in the potential hazard.

Evaluation of foreseeable effects

No significant biological effects expected from expression of reporter genes. The immunomodulatory genes may have potential to induce local inflammatory responses in the event of contamination, and would be expected to enhance immune responses against the infected cells. Systemic or long term adverse effects are unlikely. The retargeting of the vectors is expected to alter the relative efficiency for entering different cell types. There is a theoretical possibility for recombination e.g. with wild type virus, which could generate a replication-competent virus carrying the modified fibre. Containment conditions and working practices will minimise this risk. Such recombination would lead to concomitant deletion of the exogenous transgenes inserted in the E1 region.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid virus containing waste is bagged and placed in a designated metal container which is removed to the autoclave. Liquid virus waste is discarded to bottles which are placed in a designated metal container for autoclaving. All liquid waste is treated by autoclaving before disposal to drain. Disposable solid waste is also autoclaved before removal as "clinical waste" by specialist contractors, with final disposal by incineration. The exceptions are:-

Sharps and pipettes are soaked in 1% Virkon for a minimum of 15 minutes before disposal in an approved sharps box. The disinfectant is discarded down the sink. Glassware used in this virus room is soaked overnight in 1% Virkon before removal for normal tissue culture wash up processing. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above.

Autoclaving achieves effectively 100% kill of GMMs. Manufacturer's data indicates efficacy of Virkon against a variety of adenoviruses at 15 concentration, and also against a number of strains of E.coli at dilutions ranging from 0.125% - 1%. We have demonstrated that 15 minutes exposure to 1% Virkon achieves >4log kill for adenoviral infected cell pellets and >6log kill of adenovirus seed stocks.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The risk assessments were circulated to the committee and discussed with the applicant at a specially convened meeting on 8/5/2001. The committee noted that the potential for homologous recombination with wild type E1 sequences to result in the inadvertent generation of replication-competent, retargeted virus, and the potential hazard of such a virus was discussed. It was noted that the HSE/ACGM Guidance contained an example of a risk assessment for a retargeted, replication competent adenovirus (RCA), which indicated level 3 containment. Following discussion, the GMSC agreed that such recombination was a rare event in complimenting cell lines, and the committee was assured that the safest practicable complimenting cells would be used. Stocks would be monitored for RCA, and any found to be contaminated would be safely inactivated. Working practices to prevent cross-contamination were described. The committee agreed that the work could be safely conducted at containment level 2 and was therefore class 2. Any unexpected results from the work that might affect the assessment of safety, and any accidents involving the viruses will be reported to the committee. Other points of detail were clarified, and the final risk assessments modified in line with these discussions. The work has therefore now been approved by the GMSC.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
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### Project Ref 67/02.1

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<th>Date Project Ceased</th>
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<th>CultureVol</th>
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<th>CultureVolume</th>
<th>Class3-4</th>
<th>Non-GMM</th>
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<tr>
<td>12/02/2002</td>
<td>TRANSIENT EXPRESSION OF EUKARYOTIC CELL SIGNALLING PROTEINS USING REPLICA LATION DEFECTIVE ADENOVIRUS VECTORS</td>
<td>04/04/2006</td>
<td>Class 2</td>
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<td></td>
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<td></td>
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</table>

Tick if notifying a connected programme of work Y

Historical Significant Changes TRANSFERRED TO GM CENTRE 116 - 4/4/06.
**Purpose of the contained use**

Studying the roles and regulation of cell signalling proteins.

**Recipient or parental organism**

Mammalian cell cultures.

**Host/vector system**

E1-deleted, replication-defective adenovirus vectors. The viruses will be generated from transfected plasmid DNA constructs, and grown, in cells that complement the E1 deficiency, e.g. HEK293 cells.

**Origin & function**

The cell signalling proteins to be expressed will be derived from human or other mammalian species. In addition to the wild type forms, constitutively active or dominant negative mutants may be generated. They will have various roles (which are under investigation) in cell signalling pathways. The risk assessments included with this notification relate to Rho family proteins, and phosphoinositol-3 kinase (P13 kinase); as detailed in the risk assessments these are regulators of cell movement and proliferation.

(Risk assessments for other cell signalling proteins expressed in similar adenovirus vectors have been approved by the GMSC as class 1; these include phospholipase D, Arf family (GTPases, PIP kinases). Similar adenovirus vectors encoding other cellular signalling proteins may also be generated and used in this connected programme of work; it is our understanding that these would not require further notification to HSE if the GMSC agrees on the basis of risk assessments that there is no significant increase in the potential hazard.

**Evaluation of foreseeable effects**

The cell signalling proteins that are to be expressed will have a variety of roles in the regulation of various cellular responses, eg inflammation, cell survival, control of apoptosis, proliferation, migration. For proteins of negligible apparent hazard, the viruses are classified as class 1. Viruses categorised as class 2 will express cell signalling proteins that have some association with harmful processes, eg. some may have been associated with a role in oncogenic transformation; however a role in cancer would only be in conjunction with many other oncogenic events, and so the degree of hazard from their transient expression in an E1-deleted adenovirus is only modest.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation applied for

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Autoclaving is preferred means of decontamination (effectively 100% kill). Liquid waste is autoclaved and disposed to drains. Disposable solid waste is also autoclaved before being removed as clinical waste, with final disposal by incineration.

The exceptions to the above are:
"Sharps", including scalpel blades, needles and disposable tips for micropipettors may be decontaminated by drawing up 1% Virkon disinfectant and soaking in Virkon for a minimum of 120 minutes. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above. Virkon has effectively 100% kill rate.

There was discussion regarding the boundary between class 1 and 2 risk assessments, reaching agreement with that shown on the risk assessments. Points of detail have been clarified, and the final risk assessments modified in line with these discussions.

### Project Containment

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<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4</td>
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### Project Ref 67/04.1

<table>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
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<th>Non-GMM</th>
<th>Project notified under transitional arrangements</th>
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<tbody>
<tr>
<td>22/03/2004</td>
<td>EXPRESSION OF HUMAN POLYCOMB PROTEINS OR OTHER TUMOUR ANTIGENS IN VIRAL VECTORS.</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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<td>N</td>
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<tr>
<td>04/04/2006</td>
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</tbody>
</table>

Tick if notifying a connected programme of work Y

Historical Significant Changes

TRANSFERRED TO GM CENTRE 116 - 4/4/06.
## Project Additional Information

### Purposes of the contained use

Identification of human tumour antigens; characterisation of immune response to tumour antigens; possible future investigation of role in cell signalling pathways.

### Recipient or parental organism

Virus vectors are used in order to achieve efficient gene transfer and expression of the tumour antigens in target cells in vitro for immunological assays. In the first instance, replication-deficient adenoviruses (E1-deleted) will be used. Future work may involve other well-established virus vectors, such as vaccinia virus (particularly for immunological assays) or replication-defective retroviruses (signalling studies).

### Host/vector system

**E1-deleted, replication deficient adenovirus.** Virus production in cells that complement E1 deficiency, eg HEK293 cells. Target cells for immune responses include human dendritic cells, fibroblasts, lymphocytes, epithelial cells.

Future work is likely to involve other virus vectors, particularly vaccinia or replication-defective retroviruses.

### Origin & function

The polycomb proteins BM1-1 and EZH2 were identified using the SEREX screening technique, using the serus of patients with hepatocellular carcinoma, and their potential utility as tumour-associated antigens will be further investigated in this project. This family of proteins are transcriptional regulators of haematopoiesis, and have been implicated in growth transformation of cells, hence may play a direct, contributory role in tumour development.

This work is leading to the investigation of other members of the polycomb family, and other tumour-associated antigens, some of which may also be involved in growth-regulatory pathways.

The main interest in this project is recognition of these antigens by the immune system and their potential as targets for cancer immunotherapy. Future work may also investigate their role in cell signalling pathways. Their potential contribution to the multistep process of oncogenesis warrants containment of these viruses at containment level 2.

### Evaluation of foreseeable effects

Stable incorporation of these genes into cells, and subsequent high level or ectopic expression of these proteins may contribute to cellular transformation processes. However, the adenoviruses carrying these constructs do not result in stable, long term expression in infected cells; they are also replication-defective and so unable to
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is treated by autoclaving typically at 130 degrees C for 60 mins, before disposal to drains.
Disposible solid waste which is or may be contaminated with GMMs is also inactivated by autoclaving at 130 degrees C for 30 mins, before removal as "clinical waste" by specialist contractors, with final disposal by incineration.

The exceptions to the above are:
"sharps", including scalpel blades, needles (with or without attached syringes), and disposable plastic tips for micropipettors, may be decontaminated by drawing up 1% Virkon disinfectant, and soaking for a minimum of 10 minutes.
Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above.
Final disposal by incineration.

Expected degree of kill:
Autoclaving achieves effectively 100% kill of all GMMs.
1% Virkon has been validated to give >10 (to the power of 6) - fold reduction in titre.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The GMSC had no adverse comments on this assessment.

Project Containment

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<tbody>
<tr>
<td>L2  Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

This project involves use of recombinant CMV viruses to stimulate T lymphocytes in vitro, from blood of virus carriers. In addition to the current batch of recombinant CMV viruses we may in future seek to undertake work with recombinant CMVs that are deficient for other novel immune evasion genes and/or different combinations of the above described immune evasion genes. Also we may wish to acquire/construct a CMV virus incorporating a non-harmful reporter gene such as green fluorescent protein (GFP).

We do not expect the hazards associated with such viruses to be any greater than the once we currently plan to work with. Prior to embarking on such new studies we would submit a new or modified risk assessment to the GMSC.

**Recipient or parental organism**

- **Host:** MRC5 fibroblasts, U373 cells (Astracytoma cell line that is permissive to CMV), endothelial cells, human fibroblasts.

**Host/vector system**

- **Vector:** Human CMV
Genetic material: 1: Cytomegalovirus genes.

CMV is a lymphotropic/epitheliotropic beta-herpesvirus that is carried as an asymptomatic life-long infection by the majority of individuals in all communities. Control of CMV infection is thought to be due to the potent cellular immune response that is detected in healthy immunocompetent virus carriers. Laboratory strains of CMV such as AD169 and Towne are highly restricted in host cell range, namely fibroblasts. This is believed to be attributed to the loss of genes over passage time in vitro (Bolovan-Fritts C, Wiedeman JA. 2001. Human cytomegalovirus strain Toledo lacks a virus-encoded tropism factor required for infection of aortic endothelial cells. J Infect Dis. 184:1252-61. Sinzger C, Schmidt K, Knapp J, Kahl M, Beck R, Waldman J, Hebart H, Einsele H, Jahn G 1999. Modification of human cytomegalovirus tropism through propagation in vitro is associated with changes in the viral genome. J Gen Virol. 80:2867-77.)

It is a general consensus that recombinant CMVs made from such parental strains will not acquire the potential to replicate in other cell types (Thomas Jones - personal communication).

The following genes are deleted in the recombinant viruses.
RV798 (all of US2 to US11),
RV35 (US6 to US11) and
RV47 (US2 to US3 only)

The functions of each gene product are described.

US2: The US2 gene product is expressed in the early phase of the viral replication cycle. This protein induces rapid degradation of newly synthesized MHC class 1 molecules, reducing the half life from over 6 hours to less than 2 minutes. It appears that US2 proteins bind to MHC class 1 molecules causing the transport of these complexes to the cytoplasm (Jones et al. 1997). After deglycosylation, both US2 proteins and MHC class 1 molecules are degraded. CMV also interferes with MHC class II expression in several ways as described elsewhere (Miller et al. 1998; Phillips et al. 1998; Tomazin et al. 1999).

US3 &US11: The US3 and US11 genes are expressed in the immediate early phase and encode for products that physically associates with MHC class 1-B2m complexes. Whereas the US3 protein causes the retention of MHC class 1 molecules in the ER(Jones et al. 1996), US11 binds to MHC class 1 molecules and directs their translocation to the cytoplasm (Wiertz et al. 1996).

US6: The US6 gene is expressed much later in the delayed or late phase and subsequently remains expressed throughout the virus life cycle. US6 interferes with peptide loading of MHC class 1 molecules in the ER (Ahn et al. 1997). This is achieved by US6 proteins transiently associating with the complex containing TAP, MHC class 1-B2m (and other proteins such as Tapasin and calreticulin). This interaction results in the inhibition of peptide translocation across the ER membrane and efficiently blocks assembly of the trimeric MHC class 1-B2m-peptide complex.

US8: Human cytomegalovirus US8 is a type 1 membrane protein that partially colocalizes with cellular endosomal and lysosomal proteins. Although US8 does not have discernible effects on the processing and cell surface distribution of major histocompatibility complex (MHC) class 1 products, it is demonstrated that US8 binds to MHC class 1 heavy chains in the endoplasmic reticulum (Tirabassi & Pleogh, J Virol 2002 Jul;76(13):6832-5).


The other genes replaced (US4, US7, US9, US10) are not fully characterized with regards to function. They appear to be cytoplasmic proteins and may have some function in immune subversion also (Huber et al. J Virol. 2002 Jun;76(11):5748-58).
The pp65 gene is deleted in the RVAd65 virus. pp65 is a structural protein located in the viral tegument. Pp65 is expressed during the late phase of viral replicative cycle. Pp65 has been demonstrated to have kinase activity and co-immunoprecipitates with a cellular kinase (Plk-1) but is dispensable for virus growth in vitro. Although it has been reported to have immune evasive properties, pp65 itself is the target of a massive immune response (both cellular and antibody mediated). It is believed that parent CMV (strain AD169) preparations contain non-infectious viral particles known as dense bodies which contain a large excess of this protein. This is thought to bias towards stimulation of T cells specific for pp65. Therefore the absence of this gene may allow for other peptides to be presented by MHC class 1 molecules and the T cell reactivities to be detected.

**Evaluation of foreseeable effects**

The genes that have been deleted are involved in either immune subversion or part of the virion structure. The consensus view of prominent CMV virologists is that their absence does not appear to alter the pathogenic capacity of CMV. In fact it is more likely that these viruses will be more immunogenic and provoke a more vigorous response than parent CMV due to the less hindered presentation of a variety of antigens. The absence of pp65 is expected to allow for the presentation of other immunodominant CMV antigens such as IE-1, pp150 and pp50. One possibility to be considered is that an enhanced immune response may also result in greater inflammation at the site of infection. It is envisaged that this will be self-limiting and restricted to the site of infection.

The presence of prokaryotic gene sequences in these recombinant viruses are not believed to increase the pathogenicity or host range of these viruses.

CMV does not immortalise human cells in vivo or in vitro - the removal of immune-subversive genes should render the virus more immunogenic, and not confer transforming properties.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid waste to be treated by autoclaving at 130 degrees C for 60 mins before disposal to drain. Disposable solid waste which is or may be contaminated with GMMs is also inactivated by autoclaving at 130 degrees C for 30 mins, before removal by a specialist company for incineration. Disposable plastic pipettes may also be decontaminated by immersion in Virkon.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**The GMSC suggested that assessment needed to highlight the risk of working with high titre CMV, and describe how at-risk individuals such as pregnant women would be protected. The highlighted sections in the assessment were added to address these points.**
**Project Containment**

**Laboratory Activities**
- L2: Yes
- L3: L4
- L2: L3

**Glass Houses**
- L2: L3
- L4: L2

**Growth Rooms**
- L2: L3
- L4: L2

**Animal Units**
- L2: L3
- L4: L2

**Large Scale Activities**
- L2: L3
- L4: L2

**Human Clinical Applications**
- L2: L3
- L4: L2

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**Project Ref** 67/trans1

- **Date Ackn'd**: 12/11/1993
- **CU2 Project Title**: STUDIES IN CELL SIGNALLING PATHWAYS
- **Class**: Class 2
- **Culture Vol Class 2**: Class 2
- **Culture Volume Class 3-4**: not applicable
- **Non-GMM Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**
- GM67/99.1, GM67/99.2, GM67/00.1 - TRANSFERRED TO GM CENTRE
- 27/01/1999, 21/07/1999, 01/02/2000

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

---

02/03/2022
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 67/trans2

Date Ackn'd 02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment.

**Project Containment**

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**Project Ref**: 67/trans3

**Date Ackn’d**: 12/11/1993

**Project Title**: EPSTEIN BARR VIRUS IMMUNOLOGY AND BIOLOGY

**Date Project Ceased**: 04/04/2006

**Class**: Class 2

**Consent Granted**: not applicable

**Project notified under transitional arrangements**: Y

**Historical Significant Changes**: GM67/00.2, GM67/99.2, GM67/99.1, GM67/95.1 - TRANSFERRED TO GM

**Historical Date of Additional Info**:
- 10/03/2000, 18/06/1999
- 27/01/1999, 19/06/1995

**Significant Change ID**

**Date of Significant Change**: 02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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02/03/2022
**Project Ref:** 67/trans4

**Date Ackn'd:** 12/11/1993  
**CU2 Project Title:** GENE THERAPY STUDY  
**Class:** Class 2  
**Culture Vol Class:** 2  
**Culture Volume Class:** 3-4  
**Non-GMM Consent Granted:** not applicable

**Historical Significant Changes:** GM67/96.1, GM67/99.1, GM67/97.2 - TRANSFERRED TO GM CENTRE 11

**Historical Date of Additional Info:** 17/05/1996, 27/01/1999, 16/04/1997, 4/4/06

**Project notified under transitional arrangements:** Y

**Withdrawn:** N

**Tick if notifying a connected programme of work:** Y

**Date Project Ceased:** 04/04/2006

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**


02/03/2022
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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<th>Large Scale Activities</th>
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Project Ref 67/trans5

Date Ackn'd 12/11/1993
CU2 Project Title ANDENOVIRUS BIOLOGY AND CELL TRANSFORMATION

Class 2
Date Project Ceased
04/04/2006

Non-GMM
Consent Granted
not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work Y

TRANSFERRED TO GM CENTRE 116 - 4/4/06.

Historical Significant Changes
TRANSFERRED TO GM CENTRE 116 - 4/4/06.

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units

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Project Ref 67/trans6

Date Ackn’d: 12/11/1993

CU2 Project Title: EPSTEIN BARR VIRUS, CELLULAR INTERACTIONS

Class: Class 2

Culture Vol: Class 2

Class Volume: Class 3-4

Non-GMM: not applicable

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: Y

Historical Significant Changes: TRANSFERRED TO GM CENTRE 116 - 4/4/06.
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [\textbf{N}]

If yes, tick to confirm that it is attached to this form [\textbf{N}]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [\textbf{N}]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 67/trans7

Date Ackn'd 12/11/1993

Date Project Ceased 04/04/2006

CU2 Project Title STUDIES ON HUMAN PAPILLOMAVIRUS IMMUNOLOGY AND BIOLOGY

Class 2

CultureVolClass2

CultureVolumeClass3-4

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work Y

Historical Significant Changes TRANSFERRED TO GM CENTRE 116 - 4/4/06

Historical Date of Additional Info

Significant Change ID 67/05.1

Date of Significant Change 06/09/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 67/trans8

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form N
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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02/03/2022
Page 2258 of 15326
GM Centre Number: 77

Data Premises Notified (Originally) 17/01/1979

Transferred from 1992 Regs? Y

Transitional Premises Class 3

Data Premises Closed

Transitional Premises Emergency Plan Required? Y

Non-GMMs Y

Withdrawn N

Name

IMPERIAL COLLEGE LONDON

Name 2

Department

SCHOOL OF MEDICINE

Campus Estate or Research Centre

ST MARYS CAMPUS

Building

District

NORFOLK PLACE

Town

LONDON

County

GREATER LONDON

Postcode

W2 1PG

Country

ENGLAND

Tel Number 0207 594 3965

Fax Number 0207 262 6299

E-mail

HSE Division LONDON

Comments

CU3 12/05/2009 - Coin Case 4163011

Date at Which Additional Info Submitted

01/03/2001 11/04/2001

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  

Tick if confidential

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<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
**Please enter comments of the GM safety committee on the risk assessment**

**Project Ref** 107/95.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Date Project Ceased</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<th>Project notified under transitional arrangements</th>
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<td>01/05/2007</td>
<td>GENETIC MANIPULATION OF MYXOVIRUSES</td>
<td></td>
<td>Class 2</td>
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Tick if notifying a connected programme of work: **N**

**Historical Significant Changes**: GM107/00.6, GM107/02.1, - TRANSFERRED FROM GM 107 - 1/5/07 - GM107/02.1a (see above).

**Historical Date of Additional Info**: 15/08/2000, 09/09/02

**Significant Change ID**: 107/02.1a

**Date of Significant Change**: 19/10/2007

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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</tr>
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Animal Units

| L2 | L3 | L4 | L2 |

Large Scale Activities

| L2 | L3 | L4 | L2 |

Human Clinical Applications

| L2 | L3 | L4 | L2 |

Project Ref 217/03.1

Date Ackn'd: 30/03/2004

CU2 Project Title: USE OF LENTIVIRUS VECTORS TO STUDY CELL GROWTH CONTROLS

Class: Class 2

CultureVolClass2: < 1 litre

CultureVolumeClass3-4

Table:

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<td>30/03/2004</td>
<td>USE OF LENTIVIRUS VECTORS TO STUDY CELL GROWTH CONTROLS</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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</table>
Deregulation of cell controls affecting proliferation and apoptosis is a sine qua non of cancer development. This programme of work will use lentivirus vectors to aid analyses of the functions of cellular and viral genes involved in cell cycle regulation and cell survival. The lentivirus vector system will enable the efficient transduction of human and murine cells grown in tissue culture with either cDNAs encoding cellular and viral genes or cassettes encoding short interfering RNAs (siRNA).

Recipient or parental organism

The lentivirus vectors to be used in this programme of work are severely disabled derivatives of human immunodeficiency virus type 1 (HIV-1). A number of genes required for HIV-1 replication and virulence have been deleted from the lentivirus sequences contained in the vector. The virus vectors are propagated as plasmids in E. coli strains. Packaged virus able to infect human, mouse and other animal species are produced by cotransfection of the lentivirus vectors with other plasmids which provide proteins required for packaging in trans. Although the packaged lentivirus is able to transduce efficiently genes cloned in the lentivirus vector plasmid, the virus is totally unable to replicate in these cells. No infectious virus can therefore be produced.

Host/vector system

The standard vectors to be used are based on deleted/mutated HIV-1 genomes, pseudotyped with a vesicular stomatitis virus (VSV) envelope (VSV-G) allowing infection of a broad range of cell types. In some cases these recombinant HIV-1 will encode a green fluorescent protein (GFP) from a bicistronic transcript allowing the identification of infected cells by fluorescence microscopy or flow cytometry. In other cases the vector will encode a gene for drug resistance (e.g., Neomycin or Hygromycin). The delivery systems to be used involve three separate plasmids that are transiently cotransfected into the human 293T cell line to produce pseudotyped, packaged viruses. One plasmid encodes the HIV-1 derived vector, one encodes the VSV-G and one provides functions in trans for packaging. Two very similar systems will be used which only differ in the details of generating infectious virus:

SYSTEM A PLASMIDS
1. pAG131 - the HIV-IRES-GFP expression construct from which env, vif, vpr, vpu genes have been deleted and the tat gene is inactivated by inverting its open reading frame. These deletions and inversion make the resulting recombinant virus completely incapable of replication in the absence of helper proteins encoded by additional plasmids. The AG131 plasmid still encodes HIV-1 Gag and Pol and will include the cDNA of interest or encode the siRNA.
2. pME-VSV-G - this is a plasmid encoding the VSV-G envelope protein for pseudo-typing the lentivirus.
SYSTEM B PLASMIDS
1. pAB286 or pHOX-GFP or pHOX with GFP replaced by the siRNA cassette from pSUPER plus target sequence [see Brummelkamp et al. (2002) Science 196, 550-553.] or a cDNA. These plasmids all encode self-inactivating (SIN) lentivirus with a 400-nucleotide deletion in the 3’ LTR. This deletion abolishes all LTR promoter activity and therefore the vectors are incapable of replication. The HIV-1 Vpr, Vif, Vpu, Nef and Tat genes are also deleted in the vector.
2. pRD1274 (pMD.G). This plasmid encodes the VSV-G envelope for pseudotyping the lentivirus.

Origin & function
The cDNAs carried by the lentivirus vectors will comprise two classes: (1) cellular and viral genes encoding proteins that promote cell proliferation and/or decrease apoptosis - such genes are generally, but not exclusively, oncogenes and (2) cellular and viral genes encoding proteins that inhibit cell proliferation and/or promote apoptosis - such genes are generally, but not exclusively, tumour suppressor genes. The siRNA cassettes will also comprise two classes: (3) siRNAs directed against cellular and viral genes encoding proteins that promote cell proliferation and/or decrease apoptosis and (4) siRNAs directed against cellular and viral genes encoding proteins that inhibit cell proliferation and/or promote apoptosis. These are described in more detail below:

(1) Lentiviruses carrying cellular and viral cDNAs encoding proteins that promote cell proliferation and/or decrease apoptosis.

Only single growth-promoting (for example EBNA-2, EBNA-3C, LMP1, RUNXI, RUNX3, Id2 and B-myb) and anti-apoptotic genes (for example Bcl-2) will be transduced in this programme of work. As cancer development is a multistep process, transduction with such genes will not transform the infected cells. This programme of work will not include viral oncogenes which have multiple oncogenic activities such as the SV40 T antigen, nor will it include clusters of genes such as HPV16 E6-E7. Genes encoding toxins will not be included in this work.

(2) Lentiviruses carrying cellular and viral cDNAs encoding proteins that inhibit cell proliferation and/or promote apoptosis.

These genes, which include tumour suppressor genes (for example p53, ASPP, RB, P130) and pro-apoptotic genes (for example Bax, Bik and Bad), are expected to inhibit cell growth.

(3) Lentiviruses encoding siRNAs directed against cellular and viral genes encoding proteins that promote cell proliferation and/or decrease apoptosis.

Short interfering RNAs (siRNA) are designed to ablate expression of a specific gene target. Ablation of oncogene expression, for example, would in many instances inhibit cell cycling or promote cell death. Expression of this class of siRNA (for example siRNAs directed against EBNA-2, EBNA-3C, LMP1, RUNXI, RUNX3, Id2, iASPP, mdm2, RAS and B-myb), would be equivalent to that outlined in (2) above. That is, cell growth is likely to be inhibited. It is noted that siRNA sequence specificity is very precise, and generally there is insufficient homology between the mRNA target sequences of mouse and human genes for cross-species interference to occur. For example, siRNAs directed against mouse genes are unlikely to affect growth of human cells.

(4) Lentiviruses encoding siRNAs directed against cellular and viral genes encoding proteins that inhibit cell proliferation and/or promote apoptosis.

Expression of siRNAs which ablate tumour suppressor gene function has the potential to promote cell growth. Lentiviruses expressing siRNAs directed against, for example, p53, ASPP, RB and p130 may predispose cells to further oncogenic events, but would not by themselves transform the cell. This programme of work will be restricted to expression of a single siRNA directed against this class of genes, or the expression of multiple siRNAs which affect the same regulatory pathway. Examples of the latter include the p53 pathway (eg ablation of p53 or ASPP would be expected to have similar consequences) and the RB pathway (eg ablation of RB and p16 INK4a is likely to be equivalent).
Evaluation of foreseeable effects

Lentivirus vectors are designed to infect efficiently both actively proliferating and resting cells of many different species and lineages. The two major foreseeable effects are accidental transduction of laboratory personnel and animal species such as mice. In this respect, it should be noted that these vectors are replication-defective and the risk is therefore limited to cells accidentally transduced with the packaged vector. The consequences for animal species are therefore negligible, since even in the extremely unlikely event that mice were exposed to the packaged vector, the virus could not propagate.

Exposure of laboratory personnel to packaged vectors containing growth promoting genes such as oncogenes or anti-apoptotic genes (see (1) above) could result in some growth advantage of transduced cells. Similarly, transduction with lentiviruses encoding siRNAs directed to growth inhibitory genes such as tumour suppressor genes and pro-apoptotic genes (see (4) above) could also result in a growth advantage. It should be noted, however, that the single genes targeted in these studies can not by themselves result in cell transformation, even in highly responsive rodent cells. Targetting of combinations of genes with the potential to cause cell transformation has been specifically excluded from this programme of work. It should also be noted that with the saacale of production to be used, it is extremely unlikely that accidental transduction will occur in stem cells at risk of carcinogenic development.

Accidental exposure of laboratory personnel with packaged vectors carrying genes that inhibit cell proliferation and/or are pro-apoptotic (see (2) above) is very unlikely to have any consequence. Similarly, transduction with lentivirus vectors encoding siRNAs directed to growth promoting genes such as oncogenes and anti-apoptotic genes (see (3) above) would only inhibit the growth of the cells infected. In principle, transduction of stem cells could inhibit their growth and prevent them participating in tissue maintenance. In practice, it is unfeasible that sufficient stem cells would be affected in this way to have any consequences for human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be collected in double autoclave waste bags and subjected to autoclaving under approved conditions. 100% kill is achieved with this procedure.

Liquid waste will be collected in polythene contains and treated for 24 hours with 1% Chloros. This achieves 100% kill.

Small laboratory spills will be treated with 70% ethanol; high concentrations of alcohol effectively kill enveloped lentiviruses (van Engelenburg et al., 2002, J. Hosp. Infect. 51:121-125). Larger spills will be cleaned with 1% Chloros. Contaminated paper towels are treated as solid waste.

Extracts will be made from tissue culture cells for biochemical and cell biological procedures (eg microscopy). The simple act of infection inactivates much of the input virus as it becomes uncoated upon entry into the cell. When making cell extracts, residual virus will be inactivated by detergent treatment (ie 1% SDS or 0.5% NP40). Studies have shown that HIV is very sensitive to inactivation even with non-ionic detergents (Ukkonen et al., 1988 Eur. J Clin. Microbiol. Infect. Dis. 7:518-523). The effectiveness of the detergent treatments will be validated by plating transduced cell extracts on indicator cells and selecting for antibiotic resistance carried by the vector. For flow cytometry and microscopy techniques, the transduced cells will be fixed with 70% ethanol or 4% formaldehyde or 4% paraformaldehyde. These treatments achieve 100% kill.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

02/03/2022
It was unanimously agreed that Containment Level 2 was appropriate in view of the slight theoretical risk to human health of transducing genes designed to alter cellular growth controls. It was noted that other groups working with lentivirus vectors had also designated their work as a Class 2 Activity.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L3 L4 L2 L3 L4</td>
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**Project Ref** 217/03.2

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>30/03/2004</td>
<td>CLONING AND MODIFICATION OF HERPESVIRUS GENOMES IN BACTERIAL ARTIFICIAL CHROMOSOMES AND GENERATION OF BAC-DERIVED VIRUS</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td></td>
<td></td>
<td>not applicable</td>
<td></td>
</tr>
</tbody>
</table>

Withdrawn N Tick if notifying a connected programme of work Y

Historical Significant Changes

Project transferred from GM 217

Project Additional Information

02/03/2022
Purposes of the contained use

The generation of bacterial artificial chromosomes (BAC) containing entire herpesvirus genomes allows the use of powerful bacterial cloning strategies to introduce precise modifications into the virus. These modified herpesvirus BACs can be transfected into mammalian cells to study the impact of these mutations in the context of the entire genome. By stimulating the lytic cycle, infectious virus can also be recovered from these transfected cells.

Recipient or parental organism

Sequences comprising the Epstein-Bar virus (EBV) and herpesvirus saimiri (HSV) A11-S4 genomes will be inserted into BAC vectors, which will then be cloned and propagated in attenuated E. coli strains DH5 and DH10. Modified BAC constructs will be transfected into mammalian cell lines to generate virus stocks. It is envisaged that HEK 293 cells will be used initially to generate EBV stocks. Replication-defective EBV will be propagated in a 293 line containing an integrated full-length EBV genome to provide replication factors in trans. HSV will initially be propagated in either owl monkey kidney cells or marmoset embryonic fibroblasts, in which the virus is spontaneously lytic. In principle, infectious virus can be generated in any mammalian cell line if the replication cycle is stimulated, and other lines may be used to propagate virus as they become available.

Host/vector system

BAC vectors are F-factor-based plasmids which are conjugated-deficient, and are therefore extremely unlikely to transfer to bacteria in the environment. The HVS A11-S4 genome has already been inserted into a BAC vector at the University of Leeds (White et al., J. Gen. Virol., in press). This HSV strain is attenuated and lacks the stpA gene required for oncogenic transformation. Details of the proposed HVS modifications are given in the accompanying risk assessment. EBV sequences to be cloned include the entire wt sequence and derivatives with modifications of immortalisation genes, insertion of non-hazardous marker genes and viral genes as specified in GM217/99.2

Origin & function

The nature of modifications to be introduced to the EBV genome using BACs are broadly the same as those reflected in previously approved protocol GM217/99.2, namely the deletion or alteration of immortalising genes, the insertion of non-hazardous marker genes or sequences and the construction of viruses that conditionally express defined viral genes. Because modifying herpesvirus BACs does not require full viral function, we will also modify or delete viral genes or structural DNA regions involved in virus latency, replication and packaging, and in the co-ordination of the viral transcription programs. The aim of our work with HVS is to identify the viral genes and DNA motifs involved in the latent and lytic replication and packaging functions of HVS, and to develop the virus as a gene delivery system. This will be achieved by 1) deletion (and subsequent complementation) of genes involved in these processes. 2) in situ modification of these genes (eg fusion to GFP or other tags; mutation of putative domains) to dissect their function. 3) Addition of marker genes (eg antibiotic resistance, luciferase) driven by various promoters to identify differences in gene expression between integrated and episomal expression systems. 4) Tagging the viral episome and arrays of Tet operator repeats to allow the visualisation of the episome in live cells. 5) Cloning human and mouse genetic loci to study expression and regulation of mammalian genes in their natural context. Such work will initially comprise analysis of MHC and immunoglobulin gene loci, but may be extended to other gene loci, however, this work will exclude cloning of oncogenic or other potentially harmful genes.

Evaluation of foreseeable effects

Propagation of BAC-transformed attenuated E. coli strains is not expected to have any adverse effect. The BAC plasmids would be exceedingly difficult to transfer to wt E. coli and other bacterial species, and in any case viral genes would not be expected to be expressed in these bacteria. Generation of virus in transfected mammalian cell lines will give rise to recombinant virus that potentially will be able to replicate in certain primate species, including man. Both EBV and HSV are gammaherpesviruses, which have a restricted host range and grow slowly in vivo. Pathogenesis associated with A strains of HVS have not been observed in man, and the A11-S4 strain to be used has been further attenuated by removal of the transforming stpA gene. EBV is normally transmitted through exchange of saliva, and it is therefore unlikely that the virus would be transmissible under Containment Level 2 conditions. As outlined above, in the event that the recombinant EBV or HSV viruses to be used in this project were accidentally transmitted to laboratory workers, the pathogenicity of the viruses would be no greater, indeed probably less, than the wt viruses.

Release of the recombinant viruses into the environment would have no adverse effect, as both infect only primates.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Waste Type</th>
<th>Treatment</th>
</tr>
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<tbody>
<tr>
<td>Solid waste</td>
<td>Collected in double autoclave waste bags and subjected to autoclaving under approved conditions. 100% kill is achieved with this procedure.</td>
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<tr>
<td>Liquid waste</td>
<td>Collected in polythene containers and treated for 24 hours with 1% Chloros. This achieves 100% kill.</td>
</tr>
<tr>
<td>Small laboratory spills</td>
<td>Treated with 70% ethanol, which effectively kills enveloped herpesviruses. Larger spills will be cleaned with 1 Chloros. Contaminated paper towels are treated as solid waste.</td>
</tr>
<tr>
<td>Extracts</td>
<td>Made from tissue culture cells for biochemical and cell biological procedures (eg microscopy). The simple act of infection inactivates much of the input virus as it becomes uncoated upon entry into the cell. Any residual virus will be inactivated by detergent treatment (ie 1% SDS, 0.5% NP40) when extracts are made. The effectiveness of detergent treatments will be validated by plating transduced cell extracts on indicator cells and staining for virus plaques or viral antigens. For flow cytometry or microscopy purposes, virus-transduced cells will be fixed with 70% ethanol, 4% formaldehyde or 4% paraformaldehyde. These treatments achieve 100% kill.</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

It was unanimously agreed that work on manipulating EBV and HVS sequences in BAC vectors in E. coli could proceed at Containment Level 1. Generation of recombinant EBV and HVS viruses by transfection of mammalian cells will be a Class 2 activity. Similarly, infection of mammalian cells in culture with the recombinant viruses will be carried out at Containment Level 2.

Project Containment

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Recombinant vaccinia virus will be grown and used in containment level 2 facilities with class II cabinets.

**Recipient or parental organism**

GMO: Recombinant vaccinia virus WR strain with disrupted TK gene and insertion of either influenza nucleoprotein or a peptide (366-374) from the influenza nucleoprotein.

TK disruption will attenuate pathogenicity of the virus.

Insertion of influenza nucleoprotein or peptide will not have any foreseeable effect.

**Host/vector system**

Recombinant vaccinia will be cultured in vitro in TK143 cells.

**Origin & function**

Influenza nucleoprotein is an RNA binding protein that encapsidates the viral genome. It comes from influenza virus.

The influenza nucleoprotein peptide comprises amino acids 366-374 of the nucleoprotein and has no intended function. It serves as a peptide epitope presented by the MHC class 1 molecule Db. It comes from influenza virus nucleoprotein.

The F5 T cell receptor is a T cell receptor that recognises the influenza nucleoprotein 366-374 peptide presented by the MHC class 1 molecule Db.
### Evaluation of foreseeable effects

Disruption of the vaccinia virus TK gene will be attenuate pathogenicity.

Insertion of the coding sequence for influenza nucleoprotein or the nucleoprotein peptide 366-374 into the vaccinia virus will allow for expression of the protein/peptide within cells of infected mice. This will stimulate a T cell response to the nucleoprotein.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Laboratory.**

- **Solid waste:** Treatment with 1% Virkon for minimum of 10 minutes. 100% kill. This has been validated on the Antec (Virkon) website. The product has been tested to be effective at 1% solution and 10 minutes contact time. The website will be monitored for any changes in specification.

- Solid waste will be placed in sterilin bags and put in specially designated grey boxes within the laboratory or cold room. When these are full, they will be tied using a specially designated yellow tag and sent for autoclaving.

- Solid waste will be autoclaved at 134 degrees centigrade for a minimum of 30 minutes. A 12 point thermocouple test is conducted every 6 months on a simulated worst case worst load to establish that an effective and validated sterilisation temperature and time is achieved in all areas of the load. An internal thermocouple monitors the chamber temperature on every run. The current autoclave program sets temperature of 134 degrees C for 30 minutes and this ensures that all parts of a worst case load will always achieve a validated lethal temperature for the microorganisms mentioned in this assessment.

- **Liquid waste:** Treatment with 1% Virkon for minimum of 1 hour. 100% kill. This has been validated on the Antec (Virkon) website. The product has been tested to be effective at 1% solution and 10 minutes contact time. The website will be monitored for any changes in specification.

**CBS**

- **Mouse bedding and cages:** These will be removed for cleaning and decontamination by CBS staff. Bedding will be autoclaved at 134 degrees C for 30 minutes and disposed of via the clinical waste route. Cages will be autoclaved at 121 degrees for 15 minutes and then wasted for re-use.

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**Is an emergency plan required according to regulation 20?**  

<table>
<thead>
<tr>
<th>N</th>
<th>Y</th>
</tr>
</thead>
</table>

**If yes, tick to confirm that it is attached to this form**  

| Y |

**Tick to confirm that you have attached a risk assessment to this form**  

| Y |

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  

| N |

---

**Please enter comments on the GM safety committee on the risk assessment**
## Project Containment

### Laboratory Activities
- L2: Yes
- L3: No
- L4: Yes

### Glass Houses
- L2: Yes
- L3: Yes
- L4: Yes

### Growth Rooms
- L2: Yes
- L3: No
- L4: No

### Animal Units
- L2: Yes
- L3: Yes
- L4: Yes

### Large Scale Activities
- L2: Yes
- L3: Yes
- L4: Yes

### Human Clinical Applications
- L2: No
- L3: No
- L4: No

### Project Ref 32/95.1

#### Date Ackn'd
11/05/1995

#### CU2 Project Title
TRANSGENIC MICE EXPRESSING WILD TYPE MUTANTS OR VARIENT HUMAN AND ANIMAL PRION PROTEINS

#### Class
Class None

#### Culture Vol Class
- Class 2: Consent Granted
  - Yes
- Class 3-4: Consent Granted
  - Not applicable

#### Project notifiable under transitional arrangements
Yes

#### Withdrawn
No

#### Tick if notifying a connected programme of work
No

#### Historical Significant Changes

#### Historical Date of Additional Info

#### Significant Change ID

#### Date of Significant Change

---

### Project Additional Information

#### Purposes of the contained use

#### Recipient or parental organism

#### Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Glass Houses</th>
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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref  32/trans1

Date Ackn'd  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4

02/03/2022  Page 2275 of 15326
EXPRESSION OF NORMAL AND VARIENT HUMAN PRION PROTEINS IN MURINE ERYTHROLEUKMIA CELLS

Date Project Ceased: 07/01/1994

Consent Granted: yes

Non-GMM Consent Granted: yes

Project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 32/trans2

Date Ackn’d 07/01/1994

CU2 Project Title

EXPRESSION OF NORMAL AND MUTANT HUMAN PRION PROTEINS IN E COLI

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 3

Non-GMM Consent Granted yes

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref: 332/01.1

Date Ackn’d: 31/03/2007

CU2 Project Title: PRODUCTION OF RECOMBINANT HERPESVIRUS EXPRESSING NATIVE PROTEINS LINKED TO A FLUORESCENT MARKER (GREEN FLUORESCENT PROTEIN).

Date Project Ceased: 05/06/2013

Class: Class 2

Culture Vol Class 2: Not Applicable

Culture Volume Class 3-4: Not Applicable

Non-GMM: Not Applicable

Consent Granted: Not Applicable

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

Historical Significant Changes: TRANSFERRED FROM GM 332 ON 31/3/07. Transferred to GM81 on 05/06/2013

Historical Date of Additional Info:

Significant Change ID:

Date of Significant Change:

Project Additional Information

Purposes of the contained use:

Recipient or parental organism:

Host/vector system:

Origin & function:
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 332/07.1

<table>
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<tr>
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<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>14/04/2010</td>
<td>Production of recombinant herpesviruses expressing native proteins linked to a</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td></td>
</tr>
</tbody>
</table>
Purposes of the contained use

Generation of herpes viruses containing natural proteins linked to a marker - Green Fluorescent Protein (GFP). This will allow detection of fusions in living cells and analysis of pathways involved in virus assembly. Use of mutated proteins linked to GFP will further aid understanding of mechanisms involved.

Recipient or parental organism

Routinely used mammalian cell lines, e.g. Vero, COS, etc. all are well characterised and authenticated with a history of safe use.

Host/vector system

HSV-1 strains, e.g. HSV-1 strain 17, Macro Plaque and tsB7.

Origin & function

Native marker genes fused to the marker gene for Green Fluorescent Protein (GFP) which is available commercially. Work involving mutated genes will also be undertaken. GFP has fluorescent activity which enables the location of the fusion protein to be determined. Insertion will be into the natural region of the gene under investigation. Gene sequences of interest are either regulatory, for transcription initiation, or are structural.

Evaluation of foreseeable effects

The inserts display no potential for a pathogenic phenotype. Expression will be identical to that of native viral genes. The recombinant virus will be identical to the parental strain except for expression of the desired candidate gene linked to GFP.

If the modification were to affect the virus it is most likely to disrupt the function for example, by the recombinant protein being to large to permit viral development. No effect on viral susceptibility to anti viral drugs such as acyclovir is envisaged.

The likelihood of an environmental hazard is negligible as herpes virus particles are labile and inactivated by drying.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste (tissues, gloves, plasticware) is autoclaved at 121°C for 15 minutes (as measured in load) to give effectively 100% kill.

Sharps (needles used in gradient harvest) are disinfected by contact with 1% Virkon overnight prior to disposal into a sharps bin. Manufacturers validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

Waste media is inactivated by addition of Virkon tablets to a final concentration of not less than 1% and left overnight. Manufacturers validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

Pipettes are decontaminated by complete submersion in 1% Virkon overnight. Manufacturers validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

If the use of glassware is unavoidable, this is decontaminated by complete submersion in 1% Virkon overnight. Manufacturers validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The assessment has been approved by the Marie Curie Research Institute Biological Safety Committee for work at containment level 2.

Project Containment

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<td>L3 L4</td>
<td>L3 L4</td>
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Please enter comments on the GM safety committee on the risk assessment

Animal Units

<table>
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<th>L3</th>
<th>L4</th>
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Large Scale Activities

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<th>L4</th>
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Human Clinical Applications

<table>
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<tr>
<th>L2</th>
<th>L3</th>
<th>L4</th>
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</table>

Project Ref 426/99.1
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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</table>

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**Project Ref  426/99.2**

**Date Ackn'd**

28/07/1999

**CU2 Project Title**

THE USE OF RETROVIRAL VECTORS TO TRANSDUCE CULTURED CELL LINES AND PRIMARY CELLS

**Class**

Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM**

Consent Granted

not applicable

Project notified under transitional arrangements [Y]

Withdrawn [N]

Tick if notifying a connected programme of work [N]

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
Project Ref 426/99.3

Date Ackn'd 06/12/1999

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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<td>L3 L3 L4 L3</td>
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Project Ref 426/trans1

Date Ackn'd 20/02/2001

CU2 Project Title WORK INVOLVING USE OF RECOMBINANT VACCINA VIRUSES: INSERTION

Class 2

CultureVolClass2 CultureVolumeClass3-4
AND EXPRESSION OF HEPATITIS VIRUS GENES

Non-GMM Consent Granted

not applicable

Tick if notifying a connected programme of work

N

Project notified under transitional arrangements

Y

Withdrawn

N

Historical Significant Changes

GM426/97.1

Historical Date of Additional Info

09/09/1997

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 77/01.1

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<th>CultureVol</th>
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<tr>
<td>29/01/2001</td>
<td>THE DEVELOPMENT OF A RECOMBINANT VIRAL ASSAY TO MEASURE THE DEVELOPMENT OF A DRUG RESISTANCE IN HIV-1 INFECTED INDIVIDUALS</td>
<td>Class 3</td>
<td>40ml</td>
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</table>

Date Project Ceased
07/09/2012

Consent Granted
yes

Project notified under transitional arrangements
N
### Project Additional Information

#### Purposes of the contained use

The resulting recombinant virus will be tested for susceptibility to antiretroviral drugs. This will be used to determine whether RT and protease from the patient isolates demonstrate evidence of anti-retroviral drug resistance.

#### Recipient or parental organism

HIV is a human retrovirus, which is able to integrate into host DNA and persistently produce virus. Infection with HIV leads to severe immunosuppression, ultimately leading to AIDS. Prolonged and sub-optimal therapy has lead to the emergence of viral strains with reduced susceptibility to antiretroviral drugs. The RT and protease genes from infected patients failing therapy will be amplified by PCR and recombined with the pol deleted HXB2 clones.

#### Host/vector system

There are 2 replication deficient clones based on the well characterised lab adapted HIV virus HXB2. Plasmid pHXB2^RT has a 777 base deletion between codons 2-261 of HXB2 RT and is cloned into the pSP64 vector. The pHXB2^Pr similarly has a 297 base deletion between codons 1-99 of protease. Both these clones have been developed by the Department of Virology at the University Hospital in Utrecht in The Netherlands.

#### Origin & function

The deletion clones have been donated by the Department of Virology at the University Hospital in Utrecht in The Netherlands. The HXB2 deletion clones have no function as they are replication incompetent. The patient derived viral inserts encode RT or protease, 2 enzymes required for HIV replication.

#### Evaluation of foreseeable effects

HIV is ACDP Hazard group 3. The recombination experiment has 2 outcomes. If recombination does not occur, the HXB2 clone remains replication deficient. Recombination of the clones with the inserts will result in replication competent infectious HIV. This virus has the biological and pathogenic properties of HXB2, except for RT or protease encoded for by the patient derived insert. As a consequence, the resulting virus may have increased resistance to the drugs mentioned in 6. The presence of resistance mutations may result in a decrease in replicative capacity. There is no evidence that these mutations will result in an increase in viral fitness compared to wild type strains.

In the event of accidental infection of a laboratory worker, therapeutic options may be limited by the presence of resistance mutations present in the recombinant virus. However, in this event there are a number of therapies available which successfully treat patients in the clinic.

The recombinant virus should not pose a serious risk to animals and plants as envelope has not been modified, therefore the virus is only capable of infecting primates.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material including plastics will be inactivated in Basol overnight and then autoclaved on site and disposed of by incineration. Any spillages will be flooded by an excess of Alcide, the degree of kill being 100%

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
The risk assessment for this project has been reviewed and revised to the satisfaction of the GM77 GMSC.

**Project Containment**

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**Project Ref 77/01.2**

- **Date Ackn'd**: 08/03/2001
- **CU2 Project Title**: GENETIC MANIPULATION OF VACCINIA VIRUS AND OTHER CLASS 2 POXVIRUSES
- **Class**: Class 2
- **Culture Volume Class 2**: < 1 litre
- **Non-GMM Consent Granted**: not applicable
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**

- Transferred to GM43 University of Cambridge 28/09/2011

**Project Additional Information**
Purposes of the contained use

Fundamental research in virology and vaccine development.

Recipient or parental organism

The genetically modified viruses will have characteristics either similar to the parent virus, or of reduced virulence.

The expression of foreign genes by vaccinia virus will not increase the hazard posed by the virus and often the insertion of these recombinant viruses is associated with a reduction in virulence because the gene is inserted into a site in the virus genome that affects virulence. The foreign proteins pose no toxic hazard and are designed to promote appropriate immune responses that will be beneficial in preventing or reducing disease caused by infectious disease and cancer.

The deletion of existing vaccinia virus genes will either be neutral or, more often, decrease virus virulence. Removal or genes affecting the ability of the virus to be released from infected cells and spread to other cells is accompanied by a dramatic reduction in virulence. Removal of genes encoding proteins that aid virus escape from the immune system also reduces virus virulence since the virus infection is better recognised and cleared by the host immune response.

Other poxviruses to be used include the orthopoxviruses cowpox and camelpox virus and the yata poxviruses tanapox and yaba-like disease virus. Cowpox virus is similar to, and often confused with, vaccinia virus and poses a similar hazard. Camelpox virus is a less well characterised orthopoxvirus that causes a severe disease in camels but does not cause disease in man. Tanapox virus and yaba-like disease virus are simian viruses that have caused rare zoonoses in man. Such zoonoses usually produce a single lesion that heals without adverse consequence.

Host/vector system

Vaccinia virus is the live vaccine used to eradicate smallpox and has been used widely as an expression vector since 1982. For cowpox virus, yaba-like disease virus and tanapox virus see comments above under section 7. All these viruses will be treated as for vaccinia virus and pose similar or reduced risk to man. All viruses will be housed in our category 2 containment laboratory and will not be released from that laboratory. The genetic manipulation of all these viruses requires construction of plasmids in bacterial vectors (level 1 GM work).

Origin & function

Each genes to be deleted from or inserted into vaccinia virus or other class 2 poxviruses are described in detail in the accompanying document.

In summary, the genes to be deleted from these viruses encode proteins that either enable the virus to be released better from infected cells, or affect interactions with the host immune system. The latter group of proteins interfere with the host response to infection, and the deletion of these genes therefore may reduce virulence and enhance immunogenicity.

Foreign genes to be expressed in these viruses are either marker proteins that enable the virus to be detected in cell culture, such as β-galactosidase or green fluorescent protein, or antigens from other micro-organisms against which an immune response is sought. These latter proteins are not toxic but are important targets for immune responses that help prevent or reduce disease.

Evaluation of foreseeable effects

The foreseeable effects of deleting genes from vaccinia virus and other class 2 poxviruses are to make the virus less fit, less virulent and less able to cause disease and survive in nature. These virus genes selected encode proteins that aid virus dissemination from infected cells or to aid virus escape from the immune system. It follows that deletion of such genes, will reduce virus virulence and fitness. The foreseeable effects of expressing foreign genes in vaccinia virus are to make the virus a candidate vaccine for different infectious diseases or cancers. The foreign proteins to be expressed pose no toxic hazard and the immune responses to them are likely to be beneficial.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material from our virus containment room will be autoclaved, or incubated in 1% (weight/volume) virkon overnight to ensure no infectious virus remains.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The local safety committee reviewed and approved the proposed work at category 2 containment in October 2000.

Project Containment

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Animal Units

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Project Ref 77/01.3

Date Ackn’d 15/03/2001

CU2 Project Title ANALYSIS OF RECOMBINANT RESPIRATORY SYNCYTIAL VIRUS (RRSV) IN AN INFECTION MODEL

Class Class 2  
CultureVolClass2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N
## Project Additional Information

**Purposes of the contained use**

rRSV was synthesized externally (as part of GM558/999). This will be safely transported to Imperial College in sealed approved containers where it will be assessed for its ability to replicate in vitro and in vivo and to elicit an antiviral immune response.

**Recipient or parental organism**

Parental organism is respiratory syncytial virus, laboratory adapted strain A2.

**Host/vector system**

The host is RSV. It does not contain any vector.

**Origin & function**

Recombinant RSV will be provided externally (see ref GM 558/999). Specific mutations were introduced into the RSV genome by site-directed mutagenesis (SDM) in order to either (i) destroy the initiation codon of the full-length G protein (Met 1) so that only secreted form of RSV G is expressed or (ii) destroy the initiation codon of the secreted form of RSV G (Met 48) so that only the anchored form is expressed. These rRSV's will be assessed in an infection model to understand the roles played by the anchored and secreted forms of the RSV attachment (G) protein in immunopathology.

It is not known which glycosylation sites on G protein are critical for infection and it is unclear whether glycosylation contributes to the immunopathology associated with RSV infection. Thus, rRSV was made in which specific N and O glycosylation sites were abolished. Some of these mutants are expected to be non-infectious in vivo and others may be similar to parental A2 strain. The effect of these mutations upon infectivity and immunopathology will be assessed. Assessments of these deletion and substitution RSVs will be carried out in an established mouse infection model at Imperial College.

**Evaluation of foreseeable effects**

Glaxo Wellcome PLC has already notified generation of these recombinant RSVs (see GM 558/999). To date, it has been shown that they have a slower growth rate and are less pathogenic in in vitro cell cultures in comparison to wild-type RSV A2.

RSV is a category II organism. All the mutants generated will have deletion or loss of function mutations resulting in reduced pathogenicity. No insertion of extraneous material will be performed. Thus, the recombinant RSVs generated should also be classified as category II.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

n/a
All cell cultures and infectious material will be disinfected with 1% virkon overnight prior to autoclaving. There will be no viable GMMs remaining after treatment.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment has been evaluated and approved by the GM77 GMSC.

Project Containment

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Project Ref 77/01.4

Date Ackn'd 16/03/2001

CU2 Project Title ANALYSIS OF IMMUNOPATHOLOGICAL EFFECTOR MECHANISMS IN AN INFECTION MODEL

Class 2

Culture Volume Class 2 < 1 litre

Project notified under transitional arrangements  N

Historical Significant Changes

Tick if notifying a connected programme of work  Y

Historical Date of Additional Info

02/03/2022
### Project Additional Information

#### Purposes of the contained use

rVV will be used to prime mice to elicit an anti-viral immune response

#### Recipient or parental organism

Parental organism is vaccinia pox virus (WR strain)

#### Host/vector system

The host is rVV. Vectors are ‘pAB174’ or ‘pVV192.76’ containing individual RSV genes, influenza genes, or bacterial B-galactosidase under the SV40 or other promoter.

#### Origin & function

RVV were a gift from Birmingham, Alabama, USA, and Madrid. RVV will be used to induce an immune response by priming in vivo, using an established mouse infection model at Imperial College.

The inserted gene products are expressed on the infected cell surface and induce an immune response. Subsequent infection with RSV allows us to study the phenotype of the immune response and the factors involved in subsequent observed immunopathology.

#### Evaluation of foreseeable effects

Vaccinia virus is a category II organism. Despite extensive characterisation in vivo and in vitro, none of the inserted genes or gene products are known to alter the pathogenicity of vaccinia virus. The gene product is not incorporated into the vaccinia virion structure, and therefore does not affect the behaviour of the virus except for the effects of disrupting the site of insertion. The inserted genes could reduce the pathogenicity in some host species.

Vaccinia virus infection is almost always self-limiting, no specific treatment is necessary (or available). This is unaffected by the inserted gene product.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cell cultures and infectious material will be disinfected with Virkon (1%) overnight prior to autoclaving (121 degrees celsius for >20 mins). There will be no viable GMOs remaining after treatment (effectively 100% kill).

#### Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y
Project Containment

Laboratory Activities  Glass Houses  Growth Rooms

L2  Yes  L3  L4  L2  L3  L4  L2  L3  L4  L2  L3  L4

Animal Units  Large Scale Activities  Human Clinical Applications

L2  L3  L4  L2  L3  L4  L2  L3  L4  L2  L3  L4

Project Ref  77/01.5

Date Ackn’d  19/03/2001

CU2 Project Title  HUMAN CYTOMEGALOVIRUS GENE REGULATION DURING THE LATENT AND LYTIC INFECTION IN HUMAN CELLS

Date Project Ceased  23/03/2006

Class absorbance not applicable

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2  1-50 litres

Non-GMM  Consent Granted

Not applicable

Historical Significant Changes

Historical Date of Additional Info

Withdrawn  N

Tick if notifying a connected programme of work  Y

Project Additional Information

Purposes of the contained use

The isolation, culture, and study of recombinant human cytomegalovirus strains with lesions in gene regulatory genes, in order to elucidate the important and essential regulatory processes which occur during human cytomegalovirus infection.
Retroviral packaging intended for the generation of human cell lines with altered characteristics, which will serve as complementing hosts for defective human cytomegalovirus strains.

**Recipient or parental organism**

Activity 1: E.coli K12 strains
Activity 2: Human cytomegalovirus, laboratory and clinical strains
Activity 3: Cultured human fibroblast cells, Amphotropic third generation packaging cell line 0NX-A
Activity 4: Cultured human fibroblast cells.

**Host/vector system**

Activity 1: Bacterial artificial chromosomes (BACs), based on the vector pBAC108L
Activity 2: Generation by recombination between human cytomegalovirus and plasmid replacement vectors based on plasmids pGEM, pBluescript or pUC. Otherwise, direct regeneration from transfected BACs (see activity 1).
Activity 3: Amphotropic and Pan-tropic retroviral vectors from third generation packaging cell lines
Activity 4: Amphotropic retroviral vectors from transiently transfected third generation packaging cell lines

**Origin & function**

Activity 1: Human cytomegalovirus, laboratory and clinical strains, complete infectious genome. Intended to allow mutagenesis in bacteria, followed by reconstitution by transfection of permissive human cells in culture.
Activity 2: Selectable marker genes gpt (from Escherichia coli), puromycin acetyl transferase (from Streptomyces alboniger) and Green fluorescent protein (from Aequorea victoria). Intended to facilitate the selection and isolation of human cytomegalovirus strains, where insertion of the selectable marker gene is coupled to genetic change, usually deletion of cytomegalovirus gene regulator genes.
Activity 3: Individual regulatory protein genes from human cytomegalovirus, to be expressed in long term retroviral vector producer cell lines based on third generation amphotropic packaging cell line 0NX-A. Individual regulatory protein genes from human cytomegalovirus, to be expressed over the long term in human cell lines, intended for the complementation and growth of human cytomegalovirus strains unable to synthesise these proteins.
Activity 4: Human cellular proto-oncogenes and known/suspected viral oncogenes from Adenovirus, Human papilloma virus, and Human cytomegalovirus. Intended to generate human fibroblast cell lines which are immortalised or resistant to apoptosis.

**Evaluation of foreseeable effects**

Activity 1: Human cytomegalovirus BACs are infectious DNAs, able to regenerate human cytomegalovirus when introduced into permissive human cells. Foreseeable effects are limited to the accidental introduction of BAC DNA into the tissue of a human handler, either by exposure of damaged skin, or direct injury. The consequences of such an event are indistinguishable from activity 2 below. Survival outside the laboratory of bacteria containing HMCV BACs is not expected, due to the defective host strains used. DNA transfer to a human host via colonisation by BAC-copntaining strains is likewise not expected, as strains used are non-replicating and non-colonising in the human host.
Activity 2: The genetic material to be added to recombinant cytomegalovirus strains is limited to genes involved in the drug selection and tagging of recombinant viruses. All of the proteins concerned have been used as marker genes for recombinant HCMV, and none are known to modify viability or pathogenicity of the virus. Further genetic changes will involve the modification of viral gene regulatory genes, either by deletion or smaller scale modification. The majority of these changes are expected to result in reduced viability of the recombinant strains. None of the changes proposed are expected to increase viability or pathogenicity, but as the whole purpose of the modification is for experiment, such events cannot be 100% excluded. All alterations will initially be made in cell culture adapter strains AD169 and Towne. These strains are known to have significant genomic deletions relative to clinical strains, and are believed to be clinically. The Towne strain has been used as a candidate vaccine strain without any associated problems. Changes will not be introduced into clinical strains of HCMV until the effects of these changes has carefully monitored in laboratory strains, and found not to result in changes which could modify viability or pathogenicity. Therefore, in all cases, the consequences of infection by the recombinant strains generated are expected to equivalently or less severe than infection by wild-type cytomegalovirus strains. Foreseeable effects would result from accidental infection of a human handler in the laboratory, and could extend to secondary human contacts. Infection of a handler would require contaminated tissue injury, or application of infectious material to mucous membranes. Infection by aerosol is considered unlikely, due to the low levels of replication achieved with human cytomegalovirus in cell culture.
Activity 3: For this activity, the vector, rather than the modified target, constitutes the main hazard. Transduction of cells in human handler by vectors designed to express human cytomegalovirus gene regulatory proteins is not expected to be harmful, and defective retroviral vectors cannot replicate once they insert into the host cell. Infection of handlers is nevertheless undesirable. Contaminated tissue injury or application to mucous membranes are the most likely routes of transmission of vector material to human handlers. This hazard will be limited by not using sharps, and the use of appropriate protective clothing, especially gloves.

A secondary hazard is the potential of packaging cell systems to regenerate replication competent retroviruses. These might resemble wild-type retrovirus, or could conceivably incorporate other genetic material, e.g., cytomegalovirus genes. Although the cytomegalovirus gene products in question are not known to be damaging to human health, this situation is nevertheless undesirable from the point of view of potential release of uncharacterised genetically modified viruses. The generation of replication competent retrovirus will be avoided by the adoption of the following procedures.

i) The use of third generation packaging cell lines, using heterologous promoters to drive separate gag-pol and env expression cassettes.
ii) Where possible, the use of transient packaging procedures, which allow only 3 days contact between vector and packaging line, thus greatly reducing the risk of the multiple recombination events required to yield replication competent retrovirus.
iii) Regular screening of retroviral producer cell lines, once established, for the production of replication competent retrovirus on feline FL3 cells.

Activity 4: For this activity, the vector, rather than the modified target, constitutes the main hazard. Transduction of cells in human handler by vectors designed to express oncogenes could result in long-term harm to the handler, although defective retroviral vectors cannot replicate once they insert into the host cell. The oncogenes in question do not act alone to transform human cells - rather they would participate in a multiple stage process. The vectors are therefore not expected to be acutely transforming after accidental infection, but could pre-dispose to longer term carcinogenesis in any cells affected. Contaminated tissue injury or application to mucous membranes are the most likely routes of transmission of vector material to human handlers. This hazard will be limited by not using sharps, and use of appropriate protective clothing, especially gloves.

A secondary hazard is the potential of packaging cell systems to regenerate replication competent retroviruses. These might resemble wild-type retrovirus, or could conceivably incorporate other genetic material, e.g., oncogenes initially retrovirus will be prevented by the adoption of the following procedures:

i) The use of third generation packaging cell lines, using heterologous promoters to drive separate gap-pol and env expression cassettes.
ii) Limitation of the work to the use of transient packaging procedures, which allow only 3 days contact between vector and packaging line, thus greatly reducing the likelihood of the multiple recombination events required to yield replication competent retrovirus.
iii) Screening of packaged vector for replication competent retrovirus on feline FL3 cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Activity 1: Liquid waste - 30 minutes treatment with 5% v/v chloros (freshly diluted), followed by disposal to drains. Solid waste - 20 minutes autoclaving of dry waste at 121 degrees C. Waste transferred to yellow clinical waste bags, and disposed by microwaving and shredding. Both procedures give effectively 100% kill. Chloros disinfectant used according to manufacturer's instructions. Thermologe strips used in autoclave, together with regular performance testing and servicing.

Activities 2, 3 and 4: Liquid waste - 30 minutes treatment with 5% v/v chloros (freshly diluted) inside microbiological safety cabinet, followed by disposal to drains. Solid waste - 30 minutes soaking in 5% chloros solution inside microbiological safety cabinet, followed by 20 minutes autoclaving of dry waste at 121 degrees C. Waste transferred to yellow clinical waste bags, and disposed by microwaving and shredding. Both procedures give effectively 100% kill. Chloros disinfectant used according to manufacturer's instructions. Thermologe strips used in autoclave, together with regular performance testing and servicing.
Proposed containment levels and procedures approved.

### Project Containment

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### Project Ref 77/02.1

- **Date Ackn'd**: 19/03/2002
- **CU2 Project Title**: INVESTIGATION OF BACTERIAL PATHOGENESIS
- **Class**: Class 2
- **Culture Volume Class 2**: < 1 litre
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: N

- **Historical Significant Changes**: transferred to GM 8 on 12/12/2005

- **Date Project Ceased**: 12/12/2005
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
### Project Additional Information

#### Purposes of the contained use

The aim of this project is to 1) construct strains of these bacteria lacking specific genes, 2) establish whether gene inactivation has affected their ability to cause disease, and 3) further characterise the proteins encoded by these virulence genes.

#### Recipient or parental organism

Escherichia coli, Shigella spp., Neisseria meningitidis, Neisseria gonorrhoeae and Haemophilus influenzae are ACDP Hazard Group 2, as they are human pathogens. The organisms will have the equivalent or less potential to cause human disease as the wild-type bacterium. The insertions will interrupt gene function and are likely to attenuate the host.

The disabled E. coli hosts are ACDP Hazard Group 1, as they do not cause human disease.

#### Host/vector system

- **Neisseria meningitidis, Neisseria gonorrhoeae, Haemophilus influenzae (Hosts)**/pMID216 and derivatives (vector)
- **Shigella and E. coli (Host)/pCR2.1, pUC and pET derivatives, pACYC184, pSTM115, pYH204, pMID216 (vectors)**

All these are plasmids which have been used previously with the corresponding microbes. They carry antibiotic resistance markers for agents not usually used to treat human infections with these bacteria.

#### Origin & function

- **Source of genetic material (inserts):** transposons and antibiotic resistance markers.
- **Source of genetic material (vectors):**
  - pCR 2.1 topo, pACYC184, pUC and pET derivatives: commercially available
  - pMID216 from collaborator
  - pYH204, pSTM115: generated in own laboratory

#### Evaluation of foreseeable effects

All the GMMs are ACDP Hazard Group 2 except the disabled E. coli strain.

The modification of the host bacteria is in the form of insertions into the chromosome that inactivate gene function. The insertions carry antibiotic resistance markers that encode for resistance against antibiotics not used for treatment of infections caused by these micro-organisms. These modifications should not pose any increased risk to human health. The environmental survivability of the GMM will be similar to the parental wild-type strains.

For disabled E. coli, the modifications is in the form of a self replicating plasmid which may contain cloned genes from the pathogenic bacteria. The plasmids confer resistance to ampicillin, kanamycin, and erythromycin. There is a wide variety of alternative agents that can be used to treat successfully infections cause by E. coli. The environmental survivability of the GMM will be similar to the parental strain.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Chemical disinfection of GMM waste except N. meningitidis:
1% virkon as per manufacturers instructions, at least 1 hour exposure. Plates and plastic are sterilised by autoclaving. Discard cycle 134 degrees C for 30 min. 100% kill. Machine and cycle validated after each cycle by thermograph; autoclaves thermocouple tested regularly (6 monthly) qualified engineer.

Autoclaving of waste contaminated by Neisseria meningitidis: Discard cycle 134 degrees C for 30 min. 100% kill. Machine and cycle validated after each cycle by thermograph; autoclaves tested regularly qualified engineer.

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Please enter comments on the GM safety committee on the risk assessment:

It was considered that work with disabled E. coli strains could be conducted in Containment Level 1 facilities. However, this work will be done in a Containment Level 2 laboratory along with the work on Neisseria gonorrhoeae, Haemophilus influenzae, Shigella flexneri, and E. coli K1. The importance of prompt reporting any incidents and episodes of diarrhoeal disease among researchers working with Shigella spp. was highlighted.

People working with N. meningitidis must complete a health questionnaire; any persons with suspected immunodeficiency must be seen by the Occupational Health doctor before working with the bacterium. It was recommended that work with N. meningitidis is carried out in a dedicated (Containment Level 3) room. Only individuals who have undergone a locally approved training programme would be allowed to work with live N. meningitidis. All person must be aware of the signs and symptoms of meningococcal infection, and should seek early medical advice should they become unwell, informing medical staff of their potential exposure to N. meningitidis at the work place. All accidents/spillages to be reported promptly to the Occupational Health Department and Department Safety Officer. A list of individuals allowed to work with the meningococcus, details of the clinical presentation of N. meningitidis infection, and guidelines for safe working is to be kept at the entrance to the designated facility at all times.

Project Containment

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

It is intended that this cell line will be used as a cell model to investigate some of the possible effects of virus replication on host gene expression.

**Recipient or parental organism**

The Acs cell line produces infectious HBV virions. However the absence of the correct receptor(s) on the surface of Acs cells means that there is no cycle of re-infection. The HBV DNA is covalently linked to cellular DNA therefore there is little or no chance that it could be mobilised. The HBV genome contained in the Acs cell is an unmodified ‘wild type’ HBV sequence, consequently the virions produced by this cell line are unmodified ‘wild type’ HBV.

**Host/vector system**

Recipient or parental organism

| Acs cell line (HepG2.2.15) |

**Origin & function**

The Acs cell line (Sells, M A., M-L. Chen, and G. Acs. 1987. Proc. Natl. Acad. Sci. USA 84:1005-1009) was made by transfecting HepG2 cells (a stable human hepatoblastoma cell line) with a head-to-tail double stranded DNA dimeric construct of the hepatitis B virus (HBV) genome. The HBV genome is stably integrated into the cellular DNA in Acs cells and the RNAs required for HBV replication are transcribed from this integrated HBV DNA.

**Evaluation of foreseeable effects**

The HBV virions produced by Acs cells could infect unvaccinated humans. HBV can cause liver damage in infected humans and chronic infection with HBV is associated with a significant increase in the risk of liver cancer (probably as a consequence of chronic liver damage and an increase in the turnover of hepatocytes). All procedures involved in the propagation of the Acs cells and the subsequent extraction of RNA will take place in a Class III safety cabinet which is tested every 6 months.
experimenter will wear the appropriate protective gowns and gloves at all times, while handling these cells. The experimenter has been vaccinated against HBV and has been shown to have good antibody levels against HBV (next testing for antibodies should take place in 5 years ie 2007). Since the HBV genome used in Acs cells is an unmodified 'wild type’ sequence it is susceptible to anti-HBV therapies.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste produced during the culturing of the Acs cells will be treated with Virkon to inactivate HBV virions. At least 1 hour in a 1% Virkon solution (ie final concentration of waste liquid plus 10% stock solution of Virkon). The manufacturer of Virkon have demonstrated that it will rapidly inactivate HBV if used under the conditions described above effectively giving 100% killing of HBV.

Plasticware used in the propagation of Acs cells and any cells that remain associated with them will be placed in plastic autoclave bags and subjected to one cycle of autoclaving in the autoclave present in the laboratory which will give 100% kill. Chart recordings will indicate that the correct autoclave cycle has taken place and the autoclave will be maintained and tested as required by the local safety rules. After autoclaving solid waste will be removed by the approved waste disposal contractor for final disposal (clinical waste will not be generated in these experiments).

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
Comments were made on the risk assessment which was submitted to the Genetic Modification Committee (GMC). The risk form was subsequently modified to incorporate these comments. Several typographic errors and absence of ticks in the appropriate boxes were pointed out. Other comment corresponding to particular sections of the completed form were:

3.1.5 (GMC comment) "Possibly should be yes as the cell now expresses a pathogen". (MMcG response) However, the modified cell line (Acs) itself is not pathogenic.

3.1.18 (GMC comment) "Does not explain why his response is no." (MMcG response) The HBV genome used is an unmodified wild type sequence and is susceptible to anti-HBV therapies.

7. (GMC comment) "It should be stated that those working with HBV are vaccinated and that the antibody titre is checked." (MMcG response) This has been added to the risk assessment.

9.2 (GMC comment) "This sharing is not ideal under COSHH as HBV poses a greater threat of persistent contamination an infection risk than HIV." (MMcG response) The agreed protocol for HBV decontamination, stated in the risk assessment (Section 5) should minimise any potential contamination. All personnel using this facility have been successfully vaccinated against HBV.

(GMC comment) "The classification of Class II is appropriate on the basis of the information provided." (MMcG comment) The appropriate entry in the risk assessment form has been made to show this classification.

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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Date Project Ceased</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>17/06/2002</td>
<td>GENETIC TOOL KIT FOR HAEMOPHILUS, ACTINOBACILLUS AND PASTEURELLA (MANNHEIMIA) (HAP) BACTERIA</td>
<td></td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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With withdrawn N Tick if notifying a connected programme of work N
### Project Additional Information

#### Purposes of the contained use

The Haemophilus, Actinobacillus and pasturella (HAP) group contains bacteria that are commensals and/or cause disease in animals or man. Those that cause disease include H. influenzae, H. ducreyi, A. actinomycetemcomitans (man) A. pleuropneumoniae, A.suis, H. parasuis (pigs) A. ligneresei (sheep), H. paragallinarum (chickens) and H. somnus (cattle). With the exception of H. influenzae comparatively little is known about the molecular basis of their pathogenicity and there is an urgent need for safe effective vaccines that, for those that exist, currently compromise bacterins and are of limited use. The genome sequences are available for some of the human pathogens, and selected species of the animal pathogens are in progress. However, it will not be possible to maximally capitalise on the availability of these genomes because of (1) the lack of suitable genetic systems for the construction of defined and random knockout mutants for comparison in surrogate models of infection and (2) the lack of reporter gene technology to facilitate the identification of promoters suitable for use in heterologous expression systems and (3) the need for suitable heterologous expression systems for novel and known vaccine antigens, as these are crucial in extending the range of live vaccines to protect against multiple pathogens. A successful outcome to the project will contribute towards the development of live and sub-unit vaccines for the prevention of the disease caused by HAP bacteria. Thus the aims of this project are to develop (1) genetic systems (2) reporter gene technology and (3) heterologous expression systems for vaccine antigens for the HAP bacteria.

#### Recipient or parental organism

<table>
<thead>
<tr>
<th>Organism</th>
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</thead>
<tbody>
<tr>
<td>Haemophilus spp.</td>
</tr>
<tr>
<td>Actinobacillus spp.</td>
</tr>
<tr>
<td>Pasturella (Mannheimia spp.)</td>
</tr>
<tr>
<td>Escherichia coli (disabled strains)</td>
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</tbody>
</table>

#### Host/vector system

<table>
<thead>
<tr>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmids and derivatives:</td>
</tr>
<tr>
<td>pUC, pACYC, pMBK1</td>
</tr>
<tr>
<td>pYG10, pVE series, pJFF224 (RF1010)</td>
</tr>
<tr>
<td>pMIDG series</td>
</tr>
<tr>
<td>Plasmids containing Tn916, Tn5 and Tn10</td>
</tr>
</tbody>
</table>

#### Origin & function

The source of the host cells will be bacterial culture collections (St Mary's, NCTC, ATCC and individual). The source of the vectors (including those containing non-toxic reporter genes) will be departmental and individual collections, commercial sources or from investigators describing natural HAP vectors. The aims of this project are to develop (1) genetic systems (2) reporter gene technology and (3) heterologous expression systems for vaccine antigens for the HAP bacteria.
The host cells are pathogens or commensals of man or animal. For pathogens gene inactivation is likely to make the strains less virulent or have no effect on their capacity to cause disease. The expression of non-toxic reporter genes or vaccine antigens is unlikely to alter the pathogenicity of the host.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Chemical disinfection of liquid GM waste - a minimum final concentration of 2% Hycolin would be used for all infectious liquid waste. The degree of 'kill' achieved has been determined empirically to be 100%. It will also be monitored on an ongoing basis by plating out random samples to establish whether any viable cells are present. Solid GM waste will be autoclaved at 134°C for 30 min. The degree of 'kill' achieved has been determined empirically to be 100%. It will also be monitored on an ongoing basis by plating out random samples to establish whether any viable cells are present.

Laboratory equipment will be monitored on a regular basis:
1. Safety cabinets are under a regular, 6 monthly, servicing and validation contract
2. Autoclaves are under a 6 monthly servicing and validation contract
3. Equipment in the laboratory is regularly serviced, and staff in the laboratories will monitor whether equipment needs further testing and repairing
4. Safety inspections will be carried out by the Departmental safety office and random external checks will also take place.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The was concern about the centrifugation of organisms, aerosols spread and spill procedures. Assurances were given that the organisms would be centrifuged in appropriate sealed containers and that spills would be decontaminated by standard laboratory procedures. Confirmation that the autoclave was on a six monthly service contract was also given.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>
**Project Ref** 77/03.1

**Date Ackn’d**
05/03/2003

**CU2 Project Title**
IMMUNE MODULATION BY DENDRITIC CELL DERIVED CYTOKINES IN RSV INFECTION AND IN SUBSEQUENT ALLERGIC SENSITISATION; TRANSFECTION OF DENDRITIC CELLS WITH CYTOKINE GENES USING LENTIVIRAL VECTORS.

**Class**
Class 2

**CultureVol**
≤ 1 litre

**Non-GMM**
Consent Granted

**Project notified under transitional arrangements**
N

**Withdrawn**
N

**Tick if notifying a connected programme of work**
N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**
We intend to define the influence of dendritic cells on RSV disease and to explore approaches to immune modulation of disease using dendritic cells. The aim of this project is to transfect murine CD11c+ cells with genes for murine IL-12, IL-10 and IL-4 in vitro to induce overexpression.

**Recipient or parental organism**
An HIV based 2nd generation lentivirus vector with viral genes deleted containing a SFFV promoter driving expression of GFP, murine IL-12, murine IL-10 or murine IL-4 will be generated. Separate batches of E. coli cells will be transformed to express one of the following 3 split plasmids: - vector plasmid (pHR-SIN-CSGW); - packaging plasmid (pCMVR8.91) encoding gag/pol, tat and rev but with deletions of the virulence genes vif, vpu and nef; and - envelope VSV-G plasmid (pMD-G). Plasmids isolated from the respective E. coli cultures will be used to infect 293T human foetal kidney cells, which will assemble the lentiviral vector which is infective, but replication defective. Such replication deficient viruses are not pathogenic in mice (Zuifferery et al. Nat. Biotechnol. 15:871-875). Following isolation of this vector from cell culture supernatant it will be used to transfect isolated murine CD11c+ cells with GFP, murine IL-12, murine IL-10 or murine IL-4. These mouse cells will not produce new virions. The split plasmids which are used in E. coli cannot cause harm to humans. The complete lentiviral vector is able to infect human cells but it cannot replicate. Spread from cell to cell or from individual to individual is not possible! There is a theoretical risk of insertional mutagenesis/overexpression of cellular genes by random integration of the vector and from expression of the inserted murine genes. The main risk is to laboratory personal directly handling the lentivirus. The risk attached to using these modified lentiviruses is minimised by the following measures: protective clothing, gloves, glasses and filter masks will be used, no use of sharps or needles.

**Host/vector system**

02/03/2022
Gene transfer by HIV-based lentiviral vectors will be used. In these vectors viral virulence genes have been deleted and split plasmids are used. The genes of interest will be cloned in our lab and inserted into the vector plasmid. The vector plasmids, a packaging plasmid and an envelope plasmid will then be introduced separately into different E. coli cells each using calcium chloride based transformation techniques. These transformed cells will be used as plasmid stocks. Plasmids will then be extracted by growing transformed E. coli in culture, and then harvested using a commercially available kit (Qiagen). DNA from these separately purified plasmids will then be used to infect 293T human foetal kidney cells, which will produce the lentivirus vector. The vector will then be purified from the culture supernatant and used to transfect CD11c+ murine dendritic cells.

We intend to use the 2nd generation lentiviral vector for the following reasons:

- The vector has been shown to transfect murine DC successfully (pers. communication by M. Collins). Whether a 3rd generation vector would be just as suitable has not been shown.

- 3rd generation lentiviral vectors which split the rev gene onto a separate plasmid tend to have lower titers following assembly in human cell lines. Lower vector titers will result in reduced infection of target cells and will diminish the chance of successful transfection of DC.

This 2nd generation vector never given replication competent recombinant (RCR) virus in Prof. M. Collins laboratory in 3 years of use. In these non-clinical studies extra safety at the risk of poor infection does not seem justified.

Both 2nd and 3rd generation lentiviral vectors are capable of infecting human cells. There are no efficient pseudotypes of HIV with envelopes that infect only mouse cells.

Origin & function

Common disabled E. coli K-12 or B derivatives (Stratagene), modified and attenuated for laboratory use including XL-1blue will be used for transformation experiments.

Inserts (murine IL-4, IL-10 and IL-12) will be generated by PCR in our lab. These DNA fragments will be cloned into a transfer vector (pHL-SIN-CSGW). Replication-defective lentiviral vectors are generated by transient transfection of 293T human foetal kidney cells with three plasmids (the transfer plasmid, a packaging plasmid and an envelope plasmid).

The plasmids pCMVR8.91, pMD-G and pH-SIN-CSGW will be obtained from Professor M. Collins, Immunology and Molecular Pathology, Windeyer Institute, University College London.

293T human foetal kidney cells will be obtained from ATCC.

Evaluation of foreseeable effects

The host strains of E. coli used are laboratory adapted and do not infect humans or propagate in humans. Laboratory adapted E. coli need the optimised conditions of a laboratory to survive. In addition, antibiotic resistance and the recombinant plasmid would quickly be lost in the outside environment if bacteria were to survive. The used strains of E. coli are commonly used for molecular cloning of a range of foreign genes. As far as is known, cloning does not affect the growth or normal physiology of the host. The inserted genes which do not code for a pathogen or a pathogenic determinant (murine interleukins IL-4, IL-10 and IL-12) will not be expressed in E. coli.

Neither lentiviral genes nor murine cytokine genes will overcome disabling mutations in bacteria.

The inserted DNA fragments encode for the murine cytokines IL-4, IL-10 and IL-12. MiL-4 and mL-10 are not active in humans, whereas mL-12 is active in human cells. No adverse effects to human health are to be expected since the murine cytokine genes will not be used in humans or human cells. Only IL-12 could be active in human cells and might stimulate an increased local immune response with inflammation following accidental inoculation. No toxic responses are to be expected and allergic reactions are highly unlikely although theoretically possible. The recombinant murine cytokines could theoretically cause harm if transformed E. coli were to infect wild populations of mice. This event is extremely unlikely since the work involving transformed bacteria is carried out in a modern category 2 laboratory in the absence of mice. Transfer of recombinant genes between mice following adoptive transfer of transfected dendritic cells is not conceivable.
The lentiviral vectors used are mobilisation defective so that even in the event of a wild-type HIV infection (which would provide tat and other deleted genes which are required for infection) the vector and its inserted gene would not be packaged into infective virus. There is therefore no risk of mobilisation of the vectors.

To keep working procedures as safe as possible, safety rules will be maintained. Sealed buckets will be used to prevent aerosol release, and these buckets will be opened in a Microbiological Safety Cabinet only. In addition, protective clothing, gloves, glasses and filter masks will be used as a protection from aerosols. In addition to that, no sharps (needles, blades, surgical instruments etc.) will be used.

Notes not apply.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Contains and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For the disinfection on solid GM waste autoclaving will be used. Quality assurance will be monitored by the use of sterilisation indicator tubes (Fisher), which indicate that the autoclave has reached required temperature. Solid waste is autoclaved, placed into yellow bags, sealed, tagged and taken out of the laboratory as clinical waste.

Virkon (2%) will be used as a chemical disinfectant for liquid GM waste. Therefore, materials will be soaked in Virkon overnight. Liquid disinfected with 2% Virkon is washed with water in excess to drains. Both these methods will lead to 100% killing of any GM organisms.

Autoclaves and safety cabinets are services every 6 months, lab coats are autoclaved before being given to St. Mary's laundry. Surfaces are cleaned by daily swabbing after use by user, centrifuges are cleaned with 5% Decon & Mikrozid on a monthly rotation list together with hoods, incubators, and water baths and solid waste is autoclaved on a daily basis by a laboratory assistant.

At the end of each experiment mouse carcasses will be placed into biohazard bags in CBS. These bags will be sealed and incinerated.

Is an emergency plan required according to regulation 20?  

Yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

Project approved.

Please enter comments on the GM safety committee on the risk assessment

Project Containment
**Project Ref** 77/03.3

**Date Ackn'd** 28/07/2003

**CU2 Project Title** TRANSFECTION OF VARIOUS CELL LINES WITH FULL-LENGTH HEPATITIS B VIRUS PLASMID CONSTRUCTS.

**Class** Class 3

**CultureVolClass2** 10-20ml

**Non-GMM** yes

**Consent Granted**

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Hepatitis B virus (HBV) does not normally infect cells in culture. Transfection of cloned DNA is therefore used to study the biology of the virus at the molecular level. This however is not a very efficient process. Nevertheless, shedding of virus particles is expected in the tissue culture fluid. This shedding does not reach the levels of virus one sees in patient sera.

**Recipient or parental organism**

The plasmid constructs to be used in the transfection experiments will contain a common genetic background of HBV, consisting of longer than genome length HBV sequences. This is necessary as the genome of the virus is circular, and when linearised, some of the open reading frames are disrupted, necessitating duplication of part of the genome. Point mutations seen in patients with chronic infection, after treatment with antiviral agents and following immunisation will be introduced by site directed mutagenesis into the construct, one at a time, or in combination.

**Host/vector system**

The plasmid vector to be used in pBluescript KS 11+. Transcription will be under the control of endogenous HBV promoters. Host cells to be used will include cell-lines of...
hepatocyte or lymphoid origin. All of these are long established laboratory cell-lines.

Origin & function

The HBV DNA cloned in the above vector was obtained from a chronically infected patient. This construct was given to us by Dr Jinlin Hou, China. Following introduction of the necessary point mutations, transfected cells will be used to establish the effect of these mutations on the replication capacity of the resulting viruses, and level of expression of viral proteins. Constructs containing mutations relating to antiviral resistance will be used to compare their replication efficiency to that of the wild-type construct, and to assess efficacy of other antiviral compounds. Finally, vaccine escape constructs will again be used to assess their replication capacity, since the surface antigen encoding region where the observed mutations occur, overlaps with the polymerase gene of the virus, and may have a knock-on effect on its function.

Evaluation of foreseeable effects

HBV infects humans and chimpanzees. Immunised individuals are protected from infection following vaccination and development of antibodies. The use of vaccine escape constructs, may pose a threat if an individual is exposed. The risk is minimised however since HBV is not transmitted by aerosols, and sharps will not be used at all. Six well plates of transfected cell cultures will be transferred from the cabinet to the incubator one at a time.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be autoclaved within the laboratory, before removal. This will be re-bagged and placed in the ante-room to the lab. From there, laboratory staff will transport it to the contractor collection point in sealed bins. The autoclave run will be monitored by means of a chart recorder, and indicator strips attached to the bags.

For disinfection of liquids, solid surfaces and spillages, Virkon at a final concentration of 1% will be used. Contact time will be as detailed in the local rules. Liquid waste will then be poured down the sink. Solid waste will be autoclaved. HBV does not grow in tissue culture and therefore cannot be monitored for its viability. However, Virkon activity against the virus has been assessed by the manufacturer.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee agreed that appropriate training for personnel involved with this project, local rules and SOPs to be drawn up with consulation from the relevant Divisional Safety Officers prior to the commencement of this work. The committee agreed with the control measures and classification given for this work: Class 3 containment level 3.
Deregulation of cell controls affecting proliferation and apoptosis is a common event in cancer development. This programme of work will use amphotropic retrovirus and adenovirus vectors to help study the functions of cellular and viral genes that affect the regulation of cell proliferation and cell survival. These viral vectors will enable the efficient transduction of human and other mammalian cells grown in tissue culture with either cDNAs or siRNA genes.

The viral vectors to be used in this work are disabled derivatives of human adenovirus type 5 or severely disabled derivatives of animal retroviruses which are packaged in helper lines that enable the vector to infect human cells. The virus vectors are initially propagated as plasmids in E.coli. Packaged virus able to infect human and other mammalian species are produced by transfection of the vectors containing inserts into cell lines which provide functions in trans necessary for replication and/or packaging of the virus. Packaged vectors are able to transduce the inserted genes into a wide range of human and other mammalian cells in tissue culture, however, the vectors are unable to replicate in these transduced cells.
### Host/vector system

Adenoviruses will be packaged in cell lines which complement the E1a and E1B defects in the vectors. One example of the packaging lines to be used is QBI-HEK-293A cells, provided by Q-BIOgene (see www.qbiogen.com). The adenovirus vectors lack both the E1 and E3 early regions. Examples of the adenovirus vectors to be used are AdenoVator and AdEasy obtained from Q-BIOgene.

Retroviruses will be packaged in highly transfectable cell lines such as HEK293T cells and NIH3T3 cells. These cells will express gag-pol genes to provide replication and packaging functions. In addition the cells will express amphotropic or polytropic envelope proteins or the VSV-G glycoprotein. These viral receptors will enable the packaged retroviruses to infect humand and other mammalian cells. Examples of the packaging lines to be used are AmoPack-293 and RetroPack PT67 provided by BD BioSciences/Clontech and the Retrovirus Packaging Kit Amphi provided by TaKaRa Biomedicals. A number of animal retrovirus vector systems will be used including MSCV, BD Retro-X Q, LXSN, MMT, pBabe, pZIP (all based on murine Mo-MuLV) and RCAS (based on avian ALV).

### Origin & function

The cDNAs carried down by the adenovirus vectors will represent cellular and viral genes that promote cell proliferation and/or decrease apoptosis - such genes are generally, but not exclusively, oncogenes. These cDNA will also be inserted into the amphotropic retrovirus vectors. Only single growth-promoting (for example EBN-A-2, EBNA-3C, LMP1, RUNX1, RUNX3, Id2 and B-myb) and anti-apoptotic genes (for example Bcl-2) will be transduced in this programme of work. As cancer development is a multistep process, transduction with such genes will not transform the infected cells. This programme of work will not include viral oncogenes that have multiple oncogenic activities such as the SV40 T antigen, nor will it include clusters of oncogenes such as HPV16 E6-E7. Genes encoding toxins will not be included in this work. These vectors will enable efficient transduction of these genes into human and other mammalian cells grown in culture, with the aim of studying their effects on cell growth and survival. In addition, genes encoding tumour suppressor genes and siRNA genes will be inserted into retrovirus vectors. Tumour suppressor genes (for example p53, ASPP, RB, p130) and pro-apoptotic genes (for example Bax, Bik and Bad), are expected to inhibit cell growth. Short interfering RNAs (siRNA) are designed to ablate expression of a specific gene target. The siRNA genes used in this work will be directed against regulators of cell growth and survival (e.g. oncogenes, tumour suppressor genes and pro- and anti-apoptotic genes). siRNA's directed to tumour suppressor genes would be expected to promote cell growth, while those directed to oncogenes would be expected to inhibit cell growth. However, it is extremely unlikely that ablation of a single gene, as proposed in this project, would have major effects on cell growth and/or survival.

### Evaluation of foreseeable effects

Adenovirus and amphotropic vectors are designed to infect efficiently cells of many different species and lineages. The two major foreseeable effects are accidental transduction with the packaged vector. The consequences for animal species are therefore negligible, since even in the extremely unlikely event that mice were exposed to the packaged vector, the virus could not propagate. It is noted that replication-competent retroviruses can be generated at low frequencies in packaging cell lines, albeit it seems likely that acquisition of viral replication functions by the vector would be at the expense of the inserted transgene. The use of these vectors will be restricted to transduction into cells in tissue culture, and the risk that infectious virus will be transmitted to animal species is minimal under Containment Level 2 conditions proposed for this project.

Exposure of laboratory personnel to packaged vectors containing growth promoting genes such as oncogenes or anti-apoptotic genes could result in some growth advantage of transduced cells. Similarly, transduction with retroviruses encoding siRNAs directed to growth inhibitory genes could also result in a growth advantage. It should be noted, however, that the single genes targeted in these studies can not by themselves result in cell transformation, even in highly responsive rodent cells. Targeting of combinations of genes with the potential to caluse cell transformation has been specifically excluded from this programme of work. It should also be noted that with the scale of production to be used, it is extremely unlikely that accidental transduction will occur in stem cells at risk of carcinogenic development. Because of the very slight risk involved, it is proposed to carry out this activity under Containment Level 2 conditions.

It is noted that insertion of certain viral oncogenes (such as HPV 16 and E7 and some EBV genes) into E1a/E1B-deficient adenovirus vectors can complement their growth defect. Experimental observations show that rescue is incomplete (Steinwaerder et al, Mol Ther. 4:211-216, 2001) and replication of the recombinant is significantly less that of wild-type virus. The recombinant virus would therefore be debilitated in its ability to spread in an infected human host. The risk associated with such recombinants is therefore no greater than that of the wild-type virus. It is therefore proposed to carry out this activity under Containment Level 2 conditions.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be collected in double autoclave waste bags and subjected to autoclaving under approved conditions. 100% kill is achieved with this procedure. Liquid waste will be collected in polythene containers and treated for 24 hours with 1% Chloros. This achieves 100% kill. Small laboratory spills will be treated with 2% Virkon (retroviruses can also be effectively treated with 70% ethanol. Studies by the manufacturer have shown that this concentration of Virkon effectively kills a wide range of viruses. Larger spills will be cleaned with 1% Chloros. Contaminated paper towels are treated as solid waste. Extracts will be made from tissue culture cells transduced by adenovirus and retrovirus vectors for biochemical and cell biological procedures (e.g. microscopy). The simple act of infection inactivates much of the input virus as it becomes uncoated upon entry into the cell. When making cell extracts, residual virus will be inactivated by detergent treatment (i.e. 1% SDS or 0.5% NP40). The effectiveness of the detergent treatments will be validated by plating transduced cell extracts on indicator cells and selecting for antibiotic resistance carried by the vector or GFP expression. For flow cytometry and microscopy techniques, the transduced cells will be fixed with 70% ethanol or 4% paraformaldehyde. As this explained, there will be little or no packaged virus in these infected cells, and any remaining virus should be inactivated by these treatments.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

It was agreed by the GM Safety Committee that Containment Level 2 was appropriate in view of the slight theoretical risk to human health of transducing genes designed to alter cellular growth controls. It was noted that ACGM Compendium of Guidance (Part 2B - Annex III recommends Class 2 for these activities with these vectors.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
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<td>L2</td>
<td>L3</td>
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Project Ref  77/07.1
**Project Additional Information**

**Purposes of the contained use**

This project will use a newly established cell culture system to investigate the replication of Hepatitis C Virus (HCV). HCV presents a large and growing health burden with an urgent medical need. At least 70% of infections lead to chronic hepatitis, resulting in severe liver disease including cirrhosis and hepatocellular carcinoma. Current estimates are that 170 million people worldwide are infected with HCV. For patients, the only therapy for HCV is treatment with a combination of interferon-α and ribavirin, which has unpleasant side-effects and is at best effective in only 40-50% of patients.

The development of HCV RNA replicons has allowed major advances to be made in understanding the replication mechanisms of HCV and has aided the discovery of potential new anti-viral drugs. However, as the structural genes are missing, the full virus replication cycle cannot be studied using these replicons. A second important development has been the description, in 2005, of a full-length HCV sequence termed JFH1, which can undergo replication, in Huh7 cells without adaptive mutations. This was important for establishing a complete virus replication cycle system as the presence of cell culture adaptive mutations in other HCV replicons has been shown to interfere with virus particle production. Consequently, transfection of full genome length JFH1 RNA into Huh7 cells leads to the production of virus particles which are then capable themselves of infecting untreated Huh7 cells.

The new HCV cell culture system is based on the JFH1 sequence, which has a 2a genotype background, whereas most other replicons have been based on genotypes 1a or 1b. In addition, replacing the JFH1 2a structural proteins with genotype 1a or 1b structural proteins results in efficient genome replication and virus particle production. These findings raise many important questions about the replication of HCV which will be addressed in this project. For example, what are the properties of the JFH1 non-structural proteins which allows it to replicate much more efficiently than any replicon previously studied? Why is it that this virus can replicate much more efficiently in 1FN cured Huh7 cells compared to untreated Huh7 cells? Is this greater level of virus replication due to increased levels of (co) receptor molecules, or to ineffective dsRNA antiviral responses in these cells (e.g. Huh 7.5 or Huh7- Lunet) or is the JFH1 virus particularly adept at replicating and or evading the cells defenses?

**Recipient or parental organism**

Plasmid DNAs that contain a copy of the HCV genome will be grown in E.Coli DH-5 alpha which does not contain T7 polymerase that is necessary for the transcription of the plasmid insert. There will therefore be no expression of the inserts in E.coli DH5-alpha.
Human hepatoma 7 (Huh7) cells, a cell line derived from human liver, will be used to propagate HCV. Additional cell lines which have been developed from Huh7 derivative cells e.g. Huh7.5 cells (which has a mutation in the RIG-1 protein) and Huh7-Lunet cells (which express increased levels of CD81) will also be used to propagate HCV. These human hepatoma cells can only be grown under standard cell culture conditions e.g. at 37°C in DMEM medium in a CO2 incubator.

Host/vector system

Purified plasmid DNAs (pJFH1, pJFH1/GND, pJFH1/DE1-E2JFHQ, pFK-Luc-JFH1 and pFK-Luc-JFH1/E1-E2) that contain a copy of the HCV genome will be transcribed with T7 polymerase in a cell free to synthesise HCV RNA transcripts. These genome length HCV RNA molecules will be used to transfect tissue culture cells (Huh7, Huh7.5 and Huh7-Lunet cells) to produce HCV virus.

Origin & function

The genomic sequence in pJFH1 was isolated and cloned from HCV virions obtained from an infected Japanese patient i.e it is the same sequence as the virus in the natural infection (Wakita T et al (2005). "Production of infectious hepatitis C virus in tissue culture from a cloned viral genome." Nature Medicine; 11:791-796). Using standard cloning and PCR methods, intergenotypic chimaeric virus sequences will be generated which combine the coding sequences of proteins and sub-domains of proteins from different HCV genotypes in a reciprocal fashion. The HCV genome is expressed as a polyprotein. This is the process to produce the functional HCV proteins. These are: C - virus capsid protein, E1 and E2 - virus membrane glycoproteins, p7 - putative ion channel proteins, NS2 - autoprotease cofactor, NS3 - serine protease/RNA helicase, NS4A - serine protease cofactor, NS4B - membranous web inducing protein, NS5A - interferon response suppression protein and NS5B - RNA dependent RNA polymerase. In the JFH1 2a genome, which produces virus particles, proteins or domains of proteins will be replaced with those from 1a or 1b genotypes. Similarly, in the 1a and 1b genomes, which don't produce virus particles. JFH1 protein sequences will be substituted. These chimaeric HCV viruses will be assessed and compared for their replication efficiency which will identify virus sequences that are important for virus production. Site directed mutagenesis of viral sequences will also be undertaken to further pinpoint regions of interest.

Evaluation of foreseeable effects

HCV will be propagated in Huh7 cell lines and could cause hepatitis if it infected human liver. The levels of HCV replication will be monitored by quantitative PCR as part of the investigation and the PI will receive this information (and all other experimental data generated by this work) immediately after it is available. There will be a re-assessment of the risks by the local GM Safety Committee if, although unlikely, any significant increases (10-20 times the level of JFH1) in HCV replication are found. Changes may alter (probably reduce) the ability of HCV to replicate in Huh7 cells. Changes to host range or tissue tropism of HCV are very unlikely. No alterations will be made to the bacterial hosts (no expression of the HCV insert occurs in these cells). If any changes to the Huh7 cells leads to significant increase in the levels of HCV replication the risks will be also re-assessed.

None of the proteins encoded by HCV are toxins, allergens or carcinogenic. There is no risk of an exchange of genetic material between HCV and other microorganisms as there is no likelihood of recombination.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (HCV cell culture Media) will be treated by the addition of Virkon (an anti-microbial disinfectant), to a final concentration of 1% and then left for 30 mins. According to manufacturers data (http://reylon.dupont.com/mainframe2.htm) this treatment results in 100% killing of HCV. Treated liquid waste will then be disposed of via a sink.

Solid waste in plastic bags (tissue culture flasks, plastic pipettes, pipette tips and eppendorf tubes) will be autoclaved at 134C, for 30 mins. This will result in 100% killing of HCV. A chart record will indicate the successful completion of the autoclave cycle and the autoclave is tested annually by 12 point thermocouple testing. The bagged
The Committee approved the classification. The project is subject to re-assessment as the work changes. The Committee advised the PI to update the risk assessment to include a proviso in the event that a virus is created that replicates much more efficiently, and to state that the project will be re-assessed if such an event occurs. The Committee queried the varying percentages of Virkon specified in the risk assessment and Code of Practice (CoP), explained that the percentage is dependent upon the specific use. The Committee requested that prior to completion, the CoP is read through and matches the risk assessment e.g. personnel list. Recommended that personnel be listed on the laboratory door rather than the CoP. Revised CoP to be sent to Safety Dept and Chair for final approval.

Please enter comments on the GM safety committee on the risk assessment

The Committee approved the classification. The project is subject to re-assessment as the work changes. The Committee advised the PI to update the risk assessment to include a proviso in the event that a virus is created that replicates much more efficiently, and to state that the project will be re-assessed if such an event occurs. The Committee queried the varying percentages of Virkon specified in the risk assessment and Code of Practice (CoP), explained that the percentage is dependent upon the specific use. The Committee requested that prior to completion, the CoP is read through and matches the risk assessment e.g. personnel list. Recommended that personnel be listed on the laboratory door rather than the CoP. Revised CoP to be sent to Safety Dept and Chair for final approval.

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<td>L4 L2</td>
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<tr>
<td>L2</td>
<td>L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 77/07.2

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<th>Date Ack'n'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
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<th>CultureVolume</th>
<th>Class3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>26/06/2007</td>
<td>Immune response to Leishmania infection.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
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</tbody>
</table>

Withdrawn N

Tick if notifying a connected programme of work N
The goal of our project is to understand host-parasite interactions and to dissect the immune mechanisms resulting in resistance or susceptibility to infection with protozan parasites of the species Leishmania.

Parental parasite strains: Leishmania major and Leishmania mexicana.

Modified parasites: L. major DsRed, L. mexicanaDsRed
L. major GFP, L. mexicana GFP
L. major LPG1-/-, L. mexicana LPG1-/- and L. major LPG2-/-, L. mexicana LPG2-/-
L.major lmcDN16-/-

All the transfected or gene deleted Leishmania parasites fall in the hazard group 2 safety category. There are no additional harmful properties associated with the inserted fluorescent labels and the gene deletions do also not represent increased risks.

For parasite modification the following vectors were used:
2) pSSUint: to introduce fluorescent label eGFP (Mol.Bio.Par. 107:271,200)
4) plmcDNA16: to delete HASP proteins (Cell.Microbiol. 3:511,2001)

One aspect of our work focuses on host-parasite interactions and for this we will use genetically modified Leishmania parasites for in vitro experiments. The modified Leishmania we will use fall into 2 groups: One group of Leishmania parasites will be modified by the introduction of fluorescent red or green labels to facilitate the tracking of the parasites in cells. In the other group of Leishmania parasites, genes are be deleted to investigate their role in the parasite life cycle or in virulance as well as in...
host-parasite interactions. All transfected or gene-deleted parasites have already been generated by other groups.

**Evaluation of foreseeable effects**

The products of the inserted genes are fluorescent labels and they are not known to have an impact on the virulance of the parasites for humans. In all likelihood the deletions will attenuate the parasites. In the unlikely event that evidence would be obtained indicating the opposite, the work would be halted pending reassessment. Gene transfer between Leishmania parasites and other microorganisms is unlikely to occur, the parasites are not free living in nature, they only survive in their sandfly vectors, infected mammalian cells or in special culture in vitro. The parasites are not transmitted by aerosols and establishment of infection can only occur as a result of direct introduction of the parasites in the skin and the bloodstream.

The hazard to the environment is low, Leishmania parasites will not survive outside the laboratory. The alterations reduce parasite development and transmission by their vectors and in all likelihood they will attenuate the parasites.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (parasite cell culture liquid) will be collected and treated for at least 10 min with 1 % Virkon before disposal. All solid waste is collected in autoclave bags. When the bags are full they will be put in solid boxes, closed and autoclaved at 134 degree Celcius for a minimum of 30 min. The autoclaved material will be placed in yellow clinical waste bags. An internal thermocouple monitors the chamber temperature on every run and in addition, indicator strips are used. A 12pt thermocouple test is conducted every 12 months to establish that an effective and validated sterilised temperature and time is achieved in all areas of the load.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
Reviewer 1:

I felt that there were essentially two distinct GM components to this project. The class 1 cell line modification that had been carried out by other groups, (but still needed to be included as storage and use is covered by the GMO contained use regs), and the Leishmania parasite modifications which formed the class 2 component. My feeling is that for clarity that should be on two separate assessments.

2.3.3.2. - Room number and type of room e.g. lab, equipment room etc
2.3.3.3d If a tube breakage is suspected before opening the centrifuge lid wait at least 30 mins for any aerosol to settle
2.3.4.2 The COP dates look a bit old, have they been reviewed recently?
2.3.6 CL2 and class 2 for the Leishmania - CL1 and class one for the cell lines.
3.1b - check if the 12 pt TC test is actually carried out every 6 months. If this is Estates maintained the records I yhave seen indicate it is usually every 12 months - which is fine
3.2b - is the autoclave in the lab?

Reviewer 2:
The categorisation is correct.

Reviewer 3:
My only comment is a suggestion. In part 2, the deletions do not increase virulence, they decrease it. It would be a good idea to cite the evidence for that. Had this only been supposition, I would have suggested a statement something like: "In all likelihood the deletions will attenuate the parasites. In the unlikely event that evidence was obtained indicating the opposite, the work would be halted pending reassessment".

Agreed: Class 2, Containment level 2

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**Project Containment**

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<td>L4</td>
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**Project Ref 77/07.3**

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<th>CU2 Project Title</th>
<th>Class</th>
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<tr>
<td>27/09/2007</td>
<td>Characterisation and intervention of human noro- and sapovirus translation and replication.</td>
<td>Class 2</td>
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The overall aim of the project is to understand the molecular mechanism of human norovirus and sapovirus genome translation and replication. Noroviruses and sapoviruses are members of the Caliciviridae family of positive stranded RNA viruses and the major cause of viral gastroenteritis in humans. Until very recently, methods for growing human noro- and sapoviruses in tissue culture were unavailable. A recent advance in the field now suggests that noroviruses replicate in a differentiated small intestinal cell line and that cell lines containing norovirus replicons can be generated at low frequency. We now wish to use these systems to characterise cellular and viral factors required for virus translation and replication.

Specifically we aim to:
1) Generate an efficient reverse genetics system to allow the recovery of genetically defined human noroviruses and sapoviruses in tissue culture
2) Examine the effect of specific mutations in viral RNA structures and viral proteins on virus replication in tissue culture
3) Generate genetically tagged human norovirus and sapoviruses containing innocuous biochemical markers such as green fluorescent protein, luciferase, antibiotic selection markers as well as affinity purification tags such as biotin acceptor sequences, TAP, HA, FLAG etc
4) Examine the effect of small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs) and RNA aptamers on human norovirus and sapovirus replication

Recipient or parental organism

- Human noroviruses & sapoviruses
e.g. MD 145 and sapovirus, as well as various other strains which may be amplified from norovirus or sapovirus positive stool samples. Human noroviruses and sapoviruses are a major cause of viral acute gastroenteritis, the symptoms of which include nausea, vomiting and diarrhoea. Virus infection is usually self limiting and readily resolves within 24-72 hours after the onset of symptoms, however approximately 25% of infected people can continue to shed virus for up to 3 weeks. Infection is rarely fatal (<0.1% fatality rate) and generally only occurs in the elderly or individuals with underlying medical conditions. Immunity to infection is short lived (<12 months). Infection is highly contagious but acute and self-limiting. The most hazardous GMM to be generated will be a wild type norovims or sapovirus. None of the additional sequences to be inserted are considered hazardous and are unlikely to impart any additional pathogenic properties to the virus. This risk assessment excludes the generation of viruses in which the tropism or virulence is likely to be greater than that of the wild type virus
- E.coli strains:
DHSalpha (and derivatives), JM1 09, NEB Turbo, BL2 1 (and derivatives), XL 10, Mach 1
- Mammalian Cells
TNT 407, 293, CHO, HT29, Caco, HeLa (and derivatives), BEK, CRFK, Cos, RAW264.7
- MVA-T7
MVA is a highly attenuated form of VACV that was used during the latter stages of the smallpox eradication campaign without adverse reaction. MVA replicates well in some avian cell lines such as chick embryo fibroblasts but is unable to replicate in most mammalian cell types and consequently is severely attenuated in mammals.
- FPV-T7
FPV-T7 is a highly species restricted avian pox virus, replicating only in primary chick embryo fibroblasts and incapable of replicating in mammalian cells and consequently is severely attenuated in mammals.
All plasmid vectors to be utilised are considered non-mobilisable:
Expression in mammalian cells: pCDNA3, pTriEXl.l, pSportl, The following plasmids will be used for episomal maintenance of RNA and protein expression constructs:
pHebo:Super - On P containing plasniid containing Pol lit promoter pMep & pCEP — Ori P containing plasmids also expressing the EBV EBNA 1 protein

Origin & function

1) Green fluorescent protein, including all derivatives will be used to monitor virus infection and localisation
2) Luciferase and derivatives will be used to monitor virus replication
3) Norovirus and sapovirus non-structural proteins. Individual regions coding for noro and sapovirus analogues of the picomavirus proteins (Norovirus nomenclature in brackets): 2A (NS1), 2B (NS2), 2C (NS3), 3A (NS4), 3B (NS5), 3C (NS6) and 3D (NS7). Possible biological properties:
a.2A (NS 1): no biological activity ascribed as yet,
b. 2B (NS2): no biological activity ascribed as yet, probably membrane bound protein involved in virus replication c.2C (NS3): Probable NTPase/Helicase, membrane anchored;
d.3A (NS4): no biological activity ascribed yet, probably membrane anchored.
e.3B (NS5): Also known as VPg. Covalently linked to the 5' end of the viral RNA. Interacts with translation initiation factors and the viral polymerase 3D. Likely to function as a peptide primer for genome replication.
3C (NS6): Chymotrypsin-like serine protease
3D (NS7): RNA-dependent RNA polymerase
4) Norovirus and sapovirus structural proteins. The major and minor capsid proteins of human noroviruses and sapoviruses. a, VP1: Major capsid protein involved in receptor binding - no other biological activity ascribed as yet
b. VP2: Minor capsid protein. Highly basic protein, probably involved in RNA binding but no biological role ascribed as yet
5) Antibiotic selection markers for puramycin, G418, Zeocin, blasticidin and hygromycin
6) shENA sequences to host cell nucleic acid binding proteins such as PCBP, NFAR, PABP, ILF3, PTB, ITAF45 will be used to inhibit the expression levels of the relevant proteins and examine their role in the virus life cycle.
7) RNA aptamer sequences directed towards various norovirus and sapovirus proteins will be used as potential method of inhibiting virus replication.
8) Affinity purification and epitope tags such as HA, FLAG, biotin acceptor motif, TAP etc. will be used to piirifS’ viral proteins and any associated factors from infected or transfected cells.

Evaluation of foreseeable effects

None of the genetic alterations to be generated are likely to increase the pathogenicity of the viruses. In cases where point mutations will be introduced into the viral genome, the majority of these will be designed to disrupt RNA:protein or protein:protein interactions required for virus replication, effectively attenuating the virus.
None of the additional biochemical tags or genetic markers to be introduced into the viral genome will increase the pathogenicity of the virus in the natural host.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste e.g. liquids containing GM viruses or E.coli cultures:
Treated with >1% final concentration of Virkon S0minutes at room temperature. 100% effective kill rate as specified by manufacturer (http://www.antechh.com/virkonpd.html). Treated waste will then be disposed of via the sink.
Solids e.g. contaminated plastics, sharps and glassware:
Waste will be autoclaved using a cycle of 121 deg C, 2 bar, 20 minutes. Treatment is monitored via a chart recorder attached to the autoclave and validated by an annual 12 point thermocouple test. Once autoclaved the waste will join the hospital clinical waste.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Following the discussion of your proposals entitled "Characterisation and intervention of human noro- and sapovirus translation and replication", (GMIC —01599.1) via virtual Committee, they request that the following amendments are made to the proposals prior to approval:

- Section 1.25. The Norvirus and sapovirus structural proteins should be listed.
- 3.1.1. The chemical inactivation with Virkon should state 1% final concentration.
- Correct few spelling errors.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

Project Containment

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Project Ref 77/08.1

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<td>24/02/2008</td>
<td>The use of lentiviral vectors to study poxvirus proteins and their cellular orthologues.</td>
<td>Class 2 1-50 Litres</td>
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Purposes of the contained use

This programme of work will use lentivirus vectors to aid analyses of the functions of viral proteins involved in poxvirus entry, morphogenesis and immune modulation as well as human orthologues of these proteins. The lentivirus vector system will enable the efficient infection of cells grown in tissue culture with lentiviruses expressing cellular and viral proteins.

Recipient or parental organism

Lentiviruses will be produced by transfection of the SV40 transformed human embryonic kidney cell line HEK 293T. Human and rodent cells grown in tissue culture will be the recipients of the lentivirus GMOs. These cells will include (but are not restricted to) BSC-i, HeLa, and TK-143 cells and other human or rodent (e.g. NIH 3T3) cell lines supplied by ATCC. In addition, some primary cells will be used including rat embryo fibroblasts. After trypsinisation these cells will express the transgene but will not produce or contain any lentiviruses and so are uninfectious.

Host/vector system

The vectors are based on mutated lentivirus genomes, pseudotyped with a vesicular stomatitis virus (VSV) envelope glycoprotein (VSV-G) allowing infection of a broad range of cell types and containing a gene for puromycin resistance to allow the selection of expressing cells. The vectors are pCMVR8.91 (encodes the lentivirus genes), pMD (encodes VSV-G), pdlNotl'MCS'R'Pk and pdlNotl'nPk'MCSR, (each contain a multiple cloning site to allow cloning of gene of interest fused to a N- or C-terminal V5 epitope tag respectively, and a puromycin resistance gene).

Origin & function

1. Poxvirus proteins involved in entry and or morphogenesis
   A33, A34, A36, A56, 85, F12, F13, E2 and K2 each have a role in the entry or exit of vaccinia virus. Each will be cloned into the lentiviral vector.
2. Poxvirus immunomodulators
   K7, B14, Ni, A46, A52, A49, C6, A41, A44, B15, B18, and v-GAAP are all non-essential for virus replication in vitro but have roles in altering the host response to infection in vivo. Each of these will be cloned separately into the lentivirus vector. In addition, uncharacterised poxvirus proteins, which have a putative immunomodulatory role, may be expressed in this way in the future.
3. Cellular orthologues of poxvirus proteins
   Many poxvirus proteins have cellular orthologues, the function of some of which is not yet fully understood, and each of these may be cloned into the lentivirus vector. This includes the newly characterised human Golgi anti-apoptotic protein (h-GAAP), which shows 73% amino acid identity to the vaccinia virus v-GAAP (Gubser et al., 2007. PLoS Pathogens 3:e17).

Evaluation of foreseeable effects

Each lentivirus vector produced will be able to infect cells, but is not able to replicate in those cells and so cannot spread to new cells. These vectors are therefore classified as replication-defective. The nature of the foreign proteins expressed by these vectors is not likely to affect the hazard group of the parent vector and will only be expressed from the infected cell.
Lentivirus vectors provide an efficient means to transduce genes into human cells. In theory, these genes can be transduced into laboratory personnel working with the packaged viruses, however, the virus is replication-defective, and so an ongoing infection with these viruses is not possible. The ability of h-GAAP to block apoptosis means that any cells transduced with h-GAP? would be resistant to cell death, which would in theory increase the chance of those cells becoming cancerous. Because the lentivirus is replication-defective, there is a very low probability that infection will occur in stem cells with the potential to become cancerous. This programme of work will not include viral oncogenes which have multiple oncogenic activities such as the SV40 T antigen, nor will it include clusters of genes such as HPV1 6 E6-E7. Genes encoding toxins will not be included in this work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste
Treated with 1% final concentration of Virkon disinfectant for a minumum of 30minutes at room temperature. 100% effective kill rate as specified by manufacturer (http://www.antechh.com/virkongd.html) Treated waste will then be disposed of via the sink.

Solid Waste
Waste will be autoclaved using a cycle of 121 deg C, 20 minutes. Treatment is monitored via a chart recorder attached to the autoclave and validated by an annual 12 point thermocouple test. Once autoclaved the waste will join the hospital clinical waste.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The use of lentiviral vectors to study poxvirus proteins and their cellular orthologues, Virology, Investigative Sciences, St Mary’s-
Following the discussion of your proposal the following amendments are requested prior to approval:
1-25: Remove the sentence; “Any other viral gene subsequently implicated in morphogenesis may also be included.” The Committee recommend that further genes be considered on a case by case basis in new (Form C, if appropriate) submissions - - .
1.3: The sentence; The ability of h-GMP to block apoptosis indicates that is may be a proto-oncogene and contribute to cancer” is too broad and needs clarification.
3.8: Tick declaration box.
Agreed: Class 2, Containment Level I IC reference number: GMIC-01698.1

Project Containment
The aim of the project is to identify the molecular mechanisms that contribute to the pathogenicity of the human T-lymphotropic virus type 1 (HTLV-1), in particular during the formation of the virological synapse (VS), and the mechanisms by which HTLV-1 spreads between T-cells. The objective of the present experiments is to test the effects of certain HTLV-1 genes on the formation and function of the VS by examining the consequences of mutations in the HTLV-1 genome.

We plan to use an infections molecular clone of HTLV-1, designated ACH, that is easily manipulated in vitro and ex vitro, and deletion mutants of this clone. The biological activity of clone ACH has been characterized in various cell lines (Kimata JT, Virology, 1994; 204(2): 656-64). The purpose of these experiments is to express the HTLV-1 molecular clone, full length or deleted of certain regions coding for specific viral proteins, to test their effect on the formation of the virological synapse and on transmission of the virions between T-cells. The altered HTLV-1 genes encode small regulatory proteins of the virus. In some cases we may include a reporter gene such as GFP, luciferase, or a short antigen tag (“flag”), or similar additional mutations which are unlikely to increase the risk to human health or the environment.

We are also planning to introduce a biarsenical labelling system in the HTLV-1 full length molecular clone. This system is based on the insertion of a small tetracysteine
(TC) motif into a protein of interest. Cells expressing the TC-tagged protein are treated with a membrane-permeable biarsenical dye [e.g. green (FIAsH) or red (ReAsH)] that fluoresces upon binding to the TC tag (Gousset K., PLoS Pathog. 2008 Mar;4(3):e1000015). This will allow us to visualise by real time fluorescence the newly formed virions without altering their intrinsic properties (virulence and infectivity).

The precise molecular effects of each of the HTLV-1 genes to be deleted or mutated are partially characterised; all the available evidence suggests that the genetic changes in HTLV-1 will severely impair or abrogate the replication and/or transmission of HTLV-1. This will be kept under continual review: if any unanticipated effects are noted from the published data, work will be halted until an assessment has been made of the risk and approval obtained from the GM committee.

Recipient or parental organism

The plasmids will be grown in E. coli K12. We will be using the following six types of construct:

1-Full length proviral clone of HTLV-1, designed ACH (Kimata DT, Virology. 1994; 204(2): 656-64).

2-The mutant Delta p30 protein is a small accessory protein. There is some evidence that it regulates the transcription of HTLV-1 Tax protein at the post-transcriptional level, but its complete actions and its main physiological role are unknown (Nicot C. et al., Nat Med. 2004; 10(2): 197-201).

3-The mutant Delta p12. HTLV-1 pt12 protein down-regulates MHC class 1 on the surface of the HTLV-1 infected cell, and so may contribute to immune escape by the virus (Fukumoto R. et al., Blood, 2008; Epub ahead of print).

4-The mutant Delta HBZ. HBZ appears to act both at the RNA level and the protein level. There is evidence that it promotes HTLV-1 persistence by driving proliferation of the HTLV-1 infected T-Cell; it may also inhibit HTLV-1 proviral expression (Barbeau B. and Mesnard JM. Int Rev Immunol. 2007; 26(5-6):283-304).

5-The mutant Delta p13. The small regulatory protein of HLV-1, p13, is targeted to mitochondria and disrupts mitochondrial morphology. As in the case of other regulatory proteins, the main physiological role of this protein is still under investigation Silic-Benussi M. et al., Proc Natl Acad Sci U S A. 2004 April 27;101(17):6629-34).

6-Insertion of a small tetracysteine motif (TC) in the HTLV-1 full length molecular clone. Cells expressing the TC-tagged protein are treated with a membrane-permeable biarsenical dye [e.g. green (FIAsH) or red (ReAsH)] that fluoresces upon binding to the TC tag (Gousset K, PLoS Pathog. 2008 Mar 7;4(3):e1000015). This will allow us to visualize by real time fluorescence the newly formed virions without altering their intrinsic properties (virulence and infectivity).

Host/vector system

The vector system is full length proviral clone of HTLV-1, designated ACH. The E. coli K12 competent cells will be used to amplify the plasmids.

Only in vitro cell lines (Jurkat, BHK21, HeLa, 293T) will be used as hosts for these constructs; no other intact organism.

Origin & function

The precise molecular effects of each of the HTLV-1 genes to be deleted or mutated are partially characterized; all the available evidence suggests that the genetic changes in HTLV-1 will severely impair or abrogate the replication and/or transmission of HTLV-1.

Evaluation of foreseeable effects

The precise molecular effects of each of the HTLV-1 genes to be deleted or mutated are partially characterized; all the available evidence suggests that the genetic changes in HTLV-1 will severely impair or abrogate the replication and/or transmission of HTLV-1. This will be kept under continual review: if any unanticipated effects are noted from the published data, work will be halted until an assessment has been made of the risk, and approval obtained from the GM committee.
<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>
| Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate) | Containment Level 2: liquid waste treated with 1% Virkon solution for a contact time of 30 minutes, as per the manufacturer’s instructions. Disposed of promptly after use through designated sink.  
Containment Level 3: Same treatment as liquid waste. The waste is autoclaved. Autoclave cycle time is 126 deg C, 30 minutes, and monitored by indicator strips; a record is kept as the chart recorder print-out. Autoclave function is tested by an annual 12-point thermocouple test of the autoclave. Autoclaved material is disposed of in clinical waste bags. |
| Is an emergency plan required according to regulation 20? | N                                                                                                                                  |
| If yes, tick to confirm that it is attached to this form | N                                                                                                                                  |
| Tick to confirm that you have attached a risk assessment to this form | N                                                                                                                                  |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N                                                                                                                                  |

Please enter comments on the GM safety committee on the risk assessment
Following the discussion of the proposal at the GM Safety Committee meeting, the committee requested that the following amendments be made to the proposals prior to approval:

Comments: CB gave a brief summary of the project.
PK queried if the plasmids would be grown in Containment Level 1 and this was confirmed by CB.
PF asked how the mutations would be handled. CB informed the committee that they expected the mutations to result in attenuation, this would be viewed continually and additional changes made via a Form C if necessary. The Committee suggested that a statement to this effect should be added to the current risk assessment rather since the HSE would most likely request this if it was not stated.
PK requested to know what type of proteins had been altered in the Delta mutants. CB stated that some were thought to be accessory proteins but the function and roles of others were unknown.
PL suggested that this information be stated on the form.

The following amendments were requested prior to approval:

Part 1: Title Type: T-cells
1.1: Add more information about the scientific goals of the project. HTLV-1 should appear in full at the first use and thereafter in abbreviated form.
1.21: Indicate what is known about the proteins of interest (delta mutants) or state that their functions/role is unknown. Add in any reporter genes e.g. GFP that may be used.
- Typo: Properties
- Change hypochlorite to Virkon.
1.22: Specify what cell lines will be used.
1.3: Make statement indicated that any mutation would be expected to result in attenuation, that this would be reviewed continually and any committee would be informed of any unexpected phenotype and any additional charges necessary to be submitted to the committee for approval via a Form C.
1.4: Tick box: containment level 3.
2.1.1.2: Type: carring.
2.2.1.1.: Rephrase this sentence ensuring that both plasmids and bacteria are considered.
2.3.2.2: Clarify rooms
2.3.5.: Advice form occupational health is required.
2.3.6: Tick box: containment level 3.
3.1.1a: Split the liquid waste section in two parts to include the specific information for CL2 and CL3 rooms. CL3 waste should specify that tissue culture media be disposed of via autoclaving and that an annual 12 point thermocouple testing of the autoclave be used for validation.
3.1.1b: State print out is used to monitor autoclave runs and give details of the type of strips used.
3.5a: Add wipe up contact time.
- Change 1% Virkon to 2%.
- Specify aluminium box will be opened in a microbiological safety cabinet in the event of a spillage.
3.5b: Remove: Eye protection is worn for use of vaccinia virus.
3.6: Clarify rooms.
Ensure that any of the above changes and the Code of Practice (which will need to be submitted to the HSE) are consistent.

Agreed: Class 3, Containment Level 3, HSE notification and written consent required prior to commencement.

Project Containment
Purposes of the contained use

Penicillium spp. and their related sexual species, Talaromyces, in particular Penicillium marneffei and Talaromyces stipitatus, are saprophytic ascomycetous fungi. In the context of a severely debilitated host Penicillium marneffei can cause a fatal disease. Healthy individuals are at negligible risk of Penicillium marneffei infection, and this is reflected in Penicillium marneffei being listed as an ACDP category 2 pathogen in countries other than the UK. In the UK Penicillium marneffei is listed as ACDP category 3a.

The aim of this work is to identify, sequence, disrupt and analyse the expression of fungal genes involved in virulence e.g. STE12 (transcriptional regulator), STE11 (MAP kinase), RIM101 (Transcriptional regulator), RAC (polarized growth regulator) and other genes as yet unidentified. To achieve this, genes will be isolated from genomic DNA by PCR, heterologous hybridisation, mutant complementation and related techniques. This will require construction of Penicillium and Talaromyces libraries in Saccharomyces cerevisiae/Escherichia coli shuttle vectors e.g. YEp24. Genes of interest will be sequenced and characterised further. Phenotypic assays e.g. the ability to grow in the presence of 0.1% SDS, low/high pH, reduced glucose and 500mM KCl, will be used to characterise the effect of inactivation of each gene. As part of this further
analysis gene disruption and replacement experiments will be performed in the modified *P. marneffei* KU70 deletion strain. This strain has a high rate of homologous gene replacement. In addition these lesions will be complemented using the *P. marneffei* homologous gene. This will be done by DNA mediated transformation of viable fungal cells (KU70 and/or wild-type) using non-mobilisable vectors carrying appropriate nutritional (for complementation of auxotrophy) or antibiotic (eg hygromycin and phleomycin) markers. Where appropriate, ablation of virulence will be assayed in a murine model.

**Recipient or parental organism**

*Penicillium marneffei* FRR2161, FRR2161 KU70 gene-deletion strain. These strains have a long prior history of safe use in Dr. Alex Andrianopoulos' laboratory ([http://www.genetics.unimelb.edu.au/Research/andr/index.html](http://www.genetics.unimelb.edu.au/Research/andr/index.html)) in Melbourne, Australia, where the fungus is classified at a ACDP category 2 pathogen according to Australian and USA legislation.

We also seek permission to use other wild-type clinical strains that have essentially the same characteristics as those described for use as controls.

**Host/vector system**

Saccharomyces cerevisiae: the majority of laboratory strains are derived from three major wild type lineages S288Cand W303. Most contain multiple auxotrophies and would not survive outside the laboratory.

*Escherichia coli*: strains in common laboratory use eg DH5alpha, XL-10 will be used as cloning tools. These strains, which are generally derivatives of the K12 strain, have a widespread and long history of safe use. They also contain numerous mutations eg thi-1 which render the strains auxotrophic and therefore unlikely to survive outside the laboratory environment.

**Origin & function**

The origins of the GM material will be from:

1. *Penicillium marneffei* will be the donor organism and will constitute 'self-cloning' as we will reinsert genes to complement genes that have been intentionally ablated to investigate loss-of-pathogenicity mutations.

2. We will also add selected tags eg GFP, TAP, HA, etc to selected genes *Penicillium marneffei* genes (full gene-set to be determined)

3. We will use heterologous antibiotic genes eg. ampR, bleR, hygR as markers of transformation.

**Evaluation of foreseeable effects**

The GMMs and gene knock-outs are disabled or are expected to result in loss-of-virulence: they are unlikely to be able to survive in the environment and the modifications proposed (gene inactivation) would not confer a selective advantage even if they were to be transferred to a wild type organism. The likelihood of hazard is therefore considered to be no greater than that of the parental strains. We will assay phenotypes in both cell-culture (macrophage cell line THP-1) and in-vivo models (mouse) and record whether genetically-modified *P. marneffei* occur that show unusual or aggressive phenotypes.

To summarise: the most hazardous GMM organism is likely to be the re-constituted wild-type organism.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
SOLID WASTE

All pipettes, loops, and pasteur pipettes used in the facility will be of the disposable type.

Solid culture media will be in plastic Petri dishes, plastic universals, or plastic bijoux bottles.

The main disinfectants to be used will be 1% Trigene liquid in liquid-contained: this disinfectant is effective against a wide range of fungal pathogens as well as P. marneffei. However, we will validate its ability to kill P. marneffei prior to the commencement of our experiments.

All solid waste will be autoclaved post-disinfection and prior to disposal.

SPECIFIC DETAILS ON HANDLING SOLID WASTE

a) Disposafe jars are placed in the safety cabinet. These will be used to contain plastics such as tips, pastettes, microscrew tubes, loops, graduated pipettes, 96well plates, 24 well plates and any plastics that won’t hold liquid/Trigene detergent very well and therefore needs containing. 24-well tissue culture plates and 96-well tissue culture plates can be disinfected with 1% Trigene and then placed in the disposafe jar. There should be NO MORE THAN 0.5L of liquid volume in the bottom of the disposafe jar otherwise the jar will not reach the desired sterilisation temperature. The Disposafe jar can then be placed into an autoclave bag and sprayed with 70% ethanol before being removed from the MSC and then autoclaved. This is classed as Disposafe jar waste.

b) Petri dishes containing agar cultures should be sealed with tape inside a Petri dish bag and then discarded into a double Sterilin bag for autoclaving. This is classed as mixed discard.

c) Used gloves, tissues and other material that has not been in direct contact with fungi should be discarded into a double Sterilin bag suspended in the white bin liner holder and then autoclaved. This is classed as mixed discard.

All solid waste materials from the laboratory must be autoclaved before final disposal. Autoclave bags must not be more than 2/3rds full. An autoclave for routine disposal is located within the laboratory. The desired temperature is 121 degrees C for 15 minutes. All waste to be autoclaved must be discarded into a thermolabile bag sitting within a thermostable Sterilin one, both securely held by the bag holder.

Disposafe jars should be placed in double Sterilin bags. In all cases the bags must not be overfilled with jars and must not be closed (hold the bag around the neck on the way to the autoclave). Once placed in the autoclave ensure that the bag neck is open before running the autoclave.

All autoclaved material will be placed in yellow clinical waste bags, tagged with the appropriate recorded tag and transported to the yellow clinical waste bins for disposal. The print out receipt for each autoclave run and tag number of each yellow waste bag will be recorded in the diary on the day of autoclave completion.

The liquid waste in the Tundish vessel from the autoclave will be disinfected with 1% Trigene for 24 hrs before being disposed of in the sink.

All autoclaves will receive annual 12 point thermocouple testing, 6 monthly servicing and annual insurance inspection.

LIQUID WASTE

Small volumes of liquid culture (10 - 20ml) will normally be grown in plastic universals/50ml falcon tubes. Larger volumes of liquid culture (up to 100ml) will be grown in 250ml plastic Erhrlenmeyer flasks that do not have vented caps.
The main disinfectants to be used will be 1% Trigene liquid or spray.

All liquid waste will be autoclaved post-disinfection and prior to disposal.

SPECIFIC DETAILS ON HANDLING LIQUID WASTE

a) Enclosed vessels such as flasks, falcons and bijoux tubes will have 1% Trigene disinfectant added to them, re-lidded and then left overnight for 24h in the cabinet (but without causing overcrowding within the cabinet). All paper waste, contaminated gloves and paper towels together with the enclosed vessels will then be placed straight into a double autoclave bag, sprayed with ethanol and then removed from the MSC and placed directly into the autoclave. This is classed as mixed discard.

All liquid waste materials from the laboratory must be autoclaved before final disposal. Autoclave bags must not be more than 2/3rds full. An autoclave for routine disposal is located within the laboratory. The desired temperature is 121 degrees C for 15 minutes. All waste to be autoclaved must be discarded into a thermolabile bag sitting within a thermostable Sterilin one, both securely held by the bag holder.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The following comments were made by the GM Safety Committee:

State GM Centre No: GM77

1.1: Name the cell lines here.
1.2: Specify the gene deletions and list the strains for which you request permission. Remove the references to E.coli and S.cerevisiae and fill in a separate GM form A for the class 1 aspect of the work.
1.3: Add ‘GMM organism will most likely be the…’
2.1.1.1: Remove: Aspergillus fumigatus ACDP 2 and Talaromyces stipitatus ACDP 1.
2.1.1.2: Remove reference to E.coli.
2.1.1.3: ‘The majority of genes inserted will be from the same organism…’ clarify this.
2.3.3.1.a: Include PRC testing.
2.3.3.3.b: Correct/reword this sentence i.e. will the buckets be sealed?
2.3.3.3.d: The tissues used to clear up any spill should be autoclaved. State that this will be the case.
2.3.4.1: Is it 1 or 2% Trigene? Please be consistent throughout the Forms and COP.

Part 3: Fill in tick box.
3.2.a: Remove ‘drop-proof’ to ‘robust’ boxes.
3.31.b: Remove reference to A. fumigatus.
3.42: Answer the question re use of sharps.

3.5.a: Rearrange sentence order in first paragraph to reflect correct order of activities; point out that spills will be disinfected, then autoclaved. Remove reference to E.coli and S.cerevisiae.

Agreed: Class 3, Containment level 3. HSE notification and written consent required prior to start of work.

Remove the references to E.coli and S.cerevisiae and complete a new GM Form A, Class 1, for this part of the work. It was decided that MF should submit the application with the animal work included despite the fact that this work is to be done at a much later point in order to prevent later complications. An explanatory note will be included in the risk assessment and in the letter to the HSE. A code of practice for the animal work will be submitted to the Safety Dept and the HSE at a later date once home office approvals have been granted. No work will start before submitting the Codes of Practice and gaining HSE approval.

IC reference number: GMIC-01888.1

All of the comments have been addressed and the required changes incorporated into the risk assessment attached to this form.

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 Yes</td>
<td>L4 L2 L3 L4</td>
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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L4 L2 L3 L4 L2</td>
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Project Ref 77/09.3
**Genetic manipulation of influenza viruses of human, swine, equine or avian origin**

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<th>CU2 Project Title</th>
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<td>Genetic manipulation of influenza viruses of human, swine, equine or avian origin</td>
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<tr>
<td></td>
<td>Class 3</td>
<td>&lt; 1 Litre</td>
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</table>

**Non-GMM**

Consent Granted: Yes

**Project notified under transitional arrangements**: N

**Withdrawn**: N

Tick if notifying a connected programme of work: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**: 77/09.3a

**Date of Significant Change**: 18/05/2019

---

**Project Additional Information**

**Purposes of the contained use**

To study the interaction between influenza viruses and their hosts in order to understand virus pathogenesis, factors that determine the biological outcome of infection and factors that restrict or expand the virus host range. In addition to develop suitable in vivo and in vitro models for testing interventions and therapies to enable better control of influenza disease.

**Recipient or parental organism**

Influenza A viruses that are reconstructed from cDNAs representing the entire genome of the virus. The parental organism for work with swine origin H1N1 influenza virus will be A/England/195/09, an isolate from a UK patient who suffered a mild upper respiratory tract infection. In addition recombinant influenza viruses containing internal genes from the vaccine strains A/PR/8/34 and surface antigens HA and NA from the swine virus will be generated and the activity of infection of ferrets with these viruses is considered to be of higher risk than their recovery which will take place under our existing class II project.

**Host/vector system**

The influenza viruses will be rescued in vitro in cell culture systems using plasmid DNAs propagated in E. Coli bacteria.

**Origin & function**

The recombinant influenza viruses will be used to infect cell cultures to study the replication capacity, host responses induced and susceptibility to antiviral drugs. In addition the viruses will be used to infect animal models including mice and ferrets to study their pathogenesis and transmission in vivo.

As described in the attached risk assessment, point mutations will be introduced into genes of swine origin H1N1
Influenza that are linked with the acquisition of resistance to antiviral drugs. The H274Y mutation in the NA gene will be introduced to England/195/09 virus. This virus will be tested for susceptibility to Tamiflu, Relenza and Peramivir. In addition this virus will be used to infect ferret to assess pathogenesis and transmissibility. A single gene reassortant virus will be generated with 7 RNA segments from England/195/09 and the NA segment from a recent human H1N1 virus that already carries the Tamiflu resistance mutation H274Y. This virus will be tested for antiviral susceptibility, virulence and transmissibility in ferrets.

### Evaluation of foreseeable effects

Infection of workers or swine is possible. Viruses in which mutations are engineered that may confer resistance to Tamiflu are expected to remain susceptible to other antiviral drugs including Relenza. Nevertheless these viruses constitute a significant health hazard and their release is undesirable. Aerosols are controlled so that infection of workers is, so far as is reasonably possible, prevented. In the unlikely event that a worker were to be infected (or exposed and potentially infected) with such a mutant, they would be treated with Relenza and isolated at home.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Liquid waste: liquid waste containing infectious virus will be treated with 1% Virkon for minimum 30 minutes inside the MSC then in 50 ml maximum volumes in plastic flasks will be autoclaved within the room. Flasks will be placed in double autoclave bags and autoclaved for 20 minutes at 135°C 3 bar. After removal from the autoclave bags will be placed inside metal tins that will sprayed out of the room with 70% methanol and disposed of a solid waste through the usual hospital clinical waste route. |
| Solid waste: solid waste contaminated with infectious virus (plastic tips, flasks) will be immersed in 1% Virkon for minimum of 30 minutes inside the MSC. After this it will be placed inside a double autoclave bag, and autoclaved within the laboratory at 135°C 3 bars for 20 minutes. Conditions of each autoclave run are stored in printed form. The autoclave undergoes a 12 point thermocouple test annually. After autoclaving the solid waste joins the usual hospital clinical waste route. |

In room B44 CBS facility there is no autoclave within the room. In this case liquid waste and solid waste will be Virkon treated as above by immersion in 1% Virkon for 30 minutes in the MSC hood, placed inside a double autoclave bag and then the whole placed in a plastic Ultima bin. The bin will be sprayed out of the room with 70% methanol and autoclaved in the CBS autoclave at 135°C, 3 bar for 20 minutes.

Animal waste from room B44 will be placed in double autoclave bag, inside an ultima bin and sprayed out of the room with 70% methanol and autoclaved in CBS autoclave as above.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y
The following comments were made by the GM Safety Committee:

Comments: WB gave a summary talk on the background to the project. MS stated that it was important to contain exposure at the primary level when performing the ferret nasal washes. AdP responded by reporting that all relevant control measures would be put into place; the experiments will be conducted with a replication of conditions utilised at Porton Down for the handling of Highly Pathogenic Avian Influenza. This will involve the use of modified downflow benches, supplemented with positive pressure respiratory protective equipment. PF asked whether a microbiological safety cabinet (MSC) would be employed. WB replied that the use of an MSC would be difficult especially as two people were required for handling of the ferrets. PK queried the means of disinfection of the RPE in order to prevent crosscontamination and spread of the viruses. AdP replied that the CoP had yet to be written but it would involve two people spraying each other's other masks in turn with 70% EtOH; RPE would be located inside the lobby and only 'clean' reagents, people, and equipment would enter the facility. PK queried contact time with 70% EtOH. AdP and WB answered that validation experiments with EtOH addressing questions such as contact time etc. had been performed by WB’s lab and would go in the CoP. PF raised concerns over the unpredictable nature of nasal washes and asked about alternative methods for virus rescue. The alternatives were discussed and the use of a MSC discounted. AdP reiterated that the downflow benches would be tested under conditions of use to check that they offered equivalent levels of aerosol containment as an MSC and that the RPE would then provide an extra level of protection against splashes. WB also commented that the use of anaesthetised animals would probably aid in safety but that the quality of data would be severely compromised in that the amount of virus rescued may be less and that it would be very difficult to anaesthetise the animals on a daily basis.

The following amendments are requested prior to approval:

1.3. Typo: 'PUC'

2.1.3. Expound the last sentence so that management controls, e.g. logging use of viruses, obligatory (& logged) UV of hoods, etc. are mentioned; the sum of these controls should “prevent” and not just "minimize the risk". It is important to be able to demonstrate should the need arise that work on incompatible viruses is not, or has not been, conducted.

2.1.1.6. If there is an increase in titre of more than 3 logs, what is the threshold above which the novel virus will be destroyed? Does this refer to the wild one? If yes, what is the normally expected titre?

2.2.1.3 & 2.2.1.4. Reword these sections as not desirable to have Tamiflu-resistance circulating in the pig population.

2.3.1.1. Are the SOPs for microscope examination of cultures the same as for Cat II work, or will there be additional precautions?

2.3.2.1. Wherever transfer of live viruses from Cat 3 labs is mentioned, a statement should be added clarifying that these transfers are only made to other approved Cat 3 labs, or unless approval is gained from the local GM Safety Committee that a particular virus(es) is free of other contaminating Cat 3 viruses and does not require category 3 containment.
Agreed: Class 3, Containment level 3. GMSC members have been asked to forward any further specific comments on the forms to YH at the earliest opportunity. HSE notification and written consent are required prior to start of work.

A Code of Practice for the laboratory as well as the animal (ferret) work is to be devised. PL suggested, unanimously agreed, that the relevant codes of practice and pertinent testing information be forwarded to the GMSC for review in order to ensure that all effective control measures have been put in place. Additionally, AdP has asked GMSC members decide and report back on the extent to which they wish to inspect and monitor this work at the aforementioned facilities.

All of the comments have been addressed and the required changes incorporated into the risk assessments attached to this form.

### Project Containment

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### Project Ref 77/10.1

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<td>Use (but not development of) of Toxoplasma gondii and strains with defects in intermediary metabolism in order to elucidate the potential role of L-DOPA synthesis in Toxoplasma-altered host behaviour</td>
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</table>

<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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</thead>
<tbody>
<tr>
<td>09/08/2017</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Not Applicable</td>
<td></td>
</tr>
</tbody>
</table>

Project notified under transitional arrangements [N]
Toxoplasma gondii is a single-celled protozoan that is an obligate intracellular ubiquitous parasite that can infect any animal and is currently found in ranges of 22-84% in human populations across the world. The most infectious stage is the extracellular sporozoite stage found in cat feces and infections are minor and self-limiting but is classified as a Class 2 pathogen in the ACDP because it can be serious for a developing fetus whose mother first contracts the disease during pregnancy or an immunocompromised person. In the laboratory we only maintain the intracellular stages.

Recipient or parental organism

Toxoplasma gondii is a single-celled protozoan that is an obligate intracellular ubiquitous parasite that can infect any animal and is currently found in ranges of 22-84% in human populations across the world. The most infectious stage is the extracellular sporozoite stage found in cat feces and infections are minor and self-limiting but is classified as a Class 2 pathogen in the ACDP because it can be serious for a developing fetus whose mother first contracts the disease during pregnancy or an immunocompromised person. In the laboratory we only maintain the intracellular stages.

Host/vector system

The vector is based on the standard non-mobilizable cloning vector pKS+ with T. gondii sequences for expression in parasites and chloramphenicol acetyl transferase as a selectable marker. There are now several hundred publications using these vectors for T. gondii transfections including ones from UK institutions. It does not contain elements for propagation in T. gondii (e.g. origins of replication, retrotransposons) and is not expected to be mobilizable.

Origin & function

Initial gene disruptions will target an enzyme in amino acid conversion (GenBank No. ACB99414 and ACB99413). There are no known hazards associated with these classes of enzymes and the products (tyrosine and L-DOPA) are normal metabolites in animals and humans. Most experiments will only utilise a gene fragment so that the chromosomal copy of the targeted gene is inactivated whilst overexpression may increase the likelihood that the infected rat is attracted to cat odour. The intended function is to alter expression levels of metabolic genes so that the effect on the parasites ability to subtly modify rat behaviour can be monitored.

Evaluation of foreseeable effects

The final GMM is likely to be of the same risk of harm as the un-modified host. It is possible that the genetic lesions in metabolic pathways will lower fitness. It is unlikely to escape the laboratory conditions due to this intracellular parasite’s requirement of a host cell and specific nutrients. In the event of escape, there is no increase in virulence and hence no increase in harm from normal exposure to these pathogens in the environment. Indeed as this parasite is common in animals and humans and
approximately 30% of the UK population have antibodies to Toxoplasma, the GMM would find it difficult to compete for a niche. Hence, there is no foreseeable harmful effect of escape from containment. This parasite can only survive as an intracellular parasite in animal cells. It has a very short lifespan outside the culture dish. Hence possible exposure is only to lab workers and, due to high exposure rate in the environment and high levels of antisera in the population, there is negligible risk to lab workers, the environment, or community at large. This project does not use the stable sporozoite form excreted by cats that is the only form that can survive outside host cells. The ecological consequences on escape are minimal to nil as this parasite in its more competent wildtype form is prevalent in the environment. It would require 10,000-100,000 viable parasites to infect a cat. If this did occur, the resulting sporozoites in the cat feces would present no greater harm than the un-modified host.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Liquid waste from culture media obtained during initial inoculation only (GMM produced and provided from Leeds University) – will be decontaminated using an equal volume of 2% Virkon and left overnight (following manufactures literature and following assays confirming killed cultures for regrowth on fresh culture media performed at Leeds university prior to commencement of study) before disposal in drains with 100 volumes of water. Any solid waste from initial culture material will be autoclaved on programme 2 (1260 for 1 hour), before being destroyed as clinical waste. Animal bedding following initial inoculation (rats do not shed T. gondii) will be autoclaved, before being destroyed as clinical waste. Animal carcasses at the end of the study will be autoclaved and destroyed as clinical waste as above. Emergency spill procedure: Liquid spill – treat with Virkon granules until deep pink, or add an equivalent volume of 2% Virkon – will be left for at least half an hour before being mopped up with plenty of water. Wearing strong gloves of the "Marigold" or similar type, solid spills will be gathered into an autoclave bag, the area affected will be wiped liberally with 2% Virkon. The treated area will be left for half an hour for the disinfectant to have time to act and the area will then be mopped up with plenty of water. Immediately after it has been collected and the affected area has been treated with Virkon, the solid waste will be taken for autoclaving, as above. For spills inside centrifuges the local Standard Operating Procedure as indicated on the equipment will be followed. All spills of genetically modified microorganisms (GMM) will be reported immediately to the local Biological Safety Officer, or local Safety Supervisor in the absence of the BSO; all spills and their mode of treatment will be recorded. Any personal injury where an infection by a GMM may be involved will be reported immediately to one of the local BSO’s or Safety superiors and the immediate advice of Occupational Health will be sought. |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
Dear Professor

Following the discussion of your proposal entitled “The role of L-DOPA synthesis in Toxoplasma-altered host behaviour”, GMIC-01953.1 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

Comments: PL gave brief summary of the proposal. The Committee had a few queries about the methods used:
- JC explained the gavage method and JW described further how the GMM was prepared in Leeds before it will be transported to the College.
- PK queried if there will be shedding and JW confirmed there will not, this is to be clarified on the proposal.
- PK also questioned who would be doing the animal exposure work, JW answered that the CBS technicians at the College would be carrying out this work.
- MS pointed out that the risk to pregnant workers needed to be addressed.
- PL suggested that all reference to latex gloves are replaced by the use of Nitrile gloves.
- JW was also asked to confirm is centrifugation will be used and to add this to the proposal if so.
- Finally a Code of Practice needs to be completed.

The following amendments are requested prior to approval:

1.1: Describe further the link between urine attraction and behavioural changes in the mice.
1.22: Please quote references.

Part 2: GMIC reference number is: GMIC-01953.1
2.1.1.2: State the hazards of the host organism, the toxoplasma wildtype; risk to humans, and pregnant workers.
2.1.1.5: Typo: „lielihood“ and double full stop at the end of the sentence.
2.2.1.1: Typo: „avrirulent“ and „hereare
2.2.1.3: Clarify this answer.
2.2.1.4: Clarify this answer.
2.3.1.1: Space between “…10ml/kg).IP infections…”
- Change from latex gloves to Nitrile
- Typo: „personell“ and „afterwhich“
- State that there is no shedding and that all bedding will be autoclaved.
2.3.3.1.c: Typo: „personal”, should be „personnel"
2.3.3.2.a: Typo: „Transportion”
2.3.3.3: Determine if centrifugation will be used and then answer the questions in the section appropriately.
2.3.3.5: Typo: „withinthe”, „personell”, „GMOS” and „afterwhich”.
2.3.3.8.c: Typo: „consumaption”
2.3.5: AS to complete this section.

Part 3: GMIC reference number is: GMIC-01953.1

Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
3.31.a: Typo: "GMM", should be "GMO"
3.31.c: Point out that this is unlikely.
3.41: "X" mark the "yes" box and remove the "X" in the "no" box. Clarify that face mask will not be worn all the time.
3.5.a: If centrifuging will be used, add details here.
   - Remove: "(Parasite maintaining?)"
3.5.b: Typo: "sterilized"
3.7.1: Remove JC and add to 3.7.2

Agreed: Class 2, Containment level 2. HSE notification and written consent are required prior to start of work. The Code of Practice will be sent with the HSE notification.

Could you please ensure that the risk assessment is revised (add GMIC number) and the amendments above addressed, the Occupational Health information should be attached. The form should then be emailed to me. Please see http://www3.imperial.ac.uk/safety/guidanceandadvice/biosafety/gmprocedures for further information.

The HSE notification form is attached to this email.

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**Project Containment**

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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 [Yes]</td>
<td>L3 L2</td>
<td>L2 L3 L4 L2</td>
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<tr>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 [Yes]</td>
<td>L3 L2</td>
<td>L2 L3 L4 L2</td>
</tr>
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</table>

**Project Ref 77/11.1**

- **Date Ackn'd**: 16/06/2011
- **CU2 Project Title**: Characterisation of acute HIV-1, SIV AND SHIV strains
- **Class**: Class 3
- **CultureVolClass2**: < 1 Litre
- **Non-GMM Consent Granted**: Yes
- **Project notified under transitional arrangements**: N

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**Historical Significant Changes**
During acute infection, HIV-1 crosses mucosal barriers and infects primary target cells e.g. CD4 T cells, macrophages and Dendritic cells. The way in which viruses responsible for transmission interact with primary target cells and tissues is not fully understood and may differ substantially from the behaviour of chronic or lab-adapted viruses. The aims of this project are

Aim 1. To define the sequence of events required for establishment of HIV-1 infection in mucosal tissue explants and primary target cells.
Aim 2. To define the viral determinants of mucosal HIV-1 transmission using in vitro models.
Aim 3. To define the earliest genomic and proteomic responses following infection of cellular and mucosal tissue models in vitro.
Aim 4. To examine possible intervention strategies that may prevent HIV transmission via mucosal routes e.g. use of microbicides.

Our Collaborators within the Centre for HIV/AIDS Vaccine Immunology (CHAVI) consortium have cloned HIV envelope genes and full length HIV-1 provirus from acutely infected patients in order to conduct these studies. Envelope genes and full length proviral clones isolated from chronically infected patients and lab-adapted viruses will be used as controls. Some constructs also contain reporter genes such as GFP or Luciferase. These viruses will be used to infect primary cells such as dendritic cells, macrophages and CD4 T cells, established immortalised cell lines, and primary tissues of vaginal, penile, rectal and tonsil origin.

As SIV infection in macaques is used as a model of HIV in humans to examine early events in infection, SIV and SHIV (HIV/SIV chimeras) strains will also be used to study transmission, pathogenesis and to test potential anti-viral compounds. Direct comparison of these related retroviruses in human tissue models will be conducted. The effect of mutations that confer resistance to antiretroviral drugs will also be studied by inducing such mutations into proviral clones, and expanding these in established cell lines. Our collaborators will conduct in vivo infection experiments in non-human primates. Our laboratory will receive blood serum and tissue samples from these experiments for analysis and storage. We will also need to culture SIV and SHIV strains in our laboratory for infection assays.

Competent E. coli strains TOP10, DH5a, STBL2, STBL3 will be used to prepare plasmid DNA. These are recognised as non-colonising and disabled, and may be considered to be equivalent ACDP hazard group 1. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. In the event of transfer of the plasmid to other bacterial strains, HIV genes would not be expressed as HIV requires numerous human proteins to complete its lifecycle, and these genes are absent from prokaryotic organisms, thus the plasmid provide no selective advantage. The virus generated would not differ from other HIV-1 strains already present within the laboratory, and so recombination would not be expected to result in a strain that would pose any higher risk than those already in use. Outside the specialised laboratory environment and

Recipient or parental organism

Competent E. coli strains TOP10, DH5a, STBL2, STBL3 will be used to prepare plasmid DNA. These are recognised as non-colonising and disabled, and may be considered to be equivalent ACDP hazard group 1. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. In the event of transfer of the plasmid to other bacterial strains, HIV genes would not be expressed as HIV requires numerous human proteins to complete its lifecycle, and these genes are absent from prokaryotic organisms, thus the plasmid provide no selective advantage. The virus generated would not differ from other HIV-1 strains already present within the laboratory, and so recombination would not be expected to result in a strain that would pose any higher risk than those already in use. Outside the specialised laboratory environment and
without cellular support HIV is unlikely to survive and thus is unlikely to be a significant environmental threat.

Constructs will be transiently expressed in human embryonic kidney (HEK) 293T cells and virus stocks produced by transfection may be expanded in primary human HIV SIV and SHIV susceptible cells (primary CD4 T cells, macrophages and DC) and immortalised T-cell lines (Jurkat, MT-4, C8166, PM-1, CEMx174-R5). Virus stocks produced in this way will be infectious, however, as the envelope genes and full length proviral clones are derived from HIV infected patients, the virus generated is not expected to differ from known HIV-1 strains in terms of tropism, pathogenicity or infectivity. Cell lines used to generate virus stocks require specialised culture conditions and will not survive outside a specialised tissue culture environment, and so would present minimal risk to the environment.

Host/vector system

Full-length proviral clones are provided in plasmids pCR-XL-Topo, pBR322, pUC18, pTZ19R, pNLFPB, and pBluescript II. Envelope genes from acutely infected patients are cloned into a pUC18-based vectors containing the HIV-1 strain NL4-3, these constitute full-length proviral clones. Single round replication incompetent (env deleted) clones will also be used, in conjunction with HIV-derived envs, and VSV-G. Note that VSV-G will only be used in conjunction with env-deleted vectors, never with full-length virus.

Origin & function

Plasmids will contain either full-length or near full-length (env-deleted) HIV genomes derived from HIV-infected patients. Some provirus clones will contain env genes from HIV positive patients inserted into the backbone of a lab-adapted HIV-1 provirus (NL4-3). The HIV genome consists of the following genes:-

- Gag - Group-specific Antigen, codes the structural HIV-1 proteins (Capsid, Matrix Nucleocapsid, and p6)
- Pol - Codes the viral enzymes reverse transcriptase, protease, and integrase
- Tat - Transactivator protein, increases efficiency virus transcription
- Rev - regulates traffic of virus RNA species out of the nucleus
- Nef - down regulates CD4
- Vif - counteracts antiviral effect of cellular APOBEC proteins.
- Vpr - Contains nuclear localisation sequence
- Vpu - Down-regulates CD4, transports Env proteins to cell surface, counteracts antiviral effect of cellular tetherin proteins.
- Env - encodes the viral envelope proteins gp120 and gp41, theses proteins mediate virus entry into target cells. The viruses received from our collaborators will contain envelope genes derived from primary HIV-1 strains.
- Env-deleted viruses will be pseudotyped with Vesicular stomatitis virus glycoprotein (VSV-G) this allows virus entry by endocytosis, thus by-passing normal entry requirements for HIV.

Evaluation of foreseeable effects

Expression of plasmids encoding a full-length HIV genome in human cells will lead to release of infectious HIV into the culture supernatant. The virus generated would not differ from other HIV-1 strains already present within the laboratory, and so recombination would not be expected to result in a strain that would pose any higher risk than those already in use. Outside the specialised laboratory environment and without cellular support HIV is unlikely to survive and thus is unlikely to be a significant environmental threat.

Expression of SIV and SHIV strains will also lead to expression of infectious virus capable of infecting human and selected non-human primate cells. As there is no vaccination available against HIV infection and no cure, this poses a significant risk to human health, and as such will be handled at containment level 3. Adherence to containment level 3 procedures with regards to PPE, no sharps policy, and adequate training by experienced staff should substantially reduce the risk of self-inoculation to effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste - Bacterial culture supernatant will be treated with a 2% (final concentration) Virkon solution for 30 minutes. Efficiency data is available from http://www2.dupont.com/RelyOn/en_US/assets/downloads/europe/Virkon_efficacy_data.pdf

Virus containing supernatant - High volumes (>250ml) of low titre liquid waste such as PBS used to wash infectious virus from cells or tissue samples will be treated with 1% (final concentration) Trigene solution for 30 minutes then disposed of via the sink. Efficiency data available from http://www.medi-mark.co.uk

High virus titre or high protein waste e.g. cells used to grow virus - Will be collected in a sealable, hard-plastic, leak-proof container, when full this will be autoclaved at 134°C for 20 minutes (cycle 3 mixed waste) total running time 1hr 30mins.

Solid waste - disposable plasticware including flasks, pipettes, tips and centrifuge tubes that had come into contact with infectious virus will be autoclaved at 134°C for 20 minutes (total running time 1hr 30mins). Re-usable plasticware e.g. flasks for growing bacterial supernatant, will be soaked in 2% Virkon solution for 30 minutes, rinsed and autoclaved. Autoclave treatment cycles are monitored and recorded with a printout, that is stored by the laboratory manager. Autoclaves are serviced every three months and have an annual 12-point thermocouple test.

After autoclaving on site, waste is sent via the clinical waste route by the specialist waste contractor SRCL (formerly White Rose Environmental Ltd) for alternative treatment to landfill. This process involves shredding of waste and autoclaving in an industrial autoclave, and resultant waste is sent to landfill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The following amendments are requested prior to approval:

Part 1: Please provide CID numbers when available and Faculty
GM Centre number is: GM77
1.1: Amend sentence, "During acute HIV infection, the HIV-1 virus crosses..."
1.2: Remove references 'such as' and specify strains or cell lines. Also provide the extent to which it is disabled.
1.4: 'X' mark the Containment and Class 3 boxes only.
2.1.1.2: Clarify or list by containment level.
2.1.1.3: Change the term 'should' or 'would' to 'will'.
2.2.1.4: "HIV genes would not be expressed..." please explain why.
2.3.2.2.a: Amend sentence, "would take place in a containment lab..."
2.3.2.2.c: Typo: 'Conatinament'. Remove, 'the' all labs...
2.3.3.1.a: Add: "containers and rotors..."Remove the last sentence.
2.3.3.2.a: Please provide a room numbers, discuss with Roberta Perelli.
2.3.3.2.b and c: Change the term 'should' or 'would' to 'will'
2.3.3.3.c: A bit confusing. Does this section mean that class 1 work can be opened on the bench in containment level 2, class 3 work will be opened in an MSC in containment level 3. Please clarify.
2.3.3.4.a: Change the term 'should' or 'would' or 'maybe' to 'will'.
2.3.3.4.b: State if the microbiological lab coats are the Howie type or another.
2.3.5: Medical Issues to be completed by Occupational Health
2.3.6: 'X' mark the Containment and Class 3 boxes only.
3.1.1.a: Change the term 'should' or 'would' to 'will'. - Remove 'overnight' change to '30 minutes'- Autoclaves can be serviced every 6 months.
- Route of disposal is through clinical waste.
3.1.1.b: Autoclaves serviced every 3 month? Seems to be very frequent please confirm that this is correct.
- Route of disposal is through on site clinical waste.
- Please provide details of the location of the autoclaves.
3.2.b: Please explain under what conditions you would use the 50ml tube.
3.5.a: Change the term 'should' or 'maybe' to 'will'.
- Adding liquid virkon to an already substantial spill make sit bigger and dilutes the virkon. The committee recommended the following method.
Inside the cabinet: Leave the cabinet on.
Absorb the spill in absorbent material e.g. paper towels or blue roll.
Spray the site of the spill liberally with 2% virkon, and leave for 20-30mins to inactivate the spill.
Mop up with additional absorbent material and disposed of all material as solid waste after use.
Lift the grill and check under the cabinet work surface for any signs of contamination/spill. If present, contain and decontaminate as described previously.

3.5.b: For any exposure incident:
1. Encourage bleeding, but do not scrub the wound: this may increase tissue damage.
2. Wash any wound or contaminated skin with soap and clean water. Cover with a sterile dressing.
3. If blood is splashed into the eye or mouth, stop & wash out immediately with tap water or saline.
4. For any exposure during normal working hours contact the duty occ health nurse for the Trust via Trust telephone extension 33063. The nurse will assess the risk over the phone and if emergency treatment for a possible HIV exposure was indicated would arrange for HIVPEP to be dispensed locally. Outside of normal working hours follow the hospitals out-of-hours procedure for sharps accidents and go straight to A&E, and inform them of the possible exposure to HIV.

- Reporting of incident: include, Safety Coordinator, and completing an accident form to be sent to the Safety Dept.

3.6: Please list building.

3.7: Please provide CID Numbers.

3.8: Declarations Tick box to be completed once amendments have been made
Agreed: Class 3 Containment level 3. This project is notifiable to the HSE

Further comments from the committee after addition of the SIV / SHIV components
1.24: Typo: should say ‘Bluescript’
1.3: Drug resistance mutations – some clarification required as to what mutations they will be working with and any implications for treatment should be stated.
Please also include a statement in Part 2 of the Form saying “that should any of the SHIV chimeras (with or without mutations associated with drug resistance) have greater replicative fitness than the wild-type strains, then GM77 will be informed and a further risk assessment undertaken”.
2.1.1.3, Typo 1st para, last line non-human primate lines

### Project Containment

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**Animal Units**

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**Large Scale Activities**

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**Human Clinical Applications**

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### Project Ref 77/11.3

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<th>CultureVolumeClass3-4</th>
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<tbody>
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<td>14/10/2011</td>
<td>Cellular and viral components in respiratory syncytial virus (RSV) assembly and budding process</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

- Scientific research

**Recipient or parental organism**

- Human respiratory syncytial virus (RSV), A2 strain

**Host/vector system**

- RSV A2 clone containing cDNA of all RSV genes (pSynkRSVline19F)
- Helper plasmids containing L, N, P and M2 genes (names of the plasmids are not available yet)
- Vector containing T7 polymerase (name not available yet)

**Origin & function**

The WT and two Matrix mutants are originally made at Monash University, Australia (Prof J lab). The origin of the viral vectors is Emory University and Vanderbilt University, USA. I will use the WT and Matrix mutant virus to study the role of Matrix gene in viral assembly and budding process. The viral vectors will be used to generate recombinant virus with additional mutations in Matrix or other structural genes to study the interaction of virus with cellular proteins during assembly and budding.

The RSV clone used to generate recombinant virus contains Green Flourescent Protein (GFP) tag.

**Evaluation of foreseeable effects**

The RSV mutant carrying mutation in the Matrix gene that I have already been working on in the past is attenuated compared to the WT strain. The mutations cause reduced virus titer since the virus can not assemble or bud efficiently from the cell. My future mutagenesis of the RSV will be based on my viral-cellular protein interaction screen and will hopefully result in even more dramatically attenuated virus that is stucked inside the host cell. All experiments will be carried out in tissue culture, and there is no intention to generate a GM animal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (e.g. tissue culture supernatent) will be inactivated by chemical disinfection. A solution of 1% Virkon disinfectant (final concentration) will be used for a minimum contact time of 30 minutes. This will achieve effectively 100% degree of kill as validated by the disinfectant manufacturer. The inactivated liquid waste will be discarded to drain.

Solid waste (e.g. disposable laboratory plasticware, paper towels etc) will be inactivated by autoclave. Cycle conditions of a minimum of 121 degrees centigrade for a minimum duration of 20 minutes will be used to achieve effectively 100% degree of kill. Each cycle will be monitored using a chart recorder attached to the autoclave. The autoclave treatment will be validated by annual multi-point thermocouple testing. Autoclaved solid waste is then discarded as clinical waste via the ‘orange’ stream.

Please enter comments on the GM safety committee on the risk assessment

Reviewer 1
1.24: Do the plasmids and vectors listed have names? Please give them if available and any information about the characteristics of the plasmids/vectors, e.g. from where they are derived, whether they are self mobilise-able or non mobilise-able or mobilisation defective.
1.25: Isn't the GFP tag an inserted gene? Even though the modification may not have been made by this group it should be noted.
2.3.4.1: The College Policy on MSC's requires that where MSC's are used for operator protection at containment level 2 that in addition to the engineers 6 monthly checks the air flow rates are tested monthly by the operators using a 100mm vane anemometer.

Reviewer 2
1.22: RSV2 strain is this wild type and fully virulent?
1.3: Attenuated - in what way? Just budding?
-Add statement saying that if any mutations lead to more virulence then GM77 will be informed and another risk assessment submitted.
2.2.13: because....the statement should be supported

2.3.3.2: What rooms
2.3.3.3: state nature of topical disinfection (also 3.5)
Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

Project Containment
Single-round retro-and-lenti viral VsV-G pseudotyped particles will be produced to transduce mammalian cell lines in order to express or ablate the expression of a gene of interest.

Many cellular processes are regulated by the addition and removal of Ubiquitin (Ub) either as single or multiple units in various types of linkage. The mono ubiquitination of histone 2A (H2A) is a key facet of DNA damage and repair and transcriptional repression by Polycomb Repressive complex 1 (PRC1). PRC1 is itself subject to ubiquitin mediated turnover by USP11. USP11 is implicated in the regulation of senescence via the effects of PRC1 on the CDKN2A tumour suppressor locus and in DNA damage response.

The major goals of this project are to gain further understanding of the interactions between USP11 and components of PRC1 and the DNA repair machinery by a variety of approaches including the production of recombinant proteins described in this CU2 and the associated risk assessment. The genes expressed or knocked down may be oncogenic.

This is not intended to be the submission of a broad assessment to cover a multitude of different projects but relates only to the work described above by Dr Maertens and her group.

Mammalian cell lines such as HeLa, 293T, U2OS, human diploid fibroblasts, mouse embryonic fibroblasts, obtained from ATCC.
Host/vector system

Single round retro- and lentiviral vectors based on the pQCXIP, pLVX-tight-puro (Clontech) vectors and the Mission shRNA lentiviral vectors (Sigma). The envelope will be provided by the pCG-VsVG plasmid and the GagPol for retroviral vectors from pCG-GagPol, for lentiviral expression, pCMVdelta8.2 (described first in Naldini L. et al, PNAS 1996).

For transient expression in mammalian cells, the following vectors will be used pHcRed1, pEGFP (clontech), pcDNA6V5Hisb (Invitrogen)

Origin & function

Origins: shRNA plasmids to make lentiviral particles will be purchased from Sigma. The genes that will be over-expressed in mammalian cells are amplified and cloned from cDNA made from RNA isolated from HeLa cells.

Intended functions of the GM involved: the genes of interest are ablated and/or overexpressed in order to study their role in tumourigenesis and the repair of DNA damage.

Inserted genes:
USP 11 ubiquitin specific protease 11, involved in removing ubiquitin from ubiquitinated proteins
Polycomb Group Proteins (PcG): Involved in regulating transcription of housekeeping and INK4a tumour suppressor locus. They exist in two distinct complexes. Polycomb Repressive Complex 2, PCR2, which initiates the repression by marking Histone H3 with a trimethyl mark on Lys27. PRC1 is then recruited and ubiquitinates histone H2A at Lys2A and Lys119, thus maintaining the repression.

Any other protein that will be identified during the course of the project to be a binding partner/substrate of USP11 and PRC1 involved in DNA damage repair and regulation of the CDKN2A tumour suppressor locus and other PRC1 target genes.

Evaluation of foreseeable effects

The vectors produced can only be used for single round infections - thus cell lines will be produced and selected that stably express a gene of interest, or an shRNA to ablate the expression of a gene of interest. These cell lines do not present any hazard and will, after selection, be cultured like any other mammalian cell lines used in the lab. There are SOPs in place to minimalise the risk of contamination during the production and use of the small scale single-round vectors. In addition the procedure involved a very limited number of technical steps, which are all contained within a class 2 MSC cabinet, the risks involved are thus minimal.

Control measures in place are sufficient to minimise risk. The final GMMs are not hazardous to humans or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

liquid waste
*supernatant of cells:
2% Virkon for minimum 30 minutes. Virkon is provided by Antech inc, their website provides validation data. The Virkon treated liquid is disposed of via the sink.
*supernatant containing viral particles:
2% Virkon for a minimum of 30 minutes. Virkon treated liquid is disposed of via the sink.
solid waste
serological pipettes and flasks are rinsed with 2% virkon prior to autoclaving.
Tips, tubes, rinsed serological pipettes and flasks are autoclaved using a solid or mixed cycle, 134C, holding time 20 min, cycle time 1.5h. Each run is validated via a printout, stored as a record are kept by the lab manager. Autoclaves are serviced quarterly and tested quarterly, annual 12 point thermocouple test and calibration. The final route of disposal is as clinical waste.
Following the discussion of your proposal entitled "Structural and functional characterization of USP11, a ubiquitin specific protease implicated in cancer", GMIC-1380 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

1.2.1: Include where the E.coli and fungal work will take place, their procedures and risk level.
2.1.1.4: Answer n/a
2.1.1.5: Answer n/a
2.1.1.6: Answer n/a
2.2.1.1: Viral vectors are „Stable”
2.2.1.2: Answer n/a
2.3.4.1: "...stored as a record and are..."

Part 3: Hatch the tick box
3.7.2: Please answer.

Comments: GM gave a brief description of the work to be carried out.
PL queried what was considered the most hazardous aspect of the work.
GM answered that producing the single vectors or an eye splash would be, however, all the safety measures were in place for this work to be carried out with care. The work would be done in class 2 microbiological safety cabinets and when transported it will be contained.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3</td>
<td>L2 L4</td>
<td>L2 L3 L4</td>
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Animal Units

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<th>Large Scale Activities</th>
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<tr>
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Project Ref 77/12.1

<table>
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<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>05/04/2012</td>
<td>Genetic Investigations of paediatric infectious diseases</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
</tr>
</tbody>
</table>
Patient blood cells (primarily PBMCs) and 3mm skin biopsy samples, as well as primary and immortalized fibroblasts and B cells will be received from our collaborator in Paris (Dr. Casanova lab). These samples will be used for DNA/RNA extraction as well as cell stimulation experiments. Infecting primary and immortalized patient cells with viruses and assessing cytokine/protein levels (by ELISA, western blot, expression arrays, qPCR, viral load determinations, cell mortality experiments etc). Retroviral transduction of cells to genetically rescue phenotypes in patient cells.

Human fibroblasts or blood cells.

HSV-1 GFP strain (Desai, P., and S. Person. 1998. J Virol 72:7563-7568) will be used to assess viral replication in various cell types (Guo et al. 2011. J Exp Med 208(10): 2083-98). Human retroviral particles will be used to transduce patient fibroblasts. The generation of these particles will be carried out in a collaborator's lab, Dr Sylvie Fabrega in Paris INREM. Briefly, retroviral vectors pseudotyped with the vesicular stomatitis G protein (VSV-G) will be generated as previously described (Barde et al. 2006. Mol Ther 13(2):382-90) by calcium phosphate transfection into 293T cells of a packaging construct, pMNold gag-pol ; a plasmid producing the VSV-G envelope (pMD.G); and each of the pLINX vectors carrying an empty, wild type or mutated form of TRIF (our particular gene of interest). As other genes are identified in this study, we may wish to generate cells retrovirally transduced with that particular gene of interest. Once this gene has been identified in the future, we will submit an amendment of risk assessment for its approval.

Human gene TRIF will be inserted into pLINX: an adaptor protein involved in the production of types I and III interferons following viral infection or double stranded RNA stimulation. May induce...
apoptosis when overexpressed.

### Origin & function

Human gene TRIF will be inserted into pLINX: an adaptor protein involved in the production of types I and III interferons following viral infection or double stranded RNA stimulation. May induce apoptosis when overexpressed.

### Evaluation of foreseeable effects

The most hazardous GMM to be constructed are the fibroblasts retrovirally transduced with wild type TRIF as there may be higher expression of this gene that may induce apoptosis. This is the reason we have opted to use a conditional promoter which will shut off transcription in the presence of doxycycline. The cells will be allowed to express TRIF only for specific assays which will occur for no longer than 48hrs. In addition these cells have been retrovirally transduced by replication incompetent retroviral particles, are stably transduced cells, and no longer produce infectious particles thus presenting no danger to the user or to the environment. HSV-GFP, similar to the wild type KOS strain, can cause herpes labialis, whitlow, zoster, keratitis and on very rare occasions encephalitis.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: Virkon minimum of 2% final concentration - 30-60 minutes to inactivate the virus/bacteria.  
Solid waste: Autoclave, sterilization at 134 degrees for 10 minutes, the successful completion of every load will be checked prior to disposal using a chart recorder attached to the autoclave.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment
Following the discussion of your proposal entitled “Genetic investigations of pediatric infectious diseases”, GMIC - 1402 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

Comments: V.SS gave a brief description of the work to be carried out. MS queried the use of the plasma and VSS confirmed she would include the UCL reference to validate this. PF inquired what the plasma did. VSS told the Committee is was used to insert the TRIF gene. MS further queried if there were possible gene mutations. VSS confirmed there were not. IH asked what the bacteria mentioned in 1.2 were. VSS explained this would be meningococcal, however, this was not part of the current work and she would remove this from the risk assessment.

The following amendments are requested prior to approval:

Principal Investigator: Please provide CID number

1.2: Remove reference to ‘bacteria’. Typo: ‘immortalized’

5.7: Typo: ‘transferred’ and ‘spil’

5.8: Typo: ‘consulation’ and ‘distrubance’

6.4: Typo: ‘autocalving’

10.1: Typo: ‘muntes’, ‘transferred’ and ‘spil’

10.2: Typo: ‘Satnford’

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

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</tr>
<tr>
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### Project Ref 77/12.2

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<tr>
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<th>CU2 Project Title</th>
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<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>03/10/2012</td>
<td>Assessment of cellular immune responses to influenza virus.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<tr>
<td></td>
<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Withdrawn N  

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

GMMS will only include recombinant influenza virus requested from Professor Wendy Barclay's laboratory. The strains that will be grown will be generated by reverse genetics and limited to GM constructs that will share sequences with the circulating influenza strains, e.g. H1N1 2009 A/England/195/09 virus and similar strains that are circulating in the population not exceeding GMM class II containment level 2. The strains to be generated are essentially wild type strains circulating in the human population with no novel genetic introductions and therefore is similar to the currently circulating wild type strains. There is no additional risk of our GMM undergoing recombination with circulating strains than in the general environment.

Recipient or parental organism

Circulating influenza viral strains including the H1N1 2009 A/England/195/09 virus and similar strains that are circulating in the population not exceeding GMM class 2 containment level 2.

Host/vector system

The vector system is the PR8/34 influenza virus. This is a laboratory strain that has been in use for over 50 years.

Origin & function

- Segment 1. polymerase basic subunit 2, PB2. Involved in viral replication.
- Segment 2. polymerase basic subunit 1, PB1. Involved in viral replication.
- Segment 3. polymerase acidic subunit, PA. Involved in viral replication.
- Segment 4. haemagglutinin, HA. Involved in viral entry and immune signalling.
- Segment 5. nucleoprotein, NP. Involved in viral replication.
- Segment 6. neuraminidase, NA. Involved in viral entry and release from cells.
- Segment 7. matrix M1 and ion channel protein M2. Involved in viral structure and uncoating respectively.
- Segment 8, non-structural protein NS1 and nuclear export protein NEP. Involved in the modulation of immune signalling and viral replication, respectively.

Evaluation of foreseeable effects

Influenza is primarily a respiratory tract infection that affects millions of people every year worldwide, mainly infecting the elderly and the young. The GM influenza virus described here is no different to currently circulating strains and consists of surface coat proteins (haemagglutinin (HA) and Neuraminidase (NA)) that enable it to bind and enter the human host cells and other core proteins such as the Matrix proteins (M1 & M2), Nucleoprotein (NP), Polymerases (PB1, PB2, PB3) and non-structural proteins (NS1, NS2 & PB1-F2) that are essential for viral replication.

There are no foreseeable effects beyond those exhibited by the wild type virus.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material will be treated prior to disposal with Virkon (2% w/v final) and left for 30mins. This will kill the GMM. The waste material is then disposed as per guidelines in the Institute with liquid waste left to drain away and any solid waste subject to sterilisation and autoclave and then disposed as clinical waste. Sharps will be disposed in sharp bins and also autoclaved before disposal.

Autoclave parameters are 121 degrees for a holding time of 15 minutes (minimum).

13. * Is an emergency plan required according to regulation 20? Yes No

Please enter comments on the GM safety committee on the risk assessment

Comments: SS gave a brief description of the work to be carried out.
The Committee has no amendments for this risk assessment.
Agreed: Class 2, Containment level 2. This project is notifiable to the HSE.
IC reference number: GMIC-1419

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## Project Additional Information

### Purposes of the contained use

Induction of highly neutralizing antibodies requires correct presentation of HIV Env glycoproteins, and important epitopes for virus neutralization may not be presented using recombinant forms of Env. In order to present conformationally correct forms of Env, we wish to generate non-infectious HIV-based Virus-Like Particles (VLPs) and use these to vaccinate small animals models and characterize the vaccine-induced immune responses.

### Recipient or parental organism

Vector stocks will be prepared in competent cells STBL2, STBL3 and related strains. Plasmids will be transfected into mammalian cells to produce VLP stocks. Mammalian cell lines HEK293 and derivatives, CHO, SIRC, RK13,

### Host/vector system

Our Collaborator, Prof. Eric Arts, has constructed an expression vector to generate non-infectious VLPs (pRECnflHIV-1). This plasmid contains the near full length (nfl) HIV-1 genome from which the 5’ long terminal repeat has been deleted. This sequence is critical for the initiation of reverse transcription required to generate infectious progeny. The CMV promoter has been inserted in pRECnfl HIV-1 up-stream of the pbs site and transcribes nfl HIV-1 RNA, which cannot support the production of infectious virus due to the deletion of 5’ LTR but can still be spliced and translated into the full set of HIV-1 proteins and assembly of virus like particles. The nfl HIV-1 RNA cannot support virus replication due to an inability to complete reverse transcription, therefore the virus like particles produced pRECnfl HIV-1 transfected cells are completely non-infectious and there is no possibility for recombination or reversion leading...
infectious viral production (Dudley DM et al, Biotechniques. 2009;46(6):458-67, Moore DM et al, Methods Mol Biol. 2005;304:369-85, Marozsan AJ et al, J Virol Methods. 2003;111(2):111-20). DNA stocks of the vector will be prepared in competent bacterial cells. The vector will be transfected into mammalian cell lines to produce Virus-like particles, and cells may also be selected using Zeocin to establish stably producing cells. VLP stocks will be used in vaccination experiments in small animal models by mucosal and parenteral routes. Vaccinations will require use of sharps.

The cloning vector is pcDNA3.1 Zeo, which expresses the inserted sequence from a CMV promoter. The insert also contains yeast centromere sequence (CEN6), the autonomously replicating sequence (ARSH4), and the betaisopropylmalate dehydrogenase (LEU2) for the maintenance of this plasmid in yeast in the absence of leucine. However yeast expression studies will not be undertaken in this project. This vector also contains an ampicillin resistance marker for selection in bacterial cells, a zeocin marker for maintenance in mammalian cells as an expression vector.

Origin & function

Inserted material contains near-full-length HIV genome, but with the 5' LTR deleted preventing replication of this vector as it is unable to reverse transcribe.

Evaluation of foreseeable effects

The most hazardous GMMs will be the VLPs themselves produced by transfection of plasmid pRECnflHIV into susceptible cells, as they will be able to enter human cells, however, as the particles are not replication competent, due to the deletion of the LTR, no spreading infection can be established, and there is no risk posed from insertional mutagenesis, as no nucleic acid will be produced. These particles would not pose an infection hazard to non-humans, and would not be able to survive outside of a specialised tissue culture environment, and thus would pose minimal environmental hazard.

Expression of the VLP plasmid will produce HIV-1 -based Virus Like Particles (VLPs), which are structurally and antigenically identical to live HIV particles. However as they lack the 5'LTR sequence that forms the promoter in eukaryotic cells, particles are unable to reverse transcribe, and will thus be non-infectious, and proteins cannot be expressed in target cells. The plasmid will only be expressed in cell lines that do not contain LTR elements, and are not capable of supporting HIV replication, so that in the highly unlikely event of complementation of the LTR deletion, there will be not expansion of infectious virus.

In the event of transfer of the plasmid to other bacterial strains, retroviral genes would not be expressed as retroviruses require numerous cellular proteins to complete their lifecycle, and these genes are absent from prokaryotic organisms, thus the plasmid provide no selective advantage. The plasmid also contains sequences CEN6, ARSH4 and LEU2 for maintenance in yeast, however, no yeast work is carried out in the areas where this plasmid would be stored and used.

The VLPs would be structurally similar to other lentiviral particles already present within the laboratory, and nucleic acids will have significant homology with full length lentiviral plasmids, and thus there is a possibility of recombination if these plasmids were co-transfected into the same cells. However, as transfection of full-length lentiviral plasmids is only permitted in CL3, and we propose production of nfl-HIV VLPs in our CL2 laboratory, there should be minimal risk of this event occurring. In the unlikely event that the wrong DNA sample is used in culture or that VLP have acquired 5'LTR form the environment this will be detected by applying a 5'LTR targeted PCR based test to both DNA stocks and any VLP culture before it is used more widely in the laboratory. Any culture giving a positive result will be immediately disposed of in the CL 3 facility following the CL3 COP.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Liquid waste: Tissue culture waste will be soaked in 2% (final concentration) Virkon solution for 30 minutes or 1% Trigene Advance before disposal down the sink.
- Solid Waste: Tips and pipettes used to handle biological samples are placed in biobins and then into autoclave bags.
- All other plasticware used to culture biological material (flasks, plates, tubes) are placed directly into autoclave bags.
- All solid waste in contact with cells/tissue will be autoclaved at 121°C for 30 minutes (cycle 3 mixed waste) total running time 1hr 45mins.
- Virkon and Trigene have been validated for use against HIV by the manufacturers and are proven to be effective at reducing the biological viable activity by at least 10^5 under the conditions specified.
- The Autoclave is serviced quarterly.
- Annually a worst case scenario mock load is prepared and placed in the autoclave. An engineer using a 12pt thermocoule load temperature NAMAS test apparatus ensures that every test point within the load achieves a temperature known to be effective at inactivating pathogenic microorganisms. At the same time the autoclaves load and chamber sensors are calibrated against the test rig.
- In all cases a minimum of a 10^5 reduction in microbiological viable activity is expected.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Following the discussion of your proposal entitled “Generation of non-infectious Virus-Like Particles for use HIV vaccine immunogens”, (GMIC-1431) at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

4.18 or 4.19: In the unlikely event that the wrong DNA sample has been used in culture or that VLP have acquired 5?LTR form the environment this will be detected by applying a 5?LTR targeted PCR based test to the VLP culture before it is used more widely in the laboratory. Any viral culture giving a positive result will be immediately disposed of in the CL 3 facility following the CL3 COP.

Comments: RS gave a brief description of the work.

KG asked how would the protein be quantified?
RS explained he used a Light Scattering quantitation device to do this.

PF queried the transport method to the centrifuge

PL pointed out that section 5.3 has sufficiently answered this.

IH checked that the buckets would be used and if they were biosealed. IH also wanted to know how RS would get the particles out of the centrifuge tube.
RS answered the particles are pelleted, not layered and needles were not involved.

MM questioned if the committee knew of anyone else handling Virus-Like Particles (VLPs) in CL2 laboratories.
RS stated the U.S. handled them in containment level 2 laboratories.
IH pointed out 5 litres of cultures grown is quite a lot.
RS explained that this is the maximum volume he could state on the form, but they would not be growing this much.
IH asked if the wave bag was leak-proof?
RS informed IH the cells would set up for 24 hours prior to infection in the wave bags in order to test for leaks.
PF queried if during the characterisation step of producing the VLPs, would you know if you had inadvertently created Replication Competent Viruses (RCV).
RS suggested putting them on susceptible cells to check. He had not included this in the risk assessment as they were not anticipating this to occur.
PF suggested starting with a check of the DNA stocks
RS confirmed that the DNA was quality controlled before transfection.

MM asked if others in the lab worked with 5?LTR sequence

PF stated that this DNA existed in the lab but there was complete separation between the VLP DNA stocks and anything that might cross contaminate or lead to the generation of RCV.

PF stated that DNA stocks were checked prior to use. PF agreed that a culture end point check would be introduced before the VLP?ss were used elsewhere to ensure that RCV had not been inadvertently generated. They would use a 5?LTR targeted Polymerase Chain Reaction (PCR) test to look for the presence of LTR in the culture. If this was detected the culture would be disposed in the CL3 facility without opening the bag.
The Committee agreed that this should be added to the risk assessment, perhaps in section 4.18 or 4.19
IH questioned if there was a risk of shedding with the animals.
RS answered that rabbits would be the animals used and the dilution factor so large that shedding of detectable VLP would be unlikely. The doses of virus are insufficient to sero convert a human even if they inoculated the entire animal dose into themselves. Additionally the particles are replication incompetent and could increase in number, the animals used were not natural hosts to HIV and did not support replication even if the virus was RCV.

Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.
Project Ref 77/13.2

Date Ackn'd 02/09/2013

CU2 Project Title Immunology of bacterial infection of the lungs

Class Class 2

Culture Vol Class 2 Non-GMM

Volume Class 3-4 Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Bacterial lung infections are a significant cause of morbidity and mortality. Particularly in individuals with underlying co-morbidities e.g. Cystic fibrosis, COPD, asthma and diabetes are more susceptible to bacterial lung infections. We are interested in the interactions between the bacteria and the host, looking at how the host tries to control the bacterial infection and how the bacteria tries to evade this. Using both in vitro and in vivo systems, we wish to define differences between healthy individuals and those with underlying co-morbidities that leads to greater disease susceptibility to bacterial lung infection. We will look at 4 bacterial pathogens. Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and non-typeable Haemophilus influenzae ntHi (hazard group 2 pathogens). All of these bacteria can be carried as part of the normal nasal/host bacterial flora. We will also look at mouse pathogen Citrobacter rodentium (ACDP hazard group 1).

Staphylococcus aureus is a major cause of hospital acquired infection, particularly bacterial pneumonia and wound infection. Bacterial pneumonia is a significant cause of morbidity and mortality. Attention has focused in particular on hospital-acquired, antibiotic-resistant, bacterial infections, a government health priority. However, there is a lack of good in vivo models of bacterial infection in the lung. This project will develop in vivo models of S. aureus infection and then investigate the immune responses to it and approaches to control it using vaccines or antimicrobial therapies. Both Methicillin Sensitive S. aureus (MSSA) and Methicillin Resistant S. aureus (MRSA) will be used.
Pseudomonas aeruginosa is able to infect a wide variety of hosts and tissues and is an excellent model for studying infection by gram-negative bacteria. It is particularly prevalent in cystic fibrosis patients. We will be using in vitro models of infection using airway epithelial cells and differentiated airway epithelia to assess the effect of altering physiological conditions on bacterial growth. We will then assess these conditions and drugs that can alter them using in vivo mouse models.

Haemophilus influenzae is serotyped according to the capsular antigens. Strains without a capsule are described as the non-typeable (ntHi). In general the non-capsulated strains are less virulent than the capsulated strains. Non-capsulated H. Influenzae is often observed in the airways of patients with chronic obstructive pulmonary disease (COPD). We are interested in extending Haemophilus influenzae vaccination to ntHi strains. We will then assess protection of vaccines using in vivo mouse models. We will also assess ntHi as an agent of co-infection with other pathogens.

Klebsiella pneumoniae is primarily a hospital-acquired bacterial pathogen that causes pneumonia, urinary tract infections and septicemia. Its success is related to its ability to form biofilms on medical devices, such as catheters. We will investigate how Klebsiella biofilm formation affects virulence and how the metabolic profile changes.

Citrobacter rodentium is a murine pathogen that belongs to a family of enteric pathogens, including enteropathogenic E. coli (EPEC) and enterohaemorrhagic E. coli (EHEC), which utilize attaching and effacing (A/E) lesions to colonize the gastrointestinal tract of the host.

Deletion mutants will be made, but genes will not be deleted if known to increase pathogenicity. Deletion mutants will be screened for the effect on pathogenicity in mouse models and if the mutants are more pathogenic than wild type they will be destroyed.

### Recipient or parental organism

Both long term lab strains and clinically isolated strains of bacteria will be used in this project. The strains below are those most commonly used for this study, but others may be used:

- **S. aureus**  
  MSSA Lab Strains including: 8325-4 (NARSA NRS135) and derivatives, Newman  
  MRSA lab strains including Sanger 252 (NARSA NRS71), no toxin producing MRSA strain will be used.

- **P. aeruginosa**  
  PAO1, PA14, PAK, PA103, TB, SG17 (Clone C)

- **ntHi Strains**  
  12. 2019, 1479, 76, 3198, 5557, 7502, 162, 176, R2868 and 9274

- **K. pneumoniae**  
  AJ218, B5055, B5055nm

- **C. rodentium**  
  ICC168, ICC180, ICC306, ICC169

### Host/vector system

The vectors used will be non mobilizable and replication incompetent and include:

1. Gene targeting by DNA plasmid complementation and homologous recombination in a non-replicative vector
2. Clean gene deletion by suicide vectors (e.g. pKNG101 or pEX series of plasmids)
3. Random transposon mutagenesis will be performed using Mariner based transposon system or Tn5 or Tn7 derivative plasmids

### Origin & function

1. Deletion mutants. Mutants will be generated or acquired with deletions of genes in areas including, but not limited to those involved in modulation of the host immune response e.g. Pseudomonas PilA, S. aureus IEC, Chp, Sak, Sc; virulence factors e.g. Pseudomonas AprA, Cif, K. pneumoniae MrkH; normal metabolic function, e.g.
**Pseudomonas gltK, Edd, Opr; or antigenicity e.g. ntHi protein D. Complementation studies may be performed to recover function.**

2. Reporter gene expression. Mutants will be generated or acquired expressing reporter genes, for example (but not limited to) luciferase or fluorescent proteins.

### Evaluation of foreseeable effects

The mutants generated will either be loss of function mutants or carry non-toxic reporter genes. It is not anticipated that bacterial strains produced in the laboratory are more virulent than strains that already exist in the environment. Indeed, many strains produced will have deletions in genes, which are required for optimal growth and virulence of the organism. Also, antibiotic resistance markers (carbenicillin, gentamycin, chloramphenicol, tetracycline, kanamycin and streptomycin markers) used in the laboratory do already exist in the host strains found in the environment and hence there is no risk of introducing new resistance genes to environmental strains.

Non GM Citrobacter rodentium is a murine pathogen and hazard group 1 in terms of pathogenicity to humans but once genetically modified it is included in this submission as a class 2 GMO under the terms of the GM contained use regulations because of the risk of infection to wild murine populations in the event of an accidental release. However it is not anticipated that any of the modified organisms will be more hazardous than wild type and as explained above many will be less virulent.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Liquid Waste:** Tissue culture waste will be soaked in 1% (final concentration) Trigene/Distel solution for 30 minutes before disposal down the sink. Alternatively Virkon (minimum 1% final concentration) can be used.

**Solid Waste:** Plasticware including all tips, flasks, plates and pipettes used to handle biological samples are placed either in biobins, or directly into autoclave bags. All solid waste in contact with cells/tissues will be autoclaved at 121°C for 30 minutes (cycle 3 mixed waste) total running time 1hr 30 mins.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
Following the discussion of your proposal entitled "Immunology of bacterial infection of the lungs", GMIC-2393 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

1.3 Room numbers for the 4th floor labs? These will need to be inspected by Safety Dept before work starts.

Done.

2.2 First line states Animal tissues. The rest of the statement list human cell lines. Please make it more clear what tissues and cell lines are in use. It is important that the risk assessment sets the limits of the work. While some statements might be generic about types of cell line (especially where there are too many to list) it must be clear that they are all of the same type or risk group.

Have increased information about the cells/tissues. Have also included PBMC. Have stated that the cells/tissues will not be greater than CL2.

2.15 It is important that the risk assessment sets the limits for the work. It may be acceptable to have generic statements about the host strains but the limits must be clearly stated for example regarding MRSA you might want to mention that no strain used will be toxin producing, if this is the case then this must be stated in the risk assessment in this section. Will you be using clinical isolates or long term lab strains? It is worth stating which early on in the risk assessment.

See response to comments below, have put both and said they are well characterised rather than lab adapted strains. Have stated that the Staph are not toxin producing/community acquired strains

Please state the ACDP bio Hazard group for each organism (2 by the look of it) done

2.16 vector and plasmid names please, if the list is too long please give some examples and set the limits of the work, e.g. Stating that they are non mobilizable, replication incompetent etc.

Done.

2.17 Please give some examples of the types of genes you will be deleting. Also make sure that you mention all the types of genes you will be altering. Your current statement just says "including" and then lists some genetic areas of functionality.

Have put in a few examples, but still would like it to be broad. Also put a statement in 2.14 to the effect that if increased pathogenicity observed strains will be destroyed and that known increases will not be made.

Again it is important that the limits are set. For example you may wish to state that no genes will be targeted whose deletion might lead to an increase in virulence, host range or pathogenicity.

Done in 2.14 and stated we will not overexpress toxins

5.1 Will use an MSC at any stage other than cleaning up in the event of a rotor spill? Yes

You identify several aerosol risk.

Item 1. Substitute should for must. Done.

Item 2 Why use flip flop tubes? If it is practical to do so, why not remove the hazard and use screw top with an O ring seal? This also reduces the risk of aerosol from flasks during centrifugation. Done.

Item 3 How will you tell if there is a leak? Have put after an imbalance

After discussion all the above will be performed in an MSC

5.2 So material is always transported in the lab in concial screw cap flasks? Never in multi well plates, eppendorfs, or petri dishes? This looks like the answer to a different question.

It was, have changed it to say: When moved within the lab, material with a liquid component will be moved in falcon tubes with sealed screw caps in tube racks or in flasks.
with screw cap lids.
Cultured biological material will be transferred from incubators to MSC in sealed vented flasks or plates with lids in secondary containers in the same laboratory. Petri
dishes will be sealed with parafilm after incubation.

5.2 - how will this be contained in transport. See above
5.3 - add the term double contained? Done
5.6 Room number for the -80 lab. Done 463
- Volume of stocks and storage containers (both primary/secondary containers)? Biological agents may be stored as glycerol stocks at -80 in lab 463. 2 aliquots of 1ml
stored in cryovials will be stored per strain. The cryovials are stored in plastic lidded boxes.

5.7 Will rotors always be opened in the MSC? Is this practical for the fixed angle rotors? For swing out rotors with larger volumes (e.g. 50ml or 15ml falcons) the buckets will
be opened in MSC. For fixed angled rotors, these will be opened on the bench or in the centrifuge.
Remove the term tissue culture cabinet and replace with microbiological safety cabinet or MSC. This applies to all uses of the term tissue culture cabinet.
Why 2% is this diluted to 1% final concentration with waste liquid? Yes
8.1 MSC - KL once per year, airflow checks using a 11mm vane anemometer twice per year be an engineer and monthly by the operators where the MSC is used to provide
operator protection Added
- shaking incubator maintenance? Annual
9.1 - is competency assessed? Yes, put by me
10. Remove the term tissue culture cabinet and replace with microbiological safety cabinet or MSC. This applies to all uses of the term tissue culture cabinet
Why 2% virkon but only 1% Trigene? Any spill will dilute this to less than 1% and below the manufacturers recommended minimum effective concentration. Changed
throughout
13 Is all the work only carried out in one lab? Section 1.3 seems to indicate more labs are in use but does not list them. Done
14 Please complete this section. It is Class 2 Done

Comments JT gave a brief description of the work. MS suggested building in a proviso to the risk assesment that no knockouts will be conducted that will knowingly
increase virulence.Added
PF advised giving a few examples of the altered genes in 2.17. Added : 1. Deletion mutants. Mutants will be generated or acquired with deletions of genes in areas
including those involved in modulation of the host immune response e.g. Pseudomonas PilA, S. aureus IEC; Chp, Sak, Sc, virulence facoters including Pseudomonas AprA,
Cif, and normal metabolic function, these include Pseudomonas gltK, Edd", Opr or antigenicity e.g. ntHi protein D. Complementation studies may be performed to recover
function.
PF increased pathogenicity…. Have mentioned this will not be done
PL suggested adding that Toxins will not be over-expressed, this should be mentioned in the form to set boundaries. He also recommended that mariner based transposon
system for future collaborations with his group. Done
MS asked if the consequences of co-infections had been addressed and to give this some thought. Co-infections unlikely due to viral work occurring in MSC and bacterial
work on bench.
PL Remove the term 'Lab adapted' and change to 'widely used' or 'well characterised' Done in 2.10 and 2.11.
IH advised adding the current room numbers to the form and when moved to new location; a Form C can be completed to update. Done
PL pointed out that the statement about markers used for making mutants, should state "genes conferring resistance to antibiotics used to treat clinical infections will not be
used as markers". Done
IH recommended adding the phrase to the hosts * not using toxin producing strains 2. Also, to provide some examples for the vectors.
TN to check the formulations of distell. Trigene highly effective against strains described .log5 reduction at 1:200
IH suggested defining at what point in the experiment would the work be carried out in the MSC. I have put in 5.1 that work will be performed on the bench.
PL queried if JT had worked with the MRSA strain on the bench
JT confirmed he had in the past, but not at ICL.
PL advised change the word 'should' to 'must' in 5.1. Done.
Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE
Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 77/14.2

In vitro passage of HIV-1 (NL4-3 strain) to determine resistance mutations associated with the use of C34-PEG4-Chol.

Date Ackn’d: 05/03/2014

CU2 Project Title

Tick if notifying a connected programme of work: No

Date Project Ceased

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The project is a laboratory based study of the resistance mutations associated with a novel antiretroviral (ARV) drug, C34-PEG4-Chol, which is due to enter a phase 1 first in human study in late 2014. C34 is a polypeptide homologue of a section of the gp41 protein of HIV-1 and therefore acts as a virus fusion inhibitor; the C34 peptide has been pegylated with four oxalythene molecules to make it more aqueous soluble (PEG4) and a cholesterol moiety added to enhance the half-life of the drug. Preliminary data suggest that C34-PEG4-Chol has very low toxicity in comparison with current ARVs. As with all other ARVs it is anticipated that the virus will be select resistance mutations which confer reduced susceptibility to the drug (characterised by signature mutations in the viral genome).

The laboratory-based aspects of this project involve induction of resistance to C34-PEG4-Chol in laboratory virus strains and characterisation of the resistance associated mutations.
Aim: The aim of the proposed laboratory project is to select resistance mutations in vitro associated with the use of C34-PEG4-Chol. 50mg of C34-PEG4-Chol product has been procured for experimentation. It is aimed to select resistance mutations in vitro by culturing HIV-1 in the presence of sublimiting concentrations of C34-PEG4-Chol. A set of 6 overlapping oligonucleotide primers have been developed to reverse transcribe, amplify and sequence the entire sequence of gp41 to elicit which mutations emerge as a result of drug exposure during culture. Having identified mutations associated with C34-PEG4-Chol, it is planned to introduce these mutant genes into a construct of the plasmid pNL4-3 through a process of chimeric virus technology (described by Fikkert et al) to generate an infectious molecular clone of HIV-1 NL4-3; this clone can then be used to determine the effect the mutations have on the susceptibility of HIV-1 to the drug.

Selection of mutations by passage in the presence of C34-PEG4-Chol are likely to confer reduced susceptibility of the NL4-3 strain of HIV-1 to the fusion inhibitor class of antiretroviral drugs; as such, should there be accidental infection of a chimeric viral construct into human, resistance to this class of drugs would ensue. These agents however, are used exceptionally rarely in clinical practice and these mutations would not have any effect on susceptibility of the virus to the other classes of antiretroviral drugs which form the cornerstone of therapy. There are not any anticipated environmental effects by in vitro passage and creating an infectious molecular clone with mutations associated with C34-PEG4-Chol.

Permissive T-cell lines (H9 cells) will be used for cultivation of infectious virus. H9 cells can also be used for transfection. TZM-bl cells will be used as a reporter cell line to measure infection of chimeric virus into these cells when cultured in the presence of the C34-PEG4-Chol drug. Note: TZM-bl, previously designated JC53-bl (clone 13) is a HeLa cell line. The parental cell line (JC.53) stably expresses large amounts of CD4 and CCR5. The TZM-bl cell line was generated from JC.53 cells by introducing separate integrated copies of the luciferase and β-galactosidase genes under control of the HIV-1 promoter. The TZM-bl cell line is highly sensitive to infection with diverse isolates of HIV-1. This was commercially purchased and not generated by this lab. Reference: Wei X, Decker JM, Liu H, Zhang Z, Arani RB, Kilby JM, et al. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. Antimicrobial agents and chemotherapy. 2002;46(6):1896-905.

Host/vector system

Retroviral based vector plasmid, pNL4-3 will be used. The original construction of pNL4-3 involved blunt end cloning of the 5’ SmaI-EcoRI fragment of proviral NY5 (5’ SmaI in flanking sequences to 3’ EcoRI) and the 3’ fragment of proviral LAV (5’ EcoRI to 3’ NruI in flanking sequences) into pUC18 at the PvuII site after removal of polylinker sites.

Origin & function

Origins: pNL4-3 plasmids will be purchased from the AIDS reagents program. HIV-1 NL4-3 is a molecularly cloned laboratory strain of HIV-1 differing from HIV-1 HXB2, the prototypical laboratory strain of HIV-1 in that the latter is defective of three nonessential auxiliary genes: vpr, vpu and nef. NL4-3 has a low ability to generate chronic virus producers and encode all HIV-1 gene products. By creating an infectious molecular clone of NL4-3 with mutations within gp41 associated with use of fusion inhibitor drugs, this will create a chimeric virus stock with resistance to C34-PEG4-Chol and probable cross resistance to other fusion inhibitor drugs however these drugs are used only exceptionally rarely to treat HIV-1 and this alteration will not change susceptibility of the virus to other classes of antiretroviral drugs.

Evaluation of foreseeable effects

NL4-3 viral stocks represent the most hazardous GMM to be constructed; whilst this is laboratory strain virus, it retains potential to cause HIV infection in human hosts. Viral titres are likely to reach levels > 1x10^6 copies/mL. The infectious molecular clone is also likely to be hazardous however may be less replication competent.
Introduction of mutations into the gp41 (Viral envelope fusion peptide) region of the HIV-1 viral genome are likely to reduce viral fitness and infectivity.

C34-resistant NL4-3 HIV will be generated during experimentation; if accidental exposure via mucosa or transcutaneous injury were to occur, the recipient would likely have a HIV infection which is resistant to fusion inhibitor drugs. This class of antiretrovirals are used extremely uncommonly in clinical practice therefore this is unlikely to have any additional clinical consequence in terms of managing such an infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste
High viral load waste will be contained in falcons/flasks, placed in sweet jars and then autoclaved.

Solid waste
Tips and pipettes used to handle biological samples are placed in sweet jars and then into autoclave bags. Other plasticware used to culture biological material are placed directly into autoclave bags (double bags). All Solid waste in contact with cells/tissue will be autoclaved at 121°C for 30 minutes (cycle 3 mixed waste) total running time 1hr 45mins.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Dear Dr Quinn,

Following the discussion of your proposal entitled "In vitro passage experiment of HIV-1 (NL4-3 strain) to determine resistance mutations associated with the use of C34, a novel fusion inhibitor for treatment of HIV-1", GMIC-2479 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

COP still mentions Roberta Perelli and Tracey Norris who have both left Imperial College. This should be updated as soon as replacements are appointed.

PI – The PI is the grant holder. Please specify who this is.

Title of the activity - Please provide something shorter e.g. In vitro passage of HIV-1 to determine resistance mutations associated with C34.

- Throughout the form amend CAT3 to Containment Level 3.

1.2: Amend temperatures to -15℃, -80℃ and 95℃.

-In point 3, amend 10 passages to 20 passages.

2.16: Is pNL4-3 the Vector? It is possible to be both a vector and a host.

2.18: Mentions viral titres of 1x10^6, section 4.21 states titres of 1x10^9. Please rectify the apparent conflict.

4.6: Sentence does not make sense "Full personal protective equipment will be used and procedures will be supervised by Dr Steve Kaye until the principal investigator is fully 'completent' (amend to Operate independently) with procedures involved and the category 3 facility and the standard operating procedures for this lab." Please amend.

4.16: Same as 2.16. Please clarify, even if it is considered to be both vector and insert or vector and host.

5.3: Please reference the inactivation of the HIV by Triton x-100 and heating in the SOP’s. Please provide this information and the validation data or other evidence that this is effective at giving at least a Log 5 reduction in biological activity.

5.7: A 30 minute waiting time is a bit short to allow settling of aerosols. It is recommend at least an hour. This will also need changing in the suite manual.

Spraying a spill with any disinfectant will dilute that disinfectant. Either apply an equal volume of twice the recommended concentration of disinfectant or absorb the spill treat the dry but contaminated surface with 1% virkon or Trigene and dispose of the absorbent material as solid waste for autoclaving. This is described in 10.1. Please make sure that the two descriptions of how to deal with a spill are the same.

- Remove Roberta Perelli's name.

5.8: as above

5.12: Please provide an SOP reference for this technique and the validation data as per 5.3

Section 11: Please provide an SOP/COP reference

Section 12: We will let the OH physician know that you have completed this section in your capacity as a Clinician with expertise in HIV.

Comments: KQ gave a brief description of the work.

PL asked if the virus was 'fitter' is it more likely to invade human cells?

KQ explained that this unlikely. HIV-1 NL4-3 would be no more virulent than wild type virus, and as a lab strain may be less virulent.

PL questioned if it would remain susceptible to conventional ART?

KQ answered yes, there would be no impact on the front line antiretroviral viral drug resistance.

KQ added that there were no sharps used and precautions were taken to create a low risk of splashes therefore the risks of accidental infection was reduced.

PL asked if any of the altered genes would make HIV-1 NL4-3 more virulent?
KQ confirmed it would not be more virulent than wild type and quite likely to be less virulent.
IH queried if C34 has been used in any other clinical setting.
KQ explained it had not been used like this before.
KG suggested increasing the 10 passages to 20 passages.
IH checked if the plasmid pNL4-3 could cause infections.
KQ informed the committee that it could start up an infection if introduced into the cell.

**Agreed:** Class 3, Containment Level 3. This project is notifiable to the HSE.

Could you please ensure that the risk assessment is revised (add GMIC number) and the amendments above addressed. I will then forward the form onto Occupational Health to complete. The completed HSE CU2 form (attached to this email) needs to be updated to include the comments from the committee, and copy of the Purchase Order (for BACs Payment) made payable to the HSE should be sent to me (s.joomun@imperial.ac.uk), I will then send it all to the HSE. Please ensure the information given in HSE Form relates to that provided in your risk assessment.

For further information, please see http://www3.imperial.ac.uk/safety/subjects/biosafety/gmprocedures

Please note that this work must be notified to the HSE and an acknowledgement receipt received prior to this work commencing.

If you have any queries, please contact me.

Kind regards,

Sarah Joomun
Administrator for Radiological and Biological Safety
Safety Department
Imperial College London
Level 4 Sherfield Building
South Kensington Campus
London SW7 2AZ

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### Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
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**Large Scale Activities**

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### Project Ref 77/14.3

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<td>Accessory genome evolution in Streptococcus pneumoniae and related species</td>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

Streptococcus pneumoniae is a common human nasopharyngeal commensal and pathogen. The species is genetically highly variable, and these differences underlie variation in antibiotic resistance, susceptibility to vaccine-induced immunity and interaction with the host. Exchange of sequence within S. pneumoniae, and with members of related species of the viridans group streptococci, has shaped its recent evolution in response to clinical interventions. Hence this project is designed to understand the importance of differences between S. pneumoniae strains, and how sequences are transferred between cells as part of the mechanisms that generate this diversity.

**Recipient or parental organism**

- Streptococcus pneumoniae strains TIGR4, ATCC 700669, R6 and other clinical isolates (class 2)
- Other viridans group streptococci (class 2)

**Host/vector system**

S. pneumoniae and viridans group streptococci may be modified with streptococcal mobile genetic elements. Streptococcal mobile genetic elements, typically integrative and conjugative elements - known to carry antibiotic resistance genes, only transmissible through direct contact with a live donor cell. Some elements can be transferred to other streptococci. These will not be modified by the addition of any genes not commonly found on naturally occurring examples of such elements. Other streptococcal mobile genetic elements do not readily transfer between species.

Streptococcal phage - only transmissible between closely-related species; not known to carry any genes hazardous to human health

**Origin & function**

Inserted genes:
- **cat** - encodes chloramphenicol acetyltransferase, which causes chloramphenicol resistance
- **tetM** - encodes a protein causing resistance to tetracycline.
- **ermB** - encodes a protein causing resistance to macrolides, lincosamides and streptogramins
mefE - encodes a protein causing resistance to macrolides
aph3' - encodes a protein causing resistance to aminoglycosides
Genes from mobile genetic elements - these may include one, or more, of the antibiotic resistance genes listed above. All mobile genetic elements used would be native to viridans group streptococci, and would not include any genes known to be hazardous to human health (e.g. toxin-encoding genes)
Altered/disrupted genes:
Genes involved in competence - these genes are required for the acquisition of DNA through transformation, and therefore their alteration and disruption will likely impair the ability of the mutant to acquire novel DNA
Bacteriocin synthesising operons - these genes are required for the synthesis of bacteriocins, which play a role in inter-strain competition. Therefore knocking out these genes would be expected to impair the strain's fitness
Restriction-modification systems - these genes protect S. pneumoniae against infection by phage, and therefore their elimination through knock-out would be expected to impair the strain's fitness
Signalling systems - genes potentially involved in cell-cell communication within S. pneumoniae populations
Other similar genes that are likely to be involved in affecting the pneumococcal population structure.

Evaluation of foreseeable effects

The most hazardous GMMs to be constructed would be those generated through exchange of DNA between cells through transformation, as this process can affect any locus in the recipient genome. Wherever possible, the recipient would be an unencapsulated S. pneumoniae strain such as R6. Only donors of the same species, or a less pathogenic one, would be used in these experiments. Donors would also be selected to have as few antibiotic resistance genes as feasible for the experiment. Experiments of this type have been safely conducted by the principle investigator previously. Other construction of GMMs would target genes for knockout, and therefore likely impair the fitness of the strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
NC gave a description of the changes to the work.
AH wanted to know if the natural competence contributed towards resistance?
NC answered that the transformation was the biggest problem.
PL asked for more information on the restriction modification systems and how they contributed towards impaired Strep pneumoniae fitness mentioned in section 2.17.
NC explained that published research has demonstrated that increased susceptibility to phage infection has led to reduced fitness.
IH asked if the encapsulated strains are more dangerous? How do identify which are encapsulated and unencapsulated? Also, what are the dangers of being exposed?
NC confirmed the strain R6, unencapsulated, is the strain they plan to use most and has been extensively sequenced. It is not known to cause serious disease.
IH asked if the other strains were encapsulated?
NC answered, yes.
IH questioned if they should be separated in the laboratory?
NC pointed out that it was unnecessary as it is difficult to transfer all of the genes required for encapsulation from one strain to another.
IH asked if they will both be in the same Microbiological Safety Cabinet at the same time?
NC confirmed they will work with both encapsulated and unencapsulated strains.
PL inquired about invasive serotypes? Were they more transmissible?
NC informed the Committee that the Serotype 1 was most invasive. Serotype 1, and other higher virulence serotypes, would be avoided as far as possible especially in experiments involving the movement of antibiotic resistance genes between strains most frequently identified in asymptomatic carriage. There are some experiments where the choice of strains is constrained (e.g. due to the presence of genes of interest or need for high-quality sequence data), and serotype 1 strains are the least drug-resistant candidate isolates. Serotype 1 does not warrant extra control measures. Where possible, non-encapsulated isolates would be used to minimise risk.
PF pointed out that the elements stated were very specific and is NC considered other ones for the future?
The Committee suggested adding the words, ‘and other similar…’ after the list in section 2.17 and to the Standard Operating Procedure to broaden three elements.
AH queried if 10 minutes for liquid waste was enough.
IH pointed out the Safety Department recommends 30 minutes.
2.17: adding the words, ‘and other similar…’ after the list in section 2.17 (altered/disrupted genes) and to the Standard Operating Procedure to broaden the application.
5.1: Can vortexing take place in the MSC?
5.1: Cultures left for 30s to settle. I have not seen this before, is it effective or necessary? If not, please remove.
7.2 and 8.1: Please let the Safety Dept know when the autoclave in VA11b is re-commissioned.
10.1: Centrifuges: The HSE has previously mentioned that centrifuges should be left for at least an hour before opening if an infectious aerosol is suspected. Now would be a good time to make this relatively minor amendment to both the Bio1 and the SOP/COP
11.2: Remove individual names to keep this section generic. Please complete the Biopersonnel Form and return to biosafety@imperial.ac.uk
Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</table>

02/03/2022
Mycobacterial diseases are the result of a complex series of interactions between pathogen and host, and there is an urgent need for new drugs, diagnostic tools and better vaccines to combat tuberculosis and other non-tuberculous infections.

In order to understand the molecular basis of the host-pathogen interactions in mycobacterial disease we will use genetic manipulation to generate strains of Mycobacterium tuberculosis, Mycobacterium bovis BCG and non tuberculous strains (e.g. Mycobacterium abscessus and kansaii) carrying defined genetic defects and/or reporter genes.

We will also work with the live attenuated defined M. tuberculosis auxotrophic mutants (Sampson et al, Infection & Immunity (2004) 72:3031–3037; Sambandamurthy et al, (2005) Infection & Immunity 73:1196-1203; Sambandamurthy et al, Vaccine (2006) 24: 6309-6320; Roy et al., Immunology Letters (2006) 103: 196-199) with targeted mutations which render them highly attenuated and suitable for use under containment level 2 conditions. The use of these strains under CL2 rather than CL3 containment will result in increased operator safety, time and cost savings and allow experiments which are otherwise not logistically feasible under CL3 conditions.
All these strains will be characterised in a range of in vitro models with the aim of identifying new drugs and drug targets, vaccine targets, diagnostic biomarkers of infection and disease and of improving our understanding of the host immune response and host-pathogen interactions of these important pathogens.

GM will be undertaken to insert plasmids (to insert/knock down genes of interest) into competent class 2 mycobacterial species including *Mycobacterium bovis* BCG, *M. abscessus, M. kansaii, M. avium, M. intracellulare, M. chelonae* by electroporation.

No additional modifications will be undertaken on the attenuated *M. tuberculosis* strains (class 2).

<table>
<thead>
<tr>
<th>Recipient or parental organism</th>
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</thead>
<tbody>
<tr>
<td><em>Mycobacterium tuberculosis</em> - class 3</td>
<td></td>
</tr>
<tr>
<td>Clinical isolates - drug sensitive and multi-drug resistant strains, but not extensively drug resistant.</td>
<td></td>
</tr>
<tr>
<td>Fully drug sensitive laboratory strains such as H37Rv, Erdman, CDC1551, H37Ra</td>
<td></td>
</tr>
<tr>
<td>Other <em>Mycobacterium</em> species - class 2</td>
<td></td>
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<tr>
<td><em>Mycobacterium bovis</em> BCG (vaccine strain), <em>M. bovis</em> BCG is naturally resistant to pyrazinamide, but otherwise sensitive to TB drugs. Other vaccine strains of BCG such as Danish Moreau, Montreal etc may also be used.</td>
<td></td>
</tr>
<tr>
<td>Non tuberculous mycobacteria reference strains/clinical isolates including</td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium abscessus</em></td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium kansaii</em></td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium chelonae</em></td>
<td></td>
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<tr>
<td><em>Mycobacterium avium</em></td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium intracellulare</em></td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium marinum</em></td>
<td></td>
</tr>
<tr>
<td>- Attenuated <em>Mycobacterium tuberculosis</em> auxotrophic mutants - class 2. These mutants will be used in experiments but no further modification will be undertaken. Refer to the risk assessment &quot;Investigating host-pathogen interactions in mycobacterial infection - class 2&quot; section 2.14 for details of these mutants and justification for their use at ACDP CL2.</td>
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<table>
<thead>
<tr>
<th>Host/vector system</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pSMT3 and derivatives - ColE1 and pAL500 origins of replication. Not mobilisable</td>
<td></td>
</tr>
<tr>
<td>pYUB854 and derivatives - OriE. Non mobilisable</td>
<td></td>
</tr>
<tr>
<td>pMV261 and derivatives - OriE Non mobilisable</td>
<td></td>
</tr>
<tr>
<td>pHAE159, pHAE87 and derivatives - Derivatives of the conditionally replicating mycobacteriophage PH101 (ts; Bardarov et al., 97)</td>
<td></td>
</tr>
<tr>
<td>pGS202, pGS201 - details at <a href="http://webhost.nts.jhu.edu/target/plasmids.aspx">http://webhost.nts.jhu.edu/target/plasmids.aspx</a></td>
<td></td>
</tr>
<tr>
<td>pKINTA and derivatives - Non-mobilisable integrating vectors</td>
<td></td>
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</tbody>
</table>
Inserting genes may be self-cloning of mycobacterial genes either in the sense orientation to allow overexpression, or in the antisense orientation to allow downregulation, under the control of either an inducible or constitutive promoter e.g. down-regulation and also overexpression of the 19kDa lipoprotein gene (Rv3762, involved in immune subversion), Acr2 gene (Rv0251c involved in bacillary defense against oxidant stress). Mycobacteria do not express any toxins and there is little risk of altering pathogenicity by this method.

Selectable markers are usually hygromycin (not clinically used as toxic to humans), kanamycin, spectinomycin, zeocin (not part of first line therapy), sometimes in combination with the sucrose and the SacB enzyme as a counterselectable marker.

Reporter genes include a range of fluorescent reporters such as GFP or mCherry, luminescent reporters such as the lux operon from Phorhabdus luminescens or Vibrio harveyi, or the luciferase genes from Firefly or Gaussia princeps.

Genes will also be inserted into Mycobacterium tuberculosis clinical isolates that are present in the reference strain H37Rv but naturally deleted in the clinical isolate. The pSMT3 vector will be used that encode the open reading frames for genes including Rv 0180 (Function unknown, probable conserved transmembrane protein), 1519 (probable conserved hypothetical protein), 3019 (Function unknown - Secreted ESAT-6 like protein EsxR, 3020 (function unknown - ESAT-6 like protein EsxS), 3516 (Could possibly oxidize fatty acids using specific components), 3517 (Function unknown - conserved hypothetical protein), 3738 (Function unknown - PPE family protein PPE66), 3739 (Function unknown - PPE family protein PPE67).

We will also use live attenuated M. tuberculosis auxotrophic mutants which are already genetically modified and have the following gene deletions and antibiotic resistance genes (no further modifications will be made in these strains):


Evaluation of foreseeable effects

Most GM Mycobacterium strains will be obtained from Dr Brian Robertson's lab, Imperial College London, in addition to constructing some other GM strains. Genetic manipulation will be undertaken to generate strains of mycobacteria including Mycobacterium tuberculosis, Mycobacterium bovis BCG and non-tuberculous mycobacteria carrying defined genetic defects and/or reporter genes.

Previous genetic manipulation will be undertaken to generate strains of mycobacteria carrying defined genetic defects and/or reporter genes. Mutants will be created by either the deletion of all or part of target genes and the introduction of a selectable marker, by double cross overs and homologous recombination. This can be done using mycobacteriophage or suicide plasmids, usually with a two-step selection procedure such as sucrose plus selection for an antibiotic marker such as kanamycin or hygromycin.

Reporters, such as genes for luminescence or fluorescence, will be introduced by electroporation of plasmid DNA which will be either maintained episomally by a mycobacterial origin of replication or will integrate into the attB phage attachment site. Reporters and mycobacterial genes may be over-expressed, or knocked down using antisense and inducible systems such as tetracycline or acetamide.

Genetic deletions in defined Mycobacterium tuberculosis clinical isolates will also be complemented by introducing the intact genes present in the reference strain Mycobacterium tuberculosis H37RV.
There is no evidence that any of the recombinant mycobacteria made in this project will be any more hazardous than parental strains.

In order to set the boundaries of the work we confirm that we will not be doing any genetic modification that involves i) the complementation of the RD1 deletion in Mycobacterium tuberculosis BCG, ii) the over expression of efflux pumps associated with drug resistance or iii) the cloning of large DNA fragments such as large insert Mycobacterium tuberculosis libraries in mycobacteria.

There is published evidence that disrupting some two-component regulators in M. tuberculosis alters virulence in immunocompromised animals; no such experiments are currently planned for mycobacteria.

If there is any increase in pathogenicity traits observed in in vitro models, then a further application will be submitted to GM77 committee at Imperial College London.

We will also use genetically modified live attenuated Mycobacterium tuberculosis auxotrophic mutants under containment Level 2. These defined M. tuberculosis strains with already targeted mutations render them highly attenuated and suitable for use under containment level 2 conditions. No further modification will be undertaken. All four of the strains carry two independent mutations, rendering them all auxotrophic for pantothenate, in combination with either leucine or lysine auxotrophy, or the deletion of the RD1 region responsible for the major attenuation of M. bovis BCG. The auxotrophic strains are well characterised and highly attenuated. Extensive safety testing has been performed on all the 4 strains: attenuated Mycobacterium tuberculosis leuD ΔpanCD hygR, attenuated Mycobacterium tuberculosis mc2 6020 ΔlysA ΔpanCD hygR, attenuated Mycobacterium tuberculosis mc2 6030 ΔRD1 ΔpanCD hygR, attenuated Mycobacterium tuberculosis mc2 7000 ΔRD1 ΔpanCD unmarked. (references Sampson et al, Infection & Immunity 2004 72:3031–3037; Sambandamurthy et al, Nature Medicine 2002 1171-1174; Sambandamurthy et al, 2005 Infection & Immunity 73:1196-1203.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste infected with mycobacteria (e.g. unwanted mycobacterial cultures or preparations originating from live mycobacterial cultures such as supernatants from infected eukaryotic cells) are discarded by adding 10% surfanios disinfectant (minimum final concentration) and leaving overnight before placing in a clear aurtoclave bag for autoclaving to achieve the desired sterilisation temperature for killing of 121 degrees Celsius for 15 minutes. This disinfection policy has been extensively validated in house at Imperial College London and data is available upon request. Autoclaved waste is then bagged in orange bags before being disposed of via clinical waste which is transported for microwaving and landfill.

All solid waste infected with mycobacteria (e.g. bacterial plates), will be placed in autoclave bags and autoclaved to achieve the desired sterilisation temperature for killing of 121 degrees Celsius for 15 minutes. Autoclaved waste is then bagged in orange bags before being disposed of via clinical waste which is transported for microwaving and landfill.

Autoclaves are serviced twice per year, and validated and insurance inspection once per year in accordance with Imperial College London regulations.

Any liquid waste which is not infected with mycobacteria (e.g. unwanted eukaryotic cells, whole blood) and is to be discarded, is treated with 2% virkon final concentration and left for 30mins-1hr before disposing via the drain.

Any solid waste items not infected with mycobacteria or blood/cells will be discarded into orange bags for disposal via clinical waste.
Following the discussion of your proposal GMIC-01147.1 and GMIC-01148.1, the committee request that the following amendments are made to the proposal prior to approval.

No comments received.

Comments from the BSO made prior to the consultation with the GM committee.

Title We will submit both Bio1’s under the same CU2 notification but what I will do is change the Title’s to “Host and bacillary correlates of pathology in human tuberculosis - class 3” and “Host and bacillary correlates of pathology in human tuberculosis - class 2” as it is two different aspects of the same project.

GMIC 1147.1

2.14 and 4.28 Crispr/Cas9 Ther is the potential for “gene drive” occurring as a result of the use of this system. I doubt this would be the case here as I think you are pretty much just using it to knock out genes and down regulate systems but it may be as well to add a line explaining why it could not happen.

2.16 Add the word “further” to the sentence “no modifications of the live attenuated M. tuberculosis ……… “

How were the M.tb deletion mutants created? Was this a GM technique or is the only genetic modification the addition of the antibiotic markers?

I don’t see reference to the use of the Crispr/Cas9 system mentioned in 2.14. You should describe it here.

2.18 and 4.18 How likely are the increases in virulence you mention here to occur and how would you notice or monitor for an increase in virulence? Basically we need to robustly justify use at CL2, the HSE will probably ask this question.

Does the fact that you are not introducing large fragment libraries into BCG reduce the likelihood of increased virulence to negligible?

Please enter comments on the GM safety committee on the risk assessment

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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 Yes</td>
<td>L4 L2 L3 L4</td>
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<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

Animal Units

<table>
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<tr>
<th>L2 Yes</th>
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</table>

Large Scale Activities

| L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 |

Project Ref 77/16.2

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>02/03/2022</td>
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</table>
Human T lymphotropic virus type 1 (HTLV-1) remains asymptomatic in over 90% of infected individuals. Approximately 5% of infected individuals develop an aggressive malignancy known as adult T cell leukaemia/lymphoma (ATLL). A smaller proportion (1% to 4% of infected individuals) develop a chronic inflammatory disease, of which the best recognized is HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Certain experiments will be carried out on cells infected with HIV-1 and compared with the results from experiments on HTLV-1 and HTLV-2. The laboratory will focus on the understanding of virus infections. The main aims of the project are i) establish an in vitro reporter system for HTLV-1 and -2 infection (=deltaretroviruses) ii) dissect the role of host factors in viral infectivity and integration site targeting; iii) investigate the efficacy of (lentiviral) integrase strand transfer inhibitors (INSTIs) to inhibit HTLV infection; iv) investigate host receptor contribution to infection efficacy; v) investigate mechanism of cell-to-cell versus cell-free infection. To investigate the efficacy of integrase inhibitors, experiments to determine IC50s will also be performed with HIV to compare the data. The laboratory will focus on the understanding of virus infections and more specifically integration. Gene delivery by retrovirus pseudoparticles: The lab produces pseudoparticles for cellular or reporter gene transduction to create stable transgene-expressing cell lines or to study heterologous viral entry in cells lines. Production of pseudoparticles is achieved by transfecting a producer cell line (e.g., 293T cells) with plasmids encoding 1) the gene of interest flanked by 5’ and 3’ retroviral long terminal repeats (LTRs) as part of a minimal packagable proviral genome 2) a plasmid encoding the retroviral gag-pol genes 3) a plasmid encoding the envelope proteins of a broadly tropic virus such as vesicular stomatitis virus (VSV). Pseudoparticles can then be used to infect cells of interest, in which LTRs mediate integration of the transgene. In addition, pseudoparticles are used to express a library of gene fragments, to express interfering small RNAs (shRNA, siRNA) as well as microRNAs (miRNA) and other non-protein coding sequences. Efficiency and biological function of the manipulated cells will be assessed following inactivation of any residual infectious particles via flow cytometry, imaging, luciferase assays, p24 ELISA and western blot analysis. Virus stocks (e.g. HTLV-1 and -2, HIV and MLV) will be produced by using recombinant plasmid based
transfection/electroporation methods using 293T or Jurkat T cell lines to produce high-titer, wild-type or recombinant viruses encoding reporter genes allowing easy identification of infected cells (e.g. fluorescent proteins). We will furthermore generate recombinant HTLV-1 and -2 with suspected drug resistance mutations (based on the homology with HIV) to determine their viral fitness in cells. Sequencing will be performed by isolating viral or cellular nucleic acid post inactivation of any residual infectious virus. Cell-free infection by HTLV occurs with extremely low efficiency (<0.001%), thus cell-to-cell transductions are performed by co-culture of the producer cell (either made by transient transfection of recombinant plasmid or by using the stably infected and producing HTLV cell lines MT-2 or C91PL) with a suitable target cell (such as HEK293T or Jurkat T cells). Co-culture of the target cells with MT-2 or C91PL will take place following gamma-irradiation of the producer cell lines following established protocols. Viral titers will be determined by qPCR (HIV and MLV) or ELISA (HTLV-1 and 2) following inactivation of infectious particles.

Recipient or parental organism

HIV (ACDP HG3) Human T-cell lymphotropic Virus type 1 and 2 (ACDP HG 3),
Disabled Lentiviral gene transfer vectors (ACDP classification 1),
disabled retroviral (MLV) gene transfer (ACDP classification 1),
disabled retroviral (HTLV) gene transfer (ACDP classification 1)
non-pathogenic E. coli, including DH5alpha, TOP10, XL1-blue and Mach1 (ACDP classification 1),
non-pathogenic insect cells (Sf9, HiFive) for protein expression (ACDP classification 1),
HEK293T (ACDP classification 2) and Jurkat T cell lines (ACDP classification 1) (and similar) for the production of retro/lenti-viral particles and infection/transduction.

Host/vector system

Lentiviral (3rd generation attenuated gene transfer vectors relying on a three-plasmid transfection system, cotransfection of pCG-VsVG (envelope), pCMVdelta8.2 (Naldini et al PNAS 1996) and commercially available pLKO (shRNA), pLentiLox3.7 and pDEST (for gene expression), pCHW-GFP (GFP reporter)), retroviral (attenuated gene transfer vectors relying on a three-plasmid transfection system; pCG-VsVG, pCG-GagPol (Ulm JW, et al Virology 2007) and commercially available pQCXIP, or pMarx-GFP), plasmids encoding infectious cDNA/DNA clones of HTLV-1 and -2. We are generating a triple plasmid based HTLV-1 and -2 vector system to produce non-replicating virus. The three plasmids will contain the following features:
1) Envelope gene expressed from CAG promoter (either VsVG or HTLV-1 and -2 specific envelope genes)
2) packaging plasmid encoding for gag-pol-pX region (NOT ENV); here no functionalLTRs are present AND the psipackaging signal has been mutated; these genes are under the regulation of the CAG promoter
3) transfer plasmid: contains: psi packaging signal, functional LTRs and transfer gene (such as EGFP, or luciferase reporter gene).

Only the RNA from the transfer plasmid will be packaged into the viral like particles, therefore these viral particles do not contain the information to produce new particles since the essential genes are absent from the genetic information packaged in the particles.

Above described mutations will be introduced into the packaging plasmid.
Plasmids for bacterial expression: commercially available pCDF-Duet1, pET28aSUMO, pGEX, pMalc2, pET20
Plasmids for eukaryotic expression: for retroviral expression pQCXIP derived, for lentiviral shRNA expression: pLKO (Sigma), gene expression: pLentiLox3.7 or pDEST system, for transient expression we use pcDNA6 plasmids.
Plasmids for baculovirus expression: pBacPackHis-StrepTEV

Origin & function
Integrase: viral gene essential for insertion of cDNA copy of viral genome into host DNA
HTLV binding partners such as protein phosphatase 2A (PP2A),
Proteins involved in DNA damage repair, chromatin segregation and others, see list in Supplementary Table of
Guide RNAs to target genes for knock-out using the CRISPR/Cas9 system (e.g. targeting the scaffold subunit of
PP2A) will be produced in vitro and transfected (nucleofected) into Jurkat cells together with commercially available
Cas9

**Evaluation of foreseeable effects**

Genetically modified microorganisms based on HTLV-1 and -2; These recombinants encode fluorescent proteins,
antibiotic resistance cassettes or luciferase reporter cassettes, used to identify infected cells in vitro or in vivo. All GM
viruses exhibit growth properties similar to or lower than the parental virus and none of the inserts are increasing
virulence. A standard read out of our experiments is the viral titre and infection efficiency, thus any change in
virulence/growth properties will be noticed. Should any GMM exhibit enhanced growth properties/virulence, GM safety
committee approval will be sought prior to any further experiments with said GMM.
HTLV GMMs encoding drug resistance mutations conferring them with resistance to INSTIs can result in drug
resistant virus infection. However, only drug-resistance mutations for integrase will be used.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Liquid waste**
Low titre liquid waste e.g. wash solutions and Class 1 GM culture will be inactivated with 1% Virkon (final
concentration after dilution with the waste) for 30 minutes prior to disposal.
High viral titre class 2 and 3 and all HG3 and Class 3 virus culture waste in the CL3 suite will additionally be
autoclaved.

**Solid waste**
Solid infectious waste will be put into a sweetie jar in the MSC cabinet, which when full will be closed in the MSC
cabinet, sprayed with 70% ethanol and put into an autoclave waste biohazard bin for autoclaving. Plastic ware that is
too big for the sweetie jars, will be put into an autoclave bag inside of the MSC cabinet, this bag will be closed with
autoclave tape and transferred to autoclavable biohazard bin.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
Following the discussion of your proposal entitled “Study of host factors that aid or restrict delta-retrovirus infection” GMIC-6945 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

Comments from the BSO review:
2.2 Is the MT-2 HTLV-1 producer cell line (ATCC) a GMO? Under the GM contained use regulations genetically modified cell lines are counted as micro-organisms. If so it needs to be discussed and explained in the GM section.
2.10 How are the point mutations created in the HTLV1 and 2. If it is not by radiation or chemical means it will be classed as a GMO and needs explanation in the GM section.
2.15, 2.16 and 2.17 I understand from the explanation in 1.2 and elsewhere in the Bio1 that there are a number of different genetic modifications being carried out.
1. Knock outs of HTLV1 and 2 using shRNA knockdown and / or CRISPR,
2. Creation of replicating recombinant HIV incorporating fluorescent marker genes,
3. Creation of non-replicating retro virus pseudo-particles from transfected producer cells lines e.g. 293T cells.
4. Creation of recombinant derivatives of HIV and HTLV which you have classified as GM Class 2 HG2 in section 4.16.
5. Not described in 1.2 but mentioned in 2.15 The use of disabled retroviral MLV and HTLV described in 2.15 as ACDP HG1 GM Class 1, (although this might be subject to the host organisms and the inserts being used). If these are the typical commercially available 3rd generation retroviral vectors then this may be true. However if these are something that you are creating yourself then the committee will need to see that the disabling mutations are very robust and cannot be overcome by a recombination event with wild type HTLV or HIV.
Has all of this been comprehensively represented in 2.15, 2.16 and 2.17? e.g. I didn’t see mention of all of the components of the systems used to create the recombinant HIV?
I found it difficult to establish which hosts were being transfected or transduced with which vector and insert. Please could you make it clearer e.g. by identifying the various host vector insert systems with numbers or letters? It is really important that the Committee and the HSE can easily identify what GMO’s are Class 3, Class 2 and Class 1 and what vectors and inserts are associated with which hosts.
In 2.15 you mention non-pathogenic E.coli and insect cells lines used to express proteins. I don’t see plasmids mentioned in 2.16 or the genetic inserts mentioned in 2.17?
Are the disabled retroviral vectors all obtained from commercial sources where the disabling mutations have been quality controlled and verified as being robust and where, subject to the nature of the host and the insert, this could form part of a Class 1 GMO system?
4.6 and 4.14 How is the filtration accomplished? E.g. pressure, vacuum or centrifugation?
4.16 Classified by who? They are not wild type organisms, so their classification is subject to risk assessment. For these recombinant derivatives of the HG3 viruses HIV and HTLV to be classified as HG2 GM Class 2 The Committee
needs to be reassured that the disabling mutation is robust and there is a negligible chance of recombination with wild type virus leading to a return to full virulence and the ability to replicate.

4.19 4.10 I may have missed it but what is pACH. I don’t see it described in previous sections of this risk assessment.

5.1 Please be more specific about what work has to take place at CL3 and what is at CL2 and what elements of the work require an MSC. E.g. all handling of replication competent infectious HIV and HTLV virus whether recombinant or wild type including replication competent viral producer cell lines and all material suspected of being infected with these viral agents will take place in CL3 within a class 2 MSC.

5.4 Category A is PI 620 not 650. Only cultures of HIV are category A HTLV is likely to be B as exposure is unlikely to lead to permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.

5.5 Cultures of HIV are category A

5.12 Is the NP-40 or sds treatment of HTLV and HIV to inactivate it known to be effective? The HSE are insisting that these methodologies are validated or at least historically proven to be effective.

Comments: GM gave a brief description of the work.
PL checked with IH if all the BSO comments had been addressed.
IH confirmed that they had been but needed some additional refinement.
PL asked what the knockout genes were and what effects will these knockouts have on the virus?
GM explained the knock out mutant could not increase virulence. Rather it would decrease the fitness of the virus and it would be less virulent.
PL suggested that the knock out genes are all listed so far as is practical and the likely effects described.
IH pointed out that the risk assessment describes how any enhanced activity will be noticed and the work stopped. IH asked how enhancements would be recognised?
GM said that viral replication would be monitored by QPCR.
IH asked about daily visual observations and whether enhanced activity could be detected this way.
GM pointed out that this could not be done by looking at the cell, they will need to test by QPCR which would be done routinely as part of the work and would be recorded.
IH suggested adding this into the risk assessment and as a routine part of the process.
PL pointed out that HEK293T may be ACDP 2. Action: IH to check
PL agreed with IH BSO comments to make it more clear which hosts were being transfected or transduced with which vector and insert.
PL emphasised comments made by virtual reviewers that any non-waste related inactivation of HG3 or Class 3 material would need to be validated or supported by scientific papers.
IH pointed out that GM had referenced papers in the Bio1 and asked that they should be forwarded to the Safety Dept as part of the package for the HSE.
PL asked that the refresher training should be carried out at least annually. The committee agreed that this could be
part of the lab teams’ annual or termly meetings to review lab activities. The following amendments are requested prior to approval:

2.18 Remove ‘Other factors…’ and replace with a list of representative examples or classes of protein.

Forward papers to the safety dept referenced in the Bio1 regarding inactivation of material removed from the CL3 for further processing.

Review the arrangements for refresher training in the Bio1 and ensure that it is noted that it is completed at least annually.

The following comments are from committee member’s virtual review:

4.16 First paragraph ‘viruses’ needs a capital letter.

5.3 The word ‘primary’ in the first paragraph has a typo.

5.2 Second paragraph ‘lysis buffer’ is joined together and needs splitting.

10.1 First and second paragraph ‘mil’ has a ‘1’ after it which needs removing. Point 1 under large spillages has a typo in the word ‘involving’ and point 3 has a typo in the word cleaning.

I have a point to note regarding the SOPs- the HSE were very keen on the validation of inactivation procedures of infected material before removing from the CL3. Although these inactivation methods have been documented in the SOPs there is no ‘in house’ evidence to show that these inactivation methods work under the experimental conditions they are being used at i.e. no results tables. So I would suggest testing these methods in house and recording results for each inactivation procedure. Also you need to document how often you will validate and re-validate these SOPs.

With regards to the COP-Section 3 ‘Training’ - the HSE were also hot on refresher training. I suggest adding a note with regards including SOP specific training for each individual and then a section on refresher training for those people out of the lab for specific periods of time or who have not performed an SOP for a length of time e.g. 6 months.

Section 8- is the use of sharps prohibited – this has a question mark after it. Please amend.

Agreed: Class 3, Containment level 3. This is notifiable to the HSE.

IC reference number: GMIC-6945

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<tr>
<td>L2 Yes</td>
<td>L3 Yes</td>
<td>L4 L2</td>
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<tr>
<td>L2 L3 L4</td>
<td>L2</td>
<td>L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4</td>
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Project Ref 77/17.1

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<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>21/06/2017</td>
<td>Modulation of host telomere homeostasis by Kaposi’s Sarcoma Herpes Virus (KSHV)</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
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</tbody>
</table>
Endothelial cells infected with wild-type KSHV cannot be readily distinguished from uninfected endothelial cells without staining for KSHV antigens (such as the nuclear expressed latency-associated nuclear antigen, LANA-1). To circumvent this inconvenience, and to also allow a platform for genetic manipulation of KSHV, a novel recombinant KSHV (rKSHV.219) was generated and propagated in the primate Vero cell line. This virus was constructed using KSHV from the JSC-1 primary effusion lymphoma cell line and was engineered to expresses the green fluorescent protein (GFP) gene from the EF-1α promoter, as a marker of latent infection, and the red fluorescent protein (RFP) gene from the PAN RNA promoter, as a lytic cycle marker. The generation of this recombinant virus made the identification of ‘rKSHV.219-infected’ cells (GFP-positive) and ‘rKSHV.219 lytic’ cells (RFP-positive) very convenient. For these reasons my group will use rKSHV.219 to study the consequences of KSHV-infection on telomere maintenance.

Our aim is to understand how viral infection contributes to the maintenance of human telomeres. For this purpose, we will use the Open Biosystems TransLentiviral pGIPZ Packaging System to create replication-incompetent lentivirus that will be used to stably transduce human HeLa, 293, U2OS, endothelial cells, and fibroblasts with shRNAs that target the genes encoding either shelterin components (TRF1, TRF2, POT1, TPP1) telomere replication factors, DDR machinery components, and others. Transduced cells will be cultured in vitro and screened for viability, for sensitivity to DNA damaging agents, and for telomere structure and function upon KSHV infection. In addition, lentiviral CRISPR/Cas9 system will also be used to generate knock-out cell lines or introduce point-mutations into mammalian genes.

Recipient or parental organism

Hazard group 2 recipients.
Wild-type KSHV from JSC-1 strain as a recipient to generate rKSHV.219 recombinant virus.
Hazard group 1 (For information)
Human and murine cells and cell lines.
We will use the Open Biosystems TransLenti Viral pGIPZ and other third generation lentiviral lentiviral vectors (including CRISPR/CAS9). The lentiviral vectors to be used are self inactivating and replication incompetent, require three different vectors for packaging (they cannot package themselves), require upstream elements and trans complementation. They cannot survive outside of closed controlled cell culture conditions and are rapidly inactivated by dehydration or other environmental insult. They also require percutaneous inoculation for transmission. Thus, the risk to human health from the use of the pGIPZ lentiviral vector is very low and the risk of any spread into the wider community is very unlikely indeed.

Origin & function

Class 2 work with recombinant KSHV. Green fluorescent protein (GFP) gene from the EF-1α promoter, as a marker of latent infection, and the red fluorescent protein (RFP) gene from the PAN RNA promoter, as a lytic cycle marker. The generation of recombinant KSHV makes the identification of 'rKSHV.219-infected' cells (GFP-positive) and 'rKSHV.219 lytic' cells (RFP-positive) very convenient. For these reasons my group will use rKSHV.219 to study the consequences of KSHV-infection on telomere maintenance.

Work using attenuated lentiviral vectors. Experiments will involve silencing, mutation, or overexpression of cellular gene products, normally being responsible for telomere homeostasis, DNA damage response, and DNA repair. The specific genes that will be targeted are shelterin components and DDR machinery factors as well as other, so far unknown, genes (cellular and viral) to be discovered as key regulators involved in modulation of the telomere chromatin by KSHV.

Evaluation of foreseeable effects

Infectious virus rKSHV.219 is the only hazardous form and is readily inactivated with standard disinfectants such as 1% virkon. GM rKSHV.219 will be no more hazardous to human health or the environment than the wild-type KSHV. In immune-competent individuals, KSHV establishes asymptomatic life-long latency following acute infection, however, it can cause Kaposi's sarcoma or pleural effusion lymphoma in individuals with compromised immunity (HIV infected patients or transplant recipients). No risk to plants or animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste
Treated with Virkon at a final concentration of 1% for 30 minutes before disposal to drains. Effective kill is in excess of a log 5 reduction in viable organisms. Information regarding virkon's effectiveness against herpes virus is available from Antec.

Solid Waste
solid waste will be placed in bin double lined with autoclave bags. The bags are sealed when 2/3rds full and
transferred to a pick up point in labs 451c or 327 for autoclaving by support staff. Autoclave waste cycle is set to hold the waste at 121 degrees for 15 minutes. The autoclaved waste is then sent for clinical waste disposal. The autoclave is validated annually with a representative waste load to ensure that these temperatures are reliably attained. Effective kill is in excess of a log 5 reduction in viable organisms. Sharps will be collected in sharpsafe bin, autoclaved and then incinerated.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Liquid Waste
Treated with Virkon at a final concentration of 1% for 30 minutes before disposal to drains. Effective kill is in excess of a log 5 reduction in viable organisms. Information regarding virkon’s effectiveness against herpes virus is available from Antec.

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Animal Units
L2 L3 L4 L2 L3 L4 L2 L3 L4

Large Scale Activities
L2 L3 L4 L2 L3 L4 L2 L3 L4

Human Clinical Applications
L2 L3 L4 L2 L3 L4

Project Ref 77/17.2

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<tr>
<td>02/03/2022</td>
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</table>
Respiratory syncytial virus (RSV) and Influenza are major causes of morbidity and mortality in early life. It is not fully understood why infections are more severe in some infants, but it is suggested that host genetics plays a role. Mammalian cells have a variety of mechanisms to reduce viral replication which are usually mediated by antiviral proteins. These can be present constitutively but a major element of the innate immune response to viral pathogens is the amplification of antiviral proteins, induced by the activity of type I interferons (IFNs).

The overarching aim of this research project is the investigation and functional characterisation of Interferon-stimulated genes (ISGs), and their role in the immune response to respiratory viral pathogens. This requires the development of cell lines deficient in the gene, and therefore protein, of interest. Further we intend to generate novel recombinant viral vectors that will be used to modulate gene expression in mouse models of infection. Following generation of modified cells or inoculation of mice with recombinant viral vectors, the application of respiratory viruses will be used to assess the role of the modified genes. This will require use of genetically-modified variants of RSV including variants encoding fluorescent/luminescent markers (GFP, RFP, luciferase, etc.) or host factors, and variants lacking certain viral genes.

This risk assessment applies to all work required to generate genetically modified mammalian cells, production and use of recombinant viral vectors, and subsequent infection in vitro or in vivo for ISG functional analysis.

**Recipient or parental organism**

1) Immortalised cell lines (Human - A549, HEp-2, Hek293T, Hek293. Monkey - Vero, Mouse - LA-4) and murine (C57BL/6, BALB/c, or similar strains) immune cells derived from bone marrow (macrophages/dendritic cells) will be genetically modified by transfection of plasmids encoding a Cas9 endonuclease. Depending on the targeting RNAs used in this study either indels or designed deletions of genes will be produced following cell-mediated repair of the DNA. The work will generally be limited to the production of cells with single genetic alterations.

2) Recombinant viral vectors - Recombinant Adeno-associated virus (AAV) and lentivirus will be produced using common triple transfection and purification protocols. Viral genomes will be produced that contain either shRNA
sequences or sgRNA along with a Cas9 cassette. Genomes will also contain a selectable or fluorescent marker including but not limited to GFP. Packaged viral genomes will be harvested, purified, and titrated using commercially sourced kits such as Clontech AAVpro Purification kit and AAVpro Titration kit. In-house qPCR assays will also be designed for titration. These vectors will then be validated in vitro for their ability to deliver shRNA or sgRNA expression before use in vivo.

3) Respiratory Syncytial Virus - Recombinant RSV (listed below) stocks have been produced previously by a collaborator and will be propagated and used for in vitro and in vivo infections to study the impact of human immune genes and viral genes on the intrinsic immune response. This will include use of these viruses in Cas9 modified cell lines and AAV/lenti infected mice to investigate impact in the context of ISG modification as described.

Murine cytokine -
RSV-mIFN-g - murine IFN-g gene inserted
RSV-mIL2 - murine IL-2 gene inserted
RSV-mIL4 - murine IL-4 gene inserted
RSV-mGMCSF - murine GM-CSF gene inserted
RSV-mIL-18 - murine IL-18 gene inserted
RSV-mIL-12 - murine IL-12 gene inserted

Additional RSV expressing alternative murine cytokines will be produced as necessary.

Reporter -
RSV-GFP - GFP gene inserted
RSV-RFP - RFP inserted

Gene deleted -
RSV-NS1ve - NS1 gene deleted
RSV-NS2ve - NS2 gene deleted
RSV-NS1NS2ve - NS1 and NS2 genes deleted
RSV-SHve - SH gene deleted

Gene mutated -
Point mutations in RSV genes to determine gene function

No recombinant Influenza will be used.

1) CRISPR-Cas9 modification of cells in vitro:
The vectors used for CRISPR-Cas9-mediated genome alteration in vitro will be non-mobilizable DNA plasmids. These will encode both bacterial selection markers (e.g. ampicillin resistance) and either additional selection markers (e.g. puromycin resistance for selection in mammalian cell culture) or encode fluorescent proteins for selection via FACS. Vectors will allow expression of the Cas9 endonuclease and Cas9-targeting guide RNA sequences. Cas9-expressing vectors may include commercially-available constructs such as pSpCas9(BB)-2A-Puro (PX459) V2.0 and pSpCas9(BB)-2A-GFP (PX458). Vectors are subject to change as required to optimise the production of the described GMMs, e.g. alternative Cas9 types, selection markers, or number of Cas9/DNA repair-targeting elements encoded on a single vector.

2) Adeno-associated virus vectors:
AAV particles will be produced in HEK293 cells via triple transfection protocols. A Recombinant AAV2-derived plasmid will be sourced from Addgene or Cambridge Biosciences (e.g. pAAV-sh(control) - Addgene #75438). This will contain the terminal repeat sequences of the AAV genome required for packaging but will not contain the Rep or Cap sequences found in wildtype AAV. This renders the particles unable to replicate. These repeats will instead flank a pol
III promoter upstream of an shRNA sequence or sgRNA sequence and a selectable marker (e.g. GFP) downstream of a pol II promoter. For CRISPR-mediated modifications Cas9 may be encoded as an additional element. For packaging of the above vector Rep and Cap elements will be supplied by an additional plasmid (e.g. pAAV-RC6 - Cambridge bioscience) and Adenovirus helper elements provided by a third plasmid (e.g. pHelper - Cambridge bioscience). These plasmids are not incorporated into the final virus particle. Recombinant AAV particles produced in this manner are able to infect dividing and non-dividing cells but are unable to become incorporated into the host genome or replicate in the host cells without the help of a co-infecting virus such as Adenovirus.

3) Lentivirus vectors:
Lentiviral particles will be produced using 3rd-generation packaging systems which split the required elements over 4 plasmids.

-> The transfer plasmid (e.g. pII3.7 - partial HIV type genome) contains the shRNA/sgRNA and a marker flanked by long terminal repeats required for packaging. These LTRs have been modified from wildtype to eliminate requirement for Tat co-activation of shRNA/sgRNA expression.
-> Packaging plasmid 1 - GAG-POL-RRE
-> Packaging plasmid 2 - REV

These two packaging plasmids encode elements required for virus replication and packaging. They are not incorporated into the virion progeny.
Endoence plasmid - The specific envelope protein to include on this final plasmid will vary and depend on the optimisation of the use of lentivirus particles in vivo for transduction. Different organs and tissues are better transduced by viruses with certain envelope proteins, e.g. GP64 has been shown to be more effective at transducing the murine lung than the classical VSV-G.

4) GM-RSV - RSV stocks as described above have been produced previously according to Bukreyev et al (J Virol. 1996 Oct;70(10):6634-41). Briefly RSV-specific gene-start and gene-end motifs were added to either end of a cDNA encoding the ORF of the gene of interest. This cassette was then inserted into the region between the G and F genes of complete positive-sense RSV antigenome. Recombinant virus was produced by transfecting HEP-2 cells with this antigenome and four plasmids encoding the N, P, L, and M2 ORFs separately.

Deletion mutants will be generated in genes involved in the type I IFN response. In addition to CRISPR-Cas9/shRNA use in vitro, AAV/lentivirus particles will be used to generate mutants and shRNA-knockdowns in vivo. Targeted genes include but is not limited to:

IFIC4L - Little characterisation
GBP1 - ISG and GTPase
GM RSV Murine cytokines involved in propagation and skewing of immune response - RSV-mIFN-g - murine IFN-g gene inserted.
RSV-mIL2 - murine IL-2 gene inserted
RSV-mIL4 - murine IL-4 gene inserted
RSV-mGMCSF - murine GM-CSF gene inserted
RSV-mIL-18 - murine IL-18 gene inserted
RSV-mIL-12 - murine IL-12 gene inserted
Additional RSV expressing alternative murine cytokines will be produced as necessary.

RSV-GFP - GFP gene inserted. Fluorescent reporter gene.
RSV-RFP - RFP inserted. Fluorescent reporter gene.

Gene deleted -
RSV-NS1ve - NS1 gene deleted. Viral gene involved in immune evasion.
RSV-NS2ve - NS2 gene deleted. Viral gene involved in immune evasion.
RSV-NS1NS2ve - NS1 and NS2 genes deleted

Gene mutated -
Point mutations in RSV genes to determine gene function
Deleted functional regions of RSV genes
No recombinant Influenza will be used.

Evaluation of foreseeable effects

1) All genetically modified human and murine cells will be loss of function mutants lacking an element of the intracellular immune response to pathogens. Human infection/colonisation by cultured cells is routinely mitigated by the use of microbiological safety cabinets, secondary containment during transport, and appropriate centrifuge spill/leak protocols. CRISPR-Cas9 vectors will be non-mobilisable. Environmental exposure of all GMMs will be limited. Any release of modified human or murine cells is unlikely to result in any damage to environmental organisms or spread of GMMs as they require supplementation with growth factors and specific temperature and atmospheric conditions.

2) AAV are generally non-pathogenic (Daya and Berns., 2008) and will be dead-end vectors unable to replicate upon infection.

3) Lentivirus vectors produced using 3rd generation systems (4-plasmid) are also unable to replicate but will retain the ability to integrate into the host genomic DNA. Modifications will not contain any known oncogenes or oncogenic sequences.

4) RSV is a human pathogen and may cause mild symptoms in humans and other primates. Human RSV is generally very species-specific. GM RSV to date have been recorded as less virulent than wt RSV. RSV may be present in mucosal secretions of experimental animals for a short time but is not able to spread to other animals due to low titre.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1) Liquid waste - Mammalian and bacterial culture waste will be treated with Distel at a final concentration of 1% for 10 minutes before disposal down the drain. RSV and Influenza have no known resistance to chemical disinfectants.

2) Solid waste - Tips and pipettes used to handle biological samples are soaked in 1% Distel for 10 minutes then placed in biobins and then into autoclave bags. All other plasticware used to culture biological material are placed directly into autoclave bags. All solid waste in contact with cells/tissue will be autoclaved at 121°C for 30 minutes.
All other solid waste such as tips, pipettes, tubes, and plasticware not used for either biological or GM material is placed in orange biobins or orange waste bags.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Reviewer 1
2.14 GMIC – 01106 is referenced in this section and is on RADAR as a risk assessment which includes recombinant GFP RSV and various cytokines for Prof Peter Openshaw. I cannot see one for Dr Tregoning. Therefore the addition of the recombinant RSV to this project under Dr Tregonings name will unfortunately require notification to the HSE as a new GM class 2 project.
2.15 Please add the RSV as a recipient species for the GFP and cytokines.
2.16 How has the recombinant RSV been produced? Is that included here?
2.17 Please add the changes made to the RSV, including inserts and deletions.
7.1 How is the RSV and influenza treated?
14.1 Why is the recombinant AAV and lentivirus GM class 2? From the risk assessment it looked as if it was headed for being GM class 1? If it is class 2 please explain more fully the hazards to human health and the environment in section 2.14 to 2.18. If these viruses are suitably attenuated and disabled and the inserts are not hazardous then I am happy for them to be class1.
Agreed that recombinant RSV is GM class 2.

Reviewer 2
Change history should be completed as this is an update
2.10 ACDP classification needs to be completed
4.4-4.6 need to be completed
5.7-5.8: spills should be reported on SALUS.
5.8: Provided RSV is not involved and given the low risk from aerosols caused by a spill of these bacterial cultures (non-pathogenic E.coli for plasmid propagation) it should be safe to open the incubator for cleaning after one hour. If RSV is not involved the safety advisors should be informed but are perhaps not needed for a decision on whether to open the incubator.
10.2 Needlestick protocol required (possibility of needlestick whilst inoculating animals)

Reviewer 4
Can you ask John to check his room numbers for the CBS facility? As I think these will need updating. Section 1.3. I don’t think the rooms are mentioned anywhere else.

Project Containment

| Laboratory Activities | Glass Houses | Growth Rooms |

02/03/2022
In this project we aim to optimise and characterise the larvae of the wax moth, G. mellonella, as a novel infection model for mycobacteria to study host-pathogen interactions and assess the antimycobacterial activity of potential antimycobacterial agents.

Mycobacterium tuberculosis is a highly successful human pathogen that currently infects a third of the world's population, causing 9 million new cases of tuberculosis and 1.5 million deaths annually. The close association of tuberculosis (TB) with the human immunodeficiency virus (HIV) pandemic, and the rising problem of multi-drug and extreme drug resistance have created difficulties for TB control programmes worldwide. Furthermore the current BCG vaccine, although providing some protection against disseminated disease in childhood, is largely ineffective in preventing TB in adults. There is thus an urgent need for a greater understanding of host and M. tuberculosis interactions, improved vaccines to prevent infection, and novel therapies to shorten the duration of treatment and target latent TB infection (LTBI). However this has in part been hampered by the current inadequate, costly and timeconsuming in vivo infection models for TB.
M. tuberculosis is challenging to study in vivo due to its complex pathogenesis and disease progression. Animal models such as mice, guinea pigs, rabbits, zebra fish and non-human primates have previously been used to study M. tuberculosis infection. However, no single animal model can replicate all aspects of M. tuberculosis pathogenesis e.g. while the mouse model is widely used, it cannot replicate granuloma formation, vital for the study of TB infection; guinea pig and rabbit M. tuberculosis models do form granulomas yet they are expensive and time consuming. In addition, there are ethical concerns about the use of mammalian models (especially non-human primates) in TB research, and they are associated with high maintenance and cost.

To overcome these issues there is an urgent need for a rapid, well-characterised, low-cost, high-throughput model that mimics the key features of M. tuberculosis pathogenesis to further understand host-pathogen interactions, that can also be used to screen novel drug candidates and assess the efficacy of vaccine candidates in early stage development. Invertebrate models such as Caenorhabditis elegans, Drosophila melanogaster and Galleria mellonella are becoming more widely used as infection models as they are considerably cheaper and ethically more acceptable. The larvae of G. mellonella has increasingly been used as an infection model to study a range of bacterial and fungal infections. G. mellonella has a wide range of advantages that have made it a successful infection model. These include its sophisticated innate immune system, comprised of cellular and humoral defences, that shares a high degree of structural and functional similarity to that of vertebrates, and its ability to discriminate between bacterial and fungal pathogens as identified by the repertoire of immune defence peptides. Other advantages include, large size (2-3cm) for easy manipulation and infection, low cost and maintenance, large group sizes can be infected for reproducibility allowing robust statistical analyses, rapid results, survival at 37 degrees Celsius (allowing human pathogens to be investigated), and no Home Office licence is required for larvae infection.

G. mellonella will be infected with genetically modified mycobacterial species to optimise and characterise the insect larvae as an infection model for mycobacteria. We will undertake a range of experiments including survival curves of both mycobacterial species and G. mellonella over time, mycobacterial survival analysis in the presence and absence of standard antimycobacterial agents and novel therapeutic compounds, histopathology staining and transmission electron microscopy (TEM), transcriptomic, lipid and proteomic analysis, IVIS in vivo imaging and primary haemocyte (G. mellonella phagocytic cells) cell culture infection assays. These studies will aim to characterise G. mellonella as a novel infection model for mycobacteria.

**Recipient or parental organism**

Mycobacterium tuberculosis - hazard group 3
- Clinical isolates- drug sensitive and multi-drug resistant strains, but not extensively drug resistant.
- Fully drug sensitive laboratory strains such as H37Rv, Erdman, CDC1551, H37Ra

Other Mycobacterium species - hazard group 2
Mycobacterium bovis BCG pasteur (vaccine strain), M. bovis BCG is naturally resistant to pyrazinamide, but otherwise sensitive to TB drugs. Other vaccine strains of BCG such as Danish Moreau, Montreal etc may also be used.

Non tuberculous mycobacteria reference strains/clinical isolates including
Mycobacterium abscessus
Mycobacterium kansaii
Mycobacterium chelonae
Mycobacterium avium
Mycobacterium intracellulare
Mycobacterium marinum

- Attenuated Mycobacterium tuberculosis auxotrophic mutants - class 2 GMO. Refer to the BIO1 risk assessment, section 2.14 for details of these mutants and justification for their use at ACDP CL2.

M. tuberculosis Bleupan (ΔleuD ΔpanCD)
M. tuberculosis (mc2 6020 ΔlysA ΔpanCD)
M. tuberculosis (mc2 6030 ΔRD1 ΔpanCD)
M. tuberculosis (mc2 7000 ΔRD1 ΔpanCD)

Host/vector system

pSMT3 and derivatives - ColE1 and pAL500 origins of replication. Not mobilisable
pYUB854 and derivatives - OriE. Non mobilisable
pMV261 and derivatives - OriE Non mobilisable
pHAE159, pHAE87 and derivatives - Derivatives of the conditionally replicating mycobacteriophage PH101 (ts;Bardarov et al., 97)
pGS201, pGS202 - details at http://webhost.nts.jhu.edu/target/plasmids.aspx
pKINTA and derivatives - Non-mobilisable integrating vectors

Origin & function

Inserted genes are self cloning of mycobacterial genes either in the sense orientation to allow over expression, or in
the antisense orientation to allow down regulation, under the control of either an inducible or constitutive promoter e.g.
down-regulation and also over expression of the 19kDa lipoprotein gene (Rv3762, involved in immune subversion),
Acr2 gene (Rv0251c involved in bacilary defence against oxidant stress). Mycobacteria do not express any toxins
and there is little risk of altering pathogenicity by this method.
Selectable markers include hygromycin (not clinically used as toxic to humans), kanamycin, spectinomycin, zeocin
(not part of first line therapy), sometimes in combination with the sucrose and the SacB enzyme as a
counterselectable marker.
Reporter genes include a range of fluorescent reporters such as GFP or mCherry, luminescent reporters such as the
lux operon from Phorhabdus luminescens or Vibrio harveyi, or the luciferase genes from Firefly or Gaussia princeps.
Genes have been inserted into Mycobacterium tuberculosis clinical isolates that are present in the reference strain
H37Rv but naturally deleted in the clinical isolate. The pSMT3 vector has been used that encodes the open reading
frames for genes including Rv 0180 (Function unknown, probable conserved transmembrane protein), 1519 (probable
conserved hypothetical protein), 3019 (Function unknown - Secreted ESAT-6 like protein EsxR, 3020 (function
unknown - ESAT-6 like protein EsxS), 3516 (Could possibly oxidize fatty acids using specific components), 3517
(Function unknown - conserved hypothetical protein), 3738 (Function unknown - PPE family protein PPE66), 3739
(Function unknown - PPE family protein PPE67). Deleted genes in M. tuberculosis and M. bovis BCG will include
genes such as the DosS/ DosR (Rv3132c and 3133c) which is essential for dormancy survival and regulation.
We will also use live attenuated M. tuberculosis auxotrophic mutants that have the following gene deletions and
antibiotic resistance genes;
M. tuberculosis Bleupan ΔleuD ΔpanCD hygR (Sampson et al, Infection & Immunity
M. tuberculosis mc2 6030 ΔRD1 ΔpanCD hygR (Sambandamurthy et al, (2005) Infection & Immunity 73:1196-1203;
M. tuberculosis mc2 7000 ΔRD1 ΔpanCD unmarked (Sambandamurthy et al, (2005) Infection & Immunity 73:1196-

Evaluation of foreseeable effects

GMMs were created previously as detailed below and under reference GM77/16.
Mutants have been created by either the deletion of all or part of target genes and the introduction of a selectable marker, by double cross overs and homologous recombination. This has been done using mycobacteriophage or suicide plasmids, usually with a two-step selection procedure such as sucrose plus selection for an antibiotic marker such as kanamycin or hygromycin.

Reporters, such as genes for luminescence or fluorescence, have been introduced into class 2 and class 3 mycobacterial strains by electroporation of plasmid DNA which will be either maintained epimysally by a mycobacterial origin of replication or through integration into the attB phage attachment site. Reporters and mycobacterial genes have been over-expressed, or knocked down using antisense and inducible systems such as tetracycline or acetamide.

Genetic deletions in defined Mycobacterium tuberculosis clinical isolates (class 3) have also been complemented by introducing the intact genes present in the reference strain Mycobacterium tuberculosis H37Rv.

Deleted genes with known function such as DosS / DosR (Rv3132c and 3133c), essential for dormancy survival and regulation, and have been deleted from Mycobacterium bovis BCG (class 2) and M. tuberculosis H37Rv (class 3). We will also use genetically modified live attenuated Mycobacterium tuberculosis auxotrophic mutants (class 2 GMO) under containment Level 2. These defined M. tuberculosis strains with already targeted mutations render them highly attenuated and suitable for use under containment level 2 conditions. No further modification will be undertaken. All four of the strains carry two independent mutations, rendering them all auxotrophic for pantothenate, in combination with either leucine or lysine auxotrophy, or the deletion of the RD1 region responsible for the major attenuation of M. bovis BCG. The auxotrophic strains are well characterised and highly attenuated. Extensive safety testing has been performed on all the 4 strains; attenuated Mycobacterium tuberculosis leuD ΔpanCD hygR, attenuated Mycobacterium tuberculosis mc2 6020 ΔlysA ΔpanCD hygR, attenuated Mycobacterium tuberculosis mc2 6030 ΔRD1 ΔpanCD hygR, attenuated Mycobacterium tuberculosis mc2 7000 ΔRD1 ΔpanCD unmarked. (references Sampson et al, Infection & Immunity 2004 72:3031–3037; Sambandamurthy et al, Nature Medicine 2002 1171-1174; Sambandamurthy et al, 2005 Infection & Immunity 73:1196-1203.

There is no evidence that any of the recombinant mycobacteria made in this project is any more hazardous than parental strains.
Any liquid non-GM waste which is not infected with mycobacteria (e.g. unwanted haemocytes in CL2 tissue culture) is to be discarded with 2% virkon final concentration and left for 30 mins-1 hr before disposing via the drain. Any GM waste will be placed in autoclave bags and autoclaved. Any solid waste items not infected with mycobacteria or haemocytes will be discarded into orange bags for disposal via clinical waste.

All G. mellonella (whether infected or uninfected with mycobacteria) will be stored double contained at -80°C for a minimum of 24 hours to achieve a kill and then will be placed in autoclave bags and autoclaved at the desired sterilisation temperature of 121 degrees C for 15 minutes. Autoclaved waste is bagged as described as above. Autoclaves are serviced twice per year, and validated and insurance inspection once per year in accordance with Imperial College London regulations.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022

Page 2399 of 15326
Following the discussion of your proposal entitled “Galleria mellonella - a novel infection model for Mycobacteria tuberculosis aimed to replace animal infection models in experimentation.” (GMIC-8826) at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

1. Move the last paragraph to the top.
1.1 Remove zebrafish from list of mammalian models.
2.16: Add vectors
2.17: Add inserts
5.9: Describe what actions will be taken should there be a needle stick injury include that Occupational Health will be contacted.

Comments: SN gave a brief description of the work.
MS asked what length of time would the larvae be holdings for?
SN answered 2 weeks.
MS queried what conditions are required for the larvae to develop into the Moth stage?
SN explained from larvae, to pupae, to moth they would require a temperature of 30-32 degrees Celsius. To prevent this, the larvae will be kept at 19 degrees Celsius, the pupae stage is 1-9 weeks and the infection will reduce maturity. This will give them enough time to notice development, at which point they will be moved to the -80 freezer for killing and then autoclaved.
MB asked if the larvae would be double contained?
SN confirmed that they would be and the petri dish would loosely taped to keep them closed.
MS checked if they will be using syringes and if they would be blunt.
SN explained they would be using Hamilton syringes. In the CL3, a sponge will be used to attach the larvae to and a disposable loop to hold them down before injecting.
MS asked if the needle part of the syringe be re-sheathed?
SN confirmed they would be.
MS asked about using a large falcon?
SN answered yes, 50ml.
MS wanted to know what was the process if there was a needle stick injury?
SN explained they would encourage it to bleed and run it under water.
MS suggested including that Occupational Health will be contacted in this process.
MB pointed out that boundaries between the strains/inserts and containment level 2/3 needed to be clear. The strain/inserts should also be listed in more details.
MB also asked what work would take place in Dr Brian Robertson’s lab?
SN will be using the IVIS in his lab.
Agreed: Class 3, Containment level 3. This is notifiable to the HSE.
Could you please ensure that the risk assessment is revised and the amendments

### Project Containment

<table>
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<tr>
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<td>L4</td>
</tr>
</tbody>
</table>

02/03/2022
The aim of this project is to evaluate the innate immune responses during Zika virus (ZIKV) infection in vitro and investigate the determinants of inter-strain differences in virus modulation of those responses. Initially based on studying ZIKV inter-strain differences in innate immune responses (ex vivo and in vitro), the project needs to encompass GM construction and analysis of inter-strain (intra-species) chimaeras.

Recipient or parental organism

Zika virus (ZIKV)

Host/vector system

Infectious cDNA clones of pathogenic Brazilian ZIKV (HG2) strains Paraiba 01/2015 (Tsetsarkin et al., 2016, 2016 Volume 7 Issue 4 e01114-16) and/or BeH819015 (Mutso et al., Journal of General Virology 2017;98:2712–2724 DOI 10.1099/jgv.0.000938)

Origin & function

Homologous inserts from other strains/isolates of ZIKV (from cDNA or constructed synthetically) or directed
mutagenesis/editting (in vitro mutagenesis or CRISPR-mediated) to achieve same outcome.

**Evaluation of foreseeable effects**

ZIKV (pathogenic field or attenuated/vaccine strains) will be recovered from cDNA after transfection of permissive eukaryotic cells.

Knock-out or mutated versions of above ZIKV strains (by in vitro mutagenesis, including CRISPR, or by synDNA replacement); some will represent intra-species, inter-strain chimeras.

The mutations are found in different pathogenic strains which appear to share nearly identical clinical and epidemiological characteristics yet display subtle differences in innate immune responses (e.g. qualitative and quantitative differences in induction of IFN responses during in vivo and ex vivo infections). The aim is to associate these subtle differences with particular amino acid substitutions. Mutant viruses will have different combinations of mutations causing no more than a handful of amino acid substitutions (our comparison focuses on the Asian lineage). As such, none of the resultant viruses is expected to be more hazardous than parental pathogenic viruses.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste:
- Low titre/Low protein: High volumes (>250ml) of low titre, low protein liquid waste (e.g. PBS used to wash virus from cells or tissue samples) will be treated with 1% Distel (final concentration) for 30 minutes, then disposed of down the sink.
- High titre or high protein (e.g. following blood separation, discarded cells from viral stock culture): centrifuge tube used for procedure to be capped and tube placed in a sealable, hard plastic, leak-proof container ("sweetie jar") also used for solid waste produced in the MSC. In the case of residual tissue samples, excess tissue will be placed in a centrifuge tube, the lid sealed in place and the tube placed in the sweetie jar. Filled sweetie jars will be sealed and autoclaved.

Solid waste:
- Disposable plasticware (pipette tips/small tubes/tissue culture plates etc): will be placed in a sealable, hard plastic, leak-proof container ("sweetie jar") maintained in the hood. Sweetie jars will contain 200ml 1% Distel. Once filled, sweetie jars will be sealed ready to be autoclaved. The 1% Distel is present in case of a spill during transportation of sweetie jars between the CL3 and autoclave facility.
- Serological pipettes: All serological pipettes will be filled with 1% Distel in a 2L beaker maintained in the hood. At the end of the work period, pipettes will be removed into an autoclave bag, sealed and double bagged prior to autoclaving.
- Sharps: All surgical blade will be removed with a tamper-proof blade remover which will be placed in a standard rigid sharps container and sealed in a double bag. These will then be autoclaved.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N
Following the discussion of your proposal entitled “ZIKV innate immunity.” (GMIC-9012) at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

1.1. Is the virus infectious to humans from culture, for example by needle stick injury, or does it need to be activated or modified by transmission through a mosquito?

1.2. How long are the primary human cell lines cultured for?

2.18. What signs of a more hazards GM virus are you looking for? This needs to go into the COP

4.6. If this is a disposable scalpel why is the blade removed? If this is normal practice how is the blade removed without risk to the user?

4.6. How long are the primary tissue samples and CD4+ T cells from unscreened material cultured for? If over 96 hours is HIV propagation possible?

4.14. Isn’t the dissection of tissue from Zika patients using sharps the most hazardous process? Unless I am mistaken and according to your own text in 4.10 It isn’t an airborne pathogen so an aerosol isn’t a primary concern.

4.18. Is the creation of the chimeric virus something that could occur naturally if African and Asian strains co infected the same human patient?

4.21. IS there a risk of airborne infection from Zika? In 4.10 you do not list this as being a route of infection.

5.3. Last paragraph missing the 3 from CL

5.7. Lab 45? Should this be 450?

5.12. With the exception of 3% paraformaldehyde have these methods been validated as effective?

5.13. Is the sorting with live virus? Is there an SOP?

6.4 3 missing after CL

6.5 and 6.6 " 3 lab " missing after CL

7.1 and 7.4. are there any large lumps of recognisable human tissue for disposal?

10.2. Check these emergency numbers especially the OH one

Amend 14.1

Agreed: GM Class 2. This is notifiable to the HSE.

---

**Project Containment**

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<td>L3 L4</td>
<td>L2 L3 L4 L2</td>
</tr>
<tr>
<td>Animal Units</td>
<td>L2 L3 L4 L2</td>
<td>L2 L3 L4 L2</td>
</tr>
<tr>
<td>Large Scale Activities</td>
<td>L2 L3 L4 L2</td>
<td>L2 L3 L4 L2</td>
</tr>
<tr>
<td>Human Clinical Applications</td>
<td>L2 L3 L4 L2</td>
<td>L2 L3 L4 L2</td>
</tr>
</tbody>
</table>
## Project Additional Information

### Purposes of the contained use

To generate knockout, complementation and other relevant mutants e.g. fluorescence reporter mutants, of potential vaccine candidates identified via reverse vaccinology 2.0 (cloning of antibodies from patients recovering from disease/healthy volunteers). Mutants will be used to unequivocally identify/validate the targets of functional antibodies and confirm these targets as potential vaccine candidates.

### Recipient or parental organism

- Streptococcus pneumoniae
- Neisseria gonorrhoeae
- Acinetobacter baumanii
- Streptococcus pyogenes (Group A Streptococcus)

### Host/vector system

- pUC-based plasmids and derivatives (Col-E1 plasmid) - pGEM-T, pBluescript etc.
- pET-based expression vectors
- pACYC184 and derivatives
- pMIDG and derivatives
- Suicide vectors (e.g. pKNG101 or pEX series of plasmids).
- Transposons e.g. Mariner based transposon system or Tn5, Tn7 derivative plasmids.

### Origin & function


The inserts will primarily be genes encoding putative vaccine candidates of pathogens listed in section 7 above identified through antibody cloning work. Inserts will be genes inactivated by disrupting the reading frame i.e. deleting the gene and eliminating presence of its product in the cell.

Evaluation of foreseeable effects

No GMO is anticipated to be any more hazardous than wild-type strains. Genes will not be deleted if known to increase pathogenicity or host range. None of the genes of current interest to my group encodes a toxin or other product anticipated to increase the toxicity of any non-virulent recipient strain. Indeed, many strains produced will have deletions in genes, which are required for optimal growth and virulence of the organism, and mutants are expected to be less virulent than wild-type recipients. Genes conferring resistance to antibiotics used to treat clinical infections will not be used as markers.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be autoclaved (134°C for 10 minutes) or treated. Tissue culture waste will be treated with Virkon (Minimum 1% final concentration) for at least 1 hour before disposal down the sink.

Solid clinical waste (excluding microbiological waste) will be discarded into orange waste bags. Solid microbiological waste will be discarded into autoclave bags and sterilised by autoclaving (134°C for 10 minutes) before disposal.

Following autoclaving, solid waste will be transferred (double-bagged) into orange clinical waste bags and disposed following the route for clinical waste.

Packaged sterilised solid waste will be stored in waste collection bins prior to disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022 Page 2405 of 15326
### Project Additional Information

**Purposes of the contained use**

Bacterial lung infections are a significant cause of morbidity and mortality. Particularly in individuals with underlying co-morbidities e.g. Cystic fibrosis, COPD, asthma and diabetes are more susceptible to bacterial lung infections. We are interested in the interactions between the bacteria and the host, looking at how the host tries to control the bacterial infection and how the bacteria tries to evade this. Using both in vitro and in vivo systems, we wish to define differences between healthy individuals and those with underlying co-morbidities that leads to greater disease susceptibility to bacterial lung infection. We will look at 4 bacterial pathogens: Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and non-typeable Haemophilus influenzae - ntHi (hazard group 2 pathogens). All of these bacteria can be carried as part of the normal nasal/ host bacterial flora. We will also look at mouse pathogen citrobacter rodentium (ACDP hazard group 1).

Staphylococcus aureus is a major cause of hospital acquired infection, particularly bacterial pneumonia and wound infection. Bacterial pneumonia is a significant cause of morbidity and mortality. Attention has focussed in particular on hospital-acquired, antibiotic-resistant, bacterial infections, a government health priority. However, there is a lack of good in vivo models of bacterial infection in the lung. This project will develop in vivo models of S. aureus infection and then investigate the immune response to it and approaches to control it using vaccines or antimicrobial therapies. Both Methicillin Sensitive S. aureus (MSSA) and Methicillin Resistant S. aureus (MRSA) will be used.
Pseudomonas aeruginosa is able to infect a wide variety of hosts and tissues and is an excellent model for studying infection by gram-negative bacteria. It is particularly prevalent in cystic fibrosis patients. We will be using in vitro models of infection using airway epithelial cells and differentiated airway epithelia to assess the effect of altering physiological conditions on bacterial growth. We will then assess these conditions and drugs that can alter them using in vivo mouse models.

Haemophilus influenzae is serotyped according to the capsular antigens. Strains without a capsule are described as the non-typeable (ntHi). In general the non-capsulated strains are less virulent than the capsulated strains. Noncapsulated H. influenzae is often observed in the airways of patients with chronic obstructive pulmonary disease (COPD). We are interested in extending Haemophilus influenzae vaccination to ntHi strains. We will then assess protection of vaccines using in vivo mouse models. We will also assess ntHi as an agent of co-infection with other pathogens.

Klebsiella pneumoniae is primarily a hospital-acquired bacterial pathogen that causes pneumonia, urinary tract infections and septicemia. Its success is related to its ability to form biofilms on medical devices, such as catheters. We will investigate how Klebsiella biofilm formation effects virulence and how the metabolic profile changes. Citrobacter rodentium is a murine pathogen that belongs to a family of enteric pathogens, including enteropathogenic E. coli (EPEC) and enterohaemorrhagic E. coli (EHEC), which utilize attaching and effacing (A/E) lesions to colonize the gastrointestinal tract of the host.

Deletion mutants will be made, but genes will not be deleted if known to increase pathogenicity. Deletion mutants will be screened for the effect on pathogenicity in mouse models and if the mutants are more pathogenic than wild type they will be destroyed.

Recipient or parental organism

<table>
<thead>
<tr>
<th>Recipient or parental organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both long term lab strains and clinically isolated strains of bacteria will be used in this project. The strains below are those most commonly used for this study, but others may be used:</td>
</tr>
<tr>
<td><strong>S. aureus</strong> MSSA Lab Strains including: 8325-4 (NARSA NRS135) (and derivatives), Newman</td>
</tr>
<tr>
<td>MRSA lab strains including: Sanger 252 (NARSA NRS71), no toxin producing MRSA strain will be used.</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong> PAO1, PA14, PAK, PA103, TB, SG17 (Clone 0)</td>
</tr>
<tr>
<td><strong>ntHi</strong> Strains 12, 2019, 76, 3198, 5657, 7592, 162, 176, R2868 and 9274</td>
</tr>
<tr>
<td><strong>K. pneumoniae</strong> AJ218, B5055, B5055nm</td>
</tr>
<tr>
<td><strong>C. rodentium</strong> ICC168, ICC180, ICC306, ICC169</td>
</tr>
</tbody>
</table>

Host/vector system

<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
<td>The vectors used will be non mobilizable and replication incompetent and include:</td>
</tr>
<tr>
<td>1. Gene targetting by DNA plasmid complementation and homologous recombination in a non-replicative vector</td>
</tr>
<tr>
<td>2. Clean gene deletion by suicide vectors (e.g. pKNG101 or pEX series of plasmids)</td>
</tr>
<tr>
<td>3. Random transposon mutagenesis will be performed using Mariner based transposon system or Tn5 or Tn7 derivative plasmids</td>
</tr>
</tbody>
</table>

Origin & function

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Deletion mutants. Mutants will be generated or acquired with deletions of genes in areas including, but not limited to those involved in modulation of the host immune response e.g. Pseudomonas PIIA, S. aureus IEC, Chp, Sak, Sc; virulence factors e.g. Pseudomonas AprA, Cif, K. pneumoniae MrkH; normal metabolic function, e.g. Pseudomonas gltK, Edd, Opr; or antigenicity e.g. ntHi protein D. Complementation studies may be performed to recover function.</td>
</tr>
<tr>
<td>2. Reporter gene expression. Mutants will be generated or acquired expressing reporter genes, for example (but not</td>
</tr>
</tbody>
</table>
limited to) luciferase or fluorescent proteins.

Evaluation of foreseeable effects

The mutants generated will either be loss of function mutants or carry non-toxic reporter genes. It is not anticipated that bacterial strains produced in the laboratory are more virulent than strains that already exist in the environment. Indeed, many strains produced will have deletions in genes, which are required for optimal growth and virulence of the organism. Also, antibiotic resistance markers (carbenicillin, gentamycin, chloramphenicol, tetracycline, kanamycin and streptomycin markers) used in the laboratory do already exist in the host strains found in the environment and hence there is no risk of introducing new resistance genes to environmental strains.

Non GM Citrobacter rodentium is a murine pathogen and hazard group 1 in terms of pathogenicity to humans but once genetically modified it is included in this submission as a class 2 GMO under the terms of the GM contained use regulations because of the risk of infection to wild murine populations in the event of an accidental release. However it is not anticipated that any of the modified organisms will be more hazardous than wild type and as explained above many will be less virulent.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste: Tissue culture waste will be soaked in 1% (final concentration) Trigene / Distel solution for 30 minutes before disposal down the sink. Alternatively Virkon (minimum 1% final concentration) can be used.

Solid Waste: Plasticware including all tips, flasks, plates and pipettes used to handle biological samples are placed either in biobins, or directly into autoclave bags. All Solid waste in contact with cells/tissue will be autoclaved at 121°C for 30 minutes (cycle 3 mixed waste) total running time 1hr 30mins.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Following the discussion of your proposal entitled "Immunology of bacterial infection of the lungs", GMIC-2393 at the last GM Safety Committee meeting, the Committee request that the following amendments are made to the proposal prior to approval:

<table>
<thead>
<tr>
<th>Amendment</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3</td>
<td>Room numbers for the 4th floor labs? These will need to be inspected by Safety Dept before work starts. Done</td>
</tr>
<tr>
<td>2.2</td>
<td>First line states Animal tissues. The rest of the statement lists human cell lines. Please make it more clear what tissues and cell lines are in use. It is important that the risk assessment sets the limits of the work. While some statements might be generic about types of cell line (especially where there are too many to list) it must be clear that they are all of the same type or risk group. Have increased information about the cells/ tissues, have also included PBMC. Have stated that the cells/ tissues will not be greater than CL2.</td>
</tr>
<tr>
<td>2.15</td>
<td>It is important that the risk assessment sets the limits for the work. It may be acceptable to have generic statements about the host strains but the limits must be clearly stated for example regarding MRSA you might want to mention that no strain used will be toxin producing, if this is the case then this must be stated in the risk assessment in this section. Will you be using clinical isolates or long term lab strains? It is worth stating which early on in the risk assessment. See response to comments below, have put both and said they are well characterised rather than lab adapted strains. Please state the ACDP bio Hazard group for each organism (2 by the look of it) done.</td>
</tr>
<tr>
<td>2.16</td>
<td>Vector and plasmid names please, if the list is too long please give some examples and set the limits of the work, e.g. Stating that they are non mobilizable, replication incompetent etc. Done.</td>
</tr>
<tr>
<td>2.17</td>
<td>Please give some examples of the types of genes you will be deleting. Also make sure that you mention all the types of genes you will be altering. Your current statement just says &quot;including&quot; and then lists some generic areas of functionality. Have put in a few examples, but still would like it to be broad. Also put a statement in 2.14 to the effect that if increased pathogenicity observed strains will be destroyed and that known increases will not be made. Again it is important that the limits are set. For example you may wish to state that no genes will be targeted whose deletion might lead to an increase in virulence, host range or pathogenicity. Done in 2.14 and stated we will not overexpress toxins.</td>
</tr>
<tr>
<td>5.1</td>
<td>Will you use an MSC at any stage other than cleaning up in the event of a rotor spill? Yes You identify several aerosol risks. Item 1. Substitute should for must. Done Item 2 Why use flip top tubes? If it is practicil to do so, why not remove the hazard and use screw top with an O ring.</td>
</tr>
</tbody>
</table>

02/03/2022
seal? This also reduces the risk of aerosol from leaks during centrifugation Done.

Item 3 How will you tell if there is a leak? Have put after an imbalance

After discussion all the above will be performed in an MSC

5.2 So material is always transported in the lab in conical screw cap flasks? Never in multi well plates, eppendrors, or petri dishes? This looks like the answer to a different question.

It was, have changed it to say: When moved within the lab, material with a liquid component will be moved in falcon tubes with sealed screw caps in tube racks or in flasks with screw cap lids.

Cultured biological material will be transferred from incubators to MSC in sealed vented flasks or plates with lids in secondary containers in the same laboratory. Petri dishes will be sealed with parafilm after incubation.

5.2 – how will this be contained in transport. See above

5.3 – add the term double contained? Done

5.6 Room number for the –80 lab. Done 463

-VOLUME OF STOCKS AND STORAGE CONTAINERS (BOTH PRIMARY/SECONDARY CONTAINERS)? Biological agents may be stored as glycerol stocks at -80 in lab 463. 2 aliquots of 1ml stored in cryovials will be stored per strain. The cryovials are stored in plastic lidded boxes.

5.7 Will rotors always be opened in the MSC? Is this practical for the fixed angle rotors? For swing out rotors with larger volumes (e.g. 50ml or 15ml falcons) the buckets will be opened in MSC. For fixed angled rotors, these will be opened on the bench or in the centrifuge.

Remove the term tissue culture cabinet and replace with microbiological safety cabinet or MSC. This applies to all uses of the term tissue culture cabinet.

Why 2% virkon but only 1% Trigene? Any spill will dilute this to less than 1% and below the manufacturers recommended minimum effective concentration. Changed to 2% throughout.

– First paragraph looks like the answer to the incubator question- have removed this and put in incubator section

- How are the flasks sealed? Screw cap. Tissue paper will not be a good secondary container in a spill.

5.10 Where are the animals infected with the bacteria, in cbs? In an MSC? Yes

5.10 - yes and no? Which is it? Yes

5.13 Any lone working? Should be as per the FoMed Lone Working Policy. Done

6.5 Use of Eye protection while working with P. aeruginosa at all times needs to be emphasised. (college has had several eye splash incidents) Put in 5.1and re-emphasised in 6.5

7.1 Virkon 2% is this diluted to 1% final concentration with waste liquid? Yes

7.2 – incomplete answer, need to answer the first question. Hopefully it is yes, Yes

8.1 MSC – KI once per year, airflow checks using a 100mm vane anemometer twice per year by an engineer and monthly by the operators where the MSC is used to provide operator protection. Added

-shaking incubator maintenance? Annual
9.1 – is competency assessed? Yes, put by me
10. Remove the term tissue culture cabinet and replace with microbiological safety cabinet or MSC. This applies to all uses of the term tissue culture cabinet.
Why 2% virkon but only 1% Trigene? Any spill will dilute this to less than 1% and below the manufacturers recommended minimum effective concentration. Changed throughout
13 Is all the work only carried out in one lab? Section 1.3 seems to indicate more labs are in use but does not list them. Done
14 Please complete this section. It is Class 2. Done
Comments: JT gave a brief description of the work. MS suggested building in a proviso to the risk assessment that no knockouts will be conducted that will knowingly increase virulence. Added
PF advised giving a few examples of the altered genes in 2.17. Added: 1. Deletion mutants. Mutants will be generated or acquired with deletions of genes in areas including those involved in modulation of the host immune response e.g. Pseudomonas PiA, S. aureus IEC; Chp, Sak, Sc, virulence factors including Pseudomonas AprA, Cif, and normal metabolic function, these include Pseudomonas gltK, Edd, Opr or antigenicity e.g. nTH protein D. Complementation studies may be performed to recover function.
PF Increased pathogenicity….Have mentioned this will not be done
PL suggested adding that Toxins will not be over-expressed, this should be mentioned in the form to set boundaries. He also recommended the mariner based transposon system for future collaborations with his group. Done
MS asked if the consequences of co-infections had been addressed and to give this some thought. Co-infections unlikely due to viral work occurring in MSC and bacterial work on bench.
PL Remove the term 'Lab adapted' and change to 'widely used' or 'well characterised' Done in 2.10 and 2.11
IH advised adding the current room numbers to the form and when moved to new location; a Form C can be completed to update. Done
PL pointed out that the statement about markers used for making mutants, should state "genes conferring resistance to antibiotics used to treat clinical infections will not be used as markers". Done
IH recommended adding the phrase to the hosts, "not using toxin producing strains." Also, to provide some examples for the vectors.
TN to check the formulations of distell. Trigene highly effective against strains described <log5 reduction at 1:200
IH suggested defining at what point in the experiment would the work be carried out in the MSC. I have put in 5.1 that work will be performed on the bench.
PL queried if JT had worked with the MRSA strain on the bench.
JT confirmed he had in the past, but not at ICL.
PL advised change the word ‘should’ to ‘must’ in 5.1 Done
Agreed: Class 2, Containment Level 2. This project is notifiable to the HSE.

---

**Project Containment**

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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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02/03/2022
## Project Additional Information

### Purposes of the contained use

#### Recipient or parental organism

#### Host/vector system

#### Origin & function

#### Evaluation of foreseeable effects

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

---

**Historical Significant Changes**

After consideration A Hemara-Wahanui said this should stay with GM77 (15/5/2006). Re: further to telephone call 16/6/06 - advised this project to be moved to GM 8. (16/6/06).
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 77/94.4a

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Tick if notifying a connected programme of work  

02/03/2022
Historical Significant Changes
GM77/95.2, GM7794.6, GM77/95.4, GM77/97.3, GM77/95.3, GM77/94.5, GM77/95.1

Historical Date of Additional Info
01/08/1995, 04/10/1994,
30/05/1995, 04/06/1997,
04/01/1995, 08/08/1994,
21/06/2002, 12/12/2005

Significant Change ID

Date of Significant Change

---

**Project Additional Information**

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

---

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 77/94.4c

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<td>28/07/1998</td>
<td>GENE CLONING IN M BOVIS BCG (GENE CLONING IN NON-PATHOGENIC MYCOBACTERIAL STRAINS)</td>
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**Historical Significant Changes**

- Transferred to GM8 on 12/12/2005

**Project Additional Information**

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 77/96.3

Date Ackn'd 19/12/1996

CU2 Project Title STUDIES OF PATHOGENICITY OF SHIGELLA FLEXNERI

Class 2

Date Project Ceased 16/01/2009

Consent Granted not applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 77/97.1

Date Ackn'd 11/04/1997

Date Project Ceased

CU2 Project Title MECHANISMS OF ANTIGEN PRESENTATION TO CYTOTOXIC T LYMPHOCYTES

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 77/99.1

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<th>CultureVolumeClass3-4</th>
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<td>05/08/1999</td>
<td>INVESTIGATION OF VIRULENCE DETERMINANTS OF HAEMOPHILUS DUCREYI</td>
<td>Class 2</td>
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Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
The aim of this project is to utilize a replication incompetent adenoviral vector to express inserted genes to elicit immune responses to the inserted gene products. The vectors under development will be based on the adenovirus family of respiratory viruses. Serotypes expected to be used include the Ad2, 4 and 5. All will be replication incompetent. Any serotype utilized will be gene E1a, E1b and E3 deleted, thereby preventing the replication of the adenovirus after cell entry. The adenoviral life cycle does not normally involve integration into the host genome, rather they replicate as episomal elements in the nucleus of the host cell and consequently there is no risk of insertional mutagenesis.

Unmodified Ad5 is classified in hazard group 2 under the COSHH regulations but the E1a gene deleted forms are classified as hazard group 1. Although recombination with an unmodified virus in vivo is a possibility the further deletion of the E3 gene renders it extremely unlikely that even in the event of such a rare recombination occurring that the recombinant will be packaged with viral coat proteins and secreted from the cell.

Host/vector system

Cells used to propagate the recombinant adenoviruses are 293 cells. These are human embryonic kidney cells that have been transformed with the adenovirus genes E1A.
and E1B, which are required for adenovirus propagation.

Origin & function

The genetic material to be inserted will be encode HIV proteins or natural murine cytokine proteins. In the case of murine derived genetic material, RNA will be isolated, first strand cDNA prepared and primers specific for the gene in question will be used in a polymerase chain reaction to amplify the target sequence. This material will then be inserted into the E3 region of the gene deleted adenoviruses. The selected genetic material will generally constitute an open reading frame of a particular protein that we expect to be fully transcribed using the host cell transcription machinery. Translation of the resulting RNA transcripts into fully functional murine protein is expected. We intend to use these expression vectors to produce physiologically relevant quantities of the murine proteins in vivo in the mouse. These proteins are expected to have various degrees of immuno-modulatory activity, although it is expected, based on current literature, that most will have no detectable effect.

HIV genetic material will be derived from current plasmid vectors that contain small parts of the HIV genome. This material can be sub-cloned from these plasmid vectors and inserted into the E3 region of the gene deleted replication incompetent adenoviral vector.

All the inserts that will be incorporated into the gene deleted adenoviral vector will not have any known oncogenic potential.

Evaluation of foreseeable effects

The adenoviral life cycle does not normally involve integration into the host genome, rather they replicate as episomal elements in the nucleus of the host cell and consequently there is no risk of insertional mutagenesis. Unmodified Ad5 is classified in hazard group 2 under the COSHH regulations but the E1a gene deleted forms are classified as hazard group 1. Although recombination with an unmodified virus in vivo is a possibility the further deletion of the E3 gene renders it extremely unlikely that even in the event of such a rare recombination the recombinant will be packaged with viral coat proteins and secreted from the cell. When injected into mice it is possible that the gene-deleted adenoviruses may come into contact in vivo with endogenous murine viruses. While it is extremely unlikely that these replication-deficient gene-deleted viruses will be transcomplimented by any known murine virus, we will utilise specific-pathogen free mice that do not harbour murine viruses of the papovaoviridae genus that contain both murine polyomaviruses as well as human papillomaviruses.

The adenovirus vaccine vector itself will consist of E1a and E3 gene deleted adenovirus and an insert consisting of a DNA sequence encoding for small parts of the HIV genome. Typically, the vaccine vector contains a DNA sequence that encodes individual viral proteins such as the env (structural envelope glycoprotein), gag (matrix and capsid structural protein), rev (regulator of viral protein expression) or tat (regulator of HIV genome transcription) molecules. These small parts of the virus are expressed in such a manner that the essential minimal components of an infectious retroviral particle will not be present, particularly the intact HIV/SIV viral genome, thus preventing any possibility of inadvertently generating an infectious viral particle. There is no perceivable risk inherent in these proteins.

Other inserted cDNA, that will encode for natural, non-modified, mouse proteins will belong to the murine cytokine/chemokine family of secreted proteins. Other insertions may include proteins that have been shown to stimulate the innate immune system, including those that act at the cell surface as well as internal cytoplasmic proteins. These proteins have not been described to have oncogenic attributes in the published literature and are indeed non-modified and identical to those proteins expressed by normal murine cells. The proteins expressed from these constructs have between 30 and 70% identity with the corresponding human cytokine. However, most are not active or have only partial activity on human cells (COPE cytokine Database).

It is not expected that any of the insertions detailed will have an effect on the tissue tropism. However, the deletion of E3 has been shown to increase the immune response due to less down-regulation of the host cell MHC Class 1. The addition of cytokine insertions may also increase the immune response. As these effects are the precise criteria under investigation in our studies, it is impossible to predict the effects of augmentation of the immune response. It is possible that the resulting inflammatory response will be harmful. However, none of the insertions will be human genes, although murine cytokine/chemokine products may have some activity upon human cells. (COPE Cytokine Database).

Evaluation of foreseeable effects

We do not intend to use any GM animals or plants.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In view of the fact that all adenoviruses used will be replication incompetent but may have undetermined pathogenicity by virtue of the gene insert, it is appropriate for all manipulations involving this virus to be undertaken within a microbiological safety cabinet. It is expected that all large scale work with these GMOs will be done in a dedicated Category 3 facility, that has an autoclave within the lab. Small scale work (less than 100ml) may also be done within a microbiological safety cabinet in a category 2 laboratory. Liquid culture waste in both circumstances will initially be aspirated within the microbiological safety cabinet into a leak-proof container with Virkon S solution. The concentration of Virkon S will be maintained at greater than 1% - a concentration that has been validated as 100% virucidal for adenoviruses as well as most other viruses. Moreover, all waste materials (solid and Virkon S treated liquid) will be autoclaved in an autoclave maintained and appropriately certified to properly inactivate infectious waste. Autoclaved material is considered to be 100% effective at sterilization. The extra precaution of performing large scale preparative work in a category 3 laboratory with an internal autoclave ensures that large volumes of Virkon S treated culture solutions (which although almost certain to be 100% killed should be treated as potentially infectious material) cannot come into contact with other laboratory staff or any non-laboratory personnel. Any small volumes of culture performed in category will be treated with the Virkon S virucide and then contained within a leak-proof container for transport to the autoclave.

Any new staff would be specifically trained in the safety aspects of this work. Since the gene deleted replication incompetent virus is transmissible by an airborne route an inward microbiological cabinet airflow would be required.

The work involves standard laboratory protocols and there are no unusual procedures that require additional containment measures.

All work with these adenoviruses will be performed within a certified category 2 or 3 laboratory.

Mice injected with the replication-deficient gene-deleted recombinant adenoviruses will be maintained within isolators. All injections and handling of these animals after injection with the recombinant adenoviruses will be carried out entirely within a microbiological class 1 cabinet. Cells, blood and tissues harvested from these animals prior to schedule 1 euthanasia as well as post-mortem will be transported from the microbiological class 1 cabinet within the animal facility to a microbiological class 11 cabinet in our class II laboratory entirely sealed within a biohazard container. The exterior of the biohazard container will be sprayed with liquid Virkon disinfectant prior to removal from the microbiological class II cabinet to ensure no GMOs survive on the exterior of the container. Post-mortem, both the mouse carcass and the cage in which the mice lived will be autoclaved in an autoclave maintained and appropriately certified to properly inactivate infectious waste. Autoclaved material is considered to be 100% effective at sterilisation. The sterilized bedding contents of the cage and the mice carcasses will then be incinerated. The isolators that contained the cages will be fumigated.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been reviewed and approved by the St George's Hospital Medical School GMSC.

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THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY (UMIST)

Name

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY (UMIST)

Name 2

BIOMOLECULAR SCIENCES

Department

Building

P O BOX 88

District

SACKVILLE STREET

Road Name

MANCHESTER

Town

LANCASHIRE

County

M60 1QD

Postcode

ENGLAND

Country

Tel Number 0161 236 3311

Fax Number 0161228 7040

E-mail

HSE Division NORTHWEST

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
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<td>LANCASHIRE</td>
<td>M60 1QD</td>
<td>ENGLAND</td>
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## Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

  - Give brief details of the genetic modification safety committee

  - Level 1 (GMMs)
  - Level 2 (GMMs)
  - Level 3 (GMMs)
  - Level 4 (GMMs)
  - Non-microbial

  - Other (please specify)

- Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 79/00.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<td>INVESTIGATION OF THE BIOLOGICAL EFFECTS OF HEPATITIS C VIRUS PROTEINS ON HEPATOCYTES</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info GM79/00.1 transferred to GM 541

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
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02/03/2022
**Project Ref** 79/01.1

**CU2 Project Title**
INVESTIGATION OF THE ROLE OF MOLECULES INVOLVED WITH CELL FATE DECISIONS

**Date Ackn’d**
11/01/2001

**Date Project Ceased**
10/09/2004

** Consent Granted**
not applicable

**Historical Significant Changes**
Gm 79/01.1 transferred to GM 541

**Recipient or parental organism**
1) Primary cells of mammalian original.
2) Immortalised cell lines.

**Host/vector system**
1) Retroviral vectors such as PMX and pMSCV (see attached risk assessment)
2) Packaging cell lines such as phoenix

**Purposes of the contained use**
To generate retroviral particles containing DNA sequences of molecules involved with cell fate decisions. To use these retroviruses to transfect mammalian primary or immortalised cells.

**Historical Date of Additional Info**
Gm 79/01.1 transferred to GM 541
Expression of retroviral vectors in packaging cell lines will generate replication-defective viruses. Once these viral particles have been used to transfect recipient cells, supernatants from these cells will be screened for the presence of infectious virus.

Origin & function

cDNA of mammalian origin coding for molecules involved with cell fate decisions
Including: notch molecules 1-4
  notch ligands jagged 1/2, delta
  notch signalling molecules delta, csl
  notch modulatory molecules - the fringe family
  mutant forms of the above molecules

Notch molecules are thought to be involved with cell fate decisions in the presence of certain cytokines, notch delays differentiation and promotes the proliferation of primitive cell types. Notch 1 is a known oncogene and truncated, constitutively active forms of notch 1 have been found in T lymphoblastic neoplasms. Molecules which potentiate notch signalling may be expected to delay differentiation also.

Evaluation of foreseeable effects

Following retroviral transfection, cells will be monitored for the presence of infectious retroviral particles on a regular basis. Once it has been established that no infectious particles are generated by host (or recipient) cells, then transfected cells will be used for experimentation. Primary cells are routinely screened for the presence of infectious agents to reduce the risk of co-infection with more than one retrovirus. Primary cells will not be used from lab workers or their family members to the risk of engraftment of transfected cells likely routes of transmission of replication-deficient virus include aerosols and sharps injuries. See attached risk assessment. Accidental inoculation with replication-deficient virus is likely to result in the destruction of virus by the host immune system and therefore long-term injury is unlikely. Experimental waste containing virus particles and transfected cells will be autoclaved to minimise any foreseeable environmental effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

See section 7. Analysis of transfected cells will be performed under category 1 conditions only when the absence of infectious viral particles has been established.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Packaging cell lines, cell culture supernatants containing retroviral particles and transfected target cells will be treated with 1% virkon solution which will destroy any retrovirus or retroviral containing cells. (A 0.05% virkon solution is sufficient for retroviral destruction according to the manufacturer). Solid waste is also treated with 1% virkon solution for a minimum of 1 hour. Both liquid and solid waste is autoclaved in order to ensure a 100% kill of GM material. Departmental autoclaves are serviced annually using a thermocouple. Refer to attached virkon data sheet for further information.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The local GMSC considered that the work using retroviral vectors required containment level 2 because of the potential oncogenic properties of the inserts. Other aspects of the project e.g. construction of plasmids in disabled Ecoli K12 strains have been assessed separately by the GMSC.

## Project Containment

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## Project Ref 79/01.2

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<td>24/05/2001</td>
<td>TRANSFORMATION OF THE MALARIA PARASITE PLASMODIUM FALCIPARUM TO INVESTIGATE ITS FOLATE MATABOLIC PATHWAY</td>
<td>Class 3</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

### Historical Significant Changes

- **Historical Date of Additional Info**: GM 79/01.2 transferred to GM 541

### Project Additional Information

**Purposes of the contained use**

The folate pathway is an essential but complex metabolic pathway in malaria parasites that is involved in DNA synthesis and amino-acid conversion. We have identified and characterised most of the genes involved in this pathway and now aim to investigate the individual roles of these genes by transformation experiments. This will encompass experiments where individual genes are disabled, to assess whether their activities are mandatory for survival, and experiments where certain of these genes are modified, to assess the role of naturally occurring polymorphisms in a controlled genetic background. We have evidence that different parasite lines differ in their ability
To make use of preformed folate from the host, and transformation provides a powerful route to investigate such a difference. This has been demonstrated in other laboratories where transformation is now routinely used to investigate basic questions about the biology of the parasite.

Recipient or parental organism

Laboratory strains of Plasmodium falciparum that have been in vitro culture for many years, both in this and numerous other laboratories will be used. The genetic modification introduced by transformation will render them more resistant to the drugs pyrimethamine, neomycin or blasticidin (used as the plasmid markers), but will have no effect on their susceptibility to a range of antimalarial drugs currently in clinical use. The risk of laboratory infection is extremely low, but such drugs will be able to clear infection rapidly. The targeted gene modifications in the folate pathway are likely to reduce their general viability relative to the untransformed host, or leave it unaffected. The modifications will not alter host specificity.

Host/vector system

The plasmid vectors to be used are derived from the commercially available pBluescript and pGEM series of E. coli vectors. These vectors are modified to include the necessary elements for targeting the genes of interest and introduced via electroporation. They are maintained by selection using the drugs mentioned above. If such selection is removed, plasmids are lost, unless integration has occurred at the target gene locus.

Origin & function

1. Plasmid sequences plus partial sequences of target P. falciparum folate pathway genes designed to disrupt the corresponding gene of the recipient cell.
2. Plasmid sequences plus sequences of target P. falciparum genes designed to alter the target gene at known polymorphic loci.
3. Plasmid sequences plus putative promoter regions of P. falciparum designed to test the effect of possible varying levels of expression on folate gene products.
4. Plasmid sequences that carry reporter activities such as Green Fluorescent Protein (GFP) or Chloramphenicol Acetyltransferase (CAT) to rapidly test for successful transformation.

All plasmids are constructed via cloning in standard disabled E.coli hosts (principally XL1-Blue, Stratagene)

Evaluation of foreseeable effects

No new potential hazards are identified as a result of the genetic modification. As with untransformed P. falciparum, the only route for transmission to humans will remain as direct inoculation into the bloodstream of the operator. The natural route of infection, via the bite of the Anopheles mosquito, is excluded by the complete absence of the latter from the working environment (mosquitoes are not bred in this Institute). Even if such a mosquito were present, it could not feed from culture flasks. Moreover, it would be unable to ingest sexual forms of the parasite for onward reproduction and transmission of the parasite, as only asexual forms will be cultured. Airborne contamination, e.g. by aerosol spread from cultures, is also not a risk with this organism, and there is no possible person to person transmission. Viability of these organisms for experimental purposes is totally dependent upon careful maintenance of stringent laboratory culture conditions and there is no known or foreseeable risk to the environment, as the parasite cannot survive in any environment outside of the tissue culture medium and the narrowly confined parameters of temperature, pH, and gas atmosphere (5% CO/5% O/90% N).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We request derogation from full Class 3 containment because the Risk Assessment indicates that the following features of level 3 containment are not required, given the absence of an airborne contamination risk when working with this organism - see section 2 (Growth and propagation of the parasite); 3 (Assessment of risks to human health), and 4 (Assessment of risk to the environment), of the Risk Assessment.

1. Fumigation, and thus a sealable laboratory.
2. Airlock entry to the laboratory.
3. Negative pressure relative to the surrounding laboratories, and HEPA filtered air extract.
4. Autoclave in the culture facility. Biological material is completely killed by verified disinfection procedures before removal from the facility in sealed plastic boxes. These
are transported directly to the nearby Departmental Autoclaving suite and the material sterilised in situ.

5. Specific measures to control aerosol dissemination.

6. Shower within the culture facility.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1) Parasite microscope slides are treated with methanol + flaming before staining with dye (also in methanol) and viewing, rendering material on the slide harmless. After use, the slide are routinely treated with bleach (Chloros, in appropriate dilution) before disposal and areas such as the microscope stage are wiped with 70% ethanol after use.

2) Disposal of solid culture materials (plastic pipettes/tips/tubes/tissue culture flasks) is by placing the items in a separate labelled double biohazard bag kept in the tissue culture room which is subsequently directly removed from autoclaving. In the case of liquid waste (culture supernatants and residual cells), treatment is for 16-24hrs in a solution of bleach (Chloros) dilute to a final volume containing 10% free chlorine (1:3 v/v) before disposal. The container is then placed for autoclaving. Spills for infected material must be attended to immediately. The volume is reduced by use of dry paper towels (which must be placed for autoclaving) followed by treatment of the affected area with diluted (1:3 v/v) Chloros bleach followed by a wipe with paper towel soaked in 70% ethanol. The latter is important, as bleach is detrimental to many surfaces including stainless steel; for such surfaces, Virkon disinfectant can be used in place of the bleach, either as a solution or as powder.

3) Degree of kill: the parasite is an extremely fastidious organism entirely dependant for viability upon careful maintenance within host red blood cells in a stringent tissue culture medium under the atmosphere of a special gas mix. Lysis of the red cells, removal of the medium, exposure to normal air (as opposed to the 5% CO/5% O mix needed for culture), increase in temperature above approx. 39C, small changes in pH away from physiological, all lead to parasite death within a very short time. As established in microscopy, Chloros lyses all host cell within a short period, penetrating the parasites and killing them 100%. It is not possible to restore a viable culture from infected red cells treated in this way. Autoclaving also kills the parasite 100%. Autoclaving is carried out in our central sterilising facility in equipment employing data logging of all autoclaving cycles. The autoclaves are also checked regularly by internal indicator tablets included in the items for disposal that confirm the proper length and temperature of the cycle. In addition, thermocouple tests of appropriate loads are carried out as part of the annual autoclave service that is carried out by the manufacturer.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project has been considered by the UMIST GMSC. The Committee believes that the containment measures and working practices described in the Risk Assessment are appropriate for this work on Plasmodium falciparum.

Project Containment

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Animal Units Large Scale Activities Human Clinical Applications

02/03/2022
Project Additional Information

**Purposes of the contained use**

The application covers a range of specific projects with the overall goal to study the regulation and mechanisms of cell wall biosynthesis and its role in yeast-hypha morphogenesis of Candida albicans.

More specific goals:
1. Role of glycosylation
2. Role of the Ras/cAMP-dependent pathway
3. Role of specific O- and N-clycosylation enzymes in cell wall structure and adhesion

**Recipient or parental organism**

Recipient organisms include:
1. *E. coli* - many multiply disabled K12 derivatives
2. *S. cerevisiae* - many multiply disabled, non-pathogenic S288C derivatives
3. *C. albicans* (CA14, ura3; CA18, ura3 ade2 abd derivatives of these strains with further disabling markers (eg. glycosylation enzymes, components of the Ras/cAMP-dependent pathway).

**Host/vector system**

Vectors include:
1. Many non-mobilisable *E. coli* vectors (including pUC 18/19, pBLUESCRIPT, lac-based expression plasmids such as pET vectors)
**2/ Many non-mobilisable \textit{S.cerevisiae} vectors (including YEps, YCps, YIps and single-copy GAL-based expression vectors)**

**3/ Specific non-mobilisable \textit{C. albicans} vectors (single-copy integrating vectors PMB7, pDDB57, integrating expression vectors regulated by methionine pEXPa)**

**Origin & function**

The genetic materials in the modifications are all derived from naturally occurring organisms of \textit{E. coli}, \textit{S. cerevisiae} and \textit{C. albicans}. More specific Candida genes include:

1/ Glycosylation genes (eg. \textit{CaSRB1}, \textit{PMT1}, \textit{PMT2}, \textit{MNN1}, 2, 3, and 4)
2/ Regulated promoters (\textit{MET3}, \textit{MAL2})
3/ Auxotrophic markers (\textit{URA3}, \textit{ADE2}, \textit{LYS2})
4/ Components of the Ras/cAMP-dependent pathway (\textit{PDE1}, \textit{PDE2}, \textit{TPK1}, 2, 3, \textit{BCY1})
5/ \textit{Candida} genomic and cDNA libraries

**Evaluation of foreseeable effects**

\textit{Candida albicans} is a human pathogen in hazard group 2. \textit{Candida albicans} causes superficial infections of mucosal epithelia (thrush). However, at no time in the last 20 years since work with \textit{Candida} began, has a member of any lab had a thrush infection caused by a lab strain. \textit{C. albicans} also causes infections in severely immunocompromised patients and these can be fatal. However, it is important to remember that \textit{C. albicans} is widely distributed, being carried by over half of the population. It rarely affects healthy individuals. IOT only becomes a potential medical problem when the immune system of an individual is impaired. Furthermore, as with most pathogenic microorganisms, the virulence of \textit{Candida albicans} strains is likely to become attenuated rather than enhanced after prolonged laboratory culturing.

The strain \textit{Candida albicans} CA1-4 (\textit{ura3}) is the standard host for DNA transformation worldwide. Its uridine auxotrophy makes it avirulent (Leberer et al. 1996, PNAS, 93, 13217). Transformation with \textit{URA3} plasmids partially restores the virulence of CA1-4, but the restoration is not complete because the genes neighbouring \textit{URA3} remain inactivated. The \textit{URA3} marker is recycled for subsequent rounds of transformation (Fonzi and Irwin, 1993, Genetics, 134, 717). Therefore by necessity, some of the transformants we create are not genetically disabled, although their virulence is lower than the wild-type strains of \textit{C. albicans} carried by most individuals.

\textit{C. albicans} CA1-8 (\textit{ura3}, \textit{ade2}) is transformed with \textit{ADE2} or \textit{URA3} containing plasmids, leaving one remaining disabling mutation in the transformants. This is sufficient to render all of the transformants completely avirulent.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

We follow all safety guidelines for operation at containment level 2. Briefly:

All waste materials will be inactivated before disposal:

* liquid waste - autoclaving at 121 degrees C for 15 minutes (proven to effectively 100% kill fungal cells). The Department of Biomolecular sciences has two large autoclaves, one of which is to be replaced in January 2001. The next thermocouple tests will be carried out on both autoclaves when the engineers are in the department to install the new autoclave. The autoclaves have always been serviced annually by BMM Weston/Drayton Castle, thermocouple testing of various loads of waste is to be included in the annual service contract. The new autoclave will have a data logging system, the old autoclave has a chart recorder to monitor the conditions. Autoclave indicating rubes are placed in bins of contaminated materials each week.

* glass ware - soaked with Virkon (see attached information provided by the manufacturer) proven to effectively 100% kill fungal cells)

* plasticware - autoclaved under the conditions shown above. Before inactivation, plastics will be double-bagged for transport to autoclaves

* sharps will be minimised, but any contaminated sharps will be placed in separate CinBins, bagged and incinerated.
Once GMMs have been inactivated, normal routes of disposal will be used. These measures will reduce the likelihood of all hazards to negligible. The overall risk is therefore effectively zero with the proposed containment and control measures.

The UMIST GM safety committee has considered this project. The work with E. coli K12 and Saccharomyces cerevisiae is Class 1 but the Committee thought that the Candida albicans experiments required containment level 2 and were thus Class 2 activities.

Please enter comments on the GM safety committee on the risk assessment:

The UMIST GM safety committee has considered this project. The work with E. coli K12 and Saccharomyces cerevisiae is Class 1 but the Committee thought that the Candida albicans experiments required containment level 2 and were thus Class 2 activities.

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Project Ref 79/04.1

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<td>31/03/2004</td>
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<td>10/09/2004</td>
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</table>

Tick if notifying a connected programme of work N

Historical Significant Changes
### Project Additional Information

#### Purposes of the contained use

The application covers various specific projects which all have the overall aim of understanding the relationship between cellular proliferation and protein synthesis. More specific goals include an investigation of the control of translation initiation in response to stress as well as an analysis of downstream effects such as flocculence and colony morphology.

#### Recipient or parental organism

Recipient organisms include:

C. albicans - strains such as CA1-4 (ura3) and CA1-8 (ura3 ade2). These strains are standard lab strains with auxotrophic phenotypes and highly attenuated virulence. These strains cannot survive without media containing high concentrations of adenine and uracil, and given the stringent practices for autoclaving liquid and solid media prior to disposal in our institute, there is no possibility that these strains could escape into the environment.

#### Host/vector system

Vectors include:

Specific non-mobilisable C. albicans (single integrating vectors and integrating expression vectors regulated by methionine).

#### Origin & function

The genetic materials are all derived from naturally occurring organisms of E. coli, S. cerevisiae, and C. albicans. More specific Candida genes include:

1/ Protein synthetic genes (e.g. GCD1, GCD6, CDC33)
2/ mRNA decay genes (e.g. DCP1, CCR4)
3/ Auxotrophic markers (e.g. URA3, ADE2, LYS2)
4/ Candida genomic and cDNA libraries

These genes have homologues in all eukaryotic organisms. As a result there could be absolutely no effect of a gene transfer because the DNA sequences included in our constructs are part of the genomes of all known eukaryotic organisms.

#### Evaluation of foreseeable effects

Candida albicans is a human fungal pathogen in Hazard Group 2. It is a micro-organism of a very low pathogenic potential that very rarely affects healthy individuals. It becomes infectious, however, in severely immunocompromised patients like HIV positive individuals or organ transplant patients. Candida albicans can cause superficial infections of mucosal epithelia (thrush). However, in the 20 or more years that C. albicans has been studied in labs, there has never been a thrush infection caused by a laboratory derived strain. In fact the lab strain CA1-4 which is the standard lab strain worldwide is auxotrophic for uracil. This strain cannot therefore survive in the absence of high concentrations of uracil and this makes the strain avirulent. It might be anticipated that transformation of this strain with plasmids bearing the URA3 gene restores the virulence of CA1-4, but the strain is still severely disabled (e.g. Chen et al., 2004. Mol Microbiol. 51:551-65). The lab strain CA1-8 is auxotrophic for both uracil and adenine. Transformation of this strain therefore would only ever complement one of these auxotrophies, thus this is sufficient to render all transformed strains avirulent. Even though these strains are avirulent, the stringent practices for autoclaving liquid and solid cultures prior to disposal eliminates the possibility of strains escaping into the environment alive.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

NA

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

We follow all safety guidelines for operation at containment level 2. Briefly:

- All waste materials will be inactivated prior to disposal.
- Liquid - autoclaving at 121 degrees centigrade for 15 minutes which has been proven to effectively kill 100% of fungal cells. The Department of Biomolecular Sciences has a large autoclave which replaced two autoclaves in January 2001. The autoclave is serviced annually by BMM Weston/Drayton Castle and thermocouple testing of various loads of waste is included in the annual service contract. The autoclave has a data logging system and autoclave indicator tubes are placed in bins of contaminated waste on a weekly basis.
- Glassware - soaked with 1% Virkon (see information provided by the manufacturer - proven to effectively kill fungal cells)
- Plasticware - autoclaved under the conditions described above. Before inactivation plastics will be double-bagged for transport to the autoclave.
- The use of sharps will be minimised, but any contaminated sharps will be placed in separate CinBins, bagged and incinerated.

Once GMMs have been inactivated, normal routes of disposal will be used. The proposed containment and control measures will eliminate the hazards, making the overall risk effectively zero (see risk assessment).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

None.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tr>
<td>L2 L3 L4 L2</td>
<td>L2 L3 L4 L2 L3 L4 L4</td>
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02/03/2022
THE INVESTIGATION OF THE BIOCHEMICAL AND BIOLOGICAL EFFECTS ON ONCOGENES ON HAEMOPOIETIC CELLS

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

<table>
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Large Scale Activities

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Human Clinical Applications

Project Ref 79/99.1

Date Ackn’d 24/11/1999

CU2 Project Title IDENTIFICATION & PHYSICAL & GENETIC CHARACTERISATION OF PLASMID STABILITY DETERMINANTS FROM GRAM-POSITIVE BACTERIA

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements [Y]

Withdrawn [N]

Tick if notifying a connected programme of work [N]

Historical Significant Changes
Historical Date of Additional Info  
GM 79/99.1 transferred to GM 541

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
### Project Containment

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#### Laboratory Activities
- Animal Units

#### Glass Houses
- Large Scale Activities

#### Growth Rooms
- Human Clinical Applications
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#### Name

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<tr>
<td>Name 2</td>
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<td>SW7 2AZ</td>
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</table>

## Premises Conditions

02/03/2022
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 3 (GMMs)</td>
<td>Level 4 (GMMs)</td>
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<td>Transgenic</td>
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<tr>
<td>Other(s)</td>
<td></td>
<td></td>
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</tbody>
</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
**Project Ref 173/04.1**

**Date Ackn’d**: 21/06/2007  
**CU2 Project Title**: Identification of genes conferring drug resistance to Ovarian cancer.

**Class**  
Class 2

**Culture Vol Class**  
< 1 Litre

**Non-GMM Consent Granted**  
Not Applicable

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**: PROJECT MOVED FROM GM 173 (21/6/07).

**Recipient or parental organism**

Commercially available replication-defective serotype V adenoviral expression system (eg Becton Dickson AdenoX system) lacking E1 and E3 genes.

HEK 293 cells which express E1 genes necessary for packaging the virus will be transfected with adenoviral DNA to generate a replication-defective adenovirus.

The virus will be used to infect primary cultures ovarian cancer and cancer cell lines.

**Origin & function**

Adenovirus as described above.
Adenovirus as described above.

**Evaluation of foreseeable effects**

No foreseeable effects are anticipated with the use of replication-defective adenovirus type V per se. Adenovirus type V is a common respiratory pathogen, and immunity in humans is believed to be lifelong. The parental virus is considered a class 1 agent.

Therefore, any hazard that arises will depend on the nature of the insert. The insert will be shRNA directed to genes identified through micro-array analysis of avarian cancers and the work to identify these genes is ongoing. A risk assessment will be conducted for each gene which we intend to knockdown, once the identity of the genes is known. However, since the genes identified by the microarray studies are expected to promote a malignant phenotype, interfering with their expression by RNAi is unlikely to promote malignancy. Nevertheless, we may chose to inhibit the expression of genes whose function is not fully understood and for which the effects are not wholly foreseeable.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not relevant.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Work will be conducted within a containment level 2 laboratory using a class 2 microbiological safety cabinet. Liquid waste will be treated with chloros at a final concentration of 3% for 12 hours prior to sterilisation by autoclave. Solid waste will be soaked in 10% chloros overnight. Both solid and liquid waste will be collected by the central sterile supplies department and sterilised by autoclave.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

<table>
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<tr>
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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
The aim of the project is to use genetically engineered Lentiviruses to produce metastatic models that express a Luciferase marker enzyme and enable the tumours to be visualised in vivo. Cell signalling pathways that are involved in maintaining the metastatic phenotype will be

---

**Project Additional Information**

**Purposes of the contained use**

To prevent exposure of the workers to Class 2 GMM.

**Recipient or parental organism**

- E.Coli (recA)
- DH5 alpha
  
  A commonly used strain with a long history of safe use.

**Host/vector system**

The following plasmids will be utilised

- pFIV-34N
- PVSV-G
- pSIF1-H1-Puro™shRNA Expression Vector

**Origin & function**

Our intention is to introduce the luciferase gene into the appropriate established tumour cell lines using an Expression system based upon a Feline Immunodeficiency Virus (FIV) obtained from a commercial source (System Biosciences Inc). The FIV lentiviral expression systems circumvent the possible recombinant issues that normally arise with the use of HIV vectors. We also intend to use the FIV-based short hairpin RNA and double promoter – siRNA Cloning and Expression Vectors that will target selected...
gene sequences and enable us to produce stable tumour cell lines.

To create functional pseudoviral particles, the lentivector containing the expression construct is co-transfected with the packaging plasmids into the producer cell line. The packaging cells replicate the expression construct and package it into the pseudoviral particles. The pseudoviral particles will then be used to infect (or transduce) target cells and express effector or reporter molecules but cannot replicate within target cells because the viral structural genes are absent and the LTRs are designed to be self-inactivating upon transduction.

Following transduction into the target cells, the expression cassette is reverse transcribed and integrated into the genome of the target cell. After integration, the expression cassette continuously and stably produces high levels of effector or reporter molecules in target cells. Target cells stably expressing the effector molecule will be isolated using the selectable marker contained in the expression vector construct (e.g. puromycin or copGFP). The transduced cells will be assayed for gene breakdown or luciferase production using specific assays, immunoblotting or by flow cytometry. Infected cells will be assayed in vitro and in vivo to determine the effect of knock down or expressed genes on their metastatic potential.

The cell lines that will be transduced are all established human tumour cell lines either derived by the Tumour Biology and Metastasis Team or from ATCC. These will include MDAMB435 (breast), DU145 (prostate), MTSP 12-H (mesothelioma).

Evaluation of foreseeable effects

The feline immunodeficiency virus (FIV) was originally isolated from cat blood. Despite common close exposure of humans to FIV through contact with domestic cats (including bites, scratches, etc), no human infection or disease has ever been associated with FIV.

The lentiviral expression systems are made with a split-genome conditional packaging system which acts as a built in device against the generation of productive recombinants. The number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev), and the corresponding proteins are expressed from different plasmids (for HIV-based packaging plasmids) lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector, to prevent generation of recombinant replication-competent virus. None of the HIV-1 genes (gag, pol, rev) will be present in the packaged viral genome, as they are expressed from packaging plasmids lacking packaging signal – therefore, the lentiviral particles generated are replication-incompetent.

The packaging plasmids are transfected into the 293TN producer Cell Line. In the unlikely event these cells were accidentally injected the risk of pathogenesis is very low due to the recognition of the cells by the immune system as being foreign.

A deletion in the enhancer of the U3 region of 3' LTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Evaluation of foreseeable events:

The feline immunodeficiency virus (FIV) was originally isolated from cat blood. Despite common close exposure of humans to FIV through contact with domestic cats (including bites, scratches, etc), no human infection or disease has ever been associated with FIV.

The lentiviral expression system are made with a split-genome conditional packaging system which acts as a built in device against the generation of productive recombinants. The number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev), and the corresponding proteins are expressed from different plasmids (for HIV-based packaging plasmids) lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector, to prevent generation of recombinant replication virus. None of the HIV-1 genes (gag, Pol, rev) will be present in the packaged viral genome, as they are expressed from packaging plasmids lacking packaging signal - therefore, the lentiviral particles generated are replication- incompetent. The packaging plasmids are transfected into the 293TN Producer Cell Line. In the unlikely event these cells were accidentally injected the risk of pathogenesis is very low.
due to the recognition of the cells by the immune system as being foreign. The infected human cells would be unlikely to be pathogenic due to rapid complement mediated lysis of these cells when recognized by the immune system as of non-self origin. Cells would not be shedding virus as they are replication-defective and therefore do not pose a risk to human health or the environment. In our risk assessment, the modified viruses should not pose a serious risk to the environment. These viruses are extremely unstable, inactivated by detergent, UV light and ethanol, and would not survive outside of the laboratory environment. Animals containing the previously infected human cells are safe to the environment as they would be unlikely to survive outside the laboratory because of their severe immune defect. There is no potential to transfer the GMO to other organisms outside of the experimentally manipulated procedures outlined.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Protective clothing and gloves will be worn at all times. All staff are trained in the good laboratory practise and are fully aware of the Institute local rules for containment level 2 laboratories. Work surfaces, including the inside of microbiological safety cabinets, will be suitably disinfected after use. Centrifugation will only be performed in sealed tubes to prevent aerosols. Sharps will not be used. Liquid waste, such as cell culture medium, which may contain virus or cells, will be rendered safe for disposal by overnight treatment with a QAC based disinfectant (Tresolin K). Plastic pipettes will be soaked in 5% Chloros prior to removal by CSSD. Solid waste will be placed in autoclave bags for collection by CSSD. Material collected by CSSD will be autoclaved on site. The cleaning, disinfecting and disposal procedures will be documented and displayed. The biological spillage procedure will be displayed and HAZ-TAB disinfectants will be available for immediate use. Supernatant will be collected and human tumour and epithelial cells will be exposed to the supernatant in conditions to promote infection. Virus preparation will be certified free of replication competent virus (see below). These cells will then be assayed in vitro and/or injected into animals to measure their oncogenic potential.

Once the lentivirus has infected the cells it should not be able to replicate. In order to test this we will plate NIH3T3 (murine) or ECV304 (Human) cells into 6 well plates (5x104/well). Next day we will add 1ml of either fresh or frozen (-70°C) viral supernatant (supernatant 1) (Vector+gene+GFP) plus 2g of polybrene (8mg/ml stock) and incubate overnight. The following day the cells are washed with medium and replaced with fresh medium. After 24 to 48 hours the infectivity will be assessed by assaying the % positive GFP cells. The cells will grow for a further 4 to 6 days (splitting cells if necessary) and overnight supernatant harvested (supernatant 2) from confluent cells. This will allow amplification of the virus.

Plate NIH3T3 or ECV304 cells in a 6 well plate (5x104/well) and leave overnight. Add supernatant 2 plus polybrene as before. In addition include as a positive control supernatant 1 plus polybrene. Wash cells next day and add fresh

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Question: why are NIH3T3 or EC304 cells being used for the viral activity assay? Are these the cell lines in which studies will be performed?

I thought human tumour cell lines were going to be used for these studies in which case shouldn’t these be the cell lines tested for viral activity?

Answer:
Your assumption is correct that no viral particles should be present. This assay is about ensuring that any viral particles there would be detected. I understand that these cell lines are highly sensitive to infection by any virus. DH5 α

It is not clear to me from the proposal what their animal models or the cell lines actually are.

I am only being vague on this issue because we have many animal models and cell lines that we hope to use and I don't wish to be limited by naming just a few.

Initially there will be MDA MB435 (breast), DU145 (prostate), MSTO 12-H (mesothelioma)

The work is categorised as containment level 2 and yet there are not any level 2 labs in the McElwain.

The proposed laboratory (room 019) has been inspected by members of ICR's Health and Safety and has been

**Project Containment**

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**Animal Units**

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**Project Ref** 173/transA

**Date Ackn'd** 13/12/1993

**CU2 Project Title**

THE INTRODUCTION AND EXPRESSION OF ONCOGENES, GROWTH FACTORS AND CYTOPLASMIC PROTEINS IN MAMMALIAN CELLS USING AMPHOTROPIC RECOMBINANT RETROVIRAL VECTORS.

**Class** Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Consent Granted** not applicable

**Non-GMM**

**Project notified under transitional arrangements** Y

**Withdrawn** N

Tick if notifying a connected programme of work N
**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
### Project Containment

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</table>

**Project Ref** 80/03.1

**Date Ackn'd** 28/05/2003

**CU2 Project Title**

PRE-CLINICAL TESTING OF MODIFIED FORMS OF HERPES SIMPLEX VIRUS-1 FOR CANCER THERAPY.

**Class** Class 2

**CultureVolClass2** ≤ 1 litre

**Consent Granted** not applicable

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Project notified under transitional arrangements** N
<table>
<thead>
<tr>
<th>Purposes of the contained use</th>
<th>Prevention of class 2 GMOs being a hazard to workers.</th>
</tr>
</thead>
</table>

| Recipient or parental organism | HSV1 vectors deleted for ICP34.5 (neurovirulence factor) and ICP47 have been engineered to express human GM-CSF and/or TNF-alpha. These vectors are designed to replicate in tumours but not surrounding tissues (considerable literature demonstrates such a phenotype for HSV1 and 2 ICP34.5 and ICP47 deleted viruses) with the additional delivery of immunostimulatory and/or radiosensitising factors. Each of the deletions in ICP34.5 and ICP47 has been shown individually to generate a non-pathogenic, though still replication competent virus. |

<table>
<thead>
<tr>
<th>Host/vector system</th>
<th>Herpes simplex virus 1.</th>
</tr>
</thead>
</table>

| Origin & function | Inserted genetic material is of human origin and has been cloned by pcr or obtained from collaborators in plasmid form. Genes to be inserted include GM-CSF and TNF-alpha. |

| Evaluation of foreseeable effects | None of the genes to be inserted is anticipated to result in harmful effects. |

| Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants) | 
| For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification) |
| Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate) | All waste generated during the activity that has been in contact with the GMM will be treated with a Virkon solution. This broad-spectrum disinfectant is effective against herpes simplex virus at a 0.5% (w/v) concentration giving terminal disinfection after 10 minutes of contact. After treatment with the above disinfectant, the waste will then be placed into clinical waste bags and burnt offsite. |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |

| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

| Please enter comments on the GM safety committee on the risk assessment | The GMSC opinion is that the viruses to be used are probably Class 1, although as risks are low, Class 1 could be considered appropriate. |
THE USE OF SPECIFICALLY DESIGNED SMALL INTERFERING RNA (SIRNA) AGAINST GENES AND ANALYSIS OF THE EFFECTS THESE HAVE ON CELLULAR PHENOTYPE.

Recipient or parental organism

Split-genome MLV-based systems (eg. Cosset, F.L. et al., J Virol. 69: 7430-6 (1995)) or lentiviral-based systems (eg. Ikeda, Y. et al., Nat Biotechnol. 21: 569-72 (2003)) to generate non-replication competent virus. These enable virus to be harvested from the supernatant of these cells in a safe and well characterised manner which can then be used to infect cells in order to get stable expression of a packaged transgene.
Host/vector system

Murine Leukaemia Virus and Human Immunodeficiency Virus - based systems.

Origin & function

The inserted genetic material will typically be of the form of the pSUPER system that delivers an siRNA-like transcript which forms a 19-base pair stem-loop structure in order to knock-down gene expression (Brummelkamp, T.R. et al., Science 296: 550-3). These will be specifically designed against a gene of from a library of 8000 siRNA vectors being widely distributed throughout the UK under the auspices of Cancer Research UK.

Evaluation of foreseeable effects

Both types of virus will be produced from split-genome packaging cells whereby the components required for virus for production have been split onto 3 separate plasmids to prevent recombination and generation of replication competent virus. The generated viruses will only have packaged the gene of interest and their tropism will be largely defined by the envelope protein expressed in the packaging cell line. For ecotropic virus this will only enable infection of rodent cells whilst the host range for amphotropic virus includes human cells.

MLV is a type-C retrovirus and none of this class have been shown to have pathogenicity in humans. HIV leads to acquired immune deficiency syndrome (AIDS). However components of the human immunodeficiency virus, which are used in the lentiviral cells, will not lead to the generation of replication competent virus. Both are unlikely to survive outside of the laboratory, where suitable growth conditions are provided.

The normal transmission route of retro and lenti viruses is either by injection or exchange of bodily fluids so rapidly degrade once outside the body. Should the packaging cells be accidentally injected, the risk of pathogenicity is very low in an immunocompetent individual due to the recognition of the cells as foreign and the subsequent induced complement mediated lysis. However only plastic disposable ware will be used, with no sharps or needles being required.

siRNA will be generated to a large number of specific targets and per se these small inserts and transcripts provide little hazard. However the targets are likely to be wide ranging and may include a range of different classes of targets in order to screen many genes as possible targets.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Work within the containment level 2 laboratory will be conducted using a class II microbiological safety cabinet. Liquid wastes, such as cell culture medium, which may contain virus or cells, will be rendered safe for disposal by overnight treatment with a QAC based disinfectant. Pipette tips, simplettes etc will be similarly soaked in disinfectant and the tips collected for disposal in solid bins by CSSD. These measures provide at least 4 magnitudes of protection against retrovirus whilst the cells are effectively killed. Plastic pipettes will be soaked in 5% Chloros prior to removal by CSSD. Material collected by CSSD will be autoclaved on site.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment
The risk assessment form has been distributed to the members of the genetic modification safety committee who have approved our use of these class 2 GMMs.

**Project Containment**

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<td>L2 L3 L4</td>
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</tbody>
</table>

- **L2** L3 L4 L2 L3 L4 L2 L3 L4
- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 80/05.1

**Date Ackn’d** 31/05/2005  
**CU2 Project Title** The function of c-Myb, its targets and partners in cancer and T-cell development, and the regulation of T-cell development by transcription factors.

**Class** Class 2  
**CultureVolClass2** < 1 Litre

**Class CultureVolClass3-4**

- **Non-GMM** Not Applicable

**Project notified under transitional arrangements**

**Withdrawn**

- **Tick if notifying a connected programme of work**

**Historical Significant Changes**

<table>
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<tr>
<th>Historical Date of Additional Info</th>
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**Significant Change ID**

<table>
<thead>
<tr>
<th>Date of Significant Change</th>
</tr>
</thead>
</table>

**Project Additional Information**

**Purposes of the contained use**

- Prevention of Class 2 GMMs being a hazard to workers.

**Recipient or parental organism**

1. Lentiviral-based systems (eg Ikeda, Y., et al, Nat Biotechnol 21:569-72 (2003) will be used to generate non-replication competent virus. These systems enable virus to be harvested from cell supernatant in a safe and well-characterised manner which can then be used to infect cells in order to get stable expression of a packaged...
transgene.

ii). MoLV production: Viral supernatant will be made from the cell line Moloney NIH3T3 clone 4. Supernatant will be harvested from these cells and used to infect neonatal mice.

Host/vector system

I) Human Immunodeficiency virus-based systems.

ii) Wild-type MoLV

Origin & function

I) Vectors: Lentiviral vectors will be HIV-derived and replication defective. For siRNA expression, we plan to use pSico (Ventura et al (2004) Proc Nat Acad Scu USA 101:10380-10385) or similar vectors, and for cDNA expression, the LV series of vectors (Pfeifer et al (2002) Proc Nat Acad Sci USA 99:2140-2145) or similar. All vectors are replication defective and are self-inactivating as they contain mutations resulting in deletion of the LTR promoter. No vectors contain the WPRE sequence.

ii) shRNA inserts: shRNAs generated against a large number of targets in the human or mouse genome will either be introduced as a library (eg Paddison et al (2004) Nature 428:427-431), or as individual sequences. They will be used to achieve knockdown of endogenous cellular genes.

iii) Other inserts: cDNAs or mutants thereof encoding a large number of genes will be individually introduced to explore the effects of their expression on cell phenotype. Inserts are likely to include genes involved in cell cycle control, differentiation, apoptosis and control of cell movement.

iv) Wild-type MoLV will be used as an insertional mutagen to infect neonatal mice for studies on cooperation between different oncogenes.

Evaluation of foreseeable effects

Lentiviral work: All virus will be produced from split genome packaging cells whereby the components required for virus production have been split onto separate plasmids to prevent recombination and generation of replication competent virus. The generated viruses will only have packaged the gene of interest and their tropism will be largely defined by the envelope protein expressed in the packaging cell line. The host range of amphotropic lentivirus will include human cells. However, whilst HIV infection leads to AIDS, the components of the HIV virus used in the lentiviral packaging cells cannot lead to the generation of replication competent virus, as shown by Ikeda et al (2003) Nat Biotech 21:569-572. The packaging line also uses the MLV4070A envelope protein rather than HIV envelope. The viruses are unlikely to survive outside the laboratory, as suitable growth conditions will not exist.

The normal transmission route of lentiviral vectors is either by injection or exchange of bodily fluids. Should the packaging cells be injected accidentally, the risk of pathogenesis is very low in an immunocompetent individual due to the recognition of the cells as foreign. Any persons involved in the project who become immunocompromised must report this immediately and may be restricted from working on this part of the project. Only plastic disposable ware will be used, and no sharps or needles are required.

The recombinant viruses are also low risk as they are replication incompetent and cannot generate more virus in infected cells or mobile endogenous viruses in either humans or mice. Similarly, due to their route of transmission these viruses are rapidly degraded once outside the body.

MoLV work: for MoLV infection of neonatal mice, virus will be infectious but ecotropic, and therefore of negligible risk to human health. For MoLV supernatant production, the virus may potentially contaminate other murine cell lines, and so it will be grown under level 2 containment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable. Injection of MoLV viral supernatant and maintenance of mouse strains only requires level 1 containment as there is no known evidence for horizontal transmission (J. Jonkers, pers comm.)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Work within the containment level 2 laboratory will be conducted using a class II microbiological safety cabinet. Liquid wastes, such as cell culture medium, which may contain virus or cells, will be rendered safe for disposal by overnight treatment with a QAC based disinfectant (Tresolin K). Pipette tips, simplettes etc will be similarly soaked in disinfectant and the tips collected for disposal in solid bins by CSSD. Plastic pipettes will be soaked in 5% Chloros prior to removal by CSSD. Sharps will not be used in connection with this work. These measures provide at least four magnitudes of protection against virus whilst the cells are effectively killed. Material collected by CSSD will be autoclaved on site.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The risk assessment form has been distributed to members of the genetic modification safety committee who have approved the use of these class 2 GMMs.

Project Containment

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<td>L3 L4 L2</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

Project Ref  80/05.2

Date Ackn’d  14/10/2005

Date Project Ceased

CU2 Project Title  An exploratory study of the safety and biological activity of oncovey (GM-CSF) in combination with radiotherapy and cisplatin in the treatment of locally advanced epithelial cancer of the head and neck

Class  Class 2

Consent Granted  Not Applicable

CultureVolClass2  < 1 Litre

CultureVolumeClass3-4

02/03/2022
The study will be a single arm, open label upward titration of up to three dose levels of OncoVEX(GM-CSF) in combination with radiotherapy and concomitant cisplatin in the treatment of patients with locally advanced stage III and IV head and neck cancer with one or more nodal metastasis in the neck (N2-N3).

Recipient or parental organism

Replication competent herpes simplex type-1 virus that is derived from HSV1 strain JS-1 (ECACC No. 01010209). The OncoVEX(GM-CSF) vector is deleted for the neurovirulence factor, ICP34.5. Deletion of this gene provides a virus that is non-pathogenic while providing the property of tumour selective replication. The vector is also deleted for ICP47, which blocks antigen presentation by blocking the transporter associated with antigen processing (TAP1 and TAP2). The vector also contains the coding sequence for human GM-CSF, a cytokine involved in the stimulation of T-cells. Human GM-CSF gene was cloned from an IMAGE clone 2340997/5808-K14 (UK HGMP Resource Centre). GM-CSF expression is under the control of the human cytomegalovirus immediate early promoter (HCMV IE). GM-CSF occurs normally in the human body. This cytokine is a licensed pharmaceutical product that is used to aid myeloid recovery following bone marrow transplant and chemotherapy, GM-CSF rarely causes anaphylaxis and cardiovascular disorders. Patients with congestive cardiac failure and renal insufficiency will be excluded from the clinical trial. However, only very low-level systemic levels of GM-CSF are expected following intra-tumoural inoculation of the virus where GM-CSF will be locally produced. Thus toxic effects associated with high levels of systemic GM-CSF are not anticipated.

The deletion of ICP47 and the expression of GM-CSF should enhance the immune response towards tumour cells. GM-CSF expression is under the control of the human cytomegalovirus immediate early promoter (HCMV IE). This is an infectious gene therapy product that replicates selectively in rapidly dividing eukaryotic cells. The parent vector is ACDP category 2 and ACGM level 2 containment is appropriate for OncoVEX(GM-CSF) vector due to the conditional replication competence of the vector, and the expression of a biologically active cytokine.

Host/vector system

Replication competent herpes simplex type-1 virus that is derived from HSV1 strain JS-1 (ECACC No. 01010209). The OncoVEX(GM-CSF) vector is deleted for the neurovirulence factor, ICP34.5. Deletion of this gene provides a virus that is non-pathogenic while providing the property of tumour selective replication. The vector is also deleted for ICP47, which blocks antigen presentation by blocking the transporter associated with antigen processing (TAP1 and TAP2). The vector also contains the coding sequence for human GM-CSF, a cytokine involved in the stimulation of T-cells. Human GM-CSF gene was cloned from an IMAGE clone 2340997/5808-K14 (UK HGMP Resource Centre). GM-CSF expression is under the control of the human cytomegalovirus immediate early promoter (HCMV IE). GM-CSF occurs normally in the human body. This cytokine is a licensed pharmaceutical product that is used to aid myeloid recovery following bone marrow transplant and chemotherapy, GM-CSF rarely causes anaphylaxis and cardiovascular disorders. Patients with congestive cardiac failure and renal insufficiency will be excluded from the clinical trial. However, only very low-level systemic levels of GM-CSF are expected following intra-tumoural inoculation of the virus where GM-CSF will be locally produced. Thus toxic effects associated with high levels of systemic GM-CSF are not anticipated.

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As regards the safety of this vector, there are a number of specific relevant points:

* The vector is deleted for ICP34.5 & ICP47, deletion of both of which renders the virus non-pathogenic.
* HSV and HSV vectors do not to integrate into the host DNA.
* The HSV thymidine kinase (TK) gene is intact in OncoVEX (GM-CSF) and expressed from the vector. This renders the viruses sensitive to anti-viral agents such as acyclovir, which can be used to block virus replication in the unlikely event that this is required.

There is no anticipated hazards should release of OncoVEX(GM-CSF) into the environment occur. However, procedures will be used to prevent contamination of the environment with OncoVEX(GM-CSF) even though (i) there is no anticipated hazard of OncoVEX(GM-CSF) to the environment and (ii) OncoVEX(GM-CSF) would not be expected to be able to survive in the environment if release occurred.

**Origin & function**

Replication competent herpes simplex type-1 virus that is derived from HSV1 strain JS-1 (ECACC No. 01010209). The OncoVEX(GM-CSF) vector is deleted for the neurovirulence factor, ICP34.5. Deletion of this gene provides a virus that is non-pathogenic while providing the property of tumour selective replication. The vector is also deleted for ICP47, which blocks antigen presentation by blocking the transporter associated with antigen processing (TAP1 and TAP2). The vector also contains the coding sequence for human GM-CSF, a cytokine involved in the stimulation of T-cells. Human GM-CSF gene was cloned from an IMAGE clone 2340997/5809-K14 (UK HGMP Resource Centre). GM-CSF expression is under the control of the human cytomegalovirus immediate early promoter (HCMV IE). GM-CSF occurs normally in the human body. This cytokine is a licensed pharmaceutical product that is used to aid myeloid recovery following bone marrow transplant and chemotherapy, GM-CSF rarely causes anaphylaxis and cardiovascular disorders. Patients with congestive cardiac failure and renal insufficiency will be excluded from the clinical trial. However, only very low-level systemic levels of GM-CSF are expected following intra-tumoural inoculation of the virus where GM-CSF will be locally produced. Thus toxic effects associated with high levels of systemic GM-CSF are not anticipated.

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The study will be a single arm open label upward titration of up to three dose levels of OncoVEX(GM-CSF) in combination with radiotherapy and concomitant cisplatin in the treatment of patients with locally advanced stage III and IV head and neck cancer with one or more nodal metastasis in the neck (N3-N3). Treatment with OncoVEX(GM-CSF) will be scheduled to commence on the day on which chemoradiotherapy begins. Patients will be admitted to the outpatients department or a day ward for all injections of virus. The injections will always be performed in a side room where the trial subjects can be segregated from other patients for safety reasons. Groups of four evaluable patients will be treated with dose levels of 10 (to the power of 6) pfu/mL on four occasions, 10 (to the power of 6) pfu/mL on one occasion followed by 10 (to the power of 7) pfu/mL on three occasions and 10 (to the power of 6) pfu/mL on one occasion followed by 10 (to the power of 8) pfu/mL on three occasions. The first dose of virus will be up to 2 mL per tumour (4 mL in total) of the dosage strength 10 (to the power of 6) pfu/mL given on the same day that treatment with cisplatin and radiation begins. Subsequent doses of virus (which will always be administered to the same nodes) will consist of a volume of up to 4 mL in weeks 3 (day 21), 6 (day 42) and 9 at a dosage strength of either 10 (to the power of 6), 10 (to the power of 7), or 10 (to the power of 8) pfu/mL depending on the dose level to which the patient is allocated. The timing of these doses is designed to avoid chemotherapy-induced nadirs in the white cell count. The initial dose strength of 10 (to the power of 6) pfu/mL will be used in order to allow the patient to become tolerant to HSV1. Each injection will then be made, under local anaesthetic, along multiple tracks in order to disperse the vector throughout the tumour. Following injection, the injected tumour will be covered with a double occlusive dressing (Tegaderm) that must be kept in place for 24 hours. The injected tumour(s) will be swabbed 24 and 48 hours after injection in order to ensure that there is no shedding of virus from the injection site. If shedding or weeping is detected, the dressing must be replaced and changed every 24 hours, with the swabs repeated every 24 hours until shedding has ceased for at least 72 hours.

During the chemoradiotherapy the following clinical safety laboratory tests will be obtained at weekly intervals (including those tests taken prior to OncoVEX(GM-CSF)
injection): Haematology (full blood count), clinical chemistry (including urea, creatinine and electrolytes, liver function tests). The following immunology laboratory tests will be obtained before the second, third and fourth injections of OncoVEX(GM-CSF): HSV-antibodies, antinuclear antibody (autoantibody). There will be a final follow-up visit one month after the week 10 investigations or one month after surgery. At this time, a physical examination will be conducted and blood samples will be obtained for haematology (full blood count), clinical chemistry (including urea, creatinine and electrolytes, liver function tests) and HSV-1 antibodies, antinuclear antibody (autoantibody). Adverse events and concomitant medications will be documented from the time of screening until the patient comes off study.

Evaluation of foreseeable effects

Treatment with OncoVEX(GM-CSF) will be scheduled to commence on the day on which chemoradiotherapy begins. Patients will be admitted to the outpatients department or a day ward for all injections of virus. The injections will always be performed in a side room where the trial subjects can be segregated from other patients for safety reasons. Before each injection, the ECOG score will be recorded. Vital signs (core temperature, heart rate, blood pressure) will be measured and recorded. The patients will be examined for the presence of active herpes labialis. If this is present, the injection must be delayed until healing occurs. Blood will be obtained for clinical chemistry and haematology. The patients will also be questioned about any symptoms that they are experiencing at the time and any medications they may be taking. Before the first injection, the node(s) that will be injected will be identified and allocated a tumour number. Where appropriate, the node(s) that will not be injected will also be identified and numbered. All nodes will be palpated and evaluated for injection site reaction prior to injection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated by trial will be autoclaved prior to being incinerated as clinical waste by the Trust wast contractor White Rose. All small materials (eg syringes, sharps) will be soaked in a virucidal agent (such as Virkon 10%) for 30 minutes prior to autoclaving.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
## GSCM - Action points

HSV-GMCSF

- HSE also aware of trial. Would like a full report to the HSE - Actioned
- Dressing type to be confirmed on patient information letter - actioned
- Final version of patient letter to be confirmed - actioned
- BG discussed concerns over blood samples taken for staff from Occ Health and where these may be stored. Also how staff with needlestick injuries will be monitored KH felt that the risk is minimal, however will confirm action. MG felt that a swab should be taken if an abnormal lesion should develop on a staff member - actioned
- KH to amend paragraph two on the first page of GMCSF study relation to deletions - actioned
- SOP's to be made available on Weston Ward, Labs and Radiotherapy - actioned

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### Project Ref  80/06.1

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Project notified under transitional arrangements

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### Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
## Purpose of the contained use

**Prevention of Class 2 GMMs being a hazard to workers**

## Recipient or parental organism

**Lentiviral-based systems** (e.g., Ikeda, Y., et al, Nat Biotechnol 21:569-72 (2003)) will be used to generate non-replication competent virus. These systems enable virus to be harvested from cell supernatent in a safe and well-characterised manner which can then be used to infect cells in order to get stable expression of a packaged transgene.

## Host/vector system

**Human Immunodeficiency Virus-based systems**

## Origin & function

Vectors: For Aurora-A cDNA expression, the LV series of vectors (Pfeifer et al (2002) Proc Nat Acad Sci USA 99:2140-2145) or similar will be used. All vectors are replication defective and are self-inactivating. Other inserts: expression on cell phenotype. Inserts are likely to be involved in cell control.

## Evaluation of foreseeable effects

Lentiviral work: All virus will be produced from split genome packaging cells whereby the components required for virus production have been split onto separate plasmids to prevent recombination and generation of replication competent virus. The generated virus will only have packaged the gene of interest and their tropism will be largely defined by the envelope protein expressed in the packaging cell line. The host range for amphotropic lentivirus will include human cells. However, whilst HIV infection leads to AIDS, the components of the HIV Virus used in the lentiviral packaging cells cannot lead to the generation of replication competent virus, as shown by Ikeda et al (2003) Nat Biotech 21:569-572. The packaging line also uses the Vesicular Stomatitis Virus (VSV-G) envelope protein rather than HIV envelope. The viruses are unlikely to survive outside the laboratory, as suitable growth conditions will not exist. The normal transmission route of lentiviral vectors is either by injection or exchange of bodily fluids. Should the packaging cells be injected accidentally, the risk of pathogenesis is very low in an immunocompetent individual due to the recognition of the cells as foreign. Only plastic disposable ware will be used, and no sharps or needles are required. The recombinant viruses are also low risk as they are replication incompetent and cannot generate more virus in the infected cells or mobilise endogenous viruses in either humans or mice. Similarly, due to their route of transmission these viruses are rapidly degraded once outside the body.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Work within the containment level 2 laboratory will be conducted using a class II microbiological safety cabinet. Liquid wastes, such as cell culture medium, which may contain virus or cells, will be rendered safe for disposal by overnight treatment with a QAC based disinfectant (Tresolin K). Pipette tips, simplettes etc will be similarly soaked in disinfectant and the tips collected for disposal in solid bins by CSSD. Plastic pipettes will be soaked in 5% Chloros prior to removal by CSSD. Sharps will not be used in connection with this work. These measures provide at least four magnitudes of protection against virus whilst the cells are effectively killed. Material collected by CSSD will be autoclaved on site.
The project has been reviewed and the classification agreed by the Genetic Modification Safety Committee.

Please enter comments on the GM safety committee on the risk assessment

The project has been reviewed and the classification agreed by the Genetic Modification Safety Committee.

**Project Containment**

**Laboratory Activities**

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**Glass Houses**

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**Growth Rooms**

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**Animal Units**

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**Large Scale Activities**

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**Human Clinical Applications**

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**Project Ref** 80/06.2

**Date Ackn’d** 07/09/2006

**CU2 Project Title**

Production and assessment of replication-selective vaccinia viruses expressing carboxypeptidase G2 for cancer gene therapy.

**Class** Class 2

**CultureVol** 1-50 Litres

**Non-GMM** Not Applicable

**Consent Granted**

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

02/03/2022
## Project Additional Information

### Purposes of the contained use

To prevent the GMM being a hazard to workers.

### Recipient or parental organism

Human and non-human cancer cell lines, which are commercially available, have been maintained in culture for many years and are not known sources of human pathogens.

### Host/vector system

We will engineer attenuated vaccinia viruses (VV) and mutations (tk-, vgf-) targeted to reduce their virulence and restrict the viral replication to tumours vs normal tissues (Puhlmann et al., 2000; McCart et al., 2001).

### Origin & function

We will insert the genes encoding for luciferase, green fluorescent protein (GFP) or carboxypeptidase G2 (CPG2) into the VV vectors. The gene products are not harmful to any organism. Gene expression will only occur in cancer cells, however, a small fraction of non-cancer cells supporting transgene expression cannot be excluded. Luciferase and GFP are commonly used reporter enzymes to monitor gene expression. Both genes are commercially available and have been used extensively without any reported adverse effects. CPG2 is a naturally occurring bacterial enzyme. The enzyme has been assessed in a clinical trial (Napier et al., 2000) and is used in the clinic as a therapeutic drug for the treatment of methotrexate poisoning. CPG2 is non-toxic. In this study, CPG2 will be used to activate a prodrug into a cytotoxic drug in cancer cells.

### Evaluation of foreseeable effects

Wild-type VV may cause disease in people with active skin disorders, during pregnancy or in immuno-compromised people. However, in this study, we will use attenuated, tumour selective VV vectors. There is no risk of insertional mutagenesis as VV spends its entire life cycle in the cytoplasm and cannot transfer its viral DNA into the genome of infected cells.

Wild-type VV is categorised as ACDP hazard group 2 and the ACGM compendium of guidance states in Part 2B Annex III "Tk-minus phenotype is believed to reduce the virulence of the virus in mice but it is debatable whether this should be taken to imply lower virulance in man, or whether this should allow a down-grading of categorisation."

VV have been used clinically as tumour vaccines (Kwak et al., 2000) and a tk-deleted VV vector has safely completed a clinical trial in patients with cutaneous melanoma (Mastrangelo et al., 1999).

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be placed in clear autoclave bags and disposed of by autoclaving on site - this has a 100% degree of kill.

Pipettes, containers and plasticware contaminated with viruses will be left completely submerged in 1% Virkon before disposal. Virkon has been validated as being effective within 5 minutes at this concentration and Virkon stocks are routinely tested by the manufacturer (http://www.antecint.co.uk/go.htm). The website will be monitored for any change.

02/03/2022
Cells and growth medium will be treated with 1% Virkon before disposal.

The Fulham Road GMSC reviewed and discussed this project they advised the work be conducted at containment level 2. Although the pathogenicity is reduced in respect of the wild type there remains a risk of infection to certain workers. The committee requested that the work conducted at the Sutton site be considered and included in the assessment.

Please enter comments on the GM safety committee on the risk assessment

The Fulham Road GMSC reviewed and discussed this project they advised the work be conducted at containment level 2. Although the pathogenicity is reduced in respect of the wild type there remains a risk of infection to certain workers. The committee requested that the work conducted at the Sutton site be considered and included in the assessment.

Project Containment

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Animal Units

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Project Ref  80/07.1

Date Ackn’d  30/05/2007

CU2 Project Title  Study of RAS GTPases and their downstream effectors during intracellular signalling in human tumours.

Date Project Ceased

Consent Granted  Not Applicable

Tick if notifying a connected programme of work  N

Historical Significant Changes  N

02/03/2022
The aim of this project is to assess the role small GTPases, in particular those encoded by the ras family of genes and their downstream effectors, play in intracellular signalling in human cancer.

All the recipient cell lines used in this study come from the American Tissue Culture Collection (ATCC) (www.atcc.org/) and include the following (tissue origin, mutation and ATCC identifier have been provided between brackets): MiaPaCa-2 (pancreatic, K-ras, CRL-1420), CAPAN-1 (pancreatic, K-ras, HTB-79), CAPAN-2 (pancreatic K-ras, HTB-80), ASPC-1 (pancreatic K-ras, CRL-1682), PANC-1 (pancreatic, K-ras, CRL-1469) SK-MEL-2 (skin, N-ras, HTB-68), A549 (lung, K-ras, CCL-185), A427 (lung, K-ras, HTB-53) and HS766T (pancreatic, wild type, HTB-134).

For packaging of the viral particles the following two cell lines will be used Human Embryonic Kidney cells HEK 293T (for use with Sigma’s Mission shRNA libraries) and Human Embryonic Kidney cells HEK 293FT (for use with Invitrogen’s BLOCK-it™ Lentiviral Pol II miR RNAi Expression system).

To the best of our knowledge all these cell lines, recipient and packaging, are routinely and widely used in laboratories around the world and are thought not to harbour any adventitious agents to human health and or environment.

The vectors will be obtained from commercial sources, including Sigma and Invitrogen. If the vectors are obtained from Sigma, these will accompany a lentiviral library known as MISSION shRNA (http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Functional_Genomics_and_RNAi.html). The library includes the following three vectors pLKO.1-puro Vector, MISSION™ shRNA Envelope Vector and MISSION™ shRNA Packaging Vector. If the vectors are outsourced from Invitrogen, they will be included in the BLOCK-it™ Lentiviral Pol II miR RNAi Expression System (Catalogue numbers K4935-00, K4936-00, K4937-00 and K4938-00). Vectors included in these kits are: pcDNA™6.2-GW/miR, and/or pcDNA™6.2-GW/miR neg control plasmid and/or pcDNA™6.2-GW/EmGFPMiR neg control plasmid, pLenti6/V5-DEST Gateway Vector, pLenti6/V5-GW/lacZ control and pDONR™221 Vector.

During this study we plan to knock-down and re-introduce the following genes (MIM between brackets):
- H-ras (190020): potential oncogene, GTPase, member of mammalian ras gene family
- K-ras (190070): potential oncogene, GTPase, member of mammalian ras gene family
- N-ras (164790): potential oncogene, GTPase, member of mammalian ras gene family
- PLCε (608414): phospholipase C epsilon, belongs to the phospholipase family that catalyzes the hydrolysis of polyphosphoinositides such as phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) to generate the second messengers IP3 and DAG
- RAF family of genes:
  - A-raf (311010), potential downstream effector for ras family of genes
  - B-raf (164757), potential downstream effector for ras family of genes
  - C-raf (164760), MAPKK which function downstream of the ras family of genes
- NORE-1A (607020), member of the ras association domain family. It functions as a tumour suppressor
- RALGDS (601619), ral guanine nucleotide dissociation stimulator
**P13Kp110β (171834), catalytic subunit alpha of P13K**

**P13Kp110γ (601232), catalytic subunit gamma of P13K**

**Evaluation of foreseeable effects**

The lentiviruses are made with a split-genome conditional packaging system. This conditional packaging system acts as a build-in device against the generation of productive recombinants. The gene of interest will be under the control of the human cytomegalovirus (CMV) promoter. The vector also contains elements to allow packaging into virions. The packaging plasmids supply the helper functions in conjunction with structural and replication proteins (in trans) required to produce lentivirus. The HEK 293T/293FT lines stably expresses the SV40 large T antigen under the control of the CMV promoter. When packaging plasmids and lentiviral vector are co-transfected into the HEK 293T/293FT cells a replication defective virus is produced. If packaging cells were injected accidentally the risk of pathogenesis is very low due to the recognition of the cells as foreign. This virus can then be used to transduce both actively cycling and dormant mammalian cells but will not replicate in the host cells. Once inside the cell, the RNA of interest can be studied. However since the genes expressed in these vectors are potential oncogenes, they themselves may cause the host cells to proliferate.

The viral particles will in principle carry DNA encoding oncogenes, but as mentioned above the risk of any potential harm to operatives can be considered negligible as lentiviruses are incapable of replication due to its split-genome conditional packaging system. The infected human cells would be unlikely to be pathogenic due to rapid complement mediated lysis of these cells when recognized by the immune system as of non-self origin. Cells would not be shedding virus as they are replication-defective and therefore do not pose a risk to human health or the environment.

Although the lentiviral particles that will be used on this study belong to the broader Retroviridae group, none of them are listed under the approved list of biological agents by the ACDP.

In our risk assessment, the modified viruses should not pose a serious risk to the environment. These viruses are extremely unstable, inactivated by detergent, UV light and ethanol, and would not survive outside of the laboratory environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Protective clothing and gloves will be worn at all times. The individual performing theses experiments is trained in good laboratory practice and is fully aware of The Institute local rules for containment level 2 laboratories. Work surfaces, including the inside of microbiological safety cabinets, will be suitably disinfected after use. Aerosol will not be generated in this project. Centrifugation will be done in sealed tubes. Sharps will not be used. Liquid wastes, such as cell culture medium, which may contain virus or cells, will be rendered safe for disposal by overnight treatment with 1% Virkon or other approved disinfectant. Plastic pipettes will be soaked in 5% Chloros prior to removal by CSSD. Solid waste will be placed in autoclave bags for collection by CSSD. Material collected by CSSD will be autoclaved on site. The cleaning, disinfecting and disposal procedures will be documented and displayed. The biological spillage procedure will be displayed and HAZ-TAB disinfectants will be available for immediate use. Supernatant will be collected and human tumour cells will be exposed to the supernatant in conditions to promote infection. Virus preparation will be certified free of replication competent virus. These cells will then be assayed in vitro to measure their oncogenic potential.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**
The Committee asked for confirmation that the project was to be carried out at CL2 and location of laboratory.

**Project Containment**

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**Project Ref** 80/07.2

**Date Ackn’d** 01/08/2007

**CU2 Project Title**
Study the role of small GTPases in signalling and tumorigenesis.

**Class** Class 2

**CultureVolClass2** < 1 Litre

**Consent Granted** Not Applicable

**Non-GMM**

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to assess the role of small GTPases, their activators and downstream effectors, in intracellular signalling in human cancers. We wish to target these genes in human and mouse cancer cell lines as well as in mouse primary cell cultures. This procedure requires the generation of replication defective recombinant
lentiviruses capable of infecting human and mouse cells. The recombinant virus will contain genetically modified viruses that will alter the expression of specific genes, which will allow to study their function in tumorigenesis.

Recipient or parental organism

HEK 293, HEK 293T and HEK 293FT (human embryonic kidney cells) will be used to produce the lentiviruses. The following cell lines will be infected with lentiviruses: A549, H441: Human lung carcinoma; Lim 1215, Human colon carcinoma; HeLa, Human cervical carcinoma, MDA-MB-231, Human breast carcinoma; HUVEC, Human umbilical vein endothelial cells.

Primary cells from mouse tissues and mouse tumours.

Host/vector system

pLKO. 1-puro Vector, mission shRNA Envelope Vector and mission shRNA Packaging vector (sigma).
BLOCK-iT Lentiviral Pol II miRNA Expression System (invitrogen).
pMD2.G and pCMV delta R8.2 plasmids (Professor Didier Trono, EPFL, Switzerland and distributed by addgene Cambridge, USA).

Origin & function

GFP: Green fluorescent protein reporter gene
H-ras, K-ras, N-ras, RAIA, RAIB: proto-oncogenes, small GTPases RAIBP1: Effector of al small GTPases
Rac1, RhoA, RhoC: Small GTPases involved in cytoskeleton remodelling
ROCK I, ROCK II: Kinases involved in cytoskeleton remodelling
MT1-MMP: Proteases involved in degradation of extracellular matrices.

The modified genes need to be introduced into appropriate cellular model systems to assess their role in oncogenic signalling. One of the more efficient model systems uses lentiviral vectors to introduce these genes into a wide variety of cell types. The vectors currently in world-wide use, are modified non-replicating RNA viruses derived from the Murine Moloney Leukaemia Virus (MoLV), the Myeloproliferative Sarcoma Virus (MPSV), the Murine Stem Cell Virus (MSCV) and third generation Lentivirus vectors (with biosafety features). They are all designed to transmit inserted genes at high titres with a choice of various selectable marker genes. The introduced genes are driven either by the viral LTR or an internal eukaryotic promoter. Some vectors allow the expression of polycystronic transcripts with internal ribosomal entry sites to allow dual expression of the introduced potential oncogenic transcript and selectable marker which may be either a drug-resistance marker or a green fluorescent protein gene. The recombinant lentiviruses will contain shRNA directed against genes such as the ones mentioned above. To address the oncogenicity of the constructs directly, we will introduce them into the appropriate human cell lines that are already under investigation in our laboratory. In the case of the lentivirus vectors, the number of HIV genes used in the system is three (gag, pol and rev). The VSV-G gene from vesicular stomatitis virus replaces the HIV envelope. Two or Three packaging plasmids will be co-transfected along with the lentivirus vector (containing the gene of interest) into the Human Embryonic Kidney (HEK) 293, 293T or 293FT packaging cell lines. The packaging line also uses the Vesicular Stomatitis (VSV-G) envelope protein rather than HIV envelope. The viruses are replication defective and are unlikely to survive outside the laboratory, as suitable growth conditions will not exist. Supernatant from the packaging cell lines co-expressing the retroviral construct of interest will be used to infect the cell line or primary cell culture of interest.

Evaluation of foreseeable effects

The lentiviruses are made with a split-genome conditional packaging system. This conditional packaging system acts as a build-in device against the generation of productive recombinants. The gene of interest will be under the control of the human cytomegalovirus. The HEK 293T/293FT lines stably express the SV40 large T antigen under the control of the CMV promoter When packaging plasmids and lentiviral vector are co-transfected accidentally the risk of pathogenesis is very low due to the recognition of the cells as foreign. This virus can then be used to transduce both actively cycling and dormant mammalian cells but will not replicate in the host cells. Once inside the cell, the RNA virus is reverse transcribed, becomes stably integrated into the host genome and the effect of expression of the gene of interest can be studied.

The viral particles will in principle carry DNA encoding proto-oncogenes, but as mentioned above the risk of any potential harm to operatives can be considered negligible as lentiviruses are incapable of replication due to its split-genome conditional packaging system. The infected human cells would be unlikely to be pathogenic due to rapid
Complement mediated lysis of these cells when recognized by the immune system as of non-self origin. Cells would not be shedding virus as they are replication-defective and therefore do not pose a risk to human health or the environment. Although the lentiviral particles that will be used on this study belong to the broader Retroviridae group, none of them are listed under the approved list of biological agents drawn by the ACDP.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In our risk assessment, the modified viruses should not pose a serious risk to the environment. These viruses are extremely unstable, inactivated by detergent, UV light and ethanol, and would not survive outside of the laboratory environment.

Protective clothing and gloves will be worn at all times. The individuals performing these experiments are trained in good laboratory practice and is fully aware of The institute local rules for containment level 2 laboratories. Work surfaces, including the inside of microbiological safety cabinets, will be suitably disinfected after use. Aerosol will not be generated in this project. Centrifugation will be done in sealed tubes. Sharps will only be used to inject cells in mice and will be disposed according to local rules. Liquid wastes, such as cell culture medium, which may contain virus or cells, will be soaked in 5 percent Chloros prior to removal by CSSD. Solid waste will be placed in autoclave bags for collection by CSSD. Material collected by CSSD will be autoclaved on site. The cleaning, disinfecting and disposal procedures will be documented and displayed. The biological spillage procedure will be displayed and HAZ-TAB disinfectants will be available for immediate use. Supermarket will be collected and human tumour cells will be exposed to the supernatant in conditions to promote infection. Virus preparation will be certified free of replication competent virus. These cells will then be assayed in vitro to measure their oncogenic potential.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

It was approved without comments.

Project Containment

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to assess the effect of blocking the expression of B-Raf and related signalling proteins on the growth of melanoma cell lines in a 3-dimensional model which more closely replicates the tumour microenvironment. We have previously shown that siRNA-mediated gene knockdown of B-Raf inhibits melanoma cell growth. However, this culture model is not compatible with siRNA oligonucleotides. Therefore, we wish to create melanoma cell lines which stably integrate shRNA sequences into their genome for long-term gene knockdown.

**Recipient or parental organism**

The recipient cell lines will be primary human melanocytes or immortalized human melanoma cell lines. Each of these cell lines are commercially available, have been maintained in cell culture for many years, are not known sources of human pathogens, and are unlikely to survive outside the laboratory environment.

**Host/vector system**

The vector to be used is a lentivirus derived from HIV-1. However the vector has been modified in several ways to ensure that the virus is replication-defective, and will not form active viral particles unless it is co-transfected into a packaging cell line with helper plasmids.

**Origin & function**

The genetic material that will be cloned into the lentiviral vector will be DNA that encodes a short hairpin RNA molecule directed against B-Raf or related genes which...
support melanoma progression. Expression of the shRNA molecule will result in gene-specific knockdown, most likely promoting growth inhibition or cell death in the transfected cells. We also propose to use a lentiviral vector encoding the bacterial Tet-repressor protein, so we can achieve inducible expression of shRNA in the cell lines under study. Once Tet-repressor and shRNA constructs have been stably introduced into cells, shRNA expression and subsequent gene knockdown will only occur if tetracycline is added to the culture medium.

**Evaluation of foreseeable effects**

The lentiviral vectors which we propose to use do have the potential for infecting any human cell. Through good lab 1’ practise (GLP) the risk of these vectors affecting the user or others in the laboratory is extremely low. In the unlikely event of infection, the viral particles will not replicate, as discussed above, The expression of these shRNA molecules is hypothesized to target the growth of melanoma cells, and would likely induce cell death. In the case of infection with a lentivirus encoding the Tet-repressor, expression of this protein would have little effect on human cells, as Tet-repressor promoter elements are not present in human genes.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We are not requesting derogation.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All transfections and infections of cells will be performed in a designated Class II biohazard safety cabinet, which will be disinfected with 1% Virkon after use. Virkon is a chemical disinfectant that kills 99.999% of organisms in less than 10 minutes. The cabinet will prevent unwanted infection of the cell cultures as well as release of the viruses into the environment. Infected cells will be grown in a designated incubator. Pipettes, containers and plasticware contaminated with viruses will be left completely submerged in 1% Virkon before disposal. Cells and growth medium will be treated with 1% Virkon before disposal according to the Institute’s Waste Index and Disinfection protocols. HIV-1 is transmitted through blood and a limited number of bodily fluids, none of which will be encountered in the course of this work. Further, sharps will not be used. To date, no one has been identified as infected with HIV due to contact with an environmental surface (see http://www.cdc.gov/hiv/resources/qa/qa35.htm). No living material infected with HIV-1 derived lentivirus will be transported other than between cabinet, microscope and incubator in the tissue culture room 5C10.1 (ACGM2), but cell extracts will be transported between room 5C10.1, laboratories 4N19-21 (ACGM1) and the equipment room on the 4th floor (ACGM1). Viral preparations will be double-contained and transported between room 5C10.1 and the —80°C freezer (room 4S4, ACGM1) for storage. In the event of a spillage, chlorine-releasing disinfectant granules (HAZ-TAB) or 1% Virkon will be used for immediate disinfection.

**Is an emergency plan required according to regulation 20?**  

| N |

**If yes, tick to confirm that it is attached to this form**  

| N |

**Tick to confirm that you have attached a risk assessment to this form**  

| Y |

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  

| N |

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**Project Containment**

02/03/2022
**Project Ref**  80/07.4  

**Date Ackn’d**  28/12/2007  
**CU2 Project Title**  Molecular control of mammary gland development.  

**Class CultureVolClass2 CultureVolumeClass3-4**  
Class 2  < 1 Litre  

**Non-GMM Consent Granted**  Not Applicable  

**Project notified under transitional arrangements**  N  

**Withdrawn**  N  

**Tick if notifying a connected programme of work**  N  

**Historical Significant Changes**  
**Historical Date of Additional Info**  
**Significant Change ID**  
**Date of Significant Change**  

**Project Additional Information**

**Purposes of the contained use**

Generation of replication-defective lentiviruses (with biosafety features) for overexpression or inhibition of genes which may play a role in mammary gland development and/or genes for labelling cells with fluorescent proteins or luciferase.

**Recipient or parental organism**

DNA will be propagated in DH5a (disabled, recombination deficient (recA)) E. coil. 

Replication defection viruses will be generated in HEK293T cells.

**Host/vector system**

Trono laboratory split genome, conditional packaging lentiviral system (pWPl, pMD2.G, p5PAX2) for overexpression studies.

Split genome lentiviral SEW expression vector, pCMVR8.91 packaging construct and pMD2.VSVG pseudotyping vector for inhibition studies.
The aim of this project is to use new methods to enable the in vivo assessment of gene overexpression or knockdown on mammary development without the need to generate transgenic or knockbut animals. The project will use viral vectors to transduce primary mouse mammary cells in culture and then transplant them back into mammary fat pads. Previously, similar work has been carried out using retroviral transduction. However, the requirement for proliferation in the target cell in order to maximise retroviral genomic integration has meant mammary epithelial cells can only be transduced with very variable, and usually poor, success. Lentiviral vectors, which do not require proliferation in the target cells, enable much higher rates of transduction to be achieved.

The expression vectors (pWPI and SEW) contain elements to allow packaging into virions. The packaging plasmids supply the helper functions in conjunction with structural and replication proteins (in trans) required to produce the lentivirus. The 293T line stably expresses the 5V40 large T antigen under the control of the CMV promoter. When packaging plasmids and lentiviral vector are co-transfected into the 293T cells a replication defective virus is produced. This virus can then be used to transduce both actively cycling and dormant mammalian cells but will not replicate in the host cells. Once inside the cell, the RNA virus is reverse transcribed, becomes stably integrated into the host genome and the effect of expression of the gene of interest can be studied.

Genes of interest to be studied include:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D4</td>
<td>a bHLH protein involved in regulating differentiation (no known oncogenic function).</td>
</tr>
<tr>
<td>Myb</td>
<td>a transcription factor involved in cell lineage switching (oncogene).</td>
</tr>
<tr>
<td>Msx2</td>
<td>a transcription factor involved in cell lineage switching (potential oncogene).</td>
</tr>
<tr>
<td>Sox6</td>
<td>a transcription factor involved in cell lineage switching (no known oncogenic function).</td>
</tr>
<tr>
<td>Ttk</td>
<td>Ttk protein kinase (potential oncogene).</td>
</tr>
<tr>
<td>Weel</td>
<td>Weel tyrosine kinase homolog (potential oncogene).</td>
</tr>
</tbody>
</table>

The vectors may also carry marker genes such as luciferase or fluorescent proteins.

**Origin & function**

There is a negligible hazard to human health from disable E. coli host strains. DH5a has a proven history of safe use, has been well characterised and is known to be free from harmful sequences.

The infected packaging cells would be unlikely to be pathogenic due to rapid complement mediated lysis of these cells when recognized by the immune system as of non-self origin. Transduced cells would not be shedding virus as they are replication-defective and therefore do not pose a risk to human health or the environment. The risk that the vector could be rescued in the mouse is negligible (there is no known means for murine retrovirus rescue of such vectors) so there is negligible risk of spread to other cages or to staff. Mouse cells will be unable to survive in the human in the event of accidental inoculation due to the species barrier and immune response.

The only risk is from lentiviral-mediated insertion of an oncogene or knockdown sequence into a human host during handling of viral supernatants. However, insertion of an oncogene into the human cells is not enough to create by itself cancer because it needs a combination of additional alterations. We will not use sequences which knockdown p53, Rb or their regulators. The vectors will be replication defective and potential exposure will be controlled by following containment level 2 local rules and use of good microbiological practice.

The viruses are unlikely to survive outside the laboratory, as suitable growth conditions will not exist. The packaging cells and any transduced cells cannot survive outside the laboratory.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Transfection of packaging cells and harvesting and testing of viral supernatants will be carried out at Level 2. Each batch of viral supernatant will be tested to ensure it is free from replication proficient virus by transducing a target cell population with the virus, waiting up to one week and then harvesting supernatants from the target cell population. These supernatants will then be used to ‘transduce’ a fresh batch of target cells. Failure to transduce the target cell population in the second round of transductions (as indicated by lack of expression of the marker gene carried by the virus) will demonstrate that the virus was unable to replicate after the first round of transduction, is therefore replication defective and no cells transduced with the virus can shed live virus. Furthermore, there is no known mechanism for endogenous mouse viruses to rescue a replication-deficient biosafety engineered lentivirus (mouse cells will be the target cells for transduction). Therefore, once target cells have been transduced with the virus and been free of viral supernatants for 24 hours, they may be considered Level 1 GMMs.
Work surfaces, including the inside of microbiological safety cabinets, will be suitably disinfected after use (Trigene). Aerosols will not be generated. Centrifugation will be done in sealed tubes. Liquid wastes will be rendered safe for disposal by overnight treatment with an approved disinfectant according the ICR Waste Index (Virkon). Plastic pipettes will be soaked in an approved disinfectant (Virkon) prior to removal by Central Services Department (CSSD). Solid waste will be placed in autoclave bags for collection by CSSD. Material collected by CSSD will be autoclaved on site. The cleaning, disinfecting and disposal procedures will be documented and displayed. The biological spillage procedure will be displayed and HAZ-TAB disinfectants will be available for immediate use. Any incidents and accidents will be reported.

Once the lentivirus has infected the cells it should not be able to replicate. All new batches of virus will be tested for replication competence. Murine NIH3T3 or 3T3-L1 cells will be transduced with fresh or frozen (-700°C) viral supernatant (supernatant 1) (virus containing the gene of interest plus either a selectable marker or GFP) plus 2 ug/ml of polybrene and incubated overnight. The following day, cells will be washed with medium and refed. After 24 to 48 hours check the number of GFP positive cells will be assessed (or the cells will be put under selection). Cells will be grown for a further 4 to 6 days (splitting cells if necessary) and an overnight supernatant (supernatant 2) harvested from confluent cells. The supernatant will be passed through a 0.45 micron filter and either used straight away or stored frozen at -700°C. NIH3T3 or 3T3-L1 cells will then be transduced with supernatant 2 plus polybrene. A positive control consisting of supernatant 1 plus polybrene will be included. The cells will be washed the next day and fresh medium added. The cultures will be checked for GFP positive cells 24 to 48 hours later (or put under selection). In the absence of virus in supernatant 2 no green (GFP positive) cells (or resistant colonies if a selectable marker is used) should be seen whereas green cells should be present in cells transfected with positive control supernatant 1.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Comment from the committee:

Is the work in the BSU really ACGMI? Surely it is ACGM 2 since you are putting cells into mice that have been infected with a virus?

The answer from the proposer:  
‘Once primary or cell lines have been transduced with a proven replication defective virus but have been free of viral supernatants for 24 hours, they can be taken from Class 2 containment. Subsequent work with cells transduced with virus can be carried out at Class 1, as they will be no more hazardous than any cell type which has been genetically modified with an alternative strategy. The virus will have been proven to be replication defective, therefore the transduced cells cannot produce more virus. There is no known method for endogenous mouse viruses to rescue such replication defective sequences, so work in the BSU with the transduced cells can also be carried out at Class 1.’

Just to expand on this, my assessment is that as the cells for transplant will have been transduced with a replication defective virus, they cannot themselves produce virus and they are no more hazardous than cells which have been stably transfected with any other sort of gene. As I say, there is no known mechanism by which endogenous viruses can rescue these sequences. My assessment of the risks of this project is that whilst working with virus producer cells and viral supernatants is level 2, once the cells have been transduced with proven replication deficient virus, the risks associated with those cells is level 1.

Project Containment
Project Ref 80/08.2

Date Ackn'd 12/06/2008
Date Project Ceased

CU2 Project Title shRNA-mediated knockdown of lysyl oxidase family gene expression in cancer cell lines.

Class 2
Culture Volume < 1 Litre

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
The aim of the project is to assess the effects of blocking the expression of lysyl oxidase family members on tumour cell growth and progression using established cancer cell lines.

Recipient or parental organism
The lentiviral vectors will be used for growth studies on established cancer cell lines: MDA-MB-231 human breast cancer cells, and B16 mouse melanoma cells. Each of these cell lines is commercially available, have been maintained in cell culture for many years and are not known sources of human pathogens. All cell types used in this study are unlikely to survive outside the laboratory environment.

Host/vector system
We will use the commercially available pLKO.1 lentiviral vector that is classified as a Biosafety Level 2. pLKO.1 is derived from the HIV-1 virus. None of the HIV-1 structural
genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced. The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.

Origin & function

The inserted genes will be various shRNA constructs that knock-down expression of LOX and LOXL2 mRNA. LOX and LOXL2 are extracellular enzymes that are required for breast cancer cell survival, proliferation, invasion and metastasis. We have previously shown that genetic knockdown will abrogate these activities. Subsequent experiments will produce shRNA constructs that target genes that co-operate with LOX and LOXL2 to promote breast cancer tumour growth and progression. Previous shRNA experiments by Dr Erler using retroviral delivery have established sequences which specifically target LOX and LOXL2, without affecting other proteins including other LOX family members.

Evaluation of foreseeable effects

Despite the inclusion of the safety features, the lentivirus produced with this system can still pose some biohazardous risk since it can transduce primary human cells. Control measures to be used will include good laboratory practice, wearing of lab coats, wearing of two pairs of gloves, and all work to be performed in a designated Class II biohazard safety cabinet, which will be disinfected with 2% Virkon after use.

The lentivirus produced with this system can infect all primary human cells, but is replication deficient. In addition, the envelope protein of I-IIV-1 has been replaced with that of VSV-1, to avoid providing resistance to host defence mechanism. HIV-1 survival outside of the intracellular compartment is extremely limited, and is unlikely to recombine with related microorganisms. Survival of the recombinant lentiviral vectors will be similar to the wild-type virus, which is extremely low outside of a cellular host, or within human blood and a limited number of bodily fluids. HIV-1 is a human pathogen, but is not known to have any pathogenicity to agricultural animals, plants or other microorganisms.

To date, no one has been identified as infected with HIV-1 due to contact with an environmental surface.

The system to be used results in the production of a replication-defective virus. The inserted gene product is highly specific for knockdown of LOX and LOXL2. Since the inserted gene will result in the knockdown of genes known to promote tumour growth/progression upon expression, it is very unlikely to result in harmful biological activity. The safety and containment procedures in place should prevent transmission of the virus to the environment by air or drains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Transfections and infections of cells will be performed in a designated Class II biohazard safety cabinet, which will be disinfected with 2% Virkon after use. The cabinet will prevent unwanted infection of the cell cultures as well as release of the viruses into the environment. Infected cells will be grown in a designated incubator. Pipettes, containers and plasticware contaminated with viruses will be left completely submerged in 2% TriGene before incineration. Cells and growth medium will be treated with 1% Virkon before disposal according to the Institute’s Waste Index and Disinfection protocols. Virkon has been validated as being effective within 5 minutes at these concentrations and stocks are routinely tested. HIV-1 is transmitted through blood and a limited number of bodily fluids, none of which will be encountered in the course of this work. No living material infected with HIV-1 derived lentivirus will be transported other than between cabinet, microscope and incubator in the tissue culture room. Once infected, the cells and cell extracts will no longer contain productive virus. In the event of a spillage, chlorine-releasing disinfectant granules (HAZ-TAB) or 1% Virkon will be used for immediate disinfection.

Virus will be produced and collected in a designated Class II biohazard safety room. All batches collected will be tested to confirm that no replication competent virus is present. Cells will be transduced in the designated room and will be kept there until they are virus-free for 24 hours. All subsequent work will be level 1. Transduced virus-free cells will be cultured in level 1 tissue culture and cell samples will be tested in the level 1 labs.
The ICR genetic modification safety committee are happy with this risk assessment.

Please enter comments on the GM safety committee on the risk assessment

The ICR genetic modification safety committee are happy with this risk assessment.

**Project Containment**

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**Project Ref**  80/08.3

<table>
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<tr>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<tbody>
<tr>
<td>17/07/2008</td>
<td>Determination of the amount of vaccinia virus, and anti vaccinia virus antibodies, in cell culture samples and clinical material.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Not Applicable</td>
<td>N</td>
<td>Project notified under transitional arrangements N</td>
</tr>
</tbody>
</table>

**Historical Significant Changes**

- Withdrawn  N
- Date of Significant Change

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

- Vaccinia virus is classified as a group 2 biological agent under ACDP. GL-ONC1 is a highly attenuated derivative of vaccinia virus Lister (LIVP) from the Institute of Research on Viral Preparations, Moscow. LIVP has been used extensively as a vaccine for smallpox during the smallpox eradication campaign. For smallpox vaccines most adverse effects were common cold-like symptoms and only rarely were severe complications seen. Typically a lesion at point of inoculation at 1 week post vaccination was seen and rarely, generalised vaccinia 5-10 days later. The virus may be transmitted to contacts of inoculated individuals. See action sheet and document. The genetic modifications found in GL-ONC1 (insertion of Luciferase, GFP, GUS and lac-Z) are not expected to alter the pathogenicity of the virus and are themselves not hazardous to human health. Vaccinia virus does not integrate into the genome and the naked DNA is not hazardous.

**Recipient or parental organism**

CV-1 cells (African green monkey kidney) will be used for vaccinia virus culture, plaque assays and the neutralisation assay. Head and Neck, melanoma, and colorectal cell lines will be used to assess the oncolytic potential of GLONC1.

**Host/vector system**

Vaccinia virus LIVP was used as a vaccine for smallpox. The host range of this virus is humans, rabbit, cows and river buffaloes.

**Origin & function**

GL-ONC1 has a number of reporter gene inserted into its genome. A Renilla Luciferase-Green Florescent Protein (GFP) fusion, Escherichia coli (E. coli) beta-galactosidase (Lac Z), E. coli glucuronidase (GUS) have been inserted into the F14.5L, thymidine kinase (tk) and haemagglutinin (ha) loci in LIVP respectively. The transferring receptor (TFR) has also been inserted in the J2R locus but is not expressed as it is in the reverse orientation to a synthetic vaccinia early late promoter. GL-ONC1 was described by Zhang et al., in 2007 under the nomenclature (GLV-1 h68). The Renilla luciferase gene originates from the Renilla reniformis (sea pansy). The Renilla luciferase gene encodes a protein that can catalyse the oxidation of coelenterazine (coelenterate-luciferin) generating light which can be visualised using an IVIS imaging system. GFP is a commonly used reporter gene originally isolated from the jelly fish Aequorea victoria. GFP has a major excitation peak at 395nm and a minor peak at 475nm and an emission peak at 509nm in the lower green portion of the visible spectrum. The luciferase-GFP fusion is not expected to have any adverse effects if expressed in humans. beta-galactosidase hydrolyses a range of sugar molecules to generate a coloured product that can be measured to determine the amount of enzyme activity. Similarly, beta-glucuronidase cleaves the substrate X-Gluc (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid), to produce colorless glucuronic acid and an intense blue precipitate of chloro-bromoindigo. Expression of beta-galactosidase and betaglucuronidase can be detected in situ or in a variety of colourmetric assays. It is not anticipated that expression of beta-galactosidase or betaglucuronidase would have any adverse effects if expressed in humans.

**Evaluation of foreseeable effects**

- GL-ONC1 has been extensively used as a smallpox vaccine. For less attenuated smallpox vaccines most adverse effects were related to common cold-like symptoms. Rarely severe complications were encountered. Typically 1 week after vaccination, vaccination lesions appear at point of inoculation, which resolve within 28 days, and rarely as a severe complication, generalised vaccinia. Vaccinia does not ordinarily cause disease in humans, it is not a natural human pathogen, disease is a rare complication of vaccination. The greatest risk of vaccination is transmittal of the virus to unvaccinated contacts or to other sites on the vaccinated individual.

- GL-ONC1 is stable for up to 6 days when dried at room temperature, although infectivity decreases exponentially with time. Vaccinia is not pathogenic for plants, microorganisms or other animals beyond its host range. Although GL-ONC1isa replicating virus the expression of the marker genes and inactivation of the ha and tk genes are likely to produce an attenuated phenotype. There is no evidence that vaccinia virus can insert its DNA into the host genome. The safety and containment procedures in place should be sufficient to prevent release of the virus to the environment by drains or air. Animals that have been infected with the GL-ONC1 are unlikely to escape from the Biological Service Unit (BSU).
Normal working practice is only to open cages in hoods (as part of laboratory animal allergy prevention) and to record the number of occupants on each cage label. In addition, lab animals are unlikely to survive in the environment as they have no natural resistance to environmental pathogens.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Biological liquid waste will be subject to inactivation with 1% Virkon for 24 hours prior to disposal to drains with plenty of water. This has been documented as being sufficient for inactivation of the virus according to RelyOn Virkon® Efficacy Data. Surface contaminated plastic waste will be rinsed with 1% Trigene laboratory disinfectant or 1% Virkon and then autoclaved prior to disposal. Pipettes and pipette tips will be treated with either 1% Virkon or 2% Trigene for 24 hours prior to autoclaving and disposal. Trigene also breaks down and inactivates DNA and RNA and is a DEFRA approved disinfectant. Trigene will also be used for decontamination of laboratory surfaces. All clinical material will be placed in plastic Sharps/Cm bins labelled with the following ‘ACGM2 waste, 5C10.1, FOR AUTOCLAVING AND INCINERATION’ in addition to standard ICR waste index. Any biological spills will immediately be treated with Virkon granules. Vaccinia virus is also susceptible to a range of chemicals including 1% sodium hypochlorite, 2% glutaraldehyde, forraldehyde and household bleach. For example, in experiments where infected cells are fixed (with formaldehyde and glutaraldehyde) prior to staining to assess lacZ expression, infectious virus will be inactivated by this procedure but the infected material will still be disposed by the approved waste route i.e. treatment with Virkon or Trigene and autoclaving.

All animal work will be performed in the BSU and in accordance with the local rules for ACDP level 2 working in the BSU. In the BSU standard cleaning and disinfection of the work area and equipment such as beakers and balances will be carried out using Virkon or Trigene. There is a theoretical risk of virus excretion therefore cages used for animal work will be of a disposable type. The cages will not be cleaned whilst occupied by animals as all studies proposed are of short duration. At the end of the work the cage in its entirety will be placed into a yellow plastic bag and the contents sealed with a plastic tie. The sealed yellow bag will be placed into a red 60 litre leak-proof plastic container supplied by a registered and approved waste contractor (Grundon Waste Management) and later will be collected by the waste contractor, who will incinerate the waste offsite. Sharps/Cm bin waste from BSU projects will be labelled with ‘ACGM2 waste, BSU, FOR INCINERATION’ and will be collected by the approved waste contractor and incinerated offsite.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee were happy that this risk assessment addressed all the issues. The contractor, Grundon Waste Management is registered to take GM waste.

Project Containment
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**Project Ref** 80/08.4

**Date Ackn'd** 24/11/2008

**CU2 Project Title** REPLICATION-DEFECTIVE LENTIVIRAL VECTORS CONNECTED PROGRAMME.

**Class**

- **CultureVol**
  - Class 2
  - < 1 Litre

**Consent Granted** Not Applicable

**Tick if notifying a connected programme of work** Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Generation of replication-defective lentiviruses (with biosafety features) for overexpression or inhibition of genes which may play a role in mammary gland development and/or genes for labelling cells with fluorescent proteins or lucifrase. The project will use viral vectors to transduce primary mouse mammary cells in culture and then transplant them back into mammary fat pads to enable the in vivo assessment of gene overexpression or knockdown on mammary development without the need to generate transgenic or knockout animals.

**Recipient or parental organism**

DNA will be propagated in DH5a (disabled, recombination deficient (recA) E.coli. Replication defection viruses will be generated in HEK293T cells. Viruses will be used to infect mouse 3T3 fibroblasts, C57MG, NMuMG, CommaD and HC11 mouse mammary cell lines (these are widely used and none are known to
carry adventitious agents) together with primary mouse mammary epithelial cells.

Host/vector system

This is a connected programme enabling different lentiviral vector systems to be used providing all conform to minimum biosafety standards, namely all must be split-genome (on a minimum of three vectors) replication defective viruses. The initial vectors to be used will be Trono laboratory split genome, conditional packaging lentiviral system \( pWPI, pMD2.G, pSAX2 \); split genome lentiviral SEW expression vector. \( pCMVR8.91 \) packaging construct and \( pMD2.VSVG \) pseudotyping vector; GIPZ lentiviral shRNA/mir system (six vector split genome VSV-G pseudotyped).

Origin & function

The aim of this connected programme submission is to establish minimum biosafety features and standards that will be used with this work so that additional vector systems being used for the same project and which meet these safety criteria may be added to this risk assessment without the need for resubmission to the HSE. For example, we have previously had approved risk assessment for two lentiviral systems, one for over-expression of genes and one for gene knockdown. However, recent collaborators who wished to work with us doing exactly the same experiments as described above, but with their gene of interest, had their over-expression and knockdown sequences cloned into different vectors. We did not have approval for these systems, even though they had equivalent third generation features with the viral elements split between three plasmids. We therefore had to spend a considerable amount of time recloning into our own vector systems. A second example of the need for this Connected Programme is that a number of viral libraries are now available through commercial companies from which genes of interest can be "cherry picked". Again, they usually have the same high biosafety features but in different packaging systems. It makes no sense to have to apply to the HSE every time one wants to use a different system form a different company.

Genes of interest to be studied include:

- ID4 - abHLH protein involved in regulating differentiation (no known oncogenic function)
- Myb - a transcription factor involved in cell lineage switching (oncogene)
- Msx2 - a transcription factor involved in cell lineage switching (potential oncogene)
- Sox6 - a transcription factor involved in cell lineage switching (no known oncogenic function).
- Ttk - Ttk protein kinase (potential oncogene).
- Wee1 - Wee1 tyrosine kinase homolog (potential oncogene).

The vectors may also carry marker genes such as luciferase or fluorescent proteins.

Evaluation of foreseeable effects

There is a negligible hazard to human health from disable E.coli host strains. DH5a has a proven history of safe use, has been characterized and is known to be free from harmful sequences.

The infected packaging cells would be unlikely to be pathogenic due to rapid complement mediated lysis of these cells when recognized by the immune system as of non-self origin. Transduced cells would not be shedding virus as they are replication-defective and therefore do not pose a risk to human health or the environment. The risk that the vector could be rescued in the mouse is negligible (there is no known means for murine retrovirus rescue of such vectors) so there is negligible risk of spread to other cages or to staff. Mouse cells will be unable to survive in the human in the event of accidental inoculation due to the species barrier and immune response.

The only risk is from lentiviral-mediated insertion of an oncogene or knockdown sequence into a human host during handling of viral supernatants. However, insertion of an oncogene into the human cells is not enough to create by itself cancer because it needs a combination of additional alterations. We will not use sequences which knockdown p53, Rb or their regulators. The vectors will be replication defective and potential exposure will be controlled by following containment level 2 local rules and use of good micro-biological practice.

The viruses are unlikely to survive outside the laboratory, as suitable growth conditions will not exist. The packaging cells and any transduced cells cannot survive outside
of the laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Work surfaces, including the inside of microbiological safety cabinets will be suitably disinfected after use (trigene). Centrifugation will be done in sealed tubes. Liquid wastes will be rendered safe for disposal by overnight treatment with an approved disinfectant according the ICR Waste Index (Virkon). Plastic pipettes will be soaked in an approved disinfectant (Virkon) prior to removal by Central Services Department (CSSD). Solid waste will be placed in autoclave bags for collection by CSSD. Material collected by CSSD will be autoclaved on site. The cleaning, disinfecting and disposal procedures will be documented and displayed. The biological spillage procedure will be displayed and HAZ-TAB disinfectants will be available for immediate use. Any incidents and accidents will be reported.

Once the lentivirus has infected the cells it should not be able to replicate. All new batches of virus will be tested for replication competence. Murine NIH3T3 or 3T3-L1 cells will be transduced with fresh or frozen (-70oC) viral supernatant (supernatant 1) (virus containing the gene of interest plus either a selectable marker or GFP) plus 2 ug/ml of polybrene and incubated overnight. The following day, cells will be washed with medium and refed. After 24 to 48 hours check the number of GFP positive cells will be assessed (or the cells will be put under selection). Cells will be grown for a further 4 to 6 days (splitting cells if necessary) and an overnight supernatant (supernatant 2) harvested from confluent cells. The supernatant will be passed through a 0.45 micron filter and either used straight away or stored frozen at -70oC. NIH3T3 or 3T3-L1 cells will then be transduced with supernatant 2 plus polybrene. A positive control consisting of supernatant 1 plus polybrene will be included. The cells will be washed the next day and fresh medium added. The cultures will be checked for GFP positive cells 24 to 48 hours later (or put under selection). In the absence of virus in supernatant 2 no green (GFP positive) cells (or resistant colonies if a selectable marker is used) should be seen whereas green cells should be present in cells transfected with positive control supernatant 1.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee reviewed this risk assessment and considered it to be well written and approved it.

**Project Containment**

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02/03/2022
## Measles virus and measles virus vectors for in vitro and in vivo study.

**Project Additional Information**

### Purposes of the contained use

Wildtype MV is listed in ACDP as a Hazard Group 2 biological agent therefore Containment Level 2 is appropriate for MV-Edm and MV-Edm derived vectors. MV does not integrate into host DNA. Naked MV DNA is also not hazardous. The manipulations to MV-Edm are unlikely to alter the tropism or pathogenicity of the virus. (NIS and GFP genes are not potentially hazardous to human health).

### Recipient or parental organism

Mouse and rat tumour models.

### Host/vector system

The MV-Edm virus and vectors are derived from the attenuated Edmonston vaccine strain of MV(MV-Edm). The genetic basis of attenuation is unknown, however, as this is a widely used vaccine strain it is known to pose no risk to health. The manipulations to MV-Edm are unlikely to alter the tropism or pathogenicity of the virus. MV has no known transforming abilities and does not integrate into the host DNA; naked MV DNA is also not hazardous.

### Origin & function

The NIS and GFP genes, driven by constitutive CMV promoters, have been inserted into the full length MV-Edm genome downstream of the viral hemagglutinin gene. NIS is an integral plasma membrane glycoprotein which acts as an ion pump that transports iodide (I-) into cells. It has been most commonly studied and discussed in...
connection with the thyroid gland, in which NIS mediates the active transport of I- into the thyroid follicular cells as the crucial first step for thyroid hormone biosynthesis. The trapping of I- in cells, mediated by NIS, allows the delivery or radioiodine (131-I) and this method has been used effectively in the clinic as an ablative therapy for thyroid cancer for many years. NIS gene delivery into non-thyroidal tumour cells is also capable of inducing accumulation of therapeutically effective doses of 131-I and therefore has potential as a therapy for extrathyroidal tumours. In addition, the accumulation of 131-I allows scintigraphic imaging of tumours in order to assess tumour progression or regression, metastatic disease and recurrence. NIS occurs normally in the human body and, if expressed in humans, is not expected to have any adverse effects. The green fluorescent protein (GFP) is a protein that derives from the jellyfish Aequoreo Victoria. It is commonly used as a reporter gene. GFP is not expected to have any adverse effects if expressed in humans.

**Evaluation of foreseeable effects**

MV has no known transforming abilities and does not integrate into the host DNA. The MV-Edm virus and vectors are derived from the attenuated Edmonston vaccine strain of MV (MV-Edm). Although the genetic basis of attenuation is unknown, this is a widely used vaccine strain that is known to pose no risk to health. The manipulations to MV-Edm are unlikely to alter the tropism or pathogenicity of the virus. Naked MV DNA is also not hazardous.

Expression of NIS and GFP genes are not potentially hazardous to human health.

The safety and containment procedures in place should prevent transmission of the virus to the environment by air or drains. It is unlikely that the mutations introduced into MV could be stably maintained outside the laboratory conditions as the virus requires a host to replicate in. Animals that have been infected with the MV vectors are unlikely to escape from the Biological Service Unit (BSU). Normal working practice is only to open cages in hoods (as part of laboratory animal allergy prevention) and to record the number of occupants on each cage label. In addition, lab animals are unlikely to survive in the environment as they have no natural resistance to environmental pathogens.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Pippettes will be disposed of into pipette bins containing 2% TriGene, which inactivates nucleic acid, and contaminated plasticware will be soaked in a 1% Virkon solution for at least 12 hours prior to removal from containment. Following removal, plasticware will be autoclaved onsite. Biological liquid waste will be treated with 1% Virkon for at least 24 hours prior to disposal to the drain with plenty of water. A 1% Virkon solution has been shown to be sufficient for decontamination of VSV in tissue. In the event of a spillage Virkon granules will be used to immediately inactivate the virus. This treatment has been shown to kill VSV vectors effectively. All cages used for animal work will be of a disposable type. The cages will not be cleaned whilst occupied by animals as all studies proposed are of short duration. At the end of the work the cage in its entirety will be placed into a yellow plastic bag and contents sealed with a plastic tie. The sealed yellow bag will be placed into a red 60 litre leak-proof plastic container supplied by a registered and approved waste contractor (Grundon Waste Management) and later will be collected by the waste contractor, who will incinerate the waste onsite.

Is an emergency plan required according to regulation 20?  
Y

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N
1) There should only be one proposer on each risk assessment. (It is fine to have just you)
Answer from Proposer:
I have changed the proposer on each risk assessment to me.

2) I am a bit confused. It states that all work will be in one room apart from transport to the freezer, but it lists rooms CBO37, 5C10.1, 4S4.1, 4S4.1, rm46.4 of BSU for use?
- I guess one of these rooms is for the irradiation part of the procedure? How will they transport the virus since it looks to me like they may actually be going from room to room? It might be useful to state what each room is being used for?
Answer from proposer:
I have removed CBO37
5C10.1 is the Category II lab
The radiation activities will take place in Room 19.2
4S4.1 and 4S5.1 refer to rooms where -80C storage will take place.
There is no designated room number in the BSU for animal work of this kind.
Answer from proposer:
The virus will be transported from room to room in a sealed tube in a sealed container.

3) I note that both of this group submissions list the BSU on the location room numbers, but neither list any BSU activities. Maybe all their locations could be reconfirmed to ensure accuracy?
Answer from Proposer:
I had originally planned to submit a separate BSU risk assessment, however, I have updated the risk assessments to include BSU activities.

4) There is mention of Room 46.4 of BSU Fulham being used for this work. I assume this is the Procedure room located just inside the barrier which is labelled Room 44. If this is the case this room is NOT a containment level 2 facility. The BSU's at Sutton and CBL do not have facilities for Category 2 containment work.
Answer from proposer:
Where animals are infected with virus all materials will be disposed of by approved routes (see risk assessment)

5) I have just one question regarding the GMO Proposal. It refers to the use of irradiation on infected cell lines. Will this occur in the Class II room or in a radioactivity area, and if it is the latter what measures, if any are needed, will be used to contain the infected cells or ensure there is no live virus in the cell culture.
Answer from proposer:
Irradiation of infected cell lines will take place in a controlled radiation area (Room 19.2). This irradiation will take place on cultures in sealed tissue culture containers.

6) 07-09 only. And also they should change MV to VSV on pages 7 and 8.
Answer from proposer:
Done
7) 07-08 only. I would like to see more details of how the virus will be produced, will scalpel blades be used for existing bands from gels, and do infected cells themselves produce infective viral particles?
Answer from proposer:
No scalpel blades will be used in the production or purification of these viruses. For virus production cells are infected with virus, harvested and subjected to centrifugation. Where needles are used for harvesting bands from sucrose gradients this will be done using a retort stand fitted with Perspex safety blocks (approved by H&S for previous virus purification protocols) produced specifically for this application.

8) 07-09 only. More detail required. How will virus be produced? Will sharps be used for cutting gels? It is stated that the virus is replication competent - will cells infected with it be transplanted into animals? Will the animals then be giving off virus and if so, will they be contained?
Answer from proposer:
See point 7) re scalpel blades. Where animals are infected with virus all materials will be disposed of by approved routes (see BSU risk assessment).

Please add the name preferably a reference for the vector to be used. Is there a bacterial host for the propagation of the viral vectors? This should be named. Could the proposer name which cancer cell lines they plan to use.

MV vectors will be obtained from the lab of Department of Immunology, Mayo Clinic, Rochester, Minnesota. The available relevant references for virus production are:


VSV vectors will be obtained from the lab, Department of Immunology, Mayo Clinic, Rochester, Minnesota. The available relevant references for virus production are:


No bacterial host is used for the propagation of measles virus.

Cell lines used will be Vero (African Green Monkey), LA7 (mouse mammary epithelial tumour), RG2 (rat glioma) and 9LlacZ (rat gliosarcoma). Other commonly used cell lines from other cancer types may also be tested. These cell lines are used routinely in the laboratory and pose no risk to human health. The cells used in this study pose no environmental hazard as they are unlikely to survive outside the laboratory.

9) If people with eczema/impaired immunity are to be excluded from working on this project, perhaps pregnant women should also be excluded??On a similar note, is it worth checking the measles immune status of staff on this project prior to exposure to this live, attenuated virus?
Answer from Proposer:
Previously the procedure for handling virus has been that pregnant women should not handle high titre stocks of virus but are permitted to handle diluted virus as the risk of infection is minimal. The measles virus and vectors used is an attenuated vaccine strain.
Investigating the effect of wild-type and mutant receptor tyrosine kinases on receptor-tyrosine kinase inhibition using retroviral vectors.

The aim of our project is to assess the effect of expression of wildtype and mutant receptor tyrosine kinase expression in paediatric and adult glioma cell-lines on cell growth, migration and RTK inhibitor sensitivity. It has previously been shown that in glionblastoma mutations in the EGFR gene have multiple effects on this and therefore we will assess if mutations in other receptor tyrosine kinases have a similar effect. We aim to express these wildtype and mutant genes using retroviral (MMLV based) vectors.

The recipient cell lines will be paediatric and adult glioblastoma cell-lines, as well as mouse fibroblast cell-line NIH-3T3. These cell-lines have been extensively characterised, have been maintained in cell culture for many years, are not a known source of pathogens, and are unlikely to survive outside the laboratory environment.
The vector that will be used is based on the Murine Moloney Leukemia virus (pBabe-puro). The vector has been modified in several ways to ensure that the virus is replication-defective, and will not form active viral particles unless it is co-transfected into a packaging cell-line with helper plasmids.

Origin & function

The genetic material that will be cloned into the retroviral vector will be DNA encoding wildtype and mutant versions of several receptor tyrosine kinases, such as EGFR, PDGFRα, PDGFRβ, IGF1R, ERBB2, KIT, VEGFR2 and MET. We also will clone fusion genes involving these receptors. The material will either be cloned from universal human reference RNA, with the mutations being introduced using site directed mutagenesis, or will be cloned from RNA extracted from patient material.

Evaluation of foreseeable effects

The retroviral vectors that we propose to use do have the potential for infecting any human cell. Through good lab practice the risk of these vectors affecting the user or others in the laboratory is extremely low. In the unlikely event of infection, the viral particles will not be able to replicate as they lack the gene encoding the envelope protein, which is required for cell entry. It is very unlikely that receptor tyrosine kinase expression will lead to cells that are able to survive outside the laboratory environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

There will be no derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All transfections and transductions will be performed in a designated Class II biohazard safety cabinet, which will be disinfected with 1% Virkon after use. Virkon is a chemical disinfectant that kills 99.999% of organisms in less than 10 minutes. The cabinet will prevent unwanted infection of the cell-cultures as well as release of the viruses into the environment. Infected cells will be grown in a designated incubator. Pipettes, containers and plasticware contaminated with viruses will be left completely submerged in 1% virkon before disposal. Cells and growth medium will be treated with 1% virkon before disposal according to the Institute's Waste Index and Disinfection protocols. Sharps will not be used. Solid waste will be collected separately from any other biological waste, using a separate waste management system which involves autoclaving the waste in the building, after which it will be disposed of as biological waste which will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee had some minor comments at the initial risk assessment:
- eye protection should be worn when working with the virus, even when working in a biological safety cabinet.
- details of method to detect helper virus formation in transduced cells were asked
- they asked to state that good laboratory practice will be used when working with oncogenic DNA

All these points were addressed in the final version of the risk assessment, which was approved and is attached to this application.
### Project Containment

#### Laboratory Activities
- L2: Yes
- L3
- L4
- L2

#### Glass Houses
- L3
- L4

#### Growth Rooms
- L2
- L3
- L4

#### Animal Units
- L2
- L3
- L4

#### Large Scale Activities
- L2
- L3
- L4

#### Human Clinical Applications
- L2
- L3
- L4

### Project Ref 80/11.1
- Overexpression and knockdown of gene and miRNA drug development targets using lentiviral vector delivery: Cancer Therapeutics connected programme

#### Date Ackn'd
- 12/04/2011

#### CU2 Project Title
- Overexpression and knockdown of gene and miRNA drug development targets using lentiviral vector delivery: Cancer Therapeutics connected programme

#### Class
- Class 2

#### Culture Volume
- Class 2
- < 1 litre

#### Non-GMM
- Consent Granted

#### Project notified under transitional arrangements
- N

#### Withdrawn
- N

#### Tick if notifying a connected programme of work
- N

#### Project Additional Information

#### Purposes of the contained use
The generation and use of recombinant lentiviruses for overexpression or knockdown of genes or miRNA that may be involved in the development of cancer. The project will use third generation lentiviral vectors to transduce a range of target cells to generate stable cell lines expressing a defined phenotype for evaluation both in vivo and in vitro.

#### Recipient or parental organism
DNA will be propagated in DH5a (disabled, recombination deficient (recA)) E.coli or Stbl3 E. coli

#### Liposarcoma cell lines:

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02/03/2022
**DDLPS: FU-DDLS-1, LPS6, LPS695, LPS853, LPS510, DDLS8817, T1000, 93-4496, 94-778, RDD8107 WDPDS: GOT3 SW 872 (HTB 92) (lacks MDM2 amplification), LiSa-2 (WLS-160, HS-18).**

**Rhabdosarcoma:**
- RH3, RH4*, RH5, RH18*, RH28, RH30, RH41, RH30-Birch*
- CCA CT10, RD*, JR-1*, CT-T, RUCH3, RUCH-2 (Botryoid), RMS-YM*, A204/HTB82, RH7, RH10, RH13, A673, Hs729T

**Ewings sarcomas**
- A673, TC-71, SK-ES-1, A4573, STAET2.1, TTC466, SKNMC, STAET1, RM82, WE68, CADOES, SJRH.

**Desmoplastic small round tumour**
- JN-DSRCT-1.

**Models:**
- C2C12 - myoblasts, osteoblasts and adipocytes.
- C3H10T1/2 - myoblasts, osteoblasts, chondrocytes and adipocytes.
- 3T3-L1 - pre-adipocytic murine cells model.
- LPS in xenograft.
- Shep, Kelly and IMR32 Neuroblastoma.
- Primary mesenchymal stem cells.
- Human tumour cell lines and established metastatic variants.
- HEK293T packaging cells.

### Host/vector system

This is a connected programme to enable the use of different lentiviral systems that are all split genome third generation. The following vectors or derivatives will be employed. Tronolab pWPI system; split genome lentiviral SEW expression vector; GIPZ and TRIPZ lentiviral shRNAmir system (six vector split genome VSV-G pseudotyped); pLVUT-TR-KRAB (Aebischer); Sigma MISSION shRNA pre-packaged lentiviral particles; pCDF cDNA Cloning and Expression Lentivectors. (System Biosciences); Retro-X Tet on Advanced Inducible Expression System (Clontech); Virapower TREX and BlockIT Inducible RNAi Lentiviral expression system (Invitrogen).

### Origin & function

The aim of this connected programme is to allow those groups employing lentiviral systems for determining the validity of gene targets in a drug development program to do so without the need for resubmission to the HSE validity of gene targets in a drug development program to do so without the need for resubmission to the HSE.

The genes of interest that are being studied include:
- **MAP4K4** - Serine/threonine kinase that may play a role in the response to the environmental stress and cytokines such as TNF-alpha.
- **MYCN** - A Proto-oncogene. Amplification of this gene is associated with a variety of tumors, most notably neuroblastomas.
- **ALK-Orphan receptor with a tyrosine-protein kinase activity**
- **HIF1alpha** - The protein is a master transcriptional regulator of the adaptive response to hypoxia.
- **ILT8** - Chemokine that functions as a chemoattractant, and is also a potent angiogenic factor.
- **GMCSF** - The protein encoded by this gene is a cytokine that controls the production, differentiation, and function of granulocytes and macrophages.
- **TIMP-1** - The proteins encoded by this gene family are natural inhibitors of the matrix metalloproteinases (MMPs), a group of peptidases involved in degradation of the extracellular matrix.
- **TNFalpha** - This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis and lipid metabolism.
- **WNT** - A proto-oncogene. These proteins have been implicated in oncogenesis and in several developmental processes, including regulation of cell fate.
- **DVL1** - The human homolog of the Drosophila dishevelled gene (dsh) encodes a cytoplasmic phosphoprotein that regulates cell proliferation.
- **SHH** - The hedgehog (Hh) signalling pathway is crucial in the development of all known animals.
- **Siah 1 and 2** - These genes encode proteins that are E3 ligases and are involved in ubiquitination and proteasome-mediated degradation of specific proteins.
- **B Catenin** - Mutations in this gene are a cause of colorectal cancer (CRC), pilomatrixcoma (PTM), medulloblastoma (MBD), and ovarian cancer.
- **THAIP1 and 2** - Threonine aldolase 1 pseudogenes.
- **bFGF** - FGF family members bind heparin and possess broad mitogenic and angiogenic activities.
- **AKT 1-3** - Serine/threonine kinases that plays a key in regulating cell survival, insulin signalling, angiogenesis and tumor formation. Akt is a downstream mediator of the PI 3-K pathway.
- **VEGF** - This gene encodes a protein that specifically acts on endothelial cells and has various effects, including mediating increased vascular permeability, inducing
angiogenesis, vasculogenesis and endothelial cell growth, promoting cell migration, and inhibiting apoptosis.
mTOR Rictor and Raptor These kinases mediate cellular responses to stresses such as DNA damage and nutrient deprivation
HSP90 isoforms. HSP90 proteins are highly conserved molecular chaperones that have key roles in signal transduction, protein folding and protein degradation.
GSK3B Participates in the Wnt signalling pathway
Aurora kinase A and B. Aurora kinases are a family of highly homologous serine/threonine kinases that play a critical role in regulating many of the processes that are pivotal to mitosis.
Pik3CA, B, D, G Lipid kinases that are responsible for coordinating a diverse range of cell functions including proliferation, cell survival, degranulation, vesicular trafficking and cell migration. Potentially oncogenic
Gly1 and 2 Threonine aldolases involved in glycine biosynthesis
Chk1 and 2 Required for checkpoint mediated cell cycle arrest in response to DNA damage
FLT1-3 These genes encode proteins that bind to VEGFR-A, VEGFR-B and placental growth factor and play an important role in angiogenesis and vasculogenesis.
RPS6KB1 and 2 Downstream effectors of the mTOR signaling pathway
PAK4 PAK proteins are critical effectors that link Rho GTPases to cytoskeleton reorganization and nuclear signaling
FOXO1 forkhead box A10/O26/04/2011 Transcription factor. Multiple targets. Might play a role in myogenic differentiation.
NELL1 NEL-1. This gene encodes a cytoplasmic protein that contains epidermal growth factor (EGF)-like repeats. The encoded heterotrimeric protein may be involved in cell growth regulation and differentiation.
PAX3-FOXO1 Fusion gene, Rhabdomyosarcoma, Transcription Factor: Multiple targets.

The following genes encode proteins that are involved in the epigenetic control of demethylating histones:
FBX10 FBXL11 FBXL19 HR HSPB JARID1A JARID1B JARID1C JARID1D JARID2 JHDM1D JMJD1A JMJD1B JMJD1C JMJD2A JMJD2B JMJD2C JMJD2D JMJD3 JMJD4 JMJD5 JMJD6 LSD1 PHF2 PHF8 UTX UTY ASH1 ASH2L CARM1 DOT1L EHMT1 EHMT2 EZH1 EZH2 LCMT2 METT10D METT11D1 METT5D1 MLL MLL2 MLL3 MLL4 MLL5 NSD1 PRDM1 PRDM2 PRDM5 PRMT1 PRMT2 PRMT5 PRMT6 PRMT7 PRMT8 SET7/9 SET8 SETD1A SETD2 SETD3 SETD4 SETD5 SETD6 SETDB1 SETD2B SETMAR SMYD2 SMYD3 SMYD5 SUV39H1 SUV39H2 SUV420H1 SUV420H2 SUZ12 WHSC1 WHSC1L1

IGF1R IGF1R homodimers are activated by IGF-I and IGF-II and mediate pre- and postnatal growth. Mediate action via PI3K.
MET MET is a cell surface receptor that is involved in initiating cell migration.
MYC A transcription factor with multiple targets and known oncogenic function.
ALK A signalling kinase critical in cell survival and proliferation.
ERK1 A signalling kinase critical in cell survival and proliferation.
EEK2 A signalling kinase critical in cell survival and proliferation
PLK1 A signalling kinase critical in cell survival and proliferation
RAF1 This gene is the cellular homolog of viral raf gene. Functions downstream of the Ras family of membrane associated GTPases to which it binds directly.
GPC3 Heparan sulfate proteoglycan involved in growth factor signalling.
GPC3 Heparan sulfate proteoglycan involved in growth factor signalling.
SULF1 Sulatase with heparin-degrading endosulfatase activity.
SULF2 Sulatase with heparin-degrading endosulfatase activity

The following miRNAs

hsa-miR-335 *hsa-miR-450b-5p hsa-miR-483-3p hsa-miR-421 hsa-miR-376a hsa-miR-369-3p hsa-miR-19a hsa-miR-495 hsa-miR-487b hsa-miR-542-3p
hsa-miR-654-3p hsa-miR-136 hsa-miR-362-3p hsa-miR-19b hsa-miR-376b hsa-miR-301a hsa-miR-323-3p hsa-miR-181b hsa-miR-432 hsa-miR-654-5p hsa-miR-
hsa-miR-502-5p hsa-miR-21 hsa-miR-182
hsa-lwt-7i hsa-miR-199a-5p hsa-miR-379 hsa-miR-9 hsa-miR-20a hsa-miR-509-3p hsa-miR-660 hsa-miR-130a hsa-miR-501-5p
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<td>hsa-miR-409-3p</td>
<td>hsa-miR-148a hsa-miR-335 hsa-miR-543 hsa-miR-17* hsa-miR-181d hsa-miR-532-5p hsa-miR-493 hsa-miR-370 hsa-miR-493* hsa-miR-25 hsa-miR-337-3p hsa-miR-501-3p hsa-miR-183 hsa-miR-450a hsa-miR-450a hsa-miR-380 hsa-miR-362-5p hsa-miR-18a hsa-miR-500* hsa-miR-532-3p hsa-miR-375 hsa-miR-483-5p hsa-miR-425 hsa-miR-424 hsa-miR-146b-5p hsa-miR-513c hsa-miR-93 hsa-miR-500 hsa-miR-382 hsa-miR-299-5p hsa-miR-433 hsa-miR-9* hsa-miR-200b hsa-miR-502-3p hsa-miR-379* hsa-miR-485-5p hsa-miR-877 hsa-miR-92a1* hsa-miR-127-3p hsa-miR-411* hsa-miR-181a hsa-miR-199b-5p hsa-miR-16-2* hsa-miR-424* hsa-miR-99b hsa-miR-100 hsa-miR-98 hsa-miR-92a hsa-miR-191 hsa-miR-15a hsa-miR-33a hsa-miR-148b hsa-miR-378 hsa-miR-22 hsa-miR-133 a hsa-miR-133a hsa-miR-193b* hsa-miR-29c hsa-miR-1 hsa-miR-95 hsa-miR-378c hsa-miR-486-5p hsa-miR-29c* hsa-miR-206 hsa-miR-378* hsa-miR-29a hsa-miR-193b hsa-miR-199a3p/5p hsa-miR-10b</td>
</tr>
</tbody>
</table>

Their functions are mostly unknown with following exceptions:

- hsa-miR-19a and b. Known oncogenic function. Activated by cMYC
- hsa-miR-181b. Involved in myogenic differentiation through modulation of HOX genes
- hsa-miR-21 known oncogenic function. Targets and represses PTEN
- hsa-miR-9 Oncogenic function. Activated by cMYC and MYCN. Promotes metastasis
- hsa-miR-20a. Oncogenic activated by cMYC and MYCN
- hsa-miR-17a, 18a, 92a and 378 Known oncogenic function activated by cMYC
- hsa-miR-25 Known onogenic function
- hsa-miR-183 Targets PTEN

Further genes and miRNAs will be added to this risk assessment in the future by means of local updates when new ones of further interest are identified.

### Evaluation of foreseeable effects

There is a negligible hazard to human health from disabled E. coli host strains. DH5a has a proven history of safe use, has been well characterised and is known to be free from harmful sequences.

The infected packaging cells would be unlikely to be pathogenic due to rapid complement mediated lysis of theses cells when recognized by the immun system as of non-self origin. Transduced cells would not be shedding irus as they are replication-defective and therefore do not pose a risk to human health or the environment. The risk that the vector could be rescued in the mouse is negligible (there is no known means for murine retrovirus rescue of such vectors) so there is negligible risk of spread to other cages or to staff. Mouse cells will be unable to survive in the human in the event of accidental inoculation due to the species barrier and immune response.

The only risk is from lentiviral-mediated insertion of an oncogene or knockdown sequence into a human host during handling of viral supernatants. However, insertion of an oncogen intot he human cells is not enough to create by itself cancer because it needs a combination of additional alterations. The vectors will be replication defective and potential exposure will be controlled by following containment level 2 rules and use of good microbiological practice.

The viruses are unlikely to survive outside the laboratory, as suitable growth conditions will ot exist. The packaging cells and any transduced cells cannot survive outside the laboratory.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Work surfaces, including the inside of microbiological safety cabinets, will be suitable disinfected after use (Trigene). Centrifugation will be done in sealed tubes. Liquid wastes will be rendered safe for disposal by overnight treatment with an approved disinfectant according to ICR Waste Index (Virkon or Chloros). Plastic pipettes will be...
soaked in an approved disinfectant (Virkon) prior to removal by Central Services Department (CSSD). Solid waste will be placed in autoclave bags for collection by CC+SSD. Material collected by CSSD will be autoclaved on site. The cleaning, disinfecting and disposal procedures will be documented and displayed. The biological spillage procedure will be displayed and HAZ-TAB disinfectants will be available for immediate use. Any incidents and accidents will be reported.

Once the lentivirus has infected the cells it should not be able to replicate. All new batches of virus will be tested for replication competence. Where appropriate with HIV based vectors this may be done by the use of a p24 assay on the tissue culture medium. Alternatively Murine NIH3T3 or 3T3-L1 cells will be transduced with fresh or frozen (-70°C) viral supernatant (supernatant 1) (virus containing the gene of interest plus either a selectable marker or GFP) plus 2ug/ml of polybrene and incubated overnight. The following day, cells will be washed with medium and refed. After 24 to 48 hours check the number of GFP positive cells will be assessed (or the cells will be put under selection). Cells will be grown for a further 4 to 6 days (splitting cells if necessary) and an overnight supernatant (supernatant 2) harvested from confluent cells. The supernatant will be passed through a 0.45 micron filter and either used straight away or stored frozen at -70°. NIH3T3 or 3T3-L1 cells will then be transduced with supernatant 2 plus polybrene. A positive control consisting of supernatant 1 plus polybrene will be included. The cell will be washed the next day and fresh medium added/ the cultures will be checked for GFP positive cells 24 to 48 hours later ( or put under selection). In the absence of virus in supernatant 2 no green (GFP positive) cells (or resistant colonies if a selectable marker is used) should be seen whereas green cells should be present in cells transfected with positive control supernatant 1.

Please enter comments on the GM safety committee on the risk assessment

Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 80/transA

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<td>08/12/1993</td>
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<td>Class 2</td>
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Purposes of the contained use

Our laboratory has active interests in the use of viral and other biotherapeutic agents, both as treatment for cancer and for improving response rates to, and toxicity of, existing cancer therapies. At present we utilise oncolytic viruses (detailed in a previous GMSC/HSE GM80/08.3 in the treatment of cancer. We also have a significant interest in radiosensitisation by both viral and standard chemical chemotherapeutic agents. Doses of radiotherapy that can be clinically administered to cancer patients are limited by local toxicity to joints and other tissues. The administration of novel radioprotective agents is one established technique to protect normal tissue against toxicity whilst allowing target tumour cells to exhibit sensitivity; an alternative mechanism is to use biotherapeutic agents which have synergy with radiotherapy to enhance the sensitivity of tumour cells to radiation. The agents which are the subject of this application will therefore be used in existing in vitro and in vivo models to assess their efficacy in protecting normal tissues (lentivirus work) or sensitising tumour cells (listeria work). This will involve testing the agents alone and in combination with radiotherapy and chemotherapy.

Note that this application is submitted as a Connected Programme. The aim of this submission is to establish minimum biosafety features and standards that will be used with this work so that additional vector systems and genes being used for the same project and which meet these safety criteria may be added to this risk assessment without the need for resubmission to the HSE.

Recipient or parental organism

Listeria monocytogenes EDGE
Class 2 Listeria monocytogenes yyCh
For the Listeria, standard plasmid vector systems designed for use in Gram positive bacteria or shuttle vectors that replicate in both Gram negative and Gram positives hosts (Listeria, Bifidobacteria and Lactococcus). The vector systems used in this project are commercially supplied, have a history of safe use and are not likely to endow any pathogenic traits.

Lentivirus:
We will use commercially available lentiviral plasmids, with appropriate packaging systems (2nd or 3rd generation or higher) or ready-made lentiviral particles. For generation of lentiviral plasmids within the institute we will propagate cDNA in DH5a (disabled, recombination deficient (recA)) E. coli or Stbl3 E.coli), which are designed to minimise recombination in lentiviral vectors. All virus will be replication incompetent and we will routinely screen stable cell lines, and produced titres of virus, for replication competence.

cDNA will be propagated in DH5a (disabled, recombination deficient (recA)) E. coli or Stbl3 E.coli), which are designed to minimise recombination in lentiviral vectors.

All the vector systems described below are split vector systems with a minimum of three elements:

1) The expression vector into which the gene of interest is cloned and which also usually contains a fluorescence and/or selectable marker. The expression vectors also contain elements to allow packaging into virions.

2) A packaging plasmid supplying the helper functions in conjunction with structural and replication proteins (in trans) required to produce the lentivirus. In some more advanced biosafety systems, these functions are also split between more than one vector.

3) A plasmid supplying an envelope protein that gives broad tropism (usually VSV-G).

VECTOR SYSTEM 1: pLV-CMV-X-GFP-2A-puro (3rd generation LV backbone with 2nd generation packaging plasmids)

VECTOR SYSTEM 2: pGIPZ SYSTEM (2nd generation)

VECTOR SYSTEM 3: pLV-fLuc (firefly luciferase) (3rd generation)

VECTOR SYSTEM 4: pLV105-eGFP (3rd generation)

Additional vector systems with the same minimal safety features will be added as required.

For listeria:

i) lux: The inserted genes of interest for each bacterial strain encode the luxABCDE operon, which is a synthetic operon encoding a fatty acid reductase complex (LuxCDE) involved in synthesis of the fatty aldehyde substrate for the bioluminescence reaction catalyzed by the LuxAB luciferase. Its function is to serve as a reporter system to monitor bacterial viability and growth both in vivo and in vitro.

ii) fLuc: Firefly luciferase gene. Expression of the luciferase gene is driven by the human cytomegalovirus immediate early gene (CMV) promoter. The reporter gene has been used extensively in research and poses no risk to animal health or the environment.
Evaluation of foreseeable effects

Lentivirus
Accidental human inoculation with lentivirus is unlikely to result in the development of any human disease, acute or chronic, as the vectors are replication deficient and any inoculated virus will be rapidly destroyed by the human immune system.

The infected packaging cells would be unlikely to be pathogenic due to rapid complement mediated lysis of these cells when recognized by the immune system as of non-self origin. Transduced cells would not be shedding virus as they are replication-defective and therefore do not pose a risk to human health or the environment. The risk that the vector could be rescued in the rat is negligible so there is negligible risk of spread to other cages or to BSU staff.

There is a small risk from lentiviral-mediated insertion of an oncogene or knockdown sequence into a human host. However, insertion of an oncogene into the human cells is not enough to create by itself cancer because it needs a combination of additional alterations. We will not use sequences which knockdown p53, Rb or their regulators. The vectors will be replication defective, potential exposure will be controlled by following containment level 2 local rules and use of good microbiological practice. The viruses are unlikely to survive outside the laboratory, as suitable growth conditions will not exist.

Listeria monocytogenes
In healthy individuals, infection by L. monocytogenes causes the disease listeriosis, which is generally a foodborne disease, with as few as 1000 organisms having the potential to invade the gastrointestinal epithelium in susceptible individuals. Clinical treatment of wild-type L monocytogenes is with ampicillin, to which these organisms are almost universally sensitive, and produces cure.

The EDGe strain is the wild-type strain and yycH is a safety-modified derivative with further increased sensitivity to ampicillin, allowing it to be easily cleared systemically following administration of the antibiotic. Routes of infection include inoculation, ingestion, inhalation and contact with mucous membranes. Individuals working with listeria will be required to wear personal protective equipment (eye guard, face mask, gloves) to reduce the risk of transmission.

DH5-alpha strain E. coli will be used to perform transformations. Hazards to human health are non-pathogenic to human health.

Superoxide dismustase 2 is involved in the regulation of cellular oxidative stress and as such is not considered an oncogene. Similarly, CTGF knockdown is unlikely to result in de-differentiation on it's own. The hazards from over-expression or knock-down are therefore, negligible. Hazards from potential oncogenes are minimal as they can rarely act as single transforming agents in normal cells.
Listeria
The inserted gene products encode a reporter system. The genetic insert is a synthetic luxABCDE operon encoding a fatty acid reductase complex (LuxCDE) involved in synthesis of the fatty aldehyde substrate for the bioluminescence reaction catalyzed by the LuxAB luciferase. It poses no risk to animal or human health. Another gene is firefly luciferase gene. Expression of the luciferase gene is driven by the human cytomegalovirus immediate early gene (CMV) promoter. The reporter gene has been used extensively in research and poses no risk to animal health or the environment.
Hazards from alteration of existing traits:
Lentivirus
The proposed vectors are reduced in pathogenicity as they lack certain wildtype elements and are replication deficient.
Listeria
L. monocytogenes EGDe is not altered in any way in regard to its pathogenicity, host range, tropism, transmission or immunity and will behave exactly as wild-type species would so poses no increased threat to human or environmental health when compared to the parental strain.
L. monocytogenes yycH is a safety-modified derivative with increased sensitivity to ampicillin allowing it to be easily cleared systemically following administration of the antibiotic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Lentivirus will be cultured using standard culture techniques in a dedicated incubator in a containment level 2 laboratory. Following this the viral particles will be concentrated and aliquoted for administration in our established in vivo model. Transport between the CL2 laboratory and the CL2 BSU facility in which our existing work takes place will be transported in 2 containers with sealed lids.
The listeria will be cultured using standard bacterial culture techniques in a dedicated incubator in a containment level 2 laboratory. Following this the bacteria will be concentrated and aliquoted for administration in our established in vivo model. Transport between the CL2 laboratory and the CL2 BSU facility in which our existing work takes place will be transported in 2 containers with sealed lids.

Animals are housed singly in Individually Ventilated Cages (IVCs).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid wastes will be rendered safe for disposal by overnight treatment with an approved disinfectant according to the ICR Waste Index (1% Virkon). Plastic pipettes will be soaked in an approved disinfectant (2% Trigene) for 24 hours prior to removal by CSSD (Central Sterile Services Department). Solid wastewill be placed in autoclave bags for collection by CSSD. Material collected by CSSD will be autoclaved on site. The cleaning, disinfecting and disposal procedures will be documented and

13. Emergency Plan - Public Register
14. Risk Assessment - Public Register
I confirm that I have attached a risk assessment to this form.
I am not claiming exemption from disclosure for sections of the risk assessment.

Summary of files attached to this notification

Attachments Uploaded
15. Comments of the genetic modification safety committee on the risk assessment - Public Register
16. Personal information for person responsible for supervision and safety of GM activities at the premises
17. Non Disclosure of Information
18. Declaration displayed.

All GMM waste material is sealed and autoclaved on-site. Animals used in procedures involving GMMs will not be permitted to rejoin the general animal population. Only staff with appropriate training will be allowed to perform procedures using GMM's in animals. All staff must wear disposable PPE during every visit. BSU waste and culled animals that have received lentiviral/listeria inoculation will be incinerated as per routine practice. There is no expected risk of recovering infectious virus or bacteria from culled animals and frozen carcasses will be incinerated within 24 hours of death.

The ICR Genetic Modification Safety Committee have approved the attached risk assessment for submission to the HSE.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

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02/03/2022
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**Name**

UNIVERSITY OF SURREY

**Name 2**

**Department**

FACULTY OF HEALTH & MEDICAL SERVICES

**Campus Estate or Research Centre**

STAG HILL, MANOR PARK

**Building**

CLINICAL RESEARCH CENTRE

**Road Name**

**District**

**Town**

GUILDFORD

**County**

SURREY

**Postcode**

GU2 7XH

**Country**

ENGLAND

**Tel Number**

01483 686 497

**Fax Number**

01483 300 374

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee

| Laboratory | Animal Unit | Growth Room | Glass House | Large Scale |
|------------|-------------|-------------|-------------|-------------|-------------|
|            |             |             |             |             |             |

02/03/2022
Level 1 (GMMs)  
Level 2 (GMMs)  
Level 3 (GMMs)  
Level 4 (GMMs)  
Non-microbial

Other (please specify)  

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref  332/01.1
Genetic manipulation of the alphaherpesvirus herpes simplex virus type 1

Date Ackn’d: 05/06/2013
Date Project Ceased: 12/02/2016

Class: Class 2
CultureVolClass2: 
CultureVolumeClass3-4: 

Non-GMM Consent Granted: Not Applicable

Project notified under transitional arrangements: ☐ No

Historical Significant Changes: Transferred from GM77 on 05/06/2013

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 81/00.1

Date Ackn'd 13/01/2000
CU2 Project Title EXPRESSION OF REPORTER GENES USING THE TRANSIENT EXPRESSION SYSTEM

Class 2
CultureVolClass2
CultureVolumeClass3-4
Consent Granted not applicable
Non-GMM

Historical Significant Changes
Historical Date of Additional Info

Tick if notifying a connected programme of work N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that it has attached a risk assessment to this form N

Tick to confirm that it is attached to this form N

Is an emergency plan required according to regulation 20? N

Project notified under transitional arrangements Y
**Project Additional Information**

**Purposes of the contained use**

Recipient or parental organism

Host/vector system

Origin & function

**Evaluation of foreseeable effects**

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
Project Ref: 81/00.2

Date Ackn'd: 13/01/2000

CU2 Project Title: INSERTIONAL MUTAGENESIS IN CAMPYLOBACTER JEJUNI

Class: Class 2

CultureVolClass2: Class 2

Consent Granted: not applicable

Non-GMM

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

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Please enter comments on the GM safety committee on the risk assessment

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Large Scale Activities

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Human Clinical Applications

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<td>&lt; 1 litre</td>
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Purposes of the contained use

The plasmid pSB367 is a pUC based vector which contains a fusion of the Salmonella dublin spvRA promotor with the luxCDABE genes from Photorhabdus luminescens. This plasmid, which has been constructed elsewhere, will be introduced into a non-pathogenic bur colonising strain of E coli (Krogfelt KA et Infect Immun 68(5):2518-24) and Salmonella Typhimurium strains to monitor rpoS mediated gene expression.

Recipient or parental organism

The recipient bacteria will be a non pathogenic gastro-intestinal E coli strain and Salmonella Typhimurium strains. The E coli strain will be non-pathogenic yet will be able to colonise the GI-tract. Salmonella Typhimurium is an ACDP group 2 pathogen which is able to colonise the gastrointestinal tract of humans and cause a self-limiting gastrointestinal illness in healthy individuals. The route of transmission is via the oral route. The GMOs will contain a pUC based plasmid which leads to the expression of luxCDABE. These are widely used reporter genes and encode a luciferase complex that results in the production of bioluminescence. Other than the production of blue green light no other foreseeable effect is likely to be associated with the GMOs. The introduction of this plasmid will not affect pathogenicity or tissue tropism.

Host/vector system

The plasmid has been constructed elsewhere (University of Nottingham). It is a pUC based vector containing an ampicillin resistance gene. In E coli and Salmonella this vector is considered to be non-mobilisable and gene transfer is thus a remote possibility.

Origin & function

This project will involve the introduction of a plasmid into E. coli and Salmonella Typhimurium. This will represent the only genetic modification carried out. The Plasmid pSB367 is a pUC based vector which contains a fusion of the Salmonella dublin spvRA promotor with the luxCDABE genes from Photorhabdus luminescens. The spvRA promotor is a non-coding region that is active in response to levels of the alternative sigma factor RpoS. The luxCDABE genes are derived from a non-pathogenic bacterium (Photorhabdus luminescens) and will function solely as a reporter gene such that cells containing the plasmid will express bioluminescence in response to levels of RpoS.

Evaluation of foreseeable effects

Bacterial luciferase catalyses the oxidation of reduced flavin mononucleotide (FMNH2) and a long chain aldehyde by molecular oxygen to yield FMN, the corresponding acid, water and light. the luxC, luxD, and luxE encode for a multi-enzyme reductase complex that produces decanal the substrate for the luciferase reaction. These genes are not implicated in pathogenicity and have no known function other than the production of bioluminescence. Consequently, the expressed gene products can be
considered to be non-harmful. The strain of E.coli, whilst not pathogenic, will colonise the GI-tract. The recipient Salmonella Typhimurium strains will be enteropathogenic and may cause a self-limiting gastrointestinal illness in health individuals if the organisms enter the mouth. However, the GMOs will not be altered in existing pathogenic traits and there is no reason to suspect that the disease caused will be different to that of the wildtype organisms.

The GMMs may survive in the environment for a limited period. However, since the modification is not likely to confer survival advantages no specific environmental hazard is likely. Animals are frequently, colonised by the non-GM recipient organisms and these are continually shed into the environment as a result of faecal contamination. Given that no specific hazard has been identified, it is not thought that the modified organisms would present a greater risk to the environment than the wildtype.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste including liquid 100 ml cultures, 1 ml samples taken for growth measurements, and solid agar plates will be inactivated using the school waste autoclave. Inactivation will use the routine cycle which operates at 121 C for 30 min. No viable cells of the GMMs will survive this process. The inactivation process is monitored by a trained technician.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

JWD asked if attenuated strains of Salmonella Typhimurium were available, and if so, could these be used to lower risk. SFP replied yes attenuated strains were available (such as LT2) but these have rpoS mutations. Since they do not produce RpoS they could not be used to monitor it's level which is the purpose of the planned experiments. The risk assessment was then approved for submission to the HSE.

**Project Containment**

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<th>Growth Rooms</th>
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The purpose of this project is to measure circadian rhythms in cultured primary cells (of human or rodent origin) by means of real-time reporter imaging. The reporter constructs will be inserted into the cultured cells by use of a lentiviral transfection system.

Recipient or parental organism

All plasmids will be grown up in a standard disabled E.coli strain (e.g. JM109, DH5alpha; ACDP Hazard Group 1). The E.coli cloning strain used (e.g. JM109, DH5alpha) will be disabled and thus presents very low risk and should not survive outside the laboratory. Transfected E.coli will become insensitive to ampicillin for selection purposes, but will be destroyed either in the recovery of the inserted plasmids or by use of a standard detergent (e.g. wiskon). No alteration in pathogenicity of E.coli host will be caused.

Lentiviral constructs will be made using commercially available HEK293T cells which are produced by stably expressing the SV40 large T antigen in a standard HEK293 cell line. The SV40 large T antigen facilitates production of high viral titres. HEK cells are commonly used human-derived cells that pose little risk and should not survive outside the laboratory. All HEK293T cells used within an experiment will be destroyed either in the recovery of the lentiviral particles or by use of a detergent.

Host/vector system

The lentiviral particles to be used are 'second generation' vectors, in which the core and enzymatic components of the virion come from HIV-1 but have been manipulated...
To delete all viral auxiliary genes (i.e. vpr, vif, vpu and nef) and thus reduce pathogenicity.

Reporter constructs are considered harmless and there is no evidence that they will alter the pathogenic nature of the lentivirus.

Origin & function

Production of lentivectors will be based around the psPAX2 (2nd generation) packaging system and pMD2G envelope plasmid, which originate from the Trono laboratory (http://tronolab.epfl.ch/page58114.html). Reporter vectors will be cloned into a standard commercial reporter vector (e.g. pGL3). Plasmid vectors will contain an ampicillin resistance gene.

Lentivectors will be based on HIV-1, but modified to reduce pathogenicity (as described under "Host/vector system" section above).

We will initially use a reporter construct produced by fusing the firefly luciferase gene to the promoter region of the mouse Bmal1 gene, which is a rare component of circadian clock. This construct has been provided by Professor Steven Brown (http://www.pharma.uzh.ch/research/chronobiology/areas/chronobiology/projects/genetics.html), University of Geneva. In future experiments, we will likely produce additional reporter constructs, which will have a similar basis, i.e. a standard, non-hazardous reporter gene controlled by appropriate mammalian promoter/enhancer elements.

Evaluation of foreseeable effects

Details of the foreseeable effects are given above. In brief,

Preparation of the lentiviral particles will pose minimal risk and would by itself come under Class 1.

Preparation of the lentiviral particles will involve transfecting a human-derived cell line (HEK293T) with a combination of plasmids. The lentiviral system to be used takes advantage of genetic manipulation to greatly reduce pathogenicity. The reporter constructs that the lentivirus will deliver to our target cells is considered harmless and thus there is very low risk associated with this work.

The final part of the work will involve using the lentivirus to transfict human and rodent primary cell cultures. These primary cells are not considered pathogenic per se. There is very small risk that human cells may contain infectious material. However, the human volunteers for our work will be put through health screens during the selection procedure and workers with human primary cultures will be encouraged to take a course of vaccination against Hepatitis B.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only cultured cells will be used in this programme of work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We will conduct this work according to the full Class 2 containment regulations.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Treatment/inactivation of liquid waste and spills during the cell culture process will be conducted by treatment with suitable detergent (e.g. Virkon) or bleach. Liquid waste will be treated for 24 hours before being washed down the sink with plenty of water.

Solid waste will be autoclaved prior to disposal.

Centrifugation in closed vessels will minimise aerosol formation.
At the end of an experiment, cells will be destroyed using standard detergent (e.g. virkon).

The GMM committee was satisfied that the lentivirus vector system proposed in this research incorporates sufficient safety features for work at containment category 2.

The main risks associated with HIV-1 based vectors are:

1. The generation of replication competent lentivirus.
   Safety measure assessment - The vector system proposed contains only the core and enzymatic HIV-1 genes with auxiliary genes deleted. It is extremely unlikely to generate the capacity of replication by recombination events. Work at containment level 2.

2. Potential for tumourgenicity and insertional mutagenesis of lentiviral vector (this has not been well defined).
   Safety measure assessment - Work at containment level 2.

The inserted reporter genes were not oncogenic.

Please enter comments on the GM safety committee on the risk assessment

The GMM committee was satisfied that the lentivirus vector system proposed in this research incorporates sufficient safety features for work at containment category 2.

The main risks associated with HIV-1 based vectors are:

1. The generation of replication competent lentivirus.
   Safety measure assessment - The vector system proposed contains only the core and enzymatic HIV-1 genes with auxiliary genes deleted. It is extremely unlikely to generate the capacity of replication by recombination events. Work at containment level 2.

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   Safety measure assessment - Work at containment level 2.

The inserted reporter genes were not oncogenic.

Project Containment

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Project Ref 81/12.1

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<th>Date Ackn'd</th>
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<td>29/06/2012</td>
<td>GENELUX - A phase I open-label, dose escalating study of the safety, tolerability, and</td>
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The primary objective is to expand the safety profile of an attenuated vaccinia virus when administered intravenously to subjects with advanced solid tumours. Secondary objectives include the detection of virus delivery to primary and/or metastatic tumours by Polymerase Chain Reaction (PCR), viral plaque assay (VPA) and immunohistochemistry; and evaluation of anti-vaccinia virus immune response (e.g. antibody responses); evaluation of viral delivery by imaging of GFP expression. A recommended dose and schedule for future investigation will be made. Any evidence of anti-tumour activity will be described. Kinetic studies of virus in serum will be conducted.

Vaccinia viruses are of high genetic stability. The whole life cycle is restricted to the cytoplasm of infected cells thus recombination with host genomes located in the nucleus are very unlikely to occur. Since poxviruses are not endemic in human populations, it is further unlikely that these viruses would recombine with a wild-type virus to produce a more virulent strain; and, despite worldwide use of the live virus vaccine, no reported adverse events due to mutations to a more aggressive phenotype have ever been reported.

Consequences of accidental exposure as auto-inoculation of mucous membranes or abraded skin may result in a benign rash; needle sticks may most likely be restricted to local reactions as development of vesicular or pustular lesion, area induration or erythema surrounding a scab or ulcer at inoculation site. Flu-like symptoms may occur.

GL-ONC1 has a number of reporter genes inserted into its genome.

- RUC-GFP: Synthetoc vaccinia early/late promoter (pE/L), followed by sea pansy Renilla reniformis luciferase and humanized Aequorea victoria green fluorescent protein fusion gene.
- LacZ: Vaccinia virus Western reserve (WR) early/late P7.5 promoter, followed by the bacterial E. coli beta-galactosidase gene.
- gusA: Vaccinia virus WR late P11 promoter followed by the bacterial E. coli beta-D-glucuronidase gene
- hTRF: Synthetic vaccinia early/late promoter (pE/L), followed by human transferrin receptor sequences in reverse orientation to the promoter, thus the hTFR is not expressed.
The inserted genes serve several purposes:
Disruption of the viral nonessential genes (F14.5L, TK and HA) by insertion of the respective genes not only attenuated the virus but also enhanced its tumour-specific targeting.

Furthermore, the three inserted diagnostic marker genes (ruc-gfp, LacZ, gusA) will be used to monitor virus replication in vivo and in vitro for the detection of virus in biopsies:
RUC-GFP allows for direct in vivo monitoring of tumour targeting, e.g. in the pleural cavity and the detection of metastases, as well as the staging of human subjects by imaging of regional lymph nodes to determine degree of metastases.

X-Gal and X-Glu allow for histopathological staining of tumour biopsies, and thus for monitoring of tumour targeting and oncolytic effects of the virus, which can be carried out in tumours that are not easily accessible by fluorescence. The procedures can also be performed in clinics that do not have this special equipment.

The circulating beta-glucuronidase will be used for monitoring the presence/release of GL-ONC1.

The vaccinia specific promoter sequences are needed for expression of the different markers.

**Evaluation of foreseeable effects**

Hazards arising from the alteration of existing pathogenic trails: GL-ONC1 is expected to have reduced virulence compared to wild-type vaccinia strains due to the insertional inactivation of a number of viral genes. It is anticipated that these deletions will render the virus tumour selective. The inserted genes do not have any expected biological activity on the human recipient.

The risk that GL-ONC1 will revert into more virulent mutants is very low, as the parental UVP strain, a descendant of the Lister strain, was attenuated by frequent passages on calf skin (more than 500 times) and was widely used as a vaccine, with excellent documented safety, during the WHO smallpox eradication program. In addition, because orthopoxviruses are not endemic in the human population, it is unlikely that GL-ONC1, as a clonal strain, will recombine with a wild-type orthopoxvirus to produce a more virulent strain; and, despite worldwide use of the live virus vaccine, no reported adverse events due to mutation to a more aggressive phenotype has ever been reported.

Potential hazards if the genes being transferred to another micro-organism: Vaccinia virus is non-pathogenic to other micro-organisms or plants, and it does not transfer its DNA to the host genome on infection. The inserted genes are marker genes to allow assessment of viral trafficking in tissue and imaging and are unlikely to be transferred to other micro-organisms or cause harm.

Consideration of the predicted properties of the GMO to determine whether there are any mechanisms by which it could represent a hazard to the environment. Samples of Good Laboratory Practice (GLP) and Good Manufacturing Practices (GMP) produced GL-ONC1 were spotted onto filter paper to examine the stability after release into environment. At the respective tie points, the virus samples were retrieved from the filter paper and the amount of active virus was analysed by plaque assay. The sponsors experimental results showed that the titres of both GLP and GMP manufactured GL-ONC1 were decreased by 99.99% within 24 hrs when released into the environment at room temperature. By days 6-7, all viruses were disintegrate. Therefore, we do not believe that virus shed from patients will be of significant environmental concerns or health concerns to the others.

Precautions are in place to isolate patients on the treatment ward at CRC which is a locked ward with swipe-card access, to avoid environmental contamination. Vaccinia virus is non-pathogenic to other micro-organisms or plants, and it does not transfer its DNA to the host genome on infection. Transfer of DNA has not been reported. Deletions of a number of genes in GL-ONC1 mean that the virus cannot replicate without a suitable host.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A
Derogation is sought to use an autoclave located in a different building to inactivate items generated in the class 2 activity within the Clinical Research Centre. It is proposed that items that require inactivation by autoclaving will be autoclaved at the Leggett Building (previously known as the Postgraduate Medical School) which is immediately adjacent to the Clinical Research Centre, approximately 80 metres away. The Leggett building is owned and operated by the University of Surrey and is on the University Manor Farm campus together with the Clinical Research Centre. The campus is monitored by the University Security and Estates department. Access to laboratory areas (including the area in which the autoclave is situated) in the Leggett Building is controlled at all times by swipe-cards, and access to the entire building is swipe-card controlled outside working hours. All items will be transported by University staff in plastic bags placed within a rigid, leak-proof box. The outside of the box will be disinfected with 1% Virkon before transport.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

(1) In this connected programme of work all activities involving storage, preparation and administration of the GMM will take place within the University of Surrey Clinical Research Centre, using the following procedures:

Virkon is used to decontaminate all areas where GL-ONC1 virus is handled, stored and transported. After patients have been discharged floors, walls, beds, mattresses and furniture in the rooms and patients' area will be wiped-down with 1% Virkon. All equipment (ECG machines, infusion pumps) will be cleaned with 1% Virkon after use.

All GMM spills will be treated in accordance with CRC Work Instruction 0903: large volume liquid spills covered with Virkon powder and then scraped up, smaller spills and all surfaces or fabrics sprayed with 1% Virkon and wiped-down. Toilets will be disinfected with bleach.

Cups and plates used by patients will be washed in 1% Virkon and then palced in the dishwasher on a high temperature cycle.

The blood samples taken for viral testing are labelled with biohazard stickers and will be handled with universal precautions against blood-borne viruses.

All PPE and potentially contaminated clinical waste, sharps, vials and infusions sets will be placed in yellow-bags or Sharpsafes and inactivated by autoclaving before leaving the site for destruction by incineration by an external contractor. The autoclave cycle will be recorded and the autoclave undergoes regular maintenance and testing for compliance with required inactivation parameters. Derogation is sought to use an autoclave in an adjacent building.

All patients linen will be inactivated by autoclaving before being sent for laundering using an external contractor.

(2) In addition, tissue biopsies will be taken from tumours at the Royal Surrey County Hospital as radiology facilities are required. All equipment will be disposable and will be disposed of in the hospital clinical sharps waste stream. Biopsy samples will be labelled with biohazard labels and handled with universal precautions against blood-borne viruses within the hospital. All biopsies will be disposed of in the hospital clinical waste stream.

Blood samples collected at Surrey Clinical Research Centre will be sent to Roayl Surrey County Hospital for analysis. Urine samples will be sent from Royal Surrey County Hospital to Frimley Park Hospital if microscopy analysis is required. Blood and urine samples will be placed within two sealed plastic bags (double bagged), which will be marked with hazard stickers, before they are transported to Royal Surrey County Hospital in a closed rigid container. The samples will be handled with universal precautions against blood-borne viruses within the hospital. Samples will be disposed of in the hospital clinical waste stream.

3) Some blood samples collected at Surrey Clinical Research Centre will be sent to the Leggett Building (formerly the Postgraduate Medical School), for analysis. Samples will be placed in two sealable plastic bags (double bagged), which will be marked with hazard stickers, before they are transported to the Leggett Building in a closed rigid container. The samples will be handled with universal precautions against blood-borne viruses within the Leggett Building. Samples will be disposed of in the laboratory clinical waste stream.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
### Project Containment

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**Project Ref** 81/14.1

- **Date Ackn'd**: 24/04/2014
- **CU2 Project Title**: Use of GM-KSHV for molecular studies of Kaposi's sarcoma-associated herpesvirus (KSHV) pathogenesis
- **Class**: Class 2
- **Consent Granted**: Non-GMM
- **Consent Volume Class 2**: < 1 Litre
- **Consent Volume Class 3-4**: Consent Granted

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

In vitro infection studies of cells with recombinant strains of KSHV for investigation into the biology and pathogenesis of the virus, including aspects such as entry and replication processes and the significance of virus-host interactions e.g. functions and mechanisms of KSHV immune proteins.

Recipient or parental organism

KSHV is a human gamma herpesvirus that is the aetiological agent of Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL). The incidence of KSHV is 3.5% in the general population of the U.S and the United Kingdom. However, in those geographical areas where KS is endemic, the incidence is much higher, e.g. - 11.5% in western Sicily and 29% in South Africa. Overwhelming serological and molecular data justify KSHV in the aetiology of KS, but infection with the virus alone is not sufficient for the development of KS and additional factors are required, including immune suppression and perhaps host genotype.

The opinion in the KSHV field is that the virus is either not transforming, or is poorly transforming. In this regard, KS is also not considered a true monoclonal neoplasm, but rather a hyperplasia in which KSHV and environmental cofactors such as inflammatory cytokines, promote cell proliferation. Late stage KS tumours may eventually develop into true sarcoma, since there is then some evidence of clonality.

Host/vector system

Donor/vector
KSHV, E. coli and associated plasmids, Aequorea victoria (green fluorescent protein & enhanced green fluorescent protein), Discosoma (Ds red fluorescent protein).

Vector
Mobilization-defective transfer vector plasmids in which one or more genes of interest can be inserted e.g. F plasmid pMBO131: F factor-based prokaryotic replicon that carries the F factor origin of replication, the chloramphenicol-resistance gene, and the partitioning proteins A and B. It is self-replicating and limits its copy number to one or two copies/cell.

Host
Disabled E. coli for plasmid construction, 293 and other cells, human and non-human (such as Vero), to generate infectious particles and to study infection processes. PEL cell lines for production of KSH. Target cells: human cell lines, primary cells of endothelial, epithelial and lymphoid origin (liver, gut, stomach, thymus, B- and T-lymphocytes, monocytes).

Origin & function

The present programme of work will include the manipulation of the KSHV bacterial artificial chromosomes (BACs) and other recombinant strains as technologies evolve to manipulate the genomes of large DNA viruses. Selected ORF's will be deleted, mutated or reintroduced and resulting recombinant virions will be purified or infection of various cell types. We will infect cells with recombinant KSHV derivatives in which one or several viral genes involved in virus infection and/or pathogenesis have been deleted/mutated or placed under an inducible promoter (e.g. -oestrogen receptor, tetraculcine inducible promoters). In case of very low virus titres, concentration by centrifugation will be performed.

Evaluation of foreseeable effects

Wild type KSHV is ACDP category 2

However, recombinant KSHV plasmids will be propagated in disabled E. coli strains derived from K-12 which are non-pathogenic, and the viral genes of the insert and hygromycin gene are not expressed in these cells. The vector and the insert are not self-transmissible and are poorly mobilisable. The genetically modified KSHV strains and derivatives in E. coli are therefore unlikely to cause any disease to humans, animals or plants. Transmission of either naked plasmid DNA or the plasmid from bacteria to humans is highly unlikely, and with expected consequences no worse than an ACDP level 2 virus already present in the population, and quite prevalent in some areas.

Experimental infection of cells in vitro with KSHV is only usually achieved at low efficiency. The infection is then latent, unless the lytic switch protein encoded by ORF50 is
provided in trans. Several published reports claim that experimental KSHV infection of endothelial cells results in extended lifespan allowing their prolonged passage in vitro. In vivo, KSHV replication in tumour cells is predominantly latent, suggesting that the proteins encoded by the latency genes may be involved in pathogenesis. None of these genes is active on its own in the endothelial cell proliferation assay. The present study encompasses these KSHV genes and the proteins they encode. The aim of the programme of work is to create mutant viruses, deficient in one or more of these genes, and to analyse their phenotype. The creation of such a mutant requires cloning of either part or all of the target gene. Once the mutant virus is constructed, a 'revertant' must be created from that mutant. Again, this process may involve cloning the gene of interest. The revertant virus is re-engineered to express the gene deleted in the mutant virus, in order to ensure phenotypic differences observed in the mutant virus are solely due to deletion of the target gene, and not to erroneous changes made elsewhere in the virus genome. In some strategies to recreate wild type virus, the mutated gene will be complemented in trans, from an expression vector transfected into the mutant-virus-producing cells.

None of the KSHV genes, or the proteins they encode, presents a high risk to workers, particularly given that KSHV is, in the worst case scenario, only poorly transforming. Nevertheless, cell transformation has been observed in studies with certain individual KSHV proteins, e.g. - K1, vGCR and viRF-1. Should a worker be exposed to one of these protein, an immune response would be anticipated, but is unlikely to confound diagnostic assays, since KSHV infection is not screened for routinely.

The antibiotic resistance genes (e.g. - hygromycin, chloramphenicol) and the reporter genes (GFP, RFP) and other selection and reporter genes are not associated with any likely hazards.

Wild type KSHV replicates of low levels in vitro. Recombinant strains are expected to replicate to similar levels.

The genes encoded by the KSHV BAC are not expressed in the bacterial host. Hence, there is no potential to increase the pathogenicity of the host. The BAC-derived virus mutants are expected to have a decreased ability to infect and replicate in target cells compared to wild type KSHV. Mutated recombinant KSHV strains are expected to be less harmful than their wild type counterparts. The reporter genes are not expected to affect the pathogenicity of the virus. None of the primary cells or cell lines used in these studies will be derived from the worker. In addition protein, even in the context of the BAC-derived virion, their inoculation into the worker will induce a strong allogenic immune response, which will protect the individual. Hence, handling of cells expressing either individual or few KSHV proteins poses no greater threat that that of culturing cell lines infected with wild type KSHV.

The reporter genes are not expected to affect the pathogenicity of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid contaminated waste is treated by autoclaving at 121°C for 15 minutes in a mixed load validated cycle.

Post autoclaving: Solid waste is further disposed of into a tiger stripe bag and sent off site for incineration via Grundons Waste Management Company. Liquid waste is disposed of down the sink with copious amounts of water. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

Liquid disinfection may also be performed by treating the solution with 1% (weight/volume) Virkon overnight at room temperature. The solution is then disposed of to the laboratory drain flushed with copious amounts of water at a maximum volume of 2L per day. This flows to the main foul drain and onto a waste water treatment facility oned by Thames Water.

Expected degree of kill:
Autoclaving achieves effectively 100% kill of all GMMs.

1% Virkon solution can be used for disinfection when necessary. 1% Virkon solution is validated for disinfection of most herpesviruses, including herpes simplex virus (HSV). Equine herpesvirus, feline herpesvirus, turkey herpesvirus and Marek's disease virus. Virkon acts by oxidative disruption of proteins and lipids vital to normal virus infectivity. By definition KSHV shares the same structure with that of those herpesviruses for which Virkon has been validated. Moreover, KSHV titres are several orders of magnitude lower than those of HSV. Taken together, these data indicate complete inactivation of KSHV is expected.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The BioSafety Committee approved the risk assessment on 26th February 2014

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<table>
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<td>L2</td>
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</table>

Animal Units

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- L4

Large Scale Activities

- L2
- L3
- L4

Human Clinical Applications

- L2
- L3
- L4

Project Ref  81/14.2

Date Ackn'd  03/12/2014

CU2 Project Title

- Understanding Campylobacter Biofilm formation by use of a Campylobacter Transposon Directed insertion site sequencing (TraDIS) mutants and other transposon mutant libraries

Class

- Class 2

CultureVolClass2

- < 1 Litre

CultureVolumeClass3-4

- Non-GMM

Consent Granted

Project notified under transitional arrangements  N

Withdrawn  N

Tick if notifying a connected programme of work  N
The aim for this project is to utilise a Campylobacter jejuni mutant libraries (obtained from Prof. Duncan Maskell and his team at the University of Cambridge) in a series of genotypic and phenotypic assays to further elucidate the role of biofilms in the pathobiology of Campylobacter infections. The libraries contain a mutant in every non-essential gene of the Campylobacter jejuni type strain NCTC 11168/M1/a wildtype C. jejuni chicken isolate. The mutants will be entered into biofilm assays and biolog phenotypic arrays.

Recipient or parental organism

The recipient organism will be Campylobacter jejuni (ACD) hazard Group 2) which is a food borne pathogen able to colonise the gastro-intestinal tract of humans and animals. Although Campylobacter jejuni is a harmless gut commensal to animals, the organism is pathogenic to humans, causing self-limiting gastro-enteritis. The organism would therefore pose a hazard to human health if ingested. The vast majority of infections in humans are caused by the consumption of contaminated poultry products. There are currently no vaccines for Campylobacter available for humans. Erythromycin and Ciprofloxacin are the preferred antibiotic treatment.

Host/vector system

The mutant libraries were constructed by using an in vitro transposon protocol. Chromosomal DNA of the bacterial strain of interest serves as the acceptor for incorporation of the mariner transposon element. During the in vitro transposon mutagenesis the mariner transposon element is sliced out of the donor plasmid (pSV006 for Campylobacter jejuni) by Himar1 transposase and inserted into TA-dinucleotide sites in the acceptor DNA. During the in vitro transposition gaps are created at the site of insertion; these are repaired by filling in by DNA polymerase followed by a ligation step. The transposon is marked with a chloramphenicol or kanamycin resistance gene.

The transposase enzyme is expressed from the pMALC9 plasmid that contains the hyperactive from of Himar1 transposase cloned into the maltose-binding protein (MBP) fusion plasmid (ampicillin resistance) in Escherichia coli BL21 pLYsS (chloramphenicol resistance). Protein expression is induced with IPTG and the E. coli cells are lysed by one freeze-thaw cycle followed by sonication. After centrifugation the Himar1-MBP fusion protein (present in the supernatant) is allowed to bind to amylose resin and after several wash steps eluted through competition with maltose. Thereafter, the purified Himar1 enzyme is ready to be used in in vitro transposon mutagenesis protocols stored at -80°C.

It must be noted that the mutant library will contain a mutant in every non-essential gene in th Campylobacter genome. This included mutations in genes which may code for regulatory proteins or enzymes that regulate the virulence of the pathogen.

The mutations caused by the insertion of transposons in this mutant library will attenuate the strain. However, given the nature of tranposons, there is a possibility that the mutation could be reversed by the excision of the transposon in the intended insertion site to another location in the genome. However, for this to occur, the specific transposases for the removal of the transposon have to be present in the bacterial cell.
The method for the construction of the mutant libraries uses a mariner-based transposon derived from Himar1. This type of transposon is active in a wide range of hosts and has simple requirements for transposition. It is possible for transposition to occur in vitro with only its purified transposase. Himar1 is active in E. coli, and hyperactive transposase mutants are used in the methodologies for the construction of these mutant libraries to provide transposases required to transposon insertion into the Campylobacter jejuni genome. Himar1 is not active in Campylobacter, therefore host transposases in the C. jejuni genome should therefore not be able to move the position of the transposon. Regardless of this, transposons by nature are not permanent mutations and extra caution should be taken to ensure attenuated GMOs do not revert to wild type.

Having a mutant in every non-essential gene will further elucidate the genes responsible for biofilm formation in Campylobacter jejuni. Again, the mutations caused by the insertion of transposons in this mutant library will attenuate the strain.

**Origin & function**

The plasmid sequence containing the transposon will with the inserted transposon contains a chloramphenicol resistance gene which is used as a selective marker. However, this antibiotic is not used for therapeutic purposes, so potential therapies are not compromised.

Gene transfer to other bacterial species is a remote possibility. Certain Campylobacter strains are naturally competent so there is a possibility that the DNA could be transferred to other Campylobacter strains of the same species. However, there would be no hazard associated with this since this process would be common in the environment as a result of natural animal carriage and faecal shedding of bacteria into the environment.

It is also likely that any mutation will reduce the organism’s ability to survive in the environment, and therefore acquiring the mutant gene will have the same effect in the recipient cell.

Gene transfer from the mutant Campylobacter strain to a wild type organism is made difficult due to the organism’s lack of ability to grow below 30°C and will not replicate in environment outside of warm blooded animals. Campylobacter are considered to be very fragile organisms and do not survive for extended periods on surfaces or the environment.

The transposon does not code for a specific toxin or other virulence gene, and will only silence the gene that it is inserted into and this will attenuate the test Campylobacter strain. However, as mentioned previously, mutations will occur in all non-essential Campylobacter jejuni genes, including ones that may regulate virulence. Thus, care must be taken when working with the organisms since certain mutants may be slightly more virulent than the wild type.

The mutations are likely to reduce the organism’s ability to survive and therefore are unlikely to increase infectivity or pathogenicity. There is no evidence to suggest such mutations will increase host range or tissue tropism. Moreover, mutations are likely to attenuate the cells ability to produce adhesins or other surface proteins to increase pathogenicity.

**Evaluation of foreseeable effects**

The plasmid sequence containing the transposon will with the inserted transposon contains a chloramphenicol resistance gene which is used as a selective marker. However, this antibiotic is not used for therapeutic purposes, so potential therapies are not compromised.

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**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No plant or animal work is planned.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste
All contaminated solid waste, such as pipette tips and petri dishes, will be placed in Disposafe containers and sealed. These will then be autoculled at 121°C for 15 minutes.
Liquid waste
Liquid waste will be collected in a glass Duran bottle and placed in a plastic autoclave bin. Volumes larger than 500ml will be split into two bottles to allow effective steam transfer at 121°C for 15 minutes.

Non-contaminated waste will be disposed of separately. After autoclaving, GMO contaminated waste will be labelled with 'UN3245' and private contractors 'Grundon Waste Management' will collect and dispose of waste by incineration.

Virkon S will be used as a surface disinfectant at 1% with a minimum contact time of 1 hour. Virkon S has been validated for disinfection of Campylobacter jejuni in a 1:100 dilution. Virkon S achieves deactivation and destruction of the target organisms through a broad-spectrum, non-selective range of oxidation reactions. 70% ethanol will also be used for a surface disinfectant.

The Biosafety committee approved this risk assessment on 26th February 2014 on the condition that minor amendments to wording were made to confirm controls used.

Please enter comments on the GM safety committee on the risk assessment

The Biosafety committee approved this risk assessment on 26th February 2014 on the condition that minor amendments to wording were made to confirm controls used.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

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CU2 Project Title: Genetic modification of class II poxviruses - Maluquer Lab (connected programme of works).

Class: Class 2
Culture Vol Class 2: < 1 litre
Consent Granted: Non-GMM

Date Project: 02/03/2022
The overall aim of this project is to modify class II poxviruses to understand how these viruses interact with the host immune system, how they manipulate cellular resources and signalling pathways, and how these contribute to virulence. The class II poxviruses to be studied are vaccinia virus and ectromelia virus.

Vaccinia virus (VACV) is the prototypic and most studied member of the Poxviridae family. VACV was used as live vaccine to eradicate smallpox, and soon after became a useful expression vector for the expression of heterologous genes. Given the high immunogenicity generated by VACV infection, recombinant VACV expressing heterologous antigens have the potential to become powerful vaccines. Ectromelia virus (ECTV) is the causative agent of mousepox. Genetically it is closely related to Variola virus and monkeypox virus. ECTV infects only mice (so far, laboratory mice only) which develop a pustular rash reminiscent of smallpox disease in humans. Given the existence of an effective animal model, ECTV is an excellent model to study smallpox and exanthematous diseases, as well as host-pathogen interactions.

VACV is considered a hazard group 2 agent. ECTV is a mouse pathogen and it is not known to infect man. For the purpose of these rules, both should be treated as hazard group 2 agents.

VACV and ECTV are lytic viruses that replicate in the cytoplasm. This implies that chances of recombination or integration into the mammalian chromosomes are extremely rare, because the viral genome does not travel to the cell nucleus and the infected cell is eventually killed by the virus. Expression of oncogenes in VACV or ECTV is not expected in this project, but it would not likely to be hazardous because the virus kills all the cells it infects and therefore there is no potential for tumour development.

There are many distinct strains of VACV. In my laboratory most work with VACV will be conducted with the Western Reserve (WR) strain of VACV, but some work is also done using other strains such as Copenhagen, Lister, and IHOJ, that are of similar or lower virulence than WR. In addition we use some attenuated strains such as v811 (a strain lacking 55 genes from the VACV Copenhagen strain) and modified vaccinia virus Ankara (MVA). Most VACV strains are classified by ACOP as hazard group 2 pathogens. but the MVA strain is a category 1 organism due to its inability to replicate in most mammalian cells. Two strains of ECTV are described, Naval and Moscow, with almost identical properties.
VACV/ECTV genes will be expressed heterologously in a wide range of cloning and expression vectors, including (1) mammalian expression and cloning vectors that are non-mobilisable and only replicative in bacterial hosts such as pcDNA3 or pCI, (2) bacterial vectors such as pET or pGEX, (3) baculovirus vectors based on Autographa californica nuclear polyhedrosis virus (AcNPV), and (4) self-inactivating (SIN) lentivirus vectors.

A baculovirus vector based on AcMNPV will be used for high level expression of structural proteins in insect sf9 cells. This vector lacks the baculovirus polyhedrin gene and is therefore non-pathogenic for insects. Gene expression is driven by the insect cell specific polyhedrin promoter, producing fusion proteins with well-characterised tags such as glutathione-S-transferase (GST) or polyhistidine to aid purification.

SIN lentiviral vectors are categorized by the Advisory Committee for Dangerous Pathogens as a hazard group 1 biological agent. SIN lentiviral vectors are 3rd generation lentiviruses where the U3 region of the 3’ LTR has been deleted. This region contains the major viral promoters and enhancers of the virus and is copied to the 5’ end of the provirus during reverse transcription. SIN constructs result then in a provirus that is devoid of U3 enhancer sequences, therefore reducing the potential for mobilisation of the vector once inserted and the potential of transactivation of cellular genes as a result of insertion.

All systems and vectors described here are well-characterised, and pose minimal hazard to human health or to the environment.

Origin & function
We intend to clone genes from VACV and ECTV and express them in a number of delivery systems (see above) to study their activity ectopically in mammalian cells. In addition, some of these genes may be expressed in bacteria or baculovirus to produce soluble protein. That material will be used to obtain reactive sera. Finally, some constructs will be used to delete genes in VACV or ECTV using genetic methods based on recombination.

Particular genes of interest involve genes of unknown function located at the termini of the viral genome. These genes are likely to be involved in virus-host interactions aimed at evading the host immune response. Consequently, recombinant viruses engineered to lack such genes are likely to be attenuated compared to its parental strain.

Evaluation of foreseeable effects
VACV or ECTV will be modified to either express reporter genes (luciferase or fluorescent proteins) or to lack expression of a natural gene. The virulence of these modified viruses is expected to be reduced or similar to its parental virus. There is no intention to insert oncogenic foreign genes in VACV or ECTV, or to introduce antigens from other pathogens.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None.

Please note that CL3 animal facilities will be used when working with EClV in the animal model. This facility is removed by geography from other facilities to ensure appropriate quarantine processes. The work is only designated as a class 2 activity.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid and liquid contaminated waste generated in the containment level 2 laboratory is treated by autoclaving at...
121°C for 15 minutes on a mixed load validated cycle. Liquid disinfection may also be performed by treating the solution with 1% (weight / volume) Virkon for at least 30 mins at room temperature. The solution is then disposed of to the laboratory drain flushed with copious amounts of water at a maximum volume of 2l per day. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

In the CI2 animal facility, solid waste is disposed of into a yellow burn bin and sent off site for incineration via Grundons Waste Management Company. liquid waste is disinfected by treating the solution with 1% (weight / volume) Virkon for at least 30 mins at room temperature before disposing of down the sink with copious amounts of water. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

In the CL3 animal facility waste will be disposed of via autoclaving at 121°C for at least 15 minutes on a validated cycle.

Post autoclaving: Solid waste is further disposed of into a yellow burn bin and sent off site for incineration via Grundons Waste Management Company. liquid waste is disinfected by treating the solution with 1% (weight / volume) Virkon for at least 30 mins at room temperature before disposing of down the sink with copious amounts of water. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

The Safety committee met on 12th November 2014 and approved this assessment. The committee requested that the assessment was reviewed by the animal facility manager to ensure specific quarantine procedures were developed for staff working on this project. The committee also requested that Occupational Health review the assessment to confirm the health surveillance required for personnel to work on this project.

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 81/15.2
Handling (storage and culture) of genetically modified ACDP Hazard Group 1 and 2 bacteria sent from other institutions

Date Ackn'd
25/06/2015

Date Project Ceased

CU2 Project Title
Handling (storage and culture) of genetically modified ACDP Hazard Group 1 and 2 bacteria sent from other institutions

Class CultureVol
Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn
N

Tick if notifying a connected programme of work
Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
81/15.2a

Date of Significant Change
01/08/2017

Project Additional Information

Purposes of the contained use

The Phenomics core facility offers expertise in phenotyping bacteria within the University of Surrey and for external establishments. This technology is applicable to both wild-type and genetically modified organisms. This risk assessment covers the handling, storage and culture of ACDP Hazard Group 1 and 2 bacteria at the University of Surrey.

Recipient or parental organism

The recipient bacteria will be ACDP hazard group 1 or 2. Genetically modified bacteria will be supplied by the collaborative institute to the University of Surrey. No genetic modifications will be undertaken at the University of Surrey. The modes of transmission will vary according to the specific bacteria. However, all experiments involving live bacteria will be conducted in line with the safety regulations relevant to dealing with ACDP 1 and 2 bacterial species.

Host/vector system

This application will include the use of any ACDP Hazard group 1 or 2 bacteria. The bacteria will depend on the proposed study being undertaken by the collaborating institute. A variety of vectors are available for use in different bacterial species. These are usually non-mobilisable or mobilisation-defective vectors from commercial or academic sources.

Origin & function

Numerous methods are employed to create genetically modified bacteria. The method selected will depend on the purpose of the studies and species of bacteria. Generation of a mutant typically involves the insertion of nonreplicating DNA (suicide plasmid, transposon, bacteriophage, PCR product), which may or may not integrate into the chromosome. Where complete ablation of the gene function is required, the mutated DNA may contain a copy of the
Evaluation of foreseeable effects

The GMO's are unlikely to have increased pathogenic potential. It is therefore anticipated that there will no ecologically disruptive effects to other organisms. All the GMO's will be worked on in containment and only one species of organism will be worked on at anyone time. However, any addition risks (if any) will be considered following a review of the GMO risk assessment for the particular mutant strains as detailed by the collaborating institute before receipt of the isolates by the University of Surrey.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No Plant or animal work is planned.

9.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid contaminated waste is treated by autoclaving at 121°C for 15 minutes on a mixed load validated cycle.
Post autoclaving: Solid waste is further disposed of into a tiger stripe bag and sent off site for incineration via Grundons Waste Management Company. Liquid waste is disposed of down the sink with copious amounts of water. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water. Liquid disinfection may also be performed by treating the solution with 1% (weight/volume) Virkon S overnight at room temperature. The solution will then be disposed of to the laboratory drain flushed with copious amounts of water at a maximum volume of 2L per day. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Biosafety committee approved this risk assessment on the 17th June 2015 on the condition that minor amendments to wording were made.
### Project Containment

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### Project Ref 81/16.1

**Date Ackn'd**: 06/04/2016

**CU2 Project Title**: Handling (storage and culture) of genetically modified ACDP Hazard 2 Oncolytic virus sent from other institutions

**Class**: Class 2

**CultureVolClass2**: < 1 Litre

**CultureVolumeClass3-4**: Consent Granted

**Non-GMM**: Consent Granted

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

The primary objective of our research projects is to investigate oncolytic inflammation, and the enhancement of antitumour immune responses seen with oncolytic virus (OV). We also wish to combine OVs with immune modulating drug such as immune-checkpoint inhibitor antibodies to explorer immunological "danger" signals using In vitro and In vivo models. All viral vectors are attenuated resulting in tumour selective tropism.

**Recipient or parental organism**

Adenovirus (replication-defective) - ACDP Hazard group 2 - Human
Adenovirus (replication-competent, attenuated) - ACDP Hazard group 2 - Human
Maraba Virus (MaraV) (replication-competent, attenuated) - ACDP Hazard Group 2 - Sand flies
Vaccinia virus (replication-competent, attenuated) - ACDP Hazard group 2 cattle
HSV-1 (replication-competent, attenuated) - ACDP Hazard group 2 - Human

Host/vector system

Adenovirus (replication-defective or competent, attenuated)
Adenoviruses were first discovered in the 1950’s and have since been studied in detail thus much is known about their biology and pathology. Ad5 has a long history of use as a therapeutic oncolytic agent both as wild-type virus and as a gene therapy vector and has an excellent safety record with many cancer patients having been treated at high doses without adverse effects [1 7-19][20, 21, 29].

General information
Type: non-enveloped double stranded DNA virus [17]. Size: 32-40kb [1 7].
Family serotype group C, human Adenoviridae (Mastadenoviridae) [17]

Mutagenic effects

Infectivity
Natural host - humans [20,21]. Other hosts - rodent, swine and rabbit [20,21 ]. Tropism - eyes, respiratory and gastrointestinal tracts [22]. Route of transmission - aerosol [22].

Transduction capability
Normal and tumour, dividing and non-dividing cells[1 8, 23, 24].

Mechanism of cell death
Necrosis or apoptosis, dependent on cell type [25J.

Pathogenesis

Health and Safety
Executive
Commonly: well-characterized, mild, self-limiting disease (mild respiratory disease, gastroenteritis, conjunctivitis) [4].
Rarely: In infants and children pertussis-like syndrome, acute febrile pharyngitis and hepatitis[22].

Toxicity (side effects of treatment)

Human - Commonly: Minimal grade 3 and no grade 4 toxicity (observed across trials in a wide variety of cancers at doses of up to 2 x 1013 viral particles) [19J. Rarely: hepatotoxicity, acute inflammatory responses and one case of morbidly with very high doses (26). Pregnancy: extremely small risk of foetal abnormalities [27J. Immunocompromised individuals: extremely small risk to the foetus [27J.

Mouse
Dose and variant-dependent hepatotoxicity in athymic and immunocompetent mice [5, 28J.

Risk to the environment - Very low/negligible
Although release of Ad5dl309, Ad5d1922-947 or Ad5 CMV GFP to surrounding areas would increase the risk of exposure to rodents, rabbits and pigs, due to their greater immunogenicity and/or compromised replicative abilities relative to wtAd5 these 3 vectors are highly unlikely to cause disease in healthy or immuno-compromised animals. As all work using these viruses will be carried out in containment level 5 facilities the risk to the environment would be considered negligible.

Adenovirus (replication-defective or competent, attenuated) - Summary

Risk to normal, healthy immuno-competent individuals Very low. Exposure to Ad5dl309, Ad5d1922-947 or Ad5-CMV GFP
is highly unlikely to result in toxicity. In comparison to wt Ad 5, Ad5dl309 is more immunogenic [29, 30].
Ad5d1922-947 is both more immunogenic and replication restricted [5]. and Ad5-CMV-GFP is unable to replicate.
Immune recognition and activation would control viral pathogenesis in all cases.
Maraba Virus (MaraV) (replication-competent, attenuated)
Maraba Virus is from the Rhabdoviridae family one of eight set type vesicular stomatitis virus (VSV).
General information
Type: ssRNA virus (negative strand) comprising a "bullet-shaped" nucleocapsid and a lipid envelope, populated by the
viral glycoprotein (G). Maraba Virus is from Rhabdoviridae family one of eight set type VSV.
Mutagenic effects
Oncogenicity: non-oncogenic [7]. Genome integration none [7]. Mutagenesis risk very low. Vertical transmission none [7].
Transduction capability
Normal and tumour, dividing and non-dividing cells [7].
Pathogenesis
No pathogenicity has been documented in humans with Maraba virus
Maraba virus is listed as a distinct species (ICTV: http://ictvdb.bio-mirror.cn/ictv/fs_rhabd.htm) within the
Rhabdoviridae and, whilst closely related to strains of VSV such as Indiana, does not cause vesicular disease with
symptoms overlapping with FMDV in cattle or other ruminants [31].
Infectivity
Maraba virus has been shown to have limited spread outside of sandflies [31] and no natural mammalian host has
been identified, with only one documented example of human seropositivity. Vectors based on Maraba virus contain
two attenuating mutations in the glycoprotein (G) and matrix (M) proteins. The M mutation limits evasion of innate
antiviral immunity but the mechanism of action of the G-protein mutation is not known. Genetic modification of MG1
comprises an insertion site between the G and M open reading frames (ORFS), resulting in expression from an
internal transcription initiation site. Attenuations mean that high titre growth of MG1 is only possible in tumour cell
lines. This property has resulted in MG1 being proposed for use as a highly selective oncolytic virus. This virus is not
listed as a SAPO
Toxicity (side effects of treatment)
Health and Safety
Executive
Genetically modified and tumor-targeted Maraba virus, MG1 (mutations in M protein L 123W and G protein Q242R),
has been established as a superior OV compared to VSV [7]. MG1 had a 100-fold greater maximum tolerable dose
than wild-type Maraba in vivo and resulted in durable cures when administered in syngeneic and xenograft models.
Direct cranial injection does result in neurotoxicity and fatal encephalitis, but this only occurs via this invasive route of
delivery Shedding and spreading of MG1 from infected Macaques is minimal, viral genomes only being detectable in
tissues via PCR, not by the less sensitive IHC or plaque assay (personal communication D. Stojdl, CHEO Research
Institute, Ottawa).
Risk to the environment: Very low/negligible
Maraba virus has been shown to have limited spread outside of sandflies [31]. Whilst closely related to strains of VSV
such as Indiana, does not cause vesicular disease with symptoms overlapping with FMDV in cattle or other ruminants
[31].
Maraba Virus (MaraV) (replication-competent, attenuated)-summary
Maraba Virus has no known natural mammalian host and pathogenesis has not been documented in humans.

Genetic modification of MG1 results in a highly selective oncolytic virus.

Vaccinia virus (replication-competent, attenuated)

Vaccinia has been studied in detail over many years and much is known about its biology and pathology. It has a long history of safe use as a live vaccine in human smallpox vaccination programs [32J, and in addition a good safety record in clinical immunotherapy trials [1 2J.

General information

Type: enveloped double-stranded DNA virus [12, 32J. Size -192kb [12, 32J Family Poxviridae [1 2, 32J.

Mutagenic effects

Oncogenicity: non-oncogenic [12J. Genome integration: none [12]. Mutagenesis risk: very low - virus remains within the cytoplasm of the host cell and has little interaction with the host cellular proteins [12]. Vertical transmission: none [12J.

Infectivity

Natural host: none. Other hosts: human, buffalo, cattle, rabbits, mice. Route of contact: possibility of aerosol transmission [33, 34][58, '59J.

Tropism

Mucosal tissues [33, 34][58].

Transduction capability

Normal and transformed cells; is capable of infecting almost all cell types including a range of tumours including melanoma, glioma, bladder and colorectal cancer.

Pathogenesis

Human: Commonly: treatable, clinically mild disease (rashes, accidental implantations) [35J. Very rarely: potentially fatal disease (post-vaccinial central nervous disease, progressive vaccina aka vaccina necrosum, eczema vaccinatum) [35J.

Toxicity (side effects of treatment)-Human

The level of vaccinia induced human toxicity is strain-dependent [8J. Commonly: Minimal grade 3 and no grade 4 toxicity (observed across immunotherapy trials in a wide variety of cancers with administration via intramuscular, intratumoural, intravesical and subcutaneous routes. Doses of up to 109 viral particles) [6, 12, 36, 37J. Very rarely: Progressive vaccinia, eczema vaccinatum, post-vaccination encephalomyelitis [37J. Immunocompromised individuals and infants: higher incidence of adverse effects (5 deaths/106 under 1 year old vaccinees versus 0.5 deaths/1061-4 year old vaccinees in 1968) [35J.

Mice: Toxicity and mortality in immuno-competent mice are dependent upon strain [66]. Extremely virulent infection with high toxicity and mortality can occur on systemic injection of high doses of wild type vaccinia into immunocompetent or nude mice but the attenuated virus highly unlikely to cause disease in healthy or immuno-compromised animals. [11 , 38J.

Hazard identification for viral vectors- vvDD-GFP & vv-GMCSF (JX-594)

Health and Safety

Executive

Risk to normal, healthy immuno-competent individuals Very low Exposure to vvDD-GFP or vv-GMCSF(JX-594 is highly unlikely to result in toxicity. In comparison to wtWR, vvDD-GFP [12J is replication restricted and vv-GMCSF (JX-594) [39J is both replication restricted and more immunogenic. Immune recognition and activation would control viral pathogenesis in all cases.

Risk to the environment: Very low/negligible
Although release of vvDD-GFP or vv-GMCSF(JX-594) to surrounding areas would increase the risk of exposure to rabbits, cows and mice, due to their compromised replicative abilities and/or greater immunogenicity [12, 39] relative to wt-WR, these 2 vectors are highly unlikely to cause disease in healthy or immuno-compromised animals. As all work using these viruses will be carried out in containment level II facilities the risk to the environment would be considered negligible.

Vaccinia virus (replication-competent, attenuated)-summary
Vaccinia has been studied in detail over many years and much is known about its biology and pathology. It has a long history of safe use as a live vaccine in human smallpox vaccination programs [35], and in addition a good safety record in clinical immunotherapy trials [12, 28]. Disease as a result of vaccination is rare and well-characterized with treatments being readily available.

HSV-1 (replication-competent, attenuated)
Herpes simplex virus (HSV) is one of the most widely clinically used oncolytic agents. Clinical experiments have demonstrated that intravascular delivery of HSV is a safe and effective way to produce oncolytic effects.

T-VEC (talimogene laherparepvec) [40]
General information
Type: enveloped double stranded DNA virus [32]. Size -152kb [32] Family Herpesviridae.
Mutagenic effects
Oncogenicity: none reported. Genome integration: none [41]. Mutagenesis risk: none [41].
Vertical transmission: none reported. HSV1 has been widely investigated for several decades with no instances or evidence of oncogenicity or vertical transmission having been reported.

Infectivity
Natural host: Human [42] Other hosts: Mice, rat, rabbit, non-human primates, guinea pig [43]
Route of transmission
Direct contact [44]
Tropism
Oral and genital mucosa, nervous system, eyes [44]
Transduction capability
Tumour cells [15, 45]
Toxicity and Pathogenicity
Mechanism of cell death: Cytolysis due to viral replication [41]
Pathogenesis
Commonly: None [15] Rarely: Pyrexia, inflammation, nausea, anorexia [46]
Toxicity (side effects of treatment)
Commonly: Grade 1 and minimal grade 2 toxicity (observed in phase 1 trial in a range of cancers at doses of up to 108 viral particles) [46]. Rarely: Low-grade hepatotoxicity, ulceration [46]
Hazard identification for JS1/34.5-/47- and OncoVexGM-CSF (T-VEC [talimogene laherparepvec])
Risk to normal, healthy immuno-competent individuals Very low Exposure to JS1/34.5-/47- or OncoVexGM-CSF (TVEC) is highly unlikely to result in toxicity as both viruses are non-pathogenic [15].
Risk 10 the environment Very low/negligible

Health and Safety
Executive
Release of JS1/34.5-/47- or OncoVexGM-CSF 10 surrounding areas would increase the risk of exposure to rodents and rabbits. However as JS1/34.5-/47- and OncoVexGM-CSF are non-pathogenic the likelihood of any infection or disease is negligible. All work carried out using this virus will be carried out in containment level II facilities thus the risk to the environment would also be considered negligible.
Adenovirus (replication-defective) - ACDP Hazard group 2
Replication-defective Ad vectors contain deletions in their E1 region, thus requiring helper cell lines which are modified to encode these regions (e.g. HEK293) in order to complete a full infectious cycle. In all other cells, these vectors are limited to single-round infections.

Adenovirus (replication-competent, attenuated) - ACDP Hazard group 2
Replication-competent Ad vectors have a long history of use as a therapeutic oncolytic agent both as wild-type virus and as a gene therapy vector. They have an excellent safety record with many cancer patients having been treated at high doses without adverse effects [2-4].

Ad5d1922-947 derived from Ad5d1309; replication is confined to tumour cells with a non-functional retinoblastoma pathway. Ad5d1922-947 lacks a functional CR2 region in the E1A protein [5, 6], rendering the virus unable to switch on host cell proliferation mechanisms in cells with a functioning retinoblastoma pathway. It combines the immunogenic properties of Ad5d1309 with an inability to replicate in normal non-proliferating cells.

Maraba Virus (MarabV) (replication-competent, attenuated) - ACDP Hazard Group 2
Marab V MGl vectors contain two stable attenuations [7]; one in the glycoprotein (G) ORF, and one in matrix (M).

Vaccinia virus (replication-competent, attenuated) - ACDP Hazard group 2
Replication-competent vaccinia virus has a long history of safe use as a live vaccine in human smallpox vaccination programs [8], and in addition a good safety record in clinical immunotherapy trials [1, 9, 10].

ddW-GFP
Genetically modified; A double-deleted, replication-restricted mutant vaccinia virus (vvDD) expressing EGFP, derived from the Western Reserve strain. The coding sequences for both thymidine kinase (TK) and vaccinia growth factor (VGF) have been deleted and EGFP inserted into the TK locus; ddW-GFP has been shown to replicate only in tumour. Created by McCart et al [11, 12].

vv-GMCSF (JX-594)
JX-594, a single-deleted, replication-restricted mutant vaccinia virus (vVDD) expressing human GMCSF gene (encoding granulocyte macrophage colony-stimulating factor or GM-CSF; driven by a synthetic early/late promoter) and lacZ gene (encoding I3-galactosidase or I3-gal; driven by the p7.5 early/late promoter) inserted into the TK gene (encoding thymidine kinase or TK) in the J segment of the Wyeth strain vaccinia virus. [8, 13] Elimination of TK from the JX-594 genome restricts viral replication to tumour cells, whereas GM-CSF production facilitates tumour immune response, and I3-gal is included for virus tracking purposes. [1, 3]

HSV-l (replication-competent, attenuated) - ACDP Hazard group 2
A genetically modified strain of HSVl in which the coding sequences for ICP34.5 and ICP47 are deleted [14]. Deletion of ICP34.5 causes the loss of viral neurovirulence and the ability to establish latency together with confining viral replication to tumour cells [14, 15]. Deletion of ICP47 prevents virus inhibition of cell surface presentation of viral antigens increasing viral immunogenicity in comparison to wtHSVl and enhances tumour-specific viral replication [16].

Evaluation of foreseeable effects
All viral vectors are attenuated resulting in tumour selective tropism.

Adenovirus
Health and Safety Executive
AdSd1922-947 derived from AdSd1309; replication is confined to tumour cells with a non-functional retinoblastoma pathway. AdSd1922-947 lacks a functional CR2 region in the E1A protein [5], rendering the virus unable to switch on...
host cell proliferation mechanisms in cells with a functioning retinoblastoma pathway. AdSd1922-947 is both more immunogenic and replication restricted [S] compared to wild type. Clinical studies with replication-competent, attenuated adenovirus have shown safety with limited toxicity.

Maraba Virus (MaraV)
Maraba MG1 vectors contain two stable attenuations [7], one in the glycoprotein (G) ORF, and one in the matrix (M). These have been demonstrated to render MG1 capable of replicating only in tumour cell lines. The M mutation limits evasion of innate antiviral immunity [7].

Vaccinia virus
Vaccinia has been studied in detail over many years and much is known about its biology and pathology. It has a long history of safe use as a live vaccine in human smallpox vaccination programs [35], and in addition a good safety record in clinical immunotherapy trials [12]. Risk to normal, healthy immuno-competent individuals: Very low Exposure to vvDD-GFP or vv-GMCSF (JX-963) is highly unlikely to result in toxicity. In comparison to wtWR, vvDDGFP [11, 12] is replication restricted and vv-GMCSF (JX-S94) [39] is both replication restricted and more immunogenic. Immune recognition and activation would control viral pathogenesis in all cases.

HSV-1
Herpes simplex virus (HSV) is one of the most widely clinically used oncolytic agent. Clinical experiments have demonstrated that intravascular delivery of HSV is a safe and effective way to produce oncolytic effects. Risk to normal, healthy immuno-competent individuals: Very low Exposure to JS1/34.S-/-47- or OncoVexGM-CSF (T-VEC) is highly unlikely to result in toxicity as both viruses are non-pathogenic [15].

Hazards to the Environment
The potential hazards of sequences within the GMO being transferred to related organisms:
Factors to consider include whether widespread dissemination of the inserted gene as a result, for example, of either gene transfer or recombination of the GMO with a wild-type microorganism, would be a matter of concern. If this is the case an important consideration will be whether, in the event of a breach of containment the GMO could survive in the environment for long enough for such a gene transfer to take place.

Hundreds of patients have been treated with oncolytic virus recombination between wild type and attenuated virus has not been documented. Contact with wild type is almost impossible in a lab environment, the wild type viruses have never been grown in the lab. If recombination were to take place the attenuate gene would be passed on to the wild type virus. This would result in the wild type virus having a tumour select phenotype and be harmless to normal tissue. Adenovirus (replication-defective or competent, attenuated) Risk to the environment: Very low/negligible. Although release of AdSd1309, AdSd1922-947 or AdS CMV GFP to surrounding areas would increase the risk of exposure to rodents, rabbits and pigs, due to their greater immunogenicity and/or compromised replicative abilities relative to wtAdS these 3 vectors are highly unlikely to cause disease in healthy or immuno-compromised animals. As all work using these viruses will be carried out in containment level II facilities the risk to the environment would be considered negligible.

Maraba Virus (MaraV) (replication-competent, attenuated). Risk to the environment: Very low/negligible. Maraba virus has been shown to have limited spread outside of sandflies [31]. Whilst closely related to strains of VSV such as Indiana, does not cause vesicular disease with symptoms overlapping with FMDV in cattle or other ruminants [31].

Vaccinia virus (replication-competent, attenuated). Risk to the environment: Very low/negligible Although release of vvDD-GFP or vv-GMCSF (JX-S94) to surrounding areas would increase the risk of exposure to rabbits, cows and mice, due to their compromised replicative abilities and/or greater immunogenicity [11, 12, 39] relative to wt-WR these 2 vectors are highly unlikely to cause disease in healthy or immuno-compromised animals. As all work using these viruses will be carried out in containment level II
facilities the risk to the environment would be considered negligible.

HSV-1 (replication-competent, attenuated). Risk to the environment: Very low/negligible

Release of JS1/34.5-47- or OncoVexGM-CSF to surrounding areas would increase the risk of exposure to rodents and rabbits. However as JS1/34.5-47- and OncoVexGM-CSF are non-pathogenic the likelihood of any infection or disease is negligible. All work carried out using this virus will be carried out in containment level II facilities thus the risk to the environment would also be considered negligible.


34. Hans, R., et al., OPTiM: A randomized phase III trial of talimogene laherparepvec (T-VEC) versus subcutaneous granulocyte-macrophage colony-stimulating factor (GM-CSF) for the treatment (Ix) of unresected stage IIIIBIC.
and IV melanoma. Journal of Clinical Oncology, 2013. 31 (18).
41. Kuruppu, D. and KK Tanabe, Viral oncolysis by herpes simplex virus and other viruses. Cancer Biology &
42. Sissons, J.G.P., Herpesviruses (excluding Epstein-Barr Viruses). Oxford Textbook of
Medicine, 2003.
43. Wagner, EK and D.C. Bloom, Experimental investigation of herpes simplex virus latency. Clinical Microbiology
Reviews, 1997. 10(3): p. 419-&.
44. Cunningham, AL., et al., The cycle of human herpes simplex virus infection : Virus transport and immune control.
45. Liu, B.L. , et al. , ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and antitumour
46. Hu, J.C.C., et al., A phase I study of OncoVEX(GM-CSF), a second-generation oncolytic herpes simplex virus
50. Morgan, R., et al., Antagonism of HOX/PBX dimer formation blocks the in vivo proliferation of melanoma. Cancer
51. Plowright, L. , et al., HOX transcription factors are potential therapeutic targets in non-small-cell lung cancer
52. Plowright, L., et al., Disrupting the interaction between Hox and PBX causes apoptotic cell death and reduces in

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Animal work will be carried out under conditions stated in the Animal Scientific Procedures Act (Pandha project
licence 70/7347) and is under constant review by the University of Surrey's Animal Welfare Ethical Review Board.
Clinical data shows that oncolytic virus are shed from patients post treatment but due to attenuation of the viral vector
reinfection of normal tissue is not possible. As per animal house procedures all bedding waste will be sent for
incineration. Derogation for disposal GM Class 2 animal waste is detailed and accepted by the HSE in GM notification
GM81/15.1. All proceduces wil l be performed in a class 2 Biosafety cabinet

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
There is no autoclave within the building, so inactivation is by a validated disinfectant. Inactivation will be via a 1 %
(WN) Virkon S for at least 24hrs at Room Temperature.
Inactivated fluids will then be flushed to laboratory drain with copious amounts of water as below for liquid Waste as
described below
Solid plastic waste will be inactivated by immersion in 1 %(WN) Virkon S for at least 24hrs at room temperature and
then placed into a yellow bag which is sealed. This bag in turn is placed into a second yellow bag and sealed.
The double yellow bag is then sealed in a burn bin containing 1-2inches of vermiculite granuales.
This is then tagged, recorded and sent off site for incineration via Grundons Waste Management Company. The burn
bins will be labelled with UN3291. Upon collection the burnbins will be placed into a 770L taylor bin and transported to
the Clinical waste incinerator. Here the taylor bin is tipped into the hopper which feeds the incineration chamber.
Waste in the primary combustion chamber burns for a minimum of six hours and then passes to a secondary

02/03/2022
A combustion chamber which reaches temperatures of 1100 degrees C. The Colnbrook facility is licenced to process class 1 and class 2 GM waste and is registered under GM reference centre number GM782. A certificate of destruction will be provided for waste sent under derogation to Grundons. Derogation for disposal GM Class 2 animal waste is detailed and accepted by the HSE as part of GM notification GM81/15.1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid disinfection will be performed by treating Liquid waste with a solution of 1% (weight/volume) Virkon S for at least 24 hours at room temperature. The Inactivated Liquid waste will then be disposed of down the laboratory Sink. The Inactivated Liquid waste will be flushed with copious amounts of water at a maximum volume of 2L Inactivated waste per day. This flows to the main foul drain and into a waste water treatment facility owned by Thames Water. Solid plastic waste is inactivated by immersion in 1% virkon for 24hrs then further disposed of into a bag, bag, bin, burn system as follows:

1. Solid Inactivated waste is placed into a bag which is sealed.
2. This bag in turn is placed into a yellow bag and sealed.
3. The yellow bag is then sealed in a burns bin containing 1-2inches of vermiculite granuales.
4. This is then tagged, recorded and sent off site for incineration via University Waste route currently Grundons Waste Management Company.

Tumour Slices will be blocked for cutting, fixed in formalin, then, disposed of in a sealed clinical waste bin that is tagged, recorded and disposed of by separated incineration in accordance with HTA rules and regulations. Via University waste route currently Grundons Waste Management Company.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 Yes</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The Biosafety university of surrey committee has approved this risk assessment on the Tuesday 19th January 2016 on the condition that minor amendments to wording were made.

02/03/2022
Project Additional Information

Purposes of the contained use
To develop live attenuated vaccine candidates for the specific protection of poultry against the diseases and pathologies caused by Avian Pathogenic E. coli (APEC) and potentially suppress the carriage of these organisms in at risk animals.

Recipient or parental organism
The recipient organisms in this study are veterinary isolates of Avian Pathogenic E. coli (APEC). These bacteria are ACDP Hazard Group 2 organisms. E. coli is a gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. Avian Pathogenic E. coli (APEC) are a diverse group of E. coli that cause a number of differing disease outcomes in susceptible birds. Pathologies associated with these organisms include airsacculitis, polyserositis, septicaemia and other mainly extraintestinal diseases. APEC are found in the intestinal microflora of healthy birds and most of the diseases associated with them are secondary to environmental and host predisposing factors.

Host/vector system
pCM433kanT is a plasmid vector that is currently within the plasmid collection at the School of Veterinary Medicine. This plasmid was developed in the Lab of Dr Mary Lindstrom at Iowa State University (Puri et al2014). The plasmid encodes the kanamycin resistance cassette and the sacB gene. pCM433kanT is propagated in E. coli DH5 alpha.
This strain of E. coli is not a pathogen, and was developed for laboratory cloning use with multiple mutations that enable high-efficiency transformations. pCM433kanT is the source of the kanamycin resistance cassette and the sacB gene from Bacillus subtilis, two genetic markers that will be used in this study for selection and counterselection of genetically transformed E. coli isolates. The gene products arising from this construct pose no direct hazard to human health.

Origin & function

The genetic material involved in this study are the kanamycin resistance gene and sacB gene from pCM433kanT as well as the genomic flanking regions of the aroA gene of various veterinary isolates of Avian pathogenic E. coli. aroA in E. coli encodes the 3-phosphoshikimate 1-carboxyvinyltransferase (also known as 5-enolpyruvylshikimate-3-phosphate synthase) enzyme catalyses the six 1h step in the biosynthesis from chorismate of the aromatic amino acids (the shikimate pathway) in bacteria. Since the shikimate pathway is not present in vertebrates but is essential for the life of plants, fungi and bacteria, it is commonly viewed as a target for antimicrobial drug development.

Using the PCR amplified flanking regions of the aroA gene as well as the kanamycin resistance cassette and sacB gene from pCM433kanT, the aroA gene will be deleted by induction of homologous recombination. This deletion method will make use of standard mutagenesis, cloning and PCR techniques and the use of suicide vectors. In detail, a vector will be constructed containing a kanamycin resistance cassette and the sacB gene from Bacillus subtilis conferring sucrose sensitivity flanked by the genomic regions -1OOObp either side of the aroA gene (encoding the phosphoserine aminotransferase gene and Zn-dependent protease genes). Separate genetic constructs will be made for each candidate isolate.

In the first instance, these genetic constructs will be transformed into the target strains from which they are derived resulting in a kanamycin-resistant, sucrose-sensitive derivative for each isolate of APEC selected. Successfully clones will be subjected to a second transformation event in order to excise the kanamycin resistance and sucrose sensitivity cassettes, resulting in a clean, unmarked deletion of aroA.

Evaluation of foreseeable effects

Deletion of the aroA gene causes the target organism to become auxotrophic i.e. to be dependent on certain aromatic compounds for growth. Aromatic amino acids are not synthesized by vertebrates, and are only present in tissues at very low and well-controlled levels. The loss of aroA gene function results in attenuation of in vivo growth without compromising potential cellular and humoral immune responses by the avian host. The mutations described in this study are specifically intended to attenuate this organisms. Deletion of the aroA gene will render these APEC isolates auxotrophic and dependent on host-derived aromatic amino acids which are not synthesized by vertebrates. Reversion or gene recovery of deletion has never been seen with aroA deletion vaccines. Therefore, the mutations described herein, will not pose a risk to human health.

The mutation described here will likely reduce the ability of these strains to survive within the environment. Deletion of the aroA gene is a disabling mutation that will abolish the ability of the wildtype avian pathogenic E. coli strains to colonise vertebrate hosts and other animals in the environment. Reversion of a deletion in aroA has never been observed.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No Larger GMOs will be used in this study

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
All solid and liquid contaminated waste is treated by autoclaving for at least 15 minutes at 121 °C on a validated cycle resulting in 100% kill.

Post autoclaving: Solid waste is further disposed of into a tiger stripe bag and sent off site for incineration via Grundons Waste Management Company. Liquid waste is disposed of down the sink with copious amounts of water. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

Liquid disinfection may also be performed by treating the solution with 1% (weight/volume) VirkonS for 16hrs at room temperature. The solution is then disposed of to the laboratory drain flushed with copious amounts of water at a maximum volume of 2L per day. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The University Biosafety committee requested additional information to confirm the classification of the activity as a Class 2. The information was accepted and incorporated into the risk assessment. Other minor changes in text were recommended regarding waste disposal to provide full clarification.

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Project Ref 81/17.1

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<th>CultureVolumeClass3-4</th>
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<tr>
<td>26/04/2017</td>
<td>A phase 3 randomized, open-label study comparing Pexa-Vec (Vaccinia GM)</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Pexa-Vec is currently in clinical development for the treatment of Hepatocellular Carcinoma. The proposed contained use will be the administration of the investigational product, in a hospital or clinic setting, by intratumoral (IT) injections to patients as part of an international, multicenter clinical trial. This clinical trial is a Phase III trial in patients with Advanced Hepatocellular Carcinoma (HCC) without prior systemic therapy. Results from this pivotal trial will determine whether Pexa-Vec followed by sorafenib increases survival duration in advanced HCC patients compared to treatment with sorafenib alone, and whether sequential dosing with Pexa-Vec followed by sorafenib has a favourable safety profile.

Approximately 40 clinical sites in the EU will enroll patients in the JX594-HEP024 (PHOCUS) study. Additional clinical sites in Australia, Canada, China, Israel, Korea (Republic of), New Zealand, Singapore, Taiwan, Thailand and the USA will also participate in the study. A total of 600 patients will be recruited in this clinical trial with an expectation to enroll 200 patients in EU countries. In the control arm, the 300 patients will not receive Pexa-Vec. After study completion, all patients will be followed up for survival. Among them, 300 patients (i.e. approximately 100 patients in EU) will receive Pexa-Vec by IT injections.

Pexa-Vec is a replicative oncolytic recombinant vaccinia virus (W) derived from the commonly used vaccine Wyeth strain, DryvaxTM. W is a member of the Poxviridae family (genus Orthopoxvirus). Multiple strains of W exist that have different levels of virulence for humans and animals. The New York City Board of Health (NYCBOH) strain, from which the Wyeth strain of the Dryvax® vaccine was derived, has low pathogenicity in humans (Fenner F. et al., 1988). W has a long and extensive history of use in humans. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous (SC) infection. As W contains antigens that stimulate an immune response that are cross-reactive with smallpox antigens, the vaccine thereby confers protection from the human smallpox disease. W may cause local reactions including erythema, edema and systemic reactions such as fever and malaise, as has been observed with conventional vaccination to smallpox. During the smallpox vaccination campaign, serious complications had occurred in less than 1 in 4,000 individuals, mainly in immunosuppressed and extremely young individuals. Pexa-Vec is even further attenuated as the thymidine kinase gene has been disrupted which makes

Recipient or parental organism

Pexa-Vec is a replicative oncolytic recombinant vaccinia virus (W) derived from the commonly used vaccine Wyeth strain, DryvaxTM. W is a member of the Poxviridae family (genus Orthopoxvirus). Multiple strains of W exist that have different levels of virulence for humans and animals. The New York City Board of Health (NYCBOH) strain, from which the Wyeth strain of the Dryvax® vaccine was derived, has low pathogenicity in humans (Fenner F. et al., 1988). W has a long and extensive history of use in humans. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous (SC) infection. As W contains antigens that stimulate an immune response that are cross-reactive with smallpox antigens, the vaccine thereby confers protection from the human smallpox disease. W may cause local reactions including erythema, edema and systemic reactions such as fever and malaise, as has been observed with conventional vaccination to smallpox. During the smallpox vaccination campaign, serious complications had occurred in less than 1 in 4,000 individuals, mainly in immunosuppressed and extremely young individuals. Pexa-Vec is even further attenuated as the thymidine kinase gene has been disrupted which makes
replication in normal cells more difficult than the small pox vaccine. Rare complications included eczema vaccinatum (patients with eczema), disseminated vaccinia rash, progressive vaccinia (in T-cell-deficient individuals) and encephalitis (1-2 per million vaccinated) (Fields B.N., 1996). Recent studies of smallpox vaccines have identified cardiac injury including pericarditis and myocarditis as a potential risk (Halsell J.S. et al., 2003).

W replication exclusively occurs in the cytoplasm thus eliminating any risk of integration of the viral DNA into the host genome (Moss B., 2007).

In terms of classification of hazard, W is considered as a Group 2 biological agent as per Directive (2000/54/EC). W is also classified as a Biosafety Level 2 (BSL-2) infectious substance by the US Centers for Disease Control and Prevention (CDC) (CDC, 2009) and as a risk group 2 organism by the US NIH Guidelines (NIH).

Host/vector system

Pexa-Vec was generated by co-transfection of CV-1 cells (Monkey kidney cells) with W (Wyeth strain obtained from the Center for Disease Control, Atlanta, Georgia) and the plasmid pSC65/hGM-CSF. The vector pSC65/hGM-CSF contains DNA sequences coding for the hGM-CSF and ~galactosidase proteins and for their respective promoters. In addition, the transgene sequences are flanked by two W genomic regions (TKL and TKR) that allow homologous recombination between the transfer plasmid and VV.

The plasmid pSC65/hGM-CSF is generated from the plasmid pSC65 which was provided by Dr. B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland (Chakrabarti 1997).

The plasmid pSC65/hGM-CSF is inserted into VV. The insertion of pSC65/hGM-CSF into VV can be detected by using colorimetric identification of plaques containing recombinants expressing ~galactosidase.

The pSC65 vector when provided by Dr. B. Moss contains the LacZ gene. The LacZ gene is a reporter gene, under control of the W p7.5 early/late promoter. The additional donor gene (i.e. gene coding for hGM-CSF) is inserted in pSC65 as follows.

The plasmid pCSF-1 (No. 39754) was obtained from American Type Culture Collection and comprises the full-length cDNA for hGM-CSF (Wong 1985). The hGM-CSF gene was cloned first into the EcoR1 site of pBLUESCRIPTII SK (Stratagene, La Jolla, California), generating plasmid pBLUE/hGM-CSF, and providing restriction enzyme sites to allow cloning of the hGM-CSF gene into the Sail and Bglli sites of pSC65. This positioned the hGM-CSF gene downstream of a synthetic promoter (PsE/L) designed by Dr Moss’ laboratory to give maximal levels of transcription during both the early and late phases of vaccinia infection (Chakrabarti 1997).

Origin & function

Pexa-Vec contains three genetic modifications compared to the wild type Wyeth strain: 1) disruption of the viral thymidine kinase (TK) gene by, 2) insertion of the human granulocyte macrophage-colony stimulating factor (hGMCSF) gene and 3) insertion of the LacZ gene.

Due to the insertion of the transgenes, the TK gene is inactivated. This decreases W virulence (Buller R. et al., 1985) by restricting viral replication to proliferating cells. This also targets dissemination of the virus to tumors (Puhlmann M. et al., 2000).

The plasmid pCSF-1 (No. 39754, American Type Tissue Culture Collection, Rockville, Maryland) contains the fulllength cDNA for hGM-CSF (Wong G.G. et al., 1985). The cDNA for the hGM-CSF gene was inserted into the TK gene to help elicit an immune response to tumor cells both at the site of viral replication and in distant metastases.

The cytokine hGM-CSF was chosen because it was the most potent stimulator of systemic anti-tumor immunity among many tested (Dranoff G. et al., 1993), probably due to its unique ability to promote differentiation of hematopoietic precursors into dendritic cells (Pardoll D.M., 1995). Dendritic cells are professional antigen presenting cells that may take up and present released tumor antigens as the tumor cells are killed by the W.

The LacZ gene is contained in the pSC65 plasmid which was provided by Dr. B. Moss, National Institute of Allergy and Infectious Diseases.
and Infectious Diseases, Bethesda, Maryland. The LacZ gene encodes for β-galactosidase which is a hydrolase enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides.

Of note, following recombination between the W and pSC65/hGM-CSF, the antibiotic resistance gene contained in pSC65 is not part of the insert. The final GMO does thus not contain any genes conferring resistance to antibiotics.

Evaluation of foreseeable effects

Pexa-Vec is non-integrative, and replicative and propagative characteristics of W have been attenuated in Pexa-Vec, which makes the virus replication dependent on actively dividing cells such as cancer cells. Therapy with a replicating virus can theoretically lead to shedding of the virus into the environment, and potentially to the public, although controls are used in this trial to minimize this occurrence. The clinical information available to date suggests that Pexa-Vec is safe at the clinical dose of 1 x 10^9 pfu (10,000-fold higher than smallpox vaccine dose) and has not spread to caregivers in contact with the treated patients. Should shedding occur, the level of exposure would be predicted to be low compared to the doses received by patients in the proposed trial, and extremely low compared to doses of non-attenuated vaccines administered to the public (e.g. vaccines against smallpox). In addition, exposed individuals over the age of 35 will likely have been previously immunized with vaccinia. In the highly unlikely event that an exposed individual were to demonstrate virus-associated toxicity, therapy could be initiated with VIG and/or cidofovir. Therefore, public health risks with this virus are extremely low and in fact should be lower than with standard vaccination procedures. To date, no reports of transmission to health care personnel from vaccinia recipients have been published. Routine barriers nursing approaches should be used per institutional guidelines for infectious organisms (e.g. such as for M. tuberculosis, Pseudomonas).

The genetic modification of the virus is not expected to result in any post-release shift in biological interactions or host range or in any known or predictable effects on non-target organisms in the environment. It is also not expected that the release of the recombinant virus would result in any increase in pathogenicity as compared to the parental virus strain and/or in any increase in the capacity to recombine with other related viruses.

Therefore, under the conditions for use in the proposed clinical trial, Pexa-Vec is not considered to represent a risk for the environment and for the public health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable,

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Under Schedule 8 of the Genetically Modified Organisms (Contained Use) Regulations 2014 Table 2 details the containment measures to be applied for non laboratory type facilities. Line 20 requires that inactivation of GMMs in contaminated material and waste is required by validated means at containment level 2. The waste is such that it cannot be disinfected and there is no available autoclave. All waste will be packaged to send directly to incineration. Therefore a derogation is requested. Please see section below detailing how waste will be handled.
The Pexa Vec literature states 'Fabrics (e.g., clothing, sheets, towels) that have touched an uncovered pustule can be laundered in hot water with detergent'.

The SCRC laundry service via White Knight washes bedding at 90°C. Current procedures allow for soiled bedding to be bagged in a red dissolvable washing bag. The bag is placed directly into the washing machine so no contact by third persons is made when handling the laundry.

The red bag system should be used for all bedding from participants in this clinical trial.

Please see attached separate document detailing the risk assessment for this derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- The waste is such that it cannot be disinfected and there is no available autoclave. The information detailed indicates how the waste will be packaged to send directly to incineration.
  - All waste will be placed inside a yellow hazardous waste bag. This bag will be sealed with a plain cable tie. The bag is then placed inside a second yellow hazardous waste bag and sealed with another cable tie. The double bagged waste is then placed inside a 30L yellow hazardous waste burn bin containing at least 2 inches of vermiculite to act as spillage absorbent material. This burn bin is sealed by clicking the lid closed. The yellow burn bin is a UN approved biohazard waste bin used for transport of waste on public highways. The maximum weight of each burn bin must not exceed 20Kg. Operationally, the yellow burn bin containing 2 inches of vermiculite in its base and 2 yellow bags (one inside each other) will be placed out at the start of each day. At the end of each day or when the container is three quarters full, the first bag will be sealed, the second bag sealed and then the lid of the burn bin closed. This will be performed in the room where the waste has been generated. Each burn bin is tagged with a uniquely numbered locking plastic tag. The tag reference is recorded.
  - The burn bin is labelled with a UN3373 label. Regular collections are arranged with the waste management contractor Grundons. During collection the unique tag reference numbers on the will be noted onto the consignment note and confirmed to the hazardous waste safety advisor in order for a certificate of destruction to be requested.

This waste must remain segregated from other burn bin waste generated from the building.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

Please enter comments on the GM safety committee on the risk assessment

The University Biosafety Committee approved this project on 12th January 2017.

The committee did request additional information to be added into the risk assessment to make more explicit the PPE requirements during administration of the product. The committee also asked about the information provided to the patient and was satisfied that the items were documented within the risk assessment.

CU

Project Containment
Project Ref 81/17.2

Date Ackn'd 27/07/2017

CU2 Project Title Impact and transmission of mobile genetic elements (MGE) carrying drug resistance genes

Date Project Ceased

Class 2

Culture Class Volume
2 Culture 1 < 1 Litre

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The goal of these studies is to i) identify and characterise sequences in mobile genetic elements (MGE) (e.g. plasmids, transposons) that promote their spread or persistence under different environmental conditions (e.g. in vitro - in planktonic culture, on surfaces, in biofilms; ex vivo - using organ culture models, bioreactor gut models; and in vivo - using mammalian and insect models of infection); ii) construct mutants harbouring deletions or insertions to disrupt sequences of interest using standard genetic techniques based on homologous recombination, CRISPR-Cas systems or transposon mutagenesis; iii) construct restoration of function mutants in knock out strains using gene complementation approaches; iv) clone and express 'marker' tags (e.g. fluorescent proteins, enzymes such as methyltransferases, short random sequences of nucleotides (barcodes)) on MGE and/or resistant bacteria to track their movement; v) insert reporter genes to study gene expression and/or protein localisation in prokaryotic and immortalised eukaryotic cell lines.
MGE harbouring drug resistance genes will be obtained from nature, either sourced from published literature or from
internal or external collections of resistant bacteria organically isolated from clinics, the environment or animals.

'Recipients' are any species that are capable of harbouring the genetically modified MGE. It is not possible to list
every potential 'recipient' (or donor) species due to the complexity of the natural microbial communities that are
typically found in settings such as the mammalian intestine, in soil or in waste-water samples, that may be used in our
studies. However, the genetic modification is unlikely to significantly affect the virulence of any particular recipient
strain but if the MGE is stably maintained, it could affect our ability to treat any infection that the recipient is able to
cause because it also naturally carries genes coding for resistance to antibiotics.

In some cases, we may use clinical isolates of Escherichia coli or other ACDP Biohazard group 2 pathogens e.g.
Klebsiella pneumoniae, both as a source of MGE carrying drug resistance genes, and as a potential recipient of
genetically-modified MGEs. Certain strains of E. coli are capable of causing infections and are typically transmitted by
the faecal-oral route of transmission. Infection could lead to signs of gastrointestinal disease (typically self-limiting
diarrhoea in otherwise healthy individuals) or extra-intestinal infections (e.g. bladder infections). K. pneumoniae is a
commensal in healthy individuals but is capable of causing extra-intestinal infections (e.g. urinary tract infections,
pneumonia) in individuals with underlying medical issues.

Recipient or parental organism

Bacterial 'donor' species are those that naturally carry MGE harbouring drug resistance genes, such as E. coli
(pathogenic and K-12 strains). In addition, Citrobacter rodentium (a natural murine pathogen) may be used as the
'host' species during in vivo mouse challenge experiments as unlike E. coli, this organism is capable of colonising the
murine intestine to high levels. However, any species within the complex microbiota capable of taking up the MGE
could potentially be a 'donor' and used in subsequent experiments.

A range of different plasmid vectors will be used during the genetic modification processes including:
1. cloning, gene editing and suicide plasmids for basic genetic manipulation in bacteria (e.g. pCVD442, pRRS,
pCRISPR-Cas systems); 2. bacterial expression plasmids for gene complementation and functional analysis studies
(e.g. pSEVA621, pBAD-TOPO), 3. plasmids carrying transposons to facilitate the insertion of marker tags (e.g. pBAM)
or the generation of mutant libraries in bacteria (e.g. EZ-TnS, pSAM) and 4. transfection vectors to allow genes of
interest to be expressed in eukaryotic host cells to aid assignment of function (e.g. pACGFPN1, pCMV-HA).

Host/vector system

The inserted gene products code for fluorescent proteins (e.g. GFP - green fluorescent protein) enzymatic activity (e.g.
methyltransferase (MTase)) or no known product (aka short nucleotide sequences or 'barcodes'). None of these are
Toxins or are oncogenic.

Genes coding for fluorescent proteins are found in nature and if expressed, confer fluorescence in target cells. Some
variants have been adapted to confer higher levels of fluorescence in target bacterial species - these are available
from the published literature.

Genes coding for methyltransferase (MTase) activity are also found in nature. These are enzymes that add a methyl
group to specific nucleotides in the genome of organisms. This results in methylation of DNA, which in turn protects it
from cleavage by cognate restriction endonucleases. This has been recognised as a mechanism via which bacteria
protect themselves from the effects of incoming foreign DNA. Changes in the methylation status of genomic DNA can
result in widespread changes in gene expression including those involved in general celluar activity and virulence in a
species-specific manner.

'Barcodes' or short sequences of nucleotides confer no product but can be used to help differentiate different sources

Origin & function
of DNA during high throughput sequencing reactions. Barcodes are chemically synthesised and have been previously described in the literature.

**Evaluation of foreseeable effects**

The genetically-modified MGE will be harboured in ACDP Hazard group 1 or 2 strains. While the inserted sequences do not encode pathogenicity determinants, it is possible that expression of the MTase could lead to change in the expression of other genes in the genome. It is not possible to predict which genes may be affected for each species (without performing the experiment) but it is highly unlikely that changes will significantly increase the virulence of the organism over its original ACDP classification.

The genetically modified MGE could be transferred to other organisms under the right conditions because this property defines this type of genetic element (i.e., that it is mobile). Transfer is most likely to occur under conditions where there is a high localised density of donor and potential recipient cells as it is normally an infrequent event. However, specific-pathogen free mice or decontaminated research-grade Galleria mellonella larvae are unlikely to contain members of the normal microbiota that are pathogenic to humans. Thus, transfer to other members of the microbiota are unlikely to pose a problem for human health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

A derogation is in place to allow disposal of waste from the animal facility without autoclaving on-site. Derogation for sending waste direct to incineration has been approved under notified assessment GM81/15.1. All waste from the animal facility is double-contained within yellow hazardous waste bags and then placed inside a yellow hazardous waste burn bin. The bin is sealed by clicking the lid shut. The outside of the bin is disinfected with 1% bleach solution (or equivalent disinfectant) and labelled with a UN3373 Category B Biological Material sticker prior to removal from the unit. The bins will only be removed from the facility when able to be handed directly to Grundons Waste management personnel, where they will be immediately placed into a 770L ‘taylor’ style bin. The waste will then be transported to Grundons Coinbrook facility where it is processed direct through their High Temperature Clinical waste incinerator. Grundon’s Coinbrook facility is licensed to process class 1 and class 2 GM waste and is registered with the HSE (GM Centre #GM782).

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid and liquid contaminated waste generated outside of the animal facility is treated by autoclaving at 121 °C for at least 15 minutes on a mixed load validated cycle.

Post autoclaving: Solid waste is further disposed of into a tiger stripe bag and sent off site for incineration via Grundon’s Waste Management Company. Liquid waste is disposed of down the sink with copious amounts of water. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

Liquid disinfection may also be performed by treating the solution with 1% (weight/volume) Virkon overnight at room temperature. The solution is then disposed of to the laboratory drain flushed with copious amounts of water at a maximum volume of 2L per day. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

In the insect larvae studies, larvae will be disposed of, after killing by placing at -80°C for 24 hours, by autoclaving at 121 °C for at least 15 minutes on a mixed load validated cycle. Larvae will be contained in sealed, plastic tubes (e.g. 50 ml falcon tubes) during freezing and autoclaving steps.

In the animal facility, any solid or liquid waste generated can be disposed of without autoclaving on-site. Derogation
for sending waste direct to incineration has been approved under notified assessment GM81/15.1. All waste is doublecontained within yellow hazardous waste bags and then placed inside a yellow hazardous waste burn bin. The bin is sealed by clicking the lid shut. The outside of the bin will be disinfected with 1% bleach solution (or equivalent disinfectant) and labelled with a UN3373 Category B Biological Material sticker. The bins will be removed from the facility and handed to Grundons Waste management personnel, where they will be directly placed into a 770L 'taylor' style bin. The waste will then be transported to Grundons Coin brook facility where it is processed direct through their High Temperature Clinical waste incinerator. Grundon's Coin brook facility is licenced to process class 1 and class 2 GM waste and is registered with the HSE (GM Centre #GM782).

Project Containment

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<th>Laboratory Activities</th>
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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 81/20.1

Date Ackn'd 22/01/2020

CU2 Project Title The role of mobile genetic elements in spreading antibiotic resistance: evolution

Class 2

CultureVolClass2 1 Litre

CultureVolumeClass3-4 < 1 Litre
The main aim of this work will be to limit the spread of antimicrobial resistance (AMR) by sensitising resistant bacteria, blocking transfer of mobile genetic elements (MGEs) or preventing bacterial transmission. For that purpose, the following steps will be performed:

- Select the most prevalent MGEs spreading critical important priority AMR genes, especially species of Enterobacteriaceae carrying carbapenemase and ESBL genes.
- Analyse fitness, transfer and evolution of selected MGEs under different conditions. This step could provide information useful to identify host or MGE sequences subject of further experiments that could involve generation of genetically modified MGEs or bacterial hosts (via deletion, disruption or insertion of sequences of interest involved in MGE biology).
- Develop a method to study in detail the evolutionary dynamics of selected MGEs in complex microbial communities. This step could include the generation of genetically modified MGEs or bacterial hosts carrying tracking genes, such as fluorescent proteins, barcode tags, etc.
- Design a CRISPR-Cas delivery vector to remodel complex communities by sensitising bacteria carrying AMR genes. To carry out this step, a broad host range vector will be genetically modified by standard genetic techniques to carry a CRISPR array with spacers targeting MGEs-derived sequences, a Cas nuclease, and a tracking gene. Both the vector and the donor bacteria used to deliver the vector could be subject to further modification to improve delivery characteristics, such as transfer frequency or host range, or to increase the potential use (e.g. expression of antigens to use as a vaccine, or addition of spacers to the CRISPR array with other targets to use as antimicrobials against pathogenic bacteria).
- Study the evolutionary dynamics of the selected MGEs in the presence and absence of the CRISPR-Cas vector to test its sensitising ability pre- and post-infection and to understand the risks associated with the emergence of resistance from single species to complex bacterial communities (such as in vitro gut models or in vivo infection models, where a high number of bacterial species within the community will be potential recipients and new donors of the CRISPR-Cas vector and MGE targeted).
The genetically-modified organism will be mainly Escherichia coli or other species of Enterobacteriaceae classified as ACDP Biohazard group 2. They are typically commensal bacteria found in the gut of healthy humans and animals. However, some strains can cause gastrointestinal disease or extra-intestinal infections, especially in immunocompromised individuals.

Potential recipients (and new donors) of genetically-modified MGEs could include different commensal bacteria found in the gut microbiome of different species (e.g. pig, chicken, horse, human faeces used in in vitro gut models, or decontaminated research-grade Galleria mellonella larvae used in animal studies). It is highly unlikely that members of the normal microbiota of these hosts will be pathogenic to humans or animals. No Biohazard group 3 or 4 organisms will be used in this work.

**Host/vector system**

Original MGEs carrying AMR genes will be obtained from nature, either sourced from published literature or from internal or external collections of resistant bacteria isolated from clinics, animals or the environment. The AMR genes naturally present in these elements could affect our ability to treat potential infections caused by the bacterial host. Modification of the MGEs or host bacteria will be performed using standard genetic techniques, such as homologous recombination, Gibson assembly or transposition, employing strains and vectors commonly used for basic genetic manipulation of bacteria (e.g. E. coli DY380, E. coli DH5α, E. coli MFD-pir, pCas, pTargetF, RP4, pRRS, pBAM, pBAD, pSEVA, pLOF, etc.).

**Origin & function**

The MGE-related gene products that will be inserted to study MGE biology, and tracking products such as fluorescent proteins (e.g. gfp from jellyfish), are not toxic, oncogenic or predicted to alter bacterial virulence found in nature and extensively used in the literature.

The Cas nuclease inserted as part of the CRISPR-Cas delivery vector is considered a “selective toxin” that will cut DNA complementary to the spacers included in the CRISPR array. The spacers will be specifically selected to target MGE-born sequences, with the aim of sensitising the host bacteria without causing bacterial death, thus avoiding high selective pressures that would increase emergence of resistance. Alternative strategies include designing CRISPR arrays to target specific pathogenic bacteria with the aim of triggering bacterial cell death of those pathogens with complementary sequences in their genomes that have received the CRISPR-Cas vector. This strategy is nowadays a common alternative to antibiotics with great potential and effectively used in the literature, and it is also widely found in many Bacteria and Archaea as a defence mechanism against viral infection.

**Evaluation of foreseeable effects**

None of the traits inserted will be pathogenicity determinants or predicted to alter bacterial virulence. It is highly unlikely that the genetic modifications introduced affect the virulence of the host bacteria, but it cannot be fully discarded (bacteria can mutate and produce more virulent variants regardless of any inserted sequence).

The presence of the MGEs harbouring antibiotic resistance genes, both the original and the genetically-modified versions, could affect the ability to treat infections caused by the bacterial hosts acquiring these vectors and increase their survival in environments containing antibiotics. The presence of the MGEs containing CRISPR-Cas systems with bactericidal effects could also increase their ability to compete with other bacteria increasing their survival.

The purpose of the broad host range vector carrying CRISPR-Cas is the decontamination of AMR genes from the highest possible diversity of bacteria present in the gut, as well as preventing the acquisition of new AMR-harbouring MGEs. The extent of spreading of these MGEs in the bacterial community will also be tested to understand the risks of transmission of specific MGEs and how to prevent it.

It is difficult to anticipate the specific bacteria that will receive the genetically-modified MGE due to the high diversity of
bacterial species in the gut, but they will be generally commensals, only able to produce infections in very specific circumstances (such as to immunocompromised individuals). All these experiments will be carried out in contained environments and all potential donors and recipients will be inactivated after each procedure. In the event of a breach of containment, the genetically-modified MGE could be transferred to other bacteria because this property defines this type of genetic element (i.e. that it is mobile). Transfer is most likely to occur under conditions where there is a high localised density of donors, potential recipient bacteria, and nutrients (like the gut). In the unlikely event that transmission occurred, the resulting genetically-modified microorganism would be able to decontaminate the environment from resistant bacteria.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid contaminated waste is treated by autoclaving at 121°C for at least 15 minutes on a mixed load validated cycle.
Post autoclaving: Solid waste is further disposed of into a tiger stripe bag and sent off site for incineration via Grundons Waste Management Company. Liquid waste is disposed of down the sink with copious amounts of water. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water. Liquid disinfection may also be performed by treating the solution with 1% (weight/ volume) Virkon overnight at room temperature. The solution is then disposed of to the laboratory drain flushed with copious amounts of water at a maximum volume of 2 l per day. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.
For the insect larvae studies, larvae will be disposed of, after killing by placing at -80°C for 24 hours, by autoclaving at 121°C for at least 15 minutes on a mixed load validated cycle. Larvae will be contained in sealed, plastic tubes (e.g. 50 ml falcon tubes) during the freezing and autoclaving steps.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University Biosafety Committee met to discuss the risk assessment on 21/10/2019. GM2019/06 was approved at GM Class 2 with no amedments or comments.
Use of genetically modified ACDP hazard group 1 and 2 bacteria within in vitro animal gut models and in vitro organ culture (IVOC) experiments, and curing of naturally occurring plasmids. Genetically modified E. coli will be used to study the transfer of antimicrobial resistance genes between bacteria in-vitro. The recipient organisms will include Eschericia coli, Klebsiella species and non-typoidal Salmonella, isolated from humans and animals. These bacteria are ACDP Hazard Group 2 organisms. E. coli, Klebsiella and Salmonella are Gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus that are commonly found in the lower intestine of warm-blooded organisms. The organisms have the potential to cause a range of diseases in both animals and humans, but the GMO organisms should not be more virulent than the parental organism.
All genetic modifications will be done by Friedrich Loeffler Institut (FLI) in Germany and strains will be sent to Surrey for use in in vitro gut models and in vitro organ culture.

Transposon mutagenesis will be performed either using a custom commercial vector such as pMOD-2 EZ-Tn5 <MCS> from Epicentre Biotechnologies, OR a Tn5 transposon and transposase on a custom conjugative or mobilisable suicide plasmid. Both methodologies will result in the Tn5 transposon and an antibiotic (ampicillin, kanamycin or chloramphenicol) resistance encoding gene being inserted into the chromosome, by a transposase enzyme, of the host species (namely non-typhoidal Salmonella serotypes in the first instance).

The Epicentre Biotechnologies method uses a linearised non-replicating piece of DNA (a “transposome”) within the delivery system, and as such the transposase gene is lost during natural replication of the bacterial cells. In the case where a custom suicide plasmid is used, this plasmid (encoding the transposase) will contain the levansucrase gene sacB, which acts as a counter selection marker to aid the selection of transposon mutant strains that have lost the plasmid vector. Cells that have acquired the Tn5 transposon will be resistant to the named antibiotic. These cells are then grown in the presence of sucrose in order to obtain Tn transposon mutant cells that have lost the plasmid vector and thus have lost the transposase and levansucrase encoding genes.

Host/vector system

The nature of genetic modification include insertion of ~100 bp random DNA barcode marker into the chromosome (at the dif locus), insertion of a transposon e.g. Tn5 into the chromosome to disrupt gene function (creating a transposon mutant library to be used for Transposon Directed Insertion Sequencing (TraDIS)), and the removal of naturally occurring extended spectrum beta-lactamase (ESBL) encoding plasmids.

DNA barcode markers are to be used to enable the identification of an E. coli strain of interest from the mixed natural population within the animal gut model, by using PCR, qPCR or colony hybridisation (using streptavidin detection) techniques. A ~100 bp random DNA sequence is firstly synthesised using biotinylated nucleotides incorporating desired restriction enzyme sites at each end. This is inserted into the delivery plasmid (pBD62 or derivative thereof) by conventional cloning, resulting in the DNA barcode being flanked by transcription terminator elements (TrrnB from E. coli and TtL3 from coliphage λ). The delivery plasmid (encoding zeocin resistance) is transferred into the E. coli strain of interest by conjugation, and is inserted into the chromosome at the dif locus via the integration element from choleraphage CTX–phi (the XBS site). A Cre recombinase encoding plasmid is then transformed into the zeocin resistance cells to remove the backbone of the delivery vector by recombination at the loxP sites. The final cells therefore contain the DNA barcode (flanked by the two transcriptional terminators to prevent any possible transcriptional read-through from adjacent promoters) and a single loxP site, flanked by dif sites.

Origin & function

The nature of genetic modification include insertion of ~100 bp random DNA barcode marker into the chromosome (at the dif locus), insertion of a transposon e.g. Tn5 into the chromosome to disrupt gene function (creating a transposon mutant library to be used for Transposon Directed Insertion Sequencing (TraDIS)), and the removal of naturally occurring extended spectrum beta-lactamase (ESBL) encoding plasmids.

DNA barcode markers are to be used to enable the identification of an E. coli strain of interest from the mixed natural population within the animal gut model, by using PCR, qPCR or colony hybridisation (using streptavidin detection) techniques. A ~100 bp random DNA sequence is firstly synthesised using biotinylated nucleotides incorporating desired restriction enzyme sites at each end. This is inserted into the delivery plasmid (pBD62 or derivative thereof) by conventional cloning, resulting in the DNA barcode being flanked by transcription terminator elements (TrrnB from E. coli and TtL3 from coliphage λ). The delivery plasmid (encoding zeocin resistance) is transferred into the E. coli strain of interest by conjugation, and is inserted into the chromosome at the dif locus via the integration element from choleraphage CTX–phi (the XBS site). A Cre recombinase encoding plasmid is then transformed into the zeocin resistance cells to remove the backbone of the delivery vector by recombination at the loxP sites. The final cells therefore contain the DNA barcode (flanked by the two transcriptional terminators to prevent any possible transcriptional read-through from adjacent promoters) and a single loxP site, flanked by dif sites.

Evaluation of foreseeable effects

Genetic modifications will not increase infectious potential or the survivability of the bacteria. No genes of known toxins, oncogenic proteins, allergens or modulators of growth or differentiation will have been added to an organism. Any antibiotic resistance genes will not be connected to first or second-line antibiotics used for treatment in humans. No genes of known adhesins, envelope proteins or capsid proteins will have been added to an organism. Bacterial strains in which DNA sequences have been manipulated are unlikely to be more virulent than the wild-type strains, and indeed in most cases will be attenuated. As the GMO is likely to be less virulent than its pathogenic progenitor it can therefore be handled at the ACDP category relevant to the wild-type.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No larger GMOs will be used in this study.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogations applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid contaminated waste is treated by autoclaving for at least 15 minutes at 121°C on a validated cycle resulting in 100% kill.
Post autoclaving: Solid waste is further disposed of into a tiger stripe bag and sent off site for incineration via Grundons Waste Management Company. Liquid waste is disposed of down the sink with copious amounts of water.
This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.
Any spill will be cleaned using liquid disinfection by treating the solution with 1% (weight/ volume) VirkonS for 16hrs at room temperature. The solution is then disposed of to the laboratory drain flushed with copious amounts of water at a maximum volume of 2L per day. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project was reviewed 18th January 2019 and was approved subject to minor amendments

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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<th>Large Scale Activities</th>
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</tr>
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Project Ref 81/21.1

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<td>12/03/2021</td>
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<td>Class 2</td>
<td>&lt; 1 Litre</td>
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The main aim is to investigate the role of extracellular DNA and trace elements on antimicrobial resistance gene transfer by transformation and conjugation. E. coli will be used as a model organism for transformation while C. difficile will be a model organism for monitoring conjugation events.

**Recipient or parental organism**

The recipient organisms include Escherichia coli, Clostridioides difficile, Yersinia species, Klebsiella species, nontyphoidal Salmonella serotypes and other ACDP hazard group 1 and 2 bacteria. Primary experiments will be conducted using E. coli and C. difficile as recipient or parental organisms.

**Host/vector system**

Several standard non-viral vector systems such as pUC18/19, pBR322, pEK499 plasmid and plasmids isolated from E. coli or other Gram-negative bacteria maybe used. The host organism is E. coli.

**Origin & function**

The origin of the GM material is from E. coli or C. difficile strains. The GM material encodes for antimicrobial resistance genes such as rifampicin resistance (rpoB), erythromycin resistance (Erm), ESBL genes, tetracycline resistance (Tn5397) and pathogenicity island (tcdB).

**Evaluation of foreseeable effects**

E. coli transformants will carry antimicrobial resistance genes. Infection with these strains may cause urinary tract infections with a strain that is either rifampicin or cefotaxime resistant. C. difficile transconjugates will carry plasmids from the parental strain encoding for antimicrobial resistance genes. Infection with these strains may cause severe diarrhoea and dehydration due to exposure to C. difficile toxins. It is possible that the mutated DNA could transfer to other bacteria, by recombination or on mobile genetic elements capable of transferring to chromosomal DNA. This could occur within the experiment or the environment. In the event of a breach of containment, the genetically-modified plasmid could be transferred to other bacteria because of its
properties as a mobile genetic element. Transfer is most likely to occur under conditions where there is a high localised density of donors, potential recipient bacteria, and nutrients (like the gut). It is unlikely for genetically modified bacterial DNA to be transferred to others when in low concentrations, there is a lack of nutrient media and without selective pressure (antibiotics/herbicides).

Most of these bacteria are typically found in the gut of healthy individuals; however, some strains e.g. E. coli can become opportunistic pathogens in certain conditions, causing gastrointestinal disease or extra-intestinal infections (e.g. urinary tract infections). Infection by C. difficile may result in exposure to C. difficile toxins causing severe diarrhoea and dehydration. Exposure to toxin A can provoke C. difficile infection symptoms. All strains will only be used in the laboratory under containment conditions with appropriate decontamination procedures. Therefore, the likelihood of environmental contamination is negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| NA |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| NA |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

For solid waste, all surfaces and equipment will be disinfected with ANIGENE HLD4V diluted at 1:100 with a contact time of 2 minutes. Plastics will be placed in a “Bio Bin”, which is sealed in the MSC. Waste will be held in autoclave bags in the laboratory for collection by the Technical Team at an agreed time (maximum of over weekend in lab). Waste will be autoclaved at 121°C for 15 minutes hold time. Autoclaved waste will then be sent for incineration by a licensed contractor via the yellow clinical waste route.

Contaminated liquid within the gut model fermentation vessels and any other liquid waste will be decontaminated by autoclaving at 121°C for 15 minutes on a validated cycle before the liquid waste is disposed of.

In the event of a spillage:

Spills caused as a result of collection and transport of faecal/gut samples will be dealt with using ANIGENE HLD4V diluted at 1:100 with a contact time of 2 minutes. Absorbent materials will be used to mop up the spill. The materials used to mop up the spill will be treated as contaminated waste and placed into autoclave waste stream for treatment. The area of the spill must be wiped with ANIGENE HLD4V diluted at 1:100 with a contact time of 2 minutes to remove any residual contamination. Any equipment that has become contaminated during the spill must be decontaminated using ANIGENE HLD4V diluted at 1:100. All significant spills will be reported on the University electronic accident reporting system.

| Is an emergency plan required according to regulation 20? N |
| If yes, tick to confirm that it is attached to this form N |
| Tick to confirm that you have attached a risk assessment to this form Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment N |
The risk assessment was reviewed on 15/12/2020 and was approved subject to minor amendments.

**Project Containment**

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**Project Ref** 81/21.2

- **Date Ackn'd**: 25/03/2021
- **CU2 Project Title**: Use of genetically modified Hazard Group 2 bacteria to enable monitoring of the bacterial response to cellular & environmental changes as single and mixed cultures
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Consent Granted**: Non-GMM
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

We are using genetically modified (GM) bacteria to enable us to monitor the bacterial response to cellular & environmental changes as single and as mixed cultures. We are running a range of projects where bacteria are exposed to different environmental (cell cultures, temperature, acidity, oxygen) and/or stress (plasma, cold pasteurisation, ultrasounds, biomaterials) conditions and thereafter we are monitoring their growth/survival and/or their metabolic activity. To do that we are using classic microbiological techniques like plate counting or flow.
cytometry and at the same time advanced microscopy techniques (fluorescent or confocal) which enable us to
determine spatially the location and distribution of bacteria. GM strains of different colours will let us generate a better
imaging when we have co-cultures (mixed cultures) of multiple bacteria. We are following this general approach for
bacteria relevant to food safety and wound healing (skin bacteria)
Current projects under this notification are titled:
Project 1 - Biofilm formation on complex 3D food model systems
Project 2 - Understanding the molecular and cellular mechanisms that phosphate glasses and cold plasma regulate to
inhibit bacterial growth.
Project 3 - Behaviour and biofilm formation of Listeria monocytogenes missing stress regulating genes of SigmaB to
various environmental conditions and induced stresses on different food models

Recipient or parental organism

Project 1: All HG2, sourced from ATCC.
P. aeruginosa ATCC 15692-GFP (previously modified)
P. aeruginosa ATCC 10145
GFP-expressing strains are used for visualising bacteria in food-based systems. Both have infection potential.
Project 2: All sourced from ECCAC
HG1 bacteria, reference strains and strains that are resistant to antibiotics:
• Escherichia coli K12_seva631_14F_2RBS_GmR_MSFGFP
• E. coli K12_seva631_14F_2RBS_GmR_EcFbFP
• E. coli K12_seva631_14F_2RBS_GmR_mcherry
• E. coli K12_seva631_14F_2RBS_GmR_CFP
• E. coli K12_seva631_14F_2RBS_GmR_YFP
• E. coli K12_seva631_14F_2RBS_GmR
• E.coil DC10b_pTH100_AmpR_sGFP (sourced from Addgene)
• E.coli DC10b_pRN11_AmpR_mCherry (sourced from Addgene)

HG2 bacteria, reference strains and strains that are resistant to antibiotics:
• Pseudomonas aeruginosa PAO1_seva631_14F_2RBS_GmR_MSFGFP
• P. aeruginosa PAO1_seva631_14F_2RBS_GmR_EcFbFP
• P. aeruginosa PAO1_seva631_14F_2RBS_GmR_mCherry
• P. aeruginosa PAO1_seva631_14F_2RBS_GmR_CFP
• P. aeruginosa PAO1_seva631_14F_2RBS_GmR_YFP
• P. aeruginosa PAO1_seva631_14F_2RBS_GmR
• Enterococcus faecalis OG1RF_pMV158_TetR_GFP (sourced from Academic Centre for Dentistry Amsterdam
(ACTA))
• Staphylococcus aureus NCTC 8325 (sourced from Public Health England)
The HG2 bacteria rarely usually affect healthy people but may cause infections including bacteraemia, endocarditis,
meningitis and infections of bones, eyes, skin, urinary, gastrointestinal and respiratory tract in people with a
compromised immune system.
Project 3: Listeria monocytogenes strains, HG2 (University of Reading)
L. monocytogenes EGD-e
L. monocytogenes 10403S
L. monocytogenes LO28
All strains have similar infectious potential to wildtype strains and can cause listeriosis if ingested.
All have had the sigB gene deletion. SigB affects the response of Listeria to oxidative stress, without this gene the
bacteria are expected to be much more sensitive to the environment and after treatment (e.g. plasma disinfection and sterilisation) it would die faster. The deletion has been conducted to enable greater understanding of the role of SigB in responses to processing techniques.

Project 1:
pUCP18-MCS-gfp-mut3 plasmid, shuttle vector encoding the GFP variant mut3 to be used with P. aeruginosa ATCC 10145. No modifications of P. aeruginosa ATCC 15692-GFP at the University of Surrey. Systems used not known, however the modifications are only GFP labelling.

Project 2:
All bacterial strains have been modified previously at other organisations. No further modifications have been or will be done in the lab. The seva831 and pMV128 plasmids that are inserted in E.coli, P. aeruginosa and E. faecalis respectively, only carry fluorescent proteins (e.g. GFP); therefore, any gene deletion on these plasmids would not revert pathogenicity features to the strains.

Project 3:
All L. monocytogenes strains have been previously modified. Systems used unknown, however the modifications are only GFP labelling. No further modifications to be done at the University of Surrey.

Project 2:
Some survival expected in the environment and the plasmid can mobilise to environmental bacteria. The recombinant plasmid contains an antibiotic resistance gene but since the work will involve small numbers of bacteria generated in cultures up to 50 millilitres in volume, the risk of transfer of antibiotic resistance to environmental bacteria is low.

Risk of infection if ingested or into open wounds is similar to wildtype strains. No expected increased infection potential in the event of exposure in any of the modified strains.

Project 3:
The L. monocytogenes SigB is responsible for stress functions. Its deletion has no safety implications, the mutants have lower stress resistance and lower virulence and are expected to be more sensitive, grow slower and die faster than the wildtype.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid cultures will be treated with either Chemegene (5% v/v) or Virkon (1% w/v) with overnight contact time. Treated culture will be poured to drain with copious amounts of water which flows to foul drain and waste water treatment facility owned by Thames Water. Solid waste is treated by autoclaving for at least 15 minutes at 121°C on a validated waste treatment cycle. Autoclaved waste is dispatched for incineration as Offensive waste (Tiger stipe) by Grundon Waste Management Company.

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tbody>
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Animal Units

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</tbody>
</table>

Please enter comments on the GM safety committee on the risk assessment

All projects were reviewed by the University of Surrey Biological Safety Committee and were approved subject to minor amendments that have subsequently been addressed.
Project 1 - Comments on transport arrangements and disinfection 18/01/2019
Project 2 - Minor clarifications of bacterial strains, inserted genes, and protocols 23/07/2019
Project 3 - Comments relating to the hazards, disinfection, scale of work, sharps and aerosol generation 29/04/2020

Project Containment

<table>
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GENETIC MODIFICATION OF MYCOBACTERIUM AVIUM AND MYCOBACTERIUM

| Class CultureVolClass2 CultureVolumeClass3-4 |
|-----------------------------|-----------------------------|
| Class 3                      |                            |
Date Project Ceased: 18/03/2015

Paratuberculosis

Non-GMM Consent Granted: yes

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Project Containment

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Project Ref 81/97.1

- Date Ackn’d: 17/11/1997
- CU2 Project Title: GENETIC MODIFICATION OF NEISSERIA SPP
- Class: Class 2
- Non-GMM: not applicable
- Consent Granted: not applicable
- Project notified under transitional arrangements: Y
- Withdrawn: N
- Tick if notifying a connected programme of work: N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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**Name**

UCB BIOPHARMA

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

216 BATH ROAD

**District**

**Town**

SLOUGH

**County**

BERKSHIRE

**Postcode**

SL1 4EN

**Country**

ENGLAND

**Tel Number**

01753 534655

**Fax Number**

01753 536632

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential
Bacteriology
Virology
Mycology
Other(s)
Parasitology
Transgenic Animals
Transgenic Invertebrates
Transgenic Fish
Transgenic Plants
Transgenic Birds
Gene Therapy
Microbiology Research

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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**Project Ref** 82/03.1

<table>
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<tr>
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Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: N

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes
Implications of cathepsin S in the pathogenesis of degenerative diseases and the interest in developing inhibitors as potential therapeutics, make this enzyme an attractive target for structural investigations for Celltech R&D Ltd.

Full length human cathepsin S will be obtained by PCR from an image clone #3610589 supplied from Research Genetics (Invitrogen).

Recipient or parental organism

Full length human cathepsin S will be obtained by PCR from an image clone #3610589 supplied from Research Genetics (Invitrogen).

Host/vector system

Initial PCR products will be ligated into TOPO vectors (Invitrogen) and then transformed into E.coli TOP10 cells (Invitrogen). Expression is unlikely at this stage. Culture volumes are small at this stage also (<5mls). Plasmid DNA will be prepared from these and sequenced. Only those cloned with the correct full-length sequences will be pursued. Other cultures will be destroyed by autoclaving at 103KPA for 20 mins. Clones that are shown to contain the correct full-length sequence of human cathepsin S will be selectively removed from the TOPO cloning vector, and ligated into suitable restriction sites within appropriate expression vectors eg pBlueBac 4.5 (Baculoviral expression system, Invitrogen) or pMT/V5-His vectors (Drosophila expression system, Invitrogen).

The baculoviral expression will be effected within suitable insect cell host cell lines eg High Five, SF9 and SF21. (All supplied from Invitrogen).

Material ligated into the pMT vectors will be used to transfect Drosophila S2 insect cells. Stable transfectants will be selected, and these will be used for expression studies. The Drosophila S2 insect cells are supplied from Invitrogen.

Origin & function

Full length human cathepsin S will be obtained by PCR from an image clone #3610589 supplied from Research Genetics (Invitrogen)

The products of the gene will be assessed using western blotting and purified, and then used in a screening programme.

Cathepsin S is a highly active lysosomal cysteine proteinase (E.C. 3.4.22.27). It belongs to the papain super family. It is a single chain enzyme, (M, 24000), with no known glycosylation sites in the mature sequence. It is unique amongst the other known lysosomal cysteine proteinases, in that it is highly stable at neutral and slightly alkaline pH.

Cathepsin S is found mainly in lymph nodes, spleen and macrophages, including rat glial cells. It is absent or expressed only at very low levels in kidney, liver, heart, pancreas, ovary and small intestine. The limited occurrence of this enzyme has prompted speculation concerning a special function for cathepsin S in normal and diseased
tissues. The high concentration of cathepsin S in rat brain glial cells may indicate an involvement of the enzyme in Alzheimer's disease. In addition, the high elastinolytic activity of cathepsin S in lung macrophages at neutral pH suggests a participation in the pathogenesis of emphysema. (Bromme 1996). This elastinolytic activity may play a role in tissue damage associated with inflammation. (Kirschke et al., 1989, Shi et al., 1992). Additionally, newly synthesised MHC class II a & B chains associate with a protein chaperone, the invariant chain (Il), which promotes the proper assembly of MHC class II complexes and their trafficking through cells and prevents untimely loading with peptides. Efficient loading of MHC class II heterodimers with antigenic peptides requires concurrent processing of both the Il chain and endocytosed proteins. Present data indicates that Cathepsin S is involved in the degradation of the Il chain and thereby regulating the convergence of processed antigen and MHC class II dimmers competent for peptide loading. In short, it facilitates antigen presentation in the MHC class II system by degradation of the Il chain (Driessen et al., 1999) and may therefore be a target for attenuation of immune response.

Implications of cathepsin S in the pathogenesis of degenerative diseases and the interest in developing inhibitors as potential therapeutics, makes this enzyme an attractive target for structural investigations.

Previous investigators have succeeded in cloning and expressing significant quantities of human cathepsin S using the baculovirus expression system. (Bromme 1996).


Evaluation of foreseeable effects

Overexpression of Cathepsin S would be unlikely to have a negative impact on the environment. Overexpression is unlikely to increase the ability of the expression hosts to survive outside of the laboratory. The baculoviral hosts (SF9, SF21 and High Five cells) and the S2 Drosophila cells have very stringent physiological requirements to sustain growth (a very narrow temperature range, and highly specific nutrient requirements), which would prevent them from proliferating in the environment. Standard microbiological containment procedures (screw top flasks fitted with 0.2 uM filters and similar filters fitted to exhaust lines for a Biostat insect cell fermenter) would prevent any release of recombinant Drosophila into the laboratory or environment. It is recognised that baculoviral particles will not be contained by such measures, but their highly restrictive host range (ie High five, SF9 and SF21 cells) dictates that this should not pose any particular risk to human health nor to the environment. Thus the risk to both human health and the environment at this stage is negligible.

It is recognised that by applying standard good microbiological practice, that this can contain any recombinant material being produced during the initial growth stages. However it is recognised that the objective of this work is to create a quantity of recombinant biomass, which in turn can be collected in a safe manner. The issue as to why this process requires containment level 2 is detailed below.

Cathepsin S has a proteolytic role in the degradation of the invariant chains, and also an elastinolytic activity resulting in tissue damage and remodelling. Expression of the recombinant full-length material results in the production of an inactive form of the protein. Activation occurs when the pH is reduced to r.r, whereupon the proenzyme is then activated. With these physiological properties in mind, it would be advisable to ensure that good microbiological practice is followed throughout this work, and precautions taken to ensure that any aerosols generated during the fermentation process be suitably contained. Growth of the recombinant GMOs can be contained by applying routine GMP (as detailed above). It is the downstream processing of any recombinant biomass, where sonication is applied to disrupt recombinant biomass, (and where aerosols would be generated) is where it is recognised that containment within a microbiological cabinet is required to prevent inadvertent inhalation of the cathepsin S. This containment can be achieved by sonication within a class 2 microbiological flow cabinet. Exhaust is filtered through HEPA filters prior to being exhausted out of the building via a stack on the roof.

02/03/2022
### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Shake flask fermentations are effected in disposable flasks. After use, contents (ie recombinant biomass) will be recovered by centrifugation and stored in polythene bags in dedicated freezers identified with biohazard warning signs. Centrifugation containers will be sterilised by autoclaving at 103Kpa for 30 minutes, and then sent for hot washing in an industrial dish washing machine. Empty flasks will be sterilised by autoclaving at 103Kpa for 30 minutes and then sent for disposal by incineration. Spent fermentation broth will be collected, and autoclaved in a Rodwell Herald autoclave location in the laboratory. (103Kpa, 30 mins). The presence of viable recombinant baculoviral particles and viable drosophila cells could be assessed by experimentation. (Previous work with recombinant baculovirus and drosophila, involving the expression of containment level 1 products has shown that this process is 100% effective at destroying viable recombinant material.) Sterilised broth will then be run to waste down the drain.

5 litre fermentations runs within the Biostat 2 fermentation vessel will be effected with 0.2uM air filters on both the air inlet and exhaust lines. This is standard practice. This provides for the same levels of protection to human health and the environment as described for the shake-flasks. Following the recovery of recombinant biomass by centrifugation, spent fermentation broth will be decanted back into the fermentation vessel, and the complete unit will be autoclaved at 103Kpa for 1 hr (extended time to accommodate for the increased volumes). Sterilised broth will then be run to waste down the drain. Broth could be tested for viable recombinant baculoviral particles and viable drosophila cells.

All disposables (ie tips, pipettes) used during the process will be soaked in a 1% w/v solution of Vircon prior to collection and subsequent autoclaving at 103KPA for 30 minutes, prior to disposal via incineration. Any spillages will be treated with solid Vircon, and material then soaked up onto paper towels, and subsequently autoclaved at 103Kpa for 30 minutes prior to disposal.

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<tr>
<th>Is an emergency plan required according to regulation 20?</th>
<th>N</th>
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<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
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<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
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**Project Containment**

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02/03/2022
Myelin/oligodendrocyte glycoprotein (MOG) is a candidate autoantigen in multiple sclerosis. Novel approaches that have the potential to interrupt either the processing and presentation or the immune effector response to this antigen may have therapeutic potential in MS, making this pathway an attractive target for Celltech R&D Ltd.

Rat MOG residues 1-125 (extracellular domain) will be obtained from a rat brain cDNA library (Clontech) by PCR.

Initial PCR products will be ligated into TOPO vectors (Invitrogen) and then transformed into E. coli TOP10 cells (Invitrogen). Expression is unlikely at this stage. Culture volumes are small at this stage also (<5mls). Plasmid DNA will be prepared from these and sequenced. Only those clones with the correct full-length sequence will be pursued. Other cultures will be destroyed by autoclaving at 103KPA for 20 mins. Clones that are shown to contain the correct full-length sequence of rat MOG, will be selectively removed from the TOPO cloning vector, and ligated into suitable restriction sites within appropriate expression vectors, e.g., pet, pCal vectors (which are mobilisation defective and utilise the T7 RNA polymerase based expression system). Vectors will then be transferred into the appropriate host cells for large-scale protein production.
Full-length rat MOG will be obtained by PCR from a rat brain cDNA library (Clontech). This will be cloned into a non-expression vector eg TA cloning vectors. After verification of the correct sequence, the gene will be transferred into a relevant mobilisation defective T7 promoter-driven expression vector. Vectors will then be transferred into the appropriate host cells for large-scale protein production eg E. coli BL21 codon plus (DE3) cells. The products of the gene will be assessed using Western blotting and purified, and then used in EAE models.

MOG is a CNS-specific, qualitatively minor myelin component expressed on oligodendrocyte surfaces and the outermost lamellae of the myelin sheaths. 1,2,3. Amongst the many myelin components proposed as specific autoantigens in MS, MOG is the only one able to induce both an inflammatory T-cell and demyelinating antibody response in a range of laboratory animal species 4,5,6,7. In MS patients, elevated autoreactive T-cell and B-cell responses have been demonstrated in blood 8,9 and CSF 10 and the presence of circulating anti-MOG Ab's may predict disease progression 11. Furthermore, post-mortem tissue from MS patients, areas of demyelination are often associated with the deposition of anti-MOG antibodies 12.

Recombinant MOG may be used for a variety of experimental purposes, such as crystallography, the generation of anti-MOG antibodies in vitro, the isolation of MOG-specific CD4+ and CD8+ T-cell lines in vitro, investigation into the MOG processing amd MHC II interaction on CD4+ T-cells, generation of encephalitogenic T-cell lines and as an antigen in animal models such as AEA. In MS patients changes in the circulating MOG-specific T-cells and in anti-MOG antibody titres have been associated with the pathophysiology of the disease and may be useful clinical markers of disease progression.

Previous investigators have succeeded in cloning and expressing significant quantities or rat MOG in an E. coli expression system 2.

Reference list:


Evaluation of foreseeable effects

Overexpression of MOG would be unlikely to have any negative impact on the environment. Over-expression is unlikely to increase the ability of the strain to survive outside of the laboratory. It is therefore estimated that both the likelihood of and the consequence of hazard to the environment is negligible. The overall estimation of risk is therefore assessed as "effectively zero". The scale of the work within the Applikon fermenters would not exceed 4*2 litres. Should all of this material be inadvertently released from the vessel, then the GMO would be contained within an aseptic access room, that has sealed floors. Material could not leach into other rooms or into the environment. Spillages would be treated with solid Vircon, and the material soaked up onto paper towels, and subsequently autoclaved at 103Kpa for 30 mins, prior to disposal.

The recombinant biomass generated will be obtained after centrifugation of the fermentation broth. This biomass will be soted at -20 degrees C, in polythene bottles or bags, depending upon the yield of the recombinant biomass. Spent fermentation broth will be autoclaved at 103Kpa for 60 mins prior to disposal down the drain.

Recombinant biomass will be resuspended in a suitable extraction buffer, and disrupted by sonication. The sonicator will be located in a class 2 microbiological flow cabinet. Sonication will only be effected when the flow cabinet is operating to ensure that any aerosols generated, do not vent into the general microbiology laboratory. Exhaust is filtered through HEPA filters prior to being exhausted out of the building via a stack on the roof. The cabinet is maintained by the manufacturers (Envair), and is serviced annually.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Shake flask fermentations are effected in reusable pyrex flasks, with single use foam bungs at the enclosures. After use, contents (ie recombinant biomass) will be recovered by centrifugation and stored in polythene bags in dedicated freezers identified with biohazard warning signs. Spent fermentation broth will be collected, and autoclaved in a Rodwell Herald autoclave located in the laboratory. (103Kpa, 30 mins). Sterilised broth will then be run to waste down the drain. Centrifugation containers will be sterilised by autoclaving at 103Kpa for 30 mins, and then sent for hot washing in an industrial glass washing machine. Empty flasks will be sterilised by autoclaving at 103Kpa for 30 minutes and then cleaned within an industrial glasswash machine.

4*2 litre fermentation runs within the Applikon fermentation vessels will be effected with 0.2 M air filters on both the air inlet and exhaust lines. This is standard practice. This provides for the same levels of protection to human health and the environment as described for the shake - flasks. Following the recovery of recombinant biomass by centrifugation, spent fermentation broth will be decanted back into the fermentation vessels, and the units will be autoclaved at 103Kpa for 1 hr. Sterilised broth will then be
run to waste down the drain. Broth could be tested for viable recombinant bacterial cells if necessary.

All disposables (ie tips, pipettes) used during the process will be soaked in a 1% w/v solution of Vircon prior to collection and subsequent autoclaving at 103Kpa for 30 minutes, prior to disposal via incineration. Any spillages will be treated with solid Vircon, and material then soaked up onto paper towels, and subsequently autoclaved at 103Kpa for 30 minutes prior to disposal.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project was presented to members of the Celltech R&D Ltd Genetic Modification Advisory Committee & Biological Safety Committee on the 22/5/2003, at a meeting convened at the request of the proposer. The biological safety officer (Dr Alastair Durrant) noted that there was a requirement for containment level 2 for processing of the recombinant biomass as a consequence of the biological activity of MOG. Advice was sought by AD from both the head of the department where the work is intended to take place and also from the site R&D director, with respect to the biological activities of the MOG, prior to AD instigating the notification process to the HSE.

Project Containment

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Animal Units
Large Scale Activities
Human Clinical Applications

Project Ref 82/05.1

Date Ackn'd CU2 Project Title
28/01/2005 CLONING, EXPRESSION AND PURIFICATION OF RAT AND HUMAN SV2 ISOFORMS

Date Project Ceased
31/07/2008

Class CultureVol Class2 CultureVolume Class3-4
Non-GMM Consent Granted
Class 2 < 1 Litre Not Applicable

Project notified under transitional arrangements N
One of the major protein constituents of the synaptic vesicle is the family of proteins SV2. They are integral glycosylated membrane proteins with an approximate size of 90Kd and are present on all synaptic vesicles, such as calcium or ATP. We have identified SV2A as the binding site of the anti-epileptic drug, Keppra. This study is intended to characterise the molecular actions of SV2 proteins and to ascertain whether the antiseizure potency of Keppra is as a consequence of modulating the function of SV2A.

PRC products (relating to SV2A, SV2B and SV2C) will be recovered from mammalian brain specific total RNA (Ambion) using specific primers.

PCR products will be typically cloned into TA or TOPO TA cloning vectors and used to transform One Shot E.coli cells. Expression of any recombinant product at this stage is very unlikely.

In addition to the above described work with native SV2 variants, mutant versions of the gene, comprised either of chimeras between the different isoforms (EG SV2A/C chimeras) or site specific mutants of the different isoforms, where individual residues are replaced, will be cloned and expressed. Recombinant genetic material at this stage will be sequenced and only when it is shown to be the correct sequence will it then be moved into an expression vector with an aim of placing it deliberately in frame with a promoter. Typical expression vectors (which are mobilisation defective) would be from the pET, pCal-S, pGEX4T2, pLV1393, pFastBac-Mam, pLentiPst, PIE, pIND, pIRES, pD40 expression series. These will then be transferred into the appropriate host cells.

Examples of expression hosts include; SF9 & T.ni (Baculoviral expression), E.coli BL21 (DE3), BL21 (DE3) pLysS, Origami (Bacterial expression), CHO, PC12, HEK293, COS-7 (Mammalian cell line expression).

PCR products recovered from mammalian brain specific total RNA (Ambion) using specific primers will be cloned initially into PCR Cloning vectors hosted by disabled expression hosts. After verification of the correct sequence, the gene will be transferred into the relevant expression vector and then used to transform or transfect host cells.

The products of the gene will be assessed using western blotting and purified, and then used in a screening programme. Defined mutants of the SV2 gene would also be cloned and expressed.

One of the major protein constituents of the synaptic vesicle is the family of proteins SV2. They are integral glycosylated membrane proteins with an approximate size of 90Kd and are present on all synaptic vesicles. The molecular action of SV2 proteins is unknown. The SV2s are twelve transmembrane proteins with a significant homology to the major facilitator superfamily (MFS) of transporters found in both bacteria and eukaryotes. Given their universal presence in synaptic vesicle, it has been proposed that the SV2s might transport a common constituent of the vesicles, such as calcium or ATP. The small gene family consists of three isoforms, designated SV2A, SV2B, and SV2C. SV2A is the most widely distributed isoform, being nearly ubiquitous in the central nervous system as well as being present in endocrine cells. SV2B is brain specific, with a wide but not ubiquitous distribution, and SV2C is even less prevalent with a very restricted localization in old brain regions like the substantia nigra and...
striatum, the midbrain, the brainstem and the olfactory bulb. SV2C was undetectable in the cerebral cortex and the hippocampus, and found at low levels in the cerebellar cortex. SV2A -/- and SV2A/B -/- knockout (KO) mice have been reported and exhibit a severe seizure phenotype, while the SV2B -/- Kos are reported to have no phenotype. No SV2C KO have been reported so far (but are being prepared by UCB). KO studies indicate that SV2A plays an important role in modulating synaptic vesicle fusion. We have identified SV2A as the binding site of LEV (Keppra) and analogues in the brain while SV2B does not recognise LBS ligands. The observed correlation between ligand binding affinity to SV2A and anti-seizure potency suggests that LEV (Keppra) and analogues might act by altering this modulatory function of SV2A.

Lynch et al., PNAS June 29 2004 101 (26) pp9861-9866

Evaluation of foreseeable effects

SV2 proteins levels are not altered in association with any pathological conditions. They are believed to play a role in regulation of exocytosis of synaptic vesicles in the CNS. The anti-epileptic drug, Keppra that target SV2 has shown a good toxiological profile in animal models and in clinical trials. Given that this activity is common to vertebrates, it is highly unlikely that overexpression of the individual native or mutant recombinant forms would be expected to have any negative impact on the environment. Overexpression is unlikely to increase the ability of the strain to survive outside of the laboratory. It is therefore estimated that both the likelihood of and the consequence of hazard to the environment is negligible. The overall estimation of risk is therefore assessed as "effectively zero".

Use of COS-7 cell lines necessitates containment level 2. The scale of the growth of these cells will be restricted to tissue culture flasks. In the event of a spillage of cells (transfer from class 2 cabinet to incubator within the same laboratory), then the scale of spillage would be minimal (<1 litre). The spillage would be treated with solid Vircon, and the material then soaked up onto paper towels, autoclaved at 103Kpa for 30 minutes and then disposed of. The laboratory has restricted access, and has sealed floors. Material could not leach into other rooms or into the environment. Exhaust from the class 2 cabinet is HEPA filtered. Spent media (after separating the biomass by centrifugation) will be treated with Vircon, prior to disposal down the drain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For work not involving COS-7 cells, good microbial practice will be followed throughout this work. All waste generated will be autoclaved prior to disposal. For work involving COS-7 cells the following procedures will be deployed. The cells will be passaged and maintained in a tissue culture laboratory within a class 2 microbiological flow cabinet dedicated for the use of COS-7 cells. Exhaust from this unit is HEPA filtered. The unit is maintained by the manufacturers (Envair) and is serviced annually. Face velocity testing and containment efficiency certificates are up to date and indicate maximal performance with respect to user protection. Transfection of the cells will occur in the class 2 hood. Cells will be maintained in sealed tissue culture vessels, and incubated in a dedicated incubator. Cell harvesting will be carried out 24-48hrs-post transfection. The culture vessels will be transferred to the class 2 cabinet, and only then will the lids be opened and the cells then scrapped from the culture vessel surface using disposable scrapers. Dislodged cells and media will then be decanted into sealable centrifuge containers and the cells pelleted by centrifugation. The containers will be returned to the class 2 cabinet. The media will be decanted directly into 1%w/v Vircon. Cell pellets will then be transferred to a -80 degrees C freezer. This process of freezing effectively kills the cells. After this stage, there is no more potential hazard from viable COS-7 cells. Subsequent work will then be carried using good microbial practices. All waste generated (irrespective of required containment level), will be autoclaving at 103Kpa for 30 minutes prior to disposal. All disposables (ie tips, pipettes) used during the process will be soaked in a 1% w/v solution of Vircon prior to collection and subsequent autoclaving 103KPA for 30 minutes, prior to disposal via incineration. Any spillages will be treated with solid Vircon, and material then soaked up onto paper towels, and subsequently autoclaved at 103Kpa for 30 minutes via to disposal.
This project was presented to members of the Celltech R&D Ltd GMSC on 13/1/2005, at a meeting convened at the request of the site HS&E Manager and BSO. It was noted that there was a requirement for containment level 2 for work involving COS-7 cell lines. Alternative expression systems were considered, however the principal scientist for the work noted that previous experiences of the expression of these proteins in the USA has shown that COS-7 cells were the most successful. Therefore, although all other aspects of the project would require class 1 containment, the use of COS-7 cells necessitate a containment level 2 and thus the project needs to be documented and treated accordingly.

**Project Containment**

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**Project Ref 82/07.1**

- **Date Ackn’d**: 15/06/2007
- **CU2 Project Title**: Use of the ViraPower Lentiviral Expression system to produce recombinant proteins and stably transduced cell lines.
- **Class**: Class 2
- **CultureVol**: ≤ 1 Litre
- **Non-GMM**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: No
- **Tick if notifying a connected programme of work**: Yes

**Historical Significant Changes**

**Historical Date of Additional Info**
Project Additional Information

**Purposes of the contained use**

To produce stable and high level expression of transgenes in mammalian cell lines and primary cells for use in the study of disease mechanisms in the areas of cancer, autoimmune diseases, inflammation and CNS. The transduced cells will be used directly in vitro for biological assays or to produce recombinant proteins. Recombinant proteins may be purified and used in procedures like ELISA assays. Stable transduced cell lines or preparations of (e.g. membranes) may be used as antigens for the immunization of laboratory animals.

**Recipient or parental organism**

Well characterised mammalian cell lines e.g. U937, HeLa, which will be transfected with the recombinant virus and used to produce stable cell lines which may then be used to immunise animals.

**Host/vector system**

The Lentiviral system is a third generation retroviral expression system which is commercially available from Invitrogen with enhanced biosafety features. This is achieved by several mechanisms including (1) reduction in the number of genes from HIV-1 (now limited to three; gag, pol and rev) (2) structural genes and other components are separated onto 4 different plasmids (3) no regions of homology between 4 plasmids to prevent recombination (4) replication incompetent virus (5) VSV-G gene used in place of HIV-1 envelope. [continued on page 10]

(6) pLenti expression vector contains deletion in 3'LTR which renders it self-inactivating after transduction of the target cell (7) no HIV structural genes present in the packaged viral genome and hence cannot be expressed in the target cell (8) constitutive promoter place upstream of 5'LTR in pLenti expression hence tat gene no longer required for production of viral RNA. These biosafety features mean that the viruses can infect cells but are unable to replicate once they have done so.

Well characterised packaging cell lines e.g. HEK293 and HEK293T will be used for the for the production of the recombinant virus containing the GOI's. Recombinant virus is liberated into the cell culture medium generating a high titre stock of replication incompetent virus.

**Origin & function**

The genes of interest which will be delivered to target cells using the lentiviral systems are of 3 types. Firstly genes that are involved in infectious disease, autoimmune diseases and CNS diseases. Secondly genes associated with cancer and thirdly genes encoding antibodies or fragments thereof. Apart from the genes above, proteins that are commonly used as reporter genes and genes used for the identification and selection of transfected cells (i.e. fluorescent...
proteins, beta-galactosidase, Luciferase or antibiotic resistance genes) will be used in this system. Genes that will be excluded from this expression system are genes encoding (a) proteins of retroviral origin and (b) toxins.

**Evaluation of foreseeable effects**

During the virus production steps using the packaging cell lines (HEK293 and HEK293FT) there is a potential for exposure of operators to virus. This could only occur through inoculation with sharps, contact with skin which is cut or scratched or by aerosol formation. Sharps will not be required for these procedures and standard biosafety guidelines followed i.e. all cuts will be covered with a waterproof dressing and gloves worn at all times. To prevent exposure by aerosol formation or splashing a class II MHC will be used for all procedures where this could arise.

The VSVg coated particles will be used to stably transduce mammalian cells. Transduction leads to integration of the transgene into the host cell DNA. It is not expected that the inserted sequences will alter the inherent properties of the host cells such that there will be an increase in the hazards associated with the host.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**Not applicable**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Not applicable**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Plastic disposable pipettes and pipette tips are soaked in a 1% w/v Virkon solution for at least 12 hours prior to autoclaving as described for other solid waste described below.

Solid Waste is collected in biohazard bins double-lined with autoclave bags. After completion of work the double-lined bags are removed and loosely taped with autolcave tape. These are placed into metal containers and then transported to the waste autoclave facility using a trolley. The waste is autoclaved using the plastic discard cycle which is 103Kpa for 30 mins. The autoclave used for waste disposal is serviced, calibrated and validated every 6 months by thermocouple. At the end of the autoclave cycle the paperwork is checked to ensure that the cycle has "passed" and the parameters have been met. The processed waste is then disposed of as non-hazardous waste.

Liquid waste is decontaminated in culture vessels or robust plastic sealed containers by the addition of Virkon to give 1% w/v solution and left for 12 hours. Once decontaminated the liquid waste is disposed of down the drains with copious amounts of cold water.

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

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Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Although the lentiviral system is well characterised and has a number of safety features the committee felt that the ability to test for replication competent virus should be evaluated. The p24 ELISA will be used to test periodically for replication competent particles.

The committee agreed that although containment level 1 would be sufficient for most of the work with the lentiviral vectors, containment level 2 was required for the expression of tumour associated genes.

All the lentiviral work will be carried out in a designated containment level 2 or animal containment level 2 laboratory.

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<td>L2 Yes</td>
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</table>

**Project Ref** 82/19.1

- **Date Ackn’d**: 13/02/2019
- **CU2 Project Title**: Use of adeno-associated viral (AAV) vector systems to transduce primary cells/cell lines for research purposes
- **Class**: Class 2
- **CultureVol**: < 1 Litre
- **Consent Granted**

**Historical Significant Changes**

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Project Additional Information**

02/03/2022
Purposes of the contained use

To enable transient expression of transgenes in mammalian cell lines and primary cells for use in the study of disease mechanisms in the areas of cancer, autoimmune diseases, inflammation and CNS. These transgenes include gene cassettes that code for siRNA/shRNA or for overexpression of wild-type or mutated forms of proteins, or for introducing genetic components that facilitate genome editing (eg. CRISPR/Cas9). Premade viral particles will be purchased from Contract Research Organisations (CROs) such as Sirion Biotech / Sigma / Horizon Discovery.

The nature of these studies is to conduct biological research experiments into the genetic functions associated with our drug targets. The benefit of using viral based vectors is that genes can be delivered into more primary cell/tissue cultures that are often very difficult to transfect via standard means. The transduced cells will be used directly in vitro for biological assays or to produce recombinant proteins. Recombinant proteins may be purified and used in procedures like ELISA assays. Transduced cells or preparations of (e.g. membranes) may be used as antigens for the immunization of laboratory animals.

Recipient or parental organism

Well characterised mammalian cell lines e.g. U937, HeLa, or primary cells ego monocytes, T cells, which will be transduced with the recombinant virus in order to overexpress transgenes of interest, which will then be used in downstream standard biological assays ego mechanism based studies on signal transduction pathways.

Host/vector system

The AAV system can be used as a vector to transduce cells resulting in transient, quantitative gene expression or knockdown, and are very efficient for infecting non-dividing terminally differentiated cells ego macrophages. Current vectors contain enhanced biosafety features: they have low immunogenicity, low integration events - episomal dsDNA genome, AAV belong to family Parvoviridae, no known link to any human illness AAV are defective, require co-infection with helper virus (eg. Herpes simplex virus) Most AAV-based vector systems to date are typically ‘gutless’ AAV-2 systems consisting of a plasmid containing the foreign DNA to be transduced into the cell flanked by AAV ITR sequences

Origin & function

The genes of interest which will be delivered to target cells using AAV vectors will include the following types:

1. genes that are involved in infectious disease, autoimmune / inflammatory diseases, CNS diseases and cancer.
2. genes encoding antibodies or fragments thereof.
3. commonly used reporter genes and genes used for the identification and selection of transfected cells (i.e. fluorescent proteins, beta-galactosidase, Luciferase or antibiotic resistance genes) will be used in this system.
4. Commonly used genes used for genome editing ego Cas9 and its variants.
5. Small gene cassettes designed to express RNA ego siRNA, shRNA and guide RNA (used in conjunction with Cas9 for genome editing).

Genes that will be excluded from this expression system are genes encoding (a) proteins of retroviral origin and (b) toxins.

Evaluation of foreseeable effects

AAV belong to family Parvoviridae, no known link to any human illness, and they are essentially defective, requiring co-infection with helper virus (eg. Herpes simplex virus). 80% of individuals are already seropositive for AAV serotype
2, with no symptoms and long lasting immunity. Wildtype AAV's are not categorised by ACDP and therefore containment level 1 is usually sufficient. The main hazards arising from AAV vectors are due to properties of any inserted genetic material and therefore necessitate additional containment measures. All work will be conducted in containment level 2 laboratories following Standard Operating Procedures. We will not be generating AAVs in house (they will be purchased from external CROs) due to the requirement for helper viruses (Herpes). There is a potential for exposure of operators to virus. This could only occur through inoculation with sharps, contact with skin which is cut or scratched or by aerosol formation. Sharps will not be required for these procedures and standard biosafety guidelines followed i.e. all cuts will be covered with a waterproof dressing and gloves worn at all times. Aerosol formation can occur during techniques such as pipetting, vortexing, centrifugation. To prevent exposure by aerosol formation or splashing a class II MHC will be used for all procedures where this could arise. Vortexing or centrifugation will be conducted in sealed and capped tubes / rotors. Spills will be dealt with according to standard procedures as detailed in the accompanying Risk Assessment. Cells will be fixed using formaldehyde before removal of material from designated room, eg Flow cytometry, FACs. For live material that is to be removed from the designated virus room, the material will be assessed for the presence of infectious AAV (using rtPCR or ELISA). People who are pregnant or immunocompromised may be more susceptible to AAV infection and associated complications. High risk individuals will be advised not to work with AAV. Ongoing training of Staff and maximised use of PPE will be conducted. Only personnel with a professional qualification, and/or experience working with viruses along with company training may work in this room. Personnel will be overseen by the lab supervisor until deemed competent.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| Not applicable |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| Not applicable |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Plastic disposable pipettes and pipette tips are decontaminated in a 1 % w/v Virkon solution for at least 20 mins prior to placement into hard yellow bins. Solid Waste is also collected in hard yellow bins, and after the work has been conducted - sealed and sent for incineration using a registered waste courier. Liquid waste is decontaminated in culture vessels or robust plastic sealed containers by the addition of Virkon to give 1 % w/v solution and left for 20 min. Once decontaminated the liquid waste is disposed of down the drains with copious amounts of cold water |

### Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Although AA V systems are well characterised and have a number of safety features, the UCB Hazardous Substances Team felt that the ability to test for replication competent virus should be evaluated. This can be achieved by infecting a permissive non-complementing cell line (e.g., HeLa) and look for signs of productive replication (cytopathic effect, plaque formation). Alternatively, PCR for AAV-specific sequences (e.g., virus L TR motifs) in cell culture samples will also periodically be conducted to quantify presence of AAV. Ongoing training of staff and suitable and adequate use of PPE will be conducted. The UCB Hazardous Substances Team agreed that although containment level 1 would be sufficient for most of the work with the AAV vectors, containment level 2 was recommended to safe guard unknown safety aspects associated with the use of different human genes (or reporter genes) overexpressed by the vectors.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<td>L4</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
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Project Ref 82/19.2

Date Ackn'd: 13/02/2019

CU2 Project Title: Use of Adenovirus vivector systems to transduce primary cells/cell lines for research purposes.

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
Project Additional Information

Purposes of the contained use

To enable transient expression of transgenes in mammalian cell lines and primary cells for use in the study of disease mechanisms in the areas of cancer, autoimmune diseases, inflammation and CNS. These transgenes include gene cassettes that code for siRNA/shRNA or for overexpression of wild-type or mutated forms of proteins, or for introducing genetic components that facilitate genome editing (e.g., CRISPR/Cas9). Premade viral particles will be purchased from CROs such as Sirion Biotech, Sigma, Horizon Discovery.

The nature of these studies is to conduct biological research experiments into the genetic functions associated with our drug targets. The benefit of using viral based vectors is that genes can be delivered into more primary cell/tissue cultures that are often very difficult to transfect via standard means. The transduced cells will be used directly in vitro for biological assays or to produce recombinant proteins. Recombinant proteins may be purified and used in procedures like ELISA assays. Transduced cells or preparations of (e.g. membranes) may be used as antigens for the immunization of laboratory animals.

Recipient or parental organism

Well characterised mammalian cell lines e.g. U937, HeLa, or primary cells e.g. monocytes, T cells, which will be transduced with the recombinant virus in order to overexpress transgenes of interest, which will then be used in downstream standard biological assays e.g. mechanism based studies on signal transduction pathways.

Host/vector system

The Adenoviral system can be used as a vector to transduce cells resulting in transient, quantitative gene expression or knockdown, and are very efficient for infecting non-dividing terminally differentiated cells e.g. macrophages. Current vectors contain enhanced biosafety features: they are replication deficient, disabled vectors (in cells that do not express viral E1/E3 proteins) - and are referred to as third generation 'gutless' vectors. Adenovirus serotype Ad5 based vectors (7.5kb capacity) will be purchased from external CROs e.g. Sirion Biotech.

Origin & function

The genes of interest which will be delivered to target cells using Adenoviral vectors will include the following types:
1. genes that are involved in infectious disease, autoimmune / inflammatory diseases, CNS diseases and cancer.
2. genes encoding antibodies or fragments thereof will also be delivered to target cells.
3. commonly used reporter genes and genes used for the identification and selection of transfected cells (e.g. fluorescent proteins, beta-galactosidase, Luciferase or antibiotic resistance genes) will be used in this system.
4. Commonly used genes used for genome editing e.g. Cas9 and its variants.
5. Small gene cassettes designed to express RNA e.g. siRNA, shRNA and guide RNA (used in conjunction with Cas9 for genome editing).

Genes that will be excluded from this expression system are genes encoding (a) proteins of retroviral origin and (b) toxins.

Evaluation of foreseeable effects

Wildtype Adenovirus is transmitted by inhalation, contact with mucus membranes (eyes, nose and mouth), fecal-oral
Transmission and waterborne transmission. However commercially available Adenovirus vectors are not wildtype, and have been engineered to contain multiple safety features (as discussed in section 7). People who are pregnant or immunocompromised are susceptible to adenovirus infection and associated complications. High risk individuals will be advised not to work with Adenovirus. There is a potential for exposure of operators to virus. This could only occur through inoculation with sharps, contact with skin which is cut or scratched or by aerosol formation. Sharps will not be required for these procedures and standard biosafety guidelines followed i.e. all cuts will be covered with a waterproof dressing and gloves worn at all times. To prevent exposure by aerosol formation or splashing a class II MHC will be used for all procedures where this could arise. Vortexing or centrifugation will be conducted in sealed and capped tubes / rotors. Spills will be dealt with according to standard procedures as detailed in the accompanying Risk Assessment. Although adenovirus is low risk GM activity (class 1), and technically could be handled at Containment level 1, due to risk associated with inserts used all work will be conducted in containment level 2 laboratories following SOPs. Cells will be fixed using formaldehyde before removal of material from designated room, e.g. Flow cytometry, FACs. For live material that is to be removed from the designated virus room, replication competency can be first assessed by infecting a permissive non-complementing cell line (e.g. HeLa) and look for signs of productive replication (cytopathic effect, plaque formation). Or PCR for E1 sequences in cell culture samples to confirm lack of replication competence. Ongoing training of Staff and maximised use of PPE will be conducted. Only personal with a professional qualification, and/or experience working with viruses along with company training may work in this room. Personal will be overseen by the lab supervisor until deemed competent.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Plastic disposable pipettes and pipette tips are decontaminated in a 1 % w/v Virkon solution for at least 20 mins prior to placement into hard yellow bins. Solid Waste is also collected in hard yellow bins, and after the work has been conducted - sealed and sent for incineration using a registered waste courier. Liquid waste is decontaminated in culture vessels or robust plastic sealed containers by the addition of Virkon to give 1 % w/v solution and left for 20 min. Once decontaminated the liquid waste is disposed of down the drains with copious amounts of cold water.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Although Adenovirus systems are well characterised and have a number of safety features the UCB Hazardous Substances Team felt that the ability to test for replication competent virus should be evaluated. This can be achieved by exposing a permissive non-complementing cell line (e.g., HeLa) and look for signs of productive replication (cytopathic effect, plaque formation). PCR for E1 sequences in cell culture samples will also periodically be conducted to confirm lack of replication competence. Ongoing training of Staff and maximised use of PPE will be conducted. The UCB Hazardous Substances Team agreed that although containment level 1 would be sufficient for most of the work with the adenoviral vectors, containment level 2 was recommended to safeguard unknown safety aspects associated with the use of different human genes (or reporter genes) overexpressed by the vectors.

Project Containment

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<thead>
<tr>
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<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L3 L4</td>
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<td>Animal Units</td>
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<td>L2</td>
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Project Ref 82/96.1

Date Ackn'd 13/02/1996
CU2 Project Title INFECTION OF T CELLS USING RECOMBINANT ADENOVIRUS (960131)

Date Project Ceased 31/07/2008

Class 2
Consent Granted not applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
**UNIVERSITY OF PORTSMOUTH**

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<th>Name</th>
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<td>023 9284 8484</td>
<td>02392 842093</td>
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Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Y
Composition:
- Chairperson
- Biological Safety Officer (BSO) acting as vice-chair in the absence of the chair
- Secretary
- The heads of school/department that undertake GM work
- One member of technical staff with GM experience by nomination of the faculty manager.
- Manager of the bio-resources unit
- One senior researcher with GM experience from each school that undertakes GM work, nominated by the respective Head of School
- Representatives from organisations affected by the GM activities conducted within the GM Centre
- The Principal Investigator or a representative from the project group intending to carry out a particular GM activity can be invited
- Representative with relevant experience by invitation
- University Health & Safety Manager

Operation:
- The committee will receive for scrutiny and review all risk assessments for GM projects.
- The committee will advise on laboratory procedures and safe systems of work.
- Safety inspections of laboratory facilities will be used to monitor the performance of the ‘The Genetically Modified Organisms (Contained Use) Regulations’.

Outcomes of the inspections will be reviewed.
- The committee will meet termly. There will be at least 3 meetings per year.

The Chair is co-opted onto the University Health and Safety committee. Minutes from the GMSC meetings are forwarded to this committee.

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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2002/03/2022
Volumes of GM bacterial (E. coli) culture range from 1 ml up to a maximum volume of 8 litre (as 12x 500ml flasks) in shaking orbital incubators. Primary and secondary cell cultures are grown in culture flasks, orbital shakers or roller bottles (normally no more than 250ml cultures). Cells are killed using virkon tablets. Plastics and reusable glassware are soaked in virkon for 24 hours and then autoclaved. Waste from all liquid cultures are either autoclaved with a cycle at 121 degC for 30 min. prior to disposal as conventional liquid waste, or treated with disinfectant powder (virkon) and then poured down a sink with dilution. Solid waste is autoclaved and then mixed with conventional waste on normal skip disposal using our contractors, Biffa. Each run is logged. Liquid spills are treated with disinfectant powder (Virkon or Haz-tab granules) or solution (Virkon or BioCleanse) according to standard procedures and contaminated waste then disposed as above. Leaks within centrifuges are decontaminated with 70% ethanol.

All autoclaves are checked for performance (including temp calibration) and safety on an annual basis. The autoclaves are also pressure checked, annually under the Pressure Regulations (and for insurance purposes). Maintenance logs are present. Continuous monitoring of temperature is possible during a cycle. The autoclaves are checked periodically (monthly) for temperature maintenance. There are plans for an in-load temperature probe to monitor the temperature, in addition to the chamber probe. Routinely, waste is checked through the use of autoclave tape to confirm the contents have indeed been autoclaved.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
All class 1 GM work will be conducted in category 1 laboratories in accordance with the general COSHH measures to control exposure to biological agents:

1) Display suitable and sufficient warning signs, including the biohazard sign.
2) Put in place appropriate decontamination and disinfection procedures.
3) Put in place the means for the safe collection, storage and disposal of contaminated waste. This includes the use of secure and identifiable containers after treatment if appropriate.
4) Test, where it is necessary and technically possible, for the presence of biological agents outside primary physical containment.
5) Set out the procedures for working with (and on-site transport of) biological agents or material that could contain them.
6) Where appropriate, make effective vaccines available to employees who are not already immune.
7) Put in place good occupational hygiene measures including the provision of appropriate and adequate washing and toilet facilities. Where appropriate, eating, drinking or smoking is prohibited in any workplace where there is a risk of contamination with biological agents.

and in accordance with GMM Level 1:

8) Surfaces will be impervious to water and resistant to acid, alkalis, solvent, disinfectants, decontamination agents and easy to clean. Hand washing facilities with taps that can be operated without being touched by hand and a supply of disinfectant soap (required for bench).
9) An autoclave will be on site.
10) Suitable protective clothing including eye protection will be required.
11) Efficient control of vectors that could disseminate disease or GMMs, e.g. rodents and insects is required where and to extent the risk assessment shows it is required.
12) Specified disinfection procedures will be in place. Effective disinfectants should be available for immediate use in the event of a spillage, required where and to extent the risk assessment shows it is required.
13) Inactivation of GMMs in contaminated material is required by validated means.
14) An observation window, or alternative, is to be present so that occupants can be seen is required where and to extent the risk assessment shows it is required.
15) Safe storage of biological agents and GMMs. Must be clearly labelled and have compliant biohazard sign is required where and to extent the risk assessment shows it is required.
GM Centre Number: 85

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</table>

Name

UNIVERSITY OF BATH

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

CLAVERTON DOWN

District

Town

BATH

County

AVON

Postcode

BA2 7AY

Country

ENGLAND

Tel Number 01225 826 826

Fax Number 01225 826 559

E-mail

HSE Division

WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

<table>
<thead>
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<th>Date Premises Closed</th>
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<th>Building</th>
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<th>Town</th>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 18/07.1

Date Ackn'd 10/04/2012
Date Project Ceased

CU2 Project Title Infection and immunity of the Bordetellae

Class 2
Culture Volume
Class 2 1-50 Litres

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N
This project is an investigation into the molecular basis for Bordetella infection and immunity. It attempts to elucidate the processes by which these bacteria colonise the host tissues, multiply within these tissues, cause pathology, transmit to other hosts and how the host immune response reacts to the bacteria. In particular, it studies the genetic and cellular basis for these processes. Data arising from this project will obviously expand understanding of the infection biology of these bacteria. However the Bordetella are models for numerous processes that are of general interest to the infection and immunity field, including control of gene expression, interactions with ciliated respiratory epithelia, mucosal immunity in the respiratory tract, host-adaptation and the evolution of virulence.

This project involves studies at the cellular level using whole bacteria and at the molecular level using purified bacterial components (proteins, nucleic acids, carbohydrates, lipids and glycolipids).

<table>
<thead>
<tr>
<th>Recipient or parental organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory strains of Escherichia coli. These strains are used for plasmid maintenance and plasmid amplification and recombinant protein expression. They are non-infectious due to stable mutations. These strains are well characterised, and used extensively world wide for these purposes.</td>
</tr>
<tr>
<td>Species of Bordetella B.pertussis and B.parapertussis are pathogens of the human respiratory tract and the causative agents of whooping cough. Infants who have yet to receive the full schedule of pertussis vaccinations are susceptible to whooping cough. Adolescents and adults for whom vaccine or infection induced immunity has waned are susceptible to chronic cough and are sources of infection of susceptible infants. Pertussis is naturally endemic in the human population. Little is known about the contribution of B.parapertussis to the burden of whooping cough disease. Thus, laboratory workers are at risk from infection but the morbidity from such infection is very low. Appropriate control measures to prevent exposure should be used (see below). B.bronchiseptica infects a very wide range of mammals but is described as a pathogen of just a few (cats, dogs, pigs), causing a relatively mild respiratory tract infection. Human infections are rare and almost always associated with immuno-compromised people or close contact with infected animals. It is not considered a risk to healthy humans. It is a DEFRA controlled organism. B.avium infects mainly turkeys and wild fowl to cause rhinotracheitis. There are no reports of avium infection in humans or mammals. The other bordetellae cause sporadic disease in humans including respiratory disease and septicaemia. Risk factors for infection by these organisms are unknown, as is their true pathogenic potential. Although infections by these bacteria are rare, appropriate control measures are sensible. Workers may spread the bacteria, particularly B.avium and B.bronchiseptica, to other susceptible animals if they are carried out of the lab on clothing or person. All workers will be made explicitly aware of the need to maintain rigorous standards of occupational hygiene to minimize the risk of this. All workers will be required to sign that they understand this risk and the control measures required to prevent it on personal safety training/risk assessment records. Importantly, horizontal gene transfer among the bordetellae is rare. Infact, B.pertussis and B.parapertussis evolved through genome reduction. Thus, they are low risk in terms of transfer of genetic material to other bacteria.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUC based plasmids, pBluescript plasmids, pEX100T, pSS2141, pET vectors, pBBR1 series vectors, pprobe series vectors.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bordetella DNA will be cloned, maintained and manipulated in lab strains of E.coli. The main purpose for this is to introduce defined mutations into the DNA and then to</td>
</tr>
</tbody>
</table>
move the mutate loci back into Bordetella to generate allelic replacement mutants. Mostly, mutations will be constructed by inserting an antibiotic resistance cassette into the Bordetella locus.

The creation of defined bacterial mutants via allelic exchange mutagenesis is a powerful technique for elucidating gene function, is a standard technique in my laboratory and forms the majority of genetic manipulation in this project. The phenotype of the wild type parental strain is compared to that of the isogenic mutants. Changes in phenotype are correlated to the genotype. This approach is a powerful technique for ascribing gene function, for elucidating the role of that gene in the biology of the host organism and is used in studies of bacterial pathogenicity.

Some Bordetella mutants will be used to infect mice to test their phenotype in an in vivo model of infection and immunity. The mouse model of Bordetella infection is well characterised and has been instrumental in understanding the infection biology of these bacteria.

In other cases, Bordetella genes will be cloned in order to express them from the recombinant plasmids ans is also used to study gene function. Complementation of mutations is used to confirm that a mutant phenotype is due to the experimentally constructed mutation and not a secondary effect or artifact. Expression of genes in heterologous hosts is used to identify possible functions of the gene by analysing the phenotypes of the recombinant, purifiable protein for in vitro studies of protein function.

Evaluation of foreseeable effects

It is considered very unlikely that using lab strains of E.coli in this way will result in a pathogenic phenotype. A vast majority of DNA constructs used will not encode functional proteins. Bordetella promoters are not well recognised in E.coli. In the very rare instances where a Bordetella encoded protein is expressed in these E.coli hosts, it is highly unlikely to convert them to an infectious phenotype. Deliberately expressed recombinant proteins will be metabolic enzymes involved in polysaccharide synthesis and not virulence factors.


There are no foreseeable adverse effects from performing in vivo work with Bordetella mutants. The bacteria will be handled in containment level two facilities and infected animals housed in isolator facilities that will not permit release of bacteria in to the environment or to other animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid and liquid waste and contaminated glassware will be inactivated by autoclaving. Autoclaves are validated by temperature probe. Contaminated surfaces will be disinfected using 70% ethanol. 70% ethanol validated by

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N

02/03/2022
Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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</table>

Project Ref 85/01.1

Date Ackn'd 17/08/2001

CU2 Project Title FUNCTION OF THE PRION PROTEIN.

Class 2 CultureVolClass2 1-50 litres

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
**Purposes of the contained use**

The aim of the investigation is to express the prion protein (mouse, chicken, tuttle, Zenopus sequences) in E.coli and extract the protein for function studies and to express the protein in mammalian cell cultures to assess the consequences of that expression for cell survival. Non-pathological mutants of the prion protein (mouse) will also be expressed in both systems to assess their effect on prion protein function.

**Recipient or parental organism**

| E.coli (JM109, AD494, XL1 Blue, XL2 Blue, DH5a) |
| mammalian cell lines, neuroblastoma, PC12, neuroblastoma/cerebellar cell fusion cell lines. |
| Primary neuronal cultures from mouse brain. |

**Host/vector system**


d

**Origin & function**

The genes are from mouse, chicken, turtle and toad. The genes will express the prion protein (prnp) or doppel protein (prnd).

**Evaluation of foreseeable effects**

The normal protein product of the prion protein gene. The level of expression will be high in E.coli and moderate to high in mammalian cells. The mutants that will be used are the following:

1. The mouse prion protein has two known alleles. Most mice express the a allele. The b allele differs at only two amino residues (being aa 108 and 189). Mutants will be made with either the b form of codon 108, the b form of codon 189 or both. These mutations are not associated with disease. Mice expressing the b allele are less susceptible to disease.

2. Deletion of codons 51-90 of mouse prion protein. This deletion removes the copper binding domain. This mutation is not associated with disease. There is no correlation with the scrapie isoform. PrP-Sc does not contain this region of the prion protein.

3. Deletion of codons 114-120. Removal of this region abolishes the ability of the prion protein to be converted to PrP-Sc. This form of the prion protein would be safer than wild type (see Holscher et al, J. Virol. 72, 1153-1159, 1998).

4. Production of a fusion protein with the green fluorescent protein expressed before codon 23 of the prion protein. The sequence of GFP would be cloned into the prion protein gene after the signal peptide coding region. This is unlikely to have harmful effects and has been done before (Lorenz et al. 1999; P161, Conference on Prion Disease held in Tubingen).

5. Deletion of codons 235-254. This part of the protein is the signal to form a GP1 anchor. As such the protein produced would not be anchored to the cell membrane. As GP1 anchoring is believed essential for conversion of the protein to the abnormal isoform then this mutant would also be "safe".

For the following reasons it is unlikely that an infectious activity would be associated with the protein expressed:

1. Despite many attempts no researcher has managed to convert recombinant prion protein into an infectious form.
2. Conversion to the abnormal isoform (PrPSc) seems to require GP1anchoring to a cell as well as contact with the abnormal isoform. Therefore recombinant material is probably far less dangerous than prion protein expressed by mammalian cells (present ubiquitously in the environment).
3. Overexpression of the prion protein does not lead to disease in mice or production of the disease specific from (PrP-Sc).
4. The mutants to be generated are all non-disease associated mutations and in some cases actually prevent the possibility of conversion.
5. The proteins to be generated will be based on the mouse and the lower vertebrate sequences. The chicken sequence has no association with disease. There is no
naturally occurring mouse transmissible spongiform encephalopathy. Scrapie is transmitted experimentally to mice from sheep brain extracts.

6. No abnormal isoform of the prion protein will be introduced into the experimental system. Therefore conversion will either be very improbable or impossible.

7. The abnormal isoform (PrP-Sc) requires the presence of prion protein expressing cells to form. The spontaneous production of PrP-Sc known to arise in diseases such as scrapie of CJD in humans takes years to be generated. The cultures to be used will only be maintained for two weeks.

8. There exists in conversion of cellular prion protein to the abnormal isoform by PrP-Sc a species barrier. This barrier implies that there is a very low probability of human cellular prion protein being converted to the abnormal isoform by mouse PrPSc.

However it is unknown under what conditions the altered isoform of the prion protein may be generated.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Prokaryotic Work**

Decontamination: All non-disposable glass and plasticware is to be rinsed with 2M NaOH (sodium hydroxide) for 15 minutes after use.

1. Liquid waste: all solutions are to be collected in Schott (borosilicate) bottles and autoclaved at a minimum of 126 degrees C for 30 minutes. After autoclaving this waste is to be mixed with Virkon (to a final concentration of 50%) and left to stand overnight. This treatment will give an effective 100% kill of viable GMMs. After this time the liquid is safe to tip down the sink.

2. Solid waste: to be collected in a doubled biohazard bag. At the end of each round of protein purification, or when it is full, it is to be autoclaved at a minimum of 126 degrees C for 30 minutes. The waste is then treated as non-hazardous industrial controlled waste and sent for landfill.

Wastes from those parts of the procedure involving 8M Urea need not be collected or treated in this manner as 8M Urea will fully denature the protein.

**Eukaryotic Work:**

Decontamination: All non-disposable glass and plasticware is to be rinsed with 2M NaOH (Sodium hydroxide) for 15 minutes after use.

1. Liquid waste: all solutions are to be mixed with Virkon (to a final concentration of 50%) and left to stand overnight. This treatment will give an effective 100% kill of viable GMMs. After this time the solutions are safe to tip down the sink.

Solid Solid waste: to be collected in a doubled biohazard bag. At the end of each round of protein purification, or when it is full, it is to be autoclaved at a minimum of 126 degrees C for 30 minutes. The waste is then treated as non-hazardous industrial controlled waste and sent for landfill.

**FOR VALIDATION OF WASTE INACTIVATION**

After bacterial cultures are treated with Virkon, samples will be taken and streaked onto bacteriological plates to determine if there are any viable bacteria. If a positive culture is found the waste material will be autoclaved at minimum of 126 degrees C for 30 minutes.

Inactivation of theoretically infectious prion protein cannot be validated as at the present time there is no evidence of any kind that recombinant prion protein can be infectious.
All researchers involved in the work and those in the vicinity are to be made aware of the control methods required. Every three months discussion of the control methods with researchers involved is carried out to ensure that the control methods are indeed carried out. The problem with this work is that the requirement for waste treatment is applied to prevent a potential threat for which there is currently no evidence.

Project Containment

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<tr>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
</tr>
</tbody>
</table>

Please enter comments on the GM safety committee on the risk assessment

Members of the Committee are satisfied that the procedures described, and carried out under the containment conditions proposed, present no significant hazard and that the risk to human health and to the environment is, therefore, very low.

Project Ref 85/03.1

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<td>16/01/2003</td>
<td>POST-EXPONENTIAL PHASE GENE EXPRESSION IN PSEUDOMONAS AERUGINOSA</td>
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<td>&lt; 1 litre</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
This project examines the contribution of environmental events leading to cessation of growth on the expression of Pseudomonas aeruginosa genes associated with survival and persistence in stationary phase. The work involves the use of some plasmid-borne lux and gfp reporter constructs (isolated in other laboratories).

The host strains are well-characterised laboratory strains of P. aeruginosa, PAO, PAK and PA103. These strains were clinical isolates dating back to the 1950s, and characterised by workers such as Bruce Holloway. P aeruginosa is an opportunistic pathogen, notably causing infection in immunocompromised and burns patients and those with cystic fibrosis. Infections are rare in otherwise healthy individuals, with eyes, middle ear and the urinary tract being most likely involved. The vectors are derivatives of pUC18 able to replicate within P. aeruginosa.

Incomplete transcriptional and translational fusions used to drive expression of gfp. Level of expression and biological activity likely to be low. Complementing sequences for rpoS, lasR/l, rhlR/I, exsA, exoS, exoT, pilU, pilT and pilA.

RpoS is a sigma factor that controls expression of genes on entry into stationary phase (J. Bacteriol 181, 3890-3897).

LasrR/I are the regulatory and synthetic components of the one of the two quorum sensing systems in P. aeruginosa. RhlR/I control the second system (Infect Immun 68,4839-4849).

ExsA controls expression of the type III secretion system (Nature Medicine 5,392-398). ExoS is and ExoT are Ras GTPase activating proteins delivered by the type III secretion system (Infect Immun 66,2607-2613; Infect Immun 68,6066-6068).

PilA is the pilus structural subunit which is secreted by the type IV secretion apparatus including the PilU and PilT components (Infect Immun 67,3625-3630).

The proteins RpoS exists in many Gram-negative organisms, so acquisition of the Pa gene would not be expected to have additional effects. Expression of components of the two quorum sensing systems would not be expected to have biological activity in other organisms. The promoter sequences to which the LasR and RhlR transcriptional
Regulators bind appear specific to the host organism. ExsA regulates expression of a type III secretion system found only in Pa. Similarly, ExoS and ExoT require the Pa type III system for secretion and this heterologous expression in another organism is unlikely to occur. Heterologous expression of PilA, PilT and PilU components could confer additional adhesive properties, however closely homologous structures are expressed by related organisms anyway.

Overall, unlikely to cause harm to healthy subjects, but P. aeruginosa is an opportunistic pathogen in immunocompromised patients. These infections are susceptible to antibiotic treatment. The plasmids are selected on the basis of resistance to carbenicillin, which is not in current clinical use, or gentamicin. Gentamicin is in clinical use.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The GMM is handled in an ACDP containment level 2 laboratory. All material leaving the laboratory is decontaminated by autoclaving at 126 degrees C for 30 min. The cycle is under dual process control. Spills within the laboratory are treated with alcoholic cetrimide or chlorine-based agents (>2500 ppm available chlorine). Surfaces are swabbed to monitor for contamination control. Disinfection procedures follow EN1276.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Agreed level 2.

Project Containment

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</tr>
<tr>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

Project Ref 85/03.2
We are studying the genetic mechanisms controlling the symbiotic interaction between Photorhabdus and its nematode host, Heterorhabditis. These studies will involve the generation of mutant strains of Photorhabdus and assaying these mutants for their ability to support nematode growth and development (symbiosis).

Recipient or parental organism

The recipient organism is Photorhabdus, a gram negative bacterium that is a member of the family Enterobacteriaceae. This bacterium is highly pathogenic to a wide variety of insect larvae whilst also maintaining a symbiotic relationship with nematodes of the family Heterorhabditidae. The bacteria-nematode complex exists naturally in most countries surveyed (including the UK) and is commercially available as a natural biocontrol agent. Photorhabdus are considered to be non-pathogenic to humans. However one species, P.asymbiotica (interestingly this species does not have a nematode partner), has been found associated with non-lethal, easily treatable infections in the US and Australia (a total of 10 cases have been reported in the last 13 years). It is difficult to see how Photorhabdus could establish systemic infections in humans as the bacteria cannot grow well at 37°C.

Host/vector system

The host/vector system used will be based on E.coli K-12 strains and pBR and pGP704-derived vectors. E.coli S17-1 will be used to deliver a kanamycin-containing cassette to Photorhabdus. The E.coli strains used are nutritionally disabled (thi mutation) and would not survive outside of the laboratory. Furthermore pGP704-derived vectors will only replicate in S17-1 as it has been engineered to contain the lambda pir protein. Therefore once this plasmid is introduced into Photorhabdus it can no longer replicate and must recombine into the chromosome. Therefore the risk to the environment is low and there is no risk to horizontal transfer of the plasmid from Photorhabdus to other organisms.

Origin & function

We will be using several transposon-based delivery systems to generate mutants. These systems have been designed to result in stable mutants. Therefore there is no risk of the antibiotic marker spreading horizontally.
The recipient/parental organisms have all been isolated from the wild. We are currently using bacteria-nematode complexes that were originally isolated in Ireland, the UK, the US and Trinidad. The bacteria from these complexes will be mutated to determine what genes are important for the maintenance of the symbiosis. This will involve combining mutant bacteria with wild-type nematodes.

**Evaluation of foreseeable effects**

Insertion mutations generated by transposons are normally associated with the loss of gene function. Therefore I expect that the vast majority of mutants generated in these studies will result in the loss of symbiosis between the bacteria and the nematode implying that these mutants will not pose any threat to the environment as they will not be able to form active complexes with the nematodes.

Some mutants will probably increase the efficiency of symbiosis (e.g. mutants in a gene encoding a repressor protein). Although these mutants would be extremely useful in our studies we have evidence that suggests that these bacteria are not able to compete with wild-type bacteria during infections of insect larvae (Joyce and Clarke (2002), Mol Microbiol in press). However it is possible that we could generate a mutant with increased symbiosis potential and such a mutant would have important applications in the commercial production of these nematode-bacteria complexes. However it is difficult to see how this mutant might have any adverse effect on the environment.

Our mutant screens will also produce strains of Photorhabdus that are attenuated or avirulent and clearly these bacteria will not be able to survive in the wild. Moreover the LD50 of Photorhabdus against our model insect, Galleria mellonella, is normally <5 bacteria/insect suggesting that it would be difficult to isolate a mutant with increased virulence. In addition the host range of the bacteria is determined by the nematode i.e. host seeking and penetration and the presence of mutated bacteria in the nematode will not affect the normal host range of the nematode.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For laboratory manipulations the use of good microbiological practice will be sufficient to limit contact with humans and the environment. All bacterial waste i.e. agar plates, contaminated plasticware, liquid cultures will be inactivated by autoclaving before disposal. The routine regime in the Department of Biology and Biochemistry at the University of Bath is 126C for 30 minutes (as specified by DEFRA). The degree of kill is expected to be 100%.

Nematodes grown with mutated bacteria are not stored and they are inactivated by heating to 60C for several hours before autoclaving. Viability can be monitored by microscopic observation. Living nematodes have a characteristic sinusoidal "swimming" motion and they are "S-shaped". Dead nematodes do not move and they are straight.

Infected insect cadavers (and uninfected insect larvae) are placed at -70C for several days before autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Project Containment

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</table>

Animal Units

L2 L3 L4 L2 L3 L4 L2 L3 L4

Large Scale Activities

Animal Units

L2 L3 L4 L2 L3 L4 L2 L3 L4

Project Additional Information

Purposes of the contained use

To deliver transcription factors involved in the development of endodermal organs to the liver, other tissues and organs cultures, studying the ability of such transcription factors to transdifferentiate (ie convert) other cell types into pancreatic B-cells capable of controlled expression and secretion of insulin. This could prove to be a viable treatment for patients with diabetes mellitus.

Adenovirus vectors were chosen because of their ability to efficiently deliver exogenous genetic material to the adult liver and other tissues.
Recipients or parental organism

Adenovirus (Ad) was first isolated from the adenoids of latently infected individuals (humans) in the 1950s and since that time at least 47 human adenovirus serotypes have been categorised. Symptoms associated with adenovirus infection vary widely. Ad5, the serotype used to construct first-generation, replication-defective adenovirus vectors, is usually associated with mild respiratory infections in children (Shenk, Adenoviridae. Fundamental Virology. Fields et al. Philadelphia, Raven Publishers: 980-1016, 1996).

Host/vector system

First-generation, replication-defective adenovirus vectors are derived from the wild type adenovirus type 5 serotype.

The adenovirus vector has a deletion within an early region of its genome known as E1. This region is essential for virus replication in vivo and infectious virus particles are only produced when the vector infects a complementing cell line such as HEK 293. These cell lines provide the E1 functions in trans allowing the production of infectious virus particles. Without this region, no infectious virus particles are produced. Both the parental and vector genomes reside within the cell as an extrachromosomal element and do not integrate into the host genome. The injection of adenovirus vectors into athymic mice results in transgene expression for over six months with minimal decrease in expression. This suggests that the adenovirus vector genome is very stable.

First-generation adenovirus vectors are contaminated with replication-competent adenovirus (RCA), arising through the homologous recombination between the vector and the producer cell DNA sequences. (Mountain, Trends Biotechnology 18: 119-128, 2000).

Origin & function

The vectors will be utilised as delivery vehicles of exogenous genetic material to the liver of adult rodents, to embryonic organ cultures and other primary cultures eg bone marrow cells.

In the first instance, the transcription factor to be utilised is a homeobox gene called Pdx1 (or its Xenopus homologue Xihbox8), which has a fundamental role in pancreas development (MacKinnon et al. Diabetologia 44, 120301214, 2001). Expression of an activated form of Pdx1 (formed by the addition to the C-terminal of a herpes simplex virus transactivation domain, VP16) has been shown to convert larval Xenopus liver cells into pancreatic cells (Horb et al. Current Biology 13: 105-115, 2003). I will continue these studies within the mature liver of adult rodents.

Other transcription factors involved in endodermal development may be used in the future.

GFP was originally isolated from the jellyfish A. victoria. The inclusion of a reporter gene will allow the easy identification of transdifferentiated cells.

Evaluation of foreseeable effects

The mammalian homeobox gene Pdx1 encodes a transcription factor involved in the early development of the pancreas and also in the functioning of the mature endocrine B-cell. (Chakrabarti and Mirmira, Trends in Endocrinology and Metabolism 14(2):78-84). A number of studies indicate that expression of this gene within ectopic sites does not invoke transdifferentiation into pancreatic cells (Grapin-Botton et al Genes and Development 15: 444-454, 2001; Heller et al. Gastroenterology 115(2): 381-387, 1998). However, studies by Horb et al. (Current Biology 13, 105-115, 2003) suggest that the addition of a C-terminal transactivation domain enables Pdx1 to succeed in this conversion. The adenovirus vector is intended to deliver and express an activated Pdx1 construct to the liver of adult rodents in an attempt to transdifferentiate mature liver cells. Other transcription factors that may be used will have a similar risk profile to Pdx1.

The vectors will also express GFP under the control of a mammalian pancreatic-specific promoter allowing transdifferentiated cells to be viewed under fluorescence. This reporter is widely used.

All human adenoviruses that have been tested are able to oncogenically transform cultured rodent cells. However, adenovirus type 5 shows no tumourigenic potential in humans or rodents. There is currently no data suggesting that Pdx1, Xihbox8, or their fusion derivatives are oncogenic. An adenovirus expressing the transgene Pdx1 has been constructed and injected into mice. There were no reports that this vector was oncogenic (Taniguchi et al. Gene Therapy 10: 15-23, 2003). VP16 fused to lymphoid enhancing factor 1 generates transcriptional regulators that induce oncogenic transformation in chicken embryo fibroblasts (Aoki et al. Proc. Natl. Acad. Sci. USA 96: 2023/2022
Adenovirus tissue tropism is very well characterised. There is no suggestion in the literature that a developmental transcription factor, such as Pdx1, will alter the vector tropism. The expression of a transcription factor will in no way alter the vector capsid (a protein coat that encapsulates the viral DNA), which is the major factor determining which cells the virus particle can infect. Numerous transcription factors have been expressed using an adenovirus with no evidence that such factors alter the tropism of the parent vector or virus.

Care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection. If the adenovirus vector entered the bloodstream, for example via an open wound, it is likely the vector would be delivered to a tissue where it is biologically active. The liver takes up adenovirus vectors injected systemically into rodents almost exclusively (Smith et al. Nature Genetics 5:397-402, 1993). The vectors we intend to construct may contain a tissue-specific promoter driving a transcription factor. This has demonstrated the potential to transdifferentiate liver cells into pancreatic cells. Entry gained via an aerosol would infect the respiratory epithelium but it is unlikely that the transgene would be expressed unless a lung promoter is used.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Good Laboratory Practice will be maintained, with gloves and lab coats worn at all times. The GMM will be handled in an ACDP containment level 2 laboratory. The work will be performed within the confines of a BioMat Class 2 Microbiological Safety Cabinet (Medical Air Technology). Air entering the cabinet is filtered through two HEPA filters before leaving the cabinet. Twice a year contractors (Bioquell Service) organised by the university Microbiological Protection Officer visit and check the unit. Fumigation will be carried out prior to inspection and containment tests will be performed once a year. All cell lines will be grown in a CO2 incubator positioned closed to the safety cabinet.

Notices will be placed on the safety cabinet and the incubator instructing users in the event of accidents and spillages. The virus is inactivated using 1% sodium hypochlorite.

All solid waste (flasks, pipettes etc.) will be placed in a red "Biohazard" bag situated next to the safety cabinet. The bag will itself be contained within a solid container (with lid) so if virus contaminated pipettes etc. pierce the bag they will remain contained. This container can then be carried to the autoclave room. The waste is decontaminated by autoclaving at 126 degrees C for 30 minutes completely destroying all adenovirus (adenovirus is inactivated at 56 degrees C).

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

The GMSC have approved and signed the risk assessment.
## Project Containment

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### Project Ref 85/04.1

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<td>Retroviral expression of proteins involved in membrane trafficking and transport</td>
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<tr>
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### Purposes of the contained use

The project will involve the construction of recombinant retroviral vectors and packaging them into retroviral particles using packaging cell lines. The retroviral particles (virions) will be used for high efficiency infection of cells in culture for the purpose of expressing recombinant proteins in mammalian/mouse/rat cells. The purpose of the proposed work is to study the effect of the expressed proteins on membrane trafficking pathways and membrane transport. The methods that will be employed when cultivating virions are described in the Retroviral Gene Transfer and Expression Manual (BD Biosciences, Clontech).

### Recipient or parental organism

The vectors that will be used to produce virions are derived from MMLV (see below). The retroviral vectors we will use can only replicate and produce virions in special
packaging cell lines (see below). The recipients of the GM virions will be cultured cell lines. Genetically modified virions that can only infect mouse/rat cell lines will be produced in Ectotropic packaging cell lines. MMLV does not naturally infect human cells. However, when it is necessary to infect cell lines such as Hela (human), Cos (monkey), the virions will be packaged in Pantropic or Amphotropic packaging cell lines (BD biosciences Clontech). Virions packaged in these cell lines have an extended host range, including human cells.

Retroviruses require close contact for transmission and their survival in the general environment is poor. Although certain scenarios can be envisaged (such as needle stick) that virions packaged in amphotropic or pantropic cell lines may infect human cells it is unlikely that the GM virions we propose to make will cause harm to human health, due to the nature of the proteins to be expressed and the replication deficient nature of the virions.

Host/vector system

The packaging cell lines are all modified HEK-293 (human embryonic kidney) packaging cell lines.
BD EcoPack2-293 cell line.
BD Amphopack-293 Cell Line
GP2-293 cell line
All are available from BD Clontech.

pLRCX retroviral vectors and related retroviral vectors (BD Biosciences, Clontech).

Origin & function

The genetic material will be cDNA encoding proteins from a variety of eukaryotic sources. For example cDNA coding for human/rat proteins involved in multivesicular body sorting, glucose transporter trafficking and for ion channels. The purpose of the project is to study the cellular function of the proteins encoded by the genetic material.

Evaluation of foreseeable effects

Once packaged by the packaging cell line the virus is replication incompetent as the structural genes are absent in the virions. The virus is derived from MMLV. The mechanism of viral attenuation is deletion of the gag pol and env genes. The separate introduction and integration of the structural genes into the packaging cell lines minimizes chances of producing a replication competent virus due to recombination events.

The inserted genes encode for proteins that are involved in membrane trafficking and/or membrane transport. There is a possibility that the expressed proteins may alter cell physiology. The project will involve studying protein function by looking for differences between cells expressing the recombinant protein (infected cells) and control cells. Viruses expressing known oncogenes will not be constructed.

Virions will be in a contained laboratory environment and will not have access to organisms other than authorised laboratory personnel.

Retroviruses require close contact for their transmission and their survival in the general environment is poor. Although certain scenarios can be envisaged (such as needle stick) that virions packaged in amphotropic or pantropic cell lines may infect human cells it is unlikely that the GM virions we propose to make will cause harm to human (or animal) health, due to the nature of the proteins to be expressed and the replication deficient nature of the virions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Good laboratory practice will be maintained. Laboratory coat and gloves will be worn at all times. Good hygiene will be maintained and surfaces will be disinfected following manipulations using virions. All procedures with recombinant retrovirus will be performed in an ADCP containment level 2 laboratory. A class 2 microbiological safety cabinet will be used when handling virions. Twice a year contractors organised by the University Microbiological Protection Officer visit and check the safety cabinets. Fumigation will be carried out prior to inspection and containment tests will be performed once a year. Access to the laboratory where retroviruses are used will be restricted to authorized personnel only and the laboratory door will be kept closed at all times except for access. All cell lines will be grown in a CO2 incubator positioned close to the safety cabinet. Notices will be placed on the laboratory door, the safety cabinet and the incubator instructing users in the event of accidents and spillages. The virus is inactivated using 1% sodium hypochlorite. Solid waste (pipettes, flasks, etc) will be placed in a red Biohazard bag within a solid container (with lid). The container can be carried to the autoclave room. The autoclave facility is situated in the building for the sterilisation of waste. Waste will be autoclaved at 126 degrees C for 30 minutes completely destroying virions. Contaminated liquid waste will be disinfected with an appropriate disinfectant (1% Virkon) for at least 30 minutes.

The genetic modification safety committee considered the risk assessment to be appropriate and that the project should be a class 2 activity.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee considered the risk assessment to be appropriate and that the project should be a class 2 activity.

### Project Containment

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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>05/07/2007</td>
<td>Assembly and use of lentiviral particles to modulate function of intracellular signalling</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
</tr>
</tbody>
</table>
### Project Additional Information

#### Purposes of the contained use

University bench research to examine the cellular role of intracellular signalling molecules in the regulation of T cell function.

#### Recipient or parental organism

Recipient cells will be T cells and T cell lines including Jurkat, Hut 78 and CEM cells.

#### Host/vector system

- **Lentivirus derived from HIV-1.** The virus has been stripped down to its minimum essential components, which have been encoded on four separate plasmids. To inactivate the virus in the unlikely event of recombination of the four plasmids, the vector itself in inactivating (SIN). This is achieved by the deletion of the genetic sequence from the 3’ long terminal repeat (LTR) encoding enhancer and promoter functions, resulting in the transcriptional inactivation of the provirus in the infected cell.


  The virus is similar to those available commercially such as Virapower vector sold by Invitrogen.

#### Origin & function

- The Lentiviral vectors will deliver modified versions of intracellular signalling molecules including the SH2 domain containing inositol phosphatasease (SHIP). Endogenous human SHIP has been modified by fusing its catalytic region to the extracellular and transmembrane regions of rat CD2 protein. This creates a constitutively active lipid phosphatase predicted to result in depletion in cellular levels of the secondary messenger PtdIns(3,4,5)P3. (See Freeburn RW et al (2002) Journal of Immunology 169 (10):5441-5450).

  Lentiviral vectors will also be assembled to deliver short interfering RNA sequences designed to oppose expression of intracellular signalling molecules including endogenous SHIP.

#### Evaluation of foreseeable effects

The rCD2:SHIP protein will metabolise the intracellular signalling molecule PtdIns(3,4,5)P3 leading to the formation of PtdIns(3,4)P2. In T cells this may modulate cellular...
responses to receptor stimulation including growth, cytokine production and chemotactic responses.

Conversely, opposing SHIP expression with short interfering RNAs would be predicted to make T cells more responsive to receptor stimulation.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Cell used for assembling viral particles and infected target cells will be maintained (seeded, transfected and fed) in a dedicated biosafety level 2 cell culture cabinet. This will be supplemented by placing a 5L beaker containing 1% solution of Virkon for immediate use treatment of wastes and contaminated items.

The cabinet will be sprayed before and after use with 1% solution of Virkon followed by 70% ethanol.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid wastes including cell suspensions, will be soaked in a 1% solution of Virkon overnight before disposal to the drain. Overnight treatment with virkon would be predicted to result in 100% killing of viral particles.

Dry waste will be placed in autoclave bags and sent for autoclave treatment.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
Dear GM advisors, I have completed the attached form as best I can, but I will need guidance on a number of points.

Page 2: ACDP Hazard category?
Page 4: Provisional containment level?
Page 5: Provisional final activity class?

Sent 07 February 2007 08:36
To:
Subject: RE: Viral Safety

We will provide guidance to your questions soon.

In the meantime, you answered yes to these questions. Please could you just add a sentence describing which tissues could be affected and by what route of transmission?

Are all potential routes of transmission of the virus known, e.g. those that may occur during a laboratory accident? ("Yes").
If yes, will the routes of transmission deliver the virus or its products to tissues where it may be biologically active? ("Yes").

Page 5, section 2B. Level of risk has not been identified in the first block. Please could you estimate that? You say that there is a potential for harmful effects of gene expression on other organisms. Could you briefly say what that could be?

Sent 07 February 2007 10:08
Subject: RE: Viral Safety

Having spent some time looking at this today, I suggest that this work be assigned to category 2. Although the lentivirus system is not strictly listed in an ACDP category, notes I have found from ACDP meetings suggest that it should be placed in hazard category 2. Also, regardless of the modification to the virus, its final containment level etc. is level 2.

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**Project Ref** 85/07.2

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<th>CultureVolumeClass3-4</th>
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<tr>
<td>07/09/2007</td>
<td>Construction and use of replication defective lentiviral and retroviral vectors.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</tbody>
</table>
**Project Additional Information**

**Purposes of the contained use**

University bench research to explore cellular function, differentiation, and development.

**Recipient or parental organism**

For example, HEK-293 or HEK-293T will be used to package the retrovirus and HEK-293FT cells will be used to package the virus. The virions produced will be used to transduce various tissue culture cell lines and also tissue in vitro or in vivo.

**Host/vector system**

Various vector systems may be used as the technology improves in transduction and safety but will almost exclusively be purchased from a scientific company. Initially, the retroviral vector system to be used will be Stratagene’s Viraport system and the lentiviral vector system Invitrogen’s ViralPower Lentiviral Gateway Expression system.

**Origin & function**

The genetic material will be cDNA thought to be involved in development or altering the phenotype of cells or tissue (e.g., transdifferentiation) or cDNA libraries (e.g., prepared from tissue like liver or pancreas). Promoters will be used to confer constitutive expression or cell/tissue specific expression. Marker proteins (e.g., GFP, lacZ) will be used either co-expressed or as fusion proteins to follow expression. Genes may be used so that some sort of selective pressure can be applied to isolate only cells that are transduced (e.g., antibiotic resistance). Also, vectors to deliver shRNA designed to specifically knock down a protein's expression may also be constructed.

**Evaluation of foreseeable effects**

To our knowledge, there is no suggestion in literature that any of the sequences that we would hope to look at will alter the vector tropism compared to if the vector was made without it present. Numerous proteins have been expressed in systems like this or similar with no evidence of the factors altering the tropism of the parent vector or virus.

The cells transduced by the virus may be altered by the genetic information we choose to include. The aim will be to study the effects of the information we include. For example, the cells/tissue may have altered phenotype/function and express selectable traits so that we can study the effects of the factors and also identify new factors.

**Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)**

The control measures for the virus to enable it to be safe and easy to use are described in detail on the proposal and risk assessment form and also in company literature.
of systems we hope to use such as Stratagene's Viraport system or Invitrogen's ViralPower Lentiviral Gateway Expression system. In brief the virions are replication
defective and or self inactivating. Important genes involved in the assembly of the virions are encoded on many different plasmids which do not require the use of stable
cell lines expressing these factors.
Good laboratory practice will be maintained. Laboratory coat and gloves will be worn at all times. Good hygiene will be maintained and surfaces will be disinfected
following manipulations using virions.
All procedures with recombinant virus will be performed in an ACDP containment 2 laboratory. A class 2 microbiological safety cabinet will be used when handling virions.
Twice a year contractors organised by the University Microbiological Protection Officer visit and check safety cabinets. Fumigation will be carried out prior to inspection and
containment tests will be performed once a year. Access to the laboratory where retroviruses are used will be restricted to authorised personnel only and the laboratory door
will be kept closed at all times except for access.

All cell lines will be grown in a CO2 incubator positioned close to the safety cabinet.
Notices will be placed on the laboratory door, the safety cabinet and the incubator instructing users in the event of accidents and spillages.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The virus is inactivated using 1% sodium hypochlorite. Solid waste (pipettes, flasks etc.) will be placed in red Biohazard bag prior to removeal from the laminar flow hood,
enclosed in a second bag outside the hood and carried to the autoclave room. The autoclave facility is situated in the building for the sterilisation of waste. Waste will be
autoclaved at 126C for 30 minutes completely destroying virions. Contaminated liquid waste will be disinfected with an appropriate disinfectant (eg 10% bleach or 1%
Virkon) for at least 30 minutes.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Our departmental GMSC fully approved the application and stated that it was appropriate to be carried out at category 2.

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02/03/2022
Tests of gene function in vertebrate development. We will contribute to this by focussing on vertebrate embryonic development particularly that of the limb. We will test gene function in the development of chick and fish embryos because these are good models for vertebrate development and the eggs develop outside the mother. We will overexpress genes that encode for secreted factors by implanting cells transfected with the gene into chick embryos using RCAS (Replication Competent Avian Sarcoma Virus) or attenuated AdV (Adenoviral Vector) systems. We will inhibit expression of genes in fish embryos using morpholinos and in chick embryos using antisense strategies based on viral delivery. Some of these approaches have already been used to gain insights into fundamental aspects of embryonic development. In addition, in some cases these experiments could provide direct links to clinical medicine, thus it is possible to study in these model vertebrate embryos, the roles of genes known to be responsible for human congenital defects, such as Holt-Oram syndrome. Furthermore many genes expressed in embryos were first identified in tumours and embryos provide an efficient way of exploring gene function that does not require killing adult animals.

1. COS (African Green Monkey) cell lines- these cells will be transfected with genes that and then used as grafts to deliver factors to embryos (see below). In addition to transiently transfected cell lines will be produced and grafted.
2. QT6 cell lines- used for same purposes as above.

These cells will be transfected with RCAS vectors containing genes of interest (these genes may be chicken sequences or sequences from other vertebrates including mouse, human and fish) and used to produce genetically manipulated virus stocks that can then be used to infect susceptible chicken embryos.
4. HEK1293 cells for adenovirus production- these well-characterised and authenticated tissue culture cell lines will be used for propagation of the disabled adenovirus vector. We will also use HEK1293 and other well-characterised mammalian cells to express proteins for cell localisation and biochemical studies.

5. Chick embryos- we will test gene function with RACS using eggs from SPAFAS (Pathogen Free) chickens in addition to commercially available eggs because such embryos are more susceptible to viral infection and we have been importing SPAFAS eggs from Germany. We will infect chick embryos at different stages in development in 3 ways: injection of high titre virus suspensions, grafting fibroblasts producing virus, electroporating RCAS constructs directly chick embryos and then letting the infection spread. For experiments with adenovirus vectors, we will use eggs from normal supplier and the same three strategies to infect embryos. We will also carry out transient transfections in both normal and mutant chick embryos by electroporating gene constructs driven by promoters such as CMV.

6. Fish embryos and fry- we will test gene function in early development by injecting morpholinos designed to block function of specific genes into single cells at cleavage stages. We will graft cells expressing secreted factors into embryos and young fry and also introduce constructs using electroporation.

3. Chicken embryonic fibroblasts and chicken DF1 fibroblast cell lines for RCAS production.

**Host/vector system**

1. Mammalian expression vectors will be used to introduce DNA into COS cells by employing special conditions such as electroporation, use of calcium phosphate crystals, lipofectamine etc.

2. RCAS vectors will be used to transfect QT6, primary chick fibroblasts, DFI cells and chicken embryos. The [retrovirus is replication competent but infects avian species and not mammals. Very high viral titres are required to infect significant amounts of tissue in chick embryos and pathogen free embryos are most susceptible. The src gene that makes this virus oncogenic in chickens has been removed and replaced with a restriction site so that transgene can be easily inserted here. Any potentially harmful effects will depend on the gene or antisense construct inserted into RCAS. Infected embryos will not usually be allowed to develop beyond 10 days of incubation and never be allowed to hatch.

3. The adenoviral vectors to be used, such as the Ad5-SVR4 system, are attenuated through deletions in both E1 and regions of the genome. The net result is that the virus is rendered replication incompetent, the potential for viral transformation is eliminated and the ability to evade the host immune system is abrogated. Furthermore these viruses do not integrate into the genome and thus cannot cause insertional mutagenesis. The site of insertion of the gene sequence to be used is the same site as the site of disablement. The types of insert will be known or unknown vertebrate gene sequences mostly chicken sequences. None of the genes expressed will complement E1 function because as far as is known only viral sequences can do this. There is also no evidence that vertebrate sequences could interfere with capsid proteins and thus alter the immune response to the virus. The genetically modified chickens will be handled as in 2. above. The worst case scenario would involve infection of a human being with a disabled adenovirus containing an oncogenic sequence despite precautionary measures to reduce aerosols etc. Normally the human being would be immune to infection but if the individual was immuno-compromised the virus might survive. However in this case the virus would only infect single cells and not be propagated to daughter cells, therefore not producing lasting oncogenic changes. Another scenario would be that the human being was already infected with normal adenovirus. In this case, even if recombination occurred to generate replicative virus, the inserted sequence would be lost and again no oncogenic thages would be produced in the host. The wildtype Adenovirus serotype 5 causes sub-clinical infections and is categorized by the ACDP as Hazard Group 2. Most human adults are immune to these serotype 5 viruses. Based on the information given above, the attenuated adenoviral (Ad5-SVR4) vector can be classified as ACDP Hazard Group 1.

4. Morpholinos act in a cell autonomous way and must be injected into indivual cells. The effect wears off after about 3 days - genetic modification is transitory- and genetically manipulated fish embryos or fry will not be allowed to reach maturity.

**Origin & function**

We will obtain sequence information about genes that have already been described or from EST databases that have been assembled from different organisms. We will also design antisense sequences to interfere with the function of specific genes. We will test the function of genes in embryonic development by either over-expressing genes or trying to inhibit their function as described above. We will assess the phenotype morphologically and also use in situ hybridisation and immunohistochemistry where appropriate to monitor any molecular changes at either mRNA or protein level.
protein level. The chick system allows localised genetic modification and this will enable us to test the functions of genes at different stages and in different regions of developing embryos. We will use this system to examine, in particular, how limb formation is initiated and limb bud outgrowth and patterning is controlled. The fish experiments should allow rapid screening for the effects of lack of gene function in a vertebrate model system and we will focus our attention on development of paired fins. The vertebrate genes that will be investigated include genes encoding growth factors, transcription factors, phosphatases, enzymes, receptors and other genes of so far unknown function.

Evaluation of foreseeable effects

We will be over-expressing products of a range of different genes including both transcription factors and secreted factors. Many of the molecules involved in embryonic development are also found in tumours. However in embryos, the production of these molecules is normally tightly regulated and tumours are not produced. Avian specific retroviruses cannot infect humans and strains of chickens differ in their susceptibility to viruses with different envelope subgroups. Most chicken strains purchased from commercial vendors are typically CIE strains and resistant to viruses with E envelope subgroup, while the viruses we will be using most often have envelope subtypes A and B (Morgan & Fekete, 1996, Methods in Cell Biology 51, 185-214). In the very unlikely release of virus from level 2 containment facility, it could potentially infect susceptible avian species if they entered the laboratory. However we have found that very high viral titres are required to infect experimental embryos even when injected and it would be impossible for avian species from outside to get into the laboratory which is secured by a series of doors.

Adenoviral vectors and host cells transfected with these vectors are considered to be equivalent to ACDP Hazard Group 2 and require Level 2 Containment because these viruses can infect humans. These adenoviral systems have been used in many laboratories to over-express, for example, both normal and dominant-negative cell adhesion molecules in chicken embryos (Nakagawa & Takeichi, 1998, Development 125, 2963-2971; see also Leber et al, 1996, in Methods in Cell Biology 51, 161-181) without any reported harmful effects on researchers. In the very unlikely event of the release of virus from the Level 2 containment facility, manipulated virus could survive for a similar length of time to wild-type virus, If the virus infected a susceptible host then expression of the recombinant genes would occur in the infected cell. Since the virus cannot replicate under normal circumstances, expression would be limited to the infected cell and, once this cell divides, to one of its daughter cells. The virus never integrates in the host genome and the episomal DNA is eventually lost through natural degradation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The chick embryos and embryos infected with genetically modified organisms will be kept in incubators in a room with a closed door off a main laboratory, which is accessed from a corridor in the research area of the building. The door to the main laboratory is locked when there is no-one working there. The fish will be kept in aquaria behind a locked door system.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste disposal
Solid waste is collected in a lined biohazard labelled bin. After work, the liner is sealed and transported directly to the autoclave facility in a dedicated container for immediate autoclaving. After autoclaving, solid waste is disposed of as normal refuse.

Liquid waste is collected in a plastic autoclavable flask. After work the sealed flask is transported in a plastic tub or bucket directly to the autoclave facility for immediate autoclaving. After autoclaving liquid waste is disposed to drains with copious amounts of cold water.

Monitoring
Disinfection: the disinfectant of choice (Virkon) is used in strict accordance with the manufacturer’s (Antec’s) guidelines.

Autoclaving: to ensure 100% efficacy, testing of the autoclave is carried out annually to monitor temperature and pressure.

Inspections: These are carried out to ensure that local rules are adhered to and that risk assessments and training is in order.
Microbiological safety cabinets: serviced and tested annually and records displayed on each cabinet.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was circulated to the committee but no comments about the procedures were raised.

Project Containment

<table>
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Animal Units

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Project Ref 85/07.4

Disruption of genes involved in protein transport in Enterococcus faecalis and Enterococcus faecium.

Class Culture Vol Class2 Culture Volume Class3-4
Class 2 1-50 Litres

Non-GMM Consent Granted
Not Applicable

Project notified under transitional arrangements  
N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Date of Significant Change

**Project Additional Information**

#### Purposes of the contained use

- University bench research to investigate the role of genes for protein translocation in *E. faecalis* and *E. faecium*

#### Recipient or parental organism

- *Enterococcus faecalis* and *Enterococcus faecium*. *Escherichia coli* will be used as an intermediate host for the construction of plasmids

#### Host/vector system

- Two systems will be used. The first is to use *E. coli* vectors that do not replicate in Gram-positive bacteria (pBR322 derivatives), clone an internal fragment of a gene of interest in that vector, and then use that to transform *E. faecalis* or *E. faecium*. The plasmid can only be maintained if it integrates into the chromosome. The second system makes use of plasmid pCJK47, which can (i) only replicate in strain that provide repA gene in trans (plasmid lacks repA gene), and (ii) is only mobilised in the presence of a helper plasmid. See Kristich et al.(2007) Plasmid 57, 131-144

#### Origin & function

- Both donor and recipient strains are *E. faecalis* and *E. faecium*. *E. coli* is used as a host for construction of vectors.

- The genes that will be disrupted or deleted are involved in the targeting and secretion of proteins. These genes include those for components of the translocase (*SecY, SecE, SecG*), signal peptidases (which remove N-terminal signal peptides of secretory proteins), foldases, and cell wall components.

#### Evaluation of foreseeable effects

- *E. faecalis* and *E. faecium* are normally part of the intestinal flora of humans. In humans, typical concentrations of enterococci in stools are up to 108 CFU per gram. They do not cause disease in healthy humans, but they are opportunistic pathogens. Enterococci have been associated with a number of infections, including urinary tract infections, bacteraemia, wound infections, and endocarditis. Most of these infections occur in hospitals. The genes that are targeted for disruption are involved in protein transport. Protein transport is an important cellular process, and disruption of these genes could affect e.g. export of cell surface proteins, which could for instance affect biofilm formation. The mutations could, therefore, have an effect on pathogenicity, although it seems most likely that it would be reduced.

- Strains resistant to antibiotics (mainly erythromycin or kanamycin) will be created. However, these resistance markers will be present on the chromosome, and not on plasmids. The probability of resistance markers being transferred to related strains is therefore low. Any plasmids used can only replicate in *E. coli* or, in the case of pCJK47, only in a specific *E. faecalis* strain that provides the repA gene in trans. That plasmid is also not mobilisable, unless mob functions are provided in trans.

- After integration, plasmid pCJK47 is removed again through a counter-selection procedure, resulting in strains that are either wild-type or have the desired mutation. These strains do not contain any antibiotic resistance gene anymore.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

02/03/2022
All solid waste will be placed in autoclave bags and sent for autoclave treatment. Autoclaves in the department are checked regularly. Liquid waste will be soaked in a 1% solution of Virkon overnight before disposal. This treatment is predicted to result 100% killing.

**Project Containment**

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<td>L3</td>
<td>L4</td>
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</table>

Please enter comments on the GM safety committee on the risk assessment

I confirm the application was seen by our GMSC and were happy for it to be classified as Class 2.

**Project Ref** 85/08.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<td>25/04/2008</td>
<td>Studying the function of putative virulence genes of S.aureus.</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Not Applicable</td>
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</tbody>
</table>

Date Project Ceased 23/11/2017

Withdrawn N

Tick if notifying a connected programme of work N

Notification transferred to GM18 23/11/2017

Historical Significant Changes

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
### Project Additional Information

**Purposes of the contained use**

Staphylococcus aureus is a major human pathogen and the purpose of our contained use will be to study the role specific genes play in diseases caused by the bacterium.

**Recipient or parental organism**

Wild type Staphylococcus aureus (ACDP category 2) will be used (e.g., strain 8325-4). E. coli strain DH5α (ACDP category 1) will be used (genotype: F-cp8O(dZAMi5 A(lacZYA-argF)U169 deoR, recA1 endA1 hsdR17(rk- mk+ phoA supE44 A- thi-1 gyrA96 relA)). Wild type Lactococcus lactis strain MG1363 (ACDP category 1) will be used.

**Host/vector system**

pUC based plasmids e.g., the pLL plasmids (Luong et al. 2007. J. Micro. Methods. 70:186-190) and the p5K plasmids (Grkovic et al. 2003. Microbiology. 149:785-794) will be used to manipulate S. aureus. For expression in L. lactis the pKS8O plasmids will be used (Massey et al. 2001. Cell. Micro. 3:839-851). For purification the pQE vectors provided by Qiagen will be used. These plasmid contain a multiple cloning site into which the gene in question can be cloned. It results in the fusion of this gene to a poly-histidine tag which facilitates easy purification of the gene product.

**Origin & function**

This project will involve functional studies of genes believed to contribute to S. aureus’ pathogenicity. Specifically it will involve the cloning of pieces of DNA amplified from the chromosome of S. aureus by PCR into plasmid vectors in E. coli. In E. coli the genes may be further manipulated for example by performing site-directed mutagenesis (Strategene kit) to identify important amino acids. Proteins may be purified at the stage using a His-tag system. Where we need to inactivate genes in S. aureus a shuttle vector will be used. These are typically pUC based plasmid into which a S. aureus origin of replication has been cloned, which allows the plasmid to replicate in both host organisms. Gene inactivation will be performed as follow: an antibiotic resistance cassette will be cloned into the middle of the gene in . coli. The resulting plasmid will be electroporated into the restriction minus, modification plus S. aureus lab strains RN4220. Here the temperature sensitive plasmid will be forced to jump into the chromosome by shifting between temperatures. From here the inactivated gene can be transduced using Phage 11 into the final recipient wild type S. aureus strain. S. aureus genes may also be cloned and expressed in the bacterium Lactococcus lactis. This bacterium is used as a heterologous expression system to study bacterial proteins in a non-invasive or non-pathogenic bacterium. Here the virulence genes will be cloned into the pKS8O plasmid.

The types of genes to be studied will be those involved in host-pathogen interactions. For example the fibronectin binding proteins, the Eap proteins. These are both involved in adherence of the bacterium to human tissues.
**Evaluation of foreseeable effects**

With good microbiological practice the host organisms pose minimal risk to human health. Expression of individual S. aureus proteins in either E. coli or L. lactis may make them behave more like this ACDP cat 2 organism but is unlikely to make them more pathogenic than S. aureus itself. There are no foreseeable hazards associated with inactivating these genes in S. aureus above what is already associated with working with wild-type S. aureus. If anything we will be making them less pathogenic. With good waste kill management and microbiological practice environmental contamination with these organisms is unlikely, however should a breach in procedure occur the genetic manipulations will make them less fit than wild type environmental organisms and they will therefore not be competitive.

The plasmid vectors used in this study are not naturally mobilisable. They contain origins of replication specific to the host strains and antibiotic resistance cassettes, but not ability to form conjugative pili. Under laboratory conditions (e.g. electroporation of high concentrations of purified plasmid into a strain) they can be made to move between E. coli and S. aureus. But as neither of these organisms (nor L. lactis) are naturally transformable and neither have the ability to form conjugative pili, the plasmids are considered non-mobilisable from this point.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Howie style laboratory coats and disposable glove will be used when handling the GMM5. Gloves will be autoclaved (131 oC, 30psi for 30 minutes) following use, and coats autoclaved and washed monthly. All disposable plastic (eg Petri dishes, pipettes, tubes etc) will be autoclaved after use. All glassware will be decontaminated by soaking in a 1% Virkon solution overnight before either disposal or washing for re-use. Liquid cultures of GMM5 will be killed by the addition of virkon powder to a 1% solution, and these will be poured down the sink after 24hours. This has been shown to be fully effective at killing all the bacteria. The GMMs will only ever be grown as pure cultures, and killed after use, minimising the risk of transfer of DNA to other organisms. Regular testing of the efficacy of our Virkon treatments will be performed using viability assays on material believed to be decontaminated. The departmental autoclave is regularly tested and serviced for efficacy.</th>
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<tr>
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<tr>
<td>If yes, tick to confirm that it is attached to this form N</td>
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<td>Tick to confirm that you have attached a risk assessment to this form Y</td>
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<td>Tick if you are claiming exemption from disclosure for section of the risk assessment N</td>
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02/03/2022
comments on first draft of risk assessment:
Further detail on the following issues is needed as requested by the HSE advisors:
1. mobilisation of the vector
2. decontamination procedures
3. genetic characteristics of strains used (i.e., DH5a genotype)
These suggested amendments were all incorporated into this application and to the attached risk assessment. The final draft was then reassessed and considered to meet local requirements.

Project Containment

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Project Ref 85/09.1

CU2 Project Title
The screening of large insert DNA library (in standard E.coli vectors) of Burkholderia pseudomallei to screen for biological activity against invertebrates including insects, amoeba and nematodes.

Class CultureVol
Class 2 < 1 Litre

Consent Granted
Not Applicable

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
To identify novel Burkholderia pseudomallei proteins involved with interactions with invertebrate hosts. This will help us understand its persistence in the environment and also inform on the evolution of virulence factors it uses in human infections. We will also sub-clone parts of cosmid inserts into plasmids such as the pUC, pET or pBAD series.

The parental organism from which the genomic DNA library is made is Burkholderia pseudomallei strain K96243. This is a class III containment Gram-negative bacterium which is a pathogen of humans. At no point will we handle the parent bacterium, only an E.coli cosmid library previously constructed by the Sanger Centre (Hinxton, UK). The library clones are not expected to have any disease causing attributes, only toxicity to invertebrates at high dose.

The vector system is the (non-conjugating) pCC1FOS Fosmid in E.coli EPI-3000-Ti (Epicentre). The library us a set of 37 plates containing ~40kb inserts and with~10X coverage of the Burkholderia pseudomallei strain K96243 genome.

The Sanger Institute recently published the 7.2 Mb genome of Burkholderia pseudomallei in collaboration with Dr. Rick Titball of the Defence Science and Technology Laboratory, Porton Down, and Dr Ty Pitt of the Health Protection Agency. The sequenced strain K96243, is a clinical isolate from Thailand, supplied by Dr S Songsivilai of Mahidol University. The genome is 7247,547 bp in size with a G+C content of 68.06% consisting of two chromosomes of genome sequence for toxicity against Lepodopteran insects, amoeba (Acanthamoeba and/or Dictyostelium) and nematodes (C.elegans) in accordance with the protocols outlined in the following publication: (Waterfield,Sanchez-Contreras, Elefherianos, Dowling, Wilkinson, et al.2008. Rapid Virulence Annotation (RVA): identification of virulence factors using a bacterial genome library and multiple invertebrate hosts. Proc Natl Acad Sci USA. 105 (41): p 15967-72). It is also possible we will sub-clone regions of certain Fosmid clones into expression plasmids in order to test the specific anti-invertebrate activities of candidate toxins.

Burkholderia can infect humans and insects and it expresses proteins used in this infection process. We will clone genes and regions of their chromosomes into normal E.coli hosts which are harmless and test the activity of recombinant expressed proteins on invertebrate hosts. Toxins and virulence factors may be active against insect or human hosts however expression levels will be low and the vectors are not sustainable away from the host. Our screening method relies upon exposure of invertebrate hosts to high doses (for them) of the GMO E.coli which present any toxic proteins expressed (rather than as a disease process per se). The only danger to a human would be through accidental injection of recombinant E.coli. Procedures for injection of GMO into insects are closely monitored using a well-tested technique in which the needlestick injury. Needles and sharps exposed to GMOs are immediately consigned to a sharps bin provided which is later incinerated. Broken glassware containing GMOs could also potentially introduce GMOs to the blood. Full care is taken when dealing with GMO bacterial cultures to avoid this, and disposable plastic vessels are used whenever possible to minimise this risk. All work involving GM material will be performed in the class II containment microbiological hood. In brief we foresee no strong risk from handling this genome library using standard class II containment microbiological techniques.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All standard microbiological procedures for recombinant E.coli are in place in our lab. Including the use of disposable gloves and lab-coats (which do not leave the lab). Any work done with these GM bacteria would be done in the Class II containment hood. We also ensure careful monitoring of what equipment and materials will be
sterilized by 70% ethanol swab or autoclaving at 126°C for 30 mins in biohazard bin/bags provided. Most importantly all recombinant R. coli will either be stored at -80 as a freezer stock in a lockable unit or autoclaved (126°C for 30 mins) in a loosely tied autoclave bag the department autoclave under the standard sterilization conditions in daily use. Bacterial cultures or any disposable materials exposed to recombinant E. coli (such as spreaders etc) will all be sterilized for disposal by autoclaving (126°C for 30 mins) in the same manner. Under certain circumstances equipment (such as plate replicators) exposed to GMMs will be sterilised by 70% ethanol. Any solutions that cannot be autoclaved will be soaked overnight in diluted commercial bleach (>20%) to kill any surviving GMMs. All work will be conducted in a class II containment microbiological hood. Material for autoclaving will be sealed bags.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The Bath University genetic modification safety committee has seen the proposal and passed it without comment. The signed from is included with this application.

**Project Containment**

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**Project Ref** 85/11.1

**Date Ackn'd** 21/09/2011

**CU2 Project Title**
The construction and characterisation of first-generation, replication defective adenovirus vectors for expression and study of proteins involved in cell function

**Date Project Ceased**

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**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N
Project Additional Information

Purposes of the contained use

Each vector will express a protein (or a modified version of the protein) or a construct designed to inhibit protein function, for example by generating short inhibitory (sh) RNA. These proteins may be involved in cellular processes such as polarity, cell proliferation or be a marker gene such as Green Fluorescent Protein (GFP). They may be under the control of ubiquitous promoters (e.g. the cytomegalovirus promoter), tissue-specific promoters or inducible promoters (e.g. tetracycline regulated systems).

These replication-defective adenovirus vectors will be used to infect mammalian cells in tissue culture, and will lead to a better understanding of how normal and transformed cancer cells establish and maintain their polarity and undergo changes in regulation of cell proliferation. This builds on similar previous work in which adenoviral vectors were extensively used safely and successfully to define mammalian cell function (see Dukes et al. Mol Bio Cell. In Press Caunt et al. J Cell Sci. 123: 4310-20, Mol. Endo. 23: 510-9, J. Biol. Chem. 283: 6241-52 and J. Biol . Chem. 281; 2701-10).

Recipient or parental organism


Host/vector system


Origin & function

First generation adenovirus vectors originate from serotype 5 adenovirus (Shenk, Adenovindae, Fundamental Virology. Fields et al. Philadelphia, Raven Publishers: 979-1016, 1996). The vectors will be used as delivery vehicles of exogenous genetic material to mammalian epithelial cells cultured in vitro either as cell monolayers or in organotypic cultures and explant tissue.

In the examination of cell polarity in isolation, we will express GFP-Vps4EQ and CHMP3-GFP fusion proteins (Dukes et al. Biochem. J. 411: 233-9). The latter two of these proteins have been shown to perturb membrane trafficking to lysosomes when expressed in a variety of mammalian cell lines. We intend to investigate whether protein trafficking defects interfere with cell polarity and proliferation. We also intend to express other mammalian proteins implicated in cell polarity and proliferation. These include Protein Kinase C iota and lethal gian larvae (Chalmers et al. Development 312: 977-86) as well as wild-type and mutated alleles of extracellular signal regulated kinase (ERK) and ERK phosphatases from the DUSP (dual-specificity phosphatases) family (namely, wild-type, catalytically inactive and non-MAPK binding variants of SUSPs, see Caunt et al. J Cell Sci. 123: 4310-20, Mol Endo. 23: 519, J. Biol. Chem. 283: 26612-23, J. Biol. Chem 283: 2701-10). We also intend to express wild-type and oncogenic variants of Ras and Raf with the oestrogen receptor ligand binding domain (Dajee et al. Oncogene 21: 1527-38 and Samuels et al. Mol. Cell Biol. 13: 6241-52) and perform targeted knock down of proteins implicated
Vps4, CHMP3 and ERK cDNAs originate from rat, but the encoded proteins have highly conserved human homologues. All DUSP, Ras and Raf cDNAs are from human origin and new cDNA clones are likely to originate from human, mouse or rat.

Either epitope tags, GFP, ZsGreen or DsRed Express fluorescent proteins will be used as fusions to track protein localisation. Destablished variants of these proteins cloned downstream of responsive promoter elements (such as Egr-1, see Caunt et al. J Cell Sci. 123: 4310-20, Mol. Endo. 23: 510-9, J. Biol. Chem. 283: 26612-23 and J. Biol. Chem. 283: 6241-52) may also be used as transcriptional reporters in combination with the vectors described above.

Evaluation of foreseeable effects

All vector systems use viral genomes which, as a minimum, the E1 region has been deleted. This prevents expression of E1a and E1b genes, which are essential for viral replication and the production of infectious virus particles (Jones and Shenk, Cell 13: 181-88).

Adenovirus (Ad) was first isolated from the adenoids of latently infected humans in the 1950s and since that time at least 47 human adenovirus serotypes have been categorised. Symptoms associated with adenovirus infection vary widely. Ad5, the serotype used to construct first generation, replication-defective adenovirus vectors, is usually associated with mild respiratory infections in children (Shenk, Adenoviridae. Fundamental Viriology. Fields et al. Philadelphia, Raven Publishers: 979-1016, 1996). The vectors are deleted for most of the E1 region and are unable to replicate and produce infectious virus particles, except when introduced to complementing cell lines, such as HEK293. Both the parental and vector genomes reside within the cell as an extra chromosomal element and do not integrate into the host genome. The injection of adenovirus vectors into athymic mice results in transgene expression for over six months with minimal decrease in expression. This suggests the adenovirus vector genome is very stable.

First generation adenovirus vectors are contaminated with replication-competent adenovirus (RCA), arising from homologous recombination between the vector and producer cell DNA (Mountain, Trends in Biotechnology 18: 119-28). Fallaux et al. (Human Gene Therapy 9: 1909-17) tested batches of adenovirus vector for their RCA content at a sensitivity of 1 RCA in 2.5x109 infectious units. Five out of nine batches tested positive.

The design of the vector systems we are using, such as the AdEasyTM XL Adenoviral Vector System (stratagene) and the improved "RapAd" system from the University of Iowa ensures that transgenes can only be inserted into the E1 region of the adenovirus backbone. The RapAd system backbone lacks the left hand inverted terminal repeat (ITR), the packaging signal and the E1 sequence. Sensitive PCR based assays detected negligible amounts of wild type viral genomes in purified recombinant preps (three E1a genomes per 109 adenovirus genomes). Further, wild-type virus remained equally low after 15 serial amplifications of virus preparation, indicating a substantial improvement compared to previous methods where low levels of wild-type virus can be amplified in serial rounds of purification, see Anderson et al. (Gene Therapy 7: 1034-38).

Care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection. If the adenovirus entered the bloodstream (for example via an open wound), it is likely the vector would be delivered to a tissue where it is biologically active. The liver takes up adenovirus vectors injected systemically into rodents almost exclusively (Smith et al. Nature Genetics 5: 397-402). If viruses were inhaled they might infect the lungs. The inserted genes encode for proteins that are involved in cell polarity and proliferation. The outcome of infection is uncertain. However, a worst-case scenario might predict that these cell may hyper/hypo proliferate or lose their epithelial morphology. These cells would probably die by apoptosis and if they did not, would be rapidly removed by the host immune system. This is normally very rapid, but if individuals were for some reason unable to raise antibodies to Ad5, this process could take two to three weeks. However, virally expressed proteins that are presented as foreign antigens will lead to the generation of cytotoxic T lymphocytes that will eliminate virally transduced cells from the host. For individuals previously exposed to Ad5 vectors, clearance will be significantly shorter than two weeks. In fact, neutralising antibodies are most likely to prevent significant transduction of host cells.

Assuming good laboratory practice is maintained, there is no potential for the transmission of naked nucleic acid. Transfection of host cells by naked nucleic acid is very inefficient and such sequences are rapidly degraded by endonucleases should they enter the bloodstream (Kawabata et al. Pharm. Res. 12: 825-30). Ad vectors infect a wide variety of human cell lines and primary cell cultures. Ad vectors have also been injected into humans via the protal vein where they subsequently infect the liver (Raper et al. Human Gene Therapy 13: 163-75).
Wild-type serotype 5 adenoviruses show no tumorigenic potential in humans or rodents. PKCi is a suspected oncogene (Zhang et al. Cancer Research 66: 4627-35). However, expressing it at high levels in the mouse colon did not cause increased proliferation or changes in differentiation (Murray et al. J. Cell Biol. 164: 797-802). Increased tumorigenesis was only seen after induction of tumours by azoxymethane. ERK is often deregulated in cancer, and this is overwhelmingly caused by upstream mutation of Ras or Raf, rather than mutation or overexpression of ERK (Roberts and Der, Oncogene. 26: 3291-310). The constitutively active mutants of these proteins are known to cause cell transformation in vitro and cause cancer when they occur as somatic mutations in patients, but the ER fusions of these proteins do not show high levels of activity in the absence of high levels of oestrogen. In addition, one mutant of ERK2 we intend to use (D319N mutation) confers resistance to phosphatases and has been shown to cause hyperproliferation of cells in Drosophila (Brunner et al. Cell 76: 875-88) and an increase in epithelial to mesenchymal transition of cells in vitro (Shin et al. Mol Cell. 38: 114-27). The other mutants of ERK we intend to use are inhibitory to ERK signalling in our assays (Caunt et al. J. Cell Sci. 123: 4310-20). There is currently no data to suggest other constructs we will make shall be oncogenic and it appears unlikely that short-term infection with any of our vectors would cause oncogenesis because of the rapid viral clearance even if directly injected at high concentration. In addition, the proteins we intend to express are unlikely to alter the antigenicity and cytotoxicity of the vector, which is defined primarily by the capsid.

Homologous recombination between the vector and the HEK293 genome or the vector and a wild-type adenovirus might lead to the production of a replicating, infectious virus capable of expressing transgenes. Co-replication of a vector and a wild-type virus might occur due to trans-complementation within a co-infected cell. Some cell lines and viruses also have the ability to complement E1 functions allowing the vector to replicate. Packaging constraints (a limit on the amount of DNA that can be encapsulated in the viral capsid) would most likely ensure that the non-essential transgenes can only be inserted into the E1 region of the adenovirus backbone. In the case of both systems, the transgene is placed adjacent to the adenovirus encapsidation signal and the left Ad5 inverted terminal repeat. Therefore, homologous recombination eith E1 from the HEK293 cells is likely to result in loss of the transgene.

In summary, the Ad vector is highly antigenic and would be cleared rapidly from the host by the immune system. A massive dose of virus would be required to cause harm to a human and the delivery of such a dose within a lab environment seems impossible given that we are not carrying out any large scale cultures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Good laboratory practice will be maintained with gloves and lab coats worn at all times. The GMM will be handled in an ACDP containment level 2 laboratory. The work will be performed within the confines of a Biomat Class 2 Microbiological Safety Cabinet (Medical Air Technology). Air entering the cabinet is filtered through two HEPA filters before leaving the cabinet. Twice a year contractors (Bioquell service) organised by the University Microbiological Protection Officer visit and heck the units. Fumigation will be carried out prior to inspection and contamination tests will be performed once a year.

All cell lines will be grown in a CO2 incubator positioned close to the safety cabinet.

Instruction notices of how to deal with accidents or spillages will be placed on the safety cabinet, incubator and laboratory COSHH handbook. The virus is inactivated using a 1% aqueous solution of hypochlorite, and emergency "spill kits" consisting of decontaminant, gloves and absorbent disposbale towels will be kept to hand in all areas where viruses are prepared and used.

All solid waste (flasks, pipettes etc.) generated in virus preparation will be placed in a "biohazard" bag next to the safety cabinet. The bag will itself be placed within a solid container (with lid) so if virus contaminated material pierces the bag it will remain contaminated by autoclaving at 126°C for 30 minutes completely destroying all adenovirus (which is inactivated at 56°C).
The Genetic Modification Safety Committee of the University of Bath reviewed and accepted the risk assessment in the form submitted with this document.

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Safety Committee of the University of Bath reviewed and accepted the risk assessment in the form submitted with this document.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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- **Animal Units**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Large Scale Activities**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Human Clinical Applications**: L2 L3 L4 L2 L3 L4 L2 L3 L4

### Project Ref 85/13.1

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<td>Class 2</td>
<td>&lt; 1 Litre</td>
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- **Non-GMM**: Consent Granted
- **Project notified under transitional arrangements**: N

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<tr>
<th>Historical Date of Additional Info</th>
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02/03/2022
Project Additional Information

Purposes of the contained use
The study of fungal virulence factors

Recipient or parental organism
Class 2 fungal species: Candida albicans, Candida spec., Sporotrich schenkii, and Cryptococcus neoformans var. neoformans C. neoformans var gattii.

Host/vector system
Vectors used to transfer fungal DNA onclude pBluescript derivatives, pSP72 derivatives, pUC19 and pUC derivatives

Origin & function
Genetic material involved originated from fungi, including the class 1 species Saccharomyces cerevisiae and Ashbya gossypii

Evaluation of foreseeable effects
We will delete or replace genes that are involved in diverse aspects of fungal virulence. Thus, we assume, that most strains created here will be less virulent due to reduced gene function

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid cultures will be treated with Dettol for 5 minutes. This is expected to kill >99% of microbes. Validation could be achieved by test plating of a de-contaminated culture prior to disposal. Solid cultures and contaminated solid waste will be collected in appropriate bins and autoclaved (126C, 30 minutes). Color-changing autoclave tape is used as the standard for a successful autoclave run. Autoclaving should eradicate all fungi completely.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The Genetic Safety Committee approved the risk assessment without further comments.

Project Containment

02/03/2022
Factors involved in the infection process of Trichophyton rubrum and other dermatophytes

Recipient or parental organism
Trichophyton rubrum (and related fungi) and Escherichia coli

Host/vector system
pUC18/19 or similar cloning vectors for E. coli will be used, and genes of interest will be cloned in E. coli. A hygromycin resistance cassette will then be inserted into these genes, and the resulting constructs will be used to transform T. rubrum. These vectors cannot replicate in E. coli, and will integrate into the genome via homologous
recombination, resulting in disruption of genes.

Origin & function

Donor organisms are T. rubrum and related organisms, and the genes of interest are those involved in the infection process. Initially we will work on putative keratin-binding proteins as well as keratinases, which are involved in early steps such as adherence. Other related genes may also be targeted.

E. coli JM109 or similar is used as a host for construction of vectors, and a hygromycin B resistance cassette (originating from E. coli) will be used as selection marker.

Evaluation of foreseeable effects

T. rubrum and other dermatophytes are the causative agents of fungal skin, hair or nail infections. Symptoms are usually mild. Genes that will be targeted for disruption include keratinases (which degrade keratin in skin, hair or nails), keratin-binding proteins, and other genes encoding components in the membrane or cell wall of the fungi. We do not seek to express the gene products in E. coli or T. rubrum - constructs are only made to create knock-out strains.

Risk of transfer of genetic material of T. rubrum mutants is very low, as only the hygromycin resistance gene is integrated into the chromosome. The plasmid itself (including e.g. the b-lactamase gene on pUC vectors) is lost during recombination of the vector with the chromosome (via a double-crossover). Presence of the hygromycin gene and absence of the remainder of the vector will be verified by PCR and/or Southern blotting. Vectors in E. coli are unlikely to transfer to other organisms, as the vectors used are non-mobilisable and risk of transfer to other organisms is low.

We aim to disrupt genes involved in the infection process, and any mutants constructed are thus less likely to cause disease. Nevertheless, the GMM could potentially cause an infection of skin, nail or hair. However, symptoms of dermatophytosis are mild. The disease is very common and is easily managed with antifungals. T. rubrum mutants will be resistant to hygromycin B, but this antibiotic is not used in humans (it is only used in veterinary medicine as an antihelminthic).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Good microbiological practice techniques will be used for this project (as it is for all projects in the laboratory), and all work with fungi will be done in a class 2 biosafety cabinet. Culturing of fungi and E. coli will only be on plates or in small-scale liquid cultures. Maximum volumes will be -1 L, but in most cases only a 5-10 ml scale is required. Fungi are normally cultured statically on agar plates. All waste will be sterilised by autoclaving, using 30 min at 1260°C. Autoclaves in the department are checked regularly.

Work surfaces are regularly cleaned with "Distel", a laboratory disinfectant suitable for fungi (and fungal spores). This disinfectant contains a mixture of quaternary ammonium and biguanide compounds, all of which have proven activity against fungi. We will also conduct our own experiments to validate the activity of this disinfectant against T. rubrum spores. We will test this using a suspension test (following pharmaceutical standards). In addition, we will spot a known number of fungal spores (10^6-10^8) on a work surface, followed by drying for 1 hour and then treatment with diluted concentrations of the disinfectant for 30 sec-1 min. Swabbing and plating on Sabouraud agar plates will then be used to check for any surviving organisms.
Comments on draft:
It all looks fine. The one thing I would add is some method of verification that sterilisation/disinfection procedures work.
DB
You might want to add a sentence saying you'll confirm that each T.rubrum targeting construct doesn't have any bla sequences
TP
Will that be done so that there is a homology region to the left and right of the hygromycin cassette? Will the gene be merely interrupted or completely deleted? Not even the vectors will be integrated, just the hygromycin cassette. The vector backbone will be lost through the double cross-over.
SG
Comments on final version:
Looks fine to me now.SG

Please enter comments on the GM safety committee on the risk assessment

Comments on draft:
It all looks fine. The one thing I would add is some method of verification that sterilisation/disinfection procedures work.
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Comments on final version:
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Project Ref 85/17.1

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<td>21/06/2017</td>
<td>Investigating specific aspects of epithelial cell biology by gene interference</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
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02/03/2022
**Project Additional Information**

**Purposes of the contained use**
Exploring the role of specific cellular proteins in regulating epithelial cell functions (mammalian cell lines) and their potential involvement in cellular processes in health and disease.

**Recipient or parental organism**
Mammalian cell lines used in laboratory research (Caco-2, HT-29, MDCK and T84).

**Host/vector system**
Replication incompetent lentivirus (commercially-available).

**Origin & function**
Lentivirus is originally derived from human immunodeficiency virus (HIV). Viral particles will be used to transduce cell lines.

**Evaluation of foreseeable effects**
We can anticipate that the possibility of transmission by direct injection is minimized as no hypodermic needles are used in any step using this vector. Aerosolizing the virus, and thus pulmonary exposure, is minimized as all studies will be performed in a bio-safety cabinet. Skin exposure will be minimized through the use of appropriate personal protective equipment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Work including viral particles handling and transduction of target cells will be carried out in a dedicated bio-safety...
culture hood. A beaker containing a 1 % of virkon solution for inactivation of virus samples and infected target cells will be placed inside the hood. Contaminated samples will sit for 24 hours in this solution before disposal to the drain.
All dry waste generated will be sent for autoclaving before disposal (127 °C for 30 min).

13. Is an emergency plan required according to regulation 21?
   DYes [ ] No [ ]
   o If 'Yes', please check to confirm that it is attached to this form

14. [ ] Please check to confirm that you have attached a risk assessment to this form (note 12)
   o Please check if you are claiming exemption from disclosure for sections of the risk assessment

15. [ ] Please enter comments of the genetic modification safety committee on the risk assessment (note 13)

---

Comment from GMSC on a draft copy of the risk assessment:
Please include some information on the types of genes that will be over expressed or knocked down. It's good to make description fairly broad, but at the moment there is no information at all so it is difficult to make any judgement on what the risk to health or the environment is.
This was addressed to the satisfaction of the GMSC members and the risk assessment approved.

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<th>Is an emergency plan required according to regulation 20?</th>
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Please enter comments on the GM safety committee on the risk assessment

Comment from GMSC on a draft copy of the risk assessment:
Please include some information on the types of genes that will be over expressed or knocked down. It's good to make description fairly broad, but at the moment there is no information at all so it is difficult to make any judgement on what the risk to health or the environment is.
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</table>

Animal Units  
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities  
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications  
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |
Investigation of the biological properties (including photoprotective and antioxidant/therapeutic) of novel molecules including iron chelators on cell cultures.

We are interested in the role of iron in cellular oxidative stress occurring in environmental conditions, cancer, pathologies notably. We plan to investigate the biological effects on cells in culture, of novel bespoke compounds aiming at protecting cells against the deleterious effects of oxidative stress.

The cell lines we plan to use in this project are all within category RG2. Examples of GM cell models systems (and relevant references) we plan to use:

1) The Friedreich's Ataxia (FRDA) model systems: FRDA is a neurodegenerative and cardiodegenerative disease caused by a mutation within intron 1 of the frataxin (FXN) gene, in the form of a GAA triplet repeat expansion (up to 1700 repeats). This mutation results in the reduction of the levels of frataxin protein, a nucleus-encoded protein which migrates to the mitochondrial compartment where it is critical for the biogenesis and delivery of iron (Fe)-sulfur clusters. Recent studies provide evidence of its role in heme biosynthesis and mitochondrial iron storage (Reviewed in Burk K. 2017 Apr 7;4:4. Cerebellum Ataxias; Chiang S et al. 2017 Aug 4. Neurochem Int). Consequently, reduction in frataxin expression leads to oxidative stress, mitochondrial iron accumulation and cell death.

We plan to use mouse kidney fibroblast cell lines derived from a transgenic mouse model (called "GAA-repeatexpansion-based human frataxin transgenic model") of this disease: Y47R cells (derived from control mice) express normal level of FXN and YG8sR cells (derived from "FA-like" mice) express significantly reduced level of FXN (from Pock's laboratory, see Anjomani Virmouni S et al., 2015 Mar;8(3). Dis Model Mech).

We also plan to use EBV-immortalized human B lymphocytes (LCLs) obtained from repositories, from healthy individuals (e.g. AG15799, GM14406, GM05398, GM03798), unaffected carriers (e.g. GM15849, GM16200, AG15799, GM14406, GM05398, GM03798),
2) The N27 rat neuronal cell line is used as a Parkinson's disease (PD) model system. The N27 cells are neurons which have been harvested from E12 rat mesencephalic tissue and immortalized with SV40, so they can conveniently be studied in vitro. N27 cell line has been extensively characterized in studies on dopamine biosynthesis, neurotoxicity and used as a dopaminergic neuron model for in vitro and in vivo studies (from Dexter's laboratory, see Di Fruscia et al. 2015 Jan;10(1). ChemMedChem).

Characteristics of the host and/or host/vector system and any associated hazards to human health:
The level of risk to human health is low.

1) FRDA model systems:
Mouse Y47R and YG8sR cell lines: The vector is a Yeast Artificial Chromosome (YAC) containing the gene encoding the human frataxin protein (FXN) with respectively a short and a long stretch of GAA-repeats. The YAC is stably integrated in the mouse cell line genome and expresses the protein frataxin, at physiological (i.e. normal) level in Y47R cell line and at much lower level (i.e. similar to what is found in FA) in YG8sR cell line. No foreseeable associated hazards.

Human EBV-immortalized human lymphoblasts cells from healthy, unaffected carriers and FRDA patients: The vector is the whole EBV genome. It can persist as episome (multiple copies of circular DNA) or can be integrated into the host's genome (Hurley EA et al. 1991;65. J. Virol.).

2) Rat N27 cell line:
The vector is pSV3-neo, an expression vector derived from pBR322 and containing the large T antigen (TAg) gene of the SV40 virus under the control of SV40 regulatory sequences, SV40 origin of replication and Amp and Neomycin resistance genes for clone selection in bacteria and mammalian cells respectively (Prasad KN et al. 1994 Sep;30A(9). In Vitro Cell Dev Biol Anim). pSV3-neo is stably integrated in the genome of the host cells (therefore not episomal). It is not able to self-replicate in mammalian cells. No complete virus can be produced from such vector within the host cell. Therefore as such represents a low danger for the worker. Host (neurons from rat mesencephalic tissue) is incapable as such to grow outside of very specific optimal conditions of nutrients, temperature, pH etc which are provided in vitro by the culture medium. An SV40 immortalized line such as N27 would not be able to colonize a worker, unless possibly if the person would be strongly immunocompromised already.

Origin & function
Source, characteristics and intended functions of the inserted gene product and hazards arising directly from its use (including an estimation of the level of expression and biological activity of the recombinant gene product):
The level of risk to human health is low.

1) FRDA cell models:
Mouse Y47R and YG8sR cell lines:
Genetic material involved: the human frataxin (with low or high GAA repeat) gene sequence cloned into a YAC.
Function: stable expression of human mitochondrial protein frataxin which is involved in heme and iron-sulfur cluster biosynthesis and mitochondrial iron storage. There is no known hazard associated with these biological functions. In Y47R and YG8sR cell lines, the human frataxin is expressed at respectively physiological and FA disease level.
Reduced level of frataxin leads to high sensitivity to oxidative stress but this only occurs under very specific and rare pathologies such as FA. In transgenic mice, artificial overexpression of frataxin by up to tenfolds has been shown to have no deleterious effects. Natural situations of overexpression of frataxin are not known.

EBV-immortalized human lymphoblasts:
Genetic material involved: the whole EBV virus. Function: cell immortalization. In vitro generated LCLs, the virus expresses all of its proteins from its ca 172kbp genome (i.e. latency III). While individual infection with the virus is possible, it is highly unlikely to be an issue, notably because the vast majority of the population are infected asymptomatically at a young age or have had infectious mononucleosis. Seronegativity amongst laboratory workers is a very unlikely event. Nevertheless, cautions in handling is required as EBV combined with various factors (e.g. immunodeficiency, environment etc) has been linked with the development of various cancers.

2) N27 cell line:
Genetic material involved: elements of SV40 virus. Function: immortalization of the rat neurons by inactivation of tumour suppressor genes. The inserted gene product SV40 TAg drives immortalization of the rat cells and is a suspected oncogene. Nevertheless, grafting of N27 cells in mice did not lead to their proliferation nor induce tumour (Adams FS et al., 1996). In vitro immortalization of primary human cells via expression of TAg is a rare event, although TAg can efficiently delay cell senescence (Fields BN and Knipe DM. 1990. Virology, 1, 2. Raven Press, New York). Therefore overall the hazard for the user can be considered low.

Evaluation of foreseeable effects

As per the characteristics and the origin of the host and host/vector systems, there is n%r low foreseeable hazard to human health associated with the handling of the GMOs described in this form. The risk for human health could come from accidental inoculation during handling. However, this is unlikely to happen as appropriate RG2 handling protocols will be followed. Also, the possibility of transmission of the GMO to the worker by direct injection is minimized as no hypodermic needles are used in any steps of the techniques employed in our laboratory. Even if accidental inoculation should happen, an infection of the worker would be unlikely unless he/she would be strongly immunocompromised already.
Risk of aerosolizing any cells (e.g. virus-immortalised), and thus pulmonary exposure, as well as spillage are minimized as all studies will be performed in a class 2 bio-safety cabinet. Skin exposure is also minimized via the use of appropriate personal protective equipment (lab coat, gloves, safety glasses).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All handling of the cells lines described will take place within a containment class 2 laboratory (building 5W, 2.20 outer and inner), within a designated tissue culture area, under Class 2 safety cabinet, and using established code of practice (SOP) for work at containment level 2 which will be sufficient to keep contact with humans and the environment to negligibly low levels.
Contaminated sharps (e.g. Pasteur Pipettes) will be disposed of in designated yellow biohazard bins for "sharps" destined for incineration. If contamination of centrifugation equipment (e.g. buckets, seals and lids) should occur, these items will be submersed in Virkon ® (as per manufacturer's recommendations of use), then thoroughly rinsed with water prior to reuse.
Any non-sharp contaminated material (e.g. disposable gloves, plastic tips and pipettes, and vessels) will be disposed of in biohazard bags destined for autoclaving. The routine regime in the Department of Pharmacy & Pharmacology at the University of Bath is 126 degC for 30m in.
Dedicated pipettes (e.g. Gilson) will be used for the class 2 work and parts potentially exposed to contamination will be regularly soaked in Virkon®.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

A number of the lines have already been described in publications by our collaborators/providers were supported by a citation or two (which is a general point) and it's not really very clearly written: the Frataxin gene (FXN) is the one in which the triplet expansion occurs in Friedrich's Ataxia but this isn't stated, its function isn't really known and it's a little misleading to say it's a mitochondrial protein - yes, it's in mitochondria, but it's encoded by the nucleus.

Amend the document in line with the above comment.

Project Containment

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Project Ref 85/18.1

Date Ackn'd 27/04/2018

CU2 Project Title Fungal functional genomics and pathogenicity

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

02/03/2022
FUNCTIONAL GENOMICS: GM fungal strains will be generated to 1) remove a native gene, 2) re-insert the same native gene (complementation), or 3) insert the native gene fused to a reporter protein such as GFP, or alternative promoter sequence. This will enable the study of gene function and localisation. The comparative analyses of the parental and GM fungal strains described below (section 7) will identify differences in the ability of GM fungal strains lacking individual fungal genes to complete different aspects of their biology. This will assist in the discovery of the function of the respective fungal gene and its contribution to fungal disease. The identification of fungal genes important to disease and mycotoxin production, plus increased knowledge of their function, will advance the development of novel strategies to control fungal borne diseases and mycotoxin contamination.

RECIPIENTS: The application of fungal functional genomics and the assessment of genes/biological processes involved in pathogenicity and mycotoxin production will include fungi from Fusaria and Aspergilli. The characteristics of each genus is described below, followed by the potential risks of the resulting GMOs.

FUSARIA: Fusarium graminearum and F. culmorum are fungal ascomycete pathogens of small grain cereals, and while other members of the genus Fusaria have been associated with infections of immunocompromised humans i.e. F. solani and F. oxysporum, this is not the case for F. graminearum and F. culmorum. However, F. graminearum and F. culmorum can produce the DON (deoxynivalenol) and ZEA (zearalenone) mycotoxins, which are harmful to humans if ingested. DON and ZEA are produced specifically during plant infection, and under specialised in vitro growth conditions. Therefore, do not present a direct risk to human health during the handling of these fungi. Fusarium species do produces conidia, and also ascospores under specific conditions. These spores can cause allergic and respiratory issues in humans, similar to those caused by constituent high level exposure to pollen and dust. F. graminearum and F. culmorum are pathogens of small grain cereals including wheat, barley and maize, reducing grain yield, quality and safety due to the contamination with the DON and ZEA mycotoxins. Some academic studies have reported the ability of F. graminearum to infect other non-host plants, namely Arabidopsis and Soybean, under laboratory settings which do not naturally occur. Due to the predicted debilitated nature of the GM F. graminearum and F. culmorum strains, it would be predicted that the native UK Fusarium populations would outcompete the laboratory derived strains. The F. graminearum PH-1 strain originates from the USA and is therefore classified as a non-native pathogen. As a result of the genomic and genetic resources available for this strain, it used by all the prominent research groups in the UK and worldwide.
APSERGILLI: Aspergillus species are ubiquitous in the environment. They have been associated with infections of immunocompromised humans or those with chronic granulomatous disease, i.e., pulmonary aspergillosis. Aspergillus species are also food spoilage microbes and can produce mycotoxins (A. nidulans- Sterigmatocystin; A. fumigatus-Gliotoxin), which are harmful to humans if ingested. Aspergillus species airborne conidia, and also ascospores under specific conditions. These spores can cause allergenic and respiratory issues in humans, similar to those caused by constituent high level exposure to pollen and dust. Aspergillus nidulans and A. fumigatus are not pathogen of plants and present no risk.

RISK OF GMO TO HUMANS: The likelihood of non-GM or GM Fusarium strains causing harm to human health after direct exposure to the fungus is very low, as it is not a human pathogen. The likelihood of non-GM or GM Aspergillus strains causing harm to human health after direct exposure to the fungus is very low, as long as they are not immunocompromised. The ingestion of plant/food material infected with Fusarium or Aspergillus strains could be harmful to human health, through the impacts of the mycotoxins. But the likelihood of this occurring is very low. The chance of inhaling allergenic fungal spores during the preparation of suspension (the starting point for most experiments) is very low, as these suspensions will be prepared under sterile conditions within a CAT2 microbiological safety cabinet.

RISK OF GMO TO ENVIRONMENT: F. graminearum, F. culmorum, A. nidulans and A. fumigatus are not reported to be a pathogen and harmful to other organisms beyond the previously discussed animals and plants. The exception being the feeding of humans/animals with grain or plant produce contaminated with fungal mycotoxin. The GM Fusarium or Aspergillus strains would have the capacity to survive and propagate in the environment, on small grain cereals and dead plant biomass within the soil or food produce. However, due to the predicted debilitated nature of the GM strains, it would be predicted that the native UK fungal populations of these pathogens would outcompete the laboratory derived strains.

RISK OF GMO WITH INCREASED VIRULENCE: None of the genetic modifications to the CAT2 fungal pathogens are envisaged to increase fungal virulence. In fact, of the 1250 F. graminearum GM strains documented within www.PHI-base.org, only 8 have increased virulence, demonstrating that it is difficult and very rare to increase fungal virulence, and therefore the risk to human health. If the loss of gene function does cause increased disease, or mycotoxin production, experimentation will cease and the GMSC committee at the University of Bath and HSE will be notified.

Host/vector system

HOST: Whole plants or excised plant tissues (such as wheat, Maize and Arabidopsis) will be used as experimental hosts to compare the disease-causing, and mycotoxin-producing, abilities of parental and GM fungal strains. These studies will occur in the CAT2 controlled environment chamber or laboratory. The containment procedure and disposal of plant material is described in sections 8 and 12.

VECTOR: The DNA transformation cassettes will be constructed within E.coli via the Gibson protocol and the pGEMT vector, which is not mobilisable. DNA transformations cassettes will be PCR amplified from the vector carrying this cassette and purified prior to Fusarium / Aspergillus protoplast transformation. The vector will not be transformed into the fungal GM strains.

Origin & function

ORIGIN: The fungal DNA sequences will originate for the host Fusarium or Aspergillus strain, and will target the transformation cassette to a specific locus of the fungal genome by homologous recombination. The selectable markers encoding resistance to Hygromycin and Geneticin are of bacterial origin, while the auxotrophic marker are from Aspergillus species. These markers will permit the selection of positive fungal transformants on selective growth media. Fluorescent reporter proteins, such as GFP, come from the jellyfish Aequorea victoria. The fungal genes under
Investigation are conserved in closely related fungi and therefore their transfer to these fungal relatives is not believed

to present a potential hazard. The Hygromycin and Geneticin resistance genes are not present in related non-GM
fungi. However, these drugs are not used to treat fungal infections in the agricultural or clinical settings. The
auxotrophic marker are already present in environmental Aspergillus strains.

Intended Function: GM fungal strains will be generated by targeted transformation, inserting a fragment of DNA
plus a selectable marker into a specific locus of the fungal genome. This will either 1) remove a native gene, 2) reinsert
the same native gene (complementation), or 3) insert the native gene fused to a reporter protein such as GFP,
or an alternative promoter sequence. This will enable the study of gene function and localisation. Additionally, sexual
crosses between different GM or native strains may be used to incorporate the GM loci into different genetic
backgrounds within the same fungal species.

Evaluation of foreseeable effects

Evaluation of GMO: The comparative experiments between the parental and GM fungal strains described below
will enable the identification of differences in the ability of GM fungal strains lacking individual fungal genes to
complete different aspects of their biology. This will assist in the discovery of the function of the respective fungal
gene and its contribution to fungal disease.

Quantification of fungal growth in vitro on solid agar plates or liquid cultures.

Quantification of sexual and asexual sporulation in vitro.

Assessment of fungal colonisation of plants (host or non-host species) / plant derived biomass and quantification
of fungal biomass within plants.

Observation of fungal infection within plant material via microscopy, including 1) stereomicroscopy to visualise
macroscopic appearance, 2) scanning electron microscopy to visualise fungal colonisation on the surface or within
plant tissues, and 3) histology of ultrathin sections of plastic embedded materials via light or transmission electron
microscopy.

Observation of the localisation of the fungal reporter proteins or immune-labelled proteins during in vitro culture or
plant colonisation via fluorescence microscopy.

Biochemical analyses (western blots) to determine protein localisation.

Quantification of fungal toxins within infected plant materials or during in vitro culture under specific conditions, via
a specific ELISA based assay.

Extraction of fungal and/or plant RNAs, proteins and metabolites for transcriptomic, proteomic and metabolomic
analyses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

CAT2 Laboratory: Only fully trained persons or trainees under the supervision of fully trained project group
members will be permitted to use the facility. Access to the CAT2 facilities will be controlled by Drs N. Brown and V.
Cervik. The handling and storage of all CAT2 fungal pathogens will only occur within the CAT2 laboratory and the
CAT2 control environment chamber, where users will wear a dedicated lab coat and disposable gloves to avoid
transfer beyond the CA T2 environment. A sticky matt will be placed at the entrance to the facility to reduce the risk of
fungal spores being transferred on shoes beyond the laboratory. Although the CAT2 fungal pathogens are usually
contained within the CAT2 microbiological safety cabinet or the CAT2 control environment chamber, if contamination
of the main laboratory occurs, then the appropriate decontamination procedure will be performed. Following
contamination of a local area, debris must be swept up and autoclaved and the area disinfected (Microsol 3 at 1 :50
dilution). If the contamination is general then all surfaces should be thoroughly vacuumed and washed down with a
disinfectant. (Microsol 3 at 1 :50 dilution). If there is considered to be any risk from spores or insect vectors, which is
unlikely to occur, then a full decontamination with Formaldehyde should be considered.
CAT2 STORAGE: Fungal pathogens can be stored doubly contained on sealed agar plates or in sealed vials, contained within boxes or within the refrigerator/freezer provided within the CAT2 laboratory. For long term storage, fungal spore suspensions be stored within a dedicated CAT2 -80°C freezer.

CAT2 MICROBIOLOGICAL SAFETY CABINET: The preparation of all CAT2 fungal pathogen spore suspension will be performed in a CAT2 microbiological safety cabinet, and all CAT2 waste will be autoclaved, protecting the user and the environment for the pathogen. After use the surfaces will be decontaminated with 70% ethanol. After changing the filters of the CAT2 microbiological safety cabinet, the used filters will be autoclaved upon removal. Annually or after a contamination event, the CAT2 microbiological safety cabinet will be decontaminated via washing the surfaces with a disinfectant (Microsol 3 at 1 :50 dilution), and a full decontamination with Formaldehyde.

CAT2 CONTROL ENVIRONMENT CHAMBER: Access to the CAT2 facility will be controlled by Dr N. Brown. Users will wear dedicated lab coats and disposable gloves, which will stay within the CAT2 facility, to avoid transfer beyond the CAT2 environment. A sticky matt will be placed at the entrance to the facility to reduce the risk of fungal spores/plant pollen being transferred on shoes beyond the laboratory. Infected plant material and any other waste from the CAT2 control environment chamber will be transferred in an autoclave bag within a sealed metal bin, which will be autoclaved directly, without opening. The CAT2 control environment chamber may be required to be decontaminated following the arrival of unwanted pests or pathogens that could increase the risk of either the licensed organisms being distributed to unintended locations within the facility, or licensed organisms being released or escaped into the general environment. Following any contamination the room should be thoroughly vacuume, all surfaces washed down with a disinfectant. (Microsol 3 at 1 :50 dilution). The room should be run at 40°C for 48 hours minimum. If there is considered to be any risk from spores or insect vectors then a full decontamination with Formaldehyde will be considered.

TRANSFER OF CAT2 MATERIALS: The transfer of conidial suspensions of any of the CAT2 fungal pathogens to the CAT2 control environment chamber, where plants are grown, will occur in a double sealed container to prevent the risk of escape. Similarly, the transfer of any fungal or plant material to the CAT2 laboratory for downstream processing or the microscopy suite will occur in double sealed container to prevent the risk of escape. All waste will be returned using the same approach to the CAT2 laboratory for inactivation and disposal.

MONITORING CAT2 USAGE: A log of authorised users of the CAT2 facilities and the CAT2 organisms being used will be kept by Drs N. Brown and V. Cervik. Users will have to read and sign the SOPs for the use of the CAT2 facilities prior to starting their independent work. Signed SOPs will be kept by Drs N. Brown and V. Cervik. Within the CAT2 facilities, log books will record the efficacy of the pathogen inactivation procedure (double kill procedure) and any incidence that require decontamination. Additionally, the dates when routine decontamination procedures were performed. An electronic record will be kept of all fungal plant experiments performed in the CAT2 growth chamber including the experimenter, project code, plus the start and end date, noting any alteration in fungal virulence.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

CAT2 WASTE DISPOSAL: Solid and liquid waste produced in category 2 facilities must be decontaminated using steam sterilisation for 30 min at 121 C. For this reason no packaging material (i.e. cardboard boxes) should be brought into the facility to keep waste to be autoclaved at a minimum. The facility will be equipped with one or more pedal bins or autoclavable aluminium bins fitted with autoclavable bags, which can be used to dispose of smaller waste items. Lab coats which need to go to the laundry, should be deposited in a sealed autoclave bag. Liquid waste should be deposited in autoclavable glass bottles to allow chemical inactivation (Microsol 3 at 1 :50 dilution) followed by steam sterilisation, i.e. a double kill microbiological procedure. The validation of the degree of kill will be confirmed.
annually via performing the double kill procedure on a spore solution and then counting colony forming units.

The University Genetic Modification Safety Committee (GMSC) asked for more information to be provided on the mobilisation of vectors within the risk assessment. The risk assessment was amended to the satisfaction of the GMSC.

Please enter comments on the GM safety committee on the risk assessment

The University Genetic Modification Safety Committee (GMSC) asked for more information to be provided on the mobilisation of vectors within the risk assessment. The risk assessment was amended to the satisfaction of the GMSC.

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Project Ref 85/18.2

Investigation of the biological processes of self-renewal and terminal differentiation in cultures of normal and neoplastic human epithelial cells

Date Ackn'd 26/10/2018

Date Project Ceased

Class 2 Culture VolClass2 < 1 Litre

Consent Granted

Project notified under transitional arrangements
Stratified epithelia (e.g. skin epidermis, oral and tongue epithelia, esophagus epithelium) are essential for our survival as terrestrial beings. They provide protection from harmful microbes and other assaults from the external environment, and retain body fluids. To withstand normal wear and tear, these epithelia constantly self-renew. This ability resides in tissue-resident stem cells, which self-renew, preserve, and repair their tissue during homeostasis and following injury. Studying the normal process of tissue homeostasis is providing us a foundation to understand how these processes go awry in human diseases, including chronic wound-healing, hyper-inflammatory disorders and epithelial cancers.

The purpose of this work is to understand the biological mechanisms which regulate the processes of stratified epithelial stem cell self-renewal, differentiation, dysplasia and neoplastic progression. We have already identified several important components of the intracellular signalling pathways (Mishra et al., 2017; Walko et al., 2017) and we now propose to manipulate these proteins using recombinant molecular biology technology to deliver selected genes to cultured normal and dysplastic/neoplastic human epidermal cells and further dissect their roles in cell fate determination.

Commonly used customised or commercially available retroviral and lentiviral vector systems will be employed to deliver gene constructs to human epithelial cells. The genes may be those directly associated with proliferation such as proto-oncogenes or genes which produce protein components of mitotic signalling pathways. Other intracellular signalling pathways are more important for initiating cell differentiation, such as the Wnt and Hippo pathways and we will analyse the proteins which contribute to them.

Stem cell fate decisions are also made in response to their “niche” or local micro-environment and cell surface protein receptors transduce this information to the nuclear transcription machinery. Integrins, Cadherins, Delta and Notch proteins are all directly involved in cell-cell or cell-niche interactions.

Over-expression of selected relevant proteins in undifferentiated epidermal cells using cDNA constructs can yield useful information about their function. However, it can also be highly informative to suppress expression of certain genes, also resulting in modified cell phenotypes and we can employ specifically designed shRNAs to target these genes.

screen identifies YAPIWBP2 interplay conferring growth advantage on human epidermal stem cells. Nature communications. 8:14744.

Recipient or parental organism

HaCaT is a spontaneously transformed aneuploid immortal epidermal cell line from adult human skin (Boukamp et al., 1988). We will also use a panel of cutaneous squamous cell carcinoma progression cell lines derived from the parental HaCaT cells. These include the benign tumorigenic HaCaT-RAS AS cells, the malignant HaCaT-RAS 11-4 cells and the metastatic HaCaT -RAS AS-RT3 cells (Boukamp et al., 1990; Mueller et al., 2001). PM1-4 are clonal epidermal cell lines derived from adult dysplastic human forehead skin (Proby et al., 2000).

Human squamous cell carcinoma lines (e.g. SCC4, SCC12, SCC13, SCC-NR, MET1-4, SCL-1, SCL-II) have been derived from tumour tissue (Boukamp et al., 1985; Proby et al., 2000; Rheinwald and Beckett, 1981; Tilgen et al., 1983). They are capable of unlimited proliferation due to spontaneous genetic mutations present in carcinoma tissue. Human oral squamous cell carcinoma lines (e.g. SJG-003, SJG-006, etc) have been derived from tumour tissue (Benaich et al., 2014; Hayes et al., 2016). They are capable of unlimited proliferation due to spontaneous genetic mutations present in carcinoma tissue.

Primary human epithelial cell cultures have been derived from normal skin biopsies (neonatal foreskin; strains Km, Kp, etc.) (Estrach et al, 2007; Mulder et al., 2012; Walko et al., 2017). They have been banked at passage 2 and are capable of limited propagation up to 10 passages.

Additional stocks of primary human epithelial cell cultures will also be established for the proposed work from surgical waste from healthy donors and tissue biopsies from patients with cancer for the proposed work once ethical approval has been obtained from the NHS.

3T3 mouse fibroblasts will be used as a feeder layer to allow human epithelial cells to be grown at clonal seeding density under conditions that preserve the stem cell state for a several passages (Rheinwald and Green, 1975).

For certain biochemical/cell biological studies we might also use well characterised human simple epithelial cell lines. These could include: HEK293T (ATCC CRL-3216), MCF7 (ATCC, HTB-22), BT474 (ATCC-HTB-20), T-47D (ATCC, HTB-33), MCF10A (ATCC, CRL-10317), MCF10F (ATCC, CRL-10318), MDA-MB-453 (ATCC, HTB-131), MDA-MB-231 (ATCC, HTB-26), MDA-MB-436 (ATCC, HTB-26), MDA-MB-361 (ATCC, HTB-27), MDA-MB-468, ATCC, HTB-132), BT549 (ATCC, HTB-122), ZR-75-1 (ATCC, CRL-1500), SK-BR-3 (ATCC, HTB-30), Caco-2 (ATCC, HTB-37).

The level of risk to human health is medium.
Phoenix ECO cells are a second-generation gamma-retrovirus producer line for the generation of helper free ecotropic retroviruses (ATCC CRL-3214)(Swift et al., 2001). This line is based on the 293T cell line (a human embryonic kidney line transformed with adenovirus E1 a and carrying a temperature sensitive T antigen co-selected with neomycin). The Phoenix ECO line stably expresses Gag-Pol proteins and an ecotropic Env protein (for infection of mouse and rat cells), eliminating the need to deliver these genes in trans by specific plasmids. Gag-Pol and Env proteins are driven by different promoters, minimizing their inter-recombination potential.

Health and Safety

Executive

AM12 cells (ATCC CRL-9641) are an amphotropic gamma-retrovirus packaging cell line derived from 3T3 mouse fibroblasts in which the gag, pol, and env genes of the helper virus are separated on two different plasmids and in which the packaging signals and 3’ long terminal repeats are removed (Markowitz et al., 1988). The HEK293T (ATCC CRL-3216) and HEK293FT (https://www.thermofisher.com/order/catalog/product/R70007) cell lines are highly transfectable derivatives of human embryonic kidney 293 cells, and contain the SV40 T-antigen (Pear et al., 1993). They will be used for packaging of lentiviruses.

The replication-incompetent gamma-retroviral vectors are designed to greatly reduce the probability of the production of replication-competent retrovirus by homologous recombination. The transfer vectors to be used (e.g. pBABE-puro, https://www.addgene.org/1764/) are derived from Moloney Murine Leukemia Virus. These widely used vectors provide the viral packaging signal, transcription and processing elements, and a target gene, but the gag, pol and env genes are not present (Morgenstern and Land, 1990). Packaging requires the use of helper-free packaging cell lines that provide the genes required for retroviral packaging and transduction in trans (Markowitz et al., 1988; Swift et al., 2001). This means that none of the structural genes are actually present in the packaged viral genome. Moreover, in the packaging cell lines Gag-Pol and Env proteins are driven by different promoters, minimizing their interrecombination potential (Markowitz et al., 1988; Swift et al., 2001). Consequently, the recombinant gammaretroviruses can transduce target cells, but the transduced target cell does not produce additional virus.

All lentiviral transfer vectors are second or third generation vectors. Lentiviral transfer vectors will include the pLenti vectors (third generation) in the ViraPower™ expression system (ThermoFisher), the SMARTvector lentiviral shRNA vectors (second generation) in the constitutivelinducible SMARTvector lentiviral shRNA expression system (Dharmacon), and pLKO.1 and TRC-pLKO lentiviral shRNA vectors (second generation) in the MISSION lentiviral shRNA expression system (Sigma)). Lentiviral packaging vectors will include the pLP1, pLP2, and pLPNSV-G vectors in the third generation ViraPower™ expression system (ThermoFisher), and the psPAX2 (Addgene, 12260, https://www.addgene.org/122601) and pMD2.G (Addgene, 12259, https://www.addgene.org/12259/) vectors for packaging of second generation lentiviral expression vectors. These vectors include a significant number of safety features:

All lentiviral transfer vectors contain a deletion in the 3’ LTR (t.U3) that does not affect generation of the viral genome in...
the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.

I- The number of genes from HIV-1 that are used in the systems has been reduced to four (Le. gag, pol, rev and tat in psPAX2, second generation system) or three (Le. gag and pol in pLP1 and rev in pLP2, third generation system).

- The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns et al., 1993; Emi et al., 1991; Yee et al., 1994).

I- Genes encoding the structural and other components required for packaging the viral genome are separated onto three (second generation systems) of four (third generation) vectors. All vectors have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).

- Although the packaging vectors allow expression in trans of proteins required to produce viral progeny (Le. Gal, Pol, Rev, Tat, Env) in the HEK293T and HEK293FT producer cell lines, none of them contain LTRs or the 4J packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.

- The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.

I- Expression of the gag and pol genes from pLP1 has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al., 1998).

- A constitutive promoter (RSV promoter) has been placed upstream of the 5'L TR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA (Dull et al., 1998).


Origin & function

The inserted genetic material will be varied. These genes may be those directly associated with proliferation such as proto-oncogenes (e.g. YAP and TAZ, WBP2, beta-Catenin, Lef/TCF) or genes which produce protein components of mitotic signalling pathways (e.g. Erk1/2, Mek1/2, Ras, Raf), or those that are more important for initiating terminal cell differentiation (e.g. TP63, Jun/Fos, Lats1/2) or controlling cytoskeletal dynamics (e.g. RhoA, RhoE, Rock1/2, Rac, Cdc42). We might also examine cell surface-associated cell-cell and cell-matrix signalling receptors including integrins, cadherins, and Notch ligand- and receptor proteins that are all directly involved in stem cell-niche interactions. In addition, we will investigate genes/proteins which yet unknown function (previously identified in genome-wide screens; Walko et al. 2017) in the normal and neoplastic epidermal (stem) cell context.
These various genes will be under the control of constitutive or cell/tissue-specific promoters. Other genes used will be reporter genes such as GFP to track pattern of expression of a protein or as a guide of the activity of a promoter. Antibiotic resistance genes will be used so that selective pressure can be applied to isolate only cells that have been transduced. We will also use vectors expressing (either constitutively or inducibly) shRNA molecules to selectively silence the expression of endogenous genes.


**Evaluation of foreseeable effects**

Human epithelial cells are derived using skin biopsies. The likelihood of adventitious pathogens will be increased if cells are sourced from populations known to have an elevated prevalence of HepB, HepC, HIV, HPV or other pathogens. Therefore, low risk patients are selected by interview prior to surgery at the time consent is given and iv drug users, those who have received blood transfusions or have a lifestyle likely to suggest a high risk of infection with blood borne viruses, are excluded. Alternatively, patient material may be screened for HIV, HepB or C prior to use in cell culture and positive samples excluded from further analysis. Prophylactic Hepatitis B vaccination will be offered to staff in contact with human cells by the Occupational Health Department.

There is a possibility that the expressed proteins may alter cell physiology and pharmacological response, as some of them may functions as proto-oncogenes or tumour suppressors. Consequently, the expression of an oncogene in the host cell will increase proliferation and survival and could induce oncogenic cell transformation. Likewise, the shRNA-mediated down-regulation of a tumour suppressor protein could cause similar effects on cell physiology. However, it is unlikely that expression of a single oncogene would cause tumours, as multiple genetic alterations (between 4 and 6) are required for inducing tumours, and safeguard mechanisms exist impeding the accumulation of these alterations in the organisms. No infected cells will be propagated indefinitely.

The viral production systems are widely used and generally considered safe. The replication-incompetent gammaretroviral- and lentiviral vectors are designed to greatly reduce the probability of the production of replicationcompetent retro- and lentivirus by homologous recombination. The potential for generation of replication-competent retro- or lentivirus is specifically addressed by the design of the vectors and by Good Laboratory Practice. The VSV-G enveloped pseudotype virus particles produced have a wide host range, which includes humans and can infect many tissues. However, each particle can only infect one cell and not give rise to further virus production. Also, expression of the VSV glycoprotein on the plasma membrane of any infected host cell would immediately present a target for the immune system. Retro- and lentiviruses are very unstable due to their fragile envelope protein hence VSV-G is used to increase stability of the recombinant viral particles. Wild type VSV-G can survive for 3-4 days in the natural environment (in saliva etc). It is therefore reasonable to assume that any escape of virus in untreated waste may be active for a few days. However, the viral particles outside of the closed controlled conditions of cell culture are rapidly inactivated by dehydration or other environmental insult, and so scope for spread via the environment is severely limited.

In the case of a laboratory accident there is a possibility the virus may infect a member of laboratory personnel. Care must be taken to avoid the spread of infectious material by aerosol (e.g. in the event of accidental release of bacterial cultures in a shaking incubator), direct contact (e.g. through contact with mucous membranes via splashes or spills) or accidental needle stick injection. If the virus vector entered the blood stream it is likely the vector would be delivered to a tissue where it is biologically active. Entry gained by an aerosol may infect the respiratory epithelium but in this and the latter case expression of the gene would depend on the promotor used.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
All procedures involving culture of human cells and handling of recombinant virus (virus particle production and infection of cells) will be performed in ADCP containment level 2 laboratories (University of Bath, Department of Biology and Biochemistry, 4 South Building, Rooms 1.38 (dedicated virus production laboratory) and 0.7 (cell culture laboratory)). Class 2 microbiological safety cabinets equipped with double HEPA filters will be used when handling virus and for culturing cells post-infection for downstream analysis. This will greatly reduce the risk of spread of infectious material by aerosol or direct contact. No hypodermic needles will be used in any steps of the techniques employed in our laboratories. Access to the virus laboratory (Room 1.38) will be restricted to authorised personnel only and the laboratory door will be kept closed at all times except for access.

Good Laboratory Practice will be maintained at all times. Skin and eye exposure will be minimised via the use of appropriate personal protective equipment (lab coat, gloves, safety goggles). Good hygiene will be maintained, and work surfaces will be regularly disinfected (ChemGene HLD4H, 1:20 dilution), but especially following manipulations using viruses.

Solid contaminated waste (plastic pipettes, flasks, etc) will be placed in a Biohazard bag prior to removal from the Class 2 microbiological safety cabinet, enclosed into a second bag outside the cabinet and carried to the autoclave room. Contaminated solid waste will be autoclaved at 126°C for 30 min, completely destroying all biological material including the viral particles.

Contaminated sharps (e.g. plastic Pasteur pipettes) will be disposed of in designated yellow Biohazard 'sharps' bins destined for incineration.

Contaminated liquid waste will be disinfected with an appropriate disinfectant (e.g. 10% bleach) for at least 30 minutes.

Spillages will be inactivated with 5% ChemGene HLD4H spray, collected with absorbent material and disposed of via autoclaving.

If contamination of centrifugation or other laboratory equipment (e.g. buckets, seals, lids, pipettes) should occur, these items will be submerged in 5% ChemGene HLD4H, then thoroughly rinsed with water prior to reuse.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No comments were made by the committee.
### Project Additional Information

#### Purposes of the contained use

The aim of the project is to utilise molecular genetic approaches to investigate the genetic basis of virulence, biofilm formation and antimicrobial resistance in a range of opportunistic bacterial pathogens (both Gram negative and Gram positive species). All species to be studied are ACDP Hazard Group 2 pathogens. A greater understanding of the genetic basis of these processes and the underlying gene regulation, will lead to novel strategies for the prophylaxis and treatment of diseases associated with these organisms. The interaction of these organisms with bacteriophage (viruses which infect and kill host bacteria) will also be studied on a genetic level, with the view to generating novel bacteriophage based antimicrobials. Because all organisms to be studied will be subjected to the same molecular genetic techniques (described in detail in the attached RA: mutagenesis, construction of gene libraries, DNA cloning, and Genetic complementation), the same attributes investigated in all organisms (virulence, biofilm formation, antimicrobial resistance, interaction with
bacteriophage), and the same hazard group assigned to all organisms, they are included within the same genetic modification risk assessment.

Recipient or parental organism

Parental organisms (all ACDP Hazard Group 2):
Proteus mirabilis,
Pseudomonas aeruginosa,
Klebsiella pneumonia,
Moraxella catarrhalis,
Acinetobacter baumannii,
Staphylococcus aureus,
Staphylococcus epidermidis,
Streptococcus pyogenes,
Streptococcus pneumoniae,
Streptococcus agalactiae,
Streptococcus bovis,
Enterococcus faecalis
Enterococcus faecium.

Recipient organisms:
Standard attenuated Eschericia coli strains, e.g. disabled K12 type cloning strains. These have a long history of safe use with no reported harmful effects. Unlikely to survive outside the laboratory or initiate infection. Hazard group 1.
Lactococcus lactis. This is also extensively used as a cloning host, and for peptide surface display. This organism is non-hazardous, non-colon ising and non-pathogenic. Unlikely to survive outside laboratory. Hazard group 1.
Bacterial species listed as arental organisms will function as recipients ONLY for self-cloning experiments (e.g. complementation - as covered in detail in the attached RA). Hazard Group 2
No activities will increase the Hazard Group of resulting GMMs above that of the original parental organisms - Hazard Group 2

Host/vector system

A range of vector systems will be used to study the organisms listed, but all share key features relevant to containment of GMMs. No vectors capable of self-transfer between bacterial species will be used. All vectors to be used require introduction through laboratory manipulation (Electroporation, transformation of chemically competent cells, conjugal transfer from specialised hosts). The vector systems to be used include narrow host range vectors, broad host range vectors, and expression vectors. All selectable markers present are naturally occurring, and already present in the wider environment. The majority of vectors to be used are commercially available and all have a history of safe use. Small insert peptide display library vectors: such as pVESS47, pHIE11, pACL 1, pUvBBAC. Vectors contain open reading frames for cell wall anchored proteins. Cloning of DNA fragments into this region results in display of peptides on bacterial cell surface. As cloned DNA is expressed from the promoter associated with the surface anchored protein, for risk assessment purposes these vectors will be treated as expression vector systems. Standard cloning vectors: Narrow host range vectors such as pUC18, pUC19, pTOP040and pGEM-T will be used. For construction of genetic libraries in standard E. coli cloning strains, narrow host range, large insert vectors such as pCC1 FOS, pCC1 BAC, pBleoBAC will also be used. Reporter gene constructs: Reporter gene vectors such as, pPL2lux, pTCV-lac, or derivatives of pUC18 and pUC19 such as p18GFP, will be utilised to construct small insert reporter gene libraries. Mini-TnS based transposons will also be developed which incorporate reporter genes such as gfp, lacZ, luxAB, or phoA during this study. Broad host range vectors: Broad host range vectors, such as pC1372 capable of replication in E. coli and L. lactis will
be used to transfer DNA between these species only. Broad host range vectors such as pUCP, and derivatives such as pSCOSBC1 (and derivatives such as pSCOSPA1), pAT28 (derivative of pUC with a broad host range Grampositive origin of replication), pRMC2 (shuttle vector permitting expression of genes from a TetR-controlled promoter), pCN34 (broad range Gram-positive expression vector), pB4 (derivative of plasmid pOT182 lacking the TnS-OT182 transposable element), or plasmids with temperature-sensitive origins of replication such as pL T06 and pKOR1 will be used to construct genetic libraries, deliver constructs for site-directed mutagenesis and undertake self-cloning based genetic complementation studies in species to be studied.

Transposons: For random transposon mutagenesis transposable elements will be maintained on narrow host range vectors in disabled E.coli or L. lactis cloning hosts, and TnS based elements will primarily be used. These will also be used in in vitro transposon experiments in which they are not maintained in any vectors. TnS based elements have been extensively used for mutation based analysis of bacterial gene function and integrate stably into target DNA molecules. Narrow host range suicide delivery vectors, such as pUTKm, and pRL27, which cannot replicate outside of specialised disabled E. coli K12 based cloning strains, such as S17.1 lambda pir and SM10 lambda pir will be used to introduce TnS transposons into P. mirabilis, Ps. aeruginosa, K. pneumoniae or S. aureus. Introduction of the pUTKm or pRL27 is accomplished by conjugation between specialised E. coli cloning stains (e.g S17.1 lambda pir and SM10 lambda pir) and recipient organisms, however the pUTKm and pRL27 plasmids do not encode the necessary genes for self-transmission, and these are encoded only on the chromosome of the disabled E. coli donor strains. Furthermore, the pUTKm and pRL27 plasmids are unlikely to replicate outside the specialised E. coli host strains.

Genetic material involved in this work will encode a wide range of functions, related to virulence, biofilm formation and antimicrobial resistance. Some genetic material may be naturally expressed in standard E. coli or L. Lactis disabled cloning strains, but this will not increase the overall hazard above that originally assigned to bacterial species to be studied (Hazard Group 2).

**Origin & function**

**Evaluation of foreseeable effects**

Four main approaches will be applied to the genetic manipulation of the organisms to be studied, and used to identify the genetic basis of virulence, biofilm formation, antimicrobial resistance, and bacteriophage interaction with host bacteria. Bacteriophage capable of infecting and killing the above organisms will also be studied using the same molecular genetic approaches:

1) Mutagenesis
2) Construction of reporter gene libraries and surface display libraries
3) DNA cloning and sequence analysis
4) Genetic complementation

For mutagenesis based strategies (1), transposon DNA carrying selectable markers or reporter genes will be inserted into the chromosomal DNA of species to be studied, or genes of interest inactivated/removed by deletion through site directed mutagenesis based on homologous recombination. Such strategies result in the generation of attenuated mutants, with a reduced fitness in respect to the wild type organisms.

For strategies based on sub-cloning DNA from the organisms to be studied (2,3), cloned DNA will be transferred to standard E. coli or L. lactis disabled cloning hosts. As the cloned DNA may contain virulence genes that are expressed in the E. coli or L. lactis hosts, there is potential to increase the pathogenicity of these organisms.

However, as these inserts will be cloned in disabled level 1 recipient organisms (E. coli and L. lactis standard cloning strains) the overall risk is low and would not result in an increase in associated hazard group above that originally assigned to donor organisms (Hazard Group 2).
Complementation studies (4) involve the transfer of DNA between wild type organisms and derived mutants. As this is a self-cloning procedure (e.g., P. mirabilis donor DNA cloned in P. mirabilis host only) there is no additional risk above that originally assigned to donor organisms, and the overall risk is negligible. Genetic complementation is employed for restoration of a known wild-type phenotype lost in mutants and will not constitute a hazard above that of the original wild-type parent.

Overall inserted and donated genetic material is highly unlikely to increase the pathogenicity of recipient organisms, when using the proposed molecular genetic approaches and host species, and the overall risk associated with the proposed work is low.

Risk to Humans: All organisms to be studied are opportunistic pathogens and do not cause infection in normal healthy individuals. These organisms are already ubiquitous in the natural environment and/or as part of the normal human microbiome. The molecular genetic approaches to be employed will focus on generation of attenuated derivatives which are reduced in fitness compared to wild type organisms.

Risk to Animals: Species to be used do not normally constitute a risk to healthy animals. Infection in animals may also be treated as for humans. Transfer of DNA to E. coli or L. lactis cloning strains may introduce virulence genes to these organisms, but they are unlikely to survive outside the laboratory or pose a significant risk to animals, and the associated hazard will not be greater than that originally assigned to the donor species (Hazard Group 2).

Risk to Plants: Ps. aeruginosa and K. pneumoniae may cause opportunistic infections in plants, but are not typically considered to be plant pathogens. Other organisms have no known ability to cause infection in plants. However, all organisms and derived GMMs will be studied in the context of human infection, and will be handled exclusively in containment level 2 laboratories. All waste will be sterilised before disposal. Therefore, it is highly unlikely that the work undertaken will pose any risk to plants.

Risk to Environment: All GMMs and donor organisms will be handled according to ACDP containment level 2 procedures and as such are highly unlikely to disseminate from the laboratory. As all GMMs created will be the result of mutagenesis procedures, self-cloning, or transfer of DNA from hazard group 2 pathogens (into hazard group 1 disabled cloning strains (E. coli and L. lactis), the resulting GMMs will not possess an ability to displace environmental strains or survive in the environment above that of the original parental species.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be collected in robust, sealed vessels in leak proof secondary containers, and all other waste collected in robust leak proof autoclavable boxes or containers with lids. Waste will be sterilised by autoclaving (121 degrees C for 15 min). Autoclaving will be monitored using standard chemical indicators and the integral diagnostic output of autoclave units, which monitor parameters of each cycle. All autoclaves are regularly serviced and performance validated. Sterilised liquid waste will be disposed of as for standard wastewater. Sterilised solid waste will be disposed of through a specialist laboratory waste contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The University of Bath GMSC has reviewed the risk assessment for this work and approved the proposed procedures. No issues were identified by the GMSC.

# Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
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## Project Ref 85/20.1

- **Date Ackn'd**: 11/11/2020
- **CU2 Project Title**: Transducing primary cortical neurons with adeno-associate viruses (AAV) containing eGFP-tagged tau constructs
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **CultureVolumeClass3-4**: Non-GMM
- **Consent Granted**: Consent Granted

<table>
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- **Withdrawn**: Yes
- **Tick if notifying a connected programme of work**: Yes

## Historical Significant Changes
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

Project notified under transitional arrangements: Yes
### Project Additional Information

#### Purposes of the contained use

The purpose of using AAV viral particles containing GFP-tagged tau is to develop a more robust neuronal model of tau pathology. Tau is thought to be a key protein involved in the disease mechanisms of Alzheimer's Disease. By creating a neuronal model of tauopathy in vitro we can investigate these pathological mechanisms as well as the ability of dietary flavonoids to reduce this pathology.

#### Recipient or parental organism

Primary cortical neurons derived from embryonic CD1 mice or human neuroblastoma-derived SH-SYSY cells are the recipient organisms.

#### Host/vector system

- **pAAV-hSyn1-GFP plasmid (Addgene #50465)** is used as the vector.
- The AAV viruses are replication incompetent as the vector lacks the genes needed for AAV replication.
- The generation of AAV particles will be outsourced and the host will most likely be HEK293T cells.
- The WPRE element has been removed from the vector plasmid and as such there is no foreseeable risk of oncogenic effects.

#### Origin & function

- The genes of interest code for: GFP, GFP-TauWT or GFP-TauP301 L. GFP, GFP-TauWT and GFP-TauP301 L have been previously inserted in pRK5-CMV or pSF-hSyn1 vector backbones, and these constructs have been validated and characterised in primary cortical neurons. Both tau genes encode for human 4RON isoforms. These genes of interest have been subcloned into the pAAV-hSyn-EGFP plasmid. The hSyn promoter is neuronal specific and confers constitutive expression.
- Tau is a microtubule associated protein which is known to be associated with Alzheimer's disease pathology. The P301 L missense mutation is known to facilitate tau pathology and is sufficient to cause the disease Frontal Temporal Dementia with Parkinsonism on chromosome 17 (FTDP-17). The purpose of transducing cells with these genes is to investigate the pathological mechanisms of tau and whether dietary flavonoids can ameliorate these effects.

#### Evaluation of foreseeable effects

Although certain scenarios can be envisaged that the recombinant viruses may infect human cells, it is very unlikely that the recombinant viruses we intend to generate will cause harm to human health, due to the containment, neuronal-specific expression and replication-deficient nature of the recombinant viruses.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Notices will be placed on the laboratory door, the safety cabinet and the incubator instructing users in the event of...
accidents and spillages. The virus is inactivated using sodium hypochlorite (bleach) at a final concentration of 10%.
Solid waste (pipettes, flasks, etc.) will be placed in a Biohazard bag prior to removal from the microbiological safety

cabinet, enclosed into a second bag outside the cabinet and carried to the autoclave room.
Waste will be autoclaved at 126°C for 30 min completely destroying the viral particles. Contaminated liquid waste will
be disinfected with an appropriate disinfectant (e.g. 10% bleach or 2% Virkon) for at least 30 minutes.
Spillages will be inactivated with 5% ChemGene HLD4H spray, collected with absorbent material and disposed of via
autoclaving.

The University of Bath GMSC has reviewed the risk assessment for this work and approved the proposed procedures.
No issues were identified by the GMSC.

Please enter comments on the GM safety committee on the risk assessment
The University of Bath GMSC has reviewed the risk assessment for this work and approved the proposed procedures.
No issues were identified by the GMSC.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

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Animal Units

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Page 2665 of 15326
### UNIVERSITY OF CAMBRIDGE

#### Name
UNIVERSITY OF CAMBRIDGE

#### Name 2

#### Department
PLANT SCIENCES

#### Campus Estate or Research Centre

#### Road Name
DOWNING STREET

#### Town
CAMBRIDGE

#### District
CAMBRIDGESHIRE

#### County
CAMBRIDGESHIRE

#### Postcode
CB2 3EA

#### Country
ENGLAND

#### Tel Number
01223 333900

#### Fax Number
01223 333953

#### E-mail

#### HSE Division
EAST AND SOUTH EAST

#### Comments

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**Data Premises Notified**
07/08/1981 (Originally)

**Transferred from 1992 Regs?**
Y

**Transitional Premises Class**

**Data Premises Closed**

**Emergency Plan Required?**

**Transitional Premises**
N

**Non-GMMs**
N

**Withdrawn**
N

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**Date at Which Additional Info Submitted**
04/09/2003
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Liquid waste (maximum volume per flask is 2 litres but typically is 1 litre or less) and solid waste (loosely packed in an autoclavable bag: max size used in the Dept. is 420 x 610 mm) are sterilized for 30 minutes in a Boxer Model 200/200L autoclave. This is fitted with a temperature probe and chart recorder to monitor the conditions during the autoclave run.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Name
GE HEALTHCARE LIMITED

Name 2

Department

Campus Estate or Research Centre

Building

Road Name
WHITE LION ROAD

District

Town
AMERSHAM

County
BUCKINGHAMSHIRE

Postcode
HP7 9LL

Country
ENGLAND

Tel Number
01494 544000

Fax Number
01494 543083

E-mail

HSE Division
EAST AND SOUTH EAST

Comments
CHANGED FROM NYCOMED AMERSHAM PLC TO AMERSHAM PLC ON 27/11/2001

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
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<table>
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<th>Large Scale</th>
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<td>Parasitology</td>
<td>Transgenic Birds</td>
<td>Microbiology Research</td>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
<td></td>
</tr>
</tbody>
</table>
Maximum culture volume used is one litre.

Waste is deactivated in one of two LTE Scientific Kestrel 150 autoclaves which are operated by trained users according to a Plant Operating Procedure. Validation of deactivation is by temperature monitoring in a timed cycle, viewed on a control panel display and chart read-out. Both autoclaves are checked and serviced once each year by qualified LTE Scientific operatives.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The genetic modification safety committee is a continuation of the committee that was on the main Cranfield campus. The members include head of the Institute of Bioscience and Technology and the Biological Safety Officer and representatives of each staff group.

<table>
<thead>
<tr>
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Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research

Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste will be treated by autoclaving to give 100% kill. Autoclaves are monitored regularly using test strips and periodically using thermocouples.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The genetic modification safety committee accepted the risk assessment and agreed that it should be forwarded with this application.
### Project Additional Information

#### Purposes of the contained use
- To improve the concentration of the heterologous protein lysozyme in a novel fermentation system coupled with optimisation of environmental and nutrient conditions.

#### Recipient or parental organism
- Aspergillus niger is classified by ACDP as Group 1. However, as it has been known to cause aspergillosis and otomycosis it is treated here as Group 2.

#### Host/vector system
- The vector is pIGR, a non-selectable G498 fusion vector, which is integrated into the fungal genome and has no transposon-like activities.

#### Origin & function
- The genetic material is the hen egg white lysozyme gene. The intention is to produce lysozyme under fermentation conditions.

#### Evaluation of foreseeable effects
- There is no known hazards associated with lysozyme and it is classified as Generally Regarded as Safe (GRAS). It is unlikely to increase the pathogenicity of the cloned host and there are no foreseeable hazards to the environment.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
- All waste will be treated by autoclaving to give 100% kill.
- Autoclaves are monitored regularly using test strips and periodically using thermocouples.

- **Is an emergency plan required according to regulation 20?** N
- **If yes, tick to confirm that it is attached to this form** N
- **Tick to confirm that you have attached a risk assessment to this form** Y
- **Tick if you are claiming exemption from disclosure for section of the risk assessment** N

**Please enter comments on the GM safety committee on the risk assessment**
- The Committee sought evidence of the pathogenicity level of the particular strain utilised. Once this was received they accepted the risk assessment.

### Project Containment

02/03/2022

Page 2675 of 15326
<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 Yes</td>
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Animal Units

Large Scale Activities

Human Clinical Applications
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Name

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Name 2

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Tel Number

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E-mail

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Comments

Date at Which Additional Info Submitted

| 02/03/2022 |

Page 2677 of 15326
### Premises Addresses

<table>
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### Project Ref: 92/01.1

<table>
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<th>Project notified under transitional arrangements</th>
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<td>TRANSFORMATION OF NEMATOPHAGOUS FUNGI VERTICILLIUM CHLAMYDOSPORIUM, PAECILOMYCES LILACINUS AND PLECTOSPHAERELLA CUCUMERINA WITH GREEN FLUORESCENT</td>
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<td>&lt; 1 litre</td>
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- Withdrawn: N
- Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Purposes of the contained use

Investigate and observe interactions between nematophagous fungi, and plant and nematode hosts in situ, by introducing visual reporter genes into the fungi.

### Recipient or parental organism

<table>
<thead>
<tr>
<th>3 nematophagous fungi</th>
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<tbody>
<tr>
<td>1) Verticillium chlamydosporium: A well characterised wild type fungus with a history of safe use.</td>
</tr>
<tr>
<td>2) Plestosphaerella cucumerina: A well characterised wild type fungus with a history of safe use.</td>
</tr>
<tr>
<td>3) Paecilomyces Lilacinus: A well characterised wild type fungus with a history of safe use, but some isolates are reported to infect humans, but isolate used in this proposal does not grow at 37 degrees C.</td>
</tr>
</tbody>
</table>

### Host/vector system

Escherichia coli bacterium carrying well defined and characterised plasmids.
Plasmids are described in detail on attached forms.

### Origin & function

cDNAs

### Evaluation of foreseeable effects

Gene sequences where any biological effect is considered highly unlikely because of the known properties of the proteins.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Work with V. chlamydosporium and P. cucumerina is anticipated to be level 1 (see risk assessment). These fungi are not pathogenic to man, nor do they produce dry spores so containment is thought to be appropriate.

For P. lilacinus work in a CL2 cabinet is appropriate for all GM material. All manipulations and sub-culturing is to be performed in a CL2 cabinet. All material to be sealed and placed in a secure secondary container for storage and transport to prevent escape.

Growth room equivalent to plant growth facility level 2. Material in growth rooms to be contained in clearly labelled sealed containers to prevent accidental escape.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All material, equipment and waste to be autoclaves.

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
After much discussion amongst committee members, it was agreed that certain parts of this proposal would probably require C2 containment. The proposal was returned to the proposing Scientist asking him to arrange this before final approval could be given.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>19/03/2001</td>
<td>DESIGN AND EVALUATION OF NOVEL VECTORS FOR WHEAT TRANSFORMATION.</td>
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### Project Additional Information

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Purposes of the contained use
Design and develop a model vector system for wheat transformation, and evaluate its transformation efficiency against conventional bombardment vectors and conditions.

### Recipient or parental organism
- E. coli BL21(DE3)pLysS
- E. coli JM109 for cloning
- Wheat

### Host/vector system
- **Expression vector:** pET11A. This vector is designed for high level protein expression under the control of T7 promoter and is operating only in E. coli strains carrying gene for T7 RNA polymerase.
- **Cloning Vectors:** pGEM-7Zf(-) and pGEM-7Zf(+). These are standard commercial DNA cloning vectors. They contain an Amp resistance marker as well as origin of replication for producing ssDNA. The T7 and SP6 promoters that flank the polylinker, are useful in in vitro transcription experiments but are generally not designed for protein expression. These vectors will be used in E. coli JM109 which do not carry genes for T7 or SP6 RNA polymerases thus avoiding any unwanted transcription or translation from these promoters.

### Origin & function
DNA sequences will be cloned into a pET11a bacterial expression vector, having ampicillin resistance and expression driven by the T7 promoter. Expression will be achieved in E. coli carrying the T7 RNA polymerase gene, inducible by IPTG. The intended function of the gene will be studied in relation to transformation.

### Evaluation of foreseeable effects
Expression host: E. coli BL21(1DE3) PlysS. Part of these mutations are useful for successful protein expression. This strain is incapable of colonising human gut. The expressed T7 lysosome destabilises bacterial cell walls making the E. coli susceptible to mechanical or chemical damage. The strain has an attenuated ability to colonise bird guts but is not able to grow and propagate. The E. coli strain is a standard cloning vector host. The mutations make it non-viable outside favourable laboratory conditions.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Bombarded wheat tissue will be destroyed by autoclaving at 121 degrees C and disposed in deep land field.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
The cloning steps involving pGEM vectors, and culturing in DH10B will be done at CL1, as this does not warrant CL2.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
- Glassware will be autoclaved in the presence of detergent (Savlon) before washing. A special glassware set will be devoted to this work.
- Liquid media will be autoclaved in the presence of detergent (Savlon).
- Centrifuge bottles and tubes will be autoclaved in the presence of detergent (Savlon) before washing.
- Plasticware will be autoclaved in autoclavable polyethylene bags and then will be disposed in deep land field.

### Is an emergency plan required according to regulation 20?
N

**If yes, tick to confirm that it is attached to this form**
N
**Project Containment**

<table>
<thead>
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- **Animal Units**
  - L2
  - L3
  - L4

- **Large Scale Activities**
  - L2
  - L3
  - L4

- **Human Clinical Applications**
  - L2
  - L3
  - L4

**CU2 Project Title**

VISUALISATION OF GREEN FLUORESCENT PROTEIN (GFP) - TRANSFORMED LEPTOSPHAERIA MACULANS DURING INFECTION OF BRASSICA NAPUS (WINTER OILSEED RAPE).

**Date Ackn'd**

21/05/2002

**Class Culture Vol Class**

Class 2

< 1 litre

**Non-GMM Consent Granted**

not applicable

**Project Additional Information**
### Purposes of the contained use

Isolates of the oilseed rape fungal pathogen, *Leptosphaeria maculans*, will be transformed with the colour variants of the green fluorescent protein (GFP). The project will compare the efficiencies of two transformation methods commonly used for fungi, PEG-mediated transformation and transformation using *Agrobacterium tumefaciens*. Transformed fungal isolates will be used to infect oilseed rape plants. Using fluorescent confocal microscopy, the progress of infection from the leaves (initial inoculation site) down the petiole and into the stem will be monitored. The response of two genotypes of the fungus (A- and B-type) to fungicide treatments will be monitored to determine whether the genotypes differ in their response to fungicides commonly used to control the disease.

### Recipient or parental organism

The recipient organism (*Leptosphaeria maculans*) is a phytopathogenic fungus which causes stem canker or blackleg disease of brassicas including oilseed rape (*Brassica napus*). Populations of the fungus consist of two main genotypes, designated A- and B-type. A-type isolates are generally considered to be more damaging to host plants than B-type although both genotypes are capable of infecting most brassica species and are common throughout the UK and continental Europe. The asexual spores (conidial) of the fungus are splash dispersed and are known to be highly infective to the host plants. Successful infection by candidia requires the host to be wounded prior to inoculation. The sexual spores (ascospores) of the fungus are considered to be the infectious stage and are airborne. *L. maculans* is not capable of crossing with other fungal species. The fungus is unable to infect humans and is not capable of growth at 37 degrees C. The fungus does not cause known allergies in humans.

### Host/vector system

For propagation of plasmids and nucleic acid sequences, to be used for the transformation of *L. maculans*, a well characterised disabled *E. coli* strain (NovaBlue) commonly used in cloning manipulations will be used. This bacterial strain is not capable of survival in the environment or in humans and consequently does not pose a risk.

The plasmid pGpd-GFP will be used for PEG-mediated transformation of *L. maculans* protoplasts. This vector has a background of pBluescript, a well characterised standard cloning vector. The vector also contains sequences coding for the *Aspergillus nidulans* glyceraldehyde-3-phosphate dehydrogenase (gpdA) promoter, enhanced GFP (eGFP; F64L and S65T mutated form) and the hygromycin B resistance cassette. Colour variants of the GFP gene will be cloned in place of the eGFP gene to enable production of transgenic *L. maculans* expressing these markers.

*Agrobacteium tumefaciens* strain LBA 4404 will be used to transform *L. maculans*. This strain could survive if released into the environment. A tumefaciens is capable of transferring genetic material to plants and fungi but is not pathogenic to, or capable of surviving in humans.

For *Agrobacterium*-mediated transformation of *L. maculans*, the vectors pcJ102 or pSJ34 will be used. These vectors are based on the Glucuronidase Plant Transformation Vector pGPTV-KAN and contain a selectable marker for kanamycin resistance in addition to the uidA (GUS) marker (which will be removed as it is not necessary for this project and the left and right border sequences of the *A. tumefaciens* T-DNA. The gpdA-eGFP-hph cassette from pGpd-GFP will be cloned between the T-DNA border sequences of these plasmids for transformation of *L. maculans*. Colour variants of the GFP gene will be cloned in place of the eGFP gene to enable production of transgenic *L. maculans* expressing these markers.

### Origin & function

**gpdA** - glyceraldehyde-3-phosphate dehydrogenase promoter from *Aspergillus nidulans* used to drive expression of eGFP in transgenic *L. maculans*.

**PtrpC-hph-TrpC** - cassette containing hygromycin B resistance gene from *E. coli* flanked by trpC promoter and terminator sequences from *Aspergillus nidulans* used as a selectable marker to enable identification of successfully transformed *L. maculans*.

**eGFP** - enhanced green fluorescent protein from *Aequoria victoria* jellyfish (and modified colour variants such as cyan and yellow forms) used to enable visualisation of transgenic *L. maculans* in infected oilseed rape plants.
### Evaluation of foreseeable effects

The genes used in this project are purely for marker purposes and none of the inserts used will increase the pathogenicity or host range of the transgenic L. maculans isolates. None of the inserts are known to cause harm to humans, none are toxic or are known human allergens. As a precaution, however, inoculation of oilseed rape plants with transgenic L. maculans spores will be done by applying droplets of spore suspensions to wounded leaves. No spraying will be necessary and this will reduce exposure to researchers.

Transgenic L. maculans isolates will be resistant to the antibiotic hygromycin. However, hygromycin is not used for control of the fungus in diseased crops and hence this characteristic will not impose any selective advantage should escape occur.

The consequences of escape of transgenic L. maculans is considered as low - the pathogen is present normally in oilseed rape crops and is routinely controlled using fungicide sprays. Only asexual conidia will be used in this project and these spores are known not to be highly infective, even to the host species. To reduce the risk of escape, however, leaves will be removed from infected plants before the fungus sporulates. The disease is mainly initiated by airborne ascospores which are only produced if the fungus is allowed to undergo sexual reproduction with a compatible isolate of opposite mating type. Isolates of only one mating type will be used in this project so as to remove the risk of sexual reproduction occurring.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid media cultures used for growth of E. coli and A. tumefaciens and solid media cultures and spore suspensions of transgenic L. maculans will be autoclaved at 127 degrees C for 50 minutes in an autoclave registered for the disposal of genetically modified organisms. 100% kill is achieved using this method and temperatures are monitored using an internal probe. Autoclave equipment is inspected annually to ensure that it reaches the correct temperature and pressure.

Oilseed rape material (leaves, whole plants, soil and material used for microscopy) infected with transgenic L. maculans will be autoclaved at 127 degrees C for 50 minutes in an autoclave registered for the disposal of genetically modified plant material. 100% kill is achieved using temperatures are monitored and recorded using an internal probe. Autoclave equipment is inspected annually to ensure that it reaches the correct temperature and pressure.

All autoclaved material is deposited into a skip, which is emptied into landfill sites by a contractor.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Comments by GMSC: Part of this project comes under a C2 classification, as such HSE will have to be notified.
### Project Containment

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- **Laboratory Activities**
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  - L3
  - L4

- **Glass Houses**
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  - L3
  - L4

- **Growth Rooms**
  - L2 Yes
  - L3
  - L4

### Project Ref 92/03.1

**Date Ackn'd**: 03/01/2003

**CU2 Project Title**: CONSTRUCTION AND EVALUATION OF STRAINS OF THE PHYTOPATHOGENIC FUNGAL SPECIES FUSARIUM GRAMINEARUM AND FUSARIUM CULMORUM WITH REPORTER GENES AND SILENCED GENES.

**Class**: Class 2

**Culture Vol**

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**Non-GMM Consent Granted**: not applicable

**Project notified under transitional arrangements**: N

**Historical Significant Changes**

**Historical Date of Additional Info**: 03/01/2003

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

### Project Additional Information

**Purposes of the contained use**

**Specific Project Purpose**

- To generate various transgenic strains of the fungus F. graminearum and F. culmorum to
  
a) Quantitate fungal biomass in plants
  b) Determine the fungal hyphal infection route in susceptible and resistant wheat cultivars and other host and non-host plant species
  c) Ascribe a function to specific fungal genes discovered through genomics.
Overall description of the work

Five types of experiments will be performed:

1. Over-expression experiments.

Normally pathogenic strains of F. graminearum and F. graminearum will be used to generate reporter strains. These reporter strains will constitutively co-express the uidA gene from E. coli, that codes for glucuronidase (GUS) enzyme activity and the GFP gene from the jellyfish Aequorea victoria that codes for the green fluorescent protein. Transgenic isolates expressing both GUS and GFP will be selected that have an equivalent growth habit and disease causing ability as the original strains. Details of the exact constructs are given in the next section, and involve either pUC or Bluescript based vectors. The selectable marker initially used for Fusarium transformation will be hygromycin antibiotic resistance. Other markers that may be used include kanamycin or benomyl resistance. Fusarium transformation is achieved using asexual spores and/or hyphal fragments and either protoplast transformation or Agrobacterium tumefaciens mediated transformation methodology (de Grott et al., 1998) Nature Biotechnology 16:839-842).

2. Gene disruption experiments by homologous gene replacement or insertions.

Homologous gene replacement will be used to substitute a gene of interest, identified through genomics/bioinformatics experimentation, with a "null" allele for this gene. At the null allele, will be placed a gene sequence coding for either a bleomycin/phleomycin or hygromycin antibiotic resistance. This will result in a complete loss of function in the gene of interest. Alternatively gene disruptions will be generated by A. tumefaciens mediated transformation of a gene cassette encoding for hygromycin resistance or by in vitro transposition of the EZ-TN transposon (source E. coli) into F. graminearum cosmids and subsequent fungal protoplast transformation.

These novel transgenic fungal isolates will be tested for their ability to (a) infect and cause disease on wheat ear, stem and root tissue, and on various tissues and organs of other host cereal plant species and non-host plant species, (b) produce trichothecene toxins in vitro and in vivo, and (c) to produce asexual conidia and sexual ascospores in vitro.

Where the loss of a specific gene's functions result in an inability or highly reduced capacity to cause disease, produce toxins or generate spores, these isolates will be used in follow up biological and genomics experiments.

Where the reverse is observed, i.e., loss of gene function causes increased disease, toxin production or spore production further experimentation will cease. The isolates will be placed in long term storage and on an annual basis DEFRA will be informed of the frequency of occurrence of this experimental outcome.

3. Transgenic Fusarium strains harbouring fungal promoter:reporter gene fusions

The experiments described in (2) above will lead to the discovery of various Fusarium genes required to invade and cause disease on wheat, to regulate toxin production and to control sexual and asexual spore formation. Follow up transformation experiments will be required to ascertain the exact spatial and temporal patterns of each gene. PUC-based and/or binary plasmid vectors will be made containing the promoter and 3' end sequence of each Fusarium gene of interest fused to a GUS, GFP or GUS:GFP fusion reporter cassette in association with an antibiotic resistance marker cassette required for fungal transformation. The plasmids will be transformed into both wild-type strains and gene knock-out strains. Transformants for further characterisation will be selected where the reporter fusion cassette resides at an ectopic location in the Fusarium genome.

4. Sexual crosses between different transgenic fungal strains.

These experiments will only be performed with F. graminearum because the species F. culmorum lacks a sexual stage. To determine the exact gene hierarchy controlling the various biological processes, it is necessary to make both double knock-out gene mutants (from 2 above), and to combine each single-gene knock out mutant with various promoter: reporter gene fusions (from 3 above) or the constitutive reporter gene construct (from 1 above). Sexual crosses in the laboratory will only involve previously well characterised transgenic strains. It is anticipated that when combining the loss of 2 functionally important genes this will result in the double mutant strain acquiring a phenotype equivalent to one of the original mutant phenotypes.
Where the reverse is observed, i.e., the loss of 2 functional Fusarium genes causes increased disease on plants, greater toxin production and/or greater spore production, further experimentation will cease. The isolate will be placed in long-term storage and on an annual basis DEFRA will be informed of the frequency of this experimental outcome.

5. Mixed fungal strain inoculations on the same plant.
It is currently unclear if each successfully penetrating Fusarium spore establishes a separate mycelial colony within plant tissue, or if a mixed infection develops in the tissue colonised. It is also unknown whether the different Fusarium species colonise to produce a mixed infection, or whether the ear becomes a mosaic of different fungal species colonies. By generating transgenic Fusarium isolates carrying different reporter gene constructs, for example the blue and red variants of GFP, it will be possible to answer these basic biological questions by conducting dual inoculation experiments. We also wish to determine if different gene-knock-out strains, can complement each other in vivo to cause wild-type disease levels. Note: perithecia containing the sexual ascospores are never produced on inoculated ears.

Work involved.
In general the work involves obtaining relevant genes (either through direct cloning from a source organism, or indirectly from third parties), production of ‘gene constructs’ suitable for fungal transformation (via sub-cloning using plasmid vectors and micro-organism hosts), transfer of ‘gene constructs’ into plasmid vectors and host strains suitable for fungal transformation, and fungal transformation. The fungal transformants once produced are then tested for various biological characteristics through laboratory, growth room and glasshouse experimentation.

### Recipient or parental organism

<table>
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<tr>
<th><strong>Fungal hosts:</strong></th>
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<tr>
<td>1) <em>F. graminearum</em> strains PH-1, 16A, 10Fg131</td>
</tr>
<tr>
<td>2) <em>F. culmorum</em> strains 97/7, 98/11, 99w.5Fc</td>
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</table>

*F. graminearum* and *F. culmorum* both cause ear blight disease of wheat and other cereal species. *F. graminearum* produces both rain splash dispersed asexual conidia and air dispersed ascospores which form within block perithecial structures (approximately 1 mm in diameter) on exposed cereal crop debris. *F. culmorum* only produces rain splash dispersed asexual conidia. The three *F. culmorum* strains to be used in these studies are of UK origin and produce water soluble trichothecene toxins. Two strains, 97/7 and 98/11, are well characterised by our group through extensive laboratory, growth room, glasshouse and field experimentation over the past 4 years. The third *F. culmorum* strain, 99w.5Fc has been used extensively at Rothamsted Research over the last 3 years by the group of Dr Bateman. The two *F. graminearum* strains, PH-1, 16A, selected for study belong to the genetic lineage group 7. This genotype is pandemic throughout the Northern Hemisphere (O’Donnell et al., PNAS (2000) 97:7905-7910). The strains PH-1 and 16A are both of USA origin and also produce water soluble trichothecene toxins. The strain PH-1 has been selected by the world’s academic community for full genomic sequencing in 2002/2003 because of the pandemic distribution of lineage group 7. Both *F. graminearum* strains have been used by our group for the past 3 years under containment facilities and are no more pathogenic to plants than the two selected *F. culmorum* strains or other natural UK isolates of *F. graminearum* and *F. culmorum*. A third *F. graminearum* strain, 10Fg131. of UK origin will also be used. This strain has been used at Rothamsted Research for the past 3 years in the group of Dr Bateman.

*F. graminearum*/*F. culmorum*: Both fungal species are exclusively pathogens of plants and cannot infect humans. Both fungal species produce water soluble trichothecene toxins when they invade plant tissue and also when grown under specific conditions in the laboratory. The trichothecene toxins produced by *F. graminearum* and *F. culmorum* species include 4-acetyl-nivalenol, nivalenol, deoxynivalenol (DON) and other acetylated derivatives. Only if infected plant parts or grain were eaten could cause illness. The target site for the DON toxin is the peptidyl transferase protein in the ribosome, which is required for protein translation. The target sites for other trichothecenes is unproven, although suspected to be in the ribosome.

*F. graminearum* and *F. culmorum* are not included in the list of fungi known to infect and colonise immuno-compromised (AIDS) human patients are are not capable of growth at 37°C. Both fungal species are present in the UK and are routinely imported in slightly infected cereal grain samples destined for human consumption. These grain samples originate from the USA, Canada and other EU countries. The two USA *F. graminearum* strains to be used in our studies belong to genetic lineage group 7. This lineage is pandemic in Europe and North America. (O’Donnell et al. (2000), PNAS 97:7905-7910). Some humans are known to have allergies to fungal spores.
**Host/vector system**

F. graminearum/F. culmorum will be used. The transformation system will be E. coli K12 derivatives and non-mobilisable vectors pUC and Bluescript. The bacterial strains are not capable of survival in the environment or in humans and consequently do not pose a risk.

For A. tumefaciens-mediated transformation of F. graminearum/F. culmorum only the disarmed A. tumefaciens strains GV3101, GV3101:VirG++, LBA 1100, LBA 4404 will be used. As binary vectors pBin19, pSLJ vector series (The Sainsbury Laboratory, Norwich, UK), pGREEN II and derivatives will be used. A tumefaciens is capable of transferring genetic material to plants and fungi but is not pathogenic to, or capable of surviving in humans.

**Origin & function**

1. Specific vectors for fungal transformation

   a) Sources for hygromycin resistance:
      pCB 1003 and pCB1004:
      Both vectors confer resistance to hygromycin B as a dominant selectable marker. The vector backbone of pCB 1003 and pCB 1004 is pUC 19 and pBCSK respectively.
      pHAI1.3 and pUCH2-8

   b) Source of benzimidazole resistance
      pBT3:

   c) Source for phleomycin/bleomycin resistance
      pMON5161

   d) Cloning vector for genomic library preparation
      pMOCosX (M. Orbach, Gene 150:159-162, 1994)
      The vector confers resistance to hygromycin and was used for genomic library preparation of F. graminarum DNA. Individual cosmids carrying 20 kb fragments can be introduced into the fungus by transformation and selection for the hygromycin resistance marker.

   e) Vectors for A. tumefaciens mediated F. graminearum/culmorum transformation
      The pGreenII/pSoup dual plasmid system (see www.pgreen.ac.uk) is used. pSoup contains the Rep A gene function for replication in A. tumefaciens and a tetracycline resistance cassette for selection in A. tumefaciens. pGreenII contains the kanamycin resistance gene for selection in A. tumefaciens, the pSA region for replication in A. tumefaciens and a polycloning site flanked by Left and Right Border sequences essential for T-DNA transfer.

**II. cDNAs and genes**

1. F. graminearum map1 deletion allele (defective protein kinase gene) for construction of non-pathogenic F. graminearum strain.
2. E. coli GUS (uidA); GFP (Aequorea victoria). various colour types avalable commercially for reporter gene constructs
3. Chloramphenicol and Ampicillin resistance (E. coli selectable markers)
4. E. coli lacZ (plasmid backbone)
5. E. coli Byg B Phosphotransferase resistance (hph) gene as fungal resistance marker

III. Gene regulatory sequences
1. Aspergillus nidulans promoters PptrpC and Pgpd and A. nidulans 3' noncoding region TrpC for expression of GUS.GFP reporter genes and for antibiotic gene expression.
2. F. graminearum TR15 and TR17 promoter sequences linked to GUS/GFP reporter genes will be used to monitor TR15/TR17 expression.

IV. E. coli and A tumefaciens cloning vectors, plasmids and transposon for in vitro transposition:
pUC19 - derivatives (pUC18, pUC8, pUC119, pPAN7), pBluescript, pCRII-TOPO, pSPORT, pGEM Teasy, commercially available EZ::TN transposon (www.epicentre.com) carrying an E. coli KAN resistance marker to generate insertions into cosmid pMOcozX.

Evaluation of foreseeable effects

E. coli/Agrobacterium: None. The product of the inserted genes and promoters are unlikely to have biological properties (activities) which may give rise to harm. None of the sequences we intend to use have homology to known toxic proteins.

F. graminearum/F. culmorum: The inserted genes include the reporter genes GUS and GFP. These two proteins are not known to be inherently harmful to humans. Similarly the fungicide (benomyl) resistance gene/protein is not known to present any direct hazard to humans. The preferred antibiotic resistance gene to be used confers hygromycin resistance. This antibiotic is sometimes used in human health care in the UK.

Gene replacement experiments are likely to produce Fusarium strains that are less fit than the wild type but there is a possibility that strains of greater pathogenicity to plants for ability to produce toxin will be generated.

The gene silencing experiments involving Agrobacterium tumefaciens mediated fungal transformation and fungal gene replacement experiments will cause random and targeted disruption/insertions of endogenous Fusarium genes. These gene knockout experiments are again likely to produce Fusarium strains that are less fit than the wild type but there is a possibility that strains of greater pathogenicity to plants or strains able to produce toxins or metabolic bi-products in greater quantities will be generated. Only isolates found to be reduced in pathogenicity, toxin production and/or spore producing ability will be selected for follow up experiments. It is extremely unlikely that these types of experiments will alter the allergenic properties associated with the fungal spores.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid media cultures used for growth of E. coli and A. tumefaciens and solid media cultures and spore suspensions of transgenic F. graminearum/F. culmorum will be autoclaved at 127 degrees C for 30 minutes in an autoclave registered for the disposal of genetically modified organisms. 100% kill is achieved using this method and temperatures are monitored using an internal probe. Autoclave equipment is inspected annually to ensure that it reaches the correct temperature and pressure.

Plant material (leaves, whole plants, soil and material used for microscopy) infected with transgenic F. graminearum/F. culmorum will be autoclaved at 127 degrees C for 30 minutes in an autoclave registered for the disposal of genetically modified plant material. Autoclave equipment is inspected annually to ensure that it reaches the correct temperature and pressure. All autoclaved material is deposited into a skip, which is emptied into landfill sites.
For the F. graminearum strains PH-1, 16A, held under a UK DEFRA import licence liquid cultures will be treated using a double kill procedure, i.e. chemical treatment prior to autoclaving because disposal to a landfill site is not an option.

This was approved subject to the availability of Class 2 containment facilities for the plant/fungal pathogen work. (Taken from the minutes of the GMSC 15 October 2002).

All of these require level 2 plant and laboratory containment. A second category 2 laboratory has been secured in the new building. There is also a possibility of a further room becoming available in the Bawden Building. Class 2 glasshouse facilities are available at Rothamsted. However, growth room containment facilities at class 2 are not yet available at Rothamsted. There is a facility at Long Ashton that can be moved to Rothamsted.

### Project Containment

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<th>Laboratory Activities</th>
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Tick if notifying a connected programme of work  

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Specific Project Purpose

- To generate various transgenic strains of the fungus *T. yallundae* and *T. acuformis* to

  a. Quantitate fungal biomass in planta

  b. Determine the fungal hyphal infection route in susceptible and resistant wheat cultivars and other host and non-host plant species.

  c. Ascribe a function to specific fungal genes discovered through genomics.

Overall description of the work

Five types of experiments will be performed:

1. Over-expression experiments.

   Normally pathogenic strains of *T. yallundae* and *T. acuformis* will be used to generate reporter strains. These reporter strains will constitutively express the GFP gene from the jellyfish *Aequorea victoria* that codes for the green fluorescent protein. Transgenic isolates expressing GFP will be selected that have an equivalent growth habit and disease causing ability as the original strains. Details of the exact constructs are given in the next section, and involve either pUC or Bluescript based vectors. The selectable marker originally used for *Tapesia* transformation will be hygromycin antibiotic resistance. Other markers that may be used include phleomycin or benomyl resistance. *Tapesia* transformation is achieved using asexual spores and/or hyphal fragments and either PEG precipitation or Agrobacterium tumefaciens mediated transformation methodology (de Grott et al., (1998) Nature Biotechnology 16: 839-842).

2. Gene disruption experiments by homologous gene replacement or insertions.

   Homologous gene replacement will be used to substitute a gene of interest, identified through genomics/bioinformatics experimentation, with a 'null' allele for this gene. At the null allele, will be placed a gene sequence coding for either a bleomycin/phleomycin or hygromycin antibiotic resistance. This will result in a complete loss of function in the gene of interest. Alternatively gene disruptions will be generated by *A. tumefaciens* mediated transformation of a gene cassette encoding for hygromycin resistance or by an in vitro transposition of the EZ:TN transposon (source E. coli) into *Tapesia* cosmids and subsequent fungal protoplast transformation.

   These novel transgenic fungal isolates will be tested for their ability to (a) infect and cause disease on wheat stem and leaf tissue, and on various tissues and organs of other host cereal plant species and non-host plant species, (b) to produce asexual conidia and sexual ascospores in vitro.

   Where the loss of a specific gene's function results in an ability or highly reduced capacity to cause disease, or generate spores, these isolates will be used in follow up biological and genomics experiments.

   Where the reverse is observed, ie, loss of gene function causes increased disease, or spore production further experimentation will cease. The isolates will be placed in long term liquid nitrogen storage and on an annual basis DEFRA will be informed of the frequency of occurrence of this experimental outcome.


   The experiments described in (2) above will lead to the discovery of various *Tapesia* genes required to invade and cause disease on wheat and to control sexual and...
asexual spore formation. Follow up transformation experiments will be required to ascertain the exact spatial and temporal patterns of each gene. PUC-based and/or binary plasmid vectors will be made containing the promoter and 3' end sequence of each Tapesia gene of interest fused to a GUS, GFP or GUS:GFP or GUS:GFP fusion reporter cassette in association with an antibiotic resistance marker cassette required for fungal transformation. The plasmids will be transformed into both wild-type strains and gene knock-out strains. Transformants for further characterisation will be selected where the reporter fusion cassette resides at an ectopic location in the Tapesia genome.

4. Sexual crosses between different transgenic fungal strains.

These experiments will only be performed with T.yallundae. To determine the exact gene hierarchy controlling the various biological processes, it is necessary to make both double knock-out gene mutants (from 2 above), and to combine each single-gene knock out mutant with various promoter: reporter gene fusions (from 3 above) or the constitutive reporter gene construct (from 1 above). Sexual crosses in the laboratory will only involve previously well characterised transgenic strains. It is anticipated that when combining the loss of 2 functionally important genes this will result in the double mutant strain acquiring a phenotype equivalent to one of the original mutant phenotypes.

Where the reverse is observed, ie the loss of 2 functional Tapesia genes causes increased disease on plants and/or greater spore production, further experimentation will cease. The isolate will be placed in long term liquid nitrogen storage and on an annual basis DEFRA will be information of the frequency of this experimental outcome.

5. Mixed fungal strain inoculations on the same plant

It is currently unclear if each successfully penetrating Tapesia spore establishes a separate mycelial colony within plant tissue, or if a mixed infection develops in the tissue colonised. It is also unknown whether the different Tapesia species colonise to produce a mixed infection, or whether the stem base becomes a mosaic of different fungal species colonies. By generating transgenic Tapesia isolates carrying different reporter gene constructs, for example the blue and red variants of GFP, it will be possible to answer these basic biological questions by conducting dual inoculation experiments. We also wish to determine if different gene-knock out strains, can complement each other in vivo to cause wild-type disease levels. Note: perithecia containing the sexual ascospores are never produced within the time scale of pathogenicity experiments.

Work involved

In general the work involves obtaining relevant genes (either through direct cloning from a source organisms, or indirectly from third parties), production of 'gene constructs' suitable for fungal transformation (via sub-cloning using plasmid vectors and micro-organism hosts), transfer of 'gene constructs' into plasmid vectors and host strains suitable for fungal transformation, and fungal transformation. The fungal transformants once produced are then tested for various biological characteristics through laboratory, growth room and glasshouse experimentation.
T. yallundae and T. acuformis are not included in the list of fungi known to infect and colonise immuno-compromised (AIDS) human patients and are not capable of growth at 37°C.

Both fungal species are present in the UK.

The two strains have previously been studied in the UK under our existing DEFRA licence (PHL 184/4031(01/2002) and currently reside in long term storage at Long Ashton research Station, Bristol. The other Tapesia strains to be used are of UK origin.

Some humans are known to have allergies to fungal spores.

### Host/vector system

T. yallundae/T. acuformis will be used. The transformation system will be E. coli K12 derivatives and non-mobilisable vectors pUC and Bluescript. The bacterial strains are not capable of survival in the environment or in humans and consequently do not pose a risk.

For A. tumefaciens-mediated transformation of T. yallundae/T. acuformis only the disarmed A. tumefaciens strains GV3101, GV3101: VirG++, LBA 1100, LBA 4404 will be used. As binary vectors pBin19, pSLJ vector series (The Sainsbury Laboratory, Norwich, UK), pGREEN II and derivatives will be used. A tumefaciens is capable of transferring genetic material to plants and fungi but is not pathogenic to, or capable of surviving in humans.

### Origin & function

1. Specific vectors for gungal transformation
   a) Sources for hygromycin resistance:
      pCB 1003 and pCB1004:

      pAN7-1
      This plasmid confers resistance to hygromycin B. It contains a 2.2kb fragment of the Aspergillus nidulans gpdA promoter upstream of the hygromucycin resistance gene and an 0.8kb fragment of the A. nifulans trpC terminator region downstream of the gene in a pUC 19 backbone. This plasmid has been extensively used for fungal transformation over the last 10 years.

      pHA1.3 and pUCH2-8

   b) Source for phleomycin/bleomycin resistance
      pBT3:
      c) Source for phleomycin/bleomycin resistance


d) Cloning vector for genomic library preparation

pMOcosX (M. Orbach, Gene 150:159-162, 1994)
The vector confers resistance to hygromycin and was used for genomic library preparation of F. graminarium DNA. Individual cosmids carrying 20 kb fragments can be introduced into the fungus by transformation and selection for the hygromycin resistance marker.

e) Vectors for A. tumefaciens mediated F. graminearum/Culmorum transformation.

The pGreenII/pSoup dual plasmid system is used. pSoup contains the Rep A gene function for replication in A. tumefaciens and a tetracycline resistance cassette for selection in A. tumefaciens. pGreenII contains the kanamycin resistance gene for selection in A. tumefaciens, the pSA region for replication in A. tumefaciens and a polycloning site flanked by left and right border sequences essential for T-DNA transfer.

II cDNAs and genes

1. T. yallundae TYG1, TYG2 (G-proteins), PKA (protein kinase A) TYSACH (stretch activated calcium channel) and adenylate cyclase alleles for construction of signal transduction deficient strains.

2. E. coli GUS (uidA); GFP (Aequorea victoria), various colour types available commercially for reporter gene constructs.

3. Chloramphenicol, kanamycin and Ampicillin resistance (E. coli selectable markers)

4. E. coli lacZ (plasmid backbone)

5. E. coli Hyg B phosphotransferase resistance (hph) gene as fungal resistance marker


III Gene regulatory sequences

1. Aspergillus nidulans promoters (PtrpC and Pgpd and A. nidulans 3’ noncoding region TtrpC for expression of GUS/GFP reporter genes and for antibiotic gene expression.

2. T. yallundae actin (TYAC) promoter sequences linked to GUS/GFP reporter genes.

IV E. coli and A. tumefaciens cloning vectors, plasmids and transposon for in vitro transposition:

pUC19 - derivatives (pUC18, pUC8, pUC119, pPAN7), pBluescript, pCR II-TOPO, pSPORT, pGEM Teasy, commercially available EZ:TN transposon carrying an E. coli KAN resistance marker to generate insertions into cosmid pMOcosX.

Evaluation of foreseeable effects

E. coli/Agrobacterium: None. The product of the inserted genes and promoters are unlikely to have biological properties (activities) which may give risk to harm. None of
the sequences we intend to use have homology to known toxic proteins.

T.yallundae/T.acuformis: The inserted genes include the reporter genes GUS and GFP. These two proteins are not known to be inherently harmful to humans. Similarly the fungicide (benomyl) resistance gene/protein is not known to present any direct hazard to humans. The preferred antibiotic resistance gene to be used confers hygromycin resistance. This antibiotic is sometimes used in human anti-cancer therapies in the UK.

Gene replacement experiments are likely to produce Tapesia strains that are less fit than the wild type but there is a possibility that strains of greater pathogenicity to plants or ability to produce toxin will be generated.

The gene silencing experiments involving Agrobacterium tumefaciens mediated fungal transformation and fungal gene replacement experiments will cause random and targeted disruption/insertions of endogenous Tapesia genes. These gene knockout experiments are again likely to produce Tapesia strains that are less fit than the wild type but there is a possibility that strains of greater pathogenicity to plants will be generated. Only isolates found to be reduced in pathogenicity and/or spore producing ability will be selected for follow up experiments. It is extremely unlikely that these types of experiments will alter the allergenic properties associated with the fungal spores.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid media cultures used for growth of E. coli and A. tumefaciens and solid media cultures and spore suspensions of transgenic T. yallundae/T.acuformis will be autoclaved at 127 degrees C for 30 minutes in an autoclave registered for the disposal of genetically modified organisms. 100% kill is achieved using this method and temperatures are monitored using an internal probe. Autoclave equipment is inspected annually to ensure that it reaches the correct temperature and pressure.

Plant material (leaves, whole plants, soil and material used for microscopy) infected with transgenic T.yallundae/T.acuformis will be autoclaved at 127 degrees C for 30 minutes in an autoclave registered for the disposal of genetically modified plant material. Autoclave equipment is inspected annually to ensure that it reaches the correct temperature and pressure. All autoclaved material is deposited into a skip, which is emptied into landfill sites.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

N
This was approved subject to the availability of Class 2 containment facilities for the plant/fungal pathogen work (Taken from the minutes of the GMSC 15th October 2002).

All of these require level 2 plant and laboratory containment. A second category 2 laboratory is planned in the new building. There is also a possibility of a further room becoming available in the Bawden Building. Class 2 glasshouse facilities are available at Ththamsted. However, growth room containment facilities at class 2 are not yet available at Rothamsted. There is a facility at Long Ashton that can be moved to Rothamsted.

**Project Containment**

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**Project Ref** 92/03.3

**Date Ackn'd** 14/10/2003

**CU2 Project Title** EXPRESSION OF POTENTIALLY NEMATICIDAL FUNGAL OR NEMATODE GENES IN PLANT ROOTS USING AGROBACTERIUM RHIZOGENES - MEDIATED TRANSFORMATION

**Class** Class 2  
**CultureVol** < 1 litre

**Non-GMM** not applicable

**Consent Granted**

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
### Purposes of the contained use

To express genes for enzymes (e.g., protease, chitinase) from Pochonia chlamydosporia and other non-pathogenic fungi, and proteins involved in cell-cycle regulation from free-living or plant parasitic nematodes (Caenorhabditis elegans, potato cyst nematodes), in suitable plant host roots, to examine any nematicidal effects. Transgenic roots will be produced using *A. rhizogenes* containing pBin-type binary vectors with the fungal or nematode genes in the T-DNA.

### Recipient or parental organism

The recipient will be non-disarmed Agrobacterium rhizogenes (containing a functional Ri plasmid). This will be transformed, using electroporation, with pBin 19-type vectors constructed in *E. coli*, containing fungal or nematode genes with plant promoters (constitutive or induced in the root cells where nematodes are feeding). The *A. rhizogenes* will be used to induce hairy roots on suitable host plants including some species of the Solanaceae group (tomato, potato, tobacco) and others, such as Lotus japonicus and Arabidopsis thaliana. The class 2 activity concerns the use of *A. rhizogenes* which is classified as a plant pathogen. The presence of fungal or nematode genes, unlikely to be expressed in this bacterium, is not expected to increase the pathogenicity or host range of *A. rhizogenes*.

### Host/vector system

* A. rhizogenes strains LBA9402 and 8196/pBin 19-type binary vectors.

### Origin & function

The origin of the genes (proteases, chitinases, cell cycle regulators) will be non-pathogenic nematophagous or entomopathogenic fungi, or free-living (*C. elegans*) or plant parasitic nematodes. The origin of the promoters to drive expression of the genes in plants will be constitutive and inducible plant promoters. The pBin19-type vectors are well characterised. The function of the genetic material is to be co-transformed into plants during hairy root induction by *A. rhizogenes*, to express fungal enzymes in these roots that can then be assayed for resistance to infection by plant pathogenic nematodes in enclosed assays. Whole plants may be regenerated from transformed material - these will be checked to ensure they are free from *A. rhizogenes* before any nematode infection assays on a larger scale are undertaken - this will no longer be a Class 2 activity.

The agrobacteria are held at Rothamsted under Plant Health Licence PHL 174/4099(02/2002) which will also cover the use of the genetically modified derivatives. Plant parasitic nematodes are held at Rothamsted under licence PHL 174A/4485(04/2003).

### Evaluation of foreseeable effects

It is highly unlikely that the fungal or nematode genes will express in *A. rhizogenes*, as they will be inserted in the T-DNA of the binary vector pBin19, with a plant promoter. There is not normally any significant expression of genes within the T-DNA in bacteria. In the unlikely event that they are expressed, it is not anticipated that they will increase. *A. rhizogenes* plant pathogenicity or host range.

The intention is to examine the effect of these genes on transfer to plants and it is anticipated that they may have some effect in preventing infection by plant parasitic nematodes.

### Confinement and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Plants will be transformed by co-culture with the *A. rhizogenes* at level 2 containment. Transformed plants will be treated with appropriate antibiotics or sterilants to kill the live agrobacteria before subsequent experiments. However, some nematode infection assays may be carried out on hairy roots on the host plants in sealed containers in a locked growth cabinet before removal of the agrobacteria has been verified.

If plants are regenerated from hairy roots they will be checked for the absence of *A. rhizogenes* before moving to the appropriate level of containment. Experiments with regenerated transgenic plants and their progeny will be performed in the GM glasshouse facilities to avoid pollen dispersal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material (bacterial cultures, plants, nematodes, growth media and disposable plasticware) from this project will be subjected to autoclaving, using a validated autoclave, prior to disposal with other laboratory waste. Glassware will likewise be autoclaved before washing and reusing. This autoclave, No. 1 in Room 238 (building 60) is checked annually. It is set on a waste sterilisation and kill cycle of 1 h at 121 °C, 15 psi, each cycle logged and if these prescribed conditions are not met, the load will be re-autoclaved.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment

The GM committee were satisfied with the risk assessments presented and the control measures specified and agreed that this work could safely be carried out as a Class 2 activity.

Project Containment

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Animal Units  

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Project Ref 92/04.1

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<td>CONSTRUCTION AND SCREENING OF METAGENOMIC LIBRARIES CONTAINING DNA FRAGMENTS OBTAINED DIRECTLY FROM UNCULTURED BACTERIA IN NATURAL ENVIRONMENTS, CLONED INTO WIDE HOST RANGE VECTORS</td>
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Non-GMM Consent Granted  

Consent Granted: not applicable

Project notified under transitional arrangements  

N
Project Additional Information

Purposes of the contained use

To construct gene libraries from bacterial genomic DNA obtained directly from environmental populations and with no prior culturing, the libraries to be screened in different, non-pathogenic, bacterial hosts for any clones that contain genes that determine phenotypes of importance in the nitrogen cycle and in certain biotechnological applications.

Recipient or parental organism

The hosts will be Escherichia coli K12 and derivatives thereof; and a wide range of bacteria including rhizobia, Paracoccus, Streptomyces spp, Bacillus subtilis, Azotobacter vinelandii, Rhodobacter spp., non-pathogenic Pseudomonas, Burholderia, Alkaligens and related "pseudomonad" spp., and Nitrosomonas spp. E coli K12 is unable to colonise the human gut and the other genera will be chosen on the basis that, as far as is known, they are unable to cause any damage to animals, plants or the environment. These strains will be hosts for Metagenomic libraries containing DNA fragments obtained directly from bacteria isolated from the environment and cloned in cosmid vectors. By their nature, such libraries and the individual recombinant plasmids within them will be very varied in their identities. Therefore, the characteristics of any given GMO are impossible to predict.

Host/vector system

DNA will be obtained, en masse, from bacterial cells in natural populations from a range of environments. The functions to be tested initially will be the ability to confer individual steps in the Nitrogen cycle - nitrogen fixation, nitrification and denitrification. However, the libraries will also be screened for genes involved in other, known bacterial transformations including the synthesis of enzymes (eg protease and lipase) of biotechnological value and for the presence of genes (eg for polyketide synthesis) involved in the synthesis of novel antibiotics. Other genes may be sought but in no case will these include functions (eg toxin production) that are immediately deemed to be hazardous. The process of cloning DNA extracted from the soil community means the frequency which particular genes are represented in the library will reflect their abundance in soil. Relatively uncommon pathogenic bacteria are very unlikely to be represented in the library and the processes will reflect their abundance in soil. Relatively uncommon pathogenic bacteria are very unlikely to be represented whereas abundant bacteria (and genes) will be well represented. This can be justified by the observation that soil is not a potent source of virulent human pathogens nevertheless common sense dictates that the soil metagenomic libraries should be treated with caution at C2. Similarly, until the frequency of different types of genes in the library is investigated, we cannot be sure that it is dominated by innocuous housekeeping genes.

Evaluation of foreseeable effects

As stated above, exact foreseeable effects are hard to determine, just as would be the case when plating out samples of soil, mud, deep ocean water etc on appropriate microbial growth media. However, the hosts to be used for the libraries are compromised, non-colonising, and will be grown in small volumes. Therefore it is extremely

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Withdrawn

Tick if notifying a connected programme of work

02/03/2022
unlikely that any large-scale escape will occur and that even in the unlikely event of a single clone having a vast selective advantage, compared to the parental strain, the numbers involved would be so low as to compare with the natural exchange of genetic material between different bacteria in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid media cultures used for the growth of E.coli will be autoclaved at 121 degrees 15 psi for 60 minutes in an autoclave registered for disposal of genetically modified organisms; 100% kill achieved using this method and temperatures monitored using and internal probe. Autoclave equipment is inspected annually to ensure it reaches correct temperature and pressure. The autoclave run is recorded so it can be repeated in case the required temperature and pressure are not achieved for the prescribed period.

All contaminated glassware and plastics will be treated either by autoclaving or with a broad-spectrum disinfectant. Liquid waste, once treated, will be disposed to drains; solid disinfected waste (plastics, agar) will be double bagged and disposed of in the general waste skip.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GM Safety Committee was satisfied that the work could be conducted safely at C2.

Project Containment

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Project Ref 92/05.1
Construction and evaluation of strains of the phytopathogenic fungal species with Mycosphaerella graminicola (Septoria tritici) reporter genes and silenced genes

Project Additional Information

Purposes of the contained use

To generate various transgenic strains of Mycosphaerella graminicola (Septoria tritici) to (a). Quantitate fungal biomass in planta.
(b). Determine the fungal hyphal infection route in susceptible and resistant wheat cultivars and other host and non-host plant species.
(c). Ascribe a function to specific fungal genes discovered through genomics and computational analyses.

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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- Date Project Ceased: 30/01/2007
- Tick if notifying a connected programme of work: N
- Historical Significant Changes
- Historical Date of Additional Info
- Significant Change ID
### Project Additional Information

#### Purposes of the contained use

Screening of candidate nematode genes on suitable host plants in a search for necrosis phenotypes.

---

#### Recipient or parental organism

Agrobacterium tumefaciens strain C58C1 containing the helper plasmid pCH32. The pCH32 plasmid carries two vir genes (vir E and vir G) and resistance to tetracyclin. This plasmid is under kanamycin selection.

---

#### Host/vector system

Expression vector pSfinx (described in full detail in Takken et al. 2000 J.24(2):275-283). This is binary, potato virus X (PVX)-based expression vector. It contains the left and right border of the T-DNA sequences. The expression of the PVX sequence, consisting of replicase, triple block and protein genes, is driven by the 35S promotor. Two SfiI sites are used for cloning of the cDNAs. The vector also contains a neomycin-phosphotransferase II sequence. Viral-encoded suppressor vector of gene silencing 35S:p19 (described in full detail in Voinnet et al. 2003 Plant J. 33:949-956). This vector contains the p19 and the HcPro sequences of the tomato bushy stunt virus inserted into the binary vector pBin61.

---

#### Origin & function

The expression library will be constructed with cDNAs from the potato cyst nematode Globodera rostochiensis, which is endemic in the UK. This library will contain the transcriptome complement of the nematode at the moment of genetic material extraction (parasitic stage J2-J3), which may include paratism-pathogenicity-factors.

We aim at selecting nematode cDNAs from the library, out of a screen on suitable plant genotypes, that elicit a cell death phenotype (hypersensitive response). Vector 35S:p19 will be co-inoculated to avoid possible gene silencing of nematode cDNAs in planta.

The putative functions of the selected cDNAs might be related to the initiation and/or signal transduction leading to cell death. Some of these cDNAs could also be recognised specifically resistant host genotypes.

---

#### Evaluation of foreseeable effects

The expression library of parasitic nematode cDNAs will be expressed by the PVX-based binary vector pSfinx. The library will then be introduced in the A. tumefaciens strain C58C1 for sorting and expression of individual clones on suitable plant genotypes in a search of nematode cDNAs eliciting visible phenotypes. The bacteria transfer the expression vector to the plant cells, and the CaMV 35C promotor drives the synthesis of infectious recombinant PVX transcripts. These transcripts subsequently initiate the formation of virus particles that infect the tissue surrounding the inoculation site.

The modified PVX-expression vector, into which the nematode cDNAs will be inserted, is not thought to cause harm to humans or animals. PVX is a plant virus with restricted range of hosts (some Solanaceae crops such as tomato and potato). Potato virus X could be virulent to other non-target host plants. The nematode genes integrated into the PVX vector are thought not to add any more risk than the vector alone.

It is extremely unlikely that the products of the G. rostochiensis genes inserted into the PVX-based expression vector have biological properties or activities which may give rise to human or animal harm. However, it is unknown if the plant cyst nematode G. rostochiensis genome encodes for any toxin that can have a deleterious effect on human health. Some of the cyst nematode genes inserted into the PVX may be pathogenicity determinants for their natural hosts, but it is very unlikely that these factors (or any other non-pathogenicity factors) would work synergistically with PVX to alter or affect this virus features.

The experiments will use modified viruses that are as virulent or less virulent than the wild type constructs. The most virulent constructs would allow the ability to survive, establish and disseminate in the environment. However, given the use of the GM containment facilities and the known fact that PVX is not transmitted by invertebrate vectors or true seed, there is only a low likelihood of hazard from these experiments.

The most severe consequence of survival, establishment and dissemination would be the mild disease caused by PVX in potato and tomato used in other experiments.
PVX infection is already widespread but does not cause serious agronomic problems even in the susceptible cultivars. Thus the worst case scenario is that there would be a low consequence of hazard. The risk of harm is therefore low. The risk will be managed by ensuring strict compliance with the containment procedures in the nematode glasshouse GH40 at Rothamsted Research.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A.

However, since the screening of the nematode expression library will be carried out in plants, GM containment glasshouse space for nematode work (GH40) will be used for this purpose. After plants are inoculated, transient expression of individual clones on plants will be carried out. Visible phenotypes will be expected to appear in the plants 2-3 weeks after inoculation of clones. Inoculated plants used for screening will be autoclaved after use following the standard operating procedures put in place for this glasshouse space and material (procedure BB_QA_Doc/0589/002 enclosed) for work with the transformed/genetically modified plants in the nematode glasshouse.

Entry to these glasshouses is restricted to users. Doors are kept locked at all times, with entry by coded entry blocks.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

PVX-based expression library. All materials (solid or liquid cultures) not used for the construction and/or screening of the library will be treated and disposed by heat treatment (autoclaved in autoclave bags).

Plants used for screening. After inoculation and selection of individual nematode clones on plants in the containment glasshouse, plants will be destroyed by autoclaving and disposed.

All selected, post-screening Agrobacterium colonies (on solid or liquid cultures) that are not for further studies will be disposed of after autoclaving.

Autoclaving conditions for disposal of all materials are 121 C, 15psi, 60 minutes. Temperature of autoclaving is monitored using an internal probe. Autoclave runs are recorded and checked. Autoclave equipment is inspected annually.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment

The GMSC was satisfied that the work could be conducted at CL2.

### Project Containment

**Laboratory Activities**

**Glass Houses**

**Growth Rooms**
Rhynchosporium secalis is a phytopathogenic fungus causing leaf scald, an economically damaging disease in barley. This project's aims are:

1) To investigate asymptomatic infection in susceptible and 'resistant' host cultivars, using strains transformed with fluorescent marker genes;
2) To investigate sensitivity to chemical control, by silencing genes that may be associated with reduced fungicide sensitivity.

Two types of experiment will be performed:

1) Isolates of the phytopathogenic fungus Rhynchosporium secalis will be transformed with visible marker genes encoding colour variants of the green fluorescent protein (GFP) and red fluorescent protein (DsRed) under the control of either constitutive fungal promoters or endogenous inducible promoters isolated from virulence factors. Transformation using Agrobacterium tumefaciens will be compared with transformation using traditional PEG-mediated procedures. Host plants and seeds will be infected with transformed isolates by application of a spore suspension and the progress of infection will be monitored by confocal microscopy under UV light.

2) Homologous gene replacement will be used to substitute a gene with a "null" allele for this gene. At the null allele, will be placed a gene sequence coding for hygromycin antibiotic resistance. This will result in a complete loss of function in the gene of interest. Alternatively gene disruptions will be generated by A. tumefaciens mediated transformation of a gene cassette encoding for hygromycin resistance or by in vitro transposition of the EZ: TN transposon (source E. coli) into cosmids and subsequent fungal protoplast transformation.
Recipient or parental organism

The recipient organism (Rhynchosporium secalis) is a phytopathogenic fungus which causes leaf blotch of barley (Hordeum vulgare L.) The fungus is unable to infect humans and is not capable of growth at 37°C. The fungus does not cause known allergies in humans. None of the micro-organisms used are capable of infecting domestic or wild animals. None of the micro-organisms used are listed in ACDP hazard groups 2, 3 or 4. The pathogen is not controlled by Defra and no licence is required. Populations of the fungus consist of two mating types (I and II). The infection is often asymptomatic in early infection and it is unknown as to how seed-borne infection plays a role in the proliferation of the fungus. The asexual spores (conidia) of the fungus are splash dispersed. The putative sexual spores (ascospores) of the fungus have yet to be identified.

Host/vector system

For cloning purposes, E.coli K12 derivatives will be used. These disabled bacterial strains are not capable of survival in the environment or in humans and consequently do not pose a risk.

For transformation, the disabled A. tumefaciens strains AGL1 and/or LBA4404 will be used. These strains could survive if released into the environment. A. tumefaciens is capable of transferring genetic material to plants and fungi, but it is not pathogenic to, or capable of surviving in humans.

Origin & function

gpdA - glyceraldehyde-3-phosphate dehydrogenase promoter from Aspergillus nidulans used to drive expression of eGFP in transgenic R. secalis - non-harmful.
PtpC- hph- TrpC - cassette containing hygromycin B resistance gene from E. coli flanked by trpC promoter and terminator sequences from Aspergillus nidulans used as a selectable marker to enable identification of successfully transformed R. secalis - non-harmful.
eGFP - enhanced green fluorescent protein from Aequoria victoria jellyfish (and modified colour variants such as cyan and yellow forms) used to enable visualisation of transgenic R. secalis in infected barley plants - non-harmful DsRed - red fluorescent protein from Discosoma (sea anemone) - non-harmful.

None of the inserts are known to cause harm to humans, none are toxic or are known human allergens. As a precaution, inoculation of barley plants with transgenic R. secalis spores will be done by applying droplets of spore suspensions to barley leaves and seeds. No spraying will be necessary and this will reduce exposure to researchers.

Evaluation of foreseeable effects

The genes used in this project are purely for marker purposes and none of the inserts used will increase the pathogenicity, host range or fungicide resistance of the transgenic R. secalis isolates. The inserted DNA does not have any potentially harmful biological activity and is not a toxin, oncogenic protein, allergen or hormone. The inserted DNA does not encode a pathogenicity determinant. The inserted DNA will not alter the host range of the pathogen. The inserted DNA encodes resistance to the antibiotics kanamycin and hygromycin - these antibiotics are not used for the treatment of laboratory-acquired infections, nor for the agricultural control of R. secalis.

The gene silencing experiment is expected to give unaltered or increased sensitivity to fungicides, since naturally-occurring R. secalis isolates lacking a functional Cyp51A gene have higher triazole sensitivity, and previous studies in Aspergillus fumigatus (Antimicrobial Agents and Chemotherapy 49 (6): 2536-2538) and Fusarium species (Phytopathology 99(5): 487-497) have found Cyp51 disruptants to have increased or unaltered sensitivity to DMI fungicides. Only cases of increased fungicide sensitivity of transformants would be tested in planta.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A: GM micro-organism only
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid media cultures used for growth of E. coli and A.tumefaciens and solid media cultures and spore suspensions of transgenic R. secalis will be autoclaved at 127°C for 30 minutes in an autoclave registered for the disposal of genetically modified organisms. 100% kill is achieved using this method and temperatures are monitored using an internal probe. Autoclave equipment is inspected annually to ensure that it reaches the correct temperature and pressure. All autoclaved material is deposited into a skip, which is emptied into landfill sites.

All R. secalis liquid cultures will be treated using a double kill procedure, i.e chemical treatment prior to autoclaving.

Plant materials (leaves, whole plants soil and material used for microscopy infected with transgenic R. secalis will be autoclaved at 127°C for 30 minutes in an autoclave registered for the disposal of genetically modified plant material. Autoclave equipment is inspected annually to

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee assigned this project as Class 2, containment level 2, with the proviso that no experiments will be done that could potentially increase the pathogenicity of the organism.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 92/10.3

Date Ackn’d CU2 Project Title Class Culture Vol Class 2 Culture Vol Class 3-4

02/03/2022
Analysis of Gaeumannomyces graminis var. tritici and Magnaporthe oryzae infection of cereal roots and visualisation of green fluorescent protein (GFP)-transformedMagnaporthe oryzae during infection of cereal roots

Purposes of the contained use

(a) To intercompare the infection biology of these two root infecting species.
(b) To use the reporter strain of the Magnaporthe oryzae fungus as a surrogate for Gaeumannomyces graminis var. tritici when investigating the genetic basis of resistance identified in various cereal species.
(c) To explore in depth the cellular and molecular basis of resistance to Magnaporthe oryzae and Gaeumannomyces graminis var tritici.

Recipient or parental organism

G. graminis var. tritici (Ggt) causes take-all disease of cereals and grasses, and M. oryzae causes rice blast disease. Both are economically damaging fungal diseases. No GM strain of Ggt will be used in experiments, only wild type isolates collected from the field. The Ggt cultures will be used to compare infection of wheat roots with wild type M. oryzae strain Guy-11 and a GFP-transformed M. oryzae strain.

Magnaporthe oryzae (previously called M. grisea) is an economically damaging fungal pathogen causing Rice Blast (Baker et al., 1997). The process of infection of rice plants by M. oryzae is typical of foliar pathogens (Xu et al., 1997). The air-borne asexual spores, conidia, attach to leaf surfaces upon contact. Under moist conditions the conidia germinate producing germ tubes that can form specialised infection structures, appresorium, that penetrate through the leaf surface. Bulbous infection hyphae colonise the initially infected cells, from these develop filamentous hyphae which then spread rapidly between plant cells causing visible lesions on the plant surface. Under high humidity mycelia within the lesion sporulate producing conidia to start the infection cycle again.

M. oryzae strains can infect a range of temperate cereals and grasses but individual strains have limited host ranges (Valent & Chumley, 1991). M. oryzae strain Guy11 (the strain stored at Rothamstead Research and to be used in experiments; Leung et al., 1988; Chao and Ellingboe, 1991) can cause disease on the leaves of rice as well as barley and the model grass species Brachypodium distachyon (Parker et al., 2009). Under the experimental conditions M. oryzae can also be inoculated onto barley and wheat roots and causes visible disease symptoms (Dufresne and Osbourn, 2001). In rice, root infections can spread to the aerial parts of the plant and so may contribute to epidemic development in the field under natural conditions (Sesma & Osbourn, 2004).

Natural infections of M. oryzae on barley have previously not been recorded (Sweigard et al., 1998) but in 2007 field infections of M. oryzae on barley were reported for the first time in Brazil (Lima et al., 2007). Under experimental conditions high relative humidity and temperature (greater than 24°C) are required for infections on barley and...
Brachypodium. Conditions in the UK are unfavourable for rice blast development so it is unlikely to pose a significant threat. *M. oryzae* is also distributed throughout the rice growing regions of Europe (including Southern France, Italy, Portugal, Spain and Hungary; (Roumen et al., 1997)) so if it was to be a threat in the UK it is likely that it would already be a problem.

References

Host/vector system
*M. oryzae* cultures will be inoculated in plant pathogenicity assays onto the roots of cultivars of hexaploid, tetraploid and diploid wheat species to compare the infection process with that of the root attacking fungus *Gaemannomyces garminis* var. *tritici*. Various other cereal species will be included as additional controls in some experiments. These will be oats, barley, rye, triticale and rice. In the field *M. oryzae* causes leaf blast disease on a range of temperate cereals but under experimental conditions it has been shown to be able to infect rice, barley and wheat roots.

Origin & function
The genetic material involved is a GFP-transformed strain of *M. oryzae*. No transformation of *M. oryzae* will be carried out as part of this project. The GFP transformed strain to be used in plant infection studies is already stored at Rothamstead. The H3:eGFP strain (Veneault-Fourrey et al., 2006) carries the eGFP gene fused to the C-terminus of the histone E11-encoding gene hH1 from *Neurospora crassa* under control of the CCG1 constitutive promoter. The strain displays fluorescent nuclei and is hygromycin resistant. The expression of enhanced green fluorescent protein (eGFP) will be used to visualise transgenic *M. oryzae* in infected wheat plants and compare the infection process in resistant and susceptible wheat cultivars.

Reference

Evaluation of foreseeable effects
There are no foreseeable harmful consequences of the release of these fungi, either for human health or the environment.

The inserted DNA in the H3:eGFP *M. oryzae* strain does not have any potentially harmful biological activity and is not a toxin, oncogenic protein, allergen or hormone. The inserted DNA also does not encode a pathogenicity determinant and will not alter the host range of the pathogen. The inserted DNA encodes resistance to the antibiotic
Hormycin - this antibiotic is not used for the treatment of laboratory-acquired infections. Finally, the inserted DNA does not encode resistance to any disease control measures such as fungicides. The genes used to transform the GFP M. oryzae strain are purely for marker purposes and none of the inserts have been shown to increase the pathogenicity or host range of the transgenic GFP M. oryzae strain. eGFP is also not harmful to humans.

Conditions in the UK are unfavourable for rice blast development so it is unlikely to pose a significant threat. M. oryzae is also distributed throughout the rice growing regions of Europe (including Southern France, Italy, Portugal, Spain and Hungary; (Roumen et al., 1997)) so if it was to be a threat in the UK it is likely that it would already be a problem. None of the micro-organisms used are capable of infecting domestic or wild animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable to this application.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste: To discard plants at the end of each inoculation experiment in a Containment Level 2 growth room/glasshouse, a microbiological lab coat and gloves will be worn. All the aerial plant parts will be cut and placed in large plastic bags for autoclave disposal. Any soil, vermiculite, pots, capillary matting and labels will be collected into autoclave bags in the large stainless steel bins. Bags weights should not exceed 5kg. Bins should only be half full before they are sealed and taken directly to the autoclave. The solid trays will be wiped down with Microsol 3 (Anachem) disinfectant as a 1 in 10 dilution in water before a new experiment is initiated. The autoclave cycle will be 121°C for 30 mins for soil or large volumes of plant material. The autoclave will be monitored to determine that sterilisation cycle has been successfully completed. Bins must be taken directly from the growth room component to the autoclave and autoclaved immediately. Waste material should be bagged for disposal within the growth room/glasshouse in which it was generated. Transport of GM and/or licensed materials for disposal must always be in double contained bags and transported in a metal box with a lid. Once the box has been placed inside the autoclave the lid is carefully removed and placed by the side of the box within the autoclave. Ensure that bags are left open to allow steam to get into the bag. If the autoclave is in use the full bags will be sealed and left in the growth room until the first opportunity to undertake the autoclaving becomes available. This entire procedure will be undertaken only by members of the research team.

Liquid waste: To discard any liquid waste a microbiological lab coat and gloves will be worn. Any waste water or Hoaglands solution (plant growth solution for hydroponics) will be poured into 1 litre flasks as 750 ml batches, situated in the deep trays, in the growth rooms/glasshouse compartments. To each flask Microsol 3 will be added to give a final dilution of 1 in 50, i.e. 15 ml per 750 of waste water. The flasks will be wiped down with Microsol 3 (1 in 50 dilution) and transferred to a cleaned tray. They will then be transported in theses clean deep trays to the autoclave for disposal with an autoclave cycle of 121°C for 30 mins. This double kill procedure should ensure no live organisms enter the drainage system.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Rothamstead Genetic Manipulation Safety Committee has carefully considered the attached Risk assessment and the appropriateness of the containment measures proposed and has given approval for the work to proceed at Containment level 2 once ratified by the HSE.
### Project Containment

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### Project Ref 92/11.1

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<td>Identification of fungal effectors and the key recognition and signalling components conferring disease resistance in cereal plant species as well as the mechanisms leading to fungal infection</td>
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<td>Class 3</td>
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Non-GMM Consent Granted: Yes

Project notified under transitional arrangements: N

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### Project Additional Information

**Purposes of the contained use**

This is a connected programme of work, comprising of the following activities (from the highest perceived risk to the lowest):

A.1 (RRes GM project 158). Use of Barley stripe mosaic virus (BSMV) vector for transient overexpression of Fusarium graminearum predicted secreted proteins (i.e. putative effectors) in the leaves and ears of cereal plant species.

A.2 (RRes GM project 157). Identification and characterisation of genes in cereal plant species required for disease and/or resistance against floral tissue invading fungal phytopathogens using BSMV-mediated Virus-induced gene silencing (VIGS)
A.3 (RRes GM project 156). Functional characterisation of genes expressed in floral tissues and/or grain and controlling yield and various other sustainability traits in cereal plant species using BSMV-VIGS (non-pathology projects).

B.1 (Rres GM project 154). Transient BSMV or Agrobacterium tumefaciens mediated overexpression of Mycosphaerella graminicola predicted secreted proteins (ie putative effectors) in the leaves of cereal plant species.

C (RRes Gmproject 155). Functional characterisation of genes in cereal plant species required for growth and development , and those involved in resistance or required for disease caused by leaf tissue invading fungal phytopathogens using BSMV-VIGS.

D (RRes GM Projects 152 and 153). Investigating the role of different plant defence signalling pathways against phytopathogenic fungal species using GM non-flowering and flowering plants.

NOTE: Only activities A.1 and A.2 will require the use of the higher level (containment level 3) facilities. All other activities will require the use of containment level 2 facilities.

There are three classes of GMOs involved in the proposed programe of work.

1) Barley stripe mosaic virus (BSMV) engineered to express secreted fungal proteins or carrying short fragments of various cereals genes in antisense orentation (activities A.1, A.2, A.3, and C). BSMV only infects plants and is not known as a hazard to human health, nor is there any reason to suppose that it could be unless specifically modified to express toxic proteins or proteins that repoduce toxic products.

2) Wild type or GM strains of the phytopathogenic fungal species Magnaporthe oryzae and Rhychosporium secalis carrying reporter genes and Mycosphaerella graminicola, Fusarium graminearum and F. culmorum with reporter genes or silenced genes (activities A. 2, C and D). All of theses fungal pathogens are solely pathogens of plants and do not infect humans. None of the pathogens, except for F. graminearum and F. culmorum, have been known to produce toxins that are harmful to humans. F. graminearum and F. culmorum produce water soluble trichothecene toxins when they invade plant tissue which may cause poisoning of humans if the infected grain is eaten.

3) Wild type, induced mutants or GM cereal plant species (all activities) and A. thaliana plants (activity D only).

In most of the projects only wild-type plants will be used as hosts. The host plant species that will be used are hexaploid wheat (Triticum aestivum), einkorn wheat (T. monococcum), barley (Hordeum vulgare), maize (Zea mays), rice (Oryza sativa), Brachypodium species, Nicotiana benthamiana and Arabidopsis thaliana. On some projects we will also use GM plants that constitutively express reporter proteins eg GFP (green fluorescent protein), either constitutively express or have reduced levels of expression due to RNAi of genes implicated in resistance to various plant pathogens (see below).

The pollen of flowering plants can be an allergen. However, it is highly unlikely that the fungal species used or BSMV will increase allergenic effects of pollen from host plants, or cause the production by the experimental plants of other unknown allergens or toxins posing health hazard to humans.

1) The genes to be inserted into BSMV for transient in planta expression are predicted secreted proteins of unknown function which have been identified in the genome sequence of the cereal plant species infecting ascomycetous fungi Fusarium graminearum (activity A.1). A subset of predicted secreted proteins from each fungal species (~200 from F. graminearum, ~200 from M. graminicola, and ~50 from R. secalis) will be selected based on expression profiling experiments and further sequence anlyses.
Limitations are also placed on the number of genes we could test based on a maximum insert size of less than 700-nt which is stable in the BSMV vector. The encoded proteins contain no recognisable homologous or functional domains and it is highly likely that they function specifically in these host-pathogen interactions. None are currently known to be toxins. These projects aim to gain new information on the functions of these fungal putative effector proteins during plant pathogenesis and/or disease resistance.

For transient gene silencing (ie VIGS), short 100-500-nt fragments of various cereals genes will be inserted into the BSMV genome in antisense orientation (activities A.s, A.2, A.3 and C). Plant inoculation with these GM BSMV is expected to induce silencing of endogenous leaf, root, flowering-tissue and/or grain-expressing genes with homology to the 'transgene' sequence carried by BSMV.

2) The reporter genes present in the GM fungal species used (M. graminicola, F. graminearum, F. culmorum, M. oryzae and R. secalis) are green fluorescent protein (GFP) from Aequorea victoria or β-glucuronidase (GUS) from E.coli. They will be used to track the presence and progression of fungal pathogen infection in host plant tissues. In addition, we will use transgenic F. graminearum, F. culmorum, or M. graminearum strains with a single endogenous gene of interest disrupted in each strain, resulting in a 'null' allele for the disrupted gene and a complete loss of gene function. The disrupted fungal genes are those required for pathogenicity or for the synthesis of toxins. Only strains found to be equal or less pathogenic on plants, or cause the accumulation of lower levels of toxins in the host plant compared to wild-type strains will be used.

The use of transgenic M. graminicola, F. graminearum and F. culmorum on non-GM plants is covered by Defra licence PHL174C/6222. In this programme of work, we propose to use these transgenic fungal species on GM plants (activity D) or in conjunction with GM BSMV on wild-type or GM plants (activities A.2, A.3 and C).

3) Most of the gene sequences transformed into the GM cereal plant species and A. thaliana plants that are to be used (activities A.2, A.3, C and D) are from the original or other plant species. These plants will either constitutively activate, or be compromised in the ability to activate, one or more of several signalling pathways involved in plant defence against phytopathogenic microbial or fungal species. Some experiments may also involve the use of GM plants transformed with either GFP or GUS reporter genes.

Evaluation of foreseeable effects

There is no anticipated risk of GM BSMV posing any harm to human or animal health as the virus is exclusively a plant pathogen. Also there are no reasons to believe that the fungal gene or plant gene sequences to be inserted into the BSMV vector may pose any risk to human or animal health. The experiments involving BSMV will pose a potential environmental hazard if BSMV-induced down-regulation of endogenous plant genes in infected plants (1) results in developmental defects severely compromising crop yield, (2) severely compromises a host defence reaction, or (3) dramatically enhances plant susceptibility to BSMV itself or to other pathogens of cereal crops. In the initial VIGS experiments the plant phytoene desaturase (PDS) gene involved in the synthesis of carotenoids will be used as a control measure the efficiency and extent of silencing. Down-regulation of this gene results in macroscopically visible photo-bleaching due to chlorophyll photo-oxidation (Kumagai et al., 1995, Proc. Natl. Acad. Sci. USA 92: 1679-1683). Accidental release of the BSMV:PDS VIGS vector may harm the environment as initially infected wheat and barley plants will have reduced photosynthetic ability, which will have a knock-down effect on crop yield.

It has been previously shown by others that sequences inserted into BSMV RNA-gamma vector are unstable as the virus replicates (Pogue et al., 2002, Annu. Rev. Phytopathol. 40: 45-74; Bruun-Rasmussen et al., 2007, Mol. Plant Microbe Interact. 20: 1323-1331). That is, passing the recombinant virus more than once to another plant usually results in either complete loss of the heterologous sequence or its significant shortening. Short host insert sequences (<120 bp) are known to be significantly less effective in BSMV-VIGS (Schofield et al., 2005, Plant Physiol. 138:2165-2173; Bruun Rasmussen et al., 2007, Mol. Plant Microbe Interact. 20: 1323-1331). Therefore, accidental release of recombinant BSMV may cause disease on the initially infected plants. However, due to instability of the insert the hazard would be eliminated in any secondarily infected plants and the virus would be no more severe than wild type virus. BSMV infection is already widespread but does not cause serious agronomic problems even in susceptible cultivars (Jackson & Lane, 1981, Handbook of Plant Virus Infections and Comparative Diagnosis, ed. E Kurstak, pp. 565-625, Amsterdam: Elsevier).

Despite the instability of the BSMV vector, long-lasting silencing extending into second-generation plants is possible (Bruun-Rasmussen et al., 2007, Mol. Plant Microbe Interact. 20: 1323-1331. This is because of BSMV's known ability to be transmitted through seed. To reduce this potential hazard due to accidental release of recombinant BSMV the main bulk of the experimentation will involve the use of vegetative stage plants only. In those experiments requiring seed production the harvested seed from
experimental plants will be securely stored in double sealed boxes. All stored seed will be recorded in the database on a shared project drive which is automatically backed-up on a daily basis.

The transgenic reporter protein-expressing fungal species are not known to be less or more fit than the corresponding parental stains. During these experiments sexual spore formation will not take place and so the risk of vertical gene transfer between the GM fungi to be used and their wild-type relatives is minimal. These reporter proteins have been expressed in a wide range of experimental systems and do not present a risk to human health. The loss-of-function transgenic mutant M. graminicola and Fusarium spp. Strains have been found to be less virulent than wild-type strains and are also likely to be at a competitive disadvantage compared to wild-type strains. In GM host plants that are compromised in their ability to resist pathogen attack, the GM fungal pathogens may be able to spread more extensively or cause more severe symptoms, cause wild-type levels of disease or cause less disease. These fungal strains are not intrinsically more pathogenic or virulent than wild-type strains in experiments on non-transgenic plants. Indeed all the GM strains are likely to be at a competitive disadvantage compared to wild-type strains, and as such present less risk to the environment than the natural wild-type strains. Co-infection of wild-type or GM fungi with BSMV onto host plants is not though to confer any additional risk, and the potential risk of transfer of BSMV-vector genome or BSMV-expressed transgenes to any of the fungal species is negligible as MSMV has not been reported to infect any plant pathogenic fungal species.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**Activities A.2, A.3, C, and D may involve the use of GM cereal plants.** The greatest risk associated with the use of GM plants in this programme of work is the requirement for flowering plants to be used. Extensive measures have been put in place to ensure that neither pollen nor seed can escape, and particular attention has been paid to ensure that flying insects are absent from the building.

All soil used in the building is kept frozen at -20°C prior to use to kill any insects and other invertebrates. All water waste from the building is treated by UV and chemical treatment before it leaves the building (see section 12).

All plants will be grown, and experiments carried out, in containment growth facilities where the access is restricted to authorised personnel. Flowering- and pathogen-inoculated plants will be handled at all times with gloved hands, all gloves being discarded directly into autoclave bags and changed before handling other plants. Any unwanted cereal plant ears will be cut off and discarded. All plants, soil, labels and waste, including microbiological waste, from these growth rooms will be bagged in the room and autoclaved before disposal. Flowers and seed pods on plants are to be bagged and discarded first before they are put into larger autoclave bags, to reduce the possibility of dispersal around the growth chamber. All unwanted grain will also be autoclaved. Plants will be grown on capillary matting in solid trays with 4-cm sides so that all water, potentially containing pollen, fine seed or pathogens, is contained. Seed from experimental plants will be stored in double sealed boxes and records of seed stored kept in an electronic database.

When experiments do not require flowering plants, they will generally be disposed of before they start to flower. When plants need to be left for longer, they will be monitored to ensure that the heads are cut off before flowers emerge.

Designated laboratory coats are provided for work in each separate containment growth room and are never removed from the facility, unless they have been contaminated, in which case they are bagged and autoclaved. Disposable overshoes are also worn in the growth rooms and disposed of into an autoclave bag when leaving the compartment in order to prevent escape of pollen and seed on footwear. In growth rooms with experiments requiring the highest level of containment (i.e. projects A.2, A.2 and A.3) two pairs of disposable overshoes, one over the other, as an extra control measure. Long hair is always kept tied back securely and a hair net is worn to prevent pollen from attaching to loose hair.

Any plant tissue that is transported to other laboratories for analyses will be transported in double sealed containers. The outside of the transportation vessel will be wiped down with 70% Ethanol or Microsol 3 (1 in 50 dilution) just prior to leaving the containment area.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We request that only activities A1 and A2 are classed as containment Class 3, and that all other activities (A3 through to D) are derogated to Class 2. The greatest risks in activities A1 and A2 are associated with the requirement for flowering plants to be used for infection with a combination of wild-type or GM BSMV and fungal pathogens, but
activities B1, B2 and C will only utilise non-flowering plants, so there will be no risk of pollen from infected and/or GM plants escaping. Although activity A3 will use flowering plants, they will not be infected by fungal plant pathogens and so the risk associated with working with fungal spores will not be present. Similarly, experiments for activity D will be carried out with flowering plants but there will be no infection with BSMV.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste material (discarded plant material, disposable trays, pots, etc) will be bagged and steam autoclaved at 121°C for 30 minutes. Cinbins with sharps will be sealed, placed in an autoclave bag and steam sterilised.

High level liquid waste (i.e. unused liquid cultures) will be treated with concentrated sodium hypochlorite for 1 hour and then autoclaved at 121°C for 30 minutes in a double-kill procedure.

Any spillage will be wiped up immediately with tissue which is then placed in an autoclave bag and steam sterilised. Contaminated, or potentially contaminated, trays and surfaces will be wiped clean with Microsol-3 disinfectant (1:50 dilution with water) - wetted tissue and the waste tissue disposed of as above.

Low level liquid waste (i.e. water used to wash trays after experiments) from the facility is collected in a sump where it is mixed with a 1:50 dilution of Microsol-3 disinfectant (chemical inactivation before being pumped through an UV irradiation chamber for 24 hours in a double-kill procedure).


All lab coats will be steam sterilised before they are removed from the contained facility laundry.

Each autoclave run in the facility is monitored to ensure that all autoclave steam sterilisation runs proceed to completeness. The effectiveness of the UV irradiation and chemical inactivation two-step kill treatment on microbial species in liquid waste from the facility, have been published in HAMMOND-KOSACK, K.E., URBAN , M., FRANKLIN, J. & TABERER, R. (2009) Construction and establishment of a containment level 3 plant growth facility at Rothamstead Research. Proceedings of the UK Controlled User's Group, 20, 2-6. This document can be accessed online at www.ceug.ac.uk/documents/proceedings-09-acro-1_000.pdf.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Rothamstead Genetic Manipulation Safety committee has carefully considered the attached Risk assessments and the appropriateness of the containment measures proposed and has given approval for the work to proceed at Containment level 3 once ratified by the HSE

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022 Page 2715 of 15326
Project Additional Information

Purposes of the contained use

This is a connected programme of work, comprising of the following activities (from the highest perceived risk to the lowest):

A.1 Identification and characterisation of chemical compounds secreted from two genetically modified strains of T. hamatum, and their role in the enhanced biocontrol and plant growth promotion of lettuce (Lactuca sativa cv. Webb's Wonderful), relative to wild type T. hamatum strain GD12.

N.B. Lettuce plants used in this project are non-GM.

Recipient or parental organism

Trichoderma species are non-pathogenic, avirulent, beneficial soil fungi of agricultural interest as organisms which can enhance plant biomass under low nutrient conditions and used as biological control agents against crop pathogens. Whilst most reports show Trichoderma strains can elicit either plant growth or biocontrol, T. hamatum is unique due to its ability to elicit both properties simultaneously. Genome sequence comparison of T. atroviride, T. virens and T. reesei showed remarkable gene conservation between the three strains (Studholme et al., 2013), but little similarity when compared with T. hamatum. This indicates the presence of novel genomic regions, comprising approximately 40% of the T. hamatum genome, which could account for its dual PGP and biocontrol capabilities. T. hamatum GD12 is the isolate intended for use in this work, which was isolated from agricultural soil in North Devon. T. hamatum GD12 is the parental strain for this work.
There are two classes of GMOs involved in the proposed programme of work.

1) An N-acetyl-β-d-glucosaminidase-deficient mutant of T. hamatum, generated by insertional mutagenesis (using a hygromycin resistance cassette) of the corresponding gene (Saprotrophic competitiveness and biocontrol fitness of a genetically modified strain of the plant-growth-promoting fungus Trichoderma hamatum GD12, Ryder et al., 2012). Disruption of chitinase enzyme expression potentiates plant growth relative to WT T. hamatum GD12, and unlike the WT, the mutant has impaired sporulation. It has been demonstrated that the mutant is a hypersecretor of a T. hamatum specific extracellular antigen, and this phenotype could also lead to plant growth promoting compound hypersecretion, which warrants chemical identification.

2) T. hamatum with a deletion of heterochromatin remodelling protein 1 (hepA). This protein is involved in the activation and repression of silent secondary metabolite clusters in the genome, and deletion of the protein leads to the subsequent de-repression of cryptic metabolomic clusters, leading to enhanced biocontrol of the strain which could occur as a result of an expanded secretome. However, the compounds responsible for the enhanced biocontrol have yet to be identified.

Host/vector system

The wild-type fungus (Trichoderma hamatum GD12) will be the host fungus for all strains used.

In some projects we will also use GM T. hamatum that constitutively express reporter proteins eg GFP (green fluorescent protein), in order to ascertain the mechanisms by which the fungus associates with plant roots to enhance plant growth.

T. hamatum is a sporulating fungus, which has never been shown to be pathogenic to humans. Moreover, it is highly unlikely that the mutant fungal species used will pose a health hazard to humans.

Origin & function

1) N-acetyl-B-glucosaminidase is a key chitinolytic enzyme secreted by fungi to unleash sequestered chitin in soils, which are significant pools of nitrogen and carbon, enabling sequestration by plants and enhancing growth. Contrary to belief, disruption of the enzyme leads to enhancement of plant growth promotion relative to the WT T. hamatum (GD12). Generation of the enzyme deficient mutant used degenerate primers NagA and NagB to amplify the gene encoding the enzyme, and was inserted into a pGEM-T vector. Insertional mutagenesis was performed using fusion-based PCR with the hygromycin resistance gene from Neurospora crassa under the N. crassa TRPC promoter.

2) Heterochromatin protein Hep1 is a highly conserved heterochromatin remodelling protein involved in the activation and repression of secondary metabolite synthesising gene clusters. Inactivation of the protein would lead to the subsequent derepression of clusters which are silent under standard laboratory conditions, which should expand the secretome of the fungus, potentially leading to enhanced antifungal activity and biological control against cosmopolitan crop pathogens. A split marker methods of homologous recombination was used to replace the hepA gene of T. hamatum with the hygromycin resistance conferring hph gene from E. coli under a Neurospora crassa promoter.

3) The reporter genes present in the GM T. hamatum are green fluorescent protein (GFP) from Aequorea victoria. They will be used to track the presence and progression of fungal interaction of host plant tissue.

Evaluation of foreseeable effects

There is no anticipated risk of GM fungi having foreseeable negative effects on plant growth, given that the T. hamatum is a non-pathogenic, avirulent, beneficial organism when grown in association with plants, and the mutants enhance plant growth moreso than the wild type strain. Also, there are no reasons to believe that the fungal gene disruption and deletion for nag and hepA, respectively, may pose any risk to human or animal health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Activities may involve the use of GM fungal species, which in the case of the hepA mutant, is a sporulating fungus, although the nag is non-sporulating. The greatest risk associated with the use of GM fungi in this study is the use of strains containing a hygromycin resistance cassette as a selectable marker, the effects of which can be attenuated through soaking material which the fungi has been in contact with in either Virkon or Microsol.

All soil used in the building is kept frozen at -20°C prior to use to kill any insects and other invertebrates. All water waste from the building is treated by UV and chemical treatment before it leaves the building (see section 12).

Fungi will be subcultured in Category 2 laboratories.

All fungi will be grown, and experiments carried out, in containment growth facilities with a HEPA filter:
Controlled growth room R62 for the culturing of fungi on solid Potato Dextrose Agar (PDA) or liquid cultures in Potato Dextrose Broth (PDB),

Environmental growth chamber C52 for the use of peat microcosms, whereby the fungus is incorporated into soil matter containing seseds of plant, where the access is restricted to authorised personnel. Fungal-inoculated plants will be handled at all times with gloved hands, with gloves being discarded directly into autoclave bags and changed before handling other plants. All plants, soil, labels and waste, including microbiological waste, from these growth rooms will be bagged in the room and autoclaved before disposal.

An instrumental part of the project is the incorporation of fungal material into sterilized peat. This involves subculturing the fungi on PDA plates, and the addition of plugs of PDA into sterilized bran inocula. After a growth period of 5 days, 8g of bran inocula is incorporated into 300g of peat, which will take place in Cat 2 facilities. 100g of this peat-bran mix is then added to 120mm x 120mm x 7mm square petri dishes, and 25 seeds of lettuce are sown into the peat. The lids are placed onto the dishes, and they are added into an environmental growth chamber at 80% humidity for a period of 3 weeks. These will be watered with deionised water on a bi-daily basis until the plants are ready for harvesting, where the roots will be rinsed with deionised water and placed in foil packaging, and baked at 70 degrees for 48 hours. Biomass measurements will then be taken. The harvesting process will take place in the HEPA filtered room, and all water and material will have Microsol added to it, and will be autoclaved. For images of plant material, a camera will be used which will be wiped with ethanol after use to get rid of spores which may have been released.

Designated laboratory coats are provided for work in each separate containment growth room and are never removed from the facility, unless they have been contaminated, in which case they are bagged and autoclaved. I would also like to request a separate laboratory coat for handling of material in the environmental chamber.

Any fungal material that is transported to other laboratories for analyses will be transported through triple containment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste material (discarded fungal material, soil inocula waste, disposable trays, pots, etc) will be disposed in autoclaved waste and autoclaved at 121 °C for 30 minutes.

Any spillage will be wiped up immediately with Ethanol tissue which is then placed in an autoclave bag and steam sterilised. Contaminated, or potentially contaminated, trays and surfaces will be wiped clean with Microsol-3 disinfectant (1:50 dilution with water)-wetted tissue and the waste tissue disposed of as above.

Low level liquid waste (i.e water used to wash trays after experiments, condensate from chilled water units, spillages inside cabinet) from the facility is collected in a sump where it is then pumped to an container that is autoclaved at 121°C for 30 minutes.
All lab coats will be steam sterilised before they are removed for laundry.

The autoclave run in the facility is monitored to ensure that all autoclave steam sterilisation runs proceed to completion.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

“DECISION: Following emails circulated on the 25th October 2016, this project have been approved at Cat 2 and subject to the Risk Assessment and SOPs provided.”

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 Yes L3 L4 L2 L3 L4</td>
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Project Ref 92/20.1

Date Ackn’d 13/11/2020

CU2 Project Title Construction and evaluation of GM strains of the wheat root pathogen Gaeumannomyces tritici and fungal antagonists with biocontrol potential (Gaeumannomyces hyphopodiodes, ilyonectria crassa and Paraphaeosphaeria sporulosa)

Class 2 CultureVolClass2 < 1 Litre

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes
**Project Additional Information**

**Purposes of the contained use**

In this project we will construct different GM strains of the wheat root pathogen Gaeumannomyces tritici to study the function of candidate genes related to virulence and also GM strains for fungal antagonists with biocontrol potential (Gaeumannomyces hyphopodiodes, Ilyonectria crassa and Paraphaeosphaeria sporulosa).

**Recipient or parental organism**

Fungal species, isolated from Rothamsted Research fields. They are found naturally in wheat roots and the roots of other wild plants. The two Gaeumannomyces species are only soilborne, and do not produce airborne spores. I. crassa and P. sporulosa produce airborne spores, but with low establishment potential outside plant roots.

**Host/vector system**

E. coli / Agrobacterium: For cloning purposes E. coli K12 derivatives will be used. For Agrobacterium mediated transformation only disabled Agrobacterium strains will be used (i.e. AGL1).

**Origin & function**

Gene disruption and gene overexpression experiments will be performed in the four fungal species mentioned above for candidate genes related to fungal pathogenicity (for G. tritici) or biocontrol related functions (for the other three beneficial fungi). Reporter strains will be generated using fluorescent marker proteins e.g. GFP and mCherry, and other protein versions derived from them. Epitope tags like Flag or HA will be also used. The selectable marker initially used will be hygromycin antibiotic resistance. Other markers that may be used include geneticin, carboxin, phleomycin or benomyl resistance.

**Evaluation of foreseeable effects**

None of the microorganisms used as recipient could cause harm to humans, animal health or the environment. All fungal cultures will be grown in the laboratory. All materials and cultures will be autoclaved or killed in bleach after use.

The inserted genes are the marker proteins GFP, mCherry and other versions derived from these ones. The molecular epitope tags are Flag and HA tag sequence. These marker proteins and tag sequences are not known to be inherently harmful to humans. Similarly the fungicide resistance genes to be used as selectable transformation markers are not known to present any direct hazard to humans. These selectable marker genes are the E. coli hph (hygromycin phosphotransferase) conferring resistance to Hygromycin, the Neurospora crassa b-tubulin gene conferring resistance to Benomyl and the Streptoalloteichus hindustanus ble gene conferring resistance to the antibiotics bleomycin/phleomycin. Hygromycin is sometimes used in human healthcare in the UK. In addition, point mutations at the succinate dehydrogenase locus will be used to provide resistan to carboxin.

The fungicides used for selection during transformation (hygromycin and phleomycin/bleomycin) are not in use for disease control purposes in agriculture. The fungicide Benomyl is only in use for specialist horticultural purposes, since resistant b-tubulin alleles occur frequently in UK agriculture. Carboxin has been used in agriculture in the past but its use is been reduced.

The potential of the vector systems to cause harm to humans and the environment is negligible. We will use only the common disabled E. coli K12 and Agrobacterium strains that are unlikely to persist in humans, animals or to survive outside culture medium. All materials and cultures will be autoclaved or killed in bleach after use.

None of the new generated mutants is expected to alter survivability or stability. In principle, based on the functional annotations of the genes to be validated with functions expected to be related to virulence, it is not expected an increase in pathogenicity by the deletion of these genes, rather the opposite, but it may be possible in some exceptional cases to obtain an increase of pathogenicity, (e.g. during the complementation of a deleted gene if this is inserted in multiple copies). All materials and cultures will be autoclaved or killed in bleach after use.
The marker proteins and molecular epitope tags are regularly used and they do not affect the plant defence mechanisms in response to fungal infection. For the new mutants generated by gene deletion, they may affect host plant defences by reducing their capacity to escape recognition by the plant, resulting in activation of plant defence mechanisms. None of the mutants is expected to decrease plant defensces. All materials and cultures will be autoclaved or killed in bleach after use.

The planned modifications are not expected to affect tissue tropism or host range.

The planned modifications are not expected to affect transmissibility of the resulting GMM.

The GMM will be performed in fungal species, which can not transfer DNA fragments between related organisms as it is the case of bacteria. This could only happen between sexually compatible organisms within the same species.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

It doesn't apply.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

It doesn't apply.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All contaminated material will be sterilised by autoclaving or other validated means before disposal. Growth media and cultures, disposable plastic and glass ware, swabs, etc. will be placed in sterilin bags in metal boxes. These will be sealed using appropriate metal lids and transferred to Room 238 for autoclaving when the autoclave is free. A validated sterilisation cycle will be used (1h 121 C 15psi).

Only small volumes of liquid waste, of a few milliliters, will be generated. Liquid waste will be also autoclaved prior to disposal, by placing flasks and bottles containing liquid waste directly in the metal boxes.

For solid waste all material will be double bagged and steam autoclaved. Sharps and broken glass will be discarded in designated Cinbins. When full, the cinbins will be sealed, placed in a double autoclave bag and steam sterilised.

After autoclaving, all the materials will be placed in black plastic sacks and taken to the skip.

**Is an emergency plan required according to regulation 20?**

Y

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

Please enter comments on the GM safety committee on the risk assessment

---

**Project Containment**
<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**GM Centre Number: 93**

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**Name**

| UNIVERSITY OF BRADFORD |

**Name 2**

| Department |

**Campus Estate or Research Centre**

| Building |

**Road Name**

| District |

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<td>01274 309 742</td>
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**HSE Division**

| YORKSHIRE AND NORTH EAST |

**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022 |
## Premises Addresses

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<tr>
<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<tr>
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Other (please specify) Tick if confidential

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<th>Bacteriology</th>
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<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</table>
Waste management is by standard autoclaving on site with probe print out for time and temperature. Any solid material is then disposed of by the clinical waste route. Culture volumes are 600 ml or less.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste management is by standard autoclaving on site with probe print out for time and temperature. Any solid material is then disposed of by the clinical waste route. Culture volumes are 600 ml or less.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref**  93/02.1

**CU2 Project Title**

IDENTIFICATION AND CHARACTERISATION OF GENES PRESENT IN DIARRHOEA-CAUSING ESCHERICHIA COLI, BUT ABSENT IN E COLI THAT DO NOT CAUSE DISEASE

**Class**

Class 2

**CultureVolClass2**

< 1 litre

**Consent Granted**

not applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
## Purposes of the contained use

To identify genes that may contribute to disease potential in *E. coli* that cause diarrhea. This will provide information for developing suitable vaccines or diagnostic tests.

## Recipient or parental organism

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Source and Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli K-12</td>
<td>K-12 derivatives DH5a (Stratagene USA), MG 1655 (Blattner et al. Science. 1997 5:277(5331):1453-74) and Top10 (Invitrogen, USA) will be used as recipients for any cloned genes. Strains SM327 and SM10 (Mobley HL et al. Mol Microbiol 1993 Oct;10(1):143-55) will be used as recipients and hosts for suicide plasmids. All these strains are recognised as non-colonising and disabled and may be considered to be equivalent to ACDP hazard group 1. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture.</td>
</tr>
<tr>
<td>MG 1655</td>
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<tr>
<td>Top10</td>
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<td>SM327</td>
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<td>SM10</td>
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</tbody>
</table>

## Host/vector system

The vectors to be used - pBluescript, pSPORT (Strategene) and pBAD (Invitrogen) - are considered non-mobilisable and can only be introduced into laboratory recipients after chemical induction of competence or by electroporation. The K-12 recipients are incapable of conjugating DNA to other strains. Strain SM10 is the only laboratory strain capable of conjugation that will be used. Gene transfer is thus a very remote possibility. This strain will only be employed in mating experiments to conjugate plasmid constructs carrying inactivated genes. All waste from the experiments will be autoclaved or incinerated to minimise the unlikely discharge of genetic material into the environment.

The construction of mutants will result in the insertion of an antibiotic resistance gene within the gene to be inactivated. As a double crossover approach will be employed, this gene is not likely to be transmissible to other organisms once the mutant has been constructed. During the construction phases, the antibiotic marker will be carried on a suicide plasmid and therefore strains that received this plasmid are unlikely to survive.

## Origin & function

All the cloned (insert) DNA will come from natural isolates of *E. coli*. They are to be cloned to identify unique loci. Vector DNA will be restricted to the well-studied plasmids pCVD442, pBluescript and pSPORT. These will be used to facilitate sequencing of cloned DNA and to construct mutants in the natural isolates that do not possess the cloned genes of interest. Antibiotic markers will be inserted into genes in the process of constructing mutants to provide a means to select strains that have lost the gene to be inactivated. Where necessary, some genes will be expressed using pBAD. Expressed genes will encode outer-membrane proteins that are not toxins. This will be verified by sequencing before expression is attempted.

## Evaluation of foreseeable effects

(a) Hazards associated with the recipient micro-organism.

### E. coli K-12

- **Hazards associated with the recipient micro-organism.**
- E. coli K-12 derivatives DH5a (Stratagene, USA), MG 1655 (Blattner et al. Science 1997 5:277(5331): 1453-74) and Top10 (Invitrogen, USA) will be used as recipients for any cloned genes. Strains SM327 and SM10 (Mobley HL et al. Mol Microbiol 1993 Oct;10(1):143-55) will be used as recipients and hosts for suicide plasmids. All these strains are recognised as non-colonising and disabled and may be considered to be equivalent to ACDP hazard group 1. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and have auxotrophic requirements which are unlikely to be satisfied outside of laboratory culture.

(b) Hazard arising directly from the inserted gene product.

- **The strains from which DNA fragments will be cloned are enteropathogenic and enteroaggregative E. coli.** These organisms are human gastrointestinal pathogens (laboratory containment level 2). At suitable doses (above 10 to the power of 6), they can cause diarrhea in a susceptible host. Susceptible hosts include mostly children and the immunocompromised. Diarrhoea caused by these organisms resolves spontaneously or can be managed with antibiotics. Even though it is not the initial object to express cloned toxins, for the purpose of the risk assessment, the recipient should be considered to be at least as pathogenic as the donor and thus will be handled with laboratory containment level 2 precautions.
Hazard arising from the alteration of existing pathogenic traits

Most of the initial cloning experiments will be conducted by a shotgun approach and therefore, the gene(s) to be cloned cannot be listed. Fragments to be cloned are to be limited to a maximum of 15 kb of contiguous wild-type DNA, limiting the possibility of cloning a virulence factor along with its accessory delivery systems. It is expected that the sequences that are cloned through this approach will require class 1 or class 2 containment levels. However, all clones to be involved in further studies will be sequenced before these studies commence and risk assessment will be reviewed should sequence analysis reveal that a toxin or other virulence factor that may pose concern has been cloned. Known virulence genes that will be studied and therefore cloned in-frame include those that encode flagellins, iron utilisation systems and adhesins. These factors enhance the pathogenicity of virulent strains but are unlikely to convert the recipient strains to pathogenic status. Nevertheless, recipients carrying these foreign virulences genes will be handled as class 2 pathogens.

It will be necessary to construct mutants in putative virulence factors. These mutants will be constructed as non-polar double cross over deletions using the suicide vector pCVD442 (Mobley HL et al Mol Microbiol 1993 Oct; 10(1):143-55). This approach provides assurance that only the target gene will be delted and that mutants will be stable. In addition, the use of a suicide plasmid creates a situation whereby strains will either be mutated successfully or fail to survive. The genotype of all candidate mutants will be verified before they are used for subsequent experiments. It is expected that mutants will have an attenuated or similar virulence to wildtype strains and they will be handled as Class 2 pathogens.

The vectors to be used - pBluescript and pSPORT (Stratagene) - are considered non-mobilisable and can only be introduced into laboratory recipients after chemical induction of competence or by electroporation. The K-12 recipients are incapable of conjugating DNA to other strains. Strain SM10 is the only laboratory strain capable of conjugation that will be used. Tene transfer is thus a very remote possibility. This strain will only be employed in mating experiments to conjugate plasmid constructs carrying inactivated genes. All waste from the experiments will be autoclaved or incinerated to minimise the unlikely discharge of genetic material into the environment.

The construction of mutants will result in the insertion of an antibiotic resistance gene within the gene to be inactivated. As a double crossover approach will be employed, this gene is not likely to be transmissible to other organisms once the mutant has been constructed. During the construction phases, the antibiotic marker will be carried on a suicide plasmid and therefore strains that received this plasmid are unlikely to survive. If genes encoding outer membrane proteins that are not toxins are identified, the genes will be cloned into pBAD to facilitate purification of these proteins. Genes cloned into pBAD are expressed only in the presence of sufficient quantities of arabinose and are repressed in the presence of glucose, a far more abundant sugar.

Consideration of the likelihood that, in the event of exposure, the GMM could actually cause harm to human health

It is unlikely that the recipient would survive in a human host, even if virulence genes were cloned in an expressible form. However, since the genetic material is to be obtained from human pathogens, recipients will be handled as pathogens and standard laboratory containment level 2 precautions will be employed throughout. Although the risk is negligible, should infection in humans occur, diarrhoea would be the most likely sequelae and should be treated with antibiotics, other than ampicillin (which is the marker on the vectors).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No GM animals or plants will be employed

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid and liquid waste containing genetically modified organisms will be collected in lidded autoclave buckets and treated in a validated autoclave. Sterile waste from the autoclave will be sent for incineration. Any accidental spillage will be treated with 2% Virkon (see manufacturers data sheets).
The Genetic Modification Safety Committee considered this application at its meeting on 25 January 2002 and made the following observations.

The risk assessment had been carefully prepared.

The work will be carried out in a dedicated research laboratory which has access card entry control and is a containment level 2 facility. There is a designated bench for the work, and all equipment is at hand. Adequate hand washing facilities are available at the end of the laboratory.

The University has a Safety Management System, ratified at the University Safety Committee meeting on Tuesday 12 February 2002.

Please enter comments on the GM safety committee on the risk assessment

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Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 93/12.1

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Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N
Studies of epigenetic regulation of skin development, regeneration and carcinogenesis using 2D and 3D skin cell culture and replication-deficient lentiviruses expressing selected transcription factors, chromatin remodellers and shRNAs targeting their endogenous gene expression. Particularly, we will over-express or suppress the following factors: Lhx2, Oct4, Sox2, Sox9, Brg1, Brm, Ezh2, Ezh1, Lsd1, Tet1/2/3, Hdac1/2, Satb1/2 and Cbx4. The lentiviral vectors based on third generation HIV derived vectors will be obtained from commercial sources (Sigma Corporation) and from collaborative labs. We will produce lentiviral particles using the lentiviral vectors co-transfected together with packaging plasmids pS Pax2 and pMD2.G (Addgene depository, Cambridge, MA, USA) into HEK293T cells. The viral particles will be used to infect established cell lines, primary cultures of mouse and human keratinocytes, fibroblasts and melanocytes as well as the whole mouse embryonic skin cultures. In some experiments, the infected primary cells will be used to establish 3D skin tissue culture in vitro. The effect of over-expression and inactivation of the epigenetic regulators on morphological, physiological and biochemical parameters in the infected 2D and 3D cell and tissue cultures will be analyzed.

Established cell lines, primary skin cell cultures, whole mouse embryonic skin cultures and 3D reconstituted skin tissue cultures will be used in these studies. They are well characterized and are used in many labs worldwide including ours. They are not hazardous to human health in general. Primary human skin cells will be isolated from ethically obtained human skin samples. Such samples could carry human bacterial or viral pathogens and will be handled by trained staff using gloves, lab coats and biological safety cabinets. The samples are collected from dermatological clinics and any derived from patients known to be carrying infections would be excluded at the point of collection. Primary mouse cells and tissue culture will be prepared from mice kept in individually ventilated cages (IVCs) in the Biological Service Facility at the University of Bradford under strict adherence to the National and University animal care and usage regulations. The animals are specific pathogen free. The hazard associated with the recipient organism is considered to be insignificant.

Expression of the selected transgenes in lentiviruses in this research programme would not change the infectious properties of the viruses that will remain non-replicating and able to infect the cells and tissues only on direct contact with viral samples at high titre. The third generation HIV based lentiviral vectors (Sigma Cat Number SHCLND) used in these studies have their envelope (env), structural (gag), regulatory (vif, rev, nef) and replication (pol) genes deleted (Zuffery et al, 1997, Nat. Biotechnol. 15, 871-885). Additionally, they have a deletion in the 3' LTR region leading to the viral self-inactivation after integration into the host cell genome (Zuffery et al, 1998, J Virol., 73, 9873). The unintended infection of the human cells and tissues with the viruses is extremely unlikely when all the protective measures are applied and would be limited only to researchers directly involved in the experiments. Any infection would not spread further due to the replication deficient nature of the lentiviral strain specifically designed for this purpose as described above. The virus will be replicated in HEK293T cells after co-transfection of the viral vector with the packaging plasmids pS Pax2.
expressing gag, vif, rev, nef and pol genes and pMD2.G expressing vesicular stomatitis virus envelope G-protein VSV-G (Addgene depository, Cambridge, MA, USA). The separation of the genes required for the viral replication and packaging into two plasmids without sequence homology with the viral vector prevents the potential recombination to produce a replication competent virus. The viral particles are not air born and rapidly lose the infecting activity at room temperature.

Origin & function

Lentiviruses expressing cDNAs for selected transcription factors (Lhx2, Oct4, Sox4, Sox9), chromatin remodelers (Brg1, Brrm, Ezh2, Ezh1, Tet1/2/3, Hdac1/2, Satb1/2, Cbx4) and shRNAs targeting their endogenous gene expression will be used in these studies. The viruses will be used to over-express and/or inactivate these factors in 2D and 3D skin cell and tissue cultures to analyze the role of such factors in skin development, regeneration and carcinogenesis.

Evaluation of foreseeable effects

Expression of the selected transgenes in lentiviruses proposed for use in this research programme would not change the infectious properties of the viruses. These will remain non-replicating and able to infect the cells and tissues only on direct contact with viral samples at high titre. The third generation HIV based lentiviruses used in these studies have their envelope (env), structural (gag), regulatory (vif, rev, nef) and replication (pol) genes removed (Zuffery et al, 1997, Nat. Biotechnol. 15, 871-885). Additionally, they have deletion in 3' LTR region leading to the viral self-inactivation after integration into the host cell genome (Zuffery et al, 1998, J Virol., 73, 9873). The unintended infection of human cells and tissues with the viruses is extremely unlikely when all the protective measures are applied and would be limited only to researches in any case. The infected cells and tissue would not spread the viral infection further due to the replication deficient nature of the lentiviral strains specifically designed for this purpose. The infection, however, can lead to pathological conditions in affected cells and tissue that could result in cell death or carcinogenesis. Carcinogenesis is very unlikely however, because the viral particles would not be able to penetrate the skin surface in most cases and reach cells susceptible to transformation. The lentiviral strains used in this programme are not air born, are rapidly loose infectious properties at room temperature and could not be spread in the environment. The probability of recombination between the recombinant lentiviruses and natural related viral strains is essentially zero and the transgene sequence could not be transferred to the related natural viruses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and small amount of liquid (no more than 10 ml) materials and reagents that were contacted with viral samples will be be collected in the contained double bagged containers and autoclaved in a validated facility to destroy viruses before disposal. The large volumes (more than 10 ml) of liquid materials and reagents contacted with the viral particles will be treated with validated bleach (Virkon) at the final concentration of 10% for 30 min to completely destroy the viruses before disposal.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
The application was considered by the University of Bradford GMM Committee on May 11th 2012. The members of the committee noted that this application was a modified version of a draft application previously considered, and that all points raised had been attended to. Some of the recombinant viruses to be used will be supplied by collaborators at another UK HE Institution, which has CU2 approval for their use, thus reference has been made to the information available on the Public Register to ensure consistency.

The Chair of the GMO committee suggested that a SOP be established for the transfer of waste from the laboratory to the designated autoclave.

### Project Containment

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### Project Ref 93/14.1

- **Date Ackn'd:** 15/09/2014
- **CU2 Project Title:** The investigation of the regulation of receptor trafficking to synapses, endocytosis and degradation and signalling pathways MAP kinase, ubiquitination, SUMOylation, O-GlcNAcylation, Nuclear translocation) in mammalian cells using replication deficient recombinant lentivuses and related systems.

- **Class Culture Volume:** Class 2 Culture Volume Class 3-4
- **Non-GMM Consent Granted:** Yes
- **Project notified under transitional arrangements:** No
- **Tick if notifying a connected programme of work:** No

### Project Additional Information

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

02/03/2022
Purposes of the contained use

For the purpose of either over-expression or knock-down studies, AAV, Sindbis and Lentivirus particles will be produced in HEK293, BHK and 293FT cells respectively. These particles will then be transduced into different mammalian cell lines. Between 1-6 days, the cells will be lysed and the signalling pathways investigated biochemically.

Recipient or parental organism

Cell lines used: HEK293, HEK293T, COS7, HEK293FT, BHK
These cell lines are well established and in use in many laboratories worldwide for epigenetic and other studies. They are not recognised as posing a hazard to human health.
Primary cells from mouse brain will be prepared from specific pathogen free mice kept in individually ventilated cages (IVCs) in the Biological Service Facility at the University of Bradford under strict adherence to the National and University animal care and usage regulations. All staff involved in animal handling will have undergone appropriate training. The Hazard associated with the recipient organism is considered to be insignificant.

Host/vector system

Several predominantly commercial systems will be used:
- AA virus vectors: pAAV.CMV.NT-3; pAAV2.1-CMV-EGFP; pAdhelper (adenovirus helper plasmid); serotype-specific plasmids pXR2 and 4.
- Lentivirus vectors: pLent6/R4R2/V5-DEST (Life Technologies); pFIV-H1/U6-Puro and pGreenPuro (System Bioscience Inc) pLenti-Easy-His Vector and pLenti-Tri-cistronic (Applied Biological Materials (ABM) Inc)
- Sindbis viral vector pSinRep5 (Life Technologies); DH-BB and DH(26S) helper DNA templates

Origin & function

Adeno-associated virus (AAV) is a non-pathogenic helper dependent parovirus with a genome of single stranded DNA. This is one of the most promising vehicles for gene delivery and can insert genetic material at a specific site on chromosome 19 with near 100% certainty. Recombinant AAV vectors have predominantly episomal gene expression and has long-term expression in terminally differentiated cells. The very low levels of pathogenicity of AAV vectors in humans have allowed them to be classified as a Biosafety Level-1 (human pathogenic hazard group 1) agent by the ACDP.

Sindbis Viruses: The purpose is to produce pseudo-virions that may be used to infect a variety of different cell lines to optimize expression in the cell line of choice or to infect large-scale cultures for production and purification of proteins. Previously many labs used Sindbis virus efficiently to everexpress proteins in primary cortical and hippocampal cells. The low level of pathogenicity of Sindbis virus in humans has allowed it to be classified as a Biosafety Level 2 (human pathogenic hazard group 2) agent by the ACDP

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Tissue culture waste will be disinfected with Trigene, Virkon or treated with powdered Chloros (Presept). Treatment will be for a minimum of 20 minutes, followed by disposal into waste bins. Specifically a fresh 2% solution of Virkon® will be used to deactivate all viruses by disinfectant for 12 to 18 hrs. Autoclaved at 121°C for minimum of 20 minutes in the designated autoclave in the BSU. Autoclaved waste will be removed by the University's Waste Contractors.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The local GMO committee were happy with the first draft of the risk assessment in terms of the procedures for the generation of the recombinant viral particles, but requested further information be included as to the genes being incorporated/cellular targets of the viruses, and a more focused overview as to the aims of the specific project. These points were attended to, and the committee was able to approve the application.

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**Project notified under transitional arrangements**: Y

**Withdrawn**: N

Tick if notifying a connected programme of work: N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form
Project Containment

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Project Ref 95/00.2

Date Ackn'd 03/08/2000

CU2 Project Title ANGIGENIC GENE THERAPY PRODUCT FOR PERIPHERAL ARTERIAL OCCLUSIVE DISEASE

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
### Purposes of the contained use

In the proposed clinical studies, Angiogenic Gene Therapy Product, consisting of a recombinant adenovirus (human serotype 5) containing the human gene for the fibroblast growth factor 4, and referred to as Ad5.1fgf-4, will be investigated as a treatment for patients with peripheral arterial occlusive disease (PAOD). PAOD is characterised by decreased peripheral blood flow, usually in the lower limbs. Angiogene is the formation of new blood vessels.

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Inactivation of infectious waste - The infectious waste will be transferred to an autoclave in the medical microbiology unit (Jenner Wing, St. George's Hospital) by firstly double bagging the waste then placing it in specially designed, autoclavable, leakproof, metal transport boxes. Inactivation of Ad5.1FGF-4 is by autoclaving at 134C for 20 minutes, giving effectively 100% kill. Autoclaving at 121C for 15 minutes would also be adequate (again, effectively 100% kill), but 134C for 20 minutes is the standard temperature and time used in the medical microbiology unit. The autoclaves are validated on at least an annual basis (including the placing of independent thermocouples at the centre of the load). For each cycle of Ad5.1FGF-4 waste, autoclave indicator tape and the integral autoclave printer readout will be used to confirm that the waste has been autoclaved.

### Is an emergency plan required according to regulation 20?

- [ ] Yes

- [ ] No

If yes, tick to confirm that it is attached to this form

- [ ] Yes

- [ ] No

Tick to confirm that you have attached a risk assessment to this form

- [ ] Yes

- [ ] No

Tick if you are claiming exemption from disclosure for section of the risk assessment

- [ ] Yes

- [ ] No
Project Ref 95/00.3

Date Ackn'd 16/10/2000

Date Project Ceased 18/12/2013

CU2 Project Title HIV - 1 PHENOTYPE DRUG RESISTANCE IN PATIENTS ANTI - RETROVIRAL THERAPY

Class Class 3

Non-GMM Consent Granted yes

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 95/01.1

Date Ackn'd 02/03/2022  | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Please enter comments on the GM safety committee on the risk assessment

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Project Ref 95/01.2

Date Ackn’d: 15/02/2001

CU2 Project Title: GENETIC TRANSFER OF THERAPEUTIC MOLECULES TO TUMOUR CELLS

Class: Class 2

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>Class</td>
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<tr>
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**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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### Project Ref 95/01.4

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**Project Ref** 95/01.5

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<tr>
<td>21/02/2001</td>
<td>CONSTRUCTION OF A DEFECTIVE ADENOVIRUS FOR HIGH LEVEL EXPRESSION OF HUMAN CYTOMEGALOVIRUS H AND OR GLYCOPROTEIN L GENES</td>
<td>Class 2</td>
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Project Additional Information

- Purposes of the contained use

- Recipient or parental organism

- Host/vector system

- Origin & function

- Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [ ]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]  

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Project Ref 95/01.6

Date Ackn'd 21/02/2001

Date Project Ceased 18/12/2013

Class 2

Non-GMM not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

EXPRESSION OF THE HUMAN CYTOMEGALOVIRUS PPUL83 GENE IN A DEFECTIVE RECOMBINANT ADENOVIRUS VECTOR

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 95/01.7

Date Ackn'd 14/05/2001
CU2 Project Title GENETIC REQUIREMENTS FOR IMMORTILIZATION OF NORMAL HUMAN
Class 2
CultureVolClass2 < 1 litre
CultureVolumeClass3-4
**Project Additional Information**

**Purposes of the contained use**

Overall purpose: (i) a better understanding of malignant melanoma development and (ii) to enable the production of immortal human melanocyte lines for a wide variety of research.

Purpose of containment: Amphotropic retroviruses containing candidate cellular immortalizing genes, including certain oncogenes, will be used to transfer several of these genes into normal human melanocytes, to investigate which combination can overcome senescence in this cell type, and to yield immortal (continuously growing) lines of human melanocytes. The retroviruses, capable of infecting human cells, present a potential oncogenic hazard to the user and to others if not contained.

**Recipient or parental organism**

The recipient 'organisms' are primary strains of normal human melanocytes, derived from skin biopsies in other laboratories in Europe and the USA and provided to us after several passages. These cannot grow in humans other than the skin donor, but all early (recently explanted) human cell cultures are considered potential sources of human pathogenic viruses and are therefore grown under ACGM Level 2 containment conditions even without genetic manipulation. They are from identified persons with no known history of viral disease, so the risk of viruses is low.

The level of hazard from these recipient cells is not altered by the retrovirally-mediated insertion of additional genes. The cells will still not be able to grow in other humans, and the retroviruses used are disabled (see below), so that no infectious virus of this kind will be produced by the recipient cells.

**Host/vector system**

(i) The viral vectors alone are not considered harmful. They are disabled retroviruses of the pBABE family, which produce infectious particles only after transfection into packaging cells (Morgenstern, J.P. and Land, H., Nucleic Acids Res. 18: 3587-3596 (1990)).

(ii) The packaging cell line is Phoenix-AMPHO, a third-generation packaging line that contains the packaging construct (genes missing from the defective virus) in two separated components, making the probability of emergence of replication-competent retroviruses through recombination negligible (Xu, X et al., Nat. Genet. 27, 23-9 (2001), and see: http://www.stanford.edu/group/nolan/retroviral_systems/phx.html).

In other words, infectious viral particles are produced by the Phoenix packaging cells only, not by other infected cells. Phoenix cells are well characterized and free of helper virus or pathogenic viruses. They would be unable to grow in another human and thus are not hazardous unless engineered to produce infectious viruses. The half-life of the infectious viruses outside cells is estimated at about 7 hours (Andreadis, S. et al. J. Virol. 74, 1258-66 (2000)), and they are not resistant to desiccation.
### Origin & function

We will not be handling any naked proviral DNA. Phoenix-AMPHO cells already transfected with viral vectors and producing infectious viruses will be provided as frozen stocks by a collaborating group who constructed the viral vectors. The genes involved will be:

(i) hTERT, the catalytic subunit of human telomerase. This has been reported capable alone of immortalizing some types of human cells, although also expressed normally by other human cells. This sequence was originally provided by Geron Inc.

(ii) Several genes that would disrupt the p16/RB1 pathway. p16 is the product of a tumour suppressor gene, a melanoma susceptibility locus in humans. It is an activator of another important tumour suppressor gene, RB1. Both p16 and RB1 have been implicated in cellular senescence and therefore it is relevant to attempt to disrupt this pathway.


### Evaluation of foreseeable effects

If the infectious form of the retrovirus, with one of the above inserted genes, did enter the human body, it would be capable of infecting cells during its lifetime (half-life around 7 hours, as mentioned). It could not pass through intact skin, but could contact and enter cells if inhaled, or via damaged skin or mucous membranes. This would constitute a small oncogenic risk. None of the individual genes to be used can cause tumours on their own, but each might do so in combination with several other sporadic genetic changes, neoplasia being a multistep process. Infected cells would have reduced by one the number of genetic alterations required to produce neoplasia. (We will not use more than one oncogene simultaneously).

The highest risk is to the operator but, if not contained, on escape to the environment, the retroviruses could infect mice or other humans. However, these viruses have only a short lifetime outside cells, as mentioned, and are not resistant to desiccation. Thus the environmental risk is relatively low.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The following control measures will be used, in accordance with the accompanying risk assessment. The experiments will be on a small scale and for research purposes only. The work here will be initiated by a researcher who has visited the laboratory of the collaborating group who are already using these vectors, to study their established containment procedures, which will be replicated here. The procedures should make the probability of infection of the operator or escape to the environment effectively zero. We will comply with ACGM and St George's recommendations for Level 2 containment. In addition to these will be the following extra measures, for all parts of the work assessed as requiring Level 2.

- To ensure that replication-competent retroviruses (RCRs) carrying oncogenes cannot be produced, the Phoenix packaging cells will be monitored for production of RCRs, according to St George's guidance notes.
- Double gloves to be used.
- No sharps to be used.
- No work to be carried out if operator has any skin damage, eczema, etc.
- Virus particles not to be concentrated; the culture medium of packaging cells to be used unaltered as source of viruses.
- The handling of naked proviral DNA is not proposed.
- No more than one insert sequence to be used at a time. Where combinations of genes are to be tested, infections of different genes will take place at least 2 weeks apart.
- Solid waste to be transported to approved autoclave in Medical Microbiology department for autoclaving, in secure secondary container.
- Any new staff to receive careful training in the precautions to be used and the nature of the hazards involved, and should have previous experience with at least Level 1 containment procedures.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

02/03/2022
Liquids such as culture medium: addition of Chloros or other strong chlorine bleach to at least 10 ppm available chlorine, overnight. Then disposal to drains.
Approximately 100% kill of GMOs (cells, retroviruses).

Solid waste: To be transported to approved autoclave in another department in the same building, for autoclaving, in secure secondary container. 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been reviewed and approved by the GMSC.

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Project Ref 95/01.8

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<td>STUDY OF THE IMMUNOGENICITY AND SAFETY OF A SINGLE ORAL DOSE OF LIVE ATTENUATED SHIGELLA DYSENTERIAE 1 VACCINE (SC599) IN HEALTHY HUMAN ADULT VOLUNTEERS</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
1. To evaluate the suitability of the Institut Pasteur attenuated live Shigella dysenteriae type 1 icsA- ent-, fep-, stxA-; HgR vaccine SC599 when given as a single oral dose as a tool for human studies of immunity to enteric pathogenic bacteria.
2. Evaluation will be made for safety and immunogenicity by immunological, clinical, microbiological, haematological and biochemical measures, in healthy volunteers aged 18 to 45 years. The immunological data may allow for further use of the vaccine in larger scale clinical studies of immune responses.
3. By using an escalating dose, to obtain data on the optimum dose of bacteria which can effectively prime for an immune response without causing diarrhoea or other significant side reactions.

Shigella are pathogenic as a result of two features:
(a) Ability to invade human gut musosa. This is the principal virulence characteristic, and is controlled by plasmid-encoded proteins.
(b) Production of Shiga toxin. In addition to its invasiveness capability, Sh. dysenteriae type 1 produces an enterotoxin (Shiga toxin). High level toxin production enhances the pathogenesis of Sh. dysenteriae 1, but is not the primary virulence characteristic. Sh. dysenteriae type 1 is classified as ACDP Hazard Group 3 by virtue of toxin production.

Shigella are infectious via the oral route - between 10 and 100 bacteria are capable of inducing infection. Infection results in dysentery (bloody diarrhoea) with toxaemia. However, Shigella do not invade systemically and positive blood culture is uncommon. Mortality from species other than Sh. dysenteriae group 1 is almost non-existent and full recovery without sequelae is the rule, except at the extremes of age or in patients with other medical conditions (hence ACDP group 2). The secretion of Shiga toxin increases the pathogenicity of Sh. dysenteriae type 1, and mortality from Sh. dysenteriae type 1 can be up to 20% if untreated (hence ACDP group 3 on the basis of toxin production). Production of Shiga toxin is also thought to be responsible for the haemolytic-uraemic syndrome which can occasionally complicate Shiga dysentery. Post infectious complications include seronegative reactive arthritis in subjects who have the HLA type B27, thought to be an autoimmune process induced by molecular mimicry of human cellular proteins by bacterial proteins.

No virulence genes have been inserted and several key virulence genes have been deleted so there is no risk of a harmful phenotype being transferred to related micro-organisms. Genetic modification of the parent organisms was targeted homologous recombination with plasmids to delete virulence genes. The risks of transfer of other plasmid-encoded natural virulence genes from Shigella dysenteriae vaccine strain to other gut micro-organisms has not been demonstrated and is therefore very remote.

The risks of transfer of plasmid-encoded natural virulence genes from other bacteria into the vaccine strain to reconstitute its virulent phenotype is very small, as the human host would have to be carrying a wild-type Shigella spp in the gut, and multiple gene deletions have been introduced. If transfer did occur then a phenotype no more severe than the wild-type would be reconstituted as the GMM has no additional genetic sequences over the wild-type strain.

Virulence has been attenuated in SC599 by the deletion of the following genes:
1. stxA-: This completely removes the active A subunit of the Shiga toxin, essentially converting the vaccine strain from Sh dysenteriae type 1 (ACDP Group 3) to Sh dysenteriae non-1 (ACDP Group 2).
2. icsA-: This interferes with the bacterial ability to spread from cell to cell within epithelia, limiting potential to cause mucosal invasion and gastroenteritis.
3. ent-, fep-: These genes are responsible for a receptor on the iron-enterochelin complex and the biosynthesis of enterochelin. They attenuate the ability of bacterial growth and division in vivo.
In previous studies with Shigella, subjects were routinely started on ciprofloxacin treatment on days 5-12 or sooner if symptoms developed. Crucially, ciprofloxacin therapy was found to promptly end faecal shedding of bacteria and resolve conical symptoms.

On the basis that the Shiga toxin functionality has been removed, we propose that the attenuated GMM SC599 be initially classified as a wild-type Sh dysenteriae non-type1: ACDP Group 2 and hence be handled at ACGM.

Containment Level 2
In fact the additional attenuations would be expected to reduce the level of risk to less than wild-type Sh dysenteriae non-type 1 and theoretically the GMM could be classified as Group 1 Level 1, but as this is the first evaluation of the actual level of attenuation in man we would await the results of this study before proposing a reclassification of this GMM.

Host/vector system
n/a

Origin & function

The only gene insertion is the mercury resistance phenotype (HgR) inserted as selection marker. This has no clinical significance, does not enhance pathogenicity, viability or the organisms 'fitness', but allows identification of mutants. No antibiotic resistance phenotype has been inserted.

No other functional DNA is inserted. Clean deletions or in activations have been made.

Evaluation of foreseeable effects

A great deal of experience has been developed with strains of S. flexneri (SC602), including community-based phase 1 studies. Amongst subjects given an attenuated Sh flexneri (icsA- iuc- [aerobactin functionality deletion]) vaccine orally, 2/15 given between 1 (to the power of 2) to 1 (to the power of 7) bacteria developed fever, and 2/3 given 3 (to the power of 8) developed fever and diarrhoea. There were no serious adverse events.

Studies in humans of oral vaccination with Sh dysenteriae type 1 bearing only a the deletion of the toxin A subunit and insertion of mercury resistance (Sh. dysenteriae 1 stxA-: HgR) have been undertaken in the USA. This strain would be recognised as only very modestly attenuated and not a suitable vaccine candidate. However, these studies revealed that none of the volunteers given 3 x 10 (to the power of 10) bacteria had symptoms, 1/4 given 3 x 10 (to the power of 3) had fever and dysentery, 1/6 given 3 x 10 (to the power of 4) had fever and diarrhoea, and 3/6 given 3 x 10 (to the power of 5) had fever or diarrhoea. We would expect the two further attenuating mutations introduced into SC599 to be employed in this study would greatly reduce reactogenicity to the levels of acceptance seen with the Sh. flexneri strains reported above.

In all these studies, subjects were routinely started on ciprofloxacin treatment on days 5-12 or sooner if symptoms developed. Crucially, ciprofloxacin therapy was found to promptly end faecal shedding of bacteria and resolve clinical symptoms.

In a community based safety trial of S. flexneri (SC602) in which subjects were not routinely given antibiotics, 36 volunteers ingested 2.5 x 10 (to the power of 4) bacteria. There were no adverse effects. Subjects were allowed home immediately after vaccination and there were no cases of cross-infection. Daily stool sampling revealed that 20 shed bacteria in stool on day 1, rising to 86% by day 4 and all that did shed had an onset by day 7. Shedding was intermittent and the longest was 33 days. All subjects received ciprofloxacin on day 35 which terminated shedding.

Studies with Sh. flexneri SC602 are underway in children under 1 year old in Bangladesh, with does escalation up to 10 (to the power of 6) bacteria, with no ill effect (P. Sansonetti, personal communications).

Thus there is evidence in the literature that Shigella species with similar or less attenuating mutations can be safety administered to humans at the doses we propose in our escalating-dose study, and at the doses likely to be involved through cross-infection without ill effect. Furthermore ciprofloxacin therapy promptly ends bacterial shedding.
and any clinical symptoms.

The removal of the toxA gene makes haemolytic-uraemic syndrome extremely unlikely, as this virulence factor is thought to be the cause of this complication.

It is possible that if a third party became infected with the vbaccine strain then they could develop Seronegative Reactive Arthritis. However studies reported above have shown that cross-infection does not occur with the vaccine strains. Also the frequency of reactive arthritis after an infectious challenge is only 1-2%, of which 80% of affected subjects are HLA B27 positive, and the frequency of HLA B27 is only about 8% of the population. Therefore the chances of a susceptible person coming into contact with an infectious dose and sustaining a post-reactive arthritis is very remote.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

A derogation from level 2 containment is requested to use an autoclave in an immediately adjacent building in the Department of Microbiology or Department of Cellular Molecular Sciences. The vaccine Institute is designed to provide a safe environment for clinical studies, but does not have an operational autoclave in the building. Double-bagging, sealing and transporting clinical and laboratory waste in rigid containers to the autoclaves in Microbiology or CMS provides a safe disposal of material and the safest environment for study subjects.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

(A) Work proposed in the Vaccine Institute:
* All laboratory waste will be autoclaved prior to disposal by incineration.
* All volunteers will be housed in single rooms. Volunteers will use disposable bedpans which will be placed in double-bagged yellow sacks and autoclaved prior to disposal by incineration. The autoclave in the Department of Microbiology will be used as this is currently used for similar clinical specimens. The discard cycle (138 degrees C/30 mins) is validated every 3 months and is validated for inactivation of infectious agents.
* 'Household' waste from vaccines (disposable plates, cups, cutlery etc.) will be disposed of by incineration in yellow bags, as for standard laboratory waste. Laundry will be processed by the Hospital contractors.

Measures to prevent person-to-person spread:
* Proper hand-washing, especially after defecation and prior to food preparation/consumption.
* Avoidance of communal food preparation during likely periods of stool excretion.
* Avoidance of contact with persons especially susceptible to infection by excluding subjects from the study with such risk factors.
* Active monitoring of vaccinees' stool carriage.
* Repeat ciprofloxacin therapy if carriage persists beyond 4 weeks.

(B) Work in the St George's NHS Trust Microbiology Laboratory
It is proposed that Standard Operating Procedures in operation in this NHS laboratory are employed as appropriate for the isolation of wild-type Shigella dysenteriae type 1.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y
The use of two autoclave bags, one inside the other, may be of value in preventing spillage from ruptured bags after autoclaving. But it should be clear that when ready for transport to the autoclave, the bags should be left open (this is required to ensure steam penetration when in the autoclave). The rigid, sealed container should be the mechanism for preventing any leaks during transport to the autoclave. The autoclave operators will release the lid of the metal container before autoclaving the container and its contents.

Given the use of the Microbiological Safety Cabinet in the laboratory within the institute for preparation of material for various studies, rigorous procedures preventing cross contamination should be included in the standard code of practice to be followed by all users of the facility. This should include effective wipe down of the internal surfaces of the cabinet with an appropriate disinfectant.

### Project Containment

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### Project Ref 95/01.9

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Purposes of the contained use

The project aims to define the genes that determine virulence and pathogenicity of the pathogenic mycobacteria: Mycobacterium tuberculosis, M. bovis, (including the attenuated strain M. bovis BCG) and M. leprae. The long-term aim of this project is to understand mycobacterial virulence and define the genes involved in determining the host-pathogen interactions, thereby providing a rational basis on which to develop new control measures for tuberculosis, and to identify novel candidates for better drugs and vaccines for human tuberculosis.

Recipients or parental organism

The Genus Mycobacteria, (gram-positive, rod-shaped bacteria of the Family Actinomycete) consists of more than 40 recognised species, but which can be put into two main groups:

i) fast growing non-pathogenic bacteria such as M. smegmatis (commonly used for genetic studies of mycobacteria);
ii) the slow growing mycobacteria which include the major human pathogenic bacteria: TB (tuberculosis causing) complex (consisting of M. tuberculosis, M. bovis, M. africanum, M. microti), M. leprae (leprosy bacillus) and the M. avium complex which have become apparent as a major opportunistic pathogens in HIV infected people.

Mycobacteria can infect most species of animals including rodents, birds and fish. M. avium sub-species paratuberculosis is also responsible for Johne's disease in cattle and is increasingly thought to be associated with the human inflammatory bowel disease: Crohn's disease. Another slow growing mycobacteria is M. ulcerans, that causes Buruli ulcers in parts of Africa.

Mycobacterium tuberculosis is by far the most important human pathogen in the Genus Mycobacterium in terms of morbidity and mortality and sub-clinically infects a third of the world's population, killing 2 million people each year. Tuberculosis can affect almost any part of the body but is commonly of the pulmonary type, where the bacilli damage the lung function to such an extent that oxygen exchange becomes insufficient for life. TB is spread via the aerosol route, but bovine TB can be intestinally acquired. Clinical TB is still rare and is still treatable with alternative antibiotic regimes.

The genetically modified mycobacteria (recipients) will have had one or more of its genes inactivated, with or without the introduction of harmless vector sequences or selectable marker genes.

Origin & function

The genes studied in this proposal will derive from parental mycobacterial species: M. tuberculosis, M. bovis, M. bovis BCG and other pathogenic and non-pathogenic mycobacteria. The genes may be involved in metabolism, global gene regulation, stress response, intracellular survival, in vivo growth and latency. Many genes in M. tuberculosis are of unknown function (~40%) and this project aims to establish the functions of some of these by observing the effect of gene disruption or introduction.

Evaluation of foreseeable effects

The genetically modified mycobacteria (recipients) will have had one or more of its genes inactivated, with or without the introduction of harmless vector sequences or
selectable marker genes. Since virulence in mycobacteria is regarded as being defined by multiple genes acting in a highly regulated and co-ordinated way, the loss or introduction of a gene will either make the GMO either less virulent or have the same virulence as the parent organism. Thus the GMM will be identical to the parental pathogen, but will have small pieces of genes altered, missing or interrupted. These experiments may therefore be regarded as "self-cloning" and the resulting GMM may well exist naturally, although not easily identified from nature. No enhanced risk is perceived for the GMM recipients over and above the hazards of the parent strains. Introduction of genes from M. tuberculosis into the non-pathogen M. smegmatis will facilitate the function of that gene to be readily determined. The recipient GMM will not have any increased potential for harm compared to the parent, because of the multigenic nature of virulence in mycobacteria. Indeed, this has been experimentally shown by many groups. Thus, GMM M.smegmatis containing one or even many genes from the pathogenic mycobacteria will not have any enhanced hazard associated with it compared to parent M.smegmatis. Reintroduction of a gene or genes known to be missing in M. bovis BCG (the attenuated, non-pathogenic vaccine strain of M.bovis) but present in M.tuberculosis back into M.bovis BCG may restore partial or complete virulence, so the GMM recipient may revert to pathogenic status. Thus, these experiments involving reversion in BCG with deleted genes will be performed as if the recipient were pathogenic: i.e. same category of containment as the donor organism: M.tuberculosis, which is a Class 3 activity and performed under containment level 3.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation is applied for in relation to the use of autoclaves sited outside the CL3 labs but within a group of laboratories which have restricted access, for the disposal of waste from ACDP Group 3 GMM pathogens:- See risk assessment part (iv), paragraph 5. The autoclaves to be used are sited within The Department of Medical Microbiology. The autoclaves meet the correct standards and are validated by annual checks and calibrated and are registered for use by the School's GMSC. The protocol for autoclaving non-GMM Group 3 organism-waste generated within the CL3 labs in the Dept. Medical Microbiology makes use of these autoclaves. Even though the autoclaves are not within the CL3 lab, the CL3 labs are sited on the same corridor as the autoclaves and within a single unit of a series of laboratories (Department of Medical Microbiology) that have restricted access from the rest of the School by security pass-locked doors.

IT IS REQUESTED That for GMM mycobacteria the same procedure for autoclaving waste within the Dept. Medical Microbiology be used. On this specific aspect, derogation from the regulations that require autoclaving within the CL3 lab, is applied for. Waste generated within the CL3 lab will be double bagged inside the Class I MSC, placed into a metal, sealable tin for transport to the autoclaves for immediate autoclaving. All waste post-autoclaving will be placed in yellow clinical waste bags and incinerated. All waste from animal (mouse and guinea pigs) infection with GMMs will be autoclaved within the separate specified building within the School for animal work using the authorised and GMSC registered autoclave in that building. The autoclave is outside the CL3 laboratory, part of a single unit of a series of laboratories that have restricted access from the rest of the School by security pass-locked doors. Autoclaved waste is then incinerated.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMM microbiological waste generated, (liquid and solid), is first treated with Hycolin within the Class I microbiological safety cabinet. This gives a 100% degree of kill. The material is drained and then double bagged in autoclavable plastic bags, removed from the MSC to a robust, sealable tins and transported to the autoclave area for immediate autoclaving. The autoclaves are annually tested, validated and approved by the School's safety committee and GMSC for use with GMM waste. Autoclave tape confirms efficiency for each run. All autoclaved waste is then disposed of by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The risk assessment has been reviewed and approved by the St. George's Hospital Medical School GMSC and a Project Form has been registered with the GMSC.

**Project Containment**

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

Purposes of the contained use

The purpose of this study is to determine murine immune responses to an outer membrane protein of Neisseria meningitidis expressed in recombinant BCG, as a potential human vaccine candidate. NadA was initially identified by whole genome sequencing of N. meningitidis, and in silico analysis (Science 2000; 287:1816-1819). By homology with other bacterial outer
membrane proteins, the gene has been identified as a novel vaccine candidate for N. meningitidis type b (J. Exp. Med. 2002 1995 (11): 1-11) and designated 'NadA' (Neisserial adhesin A).

There is currently no vaccine available against N. meningitidis type b, which is the cause of about 45-80% of cases of meningococcal meningitis in Europe. Cross reactivity of the polysaccharide capsule of N. meningitidis type b with human antigens has made the development of a classical polysaccharide vaccine problematic. Current strategies involve identifying surface exposed proteins that can be the target for antibody and complement binding, resulting in bactericidal activity. The adhesins are a family of outer membrane proteins which mediate bacterial adherence via non-fimbrial attachment. They also confer a degree of serum resistance by unknown mechanisms. By sequence homology with known adhesins, one gene identified from the genome of N. meningitidis has been selected as a potential vaccine candidate. This antigen has been distributed within an EU-funded collaborative programme, to identify novel mucosal vaccines. The antigen is being expressed in a number of model systems including rBCG. We have experience of expressing antigens in BCG and determining murine models of immune responses. Our collaborators have extensive experience of developing expression systems in rBCG, and have expressed NadA in recombinant BCG which we now wish to evaluate in a murine model of immunogenicity.

Recipient or parental organism

BCG is used as a human vaccine for neonates, children and adults. It has a long track record of safety. It is an ACDP HG2 pathogen. It can cause idenitis, retropharyngeal adenitis when given orally in high doses. If administered parenterally to immunodeficient persons it can cause disseminated BCGosis. All these complications are rare and amenable to antibiotic therapy.

Host/vector system

Two vectors have been constructed:
(a) Replicative: based on pAL5000 a plasmid from mycobacterium fortuitum containing a mini mycobacterium - E. coli shuttle plasmid constructed by using the gene for Kan (Tn903) as a selective marker (J Bacteriol 1990; 172(5):2793-2797). NadA was cloned into pGEMT easy by BamHI/Asp718 digest of a PCR product obtained from a N. meningitidis NadA template.
(b) Integrative: Using a vector derived from M. smegmatis mycobacterial phage Ms6 (Microbiol 1998; 144:3397-3406)., pAV6950 E. coli replicative/mycobacterial integrative shuttle vector, contains the attachment site and the gene coding for integrase of Ms6; contains Kan from Tn5 (Infect immun 2002; 70(1):303-314. The expression vector is transferred from the replicative vector to this vector by restriction digest.

These vectors have been electroporated into BCG and protein expression confirmed by Western blot. The rBCG are being cultured into vaccine preparations, suitable for use in murine models of immunisation at SGHMS.

Origin & function

NadA encodes for a protein of Neisseria meningitidis, that has sequence homology with a range of 'adhesins' such as UspA1 and UspA2 proteins of Moraxella catarrhalis, YadA protein of Yersinia and EibF of E. coli. These proteins are anchored in the outer membrane of host bacteria, and have a structure, as follows (taken from EMBO J 2000; 19(22):5989-5999, see also J. Exp. Med. 2002 195 (11): 1-11):

The head region confers resistance to complement binding and bacterial lysis by antibody and complement. It may also increase bacterial adhesion to epithelial cells. The exact mode of this functionality is unknown. Antibodies raised against these regions are bactericidal and therefore excellent vaccine candidates.

Evaluation of foreseeable effects

It is not expected that virulence will be conferred on recombinant BCG by the expression of NadA for the following reasons:-
1. Serum sensitivity is not relevant to mycobacteria, as bacterial killing is mediated via macrophage uptake and intracellular killing. There is no evidence that antibody and complement play any significant role in mycobacterial killing.
2. The mycobacterial cell wall has a different structure, and it is unlikely that the NadA complex could be exported and insert into the cell wall of rBCG efficiently.
3. The cloned gene contains only the sequence from the first mature codon up to codon 350. This excludes the N terminal leader sequence making export of the protein unlikely. The C terminal anchor component is also completely missing, making the stable insertion into the bacterial membrane impossible.
4. As the cloned protein will be incapable of stable insertion into the bacterial membrane, no increased cell adhesion can occur.
This the NadA protein is incapable of exerting its virulence property in rBCG, but the antigenic head and stalk regions are present to induce an antibody response.

(a) Hazards arising from the alteration of existing pathogenic traits (eg. alteration of host range or tissue tropism): None. The shuttle vectors contain a kanamycin resistance gene, but this antibiotic is not used routinely in the treatment of BCG infection in susceptible hosts. The antibiotic sensitivity of the recipient BCG is known (INAH, rifampicin and ethambutol sensitive).

(b) The potential hazards of sequences within the GMM being transferred to related micro-organisms: Unlikely. Should transfer occur, the resultant protein will still be deficient for insertion into bacterial membrane, and therefore unable to exert its virulence properties which requires stable expression on the bacterial surface.

(c) Consideration of the likelihood that, in the event of exposure, the GMM could actually cause harm to human health. No additional harm to human health is expected above that of the parent BCG.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the infected waste will be autoclaved and the used pipettes and other materials will be soaked overnight in 5% hycolin before autoclave.

The mice will be kept in standard cages in the BRF. The policy in place for disposing of animal carcasses from the BRF will be followed: Animals will be double-bagged for autoclaving using autoclavable bags (transparent with blue writing). After autoclaving they will be put in yellow bags for disposal via BRF contractor and labelled as 'waste for incineration'.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been reviewed and approved by the St George's Hospital Medical School GMSC.

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<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<td>13/08/2002</td>
<td>PRE-CLINICAL TESTING OF MODIFIED FORMS OF HERPES SIMPLEX VIRUS FOR VACCINATION PURPOSES.</td>
<td>Class 2</td>
<td>1-50 litres</td>
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**Date Project Ceased** 18/12/2013

**Withdrawn** N

Tick if notifying a connected programme of work N

**Non-GMM Consent Granted** not applicable

Project notified under transitional arrangements N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Pre-clinical testing of modified forms of herpes simplex virus for vaccination purposes

**Recipient or parental organism**

Herpes simplex virus strains with attenuating mutations but in which replication competence is retained. Attenuating mutations remove HSV genes, which usually inhibit immune responses and also render the virus non-pathogenic. These genes are VHS and/or ICP47 and may additionally include further mutations in ICP34.5, UL43, U35 and/or vmw65. The modified HSV strains also have genes encoding non-HSV antigens inserted encoding immune modulatory molecules such as cytokines and non-oncogenic differentiation antigens.

In view of the replication competency of some of the HSV vectors, all experimental work will be conducted under class 2 conditions.

The work aims to induce immune responses to the delivered antigents and no untoward effects are anticipated. The mutations to the virus have previously been shown to render the virus non-pathogenic and the genes to be delivered are not anticipated to be harmful according to previous work published in the literature.

**Host/vector system**

Herpes simplex virus 1 or 2
Insert genetic materials is of either human or rodent origin and has been cloned by PCR or obtained from collaborators in plasmid form. Genes to be inserted include tumour antigens such as MART-1, MAGE-1, tyrosinase, gp100, Muc-1, PS1, CEA for the development of anti-tumour vaccines. Antigens from a variety of infectious agents for vaccine development purposes will also be used as will human or rodent forms of various immunomodulatory molecules e.g. GM-CSF, IL12, CD40L, B7.1, RANTES. The immunomodulatory genes are aimed, eventually, at the enhancement of the immune response to delivered antigens.

### Evaluation of foreseeable effects

None of the genes to be inserted are anticipated to result in harmful effects. No genes with oncogenic activity will be used.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated during the activity that has been in contact with the GMM will be treated with a 1% Virkon solution for a minimum of 30 minutes. This broad-spectrum disinfectant is effective against herpes simplex virus at a 0.5% (w/v) concentration giving terminal disinfection after 10 minutes of contact. 1 (ref: Hernández A, Martro E, Matas L, Martín M, Ausina VJ. Assessment of in-vitro efficacy of 1% Virkon against bacteria, fungi, viruses and spores by means of AFNOR guidelines. Hosp Infect 2000 Nov;46(3):203-9). Solid waste will be placed in autoclave bags, autoclaved then incinerated. Liquid waste will be collected in glass bottles, treated with 1% virkon then autoclaved prior to disposal in a sink in running water for maximal dilution.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
A number of replication competent and replication incompetent viruses will be used all based on the laboratory strain of HSV1, strain 17+. An essentially wild type virus contains the marker gene GFP (green fluorescent protein) inserted in the UL43 (non-essential) region of the HSV-1 genome. This virus is to be classified as class 2.

The other viral vectors to be used contain an attenuating mutation by the deletion of ICP4. These are not replication competent and will be classified as class 1 (but all experimental work will be performed under class 2 conditions where all HSV work will be conducted) due to their replication incompetency, a helper cell line is required for virus production.

Some of the replication-incompetent HSV strains also have genes encoding non-HSV tumour-associated antigens. These consist of a number of melanoma-associated genes (gp100, MART-1, MAGE) and/or the gene encoding CD40L. These genes are not oncogenic and do not affect the replication potential of the viral vectors. These viruses will also be classified as class 1.

The GMSC has reviewed the risk assessment and accepts that classification of class 2 for HSV constructs that are able to replicate and class 1 for those that are replication-incompetent.

### Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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#### Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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</thead>
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### Project Ref 95/02.4

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<td>CHARACTERISATION OF HORIZONTAL TRANSFER OF DNA INTO STAPHYLOCOCCUS AUREUS</td>
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**Withdrawn** | N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**
## Project Additional Information

### Purposes of the contained use

We wish to identify and characterise mechanisms involved in the uptake of DNA by S. aureus. This includes transformation, transduction and conjugation of DNA from other bacterial genera into S. aureus. It also includes investigating S. aureus factors that inhibit or enhance these processes. The DNA to be introduced into S. aureus will contain antibiotic resistance genes, as they are useful as selective markers. Understanding how S. aureus acquire foreign DNA will allow us to make predictions about how S. aureus acquire resistance to antibiotics, how virulence genes move between strains of S. aureus, and will allow us to construct new tools for the genetic manipulation of clinical S. aureus strains.

### Recipient or parental organism

S. aureus is a Hazard Group 2 organism. It is found as a commensal of the human nose in 30% of the population. If introduced into a wound, it is capable of causing disease, particularly if the host is immunocompromised. However, it is not considered a risk for laboratory workers. In hospitals, methicillin resistant strains (MRSA) are widespread and infections caused by these isolates can be more difficult to treat, although vancomycin can be used. Intermediate level vancomycin resistant strains (VISA) pose a new threat, but are rare.

### Host/vector system

n/a

### Origin & function

Firstly, we wish to construct libraries of S. aureus mutants using the transposon Tn917 (erythromycin resistant) which randomly integrates into the S. aureus chromosome. To do this, a plasmid containing the transposon is moved into S. aureus, and forced to insert by temperature and erythromycin selection. The resultant library of strains will each have the Tn917 inserted in a unique locations and have a unique phenotype, although none are likely to be more pathogenic or hazardous than the parent. Tn917 is a naturally occurring transposon isolated from S. aureus.

Secondly, we wish to introduce foreign DNA into S. aureus. Specifically, this will be

1. Plasmids propagated in E. coli and introduced by electroporation (pulsing S. aureus with an electric current). The plasmids used will be standard shuttle vectors composed of E. coli cloning vectors with a S. aureus origin of replication and a selectable antibiotic resistance marker.
2. Plasmids and transposons derived from Enterococci and Streptococci, and introduced by conjugation. The plasmids are naturally occurring and carry antibiotic resistance genes on the plasmid or on transposons integrated into the plasmids. The plasmids also carry genes necessary for conjugation. Similar plasmids and transposons are found naturally in S. aureus.
3. Chromosomal DNA and plasmids from other staphylococci, including other S. aureus isolates. This will be introduced by transduction using naturally occurring generalised transducing bacteriophages.

In all cases, foreign DNA will contain an antibiotic resistance gene that can be used as a selectable marker. They will encode resistance to erythromycin, tetracycline or chloramphenicol. None of these antibiotics are used routinely in the clinical treatment of S. aureus infection. Naturally occurring resistance to these antibiotics is widespread in S. aureus.

### Evaluation of foreseeable effects

The antibiotic resistance genes we plan to move are:

- erythromycin
- tetracycline
- chloramphenicol
All of these resistance genes are found naturally in S. aureus and are widespread. The genetic elements carrying these resistance genes are derived from S. aureus itself or are closely related to elements found in S. aureus. Therefore, mutants or recipients are not predicted to be a greater hazard than naturally occurring strains. None of our new strains will be multiply resistant, that is, they will always be susceptible to antibiotics currently used to treat clinical infection, such as b-lactamases or vancomycin.

Toxin genes and virulence factors genes will be moved between strains of S. aureus. S. aureus produces more than 100 known or putative toxin genes, and different strains have unique combinations and novel variants. These genes are often encoded on mobile genetic elements and move between strains naturally in the environment by transduction. Our experiments are designed to mimic this process, and will not generate new strains with any increased hazard.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Level 2 containment, with waste material to be autoclaved (effectively 100% kill) prior to incineration. Autoclaves are validated for effective waste treatment weekly by internal engineers and quarterly by external engineers. Autoclaved waste is incinerated.

An alternative procedure that has been tested for liquid waste is disinfection with Hycolin. Hycolin is added to a final concentration of 1% and left overnight before washing down the laboratory sink. This method has been validated for use with S. aureus in our laboratory. Cultures of 1 x 10^9 (to the power of 9) bacteria per ml were treated with Hycolin overnight, diluted up to 100-fold, and plated onto agar. No viable bacteria were recoverable. Dilutions of 0.5% and 0.25% Hycolin were equally effective.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

This risk assessment has been reviewed and approved by the St. George's Hospital Medical School Genetic Modification Safety Committee.

Project Containment

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Animal Units: Large Scale Activities: Human Clinical Applications
The aim of this project is to utilize a replication incompetent adenoviral vector to express inserted genes to elicit immune responses to the inserted gene products.

The vectors under development will be based on the adenovirus family of respiratory viruses. Serotypes expected to be used include the Ad2, 4 and 5. All will be replication incompetent. Any serotype utilized will be gene E1a, E1b and E3 deleted, thereby preventing the replication of the adenovirus after cell entry. The adenoviral life cycle does not normally involve integration into the host genome, rather they replicate as episomal elements in the nucleus of the host cell and consequently there is no risk of insertional mutagenesis.

Unmodified Ad5 is classified in hazard group 2 under the COSHH regulations but the E1a gene deleted forms are classified as hazard group 1. Although recombination with an unmodified virus in vivo is a possibility the further deletion of the E3 gene renders it extremely unlikely that even in the event of such a rare recombination occurring that the recombinant will be packaged with viral coat proteins and secreted from the cell.

Cells used to propagate the recombinant adenoviruses are 293 cells. These are human embryonic kidney cells that have been transformed with the adenovirus genes E1A and E1B, which are required for adenovirus propagation.
The genetic material to be inserted will be encode HIV proteins or natural murine cytokine proteins. In the case of murine derived genetic material, RNA will be isolated, first strand cDNA prepared and primers specific for the gene in question will be used in a polymerase chain reaction to amplify the target sequence. This material will then be inserted into the E3 region of the gene deleted adenoviruses. The selected genetic material will generally constitute an open reading frame of a particular protein that we expect to be fully transcribed using the host cell transcription machinery. Translation of the resulting RNA transcripts into fully functional murine protein is expected. We intend to use these expression vectors to produce physiologically relevant quantities of the murine proteins in vivo in the mouse. These proteins are expected to have various degrees of immuno-modulatory activity, although it is expected, based on current literature, that most will have no detectable effect.

HIV genetic material will be derived from current plasmid vectors that contain small parts of the HIV genome. This material can be sub-cloned from these plasmid vectors and inserted into the E3 region of the gene deleted replication incompetent adenoviral vector.

All the inserts that will be incorporated into the gene deleted adenoviral vector will not have any known oncogenic potential.

**Evaluation of foreseeable effects**

The adenoviral life cycle does not normally involve integration into the host genome, rather they replicate as episomal elements in the nucleus of the host cell and consequently there is no risk of insertional mutagenesis. Unmodified Ad5 is classified in hazard group 2 under the COSHH regulations but the E1a gene deleted forms are classified as hazard group 1. Although recombination with an unmodified virus in vivo is a possibility the further deletion of the E3 gene renders it extremely unlikely that even in the event of such a rare recombination occurring that the recombinant will be packaged with viral coat proteins and secreted from the cell. When injected into mice it is possible that the gene-deleted adenoviruses may come into contact in vivo with endogenous murine viruses. While it is extremely unlikely that these replication-deficient gene-deleted viruses will be transcomplemented by any known murine virus, we will utilise specific-pathogen free mice that do not harbour murine viruses of the papovaviridae genus that contain both murine polyomaviruses as well as human papillomaviruses.

The adenovirus vaccine vector itself will consist of E1a and E3 gene deleted adenovirus and an insert consisting of a DNA sequence encoding for small parts of the HIV genome. Typically, the vaccine vector contains a DNA sequence that encodes individual viral proteins such as the env (structural envelope glycoprotein), gag (matrix and capsid structural protein), rev (regulator of viral protein expression) or tat (regulator of HIV genome transcription) molecules. These small parts of the virus are expressed in such a manner that the essential minimal components of an infectious retroviral particle will not be present, particularly the intact HIV/SIV viral genome, thus preventing any possibility of inadvertently generating an infectious viral particle. There is no conceivable risk inherent in these proteins.

Other inserted cDNA, that will encode for natural, non-modified, mouse proteins will belong to the murine cytokine/chemokine family of secreted proteins. Other insertions may include proteins that have been shown to stimulate the innate immune system, including those that act at the cell surface as well as internal cytoplasmic proteins. These proteins have not been described to have oncogenic attributes in the published literature and are indeed non-modified and identical to those proteins expressed by normal murine cells. The proteins expressed from these constructs have between 30 and 70% identity with the corresponding human cytokine. However, most are not active or have only partial activity on human cells (COPE cytokine Database).

It is not expected that any of the insertions detailed will have an effect on the tissue tropism. However, the deletion of E3 has been shown to increase the immune response due to less down-regulation of the host cell MHC Class 1. The addition of cytokine insertions may also increase the immune response. As these effects are the precise criteria under investigation in our studies, it is impossible to predict the effects of augmentation of the immune response. It is possible that the resulting inflammatory response will be harmful. However, none of the insertions will be human genes, although murine cytokine/chemokine products may have some activity upon human cells. (COPE Cytokine Database).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

We do not intend to use any GM animals or plants.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
In view of the fact that all adenoviruses used will be replication incompetent but may have undetermined pathogenicity by virtue of the gene insert, it is appropriate for all manipulations involving this virus to be undertaken within a microbiological safety cabinet. It is expected that all large scale work with these GMOs will be done in a dedicated Category 3 facility, that has an autoclave within the lab. Small scale work (less than 100ml) may also be done within a microbiological safety cabinet in a category 2 laboratory. Liquid culture waste in both circumstances will initially be aspirated within the microbiological safety cabinet into a leak-proof container with Virkon S solution. The concentration of Virkon S will be maintained at greater than 1% - a concentration that has been validated as 100% virucidal for adenoviruses as well as most other viruses. Moreover, all waste materials (solid and Virkon S treated liquid) will be autoclaved in an autoclave maintained and appropriately certified to properly inactivate infectious waste. Autoclaved material is considered to be 100% effective at sterilization. The extra precaution of performing large scale preparative work in a category 3 laboratory with an internal autoclave ensures that large volumes of Virkon S treated culture solutions (which although almost certain to be 100% killed should be treated as potentially infectious material) cannot come into contact with other laboratory staff or any non-laboratory personnel. Any small volumes of culture performed in category will be treated with the Virkon S virucide and then contained within a leak-proof container for transport to the autoclave.

Any new staff would be specifically trained in the safety aspects of this work. Since the gene deleted replication incompetent virus is transmissible by an airborne route an inward microbiological cabinet airflow would be required.

The work involves standard laboratory protocols and there are no unusual procedures that require additional containment measures.

All work with these adenoviruses will be performed within a certified category 2 or 3 laboratory.

Mice injected with the replication-deficient gene-deleted recombinant adenoviruses will be maintained within isolators. All injections and handling of these animals after injection with the recombinant adenoviruses will be carried out entirely within a microbiological class 1 cabinet. Cells, blood and tissues harvested from these animals prior to schedule 1 euthanasia as well as post-mortem will be transported from the microbiological class 1 cabinet within the animal facility to a microbiological class 11 cabinet in our class II laboratory entirely sealed within a biohazard container. The exterior of the biohazard container will be sprayed with liquid Virkon disinfectant prior to removal from the microbiological class II cabinet to ensure no GMOs survive on the exterior of the container. Post-mortem, both the mouse carcass and the cage in which the mice lived will be autoclaved in an autoclave maintained and appropriately certified to properly inactivate infectious waste. Autoclaved material is considered to be 100% effective at sterilisation. The sterilized bedding contents of the cage and the mice carcasses will then be incinerated.

The isolators that contained the cages will be fumigated.

The risk assessment has been reviewed and approved by the St George's Hospital Medical School GMSC.
The study has been set up to assess the safety, biodistribution and biological activity of OncoVEX GM-CSF. The study involves intratumoural injection into cutaneous or subcutaneous nodules.

The oncovex human GM-CSF vector (Oncovex hGM-CSF) is a replication competent herpes simplex type-1 virus which will be tested in a phase 1 clinical trial for its safety, biodistribution and efficacy in a variety of solid tumours. The strain of HSV which Oncovex is based on is JS-1 (ECACC No. 85011433). The Oncovex GM-CSF vector is deleted for the neurovirulence factor, ICP34.5 which is essential for pathogenicity. This deletion allows the vector to selectively replicate in dividing cells. The vector is also deleted for ICP47, which blocks antigen presentation to MHC class I and II molecules by blocking the transporter associated with antigen processing (TAP1 and TAP2). The vector also contains the coding sequence for human GM-CSF, a cytokine involved in the stimulation of T-cells. The deletion of ICP47 and the expression of GM-CSF should enhance the immune response towards tumour cells. GM-CSF expression is under the control of the human cytomegalovirus immediate early promoter (HCMV IE). This is an infectious gene therapy product that can only replicate in rapidly dividing eukaryotic cells.

Reference:


Host/vector system

HSV strain JS1/34.5-/47-/CMMVGM-CSF (OncoVEX GM-CSF)

Origin & function

The hGM-CSF gene was cloned from an IMAGE clone 2340997/5808-K14 (UK HGMP Resource Centre). GM-CSF is a potent cytokine responsible for the differentiation and proliferation of dendritic cell precursors and therefore is a potent immune stimulator. Thus if the vectors were to enter a human it would be anticipated to give an improved immune response against HSV.

Evaluation of foreseeable effects

GM-CSF should enhance the immune response towards tumour cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All waste will be placed in a sharps bin or bag and placed in a second bag and securely tied. These will then be placed in an external container in order to prevent external spillages and will be identified as potentially containing genetically modified organisms. The containers will then be transferred to a designated and approved autoclave in the Medical School premises which is a separate building on the same campus as the Vaccine Institute where the trial will be conducted. Air-Sea Biopack-2 (Biojar) containers will be used (Air Sea Containers LTD, Birkenhead) for transport of solid waste. The waste will be placed in autoclave bags, autoclaved then incinerated. The containers will be decontaminated with the application of 1% Virkon.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated during the activity that has been in contact with the viral vector will be treated with a 1% Virkon solution for a minimum of 30 minutes. This broad-spectrum disinfectant is effective against herpes simplex virus at a 0.5% (w/v) concentration giving terminal disinfection after 10 minutes of contact. 1 (ref: Hernndez A, Martro E, Matas L, Martin M, Ausina VJ. Assessment of in-vitro efficacy of 1% Virkon against bacteria, fungi, viruses and spores by means of AFNOR guidelines. Hosp Infect 2000 Nov;46(3):203-9). All waste will be placed in a sharps bin or bag. These will then be placed in an external container in order to prevent external spillages and will be identified as potentially containing genetically modified organisms. The containers will then be transferred to a designated and approved autoclave in the Medical School premises. The containers will be placed in autoclave bags, autoclaved then incinerated. Liquid waste will be collected in glass bottles, treated with 1% Virkon then autoclaved prior to disposal in a sink in running water for maximal dilution.
The risk assessment has been reviewed by the St. George's Hospital Medical School GMSC. The committee accepted the statement concerning preferential replication in tumour cells but raised the possibility of virus production from some normal cells. They requested further information about the timing of the return of test results and the discharge of patients. The committee was satisfied that these issues would be dealt with effectively.

**Project Containment**

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**Animal Units**

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**Non-GMM Consent Granted** yes

**Project notified under transitional arrangements** N

**Historical Significant Changes** N

**Historical Date of Additional Info**

**Significant Change ID**

02/03/2022
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**
The aim of this project is to exchange specific amino acids in *P. falciparum* transporters and to investigate (1) altered drug susceptibilities of parasites to anti-malarials, and (2) to study the basic physiology of transporters in the parasite.

**Recipient or parental organism**
The vectors used are generated on a puc 18-backbone and contain a *P. falciparum* specific selectable marker. After transfection they replicate episomally for about 2 months, until integration as single-site crossover into the parasite genome occurs. Since human red blood cells do not contain a nucleus no integration into human host cells is expected. *P. falciparum* is the protozoon parasite responsible for the most severe forms of human malaria. Following infection via the bite of an Anopheline mosquito the parasite invades hepatic liver cells and red blood cells. This leads to a variety of well-documented clinical symptoms. Treatment with modern antimalarials is highly successful. Natural infection with *P. falciparum* is only through the bite of an infected Anopheline mosquito. Cases of malaria have also been reported as a consequence of transfusion with contaminated whole blood. *P. falciparum* needs human red blood cells to multiply and is considered to have extremely limited survivability in the environment. Transmission of asexual blood-stage parasites requires injection into the blood stream. There is no risk of infection through aerosol. The only significant risk of infection in the laboratory is via needlestick or similar penetrative injury with culture material containing infective blood-borne stages of the parasites.

It is not thought that *P. falciparum* would establish itself in the environment, since its asexual replication is dependent on red blood cells. In any case, it would not in itself, be hazardous to the environment. Spread in the environment would require transmission by the Anopheline mosquito vector. This requires strict laboratory conditions (controlled temperature, humidity and feeding) for 7-10 days. Furthermore, the disease vector is absent from the environment, since our climate doesn't meet its requirements for survival.

**Host/vector system**
Cells used to propagate transfected *Plasmodium falciparum* are human red blood cells. These cells are required for routine *P. falciparum* propagation.

**Vector**: pH22Y, pUC18 backbone.

**Origin & function**
The vector transfected into the parasite will lead to an exchange of specific amino acids in transporters of *P. falciparum*. Cultures will be propagated until recombination occurs. DNA will be extracted and primers specific for the gene sequence used to amplify the target sequence and evaluate recombination. Further evaluation steps include Southern blot, RNA isolation and RT-PCR as well as protein extraction followed by Western blot. Once successful integration has been proven, modified parasite lines will be subjected to drug assays to test for possible altered drug sensitivities. There are no indications that modified parasites have an increased pathogenic potential or are harmful to humans.

**Evaluation of foreseeable effects**
During its asexual blood stage life cycle *P. falciparum* replicates within the human red blood cells. It is categorised as a Hazard Group 3 pathogen. However, it falls under an exemption for class 3 as stated by the Advisory Committee on Dangerous Pathogens (Fourth Edition, 1995). Integration of genetic material in vitro experiments is only expected to occur into the parasite genome. In vivo asexual blood stage parasites only invade nucleus-free human red blood cells, making the event of integration of genetic material into the human host genome highly unlikely. Furthermore, it is not expected that genetic modification of *P. falciparum* will have an effect on the tissue tropism and invasion behaviour of the parasite.

Sharp objects (needles, glass, metal) are banned in a malaria culture lab. In the unlikely event of sharps or needle injuries and incorporation of *P. falciparum* into the
bloodstream, general safety measures have to be taken immediately (see also risk assessment P. falciparum culture). This involves reading of blood films in short term intervals and monitoring of potential development of fever. There are no indications that genetically modified parasites are more virulent or invasive. If suspected infection did occur then diagnosis could be quickly carried out and the necessary drug or drug combination prescribed. Infection would be quickly cleared and would not recur. The species of human malaria parasite that recur cannot be cultured in the laboratory. 

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

We do not intend to use any GM animals or plants.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

P. falciparum is listed as an exempted category 3 by the Advisory Committee on Dangerous Pathogens (Fourth Edition, 1995). It has full derogation by the HSE due to its low risk of laboratory required infection, negligible risk of spread in the community and relative ease and success of drug treatments available. Taken this into account we would like to ask specifically for derogation of the following control measures: a) entry via airlock, B) negative pressure related to pressure of immediate surroundings, c) requirements for HEPA filters, d) autoclave in laboratory suite, e) shower, f) footwear as protective clothing.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid culture waste will initially be aspirated within the microbiological safety cabinet into a leak-proof container with 1% Virkon solution, being adjusted to a final concentration of 1% at the end of work, and left there until the next morning. This is an extended precaution since malarial parasites are extremely sensitive to culture conditions. Even a slight rise in pH of the culture medium will kill them. Minor spillage is treated with 70% Ethanol spray, which kills the organism immediately. Spillages of infective material (ie in the incubator or safety cabinet) shall be contained to prevent their spread, and then covered with Virkon powder. It will be left until the liquid is absorbed. The powder/spillage mixture is scraped into a receptacle for disposal. Afterwards the area will be rinsed and disinfected with 1% Virkon. There must always be a fresh supply of 1% Virkon readily available in case of spillage or accidents. Solid waste is placed directly into leak proof boxes. These are sealed for transport and autoclaved following recommended procedures for ADCP category 3 containment. Autoclave tape indicates that the process has occurred. Autoclaved material is considered to be 100% effective at sterilisation.

Any new staff would be specifically trained in the safety aspects of the work. The vector nor the genetically modified parasites are transmissible by an airborne route.

All work with P. falciparum will be performed under derogated category 3 standards within a certified category 2 laboratory.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been approved by the local GMSC after modification.

Project Containment

02/03/2022
THE GENERATION OF GENE KNOCKOUTS IN MYCOBACTERIUM AVIUM-INTRACELLULARE COMPLEX (MAC)

This project aims to define factors that determine virulence in mycobacteria belonging to the Mycobacterium avium-intracellular Complex Systems studies will include genes involved in metabolism, global gene regulation, stress response, intracellular survival, in vivo growth and latency. The aim is to produce strains of mycobacteria that have had individual genes inactivated either by removal or by interruption with transposon sequences. Mutants will also be complimented with the deleted gene in an attempt to restore parental function/phenotype. The mutants produced will be studied in vitro, and also used in murine infection models. The long-term aim of this work is to understand mycobacterial virulence thereby allowing the development of new control measures for MAC related diseases, including new drugs and better vaccines.

The Mycobacterium avium-intracellulare Complex (MAC) includes Mycobacterium avium subspecies avium (MAA), Mycobacterium avium subspecies paratuberculosis (MAP), Mycobacterium avium subspecies silvaticum (MAS) Mycobacterium avium subspecies hominisuis (MAGH) and Mycobacterium intracellulare (MI). However some human and environmental isolates of MAC do not fall within any of the designations listed above. Such isolates can exhibit genotypic features of two or more different MAC
species, and are usually given designations such as "MACx". The existence of such intermediate types suggest that MAC organisms are a continuum of species and subspecies however the pathogens MAA, MAP and MI are distinct in many significant ways, including environmental niches, host preferences, and clinical manifestations. Available epidemiological evidence indicates that humans are infected by MAC living in the environment, not via person-to-person transmission. Although viable MAC cells can occasionally be cultured from the stool of AIDS patients, there is little evidence to suggest that infective populations of the bacteria commonly make their way back to the environment. Therefore, the populations of MAA and MI cells to which humans are exposed are likely to not have human environments in their recent evolutionary histories. MAP is the cause of chonic enteritis in many animals including primates, whilst MAA and MI infect a variety of mammals and birds in serovar-specific fashion. Although there is little evidence for direct transmission from animals to humans, some serovars and strain types can be recovered from both, consistent with the possibility that virulence mechanisms are maintained in animal reservoirs. It is possible that some strains found in the environment are especially infectious to humans, while others may be relatively harmless. This compromises the predictive value of environmental monitoring efforts that assume uniform levels of infectivity and suggests that all MAC require a similar risk classification. Some MAC are undoubted opportunistic pathogens of humans, often requiring a dysregulation of the normal immune response to illicit pathobiological effects. MAA and MI are associated with cervical adenitis in children and as a complication of other immunological disorders including AIDS. A cell wall deficient form of MAP has been found intracellularly in macrophages associated with the gut wall of up to 92% of patients with Crohn's disease and 26 of controls. MAP is also the cause of orofacial granulomatosis. MAA, and MI are currently officially classified as ACDP as a Hazard Group 3 whilst MAP are classified only as ACDP Hazard Group 2 and MAS and MAH are not listed - but are presumably included in MAA. This laboratory however considers that, in the absence of further evidence, all MAC should be classified as Hazard Group 3 and has assumed this level of risk for this assessment. In a few cases, a rapid growing non-pathogenic host (Mycobacterium smegmatis strain MC2 155) will also be used to study the function of single genes in isolation.

Host/vector system

Knockout mutants will be generated by two methods: 1) Double crossover homologous recombination in host mycobacteria using a suicide shuttle plasmid (eg pNIL) that has no mycobacterial origin of replication but contains selection maintenance genes. The plasmid has a hygromycin resistance gene, which is lost in the final generation of the GMO knock-out as a result of counter selection (single-crossover plasmid DNA containing bacteria get killed) using sacB and sucrose selection. However, the intermediate transformants or single cross-over recombinants will have the plasmid with hygromycin gene. Hygromycin is commonly used as a selective marker in mycobacteria; it is not used as an antibiotic in clinical settings. ii) Transposon mutagenesis using mycobacteriophage (L5) delivery of a transposon (with Hygromycin selectable marker) to induce stable integration into the target genes causing disruption of target gene transcription. Complementation mutants of the knocked out gene will be generated by transformation with a simple non-hazardous plasmid (eg pAGAN, pSODIT, pSMT3) containing the wild-type gene. These constructs contains the mycobacterial origin of replication and can be sustained in the host. They also have hygromycin and/or kanamycin antibiotic resistance marker genes. These complemented mutants with genes from the parent strain will have the same hazards as, or less than the parent wild-type organism.

All MAC mutants will be treated as hazard group 3 organisms, exactly as the donor or wildtype recipient and therefore there is no expected increase in exposure hazard risk and no increased disease risk.

Origin & function

This project will be techniques to knockout the transcription of a variety of existing mycobacterial genes or to replace existing genes with modified 'inactivated' genes and will only be introducing 'new' non-self genes functioning as selective markers for each mutant. These include single genes involved in host metabolism, global gene regulation, stress responses, intracellular survival, in vivo growth and latency. The virulence of mycobacteria not determined by a single gene, thus single gene knockouts are not expected to increase virulence. The introduction of altered "self" genes back into the donor to create modified recipient organisms may be regarded as self-cloning.

Evaluation of foreseeable effects

It is highly unlikely that the deletion of a mycobacterial gene will increase the virulence of mycobacteria. However there are several hypothetical possibilities which may result in the phenotype of the GM MAC being altered in favour of increased pathogenicity by directed knockout of host genes. These include:

Deletion of a repressor gene for virulence determinants resulting in genes normally only expressed during a particular phase of infection to cause damage or immune evasion being switched on permanently. An increased hazard, in these cases is unlikely as the mechanism of pathogenicity in intracellular mycobacteria is based on the ability to switch multiple sets of genes on and off at specific times and in specific locations.
Deletion of a growth repressor.
This is highly unlikely to increase the hazard of these organisms as the slow growth rate of mycobacteria is considered to be controlled by the law of mass action and the number of ribosomes as a ratio of the availability of tRNA species and translation initiation factors. Thus multiple genetic manipulations would be required to influence growth rate.

Deletion of a deominant antigen involved is immune recognition, thereby allowing the bacteria to avoid recognation and cause more disease.
This is again highly uniquely as immune recognition is a multiple-antigen-dependent event and others have shown that deletion mutants of dominant antigens in other mycobacteria (eg Mycobacterium tuberculosis) have reduced or unaltered virulence but not increased.

The pathogenicity of mycobacteria is not fully understood but is widely shown not to be a single gene function; rather mycobacterial virulence is a multi-genec property of the bacteria. Virulence and pathogenicity of mycobacteria is determined by the host-pathogen interaction, with host immunity playing a central role, which is independent itself on human genetica, nutritional status and other factors. Thus disease, or the potential for harm to host, is a complex and multi-factorial process, in which gene sets play synergistic roles with other gene sets.

Accidental exposure to GM MAC is likely to give risk to infection with disease occurring at an incidence not greater than the wild type organism. The GM MAC is treatable in the same way as the wildtype organism with antibiotic treatment. Providing genes are not transferred from drug resistance strains of donor mycobacteria, which we shall not use as recipients, then any recipient GM MAC will be treatable in the same way as the fully drug-sensitive donor strain.

Horizontal (lateral) transfer of genetic material in mycobacterial populations is minimal. Mycobacterial plasmids do occur rarely and conjugal transfer has been observed in M.smegmatis (a fast-growing non-pathogenic mycobacteria). The importance of natural horizontal transfer in mycobacteria has not however been established. Introducing DNA into mycobacteria is a very inefficient process in the laboratory with very low frequencies of recombination. So horizontal transfer would be a very rare event within the Genus Mycobacteria. Transfer of mycobacterial genes from the GM MAC to other micro-organisms would be highly unlikely to occur, and alter the properties of the recipient bacteria so as to increase its hazard. Harbouring of extra genomic material in bacteria is usually associated with decreased fitness thereby producing a counter selection against any transformant.

Recombinant mycobacteria to be generated in this project are thus considered to be no more hazardous, and most knockouts to be much less so, than the donor wild-type parental organism. The knockout strains will not contain mycobacterial genes absent from wild type natural strains of pathogenic mycobacteria.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Animal infection with GM MAC will be performed by tail vein or intraperitoneal inoculation under CL3 conditions within a Class 1 MSC, as for fully virulent wildtype organism. This will take place in a specialised CL3 containment Suite. All waste will be autoclaved within the BRF suite and then incinerated. Any material from GM MAC infected animals that is required for further experiments will be performed in the departmental laboratory. CL3 suite. This will involve transport of GM MAC material from the BRF CL3 to the departmental CL3, where it will be treated. The transport will follow strict transport safety guidelines for ACDP Group 3 pathogens, samples will be double bagged and placed in unbreakable and sealed metal transport containers and carried to the CL3 labs, where it will be unloaded within the Class 1 MSC.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All Containment Level 3 (CL3) work will be performed in the specialised CL3 laboratories under the ACDP guidance for Hazard Group 3 micro-organisms. All work will be performed with live GM MAC within a Class 1 Microbiological Safety Cabinet (MSC) following identical protocols as for wild type MAC. GM MAC for culture will be transferred to incubators within the CL3 suite to another CL3 suite will be followed as for group 3 organisms, following current transport safety guidelines.

Waste from work carried out in the Class 1 MSC will be initially treated with 1% Virkon overnight before autoclaving. All waste will be sterilised by autoclaving followed by
incineration. The autoclaves to be used are sited within the CL3 suite. The autoclaves meet the correct standards and are validated by regular checks and calibration. Restricted access to all CL3 facilities is made by security pass-locked doors and to registered CL3 trained personnel.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been reviewed and accepted by the local GMSC.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref** 95/08.1

Date Ackn'd: 15/02/2008

Date Project Ceased

Project notified under transitional arrangements N

Consent Granted

Non-GMM

Not Applicable

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Exploring the RhoA-actin, Dyrk and MAP4K4 signalling pathways using lentiviral vectors.
Lentiviruses have the ability to infect human cells, with the subsequent expression of the inserted foreign genes and shRNAs, given that the HIV-envelope protein has been replaced with VSV-G (virus stomatitis envelope) which can infect many different cell types. All progeny cells also express these genes, but are replication defective (no new lentiviral particles can be produced from the infected cells). The purpose of the contained use is therefore to minimize the risk of exposure to the lentiviral particles.

Lentiviral particles will be produced in 293FT cell line (Invitrogen) by transient transfection of vector DNA. The resulting lentiviruses will then be transduced (infected) into eukaryotic cells in vitro, primarily mouse NIH3T3 fibroblasts, mouse skeletal muscle C2C12 cells, human breast adenocarcinoma MDA-MB-231 cells, and primary human mesenchymal stem cells.

Two different lentiviral vectors will be used. Plasmids pSIN and pHR will be used for the cloning of cDNAs and commercially available pLKO.1-puro plasmid (SIGMA-ALDRICH) for shRNA expression. Both lentiviral vectors are secured by gene removal such that no new particles can be produced from infected cells (replication-deficient). Vector DNA will be transformed and purified from bacterial TOPO1O cells (Invitrogen).

Several cDNAs will be cloned into the lentiviral vectors. These include components of the: A) RhoA-actin signalling pathway (i.e. the small GTPases RhoA, Rac, cdc42, the actin remodeling proteins ROCK, LIMK, cofilin and ERMs, and the downstream transcriptional activators MALIMKL1, MALI 6/MKL2 and the novel transcription factor encoded by 8C039093; b) Dyrk signalling (i.e. wild type and kinase-dead Dyrk) and its substrates (i.e., nuclear matrin MATR3, RNA-binding protein hnRNPU and the regulatory subunit of PKA (PRKAR1A); c) MAP4K4 signalling pathway (i.e. wild type and kinase-dead MAP4K4, the small GTPases Rab2 and GBP3, and MEKK1 kinase). These cDNAs will be either subloned into the lentiviral pH/pSIN vectors by restriction enzyme digestion or PCR from mammalian expression vectors currently available in the lab (pUC1S-derivative pEP plasmids, or pACT2-NIH3T3 library from Clontech)

Commercial pLKO1-puro plasmid (SIGMA-ALDRICH) will not be genetically manipulated as they already contain the shRNA against the above mentioned cDNAs.

The lentiviral vectors are replication defective. There is the theoretical risk of recombination with wild-type HIV-1 or HIV-2, although this has never occurred. The lentiviral vectors will be pseudotyped with the vesicular stomatitis viral envelope (VSV-G) and as a result could infect several human cell types, with the theoretical risk that a laboratory worker could be infected (i.e. through wounds or by spillage to the eye) and the transgene could be expressed. There is a low risk that the expressed proteins/shRNAs could give the infected cells an increased proliferative capacity. Although our proteins of interest have not been reported to behave neither as oncogenes nor tumor suppressors, they can affect cell proliferation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

not applicable
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (tissue culture media containing most of the lentiviral particles) will be treated with 1% freshly prepared Virkon (DuPont), according to the manufacturers recommendations within the Class II containment laboratory. Virkon is fully active against lentiviruses and its activity will not be reduced by the conditions of use. Solid waste (i.e. pipettes, tips and tissue culture plastic ware) will be deposited in a Sharpsafe box, the box sealed according to the manufacturers instructions and taken to be autoclaved within the building using a secondary container. Autoclaving will be performed in a validated autoclave cycle (100% kill degree) prior to incineration.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Institution's Genetic Modification Safety Committee asked for additional information regarding cloning procedures and servicing of the Class II safety hood. This information was incorporated into the attached risk assessment, which was subsequently seen and approved by the GMS committee.

Project Containment

<table>
<thead>
<tr>
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Animal Units

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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 95/14.1

<table>
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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>03/03/2014</td>
<td>Investigating new therapeutic interventions in the replication and pathogenesis of human cytomegalovirus</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<td>Not Applicable</td>
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Project Additional Information

Purposes of the contained use

To determine what viral and cellular factors are required for the replication and pathogenesis of human cytomegalovirus.
To determine what existing and novel drugs can be using in preventing human cytomegalovirus replication and pathogenesis.

Recipient or parental organism

Human cytomegalovirus

Host/vector system

Human

Origin & function

A laboratory strain of the herpesvirus human cytomegalovirus, AD169, will be modified. Modifications will be made by either of two methodologies.
Method 1: The AD169 genome has been cloned in to a bacterial artificial chromosome (BAC). This BAC is contained within the E.coli laboratory strain GS1783. Red-two step recombination of PCR products with BACs in GS1783 E. coli will be used to modify the nucleotide sequence of the AD169 genome. BACs will be transfected into human cell cultures to generate genetically modified virus. Genetically modified virus containing the desired mutations will be used to infected human cells in subsequent experiments. Mutations in the human cytomegalovirus genome contained within the BAC will be made so as to;
(i) introduce reporter genes including green fluorescent protein or luciferase
(ii) introduce marker peptides including FLAG, His6 or Myc tags into viral open reading frames
(iii) delete open reading frames from the viral genome
(iv) express HCMV genes from ectopic sites within the HCMV genome
(v) modify viral open reading frames so that the virus becomes resistant to the action of anti-viral drugs.
Method 2: AD169 will be cultured in human cell cultures under the selective pressure of anti-viral drugs to generate drug resistant viruses. Drug resistant mutations should be in viral open reading frames nessesary for a particular drugs mechanism of action. Drugs used to generate drug resistant viruses will include bacteriostatic and anti-proliferative agents, kinase and phosphatase inhibitors, or nucleoside and non-nucleoside inhibitors of viral replication.

Evaluation of foreseeable effects

E.coli bacterial strain GS1783 containing a bacterial artificial chromosome (BAC) encoding HCMV genome AD169 will be used to modify the HCMV virus by red two step recombination. Red two step recombination will not alter the biological properties of either the bacteria or BAC used.

Neither insertion of marker genes or tags nor deletion of viral open reading frames will result in virus with altered traits such as pathogenicity, host range, tissue tropism and mode of transmission. However, mutation of virus so that it is resistant to drug treatment will take place.
No GM plants or animals will be used

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Low levels of any known disinfectant can inactivate human cytomegalovirus. All samples containing virus will be exposed to 1% Virkon for at least 1 hour before disposal.
All tissue culture plastic will be autoclaved before disposal.
All glass ware used will be autoclaved before re-use.

Project Containment

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Animal Units

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Project Ref 95/14.2

Date Ackn’d 01/04/2014

CU2 Project Title Studying Pseudomonas aeruginosa infections and development of novel treatment options

Date Project Ceased 02/03/2022

Class CultureClassVolClass2 CultureVolumeClassConsent Granted

Class 2 < 1 Litre Non-GMM Consent Granted
Bacterial lung infections are a significant cause of morbidity and mortality. Particularly in individuals with underlying co-morbidities e.g. Cystic fibrosis, COPD, asthma and diabetes are more susceptible to bacterial lung infections. We are interested in two aspects here 1) the interactions between the bacteria and the host, looking at how the host tries to control the bacterial infection and how the bacteria tries to evade this. Using both in vitro and in vivo systems, we wish to define differences between healthy individuals and those with underlying co-morbidities that leads to greater disease susceptibility to bacterial lung infection. The 2) aspect we are interested in is the development of novel antibiotics that can kill P. aeruginosa either planctonic or in a biofilm setting.

P. aeruginosa can be carried as part of the normal nasal/ host bacterial flora. The bacterium is able to infect a wide variety of hosts and tissues and is an excellent model for studying infection by gram-negative bacteria. It is particularly prevalent in cystic fibrosis patients. We will be using in vitro models of infection using airway epithelial cells and differentiated airway epithelia to assess the effect of altering physiological conditions on bacterial growth. We will then assess these conditions and drugs that can alter them using in vivo mouse models. We would like to use gene modified P. aeruginosa in order to better follow and monitor infections with the bacterium. We will use luminescent and fluorescent strains of P. aeruginosa and nutrient transporter KO strains. The strains in use will be lab derived strains and no clinical samples or community acquired strains or toxin producing strains will be used. In addition, for screening for novel antibiotics we would also like to use P. aeruginosa with luminescence, as a reporter gene, to get a fast read out for killing activity.

Recipient or parental organism

Pseudomonas aeruginosa PAO1

Host/vector system

- Tn5-luxCDABE transposon
- GFP
- knock out's

Origin & function

Luminescence Reporter Gene -Tn5-luxCDABE stemming from Photorhabdus luminescens
Fluorescence Reporter Gene GFP and derivates stemming originaly from Aequorea victoria

Nutrient (predominantly carbohydrate) transporter/utilisation KO
e.g. FruA, FruR, oprB, carbohydrate kinase, permease of ABC sugar transporter, binding component of ABC sugar transporter, zwf g-6-phosphate dehydrogenase.
P. aeruginosa is a Gram-negative bacteria in the class of Gamma Proteobacteria and in the order of Pseudomonadales. Pseudomonads are ecologically important, since they are capable of breaking down many different molecules, such as pesticides, toxic chemicals, plant and animal materials in soil and water. Pseudomonas aeruginosa is a versatile bacterium that grows in soil, marshes, and coastal marine habitats, as well as on plant and animal tissues. P. aeruginosa is an aerobic, rod-shaped bacterium (K. J. Ryan, C. G. RAY, Sherris Medical Microbiology 4th ed. (MCGRAW-HILL, New York 2004). P. aeruginosa is not an obligate parasite, it is an opportunist, initiating infections in individuals whose resistance is low, e.g immune suppressed patients or patients with large burn wounds. Especially in patients with cystic fibrosis (CF) P. aeruginosa is hard to treat. The lung environment of these patients appears to provide a unique environment, allowing persistent bacterial colonization. Clinical data indicates that P. aeruginosa is the fourth leading cause of nosocomial infection and the foremost cause of hospital-acquired pneumonia. Into the wildtype strain of P. aeruginosa PAO1 glucose knockout mutants or transposon mutant with a luminescence or fluorescence gene will be introduced. Reporter gene like GFP, YFP, RFP or luminescence are well established and used in the background of many different microorganisms as well as eucaryotic cell lines. To our best knowledge and assessment, introducing a reporter into PAO1 will not increase the hazard towards the environment as well as to animals or humans. In addition, glucose knockout mutants will be of an disadvantage of the GMM compared to the wild type strain and there is no evidence that the knockout will show any improved virulence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All wastes will be autoclaved at the facilities at SGUL

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The committee was satisfied with the information provided and agreed that the project could be conducted safely as described.

Project Containment

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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Project Ref 95/14.3

Date Ackn’d 14/07/2014

CU2 Project Title Genetic manipulation of mammalian cells in culture to study cellular signalling mechanisms in normal development and disease

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

N

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Development of vectors (viral and plasmid) and molecules for the study of cellular signalling processes in normal development and disease. This will involve transgene over-expression, gene knockdown, cellular reprogramming and directed differentiation. All gene modifier vectors are disabled and lack essential genes which prevent their autonomous replication. All require specific packaging cell types to produce a viral particle which is replication deficient and cannot multiply or produce progeny. All vectors will have no effect to the environment as they will be maintained and contained under strict laboratory conditions.

Recipient or parental organism

Recipient Cells

Mammalian cell lines (for example 293T, 3T3, CHO, HepG2, Huh7, U2OS, ela, PC3, DU145).
Primary human and mammalian cells (for example fetal and adult fibroblasts, blood cells, and vascular and lymphatic endothelial cells).
Human and mouse embryonic stem cells and adult stem cells either obtained commercially or from collaborators and repositories or from biopsy material (for example; mesenchymal stem cells, cardiac and vascular stem cells, pancreatic/hepatobiliary progenitors).

Human embryonic cells and primary cells or tissues are obtained by donation after pathogen screening (HIV, Hepb/c Human primary cells are donated from unscreened patients and are quarantined pending the pathogen screening as described prior to genetic modification.

Viral Vectors

Retroviral vectors used are derived from Moloney murine leukaemia virus (MMLV), a mouse virus therefore chances of mobilisation by recombination with endogenous
Viruses in a human are practically impossible. Furthermore, the vectors contain no MMLV protein coding sequences and so are completely replication disabled and the chances of recombination are minimised.

Lentiviral vectors derived from HIV1, SIV, EIAV and FIV are based on genome integrating viruses. Like retroviruses the severely deleted genomes contain no endogenous protein coding sequences and the 3' Long Terminal Repeat (LTR) contains a self-inactivating mutation that prevents the replication cycle initiating after reverse transcription of the RNA viral genome.

Adenoviral vectors (serotypes Ad2/5) are disabled by mutations to the E1a and E3 genes that are fundamental to the early stages of viral replication. Although adenoviruses are human pathogens infection does not result in serious illness and as such are a low risk to humans. The viral genome is transient in infected cells (does not naturally integrate into the host genome) and so there is minimal risk of insertional mutagenesis.

Adeno-associated Viral vectors (AAV) are disabled by mutations to the Rep gene which is necessary and fundamental to replication. Furthermore, AAV requires the expression of adenoviral genes to facilitate its replication. Recombinant forms of AAV will integrate into the host cell genome at very low frequency and so the chances of insertional mutagenesis are low.

### Host/vector system

#### Origin & function

Transgenic material - Human and mammalian cDNAs of transcribed genes involved in aspects of cellular transformation, self-renewal, differentiation, proliferation, senescence, apoptosis and metabolism will be expressed from pol ll promoters. These genes would include transcription factors, signalling factors, signalling molecules, enzymes, growth factors and cytokines.

Such genes would include but are not limited to:

- **Transformation:** hTERT, BMI-1, C-MYC
- **Self-renewal:** OCT4, Nanog, SOX2, LIN28, KLF4, STAT3
- **Differentiation:** Wnt, PI3K, Notch, Hedgehog, TGFbeta, BMP, MAPK, ERK, JNK, RAS, NFkB
- **Cell adhesion, migration & communication:** ICAM-1, VCAM-1, Selections, ThoGTPases, Ezrin, PDK1, Cadherins, B-Catenin.
- **Hypoxic Responses:** HIF, ARNT, C-MET
- **Innate Immunity:** TLRs, TBK, NFkB, IRF3, IRF7

The above gene targets will also be subject to gene knockdown with siRNA and miRNA expressed from pol ll/lll promoters.

Bacterial Cre-recombinase for removal of LoxP flanked gene sequences.

Reporter genes will also be expressed from endogenous and viral promoters with or without additional enhancer elements either constitutively or conditionally to mark cells or quantify surrogate gene expression. These will include;

- Fluorescent- e.g GFP, YFP, CFP, dsRed, mCherry.
- Luminescent- e.g.firefly and renilla, guassia, vargula luciferases.
- Biochemical marker genes e.g. CAT, AFP

### Evaluation of foreseeable effects

The recombinant viral vectors are all highly disabled vectors.

Lentivirus/retrovirus: These self-inactivating vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. Whilst the envelope pseudotyping extends the cellular tropism and confers greater stability the viral vector will always be contained within a class II safety cabinet thus user exposure to liquid aerosol is
The exposure of non-human hosts to pseudotyped vector could result in initial infection but, again seroconversion is highly unlikely.

Adenoviral vectors of non-human hosts to pseudotyped vector could result in initial infection but, again seroconversion is highly unlikely.

Adenoviral vectors are deleted of essential replication/packaging genes that are supplemented by an established packaging cell line (293T).

Adeno-associated Virus is defective by nature and has a replication disabled genome and is incapable of replication without the presence of helper adenovirus proteins. In this instance the vectors used would require the provision of cap and rep genes in trans in order to replicate and disseminate. There is no significant chance of reversion to the animal species therefore it highly unlikely that such a virus, if released into the environment, would represent a significant risk. Furthermore, most AAVs proposed for use constitute AAV5 genomes pseudotyped with other AAV envelopes so would never occur in nature and thus would be incapable of reversion.

The majority of the gene products expressed are expected to affect cellular properties such as proliferation, apoptosis, migration, adhesion and fate. Also, some have known or suspected oncogenic or tumour suppressor properties.

Gene products in plasmid form exist in non-mobilisable constructs and are therefore of minimal threat as expression would most likely be restricted to the infected cell, which in itself would be rejected by the host immune system.

Sharps will not be used in the production or handling of any of the viral vectors described in this project.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
<thead>
<tr>
<th>None</th>
</tr>
</thead>
</table>

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

<table>
<thead>
<tr>
<th>N/A</th>
</tr>
</thead>
</table>

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All viral production and handling procedures will occur in containment level 2 laboratories.

Non-viral work will occur in Class 1 conditions.

Liquid Waste will be treated with freshly prepared 1% Virkon or 10% hypochlorite solution for at least one hour prior to disposal via the sink. Solid waste will be autoclaved at 134°C on a destruct cycle prior to be placed in Tiger striped bags for disposal. Sharps will be placed in yellow sharps boxes that will be first autoclaved then removed by the waste contractor for incineration. Cryovials containing virus will be stored in specified cryofreezer and opened only in Class II microbiological safety cabinets. Spillages and working surfaces will be cleaned with 2% Distel or 1% Virkon and with 70% ethanol.

Solid waste that has not been autoclaved will be placed in clearly labelled biohazard double bags will be incinerated as clinical waste.

Hazard conditions consist of possible spillages, aerosol spray when opening cryovials.

Work is restricted to determined areas (containment level 2 laboratories), disinfecting solutions will always be available, working guidelines are clearly posted, unauthorised personnel is not permitted. Strict adherence to Category II working practices. Reporting in case of accident is according to College procedures using the online or paper accident incident forms or by contacting the Safety, Health and Environment Office directly on extn. 5365.

Training to work with class II GMM's will include Instruction of the guidelines regarding dressing code, how to treat spillages and disinfection with 2% Distel, 1% Virkon and
70% ethanol. Documented spillage procedures are clearly established. In case of accident with sharps, College procedure with blood borne viruses will be followed. Autoclaves are regularly serviced and checked. Autoclaved material is checked using autoclaving tape and spore strips to assess has reached temperature for disinfection.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Risk assessment GM292 has been assessed by the SGUL GM safety committee and, after minor revisions based on clarifications of the specific area of work, the revised document was approved.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>Large Scale Activities L2 L3 L4 L2 L3 L4</td>
<td>Human Clinical Applications L2 L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 95/16.1

Date Ackn'd

10/02/2016

CU2 Project Title

Production of HIV pseudoviruses for neutralizing assays

Class CultureVolClass2 CultureVolumeClass3-4

Class 3 < 1 Litre

Non-GMM Consent Granted Yes

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work Y
**Project Additional Information**

**Purposes of the contained use**

Envelope (Env)-pseudotyped HIV-1 will be produced in HEK 293T/17 cells by chemical means. This generates pseudovirus particles that are able to infect cells but are generally unable to produce infectious progeny virions due to the absence of a complete genome. This single round of infection is readily detectable in genetically engineered cell lines (e.g. TZM-bl) that contain a Tat-responsive reporter gene, such as luciferase.

**Recipient or parental organism**

- Human Embryonic Kidney 293T/17 cells [used for transfections]
- TZM-bl cells [Obtained from NIH AIDS Research and Reference Reagents Programme or its UK equivalent the National Institute for Biological Standards and Control (NIBSC)]; Parental HeLa cell line (JC.53) stably expresses large amounts of CD4 and CCR5. They constitutively express CXCR4. The TZM-bl cell line was generated from JC.53 cells by introducing separate integrated copies of the luciferase and β-galactosidase genes under control of the HIV-1 promoter. Cell line is highly sensitive to infection with diverse isolates of HIV-1
- Bacteria DH5a [used for making plasmids]

**Host/vector system**

- an Env expression plasmid (e.g., pcDNA 3.1D/V5-His-TOPO-Env)
- a backbone vector (e.g., pSG3ΔEnv) that expresses the entire HIV-1 genome except Env. pSG3Δenv was derived from pSG3.1 by SpeI partial digestion, Klenow filling of 3' recessed ends and religation. This has introduces four nucleotide insertion mutation (CTAG) in env and a translation stop codon (TAG) after amino acid residue 142 (Wei et al. 2002; Antimicrob Agents Chemother 46: 1896-1905).

Plasmids for pseudovirus will be provided by the NIH AIDS Research and Reference Reagents Programme or its UK equivalent the National Institute for Biological Standards and Control (NIBSC). No genetic manipulation will be required in SGUL.

**Origin & function**

<table>
<thead>
<tr>
<th>HIV-1</th>
<th>Gag [Group-specific Antigen, codes the structural HIV-1 proteins (Capsid, Matrix, Nucleocapsid, and p6)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pol</td>
<td>[Codes the viral enzymes reverse transcriptase, protease, and integrase]</td>
</tr>
<tr>
<td>Tat</td>
<td>[Transactivator protein, increases efficiency virus transcription]</td>
</tr>
<tr>
<td>Rev</td>
<td>[Regulates traffic of virus RNA species out of the nucleus]</td>
</tr>
<tr>
<td>Nef</td>
<td>[Down-regulates CD4]</td>
</tr>
<tr>
<td>Vif</td>
<td>[Counteracts antiviral effect of cellular APOBEC proteins]</td>
</tr>
</tbody>
</table>
### Vpr
Contains nuclear localisation sequence

### Vpu
Down-regulates CD4, transports Env proteins to cell surface

### Env
Encodes the viral envelope proteins gp120 and gp41, these proteins mediate virus-entry into target cells.

### TZM-bl
 Reporter [e.g. luciferase, β-galactosidase or GFP]

### Evaluation of foreseeable effects

Human Immunodeficiency Virus (HIV) can cause a currently incurable disease in humans which may be fatal. The virus is categorized as a BioHazard Group 3 pathogen by the Advisory Committee on Dangerous Pathogens (ACDP). However the risk of transmission of the infection in the laboratory work environment is an exceedingly rare event (ACDP/92/P5b). The primary route of infection for lab workers is through contact with open body sites (e.g. cuts, braised mucosal surfaces, eyes, blood to blood contact and through needle stick injuries). Aerosol infection is considered to be a very low risk to lab workers. The risks may be greater when handling purified, concentrated or bulk quantities of virus under laboratory conditions where the virus is present in concentrations far exceeding that found in normal body fluids.

Env-pseudotyped virus has a four nucleotide insertion mutation (CTAG) in the Env gene and a translation stop codon after amino acid residue 142 that renders the Env defunct. However, it is important to note that Env-pseudotyped viruses are handled and treated the same as live HIV-1 cultures since replication competent virus could be present. Sequence analysis indicates that recombination can occur that repairs the defect in the backbone plasmid(s) lacking Env, thus rendering the virus replication-competent. The recombination event occurs at a relatively low frequency and the proportion of the pseudovirus that is replication-competent is low (Abram et al. 2010. Journal of Virology. 84:9864-9787). When recombination occurs that renders the virus replication-competent, the virus generated is predicted to behave identically to wildtype HIV-1.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Sweetie jar for discard of tips, plates, eppendorf tubes and small volumes of liquid inside the safety cabinet (please refer to Appendix A). Sweetie jar will ultimately be autoclaved using a destructive cycle at 134°C.

### Is an emergency plan required according to regulation 20? N

### Tick to confirm that it is attached to this form N

### Tick to confirm that you have attached a risk assessment to this form Y

### Please enter comments on the GM safety committee on the risk assessment

The committee required the preparation and other work with the pSG3Δenv plasmid to be performed under conditions that protect against hazardous naked DNA, under COSHH (wearing of protective gloves, covering of cuts by suitable dressings and avoiding the use of sharps). This was included in procedures and the committee was satisfied with the information provided and agreed that the project could be conducted safely as described.
Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 | L2 | L2
Yes | L3 | L3
L4 | L4 | L4
Animal Units | Large Scale Activities | Human Clinical Applications
---|---|---
L2 | L2 | L2
L3 | L3 | L3
L4 | L4 | L4

Project Ref 95/19.1

Date Ackn'd 29/01/2019

CU2 Project Title
Infection of tissue culture cell lines, including placental cell lines, with GM Zika virus

Class 2
Culture Volume Class 3-4
< 1 Litre

Non-GMM Consent Granted
Not Applicable

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
We wish to understand replication and pathogenesis of Zika virus, with an emphasis on infection of human trophoblast cells.

Recipient or parental organism
A range of cell lines, including human trophoblasts, will be transfected with viral genes. It should be noted that several experiments will entail making replication defective lentiviral particles in order to express Zika virus RNA and proteins upon transduction of human cells.

**List of vectors:**
- pREST
- pcDNA
- pcDNA3.2/V5-DEST
- pcDNA3.2/V5-DEST
- pLenti6.3 - a second generation lentiviral system where the deletion of lentiviral sequences do not allow this vector to be mobilized by other lentivirus or any other virus.

**Origin & function**

We intend to clone and/or create recombinant versions of the following Zika virus genes, whose functions are described:
- **C** - virus capsid
- **prM** - virus maturation factor
- **E** - virus envelope fusion protein
- **NS1** - viral interferon/immunity antagonist
- **NS2A** - viral assembly factor
- **NS2B** - viral assembly factor
- **NS3** - viral protein protease
- **NS4A** - viral genome replication factor
- **NS4B** - viral genome replication factor
- **NS5** - viral RNA polymerase

The recombinant versions will be tagged with fluorescent markers such as Green Fluorescent Protein.

**Evaluation of foreseeable effects**

This activity involves - in the first instance - the infection of cells with a Zika reporter virus that expresses firefly luciferase (Royle et al, Vet J, 2016). Based on the infection experiments we will then perform a range of experiments to study viral RNA and protein function. Initially, this involves modifying the aforementioned viruses to carry other reporter genes, in particular green fluorescent protein. We will also test viral RNA and protein function by introducing mutations that inhibit viral RNA and/or viral protein synthesis or viral protein function.

Ultimately, no GM organism will be produced that is more hazardous than the wild type viruses or any replication defective lentivirus. Only loss of function mutations are to be studied (viral genes replaced by Green Fluorescent Protein (GFP) or other markers or viral genes, or fragments of them, tagged with GFP).

Regardless of which strain is being studied, Zika virus is characterized as a hazard group 2 pathogen. Human infection of the flavivirus Zika virus is typically caused by transmission to humans of virus by infected biting insects, such as certain species of mosquito. Zika virus can be transmitted between humans horizontally via blood-blood contact, sexual intercourse. Also, Zika can be transmitted from mother to foetus across the placenta during pregnancy and, potentially, from mother to child during birth or post partum (via breast milk or salivary exchange). Zika virus infection can be cleared by
immuno-component adults. The greatest risk to human health is in infection of individuals who are pregnant or may become pregnant, where virus infection in the foetus can result in microcephaly and associated developmental disorders, or those who are immunosuppressed, where infection can cause various inflammatory illnesses. There are no vaccines available for Zika. However, infection can be treated with broad spectrum anti-viral agents such as favipiravir, ribavirin, and 5-fluorouracil. Moreover, virus infection can be treated with non-specific agents such as interferon. The laboratory use of Zika virus in the tasks covered by our risk assessment is not part of the viruses normal transmission route and, therefore, viruses are highly unlikely to be laboratory acquired, even if the measures detailed in this assessment are not used. Also, the insect vector for Zika virus is not found in the UK or Northern Europe.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No plant or animal GMO will be created nor will plants or animals be infected with GMMs.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Material will be disposed of in accordance with SGUL guild lines and local rules. These are detailed in COSHH Risk Assessment Number I&l/BLS/004. Low level of any known desinfectant or a 70% ethanol solution can inactivate enveloped viruses, including Zika virus and lentiviruses. Briefly, infected liquid material will be deactivated in 1% bleach or 1% Virkon. Solid material in which infected material was used will be autoclaved on a destrcution cycle (134°C for 20 min) giving and effective 100% kill using the autoclave adjacent to the tissue culture laboratory (J2.125). This autoclaves is validated on an annual basis (including the placing of independant thermocouples at the centre of the load).

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment
The overarching goal of our research is to generate lentiviral particles pseudotyped with variants of the coronavirus Spike glycoprotein in order to investigate its function and evolution and use a well-established approach (shRNA-mediated knock down) to identify key players in infection.

Pseudotyping with the glycoprotein of interest:

Whilst retroviruses possess the ability to infect and replicate in a range of cell types, tropism and host range is determined by the specificity of the surface glycoproteins encoded by the env gene. To alter or investigate the host cell tropism of a retroviral or lentiviral vector, the env gene may be modified or exchanged with the surface glycoprotein from another virus. This process is known as pseudotyping and envelope proteins such as gibbon ape leukemia virus envelope (GALV-env) or vesicular stomatitis (VSV)-G protein are commonly used to produce pseudotyped viral particles capable of infecting cells from different host species.
In our work, lentiviral vectors will be pseudotyped with envelope proteins such as GALV-env or VSV-G or variants of the coronaviruses Spike glycoprotein. Genetic load: reporter gene or silencing RNA

The lentiviral particles can be loaded with a reporter gene (such as GFP, GST and luciferase) or interfering sequences (i.e. RNA designed to silence specific genes of interest). In addition, the genetic material may contain an epitope tag (such as Myc, His, Strep, V5). Genetic material may also contain a selectable drug marker e.g. the pac gene encoding a puromycin N-acetyl-transferase (PAC) which confers puromycin resistance and enables selection of transduced cells.

Cell lines of mammalian origin (including human, simian, murine origin) will be transduced with recombinant vectors to enable the study of the phenotypic and functional effects of the ectopically expressed glycoprotein(s) or to silence expression of target host genes.

Recipient or parental organism

Mammalian cells (including HeLa, Vero) will be transduced with pseudovirus for transient expression of a reporter gene (GFP, luciferase) in order to investigate the properties of SARS-CoV-2 Spike proteins; in separate experiments, mammalian cells (including HeLa, Vero) will be transduced for transient expression of a silencing hairpin to investigate cascades, including signalling cascades that regulates protein expression (including ACE2 expression).

Host/vector system

List of vectors. (cover names, mobilisation regions, and any disabling mutations.)

- p8.91 packaging plasmid, contains HIV-1 gag-pol, rev (Zufferey et al., 1997). It will be obtained as a gift from Dr Dalan Bailey, The Pirbright Institute, UK.
- pMD.G contains VSV-glycoprotein (Naldini et al., 1996). It will be obtained as a gift from Dr Nigel Temperton, University of Kent.
- pCDNA3.1_Spike and pCAGGS_Spike contain Spike protein from SARS-CoV-2. They will be obtained as a gift from Dr Dalan Bailey, The Pirbright Institute, UK.
- pMD.G.2-WEAU contains the Envelope glycoprotein from HIV-1 isolate WEAU_D15_410 and pMD.G.2-MLV contains the Envelope glycoprotein from MoMLV. Both will be obtained from Dr Laura McCoy, University College London.
- Reporter plasmids (pCSFLW) contain Luciferase or GFP (reporter gene) (Zufferey et al., 1997, 1998) a gift from Dr Dalan Bailey, The Pirbright Institute, UK.
- pCSRQ silencing plasmid (Clontech), contains silencing hairpins.

Example of three-plasmid system with the AIM of investigating Spike function
1. p8.91 packaging plasmid
2. pCSFLW-Luciferase
3. pCDNA3.1_Spike
A Positive Control particle will be generated as:
1. p8.91 packaging plasmid
2. pCSFLW-Luciferase
3. pMD.G (VSV-glycoprotein)

A Negative Control particle ("bald", i.e incapable of cell entry) will be generated as:
1. p8.91 packaging plasmid
2. pCSFLW-Luciferase

Example of three-plasmid system with the AIM of knocking down a host gene
1. p8.91 packaging plasmid
2. pCSRQ silencing plasmid
3. pMD.G (VSV-glycoprotein)

OF NOTE: particles pseudotyped with SARS-CoV-2 Spike will only be used in combination with a reporter gene, GFP or luciferase, and NOT in combination with a silencing hairpin.

Origin & function

Sequences for coronavirus Spike proteins, including SARS-CoV-2, will be obtained from open databases (including the UK COVID-19 Genomics UK Consortium), while silencing hairpins are designed using commercially available software (e.g. GeneArt, Life Technology; Clontech). Sequences will be either custom synthesised commercially (GeneArt, Life Technology) or will be generated using standard molecular biology techniques.

Evaluation of foreseeable effects

Hazards associated with the recipient microorganism:
The generation of pseudoviruses will involve initial amplification of packaging, load and envelope plasmids using competent bacteria and commercially available plasmid purification kits. All bacteria used for such amplification will be standard commercially-available ACGM class 1 strains of Escherichia coli (E.coli) such as JM109, DH5alpha, and TOP10 which do not pose a hazard to human health. Genes encoded by the plasmids are unlikely to be expressed in such strains of E. coli. The bacteria and genetic material will be handled as per COSHH and RAs that mitigate risks to human health to acceptable levels. Briefly, all waste will be discarded after neutralisation (Virkon/Chemgene for liquid and autoclaving for solid waste); staff are required to wear personal protective equipment (including Howie lab coat, gloves) and follow general laboratory code of practice (Local Rules) following appropriate training.
The so generated pseudoparticles will be able to enter mammalian cells, including human cells, and insert their proviral DNA into the genome. This may possibly disrupt the normal pattern of gene expression within a transduced cell. However, the pseudoparticles are replication-defective and would not then proceed to disseminate. Importantly, the cells (including
Transduced ones) and the pseudoparticles will be handled as per Containment Level 2 COSHH and RAs that mitigate risks to human health to acceptable levels. Briefly, all material will be handled in a MSC Class II; centrifugation will be performed with buckets with lids and o-rings; waste discarded after neutralisation (Virkon/Chemgene for liquid and autoclaving for solid waste); staff are required to wear personal protective equipment (including Howie lab coat, gloves) and follow general laboratory code of practice (Local Rules), following appropriate training.

Hazards arising directly from the gene products:
None of the viral genes used or modified in our studies encode a toxin, oncogenic protein, allergen, modulator of growth or differentiation or any other protein which may result in a potentially harmful biological activity. We know of no known harmful biological activity of over expression of any of the viral genes studied here.

Hazards arising from the alteration of existing traits:
The inserted gene product(s) will be reporter genes, including GFP and luciferase, which are not known or suspected to interact with host cellular processes/pathways and/or are virulence factors. The gene product may also be an interfering RNA molecules designed to silence specific genes of interest. These products may interfere with normal cellular pathways if workers get into direct contact with the viral particles. This risk will be minimised by handling the particles at Containment Level 2. In the highly unlikely event of viral particles getting into contact with skin, the skin will be washed with soap and warm water. An incident report will be filed with the Safety, Health, and Environment Office. No sharps will be used in any procedure.

Hazards arising from the transfer of the inserted sequence to other organisms:
Endogenous retroviruses have been described in many animal species. However, recombination with exogenous sequences is only likely to occur when high levels of DNA are present in the same cell and this will not occur during experimental transduction of cell lines (human HEK 293T cells are not known to express endogenous retroviruses).

All cells (including transduced ones) and the pseudoparticles will be handled as per Containment Level 2 COSHH and RAs that mitigate risks to human health and to the environment to acceptable levels. Risks to the environment are minimal as the vectors are replication deficient and very unlikely to remain viable for prolonged periods of time outside ideal culture conditions. The control measures described above to mitigate risks to human health will mitigate any risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No larger GMOs will be created

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids that have been into contact GMM is disposed in an autoclave bag and autoclaved using the equipment available in the CL3 suite. The autoclaved waste is no longer hazardous but will be disposed via clinical waste (orange bags) route.
Liquid waste is decontaminated by incubation with Virkon (1% final concentration) or chemgene (1:50) for at least one hour. The decontaminated liquid is then disposed in the CL2/3 laboratory sink.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment
Thank you,

I agree with your three comments. The comments add clarity to the risk assessment and should be added to the text. The overall risk of the project remains changed because viral particles will be handled at CL2, which provides sufficient mitigation.

Sent: 26 February 2021 15:59
Subject: Re: New GMM Risk Assessment: GM334 Gaining insight into the function and evolution of the Spike glycoprotein of coronaviruses using a replication-defective pseudovirus system

All -

I have little to add for a virological standpoint. At this junction my only comments are that there is little information about what spike mutants might be used now and in the future. It might be wise include language that covers "the study of mutants with known differences in transmission and pathogenesis compared to wild type" or similar. Plus, a general point that should be made with all lentivirus work; insertional mutagenesis of humans can occur regardless of what lentivector is they are exposed to. That point should be emphasised (I hope I did not miss it!), but should not alter this application in a substantial way.

01/03/2021

Dear All,

I agree with A's assessment and comments made by B. My only small point would be that the question as to whether or not the work can be done by a pregnant worker is not exactly answered - is a definitive answer required prior to notification?

Best wishes,

01/3/2021

Dear all

I also agree with A and B's comments about the categorisation of the work. With regard to immune-compromised people and pregnant individuals, I think it would be best to say that the person should opt of the work unless suitable anti retro viral drugs are available. While the risk assessment says that people will be referred to Occupational Health (OH) for assessment, OH could send the person back to the committee as would say that they are not experts in the work being undertaken.

I know that this may seem draconian given that no infections have been reported due to VSV and that it was used in an Ebola vaccine https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7045819/ but I think it would be best to adopt a precautionary principle.
Regards,

Thank you,
P I think you raised an important point.
I agree with C that immune-compromised people and pregnant women should opt out of work. Not that sure about the anti-retroviral part as it sound too uncertain (to me). To add to the mix, women can become pregnant without knowing it. All this needs to be clarified on the RA before we approve.
I will wait until Thursday, I am hoping to collect more comments, and then request E to deal with the corrections/comments.
Best,
02/03/2021
Dear all,
I agree that this should be a CL2 project. The relevant mitigation for lentivirus production seems to be in place and the biggest unknown is the effect of mutations in the SARS-CoV2 spike protein, but this will be packaged into the replication defective virus and will carry only reporter genes. Although these viruses are likely to be taken up efficiently by relevant cell types, there are no known harmful effects of these reporters.
I wonder whether there’s a need to use shRNA/VSV for gene silencing and whether a CRISPR/Cas9 system using transfection might be a safer approach and potentially more effective?
It’s not clear to me what the increased risks might be for women during pregnancy, but an opportunity to discuss the proposed work with OH on an individual basis might be important to determine if any further mitigation is needed for that person.
Best wishes,

Thank you, You hit the nail in the head with your comment about replacing Lentis with CRISPR; One of the steps in the hierarchy of controls is “to replace the dangerous with the less dangerous”. I will suggest E to consider this. However, in my humble lab experience, lentiviruses are very robust and efficient knocking down genes while CRISPR is far fiddlier. This will have to be weighted depending on the long-term goals of the project and the tools already available to E
I will add your example on the GMO guidance webpage.
Best,

---

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2021
This project aims to study the bacterial fitness of GBS isolates by investigating genes associated with adhesion, invasion, virulence, colonisation and disease using knock in and knock out variants in growth, adherence and invasion assays. To set up the series of functional assays, we would like to ensure the bacteria can be visualised and traced for the different assays we will carry out, such as flow cytometry and microscopy, which requires the bacteria to contain a fluorescent protein marker provided by Prof. Spellerberg at the University of Ulm, Germany.

### Recipient or parental organism
- Clinical Group B Streptococcus isolates
- Group B Streptococcus serotype reference strains (e.g. NCTC 9993) obtained from PHE
- Commercially available ATCC Group B Streptococcus laboratory strains (e.g. COH1(ATCC BAA-1176))

### Host/vector system
- pAT28 with a multiple cloning site to allow cloning of fluorescent proteins
- pBSU409 with a spectinomycin antibiotic resistance gene
- pHY304 temperature sensitive integrating vector with a chloramphenicol resistance gene
- pDCerm shuttle vector for complementation
- and other commercially available plasmids

### Origin & function
- **gfp:** encodes for green fluorescent protein used as a marker protein
yfp: encodes for yellow fluorescent protein used as a marker protein
rfp: encodes for red fluorescent protein used as a marker protein
mcherry: encodes for red fluorescent protein used as a marker protein
and other similar fluorescent proteins

Several genes identified by ourselves and other groups which control the function of protein synthesis, transport, energy metabolism and cell regulation amongst others will be investigated. The genetically modified GBS strains will not be in any way more virulent than wild type strains as we are not over-expressing or introducing gain-of-function mutations. Only mutations hindering fitness will be selected.

Evaluation of foreseeable effects

The genetically modified GBS strains will not be in any way more virulent than the wild type strains for humans or the environment as we are not over-expressing or introducing gain-of-function mutations. Only mutations hindering fitness will be selected.

Hazards associated with the recipient microorganism:
GBS (Group B Streptococcus, Streptococcus agalactiae) is a common commensal bacterium found in 20% of humans, worldwide, and does not tend to cause disease in healthy adults. GBS is also a pathogen found in fish, cows, and various reptiles.
GBS colonises the genitourinary and gastrointestinal tracts of humans. It is spread from mothers to infants during pregnancy during birth or post-partum. Infants under 3 months old are the most at risk. GBS infection can cause meningitis, sepsis, or pneumonia. Disease in infants below 7 days old is called early-onset disease, with the primary risk being maternal colonisation. Disease in infants aged 7-30 days is described as late-onset disease, the risk factors are not well-characterised. Elderly people with co-morbidities also have a higher risk of GBS infections.
GBS is treatable with antibiotics – penicillin is the first line of treatment, or alternatively, clindamycin or erythromycin. Resistance against penicillin is not common and remains the most effective treatment. Pregnant women can be screened for GBS by a risk-based assessment in the UK (or culture based through private clinics) and if deemed at risk of GBS carriage then will be given intrapartum antibiotic prophylaxis (IAP) at 35-37 weeks gestation. IAP has successfully shown that it can prevent early-onset disease.
Genetic modification of the bacterial genome is likely to create a loss-of-function phenotype. There is a possibility of cells being co-infected with the wild type strain during complementation assays to validate the loss-of-function mutations, restoring it to a wild type strain. There will only be restoration of the GMM, there will not be a gain-of-function for it to be more virulent than the wild type.

Hazards arising directly from the gene products:
All vectors used will carry an antibiotic cassette that is not used to treat GBS infections clinically, therefore preventing the risk of untreatable GBS if the pathogen escapes.

Hazards arising from the alteration of existing traits:
Gain of function mutations will not be selected. Only mutations hindering fitness will be selected.

Hazards arising from the transfer of the inserted sequence to other organisms:
Gene transfer or recombination of the GMM with another organism could occur, however, we will take precautions to minimise the risk of this happening. This includes only working with the target organism (GBS) in a microbiological safety cabinet and ensuring surfaces are thoroughly disinfected with 70% ethanol and Virkon 1% before and after. We will also ensure GMM waste is disposed of, separate to non GMM laboratory waste.

Hazards to the environment:
GBS can replicate in humans, cows, fish and reptiles but not in plants. GBS is not regulated under SAPO.
None of the genes manipulated encodes a plant or animal toxin.
We do not anticipate that manipulation of genes to cause loss-of-function will be hazardous to animals or plants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Material will be disposed of in accordance with SGUL guidelines and local rules. Solid waste will be placed in Bio-Bin Fold Flat and autoclaved at a destruct cycle at 134 degrees C for 30 minutes, liquid waste will be immersed in Virkon 1% for at least one hour, or alternatively autoclaved. 1% v/v virkon (and 70% ethanol) are highly effective at disinfecting GBS (Streptococci) [ref: https://www.fishersci.co.uk/webfiles/uk/web-docs/SLSGD05.PDF]. All samples containing GBS will be disinfected with virkon and surfaces cleaned with 70% ethanol as described.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Dear GMSC,

My assessment of GM339

I agree that this GM risk assessment for work with Group B Streptococcus should be regarded as a Class 2 activity. All the precautions to mitigate risk, worker exposure and waste disposal are adequate for this organism. Convincing arguments to support the view that any manipulation proposed will not enhance virulence or create more risk to the environment than the wild type. The use of Virkon at 1% for 1 hour is appropriate and a well-established disinfecting conditions for this bacteria. Disposal of waste by autoclaving using the discard cycle should stipulate 134oC for 30 minutes and not as in the document as 132oC. Please can this be changed as in amended RA – attached. Use of PPE and MSC Class II is appropriate and risk to workers has been considered. Pregnant workers are at no greater risk and has been considered in the risk assessment. Immunocompromised workers will not handle live organisms.

A well-considered risk assessment for Class 2 activity with all appropriate measures and risks clearly described.

Dear All,

From the information provided I agree that this should be regarded as a Class 2 activity. My only question would be whether more information should be provided on the actual changes that will be made, other than the fact that they are likely to be loss of function.

Best wishes,

Thank you for contributing to the discussion. Regarding information about the actual changes that will be made, I would argue that the researchers are trying to make the assessment as broad as possible. This was a suggestion that a researcher used to make. They do mention that “For investigation of virulence genes in GBS and the function and significance of mutations of the DNA and at the protein level, we will introduce mutations (knock in and knock out) that inhibit bacterial replication and/or protein synthesis/function”. I agree with the strategy of not mentioning any particular gene or pathway but I could ask what conditions will they use to select these mutations. As requesting “Please state that you will only select mutations that will hinder bacterial fitness” Is this a suitable compromise?

Best,

I agree with approving the risk assessment as a Class 2 activity and have no further comments beyond those mentioned.

Best wishes,

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</thead>
</table>

02/03/2022

Page 2805 of 15326
Project Ref 95/95.3

Date Ackn'd 12/04/1995

Date Project Ceased 18/12/2013

CU2 Project Title CLONING OF MYCOBACTERIUM TUBERCULOSIS GENES WHICH ARE UPREG. BY INVASION OF CAPILLARY ENDOTHELIAL CELLS

Class 3

Non-GMM yes

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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**GM Centre Number: 96**

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**Emergency Plan Required?**

**Name**

**UNIVERSITY OF LONDON**

**Department**

**BIOLOGY**

**Campus Estate or Research Centre**

**Building**

**Road Name**

**PRINCE CONSORT ROAD**

**Town**

**LONDON**

**County**

**GREATER LONDON**

**Postcode**

**SW7 2BB**

**Country**

**ENGLAND**

**Tel Number**

0207 589 5111

**Fax Number**

0207 594 5439

**E-mail**

**HSE Division**

**LONDON**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
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<td>Level 3 (GMMs)</td>
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02/03/2022
Level 4 (GMMs)

Non-microbial

Other (please specify)  

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<tr>
<td>Mycology</td>
<td>Transgenic</td>
<td>Transgenic</td>
<td>Other (please specify below)</td>
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</tbody>
</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

Project Ref 96/01.1

Date Ackn'd 12/02/2001  
CU2 Project Title DEVELOPMENT AND USE OF ADENOVIRAL VECTORS  
Date Project Ceased 02/03/2022  
Class  
Culture VolClass 2  
Culture Volume Class 3-4

Consent Granted not applicable
Historical Significant Changes
transferred to GM8 on 13/2/2006

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 96/01.2

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Withdrawn N

Historical Significant Changes transferred to GM8 13/02/2006

Historical Date of Additional Info 13/02/2006

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

Laboratory Activities Glass Houses Growth Rooms
L2 L3 L4 L2 L3 L4 L2 L3 L4
Animal Units Large Scale Activities Human Clinical Applications
L2 L3 L4 L2 L3 L4 L2 L3 L4

Project Ref 96/01.4

Date Ackn'd CU2 Project Title
14/09/2001 TO DEFINE THE INTRACELLULAR SIGNALLING EVENTS THAT CONTROL
DIFFERENTIATION, SURVIVAL AND PROLIFERATION OF LYMPHOCYTES.

Date Project Seased
13/02/2006

Withdrawn N

Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**

We aim to define the intracellular signalling events that control differentiation, survival and proliferation of lymphocytes.

**Recipient or parental organism**

We will transfec E.Coli K12 and derivatives with Blusecript containing mouse cDNAs encoding components of lymphocyte signalling pathways. We will introduce these cDNAs into pBabe retroviral vector and transfect into rodent-specific packaging cells, for infection of mouse cells and tissues with these cDNAs in vitro. Although expression of the genes of interest in the retroviral vector may affect mouse lymphocyte development and differentiation in vitro, the retrovirus cannot infect human cells and so would have no effect on human tissues and will not be oncogenic. Nevertheless, as some of the genes of interest have been shown to be oncogenes in the mouse (eg. Smoothened), we will carry out the work under Class II, Containment level II conditions.

**Host/vector system**

- E.Coli K12 type (strains JM101 and derivatives or XL1 and derivatives)
- GP+E retroviral packaging line and derivatives (packaging for infection of rodent cells only: Morgenstern and Land, 1990, Nucl. Acids Res. 18, 3587-3596)
- Bosc retroviral packaging line and derivatives (Packaging for infection of rodent cells only)
- Primary mouse cells and tissue
- Mouse cell lines

**Vectors:**

- Blusescipt and derivatives
- Puc and derivatives
- pBabe and derivatives
- MIGR1 and derivatives

**Origin & function**

- For E.Coli: Commercial (Strategene) or other labs
- For mouse cell lines: other labs
- For primary mouse tissues: mice from CBS, Imperial College
- For retroviral packaging cell lines: other labs
The genetic material will be PCR products from mouse (geneomic or cDNAs, generated by ourselves or from other labs). Vectors will be obtained commercially or from other labs.

The function of these genetic manipulations is to study T cell differentiation in vitro.

Evaluation of foreseeable effects

We hope the genes we introduce into mouse T cell cultures will interfere with or alter T cell differentiation in cell cultures, and thus enable us to study T cell differentiation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be inactivated using Virkon 1% for at least 3 minutes (according to the manufacturer's recommendations). This will give 100% kill. The material will then be autoclaved, reaching 121 degrees C for 15 minutes. The autoclave will be monitored by autoclave printouts of the status of each run and 6 month checks by external contractors to make sure the correct temperature is reached. The waste will then be removed by a College approved contractor for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee made the following statements:

1. “We are concerned about the potential oncogenic properties of the insert cloned in the vectors. Therefore we recommend that the production and handling of large quantities of DNA are carried out under Class II conditions to minimise exposure to high amounts of DNA with oncogenic potential. The Committee also requests that the packaging cell line used for generating the infectious pBabe does not generate viruses with residual infection for human cells. Could references be provided please”.

2. “Please state the required contact time required for Virkon to be effective”.

Project Containment

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**Project Ref** 96/01.5

**Date Ackn’d** 07/11/2001  
**CU2 Project Title** STUDY OF THE MECHANISM OF HOST CELL INVASION BY TOXOPLASMA GONDII USING REVERSE GENETICS TOOLS

**Date Project Ceased** 13/02/2006  
**Class** Class 2  
**CultureVolClass2** < 1 litre  
**Consent Granted** not applicable

**Non-GMM**  
**Project notified under transitional arrangements** N

**Withdrawn** N

**Historical Significant Changes** Transferred from GM96 to GM8 on 13/02/2006

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

The aim project is to study the mechanism of host cell invasion by the protozoan parasite Toxoplasma gondii by analysing the interactions between three classes of invasion molecules that are expressed in T. gondii. These molecules include molecular motors called myosins, adhesins called microneme proteins and aspartyl proteases. Using stable transfection technique, we propose to disrupt the genes coding for these invasion factors. In parallel, we will express deletion mutant and GFP fusions of these proteins to study their biogenesis. The biochemical analysis of the motor proteins will necessitate the purification of these proteins from toxoplasma gondii.

**Recipient or parental organism**

Toxoplasma gondii tachyzoites from RH and Prugniaud strains as well as the attenuated strains ts-4 mutant derived from RH strain. Toxoplasma gondii is a mild pathogen infecting human and animals. In human, this parasite is pathogen only under two circumstances: infection by immunosurpressed patients and pregnant women. Toxoplasma gondii acute infection can be treated with antifolates drugs combined to antibiotics of the class of macrolides or atovaquone. The genetic modifications proposed in our project are not anticipated to increase the virulence but rather to impair the ability of recombinant parasites to invade host cells.

**Host/vector system**

The vectors to be employed in the proposed experiments are all derived from the commercially available bluescript from Stratagene. Most of the constructs are designed to express modified proteins or to generate knockout by homologous recombination in Toxoplasma gondii. These vectors are all described in the literature and the corresponding publications are listed in the risk assessment protocol.
The selectable marker genes are including chloramphenicol acetyl transferase, hypoxanthine-xanthine-guanosine-phosphoribosyltransferase, bleomycin. The reporter genes include beta-galactosidase, green fluorescent protein and luciferase. Other bacterial genes: tetracycline repressor. Toxoplasma gondii genes and their homologues in other apicomplexa: Myosin heavy chains, myosin light chains, microneme proteins and proteases. The flanking sequences controlling expression of the transgenes are derived from Toxoplasma gondii tubulin, major surface antigens (SAG1, SAG4) genes.

Origin & function

the genetic material originates from Toxoplasma gondii genes or homologues from other Apicomplexa parasites and E.coli. Genes originating from Toxoplasma gondii and apicomplexa:
Adhesins proteins secreted by the micronemes at the time of invasion. MIC1 to MIC9
Molecular motors including myosin light chain and myosin heavy chains and docking proteins: Myosin A to Myosin E, MLC MADP
Aspartyl proteases. AP1 and AP2
Functions: Components of the invasion machinery
Gene originating for other organisms: Beta galactosidase (LacZ), tetracycline repressor (tetR), green fluorescent protein (GFP), chloramphenicol acetyl transferase (CAT)
Functions: Selectable marker to disrupt genes by homologous recombination (CAT), reporter genes (LacZ) and Creation of chimeric fusion to follow the traffic of protein (GFP) and modulation of gene expression (tetR).

Evaluation of foreseeable effects

1. The genes coding for these proteins will be disrupted in Toxoplasma gondii by homologous recombination or by conditional knockout using the inducible tetracycline based gene expression system. We anticipate an alteration of the ability of parasites to infect cells.
2. These genes will be expressed as mutants: site specific point mutations, deletions or GFP fusions of these genes will be used to generate transgenic parasites. No change in virulence is expected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Toxoplasma gondii is a micro-organism.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Propagation of Toxoplasma gondii tachyzoites in tissue culture must take place in a containment level 2. All the proposed experiments will take place in a cell culture equipped laboratory located in containment level 3. The room and equipment fulfills all the safety requirements. The room will not be shared with and no containment level 3 activity will take place there. The manipulation of live parasites will be done in a safety cabinet. Wearing gloves and laboratory coats are requested. No material or equipment can leave to zone before decontamination or autoclaving. Both liquids and solids wastes will be autoclaved in the Containment 3 zone. Autoclaving: 30 mins at 121C at 15p.s.i (1.1kg/sq cm. Monitoring: Autoclave performance is monitored continuously by means of the autoclave printout (including a multipoint thermocouple test) annually by an independent company. Work carried out in the Containment level 3 laboratory is autoclaved within the containment room prior to removal to the main autoclave for a second round of autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
the safety committee agreed that the project involving Toxoplasma gondii should be conducted in a containment level 2 and that the genetic modifications are not anticipated to change the category of the containment. They agreed that the project will be carried out in a fully equipped cell culture room located in the containment level 3 area since it was the only room available. This room will be exclusively used by the members of the group of D. Soldati. Cell culture will be restricted to Toxoplasma gondii and the host cells to propagate the parasites.

The Committee has suggested to use blunt needles for the rare cases the manipulation of seringes will be necessary. The chemical inactivation of solid wastes before autoclaving has been suggested but since Toxoplasma gondii is not an aerosol borned pathogen, this measure will not be necessary.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2</td>
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<td>L3 L4</td>
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**Project Ref 96/01.6**

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<td>THE EFFECT OF GENE KNOCK-OUTS ON GENE EXPRESSION AND STATIONARY PHASE SURVIVAL OF MYCOBACTERIUM BOVIS BCG</td>
<td>13/02/2006</td>
<td>Class 2</td>
<td>≤ 1 litre</td>
<td>not applicable</td>
<td>N</td>
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Withdrawn N Tick if notifying a connected programme of work N

Historical Significant Changes trasferred to GM8 13/02/2006

Historical Date of Additional Info 13/02/2006
**Project Additional Information**

**Purposes of the contained use**

In this project we wish to establish the role of metabolic and regulatory genes in the stationary phase survival of Mycobacterium bovis BCG. We propose to make gene knock-outs of metabolic and regulatory genes (for example transporter genes, two-component systems) and investigate the effect of these mutations on stationary phase survival and gene expression. We propose to use a single integrating vector to compliment the knock-out mutants. We plan to use macrophage infection studies to establish the effect of the knock-out mutations on virulence/infectivity.

**Recipient or parental organism**

Mycobacterium bovis BCG is an ACDP class 2 organism. It is widely used as a vaccination strain.

**Host/vector system**

The vectors that will be used in the project use kanamycin or hygromycin as selectable marker and are non-mobilisable. We will use reporter gene vectors containing XyIE or GFP as reporter genes, single integrating vectors and knockout vectors. All vectors contain an origin of replication for E. coli and mycobacteria, except for the knock-out vector, which only has an origin of replication for E. coli.

**Origin & function**

All vectors have been constructed and validated in our laboratories. To create the knock-out and complimenting vectors we have used genomic DNA from M. bovis BCG and M. tuberculosis. The genomic DNA of M. bovis BCG originated from our laboratories, while the M. tuberculosis genomic DNA was from a collaborating department within Imperial College.

**Evaluation of foreseeable effects**

M. bovis BCG is an attenuated vaccination strain, unlikely to survive outside the laboratory. We intend to knock-out genes that are involved in regulatory and metabolic functions. For the genes we propose to knock-out, homologues are described in other organisms and in these organisms the mutation caused the organism to become less virulent. In our laboratories we have made knock-out mutants of genes with metabolic functions in M. smegmatis (a non-pathogenic Mycobacterium species), and it was found that these mutants are less viable than wild-type strains. We expect that we will see a similar effect in M. bovis BCG. Mycobacteria are environmental organisms, and gene transfer between mycobacteria and other organisms has been described. However our vectors are non-mobilisable and need antibiotic selection to be sustained in the organism, it is therefore unlikely that the genetic material will establish itself into another population outside the laboratory.

M. bovis BCG is a difficult strain to maintain in the laboratory, with very specific culture methods. Mycobacteria are also sensitive to UV light. If inadvertently genetically modified M. bovis BCG is introduced to the environment, it is unlikely that the organism will survive. In the unlikely event that the genetically modified M. bovis BCG causes infection, the use of kanamycin and hygromycin as selectable markers, will not affect the treatment since the organism will stay full sensitive to Isoniazid and Rifampicin.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid GM waste is autoclaved and this should give a 100% kill, as established by Collins, CH (1993), Laboratory Acquired Infections, 3rd edition, pp 146 et seq.. All liquid GM waste is disinfected with a 2% final concentration of Hycolin for at least 16 hours. This should give a 100% kill, as established by Collins, CH (1993), Laboratory Acquired Infections, 3rd edition, pp 146 et seq..
We will monitor this on an ongoing basis by plating out random samples to establish whether any viable cells are present.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

The GM safety committee approved the project as Class 2, containment level 2. However the committee was concerned about the use of kanamycin as a selectable marker, as this may interfere with susceptibility of the GMO to aminoglycosides such as amikacin. We have submitted evidence, supported by experts in the field of mycobacteria, that amikacin and/or kanamycin are rarely used in the treatment of mycobacterial infection; that M. bovis BCG is fully sensitive to Rifampicin and Isoniazid; and the genetic exchange between mycobacteria is very difficult, therefore making the possibility of transferring resistance from M. bovis BCG to M. tuberculosis very remote. The GM safety committee was very satisfied with our response and granted approval of Class 2, containment level 2.

Project Containment

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<td>L4</td>
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</table>

Animal Units

| L2 | L3 | L4 | L2 |

Large Scale Activities

| L2 | L3 | L4 | L2 |

Human Clinical Applications

| L2 | L3 | L4 | L2 |

Project Ref 96/02.2

Date Ackn'd 24/07/2002

CU2 Project Title

REPLICATION OF PLANT VIRUSES AND VIROID

Date Project Ceased 13/02/2006

Class Consents

Class 3

Consent Granted

yes

Withdrawn N

Tick if notifying a connected programme of work Y

02/03/2022 Page 2822 of 15326
### Project Additional Information

#### Purposes of the contained use

Study of the RNA polymerases and transcription factors involved in the replication of chrysanthemum chlorotic mottle viroid, potato spindle viroid, barley yellow dwarf virus and cereal yellow dwarf virus.

#### Recipient or parental organism

- Chrysanthemum chlorotic mottle viroid
- Potato spindle tuber viroid
- Barley yellow dwarf virus
- Cereal yellow dwarf

#### Host/vector system

Escherichia coli K12 derivatives, such as DH5 alpha/ pUC-derived vectors, such as pBluescript, and, for the virus studies, expression vectors, such as the pET and PQE series of vectors.

#### Origin & function

- Chrysanthemum chlorotic mottle viroid (infected chrysanthemum plants in Spain, USA)
- Potato spindle tuber viroid (infected potato plants in Germany, USA)
- Both viroids will be used to study interactions with the cellular RNA polymerases which replicate them.

- Barley yellow dwarf virus (Infected barley and other cereal plants in UK, USA)
- Cereal yellow dwarf virus (infected barley and other cereal plants in UK, USA)
- The RNA of both viruses will be used to study interactions with the cognate virus-encoded RNA polymerases which replicate them.

#### Evaluation of foreseeable effects

Sequences alterations in the viroid or virus RNA which interfere with the ability to bind the cognate RNA polymerase will be likely to reduce the infectivity of the viroid or virus RNA to plants or plant cells.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Application for derogation of some containment level 3 measures to containment level 2 for the work involving chrysanthemum chlorotic mottle viroid and potato spindle tuber viroid

Table 1a Measure 2 Laboratory: sealable for fumigation
There is no risk to human health as viroids do not infect humans. Any accidental spillages within rooms 418 and 426 can be adequately contained by the disinfectant measures described in the standard operating procedure (attached)

Table 1a Measure 5 Negative pressure relative to the pressure of the immediate surroundings
Viroid-infected plants will not be allowed to flower. Hence there is no possibility of viroid dispersal via pollen or seed. No other parts of the plants are airborne. Plant residues will not be allowed to dry out, so that there will be no possibility of viroid transmission in dust arising from dried infected plant residues. Immediately after use, all viroid-infected plant residues will be placed in sealed containers prior to disposal by autoclaving and incineration. Precautions are in place to prevent the generation of aerosols. For details please see the standard operating procedure (attached). There will be no susceptible plants in the vicinity of rooms 418 and 426.

Table 1a Measure 6 Extract and input air from the laboratory should be HEPA filtered
Viroid-infected plants will not be allowed to flower. Hence there is no possibility of viroid dispersal via pollen or seed. No other parts of the plants are airborne. Plant residues will not be allowed to dry out, so that there will be no possibility of viroid transmission in dust arising from dried infected plant residues. Immediately after use, all viroid-infected plant residues will be placed in sealed containers prior to disposal by autoclaving and incineration. Precautions are in place to prevent the generation of aerosols. For details please see the standard operating procedure (attached). The air intake into the Sir Alexander Fleming Building is filtered to exclude insects and dust.

Table 1a Measure 7 Microbiological cabinet/enclosure
Viroids are not infectious to humans and do not encode any proteins. Hence a microbiological safety cabinet will not be required. Furthermore precautions are in place to prevent the generation of aerosols (see attached standard operating procedure). There will be no plants in rooms 418 and 426 other than those which it is desired to infect as part of the research project.

Table 1a Measure 8 Autoclave required in the lab suite
There is no possible risk of contaminating the environment in transporting infectious viroid-containing material from rooms 418 and 426 to the autoclave. Liquid waste will be absorbed on a clay-based or wood (cellulose)-based absorbent and treated as for solid waste. Solid waste will be placed in sealed containers. The sealed containers will be placed in autoclave bags, the tops of which will be folded over and taped. The autoclave bags will be placed in autoclavable buckets on which lids will be placed. The buckets will be transported to the autoclave in contained trolleys. On reaching the autoclave, the buckets will be removed from the trolley, their lids removed and the tape removed from the autoclave bags. However the containers containing the infectious viroid material will remain sealed. The white buckets will then be placed in the autoclave. During the autoclave cycle, the specially designed sealed containers will melt allowing access of the steam to the viroid waste material. After autoclaving the waste will be placed in clinical waste sacks prior to incineration.

Table 1a Measure 14 Efficient control of disease vectors which could disseminate the GMM
Viroids are not transmitted by aphid vectors when the aphids feed on plants infected by only viroids. However potato spindle tuber viroid (PSTVd) has been reported to be cotransmitted with potato leafroll virus (PLRV) by aphids feeding on potato plants infected with both PSTVd and PLRV. Several precautions are in place to prevent plants becoming infected adventitiously by aphids which may be carrying PLRV, although it is extremely unlikely that there are aphids carrying PLRV in the vicinity of Imperial College because there are no potato-growing areas in the vicinity of the College. (a) No aphids will be used in the project and there are no other projects involving aphids being carried out in the Sir Alexander Fleming Building. The air exchange system in the level 6 glasshouses, where the healthy uninfected plants are grown, is protected by apid-proof mesh. In the four years that the glasshouse have been in operation, no aphids have been detected. If any aphids were detected, they would be immediately destroyed by application of an aphicide. (b) Plants will be examined for symptoms of virus infection, prior to viroid inoculation. Any putatively virus-infected plants will be destroyed by autoclaving and incineration. (c) Aphids have never been found in rooms 418 and 426 and indeed are unlikely to enter the Sir Alexander Fleming Building because the air
Intake is filtered and there are no windows opening to the outside of the building. However as an additional precaution, sticky insecticidal tape will be placed in rooms 418 and 426.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
<thead>
<tr>
<th>Solid waste will be autoclaved at 121 degrees C, 15 psi, 30 min, followed by incineration (100% kill)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid waste will be soaked up with a clay, such as bentonite, and then autoclaved at 121 degrees C, 15 psi, 30 min, followed by incineration (100% kill).</td>
</tr>
<tr>
<td>Liquid spillages will be treated with Microsol 3 or Virkon, as recommended by the manufacturer, for 10 min, and then soaked up with paper towels which will then be autoclaved at 121 degrees C, 15 psi, 30 min, followed by incineration (100% kill).</td>
</tr>
<tr>
<td>Full details are described in section (n) of the attached Standard Operating Procedure. A chart print out, giving the temperature and pressure, is provided with every autoclave run. The autoclaves are serviced and the temperature and pressure validated by external contractors every 6 months. Before starting the work, a check that the temperature at the centre of the solid waste reaches the desired value will be carried out by placing a vial of Bacillus stereothermophilus at the centre of a typical solid waste load and carrying out the autoclave procedure. The viability of the B. stereothermophilus will then be tested.</td>
</tr>
</tbody>
</table>

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<table>
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<tr>
<th>Is an emergency plan required according to regulation 20?</th>
<th>Y</th>
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<td>Tick to confirm that you have attached a risk assessment to this form</td>
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<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>N</td>
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</table>

Please enter comments on the GM safety committee on the risk assessment
Class 2, Containment level 2 is recommended for Part 3 (Replication of Viruses Causing Yellow Dwarf Disease of Cereals and Part 4 (Expression of Replication Proteins of Cereal Yellow Dwarf and Barley Yellow Viruses).

Class 3, Containment level 3 is recommended for part 1 (RNA polymerase and transcription factors in hammerhead viroid replication) and Part 2 (replication of viruses causing yellow dwarf disease of cereals), subject to application for derogation of measures 2, 5, 6, 7, 8, and 14 as described in section 11. The following CL3 containment measures from Tables 1a and 1b of schedule 8 of the CU2000 Regulations will be in place to prevent dissemination of the viroid GMMs.

Table 1a Measure 1. Laboratory suite isolation.
Growth room 426 and laboratory 418 will be dedicated to work with viroids only. Containment for movement of potentially between rooms will be as follows. Infected plants will be harvested and transferred in sealed bags placed in buckets with lids from room 426 to laboratory 418 where they will be homogenised in a sealed container and processed for isolation of viroid RNA and RNA polymerase-viroid RNA complexes. All subsequent operations with material containing infectious viroid RNA will be carried out in room 418, except for centrifugation. For centrifugation, suspensions containing infectious viroid RNA will be placed in closed centrifuge tubes or bottles in room 418. These will be placed in the rotor which will be closed with the rotor lid and transferred to the centrifuge area of room 433 in a closed trolley. After centrifugation, the rotor will be transferred to room 418 in a closed trolley. The rotor lid will be removed and the tubes will be removed from the rotor. The rotor will be sterilised by treatment with the disinfectant Microsol3 (1:10 dilution) for 10 min (gloves and eye protection must be worn when handling Microsol), followed by washing and deionised water before being returned to the centrifuge area of room 433. The disinfectant and washings will be collected for disposal by autoclaving and incineration, as described for liquid waste in section (n) of the Standard Operating Procedure (attached). Infected sap, infected plant material, purified viroid RNA and recombinant viroid cDNA clones will be stored in a fridge and freezer in room 418. Glycerol stocks of E.coli containing recombinant DNA clones of CChMVd or PSTVd will be kept in racks placed in containers with lids in a -80 degree C freezer in room 444. For propagation, the clones in the containers will be placed in buckets with lids and transported to room 418.

Access to laboratory 418 and growth room 426 will be limited to the authorised workers (see attached risk assessment). Cleaners will not be allowed in these rooms which will be cleaned by the authorised laboratory personnel. If repair work is required to the services in these rooms, all infectious material will be removed from surfaces which will be disinfected by treatment with Microsol or Virkon before access by repair personnel is allowed. All repair work in these rooms will be supervised by one of the authorised laboratory personnel.

Table 1a Measure 2. Laboratory floor.
The floors of rooms 418 and 426 are easy to clean and can be treated with disinfectants, such as Microsol or Virkon, in case of accidental spillages.

Table 1a Measure 12.
Protective overshoes will be worn for all work in laboratory 418 and growth room 426.

Table 1a Measure 18
Laboratory 418 and growth room 426 will where reasonable practicable have their own equipment. When this is not reasonably practical, such as the centrifuges and the -80 degree C freezer for storage of glycerol stocks of E. coli carrying recombinant plasmids, dedicated systems will be in place to safely transfer potentially infective material as described above (see Table 1a Measure1).
Table 1b Measure 5.
Possible transmission of viroids through seed and pollen will be prevented by not allowing infected plants to flower. To prevent airborne dissemination of infected plant residues, viroid-infected plant waste will not be allowed to dry out and will be disposed of immediately after each experiment as solid waste as described in section (n) of the attached Standard Operating Procedure. The additional measures which are not specific to CL3 will also be employed.

Table 1a Measure 13.
Gloves will be worn at all times for work with viroids in laboratory 418 and growth room 426.

Table 1a Measure 21.
Staff have received training on the risks posed by the activity and the containment measures required to prevent the dissemination of the GMM. Written records of the staff training have been kept. Further training will be given as necessary and record kept of it.

Emergency Plan
Consideration has been given to the establishment of an Emergency plan to deal with any reasonably foreseeable accident. Treatment of accidental spillages up to the maximum culture volume of 1 litre are dealt with in the Standard Operating Procedure.

Fire is the only other foreseeable hazard which might arise. A fire emergency plan is attached as Appendix 1 to Standard Operating Procedure.

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### Project Ref 96/99.1

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Please enter comments on the GM safety committee on the risk assessment

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

Project Ref 96/99.2

Date Ackn'd 13/10/1999

CU2 Project Title
THE DEVELOPMENT OF DNA-MEDIATED TRANSFORMATION SYSTEMS OF ERYsiphe SPP.

Class 2
Non-GMM Consent Granted
not applicable

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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**Name**

**THE PIRBRIGHT INSTITUTE**

**Name 2**

**COMPTON LABORATORY**

**Campus Estate or Research Centre**

**Building**

**Road Name**

**District**

**Town**

**County**

**Postcode**

**Country**

**Tel Number**

01635 578411

**Fax Number**

01635 577237

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- [ ] Level 1 (GMMs)
- [ ] Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)  

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 628/01.1

**Date Ackn’d** 20/09/2006  **CU2 Project Title** PRODUCTION OF REPLICATION DEFECTIVE RECOMBINANT RETROVIRUSES  **Class** Class 2

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Page 2835 of 15326
<table>
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| Historical Significant Changes | TRANSFERRED FROM GM 628 - 22/9/06. |

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref 628/01.2**

**Date Ackn’d**

20/09/2006

**CU2 Project Title**

PRODUCTION OF RECOMBINANT PROTEINS IN BACTERIA

**Class**

Class 2

**Culture Vol Class**

Class 2

**Culture Volume Class**

Not Applicable

**Non-GMM Consent Granted**

Not Applicable

**Project notified under transitional arrangements**

Y

**Historical Significant Changes**

TRANSFERRED FROM GM 628 - 22/9/06

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref** 628/01.3

**CU2 Project Title**

IMMORTALISATION OF MURINE CHONDROCYTES USING SV40 LARGE T-ANTIGEN-ENCODING RETROVIRUS.

**Date Ackn’ed**

20/09/2006

**Date Project Ceased**


**Historical Significant Changes**

TRANSFERRED FROM GM 628 - 22/9/06

**Project Additional Information**

**Purposes of the contained use**

A murine chondrocyte cell line would provide a source of joint specific antigens including the model antigen Flu nucleoprotein (NP) that is expressed in the chondrocytes of CIINP14 mice. As reactive arthritis is likely to result from activation of MHC class 1 and class II-restricted T-cell responses (as well as possibly antibody responses), an immunogen that includes multiple relevant antigens may enhance the development of joint-specific disease. Immunisation with NP +ve chondrocytes would provide a known antigen, the response to which could be followed with tetramers etc., as well as uncharacterised antigens that may be more potent targets in the joint.

**Recipient or parental organism**

Murine chondrocytes expressing either wild-type or a temperature sensitive mutant of SV40 Large T-antigen. As chondrocytes will not produce recombinant virus or survive outside tissue culture the foreseeable effect on human health/safety or the environment is negligible.

**Host/vector system**

2 cells to be used in this study are transfected with pZipNeo SV(X) vectors. These contain the sequence required for viral packaging deleted from PMOV- but replace the viral gag, pol and env genes with an inserted transgene (see section C for description of cloning vector). These transfected cells are capable of producing helper-free,
recombinant retrovirus (Cepko et al., 1984. Cell, 37:1053-1062). In this study, pZipNeo SV(X)1 vectors incorporate either Large T-antigen or a temperature-sensitive version of this gene which is derived from Simian Virus (SV)40.

Origin & function

SV40 Large T-antigen (from the DNA tumour virus simian virus 40), tsTAg (temperature sensitive mutant of the SV40 Large T-antigen gene). The SV40 large T-antigen is found in early region of the virus together with the small T-antigen which together are sufficient for transformation of cell lines. Studies have shown that the large T-antigen alone is sufficient for establishment of primary cells but that these cells do not display a transformed phenotype hence the intention in this study to transform chondrocytes.

Evaluation of foreseeable effects

As mentioned immortalised chondrocytes and 2 producer cells will not survive outside tissue culture. They are unlikely to encounter murine hosts other than in experimental conditions in the case of chondrocytes. Recombinant retrovirus will not infect human cells and is helper-free therefore can not replicate in murine hosts. However infection of mice could theoretically result in transformation of cells although this is unlikely as SV40 Large T-antigen transduced cells are non-tumourigenic in murine hosts. The cells will be handled as category 2 agents for all in vitro experiments, limiting the potential of exposure to the environment to a negligible level.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solutions that have been in contact with retrovirus, 2 cells or transformed chondrocytes will be treated with virkon disinfectant. All exposed materials will be autoclaved before disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee wish to bring to the proposer's attention paragraph 20 in AnnexIII "Guidance on commonly used viral vectors" to demonstrate the absence of PCRs in vector stocks from the HSE's Compendium of Guidance. Also proposer's attention is requested to observe precautions for handling oncogenic DNA (mandatory wearing of gloves, avoidance of sharps).

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02/03/2022 Page 2840 of 15326
In order to aid our studies of the human disease reactive arthritis which may be induced as a result of salmonella infection, we need to study the interaction of salmonella infected cells with antigen specific T cells. Model antigens, to which we have specific T cells for in the laboratory, will be expressed in salmonella. T cell responses to salmonella infected cells can then be assessed.

Recipient or parental organism

Strains of Salmonella typhimurium, some of which are attenuated.

Host/vector system

Non-mobilisable or mobilisation defective plasmid vector systems, using prokaryotic promoter system for expression of recombinant antigens.

Origin & function
In vitro and in vivo experimental use at containment level 2, for induction of immune responses and models of human disease.

**Evaluation of foreseeable effects**

Transformation of Salmonella with recombinant DNA plasmid vectors is not expected to increase virulence or infectivity of Salmonella. The organisms will be handled as category 2 organisms for all in vitro and in vivo experiments, limiting the potential of exposure to the environment to a negligible level.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solutions that have been in contact with Salmonella will be treated with bactericidal disinfectant. All infected materials will be autoclaved before disposal.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The committee has discussed the possibility of induction of arthritis - however this should be no worse than with wild type Salmonella in non-transgenic mice and humans.

Work has been approved for use at containment level 2.

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**Project Ref** 628/03.1
Recombinant adenovirus vectors encoding the S and N genes of the SARS virus under the control of the CMV promoter will be used to study the effect of these proteins in vivo. The recombinant adenovirus vectors will be administered to mice and tissues from treated mice will then be removed to examine the host immune response. In some experiments mice treated with one of these vectors will then be infected with virus or bacteria, for example influenza virus or salmonella. The aim is to be able to examine the effect of the S and N genes from SARS on the host immune response.

The recombinant adenoviruses will be obtained from another investigator Dr Tripp, in the Centres for Disease Control and Prevention in Atlanta, GA. The recombinants were made in his laboratory using the commercial AdEasy vector system from Invitrogen.

The SARS proteins S (spike) and N (nucleocapsid) are from the Urbani strain of SARS isolated by the CDC in Hong Kong. This strain was sequenced by the CDC and the S and N genes were then inserted into the adenovirus vectors in the CDC in Atlanta.

It is presently unknown whether the S or N proteins have any transforming potential or any harmful sequences. None have been reported.

Adenoviruses are associated with a number of mild disorders. The pathology results primarily from inflammation and loss of infected epithelial cells. Serotype 5 which will be used here commonly causes upper respiratory infections in the elderly and in young children. Adenoviruses are Biosafety Level II pathogens.
The Adenovirus vectors do not express any harmful agents. The E3 region which encodes proteins involved in modulating the immune response of infected cells has been deleted. The recombinant adenoviruses are replication deficient due to a large deletion in the E1 gene.

**Origin & function**

The recombinant adenoviruses will be obtained from another investigator Dr Tripp, in the Centres for Disease Control and Prevention in Atlanta, GA. The recombinants were made in his laboratory using the commercial AdEasy vector system from Invitrogen.

The SARS proteins S and N are from the Urbani strain of SARS isolated by the CDC in Hong Kong. This strain was sequenced by the CDC and the S and N genes were then inserted into the adenovirus vectors in the CDC in Atlanta.

These constructs will be used to examine the effects of the S and N proteins of SARS on the host immune response. The vectors will be administered to mice and at certain time-points thereafter the mice will be sacrificed and tissues will be extracted.

**Evaluation of foreseeable effects**

The possibility that replication competence could be restored by rescue of the E1 deletion from the helper cell line or from wild-type adenovirus cannot be excluded. However even if this were the case, it is unlikely that the virus would be more pathogenic than the wild type adenovirus.

The inserted genes would not be expected to render the recombinant viruses able to replicate or to affect the tropism of the virus. Variations of S protein among strains of coronaviruses are responsible for host range and tissue tropism. However, as adenoviruses are not enveloped it is unlikely that the S protein of SARS will affect the tropism of the recombinant adenoviruses. It is unknown how the expression of the N and S genes of SARS will affect the host cells, as no work has been carried out examining this. So far there are no reports that either S or N protein is associated with toxicity or oncogenesis. Also neither is known to be closely homologous to human proteins against which autoimmune responses would be likely to be induced.

The possibility that the inserted sequences may be transferred to other related organisms is remote. The only potential risk may occur if the S or N genes of SARS were transferred to another corona virus. In that case potential problems may occur but the resulting virulence or pathogenicity if such an event occurs is not known.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the animal, culture and tissues culture waste will be disposed of by incineration according to the normal rules for Biosafety level II biological waste disposal. Liquid waste will be added to Virkon (final concentration 1%) and left for 24 hours. No microorganisms will be viable afterwards. Solid waste will be put in a metal box, sealed with autoclave tape, autoclaved at 134 degrees C for 30 minutes, before being sent to an on-site clinical waste incinerator. The degree of kill is 100%.

Is an emergency plan required according to regulation 20? ☒

If yes, tick to confirm that it is attached to this form ☒

Tick to confirm that you have attached a risk assessment to this form ☒
Please enter comments on the GM safety committee on the risk assessment

The local GMSC agree with this risk assessment Ref 2003/011

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### Project Ref 628/03.2

**Date Ackn’d**: 20/09/2006

**CU2 Project Title**: IN VITRO ANALYSIS OF THE INTERACTION OF HEPATITIS C VIRUS PSEUDOPARTICLES (HCV PP) WITH CELL LINES AND HUMAN PERIPHERAL BLOOD MONOCULAR CELLS (PBMC) SUBSETS.

**Date Project Ceased**: 10/08/2011

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**: TRANSFERRED FROM GM 628 - 22/9/06, Transferred to GM553 10/08/2011

**Historical Date of Additional Info**: 25/07/2007

### Project Additional Information

**Purposes of the contained use**: In order to aid our previous studies of the interaction of the hapatitis C (HCV) E2 glycoprotein with different PBMC subsets and the possible immunomodulatory consequences of this interaction, we would like to carry out studies the HCV pp, which express heterodimers of E1 and E2 on their surface. The proposed work would...
include in vitro analysis of interaction of HCVpp with cell lines and PBMC subsets and investigation of whether HCVpp are able to modulate the activation/functions of different immune system cell types (derived from human peripheral blood), using a variety of in vitro immunological assays.

**Recipient or parental organism**

Murine leukemia virus (MLV), which is a murine retrovirus classified as a category 1 pathogen.

**Host/vector system**

The vector packaged into the pseudotyped MLV particles encodes the marker protein green fluorescent protein (GFP). This is not thought to have any harmful properties. The particles will be pseudotyped with the HCV E1 and E2 proteins. These are not thought to be toxic or oncogenic, but there is some evidence to suggest that the E2 protein may be able to modulate the activation/functions of human lymphocyte subsets. However it is very unlikely that accidental exposure to a small quantity of HCV pp (which are not able to replicate) would have any harmful immunomodulatory effects.

**Origin & function**

The HCVpp will be provided by our collaborators, the Institute Pasteur in Lille, France; they will not be produced here. The method by which the HCVpp are generated is described in Bartosch et al, HJournal of Experimental Medicine, 197:633-642 (2003).

**Evaluation of foreseeable effects**

Pseudotyping of the MLV-based particles with the HCV E1 and E2 proteins results in the particles having a tropism different to that of ecotropic MLV. HCVpp have been shown to bind to and enter human cells expressing what are thought to be HCV receptors (CD81 and other proteins) [Bartosch et al, Journal of Experimental Medicine, 197:633-642, 2003].

This alteration in tropism increases the potential for disease to be induced in humans, although the risk of this still remains very low. Although able to infect human cells, the pseudoparticles would not replicate therein, and expression of GFP within the transduced cells would be unlikely to have deleterious effects. However, it is possible that integration of the MLV vector could lead to modulation of host cell gene expression, and potentially to transformation of the cell.

It is very unlikely that sequences from the GFP-expressing MLV vector within the HCVpp would be transferred to another micro-organism during the course of the planned in vitro experimental work; and even should this occur, it is unlikely to be hazardous, as this vector only encodes GFP.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All equipment and waste that has been in contact with HCVpp will be disinfected using procedures approved for destroying the infectivity of lipid-enveloped viruses.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

02/03/2022
The committee recommended that each batch of HCVpp should be tested for infectivity on a murine cell line, and with this provisionally approved the work to be done at containment level 2.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Conf</td>
<td>L3 L2 L3</td>
<td>L4 L2 L3</td>
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<tr>
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<td>L3 L2 L3</td>
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<tr>
<td>Human Clinical Applications</td>
<td>L4 L2 L3</td>
<td>L4 L2 L3</td>
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**Project Ref 628/04.1**

- **Date Ackn'd**: 20/09/2006
- **CU2 Project Title**: USE OF A SERIES OF DERIVATIVE INFLUENZA VIRUSES TO INVESTIGATE THE EFFECTS ON VIRULENCE IN MICE.
- **Class**: Class 2
- **CultureVol**: < 1 Litre
- **Consent Granted**: Not Applicable
- **Non-GMM**:  
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: TRANSFERRED FROM GM 628 - 22/9/06

**Project Additional Information**

Purposes of the contained use

A recombinant influenza virus of subtype A/Victoria/3/75 (H3N2) has been generated by reverse genetics at the University of Reading. In addition, a series of derivative
viruses based on A/Victoria/3/75 have been generated in which the open reading frame of the protein PB1-F2 has been deleted, truncated or extended. PB1-F2 is believed to induce apoptosis in immune cells (Chen et al., 2001). We aim to carry out apoptosis studies and mouse model studies to determine whether alterations in PB1-affect virus virulence.

A recombinant influenza virus of subtype B/Beijing/87 has also been generated at the University of Reading, as well as an isogenic virus in which the NB protein has been deleted. The NB protein is believed to function as an ion channel and, although dispensable in vitro, is believed to promote efficient viral growth in mice (Hatta and Kawaoka, 2003). We would like to confirm and extend these observations.

Influenza viruses infect humans and avians and cause a respiratory infection. Transmission is by respiratory infection via aerosols. The recombinant influenza viruses are unlikely to cause harm greater than their parental strains, which are themselves laboratory passaged and thus attenuated versions of naturally occurring influenza viruses, classified as class 2 pathogens. Both A/Victoria/3/75 and B/Beijing/87 have been used extensively in laboratories worldwide with no history of infection of workers or adverse effects as detailed in the following publications and citations therein:

In the event that the A/Victoria/3/75 virus does not produce obvious symptoms in mice, a virus with A/Panama/99 antigens and the same internal genetic backbone will be used. A/Panama/99 surface antigens were used to generate large quantities of vaccines throughout the world in recent years and no adverse affect are associated.

Recipient or parental organism

Influenza viruses infect humans and avians and cause a respiratory infection. Transmission is by respiratory infection via aerosols. The recombinant influenza viruses are unlikely to cause harm greater than their parental strains, which are themselves laboratory passaged and thus attenuated versions of naturally occurring influenza viruses, classified as class 2 pathogens. Both A/Victoria/3/75 and B/Beijing/87 have been used extensively in laboratories worldwide with no history of infection of workers or adverse effects as detailed in the following publications and citations therein:

In the event that the A/Victoria/3/75 virus does not produce obvious symptoms in mice, a virus with A/Panama/99 antigens and the same internal genetic backbone will be used. A/Panama/99 surface antigens were used to generate large quantities of vaccines throughout the world in recent years and no adverse affect are associated.

Host/vector system

Not applicable.

Origin & function

A recombinant influenza virus of subtype A/Victoria/3/75 (H3N2) has been generated by reverse genetics at the University of Reading. In addition, a series of derivative viruses based on A/Victoria/3/75 have been generated in which the open reading frame of the protein PB1-F2 has been deleted, truncated or extended. PB1-F2 is believed to induce apoptosis in immune cells (Chen et al., 2001). We aim to carry out apoptosis studies and mouse model studies to determine whether alterations in PB1-affect virus virulence.

A recombinant influenza virus of subtype B/Beijing/87 has also been generated at the University of Reading, as well as an isogenic virus in which the NB protein has been deleted. The NB protein is believed to function as an ion channel and, although dispensable in vitro, is believed to promote efficient viral growth in mice (Hatta and Kawaoka, 2003). We would like to confirm and extend these observations.

Evaluation of foreseeable effects

All mutations in influenza viruses to be used in this study will be attenuating for replication and therefore the hazards will be reduced. This includes the virus strain in which the PB1-F2 gene will be extended by 3 amino acids in order to retain the length of the wild-type gene. There is no expected alteration in host range since neither of these genes is associated with that trait. It is possible that the types of cells affected following infection with the PB1-F2 deletant will be reduced rather than extended.

Since influenza virus has a segmented genome, there is a possibility that reassortant viruses containing genes from the mutants might be generated within a laboratory worker who was coinfected with a wild type strain. We consider this unlikely, but advise all workers to refrain from experiments if influenza infection is suspected.

If reassortment did occur, the PB1-F2 deletion introduced into A/Victoria/75 is not associated with virulence and is likely to be an attenuating mutation. The virus harbouring deletion of NB from B/Beijing/87 is reported to be highly attenuated and cannot replicate efficiently in vivo (Hatta and Kawaoka). Therefore regardless of the genetic background of the virus harbouring these changes, we expect them to be attenuating.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the animal, culture and tissues culture waste will be disposed of by incineration according to the normal rules for Biosafety Level II biological waste disposal. Liquid waste will be added to Virkon (final concentration 1%), and left for 24 hours. No microorganisms will be viable afterwards. Solid waste will be put in a metal box, sealed with autoclave tape, autoclaved at 134 degrees C for 30 minutes, before being sent to an on-site clinical waste incinerator. The degree of kill is 100%.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The local GMSC are happy with this risk assessment (Ref 2003/018) provided the requirements in the associated risk assessment for influenza A and B viruses are adhered to.

Project Containment

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Project Ref 628/04.2

Date Ackn'd 20/09/2006

CU2 Project Title USE OF NOVEL REVERSE GENETICS-GENERATED INFLUENZA VIRUS (RG-X31-SWINE PB1) TO INVESTIGATE THE ROLE OF VIRUS-INDUCED APOPTOSIS

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4 Not Applicable

Non-GMM Consent Granted Not Applicable

Date Project Ceased 02/03/2022
Influenza viruses induce apoptosis in cultured cells and in vivo. We have recently shown that influenza virus detrimentally affects early lymphoid progenitor cells by inducing apoptosis mediated by TNFalpha/LTalpha. An alternative reading frame of the PB1 gene called PB1-F2 of the influenza virus has been implicated in inducing apoptosis in vitro. We want to determine whether PB1-F2 is responsible for the apoptosis that occurs in the bone marrow (or other tissues) following infection of mice with influenza virus. We therefore wish to use a novel influenza virus (RG-X31-swine PB1) that has been generated by reverse genetics (RG) to examine these questions. The PB1-F2 open reading frame (ORF) is present in nearly all human influenza A strains but is absent from swine influenza isolates. Therefore by replacing the PB1 of the wild-type x 31 (A/H3N2) virus which contains the PB1-F2 ORF with the PB1 from a swine influenza virus (A/swine/NorthCarolina/44173/00) which does not, we will be able to address these questions. Mice will be infected with the wild type/recombinant virus and sacrificed at certain time-points to assess the degree of cell death in various organs and any other differences in pathogenicity caused by the recombinant virus.

The RG influenza virus RG-x31-swine PB1 will be obtained from St Jude Children's Research Hospital, Memphis TN, USA. The viruses are being made in the laboratory for our use.

Influenza viruses infect humans and avians and cause a respiratory infection. Transmission is by respiratory infection via aerosols. The strain of influenza virus is a reassortant which has the surface proteins of A/Aichi/68 and the internal genes of A/PR/8/34. It is a laboratory strain which is commonly used in immunological studies and is BioSafety Level 2 pathogen. The recombinant influenza virus is unlikely to cause harm greater than the parental strain, which is itself laboratory passaged and thus an attenuated version of a naturally occurring influenza virus.

Recipient or parental organism

Influenza viruses infect humans and avians and cause a respiratory infection. Transmission is by respiratory infection via aerosols. The strain of influenza virus is a reassortant which has the surface proteins of A/Aichi/68 and the internal genes of A/PR/8/34. It is a laboratory strain which is commonly used in immunological studies and is BioSafety Level 2 pathogen. The recombinant influenza virus is unlikely to cause harm greater than the parental strain, which is itself laboratory passaged and thus an attenuated version of a naturally occurring influenza virus.

Origin & function

We have recently shown that influenza virus detrimentally affects early lymphoid progenitor cells by inducing apoptosis mediated by TNFalpha/LTalpha. An alternative reading frame of the PB1 gene called PB1-F2 of the influenza virus has been implicated in inducing apoptosis in vitro. We want to determine whether PB1-F2 is responsible for the apoptosis that occurs in the bone marrow (or other tissues) following infection of mice with influenza virus. We therefore wish to use a novel influenza virus (RG-X31-swine PB1) that has been generated by reverse genetics (RG) to examine these questions. The PB1-F2 open reading frame (ORF) is present in nearly all human influenza A strains but is absent from swine influenza isolates. Therefore by replacing the PB1 of the wild-type x 31 (A/H3N2) virus which contains the PB1-F2 ORF with the PB1 from a swine influenza virus (A/swine/NorthCarolina/44173/00) which does not, we will be able to address the degree of cell death in various organs and any other differences in pathogenicity caused by the recombinant virus.
Evaluation of foreseeable effects

No hazards should arise from the alteration of wild-type x 31 to RG-x31-swine PB1. Neither virus growth nor tropism should be affected. The surface proteins HA and NA have not been altered. Reverse genetics enables a recombinant virus to be produced in a much safer way than previously because one has complete control of the genes being transfected to produce the recombinant virus. Therefore there is no possibility that other influenza virus genes can be introduced. It is expected that the inclusion of the swine PB1 gene would either not change the pathogenicity/virulence of x31 or make it less so.

Little is known about the new PB1-F2 protein as it has only recently been identified. It is not essential for viral replication in vitro. The evidence to date suggests its involved in modulating the host immune response to influenza A by caused death of immune cells. A synthetic version of PB1-F2 induces apoptosis in vitro and targeted mutations which interfere with expression of PB1-F2 induce less apoptosis than those that express PB1-F2.

There is no evidence that the PB1-F2 gene causes toxicity or has oncogenic properties.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the animal, culture and tissues culture waste will be disposed of by incineration according to the normal rules for Biosafety Level II biological waste disposal. Liquid waste will be added to Virkon (final concentration 1%), and left for 24 hours. No microorganisms will be viable afterwards. Solid waste will be put in a metal box, sealed with autoclave tape, autoclaved at 134 degrees C for 30 minutes, before being sent to an on-site clinical waste incinerator. The degree of kill is 100%.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form N

Please enter comments on the GM safety committee on the risk assessment

The local GMSC are happy with this risk assessment (Ref 2003/017) provided the requirements in the associated risk assessment for influenza A and B viruses are adhered to.

Project Containment

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</table>
Influenza viruses induce apoptosis in cultured cells and in vivo. We have recently shown that influenza virus detrimentally affects early lymphoid progenitor cells by inducing apoptosis mediated by TNFalpha/LTalpha. The NS1 gene of influenza virus has been implicated in inducing apoptosis in vitro. We want to determine whether the NS1 gene is responsible for the apoptosis that occurs in the bone marrow (or other tissues) following infection of mice with influenza virus. We therefore wish to use a novel influenza virus that has been generated by reverse genetics (RG) to examine these questions. The NS1 gene in wild-type x31 will be replaced by the NS1 from the H5N1/97 virus, in order to assess whether a greater degree of cell death in bone marrow, thymus and other organs occurs as a result. Mice will be infected with the wild type/recombinant virus and sacrificed at certain time-points to assess the degree of cell death in various organs and any other differences in pathogenicity caused by the viruses.

The strain of influenza virus that will be the recipient of the foreign NS1 gene is x31 (H3N2). This strain of influenza virus is a reassortant which has the surface proteins of A/Aichi/68 and the internal genes of A/PR/8/34. It is a laboratory strain which is commonly used in immunological studies and is a BioSafety Level 2 pathogen. Research to date suggests NS1 is a virulence factor which plays a major role in inhibiting the IFN-mediated antiviral responses of the host. NS1 is a non-structural protein of influenza virus. It is an RNA binding protein which has been implicated in several regulatory functions during the influenza virus replication cycle:
1. It inhibits host mRNA polyadenylation. 2. It inhibits the nuclear export of mRNAs by binding to their poly (A) tail. 3. It inhibits pre-mRNA splicing. 4. It prevents IFN-mediated antiviral responses by binding to ds RNA. 5. It has been suggested to play a role in regulation of viral RNAPolymerase activity. 6. It is able to stimulate the
Translation of specific viral mRNAs.

There is no evidence that the NS1 gene causes toxicity or has oncogenic properties. Recombinant virus will be produced that replaces the NS gene or x31 with the NS gene of H5N1/97.

Background: The H5N1/97 viruses transmitted to humans in 1997 and were highly virulent. Reactive hemophagocytic syndrome with elevated concentrations of proinflammatory cytokines were reported. Lethal H5NI viruses (unlike other human, avian and swine viruses) are resistant to the antiviral effects of IFNs and TNFalpha. This resistance is associated with NS1 and is thought to be the mechanism of the high virulence of H5NI viruses in humans.

RG enables a recombinant influenza virus to be produced in a much safer way than previously (reassortment) because one has complete control of the genes being transfected to produce the recombinant virus. Therefore there is no possibility that the HA or other genes from the H5N1 can be included in the recombinant virus. The tropism or host range of RG-x31-156NS1 should not therefore be different from wild type x31. It would be expected that the inclusion of the H5N1, NS1 gene into x31 would make the resulting RG-x31-156NS1 virus more pathogenic (perhaps greater loss of body weight, more prolonged virus shedding) as a result of increased virus replication due to the resistance of the NS1 to aniviral cytokines.

Host/vector system

Not applicable.

Origin & function

The RG influenza virus will be obtained from Dr Richard Webby, St Jude Children's Research Hospital, Memphis TN, USA. The viruses are being made in his laboratory for our use.

Influenza viruses induce apoptosis in cultured cells and in vivo. We have recently shown that influenza virus detrimentally affects early lymphoid progenitor cells by inducing apoptosis mediated by TNFalpha/LTalpha. The NS1 gene of influenza virus has been implicated in inducing apoptosis in vitro. We want to determine whether the NS1 gene is responsible for the apoptosis that occurs in the bone marrow (or other tissues) following infection of mice with influenza virus. We therefore wish to use a novel influenza virus that has been generated by reverse genetics (RG) to examine these questions. The NS1 gene in wild-type x31 will be replaced by the NS1 from the H5N1/97 virus, in order to assess whether a greater degree of cell death in bone marrow, thymus and other organs occurs as a result. Mice will be infected with the wild type/recombinant virus and sacrificed at certain time-points to assess the degree of cell death in various organs and any other differences in pathogenicity caused by the viruses.

Evaluation of foreseeable effects

The possibility that the inserted sequences may be transferred to other influenza viruses is remote. The only potential risk may occur if the RG-x31-156NS1 virus was grown in eggs or tissue culture at the same time as other influenza viruses so that reassortment occurs. The possibility of this is remote.

In the event of exposure the virus may cause infection in humans. One may expect the infection to be more virulent than wild type x31, due to the expected increase in resistance to anti viral cytokines. In event that any person is exposed to the virus a prearranged protocol will be followed (see attached protocol called The Edward Jenner Institute for Vaccine Research: Protocol for staff working with influenza A viruses shown to have a higher risk of transmission to humans).

All work with the recombinant influenza RG-x31-156S will be carried out in a BioSafety Level 3 facility. (An almost identical virus has recently been made in Dr Webby’s department in St.Jude CRH but on the backbone of A/PR8/34 which is more virulent than x31. The work is carried out in BioSafety Level 3 facilities, Nat.Med. 8:950-954, Sept 2002). Immunosuppressed individuals or those with chronic respiratory problems will not work with these recombinants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Culture volumes approximately 100ml

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid animal, culture and tissues culture waste will be disposed of by autoclaving followed by incineration according to the normal rules for Biosafety Level III biological
waste disposal. Liquid waste will be added to Virkon Final concentration 1% for 24 hours) or chlorine compounds (0.2-3 for 10-30 minutes). No micro-organisms will be viable afterwards. Solid waste will be put in a metal box, sealed with autoclave tape, autoclaved at 134 degrees for 30 minutes, before being sent to an on-site clinical waste incinerator. The degree of kill is 100%

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
N

Please enter comments on the GM safety committee on the risk assessment

The local GMSC agree with this risk assessment Ref 2003/016/

Project Containment

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<th>Glass Houses</th>
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Animal Units

<table>
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<th>Animal Units</th>
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<td>L2 L3 L4</td>
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Project Ref 628/96.1

Date Ackn’d 20/09/2006

CU2 Project Title DELIVERY OF IMMUNOGENS INTO DENDRITIC CELLS USING RETROVIRUS VECTOR

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes TRANSFERRED FROM GM 628 - 22/9/06.
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Animal Units**

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**Large Scale Activities**

| L2 L3 L4 L2 |

**Human Clinical Applications**

| L2 L3 L4 |

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**Project Ref** 628/97.1

- **Class**: Class 2
- **CultureVolClass2**: Class 2
- **CultureVolumeClass3-4**: Not Applicable

**Non-GMM Consent Granted**: Not Applicable

**Project notified under transitional arrangements**: Y

**Historical Significant Changes**

TRANSFERRRED FROM GM 628 - 22/9/06.

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**Date Ackn’d**: 20/09/2006

**CU2 Project Title**: PRODUCTION AND USE OF RECOMBINANT VACCINIA VIRUSES

**Date Project Ceased**: 

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref** 628/99.1

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**Historical Significant Changes**

TRANSFERRED FROM GM 628 - 22/9/06.

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 97/00.6

Date Ackn'd: 11/08/2000

CU2 Project Title: CHARACTERISATIONS OF HUMAN & BOVINE RESPIRATORY SINCYTIAL VIRUS

Class: Class 2

CultureVolClass2

CultureVolumeClass3-4

02/03/2022
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### Project Additional Information

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 97/01.1

Date Ackn’d 18/01/2001
CU2 Project Title CPN 7E IMMUNOLOGY - CYTOKINES

Date Project Ceased 28/07/2015

Class 2
CultureVolClass2
CultureVolumeClass3-4

Non-GMM Consent Granted
Consent Granted
Project notified under transitional arrangements Y

Historical Significant Changes
Transferred to GM53 28/07/2015

Withdrawn N
Tick if notifying a connected programme of work N

Historical Date of Additional Info
Significant Change ID
Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
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**Project Ref:** 97/01.2

**Date Ackn'd:** 18/01/2001

**CU2 Project Title:** CPW7B IMMUNOLOGY - TRANSFECTED CELL LINES

**Class:** 2

**CultureVolClass2:**

**CultureVolumeClass3-4:**

**Non-GMM Consent Granted:** not applicable

**Project notified under transitional arrangements:** N

**Withdrawn:** N

**Tick if notifying a connected programme of work:** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Date Project Ceased
28/07/2015

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements Y

Historical Significant Changes
Transferred to GM97 28/07/2015

Tick if notifying a connected programme of work N

Withdrawn N

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022  
Page 2867 of 15326
Project Ref 97/01.5

Date Ackn'd 18/01/2001

CU2 Project Title CPW6.5 RNA VIRUSES - PICORNAVIRUS

Class 2

Consent Granted not applicable

Non-GMM

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 97/01.6

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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- **Animal Units**
  - L2 L3 L4 L2 L3 L4

- **Large Scale Activities**
  - L2 L3 L4 L2 L3 L4

- **Human Clinical Applications**
  - L2 L3 L4

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### Project Ref 97/01.7

- **Date Ackn’d**: 18/01/2001
- **CU2 Project Title**: CPW4.4 LARGE DNA VIRUSES - BACULOVIRUSES
- **Class**: Class 2
- **Consent Granted**: not applicable
- **Non-GMM**: Project notified under transitional arrangements

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Information

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#### Additional Information

- **Date Project Ceased**: 06/01/2016
- **Historical Significant Changes**: Transferred to GM53 on 06/01/2016

#### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 97/01.9

Date Ackn'd | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4 |
-------------|-------------------|-------|------------------|-----------------------|
20/04/2001   | TO INVESTIGATE THE INTERACTION OF THE VACCINE STRAIN | Class 2 | < 1 litre | |

02/03/2022  Page 2874 of 15326
**Project Additional Information**

**Purposes of the contained use**

An investigation of the interaction in vitro of BCG with Antigen Presenting Cells (APC). Mycobacterium bovis BCG-gfp will be used to investigate uptake of the organism by individual APC and track the bacterium and determine the effect on the function of the eucaryotic cells. HSP genes will be rendered inactive in order that the function of the molecules can be determined in relation to survival of bacteria in the eucaryotic cell. How removal affects how the bacterium affects the biological properties of the antigen presenting cell will also be assessed.

**Recipient or parental organism**

Mycobacterium bovis BCG vaccine strain.

**Host/vector system**

Plasmid pSMT1 carrying the gfp gene of jellyfish, cloned under the control of the BCG heat shock protein-60 (hsp60) promoter on a mycobacterial shuttle vector (pOLYG) BCG that has had HspR gene, or other genes, replaced with a defective copy of the gene using a non-replicating "suicide" vector. Selection of mutants is using the hygromycin gene from Streptomyces hygroscopicus that replaces the HspR gene and allows selection of recombinants in which a double cross over event has resulted in exchange with the wild type gene (Husson et al 1990, J. Bacteriol 172: 519-24; Garbe et al Microbiology 140: 133-8).

**Origin & function**

M. bovis BCG is the vaccine strain that has been used extensively in humans. The gfp will fluoresce under appropriate conditions. The labels are used to detect individual bacteria in cells or tissues. Inclusion into M. bovis-BCG or M. tuberculosis has been shown not to increase their virulence or the effect on antigen presenting cells (Luo et al 1996, Clin Diag Lab Immunol 3: 761-8). Deletion of HSP genes from BCG will enable the function of the gene in the interaction of the organism with antigen presenting cells to be determined in vitro.

**Evaluation of foreseeable effects**

The production of BCG with gfp will be at other establishments. M. tuberculosis or BCG marked in the same way have been produced previously. No changes have been evident when they have been tested for changes in virulence or effects on how they effect cytokine production or surface molecule expression by antigen presenting cells in vitro (Luo et al 1996 Clin Diag Lab Immunol 3: 761-8). BCG as the established vaccine strain for human use is avirulent and no effect on virulence is foreseen. The aim of targeting and removing HSP genes is to determine the properties of the encoded molecules and how they affect pathogenicity in models involving the interaction...
with antigen presenting cells in vitro. The hypothesis is that removal will affect the survival of bacteria in eucaryotic cells and will be detrimental for the bacteria. Deletion of the targeted genes from the vaccine strain of BCG is predicted to cause further attenuation, or be lethal to the bacillus. Hygromycin has no clinical application in the treatment of tuberculosis, or as far as we are aware the treatment of other bacterial infections. The organisms will be handled as ACDP category 2 pathogens for all experiments that are all in vitro thus reducing the potential of environmental exposure to negligible levels.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated or potentially contaminated category 2 laboratory waste is autoclaved or incinerated (100%). Pipettes and similar objects are disinfected, and surfaces wiped, with phenolic disinfectant or ethanol.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

If there is any indication of increased virulence in either of the mutants the work will pause for re-assessment and notification to the HSE. Approval is given on the understanding that no animal work is involved.

Project Containment

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Project Ref  97/02.1
Purposes of the contained use

Activation of bovine and human plasminogen is an attribute common to a variety of streptococcal and staphylococcal species and is considered a putative virulence mechanism. We propose identification of domains/residues of bovine and human plasminogens critical to their interactions with subsequent activation by such bacterial plasminogen activators. This will be achieved through the study of the interaction between recombinant mammalian plasminogen derivatives and bacterial plasminogen activators.

Recipient or parental organism

Recipient: proprietary Escherichia coli K12 host strains
Parent/Donor organism: human and bovine.

Host/vector system

Bovine and human nucleic acid encoding plasminogen or plasminogen derivatives will be cloned into inducible expression vectors (for example pET or pCAL) maintained within Escherichia coli K12 bacterial host strains (for example BL21 derivatives).

Origin & function

Cloned bovine and human plasminogen and plasminogen derivatives including chimeric human-bovine molecules will be overexpressed in E. coli hosts from where the protein will be extracted and purified for analysis.

Evaluation of foreseeable effects

The GMOs will be capable of inducible overexpression of cDNA encoding bovine and human plasminogen and derivatives thereof. As such they will produce precursor forms of the mammalian serine protease plasmin. The downstream effects of plasmin production include dissolution of fibrin clots (thrombolysis), cell migration, activation of further metalloproteinase cascades. For this reason under normal physiological conditions, plasminogen activation and the activity of plasmin are tightly regulated in mammals. Specific inhibitors of plasmin (alpha 2-antiplasmin - the primary inhibitor of plasmin-mediated fibrinolysis and alpha 2-macroglobulin) prevent indiscriminate protease activity. In the unlikely event of internalisation and subsequent activation by either mammalian or bacterial plasminogen activators the functional recombinant
moiety plasmin would still be highly likely to be subject to the inhibitory effects of alpha 2-antiplasmin and alpha 2-macroglobulin. The GMOs are unlikely to present any risk to human health. The capacity of the E. coli host strains to survive and/or transfer genetic material to other organisms is low. Release to the environment is thought unlikely to be of significance as human and bovine plasminogen are already present in the environment (mammals) and have no known toxic activity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste eg plastic ware, gloves and paper waste etc will be placed within autoclave bags prior to removal via a locked storage facility to the site incinerator facility.

Contaminated glass and non disposable plastic ware eg culture vessels, centrifuge pots etc will be housed within metal autoclave tins marked with autoclave tape and autoclaved at 134 degrees C for 30 minutes. Glass pipettes will be decontaminated by overnight immersion in disinfectant (2.5% chloros solution) prior to removal to wash-up facility. Both procedures kill 100% of E. coli.

Liquid waste eg spent culture fluid will be contained in <400 ml amounts within 500 ml glass bottles. The bottles are housed within metal autoclave tins marked with autoclave tape and an additional note to signify the large volumes therein, prior to removal to be autoclaved at 134 degrees C for 30 minutes to accomplish a 100% kill.

Risk assessment passed.

Please enter comments on the GM safety committee on the risk assessment

Risk assessment passed.

Project Containment

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### Project Additional Information

**Purposes of the contained use**

Identification by a genetic screen, of proteins encoded by fowlpox virus, that are able to rescue the replication of vaccinia MVA, deleted for E3L, in primary chick cells and are thus able to modulate the avian type I interferon response.

**Recipient or parental organism**

Vaccinia MVA is a host-restricted derivative of vaccinia virus Ankara. During extensive passage in avian culture it underwent extensive deletion, including the loss of almost all the known vaccinia immunomodulator genes. The MVA genome sequence is known. Originally thought capable of replicating only in avian cells, MVA has been shown to replicate in BHK21 cells and in primary (or finite) fibroblast cells from mammals but not from primates.

The recipient will be further modified to delete its remaining known immunomodulator (equivalent to vaccinia virus E3L) which has been reported to prevent its replication in avian (but not BHK21) cells. MVA is approved for use as a class I virus - the deletion is expected to render it even more reliably attenuated.

**Host/vector system**

Fowlpox virus genes will be inserted, singly or in combination, into the genome of MVAdeltaE3L, at the site of one of the known deletions, by recombination with genomic fragments of fowlpox virus, selecting for recombinants carrying the E. coli Gpt gene with mycophenolic acid.

**Origin & function**

The genes inserted into MVAdeltaE3L will be derived from the attenuated vaccine strain of fowlpox virus, HP1-438 FP9. This plaque purified strain has been completely sequenced. Like all fowlpox viruses, it is unable to replicate in mammalian cells, including those in which MVA can replicate. It is intended that the genes should restore to MVAdeltaE3L the ability, possessed by parental MVA, to replicate on avian cells. As E3L is known to interfere with the mammalian type I interferon response, by binding
dsRNA thus preventing activation of PKR or 2′5′ OAS, it is expected that the rescuing genes will encode the [as yet unidentified] fowlpox proteins responsible for interfering with avian type I interferon.

**Evaluation of foreseeable effects**

Extract from CPW 4a: Modified Virus Ankara (MVA) [is] an attenuated vaccinia strain that has had multiple regions of the genome deleted. This strain has been demonstrated not to replicate in a range of primary human cells, replicates in avian cells and, until recently, was not thought to replicate in mammalian cells. However, recent work has demonstrated replication of MVA in BHK (baby hamster kidney) cells [as well as in other primary mammalian, but not primate, cells]. MVA has been used as a vaccine without adverse reactions in immune compromised individuals. The ACGM has indicated that MVA may be considered for use at ACGM level 1 depending on the nature of the insert. MVAdeltaE3L is likely to be yet further attenuated. Though able to replicate in BHK21 cells, it cannot replicate in CEFs and, like parental vaccinia virus with E3L deletions (Beattie, E. et al (1996) Virus Genes 12: 89-94), is unlikely to be able to replicate in Vero, HeLa, and murine L929 cells.

There are no known hazards arising directly from inserted gene products derived from the completely sequenced FPV genome.

It is extremely unlikely that insertion of FPV genes into MVAdE3L will restore and enhance the virulence of MVA such that it will pose a threat to human health. MVA lacks multiple genes that are believed to be involved in pathogenicity and is extremely attenuated. FPV is unable to replicate at all in any mammalian cells (including those in which MVA can replicate). Moreover, the gene library recombinants will only be made using the attenuated form of FPV (FP9), which appears fully competent for avIFN-resistance, not the virulent form, so that specific virulence genes are likely to be absent. We cannot rule out some increase in virulence but it is unlikely even that this would even be raised even to the level of parental vaccinia viruses. In light of this possibility, we propose to perform the experiments as for parental vaccinia virus (at Cat. 2).

It is extremely unlikely that insertion of a few FPV genes into MVAdE3L will render the recombinants pathogenic for poultry or other birds. This is due to the multiple deletions in MVA and the lack of pathogenicity of even parental vaccinia for poultry. Furthermore the gene donor will be the completely attenuated (Mayr, A. & K. Malicki (1966) Zentralblatt fur Veterinarmedizin B. B13: 1-13) strain of FPV (FP9), which has been completely sequenced and carried 120 mutations relative to virulent FPV.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- **Liquid waste.** In a contained vessel, Virkon added to give a final concentration of 1%. Left for 24 hours and then disposed of down the drain. Degree of kill 100%.

- **Solid waste.** All contaminated material and solid waste will be placed in a metal autoclave box, sealed with autoclave tape (to indicate sterilisation has been effective) which is then autoclaved at 134 C for 30 minutes, before waste is sent to the on-site clinical waste incinerator for eventual incineration. Degree of kill 100%.

**Is an emergency plan required according to regulation 20?**

- **N**

**Tick to confirm that you have attached a risk assessment to this form**

- **Y**
In order to investigate the relative contribution of RSV proteins to the host range phenotype of RS viruses, the replication of recombinant BRSV, in which the surface glycoproteins or the non-structural gens have been replaced either alone or in combination by those from HRSV, will be investigated in different bovine and human primary cell cultures and cell lines. In addition, the replication of these chimeric viruses in cattle and mice will be investigated.
Characterisation of the role of different RSV proteins in determining the host range phenotype of RS viruses may lead to the development of a small animal model of BRSV and/or the development of attenuated RSV vaccines for man and cattle.

Recipient or parental organism

Bovine respiratory syncytial virus.

Host/vector system

Bovine RSV in which the F, G, SH, NS1 and/or NS2 genes have been replaced with those from human RSV.

Origin & function

Bovine RSV in which the F, G, SH, NS1 and/or NS2 genes have been replaced with those from human RSV have been made by collaborators.

The F protein mediates virus attachment and penetration of cells.
The G protein mediates virus attachment and may also have other, as yet unidentified, functions.
The function of the SH protein is not known.
The NS proteins are involved in aspects of virus replication, in resistance to the antiviral effects of type I interferons, in regulating the induction of type I interferons and may also have other, as yet unidentified, functions.

Evaluation of foreseeable effects

HRSV is a major cause of respiratory disease in young children and BRSV causes a similar disease in young calves. Although closely related, with greater than 80% amino acid identity for 8 of the 11 proteins, BRSV and HRSSV display a restricted host range in vivo. However, HRSSV and BRSV have an overlapping host range in cell culture, in vitro. Replacement of BRSV genes by genes from HRSSV may alter the host range phenotype of the virus. Thus the chimeric BRSV may be able to infect humans, chimpanzees, cotton rats and/or mice and may replicate more efficiently in primate cells than the parental BRSV. It is possible that some of the chimeric BRSV may replicate less efficiently than the parental virus in some cells or animals. If replication is poor the chimeric virus may adapt and evolve giving rise to virus with altered pathogenicity.

HRSSV is ubiquitous in the human population, the majority of whom have experienced one or more HRSSV infections and therefore have some pre-existing immunity. HRSSV infection in adults is usually restricted to the upper respiratory tract. However, HRSSV infection in young infants, the elderly or in immunosuppressed individuals can be severe. Similarly infection with BRSV is widespread in the cattle population and reinfection is usually restricted to the upper respiratory tract. If chimeric rBRS viruses were to infect an adult, pre-existing immunity to the F protein of HRSSV, which is the major protective antigen of the virus, is likely to limit the replication of the chimeric virus. Thus there is only 19% amino acid difference between the BRSV and HRSSV F proteins and neutralising monoclonal antibodies specific for the F protein recognise both the HRSSV and the BRSV F proteins. These anti-F mAbs protect against experimental HRSSV infection in mice, HRSSV in children and BRSV infection in calves. Bovine sera will neutralise HRSSV and human convalescent sera neutralise BRSV. However, the neutralisation titres are higher against homologous viruses. Intramuscular injection of cattle with HRSSV will protect against BRSV infection and infection of cotton rats with BRSV will protect against HRSSV infection. Infection of adults with the GMM is therefore unlikely to produce disease any more severe than a cold. In contrast, infants and immunosuppressed individuals are at risk of developing bronchiolitis and pneumonia if they became infected.

The chimeric viruses may be more hazardous to humans than wild-type BRSV, but are unlikely to be as hazardous to humans as wild-type HRSSV.

Since the organisms will be handled as ACDP category 2 pathogens for all experiments, the likelihood of environmental exposure is small. Furthermore, RS viruses do not replicate in the environment outside of the host animal and would not be expected to survive for long in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
RS viruses are labile viruses and are readily inactivated. All contaminated or potentially contaminated category 2 laboratory waste is autoclaved or incinerated (100% kill).

Pipettes and similar objects are disinfected, and surfaces are wiped, with hypochlorite solution or 70% ethanol (100% kill). Waste animal bedding and small animal boxes will be autoclaved (100% kill). Large animal rooms will be fumigated with formaldehyde (100% kill). Animal carcasses will be incinerated (100% kill).

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Initially, the proposed genetic modification work was brought to the attention of the Genetic Modification Safety Committee in the form of an amendment to be considered as part of a previously notified CU2 project in 2000 (re-notified under transitional arrangements in 2001) but the Committee felt this was inappropriate and had requested to the project proposer that a new full risk assessment must be carried out and suggested some amendments. This was reviewed by the Committee and is the risk assessment attached here.

Project Containment

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Project Ref  97/04.3

Date Ackn’d  28/06/2004

Date Project Ceased  28/06/2004

CU2 Project Title  Characterisation of recombinant bovine respiratory syncytial viruses (BRSV) expressing different forms of the bovine virus diarrhoea virus (BVDV) E2 protein.

Class  Class 2

Cultures Vol Class 2  < 1 Litre

Cultures Volume Class 3-4  Not Applicable

Non-GMM Consent Granted  Not Applicable

02/03/2022  Page 2883 of 15326
Purposes of the contained use

To investigate the ability of a rBRSV expressing the E2 protein of BVDV to protect against BRSV and BVDV infection in calves. The replication of rBRSV expressing different forms of the BVDV E2 protein will be investigated in different bovine primary cell cultures and cell lines. In addition, the replication of these viruses in cattle will be investigated.

Analysis of the virulence of rBRSV expressing BVDV E2 proteins and the immune response and level of protection against subsequent challenge with BRSV &/or BVDV E2 proteins and the immune response and level of protection against subsequent challenge with BRSV &/or BVDV will aid the development of a live attenuated vaccine that will protect against both BRSV and BVDV infection.

Recipient or parental organism

Bovine respiratory syncytial virus (BRSV), strain ATCC A51908Tue. BRSV A51908Tue rescued from cDNA is attenuated in young calves (Valarcher et al., 2003, J. Virol. 77:8426-8439) The virus grows more efficiently in the upper respiratory tract than in the lower respiratory tract of calves, inducing mild pulmonary pathology, but not clinical signs of respiratory disease.

Host/vector system

BRSV expressing various forms of the bovine viral diarrhoea virus (BVDV) E2 protein, which have been constructed in the laboratory, Institut fur Virologie, Tierarztliche Hochschule Hannover, Hannover, Germany:

1. rBRSV expressing the native BVDV E2 protein, which is retained within the endoplasmic reticulum (1)
2. rBRSV expressing a chimeric protein consisting of the BVDV E2 protein with the membrane anchor and cytoplasmic tail of the vesicular stomatitis virus (VSV) G protein to produce expression of the E2 protein on the basolateral side of the plasma membrane (1)
3. rBRSV expressing a chimeric protein consisting of the BVDV E2 protein with the membrane anchor and cytoplasmic tail of the VSV G protein, but with a mutation in the cytoplasmic tail to produce expression of the E2 protein at the apical plasma membrane.
4. rBRSV expressing a transmembrane deleted form of the BVDV E2 protein in an attempt to produce a soluble form of E2. However, the E2 protein cannot be detected in supernatant from cells infected with this virus and appears to be localised in the endoplasmic reticulum.

Origin & function

Bovine RSV expressing BVDV E2 genes have been made by collaborators in Germany. The BVDV E2 glycoprotein mediates virus attachment to cells and is a major protective antigen of BVDV, inducing neutralising antibodies.
Evaluation of foreseeable effects

RS viruses are labile viruses and are readily inactivated. All contaminated or potentially contaminated category 2 laboratory waste is autoclaved or incinerated (100% kill).

Pipettes and similar objects are disinfected, and surfaces are wiped, with hypochlorite solution or 70% ethanol (100% kill).

Waste animal bedding will be incinerated (100% kill). Large animal rooms will be fumigated with formaldehyde (100% kill). Animal carcasses will be incinerated (100% kill).
The GMSC have reviewed and agree with the risk assessment.

**Project Containment**

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**Animal Units**

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**Project Ref 97/06.1**

- **Date Ackn'd**: 19/05/2006
- **CU2 Project Title**: Detection of immune responses to mycobacterial antigens using recombinant adenovirus.
- **Date Project Ceased**: 28/07/2015
- **Class**: Class 2
- **Culture Vol Class 2**: < 1 Litre
- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**

- Transferred to GM53 28/07/2015
### Purposes of the contained use

As part of the current programme on immunity to TB in cattle, we will evaluate immune responses and protective efficacy of recombinant adenovirus expressing antigen (Ag) 85 of Mycobacterium bovis. The objectives of the project are:

1. To evaluate the immune responses elicited in cattle by the recombinant human adenovirus type 5 expressing Ag85A (Ad85A) of M. bovis.
2. To evaluate the use of recombinant the Ad85A for in vitro stimulation of T-cells.

### Recipient or parental organism

No construction of vector will take place at IAH as the GMO will be supplied by our colleagues at Veterinary Laboratory Agency. 
E.coli DH5alpha or equivalent. 
Recombinant, replication deficient human type 5 adenovirus. 
The vector is pACCMV or equivalent, such as pjW24. This is a widely used shuttle vector for producing recombinant adenoviruses.

### Host/vector system


Recombinant DNA will be placed between the cytomegalovirus promoter and the SV40 poly(A) sequence.  
Guest DNA will be placed in frame with the signal peptide of the human tissue plasminogen activator.  

### Origin & function

Mycobacterial DNA encoding Antigen 85A (Ag85A). 
The gene encoding Ag85A will be inserted into the adenovirus vector mentioned above for expression in mammalian hosts for expression and immunization.

### Evaluation of foreseeable effects

Recombinant Ag85A expressed in adenovirus vectors will induce Ag85A specific immune responses in immunised hosts. This construct has been shown to elicit immune responses in cattle (Vordermeier, H.M. Infect Immun 74:1416-1418).

Due to its attenuation, no new virus particles will be produced in the host. Ag85A has no known toxic effects, it is not a known virulence gene and it is not expected to confer replication competency to the adenovirus vector.

The adenovirus to be used in these experiments is human type 5, which is a double-stranded DNA virus. Wild-type, unattenuated adenovirus most commonly causes self-limited infections in the respiratory tract, causing the "common cold" and the gastrointestinal tract, causing diarrhoea.

Wild type adenovirus have been safely used as oral vaccines to prevent adenoviral infections to more than 10 million people and is also being given to military personnel in the USA and Canada (Rubin B.A. and Rorke, L. B. Adenoviral vaccines. In: Plotkin & Mortimer (Eds). Vaccines. (1988) W.B. Saunders. Philadelphia, pp 492).

The vector to be used in these experiments will have deletions in the E1 and E3 regions of the adenoviral genome and has been described by Wang, J. et al. J Immunol 173:6357-6365. In brief, the Ag85 sequence is in frame downstream of human tissue plasminogen activator signal peptide, which in turn is downstream of the murine cytomegalovirus promoter. 3' of the Ag85 sequence will be the SV40 poly (A) signal. The E1 region is essential for replication and propagation and its removal renders the virus replication deficient. Deletion of the E3 region is not essential for viral growth, but its removal allows the insertion of guest genes in this region. (Trapell, B. Adenoviral vectors for gene transfer. Advance Drug Delivery Rev. (1993). 12:185; Bramson, J.L. et al. The use of adenoviral vectors for gene therapy and gene transfer in vivo. Curr.
The recombinant adenoviral vector has been evaluated in a number of clinical trials and found to be safe. The safety and efficacy data from both clinical and pre-clinical studies using recombinant adenoviral gene transfer vectors have widely been reported (Zhang, W. W. Development and application of adenoviral vectors for gene therapy of cancer. Cancer Gene Ther. (1999). 6:113).

The main hazard with adenovirus would be infections of humans and other animals, but no detrimental effects are expected on either. Exposure monitoring by eg. Serological testing of animal carers, etc. is not necessary. These tests would be unable to distinguish between wild type viruses and the attenuated virus used in this study. Wild type adenovirus is a common cause of mild upper respiratory infections worldwide and many of us will already have seroconverted. The hazard is also minimal given the replication deficiency of the construct. We intend to reduce even this minimal risk by working with the construct at ACGM level 2.

Given the replication deficiency and the containment level at which we intend to work, the likelihood of release of the recombinant vector to the environment is effectively zero. There is no production of new viral particles in vaccinated hosts.

All work under this proposal will be carried out at HG 2 containment. No additional hazards over those of wild type adenovirus are foreseen. Cattle inoculated with recombinant adenovirus will be housed in appropriate category 2 containment facilities at all times during the experiment.

Accordingly this proposal seeks approval for a confirmed containment level 2 for work to be carried out under this proposal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste materials generated by these experiments will be inactivated mainly by autoclaving at 121C for 30 min and/or by incineration. Where necessary, materials will be soaked with 1% solution of Virkon for a minimum of 20 minutes.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

None.

Project Containment

02/03/2022
Project Ref 97/06.2

Date Ackn'd 23/08/2006

CU2 Project Title Characterisation of recombinant (r) Sendai viruses expressing wild-type or mutant forms of the F (fusion) and/or G (attachment) glycoproteins of respiratory syncytial virus

Class 2

CultureVol Class 2 < 1 Litre

Consent Granted Not Applicable

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Historical Date of Significant Change

Project Additional Information

Purposes of the contained use

To investigate the ability of a rSeV expressing the F and/or G proteins of RSV to protect against RSV infection in mice. The virulence and immunogenic potential of SeV expressing RSV F and/or G proteins will be investigated in mice. Since SeV is being considered as a potential live virus vaccine for use in man to protect against human parainfluenza virus I infection, SeV may also be of value in the development of a live, attenuated viral vaccine against RSV.

Recipient or parental organism

Sendai virus (strains H and Z) rescued from cDNA.

Host/vector system

The RSV F gene has been inserted between the N and P genes of SeV (gene order of wild-type SeV is N-P/C-M-F-HN-L) and has been provided by Queen's University, Belfast. Although the RSV F protein is a major attachment protein and mediates the formation of multinucleated giant cells, it does not contain the amino acid sequence
Since the organisms will be handled as ACDP category 2 for all experiments, the likelihood of environmental exposure is small. SeV does not replicate in the environment

same considerations are likely to apply as for humans.

House will have serious consequences from respiratory infections in experimental animals. There is no information on the susceptibility of other animals to SeV and the genetically manipulated SeV into the environment is unlikely to have a major impact on the wild rodent population. However, accidental exposure of rodents in the Animal show variations in susceptibility to infection, due to both genetic differences and to pre-existing immunity from previous exposure to wild-type virus. Therefore, release of genetically manipulated SeV into the environment is unlikely to have a major impact on the wild rodent population. However, accidental exposure of rodents in the Animal

bronchiolitis and pneumonia requiring hospitalisation in approximately 1-2% infants. Although re-infection with RSV is common throughout life, clinical disease is usually

tropism of rSeV-F are not expected to be different from that of wild-type Sev. In view of the level of pre-existing immunity to SeV (as a result of HPIV-1 infection) and to host-range restriction resulting from virus evolution in mouse versus man, there have been no confirmed cases of SeV human disease. Studies in seronegative African green monkeys inoculated I.n. with SeV showed that virus could replicate in the upper respiratory tract for approximately 4 days without any evidence of disease. There was also evidence of virus replication in the lower respiratory tract, but again without any evidence of disease. Since, in the absence of the correct amino acid sequence in the RSV F protein, it is unlikely that it will be incorporated into the virion, the virulance, host-range or cell tropism of rSeV-F are not expected to be different from that of wild-type Sev. In view of the level of pre-existing immunity to SeV (as a result of HPIV-1 infection) and to RSV, rSeV-F would be unlikely to cause disease in humans. Furthermore, the majority of humans are infected with RSV during their first year of life and causes bronchiolitis and pneumonia requiring hospitalisation in approximately 1-2% infants. Although re-infection with RSV is common throughout life, clinical disease is usually restricted to common cold-like symptoms in adults.

The genetically manipulated Sevs are unlikely to be more pathogenic than wild-type viruses, which are probably present in the rodent population. Virulent strains of the virus can cause pneumonia and death in rodents. Although SeV can persistantly infect neonatal, but not suckling, weaning or adult mouse brains when inoculated intracerebrally, the virus does not infect the CNS following respiratory exposure. Ferrets, rhesus and cynomolgous monkeys and marmosets are susceptible to experimental respiratory infection. Since there is a wide range of variability in susceptibility to infection in different strains of mice, wild, outbred rodents are also likely to show variations in susceptibility to infection, due to both genetic differences and to pre-existing immunity from previous exposure to wild-type virus. Therefore, release of genetically manipulated SeV into the environment is unlikely to have a major impact on the wild rodent population. However, accidental exposure of rodents in the Animal House will have serious consequences from respiratory infections in experimental animals. There is no information on the susceptibility of other animals to SeV and the same considerations are likely to apply as for humans.

Since the organisms will be handled as ACDP category 2 for all experiments, the likelihood of environmental exposure is small. SeV does not replicate in the environment
outside of the host animal and since it is a labile virus, it would not be expected to survive for long in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Wild-type and recombinant Sendai viruses will be handled at category 2 with additional containment for animals in the Animal House to prevent spread to other animals under experiment, where it may influence the results of others. Therefore the mice will be housed in isolators and all inoculations and post mortems will be carried out in a class II microbiological safety cabinet.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Sendai virus is a labile virus and is readily inactivated. All contaminated or potentially contaminated category 2 laboratory waste is autoclaved or incinerated (100% kill). Pipettes and similar objects are disinfected, and surfaces are wiped, with hypochlorite solution or 70% ethanol (100% kill). Waste animal bedding will be incinerated (100% kill). Large animal rooms and isolators will be fumigated with formaldehyde (100% kill). Animal carcasses will be incinerated (100% kill).

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The GMSC requested that the researcher submits the proposed work as a new class 2 notification and are happy with the proposed work.

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Project Ref  97/07.1

Date Ackn'd  02/03/2022  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4
Generation of HIV-1 from infectious molecular clones and its in vitro use.

Date Project Ceased: 10/08/2011

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: Transferred to GM553 10/08/2011

Historical Date of Additional Info

Significant Change ID: GM97/07.1a

Date of Significant Change: 21/05/2010

**Project Additional Information**

**Purposes of the contained use**

Human immunodeficiency virus type 1 (HIV-1) is a lentivirus that causes a persistent infection in humans associated with the development of an acquired immunodeficiency syndrome (AIDS) which, if untreated, is ultimately fatal. We are studying innate and T cell responses in individuals who have repeatedly been exposed to HIV but have no developed fully-seropositive infection (and unexposed control individuals); and in patients recently infected with HIV who naturally contained viral replication with differing efficiency. Our objective is to identify viral and immune correlates of good control of virus replication, to inform HIV vaccine design. As part of these studies, we propose to compare the relative susceptibility of PDMCs from different individuals to in vitro infection with different HIV isolates, and then go on to dissect mechanisms contributing to differences in in vitro viral replication (focusing particularly on the role of host innate effector mechanisms in inhibiting viral replication).

Some experiments will be carried out with uncloned HIV stocks (including primary virus isolates derived from different infected individuals); but we would also like to work with viral stocks prepared from proviral DNA clones, both for consistency, and to enable identification of viral genetic determinants of differences in in vitro replication capacity.

**Recipient or parental organism**

Plasmids containing infectious molecular clones of HIV will be grown up in disabled host bacteria such as the E.coli K12 derivative DH5α; and then transfected into a eukaryotic cell line such as 293T (a human kidney cell line).

The bacteria to be used are non-pathogenic and unlikely to survive outside culture media or disseminate themselves in the environment. There should be no expression of HIV gene products with these bacteria; but should the plasmid DNA somehow gain entry into dividing eukaryotic cells, there would be potential for HIV production from these cells (HIV RNA production being driven from the LTRs).

293T cells are a highly transfectable derivative of the 293 cell line (an adherent cell line of epithelial morphology derived from human kidney, which contains some adenovirus 5 DNA sequences) into which the temperature sensitive gene for SV40T antigen was inserted. Due to the presence of adenoviral and SV-40 T sequences, which confer the potential to form tumours, this cell line should be handled at containment level 2.

Transfection of these cells with molecular clones of HIV will result in production of HIV.

HIV is a lentivirus that is known to infect humans (via routes including the blood and via mucosal surfaces), and establishes a lifelong persistent infection ultimately...
associated with the development of AIDS. HIV-1 is classified as an ACDP category 3 pathogen.
Many of the infectious molecular HIV clones to be used will be composed of sequences derived from different HIV isolates - ie will result in the generation of genetically modified (chimeric) virus.

Host/vector system

We initially plan to work with plasmid pNL4.3, which contains an infectious molecular clone derived from the NY5 and LAV HIV isolates (Adachi et al. J Virol 59 284-291, 1986) and generates a CXCR4-utilising virus; and a matched clone (pNL4.3BaL,ecto) in which the gp160 ectodomain has been replaced with that of HIV BaL (a CCR5-utilising virus isolate). Details of these HIV molecular clones (both of which are contained in the plasmid pUC18) are provided in the two accompanying pdf files. In future, infectious clones of other HIV isolates and chimeras containing other gp160s or gp 160 fragments may also be used. Some clones may also include marker proteins such as GFP. All the viruses generated from these clones will be of similar or lower pathogenicity to those detailed above. These clones will be obtained from repositories or external collaborators; we do not propose to generate or modify infectious molecular clones of HIV ourselves.

Origin & function

As detailed above, we plan to work with infectious molecular clones derived from CXCR4 and CCR5-utilising HIV isolates. These will include chimeric clones, in which the env gene (or fragments thereof) may be derived from different HIV isolates. Some clones may also contain the marker protein GFP. Infectious HIV will be derived from these clones. Viruses expressing different envelope glycoproteins (the virion surface protein that mediates attachment and entry into host cells) will be generated from different chimeric clones.

Evaluation of foreseeable effects

1. Growth of plasmids containing infectious molecular clones of HIV in E.coli. Bacteria
The plasmids we will work with will contain full-length molecular clones of HIV. Plasmid stocks will be prepared by growth in disabled host bacteria such as the E.coli K12 derivative DH5α.
The pathogenicity of the host bacteria in which the plasmids are to be grown is very unlikely to be affected by the presence of the plasmids. There should not be any expression of HIV genes within the bacteria.
The HIV molecular clones will be contained within the pUC18 plasmid, which is a non-mobilisable vector. Plasmid sequences are thus unlikely to be transferred to other bacteria.
E.coli bacteria containing HIV molecular clones are very unlikely to cause harm to human health. They should not express any HIV gene products, and the presence of the plasmid DNA is not expected to affect their pathogenicity.
It is very unlikely that E.coli bacteria transfected with HIV molecular clones would survive in the environment; and since the plasmid DNA is non-mobilisable, it would also be very unlikely that this would be transferred to other bacteria. These GMOs thus pose an extremely low risk to the environment.

It is unlikely that plasmid DNA contained within the bacteria would be transferred into human cells and initiate HIV production. Nonethe less, this could potentially occur, and is a risk that should be considered. Given the (extremely low) risk that plasmid DNA encoding full-length molecular clones of HIV could conceivably enter replication-competent human cells and initiate production of infectious HIV, it is proposed that plasmid DNA and bacteria containing it should be handled under containment level 2 conditions, to minimise the risk of an infectious exposure incident occurring.
Plasmids containing HIV molecular clones and bacteria containing these plasmids will thus be worked with in category 2 containment laboratories. Gloves and eye protection will be worn at all times when handling these reagents (to minimise the risk of contact with skin or mucosal surfaces). The use of glass and of sharps (eg needles) will be avoided where possible, to minimise the risk of sharps injuries that may result in exposure to plasmid DNA. Equipment and work-surfaces will be decontaminated after use using a suitable a chlorine-containing disinfectant, and all waste will also be treated with disinfectant and/or autoclaved.

2. Transfection of eukaryotic cells with full-length molecular clones of HIV to generate infectious virus
Plasmids containing full-length molecular clones of HIV will be transfected into eukaryotic cells (eg 293T cells), which will then produce infectious HIV particles. Upon transfection of the plasmids into 293T cells, the pathogenicity of the cells themselves is unlikely to change. There is also very little risk of plasmid sequences being transferred from transfected 293T cells to other cell lines.
293T cells transfected with HIV molecular clones would also be very unlikely to survive in the environment.
However 293T cells transfected with HIV molecular clones will produce infectious HIV, which could initiate human HIV infection. These cells and the viruses derived from them thus present a high risk to human health - equal to that of naturally-occurring HIV isolates. Transfection of plasmids into eukaryotic cells and all subsequent work with transfected cells with thus be performed under in category 3 containment laboratories, following the category 3 laboratory code of practice.

3. Use of HIV viruses derived from infectious molecular clones

HIV is a lentivirus that is known to infect humans (via routes including blood and via mucosal surfaces), and establishes a lifelong persistent infection ultimately associated with the development of AIDS. HIV-1 is classified as an ACDP category 3 pathogen.

Some of the HIV clones we propose to work with are chimeric clones containing partial/entire env genes from one HIV isolate in the backbone of another. As gp160 is the virion envelope glycoprotein, its sequence does affect virus interaction with host cell receptors and hence viral tropism and pathogenicity. However it is not expected that any of the chimeric viruses we will generate will exhibit a level of pathogenicity greater than that of the group of primary HIV isolates from which their component sequences were derived.

HIV sequence could potentially be transferred from the chimeric viruses that we generate from molecular clones to other HIV viruses, if replication competent cells were to become co-infected with the chimeric virus and another HIV isolate. However this should not result in the generation of recombinant viruses any more pathogenic than those that could be generated naturally by recombination between the parental virus from which the chimeric virus was derived and the HIV isolate concerned.

The HIV virions we generate would pose a similar risk to the environment as that posed by natural HIV isolates. HIV virions are extremely labile- but if laboratory staff were to become infected, they could potentially transmit virus to other people. To minimise this risk, in the event of any potential HIV exposure incident, the staff involved would be tested for HIV infection, and also receive antiretroviral therapy to try and prevent full-blown infection from occurring.

HIV viruses derived from infectious molecular clones will only be used for in vitro experimental work. All work involving these viruses will be performed in category 3 containment laboratories, following the category 3 laboratory code of practice.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from bacterial cultures will be treated with 1% bleach for > 30 minutes prior to discarding (100% kill). Solid waste will be disinfected by autoclaving or incinerated (100% kill). Equipment and work-surfaces will be decontaminated after use using a suitable chlorine disinfectant.

Liquid waste from the category 3 containment labs will be treated with a suitable disinfectant (eg.1% Virkon for > 30 minutes) prior to discarding (100% kill). Solid waste will be disinfected by autoclaving (100% kill). Equipment and work surfaces will be decontaminated after using a suitable disinfectant (eg 70% ethanol).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
As part of the current programme on immunity to TB in cattle, we will evaluate immune responses and protective efficacy of the urease deficient recombinant BCG expressing listeriolysin (Hyl) of Listeria Monocytogenes (AueChly+ rBCG). The objectives of the project are:

1. To evaluate the immune responses elicited in cattle by the AureChly+ rBCG.
To evaluate the use of recombinant BCG AureChly+ rBCG for in vitro stimulation of T-cells

Recipient or parental organism

BCG is currently the only vaccine licensed for use against human TB. It is administered to newborn babies and as such it is a safe vaccine (Brewer, T. F. 2000. Preventing tuberculosis with bacillus Calmette-Guerin vaccine: a meta-analysis of the literature. Clin Infect Dis 31 Suppl 3:S64-67). However, BCG could provoke a serious, life-threatening infection in immune compromised individuals.

AureC BCG is a urease negative derivative of BCG which was created by the insertion of an ureC gene disrupted by a kanamycin marker (aph) (aph::UreC) in a suicide vector to replace the native ureC gene (Reyrat, J.M., G. Lopez-Ramirez, C Ofredo, B Gicquel, and N. Winter. 1996).


The cloning of LLO into AureC BCG (AureC hly+ rBCG), which lacks urease, did not result in increased virulence. The construct was shown to be less virulent for immune deficient SCID mice than parental BCG. Cloning and expression of Hyl did not endow BCG with the ability to lyse macrophages (Grode, L. P. Seiler, S. Baumann. J. Hess. Brinkman, A.N Eddine, P. Mann, C. Goosmann, S. Bandermann, D. Smith G, J Bancroft, J.-M.Reyrat, D. van Soolingen, B. Raupach, and S.H.E Kaufmann. 2005 Increased vaccine efficacy against tuberculosis of recombinant Mycobacterium bovis bacilli Calmette-Guerin mutants that secrete listeriolysin. J. Clin. Invest. 115;2472-2479).

The main hazard with AureC hly+ rBCG would be infection of humans and other animals, but no detrimental effects are expected on either. All genetic modifications of BCG to date have attenuated this organism further and have not restored its virulence. However, wild-type BCG can cause disease in immunocompromised hosts. Such opportunistic infection is amenable to drug treatment in humans. The hygromycin resistance marker has been chosen because they are not used in human medicine.

The recombinant AureC hly+ rBCG will be undergoing human clinical trials in Germany in late 2007.

Accordingly, no harmful effects to healthy, immune competent individuals are envisaged.

All work under this proposal will be carried out at containment level 2 by personnel trained to work at this level of containment. No additional hazards over those of BCG are foreseen.

Host/vector system

The attenuated Mycobacterium bovis bacillus Calmette-Guerin (BCG) is currently the only vaccine licensed for use against human tuberculosis. It is administered to newborn babies and as such it is considered a safe vaccine licensed for use against human tuberculosis. It is administered to newborn babies and as such it is considered a safe vaccine (Brewer, T. F 2000. Preventing tuberculosis with bacillus Calmette-Guerin vaccine: a meta-analysis of the literature. Clin Infect Dis 31 Suppl 3:S64-67). AureC BCG is a urease negative derivative of BCG which was created by the insertion of an ureC gene disrupted by a kanamycin marker (aph) (aph::UreC) in a suicide vector to replace the native ureC gene (see figure below) (reyrat, J, F Berthet, and B Gicquel. 1995 The Urease Locus of Mycobacterium bovis Bacillus Calmette-Guerin. PNAS 92:8768-8772.).

Schematic organisation of the ureC disrupted gene used for allelic exchange. Open reading frames are represented by blocks. Relevant restriction sites are indicated (P, Pst I, B, BamHI, S, Sac I). The aph gene on a 1.3-kbp BamHI fragment was inserted into the BamHI site of ureC and is represented as a black rectangle. The solid bar represents the suicide vector pBluescript K S+. Primers used for amplification by PCR of the structure resulting from double crossover are depicted by arrows P3 and P4 (from Reyrat, J. F. Berthet, and B Gicquel. 1995. The Urease Locus of Mycobacterium tuberculosis and its utilization for the Demonstration of Allele Exchange in Mycobacterium bovis Calmette-guerin. PNAS 92:8768-8772).


The hazard associated with the recipient microorganism are not expected to be greater than those expected from the vaccine against TB, BCG, which does not cause harm to immune competent individuals.

**Origin & function**

Listeriolysin O (LLO) is a cholesterol-dependent cytolysin that is an essential virulence factor of Listeria monocytogenes. LLO pore-forming activity is pH-dependent; it is active at acidic pH,(6), but not at neutral pH. LLO’s pore-forming activity is controlled by a rapid and irreversible denaturation of its structure at neutral pH at temperatures > 30 degrees C. Schuerch, D. W. E. M Wilson-Kubalek, and R.K Tweten, 2005. Molecular basis of listeriolysin under normal conditions. LLO induces a number of host cell responses, such as cell proliferation, activation of MAP kinase pathway in epithelial cells, modulation of internalization via calcium signalling and cytokine expression in macrophages (Vazquez-Boland JA, et al.,2001. Listeria pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 14(3):584-640.

The cloning of listeriolysin into BCG allows the increased permeabilization of the phagosomal membrane to allow greater leakage of BCG antigens into the cytoplasm and therefore greater processing of Mycobacterial antigens through the cytoplasmic compartment which primes for CD8+ T-cell responses. The cloning of listeriolysin into AureC BCG (AureChly+rBCG), which lacks urease, did not result in increased virulence. The construct was shown to be less virulent for immune deficient SCID mice than parental BCG. Cloning and expression of Hyl did not endow BCG with the ability to lyse macrophages (grode, L Peiser, S Baumann, J. Hess, V. Brinkmann, A.N. Eddine, P. Mann, C. Goossman, S. Bandermann, D. Smith, G.J Bancroft, J. M reyrat, D. van soolingen, B. Rapupach, and S.H.E Kuafmann. 2005. Increased vaccine efficacy against tuberculosis of recombinant Mycobacterium bovis bacilli Calmette-Guerin mutants that secrete listeriolysin. J. Clin. Invest. 115:2472-2479).


**Evaluation of foreseeable effects**

The main hazard with AureChly+rBCG would be infection of humans and other animals, but no detrimental effects are expected on either. The recombinant AureChly+rBCG will be undergoing human clinical trials in Germany in late 2007.

All genetic modifications of BCG to date have attenuated this organism further and have not restored its virulence.

It is not possible to state what effect of this construct would be on immunocompromised people; BCG is contra-indicated for immune-deficient people. However, the cloning of LLO into AureCBCG(AureChly+rBCG), which lacks urease, was shown to be less virulent for immune deficient SCID mice than parental BCG. Cloning and expression of Hyl did not endow BCG with the ability to lyse macrophages(Grodé, L P. Seiler, S Baumann, J. Hess, V. Brinkmann, A.N Eddine, P, Mann, C. Goosmann, S. Bandermann, D Smith, G. J Bancroft, J-M. Reyrat, D. van Soolingen, B. Raupach , and S. H.E Kaufmann. 2005. Increased vaccine efficacy against tuberculosis of recombinant Mycobacterium bovis bacilli Calmette-Guerin mutants that secrete listeriolysin. J Clin Invest, 115:2472-2479).

Nevertheless, should an immune deficient person become infected with the construct and develop clinical signs, such infection would be amenable to antibiotic treatment; the hygromycin resistance gene inserted into the chromosome of BCG during the creation of the AureC BCG poses no additional risk in this respect since the drug resistance marker had been chosen because they are not used in human medicine.

Health screening measures are in place at IAH to prevent immunocompromised individuals working with ACDP class 2 organisms. Accordingly, no harmful effects to healthy, immune competent individuals are envisaged.

The risk to the environment is low. The genetic lesions present in BCG render the organism unable to persist or cause disease in immunocompetent hosts (including humans) so it is unlikely that BCG would be able to establish, diseminate or displace other environmental organisms to a significant extent. The greatest hazard associated with exposure of animals to BCG is that cattle infected with BCG become positive for the tuberculin skin test. Although BCG does not persist in cattle and causes no disease, animals that become tuberculin positive through exposure to BCG would be deemed tuberculosis by the current TB screening measures in the UK and would be slaughtered as a result (please note that cattle held at IAH for experimental purposes are not subject to this routine TB surveilence). The greatest hazard is therefore an economical one for cattle kept on potential farms surrounding IAH Compton. However, there are no commercial cattle holdings in close contact to the CL2 cattle not result in faecal, aerosol or nasal BCG shedding, which is necessary for such an event to occur. The materials and consumables used for the vaccinations will be also incinerated. Cattle will be killed at the end of the experiment and incinerated, thus preventing any exposure of the environment due to BCG persisting in lymph nodes, or other internal organs.

The work to be carried out involves the injection of bacteria into cattle to determine the immune responses induced by this vector. To determine immune responses to the construct we may also use bacterial cultures grown in this laboratory to infect cells in vitro for antigen presentation. For in vitro experiments it is envisaged that the maximum volumes of bacterial culture required will be 100ml. It is extremely unlikely that the mutants of BCG will be released from the laboratory since the bacilli will be stored, analysed and dispensed in a Containment Level 2 (CL2) laboratory by trained workers. The inoculum will then be transported in secure containers to the CL2 cattle accommodation where the vaccinations will take place. Once inoculated (subcutaneously), the vaccinated animals will serve essentially as primary containment; the vaccination route means that the bacteria will be sequestered within the lymphatic system, which makes excretion into the environment highly unlikely. The construct will be stored at -80 degrees C in a secured container and when required, the construct will be transported in a secure container to the CL2 laboratory.

Accordingly this proposal seeks approval to confirm containment level 2 for work to be carried out under this proposal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste materials generated by these experiments will be inactivated mainly by autoclaving at 121 degrees c for 30 min and/or by incineration. Where necessary, materials will be soaked overnight with a 1% solution of Virkon and then disposed by incineration. At the end of the experiment animal carcasses will be incinerated. These inactivation methods achieve 100% kill of BCG.

This request and accompanying assessment has been reviewed extensively by the Institute for Animal Health's genetic modification committee at Compton. We are satisfied with the risk assessment of the proposed work.

Please enter comments on the GM safety committee on the risk assessment

This request and accompanying assessment has been reviewed extensively by the Institute for Animal Health's genetic modification committee at Compton. We are satisfied with the risk assessment of the proposed work.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 97/08.1

Date Ackn'd 22/12/2008

CU2 Project Title Development of Eimeria tenella as a vehicle for the expression of heterologous coding sequences.

Date Project Ceased 31/03/2011

Consent Granted Yes

Project notified under transitional arrangements No

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Objective : To transfect Eimeria tenella with constructs designed to express sequences coding for heterologus protozoan, bacterial and viral antigens to facilitate assessment as a vaccine delivery vehicle.

Recipient or parental organism

The Eimeria species, including E. tenella, have been rated by the Advisory Committee On Dangerous Pathogens (ACDP) as requiring a containment level of 1. The Eimeria species are completely host-and, in examples, tissue-specific: E.tenella specifically parasitizes the chicken caecae and poses no risk to human health or safety.

Host/vector system

The commercially available vector pGEM-Teasy (Promega) will be used throughout these studies, together with a series of vectors developed for the purpose of transfecting Eimeria species parasites (Clark et al 2008. mol Biochem Parasitol 162:77-86) The XL1-Blue MRF Laboratory Escherichia coli strain (deficient in all known restriction systems [(mcrA183, (mcrCB-hsdSMR-mrr)173], endonuclease(endA) and recombination (recA) deficient will be used throughout these studies.

Origin & function

DNA templates encoding the Eimeria maxima SAG, Campylobacter jejuni CjaA and Avian influenza hemagglutinin antigens (HA) are already available within the IAH in plasmid stocks (derived by PCR amplification from gDNA or directly synthesised). Thus, there is no requirement to handle either C. Jejuni or the Avian influenza virus. Amplification and manipulation of E. maxima, An ACDP hazard group 1 pathogen, is routine within the IAH. All antigens will be expressed under the control of eimerian stage-specific promoters, limiting antigen production to defined steps of the transfection process which will be controlled as described in section 12.

The primary function of these studies is to generate lines of E. tenella that express heterologous antigens in order to assess the parasite’s potential as a vaccine delivery vehicle. Thus, once created, each line will be assessed for expression of the target antigen (i.e. by RT-PCR and immunolabelling) prior to use to immunise SPF chickens within the IAH experimental facility to monitor correlates of immune protection. Added value will be gained through the phenotypic assessment of all parasite lines generated for divergence from the recipient strain.

Evaluation of foreseeable effects

The Eimeria species are antigenically complex micro-organisms and their interaction with the host is mediated by a complex series of proteins expressed in a sequential manner. All three candidate coding sequences described in this application are considered likely to contribute to host/pathogen interaction in their natural context but whilst the application of such non-self proteins might elevate the potential for novel recipient tropism within the chicken, the addition of a single protein to such a complex process is unlikely to have a significant impact. Once cloned, these sequences will be expressed under the control of one or more stage specific promoters, limiting expression to the sporozoite and/or merozoite lifecycle stages.

I) E.maxima surface antigens (SAGs). Preliminary unpublished data suggest that SAG proteins expressed by E.tennella mediate parasite/host interaction but not invasion. Single parasite RT-PCR has revealed the concurrent expression of seven distinct SAG proteins. Whilst addition of a single novel SAG gene has the potential to modify the recipient parasite’s ability to attach within the host, the possibility of a significant change in tropism is limited.
ii) C. jejuni CjaA antigen. The C. jejuni CjaA antigen has been shown to localise to the inner bacterial membrane where it is thought to function as a component of the ABC transporter system. CjaA acts as a receptor for the amino acids glutamine and cysteine. Thus, whilst CjaA could affect Eimeria/host interaction, expression on the surface of one or more zoite stages of the E. tenella lifecycle via a GPI anchor and not as an internal component of the cell wall will minimise the influence of any possible effects. Expression of the native CjaA under the control of its own promoter in a Salmonella Typhimurium crp-cya-strain previously yielded no increase in virulence or persistence during infection in chickens (Wyszynska et al. 2004. Vaccine 22:1379-89).

iii) Avian influenza HA's have been shown to play an essential role in mediating host cell entry. Pathogenicity among avian influenza isolates has been linked to the presence of a polybasic amino acid sequence at the HA cleavage site. The HA coding sequences to be used will be obtained by PCR amplification from existing artificially synthesised plasmid constructs which lack a polybasic amino acid sequence at the HA cleavage site. HA antigens have previously been delivered to chickens using viral vectors without severe repercussions (eg fowl pox virus, Newcastle disease virus).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory Eimeria species parasites have never been known to infect humans, thus laboratory handling of all stages of unmodified parasites is appropriately carried out with Hazard Group 1 containment. The transfection process introduces DNA by electroporation into Eimeria sporozoites, a stage of the lifecycle that exhibits severely limited environmental persistence and can only initiate infection following direct cloacal or oral (after bicarbonate gavage) inoculation. Following genetic transformation, the only stage of the parasite that requires additional containment and disposal measures is the oocyst. After recovery, oocysts will be stored in clearly marked, sealed bottles within a designated refrigerator. Bottles of transformed oocysts will be opened only in fumigatable rooms and all manipulations of these oocysts will be carried out in these rooms. Purified sporozoites will be removed to the laboratory for further experiments and these can be safely handled (as outlined above). Doses of oocysts may be removed, double bagged and in sealed containers, and taken to the EAH for infection of animals. All glassware, plasticware, paperware and liquids that contain oocysts, or are potentially contaminated with oocysts, will be fumigated with ammonia before autoclave sterilization.

Experimental Animal House. The passage, handling and disposal of oocysts (the environmentally stable stage of the eimerian lifecycle) will be carefully controlled. Animals are housed in strict isolation and cultured parasites are recovered in rooms which are then sealed for fumigation with ammonia, which destroys oocysts. The EAH facilities used are kept dry throughout all trials to limit oocyst survival and are ammonia fumigated in situ, together with all cages and any waste, at the conclusion of each experiment. All solid waste is removed after fumigation in sealed bags for incineration. Foot baths are removed before each trial commences and water traps are installed. All waste water is removed in sealed containers for autoclaving prior to disposal. Post fumigation all rooms are cleaned and re-fumigated shortly before re-stocking.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment was reviewed by the IAH Compton sites Genetic Modification Safety Committee. It was passed with no problem.
### Project Containment

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### Project Ref 97/09.1

- **Project Title:** GENERATION OF HCVcc FROM INFECTIOUS MOLECULAR CLONES, AND ITS IN VITRO USE
- **Class:** Class 3
- **Culture Volume:** 60ml
- **Date Ackn'd:** 05/03/2009
- **Date Project Ceased:** 10/08/2011
- **Consent Granted:** Yes
- **Historical Significant Changes:** Transferred to GM553 10/08/2011

### Project Additional Information

**Purposes of the contained use**

Hepatitis C Virus (HCV) is a Hepacivirus belonging to the family of the Flavividae that causes a persistent infection in about 80% of infected individuals. The primary site of infection is the liver, where chronic infection can lead to liver cirrhosis and hepatocellular carcinoma (HCC). The virus has also been found in peripheral blood mononuclear cells (PBMCs) and the central nervous system (CNS).

We are studying the modulation of immune functions and infection of different PBMC subsets by the virus, to understand how HCV establishes and maintains a persistent infection.

As HCV isolates cannot be grown in cell culture, these experiments will be carried out with the HCVcc generated from different molecular clones (as detailed below).

**Recipient or parental organism**
The E.coli K12 derivative will be DH5α will be transduced with the plasmids containing the HCV molecular clones and cultured. The isolated plasmid DNA from these bacteria such as the E. coli K12 derivative DH5α will be used to produce plasmids containing infectious molecular clones of HCV; these will then be transfected into a hepatocyte cell line such as Huh-7.5.

The bacteria to be used are non-pathogenic and unlikely to survive outside culture media or disseminate themselves in the environment.

The Huh-7 cell line and its derivatives (Huh-7-L6, Huh-7-L6f) are derived from a hepatocellular carcinoma (HCC). The Huh-7.5 cell line (used for production of HCVcc) is derived from the Huh-7 cell line but contains an inactivating mutation in the RIG-1 gene. RIG-1 is the intracellular sensor for dsRNA and its inactivation makes the Huh-7.5 cell line highly permissive to HCV replication.

When uninfected, this cell line should be handled at containment level 1.

Many of the infectious molecular HCV clones to be used will be composed of sequences derived from different HCV isolates - i.e. will result in the generation of genetically modified (chimeric) virus.

HCV is a Hepacivirus that is known to infect humans, via routes including the blood and via mucosal surfaces. It establishes a lifelong persistent infection in about 80% of infected individuals that is ultimately associated with diseases such as liver cirrhosis and HCC. HCV is classified as an ACDP hazard group 3 pathogen.

Until recently, HCV could not be grown in cell culture, so the use of HCV replicons, HCV proteins or HCV pseudoparticles (HCVpp) was necessary. The discovery of a clone called JFH-1, isolated from a Japanese patient with fulminant hepatitis, revolutionised the field, because it was the first clone that could replicate and produce infectious virus in cell culture and led to the production of what is called HCVcc (cell-culture derived HCV).

Currently, there are only a few HCV clones that can replicate and produce infectious virus. These include H77 (genotype 1a), Con1 and HCV-CG1b (genotype 1b), JFH-1 (genotype 2a) and a few structural chimeras of JFH-1 (H77-JFH-1, Con1-JFH-1, 452-JFH-1 and J6-JFH-1). We initially plan to work with the HCVcc chimera J6-JFH-1, which generates higher infectious titres compared to the JFH-1 clone, but also plan to use the original JFH-1 clone, the chimeras listed above and other structural chimeras (JFH-1 viruses containing glycoproteins from other viruses) in the future. Details of these HCVcc constructs are provided in the attached table.

We do not propose to generate or modify uninfected molecular clones of HCVcc ourselves. However we will grow up bacteria (E. coli derivatives) that harbour plasmids containing HCVcc molecular clones supplied to us by other investigators (Ralf Bartenschlage, Arvind Patel). There should be no expression of HCV gene products within these bacteria; but should the plasmid DNA somehow gain entry into dividing eukaryotic cells, there would be potential for HCV production from these cells. Viral stocks will be generated by transfecting plasmids containing proviral DNA clones into Huh-7.5 cells or other hepatocyte cell lines, collecting the supernatant, and finally concentrating the virus.

These viral stocks will then be used in in vitro assays.
safety cabinet, to minimise the risk of infectious exposure incident occurring. Plasmids containing HCV molecular clones and bacteria containing these plasmids will thus be worked with in category 2 containment laboratories. Gloves and eye protection will be worn at all times when handling these reagents (to minimise the risk of contact with skin or mucosal surfaces). The use of glass and of sharps (e.g. needles) will be avoided where possible, to minimise the risk of sharps injuries that may result in exposure to plasmid DNA. Equipment and work-surfaces will be decontaminated after use using 70% Ethanol and all waste will also be treated with 5% Virkon over night and/or autoclaved (see attached reference for minimal contact times in order to inactivate HCV and general disinfection procedures used in our containment level 3 laboratory).

2. Transfection of liver cells with full-length molecular clones of HCV to generate infectious virus

Plasmids containing full-length molecular clones of HCV will be transfected into liver cell lines (Huh-7.5 cells), which will then produce infectious HCV particles. Upon transfection of the plasmids into Huh-7.5 cells, the pathogenicity of the cells themselves is unlikely to change. There is also very little risk of plasmid sequences being transferred from transfected Huh-7.5 cells to other cell lines. Huh-7.5 cells transfected with HCV molecular clones would also be very unlikely to survive in the environment. However Huh-7.5 cells transfected with HCV molecular clones will produce infectious HCV, which could initiate human HCV infection. These cells and the viruses derived from them thus present a high risk to human health - equal to that of naturally-occurring HCV isolates. Transfection of plasmids into liver cell lines and all subsequent work with transfected cells will thus be performed under in containment level 3 laboratories, following the category 3 laboratory code of practice.

3. Use of HCV viruses derived form infectious molecular clones

Many of the infectious molecular HCV clones to be used will be composed of sequences derived from different HCV isolates-i.e. will result in the generation of genetically modified (chimeric) virus. HCV is a Hepacvirus that is known to infect humans, via routes including the blood and via mucosal surfaces. It establishes a lifelong persistent infection in about 80% of infected individuals that is ultimately associated with diseases such as liver cirrhosis and HCC. HCV is classified as an ACDP hazard group 3 pathogen. Some of the HCV clones we propose to work with are chimeric clones containing structural genes from one HCV isolate in the backbone of another. As E1 and E2 are the virion envelope glycoproteins, their sequences affect virus interaction with host cell receptors and hence viral tropism and pathogenicity. However it is not expected that any of the chimeric viruses we will generate will exhibit a level of pathogenicity greater than that of the group of primary HCV isolates from which their component sequences were derived. The viruses derived from them would pose a similar risk to the environment as that posed by natural HCV isolates. Being enveloped viruses, HCV virions are extremely labile - but if the laboratory staff were to become infected, they could potentially transmit virus to other people. To minimise this risk, in the event of any potential HCV exposure incident, the staff involved would be tested for HCV infection, and also receive antiviral therapy to try and prevent full-blown infection from occurring. HCV viruses derived from infectious molecular clones will only be used for invitro experimental work. All work involving these viruses will be performed in containment level 3 laboratories, following the category 3 laboratory code of practice.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from bacterial cultures will be treated with 5% Virkon for over night prior to discarding. Solid waste will be disinfected by autoclaving or incinerated. Equipment and work-surfaces will be decontaminated after use using a suitable chlorine-containing disinfectant. Liquid waste from the category 3 containment labs will be treated with a suitable disinfectant (eg. 5% Virkon over night) prior to discarding. Solid waste will be disinfected by autoclaving. Equipment and work-surfaces will be decontaminated after use using a suitable disinfectant (eg. 70% ethanol).
The Institute for Animal Health's Genetic Modification Safety Committee has reviewed and approved this work and associated risk assessment.

Please enter comments on the GM safety committee on the risk assessment

The Institute for Animal Health's Genetic Modification Safety Committee has reviewed and approved this work and associated risk assessment.

### Project Containment

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Human Clinical Applications

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### Project Ref 97/09.2

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<tr>
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<td>Induction of immune responses in cattle using Modified Vaccinia Ankara virus expressing mycobacterial antigens</td>
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Date Project Ceased 28/07/2015

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Transferred to GM53 28/07/2015

Historical Date of Additional Info

Date of Significant Change 17/06/2010

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The aim is to assess the potential of new vaccination strategies in protection against bovine TB. Prime-boost strategies utilising MVA expressing mycobacterial antigens have been used in human studies and show enhanced immunogenicity compared to conventional vaccines (McShane et al, Nature Med 2004)

Cattle will be vaccinated with BCG (prime) and boosted at defined intervals with MVA expressing immunodominant antigens from Mycobacteria. Immune responses will be assessed in vaccinated cattle to assess vaccine efficacy. This vaccination strategy is expected to induce broad protective immunity.

#### Recipient or parental organism

Modified vaccinia virus ankara (MVA) expressing M. tuberculosis antigens will be produced by infection of permissive chick embryo fibroblast cell and transfection with a shuttle vector containing the recombinant genes plus a marker gene (green fluorescent protein). The shuttle vector recombines with the viral genome inside cytoplasm of the cell. The MVA viruses will be manufactured at Jenner Institute Vector Core facility at Oxford University.

#### Host/vector system

Modified Virus Ankara (MVA) is an attenuated vaccinia strain that, by extensive passage in chick embryo fibroblasts, has had multiple regions of the genome deleted. This strain has been demonstrated not to replicate in a range of primary human cells and mammalian cells, but has recently been shown to replicate in a baby hamster kidney cells. Genes from M. tuberculosis will be expressed in a shuttle vector under the control of the MVA 7.5 promoter.

#### Origin & function

Mycobacterial DNA encoding Ag85A, TB9.8, TB10.4 and Acr2. The genes have been cloned at the Jenner Institute, Oxford.

The proteins to be expressed (Ag85A, TB9.8, TB10.4 and Acr2) are immunodominant proteins produced by Mycobacteria species during in vivo infection, these are major targets for T cell recognition.

The aim is to test the recombinant MVA expressing these proteins as part of vaccination strategies in cattle: by incorporating immunodominant proteins into the vaccine the protective T cell response should be enhanced leading to immunity from infection.

#### Evaluation of foreseeable effects

MVA is highly attenuated and can only form infectious particles in permissive avian cells.

MVA expressing mycobacterial antigen 85A has been used in phase II human vaccine trials and has an excellent safety record (Hawksridge et al, J Infect Dis 2008).

No infectious particles can be generated in human or animal cells and therefore MVA cannot cause infection or disease.

MVA cannot disseminate in the environment due to its replication deficiency.

None of the expressed genes are oncogenic or toxic and are not expected to increase virulence of MVA.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Laboratory procedures: blood samples from MVA immunised cattle will be handled at containment level 2 within class II microbiological safety cabinets according to IAH vaccinia virus rules. Liquid waste will be treated overnight with 2% Virkon prior to disposal into drains. Solid waste (plasticware) will be autoclaved (121°C, 30 min) prior to incineration. The safety cabinets will be disinfected with 2% Virkon wipe down at the end of any experimental procedure.

Animal housing procedures: animals will be housed at containment level 2 in locked accommodation with restricted access. All personnel handling MVA immunised animals will be required to wear protective accommodation with restricted access. All personnel handling MVA immunised animals will be required to wear protective clothing and gloves, which will be disinfected by wash down with 2% Virkon following contact with animals. Walls and floors within the animal accommodation will be scrubbed with 2% virkon solution (minimum 10 minutes); wash down will be into drains with traps which contain 2% virkon (held for minimum of 10 minutes). Solid waste will be covered with 2% virkon for >30 minutes prior to removal for incineration. All animals will be culled at the end of the experimental period and the carcasses incinerated.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This assessment has been reviewed and passed by the IAH Compton BAGMSC

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Project Ref 97/10.1

Date Ackn'd 26/07/2010

CU2 Project Title The determinants of replication, transmission and pathogenicity of avian influenza

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

02/03/2022  Page 2907 of 15326
The purpose of these experiments is to increase our understanding of the viral determinants for host adaptation, increased transmissibility and virulence of influenza viruses (AI) of poultry and mammalian species. This may lead to improved vaccines and better control measures against avian influenza infections in poultry and hence reduction of the viral load in the environment. We will use the reverse genetics system to produce recombinant influenza viruses derived from wild-type animal viruses to define which polypeptides, combination of polypeptides or particular amino acid residues are responsible for increases in either virus-host interactions, increased virus attachment and replication of viruses in different avian or mammalian cells and in animal models.

Recipient or parental organism

Recombinant low pathogenicity influenza viruses will be generated by transfecting eukaryotic cells (avian or mammalian) with the plasmids pHW 2000 or pHWSccdB containing PB2, PB1, PA, HA, NP, NA, M and NS genes from low pathogenicity influenza A viruses, specifically;

1. A/chicken/Italy1279/00 (H7N1)
2. A/chicken/Pakistan/UDL-1/08 (H9N2)
3. A/turkey/Turkey/1/05 (H5N1). The HA gene derived from this virus will be modified to delete polybasic amino acids at the cleavage site. The GMP construct will have a mono basic HA cleavage site identical to that present in the low pathogenicity phenotype of H5N1 avian influenza viruses.
4. A/PR/8/34. This virus will be used as recipient of HA, NA and PB2 genes derived from the H7N1 and H9N2 and H5N1 viruses described above (number 1-3).

Host/vector system

cDNA clones will be made in E. coli and plasmid DNA containing influenza virus genes will be transfected into cells of eukaryotic origin (avian or mammalian) to rescue influenza virus

Origin & function

The recombinant viruses produced will be used in tissue culture and animal models to study the effect of specific changes in their replication capacity, pathogenesis and transmission.

Alterations in amino acids will be introduced by site directed mutagenesis in the genes that encode polypeptides of haemagglutinin, neuraminidase and polymerase (PB2) genes. These will mimic strains that have emerged during the adaptation of avian influenza viruses found in waterfowl which act as reservoir for viruses affecting poultry.
As described in the attached risk assessment, the changes in the HA gene will be restricted to those observed in the field isolates. These include the variation in the potential glycosylation sites in the HA and deletion of amino acids in the stalk length of the NA gene.

**Evaluation of foreseeable effects**

The characteristics that we plan to introduce are those observed in viruses isolated from field outbreaks in poultry and are not foreseen to be any more hazardous than parental strains; thus we propose to handle these viruses in the same way as we handle the parental strains.

To minimise the risk of infection to workers, the engineered viruses will be worked on in a Class II Microbiological Safety Cabinet. Furthermore additional procedures will be used to ensure minimal risk to the operator during transfer from the cabinet to incubators, freezers and autoclaves. Individual workers will wear disposable gloves and appropriate protective clothing at all times when handling influenza viruses.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste containing infectious virus will be treated with 1% Virkon for a minimum of 30 minutes inside the Class II Microbiological Safety Cabinet (Class II-MSU), then a 50-100 ml maximum volume (in plastic flasks) will be placed into double autoclave bags (double autoclave bags will be made by inserting one autoclave bag inside the other one, this will create two protective plastic layers of the autoclave bags which further reduce the chances of any potential leakage of the contaminating material inside the autoclave bags) and into the designated metal tins, transferred to Microbiological Services (within the same building) and autoclaved at 135° C for 30 minutes prior to disposal by incineration.

Solid waste:

Solid waste contaminated with infectious virus (plastic tips, flasks) will be immersed in 1% virkon for minimum of 30 minutes inside the Class II-MSU, then placed in double autoclave bags, put in a secure metal tin and autoclaved as described above and disposed by incineration.

Other solid waste such as eggs will also be disposed of by bagging in double autoclave bags within the ClassII-MSU, put in a secure metal tin and autoclaved as described above and disposed of by incineration.

The autoclave is serviced quarterly and annually validated; records are kept and examined.

The Class II-MSU are tested and validated every six months during a scheduled maintenance period.

Animal waste in the animal facility (EAH corridor D) will be placed in the autoclave bags and sprayed with 1% virkon before being removed for autoclaving. They will then be autoclaved within EAH corridor D building before disposal by incineration.

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y
The proposal has been extensively reviewed by the local Biological Agents and Genetic Modification Safety Committee and is now considered to be acceptable for notification.

Please enter comments on the GM safety committee on the risk assessment.

The aim of the project is to evaluate immune responses and protective efficacy induced by recombinant Newcastle disease virus (rNDV) expressing either bovine (B) or...
human (H) respiratory syncytial virus (RSV) fusion (F) glycoprotein (rNDV-F) in calves. The rNDV has been produced by Dr*, Mt Sinai School of Medicine, New York, USA. The RSV F gene has been inserted between the P and M genes of the Hitchner B1 strain of NDV. Calves will be inoculated intranasally (i.n.) or i.n. and intratracheally (i.t.) with rNDV-F and analysed at intervals for excretion of NDV, induction of RSV-specific immune responses, and clinical signs of disease. Vaccinated calves will be challenged with BRSV to determine the effect of vaccination on BRSV replication in the nasopharynx and lungs, and the development of pulmonary pathology.

Recipient or parental organism

The Newcastle disease virus (NDV) to be used is the lentogenic, avirulent Hitchner B1 strain, which is a licensed live vaccine virus, available for use in the UK.

Host/vector system

NDV is an avian paramyxovirus serotype 1 (APMV-1), and belongs to the Avulavirus genus within the Paramyxoviridae family. NDV is classified as ACDP CL2 and has been reported to cause short-lived conjunctivitis and 'flu-like symptoms in man. Many of the reported instances of NDV infection in humans have been the result of direct inoculation into the eye, either by laboratory workers or those handling vaccines. Reports of 'flu-like illnesses associated with NDV are rare. Both virulent (mesogenic) and avirulent (lentogenic) strains of NDV appear to be equally able to cause conjunctivitis in humans. However, both mesogenic and lentogenic strains of NDV have been tested in man as oncolytic viruses and cancer vaccines with no adverse effects, signs of evolution towards human pathogenicity, recombination or transmission from human to human. Furthermore, virus could not be recovered from either the nose or tracheal lavage of African green monkeys that had been inoculated intranasally and intratracheally with a lentogenic strain of NDV expressing the HN protein of human parainfluenza virus-3 (HPIV3).

Lentogenic strains of NDV are used as live vaccines in poultry. The Hitchner B1 strain is not classified as a specified animal pathogen by Defra (see page 133 of http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcamp/part2.pdf) and is licensed for use in the U.K. Statutory action to control NDV is taken only for strains which are classified as "virulent" (http://archive.defra.gov.uk/foodfarm/fermanimal/diseases/vetsurveillance/profiles/documents/sp-newcastle disease.pdf). NDV does not appear to infect cattle and vaccine strains of NDV are highly attenuated in experimentally infected calves. Although, NDV has been isolated from pigs with an influenza-like illness, experimental inoculation of piglets with a strain of NDV that resembles the B1 strain did not produce any clinical signs of disease.

Origin & function

The RSV F protein mediates fusion of the virus envelope with the cell membrane and fusion of infected cells with adjacent uninfected cells. The F protein is the major protective antigen of RSV and does not contain any potentially toxic or oncogenic sequences. Inoculation of mice or cattle with eukaryotic expression plasmids expressing the RSV F gene under the control of the HCMV promoter does not result in any harmful effects. There is no evidence that the RSV F protein activates TLR4 and induces the production of pro-inflammatory cytokines. However, no adverse effects have been seen following vaccination of small laboratory animals or cattle with recombinant virus vectors expressing the F protein.

Evaluation of foreseeable effects

RSV and NDV are related iruses of the Paramyovidae family and cause respiratory disease in their respective host. rNDV-F expresses the native NDV F and HN proteins, which are involved in viral attachment and invasion of cells, and does not incorporate the RSV F protein into the virion. Therefore, expression of the HRSV or BRSV F protein will not be expected to alter the host range or tissue tropism of NDV. Furthermore, a lentogenic strain of NDV expressing the HN protein of HPIV3, which is more closely related to NDV than RSV, was highly attenuated in the nose and lungs of African green monkeys that had been inoculated intranasally and intratracheally with high titres of the recombinant virus.

If accidentally released into the environment, rNDV-F could infect susceptible individuals, but is unlikely to cause disease in livestock or poultry. Thus, following i.n. and i.t. inoculation of calves with high doses of a lentogenic strain of NDV expressing the bovine herpesvirus-1 (BHV-1) gD protein, NDV was not isolated from nasal swabs of any of the animals and none of the animals developed clinical signs of disease, even when the gD protein was incorporated into the NDV virion. Furthermore, there is evidence that insertion of a foreign gene between the P and M genes of lentogenic strains of NDV attenuates the virus further for poultry. Thus, rNDV (Hitchner B1 strain) expressing an influenza virus HA gene is attenuated in embryonated chicken eggs, in contrast to the parent wild-type. Similarly following oculonasal inoculation of 1-week-old SPF chickens with 10^6 EID50 of a lentogenic vaccine strain of NDV expressing influenza virus H5 HA gene, replication of rNDV-H5 was attenuated in the lungs and oropharynx compared with the control rNDV, rNDV-H5 was not detected in cloacal swabs, and there were no clinical signs of disease.
Recombination between single-stranded, negative-sense RNA (ssRNA(-)) viruses such as NDV is rare. The ssRNA(-) genome of NDV is found exclusively in a RNAse-resistant nucleocapsid that also contains the viral polymerase. Based on studies with other ssRNA(-) viruses, the potential for intermolecular recombination during mixed infection with closely related parainfluenzaviruses appears to be very rare. However, analysis of the genome of NDV isolates became infected with the GMM and a closely related virus and recombination occurred, transfer of the RSV F gene is unlikely to alter the tropism or virulence of the related micro-organism as virulence of paramyxoviruses is multigenic with the GMM and a related virus, it may be possible that pseudotype viruses could be produced in which the genome of one may be packaged in a virion which includes the surface glycoproteins expressed by the GMM. Such virus particles are unlikely to have an altered cell tropism as they infect similar pulmonary epithelial cells, and their progeny would be that encoded by the viral genome.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated laboratory plastics will be incinerated and contaminated glassware will be autoclaved. Contamine glass pipettes will be decontaminated with 2% hyperchlorite. Spillages and contaminated surfaces will be decontaminated with 2% Virkon for a minimum of 10 minutes. (http://indianjournals.com/ijor.aspx?target=ijor:ijps&volume=45&issue=1&article-021) (100% kill).

Waste animal beddig will be incinerated (100% kill). Large animal rooms will be fumigated with formaldehyde (100% kill). Animal carcasses will be incinerated (100% kill).

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

This assessment was reviewed and approved by the Biological Agents and Genetic Modification Committee, who agreed it should be modified as a stand-alone activity

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02/03/2022
The purpose of these experiments is to determine if the PB1-F2 protein which is extensively conserved in avian influenza strains is a virulence factor in the infection of poultry by influenza viruses. This will help understand this protein's interactions with the host and elucidate whether the PB1-F2 protein is a valid target for anti-viral strategies. We will use reverse genetics to generate avian influenza strains that express the PB1-F2 protein and isogenic viruses that do not and assess their fitness and probe their interactions in vitro and in vivo using poultry lines.

We will generate recombinant avian influenza viruses which possess:
- The surface proteins (HA & NA) of the attenuated vaccine strain A/PR/8/34 (H1N1) and the internal genes (M, NS, PB2, PA & NP) of either;
  1) A/Turkey/Turkey/01/05 (H5N1)
  2) A/Turkey/England/50-92/91 (H5N1), or
  3) A/Chicken/Pakistan/UDL-1/08 (H9N2).

This will generate 2:6 recombinant influenza viruses safe to use at containment level 2. These viruses will then have various mutations made to them in the PB1 segment to truncate or abolish the expression of the PB1-F2 protein which is in a +1 frame on the PB1 gene. At no time with the HA & NA if H5N1 influenza viruses (either LPAI or HPAl) be rescued in conjunction with these genes. Therefore no complete H5N1 viruses will be generated in this process.

These viruses will be assessed for replication, innate immune response induction, pathogenicity and transmission in vitro in cell lines and ex vivo tissue sections as well as
in vivo in poultry species.

**Host/vector system**

cDNA clones will be generated in E. coli and plasmids containing the above mentioned influenza genes will be transfected into cells of eukaryotic origin (avian or mammalian) to rescue infectious virus.

**Origin & function**

The following cDNA constructs will be provided by Professor W B of Imperial College London, these plasmids have been sequenced; HA & NA from A/PR/8/34, M, NS, PB1, PB2, PA and NP from A/Turkey/Turkey1/05 and A/Turkey/England/50-92/91.

The cDNA constructs M, NS, PB1, PB2, PA and NP for generation of the A/Chicken/Pakistan/UDL-01/2008 will be generated by de novo synthesis by GeneArt (Invitrogen) according to our sequences.

Alterations in the nucleotide sequence of the various PB1 genes will either truncate the expression of PB1-F2 or completely abolish its expression. These alteration will be generated by site directed mutagenesis of the PB1 gene segments. These alterations are not expected to increase the pathogenicity or alter the host range of these viruses.

Once generated these viruses will be used in vitro to assess in avian derived cell lines by measuring replication, innate immune response antagonism and looking at interacting partners and then taken in vivo in poultry species to assess fitness in the whole animal model and whether transmission is effected.

**Evaluation of foreseeable effects**

The hazards directly associated with this class of GM influenza virus are small because they bear the surface antigens of the attenuated vaccine strain PR8 virus and thus are highly unlikely to infect a human or transmit. The main hazard would be from inadvertent co-infection of a cell culture with these viruses and other viruses that bear human or avian influenza surface antigens. This will be minimized by either working with these viruses in a separate MSC and incubator than used for other routine work with influenza viruses or fumigation of the MSC prior to use.

Clear signage on the outside of the hood will be used to indicate the last time the hood was used and which virus was used. We eliminate the possibility that workers are 'mixing vessels' by our OH policy of seasonal influenza vaccination. These viruses remain susceptible to both neuramidase inhibitor used routinely as anti-viral treatment for human influenza infection. Mutation of the PB1 gene segment to remove the expression of PB1-F2 or truncate it should reduce any risk from these viruses as PB1-F2 is thought to be a virulence factor.

The hazards to the environment are small from these GM influenza viruses due to the HA & NA from the vaccine strain PR8. This HA & NA combination should be capable of infecting avian species but the control measures in place in both the CL2 in vitro lab (G1B) and EAH are such that the virus will be contained appropriately. This HA & NA combination will render these viruses low pathogenic in nature in avian species. Manipulation in G1B will be carried out exclusively inside a class II MSC, transportaion will be under double containment, liquid waste will be decontaminated via contact with 1% Virkon for at least 10 minutes and all associated waste directed to autoclave. Poultry infected with these viruses inside the EAH will be done so in containment level 2 rooms and housed inside isolators. Every room in EAH has rodent barriers the input and output air is filterd and all waste and drainage liquid is autoclaved. Poultry to be infected will be screened for pre-infection by Influenza virus by looking for the presence of Influenza antigens or antibodies. Experiments will not be prformed in animals previously infected with Influenza virus preventing the chance of a natural reassortment event in poultry. It is highly unlikely that the animals would be exposed to Influenza prior to the experiment as all poultry are reared on site at IAH under good biosecurity. Mutation of the PB1 gene segment to remove the expression of PB1-F2 or truncate it should reduce any risk from these viruses as PB1-F2 is thought to be a virulence factor.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
**Stewart building lab G1B - in vitro assays**

In vitro assays with live virus will be carried out exclusively in a class II MSC cabinet. Cabinets are routinely serviced and tested on an annual basis by an outside contractor and service records are maintained. The risk to laboratory workers can be virtually eliminated by carrying out all work with potentially infectious samples, virus or infected material, in a class II microbiological safety cabinet (MSCII).

- All workers will wear appropriate protective clothing.
- All work in the MSCII will be carried out in benchcote.
- All items will also be sprayed with 70% Ethanol before removal from the MSCII. Items which cannot be directly sprayed with ethanol before being removed from the MSCII will be placed in plastic storage containers prior to removal, the containers will be sprayed before removing from the MSCII.
- Disposable equipment should be used where possible and filtered tips should be used on pipettes.
- Contaminated waste from the hoods will either be;
  (a) soaked in 1% Virkon for at least 10 minutes and then disposed of through the incineration route for laboratory waste or
  (b) disposed of directly into a double bagged autoclave bag which will be sealed with tape inside the hood and placed directly into an autoclave tin. The autoclave tin will be marked with G18 and INFLUENZA VIRUS before being taken to microbiological services for autoclave.

Infected material will be transported from the MSCII to the destination within a sealed container. e.g. plastic storage containers to incubator, sealed rotors to centrifuges. All items will be sprayed with 70% Ethanol before removal from the MSCII.

Infected tissue culture will be incubated in a designated CO2 incubator. Cells will be transferred to the incubator in a sealed container, once in the incubator the lid will be unsealed and removed to allow circulation of CO2. This will prevent spills and drips whilst inside the incubator also.

Influenza viruses are routinely grown in 10-day old embryonated hen's eggs

- Allantoic fluid will be harvested within the MSCII by cracking the top of the egg shell (placing a disposable pipette tip in the hole made previously by the egg gun) and removing the disc of shell with blunt forceps. The embryo is pushed to one side with disposable equipment (pipetter or wooden spatula), the allantoic fluid is removed using a jumbo pastette or disposable pipette and placed into 50ml falcon tubes.
- Eggs are discarded into a double autoclave bag within the MSCII which is sealed then placed into a metal tin for autoclaving. The tin should then be marked with contents (EMBRYONATED EGG WASTE) and taken immediately to microbiological services for processing.
- Any non-disposable instruments (e.g. metal forceps will be left in 70% Ethanol for 30 minutes and then sent for autoclaving).

All infective material will be autoclaved, incinerated or otherwise rendered sterile, unless being transported to another CL2 facility or being put to storage. Infective material will not be voided through the drain system, but inactivated with either autoclaving or using 1% Virkon. Where possible disposable consumables will be used. Non-disposable items will be soaked in 1% Virkon for the required contact time, any attached labels removed and transferred to the Microbiological Services Department (in the same building) in labelled lidded metal tins for autoclaving and disposal.

---

**Is an emergency plan required according to regulation 20?**  N

**If yes, tick to confirm that it is attached to this form**  N

**Tick to confirm that you have attached a risk assessment to this form**  Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  N
The aim of the project is to understand the biology of recombinant Newcastle disease virus (NDV) vaccine strain (LaSota) expressing either fusion 9F) or haemagglutination-neuraminidase (HN) genes, single or jointly, closer to the promoter-proximal region of the genome. Growth kinetics of these constructs will be evaluated in the chicken and duck cell culture systems. Having an improved NDV vaccine candidate in hand, genes of viruses will be introduced into this recombinant La Sota strain. The
V protein, which is a non-structural protein of NDV, will be knocked out from vaccine strain of NDV to investigate the effect on the pathogenesis of the virus. The NDV infectious clone used in this work is tagged with GFP marker and has been produced by Dr Ben Peeter at central veterinary laboratory, The Netherlands. The modified viruses along with vaccine strain of NDV (La Sota) will be used to infect chicken and duck cell-lines to understand the role of these proteins playing in the replication and expression of the surface glycoproteins. The rescued viruses will be inoculated in 9-days old embryonated eggs to propagate and to make a stock. The same stocks will be used for quantification and titration purposes.

Recipient or parental organism

The vaccine strain of NDV to be used in this proposal is the lentogenic, avirulent (non-pathogenic) La Sota strain, which is a licensed live vaccine virus and is being used in Major endemic countries. NDV has extensively been used as vaccine vector for both veterinary and medical viruses with satisfactory results without change in the pathogenicity of the vaccine strain of NDV (Bukreyev, et al. 2005). Moreover, the genomic rearrangement presented in this work has already been made in other paramyxoviruses of medical origin such as vesicular stomatitis Virus and Respiratory Syncytial Virus (Krempl At Al 2002). Such genetic rearrangements did not effect the pathogenicity of these medical viruses (Wertz, Et al 1998). It is therefore plausible to expect that such rearrangement in NDV, a similar virus but of veterinary importance, will not effect its pathogenicity.

Host/vector system

The NDV is an avian paramyxovirus serotype 1 (APMV-1), and belongs to the Avulavirus genus within the Paramyxoviridae family. Vaccine strains of NDV are classified as ACDP CL2 and has been reported to cause short-lived conjunctivitis symptoms in man (Capua and Alexander, 2004). Many of the reported instances of NDV infection in humans have been the result of direct inoculation into the eye, either by laboratory workers or those handling vaccines. However, reports of illnesses associated with NDV are rare. Both virulent (mesogenic) and avirulent (lentogenic) strains of NDV appear to be equally able to cause conjunctivitis in humans. However, both mesogenic and lentogenic strains of NDV have been tested in man as oncolytic viruses and cancer vaccines with no adverse and effects (Zamarin and Palese, 2012). Signs of evolution towards human pathogenicity, and recombination or transmission from human to human. Furthermore, virus could not be recovered from either the nose or tracheal lavage of African green monkeys that had been inoculated intratracheally with a lentogenic strain of NDV expressing the HN protein of human parainfluenza virus-3 (HPIV3) (Bukreyev, et al 2005).

Lentgenic strains of NDV are used as live vaccines in poultry. The hitchner B1 strain, which is very similar to LaSota strain in terms of pathogenicity and genome characteristics, is not classified as a specified animal pathogen by Defra and is licensed for use in the UK. NDV does not appear to naturally infect cattle, and vaccine strains of NDV are highly attenuated in experimentally infected calves. Although, NDV has been isolated from pigs with an influenza-like illness, experimental inoculation of piglets with a strain of NDV did not produce any clinical signs of disease. Genomic rearrangements will likely to attenuate the vaccine strain of NDV as it did in medical viruses such as vesicular stomatitis Virus and Respiratory Syncytial Virus (Krempl et al 2002; Wertz et al 1998).

Origin & function

Three Genes, Presented in this proposal, will be modified. Briefly, (i) the HN protein determines the host tropism by binding to sialic acid receptor of the host. (ii) the F protein mediates fusion of the NDV envelope with the cell membrane and fusion of infected cells with adjacent uninfected cells. Both HN and F protein are the major protective antigens of NDV and do not contain any potentially toxic or oncogenic sequences. (iii) the V protein is a non-structural protein which is known to involve interferon regulations activities.

Evaluation of foreseeable effects

Genomic rearrangement may lead to attenuation of the LaSota vaccine strain of NDV as it has been experienced with viruses of same family. Since the same gene of LaSota vaccine strain will be moved to promoter proximal region, it is unlikely that the resultant virus will change any tropism. Moreover, all the modifications, presented in this protocol, will be made only in the vaccine strains of NDV. Based on the reported studies, it is very less likely that these recombinant NDV vaccine viruses will have increased virulence (Wertz et al 1998; Krempl et al 2002). Examples are available indicating the expression of any foreign gene in NDV can lead to attenuation in the animals that had are available indicating the expression of any foreign gene in the NDV can lead to attenuation in the animals that had been inoculated intranasally and intratracheally with hightitres of the recombinant virus (Bukreyev, A E et al 2005).

Recombination between single-stranded, negative-sense RNA ssRNA(-)) viruses such as NDV is rare. The ss RNA9(-) genome of RDV is found exclusively in a
RNAse-resistant nucleocapsid that also contains the viral polymerase. Based on studies with other ssRNA(-) viruses, the potential for intermolecular recombination during mixed infections appears to be very rare. However, analysis of the genome of NDV isolates from China suggests that homologous recombination of NDV can occur naturally, which is a rare event.

In the unlikely event that this genomic rearrangement will alter the tropism of the NDV since gene of the same vaccine will be moved to promoter region, Virulence is unlikely to increase because pathogenicity in NDV is determined by the cleavage site in the F protein and we are not intended to mutate cleavage site in this proposal. Moreover, such rearrangements in other paramyxoviruses including vesicular stomatitis virus and respiratory syncytia virus have not increased the virulence of the recombinant viruses but has induce the protective immune responses, collectively, indicating least possible hazard associated.

The V protein of NDV is associated with interferon antagonism. Generation of LaSota NDV, Not expressing V Protein, is very much likely to be attenuated due to immune responses produced against NDV in infected cells. Therefore, there appears to be no adverse forseeable effects with this modification.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated laboratory plastics will be decontaminated by 1% Virkon for 30 minutes prior to disposal by incineration and contaminated glassware will be autoclaved. Any tips, flasks, or there plastic wear that has been exposed to virus will also be decontaminated prior to disposal by incineration. Spillages and contaminated surfaces will be decontaminated with 2% Virkon for a minimum of 30 minutes.

**Project Containment**

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Animal Units Large Scale Activities Human Clinical Applications

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Reviewed by the BAGMSC and Dr M B (a paromyxovirus research group leader) on 26/11/13 and approved at GM2.
The use of recombinant Murine Cytomegaloviruses as vaccine vectors

To develop the use of MCMV as vaccine vectors against human and livestock diseases.

Recipient or parental organism
Mice

Host/vector system
Murine cytomegalovirus

Origin & function
Nucleoprotein (NP) and matrix (M1) proteins of X31 Influenza Virus.
The intended function is to induce a durable immune protective immune response against Influenza viruses.

Evaluation of foreseeable effects
MCMV does not pose any harm to human health. MCMV has been shown to infect human cells in vitro, but infection leads to an abortive replication cycle. Accidental infection could induce immune response to MCMV and inserted transgenes, which would not be harmful.

Release of rMCMV into the environment is very unlikely due to the standard procedures employed in the lab or animal house. However even if such an event occurred,
although rMCMV could infect wild mice (prevalence of wild type MCMV infection in wild mice is 65-90%) this is unlikely to occur because rMCMVs are attenuated and naturally infected wild mice should be cross protected against rMCMV. Furthermore even if infection did occur, attenuated rMCMVs are very unlikely to cause any pathology. The risk of infection of any other species is extremely low.

MCMV is an enveloped virus and therefore has poor survival in the environment. MCMV transmission is thought to require direct contact with contaminated excreta/secretions and cross-contamination between cages has so far not been reported.

The inserted genes are internal proteins from Influenza viruses to which humans are frequently exposed and which are known to induce immune responses. As stated above, because the risk of rMCMV infecting humans is minimal the risk of recombination between the transgene and human Influenza Virus is extremely low. Modified vaccinia Ankara or Adenovirus expressing NPM1 has been administered already to mice, pigs, chicken and humans without ill effects and these genes are present in the influenza vaccines, administered to humans.

In the case of the MCMV infected animals being challenged with Influenza virus there is no increased risk of these animals excreting a virus that could be transmissible to people compared to any other mouse strain. We shall use either A/PR/8/34 or PR8 based viruses. These challenge viruses pose very little risk for humans since PR8 is poorly infectious in people. The animals will be handled in isolators so that the risk is minimal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated laboratory plastics will be incinerated and contaminated glassware will be autoclaved. Spillages and contaminated surfaces will be decontaminated with 2% Virkon for a minimum of 10 minutes. All waste will be disposed following MSD-113.

All waste and rubbish from the animal house should be placed in an autoclave bag. This should then be double bagged and sprayed with 1% Distel before it is removed from the room. The waste should then be autoclaved before being placed in yellow clinical waste bags. These yellow bags should then be placed in the yellow bins for incineration. Staff handling the mice will shower out on exit of the unit.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Reviewed and approved at BAGMSC 8/7/14 as a GM2 project

Project Containment
Project Ref 97/15.1

Date Ackn’d 01/04/2015

Date Project Ceased 28/07/2015

CU2 Project Title
Creation and use of reverse genetics systems to understand virion formation and genome encapsidation mechanisms exploited by endemic avian viruses

Class 2
CultureVolClass2 Class CultureVolumeClass3-4
< 1 L

Non-GMM
Consent Granted

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Transferred to GM53 28/07/2015

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 97/96.1

Date Ackn'd 01/02/1998

CU2 Project Title RNA VIRUSES: PARAMYXOVIRUSES (CPW 6.9)

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

02/03/2022
**Date Project Ceased**

- Withdrawn: N
- Tick if notifying a connected programme of work: N

**Historical Significant Changes**

- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Non-GMM Consent Granted**

- not applicable

**Project notified under transitional arrangements**

- Y
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref 97/98.1

Date Ackn’d 12/06/1998

CU2 Project Title INFECTIOUS BOVINE VIRAL DIARRHOEA VIRUS FROM CLONED CDNA

Date Project Ceased 28/07/2015

Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Transferred to GM53 28/07/2015

Date of Significant Change 02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
## Project Ref 97/98.10

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### Date Project Ceased

06/01/2016

### Withdrawn

N

### Tick if notifying a connected programme of work

N

### Historical Significant Changes

Transferred to GM53 on 06/01/2016

### Project Additional Information

#### Purposes of the contained use

- [ ]

#### Recipient or parental organism

- [ ]

#### Host/vector system

- [ ]

#### Origin & function

- [ ]
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Large Scale Activities

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Human Clinical Applications

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Project Containment

Project Ref 97/98.11

Date Ackn'd 01/02/1998
CU2 Project Title PROTOZOAN PARASITES (CPW 3)
Class 2
CultureVolClass2
CultureVolumeClass3-4

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Date Project Ceased

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Withdrawn

N

Non-GMM Consent Granted

Project notified under transitional arrangements

Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 97/98.2a

Date Ackn’d: 01/02/1998
CU2 Project Title: LARGE DNA VIRUSES: AVIPOX VIRUS (CPW 4.2)
Date Project Ceased: 
Class: Class 2
CultureVol: Class 2
CultureVolume: Class 3-4
Non-GMM Consent Granted: not applicable
Project notified under transitional arrangements: Y

Withdrawn: N
Tick if notifying a connected programme of work: N
Historical Significant Changes: GM97/99.2, GM97/00.8
Historical Date of Additional Info: 21/05/1999, 10/08/2000.
Significant Change ID: 
Date of Significant Change: 

21/05/1999, 10/08/2000.
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? \( N \)

If yes, tick to confirm that it is attached to this form \( N \)

Tick to confirm that you have attached a risk assessment to this form \( \) \( N \)

Tick if you are claiming exemption from disclosure for section of the risk assessment \( N \)

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
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02/03/2022
**Project Ref** 97/98.5

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- **Non-GMM** Consent Granted: yes
- **Project notified under transitional arrangements**: Y
- **Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Is an emergency plan required according to regulation 20? \( \text{N} \)

If yes, tick to confirm that it is attached to this form \( \text{N} \)

Tick to confirm that you have attached a risk assessment to this form \( \text{N} \)

Tick if you are claiming exemption from disclosure for section of the risk assessment \( \text{N} \)

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 97/98.7

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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- **Human Clinical Applications**: L2 L3 L4

### Project Ref 97/98.9

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**Historical Significant Changes**
- GM97/00.4, Transferred to GM53 28/07/2015

**Significant Change ID**
- 10/04/2000,
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

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Name
UNIVERSITY OF CAMBRIDGE

Name 2

Department
BIOCHEMISTRY

Campus Estate or Research Centre
THE DOWNING SITE

Road Name
TENNIS COURT ROAD

Town
CAMBRIDGE

County
CB2 1QW

Country
ENGLAND

Tel Number
01223 333600

Fax Number
01223 333345

Comments

Date at Which Additional Info Submitted
02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Kid protein is toxic to mammalian cells and Kis protein protects from this effect. When they are expressed together in mammalian cells, death or cell survival depends on the ratio between Kid and Kis that cells are able to sustain. This is potentially a therapeutic or experimental tool. The ratio can be controlled at the level of transcription, mRNA stability and protein stability. We want to exploit the genetic differences between normal cells and cancer cells to regulate, differentially, the Kid-to-Kis ratio in these cells, so that cancer cells are killed and normal cells are protected from toxicity.

Recipient or parental organism

Murine and human cells (primary cells, cancer cell lines and immortalized cell lines)

Host/vector system

1) E. coli, laboratory K12 derivatives/pUC based vectors;

2) Vertebrate tissue culture cells, human and mouse, including as examples 293T cells, murine-embryo-fibroblasts and HeLa and other human cancer cell lines and immortalised normal cells; and primary cultures of human and mouse cells.

3) Adenoviral vectors and Lentiviral vectors (the latter with polytropism through viral pseudo-typing using VSV-G protein)

3.1.) Adenoviral system: The adenoviral system to be used employs a well-characterised vector and packaging cell lines (Refs below). Second generation replication defective human adenovirus serotype 5 derived adenoviral vectors will be used for expression of Kis and/or Kid proteins. Vectors are rendered replication defective by the deletion of the E1 and E3 genes. The E1 gene is essential for the assembly of infectious virus particles and is complemented in vivo by an adenovirus packaging cell. The E3 gene encodes proteins involved in evading host immunity and is dispensable (Luo et al, Nature Prot., 2 (5): 1236 (2007))

3.2.) Lentiviral systems: the two types of viral system to be used employs two well-characterised lentiviral vector and packaging cell lines (Refs below).

3.2.1.) Type A (eg. pLenti6-UbC-V5DEST from Invitrogen):

- The number of genes from HIV-1 that are used in this system has been reduced to three (i.e. gag, pol, and rev).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replicant-competent virus (Dull et al., J Virology 72 8463-8471 1998).
- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain LTR's or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced,
- The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.
Expression of the gag and pol genes from pLP1 has been rendered Rev-dependant by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev.

A constitutive promoter (RSV promoter) has been placed upstream of the 5 LTR in the lentiviral vector to offset the requirement for Tat in the efficient production of viral RNA. The 3LTR region has a deletion that promotes self-inactivation of the viral genome upon integration in the host genome.

3.2.2. The lentiviral packaging systems mentioned above (type A) is a third generation versions that utilize split-genes to provide the viral packaging elements on individual plasmids that physically separate the viral envelope, env (usually VSV-G), sequence from the gag-pro-pol sequences. These split-gene packaging strategies reduce the risk of generating RCL because multiple recombination events are necessary to create a virus that harbors the sequences required for independent replication.

Type B (Clontech’s Lenti-X HT) Packaging System also uses a split-gene packaging strategy, but adds another level of safety by further uncoupling pol (RT and IN) from gag-pro. The result is that gag, pol and env reside on three physically distinct entities, rather than the standard two. This approach further reduces the possibility of creating RCV to a level below that of standard 3rd generation packaging systems, because extra recombination events are required to create such viruses. In fact, the emergence of RCV is undetectable from systems using this approach (Wu, X., et al. (2000) Mol. Ther. 2 (1):47-55). These improvements significantly increase the safety profile of our Lenti-X HT Packaging System.

Clontech’s Lenti-X Vectors contain less than one-third of the wild-type HIV-1 genome. These wild-type sequences mainly consist of the viral LTRs and packaging signal. All essential replication genes have been completely removed and are instead supplied as separate DNA entities in the Lenti-X HT Packaging Mix (described above).

In brief, the number of genes from HIV-1 that are used in type B system has been reduced to four (i.e. gag, vpr-pol, rev and tat).

- Genes encoding the structural and other components required for packaging the viral genome are separated onto five plasmids. This reduces that incidence of replication competent lentivirus to levels below that of the system A above.
- Although the four packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain HIV-1 LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
- The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.
- Expression of the gag, tat and rev genes from the packaging plasmids is directed by tetracycline dependent promoters (PrTRE). Therefore their expression requires supplying TetR-VP16 in trans, and it is only active in the absence of doxycycline in the growth medium.
- In this system, expression of tat is required in the packaging cell line, because the lentivirus vector contains an intact HIV-1 5LTR, which allows very high titers in viral preparations.

Origin & function

The overall purpose is to introduce Kis and Kid protein expression into mammalian cells, control that expression and monitor expression. Inserts will encode reporters, such as EGFP, Ds Red; transcriptional regulators such as TetR; prokaryotic genes encoding ribonuclease Kid and its counteracting protein Kis; and transcription and post-transcription-regulating sequences of prokaryotic and mammalian origin.

Genes Kis and Kid will include wildtype, inactive mutants and fusion variants (e.g. fused to unstable protein domains), and they will be expressed under the control of viral and mammalian promoters, both constitutive (e.g. PrCMV, PrUbC, PrEF-Ialpha) and/or repressible (e.g regulated by TetR).

Evaluation of foreseeable effects

Some of the virus vectors will kill infected mammalian cells, so some of the vectors will be toxic to human and mammalian cells. Also because of the presence of the WPRE element in the lentivirus vectors, the lentivirus constructs might transform infected mammalian cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Adenovirus vectors: (Risk assessment H2008-009)

This individual RA H2008-009 is an assessment for adenovectors, and so the main issue is whether, and in what way, the inserts are hazardous to human cells. Oncogenic inserts would be Class 2, most other inserts Class 1. As some of the inserts are toxic, we have chosen precautionary Class 2 containment for the active toxic insert work only. The majority of the work proposed will be concerned with establishing control of expression and this can be done with inactive mutants, in Class 1.

The principal concern here is that, for some of the inserts, the virus generated and the packaging/producer cells are effectively toxins that could kill one human cell per virus particle, though the act of killing the cell would disable the virus. Handling and storing the producer cells and supernatants is therefore similar to handling cholera toxin, ricin, or phalloidin. Those involved must be made aware of this.

A subsidiary concern would be propagation of the virus, but the virus is sufficiently disabled to eliminate this. E1/E3 deleted adenovirus is generally accepted as disabled and, with wild-type adenov being HG2 this allows the mutant to be HG1. Once the virus has infected a target cell those cells pose no particular hazards, since the toxin involved is intra cellular.

Lentivirus (RA H2009-002)

The issues in this work are (i) the expression of the toxin Kid in lentiviral vectors, and (ii) the lentivirus vectors themselves, which, if they carry the complete WPRE, are potentially oncogenic and current guidance is to use them in CL2 regardless of their tropism. Both these have been considered by us before. (i): toxin-expressing human host range virus is toxic to cells in a similar way to a strongly toxic lab reagent and should be treated as such. They are borderline CL1/CL2 but we have previously chosen to recommend CL2. (ii) The lentivirus work does require CL2 when the vectors incorporate the putatively-oncogenic complete WPRE element. The E.coli work raises no particular issues as the vectors do not express in bacteria.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.

The construction work in E. coli is correctly assessed as Class 1.

Project Containment
### Project Ref  98/00.1

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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<td>L3</td>
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Project Ref 98/02.1

<table>
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<tr>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>04/02/2002</td>
<td>DEVELOPMENT OF ANTI-ANGIOGENIC STRATEGIES FOR THE TREATMENT OF</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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## Project Additional Information

### Purposes of the contained use

| Preparation of DNA plasmid vectors in prokaryotes for delivery of DNA into mammalian cells in vitro. |
| Expression of cloned DNA in mammalian cells and generation and use of recombinant adenoviral vectors. |

### Recipient or parental organism

| In vitro and in vivo transfection and infection. |

### Host/vector system

| Replication-deficient adenovirus encoding specific genes |

### Origin & function

| Human and/murine promoter/enhancer fragments under the control of which will be placed various human and/or murine genes to be expressed in a tissue-specific manner. |
| B-galactosidase (galactosidase cleavage from substrate) to act as a reporter. |

| These genes/gene fragments will be used to generate plasmids suitable for transfection into mammalian cells in vitro for expression and functional analysis and for subsequent use in adenoviral vectors. |

### Evaluation of foreseeable effects

| Likelihood of microorganism survival outside laboratory environment is negligible. Defective E. coli strains are used throughout. DNA will not survive in the environment. Vectors could not replicate in mammalian cells in the unlikely event of entry. |
| The basic strategy directed towards eventual use in humans utilises adenoviral vectors that have already been extensively tested in human clinical trials with no reported adverse effects. |

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The work proposed is in two categories:
Class 1 activities (preparation of DNA plasmid vectors in prokaryotes for delivery of DNA into mammalian cells in vitro)
Class 2 activities (expression of cloned DNA in mammalian cells and generation and use of recombinant adenoviral vectors).

For Class 1 activities solid waste will be autoclaved to achieve 100% killing (autoclaves undergo annual validation tests); Liquid waste will be treated with 1% Virkon (w/v) for minimum of 12 hours, as per the manufacturer's instructions.
For Class 2 activities solid waste will be autoclaved to achieve 100% killing (assayed periodically by microbiological analysis: autoclaves undergo annual validation tests); liquid waste will be treated with 1% Virkon (w/v) for minimum of 12 hours as per the manufacturer's instructions [Ref: www.anticint.co.uk/main/virkons.htm] to achieve 100% killing, assayed periodically by microbiological analysis.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

The committee were satisfied that the experiments carried out under the conditions proposed present no significant hazard.

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<td>L3 L4</td>
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<tr>
<td>Human Clinical Applications</td>
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</tbody>
</table>

Project Ref 98/02.2

Date Ackn'd 02/03/2022
**Project Additional Information**

**Purposes of the contained use**
To discover the signalling mechanisms by which the pathogen responds to the host with the possibility of discovering new antibiotic strategies.

**Recipient or parental organism**
Bacillus cereus, Bacillus thuringiensis, Bacillus subtilis Non pathogen Group 2  
E. coli K-12 or B derivatives (TG1, TG2, JM101, JM109, XL1-Blue) Pathogen derivative Group 2 disabled host.

**Host/vector system**
Host Cells: Bacillus cereus, Bacillus thuringiensis, Bacillus subtilis Non pathogen Group 2.  
E. coli K-12 or B derivatives (TG1, TG2, JM101, JM109, XL1-Blue) Pathogen derivative Group 2 disabled host.  
Vectors various non mobilisable bacterial plasmids (pGEM, pSelect, p Bluescript; pUC series; pC194, pE194, pBR327, pID408).  
Various non-mobilisable vectors. ( GT10, GT11, ZAP; M13 derivatives).

**Origin & function**
This project will study Bacillus cereus and Bacillus thuringiensis virulence factors and their role in pathogenesis. The genetic modification will be limited to endogenous gene inactivation to investigate cellular function, and transposon tagged mutagenesis to identify genes that are essential for infection of the insect or animal host and/or response to stress conditions. Using in vitro cell culture models, in vivo models and an insect model the project will employ a variety of strategies including in vivo selection techniques (eg signature tagged mutagenesis) and RNA arbitrarily primed PCR, to screen for promoters that are selectively activated during the infective phase. The genes controlled by these promoters will then be identified and an attempt made to discover the signalling mechanisms by which the pathogen responds to the host microenvironments and to map the genetic control pathways directing the sequence of events during infection and death of the host. With the exception of certain plasmid cloning vehicles specified above and their associated antibiotic resistance genes (CHLORAMPHENICOL or AMPICILLIN or ERYTHROMYCIN or KANAMYCYIN or LINCOMYCIN) no exogenous gene will be introduced into Bacillus cereus and Bacillus thuringiensis. The procedure may therefore reasonably be categorised as self-cloning.
Evaluation of foreseeable effects

The only genes to be inserted are contained on the plasmid cloning vehicles specified above including their associated antibiotic resistance genes (CHLORAMPHENICOL or AMPICILLIN or ERYTHROMYCIN or KANAMYCYIN or LINCOMYCIN). No other exogenous gene will be introduced into Bacillus cereus and Bacillus thuringiensis. The procedure may therefore reasonably be categorised as self cloning. Therefore it is not considered that any inserted gene has a biological activity which can act directly to cause harmful effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste generated is autoclaved using an Astell Scientific Swiftlock Programmable autoclave with a program of 15 minutes pulsed free steaming and 30 minutes at 132 degrees C.

Liquid-based waste materials is treated by soaking in 1% final concentration of Virkon for a minimum of 12 hours before disposal via the drains.

Solid biological material is securely packaged and disposed of by incineration via a registered contractor.

Infected insect waste is disposed off under MAFF (DEFRA) approved guidelines.

Infected solid biological waste will be disposed of by autoclaving with a program of 15 minutes pulsed free steaming and 30 minutes at 132 degrees C followed by incineration via a registered contractor.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

The committee agreed that the work posed no significant hazard when carried out with the precautions and containment proposed.

Project Containment

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02/03/2022
## Project Ref

### 98/03.1

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<td>25/07/2003</td>
<td>MOLECULAR BIOLOGICAL AND GENETIC ANALYSIS OF THE MECHANISMS WHICH CONTROL VIRULENCE AND RESPONSE TO ANTIBIOTICS IN PSEUDOMONAS AERUGINOSA.</td>
</tr>
</tbody>
</table>

### Date Project Ceased

- **Class**: Class 2
- **Consent Granted**: not applicable

### Non-GMM

- **Project notified under transitional arrangements**: N

### Withdrawn

- **N**

### Project Additional Information

#### Purposes of the contained use

The project seeks to exploit this newly acquired wealth of information with the aim of developing new anti-pseudomonal drugs.

#### Recipient or parental organism

**SPECIFIC P. aeruginosa STRAINS TO BE USED**

- PAO-1 (wild-type elastolytic prototroph)
- PAO-R1 (non-elastolytic lasR::TcR, SmR derivative of PAO-1)
- PAO-JP2 (non-elastolytic lasl rhll double mutant derived from PAO-1, HgR, TcR)
- PAO-JP3 (non-elastolytic rhll mutant of PAO-1)
- PDO111 (non-elastolytic rhIR mutant of PAO-1)

#### Host/vector system

P. aeruginosa is an opportunistic human pathogen which is responsible for an increasing number of nosocomial infections. It is resistant to most commonly used antibiotics when applied at conventionally effective therapeutic concentrations. The project seeks to exploit this newly acquired wealth of information with the aim of developing new anti-pseudomonal drugs. Although the project is based in my lab, at various stages it will also involve collaborations with the Biochemistry Dept and Chemistry Dept - drug development. No organisms will leave the secure area during these collaborations. The theme of this proposal is two-fold. First, we want to identify gene products that are elevated in PAO-1 as a result of exposure to antibiotics. This will be tested by inactivating the up-regulated genes and looking for increased susceptibility to antibiotic treatment. We hope to exploit the proteins identified in this way as targets for the development of new antibacterial therapies. Second, we want to continue our investigations into the mechanism(s) that control quorum sensing in pseudomonas. These studies mostly involve introducing pre-evaluated reporter gene constructs into PAO-1 and its derivatives or over-expression/purification of PAO-proteins.

**Origin & function**

Our mutants should be impaired for virulence, and be less able to survive in the field than the wild-type organism. The antibiotic resistance-cassettes being used should not affect the treatment of any infection caused by PAO-1 or its mutant derivatives.

There is always a possibility that the random mutagenesis (or even the targeted one - less likely though) will inactivate a repressor of virulence and therefore give rise to hyper-virulent Pseudomonads. However, this hyper-virulence is unlikely to confer upon the organism better survival characteristics, since by definition, the wild-type carries the optimal complement/expression level of genes to ensure survival in the field/host. Any hypervirulent strains that are identified will be destroyed.

**Evaluation of foreseeable effects**

Not applicable.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste and spent cultures will be autoclaved initially (as per above settings). The contaminated glassware following autoclaving will be soaked for 12 hours (completely immersed) in 1% Virkon before rinsing and sending for washing. Solid waste will be autoclaved as per above. The above HSE-approved SOP is highly effective and was designed for disposal of a comparable level 2 pathogen (Serratia marcesens) which is worked on in an adjoining lab within the level 2 secure area of the department.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The committee are satisfied that the project posed no significant hazard, when carried out with the starins, precautions, and containment proposed.

**Project Containment**

02/03/2022
### Project Additional Information

**Purposes of the contained use**

The project is concerned with understanding the mechanisms involved in lineage selection upon stem cell differentiation in the epidermis.

**Recipient or parental organism**

The work requires the use of human keratinocytes which are difficult to transfect by standard methods. Recombinant retroviral infection is therefore needed as a means of delivering the appropriate cDNA constructs into the cells. Human keratinocyte cell lines will be derived from a reliable source of normal infant skin which has been surgically removed from screened healthy individuals having a negligible risk of contamination with adventitious human pathogens.

**Host/vector system**

Constructs will be cloned into the non-mobilisable viral plasmid vector pBabe (puro) and derivatives based on Moloney Murine Leukaemia Virus (Morgenstern and Land 1990, Nucleic Acid Research 18 3587-3596).
RNAi constructs will be cloned into pRETRO SUPER and its commercially available derivatives.

Origin & function

All DNA will be propagated in E Coli (e.g DH10B or derivatives).

The plasmid will first be transfected into an ecotropic packaging cell line. Phoenix-eco, and the retrovirus produced will be harvested over 1-2 days.

The helper-free retrovirus producer cell line Phoenix-eco, was developed by Dr. G. Nolan at Stanford University, (http://www.stanford.edu/group/nolan/index.html) and has been used by over 2500 labs. Phoenix-eco cells express the gag-pol and envelope genes using different non-Moloney promoters to minimize both recombination and inter-typic recombination potential. This cell line has a history of safe usage.

This ecotropic retrovirus will then be used to stably infect a second packaging cell line, GP + envAm12, which produces disabled amphotropic retrovirus. The mouse cell line GP +enAm12 has been widely used for the production of amphotropic virus. Tests for the safety of the GP + envAm12 packaging line showed no evidence for the generation of wild type virus and it has a history of safe usage. Markowitz, Goff and Bank. Virology 167, 400-406 (1998). Defective amphotropic virus would then be used for the infection of human keratinocytes. Transduced cell would be selected and maintained in medium containing an appropriate antibiotic before further use in experiments.

All genes will be inserted downstream of the viral LTR with the intention of achieving a moderate level of expression in recipient cells. Infection of keratinocytes will be by transfer of supernatant culture fluids with any concentration of the virus particles. Recipient human keratinocytes expressing the individual genes under study will be created which are likely to have some growth advantage or induce proliferation in an autocrine or paracrine manner.

Evaluation of foreseeable effects

Bacterial strains of Ecoli such as DH10B and its derivatives are not capable of colonising a healthy individual.

The amphotropic retrovirus is able to infect cells from a broad host range, including humans but can not replicate unless it recombines with endogenous retrovirus. Exposure of an individual to amphotropic retrovirus, by possibly a break in the skin, could result in a localised transfer of the pathogenic properties of the particular gene. None of the cell lines would be expected to remain viable outside the controlled culture conditions and we do not anticipate that any of the genetic manipulations we are proposing would alter this.

It is predicted that over-expression of oncogenes and signalling molecules could lead to an increased rate of cell division although tumour progression and disease in the whole organism is unlikely. The major possible source of hazard to the environment would be accidental escape of high titre virus solutions expressing cDNAs, which could then infect rodents. Retroviruses require close contact for their transmission and their survival in the general environment is poor due to their fragile envelope. The vector is non-mobilisable and the successful infection of a cell would include stable integration of the viral genes into host DNA. Although the amphotropic virus would be able to infect humans it could not replicate unless it recombined with endogenous retrovirus. Even then it is unlikely that expression of a single oncogene or inactivation of a tumours, as multiple genetic alterations (between 4 and 6) are required for inducing tumours, and a safeguard mechanisms exist impeding the accumulation of these alterations in the organisms (i.e. single events like Ras or myc expression instead of unrestrained proliferation, induce growth arrest or apoptosis) (Hanahan & Weinberg, Cell 100, 57-70; 2000).

The use of sharps will be strictly controlled and avoided wherever possible. Needles and scalpels will not be permitted. Plastic Pasteur pipettes will be used for aspirating culture medium.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals or plants will be used.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be sealed in double autoclave bags, and autoclaved with steam at 127°C for 25 minutes. Effectively 100% kill. Autoclaves are routinely monitored and records are kept by the department.

All liquid waste will be inactivated by treatment with Virkon at a final concentration of 1% for 12 hours before disposal via the drains. Effectively 100% kill as determined by the manufacturer.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee noted that the consequences are unknown of infection of cells of the worker by the modified virus, and have the potential to lead to cell proliferation. However, the committee felt that the risk is extremely low, because of the method of handling the virus including the avoidance of sharps. Secondly, the rate of infection is likely to be very low as the virus has a low infectivity. This is clear since in vitro infection is increased by optimisation of conditions using eg lipophilic substances. Thirdly, the virus genome will only integrate in proliferating cells, which are relatively rare in the skin.

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Project Ref 98/07.2

Date Ackn’d CU2 Project Title
23/05/2007 Organelle function in Plasmodium.

Class CultureVol
Class 2 ≤ 1 Litre

Class CultureVolumeClass3-4

Page 2953 of 15326
### Project Additional Information

#### Purposes of the contained use
The project aims to study the function of the apicoplast and mitochondrion of the malaria parasite, *Plasmodium falciparum*.

#### Recipient or parental organism
*Plasmodium falciparum* will be cultured in human blood, obtained from the National Blood Service (screened, and of transfusion grade) or from blood donors at the Department of Biochemistry, University of Cambridge. Dead, fixed *Plasmodium* cells will be observed under light, confocal and electron microscopes.

#### Host/vector system
*Plasmodium* vectors containing *E. Coli* and *Plasmodium* origins of replication, two selectable markers and GFP.

#### Origin & function
In order to determine the localisation of proteins, *Plasmodium* genes/targeting regions upstream of genes will be cloned into *E. coli* vectors immediately upstream of green florescence protein gene (GFP, originally from jellyfish, or similar reporter genes). These vectors will also contain selectable markers and origins of replication for *E. coli*, as well as selectable markers and origins of replication for *Plasmodium*. These vectors will be transfected into *Plasmodium*. This will allow the expression of *Plasmodium* proteins fused with GFP in *Plasmodium* cells.

*P. falciparum* genes manipulated will be for components of the apicoplast or the mitochondrion. They will include nuclear and organellar genes. Genes for housekeeping *Plasmodium* proteins of the cytoplasm or nucleus will be used as controls.

*P. falciparum* will be transformed with antibiotic resistance proteins for selection (geneticin, blasticidin-S from *Aspergillus terrus*, zeocin, nourseothricin, phosphinothricin, puromycin or WR99210 (anti-folate)).

No human genes will be manipulated.

#### Evaluation of foreseeable effects
*Plasmodium falciparum* is the causitive agent for human malaria. There is therefore a risk of catching malaria.
The blood in which the parasite is grown will be of human origin obtained from the National Blood Service or known donors within the department of Biochemistry. Blood from the National Blood Service has been screened for blood-borne disease, such as Plasmodium, hepatitis B and HIV.

The inserted/deleted Plasmodium genes will not be harmful towards humans. The genes affected encode proteins effecting key biochemical processes within the Plasmodium cell. The inserted/deleted genes will not make the P.falciparum parasite more pathogenic. It is extremely unlikely that the host or the infectivity will change. The modifications will most likely cause the organism to be less fit than cells without the genetic modification.

The expression of GFP will not make the P.falciparum parasite more pathogenic, or change infectivity, and will most likely cause the organism to be less fit than cells without the genetic modification.

The antibiotic resistance genes are not toxic. The insertion of antibiotic resistance genes will not affect the selection or efficacy of drugs used for treatment of malaria (genes conferring resistance to geneticin, blasticidin-S, zeocin, nourseothricin, phosphinothricin, puromycin or WR99210 to be inserted). Apart from geneticin, none of these are listed in the British National Formulary as treatments for any human disease, and geneticin is not recommended as an anti-malarial.

containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals or plants will be used.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

justification for containment level 2.

P.falciparum is a group 3 pathogen, and as such may require containment level 3. However, the HSE advisory committee on dangerous pathogens, annex 1 lists biological agents that may be used at less than minimum containment conditions required by COSHH. Containment levels can be lowered because of

1. The nature of the work
2. The nature of the biological agent.

P.falciparum is listed as an agent to which this derogation may apply.

Risk assessments would indicate that this it is possible to use containment level 2 for the culturing of P.falciparum in this project for the following reasons:

1. The clonal strain of P.falciparum that has been in continuous blood-stage culture for 20 years. It has known anti-malarial drug sensitivities and a fully sequenced genome. It is not resistant to the major anti-malarial drugs used.
2. Transmission of Plasmodium is by female mosquito. In the wild, the mosquito lands on human skin, pierces the skin and takes up blood. All our blood cultures will be in petri-dishes, so any mosquito that did enter would be unable to feed, so could not acquire parasite. In addition, the strains under study do not regularly form gametocytes. Only the gametocyte form of the parasite life cycle is taken up by the mosquito.
3. The natural mosquito species carrier of P.falciparum is not present in the UK. The nearest population is in the Mediterranean. 
4. Although the UK did have malaria until 1919, this was caused by Plasmodium vivax, which is transmitted by a different mosquito species (Anopheles atroparvus) which is unable to transmit P.falciparum.
5. The laboratory in which we wish to culture P.falciparum is internal within other laboratories, locked, and has no windows.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste (used culture dishes) will be first allowed to soak in 1% Virkon or 10% bleach for 12 hours, followed by autoclaving or incineration. Liquid waste will be treated with 10% bleach or 1% Virkon for 12 hours to kill the parasite, before disposal down the drains.
Please enter comments on the GM safety committee on the risk assessment

CJH01/2007 - Organelle function in Plasmodium. The committee discussed that the organism being used is ACDP group 3.

The committee decided the assessment proposal to reduce aspects of the containment level down to level 2.

1. The organism requires a specific species of mosquito to be transferred to humans. There are none of these insects present.
2. The organism used is a characterised one that has been used for 20 years in culture and is susceptible to standard antimalarial drugs.
3. No sharp implements (needles) would be used in any of the procedures. Glass coverslips will be used only rarely.
4. The proposed culture room is isolated within the building, and is to be kept locked.

The committee agreed that it would be acceptable to derogate the containment to level 2.

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- Animal Units
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- Large Scale Activities
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- Human Clinical Applications
  - L2 L3 L4

Project Ref 98/09.1

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<td>16/07/2009</td>
<td>Structural and molecular studies of the remodelling of human erythrocytes by Plasmodium falciparum</td>
<td>Class 2</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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- Date Project Ceased
- Withdrawn N

Historical Significant Changes

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project notified under transitional arrangements N

Historical Significant Changes
**Project Additional Information**

**Purposes of the contained use**

The project aims to study the role of proteins expressed by the malaria parasite, *Plasmodium falciparum*, in modification of the human erythrocyte cytoskeleton and in clustering of adhesive proteins on the erythrocyte surface.

**Recipient or parental organism**

*Plasmodium falciparum* group 3

*Plasmodium falciparum* will be cultured in human blood, obtained from the National Blood Service (screened and of transfusion grade) or from blood donors at the Department of Biochemistry, University of Cambridge. Dead, fixed *Plasmodium* infected erythrocyted will be observed under light, confocal and electron microscopes.

**Host/vector system**

*Plasmodium* vectors containing *E.coli* and *Plasmodium* origins of replication, ampicillin resistance for selection in *E.coli* and DHFR for selection in *Plasmodium*, as described in:


**Origin & function**

In order to investigate the role of specific protein, and regions of these proteins, mutated parasite lines will be constructed. For protein deletion, the DHRF gene (allowing pyrimethamine or WR99210 resistance) will be cloned in to pGem-based vectors, flanked by sequences either side of the gene of interest. This cloning will be carried out in standard *E.coli* cloning strains. These vectors will be transfected into *Plasmodium falciparum* and selection carried out using pyrimethimine resistance. A similar vector will be used to insert truncated versions of the proteins, allowing investigation of the roles of different regions of the proteins. Dead, fixed cells will be studied by electron, light and confocal microscopy and proteins and nucleic acids will be extracted from modified cells for standard proteomic and molecular biology analysis.
Evaluation of foreseeable effects

The inserted and deleted Plasmodium genes do not code for proteins that are harmful to humans. They will not lead to a selection advantage for the parasite but are likely to lead to reduced pathogenicity.

The DHFR gene is not toxic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No plants or animals used

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Justification for containment level 2

P. falciparum is a group of 3 pathogen, normally requiring containment level 3. However, the HSE advisory committee on dangerous pathogens, annex 1 lists biological agents which may be used at less than the minimum containment conditions required by COSHH. Containment levels can be lowered because of

1. The nature of the work
2. The nature of the biological agent

P. falciparum is listed as an agent to which the derogation may apply.

Risk Assessments would indicate that it is possible to use containment level 2 for the culturing of P. falciparum in this project for the following reasons:

1. We are culturing a clonal strain of P. falciparum that has been in continuous blood-stage culture for 20 years. It has known anti-malarial drug sensitivities and a fully sequenced genome. It is not resistant to the major anti-malarial drugs on the market.
2. Transmission of Plasmodium is by female mosquito. In the wild, the mosquito lands on human skin, pierces the skin and takes up blood. All our blood cultures will be in petri-dishes, so any mosquito that did enter would be unable to feed, so could not acquire the parasite. In addition, the strains under study do not regularly form gametocytes. Only the gametocyte form of the parasite life-cycle is taken up by the mosquito.
3. The natural mosquito species carrier of P. falciparum is not present in the UK. The nearest population is in the Mediterranean.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste (used culture dishes) will be first allowed to soak in 1% virkon or 10% bleach for 12 hours, followed by autoclaving or incineration. Liquid waste will be treated with 10% bleach or 1% virkon for 12 hours to kill the parasite, before disposal down the drains.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
MKH05/2009 - The Committee queried the effect of introducing and deleting genes on the ability of the organism to be more hazardous - however, it is shown that this would not be the case. It was agreed that the work would be carried out in an existing facility which has already been approved by the HSE - "Organelle function in Plasmodium".

1. The organism requires specific species of mosquito to be transferred to humans, of which there are non present
2. The organism used is a characterised one that has been used for 20 years in culture and is susceptible to standard anti-malarial drugs
3. No sharp implements (needles, glass coverslips) would be used in any of the procedures

The committee agreed that providing the work was to be carried out in Room 311 as described with assessment procedures acceptable to both the School Safety Officer and the Department's Biological Safety Officer, it would be acceptable to derogate the work to Class 2. Training would be undertaken according to the procedures already in place.

Project Containment

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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Project Ref 98/10.1

Date Ackn'd 07/09/2010
CU2 Project Title Oncogenic and tumour development and maintenance

Class Class 2
CultureVol < 1 Litre
Non-GMM Consent Granted

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
### Project Additional Information

#### Purposes of the contained use

The project aims to understand the contribution of a number of oncogenes (principally MYC) and tumour suppressor genes such as p53 to tumourigenesis.

#### Recipient or parental organism

<table>
<thead>
<tr>
<th>Organism</th>
<th>Characteristics</th>
<th>Notes</th>
</tr>
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<tbody>
<tr>
<td>E. coli K12 derivatives</td>
<td>(bacterial host for plasmid are disabled, non-colonising, non pathogenic to humans and unlikely to persist in humans or survive outside lab culture. Examples: DH4a, JM109, SCS110, HB101. ACDP hazard group 1.</td>
<td></td>
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<tr>
<td>Yeast (Saccharomyces cerevisiae)</td>
<td>- are non-pathogenic, especially disabled and the strains used all have mutations within genes required for amino acid synthesis. They will not survive outside of the laboratory culture. ACDP hazard group 1.</td>
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</tr>
<tr>
<td>Gamma retroviruses</td>
<td>- MuLV derived, replication defective. The vectors used are based upon the Moloney murine leukaemia virus (MoMuLV). ACDP HG 1. These vectors while retaining the packaging signal are deleted for the viral gag, pol and env genes rendering them replication defective. Examples: pBabe, pWZL. Infective virus can only be produced following transfection of the retroviral vector into a &quot;Packaging&quot; cell line - one that is engineered to complement the deleted genes (for example, Phoenix, Bosc23). In addition, the packaging cell line contains the gag/pol and env genes on separate DNAs rendering the possibility of generating replication competent virus via recombination very low. While risk of recombination leading to production of retroviral particles competent for replication (RCR) is minimised, viral batches may be tested for the absence of RCR by a variety of techniques such as serial transduction, genomic, Q-PCR, detection of reverse transcriptase (pol) activity. Vectors produced may be ecotropic or amphotropic. The latter are able to infect human cells but they pose an infectious risk via the percutaneous route only. They are considered class 1 GMMs.</td>
<td></td>
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<tr>
<td>Lentiviruses</td>
<td>- Vectors are derived from HIV-1 ACDP HG 3. However they are replication defective and considered class 1 GMM. In order to minimize the likelihood of recombination events leading to production of viral particles competent for replication (RCR), the native HIV-1 virulence genes. This lentiviral production system incorporates two key safety features. First, HIV-1 virulence genes Vif, Vpr, Vpu and Nef have been deleted from the helper plasmid. Moreover, the vector itself is self-inactivating (SIN vector) i.e deleted of the 3' LTR U3 region encompassing native viral promoter and enhancer. Replication of the 3' LTR in 5' upon reverse transcription leads to a provirus devoid of viral promoter activity. The absence of RCR will be confirmed by transduction into HCT-116 cells (ATCC, #CCL-247) and the presence of the HIV-1 p24 capsid protein determined by ELISA (Perkin Elmer, #NEK050B001KT) after 14 days in culture. Absence of p24 in transduced HCT-116 supernatant will confirm that the lentivirus preparation is free of replication-competent virus. The vectors will be 'pseudotyped' with the VSV G envelope glycoprotein replacing the natural HIV envelope glycoprotein; this will greatly expand the host range and may represent an enhanced risk of infection by contact or aerosol routes.</td>
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<tr>
<td>Adenoviruses</td>
<td>- Human adenoviruses are associated with mild respiratory or gastro-enteric infections, ACDP HG 2 The Ad5 derived vector is replication defective and considered as a class 1 GMM. The vector is deleted of the E1 and E3 regions and production is dependent on growth in an E1 complementing cell line.</td>
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<tr>
<td>AAV (Adeno-associated virus)</td>
<td>- AAV is not associated with human disease ACDP HG 1. The AAV vector is replication defective and is produced in the absence of helper virus. Of note, AAV-vectors produced in an identical manner described have been previously used safely in both dogs (Herzog et al, Nat Med 199, 5 56-63) and humans. Insertional mutagenesis is theoretical possibility although this has never been observed when using an AAV vector system. Class 1 GMM.</td>
<td></td>
</tr>
<tr>
<td>Characterised mammalian cell lines</td>
<td>are well described in the literature and are considered especially disabled, non-colonising, non-pathogenic and unlikely to survive outside lab culture. ACDP HG 1.</td>
<td></td>
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<tr>
<td>Human primary cultures</td>
<td>are considered especially disabled, non-colonising and unlikely to survive outside lab culture, ACDP HG 1. Nevertheless, they originate from primary human tissue that may contain known or unknown adventitious pathogenic agents. Documented clinical serology will, where possible, be associated with clinical primary samples. Primary cultures from human tissue will always be handled inside a class II microbiological safety cabinet by experienced worker(s) and cultivated in dedicated labeled incubators. No culture of human blood-derived cells is anticipated. Nonetheless, cultures from human tissue will be handled at Containment Level 2.</td>
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</table>
Plasmid vectors - all non-mobilisable pUC derivatives (eg. pTP6, pBSKS, pCDNA3, pLKO, pGEM, pZEG)

Retroviruses - see above. These recombinant viruses derive from the MuLV (Moloney Murine Leukemia Virus) that belongs to the Retrovirus family. They are RNA viruses that stably integrate into the host genome by activity of the viral integrase after reverse transcription resulting in long term expression. Such vectors have been used for more than 20 years and have proved to be a robust and efficient strategy for expression of genes in mammalian cells. Retroviral vectors may include coding sequences for type 1 (cDNA), type 2 (shRNA), type 3 (reporter) or type 4 (antibiotic resistance) inserts. These sequences could be expressed individually by separate vectors or in combination as polycistronic transcripts (cDNA separated by IRES) in the same vector. Gamma-RV particles may be pseudotyped by the VSV-G envelope protein.
Examples: pBabe, pWZL.

Lentiviruses - see above. These recombinant viruses derive from the HIV-1 (Human Immunodeficiency Virus type 1) that belongs to the Retrovirus family. They are RNA viruses that stably integrate into the host genome after reverse transcription and action of the viral integrase resulting in long term expression. Recombinant lentiviral vectors have been widely used for more than 10 years to achieve strong and stable transgene expression in a wide variety of mammalian cells. Compared to gamma-RV, they offer the advantage of being able to efficiently transduce quiescent cells. Moreover, knowledge of the native HIV-1 genome has allowed modifications of recombinant lentiviral vectors to enhance biosafety features (deletion of virulence genes, removal of internal native promoters). Lentiviral vectors may include coding sequences for type 1 (cDNA), type 2 (shRNA), type 3 (reporter) or type 4 (antibiotic resistance) inserts. These sequences could be expressed individually by separate vectors or in combination as polycistronic transcripts (cDNA separated by IRES) in the same vector. Moreover, in order to improve the expression cassette for specific use (lineage specific expression, inhibition of transgene silencing), type 5 (functional non coding sequences) inserts could be used to modify the original lentiviral backbone. These modifications will not involve any modifications of the safety features of the original recombinant lentiviral backbone nor the production method (isolation of complementing activities for viral production on 3 separate plasmids). Vectors with some forms of WPRE have been reported to be associated with tumour development in animal models. Lentiviral particles will be pseudotyped by the VSVG envelope protein.

Adenoviruses - See above Ad5 derived, replication defective. These vectors do not integrate so expression is of a more transient type. The genes of interest are positioned in the site of disablement (E1 deletion).

AAV (Adeno-associated virus) - See above. The AAV vector is replication defective and is produced in the absence of helper virus. The vectors are integrative so can result in long term expression

Expected biological activity of inserted DNA/RNA:

cDNAs (Type 1 inserts) code for wild type and mutant transcription factors, signal transducers, growth factors and receptors (and dominant interfering proteins) of mammalian (including human) origin that are expected to play a role in tumourigenesis. Some of these cDNAs encode oncogenes.

Type 2 inserts are non-coding (miRNA, shRNA), derived from mammalian sequences and designed to interfere with expression of endogenous genes including those represented in type 1 inserts.

Reporter genes (type 3 inserts) of prokaryote origin code for proteins with fluorescent or enzymatic properties allowing detection of genetically modified cells. They are not known to have any deleterious effects when expressed.

Selection genes (type 4 inserts) of prokaryote origin produce enzymes capable of inactivating specific antibiotic families. Expression of these genes will allow selection of genetically modified cells. They are not known to have any deleterious effects.

Functional non-coding sequences (Type 5 inserts) of prokaryote, avian or mammalian origin will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES). They will be used (independently or in combination) into viral expression cassettes to achieve best control of transgene expression depending on targeted cells.
Neutral non-coding sequences (Type 6 inserts) of mammalian origin will be used in particular for gene targeting projects to promote homologous recombination at defined genomic loci (intronic/exonic genomic sequences) They should not have biological effect by themselves.

**Evaluation of foreseeable effects**

1. **E.coli containing plasmid vectors** containing a variety of inserts (sometimes more than one type). The plasmid vectors are all non-mobilisable pUC derivatives and E.coli host strains are all disabled, non-colonising, non-pathogenic K12 derivatives. None of the sequences introduced are expected to alter the disabled properties of the host so these GMMs are considered to be Class 1.

2. **Recombinant retroviruses and lentiviruses**. The vectors used are all replication defective “3rd generation” plasmids. Both ecotropic and amphotropic viruses will be generated, some of which encode oncogenic sequences; insertional mutagenesis is a possibility. Consequently, these viruses are regarded as Class 2.

3. **Recombinant adenoviruses**. The vectors used are based upon human type 5 adenovirus, but are deleted within the E1 and E3 regions of the adenoviral genome. This makes the vectors both replication-deficient and with the removal of the E1a and E1b genes (E1 deletion) unable to transform cells since they cannot activate early phase gene expression. Adenoviruses are not integrated into the host cell genome (unlike retroviruses and lentiviruses), provide only transient expression of the encoded sequences, do not replicate and spread to infect neighbouring cells (for example, in an intact tissue) and are usually rapidly eliminated. They are considered to be Class 1.

4. **Viral packaging cell lines** (for example, PA317, Phoenix) are transfected with plasmids to complement (in trans) activities required for generation of infectious virus but absent from the retroviral constructs. These activities (gag, pol and env) are separated on 2 or 3 different plasmids to restrict the possibility of replication competent virus. Transfection or infection with viral vectors results in the production of virions with an ecotropic or amphotropic host range dependent on the tropism endowed by the packaging cell line. Since these cells are used to generate infectious (but replication-defective virus) they will be considered as Class 2. Production of recombinant lentiviral particles will be achieved by transient co-transfection of 3 plasmids into HEK-293 cells by calcium phosphate precipitation.

5. **Established mammalian cell lines** will be transfected or virally infected with GMMs described in 1-3 above. The cell lines generated will have no increased capacity to survive outside of the laboratory environment and can be considered as Class 1. However, where primary human cell cultures are used - all GM derivatives will be handled at Containment Level 2.

6. **Yeast**. The yeast strains used all have mutations within genes required for amino acid synthesis and are therefore disabled. They will not survive outside of laboratory culture. The host yeast will be transformed with pUC-based non-mobilisable plasmids using lithium acetate/PEG/heat shock. None of the sequences used can alter the disabled nature of the hosts so they are considered as Class 1.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Established mammalian cell lines (including those derived from human tissue) are normally regarded as ACDP 1. They pose very little hazard and are unable to survive outside the laboratory. Primary human tissue and cell cultures will also be used in this study. Fresh human tissue from screened and unscreened individuals may therefore contain adventitious biological agents such as HIV, Hep B. Fresh tissue and primary cultures are regarded as ACDP 2 and will be handled under containment level 2 conditions. Although fresh human blood may be used there will be no attempt to culture blood cells.

All fresh human tissue and derived primary cultures will be handled under containment level 2 conditions. Personnel will be specially trained in handling such tissue especially where dissection is required. Personnel involved will be referred to University Occupational health for Hep B vaccination. Culture of human blood cells will not be attempted.

We will also express wild type and mutant alleles of oncogenes in rodents. Some of these oncogenes will be conditional alleles, only having activity when the animals are administered the specific ligand. In addition, we will genetically interfere with the sequence and/or expression of certain tumour suppressors (notably p53) in rodents. While these manipulations may generate tumours in the experimental animals there is a very low risk that the genetically manipulated animals are an increased risk to humans (eg by biting/scratching) and all workers will be specially trained in handling rodents. Due to the tight security of biofacility establishments precluding escape of genetically modified rodents, there is no risk to other animals or the environment.

Recombinant Adenoviral and AAV vectors will be used in animals. Where injections are employed extra care will be taken with the use of needles. Only trained personnel will conduct this procedure. It is anticipated that this procedure will give rise to any increased risk of infection due to bites. In addition, the viral vectors used are...
replication-defective (see above). No shedding in saliva or excreta is anticipated.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste is autoclaved (132°C, 15 mins). Liquid waste is treated with 1% Virkon (final concentration) or 5000ppm Klorsept 87 for at least 12 hours before disposal down the sink. Biological material (eg mouse tissues) is double bagged and incinerated via the Gurdon biofacility. Autoclaving: effective 100% kill, regularly monitored by microbiological testing. Certified testing of autoclave carried out annually. Liquid waste: Virkon gives >10^5 reduction in viable E. coli in 5 minutes and ≥ 4.25 log 10 reduction in lentivirus titre in 10 minutes providing effective bactericidal and virucidal activities when used as per the manufacturer's instructions. (Data supplied by the manufacturers, Dupont). Incineration: Effectively 100% kill.

Routine disinfection
All surfaces wiped with Trigene and 70% IMS. Centrifuge bowl, buckets and inserts wiped with Trigene and/or 70% IMS.

Spillage
Small amounts treated with Virkon wiped with disposable tissue and placed in autoclave bag for autoclaving. Larger spills: carefully add Virkon powder (do not inhale), leave for several hours, absorb onto tissue, bag and autoclave. Spills in orbital shaker are treated in the same way although it may be necessary to remove the orbital platform to access the spillage. Spills in centrifuge buckets should be treated with Trigene or Virkon (depending on the manufacturing material of the bucket) as above. In all cases the spill should be notified to local workers and the appropriate safety adviser. Regular testing for viable microorganisms - for example, colony growth on agar plates.

We use disposable scalpels, needles and glass pipettes. All are disposed of in “Sharps bins” that are collected for disposal by incineration

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

GIE01/10 - Oncogenes and tumour suppressors in tumour development and maintenance: The Committee discussed this assessment and took into consideration comments made in an email from Dr H (attached). The assessment was passed at Level 2. HSE form to be completed and notified. The assessment was circulated to DR K F (Clinical School Safety Advisor) for any further comments on the project. There were a few minor alterations and a request that training in the CL2 lab would be recorded.

Project Containment

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

To determine the pathway for potential fuel molecule production in Klebsiella and related species, and to manipulate the productivity of the pathways.

**Recipient or parental organism**

Klebsiella bacteria:
- Klebsiella (local species) - most similar to Klebsiella pneumonia
- ACDP Hazard Group 2 for these species generally, but the isolates 8017 and 418 are not recognized as pathogenic.

E. coli DH5alpha Group 1

**Host/vector system**

Plasmid vectors

pUC19, pKmobsacB, pUC19Km, pUM24, pKO3/pKOV. These vectors can replicate in E. coli DH5alpha. All plasmid constructs will be made in E. coli DH5alpha.
pKmobsacB, pKO3/KOV and pUC19-based vectors will be transformed into Klebsiella species. These are suicide plasmids in these species and therefore cannot replicate in these strains when cultured on media containing sucrose.

### Origin & function

N/A

### Evaluation of foreseeable effects

Final GMMs will be strains with genes in categories indicated disrupted or overexpressed. Genes encoding possible fuel molecule biosynthesis genes will be deleted in Klebsiella (local species) to determine whether they encode enzymes involved. These genes will then be expressed in E. coli to determine whether the fuel molecules can be produced in an industrial microbial strain.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste is autoclaved (132°C, 15 mins). Liquid waste is treated with 1% Virkon (final concentration) or 5000ppm Klorsept 87 for at least 12 hours before disposal down the sink. Biological material (e.g. mouse tissues) is double bagged and incinerated via the Gurdon biofacility. Autoclaving: effective 100% kill, regularly monitored by microbiology testing. Certified testing of autoclave carried out annually. Liquid waste: Virkon gives >10^5 reduction in viable E. coli in 5 minutes and > or equal to 4.25 log 10 reduction in lentivirus titre in 10 minutes providing effective bactericidal and virucidal activities when used as per the manufacturer's instructions. (Data supplied by the manufacturers, Dupont). Incineration: Effectively 100% kill.

Routine Disinfection

All surfaces wiped with Trigene and 70% IMS. Centrifuge bowl, buckets and inserts wiped with Trigene and/or 70% IMS.

Spillage

Small amounts treated with Virkon, wiped with disposable tissue and placed in autoclave bag for autoclaving. Larger spills: carefully add Virkon powder (do not inhale), leave for several hours, absorb onto tissue, bag and autoclave. Spills in orbital shaker are treated in the same way although it may be necessary to remove the orbital platform to access the spillage. Spills in centrifuge buckets should be treated with Trigene or Virkon (depending on the manufacturing material of the bucket) as above. In all cases the spill should be notified to local workers and the appropriate safety adviser. Regular testing for viable microorganisms - for example, colony growth on agar plates. We use disposable scalpels, needles and glass pipettes. All are disposed of in "Sharps bins" that are collected for disposal by incineration.

**Is an emergency plan required according to regulation 20?**  

| N |

If yes, tick to confirm that it is attached to this form

**N**

Tick to confirm that you have attached a risk assessment to this form

**Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment

**N**
Please enter comments on the GM safety committee on the risk assessment

Dr W investigated this assessment which is based on an isolate found in the laboratory and this was identified to be a Klebsiella. The work would be carried out at Containment Level 2 in the W laboratory. It is suggested that the clinical isolates mentioned in the assessment are not used in the project and removed. As the work will involve GM, the HSE will be informed and a CU2 form will need to be completed and approved before work can be started.

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Project Ref 98/13.2

Date Ackn'd 08/05/2013

Date Project Ceased

CU2 Project Title Establishment of stable mammalian cell lines expressing small G proteins and their effector proteins

Class Culture Volume

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The aim of this project is to investigate the role of small G proteins (both wt and activated) and their effector and regulatory proteins in the control of signal transduction.
pathways that are deregulated during cancer progression. The genes encoding these proteins will be cloned into transfer vectors and then recombined into viral vectors. This will include the use of a number of different plasmid and viral vectors (retrovirus and lentivirus) for transduction of mammalian cells. These will be used to establish stable mammalian cell lines.

Recipient or parental organism

Characterised mammalian cell lines are well described in literature and are considered especially disabled, non-colonising, non-pathogenic and unlikely to survive outside lab culture. ACDP HG1. These include: Transformed human cell lines: NIH3T3, HeLa, HEK293T, MCF7, HB4a, MDA-MB-361, MDA-MB-231, HCC1806, MCF71, MCF10a, T47D, DU145, U205, LNCaP, BT474, Cal51 (Class I: extensively documented)

Transformed monkey cell lines: COS 1 and 7

Rodent cell lines: NIH3T3, CHO and Rat-1

Host/vector system

E. coli K12 strains: XL1 Blue MRF', XL10, DH5a, TOP10, TOP10F'. All are disabled, non-colonising, non-pathogenic to humans and unlikely to persist in humans or survive outside lab culture. ACDP hazard group 1.

Gamma retroviruses - MuLV derived, replication defective. The vectors used are based upon the Moloney murine leukaemia virus (MoMuLV). ACDP HG1. These vectors while retaining the packaging signal are deleted for the viral gag, pol and env genes rendering them replication defective. Examples: pBabe, pWZL. Infective virus can only be produced following transfection of the retroviral vector into a "packaging" cell line - one that is engineered to complement the deleted genes (for example, Phoenix, Bosc23). In addition, the packaging cell line contains the gag/pol and env genes on separate DNAs rendering the possibility of generating replication competent virus via recombination very low. While the risk of recombination leading to production of retroiral particles competent for replication (RCR) is minimised, viral batches may be tested for the absence of RCR by a variety of techniques such as serial transduction, genomic, Q-PCR, detection of reverse transcriptase (pol) activity. Vectors produced may be ecotropic or amphotropic. The latter are able to infect human cells but they pose an infectious risk via the percutaneous route only. They are considered class 1 GMMs.

Lentiviruses - Vectors are derived from HIV-1 ACDP HG3. However they are replication defective and considered class 1 GMM. In order to minimize the likelihood of recombination events leading to production of viral particles competent for replication (RCR), the native HIV-1 genome has been isolated onto 3 plasmids; a helper plasmid coding for Gag (matrix and capsid proteins), Pol (enzymes), Tat and Rev genes. This lentiviral production system incorporates two key safety features. First, HIV-1 virulence genes Vif, Vpr, Vpu and Nef have been deleted from the helper plasmid. Moreover, the vector itself is self-inactivating (SIN vector), i.e. deleted of the 3' LTR U3 region encompassing native viral promoter and enhancer. Replication of the 3' LTR in 5' upon reverse transcription leads to a provirus devoid of viral promoter activity. The absence of RCR will be confirmed by transduction into HCT-116 cells (ATCC,#CCL-247) and the presence of the HIV-1 p24 capsid protein determined by ELISA (Perkin Elmer, # NEK0508001KT) after 14 days in culture. Absence of p24 in transduced HCT-116 supernatant will confirm that the lentivirus preparation is free of replication-competent virus. The vectors will be 'pseudotyped' with the VSV envelope glycoprotein replacing the natural HIV envelope glycoprotein; this will greatly expand the host range and may represent an enhanced risk of infection by contact or aerosol routes.

Origin & function

Rho family G proteins (full length, with and without activating and other mutations): Cdc42, Rac1, 2 and 3, RhoA, B, C, D, F and G, Rho 6, 7 and 8, TC 10 and TCL

Ras family G proteins (full length, with and without activating and other mutations): RaIA andB, K-Ras, H-Ras, N-Ras, Rap1A (Human and Simian)

Effector Proteins (full length and domains): ACKs (Human), PAKs (Rat), WASPs (Human), IQGAP (Human), IMP (Human and frog), PRK1, 2 AND 3 (Human), Rhotekin 1 and 2 (Human), Rhosphilin 1 and 2 (Human), Citron (Human), PLC-51 (Human), ZONAB (Human), RLIP-76 (Human), Filamin (Human), Exo84 (Rat), Exo70 (Human), Sec5 (rat), Raf (Human), ROCK (Human), BORGs (Human), SPECs (Human), PI3Kinases (Human), Grb10 (Human), Nck (Human), STATs 1-6 (Human), DLAT (human), COPB (Human), PREPL (Human), DDX49 (Human), BPOZ (Human), Tensin (Human) and TOCA1 (Human).

Regulator Proteins: RhoGAP (Human), p120GAPRas (Human), PIX (Human), RhodGDI-3 (Human), Vav3 (Human), Epsin (mammalian).
### Evaluation of foreseeable effects

Mobilisation defective or non-mobilisable vectors are used in all E. coli work. All E. coli strains use would not survive outside the lab and would not be able to colonise humans.

Recombinant infectious retroviruses and lentiviruses are all replication defective. Such viruses, even if released to the environment, are unable to survive very long. However, since these viruses are capable of infecting animals (including humans) and integrating into the host cell genome (including inserted sequences) there is a possibility that they can persist in an individual. However, viral titres required for infection are extremely unlikely to be achieved outside the laboratory.

The mammalian cell lines used cannot survive outside laboratory conditions. The most significant hazard with cell culture is contamination with pathogenic viruses. No primary cells will be used and all cell lines will be grown under containment level 2 conditions. The genes and vectors that we will utilise have been used extensively by the scientific community with no reported risks.

During the cloning process the DNA fragments are inserted into sites of limited promoter activity or no activity.

Modest expression of inserted DNA will be carried out in mammalian cells using transfection with plasmids containing strong eukaryotic promoters (e.g. retroviral/lentiviral promoter/enhancer sequences). There is no intention to maximize expression in mammalian cells. These GMMs do not express inserted DNA outside a host organism. Although they are capable of infecting animals (including humans) and, in the case of retroviruses and lentiviruses persisting in the host genome, the risk of infection is negligible. All such GMMs will be handled under class 2.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid bacterial waste generated is autoclaved using a Fedagari SPA Programmable autoclave with a program of 15 minutes pulsed free steaming and 30 minutes at 132°C.

Liquid-based waste materials are treated by soaking in 1% final concentration of Virkon for a minimum of 15 min before disposal via the drains.

Solid tissue culture material is securely packaged and disposed of by incineration via a registered contractor. Liquid-based waste materials are treated by soaking in 1% final concentration of Virkon for a minimum of 15 min before disposal via the drains.

### Is an emergency plan required according to regulation 20?

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

---

02/03/2022
2012.30 (vii) DO/HRM04/2012 - Establishment of stable mammalian cell lines expressing small G proteins and their effector proteins. The project involves G proteins and uses mammalian expression systems. It was pointed out that retroviruses and lentivirus must be used in Containment Level 2 laboratories. Work would not use any primary human cell lines, only established cell lines would be used. It is also noted that the work is similar to Professor E's. The assessment required to be revised to reflect this and then would need a CU2 form for HSE. An agreed statement for the work would be required before sending. CF

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref** 98/13.3

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<td>Studies on human infective African trypanosomes</td>
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Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**
To determine the molecular mechanisms of human infectivity of African trypanosomes through the production and analysis of transgenic cell lines expressing modified versions of the genes necessary for human infectivity.

**Recipient or parental organism**
Laboratory adapted isolates of Trypanosoma brucei brucei will be modified through the expression of transgenes that will confer human infectivity. Human infective Trypanosoma brucei rhodesiense (ACDP hazard group 2) and Trypanosoma brucei gambiense (ACDP hazard group 3) will be used as control but will not be genetically modified.

**Host/vector system**
Trypanosome-specific vectors for: (i) the inducible or constitutive expression of transgenes, (ii) inducible RNAi knockdown of gene expression, and (iii) targeted deletion of genes. Introduced genetic material will originate from Trypanosoma brucei rhodesiense or bacterial plasmids including reporter genes.

**Origin & function**
Some of the transgenic Trypanosoma brucei brucei will become infective to humans but will be no more virulent than Trypanosoma brucei rhodesiense. As such, they will be capable of causing human disease if introduced into an open wound, there is no aerosol transmission route. The resulting disease is readily diagnosed and can be successfully treated. A risk assessment, including SOPs, to mitigate the chances of this happening. Accidental escape from the laboratory is extremely unlikely as the trypanosomes cannot survive outside complex tissue culture medium.

**Evaluation of foreseeable effects**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
I would like to apply for the derogations of some containment level 3 measures to level 2 as detailed on the attached page in the fourth column.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid and liquid waste will be autoclaved following a SOP. This results in 100% kill that has been validated experimentally. The Department of Biochemistry has dedicated autoclaves and records of each autoclave run are kept.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
The committee agreed that this work could be carried out in the laboratories used for Trypanosome work, as they conform to CL3 with derogations justified by the researchers. The committee were satisfied that the standard operating procedures and training instructions for postdoctoral workers involved in the project were satisfactory.

**Project Containment**

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**Project Ref 98/14.1**

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Non-GMM Consent Granted

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Toxoplasma gondii is a single-celled parasite that infects and can cause disease in humans and livestock. Our interests lie in understanding numerous aspects of the basic cell biology of these parasites, with an ultimate view to elucidating treatment options and druggable targets in these parasites. We focus on various aspects of parasite cell biology and metabolism, such as how the parasite invades host cells and how the parasite assembles daughter parasites. An important approach to our study of T. gondii.
biology is to learn about the location and function of proteins in *T. gondii*. We will use gene technology to tag proteins of interest with traceable markers (such as the green fluorescent protein and epitope tags) to study the expression and location of proteins of interest. To study protein function we will use gene technology to control the abundance of proteins of interest either by regulating how much protein is made using inducible promoters, controlling the stability of the protein with destabilising domains, or removing the genes for proteins of interest. Some gene products will be expressed in the host cells and directed to the parasites via attached cell penetrating peptides. All GMOs will be used for in vitro culture studies only, and no GMOs are expected to have increased virulence, pathogenicity or transmissibility of the parasite. None of the proteins to be studied is known to code for a toxin.

*T. gondii* is grown in in vitro cultures of standard mammalian tissue culture lines (such as Human Foreskin Fibroblast (HFF) and Vero cell culture). Shuttle vector cloning will occur in *E. coli*, and genes expressed in both the parasites and host cells.

**Recipient or parental organism**

A. *Escherichia coli* (nonpathogenic strains such as XL-1 Blue, DH5a etc) - Class 1

B. *Toxoplasma gondii* wild type strains, and mutant derivatives: ΔKu80 (lacking the Ku80 gene for non-homologous recombination), TATiΔKu80 additionally containing variants of the *E. coli*-derived tet repressor (TetR) protein (see Meissner et al, Science 298(5594): 837, 2002), Δhxgprt (enabling restoration of mycophenolic acid selection as a selectable marker) - Class 2

C. Mammalian cells: Well characterized mammalian cells in culture, e.g. HFF, vero, NIH/3T3, BHK, 239T, RAW264

**Host/vector system**

Standard *E. coli* cloning vectors such as puC series, pGEM-T-Easy, BACs, cosmids and Lambda ZAPII

*T. gondii* vectors used include:

- pCTG vector and derivatives. pCTG contains a chloramphenicol-resistance marker and a GFP expression cassette. Derivatives include vectors containing resistance cassettes such as those for phleomycin, mycophenolic acid and pyrimethamine resistance as well as fusion proteins such as RFP (DsRed, mCherry and other variants), YFP and other GFP-derived fluorescent protein variants, HA, c-myc, mDHFR, destabilization domain and Ty1 epitope tags. Genes-of-interest may be driven by inducible promoters that include TetR-binding operator sequences (tetO), or by a range of parasite-specific promoter sequences. See van Doore et al, PNAS, 105(36): 13574, 2008 for a description of the base vector.

- pTCY vector and derivatives. pTCY contains chloramphenicol-resistance marker and a YFP expression cassette that is used as a negative selection marker. Derivatives of this vector are used primarily in generating knockouts of genes-of-interest in Toxoplasma or in generating homologous integration events. See van Dooren et al, PNAS 105(36): 13574, 2008 for a description of the base vector.

- pPR2-HA3 vector and derivatives. PPR2-HA3 contains a pyrimethamine-resistance cassette and is used for gene replacements. See Sheiner et al, PLoS Pathog, 7(12): e1002392, 2011) for a description of the pPR base vector that is further modified to include a 3xHA (haemagglutinin) epitope tag and additional restriction endonuclease sites.


- PSB ad TOX cosmids modified by recombineering to introduce resistance cassettes such as those for pyrimethamine, chloramphenicol, phleomycin and mycophenolic acid resistance cassettes and epitope tags. The recombineering procedure and base vectors are described in Brooks et al, Cell Host Microbe, 7(1): 62, 2010.

- loxP flanking sequences will be used to induce Cre recombinase excision, and CRISPR/Cas9 sgRNAs will be used to direct Cas9 excision.

Standard mammalian expression vectors such as the pCAG series will be used for protein expression in the host cell systems.

**Origin & function**
Standard genes used as selectable markers in bacteria (e.g. ampicillin, kanamycin).

The source and functions of some of the commonly used genes responsible for drug-resistance phenotypes in Toxoplasma gondii are as follows:
- mutant Toxoplasms DHFR gene (pyrimethamine resistance): a drug-resistant form of dihydrofolate reductase/thymidylate synthase, which reduces dihydrofolic acid to tetradihydrofolic acid.
- the Streptomyces BLE gene (phleomycin resistance): binds with high affinity to phleomycin drug to prevent it from interfering with an organism's DNA.
- E. coli-derived chloramphenicol acetyltransferase (chloramphenicol resistance): acetylates chloramphenicol drug which prevents the drug from binding to the prokaryotic ribosome (in the case of Toxoplasma, likely the apicoplast ribosome), its site of action.
- Toxoplasma HXGPRT (mycophenolic, xanthine and guanine, providing the parasite with a means of salvaging purines. In the context of Toxoplasma genetic modification, its presence allows the bypass of a second mode of purine salvage that occurs via inosine 5'-monophosphate dehyrogenase, an enzyme sensitive to mycophenolic acid. In the case of negative selection, HXGPRT converts the drug 6-thioxanthine to toxic nucleotides that become incorporated into the parasite's DNA.
- Toxoplasma UPRT (FUDR sensitivity): adds a phosphoribosyl group to the pyrimidine base uracil, thereby functioning in pyrimidine salvage. UPRT catalyses the conversion of FUDR to its toxic nucleotide, which interferes with RNA and DNA synthesis.

Tetracycline-controlled expression utilizes modified T. gondii promoters that contain tet operator sequences derived from E. coli, in cell lines expressing variants of the E. coli-derived tet repressor (TetR) protein (see Meissner et al, Science 298(5594): 837, 2002; van Poppel et al, Int J Parasitol 36(4): 43, 2006). Other promoters used in transgene expression are derived for T. gondii. Cre Recombinase, and Cas9 (of the CRISPR/Cas9 system) will be expressed in both the parasites, and the host cells with cell penetrating peptides attached (e.g. Penetratin, TAT).

Standard reporter proteins will be used including: fluorescent proteins (GFP and derivatives); epitope tags (e.g. HA, cMyc, Ty); enzyme reporters (e.g. BirA* (mutant biotin ligase), APEX (a modified ascorbate peroxidase))

Genes for T. gondii proteins of interest to this research project will be manipulated.

No human genes will be used, and no genes are expected to increase the virulence of the parasites, or produce toxins.

**Evaluation of foreseeable effects**

T. gondii cells will be made with the following final genetic outcomes:
- Knock out of an endogenous gene product
- Endogenous gene product under the control of a regulatable promoter or inducible destabilization domain.
- Reporter protein fusion of an endogenous gene product
- Generate mutant gene replacement products

Mammalian cells expressing proteins able to be directed to the intracellular T. gondii parasites via cell penetrating peptides to mediate gene editing events (e.g. recombination, DNA cutting).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be incinerated or autoclaved using a programmable autoclave with a program of 15 minutes pulsed free steaming and 30 minutes at 132°C. Liquid waste will be treated with bleach (1% sodium hypochlorite) or virkon for 12 hours to kill the parasite, before disposal down the drains.
All of these methods have been shown to kill T. gondii parasites.

Microbiological testing has shown that hypochlorite kills T. gondii. This is the standard means of killing the parasite in T. gondii research laboratories. Virkon is also routinely used in T. gondii laboratories.

Autoclaving: Effectively 100% kill at this programme as shown by microbiological testing. Certificated testing of all autoclaves is carried out annually and records are held by the Department. Printed readouts from each run are retained to ensure temperatures within the autoclave were maintained during the cycle.

Incineration: Effectively 100% kill.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Minute 2014.16 from safety committee meeting 12 May 2014
RW01/2014 - The Biology of Toxoplasma gondii parasites.

The organism is not transmissible human to human but is transmissible from a cat to a human, and from culture to human by e.g. needlestick. The Committee requested Dr W to attend the meeting to discuss concerns about methods of disinfection (bleach, 80% ethanol) which will be modified to proprietary chemicals such as trigene, and the use of sharps. The latter will be reduced to a minimum and only blunt needles will be used. The assessment is very comprehensive with an older assessment from Australia included. Dr W would also produce a standard operating procedure document to go with the assessment (now provided). The Committee then agreed it to be Level 2 and the HSE would be notified. Correspondence between Mr E, Dr W and Professor D regarding the importation of the material was available and Dr W will be implementing this as soon as notification has been agreed by HSE.

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02/03/2022  Page 2974 of 15326
Project Ref 98/99.1

Date Ackn’d 25/05/1999

CU2 Project Title

STUDIES ON GENE EXPRESSION AND THE CELL SURFACE OF TRYPANOSOMA BRUCEI

Date Project Ceased

Class Class 2

CultureVolClass2

CultureVolumeClass3-4

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 98/99.2

Date Ackn'd 20/07/1999

Date Project Ceased 27/05/2002

STUDIES OF RESISTANCE TO GLYCOPEPTIVE & B-LACTAM ANTIBODIES IN GRAM-POSITIVE BACTERIA

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Withdrawn N

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 98/99.3

**CU2 Project Title**

CLONING ERWINIA GENES AND SERRATIA GENES IN E.COLI ERWINIA AND SERRIATIA

**Date Ackn'd** 20/07/1999

**Date Project Ceased**

---

**Class** Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM** not applicable

**Consent Granted**

Project notified under transitional arrangements **Y**

**Withdrawn** **N**

Tick if notifying a connected programme of work **N**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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<td>25/07/1999</td>
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YERSINIA AND MUTOGENESIS VIA LAMBDA VECTORS CARRYING TRANSPOSONS

Date Project Ceased

Tick if notifying a connected programme of work

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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### Project Ref 98/trans1

- **Date Ackn’d**: 22/02/2001
- **CU2 Project Title**: MECHANISMS OF SURVIVAL AND DEATH IN EUKARYOTIC CELLS USING ADENOVIRAL VECTORS, FORMERLY GENES CLONED INTO DISABLED ADENOVIRUS
- **Class**: Class 2
- **Non-GMM Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y
- **Tick if notifying a connected programme of work**: N

## Historical Significant Changes

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
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**Name**

ROYAL FREE & UNIVERSITY COLLEGE MEDICAL SCHOOL

**Name 2**

UNIVERSITY COLLEGE LONDON

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

POND STREET

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

NW3

**Country**

ENGLAND

**Tel Number**

0207 794 0500

**Fax Number**

0207 794 3505

**E-mail**

**HSE Division**

LONDON

**Comments**

GM CENTRE CLOSED AND ALL WORK TRANSFERRED TO GM14

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

**Level 1 (GMMs)**

**Level 2 (GMMs)**

**Level 3 (GMMs)**
Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential

Bacteriology

Parasitology

Transgenic

Birds

Microbiology

Research

Virology

Transgenic

Animals

Transgenic

Fish

Gene Therapy

Mycology

Transgenic

Invertebrates

Transgenic

Plants

Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 99/00.1

Date Ackn'd

28/11/2000

Date Project Ceased

02/03/2022

CU2 Project Title

GENE ADDITION AND GENE CORRECTION STRATEGIES FOR DYSLIPOPROTEINAEMIA

Class

CultureVol

Class 2

Class 2

CultureVolume

Class2

Class3-4

Non-GMM

Consent Granted

not applicable
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
<td>Human Clinical Applications</td>
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Project Ref 99/03.1

Date Ackn'd: 08/08/2003

Date Project Ceased: 14/10/2008

CU2 Project Title: TO DELIVER T CELL CO-STIMULATORY GENES AND CYTOKINE GENES OR GENES DESIGNED TO ENHANCE NATURAL KILLER CELL ACTIVITY, TO TUMOUR CELLS FOR BREAST CANCER, OVARIAN CANCER OR LEUKAEMIA

Date of Significant Change: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Withdrawn: N

Non-GMM Consent Granted: not applicable

Purposes of the contained use

We are developing a number of gene based therapies to try to overcome the lack of recognition by autologous immune effector cells to the patients tumour. We are...
working with breast, ovarian and leukaemic models of disease. We propose to use combinations of the genes encoding CD80, IL-12 and the adenoviral type 5 E1A protein.

Recipient or parental organism

E. coli (including strains TG1, DH5alpha and JM109)
Human cell lines including 293, 293T, HeLa, MCF7, MDA231, BT474, CaOv, OVCAR and SKOv.
Murine cell lines including TS/A, 4T1 and SCK.
Patient derived tissue (material obtained from patients undergoing treatment for their disease, after appropriate consent) including breast, ovarian and leukaemic samples together with peripheral blood.
Transgenic mice eg the Ocomouse (Her2/neu transgenic)
immunocompromised mice (eg SCID)
Normal mice including strain Balb/C and A./J

Host/vector system

Vectors including plasmids based on the mammalian expression plasmid pBK-CMV (Stratagene) encoding human and murine CD80 and IL-12 and Adenoviral E1A, either separately or in combination.
Lentiviral constructs including plasmids pLenti6/V5, pLP-1, pLP-2 and pVSV-G (Invitrogen).
pUC 18/19 general cloning vector.

Origin & function

All human and murine genes were cloned using appropriate mRNAs derived from appropriately stimulated cells.
Adenovirus E1A was cloned from wild type Ad5 virus obtained from the ATCC (United States).
The function of these genes is detailed in Section 5 above and in Delivered Genes in Section below.

Evaluation of foreseeable effects

Recipient organisms. The inherent risks in using our bacterial strains are very low. All are commercially available and are disabled so that they can neither survive outside the laboratory nor transfer or recombine genetic material.
Our wild type mammalian cell lines are also commercially available and have been grown in continuous culture for many years. They have not been found to contain any adventitious agents.
The normal mice and transgenic mice are commercially sourced. We do not anticipate any additional risks to the environment when using these mice in our experiments.
The SCID mice and severely immuno-compromised and so would be unable to survive outside the laboratory.

Vectors. The antibiotic resistance markers carried in our plasmids theoretically could be transferred to wild type bacteria causing them to gain resistance to kanomycin and penicillin.
For lentiviral gene transfer, a minimal vector is used which has three levels of safety incorporated in it. These deletions render the virus replication defective and incapable of producing infectious viral particles in the target tissues. However there is a very small but possible effect that there may be recombination between wild type viral sequences, latent in the patient derived tissues, and the recombinant vectors. It is considered that this possibility is extremely remote.

Delivered genes. We propose to use the cDNAs encoding CD80, a T cell ligand which is usually expressed at low levels on ‘professional’ antigen presenting cells such as macrophages and dendritic cells. We also propose to use the antigen presenting cell derived cytokine Interleukin-12 (IL-12) which plays a central role in the immune system, principally for its ability to promote the development of CD4+ T helper (Th1) cells from naive T cells.
Additional experiments will use the Adenovirus E1A protein. E1A has pleiotropic effects on cells including, for the 243 amino acid variant, tumour suppressive activity in some human cell lines.
GMOs will not be delivered directly to animals but GMOs will be used to infect cells which will be checked for expression and these cells containing disabled GMOs will be injected into the animals.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All of the GM animals will be housed in cages separate from their non GM controls. They will not be allowed to mix. All animals will be of the same sex (female) and therefore will not have the opportunity to breed. At no point will the GM and non GM animals come into contact when they are alive.

All animals will be housed in the Comparative biology unit at the Royal Free Hospital which can be accessed only by authorised personnel.

The Comparative Biology Unit (CBU) where the mice will be housed has been purpose built and containment was an important design consideration. There are several levels of containment: the cages are secure and escapes are extremely infrequent. An animals that escapes from its cage would have to pass through two doors before being outside the Unit. The doors are close fitting and rodent barriers are strategically placed which are designed to prevent this happening. Further, in contrast with micro-organisms, all mice are accounted for by counting on a daily basis.

The SCID animals, due to their immune deficiency will be housed separately in cages with filtered airflow. In addition to the containment described above, these animals are further contained by being housed in a unit within double air-locked door.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material contaminated with genetically modified organisms (GMOs) or naked DNA will be rendered non-infective before disposal according to the Royal Free Campus arrangements and the Departmental Code of Practice for GMO work.

Complete inactivation of viable GM material is achieved by chemical disinfection or autoclaving.

Solid waste is disposed of in plastic bags designated for autoclavable waste. Sharps (e.g. pipette tips) are collected in containers suitable for protection of the bags and for subsequent autoclaving. Sharp use is actively discouraged in the Flow Hood. Lids are loosely sealed to ensure the appropriate penetration of steam. Where applicable, solid waste contaminated with GMOs eg tissue culture flasks are treated with Chlorine ions at 10 000 ppm (Milton Fluid) before disposal in autoclavable waste. The bags are collected regularly and disposed of by the appropriate Hospital authorities at 121 degrees C for 20 minutes.

Liquid waste containing GMOs eg tissue culture supematant, is treated with free chlorine at 10 000 ppm (Milton liquid)

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment
I understand that, after consultation between the Assistant Biological Safety Officer of the Royal Free Hospital and a member of the HSE, it was felt that this is a borderline case between Class 1 and Class 2.

The local safety committee has seen the proposal, submitted and provisionally approved as a Class 2 project. Therefore I am required to submit the project to the HSE for ratification as a Class 2 project.

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INVESTIGATION OF THE ONCOLYTIC ACTIVITY OF ATTENUATED MEASLES VIRUS (MV) EXPRESSING GROWTH FACTORS.

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Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

N

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED

Date of Significant Change

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Propagation and in-vitro characterisation of attenuated MVs expressing murine and human GM-CSF and IL-12 in-vivo characterisation in mice transgenic for the human...
**MV receptor, CD46. Determination of in-vivo oncolytic effects in relevant tumour models.**

**Recipient or parental organism**

Attenuated (vaccine) strain of MV

**Host/vector system**

Vector: cDNA encoding murine and human growth factors expressed as additional transcription units of attenuated MV.

Hosts:
1. For DNA preparation, K12 strains of E. Coli will be used, typically TOP10 strain.
2. Attenuated (vaccine) strains of MV will express the growth factors.
3. Established mammalian cell lines will be infected by the modified viruses: 293 (human embryonal kidney) Vero (African green monkey kidney), Raji, DOHH2, (human B cell lymphoma). 293-3-46 (modified 293 cells expressing attenuated MV proteins, intended for the rescue of infectious MV from the full-length anti-genomic plasmid).
4. Mice transgenic for the human MV receptor, CD46 and SCId mice bearing humaning tumour xenografts will be intentionally infected by these viruses by various route of infection.

**Origin & function**

Origins:
- cDNA encoding GM-CSF cDNA originally purchased from commercial sources (In-vivogen (murine) and ATCC (human)).
- Full-length MV plasmid obtained from Dr Roberto Cattaneo, Mayo Clinic, Rochester, MN, USA. This plasmid and its use to rescue infectious MV owas originally described in Radecke F, et al Rescue of measles viruses from cloned DNA. EMBO J. 1995, 14:5773.
- MV GM-CSF constructed in the laboratory of the applicant from starting materials above (Grote et al Cancer Res 2003; 63 6463)
- MV-IL-12 obtained from Dr Roberto Cattaneo (Singh M, Billeter MA. A recombinant measles virus expressing biologically active human interleukin-12. J Gen Virol. 1999,8:101)

Intended functions:
- Infectious MV expressing murine GM-CSF has been used by the applicants laboratory as an oncolytic agent in murine models (Grote et al Cancer Res 2003;63 6463) and is shows greater efficacy than the unmodified parental MV by virtue of stimulating a neutrophil infiltration of the tumors. We are currently further assessing its activity and mode of action. MV expressing human GM-CSF will be used to evaluate the effect on various human cell lines in-vitro. MV expressing murine and human IL-12 will be used similarly.

**Evaluation of foreseeable effects**

We and others have previously worked with attenuated MVs expressing growth factors and have had the opportunity to evaluate their effects in 2 different in-vivo models. A transgenic murine model expressing human CD46 (which has been accepted by the USA Food and Drug Administration as a suitable model in which to evaluate the toxicity of genetically modified MVs about to enter the clinic in phase 1 human studies) has been used to study the in-vivo effects of attenuated MV expressing murine GM-CSF. Second, a non-human primate model h as been used to evaluate an MV expressing human IL-12. In this application, we are thus able to consider the theoretical effects of accidental infection with a growth factor expressing, replicating attenuated MV in the light of in-vivo data. Naive mice were infected by various routes using MV expressing murine GM-CSF and suffered no ill effects. A low level of GM-CSF was detected in the blood at day 3 post-infection, which returned to background levels after that time. No effect was observed on the blood counts. Infection was terminated by an immune response. (Grote et al Cancer Res 2003-63 6463). MV
expressing human IL-12 has been administered to non-human primates without ill effects. The virus was eliminated by an immune response (R Cattaneo, personal communication).

Potential Effects on Human Health
The vaccine strains of MV themselves are not pathogens in immune competent individuals, but can cause fever and sometimes a mild rash in approximately 10% of vaccinees. Should humans become accidentally infected by attenuated MVs expressing a growth factor, it is considered that the virus would propagate to a limited extent, allowing some local production of growth factor before being limited by an immune response. By extrapolation from the animal data, it would not be expected that sufficient quantities of growth factor would be produced to cause systemic effects. All workers will be ensured to be immune to MV. Additionally, should all containment be breached, most adult individuals in the Western world, either by prior infection of vaccination. In the very remote case of an unimmunised individual being infected unintentionally, an immune response would be expected to limit infection albeit slightly slower than in a previously immune individual.

Growth factors such as GM-CSF and IL-12 are known to have pleotropic effects on human cells and their functions. Both of these growth factors have been used clinically and their range of effects are well characterised in humans in a variety of clinical scenarios. GM-CSF is a licensed product in fairly common clinical use. IL-12 has also been evaluated in clinical study. At very high doses, however, both GM-CSF and IL-12 can have harmful effects. Our animal data suggested that such toxic dose levels are not achievable when the growth factors are expressed from an attenuated replicating MV.

Bacterial vectors:
MV is a negative strand RNA virus, cDNA encoding the anti-genomic MV plasmid is not infectious, nor can it be used alone to generate infectious virus. Thus there is no foreseeable harm to human health from propagating standard laboratory strain of E coli harbouring MV plasmids since no expression will occur.

Cell lines:
When mammalian cell lines are infected by MVs expressing growth factors, infectious virus is released from the cells, although most of the virus remains cell-associated. Growth factor is secreted into the medium. Concentrations of GM-CSF in the medium are in the ng/ml range. MV-infected cells are the source of stocks of virus to be generated by freeze-thawing of infected cells. Virus concentration in stocks is usually about a million to ten million plaque forming units per millilitre. Average volumes harvested are around 1 to 3 ml. Sharps are not used when working with the cells, accidental inoculation with virus-infected cells is unlikely.

Mice:
Mice do not excrete MV in appreciable quantities. In the event of a worker being bitten by a mouse infected with MV expressing murine GM-CSF, it is unlikely that any harm would ensue, since the worker would be immune to MV, as discussed above. Additionally, where murine experiments take place, the murine growth factor is expressed. In the case of GM-CSF, there is little or no activity of the murine factor at the human receptor.

Potential Effects on the Environment
Bacterial Vectors
There is no foreseeable risk to the environment in the event of release of bacterial vectors.

Cell Lines
Would not survive outside the controlled environment of the laboratory and thus present no risk to the environment.

Mice
The only natural host for MV is humans. Normal mice are not infectable by MV, hence there is no risk to other mice in the facility nor to animals outside the facility, should a total breach of containment occur. Should MV-infected SCID mice escape into the environment, they would not survive beyond the confines of the laboratory and rapidly perish. Should the MV-infected SCID mice escape into the environment, they would not survive beyond the confines of the laboratory and rapidly perish. Should the MV-infected CD46 transgenic mice escape to the environment, they would also prevent no foreseeable threat. They would not be able to infect normal mice with MV. If by chance, these mice bred with local mice, and lost homozygosity for CD46, they would no longer be infectable by MV. It should also be noted that CD46 transgenic mice
CANNOT BE INFECTED BY WILD TYPE MV, which uses SLAM and not CD46 as a receptor. Finally, the mouse escape policy from the CBU at the RFH, which demonstrates the very unlikely nature of such an escape, is appended.

Spillage of virus stocks
In the very unlikely event of a pill outside the laboratory, where decontamination procedures for some reason were not able to be applied, which is extraordinarily unlikely, survival outside a host is brief. MV is inactivated by heat and light. Aerosols remain infectious for approximately 30 minutes and survival on surfaces is less than 2 hours. The virus is very unlikely to encounter wild-type strains, for the same reason, and also because in general such strains are usually not circulating in the community. However, considering this very remote possibility, MV is extremely genetically stable and recombination is very unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste generated will fall into the categories below:

Contaminated tissue culture plastics.
Solid waste, for example plastics will be decontaminated within the hood with 1% Virkon before disposal within autoclave bags to be autoclaved before eventual disposal according to the institutional system.

Plastic pipettes and pipette tips.
Will be placed into a rigid container within the hood and eventually, the capped container will be autoclaved before disposal, as above.

Liquid waste
Will be decontaminated with 1% Virkon or 1% sodium hypochlorite before disposal in the drains.

Animal carcasses
Animal carcasses will be disposed of within autoclave bags and autoclaved before disposal according to the institutional system.

Sharps (used during inoculation of animals)
Will be disposed of within an approved sharps container.

Virkon or sodium hypochlorite can destroy the virus so that no detectable virus remains after contact with the agents. No special monitoring will be necessary to monitor decontamination.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Comment: Will any primary cells be used?
Reply: There are no immediate plans to use primary cells.

Comment: Consider any risk from various MV-infected mice to humans or the environment.
Reply: possible risks to staff working with MV-infected mice.

Work carried out over the past 4 years at the Mayo Clinic did not require veterinary technical staff performing general housekeeping duties with mice used for similar projects be subjected to the same Occupational Health concerns as staff employed specifically for the projects. Mice infected with vaccine strains of MV do not excrete the virus to any quantifiable extent. In fact, it is rarely even possible to re-isolate the virus directly from MV-infected tissues (for example, lung, spleen) of CD46 transgenic mice by co-culture of tissue with highly permissive cells. Thus, the risk of workers handling the mice and cages for husbandry purposes actually coming into any contact with the virus can be supposed to be extremely low. The case of a person being bitten by a GM MV-infected animal has been considered in this assessment. This situation was discussed at length during the establishment of the policies at the applicant's previous institution. It was felt that no specific action would be needed in the case of an appropriately vaccinated worker, other than general measures, appropriately recording the incident and monitoring the health of the worker. In fact, this incident did occur, approximately 2 years ago, during the applicant's previous post. No harm ensued.

Comment: If new workers join the project, will these issues be addressed through the occupational Health Dept?
Reply: Yes, when new workers join the project, the issue of MV immunity will be addressed through Occupational Health.

Comments:
- Pipettes, tips, culture plates etc. do not require disinfection prior to autoclaving.
- Dry discard containers with screw caps should be used for tips rather than discard pots.

Reply: Comments are duly noted and will be acted upon.

Comment: Can project staff be named?
Reply: A technician has now been appointed and named.

Comment: Does vaccine mediated immunity protect equally well against the proposed genetically modified strains?
Reply: Murine data from our lab suggest that immunity to the GM-CSF-expressing genetically modified strain of MV is the same in magnitude and timing as to the unmodified MV.

? has sent these comments in after closure regarding your projects.

Comment: It will be important to monitor levels of anti MV immunity in people on the project and in people closely associated with it and to have a program of vaccination in place to boost levels of low response.

Reply: Titres will be checked at starting and if inadequate (<25iu/ml), project workers will be immunised. Regarding further boosting: immunity to MV after natural infection or adequate vaccination is generally accepted to be lifelong. It should not be necessary to boost. There are no data on which to base a scheme of re-testing and possible re-vaccination, this would have to be empirically determined. If it is deemed necessary, I would consult with virology and vaccine experts to try and devise an appropriate scheme.

Project Containment
**Project Additional Information**

**Purposes of the contained use**

Primary human cells will be isolated from patients: either liver taken at resection or time of transplant, placenta, amnion, bone marrow or peripheral blood, will be immortalised and transformed either by retroviral vector mediated, lentiviral mediated, lipofection, or by electroporation introduction of genes associated with cell cycle and senescence; some of these will be oncogenes. The resulting cells will be grown in vitro, to establish their proliferative and differentiative properties. Suitable cell lines may be chosen as the biological component of a bio-artificial liver machine (should this project be successful their use in a bioartificial liver would be the subject of a future application). A subsequent addendum to this application will be made if and when cell lines have been generated to test the proliferative capacity of these cells in vivo, by injection into nude mice; this will be in conjunction with a home office licence application to use nude mice.

**Recipient or parental organism**

Retroviral viral vectors based on murine moloney leukaemia virus: Vectors lack pol and env sequences and only encode a short sequence of ATG negative gag. Third generation Lin X (derivative of 293T) packaging cell lines will be used to produce replication-incompetent virus. The gag, pol and env genes are integrated into the genome.
or expressed from a Bovine Papiloma Virus based episomal vector that contains no regions of homology to the MMLV based vectors. Three separate recombination events would be required to produce replication-competent virus. The cell line has been tested exhaustively for production of replication competent virus; none was detected. The combined choice of the packaging cell lines and the replication defective nature of the vector used will prevent the production of helper/replication competent virus by the transduced cells, reducing the risk to the environment. Lentivirus vectors: cPPT-CMV and pCMV 8.91plasmid) vector is an HIV based vector described in full by Zennou et al (2000). The replication defective virus is produced in TRANS with structural components supplied by the pCMVr8.2 helper plasmid carrying gal, pol, tat, and rev. The vector is pseudo-typed with VSV-G, supplied in trans by the plasmid pMDG. At least 2 independent recombination events with a helper free genome would be required to recreate a viable pathogenic genome.

The second lentiviral gene delivery system involves three plasmids that are transfected into virus producing cell line, the 293t cell line. The lentiviral system is secured by gene removal such that no new particles can be produced from infected cells. This system functions as the host 293t cell express.

1. The viral envelope protein (CVSV-G envelope) from pVSVg.
2. The modified HIV-1 genome with the transgene (modified HIV-1 with deleted envelope and accessory genes)
3. The HIV-1 genome (from the pCMV plasmid 8.91)

The vector can stably integrate into DNA for sustained long-term expression of the transgene. The vector has the ability to infect human primary and stem cells, with subsequent expression of the foreign gene in these cells and their progeny. No new HIV-1 virions can be synthesised by the infected cells due to the deletions in the genome - and they are termed self-inactivating. The VSV-G envelop allows a broader cell target range than HIV1 and is included for this reason.

Host/vector system

Hosts: E.coli strains DH5alpha, DH10B, mammalian cells; primary epithelial and mesenchymal cells derived from human liver, peripheral blood, human placenta and amnion, human stem cells Hepatocyte cell lines Hep G2, human fibroblasts and lines ImR90, packaging cells LinX A and LinX E, 293T cells.

Vectors: pBabe, pWZL, MARX vectors (Genetica Inc, Cambridge, MA USA); LN series (LN, LNSX, LNCX, Miller et al 1989); cPPT-CMV-(eGFP); cPPT-CMV-(hTERT); pCMV 8.91

Origin & function

hTERT (telemerase), myc oncogene E7 oncoprotein, p53 tumour suppressor, E1A (early region 1A adenoviral sequence), mdm2oncogene, cyclin D1, p16 and p189 cyclin dependent kinase inhibitors, SV40 large t antigen, GreenFluorescentPortein, B-galactosidase.

All oncogene-virus constructs will be obtained from Wolfson Institute for Biomedical Research, UCL.

Evaluation of foreseeable effects

Primary human cells may contain adventitious agents and should be handled in containment level 2 according to COSHH. The other human cell lines are established long term lines ana can be regarded as belonging to ACGM activity class 1. The linX amphotropic virus is incapable of replication outside of its packaging cell for following reasons. Third generation LinX (derivative of 293T) packaging cell lines will be used to produce replication-incompetent virus. The gag, pol and env genes are integrated into the genome or expressed from a BPV based episomal vector that contains no regions of homology to the MMLV based vectors. Three separate recombination events would be required to produce replication-competent virus. The cell line has been tested exhaustively for production of replication competent virus; none was detected. The combined choice of the packaging cell lines and the replication defective nature of the vector used will prevent the production of helper/replication competent virus by the transduced cells, reducing the risk to the environment.

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The vector can stably integrate into DNA for sustained long-term expression of the transgene. The vector has the ability to infect human primary and stem cells, with subsequent expression of the foreign gene in these cells and their progeny. No new HIV-1 virions can be synthesised by the infected cells due to the deletions in the genome - and they are termed self-inactivating. The VSV-G envelop allows a broader cell target range than HIV1 and is included for this reason.

The HIV-1 vectors being used are attenuated and cannot replicate once cells have been infected. The transgene has the potential to be expressed in infected human cells. Because some of the transgenes are potential oncogenes they could, when expressed in cells, give the cells the ability to proliferate. The vector does not replicate, reducing the risk of harm significantly. Aerosol contamination is not possible - the virus can only infect when normal barriers (skin/mucosal membranes) have been penetrated. All work will be carried out in a containment level 2 laboratory, with protective clothing and no sharps inside the safety cabinets. The vector has a small and insignificant potential to become replication competent if combined with wild-type vector. We prevent this by having no live HIV-1 experimentally in the laboratory. All workers on the project will be required to attend Occupational Health department to discuss the risk potential. The overall risk of infection and harm is therefore low.

The project involves the generation of cells with a prolonged cellular lifespan; it is possible that one or several oncogenes could be enriched in some of the cell lines. Genes could belong to any of the categories above but only genes involved with senescence, cell cycle and differentiation will be selected for. Several oncogenes will be used as controls for the experiments. The effect of an oncogene or tumour suppressor on different cell types is very difficult to predict. For example, myc immortalises prostate epithelial cells but causes terminal differentiation in keratinocytes. Introduction of a single oncogene, however rarely results in malignant transformation. The hazards involved in naked oncogenic DNA will be addressed according to COSHH. Specifically naked oncogenic DNA will be handled in accordance with the "Advisory Committee on Genetic Modification Compendium, Part 3A - Annex 1. Containment level 2 is appropriate. The risk from the viral vectors themselves are minimal.

The main risk is introduction of an amphotropic virus containing an oncogenic sequence into the cells of a worker via a needlestick injury. This may result in the infection of the workers' cells and expression of the oncogene. The number of cells is likely to be small. Entry would have to take place through broken skin or mucosal tissue. The virus is incapable of replication once in the cells and the introduction of a single oncogene into a small number of cells is unlikely to result in tumour formation. This is before we consider the protective measures afforded by level 2 containment required and the class II cabinet that work must be conducted in, without the use of sharps in the hoods. The other risk is that transformed human cells may be introduced via needlestick or mucosal spillage. Because the cells are not derived from the worker they are unlikely to survive for long after entry.

There is no foreseeable risk to the environment in the event of the release of the bacterial vectors or GMMs. The transformed cell lines would not be able to survive outside of the laboratory and also present no risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Containment level 2: negative pressure relative to the pressure of the immediate surrounds: "required where and to the extent the risk assessment shows it is required". The tissue culture laboratories are constructed to Medicines Control Agency specifications and are therefore at a slight positive pressure with respect to their immediate surroundings, the anteroom. The anteroom is negative pressure with respect to the corridor (ie with respect to outside the tissue culture suite). Cell cultures will be manipulated only in Class II microbiological safety cabinets which are at negative pressure. In the event of a breach of containment outside the safety cabinet, GMM cell lines would not survive and would not pose a risk by the respiratory route.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Cell culture: All work with mammalian cells will be manipulated in Class II safety cabinets in designated tissue culture laboratories. All laboratory areas are designated containment level 2. Level 2 procedures will be observed.

Liquid waste: Liquid waste is treated with 1% Virkon or 1% Presept overnight and autoclaved before being discarded down the sink.

Solid waste: non-sharps plastics will be decontaminated within the class II cabinet, before autoclaving at 131 degrees C for 30 min and subsequent discarding as clinical waste.

Plastic pipettes and tips are decontaminated in a rigid but autoclavable container before disposal as per other solid waste.

Sharps: Sharps put into a sharps bin for off-site incineration.

<table>
<thead>
<tr>
<th>Name of disinfectant</th>
<th>concentration</th>
<th>Contact time (approx)</th>
<th>Level of kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virkon</td>
<td>3%</td>
<td>60 mins</td>
<td>below detectable level</td>
</tr>
<tr>
<td>Virkon</td>
<td>1%</td>
<td>overnight</td>
<td>below detectable level</td>
</tr>
<tr>
<td>Presept</td>
<td>1%</td>
<td>overnight</td>
<td>below detectable level</td>
</tr>
</tbody>
</table>

The expected level of kill should be included. This need not be precise eg "approximately 4 logs reduction in viability" or "below detectable level" is acceptable.

#Contact time should be at least one hour.

Autoclave: Length of sterilisation cycle (min): 30
  Temperature of sterilisation cycle: 131 C

This is checked using autoclave tape and by inspection of the printout of each autoclave cycle.

Note: If no validation for a disinfectant exists (ie the degree of kill for a particular organism is unknown) then laboratory testing could be required to establish its efficacy.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered
- Containment level is sufficient
- that sharps injury may be considered serious when handling immortalised cells
- the consideration of using temperature sensitive antigens instead of wild type
- the handling of one oncogene containing cell line at one time
- the production of transient cultures rather than stable cell lines for vector production
- the storage of vectors and cells
- risk to health of accidental insertion of the gene sequence into humans
- checks in place to ensure that staff are immunocompetent
- the laboratory facilities and procedures

FOR FULL DETAILS - PLEASE SEE THE WRITTEN NOTIFICATION
Project Containment

Laboratory Activities

L2 Yes L3 L4 L2 L3 L4 L2 L3 L4

Animal Units

L2 L3 L4 L2 L3 L4 L2 L3 L4

Glass Houses

L2 L3 L4 L2 L3 L4

Large Scale Activities

L2 L3 L4

Growth Rooms

L2 L3 L4

Human Clinical Applications


Project Ref 99/04.2

DateAckn'd 02/09/2004

CU2 Project Title

PROPOGATION OF RECOMBINANT VACCINIA VIRUSES EXPRESSING TWO HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) PROTEINS

Date Project Ceased 24/04/2012

Class 2

Culture Volume

1-50 Litres

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recombinant vaccinia viruses (NYCBH strain) expressing two HSV-2 genes (UL-7 and UL-46) will be used to infect BSC-40 cells and the cell lysates used in immunological assays. These HSV genes encode viral tegument proteins which are present in the virus particle as well as infected cells and have recently been shown to be targets of the cellular immune response. The viruses have already been prepared by University of Washington, USA. The ability of the vector to replicate is not affected by the insertion of either of the two HSV-2 genes.

Recipient or parental organism

The two HSV-2 genes encoded by the recombinant vaccinia viruses are highly unlikely to alter the tropism of vaccinia or to enhance (or diminish) its inherent pathogenic
potential. The tropism of vaccinia virus will not be affected by the expression of these two HSV proteins, which although present in HSV-2 virus particles, are internal in that structure and not known to play a role in the attachment and receptor-mediated entry of HSV-2 into target cells.

Host/vector system

Homologous recombination is a recognised natural event in the replication of poxviruses and although we may grow wild type vaccinia virus the possibility of erroneously co-infecting cells resulting in the acquisition of the HSV-2 genes is extremely low. The two HSV-2 genes would not be predicted to alter the tropism and replication properties of the vaccinia vector alone. Vaccinia virus is not present in the human population and therefore in the case of an individual becoming infected with recombinant vaccinia, there would be no opportunity for wild-type virus to acquire the inserted genetic sequences. Vaccinia virus can infect a number of animal species and any risk of release of virus into the environment by an animal source will be avoided by the physical containment of the work within a restricted access (keycode entry) category 2 containment laboratory. Acquisition of the HSV-2 sequences could not occur in a related micro-organism in the environment as wild-type vaccinia virus is not present in any animal species in the UK. No genetic modification work will be carried out, as the recombinant construct will only be propagated in BSC-40 cells.

Origin & function

Recombinant vaccinia expressing two HSV proteins obtained from our collaborator will be grown in BSC-40 cells and the lysates will be used for immunological assays.

Evaluation of foreseeable effects

Vaccinia virus, classified as a hazard group II pathogen, may cause severe disease in people with active skin disorders such as eczema or psoriasis or in immunocomprised individuals such as those with HIV. The addition of an HSV regiment protein gene in the recombinant vaccinia would not be predicted to enhance or diminish its ability to cause disease in comparison to the vaccinia virus vector alone (NYCBH strain). All the work will be carried out at containment level 2, in a single laboratory with restricted access (keycode entry, laboratory personnel only, the Department of Virology has swipe card entry system) and appropriate signs will be posted to indicate vaccinia work is being conducted, outlining persons who are potentially at risk. All members of the department will be informed. Vaccinia virus is not present in the human population and therefore in the case of an individual becoming infected with recombinant vaccinia, there would be no opportunity for wild-type virus to acquire the inserted genetic sequences.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General disinfectant will be 70% ethanol (contact time, 5 min). Bench disinfectant to be used will be Chloros at 1000 parts per million. All liquid and solid/plasticware waste will be double-bagged in autoclave bags and transported in metal tins to the autoclave facility housed within the Department of Virology. For plasticware, autoclave conditions are 10 min sterilisation cycle at 134 degrees C and for fluids, 25 min at 123 degrees C. Each run on the autoclave is validated (pass/fail) and written records kept and the autoclave is services every 6 months by specialist engineers. Spillage procedures are displayed in the laboratories where the work will be carried out (titled 'Disinfection procedures for GMO spillages'). No sharps will be used, and aerosols will be kept to a minimum as all handling of the viral cultures will be in a class II microbiology safety (serviced every 6 months).

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  

See attached.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Yes</td>
<td>L2</td>
<td>L3</td>
</tr>
</tbody>
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<table>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
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<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

**Project Ref  99/07.1**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
<th>Consent Granted</th>
</tr>
</thead>
<tbody>
<tr>
<td>28/12/2007</td>
<td>Construction of recombinant herpesviruses (herpes simplex virus (HSV) and human cytomegalovirus (HCMV) and their use in studies of herpes virus entry and replication.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Not Applicable</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Non-GMM</th>
<th>Tick if notifying a connected programme of work</th>
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<tr>
<td>24/04/2012</td>
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<tr>
<td>PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED</td>
<td></td>
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</table>

**Project Additional Information**

- Purposes of the contained use

To gain an understanding of the entry pathways of herpesviruses and the roles played by individual viral proteins in mediating virus entry.
Recipient or parental organism

<table>
<thead>
<tr>
<th>Recipient/parental viruses:</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV and HCMV are category 2 human pathogens. They can be propagated and handed under category 2 laboratory condition &amp; They are routinely cultured in this laboratory under category 2 condition. Both viruses are common in the general population and are clinically benign in immunocompetent individuals. Close contact is generally required for transmission of HSV and HCMV. Laboratory associated infection with HSV might occur if virus is introduced to the eyes (e.g. by splashing) This risk is minimised by wearing eye protection and working in a class 2 hood. Laboratory associated infection with HCMV is considered to be highly unlikely. Environmentally, these viruses pose a very low risk. Herpesviruses are enveloped and consequently are labile. Infectivity is completely destroyed by dessication, which is likely to occur in the event of a small laboratory spill. Neither virus has any natural host other than humans, so persistence in the environment, such as in rodents or insects, is considered to be very unlikely. All the viruses covered by this application retain the necessary genes to confer sensitivity to the licensed anti-viral drugs.</td>
</tr>
</tbody>
</table>

Host/vector system

<table>
<thead>
<tr>
<th>Characteristics of GMO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>The recombinant viruses fall into two groups; i) Those in which a gene (or genes) has been disrupted by addition of a stop codon or deleted and replaced with betagalactosidase, green fluorescent protein (GFP), or derivative, or other marker gene; these viruses are used to probe the function of the deleted gene product. ii) Those in which GFP (or derivatives) is fused to one or more viral proteins; Incorporation of GFP into these viruses means that they are fluorescent. These viruses are used to track the pathways used for entry into the cell.</td>
</tr>
</tbody>
</table>

Forseeable effects

A: Health

i) Deletion mutants: There is no reason to suppose that the pathogenicity of these viruses is, or will be, increased. Some of the viruses are attenuated in animal models, others are equivalent to wild type. None is known to have enhanced pathogenicity. ii) Fluorescent viruses; In all cases, these viruses are indistinguishable from wild type (other than the presence of the transgenes), indeed they would be of no use experimentally if they did not mimic wild type virus. Summary; The viruses are considered to be either equivalent to wild type virus or less pathogenic than wild type virus. HSV and HCMV are category II pathogens, thus handling of the recombinant viruses under category II laboratory conditions is considered to be sufficient for containing the risk to health. In general, close contact is required to infect an individual with HSV or HCMV. The risk of this is minimised by handling the viruses under category II laboratory conditions. Wild type HSV and HCMV are handled routinely and safely in the laboratory in this way. |

B: Environment

Herpesviruses are encased in a lipid envelope and thus are labile. They are rapidly inactivated by dehydration or mild detergent. They are very unlikely to persist in the environment. Neither HSV nor HCMV is known to have any natural non-human hosts, (although HSV can infect laboratory rodents). This lack of natural hosts combined with the environmental instability of the virus particle makes inadvertent transmission to rodents or other wild animals very unlikely. |

Origin & function

<table>
<thead>
<tr>
<th>Origin of viral genomic DNA:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant viruses are constructed by homologous recombination. For HCMV the starting material is a bacterial artificial chromosome (BAC) clone of the HCMV genome. For HSV, purified wild type viral DNA is used. In each case, virus is reconstituted by transfection into a producer cell. All procedures in the protocol prior to the transfection are carried out in E.coli and as such present no infection risk. Herpessiral genomic DNA can initiate virus production when introduced into a target cell. However, this is very unlikely to happen to a laboratory worker during laboratory manipulation &amp; Sharps are not used and good laboratory practice means the risk is negligible. The viruses fall into two categories: i) Deletion mutants: in which a viral gene is deleted and replaced by marker gene expression cassette encoding betagalactosidase, GFP (or derivatives), or another</td>
</tr>
</tbody>
</table>
fluorescent protein. In these viruses the function of the inserted genetic material is a) to facilitate selection of recombinant viruses and b) to use as a marker of infection.

ii) Fluorescent viruses: in which one more viral genes is fused in frame with GFP (or derivatives), or other fluorescent protein-encoding genes. These viruses are intrinsically fluorescent and are in all other respects comparable to wild type.

In these viruses the function of the inserted genetic material is to make the virus particles fluorescent, while retaining other wild type characteristics, so that the viruses can be used in fluorescence-microscopic studies of viral entry pathways.

Origins of the inserted genetic material:
Beta-galactosidase: this gene encodes an enzyme that digests lactose or other related substrates. It is widely used in molecular biology. The gene originates from E.coli.
GFP (and related fluorescent proteins): these genes are obtained from jelly fish (Aequoria victoria). They form distinct globular domains that can be fused to other proteins retaining function and rendering those proteins fluorescent, They are widely used in molecular biology.

Evaluation of foreseeable effects

These are detailed in the above sections.

Summary: HSV and HCMV are category II pathogens and so can be handled safely in the laboratory under category II working practice. Close contact is required for infection to occur. Laboratory associated infection is a small risk and this is reduced by the use of appropriate protective clothing.

None of the recombinant viruses is anticipated to be more pathogenic than the parental virus.

HSV and HCMV are rapidly inactivated by dessication and neither virus has a natural non-human host so the risk to the environment in the event of a spill is judged to be minimal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Herpesviruses are 100% inactivated by autoclaving.
All GMM waste, solid (eg flasks, tubes, tips, plates) and liquid (eg culture medium, used virus aliquots) is autoclaved in the department. (Standard discard cycle is 135 degrees C, 3150 mBar, 10 minutes)
Temperature and pressure parameters of each autoclave cycle are recorded and reviewed. Additionally, Browns tubes and autoclave tape are used to assess each run.
The autoclave is maintained to a high standard, sufficient for HPA accreditation, by regular servicing and testing with a 12 point thermocouple. Records are kept in the Department.
All autoclaved waste is placed in yellow biohazard bags and taken for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This project (local (GM99) reference: F1C36/07) received approval from a panel of advisers representing the Centre GM Safety Committee at a meeting held on Thursday 8 November 2007. The panel agreed with the classification of the activity and further agreed that the precautionary measures stated in the risk assessment were suitable for these laboratory procedures.

### Project Containment

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<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
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</table>

#### Project Ref 99/09.1

- **CU2 Project Title**: GENERATION OF RECOMBINANT HIV-1 TO STUDY THE EFFECTS ON VIRUS PHENOTYPE OF VARIATIONS WITHIN VIRAL GENES ENCODING PROTEINS TARGETED BY ANTIRETROVIRAL THERAPY (POL, GAG AND ENV)

- **Class**: Class 3
- **Culture Volume Class 2**: <150 ml
- **Non-GMM**: Consent Granted
- **Consent Granted**: Being Processed
- **Tick if notifying a connected programme of work**: N
- **Historical Date of Additional Info**: Project transferred to GM554
- **Significant Change ID**: 99/09.1a
- **Date of Significant Change**: 25/03/2013

### Project Additional Information

- **Purposes of the contained use**: To study the effect of mutations encoding antiretroviral resistance upon replicative function and drug susceptibility
- **Recipient or parental organism**

02/03/2022
Introduction

Wild type HIV-1 (wt HIV-1): Wild type HIV-1 describes infectious HIV-1 viruses that do not exhibit resistance to therapeutic drugs and lack the according resistance mutations. These are produced by eukaryotic cells transfected with plasmids such as pHXB2D, pNL4-3 and pMJ4. All plasmids contain single retroviral genomes (proviruses) derived from patient isolates or laboratory strains and produce infectious viruses when transfected into permissive cells.

HIV-1 vectors: HIV-1 vectors are plasmid clones of HIV-1 proviruses derived from lab strains which are NOT replication-competent. Those vectors used within this project have deletions within their genes to prevent production of infectious virus. Such deletions will be contained within either the gag (protease), pol (RT) or env genetic regions. Such vectors also carry a resistance gene e.g. ampicillin, in order to enable the selection of vector containing prokaryotic cells. The risk from these vectors is minimal in prokaryotic cells e.g. E. coli, as the HIV-1 genes are unable to be expressed. As a result work involving these vectors may be carried out under ACGM class 1 conditions as the clone itself is non-infectious. Once transfected into an appropriate cell line in the presence of full length genes (for which the vector is defective) infectious virions are able to be produced. Vectors used within this investigation will be defective due to deletions in the gag, pol and env genetic regions.

Drug resistant HIV-1: Genes of interest encoding mutations will be amplified from clinical material derived from patients exhibiting resistance to drugs after therapy, or generated by site specific mutagenesis upon wt genetic material. These mutations within the gene of interest will encode for an HIV-1 with a phenotype of resistance to a particular, or a combination of, antiretroviral drugs.

Recombinant viruses: Recombinant viruses will be constructed by homologous recombinations between the HIV vectors (with deletions in the appropriate genes) and genetic material derived from either laboratory strains (simulating wild type virus) or 'normal' genes in the context of the same viral background. The presence of both components is required within the appropriate cells before recombination can take place.

Risk of infection

Levels of infectious recombinant virus produced (under containment conditions) during this investigation are anticipated to fall within the ranges measured in the blood of persons infected with HIV-1, although many viruses with drug resistance mutations replicate at reduced rates compares to wt virus. The risk of HIV-1 infection following exposure to infected blood through sharp injury is estimated to be around 0.3% , whereas that associated with a splash exposure with blood is in the region of 0.09%. This in absence of Post - Exposure Prophylaxis (PEP).

Management of Infection

If exposure does occur, it is managed through the administration of PEP constituting a four week course of antiretroviral drugs. Although the success rate of this course is high, it is not 100%. Some recombinant and wild-type viruses contain mutations which render them of increased susceptibility to particular antiretroviral drugs. Some resistance mutations may affect more than one drug; however there are sufficient drug combinations currently available against drug resistant HIV-1.

Other risks

The potential integration of a complete retroviral vector into the host genome may also have the potential to affect host cellular function i.e. insertional mutagenesis, leading to the possibility of tumour formation, subsequent to exposure to wt lentiviral vectors and HIV-1 lab strains. Integration of complete lentiviral vectors into host cell genomes has been recently described however the overall likelihood of such events is thought to be low (J. Virol. 2008 Oct 22). Additionally, it may be speculated that the transduction of eukaryotic cells with expression vectors, or infection of such cells with recombinant HIV-1 or retroviral vectors may also result in the immortalisation of the cell line with altered properties. Such cells may retain the ability to establish tumour formation within exposed laboratory workers following a significant exposure although the likelihood of such an event is considered extremely remote. Even in such a rare occurrence, the chances of such cells becoming established and productively proliferating within the worker would be further limited by immune defence mechanisms such as MHC mismatching. Furthermore, mutations within the env region may result in the altered tropism of viruses. Such events as these however would be unlikely to influence the susceptibility of the virus for particular drug therapies in the event of
Environmentally, these viruses pose a minimal risk. HIV-1 is an enveloped particle and is consequently labile. There is no reason to suspect that mutations will result in structural changes to progeny virions, thus it is unlikely that any changes to viral genes will affect its interactions within the physical environment. Infectivity of wt HIV-1 is completely destroyed by desiccation or disinfection and waste disposal protocols implemented within the containment level 3 (CL3) laboratory, the chance of residual virus remaining infectious is unlikely.

Virus producing cell lines

The cell lines, MT4 and C8166 that will be used to propagate the virus in vitro are persistently infected with HTLV-I (although C8166 cells carry, but do not express the HTLV-I genome). C8166 is derived from umbilical cord blood cells (Virology. 1983; 129:51) whereas MT4 is a human T cell lymphoma cell line (Acta Microbiol Hung. 1992; 39(3-4):271-9). Both have been immortalised by HTLV-I virus. Cell lines were chosen as they exhibit characteristic morphologies when infected by HIV-1. There is a risk of exposure to HTLV-I when working with MT4 cells, although the virus is known to be highly cell-associated and the chance of infection is low. Additionally, the risk of HTLV-I virus particles recombining with lentiviral vectors is minimal, as is the likelihood of recombination with recombinant HIV.

In summary: the main consequence of exposure to vectors engineered to express viral genes is the potential transfer of GMM to the laboratory worker. Laboratory associated exposure to the recombinant virus would carry similar implications as exposure to the 'non-resistant' wild type HIV-1 (grown from clones in cells) and drug resistant HIV-1 (derived from patient material). In spite of any such mutations effective drugs remain available to treat infection with recombinant viruses. Additionally, such risks working with such GM organisms are further reduced by good working practice and adherence to SOPs under CL3 conditions. The availability of PEP further reduces the risk of infection after a significant exposure. Therefore the overall likelihood of infection is very low. Should infection occur despite PEP then consequently the health risks are those associated with wt HIV-1 infection.

**Host/vector system**

**Characteristics of the GMO**

Recombinant viruses will fall into three groups:

1) Those in which inserted regions, cloned directly from patient samples, contain mutations which encode for resistance to particular (or a combination of) antiretroviral drugs.

2) Those in which inserted regions are cloned directly from wild type HIV-1 laboratory strains such as pHXB2.

3) Those in which inserted regions contain mutations generated in vitro which represent intermediate 'halfway' stages between drug resistance and wild type

**Forseeable effects**

1) Health

There is no reason to expect that viruses generated by this work will be more pathogenic than 'non-resistant' wtHIV-1 virus. There is also the possibility that exposed laboratory workers may acquire a mixed infection (of both resistant and non-resistant HIV-1) although this risk is reduced as the viruses will be cultured under CL3 containment conditions however any such exposure to mixed viral populations is considered to pose no greater danger than infection by either virus individually. Although resistance mutations may affect more than one drug there are considered to be sufficient numbers of drugs, with different mechanisms of action, currently available for application against drug-resistant virus.

2) Environment

The presence and persistence of recombinant viruses within the environment is extremely limited and therefore adherence to disinfection and waste disposal protocols implemented within the CL3 laboratory further reduces the chances of viruses' surviving within the environment.
Cells proposed for use within this project will fall into 2 classes:

i) Prokaryotic cells required for the propagation of HIV-1 vectors. These include E. coli DH5α, TOP10 and equivalent cells.

ii) Eukaryotic cells required for construction and propagation of recombinant HIV-1. These include 293T, MT4, C8166 and equivalent cells.

Origin of viral genomic genetic material

Several HIV-1 vectors are intended for use within this investigation. Genetic regions of these vectors will be replaced with regions of interest in order to study the effects regarding susceptibility to certain antiretroviral drugs and on viral replication.

The pHIVΔRTBstEII vector contains the complete provirus of the HIV-1 laboratory strain HXB2D with a 1.4kb deletion within the RT region. The clone is non-infectious but when transfected into an appropriate cell line with full length RT coding sequence provided in trans infectious virus is produced by homologous recombination. All procedures within the protocol prior to transfection are carried out in E. coli and present no infection risk. This vector is unable to initiate virus production when introduced into a target cell in the absence of a full length RT coding sequence and therefore is unlikely to regain wild type phenotype in the absence of RT. This minimises the level of risk to the laboratory worker to the vector during laboratory manipulations. Sharps are not used and good laboratory practice means the risk to workers is negligible.

This plasmid and its equivalents [containing deletions/mutations within the gag (protease), pol (Integrase) and env] will be used to determine the impact of mutations within these regions upon resistance

All molecular clones used within this investigation will be obtained from the National Institute of Health AIDS Research and Reference Reagent program (www.aidsreagent.org)

Origins of the genetic material to be inserted into replication-deficient vectors

Inserts encoding for drug resistance: Genes of interest encoding mutations will be amplified from clinical material derived from patients exhibiting resistance to drugs after therapy. These mutated genes of interest will encode for an HIV-1 with a phenotype of resistance to a particular, or a combination of, antiretroviral drugs.

Inserts coding for 'non-resistant' wild type HIV-1: Genes of interest (gag, pol and env) will be cloned from the HIV-1 laboratory strains pHXB2D, pNL4-3 and pMJ4. All three plasmids contain proviruses derived from patient isolated and laboratory strains which produce infectious virus when transfected into appropriate cells. Such inserts will encode for an HIV-1 virus without a resistance phenotype.

Inserts containing intermediate mutations for HIV-1 drug resistance: Genes of interest will be cloned from the HIV-1 laboratory strains and isolates. Sequences will then be altered using in-vitro mutagenesis to induce partial sequence identity to drug resistance motifs, simulating intermediate stages in the development of drug resistance to particular (or a combination of) antiretroviral drugs.

Cloning vectors: Theses are commercial vectors used for the purpose of cloning and amplifying regions of interest and include pCR2.1TOPO derivatives, pUC vectors, pBLUESCRIPT vectors, pcDNA3.1, pCMV/Zeo and pEGFP-1.

Evaluation of foreseeable effects

These are detailed in the above sections.

To summarise: The main risk is accidental exposure of laboratory workers to infectious recombinant and wild-type HIV-1.
The handling of bacterial cultures and plasmids for cloning is carried out under ACGM class I conditions and therefore poses little risk, if any. At this stage of the work the risk of exposure of workers to infectious virus is minimal.

The HIV-1 vectors are derived from well characterised laboratory strains of HIV-1. These are not able to form infectious particles unless in the presence of a full length RT region in trans (either wild type or mutated). Such an event would ONLY occur within the contained environment of the CL3 suite. An additional foreseeable risk is the oncogenic potential of the vector to the host genome upon potential exposure. The probability of such an event is extremely low and in this respect these vectors pose a minimal risk.

The defective HIV-1 vectors can ONLY form replication-competent viruses when both the HIV-1 vector and genetic region of interest are present within the cell. Recombination in the absence of either is extremely unlikely, as is the chance of spontaneous recombination with retrovirus genomes within, or associated with the aforementioned cell lines. The presence of replication competent recombinant HIV-1 poses the main risk to laboratory workers, although the risk is no more significant than that posed by handling wild type HIV-1 virus. The main risk of exposure in the laboratory is via cuts and abrasions with contaminated sharp instruments, or splash exposures as HIV-1 is not readily transmitted by aerosol. Through adherence to CL3 working guidelines and good laboratory practice, this risk is significantly reduced. The use of PEP after accidental exposure makes the risk of an established infection very low. Should infection occur, the recombinant viruses are unlikely to be any more pathogenic than those circulating in infected patients. No genes of the source strain of HIV-1, from which the lentiviral vector has been derived, have been shown to be oncogenic or teratogenic.

The potential risk of exposure to HTLV-I during this work has been discussed previously, however as work with this cell line is confined to the CL3 suite; the risk of exposure to this virus is minimal.

Environmentally, providing correct guidelines upon disinfection and waste are followed there are no foreseeable risks posed by either the wild type or recombinant viruses due to their nature as enveloped viruses.

Container and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory workers are responsible for the following procedures:

Class 1 GMM products include HIV-1 genetic regions of interest derived from clinical samples or laboratory strains which have been cloned into plasmid vectors and the production of replication defective viral vectors.

Method of Class I GMM waste disposal

All GMM waste both solid (e.g. flasks, tubes and plates) and liquid (e.g. culture medium) is double bagged into clear autoclave bags and placed into metal autoclave tins for autoclaving. Autoclaving conditions follow the NHS diagnostic guidelines and adhere strictly to the requirements of the Clinical Pathology Accreditation. Standard autoclave cycle conditions are 135°C at 3150 mBar for 10 minutes. Temperature and pressure parameters of each autoclave cycle are recorded and reviewed. Additionally, Brown's tubes and autoclave tape are used to assess each run.

Spillages are dealt with using KLORSEPT granules. Disinfected spillages are then absorbed onto paper towels which are then autoclaved.
Methods of Class 3 GMM waste disposal

Class 3 waste products will include cell culture medium containing antiretroviral drugs, cells and wt/recombinant HIV-1 virus.

All CL3 GMM liquid waste is emptied from plastic culture flasks into a sweetie jar within the MSC containing 2x6g sachets if Vernagel gelling agent. All CL3 GMM solid waste (e.g. flasks, tubes, tips and plates) are then immersed in a container of 10% Trigene Advance located within the microbiology safety cabinet and allowed to disinfect for at least 16 hours. Lids are sealed upon 2L lidded containers and liquid contents are allowed to gel before being double bagged, and loosely swan-necked prior to disinfection and placed within a sealable plastic autoclave bucket.

After disinfection of solid waste is allowed to take place the container is removed from the hood. Disinfected solid waste is poured into a sieve placed over a sink whilst disininfected liquid waste is poured down the sink and washed down the mains drainage with copious amounts of water. All disposable solid waste from the sieve is placed into double autoclave bags which are disinfected and swan-necked loosely. Laboratory plasticware including jugs are double bagged and loosely sealed by swan-necking prior to further disinfection. Bagged and sealed plastic ware (i.e. beakers and jugs) are then placed in a sealable metal tin for autoclaving. The remaining disinfected solid waste is placed (using the sieve) into double autoclave bags. These are loosely sealed prior to disinfection and placed into sealed yellow autoclave bins.
After each work session all surfaces are disinfected with 5% Trigene Advance in order to eliminate any residual cirus. At the end of each work day, autoclave bins and sealed metal tins are surface disinfected with 5% Trigene Advance before they are placed in a lockable trolley by a trained member of the CL3 team, and transported directly to the local autoclave (within the department) for immediate incineration under standard autoclaving cycles. No GMOs will remain viable after treatment.

After autoclaving, sealed plastic autoclave bins will be labelled and sent off site for incineration. Metal transport tins and any plastic ware from the CL3 are returned directly to the CL3 suite in order to prevent them entering general laboratory circulation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Part of this work have been approved by the Royal Free & University College Medical School (Hampstead) GM99 Committee on the 28th November, 2007. Document references include HC40/07 and HC37/07.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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<tr>
<td>L3</td>
<td>L4</td>
<td>L2</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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The projects covered under this application aim to exploit lentiviral vectors as gene delivery systems for immunotherapy. To this end we have 2 complementary approaches. The first involves the use of lentiviral vectors to deliver immune-modulating (for example, T cell receptors, co stimulatory molecules or cytokines) into immune cells. Genes encoding immune modulating genes will be cloned into lentiviral vectors, and lentiviral particles will be used to transduce mouse or human immune cells or haematopoietic cells for the production of modified immune cells which can be used for immunotherapy of cancer. The second approach involves using lentiviral vectors for vaccination strategies. In this case lentiviral vectors encoding model or tumour-associated antigens are used to immunise mice. It has previously been shown that lentiviral vectors are very potent immunising agents and we will be investigating how the immune responses are activated, and whether they would be appropriate immune responses to control an emerging tumour, for example in an in vivo model of leukaemia.

GMO is the lentivector: 3rd generation HIV vector with virulence genes deleted, on split plasmids
Lentiviral particles will be used to transduce a range of cells in the laboratory: standard laboratory cell lines (e.g. 293T, HeLa, NIH3T3 etc.). Human and mouse haematopoietic cells (in particular T cells and Dendritic cells as well as other immune cells).

The viral vectors themselves are not pathogenic to humans, all virulence genes have been deleted from the original parent viral sequences. However, since these vectors have the potential to integrate into human DNA there is a low risk that infection could damage the health of the laboratory worker directly handling the viruses. We are planning to use self-inactivating (SIN) vectors to make viral particles that have been shown to significantly reduce the risk of insertional mutagenesis. In addition lentiviral vectors have been demonstrated to insert preferentially into coding regions of DNA, rather than the more risky promoter or control regions. Therefore, when considering all
these factors the risk to humans is very low.

Because the lentiviral vectors are replication deficient and undergo a single infection of initial target cells, there is no risk of spread to other recipients. The vectors will only be used as an antigen delivery system to immunise, once the viral vector is injected into mice the immunised animal is not considered genetically modified and the viral vector no longer exists. Thus, there is no risk from this point.

A WPRE element is included in these vectors to aid translation of the inserted gene. This encodes a putatively oncogenic protein (protein X) which has been mutated in all vectors. The X protein could potentially be a risk factor, but the use of vectors with non-functional X proteins negates this risk.

The vector particles are produced with a broad host specificity by using the Vesicular Stomatitis virus (VSV) G protein or other coat proteins. This may increase the susceptibility of different cells to the virus, but will not increase the risk of insertional mutagenesis.

**Host/vector system**

Vectors are standard microbiology plasmids. All cloning is performed using commercial disabled bacterial strains that are not harmful to health or the environment. The promoters used for gene expression in the lentivector are not generally expressed in bacteria.

pUC derivatives or standard molecular biology cloning vectors such as the following, or related vectors: pCMVR8.91 (packaging plasmid), pMD-G (VSV-G envelope plasmid) & pHRSIN (vector plasmid Dermaison et al (2002) Human Gene Therapy 13:630-640.).

Some vector plasmids obtained from Prof. M C’s laboratory at UCL may contain sequences to enhance expression in transduced cells (vFLIP latent gene expressed by KSHV) such as dendritic cells or T cells. Human and mouse genes of the NF-kB pathway. MAPK activators: MKK6EE kinase (p38 activator), MEK1 N3 ED kinase (ERK activator) and MKK7-JNK1 (JNK1 activator.)

**Origin & function**

The genetic material will be of bacterial origin for cloning as follows:

HB101, DH5alpha & other common disabled E. coli K-12 or B derivatives. Only disabled bacterial strains will be used.

The inserted sequences may be amplified originally from mouse or human genomic DNA or cDNA before cloning into bacteria.

For work investigating lentivectors as immunisation agents, the majority of the inserted nucleic acid encodes model antigens. These are inert gene products that do not have a biological function in the transduced cells, and will not effect growth and survival of cells receiving these vectors. For the delivery of immunomodulating genes there are genes encoding immune modulating proteins which may alter the immune function of the cells they are expressed in, but it is highly unlikely that this would lead to transformation of the cells in question.

Genes such as the vFLIP latent gene from KSHV or MAPK activators may occasionally be incorporated into lentiviral vectors to enhance immune responses to the inserted gene. It is not expected that these genes will alter the biological activity of inserts encoded in the same vector. However these sequences do have the potential to effect expression of an oncogene or tumour progression factor depending on the site of integration of the vector.

A WPRE element is also included in the vector plasmid to aid translation of the inserted gene or sequence. This encodes a putatively oncogenic protein (protein X) which has been mutated in all vectors and is not functional.

The model antigens, genes encoding immune modulating genes and mutated WPRE element are unlikely to cause harm if accidentally transferred into humans. We do identify an increased risk when using KSHV or MAPK activating genes. It is possible that in the unlikely event of a breach of containment, accidental injection into workers may result in the undesirable insertion of these genes next to an oncogene. However, this risk on insertion at a site such as this is extremely low and it is unlikely that any one gene has the capacity to act as an oncogene on its own.
Evaluation of foreseeable effects

For generation of lentiviral vectors only disabled bacterial strains will be used and we do not foresee any effects.

The most hazardous GMMs are the HIV-based vectors. The most hazardous step and the only risk that we identify at the moment is injection of the transducing particles into mice because this involves the use of needles and the risk of personal injury.

Containment and control measures for GMOs that are not micro-organisms (e.g. GM animals & plants)

All GMOs in this assessment are micro-organisms.

For immunisation of mice with lentiviral particles it is requested that the animals are injected in a level 2 designated room, but outside a laminar flow hood. Lentiviral particles are extremely sensitive to desiccation and no risk associated with infection due to air-borne particles has been identified. Injection of mice while in a laminar flow hood restricts the movement and in fact increases the risk of needle stick injuries due to the difficulty of manouevring in the confined space. The site of injection is sprayed with ethanol to inactivate any lentiviral particles leaking from the injection site (though this volume is typically less than 10-20 l).

Once the lentiviral particles are injected into mice, the inserts will be permanently integrated into the genomes of host cells, and no new viral particles will be produced. Therefore, subsequent to immunisation the mice are not considered genetically modified organisms and no risk is associated with their handling and housing. All waste from the housing of mice is disposed of by incineration and this therefore remains the appropriate method of disposal for waste from animals immunised with lentiviral vectors. Likewise cadavers will also be disposed of by incineration according to standard UCL procedure.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMM/1: line 1.29 or GMM/2: lines 2.13 (as applicable)

For routine cleaning, all surfaces will be wiped down with 70% alcohol after use which is known to be effective against enveloped viruses. Liquid GMM waste will first be disinfected using Presept overnight (effervescent disinfection tablet containing Troclosene Sodium) to give a final concentration of 2500ppm available chlorine (1x2.5g tablet/500ml waste) before being disposed of down the sink. This is a standard procedure and viability is below detectable levels. Solid GMM waste will be placed in autoclave bag, and autoclaved via the central collection service for GM waste.

The autoclave sterilizing cycle is carried out at 135°C for 5 minutes. All sharp objects, such as pipette tips are placed in a rigid plastic container prior to disposal in autoclave bags. Needles (without dissociation from the attached syringes) are placed in sharps bins which are sealed before being added to the autoclave bag.

It is not possible to estimate a degree of kill for a non-replicative virus. However, autoclaving and 2500ppm available chlorine is sufficient for a 100% inactivation of enveloped viruses.

For immunisation of mice all needles will be disposed of in dedicated sharps bins (without dissociation from the attached syringe). The sharps bins are then placed in autoclave bags and autoclaved via the central collection service for GM waste.

Note that in the event of spills, 70% ethanol will be used for surface disinfection. This agent is effective and widely used for inactivating enveloped viruses. Absorbent tissues will be used to mop the affected area and will then be disposed of by autoclaving as described above.

Temperature and pressure parameters for each autoclave run are recorded on a printout and reviewed. The autoclave is checked weekly and a 12 point thermocouple test is performed annually. The autoclave validation records are audited annually and a certificate is issued.

All solid waste (including sharps) are placed in autoclave bags and collected from the department to be taken to the central autoclave. The bags are transported to the
autoclave in a rigid, lidded container.

1.10-1.13 These sections seem to be rather generic with no real detail as to what genes they will be inserting (described for example as "immuno-modulating genes"), what micro RNA/siRNA sequences they will be inserting, which cell types will be used etc?

1.10 Immuno-modulating genes such as T cell deceptors, co-stimulatory molecules, cytokines/chemokines and the receptors for cytokines/chemokines will be studied for their function in the engineered cells. MicroRNA or siRNA sequences to control expression of inserted sequences in specific cell types. For example, the microRNA mir142 restricts expression of upstream genes to non-haematopoietic cells only (Brown et al (2006) Nat Med 12:585). Alternative micro RNA sequences may be used to allow expression only in immune cells, especially dendritic cells. SIrNA would be used to specifically down-modulate protein levels of a gene of interest in transduced cells, for example, a co-stimulatory molecule or cytokine or the endogenous T cell receptor genes.

1.12 Some vector plasmids obtained from Prof. M Cs' laboratory may contain sequences to enhance expression in transduced cells such as dendritic cells or T cells.

1.13 Cells: standard laboratory cell lines (e.g. 293T, HeLa, NIH3T3 etc.). Human and mouse haematopoietic cells (in particular T cells and Dendritic cells as well as other immune cells).

1.25 They indicate the autoclave waste will be "autoclaved later" is there any issue with waste hanging around to be autoclaved and how long "later" actually would be?

All GM waste is placed in Autoclave bags which are changed on a regular basis. All autoclave bags are kept in rigid containers. All sharps are placed in lidded containers. They are then collected by the porter, who autoclaves them on the service floor. This is what was meant by autoclaved later.

GMM2

2.16 Question: Does you laboratory operating procedure permit the use of sharps (including glass pipettes)?

Answer: No. But they intend to use sharps to inject the lentiviral vectors into mice or does the question relate only to the portion of the work which will be done in the lab rather than in CBU.

Yes, we meant that no sharps are used in the laboratory setting, but of course they are used in the animal house.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2</td>
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Withdrawn | Tick if notifying a connected programme of work
N  | N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

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<thead>
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Animal Units  
Large Scale Activities  
Human Clinical Applications

**Project Ref** 99/93.2

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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
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Tick if notifying a connected programme of work  

Withdrawn  

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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**Project Ref**  99/99.1

- **Date Ackn'd**: 07/12/1999
- **CU2 Project Title**: GENE TRANSFER OF CD80 AND IL-12 TO ACUTE MYELOID LEUKAEMIA AND BREAST CANCER CELLS USING ADENOVIRUS
- **Date Project Ceased**: 23/03/2007
- **Class**: Class 2
- **Culture Vol Class 2**: not applicable
- **Culture Volume Class 3-4**: not applicable
- **Non-GMM**: not applicable
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: PROJECT CLOSED 23/3/07.
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**
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Recipient or parental organism

Host/vector system

Origin & function

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If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
DUNCAN GUTHRIE INSTITUTE OF MEDICAL GENETICS

Name

YORKHILL NHS TRUST

Department

MEDICAL GENETICS

Building

Campus Estate or Research Centre

Road Name

District

YORKHILL

Town

GLASGOW

County

EAST RENFREWSHIRE

Postcode

G3 8SJ

Country

SCOTLAND

Tel Number

0141 201 0360

Fax Number

0141 357 4277

E-mail

HSE Division

SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

### Level 1 (GMMs)

### Level 2 (GMMs)

### Level 3 (GMMs)

### Level 4 (GMMs)

### Non-microbial

Other (please specify)  

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### Data Premises Notified

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### Transferred from 1992 Regs?

| Transferred from 1992 Regs? | Y |

### Transitional Premises

| Transitional Premises | 2 |

### Data Premises Closed

| Data Premises Closed | N |

### Transitional Premises

| Transitional Premises | N |

### Emergency Plan Required?

| Emergency Plan Required? | N |

### Level

| Level | 2 |

### Non-GMMs

| Non-GMMs | N |

### Withdrawn

| Withdrawn | N |

### Name

| Name | UNIVERSITY OF EAST ANGLIA |

### Name 2

| Name 2 | |

### Department

| Department | SCHOOL OF BIOLOGICAL SCIENCES |

### Campus Estate or Research Centre Building

| Building | |

### Road Name

| Road Name | UNIVERSITY PLAIN |

### District

| District | |

### Town

| Town | NORWICH |

### County

| County | NORFOLK |

### Postcode

| Postcode | NR4 7TJ |

### Country

| Country | ENGLAND |

### Tel Number

| Tel Number | 01603 592199 |

### Fax Number

| Fax Number | 01603 593474 |

### E-mail

| E-mail | |

### HSE Division

| HSE Division | EAST AND SOUTH EAST |

### Comments

| Comments | |

### Date at Which Additional Info Submitted

| Date at Which Additional Info Submitted | 12/01/2004 | 2022 |

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02/03/2022
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- **Bacteriology**
- **Parasitology**
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Tick if you are claiming exemption from disclosure for sections of the risk assessment
Please enter comments of the GM safety committee on the risk assessment

Project Ref  101/01.1

Date Ackn'd  15/02/2001  CU2 Project Title  THE STRUCTURE AND MECHANISM OF PROTEINS INVOLVED IN RESPIRATORY REDUCTION OF TETRATHIONATE AND THIOSULFATE IN SALMONELLA ENTERICA

Date Project Ceased  14/12/2001

Class  Class 2  CultureVolClass2  Consent Granted  not applicable

Non-GMM  Project notified under transitional arrangements  Y

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects
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**Project Ref**  
101/01.2

**Date Ackn'd**  
15/02/2001

**CU2 Project Title**  
THE CLONING AND EXPRESSION OF ELECTRON TRANSPORT PROTEINS FROM KLEBSIELLA OXYTOCA

Class  
Class 2

**CultureVolClass2**  

**CultureVolumeClass3-4**  

Non-GMM  
Consent Granted  
not applicable

Date Project Ceased

02/03/2022
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Project Containment

Laboratory Activities: L2 L3 L4 L2
Glass Houses: L3 L4 L2 L3 L4
Growth Rooms: L2 L3 L4
Animal Units: L2 L3 L4
Large Scale Activities: L2 L3 L4
Human Clinical Applications: L2 L3 L4

Project Ref 101/02.1

Date Ackn'd: 12/08/2002
CU2 Project Title: CONSTRUCTION OF RECOMBINANT POTATO VIRUS X(PVX) CLONES EXPRESSING GENES FOR CLADOSPORIUM FULVUM EXTRACELLULAR PROTEINS (ECPS) AND AVIRULENCE (AVR) PROTEINS.

Class: Class 2
CultureVolClass2: < 1 litre
Consent Granted: not applicable

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The interaction between tomato and the leaf mould pathogen C. fulvum is being investigated. A large number of C fulvum secreted proteins are recognised by specific tomato disease resistance proteins (Cf proteins) to activate a plant defence response. The purpose of this project is to construct vectors that facilitate transient expression...
of the ECP1, ECP2, ECP4, ECP5, Avr4 and Avr9 genes in tobacco and tomato to investigate their capacity to induce a hypersensitive plant defence response (HR). The assay provides a robust and sensitive procedure for detecting Cf resistance gene function and will obviate the need to perform conventional pathogen inoculation tests.

Recipient or parental organism

The C. fulvum Avr and ECP genes will be cloned into potato virus X (PVX). A cDNA copy of PVX cloned into the pGREEN vector (see www.pgreen.ac.uk) containing cloned ECP or Avr genes will be maintained in E coli DH5a or DH10B which are both K12 derivatives. For infection of plants the recombinant PVX clones will be introduced into Agrobacterium tumefaciens strain GV3101 containing the pGREEN helper plasmid pSOUP. Potato virus X is a mechanically transmitted plant virus whose host range is restricted to solanaceous plants such as tomato, potato and tobacco. PVX is unable to infect animals. The A tumefaciens strain GV3101 is non-pathogenic to humans, and other animals.

Host/vector system

Recombinant PVX will be introduced into tobacco and tomato seedlings by inoculation with Agrobacterium. With respect to plants, A. tumefaciens GV3101 is a 'disarmed' strain that is unable to cause disease. The ECP and Avr proteins are cysteine-rich pathogenicity determinants for C fulvum infection of tomato, its only known host. These fungal proteins are considered unlikely to enhance the pathogenicity of bacterial or viral pathogens such as Agrobacterium and PVX. The major environmental concern regards the possible escape of Agrobacterium containing cloned PVX, which is normally only mechanically transmitted. In the form of a recombinant plasmid in Agrobacterium this provides a potentially novel route of infection to a broad range of plants that are susceptible to Agrobacterium infection. The consequence of expressing recombinant PVX expressing Avr4 or Avr9 in tomato plants that express the cognate resistance protein (Cf-4 and Cf-9) is plant death. However, studies have shown (Thomas et al., 2000) that recombinant PVX is less virulent and cloned inserts are unstable and rapidly lost. Cloned inserts are therefore unlikely to be propagated. No deleterious effects of expressing the other ECP genes in tobacco and tomato are envisaged.

Origin & function

The ECP and Avr genes have been isolated from the plant pathogenic fungus Cladosporium fulvum. The ECP and Avr proteins are secreted into the leaf apoplast during infection and most likely function as pathogenicity determinants. These secreted proteins are recognised by specific tomato disease resistance proteins (Cf proteins) to activate a plant defence response. The purpose of this project is to construct vectors for transient expression of the ECP1, ECP2, ECP4, ECP5, Avr4 and Avr9 genes in tobacco and tomato to investigate their capacity to induce a hypersensitive plant defence response (HR).

Evaluation of foreseeable effects

In the case of the bacterial hosts that will be utilised the strains are E coli K12 derivatives that are unable to grow in nutrient-poor conditions ie they are unlikely to grow outside normal laboratory conditions. Potato virus X is a mechanically transmitted plant virus whose host range is restricted to solanaceous plants such as tomato, potato and tobacco and is unable to infect animals. Therefore, all of the recombinant clones are extremely unlikely to present a greater hazard to human health than the non-modified hosts ie equivalent to ACDP hazard group 1.

The major environmental concern regards the possible escape of Agrobacterium containing cloned PVX, which is normally only mechanically transmitted. In the form of a recombinant plasmid in Agrobacterium this provides a potentially novel route of infection to a broad range of plants that are susceptible to Agrobacterium infection. The consequence of expressing recombinant PVX expressing Avr4 or Avr9 in tomato plants that express the cognate resistance protein (Cf-4 and Cf-9) is plant death. However, studies have shown (Thomas et al., 2000) that recombinant PVX is less virulent and cloned inserts are unstable and rapidly lost. Cloned inserts are therefore unlikely to be propagated. No deleterious effects of expressing the other ECP genes in tobacco and tomato are envisaged.

In theory, Agrobacterium could survive in the environment. However, in the absence of appropriate selection, and a potential fitness advantage, the recombinant plasmids are unlikely to be maintained. ecological studies have demonstrated that only natural non-pathogenic strains are successful saprotrophs, implying that naturally pathogenic strains are poorly adapted to survival in the absence of the host. Therefore a disarmed pathogen A tumefaciens strain GV3101 is unlikely to survive for long in the environment. In addition, the pGREEN vector has been specifically chosen for these experiments since its mobilization functions have been removed, and it could not be transferred to other agrobacterium species, and it's replication is dependent upon functions located on the plasmid pSOUP.

The chances of transforming indigenous species with Agrobacterium is therefore extremely unlikely tiven the level of containment that will be applied (see below). The
chances of plasmid maintenance are also extremely low. If indigenous plant species were transformed by Agrobacterium, PVX infection is unlikely to occur since the host range is restricted to solanaceous species. The cloned ECP sequences are unlikely to be maintained in PVX since they significantly reduce viral fitness, and in any case, no deleterious effects, or fitness benefits, of ECP expression are envisaged in plants.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Tomato or tobacco seedlings will be grown in containment level 2 plant growth rooms and away from glasshouse facilities that contain other tobacco and tomato plants. Two doors separate each of the rooms from the corridor and access is restricted to authorised personnel only. Dedicated laboratory coats are worn at all times and are not taken beyond the outer door (except for sterilising and cleaning). No plants will be allowed to grow beyond the two-leaf stage and no plant material will be removed from the growth rooms for analysis. All plant material will be sealed in appropriate bags before removal from the growth rooms and transporting to the autoclave for destroying.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For E.coli and Agrobacterium work all manipulations will be performed in a containment level 2 laboratory with the appropriate handling procedures. All contaminated material will be destroyed by autoclaving (130°C for 15 minutes) before disposal down a drain.

For PVX-infected plant material appropriate disinfection and waste management procedures will be instituted in the growth rooms. All plants, growth media, plant pots and seed trays will be bagged within the growth rooms and then destroyed by autoclaving (130°C for 20 minutes) using a facility located on the same floor. All autoclave plant material will then be disposed of in a landfill site. Growth rooms will be maintained to a high level of cleanliness. Periodically rooms will be emptied and surfaces will be disinfected with bleach. These measures will reduce the likelihood of all hazards to negligible.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

**Project Containment**

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</table>

All concerns raised by Committee members have been addressed by the current version of the risk assessment.

02/03/2022
We will study genes and their enzyme products that mediate nitric oxide resistance mechanisms in Salmonella enterica LT2a. We will also study regulatory genes encoding nitric oxide responsive transcription factors that are responsible for activating the expression of nitric oxide resistance genes.

The GMOs are (i) S. enterica LT2a-derived strains containing mutations in the nitric oxide resistance pathways mediated by flavohaemoglobin (Hmp), flavorubredoxin (NorV) and nitrite reductase (Nrf), and mutations in regulatory genes responsible for activating expression of these pathways (norR and fnr); (ii) mutant strains complemented with the cognate genes expressed from multi-copy plasmids; (iii) E. coli K12 derivatives containing S. enterica structural and regulatory genes involved in nitric oxide resistance carried on multi-copy plasmids, in some cases under the control of inducible promoters.

The expressed proteins in (ii) and (iii) are involved in mediating resistance to nitric oxide (NO), by reducing it to nitrous oxide or ammonia. Enteric pathogens such as S. enterica encounter NO in the phagolysosome of activated macrophages and neutrophils. There is some evidence to suggest that NO resistance mechanisms may confer a selective advantage on Salmonella strains growing in the host environment. S. enterica LT2a is a laboratory-attenuated strain, has low colonising potential for humans and animals and is not useful for pathogenicity studies. S. enterica LT2a does not contain toxin or invasion genes, and thus in a human or animal host is incapable of crossing the intestinal epithelium and never gets as far as the blood-and lymph-norne phagocytic cells. Thus the expression of NO resistance genes in complemented mutants of this strain is deemed highly unlikely to produce a phenotype that is very harmful to humans or animals. Likewise, E. coli K12 strains are recognised as non-colonising, and the risks associated with expressing S. enterica genes in E. coli K12 are deemed negligible. S. enterica LT2 strains have been widely used for more than 30 years as model organisms in which to study basic biological processes. Numerous S. enterica genomic and expression libraries have been constructed and maintained in E. coli.
K12 strains. It is, therefore, likely that any hazardous effects of expressing LT2 genes in E. coli would, if they exist, have been identified by now.

**Host/vector system**

E. coli K12 strains are recognised as non-colonising and disabled and may be considered to be equivalent to ACDP hazard group 1. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and most have auxotrophic requirements which are unlikely to be satisfied outside laboratory culture. S. enterica is an ACDP hazard group II organism. Strain LT2 is an attenuated derivative, which does not express toxin or invasion genes, and is not regarded as pathogenic towards humans.

The vectors used are either non-mobilisable 9pUC series, pLitmus series, pBluescript series, Supercos, pREP4, pT7 series) or mobilisation-defective (pET series, pQE series, pBR322 derivatives).

**Origin & function**

Genes from S. enterica LT2a coding for functions involved in nitric oxide metabolism, specifically flavohaemoglobin, flavourubredoxin and nitrite reductase, and the regulators of their expression. Mutants defective in these systems will be constructed by a PCR-based methodology, and the mutations will be complemented by the cognate genes expressed from their own promoters on multicopy plasmids. In some cases, expression of genes will be from promoters (for example paraBAD) that allow regulatable expression; though over expression will not be attempted. E. coli K12 strains will be used as intermediates in cloning procedures.

**Evaluation of foreseeable effects**

Enteric and other bacterial pathogens encounter nitric oxide (NO) synthesised by the inducible NO synthase in activated macrophages. NO is thought to be made in response to bacterial infection, and is part of the host's non-specific defence mechanisms. Pathogens have therefore evolved mechanisms to counter the toxic effects of NO, those that have been characterised include flavohaemoglobin (HmP), flavourubredoxin (NorV) and nitrite reductase (NrF). The objective of this study is to construct S. enterica mutants defective in one or more of these pathways and to test the sensitivity of strains to NO in pure culture. To complete the analysis, mutants will be complemented with their cognate genes expressed from non-mobilisable or mobilisation-defective plasmids. E. coli K12 strains will be used as intermediates for cloning procedures. The use of non-mobilisable and mobilisation defective plasmids will ensure that the NO resistance genes are most unlikely be transferred to other pathogens or potential pathogens in which they could conceivably confer a selective advantage. The use of attenuated strains of S. enterica and E. coli will ensure that moderately enhanced expression of NO resistance genes (from multi-copy plasmids) will not confer a selective advantage, since these strains are incapable of invasion and colonisation in animal hosts. Our S. enterica mutants will be made available to collaborators, who will transfer the mutations into other strains by phage-mediated transduction and will conduct pathogenicity trials. Risk assessments and notification of these future aspects of the work will be prepared separately.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste medium from small-scale cultures (<0.5 litres) is autoclaved. All contaminated glassware and plastics are treated either by autoclaving or with a broad-spectrum disinfectant. Liquid waste, once treated, is disposed to drains; solid disinfected waste (plastics, agar) is incinerated. The methods of waste disposal have been validated and it is known that no viable GMOs remain after treatment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
All concerns raised by Committee members have been addressed by the current version of the risk assessment.

**Project Containment**

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**Project Ref** 101/03.1

**Date Ackn’d** 04/03/2003

**CU2 Project Title** CONSTRUCTION AND SCREENING OF METAGENOMIC LIBRARIES CONTAINING DNA FRAGMENTS OBTAINED DIRECTLY FROM UNCULTURED BACTERIA IN NATURAL ENVIRONMENTS, CLONED INTO WIDE HOST-RANGE...

**Class 2**

**CultureVolumeClass2** < 1 litre

**Consent Granted** not applicable

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

To construct gene libraries from bacterial genomic DNA obtained directly from environmental populations and with no prior culturing. The libraries to be screened in different, non-pathogenic, bacterial hosts for any clones that contain genes that determine phenotypes of importance in the nitrogen cycle and in certain biotechnological applications.

Recipient or parental organism

The hosts will be Escherichia coli K12 and derivatives thereof, Rhizobium leguminosarum, Paracoccus, Streptomyces spp, Bacillus subtilis, Azotobacter vinelandii, Rhodobacter spp and Nitrosomonas spp. E. coli K12 is not recognised as a colonising strain of this species and the other genera are, as far is known unable to colonise or cause any damage to animals, plants or the environment. These strains will be hosts for Metagenomic libraries containing DNA fragments obtained directly from bacteria isolated from the environment and cloned in cosmid vectors. By their nature, such libraries and the individual recombinant plasmids within them will be very varied in their identities. Therefore, the characteristics of any given GMO are impossible to predict.

In terms of the effects, therefore, of any given GMO, the worst-case scenario might be that inadvertently, a region of DNA that has the determinant for an unknown toxin is cloned and that the GMO that harbours that gene is accidentally released, such that it, or the cosmid carrying the gene has at a selective advantage, causing a significant increase in the population size of bacterial strain that carried it. However, one has to say that the list of possible detrimental gene functions found in bacteria in natural communities is limited only by one's imagination. This is particularly the case here since the genes in the library will, in many cases, be from bacteria that have not even been cultured and are therefore totally uncharacterised.

Although this sounds rather scary, in reality, it is no more so than when uncharacterised environmental samples are plated out and/or bacteria are cultured in laboratory conditions after enrichment cultures. Increasingly such environmental sampling occurs, using a very wide range of different sites, from deep smokers to ovine rumen, and employing very varied and sometimes sophisticated methods to encourage the in vitro culture of taxa that are normally recalcitrant to culture. In the proposed study, the environmental bacteria themselves will NOT be grown, but their DNA will be present in defined bacteria with no known harmful properties. Therefore any risk would have to be in terms of the massive selective advantage that any newly acquired gene might confer. Given that there is compelling evidence for large-scale alter gene transfer among very unrelated bacterial taxa, if the transfer of a single gene did confer massive selective advantage then this would have occurred in any event. Note, too that the volumes of GMOs will be no more than 10 mls, that they will be handled under Category 2 conditions, more regorous than would be the case for bacterial samples directly obtained from all environments with the exception of diseased animals.

Host/vector system

The hosts will be members of the genera shown above. They are not pathogenic to humans or animals and are indeed, very severely inhibited for growth in the mammalian GI tract. They have no known detrimental effects on the environment. However, we will use auxotrophic or otherwise debilitated mutants of these bacteria, further compromising their fitness in most environments.

The vectors will be based on the P1-group wide host-range vector pLAFR1. This has a wide host-range among bacteria, but is itself completely transfer-defective. For any transfer to occur, it also requires an unusual, mobilising plasmid to be transferred by conjugation. In some cases "hybrid" vectors, based on pLAFR1 but also containing sequences that allow the plasmid to be stably incorporated into the genome of non-pathogenic, auxotrophic Streptomyces spp will be used allowing the libraries to be mobilised into these bacteria.

Origin & function

DNA will be obtained, en masse, from bacterial cells in natural populations from a range of environments. These will include unpolluted marine and other aquatic samples, soils, the epidermal surfaces of healthy animals and plants.

The functions to be tested initially will be the ability to confer individual steps in the Nitrogen cycle - nitrogen fixation, nitrification and denitrification. However, the libraries will also be screened for genes involved in other, known bacterial transformations including the synthesis of enzymes (eg protease and lipase) of biotechnological value and for the presence of tenes (eg for polyketide synthesis) involved in the synthesis of novel antibiotics. Other genes may be sought but in no case will these include functions
(eg toxin production) that are immediately deemed to be hazardous.

**Evaluation of foreseeable effects**

As stated above, exact foreseeable effects are hard to determine, just as would be the case when plating out samples of soil, mud deep ocean water etc on appropriate microbial growth media. However, the hosts to be used for the libraries are compromised, non-colonising and will be grown in small volumes. Therefore it is extremely unlikely that any large-scale escape will occur and that even in the unlikely event of a single clone having a vast selective advantage, compared to the parental strain, the numbers involved would be so low as to compare with the natural exchange of genetic material between different bacteria in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste medium from small-scale cultures (<10 ml) will be autoclaved. All contaminated glassware and plastics will be treated either by autoclaving or with a broad-spectrum disinfectant. Liquid waste, once treated, will be disposed to drains; solid disinfected waste (plastics, agar) will be double-bagged in secured containers then incinerated. The methods of waste disposal have been validated and it is known that no viable GMOs remain after treatment.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

Please enter comments on the GM safety committee on the risk assessment

The Committee recommended "Category 2" on the basis that this work involves the use of a wide host-range vector.

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### Project Additional Information

**Purposes of the contained use**

The aims of the project are to

- Express the above genes that are then studied in further biochemical, cell biology and spectroscopic study
- Study the effects of the expression of these genes on gene expression in host cells
- Use RNAi technology to knockout the endogenous expression of the above genes.

**Recipient or parental organism**

All adenovectors to be used in this study are E1-deleted first generation adenoviral vectors based on the pJM17 system (NMcGrory, W. J., Bautista, D. S. and Graham, F. L. A simple technique for the rescue of early region 1 mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. This renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely rested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group I with containment level I as the minimum requirement. However, all the inserted genes are biologically active. The inserts are eukaryotic cDNA. The insert genes cannot be reasonably assessed as conferring no increased risk since all the genes of interest in this study are components of biologically active cell growth signalling pathways. Because, however, the vector is replication deficient and reacquisition of replication competence by homologous recombination with wild-type adenovirus would lead to deletion of the insert, the GMO can be considered to pose minimal risk to human health and the environment. The transgene is inserted at the E1 site where the transgene is unlikely to confer increased risk to human health or to the environment. The modifications do not confer a selective advantage even if transferred to wild type virus or host cells. Therefore the hazard is negligible. All transgenes will be under the control of the CMV promoter. There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the adenovirus will be inactivated almost immediately by the
immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the animal than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use.

**Host/vector system**

The adenoviral vectors are generated by homologous recombination between pJM17 and shuttle vectors containing transgene expression cassette and flanking E1 sequences. Following homologous recombination in helper 293 cells (which express the helper E1 function in trans), replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses.

**Origin & function**

The adenoviral vector DNAs are standard and originated from the laboratory of Dr F Graham (McGrory, W. J. Bautista, D. S. and Graham, F. L.: A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vitro and in vivo to generate high-level gene expression in all cells transduced by the adenovirus. All transgenes have been constructed from full length cDNAs obtained either from the investigators own laboratories or from other research institutes and verified prior to subcloning into the relevant vectors.

**Evaluation of foreseeable effects**

There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the adenovirus will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the animal than most expression vectors as they will definitely not replicate or integrate into the host genome.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All measures as per HSE containment level 2.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Experiments will be carried out so as to limit possible exposure to virus (minimise amounts made, good laboratory practice, gloves worn, sharps avoided, enclosed containers and avoid creation of aerosols etc.). Contaminated tips/gels etc will be disposed of by the appropriate disposal methods. Spillages, if they occur, will be cleaned up immediately and the cleaning materials (tissues etc.) disposed of as for contaminated waste. All waste materials will be routinely autoclaved. All liquid waste is treated with chlorine-based disinfectants for at least 24 hours prior to disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Minor changes and points of clarification were suggested by local GMO Committee and the proposal was then accepted.

**Project Containment**

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**Project Ref 101/03.3**

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</table>

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

For the genetic transformation of the pathogen Trypanosoma brucei.

Project follows my discovery of membrane heterogeneity in the Trypanosoma brucei glagellum, in particular concentration of "so called" lipid rafts. Such domains may represent a drug target. I am proposing a functional evaluation of the role of these structures in parasite biology and an evaluation of their potential for as drug targets utilizing genetic transformation to modulate gene expression.

**Recipient or parental organism**
GMOs generated are anticipated to be attenuated from the parental type, perhaps conditionally lethal due to the key roles the enzymes targeted play in lipid homeostasis. They do not foreseeably represent an increased risk to the environment compared with the parental strains. The parental strains we will employ are not normally infectious to humans and so represent a minimal risk to human health. T. brucei has an obligate dependence on the tsetse fly for transmission; tsetse are not indigenous to Britain and would therefore be unable to survive in the cooler climate. In the absence of this vector, the parasite is of little risk to the environment. Parasite should be readily contained by ACDP2 provision.

Host/vector system

Host: Trypanosoma brucei Tb 427, Tb 927
Vectors: pZJM, pLEW82, pHD1, pHD496

Origin & function

Insert: tbSMT (sphingomyelin transferase), tbNMT (myristoyl transferase) and tbLS (lanosterol synthase) are considered house-keeping genes, they are derived by amplification and cloning of the coding genomic DNA. Genomic DNA was prepared directly from parasite cultures (at Northwestern University, Chicago). All genes have key roles in lipid metabolism which are likely closely regulated. Perturbing expression of these genes is likely to result in extreme damage to parasite homostasis and likely cell death. The choice of vectors makes it very unlikely that inserts could be expressed in humans since the promoters are parasite polymerase specific. For expression, the requirement for trans-splicing in order to be translated cannot be met in human cells. Moreover for ablation, RNAi constructs will not be of close enough sequence to the human homologues to cause ablation.

The purpose of this project is to use the whole coding sequence or gene fragments for RNAi to modulate the production of raft associated molecules and hence raft stability. In this way it is hoped to derive specific information about the roles rafts are actually fulfilling to trypanosome biology.

Evaluation of foreseeable effects

Host: Trypanosoma brucei brucei is not infectious to man, but is morphologically indistinguishable from subspecies that are and is therefore considered as a potential human pathogen. It is designated by COSHH as ACDP2. We will culture bloodstream and procyclic forms. Only bloodstream forms are infectious. Risk of is solely by direct introduction of parasites into the blood, aerosol transmission has never been demonstrated.

Vectors: The stable vectors used contain neomycin (pHD496) or hygromycin (pZJM, pLEW82) cassettes and are integrated into the Ribosomal spacer region of the genome by homologous recombination. Neither of the drugs for selection are used in the treatment of disease caused by this organism. Integration of the vector is considered to have a likely negative effect on organism survival as evidenced by the spontaneous loss of drug resistance in cultures over time when selective pressure is released. The transient pHD1 plasmid contains a GFP fusion cassette. GFP expression generally has a negative effect on organism survival due to the generation of toxic free radicals during fluorescence.

All vectors can propagated in E. coli and express the bacterial resistance marker Ampicillin for selection. The bacterial promoter will not drive transcription of the inserted genes. In any case, if the inserts were transcribed, the requirements for postranscriptional modification of the insert are incompatable with E. coli and translation could not occur.

The severely disabled DH5a strain of E. coli will be used for propagation of plasmids. This commonly used laboratory strain is auxotrophic and although its ability to colonize humans and animals has not been assessed to my knowledge, however, it is likely to be considerably less fit than wild type E. coli.

Insert: tbSMT, tbNMT and tbLS are considered house-keeping genes. All have key roles in lipid metabolism which are likely closely regulated. Perturbing expression of these genes is likely to result in extreme damage to parasite homostasis and likely cell death. The choice of vectors makes it very unlikely that inserts could be expressed in humans since the promoters are parasite polymerase specific. For expression, the requirement for trans-splicing in order to be translated cannot be met in human cells. Moreover for ablation, RNAi constructs will not be of close enough sequence to the human homologues to cause ablation.

GMO: Since expression of the inserts proposed is likely to be detrimental to the parasite GMO's produced during this project are extremely unlikely to prevent a greater
hazard to human or animal health. (ie equivalent to ACDP hazard group 2). The major environmental concern regards possible escape of T. brucei bloodstream forms into UK domestic stock and wildlife. In order to infect a susceptible animal, the parasite would need to be introduced directly into the blood of such an animal. It is difficult to imagine a scenario in which this could occur. Moreover, T. brucei is transmitted through an obligate cycle within the tsetse fly. In the absence of the tsetse fly infection cannot be propagated. Tsetse flies are restricted geographically to subsaharan Africa and there are, to our knowledge, no tsetse flies in East Anglia in research facilities or otherwise. Moreover, it is highly improbable that released tsetse could survive in the British Climate. In the absence of tsetse fly, T. brucei release does not represent a significant risk to the environment. Further even in Africa, the GMOs produced by the project would be subject to a severe fitness penalty exerted by the integration of insert and selection genes upon these organisms and making their survival doubtful.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Culture of organisms will be within designated, closed and clearly marked incubators within the laboratory. Spillages of pathogene will be cleaned up immediately with absorbent tissue soaked with 1% chloros, detergent or 70% ethanol. Reagents to which trypanosomes are especially susceptible. In accordance with standard disinfection procedures for these organisms.

For disposal of infectious material, liquids will first be mixed with Chloros to 1% final concentration (visually confirmed by bleaching effect). Within the laboratory, contaminated waste will be contained and as biohazard waste. This includes disposable contact materials. Biohazard waste will be autoclaved prior to disposal, in accordance with microbiological waste management procedures. All contact areas will be cleaned and disinfected.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All concerns raised by Committee members have been addressed by the current version of the risk assessment.

Project Containment
THE TAT-DEPENDENT AMIDASES OF E. COLI K-1 AND THEIR ROLE IN VIRULENCE

We already know that pathogenic E.coli K-1 and pathogenic Pseudomonads lacking active twinarginine transport (Tat) systems are attenuated. We also know that E.coli tat mutants have defects in outer membrane assembly due to problems in targeting cell-wall amidases. We wish to determine whether the lack of active Tat-dependent amidases is the predominant contributing factor which results in the avirulence of tat mutant E.coli strains.

E.coli contains three cell-wall amidases AmiA, AmiB, and AmiC. We have already constructed unmarked deletions of each in E.coli K-12 using the well-established pMAK705 system. We will use our pre-existing pMAK705-based deletion constructs to generate gene knockouts in E.coli K-1. In collaboration with Dr Christoph Tang (Centre for Molecular Microbiology and Infection, Imperial College, London) the modified E.coli K-1 strains will be tested for their ability to infect a mouse model system. (NB. No virulence testing will be done at UEA)

Host: Escherichia coli K-1 is classed as an ACDP category 2 organism. It does not express verocytotoxin. It is a common cause of neonatal meningitis and it invades the bloodstream from the nasopharynx or GI tract. Note that such invasion is rare even in neonates and healthy adults do not suffer from this condition. Mutant strains of E.coli K-1 generated by this project are most likely to have drastically reduced virulence.

Recipient or parental organism

Host/vector system

Vector: the vector used (pMAK705, chloramphenicol resistant, temperature sensitive for replication) is a derivative of pBR322 and is thus mobilization defective. It is extremely unlikely that pMAK705 will be transferred to other bacteria. The antibiotic resistance gene of pMAK705 is only transiently transferred to the chromosome of the host strain in the early stages of the mutant construction protocol. None of the mutant strains constructed will be chloramphenicol resistant.
Inserts: All mutant strains will carry unmarked gene deletions. There will be no insertion mutations resulting from this work.

Origin & function

this project does not involve the use of ‘foreign’ DNA in the host organism. All GMO’s will lack genes encoding native amidase enzymes.

Evaluation of foreseeable effects

Final GMO: Manipulated strains are most unlikely to be enhanced in terms of their virulence. The available literature suggests strongly that the mutant strains will be severely attenuated and thus probably non-pathogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cultures will be grown in small volumes of liquid medium (10 ml or less, generally 5 ml) and on solid medium in the form of petri-dishes. All solid media will be inactivated by extensive heat and high-pressure treatment in an autoclave. This kill method is 100% effective and will result in no viable organisms remaining. Liquid cultures can also be killed absolutely by this method. Alternatively liquid cultures will be treated with broad-spectrum disinfectants. No viable organisms will survive this treatment. Killed liquid is suitable for disposal through the general drainage system. Killed solid waste is suitable for landfill disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All concerns raised by Committee members have been addressed by the current version of the risk assessment.

Project Containment

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Animal Units: Large Scale Activities: Human Clinical Applications

02/03/2022
**Project Ref** 101/04.2

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<td>GENERATION OF AN E.COLI HEAT LABILE TOXIN B SUB-UNIT VARIANT FOR USE AS A GALACTOSE SENSOR.</td>
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<th>Consent Granted</th>
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<tbody>
<tr>
<td></td>
<td>not applicable</td>
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</table>

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

To generate a non-toxic E. coli heat labile toxin B (LT-B) sub-unit variant in a class 2 Vibrio strain. This host is being used to ensure no contamination from the wild-type E. coli host protein, which might arise from over-expression of the desired LT-B variant in E. coli. Only the A sub-unit of LT-B is toxic per se; the B sub-unit is simply employed in nature to permit adhesion of the A sub-unit to cell surfaces prior to endocytosis.

**Recipient or parental organism**

The host, Vibrio sp60 (a non-pathogenic marine bacterium), is a class 2 microorganism. It is non-pathogenic to humans, animals and plants. Should any breach of containment occur, survival of this marine microorganism outside the containment lab would be expected to be transient and unlikely to have an impact on the environment.

**Host/vector system**

This study will use a pre-existing vector and host system that has been described in the literature (pTRH101R in vibrio sp60; Vaccine, 1999; 17, 1442-1453) for the production of the so-called LT-B Pk-cys fusion protein. The vector being used is non-mobilisable, reducing possible hazards from gene transfer, and confers resistance to ampicillin. The expressed protein is non-toxic. Transfer of a single gene from Vibrio sp60 is unlikely to confer a pathogenic phenotype on another host.

**Origin & function**

This study will use a pre-existing vector and host system that has been described in the literature (pTRH101R in Vibrio sp60; Vaccine, 1999; 17, 1442-1453) for the production of the so-called LT-B Pk-cys fusion protein.
Evaluation of foreseeable effects
The final GMO is unlikely to be more hazardous than the host (i.e., it is expected to be non-pathogenic) and, should any breach of containment occur, survival outside the containment lab would be transient and unlikely to have an impact on the environment.

Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Non applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste medium from cultures will be autoclaved. All contaminated glassware and plastics will be treated either by autoclaving or with a broad-spectrum disinfectant. Liquid waste, once treated, will be disposed to drains; solid disinfected waste (plastics, agar) will be double bagged in secure containers then incinerated. The methods of waste disposal have been validated and it is known that no viable GMOs remain after treatment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The committee recommended “Class 2” on the basis of classification of the parent organism.

Project Containment

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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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Project Ref 101/07.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4
02/03/2022
Systems approach to dissect the regulatory mechanisms Salmonella enterica employs to respond to its environment.

Project Additional Information

Purposes of the contained use

Mutations in genes known or proposed to be involved in the response of Salmonella to its environment will be constructed by inserting linear DNA fragments often containing a selective antibiotic marker. These mutations will be complemented by transformation of these strains with a low copy number plasmid harbouring the gene deleted from the chromosome. Complementation may require subcloning in E.coli K12 derivatives, Promoters of several Salmonella genes will also be fused with reporter genes and these fusions will be inserted into the chromosome.

Recipient or parental organism

Serovars of Salmonella enterica are classified as group 2 pathogens, as determined by the ACDP. E.coli K12 derivatives are classified as ACDP hazard 1, since these strains are considered non pathogenic and contain several auxotrophic mutations they are not expected to survive in the environment.

Host/vector system

Complementation will employ a low copy-number (5-10 copies per cell) such as pSC101-derived, non-mobilisable, ampicillin-resistant plasmid, pWKS30, containing the gene that has been deleted from the chromosome. Whilst cloning the various DNA fragments, we will initially employ the widely available general-purpose cloning vectors such as pUC18 and 19. These vectors are non-mobilisable, as they lack a bom site.

Origin & function

All genetic material will derive from serovars of Salmonella enterica or from the vectors/reporter genes described. The overall intended function of manipulations in both sets of experiments is to study the environmental regulation of genes in these strains. In all cases, genes of interest will be mutated, or their promoters will be fused to a reporter gene. We will be performing detailed phenotypic analyses to determine the consequences of the mutations. We will perform in vitro tissue-culture based assays to study the interactions of the wild-type and mutant strains with cultured cell lines. We will investigate host-pathogen interactions in murine Salmonella infection models. We will isolate RNA from the bacteria to measure gene expression by micro array and RT-PCR, and use protein-based techniques such as SDS-PAGE and Western blotting to confirm results obtained by RNA analysis. In some cases the mutants will be grown in continuous culture systems. Cellular differences in the level of GFP expression will be studied.
by fluorescence microscopy and Fluorescence Activated Cell Sorting (FACS).

**Evaluation of foreseeable effects**

We will be mutating genes though to control environmental gene regulation of Salmonella. In most cases these genes are also found in all non-pathogenic strains of Escherichia coII. As such, they do not contribute to pathogenesis in the absence of other factors and are therefore not considered to be virulence factors. Although subsequent complementation in pWKS3O, will increase the copy-number of the genes and could conceivably result in higher levels of the cognate proteins, significant over expression is unlikely, since these genes are subject to negative feedback regulation. Where Salmonella specific genes are mutated it is extremely unlikely that the virulence will be significantly higher than the isogenic parent and as such will still be a category 2 organism.

The system for introducing linear DNA constructs into the bacterial chromosome involves the presence of the temperature-sensitive plasmid, pKD46. None of the genes encoded by the plasmid are considered likely to enhance the virulence of the strains harbouring them. Since plasmid pKD4S and its derivatives cannot replicate at 37°C the plasmid would normally be lost following ingestion of the bacteria by a mammalian host. We are not aware of any potential for this plasmid to be mobilised and transferred to other strains although the theoretical possibility does exist whilst the strains harbouring the plasmid are in the gut. Thus, there is a minimal risk of antibiotic resistances encoded by this plasmid being transmitted to other strains. The alternative allelic exchange system proposed in both projects will be performed using the suicide vector pCVD442. The fusions can also be cloned onto a ColEl or pMBI replicon such as pBR322. The pBR322 vector is mobilisation defective and will only contain the promoter sequences for virulence genes. Although pBR322 is not conjugable itself, the plasmid could be mobilised in the presence of a helper plasmid. The risk is considered very low. Moreover, the contents of the plasmid would not increase the pathogenic potential of a recipient strain.

(continued on separate sheet)

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

“All GMOs will be contained in the appropriate conditions relative to the individual GMO. Where CMOs are combined the most severe containment conditions applicable will be used, which in the case of this notification will be in a category two facility. CMOs which have not been constructed in house will be obtained from regulated suppliers and transported under containment conditions. At the end of the experiment all CMOs will be disposed through autoclaving and/or incineration”.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Infectious doses for use in the murine model will be prepared in BIG and transported to the DMU in secondary containment. Once infected, the mice will not be moved from the designated category 2 area until the end of the experiment. During the experiment, animal waste and bedding will be autoclaved before being incinerated. Because rodents tend to eat their stools, water bottles can be contaminated when infected animal drink. Therefore, spillage of water from these bottles should be treated as potentially contaminated as soon as animals are infected. If a spillage occurs they will be treated as liquid spills as detailed below. At the end of the experiment, the mice will be humanely killed and then dissected to remove organs for further analysis. Organs will be transferred to sealed containers and transported to 80°C for further processing. The remains of the animals and any materials in contact with the infected animals will be autoclaved and incinerated. This approach reflects “best practice” used at the Cambridge University Veterinary School and the Hammersmith Hospital, London.

1. Liquid spills

Small spills of liquid cultures shall be treated for 10 minutes with an approved disinfectant, 1% solution Virkon. This concentration had been validated by the manufacturers and in house testing with the strains used. For large spills, add undiluted disinfectant to achieve the correct final concentration (1%). The treated area should be mopped up with paper towels which should be sent for autoclaving.

(Continued on separate sheet).
Minor changes and points of clarification were suggested by the local GMO committee and the proposal was then accepted.

Please enter comments on the GM safety committee on the risk assessment:

Minor changes and points of clarification were suggested by the local GMO committee and the proposal was then accepted.

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**Animal Units**

<table>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L3 L4</td>
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**Project Ref** 101/08.1

- **Date Ackn’d:** 05/06/2008
- **CU2 Project Title:** Use of lentivirus vectors for expression of cellular and viral proteins in eukaryotic cells.
- **Class:** Class 2
- **Culture Vol:** < 1 L
- **Non-GMM Consent Granted:** Not Applicable
- **Consent Granted:**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
Project Additional Information

Purposes of the contained use

Lentiviruses will be used for high level expression of viral and cellular proteins in cells in order to image their subcellular localization, find host proteins which bind to them and their interaction with molecular signal transduction pathways. Lentiviruses will also be used for delivery of short-hairpin RNAs (shRNA) to suppress expression of cellular proteins for functional evaluation of their role, e.g. FGFR-receptors (FGFR5), proteases, signalling proteins.

Recipient or parental organism

Mammalian primary cells and cell lines from human, monkey, mouse, origin, including epithelial, vascular endothelial, muscle, HeLa, Vero, 293FT, U87, U251 PC3, DU145, LNCaP, MCF-7, CHO, MDA-MB231, SW1353, MRC-5 cells, 1OT1/2 and 3T3, myeloblastic cell line, Eol-1, TF-i, HL-60, THP-i; airway epithelia, AS49 and RPMI 2650; monocytic and macrophage. Chicken embryo fibroblasts. E. coli DH5 and TOP10

Host/vector system

Defective lentiviruses are generated using a lentivirus vector plasmid with viral genes replaced by inserted gene driven with either a SFFV promoter or CMV promoter, and retaining LTR for integration. This is co-transfected with envelope pVSV-G, encoding the VSV envelope proteins under the control of the CMV promoter, and pCMVDR8.91 encoding replication proteins (gag-pol). The resulting virus secreted from 293 producer cell line is defective in packaging signal and will be used for infection of recipient cells

Origin & function

Provides stable, long-term expression of a target gene. Target genes include reading frames that encode viral proteins from the flavivirus classical swine fever virus (CSFV), including the N terminal autoprotease (Npro) and other nonstructural proteins (NS2, 3, NS4a and band NS5a and b). Foot and mouth virus non-structural proteins 2C, 2BC, 3A which anchor the viral replicase to membranes and has no enzyme activity. Reading frames encoding viral proteins from the coronavirus IBV (infectious bronchitis virus) Cellular proteins that regulate autophagy (LC3, p62, mTOR, Raptor, Rictor) and genes from the NF-kappa B and interferon signaling pathways (including NF-kappaB, I-kappa B, IRF3, IRE 7, I kappa B kinases)

Proteins involved in extracellular proteolysis and regulatory factors controlling their expression, activation or function, including matrix metalloproteinases (MMPs), adamalysins, tissue inhibitors of metalloproteinases (TIMPs), serine proteases and serpins, receptors associated with binding of proteases (integrins, urokinase plasminogen activator receptor, uPAR), CD44, LYVE-i, Prox-i and other transcription factors. Human Siglecs (sialic acid binding immunoglobulin like lectins; a putative regulator of receptor signaling threshold) Human calcium activated chloride channels, CLAC1/2/3. Ficolins L/H and M which are innate immune lectins. Components of the Wnt,Notch and BMP signaling pathways (e.g. APC, beta-catenin) including gain/loss of function receptors. Components of the TNF receptor signaling pathway (eg FLIP, HO-i) and gain/loss of function mutants. BCL-abl kinase and dominant negative mutants.

Evaluation of foreseeable effects

Human Health: Risk of human infection is very, very low.

Host cells: The mammalian cell hosts are non-pathogenic and unlikely to survive outside cell culture. It is recognized that lentivirus vectors can in principle cause insertional mutagenesis in the cells that they enter, but the likelihood of a worker infecting themselves and causing a hazard in this manner is extremely low.

Vector: The replication incompetent lentivirus is not a risk as it will not infect further cells. That the lentivirus has a genome split into 3 plasmids means that recombination between packaging helper and vector is very low and the potential for generation of replication competent lentivirus is extremely reduced. The virus envelope is from VSV and there is no I-III envelope protein in the system and the minimal required HIV functions are from the helper plasmid that contains no packaging or LTR sequences. There is no chance that it will recombine with HIV from a HIV infected worker

Insert: The inserted transgenes are viral proteins with no known homologues in mammals and they are not oncogenes. The CSFV Npro protein can inhibit innate immune responses by targeting interferon response factor 3 for degradation. Since this gene will be expressed from a defective lentivirus vector, it will not self-replicate. The inserted genes which are cellular are not oncogenes, except for some of Wnt/Notch/BMP signaling pathway components are oncogenes (e.g. beta catenin) or tumour suppressor genes (e.g. APC). Lentiviral delivery of such genes in vitro is practiced worldwide. Tumourigenesis in humans is a multistep process and requires multiple genetic changes. Thus, the risk for tumourigenesis, should an individual become infected, is expected to be very low. TNF signaling pathways include apoptotic and...
anti-apoptotic genes which affect proliferation or cytotoxic mechanisms within cells. None of the TNF pathway genes targeted are classed as oncogenes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GMO contained in Cat 2 labs. Plasticware and waste are autoclaved. Liquids are inactivated using 1% Trigene. All procedures performed in category 2 tissue culture hoods in a designated tissue culture laboratory. Workers wear lab coats, nitrile gloves. Spilled liquid to be treated with 1:10 Trigene.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be decontaminated in 1% Trigene for 60 minutes which has been shown to inactivate 100% of lentivirus. All plasticware and waste products will be autoclaved to provide 100% kill. No GMQ will survive treatment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Minor points of clarification were raised by the local GMO committee and the proposal was then accepted.

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Animal Units

Large Scale Activities

Human Clinical Applications

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<td>08/06/2009</td>
<td>Culture, treatments and screening of trypanosomatid parasites</td>
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<tbody>
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<td>Class 3</td>
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</table>
**Project Additional Information**

**Purposes of the contained use**

For contained handling of these parasites

**Recipient or parental organism**

Trypanosoma brucei ssp,
Trypanosoma cruzi,
Trypanosoma rangeli,
Leishmania sp.

**Host/vector system**

For T. cruzi, T. rangeli and Leishmania sp. pTEX plasmid,
For Leishmania only pXG-GFP plasmid,
For T. cruzi, T. rangeli pCos TL
For T. brucei pLEW82/pHD series

**Origin & function**

pTEX, pCosTL - LSHTM, University of London (Kelly lab)
pXG - Washington University (Beverley lab)
pLEW82/pHD series Oxford University (Gull lab)

In all cases the plasmids will be used for the expression of CDNAs with the purpose of either localizing (using GFP or epitope tags), reducing (by knockdown or knockout) or modulating expression of parasite proteins in order to infer function.

**Evaluation of foreseeable effects**

All experiments involving construction and establishment of New GMO's will be individually risk assessed by the Wolfson Pathogen Unit Management Committee. In general terms expression of endogenous proteins with epitope tags or fluorescent proteins can be expected to be functionally neutral and in the case of fluorescent proteins deleterious to the well being of the parasite due to the increased levels of free radicals and misfolded protein generated. Similarly modulating protein expression particularly...
by knockdown or knockout of endogenous proteins is normally deleterious to the parasite and is frequently fatal. Understanding how fatality occurs can be highly informative and where fatality occurs this is indicative that the gene targetted may encode a potential drug target.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We will utilize a containment level 3 suite for the handling of these organisms according in line with ACDP guidance and according to the Standard Operating Protocols provided. Trypanosoma cruzi, Leishmania donovani and Trypanosoma brucei rhodesiense are HG3 trypanosomatid parasites and it is appropriate to contain them in this manner, however, because of their large size (circa 10 microns in length) they are not readily aerolized. In light of this we request derogation of the requirement for working at negative pressure with these organisms.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Decontamination is with 0.1% chloros or 2% trigene. Waste is placed in a steel box lined with a clear autoclave bag. And is subjected to pass through autoclaving. Once cool, the autoclave bag is removed from the box and placed in a black bin bag weighed and labelled with researcher details. The bag is then placed with incineration waste in the wheelie bins of the Disease Modeling Unit and is collected by Vet Speed and taken to Cambridge Pet Crematorium for incineration.

Please see attached SOPs and publication for specific detail.

**Is an emergency plan required according to regulation 20?**  N

**If yes, tick to confirm that it is attached to this form**  N

**Tick to confirm that you have attached a risk assessment to this form**  Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  N

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02/03/2022
Prior to review by the UAE Biohazards and Genetic Modification Subcommittee had the protocols reviewed by the external specialists and specialists on the Wolfson Pathogen Unit Management Committee.

Upon their review of the finalized protocols
The UEA Biohazards and Genetic Modification Subcommittee 10/03/08 noted that:

A new paragraph will be entered into the Standard operating procedures to cover who is allowed to access the CL3 Suite.

Training for using the CL3 suite has taken place at Portland Down and all PIs will undertake this Portland Down CL3 course.

A list of trained staff should be provided to Occupational Health to assess health surveillance requirements.

Advice will be offered to estates on who should be allowed to access the Plant Room above the CL3 Suite.

Following appropriate consultation with Estates a protocol on Plant Room access will be written.

These recommendations have been attended to in the submitted documentation.

**Project Containment**

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**Laboratory Activities**

- L2: Yes
- L3: Yes
- L4: L2 L3 L4

**Animal Units**

- L2: L3 L4 L2 L3 L4

**Large Scale Activities**

- L2: L3 L4 L2 L3 L4

**Human Clinical Applications**

- L2: L3 L4

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**Project Ref** 101/10.1

- Date Ackn'd: 17/02/2010
- CU2 Project Title: Production of the alphavirus Sindbis Virus from an infectious cDNA clone
- Date Project Ceased: 
- Class: Class 2
- Culture Vol: ≤ 1 Litre
- Consent Granted: Non-GMM
- Project notified under transitional arrangements: N
- Withdrawn: N
- Tick if notifying a connected programme of work: N

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Generation of Sindbis virus RNA in mammalian cells to study the sites of replication and the production of double-stranded RNA. Cellular responses to double-stranded RNA produced during infection will be studied, including RNAi, interferon and autophagy pathways.

**Recipient or parental organism**

Mammalian cells including HEK293, MCF7 cells, Vero, BHK cells

**Host/vector system**

A plasmid cDNA containing a SP6 RNA polymerase promoter upstream of the structural and non-structural proteins of Sindbis virus SinTTP1011 and Sin DSTE12Q.

Sindbis virus RNA transcripts are synthesized from SP6 RNA polymerase promoter.

Sindbis viruses are enveloped particles with an icosahedral capsid. Its genome is a single stranded RNA approximately 11.7kb long. It has a 5' cap and 3' polyadenylated tail therefore serves directly as mRNA in a host cell. The genome encodes four non-structural proteins at the 5’ end and the capsid and two envelope proteins at the 3’ end. The genomic RNA is partially translated at the 5’ end to produce the non-structural proteins which are then involved in genome replication and the production of new genomic RNA and a shorter sub-genomic RNA strand. This sub-genomic strand is translated into the structural proteins. The viruses assemble at the host cell surfaces and acquire their envelope through budding.

The RNA is self-replicating and provides for transcription in a host cell.

**Origin & function**

The infectious clone is a cDNA copy of a functional Sindbis virus RNA genome cloned into a plasmid with a SP6 promoter. TOTO1101 is partly shortened at 5’ and 3’ end from the full-length cDNA clone HRSp (see Rice et al J.Virol. 1987). It is self-replicating and not disabled. SinDSTE12Q is in a plasmid derived from TOTO 1101 with a modified polylinker.

The function is to provide a replicating RNA virus in mammalian cells. Double-stranded RNA is generated as an intermediate during viral replication. Infected cells die by apoptosis 24 hours post-infection.

**Evaluation of foreseeable effects**

Human health: Sindbis virus infects Humans, mammals, birds. The infectious dose is not known.

The mode of transmission is by the bite of an infectious mosquito.

INCUBATION PERIOD: less than 7 days.
COMMUNICABILITY: No evidence of person-to-person transmission documented

SYMPTOMS: Self-limiting febrile viral disease. Sudden onset of fever, rash, arthralgia or arthritis, lassitude, headache and myalgia; rash may precede or follow joint
manifestations by 1-2 days; exanthem on trunk progressing to face, legs, palms, soles and lasts on average 10 days; sign of jaundice and myocardial damage are reported
but rare; often no recognized clinical disease manifestations

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Biosafety level 2 practices and containment facilities will be used for all activities involving the virus. Infected cells die by apoptosis 24 hours post-infection

PROTECTIVE CLOTHING:
Laboratory coat; gloves.

STORAGE: In sealed containers that are appropriately labelled at -80C

PHYSICAL INACTIVATION: Sensitive to heat, UV and gamma irradiation and freezing

SURVIVAL OUTSIDE HOST: Survives in blood up to 2 days at room temperature. Avoid contact with mucus membranes.

Needles will no be used. Cultures will be grown in tissue culture flasks (T75 cm3) or dishes (P100s) in DMEM tissue culture media containing 5% FCS. The cultures will be
grown in designated incubators.

Liquids are inactivated using 1% Trigene and 70% ethanol. All procedures will be performed in category 2 tissue culture hoods in a designated tissue culture laboratory.

DISPOSAL: Decontamination all wasted before disposal; steam sterilization, incineration, chemical disinfection

All liquid waste will be decontaminated in 1% Trigene for 60 minutes which has been shown to kill 100% Sindbis virus. All plastic ware and waste products will be
autoclaved to provide 100% kill.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tr>
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02/03/2022
Project Ref 101/11.1

Date Ackn'd 20/07/2011

CU2 Project Title Acathamoeba castellanii: analysis of putative P2 receptors

Date Project Ceased 20/07/2011

Class 2

Culture Vol Class 2

< 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? □

If yes, tick to confirm that it is attached to this form □

Tick to confirm that you have attached a risk assessment to this form □

Tick if you are claiming exemption from disclosure for section of the risk assessment □

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Project Ref 101/13.1

Date Ackn'd: 06/03/2013

Date Project Ceased

Withdrawn □

Tick if notifying a connected programme of work □

Use of replication deficient, recombinant adenoviruses for transfer of genetic material encoding cellular, viral and green fluorescent protein into eukaryotic cells and tissues

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements □
This risk assessment consolidates closely related research projects which use adenovirus vectors for delivery of genetic material to mammalian cells. The aims are to investigate diseases for translational medicine research. The research projects relate to human health and biomedicine, to further our understanding of the cell and molecular mechanisms underpinning several human diseases.

In particular we will use recombinant adenoviruses to express recombinant proteins in primary organs and organoids generated from gut, intestinal crypts, kidney cysts, slow and fast twitch mouse and rat muscle fibres, and in muscle in vivo by intramuscular injection of mouse tibialis anterior.

We will investigate:

- the innate immune responses to expressed viral proteins from FMDV, IBV and CSFV encoded by less than 10% of the viral genome using proteins fluorescently-tagged with GFP/RFP
- Tetracyclin inducible expression of viral proteins.
- Express inhibitors of protein expression such as shRNAs and siRNA targeted against innate immune autophagy proteins and DNA repair system components.
- The role of DNA repair systems in aging and cataract formation
- Express recombinant cellular proteins include protein fused to fluorescent tags eg: GFP, RFP, includings cytokines, defensins, inflammatory proteins, interferon signal transduction proteins, NF-kB signal transduction proteins, RNA helicases, autophagy proteins including ATG5, 16, LC3, p62 sequestasome, DNA repair proteins tagged with GFP, RFP DNA repair proteins including Ku70, Ku 80 and Lig4
- Gene reporter constructs including inducible promoter elements fused to GFP/luciferase promoter luciferase/GFP contracts
- Express Cre Recombinase to excise sequences between LoxP sites in transgenic cells and tissues

Recipient or parental organism

- Mammalian cell lines (HEK293), HeLa, MEFs, Vero, Raw 264, FHL 124 BHK, CHO
- Primary human crypt biopsy tissue
- Mouse small and large intestine crypt and organoids cultures
- Kidney cysts
- Mouse brain tissue and muscle
- Porcine lens cultures
- Donor human lens tissue culture
- Fast and slow twitch muscle isolated from rat and mouse

Host/vector system

A Easy-1 Invitrogen ViraPower system
Adeno-X (Clontech system)
Adenovirus Type 5
The function of the adenovirus genome is to efficiently transduce a wide variety of mammalian cells. It can infect resting cells, proliferating cells, undifferentiated cultured cells, and differentiated cells. The gene of interest is highly and transiently expressed.

Origin & function
The function of the adenovirus genome is to efficiently transduce a wide variety of mammalian cells. It can infect resting and proliferating cells, differentiated and undifferentiated cells. The gene of interest is highly and transiently expressed.

Evaluation of foreseeable effects
Recombinant Adenovirus vector contains mammalian and viral proteins protein. The recombinant Adenovirus is replication deficient and therefore can only replicate in cells which carry complementing regions of the E1 genes. It will not replicate in other in vivo or in vitro cells. Furthermore since the modified virus is replication deficient it is less pathogenic than the wild type and there is minimal capacity for colonisation. If it is exposed to the environment it is unlikely to survive for extended periods. Human Adenovirus Type 5 is ubiquitous causes only a mild respiratory disease in humans which is self limiting and does not require any specific treatment. The Adenovirus viral vectors are replication defective owing to the removal of the E1 and the E3 regions. The genetic inserts pose no risk to animal/plant health or the environment. Modified adenovirus may mimic some of the characteristics of the wild-type or may pose a risk to immune-compromised individuals causing only a mild respiratory disease in humans which is self limiting and does not require any specific treatment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Personnel will wear lab coats and gloves, which will be disposed of in waste to be autoclaved. Recombinant Adenovirus can only replicate in complementing cells such as Ad-HEK293 and consequently poses a low risk to animal/plant health and the environment. Level 2 containment measures will be in operation and the principles of Good Microbiological Practice will be applied. All surfaces will be decontaminated in 10% Trigene. In conjunction with this access to the laboratory will be restricted when work with infectious agents is in progress. Persons at increased risk of acquiring infection or for whom infection may have serious consequences will not be allowed to enter the laboratory. A biohazard sign will be posted at the laboratory entrance bearing appropriate information including the agent(s) in use, containment level, the investigator's name and telephone number, personal protective equipment requirements and exiting procedures if any.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Virus will be inactivated in 10% Trigene. All plastics and in contact items will be left in 10% trigene detergent for 30 minutes before placing in clear bags in yellow boxes and autoclaving. Plasticware will be autoclaved. Liquids are inactivated using 10% Trigene. Spilled liquid will be treated with 1:10 Trigene. All of these are validated methods of adenovirus destruction.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form 
Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>L3 L4 L2 Yes</td>
</tr>
</tbody>
</table>

Animal Units

| L2 Yes | L3 L4 L2 | L3 L4 L2 | L3 L4 |

Large Scale Activities

| L2 Yes | L3 L4 L2 | L3 L4 L2 | L3 L4 |

Human Clinical Applications

| L2 Yes | L3 L4 L2 | L3 L4 L2 | L3 L4 |

Project Ref 101/15.1

Date Ackn’d: 11/06/2015

CU2 Project Title: Characterisation of P. aeruginosa and its effect on biofilm formation

Date Project Ceased:

Class: Class 2

Culture: < 1 Litre

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Mutations in the genes, mexT, PA5017, PA0296, PA0602 and wspF will be individually genetically engineered into P. aeruginosa PA01 (ATCC 15692). They will be studied to understand the phenotype of these mutations and effect on antibiotic susceptibility, growth and biofilm formation.

The experiments entail standard molecular biology techniques which carry no special risk and all are conducted in...
accordance with health and safety guidelines of UEA. GMOs will be cultured at 37°C for 24-48 hrs in a maximum
volume of 10 ml. Antibiotic susceptibility and growth will be analysed using standard growth assays in a 96-well plate.
Biofilm flow cell experiments will require seeding flow cell channels (a microscope slide sized area) with P. aeruginosa
for a limited period of time (4 hours max) and passing growth medium over this to study biofilm growth (7 days max).
The GM organisms will be handled at all times within a C12 laboratory and so escape into the environment is
minimised. Survival in the environment is not expected to be increased over wild type and the risk to humans is low -
(it is an opportunistic pathogen).

Recipient or parental organism

P. aeruginosa is not considered a primary pathogen but rather an opportunistic agent in hospital acquired
infections. It exists as a normal inhabitant of human intestinal flora and is also associated with infection within an
immunocompromised host. Additionally, it is non-spore forming and is widely dispersed in the hospital and
environment. P. aeruginosa PA01 is a laboratory strain that was originally isolated from a wound in 1954 in
Melbourne, Australia. This strain is capable of survival in the environment but the risk to human health is no worse
than any other P aeruginosa strain, and containment at level 2 is appropriate.

Host/vector system

The vector called pTS is grown in E.coli. The vector contains a tetracycline marker to allow positive selection, sacB, a
counter selecti marker and a ColE1 origin in which pre ents replication of the plasmid in P. aeruginosa. The
tetracycline marker will be lost during the genetic engineering process and therefore poses no additional safety
risks compared to wild type P. aeruginosa.

Origin & function

Evaluation of foreseeable effects

Mutations genetically engineered into P. aeruginosa PA01. Gene names and positions are from P. aeruginosa PA01
(GenBank accession no. NC002516.2)
mexT deletion, TGGCCAGC (Position:2807693)  
mexT insertion, TCE (Position:2807965)  
mexT deletion, TGGCCAGC (Position:2807693) together with the mexT insertion, TCE (Position:2807965)  
Cag/Tag in PA5017 (Position: 5642054)  
tTcLtC within PA0295 (Position:332051)  
GtvAt in PA0602 (Position: 663845)  
cAg/cGg in wspF (Position:4144991)  
Mutants will be created that will have an effect on biofilm phenotypes. As mentioned in the risk assessment, mutants
created with the mexT gene deletion may alter antibiotic susceptibility to chloramphenicol, norfloxacin (and possibly
other fluoroquinolones). Production of virulence factors (pyocyanin and type three secretion) and swarming
characteristics in P. aeruginosa will be reduced (Luong et al., 2014). The TCC insertion has not been characterized
however it is known that mutations within the mexT gene alter expression of the MexEF-OprN efflux pump which can
result in reduced antibiotic susceptibility to norfloxacin and chloramphenicol (Maseda et al, 2000). All GM strains
remain susceptible to several clinically relevant antibiotics including gentamicin. Norfloxacin and chloramphenicol are
not commonly used to treat P. aeruginosa infections (Driscoll et al., 2007). Changes in the PAS017 gene may affect
flagella motility (Kulasekara et al., 2013) and hence biofilm dispersal. PA0295 and PA0602 are putrescine-binding
periplasmic proteins and mutations within these genes may have an effect on transport of small molecules across the
membrane (Imperi et al., 2009). P. aeruginosa engineered with the wspF mutation is likely to show increased biofilm production (D’Argenio et al., 2002). The mutants that will be created were initially identified as mutations in variants of the laboratory strain, P. aeruginosa PA01, using comparative genomic studies. The consequences, of the harmful effect (were it to occur) is most likely to be no worse than wild type P. aeruginosa. All mutants will be handled in a containment level 2 laboratory. P. aeruginosa is capable of survival in the environment but the risk to human health of the GM isolates is no worse than for P. aeruginosa wild type, and containment at level 2 is appropriate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated glassware, bacterial cultures and contaminated disposables e.g. Pipette tips, tissue paper, culture vials and plates, etc., will be sealed and disposed of in yellow biological waste bins, to be autoclaved on a kill cycle at 126 degrees Celsius for 20 min. Processed waste in autoclave bags are over wrapped in black bags and placed into a 'e wheelie bin in the autoclave lab for disposal as landfill. Processed glassware will be emptied and washed. t ,~ase see the attached SCRE yellow box standard operating procedure and risk assessment. Sharps materials are disposed of in cin-bins (UN 3291 compliant). Cin-bins are removed by the registered waste contractor Novus Environmental.

Accidental spillages (less than 0.5 mLs) will be cleaned with Actichlor disinfectant (Ecolab, Leeds, UK) at a final concentration of 1000 ppm, wiped with a clean paper towel immediately and discarded in the biological waste bin. Larger spills (over 0.5 mLs - 10mLs) will be treated with Actichlor to a final concentration of 1000 ppm. This will be covered with a blue paper towel, appropriately labeled and left overnight before being wiped with a clean paper towel and discarded in the biological waste bin.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No comments were provided.

Project Containment

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

Lentivirus will be used for overexpression of proteins of interest in cells to image subcellular localization and find host binding proteins by pull-down analysis. Lentivirus will also be used for delivery of small RNAs (shRNA and miRNA) for functional evaluation of cellular pathways.

**Recipient or parental organism**

The lentivirus will be used to infect human cell lines and primary samples in vitro.

**Host/vector system**

Host: The mammalian cell hosts are non-pathogenic and unlikely to survive outside cell culture. It is recognized that lentivirus vectors can in principle cause insertional mutagenesis in the cells that they enter, but the likelihood of a worker infecting themselves and causing a hazard in this manner is extremely low. Primary human lymphocytes,
monocytes, stromal cells, adipocytes, and leukaemia cells will be screened for HIV at the Norfolk and Norwich Hospital before being brought into the laboratory. Vector: The replication incompetent lentivirus is not a risk as it will not infect further cells. That the lentivirus has a genome split into 3 plasmids means that recombination between packaging helper and vector is very low and the potential for generation of replication competent lentivirus is extremely reduced. The virus envelope is from VSV and there is no HIV envelope protein in the system and the minimal required HIV functions are from the helper plasmid that contains no packaging or LTR sequences. There is no chance that it will recombine with HIV from a HIV infected worker.

Origin & function

Inserts:
Components of the TNF receptor signaling pathway (e9 FLIP, HO-1) and gain/loss of function mutants. Specific shRNA and miRNA sequences for various proteins of NFkB and NRF2 pathways. Fluorescent proteins (e9 GFP, eGFP, emGFP, OsRed, mCherry)

Evaluation of foreseeable effects

Defective lentiviruses will be generated using a lenivirus vector plasmid which contains the LTR from HIV or MLV with SFFV, CMV orland U6 promoters. This plasmid will be co-transfected with packaging plasmids: pVSV-G (encoding the VSV envelope proteins under the control of the CMV promoter) and pCMVDR8.91 (encoding replication proteins). The resulting virus secreted from 293T/FT producer cells is defective in packaging signal and will be used for infection of recipient mammalian cells listed above. No productive virus that is infectious will be created. Fluorescent proteins is non-toxic and have been used in many laboratories over years with no adverse effects. TNF signaling pathways include apoptotic and anti-apoptotic genes which affect proliferation or cytotoxic mechanisms within cells. None of the TNF pathway genes targeted are classed as oncogenes. The genes that will be targets for knock down by lentiviral delivery of shRNAs and miRNAs play functional roles in the cell-matrix interactions of mammalian cells. As such they have effects on cell growth, but their knockdown would in general be expected to compromise growth or other aspects of cell behavior. However, some of the proteins could act in certain cell types as tumor suppressor functions, and their knock-down could therefore pose a potential risk if a worker infected himself/herself. However, cancer formation is a multi-step process in humans requiring at least six genetic changes, so it is highly unlikely that an infection would contribute to tumourigenesis. Also, as the lentiviral vectors are replication-defective they would not propagate in an infected worker.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All contaminated items will be left in contact with min 1% Chemgene or any other approved detergent for 5 minutes before autoclaving. Liquids are inactivated using 1% Chemgene or 70% ethanol. If contamination of lab coat occurs it will be autoclaved prior to laundering. Liquids will be incubated in 1% Chemgene solution for 5-10 minutes, then will be poured down laboratory sink with copious water. Solid wastes will be disposed of via the 'yellow box' route (autoclaved prior to disposal by landfill). Small spills (less than 10ml) will be mopped up with disposable towels soaked in 10% Chemgene; big spills (up to 1 L)
will be mopped with absorbent material from the spill kits which available from room 214/1.42 and outside 214/27. Contaminated surfaces will be sprayed with 2% Chemgene spray, left for 1 minute and wiped with disposable towels. Any towel and absorbent material waste from spillages will then be disposed of in a yellow box and autoclaved. Plastic ware, tips, gels and other contaminated items will be autoclaved directly before disposal. Needles or sharps will not be used

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**Project Containment**

**Project Ref** 101/17.1

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<td>10/02/2017</td>
<td>Construction and sequencing of transposon insertion libraries of staphylococci</td>
<td>Class 2 &amp; 3</td>
<td>&lt; 1 Litre</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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</table>

**Withdrawn** No

Tick if notifying a connected programme of work No
Staphylococci are clinically-relevant bacteria that can form biofilms. The aim of this work is to construct and sequence transposon libraries of staphylococci to increase our understanding of the genes involved in biofilm formation. The work will be conducted in accordance with the UEA health and safety guidelines and GM organisms will be handled within a CL2 laboratory according to the contained use guidelines.

Coagulase-negative staphylococci and (coagulase-positive) Staphylococcus aureus are part of the normal skin flora and are widely-dispersed in the environment including hospitals. The parent strains were obtained from hospitals and the natural environment and are able to survive outside of the laboratory. These bacteria are not considered primary pathogens and are typically associated with infection in an immunocompromised host and infections can be treated with antibiotics. Staphylococci are non spore forming bacteria and will be contained within a CL2 laboratory. Overall, the risk to healthy individuals is low.

Recipient or parental organism

Coagulase-negative staphylococci and (coagulase-positive) Staphylococcus aureus are part of the normal skin flora and are widely-dispersed in the environment including hospitals. The parent strains were obtained from hospitals and the natural environment and are able to survive outside of the laboratory. These bacteria are not considered primary pathogens and are typically associated with infection in an immunocompromised host and infections can be treated with antibiotics. Staphylococci are non spore forming bacteria and will be contained within a CL2 laboratory. Overall, the risk to healthy individuals is low.

Host/vector system

1) A Tn5 transposon (containing an antibiotic marker) and suitable transposase (e.g. Epicentre Tn5 system)

2) A Tn5 transposon (containing an antibiotic marker) and transposase on a conjugative plasmid

The transposon systems in 1 & 2 will be maintained in HG1 E. coli or HG1 staphylococcus strains before introduction into target staphylococcus strains for construction of libraries.

3) A Tn5 transposon (containing an antibiotic marker) and transposase on a mobilisable plasmid.

The transposon will be introduced into target strains from a staphylococcus donor with transfer genes encoded in the genome as well as a sacB counter selection gene integrated into the genome.

Origin & function

Host-vector systems 1 & 2) Transposons are naturally-occurring mobile DNA elements that are inserted into DNA by a transposase. A transposon will be used as linear DNA with a suitable transposase which allows direct introduction into the target strain for insertion into the genome. A transposon (e.g. Tn5) my also be used on a conjugative plasmid alongside the corresponding transposase for introduction into an intermediate Staphylococcus
strain for subsequent transfer by conjugation to the target Staphylococcus strain for insertion into the genome. The selective marker on the transposon enables selection of bacteria that contain the transposon. The genomic insertions will allow investigations into the function of genes.

Host system 3) A transposon may be used to introduce genes into a HG1 Staphylococcus strain to generate a donor for transfer of transposons to other Staphylococcus strains. The sacB counter selection gene in the genome of the donor strain confers sensitivity to sucrose and allows selection against the donor after DNA transfer. The transfer (tra) genes in the genome allows the transfer of mobilisable plasmids to target strains.

Evaluation of foreseeable effects

Staphylococcus are part of the skin microbiome and the risk posed to healthy individuals is low. Transposons are naturally occurring mobile DNA elements that will be modified to contain a resistance marker found naturally within the staphylococcal population. The transformed staphylococci will carry additional resistance genes and may provide a reservoir of mobilisable resistance genes for other staphylococci. However, constructs and GM strains will carry a maximum of two natural resistance genes and all transformed strains will remain susceptible to many antibiotics normally used for treatment of staphylococcal infection, including; vancomycin and metacillin and the risk from transformed strains is low.

A staphylococcus transposon donor strain will contain transfer genes to allow transfer of mobilisable plasmids such as those that already exist within the population. The donor strain will also allow the transfer of the target transposon plasmid which facilitates the introduction of resistance genes, on plasmid ids, into the genome of the recipient Staphylococcus strain. Conjugal and mobilisable plasmid ids and the resistance genes in this work already exist within these bacteria. The antibiotic markers will be limited to two natural resistance genes ensuring that antibiotics used for treatment of infections remain efficacious. The sacB counter selection gene in the plasmid donor strain confers sensitivity to sucrose which is most likely to reduce the fitness of the bacterium.

The resulting transposon mutant libraries may contain bacterial cells with upregulated genes as well as inactivated genes; this may alter phenotypes such as biofilm formation. This work will initially be performed with isolates of coagulase-negative staphylococci which can rarely cause infection in healthy individuals. Transposon insertion libraries of Staphylococcus aureus may then be constructed which could potentially upregulate virulence genes. Staphylococcus aureus infections can be more severe but typically only affect immunocompromised individuals. We will assess the antimicrobial resistance profile of Staphylococcus aureus strains prior to transposon mutagenesis work and only proceed with strains susceptible to multiple antibiotics. The staphylococcus aureus mutants will contain an additional natural resistance gene and no non-native genes and are not expected to exceed HG-2.

The consequences, of the harmful effect (were it to occur) is most likely comparable to wild-type Staphylococcus. Staphylococcus can survive in the environment but the risk to human health of the GM isolates is likely to be comparable to variants in the natural population, mutants will be handled in containment at level 2 which is appropriate for this work.

Evaluation of foreseeable effects

Staphylococcus are part of the skin microbiome and the risk posed to healthy individuals is low. Transposons are naturally occurring mobile DNA elements that will be modified to contain a resistance marker found naturally within the staphylococcal population. The transformed staphylococci will carry additional resistance genes and may provide a reservoir of mobilisable resistance genes for other staphylococci. However, constructs and GM strains will carry a maximum of two natural resistance genes and all transformed strains will remain susceptible to many antibiotics normally used for treatment of staphylococcal infection, including; vancomycin and metacillin and the risk from transformed strains is low.

A staphylococcus transposon donor strain will contain transfer genes to allow transfer of mobilisable plasmids such as those that already exist within the population. The donor strain will also allow the transfer of the target transposon plasmid which facilitates the introduction of resistance genes, on plasmid ids, into the genome of the recipient Staphylococcus strain. Conjugal and mobilisable plasmid ids and the resistance genes in this work already exist within these bacteria. The antibiotic markers will be limited to two natural resistance genes ensuring that antibiotics used for treatment of infections remain efficacious. The sacB counter selection gene in the plasmid donor strain confers sensitivity to sucrose which is most likely to reduce the fitness of the bacterium.

The resulting transposon mutant libraries may contain bacterial cells with upregulated genes as well as inactivated genes; this may alter phenotypes such as biofilm formation. This work will initially be performed with isolates of coagulase-negative staphylococci which can rarely cause infection in healthy individuals. Transposon insertion libraries of Staphylococcus aureus may then be constructed which could potentially upregulate virulence genes. Staphylococcus aureus infections can be more severe but typically only affect immunocompromised individuals. We will assess the antimicrobial resistance profile of Staphylococcus aureus strains prior to transposon mutagenesis work and only proceed with strains susceptible to multiple antibiotics. The staphylococcus aureus mutants will contain an additional natural resistance gene and no non-native genes and are not expected to exceed HG-2.

The consequences, of the harmful effect (were it to occur) is most likely comparable to wild-type Staphylococcus. Staphylococcus can survive in the environment but the risk to human health of the GM isolates is likely to be comparable to variants in the natural population, mutants will be handled in containment at level 2 which is appropriate for this work.
ChemGene HLD4-L will be used for disinfection and works by denaturing nucleic acids. ChemGene is biodegradable with no known health hazards. ChemGene disinfectant will be used at 5% concentration for bio-hazard contamination (small culture splash/spillage, < 5 mL) by washing surfaces with paper towels and allowing surfaces to dry before disposal of contaminated materials by autoclaving. For larger spills (> 5 mL): If aerosols may have formed, leave the area for 10 minutes and ensure no other staff enter. Culture should be covered in absorbent material soaked in 10% ChemGene which is then collected in an autoclave bag within a ‘Yellow Box’ for autoclaving. Surfaces should be treated with 2% ChemGene, left for 1 minute and wiped with disposable towels. Contaminated materials will be autoclaved as below.

For general disinfection, ChemGene will be used at 2% concentration. ChemGene is fast-acting and an effective kill should occur in 20 minutes. ChemGene is active against Staphylococcus and E. coli and is a suitable disinfectant for this project. Reference - http://www.medi-mark.co.uk/.

Biological waste, contaminated glassware and contaminated disposables will be autoclaved prior to disposal. Autoclave cycles: 126°C for 10 minutes for plastic discard, 126°C for 20 minutes for mixed/fluid discard, 134°C for 5 minutes for fabrics. The autoclave service and validation is organised by facilities management. Biological waste disposal will proceed according to the ‘Yellow Box’ SOP. Sharps will not be used with viable bacteria.

Please enter comments on the GM safety committee on the risk assessment

No comments were provided.

Project Containment

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Human Clinical Applications

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Project Ref 101/17.2
To study genomic events in S. Typhi using a safer attenuated derivative suitable for use at CL2.

S. Typhi BRD948 (aroC aroD htrA, also known as CVD908-htrA) harbours three independent, non-reverting mutations that have been fully sequenced and shown to be inactivating. It has been extensively studied in human volunteers and shown to be a safe and highly attenuated vaccine candidate. It fails to colonise the human host, and (as the parent S. Typhi) is unable to colonise plants or animals.


BRD948 is fully antibiotic sensitive.

All DNA is derived from S. Typhi Ty2, with no imported DNA. BRD948 harbours 3 specific DNA deletions intended to stably attenuate the strain.

BRD948 has been shown to be highly attenuated in multiple studies of human volunteers. This organism should not lose its attenuated phenotype as the genetic lesions have been well characterised in multiple in vivo and in vitro systems. The derivatives have been used by different groups in the UK for many years safely at CL2. BRD948 was originally recognised by HSE as disabled in 1993. Although BRD948 is designed to induce an immune response in humans it is most unlikely that such a response could be harmful, particularly as this immunisation favours a Th1 (non-allergic) response. Also, at least 10^8 bacteria in acid neutralising buffer are normally ingested to get any type of detectable response.
The microorganism is derived from Salmonella Typhi, but will be derogated to CL2 as it is highly attenuated and unable to colonise the human host. This derivative has an excellent safety record being handled at CL2 with no evidence of human infection either in the UK or abroad. This microorganism will be handled at CL2 at all times.

Disinfectant regime:

ChemGene HLD4-L will be used for disinfection and works by denaturing nucleic acids. ChemGene is biodegradable with no known health hazards.

ChemGene disinfectant will be used at 5% concentration for bio-hazard contamination (small culture splash/spillage, < 5 mL) by washing surfaces with paper towels and allowing surfaces to dry before disposal of contaminated materials by autoclaving. For larger spills (> 5 mL): If aerosols may have formed, leave the area for 10 minutes and ensure no other staff enter. Culture should be covered in absorbent material soaked in 10% ChemGene which is then collected in an autoclave bag within a “Yellow Box” for autoclaving. Surfaces should be treated with 2% ChemGene, left for 1 minute and wiped with disposable towels. Contaminated materials will be autoclaved as below.

For general disinfection, ChemGene will be used at 2% concentration. ChemGene is fast-acting and an effective kill should occur in 20 minutes. ChemGene is active against Salmonella and is a suitable disinfectant for this project. Reference - http://www.medi-mark.co.uk/.

Biological waste, contaminated glassware and contaminated disposables will be autoclaved prior to disposal. Autoclave cycles: 126°C for 10 minutes for plastic discard, 126°C for 20 minutes for mixed/fluid discard, 134°C for 5 minutes for fabrics. The autoclave service and validation is organised by facilities management. Biological waste disposal will proceed according to the “Yellow Box” SOP.

Sharps will not be used with viable bacteria.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No comments

Project Containment

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**Project notified under transitional arrangements**

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

To investigate fundamental properties of Pseudomonas such as gene regulation, gene and genome copy number, features involved in virulence and pathogenesis, persistence and antimicrobial resistance.

**Recipient or parental organism**

The following Hazard Group 2 bacteria:
- Wild-type isolates of Pseudomonas species
- Laboratory strains of Pseudomonas aeruginosa, such as PA01, PA14
- Engineered P. aeruginosa strains (e.g. with mexT deletion/insertions).

The following Hazard Group 1 bacteria:
- Wild-type isolates and laboratory strains of Pseudomonas species
- Attenuated laboratory Escherichia coli strains such as DH5a, BL21, K12
- Engineered E. coli laboratory strains for conjugal transfer of plasmid DNA such as E. coliST18.

**Host/vector system**

Initial vectors to be used are listed below, but other vectors with similar properties, hazards and risks to those listed below may be used.

**Cloning vectors:**
- pGEM-T vectors
- pUC19
- TOPO vectors

**Expression vectors:**
- pGEX vectors
- pET vectors
**Custom Tn5 transposon mutagenesis vectors such as:** · pMOD-2 EZ-Tn5 <MCS> from Epicentre Biotechnologies · pALMAR3 · p298

### Origin & function

To be inserted into wild-type and laboratory strains of Pseudomonas aeruginosa and others such as Pseudomonas putida:

Transposons (e.g. Tn5, Himar) for insertion into target DNA (when introduced alongside a suitable transposase).

- Primer binding sites are included in the transposon for sequencing the resulting insertion libraries. The transposon will be modified to include a custom antibiotic resistance gene that naturally occurs in Pseudomonas. Transposons may also be modified by the addition of promoters of varying strength or fluorescent proteins to explore differences in expression,

- Variants of bacterial genes interrupted by the insertion of a selectable marker, such as naturally-occurring antibiotic resistance genes or other selectable markers such as tellurite resistance, to create gene knock-outs.

- Variants of bacterial genes altered, for example by site-directed mutagenesis, to replace or remove key sites in order to reduce or abolish the activity of the gene product.

Genes that can be used for counter-selection e.g. sacB

To be inserted into attenuated laboratory E. coli:

- Transposon mutagenesis vectors carrying transposons (e.g. Tn5, Himar)

- Variants of bacterial genes interrupted by the insertion of a selectable marker, such as naturally-occurring antibiotic resistance genes or other selectable markers such as tellurite resistance, to create gene knock-outs.

- Variants of bacterial genes altered, for example by site-directed mutagenesis, to replace or remove key sites in order to reduce or abolish the activity of the gene product.

Genes taken from Pseudomonas isolates known or thought to confer a phenotype of interest, such as antibiotic resistance.

### Evaluation of foreseeable effects

**Host strains:**

- Hazard group 1 bacteria are unlikely to cause human disease in healthy individuals, Hazard Group 2 bacteria can cause human disease but it is unlikely to spread into the community and effective prophylaxis and/or treatment is usually available.

**Identified risks:**

- Risk of infection due to inhalation of aerosol containing bacteria.
- Risk of infection through the skin due to contact of bacteria with cuts or abrasions on the skin.
- Risk of infection due to ingestion of bacteria
- Risk of environmental contamination through accidental release

**Severity of harm from Hazard Group 1 organisms is negligible in healthy individuals. Healthy individuals can be harmed by Hazard Group 2 organisms, but effective prophylaxis and/or treatment is generally available. Hazard Group 2 Pseudomonas species (i.e. Pseudomonas aeruginosa) are opportunistic nosocomial pathogens. They exist as a normal inhabitant of human intestinal flora and are widely dispersed in the environment. As opportunistic pathogens, they require an immunocompromised host in order to establish an infection, and as such the risk to individuals working in the containment level 2 laboratory is low.**

No hazards are associated with routine E. coli DH5α cloning strains which are E. coli K-12 derivatives. E. coli K-12 has a history of safe use, limited ability to survive in the human gut and does not produce toxins (HG1). E. coli ST-18 is an auxotrophic strain encoding conjugal transfer genes for mobilisation of DNA. This auxotrophic strain has limited ability to survive unless cultures are supplemented with 5-aminolevulinic acid.

**Vector/inserts:**
Vectors used in this work include routinely used commercial vectors. These vectors are not known to present a hazard. Other custom vectors may be mobilisable. Conjugative and mobilisable plasmids naturally occur within Pseudomonas populations and the resistance genes selected in this work already exist within these bacteria. The transposon mutagenesis vectors contain a single standard antibiotic selection gene (e.g. ampicillin) for propagation in E. coli. Transposon inserts will carry a naturally-occurring antibiotic resistance marker for selection of Pseudomonas (e.g. tetracycline) and an outward facing promoter. Constructs will carry a maximum of two resistance genes which poses minimal risk in the spread of environmental resistance. These antibiotics are not used for the treatment of Pseudomonas infections.

Genetically-modified organisms:
Transposon-transformed Pseudomonas will carry a resistance gene cassette (e.g. tetracycline). Mobilisable resistance genes occur naturally and the risk from the transformed strains is low. All transformed strains will remain susceptible to many antibiotics normally used for treatment of Pseudomonas infection, including beta-lactams and aminoglycosides. The resulting transposon mutant libraries may contain bacterial cells with activated genes as well as inactivated genes; this may alter phenotypes such as biofilm formation. However, any given transposon-mutated cell will likely only contain a single altered gene. It is therefore unlikely that any changes in gene regulation and subsequent phenotype will differ from those that can occur naturally. Pseudomonas aeruginosa infections can occur in immunocompromised individuals but are unlikely in healthy individuals. We will assess the antimicrobial resistance profile of Pseudomonas aeruginosa strains prior to transposon mutagenesis work and only proceed with strains susceptible to clinically-relevant antibiotics. The transposon mutants will contain an additional natural resistance gene and no non-native genes and are not expected to exceed HG-2.

Wild-type or laboratory strains of Pseudomonas will be transformed with functional gene sequences that have been modified to reduce or abolish their function, or with interrupted gene sequences or selective markers. These procedures will result in GMOs that in most cases are reduced in their ability to cause infection, or are phenotypically indistinguishable from the unmodified parent strains. As such these modified bacteria will pose either the same, or a reduced, hazard relative to their un-modified parents.

Attenuated lab E. coli may be transformed with functional or non-functional Pseudomonas gene sequences of interest. In the case of functional gene sequences, this may alter the E. coli phenotype to make them, for example, more resistant to a limited number of antibiotics or produce more biofilm, than their parent. As the E. coli are only being transformed with individual Pseudomonas genes, it is unlikely that they will substantially alter their phenotype relative to the unmodified parent. E. coli will also be transformed with modified genes that have been modified so as to reduce or abolishing the function of the gene product. These genes are therefore expected to have a minimal effect upon the phenotype of the E. coli. These laboratory strains of E. coli are attenuated, and as such would be out-competed and not survive in a non-laboratory environment. Therefore, even in the case of carrying functional gene sequences, the E. coli are unlikely to cause a greater hazard than their Hazard Group 1 parents. However, as a precaution all work will be conducted at Containment Level 2 and the E. coli treated as if they were Hazard Group 2 organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The autoclave located in the BCRE CL2 main laboratory will be used to remove contaminated waste. Biological waste should be disposed of via the 'Yellow Box' route (as per the SOP) for steam sterilisation prior to
disposal. The waste will be contained in an unsealed autoclave bag and autoclaved once 1/2 full. Glassware containing contaminated cultures should be no more than half full. Yellow Boxes must have a biohazard label and the originators initials and lab must be recorded. Autoclave tape must be used in every run.

Autoclaving:
126°C for 20 minutes for plastic discard
126°C for 20 minutes for mixed/liquid discard
134°C for 5 minutes for fabrics.
The autoclave service and validation is organised by facilities management.
The autoclave runs are performed by trained staff.
Autoclave cycles will be monitored using the chart recorder to show a cycle has performed correctly.
Autoclaves are maintained and serviced by the facilities management in BCRE.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

No concerns were raised.

Project Containment

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Project Ref 101/19.1

Date Ackn'd 27/03/2019  
CU2 Project Title Manipulation of human induced pluripotent stem cell gene expression

Consent Granted

Class 2  
< 1 litre
The availability of human pluripotent stem cells (hiPSCs), capable of self-renewal with the potential to differentiate into multiple cell types, offers a powerful disease modelling tool. We will utilise advances in gene editing technologies that can manipulate gene expression to gain deeper insights into the molecular and cellular pathophysiological mechanisms underlying inherited diseases.

### Recipient or parental organism

The following Hazard Group 1 bacteria:
- Laboratory strains of E. coli, such as Ten10

The following Hazard Group 2 mammalian cells:
- Human fibroblasts
- Human pluripotent cell lines

### Host/vector system

Initial vectors to be used are listed below, but other vectors with similar properties, hazards and risks to those listed below may be used.

**Expression Vectors**
- dCAS9-VP64_GFP
- MS2-P65-HSF1_GFP
- CTS CytoTune KOS
- CTS CytoTune hL-Myc
- CTS CytoTune hKlf4

**Cloning Vectors**
- sgRNA(MS2) cloning backbone

### Origin & function

To be inserted into laboratory strains of E. coli and others such as Top10.
Vectors (e.g. dCAS9-VP64_GFP) to be inserted into laboratory strains of E. coli for replication. Selection will occur through antibiotic resistance e.g. Ampicillin.
To be inserted into mammalian cells, such as fibroblasts and iPSCs.
sgRNA(MS2) uses a short 20bp sequence to guide dCAS9-VP64_GFP bind to a DNA target site, combine with MS2-P65-HSF1_GFP activator helper complex to modify gene expression. This system can be used to enhance gene expression at target site in genome. GFP fluorescence can be used to identify successfully transfected cells.
Sendai virus non-integrating expression vectors that include the four Yamanaka factors, Oct3/4, Sox2, Klf4, and L-Myc (CTS CytoTune KOS, CTS CytoTune hL-Myc, CTS CytoTune hKlf4) to be inserted into mammalian cells such as fibroblasts to reprogram them into iPSCs.

Evaluation of foreseeable effects

No hazards are associated with routine E. coli cloning strains which are E. coli K-12 derivatives. E. coli K-12 has a history of safe use, limited ability to survive in the human gut and does not produce toxins (HG1).
Modified mammalian cells are a biohazard if inhaled, swallowed or absorbed through skin or needle stick. Therefore a risk of disease to humans and hazard group 2.
Plasmids contain an antibiotic selection gene (e.g. ampicillin) for propagation in E. coli, and an alternative antibiotic resistance marker for selection in hiPSCs (e.g. Puromycin). Constructs will carry a maximum of two resistance genes which poses minimal risk in the spread of environmental resistance. There is minimal risk of the plasmid being transferred to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The autoclave located in the BCRE CL2 main laboratory will be used to remove contaminated waste.
Biological waste should be disposed of via the 'Yellow Box' route (as per the SOP) for steam sterilisation prior to disposal. The waste will be contained in an unsealed autoclave bag and autoclaved once 1/2 full. Glassware containing contaminated cultures should be no more than half full. Yellow Boxes must have a biohazard label and the originators initials and lab must be recorded. Autoclave tape must be used in every run.
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The autoclave service and validation is organised by facilities management.
The autoclave runs are performed by trained staff.
Autoclave cycles will be monitored using the chart recorder to show a cycle has performed correctly. Autoclaves are maintained and serviced by the facilities management in BCRE.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Minor points of clarification were raised by the Biological hazards and GM committee and the proposal was then accepted.

Please enter comments on the GM safety committee on the risk assessment

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

**Project Containment**

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

Level 1 (GMMs)

Level 2 (GMMs)
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref** 105/01.1

<table>
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<tr>
<th>Date Ackn'd</th>
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EXPRESS PROTEINS WHICH ARE INVOLVED IN SIGNAL TRANSDUCTION IN CULTURED CELL LINES AND MURINE LYMPHOCYTES IN VITRO

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<th>Date Project Ceased</th>
<th>Non-GMM Consent Granted</th>
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Withdrawn N Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

To efficiently infect non replicating cells in vitro so that proteins or modified proteins which participate in signal transduction can be studied.

**Recipient or parental organism**

Recombinant vaccinia virus deficient in thymidine kinase. Has potential to infect humans. Immunodeficient or individuals with skin diseases will not work with virus. Expressed proteins are unlikely to alter growth/survival characteristics of infected cells as virus causes cell lysis.

**Host/vector system**

Recombinant vaccinia virus deficient in thymidine kinase to attenuate replicative capacity and virulence will be used to infect monkey kidney cells human and murine B and T lymphocyte tumour lines and murine primary lymphocytes.

**Origin & function**

cDNAs encoding human or mouse Vav proteins as well as mutated forms of Vav proteins in which specific domains have been rendered non functional either by truncation, deletion or point mutation. These mutants will be used to infect lymphocytes purified from mice which lack Vav protein expression.

**Evaluation of foreseeable effects**

Vaccinia Virus (VV) infection would be expected to result in sub-cutaneous disease, but this should be rapidly resolved by the immune system of non-immunocompromised individuals. Infection may give rise to mild fever. As the virus replicates poorly, systemic infection or transfer to other individuals is extremely low. Infection of certain areas, such as the eye or the skin of patients with eczema can lead to severe complications, as can infection of immunodeficient individuals. The virus is not spread by aerosols and we are not proposing to inject the virus into animals therefore needle stick injuries, which could lead to infection will be avoided. The so-called oncogenic forms of Vav have been defined on the basis of altered morphology of 3T3 cells and have not been shown to give rise to tumours that persist in nude mice. In any event since VV infection leads to cell death, transformation is unlikely to occur. The inserts will affect signal transduction within infected cells, but we anticipate these will be deleterious for cell survival. Mis-expression of signalling proteins is unlikely to manifest systemic effects. Moreover as infections with thymidine kinase negative VV are generally self limiting, the consequences of foreign gene expression are likely to be transient. The expressed genes are not anticipated to increase virulence. The virus has the potential to infect animals but not plants, and there are documented cases of VV transmission from humans to domestic animals, but VV has been shown to be very poorly transmitted in the wild. Recombinant VV are expected to be attenuated and transmit poorly between individuals or animals. Infected cell lines and primary lymphocytes will not thrive outside of the laboratory environment. VV can survive for a few days in dried material. The potential route for transmission to the environment
could occur via waste disposal. VV will be handled in Class II biological safety cabinets and the waste will be inactivated before disposal by autoclaving.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Tissue culture waste/bug waste etc is autoclaved at 126 C for 10’ at 2 bar. This programme is installed in the autoclave and cannot be changed by the Operator. All details of each run are printed out and kept for reference. The waste is then removed from the building, stored in a cold store until such time that it is transported to an off-site incinerator. These parameters are those suggested by the MRC and other safety groups.

Liquid waste (media) is autoclaved (bottled), then the contents are disposed of down the sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee considered the proposal to be at Class 2. Full containment level 2 should be applied.

The Committee also emphasised the need to ensure that immunologist individuals or those with skin diseases do not work with viruses.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 105/05.1
Purposes of the contained use
The overall aim is to produce lentiviruses that direct the expression of small hairpin RNAs (siRNAs) that interfere with the expression of the catalytic subunits of class I phosphoinositide 3-kinases (PI3Kinases) and thereby lower the levels of expression of these subunits in the mammalian cells into which they have been introduced.

Recipient or parental organism
The recipient organism will comprise several well characterised mammalian cell lines maintained in tissue culture; these are the HeLa, PLB-985, MDA-MB-231 and MDA-MB-468 cell lines.

Host/vector system
The lentiviral vector (pLV-TH) system is derived originally from human T-cell lymphotropic (HTLV) but has been extensively modified to increase its biosafety characteristics. In essence this is a 'third generation' system designed to minimise the risk production of viable virus in the destination cell type.

Origin & function
The inserted DNA is synthetic and directs the synthesis of short RNA sequences that are complementary to the target PI3Kinase subunit mRNAs.

Evaluation of foreseeable effects
The lentiviral vector itself engineered such that it cannot replicate in the recipient cells. Therefore any productive infection of host cells is very unlikely to occur. However, as the GMM is capable of infecting human cells, the nature of the insert is of importance.

The insert does not encode an oncogene or toxin protein. Therefore, whilst it is possible that production of the siRNA could disrupt normal function in a single infected cell, it is unlikely that this effect will propagate further.

The control measures in place to protect human health should result in minimal release of the GMM and therefore little associated environmental effects. Although the lentiviral vector is capable of infecting a wide range of mammalian cells the virus will be incapable of replicating in those cells and therefore no spread of the GMM can be visualised.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be autoclaved using an autoclave in the same building prior to disposal.

Liquid waste will be inactivated by the addition of Virkon to a final concentration of 1% (w/v) prior to disposal.

Both of these disposal methods will achieve 100% inactivation of the GMM.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The local GMSC approved the risk assessment at a meeting on 4th July 2004.

Project Containment

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Animal Units

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Project Ref 105/05.2

Date Ackn'd 06/12/2005

CU2 Project Title Use of lentivirus to deliver shRNAs directed against regulators of cell death in human

Class 2

Culture Volume Class 2 < 1 Litre
The overall aim is to produce lentiviruses that direct the expression of small hairpin RNAs (shRNA) that interfere with the expression of members of the Bcl-2 family of proteins and also others that interfere with the expression of components of MAP Kinase signalling pathways, notably the oncogenic form of B-RAF.

The recipient organism will comprise several well characterised tumour cell lines maintained in tissue culture: these are Colo205, HT29, Co115, LS411, RKO, HCT116, SW620, BE, Lovo, SW48, SW480 and CCL39, also the adenoma cell lines, PC/AA/C1 and S/CG/C2.

The lentiviral vector (pLV-TH) system is derived originally from Human T-cell lymphotrophic virus (HTLV) but has been extensively modified to increase its biosafety characteristics. In essence this is a "third generation" system designed to minimise the risk of production of viable virus in the destination cell type.

The inserted DNA is synthetic and directs the synthesis of short RNA sequences that are complementary to the target mRNAs.

The lentiviral vector itself is engineered so that it cannot replicate in the recipient cells. Therefore any productive infection of host cells is very unlikely to occur. However as the GMM is capable of infecting human cells the nature of the insert is of importance.

The inserts do not encode oncogenes or toxic proteins. They actually encode shRNAs directed against various proteins involved in the regulation of apoptosis. One group contains shRNA directed against components of the MAP Kinase signalling pathway, in particular an oncogenic form of B-RAF, and so if anything the insert would be predicted to have an anti-oncogenic effect. Whilst it is possible that shRNA against any normal protein could disrupt normal cellular function, the B-RAF shRNA is specific for a point mutated form of B-RAF and so, due to mismatch, shouldn't impact on the expression of normal B-Raf protein. In addition, successful knockdown of components of the MAPK pathway would be expected to reduce cell survival and so any infected cell would be more likely to die and therefore be unable to propagate further. The other constructs are directed against members of the Bcl-2 protein family. These are regulators of apoptosis and in some cases are themselves regulated by the MAPK pathway downstream of B-RAF. Successful shRNA-mediated knockdown of these proteins might provide a survival signal but this would be cell autonomous, being confined to a single infected cell; any effect would not propagate further due to the fact that the construct is replication defective and lacks key genes (such as Tax) which would be
required to facilitate survival and proliferation of infected cells. In addition, it is close to impossible to envisage these inserts being transferred to other organisms except by recombination with WT HTLV.

The control measures in place to protect human health should result in minimal release of the GMM and therefore little associated environmental effects. Although the lentiviral vector is capable of infecting a wide range of mammalian cells the virus will be incapable of replicating in those cells and therefore no spread of the GMM can be visualised.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be autoclaved prior to removal for off-site incineration i.e. the waste is inactivated on site.
All solid waste will be treated with Virkon (final concentration of 1% (w/v) prior to being sealed in a burn bin and disposed of by incineration.
Liquid waste will be inactivated by the addition of Virkon to a final concentration of 1% (w/v) prior to disposal.
Both of these disposal methods will achieve 100% inactivation of the GMM.

Is an emergency plan required according to regulation 20?  
No

If yes, tick to confirm that it is attached to this form

No

Tick to confirm that you have attached a risk assessment to this form

Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment

No

Please enter comments on the GM safety committee on the risk assessment

The local genetic modification safety committee approved the risk assessment at a meeting on 29th November 2005.

Project Containment

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<td>L2 L3 L4 L2 L3 L4</td>
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</table>
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to use a lentiviral vector system to introduce the neuroprotective factor WldS and some mutants and potential modifiers of this factor into mammalian neurons in primary culture in order to verify the importance of specific sequences of the WldS protein in conferring the protective phenotype.

**Recipient or parental organism**

The recipient organism will comprise various characterised mammalian primary neurons maintained in tissue culture.

**Host/vector system**

The lentiviral vector system is derived originally from HIV-1. However the vector has been extensively modified (a "third-generation system) to increase its biosafety characteristics. In particular the system is designed to reduce the risk of production of viable virus in the destination cell type.

**Origin & function**

The inserted DNA is synthetic and directs the synthesis of a neuroprotective factor for axons, WldS, or mutants or potential modifiers of this factor that will lose or maintain the capability to induce neuroprotection.

**Evaluation of foreseeable effects**

The lentiviral vector itself is engineered such that it cannot replicate in the recipient cells. Therefore any productive infection of host cells is very unlikely to occur. However as the GMM is capable of infecting cells, the nature of the insert is of importance.

The insert does not encode either an oncogene or a toxic protein. High levels of expression of the inserts in mammals (mice and rats) in vivo are harmless. Therefore, it is unlikely that the insert will be harmful.
The control measures in place to protect human health should result in minimal release of the GMM and therefore little associated environmental effects. Although the lentiviral vector is capable of infecting a wide range of mammalian cells the virus will be incapable of replicating in those cells and therefore no spread of the GMM can be visualized.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization is GMGM898) according to disposal notification GM 105/4.1.

The disposal method is expected to achieve 100% inactivation of the GMM.
The data sheets describing inactivation by Virkon are attached.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The genetic manipulation safety committee approved the proposal at a meeting on 20th June 2006.

### Project Containment

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Project Ref 105/06.2
**Project Additional Information**

**Purposes of the contained use**

The purpose is to study the effect of modulating the levels of proteins involved in intracellular signalling pathways or transcriptional regulation in either mammalian cell culture or in transgenic mice either by protein over-expression or the use of siRNA or antisense RNA technologies to reduce mRNA levels of the corresponding proteins.

**Recipient or parental organism**

The recipient organisms will comprise either various characterized mammalian cell lines maintained in tissue culture or single cell embryos.

The mammalian cell lines fall into two types:
- A packaging/helper cell line into which lentiviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.
- Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines i.e. a negligible risk.

The single cell embryos are derived from standard mouse or rat laboratory strains. Following infection and culturing, the embryos will be extensively washed to remove free virus before transfer to recipient mothers. As such the embryos pose no more risk than that associated with culturing other mammalian cells.

In particular it should be noted that following implantation the embryos develop normally to term, and because of the nature of the lentiviral vector, no active viral particles will be made i.e. neither the mother nor offspring will be producing infectious virus.

**Host/vector system**

The lentiviral vector systems are derived originally from HIV-1. However the vector has been extensively modified (a "second-generation" system) to increase its biosafety characteristics. In particular the system is designed to reduce the risk of production of viable virus in the destination cell type. However it should be noted that the lentiviral vector systems carry the potentially oncogenic woodchuck post-transcriptional regulatory element (WPRE) sequence which has been implicated in causing cancer.
Origin & function

The inserted DNA are of three types:

a) Synthetic DNA sequences are designed to code for short RNA sequences (siRNA sequences) to cause a targeted reduction of the levels specific mRNAs and hence proteins involved in intracellular signalling pathways or transcriptional regulation in mammalian cells.

b) Mammalian cDNA sequences inserted into the lentiviral vectors to result in the synthesis of "antisense" RNAs complementary to specific mRNAs encoding proteins involved in intracellular signalling pathways or transcriptional regulation in the cell - resulting in selective reduction in the levels of those mRNA species and hence proteins in mammalian cells.

c) Mammalian cDNA sequences designed to result in over-expression of encoded proteins involved in intracellular signalling pathways or transcriptional regulation in mammalian cells.

Evaluation of foreseeable effects

The lentiviral vector itself is engineered such that it cannot replicate in the recipient cells. Therefore any productive infection of the host cells is very unlikely to occur. However as the GMM is capable of infecting human cells, the nature of the insert is of importance. Whilst it is possible that production of individual siRNAs or antisense RNAs could disrupt normal function in a single infected cell, it is unlikely that this effect will propagate further. The worse case scenario might be the reduction in the levels of a protein that normally facilitates cell apoptosis i.e. a pro-apoptotic protein. In this circumstance, cells that would normally die could survive. Even in this case the risk of serious effect is unlikely to be high as firstly, before cells could begin to proliferate in an uncontrolled manner, accumulation of additional mutations would need to occur, and secondly a large body of previous work on siRNAs suggests that the overall level of "knockdown" achieved does not generally exceed 80% and hence complete growth control is unlikely to be lost. More caution is required with respect to the lentiviral vectors directing expression of mammalian proteins. The worst case scenario is likely to be expression of a potential oncoprotein. Clearly this could result in transformation of infected cell. However, over-expression of proto-oncogenes in primary mammalian cells tends to result not in transformation; instead the result is either the induction of cellular senescence or the activation of apoptotic pathways resulting in cell death. Moreover, as the lentiviral vector itself carries the WPRE sequence that itself may have oncogenic potential, it seems likely that even in this worst case scenario that the recombinant virus would be more dangerous than the parental lentiviral vector.

The control measures in place to protect human health should result in minimal release of the GMM and therefore little associated environmental effects. Although the lentiviral vector is capable of infecting a wide range of mammalian cells the virus will be incapable of replicating in those cells and therefore no spread of the GMM is visualized.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The activity is restricted to the infection of cultured single-cell mouse or rat embryos. As such the containment and control measures will be exactly as described for infected mammalian cells in culture.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization is GM 898) according to disposal notification GM 105/04.1).

This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The local genetic modification safety committee approved the risk assessment at a meeting on 13/10/06. The committee discussed the transgenic rodents that would be finally generated from the lentiviral infected embryos. It was felt that for most transgenic animals, that this was likely to be a Class 1 activity and therefore need not specifically assessed in their proposal.

Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 Yes L3 L4 | L2 L3 L4 | L2 L3 L4

Animal Units | Large Scale Activities | Human Clinical Applications
---|---|---
L2 L3 L4 | L2 L3 L4 | L2 L3 L4

Project Ref 105/08.1

CU2 Project Title: Immunity to bacterial and fungal pathogens (Aspergillus fumigatus, Listeria monocytogenes, Helicobacter pylori, Salmonella typhimurium, Staphylococcus aureus - containing genes coding for marker proteins) using mouse models.

Date Ackn'd 04/01/2008

Date Project Ceased

Class 2 CultureVolClass2 < 1 Litre

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work Y

02/03/2022
### Project Additional Information

#### Purposes of the contained use

Mice will be infected with various GM bacteria or fungi (altered to express marker proteins) and their ability to clear the infections and to raise effective immune responses will be monitored.

#### Recipient or parental organism

- **Aspergillus fumigatus** Allergens, aspergillosis ACOP 2
- **Listeria monocytogenes** Septicaemia; fever; diarrhoea ACDP 2
- **Helicobacter pylori** Gastritis; peptic ulcer ACDP 2
- **Salmonella typhimurium** Enteritis ACDP 2
- **Staphylococcus aureus** Toxin production abscesses, boils, and it can infect wounds ACDP 2

#### Host/vector system

r61A coding for various marker proteins will be introduced into the micro-organism either as a plasmid (with standard specific promoters for each micro-organism) or inserted into the genome of the micro-organism by homologous recombination. Genes allowing for selection of the GM micro-organism (e.g. kanamycin resistance) would also be included. N.B. The antibiotic resistance gene would not correspond to the antibiotic normally used to treat each pathogen.

#### Origin & function

cDNA coding for various proteins that can be used as 'markers' for the presence of the pathogen, for example, ovalbumin (a component of chicken eggs) and Luciferase (a gene from the firefly that can emit light).

#### Evaluation of foreseeable effects

The cDNAs introduced into the pathogens code for proteins which present negligible hazard to humans or animals. 'Infections with all organisms are treatable (see attached risk assessment information sheet). Antibiotic selection markers will not correspond to antibiotics normally used to treat that organism- The control measures in place to protect human health should result in minimal release of the GMM and therefore little associated environmental effects. Although the named micro-organisms are capable of infecting humans and animals the standard operating procedures described in the attached risk assessment will minimise their escape from containment and minimise the risk of exposure of workers to these pathogens.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste material will then be placed in bins, autoclaved and then incinerated by an off-site incineration company (Vetspeed Their GM authorization is GM898) according to disposal notification GM105/41.

This disposal method is expected to achieve 100% inactivation of the GMM.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
The local genetic modification safety committee approved the risk assessment at a meeting on 18/12/2007.

Please enter comments on the GM safety committee on the risk assessment:

The local genetic modification safety committee approved the risk assessment at a meeting on 18/12/2007.

Project Containment

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Animal Units

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Project Ref 105/08.2

Retroviral mediated introduction of DNA sequence encoding proteins or RNA species into mammalian cells in culture.

Date Ackn'd 17/09/2008

CU2 Project Title

Retroviral mediated introduction of DNA sequence encoding proteins or RNA species into mammalian cells in culture.

Class CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
### Purposes of the contained use

The purpose is to study the effect of modulating the levels of proteins expressed in mammalian cells in culture either by protein over-expression or reduction in the levels.

### Recipient or parental organism

The recipient organisms will comprise various characterized mammalian cell lines maintained in tissue culture. The mammalian cell lines fall into two types:

a) A packaging/helper cell line into which retroviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines i.e. a negligible risk.

### Host/vector system

The retroviral vectors which will be used are derived from either Moloney Murine sarcoma Virus (MoMLV), Mouse Mammary Tumour Virus (MMTh) or Feline Leukaemia Virus (FeLV), all of which are ACDP Hazard Group 1 biological agents.

On the other hand, the virus will be packaged by transfecting transfer vector into specific amphoteric ‘helper’ cell line (gag, poland any stably incorporated into host chromosome cells). **NOTE**: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types.

This means that the viruses produced for this experiment could potentially infect a number of species, including man.

However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle:

- The genes encoding structural and other components of the viral genome have been separated. These genes have been engineered to minimise the risk of recombination that could lead to production of a replication-competent virus.
- The packaging cell lines allow expression of proteins, required to produce progeny virus: But the transfer vector is the only genetic material transferred to the target cells, consequently these cells cannot produce the proteins which are essential for viral assembly and infectivity.
- Second, third generation or Self INactivating vectors retrovirus vectors will be used in all experiments (see SACGM compendium of guidance part2, section 2.11 (Retroviruses) ppl 17

### Origins and intended functions of the genetic material Involved

- **a.** Synthetic DNA sequences designed to code for short RNA sequences (5iRNA sequences) to cause a targeted reduction of the levels specific mRNAs and hence proteins in mammalian cells.
- **b.** Mammalian cONA sequences inserted into the retroviral vectors to result in the synthesis of ‘antisense’ RNAs complementary to specific mRNAs in the cell — resulting in selective reduction in the levels of those mRNA species and hence proteins in mammalian cells.
- **c.** Mammalian cONA sequences designed to result in over-expression of encoded proteins in target mammalian cells.

### Evaluation of foreseeable effects

Although these retroviruses are categorized at ACDP 1, a number of factors could increase the containment that will be required to work with them. We therefore need to consider worst case scenarios:

- The virus is packaged using an amphoteric system to allow transfection of human cells and this may have knock on effects when considering...
i) Biological properties of the gene product
ii) The expression characteristics and
iii) Provirus insertion (see below)

i) In the case of the introduction of retroviruses carrying DNA sequences encoding either antisense or RNA species into cells it is possible to envisage a scenario in which the knockdown of the mRNA targeted by the siRNA or antisense RNA, and hence the reduction in level of the encoded protein will have an effect on cell metabolism. Perhaps the most extreme example of this would be the reduction in levels of a protein that normally facilitates cell apoptosis, i.e., a pro-apoptotic protein. In this circumstance, cells that would normally die could survive. Even in this case the risk of a serious effect is unlikely to be high as firstly, before cells could begin to proliferate in an uncontrolled manner, accumulation of additional mutations would need to occur, and secondly a large body of previous work on siRNAs suggests that the overall level of knockdown achieved does not generally exceed 80% and hence complete growth control is unlikely to be lost. More caution is required with respect to retroviruses encoding mammalian proteins. The worst case scenario is likely to be expression of a potential oncprotein. Clearly this could result in transformation of infected cells. However, over-expression of proto-oncogenes in primary mammalian cells tends to result not in transformation; instead the result is either the induction of cellular senescence or the activation of apoptotic pathways resulting in cell death.

ii) The promoter could be highly active in a wide range of cell types (e.g., the CMV promoter).

iii) (From the SACGM compendium of guidance part 2, page 121) ‘The effects of integration upon the infected cell should be considered. For instance, promoter sequences present in the provirus might activate genes adjacent to the integration site or, alternatively, insertion may disrupt genes and prevent their expression. Therefore, retrovirus infection might induce permanent changes in a cell, resulting in tumourigenesis. It is recognized that, in humans, this appears so far only to have occurred in the context of deliberate transduction of large numbers of stem cells with a retrovirus vector. Furthermore, the transferred gene enabled the cells to proliferate in response to cytokines, and the many ensuing cycles of cell replication may have allowed additional, co-operating events to occur. High-titre inoculations are required to establish a clinically significant level of infection or gene transfer, and accidental infections of this magnitude are unlikely during standard laboratory-based manipulations of retroviruses. Nevertheless, retrovirus vectors have been shown to have transforming properties in vivo and a cautious approach to handling them is advised.’

Whilst it is possible that production of individual siRNAs or antisense RNAs could disrupt normal function in a single infected cell, it is unlikely that this effect will propagate further. The worse case scenario might be the reduction in the levels of a protein that normally facilitates cell apoptosis, i.e., a pro-apoptotic protein. In this circumstance, cells that would normally die could survive. Even in this case the risk of a serious effect is unlikely to be high as firstly, before cells could begin to proliferate in an uncontrolled manner, accumulation of additional mutations would need to occur, and secondly a large body of previous work on siRNAs suggests that the overall level of knockdown achieved does not generally exceed 80% and hence complete growth control is unlikely to be lost.

More caution is required with respect to retroviral vectors directing expression of mammalian proteins. The worst case scenario is likely to be expression of a potential oncprotein. Clearly this could result in transformation of infected cells.

The control measures in place to protect human health should result in minimal release of the GMM and therefore little associated environmental effects. Although the lentiviral vector is capable of infecting a wide range of mammalian cells the virus will be incapable of replicating in those cells and therefore no spread of the GMM is visualized.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The activity is restricted to the infection of cultured single-cell mouse embryos. As such the containment and control measures will be exactly as described for infected mammalian cells in culture.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material will be inactivated by treatment with 1% (w/v) Virton solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed — Their GM authorization is GMGMS98) according to disposal notification GM1OS/41. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached. The activity is restricted to the infection of cultured single-cell mouse embryos. As such the containment and control measures will be exactly as described for infected mammalian cells in culture.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The local genetic modification safety committee approved the risk assessment at a meeting on Monday 28th July 2008.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 105/15.1

Date Ackn’d 25/06/2015  
CU2 Project Title Influenza A (H1N1) virus morbidity

Class 2  
CultureVolClass2 < 1 Literature

Non-GMM  
Consent Granted

Date Project Ceased 02/03/2022
Purposes of the contained use

Mice will be infected with various GM strains of influenza (altered to express marker proteins) and their ability to clear the infections and to raise effective immune responses will be monitored.

Recipient or parental organism

The recipient organisms will comprise of either:
(a) characterized mammalian cell lines maintained in tissue culture for transfection, growth and rescue of virus
(b) C57BL/6 and transgenic mice for virus morbidity studies

Host/vector system

Orthomyxoviridae, Influenza virus type A, Subtype H1 N1 (for strains see below) - All ACDP 2
• The influenza type A, subtype H1N1 and strain (WSN-OVA) ANVSN/33 virus containing the OVA 257-264 peptide was generated by reverse genetics (see D.J Topham; M.R. Castrucci; F.S. Wingo; G.T Belz and P.C. Doherty (2001) The role of antigen in the localization of naive, acutely activated and memory CD8+ T-cells to the lung during influenza pneumonia. J. Immunology, 167: 6983)
• For the generation of OVA variants flu viruses E1 (EIINFEKL) and G4 (SJIGFEKL) same reverse genetics principle was used to produce the engineered viruses (see Denton, A E et al. Affinity thresholds for naive CD8+ CTL activation by peptide and engineered Influenza A viruses. J. Immunol. 187, 5733-5744 (2011)).
• The influenza type A, subtype H1N1 virus and strain (NS1 -GFP ) AlPRl8/34 was generated using standard reverse genetics techniques of the virus NS1 gene (see Manicassamy, Bet al. Analysis of in vivo dynamics of influenza virus infection in mice using a GFP reporter virus. Proc.Nati Acad. Sci. USA 107, 11531-11536 (2010)).
• The influenza type A, subtype H1 N1 and strain (NanoLuc) AlCalifornia/04/2009 virus was generated by insertion of Gaussian luciferase gene into the NA segment of influenza virus by genetic modification. Weiqi, Pet al. Visualizing influenza virus infection in living mice. Nat. Commun. 4, 2369 (2013). Virus packaging will not be altered. The GM virus classification will be SACGM 2 (see Beare, A. S., G. C. Schild, and J . W. Craig. 1975. Trials in man with live recombinants made from AlPRl8f34 (HO N1) and wild H3 N2 influenza viruses. Lancet ii:729-732). Also, the Advisory committee on dangerous pathogens document - "Advice on Experimental working with Influenza Viruses of Pandemic Potential" - states that subtype/strains are not sufficiently different from currently circulating human viruses should be handled at higher containment - this does not apply to the subtype/strains to be used in these experiments.
Origin & function

Chicken Ovalbumin; Jelly fish (Aequorea Victoria) GFP; Marine copepod (Gaussia princeps) luciferase
Ovalbumin sequence 257-264 (SIINFEKL) and it's variants EIINFEKL (E1) and SIIGFEKl (G4); GFP gene as reporter; Gaussian Luciferase gene as reporter.

Evaluation of foreseeable effects

We classify the work with these H1 N1 influenza virus strains as SACGM 2 for the following reasons:
Strain AIWSN/33 is classified as BSL-2 by the ATCC
Strain A/PRJ8/34 is classified as BSL-2 by the ATCC
Strain A/California/04/2009 - is a currently circulating virus
Also, the Advisory committee on dangerous pathogens document - 'Advice on Experimental working with Influenza Viruses of Pandemic Potential' - states that, . Viruses with pandemic potential should be handled at higher containment (at CL3 or above as determined by risk assessment). These include subtypes H2, H1 and H3 if, in the case of the latter two, the viruses being handled are sufficiently different from currently circulating human viruses to warrant an assumption of little or no cross-reactive immunity in the human population.’
Since the H1 N1 virus strains we will be using are ‘currently circulating’ ones then this indicates containment level 2 will be adequate.
In addition the inserts are all known to be harmless.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For cells in culture the containment and control measures will be exactly as described for infected mammalian cells in culture.
For mouse infections all work will be carried out in a containment 2 area within the biological services unit. Mice will be handled within MSC II cabinets and housed in individually ventilated (HEPA filtered) sealed cages.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization is GMGM898) according to disposal notification GM105/4.1.
This disposal method is expected to achieve 100% inactivation of the GMM.
The data sheets describing inactivation by Virkon are attached

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The local genetic modification safely committee approved the risk assessment at a meeting on Wednesday 27th May 2015.

**Project Containment**

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**Project Ref** 105/17.1

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<td>Sendai virus-based Reprogramming of human Peripheral Blood Mononuclear Cell (PBMNC), fibroblasts or other somatic cell types for induced Pluripotent Stem Cell (iPSC) generation.</td>
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<td>&lt; 1 Litre</td>
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**Project Additional Information**

**Purposes of the contained use**

Induced pluripotent stem cells (iPSCs) are genetically reprogrammed somatic cells which exhibit a pluripotent stem cell-like state similar to embryonic stem cells. iPSCs can be derived by inducing selected gene expression via various methods including sendai virus-mediated gene transduction and chemical induction.
Recipient experimental systems are human peripheral blood mononuclear cell (PBMC), fibroblast– or other somatic
cell types. SeV vectors used in this kit consist of viral proteins NP, P, M, F (activated), HN, and L, and the SeV
genome RNA, from which the F gene is deleted. Because SeV infects cells by attaching itself to cell surface receptor
sialic acid, present on the surface of many cell types of different species, the vectors are able to transduce a wide
range of cells. However, they are no longer capable of producing infectious particles from infected cells, because the
viral genome lacks the F-gene. In addition, the presence of functional mutations such as temperature sensitivity in the
amino acid sequence of several SeV proteins (SeV/TS F, SeV/TS12 F, and SeV/TS15 F) renders the vectors easily
removable from transduced cells.

Host/vector system

The CytoTune™-IPS 2.0 Sendai Reprogramming Kit is a non-integrating system that uses Sendai virus (wild type
ACDP 1) vectors to reprogram somatic cells into induced pluripotent stem cells (iPSCs). The CytoTune TM-IPS 2.0
Sendai Reprogramming Kit contains three CytoTune™ 2.0 reprogramming vectors that are used for delivering and
expressing key genetic factors necessary for reprogramming somatic cells into iPSCs (see below).

CytoTune® Sendai hOct3/4 (Human Oct3/4; NM_002701.4) Pluripotency
CytoTune® Sendai hSox2 (Human Sox2; NM_003106.2) Pluripotency
CytoTune® Sendai hKlf4 (Human Klf4; BC029923.1) Proliferation
CytoTune® Sendai hc-Myc (Human c-Myc; K02276.1) Proto-oncogene, Proliferation

Origin & function

Human transcription factor genes used to reprogram human fibroblasts and PBMCs to produce iPSCs including:
Oct4
Sox2
Klf4
c-Myc (a known oncogene linked with several forms of cancer)
Sendai virus vector is a Cytoplasmic RNA vector. Sendai virus vector replicates its RNA genome and produces
proteins exclusively in the cytoplasm. It does not enter cell nucleus. In principle, the virus should not alter the
chromosomes in the cell nucleus.

Evaluation of foreseeable effects

SeV vectors used in this kit consist of viral proteins NP, P, M, F (activated), HN, and L, and the SeV genome RNA,
from which the F gene is deleted. Because SeV infects cells by attaching itself to cell surface receptor sialic acid,
present on the surface of many cell types of different species, the vectors are able to transduce a wide range of cells.
However, they are no longer capable of producing infectious particles from infected cells, because the viral genome
lacks the F-gene. In addition, the presence of functional mutations such as temperature sensitivity in the amino acid
sequence of several SeV proteins (SeV/TS F, SeV/TS12 F; and SeV/TS15 F) renders the vectors easily removable
from transduced cells. . . .J

Regarding the sequences being carried by this vector, some transcription factors are oncogenic e.g. c-Myc is a known
oncogene linked with several forms of cancer, therefore to minimise any potential risk of this oncogene getting into those
working with this system use SACGM containment Level 2

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Instead of autoclaving waste in the building, we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM1 05/4.1 (see also above section 6 above for description). The waste disposal system used by the Babraham Institute is safe and has been running since October 2004. All liquid waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization is GM898) according to disposal notification GM105/4.1. Waste from our GM work at Class2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinicalGM waste incinerator contractor will be made three times a week to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container, means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RT A, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Solid waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinicalGM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

Project Containment

Laboratory Activities

Glass Houses

Growth Rooms

02/03/2022
Adeno Associated Virus (AAV) mediated introduction of DNA sequences encoding proteins or RNA species into mammalian cells in culture and the organs/tissues of mice

The purpose is to study the effect of modulating the levels of proteins expressed in mammalian cells in culture or mouse organs/tissues either by protein over-expression or reduction in the levels.

The recipient organisms will comprise various characterized mammalian cell lines maintained in tissue culture or experimental strains of mice and transgenic mice.

The mammalian cell lines fall into two types:

a) A packaging/helper cell line into which AAV DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and
as such are no more dangerous than the parental cell lines i.e. a negligible risk.

**Host/vector system**

The AAV vector technology that we will use is based on a non-enveloped single-stranded DNA virus (Adeno Associated Virus - which are ACDP Hazard Group 1 biological agents) as vehicles to efficiently deliver and express genes in mammalian cells.

AAV viruses are taken up by endocytosis, released for transcription and expression following migration to the nucleus. Given the low pathogenicity of the recipient virus, the major hazards that will be posed by recombinant AAV vectors will depend upon the properties of the inserted genetic material and any products that it may encode.

**Origin & function**

Selectable markers – examples (but not restricted to);
- Ampicillin resistance: E.coli derived
- Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
- Puromycin resistance (PAC) : Puromycin acetyl transferase is derived from Streptomyces alboniger
- Reporter proteins such as (but not restricted to);
- Fluorescent proteins as reporters e.g. GFP derived from the jellyfish Aequorea victoria and variants of this
- Luciferase – class of oxidative enzymes used in bioluminescence, renilla luciferase derived from the Sea pansy (Renilla reniformis), firefly luciferase derived from the firefly Photinus pyralis etc.

Open reading frames, cDNAs and gene sequences encoding proteins of interest and / or shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding proteins of interest) – from various mammalian species, especially human and mouse.

This could include the expression of potentially harmful genes e.g. encoding known proto-oncogenes or genes with known oncogenic mutations or cytokines that can contribute to cellular transformation.

**Evaluation of foreseeable effects**

The AAV vectors employed in this protocol exhibit broad tropism and potential to infect human. Most work with AAV vectors would take place at containment 1 because:

i) Adeno-associated viruses (AAV) belong to the family Parvoviridae and there is no known link to any human illnesses. AAVs appear to be defective, requiring coinfection with a helper virus (for example Adenovirus or Herpes simplex virus) in order to replicate and this has led to their classification as Dependoviruses, a discrete genus within this family.

ii) The AAV-based vector systems we will use are ‘gutless’ AAV systems (of various serotypes) consisting of a plasmid containing the foreign DNA to be transduced into the cell flanked by AAV ITR sequences. Cloned rep and cap genes or expression of Adenoviral genes required for AAV replication (ie E1, E2A, E4Orf6 and VA RNA) supply helper functions.

iii) The ‘gutless’ nature of the AAV vectors we will use also mean that integration into the AAVS1 locus on human chromosome 19 will not occur, due to the lack of rep sequences in the vector backbone needed to target the genome to this locus. Long-term expression of genes transduced using AAV vectors is seen and this is thought to be mainly due to the maintenance of episomal genomes. However, the characteristics of AAV may thus have knock on effects when considering:

- Biological properties of the gene product
- The expression characteristics
  - In the case of the introduction of AAV carrying DNA sequences encoding either antisense or RNA species into cells it is possible to envisage a scenario in which the knockdown of the mRNA targeted by the siRNA or antisense
RNA, and hence the reduction in level of the encoded protein will have an effect on cell metabolism. Perhaps the most extreme example of this would be the reduction in the levels of a protein that normally facilitates cell apoptosis i.e. a pro-apoptotic protein. In this circumstance, cells that would normally die could survive. Even in this case the risk of a serious effect is unlikely to be high as firstly, before cells could begin to proliferate in an uncontrolled manner, accumulation of additional mutations would need to occur, and secondly a large body of previous work on siRNAs suggests that the overall level of “knockdown” achieved does not generally exceed 80% and hence complete growth control is unlikely to be lost.

ii) More caution is required with respect to AAV encoding mammalian proteins. The worst case scenario is likely to be expression of e.g. a potential oncprotein or cytokine. Clearly this could result in transformation of infected cells. However, over-expression of proto-oncogenes in primary mammalian cells tends to result not in transformation; instead the result is either the induction of cellular senescence or the activation of apoptotic pathways resulting in cell death.

iii) The promoter could be highly active in a wide range of cell types (e.g. the CMV promoter). (From the SACGM compendium of guidance part 2, page 121) The effects of integration upon the infected cell should be considered. For instance, promoter sequences present in the provirus might activate genes adjacent to the integration site or, alternatively, insertion may disrupt genes and prevent their expression. The ‘gutless’ nature of most AAV vectors means that integration into the AAVS1 locus on human chromosome 19 will not occur, due to the lack of rep sequences in the vector backbone needed to target the genome to this locus. Non-targeted proviral insertion is seen with AAV vectors. However, unlike the insertion events peculiar to retroviral life cycles, insertion of gutless AAV vector genomes is a passive mechanism that occurs at naturally occurring chromosomal breakpoints. Approximately 10% of all double stranded genomes are thought to integrate into host chromosomes in this way, and appear to passively target regions of transcriptionally active chromatin. Therefore the nature of most AAV vectors means that integration into the AAVS1 locus on human chromosome 19 will not occur, due to the lack of rep sequences in the vector backbone needed to target the genome to this locus. Non-targeted proviral insertion is seen with AAV vectors. However, unlike the insertion events peculiar to retroviral life cycles, insertion of gutless AAV vector genomes is a passive mechanism that occurs at naturally occurring chromosomal breakpoints. Approximately 10% of all double stranded genomes are thought to integrate into host chromosomes in this way, and appear to passively target regions of transcriptionally active chromatin.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For cells in culture the containment and control measures will be exactly as described for infected mammalian cells in culture.

For mouse infections all work will be carried out in a containment 2 area within the biological services unit. Mice will be handled within MSC II cabinets and housed in individually ventilated (HEPA filtered) sealed cages.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:

1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed – Their GM authorization is GM898) according to disposal notification GM105/4.1.
Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from Class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor’s facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed – Their GM authorization is GMGM898) according to disposal notification GM105/4.1. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The local genetic modification safety committee approved the risk assessment at a meeting on Thursday 12th March 2020

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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</table>
## Project Additional Information

### Purposes of the contained use

Studying the roles and regulation of cell signalling proteins.

### Recipient or parental organism

Mammalian cell cultures.

### Host/vector system

E1-deleted, replication-defective adenovirus vectors. The viruses will be generated from transfected plasmid DNA constructs, and grown, in cells that complement the E1 deficiency, e.g. HEK293 cells.

### Origin & function

The cell signalling proteins to be expressed will be derived from human or other mammalian species. In addition to the wild type forms, constitutively active or dominant negative mutants may be generated. They will have various roles (which are under investigation) in cell signalling pathways. The risk assessments included with this notification relate to Rho family proteins, and phosphoinositol-3 kinase (P13 kinase); as detailed in the risk assessments these are regulators of cell movement and proliferation.

(Risk assessments for other cell signalling proteins expressed in similar adenovirus vectors have been approved by the GMSC as class 1; these include phospholipase D, Arf family (GTPases, PIP kinases). Similar adenovirus vectors encoding other cellular signalling proteins may also be generated and used in this connected programme of work; it is our understanding that these would not require further notification to HSE if the GMSC agrees on the basis of risk assessments that there is no significant increase in the potential hazard.)
The cell signalling proteins that are to be expressed will have a variety of roles in the regulation of various cellular responses, eg inflammation, cell survival, control of apoptosis, proliferation, migration. For proteins of negligible apparent hazard, the viruses are classified as class 1. Viruses categorised as class 2 will express cell signalling proteins that have some association with harmful processes, eg. some may have been associated with a role in oncogenic transformation; however a role in cancer would only be in conjunction with many other oncogenic events, and so the degree of hazard from their transient expression in an E1-deleted adenovirus is only modest.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

autoclaving is preferred means of decontamination (effectively 100% kill). Liquid waste is autoclaved and disposed to drains. Disposable solid waste is also autoclaved before being removed as clinical waste, with final disposal by incineration.

The exceptions to the above are:
"Sharps", including scalpel blades, needles and disposable tips for micropipettors may be decontaminated by drawing up 1% Virkon disinfectant and soaking in Virkon for a minimum of 120 minutes. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above. Virkon has effectively 100% kill rate.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

here was discussion regarding the boundary between class 1 and 2 risk assessments, reaching agreement with that shown on the risk assessments. Points of detail have been clarified, and the final risk assessments modified in line with these discussions.

Project Containment

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Project Additional Information

The reversible phosphorylation of tyrosine residues on proteins serves as a critical switch in the regulation of fundamental cellular processes including growth, adhesion and movement, and is controlled by the antagonistic actions of protein tyrosine kinases and protein tyrosine phosphatases (PTPs). PTPs function as critical "off" switches, antagonising oncogenic kinase signaling, and can initiate signaling cascades in their own right. The molecular mechanism of tyrosine phosphatase catalysis has been well characterised, however, the extracellular regulation and relevant substrates of many receptor tyrosine phosphatases have yet to be discovered. Recent technical developments in areas such as gene editing, organoid culturing, proteomics, and next generation sequencing mean that now is the perfect time to reveal physiological roles and regulatory mechanisms of this important protein family. We aim to understand the functional role of PTPs (specifically PTPRL) in the modulation of signal transduction, protein function and cellular/tissue responses; and their impact on physiological or disease states.

In order to ascertain such information, we will need to alter the expression of candidate genes involved in such processes, using knock-down or overexpression approaches; in addition, to manipulating their function using knock-in constructs, which harbour specific mutations, deletions and/or fusions. Although such manipulation (at least transiently) of cellular proteins structure or function in culture-adapted cell lines can be achieved using standard transfection protocols (lipofection-based, PEI or electroporation), lentiviral methods will allow long-term (stable) expression in difficult to transfect cell lines, and isolated primary cells and/or tissues.

Recipient or parental organism
Various established mammalian and human cell lines. Various primary mammalian and human cell lines. Murine tissues/explants cultured ex vivo.

Host/vector system
Lentiviral vectors based on replication-disabled HIV.

Origin & function
cDNA and/or shRNA offor genes involved in regulating the phosphorylation state of various proteins involved in signal transduction and/or the modulation of cellular function (including but not limited to members of the PTP (protein tyrosine phosphatase) family of proteins, specifically PTPR). Marker and reporter constructs that bear detectable tags for our gene of interest, such tags include but are not limited to GFP, c-myc, BirA and FLAG. Plasm ids involved in CRISPR-Cas9-mediated gene editing, which encode Cas9 variants, guide RNAs targeting our gene of interest and donor template DNA (containing a desired mutation, fusion or marker/reporter). Finally, we will target newly identified gene products that modulate or are a downstream target of protein tyrosine phosphatase activity.

Evaluation of foreseeable effects
We expect the majority of our constructs to increase decrease or otherwise alter protein phosphorylation, signal transduction and protein, cellular or tissue function; however, as the specific role of PTPs is ill-defined the exact outcome is uncertain. Despite this, PTPs are linked to regulating kinase signal transduction and therefore could possibly act as tumour suppressors. Conversely, we would not expect any cellular effects from the expression of markers or reporters.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Culture media will be disinfected with virkon or trigene
Leftover packaging cells and contaminated plastic ware will be discarded in a double bagged clear biohazard bag, sealed with red and white tape and placed in a Cfl2 waste bins for autoclaving

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
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Project Containment
GM Centre Number: 107

Data Premises Notified (Originally) 17/06/1988
Transferred from 1992 Regs? Y
Transitional Premises Class 2
Data Premises Closed Transitional Premises
Emergency Plan Required? N Non-GMMs Y
Withdrawn N

Name
UNIVERSITY OF READING

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

District

Town

County

Postcode

Country

Tel Number 01189 318888 Fax Number 01189 316765

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
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<tbody>
<tr>
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02/03/2022
### Levels of GMMs

- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)

### Non-microbial

- Other (please specify)

### Bacteriology
- Parasitology

### Virology
- Transgenic Animals

### Mycology
- Transgenic Invertebrates

### Other(s)

### Other (please specify below)
- Microbiology Research
- Transgenic Birds
- Gene Therapy
- Transgenic Fish
- Other (please specify below)

---

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 107/00.4

<table>
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THE GROWTH, SURVIVAL AND PATHOGENICITY OF SALMONELLA

Date Project Ceased: 16/08/2000

Non-GMM: not applicable

Consent Granted: Y

Project notified under transitional arrangements: Y

Historical Significant Changes: superceded by GM107/13.2

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref 107/01.1

Date Ackn’d 20/07/2001

Date Project Ceased 22/07/2021

CU2 Project Title STUDIES ON WILD TYPE MOUSE PRION PROTEIN

Class Culture Vol

Class 2 1-50 litres

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Withdrawn N

Tick if notifying a connected programme of work N
### Project Additional Information

#### Purposes of the contained use

Study of the function of the normal prion protein. This protein is found on the surface of all mammalian neurones, but its function is unknown, although increasing evidence suggests it may be involved in copper metabolism. We are conducting research into the possible roles of the protein using "normal" mouse prion protein as our model system. We express the protein in moderate quantity using a bacterial recombinant expression system. Following extraction, the protein is incubated with copper and its ability to bind the metal ion is measured. Part of our studies are to isolate antibodies to the protein to act as probes for the three dimensional structure of the protein. We expect to generate more data on the possible physiological roles of normal mouse prion protein. From this, we may deduce a mechanism for how the disease form of the protein causes the pathogenicity evident in the Spongiform Encephalopathies.

A broad range of agents that bind to the normal form of the prion protein may include some that have potential as diagnostics for the disease form so the generation of a panel of antibodies that bind to the wild type form is part of the proposed work.

#### Recipient or parental organism

The host for cloning the prion gene will be E.coli HB101 or E.coli XL-1 blue both non-colonising K12 derivatives.

For expression of wild type mouse prion protein, plasmids will be introduced into E.coli AD494 or BL21 (Novagen) for expression based on the T7 promoter.

#### Host/vector system

The recipient plasmid vector is a pET series vector (see www.novagen.com). The vector is based on a small multicopy non-mobilisable vector and carries the ampicillin resistance marker gene. Vector can replicate in most E.coli strains, although it is normally lost in the absence of antibiotic pressure. Expression of the prion protein cannot occur unless T7 polymerase is present through the use of specific E.coli recipients.

#### Origin & function

The incoming sequence is the Mouse prion gene (a allele) (Accession number MMPRP) - the complete coding region. No function, harmful or otherwise has been mapped to this gene product.

#### Evaluation of foreseeable effects

No harmful effects foreseen: cloning of prion protein gene sequence in E.coli XL-1 Blue or HB101 will not result in expression of the prion protein gene. Expression in E.coli BL21 or its derivative AD494 will result in prion protein accumulating in the cells, but these will be lysed after induction to release the protein for purification and study. No harmful effects have been ascribed to normal prion proteins, which are present in large quantities in the central nervous system of all mammals. Containment level 2 will suffice to prevent any dissemination of GMMs (including those residual recombinant E.coli actually containing the mouse prion protein that have escaped lysis) into the environment. Normal prion protein, unlike the protease-resistant isoform associated with "prion diseases" such as Bovine Spongiform Encephalopathy (BSE) or Creutzfeld-Jakob Disease (CJD), is fully sensitive to normal laboratory inactivation procedures.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste to be autoclaved. Waste will consist only of culture fluids and their containers. Autoclave operation routinely monitored for temperature/pressure achieved during
operation. 100% kill expected for routine operation. After autoclaving, waste to be disposed of in normal waste stream following this route of inactivation.

The advice in the Compendium of Guidance (Part 2A, Annex III) is clear that Containment level 2 is required, ie the activity is Class 2.

The Genetic Modification Safety Committee agreed with the assessment that the proposals present negligible risk to those involved, but took no note of this ACGM guidance, and agreed that Containment Level 2 was required.

**Project Containment**

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**Project Ref** 107/03.1

**Date Ackn'd** 17/09/2003

**Date Project Ceased** 05/01/2006

**Functional Analysis of Calicivirus Replication and Host Cell Interactions**

**Consent Granted** not applicable

**Historical Significant Changes** N
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

The human enteric caliciviruses are a major cause of viral gastroenteritis and as such pose a serious problem even in the developed world. A better understanding of how these viruses replicate and interact with their target cells is required so that we can better control outbreaks. Currently human caliciviruses cannot be grown in the lab hence in order to fully understand these viruses we need to use related animal caliciviruses that can be grown in the lab and genetically manipulated. This research aims to use the feline calicivirus as a model to dissect how the virus interacts with the host cell and how this interaction allows the virus to replicate.

Using a reverse genetic approach we are able to genetically manipulate the feline calicivirus genome to introduce mutations into the viral genes. The effects of these mutations will then be studied by looking at the phenotype of the mutant viruses. Using standard molecular approaches we also wish to identify regions of the virus genome that interact with host cell proteins and how this interaction controls genome translation and replication. The biochemical properties of calicivirus proteins will also be analysed in order to get a better understanding of how the virus replicates its genome - in particular we wish to understand how the viral RNA dependant RNA polymerase recognises the viral genome and subsequently replicates it to produce progeny virus.

We hope to achieve the following goals:
1) Understand how these viruses translate their genome into proteins by using reporter gene translation assays.
2) Characterise how the viral RNA polymerase replicates the viral genome by purifying the protein and biochemically analysing its various properties in vitro.
3) Develop cell based and cell free replication systems for these viruses to allow us to study the effects of mutations in viral genes.

Recipient or parental organism

Parental strains/ostrains to be used: Feline calicivirus (strain Urb) and human caliciviruses (HuCVs, ie Norwalk, Southampton and Manchester virus) - all ACDP HG2 pathogens.

Disabled E. coli strains DH5a, JM109 and BL21(DE3).

Disabled yeast strain EGY48 for use in yeast genetic screens.

Recombinant feline caliciviruses are unlikely to cause any greater harm than the parental strains as the mutations being introduced are likely to have a detrimental effect on replication (if any). 

Alterations of the major structural protein (orf2), thought to be main determinant of virus tropism [how these viruses infect a host cell], are specifically excluded from this project. (This exclusion however does not apply to the introduction of reporter genes into the orf2 sequence: such an insertion would replace the major part of the orf2 sequence and render the gene non-functional for virus particle formation).

In the cases where expression of calicivirus proteins occurs in E. coli or yeast, all the bacterial and yeast strains used are highly lab-adapted and carry mutations requiring the use of highly supplemented growth media. (Such strains cannot therefore survive outside the laboratory environment.)

Host/vector system

All plasmid vectors will be non-mobilisable pUC derivatives or pACT2 derivatives for propagation in yeast (non-mobiliseable, 2u origion of replication), all carrying antibiotic resistance or auxotrophic complementation markers.

Origin & function
Calicivirus genes both as altered and as mutated forms generated by PCR mutagenesis. All viral genes will be derived from either feline calicivirus (Strain Urbana from infectious virus or cloned cDNA) or from cloned non-infectious cDNA copies of human caliciviruses eg Norwalk or Southampton virus. All caliciviruses to be used are classified as category 2 pathogens - NOTE THAT THIS PROPOSAL SPECIFICALLY EXCLUDES THE USE OF RABBIT HAEMORRHAGIC DISEASE VIRUS (RHDV).

Specifically:
1) Human and feline calicivirus non-structural proteins, as both mature and precursor forms, the product of open reading frame 1 (orf1).
2) Human and feline calicivirus capsid (orf 2) and the minor structural protein encoded by orf 3.
3) Cellular proteins (of mammalian origin) known to interact with the calicivirus RNA genome. These include poly-C binding proteins, poly-A binding protein, poly-pyrimidine binding protein.

Reporter genes such as green fluorescent protein and its derivatives; luciferase and B-galactosidase.

**Evaluation of foreseeable effects**

Recombinant feline caliciviruses are unlikely to cause any greater harm than the parental strains as the mutations being introduced are likely to have a detrimental effect on replication (if any). Alterations of the major structural protein (orf2), thought to be main determinant of virus tropism [how these viruses infect a host cell], are specifically excluded from this project. (This exclusion however does not apply to the introduction of reporter genes into the orf2 sequence: such as insertion would replace the major part of the orf2 sequence and render the gene non-functional for virus particle formation).

In the cases where expression of calicivirus proteins occurs in E. coli or yeast, all the bacterial and yeast strains used are highly lab-adapted and carry mutations requiring the use of highly supplemented growth media. (such strains cannot therefore survive outside the laboratory environment).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Lab benches will be swabbed daily with Tegodyne. Disposable plastic culture vessels (etc.) will be used where possible and autoclaved after use and before discard. All contaminated glassware will be treated with Virkon before autoclaving. Unless disposable, glassware then washed and sterilised for further use.

Powdered Virkon will be used directly to treat virus spills, before mopping up with paper towels. (Towels then enter routine waste stream, and autoclaved before disposal). Liquid waste treated with Virkon, and then autoclaved before disposal to drain. Autoclave operation routinely monitored for temperature/pressure achieved during operation; temperature of 134 degrees used on routine discard cycle; 100% kill expected under conditions of operation. Autoclaved solid waste sent to landfill.

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The committee agreed that Containment level 2 was appropriate to minimise the risks from the proposed activity, and supported its notification to the Competent Authority.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
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<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
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<td>L2</td>
<td>L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

#### Project Ref 107/08.1

- **Date Ackn’d**: 08/02/2008
- **CU2 Project Title**: Molecular analysis of Staphylococcus aureus and Streptococcus pyogenes physiology.
- **Class**: Class 2
- **CultureVolClass2**: 1-50 Litres
- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

#### Project Additional Information

**Purposes of the contained use**

This project aims to identify components of S. aureus and S. pyogenes that allow each organism to persist in the host environment. By assaying mutants for loss of function using in vitro model conditions, the contribution of various components to survival under stress conditions can be determined. It offers the potential for discovery of novel drug targets and strategies for treatment and prevention of S. aureus and S. pyogenes infections.

**Recipient or parental organism**
Host/vector system

Suicide vectors: pAZIO6, pMUTIN, pAULA, pMAD for use in S. aureus and S. pyogenes.
Transducing bacteriophage for S. aureus: phili, phi85
Vectors for L. lactis: pKS8O, pNZ8037; Vectors for E. coli: pUC1 8, pET2I

Origin & function

Plasmids are to be constructed and maintained in E. coli. Stable chromosomal insertions are constructed in both S. aureus and S. pyogenes by transposon mutagenesis and insertional vectors using common protocols. Antibiotic and heavy metal resistance (Cadmium, Erythromycin, Spectinomycin, Kanamycin and Tetracyclin) which are commonly used in both S. aureus and S. pyogenes will be used to construct stable chromosomal mutations where appropriate.

Methicillin-resistant strains of S. aureus [MRSA] will not be used.
Various genes of S. aureus and S. pyogenes will be expressed in E. coli and Lactococcus lactis for purposes of protein overexpression. No toxin or antibiotic resistance genes will overexpressed.

Evaluation of foreseeable effects

All E. coli clones are in a standard disabled background. Therefore they pose a low risk. L. lactis is a non-pathogenic (food grade) species.
The S. aureus and S. pyogenes mutant strains created are all stable insertions or deletions creating mutations. These are likely, if anything, to render the organism less able to survive and proliferate. Insertions are marked by antibiotic resistance markers (Erythromycin, Spectinomycin, Kanamycin and Tetracyclin) which are commonly used both S. aureus and S. pyogenes. Both organisms are opportunistic pathogens and all strains will pose no greater risk than their respective parents.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Normal microbiological practices. All contaminated waste will be autoclaved; discard autoclave has pressure/temperature probes for in-cycle monitoring. This results in total kill with no risk. Waste will consist of contaminated plasticware and glassware; culture plates and culture media; treatment by disinfection will not be used as a sole means of inactivating any viable GM Ms.
Waste will be collected from laboratories by trained staff, who will be fully informed of any risks associated with contents of the waste being collected, Waste to be stored safely pending autoclaving if not autoclaved immediately after collection.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The members of the GMSC [known in the University as the Sub Committee for Biological Safety] agreed with the risk assessment for these proposals, and agreed that the project should be approved and notified to HSE.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
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<td>Human Clinical Applications</td>
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Project Ref  107/10.1

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<th>CU2 Project Title</th>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Date Project Ceased</th>
<th>Project notified under transitional arrangements</th>
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<tbody>
<tr>
<td>04/03/2010</td>
<td>Structure, Growth properties and identification of antivirals specific to enveloped RNA viruses</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Non-GMM Consent Granted</td>
<td>22/07/2021</td>
<td>N</td>
</tr>
</tbody>
</table>

Withdrawn N

Tick if notifying a connected programme of work N

Project Additional Information
### Purposes of the contained use

The purpose of this research is to understand how RNA viruses multiply in cells and form infectious particles for transmission with the aim of designing and testing new therapeutics to target these processes.

This study will investigate how virus particles are formed and devise new ways to prevent this from happening. How these viruses are able to grow inside cells will also be studied to determine precisely where and when the essential steps of the virus life cycle take place. Proteins from viruses will be expressed in mammalian cell lines by cloning into an expression plasmid in order to study the structure of virus-like particles by electron microscopy.

Using a reverse genetics approach Whale and mouse coronaviruses will be genetically manipulated to introduce mutations into the viral genes. The effects of these mutations will be studied by looking at the phenotype of the mutant viruses.

### Recipient or parental organism

#### Parental organisms:
- Arenavirus: Tacaribe, Pichinde, Junin Candid 1 vaccine strain
- Arterivirus: Equine arteritis virus
- Alphavirus - sinbis replicon
- Coronavirus, Human strains NL63, 229E, OC43, HKU1; Avian infectious bronchitis virus, Porcine TGE virus, Feline coronavirus and Whake SW1. Mouse hepatitis virus (temperature sensitive mutant)
- Torovirus: Eqire, Bovine and Porcine strains.

All viruses are ACDP HG2 with the exception of the vaccine strain of Arenavirus Junin. Junin Candid 1 vaccine strain is not listed on the ACDP list. Junin is HG4, however the vaccine strain has been classified as HG2 in the USA and has been successfully in the human population in Argentina - it is therefore proposed that this vaccine strain can be treated as HG2.

#### Recipient organisms:
- Escherichia coli K12 - Hazard group 1
- Human cell line HEK-293T - for virus and virus-like particle production
- Monkey cell line Vero-E6 - for virus growth and virus-like particle production
- Mouse cell lines DBt, 17Cl-1, L and OBL21a cells - for murine coronavirus growth

E. coli K12 is a highly lab adapted strain and cannot survive outside the laboratory environment. All mammalian cells are immortalised cell lines and cannot survive outside the laboratory environment.

### Host/vector system

- Non-infectious Sindbis virus replicase - for rescue of deletion mutants of equine arteritis virus.
- pCAGGS expression plasmid for the expression of viral proteins, cultured in both E. coli and mammalian cell lines.

Equine arteritis virus cDNA clone - for production of virions and virus-like particles.
- Mouse hepatitis virus cDNA clone - reverse genetic studies on replicase function.
- Whale coronavirus SW1 cDNA clone - reverse genetic studies on replication and pathogenesis.
CDNA clones are non infectious copies of the RNA genome.

Origin & function

For the production of Virus like particles in mammalian cells Arenavirus genes encoding Z, GP-C and NP proteins; Arterivirus genes encoding GP4 and GP2B proteins; Coronavirus genes encoding M, E, N, S and HE proteins and Torovirus genes encoding M, N, S and HE proteins will be cloned into pCAGGS. The virus like particles are not infectious.

Alphavirus (Sindbis replicon) and arterivirus will be co-transfected into a mammalian cell line to drive the expression of viable viruses.

Whale Sw1 and mouse hepatitis viruses will be mutated using a reverse genetics with debilitating mutations to the RNA replication machinery.

Alphavirus (Sindbis replicon) and arterivirus will be co-transfected into a mammalian cell line to drive the expression of viable viruses.

Evaluation of foreseeable effects

Virus like particles produced from the expression of arenavirus, coronavirus and torovirus are not infectious and are unlikely to cause any harm to the host.

The genetic modifications to MHV and SW1 coronaviruses will consist of:
1. Mutations to key replicative enzymes and motifs which are expected to attenuate virulence, and
2. Mutations which have been deliberately selected based on a debilitated or temperature-sensitive phenotype and engineered into infectious cDNA clones.

The hazards of GM strains should be less than for parental strains in both cases as the mutations introduced are likely to have a detrimental effect on replication.

Coronaviruses are highly host-restricted: MHV has only been found in mice and SW1 has only been found in one whale. Pathogenic MHV is known to circulate widely in Britain, and so these debilitated viruses are unlikely to survive in the wild in the event of an accidental release in competitions with the wild type. The university where this work will take place is not near and not directly connected with the ocean, hence accidental release of debilitated coronavirus SW1 is unlikely to spread the infection.

We will also produce both infectious and non-infectious equine arteritis virus particles using a Sindbis expression system in mammalian cells. Equine arteritis virus has, to date, only been detected in equids such as horses, and is unlikely to pose any health risk to humans. The viruses produced are less likely to produce infection in equids and there are no host reservoirs in the near vicinity of the campus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste will be autoclaved in a discard autoclave with the temperature probes for in-cycle monitoring. This results in total kill with no risk. Waste will consist of contaminated plasticware, culture plates and media. Treatment by disinfection will not be used as a sole means of inactivating any viable GMMs.

Waste will be placed in designated containers and collected from laboratories by trained staff, who will be fully informed of any risks associated with contents of the waste being collected. Waste will be stored safely pending autoclaving if not autoclaved immediately after collection.
Safety cabinets used for viral culture will be disinfected after use with Virkon and ethanol.

The members of the GMSC (known in the University as the Sub Committee for Biological Safety) asked for minor amendments to be made to the risk assessment. The committee agreed with the procedures specified in this risk assessment for this proposal and agreed that the project should be approved as a Class 2 project and notified to HSE.

### Project Containment

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<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2</td>
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<td>L4</td>
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</table>

- **Animal Units**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Large Scale Activities**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Human Clinical Applications**: L2 L3 L4 L2 L3 L4

### Project Ref 107/10.2

- **Date Ackn'd**: 27/05/2010
- **CU2 Project Title**: Site directed mutagenesis, sequence deletion and insertion experiments, using an infectious clone of tick-borne encephalitis virus
- **Class**: Class 3
- **Culture Volume Class 2**: 500ml
- **Consent Granted**: Yes

### Project Details

- **Date Project Ceased**: 22/07/2021
- **Withdrawn**: N
- **Historical Significant Changes**: N

**Tick if notifying a connected programme of work**: N
This project replaces GM151/05.1

**Project Additional Information**

**Purposes of the contained use**

Using genetic engineering techniques (point mutations and gene deletions) this project will try to produce TBEV viruses with reduced virulence in mice models with an aim of producing a potential live attenuated human vaccine.

The project also will attempt to alter the transmissibility of the TBEV (Siberian subtype) Vasilchenko. Strain between infected and non-infected ticks co feeding on mice by substitution genes or partial genes from the related TBEV (European subtype) Hypr strain.

**Recipient or parental organism**

**Parental Organisms:**
- Flavivirus: Tick borne encephalitis virus - strain Vasilchenko (Siberian sub-type) and strain Hypr (European sub-type)
- All viruses are ACDP Hazard Group 3.

**Recipient organisms:**
- Cell cultures (of porcine, human, hamster, mouse and tick origin) - Hazard Group 1
- Eschrichia coli AbleK, XL blue, DH5α and sure strains - Hazard group 1
- Spodoptera frugiperda cells (of insect origin) - Hazard group 1
- The E. coli strains used are highly lab adapted and cannot survive outside the laboratory environment.
- The S. frugiperda cells are immortalised cell lines derived from insect larva and cannot survive outside the laboratory environment.
- Other cellular lines of mammalian origin are common laboratory stocks with fastidious media requirements (listed as recipient organisms) and also cannot survive outside the laboratory situation.

**Host/vector system**

- Baculovirus AcMNPV (Hazard Group 1) - polyhedrin deleted mutant
- Plasmids pBR322, pUC118 and pTnEX1,1 - all non mobilisable small plasmids cloning vectors

**Origin & function**

The following are individual pieces of the coding region of TBEV that will be used to study individual protein structure and function; Capsid C, premembrane prM, membrane M, envelope E, nonstructural NS1, NS2A, NS2B, NS3, NS4Am, NS4B ans NS5, and untranslated regions 5'UTR of Vs and Hypr viruses. All are cloned into PBR322 or pUC118 plasmids or baculovirus AcMNPV.

These genes were derived from full length clones of the Vs and Hypr viruses. The infectious clones are cDNA copies of TBEV genomes that were previously contructed for the Vs and Hypr strains of the TBEV. The Vs infectious clone is called pGGVs and contains an entire copy of the Vs virus genome cloned in pBR322 vector.
The infectious clone for Hypr has not been constructed as a single-cloned molecule: instead the structural and nonstructural parts of the Hypr virus genome are cloned into two different plasmids both based on the pBR322 vector.

These plasmids are intended to be used to rescue the recombinant TBEV strains from infectious clone, with genetically modified individual genes listed above. The recombinant viruses will be constructed with the purpose to test the effect of the individual genes listed above on virus transmission in ticks.

The work with ticks will be carried out in Bratislava according to our long-term collaboration.

The genetically modified viruses will be used in cell culture to estimate the effect of individual genes (listed above) on the cytopathogenesis of TBEV and also for microarray analysis.

**Evaluation of foreseeable effects**

Experience gained from the early part of this work which was carried out in the Containment level 3 facilities at The Centre for Ecology & Hydrology, Oxford, where the applicant was formerly employed, is that the recombinant genetically modified viruses were never more virulent than parental viruses which are classified as ACDP Hazard Group 3 pathogens. All recombinant viruses rescued from the infectious clones previously showed either the same or a reduced replication rate in comparison with the parental viruses. Therefore no unusual adverse effects are expected in future experiments.

The natural host for the TBEV is the tick; the transmission of virus to humans in nature occurs by tick bite and this is not expected to occur in the laboratory setting (no ticks; no tick hosts in area).

The TBEV is not contagious for transmission between humans, it is not transmitted by aerosol or direct contact.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All contaminated waste will be autoclaved at 134 C for 30 mins in a discard autoclave with temperature probes for in-cycle monitoring. This results in total kill with no risk. Waste will consist of contaminated plasticware and pre-treated media. Treatment by disinfection will not be used as a sole means of inactivating any viable GMMs.

Waste will be pre-treated to a final concentration of 1% Virkon disinfectant and placed in designated containers, these will be transferred by the laboratory workers to the autoclave, loaded and the cycle started immediately. Records of cycle completion will be kept. A second autoclave is available as back-up.

Class I microbiological safety cabinets used for viral culture will be disinfected after use with 1% Virkon and 70% ethanol and will be fumigated prior to maintenance.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y
The members of GMSC (known in the University as the Sub Committee for Biological Safety) noted that this project had previously been approved by the Centre for Ecology and Hydrology GM committee and notified to the HSE and asked about arrangements for fumigating the containment level 3 laboratory and waste disposal.

The committee agreed with the results of the risk assessment for this proposal and agreed that the project should be approved as a Class 3 project and notified to HSE for consent.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Laboratory Activities

- Animal Units
- Large Scale Activities
- Human Clinical Applications

Project Ref 107/12.1

<table>
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<tr>
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<th>CU2 Project Title</th>
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<tbody>
<tr>
<td>12/07/2012</td>
<td>Effect of ginsenosides on neuronal activity</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
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</table>

Date Project Ceased

- Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

Project Additional Information
### Purposes of the contained use

The scope of the project is to study biologically relevant ginsenosides or metabolic derivatives in electrophysiological models of neuronal activity, to identify any effects on this activity that might correlate in any way to cognitive effects. Genetically modified human neuronal cells (ReNCell VM) will be used to establish an electrophysiological model of neuronal activity by culture on multi-electrode arrays (MEAs) enabling recording of electrical activity in response to the ginsenosides.

### Recipient or parental organism

| ReNcell VM cell line derived from human brain tissue |

### Host/vector system

ReNcell VM is a commercially available cell line derived from human foetal ventral mesencephalon and have been immortalised by the manufacturer by the incorporation of the v-myc oncogene by retroviral transduction with the murine moloney leukemia virus. The maternal donor has been screened for HepB, C and HIV and the risk of infection is considered to be low.

### Origin & function

No further modification of this cell line is proposed. Pre-modified cells will be screened for electrical activity in response to ginseng extract.

### Evaluation of foreseeable effects

No further genetic modification will occur. The neuronal cell line has been immortalized by the incorporation of the v-myc oncogene. Removal of growth factors

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid waste and contaminated glassware and laboratory plastics (pipette tips etc) will be treated with 2% Trigene for 1 hr prior to autoclave treatment at 134 C for 30 mins in a discard autoclave with temperature probes for in cycle monitoring. This results in total kill with no risks. Treatment by disinfection will not be used as a sole means of inactivating waste.

The special electrode culture dishes will be treated with 2% trigene for 1hr and will then be treated with 70% ethanol, cleaned with detergent and sterilized for re-use, in line with manufacturers instructions. These dishes cannot be autoclaved.

**Is an emergency plan required according to regulation 20?** N

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

**Tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
The panel met with the principal investigators on the 14th of June 2012 and the panel were talked through the proposal.

BW explained that the funding body did not support research that was animal based and a human cell lines was needed for this study. Un-modified neurons do not survive for the extended culture periods required for these studies and so a genetically modified immortalized cell line would be required.

The host cell line is an undifferentiated neural cell line taken from foetal neural tissue. The maternal blood has been screened for HIV and other blood borne viruses, however, the cells themselves have not been screened and there is a low possibility that the cell lines could contain infectious material. The panel agreed that a precautionary approach should be used and Containment Level 2 facilites and that a no-sharps policy would be required.

The vector is a Mouse moloney leukaemia virus retroviral vector. Wild type MMLV is classified as ACDP Hazard Group 1. It is not clear, in the published literature or the information provided by the manufacturer whether this vector is replication deficient or whether the normal MMLV env gene has been replaced with an amphotropic env gene which can allow infection of human cell lines. Thus a cautious approach should be followed with a class 2 GM classification.

The gene insert is the human v-myc oncogene. Whilst no naked DNA will be used in this project there is a low risk of a tumour formation in the event that these immortalised undifferentiated cells are inoculated into the worker. The panel agreed that this cell line would require Containment Level 2 facilities and that a no-sharps policy would be required.

In light of these factors the technical review panel agreed that the proposal should be classified as a Class 2 GM activity.

Control measures BW and IS talked the panel through the control measures that will be implemented. Work will be carried out in a class II microbiological safety cabinet the Hopkins Containment Level 2 laboratories. No sharps (needles, scalpels or glass pipettes) will be used when working with this material. Solid Waste will be treated with 2% trigene for 2hrs before autoclaving). Liquid waste will also be treated with 2% trigene (final concentration) before autoclaving. The special culture plates will be disinfected with 2% trigene before washing and sterilisation with ethanol.

The panel agreed that these measures are proportionate to the level of risk and recommend approval of this project as a GM Class 2 activity to the SCBS, subject to minor modifications to the risk assessment. The GM safety committee agreed with this review.

**Project Containment**

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<td>L2</td>
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**Project Ref** 107/13.1

**Date Ackn'd** 18/04/2013

**CU2 Project Title** Regulation of Neuronal and Cardiovascular cell Functions by Signal Transduction

**Class** Class 2

**Culture Vol Class** < 1 Litre

02/03/2022 Page 3132 of 15326
**Project Additional Information**

**Purposes of the contained use**

The overall aim of this programme is to discover the molecular pathways that control cardiovascular and nervous systems and their development. We are particularly interested in how certain proteins present within cells generate signals to control cell function. By elucidating the molecular mechanisms we can start to understand how the body works and what occurs during inflammation, cardiovascular and neurodegenerative diseases and in cancers. This will also allow us to find new therapeutic targets for the treatment of human disease.

The use of genetically modified microorganisms (GMMs) is essential if the goals of this research are to be realized.

**Recipient or parental organism**

Recipient organisms will include mammalian and primate cell lines from established culture collections such as epithelial cells (e.g. HEK293, CHO) and endothelial cell lines (e.g. HMEC-1).

In addition primary neuronal, cardiac and vascular cells and stem cells from rats and mice will be used. These host cells pose no threat to human health.

**Host/vector system**

**Adenovirus vector System**

The vector system is derived from the human adenovirus. The Early Region (ER) viral gene, which is essential for replication and transcription, has been deleted to make the virus safe. The E3 gene has also been deleted to allow insertion of genes of interest. The vector system consists of two plasmids. The first is a shuttle vector (pShuttle) into which the gene of interest is inserted. The second (pAdEasy-1) contains the adenovirus genome. Then using the efficient homologous recombination machinery of E. coli (e.g., BJ5183), the gene of interest is then inserted into the adenovirus genome containing plasmid. The resulting plasmid can then be used to generate infection-competent adenovirus.

Commercially available plasmids will be used e.g., pAd easy-1 and pShuttle (Stratagene) or p:E1sp1A. The plasmids are non-mobilisable themselves pose no threat to human health.

The virus produced will be a virus competent for infection, but not for replication. To become replication competent, the virus would have to recombine with wild-type virus. The probability of reversion to wild-type virus is expected to be negligible.
Adeno-associated Virus System

The vector system is derived from adeno-associated viruses, which are defective paroviruses and depend on essential helper functions provided by other viruses, such as adenovirus virus, for efficient viral replication and propagation. However E2A, E4, and VA RNA, cap and rep). The plasmids are non-mobilisable themselves pose no threat to human health.

Infection competent but replication incompetent adeno-associated viruses will be assembled by lipid-mediated transient transfection of HEK293 cells (or derivatives there of). An essential gene (E1) for the assembly of infection competent virus is expressed by HEK293 cells.

The virus produced will be a virus competent for infection, but not for replication. The virus particles are highly stable and thus, even low amounts should be identified and decontaminated appropriately. The risk to human health is low.

Lentivirus System

The vector system is derived from the human immunodeficiency virus (HIV-1). The virus has been stripped down to its minimum essential components, which have been encoded on four separate vectors. To inactivate the virus, in the unlikely event of recombination of the four plasmids, the vector is self-inactivating. This is achieved by the deletion of genetic sequence from the 3’ long terminal repeat encoding enhancer and promoter functions, resulting in the transcriptional inactivation of the provirus in the infected cell. The threat to human health is negligible.

Alternatively, commercially available plasmids will be used, that will serve the same purpose. The plasmids are non-mobilisable themselves pose no threat to human health.

Retro-X system (Clontech): The viral vector system is derived from the Moloney murine leukaemia virus (MMLV). The vectors are self-inactivating bicistronic expression vectors designed to express a target gene along with an antibiotic selection marker without the risk of promoter interference from the 5’ LTR. The self-inactivating feature of the vectors is provided by a deletion in the 3’ LTR enhancer region (U3). During reverse transcription of the retroviral transcript in the infected cell, the inactivated 3’ LTR is copied and replaces the 5’ LTR, resulting in inactivation of the 5’ LTR promoter (CMV).

Infective competent but replication competent lentiviruses will be assembled by lipid-mediated transient transfection of four plasmids into the packaging cell lines, including HEK293-based (HEK293T (human embryonic kidney cells, HEK293FT) and NIH/3T3-based.

The virus produced will be a virus competent for infection, but not for replication. The virus has been pseudotyped to utilize the envelope protein from the vesicular stomatitis virus glycoprotein (VSV-G). This is common practice with lentiviruses and will make the virus amphotropic i.e. broad tissue tropism. It is expected that host-defence mechanisms would be effective against this replication incompetent virus. The probability of reversion to wild-type virus is expected to be negligible.

Human and mammalian genes from the following categories:

A) Cell-surface receptors e.g., G protein-coupled receptors such as protease-activated receptors, cannabinoid receptors and neuropeptide receptors

B) Ion Channels e.g., transient receptor potential channels, voltage-gated ion channels, potassium channels.

C) Transporters e.g., glutamate transporters.

D) Regulatory proteins e.g., glutamate transporters.

E) Signalling proteins e.g. extracellular-regulated protein kinases, ROCO proteins.

F) Peptidases e.g., trypsins, endothelin-converting enzyme-1, matrix metallopeptidases.

G Regulators of cell cycle and survival e.g., B1, Bcl2

H) regulators of gene expression e.g., RNA processing factors, post-transcriptional regulators (e.g., exon junction complex proteins).

I) Control proteins e.g., fluorescent proteins i.e. GFP, YFP, RFP.
| J) Focal adhesion, cytoskeletal, metabolic, circadian and nucleocytoplasmic shuttling proteins. |
| K) Proteins involved in interpreting the cellular microenvironment e.g. integrins, Roc, Rho. |

**Evaluation of foreseeable effects**

Bacteria: The plasmid vectors are non-mobilisable and therefore are unlikely to escape from the host cells. Since the inserted plasmid DNA is under the control of the CMV promoter, the inserted gene will not, in any case be expressed by the bacteria. For those bacteria that are used to produce proteins, these will require IPTG to induce expression and hence these proteins will not in any case be expressed by the bacteria. For those bacteria that are used to produce proteins, these will require IPTG to induce expression and hence these proteins will not be expressed outside of the laboratory environment. The pathogenicity and toxicity of the bacteria to humans will not be altered.

Viral vectors: All viral vectors are infectious but have been modified to be replication deficient. The likelihood of ability to cause harm to humans is low.

Mammalian cells/ tissues: These cells and tissues would already express many of the genes to be introduced. The cells may become hyper-responsive to certain agonists or have exaggerated signalling responses. The likelihood of this changing the pathogenicity or toxicity to humans is negligible.

The genetically modified viral vectors are replication deficient and would not be able to be transmitted in the environment. The bacteria and final host cells/tissues are unable to survive outside the laboratory environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste will be autoclaved in a discard autoclave with temperature probes for in-cycle monitoring. This results in total kill with no risk. Waste will consist of contaminated plasticware, culture plates and media. Treatment by disinfection will not be used as a sole means of inactivating any viable GMOs. Waste will be pre-treated with 5% Biocleanse solution before being placed in designated containers and collected from laboratories by trained staff, who will be fully informed of any risks associated with contents of the waste being collected. Waste will be stored safely pending autoclaving if not autoclaved immediately after collection.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
The proposal was reviewed by a technical review panel on behalf of the SCBS (acting as GM committee). The reviewers agreed with the concept of a connected programme constituting an umbrella proposal and risk assessment with each Principal Investigator submitting a more specific application to join the project to be assessed by the GM committee.

The reviewers agreed that due to the route of infection of adenoviruses being aerosol, there was a need to control aerosols using microbiological safety cabinets. The reviewers agreed that this work constituted class 2 GM activity subject to the following revisions to the risk assessment:

- Risk assessment needs to clarify exclusion of sharps
- Recommendation to wear safety glasses to prevent mucocutaneous transmission.
- Procedures for centrifuges need clarification (specify the opening of centrifuge buckets and rotors in safety cabinet).

The reviewers discussed the intended work with lentiviral vectors, and concluded as the vectors have an extended host range due to VSV-G (i.e., could deliver to human cells e.g., through broken skin or eyes), and due to the relatively high titers proposed, classification of Class 2 would be appropriate. The need identified to restrict use of sharps and use of gloves to prevent percutaneous/mucocutaneous transmission results in classification of class 2.

Subject to minor modification and clarification, the reviewers recommended the SCBS committee approve this project as a Class 2 activity. The SCBS committee agreed with this approval.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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</tr>
</thead>
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<td>L2 L3 L4</td>
</tr>
<tr>
<td>Human Clinical Applications</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

### Project Ref 107/13.2

- **Date Ackn'd**: 26/11/2013
- **CU2 Project Title**: Studies of aspects of key bacterial pathogens affecting virulence and environmental survival

### Class CultureVolume

- **Class CultureVolumeClass2 CultureVolumeClass3-4**
  - Class 2
  - < 1 Litre
  - Non-GMM Consent Granted

### Project notified under transitional arrangements

- **N**

### Significant Change ID

- **107/13.2a**
Project Additional Information

Purposes of the contained use

The overall aims of this connected programme are:

Metal metabolism and pathogenicity.
Since iron uptake and control are key aspects of bacterial survival and pathogenicity, this aspect will focus on potential (and defined) iron transport and iron homeostasis genes in B. cepacia, P aeruginosa and P. syringae, E. coli O157 (stx-), S. typhimurium and S. gallinarium and E. coli K-12. Modifications will involve gene knock out by replacement or integration, generation of gene fusions to study expression, topology, interaction, and protein function. Some modifications will be plasmid based, others will be chromosomally integrated. Modified genes will be assembled in plasmid vectors (using E. coli K-12) before re-introduction into the source strain. Expression in response to metals and metal-regulators will be studied in E. coli mutants lacking key regulators (e.g. Fur) as well as in the original host strains. Genes will also be introduced into plasmids to enable complementation of mutants and for gene amplification. A range of common/standard antibiotic-resistance markers will be employed and inserted into the above strains. Effects of mutations on pathogenicity and/or survival will be examined in tissue culture, by invertebrate infection, by gut-model colonisation, by soil colonisation or by plant colonisation.

Plant/soil colonisation.
E. coli O157 is a potentially deadly foodborne pathogen. Infection is often achieved through contamination of food plants. E. coli O157 (and related pathogens) is able to colonise, survive and propagate on crop plants which mediates its maintenance within the herd and enhances transmission to the human host. E. coli O157 (stx-) mutants will be used to study colonisation factors on a number of plant systems. In addition, directed mutations will be generated in candidate plant colonisation genes. Phenotypes will be confirmed by complementation. Plant-adapted strains will also be subjected to targeted gene knockout and transfer of candidate-plant-colonisation genes to non-adapted strains. Mutations in metal metabolism genes will be studied. EAEC and Salmonella plant colonisation experiments will also be performed, as above.

Recipient or parental organism

E. coli O157 stx deletion mutants, Entericaggregative E. coli strains, S. typhimurium, S. gallinarium, S. enterica complex.
Burkholderia cepacia complex
Pseudomonas aeruginosa, Pseudomonas syringae, Pseudomonas fluorescens
Environmental isolates including Erwinia, Xanthomonas, Pantoea and Citrobacter species.

Host/vector system

A range of broad host vectors will be utilized in this project for cloning together with plasmids for reporter and expression studies

Origin & function

Various genes from the species listed above which are suspected of influencing the pathogenicity of bacteria or their ability to survive in the environment.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
For limited experiments a plant growth room will be required. In order to autoclave the solid waste the autoclave in a nearby building (<10 meters away) will be utilized. Waste will be transferred in sealed secondary containment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste will be autoclaved in a discard autoclave with temperature probes for in-cycle monitoring. This results in total kill with no risk. Waste will consist of contaminated plasticware, culture plates and media.

Treatment by disinfection will only occur in limited circumstances where re-usable containers cannot be autoclaved. Containers will be immersed in Virkon at a final concentration of 2% for 16-18 hrs.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The proposal was reviewed by a technical review panel on behalf of the SCBS (acting as GM committee). The project to be assessed by the GM committee.

The review panel thought that the use of a E. coli 0157 mutant was justified and that the deletion of the stx from the strains (confirmed by sequencing) was consistent with a class 2 designation.

The panel agreed that none of the four projects targeted known virulence factors and there were no plans to enhance pathogenicity.

The review panel noted that the bacteria isolated from the environment would be isolated and characterised prior to any GM activity and that no enrichment for human pathogens would take place.

The panel discussed the infection models proposed with the principal investigators in some detail and agreed that the control measures were appropriate.

Subject to minor modification and clarification the reviewers recommended the SCBS committee approve this project as a class 2 activity. The SCBS committee agreed with this approval.

Project Containment

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The overarching aim of our research is to understand the molecular pathways that occur in haematopoietic and endothelial cells to help us to understand their role in haemostasis, thrombosis, inflammation and vascular development. This research is hampered by the absence of a nucleus in platelets and the difficulty to transfect haematopoietic and primary endothelial cells with exogenous DNA constructs using standard molecular biology procedures. To overcome this obstacle we propose using the lentiviral system (third generation defective) to drive infection as an alternative to transfection. We will also use this approach in endothelial cells due to the high levels of infection which can be achieved. We will use this approach to express fluorescently tagged wild type proteins of interest, wild type and mutant proteins of interest and to knock down the expression of specific gene-products using RNA-interference, so that we can address the important roles of individual proteins involved in haematopoietic and endothelial cells. This programme covers the generation of lentivirus both to ectopically express protein as well as to knock down protein expression in both human and mouse derived haematopoietic cells or other primary cells.
Human cell lines (e.g. Dami, Hel, Meg-01) will be transduced with lentiviral particles to assess protein knock down and to investigate function of expressed full length or mutated proteins.

Primary vascular and lymphatic endothelial cells (ECs) and related cell lines (e.g. HMEC-1 and MCEC-1) will be transduced with lentiviral particles to assess protein knock down and to investigate function of expressed full length or mutated proteins.

Host/vector system

A third generation lentiviral system will be used. This is a four plasmid system. It is considered highly unlikely that recombination among all the vectors to generate replication competent viruses will occur. (Reference: A third generation lentiviral vector with a conditional packaging system, Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L. J Virol. 1998 Nov; 72(11):8463-71).

Risk of regenerating replication competent virus or the parental virus (HIV). In third generation lentiviral vectors all the regulatory/accessory proteins (Rev, Tat, Vit, Vpr, Vpu and Net) are removed, except for Rev. Rev acts at the post- transcriptional level and is necessary for HIV gag/pol expression. Rev binds to an RNA motif (Rev responsive element [RRE]) and facilitates the cytoplasmic export of gag/pol messenger. As an extra safety measure Rev is placed on a separate vector (RSV-Rev) from the transfer vector. Only 700 bp of the HIV envelope protein are present in the transfer vector, plus RRE and the packaging signal. The packaging construct contains the minimal RRE of 374 bp and the gag/pol genes. Through deletions in both L TR's and the absence of 5 of the 6 accessory proteins, including the replication essential Tat it is highly unlikely that replication competent virus is produced. In published studies replication competent virus has never been detected. The HIV-envelope protein is replaced by non-retroviral envelope protein (VSV-G). It is highly unlikely that this will be incorporated in a new hybrid replicative virus. It is impossible that wild-type HIV will be formed, because of the omission of 5 accessory proteins and HIV-env. The lentiviral vectors to be used in this study are third generation lentiviral vectors purchased from Addgene and Biosettia.

Origin & function

Fluorescent tags derived from jellyfish and coral (Section A).

Mouse and human genes of interest will be cloned from either previously cloned cDNA or form sourced IMAGE clones of wild type sequences. We will use wildtype and mutant forms of the proteins, which may be truncated or contain point mutations (Section B).

Short hairpin (shRNA) will be designed to knock down the expression of genes of interest. Clones will be purchased from Open Biosystems or other commercially available libraries (Section C).

Section A

Green Fluorescent Protein (GFP) and derivatives from Aequorea coerulescens
OsRed and derivatives from Discosoma Sp.
mEos2, a derivative from Eos from Lobophyilia hemprichii

These molecules serve as reporters and change in fluorescence upon exposure to various wavelengths of light

Fluorescent bioprobes which enable the visualisation of signalling events.

Section B

Human and mouse wild-type and mutant forms of:
The lectin receptor CLEC-2: the receptor for endothelial cell protein Podoplanin
Haematopoetic receptors: including GPVIIIFcRy, FcyRIIA, G6f, G6f-like
Actin regulatory proteins: Involved in the regulation of the morphology of haematopoietic and endothelial cells.
Signalling proteins (e.g Syk, Tec family kinases, SLP-76, PLCy2) are implicated in the assembly of signalling
complexes and function of haematopoietic cells and endothelial cells.
Adapter molecules (e.g. Spred family proteins) may be implicated in receptor signal transduction in haematopoietic 
and endothelial cells.
Inhibitory signalling molecules: Protein implicated in the inhibition of cell function such as phosphatases or ITIM 
bearing receptors
Podoplanin, ERM family of proteins, Small GTPases: Podoplanin is the endogenous ligand for the platelet receptor 
CLEC-2 and has been reported to promote cell migration through its interaction with small GTPases and ERM family 
members.
Integrins: They are involved in cell adhesion and signalling
Focal adhesion associated proteins: These provide a structural link between the actin cytoskeleton and the 
extracellular matrix.
Receptor kinases and their ligands (e.g. Eph kinases and ephrins)

Evaluation of foreseeable effects

Viral vectors: viral vectors are infectious but have been modified to be replication deficient. The likelihood of ability to 
cause harm to humans is low.
Mammalian cells: These cells would already express many of the genes to be introduced/knocked down. The 
likelihood of this changing the pathogenicity or toxicity to humans is negligible and is not thought to result in increased 
cell proliferation.
The genetically modified viral vectors are replication deficient and would not be able to be transmitted in the 
environment. The bacteria and final host cells are unable to survive outside the laboratory environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The proposed location for this work is in two buildings, the Harborne building and the Lyle building. These two 
buildings are connected by an internal bridge corridor. The Lyle building does not house its own waste autoclave and 
the intention is for waste to be transported in sealed containers from the Lyle 1st floor to the Harborne ground floor 
waste autoclave via the internal corridors.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste will be autoclaved in a discard autoclave with temperature probes for in-cycle monitoring. 
This results in total kill with no risk. Waste will consist of contaminated plasticware, culture plates and media. 
Treatment by disinfection will not be used as a sole means of inactivating any viable GMMs.
Waste will be pre-treated with 2% BioCleanse solution before being placed in designated containers and collected 
from laboratories by trained staff, who will be fully informed of any risks associated with contents of the waste being 
collected. Waste will be stored safely pending autoclaving if not autoclaved immediately after collection.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
The committee reviewed the risk assessment in January 2016 - the committee thought the risk assessment was very well thought through and approved subject to minor amendments (use of bioclense rather than trlgene as a disinfectant). The committee thought the classification was borderline class 1/2 but that it warranted class 2 due to the relatively high titres, the need for gloves and no sharps policy.

**Project Containment**

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 107/21.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Consent Granted</th>
</tr>
</thead>
<tbody>
<tr>
<td>05/03/2021</td>
<td>Characterisation of Cancer Electrophysiology and the Evolution of Brain Metastases</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

- **Project notified under transitional arrangements**
- **Withdrawn**

**Project Additional Information**
**Purposes of the contained use**

The project aims to simulate the evolution of human brain metastases in vitro, using different cancer cell lines representing types of cancer that commonly metastasise to the brain. To study this evolution, we will culture these cell lines in hippocampal brain slices, the environment a metastasis would have to adapt to. Cells will be re-cultured this way repeatedly to establish successively evolved generations. Studying the evolution of these cells can give us more insight into the necessary adaptations the cells must have in order to metastasise to the brain. This characterisation will involve electrophysiology, calcium imaging (2photon) and can be later matched with genomic data and lead to new targeted therapies specific to cancer type.

In order to study the cancer cell electrophysiology whilst in the slices as well as retrieve them after culturing, the cells must be permanently tagged. For this reason we will need to create genetically modified populations of cancer cell lines, using lentiviruses containing a vector for red fluorescent protein (RFP), a fluorescent tag. Lentiviruses can be used to insert a desired gene into a cell population with long-term gene expression. The stability will mean the cells can be transfected once to last during the long-term evolution of the cancer cells. Cancer cells from a selection of cell lines will be the main tools of the project and will be transfected only to express RFP as all other functions and morphology are to be studied.

**Recipient or parental organism**

Mammalian cell lines (human): ACDP Category 1 or 2  
Lenti-X 293 (human embryonic kidney cells)  
Cancer cell lines including:  
U87-MG (human epithelial neuroglioma cell line)  
SK-MEL-28 (human malignant melanoa cell line)  
MDA-MB-231 (mammary gland adenocarcinoma cell line)  
MCF-7 (mammary gland adenocarcinoma cell line)  
A549 (lung carcinoma cell line)  
HT1080 (fibrosarcoma cell line)  
DU145 (prostate cancer cell line)

The inserted genes will be control proteins e.g., fluorescent proteins i.e. GFP, YFP, RFP, mCherry.

**Host/vector system**

**Description of Vector:**  
Expression vectors (CMV, RSV promotors) required for viral particle production including:  
Lentivirus (pCMV-dR8.74 packaging plasmid, pMD2.G envelope plasmid and pLenti6.3-CMV-mCherry2 transfer plasmid).

Plasmids do not contain mobilization genes and are rapidly lost upon removal of selecting antibiotic.

**Lentivirus:**  
1) The viral vector system is derived from the human immunodeficiency virus (HIV-1). The virus has been stripped down to its minimum essential components, which have been encoded on three separate vectors. To inactivate the virus, in the unlikely event of recombination of the three plasmids, the vector is self-inactivating. This is achieved by the deletion of genetic sequence from the 3' long terminal repeat encoding enhancer and promoter functions, resulting in the transcriptional inactivation of the provirus in the infected cell.

2) The viral vector system is derived from the Moloney murine leukaemia virus (MMLV). The vectors are self-inactivating bicistronic expression vectors designed to express a target gene along with an antibiotic selection marker without the risk of promoter interference from the 5’ LTR. The self-inactivating feature of the vectors is provided by a deletion in the 3’ LTR enhancer region (U3). During reverse transcription of the retroviral transcript in the infected cell, the inactivated 3’ LTR is copied and replaces the 5’ LTR, resulting in inactivation of the 5’ LTR promoter (CMV).

Mammalian cells/tissues: These cells will be modified to express a fluorescent tag which is not expressed naturally by these cells. However the likelihood of this changing...
the pathogenicity or toxicity to humans is negligible.

Origin & function

Lentivirus System:

GMMs required for Propagation of Plasmids
E. coli used for cloning and propagation of plasmids are standard, inherently safe and pose no threat to human health. Sub-strains of E. coli will include MACH1, DH5α, TOP10, GM2163, JM109, XL1-Blue, Stbl 3. The threat to human health is negligible.

Description of Vector System
The vector system is derived from the human immunodeficiency virus (HIV-1). The virus has been stripped down to its minimum essential components, which have been encoded on three separate vectors. To inactivate the virus, in the unlikely event of recombination of the three plasmids, the vector is self-inactivating. This is achieved by the deletion of genetic sequence from the 3' long terminal repeat encoding enhancer and promoter functions, resulting in the transcriptional inactivation of the provirus in the infected cell. The threat to human health is negligible.

Plasmids for virus particle production:
- pCMV-dR8.74 packaging plasmid (pCMVR8.74 was a gift from Didier Trono (Addgene plasmid # 22036; http://n2t.net/addgene:22036; RRID:Addgene_22036))
- pMD2.G envelope plasmid (pMD2.G was a gift from Didier Trono (Addgene plasmid # 12259; http://n2t.net/addgene:12259; RRID:Addgene_12259))

Plasmids required for gene expression:
- pLenti6.3-CMV-mCherry2 transfer plasmid (pLenti6.3 (Invitrogen) with the addition of mCherry2 gene).

Alternatively, commercially available plasmids will be used, that will serve the same purpose. The plasmids are non-mobilisable themselves pose no threat to human health.

Preparation of the Virus:
Infective competent but replication incompetent lentiviruses will be assembled by cationic polymer or lipid-mediated transient transfection of three plasmids into the packaging cell lines, including HEK293-based (HEK293T (human embryonic kidney cells)).

Description of Virus Produced:
The virus produced will be a virus competent for infection, but not for replication. The virus has been pseudo-typed to utilize the envelope protein from the vesicular stomatitis virus glycoprotein (VSV-G). This is common practice with lentiviruses and will make the virus amphotropic i.e. broad tissue tropism. It is expected that host-defence mechanisms would be effective against this replication incompetent virus. The probability of reversion to wild-type virus is expected to be negligible.

Description of Genes to be Expressed:
Genes to be expressed will be fluorescent marker proteins, ion channels, transporters and associated regulatory proteins. Some of these genes may have been modified to be fused to reporter systems to facilitate biochemical and microscopical detection e.g. by addition of epitope tags or fusion with fluorescent proteins. None of the genes inserted will be oncogenic or growth factors. Integration of the viral genome into the host genome is not specific.

Description of Final Host:
Host cells will include epithelial cells including HEK293 and cancer cell lines including U87-MG, SK-MEL-28, MDA-MB-231, MCF-7, A549, HT1080 and DU145. These host cells pose no threat to human health.

Evaluation of foreseeable effects

A) On human health:
Lentivirus System
On escape to the environment, the VSV-G viral envelope will enable the virus to infect all cell types it comes into contact with, including skin cells and cells of the respiratory tract. However, as the virus is replication incompetent, there is potential for one round of infection only. Thus, the risk to human health is low.

B) On the environment:

Lentivirus System
The virus can potentially infect all animals it comes into contact with. However, as the virus is replication incompetent, there is potential for one round of infection only. Thus, the risk following release to the environment is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste (liquid, sharps and solid) will be placed in Biocleanse solution (3-5% final volume; located inside the cabinet) for 1-2 hours. All waste will then be autoclaved (121 C, 30 min, 15 psi) before disposal. Autoclaved liquid waste will then be disposed to drain. Biocleanse is a non-irritant, non-toxic, non-corrosive and non-hazardous bactericidal, fungicidal, virucidal, mycobacterial and sporicidal agent.

Expected degree of kill - Autoclaving waste should kill 100% of GMM's.

Spillages will be wiped up immediately and tissues disposed by autoclaving. The affected area will then be wiped with Biocleanse (diluted 5% with dH2O) followed by 70% alcohol.

Major spillages inside cabinets may require the cabinet to be fumigated (to be arranged via Building manager/AHSC).

(AHSC = Area Health and Safety Coordinator)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment was reviewed by the Sub-Committee on Biological Safety (acting as GMSC) at it's Meeting on 23rd Jun 20. The committee noted that Sections 3a.6 and 3a.12 required completion and also the risk assessment required full sign off. These sections were completed post this meeting. The completed signed off risk assessment was approved, subject to HSE notification at it's meeting held on 8th Oct 20.
**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Laboratory Activities**
- Glass Houses
- Growth Rooms

**Animal Units**
- Large Scale Activities
- Human Clinical Applications

---

**Project Ref** 107/95.1

**Date Ackn'd** 06/10/1995

**CU2 Project Title**
GENETIC MANIPULATION OF MYXOVIRUSES

**Date Project Ceased** 01/05/2007

**Class**
- Class 2

**CultureVolumeClass2**
- not applicable

**Non-GMM Consent Granted**

**Project notified under transitional arrangements** Y

**Historical Significant Changes**
GM107/00.6, GM107/02.1, TRANSFERRED TO GM 77 1/5/07.

**Historical Date of Additional Info**
15/08/2000, 09/09/02

**Significant Change ID**
107/02.1a

**Date of Significant Change**
19/10/2007

---

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 107/99.4

Date Ackn'd 02/03/2022  
CU2 Project Title  
Class  
CultureVolClass2  
CultureVolumeClass3-4
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**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**
- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 107/trans1

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<tr>
<td>30/01/2001</td>
<td>&quot;ASSEMBLY OF THE F1 CAPSULAR ANTIGEN OF YERSINIA PESTIS&quot;</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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02/03/2022

Page 3150 of 15326
Project Ref 107/trans2

Date Ackn'd  30/01/2001
Date Project Ceased

CU2 Project Title
"CLONING, MUTAGENASIS AND GENOMIC REPLACEMENT OF GENES INVOLVED IN EXTRACELLULAR SECRETION FROM AEROMONAS SALMONICIDA"

Class  Class 2
CultureVol 0

Consent Granted  not applicable

Tick if notifying a connected programme of work  N
Project notified under transitional arrangements  Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

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Please enter comments on the GM safety committee on the risk assessment

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**Project Additional Information**

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment.

**Project Containment**

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**Project Ref 107/trans5**

- **Date Ackn’d:** 28/03/1995
- **CU2 Project Title:** OMPA GENES OF AEROMONAS SALMONICIDA AND SURFACE EXPRESSION VECTORS
- **Class:** Class 2
- **Consent Granted:** not applicable
- **Project notified under transitional arrangements:** Y

**Historical Significant Changes:**

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**Significant Change ID**

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref** 76/98.2

**Date Ackn'd** 07/07/1998

**CU2 Project Title**

EXPRESSION OF THE GFP GENE FROM AEOUORIA VICTORIA FOR USE AS A REPORTER CELL GROWTH IN SALMONELLA TYPHIMURIUM

**Class** Class 2

**CultureVolClass2**

**Consent Granted**

not applicable

**Project notified under transitional arrangements** Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications
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**Name**

ASTRAZENECA UK LTD

**Name 2**

**Department**

RESEARCH

**Building**

BLOCK 52 MERESIDE

**District**

ALDERLEY PARK

**Town**

MACCLESFIELD

**County**

CHESHIRE

**Postcode**

SK10 4TG

**Country**

ENGLAND

**Tel Number**

01625 514707

**Fax Number**

01625 517947

**E-mail**

**HSE Division**

NORTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Level</th>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

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<th>Virology</th>
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<th>Gene Therapy</th>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

### Project Ref 108/00.1

<table>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism
Host/vector system

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**Project Ref**: 108/00.2

**CU2 Project Title**: CLONING OF WILD-TYPE AND DOMINANT NEGATIVE VERSIONS OF THE PROTEIN KINASE KINASE X IN BACTERIAL, EUKARYOTIC AND ADENOVIRAL VECTORS FOR EXPRESSION IN E.COLI AND HUMAN CELLS

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**Non-GMM**: not applicable

**Consent Granted**: no

**Project notified under transitional arrangements**: Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Project Ref 108/00.3

Date Ackn'd 18/10/2000

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

EXPRESSION OF NATIVE AND MODIFIED KINASE XX AND SUBSTRATE FAMILY MEMBERS IN MAMMALIAN, E.COLI AND INSECT CELL LINES

Class 2

Consent Granted not applicable

Class CultureVolClass2 CultureVolumeClass3-4

Non-GMM

02/03/2022  Page 3164 of 15326
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment
Project Ref 108/01.1

Date Ackn’d 14/02/2001

Date Project Ceased

CU2 Project Title
GENERATION OF SEMLIKI FOREST VIRUSES EXPRESSING NEUROPEPTIDE AND ION CHANNEL PROTEINS AND EVALUATION OF EXPRESSION IN MAMMALIAN CELL LINES.

Class 2
Culture Volume
≤ 1 litre

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
We wish to evaluate the Semliki Forest Virus (SFV) as a system for high level expression of heterologous genes in mammalian cells. This work would initially involve the expression of B-gal as a test protein standard cell lines including CHO.

From the literature SFV has been noted as particularly useful for expression of receptors and ion channels as expression takes place early in the virus replicative cycle while the cells are still intact and able to efficiently process proteins bound for the cell membrane. The aim of this experimental work extends to the expression of two receptors and one ion-channel protein in SFV for comparison of protein levels with current expression systems.

Recipient or parental organism
BACTERIAL HOSTS E coli HB101; DH5 alpha
There is a large body of literature to support the view that the K-12 strain of E. coli survives poorly outside the laboratory, particularly when faced with competition from other microflora, and has limited capacity for replication. These findings are similar to those in which the survival in the animal gut has been measured and found to be poor. Available data is consistent with the view that the K-12 strains of E. coli used in genetic modification work pose a negligible risk to both human health and safety and the environment. E. coli K-12 organisms are considered disabled in the guidance given by ACGM/HSE/DETR. Recombinant strains do not have greater capacity to survive in the environment than non-recombinants and meet the requirements for classification of the organisms as Risk Class 1.

MAMALIAN CELLS (NON-HUMAN DERIVED) BHK-21 (C13); CHO cells
Cultured animal cell lines are not free living organisms found in nature, but have been produced in the laboratory from animal tissues. Their highly specific growth requirements render animal cell lines incapable of survival outside the laboratory. They pose no risk to the environment and the laboratory procedures used to transfer genetic material into animal cells in the laboratory are depend upon conditions not found in the environment. Transfer of genetic material to another organism via an adventitious agent present in an accidentally released recombinant is theoretically possible. However, this would require the sequential occurrence of a number of separate events each of low probability. The gene products encoded by the introduced DNA are often pharmacologically active and/or may effect cell growth regulation. In some cases, they may have oncogenic properties. Their strict requirements for survival and growth result in negligible environmental risk from the cells themselves. The cells to be used in the work described in this proposal meet the criteria for an especially disabled host and the requirements for classification of the organism as Risk Class 1. Such cells cannot survive outside of laboratory culture conditions so that in themselves they pose no risk to the environment.

HUMAN CELLS HEK293
Human cell lines can be considered as especially disabled hosts provided that the cell line is unable to colonise the worker (ie. not their own cells) although there may be a low risk associated with human cancer cells. The environmental risk associated with human cell lines is considered negligible. The human cell lines described in this work meet the criteria for an especially disabled host (with respect to the GMO Regulations) and the requirements for classification of the organism as Risk Class 1. Their strict requirements for survival and growth result in negligible environmental risk from the cells themselves. However, because these cell lines are of human origin it follows that if they were to contain adventitious agents which are potentially harmful, it is possible those adventitious agents would have a host range including man. The scientist intending to use a human cell line should ensure that suitable and sufficient information is available on the previous history of the line. The presence of adventitious agents may require additional precautions for the protection of human health or the environment but unless these are directly related to the genetic modification, these should be considered under other relevant legislation and need not be taken into account in risk classification for the purposes of the Genetically Modified Organisms (Contained Use) Regulations 2000.

Host/vector system
Hosts/Vectors - Cloning Vector: pSFV-1 Host: HB101; DH5a
Additional Information for Recombinant Virus Particles
Intended virus(es): Alphavirus
"Insert" Vector(s): pSFV1 RNA Vector
Helper Plasmids: pSFV2-helper
Virus Producer Cell Line: BHK-21 (C13)cells
Expression Host Cells: HEK293, CHO

Origin & function
Details of the insert: The lac Z gene is a 3.5 kbp cDNA fragment of bacterial origin which was obtained originally from Clontech and cloned in-house vector, pGEN neo K, from which it was excised for this study, by digestion with Not 1. Full length clones of receptor and ion-channel protein from several human tissue libraries have been identified in the Incyte database. These will be obtained and full length cDNA suitable for cloning will be obtained by PCR amplification.
Nature of Gene Produce: B-galactosidase is an E. coli enzyme which catalyses the hydrolysis of lactose to galactose and glucose. The enzyme is induced by the presence of substrate in the medium; the induction is part of an adaptive process which enables E. coli to utilise alternative carbohydrates. The neuropeptide receptors can be activated by specific peptide ligands resulting in changes in intracellular cAMP or iCa2+. Expression of the proteins is restricted to brains and corticoadrenal tissue and hypothalamus in particular. Changes in intracellular levels of these second messengers in specific brain neurons are then translated into physiological responses. The ion channel protein is involved in proliferation through effects on membrane potential, Ca2+ signalling and chemokine secretion. The receptors and ion channel should be produced in an active form when introduced into mammalian cells. The receptors should undergo activation by the appropriate ligand.

Evaluation of foreseeable effects

The receptors can be activated by specific peptide ligands resulting in changes in intracellular cAMP or iCa2+. Expression of the proteins is restricted to brains and corticoadrenal tissue and hypothalamus in particular. Changes in intracellular levels of these second messengers in specific brain neurons are then translated into physiological responses. The ion channel protein is involved in proliferation through effects on membrane potential, Ca2+ signalling and chemokine secretion. The effects of over-expression of these proteins following accidental infection of a worker by activated cirrus stock are impossible to predict with certainty but there are no reports in the literature of ligand independent signalling or oncogenic transformation. Sufficient levels of circulating ligand would need to be present in peripheral tissues to activate the receptors.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

There are no special measures to be adopted for rodent or insect vector control because the recombinant organisms used on site are not likely to be transmitted by rodents or insects and moreover, the location of the recombinants in liquid nitrogen or freezers, the only activity associated with this proposal, results in these being inaccessible to such vectors. All other containment level 2 measures will be adopted with the exception of an autoclave for waste inactivation being present in every building. An exemption is requested from this requirement. An autoclave request - autoclave not present in every building. An autoclave for waste inactivation is not present in every building on site where these activities are conducted. An exemption is requested for not applying these measures. The justification for this is that the waste management measures on site (see Section 12) provide an equal standard of containment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management procedures at Alderley Park make use of a central autoclave facility with suitable measures for collection and transport from the site of production to the autoclave. Briefly, the standard site procedures for disposal of relevant waste are as follows: (a) Liquid wastes: In general, the site procedures involve treatment of infectious liquid waste with 2.5% Chloros or 1% Virkon (final concentrations) for at least 30 minutes. This treated liquid mixture is then flushed to drain using copious amounts of water. Hypochlorite-based solutions are effective in inactivating the SFV particles (manufacturer's validation data available and attached - 100% inactivation using 1.7% v/v of household bleach and 2 x 105 infectious units of SFV. Mammalian cells require highly specific medium to survive and grow in vitro are inactivated rapidly outside of culture medium. Any of the commonly used disinfectants will accelerate that process yet further. The K12 bacterial trains are rapidly inactivated by 2.5% v/v household bleach or 1% Virkon. Disinfection data for bacteria indicate kill in excess of 8 logs.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y
ATCC recommends Biosafety Level 2 for HEK293 cells so that it may not be entirely appropriate to indicate that there are no harmful effects associated with the recipient micro-organisms. However, since the risk, if any, is associated primarily with potential adventitious agents, it need not to be taken into account for the risk classification purposes although it should be addressed in a separate COSHH assessment. Since work with mammalian cells is routinely carried out at CL2 in a Class 2 safety cabinet, the appropriate control measures required by COSHH will be in place.

**Project Containment**

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<td>L4</td>
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**Laboratory Activities**
- Glass Houses
- Growth Rooms

**Project Ref** 108/01.10

**Date Ackn'd** 12/04/2001

**CU2 Project Title**
CLONING AND EXPRESSION BIOLOGICALLY ACTIVE CHEMOKINE (DESIGNATED CHEMOKINE X) AND, N-TERMINAL DELETION MUTANTS OF CHEMOKINE X AND ITS NATIVE RECEPTOR IN E COLI AND MAMMALIAN CELLS

**Class**
- CultureVolClass2
- Class 2

**Consent Granted**
- not applicable

**Project notified under transitional arrangements**
- N

**Project Additional Information**

**Purposes of the contained use**
Generation of mammalian cells expressing recombinant chemokine X or its receptor and development of binding assays using either whole cells or membrane
preparations to detect the binding of full length chemokine X. Full-length chemokine X and N-terminally deleted chemokine X (a natural antagonist of chemokine X receptor signalling) are required as ligands in the assay. It is planned to express chemokine X and truncated mutants in E coli and mammalian cells. The receptor for chemokine X will be expressed in mammalian cells.

Recipient or parental organism

Cloning Host: E coli strain DH5 alpha - a disabled E coli K12 strain. Genotype: F^-supE44 hsdR17 recA1 gyrA96 endA1 th^-1 relA1 deoR prophage80d(lacZYA^-argF)u169(m80lacZdM15)

Bacterial Expression Host: MSD 2052: - an E coli B strain commonly used for expressing proteins with T3 & T7-type vectors. Following advice from HSE in May 2000 that BL21 can be regarded as disabled, the same is likely to apply to this strain which is a derivative. Source: Novagen 69443-1. Genotype: F^-ompT, (rB^- mB^-). Phenotype: B strain, lacks lon protease, lacks ompT protease DE3 lysogen sensitive to rifampicin, compatible with pET vectors.

Mammalian Cell Expression Hosts: CHO; MEL

CHO: Chinese hamster ovary cells posing negligible risk and safety handled at CL1
MEL: Murine erythroleukaemia cell line. Differentiate into erythrocytes upon addition of DMSO. CL1 appropriate for operator and environmental protection.

Host/vector system

Bacterial Cloning Vector: pCDNA. nic^-/mob^- and tra^-. Therefore non-mobilisable unless a derivative modified in these respects (In which case a new, separate entry should be made). Amp^r and either Neo^r, Hyg^r, or Zeocin^r. As the pCDNA series of vectors have both CMV and T7 promoters, it may be used for cloning in non-expressing strains such as HB101, DHS alpha which do not express T7 RNA polymerase and also for expression in a host strains which constitutively expresses T7 RNA polymerase. Contains SV40ori sequence as well as bacterial origin of replication permitting replication in mammalian cells expressing SV40 T.

Bacterial Expression Vectors:

pAT153: a non-mobilisable plasmid vector. ds-DNA 3658 b.p. Copy Number: 15 copies/cell. Constructed by removal of Haell-fragments B and H (pos. 1649-2353) from pBR322. There is conflicting information on the structure of pAT153. Some 200 bases may be erroneously missing from this sequence. pAT153 has properties comparable to pBR327. The bom or Mob site is lost. pAT153 has somewhat higher copy number than pBR322. pAT153 is derived from pBR322 by deletion of a 622 bp Haell fragment, bases 1730-2352.

pT7#3.3: The backbone of the vector is pZEN 0042 (pTB357): this vector contains the tetA/tetR inducible tetracycline resistance sequence from plasmid RP4 and the cer stability sequence from plasmid pKS492 in a pAT153 derived background - itself non-mobilisable. The T7 expression cassette contains a T7 gene 10 promoter flanked by two operator sequences (lac), the T7 gene 10 5' untranslated region including ribosome binding site, a tRNAarg5 transcriptional reporter element downstream of the expressed gene and a downstream T7 gene 10 terminator sequence. A T4 terminator sequence is also positioned upstream of the expression cassette and the vector contains the lac 1 gene. None of these sequences are known to increase the stability of the vector in the environment nor are they known to be mobilisable or oncogenic.

Mammalian Cell Expression Vectors: pCDNA series; pEV

pCDNA: nic^-/mob^- and tra^-. Therefore non-mobilisable unless a derivative modified in these respects (In which case a new, separate entry should be made). Amp^r and either Neo^r, Hyg^r, or Zeocin^r. As the pCDNA series of vectors have both CMV and T7 promoters, it may be used for cloning in non-expressing strains such as HB101, DHS alpha which do not express T7 RNA polymerase and also for expression in a host strains which constitutively expresses T7 RNA polymerase. Contains SV40 ori sequence as well as bacterial origin of replication permitting replication in mammalian cells expressing SV40 T. pEV: nic^-, mob^-, tra^-, therefore non-mobilisable. Ampicillin and Neomycin resistance genes. beta-globin & TK (mammalian promoters. Additional sequences include the beta-globin Locus Control Region.

Origin & function

The chemokine X cDNA will be cloned from human peripheral blood mononuclear leukocytes which have been stimulated with LPS. N-terminally deleted mutants will be generated by PCR using full length chemokine X as template. The native receptor will be cloned from the human monocyte tumour cell line THP-1. No known regulatory or transposable elements are present in the chemokine X or chemokine X receptor cDNAs.

Chemokines are a class of proteins involved in the recruitment and activation of leucocytes to areas of inflammation. Chemokine X belongs to the beta-chemokines sub-family and is more specific than other members of the family, in that it acts predominantly on a subset of leucocytes.
Themokine X mediates chemotaxis of cells (extravasation from the blood stream through vascular endothelial cells into tissues) and subsequent activation. Chemokine X (and all of the other chemokines discovered so far) act by binding to seven trans-membrane helix (STH) receptors on leukocytes. This binding triggers a series of signalling mechanisms Ca++ flux. Full length chemokine X is the natural ligand for the chemokine X-receptor expressed on the surface of leucocytes and interaction between the ligand and receptor results in changes in calcium flux within mononuclear cells. N-terminally deleted mutants of chemokine X are known to be antagonistic and compete with full-length chemokine X for binding to the chemokine X receptor.

Evaluation of foreseeable effects

The expressed genes are chemokine X, N-terminally truncated chemokine X and chemokine X receptor. These are naturally occurring molecules that have a role in the recruitment of leukocytes to areas of inflammation and the subsequent activation of leukocytes causing their differentiation into macrophages. Injection of purified chemokine X into animal tissues has been shown to initiate a slight inflammatory exudate formation (local) which subsides within several hours. This suggests that exposure to chemokine X could cause a local, short-lived immune reaction. Themokine X and mutants are soluble proteins and would be secreted into the culture media of MEL and CHO cells and should be fully-processed active proteins. E coli expressed chemokine X would require refolding and purification from inclusion bodies. There is a literature precedent for this but the proteins are unlikely to be active due to a lack of post-translational modification.

Mammalian cell generated chemokine X should be active and the highest level of expression would be expected in MEL cells with supernatants containing up to 2mg/L active protein. The highest risk of operator exposure is from accidental inhalation of aerosols when handling cell supernatants. There is no direct evidence in the literature that inhalation of chemokine X would cause an inflammatory response although there is indirect evidence linking raised chemokine X levels in lung homogenates to infiltration of leukocytes in a model of SEA-induced allergic airway inflammation in mice. There is also indirect evidence from studies with eotaxin (another chemokine that recruits eosinophils) in guinea pigs that inhalation of approx 2ng increases the sensitivity to histamine and 20ng results in eosinophilia (Am J Resp Crit Care Med 2000, 161: 1844-9). Inhalation of MEL supernatants could provide an equivalent level of exposure to chemokine X in man.

Chemokine X receptor requires expression on a cell surface in order to contribute to an inflammatory response.

Exposure to excess chemokine X receptor could possibly cause the mopping up of endogenous chemokine X but would not result in an immune reaction.

The likelihood of potentially harmful effects being realised as a consequence of exposure of the operator or environment to the final GMO is judged to be low because of the low probability of exposure to significant amounts of culture medium. With the use of a safety cabinet, this risk should be negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All CL2 measures will be applied with the exception of the requirement for an autoclave to be available in each building where activities are carried out. The waste management procedures used at Alderley Park are judged to provide an equivalent level of protection. The measures used are included in the section below dealing with waste management. An exemption from the requirement for an autoclave to be present in each building is therefore requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

NORMAL LABORATORY OPERATIONS:

Waste management procedures at Alderley Park make use of a central autoclave facility with suitable measures for collection and transport from the site of production to the autoclave. Briefly, the standard site procedures for disposal of relevant waste are as follows:

- **a)** Liquid wastes: In general, the site procedures involve treatment of infectious liquid waste with 2.5% Chloros or 1% Virkon (final concentrations) for at least 30 minutes. This treated liquid mixture is then flushed to drain using copious amounts of water. In some instances other specified validated disinfectants are used for specific purposes including Savlon hospital concentrate. 70% ethanol is commonly employed for routine surface decontamination.
- **b)** Solid wastes (other than sharps): are placed in an autoclave bag and when sufficiently full, the neck is twisted closed and the bag placed in a second autoclave bag which is sealed with autoclave tape. The bag is then placed in a specified location in the laboratory, pending collection. The closed bags are collected daily by Shift Technicians who transport them in robust wheeled and sealed autoclave bags back to the central site.
lidded containers to an on-site autoclave for inactivation in an autoclave located in a specialised facility on site. This procedure has been validated using a 12-point
thermomcouple procedure in which probes are placed in a representative load. After autoclaving, waste is collected by a contractor for final disposal by off-site incineration.
(c) Sharps are placed in suitable purpose-designed robust containers with a lid and when full are placed for collection and final disposal by incineration by our waste
contractor.

Measures for disinfection of any accidental spills are set out in the Alderley Park Biosafety Manual and are based on appropriate use of the above disinfectants. Briefly,
liquid spills are covered with paper towels and disinfectant added from the perimeter. After the specified period required for inactivation, the towels are removed into a
container for final collection and final disposal by either autoclaving as described above, or where the disinfectant is incompatible with that process, by incineration off-site.
All waste materials are transported in sturdy leak-proof containers.

Disinfection procedures lead to at least an 8 log reduction in viable bacterial cell count. The disabled bacterial host strains used do not survive well in the environment even
if untreated. The viable count in liquid waste samples after disinfection is estimated as 0-10/ml and is likely to be at or close to the lower end of that range. With the
associated water used to flush treated liquid waste to drain, the concentration of any bacteria in such waste is negligible.

The mammalian cells used in this work cannot survive outside a narrow range of temperature and nutrient conditions and even untreated it is anticipated that none would
survive soon after disposal to drain. Given that all relevant liquid waste is treated with either 2.5% bleach (hypochlorite) or 1% Virkon, the survival rate is effectively zero.

Large-scale work.
Standard operating procedures include steam sterilisation of the fermenters at 121 degrees C for 10 minutes. The contents are then discharged to drain via the harvest
valve. The system has been validated with host strains as it is generally considered that these are more resistant to killing than recombinant organisms, particularly as
none of the recombinants are known to express proteins as a result of the modification which would confer pathogenic traits or resistance to killing by heat or by the
disinfectants utilised in this area.

Validation has been carried out on both E coli K12 and B (BL21) derivative strains, the latter in November 2000. After growth in a yeast extract-glycerol based medium
(HYE) to an OD(550nm)>100, samples are collected aseptically. The viable cell count is determined by plating of dilutions on L agar plates followed by overnight culture at
37 degrees C and counting of colonies which subsequently develop.

Samples are also taken following sterilisation procedures as indicated above. Aliquots are diluted and plated as described above and also used to inoculate 75 ml L-broth
liquid cultures which are incubated at 37 degrees C.

Typical viable populations at maximum density during such grows are 10 (to the power of 11) cfu/ml.
No colonies were observed after streaking out 10ul of the sample or after plating of a 1:100 dilution. No growth was observed in liquid cultures.

Residual waste from harvesting operations is routinely treated in a dunk tank in a Class 1 safety cabinet with Savlon Hospital Concentrate used at a dilution of 1% v/v.

Validation of this procedure for E coli strains has been carried out using concentrations of disinfectant varying from the recommended 1% to a maximum of 5%. These
concentrations are prepared by addition of appropriate volumes of concentrate to 2 litre samples of live culture dispensed in Duran bottles and left without lids overnight in a
Class 1 Safety Cabinet.

A sterile diluent is used with Asalectin (3g/l) to neutralise the disinfectant in 10 ml aliquots of the cultures in Universal tubes. 100 ul aliquots of dilutions ranging from 10 -2 to
10 -10 are then spread onto L agar plates which are then incubated at 37 degrees C overnight. No colonies were detected on any of the plates.

These data support the conclusion that the disinfection procedure leads to more than an 8 log reduction in the number of viable bacteria. For the in situ fermentor
sterilisation, this figure is greater than a 10 log reduction.

Periodic airborne monitoring has also been carried out in the fermenter hall. Data over a period of 18 months consistently resulted in the concentration of process organisms being below the limit of detection (3cfu/m (to the power of 3)) although total counts in the order of 10 (to the power of 2) (non-process organisms) were detected.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Approved at risk class 2 with the view being expressed that was taking a very precautionary approach.

**Project Containment**

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Animal Units
Large Scale Activities
Human Clinical Applications

**Project Ref 108/01.2**

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<td>16/02/2001</td>
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Non-GMM Consent Granted
Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

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**Project Additional Information**

**Purposes of the contained use**

It is necessary to store for possible future use, recombinant organisms generated at Alderley Park or obtained from elsewhere. Facilities at Alderley Park include freezers where stocks of recombinant bacteria are maintained as glycerol suspensions at -80°C and also liquid nitrogen tanks for long-term storage of recombinant mammalian and insect lines.

**Recipient or parental organism**

Some cell lines may obtain adventitious agents so that laboratory activities involving these may require measures from containment level 2. The requirement for these measures is based on the provisions of COSHH rather than the GMO Regulations.

**Host/vector system**

Vectors may include some which are potentially mobilisable in an appropriate host background (i.e. mobilisation-defective) and which include antibiotic resistance genes for use as selection markers. Some vectors include transposons or encode transposase functions. They include non-expression and expression vectors. Expression vectors include those designed for expression in bacterial, yeast, mammalian and insect systems and include vectors used in the generation of recombinant viral particles. The resulting viral particles are all attenuated and replication defective as described in the original proposals for their use.

**Origin & function**

Source of Nucleic Acid - Inserts are from a wide variety of sources including rodent and human tissues and including both genomic and cDNA sequences.

Nature of Gene Product - The properties of the proteins covered by this proposal as they exist in the native organisms cover a wide range of known protein functions including enzymes, receptors, and other proteins involved in regulation of normal cellular activities including cell growth and division. Details are set out in separate risk assessments, records of which are kept in electronic or paper format, and which cover the actual use of the recombinant organisms for activities other than storage.

**Evaluation of foreseeable effects**

Some recombinant organisms are able to express products which may be oncogenic, toxic or allergenic. Where cells are capable of producing recombinant viral particles, these may be able to deliver to human cells, sequences able to express biologically active molecules. There may be harmful effects associated with these when expressed in an unregulated manner in cells in which expression is normally subject to tight control, or where they are expressed in cells in which they do not normally occur at significant concentrations. Where cells are designed to produce recombinant viral particles these are all attenuated and with the exception of recombinant baculoviruses, the viral particles are not replication competent. The likelihood of potentially harmful effects being realised as a consequence of exposure of persons to the final GMO is judged to be low. None of the organisms would be classified as greater than risk class 2 in normal operations and most are risk class 1. Having regard to the restriction of the activities covered by this proposal to storage only, and the conditions under which they are stored as described elsewhere in this proposal the potential for dissemination is negligible. Given the nature of the organisms, the negligible risk of dissemination for these storage activities, and the absence of any special features of the environment which may affect the risk, the risk of environmental harm is judged to be negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

02/03/2022
There are no special measures to be adopted for rodent or insect vector control because the recombinant organisms used on site are not likely to be transmitted by rodents or insects and moreover, the location of the recombinants in liquid nitrogen or freezers, the only activity associated with this proposal, results in these being inaccessible to such vectors. All other containment level 2 measures will be adopted with the exception of an autoclave for waste inactivation being present in every building. An exemption is requested from this requirement.

Exemption request - autoclave not present in every building - An autoclave for waste inactivation is not present in every building on site where these activities are conducted. An exemption is requested for not applying these measures. The justification form this is that the waste management measures on site (see section 12) provide an equal standard of containment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management procedures at Alderley Park make use of a central autoclave facility with suitable measures for collection and transport from the site of production to the autoclave. Briefly, the standard site procedures for disposal of relevant waste are as follows: (a) Liquid wastes: In general, the site procedures involve treatment of infectious liquid waste with 2.5% Chloros or 1% Virkon (final concentrations) for at least 30 minutes. This treated liquid mixture is then flushed to drain using copious amounts of water. In some instances other specified validated disinfectants are used for specific purposes including Savlon hospital concentrate. 70% ethanol is commonly employed for routine surface decontamination. (b) Solid wastes (other than sharps): are placed in an autoclave bag and when sufficiently full, the neck is twisted closed and the bag placed in a second autoclave bag which is sealed with autoclave tape. The bag is then placed in a specified location in the laboratory, pending collection. The closed bags are collected daily by Shift Technicians who transport them in robust wheeled and lidded containers to an on-site autoclave for inactivation in an autoclave located in a specialised facility on site. This procedure has been validated using a 12-point thermocouple procedure in which probes are placed in a representative load. After autoclaving, waste is collected by a contractor for final disposal by off-site incineration. (c) Sharps are placed in suitable purpose-designed robust containers with a lid and when full are placed for collection and final disposal by incineration by our waste contractor. Measures for disinfection of any accidental spills are set out in the Alderley Park Biosafety Manual and are based on appropriate use of the above disinfectants. Briefly, liquid spills are covered with paper towels and disinfectant added from the perimeter. After the specified period required for inactivation, the towels are removed into a container for final collection and final disposal by either autoclaving as described above, or where the disinfectant is incompatible with that process, by incineration off-site. All waste materials are transported in sturdy leak-proof containers.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

02/03/2022
## Project Additional Information

### Purposes of the contained use

To deliver cDNAs encoding known G-protein coupled receptors to murine and human cell lines to facilitate target identification.

### Recipient or parental organism

**Cloning Hosts and Vectors**

- **Hosts**: E coli strains DH5 alpha and STBL2
  - **DH5 alpha**: Disabled E coli K12 strain. Genotype F-supE44 hsdR17 recA1 gyrA96 end/A1 th-1 relA1 deoR; prophage80d(lacZYA-argF)u169(m801acZdm15)
  - **STBL2**: Disabled E. coli K12 strain. Genotype F-mcrA del(mcrBC-hsdRMS-mrr) recA1 end A1 gyrA96 thi supE44 relA1 lambda-del(lac-proAB).

- **Mammalian Expression Host Cells**: CHO, HEK293, HeLa, NIN3T3 fibroblast

- CHO cell line initiated from a biopsy of an ovary of an adult Chinese hamster by T T Puck in 1957 posing negligible risk which can be safely handled at CL1.

- HEK-293: Human embryonic epithelial cell line stably expressing the transforming gene of human Type 5 Adenovirus. The HEK cell line supplied by ATCC is stated to be tumorigenic in nude mice and to require minimum containment at Biosafety Level 2 on the basis of the partial adenoviral sequences. There is no risk of generating...
adenovirus by recombination associated with the proposed GM activities.

HeLa: human tumour cell line. Reverse transcriptase negative. Contains residual human papilloma virus sequence with associated potential for complementation of the E1a deletion in recombinant Adenoviral systems. ATCC recommend handling at Biosafety Level 2. CL2 may therefore be required for operator protection but the modifications involved in this study are not likely to generate a risk independent or additional to that of the unmodified host line.

NIH-3T3 fibroblast cell line is a common laboratory cell line derived from murine fetal fibroblasts.

Infected cells will be regarded as stable cell lines following removal of virus containing supernatant and selection for the appropriate antibiotic marker (i.e. - G418, Hygromycin, etc.) as the integrated provirus is not able to mobilise.

Virus Producer Cell Lines: 293T-Phoenix-eco; 293T-10A1 ampho

PhiNX-Eco cells, used to produce ecotropic virus. These viral particles will infect only murine cells. PhiNX-Eco (Phoenix) are 293T cells with stable integrations of gag-pol and ecotropic env at different chromosomal loci (3rd generation packaging cells).

293-10A1 cells (Imgenex, San Diego, CA) used to produce polytropic virus. The 293-10A1 cell line contains the retroviral structural genes (gag, pol, env) on a single cDNA integrated into the host genome. While this system is not preferred, due to an increase risk of generating helper virus, it is the only stable packaging cell line currently available to AZ for generation of amphotropic virus, and will be replaced when 3rd generation systems are obtained (Phi-NX-ampho, under negotiation with Stanford University). Viral production is transient (48-72 hr) and presence of helper virus has not been detected, according to the supplier (Imgenex, CA) so actual risk for replication competent retrovirus should be considered as low. Human embryonic kidney 293T (HEK-293) cells, used to produce amphotropic or ecotropic virus by transient transfection of plasmids separately expressing gag-pol and env (amphotropic env from 4070A MuLV; eco from MMLV). In addition to using stable packaging cell lines to generate infectious virus, plasmids containing the structural genes can be transiently transfected into 293 cells along with the recombinant retroviral vector containing the gene of interest. pC-pl and pC-ampho packaging plasmids contain the MMLV gag, pol and env genes in a single cDNA, under the control of the CMV promoter. These constructs have been engineered for safety by a deletion of the enhancer region of the 3'LTR, which would render any helper virus unable to replicate. These constructs are used transiently (24-48hr), therefore the risk of generating helper virus is greatly reduced over that found in long-term culturing of virus producing cell lines.

Host/vector system

Cloning: E. coli strains DH5 alpha and STBL2 will be used for initial cloning of the vectors 293T-Phoenix-eco; 293T-10A1 ampho will be used with the following "Insert" Vector(s): pBMNZ; pGBMNZ; pBMN-SIN; pCIVA; pDIVA.

Virus Producer Cell Lines: 293T-Phoenix-eco; 293T-10A1 ampho will be used with the following "Insert" Vector(s): pBMNZ; pBMN-IP; pCIVA; pDIVA.

The pBMNZ series of vectos are high packaging efficiency vectors constructed by Gary Nolan (Stanford) and are widely used in commercial and academic laboratories. The "SIN" designation indicates a Self-Inactivating vector. During integration of the provirus the 5' LTR is inactivated (as well as the 3'LTR), which minimises the possibility of activating adjacent cellular genes. pGBMNZ is pBMNZ with GFP inserted into the vector backbone so that transfection efficiency of packaging cell lines can be easily monitored. pCIVA and DIVA are derived from pBabe-puro, which is the most commona series of retroviral vectors in use. pLNCX-2 was created in the Mulligan lab and is another commonly used retroviral vector. Derivates are modifications that modify components of the viral construct that do not alter tropism or generation of infection competent virus. For example, replacing the Neo gene with Hygro, or inserting the GFP gene into the backbone, as in pGBMNZ.

Mammalian Expression Host Cells: CHO, HEK293, HeLa, NIH3T3 fibroblasts will be used with the vectors pBMNZ; pBMN-IP; pBMN-SIN; pGBMNZ; pDIVA; pCIVA and also for expression following transfection with recombinant viral vectors produced as described above.

Origin & function

Details of the insert:
The DNA inserts will be obtained from either clones generated by Incyte (or as RT-PCR products from rat mRNA).

Nature of Gene Product:

Stimulation of G-protein coupled receptors by specific peptide ligands results in changes in intracellular cAMP, iCa++, or inositol phosphate levels. Changes in intracellular levels of these second messengers in specific cells are then translated into physiological responses.

In addition to the specified receptors other G-protein coupled receptors identified by either literature or in silico work, as being involved in the regulation of similar behavioural-associated pathways would also be considered for expression and target evaluation in this system.

The G-protein-coupled receptors will be expressed in an active form when introduced into a mammalian cell. As the receptors being used in these experiments are full-length, native receptors, ligand dependent stimulation will be maintained.

Evaluation of foreseeable effects

Recipient micro-organism(s): Some of the human cell lines are tumorigenic and contain partial viral sequences as described above. Recommendation to work at CL2 will be observed.

Vectors: The viral particle vectors can infect mammalian cells. Although replication defective, they can deliver the insert gene to, and express that gene in, cells which may be infected as the result of accidental exposure. Since the expressed gene products are able to alter intracellular signalling pathways when exposed to ligand, it is possible that cells expressing these genes at levels greater than those found in normal cells may be adversely affected. The nature and severity of such effects in individual cells is however difficult to predict.

The resulting genetically modified micro-organism(s):

Cells producing infectious viral particles will pose the same hazard as the particles themselves as considered above, although the titre of particles, and therefore the associated risk, will be lower than in concentrated vector stocks. The likelihood of potentially harmful effects being realised as a consequence of exposure to the operator or environment to the final GMO is judged to be low. The viral particles used in this GMO application are replication defective, with the only route for generating Replication Competent Retrovirus (RCR) being homologous recombination with constructs used in packaging of the viral transcripts. In every instance the risk to the operator is minimised by utilising viral vectors engineered for safety in a transient system. The replication defective nature of these vectors means that the extent of cell/tissue affected will be restricted to those cells originally infected. Given the routes of transmision of retroviral vectors, normal host responses to exposure and the anticipated titre of viral particles being worked with, the overall risk is judged to be low. Nevertheless, to reduce this risk to negligible levels, it is judged that containment level 2 measures should be used. Use of sharps is also strictly prohibited when handling infectious viral supernatants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All CL2 measures will be applied with the exception of the requirement for an autoclave to be available in each building where activities are carried out. The waste management procedures used at Alderley Park are judged to provide an equivalent level of protection. The measures used are included in the section below dealing with waste management. An exemption from the requirement for an autoclave to be present in each building is therefore requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management procedures at Alderley Park make use of a central autoclave facility with suitable measures for collection and transport from the site of production to the autoclave. Briefly, the standard site procedures for the disposal of relevant waste are as follows: (a) Liquid wastes: In general, the site procedures involve treatment of infectious liquid waste with 2.5% Chloros or 1% Virkon (final concentrations) for at least 30 minutes. This treated liquid mixture is then flushed to drain using copious amounts of water. In some instances other specified validated disinfectants are used for specific purposes including Savlon hospital concentrate. 70% ethanol is commonly employed for routine surface decontamination. (b) Solid wastes (other than sharps): are placed in an autoclave bag and when sufficiently full, the neck is...
twisted closed and the bag placed in a second autoclave bag which is sealed with autoclave tape. The bag is then placed in a specified location in the laboratory, pending collection. The closed bags are collected daily by Shift Technicians who transport them in robust wheeled and lidded containers to an on-site autoclave for inactivation in an autoclave located in a specialised facility on site. This procedure has been validated using a 12-point thermocouple procedure in which probes are placed in a representative load. After autoclaving, waste is collected by a contractor for final disposal by off-site incineration. (c) Sharps are placed in suitable purposed-designed robust containers with a lid and when full are placed for collection and final disposal by incineration by our waste contractor.

Measures for disinfection of any accidental spills are set out in the Alderley Park Biosafety Manual and are based on appropriate use of the above disinfectants. Briefly, liquid spills are covered with paper towels and disinfectant added from the perimeter. After the specified period required for inactivation, the towels are removed into a container for final collection and final disposal by either autoclaving as described above, or where the disinfectant is incompatible with that process, by incineration off-site. All waste materials are transported in sturdy leak-proof containers. Disinfection procedures lead to at least an 8 log reduction in viable bacterial cell count. The disabled bacterial host strains used do not survive well in the environment even if untreated. The viable count in liquid waste samples after disinfection is estimated as 0-10/ml and is likely to be at or close to the lower end of that range. With the associated water used to flush treated liquid waste to drain, the concentration of any bacteria in such waste is negligible. Given that retroviruses do not in general survive well in the environment and are readily inactivated by a number of disinfectants including hypochlorite based agents and Virkon (Antec) both of which are specified for use at Alderley Park. The titres of retroviruses are low and even in vector stocks are not anticipated to be greater then 10 7/ml. Routine titres in discard flasks would be two orders of magnitude less than this. The volumes of liquid waste disposed of in any experiment do not exceed 10 ml. Under the conditions of use, the disinfectant produces a log kill of at least 10 5, so that discard contains less than 10 2 infectious particles per ml. Copious volumes of water accompany disposal of waste to drains so that the titre of infectious particles presents a negligible risk of harm to human health or to the environment.

The mammalian cells used in this work cannot survive outside a narrow range of temperature and nutrient conditions and even untreated it is anticipated that none would survive soon after disposal to drain. Given that all relevant liquid waste is treated with either 2.5% bleach (hypochlorite) or 1% Virkon, the survival rate is judged to be zero.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

The proposal was approved at risk class 2. Comments were made about the potential normal tissue distribution of receptors and ligands in the context of the potential for effects following accidental exposure. The details of these comments have been provided to the regulators in a confidential version of this document. The request for non-disclosure of this is based on the grounds that the information may disclose our position to competitors to an extent which could harm AstraZeneca's legitimate commercial interests.

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Animal Units

Large Scale Activities

Human Clinical Applications
Project Additional Information

Purposes of the contained use

As Jurkats and human T cells are refractory to non-viral transfection and to transfection with standard retroviral vectors, we wish to investigate the suitability of a Lentiviral vector system for transfection of these and other refractory cell types. A comparison is proposed of two-vector (SIN) and three vector lentiviral systems for future use in delivering inserted genes for expression in non-dividing cells. No candidate genes will be expressed initially with the insert restricted to an expressible mouse CD8a:EYFP fusion protein. This fusion protein is composed of the extracellular and transmembrane region of mouse CD8 fused to EYFP and is designed to facilitate magnetic purification of transduced cells from untransduced cells using mCD8 antibody coated magnetic beads.

Recipient or parental organism

HB101: A disabled E coli K12 derivative. Genotype: F-mcrB mrr hsdS20(rB,mB) recA13 supE44 ara14 galK2 lacY1 proA2 rps(L20(Smr) xy15 lamda-leu mtl1. No harmful characteristics.

HEK-293: Human embryonic epithelial cells stably expressing the transforming gene of human Type 5 Adenovirus. DNA from both the right and left ends of the viral genome is present but no adenovirus work is associated with this proposal. The HEK cell line supplied by ATCC is stated to be tumorigenic in nude mice and to require minimum containment at Biosafety Level 2. Collectively, this information indicates a requirement for CL2 although when making a risk assessment for the purposes of classifying GMOs according to the GMO (Contained Use) Regulations 2000, the presence of adventitious agents can be ignored.

Primary human T cells should be treated as potentially infected unless there is clear evidence to the contrary. This requires the adoption of CL2 measures as part of the "Universal Precautions" approach. Culture of cells derived from known or suspected cases of HIV infection beyond 100 hours of culture may require a high standard of containment. The cells used in this study will be restricted to low risk sources.

Jurkat E6.1 - ECACC accession number 88042803. The cells are derived from a human leukaemic T cell lymphoblast. This a well characterised and authenticated cell
Cloning: HB101 host with the following vectors:
PfV1-delta GPEVVVN-ires-mCD8a:EYFP Mobilisation-defective mammalian expression vector containing the ampicillin resistance gene for selection in E.coli and the bacterial original of replication. These sequences have been derived from pBluescript(KS+). This vector on its own will not produce infectious virus; two additional vectors are required. tat,rev, LTRs, mCD9a and EYFP genes will not be expressed, but will be propagated in E.coli. The 5 prime LTR viral promoter will not function efficiently in prokaryotic hosts, therefore active protein would not be expected to be produced in E.coli.

Pcsin-deltaGPEVVNT-CMV-ires-mCD8a:(EYFP or ZsGreen or ZsYellow or AsRed or AmCyan) +WPRE or -WPRE Mobilisation-defective mammalian expression vector containing the ampicillin resistance gene for selection in E.coli and the bacterial origin of replication. These sequences have been derived from pBluescript(KS+). This vector on its own will not produce infectious virus; two additional vectors are required. rev, mCD8a, EYFP and NFPS will not be expressed, but will be propagated in E.coli. The 5 prime CMV I. E promoter (which replaced the 5’ LTR) will not function efficiently in prokaryotic hosts, therefore active protein would not be expected to be produced in E.coli.

Pcsin-deltaEVVVNT-CMV-ires-mCD8a:(EYFP or ZsGreen or ZsYellow or AsRed or AmCyan)+WPRE or -WPRE Mobilisation-defective mammalian expression vector. The vector contains the ampicillin resistance gene for selection in E.coli and the bacterial origin of replication. These sequences have been derived from pBluescript(KS+). This vector on its own will not produce infectious virus, one additional vector is required. gag, pol, rev, mCD8a, EYFP and NFPS will not be expressed, but will be propagated in E.coli. The 5 prime CMV I. E promoter (which replaced the 5’ LTR) will not function efficiently in prokaryotic hosts, therefore active protein would not be expected to be produced in E.coli.

Viral producer host and vectors: Three Vector System:
Pcsin-deltaGPEVVNT-CMV-ires-mCD8a:(EYFP or ZsGreen/Yellow or AsRed or AmCyan)+WPRE or -WPRE. In addition to the deletions referred to above, this vector has tat and LTRs disabled. tat is a potent transactivator of HIV and its removal reduces concerns about its involvement in Kaposi’s Sarcoma. By abolishing the transcriptional activity of the LTR, the production of full length vector RNA in transduced cells is abolished. This minimises the risk that replication competent retroviruses (RCR) will emerge.
PcH-IHIV-PV:gag: product yields 5 proteins (matrix, capsid, p2, nucleocapsid and p6) involved in encapsulation of the viral RNA. pol: yields the protease (responsible for proteolytic maturation of the core HIV-1 proteins) reverse transcriptase (RT): produces dsDNA provirus copy upon infection of host cell.integrate: facilitates insertion of the provirus DNA into the host cell chromosomes.
VpCas-VSG-G encodes the VSV-G (vesicular stomatitis virus G protein) envelope protein required for pseudotyping. This renders the lentivirus infectious via non-specific adsorption to host cell glycoproteins. The result is a retrovirus with an extremely broad host cell range and lentivirus particles that are able to transduce human T-cells and Jurkats more efficiently. SIN Two Vector System:
Pcsin-deltaEVVVNT-CMV-ires-mCD8a:(EYFP or ZsGreen or ZsYellow or AsRed or AmCyan)+WPRE or -WPRE. This system is similar to the above but the gag-pol genes will not be supplied in trans by a separate vector. This increases the chances of recombination, but the fact that the transcriptional activity of the LTR has been abolished reduces these concerns, because the production of full-length vector RNA in transduced cells is also abolished.
PvPack-VSV-G(see above).

Mammalian expression systems: Primary human T-cell cultures and Jurkats cell lines transfected with the recombinant viral particles described above.

Recombinant viral vectors in expression hosts: Production of marker genes (fluorescent proteins)

Mammalian expression systems: Primary human T-cell cultures and Jurkats cell lines transfected with the recombinant viral particles produced in HEK293 cells transfected with the above.

**Origin & function**

**EYFP:** encodes a mutant form of the jelly fish green fluorescent protein that has been approved and used extensively on site and will be cloned immediately upstream of the IRES sequence. It is expressed in order that FACS analysis can be carried out to calculate transduction efficiency.

**mCD8a:** made up of the extracellular and transmembrane region of mouse CD8. It is expressed in order to facilitate magnetic purification of transduced cells from untransduced cells using mCD8 antibody coated magnetic beads.
The mCD8a ND EYFP will be expressed as a fusion protein. The AmpR gene confers resistance to ampicillin and is required for propagation of the vectors in E.coli. The rev gene product facilitates RNA export to the cytoplasm and the tat gene product activates the LTR promoter. The following are genes carried on pCI-(HIV1)-PV: 
- gag: product yields 5 proteins (matrix, capsid, p2, nucleocapsid and p6) involved in encapsulation of the viral RNA.
- pol: yields the protease (responsible for proteolytic maturation of the core HIV-1 proteins).
- reverse transcriptase (RT): produces dsDNA provirus copy upon infection of host cell.
- integrase: facilitates insertion of the provirus DNA into the host cell chromosomes.
pVPack-VSV-G encodes the VSV-G (vesicular stomatitis virus G protein) envelope protein required for pseudotyping. This renders the lentivirus infectious via non-specific adsorption to host cell glycoproteins. The result is a retrovirus with an extremely broad host cell range and lentivirus particles that are able to transduce human T-cells and Jurkats more efficiently.

**Evaluation of foreseeable effects**

**Identification of Potentially Harmful effects:**
(i) the recipient micro-organism(s) - possible adventitious agents in human primary cell culture - COSHH matter to be controlled by the adoption CL2 according to the University Principles approach.
(ii) the vector(s) - there is a risk associated with handling naked DNA because the LTR would be able to drive expression of functional viral genes such as tat in mammalian cells. The risk to the operator is minimised by safe handling of the DNA i.e. all operators will wear gloves and needles or sharps will not be used and the generation of aerosols will be avoided.
(iii) the resulting genetically modified micro-organism(s) - the recombinant viral particles are infectious with a broad animal host range including man. Most of the expressed proteins are unlikely to be deleterious but where tat expression is possible, the association between the tat gene product and Kaposi's sarcoma suggest that such expression may be harmful. (Blood, 1999, vol94, pp663-672) although the mechanisms involved are unclear. A number of papers over the past year indicate that the key factor underlying Kaposi's sarcoma is likely to be infection with HHV-8, although there are still some indications that the tat gene product may have synergistic effects in association with HHV8.

The risk of accidental production of replication competent retroviruses is negligible in these systems. However, the pseudotyping with VSV-G protein is predicted to increase survival of the viral vector and may permit transmission via an airborne route in addition to the usual route of direct contact (e.g. percutaneous injury). The use of sharps will be prohibited and wearing gloves will be mandatory. The production of aerosols will be minimised to prevent accidental contamination of surfaces. Given the broad anticipated host range of the vector, there is the potential for similar harm to animals which may be exposed to the recombinant viral particles as that described above regarding risk to human health. However, the proposed containment measures should prevent such exposure. Even if such exposure should occur, the replication-defective status of the particles would restrict any harm to the animals which were directly exposed and the vector could not be transmitted beyond any animals directly affected. There are no special features of the environment which is likely to be exposed, which affect the risk. All activities which can be adequately controlled by standard laboratory procedures. The overall risk to the environment is thus negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All CL2 measures will be applied with the exception of the requirement for an autoclave to be available in each building where activities are carried out. The waste management procedures used at Alderley Park are judged to provide an equivalent level of protection. The measures used are included in the section below dealing with waste management. An exemption from the requirement for an autoclave to be present in each building is therefore requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste management procedures at Alderley Park make use of a central autoclave facility with suitable measures for collection and transport from the site of production to the autoclave. Briefly, the standard site procedures for disposal of relevant waste are as follows: (a) Liquid wastes: In general, the site procedures involve treatment of
infectious liquid waste with 2.5% Chloros or 1% Virkon (final concentrations) for at least 30 minutes. This treated liquid mixture is then flushed to drain using copious amounts of water. In some instances other specified validated disinfectants are used for specific purposes including Savlon hospital concentrate. 70% ethanol is commonly employed for routine surface decontamination. (b) Solid wastes (other than sharps): are placed in an autoclave bag and when sufficiently full, the neck is twisted closed and the big bag placed in a second autoclave bag which is sealed with autoclave tape. The bag is then placed in a specified location in the laboratory, pending collection. The closed bags are collected daily by Shift Technicians who transport them in robust wheeled and lidded containers to an on-site autoclave for inactivation in an autoclave located in a specialised facility on site. This procedure has been validated using a 12-point thermocouple procedure in which probes are placed in a representative load. After autoclaving, waste is collected by a contractor for final disposal by off-site incineration. (c) Sharps are placed in suitable purpose-designed robust containers with a lid and when full are placed for collection and final disposal by incineration by our waste contractor. Measures for disinfection of any accidental spills are set out in the Alderley Park Biosafety Manual and are based on appropriate use of the above disinfectants. Briefly, liquid spills are covered with paper towels and disinfectant added from the perimeter. After the specified period required for inactivation, the towels are removed into a container for final collection and final disposal by either autoclaving as described above, or where the disinfectant is incompatible with that process, by incineration off-site. All waste materials are transported in sturdy leak-proof containers. Disinfection procedures lead to at least an 8 log reduction in viable bacterial cell count. The disabled bacterial host strains used do not survive well in the environment even if untreated. The viable count in liquid waste samples after disinfection is estimated as 0-10/ml and is likely to be at or close to the lower end of that range. With the associated water used to flush treated liquid waste to drain, the concentration of any bacteria in such waste is negligible. The recombinant viral vector particles are anticipated to be readily inactivated by the hypochlorite based agents and Virkon (Antec) both of which are specified for use at Alderley Park. Both lentiviruses and VSV, the parent viruses for the construct, are known to be susceptible. The titres of the recombinant particles are anticipated to be not greater than 10-7 IU/ml and the total volume in any one experiment will be approximately 100 ml. Viral particles are to be collected by centrifugation at 23000g for 2 hours. It is anticipated that only a very small proportion will remain in the discard supernatant. Reports obtained from the manufacturer of Virkon suggest that this is an effective disinfectant against lentiviruses under the recommended conditions of use (reports typically indicate at least 5 logs reduction in infectious dose). The volumes of liquid waste disposed of in any experiment are expected to be in the order of 100 ml. Assuming that 1% of the vector particles remain in the supernatant, this would leave the numbers of surviving particles at less than 1 per ml. Given the infectious dose and the dilution of any disinfectcd waste when discharged to drain, the presence of any residual infectious particles is judged to present a negligible risk of harm to human health or to the environment. The mammalian cells used in this work cannot survive outside a narrow range of temperature and nutrient conditions and even untreated it is anticipated that none would survive soon after disposal to drain. Given that all relevant liquid waste is treated with either 2.5% bleach (hypochlorite) or 1% Virkon, the survival rate is judged to be zero.

Is an emergency plan required according to regulation 20? N  
If yes, tick to confirm that it is attached to this form N  
Tick to confirm that you have attached a risk assessment to this form Y  
Tick if you are claiming exemption from disclosure for section of the risk assessment N  

Please enter comments on the GM safety committee on the risk assessment
Approval was granted for work at Containment Level 2 with the additional measures specified above. Comments about the transmissibility and survival made by the Committee have been incorporated into the proposal.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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02/03/2022
**Project Ref** 108/01.5

**Date Ackn’d** 11/04/2001

**CU2 Project Title** GENERATION OF RECOMBINANT ADENOVIRUS CODING FOR A ZINC FINGER PROTEIN (ZFP), WHICH REGULATES ENDOGENOUS HUMAN ESTROGEN RECEPTOR ALPHA (HERA) TRANSCRIPTION IN MAMMALIAN CELLS.

**Class** Class 2

**Consent Granted** not applicable

**Non-GMM** Y

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info** 02/03/2022

**Recipient or parental organism**

**Cloning Hosts:**
- DH5alpha: Disabled E. coli K12 strain. Genotype: F^-supE44 hsdR17 recA1 gyrA96 endA1 th^ - relA1 deoR prophage80d(lacZΔM15) -argF)u169(m80lacZD15)
- DH10B: Disabled E. coli strain. Genotype: F^- mcrA(mrr^-hsdR17mcrBC) prophage 80dlacZD15 lacX74 deoR recA1 endA1 AraD139d(ara,leu)7697 galU galK lambdab^- rpsL nupG
- BJ5183 Especially disabled E. coli K-12 derivative with partial recombination capability to allow the recombination of pAdEasy-1 and pAdTrack-CMV. It is ampicillin and kanamycin sensitive.

**Virus Producer Cell Line:** HEK293

HEK 293 is a human embryonic kidney cell line of widespread use. It has the adenovirus E1A region integrated into its genome stably expressing the transforming gene of human Type 5 Adenovirus and thus provides complementation to E1A deficient adenovirus constructs allowing their replication - this is used as the packaging cell line.
DNA from both the right and left ends of the viral genome is present. When used as a helper cell line production of recombinant adenoviral particles, production of wild-type virus (an ACDP Hazard Group 2 pathogen) can occur albeit at a low frequency. Two specific non-homologous recombination events would be required to generate replication-competent virus. In a supemant rescue assay performed on the virus stock to check for replication competent adenovirus (ref: Dion et al J. of Virol Methods 1996 vol56 p99-107) no replication competent virus was detected in 1x10e9 infectious particles. If virus stocks are repeatedly passaged in HEK293 cells replication-competent virus can appear after 9 passages. Methods to produce virus using pAdEasy-1 only require a maximum of three passages in HEK293 cells. Given this data, the risk of generating wild-type adenovirus is considered to be negligible using these procedures.

Expression Host Cells: HEK293, MCF, T47D
HEK-293: see description above.
MCF-7: The MCF7 line is derived from a human mammary gland adenocarcinoma and retains several characteristics of differentiated mammary epithelium contains the Tx-4 oncogene. CL2 following UKCCCR guidelines.

T47D cells: ECACC Ref No: 85102201 Established from the pleural effusion of a ductal carcinoma of the breast of a 54 year old female. The cells carry receptors for a variety of steroids. ATCC refer in an entry for a mouse myeloma cell line (HB-8630) to human mammary tumor virus particles isolated from the T47D clone-10 mammary cancer cell line. CL2 following UKCCCR guidelines. No reason to suspect that the modification will have any effect on the hazard posed by any such endogenous virus.

Host/vector system

Cloning Vectors (used with DH5alpha, DH5alpha, DH10B and BJ5183 bacterial hosts):
pShuttle: this is identical to pAdTrack-CMV except it does not contain a CMV promoter or the GFP element. It is used in conjunction with pAdEasy, to generate recombinant adenoviral plasmids in E. coli. The resulting recombinant plasmid can be used to generate infective but replication-defective adenoviral particles following transfection into suitable mammalian host cells (ie. HEK 293 cells) able to provide the E1A sequence in trans. PadTrack-CMV is a 9.2 kbp vector obtained from Bert Vogelstein (He et al PNAS 1998 vol 95 p2509-2512) and is used in conjunction with pAdEasy-1 to generate recombinant adenoviral plasmids in E. coli. The resulting recombinant plasmid can be used to produce infective but replication-defective adenoviral particles following transfection of suitable mammalian host cells (eg HEK293) above to provide the E1a sequence in trans. pAdEASY-1: A33.4 kbp vector obtained from Bert Vogelstein (He et al PNAS 1998 vol 95 p2509-2514) which contains all the adenovirus type 5 sequences except nucleotides 1-3533 (encompassing the E1 genes) and 28, 130-320, 820 (encompassing E3 region). The vector also contains an ampicillinR gene and an origin of replication for pBR322, hence the plasmid is mobilisation defective (Hom+ Tra- Mob-). The vector is used in conjunction with pAdTrack vectors to generate recombinant adenoviral plasmids in E. coli. (eg when co-transformed into a bacterial host with the pAdTrack-CMV shuttle vector containing an insert sequence, recombination of the host leads to production of a recombinant pAdEasy-1 containing insert sequences. That plasmid can be used to transfect appropriate mammalian cells (eg HEK293) to generate recombinant E1α-deletion adenoviral particles. Additional information for Recombinant Viral Vectors

"Insert" Vector(s): recombinant pAdEASY-1 used with packaging cell line (HEK) and resulting viral particles used to transfect mammalian expression host cells (MCF-7 and T47D).

Origin & function

This is an artificial construct containing: a nuclear localisation signal - NLS (derived from SV40 large T), hER2.0/1.1 ZFP DNA binding domain (synthetic), either a KRAB transcriptional repression domain (derived from Drosophila Kruppel) or a VP 16 transcriptional activation domain (derived from a fragment of Herpes Simplex Virus) and a FLAG domain (synthetic) in pcDNA3. This artificial construct is under the control of the Tet Repression system (Tet Off). This comprises two cassettes which have been PCRd from commercially available vectors: Cassette 1 consists of a Tet repressor under the control of a CMV IE promoter linked to VP16 followed by an IRES-EGFP element and Bovine growth hormone Poly A. Cassette 2 comprises a 7 x Tet Operon with a CMV IE minimal promoter, followed by a multiple cloning site (into which the above ZFP constructs will be inserted) and an SV40 Poly A. The two cassettes are inserted back to back into the pSHUTTLE expression plasmid. (See Vogelstein et al. PNAS., Vol. 95, pp. 2509-2514, March 1998) The two proteins to be expressed are artificial constructs. Each consists of zinc fingers which are expected to bind specifically to an 18-bp sequence in the hERα promoter. In the presence of tetracycline their expression will be switched off. However, in the absence of tetracycline they are expected to be highly expressed. The proteins will contain a Nuclear Localisation Signal and will therefore be targeted largely to the nucleus of hER2.0/1.1 NKF/NVF expressing cells, where the KRAB transcriptional repressor domain and VP16 transcriptional activator domain will assert their effects on hERα transcription within 1kb of the hERα Sp1 binding site. The expressed proteins are expected to be biologically active but are designed to exert their effects only in cells expressing endogenous hERα.
Since ERa is a transcription factor, over-expression will have no effect unless the cells are exposed to estrogens, in which case the consequence will be to activate expression of estrogen responsive genes.

**Evaluation of foreseeable effects**

Taking a very precautionary approach, the likelihood of potentially harmful effects being realised as a consequence of exposure of the operator or environment to the viral vectors was judged to be low. Risks associated with other GMOs are judged to be negligible.

The greatest risk of accidental infection arises during the preparation of concentrated virus stocks. Since the use of sharps may be necessary in the viral purification steps, there is chance of the user being injected with virus particles containing the genetic material. It is conceivable that infection may induce an immune response against the viral vector if large numbers of cells are infected initially. After aliquotation only small aliquots are handled at any one time and effectively, the complete aliquot would have to be ingested to generate a significant infection.

There is no evidence that the expression of the inserted gene could have an additional harmful effect in vivo. It is possible that infection with the virus containing the constructs may lead to a localised expression of ZFPs. Constructs to be expressed will be biologically active and are designed to exert their effects only in cells expressing endogenous hERa.

They are designed to bind specifically to the human ER alpha promoter where they will act as either inhibitors or activators of ERa transcription. Since ERa is a transcription factor, over-expression will have no effect unless the cells are exposed to estrogens, in which case the consequence will be to activate expression of estrogen responsive genes. Most cells which are exposed to estrogen express ERa at a level sufficient to activate gene expression on exposure to ligand, thus overexpression of ER will be of little consequence. It is difficult to predict the effects of switching off hERa expression in cells which normally express the protein. Since the function of these ZFPs is to up or down-regulate ERa expression, it is possible that infection may lead to ERa-responsive genes being switched on or off in the immediate area.

Since the ZFP sequences are to be introduced into viral vectors designed to target human cells, it will be necessary to control exposure to viral particles containing this genetic material. However, since the virus is replication-defective, the risk of widespread infection is very low. Thus, expression of the inserted genetic material should be restricted to cells which have received the initial dose of the virus.

The protein is very unlikely to bind productively to other functional Sp1 sites in the human genome than that referred to above since the Sp1 binding site comprises only 8 of the 18bp required for binding. However, binding to other sites with sufficient homology may be possible. There is evidence in the literature (liu et al, PNAS 94, 5225-5530, 1997) which suggests that the binding of synthetic ZFPs to their target sequences may not be absolute, and that there may be a risk of the ZFPs binding to sequences other than those intended. Since these synthetic ZFPs have transcriptional activation and repression domains attached to them, it is possible that when they are introduced into a viral vector which targets human cells, there is the potential for inadvertent transcriptional activation of oncogenic sequences or inhibition of tumor suppressor sequences, respectively. The protein is expected to be immunogenic but autoimmune effects are not likely because they are artificial proteins that are not present normally in humans.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All CL2 measures will be applied with the exception of the requirement for an autoclave to be available in each building where activities are carried out. The waste management procedures used at Alderley Park are judged to provide an equivalent level of protection. The measures used are included in the section below dealing with waste management. An exemption from the requirement for an autoclave to be present in each building is therefore requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste management procedures at Alderley Park make use of a central autoclave facility with suitable measures for collection and transport from the site of production to the autoclave. Briefly, the standard site procedures for disposal of relevant waste are as follows: (a) Liquid Chloros or 1% Virkon (final concentrations) for at least 30
minutes. This treated liquid mixture is then flushed to drain using copious amounts of water. In some instances other specified validated disinfectants are used for specific purposes including Savlon hospital concentrate. 70% ethanol is commonly employed for routine surface decontamination. (b) Solid wasts (other than sharps) are placed in an autoclave bag and when sufficiently full, the neck is twisted closed and the bag placed in a second autoclave bag which is sealed with autoclave tape. The bag is then placed in a specified location in the laboratory, pending collection. The closed bags are collected daily by Shift Technicians who transport them in robust wheeled and lidded containers to an on-site autoclave for inactivation in an autoclave located in a specialised facility on site. This procedure has been validated using a 12-point thermocouple procedure in which probes are placed in a representative load. After autoclaving, waste is collected by a contractor for final disposal by off-site incineration. (c) Sharps are placed in suitable purpose-designed robust containers with a lid and when full are placed for collection and final disposal by incineration by our waste contractor.

Measures for disinfection of any accidental spills are set out in the Alderley Park Biosafety Manual and are based on appropriate use of the above disinfectants. Briefly, liquid spills are covered with paper towels and disinfectant added from the perimeter. After the specified period required for inactivation, the towels are removed into a container for final collection and final disposal by either autoclaving as described above, or where the disinfectant is incompatible with that process, by incineration off-site. All waste materials are transported in sturdy leak-proof containers.

Disinfection procedures lead to at least an 8 log reduction in viable bacterial cell count. The disabled bacterial host strains used do not survive well in the environment even if untreated. The viable count in liquid waste samples after disinfection is estimated as 0-10/ml and is likely to be at or close to the lower end of that range. The associated water used to flush treated liquid waste to drain, the concentration of any bacteria in such waste is negligible.

Adenoviruses are inactivated by a number of disinfectants including hypochlorite based agents and Virkon (Antec) both of which are specified for use at Alderley Park. The titres of recombinant adenoviral vectors in concentrated stocks can approach 10 pfu. Routine titres in discard flasks would be at least two orders of magnitude less than this and probably substantially less than this. Contaminated liquid waste is diluted into at least a 10-fold excess of Virkon such that the final concentration of the disinfectant is at least 1%, the concentration recommended by the manufacturer. The maximum concentration of viral particles in the disinfectant solution is estimated to be 10 (to the power of 6) particles per ml. Under the conditions of use, the disinfectant produces a log kill of at least 10 (to the power of 5), so that the material discarded to drain contains (less than or equal to) 10 infectious particles per ml. Copious volumes of water accompany disposal of waste to drains so that the titre of infectious particles is less than 1 per ml. The presence of any residual infectious particles, which are anyway replication defective, thus presents a negligible risk of harm to human health or to the environment.

The mammalian cells used in this work cannot survive outside a narrow range of temperature and nutrient conditions and even untreated it is anticipated that none would survive soon after disposal to drain. Given that all relevant liquid waste is treated with either 2.5% bleach (hypochlorite) or 1% Virkon, the survival rate is effectively zero.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Approved as risk class 2 following a precautionary approach.

Project Containment
Hepatocyte growth factor (HGF) and its receptor c-met are both known to be upregulated in a number of human cancers including breast cancer and contribute to disease progression; HGF is normally produced by cells of mesenchymal origin and acts in a paracrine manner on epithelial cells expressing c-met. In normal physiology, HGF/c-met provide one mechanism for controlled cell proliferation and expression of other gene products that effect tissue repair processes including, cell migration and proliferation, and expression of proteolytic enzymes to facilitate tissue remodelling. The recombinant MDA MB435 H1 cell line which expresses high levels of HGF, has a more aggressive phenotype than the parental cell line, and gives rise to substantially more lung metastases when implanted into the mammary fat pad. The cell line will be grown in vitro and implanted in vivo for use in the study of tumour invasion and angiogenesis.

Recipient or parental organism

MDA MB435: One of several human breast tumour cell lines (see also MDA MB231) in routine use as xenograft models. Tumourigenic in animals, although not likely to survive outside of defined culture medium.
MDA MB435 cells have been modified by the introduction of the human hepatocyte growth factor gene in an expression plasmid, pBAT which contains a full length (~2.2kb) cDNA encoding HGF. Expression is driven from sequences in that plasmid which encode the chicken beta actin promoter. The cells have also been transformed with another plasmid, pSV2neo, which encodes sequences for neomycin resistance (for in vitro selection). The expression construct allows for the secretion of a pro-HGF molecule which is proteolytically cleaved to the active heterodimer.

Origin & function

The expression plasmid pBAT contains a full length (~2.2kb) cDNA encoding human hepatocyte growth factor. The cDNA sequence contains the appropriate signal sequences for processing of secreted proteins. The transfected cells have been shown to secrete HGF as reported by Meiners et al (Oncogene. 1998 Jan 8;16(1):9-20). It is anticipated that the product will be produced in an active form. Hepatocyte growth factor (HGF) is a 92kDa heterodimeric growth factor, secreted as a single chain pro-form from cells of mesenchymal origin. Proteolytic cleavage to the mature heterodimer is thought to occur at sites of tissue damage in the adult, and is associated with tissue remodelling in the developing embryo. HGF binds to and activates the tyrosine kinase receptor c-met, the expression of which is largely confined to epithelial cells. Thus in the adult body, HGF is secreted as a pro-form and is activated as required for tissue repair, and through activation of c-met, initiates epithelial pathways needed for repair processes such as cell migration, proliferation and the secretion of proteolytic enzymes. The intended function is to confer relevant properties on the cell line as a model for the study of tumour invasion and angiogenesis.

Evaluation of foreseeable effects

The modification is anticipated to confer a more aggressive and more metastatic phenotype on the recombinant than is exhibited by the parental cell line. It was judged unlikely that any harmful effects other than localised immune response would occur if in the worst case scenario of accidental injection while transplanting the tumour cells into mice. The probability of the cells establishing a tumour in a healthy non-syngeneic person in the case of accidental inoculation was judged to be negligible. The consequences of handling the cells detailed herein are no more severe than those considered for many other human tumour cell lines. The UKCCCR recommend that all work with human tumour cells is conducted at level 2 but the Health and Safety Executive (HSE) have indicated that this requirement can in general be treated as a COSHH issue and disregarded for the purposes of risk classification. However, after consultation, HSE have indicated that as a human tumour cell line, with anticipated increased aggressiveness and metastatic potential associated with the modification, and the potential for accidental inoculation, this work should be categorised as risk class 2. The resulting risk categorisation has arisen as a consequence of this advice.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All other relevant measures required at Cl2 will be applied except for the presence of an autoclave in the building. At Alderley Park, autoclaves are not present in each and every building where work with Risk Class 2 organisms may be conducted. In some buildings, autoclaves are present, but are available only for media and glassware sterilisation. As described in full below, the waste management system involves a central autoclave facility and a collection and transport process which we judge to provide an equivalent level of protection.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management procedures at Alderley Park make use of a central autoclave facility with suitable measures for collection and transport from the site of production to the autoclave. Briefly, the standard site procedures for disposal of relevant waste are as follows: (a) Liquid wastes: In general, the site procedures involve treatment of infectious liquid waste with 2.5% Chloros or 1% Virkon (final concentrations) for at least 30 minutes. This treated liquid mixture is then flushed to drain using copious amounts of water. In some instances other specified validated disinfectants are used for specific purposes including Savlon hospital concentrate. 70% ethanol is commonly employed for routine surface decontamination. (b) Solid wastes (other than sharps): are placed in an autoclave bag and when sufficiently full, the neck is twisted closed and the bag placed in a second autoclave bag which is sealed with autoclave tape. The bag is then placed in a specified location in the laboratory, pending collection. The closed bags are collected daily by Shift Technicians who transport them in robust wheeled and lidded containers to an on-site autoclave for inactivation in an autoclave located in a specialised facility on site. This procedure has been validated using a 12-point thermocouple procedure in which probes are placed in a
representative load. After autoclaving, waste is collected by a contractor for final disposal by off-site incineration. (c) Sharps are placed in suitable purpose-designed robust containers with a lid and when full are placed for collection and final disposal by incineration by our waste contractor.

Measures for disinfection of any accidental spills are set out in the Alderley Park Biosafety Manual and are based on appropriate use of the above disinfectants. Briefly, liquid spills are covered with paper towels and disinfectant added from the perimeter. After the specified period required for inactivation, the towels are removed into a container for final collection and final disposal by either autoclaving as described above, or where the disinfectant is incompatible with that process, by incineration off-site. All waste materials are transported in sturdy leak-proof containers.

Disinfection procedures lead to at least an 8 log reduction in viable bacterial cell count. The disabled bacterial host strains used do not survive well in the environment even if untreated. The viable count in liquid waste samples after disinfection is estimated as 0-10/ml and is likely to be at or close to the lower end of that range. With associated water used to flush treated liquid waste to drain, the concentration of any bacteria in such waste is negligible.

The mammalian cells used in this work cannot survive outside a narrow range of temperature and nutrient conditions and even untreated it is anticipated that none would survive soon after disposal to drain. Given that all relevant liquid waste is treated with either 2.5% bleach (hypochlorite) or 1% Virkon, the survival rate is effectively zero.

Approved at risk class 2 after consultation with the Health and Safety Executive. The risk classification of this particular proposal posed some particular difficulties. For this reason, the advice of HSE was sought. Their view was that the potential increased hazard associated with the modification, in conjunction with the UKCCR recommendation regarding CL2 measures as a minimum requirement for work with human tumour cells meant that risk Class 1 was inappropriate.

**Project Containment**

<table>
<thead>
<tr>
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</tr>
</thead>
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</tr>
<tr>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 108/01.7
Adenoviral mediated expression of human AKT that has been modified such that it is no longer oncogenic. The aim of the experiment is to deliver modified human AKT to all cells in a culture and measure the effect on apoptosis, cell proliferation and protein phosphorylation profiles.

Recipient or parental organism

CLONING HOSTS:
DH10B: Disabled E coli strain. Genotype: F^-mcrAd(mrr^-hsdRMS^-mcrBC) prophage 80lacZdM15 lacX74 deoR recA1 araD139 ara,leu)7697 gaiU galKlamdEl^-rpsL nupG.

BJ5183 Especially disabled E coli K-12 derivative with partial recombination capability to allow the recombination of pAdEasy-1 and pAdTrack-CMV. It is ampicillin and kanamycin sensitive.

EXPRESSION HOSTS:
A549: Authenticated human lung tumour cell line. ATCC Number: CCL-185. Reverse Transcript: negative. ATCC recommend BL1


Calu-6: ATCC Number: HTB-56. Derived from a human anaplastic carcinoma, probably lung. No special risk factors known with respect to patient origin. Tumorigenic in nude mice; forms poorly differentiated carcinoma; epithelial morphology in vitro. References date back to 1975. ATCC refer to Biosafety Level 1 but CL2 may be more appropriate given the tumorigenicity (albeit only cited in respect of nude mice).

HeLa: Human tumour cell line. Reverse transcriptase negative. Contains residual human papilloma virus sequence with associated potential for complementation of the E1a deletion in recombinant Adenoviral systems. ATCC recommend handling at Biosafety Level 2.

HT29: ATCC Number: HTB-38. Human colorectal adenocarcinoma; Tumorigenic in nude mice; forms well differentiated adenocarcinoma consistent with colonic primary (grade 1); tumors also from in steroid treated hamsters. No virus particles observed. Oncogene: myc +; ras +; myb +; fos +; sis +; p53 +; abl -; src -. The line is positive for expression of c-myc, K-ras, H-ras, N-ras. N-myc oncogene expression was not detected. The p53 antigen is overproduced. ATCC recommend BioSafety Level 1.

LNCaP: clone FGC [LNCaP.FCG] Available from ATCC. References date from at least 1980. Tissue: human carcinoma; prostate; from metastatic site: left
supraclavicular lymph node. Tumorigenic in nude mice. 

MCF7: The MCF7 line is derived from a human mammary gland adenocarcinoma and retains several characteristics of differentiated mammary epithelium Contains the Tx-4 oncogene. 

OVCA-3: The NIH:OVCA-3 line was established in 1982 by T C Hamilton, et al. From the malignant ascites of a patient with progressive adenocarcinoma of the ovary. Tumorigenic in nude mice. Described by ATCC as appropriate for work at Biosafety Level 1. 


PC-3: ECACC Ref No: 90112714. Established from a grade 4 prostatic adenocarcinoma from a 62 year old male Caucasian. 

Cloning Vectors: 

pAdTrack-CMV: A 9.2 kbp vector obtained from Bert Vogelstein (He et al PNAS 1998 vol 95 p2509-2514) and is used in conjunction with pAdEasy-1 to generate recombinant adenoviral plasmids in E. coli. The resulting recombinant plasmid can be used to produce infective but replication-defective adenoviral particles following transfection of suitable mammalian host cells (eg HEK293) able to provide the E1a sequence in trans. 

pAdEASY-1: A 33.4 kbp vector obtained from Bert Vogelstein (He et al PNAS 1998 VOL 95 P2509-2514) which contains all the adenovirus type 5 sequences except nucleotides 1-3533 (encompassing the E1 genes) and 28, 130-30,820 (encompassing E3 region). The vector also contains an ampicillinR gene and an origin of replication from PBR322, hence the plasmid is mobilisation defective (Bom+ Tra- Mob-). The vector is used in conjunction with pAdTrack vectors to generate recombinant adenoviral plasmids in E. coli (eg when co-transformed into a bacterial host with the pAdTrack-CMV shuttle vector containing an insert sequence, recombination in the host leads to production of a recombinant pAdEasy-1 containing insert sequences. That plasmid can be used to transfect appropriate mammalian cells (eg. HEK293) to generate recombinant E1a-deleted adenoviral particles. 

Recombinant Virus Particles: Recombinant adenoviral vector based on: 

"Insert" Vector(s): recombinant pAdEASY-1; Virus Producer Cell Line: HEK293. 

HEK-293: Human embryonic epithelial cells stably expressing the transforming gene of human Type 5 Adenovirus. DNA from both the right and left ends of the viral genome is present. When used as a helper cell line production of recombinant adenoviral particles, production of wild-type virus (an ACDP Hazard Group 2 pathogen) can occur albeit at a low frequency. Two specific non-homologous recombination events would be required to generate replication-competent virus. In a supernatant rescue assay performed on the virus stock to check for replication competent adenovirus (ref: Dion et al J of Virol Methods 1996 vol56 p99-107 no replication competent virus was detected in 1x10e9 infectious particles. If virus stocks are repeatedly passaged in HEK293 cells replication-competent virus can appear after 9 passages. Methods to produce virus using pAdEasy-1 only require a maximum of three passages in HEK293 cells. Given this data, the risk of generating wild-type adenovirus is considered to be negligible using these procedures. The HEK cell line supplied by ATCC is tumorigenic in nude mice and ATCC recommend minimum containment at Biosafety Level 2. 

The resulting recombinant viral particles will be used for transfection of, and subsequent expression of products in, the mammalian host cells referred to above. 

Origin & function 

Origin of insert: The expression vector containing the modified human AKT construct was obtained from Dundee University. The original insert was derived initially by PCR from a human cdNA library. The modification is of the coding sequence such that the resulting protein will contain alanine residues in place of threonine at 308 and serine at 473 and will therefore be inactive. 

Intended function: When native AKT is expressed the phosphorylation of Threonine 308 and Serine 473 act synergistically to activate AKT. Here the modified human AKT protein contains alanine residues in place of Threonine 308 and Serine 473 and therefore when expressed should not be phosphorylated at these residues hence should not be active. The modified human AKT protein should act as a dominant-negative when expressed in mammalian cells and inhibit endogenous AKT, with possible effects.
on cell proliferation, apoptosis and intracellular protein phosphorylation profiles. These effects are those that are to be analysed as part of this work however effects on other pathways eg glycogen synthesis pathway are also possible. The modification of the threonine and serine residues to alanine residues results in a functional protein that acts as a dominant negative. The protein is not active as a kinase. The activity anticipated is inhibition of endogenous AKT kinase.

**Evaluation of foreseeable effects**

Taking a very precautionary approach, the likelihood of potentially harmful effects being realised as a consequence of exposure of the operator or environment to the viral vectors was judged to be low. Risks associated with other GMOs are judged to be negligible.

The greatest risk of accidental infection arises during the preparation of concentrated virus stocks and aliquotation. Before that the virus is present in a very diluted state and large volumes would have to be involved to produce a significant infection. After aliquotation only small aliquots are handled at any one time and effectively, the complete aliquot would have to be ingested to generate a significant infection.

It is conceivable that infection may induce an immune response if large numbers of cells are infected initially. There is no evidence that the expression of the inserted gene could have an additional harmful effect in vivo. The protein should act as a dominant negative when expressed in mammalian cells and inhibit endogenous Akt, with possible effects on cell proliferation, apoptosis and intracellular protein phosphorylation profiles. Normal AKT is expressed in all tissues throughout the body. Hence in tissues that require cell division for normal function, in theory, if the handling of the dominant negative virus stock resulted in an accidental infection eg. through aerosols, this could lead to an abnormal hypoplastic status. The risk of this however is considered to be very low due to the containment measures put in place. Because normal AKT appears to have several functions within the cell an accidental infection could also result in the perturbation of other pathways apart from proliferation, but again this risk is considered to be very low. Because AKT is an intracellular serine/threonine kinase and is highly unlikely to be secreted by the host cells, the target is inaccessible in cells other than those in which it is expressed. Any effects would thus be restricted to the cells initially infected.

A further possible risk which has also been taken into consideration is the chance of homologous recombination between the insert sequence and the wild-type gene following transfection of the HEK293 cells to produce a replication-defective recombinant but one able to express the non-mutated form of the gene. If this did occur and if such expression was abnormally high or non-regulated the resulting expressed protein could be oncogenic. The chance of this happening is very low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Measures for which an exemption is claimed: All CL2 measures will be applied with the exception of the requirement for an autoclave to be available in each building where activities are carried out. The waste management procedures used at Alderley Park are judged to provide an equivalent level of protection. The measures used are included in the section below dealing with waste management. An exemption from the requirement for an autoclave to be present in each building is therefore requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste management procedures at Alderley Park make use of a central autoclave facility with suitable measures for collection and transport from the site of production to the autoclave. Briefly, the standard site procedures for disposal of relevant waste are as follows. (a) Liquid wastes: In general the site procedures involve treatment of infectious liquid waste with 2.5% Chloros or 1% Virkon (final concentrations) for at least 30 minutes. This treated liquid mixture is then flushed to drain using copious amounts of water. In some instances other specified validated disinfectants are used for specific purposes including Savlon hospital concentrate. 70% ethanol is commonly employed for routine surface decontamination. (b) Solid wastes (other than sharps): are placed in an autoclave bag and when sufficiently full, the neck is twisted closed and the bag placed in a second autoclave bag which is sealed with autoclave tape. The bag is then placed in a specified location in the laboratory, pending collection. The closed bags are collected daily by Shift Technicians who transport them in robust wheeled and lidded containers to an on-site autoclave for inactivation in an autoclave located in a specialised facility on site. This procedure has been validated using a 12-point thermocouple procedure in which probes are placed in a representative load. After autoclaving, waste is collected by a contractor for final disposal by off-site incineration. (c) Sharps are placed in suitable purpose-designed robust containers with a lid and when full are placed for collection and final disposal by incineration by our waste contractor.

Measures for disinfection of any accidental spills are set out in the Alderley Park Biosafety Manual and are based on appropriate use of the above disinfectants. Briefly,
Liquid spills are covered with paper towels and disinfectant added from the perimeter. After the specified period required for inactivation, the towels are removed into a container for final collection and final disposal by either autoclaving as described above, or where the disinfectant is incompatible with that process, by incineration off-site. All waste materials are transported in sturdy leak-proof containers.

Disinfection procedures lead to at least an 8 log reduction in viable bacterial cell count. The disabled bacterial host strains used do not survive well in the environment even if untreated. The viable count in liquid waste samples after disinfection is estimated as 0-10/ml and is likely to be at or close to the lower end of that range. With the associated water used to flush treated liquid waste to drain, the concentration of any bacteria in such waste is negligible. Adenoviruses are inactivated by a number of disinfectants including hypochlorite based agents and Virkon (Antec) both of which are specified for use at Alderley Park. The titres of recombinant adenoviral vectors in concentrated stocks can approach 10 (to the power of 11) pfu. Routine titres in discard flasks would be at least two orders of magnitude less than this and probably substantially less than this. Contaminated liquid waste is diluted into at least a 10-fold excess of Virkon such that the final concentration of the disinfectant is at least 1% the concentration recommended by the manufacturer. The maximum concentration of viral particles in the disinfectant solution is estimated to be 10(to the power of 6) particles per ml. Under the conditions of use, the disinfectant produces a log kill of at least 10(to the power of 5), so that the material discarded to drain contains <10 infectious particles per ml. Copious volumes of water accompany disposal of waste to drains so that the titre of infectious particles is less than 1 per mil. The presence of any residual infectious particles, which are anyway replication defective, thus presents a negligible risk of harm to human health or to the environment. The mammalian cells used in this work cannot survive outside a narrow range of temperature and nutrient conditions and even untreated it is anticipated that none would survive soon after disposal to drain. Given that all relevant liquid waste is treated with either 2.5% bleach (hypochlorite) or 1% Virkon, the survival rate is effectively zero.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Following a precautionary approach, the proposal was approved at risk class 2 following provision of further information requested from the proposer which has been incorporated into the above.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Project Ref  108/01.8
GENERATION OF RECOMBINANT ADENOVIRUS CODING FOR A NATIVE AND MODIFIED HUMAN KINASE AND TRANSIENT EXPRESSION IN MAMMALIAN CELLS.

Purposes of the contained use
To deliver the kinase gene to all cells in a culture and to measure the effect of expression of this gene on transcript expression profiles.

Recipient or parental organism
Cloning hosts:
Disabled E. coli K12 strains:
DH5alpha: Genotype: F^-supE44 hsdR17 recA1 gyrA96 endA1 thi-1 relA1 deoR pro phage 80d(lacZYA^-argF)u169(m801acZdM15)
DH10B: Genotype: F^-mcrAd(mrr^-hsdRMD^-mcrBC) prophage 80dlacZdM15 dlacX74 deoR recA1 endA1 araD139d(ara,leu)7697 galU galKlambda^-rpsL nupG

BJ5183 Especially disabled E. coli K-12 derivative with partial recombination capability to allow the recombination of pAdEasy-1 and pAdTrack-CMV. It is ampicillin and kanamycin sensitive.

Virus Producer Cell Line: HEK293
Expression Host Cells: HeLa, primary human aortic endothelial cells (HAEC); primary human umbilical vein endothelial cells (HUVEC)

Host/vector system
pAdrack-CMV: A 9.2 kbp vector obtained from Bert Vogelstein (He et al PNAS 1998 VOL 95 P2509-2514) and is used in conjunction with pAdEasy-1 to generate recombinant adenoviral plasmids in E.coli. The resulting recombinant plasmid can be used to produce infective but replication-defective adenoviral particles following transfection of suitable mammalian host cells (eg HEK293) able to provide the E1a sequence in trans.

pAdEASY-1: A 33.4 kbp vector obtained from Bert Vogelstein (He et al PNAS 1998 vol 95 p2509-2514) which contains all the adenovirus type 5 sequences except nucleotides 1-3533 (encompassing the E1 genes) and 28, 130-30,820 (encompassing E3 region). The vector also contains an ampicillinR gene and an origin of replication from pBR322, hence the plasmid is mobilisation defective (Bom+ Tra-Mob-). The vector is used in conjunction with pAdTrack vectors to generate recombinant adenoviral plasmids in E.coli (eg when co-transformed into a bacterial host with the pAdTrack-CMV shuttle vector containing an insert sequence, recombination in the host leads to production of a recombinant pAdEasy-1 containing insert sequences. That plasmid can be used to transfect appropriate mammalian cells (eg. HEK293) to generate recombinant E1a-deleted adenoviral particles.
Additional information for Recombinant Virus Particles.

Intended virus(es): Adenovirus

*Insert* Vector(s): recombinant pAdEASY-1.

PadE

Inserts originate from human cDNA from the Incyte collection. The proteins encoded by the inserts are serine/threonine kinases which are involved in a signalling pathway which, in response to extra-cellular factors, activates a transcription factor leading to ultimate expression of pro-inflammatory genes. The precise role of the kinases in linking the various extracellular signals with pro-inflammatory gene expression is unclear, though expression of a dominant negative mutant of these kinases is anticipated to inhibit - activation of a key pathway. Expression of the kinases will allow associated transcript profiles to be studied.

**Origin & function**

Generation of wild-type virus: Human embryonic epithelial cells stably expressing the transforming ene of human Type 5 Adenovirus are used to allow replication of viral particles by complementation of the vector defect. DNA from both the right and left ends of the viral genome is present. When used as a helper cell line production of recombinant adenoviral particles, production of wild-type virus (an ACDP Hazard Group 2 pathogen) can occur albeit at a low frequency. Two specific non-homologous recombination events would be required to generate replication-competent virus. In a supernatant rescue assay performed on the virus stock to check for replication competent adenovirus (ref: Dion et al J of Virol Methods 1996 vol56 p99-107 no replication competent virus was detected in 1 x10e9 infectious particles. If virus stocks are repeatedly passaged in HEK293 cells replication-competent virus can appear after 9 passages. Methods to produce virus using pAdEasy-1 only require a maximum of three passages in HEK293 cells. Given this data, the risk of generating wild-type adenovirus is considered to be negligible using these procedures.

Recombinant adenoviral particles:

The vector is a recombinant adenovirus type 5 capable of infecting human cells expressing adenovirus receptor in a non-productive manner, ie no replication of the virus is possible, however, for a limited period of time, the inserted gene will be expressed in infected cells at potentially high levels. No systemic adverse effect is expected should local infection occur although a localised immune response is conceivable.

The inserts encode serine/threonine kinases which are involved in the signalling pathway which, in response to extra-cellular factors, activates a target transcription factor leading to expression of pro-inflammatory genes. The precise role of these kinases in linking the various extracellular signals with pro-inflammatory gene expression is unclear, although expression of dominant negative mutants is anticipated to inhibit cytokine-mediated activation of the relevant pathway.

The greatest risk of accidental infection arises during the preparation of concentrated virus stocks and aliquotation. Before that the virus is present in a very diluted state and large volumes would have to be involved to produce a significant infection. After aliquotation only small aliquots are handled at any one time and effectively, the complete aliquot would have to be ingested to generate a significant infection. It is conceivable that infection may induce an immune response if large numbers of cells are infected initially. There is no evidence that the expression of the inserted gene could have an additional harmful effect in vivo. However, in vitro, certain cell types are sensitised to cytokine mediated apoptosis in the presence of inhibitors of the target a key constituent of the relevant pathway (ie dominant-negative form of the kinase of interest). Thus, both a large number of cells susceptible to sensitisation would have to be infected and a high concentration of cytokine would have to be present to induce apoptosis so this particular risk is negligible to low.

The only variants of the kinases which are being studied are the dominant negatives. There are no constitutively active forms proposed or made. Endothelial cells in tissue culture can be sensitised to apoptosis after cytokine treatment when all activity of the critical pathway target is inhibited. It is not clear whether this would also happen in vivo and whether inhibition of one kinase by a dominant negative would completely abolish activation of the target. The cytokine level required in vitro is much higher than would ever occur in vivo, with the exception of local production. In order to get an adverse effect, local cytokine production and recombinant adenovirus infection would have to occur simultaneously which is highly unlikely. In addition, apoptosis in vivo will be occurring naturally and it is likely that the effect of infection at anticipated levels even in the case of accidental exposure, will lead to marginal effects at worst.
The kinases are ubiquitously expressed but normally inactive unless the cells are stimulated by cytokines or bacterial lipopolysaccharide. It is unclear which isoform is rate limiting in which cells. The only imaginable effect would be if high amounts of wild-type kinase were expressed such that there could be activation of the target which would trigger an inflammatory response if enough cells were activated at the same location. The dominant-negative kinase would not have any effect in normal cells unless these cells were triggered to activate the target which one would then expect to be inhibited and suppress whatever process was triggering activation of the target.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All CL2 measures will be applied with the exception of the requirement for an autoclave to be available in each building where activities are carried out. The waste management procedures used at Alderley Park are judged to provide an equivalent level of protection. The measures used are included in the section below dealing with waste management. An exemption from the requirement for an autoclave to be present in each building is therefore requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste management procedures at Alderley Park make use of a central autoclave facility with suitable measures for collection and transport from the site of production to the autoclave. Briefly, the standard site procedures for disposal of relevant waste are as follows:

a) Liquid wastes: In general, the site procedures involve treatment of infectious liquid waste with 2.5% Chloros or 1% Virkon (final concentrations) for at least 30 minutes. This treated liquid mixture is then flushed to drain using copious amounts of water. In some instances other specified validated disinfectants are used for specific purposes including Savlon hospital concentrate. 70% ethanol is commonly employed for routine surface decontamination.

b) Solid wastes (other than sharps): are placed in an autoclave bag and when sufficiently full, the neck is twisted closed and the bag placed in a second autoclave bag which is sealed with autoclave tape. The bag is then placed in a specified location in the laboratory, pending collection. The closed bags are collected daily by Shift Technicians who transport them in robust wheeled and lidded containers to an on-site autoclave for inactivation in an autoclave located in a specialised facility on site. This procedure has been validated using a 12-point themocouple procedure in which probes are placed in a representative load. After autoclaving, waste is collected by a contractor for final disposal by off-site incineration. (c) Sharps are placed in suitable purpose-designed robust containers with a lid and when full are placed for collection and final disposal by incineration by our waste contractor.

Measures for disinfection of any accidental spills are set out in the Alderley Park Biosafety Manual and are based on appropriate use of the above disinfectants. Briefly, liquid spills are covered with paper towels and disinfectant added from the perimeter. After the specified period required for inactivation, the towels are removed into a container for final collection and final disposal by either autoclaving as described above, or where the disinfectant is incompatible with that process, by incineration off-site. All waste materials are transported in sturdy leak-proof containers.

Disinfection procedures lead to at least an 8 log reduction in viable bacterial cell count. The disabled bacterial host strains used do not survive well in the environment even if untreated. The viable count in liquid waste samples after disinfection is estimated as 0-10/ml and is likely to be at or close to the lower end of that range. With the associated water used to flush treated liquid waste to drain, the concentration of any bacteria in such waste is negligible.

Adenoviruses are inactivated by a number of disinfectants including hypochlorite based agents and Virkon (Antec) both of which are specified for use at Alderley Park. The titres of recombinant adenoviral vectors in concentrated stocks can approach 10 (to the power of 11) pfu. Routine titres in discard flasks would be at least two orders of magnitude less than this and probably substantially less than this. Contaminated liquid waste is diluted into at least a 10-fold excess of Virkon such that the final concentration of the disinfectant is at least 1%, the concentration recommended by the manufacturer. The maximum concentration of viral particles in the disinfectant solution is estimated to be 10 (to the power of 6) particles per ml. Under the conditions of use, the disinfectant produces a log kill of at least 10 (to the power of 5), so that the material discarded to drain contains <10 infectious particles per ml. Copious volumes of water accompany disposal of waste to drains so that the titre of infectious particles is less than 1 per ml. The presence of any residual infectious particles, which are anyway replication defective, thus presents a negligible risk of harm to human
health or to the environment. The mammalian cells used in this work cannot survive outside a narrow range of temperature and nutrient conditions and even untreated it is anticipated that none would survive soon after disposal to drain. Given that all relevant liquid waste is treated with either 2.5% bleach (hypochlorite) or 1% Virkon, the survival rate is effectively zero.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

Approved at risk class 2 following provision of further information requested from the proposer and which has been incorporated into the above.

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Large Scale Activities

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Human Clinical Applications

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Project Ref 108/01.9

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Non-GMM Consent Granted

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Project notified under transitional arrangements  N

Tick if notifying a connected programme of work  Y

02/03/2022
Expression of ligand-activated hormone receptors in human cells to evaluate effects of compounds with potential antagonist properties and to determine receptor selectivity.

**Recipient or parental organism**

Disabled E coli K strains:
- DH5alpha: Genotype: F^-supE44 hsdR17 recA1 gyrA96 endA1th^1 relA1 deoR phage80d(lacZYA^-argF)u169(m80lacZdM15)
- DH10B: Genotype: F^-mcrAd(mrr^-hsdRMS^-mcrBC) prophage 80dlacZdM15 dlacX74 deoR recA1 endA1 AraD139d(ara,leu)7697 galU galKlambda^-rpsL nupG
- BJ5183: Disabled E coli K-12 derivative with partial recombination capability to allow the recombination of pAdEasy-1 and pAdTrack-CMV. It is ampicillin and kanamycin sensitive.

Virus Producer Cell Line: HEK293 Human embryonic epithelial cells stably expressing the transforming gene of human Type 5 Adenovirus. DNA from both the right and left ends of the viral genome is present. When used as a helper cell line production of recombinant adenoviral particles, production of wild-type virus (an ACDP Hazard Group 2 pathogen) can occur albeit at a low frequency. Two specific non-homologous recombination events would be required to generate replication-competent virus. In a supernatant rescue assay performed on the virus stock to check for replication competent adenovirus (ref: Dion et al J of Virol Methods 1996 vol56 p99-107 no replication competent virus was detected in 1x10e9 infectious particles. If virus stocks are repeatedly passaged in HEK293 cells replication-competent virus can appear after 9 passages. Methods to produce virus using pAdEasy-1 only require a maximum of three passages in HEK293 cells. Given this data, the risk of generating wild-type adenovirus is considered to be negligible using these procedures.

Expression Host Cells:
- HeLa: Human tumour cell line. Reverse transcriptase negative. Contains residual human papilloma virus sequence with associated potential for complementation of the E1a deletion in recombinant Adenoviral systems. ATCC recommend handling at Biosafety Level2.

Host/vector system

Cloning Vectors used with DH5alpha; DH10B; BJ5183
- pAdrack-CMV: A 9.2 kbp vector obtained fromm Bert Vogelstein (He et al PNAS 1998 VOL 95 P2509-2514) and is used in conjunction with pAdEasy-1 to generate
recombinant adenoviral plasmids in E coli. The resulting recombinant plasmid can be used to produce infective but replication-defective adenoviral particles following transfection of suitable mammalian host cells (eg HEK293) able to provide the E1a sequence in trans.

PADEASY-1: A 33.4 kbp vector obtained from Bert Vogelstein (He et al PNAS 1998 vol 95 p2509-2514) which contains all the adenovirus type 5 sequences except nucleotides 1-3533 (encompassing the E1 genes) and 28, 130-30,820 (encompassing E3 region). The vector also contains an ampicillinR gene and an origin of replication from pBR322, hence the plasmid is mobilisation defective (Bom+ Tra- Mob-). The vector is used in conjunction with pAdTrack vectors to generate recombinant adenoviral plasmids in E. coli (eg when co-transformed into a bacterial host with the pAdTrack-CMV shuttle vector containing an insert sequence, recombination in the host leads to production of a recombinant pAdEasy-1 containing insert sequences. That plasmid can be used to transfect appropriate mammalian cells (eg. HEK293) to generate recombinant E1a-deleted adenoviral particles.

Additional Information for Recombinant Virus Particles

*Insert* Vector(s): recombinant pAdEASY-1
Virus Producer Cell Line: HEK293
Recombinant viral particles used for transfection of mammalian expression host cells.

### Origin & function

**Source of Nucleic Acid:** Human cDNAs

**Intended functions:** The receptors are ligand-activated hormone receptors which when activated regulate expression of target genes. Endogenous expression of the receptors is limited to only some cell types. Expression of the receptors in mammalian cells has been shown to yield biologically active proteins with potential for evaluation of the effects of compounds with potential antagonist properties and to determine receptor selectivity.

**Expression of the receptors in mammalian cells has been shown to yield biologically active proteins.** There is the potential for accidental infection with the recombinant viral particles. The greatest risk is judged to arise during the preparation of concentrated virus stocks and aliquotation. Before that the virus is present in a very diluted state and large volumes would have to be involved to produce a significant infection. After aliquotation only small aliquots are handled at any one time and effectively, exposure to the complete aliquot would have to be ingested to generate a significant infection. Since the vector is not replication competent, any harmful effects will be limited to cells directly infected following accidental exposure. Depending on the titre, this could be a greater or lesser number. It is conceivable that infection may induce an immune response if large numbers of cells are infected initially. There is no evidence that the expression of the inserted gene could have an additional harmful effect in vivo. There is no evidence that expression of the receptor is deleterious to cells (ie. transient expression of the receptor in cultured cells in the presence of hormone does not kill cells nor does it induce oncogenic effects. However, induction of inducible genes may change the normal physiology of a cell. It is not known how extensive exactly those changes would be because it is uncertain whether receptor expression alone is sufficient for induction of - hormone-dependent genes. Some genes may be inhibited by the presence of the receptor which may change the physiology of the cell to an unknown extent. The recombinant adenoviral vector could confer similar altered properties on cells accidentally infected with this vector. Although expression of the receptor in cells which may be accidentally infected is likely to be transient, it could persist for days/weeks. Taking a very precautionary approach, the likelihood of potentially harmful effects being realised as a consequence of exposure of the operator or environment to the viral vectors was judged to be low. Risks associated with other GMOs are judged to be negligible.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All CL2 measures will be applied with the exception of the requirement for an autoclave to be available in each building where activities are carried out. The waste management procedures used at Alderley Park are judged to provide an equivalent level of protection. The measures used are included in the section below dealing with waste management. An exemption from the requirement for an autoclave to be present in each building is therefore requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste management procedures at Alderley Park make use of a central autoclave facility with suitable measures for collection and transport from the site of production to
the autoclave. Briefly, the standard site procedures for disposal of relevant waste are as follows:

a) Liquid wastes: In general, the site procedures involve treatment of infectious liquid waste with 2.5% Chloros or 1% Virkon (final concentrations) for at least 30 minutes. This treated liquid mixture is then flushed to drain using copious amounts of water. In some instances other specified validated disinfectants are used for specific purposes including Savlon hospital concentrate. 70% ethanol is commonly employed for routine surface decontamination.

b) Solid wastes (other than sharps): are placed in an autoclave bag and when sufficiently full, the neck is twisted closed and the bag placed in a second autoclave bag which is sealed with autoclave tape. The bag is then placed in a specified location in the laboratory, pending collection. The closed bags are collected daily by Shift Technicians who transport them in robust wheeled and lidded containers to an on-site autoclave for inactivation in an autoclave located in a specialised facility on site. This procedure has been validated using a 12-point themocouple procedure in which probes are placed in a representative load. After autoclaving, waste is collected by a contractor for final disposal by off-site incineration. (c) Sharps are placed in suitable purpose-designed robust containers with a lid and when full are placed for collection and final disposal by incineration by our waste contractor.

Measures for disinfection of any accidental spills are set out in the Alderley Park Biosafety Manual and are based on appropriate use of the above disinfectants. Briefly, liquid spills are covered with paper towels and disinfectant added from the perimeter. After the specified period required for inactivation, the towels are removed into a container for final collection and final disposal by either autoclaving as described above, or where the disinfectant is incompatible with that process, by incineration off-site. All waste materials are transported in sturdy leak-proof containers.

Disinfection procedures lead to at least an 8 log reduction in viable bacterial cell count. The disabled bacterial host strains used do not survive well in the environment even if untreated. The viable count in liquid waste samples after disinfection is estimated as 0-10/ml and is likely to be at or close to the lower end of that range. With the associated water used to flush treated liquid waste to drain, the concentration of any bacteria in such waste is negligible.

Adenoviruses are inactivated by a number of disinfectants including hypochlorite based agents and Virkon (Antec) both of which are specified for use at Alderley Park. The titres of recombinant adenoviral vectors in concentrated stocks can approach 10^11 pfu. Routine titres in discard flasks would be at least two orders of magnitude less than this and probably substantially less than this. Contaminated liquid waste is diluted into at least a 10-fold excess of Virkon such that the final concentration of the disinfectant is at least 1%, the concentration recommended by the manufacturer. The maximum concentration of viral particles in the disinfectant solution is estimated to be 10^6 particles per ml. Under the conditions of use, the disinfectant produces a log kill of at least 10 (to the power of 5), so that the material discarded to drain contains <10 infectious particles per ml. Copious volumes of water accompany disposal of waste to drains so that the titre of infectious particles is less than 1 per ml. The presence of any residual infectious particles, which are anyway replication defective, thus presents a negligible risk of harm to human health or to the environment. The mammalian cells used in this work cannot survive outside a narrow range of temperature and nutrient conditions and even untreated it is anticipated that none would survive soon after disposal to drain. Given that all relevant liquid waste is treated with either 2.5% bleach (hypochlorite) or 1% Virkon, the survival rate is effectively zero.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Approved at risk class 2 following provision of further information requested from the proposer and which has been incorporated into the above.
Purposes of the contained use

Development and application of recombinant viral vectors for target identification, drug discovery and development using procedures involving:

- production of human sense or anti-sense cDNA or genomic libraries for screening using functional cloning studies with transfected mammalian cells. The latter will be recombinants of the following types:
  - reporter constructs (eg genes encoding marker proteins such as fluorescent proteins, linked to specific promoters eg associated with various cell signalling pathways).
  - library constructs eliciting biological effects, such as responses to specific hormones or other signalling molecules, which will be monitored after transfection.
- production of recombinant mammalian cells expressing wild-type and altered human genes to investigate mechanism of action of the gene products by monitoring the
effects of specific alterations on the predicted biological effect(s).

* production of recombinant mammalian cells expressing products of defined human genes of suspected by uncertain function to investigate the nature of potential substrates (e.g., where protein substrates are suspected, by studying incorporation of labelled precursors into those molecules in control and transfected cells).

* production of recombinant mammalian cells incorporating human transcription factors which may have a role in differentiation and monitoring the recombinants for subsequent development of specific differentiation markers.

* production of recombinant cells expressing tagged human proteins to investigate protein-protein interactions by immunoprecipitation and identification of tag-associated proteins.

In some cases, expression of products will be regulated using specific inducible expression systems (e.g., tetracycline-responsive elements).

Recipient or parental organism

Initial cloning studies will be carried out using E. coli strains such as DH5a, BJ5183 or DH10B which are all disabled as defined by ACGM and are unable to survive in the environment outside of laboratory culture conditions for extended periods. They are not pathogenic nor are they likely to colonise the gut of humans or of animal species.

A variety of human and rodent cells will be used as mammalian expression hosts. Human cells will be primary cell cultures and established cell lines. Any harm associated with these cells is restricted to potential adventitious agents or, in the case of human tumour cells, to the very low risk of establishment in the case of accidental inoculation. None of the cells to be used are derived from persons who will be working with these so that this risk is judged to be very low indeed. None of the mammalian cells are able to survive in the environment.

In some cases, mammalian cells will be used for the generation of recombinant adenoviral or retroviral particles. These particles can infect a wide range of mammalian cells but they are not replication competent so that any effect is restricted to cells which are initially transfected. Given the volumes and anticipated titre in culture media or in suspensions of the viral particles titres there are no likely environmental effects.

Accidental exposure of persons working with viral vector systems could lead to infection of a limited number of cells in exposed tissues. In such cases, foreseeable effects due to viral components of the vectors are restricted to inflammatory effects associated with immune responses. These are likely to be mild and transient given foreseeable accidental exposure. The effects associated with expression of the insert sequences are less predictable but if any, would be anticipated to vary according to the specific insert as set out below.

Host/vector system

The specified bacterial hosts will be used with the following vectors:

pLNCX
pLPCX
pCI
pVPACK
pAdEasy-1
pAdTrack-CMV
pAdTrack
pCDNA
pRevTet-On
pVPACK-VSV-G
pVPACK-ampho
pVPACK-10A1
Mammalian cells will be modified using either recombinant viral vectors (please see below) or directly with the following:

- pAdTrack-CMV
- pCDNA

Replication-defective recombinant tetroviral or adenoviral particles will be used to transfect the mammalian cells hosts. These particles are constructed using the following vectors in appropriate packaging cells or in cells otherwise complementing the replication defects:

<table>
<thead>
<tr>
<th>VECTORS CONTAINING INSERT SEQUENCES</th>
<th>HELPER PLASMIDS</th>
<th>PRODUCER CELL LINE</th>
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</thead>
<tbody>
<tr>
<td>pRevTet-on</td>
<td>pVPACK-VSV-G</td>
<td>293T</td>
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<tr>
<td></td>
<td>pClneo-GALV</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pVPACK-gp</td>
<td></td>
</tr>
</tbody>
</table>

| pBMN-CIP-SIN                        | pVPACK-VSV-G    | 293T               |
|                                     | pClneo-GALV     |                    |
|                                     | pVPACK-gp       |                    |
|                                     | pVPACK-10A1     |                    |
|                                     | pVPACK-ampho    |                    |

| pLNCX/pLPCX                         | pVPACK-VSV-G    | 293T               |
|                                     | pClneo-GALV     |                    |
|                                     | pVPACK-gp       |                    |
|                                     | pVPACK-10A1     |                    |
|                                     | pVPACK-ampho    |                    |

Recombinant pAdEasy-1  293

pLNCX: A non-mobilisable expression vector, derived from Moloney murine leukemia virus (MoMuLV), and Moloney murine sarcoma virus (MoMuSV) designed for retroviral gene delivery and expression. Upon transfection into a packaging cell line, pLNCV can transiently express, or integrate and stably express, a transcript containing psi+ (the extended viral packaging signal), the gene of interest, and a selectable marker. The 5' viral LTR in this vector contains promoter/enhancer sequences which control expression of the neomycin resistance (Neo r) gene for antibiotic selection in eukaryotic cells. A gene of interest can be cloned into the multiple cloning site immediately down stream of the human cytomegalovirus (CMV) immediate early promoter (PCMV). pLNCX also includes the E coli ColE1 origin of replication and ampicillin resistance gene for propagation and selection, respectively, in E. coli.

pLPCX: as for pLNCX but with a puromycin selection marker replacing neomycin resistance.

pCl: mobilisation-defective vector (contains minimal pBR322 nic site) from Promega. Strong CMV IE enhancer sequence, an optimized chimeric intron composed of the
donor site from the first intron of the human beta-globin gene and acceptor site from an immunoglobulin gene intron which increases expression from some cDNAs. Also includes SV40 late polyadenylation signal. These three elements combine to yield strong, constitutive expression of the cloned genes in mammalian cells. pC-neo (5474 bp) also contains neo r gene as a dominant selectable marker driven from the SV40 enhancer/early promoter. The vectors also contain a T7 promoter, an f1 origin of replication, and an Amp r gene.

pAdEasy-1: 33.4 kbp mobilisation defective vector (he et al PNAS 1998 vol 95 p2509-2514) which contains all the adenovirus type 5 sequences except nucleotides 1-3533 (encompassing the E1 genes) and 28, 130-30,820 (encompassing E3 region). The vector also contains an ampicillinR gene and an origin of replication from pBR322. The vector is used in conjunction with pAdTrack vectors to generate recombinant adenoviral plasmids in E. coli (eg when co-transformed into a bacterial host with the pAdTrack-CMV shuttle vector containing an insert sequence, recombination in the host leads to production of a recombinant pAdEasy-1 containing insert sequences. That plasmid can be used to transfect appropriate mammalian cells (eg HEK293) to generate recombinant E1a-deleted adenoviral particles.

pAdTrack-CMV: 9.2 kbp (He et al PNAS 1998 vol 95 p2509-2514) and is used in conjunction with pAdEasy-1 to generate recombinant adenoviral plasmids in E. coli. The resulting recombinant plasmid can be used to produce infective but replication-defective adenoviral particles following transfection of suitable mammalian host cells (eg HEK293) able to provide the E1a sequence in trans. The vector contains a polylinker surrounded by adenoviral sequences ("arms") that allow homologous recombination with pAdEasy-1. The left arm contains Ad5 nucleotides 34,931-35,935 and inverted terminal repeat and packaging signal sequences (nucleotides 1-480 of Ad5) required for viral production in mammalian cells. The right arm contains Ad5 nucleotides 3534-5790. The plasmid has a kanamycinR gene from pZero2.1 (Invitrogen) and an origin of replication from pBR322, hence the plasmid is mobilisation-defective (Bom+ Tra- Mob-). The vector contains a CMV promoter for expression of heterologous genes and a GFP expression cassette driven by a second CMV promoter, to track virus production. Potential for generation of "wild-type" Type 5 Adenovirus (a Category 2 human pathogen) in cells expressing E1a gene (eg HEK 293).

pAdTrack: 8.3 kbp shuttle vector (He at al PNAS 1998 vol 95 p2509-2514) and is used in conjunction with pAdEasy-1 to generate recombinant adenoviral plasmids in E. coli. The resulting recombinant plasmid can be used to produce infective but replication-defective adenoviral particles following transfection of suitable mammalian host cells (eg. HEK293) able to provide the E1a sequence in trans. The vector contains a polylinker surrounded by adenoviral sequences ("arms") that allow homologous recombination with pAdEasy-1. The left arm contains Ad5 nucleotides 34,931-35,935 and inverted terminal repeat and packaging signal sequences (nucleotides 1-480 of Ad5) required for viral production in mammalian cells. The right arm contains Ad5 nucleotides 3534-5790. The plasmid has a kanamycinR gene from pZero2.1 (Invitrogen) and an origin of replication from pBR322, hence the plasmid is mobilisation-defective (Bom+ Tra- Mob-). The vector lacks the CMV promoter present in pAdTrack-CMV promoter, to track virus production. Potential for generation of "wild-type" Type 5 Adenovirus (a Category 2 human pathogen) in cells expressing E1a gene (eg HEK 293).

Recombinant pAdEasy-1: a recombinant plasmid containing human adenovirus type 5 sequences except for the E1A sequence. When transfected into a suitable producer cell line providing the E1 function in trans (eg HEK 293 cells), recombinant infectious viral particles are produced although these are not replication competent. There is a low but finite probability of recombination between the vector and the endogenous E1a sequences. In such circumstances the insert sequences would be lost from the resulting a devirus genome to produce a recombinant infectious "wild-type" human Type Adenovirus (an ACDP Hazard Category 2 pathogen).

pCDNA: Non-mobilisable vector unless a derivative modified in these respects Amp r and either Neo r, Hyg r, or Zeocin R. As the pCDNA series of vectors have both CMV and T7 promoters, it may be used for cloning in non-expressing strains such as HB101, DH5 alpha which do not express T7 RNA polymerase and also for expression in a host strains which constitutively expresses T7 RNA polymerase. Contains SV40ori sequence as well as bacterial origin of replication permitting replication in mammalian cells expressing SV40T.

pShuttle: identical to pAdTrack-CMV except it does not contain a CMV promoter or the GFP element.

pShuttle-CMV: identical to pShuttle but containing an CMV promoter and an SV40 polyadenylation signal flanking the multiple cloning site.

pBMN-CIP-Sin: SIN derivative of retroviral vector pBMBNZ with an EMCV IRES-puro fusion for selection of infected cells with puromycin. Latter has MMLV pol and eng
genes replaced with a multicloning site, allowing the insertion of heterologous DNA fragments. The retroviral packaging sequence ( ) is retained which allows incorporation of the vector into retroviral particles when co-transfected with suitable helper vectors or when used with suitable packaging cell lines stably expressing helper functions. The full-length Moloney LTRs are retained. The "SIN" designation indicates a self-inactivating vector. During integration of the provirus the 5’ LTR is inactivated (as well as the 3’LTR), which minimises the possibility of activating adjacent cellular genes. Mobilisation-defective in E. coli.

pRevTet-On. Mobilisation-defective 7.6 kb retroviral vector expressing the rtTA protein from a CMV promoter. The vector is derived from pLNCX, a Moloney murine leukemia virus (MoMuLV)-based vector. The 5’ viral LTR controls expression of the transcript that contains the viral packaging signal and the neoR gene for selection in mammalian cells. pRevTet-On also includes the ampR gene.

pCineo-GALV: Non-mobilisable pCI-neo based vector (pGEM non-mobilisable backbone). Plasmid contains a strong CMV IE enhancer driving expression of the full length GALV gene, an optimised chimeric intron composed of the donor site from the first intron of the human beta-globin gene and acceptor site from an immunoglobulin gene intron which increases expression from some cDNAs. Also includes SV40 laste polyadenylation signal. These three elements combine to yield strong, constitutive expression of the GALV gene in mammalian cells.

pVPACK vectors: Mobilisation-defective CMV-based vectors constructed to express retroviral structural components (gag, pol and env) in mammalian cells. The vectors include a gag-pol-expressing vector that is cotransfected with the retroviral expression vector together with one of four envelope (env)-expressing vectors. Because all of the cis and trans elements required to produce infectious virus are separated onto three plasmids, with minimal or no sequence overlap between the plasmids and most MMLV retroviral vectors, the probability of generating replication-competent retrovirus (RCR) by homologous recombination is greatly reduced. The retroviral elements have been cloned into the MCS in the following cassette: CMV-IE promoter-MCS-IRES-Puro-bGH plyA. The pVPACK vectors also contain the amp resistance gene and CoIE1 origin of replication from pBR322.

* pVPACK-gp: 10.7 kbp vector encoding MMLV gag-pol protein.
* pVPACK-VSV-G: 6.4 kbp vector encoding VSV-G protein. VSV-G recognises a phospholipid that is present on all cell types, which theoretically allows any mitotic cell to be efficiently infected. Additionally, VSV-G confers stability on the resulting viral particles which are potentially more robust than traditional eumphotropic retroviral vectors.
* pVPACK-ampho: 6.4 kbp vector encoding amphe env protein.
* pVPACK-10A1: 6.4 kbp vector encoding 10A1 env protein which allows the same cell-surface receptor to be recognised as the amphotropic env plus a second receptor; it can essentially infect any cell that an amphotropic virus can infect and, in some cases, with a higher efficiency.

Origin & function

Human sense or anti-sense cDNA or genomic libraries for screening using functional cloning studies with transfected mammalian cells. The latter will be recombinants of the following types:

* reporter constructs (eg genes encoding marker proteins such as fluorescent proteins, linked to specific promoters eg associated with various cell signalling pathways).
* library constructs eliciting biological effects, such as responses to specific hormones or other signalling molecules, which will be monitored after transfection.

Defined wild-type and altered human genes to investigate mechanism of action of the gene products by monitoring the effects of specific alterations on the predicted biological effect(s).

Defined human genes of suspected but uncertain function to investigate the nature of potential substrates (eg where protein substrates are suspected, by studying incorporation of labelled precursors into those molecules in control and transfected cells).

Human genes encoding transcription factors which may have a role in differentiation to monitor recombinants for subsequent development of specific differentiation markers.

Human genes modified to encode tags (eg FLAG, HIS-6; c-myc) human proteins to investigate protein-protein interactions by immunoprecipitation and identification of
Evaluation of foreseeable effects

Reporter constructs (e.g., genes encoding marker proteins such as fluorescent proteins, linked to specific promoters e.g., associated with various cell signalling pathways) are unlikely to lead to a harmful outcome since there are many reports of expression of such proteins in vitro and in vivo without obvious harmful effects.

For inserts encoding proteins predicted to elicit biological effects, such as responses to specific hormones or other signalling molecules, expression of these following accidental exposure is likely to lead to effects similar to those predicted for cells which have been experimentally transfected. Where accidentally exposed cells already express the products, the effects are anticipated to be minimal unless the level of expression associated with the vectors is significantly higher than that occurring naturally. In the case of anti-sense constructs, or where mutations are introduced which are anticipated or known to reduce or abolish specified activities of the associated products, then reduction of the activity of the corresponding naturally occurring gene products is anticipated, although this is likely to be transient and of variable degree.

Where inserts encode human transcription factors which may have a role in differentiation it is possible that accidental transfection could, depending on the susceptibility of cells exposed, lead to re-differentiation of those cells to the corresponding phenotype.

Where expression of products is regulated using specific inducible expression systems (e.g., tetracycline-responsive), then expression is the case of accidental exposure is likely to be very limited in the absence of the inducers. In most systems, significant levels of expression are not anticipated unless there is coincident exposure to inducers (e.g., individuals receiving tetracycline therapy in the case of the tetracycline-responsive system).

It is possible that where the function of the inserts is unknown or only partly known, that effects on proliferation including potential oncogenic effects, could be associated with some of these sequences. However, oncogenesis appears in general to involve more than one contributing change from the normal condition and it is unlikely that exposure would lead to this outcome although this cannot be excluded completely. The likelihood of accidental exposure leading to harm is judged to be negligible with the adoption of risk class 2 measures including the use of safety cabinets for work where aerosols may be generated.

Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

An autoclave for inactivation of waste is not present in every building where the relevant laboratories are located. However, all relevant waste is contained in double autoclave bags which are collected from the laboratories in sturdy wheeled containers provided with lids. These are transported to a centrally-located on-site autoclave for inactivation. No special measures will be used to control rodents or insects. Adequate control is judged to be in place with the existing standard facilities, particularly having regard to the nature of the recombinant organisms and vectors and the negligible risk of these being disseminated in the environment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid wastes (other than sharps): are placed in an autoclave bag and when sufficiently full, the neck is twisted closed and the bag placed in a second autoclave bag which is sealed with autoclave tape. The bag is then placed in a specified location in the laboratory, pending collection. The closed bags are collected daily by Shift Technicians who transport them in robust wheeled and lidded containers to an on-site autoclave for inactivation in an autoclave located in a specialised facility on site. This procedure has been validated using a 12-point thermocouple procedure in which probes are placed in a representative load. Annual independent thermocouple testing of the autoclave cycle is carried out by a specialist contractor. After autoclaving, waste is collected by a contractor for final disposal by off-site incineration. Sharps are placed in suitable purpose-designed robust containers with a lid and when full are placed for collection and final disposal by incineration by our waste contractor.

Liquid waste associated with mammalian cell culture and viral vectors is treated with 1% Virkon (final concentration) for at least 30 minutes. This treated liquid mixture is...
then flushed to drain using copious amounts of water. Liquid waste associated with recombinant bacteria is treated with 1% Virkon or 2.5% Chloros (hypochlorite; - 25000 ppm available Cl final concentration.

In a validation test with GFP serving as a marker, treatment of GFP expressing retroviral particles for 5 minutes at room temperature at Virkon concentrations as low as 0.2% reduces CFU to levels comparable to background fluorescence. A 1% solution (ie 50x) is routinely used to treat waste overnight at room temperature and provides for safe, effective destruction of retroviral particles. The kill rate is effectively 100%.

The following outlines an investigation into the use of Virkon for treatment of adenoviral waste products:

B-gas adenovirus stock = 4x10(9)/ml in DMEM + 10% foetal bovine serum.

The following treatments and controls were produced:

(a) 25 ul stock (1x10 8 virus) + 25ul 4% Virkon
(b) 25 ul stock (1x10 8 virus) + 25ul 2% Virkon
(c) 25 ul stock (1x10 8 virus) + 25ul 1% Virkon
(d) 25 ul stock (1x10 8 virus) + 25ul PBS (positive control)
(e) 25 ul DMEM(10%FBS) + 25ul 4% Virkon
(f) 25 ul DMEM(10%FBS) + 25ul 2% Virkon
(g) 25 ul DMEM(10%FBS) + 25ul 1% Virkon
(h) 25 ul DMEM(10%FBS) + 25ul PBS

After 5 minutes at room temperature, 2.5 ul (equivalent to 5x10(6) virus) aliquots were added to 1x10(6) HEK 293 cells in 5 ml DMEM + 10% FBS. These cells allow adenovirus to replicate so should amplify any infective particles. Final Virkon concentration in test culture was 0.0005%. Cells were stained for B-gal expression after 48 hrs incubation at 37 degrees C.

Results
(a) No blue cells
(b) No blue cells
(c) No blue cells
(d) 100% blue cells (cytopathic effect of adenovirus replication also noted as anticipated in HEK 293 cells)
(e) No blue cells
(f) No blue cells
(g) No blue cells
(h) No blue cells

The sensitivity with viral titre used allows conclusion that at least a 4 log reduction is achieved but the actual value is likely to approach 100% kill.

This data confirms for recombinant viral systems, the claims from the manufacturer for the efficacy of Virkon in respect of the corresponding wild-type viruses.

The mammalian cells used in this programme are highly dependent on the availability of appropriate nutrients and environmental factors. They will not survive even brief periods in water much less in the disinfectant solutions used for this work.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

02/03/2022
Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>

Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tr>
<td>L2</td>
<td>L3</td>
</tr>
<tr>
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Project Ref 108/09.1

Date Ackn'd: 16/04/2009

CU2 Project Title: Knockdown of a number of receptors using lentiviral transduction particles in a variety of mammalian cell types

Class: Class 2

CultureVol: < 1 Litre

Non-GMM: Consent Granted

Project notified under transitional arrangements: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use
To knockdown the protein of interest and determine effects on phenotype of cells.

Recipient or parental organism
A variety of mammalian cell lines

Host/vector system
Commercially available lentiviral particles. These Self-inactivating replication incompetent viral particles are produced in HEK293T packaging cells by co-transfection with packaging plasmids. The method consists of three plasmid system consisting of: A packaging vector which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions. The vesicular stomatitis virus G-protein envelope vector, which provides the heterologous envelope for pseudotyping, and the transfer vector, which contains the sequence of interest as well as the cis acting sequences necessary for RNA production and packaging. The multi-plasmid approach results in no single plasmid containing all the genes necessary to produce packaged lentivirus. Resulting particles are replication-incompetent and deletion in the U3 portion of the 3' LTR eliminates the promoter-enhancer region, further negating the possibility of viral replication. The system has also removed virulence genes which are not necessary for packaging. Transduction particles encoding sequences for the knockdown of the protein of interest, as well as positive and negative controls will be ordered.

Origin & function
Lentiviral particles are commercially available. Particles are to be used to knockdown the receptor at the mRNA level and assess any phenotypic effects.

Evaluation of foreseeable effects
Lentiviral particles are commercially available. Particles are to be used to knockdown the receptor at the mRNA level and assess any phenotypic effects. The protein of interest is a receptor whose function is executed via the binding of ligand. Activation of this receptor family induces signalling cascades that result in multiple biological responses, including cell proliferation, angiogenesis, differentiation, migration, and survival. It is unclear which of these phenotypic effects will be affected by knockdown of the protein of interest. Phenotypic effects are likely to be cell type specific. Low potential hazard should be associated with the expression of the knockdown product in cell lines. Cell lines may show effects on phenotypes mentioned above. But there are no reported effects on wound healing in the literature from knockout studies. Null mice show abnormalities in the lung but no aerosol producing activity is going to be used here, risk of lung toxicity is low. Mice also exhibit liver abnormalities, of cholesterol and bile acid synthesis, but it is very unlikely that the lentivirus would be exposed to a hepatocyte as no sharps are being used. There are no abnormalities of the immune system in null mice, hence if accidental ingestion of the lentivirus did occur and infect immune cells, immune system would not be compromised and cells would be cleared from the system. It is anticipated that the product will be correctly folded and active. The genes of interest are not a known oncogenes. Expression of the insert in the recombinant organisms is unlikely to affect the pathogenicity of the expression hosts. Contact of the replication deficient lentivirus will be avoided using appropriate containment measures as transduction of the cells of the worker could result in an unknown effect. In the worst case scenario, a replication competent lentivirus capable of knocking down the kinase is produced and then infects an individual via body fluid contact (e.g. sharps contact) or ingestion/inhalation. The individual would then be infected with a potentially damaging lentivirus with unknown effects. The virus would be transmissible by body fluid contact. The likelihood of this is negligible due to the system using 3 plasmids for production of the virus, i.e. no single vector contains all the genes necessary to produce packaged lentivirus. The particles are made using features of 2nd and 3rd generation lentiviral packaging systems. Genes for replication and structural proteins are absent in the packaged viral genome since these genes are supplied by other plasmids in the packaging cells. The viral genome contains only the region between the 5’ and 3’ LTRs of the promoter, hairpin sequence, puromycin resistance gene. In addition, the lentiviral vector contains a self-inactivating 3’ LTR that renders it unable to produce infectious virus once it integrates into the host chromosome. There are no known incidents of third generation systems producing replication competent virus - cell lines transfected with these particles can be monitored for replication competency using commercial p24 titer assays. A more likely scenario is that a worker contaminates themselves with a replication deficient lentivirus. The likelihood of harm to human health due to this is low because the lentiviral particles will elicit an immune response, hence will be rapidly removed by the immune system. Workers will also be using appropriate containment methods i.e. class 2 cabinet for pipetting all virus-containing supernatants. When pipetting virus-containing supernatants and transferring plates or flasks to and from the laminar flow hood, the production of aerosols will be avoided. All plasticware used to handle liquid containing virus particles will be disinfected with virkon solution. Media and other liquids which may contain infectious vector will also be inactivated before disposal. Although not required, all work with lentiviral particles will be done in a restricted access lab. The use of sharps with this work is also prohibited, reducing the risk of accidental exposure.
To body fluids and gloves will be worn throughout. All work is small scale.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures required at CL2 will be applied except for the presence of an autoclave in the building. At Alderley Park autoclaves are not present in all buildings where risk class 2 work takes place. In some buildings, autoclaves are present but are only available for media and glassware sterilisation. The waste management facility has a central autoclave facility and a collection and transport process, which we judge to provide an equivalent level of protection.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple bagged in biohazard waste bags, suitably labelled and autoclaved. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in the decontamination process. As a result there will be no viable GMMs remaining in the solid waste. All liquid waste will be disinfected with 1% virkon. Solutions will be left soaking for 2 hours before disposal via the drain with copious amounts of water. Procedures and relevant training and safety inspections are in place to ensure that all workers deal with waste correctly as specified above.

Project Containment

<table>
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Animal Units

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<td>L3 L4</td>
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Project Ref 108/10.1

Date Ackn’d CU2 Project Title

02/03/2022
The transfer of assays for certain enzymes from Invitrogen, who are currently
developing these assays, to in house cellular assays science area............

Date Project Ceased

24/03/2010

Class 2 ≤ 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Date of Additional Info

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Project Additional Information

Purposes of the contained use

The transduced cells will be used to identify inhibitors of the enzymes. Cells are lysed post compound dosing and a signal is detected using a FRET assay.

Recipient or parental organism

Human osteosarcoma cell line (ATCC HTB-96). No adventitious agents believed present. Note that Class 2 containment recommended by ACDP/UKCCCR but this can be considered a purely COSHH issue unless any modification is anticipated to increase any hazard. J. Ponten and E. Saksela derived this line (originally 2T) in 1964 from a moderately differentiated sarcoma of the tibia of a 15 year old girl. Viruses were not detected during co-cultivation with WI-38 cells or in CF tests against SV40, RSV or adenoviruses. Mycoplasma contamination was detected and eliminated in 1972.

Host/vector system

Non-mobilisable vector from Life Technologies based on pFastBac (see also Condreay et al, PNAS 96, 127-132, 1999). The AcNPV polyhedrin promoter has been replaced by the CMV IE enhancer/promoter. The mini-Tn7 element, which is transposed from pFASTBAC-1 into the bMON14272 vector in E coli DH10Bac cells, also contains a gentamycinR gene, multiple cloning site and SV40 poly A signal inserted between the left and right arms of Tn7. In addition the plasmid contains an ampicillinR gene and a pUC-based non-mobilisable vector backbone. The generation of the recombinant "Bacmid" requires the presence of a helper plasmid (pMON7124) encoding a transposase which functions in trans. The recombinant "Bacmid" can be used to transfect insect cells to produce recombinant polyhedrin- Baculovirus particles.

Origin & function

There are 2 substrate genes of interest to be expressed as a fusion protein with GFP. In addition there are both wild type and cysteine mutant (reduced/no activity) versions of the target enzymes. GFP is Green fluorescent protein that is widely used as a fusion protein, and whose only known function is fluorescence.

The two substrate genes are involved in cell growth, proliferation and apoptosis. Enhanced levels of both are upregulated in many tumours.

Evaluation of foreseeable effects

Potential hazards could come from the use of Baculovirus particles and the expressed enzymes/substrates, where the operator accidentally inhales, injects or digests the GMO and where it could come to the potential expression of the proteins as a gene product in the worker's cell or gut flora. Bacmam virus particles are only actively replicating in insect cells, although they are known to transduce mammalian cells and cause expression of the transduced genes in mammalian cells. Expression of some
of these enzymes in mammalian cells is expected to increase the levels or activity of their substrate proteins, which could result in increased tumourigenicity. Overexpression of the two substrates may lead to increased proliferation in certain cells. Due to the combination of the overexpression genes increasing proliferation, the lack of clarity around the required virus dose to cause a transformation in lung epithelium and lack of evidence how well complement can clear Bacmam virus from lung epithelium, the work flows will be enclosed in class II cabinets.

The overall risk of this work can be considered to be low as recombinant baculo virus particles have the potential to transduce mammalian cells, but the risk around the virus remains negligible, since the baculovirus is incapable of replicating in mammalian cells, as they do not contain the necessary transcription factor for viral replication. The efficacy of transfection in vivo appears to be limited primarily by complement depending inactivation of the baculo particles and is therefore much less efficient than in vitro. In respect to accidental uptake of the cells, the immune system is expected to inactivate and remove any foreign cell from the body. Nevertheless, due to the combination of the overexpression of a gene increasing proliferation, the lack of clarity around the required virus dose to cause a transformation in lung epithelium and lack of evidence how well complement can clear Bacmam virus from lung epithelium, the final GMO is classified as class 2

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All measures required at CL2 will be applied except for the presence of an autoclave in the building. At Alderley Park autoclaves are not present in all buildings where risk class 2 work takes place. In some buildings, autoclaves are present but are only available for media and glassware sterilisation. The waste management facility has a central autoclave facility and a collection and transport process, which we judge to provide an equivalent level of protection.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid GMM contaminated waste will be triple bagged in biohazard waste bags, suitably labelled and autoclaved. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in the decontamination process. As a result there will be no viable GMMs remaining in the solid waste. All liquid waste will be disinfected with 1% virkon. Solutions will be left soaking for 16 hours before disposal via the drain with copious amounts of water. Procedures and relevant training and safety inspections are in place to ensure that all workers deal with waste correctly as specified above.

**Is an emergency plan required according to regulation 20?**

- [ ] Y

- [ ] N

If yes, tick to confirm that it is attached to this form

- [ ] Y

- [ ] N

Tick to confirm that you have attached a risk assessment to this form

- [ ] Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

- [ ] Y

Please enter comments on the GM safety committee on the risk assessment

This proposal was approved by the GMSC on 4/2/10 as class 2

**Project Containment**

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### Project Additional Information

**Purposes of the contained use**

This proposed work aims to validate Lentiviral delivery as a means of rapidly generating stable mammalian cell lines for screening applications. Two established receptor assays will be transferred into the Lentiviral system.

**Recipient or parental organism**

HEK-293: Human embryonic kidney cells stably expressing the transforming gene of human Type 5 Adenovirus. DNA from nucleotides 1 to 4344 [Louis N et al Virology 1997 Jul 7;233(2):423-9] is integrated into chromosomes 19. When used as a helper cell line for production of recombinant adenoviral particles, production of wild-type virus (an ACDP Hazard Group 2 pathogen) can occur albeit at a low frequency. Two specific non-homologous recombination events would be required to generate replication-competent virus. In a supernatant rescue assay performed on the virus stock to check for replication competent adenovirus (ref: Dion et al J. of Virol Methods 1996 vol56 p99-107 no replication competent virus was detected in 1x10e9 infectious particles, If virus stocks are repeatedly passaged in HEK293 cells replication-competent virus can appear after 9 passages. Methods to produce virus using pAdEasy-1 only require a maximum of three passages in HEK293 cells. Given this data, the risk of generating wild-type adenovirus is considered to be negligible using these procedures. HEK293 cells have an unexpected relationship to neurons [Shaw, G et al; FASEB J. 2002 Jun;16(8):869-71], a finding that may require reinterpretation of many previous studies in which it was assumed that HEK293 cells resembled more typical kidney epithelial cells. The HEK cell line supplied by ATCC is stated to be tumorigenic in nude mice and to require minimum containment at Biosafety Level 2. Collectively, this information indicates a requirement for CL2. When making a risk assessment and classification for the purposes of the GMO.
The CHO-K1 cell line (ATCC CCL-61) was derived as a subclone from the parental CHO cell line initiated from a biopsy of an ovary of an adult Chinese hamster by T.T. Puck in 1957. The cells require proline in the medium for growth because they cannot convert glutamic acid to glutamic gamma-semialdehyde, leading to proline deficiency.

If well authenticated, these cells pose negligible risk and can be safely handled at CL1.

### Host/vector system

Lentiviruses belong to the HIV group of retroviruses. These are naturally occurring RNA viruses packaged into a capsid and a membranous envelope. Upon infection of the target cell the RNA is reversed transcribed and integrates randomly into the host genome. Lentiviruses contain none genes: three structural genes, gag (encodes virus core: capsid, matrix, nucleocapsid), pol (encodes enzymes: reverse transcriptase, integrase, protease, RNaseH) and env (encodes virus envelope); two regulatory genes, tat (upregulates viral transcription, binds TAR-responsive RNA element TAR) and rev (regulates viral RNA transport and splicing, binds rev responsive element RRE); and four accessory genes, (vif,vpr,vpu and nef) which are critical for in vivo replication and pathogenesis. Both pTZV and pTRIPZ lentiviral particles are designed to infect mammalian cells so there is a potential risk for human transduction. However, the virus is being provided by Open Biosystems in a ready-to-use format and will not be propagated on site. Furthermore, work will be carried out in a class II laminar flow hood due to the usage of tumour cell lines and no needles will be used. The lentiviral vectors from Open Biosystems have been designed to maximise their biosafety features. The envelope of HIV-1 has been completely removed from the vector and replaced with the VSV-G gene from Vesicular Stomatitis Virus to pseudotype (envelope) the virus particles. To minimise the threat of producing recombinant and new replication-competent virus, the genes are separated onto five different plasmids and contain no significant areas of homology. The expression vector contains a deletion in the 3' LTR that results in self-inactivation of the virus after transduction of the target cell. Additionally, the four packaging plasmids may allow expression in trans of the proteins required to produce virus but none of the plasmids contain LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are present in the packaged DNA.

### Origin & function

FGFR4: Fibroblast growth factor receptors of (FGFRs) are a family of 4-transmembrane receptor tyrosine kinases with functions executed through interactions with the FGF family of ligands. FGFR activation induces signalling cascades that result in multiple biological responses, including cell proliferation, angiogenesis, differentiation, migration and survival. FGFR4 is the weakest activator of the MAPK cascade and appears to have roles in cell motility, proliferation and tissue remodelling. (Sahadevan et al, J. Pathology (2007) 213, p82-90; Wang et al, Clinical cancer research, (2004) Vol 10, p6169-6178). PPARdelta: Human nuclear hormone receptors consist of a ligand-binding domain and DNA binding domain. Upon ligand binding, receptors are activated by either moving from the cytoplasm to the nucleus where they bind specific DNA sequences or through the induction of a conformational change that results in activation of the receptor already bound to its target sequence. PPAR agonists bind selectively to PPARα, causing it to activate specific DNA response elements and modulate gene transcription. The hybrid GAL4-PPARδ protein can be activated by PPAR ligands to modulate gene transcription from promoters that interact with the GAL4 DNA binding domain. Outcomes of PPARδ activation include induction of angiogenesis in endothelial cells, proliferation of hepatic stellate cells, increasing oxidative metabolism and promotion of adipocyte differentiation. The construct to be expressed in this proposal is a truncated form of PPARδ fused with a GAL4 DNA binding domain. This modification ensures that only genes linked to GAL4 responsive promoters will be induced by GAL4-PPARδ. Luciferases, derived from Renilla reniformis, Photinus pyralis or Pyrophorus piargophthalmalaries, catalyse the production of light using the substrates luciferin and ATP. In the absence of luciferin Luciferase has no known adverse effect.

### Evaluation of foreseeable effects

The final GMO cell lines could colonise the operators airways or colon if inhaled or ingested. FGFR4 is a 4-transmembrane receptor tyrosine kinase with functions executed through interactions with the FGF family of ligands. FGFR4 is the weakest activator of the MAPK cascade. It appears to have roles in cell motility, proliferation and tissue remodelling, and as such should be viewed as an oncogene. PPARδ is a nuclear hormone receptor that mediates activation and or repression of various downstream target genes. Known outcomes of PPARδ activation include induction of angiogenesis in endothelial cells, proliferation of hepatic stellate cells, increasing oxidative metabolism and promotion of adipocyte differentiation. If transferred to the cells of an operator this could lead to aberrant gene activation. However, the particular construct to be expressed is a truncated form of PPARδ fused with a GAL4 DNA binding domain. This modification ensures that only genes linked to GAL4 responsive promoters will be induced by GAL4-PPARδ overexpression or by activation of GAL4-PPARδ ligands. There is some literature evidence to suggest that GAL4 can activate transcription from a minimal TATA promoter in HeLa cells so it cannot be ruled out that it could activate endogenous promoters in mammalian cells. The construct will be
co-expressed with a UAS Luciferase reporter gene, leading to expression of Luciferase. Luciferases will cause the production of light using the substrate luciferin and ATP and in the absence of luciferin will have no known effect. Fluorescent protein expression will cause cells to emit a coloured light. EGFP is not known to cause any adverse effects in cells, it is unlikely that inhalation or ingestion of the final call line by a worker could result in delivery of any of the constructs to an appropriate site of action to realise any of these potential effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures required at CL2 will be applied except for the presence of an autoclave in the building. At Alderley Park autoclaves are not present in all buildings where risk class 2 work takes place. In some buildings, autoclaves are present but are only available for media and glassware sterilisation. The waste management facility has a central autoclave facility and a collection and transport process, which we judge to provide an equivalent level of protection

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple bagged in biohazard waste bags, suitably labelled and autoclaved. This solid waste will be compromised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in the decontamination process. As a result there will be no viable GMMs remaining in the solid waste. All liquid waste will be disinfected with 1% virkon. Solutions will be left soaking for 2 hours before disposal via the drain with copious amounts of water. Procedures and relevant training and safety inspections are in place to ensure that all workers deal with waste correctly as specified above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This proposal was approved by the GMSC on 16/02/2010 as class 2

Project Containment

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02/03/2022
Project Ref 108/10.3

Date Ackn'd 23/11/2010

CU2 Project Title Validation of new targets in a Pol Beta mutated cell line

Class 2 CultureVolClass2 < 1 Litre

CEased

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To identify potential new targets that would combine well with a base excision repair deficiency in DNA polymerase β (POLβ). Specifically to test the hypothesis that homologous recombination proteins are synthetic lethal with a dominant negative truncated version of Polβ, which mimics a tumour associated variant of Polβ.

Recipient or parental organism

A549 human lung carcinoma cancer cell line (ATCC#CCL-185)

Host/vector system

Mammalian Cell Expression Vector:
LZRS-MS-IRES-GRP. Non-mobilisable Episomal retroviral expression vector containing a IRES element and gene encoding neo resistance. The vector also contains full sequence 5’ and 3’ LTR regions. (refs: based on original plasmid described in Human gene therapy 7 1405-1413 1996 and Experimental cell res. Vol 312 no 17 p3336 2006). Mammalian Cell Expression host:
A549 (lung) cell line

Origin & function

All details of construction are within the following paper: Vens et al., Nuc. Acid Res, 2002 vol 30, no. 13, 2995-3004. Briefly, a cDNA encoding a 14-kDa truncated N-terminal sequence of POLB was generated by PCR of the pKSPOLB plasmid and cloned into LZRS-MS-GFP retroviral expression vector. Green fluorescent protein (GFP) is also expressed from the LZRS-MS vector.

DNA Polymerase Beta us a 39kDa single polypeptide protein that has been extensively characterised. Pol Beta catalyses the extension of DNA strands by the addition of
nucleotide triphosphates. Pol Beta is a distributive enzyme with no intrinsic proof-reading exonuclease activity. Pol Beta is required in the Base Excision Repair (BER) pathway of DNA repair and is responsible for replacing a single or a few nucleotides after removal of damaged bases by other elements of the bER machinery. Specifically, Pol Beta contributes to the repair of DNA damage occurring by alkylating agents Pol Beta also contains an N-terminal dRP lyase domain that catalyses the release of 5'-terminal deoxyribose phosphate residues from incised abasic sites which are intermediates in the BER process. Pol beta has DNA binding activity. The 14 kDa truncated version of Polβ is expressed from the LZRS-MS-GFP vector (please note that matched cells are stably transected with empty LZRS-MS-GFP vector) in stably transduced A549 lung cells, with the intention of recapitulating a tumour associated mutation. Truncated Polβ retains its' DNA binding activity, but is polymerase inactive and so behaves in a dominant negative (DN) fashion, preventing completion of BER.

Evaluation of foreseeable effects

Potentially Harmful effects associated with
(i) the recipient micro-organism(s) No  
(ii) the vector(s) Yes  
- Contains retroviral elements, notably intact 5' and 3' LTR regions  
(iii) the donor micro-organism(s) N/A  
(iv) the resulting genetically modified micro-organism(s) No

The 14kDa DN-PolB is a tumour associated variant of PolB and is thought to contribute to tumour progression through facilitating the accumulation of mutations and preventing correct repair via base excision repair pathway. However this form has also been described in 'normal' tissue, and it is suggested that DN-PolB may play a regulatory role at moderate expression levels (Ref: Starcevic et al., 2004 vol 3, no. 8, 998-1001). Hence overexpression may result in increased cellular proliferation of the final GMO and consequently could increase the chances of colonisation of lung epithelia if exposed. Due to the nature of the work, which uses small culture columns (maximum size T175 flask), in tissue culture hoods, the chance of contact with cells is minimal. Furhtermore, in a non-immunocompromised individual any foreign agents are expected to be cleared efficiently.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures required at CL2 will be applied except for the presence of an autoclave in the building. At Alderley Park autoclaves are not present in all buildings where risk class 2 work takes place. In some buildings, autoclaves are present but are only available for media and glassware sterilisation. The waste management facility has a central autoclave facility and a collection and transport process, which we judge to provide an equivalent level of protection.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

02/03/2022
This proposal was approved by the GMSC on 27/10/10 as Class 2

### Project Containment

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### Project Ref 108/11.1

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Non-GMM Consent Granted

Project notified under transitional arrangements

### Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units   Large Scale Activities   Human Clinical Applications
The use of Human umbilical cord vein endothelial cells (HUVECs) stably expressing either GFP or a form of a target human gene that is resistant to siRNA to the target gene can be confirmed that the phenotypic effects seen with the siRNAs are specific and not as a result of off target effects.

The aim of the current experimental programme is to rescue gene expression using endothelial cells that are stably expressing an siRNA resistant to the gene. By doing this it can be confirmed that the phenotypic effects seen with the siRNAs are specific and not as a result of off target effects.

The target protein functions to inhibit caspase activation, thereby leading to negative regulation of apoptosis or programmed cell death. This has been shown by disruption of the target protein induction pathways which leads to changes in apoptosis and tumour growth. The target protein is expressed highly in most human tumours and fetal tissue, but is completely absent in terminally differentiated cells. This fact therefore makes this protein an ideal target for cancer therapy as cancer cells are targeted while normal cells are left alone.

Within this programme 2 GMOs should be considered as being a potential hazard. The first is the cell line generated that over expresses the target gene.
poses are that it may be ingested or inhaled and result in colonization within the lungs or other epithelial cells and result in growth of the transduced cells. The second GMO is the virus particle which has the potential to enter the body of the operator through the skin, via ingestion or through inhalation and the consequences of this could be transduction and proliferation of the transduced operator cells. The target protein is an anti-apoptotic protein highly expressed during fetal development and cancer cell malignancy. The target protein controls the G2/M phase of the cell cycle by inhibiting apoptosis and promoting cell division. The target protein is known to be expressed across most tumour cell types and is at the same time absent in normal non-malignant cells. The target protein can be regarded as an oncogene as it is aberrant over expression in most cancer cells contributes to the phenotype of the cancer cells being more resistant to apoptotic stimuli and chemotherapeutic therapies and, thus, allowing for continued proliferation and survival. The potential hazard of over expressing the target protein would therefore be to enhance cell survival through increased cell division and decreased apoptosis resulting in the possibility of the cells acquiring a cancer cell phenotype.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures required at CL2 will be applied except for the presence of an autoclave in the building. At Alderley Park autoclaves are not present in all buildings where risk class 2 work takes place. In some buildings, autoclaves are present but are only available for media and glassware sterilisation. The waste management facility has a central autoclave facility and a collection and transport process, which we judge to provide an equivalent level of protection.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Media will be aspirated directly into virkon and the cells will be fixed with 4% formaldehyde prior to be visualised. All cell culture will be carried out in a class 2 cabinet and the operator will wear PPE at all times.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved as class 2 by GMSC on 23/08/11

Project Containment

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</table>
The objective of this work is to generate a stable mammalian cell line that over expresses CYP17 in a cell line that is null for CYP17 expression. The cell line once established will be used for selection studies against cell lines that express the Androgen Receptor.

LnCAP cells LNCaP clone FGC [LNCaP.FGC] Available from ATCC. References date from at least 1980. Tissue: human carcinoma; prostate; from metastatic site: left supraclavicular lymph node. Tumorigenic in nude mice. Products: human prostatic acid phosphatase; prostate specific antigen. Receptors Expressed: androgen; estrogen. Morphology: epithelial. These cells are responsive to 5-alpha-dihydrotestosterone (growth modulation and acid phosphatase production). The cells do not produce a uniform monolayer, but grow in clusters which should be broken apart by repeated pipetting when subcultures are prepared. They attach only lightly to the substrate, do not become confluent and rapidly acidify the medium. Growth is very slow.

Lovo ATCC CCL-229. Human colonic adenocarcinoma derived in 1971 from a metastatic left supraclavicular region colorectal tumour. The line is positive for expression of c-myc, K-ras, H-ras, N-ras, Myb, sis and fos oncogenes. Tumor specific nuclear matrix proteins CC-3 and CC-4 are also expressed.

Retroviral transduction system based on the Moloney Murine Leukemia Virus (MMULV) this consist of expression construct, a construct to provide the packaging and envelope proteins which require the transfection of HEK 293 cells for the generation of virus that can then be used to transduce the host cells.

pBMN CIP SIN Non-mobilisable vector is based upon the pBMNZ backbone, and contains an MMLV 5' LTR, packaging sequence (Psi), powerful CMV promoter, a multi-cloning site, EMCV IRES-puro fusion for selection of infected cells with puromycin, and the MMLV 3'LTR. All viral structural genes have either been removed or
rendered inactive by the introduction of stop codons with the open reading frames. There is minimal or no sequence overlap between "insert" vector and the others used for virus production (gag/pol, and envelope). This makes this transient system much safer than the majority of stable producer cell lines or vector-based systems for which there is a large degree of homology between the packaging vector(s) and the retroviral expression vector. In these latter systems there is a relatively high probability of production of replication-competent retrovirus (RCR) due to homologous recombination between the vectors. In addition, pBMN-CIP-SIN is also a self-inactivating (SIN) retroviral vector. This is generated by a deletion in the 5'LTR rendering the 5'LTR and 3'LTR retroviral promoters inactive in infected cells. In addition to reducing the chances of insertion activation of endogenous genes, this feature also reduces further the chances of generating replication competent virus.

pVPACK GAG POL Mobilisation defective pVPACK-gp from Stragene is a 10.7 kbp pUC18 based vector which contains a nic sequence. Expression of the retroviral gag-pol protein is driven by a CMV promoter. Downstream lies an IRES-hisD sequence for selection if required and a bGH polyA signal. Used with other plasmids in packaging cells to generate retroviral vectors.

pVPACK VSVG Mobilisation defective pVPACK-VSV-G from Stragene is a 6.4 kbp pUC18 based vector. Expression of VSV-G protein is driven by a CMV promoter. Downstream lies an IRES-puro sequence for selection if required and a bGH polyA signal. Such particles are anticipated to have a wide tropism in terms of host and tissue tropism and to be more stable than conventional vectors with other types of glycoprotein.


Origin & function

Lung alveoli contains two epithelial cell types, type 1 and type2 pneumocytes (PTI and PTII). PTII serve as stem cells to produce more PTI cells. During development the Androgen receptor has been demonstrated to effect PTII branching morphogenesis and lung maturation. The adenocarcinoma lung cell line A-549 (transformed PTII cells) cells have the potential to synthesize and secrete testosteone in culture with foetal calf serum. (Endocrinology 141: 2786–2794, 2000) and have demonstrated changes of gene expression upon the administration of androgen Mikkonen et al (Molecular and Cellular Endocrinology 317 (2010) 14–24). Mikkonen et al have also demonstrated that mice treated with androgen altered murine lung gene expression profiles; for example, by up-regulating transcripts involved in oxygen transport and down-regulating those in DNA repair and DNA recombination. Several of these genes might be important in the establishment of a malignant phenotype such as such as oxygen utilization and apoptosis. The gene TMPRSS2 was also up regulated upon androgen exposure in mice and a direct target in A549 cells, this gene is also an Androgen receptor target in prostate cells (Wang et al., 2007 Mol. Cell 27,380–392). Lung carcinoma tumours and normal adjacent tissue showed AR immunoreactivity. Reports also indicate the Estrogens pathway is important in lung cancer development (Marquez-Garban et al., 2007 Steroids 72, 135–143. Androgen-dependent up-regulation in murine lung of Angpt14 was also demonstrated and this has been implicated in lung cancer progression and metastatic potential (Padua et al., 2008 Cell 133, 66–77). The potential over expression of CYP17A in lung cells may result in DHEA production. DHEA stimulates the production of estrogens, testosterone, progesterone, cortisone, and other steroid hormones which may result in an androgen response in the lung.

Evaluation of foreseeable effects

Lung alveoli contains two epithelial cell types, type 1 and type2 pneumocytes (PTI and PTII). PTII serve as stem cells to produce more PTI cells. During development the Androgen receptor has been demonstrated to effect PTII branching morphogenesis and lung maturation. The adenocarcinoma lung cell line A-549 (transformed PTII cells) cells have the potential to synthesize and secrete testosteone in culture with foetal calf serum. (Endocrinology 141: 2786–2794, 2000) and have demonstrated changes of gene expression upon the administration of androgen Mikkonen et al (Molecular and Cellular Endocrinology 317 (2010) 14–24). Mikkonen et al have also demonstrated that mice treated with androgen altered murine lung gene expression profiles; for example, by up-regulating transcripts involved in oxygen transport and down-regulating those in DNA repair and DNA recombination. Several of these genes might be important in the establishment of a malignant phenotype such as such as oxygen utilization and apoptosis. The gene TMPRSS2 was also up regulated upon androgen exposure in mice and a direct target in A549 cells, this gene is also an Androgen receptor target in prostate cells (Wang et al., 2007 Mol. Cell 27,380–392). Lung carcinoma tumours and normal adjacent tissue showed AR immunoreactivity. Reports also indicate the Estrogens pathway is important in lung cancer development (Marquez-Garban et al., 2007 Steroids 72, 135–143. Androgen-dependent up-regulation in murine lung of Angpt14 was also demonstrated and this has been implicated in lung cancer progression and metastatic potential (Padua et al., 2008 Cell 133, 66–77). The potential over expression of CYP17A in lung cells may result in DHEA production. DHEA stimulates the production of estrogens, testosterone, progesterone, cortisone, and other steroid hormones which may result in an androgen response in the lung.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures required at CL2 will be applied except for the presence of an autoclave in the building. At Alderley Park autoclaves are not present in all buildings where risk class 2 work takes place. In some buildings, autoclaves are present but are only available for media and glassware sterilisation. The waste management facility has a central autoclave facility and a collection and transport process, which we judge to provide an equivalent level of protection.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Passed class 2 on 23/08/11

Please enter comments on the GM safety committee on the risk assessment

Passed class 2 on 23/08/11

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<td>L2 L3 L4 L2 L3</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

Animal Units

- L2
- L3
- L4

Large Scale Activities

- L2
- L3
- L4

Human Clinical Applications

- L2
- L3
- L4

Project Ref 108/15.1

Date Ackn’d 07/01/2015

CU2 Project Title Knockdown of the FOXO family of transcription factors using lentiviral shRNA particles in mammalian cell lines

Class 2

CultureVol Class 2

Class 2

Consent Granted

< 1 Litre

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick if you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Is an emergency plan required according to regulation 20? N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
To understand the mechanisms by which signalling pathway synergy is observed, preliminary data has shown differential regulation of the FOXO proteins following intra-pathway combinations in vitro. To determine whether this regulation of FOXO is pertinent to the observed phenotype, there is a need to effectively knockdown the FOXO proteins in mammalian cells prior to signalling pathway inhibition. As stable and efficacious knockdown is required, a lentiviral shRNA approach would be desirable. Ready to use VSV-G pseudotyped lentiviral particles will be purchased from Sigma. It is not planned to generate viral particles in house under this proposal.

Recipient or parental organism

Human breast and prostate cancer tumour cell lines. HCC70, MOA-M8-468, PC3

Host/vector system

pLKO.1 lentiviral system purchased from Sigma. MISSION TRC viral particles are produced using a 3 plasmid system. Features of the pLKO.1-puro vector allow for transient or stable transfection of the shRNA as well as production of lentiviral particles. Stable gene silencing is selected using the puromycin selectable marker while self-inactivating replication incompetent viral particles can be produced in packaging cells (HEK 293T) by co-transfection with compatible packaging plasmids. pLKO.1-puro Vector Description and Features; cplCTE - Central polypurine tract / constitutive transport element hPGK - Human phosphoglycerate kinase eukaryotic promoter puroR - Puromycin resistance gene for mammalian selection SINLTR 3' - self inactivating long terminal repeat f1 ori f1 - origin of replication ampr - Ampicillin in resistance gene for bacterial selection pUC ori - pUC origin of replication 5' LTR - S'lon9 term inal repeat Psi - RNA packaging signal RRE - Rev response element

Origin & function

Mission TRC lentiviral particles contain short-hairpin RNA (shRNA), an RNA molecule of sense and antisense sequences connected by a short spacer of nucleotides that enables the molecule to form a loop structure. When expressed, it is transported from the nucleus into the cytoplasm, where it's processed by the Oieer enzyme into 21-23 nucleotide siRNA duplexes. The shRNA is able to enter the RNAi pathway and result in gene silencing by blocking translation. The shRNA sequence is designed to be selective towards the gene that is to be targeted, through binding to the complementary sequence of mRNA, reducing the number of off-target effects. Targeting of FOXO mRNA using shRNA will result in the loss of FOXO protein expression. Transduction particles encoding sequences for FOXO shRNA knockdown, as well as non-targeting shRNA negative control and green fluorescent protein (GFP) expressing positive control will be ordered. The GFP positive control is a plasmid that contains GFP cDNA that upon integration in the target genome, GFP mRNA will be transcribed which will result in the expression of GFP protein in the cell. GFP is a fluorescent protein which is naturally found in jelly fish and has been expressed in a wide variety of organisms with no toxic effects. The Forkhead box (FOXO) proteins (FOX01, FOX03 and FOX04) are transcription factors that play an important role in response to activation of the PI3K signalling pathway. AKT, a key node in the PI3K signalling axis directly phosphorylates and inactivates the FOXO proteins through sequestration of FOXO in the cytoplasm. In the absence of PI3K signalling or through targeted inhibition of the PI3K pathway, the FOXO proteins are dephosphorylated and translocate to the nucleus with they are able to bind to and regulate their target genes. Studies
have demonstrated that regulating the activity of the FOXO proteins is critical for a complete PI3K signalling response.
GFP is a protein composed of 238 amino acid residues that exhibits bright green fluorescence when exposed to light in the blue 10 ultraviolet range. GFP is considered to be an inert, non-toxic protein and is frequently used as a reporter protein to monitor gene expression levels.

Evaluation of foreseeable effects

There are 2 GMOs to consider. The first is the VSV-G pseudotyped lentiviral particles that present the potential risk of transducing the operator own cells. If infected with the virus, there is a risk that the FOXO shRNA can target operators mRNA to knockdown FOXO protein. It is unclear what effect that FOXO KO would have on the operators cells. The second GMO for consideration are the modified cell lines. FOXO KO has the potential to alter the growth properties/transforming ability of the cancer cell lines. This could potentially be manifested in an increased ability to survive.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals or plants are proposed to be used

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures required at CL2 will be applied except for the presence of an autoclave in the building. At Alderley Park autoclaves are not present in all buildings where risk class 2 work takes place. In some buildings, autoclaves are present but are only available for media and glassware sterilisation. The waste management facility has a central autoclave facility and a collection and transport process, which we judge to provide an equivalent level of protection.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple bagged in biohazard waste bags, suitably labelled and autoclaved. This solid waste will be comprised mainly of plasticware used in the culturing of the organisms and contaminated tissues used in the decontamination process. As a result there will be no viable GMMs remaining in the solid waste. All liquid waste will be disinfected with 2% virkon. Solutions will be left soaking for 2 hours before disposal via the drain with copious amounts of water. Procedures and relevant training and safety inspections are in place to ensure that all workers deal with waste correctly as specified above.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This proposal was approved by the GMSC on 17 Nov 14 as class 2 Contained
**Project Containment**

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<td>L4 L2 L3 L4</td>
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**Project Ref** 108/15.2

Date Ackn'd: 20/05/2015

CU2 Project Title: Genome wide screening using CRISPR-Cas9 modified gene editing in Cell Based Assays

Class: Class 2

CultureVol: 1-50 Litres

Class CultureVol: Class 2 1-50 Litres

Consent Granted

Non-GMM

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

The aim of this programme of work is to use CRISPR-Cas9 mediated genome wide screening to find and validate potential therapeutic targets. Libraries of CRISPR gRNA against any and all coding and non-coding regions of the human genome targets will be screened against a number of cell based assays in the presence of the protein Cas9. The CRISPR gRNAs and the Cas9 protein will be delivered to cells using a lentiviral delivery system. The CRISPR gRNA vector contains a puromycin resistance gene under control of the EF-1a promoter to allow selection. CRISPR gRNA lentiviral particles can be provided in single shot, 96 or 384well micro plate format. The ready to use lentiviral particles that make up the library are produced using the third generation lentiviral packaging system that has been
designed to produce replication incompetent virions and provide biological containment. Application of CRISPR/Cas9
gRNA targeting could have the following effects on gene transcription; deletion, repression or upregulation. It is not
planned to generate viral particles in house under this proposal.

Recipient or parental organism

Mammalian cell lines, including human tumour cell lines and induced pluripotent stem (iPS) cell derived cell lines, that
may or may not be expressing reporter constructs, e.g. beta lactamase and luciferase. When appropriate, tumour cell
lines will be handled at Biosafety Level 2 to comply with appropriate COSHH Risk Assessment (UKCCCR Guidelines
for the Use of Cell Lines in Cancer Research, British Journal of Cancer (2000) 82(9),1495-1509). The iPS cell lines
are derived from adult somatic tissue and generated using non viral reprogramming methods.

Host/vector system

The CRISPR gRNA and Cas9 lentiviral particles used in this proposal are each produced using the pLenti-CRISPR
transfer vector and three separate packaging plasmids, pLP1, pLP2 and pLP/vSVG. The transfer vector contains
sgRNA scaffold under the control of a U6 promoter and puromycin resistance gene driven by an EFS promoter. The
vector also contains a WPRE insert at approximately 500bp in size with a deleted X Protein locus. Ready to use VSV
G pseudotyped lentiviral particles for the CRISPR-Cas9 system are provided at high titer in the format of separate
Cas9 (-10^7 pfu/mL) and gRNA (-10^6 pfu/mL) expression vectors. The lentivector Cas9 construct expresses
human-codon optimised Cas9 Wild-type nuclease, while the lentivector gRNA expression constructs contain
specifically designed gRNAs driven by U6 Pol III promoters for robust expression. The gRNA sequences have been
designed using bioinformatic algorithms to confer specificity of Cas9 to a specific locus.

Origin & function

The pre-packaged ready to use pseudoviral particles for expression of Cas9 wild-type nuclease along with viral
particles for expression of the gRNA library can be co-infected into the target cells for specific gene knock out,
repression or regulation. The expression of gRNA targeting sequence introduces double strand breaks via the Cas9
endonuclease system that result in homologous and/or non-homologous end joining often resulting in small insertions
and/or deletions which can disrupt or knockout the gene.

The control CRISPR particles used to optimise the transduction and selection conditions will express emGFP. emGFP
is a derivative of the fluorescent protein GFP from the jellyfish Aequorea victoria. The fluorescent protein is widely
expressed in mammalian cells and is unknown to have any harmful or toxic effects. The CRISPR negative control
particles used in this work express EmGFP linked to puromycin resistance through a self-cleavage P2A peptide and
scrambled gRNA expression driven by a human U6 promoter. The scrambled gRNA is designed not to cause gene
modification when combined with the Cas9 nuclease. The CRISPR positive control particles used express EmGFP
linked to puromycin resistance through a self-cleavage P2A peptide and express gRNA targeting human
hypoxanthine-guanine phosphoribosyltransferase 1 (HPRT1). When combined with the Cas9 nuclease gRNA
targeting HPRT1 will result in modification of this gene and loss of HPRT1 protein expression in the cell.

The delivery system uses a separate lentiviral vector to express Cas9 within the cell. The Cas9 lentivector expresses
human-codon optimised Cas9 wild-type nuclease. Cas9 is a nuclease; an enzyme specialised for cutting DNA, with
two active cutting sites, one for each strand of the double helix. The gRNA interacts with Cas9, directs the nuclease to
the specific complimentary DNA sequence. Arrayed libraries of CRISPR gRNAs contain multiple copies of gRNAs
against a specific target in the genome in each well. Pooled libraries contain gRNAs against multiple different
genome targets in the same well.

The lentiviral library is designed to deliver distinct gRNA constructs targeted to maximise gene perturbation and
minimise off target effects. gRNA expression is driven by U6 promoter and the insert also contains a puromycin resistance selection motif.

Evaluation of foreseeable effects

There are 2 GMOs to consider. The first is the VSV-G pseudotyped lentiviral particles that present the potential risk of transducing the operator own cells. Application of gRNA and Cas9 lentiviral vectors has the potential to cause DNA damage via double strand breaks and initiate cellular homologous or non-homologous end joining repair resulting in insertions and/or deletions that could disrupt or knock-out the targeted gene. Application of CRISPRiCas9 gRNA targeting could have the following effects on gene transcription; deletion, repression or upregulation. This is difficult to predict but could potentially alter the cell phenotype, for instance increasing the ability to survive or proliferate. There is the potential of off-target DNA double strand breaks following transduction with the CRISPR gRNA particles however the reported incidence of this is low when compared to other modification technologies. The second GMO for consideration are the modified cells created following transduction. Potential gene disruption could alter the growth properties/transforming ability of the cells. This could potentially be manifested in an increased ability to survive.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals or plants are proposed to be used.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Alderley Park, Macclesfield (address no. 1):
Autoclave is not present in the building where this class 2 work will take place.
In some buildings of Alderley R&D, autoclaves are present but are only available for media and glassware sterilisation.
The on-site waste management facility has a central autoclave facility. There is a collection and transport process, which we judge to provide an equivalent level of protection, as described in answer to question 12. Class 2 solid waste will be deactivated within the confines of the laboratory, prior to disposal.
Building 310, Cambridge (address no.2):
No derogation required. All containment requirements are satisfied. Unlike Alderley Park, Building 310 has an autoclave present within the building.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Alderley Park, Macclesfield (address no. 1):
Class 2 solid waste will be chemically deactivated before leaving the laboratory, which is a restricted access room. The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. To deactivate, 2% virkon strength for a minimum contact time of two hours will be implemented. As a result, there will be no viable GMMs remaining in the solid waste.
All solid GMM contaminated waste will then be at least double bagged into biohazard autoclave waste bags, and secured with autoclave tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste technician will transfer the bags using medibins and a specifically designed wheeled clinical waste carrier. A bespoke 1.5 tonne lorry is used to take the carrier the short distance to an on-site and enclosed waste facility where the waste is transferred into the building and into the autoclave for 100% sterilisation.
All liquid waste will be disinfected with 2% virkon. Solutions will be left soaking for 2 hours before disposal via the
Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with waste protocols correctly as specified above.

Building 310, Cambridge (address no.2):

Class 2 solid waste will be chemically deactivated before leaving the laboratory, which is a restricted access room. The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. To deactivate, 2% virkon strength for a minimum contact time of two hours will be implemented. As a result, there will be no viable GMMs remaining in the solid waste.

All solid GMM contaminated waste will then be at least double bagged into biohazard autoclave waste bags, and secured with autoclave tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste technician will transfer the bags using medibins and a specifically designed wheeled clinical waste carrier to the autoclave within the building for 100% sterilisation.

All liquid waste will be disinfected with 2% virkon. Solutions will be left soaking for 2 hours before disposal via the drain with copious amounts of water.
Use of lentiviral CRISPR Cas9 gene editing systems for in vitro cell line studies to support drug discovery programmes

The aim of this programme of work is to use CRISPR Cas9 gene editing to find and validate potential therapeutic targets for drug discovery purposes. The intention is to modulate the activity of a variety of different human genes by single or multiple modifications in a range of cell lines maintained under tissue culture conditions. There are no plans to modify whole organisms. CRISPR gRNA targeted to specific coding and noncoding regions of the human genome will be screened in a number of cell based assays in the presence of the protein Cas9. The CRISPR gRNAs and the Cas9 protein will be delivered to cells using lentiviral delivery systems. The vectors will contain an antibiotic resistance gene such as puromycin and/or a standard fluorescent marker driven by an established mammalian promoter to allow for selection. The lentiviral particles will be supplied ready to use by an external commercial suppliers using packaging plasmids to produce VSV-G pseudotyped replication incompetent virions with suitable biosafety features. Application of CRISPR/Cas9 gRNA targeting could have the following effects on gene transcription in the final GMOs: deletion, repression, or upregulation. It is not planned to generate viral particles in house under this proposal. The CRISPR gRNA and Cas9 lentiviral particles used in this proposal are each produced using 3rd generation transfer vectors and packaged with either 2nd generation or 3rd generation packaging systems. The transfer vectors used are all commercially available and include 2 approaches: a two vector system where the Cas9 and gRNA are on separate vectors and a single vector system where both are contained on one vector. For the former, transfer vectors such as pLenti-CRISPR, pLKO.1 and pTRIPZ will be used to express either Cas9 from a conventional mammalian promoter or sgRNA scaffold under the control of a U6 promoter, along with a selectable resistance gene driven by a similar promoter. Vectors also contain a WPRE insert either with or without the x protein promoter. For the single vector system both Cas9 and gRNA are expressed from one transfer vector using one of a range of conventional mammalian promoters and U6 RNA promoter respectively. Ready to use VSV-G pseudotyped lentiviral particles for the CRISPR-Cas9 system are provided at high titre in the format of separate Cas9 expression vectors. The gRNA sequences will be designed using bioinformatic algorithms to confer specificity of Cas9 to a specific locus. This proposal covers the use of commercial reagents: there is no intention to generate viral particles.

Recipient or parental organism

Mammalian cell lines, including human tumour cell lines and induced pluripotent stem (iPS) cell derived cell lines, that may or may not be expressing reporter constructs, e.g. beta lactamase and luciferase. When appropriate, tumour cell lines will be handled at Biosafety Level 2 to comply with appropriate COSHH Risk Assessment (UKCCCR Guidelines for the Use of Cell Lines in Cancer Research, British Journal of Cancer (2000) 82(9), 1495-1509). The iPS cell lines are derived from adult somatic tissue and generated using non viral reprogramming methods.
The CRISPR gRNA and Cas9 lentiviral particles used in this proposal are each produced using 3rd generation transfer vectors, all of which are commercially available and include 2 approaches: a two vector system where the Cas9 and gRNA are on separate vectors and a single vector system where both are contained on one vector. For the former, transfer vectors such as pLenti-CRISPR, pLKO.1, pLV-CE and pTRIPZ will be used to express either Cas9 from a conventional mammalian promoter or sgRNA scaffold under the control of a U6 promoter, along with a selectable resistance gene driven by a similar promoter. For the single vector system the transfer vector will be pLV-U6g-EPGC where both Cas9 and gRNA are expressed from one transfer vector using one of a range of conventional mammalian promoters and U6 RNA promoter respectively. Transfer vectors will contain all or some of the following elements: Chimeric 5’ LTR, U3 truncated 3’ LTR, cPPT/CTS (central polyuridine tract/central termination sequence), Ψ (packaging signal), RRE (Rev-response element), IRES (encephalomyocarditis virus internal ribosome entry site), mammlian promoter (CMV, CAG, RSV) and WPRE insert either with or without the WHV x protein promoter. All transfer vectors will be packaged in HEK293T cells by the supplier using a range of commercially available 2nd and 3rd generation packaging plasmids with minimal viral components and include pVPackg, pVPackVSVG, pMDLg/pRRE, pMD2.G and pTLA1 series. Particles will be packaged with VSV-G pseudotyped envelopes. Ready to use lentiviral particles for the CRISPR-Cas9 system are provided at high titre (10^7-10^8 pfu/ml). The gRNA sequences will be designed using bioinformatic algorithms to confer specificity of Cas9 to a specific locus. This proposal covers the use of commercial reagents: there is no intention to generate viral particles within the scope of this notification.

**Origin & function**

Cas9 is a bacterial nuclease specialized for cutting DNA, with two active cutting sites, one for each strand of the double helix. The gRNA interacts with Cas9 and directs the nuclease to the specific complimentary DNA sequence. The intention is to utilise different forms of Cas9 in the target cells. These include, S. pyogenes (sp) Cas9 (traditional most well known Cas9) (UniProt: Q99ZW2 (CAS9_STRP1)), S. pyogenes (sp) Cas9 D10A (Ran et al 2013, Cell 154:1380-1389), spCas9-HF-1 (Same functions of sp.Cas9 but more specific repaired in the cell by homology directed repair (HDR), using the intact strand as the template. HDR has high fidelity and rarely results in errors. Two adjacent, opposite strand nicks can cause a double strand break (DSB) and trigger error-prone non-homologous end joining (NHEJ) repair; however, in the presence of a repair template, the double nicks can be repaired by HDR. Double nicking greatly reduces unwanted off-target effects. The gRNA interacts with Cas9, leads the nuclease to the specific complimentary DNA sequence to cause a single or double stranded cut in the DNA. In the absence of the gRNA the Cas9 should not cut the DNA. The gRNAs will be expressed as RNA and are designed to optimise specificity using proprietary supplier controls to target Cas9 to specific genes. Dependent on precise gene function, perturbation by Cas9/gRNA could result in altered function within the final GMO cell line.

rtTA3 expression will be utilised for inducible expression in the TRIPZ vector and is derived from tTA, a Tet-off regulatory protein comprising a fusion of 1-207aa of the Tet repressor protein and the C terminal 127aa of Herpes Simplex VP16 activation domain. rtTA3 is mutated at 4aa in the TetR domain reversing the functionality of the repressor such that binding of Tetracycline is required for operator binding [Gossen et al 1994 Curr. Opin. Biotechnol 5:516-520]. In the presence of tetracycline, rtTA3 will bind the TRE elements in the Tet Operator and increase expression of genes under the regulation of this control region.

Fluorescent reporter proteins GFP, eGFP, RFP, CFP, AsRed and YFP will be expressed as markers for downstream analysis. GFP is a fluorescent protein from the jellyfish Aequorea victoria. eGFP, CFP and YFP have been generated by GFP mutations to improve or alter spectral characteristics. RFP is a synthetic variant of fluorescent protein originally isolated from Discosoma coral. AsRed2 is a fluorescent protein from the Anemonia sulcata red fluorescent protein. These fluorescent proteins have been widely expressed in mammalian cells and are not known to have any harmful or toxic effects.

Puromycin, kanamycin, blasticidin and neomycin resistance gene products are classified as antibiotic resistance genes. These have been widely expressed in mammalian cells and are not known to have any harmful or toxic effects.

**Evaluation of foreseeable effects**

There are 2 GMO hazard sources to consider: the virus and the final cell line GMO.

Firstly, although all viral particles will be replication defective, the presence of the WHV x protein promoter in the WPRE fragment of some transfer vectors has been associated with a transforming phenotype (Themis et al, Molecular Therapy 12(4):763-71, 2005). Whilst it is anticipated that this hazard will only require Containment Level 2 control measures, systems devoid of these potential viral hazards will be the preferred choice where possible. Studies in mice have shown that while some vectors such as those based on MuLV vectors are genotoxic (due to insertional mutagenesis), however lentiviral vectors are not known to be associated with genotoxicity (Montini, et.)
al., Nature Biotechnology, 2006). Furthermore, all of the viral vectors proposed for use have a number of safety features which include a deletion in the enhancer of the U3 region of 3'LTR ensuring self-inactivation; chimeric 5'LTR containing mammalian promoter to decrease the risk of viral LTR activity and necessity of Tat expression; absence of accessory genes (vif,vpr,vpu,nef); the vector and helper constructs contain no significant areas of homology, minimizing their chance for recombination; HIV-1 structural genes (gag, pol, rev) will not be present in the transduced cells. All lentiviral particles are hence replication-incompetent. Exposure to gRNA and Cas9 lentiviral vectors has the potential to cause DNA damage via double strand breaks and initiate cellular nonhomologous end joining repair resulting in insertions and/or deletions that could disrupt or knockout the targeted gene. The resulting modifications could have the effect of deletion, repression or upregulation on the target gene. This could potentially alter the cell phenotype, for instance increasing the ability to survive or proliferate. There is also the potential of off-target DNA double strand breaks following transduction with the CRISPR gRNA particles, however the reported incidence of this is extremely low. Hence exposure of the operator to any virus particle by inhalation or direct inoculation into the circulation could potentially pose a risk. Consequently appropriate containment level 2 control measures will be used to minimise exposure: Class II Microbiological Safety Cabinet, double gloves, suitable and sufficient warning signs, proper waste deactivation and disposal, minimised aerosol production and in a restricted access laboratory. All staff will be trained in safety procedures and use of lab equipment. Volumes of high titre virus-containing liquids (up to 10^8 pfu/ml) will be no greater than 50ul in any one experiment. Plasticware/media/other liquids will be inactivated with a validated procedure or placed in a dedicated waste container for on site autoclaving and then disposed under COSHH regulations. For the final cell lines these will be monitored using a commercial p24 ELISA assay and demonstrated devoid of viral particles prior to moving to BCL1 containment. Plates handled outside of a Class II Microbiological Safety Cabinet will be lidded and effectively double sealed to minimise spillage risk. Cell cultures are considered to be disabled hosts as they will not survive outside of a laboratory and are not able to colonise the operators

The second consideration is the modified cells created following transduction. Potential gene disruption could alter the growth properties or transforming ability of the cells. This could potentially be manifested in an increased ability to survive. Consequently exposure by inhalation, ingestion or in the circulation could potentially lead to an increased risk of colonisation. However cell cultures are considered to be disabled hosts as they will not survive outside of culture conditions and any exposure will result in clearance by the operators immune system. As a consequence final GMO cell lines are very unlikely to colonise an immune competent operator.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals or plants are proposed to be used.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For both addresses:
Class 2 solid waste will be chemically deactivated before leaving the laboratory. Access to the laboratory is restricted. The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. To deactivate, 2% virkon strength for a minimum contact time of two hours will be implemented. As a result, there will be no viable GMMs remaining in the solid waste. All solid GMM contaminated waste will then be at least double bagged into biohazard autoclave waste bags, and secured with autoclave tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste technician will transfer the bags using medibins and a specifically designed wheeled clinical waste carrier to the autoclave within the building for 100% sterilisation. All liquid waste will be disinfected with 2% virkon. Solutions will be left soaking for 2 hours before disposal via the drain with copious amounts of water

Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with waste protocols correctly as specified above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
This proposal was approved by the AstraZeneca GMSC on 29th June 2016 as Risk Class 2

### Project Containment

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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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### Project Ref 108/16.2

**Date Ackn’d:** 25/11/2016  
**CU2 Project Title:** Cell engineering for purification and molecular cargo loading of extracellular vesicles with CRISPR/Cas9 components for in vitro genome editing  
**Class:** Class 2  
**Culture Vol:** 1-50 Litres

### Project Additional Information

**Purposes of the contained use:**  
The aim of this programme is to engineer cell lines for production of Extracellular Vesicles (EVs), including exosomes, loaded with different molecular cargo of interest. Initially the cell lines will be...
engineered to load Cas9 and CRISPR gRNA, separately or in combination, into EVs as well as modifying EV-associated proteins to facilitate affinity purification. Loading of functional Cas9 and CRISPR gRNA against various human genome targets will be assessed with various cell based assays in the presence of the produced EVs. The generation of cell lines for CRISPR/Cas9 modification is covered in 'Generic GMO number 6: 20160321101713, and we will be using many of the same Cas9 variants, cell lines and vectors detailed in that proposal.

Recipient or parental organism

Mammalian tumour and non tumour cell lines Mammalian cell lines, including human tumour cell lines and induced pluripotent stem (iPS) cell derived cell lines. When appropriate, tumour cell lines will be handled at Biosafety Level 2 to comply with appropriate COSHH Risk Assessment (UKCCCR Guidelines for the Use of Cell Lines in Cancer Research, British Journal of Cancer (2000) 82(9),1495-1509). These cell lines are generated in house [or by an external contractor] using standard cloning vectors and expression vectors and hosts. It is anticipated that the insertion and expression of the target will not increase the inherent risk posed by the parental cell line used. All details will be recorded in the Generic GMO spreadsheet and assessed by a competent scientist.

Host/vector system

pCDNA3 series 5-6kb mobilisation-defective vectors containing pBR322 nic sequence. CMV promoter and T7 promoter allows cloning without expression in E coli not containing T7 polymerase and also constitutive expression in mammalian cells. Contains SV40 ori allowing replication in cells expressing SV40T. Other features include ampicillin resistance gene and hygro, neo or zeo resistance genes; SV40 poly and ColE1 origin.
pTOIC This is an in-house generated episomal vector based on pBluescript backbone with pUC origin of replication for bacterial propagation. Non mobilisable. Contains PGK promoter driving a neomycin resistance gene. Contains a TRE3G inducible promoter driving expression of Cas9 (s.pyrogenes) and EmGFP with an intervening T2A peptide element and BGH polyadenylation signal. Contains Tetracycline transactivator (Tet-ON-3G) gene driven by a CAGG promoter with an SV40 polyadenylation site. Incapable of replication in mammalian cells. For growth in E.Coli, it contains a pMB1 origin of replication and ampicillin resistance. azPGE Series PL-16-0157 azPGE- pEF1_Cas9_T2A_PuroR. This is an inhouse generated episomal vector based on pBluescript backbone with pUC origin of replication for bacterial propagation. Non mobilisable. These are the promoters that can by driving Ca9 expression: CMV, EF1a, SV40, PGK, TRE, TRE3G (Clonetech) promoter driving expression of Sp Cas9 (UniProt: Q99ZW2 (CAS9_STRP1)), Sa Cas9 (UniProt: J7RUA5 (J7RUA5_STAAU)) and SpCas9-HF1 (Addgene: Plasmid #72247). It may contain the following: Antibiotic resistance genes: neomycin, puromycin, kanamycin and blasticidin. Fluorescent proteins: AsRed2, eCyFP or eCFP, eGFP and GFP. ZFN and
obligate sites that recognize ZFNs to enable integration of DNA into AAVS1 by ZFN. T2A peptide elements can be used to allow post translational cleavage of two proteins transcribed from the same promoter and a BGH polyadenylation signal. It may also contain a U6 promoter for eukaryotic expression of gRNA. Incapable of replication in mammalian cells. For growth in E.Coli, it contains a pMB1 origin of replication.

pMLu Vector for RNA expression driven by U6 promoter.

Origin & function

All insert sequences will be generated by routine gene synthesis using external providers. Where appropriate, protein sequences for inserts will be obtained from Uniprot. Inserts include FKBP, FRB, CIB1, CRY2, PHYB, PIF3, PIF6, HO1, PoyA, VVD, MS7, PP7, CAS9, CRE, GYRB, CD9, CD63, RAB5, and RAB1. CRISPR gRNA and protein insert sequences will be cloned into standard mammalian expression vectors e.g. pcDNA3.1 or pMLu.

Evaluation of foreseeable effects

There are 2 GMO hazard sources to consider; the final EV producing cell line GMO and the Cas9 and/or gRNA loaded EVs. Although it is not anticipated, modified cells for EV production could possess an increased survival ability and improved proliferation rate and hence may present additional hazards to operators if ingested, introduced into the circulation or inhaled as an aerosol. Exposure to Cas9 and gRNA loaded EVs has the potential to cause DNA damage via double strand breaks and initiate cellular non-homologous end joining repair resulting in insertions and/or deletions that could disrupt or knock-out the targeted gene. There is also the potential of off-target DNA double strand breaks following exposure with the Cas9 and gRNA loaded EVs.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals or plants are proposed to be used

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Class 2 solid waste will be chemically deactivated before leaving the laboratory, which is a restricted access room.
The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. To deactivate, 2% virkon strength for a minimum contact time of two hours will be implemented. As a result, there will be no viable GMMs remaining in the solid waste.
All solid GMM contaminated waste will then be at least double bagged into biohazard autoclave waste bags, and
secured with autoclave tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste technician will transfer the bags using medibins and a specifically designed wheeled clinical waste carrier to the autoclave within the building for 100% sterilisation. All liquid waste will be disinfected with 2% virkon. Solutions will be left soaking for 2 hours before disposal via the drain with copious amounts of water. Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with waste protocols correctly as specified above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This risk proposal was approved by AstraZeneca GMSC on 15/11/2016

Project Containment

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Project Ref 108/16.3

Date Ackn'd: 25/11/2016
CU2 Project Title: Use of the BacMam/multiBacMam as a delivery system for genomes editing applications

Class 2, Culture Volume Class 2, Consent Granted

Date Project 02/03/2022

Page 3238 of 15326
This proposal covers the engineering of the BacMam system (commercially available Thermofisher Scientific) or MultiBacMam system (Sari et al. 2016, AZ external collaboration -Imre Berger-Bristol University) for precise genome editing by including CRISPR/Cas9 and its derivatives, Cpf1 and Zinc Finger Nucleases that can be used to insert sequences into and/or modify the genome in cell lines. Moreover, transiently expressing catalytically dead Cas9 molecules fused to epigenetic transcriptional regulator can be used (CRISPRi/CRISPRa, Thakore et al. 2016). The aims of this GMO are: Firstly that base vectors listed in this GMO that already have Cas9 +/- gRNA and a fluorophore, can be used for subcloning of the gene editing system into the transfer vector of the BacMam system or MultiBacMam system for later assembly and production of Baculovirus particles in insect cells. Secondly, the engineered Baculoviruses will be used to transduce cell lines. If a gene is targeted either from the start before the work is carried out, this should be individually risk assessed, discussing the risks of targeting that specific locus. The BacMam Baculoviral particles will be produced in house in UK and supplied ready to use replication incompetent virions able to transduce mammalian cells via the vesicular stomatitis virus G envelope glycoprotein (VSV-G pseudotype). Baculoviral particles derived from MultiBacMam system will be produced by Imre Berger research laboratory in Bristol and supplied ready to use as pseudotyped (with envelope glycoproteins to increase transduction efficiency in mammalian cells; e.g. VSV-G, gp64, gp120/gp41) non-pseudotyped replication incompetent virions. MTA is already in place. Strep-tag will be fused to the baculoviral protein gp64 for large scale virus purification by affinity chromatography. Application of CRISPR/Cas9 gRNA targeting could have the following effects on gene transcription in the final GMOs: deletion, repression, or upregulation. Viral particles used in this proposal are produced using commercial and non-commercial transfer vectors. In both the BacMam and MultiBacMam systems the CRISPR gRNA and gene editing nuclease are present in a single vector set-up, pCMV-DEST (BacMam) or in house modified AzPGE series (MultiBacMam). Both types of transfer vectors include Tn7 sites for transposition of the CRISPR modules into the Baculovirus genome (Bacmid) which is then used to produce viral particle in insect cells. Both Cas9 and gRNA are expressed using a range of conventional mammalian promoters and U6 RNA promoter respectively. pCMV-DEST vector also contain a WPRE insert. Ready to use pseudotyped/non-pseudotyped baculoviral particles for the precise genome editing can be provided at high titre or as diluted supernatant ready to use. The gRNA sequences will be designed using
bioinfomatic algorithms to confer specificity of Cas9 to a specific locus. This proposal cover the use of commercial and non-commercial reagents. Baculoviral particles will be used to make CRISPR/ZNF modifications for in vitro assays in mammalian cell lines.

Recipient or parental organism

S. frugiperda (Sf9/Sf21/expresSF+) Includes both Sf9 and Sf21 cell lines. Cells derived from Spodoptera frugiperda (the Fall Armyworm). The larvae of this moth are an economic pest of cotton every year in extreme southern areas of the USA. Insect cell cultures can be regarded as especially disabled hosts as they represent no intrinsic hazard to human health or the environment unless they are contaminated with adventitious agents. Sf9 was cloned by G.E. Smith and C.L. Cherry in 1983 from the parent line, IPLB-SF 21 AE, which was derived from pupal ovarian tissue of the fall armyworm, Spodoptera frugiperda, by Vaughn, et al., in 1977. “expresSF+” cells are a serum-free cell-line derived from Sf9 by Protein Sciences Corporation using a series of stringent selection steps in serum-free medium supplemented with insulin.

Mammalian Cell Lines Mammalian cell lines, including human tumour cell lines and induced pluripotent stem (iPS) cell derived cell lines, that may or may not be expressing reporter constructs, e.g. beta lactamase and luciferase. When appropriate, tumour cell lines will be handled at Biosafety Level 2 to comply with appropriate COSHH Risk Assessment (UKCCCR Guidelines for the Use of Cell Lines in Cancer Research, British Journal of Cancer (2000) 82(9),1495-1509). The iPS cell lines are derived from adult somatic tissue and generated using non viral reprogramming methods.

Host/vector system

BACMAM pCMV-Dest pCMV-Dest Vector contains a CMV promoter to drive constitutive expression of the target gene. It also contains VSV-G elements, which enable baculovirus to have high transduction efficiency, and a WPRE element, which elongates transient expression in mammalian systems. This WPRE sequence also contains a 3’ fragment corresponding to the promoter and 5’ coding region from the WHV X protein. The X protein promoter is associated with tumourogenesis in certain settings. To facilitate successful cloning, AmpR and gentamicin have been incorporated for positive selection, as well as Cm(R) (chloramphenicol resistance gene) for negative selection.

pFL/ pKL multiBacMam series These are plasmids generated by Imre Berger laboratory (Bristol University). There can be promoters that can be driving Ca9 expression: CMV, EF1a, SV40, PGK, TRE, TRE3G (Clonetech) promoter driving expression of Sp Cas9 (UniProt: Q99ZW2 (CAS9_STRP1)), Sa Cas9 (UniProt: J7RUA5 (J7RUA5_STAAU)) and SpCas9-HF1 (Addgene: Plasmid #72247), sp.Cas9 D10A Nickase (Ran et al 2013, Cell 154:1380-1389), Cpf1 (Zetsche et al.; 2015), Cas9 fusion to cytidine deaminase (Komor et al. 2016), Cas9 fusions to epigenetic/gene regulation domain (CRISPRi/CRISPRa). It may contain the following: Antibiotic resistance
genes: neomycin, puromycin, gentamicin, chloramphenicol, spectinomycin, zeocin, kanamycin and blasticidin. Fluorescent proteins: AsRed2, TurboRFP, eCyFP or eCFP, eGFP and GFP. ZFN and obligare sites that recognise ZFNs to enable integration of DNA into AAVS1 by ZFN. T2A peptide elements can be used to allow post translational cleavage of two proteins transcribes from the same promoter and a BGH polyadenylation signal. They may also contain a U6 promoter for eukaryotic expression of gRNA. Incapable of replication in mammalian cells. For growth in E.Coli, it contains a ColE1 origin of replication. They may have Tn7 transposon for direct integration into the MultiBac bacmid by Tn7 transposition. They may have a loxP imperfect inverted repeat suitable for fusion via loxP to the pSPL/pUCDM multiBacMam plasmid series.

The PGE constructs used for subcloning into the BacMam system or multiBacMam system are made and sequenced within AZ. These vectors can be modified to use alternative versions of Cas9 that have the same function with equal or reduced on and off-target activity including: 1) S. pyogenes (sp) Cas9 (traditional most well known Cas9) (UniProt: Q99ZW2 (CAS9_STRP1)) 2) S. pyogenes (sp) Cas9 D10A (Ran et al 2013, Cell 154:1380-1389) 3) spCas9-HF-1 (Same functions of sp.Cas9 but more specific version with less off target effects caused by mutations in (N497A/R661A/Q695A/Q926A). (Addgene: Plasmid #72247). 4) Staphylococcus aureus (sa) Cas9 (Cas9 from a different strain, has very similar function but smaller). 5) Cpf1 from Acidaminococcus and Lachnospiraceae, Cpf1 is a single RNA-guided endonuclease lacking tracrRNA, and it utilizes a T-rich protospacer-adjacent motif. Moreover, Cpf1 cleaves DNA via a staggered DNA double-stranded break (Zetsche at al, 2015). 6) catalytically dead mutant of the Cas9 protein fused to transcriptional/epigenetic regulators (CRISPRi/CRISPRa, e.g. p300, vp64, dnmt3a Thakore et al. 2016) 7) catalytically dead mutant of the Cas9 protein fused to cytidine deaminase (AID, APOBEC3G, APOBEC1, CDA1) to mediate the direct conversion of cytidine to uridine, thereby effecting a C to T (or G to A) substitution (Komor et al. 2016), Cas9 fusions to epigenetic/gene regulation domain (CRISPRi/CRISPRa). These vectors can also be modified to include the following if desired a fluorescent proteins: AsRed2, TurboRFP, eCyFP or eCFP, eGFP and GFP. The vectors that contain a gRNA sequence will also be modified to change the 20 bp targeting sequence depending on the project. The vectors may contain tetracycline regulated expression of genes from tetracycline responsive promoters. The Strep-tag is a synthetic peptide consisting of eight amino acids (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys). This peptide sequence exhibits intrinsic affinity towards Strep-Tactin, a specifically engineered streptavidin
There are 2 GMO hazard sources to consider: the virus and the final cell line GMO. Bacmam is designed not to integrate into the host genome, but there is at least one report of stable modification with earlier versions of this vector and hence the potential for integration (Condreay et al. 1999 Proc. Natl. Acad. Sci. USA Vol. 96, pp. 127-132). pBacmam also contains a WPRE gene which also encodes the Sprime end of the WHV X protein and includes the upstream promoter, associated with a transforming phenotype (Themis et al 2005 Molecular Therapy Vol. 12, No. 4, pp763-771), carrying a low but perceivable risk of a transforming operators cells. Whilst it is anticipated that this hazard will not require any control measures above that for CL2, systems devoid of these potential viral hazards will be the preferred choice where possible. Fluorescent proteins listed in this GMO have been stably expressed in a range of cell types with no hazardous effects observed. Strep-tag fusion proteins have been stably expressed in a range of cell types with no hazardous effects observed. Addition of the Strep-tag to the baculoviral protein gp64 is not predicted to alter infectivity of the baculoviruses in insect cells or transduction efficiency in mammalian cells. However, Strep-tag might slightly diminish the virus titre obtained from insect cells. Application of gRNA and Cas9 (or other gene editing nucleases) or ZNF baculoviral vectors has the potential to cause DNA damage via double strand breaks and initiate cellular nonhomologous end joining repair resulting in insertions and/or deletions that could disrupt or knockout the targeted gene. There is an intention to use a range of targets that could potentially affect the operator when exposed to the BacMam virus. The main hazards that should be considered is anything that may increase the risk of the cell surviving if it were accidently exposed to someone such as modification of tumour suppressor or promoting genes or insertion of viral components. Deletion, disruption or up-regulation of genes that may cause oncogenic transformations such as tumour suppression or increased cytotoxicity in the cell can be potentially harmful if exposed to operator, or the environment. Consequently exposure to viral particle by inhalation, ingestion, or in the circulation could lead to an increased risk of colonisation. It is anticipated that the risk to the operator and the environment is low due to the contained use of the resultant GMOs within a designated Class 2 laboratory and by trained personnel. There is the potential of off-target DNA double strand breaks following transduction with the CRISPR gRNA particles, however the reported incidence of this is low when compared to other modification technologies. Cas9 should only be active when the full gRNA is present therefore it should not cut in the absence of gRNA or with the target specific sequence cut out. Thus, potential inoculation with a cell line where a functional gRNA has not been used does not result in a greater risk than the parental cell line. In CRISPRi/CRISPRa off-target gene activation/repression is negligible even in the presence of gRNA. In base editing PGE, there is a potential risk of off-target mutations near the double strand break
(Yamanaka et al. 1996, Robbiani & Nussenzweig 2013, Lawrence et al. 2013, Alexandrov et al. 2013). In Cas9 fusion proteins Cas9 guided targeting is not affected. The antibiotic resistance proteins listed in this GMO have been stably expressed in a range of cell types with no hazardous effects observed.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No animals or plants are proposed to be used

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Class 2 solid waste will be chemically deactivated before leaving the laboratory, which is a restricted access room. The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. To deactivate, 2% virkon strength for a minimum contact time of two hours will be implemented. As a result, there will be no viable GMMs remaining in the solid waste. All solid GMM contaminated waste will then be at least double bagged into biohazard autoclave waste bags, and secured with autoclave tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste technician will transfer the bags using medibins and a specifically designed wheeled clinical waste carrier to the autoclave within the building for 100% sterilisation. All liquid waste will be disinfected with 2% virkon. Solutions will be left soaking for 2 hours before disposal via the drain with copious amounts of water. Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with waste protocols correctly as specified above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This proposal was approved by the AstraZeneca GMSC on 15/11/2016 as a GMO class 2
Project Containment

Laboratory Activities Glass Houses Growth Rooms
L2 Yes L3 L4 L2 L3 L4 L2 L3 L4

Animal Units Large Scale Activities Human Clinical Applications
L2 L3 L4 L2 L3 L4 L2 L3 L4

Project Ref 108/18.1

Date Ackn'd 19/04/2018
CU2 Project Title Connected programmes of work: Use of lentiviral, retroviral and Bacmam vector systems for modification of mammalian cell lines

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 1-50 Litres
Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID 108/18.1a
Date of Significant Change 03/02/2020

Project Additional Information

Purposes of the contained use

This risk assessment addresses the generation and use of genetically modified lentiviral, retroviral and Bacmam particles encompassing GM risk class 2 activities assigned by the AstraZeneca UK GMSC to be within scope of this ‘Connected Programme of Work’. The aim here is to use 2nd and 3rd generation lentiviral and retroviral delivery systems or current Bacmam systems, to achieve single or multiple genetic mutations by utilising shRNA, CRISPR/Cas9 (and derivatives thereof) or overexpression techniques, in a range of mammalian cell lines in order to facilitate drug discovery projects. The extent of the planned work is limited to in vitro studies. For retroviral and lentiviral systems approaches include both transfection of plasmid vectors into eukaryotic packaging cell lines such as HEK-293T to generate replication-defective viral particles and, use of such replication-defective viral particles (generated at AstraZeneca or elsewhere) to transduce mammalian cell lines. Bacmam studies will adopt the similar approaches but will utilise packaging in an insect cell line and also may require an additional initial step of Bacmid generation in a disabled E.coli strain. Target cell
lines may comprise established and primary mammalian cell lines not known or suspected to contain pathogens at Hazard Group 3 or above. This could include human, rodent and primate sources.

Recipient or parental organism

Lentiviral and retroviral particles (or vectors) based on a range of human virus (HIV) or animal virus (MMLV, MMTV and MSCV). All vector systems will be split to minimise possibility of replication and where packaging cell lines are to be used these will be consistent with 2nd generation or later systems. Hence these viral vectors are extremely unlikely to be able to replicate in human cells or in the wider environment and are unable to present the pathogenesis associated with the originating pathogen. Viral particles may be pseudotyped to effect different tropisms by the use of ecotropic and amphotropic envelope proteins, including VSV-G. Other viral sequences may be present to improve efficiency of these molecular tools such as wild-type or mutated forms of the Woodchuck Hepatitis Virus Post-transcriptional Response Element (WPRE) which enhance transgene RNA stability. Bacmam vectors are modified forms of baculovirus vector series in which the AcNPV polyhedrin promoter has been replaced by the CMV IE enhancer/promoter and along with a WPRE element represents the only mammalian elements present.

Target cell lines for transduction may comprise established and primary mammalian cell lines not known or suspected to contain pathogens at Hazard Group 3 or above. This could include human, rodent and primate sources.

Host/vector system

For lentiviral and retroviral systems, viral particles will be generated by introducing plasmids containing elements of the virus genome into established packaging cell lines, eg. human embryonic kidney (HEK293) cells. There may be instances where the essential viral packaging genes will be expressed stably by the packaging cell, with a single plasmid carrying the vector backbone and transgene being introduced by transfection, and in other cases the viral functions and the vector backbone/transgene will be encoded on separate plasmids which will be transfected together. For Bacmam systems, since bacmids are non-infectious, this aspect of vector production is not subject to scrutiny for CL2 control measures. Packaging which will be achieved by transfection of isolated bacmid constructs of insect cells and the subsequent use of bacmam particles will be covered by this notification.

Such viral vectors will then be used to transduce a range of human and animal cell lines. Target cell lines for transduction may comprise established and primary mammalian cell lines not known or suspected to contain pathogens at Hazard Group 3 or above. This could include human, rodent and primate sources.

Origin & function

As this is a connected programme of work, the genetic material to be introduced will be from a range of origins and have a variety of functions. This will include putative and known oncogenes and tumour-suppressor genes, cytoskeletal proteins, short hairpin RNA (shRNA) molecules intended to inhibit the expression of a range of genes including those known or suspected to be involved in pathways of oncogenesis, and genes intended to induce immortalisation and the extension of the proliferative lifespan of cells. CRISPR/Cas9 systems may be expressed which are intended to cause double-stranded breaks in DNA, leading to mutation by non-homologous end-joining or insertion of specific mutations or tags with homology-directed repair constructs. Variants of the CRISPR/Cas9 system include, S. pyogenes (sp) Cas9, spCas9-HF-1, saCas9, catalytically dead mutants of Cas9 fused to effectors such as cytidine deaminase or transcriptional/epigenetic regulators (CRISPRi/CRISPRa) and non Cas9 modifiers such as Cpf1. This connected programme does not include the use of viral vectors expressing toxin genes or major pathogenesis factors.

Evaluation of foreseeable effects

For retroviral and lentiviral vectors, use of second generation (or later) viral vector systems in which all accessory genes have been deleted, and where gag, pol, env and rev genes are provided in trans (either on additional accessory plasmids or integrated into the genome of packaging cell lines), means that the risk of production of replication-competent or infectious virus is extremely low. In addition the use of self-inactivating (SIN) viral vector systems in which promoter and enhancer elements have been removed from the long terminal repeats (eg U3 deleted 3'LTR for lentiviral transfer vectors), reduces the risk of transcriptional activation of genes proximal to the insertion site, but there remains the possibility gene activation by constitutive promoter/enhancer sequences within the inserted genetic material. Insertion of the viral genome could also result in disruption of genes involved in cellular regulation (e.g. tumour-suppressor genes). WPRE sequences used to increase the levels of expressed mRNA will be present in many vector systems. Although some versions of these elements also express a promoter fragment of the Woodchuck Hepatitis Virus X protein for which there is published evidence for its association with oncogenic activity (Höhne et al., EMBO 9, 1137-1145.1990; Schuster et al., Oncogene 19, 1173-1180. 2000), it is anticipated that this would not result in a need for a higher containment level (>CL2). Nevertheless to improve safety considerations, the preference will be for vectors...
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No animals or plants are proposed to be used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals or plants are proposed to be used.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For address 1:
Class 2 solid waste will be chemically deactivated before leaving the laboratory. Access to the laboratory is restricted. The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. To deactivate, 2% virkon strength for a minimum contact time of 2 hours will be implemented. As a result, there will be no viable GMMs remaining in the solid waste. All solid GMM contaminated waste will then be at least double bagged into biohazard waste bags, and secured with tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste technician will transfer the bags using medibins and a specifically designed wheeled clinical waste carrier for offsite disposal. All liquid waste will be disinfected with 2% virkon for a minimum contact time of 2 hours before disposal. Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with waste protocols correctly as specified above.

For address 3:
Class 2 solid waste will be chemically deactivated before leaving the laboratory. Access to the laboratory will be restricted. The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. To deactivate, 2% virkon strength for a minimum contact time of 2 hours will be implemented. As a result, there will be no viable GMMs remaining in the solid waste. All solid GMM contaminated waste will then be at least double bagged into biohazard autoclave waste bags, and secured with autoclave tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste technician will transfer the bags using medibins and a specifically designed wheeled clinical waste carrier. A bespoke 1.5 tonne lorry is used to take the carrier the short distance to an on-site and enclosed waste facility where the waste is transferred into the building and into the autoclave for 100% sterilisation. All liquid waste will be disinfected with 2% virkon. Solutions will be left soaking for 2 hours before disposal via the drain with copious amounts of water. Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with waste protocols correctly as specified above.

For addresses 2 and 4:
The majority of Class 2 solid waste will be inactivated using onsite autoclave facilities. Some Class 2 solid waste may be chemically deactivated before leaving the laboratory. Access to the laboratory is restricted. The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. Where chemical deactivation is adopted, 2% virkon strength for a minimum contact time of 2 hours will be implemented. As a result, there will be no viable GMMs remaining in the solid waste. All solid GMM contaminated waste will then be at least double bagged into biohazard autoclave waste bags, and secured with autoclave tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste technician will transfer the bags using medibins and a specifically designed wheeled clinical waste carrier to the autoclave within the building for 100% sterilisation. All liquid waste will be disinfected with 2% virkon. Solutions will be left soaking for 2 hours before disposal. Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with waste protocols correctly as specified above.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
Our work consists of the use of genetically engineering, attenuated (or weakened) vaccine strains of vaccinia virus (VACV) to express protein “payloads.” The goal of this work is for the virus to target and specifically replicate within tumour cells while expressing these protein payloads. We will use in vitro cell assays and in vivo mouse (via intravenous injections) model to study the ability/efficacy of these payloads and delivery vehicles (VACV) for impairing tumour growth.

In vitro cell lines infected will include human and mouse immortalized and primary non cancer and cancer cell lines. ADCP categories will vary depending on the cell type.
and can be known or unknown. Potential contaminants pathogens might be human pathogens. For example, human cervical cancer cell lines and human head and neck
tumour cell lines harbour HPV (human papillomavirus) genomic material. Primary human samples are generally screened for the presence of the high risk human
pathogens hepatitis virus and HIV by the supplier. Samples containing such pathogens should not be used. Note: Medium hazard cells and Activity Class 2 cells present a
greater risk than low hazard cells, usually due to their potential to harbour human pathogens.

Standard laboratory mouse strains including C57/BL6J, BALB/c, NSG, nude/SCID are all purchased from approved vendors with appropriate health screen information.

**Host/vector system**

The host/vector system is a double-deleted Copenhagen strain of vacinia virus (VACV). The “double-deleted” refers to the targeted deletion/disruption of the viral I4L and
J2R gene products which encode for the viral ribonucleotide reductase and viral thymidine kinase genes, respectively. Together these two modifications render this strain
highly attenuated and restrict virus replication to metabolically active tumour cells (Foloppe et al. PMID 31011628). VACV has no known animal reservoir but can infect
humans and other mammals. A large number of virus strains exist and have been used (including the wildtype Copenhagen strain) as human vaccines for the eradication
of smallpox disease. The engineered Copenhagen strain used in these studies are highly attenuated and virus replication is restricted to human tumour cells.

**Evaluation of foreseeable effects**

While the host reservoir in nature is unknown, vaccinia virus is a human pathogen but is categorized on the HSE’s approved list of biological agents as “buffalopox”
vaccinia virus” and is considered a hazard group 2 agent by ACDP classification. The specific recombinant VACV strain of Copenhagen origin does not replicate in mice
unless a human tumour is present. Vaccinia virus (VACV) infections in humans are typically mild and asymptomatic. Wild-type and engineered VACV have generally
shown only mild toxicity in trials where they have been evaluated as oncolytics. Side effects of VACV infection can include mild skin reactions (including the formation of
lesions with the potential for virus shedding through infected cells lysis), transient fever, and malaise. VACV has the longest and most extensive history of use in humans.
Accidental exposure to VACV may occur through ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes or broken skin with infectious
particles. When working with wildtype, non-attenuated vaccinia strains ocular exposure is of particular concern due to the immune-privileged status of the eye. Many
different strains of vaccinia virus has been used as smallpox vaccines and administered by local routes of administration in hundreds of millions of humans in the 19th and
20th centuries, giving unprecedented information on VACV behaviour in humans, with the identification of populations which are at risk for rare but serious adverse events
and the measures to respond to these (Cono et al. PMID 12617510; Kretzschmar et al. PMID 16933957). Following injection into the skin, the subcutaneous infection by
the virus is brief and limited. As VACV contains antigens that stimulate an immune response that are cross-reactive with smallpox antigens, the vaccine thereby confers
protection from the human smallpox disease.

1. Viral vector: The genetic modifications described above will not increase or enhance the pathogenicity/ virulence / survivability of the parental double-deleted
Copenhagen strain of VACV nor will it permit these recombinant viruses to outcompete with wildtype vaccinia virus in nature. These vaccinia viruses remain attenuated
with the genetic modifications as outlined above. These specific vaccinia viruses are restricted to replication in only human tumour cells.

2. Engrafted animals: there is a theoretical risk of transmission through biting but the risk as assessed is negligible

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Animals treated with vaccinia constructs will be housed in IsoCage red line indivdually Ventilated Cages red line cages and all handling of animals for husbandry or
experimental tasks (dosing, measuring, sampling) will be carried out in biological safety cabinets with the user wearing suitable PPE, including gown, gloves, surgical mask,
and safety glasses. Routine cleaning of cages post usage. Dirty cages must be broken down within the Biosafety cabinet and placed into paper autoclave bags, sealed with
contents including enrichment and bedding included and sent for heat treated sterilisation (autoclave) prior to the cleaning and washing processes. The cages are not left
for an extended time period, e.g. overnight, but autoclaved as soon as possible on leaving the room. All animal carcasses will be placed in a double containment in a Class
2 microbiology safety cabinet, the outside containment will be treated with 1% Virkon solution before either being sent directly for incineration or stored for a short time (less
than 24hrs) at -20, before subsequent incineration. Animals harbouring human tumours that are infected with these vaccinia constructs can theoretically transmit virus to
cage mates who also harbour human tumours but again this risk is negligible and any infected animals will be housed in separate caging, appropriate to mitigate such
Transmission. Local Emergency procedures with regards to a spill, escape of an animal, needlestick injury including Occupational Health assessment and medical treatment are already in place, available to staff and rehearsed regularly.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be inactivated prior to disposal as Non-Infectious Healthcare Waste (EWC 18 01 04) or where inactivation is not possible as Infectious Healthcare Waste (EWC 18 01 03). Inactivation should be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).
- Liquid waste inactivated in 1% final Virkon for >30minutes
- Solid waste inactivated in 1% final Virkon for >30minutes

Routine cleaning of cages post usage. Dirty cages must be broken down within the Biosafety cabinet and placed into paper autoclave bags, sealed with contents including enrichment and bedding included and sent for heat treated sterilisation (autoclave) prior to cleaning and washing processes. The cages are not left for an extended time period, e.g. overnight, but autoclaved as soon as possible on leaving the room. All animal carcasses will be placed in a double containment in a Class 2 microbiology safety cabinet, the outside containment will be treated with 1% Virkon solution before either being sent directly for incineration or stored for a short time (less than 24hrs) at -20, before subsequent incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<thead>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 108/trans1

02/03/2022  Page 3250 of 15326
CULTIVATION OF DISABLED PROKARYOTIC, LOWER EUKARYOTIC AND MAMMALIAN CELL LINES EXPRESSING ONCOGENIC OR POTENTIALLY ONCOGENIC SEQUENCES

Project Additional Information

Purposes of the contained use
To test whether the KRAB transcriptional repression domain (derived from Drosophila Kruppel) or VP16 transcriptional activation domain (derived from a fragment of Herpes Simplex Virus) fused to synthetic Zinc Finger Proteins targeted to the human estrogen receptor a promoter are capable inhibiting or activating expression of the human estrogen receptor.

Recipient or parental organism

Cloning hosts:
DH5alpha: Disabled E coli K12 strain. Genotype: F supE44 hsdR17 recA1 gyrA96 endA1 th 1relA1 deoR proophage80d (lacZYA argF)u169(m80lacZdM15)
DH10B: Disabled E coli strain. Genotype: F mcrAd(mrr hsdRMS mcrBC) proophage 80dlacZdM15 diacX74 deoR recA1 endA1 AraD139d(ara,leu)7697 galU galKlambda - rpsL nupG
BJ5183 Especially disabled E. coli K-12 derivative with partial recombination capability to allow the recombination of pAdEasy-1 and pAdTrack-CMV. It is ampicillin and kanamycin sensitive.

Virus Producer Cell Line: HEK293
HEK 293 is a human embryonic kidney cell line of widespread use. It has the adenovirus E1A region integrated into its genome stably expressing the transforming gene of human Type 5 Adenovirus, and thus provides complementation to E1A deficient adenovirus constructs allowing their replication - this is used as the packaging cell line. DNA from both the right and left ends of the viral genome is present. When used as a helper cell line production of recombinant adenoviral particles, production of wild-type virus (an ACDP Hazard Group 2 pathogen) can occur albeit at a low frequency. Two specific non-homologous recombination events would be required to generate replication-competent virus. In a supernatant rescue assay performed on the virus stock to check for replication competent adenovirus (ref. Dion et al J. of Virol Methods 1996 vol56 p99-107) no replication competent virus was detected in 1x10e9 infectious particles. If virus stocks are repeatedly passaged in HEK293 cells replication-competent virus can appear after 9 passages. Methods to produce virus using pAdEasy-1 only require a maximum of three passages in HEK293 cells. Given this data, the risk of generating wild-type adenovirus is considered to be negligible using these procedures.

Expression Host Cells: HEK293, MCF, T47D
HEK-293: see description above.
MCF-7: The MCF7 line is derived from a human mammary gland adenocarcinoma and retains several characteristics of differentiated mammary epithelium contains the Tx-4 oncogene. CL2 following UKCCR guidelines.

T47D cells: ECACC Ref No: 85102201 Established from the pleural effusion of a ductal carcinoma of the breast of a 54-year old female. The cells carry receptors for a variety of steroids. ATCC refer in an entry for a mouse myeloma cell line (HB-8630) to human mammary tumor virus particles isolated from the T47D clone-10 mammary cancer cell line. CL2 following UKCCR guidelines. No reason to suspect that the modification will have any effect on the hazard posed by any such endogenous virus.

Host/vector system

Cloning Vectors (used with DH5alpha; DH10B and BJ5183 bacterial hosts): pShuttle: This is identical to pAdTRack-CMV except it does not contain a CMV promoter or the GFP element. It is used in conjunction with pAdEasy, to generate recombinant adenoviral plasmids in E. coli. The resulting recombinant plasmid can be used to generate infective but replication-defective adenoviral particles following transfection into suitable mammalian host cells (e.g HEK 293 cells) able to provide the E1a sequence in trans. PadTrack-CMV is a 9.2 kbp vector obtained from Bert Vogelstein (He et al PNAS 1998 vol 95 p2509-2514) and is used in conjunction with pAdEasy-1 to generate recombinant adenoviral plasmids in E. coli. The resulting recombinant plasmid can be used to produce infective but replication-defective adenoviral particles following transfection of suitable mammalian host cells (e.g. HEK293) above to provide the E1a sequence in trans.
pAdEASY-1: A33.4 kbp vector obtained from Bert Vogelstein (He et al PNAS 1998 vol 95 p2509-2514) which contains all the adenovirus type 5 sequences except nucleotides 1-3533 (encompassing the E1 genes) and 28, 130-30, 820 (encompassing E3 region). The vector also contains an ampicillinR gene and an origin of replication from pBR322, hence the plasmid is mobilisation defective (Bom+ Tra- Mob-) The vector is used in conjunction with pAdTrack vectors to generate recombinant adenoviral plasmids in E. coli (eg when co-transformed into a bacterial host with the pAdTrack-CMV shuttle vector containing an insert sequence, recombination in the host leads to production of a recombinant pAdEasy-1 containing insert sequences. That plasmid can be used to transfact appropriate mammalian cells (eg. HEK293) to generate recombinant E1a-deleted adenoviral particles.

Additional information for Recombinant Viral Vectors

"Insert" Vector(s): recombinant pAdEASY-1 used with packaging cell line (HEK) and resulting viral particles used to transfact mammalian expression host cells MCF-7 and T47D.

Origin & function

This is an artificial construct containing: a nuclear localisation signal - NLS (derived from SV40 large T), hER1.0/1.1ZFP DNA binding domain (synthetic), either a KRAB transscriptional repression domain (derived from Drosophila Kruppel) or a VP16 transscriptional activation domain (derived from a fragment of Herpes Simplex Virus) and a FLAG domain (synthetic) in pcDNA3. This artificial construct is under the control of the Tet Repression system (Tet Off). This comprises two cassettes which have been PCRd from commercially available vectors: Cassette 1 consists of a Tet repressor under the control of a CMV IE promoter linked to VP16 followed by an IRES-EGFP element and Bovine growth hormone Poly A. Cassette 2 comprises a 7 x Tet Operon with a CMV IE minimal promoter, followed by a multiple cloning site (into which the above ZFP constructs will be inserted) and an SV40 Poly A. The two cassettes are inserted back to back into the pSHUTTLE expression plasmid. (See Vogelstein et al. PNAS., Vol 99, pp. 2509-2514, March 1998)
The two proteins to be expressed are artificial constructs. Each consists of zinc fingers which are expected to bind specifically to an 18-bp sequence in the hERa promoter. In the presence of tetracycline their presence will be switched off. However, in the absence of tetracycline they are expected to be highly expressed. The proteins will contain a Nuclear Localisation Signal and will therefore be targeted largely to the nucleus of hER2.0/1.1 NKF/NVF expressing cells, where the KRAB transcriptional repressor domain and VP16 transscriptional activator domain will assert their effects on hERa transcription within 1kb of the hERa Sp1 binding site. The expressed proteins are expected to be biologically active but are designed to exert their effects only in cells expressing endogenous hERa. Since ERa is a transcription factor, over-expression will have no effect unless the cells are exposed to estrogens, in which case the consequence will be to activate expression of estrogen responsive genes.

Evaluation of foreseeable effects

Taking a very precautionary approach, the likelihood of potentially harmful effects being realised as a consequence of exposure of the operator or environment to the viral vectors was judged to be low. Risks associated with other GMOs are judged to be negligible.

The
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Date at Which Additional Info Submitted: 21/06/2001
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify) Tick if confidential

02/03/2022 Page 3255 of 15326
Bacteriology  Parasitology  Transgenic
Transgenic  Microbiology
Birds  Research

Virology  Transgenic
Transgenic  Gene Therapy
Animals  Fish

Mycology  Transgenic
Transgenic  Other (please
Invertebrates  specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum volume that could be released at any one time: 15,000 L.
Validation method: Laboratory experiments to produce kill curves followed by on confirmation on plant.
Monitoring: pH and temperature controls and monitors. Viable counts on effluent.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

Project Ref  109/06.1

Date Ackn'd  13/06/2006
Date Project Ceased  10/06/2021
Withdrawn  N

Growth of a vibrio species expressing the B subunit of E.coli enterotoxin into cell culture supernatant.

Class  CultureVol
CultureVol
Class 2  1-50 Litres

Non-GMM Consent Granted  Not Applicable
Project notified under transitional arrangements  N

Tick if notifying a connected programme of work  N
### Project Additional Information

**Purposes of the contained use**

Growth of a non pathogenic vibrio species up to 1L shake-flask volume expressing the non toxic B subunit of E.coli enterotoxin and recovery of the expressed protein from the cell culture supernatant.

**Recipient or parental organism**

Marine vibrio strain (considered non pathogenic to humans)

**Host/vector system**

An IPTG induced expression vector carrying the gene for the non-toxic B subunit of E.coli enterotoxin.

**Origin & function**

E.coli Enterotoxin B subunit expressed in a vibrio species.

Therapeutic uses,
- Allergy eg Allergic Rhinitis
- Autoimmune Disease eg Rheumatoid Arthritis
- Vaccine Adjuvant

**Evaluation of foreseeable effects**

No hazards have been identified.

The introduction of the B subunit genes of the E.coli enterotoxin into the vibrio organism is unlikely to alter any existing pathogenic traits.

The host cell line is considered non pathogenic to humans.

The B subunit of E. coli enterotoxin has been extensively evaluated in pre-clinical pharmacology studies without any adverse effects. As all vibrio species are assigned as biological hazard class 2 organisms by ACDP all project work (cell culture, primary recovery and purification) with the organism will be carried out in microbiology laboratories working to containment level 2.

All potentially contaminated waste will be autoclaved prior to disposal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Following fermentation and primary recovery the culture supernatant will be 0.22 um filtered. No viable GMO will be present following this stage but samples will be tested for viable counts.

It is unlikely that the vibrio organism would survive in the environment and as the organism is already present in the wider marine environment the consequence of release of the organism into the environment is negligible.

All potentially contaminated waste will be autoclaved prior to disposal and uplifted from the site by an approved contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The assessment was modified to account for comments from the GMSC. The modified version was approved.

Project Containment

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<td>L3 L4 L2 L3 L4</td>
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Animal Units

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Project Ref 109/21.1

Date Ackn’d 30/06/2021

CU2 Project Title Use of a genetically modified HEK293 cell line from Cisbio (commercial supplier) for analytical purposes (QC testing).

Date Project Ceased 02/03/2022

Class CultureVolumeClass2 CultureVolumeClass3-4

Class 2 < 1 litre

Non-GMM Consent Granted

Consent Granted Not Applicable
Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Quality control testing - the GMO is provided as a component within a commercial bioassay kit. The bioassay kit is used to determine the binding of affinity of IgGl mAbs to the CD32B Fc receptor stably expressed on HEK293 cells.

Recipient or parental organism

HEK293 cells from the American Type Culture Collection (ATCC. CRL-1573).
The parent HEK293 cell line contains adenovirus, which is deemed ACDP Hazard Group 2.
Where a cell line is deliberately infected with a biological agent or where it is likely that the cell line is contaminated with a particular agent, the containment level used must be appropriate for work with that agent - SACGM recommended baseline containment measures for work with cell cultures. Adenoviruses main route of transmission is via infected aerosol particles therefore all operations involving the cell line will be performed inside a Class II MSC thereby mitigating this risk. Adenoviruses can cause mild to severe illness, though serious illness is less common.
People with weakened immune systems, or existing respiratory or cardiac disease, are at higher risk of developing severe illness from an adenovirus infection. no such individuals will handle these cells.

Host/vector system

Initially amplified in the intermediate host E.coli DH5Alpha, the plasmid p-SNAP FcgRIIB containing the CD32B transgene was transfected into HEK293 cells using lipofectamine transfection reagent by Cisbio (commercial supplier). The plasmid DNA (CD32B transgene) is non mobilisable and there is no mechanism for independent replication following transfection. Consequently, existing pathogenic traits are not deemed to be altered from the transfection process. with the GMO considered a BSL-2 agent.

Origin & function

The physiological function of CD32B is to regulate the activation state of the expressing cell through interaction with other cell surface receptors. The CD32B gene incorporated into the HEK293 cell line is not deemed to have an impact on existing pathogenic traits; the transformation carried out to generate the recombinant DNA is assessed by Cisbio (cell line supplier) as containment level 1, with CD32B non-infectious for human health. Furthermore, “The SACGM Compendium of guidance Part 2: Risk assessment of genetically modified microorganisms” (p64) provides guidance on hazards posed by genetic insertion. The compendium demonstrates that if the gene is already present in nature (e.g. mammalian cells), the impact of transfer will be diminished. Considering that CD32B is a naturally occurring immune checkpoint Fc receptor, present in B cells of the human immune system, the risk to human health from
CD32B expression can be effectively judged as zero (i.e. it is non-toxic).
The GMO will be used at small scale for the performance of Quality Control bioassay analysis. The cells will be
utilised as "thaw-to-plate" cells; the cells are provided in single use aliquots by Cisbio, with the aliquot of cells thawed
and immediately applied in the bioassay. Consequently, there will be no propagation of the cell line at FUJIFILM
Diosynth Biotechnologies UK Ltd.

**Evaluation of foreseeable effects**

The parent HEK293 cell line is classified by ATCC as Biosafety Level-2 (BSL-2) (ACDP Hazard Group 2) as it
contains adenovirus genetic material. The adenovirus infection has not been altered during the lipofectamine
transfection process performed by Cisbio, with the GMO assigned BSL-2 status by the supplier. The plasmid DNA
(CD32B transgene) is non mobilisable and there is no mechanism for independent replication following transfection.
Consequently, existing pathogenic traits are not deemed to be altered from the transfection process, with the GMO
considered a BSL-2 agent.

The cells will be utilised as "thaw-to-plate" cells; the cells are provided in single use aliquots by Cisbio, with the aliquot
of cells thawed and immediately applied in the bioassay. Adenovirus' main route of transmission is via infected
aerosol particles however all operations involving the cell line will be performed inside a Class II MSC thereby
mitigating this risk, the cells will be chemically inactivated and discharged into yellow biohazard waste containers for
incineration, on completion of the testing. Furthermore, all materials and equipment exposed to the biological agent
(e.g. the Class II MSC) will be disinfected with chemical cleaning agents, designed to destroy viral particles. In
consideration of these practices, the risk of wider environmental containment being breached, can be effectively
judged as low risk. Additionally, the cell line will be handled in accordance with Biosafety Level-2 (BSL-2) guidelines
and as per laboratory practices for FUJIFILM Diosynth Biotechnologies UK Ltd BSL-2 Bioassay Laboratory.

The CD32B gene incorporated into the HEK293 cell line is not deemed to have an impact on existing pathogenic
traits; the transformation carried out to generate the recombinant DNA is assessed by Cisbio as containment level 1,
with CD32B non-infectious for human health. Furthermore, "The SACGM Compendium of guidance Part 2: Risk
assessment of genetically modified microorganisms" (p64) provides guidance on hazards posed by genetic insertion.
The compendium demonstrates that if the gene is already present in nature (e.g. mammalian cells), the impact of
transfer will be diminished. Considering that CD32B is a naturally occurring immune checkpoint Fc receptor, present
in B cells of the human immune system, the risk to human health from CD32B expression can be effectively judged as
zero (i.e. it is non-toxic).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

NA

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

NA

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The BSL 2 laboratory has a dedicated procedure for cleaning of the cell assay laboratory in addition to a cleaning
procedure for contamination in the laboratory.

The cell line is extremely unlikely to survive outside of assay media due to sensitivity to temperature, pH and culture
conditions, nonetheless the cells and disposable assay materials (e.g. assay plates and laboratory consumables) will
be chemically inactivated using Chemgene (anti viral disinfectant that denatures DNA & RNA) and discharged into
yellow biohazard waste containers for incineration on completion of the testing. These biohazard bins are locked and
disposed of by a licenced contractor (https://www.stericycle.co.uk) maintaining the cradle to grave containment

02/03/2022
approach. (assumed degree of kill of 100%). Furthermore, all materials and equipment exposed to the biological agent (e.g. the Class II MSC) will be disinfected with chemical cleaning agents, designed to destroy viral particles (chemgene).

Following testing by the department of Biochemistry at Cambridge University, HLD4 formulation was proven to precipitate I denature double stranded DNNRNA. Adenovirus genome is double stranded DNA.

(https://www.scientificlabs.co.uk/handlers/libraryFiles.ashx?filename=Technical_Data_Sheets_C_CLE3360_A.pdf)

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 109/21.2

Date Ackn'd 10/09/2021

CU2 Project Title

This notification is aimed to cover work with the Hek293 cell line and the production, purification and analysis of Adeno Associated Virus

Date Project Ceased

Class | CultureVolClass2 | CultureVolumeClass3-4 |
Class 2 | < 1 Litre |

Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

02/03/2022
Project Additional Information

**Purposes of the contained use**

The laboratories are to be used for the growth and expansion of mammalian cell lines (primarily HEK293) and the production, purification and viral testing of Adeno Associated Virus. The cell culture may be perfomed using either adherent or suspension culture methods. The viral vectors will be produced by transient transfection or infection (from a viral stock) and once produced the vectors will be purified using chromatography, tangential flow filtration (TFF) and filtration (both depth and sterile filtration). The purified vectors will be analysed to determine the amount of virus and the quality of the vector produced using a number of biochemical and chemical techniques.

**Recipient or parental organism**

The parental organism is a Human Embryonic Kidney cell line (HEK293) that has been procured from OXgene and is a clonal suspension HEK cell line that has been optimised for the production of adeno associated viral vectors by triple transfection. The cells have not undergone additional genetic modification beyond the addition of some adeno viral sequences which were used to create the original HEK293 cell line in 1977. These cell have been used extensively in the production of viral vectors for many years.

**Host/vector system**

The adeno associated viral vector production utilises a plasmid triple transfection system. This vector system has been designed to ensure that the vectors that are produced are not able to replicate. In triple transfection a three plasmid system is used, in which one plasmid (rep/cap plasmid) codes for the structural components of the vector, the second plasmid (Helper plasmid) codes for the sequences from an adenovirus that are required for generation and assemblay of the vector, and the third plasmid (GOI or transgene plasmid) codes for the genetic information that will be encapsulated in the viral vector. The product coded by the transgene plasmid and the rep/cap plasmid will vary depending on the intended cellular target and disease that is being treated.

**Origin & function**

All the genetic material involved will be generated from a recombinant source; all the plasmids used for producing the viral vectors will initially be made by gene synthesis and then subsequently produced at a larger scale via fermentation in E.coli and subsequent purification at either Fujifilm Diosynth Biotechnologies or supplied by a customer. All materials used are well characterised and undergo safety testing prior to use or receipt at FDB.

The function of the plasmids used is to allow the production of AAV particles that contain a specific gene sequence.
Evaluation of foreseeable effects

The HEK293 cell line is a well-characterised and tested cell line that has been used in clinical research and production of therapeutics since the 1970s, and is therefore of low risk to human health. The primary concern comes from the presence of Adenoviral DNA within the cells. Adenoviruses are infectious to humans and cause human disease, however the HEK293 cell line doesn’t contain the full adenoviral sequence and cannot produce adenoviruses in the absence of an adenoviral infection. The cells are an immortalised cell line that has been shown to be oncogenic in mice at a high passage number. Whilst this may be a potential risk to human health, the cells will not be taken to a high number of passages from thaw and is therefore very unlikely to pose an oncogenic risk.

The AAV construct is specifically designed to be non-replication competent. The construct is produced by triple transfection of three plasmids to produce AAV vectors which is structurally the same as a wild type AAV but only contains an expression cassette that codes for the GOI and none of the Rep Cap or helper sequences that would be required for viral replication. Whilst these AAV vectors are not able to replicate, they can transduce specific cell lines, depending on the serotype. Therefore there is a risk that should an exposure event occur, an exposed individual could transiently make the product coded for by the GOI. Whilst in some cases the GOI product will be harmless, when a different GOI is used an assessment will be made to assess the potential risk. Where the GOI poses a risk to individuals, additional controls (if deemed necessary by the risk assessment) will be put in place to ensure the safety of the users and subsequent HSE notification will be made if the work is deemed higher than GM activity class 2.

The cell line is extremely unlikely to survive outside of culture media due to sensitivity to temperature, pH and culture conditions, therefore it is unlikely to be a risk to the environment. AAV is a relatively hardy virus; non-enveloped DNA virus and therefore relatively stable and resistant to dehydration. It is therefore possible that it could survive for extended periods in the environment. However, due to the design of the vector and the lack of modification to the structure of the viral capsid, it is not able to replicate and is not known to be able to cause disease or replicate in animal hosts. Therefore they are unlikely to pose any significant risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste products that have potential to contain GMM will be treated with either Chemgene at a 10% v/v concentration or 10% v/v Sodium Hypochlorite, for up to 1hr. Chemgene has demonstrated virucidal activity against Parvoviruses (<4 log), Adenoviruses (3.68 log), and Lentiviruses (4.5 log), making it suitable for use as a disinfectant on all the viruses that could be made in the labs. They will then be autoclaved or placed into biohazard bins for incineration. These biohazard bins are locked and disposed of by a licenced contractor (https://www.stericycle.co.uk) maintaining the cradle to grave containment approach. (assumed degree of kill of 100%). Furthermore, all materials and equipment exposed to the biological agent (e.g. the Class II MSC) will be disinfected with chemical cleaning agents, designed to destroy viral particles (chemgene). Following testing by the department of Biochemistry at Cambridge University, HLD4 formulation was proven to precipitate / denature double stranded DNA/RNA.
Please enter comments on the GM safety committee on the risk assessment

Assessment circulated to GMSC on Jul 30th 2021.
1. Please see my comments on the attached document. I think the containment classes stated make sense to me. This looks like class 2 containment will be required.
   I'll be honest, I think I might need some graphics to explain the molecular biology content in more simple terms as there seems to be a lot here. I would need it more if I was to be convinced to lower it to class 1 containment. I would like to see what other members of the committee think.
2. I agree, I think there is a reasonable amount of evidence that the HEK's are probably class I but since PHE and FDBT handle them as class II it makes sense to follow that precedent.
3. In terms of the GMO RA, I've added a couple of comments. There is a bit of jargon that either needs explaining or rephrasing and a potentially superfluous/confusing passage which doesn't seem to add anything.
4. I agree with keeping it as a Class II. I also agree with the use of graphic and simplistic explanations.
5. Hi All, I agree as well! Class 2 based on the current information.
6. I agree with class II on current understanding especially if PHE use that classification.
7. I support this assessment. You need to add your details to section 8 - ‘assessment prepared by:’

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<td>L2 Yes L3 L4</td>
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Project Ref  109/21.3

Date Ackn'd 02/03/2022
Cloning and expression of a recombinant protein in CHO cell lines

The laboratories are to be used for the transfection, growth and expansion of CHO cells to enable stable cell lines expressing the recombinant proteins to be generated. The cell culture will be performed with suspension cell culture methods. Stable cell lines will be generated using plasmid DNA which will be transfected into cells using electroporation methods and then selected using a DHFR selectable marker. These recombinant cell lines will be used to generate recombinant proteins (using shake flasks) which will be purified and analysed.

The parental organism is an immortalised mammalian cell line, specifically a Chinese hamster ovary (CHO) cell line (CHO-DG44). The cells have not undergone any additional genetic modification and have been used for the production of recombinant proteins for many years. The CHO cell line comes from a GMP cell bank that has been tested for the presence of adventitious agents. It is a well characterised cell line with a low risk of endogenous infection with a biological agent presenting no apparent harm to laboratory workers (CL1).

The inserted DNA sequence covered by the risk assessment is an immunomodulatory protein, specifically the expression of the gene expressing a xxxxxxxx. The xxxxxxxxx has been engineered to bind human leukocyte antigen. The DNA for the xxxxxxxx to be expressed will be synthesised by commercial gene synthesis and then cloned into expression vectors. All materials used are well characterised and undergo safety testing prior to use or receipt at FDB. The function of the plasmids is to allow expression of the gene encoding the xxxxxxxxx product. The product plasmid is not designed to replicate in mammalian cells. The stable cell line will have linearised plasmid DNA.
integrated into the host cell genome. However, there are no mechanisms within the plasmid to enable transposition to other sites or replicons within the host i.e. the plasmid is non mobilisable.

**Evaluation of foreseeable effects**

The CHO DG44 cell line is well characterised and tested cell line that has been used in the production of therapeutics since the 1970s and is therefore of low risk to human health. The cell line is extremely unlikely to survive outside of the culture media due to sensitivity to temperature, pH and culture conditions, therefore it is unlikely to be a risk to the environment.

The primary concern comes from the GOI which may cause a risk to human health. The antibody being expressed is biologically active at low doses. This means that the acceptable daily exposure (ADE) limits for this molecule is low and the secretion of active molecule from the GMM could make the recombinant cell line a risk to workers via internalisation (principally via inhalation). Cell lines will be handled in accordance with containment level-2 guidelines and all staff working with these cell lines will follow site safety procedures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste containing the GMM will be treated using > 1% v/v chemgene for > 2 hours (internal study has shown this achieves 100% cell death). This will then be either disposed of via the drain to the public sewer or placed into biohazard bins for incineration.

Solid waste that has been exposed to the GMM will be placed into biohazard bags before being put into biohazard bins for incineration. The biohazard bins are locked and disposed of by a licenced contractor (https://www.stericycle.co.uk) maintaining the cradle to the grave containment approach. Assumed degree of kill of 100%.

Furthermore, any equipment and materials exposed to the GMM (e.g class II biological safety cabinet) will be disinfected with chemical cleaning agents as described above.

An autoclave is present in the building where the work will take place. The autoclave is on a service contract and is inspected annually where the autoclave is tested and verified and a calibration is performed. Autoclave tape is used as a visual aid to show whether run has been successful and records of each run are kept in a logbook.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
Some changes required to the initial assessment to clarify molecular biology stages, waste disposal and product information. Committee agreed with GMM Activity class 2 classification and controls applied. Risk assessment approved by the GMSC on 26/10/2021.

Project Containment

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</table>

Animal Units

- Large Scale Activities
- Human Clinical Applications

Project Ref 109/21.4

Date Ackn'd 03/12/2021

CU2 Project Title

This notification is aimed to cover work with CHO cell lines and the expression, purification and analysis of biopharmaceuticals which are potentially harmful to human health.

Date Project Ceased

Class 2

CultureVol

Class2 1-50 Litres

Consent Granted

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The laboratories are to be used for the growth and expansion of CHO cells to enable stable cell lines expressing the GOI under test to be generated. The cell culture will be performed with suspension cell culture methods. Stable cell lines will be generated using plasmid DNA which will be transfected into cells using electroporation methods and then
selected using a DHFR selectable marker. These recombinant cell lines will be used to generate protein (using shake flasks or bioreactors) which will purified and analysed. The notification has been submitted as a connected programme of work (more than one contained use at a single notified premises) at FDBK (Billingham & Wilton) as the scientific goals align i.e Mammalian cells are genetically modified to express recombinant proteins which will be purified and analysed as biotherapeutics. The cell line and vectors used will not change for this programme, the only variable is the gene of interest (GOI) product. A risk assessment for each GOI will be undertaken and reviewed by FUJIFILM Diosynth Biotechnologies GMSC to ensure the risk does not exceed GM activity class 2. If the risk exceeds the original classification or if additional controls are required, a new contained use notification will be made.

Recipient or parental organism

The parental organism is an Immortalized mammalian cell line, specifically a Chinese hamster ovary (CHO) cell line (CHO-DG44). The cells have not undergone any additional genetic modification and have been used for the production of recombinant proteins for many years. The CHO cell line comes from a GMP cell bank that has been tested for the presence of adventitious agents. It is a well characterised cell line with a low risk of endogenous infection with a biological agent presenting no apparent harm to laboratory workers (CL1).

Host/vector system

CHO-DG44 DHFR selection system The plasmid backbones are non-mobilisable. Introduction of plasmids into cells will be accomplished by DNA-mediated transfection.

Origin & function

The inserted genes will be immunomodulatory proteins such as high potency fusion proteins, bispecific and trispecific proteins which are biologically active at low doses. There are no plans to work with active toxins, allergens, Oncogenes or hormones. It is possible that work may be performed to express cytokines and growth factors. Examples of such proteins are (but not limited to):
- Vibecotamab (XmAb®14045), a tumor-targeted bispecific antibody (BiAb) that contains both a CD123 binding domain and a cytotoxic T-cell binding domain (CD3).
- Aflibercept is a recombinant fusion protein that acts as a soluble decoy receptor and binds to vascular endothelial growth factors A and B (VEGF-A, VEGF-B) and placental growth factor (PIGF). Aflibercept inhibits the activation of VEGF receptors and the proliferation of endothelial cells, thereby inhibiting the growth of new vessels that supply tumours with oxygen and nutrients.
- Rilonacept is a dimeric fusion protein consisting of the ligand-binding domains of the extracellular portions of the human interleukin-1 receptor component (IL-1R1) and IL-1 receptor accessory protein (IL-1RAcP) linked in-line to the fragment-crystallizable portion (Fc region) of human IgG1 that binds and neutralizes IL-1.
- Eloctate is a recombinant Antihemophilic Factor, Fc Fusion Protein, it is a recombinant DNA derived, antihemophilic factor indicated in adults and children with Hemophilia A (congenital Factor VIII deficiency).
- Glofitamab is a bivalent CD20-Targeting T-Cell–Engaging Bispecific Antibody

The most potent of these being vibecotamab which has been assessed in the risk assessment. Each molecule will be assessed in the same level of detail outlined in this Risk assessment prior to experimental work. The vectors used are designed to allow the expression of the gene of interest. The DNA for proteins to be expressed will be synthesised by commercial gene synthesis and then cloned into expression vectors. All materials used are well characterised and undergo safety testing prior to use or receipt at FDB.
The function of the plasmids is to allow expression of the GOI. The product plasmid is not designed to replicate in mammalian cells. The stable cell line will have linearised plasmid DNA integrated into the host cell genome. However, there are no mechanisms within the plasmid to enable transposition to other sites or replicons within the host i.e. the plasmid is non mobilisable. CPW boundaries will be implemented to exclude any higher risk activities-A risk assessment for each GOI will be undertaken and reviewed by FDB's GMSC to ensure the risk does not exceed GM activity class 2. If it is deemed to increase the activity above Class 2 then the activity will not be permitted.

Evaluation of foreseeable effects

The CHO DG44 cell line is well characterised and tested cell line that has been used in the production of therapeutics since the 1970s and is therefore of low risk to human health. The cell line is extremely unlikely to survive outside of the culture media due to sensitivity to temperature, pH and culture conditions, therefore it is unlikely to be a risk to the environment.

The primary concern comes from the GOI which may cause a risk to human health. Examples of the proteins being expressed are bispecific and trispecific molecules which are biologically active at low doses. There are no plans to work with active toxins, allergens, Oncogenes or hormones. It is possible that work may be performed to express cytokines and growth factors. Each new molecule will be assessed with respect to their potency prior to the initiation of any experimental work in the FDBK laboratories which has the potential to expose the operator to the substance, via any route. The assessment is performed by the Contamination Control Group, who will generate both a provisional Acceptable Daily Exposure limit (ADE) and a provisional Operator Exposure Limit (OEL). The assessment will be approved by the EHS manager prior to issue. The potency as indicated by the OEL will determine the control measures that are required to constrain operator exposure to below safe health based limits. Using this molecule information, any potentially harmful effects associated with the GOI, in which the transgene does encode a cytokine or growth factor (or other potentially harmful insert) will be taken into account in the GMO risk assessment. If the risk assessment (taking into account the nature of the work) determines the expressed product/API is harmful to human health and safety at any point in the process then it will be carefully considered when assigning an appropriate level of containment, FDBK would not work with anything above GM activity class 2.

Cell lines will be handled in accordance with containment level-2 guidelines and all staff working with these cell lines will follow site safety procedures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste containing the GMM can be autoclaved or treated using > 1% v/v chemgene for > 2 hours (internal study has shown this achieves 100% cell death). This will then be either disposed of via the drain to the public sewer or placed into biohazard bins for incineration.

Solid waste that has been exposed to the GMM will be placed into biohazard bags before being put into biohazard bins for incineration. The biohazard bins are locked and disposed of by a licenced contractor ([https://www.stericycle.co.uk ] maintaining the cradle to the grave containment approach. Assumed degree of kill of 100%.

Furthermore, any equipment and materials exposed to the GMM (e.g class II biological safety cabinet) will be
disinfected with chemical cleaning agents as described above. An autoclave is present in the building where the work will take place. The autoclave is on a service contract and is inspected annually where the autoclave is tested and verified and a calibration is performed. Autoclave tape is used as a visual aid to show whether run has been successful and records of each run are kept in a logbook.

CRS by inhalation appears to be a real risk and CRS looks unpredictable in severity depending on the individual. Xx Not sure if this is helpful but the NIH have shared a paper from the UK which recommends class 2 minimum for what they call Cytotoxic Agent Research Laboratories (CARL): https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7720356/

From everything I have read in the attached documents, HSE guidance and online info on CRS I would be recommending containment level 2 controls even though the cell line is well characterised and level 1.

I agree with xx that it should be Containment Level 2 due to the nature of the API.

xx, xx, xx, xx, xx xx: I support this assessment.

xx. I agree with earlier comments from xx. I think there are useful suggestions in the paper which if not already included in the SHESOP should be included in the RA for this project and rolled into future SHESOP updates.

The committee agreed there was scope for a CPW submission.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
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<td>L2 Yes</td>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2</td>
<td>L3</td>
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Project Ref 109/22.1

Date Ackn’d: 24/02/2022  Use of a genetically modified HEK293 cell line from InvivoGen (commercial supplier)

Class: Class 2  CultureVolClass2: < 1 Litre  CultureVolumeClass3-4: 

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Purposes of the contained use

Quality control testing - the GMO is provided as a component within a commercial bioassay kit. The bioassay kit is used to determine the in vitro biological potency of IFNa/b through the SEAP reporter gene stably expressed on HEK293 cells.

Recipient or parental organism

The parent HEK293 cell line is classified by ATCC as Biosafety Level-2 (BSL-2) as it contains Adenovirus 5 (ATCC, Cat# CRL-1573), however only left end sequences of the viral genome are present. The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment from nts 1 to 4344 is integrated into the chromosome.

Host/vector system

The HEK-293 cell line has been genetically modified by InvivoGen to express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the type I interferon pathway. The receptors are added by simple transfection of plasmids using a cationic lipidic transfection agent (the plasmids are not linearized before transfection). There is no mechanism within the plasmid (SEAP transgene) for independent replication following transfection. Consequently, existing pathogenic traits are not deemed to be altered from the transfection process, with the GMO considered a BSL-2 agent.

Origin & function

SEAP removes phosphates from a variety of molecules. It is a common tool in biology and although is classified as an irritant, the concentration in the culture shall be low and highly unlikely to cause irritation to process operators. The SEAP gene incorporated into the HEK293 cell line is not deemed to have an impact on existing pathogenic traits; Furthermore, “The SACGM Compendium of guidance Part 2: Risk assessment of genetically modified microorganisms” (p64) provides guidance on hazards posed by genetic insertion. The compendium demonstrates that if the gene is already present in nature, the impact of transfer will be diminished.
The parent HEK293 cell line is classified by ATCC as Biosafety Level-2 (BSL-2) (ACDP Hazard Group 2) as it contains adenovirus genetic material. Despite the GMO containing Adenovirus 5 viral DNA, the risk of Adenovirus 5 (Ad5) infection to process operators is deemed to be low as the GMO is unlikely to produce active (replicating) or infectious Adenovirus 5.

Adenovirus’ main route of transmission is via infected aerosol particles however all operations involving the cell line will be performed inside a Class II MSC thereby mitigating this risk, the cells will be chemically inactivated and discharged into yellow biohazard waste containers for incineration, on completion of the testing. Furthermore, all materials and equipment exposed to the biological agent (e.g. the Class II MSC) will be disinfected with chemical cleaning agents, designed to destroy viral particles. In consideration of these practices, the risk of wider environmental containment being breached, can be effectively judged as low risk. Additionally, the cell line will be handled in accordance with Biosafety Level-2 (BSL-2) guidelines and as per laboratory practices for FUJIFILM Diosynth Biotechnologies UK Ltd BSL-2 Bioassay Laboratory.

The SEAP gene incorporated into the HEK293 cell line is not deemed to have an impact on existing pathogenic traits; the cell line was transfected using cationic lipid agents for delivery of the plasmid DNA, with stable clones maintained under selection pressure. Additionally, the HEK-Blue™ IFN-α/β cell line has demonstrated a high degree of stability (up to 20 passages post thaw) from the vendor (InvivoGen). No mechanisms exist within the host organism to enable transposition to other sites or replicons within the host. Consequently, the transformation carried out to generate the recombinant DNA is extremely unlikely to have affected pathogenicity, with the GMO incapable of replicating or producing infectious Adenovirus 5. Furthermore, ‘The SACGM Compendium of guidance Part 2: Risk assessment of genetically modified microorganisms’ (p64) provides guidance on hazards posed by genetic insertion. The compendium demonstrates that if the gene is already present in nature (e.g. Human placenta), the impact of transfer will be diminished.

Evaluation of foreseeable effects

Not applicable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The BSL 2 laboratory has a dedicated procedure for cleaning of the cell assay laboratory in addition to a cleaning procedure for contamination in the laboratory. The cell line is extremely unlikely to survive outside of assay media due to sensitivity to temperature, pH and culture conditions, nonetheless the cells and disposable assay materials (e.g. assay plates and laboratory consumables) will be chemically inactivated using Chemgene (anti viral disinfectant that denatures DNA & RNA) and discharged into yellow biohazard waste containers for incineration on completion of the testing. These biohazard bins are locked and disposed of by a licenced contractor (https://www.stericycle.co.uk) maintaining the cradle to grave containment approach. (assumed degree of kill of 100%).

Furthermore, all materials and equipment exposed to the biological agent (e.g. the Class II MSC) will be disinfected with chemical cleaning agents, designed to destroy viral particles (chemgene). Following testing by the department of Biochemistry at Cambridge University, HLD4 formulation was proven to precipitate / denature double stranded DNA/RNA. Adenovirus genome is double stranded DNA, (https://www.scientificlabs.co.uk/handlers/libraryFiles.ashx?filename=Technical_Data_Sheets_C_CLE3360_A.pdf)
Autoclaves are accessible in both buildings where the contained use will be carried out i.e both Billingham and Wilton.

The biosafety committee had no comments on the risk assessment and supported the approach and assignment of GM activity class 2 /containment level 2. The risk assessment was signed off on the 24th Jan 2022.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if an emergency plan is required according to regulation 20? N

Tick to confirm that it is attached to this form N

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

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<th>Growth Rooms</th>
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02/03/2022
UNIVERSITY OF HERTFORDSHIRE

Name 2

Biological Sciences

SCIENCE BUILDING

College Lane

Hatfield

Hatfield

AL10 9AB

ENGLAND

01707 284550

01707 285258

EAST AND SOUTH EAST

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

1. The maximum culture volume to be released at any one time - 250 cm³.
2. Waste deactivation. Autoclaving at 121°C 20 minutes.
3. The procedure has been validated by the use of independently calibrated temperature probes, temperature sensitive strips and test cultures.
4. The procedure is monitored by a calibrated temperature probe inserted in an equivalent vessel to those being treated and in the area of the autoclave which is the last to reach the set point temperature. Each disposal run is logged and the record of the temperature profile signed and retained.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 110/19.1

**Date Ackn'd** 14/03/2019

**CU2 Project Title** Deletion of lysogenic phages from Clostridium difficile

**Class** Class 2

**CultureVol** < 1 Litre

**Class CultureVol** Class 2 CultureVolume < 1 Litre

**Consent Granted**

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
The purpose of the contained use in this project is to create isogenic mutants of C. difficile by removing (deleting) phage sequences from bacterial host genomes, enabling comparison of growth, virulence, phage susceptibility and gene expression between deletion mutants and wild type strains in vitro.

E. coli and C. difficile will be recipients of genetic modification. E. coli strains derived from disabled K12 (e.g. DH5alpha and TOP10) will be used for the construction and propagation of recombinant plasmids, and CA434 (i.e. E. coli HB101, which is derived from disabled K12 and carrying the IncPbeta conjugative plasmid, R702) will be used for the transfer of recombinant plasmids to C. difficile through conjugation [1].

C. difficile is an anaerobic spore forming bacterium in hazard group 2; toxigenic strains can cause C. difficile infection in humans and neonatal piglets under certain conditions while non-toxigenic strains are largely known not to cause disease, with evidence of protection against toxigenic strain infection in animal models and piglets [2-4]. Almost all toxigenic and non-toxigenic strains, either with known genome sequences or not, contain phage sequences/phages. The significance of phages is not well-established, although there is some evidence of phage-mediate toxin-regulation [5] and antibiotic gene transfer [6], hence the purpose of this project.

References
Host/vector system

pMTL8000 series and its derivatives, such as pMTL-SC7315 and pMTL-SC7215 are widely used for cloning and genetic manipulation of genes in C. difficile [1, 2]. The vector encodes a traJ gene for conjugation between E. coli and C. difficile, a CoIE1 for replication in E. coli, different replicons for replication in C. difficile strains, and either catP, ermB, or tetA encoding chloramphenicol resistance, erythromycin resistance, or tetracycline resistance, respectively for selection of C. difficile transconjugants [1].

Plasmids in this series can integrate into the chromosome by homologous recombination via a designed sequencespecific cassette. However for the purpose of this project, double crossover mutants will be selected for where the plasmid will be lost. Such mutants will be selected for using either codA for counter selection of C. difficile transconjugants on 5-fluorocytosine [2], or a CRISPR-based system selection, which is lethal to cells that do not undergo homologous recombination [3, 4]. Either codA, or a cassette containing sgRNA and either Cas9 or Cas12a gene will be cloned into the vector backbone as previously described [2, 3] [4].

References


Origin & function

The genetic material involved are of bacterial and phage origin. The pMTL8000 plasmids and antibiotic resistance gene markers are derived from C. difficile plasmids and transposons. The allele exchange cassette or sgRNA sequence will be derived from phage. The intended functions are to enable replication of plasmids within C. difficile, selection of transconjugants, and deletion of phage genomes by gene editing or allelic replacement.

Deletion of prophage genomes can be achieved either through a one-stage process if using a CRISPR-Cas approach, or a two-stage process if using counter-selection methods. The CRISPR-Cas approach involves selecting for viable transconjugants which have the intended deletion in the genome. The Cas9 or Cas12a gene sequences are derived either from E. coli or Streptococcus pyogenes [1, 2]. Counter selection method involves first selecting for singlecrossover integration followed by isolation of double-crossover excision events using replicative-defective plasmid that carry a counterselection marker, codA, derived from E. coli. CodA confers sensitivity to 5-fluorocytosine (FC), so that double-crossover recombinant clones which have lost the recombinant plasmid will be resistant to FC [3].

References

The E. coli strains are equivalent to hazard group 1. GM E. coli strains will not be pathogenic, and recombinant plasmids they harbour will not encode any virulence genes. Hence it is not expected to cause harm to other organisms. There should not be any hazard associated with E. coli transfer of the plasmid to other C. difficile or bacteria, as the allelic exchange cassette for deletion is sequence specific to the targeted phage genome. The plasmid is transferred at very low frequencies and is unstable in the intended host (C. difficile) [1, 2].

The strains used in this project will be toxigenic (e.g. CD630, R20291, LiBA-5763, CD843) and non-toxigenic (e.g. CD062, CD80, CD37) strains which contain phage sequences [3-6]. There should not be any foreseeable effects that are significantly different from wild-type C. difficile infection arising from the deletion of prophage genomes from C. difficile genomes. Prophage genomes do not have pathogenic traits, and they are not integrated into virulence genes so that prophage deletion will not result in a functional virulent gene. Prophage deletion is expected to result in reduced virulence, transmission abilities, and horizontal gene transfer, based on current literature and hypotheses. Disease transmission is by exposure to C. difficile spores, hence environmental contamination can only occur if spores were not inactivated before leaving the containment facility. This would be prevented by waste management procedures outlined in the section below.

References

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMS - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid waste generated from the laboratory will be sealed in plastic bags and decontaminated by autoclave for 30 minutes at 124°C for 100% kill prior to disposal off-site by University approved procedures. The types of solid waste include: agar cultures, disposable pipette tips and serological pipettes, disposable plastic tubes and bottles, paper towels used to wipe down benches with 10% hypochlorite or 1 % Anigene. All liquid waste generated from the laboratory will be decontaminated by autoclave as described above. Autoclaved liquid waste will then be poured down a laboratory sink and flushed with water. The types of liquid waste include liquid cultures, small volumes (<1 mL) of bacterial resuspensions in water used for boiling prior to PCR, small volumes (<1mL) of chemically lysed bacterial cells used for genomic DNA extractions. Autoclaving is the most effective method of sterilisation and is expected to kill 100% of C. difficile spores. This was
validated by spiking 10 mL and 100 mL of brain heart infusion broth (BHIB) with 10^{18} cfu/mL and 10^{17} cfu/mL, respectively, of C. difficile spores (ribotype 078 strain). Viable counts of both cultures were done on pre-reduced Brazier's agar. The liquid cultures were decontaminated by autoclaving at 124 degrees C for 30 mins. Viable counts of both cultures were carried out again, and no colony growth was detected after incubation for 72h. Decontaminated cultures were also inoculated in 1 mL volumes to 9 mL of fresh (pre-reduced) BHIB and incubated for 72h. No growth was observed, as determined by 00600 readings.

1. The latest version of the University's Biosafety management document (version 6) should be referred to.
2. GM cultures should be stored in a locked fridge and freezer to further restrict access to users directly involved in this work. Archived bacterial stocks should be stored in a locked deep freezer to reduce access to people not involved in this project. Hence a lockable fridge/freezer will be purchased to be housed in 2J013, and glycerol stocks will be stored in a lockable deep freezer in 1J006.
3. A training record should be kept with training details and date.
4. The method of transporting GMO between labs should be outlined.
5. It should be made clear that students who are at risk of C. difficile infection (COI) will not be allowed to work with the pathogen, even though the groups at risk of COI (e.g. hospitalised patients, the elderly above 65 years old, immunocompromised and on antibiotic therapy) are unlikely to undertake research.

The risk assessment has been amended taking into account the committee's comments

Please enter comments on the GM safety committee on the risk assessment

1. The latest version of the University's Biosafety management document (version 6) should be referred to.
2. GM cultures should be stored in a locked fridge and freezer to further restrict access to users directly involved in this work. Archived bacterial stocks should be stored in a locked deep freezer to reduce access to people not involved in this project. Hence a lockable fridge/freezer will be purchased to be housed in 2J013, and glycerol stocks will be stored in a lockable deep freezer in 1J006.
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Name
GLAXOWELLCOME OPERATIONS LTD

Name 2

Department

Campus Estate or Research Centre

Road Name
NORTH LONSDALE ROAD

District

Town
ULVERSTON

County
CUMBRIA

Postcode
LA12 9D

Country
ENGLAND

Tel Number
01229 482408

Fax Number
01229 482257

E-mail

HSE Division
NORTH WEST

Comments

Date at Which Additional Info Submitted
02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

**Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

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Cultures (including samples) of less than 10 litres to be destroyed by autoclaving using a validated method prior to disposal to drain.

Cultures (including samples) of more than 10 litres to be treated by steam injection to achieve greater than 110 degrees C for more than 40 mins. Prior to disposal to drain. The method to be validated for its effectiveness for the specific application.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 116

Data Premises Notified (Originally) 07/05/1982

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

UNIVERSITY OF BIRMINGHAM

Name 2

Department

MEDICAL SCHOOL

Building

District

EDGBASTON

Campus Estate or Research Centre

EDGBASTON CAMPUS

Road Name

Town

BIRMINGHAM

County

MIDLANDS

Postcode

B15 2TT

Country

ENGLAND

Tel Number 0121 414 5251

Fax Number 0121 414 3309

E-mail

HSE Division MIDLANDS

Comments

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</table>
**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 1 (GMMs)</td>
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- **Bacteriology**
  - Parasitology
  - Transgenic Birds
- **Virology**
  - Transgenic Animals
  - Transgenic Fish
- **Mycology**
  - Transgenic Invertebrates
  - Transgenic Plants
- **Microbiology**
  - Research
- **Gene Therapy**
  - Other (please specify below)
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.

---

**Project Ref** 116/00.1

**Date Ackn’d**: 29/03/2000

**CU2 Project Title**: TO CREATE ANTIGEN PRESENTING CELLS EXPRESSING MUTANT FORMS OF HLA-DQ6

**Class**: Class 2

**Culture Vol Class**: Class 2

**Culture Volume Class**: 3-4

**Non-GMM Consent Granted**: not applicable

**Project notified under transitional arrangements**: Y

**Withdrawn**: N

**Historical Significant Changes**

**Historical Date of Additional Info**: 3/6/2000

**Significant Change ID**: 30

**Date of Significant Change**: 3/6/2000

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<th>Large Scale Activities</th>
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02/03/2022
TO CREATE TELOMORASE-IMMORTALISED HUMAN CELL LINES
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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**Project Ref 116/00.3**

- **Date Ackn'd**: 28/04/2000
- **CU2 Project Title**: ATP INDUCES MICROBACTERIAL KILLING OF MACROPHAGES BY PROMOTING PHAGOSOME LYSOSOME FUSION
- **Class**: Class 2
- **Culture Vol Class 2**: Class 2
- **Culture Volume Class 3-4**: Class 2
- **Non-GMM**: not applicable
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

- **Historical Significant Changes**: GM116/04.1
- **Historical Date of Additional Info**: 30/01/2004
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**  
N  

**If yes, tick to confirm that it is attached to this form**  
N  

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N  

**Please enter comments on the GM safety committee on the risk assessment**

**Project Containment**
Project Ref  116/00.4

Date Ackn'd  19/06/2000

Date Project Ceased

CU2 Project Title  EXPRESSION OF TOLOMERASE & DOWN REGULATION OF THE RETINOBLASTOMA GENE IN HUMAN INTRAHEPATIC BILINARY EPITHELIAL CELLS

Class  Class 2

Culture Vol Class 2

Consent Granted  not applicable

Project notified under transitional arrangements

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 116/01.1

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<td>19/02/2001</td>
<td>TRANSFECTING, EXPRESSION VECTORS OF ONCOGENES/ TUMOUR</td>
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**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**
- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

**Project Ref 116/01.10**

**CU2 Project Title**

TO GENERATE STRAINS OF MYCOBACTERIUM BOVIS BCG WITH DELETIONS FOR GENES ASSOCIATED WITH MYCOBACTERIAL PERSISTENCE.

**Class** Class 2

**CultureVol** < 1 litre

**CultureVolumeClass** not applicable

**Non-GMM**

**Consent Granted**

**Project notified under transitional arrangements**

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

The research is concerned with the study of dormancy/persistence in M. tuberculosis, the causative agent of tuberculosis. Anarabiosis, host fatty acid metabolism, cell wall thickening, synthesis of a 16-kDa-crystallin protein and associated transcriptional regulators/signalling pathways have all been postulated to play a role in the ability of M. tuberculosis to persist in the host for long periods of time and cause reactivation disease.

To study the role these genes may play in persistence, deletion mutants in the related vaccine strain, M. bovis BCG, will be made where the gene of interest will be replaced with an antibiotic resistance gene. This will help determine the effect of knocking out specific genes on the ability of the mutant strains to grow anaerobically, survive in macrophages in vitro, generate a thickened cell wall, etc. This should help elucidate a particular gene's contribution to the phenomenon of mycobacterial persistence.

Recipient or parental organism

Host: M. bovis BCG is an ACDP class 2 organism and can be worked with on the open bench. The organism is an attenuated strain of the pathogenic species M bovis and since the 50s has been given in a live form intradermally as a vaccine to protect against tuberculosis. It has a history of safe use.

Recipient or parental organism

The vector is a suicide plasmid pXii82C15 which is identical to the non-mobilisable pGEM3Z(+) f vector except that it contains a minimal (750bp) pAL500 mycobacterial origin of replication to enable the vector to replicate once in the host following transformation. The single round of replication provides time for the homologous sequences carried on the vector and on the wild type chromosome to recombine. As with pGEM3Z(+)f, the suicidevector carries an ampicillin resistance coding region. The vector has also been modified to carry two counter selectable marker genes. These are the E. coli lacZ which encodes B-galactosidase to determine blue/white colour selection of mutants and the B. subtilis sacB which encodes levansucrase which converts sucrose to levans which is harmful to bacteria and leads to the death of transformed bacteria when they are plated onto agar containing 10% sucrose. Thus sucrose sensitivity acts as a second counterselectable marker for positive selection of allelic exchange events.

Host/vector system

Donor nucleic acid sequences will be obtained from M. bovis BCG which is an attenuated strain of M. bovis. Since the 1950s it has been used as a vaccine against tuberculosis. The genetic basis for its attenuation is now known. It has three deletions (RD1-RD3) relative to its progenitor M bovis, and 10 deletions (RD1-RD10) relative to the human pathogen M. tuberculosis. Among the known products encoded by the loci which are deleted in M bovis BCG and contribute to its attenuation are an invasin, several phospholipases, glycine-rich polymorphic proteins, the ESAT-6 secreted proteins and an epoxide hydrolase. The DNA sequence encoding resistance to the antibiotic hygromycin, which is not used therapeutically, will be obtained from the non-pathogenic soil actinomycete, Streptomyces hygroscopicus.

The aim of the work is to knock out/delete the function of the gene pertinent to the study of mycobacterial persistence by replacing it with the hygromycin resistance gene. The insert present on the vector to be transformed will comprise the hygromycin resistance gene with several kilobases of genomic M bovis BCG DNA flanking the gene on either side. This is needed to enable the homologous sequences present on the wild type chromosome and the GMM to recombine homologously and replace the gene of interest with the hygromycin resistance gene following transformation into the M bovis BCG host. Genes of interest to be replaced will include those postulated to play a role in mycobacterial resistance such as the hspX gene encoding the 16-kDa-crystallin protein, genes encoding the biochemical components of the thickened cell wall, and genes encoding transcriptional regulators and components of signalling pathways involved in sensing low oxygen conditions and the presence of nitric oxide.

Evaluation of foreseeable effects

There should be no effect on human health. M bovis BCG is administered to humans in the form of a live vaccine and knocking out/deleting genes involved in mycobacterial persistence should, if anything, lead to attenuation of the mutant strains and lessen the organism's ability to survive/persist in human tissue. It is highly unlikely that the GMO would be able to colonise or infect humans, but if it did, an infection caused by the GMO could be treated readily with the antimycobacterial drugs rifampicin and isoniazid. The drug target for these agents would still be intact in the GMOs as they are not involved in persistence and would therefore not be targeted for
deletion studies. There is negligible risk of accidental release and negligible risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

no derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures are to be autoclaved prior to disposal. Degree of kill - effectively 100%
Laboratory consumables such as tips and loops will be soaked in 2% stericol overnight, drained and placed in sharps box for disposal by incineration. Phenolic compounds such as Stericol are effective against tubercle bacilli at concentrations of between 0.2-3% within 20-30 minutes contact time (Vesley and Lauer in Laboratory Safety - Principles and Practices, 2nd Edition 1995, eds Fleming, Richardson, Tulis and Vesley, ASM Press, Washington DC).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The local GMSC has approved this activity.

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Project Ref 116/01.11

Date Ackn'd 11/05/2001

CU2 Project Title OVEREXPRESSION OF AKT, TIE 1 & 2, ANGIOPOIETIN, VEGFS,

Class 2

CultureVolClass2 < 1 litre

CultureVolumeClass3-4
To investigate the role of vascular endothelial cell growth factor family (VEGF-A-D and placenta growth factor/PIGF) and their cognate receptors (Flt-1, Flt-4 and KDR) and the Tie receptor tyrosine kinases (Tie-1 and Tie-2) and their ligands the angiopoietins (Ang 1-4), in both physiological and pathological angiogenesis. Replication defective adenovirus will be generated to express wild type or dominant negative mutants of these growth factors and receptors to investigate their activity in vitro. Endothelial, trophoblast and other cell lines will be transfected with adenovirus and their activity examined in various assays, eg proliferation, migration and tube formation. In order to delineate the signalling pathways activated by these receptors we will use replication defective adenovirus expressing wild type or dominant negative mutants of signalling molecules (Akt/PKB, HO-1 and PKC family) that lie downstream of these receptors.

Recipient or parental organism

Host: Embryonic kidney 293 cells which complement the replication deficiency; these cells have adenovirus tumour genes that complement the replication deficiency in the adenovirus.

Host/vector system

Vector: Adenovirus Type 5, E1 and E3 deleted. The site of insertion is the E1 site of an adenovirus Type 5 E1 replacement vector, eg pACCMVpLpA. The plasmid contains the CMV promoter for efficient expression of the effector genes. Recombinant (Replication defective) virus will be produced following transfection of the resultant plasmids into human embryonic kidney 293 Cells, together with a plasmid clone of E3-deletion partial adenovirus genome, which allows recovery of the virus by homologous recombination. Recombinant adenoviruses will also be produced by homologous recombination in a bacterial system (E.coli strain BJ5183) as described by Chartier et al (J. Virol 70:4805 - 4810, 1996). The adenoviral DNA and "shuttle" plasmid (pAD.CMV-Link.1) with the effector gene are recombined in the above mentioned bacterial cells. Linearised DNA (encoding the replication defective adenovirus and effector gene) will transfected into HEK 293 cells and the virus recovered by purification. The shuttle plasmid has a gene cassette that replaces the E1 adenoviral sequences with a CMV promoter, multiple cloning site and SV40 polyadenylation signal. This cassette is flanked by human adenovirus type 5 sequences consisting of 0-1 map units for the left arm and 9-16 map units for the right arm.

Origin & function

Donor: Mouse and/or human cDNA cloned by PCR.

The genetic material includes AKT, VEGFs, HO-1, Tie 1 and Tie 2 angiopoietin, PKC family.

To investigate the role of vascular endothelial cell growth factor family (VEGF-A-D and placenta growth factor/PIGF) and their cognate receptors (Flt-1, Flt-4 and KDR) and the Tie receptor tyrosine kinases (Tie-1 and Tie-2) and their ligands the angiopoietins (Ang 1-4), in both physiological and pathological angiogenesis. Replication defective adenovirus will be generated to express wild type or dominant negative mutants of these growth factors and receptors to investigate their activity in vitro. Endothelial, trophoblast and other cell lines will be transfected with adenovirus and their activity examined in various assays, eg proliferation, migration and tube formation. In order to delineate the signalling pathways activated by these receptors we will use replication defective adenovirus expressing wild type or dominant negative mutants of signalling molecules (Akt/PKB, HO-1 and PKC family) that lie downstream of these receptors.

Recipient or parental organism

Host: Embryonic kidney 293 cells which complement the replication deficiency; these cells have adenovirus tumour genes that complement the replication deficiency in the adenovirus.

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Origin & function

Donor: Mouse and/or human cDNA cloned by PCR.

The genetic material includes AKT, VEGFs, HO-1, Tie 1 and Tie 2 angiopoietin, PKC family.
Ad5 infects humans and is classified as an ACDP level 2 pathogen. In adults it does not normally cause disease as most adults will have been exposed to one of the common subgroup C viruses (which includes Ad5) by adolescence. Ad5 primarily infects the respiratory tract, although it is shed via the gut and can probably replicate therein. The vectors used in the present study will be replication defective (by virtue of the E1 deletion) and are therefore regarded as safer than the wild type virus. The possibility that replication competent virus may arise by recombination with the permissive cell line is recognised, although this does not occur with high frequency.

The presence of replication competent virus can be detected by infecting a cell line that does not complement the E1 deficiency, such as HeLa cells, than harvesting after 5-7 days and testing the lysates for plaquing or CPE on HeLa or A549 cells. We will test for replication competent virus on the initial batch of adenovirus prepared from a seed stock. If any contamination is found in this batch of adenovirus prepared from the seed stock, both adenovirus and seed stock will be discarded.

The recombinant virus will not be capable of autonomous spread in the event of accidental operator infection. In the unlikely event that an operator received and infective dose of virus via the respiratory tract, it would only cause a productive infection if the operator had a pre-existing infection with wild type virus and the same cells took up the recombinant virus. Sub group C serotypes are known to persist in adenoids and tonsils following primary infection, but the mechanism by which the virus is maintained is not clearly understood. The number of virus containing cells is low, and most virus remains cell associated and can only be detected after prolonged incubation of the tissue in vitro. Thus the risk of double infection with recombinant and wild type virus is considered to be very low. The possibility of co-infection in the gut should be considered. While it is known that, in some instances, there is prolonged shedding of sub group C viruses via the alimentary canal, there is no evidence to suggest that there is long term persistence of the virus so the opportunity for coinfection of a single cell with both wild type and recombinant virus is considered remote.

The effect of the insert should also be considered: the virus will be capable of expressing the protein in human cells infected, but will not be capable of autonomous spread as the virus is replication defective, localising the effect. With good laboratory practice the risk will be minimised. The most likely source of accidental exposure will be from aerosolisation. The virus will be handled in a Class II safety cabinet, thus minimising the risk.

Any exposure to the incoming virus would be likely to elicit an immune response. The major capsid protein is the major neutralising antigen; there would be a primarily type 5 response. Most individuals have a pre-existing immunity to Ad5 and a secondary immune response to the recombinant virus would be expected.

Environmental considerations: The recombinant adenovirus is replication defective and will not have an extended host range, therefore the potential hazard to the environment is negligible. Accidental release is unlikely from the containment conditions.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be collected in an autoclave bag for transfer to autoclave in a robust container. The waste will then follow the clinical waste route, being taken away by an approved contractor for ultimate disposal by incineration. Degree of kill - effectively 100%.

Contaminated liquid waste will be collected in screw capped pots containing 1% Virkon. Contaminated glassware, plastic tips will be submerged in 1% Virkon for at least 30 minutes. Following submersion in Virkon plastic tips will be disposed of in a sharps bin to be sent for incineration.

1% Virkon will be used for disinfection. Exposure to a 1% solution of Virkon for 15 minutes has been shown to inactivate adenovirus infected cell pellet by >4 log scale. A 1% solution also inactivates adenovirus seed stock by >6 log scale (Institute of Cancer Studies, University of Birmingham).
Please enter comments on the GM safety committee on the risk assessment

Query: The proposers do not seem to be aware that 293 cells contain adenovirus tumour genes, or at least it is not explicit that they are.

Response: Assessment amended to make this clear.

Query: Original assessment stated that Virkon, hypochlorite and SDS would be used for disinfection - the efficacy of these was questioned by a number of GMSC members.

Response: Virkon chosen as disinfectant. Has been tested against adenovirus by Institute of Cancer Studies.

Query: Are applicants going to monitor batches of virus for presence of wild type virus?

Response: Presence of wild type virus to be monitored per seed stock prepared for fresh batch of virus. To be done using a non-complementing cell line and checking for the lytic cycle.

Query: What harm would the inserts do if the recombinant got into a person and were expressed.

Response: There is a small risk of the insert expressing in the case of accidental operator exposure although the effect will be localised as the viruses are not replication competent.

Query: How are Class II cabinets vented? Into lab through double HEPA filter?

Response: Yes, through two HEPA filters into lab.

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Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<td>L3 L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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The genes encoding the \(\alpha\) and \(\beta\) peptide chains of the HLA-DQ6 molecule (DQA1*0102 and DQB1*0602 respectively) have been cloned into the pALTERMAX vector (Promega) and subjected to site-directed mutagenesis to create 10 mutant alleles (previously approved work). We now plan to use these mutant alleles to create a range of artificial antigen-presenting cells expressing variant forms of the DQ6 molecule. The alleles will be subcloned into the pClneo plasmid expression vector (Promega) and the suitable pairs of cloned alleles will be introduced into the HLA class II deficient B lymphocyte cell line, BLS-1, by electroporation. The antigen-presenting cells will then be used in functional studies, investigating the ability of the mutant molecules to a) bind to peptides derived from antigens relevant to the pathogenesis of Type 1 diabetes and b) activate DQ6-restricted T cell clones. This assessment relates to the gene transfer into the BLS-1 line.

**Recipient or parental organism**

Host: Initial host - E. coli strain DH5x - disabled, non-colonising laboratory adapted host. Non-pathogenic to humans. Final host - EBV transformed B lymphoblastoid cell line, BLS-1. This is derived from a human with a bare lymphocyte syndrome, a genetic disorder which precludes the expression of HLA class II molecules on the cell surface. The cell line is unable to colonise the workers as it will provoke a host - versus - graft immune response (BLS-1 is HLA class I incompatible with both staff members who will be working with it). The cell line is considered to pose a potential risk of infection with EBV.

**Host/vector system**

Vector: pClneo plasmid expression vector (Promega). Contains SV40 enhancer and early promoter regions including SV40 origin of replication, f1 origin of replication, CMV immediate early enhancer/promoter region for constitutive expression of cloned gene in mammalian cells, ampicillin resistance gene and neomycin phosphotransferase gene. pClneo is based on the pUC series of vectors and is therefore non-mobilisable.
Donor: Wild type inserts were cloned by RT-PCR from EBV transformed human B lymphoblastoid cell line. MGAR (from European Collection of Cell Cultures, Porton Down) MGAR cells were initially derived from a healthy human donor.

Genetic Material: Full length cDNAs (DQA1*0102 and DQB*0602) encoding the x and B peptide chains respectively of the DQ6 molecule, plus ten full length cDNAs containing single point mutations created by site-directed mutagenesis ("DQA1*0102 mutants and 8 DQB1*0602 mutants). Individual peptide chains have no biological function but combine to form functional DQ heterodimer. DQ molecule involved in binding antigenic peptides and activation of antigen specific and allo-specific CD4+ T cells. All cDNAs encode signal peptide for cell surface expression of heterodimer.

Evaluation of foreseeable effects

In DH5x - the DQA1 and DQB1 genes will be inserted separately into these host cells. The individual genes are incapable of generating functional protein. Furthermore, these cells lack the accessory molecules necessary for the correct assembly and folding of the peptide chains. We do not know whether any peptide will be secreted, but it will be non-functional.

In BLS-1 - intact functional DQ heterodimers will be expressed under the control of the CMV immediate-early promoter/enhancer. Previous studies using this cell line have shown the expression levels of DQ to be similar to those observed in normal B cell lines. CDNAs carry signal peptides for cell surface expression of the x and B peptide chains - no protein should be secreted from the cells.

Risk to human health - Cloning work: The host bacterial strain is non-pathogenic to humans. Genetic modification will not pose any risk to health as no functional protein will be expressed and the pathogenicity of the host will not be altered (except conferring resistance to ampicillin).

Expression work: The only risk to human health will be related to the EBV transformation of the BLS-1 cell line, which poses an additional risk of EBV infection. Genetic modification will not pose any additional risk - the manipulation of BLS-1 will create a cell line which expresses a combination of HLA class I and class II molecules which does not occur naturally. Thus it will not be possible for any worker (present or future) to be HLA compatible with the GMOs at all class I and class II loci. Any mis-match will result in a host-versus-graft rejection response which will have no adverse effects on the exposed individual. We have a record of the HLA profile of staff in the department and all new workers are routinely HLA typed. We will therefore be able to identify any subjects who are class I compatible with the unmodified BLS-1 cells and may therefore be at risk of colonisation.

Risk to Environment: B lymphocyte lines will die outside correct culture environment. No potential hazards associated with GM bacterial cells. Potential hazard to environment is low. Likelihood of release is also low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated liquid waste to be collected in screw capped waste pots containing 1% Virkon and autoclaved. Contaminated Disposable plasticware to be placed in autoclave bags and placed in a robust container for transport to autoclave. Contaminated plasticware such as pipettes and tips will be submerged in 1% Virkon overnight prior to disposal in autoclave bags (pipettes) or sharps bins (tips). All autoclaved/treated solid waste is bagged and ultimately taken away for incineration by a contractor (Eurocare, Birmingham).

Contaminated glassware to be soaked overnight in 1% Virkon.

Autoclaving - effectively 100% kill. Please see attached data on in-house tests on Virkon.
The assessment was circulated to the Medical School GMSC and approved without adverse comment. One query was received on the efficacy of SDS as a disinfectant, which had been included in the original assessment. The investigator amended the assessment to exclude use of SDS and to only use Virkon instead.

### Project Containment

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**Project Ref 116/01.13**

**Date Ackn'd**

30/05/2001

**CU2 Project Title**

RECOMBINANT ADENOVIRUS VECTORS WITH B7.1, FOX, AKT/PKB INSERTS TO STUDY THYMOCYTE DEVELOPMENT

**Date Project Ceased**

**Class**

Class 2

**CultureVolClass2**

< 1 litre

**CultureVolumeClass3-4**

**Non-GMM**

Consent Granted: not applicable

**Project notified under transitional arrangements**

N

**Historical Significant Changes**

Significant change January 2009

**Historical Date of Additional Info**

**Significant Change ID**

116/01.13.a
**Project Additional Information**

**Purposes of the contained use**

The ability to mount T-cell mediated responses to viruses and other pathogens requires establishment of a pool of functionally competent peripheral T-cells. For this to occur haemopoietic stem cells must be recruited to the thymus where, under the influence of the thymic microenvironment, with a particular importance of thymic epithelium, they undergo a programme of differentiation and selection. Disfunctional epithelium can stop further thymocyte development, resulting in the lack of mature T-cells in the periphery and consequently causing severe immune deficiency. The aim of this work is to gain a better understanding of the role of epithelium in T-cell development, which knowledge will be useful in devising strategies to boost or restore thymic T-cell output where this has been compromised by disease, ageing or toxic therapies. Since epithelial cells are not or very poorly dividing, the use of adenoviral vector systems is necessary. Commercially available kits from Q-Biogene and Takara will be employed to create recombinant virus vectors able to express genes regulating thymocyte differentiation and development. The recombinant adenoviruses will lack the E1 gene which makes them incapable to propagate in ordinary cells. MHC class II epithelial cells will be selected from the thymi of Balb/c mice and incubated with the viral supernatant overnight, then GFP expression will be detected by FACS and positive cells will be selected and reaggregated with thymocytes and fibroblasts. Our aim is to increase the expression of adenovirally-transduced genes in mouse thymic epithelium and assess the role of the genes in question (B7.1, Fox and Akt/PKB) in the regulation of thymocytes development.

**Recipient or parental organism**

**Hosts:**
1) Standard E.coli strains eg. DH5a. Disabled host with a non-mobilisable vector. Inserts are under control of eukaryotic promoters, therefore expression is negligible. Containment level requirements are already in place within the laboratory.
2) Packaging cells to produce virions Ad-5-transformed human embryo kidney line 293 supply E1 functions in trans from chromosomally integrated genes.
3) Target cells: primary mouse (Balb/c, C57) epithelial cells will be MHC class II purified and reaggregated in vitro.

**Host/vector system**

**Vector:**
The vector will be an adenoviral shuttle vector in which an expression cassette can be inserted. Upon homologous recombination in bacteria with a plasmid containing the E1- E3 Ad5 genome, a new plasmid is generated in which the expression cassette is inserted into the original E1 region of the adenovirus genom.

**Origin & function**

**Donor:** Healthy, mouse embryonic (15 day) thymus cDNA, genes are involved in normal development of mouse thymocytes and epithelium.
Genetic material: Fox, B7.1: wild type mouse proteins involved in the regulation of Tcell development and maturation Akt/PKB: wild type signalling molecule involved in the regulation of cellular survival.
GFP: fluorescent protein which has not been shown to have pathogenic properties.

**Evaluation of foreseeable effects**

Human Health considerations: The plasmid constructs in E.coli have no significant implications for health & safety. Expression of Fox, B7.1 and Akt/PKB in cells in vitro presents no greater hazard than the unmodified cells. Non of the expressed proteins are known to have any toxicity or transforming activity or cause allergy. The genetically modified adenoviral vectors are designed to result in efficient expression of the above proteins in the epithelium and in turn in vitro reaggregated with various subpopulations of primary mouse thymocytes and their effect on epithelial cell maturation. Adenoviruses are relatively stable at room temperature and are naturally infectious to the respiratory tract as aerosols. If accidental inoculation occurred, it might cause flu like symptoms, and the viral DNA would be introduced into a number of cells giving transient, high level expression of the transgene over a period of a few days to weeks. However, since the virus will be unable to replicate and any expression of the mouse genes regulating thymocyte development would be eventually lost from the infected cells (the process fastest in dividing cells) and further spread would not occur. In small proportion of infected cells chromosomal integration of the vector might
give the potential for long-term maintenance and expression. However, chromosomal integration of adenoviral sequences is a rare event, in the region of one integration in 10 (to the power of 4) exponentially growing infected cells. Although the number of cells undergoing replication in the respiratory tract at any one time is not precisely known, it is generally accepted that the majority of cells in the epithelium are post-mitotic, on the differentiation pathway. It is our opinion that a considerable intake of virus would have to occur to produce an integration event in vivo, much greater than the minimal in vitro transforming dose of 1 x 10 (to the power of 5) pfu.

The majority of people undergo primary infection with AD5 in childhood, and both humoral and cellular combined to prevent further productive infection in the adult. Such immunity will reduce viral entry into cells and hasten the immune destruction of infected cells in the event of accidental infection with the recombinant virus. The recombinant virus will not be capable of autonomous spread in the event of accidental operator infection. In the unlikely event that an operator were to receive an "infectious dose" of recombinant virus and the same cells would concurrently be infected with wild type virus could lead to replication of the virus. The risk of double infection of the same cells with recombinant and wild type adenovirus at the same time is considered to be very low.

The possibility that replication competent adenivirus might arise (at low frequency) by recombination within the permissive cell line is recognised. Protocols have been published for monitoring this phenomenon, and virus stocks are routinely screened on non-permissive cells to assess their status, indeed, the presence of significant levels of RCA would become obvious in the experimental use of the virus. The transgenes will be inserted in the E1 region of the virus, and so become obvious in the experimental use of the virus. The transgenes will be inserted in the E1 region of the virus, and so in the event of homologous recombination to restore E1 functions, the B7.1, Fox and Akt/PKB gene will be lost from the viral genome.

Environmental considerations: Gene transfer to both humans and mice is a possibility. The hazard is low as the non-replicative adenoviral particles are relatively stable at room temperature but it would require high exposure for infection. Even high level exposure could only cause temporary, mild flu-like symptoms. Supernatants will be contained within a Class II microbiological safety cabinet, and live animals will not gain access to the work area. Workers will wear protective clothing and will handle the virus in the safety cabinet in a containment level 2 lab, thus preventing the virus escaping into the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All tissue culture plastics and liquid waste generated during the production of adenoviral supernatants will be autoclaved before disposal. Solid waste is then removed by a contractor for ultimate disposal by incineration. Autoclaving achieves effectively 100% kill of all GMMs. Pipette tips will be treated with Virkon then autoclaved before being removed as clinical waste and incinerated. 1% Virkon is effective against adenovirus according to manufacturer's information: This indicated efficacy of Virkon against a variety of adenoviruses at 1% concentration, and also against a number of strains of E. coli at dilutions ranging from 1% to 0.125%. It has been demonstrated that 15 minutes exposure to 1% Virkon achieves >4log kill for adenovirus infected cell pellets, and >6log kill of adenovirus seed stocks.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
GMSC raised issue of monitoring for recombination. This has been addressed in the assessment. Also, request for provision of validation information on Virkon - this has now been added. GMSC requested that consideration be given to the procedure for dealing with a 10ml spill of virus stock in the open lab. This has been addressed in the Emergency Action section. The GMSC has approved the assessment.

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**Project Ref** 116/01.16

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<td>GM ADENOVIRUS WHICH EXPRESSES FGF-2</td>
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<td>≤ 1 litre</td>
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**Historical Significant Changes**

<table>
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<tr>
<th>Historical Date of Additional Info</th>
<th>Significant Change ID</th>
<th>Date of Significant Change</th>
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**Project Additional Information**

**Purposes of the contained use**

To investigate the use of fibroblast growth factor-2, expressed, e.g. in target neuronal cells, to provide neurotrophic signals to CNS neurons and thus facilitate the survival and regeneration after nerve tract lesion. In some experiments, FGF2 will also be used in combination with other neurotrophic molecules, e.g. neurotrophin (NT-3), brain derived neurotrophic factor (BDNF), nerve growth factor (NGF) or cytokines, e.g. transforming growth factor-beta (TGF-b) or cytokine antagonists (e.g. decorin).
Vectors will initially be constructed as plasmids in E. coli. These may be transfected into mammalian cells, leading to expression of the FGF-2 (but without any generation of virus). However, in order to facilitate into mammalian cells, leading to expression of the FGF-2 (but without any generation of virus). However, in order to facilitate gene transfer to a number of cell lines, and to model possible therapeutic use, a replication defective adenovirus expressing FGF-2 will also be generated.

**Recipient or parental organism**

**Host:** Plasmids grown in standard disabled laboratory strains of E. coli. Mammalian cells used for viron production will be Ad-5 transformed human embryo kidney line 293, or similar lines, e.g. 293T cells (a derivative which also expresses SV40 T antigen), HER AdS E1a; 911 (another HER/human embryo retinal line); PER.C6; all supply E1 functions in trans from chromosomally integrated genes. Eventual target cells for the GM virus will be established neuronal and glial cell lines and primary neural material. The route to construction of the adenoviral vector plasmids carrying FGF2 etc, involved intermediate plasmids which are suitable for DNA transfection into mammalian cells (e.g. carrying the neomycin G418 s selectable marker), so this will also be done in order to select derived mammalian cell lines which stably express the constructions. Such cells will not be capable of producing the GM virus.

**Host/vector system**

**Vector:** Initial assembly of the constructs will be in standard plasmid vectors, based on pUC19 and pBR322. The virus to be produced from the plasmid constructs in helper mammalian cells will be replication dwefective, GM human adenovirus type 5 (E1 deleted). Our vectors in current use are also E3 deleted, thus removing adenoviral genes which play various roles in evasion of the host immune response.

**Origin & function**

**Donor:** Human FGF2 open reading frame PCR amplified from a human brain library. GFP from jellyfish, although we intend to use EGFP, a modified version with modified spectral characteristics and optimised for expression in mammalian cells. Genetic material: Fibroblast growth factor-2 is a heparin binding growth factor which occurs in several isoforms resulting from alternative initiations of translation: an 18kD cytoplasmic isoform and four larger molecular weight nuclear isoforms (22, 22.5, 24 and 34kD). FGF-2 has pleiotropic roles in many different cell types and tissues; it is a motogenic, angiogenic and survival factor which is involved in cell migration, cell differentiation and a variety of development processes. Although devoid of signal peptide, it could be secreted. It acts mainly through a paracrine/autocrine mechanism involving high affinity transmembrane receptors and heparin surfate proteoglycan low affinity receptors, but also through a still unknown intracrine process(es) on intracellular targets. FGF-2 has many biological functions which are probably isoform specific. FGF2 is expressed at very low levels and sequestered by many mesenchymal cells, and at high levels by glia and some neuronal populations in the CNS. In the CNS FGF2 is transiently up-regulated after injury and its limited availability is thought to limit the potential for neuron survival and axon regeneration after nerve tract injury. Its receptor FGFR1 is also expressed by responsive cells after injury, and the interaction of FGF2 with FGFR1 is thought to stimulate gliosis, neuron survival and the abortive axon regeneration. Despite its potential pleiotropic activities, FGF2 is not reported to be oncogenic, and seldom acts alone to initiate cellular responses, requiring co-factors for full activity.

**Evaluation of foreseeable effects**

**Human Health Considerations:** The plasmid constructs in E. coli have no significant implications for health and safety. Expression of FGF2 in cells in vitro presents no greater hazard than the unmodified cells, (cells derived from the operator will not be used, thus there is no likelihood of colonisation). Adenoviral vectors carrying the FGF2 gene will be able to infect a broad range of human and other mammalian cells, including cells in the operator in the event of accidental contamination, leading to expression of the transgene. The expression of FGF2 is not expected to have any direct consequences for the expressing cell, i.e. it is not known to be toxic or have any signalling functions within the expressing cell. FGFR1, the receptor for FGF2, is reported to be expressed on some mesenchymal cells, allowing stimulatory signals to be provided by this route, facilitating the subsequent activation and proliferation of the antigen-stimulated cells. At the extreme it is possible that this might promote limited motosis. However, this is considered unlikely, as it would depend on the prior presence of receptor bearing cells primed to replicate. Also, under normal physiology, the ligand has a high affinity for heparan sulphate proteoglycans (a component of the extracellular matrix of all tissues) which acts to mop up soluble FGF2, sequestering it and blocking its signalling ability through its receptor. This ligand sequestration is a natural regulatory phenomenon for this class of ligands, and to be biologically active the ligand needs local activation by specific proteases. It is unlikely that this would occur to any significant degree.
Adenoviruses are relatively stable at room temperature and are naturally infectious to the respiratory tract as aerosols. Although it is envisaged that most of our experiments will use adenovirus entrapped in a solid matrix, thereby minimising the aerosol risk, if accidental inoculation were to occur, the viral DNA would be introduced into a number of cells giving transient, high level expression of the transgene over a period of a few days to weeks. However, the GM virus will be replication defective and following an accidental inoculation, further spread would not occur. With time the genome would be lost from the vast majority of infected cells, or the infected cells may be eliminated by immune responses against adenoviral antigens. In a small proportion of infected cells chromosomal integration of the vector might give the potential for long term maintenance and expression. However, chromosomal integration of adenoviral sequences is a rare event. Although the number of cells undergoing replication in the respiratory tract at any one time is not precisely known, it is generally accepted that the majority of cells in the epithelium are post-mitotic, on the differentiation pathway. It is our opinion that a considerable intake of virus would have to occur to produce an integration event in vivo.

The majority of individuals appear to undergo primary infection with Ad5 in childhood, and both humoral and cell-mediated immunity combine to prevent further productive infection in the adult. Such immunity will reduce viral entry into cells and hasten the immune destruction of infected cells, in the event of accidental human contamination with the recombinant virus. The recombinant virus will not be capable of autonomous spread in the event of accidental operator infection. In the unlikely event that an operator were to receive an infectious dose of virus via the respiratory tract, it would only lead to replication of the GM virus if they had a concurrent infection with wild type virus, and the same cells took up both wild type and recombinant viruses. Sub-group C serotypes (including Ad5) can persist in the adenoids and tonsils following primary infection, but the mechanism by which the virus is maintained is not known. However, the number of virus-containing cells is very low, and most virus remains cell-associated, and can only be detected after prolonged incubation of the tissue in vitro. Thus the risk of double infection with recombinant and wild types virus is considered to be very low.

Environmental considerations: Human adenoviruses are not known to infect other species in the wild. Experimental administration of Ad5 wt virus to cotton rats can cause pneumonia when administered at high doses, given intranasally; but cotton rats are not indigenous to the UK. Mice can only be killed by high virus doses delivered intravenously or intraperitoneally; the virus goes to the liver and causes primarily toxic damage. The recombinant virus is replication defective and will not have an extended host range. Apparent level of potential hazard to the environment is negligible. Potential hazard to species in the environment from E coli containing the plasmids associated with this work also appears negligible, due to minimal intrinsic hazard and poor ability of the strains to survive in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is treated by autoclaving at 130C for 60 minutes before disposal to drains. Disposable solid waste which may be contaminated with GMMs is also autoclaved before removal as "clinical waste" by specialist contractors with final disposal by incineration. Exceptions to the above are: "Sharps", including scalpel blades, needles, and disposable plastic tips for micropipettors, may be decontaminated by drawing up Virkon disinfectant and soaking in Virkon for 15 minutes. Disposable plastic pipettes may also be decontaminated with Virkon in this way. Autoclaving achieves effectively 100% kill of GMMs. Manufacturer's information indicates efficacy of Virkon against adenoviruses at 1% concentration, and also against a large number of E coli strains at dilutions ranging from 1% to 0.125%. We have demonstrated that 15 minutes exposure to 1% Virkon achieves >410^10 kill for adenovirus infected cell pellets and >610^10 kill of adenovirus seed stocks.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y
Please enter comments on the GM safety committee on the risk assessment

One comment from the GMSC: "Does the transgene always go into the Ad E1 region?" The response from the investigator was yes, it does.
The GMSC have approved this assessment.

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Project Ref 116/01.17

Date Ackn'd: 27/11/2001

CU2 Project Title: CHARACTERISATION OF VIRULENCE, STRESS-INDUCED AND ANTIBIOTIC-RESISTANCE DETERMINANTS FROM SELECTED PATHOGENIC BACTERIA OF HUMANS AND ANIMALS.

Class: Class 2

Culture Volume: Class 2 ≤ 1 litre

Non-GMM: not applicable

Consent Granted: not applicable

Project notified under transitional arrangements: N

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

We wish to submit a series of assessments that form a connected programme of work that includes several laboratories, several pathogens and several activities.
work focuses on the characterisation of proven or potential virulence, stress-induced and antibiotic-resistance determinants from human bacterial pathogens that fall into ACDP category 2. These include:

- Salmonella enterica (non-typhoidal serovars and the attenuated vaccine strain Ty21a)
- Echerichia coli (non-vero-toxin-producing)
- Campylobacter jejuni
- Corynebacterium diphteriae
- Staphylococcus aureus and coagulase-negative staphylococci
- Bacteroides fragilis, Clostridium difficile
- Mycoplasma pneumoniae, Shewanella putrefaciens
- Mycobacterium bovis BCG
- Streptococcus pyogenes, S. pneumoniae, S. agalactiae and viridans group streptococci (eg. S. mitis, S. oralis)
- Pseudomonas aeruginosa, Pasteurella multocida, Actinobacillus actinomycetemcomitans, Yersinia pseudotuberculosis Enterococcus faecalis and E. faecium, Neisseria gonorrhoeae.

Specific activities include:

1. Cloning of DNA fragments from pathogenic bacteria in non-mobilisable vectors in laboratory strains of E. coli with a view to sequencing or mutating the fragments. No attempt will be made to express the foreign DNA.
2. Creation of mutants in wild type strains by random transposon mutagenesis using eg. mud-lac and resulting in insertional inactivation, linked in some cases with the creation of transcriptional fusions to reporter genes.
3. Creation of mutants of pathogens by allele replacement using a suicide vector (or insertion duplication vector in streptococci).
4. Allele replacement using a gene that expresses a marker protein which is highly unlikely to have any biological effect (such as beta-galactosidase, luciferase, green-fluorescent protein) into a pathogen to monitor gene expression under different environmental conditions.
5. Expression of proteins at a high level in a disabled host bacterium to enable further chemical/biochemical characterisation of the protein.
6. Expression in the strain of its origin of a gene from a pathogenic bacterium running off its own promoter on a shuttle vector, so as to complement a mutant phenotype.
7. Expression of antibiotic resistance determinants in a disabled host bacterium to enable further characterisation of the resistance mechanism.

**Recipient or parental organism**

| Activity 1 | Disabled laboratory strains of E. coli including or similar to those listed in Part 2a, Annex II of the ACGM Compendium of Guidance |
| Activity 2 | Wild type pathogenic bacteria as described above |
| Activity 3 | Pathogenic bacteria as described above |
| Activity 4 | Pathogenic bacteria as described above |
| Activity 5 | Disabled lab strains of E. coli |
| Activity 6 | Lab strains of E. coli then pathogenic bacteria as described above |
| Activity 7 | Disabled lab strains of E. coli |

**Host/vector system**

| Activity 1 | Any non-mobilisable or mobilisation-defective vector including or similar to those listed in Part 2a, Annex II of the ACGM Compendium of Guidance |
| Activity 2 | N/A |
| Activity 3 | Non-mobilisable or mobilisation defective vectors that: can be propagated in crippled E. coli strains but not in the target species, eg pUC replicates in crippled E. coli but not in C. jejuni; show temperature sensitive replication in E. coli eg pNIL and pGOAL series; recombine with the target gene on the chromosome to cause insertional duplication of the plasmid in S. pneumoniae, eg p326(cat)pDL278(spc),pCR2(erm) |
| Activity 4 | A suicide vector |
| Activity 5 | Non mobilisable vectors, eg pGEX4T3, the pET and pCAL vectors, that express the protein, either from strong promoters or from the gene's native promoter in |
Its native form or as part of a his-tagged, calmodulin-binding peptide-tagged or similar fusion to an innocuous protein.

Activity 6 - Non-mobilisable or mobilisation defective shuttle vectors which carry origins of replication that work in both E. coli and a given other pathogen (eg C.diptheriae).

Activity 7 - Non-mobilisable or mobilisation defective vectors including or similar to those listed in ACGM Compendium of Guidance.

### Origin & function

Donors of material for activities 1-7 as listed above.

Activity 1 - Pathogenic bacteria as described above.

Activity 2 - Well characterised transposable elements, eg for Salmonella - Mud-lac; For pseudomonas - mini-Tn5 and its derivatives mini-Tn5-luxCDABE, mini-Tn5-gfp, mini-Tn5-lac; For Streptococci and Enterococci Tn916, Tn917, ISS1 and derivatives.

Activity 3 - Pathogenic bacteria as described above

Activity 4 - Depends on marker (eg. E. coli for beta-galactosidase, firefly or luminesent vibrios for luciferase, jellyfish for GFP.

Activity 5 - Pathogenic bacteria as described above.

Activity 6 - Pathogenic bacteria as described above.

Activity 7 - Pathogenic bacteria as described above.

Genetic material used in activities 1-7 listed above.

Activity 1 - DNA fragments encoding various genes. Examples include Salmonella DNA fragments amplified from next to transposon insertions or long PCR fragments that differ between different strains of pathogenic bacteria.

Activity 2 - N/A - chromosomal material will be disrupted.

Activity 3 - DNA fragments encoding various genes.

Activity 4 - A gene that expresses a marker protein which is highly unlikely to have any biological effect (eg beta-galactosidase, GFP, luciferase).

Activity 5 - DNA fragments encoding various genes. The worst case scenario is expression of genes encoding subunits of known or potential toxins from Group 3 pathogens, eg the catalytic subunit of the Shiga like toxin from E. coli 0157, the catalytic subunit of the recently described typhi toxin.

Activity 6 - DNA fragments encoding various genes.

Activity 7 - Antibiotic resistance genes expressed from their native promoters.

### Evaluation of foreseeable effects

#### Human Health considerations:

Activity 1 - No risk to human health, this part of the work falls into containment level 1.

Activity 2 - The disruption of putative virulence genes and their fusion to reporter genes will produce a GMO which will be no more hazardous than the wild type and which can therefore be handled at the ACDP category relevant to the wild type - ie level 2.

Activity 3 - The disruption of putative virulence genes and their fusion to reporter genes will produce a GMO which will be no more hazardous than the wild type and which can therefore be handled at the ACDP category relevant to the wild type - ie level 2.

Activity 4 - The introduction of the gene for the marker protein and disruption of the resident gene will produce a GMO which will be no more hazardous than wild type and which can therefore be handled at the ACDP category relevant to the wild type - ie level 2.

Activity 5 - Using the Brenner scheme the damage is set at 10 (-3) in line with discussion on ricin in Part 2A annex 1 of the ACGM Guidance. Exactly similar considerations apply to bacterial toxins, ie the catalytic subunit of any toxin cannot exert any effect unless delivered in soluble active form to the appropriate compartment of the appropriate cells within the human body.

This cannot happen here as the necessary delivery mechanisms (eg a secretion system for getting out of the cell, a binding domain for attaching to cells and mediating entry) will be absent from the cloning host, so we feel justified in assigning a value of 10 (-3). We will never express the binding and toxic domains in the same cell. A possible exception is the class of virulence determinants known as "autotransporters", under study by Ian Henderson. Here the determinants necessary for export from the bacterial cell are contained within the same protein as the toxic moiety. However, even the most toxic of these, the enterogaegregative E. coli plasmid-encoded toxin PET, is unable to damage host cells or organ transplants when expressed at low levels in disabled hosts so we feel justified in assigning a damage value of 10 (-3), and probably more appropriately 10 (-6) even though these proteins can be exported from the bacterial cell.

Activity 6 - The shuttle vector simply re-introduces a characteristic that was already present in the wild type and had then been removed by mutagenesis. The procedure will
Environmental considerations:

Activity 1 - All waste is autoclaved before leaving the building so it is very unlikely that GMOs or constructs will escape into the environment. If by some chance they did escape the disabled host would not be able to survive and the non-mobilisable vector would not be able to leave the host. Any DNA released into the environment would be similar to that which occurs during the usual processes of decay. The environmental impact would therefore be negligible.

Activity 2 - All waste is autoclaved before leaving the building so it is very unlikely that GMOs or constructs will escape into the environment. Transposon mutagenesis is a naturally occurring process and all the species we are interested in harbour transposable elements. Any disruption or alteration of gene function as a result of transposon insertion is therefore already likely to have occurred in the natural environment. We anticipate that insertional mutagenesis will result in a less fit phenotype which will be outcompeted by the wild type if it happened to escape into the environment. Transciptional fusions will be made only to reporter genes with no conceivable environmental effect. Although the transposons will carry antibiotic resistance genes as selectable markers, these will not, in the unlikely event of escape, present an increased risk to the environment because the genes used do not encode resistance to drugs used therapeutically against the host species; these resistance determinants are already abundant in naturally occurring isolates of the host species; in the case of mud-lac, the element carrying them is no longer transposable.

Activity 3 - As all waste is autoclaved before leaving the building it is highly unlikely that any GMOs or constructs will escape into the environment. Our allele replacement will be done using non-mobilisable vectors that

- can be propagated in crippled E. coli strains, but not in the target species, e.g. pUC18 replicates in E. coli but not in Campylobacter jejuni (Biotechniques 1999 Jan; 25(6):50-2, 54, 56 Karlyshev et al)
- show temperature-sensitive replication in E. coli (e.g. pK03 used in E. coli and Salmonella: http://arep.med.harvard.edu/labgc.pko3.html).

In such cases, as the construction of a disrupted gene occurs in a non-mobilisable vector in a disabled host, the resultant GMO would not be able to survive in the external environment and the non-mobilisable vector would not be able to leave the disabled host. Therefore the environmental effects would be negligible. Once the allele replacement is complete, the resulting GMO, carrying a disrupted gene, is likely to be less fit than the wild type and so will be out-competed by it, were it to escape into the environment (see arguments about natural mutations above). Allele replacement requires the introduction of antibiotic resistance genes into the target strain, both during the interim stage when the plasmid is integrated in the chromosome, and in the final stage when the disrupted allele has replaced the wild type allele. In this situation, similar constraints apply as in transposon mutagenesis - these antibiotic resistance genes will not, in the unlikely event that they escape, present an increased risk to the environment because:

- We will use only those genes that DO NOT encode resistance to drugs used therapeutically against the host species (e.g. ampicillin is not used to treat campylobacteriosis)
- These resistance determinants are already abundant in naturally occurring isolates of the host species (e.g. chloramphenicol resistance in Salmonella typhimurium DT109: Arcangioli et al. J Med Microbiol. 2000 Jan;49(1):103-10.)
- They are not lodged on a transposable or mobilisable element.

Activity 4 - As all waste is autoclaved before leaving the building it is highly unlikely that any GMOs or constructs will escape into the environment. Transcriptional fusions will be made only to reporter genes with no conceivable environmental effect (genes for beta-galactosidase, luciferase, or green-fluorescent protein). However, as an interim step, antibiotic-encoding suicide vectors may be used (see discussion above).

Activity 5 - As all waste is autoclaved before leaving the building it is highly unlikely that any GMOs or constructs will escape into the environment. The vectors that are used are non-mobilisable and will be propagated only in disabled lab strains of E. coli. If by some slim chance they were to escape, the disabled host would not be able to survive in the external environment and the non-mobilisable vector would not be able to leave the disabled host. The environmental impact would therefore be negligible.

Activity 6 - As all waste is autoclaved before leaving the building it is highly unlikely that any GMOs or constructs will escape into the environment. Our complementation work will be done using non-mobilisable or mobilisation-defective vectors that are initially propagated in crippled E. coli strains. This initial propagation of a non-mobilisable or mobilisation-defective vector in a disabled host means that the resultant GMO will not be able to survive in the external environment. The environmental effects of such a GMO escaping will thus be negligible. Also as the non-mobilisable vector will not be able to leave the disabled host, gene release into the environment could occur only in a way that parallels natural decay processes.

Once the shuttle vector is inside a pathogenic strain there is a possibility that it could create a GMO capable of surviving in the external environment. However, this GMO
will be no more fit than the wild type if the complementation of the mutant phenotype works and will probably be less fit if it does not. In the extremely unlikely event that it might escape from the laboratory, it will therefore present no increased environmental risk over and above that presented by the wild type pathogen. Also as the non-mobilisable vector will not be able to leave the disabled host, gene release into the environment could occur only in a way that parallels natural decay processes. The shuttle vector might be construed as providing a novel means of gene transfer between the pathogen and E. coli. However, any genes transferred in this way are unlikely to be expressed in a new organism, as there are considerable barriers to the expression of heterologous genes in most bacteria (eg. many Campylobacter genes are not expressed off their own promoters in E. coli: Wosten et al J Bacteriol 1998 Feb; 180(3):594-9). Furthermore, as the shuttle vectors that we will use are non-mobilisable or mobilisation-defective, they will not be able to escape easily from their hosts, should these by some unlikely eventuality find their way into the environment.

Activity 7 - As all waste is autoclaved before leaving the building it is highly unlikely that any GMOs or constructs will escape into the environment. The vectors that are used are non-mobilisable and will be propagated only in disabled lab strains of E. coli. If by some slim chance they were to escape, the disabled host would not be able to survive in the external environment and the non-mobilisable vector would not be able to leave the disabled host. In addition, in the absence of selective pressure the antibiotic resistance genes will not confer any increase in fitness. The environmental impact would therefore be negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste (aside from disinfected fluids) will be autoclaved - Kill rate effectively 100%

Two general purpose chemical disinfectants are used: Chlorine based disinfectants (Haztabs and Chloros) and Microsol. Chlorine based disinfectants should be used at 25000ppm available chlorine and material should be left to soak overnight before disposal. Disinfected liquids may then be disposed of to drains and solids to the appropriate waste bin. Alternatively, 10% Microsol may be used.

Validation data for Microsol.

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<tr>
<th>Microorganism</th>
<th>Log Reduction</th>
<th>Dilution</th>
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<tbody>
<tr>
<td>* Salmonella enterica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Escherichia coli (non-vero-toxin-producing)</td>
<td>&gt;6.00</td>
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</tr>
<tr>
<td>* Campylobacter jejuni</td>
<td>4.78</td>
<td>1:50</td>
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<tr>
<td>* Corynebacterium diphtheriae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Staphylococcus aureus</td>
<td>6.01</td>
<td>1:200</td>
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<tr>
<td>* Streptococcus pyogenes</td>
<td></td>
<td></td>
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<tr>
<td>* S. pneumoniae</td>
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<tr>
<td>* Bacteroides fragilis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Clostridium difficile</td>
<td>4.55</td>
<td>1:50</td>
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<tr>
<td>* Mycoplasma pneumoniae</td>
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<td>* Shewanella putrefaciens</td>
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</tr>
<tr>
<td>* Mycobacterium bovis BCG</td>
<td>5.63*</td>
<td>1:2</td>
</tr>
<tr>
<td>* 30 mins exposure</td>
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Data has not been included for all the organisms listed, as not all of these organisms will be used in the first instance. However, data for these other organisms will be obtained and added when they are used.

2% Stericol or Hycolin can be used for Mycobacteria. These phenolic compounds have been shown to be effective against tubercle bacilli at concentrations between 0.2-3% with a 10-30 mins contact time (Vesley and Lauer in Laboratory Safety - Principles and Practices 2nd Edition 1995, eds Fleming, Richardson, Tulis and Vesley).
GMSC was primarily concerned with the generic nature of the programme and needed reassurance that any future changes to the work would continue to be brought to the Committee for discussion and approval. The GMSC has approved this application.

**Project Containment**

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref** 116/01.2

**RETROVIRAL TRANSDUCTION OF HAEMOPOETIC PROGENITOR CELLS WITH HYDROXOSTEROID DEHYDROGENASE ENZYMES**

Date Ackn'd: 19/02/2001  
Date Project Ceased:  
Withdrawn: N

Class 2

Non-GMM:  
Consent Granted: not applicable

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref**  116/01.3

**Date Ackn'd**  19/02/2001

**CU2 Project Title**

GM ADENOVIRUS CONTAINING EXPRESSION CASSETTE
CMV-IEHHU/CMV-IEP-YO FOR USE IN CYTOTOXICITY ASSAYS

**Class**  Class 2

**CultureVolClass2**  

**Consent Granted**  not applicable

**Project notified under transitional arrangements**  Y

**Withdrawn**  N

**Tick if notifying a connected programme of work**  N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 116/01.4

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Date Project Ceased

PROPAGATION IN E COLI

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

## Project Containment

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Withdrawn N
Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 116/01.6

Date Ackn’d 19/02/2001

CU2 Project Title EXPRESSION OF HBV PROTEINS IN BACULOVIRUS

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref 116/01.8**

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<th>CultureVolumeClass3-4</th>
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<th>Project notified under transitional arrangements</th>
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Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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</table>
**Project Ref** 116/01.9

**Date Ackn’ed** 09/03/2001

**CU2 Project Title**
PATHOGENESIS OF ENTEROPATHOGENIC E. COLI (EPEC) / VEROCYTOTOXIGENIC E. COLI (VTEC)

**Date Project Ceased**

**Class** Class 2

**CultureVolClass2** < 1 litre

**CultureVolumeClass3-4**

**Non-GMM** not applicable

**Consent Granted**

Project notified under transitional arrangements N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

EPEC/VTEC adhere to, and colonise the human intestinal mucosa, resulting in a diarrhoeal disease syndrome. Acquired virulence properties are necessary to promote a range of pathogenesis events; initial attachment, subversion of host cell signalling and ultrastructural changes to the epithelial surface resulting in the formation of histopathic lesions, termed attaching and effacing lesions. In this study, using established adhesion assay models the bacterial/host cell interaction phenotype is studied using a variety of EPEC strains and transformed human carcinoma cell lines.

**Recipient or parental organism**

commercially available cell lines

**Host/vector system**

multiple plasmids. List available attached to risk assessment Some examples are pCVD438, pMSD2, pCVD462...

**Origin & function**
over 100 mutants of wildtype enteropathogenic E.coli with deletion mutations

**Evaluation of foreseeable effects**

Wild type EPEC/VTEC pose key virulence factors which are expressed as either proteins on the surface of the bacteria or soluble proteins secreted from the bacteria and translocated into the host cell via attachment fimbriae. EPEC/VTEC deletion mutants are lacking one or more virulence factors, via the knockout of relevant genes. Plasmid transformants have the gene inserted back into the deletion mutant restoring the strain to the original genetic capability. Hep-2, T84, Caco-2 cell lines are commercially available transformed lines from nasal pharyngeal (Hep-2) and intestinal epithelium (T84 Caco-2) cancers.

Wild type EPEC are able to cause diarrhoea in humans; however, in this laboratory under the small scale risk criteria a low level of risk is associated. Deletion mutants with the removal of one or more virulence genes possess a level of risk far lower than wild type. The phenotype of the transformant is often attenuated and is not fully expressed to the same level of wild type strains. No deletion mutant/transformant possess virulence levels or present an infective risk higher than that of the wild type strain. The majority of laboratory activities in this case fall into containment level 1, with some in level 2. Containment level 2 is applied to ensure that there are sufficient control measures in place for any event that may occur in the laboratory environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation - Full level 2 applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All GM waste is autoclaved at 121 degrees C for 50 minutes. Degree of kill - effectively 100%
Decontaminated glassware then undergoes usual wash-up procedures. Disposable plastic waste is placed into yellow incineration bags which are placed in designated areas for collection and disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC put several questions to Dr Knutton regarding this project and were content with his response.

**Project Containment**

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### Project Additional Information

#### Purposes of the contained use

Retroviral vectors (pBABE) containing human HIF2 a cDNA that have been produced elsewhere will be used to produce retroviral particles via transfection of a phoenix producer cell line. A number of human cell lines will be infected with these retroviruses and the effect on the expression of possible target genes will be analysed at the transcriptional and translational level. A number of other retroviral vectors containing cDNAs involved in these pathways (e.g., VHL, HIF1 a) may also be produced by subcloning pre-existing cDNAs available in the laboratory.

#### Recipient or parental organism

**Hosts:**

1. Disabled E. coli strains such as DH5a, JM109. Inserts are under the control of eukaryotic promoters, therefore expression is negligible.
2. Packaging cell Phoenix line (based on human embryonic kidney cell line 293 transformed with adenovirus E1 gene carrying a temperature sensitive T antigen co-selected with neomycin). Phoenix is a second-generation retrovirus producer line for the generation of helper free retroviruses. Different promoters for gag-pol and envelope were used to minimise their inter-recombination potential. Gag-pol was introduced with hygromycin as the co-selectable marker and the envelope proteins were introduced with diphtheria toxin 1 resistance as the co-selectable marker. The production of replicative virus through recombination has not been observed. The inserts should not affect that. The cell line has been tested for helper virus production and has been established as being helper virus free. The vector will be transfected in the Phoenix line and the medium containing the retroviral virions will be harvested 24 and 48 hours later, aliquoted and stored at -80 degrees C.
3) Cell lines derived from human kidney, colon, lung and breast.

**Host/vector system**

**Vector:**
The vector will be a retroviral shuttle vector, pBABE (Mo MuLV based vector, containing LTR, gag, cloning sites BamHI, EcoRI and selection by puromycin or neomycin transcribed from a SV40 promoter) are based on standard cloning plasmids, but have also the information for the encoded sequences to be packaged into viral particles when introduced into special packaging cell lines. The vectors themselves do not possess the genes required for the production of infectious virus. The vectors contain drug resistance markers for both pro and eukaryotic selection.

**Origin & function**

**Inserts:** Hypoxia inducing factor 1 and 2 alpha; succinate dehydrogenase subunit B; VHL; Ras Association domain Factor Isoform A (RASSF1); BLU, SLIT2 - genes involved in regulation of tumorigenecity.
**Donor:** Human cDNA from healthy tissue, originally gifts, therefore unsure of origin.

**Evaluation of foreseeable effects**

**Effects on human health:** The shuttle vector or the produced retrovirus with insert poses a negligible risk for infection. The retroviral virions, produced in the Phoenix line, can only infect dividing cells, therefore infection requires special conditions and is highly unlikely to happen in the event of an accident. No sharps will be used and the work will be carried out in a Class II safety cabinet to reduce the risk of accidental infection.

The shuttle vectors which have the non-mobilisable insert under the control of eukaryotic promoters do not possess the genes required for the production of infectious viruses. The retroviral virions, produced in the Phoenix line, can only infect dividing cells, therefore infection requires special conditions. This is unlikely to happen when the appropriate safety measures are taken.

Furthermore, in the unlikely event of infection the retroviral virions are proliferation defective. The insert coding the expression proteins HIF1a and HIF2a are putative oncogenes, having a role in angiogenesis, although they will have negligible toxicity or transforming activity. All other stated inserts are recognised or putative wild-type tumour suppressor genes and have no known tumour promoting properties.

**Environmental hazards:** The potential hazard is considered to be negligible as the retrovirus particles are very unstable and the virus is non-replicative. Retoviral supernatants will be contained within the tissue culture area. Live animals will not gain access to the work area.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - full level 2 containment applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Tissue culture plastics will be autoclaved before disposal. Pipettes are to be soaked in Virkon before autoclaving. Small volumes of liquid containing viral particles will be used immediately or treated with Virkon. Virkon will be used at 1% concentration although 0.5% Virkon is effective against retroviridae (Ref given in risk assessment).

*Sacrificial* viral supernatants will be put through disposal protocols and tested for viability by reinfection of cell lines.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The GMSC requested more information on the inserts and the potential risk to human health. Some of the comments included: What are BLU and SLIT2?

Even though the recombinant retroviruses may not be able to replicate in non-helper cells, they can still enter and express inserts. Since the inserts are putative oncogenes, has the effect of this been considered?

Cloning in retrovirus does not address the potential risk of virus transferring a potentially hazardous gene in the event of an accident. The applicants assume that the failure of the virus to replicate means that the construct is not hazardous.

Page 1 title section: The function or putative function of the genes identified needs to be described to allow an assessment of risk to be made.

The assessment was amended to take account of these comments. This assessment has now been approved by the GMSC.

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Animal Units: L2 L3 L4 L2 L3 L4
Large Scale Activities: L2 L3 L4 L2 L3 L4
Human Clinical Applications: L2 L3 L4 L2 L3 L4

Project Ref 116/03.1

Date Ackn'd: 11/06/2003
CU2 Project Title: INVESTIGATION INTO THE LIVER REPOPULATING CAPACITY OF HUMAN LIVER STEM LIKE CELLS.

Date Project Ceased: 19/11/2004

Class: Class 2
Culture Class: < 1 litre
Consent Granted: not applicable

Withdrawn: N
Project notified under transitional arrangements: N
Project Additional Information

Purposes of the contained use

The study will focus on the potential of human liver stem cells as alternative sources of functional human hepatocytes and biliary epithelial cells. Isolated human liver stem cells will be stably transduced with a recombinant lentivirus expressing the gene for Green Fluorescent Protein. The potential of these marked cells to repopulate acutely injured livers will be assessed by cell transplantation experiments in NOD/SCID mice (but not here in the UK).

Recipient or parental organism

Hosts. Standard disabled lab strains of E. coli as DH5a and JM109 will be used for growth of plasmids. Manipulation of large plasmids may involve homologous recombination in strain BJ5183. Human cell line 293T will be used for both initial rescue of virus from transfected DNA, and for virus, propagation. Primary human liver stem cells (which are non-permissive for production of replication deficient lentivirus), will be transduced with recombinant lentivirus expressing GFP.

Host/vector system

Vectors: Plasmids; Third generation lentiviral vector (replication defective). Please see assessment for details.

Origin & function

Sequence encoding Green Fluorescent Protein which functions as a reporter molecule that fluoresces upon exposure to uv light.

Evaluation of foreseeable effects

Risks to human health: The lentiviral vectors used in this study are third generation lentiviral vectors. In these third generation vectors all six regulatory/accessory proteins (Rev, Tat, Vif, Vpr, Vpu and Nef) are removed, except Rev. Rev acts as the post-transcriptional level and is necessary for HIV gag/pol messenger. As an extra safety responsive element [RRE]) and facilitates the cytoplasmic export of gag/pol messenger. As an extra safety measurement Rev is placed on a separate vector (RSV-Rev) beside transfer vector. Only 700 bp of the HIV envelope protein are present in the transfer vector, plus RRE and the packaging signal. The packaging construct contains the minimal RRE of 375 bp and the gag/pol genes. Through deletions in both LTRs and the absence of 5 of the 6 accessory proteins, including the replication essential Tat it is highly unlikely that replication competent virus is produced. In published studies and from our own experience with these vectors replication competent virus has never been detected. The HIV-envelope protein is replaced by non-retrovirally envelope protein (VSV-G). It is highly unlikely that this will be incorporated in a new hybrid replicative virus. It is impossible that wild-type HIV will be formed, because of the omission of 5 accessory proteins with HIV-env.

Environmental considerations: Proteins will not be expressed in bacteria used for plasmid propagation. Bacteria used for propagation are attenuated strains that will not survive in the environment. The recombinant virus is replication defective and will not have an extended host range, the GFP transgene is innocuous, so there is negligible potential hazard to the environment. Containment conditions specified should prevent environmental release of virus or E. coli carrying the associated plasmids; and the virus is replication defective.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is treated by autoclaving at 130 degrees centigrade for 60 mins before disposal to drain. Disposable solid waste which is or may be contaminated with GMMs is also inactivated by autoclaving at 130 degrees centigrade for 30 mins, before removal as clinical waste by specialist contractors, with final disposal by incineration. Autoclaving achieves 100% kill. Warning lights, and chart recorder, indicate whether or not the autoclave run has achieved the appropriate temperature for the required time. The autoclave is run by trained staff, who are instructed to treat waste as still contaminated if the autoclave run was not to specifications. Under such circumstances the waste would be autoclaved again, after rectifying any reason for the malfunction.

The autoclave is inspected and serviced on a regular basis. Autoclave is regularly tested with Brown's tubes.

Sharps, including scalpel blades, needles and disposable plastic tips, may be decontaminated by drawing up Virkon disinfectant, and by soaking in Virkon for a minimum of 15 minutes. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above. Manufacturer's information indicates efficacy of Virkon against strains of E. coli at dilutions ranging from 1% to 0.125%. The manufactureres of Virkon (Antec International Ltd) have also commissioned studies on the virucidal effect of Virkon on Human Immunodeficiency virus and found that a 0.5% solution of Virkon inactivated 10^66 infectious doses (ID50) of HIV-1 per ml within 10 minutes at room temperature.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
1. I am not sure that the statement that pMDLg/pRRE expresses no gag/pol has ever been convincingly demonstrated. Perhaps it is better to say that only very low or undetectable levels will be anticipated.
2. The applicant states that he has never detected replication competent virus. How has he looked for it? Has it ever been detected?
3. Is there validation data for Virkon against lentiviruses?
4. Is the KI discus test carried out annually rather than six monthly?
5. The autoclave should be additionally tested with Brown's tubes on a regular basis.

All of the above have been addressed in the assessment. The GMSC have approved this assessment.

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**Project Ref** 116/03.2

- **Date Ackn'd:** 13/06/2003
- **CU2 Project Title:** ALLELIC VARIATION IN HLA-DQ GENE EXPRESSION

**Date Project Ceased:**

- **Class:** Class 2
- **CultureVolClass2:** < 1 litre
- **CultureVolumeClass3-4:**
- **Non-GMM:** Consent Granted
  - **Consent Granted:** not applicable
- **Project notified under transitional arrangements:** N

**Tick if notifying a connected programme of work:** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

02/03/2022
### Purposes of the contained use

The study will investigate the promoter activities of a range of HLA-DQA1 and -DGB1 alleles associated with susceptibility to, and protection from, autoimmune type 1 diabetes.

### Recipient or parental organism

**Hosts:** Initial host - E. coli JM109, a disabled lab adapted host, non pathogenic.

Final host - EBV transformed lymphoblastoid cells lines, MGAR, COX, BSM, EMJ and HHK. These cell lines are derived from healthy human subjects with different HLA-DG genotypes. They are commercially available and are not derived from workers in the department of Medicine. The cell will be unable to colonise workers as they are incompatible at the HLA class 1 and class II loci and will therefore provoke a host-versus-graft immune response. There is a potential infection risk from the EBV but the proposed GM is not expected to alter this pathogenicity. The DG promoters are very weak eukaryotic promoters and will not be integrated into the genome of the host cells.

### Host/vector system

**Vectors:** pCAT3 Basic reporter vector contains f1 origin of replication, ampicillin resistance gene and chloramphenicol acetyltransferase gene. pCAT3 control vector also contains SV40 promoter and SV40 enhancer sequences.

pSVB-Gal contains SV40 promoter/enhancer. E. coli gpt promoter located upstream of the lac Z gene and ampicillin resistance gene. All vectors are derived from the pUC series and are non-mobilisable.

### Origin & function

**Source:** Five EBV transformed human B lymphoblastoid cell lines, homozygous for different DG types (MGAR, COX, BSM, HHK, EMJ). All cell lines are from the European Collection of Cell Cultures, Porton Down, and are all initially derived from healthy human donors.

**Genetic sequences:** 600bp fragments of the proximal promoter region of the following HLA-DQ alleles - DGA1*0102, DGA1*0103, DGA1*0301, DGA1*0501, DGB1*0201, DGB1*0302, DGB1*0602, DGB1*0603, DGB10604 - cloned upstream of the reporter gene encoding chloramphenicol acetyltransferase. All wild type sequences.

### Evaluation of foreseeable effects

**Human Health:** The host bacterial strain is non-pathogenic to humans. Genetic modification will not pose any risk to health as no expressed protein will be secreted from the cells and the pathogenicity of the host will not be altered (except conferring resistance to ampicillin).

Expression in human lymphocytes - the primary risk to health will be related to the EBV transformation of the host cell lines, which poses an additional risk of EBV infection. Genetic modification of these cells will not pose any additional risk as no protein products will be secreted and the GMOs created will be transient (they will be lysed for analysis 48 hours after transfection). The cell lines are HLA incompatible with the workers and will therefore provoke a host-versus-graft immune rejection response, preventing colonisation. A record is kept of the HLA profile of staff working in the department of Medicine and all new workers are routinely HLA typed. New subjects at risk of colonisation by the cell lines can therefore be identified and prevented from working with the cells.

Immunocompromised individuals and pregnant women will not be permitted to work with the transformed lymphocyte lines due to the risk of EBV infection.

**Environmental considerations:** B lymphocyte lines will die outside correct culture environment. No potential hazards associated with GM bacterial cells - disabled, non-colonising, laboratory adapted host.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**N/A**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No application for derogation

02/02/2022
Liquid waste contaminated with GMMs will be decanted into waste pots containing Vernagel to solidify the waste. This waste will then be inactivated by autoclaving (100% kill). Solid waste (excluding serological pipettes) autoclaved prior to disposal via clinical waste route - ultimately incinerated. Serological pipettes to be soaked overnight in 1% Virkon, drained and sealed in bags for autoclaving. Autoclave regularly tested. Brown's tubes used every week to check autoclave performance.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

1. Who are the donors of the healthy B-cell lines mentioned in the section “Name and Characteristics of the Host”? The cells from other workers within the Dept. of Medicine should not be cultured.

2. 70% ethanol should not be used to decontaminate the safety cabinet due to the fire/explosion risk. Trigene would be safer.

3. Putting Virkon treated waste through the autoclave may corrode the machine.

4. Has 0.5% SDS been validated as a disinfectant?

The above points have been addressed. SDS is no longer to be used as a disinfectant. The GMSC has approved this assessment.

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Project Ref 116/04.2

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
In order to investigate the signalling properties of the largely endothelial-restricted vascular endothelial cell growth factor (VEGF) receptors (VEGFR-1/Flt-1, VEGFR-2/KDR, VEGFR-3/Flt-4 and neuropilins) and Tie receptor tyrosine kinases (Tie-1 and Tie-2) we will produce recombinant replication-deficient retroviruses encoding chimaeric receptors and wildtype/inactive mutants of downstream signalling intermediates.

Recipient or parental organism

Host - The two retrovirus systems will be packaged in (I) HEK293T cells or (ii) GP+E86 or gp envE86 packaging cells Plasmids will be amplified in E.coli (e.g. DH5)

Host/vector system

Replication-defective retroviruses derived largely from Maloney murine leukaemia virus (MuLV). These will be generated using two systems: (I) The MFG retrovirus vector uses a series of 3 plasmids pMD.MLV (gag-pol), pMD.G(env) encoding the cDNAs of proteins required for viral packaging, and pMMP into which sequences of interest are inserted. (ii) LNCX plasmid contains a cytomegalovirus (CMV) immediate early promoter which drives the expression of the inserted gene, and lacks gag, pol, and env genes which are provided by packaging cells.

Origin & function

Human and mouse
EGFR-1, c-fms, VEGFR-1-3, Tie-1/-2(Tek), neuropilin, RhoA-N19, h*ARK1, dn-p85, Rac-N17, Phospholipase D (PLD), LacZ and enhanced-GFP. These include chimaeric receptors and proteins which alter cell signalling. See assessment for greater detail.

Evaluation of foreseeable effects

The chimaeric receptor inserts harness the EGF and c-fms receptor extracellular domains and the intracellular portions of VEGF, neuropilin and Tie receptors. The wild-type VEGFRs, neuropilins and Tie receptors are not associated with cell transformation. However, they may promote malignant progression indirectly. Although it is unlikely that these viruses would pose an oncogenic risk, differences in the abundance and distribution of the EGF and CSF-1 activating ligands compared with the natural ligands and their uncharacterised activity in cell types that would not normally express them should be taken into consideration. The mutant/inactive signalling proteins are well established (e.g. RhoA-N19, Rac-N17) inhibitors of cell signalling pathways some of which are associated with cell transformation and some have been shown to inhibit
cell transformation and malignant progression. Although it is unlikely that these inhibitors would present a risk of cell transformation they could disrupt normal cell function. Retroviruses expressing LacZ and E-GFP are not associated with toxicity or cell transformation. Amphotropic retroviral vectors facilitate efficient gene transfer into replicating human cells and following infection the viral genome is incorporated into the target cell chromosomes. There is a potential risk of insertional mutagenesis or enhancement of host cell expression of genes adjacent to the integration site associated with retroviral infection. There is also a potential risk that virus stock may become contaminated with replication-competent retroviruses. These viruses would be considered to pose a greater risk of malignant transformation given their potential to replicate and spread. However, it is unlikely that replication-competent retrovirus would be generated. Amphotropic retroviruses have a broad host range and are therefore capable of infecting animals in the wild. However, the risk is extremely low as retroviruses are readily inactivated and will not survive for long in the environment. The potential for an environmental hazard would be dependant upon the unlikely generation of recombinant replication competent retrovirus. Outside the tissue culture environment the retrovirus packaging cell lines and infected target cells will not be viable. The plasmids encoding the retroviral genes and inserts are mobilisation-defective in their E.coli hosts. The plasmids do not contain bacterial promoter sequences and so the inserts will not be expressed. The disabled host bacteria will not survive long in the environment.

containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

TriGene will be used to disinfect contaminated plastics, liquid waste and working areas. TriGene will be used at 1:50 dilution which is more than sufficient to inactivate retroviruses based upon the manufacturer's information even under heavy soil conditions (see below). Contaminated plastics will be immersed for at least one hour in TriGene solution and then autoclaved at 130°C for 60 mins before disposal. Solid waste will then be removed as "clinical waste" and incinerated by specialist contractors and liquid waste washed down the drain.

Expected degree of kill:

Autoclaving will effectively kill 100% of all GMMs

According to the manufacturer's information (MediChem Int.) TriGene inactivates HIV, FIV and the Feline Leukaemia virus (FeLV) at 1:200 (EN 1650) and 1:400 (EN 1276) dilution and therefore should inactivate similar retroviruses.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Because of the 2 classes of GMM created in this work the GMSC expressed concern at "mixed" working and highlighted this as an issue to be monitored. The GMSC has approved this assessment.

Project Containment
Project Ref 116/05.1

Date Ackn'd 28/01/2005
Date Project Ceased 16/09/2017

CU2 Project Title
Genesis of lentiviral Hepatitis C Virus (HCV) pseudotypes and Genesis of HCV DNA and RNA* (*which will eventually lead on to work at CL3 - this is to be submitted as a separate notification in due course when the facilities are ready locally)

Class 2
CultureVol
< 1 Litre
Consent Granted Not Applicable

Non-GMM

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work Y

Historical Significant Changes Transferred to GM553

Project Additional Information

Purposes of the contained use
Genesis of lentiviral HCV pseudotypes: To investigate how HCV infects liver cells and the role of the humoral immune response plays in controlling HCV replication. This assessment covers the generation of lentiviral pseudotypes using a disabled lentiviral vector by transient expression of HCV gpl from an independent promoter. Genesis of HCV DNA and RNA: This seeks to generate synthetic HCV strain JFH RNA for subsequent recovery at CL3.

Recipient or parental organism
Genesis of lentiviral HCV pseudotypes: Hosts include E. coli (Top 10 strains, Invitrogen); 293T embryonal kidney cell line; Human hepatoma cell lines (HepG2, Huh-7, HepH, HH29); Primary hepatocytes.

Genesis of HCV DNA and RNA: E. coli (JM 109 derivative strain, Top10, Invitrogen).

Host/vector system
Genesis of lentiviral HCV pseudotypes: Vectors - pCDNA3 and pNL4.3R-E-lentiviral vector (based on a lab strain of HIV-1 from which the vpr and envelope genes have been deleted).

Genesis of HCV DNA and RNA: Vector - pUC19 (Stratagene).

Origin & function

Genesis of lentiviral HCV pseudotypes: Insert - HCV envelope gps - The E1E2 gps mediate viral attachment and fusion of the viral and host cell membranes necessary for infection of a target cell. The E1E2 region will be PCR amplified from clinical material and synthetic start and stop codons added to enable expression. Donor - Human (plasma samples and liver biopsies).

Genesis of HCV DNA and RNA: Insert - HCV DNA encoding the full length genomic sequence is not infectious for chimpanzees. However, synthetic RNA derived from plasmid DNA can lead to infection and seroconversion if delivered directly into the liver of a chimpanzee. The JFH strain of HCV will be used at this time. Donor - Human (plasma samples and liver biopsies).

Evaluation of foreseeable effects

Genesis of lentiviral HCV pseudotypes: 1) Transfected ‘producer’ 293T - Transfection of the pE1E2 expression constructs into 293T cells will result in the expression of HCV gps, which pose minimal risk to human health. Co-transfection of 293T cells with the lentiviral pNL4.3R-E- and vectors encoding the viral gps (HCV, VSV and MLV) will lead to expression of the reporter gene luciferase and the HIV proteins (gag, pol) which will drive assembly of pseudotypic particles. None of these viral proteins or luciferase have been associated with toxicity. 2) Lentiviral pseudotype infected target cells - The pseudotypes can only undergo a single cycle of infection and so the ‘infected’ target cells do not release new rounds of progeny virus. These cells do express lentiviral proteins and luciferase, which have no reported toxicity for humans. Accidental exposure by injection of these antigen expressing cells could lead to an immune response to the viral expressed genes. Whilst not harmful the detection of these immune responses is used in diagnosis of HIV infection, and so could lead to a false positive. Risks will be contained by handling materials at CL2. Environmental risks - The E. coli hosts used are disabled and require a supplemented media for growth. The 293T and hepatoma cell lines are only viable within tissue culture media and in the presence of CO2. The pseudotyped viral particles are not desiccation resistant and are incapable of replication. No risk to the environment.

Genesis of HCV DNA and RNA: HCV JFH plasmids bearing E1E2 sequences will constitute new GMOs. The E1E2 ORF(s) will be derived from naturally occurring sequences present within infected patients and will be unlikely to possess biolocal properties not already present within nature. DNA encoding HCV genomic length sequences are not infectious for chimpanzees and constitute minimal risk to human health. Synthetic HCV genomic RNA transcripts can be infectious for the chimpanzee if delivered intrahepatically. Other routes of delivery have not led to infection. Consequently the risk to human health at generating the RNA is minimal and can be contained at CL2. All experiments involving the delivery of the RNA into mammalian cells will take place at CL3 (to be submitted in due course). Environmental risk - The E. coli strains are disabled and are transfected using non-mobilisable vectors. The naked DNA generated by in vitro transcription will be unprotected from environmental RNAases and will degrade rapidly if released.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Surfaces to be wiped with 10% Trigene which has been shown to be an effective agent for inactivation of a wide range of enveloped viruses. Disposable plasticware will be rinsed in 10% Trigene, autoclaved and disposed of as clinical waste by incineration. Disposable gloves, paper towels etc will be deposited in autoclave bags and autoclaved before disposal.
Centrifuges and other equipment will be disinfected by wiping with 10% Trigene. If necessary the buckets can be totally immersed in this disinfectant. Autoclaving of waste achieves 100% kill.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Originally the assessments were circulated in a different format with different titles, but the GMSC felt that those assessments were repetative and lacking in clarity. Comments were passed back to the PIs and meetings were held to discuss the comments in detail. The assessments were revised and recirculated for final approval. The GMSC has now approved the work.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 116/05.2

Date Ackn'd 24/03/2005  

Date Project Ceased 16/09/2017  

Withdrawn N

Tick if notifying a connected programme of work  

Class Culture Class2 Culture Volume Class3-4  

Class 3 <50mls  

Consent Granted Yes  

Project notified under transitional arrangements N

CU2 Project Title  

Cell culture propagation of Hepatitis C virus:
**Project Additional Information**

**Purposes of the contained use**
This proposal seeks to deliver HCV strain JFH RNA into human liver derived cells and to characterise the resulting particles. This follows on from the recently notified Class 2 proposals. "Genesis of HCV virus DNA and RNA" & "Genesis of lentivirus pseudotype particles bearing HCV glycoproteins". This proposal includes expression of HCV strain JFH in human liver cells. Translation of viral RNA will lead to expression of HCV proteins, particle assembly and release.

**Recipient or parental organism**
As section below.

**Host/vector system**
Host: Primary and transformed human liver cells: screened and found to be negative for known human pathogens (HIV, HBV etc). After RNA transfection, the cells will be monitored for HCV infection for up to 14 days before being discarded.
Vector: The JFH strain of HCV is the only known HCV sequence capable of autonomous replication in hepatoma cell lines without adaptive mutation. Recent experiments suggest that the JFh strain can assemble and release viral particles at low titre in cell culture, which can be passaged in human cells. It is not known if these particles are infectious for humans.

**Origin & function**
Donor: HCV within clinical material from infected patients.
Genetic material: The investigators plan to transfect synthetic HCV RNA generated by run off transcription into liver cells to generate virus particles. Construction of these HCV genomes has been described in previously notified proposals. Plasmid DNA encoding the full length HCV genomic sequence is not infectious for chimpanzees. However, synthetic RNA derived from this DNA can lead to infection and seroconversion if delivered directly into the liver of a chimpanzee (Kolykhalov 1997).

**Evaluation of foreseeable effects**
Hazards to human health: Transfection and delivery of HCV RNA transcripts into human liver cells will lead to the expression of viral proteins which will result in the assembly of viral particles. Plasmid DNA encoding HCV JFH with heterologous E1E2 sequences will constitute new GMOs. The E1E2 ORF(s) will be derived from naturally occurring sequences present within infected patients and will be unlikely to possess biological properties not already present in nature. Synthetic HCV genomic RNA transcripts can be infectious for the chimpanzee if high doses are delivered intra-hepatically. Other routes of HCV RNA delivery have not led to infection. All experiments involving the delivery of genomic RNA into mammalian cells will take place under CL3 containment.
Environmental Hazards: The GMOs generated by this work pose no threat to the environment. The eukaryotic cells and cell lines used will be unable to survive in the external environment. The naked DNA used in electroporation will be unprotected from environmental RNAases and would be degraded rapidly if released into the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - Fu

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 116/05.3

Date Ackn’d 08/07/2005

CU2 Project Title Cellular transduction in vitro and in vivo with engineered second generation……

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 ≤ 1 Litre
**Project Additional Information**

**Purposes of the contained use**

The laboratory is dedicated to researching potential therapeutic targets for central nervous system (CNS) repair and the use of gene therapy in the CNS. The aim of this project is to analyse the efficiency of gene delivery to neurons using a lentiviral vector based on HIV-1.

**Recipient or parental organism**

Host - Virus will be produced in 293T cells (do not contain any complementing genome). Infectivity of the virus will be tested on 911/HeLa cell lines (viral titering). The engineered viruses will be tested for infectivity and transgene expression initially in vitro in established cell lines including PC-12 and C6 glioma cells and rat and mouse dorsal root ganglia (DRG) and retinal ganglion primary neuronal cell cultures. These will be used to transduce neurons in vivo in rat and mouse CNS injury models by injection into a number of sites, or delivery within a fibrocellular matrix (gene activated matrix) implanted into these sites.

**Host/vector system**

Vector - GM Lentivirus based on HIV-1 backbone with the ability to integrate the vector provirus into the host genome. This virus is multiply attenuated (deletions of vpu, vif, vpr and nef virulence genes and the env gene) and incapable of replication in the host. A 400bp deletion in the U3 region of the 3'LTR renders the virus self-inactivating (Zufferey et al.1998, j.Virol.72 (12) p 9873). The vector is pseudotyped with the vesicular stomatitis virus glycoprotein G (VSV-G) coat replacing the wild-type env encoded coat, to produce an amphotropic virus or with Rabies G protein to produce a predominantly neuronal tropic virus.

**Origin & function**

Genetic material - Aequorea Victoria Jellyfish; Green Flourescent Protein (GFP) - reporter gene. Human; Nerve Growth Factor, Neurotrophin 3, Brain Derived Neurotrophic Factor, Ciliary Neurotrophic Factor, Fibroblast Growth Factor type 2 - to stimulate neurite outgrowth from transduced neurons. Woodchuck Hepatitis Virus; Post-transcriptional Regulatory Element - WPRE (with a deletion in the X-protein encoding region - for increased mRNA handling and robust expression. shRNA sequences (based on rodent mRNA) homologous to components of axonal growth cone, inhibitory pathway components including: low affinity neurotrophin receptor p75NTR, TNF, Related receptor TROY, LINGO-1, RhoA small GTPase, Rho activated kinase (ROCK) - to give knockdown by RNA interference, disrupting the cascade and preventing inhibition of neurite outgrowth by myelin. Caspase 3 shRNA - to knockdown caspase 3 and impede neuronal apoptosis. shRNA sequences will be chemically synthesised.

**Evaluation of foreseeable effects**

Vector: Insertional mutagenesis: The insertion of HIV-1 provirus into the host is widespread, can be multiple and can involve activation of transcription in adjacent host
genes (due to strong LTR promoters), resulting in insertional mutagenesis and potential activation of oncogenes depending on the site of insertion. Despite the latter possibility, lentiviruses are not usually oncogenic. The risk of insertional mutagenesis has been addressed by using a SIN vector but there is the potential ability of the internal promoter used (of which CMV and Sffv are strong promoters) to read-through the inserted genome and activate transcription of adjacent genes. Germline integration should not occur.

Recombination and formation of replication competent retrovirus (RCR): Recombination resulting in wild-type HIV-1 cannot occur. Vector design uses 3 plasmids to reduce the occurrence of recombination of a functional genome during production. Production of a replication competent GM virus is highly unlikely. Testing of the system for RCR has previously shown absence of detectable helper virus. Importantly, in the absence of RCR the virus will not disseminate. Immunogenicity: Lentiviral vectors used so far have shown minimal immune response to their introduction, VSV-G toxicity has not been reported and Rabies G vectors in the CNS have shown minimal immune response, nontoxicity, plus unaltered physiological profiles.

Inserts: SIN vectors should prevent expression of anything external to the transgene cassette in all cells. Risks due to the GFP insert are minimal. The risks of neurotrophin and FGF-2 inserts are deemed to be low, these genes have not been shown to be tumorigenic in previous studies despite their cellular effects, the risks remains but is reduced by the restriction of dissemination of the virus and the reduction (to minimal) of exposure when working within safe guidelines. All shRNA sequences except RhoA, ROCK and Caspase3 should pose effectively zero hazard if accidental inoculation occurred. The former 3 do pose a risk of tumorigenic effects especially inhibition of caspase 3, the risk is reduced by the sequence specific homology required for siRNA action to occur. We have acknowledged GTAC/SACGM advice given following the report of liver tumours in mice treated with lentiviral vectors. Appropriate measures will be taken in our animal experimentation work and in this vector system WPRE is represent with a large deletion in the coding region for Xprotein (to address it's potential oncogenic effect), it should not be necessary to suggest a containment level above 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Freshly mixed 1% Virkon. Followed by 70% Ethanol will be used for regular decontamination of all surfaces, transport and storage containers, incubator shelves and for the decontamination of titanium centrifuge buckets (suitable for this rotor).

1% Virkon has been proven active against HIV-1, Rabies and parent Rhabdoviridea (VSV).

All disposable pipettes, pipette tips, flasks, tubes and culture dishes that have been in contact with viral media will be transferred to a fresh 1% Virkon solution after use where they will be left to immerse for a least 20 minutes before transferring to a sharps container for incineration (tips only) or 2 (doubled up) autoclave bags for autoclaving (130oC for 30 minutes) and removal as "clinical waste" by specialist contractors, with final disposal by incineration. Protective clothing will be immediately transferred to autoclave bags after use.

Media used will be removed by a vacuum pump in the biological safety cabinet, which will then be used to aspirate 1% Virkon for disinfection. Media from the vacuum pump tank will be autoclaved at 130oC for 60 mins prior to disposal into allocated drains. Any media spills will be cleaned up immediately by use of Virkon powder to absorb the spill followed by wiping the surface with 1% Virkon and 70% Ethanol.

Surgical instruments and micropipette injection tips will be transferred to 1% Virkon immediately after use and packaged appropriately for incineration (sharps container for tips) or in the case of instruments for decontamination by autoclaving in BMSU.

The operating room will be decontaminated using 1% Virkon.
The GMSC felt the assessment was extremely thorough and approved it without adverse comment.

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**Project Ref** 116/05.4

- **Date Ackn'd**: 13/10/2005
- **CU2 Project Title**: Characterisation of antibiotic resistance determinants from bacterial pathogens of humans and animals.
- **Class**: Class 2
- **CultureVolume**: ≤ 1 Litre
- **Non-GMM**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Containment**

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**Project Additional Information**

**Purposes of the contained use**

The researchers wish to submit a series of assessments that form a connected programme of work that includes several pathogens and several activities. The work focuses on the characterisation of proven or potential antibiotic-resistance determinants from human and animal bacterial pathogens that fall into ACDP category 2. These include:

- Salmonella enterica (non-typhoidal serovars)
- Escherichia coli (non-vero-toxin-producing)
- Campylobacter jejuni
- Staphylococcus aureus and coagulase-negative staphylococci
- Bacteroides fragilis
- Mycobacterium bovis BCG
- Streptococcus pyogenes, S. pneumoniae, S. agalactiae and viridans group streptococci (eg S. mitis, S. oralis)
- Pseudomonas aeruginosa

**Recipient or parental organism**

Host organisms: Non-pathogenic laboratory strains of E. coli (eg K12), and pathogenic bacteria as described below.

Enterobacteriaceae (salmonella, E. coli, Campylobacter spp) are capable of causing diarrhoeal disease which is usually self-limiting within a few days, symptoms can include; diarrhoea, vomiting and abdominal cramps. Rarely these species can cause systemic disease and bacteraemia.

Staphylococcus aureus is capable of causing skin and wound infections which can be self limiting or may be prolonged. S. aureus is capable of causing severe systemic infections if it enters the bloodstream. Symptoms of skin infections include rashes, abscess and spot formation.

Bacteroides fragilis is an obligately anaerobic pathogen capable of causing deep wound infections.

Streptococci are capable of causing respiratory tract infections which may be self limiting within a couple of weeks or may become chronic and require antibiotic treatment to resolve them. Symptoms include coughing, shortness of breath and chest pain.

Pseudomonas aeruginosa is a versatile pathogen capable of causing a range of infections, particularly of the respiratory tract. P. aeruginosa can also cause systemic infection. Symptoms of the respiratory infections include coughing, shortness of breath and chest pain.

Mycobacterium bovis can rarely cause tuberculosis, symptoms are prolonged and can include fever, chills, weight loss, abdominal pain, diarrhoea or constipation.

**Host/vector system**

Vectors: Non-mobilisable or mobilisation defective vectors including, or similar to those in Part 2A, Annex II of ACGM Compendium of Guidance.

Non-mobilisable or mobilisation defective vectors that 1) can be propagated in crippled E. coli strains, but not in target species, eg pUC18 replicates in E. coli but not Campylobacter jejuni; 2) show temperature sensitive replication in E. coli, eg pK03 used in E. coli and Salmonella.

Non mobilisable vectors (eg pGEX4T3, the pET and pCAL vectors) that express the protein, either from promoters (eg tac) or from the gene's native promoter in its native form or as part of a his-tagged, calmodulin-binding peptide-tagged or similar fusion to an innocuous protein.

Non-mobilisable or mobilisation defective shuttle vectors which carry origins of replication that work in E. coli and a given other pathogen.
**Origin & function**

Source - Pathogenic bacteria as described above in Host section.
Genetic Material - DNA fragments encoding antibiotic resistance genes.

Source - E. coli, Firefly or luminescent vibrios, jellyfish
Genetic material - Genes that express marker proteins, which are highly unlikely to have any biological effect - Beta galactosidase, luciferase, Green Fluorescent Protein.

**Evaluation of foreseeable effects**

Human Health - Where host strains are non-pathogenic, expression of antibiotic resistance genes in these hosts will not increase pathogenicity. Elsewhere the risks are those associated with the wild type host - the genetic manipulations proposed should not increase the virulence of the resulting GMMs. In some areas of the work the GMMs created will become less susceptible to some antibiotics, although pathogenicity is likely to remain unaltered.

Environment - Each activity has its own environmental risk assessment within the assessment. In all cases the risk to the environment is very low or negligible. Please see assessment for discussion on each activity.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All bacterial cultures and contaminated solutions will be autoclaved. Autoclave runs are monitored. 100% kill rate. Waste from the autoclaving process is taken away from incineration as hazardous waste by approved contractor.

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<tr>
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Please enter comments on the GM safety committee on the risk assessment

Specific comments from one reviewer are attached. These have been addressed within the assessment by the PI. Other concerns raised by the GMSC included the broad nature of the proposal, and how to successfully monitor the work. It is anticipated that this proposal will catch all previously notified work, and that the Principal Investigator will submit any additional activities to the GMSC for approval in future.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022 Page 3347 of 15326
**Project Ref** 116/05.5

**Project Additional Information**

**Purposes of the contained use**

Human endothelial cells of various origins will be immortalised using a dual vector system that is controlled by temperature. Use will be made of a system that has already been optimised and established in other laboratories for this cell type. The vectors are Babe hygro-hTERT, an amphotropic murine leukemia virus containing the human telomerase gene (cell survival gene) and U19tA58LT virus containing the SV40T antigen (immortalising gene construct).

**Recipient or parental organism**

HOST - Primary human endothelial cells from a variety of sources. Commercially available from donors screened for infectious agents and unrelated to operators.

**Host/vector system**

VECTOR - pBabe hygro=mammalian expression, retroviral, resistant to ampicillin, high copy. The pBabe retroviral vector constructs transmit inserted genes at high titres and express them from the Mo MuLV Long Terminal Repeat (LTR). pBabe- hygro-hTERT was constructed by inserting the EcoRI-to-Sall fragment containing the full length hTERT cDNA from pCI-Neo hTERT into the EcoRI-Sall sites of pBabe-hygro.

Packing cell line for babe hygro hTERT is TEFLY-A derived from human rhabdomyosarcoma TE61 cells containing CeB and AF plasmids containing vector core and...
envelope proteins respectively. The U19tsA58LT vector was constructed by using the pZipNeoSV(X)1 backbone and encodes a full length LT cDNA (SV40 T antigen) from U19tsA58 and confers resistance to G418. Expression conditional on temperature as inactive at 37°C. Amphotropic packing cell line is PA317, a mouse fibroblast with herpes TK gene. Both vectors will be obtained from an external source already constructed and therefore no further manipulation will be required. Retrovirus is replication deficient.

Origin & function

OTGIN - Full length hTERT cDNA is the human telomerase gene. Telomerase is an enzyme which replicates the terminal sequence of eukaryotic chromosomes, namely the telomeras. Cells which have an unlimited replicative capacity such as male germ cells and the majority of human cancers have high levels of telomerase activity. SV40 is derived from the Polyomaviruses Simian Vacuolating Virus 40. The SV40 large T antigen is an early protein that interacts with the p53 and Rb tumour suppressor proteins.

Genetic material - U19tsA58L is a combination of 2 mutants, U19 which encodes a LT (Large T antigen) that does not bind specifically to SV40-origin DNA sequences, and tsA58 which encodes a thermolabile LT antigen that is wild type at 33.5°C, but inactive at 37°C.

Evaluation of foreseeable effects

Human Health - Constructs will be obtained pre-made and therefore no manipulations will be required other than infection of host cells. Minimal risk from ingestion as U19tsA58 thermolabile and inactive at 37°C some risk by skin contact where body temperature lower. Human telomerase activity can only be reconstituted by both the essential RNA subunit, hTERC, along with the catalytic protein component coded for by the hTERT gene, which are controlled at the transcriptional level. Retrovirus is replication deficient and so can not form viable progeny. Cells expressing hTERT and SV40 T antigen are only tumourigenic when contain oncogenic Ras (Neoplasia 2002 Nov-Dec;4(6):493-500) so this significantly lowers risk. Self inoculation with a significant dose of retrovirus could result in tumour formation but would probably require a large number of cells to be infected. Retrovirus is unable to infect non-dividing cells so this lowers likelihood of reversion to generate replication competent virus - minimised in FLY packaging cells as the gag-pol is separated from the env on different plasmids and use heterologous promoters rather than viral LTR promoters. Little or no evidence of generation of replication competent retrovirus. PA317 cells are able to generate replication competent virus but if are handled at level 2 this should provide sufficient operator protection. Cells to be transformed will not be derived from the operator or any members of staff affiliated to the laboratory. Therefore if inoculation due to sharp injury were to occur they would be susceptible to a strong allogenic immune response and so be rapidly eliminated.

Environment - Retrovirus is replication deficient and therefore unable to produce viable progeny, additionly retroviruses have a short half-life. The system is thermolabile and inactivated at 37°C. The host cells to be used are primary cultures of human cells which are unable to survive outside of controlled cell culture conditions. Therefore it is considered that release of any of the components will not harm the environment. Negligible risk. If accidental release were to occur it would be in very small quantities and the ramifications would be negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be put into capped pots and autoclaved. Plastic waste, paper waste and pipette tips in Diposafe jars will be autoclaved. Waste is then removed by clinical
waste contractor for incineration.

When the proposal was originally circulated to the GMSC it lacked considerable detail. The attached comments reflect the requests for additional information. The assessment was expanded to address the issues raised and then recirculated to the GMSC. A few more suggestions came in from the committee in response, and the enclosed assessment is the final version.

Please enter comments on the GM safety committee on the risk assessment

When the proposal was originally circulated to the GMSC it lacked considerable detail. The attached comments reflect the requests for additional information. The assessment was expanded to address the issues raised and then recirculated to the GMSC. A few more suggestions came in from the committee in response, and the enclosed assessment is the final version.

Project Containment

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Project Ref 116/06.1

Date Ackn'd 27/07/2006

CU2 Project Title Genetic manipulation of non-pathogenic E.coli K12 and class 2 E.coli O157 sakai strain.

Date Project Ceased

Class 2

Culture Vol Class 2 1-50 Litres

Non-GMM Not Applicable

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Tick if notifying a connected programme of work N
**Project Additional Information**

### Purposes of the contained use

The work investigates the basic molecular biology (e.g. gene sequences, regulation of gene expression etc) and molecular microbiology of E.coli K12 and E.coli O157 (Sakai). ONLY these strains will be used in this project. Aims and objectives include: 1) To generate reproducible expression and metabolic data on acid stress response in E.coli. 2) Construction of mathematical models representing E.coli K12 and E.coli Sakai response to acid. 3) Experimental verification of the models generated. 4) Refinement of the initial computational models.

### Host/vector system

**Host:** Host strains for recombinant DNA work will be standard laboratory-adapted E.coli K-12 and the E.coli O157 Sakai. For expression of e.g. known virulence factors at high levels, highly disabled strains such as recA-hosts will be used, whereby risk of mobilisation of sequences is minimised. For mutagenesis experiments, either E.coli K12 or Sakai will be used and genes of interest replaced by modified alleles which will inactivate the gene in question. Strains of E.coli used will normally be those for which whole-genome DNA sequences are available.

**Vector:** Vectors will generally be pUC, pBluescript and related non-mobilisable ColE1-based standard vectors, or lambda phage-based cloning vectors, or standard low copy number vectors such as pACYC or pSC101 and derivatives. They may include high level expression systems such as in the Novagen pET system in which expression is tightly controlled via T7 RNA polymerase.

### Origin & function

**Source of genetic material:** Sources are either whole genomic DNA or PCR-amplified known sequences, from Escherichia coli K12 and Sakai. In general source DNA will be taken from an organism for which a whole genome sequence is known. No organisms requiring containment level greater than 2 will be used.

**Function of genetic material:** Sequences used may be any gene or sequence including gens of known or unknown function, including virulence factors. Genes may be partial or complete and may include expression signals. Libraries may be constructed for limited purposes e.g. two-hybrid screening. Genes identified as putative factors associated to response to acid stress will be transferred between the two strains. Such experiments would not result in an increased containment level.

For mutagenesis work, sources include widely used and well characterised antibiotic resistance marker cassettes of known sequence, e.g. carrying resistance markers for kanamycin or chloramphenicol which are not used clinically or are or minor clinical significance only. These marker sequences are very widely used for such mutagenesis.
experiments and may be capable of full expression under control of their own or vector or host-derived expression signals. Where practicable, antibiotic resistance markers will be deleted after creation of mutagenised alleles, to leave an unmarked lesion in a strain of wild type antibiotic susceptibility. In general kanamycin resistance genes will be used in preference to chloramphenicol in view of the limited clinical usefulness of the latter antibiotic. In E.coli mutagenesis, the method of Datsenko and Warner (PNAS 97, 6640-6645, 2000) will be the method of choice and after excision leaves no permanent resistance marker.

Evaluation of foreseeable effects

Risks to Human Health: The level 3 containment requirement, and indeed the HSE definition for EHEC strains of E.coli and other bacteria such as Shigella spp expressing the toxin, is determined solely by the presence and expression of the stx genes (stx 1 and/or stx 2) encoding Shiga-like STX or Verocytotoxin VT. The toxin-deleted strains which are the subject of this assessment are thus by definition not EHEC and fall within containment level 2. They are nevertheless potentially capable of causing enteric or other infections or colonising the human body whether as members of the mucosal or enteric microflora. However, the diseases caused are rarely life threatening to those in normal health, are self limiting or readily treatable. E.coli K12 is a non-pathogenic strain which does not pose a safety threat. The parental pathogens concerned in the work generally express virulence factors involved in some or all of the above effects on health. GMMs based on E. coli hosts may acquire a limited number of such characteristics which in theory might enhance their pathogenic properties, but these are unlikely to comprise more than one or a small number of virulence attributes in any one instance and are not therefore likely to create new virulence phenotypes more pathogenic than the parent organism from which the cloned sequences originated (full virulence of pathogens is known generally to be the sum of expression of a number of virulence related genes).

Site-directed mutagenesis of parental pathogenic organisms by allelic replacement with disabled genes, based on engineered insertional inactivation with or without simultaneous partial or complete deletion of the wild type gene will utilise antibiotic resistance markers of no or limited clinical value such as kanamycin or chloramphenicol. Such constructs will be less virulant than wild type due to deletion of known or probably virulence determinants, and hence will be of reduced impact as pathogens and not likely to pose clinical problems in the unlikely event of an accidental infection of humans.

Environmental risk: The GMMs cannot be considered likely to express virulence or other factors that would be potentially harmful to the environment. No harmful effects are foreseen.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Inactivation will be by autoclaving of all wastes (including any small quantities of disinfectant - treated residues e.g. paper towels used for wiping up small spills) contaminated by GMMs, at a minimum of 121°C for 15 mins, validated by monitoring of internal chamber temperature and automatic printout of every autoclave run. Validation will be carried out using Bacillus subtilis spore strips and autoclave tape used on every sample being autoclaved. This method is widely acknowledged to guarantee complete killing of all known bacteria. Wastes from autoclaving will be disposed of to the drains (liquids) or to clinical waste incineration.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form 

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N
The original assessment was broader than the attached version and was felt to be too broad by the GMSC. The investigators decided to use only K12 and sakai strains of E.coli for this work as a result of the GMSC's comments:

* The objectives of this project need to be more clearly spelt out, and the host organisms need to be defined. The committee feels the organisms in use should be specified, rather than suggesting that "level 2 organisms may be the host".

There was also a concern at the number of facilities listed for use in the project and the GMSC needed reassurance that the project was going to be well managed and closely supervised.

**Project Containment**

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**Project Ref 116/06.2**

**CU2 Project Title**
Molecular studies of the genes and gene products of Kaposi's sarcoma-associated herpesvirus (KSHV), the virus also known as human herpesvirus (HHV)-8.

**Class**
Class 2

**CultureVol**
1-50 Litres

**Non-GMM Consent Granted**
Not Applicable

**Project notified under transitional arrangements**
No
**Project Additional Information**

**Purposes of the contained use**

To understand the molecular mechanisms governing KSHV gene expression and the processes by which KSHV gene products regulate host cell functions, such as cell cycle and immunological activities.

**Recipient or parental organism**

Three types of KSHV strain will be studied: (i) Wild type (WT) virus produced from naturally infected primary effusion lymphoma cells or other natural sources. (ii) Virus specifying either green fluorescent protein (strain rKSHV.152) or enhanced-GFP (E-GFP) and Ds red fluorescent protein (strain rKSHV.219) or other innocuous reporter and antibiotic resistance genes. (iii) Virus derived by bacterial artificial chromosome technology, either wild type (strain BAC36) or deleted for specific KSHV open reading frames to assess their contribution to KSHV pathogenesis. Additionally, recombinant replication-defective adenovirus and retrovirus constructs expressing KSHV genes will be used to investigate the function of specific genes and plasmids carrying KSHV genes will be grown in E.coli and transfected into mammalian cells in tissue culture.

**Host/vector system**

Disabled E.coli for plasmid construction. Eukaryotic cells such as 293 and other cell types, human and non-human, to generate infectious particles of WT and genetically modified KSHV and to study infection processes. Primary effusion lymphoma (PEL) cell lines for production of KSHV. Target cells: human cell lines, primary cells of endothelial, epithelial and lymphoid origin (liver, gut, stomach, thymus, B-and T-lymphocytes, monocytes). E1-deleted, replication-defective adenovirus vectors will be produced in cells such as 293 cells that complement the E1-deficiency of the vector; transgenes will be inserted at the site of E1 deletion of the vector. For generation of retrovirus vectors, packaging cell lines in which the gag-pol and env genes are separated will be used, to minimise the possibility of recombination leading to replication-competent virus.

**Origin & function**

Genetic material will be derived from KSHV.
Recombinant vectors will be used for projects including:
(i) Generating restriction fragments for use as DNA & RNA probes.
(ii) Studies of KSHV promoter regulation.
(iii) Functional studies of KSHV proteins purified from bacterial cells.
(iv) Transient and stable expression of wild type and mutant KSHV proteins in eukaryotic cells in studies of their function.
(v) Functional studies of KSHV proteins purified from stable eukaryotic cells.
(vi) Recombinant adenovirus vector expression of wild type and mutant KSHV proteins in eukaryotic cells in studies of their function.
(vii) Recombinant retrovirus vector expression of wild type and mutant KSHV proteins in eukaryotic cells in studies of their function.
(viii) Recombinant KSHV virion production and purification for infection studies.
(ix) Recombinant KSHV virion production and purification for functional studies of virus and virus-infected cells.

**Evaluation of foreseeable effects**

KSHV is a human gammaherpesvirus. The incidence of KSHV is 3.5% in the general population of the U.S. and the United Kingdom. However, in those geographical areas where Kaposi’s sarcoma (KS) is endemic, the incidence is much higher, e.g. - 11.5% in western Sicily and 29% in south Africa. KSHV cannot survive outside the only known host, human beings; transmission between individuals requires direct contact and probably occurs via saliva. Overwhelming serological and molecular data implicate KSHV in the aetiology of KS, but infection with the virus alone is not sufficient for the development of KS, and additional factors are required, including immune suppression and perhaps host genotype.

Current opinion in the KSHV field is that the virus is either not transforming, or is poorly transforming. In this regard, Kaposi’s sarcoma, the tumour with which KSHV is most confidently identified as the aetiologic agent, is also not considered a true monoclonal neoplasm, but rather a hyperplasia in which KSHV and environmental cofactors such as inflammatory cytokines, promote cell proliferation. Late stage KS tumours may eventually develop into true sarcomas, since there is then often some evidence of
Several published reports claim that experimental KSHV infection of endothelial cells results in extended lifespan allowing their prolonged passage in vitro. In vivo, KSHV replication in tumour cells is in predominantly latent, suggesting that the proteins encoded by the latency genes may be involved in pathogenesis. The present studies encompass these KSHV genes and the proteins they encode. Present information on these transforming proteins can be summarised as follows:

LANA: this protein is expressed in KSHV-associated tumours. It functions to maintain the KSHV episome and might also serve as a transcription activator. LANA interacts with p45 and represses its transcriptional activity and can modulate the E2F-pRB pathway. It may have cell-transforming activity.

v-cyc is expressed in Kaposi's sarcoma lesions and in body cavity lymphomas. It activates the G1/S and G2/M cell cycle transitions by interacting with the cellular cyclin-dependent kinase 6 (cdk6) resulting in inactivation of Rb by phosphorylation. As a result, the E2F transactivator is released from Rb. V-cyc is resistant to the cell cycle inhibitors p16, p21 and p27. This facilitates transition from G1 to S and G2 to M. Transfection of v-cyc in quiescent fibroblasts leads to G1/S transition.

K1: has transforming functions in conventional rodent transformation assays. It recruits the Syk, vav, ans PI3 kinases to the cell membrane. Expression in vivo has not been demonstrated so far. Modulates B cell receptor expression.

Kaposin: stabilises certain cytokine transcripts.

V-GCR: encodes a chemokine receptor homolog. It includes the expression of the angiogenic factor VEGF and can transform cells.

V-IRF-1: inhibits interferon-induced signaling and transforms rat fibroblasts. No expression of this protein in the majority of KSHV-associated tumours in vivo.

The rKSHV.152 and rKSHV.219 strains replicate to similar, low levels as wild type virus and are not expected to present any greater hazard. The genes encoded by the BAC 36 are not expressed in the bacterial host. Hence, there is no potential to increase the pathogenicity of the host. Ther BAC 36-derived virus mutants are expected to have a decreased ability to infect and replicate in target cells compared to wild type KSHV. Mutated recombinant KSHV strains are expected to be less harmful than their wild type counterparts. The reporter genes are not expected to affect KSHV pathogenesis.

None of the KSHV genes, or the proteins they encode, presents a higher risk to workers particularly given that KSHV is, in the worst case scenario, only poorly transforming (see above). Nevertheless, cell transformation has been observed in studies with certain individual KSHV proteins, e.g. - K1, vGCR and vIFR-1 and level 2 containment should be used to minimise the chance of accidental contamination of workers with adenovirus or retrovirus constructs expressing KSHV genes. Should a worker be exposed to one of the proteins, an immune response would be anticipated, but is unlikely to confound diagnostic assays, since KSHV infection is not screened for routinely. Retroviral vectors allow efficient gene transfer, resulting in chromosomal integration, in the target cells. Infection is dependent on proliferation of the target cells, and has somw associated risk of insertional mutagenesis. Note that such insertional mutagenesis has resulted in oncogenic activation and leukemia in some patients in a gene therapy trial for X-SCID, however this was in the context of deliberate administration of very high doses of virus expressing the cytokine receptor gamma chain to haematopoietic stem cells. The lack of such adverse effects in most other gene therapy trials using retroviruses indicates that the chromosomal insertion of replication-defective retroviruses is generally unlikely to cause overt harm. Any accidental inoculation would result in many orders of magnitude fewer insertional events than in gene therapy clinical trials, and the level of risk is therefore much lower. Also, due to the (very slight) possibility of replication competent retrovirus, non-producer cells that have been infected with the virus should continue to be handled under conditions that prevent human contamination, at least until it has been proven that they are not producing (replication-competent) virus.

Expression of KSHV genes in mammalian cells is not expected to confer additional biological hazards. With the exception of ORF K9, encoding vIRF and ORF 74, encoding vGCR encoding a G protein coupled receptor (GPCR), none of the KSHV genes are active in a conventional rodent cell transformation assays. Expression of vGCR protein can purportedly lead to transformation of primary endothelial cells, but the risks of such cells becoming accidently inoculated into a worker is low, as is the risk of malignant transformation occurring in such individuals. None of the primary cell or cell lines used in these studies will be derived from the worker. Therefore, regardless of either the presence or absence of an expressed KSHV protein, their inoculation into the worker will induce a strong allogenic immune response, which will protect the individual. Therefore, handling of cells expressing either individual or few KSHV proteins poses no greater threat than that of culturing tumour-derived cell lines. However, this work is also classified as level 2 since in some experiments there will be a theoretical possibility that the transfected constructs could recombine with KSHV in the cell to generate GM KSHV, the potential hazards of which are discussed above.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation. Full CL2 applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Virkon solution is validated for disinfection of most herpes viruses, including Herpes Simplex virus (HSV) and Cytomegalovirus (CMV). KSHV titres are several orders of magnitude lower than HSV titres, so that complete inactivation of this virus is expected.

All liquid waste is treated by autoclaving at 130°C for 60 mins, before disposal to drains.

Disposable solid waste which or may be contaminated with GMMs is also inactivated by autoclaving at 130°C for 30 mins, before removal as "clinical waste" by contractors, with final disposal by incineration.

The exceptions to autoclaving are:

"Sharps", including scalpel blades, needles (with or without attached syringes), and disposable plastic tips for micropipettes, may be decontaminated by drawing up Virkon disinfectant, and soaking in Virkon for a minimum of 10 minutes.

Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above.

Expected degree of kill:

Autoclaving achieves effectively 100% kill of all GMMs.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The main issue raised by GMSC members concerned the potential transforming ability of KSHV. One reviewer noted that Fields Virology spoke of "KSHV-induced cell transformation", and stated that "direct infection and transformation of primary bone marrow endothelial cells has also been reported". The assessment clearly identifies the association with Kaposi's sarcoma, but emphasises the current opinion of experts in the field that the virus is, at worst weakly transforming. Nevertheless, a precautionary approach has been accepted that the virus, or certain genes from it, may be able to contribute to malignancy. The risk assessments have been amended to the satisfaction of the GMSC, and the work has been given approval.

Project Containment

Laboratory Activities

Glass Houses

Growth Rooms

02/03/2022
**Project Ref**  116/06.3

**Date Ackn'd**  04/01/2007

**Date Project Ceased**

**CU2 Project Title**  Analysis of virulence regulation in Streptococcus agalactiae

**Class**  Class 2  
**Culture Vol Class**  2

**Culture Volume**  < 1 Litre

**Non-GMM**  Not Applicable

**Consent Granted**

**Project notified under transitional arrangements**  N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

Mutants will be created in the human pathogens, *S. agalactiae*, and these will be assessed in a variety of phenotypic assays to identify those mutants that have reduced virulence. The genetic basis of the reduced virulence will be determined and this information used in downstream research to develop novel inhibitors and create attenuated vaccine strains.

**Recipient or parental organism**

Host- Disabled laboratory strains of *E. coli*, w/t *Streptococcus agalactiae*

**Host/vector system**

Vectors - *E. coli*-S. agalactiae shuttle vectors: Suicide vectors/pWV01 derivatives for introducing gene deletion constructs into the chromosome of *S agalactiae*. 
pJRS233, pVE6007, pAT28, pHY304, pG+host5, pCAM45

**Origin & function**
Donor- Strepococcus agalactiae

Genetic material - The inserts are kanamycin [Kan R], chloramphenicol [CmR], erythromycin [EmR] and spectinamycin [SpcR] resistance genes:


S.agalactiae infections are prevented by the administration of Intrapartum Antibiotic Prophylaxis (IAP) - penicillin, or clindamycin in penicillin allergic people. No strain has ever been reported to be resistant to penicillin, however strains are commonly found to be penicillin tolerant. This finding has not changed the UK or US recommendations to use penicillin alone for prophylaxis.

S.agalactiae infections are treated with either penicillin or a third generation cephalosporin, such as cefotaxime. Some wild-type strains are resistant to erythromycin and kanamycin through the acquisition of wild-type transposons, such as Tn916 and Tn917. Thus, these antibiotics are not routinely used or recommended for treatment. Chloramphenicol is not routinely used for the treatment of bacterial infections in babies because of adverse side effects. Infection recurrence has been reported in some babies infected with penicillin tolerant S.agalactiae. In such instances, a combination of penicillin and gentamicin are used for treatment. We therefore will not create penicillin or gentamicin resistant GMO strains.

Human Health risks: Wild-type S. agalactiae is highly adapted to colonising mammalian hosts. 10-30% of people at any one time are colonised with wild-type S.agalactiae. Invasive disease by the wild-type organism in healthy immune competent adults and children is a rare event. Where disease does arise, it predominantly occurs in newborn babies (~1:2,000 newborn babies have proven infection in the UK). In the majority of pregnant mothers vaginal colonisation is a benign event, however in a small minority vaginal colonisation is followed by bacterial invasion of the amniotic cavium and the fetus becomes secondarily infected initially with pneumonia, and secondarily with septicaemia. Laboratory staff who work with S.agalactiae will be educated/counseled about colonisation in pregnancy, and will be in an informed position to arrange Screening and IAP through their Midwife/Obstetrician.

Genetically modified S.agalactiae strains, with disruptions of genes known to encode virulence determinants or virulence regulators, are likely to be less fit at colonising and causing disease in man. For instance, wild-type S.agalactiae expresses a polysaccharide capsule containing sialic acid, surface proteins involved in adhesion to host cells, surface immune evasion molecules such as IgA protease, and it secretes toxins known to injure host cells. The expression of these proteins and structures is regulated by a network of interacting regulators. We aim to disrupt the regulators that we predict to be involved in expression of these virulence determinants. The actual genes we will disrupt have been chosen because homologues in the closely-related pathogen Streptococcus pyogenes are known to enhance the expression of virulence determinants, and regulatory mutants of this bacterial species have reduced virulence. Thus, we would expect that the mutants that we intend to create will have a reduced capacity to cause disease. The main risk to laboratory staff would therefore come from handling the wild-type strain. There is a tiny theoretical risk that a GMO could be created that has an enhanced ability to cause human disease. However, we will not create GMO strains that are resistant to the antibiotics commonly used for treatment. It is extremely unlikely that mutations of the virulence regulators would create a Class 3 GMO. Such mutations must arise naturally in the wild-type organism, and yet we have not seen the natural emergence of a category 3 strain.

See additional sheet for Environmental risk section.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Inactivation will be by autoclaving of all wastes (including any small quantities of disinfectant - treated residues e.g. paper towels used for wiping up small spills) contamination by GMOs, at a minimum of 121°C for 15 mins, validated by monitoring of internal chamber temperature and automatic printout of every autoclave run. This method is widely acknowledged to guarantee complete killing of all known bacteria. Wastes from autoclaving will be disposed of to the drains (liquids) or to clinical waste incineration.

**Kill = 100%**

Comments from GMSC included:

No large spills procedure defined or detailed.

Should pregnant women avoid doing these experiments? If so it needs to be stated.

Is it safe for people who are allergic to penicillin to be exposed to these bugs? (it leaves only 1 possible type of antibiotic for treatment, according to this?).

More detail requested throughout, e.g. what is the likelihood/consequences/symptoms of a virulent infection and what are the modifications being made to the virulence genes.

If a more virulent GMM were generated, what additional procedures would be in place to contain it - would it be a cat 3 organism?

All of these queries have been addressed and the assessment has been approved by the GMSC.

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Project Ref 116/07.1
The study of signalling pathways in platelets has provided important information on the events that underlie their role in haemostasis and thrombosis. Megakaryocytes can be used to analyse signalling pathways in platelets but such studies are limited by the inability to transfected megakaryocytes with exogenous DNA constructs. It is proposed that the lentiviral system is used to drive infection as an alternative to transfection. This approach will be used to express mutant proteins of interest & knock down the function of specific gene products using RNA interference.

Recipient or parental organism

Host - Virus to be produced in human 293T cell line; Mouse primary megakaryocytes and the immortalised mouse megakaryocyte cell line HPC-7 (non-permissive for the production of replication deficient lentiviruses)

Host/vector system

Vectors - pWP1; pLVTHM; pMDLg/pRRE-rev; pMD2.g; pCMV-GIN-ZEO third generation lentiviral vector. Details given in assessment.

Origin & function

Source - Aequora victoria (jellyfish); Mouse

Genetic material - Green Fluorescent Protein; Mouse forms of I) wild type and mutant forms of immunoglobulin, lectin and tetraspanin transmembrane proteins and named signalling proteins; ii) short hairpin oligonucleotides against membrane receptors and signalling proteins named under (I) The Ig receptors are PECAM-1, G6b-B, G6F and CPV1; the lectin receptor is CLEC-2; the tetraspanins are CD9, CD63, CD151, TSSC6. The signalling proteins are Grb2, SHP-1, SHP-2; OPAL1, HS-1, cortacin, Arp2, Scar-1, Scar-2.

Future genes of interest, not named here, but with that function in similar signalling pathways, will be added to a list at the end of this assessment for periodical review by the local GMSC.
A) Risk of regenerating replication competent virus or the parental virus (HIV). In the third generation lentiviral vectors all six regulatory/accessory proteins (Rev, Tat, Vif, Vpu and Nef) are removed, except Rev. Rev acts at the post-transcriptional level is necessary for HIV gag/pol expression. Rev binds to a RNA motif (Rev responsive element [RRE] and facilitates the cytoplasmic export of gag/pol messenger. As an extra safety measurement Rev is placed on a separate vector, plus RRE and the packaging signal. The packaging construct contains the minimal RRE of 374bp and the gag/pol genes. Through deletions in both LTR's and the absence of 5 of the 6 accessory proteins, including the replication essential Tat, it is highly unlikely that replication competent virus is produced. In published studies and from Professor Alastair Strain's (Birmingham Biosciences) experience with these vectors, replication competent virus has never been detected. The HIV-envelope protein is replaced by non-retroviral envelope protein (VSV-G). It is highly unlikely that this will be incorporated in a new hybrid replicative virus. It is impossible that wild-type HIV will be formed, because of the omission of 5 accessory proteins and HIV-env.

The lentiviral vectors to be used in this study are third generation lentiviral vectors kindly provided by Dr Didier Trono or purchased from Open Biosystems.

Evaluation of foreseeable effects

A) Risk of regenerating replication competent virus or the parental virus (HIV). In the third generation lentiviral vectors all six regulatory/accessory proteins (Rev, Tat, Vif, Vpu and Nef) are removed, except Rev. Rev acts at the post-transcriptional level is necessary for HIV gag/pol expression. Rev binds to a RNA motif (Rev responsive element [RRE] and facilitates the cytoplasmic export of gag/pol messenger. As an extra safety measurement Rev is placed on a separate vector, plus RRE and the packaging signal. The packaging construct contains the minimal RRE of 374bp and the gag/pol genes. Through deletions in both LTR's and the absence of 5 of the 6 accessory proteins, including the replication essential Tat, it is highly unlikely that replication competent virus is produced. In published studies and from Professor Alastair Strain's (Birmingham Biosciences) experience with these vectors, replication competent virus has never been detected. The HIV-envelope protein is replaced by non-retroviral envelope protein (VSV-G). It is highly unlikely that this will be incorporated in a new hybrid replicative virus. It is impossible that wild-type HIV will be formed, because of the omission of 5 accessory proteins and HIV-env.

The lentiviral vectors to be used in this study are third generation lentiviral vectors kindly provided by Dr Didier Trono or purchased form Open Biosystems.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - full containment level 2 applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is treated by autoclaving at 135°C for 15 min, before disposal to drains. This procedure has been approved at the University of Birmingham for Professor Alistair Strain's laboratory previously.

Disposal solid waste, including sharps in the appropriate biohazard sharps bins, which is or may be contaminated with GMMs, is also inactivated by autoclaving at 135°C for 15 min, before removal as "clinical waste" by specialist contractors, with final disposal by incineration. The autoclave is also tested with Brown's tubes on a regular basis.

Expected degree of Kill:

Autoclaving achieves effectively 100% kill of all GMMs

The manufactures of Virkon (Antec International Ltd) have commissioned studies on the virucidal effect of Virkon on Human Immunodeficiency virus and found that a 0.5% solution of Virkon inactivated 106 infectious doses (ID50) of HIV-1 per ml within 10 minutes at room temperature. Since we are only using stocks of up to 105, Virkon should be effective.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
# Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
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<td>L2 L3 L4 L2 L3 L4</td>
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- **Laboratory Activities**
  - L2: Yes
  - L3
  - L4

- **Glass Houses**
  - L2
  - L3
  - L4

- **Growth Rooms**
  - L2
  - L3
  - L4

**Animal Units**

- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

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**Project Ref 116/07.2**

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<th>CultureVolumeClass3-4</th>
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<td>15/05/2007</td>
<td>Structural and functional analysis of Proline Rich Homeodomain.</td>
<td>Class 2</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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</tbody>
</table>

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**

**Purposes of the contained use**

The Proline Rich Homeodomain (PRH) protein is also known as Hex, plays a variety of roles in the development and differentiation of mammalian cells, including haematopoieses. It is a transcription factor that can regulate translation and cell proliferation. Loss of PRH activities or expression is associated with myeloid leukaemias suggesting that PRH is a tumour suppressor protein in myeloid lineages. The work focuses on understanding the mechanism of action of PRH as a transcription factor.
Hosts - Bacterial work: E. coli K-12, JM101, XL1 and E. coli BL21. Yeast work: S. cerevisiae, strains MAV203; Mammalian cell lines for transient transfection: Human K562 blasts, HL60, avian human Jurkat cells, human HeLa avian BM2 cells, Simian Cos-7 cells, Murine HPC-7 and BM-HPC-5 haematopoietic stem cell lines, HUVEC cells - Primary human cell line Adenoviral infection of Cos-7 cells and human and mouse haematopoietic cell lines with Ad-PRH.

Host/vector system

Vectors - Bacterial expression vectors: pKK233-3, pGex, pTrcHis Yeast expression vectors pACT-1, pAS2-1, pGAD, pGB Mammalian expression vectors, pMUG1, pcDNA3, pCMV, pEGFPc1

Origin & function

Source of genetic material - PRH/Hex (human, mouse or chicken) cDNA cDNAs corresponding to PRH-interacting proteins including TLE corepressor protein, Casein kinase 2, POG 1 (Proliferation of Germ cell 1) HC8 (proteasome subunit), ZnF127 (zinc finger protein), p32

Function - PRH is a homeodomain protein that is involved in the regulation of transcription, translation and cell proliferation. The insert is either the cDNA for the full length protein, or the coding sequence corresponding to subdomains of the PRH protein, or the coding sequence for mutant PRH proteins. Additionally proteins that interact with PRH (see above) are used as inserts for expression in bacterial, yeast and mammalian cells. The cDNAs for these proteins derive from a two hybrid screen for PRH interacting proteins.

Evaluation of foreseeable effects

Human health risk - Bacteria and Yeast - Disabled strains of E. coli and non-pathogenic yeast expressing PRH are unlikely to be a hazard because they are unlikely to colonise humans. Some homeodomain proteins are known to be capable of intracellular transfer. It is not known whether PRH has this property but to prevent this outcome purified recombinant PRH proteins are always handled whilst using gloves. Even if PRH protein were to enter human cells it is unlikely that this would be sufficient to cause cancer. PRH proteins have only been shown to have an oncogenic effect in one study when they were retrovirally expressed, stably and at high levels. However a number of studies have also shown that high level expression of PRH leads to haematopoietic cell death and would therefore not lead to leukaemia. Transient transfection studies in cell lines - As mentioned above these studies are unlikely to cause cancer because PRH protein in these cells is not expressed stably. Transfected cells also present a low risk, since (if people are not working with their own cells), colonisation of the operator would probably be prevented by an allogenic immune response. Adenoviral-PRH infection of cell lines - The adenovirus can infect many human cells efficiently and it is possible that Ad-PRH that is inhaled or taken into the bloodstream or epidermal tissues by needles or injury could infect cells. I would expect the encoded protein to be expressed locally at the site of delivery, which may have effects on cell function/growth control. Effects will depend on cell type, and infection would be transient; however in view of the biological activities of the protein, particularly the risk of oncogenicity, this probably does warrant level 2 containment during work with the virus. Environmental Risks - The bacterial strains used are disabled and cannot exist for long in the external environment. Similarly the adenovirus is replication defective and can only survive with the specific host cell. Therefore, neither the bacteria nor the virus pose a threat to the environment and so environmental hazard is negligible. The following are PRH partner proteins as identified by a yeast two hybrid screen. They are not expected to result in any significant human harm when expressed in bacteria, yeast or transiently transfected into mammalian cell lines. Casein kinase 2 beta subunit is the regulatory subunit of CK2 and a PRH interacting partner protein. CK2 overexpression can be oncogenic in mammalian cells but over-expression of the regulatory subunit in isolation is as far as I am aware not known to have this effect. Bacterial, yeast or transient transfection studies in mammalian studies with the regulatory CK2 subunit are unlikely to have deleterious effects on human health. POG1 (Proliferation of Germ cell 1), FanCD2. The loss of this protein is related to loss of proliferation by germ cells. Also mutation of this ubiquitin ligase contributes to Fanconi's anaemia. HC8 (proteasome subunit) is a component of the 20S proteasome subunit and interacts directly with PRH in cells and in vitro. The function of this interaction is not known.
ZnF127/Makorin 3 Imprinted/Ring Finger protein associated with Prader-Willi syndrome.
P32 multifunctional protein, transcriptional corepressor protein and repressor of splicing.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| No derogation - full CL2 applied |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All tissue culture plastics (135°C, 15 min) and liquid waste (121°C, 20 min) generated during the production of adenoviral supernatants will be autoclaved before disposal. Autoclaving achieves effectively 100% kill of akk GMMs. Small volumes of liquid containing the viral particles will be used immediately. Pipette tips will also be treated with 1% Virkon then autoclaved before removed as clinical waste and incinerated. 1% Virkon is effective against adenoviruses according to the manufacturers information.

*Expected degree of kill:*
The manufacturers' information indicates efficacy of Virkon against a variety of adenoviruses at 1% concentration, and also against a number of strains of E. coli at dilutions ranging from 1% to 0.125%. It has been demonstrated that 15 minutes exposure to Virkon achieves >4log10 kill for adenovirus-infected cell pellets, and >6log kill of adenovirus seed stocks.

| Is an emergency plan required according to regulation 20? Y |
| If yes, tick to confirm that it is attached to this form N |
| Tick to confirm that you have attached a risk assessment to this form Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment N |

**Please enter comments on the GM safety committee on the risk assessment**

The section on human health effects was inadequately completed when the assessment was first submitted. The assessment was returned and additional information requested. In some circumstances PRH appears to function as an oncogene, and therefore the possibility that work could cause cancer was an issue requiring further elaboration.

Other proteins were mentioned in the original assessment and information on the activities of these was also requested (included on the additional sheet with this CU2 form).

The revised version of the assessment was submitted and approved without further comment by the GMSC.

**Project Containment**

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<thead>
<tr>
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02/03/2022

Page 3364 of 15326
Project Additional Information

Purposes of the contained use

1) Stable introduction of constructs to express short hairpin (sh) RNAs in cells, to target a variety of cellular or viral RNAs for degradation by RNA interference.

2) Stable introduction of constructs into cells to express genes of interest.

Recipient or parental organism

Human or other mammalian cell lines, including tumopur cell lines; primary human or rodent cells.

Host/vector system

Retrovirus vectors will be deleted for gag, pol and env genes, and produced in "split" packaging systems (e.g. FLY-A13 or FLY-RD18; Pheonix), in which the gag-pol and env genes are expressed from separate constructs, reducing the risk for regeneration of replication-competent recombinants to an extremely low level. Some vectors are also "self-inactivating" (SIN), with a deletion within the 3' U3 region which allows the vector to go through only a single round of infection after packaging. The envelope proteins used will allow infection of human cells, e.g. murine amphptropic, feline RD114, VSV-G.
Lentivirus vectors will be at least 3rd generation as described in the HSE/SACGM Guidance, i.e. the vectors being deleted for all viral genes, and separated onto at least 2 or 3 plasmids. The HIV envelope protein will not be used, all currently planned work will use the VSV-G envelope glycoprotein. Alternative systems may be used, such as the Invitrogen or Trono lab systems. All lentivirus vectors used will be self inactivating (SIN).

Origin & function

Genes of initial interest include human telomerase, T-cell receptors, and EBV latent genes, and this list will increase.

Evaluation of foreseeable effects

The effects of downregulating expression of a targeted gene will depend upon the particular gene, and could range from no discernable effect to marked phenotypic changes. Similarly, the effects of expressing a transferred gene could range from negligible to profound. In both situations, we consider the "worst case scenario" would be to bring about changes that could contribute to transforming a normal cell into a malignant cancer cell. For example, either shRNA-mediated downregulation of a tumour suppressor gene, or expression of either an oncogene or a gene associated with cellular immortalisation such as telomerase, could have such an effect. Although conversion of a cell from a normal to a cancerous state is believed to depend upon the accumulation of multiple changes affecting growth control and cellular checkpoints, making it unlikely that accidental worker inoculation and infection of a modest number of the cells with the viruses would lead to cancer, the seriousness of this potential consequence warrants the control measures afforded by level 2 containment to minimise the potential for accidents.

Since retroviruses and lentivirus vectors insert their genomes into the chromosomal DNA of infected cells, there is an associated risk of insertional mutagenesis. There have been examples of leukaemias apparently resulting from insertional activation of an oncogene in human gene therapy clinical trials; however these have only arisen in a minority of patients receiving such vectors, and involving doses many orders of magnitude greater than would occur in any foreseeable accident. Also, liver tumours have been reported following systematic injection of certain lentivirus vectors in foetal or neonatal mice. It is unclear whether these were caused as a result of insertional mutagenesis/oncogene activation, or whether the WPRE present in some of the vectors may have contributed an oncogenic protein fragment. Although standard retrovirus vectors can only affect replicating cells, lentiviruses can also infect non-dividing cells, and thus would be expected to result in a greater level of cell transduction following accidental worker exposure. Use of certain envelope proteins, such a VSV-G to pseudotype the viruses, can also increase the stability of the viruses to some extent, and is done precisely because it can result in more efficient infection of human cells.

Although this in principle increases the risk to workers, in practice it may be balanced by the fact that lower levels of virus are needed for the experiments that would otherwise be the case. Thus, although it must be recognised that accidental worker inoculation, either to mucosa or via percutaneous exposure could lead to stable gene transfer to cells in the body on balance it appears unlikely that a foreseeable accident could result in a level of worker exposure that would likely to result in tumourigenesis. Use of level 2 containment is considered appropriate to control the risks.

The possibility of generating replication-competent retroviruses or lentiviruses should also be considered. Replication competent retroviruses in their natural host species are associated with tumourigenesis, via insertional activation of oncogenes. The split packaging cells to be used for retrovirus generation and "3rd generation" systems for lentivirus production make it very unlikely that replication-competent viruses could be produced, and such systems have a history of safe use. Use of SIN vectors adds a further level of safety, since this effectively limits the vector to a single infectious cycle.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid and liquid waste normally inactivated by autoclaving. Surface decontamination using 1% Virkon.
Autoclaving gives 100% kill.

The GMSC agreed with the classification of the work. Some comments were made, e.g. further information requested on the characteristics of the vectors. This information was added and the proposals have been approved by the committee.

Please enter comments on the GM safety committee on the risk assessment

The GMSC agreed with the classification of the work. Some comments were made, e.g. further information requested on the characteristics of the vectors. This information was added and the proposals have been approved by the committee.

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<th>Animal Units</th>
<th>Large Scale Activities</th>
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### Project Ref 116/08.1

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<tbody>
<tr>
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</table>
**Purposes of the contained use**

The principle aim of our work is to identify novel genes that are involved in blood vessel formation. Such genes have great potential as targets with which to either enhance or disrupt vessel formation. As an example, the disruption of new vessel formation has led to the discovery of new and more effective cancer drugs such as Avastin. The endothelium is a rich source of novel genes and aim is to provide new targets to enable the development of better treatment.

**Recipient or parental organism**

The hosts are cells in culture. The primary cells are either human or murine endothelial cells. After infection they will be studied up to week in a variety of different assays pertinent to new vessel formation in vivo. These include proliferation, migration and tube forming assays.

**Host/vector system**

The vectors are genetically modified viruses (adenovirus and lentivirus) that contain the exogenous gene of interest. The virus is replication incompetent.

**Origin & function**

The gene material will either be cloned out of endothelial cells or obtained from a gene bank such as an image clone. The aim of the experiments is to determine the function of the genes by introduction via virus into appropriate reporter systems. Examples of endothelial genes are LYL1, SOX7, SCM8, RhoJ, Robo4, Sphki, KCTD1 5, LRRRC6C, PCDHI2, Cl2orfll, ECSM2, GBP4, IKBKE, MED28. In view of the fact that our aim is to identify novel genes involved in angiogenesis there will be a continuous stream of new ones.

**Evaluation of foreseeable effects**

There are two aspects that should be considered with respects to effects of these experiments on human health and these are the firstly the potential for virus produced to infect the user and the consequence of this and secondly the potential for the virus to replicate after infecting the target cells.

Adenoviral vectors -

Adenovirus is able to infect all human cells and is naturally infectious to the respiratory tract. Cells infected with the genetically modified virus would express high levels of the insert gene/transgene. However this expression would be transient, lasting a few days or in some cases possibly a month and infected cells would be targeted by the individual's immune system, which may already have been primed via exposure to wild type adenovirus found in the environment. The integration of adenoviral DNA into human chromosomes is a very rare event and so it is very unlikely this would occur in an infected individual. We propose to look at the function of a variety of genes that are expressed by endothelial cells and which we hypothesise may play a role in angiogenesis, and in some cases specifically tumour angiogenesis. Some genes express proteins are more well characterised than others, see above and so in some cases it is not possible to anticipate how expression of the gene would affect the biology of an infected cells. Induction of angiogenesis can be harmful to health, for example inducing angiogenesis in the eye may contribute to blindness, and angiogenesis induced in an unvascularised incipient tumour may promote its tumorigenesis. Due the transient nature of transgene expression after infection with the genetically modified adenovirus and the very low probability of integration of the transgene into the host genome, we believe the long term consequences of accidental infection with these genetically modified adenoviruses to be minimal. It is however of the utmost importance to use strict precautionary measures to prevent this from occurring and to stringently decontaminate all areas in contact with the virus to protect all individuals from infection with adenovirus produced.
Adenoviral vectors cont.
The genetically modified adenovirus is replication deficient (it is lacking the El region). Thus infected cells would not be able to produce more virus carrying the transgene. The only way that it could become replication competent would be via recombination with a wild type virus. This may occur if there is concurrent infection of the genetically modified virus with infection with wild type virus or in there is infection of cells which are persistently infected with wild type virus. The latter is rare but has been seen in the adenoids. The genetically modified virus is constructed with the transgene being placed in the El region such that were recombination to occur to produce a replication competent virus then this would necessarily result in the elimination of the transgene and its promoter. The genetically modified virus also has the E3 region removed which contains genes which encode proteins involved in immune evasion, thus the genetically modified virus should elicit a stronger immune response against it than its wild type equivalent.

Lentiviral vectors -
Since lentivirus is able to infect all human cells the virus produced would potentially be able to infect the user if inhaled, swallowed or introduced into the blood stream. This could result in the integration of transgenes described above into the genome and could result in lifelong existence with in the genome and expression of this transgenic DNA. While we cannot be certain what would result from this, given the role of many of our genes of interest in cell signalling and angiogenesis they may promote angiogenesis in situations that may be harmful to human health. For example inducing angiogenesis in the eye may contribute to blindness, and angiogenesis induced in a unvascularised incipient tumour may promote its tumorigenesis. It is theoretically possible that insertion of the lentiviral DNA close to an oncogene may result in its constitutive activation leading to a transformation event. The use of self-inactivating (SIN) lentivirus vectors appears to reduce this risk relative to that of using the MoMuLV-type retroviruses. It is therefore of the utmost importance to use stringent precautionary measures to prevent this from occurring and to stringently decontaminate all areas in contact with the virus to protect all individuals from infection with lentivirus produced.

A number of strategies have been used by the third generation lentiviral vector system to severely minimise the chance that infected cells could themselves produce infective particles. These safeguards include the removal of 5 out of the six ieyulaLulu'ajuuesuly piciteirs rev, i at, vir, vpr, vpu anu (NeT), only rnv remains as ins is requirea ror me expression gag/pol and as a safety measure, it is encoded on a separate plasmid from both gag/pol and the transgene. Only 700 bp of the HIV envelope protein are present in the transfer vector, plus Rev responsive element [RRE] and the packaging signal. The packaging construct contains the minimal RRE of 374 bp and the gag/pol genes. Through deletions in both LTR's and the absence of 5 of the 6 accessory proteins, including the replication essential Tat it is highly unlikely that replication competent virus is produced. In published studies and from Birmingham Biosciences experience with these vectors replication competent virus has never been detected. The HIV-envelope protein is replaced by non-retroviral envelope protein (VSV-G). It is highly unlikely this will be incorporated in a new hybrid replicative virus. It is impossible that wild-type HIV will be formed, because of the omission of 5 accessory proteins and HIV-env.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

no derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste is treated with Trigene at 1/1 00, then autoclaved and incinerated. Liquid waste is disinfected with Trigene at 1/100 dilution prior to autoclaving. Mouse carcasses will be incinerated. Trigene is effective against adenovirus according to the manufacturers information.

Liquid waste is treated with Trigene at to give a final dilution of 1/100 followed by autoclaving at 135°C for 15 mm, before disposal to drains. Disposable solid waste, including pipette tips which are stored in sealed plastic jars are inactivated by autoclaving at 135°C for 15 mm, before removal as “clinical waste” by specialist contractors, with final disposal by incineration Sharps, including needles will be soaked in trigene at 1/100 prior to disposal in appropriate sharps bins. The autoclave is also tested with Brown s tubes on a regular basis, Expected degree of kill: . Autoclaving achieves effectively 100% kill of all GMMs. Trigene at 1/100 kills 100% human adenovirus type 5 according to the manufacturer’s instructions.
Lentivirus assessment - some comments/suggestions from GMSC

In the box "effects on human health" (p6) it would be worth mentioning that there is the small but finite risk that the lentivirus insertion could result in activation of cellular genes (eg Oncogenes) close to the insertion site although use of SIN lentivirus vectors appears to reduce this risk relative to that of using MoMuLV-type retroviruses. You list a number of genes at least one is an oncogene, others may promote neovascularisation. Will these viruses always be used singly, or is it possible that several could be use for co-infection? Potentially, this could deliver the many different events required for full tumourigenesis in one needlestick injury. Please either exclude this, or draw attention to the potentially greater hazard of such multiple use.

In the box "interim containment level" on P8, I think the statement "we do not consider the GFP, endothelial expressed transgenes or the short oligonucleotides to significantly increase the potential hazard, thus we classify the replication-defective lentivirus vector with this transgene as class 2" to be incorrect. Introducing an oncogene, etc does pose a greater risk than an empty SIN-lentivirus vector. I would say something more along the following lines: although the expression of some of these transgenes (eg Xyz is an oncogene) increases the potential hazard, the risks can still be controlled by use of containment level 2 measures; the additional features of containment level 3 are not required to reduce the risk to an acceptable level.

Is this risk assessment also intended to cover the initial preparation of the plasmids in E.coli? If so, it requires modification to make this clear, although the level of hazard from these is lower, and so containment level 1 is likely to be sufficient for the bacterial stages of the work.

Adenovirus assessment - GMSC comments/suggestions

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<tr>
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Project Ref 116/09.1

Date Ackn'd: 02/03/2022
Using lentiviral vectors to achieve the need for efficient gene transfer in many cell types, including lymphocytes; the need for stable expression of introduced genes, or knock-down of endogenous gene expression via shRNA and inhibitory RNA mechanisms. This has a wide application in studies of the immune response, e.g. Recombinant lentivirus vectors with T cell receptors, B cell receptors, cytokines, MHC class I and II, HDAC, MPO and PR3 proteins to allow stable production of proteins for clinical laboratory analysis and antigen processing and presentation of cell receptors.

Recipient or parental organism

Hosts - Disabled laboratory strains of E. coli (including Stbl3, DB3.1, DH5α,HB101, Top10, and XL10-gold).

293HT cells for lentivirus production.

Target: Human cell lines (e.g. 293T cells, Jurkat T cells and other established human cell lines) and Chinese Hamster Ovary cell line, primary human T and B cell lines. Primary and established human tumour cell lines will also be transduced.

Host/vector system

Lentiviral vectors - pLVX-IRES-Puro vectors (Clontech), pJZ189/pTRIP vector, pH′SIN vector, or other comparable vectors. Replication defective - but are infectious lentivirus vectors, with the potential to infect human cells, e.g. in the event of accidental exposure of the operator. This potential hazard arises in part from the fact that lentivirus vectors insert into the host cell genome, which is potentially mutagenic; and in part from the fact that this could result in long-term expression of the gene inserts.

Although the lentivirus vectors are derived from HIV, the vector systems to be used are all 3rd generation systems which divide the helper functions onto multiple plasmids designed to minimise the possibility of generating a replication-competent virus through recombination, and do not use the HIV envelope protein. Additionally, all the vectors adopt the „self inactivating“ (SIN) design, which restricts the viruses made from the packaging cells to a single cycle of infection.

Origin & function

The following genes are to be included at this stage, but this list may grow, to include other inserts of similar or lower hazard.
 recognised a bound peptide antigen in association with MHC molecules on the surface of all nucleated cells and can signal to initiate an immune response.

**B cell Receptor** – Recognises and binds to antigens to begin the process of mounting a humoral immune response.

**Immunoglobulins** – Recombinant immunoglobulin constructs (e.g., IgG) closely related to B cell receptors.

**Cytokines** – Represent a large group of immunological mediators controlling cellular communication. These are a diverse group of proteins including Interferons, Interleukins, and Chemokines. These have a broad range of biological activity including inducing cell proliferation, activation, differentiation, and cell death. Those of immediate interest include IL1, IL6, IFN gamma, TNF alpha, and related proteins involved in inflammatory processes; IL2, IL4, IL7, IL15 and IL21 (lymphocyte stimulating cytokines); GM-CSF, IL4, IL1 (roles in activating antigen-presenting cells).

**Neutrophil Granule components** – Myeloperoxidase (MPO) and Proteinase 3 (PR3) are enzymes found in phagocytes and a disease biomarker for microscopic polyangiitis.

**Antigen Processing Molecules** – Regulates the processing of antigens by antigen-presenting cells. These include MHC molecules listed below, TAP, Tapasin, ERp57, ERAAp, Proteasome, Cathepsin, Invariant Chain, HLA-DM and HLA-DN

**MHC Class I** – Found on all nucleated cells, presents self or foreign peptides to CD8 T cells. In humans there are 6 genes (HLA-A, HLA-B, HLA-Cw, HLA-E, HLA-G and HLA-F). This family of molecules are extremely polymorphic with a large number (>1,000) of alleles. We will focus on a number of common alleles such has HLA-A*0201, HLA-B*0702 and HLA-A*0101.

**MHC Class II** – Found on professional antigen-presenting cells, presents self or foreign peptides to CD4 T cells. There are 3 genes in this family (HLA-DR, HLA-DP and HLA-DQ) which, like MHC Class-I molecules, are very polymorphic.

**Reporter Molecules:** Fluorescent Proteins reporter genes (e.g., Green Fluorescent Protein, Red Fluorescent Proteins), bioluminescent proteins (Luciferase) and antibiotic resistance genes (Puromycin Resistance, Hygromycin Resistance, Zeocin Resistance) are widely used in basic and clinical research to label, select and characterise cells of interest. None of these genes have been shown to have pathogenic properties.

**HDACs:** (Histone Deacetylase) HDAC1-HDAC11 are a family of transcriptional regulators which are particularly active in controlling immune cells activation and differentiation.

**siRNA and shRNA** – These constructs will be used to reduce the expression of genes listed above, to determine the function of these genes.

In the future, the work may expand to include other leucocyte surface antigens (e.g., CD4, CD8, CD27, etc)

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**Evaluation of foreseeable effects**

The plasmid constructs in E. coli have no significant implications for health & safety.

The major potential hazards are associated with the production of infectious lentivirus vectors, with the potential to infect human cells, e.g. in the event of accidental exposure of the operator. This potential hazard arises in part from the fact that lentivirus vectors insert into the host cell genome, which is potentially mutagenic; and in part from the fact that this could result in long-term expression of the gene inserts.

Although the lentivirus vectors are derived from HIV, the vector systems to be used are all 3rd generation systems which divide the helper functions onto multiple plasmids designed to minimise the possibility of generating a replication-competent virus through recombination, and do not use the HIV envelope protein. Additionally, all the vectors adopt the “self inactivating” (SIN) design, which restricts the viruses made from the packaging cells to a single cycle of infection. See assessment for further details.

Potential hazards may also arise from the nature of the genes inserted into the vectors.

If cells in the body were to express introduced allogeneic MHC genes, following accidental exposure to the lentivirus vector, these cells would be rapidly eliminated by both the adaptive and innate arms of the immune response. Over-expression, or knock-down of the genes involved in antigen processing could modulate the immunogenicity of the cells, most likely to make the cells subject to attack by the adaptive or innate effector arms of the immune response.

It is highly unlikely that expression of the TCR, BCR or Ig constructs in a small number of cells would result in immunological activity that would be harmful. Expression of cytokines from infected cells following an accidental exposure could in principle induce local inflammation and immune activation. The worst case scenario is that infection of a T cell with lentiviruses expressing growth-promoting cytokines could lead to autocrine stimulation, which could contribute to lymphoproliferative disease, although we consider this a very remote possibility, considering the likely scale of accidental exposure.

PR3 and MPO are normally present in serum, and it appears unlikely that their expression within a small number of accidentally infected cells following operator exposure would be clinically significant.
HDAC expression has potential to alter post-translational modifications and the pattern of gene expression in cells. It is difficult to predict the consequences of this; however, the HDAC activity would be predicted to remain under control of normal signalling pathways in the cells.

In conclusion, in addition to the potential risks related to insertion mutagenesis/gene activation by the viruses, there appears a low level of risk from some of the gene inserts of contribution to inflammatory or lymphoproliferative disease. It is therefore appropriate to adopt precautions to minimise the likelihood of exposure, including avoidance of sharps, use of protective gloves, working with virus within class 2 cabinets, and ensuring an appropriate level of training.

Environmental risk - Gene transfer to other species is a possibility, however the in-built safety features, in particular lack of replication, potentially limit any environmental concern. The hazard is low: the non-replicative lentiviral particles are stable at room temperature for a couple of hours but it would require high exposure, and often simultaneous exposure to other factors (for example growth factors) for infection. The virus will be handled in a CL2 facility, and a safety cabinet will be used, so the likelihood of release to the environment is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - full CL2 applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All tissue culture plastics and liquid waste (135 oC, 15 min) generated during the production of lentiviral supernatants will be autoclaved before disposal. Autoclaving achieves effectively 100% kill of all GMMs. Small volumes of liquid containing the viral particles will be used immediately or Bleach treated or Microsol treated. Pipette tips will also be treated with 10% Microsol then autoclaved before removed as clinical waste and incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Further information was requested on the potential hazard of the inserted genes, as the assessment dwelt heavily on the hazards of the vector. This has now been addressed and the GMSC has approved this proposal.

Project Containment

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The aim is to understand the functional organisation and dynamics of neuronal networks in the mammalian brain. Quantification and characterisation of the synaptic partners of individual neurons is crucial for the development of realistic models of neuronal networks and a quantitative understanding of the functional operation of those networks. Changes in synaptic connectivity in chronic experimental models of neurological disease play a major role in determining the mechanisms of the abnormal neuronal dynamics of diseases such as epilepsy.

Quantifying the connectivity within neuronal networks requires the ability to label a single neuron, and for that label to be transported across synapses in one direction in a manner that prevents onward propagation to other neurons in the network. The labelled tissue can then be studied histologically and/or electrophysiologically. Viruses are the only tracing tools that have the properties that allow labelling across synapses without weakening of the signal, and control production to prevent onward propagation across multiple synapses in series. A review of the literature shows that this has only been demonstrated in rabies virus and in Bartha virus, an attenuated strain of the Aujeszky disease or pseudorabies virus (PRV). Both rabies and Bartha only propagate in a retrograde direction, unlike other viruses used for tracing neuronal pathways, such as herpes simplex and lentivirus, which propagate in both directions. Rabies virus is highly restricted DEFRA group 4 Specified Animal Pathogen and most definitely is not considered here. Despite the similar names, PRV and rabies are completely unrelated: PRV is a DNA virus of the α-herpesvirus subfamily and rabies is an RNA virus. Wild type PRV is SAPO Group 2, suitable for work in conventional laboratories; attenuated strains pose a smaller risk. The proposed work uses a TK-deficient version of a vaccine strain of attenuated PRV.

The TK-deficient Bartha virus will be grown in PK-15 (pig kidney) cells in culture. These cells produce the thymidine kinase required by the virus to replicate. They are an established cell culture line that has been used extensively without reported complications.
The highly disabled virus and TK construct will be injected into brain. Replication of the virus will be in the single neuron loaded with the TK construct and a limited number of glia close to the injection site. As soon as it is possible to perform these experiments the extent of the distribution of the virus in glia following intracerebral injections will be determined and it will be reported back to the appropriate committees if the virus appears in glia further than 1 mm from the injection site. Vaccine strains of PRV with gE and TK deletions have been shown to cause minimal local pathology at injection sites and are not shed. Injection of GFP expressing tk-Bartha virus into the brain show the virus is restricted to the injection site and to anatomically connected neuronal pathways. The TK genes from HSV and PRV lack sufficient sequence homology to allow recombination of TK from the construct into the TK-deficient Bartha virus. Even in the unlikely event of recombination of TK into TK-deleted PRV-Bartha, the resulting virus would effectively be the vaccine strain of PRV-Bartha.

Host/vector system

Vector: TK deficient Bartha virus, with or without fluorescent protein expression cassette or Ca2+ indicator and/or "timer" sequence; Expression cassettes for pgk-HSV-TK and amplicon-fluorescent proteins delivered by injection.

- PRV/Bartha virus. The normal host of the wild-type pseudorabies virus is the pig, but it can also infect rodents, dogs and cows, but not humans or other higher primates. Bartha is a vaccine strain with: a deletion in the unique short (US) regions including the glycoprotein (g)I, gE, US9 and US3 genes, and point mutations in the gC, gM, UL21 and US3 genes; there is no evidence of restoration of virulence despite widespread use of the vaccine in agriculture in mainland Europe and elsewhere, which has been attributed to the dispersion of the mutations across the genome.
- Recombinant Bartha-fluorophore virus was generated by co-transfection of linearised targeting plasmids with the full-length DNA of the parental virus by electroporation of actively growing PK-15 cells. Fluorophore expressing plaques were selected for production. Recombination was performed in Hungary, and is not proposed under this application. Ca2+ indicator and timing signal were inserted as described in Boldogkoi et al 2009.
- TK/fluorophore cassette. PGK promoter driven TK
- Amplicon ampiclon consists of pUC18 vector into which has been cloned a 1.9 kbp Bam HI-Sac I fragment of PRV containing its origin of replication (oris), and a 1.5 kpb Nar I fragment (from circularized PRV) containing the pac1 and pac2 packaging signals (from the two ends of the linear PRV genome) in head-to-tail arrangement, as in rolling-circle replicating form of PRV.mem Cheery or memGFP is inserted into the genome.
- Neuron and glia. The amplicon and virus will co-infect a single neuron in the brain. The amplicon replicates by the rolling circle mechanism of the virus, and concatamers are packaged into virus particles which allow transfer to the presynaptic neurons. The several glia that take up the TK-deleted Bartha virus will allow replication as a result of the host cells' TK expression, but the viruses do not transfer between cells.

Origin & function

The following genes are to be used initially, but other reporter genes for other aspects of neuronal function may be used in future. These will be of similar or lower hazard than those included here. Any additional genes will be considered by the local GMSC and will be notified if it is considered that they constitute a significant change to the proposal:

- Green Fluorescent Protein: fluorescent protein gene.
- mem-Cherry: membrane bound fluorescent protein gene.
- mRFP: fluorescent protein gene.
- Herpes simplex virus TK gene: produces thymidine kinase, in construct with eukaryotic regulatory sequences (PGK promoter, SV40 polyadenylation sequences).
- TN-L15: truncated chicken skeletal muscle troponin C in which the N-terminal amino acid residues 1-14 are deleted
- VAMP-GFP: Vesicle associated membrane proteins fused with GFP
- MARCKS-EGFP: a cytosolic protein kinase C substrate fused with EGFP

- Original donor for Green Fluorescent Protein was the jellyfish Aequorea victoria.
- Original donor for DsRed was Discosoma species (disc or mushroom anemones found in coral reefs); mRFP and mCherry are derivatives of DsRed with improved fluorescence characteristics.
- Original donor for TK was Herpes simplex.
- Original donors for TN-L15 were chicken skeletal muscle, human cardiac muscle.
The Bartha virus strain is an attenuated form of PRV which is used for vaccination of pigs in many parts of the world. Unattenuated PRV causes Aujeszky Disease in pigs, and is a notifiable animal pathogen in the UK, rated as SAPO 2 by DEFRA. Unattenuated PRV also infects other species, including rodents, dogs and cows. The most obvious symptoms are severe pruritus leading to self-mutilation. PRV does not cause disease in humans and other higher primates, but can cause local itch after needle stick injury. The risk from the disabled vaccine strain is minimal.

The Bartha strain has several recognized mutations, including a deletion in the unique short (US) region encompassing the glycoprotein (g)I, gE, US9, and US2 genes and point mutations in the gC, gM, UL21, and US3 genes.5,6,7,8 These mutations explain (a) its reduced virulence and (b) crucially for the present use, the loss of its propagation from presynaptic to postsynaptic neurons, with preservation of propagation from postsynaptic to presynaptic neurons. Vaccine strains with gE and TK deletions produce “slight lesions … in target tissues of vaccinated animals” and were not shed. Bartha virus has been used extensively for vaccination against Aujeszky’s disease in many parts of the world, with no evidence of restoration of virulence, which has been attributed to the large size of the deletions and their distribution across the genome. This leads us to conclude that the Bartha strain has few, if any, pathological effects on pigs, and minimal risk of reversion to virulence, which argues that restrictions of staff contact with farms are not necessary to protect animal health. However, we will adopt such restrictions to avoid the miniscule risk of causing pigs to become seropositive which would trigger slaughter given the absence of any UK policy on genotyping that would identify the vaccine strain.

Bartha virus can cause CNS disease in mice, with no peripheral nervous system symptoms. These studies used 10^6 PFU of virus applied to the skin, leading to symptoms by 220 hours for the Bartha strain (c.f. 7 hours for unattenuated PRV). The LD50 for the Bartha strain applied to the skin was 7.4 x 10^5 PFU.

The strain of Bartha virus used here has been further attenuated by deletion of the thymidine kinase (TK) gene. We know that this virus differs from Bartha in failing to replicate in non-mitotic cells, including neurons. The virus does replicate in glia in the immediate vicinity of the injection site, but there is no propagation between glia or between glia and neurons. The restoration of full PRV virulence would require the acquisition of four specific genes, which is a vanishingly small risk. However, on the precautionary principle, we will contain the virus and infected materials, and will decontaminate all materials produced during these experiments. The risk to the environment is therefore low. The virus is not shed and is limited to the injection site and synaptically connected neurons after injection. However the pathology caused by TK-positive Bartha virus after peripheral injection is limited to the brain, and will not apply in the TK-deficient Bartha used here because it cannot replicate in neurons and it has minimal, if any, propagation between glia.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022

Page 3376 of 15326
This application has been subject to intense scrutiny by the local GM Committee and the University's Biological Hazards Committee. The Committees requested additional proof of the attenuation of the PRV, and asked for a more detailed explanation of how the virus replicated in the neuron using the TK construct. As this proposal is also being submitted to Defra as a SAPO licence application the committees requested additional information on the control measures applied.

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### Project Ref 116/10.1

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### Project Additional Information

- Historical Significant Changes
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

Tick if notifying a connected programme of work N

Project Additional Information

02/03/2022 Page 3377 of 15326
Embryonic stem (ES) cells have the potential to differentiate into all cells within the body. Whilst this property of ES cells holds great promise for regenerative therapy, where they can be differentiated into any cell-type and transplanted into recipients, it is also a safety issue because these cells will form teratomas when transplanted into recipients. Our current research focuses on the differentiation and transplantation of ES-derived hepatocyte-like cells (ES-HLCs). We intend to use "3rd generation" lentiviruses expressing genes which we believe will affect engraftment of ES-HLCs, or improve their safety. The other arm of this research is aimed at identifying molecules which may improve the efficiency of the transplantation of human and murine hepatocytes and ES-derived hepatocyte-like cells. It is our intention to express genes which encode cell surface receptors and adhesion molecules to assess the impact of these proteins on these cells migration and adhesion, respectively.

Recipient or parental organism
Human and mouse cells (eg ES cells, primary hepatocytes and hepatocarcinoma cell lines, primary non-parenchymal cells)

Host/vector system
Retroviral/Lentiviral vectors - for this piece of work 3rd generation lentivirus vectors, e.g. pLenti6 from Invitrogen.

Origin & function
Genes involved in adhesion (ieCD44 and integrins) and migration (ie chemokine receptors). Additional specific genes are to include tmpk, eGFP, osteopontin and gaussian luciferase (gLUC). The addition of new genes will be considered by the local GMSC and changes notified to HSE if it is felt likely they fall outside the scope of the connected programme.

This particular project involves TMPK with F105Y mutation, green fluorescent protein (GFP), CXCR3, CD44 integrin α1, CD47, Gaussian luciferase (Gluc)

Evaluation of foreseeable effects
The plasmid construct in E.coli have no significant implications for health and safety.

Integrins, CD44 and OPN have been implicated in accelerated tumorigenic processes. CD47 has been found to be highly expressed on cancer cells and has been demonstrated to allow cells to evade scavenging by macrophages.

It is very unlikely that naked DNA incorporating genes with oncogenic potential could be directly introduced through the skin, but operators are advised to wear gloves when handling large amounts of such DNA, or when there may be a significant risk of skin contact; and to take particular care in the rare instances when sharps might be used.

Infection to the respiratory tract is relatively poor as a result of aerosol dispersal, however this cannot be entirely ruled out with high viral titre. If accidental inoculation occurred, the viral DNA would be introduced into a number of cells where chromosomal integration might give potential for long-term high expression of the transgene.

The main hazard is associated with the lentiviruses that will be generated in packaging cells. The viruses will be capable of infecting human cells, with the consequent insertion of the vector genome into chromosomal DNA. This raises the possibility of oncogene activation at the site of integration. Consideration of the experiences from human gene therapy clinical trials suggest this risk is low, as any accidental exposure would involve many logs fewer virus particles, nevertheless precautions to avoid exposure are appropriate.

There is also the prospect of the generation of replication competent virus. However, we will be using 3rd generation lentivirus systems which are self-inactivating and replication defective which will greatly reduce the chance of generating replication competent virus.

The final cell lines stably transduced with the lentivirus vectors should not pose a significantly greater hazard than that of the untransduced cells, as both would be subject to alloreactive immune responses.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No derogation. Full CL2 applied.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is treated by autoclaving at 130°C for 60 mins. before disposal to drains.
Disposible solid waste which is or may be contaminated with GMMs is also inactivated by autoclaving at 135°C for 15 mins, before removal as "clinical waste" by specialist contractors, with final disposal by incineration.
The exceptions to the above are:
"Sharps", including disposable plastic tips or micropipetteors, may be decontaminated by drawing up Virkon disinfectant, and soaking in Virkon for a minimum of 10 minutes. Disposable plastic pipette may also be decontaminated by immersion in Virkon as above.

Expected degree of kill:
Autoclaving achieves effectively 100% kill of all GMMs.
The manufacturer's information indicates efficacy of Virkon against HIV (at dilutions from 1:100 - 1:1400)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Comments were fairly minor and merely requested clarification in most instances. For example, further information was requested on the use of needles - weather this involved infectious virus, or stably transduced cells. Also, a request for information on the volumes involved in the project. Reportin arrangements for accidents were also amended.

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Purposes of the contained use

The generation of patient-specific pluripotent stem cells through reprogramming of adult somatic cells has become a major goal in stem cell science. Reprogramming, that is, taking a cell that has a determined function in the adult and artificially altering its pattern of expressed genes so that the earliest stages of development are recapitulated, can be achieved by introducing three or four transcription factors that are known to be associated with the maintenance of pluripotency in embryonic stem (ES) cells. As a result of the enforced alteration in gene expression, a small proportion of the somatic cells adopt a phenotype that is highly similar to that of ES cells, and as such these cells have been termed induced pluripotent stem (iPS) cells. Reprogramming of mouse or human cells into iPS cells has been achieved by the introduction of four transcription factors, Oct4, Klf4, Sox2 and c-Myc and it is widely accepted that the cells generated share many of the characteristics of ES cells. iPS cells have already been generated from patients with a number of inherited conditions, and in some cases these have been differentiated into the cells thought to underlie the effect of the genetic defect.

Introduction of the reprogramming transcription factors requires the use of lentiviral vectors, which have to be packaged in viral coat proteins so that they can infect either mouse or human cells. The GMM involved consists of a highly inactivated HIV-derived vector that is packaged into infectious viral particles by co-transfection into cells together with separate vectors expressing the viral core and envelope proteins (a "5-component" system). There is no risk of viral spread. Lentivirus encoding reprogramming transcription factors will be used to infect primary mouse or human fibroblasts. A second round of infection with a lentivirus encoding Cre recombinase will be used to delete the reprogramming lentivirus from the resultant iPS cells.

Recipient or parental organism

Hosts: Plasmids grown in standard disabled laboratory strains of E. coli
293T cells for lentivirus generation
Mouse fibroblasts (tail tip derived or embryonic day 14) and human fibroblasts (foreskin or adult skin biopsy derived)
Vector: Five component system (HIV-derived), transfected into 293T cells to allow assembly and packaging:
- Lentiviral vector (STEMCCA or integrase-negative Cre vector)
- HIV gag/pol expressing plasmid
- VSV-G envelope protein expressing plasmid
- Rev expressing plasmid
- Tat protein expressing plasmid

The requirement for transfection of five plasmid vectors into the packaging cells makes accidental transfer to other cells essentially impossible. Since the lentiviral vectors incorporate deletion of enhancer/promoter elements from the 3’ LTR they are self-inactivating (SIN), essentially eliminating any possibility of secondary spread, even if helper functions were present. Note that other HIV accessory proteins, and HIV envelope protein, not used so no possibility to generate HIV.

Donor: Mouse and human cDNAs cloned by RT-PCR from mRNA derived from various tissues and Cre sequences derived from E.coli bacterial genomic DNA

Transcription factor genes (mouse/human Oct4, Sox2, Klf4, c-Myc and B-Myb): involved in maintenance of the pluripotent stem cell state. These have specific roles in pluripotent stem cells as well as activities in a range of other cell types:
- Oct4: pluripotency factor expressed in ES cells and during early embryonic development
- Sox2: pluripotency factor expressed in ES cells and during early embryonic development
- Klf4: pluripotency factor expressed in ES cells and during early embryonic development
- c-Myc: pluripotency factor, also involved in proliferation control in a wide range of cell types
- B-Myb: possibly a pluripotency factor, but known to be associated with proliferation in a wide range of cell types

Bacterial Cre recombinase: acts on target sequences to limit bacteriophage infection. As used here it will promote excision of the targeted genes that are flanked by loxP sites.

Evaluation of foreseeable effects

Negligible risk from the plasmid vectors in E. coli, where the genes should not be expressed, and no generation of the lentivirus will be possible.

The greatest potential hazards are associated with the infectious lentivirus vectors. The vector system is designed to minimise the chance of recombining helper functions into the virus, so that the potential for generating replication-competent lentivirus is essentially zero. Furthermore, the vector is SIN, which would prevent the possibility for further mobilisation.

There is a potential hazard from insertional mutagenesis/oncogene activation, in the event of accidental human contamination/infection. This hazard may be lower than for retroviruses due to different insertion site preference, nevertheless a theoretical possibility. The risk is low if only a small number of insertion events are involved.

There is a potential hazard arising from insertion of the genes into human cells, most significantly in the case of the oncogene c-myc. It is unlikely that infection of a small number of cells would cause cancer, since oncogenesis would be a multistep process. In addition, the immune system would be likely to eliminate cells acquiring aberrant gene expression.

Lentivirus infection of human cells could occur through mucosal contamination or percutaneous injury, hence there is a requirement to minimise these risks. Aerosol route of infection may be possible at very high titres, although this not considered to be a common route of retrovirus/lentivirus transfer.

In contrast to the potential hazards presented by the infectious virus, there should be minimal risk from stably transduced target cells, since if there has been sufficient time and medium changes so that no original virus remains then the chance of replication-competent virus being present is virtually zero. Mouse cells are not a risk to humans; the human target cells WILL NOT BE DERIVED FROM THE OPERATOR OR ANYONE WITH ACCESS TO THE CELLS. Hence in the event of accidental human inoculation with the transduced cells, they should be rapidly eliminated by an allogeneic immune response.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - full CL2 applied
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfected liquid waste will be autoclaved (134 degrees/15 mins.). Similarly treated solid waste will be put into biohazard bags for incineration

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

The GMSC felt that the assignment to class 2 was appropriate, given the use of a lentivirus vector capable of infecting humans and the nature of the transgenes, especially considering the oncogenic potential of myc. There were some minor comments, requesting further information on some points

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Project Ref 116/10.3

Date Ackn'd  28/09/2010

Date Project Ceased

Withdrawn  N

Tick if notifying a connected programme of work N

Historical Significant Changes

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

The focus of the group is the role of chemokines and chemokine receptors in immune regulation. Chemokines and their receptors govern the chemotaxis of cells and influence central biological processes including organ-organization and immune responses. Part of the work concerns the role of chemokines and chemokine receptors in the regulation of the host immune responses to tumour growth. The expression of these molecules both on the tumour cells and on the immune cells can influence the development of a protective immune response. The use of retroviral vectors enables the transfer of genes to cells that are difficult to transfect by conventional methods and will be an essential tool for the work.

#### Recipient or parental organism

- **Hists** Stbl3, DB3.1, DH5ca, Top10, and XL10-gold competent cells
- **293FT**, Phoenix amphi and Phoenix ecco 293 cells
- **Target**: Primary cells and immortalized cell lines. Human and mouse

#### Host/vector system

- **pLNCX2**, pLEGFP-C1/N1, pRetroX-IRES-ZsGreen1, pRetroX-IRES-DsRedExpress (Clontech recombinant retroviral vector system). Replication defective.
- MigRI and MigRII retroviral vectors developed in the lab of W.S. P, Department of Pathology and Laboratory Medicine, University of Pennsylvania, PA. Origene HuSH-29 shRNA Vectors or equivalent. Replication defective.
- Other virus vectors may be used in future, e.g. lentivirus, adenovirus, and these will be assessed by the GMSC. If it is considered that the nature of the hazard or the risk level is altered in any way, further notifications will follow.

#### Origin & function

Initially the work will involve the following inserts, though others of a similar nature may be used. Any changes to the assessment, through the addition of inserts, will be assessed by the GMSC and where appropriate, notified to HSE.

- **CCR1-CCR11**: chemokine receptors expressed on a wide variety of cells. Influences chemotaxis.
- **CXCR1-CXCR7**: chemokine receptors expressed on a wide variety of cells. Influences chemotaxis.
- **CCL1-CCL28**: ligands for CC-receptors. Influences chemotaxis.
- **CXCL1-CXCL16**: ligands for CXC-receptors. Influences chemotaxis.
- **DARC, D6 and CCR2**: chemokine receptors. Regulate chemotaxis.
GFP Zs-Green1 and EGFP and DsRed Express etc.: selection of fluorescent proteins which have not been shown to have pathogenic properties. They are used to allow gene expression detection.

Firefly luciferase: gene from which allows luminescence detection. No pathogenic properties.

ShRNA sequences: the shRNA expression cassettes consist of a 29 bp target gene specific sequence, a 7 bp loop, and another 29 bp reverse complementary sequence, all under human U6 Promoter. A termination sequence (TTTTTT) is located immediately downstream of the second 29 bp reverse complementary sequence to terminate the transcription by RNA Pol III. The gene-specific shRNA cassettes are sequence-verified to ensure its match to the target gene.

Evaluation of foreseeable effects

Human Health: E. coli - Disabled host with a non-mobilisable vector, requiring CL1. Inserts are under control of prokaryotic promoters, therefore expression is negligible. Neither the vectors with insert nor the produced retrovirus with insert poses a risk for infection. The retroviral virions, produced in the Phoenix line, can only infect dividing cells, therefore effective infection requires special conditions and therefore highly unlikely to happen wearing protective clothing. Furthermore, at the unlikely event of an infection, the retroviral virions are proliferation defective and the insert, coding that the expressed proteins might affect immune responses in a localised area, but they are not oncogenes and are not known to have any toxicity or transforming activity.

Environmental risk: Potential hazard is NEGLIGIBLE, since the retrovirus particles are very unstable and even accidental exposure would not cause pathogenesis. Also, the virus is non-replicative. Retroviral supernatants will be contained within the tissue culture hood and incubator and live animals will not gain access to the work area.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - Class 2 applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All tissue culture plastics and liquid waste generated during the production of retroviral supernatants will be autoclaved before disposal. Autoclaving achieves effectively 100% kill of all GMMs. Small volumes of liquid containing the viral particles will be used immediately or Microsol/Trigene treated.

Pipette tips will also be treated with 10% Microsol then autoclaved before removed as clinical waste and incinerated.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The project was initially classified as Class 1, but the use of gloved when handling the viruses was considered a sensible precaution, likewise use of a safety cabinet and some access restrictions. It was therefore regraded to Class 2.
Project Additional Information

Purposes of the contained use

Our two groups are interested in internalisation and trafficking processes within mammalian cells. Specifically, we have major programmes of research on phagocytosis and the internalisation and trafficking of manufactured nanoparticles, in particular metal oxides such as CeO2 or ZnO. Thus, we will be employing in-vitro models of lung epithelium and blood brain barrier, as well as primary and cell line phagocytic cells (monocytes, neutrophils, macrophages and dendritic cells) to study these cellular processes. In order to exploit these models, we need to express exogenous transgenes (non of which are oncogenic or otherwise toxic), such as tagged and mutant proteins, in primary cells. However, as primary cells are generally resistant to conventional transfection methods we will need to employ lentiviral transduction in these studies.

Recipient or parental organism

Virus will be produced in the human embryonic kidney HEK293T cell line. Functional virus will be used to transfect either primary (peripheral blood monocyte derived) human macrophages, or macrophage-like cell lines including THP1 (human) or RAW/J774.A1 (mouse) as well as epithelial cells (e.g. HUVECs)

Host/vector system
Third generation lentiviral vectors (replication defective) pWPI, pLVTHM and/or pLVX-EF1α-IRES-Puro; packaging vector pMDLg/pRRE; pRSV-Rev expressing the HIV-rev gene; and envelope vector pMD2.G

**Origin & Function**

Origins: Human and mouse cDNA

Commercially available fluorescent tags such as GFP, mCherry, Cerulean etc.

Genetic material: GFP, mCherry, Cerulean.

WASH actin associated protein, WASP actin associated protein, Exo70 marker of exocytosis, Rab5 GTPase, Rab7 GTPase, Rab11 GTPase, Atg9 autophagosome marker, Lysosomal associated membrane protein (LAMP), CD63, Cathepsin D, and vacuolar STPase, as well as markers and inhibitors for endocytosis pathways (e.g. clathrin, eps 15 (EH29), caveolin 1, caveolin 1 (Y14F), dynamin (WT), dynamin (K44A)). Both mouse and human homologues of such genes will be utilised. Any additional genes of future interest, which function in similar signalling pathways, will be added to a list at the end of this assessment for periodical review by the GMSC - theses additional genes will be low-risk genes only, i.e. they will not include, for example, active human oncogenes or microbial toxins.

**Evaluation of foreseeable effects**

The sequences we propose to study have no known role in tumorigenesis, toxicity or other processes deleterious to the host and thus present low risk in their own right. However, it is theoretically possible that insertion of the lentiviral DNA close to an endogenous oncogene may result in its constitutive activation leading to a transformation event, although the self-inactivating (SIN) lentivirus vectors that we will use appear to reduce this risk relative to that of using the MoMuLV-type retroviruses. It is therefore critical to use strict precautions to prevent user exposure to these lentiviral constructs from occurring and to stringently decontaminate all areas in contact with the virus.

With regard to the possibility of lentiviral replication after target cell infection, a number of strategies have been used by these so-called 'third generation' lentiviral vector system to minimise the chance of infective particles being produced by infected cells. These safeguards include the removal of five out of the six regulatory/accessory proteins; Tat, Vif, Vpr, Vpu and Nef. The sixth, Rev, is retained since it is essential for the expression of gag/pol, but is encoded on a separate plasmid from both gag/pol and the transgene as an additional safety measure. Only 700 bp of the HIV envelope protein are present in the transfer vector, plus Rev responsive element [RRE] and the packaging signal. The packaging construct contains the minimal RRE of 374 bp and the gag/pol genes. Through deletions in both LTRs and the absence of five of the six accessory protein, including the replication essential Tat, it is highly unlikely that replication competent virus would be produced. Such vectors have now been used globally for many years, but to the best of our knowledge no users have ever detected replication competent virus.

**Risks to Environment:** The recombinant virus is replication defective and does not have an extended host range so would be unlikely to spread to a large number of organisms if it were potentially released. The fluorescent proteins are not considered to present any potential threat. Transgenes encoded by the lentivirus may be able to modify the behaviour of any cells they infect, but this would only occur if the virus came in direct contact with mammalian cells, which would be an unlikely scenario. Should this occur, the replication defective nature of the virus would ensure that this is a self-limiting process.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation - CL2 applied

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste is treated with 1% Virkon, followed by autoclaving at 121°C for 15 min, before disposal to drains. The autoclave is serviced and calibrated on a regular basis. Disposable solid waste, which is or may be contaminated with GMMs, is also inactivated by autoclaving at 121°C for 15 min, before removal as "clinical waste" by specialist contractors, with final disposal by incineration.
Expected degree of kill:
Autoclaving is expected to achieve a 100% kill of all GMMs.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment
Aside from some minor clarifications on waste treatment, the other main issue raised related to the handling of breakages in the centrifuge so an additional emergency protocol has been added under this section in the risk assessment. There were no other concerns raised

Project Containment

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Project Ref 116/13.1

Date Ackn’d 05/03/2013
CU2 Project Title Functional characterisation of loci from pathogenic and non-pathogenic bacteria
Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 Litre
Non-GMM Consent Granted

Project notified under transitional arrangements N
Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
Project Additional Information

Purposes of the contained use

This work focuses on bacterial pathogenesis, physiology and genomics, with particular interests in gene regulation and chromosome organisation. The group primarily work with non-pathogenic and pathogenic (HG2) Escherichia coli strains. They may also investigate other category 2 pathogens, including strains of Vibrio cholerae, or Yersinia spp but will never genetically alter these organisms. Thus, they will only genetically modify E. coli (both harmless and pathogenic strains). These modifications will involve general cloning, expression and characterisation of selected genetic determinants.

All organisms that the group will handle fall within ACDP category 1 or 2. However, on occasion they may also handle ONLY DNA from strains within taxonomic groups that fall within category 3, such as Yersinia pestis. They never work with these organisms directly and have no intention to work with genes encoding toxins or other potentially harmful proteins. DNA from these category 3 organisms will only be cloned into hosts that are handled at level 1 (e.g. E. coli). They stress that they DO NOT handle any category 3 organisms, nor will they ever make any genetic modifications to such organisms.

Activity 1: Cloning of bacterial DNA in disabled E. coli strains;
Activity 2: Allele replacement;
Activity 3: Gene expression in disabled E. coli strains;
Activity 4: Gene expression in wild type ACDP 1 and 2 organisms or mutant organisms created under Activity 2.

Recipient or parental organism

Commonly used well-characterised disabled laboratory strains of E. coli from commercial or academic sources (e.g. DH5alpha, JM109, Top10, MG1655).

Also ACDP HG2 strains of E. coli.

Host/vector system

They wish to include within this assessment any well-characterised commercially or academically available non-mobilisable or mobilisation defective
derivatives of the vectors listed in the Compendium Of Guidance. In addition, for transposon mutagenesis, they may rely on transduction with well-characterised lambdoid phages to introduce the transposon.

**Origin & function**

Activity 1 in assessment: E. coli, V. cholera, Y. enterocolitica - DNA fragments, typically non-coding promoter regions, but also genes encoding non-harmful proteins.

Activity 2: ACDP HG 1 and 2 strains of E. coli - PCR products incorporating genes from these bacteria, with or without antibiotic resistance genes or marker genes. In the case of transposon mutagenesis, well-characterised transposable elements that carry reporter genes.

Activity 3: ACDP HG1 and 2 strains of E. coli - DNA fragments encoding different genes (none associated with toxin production).

Activity 4: ACDP HG1 and 2 strains of E. coli - DNA fragments encoding various genes (never encoding toxin production or harmful products).

**Evaluation of foreseeable effects**

Activity 1: Transformed E. coli are disabled and non-pathogenic, so should not carry any risks. Transgene plasmids will confer antibiotic resistance but not to any clinically relevant antibiotics. As all waste is autoclaved before leaving the building it is highly unlikely that any GMOs or constructs will escape into the environment.

If by some slim chance they were to escape, the disabled host would not be able to survive in the external environment and the non-mobilisable vector would not be able to leave the disabled host. Any release of DNA into the environment would be similar to that which occurs during the usual processes of decay. The environmental impact would therefore be negligible.

Activity 2: Transformed E. coli are disabled and non-pathogenic, so should not carry any risks. Transgene plasmids will confer antibiotic resistance but not to any clinically relevant antibiotics. As all waste is autoclaved before leaving the building it is highly unlikely that any GMOs or constructs will escape into the environment. Allele replacement requires the propagation of antibiotic resistance genes, during the interim stage when plasmids are introduced into the target strain and in some cases in the final stage, when the disrupted allele has replaced the wild type allele. In either situation, these antibiotic resistance genes will not, in the unlikely event that they escape, present an increased risk to the environment because:

- as noted above, they will use genes that do not encode resistance to drugs used therapeutically against the host species (e.g. ampicillin is not used to treat E. coli enteritis)
- These resistance determinants are already abundant in naturally occurring isolates of the host species.

Once the allele replacement is complete, the resulting GMO, carrying a
disrupted gene, is likely to be less fit than the wild type and so will be outcompeted by natural isolates, were it to escape into the environment. As noted above, any marker genes used with have no environmental effects.

Activity 3: Transformed E. coli are disabled and non-pathogenic, so should not carry any risks. Transgene plasmids will confer antibiotic resistance but not to any clinically relevant antibiotics. The expression of the proteins of interest in bacteria is very unlikely to increase their pathogenicity. As all waste is autoclaved before leaving the building it is highly unlikely that any GMOs or constructs will escape into the environment. The vectors that are used are non-mobilisable and will be propagated only in disabled lab strains of E. coli. If by some slim chance they were to escape, the disabled host would not be able to survive in the external environment and the nonmobilisable vector would not be able to leave the disabled host. The environmental impact would therefore be negligible.

Activity 4: Among diarrhoeagenic E. coli strains, only those carrying the verotoxin are classified as category 3 pathogens. The group DO NOT use these strains in their laboratory. The distinction between these strains and other related ADCP strains is made on the grounds that these strains have the potential to cause illness that is life-threatening and can be difficult to treat. In contrast, the ADCP 2 pathogens they use in the laboratory (principally E. coli strains) elicit only self-limiting local pathology and are responsive to antibiotics. Thus, altering the expression levels or copy number of any genes from these category 2 strains and/or expressing them within category 2 strains other than those from which they originate, cannot conceivably enhance the pathogenic potential of the host strain sufficient to warrant reclassification as category 3 pathogens. Furthermore, even if, in the very worst case, some enhancement of virulence were seen, the strain would remain treatable with antibiotics. Thus, they conclude that this work can be carried at containment level 2. As all waste is autoclaved before leaving the building it is highly unlikely that any GMOs or constructs will escape into the environment. Their complementation work will be done using non-mobilisable or mobilisation-defective vectors that are initially propagated in crippled E. coli strains. This initial propagation of a non-mobilisable or mobilisation-defective vector in a disabled host means that the resultant GMO will not be able to survive in the external environment. The environmental effects of such a GMO escaping will thus be negligible. Also as the non-mobilisable vector will not be able to leave the disabled host, gene release into the environment could occur only in a way that parallels natural decay processes. SEE ASSESSMENT FOR FURTHER DISCUSSION

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None
Inactivation will be by autoclaving of all wastes (including any small quantities of disinfectant–treated residues e.g. paper towels used for wiping up small spills) contaminated by GMMs, at a minimum of 121°C for 15 mins, validated by monitoring of internal chamber temperature and automatic printout of every autoclave run. This method is widely acknowledged to guarantee complete killing of all known bacteria and they regularly check the killing efficiency of the autoclave by attempting to grow autoclaved bacterial cells. Wastes from autoclaving will be disposed of to the drains (liquids) or to clinical waste incineration.

**Project Containment**

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**Project Ref** 116/13.2

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<td>02/03/2022</td>
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The aims of the study are to investigate the mechanisms of replication of RNA viruses and how they subvert the cellular machinery to achieve this goal. Recombinant viruses will allow us to analyse this process in greater detail and is fundamental to this research strategy and its aims. The long term aims are the improvement of fundamental knowledge in the area of RNA virus replication and the future development of vaccines and antivirals.

The GMOs are viruses derived from DNA plasmid copies of RNA viruses, specifically non-segmented RNA viruses from the Mononegavirales order such as Measles virus. The target viruses are all classed as ACDP2 or lower and will not include ACDP3+ or SAPO restricted pathogens from this Order. The GMOs will also include pseudotyped replication defective lentiviruses that are capable of transducing mammalian cells to overexpress viral or host genes e.g. to over-express genes relevant in studying pathogenesis. The recipient hosts for these GMOs are mammalian cells (primary derived or immortalised cell lines) and/or mice (lab-strains or transgenic derivatives). Bacteria (commercial non-pathogenic strains) will also be used to replicate the DNA clones prior to manipulation.

The vector system for these studies is based on the use of full length DNA clones of RNA virus genomes. The non-segmented RNA virus genomes of ACDP2 (and lower) pathogens are cloned downstream of promoters that can drive transcription of a genome like RNA. This RNA, in the correct context, and with the correct support, is capable of producing infectious virus. The plasmid backbones used for this system are based on standard commercially available versions. The lentiviral system for over-expression of chosen genes is based on the commercially available ViraPower based system. A replication defective and truncated HIV1 genome is used as a vector to generate pseudotyped particles capable of infecting (non-productively) target cells. Integration and selection is anti-biotic mediated however, no nascent virus can be produced.

The genetic material (viral or cellular) will be sourced from biological isolates of these RNA viruses (Mononegavirales of ACDP2 and lower classification only), from mammalian cell-lines, from primary cell cultures or through in vitro commercial genetic synthesis. All ethical approval for the receipt of the relevant clinically associated samples will be in place prior to the commencement of work. Importantly, the genetic material will be used to generate recombinant RNA viruses with equivalent or reduced pathogenesis relative to the parental strain. Also, the pseudotyped lentiviruses are incapable of producing productive progeny virus. Recombinant tagged/mutant viruses will
be used to determine the molecular basis for pathogenesis both in vitro and in vivo (in mice only).

**Evaluation of foreseeable effects**

Recombinant viruses: Only ACDP2 class 1 and 2 pathogens from the Mononegavirales order will be ‘rescued’. Work with ACDP3+ and SAPO restricted pathogens will not be carried out. The viruses rescued will be designed in such a way as to not increase pathogenesis, relative to the parental strain. This will obviously be confirmed using in vitro tests under ACDP2 conditions in the lab. The foreseeable effects to the organism of such mutations and/or addition of tags (fluorescent etc) are therefore to either maintain its virulence or attenuate it. The risks to the environment and public are therefore effectively zero. This bears true for the recombinant lentiviruses as well as these are replication competent and incapable of producing nascent virus after the primary infection step.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Transgenic mice will be used for some of the proposed work, where virus is used to infect mice or transgenic mice. These will be held in a secure Animal facility, with the use of isolators to house the infected mice. Agreed procedures will be in place for the removal of carcasses.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation - full CL2 applied

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

GM MeV in waste will be inactivated with 1% Distel (formerly Trigene) or 1% Virkon, which has been shown to be an effective agent for inactivation of a wide range of enveloped viruses including Measles. Measles is also susceptible to 70% Ethanol.

All biological waste generated in the IBR is then autoclaved at 135°C for 15 mins under pressure before removal as clinical waste by licensed contractors. Nothing is released to the environment. All autoclave runs are monitored and recorded by trained staff - any failed runs are treated as infectious and re run.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

**Project Containment**

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02/03/2022
This work will investigate the physiology, gene regulation and behaviour of class 2 ACGM microorganisms that are relevant to the process engineering industries. Example industries include but are not limited to the manufacture of fast-moving consumer goods, pharmaceuticals, medical devices and food. Microorganisms play roles in these industries in terms of both positive and negative impact. Positive impacts from organisms would include processes that utilise microorganisms to generate specific products. Negative aspects usually involve microorganisms contaminating process plant or products, either during manufacture or use. In both cases, understanding the microorganisms can aid improving the processes and products. The genetic modifications will fall under one of the following categories:

a) Whole-cell tagging with GFP or a functionally similar fluorescent protein in order to visualise the organisms using e.g. microscopy for flow cytometry;

b) Use of promoter-fluorescent protein reporter fusions to allow measurement of gene expression using e.g. microscopy for flow cytometry;

c) Gene knockouts, to determine the effects on bacterial physiology, viability, behaviour etc.;
d) Complementation of gene knockouts; and
e) Expression of heterologous genes from organisms of similar or lower ACDP hazard level, to determine effects on bacterial physiology and behaviour.

Recipient or parental organism

A variety of bacterial species at ACDP class 2, initially including:
Pseudomonas aeruginosa
Escherichia coli ACDP class 2 strains (eg UPEC, EHEC - non-toxigenic strains only).
Additional bacterial species at ACDP class 2 will be used as the project progresses. Each new species will be assessed.
Species that are ACDP HG2 but pose an elevated risk from aerosolisation (e.g. respiratory pathogens that infect healthy individuals, such as Neisseria meningitidis) will NOT be used.

Host/vector system

Examples of vectors will include:
For Pseudomonas: pME6032 - shuttle vector, pVS1 (For Pseudomonas) and p15A (for E. coli) ori, tetracyclin resistance. No mob gene.
pVS1/p15A-based GFP promoter probe vectors such as pPROBE-KT.
pBBR-based GFP promoter probe vectors such as pPROBE-TT. (pBBR is mobilizable; doi: 10.1094/MPMI.2000.13.11.1243)
For E. coli: standard cloning vectors with colE1, pMB1, p15A, pRW origins of replication, antibiotic resistances such as AmpR, TetR, KanR, ChlR.
The use of broad host range vectors is unfortunately needed due to the range of species covered in this assessment.
Non-mobilisable plasmids will be used where possible, limiting potential spread.

Promoter-reporter gene fusions may also be chromosomally integrated

Origin & function

Donor:
Health and Safety Executive
(a & b) Fluorescent proteins: The jellyfish Aequorea victoria for green fluorescent protein (GFP); The coral Discosoma sp. for red fluorescent proteins; The plant Arabidopsis thaliana for the flavin-based Fluorescent protein (FbFP) iLOV; The freshwater algae Chlamydomonas reinhardtii for the FbFP CreiLOV.
(c) Antibiotic resistance genes would be sourced from a variety of organisms / plasmids.
(d) Complementation would involve resupplying the knocked out gene on a plasmid and so the donor would be the same species as the host.
(e) Heterologous genes would be taken from ACDP class 2 bacteria.

Inserts:
(a & b) Aequorea victoria gfp and mutated derivatives (eg eGFP, YFP); Discosoma sp. OsRed and derivatives (eg mRFP, mCherry); Arabidopsis thaliana FbFP iLOV and Chlamydomonas reinhardtii CreiLOV.
(c) Antibiotic resistance genes would be used to confirm chromosomal mutations, eg AmpR, KanR.
(d) Complementation of knocked out genes - various genes.
Various heterologous genes, but none that are known virulence factors or could be expected to increase virulence.

**Evaluation of foreseeable effects**

Human health: The GMMs generated in this study are not expected to pose any greater risk to healthy humans than their non-GMM parent strains. Experiments will be designed to minimise risk.

(a & b) Expression of GFP or functionally similar molecules is not likely to increase virulence; increased metabolic burden will likely decrease overall fitness and thus decrease virulence.

(c) Knockout of genes will not be likely to increase virulence. No genes known to be negative regulators of virulence functions will be knocked out.

(d) During complementation experiments no virulence factors will be overexpressed from plasmids. Host proteins may be expressed at slightly higher levels from plasmids than from the host chromosome, but assessment will be made in each case to ensure that no genes known to give rise to increased virulence are overexpressed.

(e) No modifications will be made that are likely to increase virulence.

As such, no genetic modifications likely to increase virulence or increase toxicity will be made.

Consideration of increasing biofilm colonization ability: Biofilm formation is a complex process involving many genes; genetic modification will be used to change biofilm structure and function in this work. However, each modification will be carefully assessed beforehand in terms of risk of increased colonisation / infection ability; modifications will not be made that are deemed to result in a hypercolonizing phenotype. All GMMs will be studied at a small scale (< 100 mL) initially; any found to have a hypercolonizing phenotype will not be grown at larger scales. All GMMs will be studied at a small scale (<100mL) initially; any found to have a hypercolonising phenotype will not be grown at larger scales.

Before growth in CDC bioreactors all GMMs will be characterised at smaller scales.

Generation of Random mutants: Generation of random gene knockout mutants using a method such as TRADIS might be used in the project. In this case, TRADIS libraries would be grown at small scale and clones of interest assessed before growth at larger scale.

Environment: Organisms: All species to be used are ubiquitous in the environment. The GMMs would typically be less fit than the wild type organisms, and therefore less able to survive in the environment.

Recombinant DNA: use of mobilisable and broad host range plasmids increases risk of transfer of recombinant genetic material, but as no virulence genes are being cloned, transfer of recombinant DNA would result in: a) transfer of fluorescence genes, which would have little effect on environmental recipients; b) transfer of antibiotic resistance genes, which would be unlikely to confer resistance to clinically useful antibiotics to organisms that would be likely to cause infection; or c) transfer of other genes encoding proteins with no virulence function, which would not be likely to result in environmental harm.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation requested

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Virkon is regularly used at the manufacturers’ recommended concentration (1%). Virkon is validated for use with P. aeruginosa and E. coli (> 5 log kill).

1 % Virkon will be used as the primary disinfectant for laboratory benches; squeeze bottles containing 1 % Virkon will be provided to treat small spillages.

Efficacy against other organisms to be used in the project in future will be checked via biological risk assessments.
Spill control for CDC bioreactors: as stated above CDC bioreactors and carboys are run on drip trays. The drip trays will contain any spills. Spills will therefore be disinfected using Virkon powder to gave 1 % concentration. Disinfected spill material will be absorbed into spill kits or paper towels and these will be placed in yellow bags and sent for clinical disposal- they will not be autoclaved.

Liquid wastes are inactivated in waste autoclaves in the Biochemical Engineering building (127 °C, 15 psi, 35 minutes). Waste inactivation is checked by the use of indicator tape. Glassware is then washed, plasticware disposed of through clinical waste route.

Degree of kill = 100%.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

It was generally felt that this was a well written assessment. The GMSC asked for some additional info, as follows:

Would it be possible to give a couple of examples of the genes that will be targeted in the KO strains and an idea of how many might be generated during the overall project. Will all the mutants be generated by recombinational insertion of a drug selection cassette or will random mutagenesis be used at some point?

It is sensible that experiments will be started on a small scale in the first instance. However what is the reason for using 60 L reactors? Perhaps this could be justified in a couple of sentences.

What action would be taken if there was a spillage from the large scale reactor?

All of the above, as well as minor queries around waste treatment, were addressed within the risk assessment.

Project Containment

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Animal Units

| L2 | L3 | L4 | L2 |

Large Scale Activities

| L3 | L4 | L2 | L3 |

Human Clinical Applications

| L3 | L4 | L2 | L3 |

Project Ref 116/19.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4
Identification of fungal effector recognition determinants in plants, and contribution to non-host resistance (NHR) against fungal pathogens and plant induced resistance in *Solanum lycopersicum* against pathogens using fluorescent proteins (FPs) to monitor disease.

Project Additional Information

**Purposes of the contained use**

Project 1. The aim of this work is to identify the determinants of fungal effector recognition in different plant species, and assess their contribution towards NHR. The pathogen used will be the ascomycete fungus *Zymoseptoria tritici*. A virus-induced gene silencing (VIGS) system based on Tobacco Rattle Virus (TRV) will be used to test ability of different plants to recognise pathogen effectors and resist pathogen infection.

Project 2. The aim of this project is to test induced resistance in different plants against GM isolates of the oomycete pathogen *Phytophthora infestans* (88069). In order to monitor in planta colonisation by the pathogen, the isolate of transgenic *P. infestans* expressing Red fluorescent proteins (RFP) will be used for these experiments.

**Recipient or parental organism**

Project 1. The recipient/parental organisms will be either wild-type (IP0323) or previously modified (IP0323~ku70, ST16(pGPD-GFP)) strains of the fungus *Z. tritici*. The IP0323~ku70 strain contains the Nptll gene conferring geneticin (G418) resistance in place of the native Ku70 gene. ST16(pGPD-GFP) contains the Hph gene for hygromycin resistance and the GFP gene for fluorophore expression. In VIGS experiments, the pTV vector system (based on RNA2 of TRV) is maintained in the Agrobacterium tumefaciens strain GV3101. For full viral infection to be initiated, TRV RNA1 must be provided by A. tumefaciens strain C58C1 carrying the vector pBINTRA6. Both A. tumefaciens strains are disarmed, non-oncogenic strains with natural resistance to the antibiotic rifampicin. Both the pTVand pBINTRA6 vectors carry the Nptll gene for resistance to kanamycin.

Project 2. The *P. infestans* isolate was originally transformed with RFP from Discosoma sp (mushroom coral).

**Host/vector system**

Project 1. The modified *Z. tritici* strains were originally produced as described above. For pTV, both pTV (TRV RNA2) and pBINTRA6 (TRV RNA1) encode the Nptll gene for resistance to kanamycin.

Project 2. The *P. infestans* isolate was originally transformed with the pTOR transformation vector which contains the NPTII gene for selection of transformants for G418 (geneticin) resistance. No hazards have been associated with the
vector.

Origin & function

Project 1. No further modification of Z. tritici strains IP0323, IP0323~ku70 or ST16 (pGPD-GFP) will be performed. For pTV VIGS experiments, short (200-400bp) segments of the plant gene to be silenced will be identified and cloned from cDNA sourced from the same plant species. Fragments will be inserted into the polylinker site of the pTV vector, and silencing initiated by Agrobacterium-mediated expression of both pTV (TRV RNA2) and pBINTRA6 (TRV RNA1) in plant tissues.

Project 2. Tandem dimer (td)Tomato Red Fluorescent Protein. Genes encoding fluorescent proteins are used in order to monitor in planta colonisation. They have a long history of safe laboratory use and no risks have been reported.

Evaluation of foreseeable effects

Project 1. None of the Z. tritici strains or pTV vectors described here are human or animal pathogens. It is highly unlikely that any further modifications (of the type described here) will produce strains that pose a hazard to humans or animals. Therefore the risk to human and animal health is considered low.

Modified Z. tritici could persist in the local environment if it were to encounter susceptible wheat plants. However, the strains used here were isolated from plants several decades ago and are therefore less-well adapted to modern field conditions than highly-virulent field isolates. The strains described here will be less virulent on modern wheat cultivars which often harbour suites of Stb resistance genes for protection against Z. tritici. Furthermore, the strains described here are fully susceptible to control by the 001 and SOH I classes of fungicides. The risk to the environment is therefore considered low.

The pTV vector system will only produce reconstituted virus when viral RNA1 and RNA2 are combined in planta. It is not anticipated that insertion of plant gene fragments into pTV will enhance virulence. TRV has a broad host range amongst dicot plants and the principal method of transmission is by nematode vectors of the genera Trichodorus and Paratrichodorus. In addition, the virus can be transmitted by seed, grafting of infected material and mechanical inoculation. The pTV vector lacks the Orf2b and Orf2c genes which are required to permit the virus to be transmitted by nematode vectors. Plants used for VIGS experiments are typically 2-8 weeks old. These plants are discarded before flowering and seed set. Infected plant materials from glasshouse experiments could theoretically initiate further infections if they were disposed of incorrectly, and put in close and prolonged physical contact to potential host plants to allow mechanical inoculation. However the inability of modified virus to spread via nematodes make it highly unlikely that accidental release would lead to persistent infection of plants outside the laboratory. The risk to the environment is therefore considered low.

Project 2. The transgene-carrying host organism is Phytophthora infestans 88069, a Dutch isolate. P. infestans is distributed worldwide, including major Solanum growing regions in Europe, North America, and South America. P. infestans primary hosts are potato (Solanum tuberosum) and tomato (Solanum lycopersicum). Stable inserts of fluorescent proteins such as tdtomato or NPTII genes do not enhance virulence, host range or survival of P. infestans.

Transgenic isolates already exist and the transgenes encoding the fluorescent proteins are stably inserted into chromosomal DNA of the host organism. Therefore, GMM transference is negligible. Transfer of via oospores is highly unlikely as only asexual phase of the pathogen will be used during experiments.

No P. infestans strain has ever been recorded as pathogenic to humans or animals, or produce toxins or allergens to mammals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Glasshouses used in this work will comply with CL2: Access restricted to authorised personnel; Negative pressure
with filtration; Control of run-off water; Dedicated PPE; All waste autoclaved before being incinerated.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - CL2 applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Project 1. All infected plant material and contaminated plasticware will be placed in biohazard bags and autoclaved at 127°C for 35 minutes prior to disposal. This treatment will destroy all microorganisms and plant cells. All contaminated glassware will be soaked overnight in 1% Distel.

Project 2. Infections will be performed by the detached-leaf/fruit inoculation method. All infected material (leaves or fruit) and laboratory material (tips, tubes, Petri dishes, etc) will be placed in biohazard bags, sealed and autoclaved before disposal. Transgenic material is autoclaved at 127°C for 35 minutes. This cycle kills all plant materials and microorganism.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Most comments related to the assessment of the Tobravirus-based vector system. The comments were generally relatively minor and requested clarification on the following points amongst others:

The use and properties of Agrobacterium;
Clarification of the specific genes in Nicotiana/Oak that are going to be silenced and what will the effect be (in addition to the vectors' genes);
Confirmation that there were no homologues of these genes present on the chromosome (eg as relics of previous infections) that could restore function by homologous recombination;
The risk of aerosolisation from syringes and pipetting;
The procedures for spills in glasshouses.
All points were addressed in the assessment.

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
Use of genetically modified microorganisms in laboratory animal infection models

**Purposes of the contained use**

This contained use notification covers the use of genetically modified CL 1 and CL2 microorganisms in murine and zebrafish animal infection models. Modifications to be studied are likely to include, but not be limited to, the expression of fluorescent tags or the disruption of putative virulence factors by approaches such as homologous recombination. Modified microbes will be introduced to wildtype or transgenic mice or zebrafish and then altered patterns of disease studied according to protocols previously approved in line with Home Office regulations.

**Recipient or parental organism**

A range of fungal and bacterial pathogenic species, including but not limited to Gram-negative bacteria (Salmonella, E. coli, Citrobacter, etc), Gram-positive bacteria (Staphylococcus, Streptococcus, etc), Mycobacteria and fungal pathogens (Cryptococcus, Candida, etc). All organisms are CL2 or below and genetic modifications to be studied are anticipated to either be neutral (e.g. expression of GFP or similar markers) or deleterious (e.g. loss of virulence factors) to the pathogen.

**Host/vector system**

A range of vector systems will be used, depending on the microorganism to be studied, but typically these are either plasmid-based expression systems (e.g. for fluorophore expression) or homologous recombination strategies (for gene 'knockout'). Vector systems to be used are typically not re-mobilisable once introduced into the host and
consequently the risk of horizontal gene transfer to endogenous microbes within the mouse/zebrafish is minimal.

Origin & function

Diverse. Genes to be studied will depend on the specific organism and project, but typically fall into the following groups:

a) 'marker' genes, such as GFP, mCherry or so forth - designed to express visible or biochemical markers that can be followed within the host organism

b) 'knockout' cassettes, designed to eliminate the expression of an endogenous gene within the microbe, such as a virulence factor

c) 'gene switch' experiments, in which, for instance, a gene from one strain of E. coli is introduced into a different strain of the same species to test its function in a different host. Such experiments are carefully considered prior to local approval being granted, in order to minimise the risk of inadvertent 'gain of function' events in which pathogenicity is increased.

Evaluation of foreseeable effects

The foreseeable consequences of each experiment are those which the experiment is designed to address - for instance, reduced disease symptoms in a mouse infection model, following infection with a 'knockout' bacterium. Inadvertent foreseeable consequences are minimal, since all of the work proposed here will be conducted within contained animal facilities. Animals are monitored carefully to ensure high standards of welfare and thus any unexpected effects of the GMO will be rapidly detected. All waste, import/export of animals and entry/exit of staff is carefully regulated from the facility and hence the likelihood of an inadvertent release of a GMO to the environment is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This notification concerns the use of GMMs within animal models. This includes lab scale work with zebrafish larvae, for example, but will also include work in animal facilities (mouse work). These facilities are designed to prevent escape. The animal work will carried out within a dedicated containment suite within the animal facility. A quarantine procedure is in place to prevent researchers entering any other part of the building (containing 'clean' animals) for 48 hours after being in the containment suite. Dedicated PPE (full suit, hair net and mask) is provided and must be worn. Shedding from the animal is possible, particularly with oral gavage. Bedding will be autoclaved and sent for disposal via the clinical waste stream for incineration.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - CL2 applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be autoclaved, bagged and sent for incineration via the University’s clinical waste contractor. Liquid waste is also autoclaved and disposed of to drain. Autoclaving achieves 100% kill. 1 % Virkon is used for disinfection within the laboratory and may also be used to treat liquid waste at 1 %.

All animal bedding and associated animal waste is autoclaved before disposal via the clinical waste stream for incineration. 100% kill.
A number of risk assessments are associated with this notification - two have already been submitted as examples to cover the bacterial and fungal aspects of the work. Most of the comments were in relation to the laboratory work, rather than the extension of the work into animal models, although more information on controls specific to the animal facility were requested.

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### Project Ref 116/19.3

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<td>Use of retrovirus and lentivirus vectors in studies involving RNA interference and CRISPR modification to regulate specific gene expression and function and for the expression of additional genes</td>
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<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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<th>Project notified under transitional arrangements</th>
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## Project Additional Information

### Purposes of the contained use

1. Stable introduction of constructs to express short hairpin (shRNA) in cells, to target a variety of cellular or viral RNAs for degradation by RNA interference.
2. Stable introduction of constructs into cells to modify genes of interest (e.g. by CRISPR)
3. Stable introduction of constructs to express wild-type or mutant proteins to examine function, or markers (e.g. fluorescent proteins)

### Recipient or parental organism

Human or other mammalian cell lines, including tumour cell lines; primary human or rodent cell lines. Cell lines may be injected into relevant animal models, such as murine models.

Vectors expressing shRNA, CAS9 and/or guide RNA sequences for CRISPR approaches, or modified genes of interest may be inoculated into relevant animal models as above.

### Host/vector system

Retrovirus vectors will be deleted for gag, pol and env genes, and produced in split packaging systems (e.g. FLY:A13 or FLY-RD18; Phoenix), in which the gag-pol and env genes are expressed from separate constructs, reducing the risk for generation of replication competent recombinants to an extremely low level. Some vectors are also "self-inactivating", with a deletion within the 3' U3 region which allows the vector to go through only a single round of infection after packaging.

The envelope proteins used will allow infection of human cells, e.g. murine amphotropic, feline RD114, VSV-G Lentivirus vectors will preferably be 3rd generation (although 2nd generation may also be used), i.e. the vectors being deleted for all viral genes, and separated onto at least 2 or 3 plasmids. The HIV envelope protein will not be used, all currently planned work will use the VSV-G envelope glycoprotein. Alternative systems may be used, such as the Invitrogen or Trono lab systems. Wherever possible, allentivirus vectors used will be self-inactivating (SIN).

### Origin & function

The shRNA constructs will be designed to inhibit expression of a variety of cellular genes through the process of RNA
interference (but may include "control" constructs, designed not to match any cellular gene). A wide variety of cellular genes will be targeted in different constructs, including tumour suppressor genes; other constructs will be designed to downregulate genes of certain viruses associated with particular human cancers, e.g. Epstein-Barr virus. CRISPR approaches may similarly be used to modify similar target genes to prevent their expression or modify their function. In addition they may be used to introduce a marker (e.g. fluorescent tag) that allows the expressed modified protein to be detected.

Retrovirus or lentivirus vectors will be constructed that express a variety of genes, either from human (or other mammalian species), or from viruses such as EBV, that are of interest for their association with certain human cancers. Genes of initial interest include human telomerase, T-cell receptors and EBV latent genes and this list will continue to increase.

(In the accompanying assessment the genes of interest include inserts currently derived from: PRMT1, PRMT5, RUVBL1, USP7, USP11, MEP50, luciferase, FOXP1, BCLAF1, THRAP3, FXR1, FMRP, SPIN1 and corresponding shRNA sequences. Vectors also contain Cre recombinase for induction of LoxP mediated recombination of floxed alleles in mice.)

Evaluation of foreseeable effects

The effects of regulating expression of a targeted gene will depend upon the particular gene, and could range from no discernible effect to marked phenotypic changes. Similarly, the effects of expressing a transferred gene could range from negligible to profound. In both situations we consider the "worst case scenario" would be to bring about changes that could contribute to transforming a normal cell into a malignant cancer cell. For example, either shRNA-mediated downregulation of a tumour suppressor gene, or expression of either an oncogene or a gene associated with cellular immortalisation such as telomerase, could have such an effect. Although conversion of a cell from a normal to a cancerous state is believed to depend upon the accumulation of multiple such changes affecting growth control and cellular checkpoints, making it unlikely that accidental worker inoculation and infection of a modest number of the cells with the virus would lead to cancer, the seriousness of this potential consequence warrants the use of control measures afforded by Containment Level 2 to minimise the potential for such accidents.

Since retroviruses and lentivirus vectors insert their genomes into the chromosomal DNA of infected cells, there is an associated risk of insertional mutagenesis. There have been examples of leukaemias apparently resulting from insertional activation of an oncogene in human gene therapy clinical trials; however, these have only arisen in a minority of patients receiving such vectors, and involved doses many orders of magnitude greater than could occur in any foreseeable accident. Also, liver tumours have been reported following systemic injection of certain lentivirus vectors in foetal or neonatal mice. It is unclear whether these were caused as a result of insertional mutagenesis/oncogene activation, or whether WPRE present in some of the vectors may have contributed an oncogenic protein fragment. Although standard retrovirus vectors can only infect replicating cells, lentiviruses can also infect non-replicating cells, and thus would be expected to result in a greater level of cellular transduction following accidental worker exposure. Use of certain envelope proteins, such as VSV-G to pseudotype the viruses, can also increase stability of of the viruses to some extent, and is done precisely because it can result in more efficient infection of human cells.

Although this in principle increases the risk to workers, in practice it may be balanced by the fact that lower levels of virus are needed for the experiments than would otherwise be the case. Thus, although it must be recognised that accidental worker inoculation, either to mucosa or via percutaneous exposure could lead to stable gene transfer to cells in the body, on balance it appears unlikely that a foreseeable accident could result in a level of worker exposure that would be likely to result in tumorigenesis. Use of CL2 is considered appropriate to control the risks.

The possibility of generating replication-competent retrovirus or lentiviruses should also be considered. Replication competent retroviruses in their natural host species are associated with tumourgenesis, via insertional activation of oncogenes. The split packaging cells to be used for retrovirus generation and use of "2nd or 3rd generation" systems...
for lentivirus production make it very unlikely that replication competent viruses could be produced, and such systems
have a history of safe use. Use of SIN vectors adds a further level of safety, since this effectively limits the vector to a
single infectious cycle.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No notifiable Large GMOs are involved in this work, although work in animal models will be carried out (e.g. murine
models).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid and liquid waste normally inactivated by autoclaving. Surface decontamination using 1% Virkon (unless
corrosion is likely, in which case Chemgene (1:100 - 1:200 dilution may be used for these applications). Autoclaving
achieves 100% kill.

Within animal facilities, bedding and associated waste is autoclaved and sent for incineration. There is no shedding
from animal. Any spills will be treated with Virkon, mopped up, placed in double yellow bags and removed for
autoclaving

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC has approved the accompanying assessment. Further information was requested on the vectors and the
inserts and it was felt that the assessment was quite complex, given the number of genes and vectors. The
assessment was rewritten to clarify these points.

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We wish to submit a series of assessments that form a programme of work that includes several laboratories, several pathogens and several activities to study the role of mobile genetic elements (plasmids and integrative conjugative elements) in the transfer of antibiotic resistance genes. This work focuses on the characterisation of mobile genetic elements carrying antibiotic resistance genes from human commensals and human pathogens that fall into ACDP category 2. Prior to GMO work, all mobile genetic elements will be sequenced to completion and excluded when genes encoding toxins are present.

The bacteria to be used include:
- Escherichia coli (non-vero-toxin producing)
- Klebsiella pneumoniae, and closely related species (K. variicola, K. quasipneumoniae) and Klebsiella aerogenes
- Salmonella enterica (non-typhoidal serovars)
- Staphylococcus aureus and coagulase-negative staphylococci
- Streptococcus pyogenes, S. pneumoniae, S. agalactiae, and viridans group streptococci (e.g. S. mitis and S. oralis)
- Enterococcus faecalis, E. faecium and other Enterococcus species
- Bacteroides fragilis and other Bacteroides species
- Clostridoides (Clostridium) difficile and other Clostridia, with the exception of Clostridium botulinum and other neurotoxin producing Clostridia
- Pseudomonas aeruginosa

Specific activities include:
1. cloning of DNA fragments (using site specific recombination) harbouring reporter constructs (genes encoding...
fluorescent proteins, including GFP, YFP, RFP and mCherry) and an antibiotic resistance marker (including erythromycin, tetracycline, ampicillin, spectinomycin, kanamycin) into mobile genetic elements that have originated from wild-type strains, including strains isolated from patients, hospitals, or the environment. Some of these elements have been extensively characterised (e.g. pKpQIL, pCT, pNDM), while others have not yet been characterised beyond their sequence.

2. conjugation, transformation or alternative methods of DNA transfer of the plasmids harbouring reporter constructs to strains of the same and different species in ACDP category 2.

3. These genetically modified mobile genetic elements within microorganisms will be used in murine and zebrafish infection models. The transfer of the modified mobile genetic elements into new bacterial hosts will be monitored using the fluorescent tags. All in vivo experiments will be performed according to protocols previously approved and in line with Home Office regulations.

### Recipient or parental organism

Hosts for activities 1-2 as listed above
Activity 1: Wild-type bacteria as described above
Activity 2 & 3: Wild-type bacteria as described above, bacteria as described above with selectable markers/genes encoding fluorescent proteins integrated on the chromosome, and other bacteria that are present in human-associated microbial ecosystems which fall into ACDP category 1 or 2.

### Host/vector system

Vectors for activities 1-3 as listed above:
Health and Safety Executive
Activity 1: Mobile genetic elements isolated from wild-type strains including hospital, patient, and environmental isolates.
Activity 2: Mobile genetic elements isolated from wild-type strains including hospital, patient, and environmental isolates.
Activity 3: Mobile genetic elements isolated from wild-type strains including hospital, patient, and environmental isolates.

### Origin & function

Donors of material for activities 1-3 as listed above
Activity 1 - Non-mobilisable vectors carrying antibiotic resistance genes and fluorescent reporter genes
Activity 2 & 3 - Conjugative plasmids developed in Activity 1 which carry antibiotic resistance gene markers and fluorescent reporter genes

### Evaluation of foreseeable effects

Human Health considerations
Health and Safety Executive
Activity 1: The integration of reporter constructs and antibiotic resistance markers in wild-type conjugative plasmids will result in a slightly increased risk to human health as the conjugative plasmid will carry an additional antibiotic resistance gene. Containment level 2 is appropriate.
Activity 2 & 3: The transfer of conjugative plasmids with a reporter construct and an antibiotic resistance marker will result in a slightly increased risk to human health as the new host strains will carry an additional antibiotic
resistance gene. Containment level 2 is appropriate.

Environmental considerations:

Activity 1: All waste is autoclaved before leaving the building so it is very unlikely that GMOs or constructs will escape into the environment. If by some chance they did escape the mobilisable plasmids will have a fitness defect, due to the cost of carrying the fluorescent reporter construct, that minimises the chance of the plasmid spreading successfully in the environment. The environmental impact of this activity is therefore negligible.

Activity 2: All waste is autoclaved before leaving the building so it is very unlikely that GMOs or constructs will escape into the environment.

Activity 3: All of the proposed work will be conducted within contained animal facilities. Animals are monitored carefully to ensure high standards of welfare and thus any unexpected effects of the GMO will be rapidly detected. Import/export of animals and entry/exit of staff is carefully regulated from the facility and hence the likelihood of an inadvertent release of a GMO to the environment is negligible. Furthermore, a quarantine procedure is in place to prevent researchers entering any other part of the building (containing 'clean' animals) for 48 hours after being in the containment suite. Dedicated PPE (Full suit, hair net and mask) is provided and must be worn. Shedding from the animal is possible, particularly with oral gavage. Bedding and all animal waste will be autoclaved and sent for disposal via the clinical waste stream for incineration, hence the likelihood of an inadvertent release of a GMO to the environment is negligible.

No notifiable Large GMOs are involved in this work. Animal model work would involve zebrafish and murine models.

Work in animal models will be carried out in the animal facility’s containment suite:

- The containment suite has a "Sanirack" system, which is HEPA-filtered for the animal and on the exhaust. Cages are sealed and, on removal from the rack, both cage and rack are sealed from the room. Cadavers and bedding are autoclaved before disposal and are then sent for incineration via the clinical waste route. Cages are also autoclaved before being processed for cleaning.
- Work can be carried out in a Class 2 safety cabinet.
- PPE includes gowns, gloves and FFP3 face masks.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All GMMs will be fully contained. No derogation requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

After use, solid media and plastic/glassware that have come into contact with GMMs will be autoclaved (50 mins, 127 degrees) and disposed of. Effectiveness of waste autoclave is regularly monitored and all runs are validated with internal temperature monitoring and reporter tape.

Two general purpose chemical disinfectants are used: Virkon (peracetic acid; CAS:79-20-0) and Chemgene, which combines a variety of active ingredients including: alcohols (CAS:68439-45-2), quaternary ammonium compounds (CAS: 68424-85-1), chlorhexidine (CAS:18472-51-0), Didecyltrimethylammonium chloride (CAS: 7173-51-5), and Bis(3-aminopropyl) Dodecylamine (CAS: 2372-82-9). It has activity against bacteria (including mycobacteria, and spore forming bacteria), fungi, and viruses.

Liquid waste containing GMMs will be inactivated with Virkon (exposed to 1 % Virkon for a minimum of 30 minutes) or Chemgene (exposed to 10% Chemgene solution for a minimum of 60 minutes). This will result in 100% kill of bacterial cells, including spores. All waste (aside from disinfected fluids) will be autoclaved as described above.
All animal bedding and associated animal waste is autoclaved before disposal via the clinical waste stream for incineration (100% kill).

It is not clear whether the resistance plasmids will still carry the "naturally occurring" resistance genes and whether these are genes for resistance against clinical drugs and whether you will therefore generate pathogenic strains with resistance against clinical compounds. It seems to me that putting these markers into the resistance plasmids should not create a danger. However, the researcher does not seem to specify how the insertion of these marks into the plasmids will be done and whether they will be inserted at a specific location and what location will be if known. Knowing how insertion is being done will indicate whether it will occur at a specific location or is random. It is conceivable that insertion at specific location could derepress certain genes which might have harmful phenotypic consequences that could be assessed with a knowledge of the plasmid.

Clarification was also requested on how this assessment linked into others within the department. Copies of these (approved and, where appropriate, notified) assessments were supplied.

Please enter comments on the GM safety committee on the risk assessment

It is not clear whether the resistance plasmids will still carry the "naturally occurring" resistance genes and whether these are genes for resistance against clinical drugs and whether you will therefore generate pathogenic strains with resistance against clinical compounds. It seems to me that putting these markers into the resistance plasmids should not create a danger. However, the researcher does not seem to specify how the insertion of these marks into the plasmids will be done and whether they will be inserted at a specific location and what location will be if known. Knowing how insertion is being done will indicate whether it will occur at a specific location or is random. It is conceivable that insertion at specific location could derepress certain genes which might have harmful phenotypic consequences that could be assessed with a knowledge of the plasmid.

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Project Containment

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Project Ref 116/20.2

Date Ackn'd 17/04/2020  
CU2 Project Title Cell Biology of Poxvirus Infection  
Class 2  
Culture Class CultureVol  
< 1 Litre
The laboratory focuses on determining the mechanisms by which Vaccinia virus subjugates host cells proteins and cellular functions. This work relies heavily on the use of recombinant viruses for cell, molecular, biochemical and virology based experimentation.

(**The following viruses will serve primarily as controls and these stocks will be stored and not used without further risk assessments being written, which will then be considered by the GMSC and be added to this connected programme of work if appropriate. They are all HG2 pathogens. They all fall under the experimental procedures outlined for vaccinia. In all cases the volumes worked with will be far below that outlined for vaccinia GMM protocols. In all cases the GM viruses are equivalent to or attenuated when compared to the respective WT virus. Those viruses are:

- Human Cytomegalovirus (HCMV): Strains: WT (AD169) IE2GFP (Viable Human Cytomegalovirus Recombinant Virus with an Internal Deletion of the IE2 86 Gene Affects Late Stages of Viral Replication) UL32GFP (Viable Human Cytomegalovirus Recombinant Virus harboring a EGFP-tagged version of UL32 in place of the endogenous copy)
- Influenza A Virus: Strains: WSN (WT H1 N1) rVII1 (Amantadine sensitive) PR8/NS1-GFP (lab strain H1 N1 with nonstructural protein 1 tagged with GFP) X31 (WT H3N2) Semliki Forest Virus: Strains: SFV-4 (WT) SFV-4-ZsG (WT that expresses ZsGreen fluorescent protein replacing non-structural protein 3) Respiratory Syncytial Virus Strains: RSVA2 (WT) RSV-RFP (WT that expresses RFP) RSV-GFP (WT that expresses GFP Simian virus 40 Strains: WT) Wild type and recombinant vaccinia viruses [strains western reserve (WR) and International health department strain J (IHDJ)] expressing EGFP, mCherry or containing viral structural proteins modified by EGFP, mCherry, HA, FLAG sequences, or a combination thereof.

Recipient or parental organism

Wild type and recombinant vaccinia viruses [strains western reserve (WR) and International health department strain J (IHDJ)] expressing EGFP, mCherry or containing viral structural proteins modified by EGFP, mCherry, HA, FLAG sequences, or a combination thereof.

Host/vector system

The function of the inserted material is to provide means of visualization, purification, and detection of virus particles or their structural constituents. The plasmids used for recombination (pJS4 and pUC19) pose no risk of direct or indirect effects, are not replication competent in mammalian cells and pose no possibility of gene transfer to host cells as they do not contain human sequences. Both pUC19 and pJS4 are mob-minus plasmids. The aforementioned tags
GFP, mCherry, HA and FLAG have been incorporated individually or in combination into individual recombinant viruses of each virus strain (WR, IHD-J and Copenhagen)

Origin & function

The GFP sequence was originally derived from Aequoraea victoria, and modified to produce mCherry. HA is derived from influenza virus and FLAG is an artificially derived sequence. All VACV genes that have been tagged are left intact. The vast majority of recombinants (-200) are in the WR background, with only a subset of recombinants made in IHD-J (-10) and Copenhagen (-20). In all cases, the recombinant viruses are attenuated or of equal replicative capacity as their WT variants as assessed by gene expression profiles, 24-h yield and virus spread (plaque assay).

Evaluation of foreseeable effects

Human Health: The wild type viruses are the most virulent. The recombinant viruses will either express fluorescent proteins to mark stages of gene expression or package fluorescent structural proteins for visualization of virus particles. All of these insertions either have no impact on virus pathogenicity or limit the pathogenicity of the virus. Vaccinia virus is used as a live vaccine against smallpox Virus, thus it is routinely used in humans. The likelihood of laboratory exposure is very limited. Exposure requires sub-cutaneous injection and does not occur via aerosol or direct contact with intact skin. To further lower the chance of exposure, only workers trained in good laboratory practice (as specified in local Codes of Practice for laboratory workers) will handle infectious material.

Environmental: Vaccinia virus poses no risk to the environment. It cannot replicate independently, is not a plant pathogen, and displays low stability under environmental conditions. In addition, vaccinia has little capacity to spread from human to human. It can be isolated in the wild from cows and buffalo yet no spread among herds or crossspecies has been reported. No vaccinia outbreak, either natural or accidental (laboratory) has been reported. Although capable of rapid spread among rodents in proximal contact (within individual cages in animal housing facilities, but not cage-to-cage) the rapid disease progression and high mortality rates limit spread within populations. (Espisito, J., Fenner, F. 2001. Poxviruses. In Fields Virology, 4th ed., Vol. 2. Lippincott-Raven, Phila. 2887-2916.)

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - full CL2 applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

5% Virkon which results in a degree of killing of 100% of Vaccinia virus on contact (Butcher and Uleato 2005, Contact inactivation of orthopoxviruses by household disinfectants), Distel (Trigene): ATS labs study 'Virucidal efficacy of a disinfectant for use on inanimate environmental surfaces' March 2007. EC50 of 1 % Distel in vaccinia stock solutions containing 1 x1 OE9 units of infectious virus/milliliter which is equivalent to 99.9% killing.

All solid material (plastic dishes) potentially containing virus will be collected in autoClave bags contained within metal housing containers and autoclaved. Smaller plastic objects (tips used to handle virus or virus containing liquid) will be inactivated by autoclaving. The vast majority of work is carried out in flasks with caps. As VACV is only delivered through wounding, round dishes in autoclave bags pose no foreseeable threat. There has never been a reported instance of VACV infection via petri dish. In addition, the fewest number of handling steps involving liquid when...
dealing with infectious material is preferred. Liquid waste will be collected in aspiration bottles containing Distel Advanced Virucidal solution (1% for at least 24h) prior to disposal. For routine infection work disposable polystyrene aspirating pipets will be utilized for aspirating virus containing media into proper disposal containers. All aspiration will be followed by disinfection of disposable polystyrene aspirating pipets through aspiration of 5% Virkon which results in a degree of killing of 100% on contact (Butcher and Uleato 2005, Contact inactivation of orthopoxviruses by household disinfectants). Autoclaving achieves 100% kill.

The GMSC was happy with the classification of the work and merely requested clarification of some points, particularly around disinfection. As described earlier, other viruses, which may be used as controls, are mentioned within this assessment, but individual assessments will be written for these viruses, should they ever be needed.

**Project Containment**

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**Project Ref** 116/20.3

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<td>25/09/2020</td>
<td>Studying host defences against Toxoplasma Gondii using genetically modified models</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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**Date Project Ceased** 02/03/2022
Toxoplasma gondii (Toxoplasma) is an obligate intracellular eukaryotic parasite that has a broad host range including humans. Latent Toxoplasma infection is widespread among the human population (approximately 30% of the world’s population is seropositive for Toxoplasma), while life threatening infection generally only occurs in individuals with weakened immune systems or neonates. Toxoplasma is able to invade any nucleated cell from warm blooded animals, inside the cell it resides within a parasitophorous vacuole (PV) – a membrane enclosed compartment derived from the host cell membrane into which the parasite inserts its own virulence associated proteins.

The long-term aim is to identify novel pathways and mechanisms which host cells use to recognise and restrict the growth of Toxoplasma within the PV. Host cell defences are able to restrict parasite replication especially if the cells are activated by the cytokine IFN-gamma.

Genetically modified Toxoplasma will help to experimentally test different aspects of the pathogen’s pathogenicity. The genetically modified Toxoplasma used will fall into two categories – fluorescently tagged Toxoplasma for microscope imaging (e.g. constitutively expressed GFP, mCherry) and Toxoplasma which have had a specific virulence associated gene deleted.

In order to probe the immune response both in vitro in tissue culture cells, we will employ the fact that Toxoplasma is amenable to genetic modification. By using parasites either knocked-out for genes that potentially influence immune recognition or infectivity, as well as parasites that transgenically express tagged versions of Toxoplasma effector genes, we will be able to pinpoint the molecular mechanisms of this recognition.

Host: Toxoplasma gondii - no genetic modification will be undertaken in the lab, but GM strains will be used.

Recipient or parental organism

N/A

Host/vector system

N/A

Origin & function

No modification carried out by this lab - strains obtained from elsewhere. Previous modifications made by other labs and now used as standard community strains routinely include fluorescent and bioluminescent proteins expressed under a constitutively active promoter for imaging applications (see below).

Toxoplasma strains genetically deleted for effector proteins or expressing transgenic effector proteins were constructed using the antibiotic pyrimethamine by other laboratories. Some of the genetically modified Toxoplasma strains will be more virulent in mice or alter the nature of the observed response in tissue culture cells as compared to their parent strains. We will not use murine experimental systems. None of the genetically modified Toxoplasma strains will ever be more virulent than any other wild-type strain available. In other words, virulence factors from one strain of Toxoplasma are expressed in another strain in order to test if these virulence factors are the specific mediators of the immune response.
Fluorescent proteins – e.g. GFP, mCherry, tdTomato. These proteins emit light at a specific wavelength when stimulated with a different lower wavelength of light. They are commonly used in Biological sciences in conjunction with fluorescent microscopy / fluorescent spectrometry to localise and quantify proteins within an organism or cell.

Bioluminescent proteins – e.g. Firefly luciferase (from Photinus sp.). Luciferase is an enzyme that produces light as a by-product of the enzymatic degradation of a substrate – for biological sciences this substrate is usually luciferin. Bioluminescence is often used as an alternative to fluorescent proteins due to its increased stability in organisms and the requirement for no excitation light.

None of the genetically modified Toxoplasma strains are expected to present an increase in hazard to humans compared to the initial parent strains used to derive the genetically modified form from. The inserted gene products are all derived from different Toxoplasma strains or encode non-infectious, non-hazardous proteins (e.g. green fluorescent protein). No Toxoplasma strains will be used that express genes toxic to human health. Toxoplasma strains knocked out for virulence factors will only ever render the modified strain as virulent as another wild-type Toxoplasma strain.

Evidence of foreseeable effects

Evaluation of foreseeable effects

None of the genetically modified Toxoplasma strains are expected to present an increase in hazard to humans compared to the initial parent strains used to derive the genetically modified form from. The inserted gene products are all derived from different Toxoplasma strains or encode non-infectious, non-hazardous proteins (e.g. green fluorescent protein). No Toxoplasma strains will be used that express genes toxic to human health. Toxoplasma strains knocked out for virulence factors will only ever render the modified strain as virulent as another wild-type Toxoplasma strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation, full CL2 applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials that have come into contact with live Toxoplasma parasites will be first disinfected with Presept solution (one 2.5g tablet in 500ml water) for at least two hours. After this time Toxoplasma is no longer viable so liquid waste can be disposed of down the sink while solid waste can be placed into the autoclave waste stream.

Is an emergency plan required according to regulation 20? Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form N

Additional information on the strains was requested and this has been supplied in the form of an Excel spreadsheet. Further information was requested on the disinfectants/concentration used and this was added. The GMSC also asked how the lab would be managed to ensure the risks associated with the agent would be communicated to all lab users. The PI has set out an action plan for this.

Project Containment

Laboratory Activities

Glass Houses

Growth Rooms
### Project Ref 116/20.4

**Date Ackn’d**
25/12/2020

**CU2 Project Title**
Identification of pathogenicity and biocontrol factors in plant-associated bacteria and fungi

**Class**
Class 2

**CultureVolumeClass2**
< 1 Litre

**Consent Granted**
Non-GMM

**Project notified under transitional arrangements**
N

**Withdrawn**
N

**Tick if notifying a connected programme of work**
N

#### Project Additional Information

**Purposes of the contained use**

This notification relates to new research to study both pathogenic and beneficial bacteria and fungi associated with plants in relation to specific disease and pest systems. For the purpose of providing clarity on the experimental approaches to be adopted, the research is broken down into two main areas (projects): Project 1: The aim of this project is to identify pathogenicity factors in fungal plant pathogens. The roles of these genes in pathogenicity will be characterised by inactivating the targeted gene via targeted double-homologous gene disruption via Polyethylene Glycol (PEG)-mediated protoplast or Agrobacterium tumefaciens-mediated transformation (AGTM). The fungal strain harbouring the inactive gene is then examined for its ability to cause disease on their corresponding plant hosts. Complementation of knock-out strains will also be performed to verify the pathogenicity gene’s role in virulence. In some instances, fungal strains will also be altered to express fluorescent proteins so that their growth can be monitored in planta infections. Project 2: This project will aim to identify the mechanisms used by the bacteria to colonise plants and insects. Gene systems within these bacteria will be identified by genetic screens. These systems will be analysed further to ascertain what function each system plays and to examine the role in bacterial fitness. Comparative analysis of other plant- and soil-associated bacteria will be done to examine the evolution of these bacteria in the plant and insect environment. This project will also include an investigation of the genetic basis of host specificity and will define factors contributing to host range of plant pathogenic bacteria. It will be examined whether epiphytic populations of the bacteria contain virulence factors that regulate their ability to persist on cultivated and wild cherry, ash, and oak as well as herbaceous model plants Arabidopsis, bean and tomato. Knock-out, or deletion, of gene candidates possibly involved in host specificity, virulence and fitness will be produced to further determine...
function. We will also explore the genetic basis of bacterial interactions with bacteriophage. This will identify the mechanisms used by the bacteria to resist infection by bacteriophage.

In future there may be toxicity studies with biocontrol strains for controlling insect pests. This would involve using aphids such as Myzus persicae and larvae of Galleria mellonella.

Recipient or parental organism

Project 1: Recipient or parental strains will be the fungal wheat/barley pathogens, from the Dothideomycetes Class. Typical examples are Zymoseptoria tritici, Bipolaris sorokianae, Pyrenophora tritici-repentis, Parastagonospora nodorum. From comparative genomics projects some sister species of these fungi may also be used for comparative purposes, such as Parastagonospora avenaria and Pyrenophora teres-teres.

Project 2: Recipient or parental strains will be the wildtype strains of plant pathogens Pseudomonas syringae from the pathovars tomato, phaseolicola, aesculi, morsprunorum and syringae (can be epiphytic and endophytic, causing leaf spot (tomato), bleeding canker (horse chestnut), halo blight (bean) and canker (Prunus species like cherry), respectively; wildtype strains of Pseudomonas savastanoi pv. fraxini, causing bacterial canker on ash, and Brenneria goodwinii, Gibbisella quercicancans, and Rahnella Victoriana, causing Acute Oak Decline (the latter three are considered opportunistic pathogens), P. avellanae: 011, 48, 51, 592, 593, 595, 596, 598 (causing bacterial canker on hazelnut); P. viridiflava: 2848 (causing leaf blight on tomato); P. cichorii 9437, 907; P. marginalis 247, 667, 949 (causing bacterial canker on lettuce, celery and chrysanthemum); P. corrugata 2445 (causing pith necrosis on tomatoes); P. agaricis 2289 and 2472 (causing drippy gill on mushrooms); P. tolaasii 2192T (causing bacterial blotch on mushrooms); P. entomophila L48 (ubiquitous in soil, aquatic, and rhizosphere, insect pathogen); Xanthomonas sp. e.g. X. campestris 8004, 8397 (causing black rot on crucifers); Erwinia sp. e.g. E. amylovora CFBP430 (causing fire blight on apples and pears); Pectobacterium sp. e.g. P. atroseptica SCRI1143 (causing blackleg of potatoes); Dickeya sp. e.g. D. capitata (causing soft rot diseases on many crops); plus other strains of the bacterial genera/species listed here would likely be isolated from the environment.

Non-pathogenic or insect-targeting environmental bacteria will include Pseudomonas sp. including fluorescens SBW25, PFO-1, PFO2, WCS365, ATCC17400, F113, P667, P702, protegens (Pf-5), chlororaphis, poae (PpR24); P. putida KT2440, PAW340; P. jessenii; P. rhizospherae; Rhizobium sp. e.g. R. leguminosarum J1300 (symbiont of legumes); Arthrobacter sp. JS443; Pantoea sp. e.g. P. agglomerans (strains carrying biocontrol marker and not clinical isolates); P. stewartii; Paenibacillus and Bacillus sp. (non-pathogenic to humans, thus NOT B. anthracis or B. cereus); naturally isolated Citrobacter sp. (not C. rodentium).

These strains are from our own (or collaborators) collections, collected from environmental samples. The bacteria listed above are classified as ACDP level 1.

Host/vector system

Project 1: For vector construction the host/vector systems are standard cloning organisms, including non-pathogenic E. coli, S. cerevisiae and A. tumefaciens. Standard non-pathogenic E. coli strains (i.e. DH5α and Top10) will be transformed with the pPAN7-1 (hygromycin, ampicilin, GFP), pPAN8-1 (bleomycin, ampicilin), ppk2 (hygromycin, kanamycin resistances, GFP) or pYAE (hygromycin, kanamycin, GFP) vectors. pYAE contains a yeast origin of replication and can be grown in S. cerevisiae strain BJ5464 (cloning intermediate). A. tumefaciens strain LBA1126, contains helper plasmid pAL1100spec (used for fungal transformation), this strain also contains resistance to rifamicin. Ppk2 and pYAE vectors can both be cloned into and replicated within A. tumefaciens. A. tumefaciens is used to transform the plant pathogenic fungi listed above.

Project 2: Standard non-pathogenic strains of Escherichia coli (principally DH5α and S17-1 or S17-1λpir and Top10) will be used as hosts for vectors and used for conjugation of the vector into recipient bacterial strains. All vectors function to carry genes (Broad or Narrow host range) or to mobilise plasmids (helpers).

Broad host range vectors: pML122; pDSK600; pJJ3200; pGfpgus; pBBR1MCS-based vectors (#1-5); pME6010, pME6030 and pME6031; pRK415; pLAFR3.

Narrow host range vectors: pULIC3; pLVETD; pUT.

Helper plasmids: pRK201; pRK2073.

Cloning vector: pCR2.1 and TOPO derivative.

Transposons: IS-Omegon-Km/hah.

pK18mobascB will be used for knock out by homologous recombination.

Origin & function

Project 1: Details of the resistance or fluorescent markers found in the plasmids are as follows: NptII encodes the neomycin phosphotransferase II enzyme which permits metabolism of the antibiotic geneticin (G418). Hph encodes the Hygromycin B phosphotransferase enzyme which permits metabolism of the antibiotic hygromycin. GFP
The patient pathogenic fungi covered under this project have never been known to infect humans or animals, therefore the risks that they pose to human/animal health are considered very low. Furthermore, the resistance genes and GFP marker that will be used to select for GM strains, have been used routinely in molecular biology for many years. GM plant pathogenic fungi that carry these markers have never been reported to harmful to human or animal health. The genetic modifications proposed are primarily knock-outs of suspected plant virulence-related genes, thus we expect that GM fungi will be less virulent than their respective WTs. Some ectopic transformants will have similar virulence levels to the WT strains. Therefore, should an accidental release of a GM strain occur it is very likely that these strains would be equal or less fit than the natural populations of fungi and would be outcompeted by natural strains. The Dothideomycete pathogens covered are pathogenic on wheat and barley and pose little to no threat to other plant species. Only splashed dispersed asexual spores will be generated for experiments in this project. These spores are not capable of being aerially dispersed over longer distances. The location of the GM work is in the CAT2 containment facilities at the School of Biosciences, which is in the centre of Birmingham city and several kilometres away from any open wheat/barley fields. Therefore, in the unlikely event of accidental environmental release the likelihood of a GM strain finding a suitable host near the Biosciences building is also deemed to be extremely low.

Project 2: The plant pathogens being used in this project are ubiquitously present in the environment (on plants/water/soil). There is no evidence that environmental Pseudomonas species (not including P. aeruginosa), or other bacterial species mentioned above are harmful or pathogenic to humans. Several strains can be toxic to some insects, but genetic modification is unlikely to increase their infectivity to insects and will not cause them to become pathogenic to humans. The E. coli strains used for cloning are non-pathogenic to humans and do not colonise the human gut. The vectors and inserts do not pose a hazard to human health and safety. None of the strains listed have any known virulence or toxic effects on animals and humans. In the highly unlikely event of accidental release, the mutants of plant pathogens or insect-toxic strains will likely have attenuated virulence and pathogenicity on plants or insects (probably be unable to propagate to the same population levels as the wildtype), while other mutants may be neutral (have no changes in their pathogenicity). Much more rarely, strains may have increased virulence, due to overexpression of a virulence factor giving more severe symptoms, but in doing so this is highly likely to incur an overall fitness cost on the bacterium and thus likely making the alteration subject to compensatory mutations that reduce virulence while increasing fitness. Therefore, control measures and maintenance of good laboratory practice within the class 2 laboratories will be in place to prevent and eliminate any dispersal of the strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation – CL2 applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Project 1 and 2: All infected plant material and disposable laboratory consumables will be placed in autoclave bags and autoclaved before disposal. Transgenic material is autoclaved at 127°C for 35 minutes. This cycle kills all plant cells and microorganisms. The temperature is monitored and recorded by a probe placed in the centre of one of the bags being autoclaved. The autoclave is regularly checked and serviced by the manufacturer. Autoclave conditions are automatically recorded and archived.
The GMSC requested further information on strains used, along with their characteristics – e.g. whether they were pathogenic, their host range, etc, and in the case of existing strains, whether these were taken from a commercial source or an existing strain collection.

They also raised questions around the likelihood that more virulent strains may be generated - for example, by knockout of a transcription factor that represses expression of a virulence factor, or whether, when inserted into E. coli, any of these genes would introduce novel functionality e.g. attachment, invasion, toxin generation.

Because the work involves targeting toxins and secretion systems, both classes of gene that committee members thought were virulence factors (certainly in human pathogens), clarification was requested on whether these were virulence factors in plant pathogens.

The committee also asked that the risk posed by transfer of broad host range plasmids to other environmental species be considered in more detail, and also requested reassurance that there was no risk to human health from any of the modifications.

There were also requests for more information on the controls used, particularly around waste treatment.

All of these points were clarified in the assessments and final versions are supplied with this notification.

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Animal Units: L2 L3 L4

Large Scale Activities: L2 L3 L4

Human Clinical Applications: L2 L3 L4

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### Project Ref 116/96.1

Date Ackn'd: 14/10/1996

AN INVESTIGATION INTO THE MECHANISMS OF ANTIBIOTIC RESISTANCE OF

Class: 2

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LABORATORY MUTANTS AND CLINICAL ISOLATES OF BACTERIA

Date Project Ceased

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

GM116/97.3, GM116/01.4

Historical Date of Additional Info

09/01/1997, 27/06/2001,

Historical Significant Changes

GM116/97.3, GM116/01.4

Historical Date of Additional Info

09/01/1997, 27/06/2001,

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref 116/97.1

Date Ackn’d 20/05/1997

CU2 Project Title STUDIES ON QUINOLONE RESISTANCE

Class 2

CultureVolClass2

Consent Granted not applicable

Project notified under transitional arrangements Y

 Withdrawn N

Historical Significant Changes GM116/98.1, GM116/98.3

Historical Date of Additional Info 23/07/1998, 05/05/1998,
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 116/98.4

Date Ackn’d 26/10/1998  
CU2 Project Title OVER EXPRESSION OF FIBROBLAST GROWTH FACTORS & DOMINANT  
Class 2  
CultureVolClass2  
CultureVolumeClass3-4

26/10/1998

OVER EXPRESSION OF FIBROBLAST GROWTH FACTORS & DOMINANT

Class 2
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<th>NEGATIVE FIBROBLAST GROWTH FACTOR RECEPTORS IN HUMAN THYROID CELLS IN CULTURE</th>
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Withdrawn N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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<td>L3 L4 L2 L3 L4</td>
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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 116/98.5

- **Date Ackn’d**: 11/01/2001
- **CU2 Project Title**: HIV PACKAGING AND VECTOR STUDIES
- **Class**: Class 2
- **Culture Vol Class 2**: not applicable
- **Non-GMM Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  [N]

If yes, tick to confirm that it is attached to this form  [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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02/03/2022
Project Ref 116/99.3

THE STUDY OF THE TOPOGRAPHICAL ORGANISATION OF AUTONOMIC NEURONES RELATED TO FUNCTIONS WITHIN THE BRAIN WHICH REGULATE HOMEOSTASIS

07/12/1999

Non-GMM not applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 116/trans1

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<th>CultureVolumeClass3-4</th>
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<tr>
<td>31/01/1994</td>
<td>GROWTH OF RECOMBINANT VACCINIA VIRUS CONTAINING SEQUENCES OF</td>
<td>Class 2</td>
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</table>
We propose to generate insertional mutants of the fungi Crytociccis neoformans and Cryptococcus gattii by means of T-DNA integration. Transgenic Cryptococcus strains will then be screened for altered virulence in the model host Caenorhabditis elegans and in the macrophage cell line J774.

Recipient or parental organism

Agrobacterium tumefaciens (primary), Cryptococcus neoformans and Cryptococcus gattii (secondary). E.coli DH5a will be used for routine cloning manipulations.

Host/vector system

Vectors – pBlN19 contains Streptomyces faecalis-derived kanamycin-resistance gene (kan R) with a prokaryotic promoter allowing selections of E.coli and Agrobacterium cells bearing the plasmid an Escherichia coli origin of replication (ori), and the two boundary sequences from the T-DNA region of the Ti plasmid. These two boundary sequences recombine with plant chromosomal DNA, inserting the segment of DNA between them into the host (Cryptococcus) DNA. Broad host range (most plant species, some fungi).

Origin & function

Nourseothricin acetyltransferase (NAT) gene controlled by C.neoformans ACT1 promoter Neomycin resistance gene controlled by C.neoformans ACT promoter. Derivates of the green fluorescent (GFP derivaties, BFP derivates, YFP) and derivates of the red fluorescent protein as well as other commonly used reporter genes.

Evaluation of foreseeable effects

Human health effects: having considered the nature of the modified organisms, the nature of the transgenes added and the nature of culturing and testing the yeast, we cannot identify and enhanced risk to human health above that normally associated with the culture of C.neoformans of C.gattii, both ACDP II organisms. The bacterial selection genes are widely used and the structural comparisons of the anti-fungal substances nourseothricin and gentamycin do not show any similarity to commonly medically used fungicides. We do not therefore anticipate additional risks to human or animal healthy from these selectable markers. The second stage of this protocol, to attempt homologous gene disruption, is intended to disable host genes. As such, transgenic strains are likely to be reduced, rather than increased, in virulence.

Environmental effects – Cryptococcus neoformans is already widespread in the UK environment (especially in soil). Accidental release would distribute Cryptococcus strains carrying a fungicide resistance gene that is used for selection of transgenic yeast or the GFP gene. As the commonly medically used antifungals show no structural similarity to these fungicides, we do not expect any complications in patients' treatment. It is unlikely that the release of GFP carrying Cryptococcus in the wild would have
any effect on the environment. Mating between cryptococcal strains is thought to be extremely rare in the wild, hence it is unlikely that these transgenes would enter the indigenous Cryptococcus population. Further insertion mutants (opposed to targeted mutants) could show increased or decreased virulence, but no spores (the infective agent) are produced in routine cultures. Spores are produced during sexual reproduction of two opposite mating types or pseudohyphal growth. Mating and Fruiting as well as pseudohyphal growth needs to be stimulated by certain environmental factors such as starvation, desiccation or pheromones. As none of these requirements are fulfilled under applied culture conditions, no spores will be produced in the lab. Bacterial and yeast cultures are to be autoclaved after use. Cryptococcus will be handled in a safety cabinet. Glycerol cultures of the GMO will be stored in a lockable freezer and access will be restricted to authorised personnel.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - Full CL3 applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable plasticware will be rinsed in 10% Trigene, autoclaved within the CL3 laboratory and disposed of via the clinical waste system, ultimately being incinerated. Disposable gloves, paper towels etc will be deposited in autoclave bags and autoclaved within the facility before disposal through the aferementioned clinical waste route. Autoclave within CL3 lab - run of 30 mins at 135C, all runs recorded.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC and local Advisory Group for the Control of Biological Hazards have scrutinised the assessment (along with the preceding Class 2 assessments) very thoroughly. Committee members met with the Principal Investigators to discuss the work on a number of occasions. Comments on this particular assessment are attached. The final assessment has taken these points into consideration.

Emergency procedures are laid out in the accompanying risk assessment. It was felt that an Emergency plan was not necessary because of the nature of the GMO in this case, but laboratory procedures are in place to deal with any accidental releases or exposures in the laboratory.

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
**Project Ref** 125/01.1

**Date Ackn'd** 04/04/2006

**Date Project Ceased**

**Class**

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**CU2 Project Title**

INVESTIGATION OF THE INFLUENZA VIRUS GENES INVOLVED IN VIRUS INDUCED APOPTOSIS

**Historical Significant Changes**

TRANSFERRED FROM GM CENTRE 125 - 4/4/06.

**Tick if notifying a connected programme of work** N

**Project notified under transitional arrangements** Y

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref**  125/01.2

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Withdrawn  N

Tick if notifying a connected programme of work  N

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMVs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 125/01.3

Date Ackn'd: 04/04/2006

CU2 Project Title: CLONING AND OVER EXPRESSION OF SOLUABLE INORGANIC PYROPHOSPHATASES IN E.COLI

Class: Class 2

Non-GMM: Not Applicable

Consent Granted: Not Applicable

Withdrawn: N

Historical Significant Changes: TRANSFERRED FROM GM CENTRE 125 - 4/4/06.

Project notified under transitional arrangements: Y

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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### Project Ref 125/01.4

#### Date Ackn'd
04/04/2006

#### Date Project Ceased

#### CU2 Project Title
EXPRESS THE ACID PROTEASE GENE OF YARROWIA LIPOLTICA IN E.COLI

#### Class
Class 2

#### CultureVolClass2

#### CultureVolumeClass3-4

#### Non-GMM
Consent Granted
Not Applicable

#### Project notified under transitional arrangements
Y

#### Withdrawn
N

#### Tick if notifying a connected programme of work
N

#### Historical Significant Changes
TRANSFERRED FROM GM CENTRE 125 - 4/4/06.

#### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 125/01.5

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<td>IDENTIFICATION OF METAL RESISTANT BACTERIA AND ARCHAEA</td>
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Withdrawn

Tick if notifying a connected programme of work

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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**Project Ref** 125/02.1

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<td>THE USE OF RECOMBINANT CORYNEBACTERIUM GLUTAMICUM AND MYCOBACTERIUM BOVIS BCG TO EXAMINE GENE FUNCTION</td>
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Non-GMM

Consent Granted

Not Applicable

Withdrawn

Tick if notifying a connected programme of work

Yes

Historical Significant Changes

TRANSFERRED FROM GM CENTRE 125 - 4/4/06.

**Project Additional Information**
Purposes of the contained use

The objectives of the programme of work are to construct and characterise mutants of Corynebacterium glutamicum and Mycobacterium bovis BCG. These mutants will be used to analyse well-defined, non-toxic, mycobacterial cell wall components and genes that contribute to the basic physiology of corynebacteria and mycobacteria.

Recipient or parental organism

Disabled laboratory strains of E. coli including or similar to those listed in Part 2A, Annex II of the ACGM Compendium of Guidance.

C. glutamicum and M. bovis BCG. M. bovis BCG is one of the oldest and most widely used live vaccines; about 3 billion doses of this attenuated M. bovis strain have been used to immunise individuals against tuberculosis worldwide. Whole genome comparisons of M. tuberculosis H37Rv and M. bovis BCG (Pasteur) have now been undertaken to determine the genetic determinants of attenuation. These reveal several (14) large deletions in the M. bovis BCG (Pasteur) genome which encompass 119 open reading frames (Behr et al., 1999 Science 28: 1520-3).

The ACDP categorisation of pathogens according to hazard and categories of containment (fourth edition, 1995), classifies M. bovis BCG as hazard group 2. However, appendix 17 (page 108) states "The BCG strains of M. bovis and the so-called 'vole bacillus' M. microti have both been used in vaccines, but if mishandled, can give rise to abscess formation. The BCG strains are included in Hazard Group 2 because of this and the extremely remote possibility of reversion to virulence although when used under clinical conditions for immunisation, laboratory Containment Level 2 is not necessary". Staff working on the programme have previously been immunised with BCG.

C. glutamicum is known for its ability to massively excrete L-glutamate. The capacity of C. glutamicum to excrete glutamate has been used for more than 40 years to produce this amino acid on an industrial scale in ever increasing quantities, around 106 tonnes/year (Hodgson, 1994, Bio/Technology 12: 152-155). The ACDP categorisation of pathogens, according to hazard and categories of containment (fourth edition, 1995), classifies Corynebacteria spp. as hazard group 2. Although, C. glutamicum has been defined as having "biological limitations" which mean that it is unlikely to survive in the gut, lung and elsewhere. This description is also generally considered to cover laboratory adapted strains (particularly multiply auxotrophic or recombination deficient mutants) as well as other non-pathogenic hosts with a negligible capacity to persist in humans and a history of safe use.

Host/vector system

Any non-mobilisable or mobilisation defective including or similar to those listed in Part 2a Annex II of the ACGM Compendium of Guidance.

The pNIL, pGOAL and pYUB854 series of vectors for constructing suicide vectors to generate marked and unmarked mutants in M. bovis BCG.

The pK18mob suicide vector will be used to generate marked mutants in C. glutamicum.

Non-mobilisable shuttle vectors that can replicate in E. coli and mycobacteria (eg pJEM15, pMV261, pVV16, pPR27 and their derivatives). These vectors contain an E. coli origin of replication (from the pUC series of cloning vectors), a mycobacterial origin of replication from plasmid pAL5000 and antibiotic resistance markers (eg Kanamycin and hygromycin).

Origin & function

Source: C. glutamicum, M. bovis BCG and M. tuberculosis H37Rv

Genetic material: DNA fragments encoding various well defined genes involved in cell wall metabolism, eg glycosyltransferases, fatty acid biosynthesis, polyketide biosynthesis, siderophore biosynthesis, p450 enzymes and peptidoglycan biosynthesis.

Evaluation of foreseeable effects

PLEASE SEE ASSESSMENT FOR DETAILS

The risk assessment deals with the risks to human health and the environment under each specific activity. Most of the work will make use of crippled lab strains and non-mobilisable vectors, although wild type strains will be used to study the effects of defined chromosomal mutations on mycobacterial cell wall physiology and other
phenotypes. The risks to human health are therefore associated with the wild type host. Environmental risks are dealt with under each activity in the assessment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full level 2 applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All potentially infected material is autoclaved prior to disposal and incineration. 100% kill of GMMs. Disinfectants such as hycolin (2% v/v) and Chloros are available for spills.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Some members of the GMSC pointed out that the original assessment contained a reference to antibiotic resistance markers, but did not then list them or discuss them further. These were added.

The GMSC asked what were the 'well defined genes involved in cell wall metabolism and cellular physiology'? Examples were added to illustrate this, as a full list would have been extremely long.

Tje assess,ent jas neem approved by the GMSC.

Project Containment

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Animal Units

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Project Ref 125/03.1
**Project Additional Information**

**Purposes of the contained use**

The work is concerned with understanding how bacterial gene expression is regulated using *E. coli* K12 as a model. The strategy is to use genetics to identify regulatory components at particular loci and then to use genetics and biochemistry to understand the interaction between these components. This involves many genetically manipulated constructs in which altered regulatory elements and regulatory components are tested. Additionally genes encoding regulatory factors are manipulated to facilitate overexpression and purification of the corresponding protein.

**Recipient or parental organism**

Hosts: Standard disabled hosts such as *E. coli* K12 strains. For mutagenesis the hosts will be standard, well characterised strains of the wild type organism.

**Host/vector system**

Vector: Vectors will generally be pUC, pBluescript and related non-mobilisable CoIE1-based standard vectors, or mabda phage-based cloning vectors, or standard low copy number vectors such as pACYC or pSC101 and derivatives.

**Origin & function**

Inserts: Sequences used may be any gene or sequence including genes of unknown function, including virulence factors. Genes may be partial or complete and may include expression signals, eg segments from the *E. coli* lac, gal, mal, ara, mel nir, and nrf operons, the fnr, crp, rpoA, rpoBC, rpoD, rpoS, fis, ihfA, ihfC hupA, hupB and hns genes. Various 'anonymous' but completely sequenced regulatory genes from K12 or the Sakai 0157 strain. Non coli genes include *B. pertussis* cyaA, *V. fischeri* lux, plus various commercially available genes that are used as tools. Libraries may be constructed for limited purposes, eg two hybrid screening.

Donor. Sources are either whole genomic DNA or PCR-amplified known sequences, from *E. coli*. In general source DNA will be taken from an organism for which a whole genome sequence is known. No organisms requiring greater than containment level 2 will be used. In some experiments the starting material will be clones from other laboratories, eg encoding lac, gfp, lux, cya or other 'tools' for molecular biology.
Effects on human health: None of the constructs cause harm beyond that of the starting host and no risk additional to that of the parental wild type organisms is envisaged.

Environmental hazards: The potential hazard is negligible as the GMMs cannot be considered likely to express virulence or other factors that would be potentially harmful to other species or the environment. Accidental release is highly unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - full level 2 containment applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be autoclaved - a process that is validated by monitoring of the internal chamber. An automatic printout of every run is produced. Achieves 100% kill. Wastes from autoclaving will be disposed of to drain or to clinical waste incineration.

The standard disinfectant used is Virkon, 1% w/v solution. This will only be used to sterilise lightly contaminated surfaces, and is not used for treatment of substantial culture volumes, or for rendering safe contaminated matter such as absorbent towels - these will be autoclaved. Studies have shown the efficacy of 1% solutions to achieve typically at least 10 (to the power of 5) reduction in counts under appropriate conditions.

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<th>Is an emergency plan required according to regulation 20?</th>
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<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
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</table>

Please enter comments on the GM safety committee on the risk assessment

The assessment has been approved by the GMSC without adverse comment.

**Project Containment**

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**Project Ref 125/03.2**

**Date Ackn'd** 04/04/2006

**CU2 Project Title** CYTOSKELETAL GENE ANALYSIS IN ENTAMOEBA AND DICTYOSTELIUM

**Class** Class 2

**CultureVolClass** 1-50 Litres

**Date Project Ceased** 03/08/2006

**Consent Granted** Not Applicable

**Project notified under transitional arrangements** N

**Withdrawn** N

**Historical Significant Changes** TRANSFERRED FROM GM CENTRE 125 - 4/4/06. TRANSFERRED TO

**Origin of genetic material:** Dictyostelium strain AX3 - a free living non-pathogenic soil amoeba which grows at 22 degrees centigrade and is harmless to crops and agriculture.

Antamoeba histolytica strain HM-1:IMSS -- standard laboratory strain. Cysts are the only infectious stage of this parasite and these can not develop or form in standard lab cultures, hence the parasite is not transmissible to humans in this setting. The cultured form, ie the trophozoite, is very fragile and susceptible to desiccation and detergents. As they are unable to persist in the environment the chance of environmental damage is remote.

Genetic material: Full length cDNAs encoding Entamoeba Arp2 and Arp3 - major skeletal proteins involved in movement and cell shape. Selectable markers conferring

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Recipient or parental organism**

Hosts. E. coli XL1-blue - disabled and slow growing;

DH5 -as XL-blue.

**Vector**. pUC18 - standard molecular biological plasmid vector.

**Purposes of the contained use**

To express cDNAs encoding Entamoeba cytoskeletal and signalling proteins in both Entamoeba and Dictyostelium cells.
G418 resistance.

**Evaluation of foreseeable effects**

**Effects on human health:**

Dictyostelium - None. It is a non-pathogenic soil amoeba, unable to grow at human body temperature and is harmless to crops and agriculture.

Antamoeba histolytica - No likely risks. It is a parasitic enteric protozoan that is responsible for amoebiasis in humans. The disease is characterised by diarrhoea/dystentery, intestinal tissue invasion and potential liver abscess formation. Epidemiology, the organism is ubiquitous and has a worldwide distribution, although prevalence is much higher in developing, disadvantaged countries and tropical regions. In contrast, rates of infection are low in the developed world, with the majority of these infections being found in specific groups (e.g. travellers from developing or high incidence areas). Symptomatic invasive amoebiasis only develops in 10% of individuals harbouring the parasite. The organism can exist as both cysts and trophozoites. Transmission is feco-oral and results from ingestion of viable cysts in contaminated food or water. Cysts can remain viable for a long time depending on environmental conditions but are extremely sensitive to desiccation. Trophozoites on the other hand are more fragile and degenerate rapidly in the external environment as well as being easily destroyed by acidic pH (e.g. stomach acid).

As the only infectious stage of the parasite, the cyst, does not form in standard laboratory cultures the parasite is not transmissible to humans in this setting. Accidental infections have never been reported, but effective drug treatments are available should this happen.

Environmental hazards: Dictyostelium - negligible hazard. The strains used are very weak due to multiple mutations and would not compete with local Dictyostelium species.

Entamoeba - The organisms are very sensitive to desiccation and detergents and are not free-living. As they are unable to persist in the environment the chances of environmental damage are remote.

The work will be carried out at CL2 and the likelihood of accidental release is very low.

E. coli XL-blue/DH5 - non-colonising, disabled lab strains, no risk to human health or the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation applied for CL2 applied. The laboratory set aside for this work is a relatively new facility and is currently being fitted with a hand wash basin.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Dictyostelium - to kill spent cultures: 1% chlorine bleach.

Entamoeba - Tubes containing liquid cultures and contaminated disposables will be placed in autoclave bags and autoclaved at 121 degrees for 50 minutes and discarded as "clinical waste" (incineration).

Agar plates, loops, tips etc will all be autoclaved. 100% kill.

All methods are more than sufficient to kill Dictyostelium which are fragile, as well as Entamoeba which are sensitive to desiccation and detergents and are not free-living.

Bacterial cultures will be killed with Virkon at the recommended dilution (1:100)
I feel information on human health should be transferred to the relevant section; the nature of the "normal laboratory clothing and protective equipment" should be defined; the autoclave used for disposal of cat 2 waste should be monitored (it is anyway, why not say so for completion?); and the disinfection regimen should be defined, i.e. is 1% chlorine bleach 1% available chlorine, and what is the actual dilution of Virkon used?

POINTS ADDRESSED IN ASSESSMENT
* There is no mention of any possible alteration in phenotype resulting from the introduction of cytoskeleton and signalling proteins in Entamoeba and Dictyostelium. I assume they will not become (more) pathogenic but perhaps this could be addressed.

* No increase in virulence/pathogenic capacity is expected. As mentioned in the application, Dictyostelium are free-living non-pathogenic soil amoebae which grow at 22 degrees and therefore do not pose a threat to humans. Likewise Entamoeba does not pose a risk to humans in this/our setting because the cyst, which is the only infectious stage of the parasite, can does not develop or form in standard laboratory cultures, hence the parasite is not transmissible to humans.

The GMSC also asked whether the Class 1 and Class 2 work should be split into two separate proposals. The investigator felt this was not necessary and not desirable because the work is one experiment, in which cDNAs encoding Entamoeba cytoskeletal and signalling proteins will be expressed in both Entamoeba and Dictyostelium cells.

The GMSC has approved this assessment.

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Project Ref 125/05.1
Investigation into the role of downstream components of the FGFR cell signalling pathway in primary human cells.

The technique of RNA interference (RNAi) will be used to knockdown FRS2 (a component of the fibroblast growth factor receptor signalling pathway) in primary human cells. Use of a replication incompetent bicistronic HIV1 based lentivirus will be applied to deliver the SiRNA into cells.

Host: Host bacteria for growth of plasmids are standard disabled lab strains of E. coli such as DB3.1 and Stbl3. DB3.1 is an E. coli strain that is resistant to CcdB effects and can support the propagation of plasmids containing the ccdB gene. One shot Stbl3 chemically competent E. coli from Invitrogen are well suited for use in cloning unstable DNA such as lentiviral DNA containing direct repeats. Human cell line HEK 293T will be transfected with the pLenti4/V5-DEST and the packaging vectors and lentivirus harvested from the supernatant. Primary human cells that are non-permissive for production of replication defective lentivirus will be transduced with recombinant lentivirus expressing GFP and RNAi gene of interest. Further work may use mouse embryo fibroblasts.

Recipient or parental organism

Source: Green Fluorescent Protein from jellyfish. Sequence encoding a small part of FRS2 (approx 21 bases) from human and hairpin sequence needed for RNAi to work effectively.

Genetic material: The sequence encoding GFP which functions as a reporter molecule that fluoresces upon exposure to uv light. Also, a partial sequence encoding a small section of the FSR2 protein and a hairpin sequence needed for the short hairpin RNAs to be produced and work effectively. FSR2 is an adaptor protein that acts downstream of FGFR.
**Evaluation of foreseeable effects**

| Human health: In third generation lentiviral vectors all six regulatory/accessory proteins (Rev, Tat, Vif, Vpr, Vpu and Nef) are removed, except Rev. Rev acts at the post-transcriptional level and is necessary for HIV gag/pol expression. Rev binds to an RNA motif (Rev Responsive Element RRE) and facilitates the cytoplasmic export of gag/pol messenger. As an extra safety requirement Rev is placed on a separate vector (pLP2) to the transfer vector. Only 700bp of the HIV envelope protein are present in the transfer vector, plus RRE and the packaging signal. The packaging construct contains the minimal RRE of 374bp and the gag/pol genes. Through deletions in both LTRs and the absence of 5 of the 6 accessory proteins, including the replication essential Tat, the biosafety of the vector is increased. In published studies replication competent virus has never been detected. The HIV envelope protein is replaced by non-retrovirally envelope protein (VSV-G). It is highly unlikely that this will be incorporated in a new hybrid replicative virus. It is impossible that wild type HIV will be formed because of the omission of 5 accessory proteins and the HIV-env. No protein is expected to be expressed in bacterial strains used for plasmid propagation. |
| Environmental Considerations: Proteins will not be expressed in bacteria used for plasmid propagation. Bacteria used for propagation are attenuated strains that will not survive in the environment. The recombinant virus is replication defective and will not have an extended host range, the transgenes are innocuous, so there is negligible potential hazard to the environment. Potential hazard to species in the environment either from the viruses, or the E. coli containing the plasmids, therefore appears negligible. Containment conditions specified should prevent environmental release of virus, or of E. coli carrying associated plasmids. |

| Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants) |
| N/A |

| For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification) |
| No derogation. |

| Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate) |
| Liquid waste will be aspirated into, and exposed to, 1% Virkon for a minimum of 30 minutes, or collected into autoclavable pots and autoclaved before disposal to drain. Disposable solid waste will be inactivated by autoclaving with final disposal by incineration. Exceptions to this are: Contaminated micropipettor tips will be soaked in 1% Virkon for a minimum of 30 minutes before being rinsed and disposed of in Sharps containers, which will be sent for incineration. Disposable plastic pipettes also treated with Virkon prior to rinsing and disposal as above. Exposure to Virkon for a minimum of 30 minutes, or autoclaving effectively kills 100% of GMMs. |

| Is an emergency plan required according to regulation 20? |
| N |

| If yes, tick to confirm that it is attached to this form |
| N |

| Tick to confirm that you have attached a risk assessment to this form |
| Y |

| Tick if you are claiming exemption from disclosure for section of the risk assessment |
| N |

**Please enter comments on the GM safety committee on the risk assessment**

No adverse comments.

**Project Containment**
## Project Ref 125/94.1

### Date Ackn'd
04/04/2006

### CU2 Project Title
TRANSFORMATION AND GENE DISRUPTION IN PLANT PATHOGENIC FUNGI

### Class
Class 2

### CultureVol
Class 2

### CultureVolume
Class 3-4

### Non-GMM
Not Applicable

### Consent Granted

### Project notified under transitional arrangements
Yes

### Withdrawn
No

### Tick if notifying a connected programme of work
Yes

### Historical Significant Changes
GM125/97.2. - TRANSFERRED FROM GM CENTRE 125 - 4/4/06.

### Historical Date of Additional Info
12/11/1997

### Significant Change ID

### Date of Significant Change

### Project Additional Information

#### Purposes of the contained use

#### Recipient or parental organism

#### Host/vector system

#### Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]
If yes, tick to confirm that it is attached to this form [ ]
Tick to confirm that you have attached a risk assessment to this form [ ]
Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

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<td>04/04/2006</td>
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02/03/2022
Date Project Ceased

Non-GMM

Consent Granted
Not Applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
GM125/95.3 - TRANSFERRED FROM GM CENTRE 125 - 4/4/06.

Historical Date of Additional Info
02/10/1995

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Project Containment

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Animal Units
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- L4

Large Scale Activities
- L2
- L3
- L4

Human Clinical Applications
- L2
- L3
- L4

Project Ref 125/97.1

Date Ackn’d: 04/04/2006

CU2 Project Title
Mutagenesis of Pathogenic bacteria: Allelic replacement using antibiotic resistance markers and antibiotic selected transposen mutagensis

Class: Class 2

Consent Granted: Not Applicable

Project notified under transitional arrangements: Y

Historical Significant Changes
TRANSFERRED FROM GM CENTRE 125 - 4/4/06.

Tick if notifying a connected programme of work: N

Tick if you are claiming exemption from disclosure for section of the risk assessment: N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Ref 125/99.1

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<td>STRUCTURE, FUNCTION AND DYNAMICS DENDROASPIN AND VARIENTS</td>
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- Non-GMM Consent Granted: Not Applicable
- Project notified under transitional arrangements: Yes

#### Project Additional Information

- **Purposes of the contained use**
  - [ ]

- **Recipient or parental organism**
  - [ ]

- **Host/vector system**
  - [ ]

- **Origin & function**
  - [ ]

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**Historical Significant Changes**

TRANSFERRED FROM GM CENTRE 125 - 4/4/06.

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**Date Ackn’ed**

04/04/2006

**CU2 Project Title**

STRUCTURE, FUNCTION AND DYNAMICS DENDROASPIN AND VARIENTS

---

**Historical Date of Additional Info**

02/03/2022
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 125/trans1

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<td>GENE CLONING, CHARACTERISATION AND EXPRESSION FROM PATHOGENIC</td>
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Date Project Ceased

Withdrawn

Historical Significant Changes
TRANSFERRED FROM GM CENTRE 125 - 4/4/06.

Project notified under transitional arrangements

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 125/trans2

Date Ackn’d: 04/04/2006
CU2 Project Title: CLONING OF MOUSE CYTOMEGALOVIRUS

Class: Class 2
CultureVolClass2: 
CultureVolumeClass3-4: 
Non-GMM: Not Applicable
Consent Granted: 

Project notified under transitional arrangements: Y

Withdrawn: N

Historical Significant Changes: TRANSFERRED FROM GM CENTRE 125 - 4/4/06.

Tick if notifying a connected programme of work: N

Significant Date of Additional Info
Significant Change ID
Date of Significant Change

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
**Project Ref** 37/07.2

**Date Ackn’d** 11/01/2013

**CU2 Project Title** MOLECULAR AND CELL BIOLOGY OF BACTERIAL RESPIRATORY PATHOGENS

**Class** Class 2

**CultureVol** < 1 Litre

**Non-GMM Consent Granted** Not Applicable

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Construction of gene detection mutants or gene replacement mutants. Analysis of phenotype of altered mutants in invitro and in vivo (animal) systems.

**Recipient or parental organism**

- Streptococcus pneumoniae
- Neisseria meningitidis
- Staphylococcus aureus

**Host/vector system**

- Introduction of linear DNA generated by polymerase chain reaction (PCR)
- Transposon Tn917 for gene inactivation
- Plasmid pMTL23 for gene inactivation in Neisseria

**Origin & function**
Modified genes from the pathogens including virulence factors such as toxins, surface proteins and signalling molecules.

**Evaluation of foreseeable effects**

All the organisms used are carried as commensals in the normal human population. All genetic alterations involve the removal of genes or protein function such that the GMO will be no more harmful to people or the environment than the parental organism. In most cases the risk will decrease in the GMO due to the genetic modification partially disabling the organism. Antibiotic resistance markers will only be used if they are associated with the normally occurring population.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

none

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

none

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

GMO Liquid cultures (volume up to 100ml) will be autoclaved. Following autoclaving cultures will be sterile. Other material (equipment etc) will be sterilized by disinfection. Animals infected with GMOs are incinerated. No GMO will be released into the environment.

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Please enter comments on the GM safety committee on the risk assessment

The GMSC assessed this proposal on 21/2/2002. The committee was satisfied that this work could be carried out under the containment indicated and that the proposal could be classified class 2.

**Project Containment**

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**Project Ref** 553/09.6
**Project Additional Information**

**Purposes of the contained use**

The use of replication defective Lentiviral vectors to modulate expression of candidate genes to assess their role in tumourigenesis

**Recipient or parental organism**

E.coli K12 or B derivatives

Mammalian cell cultures - especially disabled hosts which should be recognised as foreign by the human immune system. Due to the presence of adventitious agents certain cell lines will be handled at Containment level 2.

**Host/vector system**

Standard Lentiviral vector systems, which produce replication defective virions. The system to be used is a third generation one. The expression vectors have a deletion in the 3' LTR which results in self inactivation. The number of HIV genes have been reduced to gag, pol, rev, tat and vpr. The envelope protein is VSV-G which allows amphotrophic infection. The genes are split onto four different plasmids. No replication competent virus can be produced.

**Origin & function**

Mammalian genes thought to be involved in Hereditary Leiomyomatosis renal cell cancer (HLRCC) such as Fumerate Hydratase, succinate dehydrogenase and G-protein coupled receptors.

Fumerate Hydratase is a nuclear-encoded protein that catalyses a step of the Krebs cycle, converting fumerate to malate. It is also thought to be a tumour suppressor as inactivated protein is seen in tumours from patients suffering from Hereditary Leiomyomatosis renal cell cancer.
Succinate dehydrogenase is an enzyme also involved in the Krebs cycle catalysing the oxidation of succinate to fumarate.

G-protein coupled receptors are transmembrane signalling receptors, which pass signals across cellular membranes to activate internal pathways.

Other genes identified during the course of the project may also be expressed/knocked down.

shRNA constructs to knock down expression of the above genes.

Standard reporter genes and antibiotic resistance genes

**Evaluation of foreseeable effects**

E. coli K12 or B derivatives are disabled hosts which cannot colonise the human gut and have a history of safe use. Genes are not expected to be expressed in these systems due to the lack of bacterial promoter.

Mammalian cell cultures are especially disabled hosts which should be recognised as foreign by the human immune system. Due to the presence of adventitious agents certain cell lines will be handled at Containment Level 2. As lentivirus integrates into the host DNA and lacks essential genes for replication, mammalian cells, which have been infected pose minimal risk.

293 cells (and various derivatives) are used to produce viral particles which are capable of one round of infection. These cells will be handled at Containment Level 2 with the use of sharps minimised.

Lentiviral particles are infectious but replication defective however they are capable of integrating into the host genome where they may cause insertional mutagenesis. Control measures are in place to minimise the likelihood of exposure however needles are required for inoculations.

Inoculations using transduced cells pose minimal risk to the worker as the lentivirus will be integrated into the host genome and replication defective. The cells should be recognised as foreign by the human immune system.

Inoculations using lentivirus capable of one round of infection poses the greatest risk due to the use of needles. Workers are trained in these procedures including the policy of not re-sheathing needles and disposing of intact needle-syringe assembly into sharps bins immediately after use.

Accidental exposure to a single gene is considered to be low risk.

Inoculations using lentivirus capable of one round of infection poses the greatest risk due to the use of needles. Workers are trained in these procedures including the policy of not re-sheathing needles and disposing of intact needle-syringe assembly into sharps bins immediately after use.

The genes themselves are involved in individual steps of the Krebs cycle and cellular signalling. Although the loss of fumarate hydratase is seen in HLRCC patients it is likely that other gene mutations are required for the progression of the disease.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste steam for landfill.

02/03/2022
Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycles as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annul validation)
Incineration, effectively 100% (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

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02/03/2022
Project Ref  559/09.1

MANIPULATION OF THE REGULATORY STATE OF HAEMATOPOIETIC CELLS

Date Ackn’d  05/11/2012

Date Project Ceased

Class 2  1-50 Litres

Non-GMM  Not Applicable

Class CultureVolClass2 CultureVolumeClass3-4

Tick if notifying a connected programme of work  N

Withdrawn

Historical Significant Changes

Project transferred from GM559

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To infect human or murine cells with retro- or lenti-viral gene delivery systems. As the result, cells will permanently harbour genes encoding (1) transcription factors, epigenetic regulatory molecules, cell cycle regulators or oncogenes, (2) small hairpin RNA, (3) RNA aptamers or (4) genes encoding peptide aptamers without any replications. These genes are continuously expressed or can be induced by addition of chemicals such as 17β-estradiol, Tamoxifen, or Tetracycline. Transduced cells will be used for DNA and RNA analysis, cultured in vitro or transplanted into immuno-compromised mice.

Recipient or parental organism

MMLV (Moloney murine leukaemia virus) or MSCV (murine stem cell virus) based replication defective, non-mobilisable retroviral vectors e.g. pBabe. These are well-established and widely used retrovirus based expression vectors with a history of safe use.

The third generation of lentiviral vectors, e.g. pHIV7, HR’CS-G and their derivatives, Drug inducible expression vectors, pLVX-Tight-Puro (Clontech) co-transfected with the regulator pLVX-Tet-On Advanced or pLVX-Tet-Off Advanced. Virus particles in this system are produced by the Lenti-X HT Packaging Mix which utilises a trans-lenti viral vector system.

Lentiviral vector systems in which shRNA constructs can be directly cloned, e.g. pKLO.1.

MSCV-PM-miR30, pPRIME-CMV-GFP-FF3, pPRIME-CMV-dsRed-FF3 or pPRIME-TET will be provided by Dr Stephen Elledge (Department of Genetics and Genomics Harvard Medical School).

MSCV-MigR1 will be provided by Dr. Warren Pear (Abramson Family Cancer Research Institute University of Pennsylvania).

MSCV-Ires-hCD2t will be provided by professor Meinrad Bussligner (IMP, Vienna).

pLVTHM and pLVET-ITR-KRAB will be provided by Dr. Didier Trono and Dr. Patrick Aebischer respectively (Ecole Polytechnique Federale de Lausanne).

pHIV7-GFP will be provided by professor John J. Rossi (Beckman Institute, CA, USA).

pLL3.7 will be provided by Tyler Jacks (MIT).
Other vectors are commercially available pBABE-puro and pSico (Addgene deposit), pMSCVneo, pMSCVhygro, pMSCVpuro and pLVX-Tight-Puro (Clontech), pKLO.1 (Sigma, Open Biosystems), pSLIK (ATCC).

A full list of components (inserts, vectors and eukaryotic cell lines) can be found as a separate attached document (Manipulation of the regulatory state of haematopoietic cells COMPONENTS.doc).

**Host/vector system**

Human and mouse cell lines including haematopoietic, fibroblastic, hepatic cells. Mouse primary cells including embryonic stem cells, haematopoietic cells and embryonic fibroblasts from wild type transgenic or knock out animals. These are categorised as belonging to biological hazard group 1.

Primary human cells such as CD34+ cells, leukaemic blast cells, myeloid cells and lymphoid cells from normal donors and patients. These are biological hazard group 2 due to the potential risk of infection from endogenous pathogens.

**Origin & function**

Generation, propagation and use of these retro- or lenti-viral expression vectors aim to introduce the coding sequence of transcription factors, oncoproteins, inhibitory RNA molecules or peptide aptamers into murine or human primary cells or established cell lines. The objective is to over-express or knock down the expression of targeted molecules or to interfere with their function into cells, and analyse alterations in the expression and structure of the target genes and cellular phenotypes.

**Evaluation of foreseeable effects**

The E.coli bacteria used are not pathogenic to humans or animals and are not expected to survive in the environment, they therefore belong to hazard group 1.

The cells which will be transduced in the project are either established cell lines, mouse primary cells or human haematopoietic cells. They are highly unlikely to be able to survive outside the culture vessel and are non-transplantable due to immuno-incompatibility. The use of Class 1 containment with good laboratory practice will be sufficient to limit hazards to human health and the environment from these cell lines. Primary cell lines are categorised as requiring containment level 2 due to the potential for adventitious agents to be present.

Packaging cells for retroviral production are either HEK 293T or NIH-3T3 derivatives and HEK 293T for lentiviruses. Prior to the induction of viral vectors or packaging vectors, they do not produce any viral products and are considered in the same way as an established cell line. Once virus vectors and packaging vectors are transfected into packaging cells, these cells are capable of producing viral particle which can infect human cells and have to be used at containment level 2. Once transduced, viral sequences are integrated into the host genome, and occasionally integrate in a position adjacent to oncogenes which thereby leads to a constitutive increase in the level of expression of these oncogenes due to the promoter/enhancer in the Long Terminal Repeat (LTR). Depending on the position, virus integration can cause the aberrant silencing or disruption of the endogenous genes. Lentiviral systems described in this assessment have a self-inactivating (SIN) mutation which abolishes LTR promoter activity so as to prevent the aberrant activation of endogenous genes. With third generation retrovirus systems, the retroviral structural genes are encoded on two different plasmids and contain additional mutations, and at least 3 non-homologous recombination events are necessary to generate replication competent virus. Consequently, they are highly unlikely to acquire a replicative ability in cells. Lenti-vector particles are generated by co-expressing the virion packaging elements and the vector genome in a细胞 as a producer. In the case of HIV-1-based vectors, the core and enzymatic components of the virus come from HIV-1, while the envelope is derived from a heterologous virus. To decrease the probability of generating replication competent recombinant (RCR) virus a second generation vector was created which has deletions in its crucial virulence factors. In order to further improve their safety a third generation of lentiviral vectors was developed in which the transgene vector contains a deletion in the 3' LTR U3 region making it self-inactivating in target cells and the packaging vector is a split genome construct with the tat gene deleted and the rev gene expressed by a separate non-overlapping construct. This vector achieves transduction of target cells as well as that observed with the previous generation. To date, neither second nor third generation lentiviral vectors have been found to generate RC virus.

Some of the inserts described in the risk assessment have been shown to have transforming activities when overexpressed. The major risk of generating viral vectors expressing normal transcriptional regulatory proteins therefore comes from either introducing abnormally high levels of a normal or mutated factor into normal cells or from the downregulation of endogenous proteins by RNAi. In some cases, this could lead to aberrant cell differentiation, proliferation, apoptosis or in rare cases, oncogenic transformation. However, the expression of these proteins is highly unlikely to change the natural immunogenic characteristics of the cells they are expressed in, which are therefore very unlikely to colonize humans due to immuno-incompatibility. Consequently, the transfection of these cells and expression of the transfected genes will not
significantly alter the capacity of these cells to persist in the environment or cause harm to either humans, animals or other aspects of the environment.

The work will be undertaken in accordance with containment level 2. This includes culture of primary cells, retroviral infection protocols and subsequent culture of any cell-lines produced that have not been screened for the presence of retrovirus. Screening of the cell-lines will be by assay of reverse transcriptase activity. The assay will be carried out within the laboratory designated for Class 2 work until cell culture-derived material has been dissociated using lysis buffer (50mM Tris, 80mM KCl, 2.5mM DTT, 0.75mM EDTA, 0.5% Triton-x-100), after which time it can be continued outside of the facility providing the principles of Good Laboratory Practice are followed. Cell-lines in which no retroviral activity has been found can be de-rated to Class 1. Access to cell culture facilities such as incubators or safety cabinets in which Class 2 work is being performed will be restricted to users registered for Class 2 work. The University Occupational Health Department will be given a list of names of all workers with access to the facility and will give these workers appropriate health surveillance.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All plastic ware that has come into direct contact with viral supernatants or cell material that has been transfected or infected, e.g. pipettes, tubes etc., will be decontaminated either by immersing in 1% Virkon or trigeine prior to autoclaving, or be taken directly for autoclaving in a sealed, watertight container. All other consumables will be autoclaved.

Tips and tubes will be placed in a plastic, sealable disposable jar in the hood that will be sealed immediately after use. The outside will be sprayed with a disinfectant, put into an autoclave bag, closed, then removed from safety cabinet, put into another autoclave bag and subsequently autoclaved. The tips/tubes will not be soaked in virkon or trigeine as the disposable jar provides an extra layer within the autoclave bag separating workers from the potentially hazardous material.

Serological pipettes will be rinsed with disinfectant by aspirating the solution a few times before disposal into a two-layered autoclave bags. Immediately after the procedure, the autoclave bag will be sealed and subsequently autoclaved.

Used culture flasks, plates, and dishes will be put into an autoclave bag, closed, the outside of the autoclave bag will be sprayed with a disinfectant, then put in another autoclave bag and subsequently autoclaved. The final route for all solid material that has been contaminated is incineration.

Liquid waste will be rendered biologically inactive by adding virkon or trigeine to give a final concentration of 1% and leaving overnight before discarding down the sink. It can either be emptied aspirated in a beaker of concentrated virkon or trigeine in the hood or by using a vacuum aspirator directly into a reservoir of concentrated virkon or trigeine. Should the latter option be employed, the vacuum pump system must be fitted with the appropriate filters (changed once a month) and the nozzle and hose will be rinsed with 1% virkon or trigeine at the end of each day.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The application for this connected program of work was considered by the LIMM Local GM Safety Committee at its 6 monthly meeting on the 14th July 2008. Copies of the risk assessment had been sent to the members of the Committee approximately 10 days in advance of the meeting. The Committee felt that the project had been well set out with a clear description of the (second and third) generation retro and lentiviral vector and host systems presented, along with a description of the potential risks associated with their proposed use. Furthermore, there was a very detailed description of the inserts proposed to be investigated.

Discussion mainly centered on where the work would be undertaken and the routes of waste disposal. The bulk of the work would be undertaken in the tissue culture room on Level 6 Wellcome Trust Brenner building. However, viral stocks would sometimes require centrifugation in an ultracentrifuge based in a different ancillary room. It was felt that so long as the centrifuge tubes containing the viral cultures were both sealed and opened in the tissue culture hoods then there was not a problem with this procedure. However, emergency decontamination provision must be put in place in the event of a tube failure. The main topic of conversation regarding waste disposal related to tip disposal. It was decided that the safest route of disposal of the tips would be via collection in DispoSAFE P.E.T. jars (Microbiological Supply Company) that would then be (double) bagged in autoclave bags by workers registered at Class 2 and then transported to Level 3 WTBB for autoclaving. After autoclaving this waste is sent for incineration as a matter of course.

The consensus of the Committee was that the work described was a Class 2 project and that the group of individual projects merited the title ‘a connected program of work’. They felt that the project could now be submitted to HSE for approval.

### Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
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**Project Ref 67/00.3**

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<td>04/04/2006</td>
<td>GENETIC ANALYSIS OF THE KAPOSI’S SARCOMA VIRUS (HUMAN HERPESVIRUS 8, HHV8) GENOME AND OF THE EPSTEIN-BARR VIRUS (EBV) GENOME CLONED ONTO A F-PLASMID VECTOR</td>
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<th>Historical Date of Additional Info</th>
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02/03/2022  Page 3468 of 15326
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
Project Ref  67/01.1

Date Ackn'd  04/04/2006
CU2 Project Title  REPLICATION DEFECTIVE ADENOVIRUS RETARGETED VIA INTEGRINS

Date Project Ceased

Class  Class 2
CultureVolClass2  < 1 Litre
CultureVolumeClass3-4

Non-GMM
Consent Granted  Not Applicable

Withdrawn  N
Tick if notifying a connected programme of work  Y

Historical Significant Changes
TRANSFERRED FROM GM CENTRE 67 - 4/4/06.

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
Laboratory - based, preclinical studies developing gene therapy for cancer

Recipient or parental organism
Adenovirus; cell lines and primary tumour material

Host/vector system
E1-deleted, replication-defective adenovirus vectors; probably E3-deleted. The wild-type fibre gene will be substituted with genetically modified derivatives intended to permit binding to integrins (e.g. by insertion of peptide motifs into the HI loop). Binding to the normal receptor (CAR) will initially not be modified, but may be ablated in later vectors. The viruses will be generated from transfected plasmid DNA constructs, and grown, in cells that compliment the E1 deficiency, e.g. HEK293 cells.

Origin & function

Page 3470 of 15326
The vector sequences are derived via plasmid cloning from adenovirus type 5; integrin-binding motifs designed based on known binding motifs. Some cancers, e.g. chronic lymphocytic leukaemia (CLL) have only low levels of the coxsackie and adenovirus receptor CAR, and so adenoviral gene transfer to these is very inefficient. Retargeting the virus by insertion of integrin-binding peptide motifs into the capsid fibre protein is expected to allow more efficient delivery of therapeutic genes to the cancer cells and, if the natural CAR-binding ability is simultaneously removed, may confer some selectivity of gene delivery to the cancer cells. A range of transgenes may be inserted into the E1 region of the viral vectors; the initial risk assessment covers reporter genes (e.g. green fluorescent protein); and the potentially therapeutic genes CD80, 4-1BB ligand, CD40 ligand, and GM-CSF (secreted or membrane-anchored).

Other potentially therapeutic genes may also be inserted under this connected programme of work including e.g. other immunomodulatory genes; prodrug activating enzymes; tumor-suppressor genes; we understand these would not require further notification to HSE if the GMSC agrees on the basis of risk assessments that there is no significant increase in the potential hazard.

Evaluation of foreseeable effects

No significant biological effects expected from expression of reporter genes. The immunomodulatory genes may have potential to induce local inflammatory responses in the event of contamination, and would be expected to enhance immune responses against the infected cells. Systemic or long term adverse effects are unlikely. The retargeting of the vectors is expected to alter the relative efficiency for entering different cell types. There is a theoretical possibility for recombination e.g. with wild type virus, which could generate a replication-competent virus carrying the modified fibre. Containment conditions and working practices will minimise this risk. Such recombination would lead to concomitant deletion of the exogenous transgenes inserted in the E1 region.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid virus containing waste is bagged and placed in a designated metal container which is removed to the autoclave. Liquid virus waste is discarded to bottles which are placed in a designated metal container for autoclaving. All liquid waste is treated by autoclaving before disposal to drain. Disposable solid waste is also autoclaved before removal as "clinical waste" by specialist contractors, with final disposal by incineration. The exceptions are:-

Sharps and pipettes are soaked in 1% Virkon for a minimum of 15 minutes before disposal in an approved sharps box. The disinfectant is discarded down the sink. Glassware used in this virus room is soaked overnight in 1% Virkon before removal for normal tissue culture wash up processing. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above.

Autoclaving achieves effectively 100% kill of GMMs. Manufacturer’s data indicates efficacy of Virkon against a variety of adenoviruses at 15 concentration, and also against a number of strains of E.coli at dilutions ranging from 0.125% - 1%. We have demonstrated that 15 minutes exposure to 1% Virkon achieves >4log kill for adenoviral infected cell pellets and >6log kill of adenovirus seed stocks.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The risk assessments were circulated to the committee and discussed with the applicant at a specially convened meeting on 8/5/2001. The committee noted that the potential for homologous recombination with wild type E1 sequences to result in the inadvertent generation of replication-competent, retargeted virus, and the potential hazard of such a virus was discussed. It was noted that the HSE/ACGM Guidance contained an example of a risk assessment for a retargeted, replication competent adenovirus (RCA), which indicated level 3 containment. Following discussion, the GMSC agreed that such recombination was a rare event in complimenting cell lines, and the committee was assured that the safest practicable complimenting cells would be used. Stocks would be monitored for RCA, and any found to be contaminated would be safely inactivated. Working practices to prevent cross-contamination were described. The committee agreed that the work could be safely conducted at containment level 2 and was therefore class 2. Any unexpected results from the work that might affect the assessment of safety, and any accidents involving the viruses will be reported to the committee. Other points of detail were clarified, and the final risk assessments modified in line with these discussions. The work has therefore now been approved by the GMSC.

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**Project Ref** 67/02.1

- **Date Ackn’d**: 04/04/2006
- **CU2 Project Title**: TRANSIENT EXPRESSION OF EUKARYOTIC CELL SIGNALLING PROTEINS USING REPLICATION DEFECTIVE ADENOVIRUS VECTORS
- **Class**: Class 2
- **CultureVolClass**: < 1 Litre
- **CultureVolumeClass**: Not Applicable
- **Non-GMM**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Historical Significant Changes**: TRANSFERRED FROM GM CENTRE 67 - 4/4/06, TRANSFERRED TO G
- **Historical Date of Additional Info**: 04/04/2006
- **Significant Change ID**: 04/04/2006
- **Date of Significant Change**: 04/04/2006

**Project Additional Information**
### Purposes of the contained use
Studying the roles and regulation of cell signalling proteins.

### Recipient or parental organism
Mammalian cell cultures.

### Host/vector system
E1-deleted, replication-defective adenovirus vectors. The viruses will be generated from transfected plasmid DNA constructs, and grown, in cells that complement the E1 deficiency, e.g. HEK293 cells.

### Origin & function
The cell signalling proteins to be expressed will be derived from human or other mammalian species. In addition to the wild type forms, constitutively active or dominant negative mutants may be generated. They will have various roles (which are under investigation) in cell signalling pathways. The risk assessments included with this notification relate to Rho family proteins, and phosphoinositol-3 kinase (P13 kinase); as detailed in the risk assessments these are regulators of cell movement and proliferation.

(Risk assessments for other cell signalling proteins expressed in similar adenovirus vectors have been approved by the GMSC as class 1; these include phospholipase D, Arf family (GTPases, PIP kinases). Similar adenovirus vectors encoding other cellular signalling proteins may also be generated and used in this connected programme of work; it is our understanding that these would not require further notification to HSE if the GMSC agrees on the basis of risk assessments that there is no significant increase in the potential hazard.

### Evaluation of foreseeable effects
The cell signalling proteins that are to be expressed will have a variety of roles in the regulation of various cellular responses, eg inflammation, cell survival, control of apoptosis, proliferation, migration. For proteins of negligible apparent hazard, the viruses are classified as class 1. Viruses categorised as class 2 will express cell signalling proteins that have some association with harmful processes, eg. some may have been associated with a role in oncogenic transformation; however a role in cancer would only be in conjunction with many other oncogenic events, and so the degree of hazard from their transient expression in an E1-deleted adenovirus is only modest.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation applied for

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Utoclaving is preferred means of decontamination (effectively 100% kill). Liquid waste is autoclaved and disposed to drains. Disposable solid waste is also autoclaved before being removed as clinical waste, with final disposal by incineration.

The exceptions to the above are:
"Sharps", including scalpel blades, needles and disposable tips for micropipettors may be decontaminated by drawing up 1% Virkon disinfectant and soaking in Virkon for a minimum of 120 minutes. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above. Virkon has effectively 100% kill rate.
here was discussion regarding the boundary between class 1 and 2 risk assessments, reaching agreement with that shown on the risk assessments. Points of detail have been clarified, and the final risk assessments modified in line with these discussions.

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**Project Ref 67/04.1**

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<td>04/04/2006</td>
<td>EXPRESSION OF HUMAN POLYCOMB PROTEINS OR OTHER TUMOUR ANTIGENS IN VIRAL VECTORS.</td>
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Tick if notifying a connected programme of work Y

Historical Significant Changes

TRANSFERRED FROM GM 67 - 4/4/06.
### Purposes of the contained use

Identification of human tumour antigens; characterisation of immune response to tumour antigens; possible future investigation of role in cell signalling pathways.

### Recipient or parental organism

Virus vectors are used in order to achieve efficient gene transfer and expression of the tumour antigens in target cells in vitro for immunological assays. In the first instance, replication-deficient adenoviruses (E1-deleted) will be used. Future work may involve other well-established virus vectors, such as vaccinia virus (particularly for immunological assays) or replication-defective retroviruses (signalling studies).

### Host/vector system

E1-deleted, replication deficient adenovirus. Virus production in cells that complement E1 deficiency, eg HEK293 cells. Target cells for immune responses include human dendritic cells, fibroblasts, lymphocytes, epithelial cells.

Future work is likely to involve other virus vectors, particularly vaccinia or replication-defective retroviruses.

### Origin & function

The polycomb proteins BM1-1 and EZH2 were identified using the SEREX screening technique, using the sera of patients with hepatocellular carcinoma, and their potential utility as tumour-associated antigens will be further investigated in this project. This family of proteins are transcriptional regulators of haematopoiesis, and have been implicated in growth transformation of cells, hence may play a direct, contributory role in tumour development.

This work is leading to the investigation of other members of the polycomb family, and other tumour-associated antigens, some of which may also be involved in growth-regulatory pathways.

The main interest in this project is recognition of these antigens by the immune system and their potential as targets for cancer immunotherapy. Future work may also investigate their role in cell signalling pathways. Their potential contribution to the multistep process of oncogenesis warrants containment of these viruses at containment level 2.

### Evaluation of foreseeable effects

Stable incorporation of these genes into cells, and subsequent high level or ectopic expression of these proteins may contribute to cellular transformation processes. However the adenoviruses carrying these constructs do not result in stable, long term expression in infected cells; they are also replication-defective and so unable to spread.

Vaccinia viruses also only result in a short period of expression of the transferred genes, before the infected cells are lysed.

Use of retrovirus vectors would result in integration of the construct into genomic DNA of successfully infected cells. However this only occurs in replicating cells; the viruses are labile and low titre, not readily transmissible other than by direct inoculation, and readily contained at level 2.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All liquid waste is treated by autoclaving typically at 130 degrees C for 60 mins, before disposal to drains. Disposable solid waste which is or may be contaminated with GMMs is also inactivated by autoclaving at 130 degrees C for 30 mins, before removal as "clinical waste" by specialist contractors, with final disposal by incineration.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The GMSC had no adverse comments on this assessment.

Project Containment

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<td>Quantifying and stimulating CMV specific T cell responses using recombinant CMV strains.</td>
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<td>&lt; 1 Litre</td>
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Tick if notifying a connected programme of work  N

Historical Significant Changes  TRANSFERRED FROM GM CENTRE 67 - 4/4/06.
This project involves use of recombinant CMV viruses to stimulate T lymphocytes in vitro, from blood of virus carriers. In addition to the current batch of recombinant CMV viruses we may in future seek to undertake work with recombinant CMVs that are deficient for other novel immune evasion genes and/or different combinations of the above described immune evasion genes. Also we may wish to acquire/construct a CMV virus incorporating a non-harmful reporter gene such as green fluorescent protein (GFP).

We do not expect the hazards associated with such viruses to be any greater than the ones we currently plan to work with. Prior to embarking on such new studies we would submit a new or modified risk assessment to the GMSC.

**Recipient or parental organism**

Host: MRC5 fibroblasts, U373 cells (Astracytoma cell line that is permissive to CMV), endothelial cells, human fibroblasts.

**Host/vector system**

Vector: Human CMV

**Origin & function**

Genetic material: 1: Cytomegalovirus genes.

CMV is a lymphotropic/epitheliotropic beta-herpesvirus that is carried as an asymptomatic life-long infection by the majority of individuals in all communities. Control of CMV infection is thought to be due to the potent cellular immune response that is detected in healthy immunocompetent virus carriers. Laboratory strains of CMV such as AD169 and Towne are highly restricted in host cell range, namely fibroblasts. This is believed to be attributed to the loss of genes over passage time in vitro (Bolovan-Fritts C, Wiedeman JA. 2001. Human cytomegalovirus strain Toledo lacks a virus-encoded tropism factor required for infection of aortic endothelial cells. J Infect Dis. 184:1252-61. Sinzger C, Schmidt K, Knapp J, Kahl M, Beck R, Waldman J, Hebart H, Einsele H, Jahn G 1999. Modification of human cytomegalovirus tropism through propagation in vitro is associated with changes in the viral genome. J Gen Virol. 80:2867-77.)

It is a general consensus that recombinant CMVs made from such parental strains will not acquire the potential to replicate in other cell types (Thomas Jones - personal communication).
The following genes are deleted in the recombinant viruses.
 RV798 (all of US2 to US11),
 RV35 (US6 to US11)
 and
 RV47 (US2 to US3 only)

The functions of each gene product are described

US2: The US2 gene product is expressed in the early phase of the viral replication cycle. This protein induces rapid degradation of newly synthesized MHC class 1 molecules, reducing the half life from over 6 hours to less than 2 minutes. It appears that US2 proteins bind to MHC class 1 molecules causing the transport of these complexes to the cytoplasm (Jones et al. 1997). After deglycosylation, both US2 proteins and MHC class 1 molecules are degraded. CMV also interferes with MHC class II expression in several ways as described elsewhere (Miller et al. 1998; Phillips et al. 1998; Tomazin et al. 1999).

US3 &US11 : The US3 and US11 genes are expressed in the immediate early phase and encode for products that physically associates with MHC class 1-B2m complexes. Whereas the US3 protein causes the retention of MHC class 1 molecules in the ER (Jones et al. 1996), US11 binds to MHC class 1 molecules and directs their translocation to the cytoplasm (Wiertz et al. 1996).

US6: The US6 gene is expressed much later in the delayed or late phase and subsequently remains expressed throughout the virus life cycle. US6 interferes with peptide loading of MHC class 1 molecules in the ER (Ahn et al. 1997). This is achieved by US6 proteins transiently associating with the complex containing TAP, MHC class 1-B2m (and other proteins such as Tapasin and calreticulin). This interaction results in the inhibition of peptide translocation across the ER membrane and efficiently blocks assembly of the trimeric MHC class 1-B2m-peptide complex.

US8: Human cytomegalovirus US8 is a type 1 membrane protein that partially colocalizes with cellular endosomal and lysosomal proteins. Although US8 does not have discernible effects on the processing and cell surface distribution of major histocompatibility complex (MHC) class 1 products, it is demonstrated that US8 binds to MHC class 1 heavy chains in the endoplasmic reticulum (Tirabassi & Pleogh, J Virol 2002 Jul;76(13):6832-5).


The other genes replaced (US4, US7, US9, US10) are not fully characterized with regards to function. They appear to be cytoplasmic proteins and may have some function in immune subversion also (Huber et al. J Virol. 2002 Jun;76(11):5748-58).

The pp65 gene is deleted in the RVAd65 virus

pp65: is a structural protein located in the viral tegument. Pp65 is expressed during the late phase of viral replicative cycle. Pp65 has been demonstrated to have kinase activity and co-immunoprecipitates with a cellular kinase (Plk-1) but is dispensable for virus growth in vitro. Although it has been reported to have immune evasive properties, pp65 itself is the target of a massive immune response (both cellular and antibody mediated). It is believed that parent CMV (strain AD169) preparations contain non-infectious viral particles known as dense bodies which contain a large excess of this protein. This is thought to bias towards stimulation of T cells specific for pp65. Therefore the absence of this gene may allow for other peptides to be presented by MHC class 1 molecules and the T cell reactivities to be detected.

Evaluation of foreseeable effects

The genes that have been deleted are involved in either immune subversion or part of the virion structure. The consensus view of prominent CMV virologists is that their absence does not appear to alter the pathogenic capacity of CMV. In fact it is more likely that these viruses will be more immunogenic and provoke a more vigorous response than parent CMV due to the less hindered presentation of a variety of antigens. The absence of pp65 is expected to allow for the presentation of other immunodominant CMV antigens such as IE-1, pp150 and pp50. One possibility to be considered is that an enhanced immune response may also result in greater inflammation at the site of infection. It is envisaged that this will be self-limiting and restricted to the site of infection.
The presence of prokaryotic gene sequences in these recombinant viruses are not believed to increase the pathogenicity or host range of these viruses.

CMV does not immortalise human cells in vivo or in vitro - the removal of immune-subversive genes should render the virus more immunogenic, and not confer transforming properties.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste to be treated by autoclaving at 130 degrees C for 60 mins before disposal to drain. Disposable solid waste which is or may be contaminated with GMMs is also inactivated by autoclaving at 130 degrees C for 30 mins, before removal by a specialist company for incineration. Disposable plastic pipettes may also be decontaminated by immersion in Virkon.

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3</td>
<td>L4 L2 L3 L4</td>
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</table>

Animal Units

Large Scale Activities

Human Clinical Applications

Project Containment

Project Ref 67/trans1

02/03/2022
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Is an emergency plan required according to regulation 20? \( \text{N} \)

If yes, tick to confirm that it is attached to this form \( \text{N} \)

Tick to confirm that you have attached a risk assessment to this form \( \text{N} \)

Tick if you are claiming exemption from disclosure for section of the risk assessment \( \text{N} \)

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<table>
<thead>
<tr>
<th>Animal Units</th>
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<th>Human Clinical Applications</th>
</tr>
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<tbody>
<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
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**Project Ref** 67/trans2

**Date Ackn’d** 04/04/2006

**CU2 Project Title** RETROVIRAL VECTORS THAT EXPRESS HER-2/NEU

**Class** Class 2

**Consent Granted** Not Applicable

**Project notified under transitional arrangements** \( \text{Y} \)

**Withdrawn** \( \text{N} \)

**Tick if notifying a connected programme of work** \( \text{Y} \)

**Historical Significant Changes** GM67/97.3 - Transferred from GM Centre 67 4/4/06.

**Historical Date of Additional Info** 25/06/1997
**Date of Significant Change**

**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
### Project Ref

<table>
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#### Animal Units
- Large Scale Activities
- Human Clinical Applications

#### Project Ref: 67/trans3

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- **Class CultureVolClass2**: Class 2
- **Consent Granted**: Not Applicable

- **Withdrawn**: N

- **Tick if notifying a connected programme of work**: Y

- **Project notified under transitional arrangements**: Y

#### Historical Significant Changes

- **Historical Date of Additional Info**: 10/03/2000, 18/06/1999, 27/01/1999, 19/06/1995

#### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [ ]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units: L2 L3 L4 L2  
Large Scale Activities: L2 L3 L4 L2  
Human Clinical Applications: L2 L3 L4

Project Ref  67/trans4

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### Date Project Ceased

- 17/05/1996, 27/01/1999, 16/04/1997

### Non-GMM Consent Granted

- Not Applicable

### Tick if notifying a connected programme of work

- Yes

### Project notified under transitional arrangements

- Yes

### Withdrawn

- No

### Historical Significant Changes

- GM67/96.1, GM67/99.1, GM67/97.2 - TRANSFERRED FROM GM CENTRE

### Historical Date of Additional Info

- 17/05/1996, 27/01/1999, 16/04/1997

### Significant Change ID

- 67/trans4a

### Date of Significant Change

- 19/07/2007

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<td>Animal Units</td>
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<td>L2</td>
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**Project Ref** 67/trans5

- **CU2 Project Title**: ANDENOVIRUS BIOLOGY AND CELL TRANSFORMATION
- **Date Ackn’d**: 04/04/2006
- **Class CultureVolClass2 Class3-4**: Class 2
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: Y
- **Historical Significant Changes**: TRANSFERRED FROM GM CENTRE 67 - 4/4/06.

**Withdrawn**: N

**Tick if notifying a connected programme of work**: Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 67/trans6

Date Ackn'd: 04/04/2006

CU2 Project Title: EPSTEIN BARR VIRUS, CELLULAR INTERACTIONS

Class: Class 2

CultureVolClass2: Class 2

CultureVolumeClass3-4: Not Applicable

Non-GMM: Not Applicable

Consent Granted: Not Applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: Y

TRANSFERRED FROM GM CENTRE 67 - 4/4/06

Historical Significant Changes:

Historical Date of Additional Info:

Significant Change ID:

Date of Significant Change:

Project Additional Information

Purposes of the contained use:

Recipient or parental organism:

Host/vector system:

Origin & function:
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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</tr>
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Project Ref 67/trans7

Date Ackn'd CU2 Project Title
04/04/2006 STUDIES ON HUMAN PAPILLOMAVIRUS IMMUNOLOGY AND BIOLOGY

Class CultureVolClass2 CultureVolumeClass3-4
Class 2
Date Project Ceased

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes
TRANSFERRED FROM GM CENTRE 67 - 4/4/06.

Historical Date of Additional Info

Significant Change ID
67/05.1

Date of Significant Change
06/09/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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**Animal Units**

- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

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**Project Ref** 67/trans8

**Date Ackn’d** 04/04/2006

**CU2 Project Title**

- CLONING OF GENES FOR VIRAL PROTEINS AND ANTIGEN PRESENTATION PROTEINS IN POX VIRUSES

**Class CultureVolClass2 CultureVolumeClass3-4**

- Class 2
- Not Applicable

**Non-GMM Consent Granted**

- Not Applicable

**Project notified under transitional arrangements** Y

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**


**Historical Date of Additional Info**

- 10/03/2000, 18/06/1999,
- 27/01/1999, 19/06/1995,
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Name

MRC HGMP RESOURCE CENTRE

Name 2

Department

UK-HGMP RESOURCE CENTRE

Campus Estate or Research Centre

WELLCOME TRUST GENOME CAMPUS

Building

HINXTON HALL

Road Name

District

HINXTON

Town

CAMBRIDGESHIRE

County

Postcode

CB10 1SB

Country

ENGLAND

Tel Number

01 223 494500

Fax Number

01 223 494512

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Centre closed down on 31/07/2005

Date at Which Additional Info Submitted

31/05/2001
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
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</table>

Tick if confidential

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>
HGMP follows site regulations on the disposal of GM material. All disposal and waste treatment eg. autoclaving, is carried out by the Sanger Centre. Autoclaved waste is collected and sent in specialised containers to the Addenbrooke's hospital site for incineration. Some waste eg. CIN bins are sent directly for incineration.

Specific details of waste disposal:

- **Culture media:** This is all containment level 1 waste. The maximum culture volume disposed of at any one time is 11. Each research group inactivates their own media waste by treating with either Tegodyne, Virkon or Proceine 40 according to manufacturers instructions and biocide screening data supplied with the disinfectants. The waste is then disposed of down the sink, flushed with copious amounts of water.

- **Culture dishes:** These are put into lined plastic tubs which are then autoclaved, prior to incineration.

- **Plastic ware (eg. Pipette tips, eppendorf tubes etc):** These are put into 51 plastic containers with lids which are then autoclaved, prior to incineration.

- **Gloves, paper towels etc. which have potentially been in contact with GM material are disposed of in yellow bags which are then sent for incineration.**

- **Sharps which have potentially been in contact with GM material are disposed of in "CIN bins" which are then sent for incineration.**

The new site will follow rules implemented by the babraham site. All GM waste will be autoclaved in a specially designed "dirty" autoclave, before being added to yellow bags and put into specialised containers, organised by the site management. This waste is then sent to The Cambridge Pet Crematorium, Thriplow Heath, Cambridge.

For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Virology</th>
<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
<th>Gene Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycology</td>
<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
</tr>
</tbody>
</table>

**Other(s)**

**Tick to confirm that you are attaching a summary of the risk assessment**

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
<tr>
<th><strong>Data Premises Notified</strong></th>
<th><strong>11/12/1979</strong></th>
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<td><strong>Transferred from 1992 Regs?</strong></td>
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<td><strong>15/01/2007</strong></td>
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<tr>
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<td><strong>Emergency Plan Required?</strong></td>
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<td><strong>Non-GMMs</strong></td>
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<td><strong>N</strong></td>
</tr>
</tbody>
</table>

**Name**

UNIVERSITY OF LIVERPOOL

**Name 2**

SCHOOL OF BIOLOGICAL SCIENCE

**Campus Estate or Research Centre**

LIFE SCIENCES BUILDING

**Road Name**

LIFE SCIENCES BUILDING

**District**

MERSEYSIDE

**Town**

LIVERPOOL

**County**

MERSEYSIDE

**Postcode**

L69 7ZB

**Country**

ENGLAND

**Tel Number**

0151 794 4411

**Fax Number**

0151 794 4401

**E-mail**

**HSE Division**

NORTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

<table>
<thead>
<tr>
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<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
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</thead>
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<tr>
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<td>UNIVERSITY OF LIVERPOOL</td>
<td></td>
<td></td>
<td>LIFE SCIENCES BUILDING</td>
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<td>ENGLAND</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<tr>
<td>Level 2 (GMMs)</td>
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</table>
Level 4 (GMMs)
Non-microbial

Other (please specify)  

Tick if confidential

Bacteriology  
Parasitology  
Transgenic
Birds

Microbiology  
Research

Virology  
Transgenic
Animals  
Transgenic
Fish

Gene Therapy

Mycology  
Transgenic
Invertebrates  
Transgenic
Plants

Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 118/01.1

Date Ackn'd 22/02/2001  
Date Project Ceased 02/03/2022

CU2 Project Title  
BACULOVIRUS EXPRESSION OF ENZYMES CATALYSING ECDYSTEROID (INSECT MOURTING HORMONE) TRANSFORMATION

Class

Culture

Volume

Class

Culture

Volume

Class

Culture

Volume

Non-GMM

Consent Granted

not applicable
15/01/2007

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

TRANSFERRED TO GM 554 - 15/1/07

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form
Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
<td>L3 L4 L2 L3</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2 L3 L4</td>
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</table>

Project Ref: 118/01.2

Date Ackn'd: 22/02/2001
CU2 Project Title: CLONING AND IDENTIFICATION OF MRNAS DIFFERENTIALLY EXPRESSED IN BREAST LESIONS
Date Project Ceased: 15/01/2007
Class Culture Class 2 Volume
Consent Granted: not applicable
Non-GMM: Project notified under transitional arrangements

Historical Significant Changes: TRANSFERRED TO GM 554 - 15/1/07

Project Additional Information

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<td>L3</td>
<td>L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tbody>
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<td>L3</td>
<td>L4</td>
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<td>L3</td>
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**Project Ref** 118/04.1

<table>
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<tr>
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<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tbody>
<tr>
<td>07/01/2004</td>
<td>IDENTIFICATION AND CHARACTERISATION OF BIOFILM-REGULATED FACTORS PRESENT IN MEMBERS OF THE ORAL MICROBIAL FLORA</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td></td>
<td></td>
<td>not applicable</td>
<td>N</td>
</tr>
<tr>
<td>Date Project Ceased</td>
<td>15/01/2007</td>
<td>Withdrawn</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tick if notifying a connected programme of work N
Cells grown in biofilms behave and respond differently to cells grown in liquid media suspensions. Understanding how these changes affect the phenotype of a biofilm-grown cell is critical to our ability to control and manipulate bacterial biofilms, which play a major role in beneficial applications like water purification and detrimental scenarios like infections from implanted devices and bio-fouling of air-conditioning systems and water distribution networks. Members of the oral bacterial flora have been extensively studied and cultured in mono- and mixed-species culture, and thus lend themselves to more sophisticated studies involving the up-regulation and down-regulation of factors that direct their adaptation to the biofilm lifestyle. In the main, the biofilm-adaptive factors identified in different bacterial strains have been associated with stress responses as well as activation of mobile genetic elements, but the regulatory factors are still not well understood, nor have all the biofilm adaptive factors been identified. The goals of the work covered by this application are to examine in detail these biofilm adaptive responses in members of a well characterised mixed oral bacterial consortium both in single and mixed culture conditions.

Recipient or parental organism

Recipient organism: K12 laboratory strains of Escherichia coli will be used for all cloning work. K12 strains are considered to be non-colonising.

Host/vector system
E. coli strains MC1061 or DH5 - a will be used to propagate shuttle and suicide vectors. E. coli strain S17 will be used to mobilise the mobilisable (but not conjugative) shuttle vectors. Various shuttle vectors proposed for use are pVA838, pDL278 and pFX3 which are nonmobilisable Streptococcal/E. coli shuttle vectors; pT-COW a mobilisable but nonconjugative E. coli/Prevotella-Porphyromonas shuttle vector; and pBR322 a non mobilisable E. coli plasmid. His-tag vectors like the pQE derivatives from Clontech for specific use in E. coli.

Origin & function
Various biofilm adaptive factors from these oral strains will be cloned and identified. Not overt attempt to express these elements will be made until they have been suitably characterised by sequencing. Identified factors that have been determined not to be toxins may be cloned into vectors for the express purposes of recombinant protein purification or expression for future studies on their role in biofilm adaptation.
Evaluation of foreseeable effects

In all cases the aim of this work is to identify factors present in ACDP cat2 organisms that are activated under biofilm growth. No overt attempts will be made to express any of these factors in E.coli until they have been identified. Although increased toxin production has been associated with other biofilm-forming organisms like Pseudomonas aeruginosa, the purpose of these studies will not be to study toxin expression, so if toxins are identified as being biofilm adaptive factors in these oral organisms they will not be targets for future study. Instead, this proposed work involves identifying other genetic elements that are strictly involved in the cells ability to adapt to the biofilm environment (i.e. global regulatory factors, mobile genetic elements, stress response facors novel membrane proteins, and other elements not directly capable of conferring virulence factors to the ACDP category 1 E.coli K12 species beyond that of the original ACDP category 2 oral organism. Therefore handling recombinant E.coli at containment level 2 conditions obviates any foreseeable effects. In the case where vectors are introduced into the oral bacteria this will be done either to mark them with an antibiotic resistance marker found on vectors suitable to those organisms or to reintroduce regulatory sequences for reporter gene analysis. In either case, the virulence of the oral bacteria will not be enhanced so work done under containment level 2 conditions, again, obviates any foreseeable effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N.A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All biological waste will be handled according to the School's strictest guidelines. All waste, with the exception of glass pipettes will be bagged or boxed in metal tin cans and be subjected to autoclaving according to the British Laboratory Standards for biological waste. These autoclaves are regularly serviced and tested by the School. This will result in a 100% kill with all non-glass waste ultimately subjected to incineration and glass waste being washed (post autoclaving) with recirculation into the general glassware pool. Glass pipettes will be neutralised in fresh bleach-based disinfectant before being washed and autoclaved as is the standard practice within the School.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

At the meeting of the safety committee on 10th July 2003 it was agreed that the project was class 2.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L2</td>
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</tr>
<tr>
<td>L4</td>
<td>L4</td>
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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
The objective is to develop our understanding of key regulatory and signalling systems such as those influencing nitrogen metabolism and the regulatory response to ambient pH in A. fumigatus. This will utilise approaches that will have successful employed with A. nidulans. In response to comparative genomic analysis as well as proteomic and transcriptomic data, molecular genetic analysis will be undertaken to investigate the function of key regulons and the putative regulatory and signalling components. An initial step will be to construct strains disrupted for specific genes identified by the upstream analysis. This will be achieved by homologous integration of deletion constructs bearing selectable markers such as Neurospora crassa pyr-4 (encoding pyrophosphorylase orotidine MP decarboxylase). Additionally we will construct epitope tagged versions of key genes to identify interacting proteins. This work involves standard cloning procedures in laboratory strains of E. coli, followed by transformation into A. fumigatus. Assessment of the effect of mutations will be through plate tests, northern and proteomic analyses of wild-type and mutant strains. The identity of transformed A. fumigatus strains will be confirmed by PCR, sequencing and Southern’s, as appropriate.

A. fumigatus is humanely to normal healthy people, but it is listed in ACDP hazard group 2. It can cause postoperative infections, aspergilloma (particularly in patients with cystic fibrosis, post-TB, asthmatics), sinusitis in normal people and eye infections. Immunosuppressed individuals are the most at risk of developing pneumonia, disseminating to other organs and serious illness. Conidia (spores can cause an allergic response in sensitive individuals. It is usually transmitted by inhalation of airborne conidia which are widely distributed in nature. Conidia can be readily isolated from most environments including air, human homes, offices, work-places and soil.

A. fumigatus is sensitive to amphotericin B, itracomazole and voriconazole. It is also susceptible to 1% sodium hypochlorite and 70% ethanol.
Standard cloning procedures will be carried out in laboratory strains of E.coli to produce deletion constructs and epitope tagged versions of regulatory genes. Standard pUC based vectors will be used for this work. These constructs or PCR amplified and gene disruption in A.fumigatus utilises cosmids (e.g. from pWe15 cosmid libraries) bearing the sequence of the gene of interest will be prepared for transformation into A.fumigatus in E.coli strains (e.g. KS272) expressing the phage Reda and Redb recombination functions. This will involve replacement of a region of the gene of interest with the sequence of a marker gene to facilitate selection of recombinants in both E.coli and A.fumigatus. Purified cosmid DNS will be transformed into A.fumigatus.

**Origin & function**

The genetic material will originate from A.fumigatus and will have a role in regulation or signalling in nitrogen metabolism or in response to pH.

**Evaluation of foreseeable effects**

Virulence is multifactorial, and so far discrete virulence determinants in A.fumigatus have not been identified. However, nitrogen and pH regulatory mechanisms have been implicated in the pathogenicity of other pathogenic fungi. Strains deleted are AfareA which have been tested for pathogenicity have reduced pathogenicity compared with the parental strain. One study has reported that a revertant from AfareA deletion, where an uncharacterised mutation led to increased expression of specific nitrogen regulated genes, had increased pathogenicity. We do not intend to create revertants in this project, and we anticipate that this project will therefore produce strains with reduced pathogenicity. The regulatory genes and signalling genes produce regulatory proteins or enzymes, which are unlikely to have a harmful biological activity alone. Experiments using isolated DNA, RNA or protein should therefore involve little risk. Cloning within laboratory strains of E.coli will not involve expression, and should also be low risk. Vectors will be capable of expression in A.fumigatus and Anidulans, but not in E.coli.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Conidia: Measures to control the spread of conidia by handling plate cultures in a Class 2 cabinet. The cabinet to be sprayed with 70% ethanol before and after use, and between strains. Plates to be taped closed and exterior decontaminated (70% ethanol) for transport between cabinet and incubator in case of dropping a plate. Use scatter plates before/after work to test that conidia are contained by these measures. Mycelium: Mycelium to be ground to a powder in cabinet. Waste mycelium will be autoclaved to ensure that it is inactivated if not previously killed by extraction procedures. Any contamination incidents decontaminated with 1% sodium hypochlorite for 30 min. Spent growth medium: Spent medium, and washes from liquid cultures to be incubated with bleach prior to discard down drain. Inform Occupational Health so they can record work with this allergenic sensitiser (if deemed appropriate).

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

At the meeting of the safety committee on 10th July 2002 it was commented that the work should take place in a pathogen handling room. The containment should be checked with a non-pathogenic or non-GM strain before worked commenced.
**Project Containment**

**Laboratory Activities**

- L2 [Yes]
- L3
- L4
- L2

**Glass Houses**

- L3
- L4
- L2
- L3
- L4
- L2

**Growth Rooms**

- L3
- L4
- L2
- L3
- L4
- L2

**Animal Units**

- L2
- L3
- L4
- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4
- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4
- L2
- L3
- L4

---

**Project Ref** 118/04.3

**Date Ackn’d**

07/01/2004

**CU2 Project Title**

STUDIES ON THE ROLE AND REGULATION OF SECRETED PROTEINS OF STAPHYLOCOCCUS AUREUS AND ENTEROCOCCUS FAECALIS

**Class CultureVolClass2 CultureVolumeClass3-4**

Class 2

1-50 litres

**Non-GMM Consent Granted**

not applicable

**Project notified under transitional arrangements**

N

**Historical Significant Changes**

TRANSFERRED TO GM 554 - 15/1/07

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**Project Additional Information**

**Purposes of the contained use**

Contained use is required to prevent dissemination of genetically altered commensal bacteria that have the capacity to cause disease.

**Recipient or parental organism**

The parental strains of the two organisms that will be used are for Staphylococcus aureus strains SH1000 (8325/4) and Newman and for Enterococcus faecalis strains OG1RF and JH2-2. These are opportunist pathogenic bacteria. These are not attenuated strains but are the commonly used strains for genetic manipulation. Staphylococcus aureus SH1000 (8325/4), Newman in conjunction with the following plasmids: pAZ106, pLTV1, pMUTIN4, pCL84, (all integrative) pCU1, pSB2035 (both replicative).
Enterococcus faecalis OG1RF, JH2-2 with/without the following plasmids: pAZ106, pLTV1, pMUTIN4, (all integrative), pSB2035, pCU1, pTCVlac, pAT28 (replicative).

Escherichia coli BL21 (DE3) in conjunction with pETderivative vectors (T7 promoter)

Host/vector system

S.aureus and E.faecalis genes will be amplified and cloned into the vectors listed in section 7 using E. coli. The plasmids produced and containing the correct inserts will be purified and used to transform E. faecalis and S. aureus. The plasmids used are for two separate types: 1) replicative and maintained extrachromosomally for functions such as gene reporters and gene complementation; 2) integrative for generating insertional replacement mutations and maintained due to recombination with chromosome.

Overexpression of proteins will only be done in E.coli BL21 (DE3) using the pET system. No overexpression of proteins will be done with either S.aureus or E.faecalis as host.

Origin & function

The S.aureus and E.faecalis strains that will be used for the proposed study are laboratory isolates that have been obtained from laboratories that are currently researching the area of host/pathogen biology. Originally, these were isolated from humans.


The intended functions are to study the role and regulation of surface and secreted proteins of these two commensal bacteria to investigate their potential roles during opportunistic infection. This will mainly be done by in vitro analysis of gene and protein expression and this will be done in coordination with a diversity of characterised mutant strains of these organisms to determine the heirarchy of gene expression for virulence and secreted protein production. Murine abscess models of infection will be used for study of staphylococcal mutants that are predicted by virtue of in vitro observations to have a role in virulence. These models of infection will be studied outwith the University of Liverpool in collaboration with others. E.faecalis mutants will be tested in invertebrate models of infection at Liverpool using the worm Caenorhabditis elegans, a well established model of infection.

Evaluation of foreseeable effects

S.aureus is a category 2 microorganism and the mutations that will be made are likely to reduce virulence of the organism compared to the parent strain. This drop in virulence may even reduce the S.aureus strain to category 1, however, due to the ability of S.aureus to colonise humans all of the work will be done in category 2 condition.

E.faecalis is a category 2 microorganism and the mutations that will be made are likely to reduce virulence of the organism compared to the parent strain. This drop in virulence may even reduce the E. faecalis strain to category 1, however, due to the ability of E. faecalis to colonise the digestive tract of human of the work will be done in category 2 conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be placed in labelled, capped glass bottles and autoclaved, which results in total kill. Any spillages will be cleaned up with 2% w/v stericol and waste will be autoclaved, again resulting in a total kill. Lab coats will be worn at all times and after work is completed hands will be vigorously washed prior to exiting the category 2 laboratory.
General laboratory plastic waste (tips, plastic containers) will be placed in the hazard bags and collected for incineration.

Caenorhabditis elegans infected with E. faecalis will be completely contained in agar plates in sealed boxes during the infection model. The plates will be autoclaved after the experiment resulting in total kill. Spillages will be cleaned up with 2% stericol, resulting in death of bacteria and invertebrates, and wiped up with absorbant tissue that will be autoclaved. The invertebrate model experiments will be performed in a designated Class 2 pathogen handling facility to protect workers.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

No specific comments were made.

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 Yes</td>
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Animal Units

| L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 | L2 | L3 | L4 |

Project Ref 118/trans1

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Project notified under transitional arrangements [Y]

Withdrawn [N]

Tick if notifying a connected programme of work [N]
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Name**

**CARDIFF UNIVERSITY**

**Name 2**

**INSTITUTE OF MEDICAL GENETICS**

**Campus Estate or Research Centre**

**Road Name**

**HEATH PARK**

**Town**

**CARDIFF**

**District**

**County**

**CARDIFF**

**Postcode**

**CF14 4XN**

**Country**

**WALES**

**Tel Number**

**029 2074 2903**

**Fax Number**

**029 2074 4869**

**E-mail**

**OSHEU@cardiff.ac.uk**

**HSE Division**

**WALES AND SOUTH WEST**

**Comments**

Centre closed and transferred to GM130 on 26/04/2005

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
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<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
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Non-microbial

Other (please specify)  

Tick if confidential

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<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Relatively small volumes (maximum 100-500 ml - very rarely 2 litres) produced in each experiment.

Surplus, used and waste culture medium treated with ActiCHLOR disinfectant tablets and/or autoclaved before disposal as clinical waste as per UWCM/Trust policy. Cell remnants treated in the same manner. All contaminated plastic ware is autoclaved.

Autoclaves operated in accordance with departmental SOP. Autoclaves regularly serviced and tested by Trust Works dept. personnel. Autoclaves subject to independent annual inspection and testing.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 121/01.1

Date Ackn'd 30/04/2001

Date Project Ceased 26/04/2005

Chemical, gum or biological agents that are hazardous to human or animal health or the environment

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 litre

Non-GMM Consent Granted

Tick if notifying a connected programme of work

Project notified under transitional arrangements

Historical Significant Changes

Project Transferred to GM130 on 26/04/2005.
To elucidate the normal and pathogenic roles of the huntingtin protein, which leads to Huntington's disease when bearing a glutamine tract in excess of 36 residues.

Huntington's disease (HD) is an autosomal dominant neurodegeneration associated with an expanded trinucleotide repeat which gives rise to a glutamine tract in the N-terminus of the protein, huntingtin. The expanded glutamine tract is selectively toxic and kills particular neuronal populations, initially the medium spiny neurons of the striatum but later other neuronal populations are affected. We wish to express both full-length and truncated huntingtin with various repeat lengths in mammalian cell cultures, along with other proteins known to interact with huntingtin. This will allow us to carry out a number of experiments to answer the following questions:

1. Do huntingtin and the interacting protein co-localise in the cells? Which sections of the transfected proteins are important to that co-localisation? Is this cell-type dependent?
2. Does the presence of the interacting protein ameliorate or enhance any observed effect of huntingtin, eg as measured by apoptosis, electrical activity or other appropriate functional assay?
3. Do variations in the interacting proteins affect the interaction with huntingtin?

The recipient or parental organisms for the standard cloning are disabled non-pathogenic E coli strains. The recipient cell lines for expression of the proteins are standard cell culture lines which cannot survive out of culture and which are kept free from any adventitious agents as far as can be determined by our routine assays for contaminating agents which are problematic in tissue culture, eg mycoplasma.

All cell transfections will be transient.

Host/vector system


Cell lines: Cos7, HeLa, HEK293, N2a, PC12, IMR32, SK-N-SH and variants of this line such as SK-N-5Y.

Vectors include pRcCMV, pcDNA3.19+/- and variants, eg pc3.1DNA HisMax and HisMyc pUni/V5-His-TOPO Echo cloning system (Invitrogen), Clontech living colour vectors (CMV promoter, GFP tags).
Evaluation of foreseeable effects

Huntingtin with an expanded polyglutamine tract is known to be toxic, particularly when expressed in truncated fragments. However, expression will be restricted to transient experiments only. The vectors used for expression in the mammalian cells will not be capable of replication in such cells and should they integrate into the host genome at low levels all the cells will be destroyed at the end of the experiment anyway. Huntingtin is ubiquitously expressed but only particular cell-types are damaged by its expression. These cells are in the brain and thus access of the vectors to the cells susceptible to damage is extremely unlikely. Should the vectors actually be transported to the appropriate cells they would be unlikely to integrate with the host genome in the absence of selection pressure and in order to manifest a problem substantial numbers of cells would need to be so affected. This is extremely unlikely. However the object of these experiments is to overexpress the proteins so promoters which give good expression levels in mammalian cells will be used, thus if the construct could integrate into the host genome of an appropriate cell then the cell would undoubtedly be compromised. HD is a late onset disease, but high repeat numbers (>50) as would be used in this experiment, give early onset disease (<20 years) and expression levels are also thought to be important in downstream cellular effects.

The toxicity of huntingtin and its truncated fragments are the reason for classifying this project as Class 2. The interacting proteins currently under study are the nuclear receptor co-repressor and endophilin 3, neither of which are known to be toxic to mammalian cells when overexpressed. Other interacting proteins may also be used but should any of these be intrinsically as toxic or more toxic than huntingtin itself, then a fresh application to work at the appropriate containment level will be made. Similarly, if the interaction observed in cells causes substantially increased toxicity of huntingtin (eg >10-fold increase in cell death at a given time-point) then shorter repeat units will be used experimentally to decrease the toxicity or again, a further application will be made to continue the work at appropriate containment levels.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Cell cultures are kept entirely within our tissue culture suite until disposed of by sterilisation and autoclaving. They would not survive outside the specialist conditions of tissue culture (37°C, 5%CO2).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Committee were concerned about the ability of the gene to cause harm if it were accidentally injected, but satisfied now that Containment Level 2 conditions and practices are sufficient. Risk Assessment now suitable and sufficient.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures, mammalian cell and microorganism, will be autoclaved in an approved and regularly tested autoclave room 2.05 Institute of Medical Genetics. In addition all disposables which come into contact with cell cultures will sterilised in freshly made solutions of Actichlor at a minimum concentration of 20,000ppm for a period of at least 2h. The tissue culture hoods used will also be disinfected with the above solution plus a 70% ethanol spray. The above procedures are already standard operating procedures in the Tissue Culture Suite in IMG.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

none.

02/03/2022
### Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<td>L4</td>
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- **Animal Units**: L2  L3  L4  L2
- **Large Scale Activities**: L3  L4  L2
- **Human Clinical Applications**: L3  L4  L2
### GM Centre Number: 124

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#### Name

UNIVERSITY OF BIRMINGHAM

#### Name 2

CHEMICAL ENGINEERING

#### Campus Estate or Research Centre

EDGBASTON CAMPUS

#### Road Name

EDGBASTON

#### Town

BIRMINGHAM

#### District

MIDLANDS

#### County

ENGLAND

#### Postcode

B15 2TT

#### Tel Number

0121 414 5251

#### Fax Number

0121 414 3309

#### E-mail

MIDLANDS

#### Comments

CENTRE MERGED WITH GM CENTRE 116.

#### Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
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<th>Level 1 (GMMs)</th>
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Tick if confidential

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Recyclable culture glassware is collected in lidded autoclavable discard drums. Drums are autoclaved for 30 mins at 121°C. Any autoclaved spent media and culture is run to drain and glassware is washed and reused.

Bioreactors with culture and spent media are autoclaved or sterilised by steam in situ according to volume at 121°C. Contents are cooled and sent to drain. With large capacity bioreactors (>20 litres) biomass is removed by centrifugation and incinerated because of environmental considerations.

Disposable contaminated plastics are collected in autoclave bags, in lidded autoclave drums and autoclaved at 121°C for 30 minutes. Bags are then put in clinical waste bags categorised and sent for incineration.

Any sharps are collected in sharps bins and sent for incineration. (This is being reviewed to include treatment before incineration).

Disinfectants are used for the cleaning of surfaces in accordance with Good Microbiological practice.

Action for spills: Absorbents are recommended to be used with disinfectants for spillages and these should be bagged and autoclaved.

Recommended disinfectants to be used according to biological material: 1% Virkon, Hibitane 1 in 200, Chloros, SBS 0.5%, Data sheets available

Validation of autoclaves and all these disinfectants is under review.
GM Centre Number: 125

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Name

**UNIVERSITY OF BIRMINGHAM**

Name 2

**BIOSCIENCES**

Campus Estate or Research Centre

**EDGBASTON CAMPUS**

Road Name

District

**EDGBASTON**

Town

**BIRMINGHAM**

County

**MIDLANDS**

Postcode

**B15 2TT**

Country

**ENGLAND**

Tel Number

0121 414 5251

Fax Number

0121 414 3309

E-mail

HSE Division

**MIDLANDS**

Comments

CENTRE MERGED WITH GM CENTRE 116 - 4/4/06.

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

  - Give brief details of the genetic modification safety committee
  - Level 1 (GMMs)
  - Level 2 (GMMs)
  - Level 3 (GMMs)
  - Level 4 (GMMs)
  - Non-microbial
  
  - Other (please specify)

  Tick if confidential

02/03/2022
### Project Ref: 116/03.1

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<td>&lt; 1 Litre</td>
<td></td>
<td></td>
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</table>

**Historical Significant Changes:** 116/03.1 TRANSFERRED FROM GM116.
### Project Additional Information

#### Purposes of the contained use

The study will focus on the potential of human liver stem cells as alternative sources of functional human hepatocytes and biliary epithelial cells. Isolated human liver stem cells will be stably transduced with a recombinant lentivirus expressing the gene for Green Fluorescent Protein. The potential of these marked cells to repopulate acutely injured livers will be assessed by cell transplantation experiments in NOD/SCID mice (but not here in the UK).

#### Recipient or parental organism

**Hosts.** Standard disabled lab strains of E. coli as DH5a and JM109 will be used for growth of plasmids. Manipulation of large plasmids may involve homologous recombination in strain BJ5183. Human cell line 293T will be used for both initial rescue of virus from transfected DNA, and for virus, propagation. Primary human liver stem cells (which are non-permissive for production of replication deficient lentivirus), will be transduced with recombinant lentivirus expressing GFP.

#### Host/vector system

**Vectors:** Plasmids; Third generation lentiviral vector (replication defective). Please see assessment for details.

#### Origin & function

**Sequence encoding Green Fluorescent Protein which functions as a reporter molecule that fluoresces upon exposure to uv light.**

#### Evaluation of foreseeable effects

**Risks to human health:** The lentiviral vectors used in this study are third generation lentiviral vectors. In these third generation vectors all six regulatory/accessory proteins (Rev, Tat, Vif, Vpr, Vpu and Nef) are removed, except Rev. Rev acts as the post-transcriptional level and is necessary for HIV gag/pol messenger. As an extra safety responsive element [RRE]) and facilitates the cytoplasmic export of gag/pol messenger. As an extra safety measurement Rev is placed on a separate vector (RSV-Rev) beside transfer vector. Only 700 bp of the HIV envelope protein are present in the transfer vector, plus RRE and the packaging signal. The packaging construct contains the minimal RRE of 375 bp and the gag/pol genes. Through deletions in both LTRs and the absence of 5 of the 6 accessory proteins, including the replication essential Tat it is highly unlikely that replication competent virus is produced. In published studies and from our own experience with these vectors replication competent virus has never been detected. The HIV-envelope protein is replaced by non-retrovirally envelope protein (VSV-G). It is highly unlikely that this will be incorporated in a new hybrid replicative virus. It is impossible that wild-type HIV will be formed, because of the omission of 5 accessory proteins with HIV-env.


**Environmental considerations:** Proteins will not be expressed in bacteria used for plasmid propagation. Bacteria used for propagation are attenuated strains that will not survive in the environment. The recombinant virus is replication defective and will not have an extended host range, the GFP transgene is innocuous, so there is negligible potential hazard to the environment. Containment conditions specified should prevent environmental release of virus or E. coli carrying the associated plasmids; and the virus is replication defective.
### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation applied for.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is treated by autoclaving at 130 degrees centigrade for 60 mins before disposal to drain. Disposable solid waste which is or may be contaminated with GMMs is also inactivated by autoclaving at 130 degrees centigrade for 30 mins, before removal as clinical waste by specialist contractors, with final disposal by incineration. Autoclaving achieves 100% kill. Warning lights, and chart recorder, indicate whether or not the autoclave run has achieved the appropriate temperature for the required time. The autoclave is run by trained staff, who are instructed to treat waste as still contaminated if the autoclave run was not to specifications. Under such circumstances the waste would be autoclaved again, after rectifying any reason for the malfunction.

The autoclave is inspected and serviced on a regular basis. Autoclave is regularly tested with Brown's tubes.

Sharps, including scalpel blades, needles and disposable plastic tips, may be decontaminated by drawing up Virkon disinfectant, and by soaking in Virkon for a minimum of 15 minutes. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above. Manufacturer's information indicates efficacy of Virkon against strains of E. coli at dilutions ranging from 1% to 0.125%. The manufactureres of Virkon (Antec International Ltd) have also commissioned studies on the virucidal effect of Virkon on Human Immunodeficiency virus and found that a 0.5% solution of Virkon inactivated 10^6 infectious doses (ID50) of HIV-1 per ml within 10 minutes at room temperature.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

N

### Please enter comments on the GM safety committee on the risk assessment

1. I am not sure that the statement that pMDLg/pRRE expresses no gag/pol has ever been convincingly demonstrated. Perhaps it is better to say that only very low or undetectable levels will be anticipated.
2. The applicant states that he has never detected replication competent virus. How has he looked for it? Has it ever been detected?
3. Is there validation data for Virkon against lentiviruses?
4. Is the KI discus test carried out annually rather than six monthly?
5. The autoclave should be additionally tested with Brown's tubes on a regular basis.

All of the above have been addressed in the assessment. The GMSC have approved this assessment.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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02/03/2022
### Project Ref 125/01.1

<table>
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<td>INVESTIGATION OF THE INFLUENZA VIRUS GENES INVOLVED IN VIRUS INDUCED APOPTOSIS</td>
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<th>Class</th>
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**Historical Significant Changes**

TRANSFERRED TO GM CENTRE 116 - 4/4/06.

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
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Project Ref 125/01.2

Date Ackn'd | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4 |
-------------|-------------------|-------|------------------|-----------------------|
15/02/2001   | THE IDENTIFICATION OF INFLUENZA VIRUS PYROGENS | Class 2 |                |                      |
Date Project Ceased
04/04/2006

Non-GMM

Consent Granted
not applicable

Tick if notifying a connected programme of work
N

Project notified under transitional arrangements
Y

Withdrawn
N

Historical Significant Changes
TRANSFERRED TO GM CENTRE 116 - 4/4/06.

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<td>L2 L3 L4 L2</td>
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**Project Ref 125/01.3**

- **Date Ackn’d**: 15/02/2001
- **CU2 Project Title**: CLONING AND OVER EXPRESSION OF SOLUBLE INORGANIC PYROPHOSPHATASES IN E.COLI
- **Class**: Class 2
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**

TRANSFERRED TO GM CENTRE 116. 4/4/06.
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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</table>

02/03/2022
Project Ref 125/01.4

EXPRESS THE ACID PROTEASE GENE OF YARROWIA LIPOLITICA IN E.COLI

15/02/2001

04/04/2006

TRANSFERRED TO GM CENTRE 116 - 4/4/06.

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 125/01.5

Date Ackn'd 15/02/2001

CU2 Project Title IDENTIFICATION OF METAL RESISTANT BACTERIA AND ARCHAEA

Class 2

CultureVolumeClass3-4

Class 2
Date Project Ceased: 04/04/2006

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Historical Significant Changes: TRANSFERRED TO GM CENTRE 116 - 4/4/06.

Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Project Ref 125/02.1

Date Ackn’d 12/09/2002
Date Project Ceased 04/04/2006

CU2 Project Title

THE USE OF RECOMBINANT CORYNEBACTERIUM GLUTAMICUM AND MYCOBACTERIUM BOVIS BCG TO EXAMINE GENE FUNCTION

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 litre

Non-GMM Consent Granted not applicable

Tick if notifying a connected programme of work Y

Historical Significant Changes
TRANSFERRED TO GM CENTRE 116 - 4/4/06.
**Project Additional Information**

**Purposes of the contained use**

The objectives of the programme of work are to construct and characterise mutants of Corynebacterium glutamicum and Mycobacterium bovis BCG. These mutants will be used to analyse well-defined, non-toxic, mycobacterial cell wall components and genes that contribute to the basic physiology of corynebacteria and mycobacteria.

The programme of work on M. bovis is illustrative rather than exhaustive so that similar work can begin on other mycobacterial ACDP category 2 organisms without the need to submit a fresh application each time. Changes to the project will, however, be risk assessed and passed to the local GMSC for approval.

A previous risk assessment titled "The use of recombinant Mycobacterium smegmatis for the biochemical/physiological analysis of cell wall products "covers our work studying the function of mycobacterial genes (from M. tuberculosis H37Rv and M. bovis BCG) using Escherichia coli K12 and M. smegmatis as hosts prior to our move from the University of Newcastle upon Tyne. This assessment was approved by the University of Newcastle upon Tyne ACGM committee in 1999 as Hazard Group 1 and was reassessed as Activity class 1 in January 2000, in revising assessments under the New Contained Use Regulations 2000. Work in the laboratory using E. coli K12 and M. smegmatis as hosts for mycobacterial sequences, including work linked with the current project, will continue to be covered by this original risk assessment and has been approved by the local GMSC at the University of Birmingham. In addition, whilst at the University of Newcastle upon Tyne approval was granted by the Microbiological Hazards and Genetic Modification Safety Advisory Committee for the project "The use of recombinant Mycobacterium bovis BCG to examine gene function" (October 2001) and assessed as containment level 2, class 2. The project was subsequently notified to the HSE and approval was granted in November 2001 at Newcastle (REF: GM540). The present assessment aims to extend cover to the use of C. glutamicum and the vaccine strain M. bovis BCG (Pasteur) as hosts (M. bovis BCG as originally proposed at Newcastle prior to our move to the School of Biosciences at The University of Birmingham.

The individual activities with this connected programme are as follow:

1. Cloning of DNA fragments from C. glutamicum, M. bovis BCG and M tuberculosis H37Rv in non-mobilisable vectors in lab strains of E. coli with a view to sequencing or mutating the fragments.
2. Creation of mutants in wild type strains of C. glutamicum and M. bovis BCG by random transposon mutagenesis resulting in insertional inactivation, linked in some cases with the creation of transcriptional fusions to reporter genes.
4. Allele replacement using a gene that expresses a marker protein which is highly unlikely to have any biological effect (such as B-galactosidase, luciferase, GFP) into a pathogen to monitor gene expression under different environmental conditions.
5. Expression of proteins at a high level in a disabled host bacterium to enable further chemical/biochemical characterisation of the protein.
6. Expression in C. glutamicum and M. bovis BCG of well defined genes from C. glutamicum, M. Bovis BCG and M tuberculosis H37Rv running off its own promoter on a shuttle vector, also to complement well-defined mutant phenotypes.
7. Expression of antibiotic resistance determinants in a disabled host bacterium to enable further characterisation of the resistance mechanism.

**Recipient or parental organism**

Disabled laboratory strains of E. coli including or similar to those listed in Part 2A, Annex II of the ACGM Compendium of Guidance.

C. glutamicum and M. bovis BCG.

M. bovis BCG is one of the oldest and most widely used live vaccines; about 3 billion doses of this attenuated M. bovis strain have been used to immunise individuals against tuberculosis worldwide. Whole genome comparisons of M. tuberculosis H37Rv and M. bovis BCG (Pasteur) have now been undertaken to determine the genetic determinants of attenuation. These reveal several (14) large deletions in the M. bovis BCG (Pasteur) genome which encompass 119 open reading frames (Behr et al., 1999 Science 28: 1520-3).

The ACDP categorisation of pathogens according to hazard and categories of containment (fourth edition, 1995), classifies M. bovis BCG as hazard group 2. However, appendix 17 (page 108) states "The BCG strains of M. bovis and the so-called 'vole bacillus' M. microti have both been used in vaccines, but if mishandled, can give rise to abscess formation. The BCG strains are included in Hazard Group 2 because of this and the extremely remote possibility of reversion to virulence although when used..."
under clinical conditions for immunisation, laboratory Containment Level 2 is not necessary”. Staff working on the programme have previously been immunised with BCG. C. glutamicum is known for its ability to massively excrete L-glutamate. The capacity of C. glutamicum to excrete glutamate has been used for more than 40 years to produce this amino acid on an industrial scale in ever increasing quantities, around 106 tonnes/year (Hodgson, 1994, Bio/Technology 12: 152-155). The ACDP categorisation of pathogens, according to hazard and categories of containment (fourth edition, 1995), classifies Corynebacteria spp. as hazard group 2. Although, C. glutamicum has been defined as having "biological limitations" which mean that it is unlikely to survive in the gut, lung and elsewhere. This description is also generally considered to cover laboratory adapted strains (particularly multiply auxotrophic or recombination deficient mutants) as well as other non-pathogenic hosts with a negligible capacity to persist in humans and a history of safe use.

Host/vector system

Any non-mobilisable or mobilisation defective including or similar to those listed in Part 2a Annex II of the ACGM Compendium of Guidance.

The pNIL, pGOAL and pYUB854 series of vectors for constructing suicide vectors to generate marked and unmarked mutants in M. bovis BCG.

The pK18mob suicide vector will be used to generate marked mutants in C. glutamicum.

Non-mobilisable shuttle vectors that can replicate in E. coli and mycobacteria (eg pJEM15, pMV261, pVV16, pPR27 and their derivatives). These vectors contain an E. coli origin of replication (from the pUC series of cloning vectors), a mycobacterial origin of replication from plasmid pAL5000 and antibiotic resistance markers (eg Kanamycin and hygromycin).

Origin & function

Source: C. glutamicum, M. bovis BCG and M. tuberculosis H37Rv

Genetic material: DNA fragments encoding various well defined genes involved in cell wall metabolism, eg glycosyltransferases, fatty acid biosynthesis, polyketide biosynthesis, siderophore biosynthesis, p450 enzymes and peptidoglycan biosynthesis.

Source: Depending on marker -E. coli for B-galactosidase, luminescent vibrios for luciferase, jellyfish for GFP.

Genetic material - A gene that expresses a marker protein which is highly unlikely to have any biological effect, eg GFP, luciferase, B-galactosidase.

Antibiotic resistance genes expressed from their native promoters.

Evaluation of foreseeable effects

PLEASE SEE ASSESSMENT FOR DETAILS

The risk assessment deals with the risks to human health and the environment under each specific activity. Most of the work will make use of crippled lab strains and non-mobilisable vectors, although wild type strains will be used to study the effects of defined chromosomal mutations on mycobacterial cell wall physiology and other phenotypes. The risks to human health are therefore associated with the wild type host. Environmental risks are dealt with under each activity in the assessment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full level 2 applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All potentially infected material is autoclaved prior to disposal and incineration. 100% kill of GMMs. Disinfectants such as hycolin (2% v/v) and Chloros are available for spills.
Some members of the GMSC pointed out that the original assessment contained a reference to antibiotic resistance markers, but did not then list them or discuss them further. These were added.

The GMSC asked what were the ‘well defined genes involved in cell wall metabolism and cellular physiology’? Examples were added to illustrate this, as a full list would have been extremely long.

The assessment was approved by the GMSC.

Please enter comments on the GM safety committee on the risk assessment

Some members of the GMSC pointed out that the original assessment contained a reference to antibiotic resistance markers, but did not then list them or discuss them further. These were added.

The GMSC asked what were the ‘well defined genes involved in cell wall metabolism and cellular physiology’? Examples were added to illustrate this, as a full list would have been extremely long.

The assessment was approved by the GMSC.

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**Project Ref 125/03.1**

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<td>04/04/2006</td>
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Withdrawn: N

Tick if notifying a connected programme of work: Y
Project Additional Information

**Purposes of the contained use**

The work is concerned with understanding how bacterial gene expression is regulated using E. coli K12 as a model. The strategy is to use genetics to identify regulatory components at particular loci and then to use genetics and biochemistry to understand the interaction between these components. This involves many genetically manipulated constructs in which altered regulatory elements and regulatory components are tested. Additionally genes encoding regulatory factors are manipulated to facilitate overexpression and purification of the corresponding protein.

**Recipient or parental organism**

**Hosts:** Standard disabled hosts such as E. coli K12 strains. For mutagenesis the hosts will be standard, well characterised strains of the wild type organism.

**Host/vector system**

**Vector:** Vectors will generally be pUC, pBluescript and related non-mobilisable CoIE1-based standard vectors, or mabda phage-based cloning vectors, or standard low copy number vectors such as pACYC or pSC101 and derivatives.

**Origin & function**

**Inserts:** Sequences used may be any gene or sequence including genes of unknown function, including virulence factors. Genes may be partial or complete and may include expression signals, eg segments from the E. coli lac, gal, mal, ara, mel nir, and nrf operons, the fnr, crp, rpoA, rpoBC, rpoD, rpoS, fis, ihfA, ihfC hupA, hupB and hns genes. Various 'anonymous' but completely sequenced regulatory genes from K12 or the Sakai 0157 strain. Non coli genes include B. pertussis cyaA, V. fiscerii lux, plus various commercially available genes that are used as tools. Libraries may be constructed for limited purposes, eg two hybrid screening.

**Donor.** Sources are either whole genomic DNA or PCR-amplified known sequences, from E. coli. In general source DNA will be taken from an organism for which a whole genome sequence is known. No organisms requiring greater than containment level 2 will be used. In some experiments the starting material will be clones from other laboratories, eg encoding lac, gfp, lux, cya or other 'tools' for molecular biology.

**Evaluation of foreseeable effects**

**Effects on human health:** None of the constructs cause harm beyond that of the starting host and no risk additional to that of the parental wild type organisms is envisaged.

**Environmental hazards:** The potential hazard is negligible as the GMMs cannot be considered likely to express virulence or other factors that would be potentially harmful to other species or the environment. Accidental release is highly unlikely.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - full level 2 containment applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be autoclaved - a process that is validated by monitoring of the internal chamber. An automatic printout of every run is produced. Achieves 100% kill. Wastes from autoclaving will be disposed of to drain or to clinical waste incineration.

The standard disinfectant used is Virkon, 1% w/v solution. This will only be used to sterilise lightly contaminated surfaces, and is not used for treatment of substantial culture volumes, or for rendering safe contaminated matter such as absorbent towels - these will be autoclaved. Studies have shown the efficacy of 1% solutions to achieve typically at least 10 (to the power of 5) reduction in counts under appropriate conditions.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The assessment has been approved by the GMSC without adverse comment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L3 L4 L2 L3 L4 L2 L3 L4</td>
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Project Ref 125/03.2

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<tr>
<td>17/07/2003</td>
<td>CYTOSKELETAL GENE ANALYSIS IN ENTAMOEBA AND DICTYOSTELIUM</td>
<td>Class 2</td>
<td>1-50 litres</td>
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**Project Additional Information**

**Purposes of the contained use**
To express cDNAs encoding Entamoeba cytoskeletal and signalling proteins in both Entamoeba and Dictyostelium cells.

**Recipient or parental organism**

**Hosts.** E. coli XL1-blue - disabled and slow growing; DH5 - as XL-blue.

Dictyostelium strains AX2 and AX3 - free living, non-pathogenic soil amoeba. Grow more slowly than wild type under non-laboratory conditions. No pathogenicity or harm to humans or livestock. Potential harm to environment is negligible. Entamoeba histolytica strain HM-1:IMSS - standard laboratory strain. Cysts are the only infectious stage of this parasite and these can not develop or form in standard lab cultures, hence the parasite is not transmissible to humans in this setting. The cultured form, ie the trophozoite, is very fragile and susceptible to desiccation and detergents. As they are unable to persist in the environment the chance of environmental damage is remote.

**Host/vector system**
Vector. pUC18 - standard molecular biological plasmid vector.

**Origin & function**

Origin of genetic material: Dictyostelium strain AX3 - a free living non-pathogenic soil amoeba which grows at 22 degrees centigrade and is harmless to crops and agriculture.

*Antamoeba histolytica strain HM-1:IMSS* -- standard laboratory strain. Cysts are the only infectious stage of this parasite and these can not develop or form in standard lab cultures, hence the parasite is not transmissible to humans in this setting. The cultured form, ie the trophozoite, is very fragile and susceptible to desiccation and detergents. As they are unable to persist in the environment the chance of environmental damage is remote.

Genetic material: Full length cDNAs encoding Entamoeba Arp2 and Arp3 - major skeletal proteins involved in movement and cell shape. Selectable markers conferring G418 resistance.
### Evaluation of foreseeable effects

**Effects on human health:**

**Dictyostelium** - None. It is a non-pathogenic soil amoeba, unable to grow at human body temperature and is harmless to crops and agriculture.

**Entamoeba histolytica** - No likely risks. It is a parasitic enteric protozoan that is responsible for amoebiasis in humans. The disease is characterised by diarrhoea/dystentery, intestinal tissue invasion and potential liver abscess formation. Epidemiology, the organism is ubiquitous and has a worldwide distribution, although prevalence is much higher in developing, disadvantaged countries and tropical regions. In contrast, rates of infection are low in the developed world, with the majority of these infections being found in specific groups (e.g., travellers from developing or high incidence areas). Symptomatic invasive amoebiasis only develops in 10% of individuals harbouring the parasite. The organism can exist as both cysts and trophozoites. Transmission is faeco-oral and results from ingestion of viable cysts in contaminated food or water. Cysts can remain viable for a long time depending on environmental conditions but are extremely sensitive to desiccation. Trophozoites on the other hand are more fragile and degenerate rapidly in the external environment as well as being easily destroyed by acidic pH (e.g., stomach acid).

As the only infectious stage of the parasite, the cyst, does not form in standard laboratory cultures the parasite is not transmissible to humans in this setting. Accidental infections have never been reported, but effective drug treatments are available should this happen.

**Environmental hazards:** Dictyostelium - negligible hazard. The strains used are very weak due to multiple mutations and would not compete with local Dictyostelium species.

Entamoeba - The organisms are very sensitive to desiccation and detergents and are not free-living. As they are unable to persist in the environment the chances of environmental damage are remote.

The work will be carried out at CL2 and the likelihood of accidental release is very low.

**E. coli XL-blue/DH5** - non-colonising, disabled lab strains, no risk to human health or the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation applied for CL2 applied. The laboratory set aside for this work is a relatively new facility and is currently being fitted with a hand wash basin.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Dictyostelium** - to kill spent cultures: 1% chlorine bleach.

**Entamoeba** - Tubes containing liquid cultures and contaminated disposables will be placed in autoclave bags and autoclaved at 121 degrees for 50 minutes and discarded as "clinical waste" (incineration).

Agar plates, loops, tips etc will all be autoclaved. 100% kill.

All methods are more than sufficient to kill Dictyostelium which are fragile, as well as Entamoeba which are sensitive to desiccation and detergents and are not free-living.

**Bacterial cultures** will be killed with Virkon at the recommended dilution (1:100)

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N
**Please enter comments on the GM safety committee on the risk assessment**

* I feel information on human health should be transferred to the relevant section; the nature of the "normal laboratory clothing and protective equipment" should be defined; the autoclave used for disposal of cat 2 waste should be monitored (it is anyway, why not say so for completion?); and the disinfection regimen should be defined, ie is 1% chlorine bleach 1% available chlorine, and what is the actual dilution of Virkon used?

**POINTS ADDRESSED IN ASSESSMENT**

* There is no mention of any possible alteration in phenotype resulting from the introduction of cytoskeleton and signalling proteins in Entamoeba and Dictyostelium. I assume they will not become (more) pathogenic but perhaps this could be addressed.

* No increase in virulence/pathogenic capacity is expected. As mentioned in the application, Dictyostelium are free-living non-pathogenic soil amoebae which grow at 22 degrees and therefore do not pose a threat to humans. Likewise Entamoeba does not pose a risk to humans in this/our setting because the cyst, which is the only infectious stage of the parasite, can/do not develop or form in standard laboratory cultures, hence the parasite is not transmissible to humans.

The GMSC also asked whether the Class 1 and Class 2 work should be split into two separate proposals. The investigator felt this was not necessary and not desirable because the work is one experiment, in which cDNAs encoding Entamoeba cytoskeletal and signalling proteins will be expressed in both Entamoeba and Dictyostelium cells.

The GMSC has approved this assessment.

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**Project Containment**

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**Project Ref** 125/05.1

**Date Ackn’d** 07/04/2005

**CU2 Project Title** Investigation into the role of downstream components of the FGFR cell signalling

**Class** Class 2

**CultureVolClass2** < 1 Litre
The technique of RNA interference (RNAi) will be used to knockdown FRS2 (a component of the fibroblast growth factor receptor signalling pathway) in primary human cells. Use of a replication incompetent bicistronic HIV1 based lentivirus will be applied to deliver the SiRNA into cells.

**Recipient or parental organism**

Host: Host bacteria for growth of plasmids are standard disabled lab strains of E. coli such as DB3.1 and Stbl3. DB3.1 is an E. coli strain that is resistant to CcdB effects and can support the propagation of plasmids containing the ccdB gene. One shot Stbl3 chemically competent E. coli from Invitrogen are well suited for use in cloning unstable DNA such as lentiviral DNA containing direct repeats. Human cell line HEK 293T will be transfected with the pLenti4/V5-DEST and the packaging vectors and lentivirus harvested from the supernatant. Primary human cells that are non-permissive for production of replication defective lentivirus will be transduced with recombinant lentivirus expressing GFP and RNAi gene of interest. Further work may use mouse embryo fibroblasts.

**Vector(s):** The viraPower Lentiviral Expression System (Invitrogen) used allows creation of a replication incompetent HIV-1 based lentivirus which can then be used to deliver and express our gene of interest in either dividing or non-dividing cells. Four vectors are involved; one viral transfer vector and three packaging vectors (see assessment for details). The method used to clone our gene of interest into the lentivirus vector is gateway cloning and for this another vector will be used, known as pDONR.

**Origin & function**

Source: Green Fluorescent Protein from jellyfish. Sequence encoding a small part of FRS2 (approx 21 bases) from human and hairpin sequence needed for RNAi to work effectively.

Genetic material: The sequence encoding GFP which functions as a reporter molecule that fluoresces upon exposure to uv light. Also, a partial sequence encoding a small section of the FSR2 protein and a hairpin sequence needed for the short hairpin RNAs to be produced and work effectively. FSR2 is an adaptor protein that acts downstream of FGFR.
Human health: In third generation lentiviral vectors all six regulatory/accessory proteins (Rev, Tat, Vif, Vpr, Vpu and Nef) are removed, except Rev. Rev acts at the post-transcriptional level and is necessary for HIV gag/pol expression. Rev binds to an RNA motif (Rev Responsive Element RRE) and facilitates the cytoplasmic export of gag/pol messenger. As an extra safety requirement Rev is placed on a separate vector (pLP2) to the transfer vector. Only 700bp of the HIV envelope protein are present in the transfer vector, plus RRE and the packaging signal. The packaging construct contains the minimal RRE of 374bp and the gag/pol genes. Through deletions in both LTRs and the absence of 5 of the 6 accessory proteins, including the replication essential Tat, the biosafety of the vector is increased. In published studies replication competent virus has never been detected. The HIV envelope protein is replaced by non-retrovirally envelope protein (VSV-G). It is highly unlikely that this will be incorporated in a new hybrid replicative virus. It is impossible that wild type HIV will be formed because of the omission of 5 accessory proteins and the HIV-env. No protein is expected to be expressed in bacterial strains used for plasmid propagation.

Environmental Considerations: Proteins will not be expressed in bacteria used for plasmid propagation. Bacteria used for propagation are attenuated strains that will not survive in the environment. The recombinant virus is replication defective and will not have an extended host range, the transgenes are innocuous, so there is negligible potential hazard to the environment. Potential hazard to species in the environment either from the viruses, or the E. coli containing the plasmids, therefore appears negligible. Containment conditions specified should prevent environmental release of virus, or of E. coli carrying associated plasmids.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be aspirated into, and exposed to, 1% Virkon for a minimum of 30 minutes, or collected into autoclavable pots and autoclaved before disposal to drain.

Disposable solid waste will be inactivated by autoclaving with final disposal by incineration.

Exceptions to this are: Contaminated micropipettor tips will be soaked in 1% Virkon for a minimum of 30 minutes before being rinsed and disposed of in Sharps containers, which will be sent for incineration. Disposable plastic pipettes also treated with Virkon prior to rinsing and disposal as above.

Exposure to Virkon for a minimum of 30 minutes, or autoclaving effectively kills 100% of GMMs.

Is an emergency plan required according to regulation 20?  Y

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

No adverse comments.

Project Containment
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**Project Ref** 125/94.1

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Tick if notifying a connected programme of work

Withdrawn N

Historical Significant Changes
GM125/97.2. TRANSFERRED TO GM CENTRE 116 - 4/4/06.

Historical Date of Additional Info
12/11/1997

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

02/03/2022
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 125/95.3

Date Ackn'd 20/02/2001

CU2 Project Title REGULATION OF GONOCOCCAL GENE EXPRESSION

Class 2

CultureVolClass2 CultureVolumeClass3-4
Date Project Ceased
04/04/2006

Tick if notifying a connected programme of work N

Withdrawn N

Historical Significant Changes
GM125/95.3 - TRANSFERRED TO GM CENTRE 116 - 4/4/06.

Historical Date of Additional Info
02/10/1995

Project notified under transitional arrangements Y

**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Project Ref 125/97.1

Mutagenesis of Pathogenic bacteria: Allelic replacement using antibiotic resistance markers and antibiotic selected transposen mutagensis

Date Ackn’d 21/10/1997

CU2 Project Title

Date Project Ceased 04/04/2006

Non-GMM Consent Granted

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Date of Additional Info

TRANSFERRED TO GM CENTRE 116 - 4/4/06.
**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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**Project Containment**

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02/03/2022

Page 3551 of 1532
Project Ref 125/99.1

Date Ackn'd 18/10/1999
Date Project Ceased 04/04/2006

CU2 Project Title STRUCTURE, FUNCTION AND DYNAMICS DENDROASPIN AND VARIENTS

Class 2
CultureVol 2
Consent Granted not applicable

Project notified under transitional arrangements

Historical Significant Changes TRANSFERRED TO GM CENTRE 116 - 4/4/06.

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Date Project Ceased**
04/04/2006

**Non-GMM Consent Granted**
not applicable

**Project notified under transitional arrangements**
Y

### withdrawn
N

### Tick if notifying a connected programme of work
N

### Historical Significant Changes
TRANSFERRED TO GM CENTRE 116 - 4/4/06.

### Historical Date of Additional Info

### Significant Change ID

### Date of Significant Change

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## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20?  
N
If yes, tick to confirm that it is attached to this form  
N
Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

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Withdrawn N  
Tick if notifying a connected programme of work N  

Historical Significant Changes
TRANSFERRED TO GM CENTRE 116 - 4/4/06.

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Name

MRC CENTRE HARWELL

Name 2

CELL MUTATION SECTION

Department

Building

HARWELL

District

DIDCOT

Road Name

OXFORDSHIRE

Town

OX11 ORD

County

Town

ENGLAND

Country

Tel Number

01235 841 000

Fax Number

01235 834 776

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

**Level 1 (GMMs)**

**Level 2 (GMMs)**

**Level 3 (GMMs)**

**Level 4 (GMMs)**

**Non-microbial**

**Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

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The maximum culture volume that could be released at any one time is 2 litres. GM waste: Liquid waste is deactivated using Domestos bleach at recommended concentrations (Domestos containers are date-stamped on receipt and bottles with dates older than 6 months not issued for lab use); Solid waste is double-bagged and disposed of by autoclaving (120 degrees /20 min) by trained staff (a signed record is kept of all autoclave runs). Monthly validation of autoclaves is made by the inclusion of Browne's tubes.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

UNIVERSITY OF GLASGOW

**Name 2**

INSTITUTE OF GENETICS & BIOTECHNOLOGY

**Department**

ANDERSON COLLEGE

**Campus Estate or Research Centre**

Building

**Road Name**

56 DUMBARTON ROAD

**District**

**Town**

GLASGOW

**County**

EAST RENFREWSHIRE

**Postcode**

G11 6NU

**Country**

SCOTLAND

**Tel Number**

041 330 5105

**Fax Number**

0141 330 4878

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)  Tick if confidential

Bacteriology

Parasitology  Transgenic

Birds

Virology

Transgenic

Animals  Transgenic

Fish

Mycology

Transgenic

Invertebrates  Transgenic

Plants

Transgenic

Birds

Transgenic

Animals

Gene Therapy

Microbiology

Research

Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref  129/03.1

Date Ackn'd  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4

02/03/2022  Page 3563 of 15326
GENETIC MODIFICATION OF HUMAN MALARIA PARASITES FOR FUNCTIONAL GENETIC ANALYSES

Date Project Ceased: 26/10/2007

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: PROJECT TRANSFERRED TO GM 37 (26/10/07)

Historical Date of Additional Info: 26/10/07

Significant Change ID

Historical Date of Additional Info

Project notified under transitional arrangements: N

PROJECT TRANSFERRED TO GM 37 (26/10/07)

Purposes of the contained use

P. falciparum is a serious human pathogen classified by ACDP as Group III* (derogation applies such that they can be handled in category II facilities, with no requirement for positive pressure of HEPA filtration, since there is no risk of airborne contamination.).

No significant additional hazards have been identified above and so it is appropriate to assign the same containment level as that required for the non-GM organism. This is categorised by ACDP as Class III, but derogation is permitted to allow work to proceed under containment level 2. A COSHH form pertaining to P. falciparum cultures has been signed by all relevant personnel.

Recipient or parental organism

The function of the inserted parasite gene product will frequently be unknown, or can only be implied through homology with other organisms. Gene knockout experiments targeting genes linked to invasion should result in parasites with lowered invasion potential. Knockouts of potential drug targets (e.g., protein kinases) are aimed at reducing the fitness of the knockout parasites, preferably to produce non-viable or severely functionally-impaired parasites. Allelic replacement of protein kinase genes with those exhibiting hypersensitivity to inhibitors should result in parasites with increased sensitivity to these inhibitors. Growth rates are not expected to be affected, other than negatively, in any of these experiments.

Host/vector system

Growth of Plasmodium falciparum in human red blood cells.

Origin & function

Stable transfection systems for the human malaria parasites have only recently become available, and provide a means to investigate gene function using gene modification or ablation ("knockout"). Transfection is achieved through electroporation of either red blood cells prior to infection by the parasite, or ring-stage parasites (asexual development cycle in the blood), using in vitro cultured parasites grown in human erythrocytes, with specific plasmid vectors such as pHRPCAT. These vectors contain a drug-selectable marker, usually the dihydrofolate resistance gene (DHFR) from pyrimethamine-resistant strains of either P. falciparum or Toxoplasma gondii, for selection with pyrimethamine, or the human DHFR for selection with the antifolate drug WR99210. We will also use plasmids based on the pHHT-TK vector 4, which allows negative selection using gancyclovir.
Transient transfection (without permanent modification of the parasite’s genome) will be performed using plasmids encoding reporter genes such as GFP, luciferase or CAT. The mRNA levels of specific genes will be targeted by RNAi.

Plasmodium genes considered for possible GM work:

* Protein kinases (a preliminary examination of the PlasmoDB database indicated the presence of approximately 60 genes of this family in the parasite’s genome, any of which can be of interest in the context of our work).
* Cyclins (pfcyc-1, -2, -3, -4)
* Apical complex proteins (esp. pFRH1, -2A, -2B, -3, and -4)

Evaluation of foreseeable effects

Any environmental risk is more likely due to the organism itself rather than the genetic modification. Because we will not be using mosquitoes, the release of the parasite into the environment is exceedingly unlikely.

The function of the inserted parasite gene product will frequently be unknown, or can only be implied through homology with other organisms. Gene knockout experiments targeting genes linked to invasion should result in parasites with lowered invasion potential. Knockouts of potential drug targets (e.g., protein kinases) are aimed at reducing the fitness of the knockout parasites, preferably to produce non-viable or severely functionally-impaired parasites. Allelic replacement of protein kinase genes with those exhibiting hypersensitivity to inhibitors should result in parasites with increased sensitivity to these inhibitors. Growth rates are not expected to be affected, other than negatively, in any of these experiments. For all new transgenic strains, growth rates will be checked as part of phenotypic characterisation.

Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Similar containment requirements are for unmodified P. falciparum. As for unmodified P. falciparum, derogation from various aspects of Level 3 containment is appropriate. The reasons for derogation relate to the fact that infection by aerosol or ingestion does not occur, and spread of the disease requires the appropriate vector species of mosquitoes, which are absent in the area where the work will occur.

We request derogation from standard level three containment on the three points detailed below. Plasmodium cannot be transmitted by aerosol. The normal route of transmission is by mosquito bite. In laboratory conditions, the only means of infection is by an accidental puncture wound, for example with a contaminated syringe needle. Also, plasmodium is exceptionally fragile. To survive outside its host species (humans and mosquitoes), it must be kept in complex media. Plasmodium dies instantly if placed in water and is also killed by drying.

1. Negative pressure relative to the pressure of immediate surrounds. Derogation requested. Plasmodium transmission by aerosol does not occur.
2. Extract and input air from the laboratory should be HEPA filtered. Derogation requested. Plasmodium transmission by aerosol does not occur.
3. Protective footwear. Derogation requested for footwear. The risk of live/infectious parasites escaping to the environment in this way is nil as they cannot survive outside the host in the environment - not necessary as this requirement is only where and to the extent the risk assessment shows it to be required.
4. Autoclave required in the laboratory suite.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste is inactivated using Hibitane prior to disposal. Solid waste for autoclaving will be double-bagged and transported to the autoclave room in a plastic drum. The culture room is kept locked and access is restricted to trained personnel. Written training and culture records are maintained. The sensitivity of new transgenic strains to killing by hibitane will be tested.
We feel this is a good and well thought out risk assessment. From an original discussion of the proposal, the committee raised a variety of questions, all of which have been answered to our satisfaction, by the principle investigators. In particular, we feel that the areas of derogation from level 3 are appropriate because of the extremely low risk of spread of plasmodium into the wider community and environment. We believe the only significant risk is to the actual individuals involved in the project where infection as a result of a puncture wound could theoretically occur. The risk of a puncture wound occurring is minimised by avoiding the use of sharps in handling transgenic plasmodium.

### Project Containment

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### Project Ref 37/99.1

- **Date Ackn’d**: 18/05/2004
- **CU2 Project Title**: MOLECULAR GENETICS OF TRYPANOSOMES AND LEISHMANIA
- **Date Project Ceased**: 26/10/2007
- **Withdrawn**: N
- **Consent Granted**: Non-GMM
- **Project notified under transitional arrangements**: Y

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GM37/00.2, **** PROJECT TRANSFERRED BACK TO GM 37 (26/10/07)****
Historical Date of Additional Info: 02/06/2000

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
# Project Containment

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Name

CARDIFF UNIVERSITY

Name 2

Department

OCCUPATIONAL SAFETY, HEALTH & ENVIRONMENT UNIT

Campus Estate or Research Centre

Building

HEATH PARK

Town

CARDIFF

Road Name

District

HEATH PARK

County

CF14 4XN

Country

WALES

Tel Number

02920 874790

Fax Number

02920 874565

E-mail

HSE Division

WALES AND SOUTH WEST

Comments

GM 121, 291, 292, 302, 312, 399, 431, 446, 482, 525, 573, 624, 645 and 693 have all merged with GM130 as of 26/04/2005.

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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02/03/2022
Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

### Other (please specify)

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<td>Mycology</td>
<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

Project Ref 121/01.1
EXPRESSION AND OVER EXPRESSION OF FULL LENGTH AND PARTIAL FRAGMENTS OF HUNTINGTIN AND ITS INTERACTING PROTEINS

Purposes of the contained use

To elucidate the normal and pathogenic roles of the huntingtin protein, which leads to Huntington's disease when bearing a glutamine tract in excess of 36 residues.

Huntington's disease (HD) is an autosomal dominant neurodegeneration associated with an expanded trinucleotide repeat which gives rise to a glutamine tract in the N-terminus of the protein, huntingtin. The expanded glutamine tract is selectively toxic and kills particular neuronal populations, initially the medium spiny neurons of the striatum but later other neuronal populations are affected. We wish to express both full-length and truncated huntingtin with various repeat lengths in mammalian cell cultures, along with other proteins known to interact with huntingtin. This will allow us to carry out a number of experiments to answer the following questions:-

1. Do huntingtin and the interacting protein co-localise in the cells? Which sections of the transfected proteins are important to that co-localisation? Is this cell-type dependent?

2. Does the presence of the interacting protein ameliorate or enhance any observed effect of huntingtin, eg as measured by apoptosis, electrical activity or other appropriate functional assay?

3. Do variations in the interacting proteins affect the interaction with huntingtin?

Recipient or parental organism

The recipient or parental organisms for the standard cloning are disabled non-pathogenic E coli strains. The recipient cell lines for expression of the proteins are standard cell culture lines which cannot survive out of culture and which are kept free from any adventitious agents as far as can be determined by our routine assays for contaminating agents which are problematic in tissue culture, eg mycoplasma.

All cell transfections will be transient.

Host/vector system
Hosts


Cell lines: Cos7, HeLa, HEK293, N2a, PC12, IMR32, SK-N-SH and variants of this line such as SK-N-5Y.

Origin & function

Full length and truncated huntingtin constructs in plasmids obtained from collaborators or generated in house. Repeat lengths of 2-150CAG (or CAA/CAG). Full-length or truncated fragments of interacting proteins, provided they do not themselves have exceptional hazards associated with them such as potential oncogenic activity, in which case a further application in the appropriate Class will be made.

Vectors include pRcCMV, pcDNA3.19+/- and variants, e.g. pc3.1DNA HisMax and HisMyc pUni/V5-His-TOPO Echo cloning system (Invitrogen), Clontech living colour vectors (CMV promoter, GFP tags).

Evaluation of foreseeable effects

Huntingtin with an expanded polyglutamine tract is known to be toxic, particularly when expressed in truncated fragments. However, expression will be restricted to transient experiments only. The vectors used for expression in the mammalian cells will not be capable of replication in such cells and should they integrate into the host genome at low levels all the cells will be destroyed at the end of the experiment anyway. Huntingtin is ubiquitously expressed but only particular cell-types are damaged by its expression. These cells are in the brain and thus access of the vectors to the cells susceptible to damage is extremely unlikely. Should the vectors actually be transported to the appropriate cells they would be unlikely to integrate with the host genome in the absence of selection pressure and in order to manifest a problem substantial numbers of cells would need to be so affected. This is extremely unlikely. However the object of these experiments is to overexpress the proteins so promoters which give good expression levels in mammalian cells will be used, thus if the construct could integrate into the host genome of an appropriate cell then the cell would undoubtedly be compromised. HD is a late onset disease, but high repeat numbers (>50) as would be used in this experiment, give early onset disease (<20 years) and expression levels are also thought to be important in downstream cellular effects.

The toxicity of huntingtin and its truncated fragments are the reason for classifying this project as Class 2. The interacting proteins currently under study are the nuclear receptor co-repressor and endophilin 3, neither of which are known to be toxic to mammalian cells when overexpressed. Other interacting proteins may also be used but should any of these be intrinsically as toxic or more toxic than huntingtin itself, then a fresh application to work at the appropriate containment level will be made. Similarly, if the interaction observed in cells causes substantially increased toxicity of huntingtin (e.g., >10-fold increase in cell death at a given time-point) then shorter repeat units will be used experimentally to decrease the toxicity or again, a further application will be made to continue the work at appropriate containment levels.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Cell cultures are kept entirely within our tissue culture suite until disposed of by sterilisation and autoclaving. They would not survive outside the specialist conditions of tissue culture (37°C, 5%CO2).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Committee were concerned about the ability of the gene to cause harm if it were accidentally injected, but satisfied now that Containment Level 2 conditions and practices are sufficient. Risk Assessment now suitable and sufficient.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures, mammalian cell and microorganism, will be autoclaved in an approved and regularly tested autoclave room 2.05 Institute of Medical Genetics. In addition all disposables which come into contact with cell cultures will sterlised in freshly made solutions of Actichlor at a minimum concentration of 20,000ppm for a period of at least 2h. The tissue culture hoods used will also be disinfected with the above solution plus a 70% ethanol spray. The above procedures are already standard operating procedures in the Tissue Culture Suite in IMG.
Please enter comments on the GM safety committee on the risk assessment

None.

### Project Containment

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- **Animal Units**
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- **Large Scale Activities**
  - L2 L3 L4 L2 L3 L4

- **Human Clinical Applications**
  - L2 L3 L4

### Project Ref 130/01.1

- **Date Ackn'd**: 01/02/2001
- **CU2 Project Title**: ANALYSIS OF GENE EXPRESSION IN HUMAN CYTOMEGALOVIRUS
- **Class**: Class 2
- **CultureVolClass2**: not applicable
- **Consent Granted**: not applicable
- **Date Project Ceased**: 
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**: 
- **Significant Change ID**: 
- **Date of Significant Change**: 

Project notified under transitional arrangements Y
**Project Additional Information**

**Purposes of the contained use**

analysis of gene regulation during viral infection of human cell lines with cytomegalovirus. Attenuated virus, with or without the addition of a GFP reporter gene, will be used to infect human cell cultures. The expression of endogenous viral and cellular genes and their protein products will be monitored using techniques such as reporter assays, protein analysis techniques and differential mRNA expression.

**Recipient or parental organism**

The GMO in question is a pBabe-SOD3 retroviral vector and the pBabe-puro (empty mock) retroviral vector control. The pBabe-SOD3 retroviral vector will not be constructed at Cardiff University, but will be a gift from Professor Thomas von Zglinicki (Institute of Ageing & Health, Newcastle University).

The pBabe-SOD3 retroviral vector and pBabe-puro (empty mock) retroviral vector are based on the pBabe series of vectors, encoding a puromycin resistance gene, transcribed from the 3'-long terminal repeat of the viral vector (Morgenstern and Land, Nucleic Acids Res. 1990; 18: 3587-3596). The pBabe retroviral vectors are based on the Moloney murine leukaemia virus (Mo MuLV), expressing the inserted genes from the Mo MuV 3'-long Terminal Repeat. The characterised, full length SOD3 cDNA obtained from Professor Stefan Marklund (University of Umeå, Sweden), has been sub-cloned into the "defective" retroviral vector plasmid, pBabe.

This vector does not contain any viral proteins and so is not replication competent. The SOD3 retroviral vector plasmid will be transfected into a "packaging" (ΨCRIP) cell line, which will thereafter secrete retroviral virosomes, capable of infecting our chronic wound and normal skin fibroblasts, without further cycles of replication. Such cell lines have been developed to reduce the risk of wild type Mo MuV generation via homologous recombination events.

**Host/vector system**

cDNA sub-cloning was previously performed using a pUC18 plasmid and E.coli HB 101 as the host strain. The SOD3 cDNA has been sub-cloned into a "defective" retroviral pBabe-SOD3 retroviral vector plasmid.

The pBabe-SOD3 retroviral vector will be transfected into a "packaging" cell line (ΨCRIP), permanently transfected with "helper virus", which provides the necessary viral components for creating infection competent virus, which will thereafter secrete retroviral virosomes, capable of coding for the inserted sequence. Such cell lines have been developed to reduce the risk of wild type Mo MuV generation via homologous recombination events. These will be able to infect our chronic wound and normal skin fibroblasts, without further replication cycles.

**Origin & function**

cDNA for the antioxidant enzyme superoxide dismutase 3 (SOD3) has previously been isolated and characterized from a human placenta cDNA library (Hjalmarsson et al, Proc. Natl. Acad. Sci. USA 1987;84: 6340-6344). cDNA sub-cloning was performed using a pUC18 plasmid and E.coli HB101 as the host strain.

Retroviral virions, capable of coding for the inserted sequence, will be used to infect our chronic wound and normal skin fibroblasts with SOD3, without further replication cycles.

**Evaluation of foreseeable effects**

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 130/01.2

Date Ackn'd 01/02/2001

CU2 Project Title INTRODUCTION OF NEUROTRANSMITTER RECEPTORS AND CELL SIGNALLING MOLECULES INTO THE MAMMALIAN BRAIN USING REPPLICATION-DEFICIENT ADENOVIRAL VECTORS WITH ENHANCED GREEN FLUORESCENT PROTEIN TAGS.

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N
Certain neurotransmitter receptors, including the N-methyl-d-aspartate (NMDA) - type glutamate receptor, are important for learning, memory and interneuronal communication within the brain. While NMDA receptors may initiate these processes, they also require intracellular signalling molecules, including protein kinases, proteases, and adapter proteins. While the role of many of these have been investigated using either traditional pharmacological techniques or genome modification, these lack both temporal and spatial specificity. The use of viral vectors will allow us to express these molecules more selectively in targeted brain regions and at specific developmental stages.
Project Containment

Laboratory Activities

Glass Houses

Growth Rooms

L2  L3  L4  L2  L3  L4  L2  L3  L4

Animal Units

Large Scale Activities

Human Clinical Applications

L2  L3  L4  L2  L3  L4  L2  L3  L4

Project Ref  130/01.3

Date Ackn'd  27/04/2001

CU2 Project Title

CONSTRUCTION OF REPLICATION DEFICIENT ADENOVIRUSES CARRYING MUTANT AND WILD TYPE IG-H3 GENE AND INFECTION OF CULTURED CORNEAS AND CORNEAL CELL CULTURE

Date Project Ceased  02/12/2015

Class  Class 2

Cultured Volume  < 1 litre

Class CultureVolume

Class 2  < 1 litre

Non-GMM  Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

ig-h3 is the gene encoding keratoepithelin, which is associated with a number of corneal dystrophies. The role of this protein is not clear. Therefore ig-h3 has been incorporated in recombinant adenovirus in order to overexpress the protein in corneal organ and cell culture and determine the effects of the protein.

Recipient or parental organism

Replication deficient adenovirus type 5

Host/vector system
Host: 293, HeLa, CRL 11516 cell lines, corneal primary epithelial cell culture, corneal organ culture.

Vector: Recombinant replication deficient adenovirus carrying wild type and mutant (R555W, R555Q) ig-h3 cDNA and -galactosidase gene.

Origin & function

ig-h3 cDNA was extracted from fibroblast cells and cloned into p E1-cl, which produced the recombinant adenovirus by co-transfection with pJM17 in cultured 293 cells. Plasmids as well as 293 cells were obtained from Microbox Biosystems Inc (Graham FL 1984 EMBO J 3:2917-22)

Evaluation of foreseeable effects

The produced recombinant adenovirus had deleted E1 and E3 viral gene, and therefore is replication deficient. This means that it is able to infect cultured cells/corneas in vitro (ie. transfer its genetic material, including transgene, to the nucleus of the cells) but unable to produce any progeny virus. Thus there are no harmful foreseeable effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Only disposable plasticware will be used for the activities that involve contact with the adenoviruses. Waste will be immersed in 3% virkon solution. According to manufacturer's instructions 1% Virkon is sufficient for complete inactivation of adenovirus. Following virkon disinfection, waste will be autoclaved at 121 degrees C for 20 minutes, conditions which result in 100% effective kill, and subsequently will be incinerated.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMBA Safety Committee has assessed the proposed project and has approved it at Class 2. The control measures to be used and the location for the project have also been approved as being suitable and sufficient.

Project Containment

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02/03/2022
VIRULENCE AND ANTIMICROBIAL RESISTANCE OF PSEUDOMONAS
AERUGINOSA, BURKHOLDERIA CEPA CIA AND PROTEUS MIRABILIS

Purposes of the contained use

Genetic manipulation of Pseudomonas aeruginosa, Burkholderia cepacia and Proteus mirabilis will be used to identify virulence factors and the basis for antimicrobial resistance in these human opportunistic pathogens. The research will lead to the development of strategies to prevent and treat infection with these bacterial opportunistic pathogens.

Recipient or parental organism

Three Gram negative bacterial species will be genetically manipulated: Burkholderia cepacia, Pseudomonas aeruginosa and Proteus mirabilis. All these species are classified as ACDP as Hazard Group II biological agents. They can all cause opportunistic infections in patients which are compromised, but do not normally cause infection in healthy individuals. In addition, Escherichia coli K12 general purpose cloning strains will be the primary recipient of recombinant DNA. These E. coli strains carry multiple mutations and have not been associated with any health or environmental risk.

Three genetic modifications methods will be used to study virulence and resistance in these bacteria:
(i) Mutagenesis. This forms the primary genetic strategy which will be employed. Mutagenesis will be carried out using bacterial transposons based on the Transposon Tn5 and by site-directed means aimed at specific gene targets involved in resistance or virulence. The resulting mutants will have an attenuated phenotype as compared to their parental strains and hence this procedure does not carry an associated genetic modification hazard.
(ii) DNA cloning and sequence analysis. In order to analyse the genomic DNA of B. cepacia, P. aeruginosa and P. mirabilis it will be subcloned. The host species used for this analysis will be standard E. coli K12 cloning strains which are safe and have been extensively used in this type of research. Narrow-host range plasmid vectors (e.g. pUC18) and phagemid vectors (e.g. M13) will be used to subclone DNA. Use of E. coli K12 as a general cloning host has proven to be a very safe means of studying the genetics of both B. cepacia and P. aeruginosa.

(iii) Genetic complementation. B. cepacia, P. aeruginosa and P. mirabilis DNA will also be incorporated into broad-host range plasmid vectors capable of replication in a wide range of Gram negative bacteria (e.g. pUCP vectors and pSCOSBC1/PA1 vectors). After construction of genetic libraries in E. coli K12, these recombinant vectors will then be used for self-cloning procedures such as the re-introduction of wild-type DNA into a corresponding B. cepacia or P. aeruginosa mutant. Self-cloning will also be used to examine expression of the cloned DNA. The broad host range vectors used for this procedure can only be introduced by electroporation and are not self-mobilizable between bacterial species. Only self-cloning of DNA will be carried out (i.e. B. cepacia DNA to B. cepacia host strains, P. aeruginosa DNA to P. aeruginosa host strains or P. mirabilis DNA to P. mirabilis host strains). Hence, the latter restrictions of the method will greatly reduce the potential risk associated with the genetic manipulation strategy.

The foreseeable effects of the genetic manipulation procedures outlined above will not lead to the creation of GMOs which possess a risk greater than that of the ACDP Hazard Group 2 parental strains.

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**Host/vector system**

**Host organisms:**
- Escherichia coli K12 cloning strains; ACDP Group 1 microorganism; E. coli will be used as the general purpose cloning host for the majority of genetic manipulation.
- Pseudomas aeruginosa; ACDP Hazard Group 2 microorganism; P. aeruginosa strains will be one of the sources of genetic material and will also act as recipient host for DNA from other P. aeruginosa strains (self-cloning only).
- Burkholderia cepacia; ACDP Hazard Group 2 microorganisms; B. cepacia strains will be one of the sources of genetic material and will also act as recipient host for DNA from other B. cepacia strains (self-cloning only).
- Proteus mirabilis, ACDP Hazard Group 2 microorganism; P. mirabilis strains will be one of the sources of genetic material and will also act as recipient host for DNA from other P. mirabilis strains (self-cloning only).

**Vector systems**
- Transposon donor plasmids - plasmids pUTMini-Tn5 derivatives, pOT182 and plasposon derivatives of pTnModOTc. All these vectors carry the transposon Tn5 on suicide donor plasmids incapable of replication outside of E. coli hosts, but capable of transient transfer of plasmids by conjugal mating procedures. They will be used to create transposon mutants of the host organisms listed above.
- pUC18 and M13 DNA sequencing vectors - narrow host range plasmid vectors (incapable of self-transfer). These vectors and their commercially available derivatives will be used for most general purpose genetic manipulation.
- pUCP derivative, pSCOSBC1 and pSCOSPA1 - broad-host plasmid vectors that require electroporation for introduction into bacteria, incapable of self-transfer. The plasmids will be used to carry DNA between p. aeruginosa strains or between B. cepacia strains.
- pEX18Tc - broad-host range suicide plasmid vectors. This vector will be used to create site-directed gene knockouts in the P. aeruginosa and B. cepacia strains. It possesses sucrose counter-selection and has been designed to facilitate gene knockouts.

**Origin & function**

DNA will derive from Pseudomonas aeruginosa, Burkholderia cepacia and Proteus mirabilis. Genetic modification will be carried out to determine the function of genes.
involved in virulence and antimicrobial resistance of these bacteria. Marked transposons will be mobilised from E. coli into P. aeruginosa, B. cepacia and P. mirabilis. Mutants which demonstrate either susceptibility to various agents or a diminished capacity for virulence will be selected using laboratory models of disease. The genes which have been disrupted by these transposon insertions will then be identified by genetic analysis. To determine if these disrupted genes play a role in the actual observed phenotype, the wild-type gene will be cloned back into the mutants; restoration of the parental phenotype will then be examined. In addition, the genes may also be mutated by site-directed mutagenesis to create unmarked, non-polar mutations, in order to clarify their role in virulence and resistance. This gene knockout and complementation approach will be used to elucidate the genetic basis for virulence, pathogenesis and overall environmental fitness in P. aeruginosa, B. cepacia and P. mirabilis.

Evaluation of foreseeable effects

The foreseeable effects of the genetic manipulation procedures outlined above will not lead to the creation of GMOs which possess a risk greater than that of the ADCP Hazard Group 2 parental strains, P. aeruginosa, B. cepacia and P. mirabilis. The GMO strains will either carry gene mutations which render themselves less fit that their parental strains. Or they will be mutants which have been complemented with wild-type DNA in order to test its true function; hence they will not be carrying "foreign DNA." E. coli K12 general cloning strains will be the host receiving DNA from other species eg. P aeruginosa, B. cepacia and P. mirabilis. Genetic manipulation of these non-pathogenic E. coli K12 cloning strains has proven very safe and has no foreseeable effects or risks.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be inactivated by autoclaving with a predicted 100% degree of kill. Liquid culture waste will be collected in sealed vessels. All other waste (tips, tubes, petri dishes) will be placed in autoclavable boxes or discard tins with lids. The waste will then be sterilised by autoclaving. Autoclave cycles will be monitored for efficacy of kill and the sterility of the waste checked at regular intervals by culture. Laboratory cleaning and small-spill disinfection will be carried out with the broad-spectrum biocidal agent, Virkon, following the manufacturer's recommended procedures and specifications.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The G MBA safety committee has assessed the proposed project and approved it as Class 2. The control measures and the location for the project have also been assessed and approved as being suitable and sufficient.

Project Containment

Laboratory Activities Glass Houses Growth Rooms
**Project Ref** 130/02.2

**Date Ackn’d** 26/11/2002

**CU2 Project Title** IMPROVED INSECTICIDAL BACTERIA

**Class** Class 2

**Culture Vol Class 2** < 1 litre

**Consent Granted** not applicable

**Non-GMM**

**Project notified under transitional arrangements**

**Withdrawn**

Tick if notifying a connected programme of work

---

**Project Additional Information**

**Purposes of the contained use**

The proposed activity involves the modification of bacteria that are insecticidal and, although their target range is highly limited and there are no significant effects on eg. vertebrates, according to legislation, these organisms are "animal pathogens". As a result, level 2 containment is indicated. Since these bacteria are not human pathogens, the purpose of containment is to protect the environment from the release of the modified organisms and not to protect the human operator from the organisms with which he/she is working. Hence, derogations are sought under section 11 of this form.

**Recipient or parental organism**

Bacillus sphaericus  
Bacillus thuringiensis  
Brevibacillus laterosporus (formerly known as Bacillus laterosporus)  
Auxotrophic Bacillus subtilis strains may be used for some aspects of the work

**Host/vector system**
### Hosts:
Bacillus sphaericus, Bacillus thuringiensis and other gram positive microorganisms

### Vectors:
E. coli/Bacillus thuringiensis shuttle vectors. Standard gram-positive vectors such as pUB110 and their derivatives.

### Origin & function
For the purpose of understanding the nature of insecticidal toxicity of the parental strains and to enhance this activity, the project envisages the use of the genes of the following origins and functions:

**Origin of material:**
Bacillus sphaericus, Bacillus thuringiensis, Brevibacillus laterosporus

**Function:**
- Insecticidal toxin gene coding regions and control regions.
- Genes with potential effect on the insecticidal virulence of host bacteria (eg possible peptide antibiotic genes).
- Genes affecting gram positive phenotype in relation to toxin synthesis (eg germination genes).
- Promoter sequences.
- Repressor proteins and control sequences.
- Origins of replication from stably-maintained plasmids from the above species.

### Evaluation of foreseeable effects
No potential hazard to human health. The source bacteria and their toxins are not human pathogens. The manipulations envisaged are intended to increase the insecticidal potency of the resulting strains by the addition of new toxin genes to complement the pre-existing armoury of these bacteria. All the genes already exist in the environment. The parental bacteria are already used extensively in public health programmes for the control of human disease vector insects.

Environmental risks: As above, no toxicity is envisaged outside the range of insects that are targets for the parental bacteria. Therefore, although there is a limited risk from the environment that the modified bacteria could survive if released, there is no perceived hazard associated. Toxins are highly specific to a very narrow range of insect species with no effects on non-target organisms. They are naturally present in the environment worldwide.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Since the organisms involved are not human pathogens, it is not necessary to protect the operator from the organisms. Therefore, the following derogations are sought:

- Negative pressure relative to immediate surroundings - not necessary
- Microbiological safety cabinet/enclosure - not necessary
- Use of gloves - not necessary
- Observation window or alternative so that lab occupants can be seen - not necessary

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste procedures:
* All GMO contaminated waste (tips, tubes, glassware, plasticware spent media etc) to be treated before disposal
Tips, eppendorfs, inoculating loops, small o/n cultures etc: Place in double thickness OPEN autoclavable bag, bearing autoclave tape strip, for later autoclaving.

- Transfer items to the designated disposal autoclave without spillage in secure outer containers
- Place autoclave bags inside autoclavable containers to collect any leakage
- Include a Thermolog S autoclave test strip in each disposal run to ensure proper function: INSIDE the container whenever possible (it is not sufficient to place the strip on top of the waste bag)
- Sterilisation is only successful if the dark bar on the indicator enters the "safe zone"
- Record the use of the test strip in the log book
- Autoclave at 15 psi for at least the following times:

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume</th>
<th>Time</th>
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</thead>
<tbody>
<tr>
<td>Glass bottles/tubes</td>
<td>100 ml</td>
<td>15 min</td>
</tr>
<tr>
<td>Glass bottles/tubes</td>
<td>500 ml</td>
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<tr>
<td>Glass bottles/tubes</td>
<td>1000 ml</td>
<td>25 min</td>
</tr>
<tr>
<td>Glass bottles/tubes</td>
<td>2000 ml</td>
<td>35 min</td>
</tr>
<tr>
<td>Plastic waste bags</td>
<td></td>
<td>45 min</td>
</tr>
</tbody>
</table>

The genetic Modification and Biological Agents Committee of Cardiff University have considered the risk assessment for this project and consider it to be suitable and appropriate and agree with the allocation of final containment level 2.

Please enter comments on the GM safety committee on the risk assessment:
The genetic Modification and Biological Agents Committee of Cardiff University have considered the risk assessment for this project and consider it to be suitable and appropriate and agree with the allocation of final containment level 2.

**Project Containment**

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<td>L2 L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2</td>
<td>L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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Project Ref 130/03.1
The project aims to determine the role of protein kinase C (PKC) in breast cancer cell growth and acquisition of tamoxifen resistance. Although PKC pharmacological inhibitors exist, there are no good isoform selective ones, so their value is limited. We have previously found that adenovirus is an efficient way of delivering dominant negative forms of PKC to cells. Here we wish to use this method to "knock-out" PKC expression in these cells and determine the effect of that on the cells ability to proliferate, invade and apoptose.

Various long-term cultures of breast cancer cell lines. These cells do not contain other viruses, so there is no risk of recombination events. Transfection with virus will only be transient and the biological hazard therefore negligible. All work with these cells is routinely carried out at Class II and this will of course be continued, and all media will be autoclaved and all lab ware treated with 5% Virkon prior to disposal.

The vector systems to be used are commercially available and well characterised. Type 5 adenovirus vectors will be used which have been rendered replication deficient due to deletion of the E1 gene region, which is a recognised safety feature. The virus will be further disabled by partial deletion of the non-essential E3 gene region. As such it is unable to replicate in the host cells. Adenovirus has no mechanism for cytolysis so in culture most viral particles remain cell associated, although they can spread to neighbouring cells, leading to plaque formation. Expression using the adenovirus vector is only transient.

Adenovirus vectors are extremely efficient in vivo delivery systems (hence the wish to use them for these experiments) and can be spread by aerosols. This potential hazard will be contained by restricting manipulations with the virus to biological safety cabinets.

The wild-type and dominant negative kinase defective protein kinase C sequences to be used were recommended by Dr Ohba, Institute of Mol Oncology, Showa University, Tokyo. The adenovirus containing these sequences were provided by Dr D Murphy of Bristol University and grown on in association with Dr Gavin Wilkinson, Dept of
Wild type PKC alpha cDNA (-4~ +2647) will be used. Kinase defective PKC-alpha dominant negatives contain a single base pair mutation at position 368 (K→R).

Wild type PKC delta cDNA (-12~ +2525) and kinase defective, dominant negative PKC delta containing a 4 bp mutation at position 376 (K→A) will be used.

These sequences will be used to infect the breast cancer cells to determine the effect of over or non-expression of these signalling molecules on cell proliferation and Tamoxifen resistance. Only the infection part of the project will be carried out under the current project.

Evaluation of foreseeable effects

There is obviously a potential hazard associated with using adenovirus recombinants to express transgenes that are overtly transforming or have a strong capacity to promote host cell proliferation. Protein kinase C is a signal transduction molecule associated with cell proliferation in a number of cellular systems. Although not thought to be the driving force behind cell replication, it does appear to act as a rheostat, enhancing proliferation/apoptotic and invasive responses. The inserted gene product could therefore potentially be harmful, however due to the transient nature of the infection, this risk is thought to be minimal. Dominant negative mutants of PKC have previously been widely used in other laboratories and by the co-applicant Dr J Assender at Bristol University. As the adenovirus is replication-deficient due to E1 and E3 deletions, they can not replicate in host cells unless E1 sequences are provided in trans (which will not be the case in this study). It is considered highly unlikely that this could happen spontaneously. Even if it did, evidence suggests that recombination would be associated with spontaneous loss of the transgene. Furthermore efficient delivery of large number of viral particles will be required to infect target cells, which is unlikely to happen, in vivo. Since the adenovirus is replication deficient, no persistent long term effects are likely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The tissue culture cabinet and any potentially contaminated areas will be disinfected with 1% Virkon according to an approved protocol. Access to the laboratory will be restricted. Areas in which the virus is stored will be clearly labelled and kept locked when not in use. The laboratory door will be kept shut, a designated lab coat worn and designated pipettes used. Hands will be washed before personnel leave the room. Virus in solution (tissue culture media and buffers such as PBS) will be destroyed by autoclaving. Plastic ware, such as pipette tips and culture dishes will be fully immersed in 5% Virkon for at least 4 h. Pipette tips will then be disposed of in sharpsafe bins. Other solid wastes, such as soaked culture dishes and paper towels will be placed in unsealed autoclavable bags and autoclaved for 1 h at 129 degrees C before putting in yellow bags. The high concentration of Virkon used for soaking tips will ensure that the high organic content of the media is overcome and 100% killings should be achieved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The University GMBA Safety Committee have approved this project as being suitable for Containment Level 2. It was noted that the present project does not involve generation of the recombinant virus. Should there be a future requirement to do this at Cardiff University formal approval for this will be required from the GMBA Committee.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tbody>
<tr>
<td>L2 Yes</td>
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**Animal Units**

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**Large Scale Activities**

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**Human Clinical Applications**

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### Project Ref 130/03.2

- **Date Ackn'd**: 08/07/2003
- **CU2 Project Title**: TRACKING THE DEVELOPMENT OF ARTICULAR CARTILAGE USING PANTROPIC REPLICATION DEFICIENT PSEUDOTYPED RETROVIRUSES.
- **Date Project Ceased**: 02/12/2015
- **Class**: Class 2
- **Culture Volume**: ≤ 1 litre
- **Non-GMM Consent Granted**: not applicable
- **Project notified under transitional arrangements**: N

### Project Additional Information

The aim of the project is to use a pantropic replication-deficient pseudotyped retrovirus to follow both the development of articular cartilage in mouse, monodelphis and chick. The project is funded by the ARC and will make use of the Clonetech Pantropic retroviral expression system (K1063-1) to stably introduce a marker gene (lac z/GFP) into a chondroprogenitor cell population at the articular surface of developing joints. The virus will be introduced into the surface zone cells of neonate joints by...
microinjection and the fate of the cells followed by histology. Additionally, cultured bovine chondroprogenitors will be infected with marker genes and the fate of infected cells traced after microinjection into developing chick wing buds.

### Recipient or parental organism

Transient transfection of GP293 cells and chondrocytes. These cells do not contain other viruses, so there is no risk of recombination events. Transfection with virus will only be transient and the biological hazard therefore negligible. All work with these cells is routinely carried out at Class II and this will of course be continued, and all media will be autoclaved and all lab ware treated with 5% Virkon prior to disposal.

### Host/vector system

**Host organism**
The 293 GP cell line derived from human Ad5 transformed embryonal kidney cell line 293, stably expressing the gag and pol proteins for Moloney Murine Leukaemia Virus (MoMLV). Retroviral vectors generated from such cells are non-infectious since the virions contain none of the envelope proteins required for cell entry.

**Vector system**
Clonetech Pantropic Retroviral Expression System (K1063-1)

### Origin & function

A commercially available kit (see above) will be used to produce replication-deficient viruses carrying innocuous markers (lac z and GFP) to follow articular cartilage development and plasticity in vivo.

### Evaluation of foreseeable effects

The kit we will use is commercially available and since the virus is incapable of making the envelope proteins outside the packaging cell line, it is replication deficient and therefore poses a limited threat. However once the virus has been co transfected in the packaging cell line with the envelope glycoprotein, the virions produced are replication deficient, but highly infective. All work will therefore be performed in class II biological safety cabinet.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The tissue culture cabinet and any potentially contaminated areas will be disinfected with 1% Virkon according to an approved protocol. Access to the laboratory will be restricted. Areas in which the virus is stored will be clearly labelled and kept locked when not in use. The laboratory door will be kept shut, a designated lab coat worn and designated pipettes used. Hands will be washed before personnel leave the room. Virus in solution (tissue culture media and buffers such as PBS) will be destroyed by autoclaving. Plastic ware, such as pipette tips and culture dishes will be fully immersed in 5% Virkon for at least 4 h. Pipette tips will then be disposed of in sharpsafe bins. Other solid wastes, such as soaked culture dishes and paper towels will be placed in unsealed autoclaveable bags and autoclaved for 1 h at 129 degrees C before putting in yellow bags. The high concentration of Virkon used for soaking tips will ensure that the high organic content of the media is overcome and 100% killings should be achieved.

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N
The University GMBA Safety Committee have approved this project as being suitable for Containment Level 2.

**Project Containment**

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**Project Ref** 130/04.1

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<td>Investigation into the function of src kinase family members in endocrine-resistant cancer - expression of src within mammalian breast cancer cells</td>
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<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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</thead>
<tbody>
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**Withdrawn** N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
### Purposes of the contained use

The aim of this project is to transfect full length and truncated (producing a functionally inactive protein) DNA encoding src kinase into endocrine-sensitive and endocrine-resistant mammalian cancer cells in order to investigate the role that src kinase plays in mediating the phenotype of endocrine resistance.

### Recipient or parental organism

Human breast cancer cells will be the recipient of the src cDNA-containing vector. These cells are in wide use and have been extensively characterised in this and other departments in terms of their protein expression and in vitro behaviour (low in vitro metastatic ability).

A possible consequence of the over-expression of active src kinase in these cells may be an increased growth rate over their parental cells. However, since these src mediated effects may require the presence of other growth factors receptors not normally present/expressed in low abundance on these cells, a phenotypic change may be absent.

### Host/vector system

src cDNA pre-cloned into a mammalian expression vector will be sourced from a reputable molecular biology company. We will choose a company that can provide src in a suitable vector with its expression under the control of the CMV promoter.

### Origin & function

Src cDNA will be supplied by a reputable company where their products have undergone rigorous testing prior to marketing. The intended function of the src DNA is for it to be expressed in breast cancer cells in order to determine its effects on celluar behaviour (such as growth). Conversely, expression of functionally inactive src (dominant negative) in these cells will aid in the identification of cellular processes in which src plays a key role.

### Evaluation of foreseeable effects

Any alterations in the growth rate of the transfectants will be determined by an in vitro growth assay. This will involve growing cells (stable transfectants) in 96-well microtitre plates for a suitable period of time (5-7 days) following which they are incubated with a growth indicator prior to lysing. Once lysed, the plates may be read on a microtitre plate reader.

The motility of src-overexpressing cells will be analysed by plating the cells on a fibronectin-coated surface and allowing migration to occur over a period of 24 hours. Following fixation of cells, migrating cells are stained and then counted using a microscope.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be disposed of by the addition of a chlorine-based disinfectant (5% w/v Virkon) for 24 hours prior to removal. Use of this chlorine-based disinfectant will also be employed for inactivation of GMMs in discarded containers and in cases of liquid spillage. This will result in 100% GMM death.

Solid waste will be treated with 1% Virkon for 24 hours to ensure 100% cell death.

In the absence of the principal investigator, maintenance and waste management of transfected cells will be performed by a qualified member of staff with the tissue culture department, appropriately trained in the handling of GMMs.
The GMBA Safety Committee has assessed the proposed project and has approved it at Class 2. The control measures to be used and the location for the project have also been approved as being suitable and sufficient.

Please enter comments on the GM safety committee on the risk assessment.

The G MBA Safety Committee has assessed the proposed project and has approved it at Class 2. The control measures to be used and the location for the project have also been approved as being suitable and sufficient.

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**Project Ref 130/05.1**

**Date Ackn’d** 18/04/2005

**CU2 Project Title** Identifying the role ESCRT proteins in the life cycle of Molluscum Contagiosum Virus using replication incompetent adenoviral vectors

**Date Project Ceased**

**Class**

**Culture Vol Class**

**Culture Volume Class**

**Non-GMM Consent Granted**

**Project notified under transitional arrangements**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**
**Project Additional Information**

**Purposes of the contained use**

MCV 1.3 Expression of selected MCV genes in mammalian cells using replication incompetent adenoviral expression vectors; MCV 1.12 Expression of cellular gene products affected by MCV infection in mammalian cells using replication incompetent adenoviral expression vectors.

**Recipient or parental organism**

HaCat human keratinocyte cell line (Fusing, DKFZ, Heidelberg, Germany)
NIKS human keratinocyte cell line (University of Wisconsin-Madison, and Stratatech, University Research Park, USA) primary keratinocytes (from tissue biopsies)
HEK 293 cells

**Host/vector system**

BD AdenoX-pCMV-Tet (delta E1/E3)
in conjunction with
BD AdenoXTet-on

**Origin & function**

**Viral genes:**
- mc007R MCV genome position: 12611-13264 217 amino acids non-globular protein
- mc002L MCV genome position: 4846-6201 451 amino acids immunoglobulin domain
- mc161R MCV genome position: 180328-181764 478 amino acids
- mc026R MCV genome position: 182041-183639 532 amino acids
- mc007R MCV genome position: 30750-31001 83 amino acids modified RING finger domain

**cell genes:**
- Aip4 aryl hydrocarbon receptor-interacting protein 4: human E3 ubiquitin protein ligase 903 amino acids AF095745
- Nedd4 neuronally expressed developmentally downregulated protein 4 911 amino acids BC032597
- Hrs hepatocyte growth factor-regulated tyrosine kinase substrate 777 amino acids D84064
- Stam 1,2 signal transducing adapter molecules (SH3 domain and ITAM motif) 1 and 2 540 amino acids U43899
- SNX sorting nexin 3 162 amino acids AF034546
- PIP3 endosome-associated FYVE-domain protein 1539 amino acids AF434817
- SNAP25 synaptosomal-associated protein, 25kDa 206 amino acids L19760

**Evaluation of foreseeable effects**

Possible novel-harmful properties of the mc007 transgene: MCV mc007 like Human Papilloma Virus protein E7 binds Rb protein. The strength of this interaction in comparison to HPV E7 and the question if this binding leads to enhanced cell cycle progression and cellular transformation is the subject of this work. The potential hazard of inserting possible oncogenes, like mc007, into replication-deficient adenoviruses is the risk of recombination events between the recombinant and wildtype viruses, to generate a replication-competent recombinant adenovirus. Increased virulence or change tissue tropism are not to be expected from the insertion. However, mc007 might have other hazardous biological properties, as yet unknown. As a safety measure, the MCV transgene will be under the control of a tetracycline promotor. It is recognised, however, that very low level of transgene expression might occur in the absence of the tetracycline inducer.

Possible novel-harmful properties of other viral transgenes: MCV genes mc162, mc002L, mc161R, and mc026R have at most an indirect effect on cell proliferation, via interference with endosomal sorting mechanisms and protein ubiquitylation. Pleiotropic effects would be probably be mediated through the EGF receptor signalling.
cascade. For mc162 preliminary experiments indicate that it induces endosomal vesicle anomalies, resulting in cytotoxicity and cell death (apoptosis). Again, these viral proteins might have as yet unknown hazardous biological properties.

As a safety measure and to avoid cytotoxicity and apoptosis, all viral transgenes will be expressed under the control of tetracycline inducible promoter.

Cellular protein to be expressed in the replication deficient AdenoX adenoviral vector system are the human proteins of the ESCRT pathway and associated E3 ubiquitin protein ligases. These include Aip4, Nedd4, Hrs, Stam, Stam2, PI3P, SNX, and SNAP25. There is no evidence that overexpression of these human cellular genes will cause increased virulence of change of tissue tropism of the recombinant adenoviruses. To the contrary, overexpression will cause endosomal vesicle anomalies most likely resulting in cytotoxicity and apoptosis.

As a safety measure and to avoid cytotoxicity and apoptosis, all cellular transgenes will be under the control of a tetracycline inducible promoter.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Us eof recombinant adenoviruses will be restricted to class II safety cabinets. Infectious waste will be disinfected, autoclaved and then incinerated. Disinfection of liquid waste occurs in at least equal volumes of 3000ppm sodium dichloroisocyanurate. Small amounts of liquid disinfected waste will be autoclaved. Large amounts of liquid inactivated waste can be discarded to main drainage after 4 hours. Inactivation of adenoviruses in the disinfection procedure will be confirmed by a cell culture assay, where appropriately diluted and pH neutralized liquid inactivated waste is inoculated into 293 cells, before discarding into mains drainage. Pipettes or pipette tips are immersed in 3000ppm sodium dichloroisocyanurate (minimum final concentration), then autoclaved and incinerated. Spillage procedures are outlined in the Level 2 containment regulations.

Previously described adenovirus susceptibility to disinfectants: susceptible to 1% sodium hypochlorite, 2% glutaraldehyde and 0.3% sodium dichloroisocyanurate. Recommended: frsh solution of any disinfectant for 4 hours.

Previously described adenovirus susceptibility to physical inactivation: sensitive to heat; 1 hour at 56 oC is sufficient to inactivate virus.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee fully agreed with assignment to Containment Level 2 and Activity Class 2. Small alterations to the original risk assessment were requested regarding control of disinfection procedure and the likelihood of recombination between MCV and adenoviruses. The alterations and additional explanation were made and are part of the attached final RA for this project, which was accepted by the committee.

Project Containment
The aim of the project is to investigate the signalling pathways downstream of activated Ras in primary human thyroid follicular cells. We wish to use a commercial lentiviral system, Virapower from Invitrogen. This is a 3rd generation system that has been modified for enhanced safety features. We will introduce various Ras mutants into thyroid cells and dissect signalling pathways involved in proliferation. Other genes used are B-RAF, Ret/PTC and p21 and eGFP.
Origin & function

The following are in pBABE neo H-Ras V12 cDNA (activated H-Ras oncogene), H-Ras V12/G37 (point mutant activating Raf/GEF pathway alone), H-Ras V12/S35 (point mutant activating Raf/GEF pathway alone), H-Ras V12/C40 (point mutant activating P13K pathway alone).


Evaluation of foreseeable effects

The viral particles produced in this system are replication incompetent and only carry the gene(s) of interest. No other viral species are produced. This also means that none of the structural HIV genes (necessary for production of viral progeny) are present in the packaged viral genome. Gag, pol, rev and envelope genes are not present in the viral genome and are therefore never expressed in the target cell, so no new virus can be produced.

The stable cell lines that are made with the lentiviral system would not contain any of the packaging proteins. These proteins are only used to package the viral particles from the 293T cells. They are provided in trans, so they are not part of the viral particle themselves, and therefore not present when the target cell is transduced and the gene of interest stably integrated.

All four plasmids have been engineered to not contain any regions of homology with each other to prevent rare, but unwanted, recombination events that could lead to the generation of a replication competent virus. The expression of the gag-pol sequence is rev-dependent, thus preventing the expression of gag-pol in the absence of rev.

The gene transfer vector pLenti6/V5 has been modified to be "self-inactivating". A deletion has been made in the 3' LTR (called "delta U3") that has no effect on the generation of viral genome for packaging in the producer cell. However once the produced virus transduced a target cell, the mechanisms of reverse transcription use the 3'LTR as a template to create the 5'LTR. The end result is an integrated viral genome that is defective in both its 5' and 3' LTRs. Thus, there is no transcription of the viral sequences in the target cell and no packagable viral genome is produced. This self-inactivation also allows long-term expression from adjacent heterologous promoters.

This means that transduction with these lentiviral vectors ends with the gene of interest integrated into the host cell genome with no production of viral particles. Tat has been completely removed from the lentivirus system. The HIV LTR (in the pLenti6 vectors) has been modified and is now a hybrid fusion between the RSV promoter and the 5' LTR, and this modification makes it a stronger promoter and removes its dependence on tat for virus production. Tat is not required in any downstream events.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All lentiviral work will take place in a dedicated Class II facility. All liquid waste is sterilised at point of use by sodium dichloroisocyanurate. All solid waste is sterilised in an adjacent autoclaving facility. Use of glass and needles will be avoided to reduce the risk of sharps injury.

Autoclave thermocouple tested annually. Also daily monitoring of efficacy of autoclave. Discussed determining efficacy of disinfectants with HSE inspector on recent visit. Relying on manufacturer's quality control and the very unstable nature of GMOs involved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

Project passed at meeting at containment level 2, and class 2 activity.

**Project Containment**

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**Project Ref**  130/06.1

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**Class**  

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**Non-GMM**  

Consent Granted: Not Applicable

Tick if notifying a connected programme of work: Y

**Project notified under transitional arrangements**  

N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The purpose of the planned GM work is to test a new therapeutic poxviral HCV vaccine in vitro and in a small animal model. We want to clone the full length HCV polyprotein of HCV type 1 strains and HCV type 4 strains into vaccinia virus MVA. We want permission to use a recombinant vaccinia virus WR containing a HCV type 1 polyprotein (vNM-HCVpp-923711; from pCVH77C-Yanagin et al., 1997) that has previously been constructed at the University of Heidelberg. The recombinant viruses are
to be tested in a transgenic mouse model of chronic HCV infection.

Recipient or parental organism

The GM organisms we want to notify the HSE about are
1. vNM-HCVpp-92311: vaccinia virus WR containing HCV typ 1 a polyprotein without regulatory sequences from pCVH77C; made at the University of Heidelberg as L (S) 2 organism
2. Vaccinia virus MVA containing polyproteins from HCV type 1 and 4 strains without regulatory sequences; planned.

Host/vector system

1. Vaccinia virus WR (Western Reserve; obtained from B. Moss 1996, contact bmoss@nih.gov).
2. Vaccinia virus MVA (obtained from ATCC in 2002 while in Heidelberg, transferred to Cardiff 2003)

Origin & function

We ask for permission to further characterise the completed vaccinia WR construct and proceed with the construction of the planned MVA constructs (particle morphology/immune EN; hostrange and induction of antiviral and anti inflammatory cytokines in eucarvotic cell lines, vaccination and challenge of Balb/c and HCV transgenic mice).

Origin of the recombinant vaccinia virus vNM-HCVpp-923711: Receptor virus: vRB12; Donor plasmid: pRB21 (Blasco and Moss, Gene 158: 157pp, 1995). Insert derived from pCVH77C (Yanagin et al., 1997): HCV-polypeptide (HCVpp); Cloning: HCV-ORG without regulatory signals (pRB-HCV-clone 9-2) Nadja Melquiot, Christoph Springfeld, J Encke, Joachim J. Bugert (2001-2002 unpublished results); Isolation of recombinant VV: 28.9.2002 by Nadja Melquiot and Joachim J Bugert (2002, unpublished results); Resulting in: vHCV-92-3 (primary isolate), vHCV-923-78, vHCV-923-7-1, vHCV-923-71-1; Final virus: vNM-HCVpp-923-711 (triple plaque purified on CV-1 (ATCC: CCL-70)-8.10.2002. This virus will be assessed regarding its hostrange and induction of an innate immune response in a number of mammalian cell lines (eg. HUH7, HepG2, CV-1, RK13, HeLa, A549). The WR recombinant HCV vaccinia virus will be used as a challenge virus will be used as a challenge virus in the mouse model to assess the efficacy of prior vaccination with cDNA and recomabt adenoviruses (separate project within the connected programme of work).

Recombinant vaccinia virus MVA will be prepared using the pLW44 donor plasmid and MVA obtained from the ATCC in chicken embryo fibroblasts made from embryonated chicken eggs. HCV typ 1 a and type 4 partial and if possible genomes will be isolated from patient material (serum) genotyped in Heidelberg University clinical centre and in the Cardiff NPHS virology lab.

HCV connected program of work: Molecular Virology of Hepatitis C virus

HCV 2.1 Maintenance of HCV full length open reading frame and subfragments in bacterial plasmid cloning vectors and donor plasmids in E. coli. HCV 2.2 Investigation of a pRB binding motif located in HCV E2: expression of full length and truncated HCV E2 protein in E. coli as a GST fusion protein. HCV 2.3 Expression of HCV full length open reading frame and selected HCV subproteins in mammalian cells using replication incompetent adenoviral expression vectors. HCV 2.4 Expression of HCV full length open reading frame and selected HCV full length open reading frame and selected HCV subproteins in mammalian cells using vaccinia virus expression vectors. HCV 2.5 Expression of subgenomic HCV replicons in mammalian cells. HCV 2.6 HCV transgenic mouse lines.

Evaluation of foreseeable effects

The most hazardous construct is likely the one with the HCV open reading frame without regulatory signals from pCVH77C cloned into replication competent vaccinia virus WR: recombinant Vaccinia Virus vNM-HCVpp-923711.

Properties of VV WR: VVWR has a broad cellular host range and infects humans and a variety of other mammals. However, VV WR has been one of the three vaccinia strains used by the WHO in the smallpox eradication campaign. The construct vNM-HCVpp-923-711 qualifies as a recombinant HCV vaccine in its own right, specifically because it excludes the HCV 5' and 3' NTR regulatory signals-making the production of infectious HCV particles containing replication competent HCV genomes impossible. To be expected are HCV - VLPs (virus like particles) or recombant vaccinia showing HCV antigen on their virion particles.

Properties of the HCV ORF insert: 1. HCV infection leads to hepatocarcinoma but the Hepatitis C virus genome does not encode known oncogenic proteins. 2. Hepatitis
C virus has a reduced susceptibility to tissue interferon action in vitro, which has been offered as an explanation for the chronic nature of the infection. 

Re 1: In contrast to natural (chronic) HCV infection, vaccinia recombinants will express potentially oncogenic proteins only for a very short period of time, because of their lytic nature. This reduces the likelihood of oncogenesis in the accidental human host to a negligible level.

Re 2: In the case of vaccinia recombinants HCV ORF, a reduced tissue interferon response triggered by HCV proteins are not relevant, because vaccinia virus encodes interferon reducing activities through at least 3 different pathways. This has no effect on the immediate eradication of the virus by the human immune system and the generation of a lasting neutralising immune response.

Properties of VV MVA: Modified vaccinia virus Ankara (MVA) has been adapted to chicken cells during 573 passages in and chicken embryo fibroblasts. MVA is effectively disabled in human cells and consequently in the human host, because the viral DNA cannot be packaged into virus particles (Krijnse-Locker et al., 2000).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Balb/c mice and transgenic HCV mice will be infected with recombinant vaccinia viruses. These experiments will take place in the Cardiff University-Biomedical services Unit and the animals will be kept in Scantainers.

For animal infection experiments, vaccinia viruses will be transported to the animal facility (in the same building, one floor up). Viruses will be in plastic vials, packed into sealable plastic bags, lined with absorbent paper towels, inside sealable plastic containers (L3 standard) with carrying handles and attached biohazard stickers. Virus suspension volumes carried in this way will not exceed 1ml. In the event of spillage, GM spillage procedures will be followed. Infection of laboratory animals will be carried out in the animal facility in class II microbiological safety cabinets (max dose 10^7 pfu).

Animals infected with replication competent VV WR will be housed in negative pressure bubble isolators for the duration of the experiments (usually 14 days).

Infection will be carried out using sharps (needles) via the footpad and the intraperitoneal (ip) route. Personnel carrying out these experiments are trained to a high level to avoid needle injuries, and are aware of all the risks involved with working with vaccinia virus.

All animal technicians taking care of the animals will be made aware of the symptoms of vaccinia infection. All care-taking procedures outside of negative pressure bubble isolators will be carried out using gloves and respiratory protection. All waste products, including water not drunk, will be autoclaved. Isolators will be disinfected with Virkon after use. Animal carcasses will be bagged inside the negative pressure bubble isolators in case of a sick animal or inside a class II safety cabinet at the humane endpoint (day 14), then frozen at -20 degrees C and later disposed by autoclaving.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Enclosed to this application are:
1. The assessment of the German Central Commission for Biological Security (Zentrale Kommission fuer Biologische Sicherheit-ZKBS) in the German language, which finds biosafety level 2 containment adequate for experiments with the recombinant vaccinia virus WR containing HCV polyprotein lacking regulatory sequences: Permission for a S2 project with the number AZ:57-8/8817.40-020/UNI.HD..52.02-13 to the construct in Germany.

2. Letter of the Heidelberg University BSA, describing the details of the project permission for Heidelberg, including the translation of the statement of the German HSE equivalent (Zentrale Kommission fur die Biologische Sicherheit at the Robert Koch Institut in Berlin) is attached to this GM proposal.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All laboratory work involving recombinant vaccinia viruses will be carried out in L2 containment level labs. All personnel will be trained in recognising vaccinia virus infections, awareness of human-to-human-transmission, and the increased risk for individuals suffering from eczema or other immune disorders (eg atopic dermatitis, severe acne, impetigo, contact dermatitis, psoriasis, chickenpox, steroid medication, pregnancy, infection from skin pathogens). The exposure route via the conjunctiva will be particularly stressed and the use of adequate protective gear enforced in the lab. All personnel will be monitored by the Occupational Health Service at the School of Medicine.

Laboratory work will be carried out in class II safety cabinets, with personal wearing gloves. Disposable plastic pipettes and pipette tips will be used. Recombinant viruses
will be used under conditions avoiding the generation of aerosols. Flasks containing virus will be kept sealed outside of the safety cabinets. Centrifugation will be carried out in sealed buckets, that will only be opened in safety cabinets. All waste materials will be collected in plastic bags in the safety cabinets, the bags will be sealed inside and then autoclaved. No sharps will be used except when inoculating animals (see point 8).

Removable equipment and interior surfaces of the safety caninets will be sprayed with Incidin PLUS (Ecolab, Swindon, UK) containing the patented active substance Glucoprotamine with wide effect spectrum against bacteria (also MRSA and TBC), fungi, papova-, adeno-, herpes-, rota- and hepatitis-B-viruses (HBV) in MADT and antigen test, as well as AIDS agent (HIV)-certified by the German Society for Hygiene and Microbiology (DGHM). Spillage of GM material will be treated with hypochloride. For the quality assurance of the disinfection and autoclaving procedures, vaccinia virus contaminated Incidin disinfected and bleached plasticware, as well as autoclaved waste material will be rinsed with a small volume of PBS and tested for rest-infectivity on CV-1 cells in a standard plaque assay.

The main concern of the Cardiff University GM committee was, what would be the effect of the insert on the wild type organism. The committee accepted the Pis interpretation on what would be the most hazardous GMO and also the letter from the University of Heidelberg BSA. The conclusion of the committee was to write to the HSE for advice.

The HSE Inspector was then involved in the safety assessment of this project. His main concern was, whether there was a possibility of a replicating HCV being produced from HCV-vaccinia recombinants (WR).

The Principal Investigator replied to the query confirming that there was no possibility for replicating HCV coming out of HCV-vaccinia infected eucaryotic cells. He explained that the part of the HCV genome to be used in this project is lacking all HCV untranslated 5' and 3' regulatory sequences, without which a packaged particle cannot replicate. Accidental infection with a particle packaging such a replication-incompetent genome would lead to immunisation. He pointed out that this was the essential point why German authorities allowed the project to be conducted under level 2 safety containment (see enclosed letter).

The BSA of Cardiff University, received no more adverse comments from the Cardiff University GM committee when she circulated the risk assessment after the principal investigators comments to the HSE Inspector's question. The GM committee recommended to submit a notification as Class 2.

## Project Containment

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02/03/2022
Project Ref 130/06.2

Biofilm formation by GFP-tagged Pseudomonas aeruginosa.

Class 2

Consent Granted

Not Applicable

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

The purpose of our experiments is to utilise the GMM Pseudomonas aeruginosa strains to assess the proportion and spatial distribution of the organisms in in vitro biofilms comprising of mixed bacterial species. Using this approach it will be possible to enhance our knowledge on the interaction of different bacterial groups with biofilms that may develop within clinical settings.

Pseudomonas aeruginosa is a pathogen listed within ACDP hazard group 2. The organism is a Gram negative bacillus, aerobic, non-sporulating and is motile by polar flagella. As a pathogen Ps. Aeruginosa greatest risk of causing disease is in the immunocompromised where it colonises individuals in their respiratory tracts or urinary tracts. This colonisation can lead to pneumonia and bacteremia; chronic respiratory infections among cystic fibrosis patients; eye infections in contact lens wearers; nosocomial infections in immunocompetent patients. Mode of transmission is by direct contact with contaminated water, aerosols or aspirations. Survival of the organism for several months in water with minimal nutrients is possible. The organisms is susceptible to many disinfectants including 1% sodium hypochlorite, 70% ethanol, and 2% glutaraldehyde. It is also sensitive to extended spectrum penicillins, aminoglycosides, cephalosporins, polymixins, fluoroquinolones. The first line antibiotic treatment is an aminoglycoside with a B-lactam pencillin. The bacterium is inactivated by moist heat (121 C for 15 min).

In terms of laboratory hazards, no acquired infections with Ps. Aeruginosa have been reported to date, there are no special hazards and the primary hazard is by accidental parenteral inoculation; direct contact of mucous membranes with infected material; inhalation of infectious aerosols and ingestion.

Recipient or parental organism

Host/vector system

27/02/2006
No vectors are utilised by the researchers as the GFP strains will be gifted from our collaborators in Denmark.

**Origin & function**

The DNA sequences inserted are those from a mini-Tn7 transposon system. The GFP expressing strain has received a pUC19 derived plasmid carrying a GFPmut3.1 variant of the Aequorea victoria GFP. In addition, this plasmid vector also contains antibiotic resistance markers (ampicillin) and a mob region. Once the mini-Tn7 transposon has been inserted, the plasmids are lost from the bacterial strains. The inserted GFP2 gene is constitutively expressed and controlled by a modified lac promoter PA1/04/03. For cfp and yfp expressing strain, the mini-Tn7 transposon system contained 740 bp PCR amplicons from commercial templates (pECFP and pEYFP; Contech).

**Evaluation of foreseeable effects**

No work in the construction of the GMO will be undertaken at Cardiff University.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Biosafety level 2 practices will be used throughout. The organisms will be cultured within an enclosed fermenter. Protective clothing in the form of Howie style laboratory coats will be worn for all experiments. All cultures will be stored in sealed containers that are appropriately labelled. Laboratory spills will be treated by gently covering with paper towels and application of a 1% sodium hypochlorite solution. Contact time of at least 30 min before clean up and disposal.

All waste material will be autoclaved within using the autoclave housed within the laboratory. A kill rate of 100% is expected from the autoclaving procedure. All autoclave waste is subsequently disposed of by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

GMSC was satisfied that Risk Assessment suitable and sufficient and, after seeking advice from HSE, that the project was a Class 2 activity.

**Project Containment**

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

The aims of the project are to determine whether the transfection of human chronic wound fibroblasts to over-express the antioxidant enzyme, superoxide dismutase 3 (SOD3), can reverse the chronic wound fibroblast phenotype and protect these cells from oxidative stress-induced ageing, allowing normal wound healing functions (proliferative life-span, cell migration, extracellular matrix turnover, etc) to be maintained. We intend to SOD3-transfect the human chronic wound and normal skin fibroblasts via retroviral infection, using the retroviral vector pBabe-puro, based on established protocols (Serra et al. J. Biol. Chem. 2003; 278: 6824-6830).

**Recipient or parental organism**

The GMO in question is a pBabe-SOD3 retroviral vector and the pBabe-puro (empty mock) retroviral vector control. The pBabe-SOD3 retroviral vector will not be constructed at Cardiff University, but will be a gift from Professor Thomas von Zglinicki (Institute of Ageing & Health, Newcastle University).

The pBabe-SOD3 retroviral vector and pBabe-puro (empty mock) retroviral vector are based on the pBabe series of vectors, encoding a puromycin resistance gene, transcribed from the 3'long terminal repeat of the viral vector (Morgenstern and Land, Nucleic Acids Res. 1990; 18: 3587-3596). The pBabe retroviral vectors are based on the Moloney murine leukaemia virus (Mo MuLV), expressing the inserted genes from the Mo MuLV 3'-long Terminal Repeat. The characterised, full length SOD3 cDNA, obtained from Professor Stefan Marklund (University of Umea, Sweden), has been sub-cloned into the "defective" retroviral vector plasmid, pBabe.
This vector does not contain any viral proteins and so is not replication competent. The SOD3 retroviral vector plasmid will be transfected into a "packaging" (ΨCRIP) cell line, which will thereafter secrete retroviral virions, capable of infecting our chronic wound and normal skin fibroblasts, without further cycles of replication. Such cell lines have been developed to reduce the risk of wild type Mo MulV generation via homologous recombination events.

Host/vector system

cDNA sub-cloning was previously performed using a pUC18 plasmid and E.coli HB101 as the host strain. The SOD3 cDNA has been sub-cloned into a "defective" retroviral pBabe-SOD3 retroviral plasmid.

The pBabe-SOD3 retroviral vector will be transfected into a "packaging" cell line (ΨCRIP), permanently transfected with "helper virus", which provides the necessary viral components for creating infection competent virus, which will thereafter secrete retroviral virions, capable of coding for the inserted sequence. Such cell lines have been developed to reduce the risk of wild type Mo MulV generation via homologous recombination events. These will be able to infect our chronic wound and normal skin fibroblasts, without further replication cycles.

Origin & function

cDNA for the antioxidant enzyme superoxide dismutase 3 (SOD3) has previously been isolated and characterized from a human placenta cDNA library (Hjalmarsson et al, Proc.Natl.Acad.Sci.USA 1987; 84: 6340-6344). cDNA sub-cloning was performed using a pUC18 plasmid and E.coli HB101 as the host strain.

Retroviral virions, capable of coding for the inserted sequence, will be used to infect our chronic wound and normal skin fibroblasts with SOD3, without further replication cycles. Transfected cells will be selected using puromycin, as this vector has been constructed to induce puromycin-resistance in cells. On transfection, puromycin-resistant clones will be selected and used in future in vitro wound healing-related experiments. The human chronic wound and patient-matched, normal skin fibroblast cell strains involved, have previously been derived from biopsies, collected with full Local Research Ethical Committee approval and informed consent from individuals (N=3), attending the Wound Healing Research Unit UHW.

Evaluation of foreseeable effects

For these experiments using retroviral gene transfer of SOD3, as SOD3 is a recognized enzymic antioxidant, we already know that the expression of SOD3 can lead to an increase in cellular proliferative lifespan and reduce oxidative stress. The present project wishes to determine whether the over-expression of SOD3 can reverse the chronic wound fibroblast phenotype and protect these cells from oxidative stress-induced ageing, allowing normal wound healing functions (proliferative life-span, cell migration, extracellular matrix turnover, etc) to be maintained. This will be undertaken using experimental techniques routinely used within the Department.

The pBabe-SOD3 retroviral vector has previously been extensively characterized by Professor Thomas Von Zglinicki's Group (Institute of Ageing & Health, Newcastle University), who constructed the retroviral plasmid (Serra et al. J Biol. Chem.2003; 278: 6824-6830). The vector is replication incompetent because it lacks the genes required to make viral capsid, and only carries the gene of interest. The vector is "packaged" in a "packaging" cell line (ΨCRIP), which produces virus capable of infecting the targets cell of interest; but without further replication cycles. Such cell lines have been developed to reduce the risk of wild type Mo MulV generation via homologous recombination events. Therefore, it is highly unlikely that homologous recombination events or transfer of genetic material between the GMO and other organisms would occur.

A potential hazard is obviously the exposure of humans to the retroviral particles. However, this will be minimized by performing all manipulations in a Class 2 Biological Safety Cabinet, in a designated laboratory facility. Access will be limited to Staff trained in ACGM level 2 procedures. These Staff will also have access to laboratory coats and disposable purple nitrile gloves (guaranteed to prevent viral particle penetration). All disposable material that has had contact with the viral particles will be chemically disinfected and autoclaved, prior to disposal. The use of sharps will be avoided to prevent accidental injury-related risks. Consequently, any risks of exposure of humans or the environment are very low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable
None. All the measures specified as requirements for Level 2 containment will be implemented.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus will be held in a Class 2 containment facility with available autoclave and disinfectant. The Class II cabinet will be cleaned before and after use. Before use, the cabinet will be cleaned with sodium hypochlorite (Effervescent Chlorine Tablets, 1 tablet/litre water, 600 ppm concentration), followed by spraying with 70% ethanol. Chlorine tablets have been previously demonstrated by the Manufacturers' to be effective against all viruses at a recommended final concentration of 250ppm.

Anything used as part of the experimental procedure will be soaked in 600ppm sodium hypochlorite overnight, prior to removal from the cabinet. These will then be autoclaved before disposal.

After use, the cabinet will be sprayed with 70% ethanol and cleaned with sodium hypochlorite, before removing any contents (including stripettes, tips, flasks, medium, etc) from the cabinet. Anything used as part of the experimental procedure will be soaked in 600ppm sodium hypochlorite overnight, prior to removal from the cabinet. These will then be autoclaved before disposal. After use, the cabinet will be sprayed with 70% ethanol and cleaned with sodium hypochlorite, before removing any contents (including stripettes, tips, flasks, medium, etc) from the cabinet.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee has unanimously agreed that this is a satisfactory risk assessment and that sufficient controls are in place to protect both human health and the environment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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Project Ref 130/06.4

02/03/2022
Project Additional Information

**Purpose of the contained use**

The project involves the growth of fibroblasts from the human progeroid syndrome Werner. These fibroblasts grow more slowly and have a much-reduced replicative life span than normal fibroblasts. The kinase inhibitor SB203580 rescues this reduced growth. The drug is believed to inhibit the MAP kinase p38 (MAPK14). The GM involves the introduction of an SB203580-resistant form of p38 into Werner syndrome fibroblasts using retroviral transfer and testing the effects of SB203580 on the resultant cells. A wild type p38 will be used as a control. To this end mutant and non-mutant forms of p38 will be cloned into a series of retroviral vectors. If we have problems cloning MAPK14 into retroviral vectors we will use pre-existing adenoviral vectors which already contain the mutant and non-mutant forms of p38. These plasmids will be co-transfected with pJM17 into 293 cells which supply an E1 helper function. Recombinant virus stocks will be plaque purified through reinfected 293 cells.

**Recipient or parental organism**

Host organisms are primary human cells, in particular fibroblasts. These cells may also have been infected with retroviruses expressing human telomerase (hTERT).

**Host/vector system**

All genes will be introduced into a series of plasmid backbones of the pBABE series of vectors. In the appropriate producer lines these produce ecotropic and amphotropic retroviruses where gene expression is driven by the MMLV LTR’s. All retroviruses are replication defective as many helper functions are supplied by the producer cell lines psiCRIP and Omega E. Cells that have stably integrated the retroviral expression cassette will be selected on the basis of drug resistance to G418 or puromycin. After infection no infectious viral particles can be produced.

The adenoviral vectors are replication incompetent due to absence of the E1 helper function. None of the genes intended for use contain or complement the E1 function. In addition, these are not capable of integrating into the host's DNA and are thus subject to rapid dilution with cell division. Homologous recombination with a wild-type adenovirus present in the host would generate competent virus, however, this recombination would result in the deletion of the inserted gene.

**Origin & function**

The project involves the growth of fibroblasts from the human progeroid syndrome Werner. These fibroblasts grow more slowly and have a much-reduced replicative life span than normal fibroblasts. The kinase inhibitor SB203580 rescues this reduced growth. The drug is believed to inhibit the MAP kinase p38 (MAPK14). The GM involves the introduction of an SB203580-resistant form of p38 into Werner syndrome fibroblasts using retroviral transfer and testing the effects of SB203580 on the resultant cells. A wild type p38 will be used as a control. To this end mutant and non-mutant forms of p38 will be cloned into a series of retroviral vectors. If we have problems cloning MAPK14 into retroviral vectors we will use pre-existing adenoviral vectors which already contain the mutant and non-mutant forms of p38. These plasmids will be co-transfected with pJM17 into 293 cells which supply an E1 helper function. Recombinant virus stocks will be plaque purified through reinfected 293 cells.

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Stress signalling kinases:

Human MAPK14 (p38)
Mutant MAPK14 (resistant to the inhibitor SB203580)

Evaluation of foreseeable effects

With regard to the hazards associated with the host/recipient the following have been considered including the pathogenicity of the host strain, virulence, infectivity and toxin production. In this case the recipients and hosts are non pathogenic.

With regard to the hazards rising directly from the inserted gene: These are non oncogenic and are downstream members of signalling pathways. In addition, the inserted MAPK14 is inactive unless activated by an intracellular signal. Activation of this pathway will affect the inserted gene in a similar fashion to the endogenous copy. With regard to the mutant form of MAPK14, the only effect will be resistance of this kinase to an exogenously applied kinase inhibitor. The function of the MAPK14 is essentially unaltered.

With regard to the hazards arising from the alteration of existing pathogenic traits: There is no increase in infectivity of pathogenicity and no disabling mutation within the recipient can be overcome due to the insertion of the foreign gene. Foreign genes do not encode pathogenicity determinants from a related organism.

Considering whether an inserted sequence that does not give rise to harmful phenotype in the recipient micro-organism could give harm as a result of natural gene transfer to another possibly related organism: This is extremely unlikely to have a harmful effect.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable to this application.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable to this application.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All retroviral and adenoviral work will take place in a dedicated Class II facility. All liquid waste is sterilised at point of use by sodium hypochlorite. All solid waste is sterilised in an adjacent autoclaving facility. Use of glass and needles will be avoided to reduce the risk of sharps injury.

Autoclave thermocouple tested annually. Also daily monitoring of efficacy of autoclave. Discussed determining the efficacy of disinfectants with HSE inspector on site. Relying on manufacturers quality control and the very unstable nature of GMO’s used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Project passed at meeting at containment level 2. Minor revision requested to clarify if MAPK14 transferred to humans. Risk assessment of adenoviral use also modified to fully cover risk to health. Risk assessment modified accordingly and signed off.

Project Containment

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Project Ref 130/06.5

Date Ackn'd 02/01/2007
CU2 Project Title Adenovirus Vector Development.
Date Project Ceased

Class CultureVol Class 2 CultureVolume Class 3-4
Class 2 1-50 Litres

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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Project Title
Introduction of interfering RNAs into mammalian cells for lowering gene expression.

Purposes of the contained use
The purpose of the project is to use retroviral, lentiviral and adenoviral vector expression of interfering RNAs in order to obtain significant reduction in the expression of specific genes (human, mouse or CMV in origin).

Recipient or parental organism
Retroviruses are viruses found throughout the animal kingdom and may be associated with oncogenesis. 
Lentiviruses are complex retroviruses that cause the transmissible immunodeficiency syndomes. 
Adenoviruses are primarily associated with mild, self limiting respiratory tract infections (common cold).

Origin & function
Deletion of the enhancer region of the 3'U3 of LTR. This results in a transcriptionally inactive vector that cannot be converted into a full length RNA. The deletion also reduces the risk of tumourigenesis via promoter insertion. 
In addition to loss of the regulatory/accessory genes (tat, vpr, vpu, vif and nef), pLKO.1 is also deficient for the gag, pol, rev and env genes of HIV-1, which must be
provided on three non-homologous plasmids expressed in a packaging cell-line.

Retrovirus: The origin of the retroviral vectors (as typified by pSUPER.RETRO and pSIREN-RetroQ-ZsGreen) requested in this application is the Murine Stem Cell Virus. The retroviral vectors are lacking the gag, pol and env genes required for viral replication. These genes are provided in trans either as stable transfectants or on separate plasmids. All packaging signals on the viral genes have been removed, preventing their packaging into recombinant virus, and retroviral genes are under control of non-homologous promoters reducing the possibility of recombination with packaging vector. These highly attenuated lentivirus and retrovirus vectors have shown sufficient biosafety for their inclusion in ongoing human clinical trials as therapeutic vectors.

Adenovirus: The recombinant adenovirus vectors are based on commercial. Replication-deficient adenovirus type 5 vectors that have been widely used worldwide in research and gene therapy protocols. All vectors will carry deletions in the adenoviral E3 region.


Evaluation of foreseeable effects

The generation of replication competent virus has to be viewed as a potential major safety issue. However, the probability of the lentiviral, retroviral or adenoviral vectors reverting to wild-type is extremely low (see risk assessments). Recombinant adenoviruses generated in these studies will be replication-deficient due to deletion in the E1 and E3 gene sequences. For replication deficient adenoviruses there is a potential risk of recombination with wild type viruses to generate replication competent virus. However, laboratory containment of replication deficient adenoviruses makes the likelihood of exposure to wild type viruses extremely remote. In summary, although the viral vectors can potentially infect a wide-range of cell types there is minimal risk of viral replication in infected cells.

Replication-deficient adenoviruses are not associated with oncogenesis due to loss of E1A and B gene products and the fact that the virus does not integrate. However, insertion mutagenesis is a potential concern for retroviral-based vectors. Retroviral integration can lead to oncogenesis but appears restricted to stem cells whereas integration of lentiviruses into the human genome is not known to result in oncogenesis.

In the context of insertional mutagenesis, an additional risk factor may influence the probability of an adverse event as the nature of the specific shRNA to be expressed from the retroviral/lentiviral vectors. The shRNAs of most concern would be those capable of achieving down-regulation of expression of known tumour suppressors. However, as tumourigenesis is recognised to require multiple events the likelihood of cellular transformation is nevertheless minimal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Recombinant viruses will at all times be maintained in an environment designed to prevent aerosol spread. All flasks containing the virus shall be kept closed except when decanting and pipetting which will be conducted in tested class II safety cabinets. Centrifugation steps shall be performed in sealed buckets that will be opened only in safety cabinets.

Liquid waste will be disinfected with Activchlor (2500 ppm sodium dichloroisocyanurate), a chlorine-based disinfectant. The expected degree of kill will be 100%. The activity of the disinfectant will be checked using starch iodide test papers. Solid waste will be autoclaved.

Contaminated pipettes will be immersed in Actichlor for a minimum of 4hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with chlorine-based disinfectant according to an approved protocol. Access to laboratories employed for virus work will be restricted.
Project Containment

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Project Ref 130/07.2

Date Ackn’d 05/07/2007

CU2 Project Title

Introduction of leukocyte cell surface proteins into mammalian cell lines using retroviral and lentiviral vectors.

Class CultureVol Class 2 CultureVolumeClass3-4

Class 2 1-50 Litres

Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Tick if notifying a connected programme of work Y
Project Additional Information

Purposes of the contained use
The purpose of the project is to use retroviral and lentiviral vector expression of leukocyte cell surface proteins in order to determine the functional characteristics of modified versions of these molecules including TCR's, MHC's, CD8 and CD4 coreceptors.

Recipient or parental organism
Retroviruses are viruses found throughout animal kingdom and may be associated with oncogenesis. Lentiviruses are complex retroviruses that cause the transmissible immunodeficiency syndromes.

Host/vector system
Mammalian cells/Retrovirus vectors (including pBABEpuro and pSIREN-RetroQ-Zs Green)
Mammalian cells/Lentivirus vectors (including pLKO.1 puro)

Origin & function
Retrovirus: The origin of the retroviral vector pSIREN-RetroQ-Zs Green is the Murine Stem Cell Virus. pBABEpuro is derived from the Mouse Leukaemia Virus. The retroviral vectors are lacking the gag, pol and env genes required for viral replication. These genes are provided in trans either as stable transfectants or on separate plasmids. All packaging signals on the viral genes have been removed, preventing their packaging into recombinant virus, and retroviral genes are under control of non-homologous promoters reducing the possibility of recombination with packaging vector.

Lentivirus: The origin of the lentiviral vector requested in this application is HIV-1. pLKO.1puro is a representative third-generation self-inactivating lentivirus vector (Dull et al, 1998, Zufferey et al, 1998, Stewart et al, 2003). The mechanism of attenuation includes: Deletion of the enhancer region of the 3' U3 of LTR. This results in a transcriptionally inactive vector that cannot be converted into a full length RNA. The deletion also reduces the risk of tumourigenesis via promoter insertion. In addition to loss of the regulatory/accessory genes( tat, vpr, vpu, vif and nef), pLKO.1 is also deficient for the gag, pol, rev, and env genes of HIV-1, which must be provided on three non-homologous plasmids expressed in a packaging cell-line.

These highly attenuated lentiviral and retroviral vectors have shown sufficient biosafety for their insertion in ongoing human clinical trials as therapeutic vectors.

Evaluation of foreseeable effects
The generation of replication competent virus has to be viewed as a potential major safety issue. However, the probability of the lentiviral or retroviral vectors reverting to wild-type is extremely low (see risk assessment). Therefore, although these viral vectors can potentially infect a wide range of cell types there is a minimal risk of viral replication in infected cells.

The only potential safety concern for retroviral-based vectors is insertional mutagenesis of essential genes. Retroviral integration can lead to oncogenesis but appears restricted to stem cells whereas integration of lentiviruses into the human genome is not known to result in oncogenesis. Although the leukocyte cell surface proteins themselves do not cause a major risk due to their natural presence on mammalian cells, their modification may in the worst case scenario induce autoimmune disease.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Recombinant viruses will at all times be maintained in an environment designed to prevent aerosol spread. All flasks containing the virus shall be kept closed except when decanting and pipeting which will be conducted in tested class 2 safety cabinets. Centrifugation steps shall be performed in sealed buckets that will be opened only in safety cabinets.

Liquid waste will be disinfected with Actichlot (2500 ppm sodium dichloroisocyanurate), a chlorine-based disinfectant. The expected degree of kill will be 100%. The activity of the disinfectant will be checked using starch iodide test papers. Solid waste will be autoclaved.

Contaminated pipettes will be immersed in Actichlor for a minimum of 4 hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with chlorine-based disinfectant according to an approved protocol. Access to laboratories employed for virus work will be restricted.

Please enter comments on the GM safety committee on the risk assessment

Agreed with the proposed activity classification.

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- **Animal Units**: L2 L3 L4 L2
- **Large Scale Activities**: L2 L3 L4 L2
- **Human Clinical Applications**: L2 L3 L4

### Project Ref 130/07.3

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<th>CU2 Project Title</th>
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<td>Viral vectors for gene delivery to skin.</td>
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<tbody>
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</table>

- **Non-GMM**: Not Applicable
- **Consent Granted**: Not Applicable

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
**Purposes of the contained use**

The outer skin layer provides a barrier to gene delivery. In this study, we will use microchannel creating devices, e.g., microneedles, to penetrate the outer barrier layer of skin to allow pathways for the delivery of genes into viable skin regions. Viral vectors will be used to drive gene expression in the viable skin. Potential therapeutic applications include cutaneous gene therapy and DNA vaccination.

**Recipient or parental organism**

- Continuous cell lines, HaCaT and A549 cells.
- Primary cell cultures of keratinocytes.
- When using explanted human skin tissue (ethical approval and informed consent in place) we will target cells in the epidermal/dermal region including keratinocytes, Langerhans cells and dermal dendritic cells.

**Host/vector system**

- Lentiviral vectors based on human immunodeficiency virus-i (HIV-i) will be generated from the pHR'SIN parental construct. There is no HIV viral protein expression from the vectors and they are multi-attenuated; all the HIV-i genes with accessory and potentially pathogenic functions have been deleted. The vectors are unable to replicate. The spleen focus forming virus (SFFV) long terminal repeat (LTR) will be used to drive expression. Vectors are self-inactivating due to a deletion in the 3' HIV-i LTR, providing another layer of safety. We use integration-deficient vectors which contain a mutated integrase that is functionally disabled.

**Origin & function**

- The transgenes expressed by the vectors are non-pathogenic and are not hazardous to human health or the environment.
- To test gene expression in skin: Reporter genes enhanced Green Fluorescent Protein (GFP) from Acquoria Victoria and beta-galactosidase.
- To test functional gene expression: cDNAs encoding Hepatitis B surface antigen will also be used as a representative therapeutic gene. As all workers are immunised against hepatitis B, this is a safe and appropriate option.

**Evaluation of foreseeable effects**

- The effluent non-integrating vectors will be able to undergo reverse transcription and nuclear entry but be defective specifically for the integration stage of the viral life cycle. This is due to mutation of the HIV integrase in order to disable its function. The maximum culture volumes used in preparation are 30mL. The vectors are inactivated by treatment with proline and autoclaving.
- The lentiviral vectors to be used in this project are based on human immunodeficiency virus-i (1-IIV-i) and will be generated from the pHR'SIN parental construct. There is
no HIV viral protein expression from this plasmid. The spleen focus forming virus (SFFV) long terminal repeat (LTR) will be used to drive expression of a reporter protein (e.g., eGFP, β-galactosidase) transgene. Furthermore, the vectors are self-inactivating due to a deletion in the 3' HIV-1 LTR, so that only the internal promoter will be active in transduced cells. Vectors will be generated with envelope from the G glycoprotein of vesicular stomatitis virus (VSV-G).

Reversion of a non-replicating lentiviral vector into a replicating form by recombination with wildtype has never been shown to occur and the multiple disabling mutations described mean that the risk of this occurring is very low.

Percutaneous or mucosal are routes of infection that will be prevented by standard category 2 procedures, such as wearing gloves, lab coat and use in category 2 cell culture hood. Even in the event of a laboratory accident, exposure has negligible chance of causing harm since the virus is unable to replicate and the transgenes used in these studies have no pathologic potential. In the event of an accident the immune system will not be compromised.

The overall likelihood that, in the event of exposure, the GM virus could cause harm to human health is effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Excised human skin will be collected and experiments contained within a designated containment 2 laboratory.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Experimental procedures will not generate aerosols. A safety cabinet will be used. Sharps use minimised and sharps disposed of in dedicated sharps containers. Sharps primarily used for skin preparation before the use of any vector.

Work to be conducted within a dedicated category 2 laboratory. Skin handling, preparation and transfection to be conducted in a category 2 laboratory. Standard decontamination of work areas including wiping with 70% ethanol, Barcidal, Presept and 1% Virkon.

Lab coats specific for use in GMM room within which they remain. Spillage and disinfection procedures in place with notifications posted in laboratory. Spillage (small volume <50mL) to be wiped immediately with WIPEX infection control cloths and area swabbed with 1% Virkon. Contaminated cloths/tissue/coats etc. to be autoclaved as usual.

Spillage (large volume >50mL) on floor. All personnel to leave room for 30 min to allow aerosol to settle. Lab coats/clothing contaminated with spillage autoclaved for disposal. Broken glass collected with autoclavable forceps and decontaminated before placing in SharpsSafe containers. Inform Safety Officer of incident.

Solid and liquid waste will be autoclaved.

Contaminated waste, including paper towels and plasticware is placed in autoclave bags and transported in designated metal bins to autoclave for 1 hr at 130°C before placing in yellow biohazard bags.

Contaminated glassware and plastic disposable pipettes soaked in Presept 2500 ppm (2 x 2.5g tablets in litre water) for more than 30 min Pipettes can then be disposed in SharpsSafe containers.

All cytocidal agents have widespread activity used at recommended manufacturer’s concentrations are validated for 100% cell kill.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

GMSC wanted further information re. Skin layers and cell lines - now received. Hep B surface antigen added but risk assessment recirculated and approved.
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### Project Ref 130/07.4

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### Project Additional Information

**Purposes of the contained use**

The aim of this project is to enable production of recombinant retrovirus vectors for the transduction of non-human cells. This protocol is predominantly for the production of rodent cells that express receptors or other molecules in molecular pathways of the immune system. Typical application of this involves for example introduction of an immune receptor into a cell for study of its role in immune recognition and cell biology.

**Recipient or parental organism**

The eukaryotic cells that will be infected with the retroviral particles include non-human cell-lines and non-human primary cells. The majority of these cell lines will be rodent cells.
The retroviral vectors are replication deficient derivatives (e.g., MMLV and MSCV). The vectors consist of plasmid backbones containing a pair of viral long terminal repeats (LTRs), which must be transfected into packing cell lines that provide the structural proteins for packaging of infectious but replication incompetent retroviral particles. The packaging cell line determines the range of target cells that may be infected with the virus particles and ecotropic packaging cells are used to prevent infection of human cells.

**Origin & function**

The recombinant genetic material will be mostly mammalian derived (predominantly rodent). It will include material encoding molecules of immune pathways (e.g., cell surface immune receptors and molecules involved in their signalling pathways) and regulators of cell division and fate (e.g., homeobox genes). This material will be used for the analysis of the function of specific immune pathways or for generating novel cell lines from primary cells, respectively. Human material for the study of immune molecules and signalling pathways will also be used.

The recombinant viral vectors will be introduced into an ecotropic packaging cell line by transient transfection. The ecotropic packaging cell line contains the viral gag-pol and env sequences necessary for the packaging of infectious replication incompetent retroviral particles that cannot infect human cells. The viral particle containing culture medium is harvested and used to infect rodent cell lines or primary cells, usually by centrifugal infection. The murine cell lines are then selected in the appropriate manner (e.g., antibiotic containing media) to allow survival of those containing integrated retroviral vector and expressing the recombinant genetic material. This protocol will generate altered non-human cells for the study of immunological process as well as growth and differentiation altered rodent primary cells.

**Evaluation of foreseeable effects**

The viral vectors are disabled:

The viral vectors are replication deficient derivatives and packaging cell lines are required for the production of virus. They lack the viral gag-pol and env sequences required for production of infective virus. Plasmid backbones containing a pair of viral long terminal repeats (LTRs), which must be transfected into packing cell lines that provide the structural proteins for packaging of infectious but replication incompetent retroviral particles. The use of ecotropic packaging cell lines ensures that human cells are not infected.

The retroviral vector cannot revert to wildtype:

The retroviral vector is free from unnecessary retroviral sequence and the viral proteins (gag-pol and env sequences) required for packaging of infectious viral particles are contained on different selectable cassettes within the packaging cell line.

Hazard to human health (e.g., infectivity and the effect of additional material on tropism or biological function):

Theoretically, virus could be delivered to humans by mucous membranes, cut skin, or via trauma (e.g., needle stick injury). However, the virus is only packaged in ecotropic packaging cells and so is not able to infect human cells. Furthermore, if any infection were possible, it would not be a productive one and limited use of viral sequence ensures that the likelihood of recombination is significantly reduced. Containment procedures prevent this. Neither the tissue/cell tropism nor host specificity of the virus will be affected by addition of foreign material. Introduction of rodent homeobox genes can alter the differentiation and proliferation of rodent haematopoietic cells and hence could be considered oncogenic, however, the use of restricted tropism and accessibility of human target cells prevents this.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste will be disinfected using bleach (>1% w/v) or virkon (>2% w/v) for greater than 1 hour. Solid waste will be autoclaved or incinerated prior to disposal. These procedures will result in complete killing of the retroviral vector.

The Committee believed that the original activity classification of 1 was incorrect and after discussion with was agreeable to changing it to 2-

**Project Containment**

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**Large Scale Activities**

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**Human Clinical Applications**

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**Project Ref** 130/07.5

**Date Ackn'd** 06/09/2007

**Testing of candidate antiviral compounds for activity against viruses: laboratory strains, vaccine strains, wildtypes and recombinant viruses expressing fluorescently tagged proteins (eg. GFP, EGFP) using classical virus titrations methods and flow cytometry.**

**Consent Granted** Not Applicable

**Project notified under transitional arrangements** N
Project Additional Information

Purposes of the contained use

The purpose of the contained use here is the development and testing of antiviral substances active against human viruses in collaboration with Welsh School of Pharmacy. Virus titers will be determined by quantitation of fluorescence from recombinant viruses expressing E/GFP in susceptible cell lines (high throughput screening). Luciferase reporters will be used for the investigation of viral entry mechanisms. The project is a connected program of work for the evaluation of antivirals against a variety of human viruses (see below).

Recipient or parental organism

The GM organisms we want to notify the HSE about are derived from ACGM L2 classified wildtype viruses and labstrains. These viruses have been modified by various investigators to express green fluorescent protein (GFP) from enhanced forms of GFP (EGEP) or firefly luciferase from (hosts and origins of GM strains see below). Modifications of this type generally attenuate the recipient virus, or do not affect its pathogenicity. There is NO evidence in the literature that expression of fluorescent tags/luciferase increases viral pathogenicity.

Host/vector system

Expression of green fluorescent protein or firefly luciferase allows rapid identification and quantitation of virus early (day 1-2) in the course of infection and with direct observation methods (IF microscopy/flow cytometry/luminometer). The fluorophores/enzymes used are GE? (enhancedEGFP) from Aequorea victoria that fluoresces green when exposed to blue light, and firefly luciferase from Photinus pyralis [Photinus-luciferin:oxygen 4-oxidoreductase (decarboxylating, ATP-hydrolyzing), EC 1.13.127].

Origin & function

AV 3.1 Recombinant measlesviruses: gfp and enhanced gfp tags Measlesvirus IC323-EGFP (obtained from Prof Yusuke Yanagi; Takeda et al., 2000; J Virol 74: 6643-6647) expresses green fluorescent protein in B95a cell syncytia. The EGFP (Clontech) gene unit has been attached to the 5’ end of the measlesvirus genome (5’ of N).

AV 3.2 Recombinant adenoviruses: gfp and enhanced gfp tags Adenovirus ad852-gfp/streplll was constructed in Professor Gavin Wilkinson's lab at the Cardiff University School of Medicine (Stanton et al., 2006; unpublished results). The replication incompetent (delta E) virus will be used to test potential antivirals, infects a wide range of cells, but replicates only in HEK293 cells.
AV 3.3 Recombinant poxviruses (vaccinia virus): luciferase, gfp and enhanced gfp tags
The firefly luciferase expressing vaccinia virus WRLuc-> will be provided by Departamento de Biologia Molecular Celular, CNB, Spain (Rodriguez, et al., 1988; PNAS 85: 1667-1671). The luciferase gene has been cloned into the TK site of vaccinia virus strain WR (Western Reserve vaccine strain).

AV 3.4 Recombinant influenzaviruses: gfp and enhanced gfp tags
We have obtained influenza virus expressing GFP from the NSI reading frame from Institute of Applied Microbiology, University of Natural Resources and Applied Life Sciences, Austria (Kittel et al., 2004; Virology 324: 67-73). This virus grows productively only in MDCK cells and embryonated chicken eggs.

AV 3.5 Recombinant herpesviruses: gfp and enhanced gfp tags
We have obtained the recombinant human Cytomegalovirus UL32-EGFP-HCMV from University of Tuebingen, Germany (Sampaio et al., 2005; J Virol 79: 2754-2767). UL32-EGFP-HCMV infects under laboratory conditions-in vitro- only human fibroblasts (e.g. MRC-5, HEL299, BJ-1).

All described viruses are currently in L2 storage and will be used to test potential antivirals after HSE permission is granted.

Evaluation of foreseeable effects
Expression of gfp does not alter host range nor pathogenicity of the recombinant virus.

The viruses used are classified ACGM L2 and will be used in L2 containment facilities, if procedures are followed there is only negligible risk of infection with the GM organisms, most of which are derived from vaccine strains (except the adeno- and cytomegalovirus recombinants).

All laboratory personnel are vaccinated against measles virus infection (Edmonston strain). The vaccine is very effective. Even with waning immunity, measles virus infections are absent in the immunized elderly (outbreaks in Germany, Italy and UK, 2003-2005). All lab personnel get yearly flu vaccinations.

Overall the risk from the use of the GFP/luciferase tagged viruses is estimated as effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All laboratory work involving recombinant viruses will be carried out in L2 containment level labs. All personal will be trained in recognizing virus infections, awareness of human-to-human transmission, and the increased risk for individuals suffering from immune disorders (e.g. atopic dermatitis, severe acne, impetigo, contact dermatitis, psoriasis, chickenpox, steroid medication, pregnancy, infection with other pathogens). The exposure route via the conjunctiva will be particularly stressed and the use of adequate protective gear enforced in the lab. All personnel will be monitored by the Occupational Health Service at the School of Medicine.

Laboratory work will be carried out in class II safety cabinets, with personal wearing gloves. Disposable plastic pipettes and pipette tips will be used. Recombinant viruses will be used under conditions avoiding the generation of aerosols. Flasks containing virus will be kept sealed outside of the safety cabinets. Centrifugation will be carried out in sealed buckets, that will only be opened in safety cabinet. All waste materials will be collected in plastic bags in the safety cabinets, the bags will be sealed inside and then autoclaved. No sharps will be used.

Removable equipment and interior surfaces of the safety cabinets will be sprayed with Incidin® PLUS (Ecolab, Swindon, UK) containing the patented active substance Glucoprotamine with wide effect spectrum against bacteria (also MRSA and TBc), fungi, papova-, adeno-, vaccinia-, herpes-, rota- and hepatitis-8-viruses (HBV) in MADT and antigen test, as well as AIDS agent (HIV)-certified by the German Society for Hygiene and Microbiology (DGHM).

Spillage of GM material will be treated with hypochloride.

For the quality assurance of the disinfection and autoclaving procedures, measles virus contaminated Incidin disinfected and bleached plasticware, as well as autoclaved...
waste material will be rinsed with a small volume of PBS and tested for rest-infectivity on appropriate cells in a standard virus detection assay.

The main concern of the Cardiff University GM committee regarding the measles virus was whether the laboratory personal was vaccinated. The committee accepted the PI's assurance that the measles-vaccination-status of the personal working in the laboratory where measles virus is used is properly controlled. Non-vaccinated personal will be asked to receive a measles virus vaccination form the Occupational Health Unit Cardiff University or be excluded from the access to the laboratory. BSA Cardiff University received no more adverse comments from the Cardiff University GM committee after the principal investigators comments regarding vaccination of personal. The GM committee recommended to submit a notification as Class 2 for the measles virus risk assessment.

Please enter comments on the GM safety committee on the risk assessment

The main concern of the Cardiff University GM committee regarding the measles virus was whether the laboratory personal was vaccinated. The committee accepted the PI's assurance, that the measles-vaccination-status of the personal working in the laboratory where measles virus is used is properly controlled. Non-vaccinated personal will be asked to receive a measles virus vaccination form the Occupational Health Unit Cardiff University or be excluded from the access to the laboratory. BSA Cardiff University received no more adverse comments from the Cardiff University GM committee after the principal investigators comments regarding vaccination of personal. The GM committee recommended to submit a notification as Class 2 for the measles virus risk assessment.

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Project Ref 130/07.6

Date Ackn'd 23/10/2007

CU2 Project Title Expression of apoptosis regulators in mouse mammary epithelial cells.

Date Project Ceased

Class 2

Culture Vol Class 2 < 1 Litre

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work Y
### Project Additional Information

#### Purposes of the contained use

| The aim of this study is to observe the effects of known apoptosis-related signalling molecules in mouse mammary epithelial cells (MECs) by conditionally expressing these genes, and dominant negative variants, in primary and transformed cultures of mouse MECs. Recombinant genes will be administered to cultured cells by commercially available lentiviral / retroviral vectors and expression will be controlled using the Tet-on gene expression system. Changes in cell survival, motility and/or adhesions will be monitored by fluorescence microscopy and biochemical [assay of cell lysates. |

#### Recipient or parental organism

| Commercial lentivirus vectors (eg. Virapower, Invitrogen) originating from pseudotypes HIV-1 based lentivirus and propagated in the attenuated E. coli strain Stbl3. |

#### Host/vector system

| A third generation HIV-1-based lentiviral vector. It is a self-inactivating (deltaU3), replication-incompetent virus that ‘once transduced is unable to package viral genome The VSV-G gene replaces the HIV-1 envelope gene :pseudotyped), packaging genes are located on three separate non-homologous plasmids and no HIV-1 structural ‘genes are present in the packaged virus- The vector is able to transduce and integrate into the genome of dividing and non-dividing primary mammalian cells with high efficiency upon contact but is non-transmissable subsequently. The most likely route of transmission is via airways and eyes. There is no risk of the infection spreading to the community via human-human contact, and the packaged virus is unstable in the general environment. |

#### Origin & function

| The following will be inserted into the vector sequence for expression in mammalian cells: murine apoptosis inducing genes belonging to BH, BIR and NF-kB gene families and apoptosis suppressing genes belonging to BH3-only, DED/DD and caspase families and apoptosis suppressing genes belonging to BH, BIR and NF-kB gene families will be cloned into a tetracycln conditional lentiviral expression vector Packaged replication defective virus will be generated in 293 cells and used to transduce target mammary epithelial cells (MECs) in culture that already carry the rTA gene necessary for Tetracycline induction of recombinant gene expression. Target primary rTA-MECs will be generated from rTA-transgenic mice while target immortalised rTAMEC lines will be generated by prior stable integration of retroviral or lentiviral rTA constructs. Thus packaged recombinant expression vector and packaged rTA vector will not be used simultaneously. Once transduced, these cells will be maintained in culture and assessed for a variety of cellular responses including apoptosis. After cellular assays are completed the infected cells will be disposed of. |

#### Evaluation of foreseeable effects

| The recombinant vectors will express mammalian apoptosis regulators (both suppressors and activators of cell death). |
Expression of the recombinant genes is dependent upon co-expression of the TetRepressor protein (which is not present in the vector) in combination with the presence of the antibiotic tetracycline (or analogue). Thus accidental infection by the recombinant vector is highly unlikely to result in the expression of the recombinant gene encoding the apoptosis regulator as neither the TetRepressor protein nor tetracycline are present. However there is a low risk that a minimal level of recombinant gene expression could result from 'leaky' expression in the absence of the inducing agents. The TetRepressor protein will be delivered to target immortalised cell lines by attenuated lentiviral or retroviral vector. This protein is non-toxic to mammalian cells in vivo and in vitro. These stable lines will be maintained and stored for use as target cells for the recombinant lentiviral expression vector. Apoptosis inhibitors are potential oncogenes, contributing to tumour progression by prolonging the life of damaged or mutated cells. Clonal expansion of these cells would likely require additional mutations in growth and apoptosis pathways prior to onset of disease. Exposure to the virus could result in low level (uninduced) expression in infected cells. The likelihood that this could result in partial resistance to cell death is low. However the integration of the virus into the genome upon transduction increases the hazard risk. Some apoptosis inhibitors may interfere with intrinsic (target) host cell response to viral infection (ie. apoptosis) but would not be expected to influence cell-mediated immune responses and are unlikely to affect global host defence mechanisms in the host organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Primary mammary epithelial cells will be derived from wild-type and genetically modified mice. All animals will be housed in the Designated animal facility within the Biosciences building, killed by schedule 1 method and transferred to the viral containment suite in closed box. Mammary glands will be dissected from the mice in class II cabinets and epithelial cells purified from this tissue. Cells will be cultured within the suite until required for gene expression studies. Carcasses will be disposed of as biohazard waste under the same procedures used by the designated animal facility. Cell lines and stable clones will be archived by freezing and storage in labelled boxes within designated -80°C freezers.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work involves standard laboratory procedures on a small scale. All cultures performed in sealed flasks and handled in Class II recirculating microbiology safety cabinets with double HEPA filters in a restricted-use culture facility under negative pressure. Gloves, facemasks and lab coats are worn within the suite. Only disposable plasticware, including filtered pipette tips, will be used for contaminated liquids etc. Once used, these will be fully immersed in Virkon for 24 hours, placed into double autoclave bags and disposed of by the School of Biosciences approved decontamination method involving autoclaving. Company product efficiency data (DuPont) and independent test data on Virkon indicate a 100% kill of all known viruses, including lentiviruses, within 10 minutes of direct contact. Work surfaces and spillages will be decontaminated by spreading Virkon. Automated pipettors and plastic pipettes will be used to transfer and dispose of contaminated liquids/media, no mechanical vacuum aspirators will be used. Contaminated liquids will be transferred to Virkon, soaked for 24hrs and disposed by sink. Subsequently, containers will be disposed of as described above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The committee queried whether or not should be Class 3 activity and instructed BSO to seek advice. Advice received and committee agreed class 2.

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**Project Ref**  130/08.1

Date Ackn'd: 18/07/2008

Date Project Ceased

**CU2 Project Title**

Production of mouse cytomegalovirus (MCMV expressing functional interleukin-10), and subsequent infection of cells and mice with the recombinant virus.

**Class**

Class 2

**CultureVol**

< 1 Litre

**Consent Granted**

Non-GMM Not Applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To model the in vivo function of homologues of IL-iC that is expressed by human herpesviruses such as Human cytomegalovirus (HCMV) and Epstein Barr virus (EBV). In particular, to study the effect of IL-10 expression by herpesviruses on tissue tropism and viral replication, and the suppression of host immunity at different sites of viral infection.

**Recipient or parental organism**
The wild type MCMV strain M97.0i will be used as a recipient for mouse IL-10, i.e. the IL-10 gene will be inserted into the M97.01 genome. The M97.01 genome is expressed by the pSM3fr plasmid (reference: Wagner et al., J Vir, 1999, 73:7056) suitable for growth in E.coli.

Host/vector system

The DNA encoding mouse interleukin-10 will be inserted into the M97.01 genome that is expressed by the pSM3fr bacterial artificial chromosome (BAC) plasmid (Wagner et al., J Vir, 1999, 73:7056). The plasmid that subsequently contains MCMV 97.01 genome and mL10 will then be grown up in Ecoli, and then the plasmid isolated and used to transfect mouse fibroblasts for production of the infectious MCMV-IL-10 virus.

Origin & function

The genetic material encodes mouse IL-10. The IL-10 cDNA will be either cloned from mouse genomic DNA, or from a plasmid where the IL-10 cDNA has already been cloned into. This cytokine is specific-specific (i.e does not bind to human IL-b receptor). IL-10 suppresses different aspects of the immune system in vivo. Therefore, MCMV expressing IL-10 will be used to infect mice to study the effect of IL-10 expression by herpesvirus in vivo on different aspects of the immune system in a variety of mucosal and non-mucosal organs in the body. In some experiments, the virus will also be used to infect cells in vitro and the effect of MCMV-IL-10 on different cell functions will be analysed.

Evaluation of foreseeable effects

MCMV-IL-10 infected mice may exhibit prolonged and/or possibly exacerbated weight loss/liquefation than wild type MCMV-infected controls. If MCMV-TL-10 does exacerbate weight loss, infected mice will be observed daily until weight loss returns to pre-infection levels, in line with our Home Office Project License (PPL 30/2442). Expression of IL-10 by MCMV may also suppress the host immune responses to the virus. Therefore, MCMV-IL-10 may display increased replication and may alter tissue tropism in the mouse. In the case of mucosal organs such as the intestines or kidneys, this elevated viral load may result in increased shedding in the urine or kidneys. The SCAN containers used to house MCMV-infected mice are suitable for housing mice infected with lung and gut-associated pathogens. Therefore, this type of housing will effectively contain MCMV-IL-10 even in the unlikely event of the virus displaying increased shedding via “novel” mucosal secretions. Furthermore, MCMV is relatively unstable in the environment, and IL-10 is a non-structural protein. Therefore the inability of the virus to persist in the environment will not be affected by the expression of IL-10 by the virus. Importantly, 11-10 is a mouse protein and does not cross-react with humans. Therefore, like “wild type” MCMV, MCMV-IL-10 also poses no threat to human health.

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The following chemical disinfectant methods have been shown to induce 100%, as tested by plaque assay for replicating MCMV. The chlorine-based disinfectant is Haztabs (2500 ppm). The efficacy of this disinfectant has been perfomed with wild-type MCMV and will be repeated with MCV-IL-10 (once constructed) prior to the commencement of the research.

Liquid waste: Empty into waste pots kept inside the flow cabinet. Allow to stand for 60 minutes in chlorine-based disinfectant before emptying down the sink with excess running water.

Solid waste: Plasticware (including tips) that has come into contact with infectious material should be submerged in the waste pot containing chlorine-based disinfectant and left to soak, as described above.

Pipettes: Rinse pipettes up and down in bleach-containing waste pot and leave to soak for 60 minutes. Then dispose of these in large sharps bin.

Spillages: Spillages should be wiped up with tissue soaked in chlorine-based disinfectant for 60 minutes. The tissue will then be disposed of in clinical waste.

Infected carcasses and bedding from cages housing infected mice: Disposed of as clinical waste by a licensed contractor.
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Project Ref 130/08.2

Date Ackn'd 22/10/2008

CU2 Project Title Introduction of interfering RNAs into eukaryotic cells using adenovirus and retrovirus vectors in order to modulate gene expression.

Class 2

CultureVolClass2 1-50 Litres

Non-GMM Not Applicable

Consent Granted

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Did you use a Category 2 Culture? Yes

Date Project Ceased

Non-GMM Consent Granted

Non-GMM

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Risk assessment suitable and sufficient after minor alterations had been made, in agreement with classification of activity.
Project Additional Information

Purposes of the contained use

Commercial systems are readily available that enable researchers to obtain interfering RNA for any cellular gene (usually human or mouse) already inserted into adenovirus and retrovirus (usually lentivirus) vectors. The application also seeks to cover in-house technology based on replication-deficient adenovirus vector being developed for this purpose. The cover is directed at the delivery of these recombinant viruses to cell culture systems in vitro. The aim being to provide knock-down expression of targeted genes so their independent function or role in a pathway can be determined.

The worst case situation identified in the risk assessment was to knock-out expression of a tumour suppressor protein (examplified by p53, Rb and PML). The intention is for individual researchers in Cardiff University to perform separate risk assessment to cover the application of this technology. Should any future assessment identify an increased hazard (even assessed at ACGM2 level) relative to that assessed, it will be subject to independent notification.

Recipient or parental organism

The vectors will be delivered to cells in culture. Replication-deficient adenovirus vectors are associated with high level transient expression (although can insert into the host genome at low frequency) can be produced in high concentration and can be spread by aerosol to contact. Retroviruses are transducing vectors that integrate into their host to provide long term expression in cell targets and their progeny. The retrovirus will normally be pseudotyped which enhances their stability and may facilitate aerosol spread. In target culture cells knock down or knock out of specific cellular or virus-encoded gene expression gene expression will be sought. Knock-out of cellular of gene expression in the target cell is most likely going to be neutral of detrimental to the cell or virus survival. Depletion of a tumour-suppressor gene is likely to promote cell transformation. Eukaryotic cells require a sophisticated mixture nutrients and environmental conditions and would not be expected to be able to survive free outside of the laboratory. Containment is most needed for viruses and cells expression interfering RNA to tumour suppressors. Exposure to such vectors could promote cellular transformation, and warrants containment.

Host/vector system

Replication-deficient Adenovirus 5 vectors including specifically: AdZ vector based on recombineering (in-house, and detailed independently), AdEasy (He et al, Proc Natl Acad USA, 955, 2509), Admax (Microbix) and Ad-HQ™ (Vector Laboratories).

The Retroviral vectors will be based on established and commercially available technologies that include Super Retro (Oligoengine, Seattle) and Psiren - Retro Q-Zs Green (Clontech), and a commercial library based on MISSION® RNAi lentivirus technology (pLKO.1 puro; Sigma). Virus will be pseudo-typed with the envelope glycoprotein from Vesicular stomatitis virus (VSV-G).

Origin & function

DNA complementory to interfering RNA will be obtained pre-made in vectors or synthesised de novo in vitro. The sequence will generally be designed to contained a short sequence complementary to the target transcript.

Evaluation of foreseeable effects

Delivery of the recombinant will express a short RNA sequence that will anneal to a target transcript so as to promote its degradation or impair expression. Knock down of anti-inflammatory response could impair the cellular immune response eg interferon response. Even with efficient vector delivery in vivo this is likely to be a restricted localised effect that could make an individual more susceptible to infection at this site. A greater hazard was considered to be the knock out of a tumour suppressor protein that could promote cell transformation. Adenovirus and pseudotyped retrovirus vectors are capable of in vivo delivery. Knock-out of a single tumour suppressor protein is by itself not likely by itself to induce a malignancy, but containment is required to avoid operator exposure.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Recombinant viruses will at all times be maintained in an environment designed to prevent aerosol spread. All flasks containing the virus shall be kept closed except when decanting and pipetting which will be conducted in tested class II safety cabinets. Centrifugation steps shall be performed in sealed buckets that will be opened only in...
The use of Sharps when handling virus preparations shall be avoided wherever possible. Contaminated pipettes will be immersed in Actichlor for a minimum of 4 hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with chlorine-based disinfectant according to an approved protocol. Access to laboratories employed for virus work will be restricted.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Recombinant viruses will at all times be maintained in an environment designed to prevent aerosol spread. All flasks containing the virus shall be kept closed except when decanting and pipetting which will be conducted in tested class II safety cabinets. Centrifugation steps shall be performed in sealed buckets that will be opened only in safety cabinets. The use of sharps when handling virus preparations will be minimised. Tissue culture media will be disinfected for a minimum of 2 h by addition of Actichlor (2500 ppm sodium dichloroisocyanurate), this process has been validated in-house for adenovirus. Contaminated pipettes will be immersed in Actichlor for a minimum of 4 hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with chlorine-based disinfectant according to an approved protocol. Access to laboratories employed for virus work will be restricted.

Risk Assessment now suitable and sufficient after alteration to include the highest risk GMM.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Risk Assessment now suitable and sufficient after alteration to include the highest risk GMM.

**Project Containment**

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**Project Ref** 130/08.3
Modification of Wnt signalling in human breast and ovarian cancer cells using lentivirus and retrovirus expression vectors.

We have evidence that inhibition of autocrine Wnt signalling by DKK, a secreted antagonist of Wnt signalling which acts at the level of ligand-receptor interaction, in breast and ovarian cancer cells decreased their proliferation and increased apoptosis. We will undertake studies aimed at assessing whether autocrine Wnt signalling plays a role in the resistance of breast/ovarian tumor cells to therapeutic agents. We had identified a sub-set of autocrine Wnt breast cancer cell lines with very high levels of the Wnt co-receptor LRP6 protein and demonstrated that down-regulation of LRP6 by a lentiviral siRNA in one of these cell lines, BC3, resulted in inhibition of uncomplexed β-catenin, suggesting that activation of Wnt signalling depends on high LRP6 levels. We will establish stable expression of Wnt pathway genes in these tumour cells and analyze the ligand effects on RNA and protein LRP6 levels. Should over-expression of Wnt in these tumour cells fail to reduce LRP6 RNA levels, this would suggest that the loss of this feed back mechanism is the cause of high LRP6 levels.

Host organisms are human cancer cell lines, in particular breast and ovarian.

All genes will be introduced into a series of plasmid backbones of the pBABE series of vectors. In the appropriate producer lines these produce ecotropic and amphotropic retroviruses where gene expression cassette will be selected on the basis of drug resistance to G4 18 or puromycin. Alternatively, the inserts will be introduced using the lentiviral vector VIRHD/EP that has been modified to be self-activating, since it lacks the genes to make both the viral envelop and the packaging, so after integration in the host cell DNA the lentivirus cannot reproduce itself.

Members of the Wnt signalling pathway, all are either human or mouse genes.

Wnt-1, Wnt-2, Wnt-3, Wnt3a, Wnt4, Wnt5b, Wnt6, Wnt7a, Wnt7b, FRP, DKK, siRNA to LRP5, siRNA to LRP6, dishevelled, Kremen 1, Kremen 2, reporter constructs for Wnt transcriptional activity: TOP-Flash, FOP-Flash in frame with either luciferase or GFP, LRP5, LRP6, Frizzled 1, dominant negative TCF4.
Please note that this notification is the first part of a connected program of work using these vector systems to introduce members of the wnt signalling system into human cancer cell lines. Any additional sections of the program will be using other members of this signalling pathway into cancer cell lines that may be other than in this notification. However, any such new gene or cell lines will be of similar types with the same overall risk associated and will undergo risk assessment via the local Genetic Modification Safety Committee for approval.

**Evaluation of foreseeable effects**

With regard to the hazards associated with the host/recipient the following have been considered including the pathogenicity of the host strain, virulence, infectivity and toxin production: in this case the recipients and hosts are non pathogenic.

Most of the inserts that will be utilized are not hazardous since they are inhibitors of the Wnt pathway that may result in a small increase in cell proliferation and decrease apoptosis. The most hazardous vectors contain different WNT genes (Wnt-3a, Wnt-1, Wnt-2). Some Wnts are classified as oncogenes, although they have not been shown to cause oncogenic transformation of cells. When Wnt is utilised it can cause a mild increase in cell proliferation, and decreased cell differentiation. However, it must be stressed that the effects of these inserts on cell proliferation are minimal and they would not significantly affect normal cells, as they should not affect proliferation barriers.

With regard to the hazards arising from the alteration of existing pathogenic traits: The tropism of the vector has been altered by the use of the VSVg protein that is not present in the parent strain to enable infection of a wider range of recipient cells. In addition, the WNTd member of the indered gene family has been shown to modify the host defence mechanism in insects: however, there is no mammalian WNTd gene and the mammalian defence mechanism differs from that in insects, so there is no similar situation in mammals and all work is done in a dedicated Class II facility. There is no increase in infectivity or pathogenicity and no disabling mutation within the recipient can be overcome due to the insertion of the foreign gene. Foreign genes do not encode pathogenicity and no disabling mutation within the recipient can be overcome due to the insertion of the foreign gene. Foreign genes do not encode pathogenicity determinants from a related organism.

Considering whether an inserted sequence that does not give rise to harmful phenotype in the recipient micro-organism could give harm as a result of natural gene transfer to another possibly related organism: This is extremely unlikely to occur and if so it is unlikely to have a harmful effect.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable to this application.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable to this application.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All retroviral and lentiviral work will take place in a dedicated Class II facility. All liquid waste is sterilized at point of use by sodium hypochlorite or actichlor. All solid waste is sterilized in an adjacent autoclaving facility. Use of glass and needles will be avoided to reduce the risk of sharps injury.

Autoclave thermocouple tested annually. Also daily monitoring of efficacy of autoclave. Discussed determining the efficacy of disinfectants with HSE inspector on site. Relying on manufacturer's quality control and the very unstable nature of GMOs used.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y
Project passed at meeting at containment level 2. Minor revision requested to clarify risk of affecting host defence mechanisms. The WNTd gene may alter host defence mechanisms in insects, but as the host defence mechanism in mammals is very different this is not thought to be a significant risk. Risk assessment modified accordingly and signed off. In addition, further details of the genes to be inserted have been added.

Project Containment

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Project Ref 130/09.1

Date Ackn'd: 15/06/2009

CU2 Project Title: Development of a diabetic/chronic wound bioassay utilising lentiviral vectors to introduce disease specific fluorescent promoter reporter constructs into human diabetic/chronic wound and normal fibroblast cell lines

Class: Class 2
CultureVol: 1-50 Litres
Consent Granted: Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Project Additional Information
Purposes of the contained use

The purpose of this project is to use a commercial lentiviral system to create stably transfected promoter-reporter cell lines for use in the screening of agents that may alter the wound healing properties of fibroblasts. Lentiviral transduction of the sequence encoding a reporter protein linked to the promoters of wounding responsive/disease specific genes into normal, diabetic wound and chronic wound fibroblast cell lines will allow the activation of wound healing responses to be assayed in disease specific and normal human fibroblast cells and thereby facilitate the screening of possible therapeutic agents.

Recipient or parental organism

Inserting the sequence encoding a fluorescent reporter protein (DsRed and pZsGreen (Clontech)) downstream of the promoter sequences of wounding responsive/disease specific human genes and also a control promoter (GAPDH), into normal (NF-hTERT), diabetic wound (DF-hTERT) and chronic wound human fibroblast (CWF-hTERT) cell lines will allow the activation of wound healing responses to be assayed in disease specific and normal fibroblast cell lines.

The ViraPower™ Promoterless Lentiviral Gateway Vector Expression system (Invitrogen) is a 3rd generation HIV-1-based lentiviral system that has been modified for enhanced safety (DuH et al., 1998, J. Virol. 72, 8463-8471) This can be utilized to transduce these promoter-reporter constructs into normal, diabetic wound and chronic wound fibroblast cell lines using established protocols provided by the manufacturer. Selection of successfully transduced cells will be by antibiotic resistance (blasticidin) to create stable clones.

Replication defective lentiviruses will be produced in a pseudoviral particle producer cell line (293FT). Virions will be generated by co-transfecting 293FT cells with pLenti6/JR4R2N5-DEST (promoter-reporter) plasmid constructs containing the recombinant lentiviral expression cassettes and the pLP1 (gag/pol), pLP2 (rev) and pLPNSV-G (VSV-G envelope) packaging plasmids which contain genes encoding structural and replication proteins required to facilitate viral packaging of the pLenti-based expression constructs.

The viral particles produced in this system are replication incompetent and only carry the aene alpha promoter of interest No other viral species are produced This &so means that none of the structural HIV genes (necessary for replication) are present in the viral genome and are therefore never expressed in the target cell, so no new virus can be produced.

The stable cell lines that are made with the lentiviral system would not contain any of the packaging proteins. These proteins are only used to package the viral particles from the 293FT cells. They are provided in trans, so they are not part of the viral particle themselves, and therefore not present when the target cell is transduced and the gene of interest stably integrated.

Host/vector system

Hosts:

E. coli - DH5 alpha for propagation of pDsRed and pZsGreen plasmids; One-shot Stbl3 for propagation of pENTRw5'TOPO® and pENTR/D-TOPO® plasmids. Pseudoviral particle producer cell line 293FT. Lentiviral particles will be used to infect DF-TERT, NF-TERT and CWF-TERT human fibroblast cell lines. Vector System: Human disease specific promoters and the reporter genes (ZsGreen and DsRed) will be individually cloned into ENTR™5'-TOPO® and pENTR™/D-TOPO® entry plasmids respectively. These plasmids will be propagated in E.coli (DH5 alpha or One-shot Stbl3). The entry plasmids containing the disease promoter sequence and reporter gene of interest will then be recombined with the pLenti6/R4R2/V5-DEST expression plasmid during a MultiSite Gateway® LR recombination reaction (Invitrogen).

The pLenti6/R4R21V5-DEST (promoter-reporter) expression constructs and the ViraPower™ Packaging Mix will then be co-transfected into the pseudoviral particle producer cell line 293FT to produce a high titer of pseudoviral particles. The ViraPower™ Packaging Mix facilitates viral packaging of pLenti-based expression constructs in 293FT cells and contains the packaging plasmids (pLP1 (gag/pol), pLP2 (rev) and pLPNSV-G (VSV-G envelope)). Promoter-reporter constructs packaged in pseudoviral particles are secreted by producer cells into culture media and will be used directly to transduce these constructs into existing DF-hTERT, NF-hTERT and CWF-hTERT human fibroblast cell lines.

The viral particles produced in this system are replication incompetent and only carry the gene(s) of interest. Following transduction into the target cells, promoter-reporter
constructs are reverse transcribed and integrated into the genome of the target cell line. None of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. There is no known toxicity/infectivity/pathogenicity associated with any of the reporter genes used.

Origin & Function

The reporter genes encoding ZsGreen and DsRed (CLONTECH) were originally derived from reef coral (specifically Zoanthus sp. and Discosoma sp. respectively). These commercially available proteins are commonly used as reporter proteins as they are non-toxic, fluorescent and not expressed by mammalian cells. When transduced into human cells, reporter gene is likely to be transcribed in a similar pattern and level to the gene from which the promoter sequence was taken. No reporter gene expression is expected in bacterial cells (used to propagate the plasmid cloning vectors) as they lack the mammalian transcription mechanism.

Human promoter sequences will be generated via PCR from extracted human genomic DNA and inserted into commercially available promoterless lentiviral expression vectors (ViraPowerTM Promoterless Lentiviral Gateway® Vector Expression system (Invitrogen)). Primers designed to facilitate amplification of the region up to 2.5kb upstream of the transcriptional start site of a number of specific human promoters will be utilised. Specific promoter sequences will then be cloned on the basis of their downstream genes pattern of gene expression (and transcriptional activation) in each of the wound healing impaired and normal human fibroblast cell lines (DF-hTERT/CWF-hTERT and NF-hTERT respectively) used in this project both under serum limited culture conditions and in response to a serum stimulus. Gene expression patterns of target genes has been determined by microarray analysis.

The identity and integrity of cloned promoter sequences will be confirmed by DNA sequencing prior to insertion into the lentiviral vector. None of the coding sequence for any of these genes will be present within any of the viral vectors.

involved in developmental regulation.

Promoter and reporter sequences will be cloned into a commercially available lentiviral vector (pLenti6/R4R21'J5- DEST). The pseudoviral particles containing promoter-reporter sequences will be utilized to infect DF-hTERT, NFhTERT and CWF-hTERT cell lines (without the need for additional replication cycles) with the insertion of promoter-reporter genes into target cell lines facilitating the monitoring the transcriptional activity of these disease specific promoters in the presence of therapeutic agents.

- Successfully transduced human fibroblast cells will be selected using antibiotic resistance. The gene encoding blasticidin S deaminase conferring blasticidin resistance is present in the lentiviral vector and will be used to create of stable (promoter-reporter) clones.

Evaluation of foreseeable effects

The viral particles produced in this system are replication incompetent and only carry the reporter gene and promoter of interest. No, other viral sequences are reproduced. This also means that none of the structural HIV genes (nepssarv for production of viral progeny) are present in the packaged viral genome. Gag, pol, rev and envelope genes are not present in the viral genome and are therefore never expressed in the target cell, so no new virus can be produced following transduction. Since packaging and replicative genes are never packaged into the viral genome infective viruses are incapable of undergoing further rounds of replication. The stable cell lines that are made with the lentiviral system would also not contain any of the packaging proteins. These proteins are only used to package the viral particles from the 293FT cells. They are provided in trans, so they are not part of the viral particles themselves, and therefore not present when the target cell is transduced and the promoter and reporter gene of interest stably integrated. As a result retroviral particle production is limited to the producer cell line.

The ViraPowerTM Promoterless Lentiviral Gateway® system is derived from HIV-1, with three genes from HIV-1 used in the system (i.e. gag, pol, and rev). Genes encoding the structural and other components for packaging the viral genome are separated onto four plasmids (i.e. three packaging plasmids and pLenti6IR4R2/V5-DEST). These genes are only present and expressed in the producer cell line (293FT) when the packaging plasmids are cotransfected. All four plasmids encoding the structural and other components required for packaging the viral genome are separated onto four plasmids (i.e. three packaging plasmids and pLenti6IR4R2/V5-DEST). These genes are only present and expressed in the producer cell line (293FT) when the packaging plasmids are cotransfected. All four plasmids have been engineered not to contain any regions of homology with each other to prevent rare, but undesirable, recombination events that could lead to the generation of a replication-competent virus.

The gene transfer vector pLenti6/R4R2N5-DEST has been modified to be ‘self-inactivating’. A deletion has been made in the 3’LTR (called ‘deltaU3’) that has no effect on the generation of viral genome for packaging in the producer cell. However, once the produced virus transduces a target cell, the mechanisms of reverse transcription use the 3’LTR as a template to create the 5’LTR. The end result is an integrated viral genome that is defective in both 5’ and 3’LTRs. Thus, there is no transcription or production of viral particles.
The DsRed and ZsGreen reporter proteins encoded in the lentiviral constructs have previously been shown to have little or no cytotoxic effects in mammalian cells. Insertional mutagenesis is known to be a low risk in retroviral vector systems, although lentiviral vectors appear to pose less of a risk to gene upregulation than retroviral vectors as they preferentially integrate into the 5' region of active genes and are thus most likely to abrogate gene expression.

A potential hazard is obviously the exposure of humans to the lentiviral particles. Exposure to these particles will be minimised by performing all manipulations in a Class 2 Biological Safety Cabinet. All procedures will be carried out whilst wearing laboratory coats and disposable purple nitrile gloves (guaranteed to prevent viral particle penetration). Known routes of transmission include needle stick injury. However, no sharps or glass will be used to minimise the risk of sharps injury, while the use of a Class 2 containment facility will reduce the likelihood of risk to individuals working with this lentivirus.

F. coli K12-derivatives used for subcloning and propagation of plasmids (e.g. DH5 alpha and Stbl3) are disabled hosts and are non-pathogenic, non-reversional and are unable to colonise the human gut. These strains are unlikely to survive outside the laboratory where they would be subjected to sub-optimal growth conditions (nutrients and temperature) and pose a very low/negligible risk to human health or the environment.

Human cell lines have a low capacity to survive, establish, disseminate and/or replace other organisms. Retroviruses are rapidly inactivated outside the host cell line and are highly susceptible to dehydration.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None. All the measures specified as requirements for Level 2 containment will be implemented.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All experiments will be carried out in a Class 2 containment facility with available autoclave and disinfectant. The Class II cabinet will be cleaned before and after use. Before use, the cabinet will be, cleaned with sodium hypochlorite (Effervescent Chlorine Tablets, 1 tablet! litre water, 600ppm concentration), followed by spraying with 70% ethanol. Chlorine tablets have been previously demonstrated by the manufacturers’ to be effective against all viruses at a recommended final concentration of 250ppm. After use, the cabinet will be sprayed with 70% ethanol and cleaned with sodium hypochlorite, before removing any contents (including stripettes, tips, flasks, medium, etc.) from the cabinet. All consumables and byproducts used during and generated as part of the experimental procedure will be soaked in 600ppm sodium hypochlorite overnight and will then be autoclaved before disposal. All liquid waste will be autoclaved prior to disposal.

Degree of kill:
Autoclaving, effectively 100% kill.

Monitoring of each run will be carried out by electronic printout schedule of autoclave cycle parameters to ensure successfully completed autoclave cycle on each occasion in addition to annual validation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The Committee required further information and a reference to explain oncogene KIT. This has since been provided and the Risk Assessment is now regarded as being suitable and sufficient.

**Project Containment**

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**Project Ref 130/09.2**

- **Date Ackn’d**: 03/12/2009
- **CU2 Project Title**: The contribution of the exosporium to survival and biocide resistance
- **Class**: Class 2
- **CultureVol**: ≤ 1 Litre
- **VolumeClass**: Class 2
- **Consent Granted**: Yes

**Project notified under transitional arrangements**: No

**Withdrawn**: No

**Tick if notifying a connected programme of work**: No

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

We propose to inactivate genes encoding the following activities: superoxide dismutase; arginase; alanine racemase and inosine hydrolase. This will be achieved using the following GM based approaches; Transposon mutagenesis using Tri917 (encodes resistance to erythromycin) and a targeted mutagenesis approach in which a Cre recombinase system is used to create deletions. Both approaches will result in the further inactivation of an already attenuated strain of B. anthracis. Erythromycin is not a first choice drug for the treatment of anthrax.
Recipients or parental organism

We propose to further inactivate the attenuated Sterne vaccine strain of Bacillus anthracis, a strain which has been approved by HSE for handling and manipulation at containment level 2 conditions. The strain lacks a major plasmid encoded virulence factor and as a consequence has been rendered harmless. The avirulence of this strain has been amply demonstrated over the years and it has been used extensively as a vaccine to protect domestic animals.

Host/vector system

The study will make use of two plasmid vectors, one for transposon mutagenesis and a second in support of the targeted mutation approach using the Cre recombinase system.

Tranposon Mutagenesis

The pLTV-1 plasmid is a temperature sensitive E.coli/B. subtilis shuttle vector plasmid carrying the transposon Tn917-LTV1 originally from Enterococcus faecalis which confers erythromycin resistance and generates lacZ transcriptional fusions when insertions occur within the appropriate orientation. While the plasmid contains additional antibiotic resistance genes they constitute selective markers already in routine use in standard cloning vectors and will be eliminated following growth at the non-permissive temperature. The plasmid contains a Cole1 origin for replication in E.coli and makes use of the temperature sensitive pE194 original replication to drive replication in Bacillus spp. And is considered non-mobilisable in this background.

Cre Recombinase mutagenesis

A non-expressed copy of the B. anthracis gene to be activated is the starting point of this method. The gene will be obtained from Pathogen Functional Genomics Resource Centre of the Craig Venter Institute in the US which provide the sequence on the non-mobilisable pUC based pENTR vector designed to prevent expression from its E.coli host DB3.1 a disable K12 derivative (invitrogen).

This gene is functionally inactivated by the insertion of the loxP-Spectinomycin-LoxP cassette in a disable E.coli K12 host. The resulting insert is then cloned into pHY304 and E.coli/B subtilis vector with a Gram positive temperature-sensitive (permissive 30oC, non permissive 37oC) replicon derived from Lactococcus facts. It is tra-andmob-and contains erythromycin and chloramphenicol as selective markers which are already in routine use in standard cloning vectors. This work is carried out in a disable K12 host. Following construction the plasmid is transformed into B.anthracis were the inactive gene replaces the functional gene on the chromosome. Finally the spectinomycin resistance marker is removed using Cre recombinase to leave behind a single loxP site within the target gene, which disrupts translation.

Origin & function

The aim of this study is to determine the inactivating B. anthracis genes on biological function. The gene targeted for inactivation have been implicated in virulence and regulation of germination and as such their inactivation is likely to further attenuate the strain. No complete biologically functional gene will be used in this study do not encode resistance to the first line antibiotic resistant markers. The resistant markers that will be used in this study do not encode resistance to the first line antibiotics used to treat anthrax and in the case of the targeted mutations using the Cre recombinase system will be eliminated from the final construct. In the event that remains of the target gene are expressed, they are unlikely to be produced in an active form which restores biological function in the inactivated gene. The following genes targeted for inactivation are homologues to genes which have been shown to play the following roles;

- Construction of exosporium - exeA gene homolog (BAS4324) attachment of exosporium to the spore coat exsY (GBAA1238) construction/ attachment of the exosporium
- Regulation of germination - Dal (BA0252) alanine racemase homolog
- Superoxide dismutase - SodA-1 (BA4499) Manganese dependent superoxide dismutase
- SoDFe(BA1489) Iron dependent superoxide dismutase
- Arginase - RocF (BA154)

Evaluation of foreseeable effects

The aim of this study is to determine the effect of inactivating specific B anthracis genes within in the attenuated Sterne vaccine strain approved for use under containment level 2 conditions. This strain will transformed with homologous fragments of B.anthracis DNA for the purpose of creating knockout mutants, the mutations can only reduce the virulence of an already a virulent strain. The target gene will be cloned into E.coli vectors that are not designed for expression. Indeed it is our experience that B. anthracis tend to be extremely AT rich and as a consequence are extremely difficult to express from laboratory strain of E.coli. The use of non-mobilise or mobilisation-defective vectors, disabled non-pathogenic auxotrophic E.coli K12 derived hosts that have very limited ability to survive outside the lab, greatly reduces the likelihood of transfer of cloned DNA fragments from B anthracis to related microorganisms. Further, even in the very unlikely event of transfer to a related micro-organism, the cloned sequences will not express a product that could have toxic or other detrimental effects if delivered to human tissues.
In the case of the transposon mutation experiments we will employ a temperature sensitive, non-mobilisable plasmid to deliver the transposon thusus removing a potential mechanism for host/gene transfer. The resistant marker that does not encode resistance to a first line antibiotic used to treat anthrax. While there is a remote possibility that host sequences flanking the transposon insertion could be mobilized following integration into and excision from the the chromosome it is extremley unlikely that the B. anthracis derived sequences will encode a product that could have toxic or other detrimental effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste and contaminated glassware will be autoclaved as per the published Cardiff University instructions. The correct operation and the validation of sterilization and discard autoclaves within in the dept is described in the SOP entitled "The Welsh School of Pharmacy Procedures for the Use of Horizontal Autoclaves for Sterilization and discard"

Laboratory surfaces likely to have been in contact with spores of the attenuated vaccine strain of B. anthracis will be wipes with 10% Sodium hypochlorite. Following a 10 minute contact time the surfaces are washed with water to remove any toxic residue. As part of good working practice all work surfaces will be wiped clean with 10% hypochlorite solution at the end of each experiment and at the end of the the working day.

Laboratory studies have confirmed the effectiveness of this decontamination for B anthracis spores.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The comments of the university of the university GM safety committee were as follows: the committee were in agreement that the project is a class 2 activity and advised that we consider the choice of disinfectant carefully so that you are sure the disfectant is appropriate for the microrganisms that you will be working with and the particular envirnoment that you will be disinfecting. We have done this.

The committee also commented that the school as a whole should be thinking about decontamination of the rooms where you will be carrying out the work, should the use of this room change. We have also done this.

Project Containment

<table>
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<td>Yes</td>
<td>L3</td>
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Project Ref 130/09.3

Date Ackn’d 03/12/2009

CU2 Project Title Development of an antibody based therapy for Clostridium difficile. The expression of Clostridium difficile protective immunogens from an attenuated Escherichia coli based expression system

Date Project Ceased

Class CultureVol

Volume

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Clostridium difficile is a significant cause of morbidity and mortality amongst hospitalized patients, costing the NHS £4000 per case to treat. The financial cost is further increased due to the high rate of relapse. Studies have shown that the stimulation of a robust antibody based immune response plays a key role in mediating protection. Animal studies have confirmed the feasibility of using antibodies to confer passive protection. The C terminal receptor binding domains of the C. difficile major virulence factors, toxin A (tcdA) and toxin B (tcdB), stimulate the production of protective antibodies in animals. In addition vegetative cell surface associated S layer proteins have been shown to induce the production of antibodies which inhibit the binding of the vegetative bacterium to human gut cells. We propose to clone and express codon optimized versions of the C terminal region in E. coli and isolate recombinant proteins. Sequences will be cloned and expressed using a commercially available 6XHis tag expression system produced by QIagen Inc, California. An external company Genscript will construct a cloning vector containin the codon optimised gene sequence. The sequences will be inserted downstream of a 6XHis affinity tag to enable subsequent purification with a Nickel column.

Recipient or parental organism

The host from which proteins will be expressed is SG13009, an attenuated K12 E.coli strain with the following phenotype NalS, StrS, RifS, Thi-, Lac-, Ara+, Mtl-, F-, RecA+, Uvr+, Lon+. As the organism is considered non pathogenic to humans and animals and due to the limited survivability of the organisms in the environment, the risk to human health and the environment is low. The use of auxotrophic strains also reduces the potential risk of the transfer of genetic material. The proteins to be expressed are the fragmented non toxic regions.

Host/vector system
The vector to be used is pUC18 an E.coli plasmid isolated from E.coli DH5α - derived from the non pathogenic E. coli K12 strain. The vector is non transferable.

**Origin & function**

- **Toxin A tcdA** - C terminal domain
- **C. difficile 630 genome nucleotides 801048 to 803975**
- **Toxin B tcdB** - C terminal domain
- **C. difficile 630 genome nucleotides 792643 to 794493**

High molecular weight S-layer protein slpA
Low molecular weight S-layer protein slpA

The C terminal domain of the toxin has been identified as the receptor binding region. It has been shown to be highly immunogenic and is non-toxic. S-layer proteins are believed to be involved in adherence.

**Evaluation of foreseeable effects**

The GMO is an attenuated autrophic E. coli strain, considered equivalent to ACDP hazard group 1. The organism will have limited survivability in the environment and as such poses a low risk to human health, animals and plants and the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste will be sterilised in a designated waste autoclave as per the published guidelines stated in the SOP entitled "The Welsh School of Pharmacy Procedures for the Use of Horizontal Autoclaves for Sterilisation and Discard".

All work surfaces will be wiped clean with 10% hypochlorite solution, following a 10 minute contact time the surfaces are washed with water to remove any toxic residues at the end of each experiment and at the end of the working day.

**Is an emergency plan required according to regulation 20?**  

| N |

If yes, tick to confirm that it is attached to this form  

| N |

**Tick to confirm that you have attached a risk assessment to this form**  

| Y |

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  

| N |

**Please enter comments on the GM safety committee on the risk assessment**

The committee queried whether or not the initial cloning of the non toxic fragments of Toxin A and Toxin B would be carried out in Cardiff and have been assured that it will not. References were also requested to show that the C terminal domain has no toxic effect. The Committee were then satisfied that the risk assessment was suitable and sufficient and agreed with the classification of the activity.
**Project Additional Information**

**Purposes of the contained use**

Parkinson's disease is a neurodegenerative disease characterised by the loss of specific dopamine neurons in the brain and the accumulation of proteinous inclusions known as Lewy bodies. In idiopathic Parkinson's disease these inclusions contain a protein called alpha-synuclein. Some genetic forms of Parkinson's disease are caused by mutations of the alpha-synuclein protein. Transgenic animal models expressing mutant alpha-synuclein do not show extensive neuronal loss or motor deficits. However, work by our collaborators in Sweden has demonstrated that administration of the recombinant adeno-associated viral (rAAV) vector containing human wild-type or mutant alpha-synuclein is toxic to the specific dopaminergic neurons in the brain affected in Parkinson's disease. We intend to use rAAV vectors to express human wildtype and mutant alpha-synuclein to create a rodent model of the degeneration observed in Parkinson's disease. The rAAV vectors will be stereotaxically injected into the brains of anaesthetised animals. We will inject the rAAV into selected areas of the basal ganglia, or associated brain regions to examine influences on motor and cognitive
function. This will enable us to identify the role that alpha-synuclein may play in synaptic plasticity and its relationship to the symptoms of Parkinson's disease. In later stages of the study we will determine the efficacy of transplantation of foetal cells to repair this damage and restore function and whether the presence of alpha-synuclein is detrimental to the development of the graft.

Recipient or parental organism

Recipients of rAAV vectors will be rats and mice. During a stereotactic surgery the vector will be injected directly into the target areas in the brain. The injections sites will be in cortical areas and within the Basal Ganglia. The virus is replication incompetent and the small titres (µl volumes) of virus transferred to the brain have no risk of excretion in the urine or faeces of injected animals.

Host/vector system

Vector system: rAAV5, rAAV6. The vectors express the transgene from either a hybrid promoter consisting of an enhancer element from the cytomegalovirus promoter, followed by the chicken b-actin promoter containing a rabbit b-globin intron, termed CBA, or the human synapsin promoter.

The functional recombinant vectors contain the coding sequence for the human wildtype or mutant (A53T, S129D, S129A and S129G) alpha-synuclein gene under the control of the promoter and the human bovine growth hormone poly A site flanked by inverted terminal repeats (ITRs). In addition, matching vectors encoding for GFP marker proteins will be used as controls.

rAAV vectors are replication incompetent (only 4% of the original DNA is left in the rAAV) and the safety of rAAV therapy has been approved by 3 clinical trials which have been completed and published (for review see Bjorklund and Kirik, Biochimica et Biophysica Acta (2009) 703-713; see also references therein).

Origin & function

The production of the rAAV vector will be carried out by our collaborators in at Lund University in Sweden. Prof. Anders Bjorklund and Prof. Deniz Kirik will provide us with a working solution of the rAAV vectors that can be used directly without any further processing.

Even though the rAAV vector is replication incompetent, the vector solution is kept free from adeno helper viruses. The production in Lund follows the so-called helper-free method, which is based on the adenovirus-free transient transfection of all elements that are required for rAAV production in host cells such as HEK293 cells (for further review of rAAV vectors and their production, please see Goncalves Manicure, Virol J. 2005 May 6;2:43).

Evaluation of foreseeable effects

The rAAV vector only contains the inverted terminal repeats (ITRs) from the original virus which make up only 4% of the total DNA. It is replication incompetent even in the presence of the adeno helper virus. The host organism will be a rodent. There will be no transmission from rodent tissues to other organisms (the rAAV vector will be injected directly into the brain). The virus is able to infect cells it comes into contact with, although human transduction efficiency is low.

It is known that overexpression of either wildtype or mutant human alpha-synuclein in the substantia nigra of rodents or non-human primates will lead to the generation of protein inclusions, which closely resemble the Lewy bodies formed in Parkinson's disease. This can lead to a progressive cell death and motor impairments. We intend to use this property of the rAAV vector to generate animal models of disease pathology. Alpha-synuclein is a naturally expressed protein whose function in the human brain is unclear. However, rare genetic mutations of this protein are strongly implicated in the development of familial Parkinson's disease. Although not scientifically determined, it is highly unlikely that these rAAV vectors could cause harm to human health. The rAAV infects human cells with very low transduction efficiency and the wildtype AAV does not cause disease in immunocompetent humans. Toxicity of the rAAV-alpha-synuclein has only been reported following direct administration of high titres into the central nervous system in animals and even in this context, only certain populations of cells appear vulnerable to its effects (e.g. the dopaminergic neurons of the ventral tegmental area are relatively resistant whilst cells in the substantia nigra are significantly affected by exposure to the viral vector). There is no evidence to suggest that wildtype or mutant alpha-synuclein is harmful if it comes in to contact with the skin or blood stream (in the case of a needle stick).

To further minimise any risks, Dr E L was trained by our experienced collaborators at Lund University in how to handle the rAAV vector and how to conduct stereotactic
surgeries with them. All future users of the facility need to be instructed in all safety regulations etc. by the chief animal technician (currently Veronica Walter) and will follow standard operating procedures.

Needle stick - the use of sharps and administration to animals through a glass capillary opens up the possibility of transmission through needle stick. Training from Swedish collaborators on how to work with the vector and two layers of gloves will minimise the risk of a needle stick.

Inhalation - opening a vial or spillage of the virus could cause an aerosol of viral particles although the risk is extremely low. All procedures requiring an open container (loading the injection syringe) will be carried out in an appropriate safety cabinet in our existing and designated virus facility. The viral vector could be transmitted through contact with the skin and eyes. These risks will be minimised following the standard operating procedure of the virus suite. Although not scientifically proven we believe that - as wildtype alpha-synuclein is normally expressed only in neurons - this contamination is unlikely to pose a health risk. Nevertheless, all precautions will be taken to avoid such an event, including minimal use of sharps and two layers of surgical gloves. rAAV vectors do not cross the blood brain barrier, therefore they do not pose a risk of transmission to the central nervous system if the exposure is peripheral.

Rodent hosts will be anaesthetised in a separate area in the virus surgical theatre. The surgical tools used for the surgery will be a dedicated set of surgical instruments that will not leave the room and sterilised therein. The surgical procedure will be performed and the animal then returned to recover in a recovery cage. Transmission through the rodent host will not occur as there is no viral replication and therefore no excretion of the viral procedure. The rAAV vector is in the brain and cannot be excreted from there. Adherence to all standard operating procedures of the facility will ensure no transmission outside of the viral suite.

The viral suite will have special colour lab coats which will not leave the room. Special shoes will be provided and a designated changing area will be marked at the entrance of the viral suite.

Standard operating procedures and emergency instructions will be clearly posted to the walls on laminated paper.

The viral suite will have special colour lab coats which will not leave the room. Special shoes will be provided and a designated changing area will be marked at the entrance of the viral suite.

Super infection with rAAVs of individuals already carrying an adenovirus is unlikely to be an additional risk factor. rAAVs are replication incompetent (only 4% of the original DNA is left in the rAAV) and the safety of rAAV therapy has been approved by 3 clinical trials which have been completed and published (for review see Björklund and Kirik, Biochimica et Biophysica Acta (2009) 703-713; see also references therein).

The virus is replication incompetent and the small titres (µl volumes) of virus transferred to the brain have no risk of excretion in the urine or faeces. The only risks are from aerosols (prevented by syringe loading in a safety cabinet) and with sharps. Use of sharps will be minimal and risk reduced by using blunt ended syringes and double layers of gloves.

Once with the vector is injected into the rats' brain, the animals do not require a level 2 housing facility and do not cause health and safety risks.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The virus is replication incompetent and the small titres (µl volumes) of virus transferred to the brain have no risk of excretion in the urine or faeces of injected animals (bedding in the holding rooms can be regularly disposed).

The only risks of virus exposure are from aerosols (prevented by syringe loading in a safety cabinet) and plastic/sharps/paper waste.

Use of 1% Virkon (contains a blend of inorganic peroxygen compound, inorganic salts, organic acid and anionic detergent). It has been proven highly effective to kill over 65 strains of virus in over 19 viral families.

Sharps: thorough spraying with 1% Virkon solution before desposition into clinical waste sharps bins for disposal as clinical waste (ready for incineration).

Plastic/paper waste: thorough spraying with 1% Virkon solution before deposition into specially marked biohazard bags and placing into containers for incineration. Pipette tips used for handling of solutions will be disposed into plastic screw bottles. The bottle will be disinfected on the outside and transferred into appropriate container for incineration.

Liquid waste will be collected inside designated bottles containing 10% Virkon and disposed for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Our application was accepted with 2 minor revisions which have already been corrected. The risk assessment application has now been fully approved (9th of July 2009).

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
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</table>

Animal Units

| L2 L3 L4 L2 | L3 L4 L2 L3 | L3 L4 L2 L3 |

Large Scale Activities

| L2 L3 L4 L2 | L3 L4 L2 L3 | L3 L4 L2 L3 |

Human Clinical Applications

| L2 L3 L4 L2 | L3 L4 L2 L3 | L3 L4 L2 L3 |

Project Ref 130/10.2

Date Ackn'd CU2 Project Title

02/03/2022
The purpose of the project is to investigate mammalian cell function by manipulating proteins involved in cell signalling and gene regulation. The particular focus is on the genetic modification of cells of the immune system particularly primary leukocytes and lymphocytes. We are interested in molecules found in all parts of the cell, for example cell receptors, kinases and transcription factors and some soluble factors.

Two approaches will be used to alter leukocyte function. Firstly, the manipulation of proteins by the expression of those proteins, or mutants thereof, in cells. This will allow us to study molecular function. Secondly, the reduction in the expression of specific mammalian genes using small interfering RNAs. So called third generation viral vectors will be used to introduce DNA encoding proteins or shRNAs into mammalian cells. For example, to enable analysis of the human and mouse genomes the RNAi Consortium has developed lentiviral shRNA libraries targeting each of the 22,000 human and mouse genes. Lentiviral vectors currently offer the most efficient means for stably introducing genetic material into quiescent cells.

This programme of work will involve the generation of a variety of genetically modified organisms.

Firstly, preliminary manipulations and amplification of DNA will be performed in disabled E.coli strains (e.g. DH5α).

Recombinant lentivirus will be generated in helper-free cell lines (for example, 293T cells).

These viruses will then be used to infect cell lines in culture and will be used to infect primary human and mouse cells (for example: macrophages, antigen presenting cells, B and T cells, fibroblasts and haematopoietic cells).

Finally, the viruses will be used to infect mice.

Purposes of the contained use

Recipient or parental organism

This programme of work will involve the generation of a variety of genetically modified organisms.

Firstly, preliminary manipulations and amplification of DNA will be performed in disabled E.coli strains (e.g. DH5α).

Recombinant lentivirus will be generated in helper-free cell lines (for example, 293T cells).

These viruses will then be used to infect cell lines in culture and will be used to infect primary human and mouse cells (for example: macrophages, antigen presenting cells, B and T cells, fibroblasts and haematopoietic cells).

Finally, the viruses will be used to infect mice.

Host/vector system

Preliminary vector manipulations will be performed in disabled E.coli strains (e.g. DH5α).
The resulting plasmids will be transiently transfected to generate HIV based lentiviruses.

**Origin & function**

This programme of work will generate genetically modified lentiviruses in order to study primary human leucocytes. Viruses containing different genetic material will be generated.

The GMOs that will be generated are disabled. The viral vectors that will be used, for example pLKO-1 or PCMV::R8.91, cannot express any viral proteins. All of the regulatory/accessory genes of HIV-1 have been removed, specifically Nef, Vpr, Vpu, Vif and Tat that are critical for viral replication and pathogenesis. The proteins required for generating virus, which does not include Nef, Vpr, Vpu or Vif, are instead supplied separately on another plasmid(s).

Furthermore, the vector is self inactivating. Self inactivation results from the introduction of a deletion in the U3 region of the 3' long terminal repeat (LTR) of the viral vector used to produce the vector RNA. During reverse transcriptase, this deletion is transferred to the 5' LTR of the proviral DNA and transcriptional activity of the LTR is lost. This reduces the likelihood that replication-competent retrovirus can originate in the vector producer or target cells.

In terms of inserts, it is important to recognise that some of these proto-oncogenes can lead to tumour formation when expressed in transgenic mice or in rodent cells. This demonstrates that they can contribute to cancer and so should be seen as having the potential to be a risk to human health.

However, it is important to note that no one gene has been shown to be sufficient to transform human cells (Hahan and Weinberg "Rules for making human tumour cells" (2002) New England Journal of Medicine, 347 (20), pp. 1593-1603). These authors published a report that shows that introduction of three individual genes was required to transform primary human epithelial or fibroblast cells (Hahan et al, Nature (199) 464-468). Historically, the assays for transformation are usually assessed from non-human cell types, for example chicken or mouse cells either in vitro or in vivo. Human cancer is now accepted to be a multi-step event due to the accumulation of multiple genetic alterations. Thus, it is extremely unlikely that any of the viruses proposed could transform human cells alone.

**Special considerations for work:**

I) Introduction of any proto-oncogene into a lentiviral vector and accidental administration to a worker would only represent a 'single hit' in a cascade and none of the proposed genes of study have been shown to be sufficient alone to transform HUMAN cells.

II) At any one time workers will work with only one proto-oncogene containing lentiviral vector. Experimental cells may be sequentially modified, but individual viral vectors will be handled in isolation to prevent accidents involving 'multiple hits'.

III) When working with human inserts in lentiviral vectors the use of sharps is strictly forbidden.

IV) Human skin has many properties as a protective barrier. However, care should be taken for researchers in which skin is compromised for whatever reason. This includes those with open wounds or those with skin problems. If the wound or problem cannot be adequately covered or protected then consideration should be given to delaying the work.

V) When infecting mice with lentiviral vectors:

- Needles are required, however lentiviral vectors with human sequences will not be used. This ensures that should accidental needle stick injury occur the viral insert sequences are foreign sequences that should be recognised by the immune system.

- The procedure rooms will be isolated from other workers to prevent accidental 'crowding' interference of others increasing the chances of needle-stick injury, which are minimised by good handling practice.

VI) Below we have specifically considered the risks to human health associated with the following 'highest-risk' genes as models of 'worst case' scenarios:

**XAP-70**

Tyrosine kinases were the first identified oncogenes. Viral forms of the proteins were shown to be involved in the growth of tumours in chickens. However, it has not been possible to derive a transformed human fibroblast cell line with any tyrosine kinase, despite the importance of these molecules, for example c-SRC, to numerous human
cancers. ZAP-70 in particular is overexpressed in cells from some patients with chronic lymphocytic leukaemia. However, the functional role of the molecule is poorly understood and it may be a marker of poor prognosis rather than an oncogenic molecule. Thus it would seem essential for this molecule to be expressed with many others to become a risk to humans.

IKK
The serine kinase, IKK, lies upstream of the NF-κB pathway which plays an important role in inflammation and cell survival. This pathway is active in some cancers, including chronic lymphocytic leukaemia. However, IKK is unable to transform cells alone. Furthermore activation of this pathway through IKK would likely cause the activation of an inflammatory response that would be likely to clear the virus and any cells infected with the virus.

Tert
While not a bona fida oncogene, Tert prevents senescence of human cells and is one of the molecules necessary to allow human cell transformation. It can cause cell immortalisation. However, by itself, it cannot cause cancer. This molecule is the focus of research within the School of Medicine and a retrovirus expressing this gene is used. The ability of lentivirus to enter non-dividing cells, the key difference between lentivirus and retrovirus, would be unlikely to provide an extra risk for Tert as Tert activity would not alter a non-dividing cell.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
The GM mice will be contained in a modern animal facility providing a specific pathogen free environment and a series of well tested physical barriers to prevent the escape of the animals to the environment and allow the routine disposal of animal corpses. Class 2 procedures are performed in a dedicated area with biological safety cabinets and mice subsequently housed in filtered cages.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Recombinant viruses will at all times be maintained in an environment designed to prevent aerosol spread. All flasks containing the virus shall be kept closed except when decanting and pipetting which will be conducted in tested class II safety cabinets. Centrifugation steps shall be performed in sealed buckets. The use of Sharps when handling virus preparations will not be permitted. Contaminated pipettes will be immersed in Actichlor, or a similar chlorine based disinfectant, for a minimum of 4hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with 70% ethanol according to an approved protocol. Access to laboratories employed for virus work will be restricted. Recombinant viruses that will be injected into mice cannot replicate and thus virus should not escape into the environment from these mice. Thus, bedding and feed can be disposed of as normal. The only risk is escape from the injection site and this will be controlled by treatment of the injection site with antiviral agents as described in the risk assessment. Carcasses of mice injected with recombinant virus will be labelled, packaged and carried appropriately offsite for incineration. These mice should not contain any live genetically modified organisms. The waste contractors are registered to accept this waste.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
The Genetic Modification Safety Committee had reservations which have been completely satisfied by the provision of further information and updating of the risk assessment.

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**Project Ref 130/10.3**

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<td>Expression of Selected MCV genes and cellular gene products affected by MCV infection in mammalian cells using lentiviral expression vectors</td>
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Withdrawn N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The purpose of the contained use are the functional analysis of MCV genes in isolation, development of a MCV genes in isolation, development of a MCV cell culture system for the analysis of innate and T cell immunity directed against MCV and the testing of antiviral substances active against MCV in this system.

The project is a collaboration between the PI and GW and BM both of Cardiff University School of Medicine, Wales, UK, FM, INRA Toulouse, France, BM, NIH, Bethesda.
The project is a connected programme of work to accommodate the variety of gene expression systems necessary to study different aspects of MCV infection.

**Recipient or parental organism**

Parental organism to most all proposed GMMs is the Molluscum contagiosum virus (MCV). MCV DNA is stored in a MCV overlapping and redundant genome fragment library available in the Pis lab and submitted to ATCC for distribution in 2009 (Bugert J. (2008) Encyclopedia of virology: molluscum contagiosum virus. 3rd ed. Oxford Elsevier. Pp319-324). MCV genes are prepared in isolation for insertion into the Host/vector system by PCR. Homologs of the MCV mc026 gene from Squirrelpox virus (SPV; gift of CM, Moredun Research Institute, Scotland) will be used in comparative functional studies.

Cellular gene products found to have molecular interactions with MCV gene products, incl. genes of the ESCRT pathway (mc162), will be expressed for functional studies.

**Host/vector system**

MCV 1.1 Maintenance of the MCV gene fragment library, cloning of MCV wildtype genome fragments, and expression of MCV gene products in E. coli: bacterial plasmid hosts are pAT153 and pACYC184; bacterial expression vectors will include pET and the pGEX derived plasmids.

MCV 1.2 Expression of selected MCV genes in mammalian cells using eucaryotic plasmid expression vectors: eucaryotic plasmid expression vectors will include the pIRESneo, pIRES-EGFP, and pcDNA3 derived plasmids.

MCV 1.3 Expression of selected MCV genes in mammalian cells using replication incompetent adenoviral expression vectors: adenoviral expression systems will include the AdX (BD Clontech), and AdEasy (Stratagene) and locally developed derivatives (G W).

MCV 1.4 Expression of selected MCV genes in mammalian cells using vaccinia virus expression vectors: VACV viruses will include the WR and the MVA strains of vaccinia virus, both obtained from Dr B M, NIH. NIAID, LVD, Bethesda, Maryland.

MCV 1.5 Expression of selected MCV genes in mammalian cells using replication incompetent retroviral expression vectors: retroviral expression systems will need to infect resting cells and use the lentiviral system (LentiShuttle - Gruh et al., Leibniz Research Laboratories, Hannover, Germany, BioTechniques, Vol. 38, No 4, April 2005, pp. 530-534) already approved for use in other labs of the Cardiff University School of Medicine.

MCV 1.6 Expression of selected MCV genes in insect cells using baculovirus expression vectors: baculovirus expression systems use the pMEL Bac A, B, C donor system for secretion of the transgene via the honeybee melittin secretion signal sequence (Bac-to-Bac Baculovirus Expression system, Invitrogen).

MCV 1.7 Cloning of selected MCV genes into Shope Fibroma Virus (SFV) donor plasmids; the SFV donor plasmid is a gift from FM, INRA Toulouse, for expression of MCV genes via recombinant SFV and while recombinant virus production and animal experiments will take place in Toulouse.

MCV 1.8 Cloning of selected MCV genes into Fowlpoxvirus (FPV) donor plasmids: expression of MCV gene in FPV in a collaboration with DrM S, Imperial College, London. Here only the plasmid construct will be made in Cardiff (L1), while recombinant virus production and animal experiments will take place in London.

MCV 1.9 Expression of cellular gene products affected by MCV infection in mammalian cells using eucaryotic plasmid expression vectors: eucaryotic plasmid expression vectors will include the pIRESneo, pIRES-EGFP, and pcDNA3 derived plasmids.

MCV 1.10 Expression of cellular gene products affected by MVV infection in insect cells using baculovirus expression vectors: cellular genes will be expressed using the pMEL Bac A, B, C donor system for secretion of the transgene via the honeybee melittin secretion signal sequence (Bac-to-Bac Baculovirus Expression system, Invitrogen).

MCV1.11 Expression of poxviral homologs of MCV genes in E. coli and eucaryotic cells: as in MCV 1-1 and 2.

**Origin & function**


Homologs of the MCV mc026 gene from Squirrelpox virus (SPV; gift of Dr C M, Moredun Research Institute, Scotland) will be used in comparative functional studies.

Cellular gene products found to have molecular interactions with MCV gene products, incl. genes of the ESCRT pathway (mc162), will be expressed for functional studies.
Evaluation of foreseeable effects

Forseeable effects and risk determined by the nature of MCV gene inserts:

MCV genes will be overexpressed in a number of viral and non-viral expression systems and purified to raise gene specific antibodies, establish an MCV ELISA for seroepidemiology and to investigate function and MCV specific T cell immunity.

Most MCV genes (89%) are MCV structural proteins with high homology to other poxviral genes and no known effects on cell cycle or host immunity.

Of the MCV nonstructural proteins to be expressed (initially mc002, mc007, mc080, mc161 and mc162 (Slam homology family), most have no known effects on cell cycle or host immunity.

Our worst case risk assessment will focus on mc007, which has been shown to bind Tb protein and to interfere weakly with the host cell cycle in a non-poxviral expression system (Mohr et al, Jvi (2008) 82: 10625-10633) and mc080 a MCV MHC class I homolog (Moss B et al., Trends Microbiol. (2000) 8:473-477).

Worst case scenarios:

MC007: MC007L gene of human pathogenic MCV encodes a mitochondrial outer membrane (MOM) protein that targets pRb and E2F-1 via a conserved LxCxE motif, which is present in many viral oncoproteins. This oncoprotein induced deregulation of the pRb pathways plays a central role in tumour pathogenesis. Through the LxCxE motifs, MC007L induces a cytosolic sequestration of pRb at mitochondrial membranes, leading to the inactivation of the protein by mislocalization. The disruption of the pRb/E2F protein complex leads to cell proliferation, induction of apoptosis, and cellular transformation (Mohr et al., 2008). Thus the interaction between MC007L and pRb provides a mechanism by which a virus can perturb the cell cycle.

MC080R: The main function of MHC class I molecules is the cell surface presentation of peptides, derived from foreign antigens, for recognition by the immune system. The presence of MHC class I molecules on the surface of cells inhibits NK-cell activation and lysis. The MC080 gene of MCV encodes a glycoprotein that is homologous to the mammalian MHC class I heavy chains. MC080 has a very long signal peptide (69 amino acids) and there is a 24.5% overall amino acid identity between MC080 and the human MHC-1 protein (Bugert et al. 2000). The MCV MHC-1 homologue thus clearly has the potential to inhibit NK recognition of MCV-infected cells by a wide range of mechanisms. NK cell cytotoxicity is controlled by a fine balance between activating and inhibitory signals, the latter generally over-riding the former. Both activating and inhibitory NOK receptors can bind HLA class I molecules. There are several known viral MHC-1 homologs, mostly in herpesviruses, and some of them have already been shown to be inhibitors of natural killer cells (Tomasec et al., 2000).

REFERENCES:

Targeting the Retinoblastoma Protein by MC007L, Gene Product of the Molluscum Contagiosum Virus: Detection of a Novel Virus-Cell Interaction by a Member of the Poxviruses. Journal of virology 82: 10625-10633.

During construction of GMM and gene expression using pro- and eukaryotic plasmid vectors:

Naked viral nucleic acid could be transmitted when using pro- and eukaryotic plasmid expression vectors and during construction of the recombinant viral genomes (via donor plasmid)

Medium risk

Adenoviral and lentiviral donor plasmids use herpes and retroviral transcription control sequences which can be recognized by the human host. The plasmids can be
expressed and the risk is comparable to the risk of the respective recombinant virus GMMs.

Very low risk:
Plasmids with procaryotic transcription signals cannot express transgenes in human cells they could Donor plasmids of baculovirus employ baculoviral transcription control elements which are not recognized in human host. In the case of poxviral donor plasmids, poxviral promoters are not recognized by the eucaryotic transcription machinery. All poxviral nucleic acids will be transcriptionally silent if accidentally transferred into the human host.

Risk of viral vectors used:
Three of the six viral expression systems proposed can cause pathology could cause pathology in the human host independent of transgene expression. The expression pattern in a range of human tissues will depend on the individual transmission and replication characteristics of the each viral expression system.

Adenovirus: can infect and replicate in a wide range of human cells, mostly via the respiratory and faecal oral routes. Wild type Adenovirus infection varies in a clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response. Wild type virus is spread directly by oral contact and droplet spread; indirectly by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person. In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection.

Baculovirus; cannot replicate in human host cells. No known pathology in humans.

Fowlpoxvirus: cannot replicate in human cells efficiently. No known pathology in humans.

Lentivirus: can infect and replicate in a wide range of dividing and resting human cells via all known transmission routes. Considering that up to 30% of the human genome is of retroviral origin, any pathological effects of the unmodified lentiviral vector would be negligible in comparison to ongoing endogenous retroviral activity in the human host (HERVs) Integration of the transgene cassette in the host genome is a deliberate effect when using lentiviral constructs. Accidental infection of a human host would likely result in integration of transgenes into host cell genomes. The outcome would be determined by the physiological/pathological effect of the transgene.

Shope Fibroma virus: cannot replicate in human host cells efficiently. No known pathology in humans.

Vaccinia virus: can infect and replicate in a wide range of human cells. Known routes for vaccinia infections are the skin, through smear infection, and the conjunctivae, through aerosols and messy accidents. The ability of attenuated vaccinia viruses to induce fever, inflammation and death in the human host is much reduced. The most at-risk-individuals are those with a defective immune system. In this case replication competent vaccinia viruses can induce eczema vaccinatum, an extensive skin condition, with a smallpox-like rash. Eczema vaccinatum encephalitis. This condition has been observed in one in a million vaccinees, when vaccination was still widely practised. Vaccinia encephalitis can be lethal, the severity of the condition is, however, tightly linked to the genetic predisposition fo the individual, eg, tendency to develop overwhelming autoimmune responses.

The ultimate compound risk of the various GMMs will be determined by the risk of the MCV viral insert. This is discussed for two examples at the beginning of this section.

The safe containment of the GMM and the prevention of transmission are the most important goals in risk prevention. The most important precautions are therefore use of class II safety cabinets in L2 laboratories, effective disinfection and destruction of waste with chemicals and use of autoclaves, complete personal protective gear in the lab, including goggles (mandatory), and to avoid the generation of viral aerosols

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**Not applicable**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
All laboratory work involving recombinant viruses will be carried out in L2 containment level labs. All personnel will be trained in recognising virus infections, awareness of human-to-human transmission, and the increased risks for individuals suffering from immune disorders (e.g., atopic dermatitis, severe acne, empetigo, contact dermatitis, psoriasis, chickenpox, steroid medication, pregnancy, infection with other pathogens). The exposure route via the conjunctiva will be particularly stressed and the use of adequate protective gear enforced in the lab. All personnel will be monitored by the Occupational Health Service at the School of Medicine.

Laboratory work will be carried out in class II safety cabinets, with personnel wearing gloves. Disposable plastic pipettes and pipette tips will be used. Recombinant viruses will be used under conditions avoiding the generation of aerosols. Flasks containing virus will be kept sealed outside of the safety cabinets. Centrifugation will be carried out in sealed buckets, that will only be opened in safety cabinets. All waste materials will be collected in plastic bags in the safety cabinets, the bags will be sealed inside and then autoclaved. No sharps will be used.

Removable equipment and interior surfaces of the safety cabinets will be sprayed with Incidin® PLUS (Ecolab, Swindon UK) containing the patented active substance Glucoprotamine with wide effect spectrum against bacteria (also MRSA and Tbc), fungi, papova-, adeno-, vaccinia-, herpes-, rota- and hepatitis-B-viruses (HBV) in MADT and antigen test, as well as AIDS agent (HIV)-certified by the German Society for Hygiene and Microbiology (DGHM). Spillage of GM material will be treated with hypochloride.

For the quality assurance of the disinfection and autoclaving procedures, virus contaminated Incidin disinfected and belached plasticware, as well as autoclaved waste material will be rinsed with a small volume of PBS and tested for rest-infectivity on appropriate cells in a standard virus detection assay.

The Cardiff University subcommittee recommended the attached risk assessment for use of recombinant vaccinia viruses in this connected program of work as exemplary.

The committee requested the PIs to elaborate on the differences between the WR and the MVA strains of vaccinia virus and the relative safety aspects, when potentially oncogenic proteins are expressed. The risk was deemed higher with the replication incompetent (in human cells) MVA, because of the longer stay-on time in infected tissues of the recombinant virus, but still not exceeding Class-2.

Furthermore regarding laboratory access, the subcommittee took note that Dr B’s virus laboratory 1TB115 is located in a NPHS secure area and immune incompetent personnel will be excluded from the access to the laboratory.

Dr R C., BSA Cardiff University, received no more adverse comments from the Cardiff University GM committee.

The GM committee recommended to submit a notification as Class 2 for the Connected program of work and attach the MCV/vaccinia virus risk assessment (MCV 1-4).
Project Ref 130/10.4

Date Ackn'd 20/07/2010

CU2 Project Title Investigation of potential therapeutic targets in myeloid leukaemia

Class 2
CultureVolClass 1-50 Litres

Non-GMM
Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Use of lentiviral gene transfer to identify new therapeutic targets in acute myeloid leukaemia

Recipient or parental organism
Replication-defective lentivirus

Host/vector system
The following expression vectors will be employed: encoding both lentiviral expression vectors and (separate) plasmids for packaging of replication-defective virus

pLenti6/TR
The vector system to be used is a third generation HIV-1 based lentiviral system, with a number of safety features designed to enhance biosafety [J. Szulc, et al., Nat. methods 3 (2): 109-116, 2006]. Distinct packaging vectors are used, none contain the psi or LTR signals. The expression vector 3'-LTR contains a deletion (deltaU3) rendering it "self-inactivating" and thus incapable of packaged virus production following transduction into the target cell. Pseudotyping is implemented with VSV-G replacing the HIV-1 envelope gene.

Evaluation of foreseeable effects

The major safety concern is the possibility of generating of replication competent virus. The vector system employed incorporates multiple safeguards to make the risk of this extremely low (see risk assessment).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Will liquid waste be autoclaved disinfected and then autoclaved

Disinfection:

Product name Actichlor
Generic Chemical Name: NaDCC hypochlorite
Expected degree of kill 1000ppm hypochlorite
How validated commercial source

Solid waste to be autoclaved and disposed of as clinical waste

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The committee had a number of queries and requests for further information, including the higher risk involving pseudotyping with the VSV-g gene. Alterations were made to the Risk Assessment which satisfied the GMSC.

**Project Containment**

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**Project Ref** 130/11.1

- **Date Ackn’d**: 26/08/2011
- **CU2 Project Title**: Optogenetic stimulation and recording of thalamocortical cellular and network activity in vivo and in vitro using viral-mediated light-sensitive channel expression
- **Class**: Class 2
- **Culture Vol**: < 1 Litre
- **Non-GMM Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**
- **Historical Date of Additional Info**: 
- **Significant Change ID**: 
- **Date of Significant Change**: 

**Project Additional Information**

**Purposes of the contained use**

Thalamocortical (TC) neurons are characterised by state-dependent firing properties. During quiet wakefulness they exhibit tonic action potential firing, whereas during non-rapid eye movement sleep they fire high-frequency bursts of action potentials riding on low-threshold Ca2+ potentials (LTCP). These LTCPs are generated by T-type...
Ca2+ channel activation and contribute to the emergence of sleep slow oscillations (<1Hz) and delta waves (1-4Hz) in thalamocortical territories. In addition to their physiological roles in sleep, T-type Ca2+ channels have been implicated in a number of pathological brain states, including absence seizures. These are characterised by a brief loss of consciousness and bilaterally synchronised 3Hz ‘spike and wave’ discharges (SWDs) occurring spontaneously in the EEG, in depth EEG recordings and in thalamic areas. Though SWDs had been suggested to be generated by dampened inhibitory (GABAergic) signalling, it has recently been shown that GABA re-uptake mechanisms are disrupted in thalamic astrocytes resulting in excessive inhibition. To further our understanding of the cellular mechanisms underlying thalamocortical oscillations of physiological and pathological states, we now need to selectively manipulate cellular and neuronal network activity using recombinant adeno-associated viral (rAAV) and lentiviral (LTV) vectors to express microbial rhodopsins (light-sensitive cation or chloride channels; 'Opsins') or engineered light-activated ligand-gated neurotransmitter channels ('LALGNCs') under cell type specific promoters. Laser light stimulation of opsins and LALGNCs offers unprecedented temporal (on a millisecond timescale) and spatial (over mm of tissue) control over the activity of discrete cell populations, and will allow us to selectively manipulate and record cellular and neuronal network activity in vitro and in vivo.

Microliter volumes of high titer virus (expressing an opsin or LALGNC) will be targeted to thalamic territories and associated cortico-limbic areas for in vivo and in vitro studies of cellular and neuronal network activity

**Origin & function**

Viruses will be produced on our behalf by a commercial Vector Core in the United States using plasmids originating from the Karl Deisseroth laboratory, available from AddGene.

rAAV and LTV plasmids will contain transgenes encoding for microbial opsins (light-activated cation channels and chloride pumps) or LALGNCs. Opsi and LALGNC transgenes will be fused with genes encoding for fluorescent proteins (eYFP or m-cherry). Fusion opsin-fluorescence transgenes will be driven by exogenous (i.e. not wildtype HIV-1) constitutive or cell-type specific promoters.

Control rAAV or LTV vectors will not carry opsin, LALGNC or optical sensor transgenes. Rather, they will encode for fluorescent proteins (i.e. eYFP or m-cherry), or scrambled DNA sequences fused to a fluorescent protein.

All rAAV vectors (pAAV-MCS) will comprise (reading sense 5' to 3'): an exogenous promoter (i.e. not from wildtype AAV2), the transgene of interest (fused to a fluorescent protein), woodchuck hepatitis B virus posttranscriptional regulatory element (WPRE; used to enhance transgene expression) and polyA sites, flanked by AAV2 ITRs. rAAV vectors will be serotyped with AAV1, 2 or 5 coat proteins.

All LTV vectors (pLECYT) will comprise (reading sense 5' to 3'): HIV-1 Psi+ element, HIV-1 Rev-response element, an exogenous promoter (i.e. not from wildtype HIV-1), HIV-1 cPPT, the transgene of interest (fused to a fluorescent protein) and WPRE, flanked by HIV-1 3' truncated long-terminal repeats (LTRs). LTV vectors will be
Evaluation of foreseeable effects

Viral vector characteristics:
The rAAV (pAAV-MCS) and LTV (pLECYT and pFCK) vectors nominated for use in this study are replication incompetent and will be used in small (µl) volumes. Neither vector type contains pathogenic wildtype viral genes, or those relevant for replication. The vectors contain only those wildtype genes necessary for production of viral core structural proteins, genome packaging and RNA transcription and packaging. The risk of reversion to wildtype is considered minimal as all pathogenic genes have been deleted in pAAV-MCS and VSV-G pseudotyped LTV vectors. The AAV2 ITRs in the pAAV-MCS vector do not share any regions of homology with viral rep/cap-gene containing plasmids, thereby preventing the production of wildtype AAV2 through recombination. Since the majority of wildtype genes (most notably the promoter and enhancer sequences contained within HIV-1 3’ LTRs) have been deleted in pLECYT and pFCK LTV vectors, any risk of recreating replication competent wildtype HIV-1 or wildtype-like viruses by recombination is excluded (see Sliva K and Schnierle B (2010) Selective gene silencing by viral delivery of short hairpin RNA. Virol J, (7): 248).

pAAV-MCS vectors (derived from wildtype AAV2) will be serotyped with AAV1, 5 or 7 coat proteins, altering the tropism of the rAAV vectors compared with wildtype AAV2 (specifically, increasing their tissue spread). The pLECYT and pFCK vectors are derived from HIV-1. All HIV-1 wildtype envelope proteins will be replaced with the vesicular stomatitis virus G-protein (VSV-G) envelope vector; pseudotyping LTV vectors with VSV-G is expected to broaden tissue tropism compared with the parent virus (i.e. wildtype HIV-1 infects only CD4+ T cells, macrophages and microglial cells, whilst VSV-G LTV vectors can infect a broad spectrum of vertebrate cells; Sliva K and Schnierle B (2010) Selective gene silencing by viral delivery of short hairpin RNA. Virol J, (7): 248).

There will be no oncogenic sequences present in rAAV or LTV vectors. LTV vectors such as wildtype HIV-1 express their genetic material through integration into the host genome (using the viral enzyme Integrate). Consequently, there is typically a risk of insertional mutagenesis or cellular oncogene activation associated with random integration of the LTV vector. However, we feel this risk will be minimal, due to the deletion of wildtype LTV promoter and enhancer sequences within the LTRs of pLECYT and pFCK vectors, rendering them ‘self-inactivating’ (SIN) (see Miyoshi H et al (1998) Development of a self-inactivating lentivirus vector. J Virol, (72): 8150-8157; Zufferey R et al (1998)).


Possible routes of transmission:
Viral vectors could be transmitted to humans through contact with the skin, eyes or airways, or transmission into the blood stream. These risks will be minimised by following the standard operating procedure of the virus suite.

Needle stick (entry into the blood) - the use of sharps provides an opportunity for transmission to humans via needle stick. Needle stick injuries will be minimised by wearing double thickness gloves and changing to fresh, sterile gloves after handling the virus. Although both rAAV and LTV vectors would be able to infect peripheral human cells they come into contact with (i.e. via a needle stick injury), rAAV vectors exhibit low human transduction efficiency and are unable to cross the blood brain barrier (BBB). Furthermore, wildtype AAV is apathogenic in humans, according to current knowledge. Although not scientifically confirmed, given the size of the particles and their VSV-G pseudotype, it is not expected that LTV vectors will pass through the BBB.

Aerosols (absorption through the eyes or inhalation) - opening or spilling a vial of virus could cause an aerosol of viral particles. All procedures involving an open container will be performed behind the screen of an appropriate Class 2 safety cabinet.

Adherence to all standard operating procedures of the virus suite will ensure no transmission outside of the facility, which has designated colour lab coats that do not leave the room. Designated shoes will be worn only in the virus suite, and changing will occur only in selected areas of the room. Standard working procedures and emergency instructions (including office and out-of-hour phone numbers) will be clearly posted on the walls of the virus suite, which will be locked at all times when not in use. The room is clearly labelled as a virus surgery suite and contains a spill kit (containing absorbent paper towels, goggles, masks, gloves and 5% Virkon solution).

We have not encountered any documentation to suggest that rAAV and LTV vectors could recombine with a wildtype organism in the environment. However, transfer of viral vectors to the environment may occur via infected equipment and aerosol production; since rAAV and LTV vectors may exhibit survival in the environment, appropriate protective clothing (masks, gloves and designated lab coats and shoes) and cleaning of the virus suite with 5% Virkon is necessary (refer to Section 12: Waste Management Strategies). rAAV vectors are relatively resistant to temperature changes, but autoclaving is sufficient to kill the virus. Conversely, HIV-1 is fragile and extremely sensitive to even small fluctuations in temperature and the presence of oxygen. There is no evidence to suggest that pLECYT or pFCK vectors exhibit enhanced environmental survival compared with their parent virus HIV-1, though it should be noted that pseudotyping with VSV-G may enhance LTV survival relative to parental HIV-1.
We consider minimal risk to human health associated with exposure to rAAV or LTV vectors since: all pathogenic genes have been deleted; promoter sequences within LTV LTRs have been deleted to minimise the risk of insertional mutagenesis and cellular oncogene activation; wildtype AAV2 is apathogenic in humans and shows low transduction efficiency; rAAV is non-integrating, thereby minimising the risk of insertional mutagenesis; both viral vectors are replication incompetent; neither viral vector is expected to alter human cellular immunity or defence mechanisms; and there are low risks associated with reversion to wildtype, due to a lack of rAAV-ITR homology with viral rep/cap-gene containing plasmids, and LTV 3' LTR truncation.

Furthermore, opsins and LALGNCs have not been associated with human disease, but rather represent useful tools for studying neuronal network activity.

rAAV vectors are relatively resistant to environmental temperature changes, though autoclaving is sufficient to kill the virus. HIV-1 is fragile and extremely sensitive to even small fluctuations in temperature and the presence of oxygen.

Although (1) there is no evidence to suggest that rAAV, pLECYT or pFCK vectors exhibit enhanced environmental survival compared with their parent viruses (though pseudotyping LTV vectors with VSV-G may enhance LTV survival relative to parental HIV-1); (2) experiments will involve only very small (2-4µl) volumes of viral suspension; and (3) rAAV and LTV vectors are replication-incompetent, all in vitro and in vivo experimental procedures involving viruses will be performed in appropriate Activity Class 2 facilities. All virally infected tissue will be contained and treated as Class 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

rAAV vectors are relatively resistant to environmental temperature changes, though autoclaving is sufficient to kill the virus. HIV-1 is fragile and extremely sensitive to even small fluctuations in temperature and the presence of oxygen.

Although (1) there is no evidence to suggest that rAAV, pLECYT or pFCK vectors exhibit enhanced environmental survival compared with their parent viruses (though pseudotyping LTV vectors with VSV-G may enhance LTV survival relative to parental HIV-1); (2) experiments will involve only very small (2-4µl) volumes of viral suspension; and (3) rAAV and LTV vectors are replication-incompetent, all in vitro and in vivo experimental procedures involving viruses will be performed in appropriate Activity Class 2 facilities. All virally infected tissue will be contained and treated as Class 2.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

All infected tissue samples will be double bagged and disposed of as clinical GM waste for incineration. All liquid waste and designated non-disposable equipment (i.e. glassware) will be thoroughly disinfected with 5% Virkon and then autoclaved. Non-biological, non-designated solid waste (i.e. cotton swabs), will be disinfected with 5% Virkon and then disposed of as clinical GM waste. Non-designated disposable sharps (i.e. glass micropipettes and microscope slides) will be disposed of in designated clinical GM waste sharps bins immediately after use. The use of sharps will be minimised. All sharps bins will be sprayed with Virkon inside and out, before being sealed and removed from the viral suite at the end of each batch of experiments. Double thickness gloves will be worn when handling the virus and associated sharps, to minimise the risk of virus to skin contact and needle stick injuries.

Because of these precautions, we do not believe that rAAV or LTV vectors would survive long enough in the environment for gene transfer to occur. Patch-clamp and calcium imaging experiments will require immersion of a microscope lens in perfusion solution that washes over tissue slices; therefore following in vitro experiments, lenses and perfusion lines will be cleaned using 5% Virkon solution. Pulled glass micropipettes used for patch-clamp experiments will be disposed of in designated clinical GM waste sharps bins.

All efforts will be made to contain potential transmission. All work will be performed in appropriate Class 2 facilities (i.e. Level 2 safety cabinets). Any viral spillages will be contained and thoroughly disinfected with 5% Virkon. To further minimise any risks associated with handling tissue injected with rAAV or LTV vectors, gloves, goggles and masks will be worn to minimise skin and eye contact, and inhalation of any potential viral aerosols.
Our application was accepted by Cardiff University's GM Safety Committee with minor revisions, which have already been corrected. The risk assessment application has now been fully approved (August 2011).

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### Project Ref 130/11.2

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<td>Role of neurotrophic factors, cell signalling, microRNA and gene transcription in neuronal development</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Project Additional Information

**Purposes of the contained use**

To enhance expression of selected proteins and miRNA in cultured primary neurons, using the Sindbis Expression System (Invitrogen).

**Recipient or parental organism**

- Primary neuronal culture

**Host/vector system**

- **DH5alpha E. coli/pSinRep5 (Invitrogen, commercial), DH-BB vector (Invitrogen, commercial)**
- **BHK21 cell line/pSinRep5 (Invitrogen, commercial) DH-BB vector (Invitrogen, commercial)**
- Primary culture neurons/viral particles

**Origin & function**

Sequences encoding the following:
- p75 receptor protein (common receptor for neurotrophins)
- super-repressor IκBα protein (inhibitor of NF-κB canonical signalling)
- siRNA to knockdown expression of endogenous p75, members of the Trk family of receptor tyrosine kinases (the other class of receptors for neurotrophins) or the CaSR (a G-protein coupled receptor that senses the level of extracellular free calcium ions).

Sequences of these proteins and miRNA are from Mus musculus

**Evaluation of foreseeable effects**

pSinRep5 is a 9951 bp vector used to generate recombinant RNA molecules for expression in infected eukaryotic cell in culture. It contains the Sindbis virus non-structural protein genes 1-4 (nsP1-4) for replicating RNA transcripts in vivo, the promoter for subgenomic transcription, and a multiple cloning site. The multiple cloning site allows insertion of the microRNA behind the subgenomic promoter for expression in BHK cells. The recombinant RNA transcripts are synthesized using the SP6 promoter and transfected into BHK cells.

DHBB [Defective Helper] deleted between BspM II and BamH I is a 6729 bp DNA template that contains the genes for the four structural proteins required for packaging of the Sindbis viral genome.

Mechanism of replication of the virus:

The vector RNA encoding non-structural proteins 1-4 (pSinRep5) and the transgene under the control of the subgenomic RNA promote, and defective helper RNA (DHBB) are obtained by in vitro transcription. The virus must be cotransfected with defective "helper RNA" to be packaged into infectious particles in BHK-21 cells. Within the cytoplasm of BHK-21 cells, defective helper RNAs have a large deletion in the nonstructural protein genes (i.e., the replicase genes) and are not self-replicating, but can be replicated and transcribed by the nonstructural proteins provided by the replicon. Helper subgenomic RNAs are translated to provide the structural proteins for replicon assembly under conditions in which helper RNAs are not packaged. Upon infection of cultured neurons used in our proposed studies, the packaged replicons derived by this approach will infect the cultured neurons, but the absence of structural protein genes prevents the generation of new particles.

The primary hazards are accidental parenteral inoculation and droplet exposure of the mucous membranes. The particles produced by transfection are replication defective (Bredenbeek et al. (1993)). The components of the Sindbis Expression System have been designed to guard against any potential health threats.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

There are'nt any procedures likely to generate aerosols.
A safety cabinet will be used and there will be no use of sharps.

Any liquid will be disinfected with Sodium Hypochlorite.
The expected degree of kill is 100% and can be validated by dialyzes of the mixture of bleach and supernatant with viral particles, add the solution to the BHK cells and incubate at 37C. The cells should not be infected.

Solid waste will be autoclaved.

The non-competent recombinant viral vectors will be generated in the basement viral suite of the Life Sciences Building. Primary neuron cultures will be infected with these vectors and grown. Then cells will be either fixed or lised for RNA extractions. We will use standard laboratory procedures.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee requires the work to be carried out in the departmental viral laboratory containment level 2. Some alterations were requested and the risk assessment was deemed to be suitable and sufficient.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
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The scientific goal is to characterise structural and functional experience dependent plasticity mechanisms in the cortex with the view of further understanding cortical processing of information, learning and consolidation of memories. Experiments will focus on identifying key regulatory molecules, some of which are linked to neurological disease, and delineating their role in plasticity mechanisms through the use of commercially available and well characterised transgenic models (for example CCR5, DISC1 and GluR1 gene knock out). In addition Cre recombinase/loxP transgenic technology will be used in some experiments to restrict transgene expression to specific tissues such as cortical layer 2/3 neurons. Mature neurons will be virally transfected using the well characterised, safe and commercially available Adenoviral Associated Virus (AAV) vector system (eg. Stratagene, UPEN, Applied Viromics for review see Buning H. et al 2008 J Gene Med). AAV are non-pathogenic viruses naturally occurring in mammals. Genetic inserts will be cloned into viral vector plasmids through the use of E. coli and HEK293 cells. Recipients are transgenic knock-out models for neurological disorders (CCR5, GluR1 and DISC1) and these will be injected with AAV-vectors for the purposes of expressing fluorescently labelled proteins. This will enable structural imaging of the infected neurons in vivo. E. coli and HEK293 cells will be used in the cloning and amplification of AAV-vectors. Adenoviral -Associated-Virus Vectors will be used for the delivery of DNA coding for fluorescently labelled proteins HEK293 AAV and E. coli cells will be used for cloning purposes of AAV vectors. Inserted genes will encompass very safe, well characterised and routinely utilised fluorescent reporter genes such as GFP, YFP, tdTomato, DsRed etc. In addition...
photosensitive receptors expressed on the cell surface such as Channelrhodopsins which are only activated upon specific photostimulation protocols. AAV vectors containing short hairpin (sh-) RNA inserts, coding for the depletion of GluR1, CCR5 and DISC1 will disrupt the expression of these genes but only in neurons directly transduced with the AAV virus (eg. GluR1 - subunit of ionotropic glutamate receptor, DISC1 gene disrupted in schizophrenia and CCR5 - chemokine receptor). Similarly gene inserts encoding for the expression of GluR1, DISC1 and CCR5 will be utilised. In the majority of cases the gene insert expression will be under the control of tissue specific promoters such as CaMKII, limiting expression to neuronal cells. Additional measures for restricting transgene expression are through the insertion of loxP sites around the gene insert, ensuring transgene will only be expressed in cells with Cre-recombinase activity. This genetic material originate from human cDNA, murine cDNA, green algae and parvovirus AAV-DNA.

Evaluation of foreseeable effects

Viral vectors will encode fluorescent proteins which will allow visualisation of specific cells through optical imaging. Inserts in viral vectors will also encode genes for GluR1, DISC1, CCR5.

GluR1 is a member of the ligand-gated ion channels family which are membrane proteins that mediate information transfer at synapses. Their function relies on the ability to respond very rapidly to the transient release of a neurotransmitter to produce a change in membrane potential of the postsynaptic cell. A subset of these ion channels are glutamate receptors, tetrameric cation-selective channels that are activated by glutamate and are abundantly expressed in the brain and spinal cord and mediate responses at the vast majority of excitatory synapses. AMPA receptors are one type of glutamate receptor, and there are four AMPA receptor genes (GluR1-4) which are transcribed to assemble the hetero-terameric receptor. We are investigating ONE of these genes which codes for the GluR1 subunit of AMPA receptors. To produce a functional receptor - dimeric-GluR1 subunits must combine with dimeric GluR2 or GluR4 subunits. Hence expression in non-neuronal tissue results in a biologically inactive protein, as GluR2 and GluR4 genes are not expressed. Only in brain tissue will the GluR1 transgene expression have any biological effect. The biological activity of GluR1 reported in the literature, suggests effects will most likely be cognitive impairment. In neurons AMPA receptors mediate forms of synaptic plasticity such as Long-Term Potentiation (LTP) and Long Term Depression (LTD) which are critical in the development of learning and memory formation and consolidation. Recent studies have demonstrated LTP is reduced in GluR1 knock-out models.

Disrupted in Schizophrenia (DISC1) mutations have been linked to a number of neurological disorders, including Schizophrenia and bipolar disorder. DISC1 is a large scaffolding protein (93 kDa) and as such, has been shown to bind numerous proteins including microtubule associated proteins, kinases (GSK3beta) and phosphodiesterase (PDE4) enzymes involved in key signalling cascades in the brain. No biological activity for DISC1 has been characterised in any other tissue than the brain. Given the wide variety of neuronal binding partners, it is not surprising that DISC1 mediates a plethora of different biological functions, both in vitro and in vivo. Some examples include regulating neuroblast migration or the proliferation of neural progenitors via an interaction with GSK-3beta. Mutations which lead to Schizophrenia have been shown to decrease Wnt signalling, a presumed affect of decreased spatially specific manner has been achieved by generation of a transgene overexpressing a truncated form of DISC1 under the control of an inducible CaMKII promoter. It is this transgenic model that will be utilized in our experiments. Schizophrenia is typically diagnosed in late adolescence or early adulthood, and the Disc1 knockdown model phenotypes relevant to Schizophrenia, including disturbed dopaminergic neurotransmission and interneuron deficits in the cortex, plus several behavioural changes, such as deficits in working memory, become apparent in young adulthood. Numerous studies have demonstrated a disruption in DISC1 is associated with Schizophrenia only at early stages of neural and circuitry development.

Taken together this information suggests the transgene rAAV encoding sh-DISC1 will only have a biological effect in brain tissue at an embryonic or early post natal stage. These biological effects would most likely be cognitive impairment.

CCR5 is a chemokine receptor and ablation of this protein has been shown to inhibit chemotaxis of a subprotein of leucocytes. In contrast increased expression of CCR5 induces pro-inflammatory effects. CCR5 is also a protein recognised by HIV for the binding and entry into cells. Therefore decreased CCR5 is shown to be protective for HIV infection. Murine CCR5 may have biological activity in leucocytes and may demonstrate altered properties of migration. In our study however we will utilize CCR5 transgenes for investigation of it's function in the brain. Disruption of CCR5 expression will most likely result in changes to cognitive function cognitive.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Transgenic models are housed in a secure facility, where all users adhere to standard operating procedures. Standard Operation Procedures include documentation of all
subjects housed in the facility, regular testing for pathogens, relevant safety procedures such as designated clothing, facemasks, gloves to be worn within the facility. The probability of transgenic models in use escaping into the environment are effectively zero, however in such a remote eventuality, subjects will be non viable in the environment, ill equipped to survive predation or acquire food, due to cognitive deficits, and have limited immunity to natural pathogens in the environment. Recombinant AAV vectors will be loaded into injection syringes in a designated viral suite, in a class 2 biosafety cabinet. Standard Operating Procedures are in place for the viral suite. Only registered users documented in training records have access to the suite. All equipment is kept within the suite and designated clothing (gowns and shoes) will be worn in the viral suite and removed prior to exit.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Genetically modified waste will be treated with chemical disinfectants, autoclaved and incinerated. Some GM subjects will be fixed with paraformaldehyde by perfusion for analysis. In these cases paraformaldehyde inactivates any infectious GM material. All tissue and carcases are disposed of in designated clinical waste bags and incinerated. Contaminated and/or biological waste is disposed of in clinical biohazard bags and autoclaved. Standard Operating Procedures for the viral unit require all surfaces and equipment to be wiped down with Virkon 1% or Trigene 4% as per manufacturers instructions. Virkon (potassium peroxymonosulphate). Sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Trigene - Halogenated tertiary amine. Both these compounds have broad spectrum activity against vegetative bacteria, spores, mycobacteria and viruses compounds have broad spectrum activity against vegetative bacteria, spores, mycobacteria and viruses.

Please see attached Trigene datasheet

All tissue and carcases are disposed of in designated clinical waste bags and incinerated. Contaminated and/or biological waste is disposed of in clinical biohazard bags and autoclaved. Standard Operating Procedures for the viral unit require all surfaces and equipment to be wiped down with Virkon 1% or Trigene 4% as per manufacturers instructions. Virkon (potassium peroxymonosulphate). Sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Trigene - Halogenated tertiary amine. Both these compounds have broad spectrum activity against vegetative bacteria, spores, mycobacteria and viruses.

Please see attached Trigene datasheet

All viral vectors to be used are susceptible to these disinfectants, according to the biosafety and manufacturers literature

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMS committee asked for clarification on a number of issues.
These have been dealt with and the committee agree that the risk assessment is suitable and sufficient.

Project Containment

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02/03/2022
The aim of this study is to employ the technology of somatic cell reprogramming to derive novel induced pluripotent stem cell lines (iPS) to generate in vitro cell models of disease. The technology involves the simultaneous transduction of somatic cells (e.g. dermal fibroblasts) with up to 5 genes responsible for conferring pluripotency in ES cells (Takahashi et al 2007, Cell 131,861-872,) Genes currently employed for iPS cell generation include Pouf5f1, Sox2, Klf4, Nanog, LN28. Genes are most effectively delivered to cells using lentiviral vectors. Once derived, iPS cells are pluripotent and can be used to derive multiple somatic cell types of interest. To model neurological disease we are also developing strategies to direct the differentiation of pluripotent cells to derive specific neuronal lineages of interest. This will be achieved by genetic programming by ectopic expression or knockdown of selected fate determining genes, which will also be effectively achieved by lentiviral delivery of genes.

Recipient or parental organism

Lentiviral vectors will be used to transduce human and mouse cell cultures in vitro. For iPS cell derivation recipient cell types will include fibroblasts, lymphocytes, or keratinocytes. The recipient cell cultures will either be derived from tissue biopsies and grown as primary or early passage cell cultures, or obtained from cell repositories such as ECACC.
We will use 3rd generation self inactivating HIV based lentiviral vector systems for gene delivery. Disabled and self inactivating viral vector particles based on a HIV system will be generated by co-transfecting HEK293T cells with four plasmids. The plasmids (described below) express a single viral gene and together (following transfection) they represent the minimal number of genes essential for propagation. This VSV-G (vesicular stomatitis virus) pseudotyped vector is self-inactivating and could not recombine with HIV, i.e. the probability of the production of replication competent vectors is extremely small.

Genes will be subcloned into:

a. Standard bacterial plasmid vectors for sequencing and propagation will be used.

B. The standard plasmids are pRRL-CMV-GFP, pRRL-synapsin-GFP, pMPDLg-PrPe, pR-REV and PMD2-VSVGP. All the genes required for producing full length viral RNA and packaging it into viable viral particles (gag-pol), rev and env) have been removed from the pRRL Lenti plasmids. Gag-pol and rev are expressed separately as is the VSV-G coat protein. The plasmids have been engineered so as to have no recombination sequences that would allow those genes to be inserted into the pRRL plasmid. This allows for a viral particle to be produced that can enter any cell, but that once inside does not have the relevant genes necessary to re-package itself or to re-generate full length viral RNA following genomic integration. In addition, following infection of the target cell, the lentivirus is self-inactivating on integration into the host genome. This is achieved because the process of reverse transcription uses the 3' long terminal repeat (3'LTR) as a template to produce the 5'LTR. The U3 region of the 3'LTR has been deleted, resulting in deletions in both 3' and 5'LTRs on integration into the host genome. These deletions are designed to ensure that the viral genome cannot be released from the host genome. Thus this system allows for expression of the gene of interest with little risk of the production of further viral particles.

In some cases we will use commercial 3rd generation lentiviral vector and packaging systems, such as ViraPower™ Lentiviral Expression System (from Invitrogen-Life technologies). These systems carry the same vector and packaging safety features as described above and have been designed for use with minimal risk and are marketed with full user manual and safety instruction.

The sequences that are to be expressed using this system fall into the following categories.

i) Full-length sequences designed to be overexpressed coding for genes that regulate stem cell pluripotency (Pou5f1, Sox2, Klf4, Lin28, Nanog); genes involved in neural fate specification (e.g. transcription factors Gsx2, Foxg1, Dlx2, Mash1, Isl1, FoxP1, Ebf1, Crip2 and growth factors SHH, WNTs). Protein sequences may be expressed:

(1) in their native form,
(2) as chimaeras where part of one protein sequence is placed inside another or
(3) with single or multiple point mutations to investigate the functions of single residues in, for instance, phosphorylation/dephosphorylation cycles or
(4) s tagged sequences where the tag is an antibody recognition sequence (V5, HIS, Flag…etc) and/or fluorescent proteins (GFP, CFP, YFP, DSRED etc).

ii) si/shRNAs designed to reduce or block the expression of proteins involved in the neural differentiation and that interact with pathways regulated by genes described in (i) above, e.g. to knockdown Pax6/Ngn2 expression. Small inhibitory and small hairpin RNAs are generated to be complementary to the mRNA of a target protein. The si/shRNA then acts as a guide for the (RNA-induced silencing complex) RISC to target and degrade its complementary mRNA.

Origin & function

The lentiviral vector and packaging systems described above generate replication incompetent infectious particles. The probability of reversion to the viral wildtype is virtually non-existent due to loss of four structural genes residing on non-homologous plasmids, and lack of a wild-type 3'LTR.

In the case of a HIV-1 infection following lentiviral integration, it has been previously shown that, even under permissive in vitro conditions, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host genome (Bukovsky et al (1999) J. Virol. 73; 7087-7092), although non-self inactivating vectors can be.

In the case of a worker already infected with HIV-1, the HIV genome will have already integrated into the host genome in infected cells. While it is conceivable that the lentiviral genome will infect the same cells and integrate into a location that will allow for homologous recombination with the native HIV-1 genome, the probability of that occurring is extremely small.

All potential routes of transmission of the virus are known. In the event of an accident, the main routes of transmission to deliver the virus or its products to tissues are airways and eyes. However direct contact of virus with cells, eg, via sharps injury, could also result in infection. So. The identifiable risk to the laboratory worker is infection by aerosol, or through sharps injury. To minimise this risk, no vacuum assisted aspiration, needles or glassware are included in procedures.

There is no potential for the transmission of the naked nucleic acid. The risk of incidental transfection of naked DNA is negligible, if it could happen genes required for replication and transmission are removed.

Evaluation of foreseeable effects

The lentiviral vector and packaging systems described above generate replication incompetent infectious particles. The probability of reversion to the viral wildtype is virtually non-existent due to loss of four structural genes residing on non-homologous plasmids, and lack of a wild-type 3'LTR.
The viral vector does infect human cells since the VSV-G pseudotyping envelope provides increased titres and broader host range, including human. Risk of human infection is nevertheless low. In order for spread of the gene of interest to occur following accidental infection (assuming that this has lead to viral integration), a series of unlikely events would have to occur.

(a) The worker would have to become infected with HIV-1 or to be already infected with the virus.
(b) The viral and lentiviral genomes would have to integrate into the host worker's genome in the same cells and in a position where they could interact to effect homologous recombination.
(c) Recombination would have to occur in just the right regions to allow for transfer of the gene of interest from the lentivirus to the HIV-1 genome, which would also involve the transfer of the HIV-1 genes to the lentiviral genome. In this case, it is just conceivable that a non-self inactivating HIV could be generated that contained the gene of interest but not the rest of the genome it requires. The other gene products could be provided in trans from the lentiviral genome that may now contain the HIV-1 genes or from other HIV-1 integrants.
(d) This could conceivably lead to the production of an infectious particle containing the gene of interest, but again no genes necessary for subsequent replication. In order for another round of infection, the process would have to begin again.

In truth, the affects of lentiviral infection in these circumstances are minor in comparison to the effects of the HIV-1 infection that would be required to effect the spread of the gene of interest. In addition, the scenario described is essentially equivalent to the rescue of the lentiviral genome from the host, which has already been shown not to occur.

Genes to be expressed from the lentiviral vectors encode for proteins with known developmental functions in that they regulate cell reprogramming and cell differentiation. We will purposefully avoid the use of oncogenes such as c-myc for cell reprogramming. Although the recombinant genes to be used have the potential to induce a pluripotent state in host cells providing a potential for growth and teratoma formation. However induction of pluripotency is a low efficiency event requiring the cotransfection of 4 vectors. Most importantly the cell reprogramming mediated by the reprogramming genes also requires very precise culture conditions that would not be present in a living host environment, only being present in stringently defined media conditions in laboratory cell culture. The genes used to regulate neural cell differentiation are not oncogenic. Thus the risk of accidental induced pluripotency and teratoma formation is negligible.

There is no reason to suspect that the tissue tropism or host range of the recombinant virus will be any different from that of the parent vector or virus since similar vectors have been used extensively and no differences in tropism have been reported.

There is also no reason to suspect that the recombinant viruses may have altered susceptibility to host defence mechanisms since no change to viral epitopes will be made that would affect viral immunogenicity or tropism. Neither vector nor inserts would have immunomodulatory function, and recombinant viruses are not likely to have any effects upon immunocompromised host beyond those normally expected with the parent virus. Taken together the recombinant viral particles will pose negligible risk to human health.

The overall likelihood that, in the event of exposure, the GM virus could cause harm to human health is low.

1. It is extremely unlikely that any worker would infect themselves with a significant dose of lentivirus as the volumes that are used for experimental infections are small (<5μl) and will be carried out in a Class II MSC. No significant aerosols will be produced by any of the procedures used and no needles need to be used, so there is no risk of needle sticks to introduce infection to the worker. All work will be carried out under category 2 containment conditions.
2. We cannot rule out the possibility that, when the lentiviral genome integrates into the host genome, it will not lead to the activation of an endogenous oncogene. However, all transcriptionally active long-terminal repeats have been removed as well as all promoter-like elements other than that required to drive expression of the transgene (eg CMV promoter). This should prevent unforeseen activation of such genes. Thus, we do not consider that the use of these lentiviral expression systems will result in a significantly increased risk of oncogenic activation over any other viral delivery system.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All viral waste will be treated by disinfection using 1% Virkon solution. The expected degree of kill is 100% in a 1% solution in 10 minutes. All disposable materials used in the course of viral handling (pipette tips, tubes, culture plates etc) will be soaked in Virkon 1% solution overnight then autoclaved as clinical waste. Procedures will use a Class II safety cabinet and all surfaces will be disinfected using Virkon spray and clinical wipes, that will similarly be disposed of as clinical waste.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

It was commented to ensure that all staff involved in the project will receive training to understand all identified potential hazards and potential consequences of viral expression and transfer of any harmful sequences. In the event of accident or emergency all relevant information pertaining to risk and inactivation would be conveyed to any emergency worker or health practitioner should a contamination incident occur. Project Risk Assessment information will be printed and filed in the lab for immediate access and reference.

There were also a number of other queries which have now been answered to the Committee's satisfaction.

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<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
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Project Ref 130/12.3

Date Ackn'd 20/04/2012

CU2 Project Title Analysis of HIV infection impact on Dendritic Cells behaviour and immune functions

Class 3

Culture Vol Class 2 1 L

Consent Granted Yes

Non-GMM

Date Project Ceased

02/03/2022
### Project Additional Information

**Purposes of the contained use**

On one hand, our projects request production of retroviral and lentiviral vectors then to transduce human primary cells and cell lines. These activities, when only vectors have to be produced and used alone, would require to be handled in a Cat2 level laboratory.

On the other hand, we would need to produce HIV viral cultures then to infect human primary cells and cell lines. Any activity involving handling and use of infectious HIV would thus have to be confined in Cat 3 level laboratory.

**Recipient or parental organism**

- For Cat 3 level laboratory activities, all our constructs will be based on HIV proviruses (pR9, pNL-4.3, pR8-Bal). Deletion or mutation of viral proteins according projects requests will be done by PCR-mediated mutagenesis on viral plasmid DNA. The overall likelihood of GM viruses causing harm to human health is medium and comparable to likelihood of parental wild-type viruses.

- For Cat 2 level laboratory activities, our constructs are HIV- or SIV-derived (SIV3), however with important structural and auxiliary gene deletions. Some other vectors are retroviral vectors based on Moloney MLV (pBABE-puro) and, like lentivectors, are lacking the gag, pol and env genes required for viral replication.

**Host/vector system**

- For Category 3 level activities, HIV proviruses and derived mutated strains, obtained upon PCR-mediated mutagenesis of viral DNA template, will be produced by transfection of producer cells (293T cells, HeLa cells) with purified viral DNA in Cat3 level premise. Viral culture will be then purified and concentrated on sucrose gradient by ultracentrifugation. Proviruses obtained will then serve to infect human primary cells and cell lines in Cat 3 level laboratory.

- For Category 2 level activities, retroviral and lentiviral vectors will be produced by transfection of 293T cells with a mix of plasmids compromising the transgene plasmid (providing the genetic material to be encapsidated into the vector thus encoding gene of interest in target cells), the packaging plasmid (encoding for the Gag and Pol gene products) and a plasmid encoding for a heterologous envelope gene product (like VSV-G). Theses highly attenuated lentivirus and retrovirus vectors have shown sufficient biosafety for their inclusion in ongoing human clinical trials as therapeutic vectors. The probability of the lentiviral vectors reverting to wild-type is extremely unlikely given the 3’LTR deletion and loss of some structural genes. Although the viral vectors can potentially infect a wide-range of cell types there is minimal risk of viral replication in infected cells. After serial passage, cells transduced with recombinant retroviruses or lentiviruses can be reclassified as category 1 mainly due to the loss of envelope required to infect neighbouring cells. However, and for a safety concern, all steps from viral vectors production to transduction will be carried out in Cat2 level laboratory.

**Origin & function**

- For Category 3 level activities, Human immunodeficiency viruses and derived mutants will serve to infect human primary cells (mainly Dendritic cells and CD4+ T cells) and cell lines (like Jurkat cell lines) to analyze the impact of infection on cell behaiour and immune functons. Some of these activities will also require the concomitant use of lentiviral vectors, to silence or overexpress specific cellular factors known to restrict HIV infection or influence HIV viral proteins trafficking. Hence, recently, inefficient HIV infection in DC has been challenged but the discovery that pre-transduction of DC with Vpx expressing SIV-derived lentivectors (Vpx-VLPs) could circumvent restriction thus leading to massive HIV infection. While the mechanism of Vpx action is still not known, this discovery allows infection to reach unprecedented levels in DC comparable.
To HIV infection routinely observed in CD4+ T cells. Thus we would need to consistently transduce DC with Vpx-VLPs concomitantly with infection in order to really assess the potential effects of high infection levels on DC immune functions.

- For Category 2 level activities, lentiviral and retroviral vectors will serve to transduce human primary cells or cell lines as above, to silence or overexpress some cellular factors or to express viral proteins fused to fluorescent tags (GFP, YFP). This will allow to analyze viral proteins trafficking and interaction with cellular host factors.

**Evaluation of foreseeable effects**

- Concerning HIV proviruses and mutated strains, even if these viruses are replication competent (so severe consequence of Hazard with acquired immunodeficiency), viral entry and spread would require a direct access to blood, internal mucosal surfaces or broken-skin which gives a low likelihood of Hazard. Hence, lentiviruses and retroviruses do not possess a capacity for air-borne or water-borne transmission and do not survive well in the environment making the possibility of environmental transmission remote. Mammalian cells infected with the viruses would not survive the osmotic challenges presented in the environment and would not survive without optimised nutrient tissue culture medium, pH and temperature.

For safety concerns, all steps from viral production to infection will be carried out in Cat3 level laboratory where the use of sharps when handling virus preparations is strictly forbidden.

- The likelihood of Genetically Modified vectors causing harm to human health is low despite the presence of an amphotropic envelope but given that the viruses are incapable of replication. Hence, integration of lentiviruses into human genome is not known to result in oncogenesis while retroviral integration can lead to oncogenesis but appears restricted to stem cells. After serial passage, cells transduced with recombinant retroviruses or lentiviruses can be reclassified as category 1 mainly due to the loss of envelope required to infect neighbouring cells. However, and for a safety concern, all steps from viral vectors production to transduction will be carried out in Cat2 level laboratory.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- All liquid wastes will be disinfected with Actichlor (2500 ppm sodium dichloroisocyanurate). A Chlorine-based disinfectant (Expected degree of kill 100%), prior to being autoclaved.

- All solid wastes will be autoclaved.

- The use of Sharps when handling virus preparations is not permitted.

- Contaminated pipettes will be immersed in Actichlor for a minimum of 4hr prior to transferring to a container for autoclaving.

- The tissue culture cabinet and any potentially contaminated areas will be disinfected with chlorine-based disinfectant according to an approved protocol.

- Access to laboratories employed for virus work will be restricted.

**Is an emergency plan required according to regulation 20?**  
N

If yes, tick to confirm that it is attached to this form  
N

02/03/2022
Upon initial submission by the applicant, the GM Safety Committee asked for the risk assessments for the Class 2 and Class 3 activities of the project to be made clearer and more distinctive, and for a number of issues to be clarified. Upon revision, the Chairman of the GMSC and a selected number of the GMSC now feel that this has been done and are satisfied that the risk assessments submitted are suitable and sufficient.

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**Project Ref** 130/12.4

**Date Ackn'd**
23/10/2012

**Date Project Ceased**
23/12/2015

**CU2 Project Title**
The Molecular Virology of Hepatitis C virus (HCV): HCV cell cycle and immune responses; comparison to HCV vaccine candidates based on poxviral and adenoviral expression systems

**Class**
Class 3

**CultureVolClass2**
< 1 Litre

**CultureVolumeClass3-4**

**Non-GMM**
Consent Granted
Yes

**Project notified under transitional arrangements**
N

**Tick if notifying a connected programme of work**
N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

Tick to confirm that you have attached a risk assessment to this form
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment
N
**Project Additional Information**

### Purposes of the contained use

We intend to investigate aspects of the innate immune response against HCV, comparing replicating HCV with recombinant poxviruses expressing HCV proteins. We will try to isolate new replicating strains and test novel antivirals for activity against HCV. For this purpose, pJFH-1, pCVH77C and chimeric HCV expressing plasmids (see below 'Construction details of the JFH1 derived plasmids') will be used to transfect/infect susceptible cell lines in a L3 containment laboratory.

Four collaborative projects will use the JFH-1 system:

I. In vitro antivirals studies in collaboration with Prof C M, Welsh School of Pharmacy. Infection of cell lines with JFH-1 and SAR studies with candidate compounds. Readout: qPCR, luciferase reporter assay and NS3/4 protease activity. Can start as soon as notified.

II. Study of innate immune responses in JFH-1 infected cells in collaboration with Prof K M and M T, School of Medicine. Infection of cell lines and investigation of novel innate immunity pathways.

Comparison of innate immune responses versus replicating HCV and recombinant poxviruses expressing HCV ORF and subunits (GM130/06.1), as a predictor of vaccine efficacy. Readout: FACS to assays to test TLR and RLR expression in HCV infected cells. Pilot experiments for grant application.

III. Investigation into restricting factors for HCV infection, including BST2/tetherin in hepatoma cells in collaboration with F B and V P, School of Medicine. Construction and use of functional deletion mutants based on the pJFH-1 plasmid. Readout: FACS, confocal microscopy, qPCR, ELISA, luciferase reporter assays. Pilot experiments for grant application.

IV. Identification of replication competent clinical strains of HCV and genetic modification of pJFH-1, pCVH77C and cDNA clones (plasmid) derived form clinical specimen as part of planned clinical fellowship (Dr M B).

Readout: FACS, confocal microscopy, qPCR, ELISA, luciferase reporter assays. Pilot experiments for grant application.

### Recipient or parental organism

HCV genotype 2a/reversed transcribed DNA sequences inserted into a pUC plasmid backbone.

HCV type 1a reversed transcribed DNA sequences inserted into a pUC plasmid backbone.

### Host/vector system

**HUH7-Lunet/CD81. Source: Bartenschlager lab, Heidelberg, Germany, Dr V L**

### Origin & function

Replication competent pJFH-1 (Wakita et al, 2005). Source: Bartenschlager lab, Heidelberg, Germany, Dr V L

HCV genotype 2a and chimeric virions released from producer cells transfected with pJFH-1 derived plasmid.

Plasmids to be established in the course of this project from replication competent clinical strains identified.

Replication incompetent plasmids based pn pCVH77C (Yanagin et al., 1997)

References:


**Evaluation of foreseeable effects**

Summary risk assessment HCV is a hazard group 3 pathogen and needs to be contained at BSL3. All HCV activities in Cardiff BSL3 are covered in an adapted HCV SOP (Blanchett and Ladell, 2010), and informed by UK and Heidelberg University cat 3 HCV working procedures.

The highest risk is associated with HCV infected materials produced in BSL3 and moved out to lower category labs for a number of preparation and readout procedures, where necessary equipment is not available in the cat 3 facility. The safety aspect is determined by the buffers used to collect and store the materials prior to moving them out of the BSL3 and the procedures used to disinfect transport containers externally.

Stability/infectivity of HCV (comparison to HIV): This has been studied in detail by Song et al, in a US/Chinese study published in VIROLOGY JOURNAL (2010) 7:40. Both HCV and HIV and sensitive to all buffers containing detergents or aldehydes, and heat over 60 centigrades for 8 minutes. UV light is sterilizing both viruses after 2 minutes exposure (wavelength= 253.7nm) with an intensity of 450μW/cm2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Ultracentrifugation: infected cell supernatants will be sealed inside the BSL3 class ll safety cabinet into ultracentrifuge rotor buckets. These will be disinfected externally, moved out of BSL3, used as transport containers, run in the ultracentrifuge and brought back into BSL3, to be opened in the safety cabinet. The samples will be inside sealed SW28 Beckmann tubes and carried in closed SW28 rotor buckets (screw cap with rubber seal). The buckets will be placed in a bucket stand and transported from the JBIOS CL3 lab, through HWRB 3rd floor to basement room inside a biohazard carrier box using the stair case. Elevators will not be used.

Flow cytometry and ELISA: equipment available in BSL3, 1st floor Henry Welcome building. Samples will be transported from the JBIOS class 3 lab, through HWRB 3rd floor inside a biohazard carrier box using the stair case. Elevators will not be used.

qPCR: the buffer used to extract nucleic acids from cells and tissues contains guanidine thiocyanate (GTC), a chaotropic salt. This buffer reduces infectivity of a wide range of viruses, including HCV, to zero (100% kill), after exposure over night and storage at minus 20 degree centigrade followed by thawing. HIV infected materials collected in this buffer are currently moved out of BSL3 as detailed in the HIV/(HCV)SOP.

Immunohistology - confocal microscopy: HCV infected cells are grown on coverslips and fixed in 3% paraformaldehyde in PBS for 30 minutes. This procedure is sufficient to disinfect tenacid vaccinia viruses and adenoviruses. The safety of this material is documented in Heidelberg University SOPs. We will confirm this with independent reinfection experiments of HCV materials in 3% PFA in BSL3, followed by qPCR readout.

Luciferase reporter assays: passive lysis buffer (PLB;PROMEGA) is used to collect and store the samples to be moved out of BSL3. This buffer reduces HCV infectivity to zero (100% kill), after exposure over night and storage at minus 20 degree centigrade followed by thawing. The safety of this material is documented in Heidelberg...
University SOPs. We have conducted reinfection experiments at L2 with the very stable and tenazid vaccinia virus in PLB. Buffer incubation followed by a single freeze/thaw cycle resulted in zero infectivity (100% kill; data of experiment Inf 12/233-3 is available upon request). Once replicating HCV is set up in CL3, we will confirm this with independent reinfection experiments of HCV materials in PLB and 3% PFA in BSL3, followed by qPCR readout. It is planned to eventually buy a luminometer to introduce into the new facility.

1. Transport of large amounts of life virus: samples inside SW28 rotor buckets for ultracentrifugation and enrichment are dropped. The buckets are made of stainless steel and will not break. 2. Samples for CL2 processing (qPCR, immunohistology, luciferase assay): samples are not correctly treated with inactivating buffers: In this case very small quantities of life virus may be present in samples. Samples for PCR will be sealed inside PCR tubes and subsequently heated repeatedly to boiling temperatures. Samples for immunohistology are sealed under coverslips with aceton-containing glue. Samples for luciferase assay will be sealed inside 96 well plates and frozen/thawed. In all cases the risk for workers to be exposed to life virus is effectively zero. As detailed in Song et al., both HIV and HCV are enveloped viruses known to be very sensitive to environmental conditions and disinfectants.

If all safety precautions are followed as detailed in the HCV/HIV SOP, the overall risk is negligible.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation applies

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Viruses will at all times be maintained in an environment designed to prevent aerosol spread. All flasks containing the virus shall be kept closed except when decanting and pipetting which will be conducted in tested class II safety cabinets. Centrifugation steps shall be performed in sealed buckets that will be opened only in safety cabinets. The use of Sharps when handling virus preparations will not be permitted. Contaminated pipettes will be immersed in Actichlor for a minimum of 4hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with chlorine-based disinfectant according to an approved protocol. Access to laboratories employed for virus work will be restricted.

Disinfectant Product Name: Actichlor (2500ppm sodium dichloroisocyanurate). Generic Chemical Name: Chlorine-based disinfectant Expected degree of kill 100%

How validated: HCV infectivity in collection buffers will be validated by a BSL3 reinfection assay using qPCR readout.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The risk assessment was discussed extensively in the GM committee, and revisions were required. In the following a listing of the required amendments/changes.

1. The revised risk assessment (version 4) for the use of replication competent HCV strains and reporter constructs, providing new information and corrections, as requested by the GM committee in the meeting on October 5, 2011.

- Name of Institute/department updated: CI3/Medmicro &ID
- Dr L identified as provider of HCV reagents in PI profile.
- CL3 training course mentioned in PI profile
Dr T removed from list of ‘staff of the project’ - no active labwork planned; collaboration planned with materials produced by B and B in the CL3 lab.
- Materials provided by L identified as pFK-JcR2a and Huh7-Lunet/CD81
- Worst case scenarios have been added for samples dropped in transport and insufficiently treated in working buffers.
- Bottom of page 11: GM virus could cause harm to human health - "High" as requested by committee.
- The list of projects in the connected program of work has been updated according to the new listing provided with RA 130/613.
- We have conducted experiments showing that the processing of samples for luciferase reporter assay renders vaccinia virus uninjective. This is a good indication that HCV infected cells treated the same way will be safe outside of CL3. Treatment of immunohistology samples with 3% PFA is a harsher disinfective treatment, so samples will be safe as well. The same procedure has been proposed by Dr B for his HIV infected cells.
- Details have been added regarding the transport of HCV infected cells inside sealed ultracentrifuge rotors to the ultracentrifuge in the basement HWRB: The samples will be inside sealed SW28 Beckmann tubes and carried in closed SW28 rotor buckets (screw cap with rubber seal). The buckets will be in a bucket stand and transported from HWRB 3rd floor to basement inside a biohazard carrier box using the staircase. Elevators will not be used.

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Project Ref 130/12.5

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Date Project Ceased 02/03/2022
Project Additional Information

Purposes of the contained use

Expression of transgenes in T cells in order to carry out short term functional analysis

Recipient or parental organism

Mouse T Cells

Host/vector system

Retroiral vector mIGR

Origin & function

Transduction of T cell in vitro for reintroduction in vivo. The T cells bearing the original T cell receptor (TCR) is known to induce diabetes in susceptible mouse strains. The transgene for the purpose of the project consisting of a modified TCR will be introduced to T cells to direct them to the pancreas.

FoxP3 is a regulatory transcription factor in mouse T cells

T cells transduced with these genes will have a regulatory phenotype designed to protect against diabetes in susceptible mouse strains

Evaluation of foreseeable effects

The inserted genes are the already rearranged T cell receptor alpha and beta chains from a mouse and there is very little possibility of recombination to create an effective protein in humans. Mouse and human foxP3 are not identical and humans have additional splice isoforms that are not present in the mouse and therefore the mouse foxP3 is highly unlikely to be an effective protein in human cells.

The DNA components are located on different plasmids in the vector and packaging cell line so there is no potential for transmission of the naked nucleic acid. The viral vector infects rodents but not human cells. The viral vector used is fragile and has a short life and will be used only in a highly controlled tissue culture environment. Once the T cells are transduced and prepared for injection into mouse hosts, there will be no free viral particles and the following activities, including injection into mice will be class I activities.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solutions will be treated with bleach before removal from tissue culture hood. All solid waste will be bagged in the tissue culture hood before removal in a sealed container to autoclave in an adjacent room.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification and Biological Agents Safety Committee agree that the Risk Assessment is suitable and sufficient and that the project is a Class 2 activity

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Project Ref 130/13.1

Date Ackn'd 17/04/2013

CU2 Project Title Construction of a GFP expressing attenuated vaccine strain of Bacillus anthracis

Class 2

CultureVolClass2 Class 3-4

< 1 Litre

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info
Bacillus anthracis belongs to a group of genetically related organisms (B. cereus, B. thuringiensis) for which the formation of an emperium spore represents a key survival strategy. The spore form confers considerable resistance to chemical and thermal insult hindering attempts to decontaminated exposed environments. Decontamination modalities for anthrax to date have centred on the use of toxic biocides (formaldehyde, chlorine dioxide) or gamma radiation. These approaches suffer from the dual handicap of toxicity to man and the environment and/or are extremely expensive. There is an urgent need for strategies which are environmentally friendly, can be used to decontaminate a range of environments with little or no toxicity to fauna or flora and are cost effective.

We have developed a simple mixture of amino acids and sugars which when applied to soil contaminated with anthrax spores is able to trick the organism into converting from a chemically resistant spore into a chemically sensitive bacterium, a process known as germination. This approach renders the organism considerably more susceptible to biocides and decontaminating agents making it possible to reduce the concentration of toxic agents required to clean up an incident.

Forcing the bacteria to germinate also renders the bacteria susceptible to the attentions of natural soil predictors such as the bacteria eating nematode Caenorhabditis elegans. In preliminary laboratory studies using sterile soil we have demonstrated that a combination of C. elegans and germinants can reduce the B. anthracis spore load of artificially contaminated soil by >95%. To progress this work we will assess the ability of C. elegans to reduce B. anthracis numbers in non-sterile soil. To assist in this task we propose to make use of a GFP expressing variant of B. anthracis by transforming a plasmid expressing GFP from Aequorea victoria (pSW4-GFPmut1), a safe, well established tag used for visualising investigated proteins in cells and bacteria, into an attenuated vaccine strain of B. anthracis. The feasibility of this approach has been demonstrated by colleagues in the United States who have kindly agreed to provide us with pDW4-GFPmut1 (Sastalla et al, Appl Environ Microbiol. 2009 Apr;75(7):2099-110). The pSW4-GFPmut1 plasmid was constructed as follows. A copy of the Aequorea victoria-derived gfpmut1 gene (Cormack et al., 1996 Gene 173:33-38.) was amplified by PCR and inserted downstream of the pag promoter in pSJ115 (Park and leppla, 2000 Protein Expression and Purification 18, 293-302) to replace the lef gene (Pomerantsev et al., 2003 Immun. 71:6591-6606).

E. coli SCS110 (Stratagene)- propagation and maintenance host: The pSW$-GFPmut1 plasmid will be transformed into E. coli SCS110 (Stratagene) which is an endA-derivative of JM110 strain which in turn is derived from E. coli K12. The SCS110 strain is ideal for preparing plasmid or phagemid DNA free of Dam or Dcm methylation so that the DNA can be restriction digested by one or more methylation-sensitive restriction enzymes. The genotype of this strain is as follows; rpsL (Strr) thr leu endA thi-1 lacY galK glaT ara tonA tsx dam dcm supE44 Δ(lac-proAB) [F traD36 proAB laclqZΔM15]. (Genes listed signify mutant alleles. Genes on the F episome, however, are wild-type unless indicate otherwise.) Given that SCS110 is a K-12 derivative and is thus considered non pathogenic for humans the risk associated with its use is low.

2. Attenuated animal vaccine strains of B.anthracis: The ultimate aim of this project is to construct a GFP expressing variant of B. anthracis which can be used to support laboratory studies. This will be achieved by transforming pSW4-GFPmut1 plasmid into an attenuated vaccine strain of B. anthracis called the Sterne strain which has been approved by the HSE for handling and manipulation under containment level 2 conditions in our laboratory.

Host/vector system

The study will make use of one plasmid, pSW4-GFPmut1, which is a GFP expressing E. coli/B. subtilis shuttle vector. In addition to carrying a copy of the Aequorea victoria-derived gfpmut1 gene (Cormack et al, 1996 Gene 173:33-38.) under the control of the pag promoter it also encodes resistance to ampicillin and Kanamycin. The plasmid has a Gram positive (pUB110) origins of replication which is non-mobilisable in B. subtilis (ACGM) and a Gram negative (pBR322 - ColEI ori) origins of replication which is mobilization defective (ACGM).
The aim of this study is to generate a GFP expressing variant of an attenuated strain of B. anthracis. GFP is a well established tag used for visualising investigated proteins in cells and bacteria. In this case the gfpmut1 gene of Aequorea Victoria (Cormack et al. 1996 Gene 173:33-38) has already been cloned into the E. coli/B. subtilis shuttle vector pSW4 under the control of the pag promoter which is expressed during vegetative bacterial growth. Expression from the pag promoter is controlled by the presence of bicarbonate ions meaning that the level of GFP expression can be regulated.

Evaluation of foreseeable effects

Although the Sterne vaccine strain of B. anthracis encodes a number of putative virulence factors it has been shown to be non-pathogenic to humans and animals (Sterne, M. et al. (1942) J. African Med. Vet. Med. Assoc). We do not expect the expression of GFP in B. anthracis to have any effect on host gene expression.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All working areas will be cleaned with hypochlorite (10,000 ppm) prior to and post working with the organisms. All work with this bacteria will take place within a Microbiological Safety Cabinet. A separate incubator will be used for these bacteria. All liquid and solid waste will be autoclaved.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Committee found the risk assessment for the project "Construction of a GFP expressing attenuated vaccine strain of Bacillus anthracis" to be suitable and sufficient.

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The aim of the project is to investigate the antibacterial activity of compounds derived from hops (Humulus lupulus L.) against Mycobacterium smegmatis (MTS) and Mycobacterium abscessus (MCAB). These bacteria belong to a group of genetically related organisms which include Mycobacterium tuberculosis a bacterium which currently infects 1/3 of the world's population and is a leading cause of death worldwide. Its success as a pathogen is due in part to its ability to survive and multiply within the macrophages of the infected individual. In contrast MTS and MCAB lack the ability to initiate infection in healthy individuals (they are classed as hazard group 2 organisms) and this coupled with their ability to grow on standard laboratory media and in macrophages at a rate considerably faster than that of M.tuberculosis makes them ideal substitute organism with which to study the development of antibacterial agents with activity against M.tuberculosis. We are thus seeking to identify new classes of antimicrobial agents which are effective against antibiotic resistant strains of M. tuberculosis. The current focus of our search is the hop plant which have been used for centuries in the brewing industry and is known to contain compounds which are inhibitory to a wide range of organisms including M. tuberculosis. In previous laboratory studies we have confirmed the bactericidal activity of hop extracts against M. smegmatis. To aid us in the task of identifying hop derived inhibitory compounds we propose to employ GFP expressing variants of both MTS and MCAB. These strain will be used to develop high throughput agar and cell culture based screening methods.

The two mycobacterial species which will be used in this study are Mycobacterium smegmatis and Mycobacterium abscessus both of which are classified as hazard group 2 organisms. Bacterial infection with Mycobacterium smegmatis have only been associated with immunocompromised individuals. The ultimate aim of the project is to construct a GFP expressing variant of M. smegmatis which can be used to support laboratory studies. The M. smegmatis strain which we propose to use in this study, ATCC 700084 has been approved the ACDP/HSE for handling and manipulation under containment level 2 laboratory conditions.
M. abscessus (MACB) lung infections are seen in cystic fibrosis patients, but not in healthy individuals. The MACB reference strain CIP104536T has been classified as a class 2 organism. Wild type strains will be provided by Dr M R of the Llandough mycobacterial reference unit (public Health Wales)

**Host/vector system**

The plasmid system which will be used in this study (pSMT3) is a GFP expressing E. coli/Mycobacterium shuttle vector. In addition to carrying a copy of the Aeouora victoria-derived gfmut1 gene (Cormack et al, 1996. Gene. 173:33-38.) under the control of the paq promoter it also encodes resistance to the antibiotic hygromycin however this is not a commonly used antibiotic for any infections arising from this bacterium. The plasmid has a Gram positive (pUB110) origin of replication which is non-mobilisable) and a Gram negative (pBR322 - ColE1 ori) origins of replication which is mobilization defective. (ACGM).

Plasmid pJV53 expresses mycobacteriophage genes (Che9c gp60 and gp61 which are homologous of E. coli RecE and RecT, encoing exonuclease and DNA-binding activities, which substantially elevate homologous recombinations of linear DNA for the production of allelic exchange mutants in mycobacterium ssp. The plasmid can be used for recombineering into M smegmatis.

**Origin & function**

The aim of this study is to generate GFP expressing variants of Mycobacterium smegmatis and Mycobacterium abscessus. The GFP gene which is derived from Aeouora Victoria (Cormack et al., 1996 Gene 173:33-38.) is a well established tag used extensively for the visualisation of proteins expression in bacteria and mammalian cells. Expression of the GFP is constitutive and is driven by the Paq promoter which also drives the expression of the hygromycin resistance gene. As far as we are aware GFP poses no danger to human, animal or plant life.

**Evaluation of foreseeable effects**

Mycobacterium smegmatis ATCC 700084 and MACB strains used are considered non-pathogenic for healthy humans while GFP has been extensively used with no reports of ill efectives and as a consequence the risk associated with use is low. They are not pathogens of animals and plants and thus no specific hazards have been identified.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All working areas will be cleaned with hypochlorite (100% v/v) prior to and post working with the organisms. All work with this bacteria will take place within a Microbiological Safety Cabinet. A separate incubator will be used for these bacteria. All liquid and solid waste will be autoclaved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee found the risk assessment for the project. "The use of natural products and bacteriophages against Mycobacterium spp". To be suitable and sufficient.
The scientific goal is to characterise structural and functional experience dependent plasticity mechanisms in the cortex with the view of further understanding cortical processing of information, learning and consolidation of memories. A large number of molecules and pathways have ben identified and shown to play a key role in synaptic plasticity. Gene defects affecting some of the associated postsynaptic proteins cause neuro-developmental disorders (also known as synaptopathies) such as Fragile X. We will study development plasticity in the primary visual cortex (V1) at the cellular level in mouse models of those disorders using two-photon laser scanning microscopy. The mice to be used carry a knock-out mutation on one of the mouse homologues of human genes associated with neurodevelopmental disorders (FMR1, DLG3) and/or they conditionally express fluorescent proteins that will allow specific populations of cells in the cortex to be identified during imaging. In addition Cre recombinase/loxP transgenic technology will be used in some experiments to restrict transgene expression to specific tissues such as cortical layer 2/3 neurons. Mature neurons will be virally...
Transfected using the well characterised, safe and commercially available Adenoviral Associated Virus (AAV) vector system (Upenn). AAV are non-pathogenic viruses naturally occurring in mammals.

Recipient or parental organism

Recipients are transgenic knock-out models for neurological disorders (FMR1, DLG3) and these will be injected with AAV-vectors for the purposes of expressing fluorescently labelled proteins. This will enable structural imaging of the infected neurons in vivo.

Host/vector system

Adenoviral-Associated -Virus Vectors will be used for the delivery of DNA coding for fluorescently labelled proteins.

Origin & function

Inserted genes will encompass very safe, well characterised and routinely utilised fluorescent reporter genes such as GFP, YFP, tdTomato, DsRed etc. In addition photosensitive receptors expressed on the cell surface such as Channelrhodopsins which are only activated upon specific photostimulation protocols. This genetic material originates from human cDNA, murine cDNA, green algae and parovirus AAV-DNA.

Evaluation of foreseeable effects

Viral vectors will encode fluorescent proteins which will allow visualisation of specific cells through optical imaging.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Transgenic models are housed in a secure facility, where all users adhere to standard operating procedures. Standard Operating Procedures include documentation of all subjects housed in the facility, regular testing for pathogens, relevant safety procedures such as designated clothing, facemasks, gloves to be worn within the facility. The probability of transgenic models in use escaping into the environment are effectively zero, however in such a remote eventuality, subjects will be ill equipped to survive predation or acquire food, due to cognitive deficits, and have limited immunity to natural pathogens in the environment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Genetically modified waste will be treated with chemical disinfectants, autoclaved and incinerated. Some GM subjects will be fixed with paraformaldehyde by perfusion for analysis. In these cases paraformaldehyde inactivates any infectious GM material. All tissue and carcasses are disposed of in designated clinical waste bags and incinerated. Contaminated and/or biological waste is disposed of in clinical biohazard bags and autoclaved.

Standard Operating Procedures for the viral unit require all surfaces and equipment to be wiped down with Virkon 1% or Trigene 4% as per manufacturer's instructions. Virkon (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Trigene - Halogenated tertiary amine. Both these compounds have a broad spectrum activity against vegetative bacteria, spores, mycobacteria and viruses.


Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The GMBA committee asked for clarification on a number of minor issues which was provided. These have been dealt with and the committee agree that the risk assessment is suitable and sufficient.

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### Project Ref 130/13.4

**Date Ackn'd** 14/05/2013

**CU2 Project Title**

Molecular regulation of mammary epithelial cells Part 1 (Generation and use of replication deficient lentiviruses; Containment Level 2 activity)

**Class** Class 2

**Culture Vol** < 1 Litre

**Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
**Project Additional Information**

**Purposes of the contained use**

To use replication-deficient third generation lentiviral vectors to deliver cDNA and knockdown constructs to mammalian cells (in particular, to mammary epithelial cells) to investigate the molecular regulation of mammary epithelial cell biology, and how this is de-regulated in breast cancer.

Having generated replication deficient lentiviruses, these will be used to transduce mammalian cell cultures. Generation of lentiviral vector constructs will be carried out under Containment Level I conditions. Generation of lentiviral supernatants and transduction of cell cultures will be carried out under Containment Level II conditions. However, providing the supernatants being used have been proven as free of replication competent virus, and the transduced target cells have been washed and free of supernatant for 24 hours, we will return cells to Level 1 containment.

Once the lentivirus has infected the cells it should not be able to replicate. All new batches of virus will be tested for replication competence. Murine NIH3T3 or 3T3-L1 cells will be transduced with fresh or frozen (-70°C) viral supernatant (supernatant 1) (virus containing the gene of interest plus either a selectable marker or GFP) plus 2ug/ml of polybrene and incubated overnight. The following day, cells will be washed with medium and refed. After 24 to 48 hours the number of GFP positive cells will be assessed (or the cells will be put under selection). Cells will be grown for a further 4 to 6 days (splitting cells if necessary) and an overnight supernatant (supernatant 2) harvested from confluent cells. The supernatant will be passed through a .45 micron filter and either used straight away or stored frozen at -70°C. NIH3T3 or 3T3-L1 cells will then be transduced with supernatant 2 plus polybrene. A positive control consisting of supernatant 1 plus polybrene will be included. The cells will be washed the next day and fresh medium added. The cultures will be checked for GFP positive cells 24 to 48 hours later (or put under selection). In the absence of virus in supernatant 2 no green (GFP positive) cells (or resistant colonies if a selectable marker is used) should be seen whereas green cells should be present in cells transfected with positive control supernatant 1.

Cell lines generated using replication-deficient virus (as opposed to short term cultures) will be tested every six months to confirm continued absence of replication competent virus. Supernatant will be harvested from confluent cells, passed through a 0.45 micron filter. NIH3T3 or 3T3-L1 cells will then be 'transduced' with supernatant plus polybrene. The cells will be washed the next day and fresh medium added. The cultures will be checked for GFP positive cells 24 to 48 hours later (or put under selection). In the absence of virus in the supernatant no green (GFP positive) cells (or resistant colonies if a selectable marker is used) should be seen. The positive control will be the cell line from which the supernatant was harvested. The negative control will be NIH3T3 or 3T3-L1 cells not treated with supernatant.

**Recipient or parental organism**

Viruses will be generated by transfection of mammalian cell lines (HEK293FT) with a minimum of three different vectors coding for envelope, expression vectors and packaging proteins.

**Host/vector system**

The vector systems we will use are split vector systems with a minimum of three elements:

1) The expression vector into which the gene of interest is cloned and which also typically contains a fluorescence and/or selectable marker. The expression vectors also contain elements to allow packaging into virions.

2) A packaging plasmid suppling the helper functions in conjunction with structural and replication proteins (in trans) required to produce the lentivirus. In some more advanced biosafety systems, these functions are also split between more than one vector.

3) A plasmid supplying an envelope protein that gives broad tropism.

The 293T line stably expresses the sV40 large T antigen under the control of the CMV promoter. When packaging plasmids and Intiviral vector are co-transfected into the 293 FT cells a replication defective virus is produced. This virus can then be used to transduce both actively cycling and dormant mammalian cells but will not replicate in the
host cells. Once inside the cell, the RNA virus is reverse transcribed, becomes stably integrated into the host genome and the effect of expression of the gene of interest can be studied.

The following vector systems will initially be used. Additional systems with the same minimal safety features will be added as required following local approval by the GMBA Committee.

VECTOR SYSTEM 1: TRONOLAB pWPI SYSTEM

The Trono laboratory lentiviral vector system (pWPI, pMD2.G and psPAX2) will be used for overexpression studies. The lentiviruses are made with a split-genome conditional packaging system. This conditional packaging system acts as a built-in device against the generation of productive recombinants. For expression studies, the gene of interest will be under the control of the human cytomegalovirus (CMV) promoter.

VECTOR SYSTEM 2: pSEW SYSTEM

The SEW expression vector, the packaging construct pCMVR8.91 and the pseudotyping construct pMD2. VSVG will be used for knockdown studies (obtained from B. Groner, Frankfurt). SEW carries a proviral cassette containing the 5' -UTR of HIV-1, followed by an eGFP sequence under the control of the Spleen Focus Forming Virus (SFFV)- promoter and a 3' - self-inactivating LTR. The desired shRNA is cloned into the 3' - LTR of SEW. Since no promoter is present in the 3' - LTR, a promoter for a small RNAs (e.g. H1 promoter: Pol III promoter) has to be cloned in front of the shRNAs before insertion into the SEW-vector. We have also established a variant of this vector in which an EF1a promoter together with a cDNA for a gene of interest can be inserted instead of an shRNA to generate an over-expression vector.

Origin & function

The vectors we are working with are artificial constructs built from a variety of elements of different origin including elements of HIV, Vesicular Stomatitis Virus and Spleen Focus-Forming Virus. The cDNA inserts will come from publicly available repositories of coding sequences. The knockdown sequences will be Synthesised to order. The coding sequences delivered by the viruses will include potential oncogenes.

Evaluation of foreseeable effects

The split vector nature of the viruses means that it is highly unlikely that recombination could occur with related viruses. If there was a loss of containment, neither the packaging cells could not survive long enough in the environment for recombination to occur. Even if widespread dissemination of an oncogene in a virus did occur, that gene would have to infect a mammalian cell already carrying defects in cell cycle checkpoint controls for it to cause cancer.

To our knowledge there are no reported occurrences of a replication-deficient lentiviral construct infecting a laboratory worker or indeed of a 3rd generation virus recombining in such a way to generate replication competent virus. The risk is assessed as Low, because of the use of potential oncogenes, however, providing SOPs and GLP are followed, the risk can be downgraded to negligible. In addition, even in the highly unlikely event of such a potentially oncogenic DNA sequence getting into a human host that does not mean it will cause cancer. Multiple genetic changes are required for tumour formation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be disinfected with Virkon (Peroxygenic acid; 1 x 5g tablet for each 550ml liquid waste; expected degree of kill >99.999 for 1% solution for bacteria, virus, yeast; See Pub MedID 11073729).
Solid waste will be autoclaved.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment

The Committee had a number of queries re the risk assessments for the parts of the connected programme of work but these have now been addressed and the risk assessments are suitable and sufficient.

## Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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## Project Ref 130/13.5

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Project notified under transitional arrangements **N**

Historical Significant Changes
### Project Additional Information

**Purposes of the contained use**

We wish to determine whether modifications of brain pathway (ERK pathway) induce therapeutic benefits in a mouse model of HD. ERK pathways will be altered by the delivery of a virus into a specific brain region for this purpose.

**Recipient or parental organism**

The recipient organism of the virus is mouse

**Host/vector system**

The vector system is a 3rd generation retrograde self-inactivating lentivirus (LV). Sequences coding gag,pol, env and rev are removed from the transfer vector and provided in trans by three helper constructs. Several lentiviral accessory genes are deleted (vif, vpr, vpu and nef). Tat gene is deleted and the tat-responsive promoter present in the 5’-LTR is replaced by an heterologous promoter in the U3 region.

**Origin & function**

The viral vectors created in the laboratory of Dr R B in the San Raffaele Scientific Institute, in Milan. The nucleic acids were purchased from Thermo scientific (http://thermoscientificbio.com?). The intended function of the viral vectors are to modify a specific molecular (ERK/MAP kinase) pathway in the brain that has been implicated in the development of pathology in a number of neurological disease states. More specifically short hairpin sequences will be used for RNA interference of the mouse ERK1 gene product.

**Evaluation of foreseeable effects**

The virus is not able to replicate and carries no pathological payload, consequently the application of the virus will have no negative consequences to the well being of the mouse or spread beyond the host to the wider environment or to other organism. As this procedure is a therapeutic application we would hope that the virus would improve the disease phenotype in the mouse as demonstrated by ERK/MAPK modifications in other animal models of disease (see Fasano and Brambilla 2011. Font Behav Neurosci 5.79)

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The virus vials will only be opened in a Class 2 microbiology safety cabinet. Any used vials and surgical sharps, glass capillaries, disposable gloves will be placed directly into a clinical waste bin which will be sealed and incinerated. The surgical area will be cleaned with virkon which has been validated as an antiviral agent.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Cardiff University Genetic Modification and Biological Agents Safety Committee agreed with the classification of the activity as Class 2. They did however have a number of queries with the local risk assessment. The queries have now been answered to the satisfaction of the committee

Project Containment

**Laboratory Activities**

- L2: Yes
- L3: L4
- L2: L3

**Glass Houses**

- L2: L3
- L3: L4
- L2: L3
- L4

**Growth Rooms**

- L2: L3
- L4

**Animal Units**

- L2: Yes
- L3: L4
- L2: L3
- L4

**Large Scale Activities**

- L2: L3
- L4
- L2: L3

**Human Clinical Applications**

- L2: L3
- L4

Project Ref  130/14.1

**Date Ackn’d**  08/01/2014

**CU2 Project Title**  Controlled Wht pathway gene expression in primary human and murine organoid and tissue culture cell lines

**Date Project Ceased**  02/03/2022

**Class**  Class 2

**CultureVolClass2**  < 1 Litre

**CultureVolumeClass3-4**  Non-GMM

Consent Granted

Project notified under transitional arrangements  N
Recent advances in 3D organoid culture systems recapitulate tissue organization, stem cell behaviour and differentiation. These culture conditions, based on the careful control of Wnt pathway activity, allow maintenance of intra-organoid cellular diversity including tissue and cancer stem cells in a variety of primary tissues including the intestine, liver and mammary gland together with tumours form corresponding issues. Organoids better recapitulate in vivo cell responses and will likely play a significant role in studies of gene function and future drug discovery where there is a major demand for systems that better predict patient responses than 2D cell culture. Human and mouse derived organoids could provide a fast, cost-effective and high-throughput alternative to animal models. For this technology to be maximally exploited, it would ideally be combined with genetic modification techniques.

This project will involve expression of site-specific (eg. Cre-LoxP) recombinases, Wnt signalling pathway regulators and signalling pathway reporters in primary 3D organoid cultures and routine tissue culture cell lines of mouse and human origin (Eg. HEK293, SW480) using aden- and lentiviral vectors. Recombinant gene expression will be controlled using lentiviral or adeno viral expression vectors and morphological and biochemical changes will be monitored by fluorescence microscopy and biochemical assays. Reporter genes will be used to assess the effects of culture conditions on intracellular signalling pathways. Inducible Cre-LoxP and transgene expression will be used to alter gene expression in the organoids and in tissue culture cell lines. Of most interest from a safety point of view are vectors that, following transduction, might alter cellular function in non-target cells (eg. Lab worker tissues) during experiments.

Replication deficient recombinant adenoviruses or lentiviruses will either be bought pre-packed or generated in house. To this end, genes involved in oncogenesis and Wnt signalling regulation will be cloned into commercially available vectors in attenuated E. coli strains (e.g. DH5a, JM109, XL-1 blue, BJ5813, SW102, STbl3). Packaged replication defective viruses will be generated in E+ helper cell lines (e.g. 293 cells and 991 cells) and used to transduce target cells.

Although a range of different adenoviral vector systems are used to generate recombinant viruses, the vectors are all based on Ad5 E1. E3 deletion mutants with essentially identical biological properties. However, we are aware that Ad5 encodes two short virus-associated RNAs (VA-RNAI & VA-RNAII) that do not encode a protein but suppress RNA interference VA-RNA transcription is driven by RNA pol III, is activated late in infection, and theoretically should not affect the use of the replication-deficient vectors for this application. Adenoviruses can infect a broad range of mammalian cells. The Adenoviral DNA genome is generally considered to be stable, remaining episomal and not integrating into the gemome of the host. However, it has been reported to integrate into the genome in rare cases with an integration rate of less than 1 x 10-7 / cell (Stephen et al. 2010). There is therefore only a remote possibility that it may activate or inactivate host cell genes.

The lentiviral vectors are third generation HIV-1 based lentiviral systems with advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and are replication incompetent once transduced. However the viruses are still able to transduce dividing and non-dividing primary human cells and will stably integrate into the host genome.

**Origin & function**

This project will involve expression of site-specific (E.g. Cre-LoxP) recombinases, Wnt signalling pathway regulators and signalling pathway reporters in primary 3D organoid cultures and routine tissue culture cell lines of mouse and human origin (E.g. HEK293, SW480) using adenoviral and lentiviral vectors.

The vectors will contain cDNA inserts of mouse, human, viral or bacterial origin or synthetic oligonucleotides. The Wnt signalling plays an important role in stem cell regulation, proliferation and cell fate. Mutations in Wnt signalling components have been linked to cancer. However, Wnt pathway dependent oncogenesis occurs normally in combination with 8-10 other genetic changes. These additional changes will not be induced by the viral vectors.

**Evaluation of foreseeable effects**

The recombinant viruses will express potential oncogenes (e.g. Wnt signalling pathway components) with the capacity to trigger unwanted proliferation in infected cells. Therefore there is a very small but identifiable risk to an infected individual of long term alteration of gene expression. However only cells that come in direct contact with the packaged virus could become infected. The route of transmission to deliver lenti- and adenoviruses is through airways and eyes. The laboratory worker is thus under risk from aerosols and through sharp injury during handling. There is no risk of the infection spreading to the community via human-human contact. The packaged virus is unstable in the general environment. And both lenti- and adenoviruses are deleted in the essential E1 gene and are thus rendered replication deficient. Vectors are also deleted in E3 and are therefore disabled with respect to their capacity to evade immune recognition.

In the case of adenoviruses, there is an extremely low risk of reversion to a wild type, since the helper cell line does not contain E3 sequences. Replication -competent adenoviruses could be generated as a result of recombination between the helper cell line and the vector. This is an inefficient process that tends to be associated with transgene deletion. Such viruses tend to grow poorly and are not fully competent.

In lentivirus, the risk of reversion to the wild type is virtually non existent as the lentivirus lacks four structural genes that reside on non homologous plasmids in the packaging line. Furthermore, the experimental vectors lack a wild type 3’ LTR that is essential for efficient viral replication.

The recombinant genes to be expressed from the viral vectors are potential oncogenes, possibly contributing to excess proliferation and prolonging the life of damaged or mutant cells. In addition, lentiviral transduction leads to the stable integration of the virus into the host cell's genome. However, as tumourigenesis is recognised to require multiple events (e.g. further mutations in growth pathways), it is unlikely that a single viral infection will result in the formation of a tumour.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Disinfection is achieved using Virkon (peroxyen compound). A 1% Virkon solution is expected to kill 100% in 10 min.
- All liquid waste will be collected and treated with a combination of autoclaving and disinfection methods.
- Pipette tips will be discarded directly into Sharpsafe container, autoclaved, then disposed as clinical waste.
- Plastic pipettes and other solid waste will be soaked in Virkon over night then autoclaved.
- All work surfaces will be treated and wiped down with Virkon.
Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Project Ref 130/14.2

Date Ackn'd 13/06/2014
CU2 Project Title Using lentiviral vectors for gene knockdown and expression in eukaryotic cells

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 ≤ 1 Litre

Non-GMM Consent Granted

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Cardiff University Genetic Modification and Biological Agents Safety Committee agreed with the classification of the activity as Class 2. They did, however, have a number of queries concerning the local risk assessment. These queries have now been answered to the satisfaction of the Committee.
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The purpose of the project is to modulate expression of genes in eukaryotic cells using lentiviral vectors. Coding sequences of the genes, ribozymes targeting these genes will be cloned into replication-incompetent HIV-1 based lentivirus which will be used to deliver and express the gene of interest in either dividing or non-dividing mammalian cells.

**Recipient or parental organism**

Amplication of constructed lentiviral vectors will be performed using E. coli. Packaging and amplification of the lentiviral particles will use 293 cells.

**Host/vector system**

Following lentiviral vectors will be used in the proposed research: ready to use lentiviral particles for fluorescent proteins (amsbio), 3rd generation lentiviral vector; Pre-made lentiviral expression particles for luciferase (amsbio), 3rd generation lentiviral vector; Promoter-less lentiviral expression vector (amsbio), 3rd generation lentiviral vector; pLenti6/V5-D-TOPO plasmid vector (Invitrogen), 3rd generation lentiviral vector.

Amplication lentiviral vectors will be performed in E. coli, whilst packaging for lentiviral particles will be carried out in 293 cell.

Investigation of the effect on tumour cells and other cell types involved in tumourigenesis, angiogenesis and metastasis including breast cancer (MCF-7, MDA-MB-231, ZR-751 and BT549), prostate cancer (PC-3, DU-145, LNCaP, CAHPV-10), Lung cancer (A549 and SK-ems-1), colorectal (HRT-18, HT-115, CACO2), osteoblast (hFOB), fibroblast (MRC-5) and vascular endothelial cells (HECV, HUVEC and HBMEC).

**Origin & function**

Synthetic cDNA of human mRNA from normal human prostate and breast tissues will be used for amplify CDs pf BMP7, Noggin, Follistatin respectively; cDNA of human placenta tissue will be used to amplify CDs of Gremlin; ribozymes containing complementary oligonucleotides of target genes, i.e. BMP-7, Noggin, Follistatin and Gremlin for knockdown studies; Label the cells with luciferase or fluorescent protein for in vitro and in vivo tumour models.

**Evaluation of foreseeable effects**

Deletion of accessory genes vpr, vpu, vif and nef in the lentiviral vectors lead to an incompetency of replication once the vectors have been transduced into target cells. In addition third generation lentivirus are deleted for the tat gene and carry SIN deletion of the 3’ LTR which results in “self-inactivation” of the lentivirus following transduction of the target cell, which can prevent adventitious activation of the vector by endogenous retroviruses (ERVs) and minimising the risk of recombination with ERVs.

The currently proposed research using lentiviral vectors will focus on the role played by TGF-beta family in bone metastasis from three common cancers, i.e. prostate cancer, breast cancer and lung cancer. The target gene/molecules include TGF-beta and bone morphogenetic proteins (BMPs), their receptors, antagonists and the corresponding intracellular signalling molecules Smads. Inserts into the lentiviral vectors will be the coding sequence of the corresponding human genes, ribozyme or interfering RNA (shRNA or siRNA) targeting the genes. To date none of these genes have been shown to be oncogenic or alter the properties of the genetically modified viral vectors. More details for the genes/molecules and the relevant risks are provided in the Excel spread sheet.

None of the genes in the currently proposed research has been shown to be oncogene. Overexpression or suppression of the expression have been performed in other studies. The alterations in their expression are not expected to result in the expression have been performed in other studies. The alterations in their expression are not expected to result in any form of substantial hazard. To date lentiviral vectors offer the most successful means of transduction in these cells, and can not be avoided.

Gremlin has been indicated as one of upregulated gene in Hereditary mixed polyposis syndrome which may contribute to tumourigenesis in large intestine (Jaeger 2012). However, as this gene is widely expressed by many different types of cells in various tissues. There is no evidence showing a direct link of Gremlin to tumourigenesis. To jeopardize human health, the knock down the expression of a tumour suppressor would have to occur within the human body. Entry into the body and human cells is not very efficient. Should knock down of a tumour suppressor gene occurs in vivo delivery, it is not likely by itself to cause cellular transformation. Additional genetic events would be required. Nevertheless, ACGM2 containment failities are designed to prevent operator exposure and are appropriate to control this potential hazard.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

In accordance with the SACGM Compendium of Guidance, GMM mammalian cells will be handled at containment level 2 and using microbiological safety cabinets. All GM virus waste will be properly inactivated by autoclaving. Spillages will be soaked with tissue paper, which will afterwards be autoclaved. Contaminated surfaces may be decontaminated with 70% Ethanol.

Only plastic pipettes will be used in the tissue culture facilities. Furthermore, all contaminated waste destined for subsequent incineration will be autoclaved. All precautions will be taken to identify procedures or devices that might be used in these projects which could cause an injury to the researchers. Where so identified safe alternative methods or equipment will be sought. Sharps will not be used.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GM virus waste will be properly inactivated by autoclaving. Spillages will be soaked with tissue paper, which will afterwards be autoclaved. Contaminated surfaces may be decontaminated with 70% Ethanol. Liquid waste will be disinfected using Chlorine-based disinfectant, the expected degree of kill is 100%. How validated the efficacy of the procedure has been demonstrated experimentally (in-house evaluation). Recombinant viruses will at all times be maintained in an environment designed to prevent aerosol spread. All flasks containing the virus shall be kept closed except when decanting and pipetting which will be conducted in tested class II safety cabinets. Centrifugation steps shall be performed in sealed buckets that will be opened only in safety cabinets. Contaminated pipettes will be immersed in Actichlor for a minimum of 4hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with chlorine-based disinfectant according to an approved protocol. Access to laboratories employed for virus work will be restricted.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The GMSC had a number of queries with the initial risk assessment presented. The risk assessment was altered as requested, answers given to the queries and the GMSC is now satisfied that the risk assessment submitted is suitable and sufficient.

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
The aims of the project is to generate induced pluripotent stem cells by viral based genetic reprogramming of somatic cells of patient and healthy individuals. Induced pluripotent stem cell (iPSC) technology is a novel method for programming somatic cells directly into pluripotent cells by introducing a defined set of transcription factors such as Oct 4, Sox2, c-Myc, and Klf4. iPSC are functionally equivalent to embryonic stem cells. Thus, via iPSCs, all somatic cells are interconnected by the reprogramming route. Studying iPSC will improve knowledge of the molecular process that occurs during reprogramming as well as to model human diseases. iPSCs offer promising donor sources for autologous cell based therapies. Patient-specific iPSCs provide a new mean to investigate the etiology of complex disorders, such as schizophrenia.

We plan to establish human iPSC lines from human fibroblasts and keratinocytes donated by patients with neuropsychiatric conditions and healthy volunteers. We will generate cortical projection neurons, cortical interneurons and dopaminergic neurons from three iPSC cells and to study the potential pathophysiological defects relevant to the neuropsychiatric diseases.

Recipient or parental organism

Replication deficient Sendai viral vector simultaneouly expressing the four reprogramming factors will be purchased as ready packaged from commercial sources (eg. CytoTune® -iPS 2.0 Sendai Reprogramming Kit from Life Technologies). The virus will be used to transduce human keratinocytes and potentially other somatic cell types of human origin.
The lentiviral vector, pSIN4 come in attenuated E. coli strains Stbl3. HEK293T cells will be used to package these vectors into virus, and the viruses will be used to transduce human fibroblasts.

### Host/vector system

| E.coli: Stbl3  | Host for viral packaging: HEK293T |
| pSIN4 lentiviral expression vector | Sigma -Aldrich 'packaging vector' |
| Sigma -Aldrich 'envelope vector' | SeV/dp Sendai viral vector system |

### Origin & function

The viral vectors will contain cDNA inserts of human origin that encode transcription factors Oct4, Sox2, Klf4 and potentially cMyc. The Oct4, Sox2, Klf4, and cMyc (OSKM) genes are ordinarily expressed in embryonic stem cells. The introduction and expression of these genes in differentiated cell type’s results in the effected cell reverting to an embryonic stem cell-like pluripotent state. The final viral vector is designed to introduce the OSKM genes to the host cell and facilitate long term stable expression of the genes for the purposes of generating iPS cell cultures.

### Evaluation of foreseeable effects

The transcription factors to be used in these experiments come in lentiviral (HIV)-plasmid (pSIN) and will be cotransfected into packaging cells (HEK293T) alongside plasmids for packaging proteins (MISSION lentiviral packaging mix, Sigma-Aldrich) to produce lentiviral particles. The lentiviral vector systems have been developed with enhanced safety features. It is a third generation lentiviral vector system, with two plasmids consisting of:

1. The packaging vector, which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions. 2. The vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector, which provides the heterologous envelope for pseudotyping.

The lentiviral vectors are third generation HIV-1 based lentiviral systems with advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and are replication incompetent once transduced. However the viruses are still able to transduce dividing and non-dividing primary human cells and will stably integrate into the host genome.

Sendai virus are based on defective and persistent Sendai virus vectors SeVdp) lacking the viral M, F and HN genes, and are non-transmittable. They replicate in the forms of single-stranded RNA in the cytoplasm of the infected cells. They do not go through a DNA phase nor integrate into the host genome. It replicates its genome exclusively in the cytoplasm and does not enter cell nucleus. Therefore, Sendai virus vector is integration free and unlikely to alter the host chromosomes like the conventional viruses.

One of the reprogramming factor (cMyc) is an oncogene with the capacity to trigger unwanted proliferation in infected cells. Therefore there is a very small but identifiable risk to an infected individual of long term alteration of gene expression. However only cells that come in direct contact with the packaged virus could become infected.

To minimize the possibility of self inoculation, personal protection measures will be taken, for example needles will not be used when handling biological agents and appropriate personal protection will be used (Lab coat, gloves, safety glasses). To minimize any possible aerosol effects of the viral stocks, appropriately capped centrifuge bottles and screw-capped tubes will be used. Virkon has been shown to completely inactivate lentivirus and Sendai virus at a final concentration of 1:100 and so will be used to thoroughly clean everything involved in these experiments.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Disinfection is achieved using Virkon (peroxynate compound). A 1% Virkon solution is expected to kill 100% in 10 min.

All liquid waste will be collected and treated with a combination of autoclaving and disinfection methods.

Pipette tips will be discarded directly into sharpsafe container, autoclaved, then disposed as clinical waste.

Plastic pipettes and other solid waste will be soaked in Virkon over night then autoclaved.

All work surfaces will be treated and wiped down with Virkon.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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**Project Ref** 130/15.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/06/2015</td>
<td>Characterization and Treatment of Mouse Models of Epithelial Carcinoma</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
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</table>
Project Additional Information

**Purposes of the contained use**

To introduce an adenoviral vector (Ad5-CMV-cre or Ad5-cre-IRES-GFP) to specific mouse tissues (ovary and lung). The vector encodes a cre recombinase enzyme and hence causes recombination between loxP sites in DNA, to delete specific genes in relevant genetically-modified mice (as listed in the connected project).

**Recipient or parental organism**

We will use GM mice with 10xP-flanked DNA sequences, such that specific genes will be deleted by introduction of the adenoviral cre. For the ovarian work, we propose to delete the tumour suppressor genes apc and pten, by intra-bursal injection of the virus. For the lung work, the tumour suppressor p53 will be deleted and the k-ras oncogene will be over-expressed, by nasal inhalation of the virus.

**Host/vector system**

Ad5-CMV-cre is a commercially-available, replication-deficient virus which is routinely used to inactivate genes in mice in a tissue-specific manner. It does so by expressing the cre recombinase enzyme in cells in to which it has been delivered, thus allowing recombination in host genes carrying 10xP-flanked sections of DNA. The virus has been attenuated due to deletion of the E1 and E3 regions of its genome. We may also use an additional vector, AdS-cre-IRES-GFP, which is identical to Ad5-CMV-cre, but has an additional marker gene (GFP) to analyse expression of the cre recombinase enzyme.

**Origin & function**

The viruses encode the gene for the cre recombinase enzyme, which originates from Bacteriophage P1. The gene will be expressed only within cells into which it has been introduced. The sole function of the cre recombinase enzyme in these cells is to cause recombination between lox? sites in host DNA, which causes deletion of genetic material between these sites (gene deletion). The lox? sites also originate from Bacteriophage P1 and have been engineered into mice via the germline. Only genes carrying these sites will be affected by the cre recombinase.
Evaluation of foreseeable effects

As the viruses to be used in this project are replication-deficient, due to deletion of their E1 and E3 genes, they are unlikely to cause any effects on humans or the environment. However, human adenovirus is known to cause mild flulike symptoms in humans and, as such, the virus will only be handled in a class II hood, either during aliquotting (in Hadyn Ellis Building viral suite) or during delivery to mice (in JBIOS animal unit, School of Biosciences).

The addition of the genetic modification (the ere recombinase gene) to these viruses is thought to have no effect on their ability to survive in host organisms or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GM mice will be housed in a conventional animal unit in standard cages.

Health and Safety

Executive

Following exposure to Adenovirus, mice will be housed in an isolator within the unit for 2 weeks, before being returned to standard caging. Cages used to house the mice within this 2 week period will be disinfected with bleach and all bedding and other disposable material will be bagged, boxed and sent for incineration.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

It is possible that aerosols may be generated during administration of the virus to the mice, hence all such work will be carried out in a class II safety cabinet. All waste generated will be inactivated by autoclaving or appropriately packaging and transporting off site for incineration (SRCL waste contractors).

Mice which have received the virus will be left to survive for at least several weeks following viral administration. Cages used to house the mice within the 2 week period following exposure to virus will be disinfected with bleach and all bedding and other disposable material used in animal care will be packaged and sent for incineration. The carcasses of any infected mice which die or are sacrificed within this period will be disposed of by incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The Committee were in agreement with the Classification assigned by Professor Clarke to the activities. A small number of alterations to the risk assessment were requested, which have now been made and the Committee is now satisfied that the risk assessments are suitable and sufficient.
Project Containment

Laboratory Activities

L2 Yes L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4

Glass Houses

Animal Units

L2 Yes L3 L4 L2 L3 L4 L2 L3 L4

Large Scale Activities

Human Clinical Applications

Laboratory Activities Glass Houses Growth Rooms

Project Ref  130/15.2

Date Ackn'd 03/09/2015

CU2 Project Title Development of oncolytic adenoviral vectors suitable for targeted cancer cell killing through selective transduction and replication in cancer cells and production of therapeutic proteins and antibodies

Date Project Ceased

Class CultureVol Class 2 Culture Volume Class 3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work

Y

Project Additional Information

Purposes of the contained use

Globally, an estimated 14 million people are diagnosed with cancer each year, accounting for ~8 million deaths. New, more efficacious treatments for cancer are therefore required urgently to combat this. The purpose of this application is to develop a programme of research seeking to develop viral vectors which can be used, ultimately clinically, as a novel means for treating cancer ("virotherapy" using "oncolytic viruses"). This can promote cell killing through (a) selective viral replication in cancer cells resulting in cellular lysis and spread of virus to surrounding cells (b) expression of an apoptosis inducing protein or cell sensitizing protein (e.g. an enzyme which can convert a nontoxic prodrug into a toxic drug) or protein/antibody designed to increase the immune recognition of the infected tumour.
cell, and (c) increased immunogenicity against the tumour cells through the presence of a viral particle within the cell, promoting an increased host anti-tumour response. With this in mind, I propose to split this connected programme of research into the following areas:

1. Generation of tropism modified adenoviral vectors.

Overview: In this section of work, I will use replication deficient adenoviral vectors available in house, based on human adenovirus serotype 5 (Ad5) to generate a bank of viruses that are "detargeted" for known tropism dictating receptor interactions, including (but not limited to) fiber: heAR interactions, penton base: cellular (Qv~3/5) integrin interactions and hexon interactions with blood clotting factor X (FX)/complement proteins/scavenger receptors. In order to "retarget" tropism more specifically to cancer cells, I will generate Ad5 based vectors incorporating peptides in key capsid proteins (fiber, hexon, penton) which target to receptors upregulated on tumour cells, including (but not limited to) EGFR, FGFR1, Qv~6 integrin, CD133, CD44, nestin. Additionally I will engineer the virus to include biotinylatable peptides so as to enable to linkage of larger targeting ligands (e.g. antibodies) onto the viral capsid. All vectors in this section of work will be encoding reporter genes (e.g. luciferase, B-Gal, eGFP), and findings from this section may be used in the design of targeted oncolytic vectors in future sections of research.

2. Use of wild type adenoviruses from several species, evaluation for cancer cell killing and genome capture for onward manipulation in BACs.

Overview: In this section of work, I will evaluate the ability and selectivity of adenoviruses from alternative adenoviral species, to infect and kill cancer cells. Specifically, I will focus on adenoviruses from species Band D which demonstrate lower seroprevalence rates compared to Ad5 (PMID: 17329340), and utilise as yet undocumented receptors. Furthermore, my preliminary data (from a previous post, some of which is now published, PMID: 17329340) demonstrated that vectors based on species 0 adenoviruses show dramatically increased levels of transduction when compared to Ad5 (widely considered as the "gold standard"). In this section of work I propose to capture the wild type viral genome in a BAC (bacterial artificial chromosome) which will enable onward engineering into a reporter gene expressing, replication deficient vector for further evaluation through recombineering technologies. Derived vectors will be assessed for receptor usage and host protein interactions, and point mutations within key proteins will be introduced to manipulate/prove these interactions. All vectors in this section will either be wild type (i.e. not encoding any transgene) or encoding reporter gene(s).

3. Directed evolution of adenoviral vectors for optimised cancer cell killing.

Overview: in this section of work, I will select for viral recombinants with improved selectivity/cell killing of specific cancer cell lines. Using the same panel of adenoviruses (as described in (2)) at high titers to force recombination and evolution of viral vectors with the best selectivity and cell killing ability in different cancer types (focussing initially on 3 types of cancer, namely Ovarian, Panreatic and Esophageal). This strategy will replicate previous research (PMID: 18560559) which generated the oncolytic adenoviral vector, ColoAd (Enadenotucirev), which is now being evaluated in phase 1/11 clinical trials in colorectal cancer following intratumoural or intravenous delivery. The best candidate chimaeric vectors will be captured within BAC based vectors for onward engineering and development as therapeutics. All vectors tested with in th is section of research will be either "wild type" (i.e. not encoding a transgene) or encoding reporter gene(s).

4. Development of oncolytic forms of adenoviruses selective for cancer cells by introducing mutations in the genome to render the virus replication selective for cancer cells.

Overview: In th is section of work, we will introduce mutations within the adenoviral genome which render the virus "oncolytic", i.e. only able to replicate within cancer cells. These include deletion of the E1 B55k protein, which has been shown to enable replication selective in p53- cells (e.g. see PMID: 9176490), delta 24 mutation in E1A protein (e.g. as utilised clinically in PMID: 23756180), and the potency enhancing T1 mutation (see PMID: 25093639), or
combinations thereof. Viral vectors tested within this section of research will be either "wild type" (not encoding any transgene) or encoding reporter genets).

5. Incorporation of miRNA binding sites or tumour specific promoter sequences to generate cancer selective viral vectors at the level of transcription/translation.

Overview: As alternative strategies to ensure tumour selective viral replication, we will 1. incorporate miRNA binding sites to silence adenoviral expression of essential early viral genes in "off target" cells (i.e. where the relevant miRNA expressed at high levels in off target cells), and/or 2. the inclusion of tumour specific promoters into the adenoviral genome to limit the expression of critical early genes to tumour cells.

6. Development of armoured virotherapies encoding biological genes that may be beneficial in cancer (e.g. that interfere with checkpoint blockade).

Overview: In this section of work, biological genes will be encoded within the selected viruses outlined in sections 1-5. This will allow assessment of efficacy both in vitro and in vivo in preclinical models. Examples of genes that might be encoded would include (but not limited to) genes which express a protein that sensitises a cancer cell to a prodrug (e.g. cytosine deaminase, nitroreductase), genes which affects host immune recognition of tumour cells (e.g. antibodies that interfere with PD-1, PD-1, CTLA-4) or tumour suppressor genes (e.g. p53).

7. Development of visualisable (detectable) oncolytic adenoviral vectors for quantitative evaluation of virotherapy efficacy in preclinical cancer models.

Overview: In this section of work we will seek to develop viruses which can be utilised to visualise oncolysis in real time using fluorescence based techniques. We will use (and compare) both replication deficient and oncolytic Ad based vectors encoding transgenes (most likely GFP) and genetically incorporate a fluorophore, e.g. mCherry in the capsid protein. This will allow us to simultaneously visualise cellular transduction (thought expression of the transgene, GFP) and viral spread (through visualisation of viral capsid protein: fluorophore fusion protein) in real time using sophisticated imaging software. Other combinations of reporter gene/fluorophore may also be evaluated.

Recipient or parental organism

Adenovirus - most work will involve serotype 5 (species C), though large sections of the work will develop a number of species Band 0 adenoviruses also (available from ATCC or ECACC), and generate chimaeric versions of these viruses.

Host/vector system

Manipulation of the adenoviral genome will be achieved by recombineering technologies in bacteria, based on red lambda genetics, and the presence of the viral genome captured within a BAC based plasmid.

Replication deficient Ad based vectors will be propagated in complementing cell lines (e.g. 293, TRex, 911, PerC6 cells), whilst replication competent viruses will be produced in either 293 cell lines or any other relevant permissive cell lines (e.g. A549 cells).

Tropism/cell killing will be evaluated in a wide range of cell lines and primary cells of human origin as well as cells from other origins (e.g. murine).

We expect to advance of leading formulations into model animal systems based on murine tumour xenograft and allograft models.

Origin & function

1. Reporter genes. (a) GFP (Green Fluorescent Protein) refers to the protein first isolated from the jellyfish Aequorea victoria and is a 238 amino acid protein (26.9 kDa) that exhibits bright green fluorescence when exposed to light in the
blue to ultraviolet range. (b) Luciferase refers to the generic term for the class of oxidative enzymes used in
bioluminescence. One example is the firefly luciferase from the firefly Photinus pyralis (62kDa protein, of 1616bp), and
this is the version we will use primarily, although we may also make use of Renilla luciferase, derived from the fancy
sea pansy, Renilla reniformis, (c) β-galactosidase, also called beta-gal or β-gal, is a hydrolase enzyme that catalyzes
the hydrolysis of β-galactosides into monosaccharides. Originally isolated from the bacterium, E. coli, the gene of β-
galactosidase, the lacZ gene, is present as part of the inducible system lac operon which is activated in the presence
of lactose when glucose level is low, (d) mCherry - originally isolated from the mushroom coral Discosoma sp, ie a
fluorophore used as a marker when tagged to molecules and cell components.

2. Prodrug sensitizing genes. In this section of work, genes will be cloned into the virus for potential Virus Directed
Enzyme Prodrug Therapy (VDEPT) approaches. This approach requires the expression of an enzyme within the
target cell that can convert a harmless prodrug into a cytotoxic drug. A table showing possible enzyme/prodrug
combinations that may be evaluated is outlined below

<table>
<thead>
<tr>
<th>Enzymes delivered</th>
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<tbody>
<tr>
<td>Herpes simplex virus thymidine kinase</td>
</tr>
<tr>
<td>Human carboxysterase</td>
</tr>
<tr>
<td>E. coli nitroreductase</td>
</tr>
<tr>
<td>Prod rug</td>
</tr>
<tr>
<td>GCV</td>
</tr>
<tr>
<td>Irinotecan</td>
</tr>
<tr>
<td>CB1954</td>
</tr>
<tr>
<td>Yeast cytosine deaminase 5-FC</td>
</tr>
<tr>
<td>Human CYP and p450 reductase Cyclophosphamide and IFA</td>
</tr>
</tbody>
</table>

3. Inhibitors of checkpoint blockade. Recent advances in understanding the role of checkpoints in immune tolerance
and the use of antibodies to interfere with these axes (so called "checkpoint blockade") have resulted in dramatic
clinical improvements using antibodies directed at CTLA-4 (Ipilimumab) and the PD-1/PD-L 1 axis (Nivolumab). In this
section of work, antibodies targeted against these key modulators of checkpoint blockade will be incorporated into the
Ad genome for overexpression studies. These DNA constructs have been made available to us in plasmid form (from
Prof Guy Ungerechts and Dr Christine Engerlaand, Heidelberg University, Germany).

4. Tropism modification. In order to alter adenoviral tropism, point mutations will be introduced into the capsid to
abrogate defined receptor interactions or other interactions known to influence viral tropism and toxicity. This will be
performed in combination with the introduction of peptides into the capsid proteins (synthetically produced, sequences
derived from bacteriophage biopanning strategies) that bind to alternative receptors (as outlined in part 1 (section 6)
of the outlined work).

Evaluation of foreseeable effects

For the wider connected programme of work, the following worst case scenarios apply:

1. Generation of tropism modified adenoviral vectors.

WORST CASE SCENARIO: The worst case scenario for this project would be that a worker would inadvertently
inoculate themselves with a huge dose of virus that was (1) targeted to an alternative receptor, and (2) harder for the
immune system to recognise and clear. In this scenario, should the virus enter the bloodstream, the biodistribution of
the recombinant virus may be altered compared to that of a wild type AdS viral capsid (which would predominantly
locate to the liver, and to a less extent the spleen). Mutations introduced to detarget the virus may also render the
virus harder for the host to clear. However, the virus is rendered replication deficient it would not form a productive
infection.

2. Use of wild type adenoviruses from several species, evaluation for cancer cell killing and genome capture for
onward manipulation in BACs.

ENVISAGED WORST CASE SCENARIO: This section of research involved the study of wild type adenovirus. The envisaged worst case scenario therefore in this section of work would be that a worker inadvertently self-innucleated with a high dose of fully replicative, wild type adenovirus. In the vast majority of cases, infection with a wild type adenovirus causes a transient, non-life threatening infections, mainly of respiratory and GI tract, although some species D adenoviruses (e.g. Ad37, Ad19a) may also cause conjunctavitis and tonsilitis. Adenoviral infections are typically transient and self limiting, with virus symptoms typically lasting 7-7 days. The only exception to this is in the immunocompromised individual, and therefore all staff working on this project will be made aware of the requirement to declare if they are using immunosuppressive drugs, and any such individual will be prevented from working with adenovirus. To minimise the potential for infection, all work will be conducted under containment level 2 conditions, including the use of class 2 level biosafety cabinets.

3. Directed evolution of adenoviral vectors for optimised cancer cell killing.

ENVISAGED WORST CASE SCENARIO: This section of research involved the study of wild type adenovirus, and chimeric adenoviruses generated by recombination between viral strains. The envisaged worst case scenario therefore in this section of work would be that a worker inadvertently self-innuclelated with a high dose of either a wild type adenovirus (as in section 2) or a chimeric adenovirus generated through the research programme. In the vast majority of cases, infection with a wild type adenovirus causes a transient, non-life threatening infections of the respiratory and GI tracts, although some species D adenoviruses (e.g. Ad37, Ad19a) cause conjunctavitis and tonsilitis. Adenoviral infections are typically transient and self limiting. As adenoviral infections can be significantly more problematic in the immunocompromised individual, as in section (2), all staff working on th is project will be made aware of the requirement to declare if they are using immunosuppressive drugs, and any such individual will be prevented from working with adenovirus. The chimeric adenoviral variants generated in this programme are unlikely to have different tropism or higher levels of virulence than the parental viral hosts in non-transformed cells, but will be selected for their ability to replicate selectively and rapidly in cancer cells, as previously published (PM ID: 18560559), and therefore this cannot be ruled out entirely. Chimeric viruses generated will be evaluated for their selectivity for replication in cancer cells (compared to non-transformed cells) and any chimeric virus not demonstrating improved selectivity for tumour cells (and therefore an improved safety profile) will not be pursued for onward studies. To minimise the potential for infection, all work wi ll be conducted under containment level 2 conditions, including the use of class 2 level biosafety cabinets.

4. Development of oncolytic forms of adenoviruses selective for cancer cells by introducing mutations in the genome to render the virus replication selective for cancer cells.

ENVISAGED WORST CASE SCENARIO: This section of research involved the study of wild type adenovirus, and viruses that have been engineered to be selective for replication only in tumour cells ("oncolytic" adenoviral vectors). The envisaged worst case scenario therefore in this section of work would be that a worker inadvertantly self-innuculated with a high dose of fully replicative, wild type adenovirus, and therefore the worst case scenario would be as stated for (2) and (3). For the scenario where a staff member self-innuclelated with an "oncolytic" adenovirus, the risk is considered to be lower than with the wild type virus, and viruses based on the described tumour-selective mutations have been trialled clinically, with a similar derivatives (H1 01) having previously gained regulatory approval in China for treatment of Head and Neck Cancer (see PMID: 16507823). An in the previous sections all staff working on this project will be made aware of the requirement to declare if they are using immunosuppressive drugs, and any such individual will be prevented from working with adenovirus. To minimise the potential for infection, all work will be conducted under containment level 2 conditions, including the use of class 2 level biosafety cabinets.

5. Incorporation of miRNA binding sites or tumour specific promoter sequences to generate cancer selective viral vectors at the level of transcription/translation.
ENVISAGED WORST CASE SCENARIO: The envisaged worst case scenario in this section of work would be that a worker inadvertently self-innociulated with a high dose of genetically modified adenovirus, where the transgene is under the control of a tumour selective promoter (so gene expression is only switched ON in tumour cells) or a miRNA binding site (to switch OFF expression of a transgene (at the mRNA level) in non-tumour cells). It is expected that these "safety switches" would lower the potential risk of pathogenesis, and therefore the relative risk is likely to be lower than those outlined above (in 2 and 3). It is likely that any individual self inoculating with such a virus would experience a transient, self limiting infection, as described above, with virus symptoms typically lasting -7 days. As previously, all staff working on this project will be made aware of the requirement to declare if they are using immunosuppressive drugs, and such individual will be prevented from working with adenovirus. To minimise the potential for infection, all work will be conducted under containment level 2 conditions, including the use of class 2 level biosafety cabinets.

6. Development of armoured virotherapies encoding biological genes that may be beneficial in cancer (e.g. that interfere with checkpoint blockade).

ENVISAGED WORST CASE SCENARIO: The envisaged worst case scenario for this section would be that a worker would inadvertently self-inoculate with a large dose of virus that was (1) replication competent, (2) expressing a transgene that has a biological function (e.g. interferes with immune tolerance), (3) shows an altered tropism compared to the parental virus. In this scenario, the worker may experience transient productive infection (although this should be restricted by the mutations that render the virus cancer selective). The infected cells may be nonnative, through the introduction of tropism modifications that render the virus more selective to tumour cells and less selective to "normal" (i.e. non-transformed) cells. Where a productive infection does occur, the infection should be transient and non-life threatening (at least in the non-immunocompromised individual, as is observed for "wild type" adenoviral infections). The expression of a biological gene product interfere with immune recognition (e.g. anti-CTLA4/PD1/PD-L 1) may positively influence the ability of the body to recognise infected cells and targeted them for clearance. As previously, all staff working on this project will be made aware of the requirement to declare if they are using immunosuppressive drugs, and such individual will be prevented from working with adenovirus. To minimise the potential for infection, all work will be conducted under containment level 2 conditions, including the use of class 2 level biosafety cabinets.

7. Development of visualisable (detectable) oncolytic adenoviral vectors for quantitative evaluation of virotherapy efficacy in preclinical cancer models.

ENVISAGED WORST CASE SCENARIO: The envisaged worst case scenario scenario in this section of work would be that a staff member were to self inoculate with a high dose of oncolytic adenovirus that is genetically labelled with a fluorophore in the capsid protein. The presence of the fluorophore in the capsid protein should not have any effect the level of risk, and therefore the risk can be considered to be more or less identical to that outlined in section (4). It is likely that any individual self inoculating with such a virus would experience a transient, self limiting infection, as described above, with virus symptoms typically lasting -7 days. As previously, all staff working on this project will be made aware of the requirement to declare if they are using immunosuppressive drugs, and such individual will be prevented from working with adenovirus. To minimise the potential for infection, all work will be conducted under containment level 2 conditions, including the use of class 2 level biosafety cabinets.

Use of wildtype adenoviruses has previously been notified by Prof Gavin Wilkinson.
All Adenovirus activities in Cardiff are considered as BSL2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Mice may be used in this study, but will not be genetically modified, rather they will be inoculated with a GMM vector, and therefore will be be treated with suitable control measures, with all animal carcasses and bedding destroyed by incineration. Human adenoviruses cannot replicate in the mouse and therefore there is no possibility of a productive
infection forming in this model. For some aspects of the in vivo work, mice may be inoculated with tumour cells, grown subcutaneously under permission granted in Home Office Project License ref PPL 30/3224.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

NIA

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Viruses will be maintained in an environment designed to prevent aerosol spread (biosafety level 2 tissue culture hoods, under negative pressure). All plastics containing the virus shall be kept sealed/closed except when decanting and pipetting which will be conducted in tested class 11 safety cabinets, and virus containing waste media will be treated with immediately by discarding into a waste chloros (bleach) solution. The use of Sharps when handling virus preparations will be minimised. Contaminated pipettes or other contaminated waste plastics will be immersed in Actichlor for a minimum of 4hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with chlorine-based disinfectant according to an approved protocol. Access to laboratories employed for virus work will be restricted.

Disinfectant Product Name: Actichlor (2500 ppm sodium dichloroisocyanurate)
Generic Chemical Name: Chlorine-based disinfectant
Expected degree of kill 100%
How validated: Adenovirus infectivity in collection buffers can be validated by reinfection assay.
For in vivo studies, all animal carcasses and bedding destroyed by inCineration, and housing will be cleaned down with chlorine based disinfecants prior to washing..

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Project 1 “Generation of tropism modified Adenoviral vectors” GM130/683, has been fully risk assessed and the risk assessment reviewed by the Committee. The Committee were satisfied that this was a Class 2 activity requiring Containment Level 2 facilities and after a number of queries were answered and alterations made to the risk assessment that the risk assessment was suitable and sufficient.

The Committee also reviewed the the CU2 form with the connected programme of work and the worst case scenarios for each project/part of the programme. They felt that each part could be carried out at Containment Level 2, with the additional control measure used of making staff aware of the requirement to declare if they were using immunosuppressive drugs to declare it as they would be prevented from working on a number of the projects. The Committee agreed that they would be Class 2 activities.

The work on each part of the programme will not be allowed to start until the detailed risk assessment for that part has been reviewed by the Committee with intermediate results from other parts of the programme and published work of others being used to inform the risk assessment. Any results indicating a higher risk requiring higher containment levels will lead to experiments being halted until risk assessments are reviewed by the Committee and appropriate control measures put in place, seeking advice from the HSE re notification of a "significant change".

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Project Ref** 130/15.3

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<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<tbody>
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<td>16/09/2015</td>
<td>Developing cytomegalovirus (CMV) as a vaccine vector</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Consent Granted</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tick if notifying a connected programme of work | N |

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
Project Additional Information

Purposes of the contained use

This notification covers a wide program of work, broken down into sections. Each section will be notified separately to the local GMBA committee for approval. At this time, only the first section of the work will be started, and a detailed risk assessment has been previously submitted to the local GMBA committee as detailed below. The broad aim of the work is as follows:

We have extensive experience working with CMV. CMV has a unique capacity to induce very high levels of polyfunctional T-cells. In the rhesus macaque model of HIV, RhesusCMV is unique in being able to protect against challenge with highly pathogenic SIV. We now seek to harness this ability to protect against cancer.

The sub-sections of this connected project are as follows:
1. CMV is a persistent virus, and will be caught by >90% of people at some point in their lives. In the connected program we have been using the tet-repressor (expressed from a cell line) to control expression of viral genes. This system will be slightly modified to produce a self-contained tet-controlled virus. This will increase the safety of CMV-based vaccine vectors by rendering viruses ‘replication-controllable’, such that they are only able to replicate in the presence of doxycycline. In addition to improving vector safety, this will also enable fundamental questions to be answered surrounding the extent to which chronic replication in vivo is required to generate this unique immune response. This section has been risk assessed in detail and the risk assessment has been reviewed by the local GMBA Committee.

2. Once the tet-regulated vectors have been tested in vitro and in vivo (in mouse), cancer antigens will be inserted into the genomes of the vectors. These viruses will be tested both in vivo and in vitro, in murine models of cancer. We will monitor both the immune response to these antigens, and the ability of these immune responses to control tumour growth and spread. We do not seek permission to perform this work at this time, but will risk assess the procedures in detail through the local GMBA committee, once the safety of the vectors has been assessed (section 1).

Recipient or parental organism

Bacterial artificial chromosome (BAC) clones of the Merlin strain of human CMV, and the smith and K181 strains of mouse CMV. These BACs contain the complete genome of a wildtype HCMV virus or the complete genome of a mouse CMV strain. We already have permission to work with these constructs as part of ongoing projects. CMV is highly species specific, thus MCMV cannot replicate in human tissue and poses little risk to humans, and is ADCP 1. The majority of the population harbour latent HCMV by later life, the virus is controlled by the immune system and does not cause symptoms. HCMV is a cause of congenital malformation if passed to the fetus during pregnancy, thus pregnant workers are given the option of avoiding work in labs where HCMV is being used.

Host/vector system

The HCMV viruses will be grown in human foetal foreskin fibroblasts. The MCMV viruses will be grown in 3T3 cells.

Origin & function

The BACs will be modified such that essential genes are only expressed in the presence of doxycycline (Dox). In the
connected program, we have placed tetracycline operator sites upstream of essential viral genes, and expressed the
tet-repressor (tetR) from a cell line. In this manner we have rendered the expression of these genes under tight doxdependent
control. In this program, we wish to make the system self-contained, by expressing the tet-repressor from
the viral backbone. Thus in the absence of Dox, the tetR binds to the tetO and transcription of the essential genes is
prevented. Since these genes are essential, the vector is rendered replication deficient. However in the presence of
doxycycline, tetR can no longer bind to tetO, and the repression is relieved. Thus the virus becomes replication
competent. In addition to significantly enhancing the safety of the vector, this also enables the construction of
replication defective viruses without the need for complementing cell lines. We may also seek to use a tetON system,
which will be phenotypically identical (i.e, the virus will be replication defective unless Dox is supplied) but differs
mechanistically. In this system, the viral gene is placed under control of a minimal promoter, and is not expressed.
The reverse tet-transactivator (rtTA) is also expressed from the viral genome. When Dox is supplied, it binds to the
rtTA and enables it to bind and activate expression from the minimal promoter.

In the second sub-section (Summary risk assessment below, final paragraph in section 7 'evaluation of foreseeable
effects'. The full detailed risk assessment will be reviewed by the gmba committee when initial work on section 1 of the
connected programme has been carried out), cancer antigens will be inserted into the MCMV/HCMV vectors. These
will cover a number of well established antigens such as 5T4, gp100 and melanA. 5T4 is an oncofetal antigen,
frequently upregulated on cancer cells. gp100 and MelanA are melanoma differentiation antigens.

Evaluation of foreseeable effects

CMV is highly species specific. It is a fragile virus that is highly sensitive to dessication, and thus is highly unlikely to
escape into the environment directly. Although it spreads between humans via infected body fluids (e.g. saliva, urine,
breast milk), spread is relatively inefficient - for example pregnant mothers frequently fail to pass it on to their children.
Spread in the community is markedly reduced by simple hygiene measures such as handwashing.
The only appreciable danger from this work arises from an accident in which a lab worker splashes a solution
containing the virus, and this results in virus accessing mucosal surfaces (e.g, the mouth) of the individual. In the case
of MCMV, replication would be abortive since MCMV cannot repl icate in human cells. Thus MCMV poses negligible
risk. In the case of HCMV, the only differences between our vectors and the parental vector (with which we have
extensive experience) is the presence of tetO sites, and an expression cassette for tetR/rtTA. The effect of these
modifications is to render the virus replication deficient. As a result, the vectors have lower risk than the wildtype virus
- the virus will be unable to spread from the site of infection, and will be unable to establish latency (which requires
gene expression, and occurs following virus spread to CD34+ myeloid progenitors in the bone marrow). However it is
possible that multiple viruses will need to be made, with tet regulation of multiple different genes tested, to determine
a configuration of tetO/tetR/rtTA that results in successful control of replication. Thus there may be viruses produced
which replicate to a similar extent as wildtype virus, despite expressing tetR/rtTA. If a lab worker were to splash a
solution of this virus on their face, and it were to reach mucosal surfaces, this virus would behave the same as a
wildtype virus - i.e. it could establish latency in the individual. The only difference to a wildtype infection is that the
virus would express tetR/rtTA. These proteins are naturally expressed in E.coli, a commensal organism, and are
widely used in vectors intended for gene-therapy applications. The only known function of tetR is to bind to tetO and
control transcription, thus there is no evidence that expression of tetR would have deleterious consequences. In
existing programs of work we have permission to express a wide variety of genes from other species (e.g. GFP,
mCherry) including bacteria (e.g. LacZ, gpt) from CMV. The risk of these new viruses is, under the absolute worst
case scenario (accidental infection of a worker with a virus in which the tet-control fails) identical to the risk of those
previous constructs.

In the second section of this project, cancer antigens will be inserted into the vectors. This will only be done once the
tet-regulated viruses have been evaluated in vivo, and demonstrated to be replication deficient. 5T4 is an oncofetal
antigen, overexpression can disrupt cell1-cell contacts and increase cellular motility. No functions associated with
expression of gp100 and melanA have been reported. All humans tested have a natural CTL response against these proteins, although this response dissipates during cancer. A human trial using a 5T4 expressing modified vaccinia ankara (MVA) vector is currently underway in Cardiff by our collaborators (AGallimore and A Godkin), and other human trials of viral vectors expressing all these proteins were all well tolerated. Given that these antigens have all been well tolerated in human trials already, there is little risk from expressing them in well characterised, replication deficient HCMV vectors. The highest risk antigen is 5T4, since there is a theoretical risk that accidental inoculation of a pregnant worker with a 5T4 expressing vector could induce immunity to 5T4 expressed on the fetus. As mentioned above, this is unlikely to cause problems since all humans have a pre-existing immune response to 5T4 anyway. However to mitigate against any potential risks, as with the 5T4 MVA vector that our collaborators (A.Gallimore and A.Godkin) already have permission to use, pregnant workers will be given the option of avoiding working in a lab where the 5T4 vector is being used. This rule is already in place, since we work with HCMV, and pregnant workers are already given the option of working in different laboratories when HCMV is being used. Once expression of the antigen has been verified, the ability of these vectors to induce a specific immune response in vitro, in humanised mice, and in murine models of cancer will be determined, as will the ability of these immune responses to protect against tumour development, and to prevent metastasis.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The virus will only ever be used at containment level 2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

CMV is completely killed by 70% ethanol, hypochlorite and autoclaving. Tissue culture flasks are autoclaved, then incinerated. All other plasticware such as pipette tips, pipettes and storage tubes, are soaked overnight in hypochlorite then autoclaved before being incinerated. All surfaces in the class 2 hood are wiped down before and after use with 70% ethanol. These methods are validated by culture.

Whilst handling virus or virus-infected mice in the animal facility, staff will wear two pairs of gloves, disposing of the outside pair once finishing handling vectors infected mice/tissue. All work (including dissections) will be performed in a Class 2 Microbiological Safety Cabinet and mice will be kept in scantainers. These procedures are currently performed routinely and have successfully contained MCMV - as shown by the absence of infection in naive mice housed in adjacent cages to infected mice.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Sub Section 1 - Risk Assessment GM130/681 has been reviewed by the Committee on several occasions with alterations/additions being made at each stage until the Committee felt that the project had been adequately risk assessed. The Committee agreed with the activity classification and the containment level required.

Sub Section 2 - The Committee reviewed the CU2 form for the connected programme of work and the worst case scenario/summary risk assessment within. The Committee are in agreement with the anticipated activity class and containment level required, however permission will not be given for the work to go ahead until the Committee has reviewed a detailed risk assessment for this work. The Committee also expects that if, during the course of the work, evidence arises pointing to risk levels going beyond what can be contained in Level 2 facilities, that they will be notified by the Principal Investigator and work will be stopped immediately. The Risk Assessment would then be updated and reviewed by the Committee to discuss the class of the activity and the containment measures required. Advice would be sought from the HSE as to whether or not a notification of significant change would be required.

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### Project Ref 130/16.1

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<td>17/11/2016</td>
<td>Using a modified rabies virus for monosynaptic tracing of neurones</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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</table>

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

There are 2 overall aims of this project:

1. **Activity Notification**
   - To generate a recombinant, glycoprotein deleted rabies viral vector pseudotyped for the avian EnvA protein, limiting infectivity to cells expressing the TVA receptor.
   - Using this recombinant vector, trace the pattern connectivity and circuit integration of iPSe derived neurons in vitro.

Recombinant rabies viruses and their wild-type counterparts are highly effective at infecting and traversing mammalian 'nervous systems, spreading transynaptically without generating cytopathology or invading surrounding unconnected cells. These features make them an ideal choice for the study of neuronal systems and structures both in vivo and invitro.

**Recipient or parental organism**

The lentiviral vector, pSIN4 come in attenuated E.coli strains Stbl3, HEK293T cells will be used to package these vectors into virus, and the viruses will be used to transduce human fibroblasts. Replication deficient / Attenuated Rabies viral vector will be purchased from a commercial source (e.g., Addgene.org, a non-profit global plasmid repository). The virus will be used to transduce human keratinocytes and potentially other somatic cell types of human origin.

**Host/vector system**

1. **Host = E coli: Stbl3**
   - Host for viral packaging: HEK293T or hiPSe derived neurons
   - Using Lentiviral Vector plasmids = pSIN lentiviral expression vector pBOB-syn-HT (Addgene ID 30456) and pBOB-synP-HT (Addgene ID 30195)

**Origin & function**

2. **Host = HEK293T or BHK-EnvA cells for use with Recombinant Rabies Vectors**
   - pSAO~G-F3 (Addgene, 10 no. 32634)
   - pcONA-SAOB19N (Addgene, 10 no. 32630)
   - pcONA-SAOB19P (Addgene, 10 no. 32631)
   - pcONA-SAOB19L (Addgene, 10 no. 32632)
   - pcONA-SAOB19G (Addgene, 10 no. 32633)

**Health and Safety**

**Executive**

The morbidity of wild type Rabies virus is due to its ability to spread from the site of infection. However, the recombinant Rabies virus is incapable of spreading from the site of infection due to the genomic deletion of the gene encoding for the viral Glycoprotein.

**Activity**

**Evaluation of foreseeable effects**

Viral Vectors are disabled

Lentivirus:
Lentiviral Expression System allows creation of a replication-incompetent, HIV-1-based lentivirus that is used to deliver and express our gene of interest in either dividing or non-dividing mammalian cells. The plasmid is pSin. It is a self-inactivating virus, with the packing genes located on three separate plasmids. Lentivirus is the more hazardous virus used in this protocol compared to Recombinant Rabies virus, but there are no foreseeable effects.

Recombinant Rabies Virus:
This Rec. Rabies system allows for the generation of viral particles able to infect but not replicate in target mammalian neuronal cell systems due to deletion of the native glycoprotein gene (G). This is also non-transmittable, as the vector in this case has the glycoprotein (G) deleted, rendering the virus incapable of producing infectious particles from infected cells without host expression of the G protein for pseudotyping.

Using this strategy due to its neuronal tropism, pseudotyped rabies virus vectors can be used to study neuronal trafficking or express endogenous genes efficiently in neurons. Lentivirus has one colour i.e. GFP in its plasmid for visualising and the Rabies has another colour i.e. RFP so the initial cell can be identified and the subsequent synapse.

Worst case scenario you may be exposed but it can\'t infect & replicate in normal human tissue due to the pseudotyping, so again no foreseeable effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection is achieved using Virkon (peroxygen compound). A 1% Virkon solution is expected to kill 100% in 10min. All liquid waste will be collected and treated with a combination of autoclaving and disinfection methods. Pipette tips will be discarded directly into sharpsafe container, autoclaved, then disposed as clinical waste. Plastic pipettes and other solid waste will be soaked in Virkon overnight then autoclaved. All work surfaces will be treated and wiped down with Virkon.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Cardiff University Genetic Modification and Biological Agents Safety Committee agreed with the classification of the activity as Class 2. They did, however, have a number of queries concerning the local risk assessment. These queries have now been answered to the satisfaction of the Committee.
The aim of this project is to increase our understanding of the contributions of specific, anatomically defined pathways in the brain that underlie various forms of learning and memory. The genes that are under study will not encode a product that could act directly to cause harmful effects (e.g. toxin gene or an oncogene), nor will they encode a product that may enable the micro-organism with enhanced pathogenic properties. Viral vectors will be used to genetically manipulate neuronal activity in discrete brain regions and in specific populations of neurons in rodents. These tools provide a wealth of opportunities for investigating the neurophysiology of a variety of learning and memory processes by allowing temporally restricted up or down regulation of neuronal activity and
Recipient or parental organism

Recipient organisms will be rats or mice.

Host/vector system

**EIAV-based lentiviral vectors:**
Self-inactivating lentiviral vectors will be used. Viral vector particles based on the equine infectious anaemia virus (EIAV). The replication competent wild type EIAV can only infect horse cells. The vectors proposed for use here will be generated by a three plasmid co-transfection system (of HEK293T cells) in which separate plasmids expressing gag/pol, envelope and genome are used as a mixed DNA preparation. These minimal plasmids have little or no common sequences therefore the probability of recombination between plasmids and production of replication competent virus is very low. The vectors are pseudotyped with a rabies-G glycoprotein making them neurotropic and allowing retrograde neuronal transport. The pseudotyped vector is self-inactivating and is extremely unlikely to recombine in a patient with HIV, i.e. the probability of the production of replication competent vectors is very small. Once injected into the rodent, the viral particles cannot re-infect any other tissues as: (i) the coat protein is lost following attachment and the DNA is incorporated into the host cell replication deficient; (ii) they are replication deficient. Hence, lentiviral vectors cannot re-infect once injected into the brain. Lentiviral vectors are enveloped RNA viruses that are relatively unstable, highly susceptible to dehydration and rapidly inactivated outside the host. Lentivirus particles have, thus, a short half-life and are unstable at room temperature.

**rAAV vectors:**
Recombinant Adenoviral associated virus (rAAV) are derived from wild type Adenovirus-Associated Viruses (wtAAV) which are non-pathogenic, non-replicative viruses which infect both humans and animals. rAAV lack wtAAV viral protein coding sequences. rAAV vectors are based on rAAV viruses which are non-pathogenic in humans and the vectors themselves are not known to cause any diseases in humans or animals. Although wild type AAV can enter mammalian cells in the presence of adenovirus and their genomes integrate into host cell DNA, AAV vector genomes remain primarily episomal in target cells and have a low (if any) frequency of integration.

In both of the above cases, the viral particles will be received from either Bristol or a recognised commercial source (e.g., UNC Vector Core), consequently in-house cell lines and cultures will not be required.

Origin & function

The scientific goals will be achieved by transducing neurons in the rat/mouse brain with viral vectors containing one or more of the following types of inserts:

a) lacZ
b) DREADDs (designer receptors exclusively activated by designer drugs)
c) A fluorescent protein such as green fluorescent protein (GFP) to tag cells.
d) A channelrhodopsin

a) LacZ is a bacterial gene derived from Escherichia coli and codes for 3-galactosidase, an enzyme, which catalyzes the hydrolysis of 3-galactosides into monosaccharides. Typically it is used to cleave x-gal and the subsequent blue colour produced is used as a reporter of activity and of cellular localization. The viral vectors can be injected into a particular brain region in normal wild-type rats. The particles are retrogradely transported and so accumulate in the brain regions that project directly to the injection site, where the transgene, lacZ is expressed. Subsequent infusion of the commercially available prodrug, Daun02, transiently inactivates just this population of neurons. This occurs as the LacZ gene, codes for ~galactosidase which catalyses the hydrolysis of Daun02 to
Daunorubicin which subsequently inactivates those cells, i.e., creates a temporary, highly selective lesion. It is then possible to behaviourally assess the impact of these inactivation taking advantage of a within-subject design. b) DREADDS are G-protein coupled receptors (GPCR) engineered to respond exclusively to synthetic small molecule ligands, rather than their natural ligand(s). DREADD constructs are typically delivered via a recombinant adeno-associated virus (rAAV) vector and are activated by systemic administration of a prodrug, such as clozapine N-oxide (CNO), enabling the precise spatiotemporal control of GPCR signalling in vivo (Urban & Roth, 2015). For the purposes of the current project a combination of DREADD constructs which, upon activation, act to either enhance or reduce neuronal firing will be used to explore the involvement of particular brain regions and/or neuronal populations in various forms of learning and memory (for an example of a similar approach see Robinson et al., 2014). c) GFP is a non-toxic protein composed of 238 amino acid residues (26.9kDa) that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. GFP traditionally refers to the protein first isolated from the jellyfish Aequorea victoria, which has a major excitation peak at a wavelength of 395 nm and a minor one at 480 nm. Its emission peak is at 509 nm, which is in the lower green portion of the visible spectrum. It is used as a means of tagging cells that have been transduced. d) Channelrhodopsins (ChR) are a subfamily of retinylidene proteins (rhodopsins) that function as light-gated ion channels found in certain algae, e.g. chlamydomonas. ChRs can be readily expressed in neurons via either lentiviral or rAAV transduction. Upon integration into the cell membrane of neurons the activity of transduced neurons can then be modulated by photostimulation in order to investigate their role within the neural network Circuitry underlying various types of learning and memory.

None of the proteins listed above have any physiological, pathological and/or pharmacological effect beyond the small number of neurons in which they are expressed. The inserts do not encode a product that could act directly to cause harmful effects (e.g. toxin gene or an oncogene). In addition, none of the proteins are secreted from eukaryotic cells and, therefore, transgene expression will be limited to discrete regions of brain tissue directly infected through injection.

Evaluation of foreseeable effects

Viral vectors could be transmitted to humans via; exposure to mucous membranes of the eyes, nose and mouth, or needle stick injury. When carrying out surgery to inject the viral particles into rodent brains the anaesthetised animals will be injected directly with a stereotaxically mounted needle, therefore needle handling is minimal and the risk of needle stick injury low. Further, the the Hamilton syringes that we use have blunt bevelled ends, making it difficult (but not impossible) to suffer a needlestick injury. A needle stick injury with a Lentiviral vector (EIAV) pseudotyped with a rabies coat protein allows transfection of most dividing and non-dividing cell types. Direct injection as a result of a needle stick injury may lead to transmission of virus to human cells, however, the minute volumes of virus that will be used in rodent experiments means the likelihood of achieving an infective dose for humans through a needle stick injury is effectively zero. The non-replicative nature of the viral vectors to be used means that any virus infection via a needle stick injury will be localised to the immediate penetration site; this would be a T-cell mediated response to the presentation of a foreign protein. rAAV vectors are based on rAAV viruses which are nonpathogenic in humans and the vectors themselves are not known to cause any diseases in humans or animals As the volumes used for infections are small <5(.11) and all handling, aside from surgeries, will be carried out in a Class II MSC, it is extremely unlikely that a worker could infect himself or herself with a viral vector. Syringes for injection will be loaded in the Class 2 hoods and then transported to the surgery suite in a sealed container lined with absorbent paper to minimize the risk of virus leaking from the syringe. Leakage is very unlikely due to the micron sized tip, which would require sufficient pressure from the syringe plunger to dispel liquid. Additionally, the surgery suite is in the adjacent room to the viral suite, with direct access between these two rooms and no need to
go out into the general corridor. Some techniques may generate aerosols (pipetting of aliquots and centrifugation) but this will all be carried out in a Class II MSC. It's worth noting that centrifugation will not be carried out to concentrate the viral particles, only to ensure all of the aliquoted volume is at the bottom of the Eppendorf tube.

Direct injection into the brain with recombinant virus during experiments means the likelihood of transmission to another animal in the animal housing unit is extremely low as the virus cannot cross the blood brain barrier and the vectors are non-replicative. The only risk of transmission through the oral/faecal route would be as a result of systemic infection, which will not occur.

A potential risk for lentiviral expression concerns release into a human population already infected with HIV, allowing for potential recombination events that would incorporate the inserted sequence into a viable, replication competent virus. This scenario is extremely unlikely, and would in any case be unlikely to cause any additional problem. Sequences within the viruses cannot be transferred to other micro-organisms without a very rare recombination event. Thus, we see no conceivable hazard associated with transfer of the expressed sequences. As mentioned above, EIAV is an enveloped RNA virus that is relatively unstable, highly susceptible to dehydration and rapidly inactivated outside the host. These particles have a short half-life and are unstable at room temperature. Therefore, the survivability of the lentiviral vectors is not thought to pose a risk to the environment. rAAV V contains no virulence factors. As the viral systems used are self-inactivating and non-replicative, it is extremely unlikely that accidental release into the environment will cause any environmental damage or risk to others.

Our experiments do not require systemic infection and animals will be injected with virus directly into the brain only. The dose of virus required to transduce brain cells via direct injection is 1000 fold lower than the dose required for systemic infection, so even in the unlikely event that a viral injection reached the systemic circulation, a systemic 'infection' is unlikely to occur. Injections will be targeted to the brain and studies report rAAV serotype 2 does not efficiently cross the blood brain barrier, therefore systemic infection is not perceived as a significant risk. Workers performing the surgical and behavioural tests are required to follow agreed procedures for the handling of animals to reduce the risk from allergens and bites, including the use of appropriate protective clothing and face masks, but there are no extra risks associated with the fact that the animal has undergone the procedures detailed. All workers using animals are required to attend an in-house animal handling course prior to starting work and are then closely supervised by experienced post-doctoral workers and group leaders until competent in all procedures. All in vivo work is carried out with the appropriate licences and permissions from UK regulatory authorities. The animals containing the genetically modified material are healthy and possess no risk to human health. Rodents are likely to survive if released from containment. However, the rodents are housed in secure cages within a secure facility and, therefore, the risk of accidental release into the environment is very low. The inability of the lentiviral vector to propagate in mammalian cells also reduces the risk. Also, as detailed above, lentiviral vectors are enveloped RNA viruses that are relatively unstable; highly susceptible to dehydration and rapidly inactivated outside the host. Lentivirus particles have, thus, a short half-life and are unstable at room temperature. Therefore, the survivability of the lentiviral vectors is not thought to pose a risk to the environment. Other than injection into the intended rodent host, no other organism is likely to be infected with the virus as vectors will be directly injected into the brain only in the designated viral surgery suite. Once introduced into an animals, the viral particles are no longer infective. Expression from the transgenes persist in the animal over a long period of time (more than a year).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid virus waste will be treated with 4% Virkon (generic chemical name: potassium peroxymonosulphate, sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers) or 10% chlorine bleach, destroying any naked nucleic acids. Solid waste will be autoclaved. Expected degree of kill 100% Following completion of the behavioural experiments, rats required for neuroanatomical studies will be perfused with 4% paraformaldehyde fixative, thereby inactivating any viral particles. Brain slices from fixed tissue will be prepared and analysed by histological techniques. Animal carcasses will be disposed of in designated clinical waste and incinerated.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC complimented the responsible person on the detail included in the risk assessment however they did have a number of queries which have now been answered. The GMSC is now satisfied that the risk assessment is suitable and sufficient.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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</thead>
<tbody>
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Project Ref 130/17.2

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<tr>
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<th>CultureVolumeClass3-4</th>
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<tr>
<td>13/01/2017</td>
<td>Engineering luciferase enzymes as reporter genes for bioluminescence imaging in biomedical research</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td>Non-GMM</td>
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Date Project 02/03/2022
Wildtype and thermostable Fluc derivatives will be cloned into a pCCl lentiviral vector fused to enhanced blue fluorescent protein 2 (EBFP2) via double serine-glycine linkers. Expression and properties will be tested by transfection of HEK293 cells, e.g. relative light yields at 250°C and 37°C, kinetics and bioluminescence spectra will be measured using the PhotonMAGER Optima (PIO) small animal optical imager (Biospace labs, Paris France). This is a kinetic and multi-wavelength imager designed for imaging up to 5 mice at a time. Subsequently, high titre virus will be produced in HEK cells by triple transfection of pCCl encoding transgenes along with gag pol and env encoding packaging plasmids. Virus titres will be assessed by transduction of HEKs with different amounts of virus and subsequent FACS of EBFP2. Different types of cell lines (e.g. HEK, Raji, Jurkat, mouse cell lines and primary cells and primary human neural stem cells) will be transduced, sorted for equivalent expression levels (by EBFP2 fluorescence) to generate stable cell lines for further study. The HEK cells provide a xenograft model for superficial imaging in mice and the Raji cells (when injected intravenously) provide a systemic lymphoma model in which the effects of blood attenuation on reporter systems can be ascertained. Transduced cell lines will be cryopreserved in small banks of 18 aliquots (each of 3-5 x 10^11 cells) prior to larger scale testing using phantoms (150mm square petri dishes containing agarose or gelatine impregnated with intralipid and/ or blood to mimic mammalian tissue scattering and absorption) and in mice. The native colour of bioluminescence of the firefly system is heavily absorbed by blood within mammals making detection and quantification of deeper signals more problematic. However, synthetic bioluminescence with redshifted analogues and engineered enzymes has the potential to overcome problems associated with blood absorption and with the novel analogue infraluciferin, there is potential to develop deep tissue dual parameter bioluminescence systems.

Vectors prepared from E.coli DH5α and BL21 DE3 plysS (Agilent Technologies, CA, USA) and utilised in recipients: HEK293 (Homo sapiens, ‘embryonic Kidney, ATCC Number: CRI-1573), Jurkat (Homo sapiens, peripheral blood lymphocyte, disease: acute T cell leukemia, ATCC number: TIB-152), Raji (Homo sapiens, tissue: lymphoblast, B lymphocyte, disease: Burkitt's lymphoma, ATCC number: CCI-86), Chinese hamster ovary (CHO- U.S. Patent Number 7,429,644) cells and also primary mouse splenocytes and primary human stem cells. Since the transgenes cause no effects besides luminescence, the risk that modified cell lines pose to Human health and the environment is low.
pCL-Fluc-EBFP2 or pCCL.GFP controls.

## Origin & function

pCL is a third generation lentiviral expression vector to allow the transfer and expression of genes in human cells and contains the hPGK promoter. This vector has a number of biosafety features (Dull et al., 1988, J. Virol. 72: 8463) and can be used to produce lentivirus after triple transfection of cells with additional packing plasmids (it also self-inactivates upon integration into host genomes). The vector backbone was kindly obtained from Dr. Riccardo Brambilla (Cardiff, UK). It is pure plasmid DNA and not live virus. The virus is derived from HIV-1 (lentiviridae) but has been ‘attenuated as described above. They exist as ssRNA and are retrotranscribed to dsDNA in host cells for subsequent integration. It is replication defective and in this work will contain no effector genes, merely reporters.

Inserted sequences: Genes derived from organisms and engineered to act as novel reporter genes: The firefly luciferase gene is originally from Photinus pyralis and contains mutations which improve its properties. Another gene is derived from the coleopteran Pyrophorus plagiopothalamus, known as ‘click beetle red luciferase’. Lastly, enhanced blue fluorescent protein 2 (EBFP2) is an engineered blue fluorescent protein.

## Evaluation of foreseeable effects

There are two major risk factors with using lentiviral (LV) delivery systems: first, the generation of replication competent infective virus and the second the chance of oncogenesis through the random chromosomal insertion of virus in a host genome. Both these risks are heavily mitigated when using third generation self-inactivating vectors such as pCL. The SIN element is caused by a 133 base pair deletion in the U3 region of the 3' TR which transcriptionally inactivates the virus after reverse transcription and chromosomal integration in cells (Miyoshi et al., 1998. Am. Soc. Microbiol. 72: 8150). Furthermore, this vector is not replication competent, i.e. does not contain gag, pol, rev or env genes for viral replication. Gagpol and env are provided on separate helper plasmids for the construction of virus (conditional packaging) (Dull et al., 1998. Am. Soc. Microbiol. 72: 8463). Other deleted genes are vpr, vif, vpu and nef and tat. To mitigate against the second risk (infection and chromosomal integration causing oncogenesis in subjects): firstly, the risks of this occurring are very low (normal immune competent individuals are unlikely to become infected). Secondly, the conditions of virus generation and manipulation are extremely well controlled and no human or animal subjects will be exposed live virus particles in this work. Immunocompromised individuals will not be permitted to work on this project. VSV-g pseudotyped virus will be used to generate stable cell lines for in vitro studies and implantation into mice. Lastly, the risk of oncogenesis depends on the transgene inserted into the vector and in this work no genes other than bioluminescence and fluorescence reporters will be inserted into vectors (and thus subsequently cell lines or mice). This further reduces the risks associated with routine IV vector use in biomedical studies. The effects of the bioluminescence and fluorescence genes are to express proteins which have the function of adenylation of fatty acids and the generation of bioluminescence with the addition of firefly luciferin, and, fluorescence, respectively. Outside of this, these genes have no other known functions. In case of accident, the routes of transmission are known and hazards associated with potential delivery of the virus or its products to tissues where it may be biologically active have been fully considered. Sharps - the use of sharps and administration of luciferin to image mice (given intraperitoneally) has the potential to cause a needlestick injury. In the unlikely event of a needlestick wound, the normal methods for treating the wound will be followed (wound disinfected and cleaned) with the addition of notification to the school health and safety representatives. The risk of needle stick injury will be reduced, by unsheathing needles only at the time of use and also not re-sheathing needles after they have been used. Lastly, needles will be disposed of by placing them into a sharps box immediately after they have been used. Needlestick injuries will be referred to the UHW occupational health (1st floor, Denbigh House in work hours, or the accident and emergency department UHW outside of work hours. Inhalation - opening a vial or spillage of the virus could cause an aerosol of viral particles. Therefore the virus will always be contained within a class II cabinet. If there
is a failure of personal protective equipment, such as gloves, the viral vector could be transmitted through contact with
the skin, transmission into the blood stream or to airways and eyes. These risks will be minimised following the
standard operating procedure of the tissue culture lab. Transmission through the mouse host will not occur as there is
no viral replication and therefore no excretion of the viral product. Adherence to all standard operating procedures of
the facility will ensure no transmission outside of the animal facility (surgery rooms).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Sharps will be placed in clinical, sealable waste sharps bins for autoclaving and disposal.
Liquid waste will be disinfected with generic chemical Virkon which contains a blend of inorganic peroxygen
compound, inorganic salts, organic acid and anionic detergent (pentapotassium •
bis(peroxymonosulphate)bis(sulphate), sulphamidic acid, sodim dodecylbenzenesulfonate. dipotassium
peroxodisuphate), which is expected to kill: 100% of microorganisms in 10 minutes with a 1% solution (validated:
Virucidal efficacy data from Dupont). It has proven highly effective over 65 strains of virus in over 19 viral families,
including the lentivirus HIV.
Solid waste to be autoclaved on site through our in-house waste management facility.
The virus is replication incompetent and the small titres (1..11 volumes) of virus infected cells implanted in mice will have
no risk of virus excretion in the urine or faeces. Animal carcasses and waste will be treated as GM waste and sent
through the appropriate school of Biosciences GM waste route regulated within JBIOS where animal carcases will be
sent for incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GMBA safety committee had some minor comments which have now been addressed. The University
GMBASC is now satisfied that the risk assessment is suitable and sufficient.

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02/03/2022
Project Ref 130/17.3

Investigating circuit integration of adult born neurons in mouse neuropsychiatric disease models using modified retrovirus and rabies virus for labelling and monosynaptic tracing.

This project has five aims:
1) To generate a retrovirus (Moloney murine leukaemia virus, MMLV) expressing the avian viral receptor (TVA), rabies glycoprotein (Rgp) and green fluorescent protein (GFP) under control of the neuron specific synapsin promoter (RVSYN-GTRgp).
2) To inject modified MMLV into the dentate gyrus of mice to highly specifically label adult born dentate gyrus granule neurons to assess their structural and functional developmental trajectory.
3) To generate a rabies virus pseudotyped for the avian EnvA protein in which Rgp is replaced by the fluorescent protein mCherry (EnvA-OG-MCh).
4) To inject rabies virus into a) the dentate gyrus of mice in combination with MMLV to trace development of synaptic connectivity and circuit integration in animal models of neuropsychiatric diseases, or to b) use viral vectors in organotypic hippocampal slice cultures with the same aims
5) To characterise the electrophysiological properties of labelled neurons (both MMLV and rabies virus) in ex vivo brain slices and slice cultures at differing developmental stages.
Replication deficient Moloney murine leukemia virus (MMLV, retrovirus) and/or replication deficient rabies virus will be injected into mice/rats to label specific cell types in the brain and to trace synaptic connections in neuronal circuits in order to investigate circuit dysfunction in neuropsychiatric disease.

MMLV will be produced using the human embryonic kidney cell (HEK293T) line and rabies viruses will be produced using the B7GG (BHK-21, baby hamster kidney fibroblast cell line).

Rabies viruses will be pseudotyped with the avian retrovirus envelope protein (EnvA) using the BHK-EnvA cell line.

Host/vector system

**Recipient or parental organism**

Replication deficient Moloney murine leukemia virus (MMLV, retrovirus) and/or replication deficient rabies virus will be injected into mice/rats to label specific cell types in the brain and to trace synaptic connections in neuronal circuits in order to investigate circuit dysfunction in neuropsychiatric disease.

MMLV will be produced using the human embryonic kidney cell (HEK293T) line and rabies viruses will be produced using the B7GG (BHK-21, baby hamster kidney fibroblast cell line).

Rabies viruses will be pseudotyped with the avian retrovirus envelope protein (EnvA) using the BHK-EnvA cell line.

**Host/vector system**

- **Host:** mouse/rat
- **Vector:** RV-SYN-GTRgp or RV-CAG-GFP MMLV virus and/or EnvA-deltaG-MCh rabies virus
- **Host:** HEK293K cells
- **Vector:** pSYN-GTRgp or pCAG-GFP (The Salk Institute), pBS-CMV-gagpol (Addgene #35614), pCMV-VSV-G (Addgene #8454)
- **Host:** B7GG (BHK-21) cells
- **Vector:** pSAD-deltaG-MCh (Addgene #32636), pcDNA-SADB19G (Addgene #32633), pcDNA-SADB19P (Addgene #32631), pcDNA-SADB19N (Addgene #32630), pcDNA-SADB19L (Addgene #32632)
- **Host:** BHK-EnvA cells
- **Vector:** deltaG-MCh rabies virus

**Origin & function**

The recombinant MMLV viruses used are:

- **Health and Safety Executive**
- **RV-SYN-GTRgp:** This virus contains genes from the retrovirus genome, green fluorescent protein (GFP) from the jellyfish *Aequorea Victoria*, the avian tumor virus receptor A (TVA) and the rabies virus glycoprotein (Rgp).
- **RV-CAG-GFP:** This virus contains genes from the retrovirus genome and the green fluorescent protein (GFP) from the jellyfish *Aequorea Victoria*.

The recombinant rabies viruses used are:

- **EnvA-deltaG-MCh:** This virus contains genes from the rabies virus genome and the red fluorescent protein mCherry from the mushroom coral *Discosoma sp.*
- **Moloney murine leukemia virus (MMLV)**
- **HEK293T cells** will be used to package recombinant DNA plasmids into virus particles and these will be used to infect cells in the rat/mouse brain (as described above).

The plasmids used for MMLV are:

- **Transfer plasmids:**
  1. **pSYN-GTRgp** (from Prof. Fred Gage, The Salk Institute, USA): a combination of green fluorescent protein (GFP), avian viral receptor TVA, rabies virus glycoprotein (Rgp). These are expressed as one transcript, linked by self-cleavable peptides, under the control of the synapsin promoter. The nuclear targeted GFP serves to identify and track infected cells, TVA allows for infected cells to subsequently be infected with rabies virus for retrograde tracing, and Rgp allows for production of rabies virus only in cells infected with this retrovirus.
  2. **pCAG-GFP** (from the Salk Institute Vector Core, The Salk Institute, USA): enhanced Green Fluorescent Protein (eGFP), under control of the CAG promoter. This will identify any dividing cells in the infected tissue, and highlight...
their morphology.

Packaging plasmid:
3) pBS-CMV-gagpol (from Addgene #35614): contains genes for the retroviral structural proteins Gag and Pol.

Envelope plasmid:
4) pCMV-VSV-G (from Addgene #8454): contains the gene for the envelope protein VSV-G from the vesicular stomatitis Indiana virus.

Rabies virus B7GG (BH K-21) cells will be used to package recombinant DNA plasmids into virus particles and these will be used to infect cells in the rat/mouse brain (as described above).

The plasmids used for Rabies virus are:
Transfer plasmid:
1) pSAD-deltaG-MCh (from Addgene #32636): The transfer plasmid contains genes from the rabies virus genome and the red fluorescent reporter mCherry gene (originally from the mushroom coral Discosoma sp.).

Packaging plasmids:
2) pcDNA-SADB19G (Addgene #32633), pcDNA-SADB19P (Addgene #32631), pcDNA-SADB19N (Addgene #32630), pcDNA-SADB19L (Addgene #32632): contain genes from the rabies virus genome.

Rabies viruses will be pseudotyped to express the avian retrovirus envelope protein (EnvA) using the BHK-EnvA cell line.

Evaluation of foreseeable effects

MMLV:
MMLV is a group VI, positive sense, single-stranded RNA (ssRNA), lipid encapsulated retrovirus from the genus gammaretrovirus. MMLV is capable of inserting into the host genome at random locations, and infection is therefore potentially oncogenic. Infection can only take place in dividing cells. Systemic infection of MMLV is normally largely asymptomatic in mammalian hosts.

The MMLV retrovirus to be used is replication deficient. In the viral production procedure the genes required to produce virions are separated onto three different plasmids namely the transfer, packaging and envelope plasmid ids. The transfer plasmid which contains the genetic information to be inserted into the infected host cell lacks the necessary genes to produce infectious virus particles. It is, therefore, a self-inactivating virus. The virus is pseudotyped with the VSV-G protein making it possible for it to infect a variety of mammalian cell types including human cells. However, due to the replication incompetent nature of the viral vector, only cells directly exposed to viral particles will be infected and further systemic infection is impossible.

Recombinant rabies virus:
Rabies Virus belongs to the order Mononegavirales, family Rhabdoviridae and genus Lyssavirus characterised by having negative stranded RNA genomes and specialising in the infection of mammalian neuronal systems. The virus is often transmitted via bites from infected animals (often hosted in bats and transmitted to domesticated pets) the pathogen will then infect peripheral nerve terminals at the site of the bite and travel throughout the central nervous system of the infected host by retrograde (post synaptic to presynaptic) infection of connected active synapses. Both wild type and recombinant rabies viruses are well known to neuroscience research. The virus specialises in infecting and spreading throughout the mammalian nervous system whilst leaving infected cell bodies largely intact. This low cytopathology enables researchers to use the vector to trace neurons and map neural connectivity.

The rabies viruses used will be disabled through two mechanisms.
1) The virus to be used in the majority of experiments including all in vivo injections will be pseudotyped with the avian envelope protein EnvA and therefore will only be able to infect cells previously engineered to express the avian viral receptor TVA and not native mammalian/human cells.
2) The virus will be engineered to lack the crucial rabies glycoprotein (Rgp) gene and will therefore be unable to...
produce the Rgp necessary to allow it to infect mammalian cells. During the rabies virus production procedure a rabies virus will be produced in and harvested from B7GG cells and used to infect BHKEvA helper cells to pseudotype the virus for EnvA. This virus will express the Rgp (derived from the helper cells) and therefore present a risk of infection to humans. However, this virus will be replication deficient since the Rgp gene is deleted from the viral genome. The likelihood of infection by this rabies virus is very low as it will be handled in a class 2 biological safety cabinet and with appropriate PPE (lab coat, safety glasses, double gloves).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated waste will be deactivated locally by chemical disinfection using Distel or Virkon followed by autoclaving prior to disposal via an appropriate route following the steps detailed below. Local deactivation by chemicals and autoclaving prior to collection and disposal by an external company (SRCL) is the preferred disposal route as this is less hazardous and involves smaller volumes.

Preferred local deactivation/disposal pathway:
1) Chemical disinfection by Distel or Virkon.
   Liquid waste (e.g. culture media) will be inactivated with Distel or Virkon® (final concentration 1 %) for at least 1 hour (typically overnight) - according to manufacturer's instructions.
   All solid waste (tissue culture plastics) will be treated with Virkon® for at least 2 hours before autoclaving.
2) Waste placed into autoclave bag.
   Chemically deactivated waste will be placed into autoclave bags and labelled GM 180104.
3) Waste autoclaved locally using the waste cycle at 134°C for 25 mins.
   Solid waste will be autoclaved in the designated autoclave located immediately outside of the viral suite before being placed in a yellow 'Tiger bag' and disposed of as 'offensive waste' (HL 180104).

The autoclave is a Priorclave 60L Benchtop, The machine is serviced annually and a twelve-point thermocouple test is performed annually. Waste material will be inactivated using the waste cycle at 134 degreeC for 25ms ins.
   Waste material will be inactivated using the waste cycle at 134 degreeC for 25ms ins.
   All staff operating the autoclave will have been trained in manual handling and are also trained in appropriate spillage procedure. 1 % Virkon® or Distel is the disinfectant to be used in case of inactivated waste being spilled in or out of the autoclave,
   The operator loads the autoclave and runs the discard cycle (134 degreeC for 25 minutes). The temperature probe is put into the waste to ensure waste in the centre of the bin is inactivated and it monitors every run.
   Once the cycle is complete the load is left to fully cool if possible. On occasion special gloves must be used to prevent burns when taking the load out.
4) Removal and disposal/destruction by SRCL.
   Once cooled, autoclaved waste is placed in yellow 'Tiger bags' and moved to the storage area in Hady Ellis building basement for disposal by SRCL as 'offensive waste' (HL 180104).

Animal waste from GM animals and/or animals injected with GM viruses will be handled using appropriate PPE (gloves and lab coat) and will be double bagged before being placed into a yellow plastic bin (GM180103) and stored in a freezer. Animal waste will be clearly labelled as ‘clinical waste’ containing GM waste. Waste will be disposed of using established local guidelines involving collection and destruction offsite by incineration by SRCL.
For chemical disinfection we will use the following products:

**Product Name: Distel**
- Expected degree of kill: 100%

How validated HLD4 disinfectants are manufactured in the United Kingdom to the highest standards conforming to ISO 9001:2008. HLD4 disinfectants are certified Sporicidal, Mycobactericidal, Virucidal, Fungicidal and Bactericidal under EN protocols. Distel is non-corrosive and non-fragranced. So this is the preferred disinfectant for use in the class II hood.

**Product Name: Virkon**
- Expected degree of kill: 100%

It is sold as tablets or powder which dissolve readily in water. It is intended to be mixed with water to form a 1% to 3% solution (by weight, i.e. 10g to 30g per litre). The pink colour is useful in that it helps gauge the concentration when preparing the Virkon®, and importantly, as the Virkon® ages it discolours, making it obvious when it needs to be replaced. The solution is generally stable for five to seven days. Virkon® has a wide spectrum of activity against viruses, some fungi, and bacteria.

The project risk assessment has been viewed by Cardiff University Genetic Modification and Biological Agents safety committee. The Committee requested some minor modifications to the risk assessment but are now satisfied that the risk assessment is suitable and sufficient. They are in agreement that the Activity is Class 2 requiring Containment level 2 facilities and conditions.

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Purposes of the contained use

The specific aims of this project are:
1) To generate recombinant, glycoprotein deleted rabies viruses pseudotyped with the avian EnvA protein.
2) To use these recombinant rabies viruses, in combination with adeno associated viruses (AAVs) expressing the TVA receptor and an optimized rabies glycoprotein, to trace synaptic connectivity in vitro and in vivo.

Recipient or parental organism

Recipient organisms for the virus will be:
- ROSA-tomRITVA ESC line, which expresses the fluorescent protein tdTomato, the rabies G protein and TVA from the ROSA26 locus and can thus be used as the starter population for neuronal tracing in vitro.
- Mouse lines that express Cre recombinase in specific neuronal populations. AAVs that express TVA, mCherry and oG proteins in a Cre-dependent manner will be injected first to generate a population susceptible to be infected by the modified rabies viruses. Modified, self-inactivating rabies virus that expresses the FLP recombinase will be injected 3 weeks later. As our animals will have been bred into a RCE:FRT background, the presynaptic neurons that receive the SiRFLP virus will become green.

Host/vector system

AAVs are defective viruses even in their wild type state. Replication can only be induced by the presence of a helper virus or under conditions of cellular stress, such as genotoxic agents or following UV irradiation. When used as a vector, the whole genome of the virus, except for the inverted terminal repeats, is substituted with genes of interest. Those recombinant viral vectors are not only incapable of replication or spread, but also largely loose the capacity to
integrate into the host's genome. This only happens through a passive mechanism at naturally occurring chromosomal breakpoints for 10% of all double stranded viral genomes. The recombinant rabies system allows for the generation of viral particles that have both restricted infectivity and spreading ability. The rabies virus cannot create any infective viral particles within infected cells due to deletion of the critical viral gene, glycoprotein gene (G). The rabies virus is also pseudotyped with EnvA protein and can only infect cells with the complementary receptor to EnvA (called TVA receptor). Mammalian cells do not natively express TVA receptor. This means that rabies virus cannot infect or spread within mammalian hosts. Hence, there is negligible hazard associated with the recombinant rabies virus if the rabies virus was accidently transmitted to another host (besides the cells in culture/ rodents). In the case of the SiR virus, the hazard is even further reduced, as the destabilised protein N suppresses the viral transcription-replication cycle. Even if the virus was to enter a cell, 2 weeks after infection the viral genome completely dissapears from the infected cell.

Recombinant rabies viruses and their wild-type counterparts are highly effective at infecting and traversing mammalian nervous systems, spreading transynaptically without generating excess cytopathology or invading surrounding unconnected cells. these features make them an ideal choice for the study of neuronal systems and structures both in vivo and in vitro.

All recombinant rabies viral vectors to be used in this project have been designed and tested by other laboratories (Wickersham et al. 2007; Wickersham et al. 2010; Wall et al. 2010; Weible et al. 2010) and have two characteristics: 1) a deletion of the glycoprotein (G) gene, a critical gene for the viral cycle. Viruses that lack the glycoprotein gene cannot spread beyond an initially infected cell. Only if that infected cell can provide the missing viral glycoprotein will the virus be able to spread retrogradely to a presynaptically connected neuron, as the new glycoprotein can be incorporated into the viral particles in a process known as pseudotyping.

2) Pseudotyping with the EnvA envelope protein of the subgroup A avian sarcoma and leukosis virus (AS LV-A). This protein interacts specifically with the TVA receptor, a protein that is found in birds, but not in mammals. In this way, the modified rabies viruses are not only incapable of spreading, but also incapable to infect any mammalian cells that do not display TVA on their surface.

As a result of the alterations explained above, the modified rabies virus can only infect and spread from cells that have been genetically engineered to express TVA and the viral glycoprotein. By labelling both the cells capable of expressing the viral glycoprotein and the recombinant rabies virus with fluorescent tags, we can specifically track the infection of the modified rabies virus from the starter cell to its presynaptic targets, and determine the patterns of connectivity from these specific cells. We will use this strategy to analyse synapse formation in vitro and in vivo.

To study synaptogenesis in vitro, we will use the ROSA-tomRITVA ESC line, which was generated by the Arenkiel lab. It expresses the fluorescent protein tdTomato, the rabies G protein and TVA from the ROSA26 locus and can thus be used as the starter population for neuronal tracing. A mix of ROSA-tomRITVA and other ESC-derived neurons will be infected with the modified recombinant rabies virus and then analysed 3 days later.

For the in vivo analyses, we will first inject AAVs into Cre-expressing mice to generate a population of neurons susceptible to be infected with the modified rabies virus. The AAVs used in this step express TVA, mCherry and oG proteins in a Cre-dependent manner. Therefore, only those neurons that express Cre will become potential starter neurons for presynaptic tracing, allowing us to analyse the presynaptic partners of those specific neurons. The modified, self-inactivating rabies virus that we will inject 3 weeks later expresses the FLP recombinase. As our animals will have been bred into a RCE:FRT background, those presynaptic neurons that receive the SiRFLP virus will become green.
There are no foreseeable detrimental effects regarding the use of the AA V and rabies viruses, beyond the intended experimental results of presynaptic neuronal labeling. The AAV vector is designed to infect mammalian cells in order to deliver the TVA receptor, mCherry and glycoprotein genes necessary for infection of these cells by the recombinant rabies vector. As the AAV will only be carrying this donor plasmid encoding for these genes and not for the viral genes needed for replication, the AAV is replication incompetent. This means the infection of the AAV is contained. In addition, AAVs are not linked to any human diseases, so even in the highly unlikely event that the virus would enter human cells, there is no reason to expect any harm to be caused. The virus is replication incompetent and the genes carried in the recombinant virus are not expected to be detrimental in any way. In addition, expression of the genes in the AAV vector is dependent on the presence of Cre recombinase, so even in the event that the virus would enter a human cell, the genes in the vector would not be expressed. Recombinant AAVs also largely lose the capacity to integrate into the host's genome, so any potential harm due to insertional mutagenesis is also extremely low.

The recombinant rabies vector will be pseudo-typed with the Env-A glycoprotein enabling binding exclusively to a TVA receptor-expressing cell. This means the vector will undergo a single replication cycle only within the neurons that have been genetically engineered to express TVA and rabies glycoprotein. From these neurons the rabies virus can only be transmitted along a single active synapse to a second neuron. After this initial transmission the virus cannot generate any infective particles and is inert because it lacks the glycoprotein gene. The rabies virus doesn't integrate into the host's genome, so there is no risk of insertional mutagenesis. The recombinant rabies virus is also incapable of spreading to neighbouring cells, so the infection would be contained to the very cells infected in the first place. Such an infection would anyway be extremely unlikely, as the pseudotyped virus can only enter cells expressing the avian TVA receptor, and mammalian cells, including human cells, do not express that receptor.

The genes inserted into the vectors (GFP, mCherry, TVA receptor, rabies glycoprotein G and FLP recombinase) are solely included to enable the entry, visualisation and single replication cycle of the recombinant rabies vector within mouse embryonic stem cell derived neurons and the mouse brain.

GFP and mCherry are widely used fluorophores for imaging cells in vitro and in vivo with no hazards associated with their use. The TVA receptor and EnvA proteins are avian in nature and are not known to produce pathology in human tissue. The rabies glycoprotein G has not been associated with pathology in human tissue and in fact has been shown to provide limited protection to rabies infection in murine models. The FLP recombinase has been used in mammalian systems for over 15 years and there is no evidence of any detrimental effects.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Live animals are kept in a secure facility and they are killed by schedule one methods before they leave the facility. For the disposal of the animals, the Joint Services’ Biohazard Disposal Route is used, which involves incineration.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste (culture media) will be inactivated with Virkon® (final concentration 1 %) for at least 1 hour - according to manufacturer's instructions.

Product Name: Virkon
Generic Chemical Name: Pentapotassium bis(peroxymonosulphate) bis(sulphate) 40 - 55%, Sodium C10-13-alkylbenzenesulphonate 10 - 12%, Malic acid 7 - 10%, Sulphamidic acid 4 - 6%, Sodium toluenesulphonate 1 - 5%, Dipotassium peroxodisulphate <3% and Dipentene <0.25%

Expected degree of kill: 100%

How is this validated? (e.g. is in-house testing carried out, or is there specific data available regarding efficacy against the organisms you are working with).

Explanation: Virkon is sold as tablets or powder which dissolve readily in water. It is intended to be mixed with water to form a 1 % to 3% solution (by weight, i.e. 10g to 30g per litre). The pink colour is useful in that it helps gauge the concentration when preparing the Virkon®, and importantly, as the Virkon® ages it discolours, making it obvious when it needs to be replaced. The solution is generally stable for five to seven days. Virkon® has a wide spectrum of activity against viruses (47 strains/clinical isolates from 35 viruses), some fungi (45 strains/clinical isolates from 17 fungi), and bacteria (300 strains/clinical isolates from 76 bacteria). It has been proven effective against all viral families, including Adenoviridae and Rhabdoviridae (rabies virus).

Solid waste to be autoclaved and/or disposed of as clinical waste.

Equipment that has been in contact with the virus (e.g. tissue culture plates, storage vials, needles) will be autoclaved. Contaminated equipment will be immersed in Virkon for a minimum of 24hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with Distel disinfectant according to an approved protocol. Access to laboratories employed for virus work will be restricted.

Disposal of animal carcases - Carcasses will be contained in a sealed yellow bin bag and kept in the freezer ready for incineration with the other GM waste.

Disposal of animal bedding - the rodents that have received the rabies viral injection into the brain do not shed the recombinant virus. This means animal bedding can be treated in the same manner as other animal waste in the facility.

Health and Safety

Executive

Disposal of animal excrement (faeces and urine) - the rodents that have received the rabies viral injection into the brain do not shed or excrete the recombinant virus. This means animal waste can be treated in the same manner as other animal waste in the facility.

Other control measures to be used:

Gloves and lab-coats will be worn, remembering the standard rule about using the ungloved hand to open doors to prevent contamination by latex or lab-materials.

The virus suite (both in HEB and Bioscience Animal Unit) has specific lab coats and they are not worn outside of this area.

In the event of a spill to clothing (which is unlikely) there is a paper suit in the spill kit available.

In the event of a spillage during transport to the autoclave, the area will be isolated and then promptly cleaned. It is recommended that a boil of disinfectant is to hand to deal with spillages. Distel or Virkon® is the disinfectant to be used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GM safety committee required evidence that Dr. M G had received the appropriate training in the specific techniques described in the risk assessment.

### Project Containment

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### Project Ref 130/19.1

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### Project Additional Information

**Purposes of the contained use**

Diarrheal disease is the second leading cause of death in children under five years of age. Multiple microbes may be responsible for these infections, but the majority of diarrheal cases are associated with bacteria called Escherichia.
coli, Salmonella and Shigella. These bacteria can be found in contaminated water and food. After they have been eaten, they colonise the gut, cause inflammation and stop food absorption leading to malnutrition, which can be fatal, especially in children in the poorest countries.

General Scientific purpose:
This form is part of a set of forms related to larger programme (see Appendix-A). The work on each part of the programme will not be allowed to start until the detailed risk assessment for that part has been reviewed by the Committee. Any results indicating there is an increased hazard will lead to experiments being halted with immediate effect. Work will not restart until a 'significant change' has been notified to the HSE and the necessary approval given.

The programme objective is to investigates the cellular and molecular basis of enteric infection by looking at host cell responses to the infection. It will use multiple approaches in order to acquire more specific knowledge on how virulence factors are working during infection. Pathogens used in this project include pathogens that exploit secretion systems and toxins for virulence: Enteropathogenic E. coli (EPEC), non-toxigenic Enterohaemorrhagic E. coli (EHEC), Citrobacter rodentium, Enteroinvasive E. coli (EIEC), Diffusely Adherent E. coli (DAEC), non-typhoid Salmonella and Shigella sonnei. These are all important diarrheagenic pathogens for humans and animals.

1- GM 130/764 - Alteration of the expression of bacterial protein in pathogens
In this section of work our aim is to delete or mutate virulence factors in the pathogenic bacteria to understand their functions. Phenotypes after infection of cultured cells or mice induced by wild type strains or mutant will be compared.

2- GM 130/765 - Bacterial and Eukaryotic proteins cloning and expression in E. coli strains
In this section of work our aim is to clone virulence factors or identified host partner proteins (from GM 130/766) in a different vector and have this vector transformed in E. coli laboratory strains. The plasmids generated will be then used for plasmid production (in order to do transfection (GM 130/767 and GM130/768) or yeast transformation (GM 130/766)), protein production (in order to analyse the biological activities of the proteins in vitro or protein-protein interactions) or for the generation or the complementation of bacterial pathogen mutants (GM 130/764).

3- GM130/766 - Identification of host partner for bacterial protein in yeast
In this section of work our aim is to identify the host target of the bacterial proteins. In order to characterize this interaction, human/mice proteins fused to the DNA-binding domain (BD) or activating domain (AD) of the Gal4 transcription factor will be co-expressed with bacterial protein fused to the BD or AD domain in Saccharomyces cerevisiae. Interaction between the two domains will allow the yeast to grow on a selective media.

4- GM 130/767 - Viral transduction of cell culture lines for virulence factor study
In this section of work our aim is to produce non-replicative virions to transduce cell lines. Plasmids generated previously (GM 130/765) containing the gene of interest will be transfected into packaging cell lines (e.g. 293T cells) to generate VSV-G pseudotyped retrovirus. The non-replicative virion will be harvested and used to transduce cells generating permanent cell lines expressing the gene of interest. Genes of interest will be bacterial effectors, binding partners of the effectors identify previously (GM 130/766), RNA interference targeting pathway playing a role during infection or fluorescent reporter to monitor the activity of transcription factor or protein stability. These cell lines can be infected with bacterial mutant generated in GM 130/764 will be then analysed using various techniques to phenotype them (e.g. Western blot, immunofluorescence).

5- GM130/768 - Protein expression and gene interference in cell culture
In this section of work our aim is to transiently or permanently change gene expression to study their function in vitro. Plasmid generated in GM 130/765 will be transfected in culture cell lines using transfection agents commercially available (e.g. GeneJuice, Lipofectamic). The gene of interest can be either a virulence factor from bacterial pathogens or proteins from the host that have been identified as a partner or involved in the signalling of the virulence factor. Most of this work will reliant on transitory express protein but in some condition permanent cell line could be
generated using selection marker (antibiotics). These cell lines can be infected with bacterial mutant generated in GM 130/764 will be then analysed using various techniques to phenotype them (e.g. Western blot, immunofluorescence).

6- Bacterial pathogen infection in transgenic mice.

In this section of work our aim is to use transgenic mice to study the molecular mechanism required for the infection by bacteria.

Recipient or parental organism

1- GM 130/764 - Alteration of the expression of bacterial protein in AIE pathogens

E. coli/Shigella cause diarrhoea which is sudden, severe, and often bloody. Other symptoms of these infections include fever, loss of appetite and stomach cramping. Symptoms of a serious infection with EHEC may include bruising, blood and bloody urine and a reduced amount of urine. Symptoms usually appear 24 - 72 hours after infection. These symptoms are linked to the expression of the Shiga toxin by these bacteria. Only non-toxigenic strains will be used which eliminates the potential sequel of Haemolytic Uremic Syndrome. No reversion has been reported for these strains in the literature.

All S. sonnei strains are of the same serotype and very closely related (Holt et al, Nature Genetics, 2012) but apparently are becoming less virulent although there is increasing antibiotic resistance. We would not be using the antibiotics recommended for treatment (Azithromycine, Ciprofloxacine and Ofloxacine) in the lab, but would be introducing standard cassettes such as carbenicillin, gentamycin, chloramphenicol, tetracycline, kanamycin and streptomycin resistance. Treatment for mild Shigella infection usually involves just rest and fluids, and the illness resolves on its own without antibiotics. In case of infection during pregnancy, women need to ensure they do not become dehydrated or experience electrolyte imbalances due to diarrhoea from the illness. In patients with acquired immunodeficiency syndrome (AIDS), prolonged, relapsing symptomatic shigellosis and lengthy carrier states have been described despite proper treatment.

Salmonella typhimurium cause watery diarrhoea. Infection is likely to be self-limiting and non-lethal in healthy adults. Antibiotics are required in less than 2 percent of cases. Immunocompromised adults are associated with invasive recurrent bacteraemia and have markedly increased mortality. Salmonellosis during pregnancy may produce severe disease and death of the foetus.

Citrobacter rodentium does not cause infection in humans, it infects mice with a similar infection profile to nontoxigenic E. coli.

All the described bacteria are all transmitted orally. Exposure is easily avoided by good laboratory practices.

2- GM 130/765 - Bacterial and Eukaryotic proteins cloning and expression in E. coli strains

The laboratory E. coli strains are non-pathogenic. The virulence factor by itself will not present any pathogenicity as the bacteria required multi-factor protein to become infectious and provide no risk for the health of humans or animals. These bacteria are classified in Risk Group 1 in biosafety guidelines. Furthermore, they have been designed so that they will not survive long outside laboratory conditions and are neither able to the recombine plasmid or cut the plasmid with endonucleases impairing their abilities to transfer the gene of interest to other bacteria.

3- GM 130/766 - Identification of host partner for bacterial protein in yeast

Saccharomyces cerevisiae commonly known as baker's yeast, is not a pathogen for humans or the environment. The strains used for this study are either auxotrophic for Histidine, Adenine, Leucine and Tryptophan or for Histidine, Leucine, Methionine and Uracil and are unlikely survive in the environment.

4 and 5 - Viral transduction (GM 130/767) and protein expression and gene interference in cell culture (GM 130/768).

Mammalian cell lines sourced from ATCC (e.g. HeLa, A549, Swiss 3T3, COS-7, RAW 264.7, THP-1, HEK 293T, U937, T84, Caco-2). History of cell line screening is provided by ATCC (MSDS and Certificate of Analysis). Manipulation of the cell lines will be done in accordance with the ATCC assigned biosafety level designations. The highest biosafety level designation identified is level 2 for HeLa cells as this cell line contains human papilloma virus
Host/vector system

1. GM 130/764 - Alteration of the expression of bacterial protein in pathogens
   All the vectors used have antibiotic resistances genes however, these antibiotics are not used for the treatment of the diseases associated. These vectors are used worldwide in the laboratory and are considered as safe.
   Bacterial complementation constructs will include pSA10 (high copy number, IPTG inducible expression), pACYC184 (medium copy vector, constitutive expression), pBAD vectors (high copy number, arabinose inducible expression)
   Mutagenesis plasmids include pKD46 (arabinose inducible expression of red recombinase) pACBSR/pAKBSR (arabinose inducible expression of red recombinase and I-SceI endonuclease)

2. GM 130/765 - Bacterial and Eukaryotic proteins cloning and expression in E. coli strains
   All the vectors used have antibiotic resistance genes however, these antibiotics are not used for the treatment of the diseases associated. These vectors are used worldwide in the laboratory and are considered as safe.
   Bacterial complementation constructs will include pSA10 (high copy number, IPTG inducible expression), pACYC184 (medium copy vector, constitutive expression) and commercially developed pBAD vectors (high copy number, arabinose inducible expression)
   Cloning vectors will include commercially available vector pGEM T -Easy or TOPO and pUC 18/19 derivatives
   Protein expression constructs will include commercially developed pACYC Duet (IPTG inducible expression), pET vectors (IPTG inducible expression), pMAL (Arabinose inducible expression) and pGEX (IPTG inducible expression).
   Transfection vectors will include pEGFP, pRK5, pHM6, pCB6, pTRE-Tight-BI and pTRE2-Pur. All these vectors are commercially available.
   Transduction vectors will include pMIX-IP and pRetroX-Tight-Puro. All these vectors are commercially available.
   Yeast expression vectors will include the commercially available vectors pYES2 (Invitrogen), pGBT9, pGAD424, pGBK7, pGADT7 (Clontech)

3. GM 130/766 - Identification of host partner for bacterial protein in yeast
   All the vectors used have antibiotic resistance genes however, these antibiotics are not used for the treatment of the diseases associated. These vectors are used worldwide in the laboratory and are considered as safe.
   Yeast expression vectors will include the commercially available vector pYES2 (Invitrogen), pGBT9, pGAD424, pGBK7, pGADT7 (Clontech)

4. GM 130/767 - Viral transduction of cell culture lines for virulence factor study
   The origin of the virus is the Moloney Murine leukaemia virus. Only 12 to 17% of the WT MMLV genome is encoded in the vector backbone to reduce the chance of viral infection. Plasmids being used all are commercially developed such that they generate replication-defective viruses (e.g. http://www.cellbiolabs.com/retroviral-cloning-and-expressionvectors).
   This is because they lack virulence genes from these viruses and only have a truncated gag, rre and modified long terminal repeats (L TRs), to optimise plasmid packaging and allow gene expression in target cells.
   Packaging will be carried out using separate 2-plasmid systems that supply the packaging proteins Gag + Pol and the VSV-G pseudotyping proteins in trans, to generate the virions that can transduce cell lines and lead to the expression of gene of interest, but, that cannot induce production of new virions.

5. GM 130/768 - Protein expression and gene interference in cell culture
   All the vectors used have antibiotic resistance genes however, these antibiotics are not used for the treatment of the diseases associated. These vectors are used worldwide in the laboratory and are considered as safe. Some of these vectors also have a selective marker for eukaryotic cells (geneticin, hygromycin B, puromycin, or blasticidin).
   Transfection vectors will include pEGFP, pRK5, pHM6, pCB6, pTRE-Tight-BI and pTRE2-Pur. All these vectors are commercially available.
   Transduction vectors will include pMIX-IP and pRetroX-Tight-Puro. All these vectors are commercially available.
### 1- GM 130/764 - Alteration of the expression of bacterial protein in pathogens

Health and Safety

**Executive**

Bacterial effector proteins translocated by bacteria via type III secretion systems (e.g. EPEC EspZ). Type III secretion is an endogenous system that bacteria use to transport bacterial proteins into host cells. Effector proteins have diverse biochemical and physiological functions. Some include kinases (e.g. Salmonella SteC), proteases (e.g. Shigella IpaJ), acetyltransferases (Yersinia YopJ), deubiquitinases (e.g. Salmonella AvrA) and others. Collectively, these proteins affect many different cellular functions including actin dynamics, cellular trafficking, cell signalling, innate sensing and some are cytotoxic upon overexpression in certain cell types (e.g. EPEC EspF). Alone, effectors have a limited effect but collectively they contribute to virulence.

We will also construct mutant strains lacking these genes. These will be anticipated to have lower to equal virulence to wild-type.

In some cases, genes of interest will be expressed in bacteria (with or without a tag). This will be done in bacterial strains already knocked out for these genes, in order to complement the mutation. If there is a virulence defect as a result of the mutation, virulence is expected to go back to wild-type levels. Higher expression using a high copy plasmid can be achieved but are not expecting to impact the virulence as higher expression decrease the efficiency of the secretion system.

### 2- GM 130/765 - Bacterial and Eukaryotic proteins cloning and expression in E. coli strains

Bacterial proteins inserted are virulence factors that are needed for the bacteria to be pathogens, but their biological activities are yet mostly unknown. However, each protein individually does not present any risk of hazard as these proteins cannot be injected into the host by the commercial bacteria as they lack the secretion systems required.

These proteins do not have any biological activity in the bacteria.

The eukaryotic genes inserted have various known or unknown activities. However, none of the proteins are secreted from the bacteria and they do not present any hazard risk.

### 3- GM 130/766 - Identification of host partner for bacterial protein in yeast

Bacterial proteins inserted are virulence factors that are needed for the bacteria to be pathogens, but their biological activities are as yet mostly unknown as their targets are inside the host. However, each protein individually does not present any risk of hazard as these proteins cannot be injected into the host by the commercial yeast as they lack the secretion system required.

The eukaryotic genes inserted have various known or unknown activities. However, none of the proteins are secreted from the yeast and they do not present any hazard risk.

### 4- GM 130/767 - Viral transduction of cell culture lines for virulence factor study

Fluorescence or tagged eukaryotic genes, including point and deletion mutants. Examples are lifeact-mTurquoise, which labels filamentous actin in live cells, and CD63-GFP that is a marker of mature lysosomes.

Reporter genes, such as luciferase or biotin ligase BirA.

Fluorescence or affinity tagged bacterial genes or mutated variants from pathogenic E. coli (e.g. EPEC and EHEC), Shigella, Salmonella spp. which include e.g. espZ. None of the genes is anticipated to create a new hazardous GMM.

Silencing RNA expression gene cassettes, e.g. to downregulate the expression of endogenous eukaryotic genes.

No new GMMs will generate a new hazardous virus or reconstitute the viral vectors.

### 5- GM 130/768 - Protein expression and gene interference in cell culture

Bacterial proteins inserted are virulence factors that are needed for the bacteria to be pathogens, but their biological activities are yet mostly unknown. However, each protein individually does not present any risk of hazard as these proteins cannot be injected into other cells.
The eukaryotic genes inserted have various known or unknown activities. The greatest potential hazard assessed would be the knock down expression of a tumour suppressor function (e.g. p53, Rb or PML). There is no obvious, substantial hazard associated with knock-down expression in a restricted population of cells. Many in vitro cell lines already have defects in tumour suppressor genes and are handled without specific precaution. Should knock down of a tumour suppressor gene occur following in vivo delivery, it is not likely by itself to cause cellular transformation. Additional genetic events would be required.

### Evaluation of foreseeable effects

1- GM 130/764 Alteration of the expression of bacterial protein in pathogens

**HAZARDS ARISING:**

**Health and Safety Executive**

The mutants generated will either be loss of function mutants or carry non-toxic reporter genes. It is not anticipated that bacterial strains, including complemented strains, produced in the laboratory will be more virulent than strains that already exist in the environment even in the case of an increased expression of the specific virulence factor due to a high copy plasmid. Indeed, many strains produced will have deletions in genes, which are required for optimal growth and virulence of the organism. In addition, antibiotic resistance markers (carbenicillin, gentamycin, chloramphenicol, tetracycline, kanamycin and streptomycin markers) used in the laboratory already exist in the host strains found in the environment and hence there is no risk of introducing new resistance genes to environmental strains.

**HARM TO HUMANS OR ANIMALS:**

The WT strain can cause potentially lethal disease in immunocompromised adults or pregnant women. As the source of infection is the ingestion, the risk is easily mitigated by good laboratory practice. EPEC, EHEC, C. rodentium, EIEC, Salmonella spp. and S. sonnei are not pathogens under DEFRA control. EPEC and EHEC can colonized some mammals in the wild. Mutation is very unlikely to revert to WT and should not induce a more severe infection than the WT as we are targeting genes that are required for the infection.

- a. C. rodentium is a mouse restricted pathogen however, wild mice are resistant to the infection.
- b. Salmonella enterica can infect a wide range of animals including dogs and cats. Mutation is very unlikely to revert to WT and should not induce a more severe infection than the WT as we are targeting genes that are required for the infection.
- c. Salmonella bongori colonises cold blooded animals including turtles however, it is not known if these bacteria are associated to any diseases in the environment. As for the other pathogens, mutation is very unlikely to revert and should not induce a more severe infection than the WT.

2- GM 130/765 - Bacterial and Eukaryotic proteins cloning and expression in E. coli strains

**HAZARDS ARISING:**

The virulence factor by itself will not present any pathogenicity as the bacteria required multi-factor protein to become infectious and provide no risk. Antibiotic resistance markers (carbenicillin, gentamycin, chloramphenicol, tetracycline, kanamycin and streptomycin markers) can be used for infection treatment however, the bacteria used have been designed so that they will not survive long outside laboratory conditions.

**HARM TO HUMANS OR ANIMALS:**

These bacteria are classified in Risk Group 1 in biosafety guidelines and not known to induce harm to human or animals.

3- GM 130/766 - Identification of host partner for bacterial protein in yeast

**HAZARDS ARISING:**

Saccharomyces cerevisiae, commonly known as baker's yeast, is not a pathogen for humans or the environment. The expression of the virulence factor or eukaryotic protein will not confer any pathogenicity. Furthermore, the strains used in this study are auxotrophic for some amino acids and cannot survive in the environment.
HARM TO HUMANS OR ANIMALS
These yeasts are classified in Risk Group 1 in biosafety guidelines and not known to induce harm to human and animals

4- GM 130/767 Viral transduction of cell culture lines for virulence factor study
HAZARDS ARISING:
Two main risks raised from the generation of this GMM. The first one is linked to the formation of Replication Competent Retrovirus and the second one is related to gene expressed in the target cells that can be oncogenic or alter the expression of oncogenic genes (p53, Tb, PML). These two risks will be mitigated by controlling the sequence homology between the various components of the vector system and by reducing the risk of exposure by using a Class 2 MSC. No sharps will be used, gloves will be worn and filtered tips will be used for all the manipulations of the virion and the virion-producing cell lines.

HARM TO HUMANS OR ANIMALS:
Recombinant retroviruses (oncoretrovirus) could potentially infect tissues via inhaled aerosols, broken skin, or needle-stick injuries. However, these viruses are defective for replication and no proliferation of the virus should occur. As the virus is defective for replication, it should not impact the host defence mechanism. However, insertional mutagenesis is still a possibility even if a remote one which could lead to alteration of the immune response or could lead to oncogenic.

5- GM 130/768 - Protein expression and gene interference in cell culture
HAZARDS ARISING:
Use of HeLa cells present a risk as these cells have the HPV virus however, the GMM generated does not have a higher risk than the wild type. The HeLa cells will be handled in containment level 2 facilities using good microbiological practise. The greatest potential hazard assessed would be the knock down expression of a tumour suppressor function (e.g. p53, Rb or PML) in a permanent cell line. There is no obvious substantial hazard associated with knock-down expression in a restricted population of cells. Moreover, many in vitro cell lines already have defects in tumour suppressor genes. Should knock down of a tumour suppressor gene occur following in vivo delivery, it is not likely by itself to cause cellular transformation. Additional genetic events would be required. Containment facilities are designed to prevent operator exposure and are appropriate to control this potential hazard.

HARM TO HUMANS OR ANIMALS:
GM cells generated do not have increased risk from existing traits. Viruses pathogenic for humans are one of the most likely biohazards presented by cell cultures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For some aspects of the in vivo work, mice may be inoculated with GM bacteria under the permission granted in Home Office project licence (application under submission)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

LIQUID WASTE:
Disinfectant Product Name: VIRKON
Health and Safety Executive
Generic Chemical Name: Oxone (potassium peroxymonosulfate), sodium dodecylbenzenesulfonate, sulfamic acid,
and inorganic buffers
Expected degree of kill: 99.999%
How is this validated? Antec supplies the independent test report. All the bacteria associated with a risk for humans that we are using were tested including Salmonella, E. coli and Shigella.

SOLID WASTE:
Autoclave
Expected degree of kill: 99.999%
How is this validate: Autoclave calibration is assessed every year as part of the normal procedure within the School of Biosciences

**Please enter comments on the GM safety committee on the risk assessment**

The connected programme outlined above was initially submitted to the University Genetic Modification & Biological Agents Safety Committee as two risk assessments. However, due to the number of different approaches being used, the committee advised Dr. Berger that it would make better sense to submit the work as 5 individual risk assessments that comprise the connected programme presented here. There is an additional GM animal form to be submitted to the University GMBA Safety Committee, which will be reviewed locally in due course, which hasn't been included with this notification paperwor.

During the process of separating the initial two risk assessments into the suggested connected programme, the GMBA Safety Committee required Dr. Berger to (1) make clear what work would be undertaken in each aspect of the programme, and (2) provide a clear explanation on how each risk assessment fits into the overall connected programme. This was not clear in the original submission but has subsequently been addressed in each of the individual risk assessment forms and in the appendix depicting the programme (appendix included in the paperwork as part of this notification).

The committee also asked for a number of minor amendments to be made to all of the risk assessments, all of which have been addressed. Typical examples of such amendments would be the reconsideration of the most hazardous GM to be constructed in GM130/764. Initially the link between the use of high copy number plasmids and a potential increase in virulence had not been considered. The committee also asked for confirmation that the plasmid vectors were non-mobilizable.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Additional Information

i) Scientific Purpose:
This application covers research aimed at understanding the interaction of the immune system and fungi. We are particularly interested in how the immune system recognises fungi via surface receptors and soluble factors. These studies will involve in vitro studies.
Aims include:
Determining the role of recognition systems in the host response to infection.
Targeting of specific receptors to modulate immune responses.
Examining the role of receptor systems in homeostasis.
Examining the dissemination of infectious organisms within the host.
Examining differences in the host response to different Candida species.

ii) Types of genetically modified microbes to be used:
This application will not involve generation of genetically modified fungi, it is rather an application to cover their use in in vitro and in vivo experimentation.
Various strains of different Candida species including C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis and C. glabrata will be used and they will be obtained from other institutions throughout the world.
Recipient or parental organism

Candida species including C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. dublinensis

Host/vector system

To generate the GM Candida the types of vectors used were specific non-mobilisable C. albicans vectors (low copy replicating and integrating vectors [YPB1, Clp10, Clp20, Clp30]; low copy replicating and integrating vectors for ectopic expression [YPB-ADHpt, pACT1]; integrating expression vectors regulate by methionine [MET3 promoter], maltose [MAL2 promoter], glucose [PCK1 promoter], or doxycycline [tet promoter and reverse tet promoter]. Some of these vectors were developed in the laboratories of Prof. Brown, Gow and Odds (Aberdeen), the suppliers of most of the strains to be used in this study, and they are being used in laboratories all over the world.

Origin & function

The genetic material was sourced from Candida, prokaryotes and recombinant reporters. NOTE: We will not modify Candida. This large scale modification has already occurred and is ongoing in the laboratories of our collaborators (e.g. Prof Gow, Aberdeen, Prof Julian Naglik):

Nature of the genetic modification:
Most experiments will involve fungi with mutations which target, either directly or indirectly, the fungal cell wall biosynthetic machinery and/or virulence factors. This will include fungi with targeted mutations in genes involved in, for example, metabolism and transcription. Fungi overexpressing fungal or foreign genes may also be used (e.g. fluorescent reporter molecules).

Examples of modified genes include:
- Chitin synthetic genes
- Mannosylation genes
- Beta-glucan synthetic genes
- Hypha specific genes
- Cell wall protein genes
- Secreted hydrolases and other virulence factors
- Transcription factors
- Signalling genes
- Calcium signalling genes
- ABC transporters
- Stress and metabolism genes
- Genes involved in programmed cell death
- Auxotrophic and dominant selectable markers
- Regulated promoters
- Sterol synthesis genes
- Expression of reporter genes (e.g. fluorescent molecules or luciferases)
- Transposon mutant libraries

These modifications will not endow the Candida species with any harmful properties, in fact they usually result in less virulent Candida.

Proposed methods:
In vitro experiments will be performed.
For example, bone marrow derived macrophages, bone marrow derived dendritic cells, neutrophils or monocytes from wild type mice or from genetically altered mice, lacking certain receptors such as C-type lectin like receptors or certain
cytokines or cytokine receptors such as IL-27R or IFNAR, will be incubated with different Candida species/strains. Immune functions such as cytokine/chemokine production and host cell signalling will be examined and Candida killing assays will be performed. For these types of assays, candida will be incubated with cells and cytokine/chemokine production will be measured by ELISA. For cell signalling experiments, cells will be lysed and cell signalling will be examined by western blot. For the candida killing assays, cells will be lysed and candida will be plated and cultured for 24h to determine the cells ability to kill candida. In some instances, cells will be prestimulated with certain cytokines such as IFN-b to increase candida killing ability.

Evaluation of foreseeable effects

C. albicans is a human pathogen in ACDP Hazard Group 2. C. albicans causes superficial infections of mucosal epithelia (thrush) (Odds [1988] Candida and Candidosis, Balliere Tindall). C. albicans can also cause systemic infections which can be fatal, but these only occur in severely immunocompromised patients. To keep things in perspective, it is important to realise that C. albicans is widely distributed. C. albicans is carried commensally at least 60% of the healthy population and most Candida infections arise as an overgrowth of the strains resident in the normal microflora. It is a micro-organism of low pathogenic potential that rarely affects healthy individuals. It only becomes a potential medical problem when an individual's immune responses are significantly impaired. Furthermore, as with most pathogenic micro-organisms, the virulence of C. albicans strains is likely to become attenuated rather than enhanced after prolonged laboratory culture.

Many of the C. albicans strains that will be used are auxotrophic and are largely avirulent. However, by necessity, in virulence studies for example, other strains will not be genetically disabled. This includes null mutations in clinical (wild) isolates. It is important to note that a large number of P. aeruginosa mutants have already been analysed in various laboratories world-wide, but there is no report of a mutation that increases the virulence of this yeast (e.g. Gow et al. [1994] PNAS 91, 6216; Leberer et al. [1996] PNAS, 93, 13217; Hube et al. [1997] Infect. Immun. 65, 3529; Sanglard et al. [1997] Infect. Immun., 65, 3539; Navarro-Garcia et al., (2001) FEMS Microbiol. Rev. 25: 245-268). This is not surprising, since all evidence to date indicates that virulence in C. albicans is a multi-factorial process at the molecular level, with no single virulence factor of over-riding importance. Therefore, mutations, even in wild isolates, are expected to have a neutral or negative effect upon virulence.

In some experiments, we will use C. albicans strains ectopically expressing specific genes, such as GFP or luciferase for example, although we may also use strains which over express cell-wall specific or other genes. We will also use strains where genes have been deleted and then reintegrated (Moyes [2016] Nature 532,7597). The ectopic expression or re-expression of these genes has no affect on virulence, and in some cases it even reduces C. albicans virulence. While all strains that will be tested will not have been fully characterised, it is important to note that the probability of increasing virulence by ectopic expression of a single gene is very low, because pathogenicity is a complex and polygenic trait requiring a high level of fitness of the C. albicans cell (Odds [1994] ASM News, 60, 313). Indeed, there is evidence that ectopic expression of factors involved in virulence traits does not increase virulence (Bailey [1997] PhD thesis, University of Aberdeen; Leng [1999] PhD thesis, University of Aberdeen; Rodaki et al. [2006]).

Experiments will also be performed with genetically modified strains of C. glabrata, C. tropicalis, C. parapsilosis and C. dubliniensis. These species are Closely related to C. albicans, they generate similar types of infections in humans, but they are considered to be less virulent than C. albicans. C. glabrata does not even form hyphae, unlike C. albicans and C. dubliniensis. It will therefore be entirely appropriate to use similar precautions to those used for our C. albicans experiments.

Thus the use of genetically modified strains of Candida strains under laboratory conditions represents minimal risk to human health. Furthermore, even upon accidental infection, normal individuals would clear the doses that are used experimentally from their bloodstream within minutes [MacCallum and Odds (2005) Mycoses 48, 151-161]. Lab workers are unlikely to be exposed to the following factors, which increase the susceptibility to systemic Candida
infections:
1. Use of cytotoxic or strongly immunosuppressive drugs (e.g. systemic corticosteroids)
2. Use of catheters/any intravascular prosthetic device
3. Age (very young or very old individuals are more prone to infection)
4. Serious trauma, particularly when the abdomen is involved
5. Abdominal surgery
6. Patients generally immunosuppressed (e.g. organ transplant recipient, neoplastic disease, severe burns)
7. Long-term use of antibiotics

Lab workers are made aware of these risk factors and are asked specifically to make their supervisors (or Occupational Health) aware if they are exposed to any such risk factors. As a precaution, on occasions where staff/students are undergoing corticosteroid treatment or exposed to some other relevant factor, they are asked to refrain from working with or around fungal pathogens during this time and for an appropriate time after cessation of treatment.

Systemic infections usually only occur in severely immunocompromised patients. Symptoms of systemic Candida infection include fever, rigors and other similar non-specific indicators of sepsis. Standard antifungal treatments include amphotericin B, azoles, and echinocandins.

Symptoms of superficial Candida infection include local rashes (e.g. oral or vaginal) and white aphthae on mucosal surfaces. Azoles or other antifungal agents provide effective antifungal treatment.

With the exception of vaginal infections, none of the above forms of Candida infection are likely to be seen in an immunologically competent, ambulatory person of the type employed as laboratory staff.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No larger GMOs will be used.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogations requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

A safety cabinet will be used, liquid waste will be disinfected with Bleach and solid waste will be autoclaved. The expected degree of kill is >99.99%. Disinfected liquid waste will be disposed of via drain. Autoclaved solid waste will be sent for disposal as Tiger Bag waste to a suitably permitted or licensed landfill site.

Laboratory and associated peripheral workers will be made aware of the risk factors involved with work with Candida.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
The Committee requested clarification on a number of issues including how the GM Candida were produced, whilst acknowledging that the planned activity at Cardiff involves use of these without further modification. The Committee now agrees that enough information has now been provided and that the risk assessment is now suitable and sufficient.

### Project Containment

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#### Project Ref 130/19.3

**Date Ackn’d**

14/06/2019

**CU2 Project Title**

Investigating the role of mutations at the 1q21.1 locus in the associated increased risk for neurodevelopmental disorders by generation of IPSCs and by direct reprogramming into neuronal lineage cells leveraging viral based imaging paradigms

**Class**

Class 2

**CultureVolClass2**

< 1 Litre

**Consent Granted**

Non-GMM

**Project notified under transitional arrangements**

N

**Withdrawn**

N

**Tick if notifying a connected programme of work**

N

### Project Additional Information

**Purposes of the contained use**

The main aim of this project is to provide tools to better understand the molecular mechanisms which underlie the increased psychiatric risk associated with the 1q21.1 copy number variant.
Copy number variants (CNVs) provide an important tool for modelling increased susceptibility to psychiatric disorders, as they are highly penetrant compared to single nucleotide polymorphisms and can be easily modelled. The simplest method to assess these mutations is to make induced pluripotent stem cells (iPSCs) from patients carrying these CNVs. These iPSCs will be created by reprogramming human fibroblasts into iPSCs using Sendai virus based reprogramming methods.

Once these iPSCs have been characterized and validated, they can be reprogrammed into different neuronal lineage cells including astrocytes, neurons, oligodendrocytes and microglia, as well as brain-specific organoids. However, current production of astrocytes and oligodendrocytes from human iPSCs using classical developmental patterning approaches is highly complex and time consuming. Therefore, we will be attempting to directly reprogram iPSCs into astrocytes, neurons and oligodendrocytes by overexpressing key transcription factors known to be critical in these cell types.

Having generated these cell types from the 1q21.1 iPSC lines these cells will then be assessed for any abnormal phenotypes. To aid in the delineation of what may be complex and multifactorial phenotypes, we will exploit the ability to selectively express markers such as GFP, RFP and luciferin within certain cell populations to track and monitor specific subpopulations within our cell systems.

Finally, having identified key mediators associated with specific phenotypes caused by the 1q21.1 CNV, we will then perform knockdown experiments to prove the precise mechanisms underlying these phenotypes and their link to the 1q21.1 CNV. These knock downs will be carried out by viral mediated shRNAs.

Recipient or parental organism

**Lentivirus:**

Plasmids required to produce the virus will be generated and modified in bacteria, specifically a lab based E. coli (either TOP10 or 10-beta). To produce the virus plasmids will be combined in HEK293T cells.

**Sendai virus:**

Purchased from Thermo Fisher Scientific under the product name CytoTune™-iPS 2.0 Sendai Reprogramming Kit

Host/vector system

**Lentivirus:**

Vectors used in this study are commercially available 2nd generation HIV-1-based lentiviral systems, engineered to maximise biosafety by separating the genes required for viral generation onto 3 separate plasmids. The key separation is that of the expression (gag, pol and rev) genes and envelop (VSV-G) gene from the packaging signal contained on the transfer plasmid therefore preventing the expression of envelope genes from integrating into the virus. Additionally, none the plasmids involved contain any of the four essential viral accessory genes required to be replication competent. Consequently, the nucleic material contained within the virus lacks the majority of genetic information to produce further viral particles. Furthermore, the transfer vectors 3'-LTR contain a deletion which prevents the transcription of the full viral genome thereby rendering the virus self-inactivating.

**Sendai virus:**

Sendai virus, in this case, is based on defective and persistent Sendai virus vectors (SeV) lacking the viral M, F and HN genes, and are also non-transmittable. They replicate in the forms of single-stranded RNA in the cytoplasm of the infected cells. They do not go through a DNA phase or integrate into the host genome. It replicates its genome exclusively in the cytoplasm and does not enter the cell nucleus. Therefore, the Sendai virus vector is integration free and is unlikely to alter the host's chromosomes like the conventional viruses.

Origin & function
Sendai Virus:
Although cMyc is a known oncogene, and the other genes are found to be up-regulated in tumour cells, Oct4, Sox2, Klf4, and cMyc (OSKM) genes are also ordinarily expressed in embryonic stem cells. The introduction and expression of these genes in differentiated cell type's results in the effected cell reverting to an embryonic stem cell-like pluripotent state rather than a tumour. The final viral vectors are designed to introduce the OSKM genes to the host cell and facilitate the stable expression of the genes for the purpose of generating iPS cell cultures. The CytoTune® - iPS Sendai Reprogramming Kit contains a temperature sensitive mutant of c-Myc that facilitates the clearance of this vector. It has been shown that c-Myc persists in the cells longer than the other vectors. However, as this virus is nonintegrating the OSKM genes will be cleared and expression will be maintained by endogenous expression.

Lentivirus:
Lentiviruses will contain transcription factors (specific to the induction of astrocytes and oligodendrocytes), imaging constructs (including GFP, RFP and Luciferin) and shRNAs to specific transcription factors. The transcription factors used in this study will be specific to the oligodendrocyte and astrocyte lineage and are not currently considered oncogenic. These vectors will alter the transcriptional regulation of a large number of genes with the aim of making induced astrocytes of oligodendrocytes. The insertion of GFP, RFP or luciferin is unlikely to have any major effect on the cells as this is a routine procedure with little to no reported side effects. Finally the use of shRNA will be limited to specific genes identified as key regulators in phenotypes associated with the 1q21.1 CNV and will only be able to knock down these specific genes with no other effect on the cells.

Evaluation of foreseeable effects

1. The CytoTune® - iPS Sendai Reprogramming Kit contains a temperature sensitive mutant of c-Myc that facilitates the clearance of this vector. It has been shown that c-Myc persists in the cells longer than the other vectors. However, as this virus is non-integrating the OSKM genes will be cleared and expression will be maintained by endogenous expression. Further checks are in place to demonstrate the clearance of this virus as by doing gene expression analysis as this is necessary for the continued use of these cell lines. Therefore, while these viruses have significant effects on the cells they are introduced into they are not maintained or propagated and therefore will not have any effect beyond the originally infected cells.

2. The iPSC cells generated do not form tumours and has clear potential to differentiate into any neural lineage cells when stimulated with specific growth factors and cultured in appropriate conditions. This approach is named developmental reprogramming and its potential for regenerative medicine is being explored. We will perform the gene expression analysis of the derived cells which will confirm the cells don't express tumor associated genes.

3. The genes and constructs which will be expressed in this study are considered non-harmful, and while the transcription factors used have a broad effect on the transfected cells, they are not considered oncogenic and will be under the control of an inducible promoter. The genes contained within the transfer plasmids will have no effect on the viral stability, activity, infectivity or resistance. Therefore, the effect of infection with these viruses is likely to be minimal other than integration of the transfer genes, with larger effects occurring when the transfer genes are induced.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Product Name: Virkon
Expected degree of kill: 100%
Health and Safety

It is sold as tablets or powder which dissolve readily in water. It is intended to be mixed with water to form a 1% to 3% solution (by weight, i.e. 10g to 30g per litre). The pink colour is useful in that it helps gauge the concentration when preparing the Virkon®, and importantly, as the Virkon® ages it discolors, making it obvious when it needs to be replaced. The solution is generally stable for five to seven days. Virkon® has a wide spectrum of activity against viruses, some fungi, and bacteria.

Product Name: Chemgene
Expected degree of kill: 100%

Chemgene is supplied as liquid and is extensively tested against viral, bacterial and fungal species and subtypes. Literature supplied by the manufacturer indicates >log4 reduction in HIV when Chemgene is used at a concentration of 1:50. In House SOP is to add chemgene at a minimum of 1:50 dilution to spent media containing viral particles in a sealed container for a minimum of 24 hours before liquid is considered decontaminated. Any plasticware which has come into contact with viral particles is disinfected as above and then autoclaved and any consumable material including gloves, spent media bottles or other material which has not come into direct contact with virus but has been used while performing work using the viruses is autoclaved before disposal.

Cardiff University GMBA Committee agreed that the activity described is a Class 2 activity requiring Containment Level 2. After some queries and subsequent additions/alterations regarding the source of the human fibroblasts and what type of Lentiviral vectors were to be used, the Committee are now satisfied with the risk assessment for this project.

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 130/20.1

Characterisation of host responses to infectious organisms

This work focuses on the use of pathogens modified to contain a reporter genes as tools to characterise host responses to and regulation of infectious organisms. A number of pathogens with a broad range of replication characteristics are included to gain a maximum understanding of host responses to infection.

Recipient or parental organism

Mice (C57BL6)/Immortalised mouse cell lines/immortalised human cell lines! Primary human cells! Primary mouse cells

Host/vector system

Herpes simplex virus (HSV) 1 or 2, Human Cytomegalovirus (HCMV) (H2B5, UL 127, Power, ts, ts66, Towne, Gery, Toledo, Towne long, Fiala-P6, FK7), Murine Cytomegalovirus (MCMV) (Smith, Rm461, MIEP-GFP RM427+, ES, Rm408, Rm427, Power, C3X, C3X-ES, ME97.01, Smith) + mutants (ie1 and 3 knockouts + revertants), Epstein Barr virus (EBV) B95.8 strain restricted to B95-1 cells, Murine Herpesvirus 68 (mHV-68), Bacterial artificial chromosomes (BACs) e.g. ZEN-Ub1 for gene knockout. Influenza A virus (Udorn/72 (H3N2) and A/WSN/33 (H1 N1 )), Human adenovirus (E1 deficient), Vaccinia (Ankara), Baculovirus expressing HCMV pp65, Lentivirus (HIV-1 derived and attenuated)

Origin & function

For reporting/ purifying cells/ viruses: Jellyfish - GFP, RFP, YFP, Firefly luciferase, Renilla luciferase, Bacterial
antibiotic resistance genes
To analyse gene function in vitro: PCR amplified human and mouse genes - Gpr84, Gpr43 Hk3, Olah and Ch25h
(No oncogenes), Lab generated short RNA sequences and hairpins
For reverting mutated mCMV: PCR generated ie1 and ie3 from mCMV
To enhance gene expression: SV40 promoter from Simian Virus - into adenovirus plasmids, CMV promoter from
hCMV - into lentiviral plasmids and adenovirus

Evaluation of foreseeable effects

Herpesviruses (ACDP Hazard Group 2 suitable for Containment Level 2 conditions)
HSV-1 and HSV-2 are naturally occurring neurotropic a-herpesviruses, and whilst morbidity/mortality can be high in
eonates or immunocompromised people, infection of healthy individuals causes a self-limiting subclinical primary
infection in healthy individuals. Neither vaccine nor anti-serum is available, and treatment of severe cases is local
or systemic use of acyclovir. Despite a high (~90%) global prevalence, HSV-1 and HSV-2 present a potential risk to
humans and are classified as ACDP Hazard Group 2.
CMV is a human J3-herpesvirus endemic in 40-100% of the population depending on geographic location/socioeconomic
status, and infection occurs largely in childhood by direct contact with infected bodily secretions. Primary
infection is largely benign in healthy individuals but causes severe opportunistic infections in the
immunocompromised and is the major cause of congenital infections which can cause severe CNS abnormalities.
There is no vaccine available, but infection can be treated by hyperimmunoglobulin, ganciclovir and valganciclovir.
CMV present a potential risk to humans and are classified as ACDP Hazard Group 2.
EBV is a human v-herpesvirus with very high (~90%) seroprevalence in UK adults. Primary infection causes
infectious mononucleosis, and infection remains latent in B-cells. EBV is associated with Burkitt's lymphoma,
nasopharyngeal carcinoma, Hodgkin's lymphoma and gastric cancer. Due to high prevalence, and very rare
occurrence of re-infection, experimental work with EBV poses no additional threat to normal exposure to the virus.
EBV present a potential risk to humans and are classified as ACDP Hazard Group 2.
MCMV is a murine-specific b-herpesvirus and poses no threat to human health and safety.
mHV68 is a murine-specific v-herpesvirus and poses no threat to human health and safety.
Human adenoviruses (HAdV) are transmitted by aerosols and faecal-orally and cause acute infections the
respiratory tract, pharyngitis, conjunctivitis, cystitis and gastroenteritis. Zoonotic transmission to humans has been
reported (from bovine, simian and feline adenoviruses) but is asymptomatic. Infection is diagnosed serologically, or
by PCR and whilst there is no vaccine available, patients with severe infection are treated with Cidofovir. HAd V are
classified as ACDP Hazard Group 2, and CL2 conditions are suitable for their growth.
Influenza A is a segmented RNA virus infecting aquatic birds, but zoonosis to humans causes highly virulent
respiratory tract infections. Season-specific vaccines, containing the currently Circulating species and
neuraminidase inhibitors (Osaltamivir and Zanamivir) are available. Influenza subtypes B, and much rarer subtype
C, also infect humans and the nature of the segmented genome means co-infection is common and there is the potential to generate strains with altered
pathogenicity/tropism due to genome re-assortment. For this reason, work
will be restricted to human virus strains, and those classified as ACDP Hazard Level 2. These will include Influenza
AIUdorn/72 (H3N2) and AIWSN/33 (H1 N1), CL2 conditions are appropriate for growth of these strains.
Lentivirus: These are genome integrating viruses which can potentially pose a hazard to human health by
integrating into host DNA. Although lentivirus synthesised from the vectors has the ability to enter all cells within the
body they are replication deficient thus pose relatively little risk. As these viruses integrate into the host genome,
they can potentially produce cancerous cells, therefore no oncogenes will be inserted to effectively eliminate this
risk.
Baculovirus is incapable of replicating in mammalian cells.
All viruses that infect humans may potentially be transmitted to workers by ingestion, injection and droplet or
aerosol exposure of mucus membranes. All work will be carried out in a class 2 biosafety cabinet with full protective equipment (lab coat, gloves, glasses). The modifications of viruses to insert reporter genes are not expected to alter virulence.

E. coli K12 and 8 derivatives, such as DH5a and DH10- used to propagate individual viral and host gene expression plasmids, are disabled laboratory adapted strains unable to survive outside culture conditions or colonise the human gut. They are generally non-hazardous to human health.

Well characterised and pathogen-permissive mammalian cell lines or primary cells will be used for the culture and propagation of wild-type and mutant pathogens under Containment Level 2. Cell lines used include HeLa cells (human epithelial cells), A549 cells (human lung epithelial cells), THP-1 cells (human monocyte), RAW 264.7 (murine macro phages), 3T3/NH (murine fibroblasts), p53 -/- MEFs and STAT1 -/- MEFs (murine embryonic fibroblasts), Mewo cells (human melanoma cells), MRC5 cells (human lung fibroblast cells), HEK-293 and 293T cells (human embryonic kidney cells), and 895-1 cells (EBV-positive lymphoblastoid cells).

Mice will be genetically modified, however the modifications are not anticipated to endow the GMO with any novel harmful properties and will not provide the GMOs with a survival advantage. Knockout of these genes will have potentially harmful phenotypic effects on the animal, which are recorded on the Jackson website. No new genetically modified mice will be created so this data is available. The likelihood of these effects spreading to the environment are effectively zero because this would require cross breeding with wild-type mice that must escape our secure animal containment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The animals are contained in specific pathogen free environments and virally infected mice will be housed in sellotainer cages all inside a secure animal facility which has barriers to prevent any animal escape if they got out of their cage. Cages are checked for mouse numbers every day. All work conducted on virus infected animals will be carried out under a biosafety level 2 cabinet in category 2 designated rooms.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be treated with 10,000 ppm Sodium Hypochlorite (HazTabs) for 4h before disposing down the sink, this treatment will kill all GMMs used in this protocol - tested by viral and cell culture attempt. All solid waste, including sharps, will be disposed of via clinical incineration. In the case of virus waste solids will be treated where appropriate with sodium hypochlorite and autoclaved before incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Minor corrections were asked for such as typo's and also in regard to where the terminology used drifted between that used in the UK and USA e.g. containment level/biosafety level. Please see section 17 for further comments.

### Project Containment

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### Project Ref 130/20.3

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<td>09/07/2020</td>
<td>Identification of T cell receptor specificity in type 1 diabetes patients as part of single cell analysis of the antigen-specific response</td>
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<td>&lt; 1 Litre</td>
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<th>Project notified under transitional arrangements</th>
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### Project Additional Information

**Purposes of the contained use**

Type 1 Diabetes is a result of the targeted attack on the insulin-producing beta cells within the pancreatic islets of Langerhans. Antigen-specific immunotherapy is one promising avenue for preventing/reversing autoimmune diseases, like type 1 diabetes, by restoring tolerance to autoantigens. However, to date, it has proved very challenging to monitor the antigen-specific response using peripheral blood samples as the frequency of relevant
cells is very low. Our group has developed a protocol to sample draining lymph nodes (LN) in humans via ultrasound guided fine needle aspiration. Lymphocytes are harvested pre- and post-intradermal treatment with islet derived peptide/antigens (GAD-alum or pro-insulin C19-A3) for phenotypic analyses. Single cell (bar-coded) RNAseq next generation sequencing is used to phenotype individual LN-derived cells. Through this technology, the T cell receptor (TCR) sequence can be determined including the appropriate pairing of alpha and beta chains. Single cell analysis also identifies if unique TCRs are expressed by 2 or more cells, indicating clonal expansion. To complete this approach to monitoring the antigen-specific T cell response, we require technology to identify the antigen specificity of T cells of interest, such as those clonally expanded. The remaining single cell RNAseq data provides the complete transcriptome of each cell which can then be matched to antigen specificity to characterise phenotype and likely function (e.g. T reg, Teff, Tcm etc). I will generate hybridomas expressing the TCR sequences identified by RNAseq from human LN. Vectors are generated with custom oligos based on patient sequencing and ligated with established packaging vectors. DH5alpha cells will be transformed. PCR and sequencing will confirm vector construction. Phoenix-ECO cells will be transfected to generate mouse stem cell virus particles with packaging and TCR which will be used to transduce 5KC mouse cells, which lack a T cell receptor. Cells expressing the vector will be selected based on CD3 expression. Peripheral blood will be used as antigen presenting cells to challenge the generated hybridomas with GAD protein and/or insulin peptide C19-A3. Testing these cells will identify the antigen specificity and phenotype of T cells in patients with type 1 diabetes undergoing immunotherapy. Of importance, if T cells specific for GAD or Insulim-are identified, these cells will be evaluated for phenotypic changes during the trial.

Recipient or parental organism

The retrovirus used in this system is murine-specific thus presenting no risk to humans. Phoenix-ECO cells will be transfected to generate mouse stem cell virus particles with packaging and TCR which will be used to transduce 5KC mouse cells, which lack a T cell receptor. The Phoenix cells are treated with mitomycin c to prevent cell replication, resulting in cell death after the initial shedding of virus. There is no risk associated with expression of T cell receptors on the transduced cells.

Host/vector system

Murine stem cell virus (MCSV) is as mouse-specific retrovirus that infects embryonic and hematopoietic cells. The vector lacks Pol/gag/env, which is provided by the packaging cell line (Phoenix-ECO). The retrovirus is mouse specific and does not infect human cells.

Origin & function

Inserted genes encode the alpha and beta variable chains of the T cell receptor based on next generation sequencing of patient samples. DNA oligos are provided from Twist Bioscience for insertion to vector. Antigen specificity will be evaluated via expression of the TCR on murine 5KC cells.

Evaluation of foreseeable effects

The virus is murine specific and does not infect human cells. The virus in replication deficient. The vector encodes for human T cell receptor variable regions which are specific for HLA and would not recognize mouse MHC.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Work will be conducted in a class 2 biological cabinet following appropriate guidelines for safe handling. Solid waste will be put in yellow bags for autoclaving. Liquid waste will be collected in bottles and treated with 10% bleach overnight to provide full inactivation of viral proteins.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The University GMBA Committee had a number of queries regarding the risk assessment and requested a small number of amendments. The queries have now been answered and amendments made. The Committee were satisfied that this work could go ahead in Containment Level 2 facilities.

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**Project Ref**  130/20.4

**Date Ackn’d**  26/08/2020

**CU2 Project Title**  Gene and epigenome editing for expanded CAG/CTG repeat disorders in human cells

**Class**  Class 2

**CultureVolClass2**  ≤ 1 Litre

**CultureVolumeClass3-4**

**Non-GMM**  Consent Granted

02/03/2022  Page 3753 of 15326
We are developing new ways of treating a family of over 20 different neurological, neuromuscular, and developmental diseases that affect over 1 in 400 people worldwide. These are caused by the expansion of tandem repeats in the genome and include Huntington’s disease, myotonic dystrophy type 1, as well as several spinocerebellar ataxias. Specifically, we will use gene editing (via CRISPR-Cas9 nickase) approaches to contract the repeat tracts, or deliver chromatin modifying enzymes that could lead to the silencing of the allele containing the expanded repeat tract. We also plan to make knockout of specific factors individually or as part of screens to understand how these processes work and thus how to make them more efficient and safer. The cDNA of the candidates that we identified (e.g., DNA repair genes) will also be overexpressed.

**Recipient or parental organism**

Human cell lines, patient-derived cells, mice, E. coli, S. cerevisiae

**Host/vector system**

Non-replicating lentiviral vectors, plasmid DNA, gutless Adeno-associated viruses

**Origin & function**

We will use these vectors to deliver inside human cells human cDNAs, as well as the bacterial Cas9 enzyme and/or a single-guide RNA. We will use genome-wide libraries and thus the genes mutated have varied roles that are explained in details in the accompanied risk assessments.

**Evaluation of foreseeable effects**

E. coli has been used extensively for molecular cloning, using the plasmids containing genes granting antibiotic resistance to ampicillin, kanamycin, chloramphenicol, tetracycline, and/or spectinomycin. The strains of E. coli used, e.g., DH5α, are recombination deficient (for example because of the presence of recA or recBCD mutations) and non-pathogenic.

S. cerevisiae will be used to construct plasmids for expression of specific recombinant DNA. This yeast strain, W303, is not pathogenic and contains a number of
spontaneously occurring auxotrophies that reduce its fitness.

Mus musculus will be generated with a Cas9 nickase knocked in at the ROSA26 locus. Another line will express a dual colour reporter for detecting changes in CAG/CTG repeat size in vivo. This reporter will also be knocked into the ROSA26 locus. This reporter will be crossed to the R6/1 Huntington mouse model. Given that the risk of using these strains of yeast and bacteria is negligible, we request that this work be class 1. The mouse strains also do not pose a threat to the health of the workers beyond the risks associated with handling mice. In addition, the mice are held in secure mouse facilities, greatly minimizing the chance that they escape into the wild. Thus, we request class 1 for these as well.

Adeno-associated viral viruses: AAVs are non-replicating by nature as they require the help of adenoviruses or herpes simplex viruses. We will use them with the Cas9 nickase or a sgRNAs packaged into them. The packaging will be outsourced to the UKDRI facility in King’s college or to an outside company. These vectors will be tested in the lab before being used in vivo. Although these viruses are considered class 1 by the HSE, we will be using them in large quantities and they can infect (but not cause disease) if a worker is already infected by helper viruses. Therefore they will be treated as class 2 agents. PPE will be used and the workers will be trained accordingly.

Lentiviral vectors: We will use lentiviral vectors that are replication-deficient and use 3rd generation packaging for reducing the risk of generating proficient viruses by recombination. Lentivirus are clearly a class II organism and will be treated as such (see below for details). The risk of infection of a worker is highest while making the viruses and early in the first few days of the experiments. Sharps will be avoided, and the viruses and infected cells will be handled in category 2 hoods. PPE will also be used and the workers will be trained accordingly.

The mice are housed in specific rooms inside the facility with at least three doors to get in and out that are card-accessible. There is no window to escape. The mice are inside the cages and are ID’ed. There is a daily check on them and thus any missing mouse will be noticed within 24 hours. There are traps that are routinely checked for function in the facility to catch any mouse that escapes the cages (eg. during cage changes). The number of people having access to the premises is limited and requires card access, reducing the chances that someone deliberately walks in to “free” the mice. Any new faces are challenged and only those that undergo a full induction by the facility manager would be allowed access. All control measures are carried out under the guidelines of Joint Services.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Centrifugation can lead to aerosol formation and containment of such activities to minimize cross contaminants is necessary. All tubes will be wiped down with ethanol prior to being opened in a category 2 hood. SOP of wiping the hood down prior to use and after use is necessary as well as use of UV light for decontamination.

Liquid waste will be disinfected using Presept tablets (2.5g), Chemogene or similar 2500ppm, sodium Dichloroisocyanurate (chlorine-based disinfectant). We expect that this will kill 100% of the viruses within 4 hours.

Recombinant viruses will at all times be maintained in an environment designed to prevent aerosol spread. All flasks containing the virus shall be kept closed except when decanting and pipetting which will be conducted in tested class II safety cabinets. Centrifugation steps shall be performed in sealed buckets and centrifuge tubes containing the virus will be opened only in safety cabinets.

Contaminated plastic pipettes will be submerged in Presept disinfectant, or a similar chlorine based disinfectant, for a minimum of 4hr prior to disposal. The tissue culture cabinet and any potentially contaminated areas will be disinfected with 70% ethanol. Waste that has been decontaminated will then be double-bagged and autoclaved.

Users will wear double gloves when handling viruses, and the outer glove removed when moving out of the safety cabinet.
Solid waste will be autoclaved.

The Committee were satisfied that the separate projects could be linked as a connected programme of work. The highest risk activities are Class 2 activities. The Committee were in agreement of the Containment level of the facilities required. The Committee had a number of queries which have been answered to their satisfaction. They also required that Professor Dion reorganise some of the forms and produce a diagram to clearly indicate the connections between the parts of the programme. These are all submitted with this notification.

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**Project Ref** 130/20.5

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<td>Role of neurodegenerative disease associated genes in mammalian/human neurons, glia and stable cell lines</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

02/03/2022

Page 3756 of 15326
This programme of work aims to investigate the pathogenic mechanisms underlying neurodegenerative disease processes implicated in Alzheimer’s disease, Parkinson’s disease and frontotemporal dementia. Our approach includes the manipulation of disease linked genes as a model system for biological investigation. We will manipulate gene expression levels (knockdown and over expression) to investigate cellular changes, as well as introduce tagged proteins to facilitate biochemical analysis.

We will manipulate expression levels of target genes in rodent primary neurons, stable neuroblastoma and microglial cell lines using constructs expressing cDNA to increase expression, or short hairpin RNA sequences (shRNA) to knock down expression of genes linked to neurodegenerative disorders (motor neuron disease, frontotemporal dementia), autophagy and mitochondria biology.

This project will only use commercially available vectors obtained from Horizon Discovery/Dharmacon, OriGene, VectorBuilder and Genscript. Manipulated cells will be investigated using a variety of techniques including morphological analysis by fluorescent microscopy, molecular biology and biochemical assays. Lentiviral vectors and particles will not be produced under this proposal.

Recipient or parental organism

- BV2 mouse neonatal microglia (RRID:CVCL_0182)
- SH-SY5Y human neuroblastoma stable cells (ATCC CRL-2266)
- HEK293 (RRID:CVCL_0045)
- Mouse embryonic (E17) cortical neurons

Host/vector system

- SMARTvector Lentiviral shRNA - Commercial short hairpin RNA expressing known vector (Dharmacon)
- pGFP-C-shLenti Lentiviral shRNA - Commercial short hairpin RNA expressing known vector (Origene)
- pLK0.1[shRNA] - U6-promoter driven Knockdown vector derived from the third-generation lentiviral vector system.

Origin & function

- LVs contain shRNA will target:
  - TBK1: TANK binding kinase 1, kinase with broad activity in immunity, mitophagy and autophagy
  - p62: autophagy adapter molecule targeting intracellular molecules for degradation
  - OPTN: autophagy adapter molecule targeting intracellular molecules for degradation
Gene expression LVs will contain human or mouse cDNAs for the below, either with or without fusion with the biotin protein ligase BirA R118G:

- ABI3; ABI Family Member 3,
- BIN1; Bridging Integrator 1,
- CD2AP; CD2 Associated Protein,
- CD33; Sialic Acid-Binding Ig-Like Lectin 3
- INPP5D; Inositol Polyphosphate-5-Phosphatase D, immune cells.
- PICALM; Phosphatidylinositol Binding Clathrin Assembly Protein,
- PILRA; Paired Immunoglobulin-like receptor
- PLCG2; Phospholipase C Gamma 2,
- PTK2B; Protein Tyrosine Kinase 2 Beta,
- SPI1; Spleen Focus Forming Virus (SFFV) Proviral Integration Oncogene
- TREM2; Triggering Receptor Expressed on Myeloid Cells 2
- MS4A6A; Membrane Spanning 4-Domains A6A.
- CLNK; Cytokine Dependent Hematopoietic Cell Linker,
- SCIMP; SLP Adaptor and CSK Interacting Membrane Protein
- MEF2C; Myocyte Enhancer Factor 2C,

In addition to the gene specific cDNA or RNAi sequences, LVs will contain combinations of the following standard viral vector components: Woodchuck hepatitis virus posttranscriptional regulatory element, Simian virus 40 early polyadenylation signal, eGFP/RFP, antibiotic resistance cassette (Ampicillin, Kanamycin, Puromycin, Neomycin), promoter (U6, CMV, EF1a) and an Internal ribosome entry sequence.

**Evaluation of foreseeable effects**

The lentiviral vectors used in this project pose a low biohazard risk as though capable of transducing human cells, however the virus is replication incompetent. Replication requires sequences coding gag, pol, env, and rev, which are removed from these third-generation vectors and would be required to be provided in trans by helper constructs. There is no evidence to suggest that the recombinant virus may alter susceptibility to host defence mechanisms. All DNA components of the recombinant virus are routinely used in biological research, and are not known to have effects on susceptibility of host immune systems. To reduce risk in this project we have considered foreseeable circumstances where there is a risk of transmission of the vectors.

**Routes of transmission:**

- Needle stick – needles will not be used in these experiments.
- Inhalation - opening a vial or spillage of the virus could cause an aerosol of viral particles. As such, all tasks where there is a risk of aerosols being produced will be carried out inside the safety cabinet.
- Skin contact - The viral vector could be transmitted through contact with the skin, transmission into the blood stream or to airways and eyes. These risks will be minimised following the standard operating procedure of the virus suite and use of double gloves to minimise risk of skin contact through piercing of gloves. The only consequence of exposure we envisage is some irritation/inflammation caused by the immune response which may be caused by the vector. Normally, if an immediate action is taken, i.e. washing the exposed area with detergents, no significant consequence will occur.

The BCL-II viral suite will have designated lab coats (green) which also will be stored in the viral room.
Standard working procedures and emergency instructions (phone numbers including after work phone numbers) will be clearly posted to the walls on laminated paper. A spill kit will be provided in the room.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Transduced cell lines that are free of infectious virus will be moved from the virus suite for microscopy/opera phenix imaging. Stable cells will also be used for immunocytochemistry and will therefore need to be moved to a fume cupboard/LEV for formaldehyde fixing.

Assuming a maximum dose of 10,000,000 TU per well and lentiviral particle half-life of eight hours (Higashikawa and Chang, 2001) it will take approximately eight days for there to be less than one viral particle per well. We will therefore use conservative 12 day cut-off before moving cells.

We will periodically (six months) test that the stable cell lines are still free from infectious virus particles by concentrating media in a spin column and testing for residual "free" virion RNA using RT-PCR and primers designed to the 5' LTR and 3' LTR region of the lentiviral vector.


Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Preferred local deactivation/disposal pathway:

1) Chemical disinfection by Chemgene or Virkon.

Liquid waste (e.g. culture media) will be inactivated with 5% Chemgene or Virkon® (final concentration 1%) for at least 1 hour (typically overnight) - according to manufacturer’s instructions.

All solid waste (tissue culture plastics) will be treated with Virkon® for at least 2 hours before autoclaving.

2) Waste placed into autoclave bag.

Chemically deactivated waste will be placed into autoclave bags.

3) Waste autoclaved locally using the waste cycle at 134°C for 25 mins.

Solid waste will be autoclaved in the designated autoclave located immediately outside of the viral suite before being placed in a yellow ‘Tiger bag’ and disposed of as ‘offensive waste’ (HL180104).

The autoclave is a Priorclave 60L Benchtop, the machine is serviced annually and a twelve-point thermocouple test is performed annually. Waste material will be inactivated using the waste cycle at 134°C for 25 mins.

All staff operating the autoclave will have been trained in manual handling and are also trained in appropriate spillage procedure. 1% Virkon® or 5% Chemgene is the disinfectant to be used in case of inactivated waste being spilled in or out of the autoclave.
As this is a closely connected programme of work there were common discussion points throughout the 3 risk assessments that make up the programme.

Minor amendments e.g. additional information asked for in Table 1.
Discussion on the suggested use of FFP3 masks in the viral suite (The GMBA advised these will not be needed in addition to the Class 2 MSC). This does not effect any local rules on face coverings/PPE that may be required in regard to Covid-19 restrictions.
Additional information (protocol included with this notification) was required to show transduced cells were virus-free when moved to CL1.

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Project Ref 130/20.6

Date Ackn'd 11/11/2020
CU2 Project Title Alteration of virulence factors in fish bacterial pathogens (GM130/779)

Class 2
Culture
Vol
Class
2
51-500 Litres

Non-GMM
Consent
Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Aquaculture is a diverse industry and the fastest growing food sector. In 2016, 110 million tons of seafood were produced worldwide with an estimated value of $243 billion. This industry, however, faces multiple challenges from global warming to increased infection rates and multi-resistant drug pathogens. Because the physiopathology of bacterial infections is still poorly understood, few preventative or curative treatments are available.

**General Scientific purpose:**

Aeromonas hydrophila, A. salmonicida, Edwardsiella spp., Vibrio anguillarum, V. salmonicida and Yersinia ruckeri are well-recognised fish pathogens that create a strong burden for the fish industry and the environment. All these pathogens are Gram negative bacteria that use different molecular mechanisms to infect their host. Unfortunately, they have a high antibiotic resistance panel, can infect humans (opportunistically) and other animals through the food chain. In humans, they will cause gastroenteritis.

All these pathogens use numerous virulence factors to infect their host including toxin and secretion systems. More specifically, type 3 secretion system (T3SS) and type 6 secretion system (T6SS) clusters have been identified by genome sequencing. These secretion systems allow pathogens to translocate bacterial proteins, named effectors, directly from the bacterial cell into the host cell cytoplasm. Following translocation, the effectors take control of cell signaling for the benefit of the adherent pathogen allowing the bacteria to invade and replicate inside the cells. But only a few effectors have been so far identified and the mechanism of action is poorly understood.

**Specific Scientific purpose and goals:**

Our aim is to delete or mutate virulence factors in the pathogenic bacteria to understand their functions. Phenotypes after infection of cultured cells in Petri dish or fish in aquarium (from 1L up to 160L) by wild type strains or mutant will be compared. Infection with GM bacteria may happen in different rooms simultaneously. Transfer of the GM bacteria from one room to another will be done using a second seal containment. Fishes tested for this project include rainbow trout, guppy (Poecilia reticulata) and zebrafish (Danio rerio).

**Type of GMM to be constructed:**

Bacterial genes of interest will be deleted from bacteria by site directed mutagenesis. Most of the bacterial genes targeted have unknown function but are suspected to be linked to the pathogenicity of the bacteria (secretion system effector, toxin, adhesins and pili). Wild type or mutated genes will be also reintroduced into the mutants by direct gene reinsertion or using plasmid (low and high copy). Random insertional mutagenesis using mini-Tn5

**Recipient or parental organism**
Edwardsia spp., Aeromonas hydrophila, A. salmonicida, Vibrio anguillarum, V. salmonicida and Yersinia ruckeri can cause gastroenteritis and infect open wounds in humans mostly in young children and people who have compromised immune systems. Workers associated with this project will be informed of the risk and will sign a risk assessment form. The pathogens are already present in the environment.

Host/vector system

All the vectors used have antibiotic resistances genes, however, these antibiotics are not used for the treatment of the associated diseases. These vectors are used worldwide in the laboratory and are considered safe. They also are not mobile and should not be transferred by conjugation.

Bacterial complementation constructs will include pSA10 (high copy number, IPTG inducible expression), pACYC184 (medium copy vector, constitutive expression), and pBAD vectors (high copy number, arabinose inducible expression).

Mutagenesis plasmids include pKD46 (arabinose inducible expression of red recombinase) and pACBSR/pAKBSR (arabinose inducible expression of red recombinase and I-SceI endonuclease).

Origin & function

Bacterial effector proteins are translocated by bacteria via type 3 and 6 secretion systems. Type 3 and 6 secretions are endogenous systems that bacteria use to transport bacterial proteins into host cells. Effector proteins have diverse biochemical and physiological functions. Collectively, these proteins affect many different cellular functions including actin dynamics, cellular trafficking, cell signalling, innate sensing and some are cytotoxic upon overexpression in certain cell types. Alone, effectors have a limited effect but collectively they contribute to virulence.

We will construct mutant strains lacking these genes or genes involved in the adhesion of the pathogen (flagella and pili).

These will be anticipated to have lower to equal virulence to wild-type.

In some cases, genes of interest will be expressed in bacteria (with or without a tag). This will be done in bacterial strains already knocked out for these genes, in order to complement the mutation. If there is a virulence defect as a result of the mutation, virulence is expected to go back to wild-type levels. Higher expression using a high copy plasmid can be achieved but with no expected impact on the virulence as higher expression decreases the efficiency of the secretion system.

Evaluation of foreseeable effects

HAZARDS ARISING:
The mutants generated will either be loss of function mutants or carry non-toxic reporter genes. It is not anticipated that bacterial strains, including complemented strains, produced in the laboratory will be more virulent than strains that already exist in the environment even in the case of an increased expression of the specific virulence factor due to a high copy plasmid. Indeed, many strains produced will have deletions in genes, which are required for optimal growth and virulence of the organism. In addition, antibiotic resistance markers (carbenicillin, gentamycin, chloramphenicol, tetracycline, kanamycin and streptomycin markers) used in the laboratory already exist in the host strains found in the environment and hence there is no risk of introducing new resistance genes to environmental strains.

HARM FOR HUMANS OR ANIMALS:
The listed pathogens are pathogenic to aquatic animals (mostly fishes) and are found in the environment however, they are not pathogens under DEFRA control.

E. tarda can infect invertebrates, amphibians, reptiles, birds and mammals, including humans, cattle, swine, dogs, and Weddell seals.

Aeromonas hydrophila can also infect some vertebrates (lizards).

Aquariums ‘infected’ with GM organisms will be housed in suitable containment tanks capable of holding the whole volume of water held in the aquariums.

As the source of infection is from ingestion, the risk is easily mitigated by good laboratory practice.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For some aspects of the in vivo work, fish may be inoculated with GM bacteria under the permission granted in Home Office project licence. Aquariums 'infected' with GM organisms will be housed in suitable containment tanks capable of holding the whole volume of water held in the aquariums.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

LIQUID WASTE:
Disinfectant Product Name: VIRKON
Generic Chemical Name: Oxone (potassium peroxymonosulfate), sodium dodecylbenzenesulfonate, sulfamic acid, and inorganic buffers
Expected degree of kill: 99.999%
How is this validated? Killing of vibrio spp. and Edwardsiella has been reported in the literature (Machen et al. 2008; Mainous et al. 2010). At the end of the experiment in an aquarium, Virkon powder will be added to disinfect the water to reach the terminal disinfection concentration (1kg/100L) during 15 min before extensive washing with water.

SOLID WASTE:
Autoclave
Expected degree of kill: 99.999%
How is this validate: Autoclave calibration is assessed every year as part of the normal procedure within the School of Biosciences

ANIMAL WASTE:
Dead infected animals will be disposed according to Cardiff University rules and regulations established for animal facilities housing animals.


Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University G MBA Committee had queries regarding the use of sharps and also regarding disinfection of large volumes but those queries have now been answered.
The Committee were satisfied that this work could go ahead in Containment Level 2 facilities

Project Containment
Purposes of the contained use

Alzheimer's disease has a strong genetic component, however much of the heritability of late-onset Alzheimer's disease is thought to be polygenic, i.e. due to multiple gene variants that individually have a weak impact on Alzheimer's disease, but when combined together have a stronger effect to increase the risk of Alzheimer's disease. To date, Alzheimer's disease polygenic risk has not been well-studied. To study the effect of Alzheimer's disease polygenic risk on cell biology, we need to capture real patient genetics in a cell model that can be cultured in a dish, and can be transformed into multiple relevant brain cell types. Therefore we propose to take blood cells donated by human patients with high polygenic risk for Alzheimer's disease, and convert ("reprogram") them into induced pluripotent stem cells (iPSC), which have the ability to be differentiated into any human cell type (i.e. pluripotency). In order to generate iPSC, four genes must be overexpressed to establish pluripotency (Oct3/4, Sox2, Klf4, cMyc), which we will achieve by transducing the blood cells with commercial Sendai virus vectors that contain the pluripotency genes. Sendai virus vectors are considered to be a high-efficiency method for establishing pluripotency of human cells, which is necessary since only limited numbers of patient samples are available.
The recipient cells are human peripheral blood mononuclear cells taken from healthy controls and Alzheimer's disease patients. The highest associated hazard is the potential for samples to harbour human blood-borne virus pathogens (e.g. HIV1), and accidental contamination of field or lab workers. Subjects are asked prior to blood collection if they have been diagnosed with a blood-borne virus, and excluded if this is the case, so the hazard applies only to subjects who have undiagnosed blood-borne virus infections at the time of the blood collection. After genetic modification, the recipient cells will transiently express the Sendai transgenes and become iPSC, with the Sendai vectors (and transgenes) completely eliminated after 5-15 passages. The risk to human health and the environment of the iPSC is extremely low, as iPSC survival and pluripotency requires specific maintenance media, and specific environmental conditions (temperature of 37 degrees Celsius and minimum of 5% carbon dioxide). If iPSC were directly injected into a human, any that survive would most likely immediately differentiate into a local specialized cell type and cease replicating. If a large number were injected into a human, teratoma formation is theoretically possible at the site of injection (Xiang et al 2019. Theranostics, 9(1): 290–310). Teratomas are self-limiting, benign tumours, and not likely to cause significant harm to health.

Host/vector system

The vector system is based on Sendai virus, in the form of the commercial kit "CTS CytoTune-iPS 2.0 Sendai Reprogramming Kit" from Life Technologies. The highest associated hazard is accidental infection of lab workers with the Sendai virus. The kit pre-packaged viruses have the ability to infect human cells, with the most likely route being mucosal exposure to aerosols. Once cells are infected, the vector lacks a fusion gene and is therefore unable to produce competent virus particles for onward transmission, except in the very unlikely event that it recombines with another RNA virus within the host.

Origin & function

The viral vectors are designed to introduce the Oct3/4, Sox2, Klf4, and cMyc transgenes to the host cell, and facilitate transient expression of the genes for the purpose of establishing induced pluripotent stem cell cultures. These genes are ordinarily expressed in embryonic stem cells, and their transient expression is sufficient to induce sustained pluripotency. There are three Sendai virus vectors that must be mixed together at the right ratio and then applied the cells to achieve iPSC reprogramming, one contains human c-Myc alone, one contains human Klf4 alone, and one is polycistronic with Klf4-Oct3/4-Sox2. The transgenes and viral vector are eliminated after 5-15 passages of the cells, with elimination confirmed by ICC and qPCR. The highest associated hazard is accidental infection of lab workers with the three viruses simultaneously when the pre-prepared kit vectors are handled, with the most likely routes being via aerosol inhalation or sharps injury, and the potential that this could cause teratoma formation at the site of infection due to cell de-differentiation and proliferation. Teratomas are self-limiting, benign tumours, and not likely to cause significant harm to health. An additional hazard is that cMyc is considered to be potentially oncogenic, thus there is a hypothetical possibility that infection with cMyc alone could cause malignant transformation of cells, although this vector will only be handled very briefly on its own since a mixture of three viruses will be added to cells. The likelihood of accidental exposure of lab workers to the Sendai vectors will be mitigated by the control measures put in place, e.g. secure storage of viruses, handling within a Class 2 biosafety cabinet with good aseptic technique, use of protective gloves and labcoat, procedures with minimal aerosol production, no use of sharp items that could puncture gloves, controlled waste disposal etc. After the transduction step there is negligible risk to human health from the vectors, since virus produced by the human cells will be fusion incompetent.

Evaluation of foreseeable effects

The GMO will originate from human blood, which carries a risk of exposing workers to undiagnosed blood-borne viruses, however the risk is low and is further minimised by wearing of appropriate PPE, handling the blood only within a Class 2 biosafety cabinet, and never using sharps in experimental procedures.

Creation of the GMO involves handling Sendai virus vectors that are capable of infecting human cells, and contains transgenes that induce pluripotency when co-expressed, including the cMyc gene that is a proto-oncogene. The risk of accidentally infecting lab workers is low and is further minimised by handling the virus only within the Class 2 virus lab facility, within a class 2 biosafety cabinet, workers wearing appropriate PPE, waste inactivation procedures, minimising production of aerosols while handling the virus/cells (e.g. not using an aspirator), and no usage of sharps.

During the reprogramming phase, the GMO human cell cultures will contain cytoplasmic Sendai vectors expressing Oct3/4, Sox2, Klf4, and cMyc genes. After 5-15 passages the vectors and transgenes will be eliminated, and elimination confirmed by ICC and qPCR. After elimination there will be no risk from the Sendai vector or transgenes. To minimise any further risk from blood-borne viruses, the induced pluripotent stem cells will be tested for the blood-borne viruses HIV1/2, Hepatitis A/B/C, and HTLV1/2, using an external pathogen testing laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (culture media) will be inactivated with Chemgene (5% at final concentration) for at least 24 hours, and then disposed down the sink. 5% Chemgene is expected to cause 100% kill of viruses and human cells. All solid waste (tissue culture plastics) will be treated with 5% Chemgene for 24 hours before disposal into the biological waste bin that is then autoclaved. Autoclaving will take place in a Priorclave 60L Benchtop autoclave (serviced annually), set at 134°C for 25 minutes, with a temperature probe in the centre to ensure that the correct temperature is achieved to fully inactivate waste. After waste bags are autoclaved and cooled, they will be placed into “Tiger bags” and moved for disposal by SRCL into the 770L Yellow Bin / disposal area in the Hadyn Ellis Building delivery compound. The GM waste is never be left unattended – the 770L yellow SRCL bins are locked.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GMBA Safety Committee raised several comments that were based on minor issues and the need for clarification of certain aspects of the assessment. The Principal Investigator was therefore asked to add this additional information to the risk assessment before final approval. The information was provided before the assessment was approved internally.

The summary of the comments is provided here:

1. All those listed on the risk assessment were asked to complete the University GM induction course.
2. The GMBA required the PI to include additional information on how bloods were to obtained and the associated risks/control measures.
3. The GMBA required information on how host cells were tested to ensure they were virus-free before being moved outside of the virus suite.
4. The GMBA asked them to revise their original residual risk level for questions 3A.8, 11 & 19.
5. The GMBA asked for information on control measures for questions 3B2/3.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
## Purposes of the contained use

Gastrointestinal (GI), liver and genitourinary (GU) cancers are leading causes of cancer-related deaths in the UK and is a huge health and financial burden. Thus, developing novel therapies and diagnostics is an urgent clinical need.

The aim of this project is to understand GI/GU/liver cancer biology from the transformation of normal cells, through stem cell activation, regeneration, tumour progression and finally metastasis to gain a comprehensive view of the disease. We have four objectives which will focus on mammalian (human/mouse) GI/GU/liver epithelial cells:

1. To test the in vivo influence of Wnt, PI3K, MAPK, AR and DDR signalling on the function of normal tissue, stem cell function and tissue damage/regeneration.
2. To develop new preclinical GEMMs of GI/liver/GU cancers that can act as accurate reporters of therapeutic activity to test new diagnostic and therapeutic strategies, and to identify new molecular biomarkers.
3. To test the cell intrinsic/extrinsic influence of genes that regulate Wnt, PI3K, AR, MAPK and DDR signalling and cell function on tumour biology including initiation, growth, progression and metastasis.
4. To use the knowledge gained in the previous aims to determine the therapeutic benefit of inhibiting newly identified targets genetically (and/or pharmacologically), for the treatment of GI/GU/liver cancers.

We will manipulate target gene expression in primary/established human and normal/genetically engineered mouse GU/GI/liver/bone/stromal epithelial cells using constructs expressing cDNA to increase expression, introduce an oncogenic mutation or express a reporter construct, and/or short hairpin RNA sequences (shRNA) or small interfering RNA (siRNA) sequences to knock down expression.

This project involves the generation of gamma-retroviruses and 3rd/4th generation lentiviruses to generate stable GMMs, as well as transient approaches using commercial reagents (e.g. lipofectamine). Manipulated cells will be investigated using a variety of techniques, including morphological analysis by fluorescent microscopy, molecular biology, ex vivo organoid culture and biochemical assays. Manipulated cells will also be investigated in vivo, to determine cellular functions of genes of interest during
tumour biology (e.g. to establish their role during tumour formation/progression/metastasis) and therapeutic intervention.

Recipient or parental organism

Established or immortal GI/GU/liver/bone/stromal cells from either:
(a) GEMM (PBCre/ PSAcre, Ptenflofl, Pik3caHR, RB1, TP53, hiMyc), or
(b) human (PC3, DU145, 22RV1, PrEC, BPH1, LNCaP, PNT1/2A , AGS, MET5a, MKN7, MKN45, MKN48, MKN28, SNU-1079, TFK1, LP1, hFOB.1.19 and BMMSCs + derivatives).

Primary derived cells from GI/GU/liver and bone/stromal cells:
(a) human PDXs/CTCs (P5460 (LN-NEPC-1), 202.1/2, 27.1/2, Maitland/ Phesse PDX lines) or
(b) GEMMs (PSACre-ERT2, AhCre, AhCre-ERT2, VillinCre-ERT2, LGR5Cre-ERT2, PBCre, Tff1-Cre, CL18-CreERT2, Col1a2-CreERT2, R26;LacZ, R26;RFP)

Host/vector system

For human cells: Viruses will be grown in E1 helper cell lines (e.g. Lenti-X 293T, HEK293T, 293FT/phoenix and 911 cells).
For mouse cells: Viruses will be grown in MMLV Gag, Pol, and Env protein positive cell lines (e.g. EcoPACK HEK293)

Replication-deficient retroviral vectors:
- pCL-Ampho
- pBABE
- pcDNA
- pMSCV
- pLPCX
- pRetrosuper

Replication-deficient 3rd/4th lentiviral vectors:
- pMD2.VSV-G
- pCMVΔR8.91 pFH11UTG
- pWPI
- pMD2.G psPAX2
- pEF
- pTRE.ITS/rtTA

Origin & function

Gene silencing via shRNAs, siRNA, and DNA sequences to enforce gene expression (wt or mutant) in the following human/mouse genes:

Non-canonical WNT pathway components:
- SCRIB; Scribble Planar Cell Polarity Protein
- PTK7; Protein Tyrosine Kinase 7
- VANGl1/2; Van Gogh-Like Protein 1/2
- CELSR1-3; Cadherin EGF LAG Seven-Pass G-Type Receptor 1-3
- ROR1/2; Receptor Tyrosine Kinase Like Orphan Receptor 1/2
- CTHRC1; Collagen Triple Helix Repeat Containing 1
- PRICKLE1-2; Prickle-like protein 1/2
- FZD1-10; Frizzled Class Receptor 1-10
- LKB1; Serine/Threonine Kinase 11
- Axin2; Axis Inhibition Protein 2
- ZNRF3; Zinc And Ring Finger 3
- ZEB1; Zinc Finger E-Box Binding Homeobox 1
RNF43; Ring Finger Protein 43
Canonical Wnt pathway components:
CTNNB1; Catenin Beta 1
TCF1-4; Transgenic T cell factor 1-4
APC; Adenomatous polyposis coli
PORCN; Protein-serine O-palmitoleoyltransferase
WNT1-19; Wingless and Int-1-19
DVL1-4; Dishevelled 1-4
CDH1; Cadherin-1 (E-cadherin)
LGRs; Leucine-rich repeat-containing G-protein coupled receptors
LRPs; Low density lipoprotein receptor-related proteins
sFRPs; Secreted Frizzled Related Proteins
MYC; MYC Proto-Oncogene, BHLH Transcription Factor
MAPK pathway components:
HRAS; Harvey rat sarcoma viral oncogene homolog
KRAS; Kirsten Rat Sarcoma virus
BRAF; v-Raf murine sarcoma viral oncogene homolog B
DDR pathway components:
TP53; Tumor Protein P53
BCL2; B-cell lymphoma 2
BCL3; B-cell lymphoma 3
BRCA1/2; Breast cancer gene 1/2
ATM; Ataxia Telangiectasia Mutated Serine/Threonine Kinase
ATR; ataxia telangiectasia and Rad3-related protein
PARP1-3; poly(ADP-ribosyl)transferase 1-3
CHEK1; Checkpoint kinase 1
RB; Retinoblastoma
PI3K pathway components:
PIK3CA; Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
PTEN; Phosphatase and tensin homolog
mTOR; Mechanistic Target Of Rapamycin Kinase
PHLPPs; PH domain and Leucine rich repeat Protein Phosphatases
SGK1-3; Serum/Glucocorticoid Regulated Kinase 1-3
FKBP5; FK506 binding protein 5
RICTOR; RPTOR Independent Companion Of MTOR Complex 2
RAPTOR; Regulatory Associated Protein Of MTOR Complex 1
RABs; Ras Related Proteins
STX1-17; Syntaxin 1-17
AR pathway components:
AR; Androgen receptor
PSA; Prostate specific antigen
Immunocompromised GEMMs:
NSG; Spontaneous Prkdc mutation, Il2rg null
NSG (KbDb): Spontaneous Prkdc mutation, Il2rg null, H2-K1, H2D1 and H2Ab1 mutations
Athymic nude mice: Spontaneous FOXn1nu mutation
In addition to gene specific cDNA/shRNA/siRNA sequences, viral vectors will also contain combinations of standard viral vector components:
Reporters: LacZ, GFP (green fluorescent protein), YFP (yellow fluorescent protein), RFP (red fluorescent protein, Cherry, dTom reporters, luciferase, and AR,
non-homologous end joining/Homologous recombinationNHEJ/HR GFP I-SceI and TET-ON/OFF constructs.
Antibiotic resistance cassette: Puromycin, Kanamycin Gentamicin, Neomycin, Hygromycin, Zeomycin
Promoter: U6, CMV, EF1a
Internal ribosome entry sequence

Evaluation of foreseeable effects

The viral vectors used in this project pose a low biohazard risk as although they are capable of transducing human cells, the virus is replication incompetent. Replication requires sequences coding gag, pol, env, and rev, which are absent from the viral vectors used, and would be required to be provided in trans by helper constructs or packaging cell line. There is no evidence to suggest that the recombinant virus may alter susceptibility to host defence mechanisms. All DNA components of the recombinant virus are routinely used in biological research, and are not known to have effects on susceptibility of host immune systems. To reduce risk in this project we have considered foreseeable circumstances where there is a risk of transmission of the vectors.

Routes of transmission:
Needle stick – needles will not be used in these experiments (needles will only be used to inject genetically modified cells into mice with cells that are free from virus).
Inhalation - opening a vial or spillage of the virus could cause an aerosol of viral particles. As such, all tasks where there is a risk of aerosols being produced will be carried out inside the Class II microbiological safety cabinet.
Skin contact - The viral vector could be transmitted through contact with the skin, transmission into the blood stream or to airways and eyes. These risks will be minimised following the standard operating procedure of the virus suite and use of double gloves to minimise risk of skin contact through piercing of gloves. The only consequence of exposure we envisage is some irritation/inflammation caused by the immune response which may be caused by the vector. Normally, if an immediate action is taken, i.e. washing the exposed area with detergents, no significant consequence will occur.
The viral suite will have designated lab coats which also will be stored in the viral room.
Standard working procedures and emergency instructions (phone numbers including after work phone numbers) will be clearly posted to the walls on laminated paper. A spill kit will be provided in the room.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All animals are contained within the JBIOS Biosciences, T2, IVC suite and Heath Park facilities at Cardiff University. Handling and disposal procedures of animals will adhere to the standard operating procedures of this facility. Waste will be disposed of by an approved waste route. Animal carcasses will be disposed of by an approved route. Good laboratory practice will be employed. Good practice standards in the animal house will be used to ensure that all door barriers are maintained.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Preferred local deactivation/disposal pathway:
1) Chemical disinfection by Chemgene or Virkon.
Liqid waste (e.g. culture media) will be inactivated with 5% Chemgene or Virkon® (final concentration 1%) for at least 1 hour (typically overnight) - according to manufacturer’s instructions.
All solid waste (tissue culture plastics) will be treated with 5% Chemgene inside the Class 2 MSC (or a sealed jar) for at least 2 hours before being drained, rinsed and disposed of (double bagged). Waste is initially autoclaved (ante-room, Level 3, HEB, adjacent to the viral suite), followed by incineration (NB: Syringe barrels are not autoclaved after soaking, draining and rinsing, instead they are disposed of in a sharps bin and sent for incineration).
2) Waste placed into autoclave bag.
Chemically deactivated waste will be placed into autoclave bags (double bagged) and labelled GM180104.

02/03/2022
3) Waste autoclaved locally using the waste cycle at 134°C for 25 mins. Solid waste will be autoclaved in the designated autoclave located immediately outside of the viral suite before being placed in a yellow ‘Tiger bag’ and disposed of as ‘offensive waste’ (HL180104) by Stericycle/SCRL. The autoclave is serviced annually and a twelve-point thermocouple test is performed annually. Waste material will be inactivated using the waste cycle at 134°C for 25 mins. All staff operating the autoclave will have been trained in manual handling and are also trained in appropriate spillage procedure. 1% Virkon® or 5% Chemgene is the disinfectant to be used in case of inactivated waste being spilled in or out of the autoclave. [NB: Chemgene is primarily used on the surface of the Class 2 MSC, as Virkon has been found to rust the hoods].

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GMBA Safety Committee required several minor corrections/additions to be made to the risk assessments before they were internally approved.

For GM130/807:
1. Several typographical errors were asked to be corrected.
2. They were asked to highlight processes where aerosols were likely to be produced and how they were to be controlled.
3. They were asked to add a brief paragraph into 3A.1 on retrovirus
4. Q3A.13 - an inconsistency in the information on virus tropism was asked to be corrected.
5. Q3B.8 - they were asked to briefly discuss the risk of recombination with other viruses

For GM130/808:
In section 2 (project description) they were asked to confirm that transduced cells administered to the animal models were virus-free. They were also asked briefly explain how the cells would be administered.

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<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
</thead>
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</table>

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<tr>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tbody>
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<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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Project Ref 130/21.3
Investigation of the effects of gene knock-down on Huntington's disease molecular phenotypes

To examine the effects of lowering the expression of specific genes on cellular and molecular phenotypes associated with Huntington's disease.

Recipient or parental organism

1. U20S (ATCC HTB-96) and 2. a series of induced pluripotent stem cells.

The U20S cells have a stable insert that expresses a human huntingtin exon 1 sequence containing an expanded CAG repeat of 18, 50 or >100 CAG, fused to p2A (66bp) followed by an in frame luciferase reporter (Nanoluc 516bp), stop codon, CMV promoter and DS-Red. The induced pluripotent stem cell lines are derived from patients with Huntington’s disease. We have several lines available with HTT exon 1 CAG starting lengths of 22, 60, 109, 125 and 180. Our main line is the HDQ109i line, derived from a juvenile patient, that originally had 109CAG and its isogenic pair, HDQ22i with 22CAG.

Host/vector system

We will infect the cells with human shRNAs against genes of the DNA repair pathways including: MSH3, MLH1, FAN1, POLQ, MLH3 and potentially others in the future. We will use the MISSION TRC shRNA clones from Merck. We will receive the lentiviral transduction particles, that are self-inactivating replication incompetent viral particles. Individual constructs are provided as a 200 μL frozen stock containing a minimum of 1.0 x 106 TU/ml.

Origin & function

We will use the MISSION shRNA lines which are lentiviral particles. These belong to the retrovirus family and are thus characterized by their ability to convert their RNA genome to DNA by reverse transcription. They contain three major coding domains: gag (encodes the structural proteins matrix, caspid and nucleocaspid), pol (encodes protease,
reverse transcriptase and integrase) and env (encodes surface and transmembrane components of the viral glycoprotein). Upon entry into the cell, the virus replicates through the interaction of cellular and viral components in order to produce a viable virus. The major concerns regarding lentiviral vectors are 1) Potential generation of replication competent lentiviruses, 2) in vivo recombination with lentiviral sequences and 3) insertion of proviral DNA in or close to active genes may trigger tumor initiation or promotion. Consequently, safety features have been built into the synthetic vectors to be used by a Contract Research Organisation.

Each MISSION® shRNA clone is constructed within the lentivirus plasmid vector pLKO.1-Puro or TRC2-pLKO-Puro followed by transformation into Escherichia coli. The pLKO.1–Puro and TRC2-pLKO-Puro vectors contain bacterial (ampicillin) and mammalian (puromycin) antibiotic resistance genes for selection of inserts in either bacterial or mammalian cell lines. Each gene is represented by multiple individual shRNA constructs or clones targeting different regions of the gene sequence. Therefore, a range of knockdown efficiency, with at least one construct from each gene set being >70%, can be expected when using these clones. This allows one to examine the effect of loss of gene function over a range of gene knockdown efficiencies. Each shRNA construct has been cloned and sequence verified to ensure a match to the target gene. We will be sent the clones in the lentiviral transduction particle format. Safety features of the recombinant replication-deficient lentivirus include:

(1) Splitting of the viral genome into three plasmids such that if the recombination between the different plasmids were to occur, no wild type HIV can arise. Furthermore, the virus is pseudotyped which cannot cross cell layers and can only infect the primary cell layer of encounter.

(2) Deleting the genes encoding accessory factors of HIV, known to be essential for HIV pathogenecity (Nef, Vif, Vpr, & Vpu).

(3) Using of self-inactivating (SIN) vectors that have inactive LTRs, thus preventing potential transcriptional activation of any (onco)genes downstream of the integration site.

The improvements in vector technology make it highly unlikely for a viral disease including HIV to occur as a consequence of an accidental exposure to a lentiviral vector. As a result, HSE have deregulated to Level 1. However, the high efficiency of transduction by these vectors could pose an increased risk for accidental introduction of potentially hazardous genes (e.g. oncogenes) into the experimenter making consideration of risk associated with the expression products a high priority. This is outlined below for the specific genes to be undertaken in the proposed work and extrapolated to other genes that may be used in the future. Any additions to the work will be notified to the University GMBA committee for review.

Scale of work

This assessment covers tasks undertaken at a scale such that less than 100 microlitres of purified lentiviral stock with a titre of up to 5x 10^9 will be handled at any one time. The equipment used in handling the virus, such as contained cell culture vessels will minimise the risks of accidental spillage. In the event that spillage does occur the quantity of a resultant spill will be such that it can be disinfected and a clean up operation performed within the confines of the laboratory where the spillage has occurred and without significant risk of escape to the environment. The University has a standard spillage protocol and a spillage kit available in the virus suite (see 12 below).

Evaluation of foreseeable effects

Describe the nature of the inserted genes and the properties of the final genetically modified viral vector.

We plan to generate stable cell lines expressing shRNA from a number of DNA repair genes of interest because they carry variants that modify the age at onset of Huntington’s disease. The read out in our U2OS system will be somatic expansion of the inserted HTT CAG repeat tract. We aim to achieve stable knock down of these genes in the cells. In each case the expression product is biologically active but not expected to have a deleterious effect because its concentration would not approach normal body levels. Only MLH1 is a known human tumour suppressor gene: the evidence for the other genes being tumour suppressors is not robust and they do not show any selection against damaging variants at the population level.
(1) MSH3

MSH3 forms an obligate heterodimer with MSH2 to form MutSα, part of the post-replicative DNA mismatch repair sequence. MutSα initiates mismatch repair through recognition of the lesion then recruiting other proteins in an ATP-dependent fashion. Defects in this gene are a potentially associated with susceptibility to endometrial cancer and Familial Adenomatous Polyposis 4, though there is no evidence of selection in human populations against deleterious or loss of function variants of MSH3.

Elevated microsatellite alterations at selected tetranucleotide repeats (EMAST) is a type of microsatellite instability that is enhanced with MSH3 reduction. Furthermore, genetic reduction or mutation of MSH3 has been reported to stabilise trinucleotide repeat expansion sequences and conversely, overexpression of MSH3 has been shown to exacerbate repeat instability. Msh3 KO in mice is well tolerated and does not shorten life span or induce oncogenic transformation.

Reduction or removal of MLH1 abrogates somatic instability of trinucleotide repeats in vitro and in vivo.

(2) FAN1

FAN1 (FANCD2/FANCI associated nuclease 1) (KIAA1018) is an enzyme that is encoded by FAN1. FAN1 is a structure dependent endonuclease that is thought to play a role in the Fanconi Anemia pathway. FAN1 protein possesses endo- and exo-nuclease functions to remove interstrand DNA crosslinks. The presence of the FANCD2/FANCI complex is unaffected by knockdown of FAN1 as it is a downstream contributor to the pathway.

Indeed, FAN1 is not confidently categorised as a FA gene given mild ICL agent sensitivity and chromosomal fragility with microdeletions affecting the FAN1 chromosomal location. Mutations in FAN1 can result in chronic kidney diseases such as recessive karyomegalic interstitial nephritis (KIN) and neurological conditions, and it has been associated with hereditary colorectal cancer due to defective DNA repair. There is no evidence of selection in human populations against deleterious or loss of function variants of FAN1.

Variants of FAN1 with increased expression appear to stabilise trinucleotide repeat expansion sequences and conversely, variants with reduced expression or function of FAN1 have been reported to exacerbate repeat instability. Fan1 KO in mice leads to renal tubular karyomegaly with polyploidy and defects in ICL repair with age, which is exacerbated by treatment with ICL-inducing agents.

Reduction or removal of FAN1 promotes somatic instability of trinucleotide repeats in vitro and in vivo.

(3) MLH1

The MLH1 gene encodes MutL homolog 1 protein and is commonly associated with hereditary non-polyposis colorectal cancer in addition to other cancer syndromes. This protein is one component of a system of DNA mismatch repair proteins that work co-ordinately in sequential steps to initiate repair of mismatch lesions in humans. MLH1 is one of two obligate heterodimer units in the MutL proteins and is common to all three isoforms: MutLα is formed of MLH1 and PMS2; MutLβ consists of MLH1 and PMS1 while MutLγ comprises MLH1 and MLH3. In addition to cancer-causing mutations, MLH1 can also be silenced through epigenetic alterations. Mlh1 KO, cKO or G67R knock in mice have high levels of molecular defects together with the presentation of cancer and fertility defects and a reduced lifespan.

To reach the tissues most susceptible to reduced MLH1 expression in the gut the lentiviral particles would need to be ingested. This is unlikely in the laboratory setting where the viral particles will be used in small quantities in an appropriate Class 2 environment. There is an association with an increased risk of other cancers including skin cancers. The skin is the most accessible organ but if the viral particles reach the skin they would be in small amounts and the skin itself would act as a barrier to reaching any deeper layers to enter cells with any replicative potential. In addition any spill would be small as we are using small quantities and can be washed off. If ingested viral particles would be broken down in the digestive tract. The risk of the MLH1 shRNA reaching the organs associated with
The MLH3 gene encodes MutL homolog 3 protein which together with MLH1 functions as an obligate heterodimer, MutLalpha. Somatic mutations in this gene occur in tumours exhibiting microsatellite instability and germline mutations have been linked to hereditary nonpolyposis colorectal cancer type 7. The MLH1-MLH3 heterodimer is an endonuclease that makes single strand breaks; and can bind specifically to Holliday Junctions. Knockout of Mlh3 has been shown to retard somatic instability of trinucleotide repeats in mouse models. It is also associated with molecular and phenotypic defects albeit to a much lesser extent than Mlh1 KO mice. The risk of the MLH3 shRNA reaching the organs associated with cancers that it might contribute to is very low. Knock out of MLH3 prevents meiosis, but the chance of the viral particles reaching the gonads in any quantity is minimal as we are using small quantities and appropriate PPE.

DNA polymerase theta is encoded by the POLQ gene and plays a role in microhomology-mediated end-joining, which is an alternative nonhomologous end-joining pathway triggered in response to double stranded DNA breaks. The polymerase activity is highly promiscuous and low-fidelity, it is also involved in somatic hypermutation of immunoglobulin genes. Pol is required for the viability of BRCA- mutated cancer cells. Polq KO in mice leads to hypersensitivity to radiation and DNA double stranded breaking agents. There is no evidence of selection in human populations against deleterious or loss of function variants of POLQ.

The viral vectors will only produce shRNAs. These give hypomorphs and not complete knock out of the gene product. None of the genes proposed to be used here are oncogenes or proto-oncogenes. The conditions of virus generation and manipulation are extremely well controlled, the virus will only be present in low volumes and no human will be exposed live virus particles in this work.

The most risk in this application is associated with knock down of MLH1. MLH1 heterodimerizes with PMS2 to form MutL alpha, a component of the post-replicative DNA mismatch repair system (MMR). DNA repair is initiated by MutSalpha (MSH2-MSH6) or MutSbeta (MSH2-MSH3) binding to a double strand DNA mismatch, then MutL is recruited to the heteroduplex. Assembly of the MutL-MutS-heteroduplex ternary complex in the presence of replication factor C and proliferating cell nuclear antigen (PCNA) activates the endonuclease activity of PMS2. This introduces single-strand breaks near the mismatch and generates new entry points for exonuclease EXO1 to degrade the strand containing the mismatch. DNA methylation would prevent cleavage and therefore assure that only the newly mutated DNA strand is going to be corrected. MutL alpha (MLH1-PMS2) interacts physically with the clamp loader subunits of DNA polymerase III, suggesting that it may play a role to recruit the DNA polymerase III to the site of the MMR. MLH1 is also implicated in DNA damage signalling, a process which induces cell cycle arrest and can lead to apoptosis. MLH1 also heterodimerizes with MLH3 to form MutL gamma which plays a role in meiosis. If the mismatches are not repaired, DNA damage accumulates. Many cells will not replicate if the damage is not repaired but in a proportion of cells the damage can eventually lead to uncontrolled growth and cancer.

MLH1 has reduced expression in hereditary non-polyposis colon cancer (HNPCC) – and people with Lynch syndrome which can be caused by loss of function or damaging mutations in MLH1 – are at high risk of HNPCC and of a number of other cancers including increased risk of cancers of the stomach, small intestine, liver, gallbladder ducts, urinary tract, brain, and skin. This is the main risk associated with reducing expression of MLH1.

In addition, to cause any substantial risk of harm the viral particles would need to replicate and these particles are replication deficient and rendered incapable of infecting cells. It is possible that recombination events could render
them replication efficient again, but more than one recombination event would need to occur as several genes required for replication have been removed, and as each recombination event is unlikely, the chances of this happening are low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This assessment covers tasks undertaken at a scale such that less than 100 microlitres of purified lentiviral stock with a titre of up to 5x 10^9 will be handled at any one time. The equipment used in handling the virus, such as contained cell culture vessels will minimise the risks of accidental spillage. In the event that spillage does occur the quantity of a resultant spill will be such that it can be disinfected and a clean up operation performed within the confines of the laboratory where the spillage has occurred and without significant risk of escape to the environment. The University has a standard spillage protocol and a spillage kit available in the virus suite (see 12 below). Maximum volumes of 100mls medium containing diluted viral particles for each experiment, though most experiments will use individual culture wells of 1-2ml of medium.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be disinfected using Chemgene HLD4L concentrate (Didecyldimethylammonium Chloride, Propan-2-ol, C9-C11 Alcohol Ethoxylate, Polymeric Biguanide Hydrochloride and Alkyl (C12-16) Dimethylbenzylammonium Chloride). This gives 100% kill. HLD4 formula is Article 95 compliant and has been developed with the Biocidal Products Regulations (BPR), REACH, CLP (Classification of Labelling and Packaging) and CDG (Carriage of Dangerous Goods) regulations. HDL4 disinfectants are certified Sporicidal, Mycobactericidal, Virucidal, Fungicidal and Bactericidal under EN protocols. Chemgene is non-corrosive and non-fragranced. This is the preferred disinfectant for use in the class II hood (2% Chemgene) as well as disinfecting wastes (5% Chemgene). Liquid waste will be inactivated with 5% Chemgene for 24 hours before disposal. Tissue culture waste will be treated with 5% Chemgene for 24 hours before disposal into a biological waste bin that is subsequently autoclaved. All waste is identified with the name of the responsible PI and the researcher responsible for it, and the GM risk assessment identifier. Solid waste is kept locked in the virus suite until autoclaved.

Solid waste will be autoclaved in a Priorclave 60L Benchtop autoclave. The machine is serviced annually and a twelve-point thermocouple test is performed annually. The autoclave includes a temperature probe to ensure that the waste reaches the appropriate temperature to inactivate all the waste. Users are trained in manual handling and in spillage procedures, PPE is worn with dedicated lab coats for the viral suite, double gloves and a spillage kit available. The autoclaved waste is disposed of through the building biological waste route which has designated bins for biological waste housed in a locked compound.
The Committee debated the classification of this activity as Prof Jones had originally proposed Class 1 however after consideration of SACGM Guidance Note 2, Section 2.2 Point 24, with the potential of downregulating targeted genes it was agreed that this was a Class 2 Activity.

### Project Containment

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### Project Ref 130/21.4

- **Date Ackn'd**: 25/11/2021
- **CU2 Project Title**: Introduction of leukocyte-homing molecules to mammalian cells using retroviral and lentiviral vectors
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Non-GMM Consent Granted**: Yes
- **Date Project Ceased**: 25/11/2021
- **Withdrawn**: No
- **Tick if notifying a connected programme of work**: Yes

### Purposes of the contained use

The aim of this research is to boost the immune system to fight cancers and viruses by improving the recruitment or ‘homing’ of white blood cells such as T-cells to diseased tissues. Adoptive T-cell therapy is already being used in the clinic to treat cancers and chronic virus infections, but it does not work in all patients. This shortfall in
GM mice are either deficient in genes or overexpress genes that prevent normal functioning of the immune system. They are generated by intranuclear injection of synthetic

house for testing, the GM cells will be tested and analysed to ensure there is no residual virus remaining. (regarding human cell targeting vectors), the genes may become active and induce immune responses. However appropriate safety measures put in place (i.e. PPE and Cat 2 safety cabinet use and SCANtainer containment of mice receiving GM cells) will render the likelihood of viral contact with operator leukocytes extremely remote. Moreover, before transfer of the GM cells to the animal house for testing, the GM cells will be tested and analysed to ensure there is no residual virus remaining.

Recipient or parental organism

Cell lines to receive the genetic material will be designated viral packaging cell lines such as HEK293T and Platinum-E cells which are widely used in the field for the production of recombinant lentiviral and retroviral particles. Cell lines to subsequently receive the harvested recombinant virus will be immortalised human or murine immune and tumour cell lines such as Molt3, Jurkat, NALM6, B16-F10, LLC and MC38. Additionally, the recombinant virus will be delivered to primary human PBMCs and primary mouse T-cells. Once insertion of the gene of interest has been confirmed in the relevant cell lines (and in vitro analysis of activities and behaviours have been ascertained), the modified cell lines will then be introduced into wild type and GM mouse models for in vivo analysis. GM mice to be used will be deficient in genes required for a functioning adaptive immune system and will of no increased risk than wild type mice.

Host/vector system

Recombinant viruses to be constructed will be based on pantropic retroviral or lentiviral delivery systems depending on the specific size, design and preferences for the relevant gene insert. Regarding retroviral platforms, all retroviral vectors to be used in this project are derived from moloney murine leukaemia virus, myeloproliferative sarcoma, and murine stem cell virus (MSGV). They contain viral LTRs on a plasmid backbone and do not produce viral proteins due to a lack of gag, pol and env genes (rendering the viral particles non-replicative). Specific ecotropic packaging cell lines are required to provide the viral proteins required for packaging of retroviral particles. Regarding the lentiviral vectors, the ones to be employed are based on human immunodeficiency virus type 1 (HIV-1). All vectors are third generation lentiviral systems with tat gene deletion. An altered 3’LTR renders the vector “self-inactivating”, and a heterologous coat protein (e.g., VSV-G) is used in place of the native HIV-1 envelope protein to enable transduction of a wider range of cell types. All lentiviral vectors to be used are devoid of replicative capacities as are lacking regulatory and accessory genes such as tat, vpr, vpu, vif and nef, gag, pol and env.

Origin & function

The genes to be introduced are associated with immune cell function and homing mechanisms in humans and mice respectively. Specifically, these include leukocyte cell surface proteins involved in antigen presentation, antigen recognition, immune cell activation, adhesion and homing (e.g., CD8, CD19, CARs, TCRs, L-selectin, L-selectin mutants) and transmembrane and intracellular signalling proteins (e.g., 4-1BB, CD3z,) which are components of the signalling platform downstream of the surface proteins. Additionally, in some instances intracellular and surface reporter genes will be introduced (e.g. luciferase, dsRED, GFP, NGFR) for the purpose of experimental monitoring and evaluation of cellular activity. GM cells will be evaluated in vitro and following administration to either wild type or GM mice.

Evaluation of foreseeable effects

The genetic cargo of the viral particles (to be delivered to the cells) will have no impact, influence, or alter the behaviour of these well-established viral delivery systems. It is anticipated that the genetic modification of the cells (via viral integration of the genes of interest) will improve immune cell recognition of antigens. Through the introduction of molecules and complexes responsible for immune cell activation, adhesion and homing it is expected that the modification will enhance the ability of immune cells to recognise and destroy their target cells. Moreover, they should have improved transport through the lymph and vasculature to target sites of tumours and/or chronic inflammation. As a result, the GMO cell lines will hypothetically have an enhanced ability to deal with chronic infection and cancerous tumours in vivo.

The highly attenuated lentiviral and retroviral vectors to be used have shown sufficient biosafety in the research field. The probability of viral vector reversion to wild-type is extremely unlikely, therefore there is minimal risk of viral replication in infected cells or other cell lines (and, as a result, will not compromise the immune system of an operator if exposed). Additionally, the recombinant viruses pose no oncogenic risk as they will not contain any oncogenes. As the genes to be introduced are involved in natural immune cell responses, there is an extremely small risk that, if successfully integrated into a worker’s leukocytes (regarding human cell targeting vectors), the genes may become active and induce immune responses. However appropriate safety measures put in place (i.e. PPE and Cat 2 safety cabinet use and SCANtainer containment of mice receiving GM cells) will render the likelihood of viral contact with operator leukocytes extremely remote. Moreover, before transfer of the GM cells to the animal house for testing, the GM cells will be tested and analysed to ensure there is no residual virus remaining.

GM mice are either deficient in genes or overexpress genes that prevent normal functioning of the immune system. They are generated by intranuclear injection of synthetic...
DNA into either embryonic stem cells or isolated blastocysts which is either incorporated into the genome (transgene) or deletes the targeted gene from the genome (knockout). GM Stem cells/blastocysts are transferred into pseudopregnant mice and offspring genotyped for the targeted gene and interbred to homogeneity. The resulting GM mice are immunodeficient (e.g. SCID, RAG knockouts) and pose no increased risk over and above wild type counterpart.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Mice will be housed in individually ventilated SCANtainers in rooms designated for Class 2 work. Mice will be handled only in Class 2 biological safety cabinets by fully trained staff wearing full PPE. Warning signs will be added to any cages that houses animals that have received GM cells. All biological and non-biological material exposed to mice received transfused GM cells will be double-bagged and disposed of by incineration. Work with mice transfused with GM cells will only be carried out by fully trained staff.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All contaminated waste will be handled in Class II safety cabinets until safely sealed. Contaminated solids (e.g. pipettes) will be immersed in a sealed container of Haz Tab solution (Triclosene Sodium Dihydrate) with 1 HazTab tablet per 100 mL used. Waste will be immersed in this solution for a minimum of 24hr prior to transferring to a container for autoclaving and/or incineration. Liquid waste will be dealt with in a similar way, whereby liquid waste containing GMM will be poured into a container housing Haz Tab solution, sealed and left for 24 hours to ensure total destruction of viral particles prior to disposal. Triclosene Sodium Dihydrate solution (Haz Tabs) is common-place in laboratories to disinfect surfaces from viral contaminants with 100% kill rate. HazTabs are already in use for this purpose in other laboratory in the Henry Wellcome Building of Cardiff University. The tissue culture cabinet and any potentially contaminated areas will be disinfected with chlorine-based disinfectant. The safety cabinets will then additionally be disinfected with 70% ethanol and a UV lamp used in the hood after each operation. Annually, the hoods are decontaminated with formaldehyde by designated personnel and serviced. Additionally, the laboratory premises are routinely inspected by health and safety officers.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

**Please enter comments on the GM safety committee on the risk assessment**

The GMBA Committee were in agreement that GM130/813 was a Class 2 GM activity, however they did have some queries and asked for clarification on a number of issues, including how the transfected cells were tested to ensure that they were virus free and control measures to be used during aerosol formation. The Committee were satisfied that the use of GM animals in GM130/816 was a Class A or Class 1 GM activity, however appropriate control measure would need to be used when the animals were injected with virus free cells. Further information was provided and the Committee were satisfied that the risk assessments were suitable and sufficient.

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02/03/2022
Project Ref 291/01.1

Date Ackn’d 26/04/2005

CU2 Project Title TO CLONE AND SEQUENCE THE RAT AND MOUSE ANALOGUES OF THE HUMAN COMPLEMENT INHIBITING PROTEIN CD59 AND TO IDENTIFY THE FUNCTIONALLY IMPORTANT DOMAINS OF HUMAN COMPLEMENT INHIBITING PROTEIN

Class Class 2

CultureVolClass2 Class 2

Consent Granted Not Applicable

Project notified under transitional arrangements

Historical Significant Changes

Project transferred from GM291 on 26/04/2005

Historical Date of Additional Info

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Withdrawn [N]  
Tick if notifying a connected programme of work [N]  

Historical Significant Changes  
Project transferred from GM291 on 26/04/2005  

Historical Date of Additional Info  

Significant Change ID  

Date of Significant Change  

Project Additional Information  

Purposes of the contained use  

Recipient or parental organism  

Host/vector system  

Origin & function  

Evaluation of foreseeable effects  

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)  

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)  

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)  

Is an emergency plan required according to regulation 20? [N]  

If yes, tick to confirm that it is attached to this form [N]  

Tick to confirm that you have attached a risk assessment to this form [N]  

02/03/2022  
Page 3782 of 15326
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Tick if notifying a connected programme of work N

Historical Significant Changes

Project transferred from GM291 on 26/04/2005

**Project Additional Information**

Purposes of the contained use

02/03/2022
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<th>Laboratory Activities</th>
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<th>Animal Units</th>
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<th>Human Clinical Applications</th>
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**Project Ref** 291/01.5

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<td>26/04/2005</td>
<td><strong>EXPRESSION AND FUNCTIONAL CHARACTERISATION OF COMPLEMENT COMPONENTS, REGULATORY PROTEINS AND RECEPTORS FROM HUMAN AND OTHER SPECIES. (GM291/AKC.BPM/11)</strong></td>
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Withdrawn N

Tick if notifying a connected programme of work N

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Project Containment

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Project Ref 291/01.6

Date Ackn'd: 26/04/2005

CU2 Project Title: PRODUCTION OF RECOMBINANT COMPLEMENT REGULATORY PROTEINS/COMPLEMENT RECEPTOR IMMUNOGLOBULIN FUSION PROTEINS. (GM291/AKC.BPM/10)

Class: Class 2

Non-GMM Consent Granted: Not Applicable

Project notified under transitional arrangements: Y

Historical Significant Changes: Project transferred from GM291 on 26/04/2005

Withdrawn: N

Tick if notifying a connected programme of work: N

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick if you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

Recombinant vaccinia viruses will be used for several purposes:

1. To immunise mice with recombinant antigens
2. To measure immunity against recombinant antigens.
3. To infect cells in vitro for the purpose of screening immune responses.

The antigens to be used represent non-functional proteins either of viral or mammalian origin.

Laboratory work involving recombinant vaccinia viruses will be carried out at ACGM Containment Level 2 with the use of a microbiological safety cabinet. In laboratory situations, sharps will not be used and workers will wear gloves at all times. In the case where animals will be inoculated with recombinant vaccinia viruses, virus will be delivered via the intraperitoneal, intravenous or subcutaneous routes. In all cases needles will be used. Workers involved in inoculation of animals will be trained to a high level to minimise the risk of needle-stick injuries. The highest inoculum used will be 10 million plaque forming units per mouse. Vaccinia virus infection does not spread within mouse or human communities. The virus does not cause persistent infection since it lyses cells upon infection. Infection with recombinant vaccinia virus may however lead to some blistering or scabbing during the acute phase of infection. Tissues recovered from infected animals will be manipulated within microbiological safety cabinets and animal carcasses will frozen and later disposed of by incineration.
The recombinant vaccinia viruses to be used have already been constructed through homologous recombination of plasmid DNA (pSC11) into the thymidine kinase gene of vaccinia virus (Western Reserve, WR). Inserted genes encode for: 1) mouse melanocyte antigens; 2) the glycoprotein of murine leukaemia virus; 3) influenza antigens; 4) antigens from lymphocytic choriomeningitis virus and 5) non-functional murine proteins. A complete list is shown in Appendix 1. These inserted genes pose no risk. Indeed, insertion of these genes into vaccinia virus WR, reduces the virulence of the virus 10,000 x (Buller et al. 1985. Nature 317: 813).

Host/vector system

The recombinant vaccinia viruses to be used have already been constructed through homologous recombination of plasmid DNA (pSC11) into the thymidine kinase gene of vaccinia virus (Western Reserve, WR).

Origin & function

Inserting genes encode for: 1) mouse melanocyte antigens; 2) the glycoprotein of murine leukaemia virus; 3) influenza antigens; 4) antigens from lymphocytic choriomeningitis virus and 5) non-functional murine proteins. A complete list is shown in Appendix 1. These inserted genes pose no risk. Indeed, insertion of these genes into vaccinia virus WR, reduces the virulence of the virus 10,000 x (Buller et al. 1985. Nature 317: 813).

Evaluation of foreseeable effects

Use of recombinant vaccinia viruses within the laboratory will be confined to a class II microbiological safety cabinet. In some situations it will be necessary to infect mice and therefore to transport viruses from the laboratory to the animal house. Vials containing vaccinia viruses will be transported from the laboratory to the animal house within sealed containers. The possibility of spillage from sealed containers is very unlikely. In the event that a spillage does occur, the spillage will be dealt with according to local rules at the University of Wales College of Medicine.

With regards to environmental risks, it is known that there was no colonisation of domestic or wild animals in the UK following widespread administration of vaccinia virus to humans in the smallpox eradication campaign, nor has the recombinant vaccinia virus that has been used to control rabies in part of Belgium and France led to colonisation of wild species. There is no reason to believe that recombinant vaccinia viruses produced in this work are any more likely to survive and become established in the environment than the wild-type virus. Thus, taking into account the control measures assigned above, the overall risks to the environment from the modified organisms produced in this work are effectively zero. Therefore no additional containment or control measures are considered necessary to protect the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables including plasticware and pipettes will be autoclaved for at least 15 minutes at 121-130 degrees C. Subsequently autoclaved waste will be disposed of by incineration with other clinical waste. Liquid waste will be treated with Hycolin for 30 minutes prior to discharge to drains.

Animal bedding will be autoclaved and animal carcasses will be destroyed by incineration.

Degree of Kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Additional detail required in environmental assessment - now added. Advice should be sought regarding avoidance of handling virus by persons with sensitive skin conditions, eg eczema. Agree with assessment as Class 2 activity requiring Containment Level 2 facilities.

### Project Containment

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### Project Ref 292/01.1

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Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Transferred from GM292 on 26/04/2005

Historical Date of Additional Info

Significant Change ID
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment
26/04/2005

THE ROLE OF MOLECULAR ABNORMALITIES ASSOCIATED WITH LEUKARIA
IN UNVERTING NORMAL HAEMATOPOIETIC DEVELOPMENT

26/04/2005

Transferred from GM292 on 26/04/2005

Non-GMM
Consent Granted
Not Applicable

Project notified under transitional arrangements

Historical Significant Changes
Transferred from GM292 on 26/04/2005

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form

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Please enter comments on the GM safety committee on the risk assessment

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**Project Ref**  302/01.1

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 302/01.2

Date Ackn’d 26/04/2005

CU2 Project Title EXPRESSION OF ADENOVIRUS RECEPTOR (CAR) BY USING A RETROVIRUS VECTOR

Class 2

Consent Granted Not Applicable

Project notified under transitional arrangements Y

Withdrawn N

Historical Significant Changes

Transferred from GM302 on 26/04/2005

26/04/2005
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Is an emergency plan required according to regulation 20? [N]
- If yes, tick to confirm that it is attached to this form [N]
- Tick to confirm that you have attached a risk assessment to this form
- Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022

Page 3798 of 15326
Project Additional Information

Purposes of the contained use

This project aims to use replication deficient adenoviral vectors to investigate intracellular signalling in cell lines and in human cells from diseased synovial tissue. This replication deficient adenoviruses can still infect cells but not replicate within them. Thus, if some person would deliberately or accidentally inhale these viruses or rub them into his eyes, a mild rhinopharyngitis (similar to a common cold) or conjunctivitus (harmless eye inflammation), lasting a few days at most, would ensue. Many people have immunity due to previous exposure, however, as this is a common pathogen. The project will not involve the production of new recombinant adenoviruses on site using vector DNA, but will involve growup of adenoviruses from imported stock using the 293 cell line, and infection of cells.

Recipient or parental organism

No in vivo studies are planned.

Host/vector system

This project aims to use replication deficient adenoviral vectors to investigate intracellular signalling in cell lines and in human cells from disease synovial tissue. These replication deficient adenoviruses can still infect cells but not replicate within them. Thus if some person would deliberately or accidentally inhale these viruses or rub them into his eyes, a mild rhinopharyngitis (similar to a common cold) or conjunctivitus (harmless eye inflammation), lasting a few days at most, would ensue. Many people have immunity due to previous exposure, however, as this is a common pathogen. The project will not involve the production of new recombinant adenoviruses on site using vector DNA, but will involve growup of adenoviruses from imported stock using the 293 cell line, and infection of cells.
into his eyes, a mild rhinopharyngitis (similar to a common cold) or conjunctivitus (harmless eye inflammation), lasting a few days at most, might ensue. There could be no other complications unless a massive dose of virus was deliberately injected intravenously. The project will not involve the production of new recombinant adenoviruses on site using vector DNA, but will involve growth of adenoviruses from imported stock using the 293 cell line, and infection of cells. These imported stocks are all from the Kennedy Institute of Rheumatology and have been carefully monitored to exclude contamination with wild-type adenovirus.

Origin & function

It is primarily planned to use

1) A replication deficient adenovirus with no insert (as a control).
2) A replication deficient adenovirus transferring the beta-galactosidase gene. This is a non-harmful molecule that is useful for determining viral infectibility.
3) A replication deficient adenovirus transferring the green fluorescent protein gene. This is a non-harmful molecule that is useful for determining viral infectibility.
4) A replication deficient adenovirus transferring the gene for I-kappa-B the natural inhibitor of NF-kappaB, an anti-inflammatory molecule.
5) A replication deficient adenovirus transferring the dominant negative form of the I-kappa-B kinase-2, a modification that would also be expected to have an anti-inflammatory effect.
6) A replication deficient adenovirus transferring the dominant negative form of the p38 MAP kinase, a modification that would also be expected to have an anti-inflammatory effect.

All of these have been constructed from plasmids at the Kennedy Institute, London. These molecules are all anti-inflammatory rather than pro-inflammatory, as would be expected if they would be any use as treatment for inflammatory diseases. No adenoviruses with oncogenic genes or sequences will be used. No animal work is planned.

The adenoviral work will take place in a category II facility with a class II vented hood designated for adenoviral work. A locked, designated -70 degree virus freezer will be used for virus storage. There is a designated incubator for infected cells.

Evaluation of foreseeable effects

All vectors used will be based on adenovirus serotype 5, and will be replication deficient due to disabled in the E1 region by an insert that prevents the packaging of viable particles. The E1 region is replaced with inserted foreign DNA. These replication deficient adenoviruses can still infect cells but not replicate within them. Thus if some person would deliberately or accidentally inhale these viruses or rub them into his eyes, a mild rhinopharyngitis (similar to a common cold) or conjunctivitus (harmless eye inflammation), lasting a few days at most, might ensue, in case the person infected did not, like the majority of the population, already possess antibodies to the wild type virus. All inserted gene products will be anti-inflammatory rather than the opposite. None will have mutagenic or oncogenic effects. They should not cause any excess risk. There will be no alteration of existing pathogenic tracts, except for the replication deficiency mentioned above, which will not cause any hazard but rather the opposite. Since all inserted gene products in this study will be anti-inflammatory rather than the opposite, and none will have mutagenic or oncogenic effects, they should not cause any excess risk compared with an adenovirus without an insert. There is thus a risk of very slight and time limited harm should a non-immune person be exposed to these adenoviral vectors.

It is known that recombinant adenovirus reverts to wild type at a low frequency (10^-7). To monitor this and exclude any risk, cytopathic assays are regularly used every 2-3 months to check for replication-competent virus. Since all inserted gene products in this study will be anti-inflammatory rather than the opposite, and none will have mutagenic or oncogenic effects, they should not cause any excess risk compared with an adenovirus without an insert.

The parental virus is ACDP Hazard Group 2. Since all inserted gene products in this study will be anti-inflammatory rather than the opposite, and none will have mutagenic or oncogenic effects, they should not cause any excess risk compared with an replication deficient adenovirus without an insert. Therefore the provisional containment level is 2.

It is not thought that these replication incompetent adenoviruses would pose a risk to animals or plants in the environment, particularly as good care is taken to protect human health. Nor would there, as these viruses will be kept in a locked freezer, be any risk of exposure in a fire, landslide or flooding.

Since the decision was made to apply containment level 2, the activity class is confirmed as Class 2.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation is made.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be autoclaved prior to incineration. The laboratory is separated from the rest of the building by means of a lockable door. Culture medium and liquid waste should be disinfected using 10% Microsol, with exposure at least 1h prior to disposal in the sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The committee was in principle satisfied with the risk assessment for this project and that the work requires Containment level 2 facilities and is class 2.

**Project Containment**

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**Project Ref** 302/01.4

**Date Ackn’d** 26/04/2005

**CU2 Project Title** GENERATION OF DEFINED DELITIONS IN THE HUMAN CYTOMEGALOVIRUS (HCMV) AND HUMAN HERPESVIRUS-6 (HHV-6) GENOMES WILL BE PERFORMED TO INVESTIGATE GENE FUNCTION

**Class** Class 2

**Culture Vol Class 2** 1-50 Litres

**Non-GMM Consent Granted** Not Applicable

02/03/2022
Generation of defined deletions in the human cytomegalovirus (HCMV) and human herpesvirus-6 (HHV-6) genomes will be performed to investigate gene function. To further analyse betaherpesvirus gene function sub-genomic or cDNA fragments will be subcloned and expressed using a range of prokaryotic and eukaryotic expression vectors.

HCMV and HHV-6 are both ACDP category 2 pathogens that are both predicted to encode a large number of open reading frames (in the order of 200). Currently functional information is available for a relatively small number of their genes. Both viruses are associated with mild disease in healthy individuals but occasionally HCMV causes severe disease in immunocompromised individuals. There is no compelling evidence to link either virus with cancer. Both virus are species-specific with productive replication being effectively restricted to human cells. Deletion of genes would usually be expected to reduce virus pathogenicity. Both viruses are expected or known to encode genes that modify cellular physiology and others that modulate the immune response.

The cloning of such genes in a replication-deficient Ad vector will generate recombinant viruses that are more readily transmitted, will provide high level expression and may induce the expression of protein that have a biological effect both in humans and animals. Adenovirus is a ADCP category 2 pathogens although the deletion of the E1 gene region will make both unable to replicate or promote efficient early phase gene expression. Being replication-deficient the recombinant viruses have limited potential for lateral spread, although this could be facilitated by a co-infection with a wt Ad. Wild type Ad type 5 is capable of productive replication in very few species and is associated with mild upper respiratory tract infection in childhood.

For the generation the HCMV genome will be used in the first instance. The HCMV strain AD169 and Toledo genomes will be used in preference. Deletion of the HHV-6 genome is planned in future but will be dependent on the development of better systems for growing the virus.
HCMV strains AD169 and Toledo are characterised laboratory isolates. The Strain AD169 genome is sequenced and published and there is extensive sequence data available for strain Toledo.

HHV-6 strain Z29 and U1102 have been fully sequenced. We intend also to investigate sequence variation in a range of fresh clinical isolates.

**Evaluation of foreseeable effects**

The expression of individual HCMV or HHV-6 genes in cells transiently or in established lines is of low potential hazard. Both viruses are expected or known to encode genes that modify cellular physiology and others that modulate the immune response. The cloning of such genes in a replication-deficient Ad vector will generate recombinant viruses that are more readily transmitted, will provide high level expression and may induce the expression of protein that have a biological effect both in humans and animals. HCMV encodes 6 gene that modulate class I presentation, two functions that modulate resistance to NK cells and both viruses encode chemokine and chemokine receptor homologues.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solution (tissue culture media or buffers such as PBS) is inactivate by adding to less than equal volume 2500 ppm Sodium Dichloroisocyanurate (Actichlor), after 4h solution can be discarded to mains drainage. Pipettes immersed in 2500 ppm Sodium Dichloroisocyanurate for 4h before transferred to plastic container for autoclaving, all plasticware is autoclaved after exposure to virus. Actichlor is effective against all viruses and te high concentration overcomes high organic content of media. Animals may be inoculated in a contained isolator or if Class I or Class II microbiological safety cabinet. All animal waste is incinerated.

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
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<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4 L3 L2 L3 L4</td>
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</table>

Animal Units Large Scale Activities Human Clinical Applications

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02/03/2022
Project Ref 302/01.5

DEVELOPMENT OF ADENOVIRUS VECTOR SYSTEMS

Date Ackn'd: 26/04/2005

CU2 Project Title: DEVELOPMENT OF ADENOVIRUS VECTOR SYSTEMS

Class: Class 2

CultureVolClass2: 1-50 Litres

Non-GMM: Not Applicable

Withdrawn: N

Tick if notifying a connected programme of work: Y

Transfered from GM302 on 26/04/2005

Historical Significant Changes

Project notified under transitional arrangements: N

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

This notification covers the development of adenovirus vectors designed to facilitate the induction/modulation of specific immune responses by transgenes expression of. Host immune responses to vector components can be proinflammatory and often over-ride or subvert potential biological effects associated with the transgene. Specific mutations in the E2, E3, E4, L5 genes will be incorporated into adenovirus type vectors to limit breakthrough expression from replication-deficient adenovirus vectors. Ad5 vectors utilises CAR as a primary receptor for virus infection. We intend to deliver transgenes to cells that do not express detectable levels of CAR (notably lymphocytes). Using established technology, we intend to modify the fibre gene so that the expressed protein will no longer recognise its native CAR receptor and so that it will recognise specific receptors present on target cells. The modified fibre will not be inserted into an adenovirus genome but expressed as a complementing function in a helper cell line. Many individuals have been exposed to adenovirus type 5 and will also generate strong immune responses on exposure to this vector. Adenovirus type 4 has been used as a live viral vaccine. Fewer individuals have seroconverted to adenovirus type 4 and it can potentially also be used in combination with Ad 5 vectors in prime:boost vaccination protocols. We wish therefore to develop replication-competent and replication-deficient adenovirus type 4 vectors. A range of tumour-associated and virus antigens will be expressed in these vectors.

Recipient or parental organism

Preliminary prokaryotic cloning steps are necessary to generate the recombinant adenoviruses. Genetically disabled E.coli K12 strains specifically developed for application in recombinant DNA cloning experiments will used. These strains have limited potential to colonise the gut or survive in the environment. Adenovirus is an ACDP category 2 pathogen. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Adenovirus type 4 infection is also associated primarily with mild, debilitating respiratory tract infections but can occasionally cause an acute respiratory disease (ARD), acute haemorrhagic conjunctivitis or
rarely a fatal non-bacterial pneumonia. To protect against infection, a non-attenuated live Ad4 vaccine has been administered to military recruits in North America. This live viral vaccine consists of an enteric-coated tablet containing live, non-attenuated, tissue culture grown Ad4 virus. The enteric coating prevents infection of the respiratory tract and when swallowed virus is only released from the tablet in the stomach where it infects the gut inducing protective immunity but not disease. Over half a million doses of this vaccine has been administered without complication and it has proved to be effective in preventing ARD. It has been proposed that this vaccine and method of administration might be used as a vector to generate an immune response against both Ad4 and the heterologous immunogens. Ad4 recombinants expressing a range of viral genes have been generated and tested in animals and humans with promising results. The Ad4 virus strain used in the vaccine virus will be used as the basis for all Ad4 vectors and recombinants constructed.

Deletion of the E1 gene region will render the adenovirus vectors replication-deficient. Replication-deficient Ad4 vectors by their nature have limited potential for lateral spread although this could be facilitated by co-infection with a wt adenovirus. Both wild type Ad4 and Ad7 exhibit a severe host restriction and are capable of productive replication in very few species other than man.

Host/vector system

E.coli strains JM109, TOP10 and XL1-blue will be used. Prokaryotic vectors include pcDNA3.1/ct-GFP-Topo, pBLUETOPO, pUC-based vectors containing the HCMV major IE promoter (pMV100), adenovirus transfer vectors pAL200 (and variants with alternative cloning sites). Commercial Ad5 vector systems will be used as the basis for vector development. From Microbix (Canada) pJM17 (contains the complete Ad5d309 genome) and the Admax TM vector system will be used. From Qbiogene (Hareford, Middx) the Ad5 based AdEasy TM vector system, which involves all recombination events be performed in E.coli, will be used.

Ad4 vectors will be generated de novo from the vaccine strain.

Replication-deficient adenoviruses will be propagated in a helper cell line expressing an E1 helper function (eg 293 or 911 cells) and then used to infect target cells in vitro or in vivo. A 293 cell line expressing E4 will be obtained from a commercial source (Microbix). Additional helper cell lines will be constructed in 293 or 911 cells.

Origin & function

A number of human and murine tumour-associated antigens will be cloned.

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<thead>
<tr>
<th>Antigen</th>
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<tr>
<td>Melan A</td>
<td>Coulie et al Immunity 2:167, 1995</td>
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<td>BAGE</td>
<td>Boel et al 178:489, 1993</td>
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<td>Castelli et al J Cell Physiol 182: 323, 2000</td>
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<td>Castelli et al J Cell Physiol 182: 323, 2000</td>
</tr>
<tr>
<td>Mage 3</td>
<td>Castelli et al J Cell Physiol 182: 323, 2000</td>
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<td>NY-ESO-1</td>
<td>Chen et al PNAS 94: 1914, 1997</td>
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<td>CT10</td>
<td>Gre et al Int J Cancer 85:726, 2000</td>
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<td>SOX</td>
<td>Tureci et al Int J Cancer 77:19, 1998</td>
</tr>
<tr>
<td>ZIC</td>
<td>Tureci et al Int J Cancer 77:19, 1998</td>
</tr>
</tbody>
</table>
| Human cytomegalovirus and Human herpes virus 6 are closely related human herpesviruses capable inducing disease in immunocompromised individuals but with no recognised association with cancer. Genes from both these viruses will be cloned into adenovirus vectors for the purpose of generating and measuring immune responses. Venezuelan Equine Encephalitis virus (VEEV) is an Alphavirus that replicates in the cytoplasm of infected cells. The virus particle contains genomic RNA packaged in a capsid consisting of core proteins and an envelope containing the glycoproteins Env1 and Env2. Two other proteins Env3 and 6k may also be found in the virus particle. These two small proteins contain the translocation signal sequence for Env1 and Env2. Being an Alphavirus, the structural proteins are translated from a subgenomic RND as a single polyprotein that is then cleaved by cellular proteases. Env2 contains a major protective epitope. Replication-competent and replication-deficient adenovirus recombinants encoding VEEVEnv3Env2/6K will be generated.
Some of the tumour-specific antigens are expressed in melanocytes and are involved in the melanin biosynthetic pathway. All such proteins are expressed either in normal testis or in melanocytes. None of these proteins in isolation have any toxic, oncogenic, carcinogenic or allergenic properties since they are all expressed in normal cells. The risk of any autoimmune disorder triggered by an overexpression of these proteins is negligible because no autoimmune effect has been reported in healthy volunteers and melanoma patients with high frequencies of cytotoxic T lymphocyte specific for these proteins. (Van Pel et al., Immunological Review. 145:229, 1995; Coullie et al., J Cancer 50:289, 1992). The immune response specific to colon carcinoma antigens is still ill defined, and very few proteins have been shown to be specifically expressed by colon cancer cells. One of the most promising family of newly characterised colon cancer proteins are the SSX proteins, which are expressed in a high proportion of colon.

The expression of individual HCMV or HHV-6 genes in cells transiently or in established lines is of low potential hazard. Both viruses are expected or known to encode genes that modify cellular physiology and others that modulate the immune response. The gene product of VEEV E3, E2 and 6k sequences are not known to be toxic, allogenic or oncogenic although E2 is associated with a strong humoral immune response. The cloning of such genes is a replication-deficient Ad vector will not generate recombinant viruses that are more readily transmitted, will provide high level expression and may induce the expression of protein that have a biological effect both in humans and animals.

This work involves standard laboratory protocols and there are no unusual procedures that require additional containment measures. A more detailed risk assessment was performed for the local ACGM safety committee.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solution (tissue culture media or buffers such as PBS) is inactivate by adding to less than equal volume 2500 ppm Sodium Dichloroisocyanurate (Actichlor), after 4h solution can be discarded to mains drainage, Pipettes immersed in 2500 ppm Sodium Dichloroisocyanurate for 4h before transferred to plastic container for autoclaving, all plasticware is autoclaved after exposure to virus. Actichlor is effective against all viruses and the high concentration overcomes high organis content of media.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved, suitable and sufficient Class 2 Containment Level 2

Project Containment
Project Ref: 302/96.4

Date Ackn'd: 26/04/2005

CU2 Project Title:
ANALYSIS OF EPSTEIN BARR VIRUS LATENT GENE FUNCTIONS (I) INSERTION OF VIRAL GENES INTO EUKARYOTIC EXPRESSION VECTORS (II) TRANSFECTION INTO MAMMALIAN CELLS

Class: Class 2

Consent Granted: Not Applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes:
Transferred from GM302 on 26/04/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units: L2, L3, L4

Large Scale Activities: L2, L3, L4

Human Clinical Applications: L2, L3, L4

Project Ref 302/99.1

Date Ackn'd 26/04/2005

CU2 Project Title INVESTIGATION OF THE ROLES OF THE MAPKK AND PI3-K PATHWAYS IN

Class 2

CultureVolClass2 Class 2

CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form

Tick if you confirm that it is attached to this form N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
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**Animal Units**

<table>
<thead>
<tr>
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<th>Human Clinical Applications</th>
</tr>
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<tbody>
<tr>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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**Project Ref** 302/trans1

**Date Ackn'd** 26/04/2005

**CU2 Project Title**

A PHASE IIA, OPEN LABEL TRIAL TO ASSESS THE SAFETY, IMMUNOGENICITY AND EFFICACY OF A PRIME-BOOST STRATEGY OF TA-CIN ADMINISTERED IN ASSOCIATION WITH TA-HPV TO PATIENTS WITH HIGH GRADE ANO-GENITAL

**Class**

<table>
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**Non-GMM** Consent Granted Not Applicable

**Project notified under transitional arrangements** Y

**Withdrawn** N

**Tick if notifying a connected programme of work** Y

**Historical Significant Changes**

GM302/95.1, GM302/97.1 This Project and significant changes transferred

**Historical Date of Additional Info**

19/05/1995, 17/07/1995, 19/06/2001
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref  312/02.1

Date Ackn’d                  CU2 Project Title
26/04/2005                     MODULATION OF SIGNALLING IN LYMPHOCYTES BY VIRAL VECTORS

Date Project Ceased

Class              CultureVolClass2              CultureVolumeClass3-4
Non-GMM              Consent Granted
Class 2              < 1 Litre
Not Applicable

Withdrawn             Tick if notifying a connected programme of work
N                       N

Historical Significant Changes
Project transferred from GM312 on 26/04/2005.

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
I wish to study the molecules involved in lymphocyte growth. This will take place in B and T-lymphocyte cell lines as well as primary human cells. The molecules chosen include ras, P13K, Jak, and STATs, all of which are activated in response to T-cell receptor or cytokines, the molecules that result in T-cell growth. Human primary lymphocytes are not easily transfected and infection remains the best way to modulate their genotype.

Recipient or parental organism
The GMO that will be generated are retroviruses based on the murine (MuLV) system containing molecules involved in lymphocyte growth and signalling.

Host/vector system
A retroviral vector system has been chosen. DNA corresponding to molecules involved in lymphocyte signalling will be introduced in the pinco retroviral plasmid. This plasmid will be introduced into the Phoenix AM cell line that allows the production of amphotropic retroviruses. Phoenix is a second-generation retrovirus producer line for the generation of helper free ecotropic and amphotropic retroviruses based on the murine (MuLV) system. The lines are based on the 293T cell line. The Phoenix cell line was chosen because it is a well established cell line that has been extensively tested for helper virus production and established as been helper-virus free. The cell line
contains two separate plasmids for the generation of the env protein and the gag-pol protein. Both the gag-pol and envelope constructs with non-moloney promoters were used to minimise recombination potention. Different retrovirus cannot be produced from these cells.

Origin & function

The genetic material for the retroviruses are derived from the murine (MuLV) system. The genes that will be introduced into these vectors are mammalian in origin. Each of these have been chosen because of their role in the manipulation of the immune system. While all of these molecules play an important role in growth and/or survival, none have been shown to induce lymphocyte malignancy alone. Wild type, active and inhibitory forms of the molecules will be studied.

Evaluation of foreseeable effects

The retroviruses by themselves do not constitute a risk. DNA corresponding to many different proteins have been described on the attached sheet. Details from each of these gene products have been given. When considering the possible hazard to human health it is appropriate to consider the gene product that has the most potential to cause harm. The virus with the most potential to cause harm is the V12-ras retrovirus. However, this has been used safely in other Departments within the University of Wales College of Medicine. This retrovirus could result in the expression of V12 in human cells. However this would only cause harm if administered in large doses or were to become infectious. However, the amounts of virus generated will be low and there are many features of the retroviral system that proscribe the ability of the retrovirus to become infectious.

Three other important factors:
(1) While V12 is an oncogene, it is unable to transform primary human cells alone. The short half life of the virus minimises the duration of any exposures. None of the other inserts are oncogenic.
(2) The pathogenicity of the retrovirus is unaffected by any of the inserts and there is unlikely to be any transfer of harmful sequences to related viruses.
(3) The viruses generated cannot cause a productive infection.

Origin & function

The genetic material for the retroviruses are derived from the murine (MuLV) system. The genes that will be introduced into these vectors are mammalian in origin. Each of these have been chosen because of their role in the manipulation of the immune system. While all of these molecules play an important role in growth and/or survival, none have been shown to induce lymphocyte malignancy alone. Wild type, active and inhibitory forms of the molecules will be studied.

Evaluation of foreseeable effects

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Origin & function

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Evaluation of foreseeable effects

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(1) While V12 is an oncogene, it is unable to transform primary human cells alone. The short half life of the virus minimises the duration of any exposures. None of the other inserts are oncogenic.
(2) The pathogenicity of the retrovirus is unaffected by any of the inserts and there is unlikely to be any transfer of harmful sequences to related viruses.
(3) The viruses generated cannot cause a productive infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste contaminated with GMMs will be treated with freshly prepared 2500 ppm sodium dichloroisocyanurate. This completely destroys the retrovirus.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Safety sub-committee found the risk assessment to be suitable and sufficient and agreed that the work was Class 2, requiring Containment Level 2 facilities.
### Project Containment

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</tbody>
</table>

**Animal Units**

| L2 | L3 | L4 | L2 | L3 | L4 |

**Large Scale Activities**

| L2 | L3 | L4 | L2 | L3 | L4 |

**Human Clinical Applications**

| L2 | L3 | L4 |

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### Project Ref 312/02.2

**Date Ackn'd**

26/04/2005

**CU2 Project Title**

EXPRESSSION OF IMMUNE REGULATORY PROTEINS BY USING AN ADENOVIRUS VECTOR

**Non-GMM Consent Granted**

Not Applicable

**Project notified under transitional arrangements**

Y

**Historical Significant Changes**

Project transferred from GM312 on 26/04/2005

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### Project Additional Information

**Purposes of the contained use**

The immune system is controlled by a complex balance between the innate and the adaptive immune responses. IL-10 is associated with shifting the balance of the immune response away from a TH1 and towards a TH2 response. Work in other centres has demonstrated that adenovirus (Ad) vectors encoding the Epstein Barr virus (EBV) interleukin 10 (IL-10) homologue is effective in suppressing inflammation in animal gene therapy models of autoimmune disease (Apparailly et al. 1998 J Immunol. 160, 5213). Recently, a functional human cytomegalovirus IL-10 homologue has also been identified (Kotenko et al. 2000 PNAS 97, 1695). We wish to use Ad recombinants encoding human, rat, EBV and HCMV IL-10 in both in vitro and in vivo assays. An Ad vector is required for efficient expression of these molecules. We intend to test the relative efficiency of the various versions of IL-10 on Dendritic cell (differentiation and maturation), CD8+ cytotoxic T cells (CTL) and NK cell function in vitro. Major histocompatibility class I molecules play a key role in functional interactions of all these three cell types and are regulated by IL-10. As part of this programme...
of work, we seek permission of express both classical (HLA A-C) and non-classical (HLA-E and HLA-G) molecules in replication-deficient Ad vectors. HLA A-C are highly polymorphic and a number of variant genes may be expressed. In contrast HLA-E and HLA-G are highly conserved but subtle variants do exist and may be expressed. The capacity of NKX2.5 to modulate myeloid cell function in vitro will be examined in a pilot study. LIR-1 is an inhibitory receptor found on cells of the myeloid lineage, T cells and NK cells that interacts with classical MHC-1 molecules and HCMV MHC-1 homologue gpUL18. We intend examining functional interaction between HCMV gpUL18 and LIR01. This notification covers the limited use of Ad recombinants designed to modulate immunological responses by transgene expression. In particular, we intend testing replication-deficient adenovirus recombinants encoding the EBV and HCMV IL-10 homologues in an established in vivo experimental model of rheumatoid arthritis.

Recipient or parental organism

Preliminary prokaryotic cloning steps are necessary to generate the recombinant adenoviruses. Genetically disabled E-coli K12 strains specifically developed for application in recombinant DNA cloning experiments will used. These strains have limited potential to colonise the gut or survive in the environment.

Adenovirus is an ACDP category 2 pathogen. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Deletion of the E1 gene region will render the adenovirus vectors replication-deficient. Replication-deficient Ad vectors by their nature have limited potential for lateral spread although this could be facilitated by co-infection with a wt adenovirus or gene rescue from the helper cell line.

Host/vector system

E coli strains JM109, BJ583 and XL1-blue will be used.

Commercial Ad5 vector systems will be used as the basis for vector development. For the construction of replication-deficient adenovirus recombinants: The Admax vector system from Microbix, Canada (pDC511, pDC512, pDC515 and pDC516, PBHGfrtDE1,3FLP, pXCXL-based transfer vectors, pJM17) will be used. From Qbiogene (Hareford, Middx) the Ad5 based AdEasytm vector system (pAdEasy-1, pShuttle, pShuttle-CMV, pShuttleCMV-LacZ) will be used. Replication-deficient adenoviruses will be propagated in a helper cell line expressing an E1 helper function (e.g 293 or 911 cells) and then used to infect target cells in vitro or in vivo.

Origin & function

Human, rat, EBV and HCMV IL-10 genes will be used to evaluate their relative capacity to modulated a range of immune responses in vitro and in vivo assays. NKX2.5 is a transcription factor associated with maturation of cardiomyocytes, its effect on myeloid cell maturation will be evaluated. HLA-A, HLA-B, HLA-C, HLA-E, HLA-5 and teh human cytomegalovirus MHC-1 homologue UL18 as test ligands of Lir-1 and to examine function of IL-10. LIR-1 is an inhibitory receptor originaly identified by high-affinity binding to synthetic gpUL18 and subsequently shown to bind a broad range of MHC-1 molecules. All DNA are cDNA clones obtained commercially or gifts from collaborators. DNA sequences are known and will be confirmed in adenovirus transfer vectors prior to generation of recombinant virus.

Evaluation of foreseeable effects

It is recognised that there is a potential hazard associated with using Ad recombinants encoding transgenes capable of modulating the immune response IL-10 is a 17-18kDa homodimer that can be secreted by T cells, macrophages and EBV- transformed B cell lines. IL10 functions by binding the IL-10 receptor. IL-10 was first recognized for its ability to inhibit activation and effector function of T cells, monocytes, and macrophages, and is a multifunctional cytokine with diverse effects on most haemopoietic cell types. The principal routine function of IL-10 appears to be to limit and ultimately terminate inflammatory responses. In addition to these activities, IL-10 regulates growth and/or differentiation of B cells, NK cells, cytotoxic and helper T cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells. hIL-10 is active on both mouse and human cells, whereas mIL-10 is effective only on mouse cells. The mature hIL-10 and EBV viral IL-10 (vIL-10) amino acid sequences are 84%
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This construct is thus expected to have a biological function following in vivo delivery. There is evidence in mouse and humans that elevated IL-10 production usually imposes some limits on the effectiveness of antipathogen immun responses, especially innate immunity and adaptive Th1 responses. This cost if often outweighed by the ability of IL-10 to protect the host from collateral damage by antimicrobial cytokines and effector molecules. IL-10 has been considered an attractive candidate for therapeutic use based on its potent in vitro immunomodulating activities and proven effects in animal models of acute and chronic inflammation, autoimmunity, cancer and infectious disease. Phase I and II clinical trials investigating safety, tolerance, pharmacokinetics, pharmacodynamics, immunological and hematological effects of single or multiple doses of IL-10 administered by intravenous (iv) or subcutaneous (sc) route. IL-10 has been tested in specific patient populations including those with Chrohn's disease, RA, psoriasis, and patients suffering from chronic hepatitis C infections. Administration of IL-10 (7 days iv) reduced the Cohrohn's disease activity index score in patients with steroid-refractory Crohn's disease and showed some clinical benefit in a larger 28-day sc safety and efficacy study in patients with chronic active Crohn's disease. Similarly, a trend towards efficacy and a good safety profile was observed when IL-10 was administered for 28 days to RA patients. Since intravenous (iv) or subcutaneous (sc) doses of IL-10 resulted in transient dose-dependent changes in white blood cell populations, including increases in total white blood cells and neutrophils.

There are a number of theoretical hazards associated with this experiment. First, as the recombinant virus is intended for in vivo use, there is the potential that the virus could spread from animal to animal and hence get into the natural population. However any in vivo injections will be local and given the fact the virus cannot penetrate more than a few layers of cells, the systemic spread of the virus should be negligible. Even in the highly unlikely event of the spread of the virus systemically, the recombinant virus is replication deficient, is not maintained in dividing cells and as such is incapable of horizontal spread throughout a population. Additionally wild-type Ad type 5 is able to undergo productive virus replication in very few non-human cells.

A second potential hazard is the exposure of humans to the recombinant virus. All manipulations of the virus will be performed in a class II biological safety cabinet in the laboratory. In vivo injections of Ad recombinants encoding recombinant genes into animal models will be carried out in an animal isolator, a negative pressure facility (i.e. in a class I cabinet) or a class II cabinet. A potential human infection will be a needle stick injury. Use of sharps will be kept to a minimum but are required for animal inoculations. In this event, gene expression from the Ad recombinant should be localised and the potential inhibition of complement localised and transient. In the unlikely scenario that the recombinant inhibitor becomes systemic, for the reasons stated above (inability to replicate without E1 complementation and the loss of the virus in dividing cells) the effect of the complement inhibitor should be transient. Also the effect of the complement inhibitor in humans should be minimal as the dose will have been designed for a rate and should therefore be at too low a level for a significant effect in humans. Horizontal spread of the recombinant virus through the human population is not likely for the same reasons as it is not likely in the rat population.

Classical and non-classical MHC molecules are widely expressed on human cells. The over-expression on non-classical HLA A-C molecules could promote antigen specific or allospecific immune responses and expression of any of the classical or non-classical HLA molecules could suppress NK recognition but should this occur the risk is minimal. LIR-1 (also known as ILT-2) is an inhibitory receptor thus its over-expression in lymphocytes or myeloid cells could suppress the function of that the target cell. The Ad5 vector infects lymphoid and myeloid cells relatively inefficiently. It is extremely unlikely that with the natural biological containment that the gene could be delivered with sufficient efficiency to exert a biological effect in man or animals. NKX2.5 transcription factor (a cell specific expression marker in cardiomyocytes) that will be subcloned into an replication-deficient Ad vector. There is no known transforming activity or toxicity associated with this gene. Use of this construct has been independently approved at ACGM 2 level elsewhere.

These recombinant viruses will be contained using ACGM level 2 procedures as stipulated in the risk assessment.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solution (tissue culture media or buffers such as PBS) is inactivate by adding to less than equal volume 2500 ppm Sodium Dichlorocyanurate (Actichlor), after 4h solution can be discarded to mains drainage. Pipettes immersed in 2500 ppm Sodium Dichlorocyanurate for 4h before transferred to plastic container for autoclaving, all plasticware is autoclaved after exposure to virus. Actichlor is effective against all viruses and the high concentration overcomes high organic content of media.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

A minor modification was made to the title for accuracy.

Notification of this project as an extension of a connected programme of work would be necessary.

Containment level 2, class 2 was appropriate

Approved

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<td>L3</td>
<td>L4</td>
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</table>

- **Animal Units**
  - L2 Yes
  - L3 | L4 | L2

- **Large Scale Activities**
  - L3 | L4

- **Human Clinical Applications**
  - L3 | L4

**Project Ref** 312/02.3

**Date Ackn'ed** 26/04/2005

**CU2 Project Title** THE DEVELOPMENT OF ADENOVIRAL VECTORS FOR THE EXPRESSION OF

**Class** Class 2

**CultureVolClass2** 1-50 Litres

**CultureVolumeClass3-4**
THE PROPOSED PROJECT INVOLVES THE CONSTRUCTION OF ADENOVIRAL RECOMBINANTS FOR THE EXPRESSION OF COMPLEMENTARY INHIBITORY MOLECULES IN CELL LINES IN VITRO AND EVENTUALLY IN ANIMAL MODELS OF DISEASE.

Purposes of the contained use

Recipient or parental organism

Preliminary prokaryotic cloning steps are necessary to generate the recombinant adenoviruses. Genetically disabled E-coli K12 strains JM109 and XL1-blue specifically developed for application in recombinant DNA cloning experiments will be used. These strains have limited potential to colonise the gut or survive in the environment. Adenovirus is an ACDP category 2 pathogen. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Deletion of the E1 gene region will render the adenovirus vectors replication-deficient. Replication-deficient Ad vectors by their nature have limited potential for lateral spread although this could be facilitated by co-infection with a wt adenovirus or recombination with the E1 gene in the helper cell line.

Host/vector system

E.coli strains JM109 and XL1-blue will be used. Prokaryotic vectors include pUC-based vectors including the Ad transfer vector pAL119. A commercial Ad5 vector systems provided by Prof Frank Graham will be used as the basis for the vector (Microbix, Canada). pJM17 contains the complete Ad5dl309 genome vector system cloned into the pBR322-based vector pBRX. Recombinant viruses will be generated following recombination in 193 cells.

Prokaryotic cloning experiments are required to manipulate the complement inhibitory genes prior to recombination into the Ad genome. Expression will not be sought in E. coli and no specific hazard has been identified for prokaryotic manipulation. Replication-deficient adenoviruses will be propagated in a helper cell line expressing an E1 helper function (e.g., 293 or 911 cells) and then used to infect target cells in vitro or in vivo.

Origin & function

The molecules of interest include human CD59, DAF (Decay Accelerating Factor), MCP (Membrane Cofactor Protein) and CR1, the rat analogues of DAF, CD59 and MCP and the rat complement inhibitor Crry. It is intended to clone these molecules in two forms: a) the natural form which includes the sequence necessary to anchor the protein to the cell surface and b) with a deletion of the sequence responsible for anchoring the protein to the membrane so that it is secreted from cells in a soluble form. Secreted forms of complement inhibitors do occur naturally. We also intend to perform mutagenesis on the predicted active sites of the complement inhibitors to examine function. Such constructs can be expected to have reduced hazard potential under most circumstances, although a dominant mutation could possibly reduce the efficiency with which complement action is inhibited.
DAF and CR1 have decay accelerating activity of the bimolecular enzyme complexes which constitute the convertase in both the classical and alternative complement activation pathways. They dissociate the C2a or the Bb subunits from the formed convertases and may also prevent the subsequent association of their procatylitic precursors C2 and B with membrane bound C4b or C3b molecules. Hence by accelerating the decay of preformed convertases and inhibiting the formation of new ones, DAF and CR1 can prevent amplification of the C3 activation on the host cell surface. Cr1 also acts as a cofactor for the cleavage of iC3b to C3c and C3d.g (iC3b can bind and activate neutrophils via CR3). CR1 also acts in vivo to mediate phagocytic binding of particles opsonized with C3b and participates in the transport of immune complexes on erythrocytes.

Like CR1, MCP acts as a cofactor for the factor-I cleavages of C3b and C4b and hence prevents the formation of active C3/C5 convertases. Although this inhibits both the classical and alternative pathways of the complement system, MCP is thought to act preferentially on the convertase of the alternative pathway.

CD59 works at the end of the complement pathway by inhibiting the assembly of the MAC (Membrane Attack Complex). It does so by incorporating into the partially formed MAC by binding to C5b8 and interfering with the subsequent binding and polymerization of C9.

The specific mechanism for Crry function is still unclear although it is thought to be a functional homologue for human MCP and/or DAF.

The main function of the complement system is its role in both the adaptive and humoral immune system in killing foreign microorganisms. Other biologically important functions mediated by the complement system include:

1) Low molecular weight anaphylotoxins C3a, C4a, and C5a which promote smooth muscle contraction and increase vascular permeability.

2) Large C4b and C4b fragments involved in the binding to the complement activator and therefore interact with specific receptors to allow efficient clearance of the activating cell of particle including virus.

3) Degradation of the fragments of C3b to iC3b, C4d.g and C3d which are also important in the clearance of the immune aggregates and the triggering of the receptor mediated activities including the regulation of the immune response.

Hence expression of the human (only) complement inhibitory proteins could result in a reduction/inhibition of systemic complement in man and potentially inhibit to varying degrees one, some or all of the above activities leading to an increased susceptibility to infection and the accumulation of immune complex precipitates.

The recombinant virus will be based on an Ad5 dl309 background with an additional deletion in the E1 gene at which site the insert will be inserted. The deletion of the E1 gene make the Ad recombinant replication-deficient and thus replication of the virus requires complementation by a helper function. 293 or 911 cells both carry an integrated copy of the Ad5 E1 gene and hence will be used for the construction and propagation of new recombinant virus. The complement inhibitory genes will be inserted at the site of the E1 deletion.

**Evaluation of foreseeable effects**

Activation of complement is known to be an important factor in numerous diseases including the development and propagation of inflammation of the rheumatoid joint. Efficient inhibition of complement could therefore ameliorate disease. This has been shown previously in the case of rheumatoid arthritis, by systemic and local inhibition of complement using complement inhibitory proteins. This project is an extension of this work but is intended to avoid repeated protein injections by assessing the viability of in situ expression of complement inhibitors using adenoviral vectors.

There are a number of theoretical hazards associated with this experiment. Firstly, as the recombinant virus is intended for in vivo use, there is the potential that the virus could spread form animal to animal and hence get into the natural population. Complement inhibitors exhibit host specificity and can be considered as a significant hazard only in the homologous species. However any in vivo injections will be local and given the fact the virus cannot penetrate more than a few layers of cells, the systemic spread of the virus should be negligible. Even in the highly unlikely event of the spread of the virus systemically, the recombinant virus is replication deficient, is not maintained in dividing cells and as such is incapable of horizontal spread throughout a population. Additionally wildtype Ad type 5 is able to undergo productive virus
replication in very few non-human cells.

A second potential hazard is the exposure of humans to the recombinant virus. All manipulations of the virus will be performed in a class 11 biological safety cabinet in the laboratory. In vivo injections of Ad recombinants containing human complement inhibitory proteins into animal models will be carried out in an animal isolator, a negative pressure facility (ie in a class 1 cabinet) or a class 11 cabinet. A potential human infection will be a needle stick injury. Use of sharps will be kept to a minimum but are required for animal inoculations. In this event, gene expression from the Ad recombinant should be localised and the potential inhibition of complement localised and transient. In the unlikely scenario that the recombinant inhibitor becomes systemic, for the reasons stated above (inability to replicate without E1 complementation and the loss of the virus in dividing cells) the effect of the complement inhibitor should be transient. Also the effect of the complement inhibitor in humans should be minimal as the dose will have been designed for a rat and should therefore be at too low a level for a significant effect in humans. Moreover there are a total of 10 known complement regulatory molecules and the transient increase of one or two of these molecules should not significantly imbalance the immune system. Horizontal spread of the recombinant virus through the human population is not likely for the same reasons as it is not likely in the rat population.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solution (tissue culture media or buffers such as PBS) is inactivate by adding to less than equal volume 2500 ppm Sodium Dichloroisocyanurate (Actichlor), after 4h solution can be discarded to mains drainage. Pipettes immersed in 2500 ppm Sodium Dichloroisocyanurate for 4h before transferred to plastic container for autoclaving, all plasticware is autoclaved after exposure to virus. Actichlor is effective against all viruses and the high concentration overcomes high organic content of media.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Assessment suitable and sufficient. Concern expressed re use of sharps. Questioned - no alternative for inoculating animals.

Project Containment

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<thead>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>

02/03/2022
### Project Additional Information

**Purposes of the contained use**

I wish to study the molecules involved in lymphocyte growth. This will take place in B and T-lymphocyte cell lines as well as primary human cells. The molecules chosen include P13K, Jak, and STATs and other molecules that modulate cell growth and gene expression. Human primary lymphocytes are not easily transfected and infection remains the best way to modulate their genotype.

**Recipient or parental organism**

The GMO that will be generated are adenoviral system containing molecules involved in lymphocyte growth and signalling.

**Host/vector system**

The adenoviral host vector systems are commonly used and well-characterised. All adenovirus vectors will be replication deficient due to deletion in the E1 gene region; this is a recognised safety feature. Repair of the deletion in the E1 gene region may occur due to homologous recombination with the E1 gene in the helper cell line (an extremely unlikely event) or following co-infection of a cell with a wild type Ad. In the unlikely event of such a recombination, it is likely to result in concomitant deletion of the transgene.

**Origin & function**

The genetic material for the adenoviral system are derived from the human Ad5 system. The genes that will be introduced into these vectors are mammalian in origin. More information is shown on the attached sheet. Each of these have been chosen because of their role in the manipulation of the immune system and gene expression. While all of these molecules play an important role in growth and/or survival, none have been shown to induce lymphocyte malignancy alone. Wild type, active and
inhibitory forms of the molecules will be studied.

Evaluation of foreseeable effects

The adenoviruses by themselves do not constitute a risk. When considering the possible hazard to human health it is appropriate to consider the gene product that has the most potential to cause harm. The virus with the most potential to cause would be the active PKB or dominant negative PML. Both of these proteins have the capacity to enhance cell survival and proliferation. However neither alone is sufficient to cause malignant transformation. Cells of the haematopoetic system are most at risk from these proteins. However, these cells are poorly infectible by adenovirus. Furthermore, the transient nature of expression following adenoviral infection makes the expression unlikely to affect human health.

As the Ad is replication deficient, ie cannot replicate in host cells unless E1 sequences are provided in trans (eg in HEK 293 cells for virus propagation). It is considered highly unlikely that this will happen spontaneously. Furthermore efficient delivery of large number of virus particles would be required to infect cells. Expression using the Ad vector is only transient and is quickly lost in dividing cells as it only integrates DNA at a low frequency. The Ad vector is unlikely to cause any permanent change in cells it infected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste contaminated with GMMs will be treated with freshly prepared 2500ppm sodium dichloroisocyanurate. This completely destroys the adenovirus.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC found the risk assessment to be suitable and sufficient.

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications
Project Ref 312/02.5

Date Ackn'd 26/04/2005

CU2 Project Title PRODUCTION OF REPLICADEFICIENT ADENOVIRUS RECOMI-NANTS ENCODING DOMINENT NEGATIVE AND WILD TYPE MUTANTS OF VARIOUS ISOFORMS OF THE SIGNAL TRANSDUCTION MOLECULE, PROTEIN KINASE C.

Date Project Ceased

Class Class 2

CulureVolClass2 < 1 Litre

Consent Granted Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Project transferred from GM312 on 26/04/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The dominant negative and wild-type protein kinase C constructs will be transfected into cells in order to determine the role of isoforms of this enzyme in cell signalling leading to volume regulation, cell proliferation and drug resistance.

Recipient or parental organism

Recipient myocytic cells are only capable of proliferating to a couple of generations before they terminally differentiate, transfection of these cells is therefore unlikely to pose a hazard, however all work with the transfected cells will be conducted in a class 2 laminar flow cabinet and all media and labware treated with Virkon solution.

Breast cancer cell lines such as MCF-7 and our Tamoxifen resistant derivative thereof are able to grow in culture for significant periods of time. Transfection with the virus however would only be transient and the biological hazard therefore negligible. These cell lines do not contain other viral particle which might recombine with the vector beginning introduced, such as has been found in cell such as Hela cells. Again all work will be conducted in a class 2 laminar flow cabinet and all media and labware treated with Virkon before disposal.

Host/vector system

The host vector system is well characterised and available commercially. All adenovirus vectors used will be replication deficient due to deletion in the E1 gene region; this is a recognised safety feature. Repair of the deletion in the E1 gene region may occur due to homologous recombination with the E1 gene in the helper cell line (an extremely unlikely event) following co-infection of a cell with a wild-type adenovirus. Such a recombination event is likely to result in concomitant deletion of the transgene, is extremely unlikely but not impossible. Such recombinations also only usually occur within a subgenus not between subgenus. Adenovirus vectors are associated with...
extremely efficient in vivo delivery and can be spread by aerosol. This hazard will be contained by restricting manipulation with the virus to biological safety caninets and sealed centrifuge rotors. Adenovirus have no mechanism for cytolysis so in culture most viral particles remain cell-associated, although they can spread to neighbouring cells, leading to plaque formation.

Origin & function

PKC- 'Gutless' tetracycline - regulated binary adenoviral vectors will be supplied (Harding et al., 1997, J. Neurochem 69 2620-3 and Harding et al., 1998, Nature Biotech 16: 553-5) (Hussain S, Assender JW, Bond M, Wong LF, Murphy D, Newby AC 2002; "Activation of protein kinase-C is essential for cytokine-induced metalloproteinase-1, -3 and 9 secretion from rabbit smooth muscle cells and inhibits proliferation." J. Biol Chem.: 277; 30: p27345-52). The original kinase defective (point mutation within the ATP binding site 275 Lys> Trp) dominantnegative mutant was obtained from Dr Moscat, Universidad Autonoma de Madrid.

PKC-a and : Wild-type and kinase defective (point mutations within the ATP binding site) dominant negative mutants of PKC-a and PKC- have been obtained from Dr Ohba, Instit of Mol. Oncology, Showa University, Tokyo (Nature 325: 161-66, 1987; Mol Cell Biol 18: 5199-5207, 1998; EMBO 13: 2331-2340, 1994). Rabbit PKC-a cDNA (-4~+2647), mouse PKC- cDNA (-13+2525/genbank:X60304), Dom neg PKC-1 cDNA (-42647 with 1 nucleotide mutation, AAGATCC>ACGATCC: 368 k>r), Dom neg PKC- cDNA (-13+2525 with 4 nucleotide mutation AAGTGTC>GCATGCC: 376 k>a). These will be used to determine the effect of over or non-expression of these signalling molecules on cell proliferation and Tamoxifen resistance in breast cancer and regulatory volume decrease in myocytes.

Evaluation of foreseeable effects

There is a potential hazard associated with using adenovirus recombinants to express transgenes that are overtly transforming or have a strong capacity to prime host cell proliferation. Protein kinase C is a signal transduction molecule associated with cell proliferation in a number of cellular systems. Although not thought to be the driving force behind cell replication, it does appear to act as a reostat, enhancing proliferation/apoptotic responses. The inserted gene product could therefore potentially be harmful, however due to the transient nature of the infection (see below), this risk is thought to be minimal. Dominant negative mutants of protein kinase C have previously been widely used in other laboratories and by the co-applicant. "Activation of protein kinase-C is essential for cytokine-induced metalloproteinase-1, -3 and 9 secretion from rabbit smooth muscle cells and inhibits proliferation". J Biol Chem.: 277 (30): p27345-52).

As the adenovirus is replication-deficient due to E1 and E3 deletions, they can not replicate in host cells unless E1 sequences are provided in trans (eg in HEK 293 cells for virus propagation). It is considered highly unlikely that this will happen spontaneously. Furthermore efficient delivery of large numbers of virus particles will be required to infect target cells, which is unlikely to happen, in vivo. Furthermore, since the adenovirus is replication deficient, no persistant long term effects are likely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solutions (such as tissue culture media or buffers such as PBS) will be inactivated by addition of more than an equal volume of sodium Dichloroisocyanate (Actichlor, 25000 ppm). After 4h, the solution will be discharded to main drains. Pipettes will be totally immersed in 2500 ppm sodium dichloroisocyanurate for 4 h before transferring to plastic containers for autoclaving. All plasticware will be autoclaved after exposure to the virus. Acticlor is a broad spectrum and rapid acting biocide, effective against all viruses. The high concentrations used here will ensure that the high organic content of the media is overcome and 100% killing should be achieved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
# Project Containment

<table>
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<th>Large Scale Activities</th>
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## Project Ref 312/02.6

**Date Ackn’d** 26/04/2005

**CU2 Project Title** MANIPULATION OF ACTIVITY OF THE INTERFERON SIGNALLING PATHWAY IN CULTURED HUMAN CELLS

**Class** Class 2

**Culture Vol Class** < 1 Litre

**Class Culture Volume** Class 3-4

**Non-GMM** Not Applicable

**Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes** Project transferred from GM312 on 26/04/2005

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

## Project Additional Information
Purposes of the contained use

The aim of the project is to manipulate the levels of activity of the interferon signalling pathways in cultured human cells, both normal and immortalised, and potentially in combination with infection with other human retroviral vectors, already covered by HSE notifications in our laboratory, such as that encoding Ras.

We have identified the interferon pathway as an important area of investigation due to our previous study of the effect of activated Ras in thyroid cells. Ras confers a limited proliferative potential to thyrocytes. However the cells eventually cease to divide. We have analysed gene expression in this arrested state using DNA microarrays and it appears that the interferon pathway is involved. This project will involve manipulating the interferon response pathways in a background where mutant Ras has already been introduced.

Interferons play key roles in mediating antiviral and antigrowth responses and modulating the immune response, particularly in response to viral infection. Interferons are proteins naturally produced by many cells in the body. The interferons are pleiotropic cytokines that are induced in response to virus infection and act in a paracrine fashion to elicit an antiviral state in nearby cells. Binding of interferons to their cell-surface receptors induces a tyrosine kinase signalling cascade that leads to the activation of latent cytoplasmic signal-transducer-and-activator-of-transcription (STAT) factors. Activated STATs then translocate into the nucleus, where they are targeted to conserved promoter-enhancer sites to induce the transcription of interferon-responsive genes, that encode for protein with potent antiviral, growth-inhibitory, anti-tumour and immunomodulatory properties. There are 3 classes of interferon: interferon alpha, beta and gamma. Interferon alpha and beta signal through the Type 1 pathway, and interferon gamma uses the closely-related Type II pathway.

We are interested in altering the levels of various members of the interferon signalling pathways, to attempt to modulate signalling of each pathway. We intend to do this by over-expression of various components of the pathways, the use of inhibitory genes, either naturally occurring (e.g. IRF2) or known dominant negative mutant alleles (e.g. of STAT 1). Cells will be studied in culture (e.g. phase contrast microscopy, the response to drug or other treatments), or killed and then subjected to cellular and molecular biology assays, such as immunocytochemistry, Western blotting, and extraction of RNA and DNA for subsequent analysis.

We may also during the course of this work generate dominant negative and loss-of-function versions of the genes specified on accompanying risk assessments and below. These will be tested in the same manner as the wild type genes, as described above.

Recipient or parental organism

Host organisms are primary human cells in particular thyrocytes. These cells may also have been infected with retroviruses expressing mutated RAS.

Host/vector system

All genes will be introduced into a series of plasmid backbones of the pBABE series of vectors. In the appropriate producer cell lines these produce ecotropic and amphotropic retroviruses where gene expression is driven by the MMLV LTRs. All retroviruses are replication defective as many helper functions are supplied by the producer cell lines psiCRIP and Omega E. Cells that have stably integrated the retroviral expression cassette will be selected on the basis of drug resistance to hygromycin, G418 and puromycin.

Origin & function

Cytokines: Interferons alpha, beta and gamma
Interferon alpha/beta receptor: Cell-surface receptor for interferon alpha and beta
Interferon gamma receptor: Cell-surface receptor for interferon gamma
Components of interferon signalling pathway: Interferon Regulatory Protein 1 (IRF1); Interferon Regulatory Protein 2 (IRF2); Interferon Regulatory Protein 3 (IRF3); Interferon Regulatory Protein 5 (IRF5); Interferon Regulatory Protein 6 (IRF6); Interferon Regulatory Protein 7 (IRF7); Interferon Regulatory Protein 8 (IRF8, ICSBP); Interferon Regulatory Protein 9 (IRF9, p48); Janus Kinase 1 (JAK1); Janus Kinase 2 (JAK2); TYK2; SHP-2 (tyrosine phosphatase); Cytosolic phospholipase A2 (CPLA2)
Components of interferon signalling pathway and transcription factors: Signal Transducer and Activator of Transcription 1 (STAT1); Signal Transducer and Activator of Transcription 2 (STAT2); Signal Transducer and Activator of Transcription 3 (STAT3); CREB -binding protein (CBP/p300)
Inhibitor of IRF-1: Nucleophosmin (NPM)

Evaluation of foreseeable effects

With regard to the hazards associated with the host/recipient the following have been considered including the pathogenicity of host strain, virulence infectivity and toxin production. In this case recipients and hosts are non pathogenic.
With regard to the hazards rising directly from the inserted gene these are non oncogenic and are downstream members of signalling pathways. Considering whether an inserted sequence, that does not give rise to harmful phenotype in the recipient micro-organism could give rise to harm as a result of natural gene transfer to another possibly related organism this is extremely unlikely to have a harmful effect.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All retroviral work will take place in a dedicated Class II facility. All liquid waste is sterilised at point of use by sodium hypochlorite. All solid waste is sterilised in adjacent autoclave facility. Use of glass and needles will be avoided to reduce the risk of sharps injury. Autoclave thermocouple tested annually, also daily monitoring of efficacy of autoclave. Discussed determining efficacy of disinfectants with HSE inspector on recent visit. Relying on manufacturer's quality control and the very unstable nature of GMOs involved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project passed at meeting at containment level 2. Minor revision requested in order to clarify justification of project. Risk assessment modified accordingly and signed off.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Project Ref 312/03.1
We intend to perform a systematic investigation of Human cytomegalovirus (HCMV) gene function by the cloning and expression of HCMV genes in cell lines, baculovirus vectors and replication-deficient adenovirus vectors. The vast majority of assays will be performed using cultured cells in vitro. Occasionally, animals will be inoculated with Ad recombinants primarily to generate antibody specific for the expressed transgene.

Recipient or parental organism

Preliminary prokaryotic cloning steps are necessary to generate the recombinant adenoviruses. Genetically disabled E. coli K12 strains specifically developed for application in recombinant DNA cloning experiments will used. These strains have limited potential to colonise the gut or survive in the environment. Continuous cell lines expressing HCMV gene is also envisaged. Prokaryotic cloning experiments, transient and continuous cell lines will be at ACGM category 1 level.

Adenovirus is an ACDP category 2 pathogen. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Deletion of the E1 gene region will render the adenovirus vectors replication-deficient. Vectors will also contain defined deletions in the non-essential E3 gene region. Replication-deficient Ad vectors by their nature have limited potential for lateral spread although this could be facilitated by co-infection with a wt adenovirus or gene rescue from the helper cell line. The use of more disable Ad vectors, specifically gutless vectors, is envisaged when feasible.

Baculovirus vectors are replication competent in insect cells but replication-deficient in mammalian cells. The vectors lack the non-essential polyhedrin gene.

Host/vector system

E. coli strains DH5a, JM109, BJ583 and XL1-blue will be used. Details of vectors for ACGM1 work is given in the risk assessment.

Commercial Ad5 vector systems will be used as the basis for vector development.

For the construction of replication-deficient adenovirus recombinants:

The Admax vector system from Microbix, Canada (pDC511, pDC512, pDC515 and pDC516, pBHGrfDE1,3FLP, pXCXL-based transfer vectors, pJM17) from Qbiogene.

We intend to perform a systematic investigation of Human cytomegalovirus (HCMV) gene function by the cloning and expression of HCMV genes in cell lines, baculovirus vectors and replication-deficient adenovirus vectors. The vast majority of assays will be performed using cultured cells in vitro. Occasionally, animals will be inoculated with Ad recombinants primarily to generate antibody specific for the expressed transgene.
Replication-deficient adenoviruses will be propagated in a helper cell line expressing an E1 helper function (e.g., 293 or 911 cells) and then used to infect target cells in vitro or in vivo.

Baculovirus Vectors: Introgen Insect Select and Novagen BacVector System. SF9 cells for virus growth of baculovirus vectors. HCMV genes may be placed under the control of the baculovirus polyhedrin promoter to provide for high level expression in SF9 cells. Genes may also be inserted into the baculovirus vector under the control of a constitutive promoter namely the HCMV major IE promoter. Baculovirus can infect (inefficiently) but not replicate in mammalian cells. Baculovirus vectors thus can thus be used as a replication-deficient gene delivery system in mammalian cells as an alternative to adenovirus vectors.

Tissue culture cells including primary fibroblasts, U373, THP-1, primary myeloid cells. Animals may be inoculated with Ad recombinants, primarily with the intention of generating polyclonal or monoclonal antibodies for the further characterisation of gene products.

Genes will be cloned from any human cytomegalovirus isolate including the laboratory strains AD169, Towne and Toledo. Sequences will also be cloned from clinical isolates. HCMV is a ubiquitous herpesvirus with the largest genome of any characterised human virus (approx. 235Kb). It is designated as an ACDP 2 agent. The virus is extremely well-adapted to its host with the vast majority of infections passing unnoticed. Between 50-100% of adults are seropositive depending on socio-economic conditions. In healthy individuals primary infection is normally asymptomatic or associated with a mild febrile infection, followed by lifelong persistence (clinically silent) in its host. The virus is thought to establish 'latency' in a subset CD34+ myeloid progenitors with reactivation accompanying cellular differentiation. However, HCMV is an occasional cause of heterophile-negative infectious mononucleosis and a major viral cause of congenital malformation. In severely immunocompromised individuals (notably late stage AIDS patients and bone marrow recipients), HCMV disease is associated with morbidity and mortality. HCMV is not believed to cause cancer nor to encode oncogenes. 'Transforming regions' of the genome have been defined but have extremely low potency (Macasrski and Courcelle, 2001; In Fields Viology, ed; DM Knipe & PM Howley pp2675-2705. Lippencott William & Wilkins, Philadelphia). Although HCMV is known to encodes a number of immunomodulatory genes, there is no compelling evidence that HCMV infections are immunosuppressive.

The complete sequence of the laboratory-adapted HCMV strain AD169 was determined by Chee et al (1990) and was predicted to encode 208 non-overlapping ORFs of greater than 80 amino acids. However, strain AD169 is known to harbour a number of defects, most spectacularly a 15 kb deletion that has since been sequenced in strain Toledo. Mocarski and Courcelle (2001) have recently updated the analysis of the HCMV genetic content and this was used as a basis for the risk assessment (attached). The complete sequencing of the HCMV clinical isolate Merlin is now being undertaken in the University of Glasgow (A. Davison, personal communication). The HCMV isolate Merlin will be used in the majority of our studies to avoid issues of genetic instability associated with laboratory-adapted strains. However, we also envisage a need to clone DNA fragments and genes from other HCMV laboratory strains and primary isolates to analyse sequence variation. The definition of HCMV ORFs by Mocarski and Courcelle (2001) is known not to be definitive. More detailed analysis will identify additional functional ORF and delete spurious ones. HCMV also encodes untranslated transcripts (eg the B2.7 gene). We seek to cover the expression of all HCMV genes including untranslated transcripts. The herpesviruses encode a core set of genes associated with nucleic acid metabolism, DNA replication, capsid assembly and the production of infectious viruses that can be readily predicted from their sequence. A number of functions associated with transcriptional regulation and immune function have been identified, some by homology to cellular proteins. However, clearly a large proportion of the HCMV genome awaits detailed analysis.

There are few antibodies available to HCMV proteins. HCMV ORFs will therefore also be expressed as fusion proteins with GFP/RFP or YFP variants, a 6xHis tag, a streptavidin-binding peptide tag, a tetracycisteine peptide tag or an Fc fusion protein to monitor expression, facilitate purification or identify binding ligands.

Most HCMV genes either have no known biological functions or encode functions that have no predictable detrimental effect when expressed in the proposed vectors. A risk analysis is performed on HCMV gene product with known functions that have the highest potential to cause harm. The virus does not encode any known oncogenes or toxins. HCMV gB is involved in virion attachment to a currently unknown cellular receptor. Recently, microarray experiments and other systems indicated that the binding
of gB to its receptor modulates the transcription of a large number of cellular genes including the induction of an interferon-life response (Simmen et al, PNAS 2001; 98: 7140). Recombinant gB thus has the potential to induce transcriptional effects when expressed. Although normally a membrane protein, secreted forms of gB would have the potential to effect cells in trans. An Ad5 recombinant expressing HCMV gB is capable inducing a neutralising immune response (Marshall et al, J. Infect. Dis, 162, 1177). Ad is not enveloped so expression of gB does not have the potential to effect Ad5 tropism. Ad5 recombinants encoding cell-associated or soluble versions of gB have the capacity to transiently modulate expression in trans following in vitro or in vivo administration. There is a potential hazard with Ad-gB recombinant viruses that should gB released from cells it has the potential to modulate transcription in adjacent cells. This effect may be similar to an interferon response, is liable to be detrimental to the cell but should be transient.

IE2 is the predominant regulatory protein in the virus. It is a promiscuous transactivator that interacts with a wide range of cellular factors (including p53 and Rb), may inhibit apoptosis, promotes cell cycle progression and cell cycle arrest in G1/S. By itself IE2 is not transforming, indeed expression of this protein in mammalian vectors has proved problematical as it appears incompatible with long term cell survival, probably due to the block in cell cycle progression. IE2 has some capacity to rescue an Ad E1a deletion but does not rescue a complete E1 deletion. An ADIE2 recombinant has been shown in microarray analysis to increase the levels of RNA moleculares that promote cell cycle progression (Song and Stinski, PNAS 99, 2836). Infection with an Ad5 1E2 construct is liable to be inconsistent with the long term survival of the cell (as is a wt Ad5 infection) but would not be expected to result in the release of virus nor cell transformation. HCMV encodes a series of additional regulators of cellular gene expression that appear to be significantly less potent than IE2; including IE1 (weak transactivator; disperses PML-bodies), pUL36 (IE) the virion proteins ppUL82 (pp71), ppUL69, pIRS1 and pTRS1. There is no clear potential for hazard associated with the expression of these molecules.

HCMV has become a paradigm for investigating virus modulation of cellular immune systems. A number of immunomodulatory genes have been characterised and it is anticipated that a significant number of uncharacterised HCMV genes will be found to interact with immune systems. The expression of genes capable of modulating immunological responses in Ad5 vector systems constitute a potential hazard.

HCMV UL111a is a functional IL-10 homologue. IL-10 used therapeutically to suppress immunological responses yet is well tolerated and in high doses could predispose to infection. Ad recombinant encoding cellular and EBV vIL-10 have been used in animal models to suppress immunopathologies. The CMV homologue is believed to be of lower potency. As a secreted virokin Ad recombinants have a theoretical potential to induce transient, localised suppression of Th1 immune responses. There is a low level potential hazard with Ad-UL111a recombinant viruses that has recently been approved at ACGM2 level in a separate notification from this laboratory. HCMV encodes a number of putative secreted immunomodulators that may be considered to represent a comparable hazard level to the UL111a gene product and a substantial number of additional secreted glycoproteins is predicted by analysis of ORFs and preliminary expression studies. Preliminary data indicates the hypervariable UL146 gene product (vCXc-1) to be an IL-8-like chemokine that induces chemotaxis of neutrophils. UL147 also exhibits cysteine spacing and weak homology to CXC chemokines. Additional uncharacterised chemokine-like ORFs are expected to be functional.

US2, US3, US6 and US11 encode glycoproteins that act on infected cells to downregulated cell surface expression of MHC-1, and thus they can be expected to promote evasion of CD8+ CTLs. The leader sequence from UL40 acts to upregulate cellular HLA-E expression and thus promote protection against attach by CD94/NKG2A+NK cells. gpUL16 promotes the intracellular retention of ligands for the NK activating ligand NKG2D and this may also impede NK recognition. GpUL18 is expressed on the cell surface where it binds the inhibitory ligand Lir-1 that is present of NK subsets, T cell subsets anad myeloid cells. The functional role of gpUL18 is controversial, it may inhibit NK and T cell subsets. Four HCMV genes (UL33, UL78, US27, US28) are predicted to encode 7TM receptors. US28 encodes a functional C-C chemokine G-protein-coupled receptor (GCR) which binds both CC and CX3C chemokines and signals in response to some. Experiment by others using an AdUS28 recombinant infecting vascular smooth muscles cells indicate that US28 expression can direct cells movement (Sgtrebnow et al, Cell 1999; 99, 511). HCMV is predicted to encode additional NK inhibitory mechanisms and additional glycoproteins that may be expected modulate the expression of existing surface glycoproteins, to promote intracellular signalling and/or promote interaction with other cells. All these genes would normally only be expected to functions only in the transgene-expressing cell and thus are of lower potential hazard. The hazard with cell surface inhibitory receptors (eg UL18) may be increased if expressed as soluble forms to a hazard level similar to that of cmvIL-10 (UL111a). There is a potential hazard associated with the expression of biologically active molecules in native and modified (soluble or epitope tagged) forms. The hazard is reduced by the vector being replication-deficient. Although, the Ad vector has the potential to provide for relatively efficient in vivo delivery of these agents, in both animal models and in clinical trials it has been difficult (although possible) to deliver transgenes with Ad vectors efficiently enough to induce therapeutic effects with immunomodulatory cytokines such as IL-2 and IL-10. Hazards associated with using replication-deficient Ad recombinants encoding HCMV immunomodulatory genes are similar to those with human cytokines. This hazard should be controlled by providing ACGM-2 containment.
Baculovirus recombinants have the potential for environmental harm due to their capacity to kill insects. Enhanced baculovirus pathogenesis/virulence as a consequence of expressing a novel transgene is therefore to be avoided or controlled. Baculovirus is an enveloped virus and HCMV protein expressed in a baculovirus recombinant have the potential to be incorporated into baculovirus virions. HCMV has an extreme restricted tropism productively infecting only certain human cell types. HCMV infection also requires a complex series of events involving a number of HCMV glycoproteins acting together (gB, gH, gL, Go). While the HCMV receptor has not been characterised, the complex nature of the HCMV infectious process makes it extremely unlikely that the expression of an HCMV gene product would be capable of modifying the tropism of the baculovirus vector. The immune system of insects and humans has diverged. The HCMV immune modulators have evolved to have very precise effects on the human immune system. It is extremely unlikely that an HCMV-encoded immune modulator would be able to mediate a significant function in insects. Soluble chemokines and virokines depend on interactions with a matched receptors. Genes involved in MHC-1 downregulation and NK evasion also depend on highly specific interactions with molecules such as the MHC-1 proteins, TAP or NK ligands that are not likely to have survived evolutionary divergence. Baculoviruses-expressing HCMV genes have an extremely low likelihood of having enhanced virulence in insects. In certain cases HCMV genes may also be inserted into baculovirus under the control of a constitutive promoter for transgene expression to mammalian cells. Specific baculovirus recombinants would then have the potential to express HCMV immune modulators in human cells as with replication-deficient Ad vectors. The potential hazard is lower because Baculoviruses infect human cells relatively inefficiently and following infection there is an absolute barrier to replication. There is extremely low likelihood of enhanced environmental hazard with these constructs, for the same reasons as given above.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Animals are inoculated in a Class 2 safety cabinet. The site of inoculation is disinfected according to agreed protocols before the animals are placed in filter-top cages. It is not anticipated that the virus will remain viable in the animal nor be secreted from the animal. Nevertheless, both the animal and waste are incinerated according to standard procedures in Biomedical Services approved by the Health and Safety Unit.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The committee queried 'whether or not the environmental Risk Assessment was sufficient for the use of baculovirus'. One has now been appended to the Risk Assessment.

The committee was happy that the containment level and classification of work assigned was appropriate. Class 1 Containment Level 1 for prokaryotic organism and cell lines. Containment level 2 for adenovirus and baculovirus.

Project Containment
### Project Information

**Project Ref:** 312/03.2

**Date Ackn'd:** 26/04/2005

**CU2 Project Title:** Identifying the functions of Epstein-Barr virus (EBV) genes using plasmid vectors and recombinant virus vectors (baculovirus, vaccinia virus, and replication-deficient adenovirus vectors)

**Class:** Class 2

**Culture Volume:** 1-50 Litres

**Non-GMM Consent Granted:** Not Applicable

**Project notified under transitional arrangements:** N

**Historical Significant Changes:** Project transferred from GM312 on 26/04/2005

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

We intend to perform a systematic investigation of human Epstein-Barr virus (EBV) gene functions by the cloning and expression of EBV genes in cell lines using baculovirus, vaccinia virus and replication-deficient adenovirus vectors. Previous work by our group has focussed mainly on the limited number of 'latent' viral genes expressed in B lymphoblastoid cell lines growth-transformed by EBV. However, the virus contains about 80-90 genes in total. Most of these are expressed only in cells entering the lytic virus productive cycle. The functions of some lytic cycle genes are quite well characterised (eg the BZLF1 immediate-early gene, encoding a transcriptional activator which initiates a cascade of viral gene expression) while the products and functions of many other genes are uncharacterised. The broad aims of this project are to investigate the mechanisms regulating induction and progress through EBV lytic cycle, and to screen for novel functions of EBV genes, particularly those which modulate immune responses. Experiments involving the use of replication-deficient recombinant adenoviruses may also include the use of a plasmid expression vector for the adenovirus receptor (CAR) to render specific cell lines susceptible to infection. Other components of the work designed to elucidate the mechanisms of EBV immunomodulating genes may also involve expression of components of the antigen processing pathway (eg HLA and TAPs). The vast majority of experiments will be performed on cell lines, but animals may occasionally be inoculated with Ad-recombinants primarily to generate antibodies specific for the transgene.
Preliminary prokaryotic cloning steps are necessary to generate the recombinant viruses. Genetically disabled E.coli K12 strains specifically developed for application in recombinant DNA cloning will be used. These strains have limited potential to colonise the gut or survive in the environment. Prokaryotic cloning experiments will be at ACGM category 1 level.

Adenovirus is an ACDP category 2 pathogen. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Deletion of the E1 gene region renders the adenovirus vectors replication-deficient, which limited the potential for lateral spread. Theoretically, lateral spread could be facilitated by co-infection and a wt adenovirus or by gene-rescue from the helper line.

Vaccinia virus is an ACDP category 2 pathogen. Recombinant viruses may retain the pathogenic potential of wild-type vaccinia. The WR strain is a replication-competent lytic virus which has the potential to cause lesions, whereas the MVA strain is non-lytic and has to be generated in chicken embryo fibroblast (CEF) or BHK-21 cells. However, neither WR nor MVA recombinants are compatible with cell survival. Insertion of foreign genes into the thymidine kinase (TK) locus results in a TK phenotype which is attenuated relative to wild-type virus. The MVA strain is already highly attenuated.

Baculovirus vectors are replication competent in some insect cells but are replication-competent in mammalian cells. The vectors lack the non-essential polyhedrin gene.

**Host/vector system**

- **Prokaryotic hosts:** E. coli strains DH5a, JM109, BJ583 and XL-blue will be used.
- **Adenovirus recombinants:** Commercial Ad5 vector systems will be used. The Admax vector system from Microbix, Canada; the AdEasy vector system from Qbiogene, Hareford, Middx. Additionally, the gutted Ad vector as described by Schneider et al (J. Virol. 2002, 76:1600). Replication-deficient adenoviruses will be propagated in a helper cell line expressing an E1 helper function (eg 293 or 911 cells) and then used to infect target cells in vitro or in vivo.
- **Vaccinia virus recombinants:** These will be made in the Western Reserve (WR) or in the Modified Virus Anraka (MVA) vaccinia strains. WR virus will be repared in TK 143 cells, while MVA will be prepared in CEF or BHK-21 cells.
- **Baculovirus recombinants:** Commercial Insect Select (Invitrogen) and BacVector (Novagen) systems. Sf9 insect cells for growth of baculovirus vectors.
- **Target cells:** Tissue culture cells, including human tumour lymphoid (eg Burkitt’s lymphoma B cell lines, and Jurkat T cell line) and epithelial cell lines (eg Hele, 293). Primary lymphoid and fibroblast cells.
- **Animals may be inoculated with Ad recombinants, primarily to generate polyclonal or monoclonal antibodies.**

**Origin & function**

Genes will be cloned from Epstein-Barr virus isolates, including B95.8 and Raji which have been used to generate a complete ‘wild-type’ sequence (EBV-wt; accession number AJ507799) of 171,823 basepairs with approximately 80-90 known or postulated genes. Epstein-Barr virus is a category 2 ACDP pathogen. It is a lymphotropic/epitheliotropic gamma-herpesvirus that is carried as a life-long persistent infection by more than 90% of adults. Despite the asymptomatic nature of EBV infection, the virus has the potential to transform one of its principal target cells, B lymphocytes, and experimental infection of B cells in vitro leads to the establishment of lymphoblastoid cell line (LCL). These LCL carry EBV predominantly as a ‘latent’ (ie non-productive) infection. In healthy EBV-positive individuals, the persistent virus infection is effectively controlled by immune mechanisms, particularly cytotoxic T cells (CTLs). Since EBV is a virus with transforming potential, one potential hazard of the vectors to be generated is the possibility that the expressed genes may be oncogenic. The role of EBV genes in cellular transformation is well studied. At least five of the ‘latent’ genes are known to be absolutely essential for the ability of EBV to transform human B cells. The main function of the EBNA1 protein is maintenance of the EBV episome during cell replication. EBNA2, EBNA3A and 3C are nuclear proteins that activate and/or repress transcription of EBV and cellular genes. LMP1 is a membrane protein that mimics a constitutively active receptor of the CD40/TNFR superfamily. Both LMP1 and EBNA3C have been defined as having oncogenic or immortalizing
properties in rodent fibroblast assays. However, all attempts to demonstrate conversion of non-malignant human cells to a full malignant phenotype with any single EBV gene have been unsuccessful. While some of this work will necessitate using EBV 'latent' genes in recombinant adenovirus or vaccinia virus expression vectors, the main objective of this project is to identify functions of the lytic cycle genes, most of which are poorly characterised, if at all. The original impetus for this work derived from a wish to identify those genes responsible for down-regulating expression of components of the antigen processing pathway in cells harbouring EBV as a productive, lytic infection. Therefore, it can be deduced that we expect some EBV genes to have immunomodulatory functions. Indeed, one well-characterised gene (BCLF1) is known to encode an IL10 homologue. However, there is no compelling evidence to suggest that EBV infection is immunosuppressive; on the contrary, it elicits potent immune-responses.

In addition to vectors expressing wild type EBV genes and mutated genes, other constructs will be required for this project. The use of adenovirus vectors on B lymphocytes, a natural host for EBV, is hampered by the fact that these cells often express only low levels of adenovirus receptor (CAR). Therefore, a human or porcine CAR-expression plasmid will be used to make stable transfectants of established cell lines to generate lymphocytes that can be efficiently infected with replication-deficient adenovirus vectors. The potential risk of the CAR expression plasmids in this context is minimal.

Some experiments, designed to establish the mechanisms by which EBV genes modulate antigen presentation, will also require the use of expression plasmids or recombinant viruses for components of the immune recognition process (eg human MHC, TAP, NK receptors).

Other experiments will use a number of reporter plasmids with specific transcription factor binding motifs to characterise signalling functions or to indicate activation of lytic cycle. Furthermore, reagents generated from related notified projects ("modulation of lymphocyte function with adenoviral vectors" - Dr Brennan; and "Analysis of Epstein-Barr virus latent gene functions" - Prof Rowe) will be used to assist elements of this project. For generation of new expression plasmids, we will use well characterised constructs, some of which are designed to incorporate 'tags' to allow detection and/or isolation of the expressed proteins. These tag sequences are unlikely to pose any significant additional hazard.

Evaluation of foreseeable effects

Most EBV genes have no known biological functions or functions that have no predictable detrimental effect when expressed in the proposed vectors. The 'latent' genes associated with the growth-transforming functions of EBV may be considered to constitute the greatest potential hazard when expressed in the proposed vectors. Both LMP1 and EBNA3C have been defined as having oncogenic or immortalising properties in rodent fibroblast assays. However, it is clear that EBV requires the co-operative action of at least 5 genes (EBNA1, EBNA2, EBNA3A, EBNA3C, and LMP1) to immortalize human B cells. Therefore, the hazard posed by accidental expression of these genes is substantially reduced. Furthermore, the adenovirus and vaccinia virus vectors should give only transient expression whereas the potentially pathogenic effects of the latent genes require persistent expression. Finally, all EBV latent genes (with the possible exception of EBNA1) are effectively targeted by cytotoxic T cell immune response. Thus, the potential hazard with regards possible transformation of human cells infected with Ad virus recombinants expressing EBV latent genes is acceptably low. This view is supported by the fact that similar work has already been approved at Birmingham (Rickinson Group) and Liverpool (Blaake group) and is being performed under category 2 containment.

The effects of most EBV lytic cycle genes on cellular gene expression and phenotype is unknown - which is the very reason for undertaking this project. However, none of the lytic cycle associated EBV genes studies have been reported to show oncogenic properties. Indeed two of the immediate-early genes (BZLF1 and BRLF1) have been expressed by others in Ad virus vectors and have been shown to selectively inhibit growth of EBV-positive tumours (J. Virol., 2002. 76:10951). Unlike many other members of the herpesvirus family, EBV encodes relatively few genes that are obvious homologues of immunomodulatory molecules. The notable exception is BCRF1 which is highly homologous to IL-10 and shares functional properties with the human cytokine. IL-10 is used therapeutically to suppress immunological responses, and is well tolerated in high doses although it may predispose to infection. Ad recombinants encoding cellular and EBV vIL-10 have been used in animal models to suppress immunopathologies. As a secreted virokine, vIL-10 Ad recombinants have a theoretical potential to induce transient and localised suppression of Th1 immune responses. Therefore, there is a low level potential hazard with Ad-BCRF1 recombinant viruses.

Adenoviruses are ACDP level 2 agents that are naturally infectious to the respiratory tract as aerosols. If accidental inoculation were to occur, the viral DNA would be introduced into a number of cells giving transient expression of the transgene over a period of a few days to weeks. However, all Ad vectors used will be replication-deficient due to deletion of the E1 and E3 gene regions; this is a recognised safety feature. Therefore, following accidental inoculation, further spread would not occur. With time, the genome will be lost from the infected cells, this process being more rapid in proliferating cells. Chromosomal integration of Ad virus sequences is a rare event (in the region of one integration in 10(4) exponentially growing cells). It is generally agreed that most cells of the respiratory tract epithelium are post-mitotic and...
on the differentiation pathway. Therefore, it is our opinion that a considerable intake of virus, much greater than $10^5$ pfu, would be required to produce a single integration event. Working practices are designed to minimise the risk of any human contamination, and the likelihood of accidental infection followed by viral integration is almost negligible. Repair of the E1 deletion of the Ad vectors could potentially occur in vitro due to homologous recombination with the E1 gene in the helper cell line, or in vivo following the unlikely event of coinfection of a single cell with recombinant Ad virus and a natural wild-type Ad virus. Such recombination events are likely to result in concomitant deletion of the transgene in the E1 locus. Generation of a replication-competent Ad carrying the whole or part of the transgene is not impossible, but extremely unlikely. It is notable that attempts to detect such complementation by dual infection in the cotton rat model gave negative results. The potential hazard of the recombinant adenoviruses should be adequately controlled by adhering to ACGM-2 containment.

Vaccinia viruses are ACDP category 2 agents which have the ability to infect a wide range of cells in human and non-human hosts. Vaccinia may cause pustular lesions at the site of infection, and can cause severe disease in individuals with active skin disorders such as eczema or psoriasis. Acute conjunctivitis may occur after inoculation into the eye but permanent eye damage is rare. Disseminated vaccinia necrosum can occur in immunosuppressed or immunodeficient individuals. Recombinant viruses may retain the pathogenic potential of wild-type vaccinia. The WR strain is a replication-competent lytic virus which has the potential to cause lesions, whereas the MVA strain is non-lytic and has to be generated in chicken embryo fibroblast (CEF or BHK-21 cells. However, neither WR nor MVA recombinants will be compatible with cell survival. Therefore only transient expression of the inserted genes will be obtained, and the additional risk posed by the inserted genes will be low. It is not clear whether EBV glycoproteins would be expressed in the vaccinia virus envelope, but it is not expected that this would alter the tropism of the virus. The potential hazards of the recombinant vaccinia viruses should normally be adequately controlled by adhering to ACGM-2 containment. However, individuals with skin disorders are at greater than normal risk for working with vaccinia viruses and should seek additional advice.

Genetically modified baculoviruses retain the ability to infect cells of the insect host species but do not efficiently infect human cells. During infection of insect cells, expression of the gene insert will occur under the control of the Polyhedrin or P10 promoters, but these promoters are not expected to be functional in human cells. It is not clear whether EBV glycoproteins would be expressed in the baculovirus envelope, but the complexity of the EBV infection process makes it extremely unlikely that insertion of a single EBV protein would alter the tropism of a recombinant baculovirus. Therefore the nature of the EBV gene insert in recombinant baculovirus itself is not expected to pose any additional risk to human health or to the environment. A case could be made for using the recombinant baculoviruses at ACGM level 1, but taking a more cautious view we propose to adhere to ACGM-2 containment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solution (tissue culture medium or buffers such as PBS) will be inactivated by adding at least an equal volume of 2500 ppm sodium dichloroisocyanurate (Antichlor). After 4 hr, the solution can be discarded to mains drainage and flushed with excess water. Contaminated pipettes are immersed in 2500 ppm sodium dichloroisocyanurate (Antichlor) for 4 hr prior to transferring to a containing for autoclaving. All plasticware is autoclaved after exposure to virus. Actichlor is effective against all viruses and the chosen concentration overcomes the high organic content of tissue culture media.

Animals may be inoculated with recombinant adenoviruses in a Class 2 microbiological safety cabinet. The site of inoculation is disinfected according to agreed protocols before the animals are placed in filter-top cages. It is not anticipated that the virus will remain viable in the animal nor be secreted from the animal. Nevertheless, both the animal and all animal waste is incinerated according to standard procedures in Biomedical Services approved by the Health and Safety Unit.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Did want care taken over RA. RA now suitable and sufficient. Agreed by Chair.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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**Project Ref** 312/95.1

**CU2 Project Title**

26/04/2005

STRUCTURE AND FUNCTION STUDIES OF NORMAL AND MUTANT THYROTROPIC RECEPTORS

**Class**

| Class 2 |

**CultureVolClass2**

| Not Applicable |

**Project notified under transitional arrangements**

Y

| Significant Change ID | Date of Significant Change |

Transferred originally from GM312 to GM302 then transferred from GM302

| Withdrawn | Tick if notifying a connected programme of work |

N

| Historical Significant Changes | Historical Date of Additional Info |

Transferred originally from GM312 to GM302 then transferred from GM302

| Significant Change ID |

| Date of Significant Change |

02/03/2022

Page 3836 of 15326
### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
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Please enter comments on the GM safety committee on the risk assessment

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Project Ref: 312/99.1

Date Ackn’d: 26/04/2005
Date Project Ceased: 

Class: Class 2
CultureVolClass2: 
CultureVolumeClass3-4: 
Non-GMM: Not Applicable
Consent Granted: 

Project notified under transitional arrangements: Y

Withdrawn: N
Tick if notifying a connected programme of work: N

Historical Significant Changes: Project transferred from GM312 on 26/04/2005

Historical Date of Additional Info: 
Significant Change ID: 
Date of Significant Change: 

Project Additional Information

Purposes of the contained use:

Recipient or parental organism:

Host/vector system:

Origin & function:

Evaluation of foreseeable effects:

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants): 

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

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Please enter comments on the GM safety committee on the risk assessment

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 312/99.3

- **Date Ackn'd**: 26/04/2005
- **CU2 Project Title**: MANIPULATION OF TELOMERASE ACTIVITY IN NORMAL AND NEOPLASTIC CELLS
- **Class**: Class 2
- **Consent Granted**: Non-GMM

- **Historical Significant Changes**: Transferred from GM312 on 26/04/2005
- **Project notified under transitional arrangements**: Yes

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

### Historical Significant Changes
Project transferred from GM312 on 26/04/2005

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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<td>FACTORS INFLUENCING THE PATTERNING AND SURVIVAL OF MAMMALIAN EPITHELIAL STEM CELLS</td>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

To identify and study the behaviour of mammalian epithelial stem cells. After the basic epithelial stem cell patterns have been established, work will examine how intrinsic stem cell patterns are altered by the forced expression of certain genes that are putatively associated with control of stem cell growth and differentiation. This project will use combined techniques of stem culture, retroviral transduction, tissue reassembly to determine: a) the size and distribution of stem cell territories in a range of human and murine epithelia of differing complexities, b) the influence of stromal/epithelial interactions in determining stem cell patterns, c) how stem cells are alterer in malignancy, and d) how stem cell behaviour is influenced by the expression of genes associated with cell differentiation and cell death.

**Recipient or parental organism**

The recipient or parental organism for the standard cloning are disabled non-pathogenic E coli strains. The recipient cell lines for expression of the proteins are standard cell culture lines which cannot survive out of culture and which are kept free from any adventitious agents as far as can be determined by our routine assays for contaminating agents which are problematic in tissue culture, e.g. mycoplasma.

**Host/vector system**

- **Host** - DH5a (bacterial cloning)
- **Vector** - pPinco

**Origin & function**

- **Source of Nucleic Acid:**
  - GFP cDNA
  - Alkaline phosphatase cDNA
  - Galactosidase cDNA

- **RAS human cDNA**
- **Bcl-2 related human cDNAs**

---

**Description of DNA to be manipulated:**

Transferred to GM130 on 26/04/2005
Modified jellyfish gene, Mammalian placenta & E.Coli - Lineage markers

Mammalian GTPase & Mammalian homologs of C.Elegans ced genes - Potential modifiers of Stem cell behaviour

**Evaluation of foreseeable effects**

To introduce modified oncogenes and anti-oncogenes into mammalian cells in culture, the cDNA of the gene in question is sub-cloned into a replication defective retroviral vector plasmid. This is transfected into a "packaging" cell line which therefore secretes retroviral virions capable of coding for the inserted sequence. These are able to infect human cells but are not capable of further cycles of replication. There is therefore no risk of subsequent spread of infection. The viruses are extremely fragile and are extremely sensitive to desiccation, the recipient cell lines are unable to survive outside culture conditions.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All cells and media which may contain infectious virus are handled by designated specially trained workers in a class II biological safety cabinet equipped with external ducting. All liquid waste will be sterilised at point of use by sodium hypochlorite, as per College and Trust sterilisaation and disinfection policy. All solid waste will be sterilised in an adjacent autoclaving facility which is regularly tested by Cardiff and Vale Trust engineers.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

Risk assessment suitable and sufficient, agree with assigned containment level and classification.

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02/03/2022
The function and the structure of the extracellular matrix (ECM) is controlled by regulatory molecules as well as by modifying enzymes which can crosslink or cleave matrix constituents. Some of these molecules have functions in the ECM and also control cells residing in the matrix by regulating the availability of cytokines or by direct effects on cells: (1) decorin (see below) can regulate collagen fibril formation as well as TGF-B availability and has direct effects on cell differentiation and survival (Kresse & Schonherr, 2000, J. Cell Physiol. 189:266). (2) Tissue transglutaminase does not only influence cell-matrix interactions but also has GRPase activity and via this activity can contribute to the regulation of intracellular signalling cascades that control cell spreading and motility (Stevens et al., 2004, J. Cell Sci. 117:3389). To investigate the contribution of these distinct functions to biological effects, we plan to use adenoviruses for the expression of wild type or mutant components in different types of cells. Adenoviral gene transfer is necessary (a) to mimic the situation in vivo (because naturally these molecules are induced and not constitutively expressed), (b) the cells used in the investigation (eg endothelial cells, primary fibroblasts, macrophages) are difficult to transfect and easily damaged and © adenoviral infection allows a reproducible induction of a specific molecule.

Small leucine-rich proteoglycans (SLRPs) are multifunctional regulatory molecules of the extracellular matrix. Members of this family are decorin, biglycan, fibromodulin and lumican. In the past, SLRPs were primarily considered as organizers of collagenous networks. More recently their interactions with TGF-B and their direct effects on cells have come into focus. We showed with the help of adenoviral vectors that decorin induces capillary formation and survival of endothelial cells, but it induced programmed cell death in tumour cells (Schonherr et al. 1999, Eur. J. Cell Biol. 78:44; Tralhao et al. 2003 FASEB J.. 17:464). Our studies on endothelial cells further indicated that protein kinase B (Akt) is involved in decorin-mediated signalling leading to cell differentiation and survival (Schonherr et al., 2001, J. Biol. Chem 276, 40687). In collaboration with Dr P. at this institution we plan to use adenoviruses that have already been prepared to investigate signalling cascades in other cell systems. These adenoviruses will allow us an in dept analysis the decorin signalling pathways(s). We will use these viral vectors to transfect endothelial cells and fibroblasts in culture. The already existing adenoviral vectors will allow us to carry out in depth analysis of decorin-induced intracellular signalling pathways(s). We plan to use adenoviral vectors containing different mutated forms of SLRPs to analyse which parts of these molecules have specific functions. Therefore, we will use the cDNAs of the SLRPs which are
available in our laboratory to construct new adenoviral vectors. The resulting replication deficient viruses will subsequently be used in different cell culture models to analyze the function of SLRP mutants.

Transglutaminases are enzymes that have extracellular as well as intracellular functions. One of the best characterised members of this family of multifunctional enzymes is tissue transglutaminase (TGase2). In the extracellular matrix, this enzyme can catalyse the formation of isopeptide bonds leading to protease resistant high molecular weight complexes, while intracellularly, the enzyme acts as a G-protein in signal transduction and mediates PKCa activation via regulation of PLC81. Recent evidence demonstrates that TGase2 controls cell morphology, differentiation and survival by modulating cell-matrix interactions. We have shown that fibroblasts deficient in TGas1 are defective in cell spreading, migration and matrix assembly (Stevens et al., 2004 J.Cell Sci. 117:3389). Recent work by us and others also showed that lack TGases2 have delayed healing of skin wounds and have a deficiency in TGR-B1 production (Szondy et al., 2003. Proc. Natl. Acad. Sci. USA 100:7812). While previous cell culture data also implicated the enzyme in the activation of latent TGF-B the mechanism has not been elucidated. At present it is unclear whether the effects in the wound healing relate to defects in fibroblasts or in cytokine release by macrophages and also which of the activities of the enzyme is are essential for normal wound healing. We plan therefore to generate different adenoviruses which contain transglutaminase and different transglutaminase to determine whether we can rescue the phenotype of cells isolated from mice lacking transglutaminase and to analyse which activity of the enzyme is relevant for which biological function.

Recipient or parental organism

Adenovirus is an ACDP category 2 pathogene. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Deletion of the E1 gene region renders the adenovirus replication deficient. Replication deficient adenoviral vectors by their nature have a limited potential for lateral spread, although this could be facilitated by co-infection with wild-type adenovirus or gene rescue from helper cells.

To generate new replication deficient adenoviruses containing transgenes preliminary prokaryotic cloning steps are necessary. Genetically disabled E. coli K12 strains specifically developed for the applications in recombinant DNA cloning experiments will be used. These strains have limited potential to colonise the gut or survive in the environment.

Host/vector system

E. coli strains JM109, TOP 10, INV a F’ and DH5a will be used.

A commercial Ad5 vector system will be used for the construction of the replication deficient adenovirus (rendered replication deficient through deletion of the complete E1a and E1b gene function and further disabled by partial deletion of E3 gene). The Ad5 based AdEasy vector system from Stratagene. This system comprises pAdEasy-1, pShuttle, pShuttle-CMV, pShuttle-CMV-LacZ (control).

Replication deficient adenoviruses will be propagated in HEK-293 cells (a helper cell line expressing the E1 helper function). The resulting adenoviral vectors will be used to infect target cells in vitro.

Origin & function

All DNA are human and mouse cDNA clones previously generated by us or gifts from collaborators. DNA sequences are known and will be confirmed prior to generation of recombinant virus.

Replication deficient adenoviruses containing wild type human decorin and variant forms of decorin and variant forms of decorin have been previously generated with the AdEasy System in Germany or the USA (Schonherr et al. 1999, Eur. J. Cell Biol. 78:44; Tralhao et al. 2003FASEB J. 17:464). New adenoviral vectors containing additional mutations in the transgene will be prepared using the same technology. In addition, adenoviral bvectors for other members of this protein family, ie human biglycan, fibromodulin, and lumican will be generated. The effect of transgenes cloned into these vectors on endothelial cells and fibroblast will be investigated using a number of established biological assays to study extracellular matrix assembly and angiogenesis. New adenoviral vectors containing mutants of human decorin using the same technology will be prepared.
Replication deficient adenoviruses containing mouse dominant negative and positive Akt were gifts from Boston USA (Fujio Y & Walsh K, 1999, J Biol. Chem. 274:16349). These adenoviruses will be used to investigate the decorin signalling in target cells in culture. In addition, replication deficient adenoviruses approved by Genetic Modification Sub-Committee (GM 312/DWT./PB/3) will be used in collaboration to investigate pathways in these cells in culture.

Replication deficient adenoviruses containing human and mouse transglutaminase or mutants thereof will be generated to determine whether we can rescue in vitro in cell culture assays the phenotype of cells isolated from mice lacking transglutaminase and to analyse which activity of the enzyme is relevant for which biological function.

Evaluation of foreseeable effects

From experiments using adenoviral gene transfer of decorin we already know that the expression of decorin leads to differentiation and survival of normal cells (endothelial cells, primary fibroblasts). In addition, we and others have shown that decorin expression in tumour cells leads to a more differentiated phenotype or programmed cell death. Therefore, the transient expression of decorin in cells in culture does not present a foreseeable hazard. Furthermore, as these experiments should mimic the situation in vivo, no large scale over-expression of the transgene is planned and thus fairly low concentrations of the adenoviruses will be used.

The generation of other adenoviral vectors containing the cDNA of molecules related to decorin is of low risk, because none of the SLRP's has been shown to have a transforming capacity. In contrast eg the lack of lumican or decorin expression in mammary tumours has been shown to be associated with a bad prognosis. In addition, over expression of biglycan in the pancreas carcinoma cells cause G1-arrest.

Potentially the largest risk is the use of the existing adenovirus containing active Akt which can enhance cell survival and proliferation. However, Akt alone is not sufficient to cause a malignant transformation of human primary cells. In addition, active Akt will only be used infrequently and only at a dose that results in activity mimicking the normal levels of activation in response to decorin. The adenovirus transducing the dominant negative form of Akt (which prevents Akt signalling) will be used to much greater extent. The potential hazard presented by these experiments is perceived to be low.

The generation of other adenoviral vectors containing the cDNA of transglutaminase which is a protein cross linking enzyme is also perceived to be very low. Transglutaminases have no transforming effect on cells. These enzymes strongly promote cell adhesion and several studies have shown that the malignant potential of cancer cell lines is reduced upon transfection with vectors directing constitutive expression of transglutaminase.

The host vector systems which will be used in the experiments are commercially available and well characterised. As the used adenoviruses are replication deficient they can only replicate in host cells which contain E1 sequences that have been deleted from the viral vector (these sequences are present eg in HEK-293, which are used for virus propagation). It is highly unlikely that the replication deficient viruses would regain spontaneously the ability for replication. In addition, large numbers of virus particles would be necessary for a sustained infection. Expression using the adenoviral vectors is only transient and is lost in rapidly replicating cells. Therefore, adenoviral vectors are unlikely to cause any permanent change in infected cells.

A potential hazard is the exposure of humans to recombinant virus. All manipulations of the virus will be done in a class 2 biological safety cabinet in the designated laboratory facility. The access will be limited to staff trained ACGM level 2 procedures. Any disposable materials that had contact with the virus will be disinfected chemically as well as by autoclaving prior to disposal. The use of sharps will be avoided to prevent accidental injury related risk. Therefore, any risk of the exposure of humans or the environment is very low.

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2500 ppm. After 4 h, solutions can be discarded. Nevertheless, the solutions will be autoclaved prior to being discarded through the main drain. Pipettes immersed in 2500 ppm sodium dichloroisocyanurate for 4h will be transferred to plastic bags for autoclaving. All plastic ware is autoclaved after virus exposure. Actichlor is effective against all viruses and the high concentration overcomes the high organic contents of media.

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

Risk assessment suitable and sufficient once additional information had been provided with the tables of vector, host and DNA.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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Project Ref 624/01.1

Date Ackn'd 15/09/2005

Date Project Ceased

CU2 Project Title INFECTION OF EUKARYOTIC CELLS WITH VACCINIA RECOMBINANTS EXPRESSING HUMAN PAPILLOMA VIRUS PROTEINS

Class 2

Consent Granted Not Applicable

Non-GMM Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes GM624/01.1 TRANSFERRED TO GM130 FROM GM624 ON 15/09/2005.
### Project Additional Information

#### Purposes of the contained use

Recombinant vaccinia virus will be used to infect target cells in vitro, normally human B-lymphocytes derived from patients with cancer of the cervix. The recombinant (TA-HPV) is derived from the Wyeth strain of vaccinia and expresses human papilloma virus 16 and 18 fusion protein E6/E7. The virus is replication-competent and is not attenuated. Infected B cells will be used as in vitro targets to monitor the success of a vaccination schedule carried out using dendritic cells loaded with E6/E7 in vivo. The purpose of the vaccination is to induce an immune response to cancer cells expressing E6/E7 in vivo. The recombinant will be obtained from the University of Wales College of Medicine and no further genetic modification of the virus will be carried out at Velindre. Patients will not be exposed to vaccinia.

#### Recipient or parental organism

The recombinant (vaccinia TA-HPV) expresses an HPV16 and 18 E6/E7 fusion protein which is produced from the two separate sequences by a modification of the E6 termination codon. It is known that expression of E6 and E7 in mammalian cells is able to induce both immortalisation and a transformed phenotype so that infection of an individual with TA-HPV must be considered to increase the risk of carcinogenicity. However, vaccinia is a lytic virus, so that all infected cells die. In addition, the E7 sequence has been modified to abolish its ability to bind Rb and hence to cause immortalisation. Both these factors are considered to reduce the risk of carcinogenicity. Vaccinia itself is a pathogen and is in ACDP Hazard Group 2. The recombinant is considered to pose an elevated risk compared to wild-type Wyth strain vaccinia.

#### Host/vector system

**Vector:** Wyeth strain vaccinia virus (TA-HPV) expressing human papilloma virus fusion protein E6/E7. The sequence has been modified to reduce Rb binding and hence the risk of immortalisation of the host cell. The virus is replication competent, unattenuated and lytic.

**Host:** Mammalian cells in vitro. These will normally be human B-lymphocytes derived from patients with cancer of the uterine cervix.

#### Origin & function

The vaccinia recombinant TA-HPV has been constructed elsewhere and will be obtained from the University of Wales College of Medicine, Cardiff. Mammalian cells infected in vitro with TA-HPV will express the human papilloma virus 16 and 18 fusion protein E6/E7. These cells will then be used as targets for T-cells derived from vaccinated patients before vaccinia-induced lysis occurs.

#### Evaluation of foreseeable effects

Pathogenicity of vaccinia: Vaccinia is in ACDP Hazard Group 2. The Wyeth strain has been widely used in vaccination programmes and its effects are well characterised.
Infection can lead to a vesicular lesion usually accompanied by a generalised infection. The lesion generally heals in about 10 days. In a recent case involving the accidental infection of a laboratory worker, lesions appeared on the finger and on a recently-pierced eyebrow. However, it is considered unlikely that infection will lead to a serious long-term sequelae. Skin penetration or mucosal contact is required for infection to occur.

Pathogenicity of TA-HPV recombinant: The recombinant expresses the human papilloma virus 16 and 18 fusion protein E6/E7. These proteins are known to have immortalisation and transforming potential; however, the E7 sequence has been modified to reduce binding to Rb which will minimise the probability of immortalisation. Also, the fusion of E6 and E7 reduces the risk of transformation. Nevertheless, the E6/E7 sequence must be considered to be potentially oncogenic so that pathogenicity of the recombinant is greater than that of the wild-type. However, the probability of carcinogenesis in vivo is reduced by cell lysis caused by vaccinia.

Hazard to the environment: Wyeth strain vaccinia has been widely used in wild animal vaccination programmes involving the release of genetically modified virus into the environment. This work has demonstrated that there is a low risk of environmental damage due to vaccinia. It is unlikely that this risk will be significantly greater in TA-HPV than in the wild type.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

NONE

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Small-scale solid and liquid waste will be generated (less than 100gm). All waste will be inactivated by autoclaving, and chemical disinfectants will not be permitted unless they are compatible with subsequent autoclaving.

Autoclaving will be carried out using a sterilizing criterion of 126°C held for 10 minutes. These conditions within the load are verified annually using positioned thermocouples. Autoclaved waste will be sealed in a yellow bag for incineration off-site as clinical waste.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Risk assessment suitable but requested more information on transport of GMO from UWCM to Velindre, and procedure for seeking emergency advice from Occupational Health Service. Additional information now provided.

Project Containment
### Project Additional Information

**Purposes of the contained use**

To examine the ability of cells over expressing the ADH gene to be damaged by excess acetaldehyde production following exposure to ethanol.

**Recipient or parental organism**

The adenovirus employed in these experiments is adenovirus serotype 5 (ad5). This virus is associated with childhood respiratory infections and most adults will have antibodies to the wild type virus. The virus has had the genes for the E1 gene (nucleotides 1-3533) completely removed. This gene is an absolute requirement for replication and so its removal makes it unable to replicate and so incapable of making infectious viral particles.

The removal of the E1 gene has the effect of minimising the pathogenicity of the virus to human contacts. This removal places the virus in the ACGM Hazard group 1 'unlikely to cause human disease'.

The gene of interest is inserted into the site of the disabling mutation. This should minimise the theoretical possibility of recombination with wild type adenovirus.
Cancer cell lines including; HBL 100, Hela, Jurkat, Daudi.

Host/vector system

Adenovirus serotype 5
The virus is made replication deficient due to deletion of E1 locus. (ref He et al; PNAS 95; 2509-2514).

Human alcohol dehydrogenase beta 2 (ADH b2) enzyme cDNA. Obtained from human liver cDNA (ref Ikuta et al PNAS 83; 634-638)

Origin & function

Adenovirus serotype 5
The virus is made replication deficient due to deletion of E1 locus. (ref He et al; PNAS 95; 2509-2514).

Human alcohol dehydrogenase beta 2 (ADH b2) enzyme cDNA. Obtained from human liver cDNA (ref Ikuta et al PNAS 83; 634-638)

Evaluation of foreseeable effects

The risk to human health is low as;
The virus has very low infectivity for human cells outside of the in vitro experimental setting.
The virus is non-replicating.
In infected cells the gene is expressed for 3-4 days only.
The gene encoded is a normal human gene expressed in many cells.
The virus and the cDNA are non-oncogenic, non-toxic and harmless to human health.
The modification to the virus will not expand the host range of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All the cells, containers, pipettes etc used in the project using the adenovirus will be disposed of via the on site autoclave. This is validated annually and has an assumed 100% kill.
Laboratory surfaces/hoods etc will be disinfected with sodium hypochlorite solution.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The committee are happy that this project poses a negligible risk to both human health and the environment. Purely for GM purposes it would be allocated to containment level 1, but as the work will involve primary human cells the project will need to be performed a category 2.
## Project Containment

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<th>Growth Rooms</th>
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### Project Ref 693/00.1

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**Historical Significant Changes**

Transferred from GM693 on 26/04/2005.

**Project Additional Information**

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>Human Clinical Applications</td>
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GLAXOSMITHKLINE PHARMACEUTICALS

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Date at Which Additional Info Submitted: 02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

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02/03/2022

Page 3858 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

All small scale laboratory waste is decontaminated before disposal by autoclaving at 121 degrees C for 20 minutes or by treatment with disinfectants. The effectiveness of the disinfectants on viability having been validated.

All the larger scale processing equipment operates to "Contained Use" within a building that has contained drains directly linked to the site effluent treatment plant. The maximum volume possibly released at any one time is 35,000 litres, due to loss of vessel integrity, but this would be totally contained within the fabric of the building and collected into the site effluent system for treatment. Historically this event has not happened during, at least, the last 25 years and all vessels are regularly maintained and insurance validated.

All SB Worthing site liquid effluent undergoes chemical treatment prior to disposal, to kill process organisms, including GMMs, to comply with current EA and EC regulations. Laboratory experiments determined the operational parameters for the effluent treatment process to ensure that GMM numbers are controlled to the requirements of the regulations.

The site operates controlled waste disposal using a detailed "What Goes Where Guide" for other solids and general waste material.

The effluent treatment plant is a state of the art facility, fully automated and controlled by a central computer with numerous plant interlocks to prevent operation outside of specified parameters. The process is continually monitored to ensure the required treatment conditions are maintained. In addition, the process has been fully validated by monitoring to determine that the organisms have not survived after treatment. Procedures are in place for this validation to be repeated on a regular basis to maintain assurance that there is no release of GMMs to the environment.

All operations are controlled by a site Quality change control procedure with annual reviews of recorded data and modifications, which are regularly assessed and approved by the site GMSC.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 132

<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
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Name

KING'S COLLEGE LONDON

Name 2

UNIVERSITY OF LONDON

Campus Estate or Research Centre

GUY'S CAMPUS

Building

8TH FLOOR GUY'S HOSPITAL TOWER

Road Name

LONDON BRIDGE

District

Town

LONDON

County

GREATER LONDON

Postcode

SE1 9RT

Country

ENGLAND

Tel Number

0207 955 2512

Fax Number

0207 955 4444

E-mail

HSE Division

LONDON

Comments

GM 132 MERGED WITH GM 386 ON 8/9/2003

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
<th>Laboratory</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 133

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<thead>
<tr>
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</table>

**Name**

INSTITUTE OF CHILD HEALTH

**Name 2**

UNIVERSITY COLLEGE LONDON

**Campus Estate or Research Centre**

**Building**

**Road Name**

30 GUILDFORD STREET

**District**

LONDON

**Town**

**County**

GREATER LONDON

**Postcode**

WC1N 1EH

**Country**

ENGLAND

**Tel Number**

0207 829 8892

**Fax Number**

0207 831 4366

**E-mail**

**HSE Division**

LONDON

**Comments**

GM CENTRE CLOSED AND ALL WORK TRANSFERRED TO GM 14

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

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Give brief details of the genetic modification safety committee

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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 133/01.1

Date Ackn'd 19/02/2001

Date Project Ceased 15/12/2006

INVESTIGATION OF THE PAX3-FKHR ONCOGENE

Class 2

Culture Vol

Class 2

< 1 litre

Non-GMM yes - conf

Consent Granted not applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info
**Project Additional Information**

**Purposes of the contained use**
To determine the effect of PAX3-FKHR protein expression in myocytes

**Recipient or parental organism**
GMOs generated from this activity have no foreseeable effects to human health or safety

**Host/vector system**
Hosts: Disabled K-12 derived E.coli SURE
Vector: Full length PAX3-FKHR cDNA has been cloned into the mammalian expression vector PBK-CMV under the control of the MyoD promoter/enhancer sequence. This construct includes a Kozak sequence, the PAX3-FKHR coding sequence, an estrogen receptor and a poly A tail. The host vector system proposed presents negligible risk to human health or the environment. ACGM Compendium of Guidance Part 2A Annex II.

**Origin & function**
PAX3-FKHR is a fusion protein generated from a chromosomal translocation, which occurs in the paediatric tumour Alveolar Rhabdomyosarcoma. Evidence suggests it is responsible for the aggressive malignant phenotype of this disease. Previous in vivo experiments have shown that PAX3-FKHR is a transcriptional activator that significantly enhances transcription and mitotic index in several cell types. This project will investigate the role of the PAX3-FKHR oncogene by expressing the abnormal protein in a model system.

**Evaluation of foreseeable effects**
The host and vector systems used to generate the constructs present minimum risk to human health and safety and could be handled adequately as a class 1 activity under level 1 containment. However, as this project involves the generation of naked oncogenic DNA it will be subject to the appropriate containment and control measures for a class 2 activity and level 2 containment. Although there is no direct evidence that direct contact with naked oncogenic DNA can lead to tumours in humans it is intended to incorporate measures which will limit accidental inoculation or transmission of these DNAs to the laboratory workers. All experiments will be carried out in the ACDP level 2 laboratories with emphasis on the following points in accordance with our local rules and recommendations given in ACGM Compendium of Guidance Part 2A-Annex III and Part 3A-Annex 1
1. Gloves will be worn at all times and changed when working elsewhere.
2. Steps are in place to limit aerosol production and aerosol contamination (filter tips)
3. Limited use of sharps and glassware to avoid accidental inoculation
4. Staff are aware of the hazards of handling oncogenic DNA

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
none

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Waste for GMMs will be handled according to our local rules. Bacterial cultures will be inactivated by chemical treatment with fresh Chloros for a minimum of 24 hours. All plastic disposables and gloves will be autoclaved and incinerated.

The risk assessment for this activity has taken into account the oncogenic potential of the DNA sequence to be inserted in the GMO. The activity has therefore been designated as Class 2. Suitable precautions to limit the risk of accidental inoculation of naked oncogenic DNA are already practiced in the laboratories designated for Level 2 containment. All other working practices described are in accordance with our local rules.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<th>Human Clinical Applications</th>
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Project Ref 133/01.2

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<tr>
<td>27/04/2001</td>
<td>THE INVESTIGATION OF P15 INACTIVATION IN CHILDHOOD ACUTE Lymphoblastic Leukaemia (ALL)</td>
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<thead>
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<td>Class 2</td>
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Non-GMM: Consent Granted

Project notified under transitional arrangements: N

Tick if notifying a connected programme of work: N
### Project Additional Information

#### Purposes of the contained use

1. To ascertain the extent and significance of inactivation of the tumour suppressor gene p15 in childhood leukaemia.
2. To investigate the relationship between p15 and other known molecular abnormalities in childhood leukaemia.

#### Recipient or parental organism

GMMs generated from this activity have no foreseeable effects to human health or safety. The hosts are all disabled or non-colonising in humans and plasmid and cosmid vectors are non-mobilisable.

#### Host/vector system

<table>
<thead>
<tr>
<th>Vectors</th>
<th>Plasmid vectors pBR327, pGEMT, pBS, PUC, Cosmid and Plasmid artificial chromosome vectors pWE15, pCyPAC1</th>
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<tbody>
<tr>
<td>Hosts</td>
<td>Disabled K-12 derived E. coli vectors: Sure, JM109, DH5a.</td>
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</table>

#### Origin & function

p15 is a known tumour suppressor involved in cell cycle regulation and its loss in human tumours is thought to contribute to the malignant phenotype. Overexpression of this gene leads to suppression of cell division and cell death. This activity involves the use of vectors containing several known oncogenes eg c-ABL (J Mol Appl Genet 1983;2(1):57-68) and chimeric gene products that have potential to induce increased cellular proliferation and have been shown to be leukaemogenic in in-vivo systems. Eg MLL/AF9 (EMBO J 1999 Jul 1;18(13):3564-74)

#### Evaluation of foreseeable effects

The host and vector systems used to generate the constructs present minimum risk to human health and safety and could be handled adequately as a class 1 activity under level 1 containment. However, as this project involves the generation of naked oncogenic DNA it will be subject to the appropriate containment and control measures for a class 2 activity and level 2 containment.

Although there is no direct evidence that contact with naked oncogenic DNA can lead to tumours in humans it is intended to incorporate measures which will limit accidental inoculation or transmission of these DNAs to the laboratory workers.

All experiments will be carried out in ADCP level 2 laboratories with emphasis on the following points in accordance with our local rules and recommendations given in ACGM Compendium of Guidance Part 2A - Annex III and Part 3A - Annex 1.

1. Gloves will be worn at all times and changed when working elsewhere.
2. Steps are in place to limit aerosol production and aerosol contamination (filter tips)
3. Limited use of sharps and glassware to avoid accidental inoculation.
4. Staff are aware of the hazards of handling oncogenic DNA.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste from GMMs will be handled according to our local rules. Bacterial cultures will be inactivated by chemical treatment with fresh Chloros for a minimum of 24 hours. All plastic disposables and gloves will be autoclaved and incinerated.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The risk assessment for this activity has taken into account the oncogenic potential of the DNA sequence to be inserted in the GMO. The activity has therefore been designated as Class 2. Suitable precautions to limit the risk of accidental inoculation of named oncogenic DNA are already practiced in the laboratories designated for Level 2 containment. All other working practices described are in accordance with our local rules.

Project Containment

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<tr>
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Project Ref 133/01.3

Date Ackn'd 02/03/2022
### Purposes of the contained use

1. To propagate conditionally immortalised cells from human normal and malformed kidneys and those affected by inherited metabolic diseases.
2. To study the effects of alterations in expression of the lysosomal membrane protein Cystinosin by transfection and antisense oligonucleotides.

### Host/vector system

- Packaging line PA317 producing a temperature sensitive SV40Tag transducing replication-defective virus
- PcDNA3.1 zeo+ expression vector (Invitrogen)
- E coli strain PS disabled but has copies of F'

### Origin & function

The vector used in this experiment is a replication deficient amphotrophic retrovirus. The vector introduces into the host genome in-frame coding sequences for neomycin resistance and a temperature sensitive Simian Virus 40T antigen (SV40TAg: tsA58 and U19 mutations). Permissive temperature is 33°C and non-permissive temperature 37°C to 39°C. Strong eukaryotic promoters derived from SV40 and retroviral LTR drive expression of both genes.

Cytinosin is an integral lysosomal membrane protein (Town et al Nature Genetics 1998:18:319-324) which is mutated in patients with nephropathic cystinosis.

### Evaluation of foreseeable effects

**SV40TAg**

The neo resistance gene is not harmful to humans but the SV40TAg must be regarded as having oncogenic potential, however this is likely to be considerably reduced in-vivo as the transduced gene has been engineered to produce a protein that is functionally inactivated at mammalian body temperature (37°C).

Transduction experiments are not to be performed at ICH.

Transduced cells will be tested for production of wild type virus before introduction into the ICH laboratories. The production of RCRs is a highly unlikely event since the vector is engineered to be replication deficient. Moreover, even if these cells were to accidentally enter the body of an operator (eg through an abrasion or be inhaled)
would be most likely be immunologically rejected on account of HLA incompatibility. It should be noted that control cells would not be derived from personnel involved in the project.

Cystinosin.
Overexpression is unlikely to be damaging. Conversely, ablation of expression may induce direct damage to a cell and reduce its life expectancy and changes of replication. Accidental inoculation of naked antisense cDNA may be of potential risk to the operator.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be disposed of according to our local rules.
All cellular and bacterial liquid waste is treated with 10% chloros before disposal.
All contaminated disposable plastic ware (tips, pipettes and tissue culture flasks) are treated with chloros, bagged immediately in autoclave bags and autoclaved and disposed of at the end of the day.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The initial transduction experiments will be carried out elsewhere. Only stable cell lines without recombinant wild type virus will be used at the ICH site.

The retroviral experiments are performed in designated class 2 facilities with restricted access.
All personnel involved in the experiments are aware of the potential risk to human health from both retroviral experiments and the handling of potentially damaging cDNA.
All waste is disposed of according to our local rules, which are appropriate for level 2 containment.

Project Containment

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02/03/2022
### Project Additional Information

**Purposes of the contained use**
To investigate the role of PAX3, the human proto-oncogene c-met and hepatocyte growth factor (HGF) in tumour progression in Rhabdomyosarcoma using Tetracycline inducible antisense constructs.

**Recipient or parental organism**
Human Rhabdomyosarcoma cell lines.

**Host/vector system**
Disabled K-12 derived E. coli SURE
Tetracycline inducible plasmids pUHG 17-1, pUHG 13-3 and pUHG 10-3

**Origin & function**
Antisense constructs to the proto-oncogene c-met and its ligand hepatocyte growth factor (scatter factor) the oncogene PAX3-FKHR will be amplified by PCR and cloned in antisense orientation into the tetracycline inducible vectors. Evidence from mouse models which are homozygous for PAX3 suggest it has an important role in the migration of immature muscle cells (Goulding et al Development 1994, 120(4):957-571). Similar phenotypes are seen in homozygous mice deficient in c-met. It is suggested that PAX3 may drive cellular migration and tumourigenesis via the transcriptional upregulation of the c-met/HGF pathway. The effect of antisense constructs in humans is not
known however possible harmful effects could conceivably occur if the naked DNA were to contaminate laboratory workers and insert into tissues in which the MyoD promoter is active (ie muscle stem cells).

**Evaluation of foreseeable effects**

The host and vector systems used to generate the constructs present minimum risk to human health and safety and could be handled adequately as a class 1 activity under level 1 containment. However, as this project involves the generation of naked oncogenic DNA it will be subject to the appropriate containment and control measures for a class 2 activity and level 2 containment.

Although there is no direct evidence that contact with naked oncogenic DNA can lead to tumours in humans it is intended to incorporate measures which will limit accidental inoculation or transmission of these DNAs to the laboratory workers.

All experiments will be carried out in ADCP level 2 laboratories with emphasis on the following points in accordance with our local rules and recommendations given in ACGM Compendium of Guidance Part 2A - Annex III and Part 3A - Annex 1.

1. Gloves will be worn at all times and changed when working elsewhere.
2. Steps are in place to limit aerosol production and aerosol contamination (filter tips)
3. Limited use of sharps and glassware to avoid accidental inoculation
4. Staff are aware of the hazards of handling oncogenic DNA.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste from GMMs will be handled according to our local rules.
Bacterial cultures will be inactivated by chemical treatment with fresh Chloros for a minimum of 24 hours.
All plastic disposables and gloves will be autoclaved and incinerated.

**Is an emergency plan required according to regulation 20?**

**If yes, tick to confirm that it is attached to this form**

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

The risk assessment for this activity has taken into account the oncogenic potential of the naked DNA sequence to be inserted in the GMMs and GMOs. Activity has therefore been designated as Class 2. Suitable precautions to limit the risk of accidental inoculation of naked oncogenic DNA are already practiced in the laboratories designated for level 2 containment. All other working practices described are in accordance with our local rules.

The risk to the environment or human health from the GMMs is negligible.
**Project Additional Information**

**Purposes of the contained use**

Primary immunodeficiencies (PID) are congenital disorders that affect the function of the immune system. In recent years, a number of genes causing PID have been cloned, which has made gene replacement therapy for these diseases a realistic alternative for bone marrow transplantation. With increased knowledge of haematopoietic stem cell (HSC) biology and improved retroviral transduction protocols, it is now feasible to use onco-retroviral vectors for efficient corrective gene transfer into HSCs of affected individuals. Since transduced self-renewing HSCs are capable of reconstituting a complete immune system, this approach potentially provides a life-long cure of the disease.
HSCs isolated from cord blood, bone marrow or mobilised peripheral blood will be transduced ex vivo in a closed system with clinical grade retroviral supernatant.

Host/vector system

Vector: Moloney Murine Leukemia Virus (MoMuLV) based retroviral vectors, containing retroviral 5’ and 3’ LTRs, packaging signal and part of the gag gene, expressing full-length human cDNAs encoding wild type PID causing genes.

Host: Human CD34+ haematopoietic cells isolated from cord blood, bone marrow or mobilised peripheral blood.

Origin & function

MoMuLV based replication deficient retroviral particles pseudotyped with the envelope of Gibbon Ape Leukemia Virus (GALV), will be used to transduce human CD34+ cells. The GALV envelope has been shown to mediate efficient transduction of HSCs. Replication deficient retroviral particles were generated in packaging cell lines, which provided retroviral gag/pol and env helper functions in trans. The MoMuLV-based retroviral vector was introduced into the packaging cell independently and replication competent retrovirus (RCR) can only be generated after three independent recombination events, which is very unlikely to occur. The production of clinical grade retroviral supernatant, with all the required safety testing, was performed by a licensed commercial organisation.

In the vector, the retroviral genome is replaced by the cDNA encoding the appropriate corrective gene, whereas only 5’ and 3’ viral LTRs, the packaging signal and part of the gag coding sequence (for optimal protein expression) are still present.

Clinical grade retroviral supernatant is provided by a commercial organisation licensed to undertake such a process and has been tested extensively for the presence of RCR. The transduced CD34+ cells will be returned to the patient and the patient will be monitored over time for RCR.

Evaluation of foreseeable effects

The patient will be monitored extensively for the possible production of PCR, though as discussed, this possibility has been minimised by the use of packaging cell lines providing the gag, pol and env functions in trans. Expression of the inserted gene will be monitored by PCR, protein and functional assays. The inserted gene is not judged to be an oncogene or growth factor which could cause potentially harmful effects. The gene is unlikely to act alongside existing characteristics of the cell as to endow the cell with altered pathogenic properties.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The work with CD34+ haematopoietic cells constitutes no higher risk than work with fresh blood or blood-related products. Patients will be screened for presence of pathogens prior to donation of cells.

To reduce the risk, all the work is performed in a Class II safety cabinet and cells will be grown in a closed system.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All processes will take place in a closed system inside a class II tissue culture cabinet.

The class II tissue culture cabinets will be swabbed with 70% IMS before and after use, and any spillages will be dealt with immediately using 1% Virkon followed by 70% IMS. In the event of a larger spill, paper towels will be used prior to disinfection.

All solid waste, such as discarded bags, pipettes, tips, towels, etc will be disposed of in autoclave bags. These will be removed when full and autoclaved prior to incineration.

The use of sharps (eg needles, Pasteur pipettes) will be avoided, however when use is unavoidable they will be safely disposed of using sharps bins.
These processes should give 100% kill.

The genetic modification safety committee was satisfied with the risk assessment and had no further comments.

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee was satisfied with the risk assessment and had no further comments.

Project Containment

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Project Ref 133/01.6

Class CultureVol Class2 CultureVolumeClass3-4

Class 2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Date Ackn'd 17/08/2001

Date Project Ceased 24/04/2012

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED

Historical Date of Additional Info
### Puriposes of the contained use

c-Jun Activation domain Binding protein (JAB1) and Geminal centre kinase Like Kinase (GLK) have been shown to interact with the tumour suppressor gene p15 in the yeast two-hybrid system. These nature and significance of these novel interactions will be tested in the mammalian system using both in-vitro and in-vivo techniques.  

**In-vitro expression:** Full length cDNA and partial constructs of GST-JAP1 and GST-GLK chimeric proteins will be expressed in bacteria and tested for interaction with endogenous p15 protein extracted from established human cell lines.  
Both proteins will also be transcribed and translated in-vitro using the rabbit reticulocyte system and tested for interaction with in-vitro translated p15.  

**In-vivo expression:** Full length cDNA and partial constructs of JAP-1 and GLK and p15 will be cloned into mammalian expression vectors and transiently expressed singly or in combination in established human cell lines.

### Recipient or parental organism

**Bacteria:** JM109, SURE, DH5a  
All bacterial hosts used in this study are K-12 derivatives and non-pathogenic in humans. They are able to survive for short periods in the gut and lung of mammals but are non-colonising. They are able to survive for a similar time in the environment. The plasmid vectors used in this study express antibiotic resistance genes eg ampicillin or neomycin. The vectors are non-mobilisable and are defective in one or more functions required to transfer to other hosts. Although transfer of antibiotic resistance to other bacterial hosts is possible, the likelihood is low.

**Cell Lines:** Hela, MM6, Jurkat, CEM  
All human cell lines are established and characterised. Conditions for growth include maintenance at a constant temperature (37 degrees C) in low CO2. They are unable to survive outside these conditions. Both parental and modified cell lines present negligible risk to human health or the environment.

### Host/vector system

**Vectors**  
pGex-6p-2, pGEMT, pCDNA3.1, pEGFP-N1, pDsRed1-N1, pGADT7  
**Hosts**  
Disabled K-12 derived E.coli vectors: Sure, JM109, DH5a

### Origin & function

**p15** full length and partial c-DNA sequences promotor region and exon 1 antisense cDNA. p15 is a known tumour suppressor involved in cell cycle regulation and its loss in human tumours is thought to contribute to the malignant phenotype. Although functional loss of p15 is associated with many human malignancies, there is no evidence to suggest that loss of p15 expression alone is a transforming event. Overexpression in mammalian cells can lead to cell cycle arrest or retardation of proliferation.  

**JAB-1** full length and partial c-DNA sequences. JAB-1 is expressed in a wide range of normal cells and is involved in several signalling pathways involving gene transcription, (JNK), cell cycle control (p27) and integrin signalling. JAB1 is also known to regulate protein levels of the cell cycle regulator p27 by promoting its degradation. Overexpression may lead to cellular proliferation, apoptosis or differentiation.  

**GLK** full length and partial c-DNA sequences. GLK is involved in cellular stress pathways (JNK). It is also expressed in a wide range of tissues. Overexpression may lead to growth retardation or apoptosis.

### Evaluation of foreseeable effects

The non-mobilisable GMMs used for this study contain full length and partial cDNA sequences designed for maximum expression in mammalian cells and as GST fusion proteins in bacteria. They are propagated in bacterial hosts, which are disabled or non-colonising in humans. Should the bacteria or GMMs be accidentally released into...
the environment their chance of survival is negligible and the potential to cause harm is effectively zero.

HUMAN HEALTH
Inserts into the pGEX vector are designed to be expressed as a fusion protein with glutathione-s-transferase (GST). The expressed proteins are unlikely to change the properties of the parental recipient. Inherent risks and outcomes are the same as the unmodified recipient. i.e. the risk to human health is negligible. Expression of single and multiple proteins from these genes in mammalian cell lines may alter their growth properties. The most likely outcomes are growth arrest and/or cell death. However, overexpression of JAB1 protein may lead to cellular proliferation in some instances. Over expression of either GLK or JAB1 may inhibit the regulatory nature of p15 allowing greater cell proliferation. Expression of antisense p15 is likely to have a similar effect. Accidental insertion of naked cDNA into humans may lead to limited protein expression, however normal immunological response would rapidly eliminate foreign DNA or protein. Modified cell lines present no additional risks or hazards compared with the parental lines.

CONTROL MEASURES
Accidental inoculation of CDNA sequences or GM cell lines into laboratory workers by stick injury or entry via cuts and abrasions may constitute a hazard and therefore use of needles and scalpels will be limited, cuts and abrasions will be covered and gloves will be worn at all times. All cell work is carried out in Class 2 Safety hoods and use of filter tips limits aerosol formation.

All experiments will be carried out in ADCP level 2 laboratories with emphasis on the following points in accordance with our local rules and recommendations given in ACGM Compendium of Guidance Part 2A-Annex III and Part 3A-Annex 1.

1. Gloves will be worn at all times and changed when working elsewhere.
2. Steps are in place to limit aerosol production and aerosol contamination (filter tips)
3. Limited use of sharps and glassware to avoid accidental inoculation.
4. Staff are aware of the hazards of handling potentially damaging cDNA.

GM human cell lines. Accidental inoculation of sequences into laboratory workers by stick injury or entry via cuts and abrasions may constitute a hazard and therefore use of needles and scalpels will be limited, cuts and abrasions will be covered and gloves will be worn at all times. All cell work is carried out in Class 2 Safety hoods and use of filter tips limits aerosol formation.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

a) Waste from bacterial cultures will be inactivated by chemical treatment with fresh Chloros for a minimum of 24 hours. All plastic disposables and gloves will be autoclaved at 121 degrees C for a minimum of 15 mins and incinerated (100% kill).

b) Cellular waste is treated with Virkon and all disposable plastic ware is bagged and autoclaved and incinerated (as above) before disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
No additional comments. Class 2 facilities are available for this activity.

**Project Containment**

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**Project Ref** 133/01.7

- **Date Ackn’d**: 05/11/2001
- **Date Project Ceased**: 24/04/2012
- **CU2 Project Title**: MECHANISMS OF MURINE HAEMATOPOIETIC CELL TRANSFORMATION AND APOPTOSIS
- **Class**: Class 2
- **Culture Volume**: < 1 litre
- **Non-GMM Consent Granted**: not applicable
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**: PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED

**Project Additional Information**

**Purposes of the contained use**

Apoptosis regulatory genes and oncogenes will be introduced into established mouse and human cell lines in order to study the effects of these genes on apoptosis and their transforming capacity. Disabled retroviral vectors will be used to transduce the cells with members of the Bcl-2-gene family and with the oncogenes, MLL-AF4,
Recipient or parental organism

E. coli K-12 multiple auxotroph XL1-Blue bacteria will be used. These bacteria are unlikely to survive in the human gut, lung or elsewhere in environment and are non-pathogenic to humans.
NIH-3T3, BaF/3 and s49.1 mouse cell lines and primary cells. 293T human cell line.
The mouse and human cell lines used are all long-term established cell lines and present no apparent hazard to workers or the environment. Work with primary mouse cells constitutes no higher risk than work with fresh tissue. Animals will be screened for the presence of pathogens prior to the donation of cells. All cells will be grown in a closed system. Despite the possibility that some of the genetic alterations planned may confer growth advantages to the cells, the derivative cell lines would not survive outside the culture conditions (i.e., in the environment) used for their propagation. In the extremely unlikely event, that the cells are accidentally introduced into humans the cells would be very unlikely to survive because host allo- and zeno-immune responses would lead to their total destruction.

Host/vector system

pcDNA3.1 and pBluescript plasmid vectors
The plasmid vectors used in this work will not cause expression of inserted genes in XL1-Blue cells and they are non-mobilisable or mobilisation-defective. It is envisaged that neither the original XL1-Blue cells nor their genetically modified derivatives will be harmful to humans, since the risks of the bacteria infecting and persisting in humans and/or transferring genetic information to other micro-organisms is very low.
pMSCV and pRwevTRE retroviral vectors. Both these vectors are disabled retroviral vectors since they do not contain the gag (structural), pol (reverse transcriptase/integrase) and env (coat glycoproteins) genes necessary for particle formation, infection and replication. Replication-incompetent retroviral particles will be made by transfecting the retroviral vectors into the ecotropic Phoenix-eco (ATCC) packaging cell line. This cell line has the gag, pol and env genes stably integrated in its genome. For both the gag-pol and the env constructs non-moloney promoters were used to minimize recombination potential and different promoters for gag-pol and env were used to minimize their inter-recombination potential. After transfection with disabled retroviral vectors, the Phoenix-eco cells produce ecotropic viral particles containing envelope proteins derived from the Moloney Murine Leukaemia virus, which are only capable of infecting murine cells. The Phoenix-eco cell line has been established as being helper-virus free) Recombination would also be limited since retroviral vectors would only be transiently transfected into packaging cell lines and the cells would be disposed of after virus harvest (48 hours after transfection), thus long-term culture of packaging cell lines containing retroviral vectors would be avoided. In the extremely unlikely event of a replication competent virus being made, this would only be ecotropic and thus would not infect humans.

Origin & function

The DNA sequences that will be used in the project belong to the Bci-2 family of apoptosis regulatory molecules or are known and presumed oncogenes.
Gene sequences: Oncogenes MLL-AF4, MLL-ENL, MLL-AF9, TEL-AML, TEL-JAK2
Apoptosis regulatory molecules Bcl-2, BclXL, BclX, Bid, Bim, Bak, Bax, Bad

Evaluation of foreseeable effects

It is expected that the gene sequences will be active in mammalian cells and affect their growth and differentiation characteristics. Measures to avoid accidental inoculation and transmission of these sequences to the laboratory workers will be adhered to. For these sequences to be dangerous to humans or other organisms in the environment, they would have to integrate into the genome of the organism in question. Although there is a small risk of this once the DNA sequences are introduced into bacterial and mammalian cells, the use of non-mobilisable and mobilisation defective vectors, disabled retroviral vectors and disabled bacteria will minimise this risk.

Although there is no direct evidence that contact with naked oncogenic DNA can lead to tumours in humans it is intended to incorporate measures which will limit accidental inoculation or transmission of these DNAs to the laboratory workers.

All experiments will be carried out in ADCP level 2 laboratories with emphasis on the following points in accordance with our local rules and recommendations given in AcGM Compendium of Guidance Part 2A - Annex III and Part 3A - Annex 1.

1. Gloves will be worn at all times and changed when working elsewhere.
2. Steps are in place to limit aerosol production and aerosol contamination (filter tips)
3. Limited use of sharps and glassware to avoid accidental inoculation
4. Staff are aware of the hazards of handling oncogenic DNA

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- The work with the genically modified primary cells and established cell lines constitutes no higher risk than work with the parental cells.
- Work with these cells is performed in a Class II safety cabinet and cells will be grown in a closed system.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
- All processes will take place in a closed system inside a class II tissue culture cabinet.
- The Class II tissue culture cabinets will be swabbed with 70% IMS before and after use, and any spillages will be dealt with immediately using 1% Virkon followed by 70% IMS. In the event of a larger spill, paper towels will be used prior to disinfection.
- All solid waste, such as discarded bags, pipettes, tips, towels, etc will be disposed of in autoclave bags. These will be removed when full and autoclaved prior to incineration.
- The use of sharps (eg. needles, Pasteur pipettes) will be avoided, however when use is unavoidable they will be safely disposed of using sharps bins.
- These processes should give 100% kill.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
- The risk assessment for this activity has taken into account the oncogenic potential of the naked DNA sequences to be inserted in the GMMs and GMOs. Activity has therefore been designated as Class 2. Suitable precautions to limit the risk of accidental inoculation of naked oncogenic DNA are already practiced in the laboratories designated for Level 2 containment. All other working practices described are in accordance with our local rules.
- Under these conditions, the risk to the environment or human health from the GMMs is negligible.

Project Containment
Investigating interactions of human cells with Neisseria meningitidis.

We are conducting a number of projects looking at inflammatory and immunological responses of human dendritic cell, neutrophils, endothelial cells in vitro to the organism Neisseria meningitidis. We will use genetically modified strains lacking a number of known virulence factors (lipopolysaccharide and capsule) to explore the relevance of their presence or absence on these responses.

Recipient or parental organism

Neisseria meningitidis serogroup B will be the recipient organism for this work. This is a gram negative organism which only infects humans. It is carried by up to 25% of the population, and can cause meningitis and/or meningococcal sepsis, particularly in children between 0-5 years, and young adults between 15-19 years old. Meningococcal disease (where there is both meningitis and sepsis) has a case fatality rate of approximately 10%.

There is currently no vaccine for this serogroup.
The strains are already transfected with linearised DNA containing an antibiotic resistance cassette, which results in the disruption of the gene of interest (lpxA and siaD). These are stable transfectants and do not harbour transmissible plasmids. The kanamycin cassettes released from puc17 and the gene of interest is cloned in proprietary cloning vector pcr2.1. The gene is then excised by restriction digest and the antibiotic resistance cassette inserted. These are subcloned in E.coli, extracted and then linearised by restriction digest.

Origin & function

The bacterial strains to be used were generated by insertional inactivation of enzymes involved in the lipopolysaccharide biosynthesis and capsulation. In general all the mutant strains involve loss of virulence compared to the wild type parent strain. For example, one strain, lpxA (1) lacks any LPS, the major virulence factor in N.meningitidis. Other strains to be used include lpxA gene under control of an isopropyl-BD-thiogalactopyranoside (IPTG)-inducible promoter allowing for controlled biosynthesis of LOS by the bacteria (2). In the presence of IPTG, this strain will have similar virulence potential to the wild strain but in the absence of external source of IPTG, bacteria harbouring sequence cannot produce LPS. Another mutant strain, salD-, lacks the enzyme required for capsule biosynthesis (3). Almost all infection with N.meningitidis are due to capsulated strains therefore this mutant is of no greater virulence than wild type and potentially less.

The mutated strains will not be modified further in any way. These were kindly provided by Dr. Peter van der Ley from the National Vaccine Institute, RIVM, Bilthoven Netherlands. The description of the mutants are available in full in the following references:


They are only intended for in vitro stimulation work and will not be modified in any other way.

Evaluation of foreseeable effects

The genetically modified strains should have no more and probably less virulence potential than their wild type derived strains. For the purpose of the projects, they will be handled in the same fashion as the wild type organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Culture volumes will not exceed 40mls but in the main are only 5mls.
- Bacteria will be inactivated for use by 20 minute exposure to 2% paraformaldehyde, or heat inactivation at 560C for 30 minutes. This is accepted practice in laboratories used to dealing with this organism. Testing for killing is not normally performed if equipment is functional; ie thermocouple thermometer in water bath is accurate and reagents are freshly prepared.
- Tissue culture incubated with live bacteria will be inactivated in either 2% formaldehyde or 4% paraformaldehyde. Liquid waste will be disposed of in 5% Hycolin (phenolic surface disinfectant), contained in pot within Class 1 cabinet. This is disposed of by laboratory staff at the end of each working day.
- Dry waste and agar plates are autoclaved.
- Sharps are incinerated.
- Small spills can be inactivated with squeeze bottle of 5% Hycolin allowed to soak for 30 minutes and mopped up with absorbent paper and autoclaved.
- All these waste management procedures are monitored and quality controlled in the Microbiology laboratory which has CPA accreditation.
All laboratory workers should have the necessary training and supervision on any work involving Genetically Modified Neisseria Meningitides and all safety precautions and measures should be placed in the laboratories carrying out this type of work. Furthermore, where possible, culture volumes should be kept to a minimum and all waste disposals should be treated with the appropriate detergent and disposed into designated clinical waste for incineration.

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 133/10.1

Identification of genes promoting lethality of MYCN-amplified neuroblastoma cells using genome-wide RNAi screening

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 1-50 Litres
Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes
PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED

Date Ackn'd 18/01/2010
Date Project Ceased 24/04/2012
Withdrawn N

Tick if notifying a connected programme of work N
Purposes of the contained use

In this research project we aim to identify genes that promote cell lethality in the most aggressive forms of neuroblastomas using shRNAmir library.

Neuroblastomas is a paediatric tumour that derives from primitive sympathetic neural precursors. Amplification of MYCN oncogene is very frequent in a neuroblastoma and is often associated with advanced disease stages with poor prognosis and resistance to therapy. Interestingly, overexpression of MYCN protein in non MYCN-amplified cells can sensitize cells to drug treatment and induce cell death. This could suggest that survival and growth of MYCN-amplified tumour cells relies on expression of genes triggered by MYCN overexpression. When certain gene becomes essential for cell survival in response to MYCN overexpression, this gene can be called "synthetically lethal" partner of MYCN. The mechanism of synthetic lethality is very attractive for the development of drugs that could selectively kill tumour cells, since targeting the synthetic lethal partner of MYCN will cause growth inhibition of MYCN-amplified aggressive tumour cells leaving normal cells relatively intact.

The shRNAmir library consists of over 70,000 pGIPZ individual lentiviral vectors. Each vector contains a unique hairpin sequence designed to target and reduce the expression of most/all genes within the human neuroblastoma cell lines with the shRNAmir library. We will follow the change in relative abundance of individual shRNA over time by microarray hybridization to detect genes essential for cell proliferation since shRNAs important to cell survival will disappear from the population. We will pick the candidate genes by comparing the lethality profile of MYCN-amplified and non MYCN-amplified neuroblastoma cell lines and will validate the candidates after screening using available human neuroblastoma cells. The group of genes common for MYCN-dependent synthetic lethality in several neuroblastoma cell lines will be selected as the potential clinical targets that can selectively kill MYCN-amplified neuroblastoma cells.

Recipient or parental organism

Recipient/Parental organisms:
- Bacterial E.coli strains - HB101, XL1-blue, Top10F, DH5alpha.

Associated risks and likeliness of occurrence:
The bacterial strains used are attenuated non-colonising strains so are incapable of causing human infection and are therefore harmless to humans. The viral vector to be used will be pseudotyped with the envelope protein VSV-G from Vesticular Stomatis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could enter human cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make a new progeny virus and establish a productive infection, its insertion into cellular DNA could result in a potentially oncogenic mutation. The recipient cells are harmless to humans.

The viral vectors are replication incompetent so therefore can not self-replicate and transfer to another host so are harmless to the environment. The cell that will eventually receive and integrate the DNA cannot survive in the environment as they are only viable under culture conditions and therefore are harmless to the environment. The bacterial strains used are attenuated non-colonising strains that cannot survive outside culture conditions and are therefore harmless to the environment. The viral vectors are replication incompetent so therefore can not self-replicate and transfer to another host so are harmless to the environment. The cell that will eventually receive and integrate the DNA cannot survive in the environment as they are only viable under culture conditions and are therefore harmless to the environment.

Host/vector system

Lentiviral vectors:
Commercial pGIPZ vectors fro Open Biosystems.

Associated Risks and likelihood of occurrence:

The viral vector used is a lentiviral vector that contains the CMV (PolI) promoter to drive expression of the hairpin cassette. The lentiviral vector does not contain the appropriate accessory elements to form a viral particle itself and therefore poses no harm to health and the environment. Packaging and envelope proteins are contained on two additional plasmids to the lentiviral expression vector and these plasmids must be introduced into the cell and expressed alongside the lentiviral vector for viable lentiviral particles to be produced.

Woodchuck hepatitis B virus post-transcriptional regulatory element (WPRE) sequence is also incorporated into the pGIPZ vector in order to enhance the stability and translation of the transcripts. This negates the need for rev in the lentiviral vector packaging systems and improves biosafety by eliminating lentiviral genes from the system. However, the WPRE has potential promoter activity and the potential to express part of the X protein form woodchuck hepatitis virus (WHV) which may be oncogenic.

There is no direct experimental evidence of oncogenic activities of the truncated fragment of X protein. Moreover, there is low probability that it would be expressed from the X promoter that is present in the WPRE since many studies have suggested that the X promoter is not functional in the absence of a second enhancer, which is not present in the WPRE. There is possibility of expression directed form other promoter/enhancer at chromosomal integration sites as well as the possibility that truncated X protein fragment has oncogenic properties. However, the likelihood of this is very low.

Origin & function

The inserted RNAi hairpins are sequences that have been derived de novo and are not from an organism. The sequences contain a 22bp sense and antisense sequence that is homologous to the gene of interest to which the hairpin is designed. These sequences are separated by a small loop sequence and flanked by mir-30 sequences. When transcribed in the cell, the RNA will form a hairpin structure which will initiate a normal cellular in vivo downstream cascade of processing events in response to foreign cellular single-stranded RNA intruder molecules. This process results in the post-transcriptional down regulation of the gene to which the hairpin sequence is designed and targeted by destroying the corresponding mRNA molecules prior to translation. The utilization of this normal endogenous pathway to down-regulate genes of interest is known as RNA interference.

Evaluation of foreseeable effects

Risks and likelihood of risks:

- The bacterial strains to be used are non colonising and incapable of surviving in the environment or causing human infection. They pose no risk to users or the environment.
- None of the DNA viral vector plasmids are harmful to health or to the environment and do not supply a survival advantage to any microorganisms. The packaging viral components are from the HIV-1 virus. HIV-2 is harmful to humans, however, the packaging vector does not contain full length HIV-2 molecule. Many components of HIV-1 that are critical for HIV-2 infection have been removed from the packaging vector. Only those essential for viral packaging remain.
- The most dangerous GMM is the lentiviral preparations of pGIPZ particles and the most dangerous step is the collection, concentration and use of this lentivirus, however this risk is minimal. Lentiviral vectors can infect human cells and therefore if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate its genome into cellular DNA. If the inserted sequence were to be expressed in humans after accidental transfer the hairpin could lead to the down-regulation to the gene to which it targets via RNA interference. This could be a gene essential for growth regulation such as a tumour suppressor gene therefore resulting in a potentially oncogenic mutation. Additionally, physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene, however the likelihood of this is very low. Theoretically the virus can also infect other mammalian cells and knockdown the gene of interest. However this is extremely unlikely as the hairpin is targeted to human genes and many hairpins are not homologous with the genes from other species. The overall risk is very low.
- Woodchuck hepatitis B virus post-transcriptional regulatory element (WPRE) in the pGIPZ vector has potential promoter activity and the potential to express part of the X protein from woodchuck hepatitis virus (WHV) which may be oncogenic. However, there is no direct experimental evidence of oncogenic activities of the truncated fragment of X protein and the likelihood that the expression of this protein would be directed from the promoter/enhancer at chromosomal integration sites in mammalian cells is very low. Any infection would not be severe as the virus is replication incompetent and therefore self-inactivating (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection. Also, it would only be a small number of cells and would not impact on the pathogenic outcome of an exogenous viral infection. Additionally, the viral particles are labile and do not survive on environmental surfaces outside the laboratory making infection unlikely.
- The cell lines used cannot survive outside the laboratory incubators and as such pose no threat to health or environment.
Precautions in place to minimize risks:
The Institute of Child Health is secured by restricted access at all entrances and additionally to all laboratory areas. Additionally, Molecular Haematology and Cancer Biology Unit has level 2 facility. All work involving viruses will be performed in class 2 cabinets in containment level 2 laboratories specified particularly for retroviral and lentiviral GMM work. All DNA plasmids containing viral proteins will be grown separately to avoid opportunity for recombination. To prevent contamination of workers by virus, when handling any GMM or GMM-derived DNA virus, no glass or other sharps will be used in the rooms where the viruses are prepared and used and workers will be protected by a lab coat and nitril gloves. Skin lesions will be covered with a bandage in addition to the protective wear described above. Specific guidelines will be in place to ensure all viral preparations are handled, labelled, stored, transported and cleaned up correctly. A separated clearly labelled compartment in the freezer to store viral supernatant will be used to prevent access to the stored materials for departmental staff and students, who are not involved in the project. All those involved in the project will be made aware of the guidelines and the associated risks as well as anyone else who may use the laboratory. Persons involved on project from other Institutes will work under the supervision of a competent trained worker from this project. No-one visiting the laboratory that is not involved in the work will work on this project. All persons entering the facility will be made aware of the safety precautions upon entering the facility. All equipment used in the level 2 laboratory will be available in other general purpose laboratories for general use so it should not be necessary for others who are not involved in retroviral or lentiviral work to enter the laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Virkon (w/v) will be used to treat liquid waste (18 hours) and also used to decontaminate any spills and for disinfection of any reusable bottles or other equipment and laboratory materials used. Liquid waste is subsequently poured down the sink after treatment with Virkon. Work surfaces will be wiped down with 1% Virkon and 70% IMS after use. Virkon is certified by the PHLS to provide 100% viricidal and GMO kill under these conditions by denaturing and inactivating viral and cellular proteins. Virkon will be used with their lifespan to ensure required kill is achieved. Solids waste will be disposed of into biological waste bags, sealed and autoclaved 132° for 15 mins by trained staff then bagged in yellow biohazard bags and removed by UCL waste services. Autoclave runs are regularly validated by waste management staff.

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

A representative group of the UCL Institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees that this work should be classified as an activity class 2 (AC2) notification.

Project Containment

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02/03/2022
The aim of this project is to generate induced pluripotent stem (iPS) cells from mouse and human fibroblasts by employing lentiviral vectors coding for 4 reprogramming genes Sox2, Oct4, Klf4 and c-Myc. iPS cell technology has the potential to transform regenerative medicine especially in the areas of stem cell transplantation and patient-specific gene therapy. Briefly, mouse and human fibroblasts will be infected with 4 different lentiviral constructs and reprogrammed to become embryonic stem (ES) cell like cells which are known as iPS cells. Once generated, these cells can be expanded at will maintaining their undifferentiated pluripotent state and can be subjected to differentiation pathways eventually leading to the formation of terminally differentiated somatic cells. As for example, these iPS cells can be induced to become different kinds of blood cells. The efficiency of reprogramming is pretty low (0.01-0.05%) and to obtain sufficient number of iPS cell generation. Upon successful execution, the iPS cells generated from this project will help us to study various genetic defects either from murine disease models or from human patient samples and would be crucial in our therapeutic approaches.

E.coli disabled strains (e.g. DH5alpha), Mammalian cell lines (established, commercially available) and mammalian primary cells. Neither of these are pathogenic to humans, nor capable of survival in the environment.
Prokaryotic plasmids, eukaryotic expression vectors, replication-deficient self-inactivating (SIN) lentiviral vectors, The lentiviral vectors used are multi-attenuated meaning it is devoid of all potentially pathogenic HIV-1 encoded functions (1.2). In addition it is replication-defective which means that the vector cannot multiply on its own unless supplied by certain proteins in trans. It is self-inactivating which means that the viral promoter in the U3 region of the 5' LTR has been disabled by genetic manipulation. Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The probability of such an event is extremely low.


Mus musculus, Homo sapiens sapiens
Production of transcription factors that will reprogram the somatic cells to become iPS cells
The vector sequences (sox2, oct4, klf4 and c-myc) code for oncogenic products which if accidentally transferred to a human host might cause tumour formation depending on the size of the inoculums but is most likely expected to be nullified by immuno-competent individual.
Sequences cannot cause harm if transferred to species in the environment; the donor organism is not pathogenic

Evaluation of foreseeable effects
The donor organism does not have apathological or harmful characteristics
Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The probability of such an event is extremely low. The most hazardous GMM are the lentivector viruses and the most hazardous step is the harvesting of the viral supernatant before infecting the target cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All GMMs are inactivated for disposal using standard biosafety level 2 procedures. Briefly, liquid and solid wastes are treated with PRESEPT effervescent disinfecting tablets (2.5g). All plastic ware used in tissue culture are autolaved. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern. Proline and IMS are used which have been shown to be 100% effective in tissue-culture related decontamination.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N

02/03/2022
A representative group of the UCL institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees that this work should be classified as an activity class 2 (AC2) notification.

**Project Containment**

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**Large Scale Activities**

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**Human Clinical Applications**

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**Project Ref** 133/10.3

- **Date Ackn'd**: 04/05/2010
- **CU2 Project Title**: Phase I/II Clinical Trial of Haematopoietic Stem Cell Gene Therapy for Wiskott-Aldrich syndrome
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Consent Granted**: Non-GMM

**Historical Significant Changes**

- **Historical Date of Additional Info**: 02/03/2022
- **Significant Change ID**: PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED

**Project Additional Information**

- **Date Project Ceased**: 24/04/2012
- **Withdrawn**: No
- **Historical Significant Changes**: PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED
- **Project notified under transitional arrangements**: No
Purposes of the contained use

To undertake gene therapy on autologous HSC from patients with Wiskott Aldrich Syndrome. The study is summarised below.

Phase 1/2 clinical trial of haematopoietic stem cell gene therapy for the Wiskott-Aldrich Syndrome
This is an open labelled, non-randomised, single centre, phase I/II, cohort study involving a single infusion of autologous CD34+ cells transduced with the w1.6_hWASP_WPRE (VSVg) lentiviral vector in up to 5 patients with WAS.

Primary Objectives
To safely administer a lentiviral gene therapy vector encoding the human WAS cDNA to stem cells from WAS patients.
To provide sustained engraftment of WASP-expressing transduced cells, reconstitution of humoral and cell mediated immunity, and correction of microthrombocytopenia

Secondary Objectives
To improve the overall health of the patient, including reduction in frequency of infections, resolution of autoimmunity, and improvement in eczema, reduction in bruising and bleeding episodes.
To evaluate the longitudinal clinical effect in terms of augmented immunity.

Recipient or parental organism
The lentiviral vector (LV) vector used in the proposed clinical trial is a 3rd generation replication-defective hybrid viral particle made by core proteins derived from Human Immunodeficiency virus type 1 (HIV-1) and the envelope of the unrelated Vescicular Stomatitis Virus (VSV). The transfer vector encodes the 12 exons of the human WAS cDNA under control of a 1.6 kb endogenous promoter sequence and leads to the integration of a 5.6 kb sequence.

Host/vector system
Lentiviral vector (w1.6_hWASP_WPRE (VSVg). There is no recommended International Nonproprietary Name (INN). The vector is pseudotyped with the VSV.G envelope.

The lentiviral vector is a 3rd generation replication-defective, self inactivating, hybrid viral particle made by core proteins derived from HIV-1 and the envelope glycoprotein of the unrelated Vescicular Stomatitis Virus (VSV). The transfer vector encodes the 12 exons of the human WAS cDNA under control of its native promoter. This lentiviral vector leads to the integration of a 5.9 kb proviral sequence into the cellular genome.
The system is based on 4 non-overlapping expression constructs in order to maximize the segregation of cis and transacting functions. The system is engineered in such a way that minimal homology regions are present between packaging and transfer vectors, thus minimizing the likelihood of homologous recombinational events and the generation of replication competent lentiviruses (RCLs).

In addition, the packaging construct is deleted of all HIV accessory proteins (vpu, vpr, nef, vif) and Tat. The conditional packaging system segregates gag/pol and rev genes in two separate plasmids. The Rev responsive element (RRE) maintained in the gag/pol plasmid makes the gag/pol gene expression rev dependant. As the transcripts of gag and pol genes contain cis acting repressor sequences, they are expressed only in the presence of Rev, expressed in trans on separate plasmid, which promotes their nuclear export and expression by binding to RRE.
The transfer vector codes for the therapeutic human WASP gene and for the sequences necessary for expression, capsidation, reverse transcription and integration of the viral genome. RRE sequences permit the nuclear export and expression of the viral RNA. In order to minimize the risk of RCL generation, the 3' LTR of the transfer vector has been deleted in the U3 region. The introduction of this 400 bp deletion abolishes the production of full-length vector RNA in transduced cells by exploiting the reverse transcriptase mechanism which generates both U3 regions from the 3' of the viral genome, thus transferring the deletion to the 5' LTR of the proviral DNA. The lentiviral particle is therefore conceived with a self-inactivating mechanism that enables the production of infective particle only in the presence of accessory plasmids.

Origin & function
The human WAS protein (WASp) is expressed exclusively in haematopoietic cells where it functions as a regulator of actin cytoskeleton reorganisation by linking various
types of signals to Arp2/3-mediated actin polymerization. Two main molecular activators of WASP are Cdc42 and PIP2. In T cells, WASP is an essential component of the signal transduction cascade initiated by T cell receptor engagement contributing to the establishment of immunological synapse and T cell activation.

Risk of transfer to environmental species is negligible, and would not be expected to cause harm

Evaluation of foreseeable effects

The potential for harm is negligible. Work will be at level 2 containment.

All personnel involved in the ex vivo transduction of HSC at ICH/GOSH are trained in GMP cell processing procedures and there competency is assessed every 6 months. Sharps will not be used for the direct manipulation of vector.

A policy is in place for accidental needlestick injuries and can then be assessed via our hospital website (section 2.2.3 http: www.ich.ucl.ac.uk/clinical_information/clinical_guidelines/cpg_guideline 00121. A copy of the document is appended

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Autoclave facilities are in an adjoining building. However, all waste will be double bagged and transported by the lab worker in accordance with a written operating procedure to the autoclave facility and this will be recorded in the batch manufacturing record. Sharps will be disposed of in appropriate puncture-proof containers

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For routine cleaning all surfaces are wiped down with 70% ethanol after use. Liquid GMM waste is first disinfected using Sanichlor (effervescent chlorine tablets) to give a final concentration of 2500ppm chlorine overnight before being disposed of down the sink. This is a standard procedure and viability is below detectable levels. Any solid GMM waste is placed in autoclave bags, labelled with the department's name and autoclaved at the ICH. All sharps are placed in puncture-proof containers and disposed of through the central collection service for contaminated waste. All spillages are cleaned using 1% sodium hypochlorite or 70% ethanol.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment
Kidney and urinary tract malformations are the commonest cause of end-stage renal failure in children, whilst progressive destruction of normal kidney tissues by cysts or interstitial fibrosis account for most cases in older patients. Very few of these conditions are monogenic but we have an increasing understanding of the processes involved in maldevelopment, cyst formation and fibrosis, which highlight potential targets for therapies. An example is the multifunctional lectin, galectin-3; this molecule is expressed in normal collecting ducts in the developing kidney and exogenous therapy reduces growth of collecting duct-derived cysts in vitro.

Lentiviruses are excellent tools for gene delivery, due to their relatively large packaging capacity and ability to infect a wide range of cell types when coated with different envelopes. Gene therapy requires a functional version of a gene to be delivered and then expressed over long periods. The aim of this project is to develop lentiviral vectors to modulate galectin-3 in the kidney.
Mammalian cell lines (established, commercially available such as human embryonic kidney 293 cells, renal proximal tubular epithelial cells, inner medullary collecting duct cells and Madin-Darby kidney cells) Mammalian primary cells (primarily kidney-derived, such as glomerular, proximal and collecting duct cells, and human fetal renal progenitor cells)

Whole kidneys in organ culture

Mice strains

The bacteria and recipient cells used in this project are not hazardous to human health because they are incapable of causing human infection. Nevertheless, we will still maintain the highest standards to prevent accidental spillage or splashing of workers, and ensure that the equipment is cleaned and disposed of correctly.

The infected mice are no greater than normal animals because the virus is not shed; hence standard animal handling precautions will be used. These currently include a gown, protective clothing, gloves and masks (to reduce mouse allergen exposure of the workers). Injection involves use of a needle which would be hazardous in a mobile, mature mouse. However, we use newborn mice which are easily immobilised by hypothermia, and the injection procedure is then carefully controlled by two workers - one places the needle into the vein and holds it in position whilst the other does the injection. These procedures will be done in quiet rooms with limited access, that meet containment level 2 standards, using disposable materials that will be removed at the end of the procedure. The area will then be cleaned and signed off as ready for future work by our personnel.

Host/vector system

Recombinant (inactivated non-replicating) lentiviral vectors. Replication deficient lentivirus particles are generated in cell lines following co-transfection of three different plasmids. These supply the self-inactivating transfer vector containing galectin-3 and viral long terminal repeat (LTR) (but lacking any expression of HIV genes; packaging and structural proteins, gag/pol; and envelope (env), vesicular stomatitis virus g (VSV-g). The vector itself is self-inactivating due to a deletion in the promoter enhancer region of the 3LTR. The only expression from the vectors is of the transgene cDNA, controlled by an internal promoter such as SFFV. Galectin-3 mRNA has been cloned into a VSV-G pseudotyped HIV-1-based vector be GeneArt, a commercial biotechnology company. This strategy is similar to the production of Mertk as described in Tschemutter M et al. Gene Ther. 2005 12:694-701.

There is a finite (albeit very small) risk that lentiviral vectors could infect human cells, but we will be using multi-attenuated lentiviruses that are replication incompetent because they lack the gag, pol or env genes. Hence, they are unable to spread even if workers are accidentally exposed by injection, ingestion or through a wound; risk of entry through these routes will be limited by wearing appropriate labwear and gloves, and completely covering open wounds. Although replication incompetent, lentivirus could theoretically insert into cellular DNA and this might result in a potentially oncogenic mutation. The infection with high titre lentiviral vectors encoding growth promoting molecules and selection in vivo for cells with altered growth potential. No adverse affects have ever been described through accidental exposure to retroviral vectors. The major risks in this project are when the lentivirus is being prepared and then injected but this will be done into immobilised mice by experienced staff, in quiet CL2 standard rooms, wearing a laboratory coat and latex or nitrile gloves.

Origin & function

Gene Sequences: cDNAs encoding human and wils-type and modified galectin-3

Origins and functions of the genetic material involved: Galectin-3 is a lectin that modulates cell adhesion, differentiation and proliferation. It is normally expressed during mammalian development in the kidney, and reactivated in diverse diseases. We predict that increased galectin-3 will reduce the severity of cyst formation and fibrosis in vivo.

Will the sequences cause harm if expressed in humans? Galectin-3 is involved in multiple cellular processes including cancer but the lectin does not have any direct oncological effects by itself, indeed treatment with recombinatn galectin-3 protein has been shown to reduce cancer metastases in mice models and the severity of several other renal diseases. Therefore, any accidental increased expression of galectin-3 is unlikely to have any detrimental effects on humans.

Will the sequences cause harm if transferred to the environment? Galectin-3 is a naturally occurring lectin and therefore highly unlikely to cause environmental harm. The potential routes of transmission or escape to the environment of the virus that may occur are known (air or liquids) and full protective measures will be taken to minimise or prevent the access of the virus to other organisms and the environment.

Evaluation of foreseeable effects

The biggest potential harm would be accidental transfer and overexpression of galectin-3 in humans by needlestick injury when the virus is being injected. These risks will...
be reduced by carefully controlling the mice, procedure and environment for the injection. We use new born mice which are easily immobilised by hypothermia, and the injection procedure is then done by two workers - one places the needle into the vein and holds it in position whilst the other does the injection. Dr ****, will teach us how to do this procedure and he has already done this over a thousand times without mishap. Even with accidental human injection, the risks are minimal because we are using multi-attenuated lentiviruses which are unable to spread in humans even if they do infect cells.

In addition, we have in place in association with UCL Biological Services various specific control measures for the lentivirus experiments to minimise exposure and have completed a separate risk assessment for all personnel involved in the animal work. The general work will be performed in a containment level 1 environment but preparation work with virus handling will be in level 2 safety cabinets and injection will be in a quiet room that meets CL2 requirements; personnel involved with the virus work will wear gloves, glasses and a mask.

In the case of a laboratory accident, such as defective/leaking plasticware or dropped samples leading to viral spillage, all the necessary procedures will be applied to eliminate any potential hazardous effects of the virus in the environment. These include: wash with virkon, detergents, and disinfectants. Both wild-type and optimised lentiviruses are sensitive to UV, temperature and other physical agents so their chances of survival are low. All of the liquid waste will be treated with virkon and autoclaved; solid waste will be autoclaved and disposed as biohazard materials.

In the case of a needlestick injury, we will contact occupational health immediately or attend an Accident and Emergency Department immediately to assess injury and treatment required for the penetrating wound. Risks from viral inoculation are minimal as described above.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. 5% Virkon or equivalent PHLS certified treatment will be used to treat liquid waste (18 hours) and also any spills and for disinfection of equipment and laboratory materials. Work surfaces will be wiped down with this and 70% ethanol after use. Sharps will be placed in sharps bins that will be autoclaved.

2. 199%

3. Virkon is certified by the PHLS to provide 100% viridical kill under these conditions. Additionally, we will ensure that only in-date products are used to maintain optimal efficiency. Autoclave runs are regularly validated by waste management staff.

4. Solid waste will be double-baged in biological waste bags and sharps in sharps bin. Both are autoclaved at 132° for 15 minutes, then collected for disposal (usually incineration) by the contractors employed by ICH to handle potentially infectious clinical waste.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment
A representative group of the UCL Institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees that this work should be classified as an activity class 2 (AC2) notification.

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**Animal Units**

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**Project Ref** 133/11.1

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**Date Project Ceased** 24/04/2012

Tick if notifying a connected programme of work N

**Historical Significant Changes**

PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED

**Project Additional Information**

**Purposes of the contained use**

The shRNAmir laboratory is available from Open Biosystems through UCL, and consists of over 70,000 individual E. coli glycerol stocks containing the pGIPZ lentiviral vector. Each stock contains a unique hairpin sequence within the pGIPZ vector designed to target and reduce expression of most/all genes within the human genome via RNAi interference. We will also use other selected shRNA sequences that we will clone into either pGIPZ or another lentiviral vector, pLL3.7, ourselves in case hairpins from the Open Biosystems stocks are ineffective, or if knockdown in non-human cell lines is required.
This project will involve maintenance, storage and use of this shRNAmir library for use in various projects. Specifically, this project will involve lentiviral production from either pGIPZ or pLL3.7 plasmids and the subsequent use of this lentivirus in cell based screening functional assays. Additionally, clones will be pooled at the bacterial, DNA and viral level to make various intermediate pools of clones to be used to transduce cells in screens and subsequently undertake functional assays.

### Recipient or parental organism

**Cell lines:**
- ATDC5, canine-MDCK, DK, D17, C2F; feline-AH927, CRFK, Mya1; Human-HeLa, TE671, HT1080, 293T, HEL, HFFF, BJ1, Jurkat, CEM, supT1, C8166, A549, LECs, tMSCs; Mouse-MDTF, NIH3T3, Avian- QT36; Marsupial- SC300; Bat Tb1u; non-human primate- FRhK4, LLCMK2, CV1, VERO, Pindak, SMLF; bovine- MDBK, IMR31; Porcine- PK15, SKL, CPK, STIOWA; Mink- mv-1-lu; Rabbit- SIRC, eREP, ratHSN, RAT2, 9L, NRK, TenRPE, human and mouse ES cell lines.
- Mouse-MDTF, NIH3T3; Avian- QT36; Marsupial- SC300; Bat Tb1u; non-human primate- FRhK4, LLCMK2, CV1, VERO, Pindak, SMLF; bovine- MDBK, IMR31; Porcine- PK15, SKL, CPK, STIOWA; Mink- mv-1-lu; Rabbit- SIRC, eREP, ratHSN, RAT2, 9L, NRK, TenRPE, human and mouse ES cell lines.
- Primary Human cells: Derived from various human tissues including peripheral blood derived lymphocytes and macrophages.

The bacterial strains used are attenuated non-colonising strains so are incapable of causing human infection and are therefore harmless to humans. The viral vector to be used will be pseudotyped with the envelope protein VSV-G from Vesticular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could enter human cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection, its insertion into cellular DNA could result in a potentially oncogenic mutation. The recipient cells are harmless to humans.

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### Host/vector system

**Lentiviral vectors:** Commercial TZV vector from Tranzyme and derivatives (including pGIPZ and pLL3.7). Lentiviral Gag/pol expression vectors p8.9 and derivatives (HIV-1).
- The viral vector used is a lentiviral vector that contains the CMV (Pol II) promoter to drive expression of the hairpin cassette. The lentiviral vector does not contain the appropriate accessory elements to form a viral particle itself and therefore poses no harm to health and the environment. Packaging and envelope proteins are contained on two additional plasmids to the lentiviral expression vector and these plasmids must be introduced into the cell and expressed alongside the lentiviral vector for viable lentiviral particles to be produced.

### Origin & function

The RNAi hairpins are sequences that have been derived de novo and which will initiate a downstream cascade of events leading to knockdown of the target gene to which the hairpin was designed.

The accessory proteins are from the HIV-1 virus.
The hairpin sequence will be transcribed and the RNA will then form a hairpin structure. The hairpin structure will induce a normal cellular in vivo downstream sequence of processing events in response to foreign cellular single-stranded RNA intruder molecules that will result in the post-transcriptional down regulation of the gene to which the hairpin sequence is targeted.

If the inserted sequence were to be expressed in humans after accidental transfer the hairpin could lead to the down-regulation of the gene to which it targets via RNAi interference. This could be a gene essential for growth regulation such as a tumour suppressor gene therefore resulting in a potentially oncogenic mutation. This, however, would not be as severe as the virus is replication incompetent and therefore self-inactivating as none of the structural genes are actually present in the packaged viral genome, such that no new virus can be produced and no other cells can be infected. Physical insertion of the virus into the genome of the host cell could potentially disrupt a tumour suppressor or oncogene, however the likelihood is very low.

VSV-G pseudotyped lentivirus can only infect mammalian cells. Potentially the hairpin could be expressed in another mammalian species and lead to the down-regulation of a growth regulatory gene such as a tumour suppressor gene resulting in a potentially oncogenic mutation. This, however is extremely unlikely as the hairpin is targeted to human genes and many hairpins are not homologous with the genes from other species. If the hairpin were to target and lead to the reduction of a gene via RNA interference it would not be as severe as the virus is replication incompetent and therefore self-inactivating as none of the structural genes are actually present in the packaged viral genome so no new virus can be produced and no other cells can be infected.

The envelope protein is VSV-G from the Vesicular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection, its physical insertion into cellular DNA could result in a potentially oncogenic mutation. This would be a very unlikely event and if so only a very small number of cells and would not impact on the pathogenic outcome of an exogenous viral infection. The packaging viral components are from the HIV-1 virus. The full length HIV-1 virus is harmful to humans, however the packaging vector used does not contain the full length HIV 1 molecule. Many components of HIV-1, that are critical for HIV-1 infection but not required for viral packaging, have been removed so the virus is attenuated.

The de novo hairpin donor sequences could potentially be expressed in cells and lead to the knockdown of certain genes leading to the increase of oncogenic potential of the virus. This would, however, only be a very small number of cells and as the virus is replication incompetent and cannot make new viral progeny, it would not impact on the pathogenic outcome of an exogenous viral infection.

Evaluation of foreseeable effects

The envelope protein is VSV-G from the Vesicular Stomatitis Virus so, if introduced into the body by injection, ingestion or through a wound, the virus could potentially enter cells and integrate into cellular DNA. Although the virus is replication incompetent (does not carry gag, pol or env genes) so it cannot make new progeny virus and establish a productive infection, its physical insertion into cellular DNA could result in a potentially oncogenic mutation. This would be a very unlikely event and if so only a very small number of cells and would not impact on the pathogenic outcome of an exogenous viral infection. The packaging viral components are from the HIV-1 virus. The full length HIV-1 virus is harmful to humans, however the packaging vector used does not contain the full length HIV 1 molecule. Many components of HIV-1, that are critical for HIV-1 infection but not required for viral packaging, have been removed so the virus is attenuated.

The de novo hairpin donor sequences could potentially be expressed in cells and lead to the knockdown of certain genes leading to the increase of oncogenic potential of the virus. This would, however, only be a very small number of cells and as the virus is replication incompetent and cannot make a new viral progeny, it would not impact on the pathogenic outcome of an exogenous viral infection.

The most dangerous GMM is the lentiviral preparations of pGIPZ particles. The most dangerous step is the collection, concentration and use of the lentivirus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

2.5% Trigene advance or Virkon (v/v) will be used to treat liquid waste (18 hours) and also used to decontaminate any spills and for disinfection of any reusable bottles or other equipment and laboratory materials used. Liquid waste is subsequently poured down the sink after treatment with Virkon. Work surfaces will be wiped down with 5% Trigene advance and 70% ethanol after use. Virkon and Trigene advance are certified to provide 100% vircidal and GMO kill under these conditions by denaturating and inactivating viral and cellular proteins. Trigene advance and Virkon will be used with its lifespan to ensure required kill is achieved. Solid waste will be double bagged in biological waste bags, sealed and autoclaved 132°C for 15 mins by trained staff then bagged in yellow biohazard bags and removed by UCL waste services. Autoclave runs are regularly validated by waste management staff.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

A representative group of UCL institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees that this work should be classified as an activity class 2 (AC2) notification

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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Project Ref 133/11.2

Date Ackn'd 23/06/2011

Date Project Ceased

CU2 Project Title Immortalization of human cells

Class CultureVol Class2 CultureVolume Class3-4

Class 2 < 1 Litre Consent Granted

Non-GMM
The aim of this project is to immortalize human cells (e.g. skeletal muscle derived myoblasts and stem cells, blood-derived stem cells and fibroblasts). Cells will be prepared from normal individuals and from patients with neuromuscular diseases (e.g. Duchenne muscular dystrophy). Myogenic cells have a limited life span in culture, which prevents their expansion to sufficient numbers for experimentation and seriously limits any potential use in cell replacement or ex vivo gene therapy. An immortalization protocol for normal human cells (e.g. myoblasts) would allow one to isolate cellular models from various neuromuscular diseases, thus opening the possibility to develop and test novel therapeutic strategies. We intend to use a method developed by M and colleagues (names removed. Aging cell. 2007 Aug; 6(4):515-23. Epub 2007 Jun 8: names removed Biochem Biophys Res Commun. 2009 Oct 16;388(2):333-8. Epub 2009 Aug 6). Cells will be infected with lentiviruses coding for hTERT and cdk-4, selected by antibiotic resistance, expanded in culture and stored in liquid nitrogen. Immortalized cells will be stored and distributed under the auspices of the MRC Centre for Neuromuscular Diseases Biobank.

Recipient or parental organism

- HIV.PGK.Puro.CMV.hTERT
- HIV.PGK.Neo.CMV.cdk4
- Human myoblasts, fibroblasts and stem cells.

The lentiviral vectors used are multi-attenuated, meaning they are devoid of all potentially pathogenic encoded functions. In addition they are replication-defective which means that the vector cannot multiply on its own unless supplied by certain proteins in trans. It is self-inactivating which means that the viral promoter in the U3 region of the 5' LTR has been disabled by genetic manipulation. Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The probability of such an event is extremely low.

The intended recipient organisms are not capable of independent survival in the environment and will not infect or transfer to other hosts.

Host/vector system

- HIV-derived lentiviruses, prepared in 293 Cells:
  - HIV.PGK.CMV.hTERT
  - HIV.PGK.Neo.CMV.cdk4

The lentiviral vectors used are multi-attenuated, meaning they are devoid of all potentially pathogenic encoded functions. In addition they are replication-defective which means that the vector cannot multiply on its own unless supplied by certain proteins in trans. It is self-inactivating which means that the viral promoter in the U3 region of the 5' LTR has been disabled by genetic manipulation. Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose...
the person to a wild type HIV-1. The probability of such an event is extremely low.

WPRE is not present in either lentivirus.

Origin & function

Mus musculus, Homo sapiens sapiens

Lentiviruses - can infect human cells.
Puro and Neo - antibiotic resistance genes.

H-TERT - catalytic subunit of telomerase, that lengthens telomeres in DNA strands. Expression of telomerase bipasses telomere-dependent senescence so that cells that would otherwise become postmitotic and undergo apoptosis exceed the Hayflick limit and become potentially immortal.

Cdk4 is the catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression; its expression prevents p16INK4a-associated premature growth arrest.

Cdk4 therefore extends life span and immortalization is induced by hTERT.

There would be a risk of cell immortalization if cdk-4 and h-TERT were introduced into human cells.

The risk of transfer to species in the environment is negligible, but would be a risk of cell immortalization if cdk-4 and h-TERT were introduced into human cells.

The lentiviruses are HIV-derived, but are replication-deficient, therefore represent a low risk.

The donor organism has no pathological or harmful characteristics

Evaluation of foreseeable effects

The lentiviruses are HIV-derived, but are replication-deficient, therefore represent a low risk.

The donor organism has no pathological or harmful characteristics.

Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type virus. The probability of such an event is extremely low. The most hazardous GMM are the lentivector viruses and the most hazardous step is dealing with concentrated viral stocks before infecting the target cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMMs are inactivated for disposal using standard biosafety level 2 procedures. Briefly, liquid waste is treated with 1% Virkon. Work surfaces are decontaminated on completion of work, or at the end of the day, or after any spill or splash of viable material, with 1% Virkon. Virkon has been shown to achieve 100% GMO kill when used as
directed. After decontamination, liquid waste is disposed of via the sink. Solid waste is double-bagged in biological waste bags, sealed, transported to the autoclave, autoclaved and then removed by waste services.

A representative group of the UCL Institute of Child Health GMSC has reviewed this project, and the forms being amended according to their recommendations, agrees this work should be classified as an activity class 2 (AC2) notification.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units

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Project Ref 133/95.1

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Tick if notifying a connected programme of work N

Historical Significant Changes PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref 133/99.1**

- **Date Ackn’d**: 16/03/1999
- **CU2 Project Title**: TRANSDUCTION EXPERIMENTS WITH MINIMAL HUMAN IMMUNODEFICIENCY VIRUS (HIV) VECTORS
- **Date Project Ceased**: 24/04/2012
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**

- PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref: 133/99.2**

**CU2 Project Title:**

TRANSDUCTION EXPERIMENTS WITH IMPROVED EQUINE INFECTIOUS VIRUS (EIAV) VECTORS

**Class:**

Class 2

**CultureVol:**

Class 2

**CultureVolume:**

Class 3-4

**Consent Granted:**

not applicable

**Project notified under transitional arrangements:**

Y

**Date Ackn'd:** 16/03/1999

**Date Project Ceased:** 24/04/2012

**Withdrawn:** N

**Tick if notifying a connected programme of work:** N

**Historical Significant Changes:**

PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED

**Historical Date of Additional Info:**

**Significant Change ID:**

**Date of Significant Change:**

**Project Additional Information**

**Purposes of the contained use:**

**Recipient or parental organism:**

**Host/vector system:**

**Origin & function:**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

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Project Containment

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<td>L4</td>
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<td>Animal Units</td>
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## GM Centre Number: 134

### Data Premises Notified (Originally)
- **Date:** 06/05/1983
- **Transferred from 1992 Regs:** Y
- **Transitional Premises Class:** 1
- **Data Premises Closed:** 24/04/2012
- **Transitional Premises:** N
- **Non-GMMs:** N
- **Withdrawn:** N

### Name
- **INSTITUTE OF NEUROLOGY**

### Name 2
- **UNIVERSITY COLLEGE LONDON**
- **Department:** NEUROCHEMISTRY

### Campus Estate or Research Centre
- **Building:**

### Road Name
- **1 WAKEFIELD STREET**

### Town
- **LONDON**

### County
- **GREATER LONDON**

### Postcode
- **WC1N 1PJ**

### Country
- **ENGLAND**

### Tel Number
- **020 278 1552**

### Fax Number
- **020 278 7045**

### E-mail

### HSE Division
- **LONDON**

### Comments
- **GM CENTRE CLOSED AND ALL WORK TRANSFERRED TO GM14**

### Date at Which Additional Info Submitted
- **02/03/2022**
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Tick if confidential

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<th>Parasitology</th>
<th>Transgenic</th>
<th>Microbiology Research</th>
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<td>Birds</td>
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HEAT INACTIVATION
This is the preferred method and should be followed where practicable. This should be used for solid waste (e.g., disposable pipettes, petri dishes, tips, disposable test tubes etc.) and small scale liquid cultures contained within disposable tubes. The waste should be transported to the autoclave without spillage in a robust metal container with a lid, that can be placed directly into the autoclave. Material should be inactivated using one of the autoclaves provided for this purpose (1966 Wing or Clinic). After autoclaving the waste should be transferred to a yellow plastic bag, tied and labelled with the laboratory of origin and transported to the waste containers at the back of the Clinic. The waste will be collected by outside contractors and destroyed by incineration.

CHEMICAL INACTIVATION
This should be used for larger volumes of liquid culture waste (up to but never exceeding 10 litres at any one time), and for treatment of glassware or other equipment that is to be reused. These should be inactivated by addition of, or immersion in, a chemical agent that is appropriate to the organism in question. A number are available of which the most commonly used are: hypochlorites (e.g., Chloros); phenolics (e.g., Stericol); alcohols (e.g., 70% ethanol); and peroxygen compounds (e.g., Virkon). Effective disinfection with any of these depends on activity (whether it is effective against the target micro-organism), concentration (use of the correct dilution/concentration in the particular circumstances, also allowing for age of the solution if this affects effectiveness), and contact (intimate contact for sufficient time). Refer to the manufacturers guidance or contact the Biological Safety Co-ordinator if you are unsure how to use a chemical agent. Once inactivation has been performed liquid waste/solutions are either disposed of via the sink, flushing with copious amounts of tap water, or stored for disposal by contractors. The latter route should be used where the chemical agent itself presents a hazard and the solution cannot be safely disposed to the drains.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 138/03.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4
GENETICALLY DISABLED MUTANTS OF LEISHMANIA MEXICANA

Date Project Ceased

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The specified purpose of the contained use is to create mutant lines of Leishmania mexicana with altered expression of specific enzymes or antigens. This is part of ongoing research aimed at identifying targets for chemotherapeutic (drugs) or immunological (vaccines) intervention against Leishmania parasites.

Recipient or parental organism

Leishmania mexicana is a parasite protozoan that causes cutaneous leishmaniasis in Central and South America. It is a Hazard Group 2 organism as defined in the ACDP code and presents a hazard to human health.

Host/vector system

The vector systems to be used are derivatives of pX63-HYG (Cruz et al. 1991, Proc. Natl. Acad. Sci. USA88: 7170-74)

Origin & function

Genes encoding surface membrane, secretory and biosynthetic enzymes and antigens of Leishmania mexicana will be isolated using standard PCR or cloning techniques and constructs assembled in vitro.

Evaluation of foreseeable effects

Any GMO created is very likely to exhibit reduced pathogenicity compared to the normal wild type organism, and in many cases this will be the deliberate purpose of the manipulation - creation of a knock-out organism lacking a key protein. The general reduction on pathogenicity results from the need for an extended period of in vitro manipulation in order to create the mutant lines, and this process invariably leads to a loss of virulence. There is no foreseeable additional pathogenicity of the recipient organism as a result of the creation of a GMO.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Waste management employs a "belt and braces" approach: disinfection followed by autoclaving. Both individual procedures are capable of achieving 100% killing of Leishmania organisms.

Specific disinfection procedures:
- Three disinfectants are used: 70% ethanol, Virkon and Stericol. All are 100% effective against Leishmania when used properly, as verified by absence of viable organisms upon microscopical examination and lack of growth in culture following treatment. Each is applied in a different way and for different purposes, as detailed below:
  i) Surface sterilisation. Hard surfaces such as the work surface in a sterile hood or benches are disinfected with 70% ethanol both before and after completion of work. Wash bottles containing 70% ethanol are used to wet a piece of blue towelling and this is wiped over the surfaces (wear gloves). The towelling is disposed into waste for autoclaving.
  ii) Pipettes. Pipettes are placed and fully submerged into pipette canisters on the floor by each sterile hood. These contain 5% (w/v) Virkon. Virkon is a pink powder, which when dissolved acts as a very effective wide spectrum disinfectant: a 1% solution can be used for general disinfection purposes and is 100% effective against Leishmania. Fresh solutions are prepared weekly as Virkon solutions are effective for a limited period of time. Pipettes are then placed into waste for autoclaving.
  iii) Slides. Microscope slide with live material are placed into large plastic jars with lids containing 5% (v/v) Stericol (a dark brown liquid). Stericol is a phenolic and had an unpleasant smell (which is why it is not used in open pipette canisters). However, it does not lose effectiveness with time. Slides are then placed into waste for autoclaving.
  iv) Cultures. Liquid cultures in flasks etc. are inactivated by addition of a small volume of neat Stericol from the wash bottle by each hood. The final concentration should be > 1% (v/v). Stericol is added to the flask, this is firmly capped and shaken to mix. The flask is then placed into waste for autoclaving.
  v) Spillages. Small spillages are suspected contamination on hands, gloves or work surfaces should be disinfected immediately with 70% ethanol. To inactivate large volume spillages add Virkon powder directly to the contaminated area, leave for 10 minutes then wash with 1% Virkon solution.

Autoclaving: Waste material from flip-top bins (paper waste) is rendered safe by autoclaving. After autoclaving, solid waste is collected and destroyed by incineration, sterile liquid waste is discharged to the drains.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee was satisfied with the risk assessment and conclusions reached. Discussion emphasised the importance of ensuring that existing Codes of Practice were adhered to and that new work was properly supervised. It was noted that any proposed future change of activities, for example, dealing with a new gene sequence would need to be carefully considered so that any possible increase in virulence of the pathogen as a result of genetic manipulation could be properly assessed.

**Project Containment**

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02/03/2022
The general aim of the project is to investigate the role of African trypanosomes (i.e. Trypanosoma brucei brucei and Trypanosoma congolense) genes that may be important or essential for this parasite to colonize, establish or produce infectious life forms in the tsetse fly vector. In addition, we are interested in genes involved in tissue tropism and migration within the fly. We are particularly interested in regulating the expression of genes encoding for a family of surface metalloprotease MSP-B, glycosylation enzymes and sugar transporters, flagellar proteins, GPI-anchored proteins, metabolic enzymes, cell cycle and differentiation and those involved in amino acid uptake and metabolism.

Trypanosoma brucei brucei strains Lister 427 (wild type), 427.29.13 (containing tet repressor and HYG in Pol1 large subunit locus, and T7 RNA polymerase, neomycin resistance gene and tet repressor in the alpha tubulin locus), 2T1 (containing T7 RNA polymerase, Tet operator, PAC (puromycin resistance gene) and a partial hygromycin resistance gene (HYG) in the rRNA locus) and AnTat1.1 Trypanosoma brucei brucei is in ACDP hazard group 2. Strain Lister 427, clone 29.13, will be used in the procyclic (tsetse midgut) form. 2T1 and AnTat1.1 strains will be cultured in the bloodstream form but AnTat1.1 will also transform to procyclic form when fed to tsetse flies.

Origin & Function

The T. b. brucei MSP-B (major surface protease B) gene, portions of this gene and its 5’ and 3’ flanking regions will be cloned for overexpression, RNAi and knockout constructs, respectively. Proline uptake/metabolism gene fragments (for RNAi), PIP39 (phosphatase involved in control of stumpy to procyclic form progression) gene portions (for RNAi), genes involved in glycosylation and glycosylphosphatidylinositol anchor addition (for RNAi). In addition to the endogenous genes investigated, the following antibiotic resistance selective markers will also be integrated as selective markers: Streptomyces alboniger puromycin N-acetyl transferase (PAC), Aspergillus terreus elasticin S-deaminase (BSD), Streptococcus lactisus bie (phleomycin resistance), Escherichia coli strain K12 neomycin phosphotransferase (G418/Neomycin resistance) and Escherichia coli hygromycin phosphotransferase (HYG). The acceptor strains for the genetic modification will already have been modified with the bacteriophage T7 RNA polymerase and T7 promoter, and the Escherichia coli tet repressor and tet operator. This allows for the transcription of the inserted genetic material to be induced by the addition of Tetracycline to the media. EGFP, derived by mutation of Aequorea victoria GFP will be used as an in vivo marker for trypanosomes in the tsetse fly.

Evaluation of foreseeable effects

It is very unlikely the genetic modification will make the trypanosomes more hazardous. Some of the modifications might make T. b. brucei more infectious to the tsetse flies. However, gene knock outs or knock down by RNAi are usually disadvantageous to T. b. brucei.

No effect on the pathogenicity to mammalian hosts is anticipated. This is because changing the transmissibility of the parasite through the tsetse fly does not change the pathogenicity of the parasites to animals or humans. T.b. brucei will remain non infectious to humans. The antibiotic resistance genes do not encode resistance to the drugs that would be used to treat T. b. brucei infections in animals, so would not impact on any potential treatment of infections.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste comprised of culture flasks, contaminated serological pipettes and tips, and infected flies and the microscope slides on which they are dissected:

(i) Contaminated serological pipettes and plastic pipette tips will be soaked in Virkon solution (minimum 1%) solution for at least 24 hours and then destroyed by incineration.

(ii) Plastics, slides and infected flies will be autoclaved then sent for incineration. Kill by heat treatment will be confirmed by viewing autoclaved material under the microscope to check for death of parasites. Printed records will confirm that the autoclave run completed satisfactorily.

Liquid T. b. brucei culture waste is decontaminated for at least 24 hours by Virkon solution (minimum 1%). A 10 minute immersion in 1% bleach (sodium hypochlorite, NaOCl) is used to decontaminate feeding trays and membranes with small quantities T. b. brucei in blood. This is sufficient to kill all the parasites (Wang et al 2008, Parasites and Vectors 1:35).
The GM committee requested additional information be given on the vectors to be used in the genetic modification of the trypanosomes so that the risk could be fully assessed including any change to patenogicity to humans.

Further information concerning waste disposal and the procedure for disinfecting spills was requested.

The committee deemed the risk assessment satisfactory with inclusion of the requested details.

Please enter comments on the GM safety committee on the risk assessment

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Project Containment

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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2 L3 L4 L2</td>
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Project Ref 138/15.1

Date Ackn'd 27/05/2015

CU2 Project Title Immortalisation of Brugia malayi cells for the development of a nematode cell line

Class 2 CultureVolClass2 ≤ 1 Litre CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes
### Project Additional Information

**Purposes of the contained use**

To induce proliferation of an immortalised cell line from *Brugia malayi* for the development of a celabased screening assay for macrofilaricides.

**Recipient or parental organism**

Isolated primary cells from *Brugia malayi* adult female worms.

**Host/vector system**

Lentivirus (Moloney Murine Leukemia Virus, MMLV) containing plasmids encoding green fluorescent protein and one of the oncogenes SV40, hTERT, HPV-16 E6/7, CD4K, p53 or MycT588A.

**Origin & function**

Commercially available vectors from Applied Biological Materials, Inc. Distributed in UK by NBS Biologicals Ltd.

**Evaluation of foreseeable effects**

Viruses are replication incompetent so cannot proliferate following transduction into the target organism, Gag/Pol/Rev deletion from the viral genome, so immortalised *Brugia malayi* cell line will not shed virus. Insertion of oncogene vectors could occur accidentally into operator but do not replicate following insertion. Work will be conducted in a class II safety cabinet and operators will wear lab coat, gloves and safety glasses to guard against splashes. Sharps will not be used to prevent puncture accidents. Neither the *Brugia Malayi* primary cells or immortalised cell line or the lentivirus cultures will be able to survive outside laboratory culture so provide minimal risk to other than laboratory personnel and the environment.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid contaminated waste is disinfected with a 10% Virkon solution, followed by autoclave treatment. Liquid media are made to 10% with Virkon and incubated overnight to ensure kill of organisms. Inactivated waste is sent for incineration.

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Comments from LSTM GSC, 06/05/15:
1. Include information on potential routes of accidental entry for the viral vectors.
2. Confirm routes of waste disposal and provide information on the efficacy of Virkon against lentivirus.
3. Review available hazard information from AMB and provide a safety data sheet if available for the lentivirus vectors.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Comments on the GM safety committee on the risk assessment

Unit Ref 138/17.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
Understanding the host-pathogen interactions in Mycobacterium tuberculosis and HIV co-infections in human immune cells

Project Additional Information

Purposes of the contained use
To define the host-pathogen relationships in immune cells containing co-infections of HIV and Mtb.
Host cells will include established cell line THP1 and isolated human immune cells: dendritic cells, neutrophils and alveolar macrophages. GM HIV-1 expressing GFP and a GM respiratory knock-out mutant of MTB expressing mCherry will be used.

Recipient or parental organism
HEK-293 cells will be used as host for the HIV-1 BAL_GFP to produce the infectious virus particles.
Mtb cell line H37RV will be used

Host/vector system
pGFP plasmid for green fluorescent protein has been inserted into the HIV-1 BAL
pENVY10 plasmid for mCherry has been inserted into M tuberculosis strain H37Rv

Origin & function
HIV-1 BAL expressing GFP is obtained from the University of Liverpool ref: GM445 (UoL)
Mtb strain H37RV expressing GFP or m Cherry is already in use at LSTM, ref: GM107 (LSTM)

Evaluation of foreseeable effects
HIV-1 BAL contains the full HIV-1 genome. Insertion of the pGFP plasmid is
not thought to affect the virulence of the strain, which is equivalent to clinical isolates. Mtb strain H37Rv is a respiratory knock-out mutant which is less virulent than wild-type Mtb. The pENVY10 plasmid encodes for a hygromycin antibiotic resistance to aid expression. Hygromycin is not an antibiotic used against Mtb so the virulence of the knock-out mutant is not affected by insertion of this plasmid.

Containment level 3 will be used for this work as this is required for both HIV and Mtb as ACDP HG3 organisms.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste for HIV and Mtb will be made to 5% with Surfanios disinfectant and incubated overnight, prior to autoclaving at 122°C for 4 hours. Solid contaminated waste is immersed in 5% Surfanios overnight followed by autoclaving at 122°C for 4 hours. Other waste from CL3 laboratory is autoclaved at 122°C for 4 hours. Following autoclave all waste is sent for incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment
Points raised at the LSTM GM review:
• SOP’s to be provided with detail of the work with HIV which can be referenced in this Risk Assessment.
• Reference risk assessment (non-GM) for the HIV work, i.e. how to work safely with HIV.
• Details of how training and instruction will be given to ensure competence to work with HIV and MTB.
• What evidence will be available to demonstrate competence of workers? Include evidence of training from University of Liverpool and at LSTM.
• Include the GM information for the GM MTB from risk assessment GM107v3 as this will be used in the project.
• Include information of the plasmids used to generate the HIV either a reference paper or plasmid map.
• Include assessment of the risk from unscreened blood as a source of MDM and DC cells.
Ensure an inventory is set up for storage of any GM HIV within the CL3 lab. Ensure adequate control and segregation from other HG3/GM organisms stored within the laboratory.

Project Containment

Laboratory Activities

| L2 | L3 | Yes | L4 | L2 |

Glass Houses

| L3 | L4 | L2 |

Growth Rooms

| L3 | L4 |

Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 138/17.2

Date Ackn’d 01/11/2017

CU2 Project Title Expression of Streptococcus exotoxins in E.coli

Class 2

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
### Project Additional Information

#### Purposes of the contained use

To confirm in vitro the efficacy of neutralising antibodies to the exotoxins

#### Recipient or parental organism

Non-pathogenis laboratory strains of Escherichia coli (Top10 and BL12DE3)

#### Host/vector system

Plasmid pDESt14 (Thermoscientific) using IPTG promoter. DNA inserts will be directly cloned into the vector through restriction sites.

#### Origin & function

Streptococcus pyrogens exotoxin genes SpeA, SpeB, SpK, Ssa and Sla will be inserted into E.coli to over express the functional exotoxins.

#### Evaluation of foreseeable effects

E.coli strains are non-pathogens. All genes have signal peptides removed to prevent secretion into growth media. Expression of exotoxins only occurs with IPTG promoter. Foreseeable effects with IPTG promoter. Expressed exotoxins will be functional with estimated LD50 of 150mg/kg.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

CL2 laboratory. Use of class II safety cabinet for liquid handling procedures. Sealed centrifuge buckets. Waste inactivated by use of 1% Virkon or autoclave then sent for incineration.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste made to 1% Virkon; solid waste autoclaved to achieve 100% kill. Virkon well documented as suitable disinfectant for E.coli. Autoclave cycle confirmed at each run. All waste sent for incineration post disinfection.

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</table>
LSTM GMM review panel met on 4/10/17 and the following comments were raised:

- Class 2 GMM accepted rather than class 1. Although lab strain of E.coli to be used and Streptococcus exotoxins are routinely used in immunology and well characterised there is potential for causing illness if contamination with the GMM.
- The room location used for the work with GMM should be added to the risk assessment.
- Indicate use of sealed centrifuge buckets/rotor within control measures, (section 4.1)
- Add information indicating the stage at which GMM is inactivated during purification of the exotoxins. Reference the SOP for purification of the exotoxins.

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Project Ref 138/19.2

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<tr>
<td>01/05/2019</td>
<td>Optimisation of antibiotic treatment for E. coli and K. pneumoniae by determination of epistatic interactions following development of multiple antimicrobial resistances</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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Class CultureVol

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Withdrawn

Historical Date of Additional Info

Tick if notifying a connected programme of work

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Page 3925 of 15326
**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The development or acquisition of antimicrobial resistance often result in a fitness cost to bacteria as resistance can be metabolically expensive, however sometimes resistance is accompanied by a fitness benefit. The overall aim of the project is to determine whether the evolutionary trajectory of the emergence of antimicrobial resistance can be predicted and taken advantage of in order to inform clinicians of particular antibiotic combinations which consistently result in unfit antimicrobial resistant bacteria. Genetic background of an isolate is of particular importance in this respect. We will select for naturally occurring mutations and / or horizontal gene transfer events in bacteria that confer resistance to various clinically used antimicrobials by growing them in subinhibitory concentrations of antibiotics (mutation) and together with suitable sensitive recipient organisms. Following this the strains will be sequenced and the mutations and acquisition events determined.

To confirm that these mutations result in antimicrobial resistance (and are not part of a compensatory mutational effects) we will do two things:

1. clone the mutated gene into a suitable vector in a laboratory strain of E. coli and replace (using homologous recombination ) or complement the wild-type gene in the original, wild-type, sensitive ancestor. This should lead to a resistant strain as seen in our evolutionary experiments.

2. will also complement / repair the mutation to demonstrate reversion to sensitivity.

Therefore the only genetic modification we are requesting to be covered in this application is the complementation of naturally occurring mutations to resistance (leading to sensitivity) and the cloning of mutant alleles in susceptible strains to replicate the naturally occurring mutations which we detect during the evolution of our strains (this will lead to resistance).

**Recipient or parental organism**

Clinical isolates of Escherichia coli and Klebsiella pneumoniae isolated from clinical samples from the UK, Malawi and Uganda. These isolates will be susceptible to the majority (if not all antibiotics) normally used to treat infections caused by the organisms.

E.coli (α-select), JM109 and BL-21 , E. coli classified as disabled or non-colonising hosts. These strains will be our general cloning strains and all construction will be carried out in these. This category of hosts has been defined by ACGM as those which are non-pathogenic, are unlikely to survive outside of culture media and have a history of safe use.

**Host/vector system**

The vectors used will be standard, non-mobilisable cloning and expression vectors for use in Escherichia coli and Klebsiella pneumoniae. These are based on a colE1 replicon and will include the following plasmids:

- pBR322 (copy number 15-20 per cell. Ref; Chang and Cohen; 1978) [PMID:149110]
- pUC18 (copy number 500-700 Ref; Messing; 1983) [PMID: 6310323]
- pET (Non-mobilisable expression vector. Novagen).

**Origin & function**

During the course of the evolution experiments we will determine naturally occurring mutations in genes resposible for resistance and fitness (e.g. in an efflux pump or a change in a ribosomal protein). In order to determine if these mutation(s) are solely resposible for resistance we will engineer this mutation in the original sensitive strain. Antibiotic resistance genes will be present in vectors (ampicillin or chloramphenicol) and genetic modification as detailed above will give rise to phenotypic resistance.
towards our antibiotic of choice. These include amoxicillin, chloramphenicol, ciprofloxacin, gentamicin, co-trimoxazole and ceftriaxone.

**Evaluation of foreseeable effects**

**Direct Effects:**
In some of the clones, where we are replicating the naturally occurring mutation to resistance, the genetic material will make the host more resistant to clinically relevant antibiotics. We will never make the strains resistant to more than two clinically relevant antibiotics thus ensuring there are multiple, clinically used antibiotic susceptibilities remaining.

**Indirect Effects:**
The genetic material may increase or decrease the fitness of the isolate compared to the ancestor strain.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste will be autoclaved with the using the “Fluid Discard” autoclave cycle. Confirmation of valid autoclave run is provided on print out from autoclave. Each run is recorded in a log book.

**Is an emergency plan required according to regulation 20?**  N

**If yes, tick to confirm that it is attached to this form**  N

**Tick to confirm that you have attached a risk assessment to this form**  Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  N

**Please enter comments on the GM safety committee on the risk assessment**

Review of this GM application by the BSC panel was conducted by e-mail during July 2018.
Comment/request was to include specific detail on the vectors/plasmids to be used in this work to allow determination of any safety issues which may arise from their use.

The risk assessment was updated to include this information.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 Yes</td>
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<th>Animal Units</th>
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02/03/2022
Using infectious clones and genetically altered arboviruses to study virus-host interactions

Purposes of the contained use

Virus sequences, wild-type and mutated, will be cloned into plasmids and transformed into bacteria for amplification (virus particles will not be produced at this stage). Plasmids will be purified and used to produce stocks of infectious virus particles in mammalian and/or insect cell cultures. Stocks of infectious virus will maintain a standard infectious viral population that can be rescued without further passage. Altered viruses will express fluorescent or luciferase proteins either as a fusion protein with a viral gene or sequentially on a separate promoter. In addition, specific mutations will be engineered into viruses to examine the genetic basis of infection in mosquitoes. We evaluate the replication, dissemination and transmission of viruses in cells or insects by observing the presence of a fluorescent or luciferase reporter that is expressed by the virus. These are standard techniques used in many laboratories.

Recipient or parental organism

Zika virus (ZIKV), Semliki Forest virus (SFV), o’nyong’nyong virus (ONNV)

Host/vector system

Whole virus genomes will be cloned into standard pUC plasmid for
amplification in E. coli, RNA polymerase promoter sequence will be added for in vitro transcription.

**Origin & function**

For GFP or luciferase labelling the sequence for either the fluorescent protein or luciferase will be added onto the structural or non-structural polyprotein sequences of the viruses. Additional mutations to the wild-type sequence will be made to assess the ability of the virus (e.g., fidelity mutants, vaccine candidates) to disseminate through the insect host or to create non-infectious, replicating particles (replicons) to view susceptible cells in mosquito tissues. Mutations will be made in both structural and non-structural sequences and will decrease virulence.


**Evaluation of foreseeable effects**

There is no increased risk associated with cloning ZIKV/SFV/ONNV whole genome into standard pUC plasmids for amplification in E. coli because transcription of viral RNA and translation of viral proteins will not be initiated so no virus particles will be produced in E. coli. There is no risk associated with cloning RNA polymerase promoter sequence as this does not produce virus particles and requires additional steps for RNA to enter cells. There is no risk associated with cloning sequence for fluorescent or luciferase protein onto the structural or non-structural polyprotein sequences as none of these encode sequences for virulence factors. Virus strains used in containment laboratory are unlikely to persist outside of the lab because there is no natural virus vector in the UK. Infected insects will not survive outside the lab as they are tropical species.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be autoclaved at 122C for 30 minutes to ensure 100% kill. Autoclave cycle will be confirmed from record printout and and recorded for each run. Liquid waste will be made to 2% Virkon solution followed by autoclaving at 122C for 30 minutes. Autoclave cycle confirmed as above.
All waste is sent for incineration

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Additional information was requested about the plasmids, vectors and the genetic modification process. Additional information was requested about the necessary control measures for working with HG2 viruses / class 2 GMM. Additional information requested about whether GMM viruses would be able to persist in the environment.

Project Containment

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Project Ref 138/20.1

Date Ackn'd 03/01/2020

CU2 Project Title Pathogenesis and treatment of intracellular pathogens

Date Project Ceased

Consent Granted

Class 3

Class Vol Class 2 < 1 Litre

Class Vol Class 3-4 < 1 Litre

Project notified under transitional arrangements N

Withdrew N

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

This work will involve the culture of a number of clinically important intracellular pathogens. We wish to gain a better understanding of what influences susceptibility to disease and to develop novel treatment strategies to improve clinical outcome.

Recipient or parental organism

- Neisseria gonorrhoeae (HG2)
- Escherichia coli (HG2)
- Listeria monocytogenes (HG2)
- Staphylococcus aureus (HG2)
- Chlamydia trachomatis (HG2)
- Mycobacterium ulcerans (HG3)

Host/vector system

1. pFCCGi plasmid (rpsM::mCherry and PBAD::EGFP); constitutive mCherry and inducible GFP expression.
2. pDiGc plasmid (rpsM::EGFP and PBAD::dsRed); constitutive GFP and inducible dsRed expression.
3. pSW002 plasmid (psbA::dsRed); constitutive dsRed expression.
4. prpsM-DsRed.T4 plasmid (rpsM::dsRed); constitutive dsRed expression.
5. pZEP17.1 plasmid (rpsM::EGFP); constitutive GFP expression.

Origin & function

Knock-out (KO) mutants will be generated by site directed mutagenesis in the LSTM laboratory.

The inserted genetic material will code for a variety of fluorescent proteins. The plasmid-borne expression of fluorescent proteins will be used to track internalisation and growth/survival by flow cytometry and confocal microscopy. These plasmids have been used previously, or designed, for applications like that proposed here (Helaine et al., 2010, Mengesha et al., 2006, Saliba et al., 2016, Wilton et al., 2017).

**Evaluation of foreseeable effects**

All KO mutants are expected to be less virulent than the wild-type. GMMs created by site-directed mutagenesis will not express key genes associated with pathogenesis. As a result, they are expected to be less able to survive and replicate and will exhibit reduced virulence compared to the wild-type.

Fitness of mutants will be assessed using intracellular assays. Evidence of increased fitness (improved invasion and/or increased intracellular replication) will be recorded. It is hypothesised that mutants which carry fluorescence expression plasmids will incur a fitness cost and will be less virulent. The impact of all modifications on growth and drug sensitivity will be determined as part of strain characterisation.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| None |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Solid waste will be disinfected using the standard discard autoclave cycle. Confirmation of valid autoclave run is provided on print out from autoclave. Each run is recorded in a log book. |
| Liquid waste for hazard group 2 organisms will be made to 1% with Virkon. Liquid waste for Mycobacterium ulcerans will be made to 5% with Surfanios. |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment

LSTM Biological Safety Committee review the project proposal and risk assessment with the following comments:
1. Confirm the hazard group of all organisms to ascertain the appropriate GM classification for work.
2. Identify activities within each laboratory.
3. Confirm the appropriate chemical disinfectant for M ulcerans.

**Project Containment**
**Project Ref** 138/20.3

**Date Ackn’d** 01/04/2020  
**CU2 Project Title** Manipulation of bacterial symbionts associated with arthropod vectors

**Class** Class 2  
**Culture Vol Class 2** < 1 Litre  
**Non-GMM** Not Applicable  
**Consent Granted** Project notified under transitional arrangements

**Withdrawn** N  
Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Bacterial symbionts of mosquitoes will be modified to investigate host-microbe interactions and paratransgenic control strategies for arthropod vectors. Bacteria will be transformed with plasmids expressing fluorescent proteins and genes conferring antibiotic resistance. Native gene over-expression will also be undertaken exploiting plasmid expression. These plasmids contain normal antibiotic resistance genes (Ampicillin, Chloramphenicol or Kanamycin) for selection on agar plates. Genome engineering approaches will also be undertaken which utilize CRISPR-Cas9 gene editing approaches in combination with recombineering. Genome modification will entail creating mutations or integrating genes into the bacterial chromosome. Paratransgenic studies will involve over-expression of dsRNA from plasmids or via integration into the genome. This work will be undertaken in bacteria that associate with mosquitoes which commonly includes bacteria from the Proteobacteria, Bacteroidetes, and Actinobacter phyla

**Recipient or parental organism**

E.coli BL21, K-12 MG1655, DH5alpha
Aeromonadaceae such as Aeromonas hydrophila
Enterobacteriaceae such as Cedecea netari, Enterobacter cloacae, Pantoea agglomerans, Serratia mercescens,
Acetobacteria such as Asaia bogorensis
Pseudomonadaceae such as Pseudomonas fluorescens Holomonadaceae such as Zymobacter palmae
Wolbachia
Leucobacter
Chysdeobacterium
Stenotrophomonas

Host/vector system

Original or modified version of commonly used vectors for bacteria including pKDsgRNA-ack (Spectinomycin resistant) and pCas9-CR4 (chloramphenicol resistance) (Reisch and Prather 2015; 10.1038/srep15096), pRed/ET (Tetracycline resistant) (Trehan et al 2016; doi: 10.1038/srep19121), pET28a/Cas9-Cys (Kanamycin Resistant) (Ramakrishna et al, 2014 10.1101/gr.171264.113.), pRAM-mCherry (Kanamycin and Rifampicin Resistant), pRAM-GFP (Kanamycin and Rifampicin Resistant), pRAM-CFP (Kanamycin and Rifampicin Resistant) (Burkhardt et al. 2011; 10, 1371/journal.pone.0029511), pBAVIK-T5-gfp(Kanamycin Resistant) (Bryksin and Matsumura 2010; 10, 1371/journal.pone.0013244), shuttle vectors such as pGEM-T easy (ampicillin resistant).

Origin & function

Native symbiotic bacteria of mosquitoes will be transformed with plasmids for gene over-expression. Genes that will be over-expressed include but are not limited to i) fluorescent proteins, ii) antibiotic resistance genes iii) bacterial genes and iv) dsRNAs

For CRISPR gene editing approaches, we will edit the genome of bacteria to mutate or integrate genes into the bacterial genome. Potential target sites for integration and mutation include bacterial genes (such as OmpA, Waal, hns, ifhB, aroD, aroG, yfB, lip, secB, lon, pdl, ycbJ and RscA) or sites within intergenic regions. Mutant bacteria will likely be less fit in mosquitoes compared to their WT relatives.

Gens to be integrate include but are not limited to i) fluorescent proteins, ii) antibiotic resistance, iii) cassette for dsRNA expression and iv) native bacterial genes. Integrated genes will be driven from native, inducible or constitutive promoters. dsRNA will target mosquito genes or viral sequences with the aim of creating mosquitoes that are incapable of harbouring or transmitting viruses.

Cloning of plasmids will be undertaken using conventional approaches in E. coli.

Evaluation of foreseeable effects

The work entails transforming native bacterial symbionts that commonly associate with mosquitoes with expression plasmids and plasmid used for genome modification. Symbionts are classified as hazard group 1 or 2. Genomic integration or plasmids possess gene(s) expressing antibiotic resistance, cas proteins, fluorescent protein, bacterial membrane or cytosolic proteins, and dsRNA fragments.

The protein expressed from the inserted genetic material might be harmful to humans if injested or inhaled. These proteins will be expressed in the controlled manner inside the bacterial cells and will not come in direct contact with the humans. The proteins expressed in the bacterial cell will have minimal effect on the environment. Antibiotic resistance may give bacteria a selection advantage but only in cases where antibiotics are present. More likely, possessing these genes would be a fitness cost for the bacteria as evidenced by studies showing the plasmids are lost from bacteria after successive passages. These antibiotic resistance genes are already present in the environment. Containment of these genes to the laboratory mitigates these risks.
Gene transfer, although unlikely, may result in novel phenotypes in these mosquito symbionts. These may alter the fitness, either positively or negatively, compared to the wild type strains. In the unlikely event of a gene transfer, containment procedures will mitigate any risk to the environment. Infections of mosquitoes are done under Category 2 containment insectaries.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Infections of mosquitoes are done under Containment Level 2 insectaries.

Infected arthropods will be held in appropriate CL2 facilities separate from non-infected arthropods. The insectaries will be separated by multiple doors (security clearance required to enter anteroom, enter another door to access insectary containing pathogens), and with measures to prevent escape and detect escapees (mesh over vents, use of white trays, light coloured walls, ceilings, etc.). Access will only be granted to personnel with documented training and with satisfactory techniques.

Infected arthropods will be kept in double containers (e.g. carton with mesh covering inside a plastic clip-top container or netted enclosure). Labels for containers will include the arthropod species, number of arthropods and pathogen. Live challenged arthropods will not be imaged. Live challenged arthropods will have saliva collected and tissues will be dissected. In these instances, arthropods will be immobilized by cold prior to manipulation. Saliva collection will only be performed on immobilized insects (anesthetized or legs/wings removed). Collected samples will be used for plaque assays and/or inactivated prior to RT-PCR.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid wastes e.g. agar media, killed insects, will be disinfected using validated autoclave cycle, followed by incineration. Liquid wastes will be disinfected by making to 1% with Virkon, or use of autoclave, followed by incineration of the disinfected waste in either case.

*Is an emergency plan required according to regulation 20?* N

*If yes, tick to confirm that it is attached to this form* N

*Tick to confirm that you have attached a risk assessment to this form* Y

*Tick if you are claiming exemption from disclosure for section of the risk assessment* N

**Please enter comments on the GM safety committee on the risk assessment**

This project was reviewed by the LSTM Biological Safety Committee via email.

The following comments were raised:
Additional information was requested about the plasmids and bacterial targets for mutation to clarify the intended work.
Confirmation of the hazard grouping of the bacterial symbionts indicates some are hazard group 2 so GM class 2 is appropriate for the work with the use of necessary control measures.
Clarification of any potential effect of the GM bacteria on human health or the environment.

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<table>
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GM Centre Number: 139

Data Premises Notified (Originally) 26/09/1983

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Emergency Plan Required?

Non-GMMs Y

Withdrawn N

Name
CHARLES RIVER LABORATORIES EDINBURGH LTD

Name 2

Department

Campus Estate or Research Centre
ELPHINSTONE RESEARCH CENTRE

Road Name

Building

District

Town TRANENT

County EAST LOTHIAN

Postcode EH33 2NE

Country SCOTLAND

Tel Number 01875 614545

Fax Number 01875 614555

E-mail

HSE Division SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<tr>
<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
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<th>Campus Estate or Research Centre</th>
<th>Building</th>
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<th>District</th>
<th>Town</th>
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: 

- **Give brief details of the genetic modification safety committee**: 

<table>
<thead>
<tr>
<th>Laboratory</th>
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<td>Level 3 (GMMs)</td>
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Other (please specify)  

Bacteriology  
Parasitology  
Transgenic  
Birds  
Microbiology  
Research  

Virology  
Transgenic  
Animals  
Transgenic  
Fish  
Gene Therapy  

Mycology  
Transgenic  
Invertebrates  
Transgenic  
Plants  
Other (please specify below)  

Other(s)  

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref**  139/06.1

**Date Ackn’d**  16/11/2006  
**Date Project Ceased**  

**CU2 Project Title**  Intramuscular Toxicity Study in Rabbits using Ad35, a preclinical safety toxicology study.  

**Class**  Class 2  
**CultureVolClass2**  < 1 Litre  
**CultureVolumeClass3-4**  

**Non-GMM**  Not Applicable  

**Consent Granted**  

**Project notified under transitional arrangements**  N  

02/03/2022  Page 3939 of 15326
**Project Additional Information**

**Purposes of the contained use**
- To provide safety data to allow vaccine to enter the clinical trial stage

**Recipient or parental organism**
- Ad35 Adenovirus

**Host/vector system**
- Replication deficient Ad35 human adenovirus vector encoding HIV-1 antigens gag, RT, In, nef and gp 140

**Origin & function**
- See above host/vector system, function is to provide immunogenicity against HIV, however the purpose of this study is to look for possible toxicity or tissue damage at the site of injection.

**Evaluation of foreseeable effects**
- Non-replicating viral vector being used to evaluate possible toxicity/tolerance effect.
- No foreseeable effects predicted.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
- N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
- Not required

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
- Inactivation (kill) will be two part but achieve 100% kill.

Animals will be fully necropsied at the end of the study. All tissues will be retained for histology and be preserved in formaldehyde which inactivate any remaining viral material. Blood samples will be frozen and retained for further analysis.

Any remaining tissues, bedding and cage debris will be double bagged, sealed, placed in a yellow euroskip then sent directly for incineration in the on-site PPC authorised clinical waste incinerator. All cages and racking will be cleaned by washing at 82C using standard detergents and cleansers.
The GMM will be supplied via the sponsor, no genetic manipulation will be carried out on the site. The predominantly closed environment and non-replicating nature of this material would imply that it would be considered a very low risk study.

Please enter comments on the GM safety committee on the risk assessment

The GMM will be supplied via the sponsor, no genetic manipulation will be carried out on the site. The predominantly closed environment and non-replicating nature of this material would imply that it would be considered a very low risk study.

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Project Ref 139/18.1

Date Ackn'd 08/02/2018

CU2 Project Title Intermittent dose studies of various adenovirus (Ad) serotype

Class 2

Culture Class Vol 2 Not Applicable

Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Project Additional Information

#### Purposes of the contained use

To provide preclinical data to allow the materials under investigation to enter clinical trials, or to provide anti-serum that will be used in subsequent testing to indicate that the materials are safe for use clinically.

#### Recipient or parental organism

Various replication deficient adenovirus (Ad) serotypes that incorporate sequence(s) of amino acids or proteins that act as antigen(s). The antigens are non-pathogenic and do not present a higher risk than the parent virus.

#### Host/vector system

Replicant deficient Ad vectors. The HSE guidance document at [http://www.hse.gov.uk/ukbiosafety/gmo/acgm/acgmcomp/part2.pdf](http://www.hse.gov.uk/ukbiosafety/gmo/acgm/acgmcomp/part2.pdf) provides full details on Ad vector strains and indicates that these may be handled at containment level 1 if there is sufficient attenuation demonstrated. All vectors in this programme of work will have been rendered replicant deficient, or attenuated. The Ad vector serotypes that will be used include (but not limited to) Ad4, Ad5, Ad26, Ad35 and Ad 48.

#### Origin & function

The supplied materials are provided from a sponsor company, there is no genetic manipulation on site. Information including a certificate of analysis and material safety data sheet is provided for each material. The intended function of the material is to provide prophylactic treatment for the prevention of diseases or the therapeutic treatment for management of diseases.

#### Evaluation of foreseeable effects

The materials are under investigation are nonreplicating/attenuated viral vectors being used to evaluate toxicity/tolerance effects in experimental animals or for anti-serum production. There is little, or no, established risk with wildtype reversion. See waste disposal section (section 12). There are no foreseeable effects predicted.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Animal waste such as bedding and cage debris will be handled as per site procedures, solid waste is sealed in bags and placed in skips that are sent for on-site incineration. Animals that are subject to terminal procedures with tissues being retained for histology and preserved in formaldehyde which inactivate any remaining viral material. Carcasses are sealed in bags and placed in specific skips.
as per site procedures and sent for on-site incineration. Blood samples are either handled on site within a central laboratory or plasma/serum are prepared and frozen for off site shipping or retained for further analysis. Off site shipping is handled by a dedicated shipping department and all local national laws are followed with regards shipping preparation and documentation. All cages and racking are cleaned on site in a custom designed cage washing facility by sterilisation/washing using standard detergents and cleansers that are known to inactivate the vector.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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<tbody>
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Project Ref 139/18.2

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<td>11/05/2018</td>
<td>In vitro and in vivo testing using test items containing a lentiviral vector</td>
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<td>1-50 Litres</td>
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Tick if notifying a connected programme of work  

Withdrawn

N

Project notified under transitional arrangements

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To provide preclinical data to allow the materials under investigation to enter clinical trials or for GMP lot release. To provide anti-serum that will be used in subsequent testing to indicate that the materials are safe for use clinically.

Recipient or parental organism

Replication incompetent lentiviral vectors that incorporate sequence(s) of amino acids or proteins.

Host/vector system

Replicant deficient lentivirus vectors. The HSE guidance document at http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part2.pdf provides full details on lentivirus vector strains and indicates that these may be handled at containment level 1 if there is sufficient attenuation demonstrated. All vectors in this programme of work will have been rendered replicant deficient, however sharps will be used therefore classification is determined as containment level 2.

Origin & function

The supplied materials are provided from a sponsor company, there is no genetic manipulation on site. Information including a material safety data sheet is provided for each material. The intended function of the material is to provide therapeutetic treatment for diseases.

Evaluation of foreseeable effects

Not applicable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory and animal waste will be handled as per site procedures, solid waste is sealed in bags and placed in skips that are sent for on-site incineration.

Animals that are subject to terminal procedures with tissues being retained for histology and preserved in formaldehyde which inactivate any remaining viral material.
Carcasses are sealed in bags and placed in specific skips as per site procedures and sent for on-site incineration.

Blood samples are either handled on-site or plasma/serum are prepared and frozen for off site shipping or retained for further analysis. Off site shipping is handled by a dedicated shipping department and all local/national laws are followed with regards shipping preparation and documentation.

All cages and racking are cleaned on site in a custom designed cage washing facility by sterilisation/washing using standard detergents and cleansers that are known to inactivate the vector.

The genetically modified materials will be supplied from a sponsor company, with no genetic manipulation being carried out on the site. The predominantly closed environment in which they will be handled and non-replicating nature of these materials would imply that they are considered a low risk. The current site procedures are considered adequate to mitigate any potential risk.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Name

UNIVERSITY OF EDINBURGH

Name 2

MRC BRAIN METABOLISM UNIT

Campus Estate or Research Centre

ROYAL EDINBURGH HOSPITAL

Road Name

MORNINGSIDE PARK

Town

EDINBURGH

County

EAST LOTHIAN

Postcode

EH10 5HF

Country

SCOTLAND

Tel Number

0131 537 6533

Fax Number

0131 537 6110

E-mail

HSE Division

SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

### Level 1 (GMMs)

### Level 2 (GMMs)

### Level 3 (GMMs)

### Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

CANCER RESEARCH UK MANCHESTER INSTITUTE

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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<th>Date Premises Closed</th>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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</table>

Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref** 141/01.1

**Date Ackn'd** 19/02/2001

**CU2 Project Title** TO EVALUATE TRANSDUCTION EFFICIENCY OF LENTIVIRUSES

**Class** Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Date Project Ceased** 25/05/2017

**Non-GMM**

**Consent Granted** not applicable

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Project notified under transitional arrangements** Y

**Historical Significant Changes** Transferred to GM541
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**Project Ref** 141/01.2

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<td>HUMAN GENE THRERAPY -MODULATION OF CELL ACTIVITIES BY ANTI-APOPTOTIC PROTEINS</td>
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| Withdrawn     | N                                                       |       |                 |                       |          |                                          |
| Historical Significant Changes | Transferred to GM541 |       |                 |                       |          |                                          |

Tick if notifying a connected programme of work  N

Project notified under transitional arrangements  Y

Historical Date of Additional Info

Significant Date of Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? \( N \)

If yes, tick to confirm that it is attached to this form \( N \)

Tick to confirm that you have attached a risk assessment to this form \( N \)

Tick if you are claiming exemption from disclosure for section of the risk assessment \( N \)

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
This project intends to utilise the well characterised ability of Pseudomonas aeruginosa to form a biofilm mode of growth to establish whether bacterial biofilms could be used as a method to transfer proteins for gene therapy usage.

Pseudomonas aeruginosa has been shown to preferentially assume a biofilm mode of growth, embedding itself in a gelatinous organic polymer matrix composed primarily of alginate. There is a lack of understanding of the in situ regulation of biofilm matrix polymer formation nor whether the genetic promoters of the biofilm could be utilised to express non-biofilm genes in a formed biofilm. This project intends to address both aspects.

The contained use activity is required as part of this study involving vectors constructed at the Paterson Institute encoding the algC or the algD gene that encodes the enzyme phosphomannomutase or GDP-mannose dehydrogenase respectively that are both critical enzymes in the formation of the biofilm under the genetic control of the tetracycline response element or their natural promoters driving a non biofilm gene.

In the first experiments we must determine the activity our algC or algD promoter that has been inserted into our non-mobilisable vector. This can only be done in the wild-type strain (PAO1) as we require all the genetic components required for biofilm formation to be intact. Furthermore PAO1 is genetically identical to the delta strains of...
P. aeruginosa (PAO1 algC and PAO1 algD) that is also being used in this study. It is hypothesised that when the transformed wild-type P. aeruginosa is grown under conditions that will allow the formation of the biofilm that expression of a non biofilm reporter gene such as B-galactosidase or green fluorescent protein will increase as this will be placed downstream of our algC or algD promoter.

We will then compare this data to that acquired by the transformation of the PAO1 alg C and the PAO1 algD (avirulent defined mutants) with the algC or algD gene under the control of the tetracycline promoter where it is hypothesised that addition of tetracycline to the medium will allow the induction of a biofilm. The final stage of this project will then look at whether the algC or algD promoter can be utilised to drive expression of non-biofilm genes within this tetracycline induced biofilm.

Recipient or parental organism

Pseudomonas aeruginosa strain PA01. The most widely used laboratory stain with complete physical and genetic maps being available and the genome sequenced. The original strain was isolated from a wound in 1955 by B Hollowoway described in detail in 1969 in Bacteriology Review. The environmental bacterium is a gram negative prototrophic bacteria that like many is an opportunistic human pathogen that causes bacteraemia in burn victims, urinary tract infections in catheterised patients and is the predominant cause of morbidity and mortality in cystic fibrosis patients.

Host/vector system

All vectors are based on non mobilisable vector systems. They will either be pUC, pGEM, pBluescript, pSP (Promega), pPROTet (Clonetech) based vectors.

Origin & function

E. coli - galactosidase gene or Aquae Victoria green fluorescent protein (GFP) under the control of the O, aerygubisa algC or algD promoter that normally promotes the expression of phosphomannomulase or GDP mannose dehydrogenase respectively in the wild type organism.

Evaluation of foreseeable effects

The algC and algDpromoters regulate the formation of the P.aerugosa biofilm and so therefore it is foreseen that when the bacteria is grown under conditions to form a biofilm the algC and algD promoters will drive expression of the downstream B-galactosidase for GFP gene.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Animals will not be used in these experiments.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g gloves, plastic ware, agar plates, cell pellets) are rendered inactive by either autoclaving or disinfection using 1% Virkon and are subsequently incinerated along with clinical waste.

Liquid waste [culture & medium - volumes <5l] is disinfected using Virkon at either 2% or 3% for a minimum contact time of 1 hour prior to disposal to sewers.

Is an emergency plan required according to regulation 20? 

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
The committee have reviewed the amendments to the risk assessment 02/614 to include the use of Pseudomonas aeuringinosa strain PA01 as a host and agree with its conclusions.

### Project Containment

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### Project Ref 141/06.1

**CU2 Project Title**
Modulating signalling through the Notch and Wnt pathways in cell culture by adenoviral, lentiviral or retroviral transduction.

**Class**
Class 2

**CultureVolume**
< 1 Litre

**Non-GMM Consent Granted**
Not Applicable

**Date**

- **Date Ackn'd**: 27/01/2006
- **Date Project Ceased**: 19/10/2006

**Withdrawn**
N

**Project notified under transitional arrangements**
N

**Historical Significant Changes**
TRANSFERRED TO GM CENTRE 541.

### Project Additional Information

**Purposes of the contained use**
To evaluate the effects of activation or inhibition of expression of the Notch and Wnt signalling pathways in mammalian cell lines and mammalian cell lines. Notch and Wnt signalling pathways are highly conserved cell-fate determination pathways, and play a central role, at the cellular level, regulating morphology, proliferation, motility and cell fate. Abnormalities in the components of these pathways are associated with a number of developmental disorders and cancer. Modulating the expression...
of the pathways will help to understand the intricacies of the signalling system and understand the effects on cell development and cancer induction. If any of these translated sequences were over expressed, after self inoculation, in a target cell it could possibly result in transformation of that cell. Although this has not been proven, there is a risk factor associated with the production of vectors expressing such sequences.

Recipient or parental organism

Adenovirus serotype Ad5-Adenoviridae are classified as ACDP hazard group 2
Lentivirus - Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3
Retrovirus - MuLV based

Host/vector system

Non-viral systems
Bacterial Host-Escherichia coli k-12 derivaties (TG1, TOPO 10 series and DH5-alpha)
Plasmid vectors-pcDNA3.1, pBluescript (Strategene) and TOPO (Invitrogen) based plasmid vectors.

Viral systems
Adenoviral vectors deleted for E1 (cell transformation and tumourgenicity) and E3 genes (non-essential genes for in vitro growth). E1 is provided in trans by 293T packaging cell line.
Adenoviral vectors deleted of all viral lytic cycle genes. Coding sequence (except for E1) provided by helper virus and E1 provided by 293T cells.
Replication defective E1 vectors in themselves can be considered unlikely to cause disease according to the HSC’s advisory committee’s compendium of guidance on genetic modification.

Lentiviral and retroviral vectors are deleted of any viral expressed sequences. Genes for packaging provided by Transient plasmid transfection in to producer cells.

Origin & function

Reporter genes, including GFP, dsRed, Luciferase, will be used to assess modulation of the notch and wnt pathways in vivo.
Genetic material of human, murine, rat and Xenopus origin will elucidate the relative contributions of wnt and notch signalling on the differentiation of mesenchymal stem cells to chondrogenic, dipogenic or osteogenic lineages.
Due to the nature of the inserted genetic material (i.e oncogenic) containment level 2 controls and procedures will be employed in this project.

Evaluation of foreseeable effects

The Adenoviral, Retroviral and Lentiviral based vectors are replication incompetent. Thus whilst they pose an infection risk to humans and other species, they are unable to initiate further rounds of replication/infection cycles.

If self-inoculation occurs with adenoviral constructs the chances of seroconversion are minimal due to double deletion of genetic material from replication deficient plasmid.
The most likely course of events is that a localised immune response will clear the inoculated pathogen without any chances of further contamination. The retroviral and lentiviral vectors contain no viral genes so are essentially “gutless” shells. The chances of seroconversion are again minimal especially as the retrovirus is a murine pathogen.

The heterologous genes do not encode viral specific proteins, nor do they interfere with the known activities of the virus and as such are unlikely to have an effect upon the basic nature of the recombinant adenovirus and lent or retrovirus.
However since they are oncogenic the appropriate containment level to be used is CL2.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable
No animals will be used in this project

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste materials (e.g. gloves, plastic ware, agar plates, cell pellets are rendered inactive by autoclaving and are subsequently incinerated along with clinical waste. Surfaces are disinfected with 1% Virkon solution.
Plastic ware that can be effectively disinfected is treated with 1% Virkon solution for a minimum of 1 hour prior to incineration.
Glassware is treated with 1% Virkon for a minimum of 1 hour prior to cleaning.
Liquid waste (culture & medium - volumes < 5l) is disinfected by the addition of Virkon to give a 1% using Virkon at either 2% or 3% for buffered solutions (i.e. culture media) A minimum contact time of 1 hour is allowed prior to disposal to sewers.

Validation

Autoclaving

The discard autoclave is subject to regular three monthly service and annual validation. Records of service and validation are kept on site.

Disinfectant

For the modified bacteria, Lentivirus/retrovirus/adenovirus, Virkon is routinely used according to the manufactureres (Antek) guidelines. Antek report a 4 log kill for retrovirus at a dilution of 1:2000 i.e. 0.05% after 30 minutes. Our protocols require 2% & 1% Virkon which is excess of the validation concentration.

For bacterial systems we have carried out in-house tests and have shown that Virkon will provide effective kill at a concentration of 1% after 10 minutes contact time. We routinely allow a minimum contact time of 1 hour to ensure complete inactivation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC assessed the project as GM Class 2.
The following points were highlighted.
The production of virus to be carried out in a dedicated virus containment room.
The recovery of viral particles/DNA by ultracentrifugation would involve the use of sharps. Only suitably trained and experienced persons allowed to carry out this specific procedure.

Project Containment
<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
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**Project Ref** 141/06.2

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<tr>
<th>Date Project Ceased</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tbody>
<tr>
<td>25/05/2017</td>
<td></td>
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<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
<th>Historical Significant Changes</th>
<th>Historical Date of Additional Info</th>
<th>Significant Change ID</th>
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<td></td>
<td>Transferred to GM541</td>
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</tbody>
</table>

**Project Additional Information**

**Purposes of the contained use**

shRNA technology will be used to reduce the expression of genes of interest in primary murine haemopoietic cells and murine and human haemopoietic cell lines. Lentiviral vectors will be used to deliver the sequences of interest. Targets will be identified through proteomic assessment of proteins involved in stem cell biology and oncogenic transformation of haemopoietic cell lines. An example of such a target is the protein FMIP which is involved in M-CSF signalling.

**Recipient or parental organism**

Lentivirus parental organism - Human Immunodeficiency virus 1 (HIV-1) is classified as ACDP hazard group 3.

**Host/vector system**

Bacterial host - Escherichia coli k-12 derivatives (e.g DH5-alpha).
Plasmid vector - pSuper and Bluescript (Strategene) or similar based vectors which are Bom-,Mob- and Tra-
**Viral vectors** - Recombinant Lentiviral vectors pseudotyped with VSV-G envelopes will be used in 293T cells.

**Origin & function**

Origins - genes of interest will arise from studies in primary murine haemopoietic cells and murine and human haemopoietic cell lines.

Genes of interest will be identified using proteomic analysis of stem cell differentiation and the process of transformation by leukaemic oncogenes. Protein profiles will be obtained by Mass Spec analysis of differentiating cells and the consequences of leukaemic oncogene expression on the proteome will be assessed. This will enable the identification of proteins that change during these processes. shRNAi will then be designed to target these genes.

An example of such a target is the protein FMIP which is involved in M-CSF signalling.

It is not intended to target known tumour suppressor genes or genes that could be inferred as such.

The project will be reviewed on a six or twelve month basis, in this regard: the exact frequency being dependent on the progress of this work.

**Evaluation of foreseeable effects**

The recombinant viral vectors are highly disabled vectors based on the HIV lentivirus. These self inactivating vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk to humans and other species, they are unable to initiate further rounds of replication/infection cycles. The chances of seroconversion are minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability it is thought there is NO significant increase of the likelihood of transfection via airborne routes of exposure. Besides the control measures identified are appropriate to guard against the associated risks.

The target genes do not encode viral specific proteins, nor do they interfere with the known activities of the virus and as such are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

However since they are oncogenic by ACGM definition the appropriate containment level to be used is CL2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

No animals will be used in this project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Solid waste materials (e.g. gloves, plastic ware, agar plates, cell pellets are rendered inactive by autoclaving and are subsequently incinerated along with clinical waste.
- Surfaces are disinfected with 1% Virkon solution.
- Plastic ware that can be effectively disinfected is treated with 1% Virkon solution for a minimum of 1 hour prior to incineration.
- Glassware is treated with 1% Virkon for a minimum 1 hour before cleaning.
- Liquid waste (culture & medium - volumes <5l) is disinfected by the addition of Virkon to give a 1% using Virkon at either 2% or 3% for buffered solutions (i.e. culture media). A minimum contact time of 1 hour is allowed prior to disposal to sewers.

**Validation**

**Autoclaving**

The discard autoclave is subject to regular three monthly service and annual validation. Records of service and validation are kept on site.
Disinfectant
For the modified bacteria, and Lentivirus, Virkon is routinely used according to the manufacturers (Antek)

The GMSC assessed the project as GM Class2.
The following points were highlighted

- The recovery of viral particles/DNA by ultracentrifugation should not involve the use of sharps.
- The project needs to be reviewed on a 6 or 12 monthly basis, depending on the progress of the work, in the context of reviewing any genes of interest which may be of uncertain function, in case this affects the risk assessment in any way (e.g. unknown tumour suppressor genes)
- It was confirmed that the expression of shRNA may block normal self renewal and differentiation. It may also lead to the induction of cell death.

Please enter comments on the GM safety committee on the risk assessment

The project contains...
Retroviral vector systems will be used to transfect well-studied cell lines either transiently or to establish stable cell lines. The project hopes to elucidate further, the role of Rho GTPases in tumourgenesis.

pLZRS is derived from the Moloney murine leukaemia virus (MMLV); the env pol sequences being replaced by the LacZ gene; which in turn was replaced with a multiple cloning site, followed by an internal ribosomal entry sequence (IRES) and either neomycin or zeocin resistance gene.

The vectors used are disabled vectors based on the Moloney murine leukaemia virus. They are used in conjunction with eco or amphotrophic versions of the Phoenix cell line. The systems used are designed such that viral particles produced in the packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication. The chances of seroconversion is minimal.

From a functional perspective RAS is a "worst case scenario" being a known oncogene. Other oncogenes will also be used.

The target genes do not encode viral specific proteins, nor do they interfere with the known activities of the virus and as such are unlikely to have any effect upon the basic nature of the recombinant virus.

However since some of the target genes are oncogenic the appropriate containment level to be used is CL2.
Solid waste materials (e.g. gloves, plastic ware, agar plates, cell pellets) are rendered inactive by autoclaving and are subsequently incinerated along with clinical waste. Surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon solution for a minimum of 1 hour prior to incineration. Glassware is treated with 1% Virkon for a minimum of 1 hour prior to cleaning.

Liquid waste (culture and medium-volumes <5L) is disinfected by the addition of Virkon to give 1% using Virkon at either 2% or 3% for buffered solutions (i.e. culture medium). A minimum contact time of 1 hour is allowed prior to disposal via sewers.

Autoclave validation-the discard autoclave is subject to annual validation. Records of service and validation are kept on site.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

The GMSC assessed the project as GM Class 2.
The following points were asked for:

References for the target gene-supplied by author as Appendix 1
The project to be reviewed on a 6 or 12 monthly basis, depending on progress of the work.

Project Containment

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<thead>
<tr>
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<tbody>
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<td>L4</td>
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02/03/2022
We have identified a number of genes that are overexpressed in childhood leukaemias. One of these, a cathepsin, appears to be involved in B-cell differentiation and may be involved in cell adhesion and/or migration. We propose to study this by (i) transducing human leukaemic cell lines with lentiviral vectors (either expression or RNAi) (ii) transducing human haematopoietic cells obtained from cord blood. We hope to show that overexpression of the protein leads to an invasive phenotype in leukaemic cell lines and altered development in HSC’s.

Recipient or parental organism
Lentivirus parental organism - Human immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3.

Host/vector system
Bacterial host - BL21 (DE3)pLysS
Plasmid vector - Bluescript plasmids (Stratagene)
Viral vectors - A minimum of a 3-plasmid system will be used. A SIN-lentivector with a pseudotyped VSV-G capsule will be derived using 293T packaging cells.

Origin & function
Origins - gene of interest - The cathepsin, asparaginyl endopeptidase (AEP).
This is expressed at various levels during the normal maturation of a lymphoblast and is not oncogenic.
The cDNA has been cloned from a human cDNA pool and will be expressed in both its active and inactive forms and shRNA will be synthesised prior to insertion.
**Evaluation of foreseeable effects**

The recombinant viral vectors are highly disabled vectors based on the HIV-1 lentivirus. These self inactivating vectors have had regulatory and accessory genes deleted ensuring viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication / infection cycles. The probability of seroconversion is minimal. The VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability. It is thought that there is NO significant increase in the likelihood of transfection via the airborne route of exposure. The control measures utilised are appropriate to guard against this eventuality and associated risks. The target gene does not encode viral specific protein, nor does it interfere with known activities of the virus and so is unlikely to have any effect upon the basic nature of the recombinant lentivirus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No animals will be used in this project at this site.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. Virkon is routinely used as per manufacturer's recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3 % solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations). The autoclave undergoes annual validation. Records are kept on site. |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment
The GMSC assessed the project as GM Class 2.

It was noted that there is considerable experience of using such lentiviral vector systems are already in the Institute. The project needs to be reviewed within 12 months, depending on the progress of the work, to ensure that the risk assessment remains valid.

Responsibility as defined in legislation lies with the Head of of research group. They have managerial control and responsibility for ensuring suitable and sufficient risk assessments are undertaken and that work is conducted in accordance with Institute Codes of Practice, which have been drawn up to ensure compliance with HSE guidance.

**Project Containment**

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Animal Units

- Large Scale Activities
- Human Clinical Applications

**Project Ref 141/07.2**

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<td>29/11/2007</td>
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<td>invasive carcinomas.</td>
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Non-GMM Consent Granted

- Not Applicable

Project notified under transitional arrangements

- N

Withdrawn

- N

Tick if notifying a connected programme of work

- N

Historical Significant Changes

- Transferred to GM541

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**
We wish to investigate the roles of non-cancer cells within tumour. It is already known that these non-carcinoma cells promote tumour growth. We intend to culture the non-cancer cells and modify expression levels of particular candidate genes using lentiviral siRNA and retroviral cDNA expression vectors. This technology will help us elucidate molecular mechanisms by which the non-carcinoma cells promote tumour growth.

Recipient or parental organism

Lentivirus parental organism- Human Immunodeficiency virus-i (HIV-1) is classified as ACDP hazard group 3 Moloney Murine Sarcoma virus (MMSV) parental organism - hazard group I. Causal agent of sarcoma in mice and rats.

Host/vector system

Bacterial host- E. Coli- XL1O Gold, Stbl3, and DH5 alpha.

VECTORS: PLKO1 siRNA lentiviral vector; pWPI lentiviral cDNA expression vector; pHAGE-CMV-eGFP lentiviral expression vector; PBabe retroviral cDNA expression vectors (puro! Neo!Hygro!Zeomycin); pWZL Blast retroviral cDNA expression vector. Retroviral expression vectors MSCV IRES GFP; pLV-rTRIKRAB-red; iDuetl 01 (tetEF. GFP. PGK. hygro) - MI NON-mobilisable. Viral vectors/ Host systems- SEE ADDITIONAL SHEET.

Origin & function

Genes of interest are: Human SDF-1/CXCL12 cDNA; human CXCR4 cDNA; human CXCR7 cDNA; humanTGF-beta cDNA; human EphB2 cDNA; human p16INK4A cDNA.

The first 4 genes and their gene products play a role in tumour progression and proliferation. EphB2 and p16INK4 play a role in the regulation of cell positioning and proliferation.

More detailed functional information for the above are given in appendix 2 of the risk assessment.

Evaluation of foreseeable effects

The recombinant lentiviral vector utilised is a highly disabled vector based on HIV. It is a self inactivating vector which has had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells ( HEK 293T) are replication incompetent. Thus whilst they pose an infection risk; in that they can integrate in to the chromosome, they are unable to pop-out and initiate further rounds of replication/ infection cycles. The probability of seroconversion is thought to be minimal.

Whilst the VSG envelope extends the cellular iropism and confers greater environmental survivability, it is thought that there is no increase in the likehood of transfection via the airborne route of exposure. Besides, the control measures utilised for this work are appropriate to guard against the associated risks.

It is worthy of note that the HIV specific envelope protein that targets CD4 T cells is exchanged for the ubiquitous VSV-g (Vesicular stomatis virus C protein), which makes it more visible to the immune system.

The target genes do not encode viral specific proteins, nor are they likely to interfere with known activities of the virus and so are unlikley to have any effect upon the baisc nature of the recombinant lentivirus.

Likewise the recombinant Moloney reirovirus used ( MMSV) is similarly disabled and is replication incompetent. MMSV is thought to be fragile and has poor environmental survivability capabilities. Again workplace precautions and controls will minimise the likehood of risk to human health and the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materilas ( e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incinercation.
Virkon is routinely used as per the manufacturer’s recommendations: Solid surfaces are disinfected with 1% Virkon solution.
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Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer’s recommendations).
The autoclave undergoes annual validation. Records are kept on site.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

The GMSC agreed with the classification of this project as GM Class 2. They asked the principal investigator (P1) to provide details of the genes of interest; in order to better consider the risks to human health and the environment. These are given in Appendix 2 and 3 of the risk assessment.

**Project Containment**

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<td>L4: L2</td>
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**Animal Units**

- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

**Project Ref** 141/08.1

**Date Ackn’d** 21/05/2008  
**CU2 Project Title** Knockdown of genes in normal haematopoietic and leukemic stem cells.

**Date Project Ceased** 25/05/2017  
**Class** Class 2  
**CultureVolClass2** < 1 Litre  
**CultureVolumeClass3-4**

**Non-GMM** Not Applicable  
**Consent Granted**

**Project notified under transitional arrangements** No
Microarray experiments previously performed have identified a number of candidate genes that may be critical regulators of normal haematopoietic and/or leukaemia stem cells (LSC5). This project seeks to evaluate the role of these genes in these cell types in murine and human model systems using genetic knockdown experiments. Briefly, murine or human bone marrow cells will be transduced using lentiviral vectors containing shRNAs and cells will then be functionally evaluated using a combination of in vitro and in vivo techniques, the latter involving transplantation of cells into mice. Three lentiviral vector systems will be used: (i) pLB, which provides constitutive expression of shRNAs from a Pol III promoter, (ii) pSicoR, which provides constitutive expression of shRNAs from a Pol II promoter with the option of irreversible Cre-mediated conditional inactivation of knockdown, and (iii) pSLIK, which provides the option of inducible knockdown from a tetracycline-responsive promoter.

**Recipient or parental organism**

Lentiviral parental organism- Human Immunodeficiency virus-i (HIV-i) is classified as ACDP hazard group 3

**Host/vector system**

Bacteria host:
- E. coli Stbl3 (for generating lentiviral system plasmid)
- E. coli DH5alpha (for all other plasmids)

Lentiviral vector systems:
- pLB- see appendix 1 risk assessment
- pSicoR- see appendix 1 risk assessment
- pSLIK- see appendix 3 risk assessment

Used with 293 FT cells

**Origin & function**

Origins- genes of interest- Human

Microarray experiments have identified the genes of interest as candidates for regulation of normal haematopoietic and/or leukaemia stem cells (LSC5).

These include:
- Bbx- an HMG-box transcription factor of unknown function
- Bmprl- a TGFβ family receptor
- Transcription factors- Myb; E2f6; Mycn
- Cell cycle regulatory proteins Cdk1,2 and 6
- RNA binding protein- Igf2bp3
- Chromatin regulatory proteins- Arid2; Cbx 5; Hmgb3; Smarc2 and 5
The recombinant viral vector vectors are highly disabled vectors based on the HIV lentivirus. They are self inactivating vectors, which have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal (Details of the origins and deletions within the lentiviral constructs are given in appendix 1 and 3 of the risk assessment).

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated risks. The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Knock-down of some of the target genes which are cell cycle inhibitors or tumour suppressors may accentuate oncogenesis. Whilst knock-down of others, which are potential oncogenes, may inhibit oncogenesis.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Knock-down of some of the target genes which are cell cycle inhibitors or tumour suppressors may accentuate oncogenesis. Whilst knock-down of others, which are potential oncogenes, may inhibit oncogenesis.

The VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of Virkon); work carried out in containment 2 laboratories or Home office inspected animal facilities; work within Class2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Whilst no transgenic mice are generated in this project; transduced cells will be transplanted in to C571 BL6 mice by intravenous injection. This procedure will be carried out by specifically trained staff only. The Biological Resource unit (BRU) houses all mice in sealed individually ventilated cages. All handling is within cabinets. The rooms are secured with sealed drains and close sealed doors. There are no open ducts within the room. Corridors are sealed with electronically locking flush fitting doors. All corridor risers are sealed. The unit is inspected regularly by Home Office inspectors to ensure that it meets the required standards.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site.

Virkon is routinely used as per the manufacturer’s recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation).

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y
A query as to the risk to workers from any free virus whilst administering transduced cells to mice was raised. This is answered in appendix 8 of the risk assessment.

The need to refer workers to the Institute GM Code of Practice and the local Virus room Code of Practice was raised. This was subsequently inserted in to section 7 vi and section 9.2 of the risk assessment.

The GMSC agreed that it was a GM Class 2 project.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
<tr>
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<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
</tr>
</tbody>
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**Project Ref 141/08.2**

Date Ackn'd: 05/06/2008
CU2 Project Title: Initiating leukaemia with primary human bone marrow cells.

Date Project Ceased: 25/05/2017

Class: Class 2
Culture Vol: Class 2 < 1 Litre

Non-GMM Consent Granted: Not Applicable

Project notified under transitional arrangements: N

**Historical Significant Changes**

Transferred to GM541

**Project Additional Information**
# Purposes of the contained use

The project aims to investigate the biology of human leukaemia stem cells (LSCs) using a xenogeneic murine model system. Briefly, leukaemia is experimentally initiated in mice by infecting primary human bone marrow cells with retroviruses which carry oncogenes that are associated with human leukaemia. The transduced bone marrow cells are then transplanted in to irradiated recipient immune deficient mice.

## Recipient or parental organism

Murine Stem Cell Virus (MSCV) viral vector system was derived from the Murine Embryonic Stem Cell Virus (MESV) and the LN retroviral vectors (first described in Hawley et al., (1992) Journal of Experimental Medicine 176:1149). The vectors achieve stable, high-level gene expression in hematopoietic and embryonic stem cells through a specifically designed 5' long terminal repeat (LTR). This LW is from the murine stem cell PCMV virus, and it differs from the M0MuLV LTR used in other retroviral vectors by several point mutations and a deletion. These changes enhance transcriptional activation and prevent transcriptional suppression in embryonic stem and embryonal carcinoma cells. As a result, the LTR drives high-level constitutive expression of a target gene in stem cells.

## Host/vector system

<table>
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<tr>
<th>Bacterial host:</th>
<th>E.coli DH5 alpha (for amplifying plasmid stocks)</th>
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</thead>
<tbody>
<tr>
<td>Viral vector system:</td>
<td>MSCV used with Phoenix A and Phoenix GP cells</td>
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## Origin & function

<table>
<thead>
<tr>
<th>Origins- genes of interest- Human</th>
<th>Oncogenes associated with human leukaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Function of genes of interest- oncogenes associated with human leukaemia</td>
<td>A full list of these genes is given in appendix 2 of the risk assessment</td>
</tr>
</tbody>
</table>

## Evaluation of foreseeable effects

The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication! infection cycles. The probability of seroconversion is minimal. (Details of the origins and deletions within the MSCV constructs are given in appendix 1 of the risk assessment). Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated risks.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus. Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of Virkon); work carried out in containment 2 laboratories or Home office inspected animal facilities; work within Class2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Transduced cells will be transplanted in to immune deficient mice by intravenous injection. This procedure will be carried out by specifically trained staff only. The Biological Resource unit (BRU) houses all mice in sealed individually ventilated cages. All handling is within cabinets. The rooms are secured with sealed drains and close sealed doors. There are no open ducts within the room. Corridors are sealed with electronically locking flush filling doors. All corridor risers are sealed. The unit is inspected regularly by Home Office inspectors to ensure that it meets the required standards.

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site.

Virkon is routinely used as per the manufacturer’s recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

Liquid waste (culture and medium) is disinfected by the addition of Viricon to give a 1% final working concentration. 2% or 3% solutions of Viricon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation).

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

A query as to the risk to workers from any free virus whilst administering transduced cells to mice was raised. This is answered in appendix 5 of the risk assessment.

The need to refer workers to the Institute GM Code of Practice and the local Virus room Code of Practice was raised. This was subsequently inserted in to appendix 6 of the risk assessment.

The GMSC agreed that it was a GM Class 2 project.

Project Containment

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<th>Growth Rooms</th>
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<td>Animal Units</td>
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<td>L2 L3 L4 L2 L3 L4</td>
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</table>

Project Ref 141/08.3
### Project Additional Information

**Purposes of the contained use**

Recombinant Lentiviruses will be used to express genes involved in stress signalling pathways in primary mouse cells and mammalian cell lines. The gene products may be involved in signal transduction eg p3BMAPK and JNK, or downstream target genes eg the dusp family of dual-specificity phosphatases. We will not use Lentiviruses to express genes that we could reasonably predict to have potentially harmful effects in humans eg oncogenes and pro-inflammatory cytokines.

The transduced primary mouse cells will be transplanted into mice and investigated in in vitro systems. Mouse, mouse primary cells and mammalian cell line responses to environmental stresses (e.g. chemotherapeutic agents) will be evaluated.

**Recipient or parental organism**

Lentiviral parental organism- Human Immunodeficiency virus-I (HIV-1) is classified as ACDP hazard groups

**Host/vector system**

Lentiviral vector System:
- pCMV-VSVG
- pMDL9/RRE
- pRSV-REV
- pRRL

Host : HEK 293 cells

**Origin & function**

cDNA5 encoding:
- p38 MAPKalpha, JNK1, JNK2, ERKI, ERK2 duspi, dusp4, dusp8, dusplO
- pten

---

**Expression of stress response pathway components using recombinant Lentivirus.**

08/07/2008

25/05/2017

Date Ackn'd

CU2 Project Title

Expression of stress response pathway components using recombinant Lentivirus.

Date Project Ceased

Class

Consent Granted

Class Culture Vol

< 1 Litre

Not Applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Historical Significant Changes

Transferred to GM541

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Page 3975 of 15326

02/03/2022
ppm2c, ppmib
These genes are signal transduction components, either kinases or phosphatases.
The origins of these genes are murine and human

**Evaluation of foreseeable effects**

The recombinant viral vector vectors are highly disabled vectors based on the -(IV lentivirus. They are self inactivating vectors, which have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication? infection cycles. The probability of ser000nversion is minimal
Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated risks. The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building ( either by autoclaving or use of Virkon); work carried out in containment 2 laboratories or Home office inspected animal facilities; work within Class2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The Biological Resource unit (BRU) houses all mice in sealed individually ventilated cages. All handling is within cabinets. The rooms are secured with sealed drains and close sealed doors. There are no open ducts within the room. Corridors are sealed with electronically locking flush fitting doors. All corridor risers are sealed. The unit is inspected regularly by Home Office inspectors to ensure that it meets the required standards.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site.
Virkon is routinely used as per the manufacturer’s recommendations:Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. My contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration.
2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers( as per manufacturer’s recommendation).

Is an emergency plan required according to regulation 20? Y
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form N
Tick if you are claiming exemption from disclosure for section of the risk assessment
The GMSC agreed that this was a GM Class2 project. They asked that the COSHI-I assessment be completed for the use of cell lines in this body of work. This has been done and is enclosed.

### Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
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### Project Ref 141/08.4

- **Date Ackn'd**: 23/12/2008
- **CU2 Project Title**: Knockdown/overexpression of genes in embryonic stem cells and its (haematopoietic) progeny and murine cell populations
- **Class**: 2
- **CultureVolClass2**: < 1 Litre
- **CultureVolumeClass3-4**: Non-GMM
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: Transferred to GM541

### Project Additional Information

**Purposes of the contained use**

Previous studies, such as microarrays, have identified a number of candidate genes that may be critical regulators of normal haematopoietic cells development. This project seeks to evaluate the role of these genes in murine model systems using genetic knockdown of forced expression experiments. Briefly, murine cell populations will...
be transduced using lentiviral vector, retroviral vectors or transfected with normal plasmids containing shRNAs or the cDNA of the gene of interest. The cells will then be functionally evaluated using a combination of in vitro and in vivo techniques, the latter involving transplantation of cells in to mice.

**Recipient or parental organism**

- Lentivirus parental organism- Human Immunodeficiency virus-1 (HIV-1 is classified as ACDP hazard group 3
- Murine stem Cell Virus- derived from the Murine Embryonic Stem Cell Virus (MESV) and the LN retroviral vectors (first described in Hawley et al, (1992) Journal of experimental medicine 176:1149

**Host/vector system**

- Recombinant lentiviral vectors pseudotyped with VSV-G envelopes, will be used with 293T cells
- MSCV used with Phoenix A and Phoenix GP cells

**Origin & function**

Candidate regulator genes for normal haematopoietic cell development will be obtained in the form of cDNA originating from murine genomic or RNA libraries

**Evaluation of foreseeable effects**

The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal (details of the origins and deletions within the MSCV and lentiviral constructs are given in appendix 1 of the risk assessment).

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated residual risks.

The target genes do not encode viral specific protein, Nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of Virkon) work carried out in containment 2 laboratories or home office inspected animal facilities; work within class2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site.

Virkon is routinely used as per the manufacturer’s recommendations –

- Solid surfaces are disinfected with 1% Virkon solution.
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- Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation).

A query as to the risk to workers from any free virus whilst administering transduced cells to mice was raised. This is answered in appendix 4 of the risk assessment.

The committee agreed it was a GM Class 2 project.

### Project Containment

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<th>Large Scale Activities</th>
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**Project Ref** 141/09.1

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<td>02/03/2022</td>
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</table>
Purposes of the contained use

Viruses have been explored as agents to target cancer either in a gene therapy setting (through the delivery of cytotoxic gene payloads or alike) or as attenuated strains which replicate within tumour cells leading to the subsequent destruction of that cell. The replication competent virus approach is being explored in the clinical setting with a number of different viruses including reovirus and adenoviruses. Whilst attractive, there are a number of potential barriers which the viruses need to efficiently overcome in order to drive tumour destruction including efficient tumour cell targeting, infection and replication. This project seeks to use gene-modified replication competent viruses to investigate whether the approach can be improved in model systems.

Recipient or parental organism

Adenovirus type 5 (Replication Competent): ACDP Hazard Group 2
The actual genetic modification work will be carried out by colleagues in The Netherlands. In this project the already modified virus will be used for in vitro and in vivo (mouse) experiments.
The modification removes the ability for the modified virus to infect via the coxsackie B adenoviral receptor (CAR).
The modification enables the virus to infect cells via the Carcinoembryonic antigen (CEA), which is highly expressed in a number of solid tumours, but only in the luminal side of the gastro intestinal tract of a normal human adult. Thus the tropism of the virus has been affected. This characteristic of the virus targeting CEA is realised by the incorporation of a 58 aa affibody specific for the tumour antigen.
The immunogenicity of the affibody is currently unknown - as such, if immunogenic, it may further drive the immune response against the virus.

Host/vector system

Not applicable - the already modified virus will be supplied by collaborators based in the Netherlands

Origin & function

Gene-modified adenovirus targeted to CEA. The gene modification involves the deletion of amino-acids within the fibre domain which disrupts the normal infective route of the virus (through CAR) and incorporates an affibody for targeting. An affibody is effectively a very small antibody type domain that is small enough to permit efficient expression on the adenoviral fibre without significantly reducing viral titre. Viruses directed to the Her2/neu antigen have been described (Myhre S et al. (2009) Gene Therapy 16:252-261). In effect, these modifications of the virus re-direct the virus to target known tumour antigens thereby improving the specificity of targeting of the virus.
Origin of the affibody.

Affibodies have been generated using combinatorial protein approaches - effectively the antigen binding domain was generated from synthesised library cDNA's and tested using a screening approach to bind antigen. The cDNA of the affibody was then cloned into the H1 loop of the adenoviral fibre domain which contains mutations destroying natural CAR binding and then introduced into the Ad5 genome by homologous recombination replacing the endogenous Ad5 wild-type fiber. The Ad5 genome is then used to generate functional viruses using standard methods.

Evaluation of foreseeable effects

The virus is essentially wild-type but with mutations of the fibre domain which prevents binding to the normal virus receptor (coxsackie adenovirus receptor - CAR) and incorporation of a 58 aa affibody specific for the tumour antigen. Initially, the target antigen used will be Carcinoembryonic antigen (CEA) which is highly expressed on a number of solid tumours but only within the luminal side of the gastro-intestinal tract of the normal human adult. The fibre attenuation prevents the normal route of viral infection and the CEA specificity will result in targeting to CEA expressing cells. These viruses have only recently been generated and part of this proposal is to test the level of specificity of infection driven by the affibody.

The affibody/fibre mutations will modify the range of cell the virus will be able to infect. Normal targeting should be severely reduced and targeting of target antigen cells increased; consequently, this will alter the tropism of the virus. The mutations will otherwise have no major effect upon the individuals except that the immunogenicity of the affibody is currently unknown - as such, if immunogenic, it may further drive the immune response against the virus.

Currently we do not have any information on the shedding of the modified virus during animal experiments. As the virus lacks CAR binding, it is possible that more virus could be shed since there isn't the extensive pool of cells to soak up the virus. However, all work using this virus will be performed in the class 2 containment room within BRU. Waste disposal procedures are stringently adhered to (see section 12).

Further viral modifications may be generated at a later date. But these will be submitted to the local GMSC for consideration.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste material (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration.

Virkon is routinely used as per the manufacturer's recommendations:-
Solid surfaces are disinfected with 1% Virkon solution.
Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration.
Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.
Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration.
2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

The autoclave undergoes annual validation. Records are kept on site.
With regard to the animal work: clarification on viral shedding and treatment of waste bedding was asked for, the information provided was supplied and incorporated in to the risk assessment.

### Project Containment

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### Project Ref 141/09.2

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<td>RNAi knockdown of genes involved in stress responses using the BLOCK-iT Lentiviral miR RNAi Expression System from Invitrogen</td>
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Purposes of the contained use

We wish to study the role of the transcription factor ATF2 in tumour cells. By knocking down the expression of ATF2 in a variety of cell lines, we will assess the contribution ATF2 makes to the transformed phenotype. Knockdown will be achieved using Lentiviral based gene transfer. Genetic modifications include the sub-cloning steps involved in the production of a Lentiviral expression plasmid, co-transfection of this expression vector together with packaging vectors into a host cell line to produce virus, and subsequently, use of Lentiviral stocks to infect target cell lines that will stably express the miRNAi of interest.

Recipient or parental organism

Lentiviral parental organism - Human Immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3

Host/vector system

Bacterial host - E.coli XL1-Blue
Plasmids - all have pUC origin of replication and all are mobilisation defective.

Lentiviral vector system- commercially available BLOCK-it Lentiviral miR RNAi Expression System from Invitrogen.
HEK 293 cells is packaging cell line.
The ’Block-It Lentiviral miR RNAi expression system’ (Invitrogen cat no K4937-00) is a four plasmid virus production system and consists of:

pLenti6/V5 DEST - packaging vector into which the target sequence of interest is sub-cloned.
pLP1 - encodes Gag (viral structural protein) and Pol (viral replication protein).
pLP2 - encodes Rev (viral transcription factor)
pLP/VSVG - encodes the VSV-G glycoprotein for production of pseudotyped virus.

Target cells for viral vector are:- Ramos, Raji, Nmalwa, SudHL4, CRL 2261

Origin & function

ATF2 - Human - both tumour suppressing and tumour promoting phenotypes have been described for ATF2 gene. It functions as a transcription factor.

Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors based on the HIV lentivirus. They are self inactivating vectors, which have had regulatory and accessory genes deleted, ensuring that viral particles produce in packaging cell lines are replication incompetent. This whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated risks.

The target gene does not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control
measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of Virkon); work carried out in containment 2 laboratories or Home office inspected animal facilities; work within Class 2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Animal work will be carried out by trained staff only. Intravenous injection of cell line satably expressing miRNA of interest only. NO live virus will be injected in to mice, therefore shedding of virus is not an issue.

The Biological Resource unit (BRU) houses all mice in sealed individually ventilated cages. All handling is within cabinets. The rooms are secured with sealed drains and close sealed doors. There are no open ducts within the room. Corridors are sealed with electronically locking flush fitting doors. All corridor risers are sealed. The unit is inspected regularly by Home Office inspectors to ensure that it meets the required standards.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-pont thermocouple tests on an annual basis. Records are kept on site.

Virkon is routinely used as per the manufacturer's recommendations:-
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Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimum contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturers recommendation).

Dead mice carcasses are disposed of via the clinical waste route.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Since the project employs a lentiviral system with a gene that shows both tumour promoting and suppressor activities it was thought GM Class 2 was appropriate and this agreed with the applicants classification.

**Project Containment**

02/03/2022
# Project Ref

**141/10.1**

## Purposes of the contained use

Human tumours are frequently found to contain activating mutations in the ras family of oncogenes. Expression of oncogenic ras in cells leads to aberrant signalling and acute activation of the AP-1 family of transcription factors. We wish to study the signalling events occurring downstream of oncogenic ras and subsequent changes in gene expression with a particular focus on ATF2-dependent gene expression. To do this we need to differentiate between events occurring early (within a few hours), after ras expression, and those occurring later (several days). This will be achieved by generating high-titre recombinant adenoviruses that express oncogenic ras. This allows the synchronous infection of all the cells in a sample followed by a rapid onset of ras gene expression (within 8 hours). Given the hazardous nature of the ras oncogene we are proposing to undertake and emplou a range of control measures, as detailed in the risk assessment.

## Recipient or parental organism

**Adenovirus:** ACDP Hazard Group 2
**Host/vector system**

Bacterial Host; E. coli X-l1 or DH5alpha

Vector systems: pCDNA series, pBluescript, pUC, pAxwit2 (TaKaRa), pAxCAwtit (TaKaRa)

Viral System: Adenovirus/Hek293 cells

Target cells: Human and murine fibroblasts and hepatoblasts, A549 and HeLa

**Origin & function**

Human cDNA encoding HRasG12V, KRasG12V. These are oncogenes frequently found to be mutated in human cancers. Their expression causes constitutive activation of MAPK and P13K signalling pathways and acute activation of the AP-1 family of transcription factors. We will carry out experiments intended to gain a better understanding of their role in tumourigenesis of interest and these cause aberrant expression and acute activation of the AP-1 family of transcription factors.

**Flp recombinase. Required to initiate a recombination event to remove the STOP cassette and allow expression of ras in cells co-infected with recombinant ras-Adenovirus**

**Evaluation of foreseeable effects**

The recombinant Adenovirus lacks the E1 region and so is replication-incompetent. However, it is possible that an adverse recombination event occurring within the 293 packaging line, (or theoretically in human tissues, since adenovirus is ubiquitous in humans), could lead to generation of a recombination-competent virus (RCV). We plan to use a well characterised PCR-based assay to screen our virus stocks for RCV (indicated by the presence of the E1a gene). We will carry out this test each time we expand the virus. Furthermore we will check our stocks by infection of either HeLa or A549 cells which do not contain the E1 region. Only if RCV is present will this lead to cytopathic effects in the infected cells.

The recombinant Adenovirus possesses a STOP cassette located upstream of ras which essentially prevents its expression. Gene expression requires removal of the STOP cassette by a recombination event mediated by Flp recombinase. Flp will be expressed by means of a second recombinant adenovirus. Therefore, ras gene expression only occurs if both the ras-expressing virus and the Flp-expressing virus co-infect the same cell. This will significantly reduce the risk of workers accidentally expressing oncogenic ras in their own tissues. We will undertake not to handle open tubes of the ras-expressing and the Flp-expressing virus stocks at the same time.

Stringent disinfection regimes are applied in the laboratory along with autoclaving of all solid contaminated waste. The use of biosafe rotors and microbiological safety cabinets are also employed to protect human health and the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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The autoclave undergoes annual validation by a third party. Records are kept on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee asked for additional information on the STOP cassette and the possibility of read-through: the techniques employed to detect replication competent adenovirus; and whether both viruses are handled at the same time. This additional information is included in the Appendices 2 and 3 of the risk assessment

Project Containment

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Animal Units

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Project Ref 141/11.1

Date Ackn'd | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4 | Date Project Ceased | Project notified under transitional arrangements |
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<td>25/01/2011</td>
<td>Use of lentiviral vectors to investigate the biological function of 5T4 and related molecules</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
<td>25/05/2017</td>
<td>N</td>
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**Project Additional Information**

**Purposes of the contained use**

5T4 and related molecules will be introduced or knocked down in cell lines to investigate their biological functions. These goals will be achieved with the use of lentiviral vectors which are able to infect dividing and non-dividing cells and stably integrate into their genome. The vectors will encode the desired genes under constitutive or inducible promoters. Gene knock down will be achieved by the use of vectors encoding for shRNA against target genes. The system for lentiviral vector production is available from Invitrogen. The system utilises SIN (self inactivating) vectors which have had regulatory and accessory genes deleted to ensure that viral particles produced in packaging cell lines are replication incompetent.

**Recipient or parental organism**

Lentiviral parental organism- Human Immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3.

**Host/vector system**

Bacterial: E. coli One Shot Stbl3
Plasmids: pLP, pcDNA, pLenti, pENTR, pDONR, pMDLg/pRRE, pRSV-Rev and pMDg.2 plasmid vectors for packaging of lentiviral particles.

**Origin & function**

Human and mouse 5T4 coding sequence cloned from cDNA of respective species and is deposited in NCBI database. The sequences of other 5T4 related genes will be cloned from cDNA obtained from human or mouse cell lines. siRNA sequences targeting genes of interest will be designed and/or purchased from Invitrogen. Fluorescent protein genes are commercially available. High expression of 5T4 is very strongly expressed in normal placental trophoblast. We therefore believe 5T4 is “Oncogenic by ACGM definition” and this is highlighted in the risk. Assessment (section 7 (iv))

**Evaluation of foreseeable effects**

The recombinant viral vectors are highly disabled vectors based on the HIV lentivirus. These self inactivating vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular topism and confers greater stability and environmental survivability it is though that there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the control measures utilised are appropriate to guard against the associated risks.

The target gene does not encode viral specific protein, nor does it interfere with known activities of the virus and so is unlikely to have any effect upon the basic nature of the recombinant lentivirus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
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The autoclave undergoes annual validation. Records are kept on site

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Committee agreed with the GM Class 2 classification of the work.
It asked if 5T4 was expressed in foetal cells. 5T4 is expressed in normal placental trophoblasts although the risk to any pregnant females is considered to be very low, due to the nature of the viral vector system used which generates replication competent viral particles. No pregnant females work on project. This will be kept under review.

Project Containment

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>

02/03/2022
Project Additional Information

Purposes of the contained use

We seek to investigate the role of normal and activated fibroblasts in tumour-stromal interactions upon knockdown or overexpression of genes of interest. Fibroblast are known to exert tumour-promotive as well as tumour-suppressive functions depending on cell and expression level context. We intend to co-culture fibroblast cells with breast cancer cell lines and analyse proliferation, migration and molecular markers with Western blot, real-time PCR and Elisa.

Recipient or parental organism

Lentivirus parental organism - Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.

Retroviral system parental organism - Moloney Murine Leukaemia virus (MMLV)

Host/vector system

Bacterial host: E. coli DH5 alpha and Stbl3

Viral vector/Host system - 3 plasmid based systems will be used

Lentiviral system is a self-inactivating lentivector system with a pseudotyped VSV-G capsule. Viral particles will be produced using HEK 293T packaging cell line.

Retroviral system: pBABE system with packaging plasmids pCMV-VSVG and pUMVC3-gag-pol. Viral particles produced are replication incompetent. System is available
from Adgene. (More detailed descriptions of vector system characteristics are given in Appendix 1 of risk assessment)

Target cell lines for recombinant virus are Fibroblast cell lines 522 and 544 (see Appendix 4 of risk assessment) and breast cancer cell lines (MCF7, T47D, ZR75.1, MDA-MB-231, MDA-MB-468). Other tumour, non-tumour epithelial and stromal cell may be investigated. Amendment and GMSC approval would be sought beforehand.

### Origin & function

Human genes TGF beta receptor2 has suspected tumour promoter and suppressor functions. See appendix 2 of risk assessment for more detail. (Obtained from colleagues in PLKO1 plasmid as a knockdown). Lentiviral vector system will be utilised to generate knockdown versions of this gene; whilst the MSCV retroviral system will be used to over express these genes in the target cells.

Cyclin D1 is a tumour promoter gene (cDNA from Adgene) will be knocked down and over expressed with the same systems as above; within the breast cancer cell lines given in section 4 of the risk assessment.

### Evaluation of foreseeable effects

The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, control measures utilised are appropriate to guard against the associated risks.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

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The autoclave undergoes annual validation by a third party company. Records are kept on site.
The committee agreed with the GM Class 2 classification.

**Project Containment**

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- **Animal Units**
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- **Large Scale Activities**
  - L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Human Clinical Applications**
  - L2 L3 L4 L2 L3 L4 L2 L3 L4

**Project Ref** 141/11.3

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<td>11/07/2011</td>
<td>The use of lentiviral vectors in the study of Rho GTPases abd Rho associated proteins</td>
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- **Date Project Ceased**: 25/05/2017
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- Transferred to GM541
- Project notified under transitional arrangements: N
# Project Additional Information

## Purposes of the contained use

The work involves the study of Rho proteins (a family of GTPases involved in many cell signalling functions), their regulators and pathway-related genes along with their mutant forms. Frequently we use either overexpression or RNAi knockdown as a method of study. We have identified some important cell lines where lentiviral transduction may be required to get acceptable expression levels either transiently or to produce stable cell lines.

## Recipient or parental organism

Lentivirus parental organism - Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.

## Host/vector system

- **Bacterial host:** E. coli NEB 10-beta (DH10B derivative) from New England Biolabs
- **Viral vector/Host system:** 3 plasmid based systems will be used from sigma and/or Invitrogen (PLkO.1 and pLenti respectively).

Lentiviral system is a self-inactivating lentivector system with a pseudotyped VSV-G capsule. Viral particles will be produced using HEK 293T packaging cell line.

**Target Cells:** Well established cell lines such as MDCK II (dog) and human cancer cell lines (e.g. breast cancer cell line MDA 231 and osteo-sarcoma cell line HOS and MG63).

## Origin & function

Cloning will typically be subcloning of existing vectors (pcDNA) or if not available from a cDNA library. This will include versions of mammalian species including human, mouse and dog. Target siRNA sequences are designed from existing databases.

## Evaluation of foreseeable effects

The recombinant Lentiviral viral vector systems consist of disabled vectors. They have had HIV regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. The systems employed will be Self Inactivating (SIN) vector systems. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the risk control measures utilised are appropriate to guard against the associated risks. These include the use of safety cabinets, biosafe rotors and prescribed disposal routes for waste, wearing of appropriate PPE and no use of any sharps.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment

The committee asked for additional information in the form of maps of the lentivector systems which are to be employed. These were supplied - see maps attached to risk assessment.

The committee agreed with the GM Class 2 classification

**Project Containment**

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**Project Ref** 141/11.4

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<tr>
<td>04/10/2011</td>
<td>Constitutive and Inducible Protein Knockdown and Overexpression of Drug Targets</td>
<td>Class 2</td>
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Novel molecular based therapeutics work by targeting enzymes, such as kinases or substrate transporters. However, these drugs frequently have off-target effects and it is important to know exactly what the mechanism of action of the drug is. To demonstrate that a drug is acting through its main target one can either knock-down the target by RNAi or overexpress a dominant negative version of the protein and show that this produces the same effect as the drug (both approaches are required for convincing proof of on target effects). This also allows for dissection of downstream pathways to ascertain the effectors responsible for drug related changes. The work needs to be carried out in a variety of cell lines and for a variety of targets due to the varied nature of Clinical and Experimental Pharmacology, which not only depends on the results obtained with different drugs but also the availability of drugs which depends on collaborations with pharmaceutical companies. Some of the cell lines we work with, mainly small cell lung cancer cell lines, are impossible to transfect with conventional transfection approaches (e.g. Lipofection or electroporation and therefore a lentiviral based approach has been chosen. As knocking-down/overexpressing some of the targets of interest may prove lethal to the cells ability to carry out inducible knock-down/overexpression is key to this proposal.

**Host/vector system**

**Bacterial Hosts:** E. coli DH5 alpha and ccdB survival 2 (genotypes are given in appendix 2 of the risk assessment).

**Plasmid Vectors:**
- pENTR/pSUPER+; pENTR/pTER+ (used for RNAi delivery in to cell lines expressing target mRNA/protein via lipid based transient transfection).
- pENTR4; pENTR4-FLAG (used for protein expression studies).
- pLenti X1 GFP-Zeo; pLenti X2 Puro; pLenti CMV TetR Blast; pLenti X 2 Puro; Lenti CMV TetR Blast; pLenti CMV Puro; pLenti CMV/TO Puro; pLenti CMV/TO GFP-Zeo; psPAX2; oMD2.G (used for lentiviral particle generation)

**Viral vector system:** HEK293 cells will be used to generate lentivirus particles

The lentivirus vector system is a Self-inactivating (SIN) vector system. Disablement characteristics of the system is given in Appendix 4 of the risk assessment.
### Wild-type and mutated (activating or inactivating mutations) genes of the following proteins

- TDP2, MCT-1, MCT-4, c-Myc, p110alpha, p110beta, AKT1, AKT2, AKT3, PDPK1, K-Ras and other PI3K interacting proteins. Also Bcl-2 family members including Mcl-1, Bcl-2, Bcl-xL, Bax, Bak, Bim, Bid, Bad Puma and Noxa.

The genetic material is mainly human in origin, but some genes may be animal in origin (mainly murine). The genetic material will be in the form of cDNA. The function or suspected functions of these genes are listed in Appendix 3 of the risk assessment. They are also listed below.

#### Biological function of proteins of interest.

**TDP2** - Resolves DNA-topoisomerase adducts induced by topoisomerase poisons such as etoposide. Overexpression may result in resistance to topoisomerase. Unlikely to be oncogenic.

**MCT-1 and MCT-4** - Monocarboxylate transporters. Involved in the influx and efflux of molecules such as lactate from cells. Unlikely to be oncogenic.

**C-Myc** - Transcription factor involved in expression of numerous prosurvival and proliferation genes. Frequently activated in cancer and known to be oncogenic when overexpressed in presence of other mutations which promote cell survival.

**K-Ras** - GTPase known to activate the prosurvival and proliferation signalling pathways PI3K and MAPK. Frequently activated in cancer and oncogenic when activated.

**P110 alpha and beta** - PI3K catalytic subunits which generate secondary messenger PIIP3. Frequently activated in cancer and oncogenic when activated.

**AKT1, 2 and 3** - Main PI3K downstream effector which promotes survival, proliferation and migration. Frequently activated in cancer but not oncogenic by itself.

**PDPK1** - Required for activation of AKT downstream of PI3K signalling. Frequently activated in cancer but not oncogenic by itself.

Other PI3P3 interacting proteins - Biological function not clear but likely to be activated in cancer and could potentially be oncogenic.

**Mcl-1, Bcl-2** - Anti-apoptotic (pro-survival) Bcl-2 family members which inhibit Bax and Bak. Frequently upregulated in cancer but not oncogenic by themselves.

**Bax and Bak** - Pro-apoptotic proteins which cause release of cytochrome C from mitochondria. Frequently downregulated in cancer but require other mutations for their tumour suppressor function to be apparent.

**Bim, Bid, Bad, Puma and Noxa** - Pro-apoptotic BH3 only proteins which inhibit anti-apoptotic Bcl-2 family members and therefore activate Bax and Bak. Frequently downregulated in cancer but require other mutations for their tumour suppressor function to be apparent.

### Evaluation of foreseeable effects

The recombinant Lentiviral viral vector system consists of disabled vectors. They have had HIV regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. The system employed is a Self Inactivating (SIN) vector systems. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the risk control measures utilised are appropriate to guard against the associated risks. These include the use of safety cabinets, biosafe rotors and prescribed disposal routes for waste, wearing of appropriate PPE and no use of any sharps.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation by a third party company. The performance test results generated during validation are kept on site.

Trigene is routinely used as per the manufacturer's recommendations:
- Solid surfaces are disinfected with 2% Trigene solution.
- Plastic ware that can be effectively disinfected is treated with 10% Trigene for a minimum of 1 hour prior to incineration.
- Any contaminated glassware is treated with 10% Trigene solution for a minimum of 1 hour prior to cleaning. Usually the contact time is longer, typically overnight.
- Liquid waste (culture and medium) is disinfected by the addition of 10% Trigene. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The committee asked for further details of the animal work which was supplied - see Appendix 7 of risk assessment. The committee asked for justification on the use of Trigene disinfectant. This was supplied - see Appendix 8 of risk assessment.

Project Containment

<table>
<thead>
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</thead>
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02/03/2022
Project Ref 141/12.1

Date Ackn'd 03/01/2012

Date Project Ceased 25/05/2017

Project notified under transitional arrangements N

Historical Significant Changes
Transferred to GM541

Historical Date of Additional Info

Significant Change ID

Project Additional Information

Purposes of the contained use
Over-expression or knockdown of kinases to study the role of various kinases in cell lines and patient samples. Genetic modifications include the sub-cloning steps involved in the production of a Lentiviral expression plasmid, co-transfection of this expression vector together with packaging vectors into a host cell line to produce virus, and subsequently, use of Lentiviral stocks to infect target cell lines that will stably express the protein of interest. Downstream analysis of stable cell lines will involve Western blotting, FCAS analysis and microscopy.

Recipient or parental organism
Lentivirus parental organism-Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3

Host/vector system
Bacterial: E.coli Oneshot stbl3; E. coli ccdB survival 2; E.coli TOP10; E.coli Oneshot Omnimax. Bacterial hosts are disabled. Genotypes of bacterial host are given in Appendix 2 of the risk assessment.

Viral vector system: Lentivirus (VSV-G) with 293FT as host. This system is a self-inactivating (SIN) vector system. The system is a 4-plasmid system. Details of the biosafety features of this system are given in appendix 4 of the risk assessment.

Target cells: ATCC catalogue lung cancer cell lines and patient B-cell lines or fibroblasts, HTCC116 DLD-1 colon cancer cell lines and additional catalogue colon and breast cancer cell lines.

The likelihood of the presence of adventitious agents in patient derived cells is considered in Appendix 7 of the risk assessment.
Biological Functions of Proteins of Interest are given below. They are all of human origin.

PKC - Protein kinase C (PKC) is a family of serine- and threonine-specific protein kinases. PKC family members phosphorylate a wide variety of protein targets and are known to be involved in diverse cellular signalling pathways PKC family members also serve as major receptors for phorbol esters, a class of tumor promoters.

MLK4 - MLK4 is a member of the mixed lineage family of kinases activated by environmental stress, cytokines and growth factors. This family of kinases lies upstream of the kinase MKK4 and these kinases are critical regulators of the JNK pathway. In addition MLKs can activate the MKK3/6-p38 pathway and the Raf/Mek/Erk pathway.

FRK - The protein encoded by this gene belongs to the TYR family of protein kinases. This tyrosine kinase is a nuclear protein and may function during G1 and S phase of the cell cycle and suppress growth.

PAK3/7 - Both are Ser/Thr p21 activated kinases that have been described to regulate the Raf-Mek-Erk pathway and play a role in regulating the cell cycle.

Abl 1/2 - Is well studied tyrosine kinase that plays a role in CML and other cancers where the kinase is constitutively activated by a translocation to create a fusion protein BCR-ABL. Activated form can be oncogenic.

Csnk1g2 - This is an undescribed kinase that is in the casein kinase family.

Evaluation of foreseeable effects

The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

While the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, control measures utilised are appropriate to guard against the associated risks. These include the use of Class II safety cabinets, disinfection of liquid biological waste and autoclaving solid waste. The use of sharps is minimised in this project. Their use is not thought to be necessary. Any use will conform to the measures outlined in the Institute's Code of Practice.

The target genes do not encode viral specific protein nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation (12-point thermocouple testing) by a third party company. Records are kept on site.
Trigene is routinely used as per the manufacturer's recommendations:
Solid surfaces are disinfected with a 1 in 50 dilution of Trigene.
Plastic ware that can be effectively disinfected is treated with a dilution of 1 in 50 dilution of Trigene overnight.
Any contaminated glassware is treated with a 1 in 50 dilution of Trigene.
The contact time is overnight.

Liquid waste (culture and medium) is disinfected by the addition of Trigene to give a working dilution of 1 in 50; and left overnight before disposal to sewers.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The committee thought that this risk assessment was comprehensive and supplied all the relevant information.

Project Containment

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Human Clinical Applications

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Project Ref  141/12.2

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Date Project Ceased  25/05/2017

Project notified under transitional arrangements  N
The aim of the project is to develop a vaccine for childhood Acute Lymphoblastic Leukemia (ALL). As BCG is safely used as a childhood vaccine against tuberculosis, a childhood ALL specific recombinant BCG vaccine will be developed and tested in a mouse model. BCG has been shown to be an effective vaccine vector due to its adjuvant properties and ability to cross-present antigens to the MHC class I processing pathway. The TEL/AML fusion protein occurs in 25% of cases of childhood ALL. T-cell peptide epitopes have been identified in this protein and will be cloned into wildtype BCG to make a recombinant vaccine.

Recipient or parental organism
Mycobacterium Bovis BCG Pasteur strain (BCG) (attenuated with a long history of safe use). ATCC 35734.
Nucleotide sequence. AF095590

Host/vector system
Plasmid vectors:
pCR3.1 (non-mobilisabe).

Bacterial hosts:
Echerichia coli DH5 alpha and TOP 10 series.
Mycobacterium Bovis BCG Pasteur strain (BCG) (attenuated with a long history of safe use). ATCC 35734

Origin & function
Genes of interest will be of human origin.
Genes of interest will be junctional peptides from the TEL-AML1 and BCR-ABL. Either the whole gene, gene fragments or peptides will be used.

The TEL-AML1 fusion peptide is a small 30aa sequence taken from the TEL-AML1 fusion protein which is an oncogenic fusion protein that is an initiating lesion in childhood ALL. However, this protein will not be used. The fusion peptide which will be cloned into the BCG and is not known to have any biological activity.

Evaluation of foreseeable effects
The BCG strain used in this project is close relative of the commercially available BCG vaccine strain which has been used to vaccinate millions of patients. This strongly suggests that there would be minimal risk from the BCG strain used in this work. The expressed tumour proteins should not be functional in the bacterial strain and given the nature of the vaccine approach, the bacterial vector with human proteins should be rapidly degraded by the innate immune system and used to prime an immune response. The risk to human health from the GMM is likely to be minimal.

The genetic insert would not alter the pathogenicity of the BCG but may impact upon deleteriously upon the growth of the bacteria since the expression of heterologous
genes is commonly associated with reduced growth rates of the individual vector.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation (12-point thermocouple testing) by a third party company. Records are kept on site.

All liquid laboratory waste will be inactivated prior to removal from the Class II microbiological safety cabinets employed for this work.

Virkon is routinely used as per the manufacturer's recommendations:-
- Solid surfaces are disinfected with 1% Virkon solution.
- Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration.
- Any contaminated glassware is treated with 1% Virkon for a minimum of 1 hour prior to cleaning. Usually the contact time is longer, typically overnight.
- Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer's recommendations).

**Is an emergency plan required according to regulation 20?**

- **N**

**If yes, tick to confirm that it is attached to this form**

- **N**

**Tick to confirm that you have attached a risk assessment to this form**

- **Y**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- **N**

**Project Containment**

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02/03/2022
Project Additional Information

Purposes of the contained use

The aim of this project is to overexpress or knock-down genes of interest using lentiviral transduction in both fibroblast and epithelial cell lines to investigate the importance of these genes in both cancer cells and the associated stromal compartment in order to investigate effects on cancer cell growth, tumour-stoma interactions in co-cultures between stromal fibroblast cells and cancer cells, and in cancer cell metabolism.

For stable knockdown experiments, lentiviral particles containing shRNA sequences targeting genes of interest will be custom made or purchased from Santa Cruz or Genocopia. The lentiviral particles are a pool of concentrated transduction ready viral particles containing target specific constructs encoding the specific shRNA under the control of a CMV promoter. Details of the viral vectors are provided in appendix 1 of the risk assessment.

For overexpression, pre-made or custom made lentiviral particles will be purchased from GeneCopoeia. The full coding sequences of the candidate genes will be inserted by the company into the viral vectors under the control of a CMV promoter and a puromycin resistance gene for selection purposes after transduction.

The viral particles are prepared by the company in the following manner. An OmicsLink™ ORF lentiviral expression plasmid (GeneCopoeia Cat. No EX-EGFP-Lv105) was constructed using GeneCopoeia proprietary RecJoin™ technology. This plasmid was co-transfected into 293Ta cells (GeneCopoeia Cat Np CLv-PK-01) with the Lenti-Pac HIV Packaging Mix (GeneCopoeia Cat. No HPK-LvTR-20). Lentivirus-containing supernatants were harvested 48 hours after transfection. Pre-made lentivirus titer ranges from 10^7 copies/ml for crude version to 10^9 copies/ml for purified lentivirus. Lentifect lentivirus production process involves stringent quality control processes including sequence verification of the insert, qRT-PCR based titer estimation, etc.

We intend to use these particles on a variety of cell lines in both monoculture and co-culture of fibroblast cells with breast cancer cell lines and look at cell metabolism.
autophagy, mitophagy, proliferation, migration and molecular markers with Western blot, immunocytochemistry and flow cytometry, as well as biochemical assays of products of metabolism such as lactate and ketones.

### Recipient or parental organism

**Lentivirus parental organism - Human Immunodeficiency virus (HIV-2) is classified as ACDP hazard Group 3.**

### Host/vector system

**Target Cells for Viral vector**

Fibroblast cell lines BJ5TA, BJ1, 218, 522 and 544

Epithelial cancer cell lines MCF7, MCF10A, MDA-MB-231, SKBR3, T47D, BT20, BT474, MBA-MB-468 (all breast cancer cell lines) see appendix 2 in risk assessment for more details of the cell lines to be used.

Other epithelial cell lines may be investigated, and amendment to the risk assessment would be obtained beforehand

### Origin & function

**Genes of interest:**

- cDNA: Human Caveolin -1 and related genes
- cDNA: Human Autophagy/mitophagy related genes - including ATG16L, LAMP, TOM-20, LC3
- cDNA: Human Lactate transporter genes including MCT1 and MCT4
- cDNA: Human Transcriptional regulator genes - Including CAPER
- cDNA: Human genes associated with glycolysis and glucose transport - including GLUT-1
- cDNA: Human genes associated with senescence such as WNT5a, FBX032 and the CDK family of genes
- siRNA: to target Caveolin and related genes
- siRNA: to target MCT1, MCT4 and related genes
- siRNA: to target CAPER and associated transcriptional regulators.

As we will be studying a variety of similar and related genes within the above families, we will review the risk assessment when we look at other genes to ensure the assessment is still valid.

For knock-down experiments - Lentiviral particles containing shRNA sequences targetting genes of interest will be purchased from Santa Cruz or Genocopia.

For over-expression custom made particles will be purchased from Genocopia.

### Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors based on the HIV lentivirus. They are self inactivating vectors, which have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated risks.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of disinfectant); work carried out in containment 2 laboratories, work within Class 2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site. Trigene is routinely used as per manufacturer's recommendations:-
Solid surfaces are disinfected with Trigene trigger spray solution.
Plasticware that can be effectively disinfected is treated with final concentration of v/v 1% Trigene for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Trigene solution for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfectd by the addition of Trigene to give a 1% final working concentration. A minimal contact time of 1 hour is allowed prior to disposal to sewers.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee agreed that this was a Class 2 project.
It was the committee's view that the assessment was adequate for the risk profile of the work

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Project Ref 141/12.4
Previous studies have identified the attraction to, and the ability to cross the bone marrow endothelium as being pivotal in the metastatic process of prostate cancer. Previous studies have identified a number of candidate genes involved in targeting and crossing the endothelial membrane towards bone marrow stroma. This project seeks to evaluate these candidate genes in established cell line models of prostate epithelial metastasis. Cell lines used will be human bone metastatic cell line PC-3 and non-bone metastatic cell line LNCaP to assess action of these genes. Cell lines will be transduced with genes of interest or their corresponding siRNA pools using lentiviral vectors and analysed using a combination of human bone marrow stroma co-culture assays which model binding, transendothelial migration, colony formation and expansion.

Recipient or parental organism

Lentivirus parental organism - Human Immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3

Host/vector system

Bacterial host: E.coli JM109


Host cell; HEK293

Target cells for viral vector:-
Human bone metastatic cell line PC-3
Non-bone metastatic cell line LNCaP
Ephrin receptors:- EphA1, EphA2, EphA4, EphB, 2EphB3, EphB4, EphB6
Eph receptors are a group of receptors activated by the ephrin and form the largest known subfamily of receptor tyrosine kinases (RTKs). Both Ephs and their corresponding ephrin ligands are membrane-bound proteins that require direct cell-cell interactions for Eph receptor activation. Subsequent downstream pathways have been shown to regulate motility, cell adhesion and cell polarity through the Rho family of GTPases. Eph/ephrin signalling regulates a variety of biological processes including the guidance of axon growth cones, formation of tissues boundaries, cell migration, and contact inhibition of locomotion (CIL). Additionally, Eph/ephrin signalling has recently been identified to play a critical role in several processes including long-term potentiation, angiogenesis, and stem cell differentiation.

Genes listed are expressed in prostate epithelial cells.

Ephrin A1, A3, A4, A5; Ephrin B1, B2, B3:- The Ephrin family are a family of proteins that serve as the ligands of the Eph receptors, which compose the largest known subfamily of receptor protein-tyrosine kinases (RTKs). Both ephrins and Eph receptors are membrane-bound proteins binding and activation of Eph/ephrin intracellular signalling pathways only occur via direct cell-cell interaction. Subsequent downstream pathways have been shown to regulate motility, cell adhesion and cell polarity through the Rho family of GTPases.
Eph/ephrin signalling regulates a variety of biological processes including the guidance of axon growth cones, formation of tissue boundaries, cell migration, and contact inhibition of locomotion (CIL). Additionally, Eph/ephrin signalling has recently been identified to play a critical role in several processes including long-term potentiation, angiogenesis, and stem cell differentiation.

2 classes of ephrin:
Ephrin A - GPI (glycosylphosphatidylinositol) linked ephrins
Ephrin B - Transmembrane linked ephrins

Gene listed are expressed in prostate epithelial cells.

Caveolin: Cav-1 Cav-2:- Family of integral membrane proteins which are the principal components of caveolae essential for receptor-independent endocytosis. Caveolins act as scaffolding proteins within caveolins have also been shown to have lipid binding properties and mediate aspects of cholesterol and fatty acid metabolism.
The caveolin gene family has three members in vertebrates: CAV1, CAV2, and CAV3, coding for the proteins caveolin-1, caveolin-2, caveolin-3, respectively.

Evaluation of foreseeable effects
The recombinant Lentiviral vector system consists of disabled vectors. They have had HIV regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is though there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the risk control measures utilised are appropriate to guard against the associated risks. These include the use of safety cabinets, biosafe rotors and prescribed disposal routes for waste, wearing of appropriate PPE and no use of any sharps.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
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Virkon is routinely used as per the manufacturer's recommendations:-
Solid surfaces are disinfected with 1% Virkon solution.
Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as manufacturer's recommendations).

The committee agreed with the classification.
It asked the author for more information about the genes of interest and these were supplied and are listed in section 7 of this form and as a table appended to the risk assessment.

Please enter comments on the GM safety committee on the risk assessment

The committee agreed with the classification.
It asked the author for more information about the genes of interest and these were supplied and are listed in section 7 of this form and as a table appended to the risk assessment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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**Project Ref 141/13.1**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>14/06/2013</td>
<td>Using fluorescent and luminescent reporters to monitor and isolate breast cancer</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</tbody>
</table>
(stem) cells in vitro and in vivo

Purposes of the contained use

Primary aim: to monitor and isolate breast cancer (stem) cells in vitro and in vivo.

Micrometastases are a major problem in breast cancer as they are resistant to standard therapies and have the potential to kill the host. It is known that breast cancer cells preferentially metastasise to the lungs, liver and bone, however the timing of this progression and the processes underpinning it are not clear. Understanding how and when breast cancer cells metastasise and how micrometastases are regulated in their environment will aid the development of new strategies to inhibit their formation or progression.

We plan to monitor cellular dissemination of breast cancer (stem) cells using in vivo imaging and isolation from mouse tissues. We have obtained bicistronic luciferase and green (GFP) and red (RFP) fluorescent cDNA vectors for lentiviral transduction from Standford University, USA (FULG and FULT vectors from (Liu et al., 2010)) for this purpose. We will transfect both breast cancer and cell lines and primary breast xenografts with the fluorescent vectors and inject them into mice. We will then monitor the spread of breast cancer (stem) cells to metastatic sites. The advantage of the bicistronic vector is that injection of luciferein can be used to detect as few as 10 cells in a metastatic site and the GFP/RFP+ cells can be localised in the excised tissues and sorted from host cells by FACS.

Recipient or parental organism

Lentivirus parental organism- Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.

Host/vector system

Bacterial: E. coli Stbl3 (from Invitrogen)

Viral vectors; pMDLg/pRRE, pRSV-Rev, pMD2.G, pCMVdelta8.91 lentiviral packaging plasmids

Lentiviral system: This is a self-inactivating lentivector system with a pseudotyped VSV-G capsule. Viral particles will be produced using the HEK293T packaging cell line.

FLUG and FULT plasmids contain multifunctional reporter genes firefly luciferase and fluorescent proteins

Target cells: Continuous human breast cancer cell lines: MCF-7; T47D; MDA-MB-231; BT474 - All from ATCC Primary human tissue samples from Christie Hospital Biobank.
The FULG and FULT plasmids are described in Liu et al, 2010 (PNAS vol. 107 p18115-18120). They are multifunctional reporter genes used to analyse disease models by linking in vivo and ex vivo assays. They contain both firefly luciferase (Luc+) for whole body tracking of cells via bioluminescence imaging, and fluorescent proteins to allow intravital imaging and ex vivo analyses. In FUKG and FULT Luc2 is fused to either enhanced GFP (eGFP) (FULG) or the red fluorescent protein tomato (FULT).

**Evaluation of foreseeable effects**

The recombinant Lentiviral vector systems consist of disabled vectors. They have had HIV regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. The systems employed will be Self-inactivating (SIN) vector systems. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the risk control measures utilised are appropriate to guard against associated risks. These include the use of safety cabinets, biosafe rotors and prescribed disposal routes for waste, wearing of appropriate PPE and no use of any sharps.

The reporter genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

**Origin & function**

The reporter genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The mice used in this study will not be GM animals. GM human tumour cells will be injected into mice. These will not have the ability to infect the animals cells.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user.

The autoclave undergoes annual validation (12-point thermocouple testing) by a third party company. Records are kept on site. It also undergoes a quarterly maintenance regime as recommended in BS2646.

Any plasticware that can be effectively disinfected is treated with 1% virkon and left overnight before entering the clinical waste route.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturers recommendations) but it is usual to leave overnight until disposal to drains.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The Committee agreed with the classification of the work as GM Class 2. It is asked for confirmation that no sharps would be used during lentiviral particle generation. This was confirmed by the research group concerned.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
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### Project Ref  141/13.2

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<th>Project notified under transitional arrangements</th>
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<td>11/09/2013</td>
<td>Use of Retroviral Transduction</td>
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<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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</thead>
<tbody>
<tr>
<td>25/05/2017</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

**Historical Significant Changes**

- Transferred to GM541

**Project Additional Information**

**Purposes of the contained use**

We seek to investigate the role of normal and cancer-activated fibroblasts in tumour-stromal interaction using knockdown or overexpression of genes of interest. Fibroblasts are known to exert tumour-promoting as well as tumour-suppressing functions dependent upon micro-environmental conditions and the metabolic state of the contributing cell types. We intend to co-culture fibroblast cells with breast cancer cell lines and analyse cell functionality (proliferation, migration, invasion, stem cell expansion) as well as biochemical states (molecular markers by western blot, real time PCR and Elisa).
Moloney Murine Leukemia Virus (MMLV)-based retroviral vectors pBabe, pWZL and pLNCX used to generate recombinant viral particles using a HEK293T based packaging cell line.

### Host/vector system

Phoenix-amphotropic HEK293T packaging cell line. Provides the env gene and protein which determines the virus particle infectivity range. In this case- amphotropic.

Retroviral vectors pBabe; pWZL and pLNCX MMLV-based retroviral vectors

Details of the host vector system are given in Appendix 1 of the risk assessment

### Origin & function

The host-vector system will produce recombinant virus particles which will then be used to infect a range of cell lines. These cell lines are human breast cancer cell lines and human and murine fibroblast cell lines.

The genes of interest comprise a wide range including those involved in proliferation, migration, invasion and stem cell expansion; as well as biochemical states. The known or suspected functions of these genes are listed in Appendix 2 of the risk assessment.

### Evaluation of foreseeable effects

The recombinant virus has its structural genes deleted, which disables the virus and so prevent its growth in the absence of complementing cell lines or co-transfection of appropriate plasmids.

The amphoteric envelope, conferred by the packaging cell line, changes cellular tropism to most mammalian cells including human. It may be able to confer greater stability and environmental survivability of the virus. However, appropriate work practices will prevent the replication incompetent viruses from being released in to the environment.

The absence of the use of sharps in this work should help minimise risks to workers

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user.

Virkon is routinely used as per the manufacturer's recommendations:-

- Solid surfaces are disinfected with 1% Virkon solution
- Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to being placed in to the clinical waste route and then goes for incineration
- Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Usually the contact time is longer, typically overnight.
- Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).
The autoclave undergoes annual validation by a third party. Records are kept on site.

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

The committee asked to be informed of the genes that the project studies, as work progressed. This was agreed by the risk assessment author.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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</tr>
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<tr>
<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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- Animal Units: L2 L3 L4 L2 L3 L4 L2 L3 L4
- Large Scale Activities: L2 L3 L4 L2 L3 L4 L2 L3 L4
- Human Clinical Applications: L2 L3 L4

Project Ref 141/15.1

<table>
<thead>
<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
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<tbody>
<tr>
<td>09/07/2015</td>
<td>Knockdown of genes in prostate normal and tumour cells</td>
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<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Class</th>
<th>CultureVol</th>
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<td>Class 2</td>
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<td>Not Applicable</td>
</tr>
</tbody>
</table>

- Non-GMM: [N]  
- Project notified under transitional arrangements: [N]  

Historical Significant Changes: Transferred to GM541
Microarray and single cells expression profiling experiments previously performed have identified a number of candidate genes that may be critical regulators of normal and tumourigenic prostate progenitor cells. Genes expressed in the stromal compartment of the prostate may also have a critically important function in the regulation of normal prostate and tumour-initiating cells. This project seeks to evaluate the role of these genes in these cell types in murine and human model systems using genetic knockdown experiments. Briefly, murine or human prostate cells will be transduced using lentiviral vectors containing shRNAs and cells will then be functionally evaluated using a combination of in vitro and in vivo techniques, the latter involving transplantation of cells in to mice. One retroviral vector system will be used: pSHAG-MAGIC2, which provides constitutive expression of the shRNA from MSCV promoter. Two lentiviral vector systems will be used: (i) pLKO.1, which provides constitutive expression of shRNAs from a Pol III promoter, and (ii) pSicoR, which provides constitutive expression of shRNAs from a Pol III promoter with the option of irreversible Cre-mediated conditional inactivation of knockdown. In some cases, we would like to ensure that the genetic alteration is restricted to specific prostate lobe/area in vivo. For this, adenovirus or lentivirus will be injected intraprostatically to genetically modified mice and their control counterparts.

Bacterial hosts: Escherichia coli Stbl3 (for use with lentiviral vector system plamids)  
Escherichia coli Dh5 alpha (for general cloning purposes)

Bacterial recipients are none-infective strains and have multiple auxotrophic requirements. Growth and survivability is unlikely out of laboratory growth medium. Transformation with plasmids used in this work will not alter this phenotype.

Target cell lines- Primary murine and human prostate cells (please see appendix 7 for cell use criteria)

Growth and survivability of modified and un-modified cells is unlikely outside of laboratory growth medium.

Lentivirus pLKO.1 and pSicoR systems: parental organism is Human Immunodeficiency virus (HIV-1) and is classified as ACDP hazard group 3. Used with HEK 293 FT cells.

More details can be found in appendix 1 and 3 of the risk assessment.

The pSHAG-MAGIC2 retrovirus system: parental organism is Murine Stem Cell Virus. Used with Phoenix-E and Plat-E cells.

More details can be found in appendix 3 and 4 of the risk assessment.

Target cells: primary murine and human prostate cells

shRNAs expressed will target the genes listed in appendix 2 of the risk assessment, for knockdown. The function of these genes in leukaemia stem cells is not known formally, however, genes such as Pten, Brca1, and ATM are cell cycle/dna damage regulators/tumour suppressors so their knockdown may accentuate oncogenesis.
### Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors based on the HIV or MSC virus. These vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

For the lentivirus, whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability it is thought that there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the control measures utilised are appropriate to guard against the associated risks.

The target genes do not encode viral specific protein, nor does they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus or MSC retrovirus.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
<thead>
<tr>
<th>Material Type</th>
<th>Treatment Method</th>
<th>Contact Time</th>
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<td>Solid waste materials (e.g. gloves, plastic ware, cell pellets etc)</td>
<td>Rendered inactive by autoclaving (100% kill) in a validated machine located in the same building.</td>
<td>1 hour prior to cleaning.</td>
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<tr>
<td>Subsequently the solid waste enters the clinical waste route and goes for incineration.</td>
<td></td>
<td></td>
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<tr>
<td>Virkon is routinely used as per the manufacturer's recommendations:</td>
<td>Solid surfaces are disinfected with 1% Virkon solution.</td>
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<td>Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration.</td>
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<td></td>
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</tbody>
</table>

The autoclave undergoes annual validation. Records are kept on site.

### Is an emergency plan required according to regulation 20?  
Y

### If yes, tick to confirm that it is attached to this form  
N

### Tick to confirm that you have attached a risk assessment to this form  
Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment  
N
The committee agreed with the classification. It asked for clarification on the regime for training workers in this work. This was supplied to the committees satisfaction. Likewise the committee asked for a standard operating procedure for dealing with breakages in centrifuges used in this work. Again this was supplied to the committees satisfaction.

Project Containment

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<tr>
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<td>L4</td>
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Animal Units

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<tr>
<th>Large Scale Activities</th>
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Project Ref 141/15.2

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<td>Investigating Transcriptional Networks in Lung Cancer</td>
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Date Project Ceased

| 25/05/2017 |

Withdrawn

| N |

Tick if notifying a connected programme of work

| N |

Historical Significant Changes

| Transferred to GM541 |

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The proposal concerns the immortalisation of primary cells that can be readily expanded in a number for experimental investigations. Specifically, the primary cells of interest will be those that comprise the tumour microenvironment. This
is a broad set of cell types but the aim of the work is to attempt to generate mixed cultures of cells that re-capitulate key aspects of the tumour microenvironment including immune suppression. Consequently, cells that would be of interest include tumour and non-tumour stromal cells including fibroblasts and the diversity of immune cells. We have expertise in disaggregating tumours and expanding T cells for in vesteation and also immortalising B cells employing EBV. We also have performed short-term tumour cell growth for co-culture assay. However, in the absence of immortalisation, cell number is usually a limiting factor for the breadth of potential experiments that can be performed. For this project, we propose to exploit the directed expression of single genes that have been shown to immortalise a range of primary cells.

Recipient or parental organism
Primary human tumour or peripheral blood cells

Host/vector system

Retroviral vectors.
Retroviral viral gene transfer vectors have been extensively engineered to eliminate all virus protein coding sequences within the vector itself. Vector particle are generated by co-expression of gag/pol and env genes with the retroviral vector within a packaging cell. The gag/pol and env genes are encoded on separate plasmids and engineered to reduce homologous sequences with the retroviral vector. This degree of engineering reduces the possibility of generating a replication-competent retrovirus to essentially zero based upon the extensive history of use of retroviral vector technology in the pre-clinical and clinical situation.

Lentiviral vectors.
Lentiviral vectors have been engineered as described below:
An enhancer deletion in the U3 region of 3’LILTR ensures self-inactivation of the lentiviral vector following transduction and integration into the target cell ’s genomic DNA.
• The number of lentiviral genes necessary for packaging, replication and transduction is limited to three (Gag/Pol/Rev), and their expression is derived from different plasmids, all lacking packaging signals. These plasmids share no significant homology to the expression vector, thus preventing the generation of replication-competent virus by recombination events .
• None of the Gag, Pol, or Rev genes will be incorporated into in the packaged viral genome, thus making the mature virus replication-incompetent.

Origin & function
The genes to be used are sourced commercially with sequences as listed below.

Immortalising genes.
SV40 large and small T antigen sequences. Full sequence available here: http://www.abmgood.com/SV40-CellImmortalization.html
hTERT. Full sequence available here: http://www.abmgood.com/hTERT-Cell-Immortalization.html#

siRNA targets to aid immortalisation:
pRB siRNA. Full sequence available here: http://www.abmgood.com/Myc-p53-Rb-Ras-Cell-Immortalization.html

Evaluation of foreseeable effects
Both retroviruses and lentiviruses are lack any potentially immune avoidance gene products that are encoding within the wild type viruses hence they are unlikely to have the same degree of immune protection as that afforded to the
wild type virus. The envelope glycoproteins lend to be derived from other viruses which possess different and, most often, increased immunogenicity profiles compared to the retrovirus lentivirus envelope glycoproteins. Indeed, the VSVg envelope commonly used to pseudotype lentiviral vectors is sensitive to complement mediated destruction in human serum. Overall, the replication deficient virus vectors are most likely to possess an increased immunogenicity profile as compared to the wild type vector suggesting that in the immune-competent individual, these vectors are more likely to be subjected to immune-mediated clearance.

For the target cells...
The genes to be expressed facilitate the modified cell to overcome cellular senescence and to allow the transduced cell to become immortalised.

The modified cells require specific conditions to survive including maintenance at 37 degrees C and cultured in incubators flushed with CO2. Outside of this environment, the cells could survive in media for a period of time but would be unlikely to expand in number. However, with evaporation, solute concentrations would build within the remaining media and the cells would either die of that level of toxicity or die through drying out. Outside of the media environment, cells would last only a short period of time (hours) before dying due to dehydration.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation (12-point thermocouple testing) by a third party company.

All liquid laboratory waste will be inactivated prior to removal from the Class II microbiological safety cabinets employed for this work. Virkon is routinely used as per the manufacturer's recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Usually the contact time is longer, typically overnight. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
This project involves over expression of the PDGFRAIB in human lung cancer cells from the ATCe. The PDGFR gene is implicated in lung cancer and we wish to introduce and express these genes in a small range of cell lines.
gene will be introduced into the cell by chemical means e.g. lipofectamine. Downstream analysis, involving cell sorting and various assays will be carried out. Other genes of interest involved in lung cancer may be used subsequently (e.g. AMPK, 81M, NIKRAS2) but the Biosafety Committee will be informed beforehand. We also intend to transfect a small range of cell lines with microRNAs. miRNAs will be introduced into the cell lines using premade Lentiviral particles resulting in integration into the cell genome. The modified cells will then be analysed using standard laboratory assays and techniques such as western blotting. Whilst we have identified the use of two miRNAs which we intend to use, other miRNAs may be used later in the project. The Biosafety Committee will be informed beforehand if this occurs. We also plan to stably silence important oncogene targets of the identified microRNAs using the shRNA lentiviral particles. We will purchase ready for use Lentiviral particles from Santa Cruz Biotechnology. Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

Recipient or parental organism

Lentiviral systems: Lentivirus parental organism- Human Immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3

Host/vector system

Recipient or parental organism

Bacterial HosU Vector system: E.coli TOPO 101 pCMV expression vectors

Viral/Host systems: Custom made lentiviral particles from Santa Cruz Biotechnology for transduction of shRNA constructs into target cells

miRNA lentivector expression systems from System Bioscience (SBI), involving co-transfection of expression and packaging vectors in HEK293 cells, resulting in pseudo-viral particles harbouring miRNA expression constructs for transduction into target cells

Target Cells: A549 lung cells from ATCC, H292 lung cells from ATCC

Origin & function

Human PDGFR Gene eDNA Clone (full-length ORF Clone), expression ready, C-HA-tagged

Vector: pCMV3-C-HA

HA Tag Sequence: TATCCTTACGACGTGCCTGACGCC

Species: Human

Gene Synonym: PDGFR- Beta and PDGFR-Alpha

Gene Bank ref 10: NM_002609.3 and NM_002609.4

Health and Safety Executive

PDGFR is implicated in lung cancer in humans. Other genes implicated in human lung cancer, such as AMPK, 81M and NIKRAS2 and genes of similar functional profile, may also be investigated.

Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors based on the HIV lentivirus. They are self-inactivating vectors, which have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control
measures utilised are appropriate to guard against the associated risks. The target genes does not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of disinfectant); work carried out in containment 2 laboratories, work within Class2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation (12-point thermocouple testing) by a third party company.

All liquid laboratory waste will be inactivated prior to removal from the Class II microbiological safety cabinets employed for this work. Virkon is routinely used as per the manufacturer's recommendations: Solid surfaces are disinfected with 1% Virkon solution.

Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Usually the contact time is longer, typically overnight.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

Please enter comments on the GM safety committee on the risk assessment
The Committee asked for more information on the lentivirus systems intended for use. This was supplied to their satisfaction. The Committee agreed with the classification of the work as GM Class 2.

**Project Containment**

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<tr>
<td>CU2 Project Title</td>
<td>Lentiviral infection of pancreatic cells and the surrounding stroma</td>
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<td>Project notified under transitional arrangements</td>
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**Project Additional Information**

**Purposes of the contained use**

Lentiviral vector packaging systems will be used to transduce cells which are otherwise difficult to modify using retroviral or other methods of transfection. This will mostly be employed within a pancreatic cancer setting, focusing on the tumour cells and surrounding stromal cells.
### Recipient or parental organism

Lentivirus parental organism- Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.

### Host/vector system

Lentiviral vector systems:-
Lentiviral vector packaging systems have divided the essential functions amongst multiple plasmids to reduce the risk of generating replication-competent lentivirus (RCL). The split-genome packaging system is designed so that multiple recombination events between the components are required for autonomous replication. Clinical trials using a split-genome packaging system have shown that this strategy effectively eliminates the creation of RCLs (see Levine et. al. PNAS, 103: 17372-17377, 2006). Commercially available 3rd generation lentiviral vector systems separate the viral envelope, env (e.g. VSV-G) from the gag-pro-pol, which encodes structural and enzymatic functions. We will be using pLB, pSicoR and pSLIK which lack gag, pol, env,tat, rev and other accessory viral genes.
We will also be using GIPZ shRNA Lentiviral Particles which are produced using the Trans-Lentiviral Packaging System. The Trans-Lentiviral Packaging system provides an even higher level of safety over 3rd generation packaging systems by further splitting the viral pol (reverse transcriptase (RT) and integrase (IN) functions) from gagpro. Because the RT and IN enzymes are provided in trans to gag-pro, additional recombination events are necessary to produce RCLs

Target cells:-
Cells will be cultured from primary animal or human sources or through immortalised cell lines. Cell lines of human origin will be verified using the Institute's human cell line authentication service. The source tissue of the cells includes pancreas, kidney, spleen, liver, breast, pleural effusion, lymphocyte, brain, pericardial effusion, ascites, lymph node and endothelium. Human patient material will be acquired via the MCRC Biobank. The Institute prohibits the collection of human tissue known, or highly likely, to be infected with a human pathogen. Any deviation from this rule must be approved by the Institute Director and Biosafety Committee.

### Origin & function

PKN2 (Protein Kinase N2) is a PKC-related serine/threonine-protein kinase and Rho/Rae effector protein that participates in specific signal transduction responses in the cell. Plays a role in the regulation of cell cycle progression, actin cytoskeleton assembly, cell migration, cell adhesion, tumour cell invasion and transcription activation signalling processes.
Other genes of similar function or implicated in the same cellular processes to that named may be investigated. But information as to their name and nature will be provided to the Biosafety Committee beforehand.

GFP (Green Fluorescent Protein) is a energy-transfer acceptor. Its role is to transduce the blue chemiluminescence of the protein aequorin into green fluorescent light by energy transfer. Fluoresces in vivo upon receiving energy from the Ca2+-activated photoprotein aequorin.

### Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors based on the HIV virus. These vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.
For the lentivirus, whilst the VSV-G envelope extends the celluar tropism and confers greater stability and environmental survivability it is thought that there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the control measures utilised are appropriate to guard against the associated risks.
The target genes do not encode viral specific protein, nor does they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration.

Virkon is routinely used as per the manufacturer's recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

The autoclave undergoes annual validation. Records are kept on site.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The committee agreed with the classification.

It asked for details of the target cells which were subsequently provided and incorporated in to the risk assessment.

Project Containment

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02/03/2022
The project aims to investigate the immunomodulatory properties of factors derived from cancer cells on innate and adaptive immune cells typically found in the tumour microenvironment. Briefly, mouse cancer cell lines will be transduced using standard retro and lentiviral vector packaging systems such as pFB, pMSCV, pRevTRE or pLKO.1 to variably induced the upregulation or downregulation of candidate factors that might impact on the ability of the cancer cells to modulate inflammation and subvert immune surveillance. The factors will typically involve inflammatory and immune mediators but might also include known oncogenes or tumour-suppressors in order to examine how specific mutations impact the ability of the cancer cells to modulate the inflammatory and immunogenic properties of cancer cells.

Recipient or parental organism
Lentiviral parental organism- Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.
Moloney Murine Leukaemia Virus parental organism is classified as hazard group 1.

Host/vector system
Lentiviral vector systems:
Lentiviral vector packaging systems have divided the essential functions amongst multiple plasmids to reduce the risk of generating replication-competent lentivirus (RCL). The split-genome packaging system is designed so that multiple...
recombination events between the components are required for autonomous replication. Clinical trials using a split-genome packaging system have shown that this strategy effectively eliminates the creation of RCLs (see Levine et al. PNAS, 103:17372-17377, 2006). Commercially available 3rd generation lentiviral vector systems separate the viral envelope, env (e.g. VSV-G) from the gag-pro-pol, which encodes structural and enzymatic functions. We will employ systems such as pFB, pMSCV, pRevTRE and PLKO.1. These systems are designed as described above with the gag and pol elements provided in trans using GP-2 or Phoenix cells.

Retraviral vector sytems:- GP2-293 (Clonetech@631458) is an HEK-derived cell line engineered to express the Moloney Murine leukemia Virus (MoMuL) essential viral packaging components by stably integrating, gag and pol genes. The viral envelope must be supplied in trans. High titre recombinant retrovirus particles are produced by transient co-transfection of pFB or pMSCV-based retraviral expression vector and a plasmid that expresses a viral envelope, such as pVSV-G. These commercially available vectors with proven safety profile are in use over many years for producing infectious but defective retroviral particles. These replication deficient VI Ps will be used to transduce the target cancer cells to study the impact of the encoded transgene expression on its immunogenic properties. The final genetically modified retroviral and lentiviral particles will be replication incompetent and therefore will not be able to propagate and establish a clinical infection. However, because they are infective they will be treated under containment level-2. Their properties are not expected to be altered by any of the inserts we will introduce into the vectors.

The potential route of transmission of the viral particles in the laboratory is mainly through skin contact that could arise from splashes, touching the objects or surfaces that are contaminated with culture media. Precautionary measures such as wearing laboratory coats and gloves and working in a laminar flow safety cabinet will prevent transmission through direct contact or inhalation of aerosol. Airborne transmissions are minimised since all cell culture work will be conducted in a class 2 microbiological safety cabinet and all centrifugation steps will be carried out in specialised sealed centrifuge buckets to prevent the generation of aerosols. Filter tips will be employed to prevent cross contamination of pipettes. No sharps or needles will be used so there is not risk of percutaneous transmissions. Accidental spillages will be dealt with by cleaning with appropriate disinfectant solutions (i.e. 1% Virkon). Any viral particle suspensions will be disposed of only after completely neutralising the virus using 1% Virkon for at least 24 hours. Contaminated consumables will be autoclaved prior to incineration.

Target Cells:- Well-characterised mouse cancer cell lines such as melanoma (8 16, 5555), colon-carcinoma (CT26) and breast-carcinoma (4T1), will be used in this project and are (or in the process to be) commercially available. B16 (ATCC® CRI6475™) and 5555 melanoma cells are a mixture of spindle-shaped and epithelial-like cells derived from C57BU6J mice. CT26 (ATCC® CRL-2638™) is an induced, undifferentiated colon carcinoma cell line derived from Balb/c mice. Product data sheets and certificate of analysis are available on the ATCC.

Origin & function

The specific gene that we are using in this project is mouse Cycloxygenase-2 gene that was supplied commercially by SourceBioscience. COX-2 is an enzyme that is responsible for formation of prostanooids. Other genes may be employed in this project encoding for molecules that may play a role in inflammation and immunity. In this event the Biosafety Committee will be notified beforehand.

Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors. These vectors have had regulatory and accessory genes deleted ensuring that viral particles produced are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication infection.
cycles. The probability of seroconversion is minimal. For the lentivirus, whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability it is thought that there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the control measures utilised are appropriate to guard against the associated risks. The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

8.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets, etc) are rendered inactive by autoelaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. Virkon is routinely used as per the manufacturer's recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer's recommendations). The autoclave undergoes annual validation. Records are kept on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee agreed with the classification of the work. The research group was asked to notify the Committee of the intended use of additional genes of interest Likewise if the work progressd in to animals (mice) then appropraite assessment would be required beforehand.

Project Containment

02/03/2022
Project Ref 141/93.1

Date Ackn’d 08/11/1993

Date Project Ceased 13/01/2003

THE PATHOGENICITY OF EPSTEIN BARR VIRUS, THE IMMUNOLOGY OF HUMAN PAPILLOMAVIRUS AND THE USE OF VIRUS VECTORS IN GENE THERAPY, IN RELATIONSHIP TO HUMAN CANCERS

Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes GM141/96.1, GM141/98.1

Historical Date of Additional Info 11/11/1996, 16/06/1998

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

It was approved without comments

Project Containment

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Project Ref 141/93.1A

Date Ackn'd 08/11/1993  Project Title TRANSFER OF GENES INTO HAEMOPOIETIC CELLS:

Class 2
Date Project Ceased
25/05/2017

Non-GMM

Consent Granted
not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
GM141/96.1, GM141/98.1, Transferred to GM541

Historical Date of Additional Info
11/11/1996, 16/06/1998,

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 80/07.3

Date Ackn’d: 23/01/2013

**CU2 Project Title**
Inducible shRNA-mediated knockdown of B-Raf gene expression in melanoma cell lines.

Date Project Ceased: 25/05/2017

Class: 2
Culture Volume: < 1 Litre

Non-GMM: Not Applicable

Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: Transferred from GM80 Transferred to GM541

02/03/2022
Project Additional Information

Purposes of the contained use

The aim of the project is to assess the effect of blocking the expression of B-Raf and related signalling proteins on the growth of melanoma cell lines in a 3-dimensional model which more closely replicates the tumour microenvironment. We have previously shown that siRNA-mediated gene knockdown of B-Raf inhibits melanoma cell growth. However, this culture model is not compatible with siRNA oligonucleotides. Therefore, we wish to create melanoma cell lines which stably integrate shRNA sequences into their genome for long-term gene knockdown.

Recipient or parental organism

The recipient cell lines will be primary human melanocytes or immortalized human melanoma cell lines. Each of these cell lines are commercially available, have been maintained in cell culture for many years, are not known sources of human pathogens, and are unlikely to survive outside the laboratory environment.

Host/vector system

The vector to be used is a lentivirus derived from HIV-1. However the vector has been modified in several ways to ensure that the virus is replication-defective, and will not form active viral particles unless it is co-transfected into a packaging cell line with helper plasmids.

Origin & function

The genetic material that will be cloned into the lentiviral vector will be DNA that encodes a short hairpin RNA molecule directed against B-Raf or related genes which support melanoma progression. Expression of the shRNA molecule will result in gene-specific knockdown, most likely promoting growth inhibition or cell death in the transfected cells. We also propose to use a lentiviral vector encoding the bacterial Tet-repressor protein, so we can achieve inducible expression of shRNA in the cell lines under study. Once Tet-repressor and shRNA constructs have been stably introduced into cells, shRNA expression and subsequent gene knockdown will only occur if tetracycline is added to the culture medium.

Evaluation of foreseeable effects

The lentiviral vectors which we propose to use do have the potential for infecting any human cell. Through good lab practice (GLP) the risk of these vectors affecting the user or others in the laboratory is extremely low. In the unlikely event of infection, the viral particles will not replicate, as discussed above, The expression of these shRNA molecules is hypothesized to target the growth of melanoma cells, and would likely induce cell death. In the case of infection with a lentivirus encoding the Tet-repressor, expression of this protein would have little effect on human cells, as Tet-repressor promoter elements are not present in human genes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We are not requesting derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All transfections and infections of cells will be performed in a designated Class II biohazard safety cabinet, which will be disinfected with 1% Virkon after use. Virkon is a chemical disinfectant that kills 99.999% of organisms in less than 10 minutes. The cabinet will prevent unwanted infection of the cell cultures as well as release of the viruses into the environment. Infected cells will be grown in a designated incubator. Pipettes, containers and plasticware contaminated with viruses will be left completely submerged in 1% Virkon before disposal. Cells and growth medium will be treated with 1% Virkon before disposal according to the Institute’s Waste Index and Disinfection.
protocols. HIV-1 is transmitted through blood and a limited number of bodily fluids, none of which will be encountered in the course of this work. Further, sharps will not be used. To date, no one has been identified as infected with HIV due to contact with an environmental surface (see http://www.cdc.gov/hiv/resources/qa/qa35.htm). No living material infected with HIV derived lentivirus will be transported other than between cabinet, microscope and incubator in the tissue culture room 5C10.1 (ACGM2), but cell extracts will be transported between room 5C10.1, laboratories 4N19-21 (ACGM1) and the equipment room on the 4th floor (ACGM1). Viral preparations will be double-contained and transported between room 5C10.1 and the —80°C freezer (room 4S4, ACGM1) for storage. In the event of a spillage, chlorine-releasing disinfectant granules (HAZ-TAB) or 1% Virkon will be used for immediate disinfection.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Name**

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**Name 2**

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**Campus Estate or Research Centre**

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**Road Name**

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**District**

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<tr>
<td>FK11 7ES</td>
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**Tel Number**

| 01259 761381 |

**Fax Number**

| 0131 529 3894 |

**E-mail**

|                     |

**HSE Division**

| SCOTLAND |

**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022 |

Page 4034 of 15326
Premises Addresses

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<tr>
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<th>Department</th>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

Virology Transgenic Animals Transgenic Fish Gene Therapy
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<th>Transgenic Invertebrates</th>
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<td>Other(s)</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

All materials are autoclaved following use. Set points for system are 121 degree C for 20 minutes at 20 psi. This is normal operating procedure whether material has been used for GM work or otherwise. At this moment in time no GM work is being undertaken.

Tick to confirm that you are attaching a summary of the risk assessment ☐

Tick if you are claiming exemption from disclosure for sections of the risk assessment ☐

Please enter comments of the GM safety committee on the risk assessment
<table>
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**Name**

HORTICULTURAL RESEARCH INTERNATIONAL

**Name 2**

WARWICK - HRI

**Department**

**Campus Estate or Research Centre**

**Building**

**Town**

WELLSBOURNE

**County**

WARWICKSHIRE

**Postcode**

CV35 9EF

**Country**

ENGLAND

**Tel Number**

02476 574455

**Fax Number**

02476 574500

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

GM145 closed and all active projects transferred to GM13

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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<thead>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 145/01.1

<table>
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<tr>
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<th>CU2 Project Title</th>
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<td>VEGETABLE AND ORNAMENTAL CROPS USING WILD-TYPE AGROBACTERIUM RHIZOGENES-MEDIATED</td>
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Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

---

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</table>
## Project Ref: 145/01.2

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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<th>Date Ackn’d</th>
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<th>Class</th>
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<td>VARIOUS STUDIES INVOLVING PSEUDOMONAS SYRINGES AND XANTHOMONAS CAMPESTRIS</td>
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- **Transferred to GM13 on closure of GM145**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Withdrawn**

- N

**Tick if notifying a connected programme of work**

- Y

02/03/2022 Page 4041 of 15326
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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Animal Units

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Project Ref 145/01.3

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<td>EVALUATE THE EFFECT OF BACILLUS THURINGIENSIS CRY O-ENDOTOXINS ON TARGET AND NON TARGET INSECTS</td>
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Date Project Ceased 02/06/2015

Withdrawn N
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N

02/03/2022
Page 4043 of 15326
Project Containment

<table>
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Project Ref 145/01.4

Date Ackn'd: 22/02/2001

CU2 Project Title: PRODUCTION OF INFECTIOUS CDNA CLONES OF TURNIP MOSAIC VIRUS.

Class: Class 2

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Historical Significant Changes: Transferred to GM13 on closure of GM145

Project Additional Information

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
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<th>Laboratory Activities</th>
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02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tbody>
<tr>
<td>26/04/2001</td>
<td>USE OF COWPEA MOSAIC VIRUS (CPMV) FOR THE EXPRESSION OF FOREIGN PEPTIDE SEQUENCES</td>
<td>Class 2</td>
<td>1-50 litres</td>
<td>not applicable</td>
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</table>

Date Project Ceased 02/06/2015

Withdrawn  N

Tick if notifying a connected programme of work  N
### Project Additional Information

**Purposes of the contained use**

CPMV is a plant virus with a bipartite RNA genome separately encapsidated in icosahedral particles. The 3-dimensional structure of the virus particles has revealed that the capsid proteins would be able to tolerate the addition of foreign sequences. Use of this property will be made for the display of epitopes from animal pathogens eg human immunodeficiency virus 1 (HIV-1) at the surface of CPMV capsids. The aim is to produce vaccines based on the expression of small immunogenic sequences via a macromolecular carrier that is non-pathogenic for animals.

**Recipient or parental organism**

Full-length cDNA copies of the genome of CPMV cloned downstream of the CaMV 35S promoter in pUC18-derived plasmids will be used as the cloning templates. Foreign sequences will be linked to the viral coat proteins.

**Host/vector system**

pUC18-based plasmids will be propagated in disabled E. coli strains (JM83 or DH5a). Purified plasmid DNA will be used to initiate an infection of the propagation host for CPMV, cowpea (Vigna unguiculata) cv Blackeye.

**Origin & function**

Synthetic oligonucleotides coding for peptide sequences which correspond to defined antigenic sites from animal pathogens, eg the envelope protein gp160 of HIV-1 will be inserted into the viral genome fragment coding for the CPMV capsid proteins. These inserts can vary in length between less than 10 and up to 100 amino-acids and are selected for their potential to elicit an immunological response in animals or humans, but are devoid of pathogenicity of their own. In addition given the synthetic nature of the DNA inserts, no propagation of the pathogens from which the sequence of these peptides is derived will be involved.

**Evaluation of foreseeable effects**

Alteration of the capsid proteins of CPMV by the addition of foreign sequences, which are neither plant nor plant pathogen-derived, has in past experiments not changed the virus host range; such alterations actually tend to produce virus particles which are less stable, less infectious and less transmissible by the crysomalid beetle vector than the wild-type counterpart.

The foreign sequences to be expressed via CPMV present no endogenous toxicity or allergenicity. Accidental induction of an immune response against the antigenic peptides grafted onto CPMV particles is unlikely given that this can only be achieved by deliberate exposure, via artificial methods (injection) of large doses of purified virus in the presence of an adjuvant.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In the laboratory.
- the E. coli strains used to propagate plasmids bearing CPMV sequences are disabled; they constitute Class 1 GMMs which will be handled in Tontainment Level 1 Laboratory facilities, according to conditions of Good Laboratory Practice.
- residual plant material from the virus extraction procedure and disposable plasticware will be autoclaved before disposal whilst reusable centrifugation bottles and tubes will be treated with the broad-spectrum phenol-based disinfectant Hycolin and with bleach which will ensure their complete decontamination.

In the glasshouse, plants will be grown on waterproof benches that will permit collection of any water effluents; accidental spillages onto floors and all collected waters will be treated with 0.8% of the commercial disinfectant Jet5, supplied by Hortichem (final concentration 0.04% peroxyacetic acid). All materials used for virus inoculation (gloves, tips, tubes etc) as well as waste products from infected plants: pots, compost, residual plant material will be collected in plastic bags which will be sealed and placed into appropriate containers for autoclaving to ensure total virus particle destruction before disposal.

Is an emergency plan required according to regulation 20? Y
If yes, tick to confirm that it is attached to this form Y
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The attached risk-assessment has been discussed and approved by the Genetic Midification and Biological Safety Committee at HRI, Wellesbourne.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 145/01.7

Date Ackn'd 05/09/2001
CU2 Project Title TRANSFORMATION OF VERTICILLIUM DAHLIAE AND IN PLANTA DETECTION
Class 2
CultureVolClass2 < 1 litre
CultureVolumeClass3-4
**Project Additional Information**

**Purposes of the contained use**

a. To develop DNA-mediated transformation systems of the plant pathogenic fungus Verticillium dahliae  
b. Introduction and expression of established marker genes hygromycin resistance (hph), green fluorescent protein (GFP), luciferase (luc), B-glucoronidase (GUS)  
c. Monitoring infection, growth and development of marked V. dahliae on strawberry plants.

**Recipient or parental organism**

Verticillium dahliae, is a plant pathogenic, soil borne fungus causing vascular wilt in a variety of plant species including cotton, tomatoes, potatoes and strawberries. It is an imperfect asexual fungus with no sexual stage and as such does not interbreed. The pathogen infects the vascular system of various crop species and produces conidia (asexual spores) in planta that do not persist outside of the host. There is effectively no air-borne dispersal phase. Sole route of infection is through contaminated soils. This point is no better illustrated by the fact that healthy plants are routinely cultured beside infected plants without any cross-contamination. Micro-sclerotia (spore resting phase) are formed in necrotic plants, these are able to survive for many years in soil, but will not be formed in proposed experiments as plants will be destroyed at advanced stages of disease.

**Host/vector system**

Two host strains of V. dahliae will be used. These are two isolates (12008, 12009) from infected strawberry plants at East Malling, UK. Transforming DNAs will in the form of closed circular plasmid or as Agrobacterium binaries and will consist of marker genes under the regulation of the Aspergillus nidulans' glyceraldehyde-3-phosphate dehydrogenase (GPD) 5'UTR promoter sequence and tryptophan (trpC) terminator sequence. Transformation of the fungi occurs by integration into the genomic DNA; vector sequence do not replicate autonomously within the host strains.

**Origin & function**

V. dahliae transformants harbouring the GFP marker will be grown in broth cultures (100 ml) to produce conidia for strawberry plant inoculations. Broth cultures will be filtered to remove mycelium and conidial suspensions used for root tip inoculations. Up to 20 plants will be infected in an experiment. After ca. 1-week crown or petiole samples will he barvested and examined by UV microscopy. All plants and soils will be destroyed before necrosis.

**Evaluation of foreseeable effects**

All activities will be performed within and effectively 'contained' within purpose built laboratories and growth cabinets at HRI-Wellesbourne.
V. dahliae modified to express the marker genes proposed is no more likely to survive in the environment than wild type strains and is unlikely to displace other organisms.

V. dahliae is a plant pathogen and is non-pathogenic for humans. The inserted marker gene sequences have no known hazard and do not offer any substantial advantage should the transgenic escape into the wider environment.

As described above the organism is contained in plants, has no air-borne dispersal mechanism and is not able to transfer genes to other isolates/species.

The plant pathogenic fungus can be controlled by standard industry practices. V. dahliae is widespread throughout Europe and within the UK. Growers normally destroy infected crops. Primary disease control method is soil fumigation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated soil, waste water and plant materials will be sterilised by autoclaving prior to disposal.

Plant pots will be held on trays to collect any run-off water. Waste water and trays will be autoclaved and/or treated with the proprietary disinfectant Virkon. Water from the growth cabinets does not empty to a general drainage system.

All contaminated laboratory consumables and cultures will be sterilised by autoclaving prior to disposal or washing.

All laboratory surfaces will be disinfected using 70% v/v ethanol, hypochlorite (1,000-10,000 ppm available chlorine) or Virkon.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The risk assessment has been discussed and approved by the Genetic Modification and Biological Safety Committee at HRI, Wellesbourne.

Project Containment

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02/03/2022
Project Ref 145/02.1

Date Ackn’d 13/03/2002
Date Project Ceased 02/06/2015

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

PVX and PEBV are both single-stranded RNA viruses with a positive-sense genome, monopartite in the case of PVX, bipartite in the case of PEBV. PVX is a well-characterised widely used transient expression vector for foreign genes. It will be used to explore the properties of proteins encoded by another plant virus, cowpea mosaic virus (CPMV). In addition heterologous phytases will be expressed in plants via PVX.

PEBV is under development as an expression vector and only reporter genes giving colorimetric reactions will be used in order to be able to assess the efficiency of the system.

Recipient or parental organism

Full-length cDNA copies of the genome of PVX in either pBluescript (bacteriophage T7 promoter) or the binary Ti vector pGreen (Cauliflower mosaic virus 35S promoter) will be used as cloning templates. Foreign genes will be expressed either from a duplicated promoter for the PVX coat protein (CP) or linked to the PVX CP.

Full-length cDNA copies of the bipartite genome of PEBV are cloned downstream of the T7 or 35S promoter in pUC-derived plasmids. Transfer of these cDNAs into the binary vector pGreen is planned in order to allow infection of plants via Agrobacterium. Reporter genes will either replace viral gene products or be added to the viral genome.

Host/vector system
pBluescript and pUC-based plasmids will be propagated in disabled E coli strains (JM83 and DH5a). pGreen-based plasmids will be transformed into disarmed Agrobacterium tumefaciens strains (eg GV3101). Either in vitro transcripts (T7 promoter-driven clones), purified plasmid DNA (35S promoter-driven clones) or Agrobacterium cultures (pGreen-based clones) will be used to mediate the infection of host plants. PVX will be inoculated onto Nicotiana benthamiana plants and PEBV will be propagated in both N. benthamianas and peas.

Origin & function

The sequences to be inserted into PVX are of two kinds:
- genes derived from the genome of CPMV. Known functions of these viral proteins are encapsidated and movement of the viral particles from cell to cell. Their expression via PVX is destined to identify potential pathogenicity-determining functions.
- genes encoding phytases of bacterial or plant origin. These enzymes liberate phosphate stored in the form of phytate making it bioavailable both to the plant and to animals, who are devoid of this enzyme (eruminants excepted).

Genes to be inserted into PEBV consist in the green fluorescent protein (GFP) or B-glucuronidase (GUS); expression of these reporter genes from the viral vector can be monitored by fluorescence or an enzymatic reaction respectively.

Evaluation of foreseeable effects

CPMV-derived proteins are by nature of their plant virus origin not expected to represent a hazard to human health. As yet unidentified properties of some of these genes may consist in pathogenicity-enhancing traits. However given the nature of the PVX vector used, their expression will only be transitory; due to duplication of the CP promoter gene in the PVX vector, foreign sequences are rapidly lost as a result of homologous recombination.

No adverse effects are expected from the expression of phytases via PVX as this type of enzyme is already present in plants although often expressed at low levels. Higher levels of phytase will allow the host plant to better utilise phosphate from soil sources. As to animals their feed is already supplemented with phytases.

GFP and GUS are widely used reporter genes and are neither expected to increase the infectivity of the recipient PEBV nor known to have toxic or allergenic effects on humans and animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In the laboratory, including propagation of recombinant plasmids in E coli and Agrobacterium, and analysis of infected plant material. Residual plant material and disposable plasticware will be autoclaved. Reusable items, eg glassware, will either be decontaminated with 20% bleach or 2% Hycolin by contact overnight.

In the glasshouse. Plants will be grown in waterproof trays in a facility with a concrete floor and presenting no connection to the general drains. Accidental spillages to the floor will be treated by addition of the commercial disinfectant Jet5 to a final concentration of >0.8%. Reusable items, including plant trays will be disinfected with 0.8% Jet5 overnight. Jet5 concentrate contains 5% peroxyacetic acid and is used according to manufacturer's recommendations.

Waste products from virus inoculation, and from plants following completion of the experiments, will be autoclaved at 121 degrees C for 30 mins to ensure complete virus particle destruction before disposal.
The attached risk assessments have been discussed and approved by the Genetic Modification and Biological Safety Committee at HRI, Wellesbourne.

Project Containment

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Project Ref 145/03.1

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Project notified under transitional arrangements N

Historical Significant Changes

Transferred to GM13 on closure of GM145

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

**CMV- 2b-GFP and CMV-GFP**

A mutant of cucumber mosaic virus (CMV) that lacks the 2b protein gene and expressing free green fluorescent protein (GFP) and the wild type CMV expressing free GFP obtained in the laboratory of Prof P Palukaitis (SCRI) will be used as a model system to determine whether 2b-less CMV isolates/mutants make better cross-protecting strains and test the hypothesis that cross-protection occurs via virus induced RNA silencing.

Virus movement in the plant will be assayed by detection of GFP under UV light.

Presence of the 2b protein will be determined by Western blotting.

Two well established markers for the silenced state in plants are 25 nt oligonucleotides (SiRNA) and increased levels of host RdRP (RNA dependent RNA polymerase) determination of whether levels of these markers are increased will be determined by Northern blotting.

**TRV-GFP**

A tobacco rattle virus (TRV) modified to remove the non-structural 2b and 2c genes and replaced with GFP driven by a second coat protein gene subgenomic promoter derived from a different tobravirus isolate provided by Dr S Macfarlane (SCRI) will be used as a control for CMV-GFP experiments. Virus movement in the plant will be assayed by detection of GFP under UV light.

Two well established markers for the silenced state in plants are 25 nt oligonucleotides (SiRNA) and increased levels of host RdRp (RNA dependent RNA polymerase) determination of whether levels of these markers are increased will be determined by Northern blotting.

**Recipient or parental organism**

CMV is one of the most important and widespread viruses of field grown horticultural crops in the world. CMV has a particularly wide host range including Cucumis sativus, Lycopersicon esculentum and Spinacia oleracea. In total it infects over 1,000 species including many weed species. CMV can be transmitted mechanically, and in few cases via seed, but the major mode of infection in vegetable crops is by aphids in a non-persistent manner.

Tobacco rattle virus belongs to one of only two genera of viruses that are transmitted by nematodes, the tobraviruses. Tobraviruses are transmitted only by nematodes belonging to the genera Trichodorus and Paratrichodorus. It can also be transmitted via seed, grafting and by mechanical inoculation. TRV has a wide natural host range including Stellaria media, Voila arvensis. Beta vulgaris. Spinacia oleracea, Capsicum annum Solanum tuberosum Narcissus pseudonarcissus. Tulipa sp. and Hyacinthus sp.

**Host/vector system**

CMV- 2b-GFP and CMV-GFP

To ensure that virus stocks are genetically uniform and satellite-free 2b-less CMV and the parental wild type CMV will be reconstituted from cDNA clones by in-vitro transcription and the resultant RNA inoculated onto tobacco to bulk up the virus for subsequent experiments.

**TRV-GFP**

Clones of RNA2 of TRV were modified to remove the non-structural 2b and 2c genes and replaced with GFP driven by a second coat protein gene subgenomic promoter, derived from a different tobravirus isolate (pea early-browning virus).

**Origin & function**

The CMV- 2b-GFP and CMV-GFP will be obtained from the laboratory of Prof P Pulukaitis (SCRI) and the TRV-GFP from Dr S Macfarlane (SCRI)

GFP will be used as a reporter gene for virus movement within the plant.
Evaluation of foreseeable effects

The 2b protein gene in virus interferes with virus induced gene silencing in plants protecting the virus against host defences. It is hypothesised that the properties of the 2b protein may play a role in the induction of cross protection. If the virus lacks a functional 2b protein and is therefore unable to prevent gene silencing it should be less pathogenic. The CMV-2b mutant is less able than the wild type virus to spread systemically and accumulates to lower levels than the wild type in inoculated and uninoculated tissues (Soards A.J. et al 2002, Mol. Plant-Microbe Interact Vol 15, No. 7 pp 647-653). There is therefore no predicted hazard arising from the deleted gene. The inserted gene GFP is well characterized and there is no evidence for it being a hazard.

TRV-GFP
The deletion of the non-structural 2b and 2c genes should not alter pathogenicity as infection can occur in the complete absence of RNA 2. The non-structural genes on RNA 2 have been shown to be necessary for transmission of tobraviruses by vector nematodes; thus constructs in which these genes have been deleted should be effectively contained in the plants under test. (Macfarlane and Popvich 2000 Virology 267 29-35). The inserted gene, GFP is well characterized and there is no evidence for it being a hazard.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Plant pots will be placed on trays or saucers in order to catch run-off water and this water will be allowed to evaporate naturally. All plant material, soil pots and labels will be autoclaved at 121 degrees C before final disposal in the skip. An autoclave is situated in the glasshouse. All trays, saucers, glass, flooring and benching will be thoroughly disinfected with Jet 5 disinfectant (8mls/L). Plant material used in the laboratory will be autoclaved at 121 degrees C along with bags, tips and any other items used during laboratory procedures. This will then be placed in the skip. An autoclave is present in the same building as the laboratory. Benches in the laboratory are impervious and will be swabbed down with 70% ethanol before and after use.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk-assessment has been discussed and approved by the Genetic Modification and Biological Safety Committee at HRI, Wellesbourne.

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms

02/03/2022  

Page 4056 of 15326
Molecular basis for acquisition and transmission of aphid picornaviruses.

The main reasons for developing genetically manipulated aphid picorna-like viruses are:

1. To develop a cell-culture based system for production of aphid picorna-like viruses
2. To identify viral genes (eg regions of the coat protein(s) which are specifically required for virus acquisition by aphids.
3. To map gene(s) which determine the host range of aphid picorna-like viruses
4. To understand role of plants in the transmission of aphid picorna-like viruses (this will include checking whether aphid picorna-like viruses can replicate in plant cells in vivo and in vitro)
Aphid viruses which would be used in this study (Aphid lethal paralysis virus - APV, Rose-apple aphid virus - RAAV, and Acyrthosiphon pisum virus - APV) form spherical virus particles containing a single stranded positive RNA genomes. Genomes of those viruses encode non-structural replication proteins containing motifs characteristic of RNA-dependent RNA polymerases, chymotrypsin-like proteases, and helicases showing similarities with homologous proteins of other so-called picorna-like viruses.

The genome of ALPV contains two long open reading frames (ORFs), which are separated by an intergenic region. The deduced amino acid sequences of the 5' ORF and 3' ORF products respectively showed similarity to the non-astructural and structural proteins of members of the insect-specific family Dicistroviridae. Infectivity experiments showed that ALPV can not only infect aphid species but is also able to infect the whitefly Trialeurodes vaporariorum, extending its host range to another family of the order Hemiptera.

The APV genome contains two large open reading frames (ORFs). The 5'-proximal part of the ORF1 encodes non-structural proteins. The 34K major structural protein is encoded by the 3'-proximal part of the ORF1, while the 66K protein contains both ORF1-(34K) and ORF2-derived sequences and is probably expressed by a (-1) translational frameshift. The 23/24K proteins most likely arise by proteolytic breakdown of the 34K protein. APV was found in pea aphid (Acyrthosiphon pisum).

Gemp,e organisation of RAAV is very similar to that of APV. APV shares a high degree of nucleic acid (78%) and amino-acid similarity (87%) with RAAV. RAAV was isolated from rose-apple aphids.

Although the deduced APV and RAAV genomic organisation in some aspects resembles that of the picornaviruses, its overall genomic organisation indicates that APV is a distinct species only distantly related to the Picornaviridae. To date, no viruses with this genome organisation have been found in other hosts except aphids.

Recipient or parental organism

pCRII-Topo (Invitrogen) is a bacterial plasmid vector which will be in disabled E. coli strains (TOP10 or DH5a)

In vitro transcription will be used to infect insect cell culture (eg Drosophila S2 cells). Recombinant viruses will be recovered from infected cells and used for the inoculation of aphids using artificial liquid diets and by surface contamination of host plants.

In vitro RNA transcripts will also be used in plant protoplast transfection experiments to determine if aphid picorna-like viruses can replication in plant cells.

Host/vector system

Reporter gene for green fluorescent protein - GFP (from the jellyfish Aequoria victoria) will be used to facilitate the construction of the recombinant viruses and to study gene function by producing gene "knockouts" and GFP fusion proteins.

Origin & function

Picorna-like RNA viruses of aphids (RAAV, APV, ALPV) are pathogens of aphids and insect specific. They are not hazardous to vertebrates, including humans. The organisation of APV, RAAV and ALPV tenomes in significantly different from that of picorna-like viruses of mammals, in terms of tenome organisation and significant sequence similarity. Therefore aphid picorna-like viruses are unlikely to cause human disease and aphid picorna-like virus-derived proteins are not expected to represent a hazard to human health.

Plant and mammalian, and insect RNA viruses (even those which belong to the same "picorna-like" supergroup) share very low level of sequence similarity with no extended stretches of similarity, which are prerequisite for recombination events between RNA genomes. Such a low level of nucleotide similarity suggests that a possibility of recombination event between animal (insect) and plant viruses even in the event of simultaneous replication of both viruses in the same cell is extremely low and will not result in generation of viable viral genome which is capable of independent replication. Given that GM picorna-like viruses which will be produced in this study will be less pathogenic, the probability of such recombination event is very unlikely.

No adverse effects are expected from the expression of GFP, which is widely used reporter genes and are neither expected to increase the infectivity of the recipient aphid picornaviruses nor known to have toxic or allergic effects on humans and animals.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In the laboratory, including propagation of recombinant plasmids in E. coli and analysis of infected cell culture, aphids and plant material.

All microbiological work is conducted in a class 2 cabinet and all waste eg Eppendorf tubes. Gilson tips etc is sterilised by autoclaving. Glassware is sterilised by autoclaving and microbiological cultures are destroyed by autoclaving. Sharps are collected in the appropriate bin, which is sent for incineration.

Cell culture work is carried out in a class 2 cabinet fitted with an UV sterilising lamp. Virus contaminated instruments are either autoclaved directly eg forceps and scrapers and contaminated glassware and centrifuge tubes are soaked in 1% "Virkon" for at least one hour which will inactivate the virus. Insects are infected in a virus handling room also equipped with UV sterilisation. The virus handling room is cleaned with 1% Virkon and then UV treated after use. Centrifuge rotors are washed in Virkon and placed under an UV lamp to inactivate any residual virus. All centrifuge gradients are prepared and loaded in the virus handling room.

Virus infected aphids are sealed into escape proof containers and are stored in designated incubators, glasshouses and CT rooms. These are opened in the virus handling room. Aphids will be transported between the designated areas in sealed containers.

Lab coats are segregated for use with viruses and labelled, gene cloning and insect rearing and are autoclaved and then laundered weekly. Hand wash facilities are in place in each room.

Recombinant virus will be handled in a Class II cabinet in Lab 30 PPB fitted with a UV lamp to destroy and residual virus or in the virus handling room in lab 13 TPB. Red lab coats will be worn and these will be autoclaved before being sent to the laundry.

Recombinant viruses and plasmids are stored at - 20 degrees C or -80 degrees C and their location recorded.

In the glasshouse.

Plants will be grown in waterproof trays in a facility with a concrete floor and presenting no connection to the general drains. Accidental spillages to the floor will be treated by addition of the commercial disinfectant "Virkon" to a final concentration of 1% Reuseable items, including plant trays will be disinfected with 1% "Virkon" overnight.

Waste products (plants, pots, gloves etc) following completion of the experiments, will be autoclaved at 121 degrees C for 30 minutes to ensure complete virus particle destruction before disposal.

Virus infected aphids are sealed into escape proof containers and are stored in designated incubators, glasshouses and CT rooms. These are opened in the virus handling room.

Infection of aphids by artificial feeding will be carried out in escape-proof containers (Lab 13 TPB adjacent to the cell culture room). After infection aphids will be transported in the sealed containers to Glasshouse Compartments CC8, or CC9 where they will be placed on plants grown in separate aphid-proof ventilated containers. Sampling of infected aphids and plants will be carried out in the glasshouse Compartments CC8 and CC9. Sampling procedure will involve (1) transfer of the aphid proof boxes (containers) to CC8/CC9 (2) collection of aphids and plant material (to Eppendorf tubes), placing the remaining plant material, pots, etc into an autoclave bag; (4) transfer of the emptied container and bagged plant material to the designated freezer (Compartment CC5) (5) 24 hr incubation in the freezer to kill aphids (6) cleaning of...
Aphid samples will be collected from the containers in the containment area CC5, counted and placed in eppendorf tubes. Gloves used in aphid handling will be autoclaved (autoclave is located in the same building, C), the lab coats will be kept in -20°C freezer. Aphids which will be left in the containers by the end of the experiment will be anesthetized by placing the entire containers with plants in -20 freezer (located in the handling compartment CC5 which is kept under negative pressure) for several hours prior to (1) collecting the remaining plant material (with pots) to autoclave bags for sterilisation and (ii) sterilisation of the containers with disinfectant. The material used in cleaning the boxes, including gloves, cloths, and paper tissues will be autoclaved alongside with the plant material. To ensure that there will be no contamination of clean plants (maintained in Compartments C8 and C9) with aphids and prevent escape of aphids to the environment, the unit will require negative pressure, airflow (direction C8/C0 - CC6 - CC5 - CC8/CC9 and airlock (CC$) in constant operation through the course of experiments. Negative pressure containment is a standard requirement for work with infected aphids (See the plan of building C above on CU2 form). Entry and exit air will be filtered by F7 filters to prevent escape of aphids (99% effective for particles of 10um).

Transfer of aphid and plant sanokes (placed in a sealed Eppendorf tubes) from the Building C to the virus handling laboratory 13 TPB will be carried out in a sealed box for additional security. Autoclaves are housed in both the glasshouse complex and the laboratory building and therefore waste will be inactivated locally without need to transfer between buildings.
Use of Pseudomonas syringae pv tomato (DC3000) to deliver oomycete effector genes into plant cells

Date Project Ceased
02/06/2015

Tick if notifying a connected programme of work
N

Historical Significant Changes
Transferred to GM13 on closure of GM145

Project Additional Information

Purposes of the contained use

a. Oomycete effector genes (from Hyaloperonospora arabidopsidis and Phytophthora infestans) will be fused to the secretory signal of the Pseudomonas syringae pv Tomato DC300 effector AvrRPS4 and transformed into Pseudomonas syringae pv Tomatoe Dc3000 isolate.

B. Arabidopsis plants will be inoculated with the modified DC300 isolate and tested to determine if the introduced effector triggers a defence response from the plant via recognition by a plant resistance gene (effector triggered immunity) or if the effector increases isolate virulence due to suppression of the plant's basal defence response (effector triggered susceptibility). The following plants may also be inoculated: Potato, Tobacco and Brassica oleracea.

Recipient or parental organism

Pseudomonas syringae pv Tomato is a gram negative, aerobic, rod-shaped bacterium, which is able to survive in soil, in debris form diseased plants and on seeds. Infection is spread by rain splash allowing entry into leaves via natural openings, such as stomata, where it multiplies in the intercellular space resulting in lesions on infected leaves.

Successful infection is dependant upon the translocation of effector molecules into the plant cell to promote disease and suppress/avoid PAMP (pathogen associated molecular patterns) triggered immunity and effector triggered immunity (Ponciano et al 2003, Jones and Dangl 2006)

Pseudomonas syringae pv Tomato is able to cause disease on Solanum Lycopersicum (tomatoe), Capsicum annuum (pepper) and Solanum melongena (aubergine) (Bradbury 1986). In addition, some strains, which include DC3000 are virulent on Arabidopsis thaliana, Brassica oleracea (cabbage, cauliflower, broccoli) and B. campestris (turnip) (Whalen et al 1991). DC3000 is also able to infect Nicotiana benthamiana (tobacco) if the effector hopQ1-1 is removed (Kvitko et al 2009). The isolate DC3000 was isolated in Ontario, Canada by Diane Cuppels and is widely used for molecular studies as it is a naturally occurring pathogen of the model plant Arabidopsis thaliana. In addition, the genomes of both DC3000 and Arabidopsis have been sequenced. Consequently, the technology we wish to use has been developed using this Pseudomonas isolate (Sohn et al 2007).

References:

Host/vector system

The host strain Pseudomonas syringae pv Tomato DC3000 will be used, which was isolated in Ontario Canada in 1986.

Oomycete effector genes will be cloned into the pEDV6 vector and transformed into DC3000. The pEDV6 vector was developed in Jonathon Jones's lab at the Sainsbury laboratory and contains the N-terminal secretory signal from the DC3000 effector AvrRPS4 and is expressed using the native AvrRPS4 promoter. This allows delivery of the oomycete gene into plants cells via the bacterium's type three secretion delivery mechanism. This vector carries gentamicin to allow antibiotic selection of transformed cells. The pEDV6 vector is a replication compromised plasmid that is unstable and lost in the absence of persistent antibiotic selection.

Origin & function

DC3000 transformants will be grown in liquid culture in volumes of no greater than 30 ml. Cultures will then be adjusted to an appropriate OD (1 - 2.5) before infiltration of leaves using a 1ml syringe (without needle) or spray inoculation using a hand held atomiser. Infected plants will be analysed after 0-7 days. All plants and soil will be autoclaved at the end of the experiment (see section 12).

Evaluation of foreseeable effects

All activities will be performed and effectively contained within purpose built laboratories and growth rooms at Warwick HRI, The University of Warwick, Wellesbourne. Pseudomonas syringae bacteria are non-sporulating and can be readily contained using good microbiological practice.

The oomycete effectors may increase, decrease or have no effect on DC3000 virulence when it comes into contact with its host plants. The modified DC3000 isolates may be able to suppress/avoid the host's basal resistance response. Conversely, the introduced effector may act as an avirulence factor and trigger an R gene mediated resistance response in a normally susceptible accession.

Whether the modified DC3000 strains could infect a new host plant has never been tested but this is unlikely to happen, as a single effector, evolved to function in a specific plant-microbe interaction, is unlikely to happen, as a single effector, evolved to function in a specific plant-microbe interaction, is unlikely to overcome the reasons why the plant is a non-host to DC3000 in the first instance. For example, where DC3000 is unable to overcome the plant's passive defence mechanisms, such as a waxy cuticle, rigid cell walls and preformed antimicrobials or effector triggered immunity.

During escape this organism could potentially transfer the oomycete effector gene to other bacteria and possibly cause the undesirable spread of resistance to gentamicin. The likelihood of these hazards occurring is extremely low due to the use of the replication-compromised plasmid that is unstable and lost in the absence of antibiotic selection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All infected/contaminated laboratory waste, discarded cultures and infected/inoculated plant material will be disposed of by autoclaving using a discard cycle at 121 C (15psi) with the centre of the load maintained at this temperature and pressure for a minimum of 30min. The autoclaved material will be emptied into a dustbin bag and taken to a dedicated skip for disposal.

Spillages or infected liquid will be absorbed using tissue and then disposed of by autoclaving (as above) and then disinfection of the area/equipment with 70% ethanol.

The Class II safety cabinets will be disinfected by spray and wiping with 70% ethanol. Growth chambers will be disinfected after use by spray wiping with 70% ethanol.

Propogator trays using in the growth chamber will be disinfected by immersion in a disinfectant solution (e.g. 10% bleach water solution) and/or spray wiped with 70% ethanol. All contaminated glassware/equipment will be disinfected after use and before cleaning by autoclaving where possible, otherwise by immersion in disinfectant solution or spray wiped with ethanol.

These measures will result in 100% kill of the pathogen

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Risk assessment approved by the Genetic Modification Safety Committee at Warwick HRI on 15/06/2009

Project Containment

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Date at Which Additional Info Submitted

| 11/05/2001 |
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#### UNIVERSITY OF CAMBRIDGE
- **DEPARTMENT OF ZOOLOGY**
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  - **N**

#### UNIVERSITY OF CAMBRIDGE
- **DEPARTMENT OF ZOOLOGY**
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: [ ]

- **Give brief details of the genetic modification safety committee**

  - **Laboratory**
  - **Animal Unit**
  - **Growth Room**
  - **Glass House**
  - **Large Scale**

  - **Level 1 (GMMs)**
  - **Level 2 (GMMs)**
  - **Level 3 (GMMs)**
  - **Level 4 (GMMs)**
  - **Non-microbial**

  - **Other (please specify)**
    - **Tick if confidential**: [ ]
    - **Bacteriology**
    - **Parasitology**
    - **Transgenic**
    - **Birds**
    - **Microbiology**
    - **Research**
For activities involving GMMs, describe the waste management measures which will apply to the activity.

The maximum culture volume that could be released at any one time is one litre. Most cultures are less than 50 ml.

Waste is deactivated by autoclaving. The deactivation method is validated and monitored using Browne Sterilizer Control Tubes and Autoclave Indicator tape, and the autoclaves are serviced every six months and their performance verified.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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#### Comments

| GM CENTRE CLOSED AND ALL WORK TRANSFERRED TO GM14 |

#### Date at Which Additional Info Submitted

| 02/03/2022 |
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

| Level 1 (GMMs) |
|----------------|----------------|
| Level 2 (GMMs) |
| Level 3 (GMMs) |
| Level 4 (GMMs) |
| Non-microbial  |

Other (please specify) Tick if confidential

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<td>Birds</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All experiments are Class 1 at present. Waste management involves autoclaving at 121 degrees C, before disposing in clinical waste. Max. culture volumes used approximately 500 mls - 1 L.

Autoclaves serviced and checked regularly.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 147/07.1

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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

PROJECT TRANSFERRED TO GM14 & GM CENTRE CLOSED

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
## Project Additional Information

### Purposes of the contained use

We wish to use third generation lentiviral vectors (PCDH family; Systems Biosciences) to express ion channel genes in neurons and neuronal cell lines. While these lentiviral vectors are deactivated using several approaches and are regarded as a very low risk for recombination (details at www.systembio.com) there remains a theoretical risk. For this reason, and to protect the target mammalian cells from contamination, as is usual for tissue culture experiments, we will conduct work in containment level 2 facilities. All live animal work will be done in containment level 2 as well.

### Recipient or parental organism

The GMOs will be lentiviral particles containing sequences encoding ion channel genes. These viruses will be recombination deficient, but will be capable of transducing individual mammalian cells (including handlers, if skin is broken). Any inoculation with these CMOs should be restricted to the subset of cells that come immediately in contact, but will not spread. The genes carried by the CMOs will be expressed in inoculated cells, but these genes (ion channels) are not dangerous to neighboring cells.

### Host/vector system

The vector system used will be the Systems biosciences PCDH lentiviral kit. These lentiviral vectors are third generation and represent a low risk of recombination or of infection. They are self-inactivated, Tat deleted, have the packaging genes split on to three separate packing vectors, and mature virus particles (the CMOs) will contain no original packaging genes. However as developments in lentiviral research provide safer alternatives we will continue to upgrade our choice of viral system, always in the direction of reduced ability to recombine and increased safety.

### Origin & function

The genetic material will be cloned cDNA from human tissues. These cDNAs will encode ion channels that are associated with inherited diseases in humans. We have a large collection of mutations in different ion channel genes (an expanding group including: SCNs, CACNA1A, KCNA1, and accessory subunits), that have been identified with patients presenting with various neurological disorders (including epilepsy, ataxia, and migraine). None of the ion channel genes are thought to be oncogenic or dangerous on their own or in combination with other genes. They are not secreted but remain in the membranes and cytosol of the cells expressing them. The genes encode ion H channels and their accessory subunits that allow cells, generally neurons or muscles, to respond to stimuli by passing ions across their membranes. We will analyse how the ability to pass ions across the membranes is altered when mutations associated with human diseases are expressed in the ion channels. All work with patient DNA is done under appropriate ethical approval and consent. The neurological nature of the diseases that we are studying, and the specialized nature of the ion channels we are studying requires that we explore the behavior of these genes in neurons and muscles. While much of our work can be done in vitro, the actual functioning of neurons and muscles in vivo, when expressing the target genes (wild-type vs. mutant) will be key to understanding how different ion channels are capable of causing different neurological disorders. We hope to investigate the ability of cells inoculated with viruses in vivo (in rodents only) to survive, propagate action potentials and to communicate with other neurons, especially when expressing ion channel H mutants that in humans have been shown to disrupt these processes.
We anticipate that the primary effects will be the limited expression of our genes of interest in the desired H mammalian cells. In general this will have modest effects on the infected cells behaviour, but should not lead to H changes in neighboring cells. It is possible that the virus particles could infect any human cells including those of H workers, if they contacted broken skin (i.e. with a sharp glass pipette or syringe needle). Such an infection with an H inactivated non-replicable virus is likely to present a very small risk to the worker. Individual cells infected by the H virus would express only the ion channel genes encoded in them and the effects of these genes would be limited to H the infected cells. However it has been shown in some circumstances where large amounts of virus are used to H infect emryonic or stem cells that tumors may be produced, although the mechanism has not been clearly linked to H the viruses. For these reasons, as well as to maintain the sterility of our tissue culture stocks, and the health of H animals during surgery, we will conduct all experiments with viral particles in containment level 2 cabinets. In H addition all plasticware, sharps and media that comes in contact with the virus particles will be disinfected with H bleach. Innoculated animals will be maintained in containment level 2 conditions and animal remains will be H disposed of in sealed containment level 2 clinical waste bags. No animals inoculated with viral particles will be H allowed to breed, and no GM animals will be generated.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Both liquid and solid waste will be treated with bleach solutions (degree of kill effectively 100% for both virus U particles and transformed mammalian cells). The effectivity of these solutions is monitored routinely by the the p1-I U indicating properties of the media, and bleach is stored in solid form to prevent inactivation. After disinfection with U bleach solid waste is disposed as clinical waste. U Surfaces in contact with media and virus will be sterilized with ethanol, and will be routinely (usually daily or, U exceptionally, weekly) UV irradiation. U Animal remains will be disposed of in sealed class 2 clinical waste bags. Live animals will be maintained in Class 2 U containment facilities, and used cages will be autoclaved before cleaning.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
In view of current understanding of the risk associated with this vector system the committee argued that containment level 2 precautions are appropriate for this purpose.

### Project Containment

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GM Centre Number: 151

Data Premises Notified (Originally) 18/01/1984

Transferred from 1992 Regs? Y

Transitional Premises Class 3

Data Premises Closed N

Emergency Plan Required? N

Transitional Premises N

Non-GMMs N

Withdrawn N

Name

NERC INSTITUTE OF VIROLOGY & ENVIRONMENTAL MICROBIOLOGY

Name 2

CENTRE FOR ECOLOGY & HYDROLOGY

Department

Campus Estate or Research Centre

Building UNIT 44C

District ABINGDON

Road Name MILTON PARK

Town OXFORD

County OXFORDSHIRE

Postcode OX1 4RU

Country ENGLAND

Tel Number 01865 281630

Fax Number 01865 281696

Comments

Date at Which Additional Info Submitted 02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.

Project Ref 151/01.1

Date Ackn'd 21/02/2001

CU2 Project Title CLONING AND SEQUENCING OF POPLAR MOSAIC CARLAVIRUS

Class CultureVol Class 2 CultureVolume Class 3-4

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
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### Project Ref 151/99.1

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<td>FATE ACTIVITY &amp; THREAT BY RALSTONIA SOLANACEARUM CASUAL AGENT OF POTATO BROWN ROT IN EUROPEAN SOILS RHIZOSPHERES &amp; WATER SYSTEMS</td>
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**Project notified under transitional arrangements**: Y

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 151/trans1

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Tick if notifying a connected programme of work [N]
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
Please enter comments on the GM safety committee on the risk assessment

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QUADRAM INSTITUTE BIOSCIENCE

NORWICH LABORATORY

GENETICS & MICROBIOLOGY

NORWICH RESEARCH PARK

COLNEY

NORWICH

NORWICH

01603 255000

01603 507723

EAST AND SOUTH EAST

02/03/2022
## Premises Addresses

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<th>Name</th>
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<th>Building</th>
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<th>Town</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 3 (GMMs)</td>
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Page 4082 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 155/01.1

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Date Project Ceased</th>
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<tr>
<td>12/08/2001</td>
<td>IN VITRO TRANSPOSITION MUTAGENESIS OF CAMPYLOBACTER JEJUNI</td>
<td></td>
<td>Class 2</td>
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Withdrawn: N

Tick if notifying a connected programme of work: N
### Project Additional Information

**Purposes of the contained use**

The construction of libraries of random insertion mutants of C. jejuni as a tool for studying genes essential for virulence and pathogenicity.

**Recipient or parental organism**

C. jejuni is an ADCP group 2 pathogen.

**Host/vector system**

No DNA capable of extra-chromosomal replication in C. jejuni is involved.

**Origin & function**

The mini-transposons to be inserted contain specific insertion sequences derived from wild type transposons. The function of these sequences is to ensure the insertion of the mini-transposon into chromosomal DNA by the action of the appropriate transposase in vitro. These insertion sequences flank a short synthetic unique DNA tag sequence and an antibiotic resistance gene. The tag sequence allows the identification of inserted mini-transposons to be confirmed by hybridisation. The antibiotic resistance genes are present to allow for selection for mutants on antibiotic containing growth media. The antibiotic genes were originally cloned from Campylobacter coli isolates. The mini-transposons are cloned into standard pUC derived cloning vectors. These vectors are grown in E. coli to generate sufficient amounts of DNA needed for the in vitro transposition reactions. The construction and growth of these vectors has been classified as Class 1 in a separate risk assessment.

**Evaluation of foreseeable effects**

The GMM's will be resistant to either kanamycin, chloramphenicol or tetracycline depending on the resistance gene inserted. Since these antibiotics are not used to treat C. jejuni infections in humans, the GMM's will not pose any increased risk due to this phenotypic change. The inserted mini-transposons are likely to inactivate any gene into which they are inserted. In most cases such mutants are likely to be less able to either survive or cause pathogenic effects.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Appropriate disinfection and waste management procedures will be carried out in accordance with Schedule 8. All contaminated plasticware is to be either incinerated directly, or following an overnight soak in 5% Hycolin disinfectant. Items will be double-bagged for transport to the incinerator. Hazardous waste will be collected weekly by a firm registered with the HSE as being a licensed hazardous waste incinerator. All other items, including vessels containing small culture volumes (up to 200ml) will be placed in lidded steel buckets for autoclaving.

The autoclave for waste has a chart recorder for monitoring load and drain temperatures. It is serviced quarterly and inspected for accuracy annually. Both incineration and autoclave procedures are expected to give a 100% kill of all GMMS.

The committee commented on the likelihood that a library of random mutants would contain mutants whose phenotype might be more pathogenic or virulent than the wild type if a negative regulatory mechanism was affected. It was agreed that this likelihood was low as in most cases gene inactivation mutants are less able to survive. It was also noted that this risk assessment in some way covers the construction of directed gene insertions in C. jejuni as the library may well contain random insertions into those desired genes.

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

### Project Containment

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#### Project Ref 155/01.2

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<tr>
<td>03/08/2001</td>
<td>PATHOGENIC STREPTOCOCCI: DEVELOPMENT OF NOVEL VACCINES, ANTIBACTERIALS AND ANTI-INFECTIVES</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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Date Project Ceased: 02/03/2022
The construction of knock-out mutants of Streptococcus pneumoniae and expression of green fluorescent protein reporter, in order to investigate mechanisms of pathogenesis. S. pneumoniae is an ACDP class 2 pathogen.

Recipient or parental organism

Category 1 disabled hosts
Lactococcus lactis (for gene cloning and expression: classified by HSE as disabled host). L. lactis strains used in this work are naturally auxotrophic and in addition lack proteases and genes for required for lactose metabolism and growth in their natural environment (ie milk).

E.coli K12 laboratory strains eg DH5α (for gene cloning; classified as a disabled strains by HSE).

E. coli BL21 for expression work (considered non-pathogenic).

Class 2
Streptococcus pneumoniae (construction of knock-out mutants to investigate mechanisms of pathogenesis and expression of green fluorescent reporter gene).

Host/vector system

pTREP and pTREXnis broad host range gram-positive vectors; non-mobilisable for cloning and expression in L.lactis. replicon = pAMB1 antibiotic resistance = erythromycin.

pET series vectors for expression in E.coli BL21 strain and its derivatives. Vector is non-mobilisable and utilises the T7 RNA polymerase promoter for high level expression. Bom (nic+) mob-, tra-.

pUC based vectors for cloning in E.coli K12 strains designated as disabled.

No vectors are used in the Streptococcus pneumoniae work; genes are disrupted by natural transformation with PCR amplified DNA containing an antibiotic resistance marker (erythromycin).
Surface antigens from *S. pneumoniae* and *S. agalactiae* lacking any signal export sequence are to be expressed in the cytoplasm of disabled laboratory strains of *E. coli* or *L. lactis*. The antigens will be purified and evaluated as cross protective vaccine antigens for example using a range of assays eg opsonophagocytosis with purified human neutrophils and cross reactivity with human sera. Specific genes will also be knocked-out in wild type strains of *S. pneumoniae* in order to assess their effects on growth and virulence. The mutant strains will be investigated using a variety of approaches eg biochemical, proteomics, microarrays and physiological methods to investigate gene function.

### Evaluation of foreseeable effects

The host strains *L. lactis* and *E. coli* K12 are disabled and the vectors are non-mobilisable. BL21 (DE3) and BLR (DE3) are *E. coli* strains for use with the T7 expression system. The ACGM newsletter 23 of Sept 1998 states that these strains can be assigned to level 1 containment.

The genes to be cloned in these hosts are derived from pathogenic streptococci (ie *S. agalactiae* (group B streptococcus) and *S. pneumoniae*).

The expression of proteins from GBS or *S. pneumoniae* in disabled *E. coli* or *L. lactis* strains will not make these host strains pathogenic even if the genes contribute to virulence in their normal host. The capacity to cause disease is multifactorial and dependent on numerous other properties such as encapsulation, capacity to grow in vivo, capacity to colonise the host etc. The genotypes and phenotypes of the *E. coli* and *L. lactis* host strains are well documented ie they lack genes encoding invasins, various adhesion phenotypes, and a range of enterotoxins. All genes for expression in these hosts will be investigated by bioinformatics tools to ensure that they do not encode factors that might conceivably cause harm. The list of sequences to be expressed and the results of the bioinformatics searches will be provided to the members of the committee before the work commences. It is intended that all genes with greater than 50% amino acid identity to genes of known function that would not cause harm to humans or the environment in a worst case scenario will be handed at category 1. All other genes mentioned above would be handled at category 2 to ensure the overall risk is negligible.

Specific genes will also be knocked-out in wild type strains of *S. pneumoniae* in order to investigate their function. These mutations are highly unlikely to increase the capacity of the strain to cause diseases since a factor will be absent from the strain that may be essential for growth under certain environmental conditions such as those found in vivo.

The genes will be disrupted by recombination with a truncated gene fragment carrying an erythromycin resistance gene following natural transformation. This antibiotic is not routinely used to treat pneumococcal infections and autonomously replicating plasmid vectors will not be used.

The overall risk of these GMO's causing harm by accidental contamination of a laboratory worker is therefore effectively zero.

As the host vector systems utilise non-mobilisable vectors and host strains are disabled the risk of disseminating genetic material in the environment is effectively zero.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not applicable.**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Not applicable.**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Appropriate disinfection and waste management procedures will be carried out in accordance with Schedule 8.

All contaminated plasticware is to be either incinerated directly, or following an overnight soak in 5% Hycolin disinfectant. Items will be double-bagged for transport to the incinerator. Hazardous waste will be collected weekly by a firm registered with the HSE as being a licensed hazardous waste incinerator.

All other items, including vessels containing small culture volumes (up to 500ml) will be placed in lidded steel buckets for autoclaving. The autoclave for waste has a chart...
recorder for monitoring load and drain temperatures. It is serviced quarterly and inspected for accuracy annually. Both incineration and autoclave procedures are expected to give a 100% kill of all GMMs.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The committee requested further information about the genes to be expressed and of any virulence factors from GBS and S. pneumoniae that might cause harm if accidentally ingested. The requirement of multiple factors such as the capsule, capacity to grow in blood and assimilate iron in vivo etc were discussed in assessing the likelihood that the possession of any one factor could make a disabled host more dangerous. Given that these surface associated proteins will be expressed intracellularly it was considered unlikely that they will increase adhesiveness of the bacteria or reduce their susceptibility to the innate defence mechanisms of humans and animals. Indeed high level expression of recombinant protein often diminishes the capacity of the bacteria to grow competitively. Furthermore, the expressed proteins often aggregate and form inclusion bodies when expressed at high level.

Project Containment

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Project Ref 155/01.3

Date Ackn'd 06/08/2001

Date Project Ceased

CU2 Project Title

DETERMINING THE FUNCTION OF PUTATIVE TRANSCRIPTIONAL REGULATORS IN CAMPYLOBACTER JEJUNI

Class CultureVolClass2 CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements [N]
Project Additional Information

Purposes of the contained use
Construction of knockout mutants and conditional mutants in Campylobacter jejuni putative gene regulators and expression of such regulators in Escherichia coli facilitating their purification.

Recipient or parental organism
Campylobacter jejuni is an ADCP group 2 pathogen

Host/vector system
Non-mobilisable E. coli vectors pBluescript II SK+ (Stratagene), pCR2.1 (Invitrogen), pCRT7NT-TOPO (Invitrogen), pCRT7CT-TOPO (Invitrogen) will be used for cloning, propagation, mutagenesis and expression of Campylobacter genes in E. coli K12 strains JM109 (Promega), TOP10F' (Invitrogen), BL21(DE3)pLysS (Invitrogen)

Origin & function
pBluescript and pCR2.1 will be used as standard cloning vectors to facilitate cloning, propagation and mutagenesis (by inverse PRC) of Campylobacter genes in E. coli. pBluescript is a standard cloning vector derived from a variety of sources and includes an origin of replication derived from the E. coli plasmid ColE1, a portion of the E. coli lacZ gene, a synthetic multiple cloning site, a gene conferring resistance to ampicillin and the origin of replication derived from the E. coli phage f1. pCR2.1 is another general purpose cloning vector that has been developed by Invitrogen to facilitate direct cloning of PCR products. In addition to the elements described above for pBluescript it, additionally, contains a promoter derived from the E. coli phage T7 and a gene encoding resistance to kanamycin. After mutagenesis an antibiotic resistance element (of Campylobacter coli origin) encoding resistance to either chloramphenicol or kanamycin will be introduced into the mutated genes and these constructs will be used to mutate the Campylobacter genome by allelic replacement. Plasmids pCRT7NT-TOPO and pCRT7CT-TOPO will be used to clone and express Campylobacter regulators. They are derivatives of pCR2.1 and, in addition to the elements described for that vector, contain a synthetic ribosome binding site, ATG initiation codon, additional codons including 6 histidine codons to facilitate purification of fusion proteins and an epitope (the Xpress epitope) that can be used to identify fusion proteins immunologically. In cases where knockout of a particular gene is not possible due to its being essential for cell viability a chloramphenicol resistance gene of Campylobacter origin, in which a synthetic iron-responsive operator sequence has been inserted will be cloned upstream of the gene of interest allowing for the controlled expression of the gene.

Evaluation of foreseeable effects
The genetically modified Campylobacter will be resistant to either chloramphenicol or kanamycin. Neither of these agents is used to treat Campylobacter infections in either man or farm animals and so this does not pose any additional risk. The mutants will, additionally, be unable to express particular regulators, which will have unpredictable effects on genes that are normally regulated either positively or negatively. It is assumed to be highly unlikely that such changes in gene expression will result in an increase in the virulence of the organism or its ability to survive in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid C. jejuni and recombinant E. coli cultures will be transferred in sealed containers to be autoclaved before disposal. Solid media on which modified C. jejuni or E. coli has been cultured will be double-bagged and sealed before being incinerated. Contaminated plastic ware will be double bagged and incinerated. A firm registered with the HSE as being a hazardous waste incinerator will collect hazardous waste weekly. Other items including glassware will be placed in lidded metal buckets for autoclaving. The autoclave has a temperature recorded (chart recorder) for monitoring load and drain temperatures. It is serviced quarterly and inspected for accuracy annually. Both incineration and autoclaving are expected to kill 100% of all genetically modified E. coli and C jejuni.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All the initial review of this proposal, the GMSC asked for a list of the genes encoding the putative regulators to be attached to the risk assessment. It was also requested that vector maps should be supplied, together with details of their origin of replication. The possibility of knocking out a negative regulator was discussed (thereby increasing transcription levels of unknown genes), but this would not necessarily cause the recombinant strain to become more hazardous, and in any event it was considered that the proposed containment level would still be adequate.

Project Containment

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Animal Units

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Project Ref 155/01.4

Date Ackn'd 17/08/2001

CU2 Project Title MOLECULAR GENETICS OF GRAM POSITIVE FOOD PATHOGENS

Class 2

CultureVolClass2 < 1 litre

Class VolumeClass3-4
This project will concentrate on changes in gene regulation of Listeria monocytogenes in response to its environment. A plasmid with a constitutive Listeria promoter controlling green fluorescent protein (gfp) expression in Listeria will allow detection protocols of this reporter gene to be optimised. A vector derived from this will be used to clone promoters that are environmentally regulated in Listeria. These plasmid derivatives will be introduced into the Listeria monocytogenes strains in the study. Plasmids based on this system can be present in Listeria and replicate as a plasmid or under temperature induced chromosomal integration resulting in gfp expression under the cloned promoter on the chromosome. The gfp expression from the various promoters will be monitored under different environmental conditions.

**Hosts:** E.coli MC1002, MC1061 and S17-1 strains, Listeria innocua and Listeria monocytogenes EDGe, ScottA and 10403S strains. 10403S is streptomycin resistant. E.coli strains used for ease of laboratory manipulation before cloned fragments such as promoters are transferred into Listeria. Listeria monocytogenes strains EDGe, ScottA and 10403S are established laboratory strains.

**Vectors:** pMJ153 is a listerial promoter probe plasmid which encodes for ampicillin and kanamycin resistance in E.coli and just kanamycin resistance in Listeria. pMJ161 is pMJ153 with an insert in the BamHI site that gives constitutive expression of gfp and chloramphenicol. The constitutive promoter is very strong and gives resistance to 100g/ml of chloramphenicol. Both of these plasmids have a temperature sensitive RepA protein and so will only replicate at 30C in Listeria. At higher temperatures pMJ161 and other pMJ153 derivatives will integrate into the chromosome at the position of the cloned promoter resulting in a single copy of gfp under the control of the promoter and an uninterrupted copy of the promoter and its listerial gene. These plasmids will replicate in both E.coli and Listeria. They are not temperature sensitive in E.coli and they can conjugate from E.coli into Listeria.

**Inserts:** Green fluorescent protein gene from Aequoria victoria mut1 allele F64L, S65T (Cormack et al.(1996) Gene 173:33-38) with promoters for the expression of green fluorescent protein (GFP) including listerial promoters such as the constitutive promoter in pMJ161, acid and oxidative stress responsive promoters cloned randomly, sigB the sigma factor that responds to general stress and the virulence promoters for internalin and haemolysin.

Listeria monocytogenes is defined as a ACDP class 2 organism requiring its growth and manipulation at containment level 2.
non-mobilisable. This will be used to investigate promoter expression, as indicated by gfp activity, in response to different environmental conditions (e.g. food preservation techniques). Gfp activity will be assayed by FACS analysis and by visual fluorescent microscopy.

Evaluation of foreseeable effects

Listeria monocytogenes is defined as an ACDP group 2 organism requiring its growth and manipulation at containment level 2.

The treatment of choice for listeriosis is ampicillin/penicillinG combined with an amino glycoside such as gentamycin. The second choice for patients who are allergic to penicillin is trimethoprim with a sulphonamide e.g. sul famethoxale. Neither of these treatments is affected by the manipulations envisaged so the GMMs should not have a selective advantage over the wild type in the event of environmental release.

The pMJ derived plasmids can replicate in E.coli and Listeria and transfer from E.coli to Listeria because there is an E.coli oriT on the plasmid. This plasmid and its derivatives do not carry Listeria mobilization functions so transfer between Listeria is highly unlikely. The antibiotic resistance genes used for vector selection do not code for antibiotics used in the treatment of listeriosis. Environmental antibiotic resistance in Listeria is generally low except for tetracycline.

The expression of reporter genes such as green fluorescent protein gene is highly unlikely to cause increased pathogenicity. So the GMMs should not be considered more pathogenic than the wild type.

However this study is designed to determine whether environmental conditions can influence gene regulation including virulence; this will be taken into consideration when handling both the wild type Listeria monocytogenes and the GMM cultures that have been exposed to stressful conditions.

Transmission to people within the laboratory is most likely by the ingestion route. Listeria monocytogenes and other GMMs generated during this study will be handled in accordance with Good Microbiological Practice and with awareness of high risk groups. The risk of contracting listeriosis is higher in certain groups such as pregnant or immunosuppressed people. Access to the laboratory will be restricted and pregnant women will not be permitted to enter. Transport to and from the laser laboratory (where gfp is assayed) will be in closed containers marked with Danger of Infection tape.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Appropriate disinfection and waste management procedures will be carried out in accordance with Schedule 8. All contaminated plastic ware is to be either incinerated directly, or following an overnight soak in 5% Hycolin disinfectant. Items will be doubled-bagged for transport to the incinerator. All other items, including vessels containing small culture volumes (up to 100ml) will be placed in lidded steel buckets for autoclaving. Small bioformentors (volume up to 1.7 litres) will be sealed, transported in a suitable secondary containment vessel and autoclaved separately. The autoclave for waste has a chart recorder for monitoring load and drain temperatures. It is serviced quarterly and inspected for accuracy annually. Both incineration and autoclave procedures are expected to give a 100% kill of all GMMs.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
Members of the GMSC asked for maps of the vectors that were to be used. After enquiries had been raised about the mobilisability of these plasmids, they were satisfied that the final classification and containment measures are adequate for the proposed project.

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Project Ref 155/01.5

Date Ackn'd 13/08/2001

CU2 Project Title REGULATORY MECHANISM AFFECTING VIRULENCE IN SALMONELLA ENTERICA SEROVARS TYPHIMURIUM AND ENTERITIDIS, ENTEROPATHOGENIC E. COLI & E COLI 0157:H7 VT- BY NUCLEOID PROTEINS

Class 2

Culture Vol Class 2 51-500 litres

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Mutations in the nucleoid-associated DNA-binding protein family of genes will be created in four enteropathogenic bacterial strains. These mutations will be complemented by transformation of these strains with a low-copy plasmid containing the gene deleted from the chromosome. Linear fragments of DNA will also be integrated into the pathogenic strains, in order to introduce mutations or reporter-gene constructs, together with antibiotic resistance markers. Promoters of several Salmonella virulence genes will be fused to a green fluorescent protein reporter (GFP) gene, and these constructs will be introduced into the two...
Salmonella serovars either by integration into the bacterial chromosome, or on a low-copy number plasmid. GFP expression will be measured during invasion of cultured mammalian cells and in the tissue of infected animals. Specific mutations affecting regulatory proteins will be introduced into the Salmonella strains harbouring the gfp fusions, and their effects upon virulence gene expression determined by measuring GFP production.

Recipient or parental organism

Salmonella enterica serovars Typhimurium LT2a and Enteritidis, and enteropathogenic (EPEC) Escherichia coli E2348/69 (O127:H6) are classified as group 2 pathogens, as determined by the ACDP. Normally, Escherichia coli O157:H7 (VTEC) is classified as group 3, requiring more stringent containment conditions. However the strain that we are proposing to work with, NCTC 12900, lacks the prophage encoding the shiga-like toxin and therefore satisfies the requirements for consideration as a group 2 pathogen. E. coli K-12 (strains DH5a and MG1655) are classified as ACDP hazard group 1, since these strains are not considered pathogenic and are not expected to survive in the environment due to having several auxotrophic requirements.

Host/vector system

1) In all 4 strains, we will be mutating genes encoding members of the nucleoid-associated DNA-binding protein family such as hns, stpA and fis. We will complement the mutations by transforming the strains with a low copy-number (5-10 copies per cell), pSC 101-derived, non-mobilisible, ampicillin-resistant plasmid, pWKS30, containing the gene that has been deleted from the chromosome. Whilst cloning the various DNA fragments, we will initially employ the widely available general-purpose cloning vectors pUC18 and 19. These vectors are non-mobilisible, as they lack a bom site.

To introduce mutations or reporter-gene constructs into the chromosomes of the pathogenic strains, we will transform linear fragments of DNA containing the mutated allele / reporter-gene construct (containing kanamycin or chloramphenicol antibiotic-resistance markers) into the strain of interest, harbouring an oriR101-based temperature-sensitive plasmid (pKD46) encoding the ?Red recombinase, ampicillin and/or chloramphenicol resistance and araC (Datsenko and Wanner, 2000). This plasmid encodes a recombinase and proteins required to inactivate the host's RecBC exonuclease V system, allowing efficient recombination of the linear DNA fragments into the host chromosome, and is induced by adding L-arabinose to the culture medium. The plasmid also encodes a temperature-sensitive RepA protein, only allowing replication of the plasmid at temperatures up to 30C.

2) We will be fusing the promoters of Salmonella virulence genes to gfp, the gene encoding green fluorescent protein (GFP) and introducing the resultant constructs in to the puPA locus, encoding a proline permease and proline dehydrogenase, located on the Salmonella chromosome. The promoters that will be fused to gfp will include ssaG, prgH, invF, spiC, spvB, nirB, rpsM, htrA, waaN and other gene promoters of future interest. Virulence gene promoters and the promoterless gfp gene will be amplified by PCR and fused by a recombinant PCR approach, and will not involve plasmid based cloning. Linear DNA constructs will be introduced into the chromosome using the oriR101-based plasmid system described above in (1).

In instances where the introduction of mutations by the RED system proves unsatisfactory, allelic exchange will be performed using the suicide vector pCVD442 (Donnenberg and Kaper, 1991). The fusions can also be cloned onto a ColE1 or pMB1 replicon such as pBR322. The pBR322 vector is mobilisation defective and will only contain the promoter sequences for virulence genes.

Origin & function

All genetic material will derive from the four enteropathogenic strains listed above, or from the vectors/reporter genes described. The overall intended function of manipulations in both sets of experiments is to study the regulation of virulence in these strains. In all cases genes of interest will be mutated, or their promoters will be fused to the gfp reporter gene.

We will be performing detailed phenotypic analyses to determine the consequences of the mutations. We will perform in vitro tissue-culture based assays to study the interactions of the wild-type and the mutant strains with cultured cell lines, isolate RNA from the bacteria to measure gene expression by micro array and multiplex primer extension analysis, and use protein-based techniques such as SDS-PAGE and Western blotting to confirm results obtained by RNA analysis.

In project (2), the phenomenon of non-genetic variation (NGV), a poorly understood mechanism whereby non-genetic differences between sister cells enables strain survival under adverse conditions, will be studied using both gfp fusions and an additional reporter gene encoding an easily distinguishable phenotype, that can be correlated with the GFP expression. This additional gene will probably encode a salmonella outer membrane protein. Cellular differences in the level of GFP expression will be studied by fluorescence microscopy and Fluorescence Activated Cell Sorting (FACS).

We will be using some of the promoter-GFP fusions, to investigate the effects of growing Salmonella in simulated food environments. This will be done by fluorescence ratio imaging microscopy (FRIM).
Genetically modified strains will be used to measure the levels of virulence gene expression in vivo in mice, by fluorescence microscopy and FACS. The regulatory mutations that will be investigated are hns, stpA, fis, and hilA. Mutations in these genes have previously been shown to attenuate virulence in Salmonella. The promoters that will be fused to gfp will include ssaG, prgH, invF, spiC, spvB, nirB, rpsM, htrA, waaN and other gene promoters of future interest.

**Evaluation of foreseeable effects**

In all 4 strains, we will be mutating genes encoding members of the nucleoid-associated DNA-binding protein family such as hns, stpA and fis. These genes are also found in all non-pathogenic strains of Escherichia coli. As such, they do not contribute to pathogenesis in the absence of other factors and are therefore not considered to be virulence factors. Although subsequent complementation, by transforming with clones of hns, fis and stpA in pWKS30, will increase the copy-number of the genes and could conceivably result in higher levels of the cognate proteins, significant over expression is unlikely, since these genes are subject to negative feedback regulation.

The system for introducing linear DNA constructs into the bacterial chromosome involves the presence of the temperature-sensitive plasmid, pKD46. None of these genes encoded by the plasmid are considered likely to enhance the virulence of the strains harbouring them.

Since plasmid pKD46 and its derivatives cannot replicate at 37 degrees C, the plasmid would normally be lost following ingestion of the bacteria by a mammalian host. We are not aware of any potential for this plasmid to be mobilised and transferred to other strains although the theoretical possibility does exist whilst the strains harbouring the plasmid are in the gut. Thus, there is a minimal risk of antibiotic resistances encoded by this plasmid being transmitted to other strains.

The alternative allelic exchange system proposed in both projects will be performed using the suicide vector pCVD442. The fusions can also be cloned onto a ColE1 or pMB1 replicon such as pBR322. The pBR322 vector is mobilisation defective and will only contain the promoter sequences for virulence genes. Although pBR322 is not conjugable itself, the plasmid could be mobilised in the presence of a helper plasmid. The risk is considered very low. Moreover, the contents of the plasmid would not increase the pathogenic potential of a recipient strain.

Plasmid pCVD442 is an ampicillin resistant, RP4-based replicon and can only be maintained in strains containing the pir gene. It lacks most of the genes necessary for its mobilisation and thus, will neither replicate nor be mobilised unless the strain harbours another RP4-based plasmid, which could complement the deficiencies in pCVD442. This plasmid also contains the sacB gene from Bacillus subtilis, conferring toxicity to the bacteria in the presence of sucrose. This will have no harmful effect upon animal hosts.

There is no reason to believe that strains harbouring pWKS30-derivatives expressing nucleoid-associated proteins will be more hazardous than the original wild-type strain. Indeed, over-expression of nucleoid-associated proteins frequently reduces fitness and growth rate. It has not been previously reported that cloning and increasing the copy-number of genes encoding nucleoid-associated proteins enhances the virulence of pathogens. Indeed, we have previously shown that increasing the copy number of fis actually represses the expression of certain virulence genes (see preprint supplied).

The mutants defective for any of the nucleoid-associated DNA-binding proteins will be, at best, attenuated for virulence and at worst, equivalent to wild-type. Although H-NS acts a repressor of virulence gene expression, the consequent derepression of several virulence factors in the hns mutant will not enhance the organisms' pathogenic potential as it has already been previously shown that such mutants are avirulent in animal models. This is believed to be largely due to the inability of the mutants to respond appropriately to the environmental stresses that are encountered in the host. Moreover, because these organisms tend to express some of their virulence determinants inappropriately, they alert the host of their presence, enabling the host to mount an early response. For this reason, we will be investigating the possibility of using these strains for the development of vaccines. Since these proteins are required by the bacteria to assist in adapting to environmental changes and stresses, these strains should have a selective disadvantage.

The mutants will contain kanamycin and/or chloramphenicol resistance genes; these are not the drugs of choice for the treatment of infections by these infectious agents and thus would present no selective advantage in the event of an accidental release. Complementation of the mutants with pWKS30-derived plasmids encoding ampicillin resistance would similarly not present a selective advantage. Likewise, ampicillin is not the choice of antibiotic for the treatment of such infections.

All in vitro work will involve standard laboratory procedures at small scale. Given the level 2 containment and control measures, it is unlikely that the GMMs will reach the environment and cause harm to humans or animals outside the laboratory.
Transmission to laboratory personnel is most likely via the oral route. Good laboratory practice and implementation of the recommendations outlined in Schedule 8 will minimise risks. This will include restricting access to authorised workers, ensuring that all areas that have been in contact with potentially hazardous microorganisms will be routinely swabbed with a recommended disinfectant, no laboratory clothing will be allowed outside the laboratory and all outdoor clothing will be stored in offices. All contaminated waste will be autoclaved and/or incinerated.

Highly qualified staff, in special category 2 containment animal research facilities, will conduct all in vivo work involving animals. Once infected, the animals will not be moved from the designated area until the end of the experiment. At the end of the experiment, the animals will be dissected to remove organs for further analysis. Organs will be transferred to sealed containers and transported to the laboratory for further processing. The remains of the animals and any material in contact with the infected animals will be autoclaved and incinerated. This approach reflects "best practice" used at the Cambridge University Veterinary School, the Hammmersmith Hospital, London and the Veterinary Laboratory Agency, Weybridge.

Salmonella is pathogenic to animals and humans, although it causes only limited gastro-intestinal infection in health individuals. Therefore, the consequence of hazard is restricted.

The Salmonella strains containing the promoter-gfp fusions are anticipated to have virulence characteristics close to wild-type levels. Introduction of the fusions are not expected to enhance the virulence properties of these strains because they only contain virulence gene promoters, not the entire structural genes.

S. enterica serovars Typhimurium and Enteritidis, EPEC and E. coli 0157 VT are classed as ACDP group 2 organisms. It is considered that the GMM strains resulting from our work will not be more pathogenic than the wild type. This would indicate a biological hazards group 2 for all strains, requiring containing level 2 precautions and hence a provisional classification of Class 2. The above precautions will reduce the likelihood of a hazardous event to negligible levels. Thus, the overall risk is effectively zero given the proposed containment and control measures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Appropriate disinfection and waste management procedures will be carried out in accordance with Schedule 8.

All in vivo work involving mice will be conducted in special category 2 containment animal research facilities. Once infected, the animals will not be moved from the designated area until the end of the experiment. During experiments, animal waste and bedding will be autoclaved before being incinerated. At the end of the experiment, the animals will be dissected to remove organs for further analysis. Organs will be transferred to sealed containers and transported to the laboratory for further processing. The whole cage will be autoclaved; its contents will be autoclaved and incinerated. The remains of the animals will subsequently be incinerated. This approach reflects "best practice" used at the Cambridge University Veterinary School, Hammersmith Hospital, London and Veterinary Laboratory Agency, Weybridge.

All contaminated plastic ware is to be either incinerated directly, or following an overnight soak in 5% Hycolin disinfectant. Items will be double-bagged for transport to the incinerator. A firm registered with the HSE as being a licensed hazardous waste incinerator will collect hazardous waste weekly.

All other items, including vessels containing small culture volumes (up to 100 ml) will be placed in lidded steel buckets for autoclaving. The autoclave for waste has a chart recorder for monitoring load and drain temperature. It is services quarterly and inspected for accuracy annually.

Both incineration and autoclave procedures are expected to give a 100% kill of all GMMs.
The original version of the risk assessment submitted to the committee lacked information on the VTEC E.coli strain NCTC12900, (ie exactly how the shiga-like toxin was no longer produced); this was supplied at a further assessment meeting (the prophage encoding the toxin is not present).

The GMSC also asked for more details on the regulation of virulence genes by the nucleoid-associated DNA - binding proteins, as the claim was made that mutants of the latter were likely to be attenuated. The modified risk-assessment states that mutations in at least three known members of this family (hns, stpA and fis) have been shown to attenuate virulence in Salmonella, and a mutation in fis markedly attenuates virulence in the EPEC strain of E.coli.

Finally, maps of the key plasmid vectors to be used were requested.

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Project Ref 155/02.2

Date Ackn’d 18/04/2002

Date Project Ceased 15/10/2008

PROJECT TITLED USE OF ADENOVIRUS TO EXPRESS TIMP’S IN A MURINE ARTHRITIS MODEL

Class 2

Consent Granted not applicable

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

The purpose of this project is to test the efficacy of tissue inhibitors of metalloproteinases (TIMPs) in a murine model of arthritis (collagen-induced arthritis). Delivery will be by intraarticular injection of a recombinant adenovirus expressing either TIMP-1 or TIMP-2. Early experiments will use virus expressing β-galactosidase as a marker to range find for dose and length of expression. TIMPs are inhibitors of the matrix metalloproteinase (MMP) family of enzymes. These enzymes, and particularly those that have collagenolytic activity, are strongly implicated in cartilage destruction in both osteo- and rheumatoid arthritis.

Recipient or parental organism

All adenovectors to be used in this study are E1-deleted first generation adenoviral vectors based on the pJM17 system (McGrory, W. J. Bautista, D. S and Graham, F. L: A simple technique for the rescue of early region 1 mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. This renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. However, all the inserted genes are biologically active. None of the transgenes are proto-oncogenes. All transgenes will be under the control of the CMV promoter. There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target infected cells. It is generally accepted that the adenovirus will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenavirus will pose less of a threat once in the animal than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use.

Host/vector system

The adenoviral vectors are generated by homologous recombination between pJM17 and shuttle vectors containing transgene expression cassette and flanking E1 sequences. Following homologous recombination in helper 293 cells (which express the helper E1 function in trans), replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses.

Origin & function

The adenoviral vector DNAs are standard and originated from the laboratory of Dr Frank Graham (McGrory, W J Bautista, D. S and Graham, F L: A simple technique for the rescue of early region 1 mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vitro and in vivo to generate high-level gene expression in all cells transduced by the adenovirus. All transgenes have been constructed from full length TIMP cDNAs obtained from other research institutes and verified prior to subcloning into the relevant vectors.
Evaluation of foreseeable effects

There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the adenovirus will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the animal than most expression vectors as they will definitely not replicate or integrate into the host genome.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory-based experiments: during aliquotting, or diluting replication-defective adenoviruses, all solid waste (plastics etc) is autoclaved prior to disposal. All liquid waste is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. Animal experiments: All instruments used in the preparation of animals for GM work will be sterilised by autoclaving. Solutions exposed to the viable GMOs will be disinfected with chlorine-based disinfectant. All plastic ware will be autoclaved prior to disposal. Animals receiving the GMO will be housed in separate cages during the procedure-kill time period. All animal carcasses will be disposed of by incineration. Animal bedding will be autoclaved prior to disposal and cages disinfected prior to being re-used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

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02/03/2022
ANALYSIS OF EFFECT ON STREPTOCOCUS PNEUMONIAE VIABILITY FOLLOWING INDUCTION OF THEIR BACTERIAL APOPTOTIC SYSTEMS (TOXIN/ANTI-TOXIN SYSTEM & IDENTIFICATION OF THE BACTERIAL CELLULAR TARGETS)

Streptococcus pneumoniae is an ADCP group 2 pathogen.

Recipient or parental organism
Streptococcus pneumoniae R61 strain is a non capsulated strain and research has shown this strain to be non pathogenic in mice. Using homologous recombination the chromosomal relB gene has been deleted and replaced by a chloramphenicol resistance gene. The BAS relE type toxin gene(s) are cloned in the non-mobilisable S. pneumoniae plasmid vector, pLS1 (Nieto et al 2000, Plasmid 43,205-213), originally derived from the S. agalactiae natural replilicon pMV158. The plasmid vector pLS1 contains a gene for erythromycin resistance.

Origin & function
The RelE type BAS toxin gene(s) from S. pneumoniae strain R61 were cloned into the plasmid vector pLS1 by our EU project partners in Spain. The toxin gene9s) are under the control of the MalR gene and are inducible by the addition of maltose to the media. Induction of the BAS toxin genes has been demonstrated to reduce bacterial host viability. Following maltose induction of S. pneumoniae R61 containing the plasmid borne BAS toxin genes, culture samples will be taken at specific time points over a period of several hours. At the time of sampling the bacterial culture aliquots will be mixed immediately with a chilled solution of ethanol/phenol. This procedure has previously been demonstrated to kill S. pneumoniae. The cell material will be stored at -80°C until required for RNA or protein isolation prior to microarray or proteomic analysis.

Project notified under transitional arrangements N
analysis. The cellular target of the toxins will be identified using biophysical, immunological and biochemical techniques.

**Evaluation of foreseeable effects**

Streptococcus pneumoniae R61 strain is a non capsulated strain and research has shown this strain to be non pathogenic in mice. Using homologous recombination the chromosomal relB gene has been deleted and replaced by a chloramphenicol resistance gene. The presence of the chloramphenicol resistance does not necessarily increase the hazard level as third generation cephalosporins and alternative antibiotics are typically used to treat human infections. Precautions will be taken to prevent accidental ingestion or inhalation of the bacteria, containment will be provided by the use of a Class II cabinet for handling of all S. pneumoniae strains. When centrifugation is required, aerosol retaining caps will be used. After centrifugation the centrifuge vials will be opened in the Biological Safety Cabinet.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Appropriate disinfection and waste management procedures will be carried out in accordance with Schedule 8. All contaminated plastic waste and small culture volumes will be placed into autoclave bags contained within lidded polypropylene buckets and autoclaved prior to incineration of the contents. All contaminated glassware will be autoclaved in lidded steel buckets. The larger volumes of culture media supernatant, generated during the time course experiments and containing ethanol/phenol mix are not suitable for autoclaving. However treatment of the samples with ethanol/phenol solution has been demonstrated locally to kill S. pneumoniae. The treated culture supernatant will be dealt with as toxic chemical waste.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

The committee accepted the risk assessment for the project work involving streptococcus pneumoniae, and suggested the proposal include any potential future work (e.g. the generation of gene mutations and the production of overexpressing strains), that may be undertaken on the material described in the proposal. However due to financial and time constraints the proposer thought it highly unlikely that further work would be undertaken.

**Project Containment**
**Project Additional Information**

**Purposes of the contained use**

Microarray experiments will be performed upon Shigella flexneri, Salmonella enterica (excluding S. Typhi, S. Paratyphi A and S Paratyphi B) and category II pathogenic strains of E. coli to observe how these organisms respond 1) to different environmental conditions and 2) to the presence of a range of mutations. To confirm the results obtained, defined mutations will be made in selected genes and a range of experiments conducted to investigate how the mutants respond to different environmental conditions and in some cases, how virulence is affected, using tissue culture - based techniques and animal models. In most instances, linear fragments of DNA will be integrated into the pathogenic strains, in order to introduce mutations or reporter-gene constructs, together with antibiotic resistance markers. On some occasions, suicide-vectors will be used to introduce mutations into a strain. Mutations or reporter genes will sometimes be moved between strains by generalised transduction. Mutations will be complemented by cloning functional copies of the genes of interest into low copy-number vectors.

Promoters of several genes will be fused to reporter genes, and these constructs will be introduced into a variety of strains either by integration into the bacterial chromosome, or on a low-copy number plasmid. Reporter gene expression will be measured during invasion of cultured mammalian cells and in the tissue of infected animals. Specific mutations affecting regulatory proteins will be introduced into the Salmonella strains harbouring the reporter gene fusions, and their effects upon virulence gene expression determined by assaying the activity of the reporter gene product or by a range of microscopic examinations.
**Recipient or parental organism**

S. enterica (except serovars Typhi, Paratyphi A and Paratyphi B). S. flexneri, category II strains of E. coli such as ETEC and EPEC are classified as group 2 pathogens, as defined by the ACDP. Normally, Escherichia coli 0157:H7 (VTEC) is classified as group 3, requiring more stringent containment conditions. However, the strains that we are proposing to work with, NCTC 12900 and EDL933 either lack the prophage encoding the shiga toxin or have had the toxin genes specifically deleted and therefore satisfy the requirements for consideration as group 2 pathogens. E coli K-12 is classified as ACDP hazard group 1, since this group of strains is not considered pathogenic and is not expected to survive in the environment due to having several auxotrophic requirements.

**Host/vector system**

We will be employing a range of plasmids and vectors, many being based upon the pUC family and are designated as disabled. As such, these vectors are non-mobilisable.

To construct some mutants, we will utilise suicide vectors based upon plasmid pCVF442 (Donnenberg and Kaper (1991) to perform allelic exchange. This vector is based upon an ampicillin resistant, RP4 - replicon and can only replicate in strains containing the pir gene. It lacks most of the genes necessary for its mobilisation and thus, with neither replicate nor be mobilised unless the strain harbours another RP4-based plasmid which could complement the deficiencies of pCVD442. This plasmid also contains the sacB gene from Bacillus subtilis, conferring toxicity to bacteria in the presence of sucrose. This will have no harmful effect upon an animal host.

Construction of most of our bacterial mutants will utilise a non-cloning strategy (Datsenko and Wanner, 2000). We will transform S. enterica, E. coli and S. flexneri with linear fragments of DNA, generated by PCR, containing the mutated allele/reporter-gene construct, flanked by two FRT recombinase sites and ~50bp DNA homologous to the sequences either side of the target gene(s). The linear DNA fragments will contain a selectable marker (kanamycin or chloramphenicol resistance). During the mutagenesis procedure, this strain will be harbouring an oriR101-based temperature-sensitive plasmid (pKD46) encoding Red recombinase under the control of the tightly regulated arabinose-inducible ParA promoter. The plasmids also encode ampicillin and/or chloramphenicol resistance and araC. The use of an arabinose-inducible recombinase and proteins (required to inactivate the host's RecBC exibyckease V system) avoids unwanted recombinatorial events under non-inducing conditions, allowing efficient recombination of the linear DNA fragments into the host chromosome. The plasmid also encodes a temperature-sensitive RepA protein, only allowing replication of the plasmid at temperatures up to 30 degrees C. None of the genes encoded by the plasmid are considered likely to enhance the virulence of the strains harbouring them. The 50 bp of DNA sequence designed to permit homologous recombination into the host chromosome are too short to encode any functional genes, are identical to the normal sequence of chromosomal DNA and will therefore not affect the virulence of the strain.

Once the gene of interest has been successfully replaced by the antibiotic resistance marker, if desired, the marker can be excised to leave a clean deletion. This achieved by transforming the strain with another temperature-sensitive plasmid, pCP20, encoding a flip-recombinase that deletes the DNA sequence between the two FRT sites (Datsenko and Wanner, 2000). Like the Red recombinase, the flip-recombinase is induced by the addition of arabinose to the culture medium. The plasmid is easily eliminated from the host strain by incubating at 37 degrees C. None of the gene products encoded by pCP20 are considered likely to affect the virulence of the host strain.

To complement the mutant phenotypes, we will be cloning wild-type copies of the gene(s) of interest into single/low-copy-number cloning vectors. These plasmids are non-transmissible and are intended to mimic the conditions within the wild-type background i.e to simply replace the missing gene functions within the strain. Cloning genes into these vectors will in some cases raise the number of copies of these genes. However, the increase in gene dosage would be modest and is considered unlikely to significantly alter the virulence properties of the host strain.

On occasions, we will use expression vectors that use a variety of inducers such as arabinose or isopropyl-B-D-thiogalactopyranoside (IPTG) to induce en of the cloned gene of interest. In the absence of the inducer, expression of the cloned gene is normally undetectable. These vectors are designed to give moderate levels of expression following induction. The copy number of the plasmids is low to medium and these vectors lack mobilisation functions.

**Origin & function**

All genetic material will derive from the strains listed above, or from the vectors/reporter genes described. The overall intended function of manipulations in all experiments is to study the regulation of virulence in these strains. In all cases, genes of interest will be mutated, or their promoters will be fused to reporter genes.
We will be performing detailed phenotypic analyses to determine the consequences of the mutations. We will perform in vitro tissue-culture-based assays to study the interactions of the wild-type and mutant strains with cultured cell lines, isolate RNA from the bacteria to measure gene expression by micro array and other RNA-based techniques, and use protein-based techniques such as SDS-PAGE and Western blotting to confirm results obtained by RNA analysis. In addition, we will perform in vivo assays in mouse models to 1) compare the virulence of different mutants, 2) measure gene expression using reporter genes fused to promoters of selected genes and 3) extract the bacteria from different organs to isolate and purify the bacterial RNA for microarray analysis.

**Evaluation of foreseeable effects**

Many genes in *S. flexneri, S. enterica* and *E. coli* encode proteins of unknown function. Microarray analysis enables us to identify many of these genes that may be expressed under specific conditions. We anticipate that whilst some mutants will retain virulence characteristics close to the parental strain, many will display attenuated phenotypes. Since all work will be formed under class 2 conditions that are already deemed satisfactory for the handling of the wild-type strains, it is therefore considered that no additional risk is likely to be posed by the mutants derived from these strains.

Plasmids pKD46 and their derivatives cannot replicate at 37 degrees C. Following ingestion of the bacteria by a mammalian host, the plasmid will eventually be lost. Apart from pCVD442, none of the plasmids that we are proposing to work with contain genes that would enable mobilisation into another strain. Plasmid pCVD442 and its derivatives lack most of the genes necessary for their conjugal transfer. The plasmids also lack the pir gene necessary for their replication and would thus be rapidly lost if acquired by another strain. Whilst the theoretical possibility exists, that the plasmids could be acquired by bacteria in the gut harbouring functional RP4-based plasmids, which would permit replication of pCVD442 and its derivatives, there will be nothing cloned into the plasmids that would enhance the virulence properties of the strain.

The antibiotic-resistance genes that we will be using encode resistance to kanamycin, chloramphenicol, tetracycline, ampicillin/carbenicillin and occasionally gentamycin. Since these antibiotics are not the drugs of choice by clinicians for the treatment of infections by these infectious agents, the presence of these resistance genes within the strains would not present a selective advantage in the event of an accidental release.

The salmonella strains containing promoter-reporter-gene fusions are anticipated to have virulence characteristics close to wild-type levels. Introduction of the fusions will not enhance the virulence properties of these strains because they only contain virulence gene promoters fused to well-characterised proteins/enzymes, known to catalyse specific, assayable, biochemical reactions. When assaying the reporter enzymes, a cell lysate will be prepared from the strains and will not contain viable organisms that could be accidentally released.

All in vitro work will involve standard laboratory procedures at small scale. Given the level 2 containment and control measures, it is unlikely that the GMMs will reach the environment and cause harm to humans or animals outside the laboratory.

Transmission to laboratory personnel is most likely via the oral route. Good laboratory practice and implementation of the recommendations outlined in Schedule 8 will minimise risks. This will include restricting access to authorised workers, ensuring that all areas that have been in contact with potentially hazardous microorganisms will be routinely swabbed with a recommended disinfectant, no laboratory clothing will be allowed outside the laboratory and all outdoor clothing will be stored in offices. All contaminated waste will be autoclaved prior to leaving the site and incinerated by authorised contractors.

All in vivo work involving mice will be conducted in special category 2 containment animal research facilities. Once infected, the mice will not be moved from the designated area until the end of the experiment. At the end of the experiment, the mice will be humanely killed and then dissected to remove organs for further analysis. Organs will be transferred to sealed containers and transported to the laboratory for further processing. The remains of the animals and any materials in contact with the infected animals will be autoclaved and incinerated. This approach reflects "best practice" used at the Cambridge University Veterinary School and the Hammersmith Hospital, London.

Culture of mammalian cells will be prepared in a dedicated tissue culture incubator in a Category 2 pathogen laboratory. Bacterial cultures will be grown in approved Category 2 pathogen laboratories. Once the mammalian cells are infected with Salmonella strains, they are to be considered as class 2 hazardous material and the containers in which they are grown will only be opened in Category 2 containment laboratories for further processing. The tissue-culture plates containing the hazardous material will be transported between the tissue-culture incubator and other Class II laboratories in sealed containers. Lysis of cultured mammalian cells harbouring
Intracellular Salmonella cells will be quickly transferred to sealed tubes for bacterial cell harvest. Tissue culture and bacterial culture waste will be collected in appropriate bags/containers and autoclaved prior to incineration.

The above precautions will reduce the likelihood of a hazardous event to negligible levels. Thus, the overall risk is effectively zero given the proposed containment and control measures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Appropriate disinfection and waste management procedures will be carried out in accordance with Schedule 8.

All in vivo work involving mice will be conducted in special category 2 containment animal research facilities. Once infected, the animals will not be moved from the designated area until the end of the experiment. During experiments, animal waste and bedding will be autoclaved before being incinerated. At the end of the experiment, the animals will be dissected to remove organs for further analysis. Organs will be transferred to sealed containers and transported to the laboratory for further processing. The whole case will be autoclaved; its contents will be autoclaved and incinerated. The remains of the animals will subsequently be incinerated. This approach reflects "best practice" used at the Cambridge University Veterinary School, Hammersmith Hospital, London and Veterinary Laboratory Agency, Weybridge.

All contaminated plastic ware is to be either incinerated directly, or following an overnight soak in 5% Hycolin disinfectant. Items will be double-bagged for transport to the incinerator. A firm registered with the HSE as being licensed hazardous waste incinerator will collect hazardous waste weekly.

All other items, including vessels containing small culture volumes (up to 200 ml) will be placed in lidded steel buckets for autoclaving. The autoclave for waste has a chart recorder for monitoring load and drain temperatures. It is services quarterly and inspected for accuracy annually.

Both incineration and autoclave procedures are expected to give a 100% kill of all GMMs.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This notification arose as a result of submitting internal Risk Assessment amendments to the local GMSC, which we assumed would be covered by our original class 2 notification to the HSE (GM 155/01.5 'Regulatory mechanism affecting virulence in Salmonella enterica serovars typhimurium and enteriditis, enteropathogenic E. coli and E. coli 0157:H7 VT, by nucleoid proteins').

However, the GMSC advised us that

1) analysis of genes that are not directly connected to virulence,
2) the construction of any mutants in these micro-organisms, and
3) the use of any reporter gene other than GFP, constituted a significant change in the direction of the research project. We were therefore obliged to submit this document as a new notification.

### Project Containment

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### Project Ref 155/08.1

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<td>Functional Metagenomics of Colonic Bacteria.</td>
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Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The complex microbiota of the gastro-intestinal tract plays a vital role in human health but its analysis by conventional microbiology is hampered by the fact that many bacterial components are difficult to culture or currently unculturable. Metagenomics offers an alternative molecular approach based on the analysis of genomic DNA extracted from the entire complex microbial community. This can be used to make clone libraries to be screened for specific sequences (by PCR and hybridisation) or functional properties. Conventionally E. coli is used as a background host for metagenomic libraries and we will make libraries with E. coli fosmids. However the main purpose of this study is to investigate the use of the Gram positive bacteria Lactococcus lactis and Lactobacillus as background hosts. We will develop the genetic tools for library construction in Lactococcus lactis and Lactobacillus. The metagenomic libraries generated will NOT be expression libraries with cloned genes expressed from a plasmid derived promoter. Expression will be from the native cloned promoter and relies on its compatibility with the transcription/translation machinery of the background host bacteria.

Metagenomic libraries derived from GI tract bacteria will be subject to a series of functional screens to identify genes that are relevant to microbiota dynamics and/or the role of the microbiota in the maintenance of health. Specific targets could include antimicrobial compounds and enzymes involved in microbial catabolism and transformation of glycosyl and glucuronyl conjugates of dietary phytochemicals. They are involved in human health maintenance but many conjugates are only active when released from their bound form in the diet by the action of gut bacteria.

**Recipient or parental organism**

E. coli 1(12 and derivatives, Lactococcus lactis MG 1363 and Lactobacillus and their derivative strains including mutants. E. coli K12 is compromised and it is unable to colonise the human gut. Lactococcus lactis and Lactobacillus are recognised as Generally Regarded As Safe (GRAS) organisms. Lactococcus lactis MG 1363 does not colonise the gut but passes through as a transient component of the microflora. The colonising ability of Lactobacillus is dependent on the species and strain used. (continued on additional comments sheet)

**Host/vector system**

Hosts will be the bacterial species described above. The vectors include E. coli fosmid and cosmid systems available commercially and a pLP712 derived vector system for use in Lactococcus. If an alternative vector was required for library construction in Lactobacillus this could be based on native plasmids or the broad host range pAMBI vector. The vector systems that we have chosen to use are NOT expression vectors. Expression of cloned genes will be reliant on their own promoter activity and compatibility with the transcription and translation machinery of the host.

**Origin & function**

icDNormetagenomic libraries will be sourced from both specific individual strains (1) and mixtures (2).

1. Specific individual strains Individual cultivated bacterial species will be obtained from the IFR or Rowett culture collections or other strain collections including commercial type strains. These strains will have been characterised to different extents ranging from primary isolation and cultivation from faecal samples up to and including the availability of full genome sequence information. We are unlikely to want to make a clone library of a known pathogenic strain. 2. DNA fragments directly from uncultured gut bacteria to generate library of GI tract bacteria DNA for library construction will be isolated from a variety of samples as appropriate :-

   a. Animal faecal samples (mice, pig, chicken, turkey)
   These samples may be collected from healthy animals or animals previously inoculated with lactic acid bacteria, commensal bacteria or infected with pathogens (eg. Salmonella, Clostridia). This will include gnotobiotic mice (germ-free) inoculated with selected organisms and mice with a defined gut flora.

   b. Human faecal samples (healthy and unhealthy)
   Healthy humans (absence of identified clinical symptoms) and human volunteers taking part in the study of gut disorders in both active and remission states (IBS, IBD, Crohn’s disease, diarrhoea). Faecal samples will be provided, stored and used as agreed with the Human Ethics Committee and the in-house IFR Code of Practice for
work with Biological Samples.
c. Samples taken from the in vitro colonic gut model.
The in vitro colonic gut model comprises a closed 3 vessel fermentor system that mimics the human colon. It is inoculated with fresh faecal material and the bacterial population stabilises. This gut model system is then maintained as a “control” or exposed to an environmental change by addition of another bacterial species or media component.
Samples taken from the gut model will be used as the source of the genomic DNA for library construction.
Screening of libraries can be sequence based and utilise PCR and hybridisation to identify homologues of known proteins. In no case will this be for genes that include functions that are thought to be potentially harmful (eg. toxin genes). Our primary aim is to use function based screening incorporating a) Activity based selection for antibiotic and bacteriocin resistance, b) Enzyme screening methods utilizing colour and fluorescence assays (eg. CMCase carboxymethyl cellulase activity) and possibly c) Antibodies specific for known proteins to detect protein expression. These approaches will be used to screen the metagenomic libraries for other genes beside those already mentioned. Lactococcus, Lactobacillus and E.coli are non pathogenic and classed as ACDP group 1 organisms. Due to the possibilities of cloning detrimental genes as described elsewhere the GMMs should be placed in a higher category (Class II) and the metagenomic library strains will be handled under category 2 laboratory conditions.

Origins and intended functions of genetic material involved
One potential problem with a metagenomic library is the scarcity of clones. We will increase our chances of finding a particular clone by the selection of active clones by activity or complementation. If the library is transformed into a mutant strain we can select for a particular activity. Selection is more efficient than screening. Alternatively, we may enrich the genomic DNA prior to library construction. Methods available include % CC DNA fractionation and enrichment for the metabolically active bacteria (growth on xenobiotics, sugars etc., stable isotope labelling with 13C (SIP) or 5-bromo-2-deoxyuridine BrdU to label DNA).

Evaluation of foreseeable effects
From a mixture of faecal bacteria we run the risk of cloning known and unknown genes which can be harmless or harmful. Potential detrimental genes include toxins, virulence and bacteriophage genes and viruses (human and animal dependent on DNA source).

The aim of these metagenomic libraries is to isolate large fragments of genomic DNA and the level of risk will vary with the source of the genomic DNA used for metagenomic cloning. In the absence of cloning biases, the frequency with which genes will be present in the metagenomic library is a reflection of their prevalence in the starting material. Relatively uncommon pathogenic bacteria are unlikely to be represented within the library whereas abundant bacteria will be well represented. However even the healthy gut (human or animal) as defined by the lack of defined clinical symptoms is a potential source of pathogenic bacteria and hence genes. In the case of animals infected with pathogens or human subjects with gut disorders the relative percentage of potentially harmful bacteria may be higher so the likelihood of cloning deleterious genes will also increase. However virulence is often multifactorial and is unlikely to be cloned in its entirety on one plasmid and be active in the library host.

Where we are cloning the genome of an individual strain the amount of prior information or characterisation available will vary. Within a metagenomic library the individual plasmids will be extremely varied so it is impossible to predict the characteristics of any given GMM.

Exact foreseeable effects are hard to determine as would be the case in the cultivation of faecal bacteria under appropriate conditions and media. Within a metagenomic library the individual plasmids will be extremely varied so it is impossible to predict the characteristics of any given GMO.

For all these reasons the gut metagenomic libraries will be cultivated under category 2 laboratory conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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<tr>
<td>Appropriate disinfection and waste management procedures wdl be carried out in accordance with Schedule 8. Good Microbiological Practice (GMP) and the in-house Code of Practice for Biological Waste Disposal will be followed. All biological waste including GMO waste is double bagged, collected from the laboratories and autoclaved. Following autodaving, the bags are sealed prior to transport to the incinerator. Hazardous waste is collected weekly by a firm</td>
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registered with the HSE as a licensed waste incinerator. All other items, including small culture volumes (up to 200m1) are placed in lidded steel buckets for autoclaving. All contaminated plasticware and solid media is bagged with the rest of the biological waste, autoclaved then incinerated as described above. Where plasticware is to be used again (eg. some centrifuge tubes and pots) it is soaked in chlorine-based detergent at an appropriate concentration overnight after which it is washed up. The autoclave is used exclusively for waste and has a chart recorder for monitoring load and drain temperatures. Additional indicator strips are put in with each load and checked at the end of every autoclave run. The autoclave is serviced quarterly and inspected for accuracy annually. Both autoclave and incineration procedures are expected to give 100% kill of all GMMs.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC required confirmation that the activity screens rely on successful promoter and transcription/translation control in the library host, but also on having all the necessary processing and transport/secretrion mechanisms as well. We are looking to capitalize on the ability of Lactococcus to express heterologous genes that come from other low GC Gram positive bacteria (eg. Firmicutes) and have no plans to use expression vectors in the future. The committee felt that It is quite likely that faecal samples will contain some bacteria with potentially harmful genes and are happy that we are assessing the probability of hazard. However the chance of producing a virulent GMM is unlikely as this would require the heterologous expression of many different genes. As we are using a system built on a native lactococcal plasmid with a cos site we are limited to DNA fragments of around 50-60kb and this will be maintained at the relatively low copy number of the parent plasmid. Although different strains/species of Lactobacilli display different gut colonisation abilities, they will all have GRAS status and many of them will be probiotic strains. The genetic modification safety committee agreed with the assessment of this research as Class 2.

Project Containment

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Project Ref 155/08.2

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
The activity involves the introduction of reporter gene and knock out mutant complementation sequences into a conserved pseudogene in Campylobacter jejuni using suicide vectors.

Recipient or parental organism

Campylobacter jejuni is a ACDP class II pathogen. The expression of reporter genes in this will not alter the hazard associated with this organism. Expression of genes complementing existing knock out mutants should merely restore the wild type phenotype.

Host/vector system

The suicide vectors are constructed in Ecoli K12 using pUCI8 derived plasmids

Origin & function

The suicide vectors contain flanking sequences derived from the Campylobacter pseudogene to allow homologous recombination to occur and commonly used antibiotic resistance marker genes to allow selection of transformants. The reporter genes to be inserted are:

- Green fluorescent protein (gfp) from Aequorea victoria
- Beta-Galactosidase (B-Gal) from E.coli
- Beta-Glucuronidase (GusA) from E.coli

The promoters used to control the expression of genes complementing knock out mutants will either be the native promoter associated with the gene to be complemented or Campylobacter derived sequences with known levels of expression.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Appropriate disinfection and waste management procedures will be carried out in accordance with Schedule 8. Good Microbiological Practice (GMP) and the in-house Code of Practice for Biological Waste Disposal will be followed. All biological waste including GMO waste is double bagged, collected from the laboratories and autoclaved. Following autoclaving, the bags are sealed prior to transport to the incinerator. Hazardous waste is collected weekly by a firm registered with the HSE as a licensed waste incinerator. All other items, including small culture volumes (up to 200ml) are placed in lidded steel buckets for autoclaving. All contaminated plasticware and solid media is bagged with the rest of the biological waste, autoclaved then incinerated as described above. Where plasticware is to be used again (eg. some centrifuge tubes and pots) it is soaked in chlorine-based detergent at an appropriate concentration overnight after which it is washed up. The autoclave is used exclusively for waste and has a chart recorder for monitoring load and drain temperatures. Additional indicator strips are put in with each load and checked at the end of every autoclave run. The autoclave is serviced quarterly and inspected for accuracy annually. Both autoclave and incineration procedures are expected to give an 100% kill of all GMMs.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC had few comments about the risk assessment, concerning the prevalence of the pseudogene target in other Campylobacter strains, for which there is little or no evidence, and a request for further details of the pseudogene target with regards to possible problems with transcription read through. Both of these issues were addressed in the final approved risk assessment.

Project Containment

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<td>L3 L4 L2 L3</td>
<td>L2 L3 L4 L3</td>
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Project Ref 155/08.3
The aim of the research is to determine how microbial pathogens invade the body and in particular the routes they take to cross boundary cells that line tissues exposed to the environment. For example, epithelial and endothelial cells that line the gastrointestinal tract and blood vessels, respectively, act as barrier cells to pathogen invasion. Using model pathogens genetically engineered to express reporter genes allows them to be "visualised" by for example, microscopy so that we can track and define their migration in living cells and tissues.

Microorganisms used will be representative of enteric pathogens (Salmonella typhimurium, campylobacter jejuni, Toxoplasma gondii) and systemic pathogens (listeria monocytogenes) engineered to express chemiluminescent (e.g luciferease) or fluorescent (e.g green/yellow and flavin mononucleotide-based fluorescent proteins) reporter genes. The reporter genes have no known effect on virulence and the GM forms will be no more harmful than parental strains.


Origin & function
The reporter genes are derived from diverse species including the firefly( luciferease), sea pansy (renilla luciferease), click beetle (fluorescent proteins), bacteria (e.g bacillus subtilis and pseudomonanas puida as sources of flavin mononucleotide-based fluorescent proteins and Vibio spp. For luciferease), algae (fluorescent proteins) and jelly fish (GFP-YFP). In the event that additional fluorescent genes (e.g Blue light photosensor (LOV) genes) are inserted into these backgrounds the IFR GMSC will assess any amendment. The genes encoding these reporter proteins which are invariably single polypeptides have been cloned and the coding sequences optimised for expression in bacteria,
The committee had some questions specially related to the Toxoplasma.
1. Will you just be using the asexual forms, tachyzoites and bradyzoites? How do you propagate your stocks in tissue culture?
2. Is toxoplasma a particular risk to pregnant women like Listeria is?
3. Are Toxoplasma gondii oocysts resistant to disinfection with Virkon? Will Toxoplasma never be in this form or is there evidence that Virkon is effective against protozoa in all stages of the life cycle?
Other general questions included classification of transport and conditions of use within special imaging facilities. Also which antibiotic resistance markers were in use?
The GMRAs related to the CU2 were approved following the satisfactory response to the committees comments.

Project Containment
### Project Ref 155/10.1

#### Date Ackn'd
25/05/2010

#### Date Project Ceased

#### CU2 Project Title
Defining the molecular mechanisms underlying the interactions of intestinal Bacteroides ssp. with the host and with other members of the gut microbiota

#### Class CultureVol
Class 2 < 1 Litre

#### Non-GMM Consent Granted
Not Applicable

#### Project notified under transitional arrangements
N

#### Withdrawn
N

#### Tick if notifying a connected programme of work
N

### Project Additional Information

**Purposes of the contained use**

Defining the molecular mechanisms underlying the interactions of intestinal Bacteroides ssp. with the host and with other members of the gut microbiota and development of commensal bacteria as heterologous expression hosts.

**Purpose of research:**

Our overall aim is to develop or use established genetic tools to study the physiology of gut commensal bacteria with a particular interest in their relationship and interactions with the host. Furthermore, we would like to develop these gut commensals into heterologous expression hosts. This work will involve the expression of heterologous genes and operons (e.g., bacterial such as sseB from Salmonella; viral such as synthetic h5f stem form influenza; antigenic protein coding genes from Yersinia such as v1 and caf1 and eukaryotic genes (e.g. microRNAs and cytokine genes)) in both Gram-negative (E. coli, Bacteroides) and Gram-positive (Lactococcus, Bacillus and Lactobacillus) bacteria and the introduction of point mutations or the generation of deletions into several genes of the commensal bacteria.

- **Gene deletion:** using site-directed mutation as well as other mutagenesis strategies. These experiments will be performed with the aim to remove native gene functions that might interfere (regulatory or metabolically) with the successful expression of heterologous genes and biosynthetic gene clusters.
- **Gene insertion:** we will introduce heterologous DNA, both in the form of replicating plasmids as well as stable chromosomal insertions with the aim to identify the
products of the inserted genes and their biological function.

Recipient or parental organism

E. coli (derivatives of K12 and other laboratory strains)
E. coli strains will be used for subcloning during the construction of gene integration vectors, expression vectors or for plasmid isolation. Derivatives of K12 are crippled and will only survive under laboratory conditions. These strains are unlikely to colonise and establish a persistent infection in the gut of a healthy individual. E. coli strains such as DH5α and BL21 do not carry the well-recognized pathogenic mechanisms required by strains of E. coli which cause "the majority of enteric infections" and therefore they are "considered to be non-pathogenic and unlikely to survive in host tissues and cause disease."

Lactobacillus are generally regarded as safe (GRAS) organisms. As natural residents of the gut they should be considered to have the potential for colonisation. All of the strains mentioned are obtained from strain collections and have been characterised by others.

Lactococcus lactis (derivatives of MG1363)
Lactococcus lactis is a generally regarded as safe (GRAS) organism used in food and feed fermentation. Lactococcal strains in use for this project will include derivatives of L. lactis MG1363 including strain UKLC10 in which the nisR and nisK regulatory genes of the nisin operon have been inserted in the bacterial chromosome. This enables nisin regulated control of heterologous genes under the control of the nisA promoter in expression vectors such as pUK200. In addition UKLC10 was engineered to be a peptidase-deficient mutant of MG1363.

Lactobacillus isolates of GI tract origin (human or animal).
Lactobacillus are generally regarded as safe (GRAS) organisms. As natural residents of the gut they should be considered to have the potential for colonisation. Strains used will be type strains of these organisms (e.g. Lb. gasseri NCIMB 11718) and/or isolates of GI tract origin (human and animal, held in the IFR collection) and may include Lb. Johnsonii F19785.

Bacillus subtilis is not a human pathogen, nor is it toxigenic like some other members of the genus. The virulence characteristics of the microorganism are low. According to Edberg (1991) either the number of microorganisms challenging the individual must be very high or the immune status of the individual very low in order for infection with B. subtilis to occur. This microorganism also falls under the Class 1 Containment under the European Federation of Biotechnology guidelines.

L. lactis (derivatives of MG1363), laboratory strains of E. coli (derivatives of K12), B. subtilis and Lactobacillus isolates of GI tract origin (human or animal) are all classified as ACDP Hazard Group 1.

Bacteroides
The Bacteroides spp. used in this study are commensal in the colon and as such should be considered to have the ability to colonise. The characteristic of this genus is that its members are anaerobes. They occur in the alimentary tract of humans and animals. These organisms are classified by ACDP as Hazard Group 2 but are generally considered non-pathogenic and have generally not been shown to have any toxic or allergenic properties. Exception is represented by some strains of Bacteroides fragilis that have been associated with wound infections, especially after surgery. Several B. fragilis virulence factors have been shown to be implicated in the development of these infections. This work will be carried out under Class II conditions.

Host/vector system

E. coli: colE1, pACYC, R↓Δori, pBBR1, pSC105
The pCR2.1 and TA type systems for the cloning of PCR fragments into E.coli are commercially available and the associated plasmids are non-mobilisable.

Lactococcus: pSH71, pWV01, pGhost, pAM↓1, pOrI,

Lactobacillus: Plasmids will be based in the replicons, listed in the Lactococcus section above, that replicate in Lactobacillus. In addition, this work will take advantage of
vectors based on intrinsic lactobacillus plasmids (e.g. p9785S and p9785L from Lactobacillus johnsonii FI9785) including the derivative expression plasmid pFI2560.

Plasmids may carry one or more of the following resistance genes: chloramphenicol (cmR), erythromycin (emR), ampicillin (ampR), kanamycin (kanR), spectinomycin (spcR), trimethoprim and tetracycline (tetR).

Additional genes may be present on some plasmids as part of a controlled expression or integration system.

Bacteroides: Plasmids will be based on Bacteroides replicons as is the case detailed in GM559/05.2. Dependent on the bacterial host these plasmids will be low or high copy for plasmid replication alternatively they could be suicide or temperature/antibiotic permissive selection vectors for chromosomal integration. Promoters will be used to express genes encoding proteins either by inducible (e.g. L. lactis; lac or nisin promoters, Bacteroides; xylanase chromosome. The reporter gene plasmids will be further adapted to act as promoter isolation vectors for the selection of stronger or inducible promoters from chosen bacterial species. Choice of promoters will be by random selection or selected using genome analysis where the genome sequence is available.

Origin & function

Selected genes (e.g. B. thetaiotaomicron lipoprotein genes mini-library, relaxase genes or phosphatase genes) will be cloned into suitable vectors and transformed into the bacterial hosts using standard transformation, electroporation, conjugation or transposon-based methods as appropriate. The decision whether to express the gene on a plasmid or to integrate into the chromosome will be dependent on the strength of expression of the gene i.e. the relative dosage effect. Further development of the system may include the use of a reporter gene for the isolation of stronger and/or inducible promoters. This may lead to the identification of better sites for chromosomal integration strategies.

PCR, splicing and cloning will be used to introduce these genes into appropriate vectors for Bacteroides, E.coli, B. subtilis, L. lactis and Lactobacillus.

Evaluation of foreseeable effects

Bacteroides spp. are generally susceptible to a variety of antibiotics including metronidazole, carbapenems, beta-lactams and chloramphenicol; moreover since they are anaerobic, their environmental survival is expected to be very limited. Lactobacillus spp. are generally susceptible to imipenem, piperacillin-tazobactam, erythromycin and clindamycin. Escherichia spp. are generally susceptible to nitrofurantoin, norfloxacin and gentamycin. Lactococcus lactis is susceptible to erythromycin, chloramphenicol, ciprofloxacin and vancomycin.

All gene products are potentially non-toxic and should not make these GMOs more harmful than the unmodified hosts. However, although most of Bacteroides spp. have generally not been shown to have any toxic effect, there is a low risk that overexpression of some of the proteins could exert some toxicity.

Lactococcus and Lactobacillus are GRAS organisms and the E. coli strains are K12 derivatives for laboratory use. B. subtilis is also routinely used in category 1 sites. The antibiotic resistance genes are all in routine use in other vectors used with these host bacteria. Bacteroides spp. organisms are classified by ACDP as Hazard Group 2 and are generally considered non-pathogenic and have generally not been shown to have any toxic or allergenic properties.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Bacteroides ssp., B. subtilis, L. lactis, Lactobacillus spp., and E. coli and the GMM’s generated during the study will be handled in accordance with Good Microbiological Practice and the correct Hazard Category. …

Appropriate disinfection and waste management procedures will be carried out in accordance with Good Microbiological Practice (GMP) and the Institute Code of Practice for Biological Waste Disposal will be followed. …

Highly qualified staff, in special category 2 containment animal research facilities, will conduct all in vivo work involving animals. Once infected, the animals will not be moved from the designated area until the end of the experiment. At the end of the experiment, the animals will be dissected to remove organs for further analysis. Organs will be transferred to sealed containers and transported to the laboratory for further processing. The remains of the animals and any materials in contact with the infected animals will be autoclaved and incinerated. This approach reflects “best practice” used at the Cambridge University Veterinary School, the Hammersmith Hospital, London and the Veterinary Laboratory Agency, Weybridge. …

Initially the RA was considered to be too generic and lacking detail - examples of the types of genes to be investigated were requested.

The committee asked for statements regarding the risks associated with the genes to be investigated to be included.

All of these points were addressed in the final RA

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Project Ref 155/20.1

Date Ackn’d 22/01/2020

CU2 Project Title Characterisation of the Lifestyles of Human Opportunistic Pathogens

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 1-50 Litres
**Date Project Ceased**

- **Non-GMM Consent Granted**
- Project notified under transitional arrangements

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

#### Purposes of the contained use

Human opportunistic pathogens, and genetically modified variants of human opportunistic pathogens, are to be used to characterise the lifestyle of said pathogens, in the pursuit of novel strategies for treatment.

#### Recipient or parental organism

The recipient organisms are all Hazard Group 2 human opportunistic pathogens. Such organisms are generally ubiquitous in the environment and/or form part of the human commensal flora, and rarely pose a problem to the immunocompetent. However, these organisms are major nosocomial infections and are a significant cause of morbidity and mortality in the immunocompromised, such as post-surgical patients, chemotherapy patients, the very young and the elderly. Examples of these organisms include: Pseudomonas aeruginosa; Staphylococcus aureus; Enterococcus faecalis; Enterobacter spp.; Acinetobacter baumannii; non-tuberculoid Mycobacterium; Haemophilus influenzae; Klebsiella pneumoniae; and, the Burkholderia cepacia complex.

Initial studies will be performed in laboratory strains of these organism which have been extensively characterised. It will be necessary to validate these findings in clinical isolates. Such isolates often have increased virulence and antimicrobial resistance. Any undertakings with clinical isolates will only be performed when sufficient characterisation has occurred to ensure that any genetic modification does not increase the virulence or significantly decrease the available treatment and disinfection options. All species will be checked against the COSHH and any work will be performed as per the precautions stipulated in that assessment.

Treatment of these organisms is limited due to the high prevalence of multi-drug resistant strains and more effort must be made to further characterise the lifestyles of these organisms so as to develop innovative and effective treatments. The laboratory strain Escherichia coli K12 (and derivatives) is often used as an intermediary, expression or cloning vector for research. They are well characterised and non-pathogenic strains of E.coli and are routinely used in research.

#### Host/vector system

Vectors will be used to introduce homologous recombination, incorporate markers and express proteins. Many vectors...
will be used over the course of this project, however non-conjugative vectors will be used and vector exchange can only be achieved through established laboratory protocols, the use of accessory plasmids, and/or the incorporation of the strain into a conjugative E. coli K12 derivative. Such exchange is therefore extremely unlikely without knowledgeable intervention. Such vectors include (but are not limited to): pGem-T and derivatives; PME based plasmids and derivatives; pUC18/19 and derivatives; pUCP18/19 and derivatives; MiniCTX and derivatives; pME3087 and derivatives; pBlueScript and derivatives; pET and derivatives and pG19 and derivatives. Transposon-based genetic modification will also be used and will include the use of the Tn7 transposon system, the Tn5 transposon system, the Himar transposon, and the Mariner transposon.

E. coli K12 derivatives will be used as intermediaries, donor organisms or protein expression vehicles.

Origin & function

In most cases the host DNA is being altered by deletion and the deleted genes restored under the control of a native, inducible or constitutive promoter. Exogenous genes may be added as a marker or created as a genetic fusion for protein characterisation or localisation. These genes include, antibiotic resistance genes, fluorescent protein genes, luminescent protein genes or, genes for enzymes that catalyse a quantifiable phenotypic change, such as β-galactosidase (lacZ). These genes are very well characterised and are used frequently by those in the art and come from a variety of different sources. Such genes would not convey any selective advantage to the host organism or increase the virulence or pathogenicity of the organism, or significantly alter treatment options.

Evaluation of foreseeable effects

Gene deletions will be made in the host organism. Such deletions will be made in genes involved in virulence and pathogenicity and as such the resulting GMO is expected to be less virulent than the wildtype. The gene defect will be restored by the inclusion of the gene under the control of a native, inducible or constitutive promoter, such that the resulting GMO will have no increased virulence or pathogenicity when compared to the wildtype.

It is possible that an unknown regulatory promotor will be deleted leading to a rise in the overall virulence of the GMO. This is not the aim of this project and such regulatory proteins often require multiple changes or deletions in metabolic pathways to significantly alter the virulence of an organism or increase the resistance of the organism to antimicrobials or disinfection procedures. It is highly unlikely that such a GMO will occur but if it does, the organism will be immediately destroyed.

Genes encoding toxins may be introduced and produced. Individual risk assessments will be carried out on each toxin as and when appropriate. For the purposes of protein expression, these toxins will only be produced by laboratory attenuated bacterial hosts and as such would not survive outside of the carefully controlled laboratory environment. They will only be made on non-conjugative vectors so that the toxin producing gene will not spread to other microorganisms. Wherever possible toxin production will be controlled using inducible promoters so that toxin production can not occur without specialised intervention.

Antibiotic resistance genes will be incorporated for the use as a selection marker for genetically altered strains. Antibiotics resistance genes used as selection markers are for antibiotics not used or not preferred for clinical treatment, such as kanamycin. No GMO will be created that render the organism resistant to current treatment options.

The GM strains generated by this work are extremely unlikely to pose any significant threat to human health or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
No genetically modified plants and animals will be created.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid biological waste will be sterilised by autoclaving at 121 °C for 20 minutes, or 30 minutes for dense loads. Liquid biological waste will be sterilised by autoclaving at 121°C for 20 minutes. Any chemically contaminated biological waste will be sent for incineration by a licenced waste contractor.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref**  559/04.2

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<th>CU2 Project Title</th>
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<td>26/07/2004</td>
<td>Use of recombinant lentiviruses to knockdown the expression of genes encoding anti-microbial proteins in intestinal epithelial cells.</td>
<td>Class 2 &lt; 1 Litre</td>
<td>Non-GMM Not Applicable</td>
<td></td>
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Date Project Ceased  02/03/2022
Purposes of the contained use

The aim of the research is to identify how the gut protects the body from invasion and disease caused by bacteria that either normally live in the gut or those that gain entry via contaminated food. Specifically, we will determine the role intestinal epithelial cells play in preventing microbes from invading the body by determining what the consequences of interfering with the expression of genes known to be important in protecting other cells of the body from invasion are on the ability of intestinal epithelial cells to prevent infection by different types of bacteria. A type of virus, Lentivirus, will be used to deliver toxic molecules to epithelial cells that will interfere with the expression of genes involved in microbial defence.

Recipient or parental organism

Lentivirus based vectors have been chosen for this study since of all available gene delivery vehicles they are most suited to the delivery of genes into primary, non-dividing, cells. The vectors to be used are replication defective, HIV-1 based, recombinant lentiviral vectors that can only be generated using a transient triple transfection system. Since all HIV coding sequences have been deleted no viral genes can be transferred to the target cells. Due to the transient and reversible effects of the Lentivirus delivered genes, the final modified cells are expected to be more harmful than the unmodified cells.

Host/vector system

The Lentivirus has been significantly modified for biosafety. Use is made of self-inactivating (SIN) vectors, which prevent promoter activity in the viral 5' LTR in the integrated provirus from in target cells, reducing the probability of insertion activation. To minimise the possibility for homologous recombination, the HIV genome is divided into three parts, cloned into separate expression plasmids and extensively modified. The following modifications have been made to prevent viral replication.

1. Packaging vector lacks both LTRs and has no viral packaging signal (y).
2. The env, tat, rev, vpr, vpu, vif and nef gniral genes have been deleted from the packaging vector.
3. Rev is supplied in trans on a different vector.
4. The vector expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion) and expresses no viral gene product.
5. Envelope (VSVG) is expressed on a separate vector.

Relevant references:
Packaging vectors:

Self inactivating LTR:
The inserted synthetic sequences will encode interfering RNA (RNAi) or short hairpin RNA (shRNA) species that will result in reduced or no expression of the target genes (eg NOD2, alkaline phosphatase, B-galactosidase) in intestinal epithelial cells. The effect of the NRAi/shRNA is expected to be transient and non-lethal and will only interfere with the cells ability to produce certain cytokines in response to challenge with infectious and non-infectious stimuli.

Origin & function

Since all HIV coding sequences have been deleted from the Lentivirus vectors to be used no viral genes can be transferred to the target cells. Due to the transient and reversible effects of the Lentivirus delivered genes, the final modified cells are expected to be no more harmful than the unmodified cells.

Evaluation of foreseeable effects

Evaluation of foreseeable effects

Since all HIV coding sequences have been deleted from the Lentivirus vectors to be used no viral genes can be transferred to the target cells. Due to the transient and reversible effects of the Lentivirus delivered genes, the final modified cells are expected to be no more harmful than the unmodified cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

There is very little risk associated with the "escape" of the Lentivirus vectors which alone or in combination cannot produce replication competent virus. Cultured primary intestinal epithelial cells cannot survive outside the culture medium and have a finite life span. (<2 weeks) in vitro. It is expected that Lentivirus-transduced cells will not survive any longer than non-transduced cells (<2 weeks).

The likelihood of the escape of treated cells is remote. Virus-transduced cells are kept in tissue culture incubators in a category 2 designated tissue culture room within a research laboratory to which only authorised laboratory personnel have access. Access to this laboratory is via locked doors that have a coded keypad locking system and access to the tissue culture room within is restricted to designated personnel and is locked when not in use. A Class 2 cabinet to BSEN 12469 specification will be used to contain virus-producing cell lines and vector-transduced cells. Sharps will be eliminated from all aspects of the laboratory work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Routine disinfection will be carried out using 1% Virkon spray.
Liquid waste will be diluted with an equal volume of 2% Virkon and left for a minimum of 16h prior to drain discard.
Solid waste will be disposed of by autoclaving at 121 degrees C followed by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Approved by chairmans action in consultation with local BSO, Faculty Safety Manager and an experienced virologist.
To be ratified by the full committee at the next meeting.

6/7/2004 - School of Biology GMAG Committee Chairman.

Project Containment
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L2 L3 L4 L2 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4

Laboratory Activities
Glass Houses
Growth Rooms
Animal Units
Large Scale Activities
Human Clinical Applications

Project Ref 559/05.2

Date Ackn'd 14/04/2005
CU2 Project Title Cloning and characterisation of the Zylanase operon promoter of Bacteroides ovatus; expression of genes encoding immunomodulatory proteins, growth factors and cytokine/growth factor antagonists by B. Ovatus under control of the Zylanase promoter and secretion of recombinant proteins.

Date Project Ceased

Class
CultureVol
Class
Consent Granted

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Transferred from GM559 October 2008

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The modified B. ovatus will be used to assess zylan-inducible expression of the genes described in Section 5. Recombinant organisms will then be used in experiments as novel immunotherapies to try and treat and prevent disease.

Recipient or parental organism

B. ovatus is ACDP Hazard Group 2. The final modified organism will be tetracycline resistant. Although a gut commensal, the likelihood of the GMM colonising humans or persisting in the environment is minimal. Expression of the genes described in Section 5 by this organism should not pose a hazard.

Host/vector system

E. coli vector pGEM is non-mobilisable. Vector pBT-2 is an E. coli-Bacteroides mobilisation-defective shuttle vector requiring E. coli strain J53/R751 for transfer. This is a suicide vector in Bacteroides, requiring integration into the genome for maintenance.
E. coli DH5a and J53/R751 are ACDP Hazard Group 1. B. ovatus is Hazard Group 2.

**Origin & function**

The zylanase promoter originates from B. ovatus. They cytokine/growth factor and antagonist genes will be cDNA copies that will be transcribed in a zylan-inducible manner in the final GMM.

**Evaluation of foreseeable effects**

The final GMM will be tetracycline resistant. Expression of genes described in Section 5 should be regulated and the modified organism must compete with resident microflora to colonise. Release of the number of viable organisms required to colonise other animals or humans is unlikely. Survival in the environment is likely to be limited. Expressed gene products are unlikely to be produced in large enough quantities to have a significant effect on humans. The modified organism should pose no greater hazard than the wild type.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All pipette tips, slides and small disposable plasticware will be disposed of in discard pots containing 2% (v/v) Tego prior to being autoclaved and discarded.
All waste cultures, media and larger plasticware will be autoclaved prior to discarding.
All glassware will be autoclaved before being washed and returned for use.
Sharps will be disposed of in a cin bin which will then be autoclaved and incinerated.
All culture spills will be mopped up with Trigene and tissue that will then be autoclaved prior to discarding.
No viable GMMs will be left after treatment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Following consideration of this application by the Microbiology GM Safety Committee at its meeting on 16 August 2004, the Committee requested some very minor, mostly typographical changes to be made. These changes have been completed to the satisfaction of the BSO.

**Project Containment**
<table>
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<th>L4</th>
<th>L2</th>
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Animal Units

Large Scale Activities

Human Clinical Applications
### MRC CELL MUTATION UNIT

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**Data Premises Notified**
- 16/03/1984

**Data Premises Closed**
- 30/09/2001

**Transferred from 1992 Regs?**
- Y

**Emergency Plan Required?**
- N

**Transitional Premises Class**
- 2

**Non-GMMs Withdrawn**
- N

**Withdrawn**
- N

**ALL GM WORK HAS TRANSFERRED TO GM4 AND THESE PREMISES HAVE NOW CLOSED**
## Premises Addresses

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<tr>
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<th>Department</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Level 2 (GMMs)</td>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 157/01.1**

<table>
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<th>CultureVolumeClass3-4</th>
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<td>06/02/2001</td>
<td>ANALYSIS AND EXPRESSION OF HUMAN DNA REPAIR AND CHECKPOINT GENE USING RETROVIRAL VECTORS</td>
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Withdrawn [Y]  

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

02/03/2022
### Purposes of the contained use

To protect laboratory works and the environment from any potential harm.

### Recipient or parental organism

Cultured mammalian cells. These are characterised cell lines with strict growth and temperature requirements and will be incapable of growth outside the culture flask.

### Host/vector system

Different retroviral vectors will be used with a range of selectable markers. In all cases the vectors are non-mobilisable ie defective for replication with no gag, pol or envelope genes. Expression of the inserts is not expected to be harmful since they are expressed in normal human cells.

### Origin & function

The repair/checkpoint genes encode proteins known to function in damage response pathways. The genes may contain mutations engineered into them.

### Evaluation of foreseeable effects

The human genes are not harmful and are non-oncogenic. Packaging lines are non-mobilisable. Expression not expected to be harmful since they are expressed in normal human cells. Human cells, to which they will be transferred, have strict growth requirements and are incapable of growth outside the culture vessel. Therefore, risk to the environment and laboratory workers is negligible.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be destroyed using Virkon. Disposable plastics destroyed by autoclaving. See attached risk assessment for further validation. Virkon has been shown to be effective against retroviruses and we consider will inactivate our low litres.

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

**Please enter comments on the GM safety committee on the risk assessment**

Committee in agreement with risk assessment.
### Project Containment

#### Laboratory Activities
- L2
- L3
- L4

#### Glass Houses
- L2
- L3
- L4

#### Growth Rooms
- L2
- L3
- L4

#### Animal Units
- L2
- L3
- L4

#### Large Scale Activities
- L2
- L3
- L4

#### Human Clinical Applications
- L2
- L3
- L4

---

### Project Ref 157/01.2

**Date Ackn'd**: 06/02/2001

**CU2 Project Title**: CLONING OF DNA REPAIR/CHECKPOINT GENES USING CDNA LIBRARIES

**Class CultureVol**: Class 2

**VolumeClass**: Class 2-4

**Non-GMM Consent Granted**: not applicable

**Consent Granted**: Y

**Project notified under transitional arrangements**: Y

---

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

### Project Additional Information

#### Purposes of the contained use
To protect the laboratory workers and the environment from any potential harm.

#### Recipient or parental organism
Cultered mammalian cells. These are characterised cell lines with strict growth requirements and are incapable of growth outside the culture flask.

#### Host/vector system

---

02/03/2022
Retroviral vectors or transfection vectors (e.g., pcDNA3).
The retroviral vectors are defective for replication with no gag, pol or env genes.
All vectors are non-mobilisable.

Origin & function

cDNA libraries derived from human or mouse cells. Hopefully the libraries will be representative of all human cDNAs. Our interest is in those expressing DNA repair and cell cycle checkpoint genes.

Evaluation of foreseeable effects

The human cDNA library could potentially include harmful genes such as oncogenes. These will however represent a low percentage of the total cDNAs in the library.
The packaging line contain no helper virus and helper functions are encoded by separate segments. The vectors are non-mobilisable and defective for replication. The inserts are however under strong promoters. The insert will not change the host range of the vector.
The mammalian recipient lines have strict growth requirements and are incapable of growth outside the culture flasks.
Taken together the environmental and human risks are minimal.

Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be destroyed using Virkon. Disposable plastics destroyed by autoclaving.
See attached risk assessment for further validation.
Virkon has been verified to be effective against retroviruses and we consider will inactivate our low litres.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Committee in agreement with risk assessment.

Please enter comments on the GM safety committee on the risk assessment

Committee in agreement with risk assessment.

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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02/03/2022
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**Name**

BIRKBECK COLLEGE

**Name 2**

UNIVERSITY OF LONDON

**Department**

CRYSTALLOGRAPHY & BIOLOGY

**Campus Estate or Research Centre**

**Road Name**

MALET STREET

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

WC1E 7HX

**Country**

ENGLAND

**Tel Number**

0207 631 6000

**Fax Number**

0207 631 6270

**E-mail**


**HSE Division**

LONDON

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

<table>
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<th>Date Premises Closed</th>
<th>Name</th>
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<td>WC1E 7HX</td>
<td>ENGLAND</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<td>Level 3 (GMMs)</td>
<td>Level 4 (GMMs)</td>
<td>Non-microbial</td>
</tr>
</tbody>
</table>

Other (please specify) Tick if confidential

- **Bacteriology**
  - Parasitology
  - Transgenic Birds
- **Virology**
  - Transgenic Animals
  - Transgenic Fish
- **Microbiology**
  - Research
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum culture volume that could be discarded is 10 litres. All cultures whether in glass or disposable containers should be autoclaved before disposal or recovery of containers. Liquid medium from which micro-organisms have been recovered should be sterilised either in the autoclave or by treatment with a suitable disinfectant before being discarded. Place Pasteur pipettes into 2.5% chlorosan in a beaker; rinse and discard after 24 hours in an appropriate container for waste glass.

The autoclave should operate at 15 lbs/ in 2 for 20 minutes following the maker's recommendations for use. Regular monitoring should be undertaken once a week using Thermolog S to confirm that the desired autoclave temperatures are attained. The autoclave should be regularly serviced.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 159/01.1**

**CU2 Project Title**

PRODUCTION OF THE VIRULENCE DETERMINANT PROTEIN PNEOMOLYSIN OF THE HUMAN PATHOGEN STREPTOCOCCUS PNEUMONIAE EXPRESSED IN A GENETICALLY MODIFIED MICRO-ORGANISM, I.E. E.COLI

**Class**

Class 2

**CultureVolClass2**

1-50 litres

**CultureVolumeClass3-4**

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info
**Project Additional Information**

**Purposes of the contained use**

To research the molecular structure and function of the pneumolysin protein from the human pathogen Streptococcus pneumoniae in the formation of oligomeric complexes during membrane pore formation.

**Recipient or parental organism**

The GMM will express at high levels the virulence determinant protein pneumolysin of the human pathogen Streptococcus pneumoniae. While the purified toxin protein will be a hazardous material, the GMM itself should not directly cause harm to human health or other organisms.

**Host/vector system**

The host: E.coli K-12 derived strain, M15 [pREP4], this is a multiple auxotroph. The vector: pKK233-2 [discontinued by Pharmacia PLC. in 1991] is a derivative of pBR322 which contains the colE1 replicon which renders the vector mobilisation defective. The combination of a disabled host and a mobilisation defective vector greatly reduces the transfer risk with this GMM to other hosts.

**Origin & function**

The host/vector system has been produced and characterised by the group of Professor Peter Andrew at the Department of Microbiology and Immunology at the University of Leicester. This GMM will be provided for culture at a small scale [6 litres maximum volume] and the expressed toxin purified for structural analysis.

**Evaluation of foreseeable effects**

Although the GMM carries a vector which will express the pneumolysin toxin in a soluble form, there are no known or recorded harmful effects to humans, animal or plants anticipated from the growth and use of this host/vector system.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
At the meeting of the genetic modification committee held on 9th May 2001 the proposed category 2 risk assessment was considered. The suggested GM risk level of category two was discussed and indicated that the GMM itself was unlikely to lead directly to harm to humans or other organisms. It was proposed that the risk assessment be forwarded to the Notifications Office at the HSE for their consideration and comments.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
</thead>
<tbody>
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**Project Ref** 159/10.1

- **Date Ackn'd**: 22/04/2010
- **CU2 Project Title**: Structural studies of the Type IV secretion system
- **Class**: Class 2
- **Culture Volume**: 1-50 Litres

- **Non-GMM Consent Granted**
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The aim is to produce the component proteins comprising the Type IV secretion system in E. coli together at high levels for structural studies. This will make the
host/vector(s) system self-mobilising at this point and probably conjugation competent. However the expressed proteins should have no adverse biological effects. The Type IV secretion system is found widely in nature, but as we will be effectively reducing the biological containment level while undertaking protein expression, we consider this as a Category 2 experiment.

Recipient or parental organism

The GMO will express the components of the Type IV secretion system. The GMO itself will not in our assessment cause harm to human health or other organisms.

Host/vector system

The recipient strain of E. coli acting as host for expression will be BL21 (DE3), TOP10. The vectors include: pASK-IBA3c_virB1-11, pRSF oriT, pHis17_tralHJK, pKM101.

Origin & function

All sequences will be PCR amplified from the plasmid pKM101.

Evaluation of foreseeable effects

Although the GMO will be expressing the complete Type IV system, there are no known or recorded harmful effects to humans, animals and plants. There are no anticipated harmful effects from the growth or the use of these host/vector systems to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Negative pressure relative to the pressure of the immediate surrounds - not required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The systems in place are designed to ensure that all contaminated waste is efficiently sterilised and only GMO's killed. There are two main routes for the sterilisation of laboratory waste to be used in this notification. All liquid and solid laboratory waste will either be incubated with 1% Virkon for at least 30 minutes using validated conditions, or will be autoclaved on the same day. Each autoclave cycle is validated with the inclusion of a thermolog S strip to monitor that the target temperature for killing the GMOs has been successfully achieved. The sterile, solid autoclaved material can then be disposed of via our normal waste route. Treated sterile liquid waste would go down the sink route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The risk assessment was circulated to all members of the College GM safety committee. All responses were positive. Some minor corrections were made. It was recommended to be forwarded to the HSE for consideration and duly signed by the Clerk of the College on 15th April 2010.
**Project Containment**

<table>
<thead>
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<th>Laboratory Activities</th>
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**Project Ref** 159/13.1

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<td>Class 2</td>
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- Non-GMM
- Consent Granted
- Project notified under transitional arrangements N

**Historical Significant Changes**

- Withdrawn: N
- Tick if notifying a connected programme of work: N

**Project Additional Information**

**Purposes of the contained use**

The aim is to express and purify the individual component proteins comprising the Type IV secretion system in Agrobacterium tumefaciens at high yields for both structural and electron microscopic studies. The production of these proteins requires the transformation of a strain of Agrobacterium tumefaciens (A348) that is not disabled, and so we consider this as a Category 2 experiment. As this is a plant pathogen, we have also obtained an import licence to work with Agrobacterium tumefaciens.

**Recipient or parental organism**

Molecular cloning of the virB operon proteins into the pBADM-11 vector using E. coli hosts in this project are assigned to GM class 1. The growth of transformed A. tumefaciens for the production of virB operon proteins in both liquid and solid cultures, and storage of frozen cell pellets in this project are assigned to GM class 2. For the...
latter work neither the pathogenicity, nor the host range or tissue tropism will be altered from wild type A. tumefaciens.

<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient host strains are;</td>
</tr>
<tr>
<td>1. Agrobacterium tumefaciens strain A348 harbouring a pTiA6NC octopine-type plasmid co-expressing VirB10-Strep from an IncP plasmid,.</td>
</tr>
<tr>
<td>2. E. coli C43(DE3): F- ompT hsdSB (rB- mB-) gal dcm (DE3).</td>
</tr>
<tr>
<td>3. E. coli BL21(DE3): F- ompT hsdSB(rB-, mB-) gal dcm (DE3)</td>
</tr>
<tr>
<td>4. E. coli TOP10: F- mcrA □ (mrr-hsdRMS-mcrBC) □80lacZ□:M15□:lacX74 recA1 ara□:139□:(ara-leu)7697 galU galK rpsL (StrR)endA1 nupG.</td>
</tr>
<tr>
<td>Recipient vector is : pBADM-11</td>
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</table>

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>The following genes will be amplified by PCR;</td>
</tr>
<tr>
<td>1. virB1 (transglycosylase).</td>
</tr>
<tr>
<td>2. virB2 (major pilus protein)</td>
</tr>
<tr>
<td>3. virB3 (inner membrane protein).</td>
</tr>
<tr>
<td>4. virB4 (ATPase)</td>
</tr>
<tr>
<td>5. virB5 (minor pilus protein)</td>
</tr>
<tr>
<td>6. virB6 (inner membrane protein).</td>
</tr>
<tr>
<td>7. virB8 (inner membrane protein).</td>
</tr>
<tr>
<td>8. virB10 (inner and outer membrane protein).</td>
</tr>
<tr>
<td>9. virB9 (outer membrane protein).</td>
</tr>
<tr>
<td>10. virB7 (lipoprotein).</td>
</tr>
<tr>
<td>11. virB11 (ATPase).</td>
</tr>
<tr>
<td>12. virD4 (ATPase).</td>
</tr>
<tr>
<td>13. virD2 (relaxase).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Evaluation of foreseeable effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli will be used to make the expression constructs. The Agrobacterium tumefaciens will be used to express the various components of the Type IV secretion system. The GMOs themselves will not, in our assessment, cause any harm to human health. The Agrobacterium tumefaciens expression constructs will retain the normal plant pathogen functions associated with this host and will not carry any additional hazard to the environment</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not applicable</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative pressure relative to the pressure of the immediate surrounds - not required</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The control systems in place are designed to ensure that all contaminated waste is efficiently sterilised and any GMOs killed. There are two main routes for the sterilisation of laboratory waste to be used in this notification. All liquid and solid laboratory waste will either be incubated with 1% Virkon at least 30 minutes or will be autoclaved on the same day using validated conditions. Each autoclave cycle is validated with the inclusion of a thermolog S strip to monitor that the target temperature for killing the GMOs has been successfully achieved. The sterile, solid autoclaved material can then be disposed of via our normal waste route. The 1% Virkon treated sterile liquid waste would go down the sink route</td>
</tr>
</tbody>
</table>
The risk assessment entitled "Structural studies of Agrobacterium tumefaciens type IV secretion system" was considered at the College GM safety committee on Thursday 2nd May 2013. The committee approved the risk assessment level at GM category 2 and agreed that it should be forwarded to the HSE for notification and consideration with the appropriate fee.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L2</td>
<td>L2</td>
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<tr>
<td>L3</td>
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<td>L4</td>
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</table>

Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
</tr>
<tr>
<td>L3</td>
<td>L4</td>
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Project Ref 159/13.2

Date Ackn'd 31/10/2013

cu2 Project Title Bacterial Plasmid Expression

Class 2

CultureVolClass2 1-50 Litres

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**

The aim is to express and purify full-length pili from E. coli for use in both structural and electron microscopic studies. Expression will be undertaken in both a standard disabled host and the Uropathogenic E. coli strain YT189_LON. The production of these pili from engineered Uropathogenic bacterium, known to cause cystitis in humans, causes us to classify these experiments as requiring GM category 2 containment.

**Recipient or parental organism**

Full-length pili are to be expressed in two different hosts; Pili are to be expressed from pPAP5 plasmid in the commercial strain HB101 for expression, and Top10 for plasmid propagation. pPAP5 carries a 9.8-kilobase (kb) EcoRI-BamHI chromosomal fragment previously constructed from isolated E. coli J96 that encodes for the production of an adhesive P pilus. This has the ability to grow a pilus and hence cause hemagluttination of human erythrocytes. Full-length pili are also to be expressed and purified from the Uropathogenic E. coli strain U189_LON phase locked on resulting in the fim promoter being constitutively on. The UT189_LON is a wild type isolate and can cause cystitis in humans. However, apart from the need for a higher level of containment and laboratory hygiene, it is not expected to have any additional harmful properties beyond its ability to cause treatable cystitis in humans.

**Host/vector system**

Recipient vector for HB101 and Top10 is pPAP5 (Lindberg et al., (1984), EMBO Journal vol. 3 no. 5 pp. 1167 - 1173).

**Origin & function**

In terms of human health risk, the expression in hosts (HB101 and TOP (both are E. coli standard laboratory strains), these are not known to be infectious. A functional pilus can be formed using this plasmid, causing hemaglutination of human erythrocytes. However these bacteria lack many other essential components for a successful urinary tract infection (Type I fimbriae, siderophores, hemolysins) and requires ampicillin in order that the plasmid is maintained in the bacteria. Expression products will be accumulated in the periplasm or the outer membrane of bacteria. The products are not oncogenic. So there is minimal risk to human health.

For the environmental risk, the E. coli standard laboratory strains show low viability outside controlled environments. Non-pathogenic strains are used. Plasmids used are mobilization defective and are lost without selection. Inserted genes do not give competition advantage to host. These pili perform an adhesive function for the bacteria, they are not conjugative pili, or an injectosome organelle and as such do not increase the chance of DNA transfer between bacterial species.

For human health, UT189_LON was derived from Uropathogenic E. coli UT189, this is an isolate from a patient with cystitis, and therefore would have the capability to cause urinary tract infection. However all standard laboratory procedures in place will keep this to a minimum risk, i.e. informing the laboratory workers, high levels of personal hygiene, use of gloves and other personal protection, regular swabbing of benches, and the daily autoclaving of waste by validated means. These control measures should therefore ensure minimal risk to human health.

For the environmental risk, UT189_LON would be a viable competitor to the wild type UT189 in infecting humans and so offers no additional risk beyond that characterised...
previously (ref: Kostakioti et al. 2012, Infect Human.; 80 (8): 2826-2834). Containment controls in place within the laboratory will minimise any potential escape to the environment and wider population.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Negative pressure relative to the pressure of the immediate surrounds - not required

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The control systems in place are designed to ensure that all contaminated waste is efficiently sterilised and any GMOs killed. There are two main routes for the sterilisation of laboratory waste to be used in this notification. All liquid and solid laboratory waste will either be incubated with 1% Virkon for at least 30 minutes or will be autoclaved on the same day using validated conditions. Each autoclave cycle is validated with the inclusion of a thermolog S strip to monitor that the target temperature for killing the GMOs has been successfully achieved. The sterile, solid autoclaved material can then be disposed of via our normal waste route. The 1% Virkon treated sterile liquid waste would go down the sink route.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment entitled "Bacteril Pius Expression" was considered by the members of the College GM safety committee. The committee approved the risk assessment level at GM category 2 and agreed that it should be forwarded to the HSE for notification and consideration with the appropriate fee.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
</tr>
</tbody>
</table>

**Project Ref**  159/15.1
The obligate intracellular bacterium Chlamydia trachomatis has been considered genetically intractable. However, there have been multiple recent reports of transformation from different laboratories using recombinant plasmids based on hybridisation of E.coli and the endogenous Chlamydia trachomatis plasmids. We intend to use these vectors to express epitope-tagged or fluorescent fusion proteins in Chlamydia trachomatis to investigate the localization of virulence effectors and components of the type III secretion system biochemically and using imaging techniques.

Recipient or parental organism

The following aspects of this project are assigned to Class 1.
- Cloning and manipulation of the vectors and their propagation in E.coli strains

The following aspects of this project are assigned to Class 2.
- Transformation and propagation of transformed C. trachomatis within cultured mammalian cells.

Host/vector system

Wild-type Chlamydia trachomatis (this is a Biosafety level 2 organism and not disabled)
E.coli DH5a and XL-1 Blue will be used for plasmid propagation and sub-cloning procedures. These are E.coli K12 derivatives and are classified as 'disabled and non-colonising' by the ACGM.

X1-Blue recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F' proAB lacIqZΔM15 Tn10 (TetR)]
DHSalpha F- phi80lacZ::M15 delta(lacZYA-argF)U169 deoR recA1 endA1 hsdR17 (r-, m+) phoA supE44 thi-1
gyrA96 relA1 lamdapASKGFPL2
pTRL2-GFP
pCDSSKO
placZ-CD55KO
Origin & function

We plan to study the localisation and function of C. trachomatis proteins encoding components of the type III secretion system (inner and outer membrane protein, ATPase, translocan and needle) and the substrates, which are delivered into the boundary membrane of the replicative vacuole (hydrophobic inclusion proteins), host cytosol or nucleus. These genes will be amplified by PCR from chromosomal DNA of C. trachomatis held in our laboratory or by gene synthesis (as some genes are exceptionally difficult to amplify by PCR). Epitope tags will be added to the sequence as part of the PCR primer or synthesised product (e.g. FLAG, Myc) when required. In the case of fusion proteins to MBP, GFP, mKate2 these will be amplified from existing commercial vectors (e.g. pEGFP-C2; Clontech) or obtained from collaborating laboratories where necessary.

Evaluation of foreseeable effects

E.coli. will be used to make the expression constructs. The Chlamydia trachomatis will be used to express the various components of the epitope-tagged or fluorescent fusion proteins to investigate the localization of virulence effectors and components of the type III secretion system biochemically and using imaging techniques. The GMOs themselves will not, in our assessment, cause any additional harm to human health above the virulence of the wild-type organism. Chlamydia elementary bodies survive for only a very short time outside their host cells (30 minutes to 1 hour), so risks from environmental release do not significantly exceed those of the wild-type organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The control systems in place are designed to ensure that any contaminated waste is efficiently sterilised and any GMOs killed. There are two main routes for the sterilisation of laboratory waste to be used in this notification. All liquid and solid laboratory waste will either be incubated with 1% Virkon for at least 30 minutes or will be autoclaved on the same day using validated conditions. Each autoclave cycle is validated with the inclusion of a thermolog S strip to monitor that the target temperature for killing the GMOs has been successfully achieved. The sterile, solid autoclaved material can then be disposed of via our normal waste route. The 1% Virkon treated sterile liquid waste would go down the sink route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The risk assessment entitled "Transformation of Chlamydia trachomatis" was considered at the College GM safety committee on Wednesday 29th October 2014. The committee approved the risk assessment level at GM category 2 and agreed that it should be forwarded to the HSE for notification and consideration with the appropriate fee.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4 L2 L3 L4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

<table>
<thead>
<tr>
<th>Project Ref</th>
<th>159/15.2</th>
</tr>
</thead>
</table>

- **Date Ackn'd**
- **CU2 Project Title**
  - Structural Studies of the Type IV secretion system
- **Class**
  - Class 2
- **CultureVolClass2**
  - 1-50 Litres
- **CultureVolumeClass3-4**
- **Non-GMM Consent Granted**
- **Project notifed under transitional arrangements**

- **Withdrawn**
- **Tick if notifying a connected programme of work**

**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The aim is to produce the component proteins comprising the Type IV secretion system in E. coli together at high levels for structural studies. This will make the host/vector(s) system self-mobilising at this point and probably conjugation competent. However the expressed proteins should have no adverse biological effects. The Type IV
secretion system is found widely in nature, but as we will be effectively reducing the biological containment level while undertaking protein expression, we consider this as a Category 2 experiment.

Recipient or parental organism

The GMO will express the components of the Type IV secretion system. The GMO itself will not in our assessment cause harm to human health or other organisms.

Host/vector system

(i) List of recipient strain(s)
E. coli C43(DE3): F-ompT hsdSB (rB- mB-) gal dcm (DE3)
E. coli C41(DE3): F-ompT hsdSB (rB- mB-) gal dcm (DE3)
E. coli BL21 (DE3): F-ompT hsdSB (rB-, mB-) gal dcm (DE3)
E. coli TOP10: F- mcrA mcr (mrr-hsdRMS-mcrBC) □ 80lacZ □ M15 □ lacX74 recA1 araI-139 (ara-leu)7697 galU galK rpsL (StrR) endA 1 nupG
E. coli BL21(DE3)Star: F-ompT hsdSB (rB-, mB-) gal dcm (DE3)
E. coli Arctic Express: E. coli B-ompT hsdS (rB- mB-) dcm+ Tetr gal endA Hte [cpn 10 cpn60 Gentr] Bacillus megaterium strain YYBm1: Δ npnM, Δ xyIA.

(ii) List of vector(s)

Origin & function

All sequences will be PCR amplified

Evaluation of foreseeable effects

Although the GMO will be expressing the complete Type IV system, there are no known or recorded harmful effects to humans, animals and plants. There are no anticipated harmful affects from the growth or the use of these host/vector systems to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Negative pressure relative to the pressure of the immediate surrounds - not required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Considered in the risk assessment, see attached.
Considered at the 6th May 2015 College GM safety committee and it was agreed that it be forwarded to the HSE as a Subsequent Class 2 activity notification for their approval with the corresponding fee.

Please enter comments on the GM safety committee on the risk assessment

Considered at the 6th May 2015 College GM safety committee and it was agreed that it be forwarded to the HSE as a Subsequent Class 2 activity notification for their approval with the corresponding fee.

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<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2</td>
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<td>L3 L4</td>
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<tr>
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</tr>
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</table>

**Name**

ROSLIN INSTITUTE (EDINBURGH)

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

**District**

**Town**

ROSLIN

**County**

MIDLOTHIAN

**Postcode**

EH25 9PS

**Country**

SCOTLAND

**Tel Number**

0131 527 4200

**Fax Number**

0131 440 0434

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

<table>
<thead>
<tr>
<th>Date</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
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<th>Road Name</th>
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<th>Town</th>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
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<tr>
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Tick if confidential

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<th>Transgenic Animals</th>
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</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 162/00.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<td>Project notified under transitional arrangements</td>
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Withdrawn | N | Tick if notifying a connected programme of work | N |

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

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**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</table>

02/03/2022

Page 4152 of 15326
**Project Additional Information**

**Purposes of the contained use**

Purpose of this project is to optimise and assess the ability of hTERT to extend the proliferative lifespan of primary cultured fibroblasts.

**Recipient or parental organism**

Primary fibroblasts.

**Host/vector system**

The retroviral vectors, pBabehTERTloxP and pBabeCreHygro, based on Moloney Murine Leukemia Virus (MuLV) and were designed to be replication defective by deleting trans-acting viral structural genes pol and env. The vectors contain open reading frame of hTERT, which is flanked by loxP sites, or Cre gene as well as selecting markers, puromycin or hygromycin resistant genes. Packaging cell lines (both ecotropic and amphotropic) are required for infecting host cells.

**Origin & function**

The retroviral vectors, pBabehTERTloxP and pBabeCREHygro, are gift from Dr. Shay, Dr. Wright and Geron Corp. (Steinert et al 2000). In these vectors, hTERT cDNA and Cre gene are under the control of the viral LTR. Expression of hTERT in the primary cultured cells is expected to extend proliferative lifespan in these cells. Cre recombinase is going to be transiently expressed in hTERT stable-transfected cells to excise the floxed hTERT cDNA from the cells so that the cells no longer express...
hTERT protein after Cre expression. Selectable makers, puromycin or hygromycin are applied for selecting transfected cells.

Evaluation of foreseeable effects

The retroviral vectors used in this work are modified MuLV which are only able to replicate in a retroviral packaging cell line. The vector RNA genome is packaged into virus particles which bud off into the culture medium. The virus stocks produced by the packaging cells are also replication-defective.

However, even if containment of the virus failed, infection of human and animals is expected to cause very limited damage as the retroviruses require close contact for their transmission and their survival in the general environment is poor. Reports suggest that hTERT alone can only extend the lifespan of human primary cells but not transform them (Bodnar et al 1998; Jiang et al. 1999; Morales et al. 1999). Cre, puromycin and hygromycin do not have cytotoxic effects. The literature/data will be checked regularly for the changes in the risk associated with the inserts (e.g. hTERT) and the risk assessment will be updated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

a) Contaminated Pasteur pipettes and tips will be disposed into a plastic sharps-safe bin located in the tissue culture hood. On a daily basis, bins will be sealed, level 2 labelled, autoclaved and incinerated, 100% kill.
b) Contaminated plastic pipettes will be placed in tripled, clear, plastic biohazard bags. These will be sealed with tags and level 2 labelled, autoclaved and incinerated as a).
c) Aspiration of all fluids used within the dedicated tissue culture hood will fed directly into a flask with haz tabs tablets, such that the maximum dilution will be 1 tablet/litre fluid, producing 2500 ppm available chloride. Biological material will be inactivated overnight (100% kill), before discarding down the sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee has reviewed the risk assessment of the proposed activity and concurs with the assignment of the activity class 2. The laboratory identified is appropriate for the application of full level 2 containment and the use of a class 2 tissue culture cabinet will further reduce the hazard to the personnel involved. In addition, the waste management arrangements will ensure that risks to other workers and to the environment are reduced to negligible levels.

Project Containment

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02/03/2022  Page 4154 of 15326
The MoMuLVsup-1 will be used to measure the effect of HP1 deletion on the De Novo methylation machinery. Briefly, ES cells, which have been deleted for HP1 gene products, will be infected with the MoMuLVsup-1. The viral DNA will integrate into the host genome. In wild-type ES cells the viral DNA becomes De Novo methylated, which can be measured by Southern Blot analysis. We aim to determine the effect of deleting HP1 function on De Novo methylation of the introduced MoMuLVsup-1 viral DNA.

Recipient or parental organism
Mouse embryonic stem (ES) cells.

Host/vector system
The retroviral vector, MoMuLVsup-1 is based on the Moloney Murine Leukaemia Virus (MoMuLV). In this vector, the bacterial sup-F gene has been integrated into the LTR (Reik et al., PNAS USA 82, 1141-1145, 1987) which allows its detection in mouse genomic DNA. The virus is replication competent so makes its own coat proteins that are required for infecting cells.
Origin & function

The MoMuLVsup-1 vector is a gift from Dr En Li (See Hong et al., Development, 122, 3195-3205, 1996; Okano et al., Cell 99, 247-257, 1999). The vector will be used, as documented in Dr Li's papers above, to measure the De Novo methylation activities in ES cells. Briefly, 3T3 cells, which express the MoMuLVsup-1 virions, will be cultured in ES cell medium. Supernatants from these cultures will be used to infect ES cells whereupon the MoMuLVsup-1 DNA will integrate into the host genome. In wild-type ES cells the viral DNA becomes De Novo methylated, which can be measured by Southern Blot analysis.

Evaluation of foreseeable effects

The MoMuLVsup-1 retrovirus is replication-competent and the vector RNA genome can be packaged into virus particles that can bud off into the medium. Appropriate procedures (see 12) will be used to contain the virus. However, even if the containment of the virus failed, infection of animals is expected to be very limited, as the retrovirus requires close contact for transmission: their survival in the general environment is also poor. With the method described, this virus has been used in different laboratories for over 15 years without deleterious effects (Reik et al., PNAS USA 82, 1141-1145, 1987; Hong et al., Development, 122, 3195-3205, 1996; Okano et al., Cell 99, 247-257, 1999).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

(a) Contaminated Pasteur pipettes and tips will be disposed into a plastic sharps-safe bin located in the tissue culture hood. On a daily basis, bins will be sealed, level 2 labelled, autoclaved and incinerated. 100% kill.
(b) Contaminated plastic pipettes will be triple sealed in clear plastic biohazard bags. These will be sealed with tags and labelled, autoclaved and incinerated as in (a). 100% kill.
(c) Aspiration of all fluids used within the dedicated tissue culture hood will feed directly into a flask with haz tab tablets, such that the maximum dilution will be 1 tablet/litre fluid, producing 2500 ppm available chloride. Biological material will be inactivated overnight (100% kill), before discarding down the sink.

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee reviewed the risk assessment of the proposed activity and concurs with the assignment of the activity to class 2. The laboratory identified is appropriate for the application of full level 2 containment and the use of a class 2 tissue culture cabinet will further reduce the hazard to the personnel involved. In addition, waste management arrangements will ensure that the risks to other workers and to the environment are reduced to negligible levels.

Project Containment

02/03/2022
USE OF ADENO- (AD) AND ADENO-ASSOCIATED VIRAL (AAV) VECTORS TO INTRODUCE AND EXPRESS TRANSGENES IN CULTURED HUMAN AND MOUSE EMBRYONIC STEM (ES) CELLS.

We intend to use the B-galactosidase marker to optimise the ability of Ad and AAV to transfect human and murine ES cells. We then plan to investigate the possibility that the preferred integration site for AAV will prove to be an appropriate site for the expression of further transgenes by integrating a 'floxed' marker gene and replacing it with a second histological marker. In the event that Ad or AAV are useful for the reliable expression of transgenes then we will use this route for the introduction of genes which direct the differentiation of ES cells to provide therapeutically useful cell types. We also plan to establish if AAV carrying homology to endogenous genes will lead to integration by homologous recombination.

We will obtain replication incompetent viral vectors as follows:
(i) adenoviral (Ad) vectors designed to deliver and express marker genes to cultured cells
(ii) adeno-associated viral vectors (AAV) designed to deliver and express marker genes and/or genes designed to cause directed differentiation to cultured cells
(iii) Ad or adeno-associated viral vectors carrying a 'floxed' marker to facilitate integration of subsequent transgenes by site-specific recombination.
Adeno-associated vectors designed to target endogenous genes

Recipient or parental organism
Human and murine embryonic stem (ES) cells.

Host/vector system
Recombinant adenovirus - Ad 5
Non enveloped, icosahedral virions of 75-80nm diameter with double stranded 37kb DNA linear genome. Replication defective recombinants have E1 and E3 deletions and can only be produced in cell lines where E1 genes are supplied in trans, such as the 293 line, which contains 17% of the left-hand side of the adenovirus genome. There is a risk of recombination events between the virus and integrated DNA fragment during scale-up, leading to production of replication competent adenovirus (RCA). Therefore, preparations of the vector are checked for RCAs. In these vectors the transgene is under the control of the CMV promoter. These recombinants fall into ACGM containment level 2.

The virus is stable and can retain biological activity after extraction with ether and/or chloroform, or withstand exposure to room temperature for 3-8 weeks. The virus can be inactivated by heat inactivation (56 degrees C/1 hour) or exposure to Mikrozid solution (A. J. Beveridge, Cat: 2A63) for 30 minutes.

Health concerns
Adenovirus does not need to be replication competent to cause symptoms such as corneal or conjunctival damage. Symptoms may also include acute respiratory illness (cold-like symptoms), pneumonia, conjunctival infection (red eye), and corneal infection leading to scarring. Skin contamination should be washed repeatedly with soap and water, with eye infections being thoroughly washed in water.

Origin & function
Selectable marker transgenes are bacterial in origin and confer resistance in culture to drugs such as G-418. Their presence is to facilitate the cloning of colonies of transfected cells. Histological markers are either bacterial (B-galactosidase) or eukaryotic in origin (luciferase, GFP). Histological markers are used to visualise transfected cells in order to track their differentiation following engraftment. Transgenes likely to affect the pattern of differentiation of ES cells are predominantly of human or murine origin and may be under inducible transcriptional control. For example certain muscle regulatory factors (myogenin, myf5 myoD) are known to direct the differentiation of some cell types to muscle.

Evaluation of foreseeable effects
Drug resistance markers may include neo, puro, hyg or cat genes. Expression markers may include GFP, luciferase, B-galactosidase or dsRed. These reporter genes have been evaluated in transgenic mice, which express the markers without showing a phenotype. (For example, see Capecchi, M.R (1989) Altering the genome by homologous recombination. Science 244:1288-1292 and Okabe M. et al. (1997) Green mice as a source of ubiquitous green cells. FEBS Lett. 5: 407, 313-9). Other transgenes may include hTERT or hTR (RNA subunit of telomerase). These transgenes are expected to extend life span in transfected tissue culture cells and the consequence of viral release to the environment must be considered. If release occurs, which should be unlikely given the working practices described below, infection of humans and animals is expected to cause very limited damage. Reports suggest cultured human fibroblasts have extended life soabs vyt are bit trbsfrined as denibstrated vt ckassucak assats sygc as faukyre if cekks ti griw ub sft agar ir ub byde nuce (Morales, C. P. et al (1999) Absence of cancer-associated changes in human fibroblasts immortalised with telomerase. Nature Genetics 21:115-118 and Bodnar, A. G. et al (1998) introduction of telomerase into normal human cells. Science 279:349-352). None of these transgenes is expected to affect cell function or survival under normal culture conditions. Some modifications will be designed to affect cell survival or survival under normal culture conditions. Some modifications will be designed to affect cell survival in vivo. In some instances such as the expression of xenogeneic surface markers, the effect will be to reduce viability. In others, such as the expression of angiogenic factors, the effect may be to enhance cellular engraftment. In both cases however, the likelihood of cells gaining access to the human body accidentally is small and would be expected to elicit a rapid immune response that would destroy the contaminating cells. The possibility of infectious virus carrying these transgenes as a consequence of accidental co-transfection with wild type virus is small but must be considered. The particular concern would be the case of transgenes encoding factors to enhance engraftment which could, in principle, lead to phenotypic consequences.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

a) Contaminated Pasteur pipettes and tips will be disposed into a plastic sharps-safe bin located in the tissue culture hood. On a daily basis, bins will be sealed, level 2 labelled, autoclaved and incinerated, which gives 100% kill.

b) Contaminated plastic pipettes will be placed in tripled, clear, plastic biohazard bags. These will be sealed with tags and level 2 labelled, autoclaved and incinerated as (a).

c) Aspiration of all fluids used within the dedicated tissue culture hood will fed directly into a flask with haz tab tablets, such that the maximum dilution will be 1 tablet/litre fluid, producing 2500 ppm available chlorine. Biological material will be inactivated overnight (100% kill), before discarding down the sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee has reviewed the risk assessment of the proposed activity and concurs with the assignment of the activity to class 2. The laboratory identified is appropriate for the application of full level 2 containment and the use of a class 2 tissue culture cabinet will further reduce the hazard to the personnel involved. In addition, the waste management arrangements will ensure that risks to other workers and to the environment are reduced to negligible levels.

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Animal Units

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<th>Human Clinical Applications</th>
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02/03/2022
**GM Centre Number: 164**

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**Name**

UNIVERSITY OF THE WEST OF ENGLAND, BRISTOL (UWE BRISTOL)

**Name 2**

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**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**
  - [ ]

- **Give brief details of the genetic modification safety committee**

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**
  - [ ]

- **Tick if confidential**
  - [ ]

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
Virology

Mycology

Transgenic Animals

Transgenic Invertebrates

Transgenic Fish

Transgenic Plants

Gene Therapy

Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 164/05.1

<table>
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Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**
To assess the uptake of anti-tumour drugs by acute myeloid leukemia (AML) cell lines by measuring light output of virally transformed cells.

**Recipient or parental organism**
Well characterised human AML cell lines, including THP-1 and KG1a

**Host/vector system**
A third-generation lentiviral vector, such as pLenti6/V5-DEST (Invitrogen)

**Origin & function**
Genes encoding bacterial luciferase enzymes and a fatty acid reductase
Genes encoding fluorescent proteins, such as GFP.

**Evaluation of foreseeable effects**
The lentivirus produced with the pLenti 6/V5-DEST system is able to transduce primary human cells and therefore poses some risk (classified as hazard group 2). However, the packaged virus lacks viral structural genes and cannot produce replication-competent virus. The genes to be inserted are widely distributed in fluorescent organisms in the environment. The products are well-characterised with a history of safe use as reporters and no harmful biological activity has been reported. The modified virus is thus not expected to be more harmful than the unmodified vector.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All culture material, reusable glassware and disposable waste will be inactivated by autoclaving at 121°C for a minimum of 30 minutes. Each load will be monitored by the use of test strips. Autoclave function is independently monitored by an external contractor on an annual basis.

**Is an emergency plan required according to regulation 20?**

Y

**Tick to confirm that it is attached to this form**

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
The risk assessment is required for a new part of an ongoing project which will involve the use of lentiviral vectors […] explained the background to this new area of work and the reasons for using these viral vectors. […] commented that the vectors described could not produce viable virus. They would be able to produce the DNA provirus, but not viral RNA. […] asked for clarification of the description of the packaging cell line. The risk assessment submitted did not fully described the E. coli strains to be used in this work; these should be included. […] questioned a sentence under disposal of waste that described the use of settle plates to monitor for contamination. It was agreed that this was not necessary or appropriate as a routine procedure. Since the project involves collaboration with another institution, it was felt that transport of materials should be considered and the procedure(s) to be followed should be detailed under section 2(d). The committee approved the risk assessment subject to the suggested changes being made to the satisfaction of the chair.

### Project Containment

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### Project Ref 164/07.1

**CU2 Project Title**

Silencing and over-expressing hormone-and stress-related genes in potato (Solanum tuberosum L.).

**Non-GMM** Grant

Consent Granted: Not Applicable

**Project notified under transitional arrangements**

N

**Project Additional Information**

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Purposes of the contained use

To study the effect of silencing or over-expressing genes involved in hormone- or stress-signalling in potato plants grown in sterile culture.

### Recipient or parental organism

- Laboratory strains of *Escherichia coli* K12
- Laboratory strains of *Agrobacterium tumefaciens*
- *Solanum tuberosum* plants grown in vitro

### Host/vector system

Plasmids pGR106/7, which are non-mobilisable binary vectors for transformation of plants using *Agrobacterium tumefaciens* and which obtain sequences derived from *potato X* (PVX) isolate UK3.

### Origin & function

Initially, we intend to silence brassinosteroid signalling genes from potato, but in future we wish to use other hormone- and stress-related genes where no change in PVX host range or pathogenicity can be predicted and where no altered interaction with toher plant pathogens is expected. These might include: plant genes encoding proteins involved in phytohormone biosynthesis, perception, signal transduction and metabolism, for example, enzymes that catalyse phytohormone biosynthetic reactions, phytohormone receptors, positive and negative regulators of phytohormone signal transduction, plant genes encoding proteins involved in stress perception, signal transduction and responses, for example, heat shock proteins, proteins up- or down-regulated after stress.

Genes encoding GFP (Green Fluorescent Protein) and PDSas (Phytoetene Desaturase) will be used as controls.

### Evaluation of foreseeable effects

The *Agrobacterium* constructs to be produced will be capable of generating modified potato Virus X (PVX), (derived from strain UK3) when they infect a susceptible plant. PVX is a plant pathogen that infects solanaceous crops such as potatoes and occurs naturally in the UK. Plants deliberately infected with *Agrobacterium* constructs in the laboratory will produce viable PVX, which could be transmitted mechanically to other susceptible plants in the absence of control measures. However, potato crops are not grown in the immediate vicinity of the laboratory or growth rooms. In the case of gene-silencing constructs, expression of endogenous plant genes might be altered in recipient plants. It is possible that this might affect the growth or survival of the infected plant, but the predominant effect is likely to be the disease caused by the virus.

There is no reason to suppose that the host range or infectivity of the modified virus will be altered compared to the recipient strain.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The plants will be cultured in vitro, in tissue culture vessels maintained in growth cabinets in a restricted access area within the faculty. When needed, the GM plants inside tissue culture vessels will be transported to the laboratory in sealed boxes via a specified route. A class 2 laminar flow hood will be used when formicpropogating the plants. This is required to ensure sterility during tissue culture and will be sufficient to control the risk to the environment. Disposable gloves and sterile material will be used when manipulating the plants. All non-disposable materials will be autoclaved before re-use. All plants will be transferred to autoclavable bags and autoclaved before disposal. All waste, including gloves, that has possibly been in contact contaminated plant material will be autoclaved before disposal. Used tissue-culture vessels will be autoclaved before cleaning. The working surfaces will be disinfected with Virkon S after the manipulation of infected plant material.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All plant material, bacterial cultures, re-useable glassware and disposable waste will be inactivated by autoclaving at 121°C for a minimum of 30 minutes. An autoclave is situated in the building and waste will be transported to the autoclave in autoclave bags contained in robust containers with lids. Each load will be monitored by the use of
test strips. Autoclave function is independently monitored by an external contractor on an annual basis.

(…) gave a short summary of the proposed work. The committee agenda agreed the main hazard was the possibility of the virus escaping and infecting another plant. The virus can be mechanically transmitted, but (…) explained that this was not a very efficient process. The transgenic plants would be grown in tissue culture vessels and would be likely to die unless very carefully transferred to soil due to humidity change. The most likely route of transmission of virus was thought to be via escape of the Agrobacteria containing viral sequences. The committee felt that the overview did not adequately explain how the virus was produced. Another point raised by members of the committee was whether the Agrobacterium strain was disarmed. (…) told the committee that it does not have the genes to cause crown Gall. (…) then went on to explain that although sharps wont be used with bacteria as stated on the risk assessment, scapel will be used when manipulating the plants. This was thought not to be an issue as PVX does not infect humans. The Chair went on to raise the issue of transporting the plants from the undercroft to the labs. This should be in sealed boxes via the route described for the transport of hazardous materials in the faculty. The Chair suggested that the risk assessment should say that a class 2 sterile hood would be used when opening the tissue culture vessels and handling the plants. The committee agreed that the work could be carried out safely under class 2 containment and approved the risk assessment subject to the changes noted and correction of typographical errors.

Please enter comments on the GM safety committee on the risk assessment

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Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 Yes L3 L4</td>
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</table>

Animal Units

<table>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tbody>
<tr>
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Project Ref 164/16.1

<table>
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<th>Date Project Ceased</th>
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<td>&lt; 1 Litre</td>
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<td>Non-GMM</td>
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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

To build a lenti-viral knock-down rescue systems to study metabolic signalling mechanisms in health and disease.

**Recipient or parental organism**

| a. | IMR-32 neuroblastoma cells. Human cells. Widely used and considered biosafety level 1. |
| b. | SH-SYSY neuroblastoma cells. Human cells. Widely used and considered biosafety level 1. |
| c. | hCMEC/D3 immortalized endothelial cells. Human cells. Widely used and considered biosafety level 1. |
| d. | L6 myocytes. Rat cell line. Widely used and considered biosafety level 1. |
| e. | MIN6/INS1. Mouse insulinoma cell lines. Widely used and considered biosafety level 1. |
| f. | HEK293TN. Human cell line. HEK293TN producer cell line is optimized for effective, high titer production of pseudoviral particles. The cell line has been transformed with the SV40 large T antigen to promote robust growth. The Neomycin resistance marker is also featured in the 293TN cell line for stable propagation. Biosafety level 2. |

**Host/vector system**

| a. | Tet-pLKO-puro – third generation lentivirus backbone plasmid. This plasmid cannot produce lentiviral particles without co-transfection with a minimum 2 packaging plasmids in mammalian cells. The plasmid confers resistance for mammalian cells to puromycin/neomycin with the Pac gene. Plasmid also contains Amp gene for bacterial ampicillin resistance. The risk of spontaneous viral generation is very low with the 3rd gen system as: |
| i. | HIV-1 gene number is reduced and separated (requires 4 recombination events for spontaneous generation); to date there have been no reports of spontaneous expression. The National Gene Laboratory (NGVL) has produced over 60 liters of HIV-1 vector and has screened supernatant and cells from different vector systems, using different assays, without detecting recombination events. |
| ii. | The expression vectors are not self-replicating - TAT is removed totally. |
| b. | pXLG3 – a third generation lentivirus backbone plasmid. This plasmid cannot produce lentiviral particles without co-transfection with minimum 2 plasmids in mammalian cells. The plasmid confers resistance for mammalian cells to puromycin/neomycin with the Pac gene. Plasmid also contains Amp gene for bacterial ampicillin resistant. |
| i. | The vector is self-inactivating – it contains a 400bp deletion in the 3'LTR, removing the TATA box and promoter activity, in common with other 3rd generation plasmids. |
c. pCMVR8.91, the packaging plasmid, in which the cytomegalovirus (CMV) immediate early gene promoter controls the expression of HIV-1 genes gag, pol (necessary for the nuclear transport of the HIV-1 pre-integration complex), tat and rev (both required for viral expression by transcriptional and post-transcriptional mechanisms).

d. pMD.G2, encoding the VSV-G coat protein, flanked by β-globin regulatory sequences, under the control of the human CMV immediate early promoter.

Note that the transfer vectors (pLKO, pXLG) are 3rd generation, whereas the packaging and envelope vectors are 2nd generation. This compromise allows the high efficiency of virus production of the 2nd generation system, whilst also maintaining the safety of the self-inactivating 3rd generation transfer vectors.

**Origin & function**

The function of the genes to be expressed or knocked down is as follows: BCAT1 or BCAT2 gene (branched chain amino acid metabolism); UBC9, SENP1-7, SUMO1-3 (regulators of protein SUMOylation); syntaxin-1/3/4, SNAP-25, Munc18 (secretory proteins); GLUT4 (glucose transporter); Neto1/2 (K+ channel regulators), GFP, mCherry, SyGCamp (fluorescent marker proteins).

**Evaluation of foreseeable effects**

The most hazardous GMO are the lentiviral particles generated. These will only be generated during a closely monitored set timepoint; spontaneous generation is extremely unlikely to occur by accident as vector formation requires transfection of an additional 2 plasmids. Random formation and recombination events resulting in infectious virus have yet to be reported despite extensive attempts.

When lentiviral particles are generated full PPE (class 2 microbiological safety cabinet, nitrile gloves, and a lab jacket) will be worn and a class 2 biological safety environment will be maintained throughout the experiment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Full PPE (class 2 microbiological safety cabinet, nitrile gloves, and a lab coat) will be used. All GMM viral manipulations will be contained within a class 2 microbiological safety cabinet. All microbiological safety cabinets are serviced and calibrated by British Clinical Services on a 6th monthly interval. All work will be performed in accordance to Biological Containment Level 2.

All work surfaces will be sprayed with 70% ethanol before work commences. All bottles and gloves when introduced or removed from the biological safety cabinet will be sprayed with 70% ethanol.

Discard containers containing 1% virkon will be used in the microbiological safety cabinet for discard of pipette tips and microcentrifuge tubes. Users will ensure all pipette tips and microcentrifuge tubes are fully submerged into the 1% virkon. Discard containers will be left overnight to ensure 100% kill and changed each morning before work commences.

Liquid waste generated will be discarded into clean duran bottles and autoclaved using a standard, calibrated discard programme (121 degrees Celsius, 15psi for 15 minutes) ensuring 100% kill.

Solid waste (pipettes and plastic cell culture flasks, bacterial agar plates) will be deposited in a biohazard autoclave bag, placed in a biohazard bin and autoclaved. A fresh autoclave bag will be used for each experiment involving lentiviral material. At the conclusion of each experiment waste will be autoclaved immediately.

All surfaces will be disinfected with 1% virkon at the end of the procedure to achieve 100% kill of any remaining microorganisms and viral particles. Small spillages will be treated with 1% virkon. A large volume (excess of 50ml) spill kit is available containing powdered virkon, absorbant material, face mask and gloves.

Cell lines transduced using the lentiviral protocol will be monitored for the presence of remaining virus by pipetting cell media onto un-transduced cells and visual monitoring (active viral products produce GFP) and ELISA assays. This will ensure the absence of active virus from transduced cells used in subsequent experiments.
The GMSC met to consider the risk assessment entitled ‘Lentiviral induction of mammalian cell lines’ submitted by Dr Tim Craig (TC). The RA was presented by TC and the committee asked for clarification on a number of points. Overall although the GMSC considered most aspects of the RA to be class 1, because some 2nd generation lentiviral packaging and coat protein plasmids being used the conclusion was to assign this RA as class 2. TC had completed part 2 of the RA identifying any potential risks and describing the measures taken to reduce these and the GMSC were satisfied with this.

After some minor corrections were addressed the RA was accepted as class 2 and the UWE RA number 2016.1 assigned to it.

Prof. Dawn Arnold, GMSC chair.

Please enter comments on the GM safety committee on the risk assessment

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Prof. Dawn Arnold, GMSC chair.

Project Containment

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Animal Units

L2 | L3 | L4 | L2 |

Large Scale Activities

L3 | L4 | L2 |

Human Clinical Applications

L3 | L4 |

Project Ref 164/96.1

Date Ackn’d CU2 Project Title

21/02/1996 CONSTRUCTION OF PARTIAL GENE LIBRARIES FOR PSEUDOMONAS SYRINGAE PATHOVARS

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted

not applicable

Tick if notifying a connected programme of work

N

Project notified under transitional arrangements

Y

Historical Significant Changes

GM164/98.3,
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
# Project Containment

## Laboratory Activities

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## Glass Houses

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## Growth Rooms

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## Large Scale Activities

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## Human Clinical Applications

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## Project Ref

**164/98.2**

### Date Ackn'd

02/11/1998

### CU2 Project Title

LUX GENES FOR MONITORING THE VIABILITY AND METABOLIC ACTIVITY OF BACTERIA

### Class

Class 2

### Culture Vol

Class 2 CultureVol

### Consent Granted

not applicable

### Withdrawn

N

### Tick if notifying a connected programme of work

N

### Project notified under transitional arrangements

Y

---

## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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GM Centre Number: 165

Data Premises Notified (Originally) 24/09/1984

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed

Transitional Premises N

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

KENNEDY INSTITUTE OF RHEUMATOLOGY AT IMPERIAL COLLEGE

Name 2

KENNEDY INSTITUTE OF RHEUMATOLOGY

Department

FACULTY OF MEDICINE

Campus Estate or Research Centre

Building

Road Name

1 ASPENLEA ROAD

District

HAMMERSMITH

Town

LONDON

County

GREATER LONDON

Postcode W6 8LH

Country ENGLAND

Tel Number 0208 383 4444

Fax Number 0208 388 1027

E-mail

HSE Division LONDON

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>Imperial College</td>
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<td>Infectious Diseases</td>
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<td>150 Du Cane Road</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 165/01.1

Date Ackn'd 15/02/2001
Date Project Ceased 31/05/2002

CU2 Project Title
ENDOGENOUS AND EXOGENOUS PROCESSING PATHWAYS IN THE PRESENTATION OF DIFFERENT EPITOPE REPERTOIRES TO T CELLS

Class Class 2
CultureVolClass2 < 1 litre
CultureVolumeClass3-4

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
**Project Additional Information**

**Purposes of the contained use**

To generate professional antigen presenting cells which process thyroid peroxidase (TPO) via the endogenous rather than the exogenous pathway, to examine the repertoire of TPO epitopes presented by these cells, and their interactions with TPO-specific T cells.

**Recipient or parental organism**

Baby hamster kidney (BHK-21) cell line. This cell line is permissive for the replication of the MVA vector, and will be used for large scale preparation of recombinant MVA-TPO vector. Dendritic cells established from anonymous donor blood packs. EBV-transformed B cell lines (LM and CX81).

**Host/vector system**

The vector system is Modified Vaccina Ankara strain (MVA), a highly attenuated strain of vaccinia virus which is unable to replicate in most mammalian cells, and has a history of safe use as a smallpox vaccine. MVA is regarded as a single round expression vector with little potential to spread.

**Origin & function**

Thyroid peroxidase (TPO) is a membrane-bound protein expressed by thyroid epithelial cells, and is a common autoantigen in auto-immune thyroiditis. Mouse TPO cDNA will be introduced into the MVA vector by means of homologous recombination in BHK-21 cells. Recombinant vector will be prepared by sequential plaque purification, followed by ultracentrifugation of BHK-21 cell lysates. The vector will be used to induce the expression of full length TPO protein in professional antigen presenting cells (either primary human dendritic cells or established B cell lines).

**Evaluation of foreseeable effects**

There is a hypothetical possibility that the accidental infection of an operative with the recombinant TPO-expressing vaccinia virus might provoke an autoimmune reaction targeting the thyroid. In a worst case scenario this could lead to hypothyroidism. The MVA vector is highly attenuated, so that there is no means of propagation of an infection. MVA alone provokes a relatively poor immune response to exogenously expressed antigens, and current vaccinations protocols combine MBA with a DNA immunisation step. The local rules for the use of vaccinia virus expressly forbid the use of sharps, and impose strict conditions for the prevention of aerosol release. The possibility of harm to human health is therefore regarded as remote. No infection of animals with recombinant virus is proposed, and contaminated waste will be disinfected in a manner known to inactivate the virus. There is therefore no significant risk to the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: Disinfection by exposure to Microsol3 at a final concentration of >2% for a period greater than 2 hours. The disinfecting reagent is certified by the manufacturers to inactivate pox-viruses under these conditions (effectively 100% kill). Disinfected liquid waste is washed down the sink.

Solid waste: Solid waste (in double thickness biohazard bags) is collected from the contained use site each day and autoclaved immediately at 134oC for 15 minutes. These conditions will achieve 100% kill. The autoclave is subject to a maintenance contract with the manufacturers, with 2 maintenance visits and 1 full KI discus test per year. Autoclaved waste is collected and disposed of by a licensed waste disposal company.
The risk assessment has been rigorously examined by the local Genetic Modification Safety Committee over a period of several months, and during this time has undergone extensive modification. The GMSC was concerned about the possibility of thyroid auto-immunity following accidental infection with the recombinant virus. Ultimately, it was agreed that the risk of this occurrence was small, but could not be discounted entirely. On the grounds of this theoretical risk it was agreed that the work should be classified as a Level 2 operation, and that local rules should provide sufficient protection for operatives and the environment. The local rules for vaccinia virus work are appended.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>Human Clinical Applications</td>
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Project Ref 165/05.1

Date Ackn'd: 27/09/2005
CU2 Project Title: Developing viral vaccines to protect against microbial disease.

Class

Consent Granted

Non-GMM: Not Applicable

Project notified under transitional arrangements: N

Historical Significant Changes

Withdrawn: N

Tick if notifying a connected programme of work: N
The principal goal of this project is to develop a vaccine to protect against microbial diseases. Vaccination is suboptimal for several diseases, such as viral infections (Influenza) and cancer. Several previous approaches to vaccination have only been effective in a percentage of the population. To fully immunise the elderly, young children and other susceptible populations, new approaches to vaccination must be explored. Vaccination with a non-replicating adenoviral vector provides the opportunity to induce strong protective immune responses while remaining relatively safe to the vaccinee. Therefore, this approach may provide the opportunity to develop an effective vaccine that will induce good immune responses across all members of the population.

Through the use of these GM Adenoviruses we may be able to develop a vaccine that is effective against disease in all target populations.

A non-replicating adenovirus will be used for vaccination.

The virus is genetically modified with commercially available and laboratory constructed vectors from the gateway system of cloning (Invitrogen). All adenoviral constructs are deleted at both the E1 and E3 regions, and are hence replication deficient. Recombinant adenovirus genomes, purified from a K12 strain of E. coli, are transfected into the packaging cell line HEK293, virus will replicate and then plaque purification is performed. A large scale grow-up is carried out, the construct is validated by PCR and western blotting, and the presence of replication competent adenovirus is checked by a plaque-forming assay using human skin fibroblasts.

These adenovirus constructs will express an antigen from a disease causing microorganism, such as influenza. The function of the genetic material from the microorganisms is to induce an immune response, but will not include toxins or other molecules that can be directly detrimental to the health of humans and other animals.

The normal tropism of adenovirus is for the upper respiratory tract; however, it is able to infect a variety of other cell types in vivo and in vitro. This virus can cause minor respiratory disease but because of the mechanisms of viral attenuation it cannot form a pool of replicative virus. Therefore disease in humans is minor. The principal routes of accidental infection are needlestick injury or aerosol production. The microbial antigenic determinants may generate an immune response in humans if exposure occurs, but this response will likely be beneficial rather than detrimental.
The antigens have a remote possibility of causing allergic responses if the recipient has been exposed previously and has become allergic. There are no direct hazards arising from the alteration of existing traits of the adenovirus. However if mobilisation of the genome between adenovirus strains occurs these inserts could be expressed by other replicative adenoviruses. These new replicative viruses could then affect immune responses through the expression of the inserts as detailed above and in 17, however this is unlikely. Mobilisation of adenovirus requires the complementation of the deleted E1 and E3 functions or recombination with endogenous adenoviral sequences to restore these to the genome. Complementation of the virus could theoretically occur following the infection of the adenovirus into hosts. However if recombination does occur, the inserted gene products will most likely be deleted due to the packaging restraints of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste
Treated by: Addition of Microsol3 to a final concentration of 10%, incubation for >1hr.
Kill is effectively 100%
This treatment is used as per the manufacturers instructions, the effectiveness of the reagent is checked on any change in procedure.
Disposal by: Sinks (down the drain after inactivation).

Solid waste
Treated by: autoclaving at 134°C for 3 minutes under vaccuum.
Kill is effectively 100%
This treatment is used as per the manufacturers instructions. A temperature recorder is attached to assure that the optimal temperature is achieved for each run. In addition, the manufacturer checks the operating parameters of the autoclave yearly by contract.
Disposal by: Waste is collected for incineration by PHS.

Animal waste
Treated by: Waste is soaked in 1% Virkon overnight and are stored at 4°C before maceration.
Kill is effectively 100%
This treatment is used as per the manufacturers instructions, the effectiveness of the reagent is checked on any change in procedure.
Disposal by: Macerated waste is disposed of down an approved drain.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y
Approved for use subject to HSE approval.
The antigen used here to induce the immune response to the pathogen has been previously used as a vaccine.

Project Containment

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Project Ref 165/10.1

Date Ackn’d 07/04/2010
Date Project Ceased 21/07/2011
Project notified under transitional arrangements N

CU2 Project Title
Lentiviral expression systems as a tool for studying intracellular signalling pathways in cells of the immune system

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 ≤ 1 Litre
Non-GMM Consent Granted

Purposes of the contained use
This is a study undertaken in order to investigate the mechanisms of pro and anti-inflammatory signaling in immune cells such as macrophages, dendritic cells and lymphocytes.
We have previously generated lentivirus particles using 3rd generation transfer vectors and a 3rd generation packaging system. This system is designed to minimise the
possibility of replication competent virus particles arising during lentivirus production, where the gag/pol and rev gene products are expressed from separate plasmids that are co-expressed with the envelope (VSVg) vector following co-transfection with the lentiviral backbone vector into packaging cells (KEK293T/17). Substitution of the HIV-1 envelope protein with the Vesicular Stomatitis Virus envelope glycoprotein (VSV G) not only results in the production of a higher virus titre with a significantly broadened host cell range but also contributes to an enhanced safety of the system. The resulting retrovirus is replication-defective. However, the improved safety features of 3rd generation packaging systems are off-set by considerably lower virus titre yields obtained with this configuration. Therefore to improve viral titres we obtained a 2nd generation packaging system from the Trono Lab (Switzerland). In this case, all the auxilliary genes are expressed from one plasmid, which also incorporates the transactivator gene tat (absent in the 3rd generation system), namely psPAX2 or PDCMV8.91. Together with the envelope protein encoding plasmid (pMD2.G) these 2nd generation packaging systems are compatible with packaging of 3rd generation lentiviral transfer vectors containing SIN LTRs and are widely used to produce small scale amounts of lentiviral particles of in vitro transduction of primary mammalian cells.

Viruses will be assembled and packaged in the HEK293T/17 cell line and the conditioned supernatants (containing mature lentivirus) will be concentrated by centrifugation and titred prior to use in experiments. Virus titres will be determined using a FACs-based assay using either GFP fluorescence assay or labelled tNFGR (truncated nerve growth factor receptor) marker genes to calculate the amount of lentivirus infectious units. This will be carried out in HT1080, Jurkat and SupT1 for 3-6 weeks and monitoring the culture supernatants for p24 expression by ELISA. A positive control for p24 protein will be incorporated in this assay.

Once generated, lentiviruses will be used to infect a range of cell lines and primary cells over a range of MOI (multiplicity of infection). In the first instance we plan to study innate immune receptors, including TREM-1, DAP12, CD38, CD164, transmembrane protein123 (TMEM123), TLRs, tyrosine kinases., IRF family membesrs, NFKB signaling components (e.g. Myd88), p38 MAP kinase, and reporter genes such as TNF-luc, IL-6-luc, Stat3-luc. All these targets represent non-oncogenic, non-secreted cell components.

Recipient or parental organism

- 293T/17 cells (highly transfectable cell line derived from the 293T cell line established from human embryonic kidney cells)
- RAW 264.7 (murine macrophage line)
- HeLa (human epithelial carcinoma cell line)
- HT1080 (human fibrosarcoma cell line)
- MonoMac6 (monocytic cell line)
- SUP T-1 (human T Lymphoblast cell line)
- HUVECS (Human Umbilical Vein Endothelial Cells)
- Primary human monocytes, acrophages, dendritic cells osteoclasts & lymphocytes derived from elutriated blood packs
- Cells derived from dissociated human synovial membrane tissue taken from rheumatoid arthritis or osteoarthritis patients (primarily fibroblasts, monocytes, macrophages, dendritic cells & lymphocytes)
- Alveolar macrophages from sarcoid patients

Host/vector system

- pENTR/U6 vector
- pENTR/4.1 vector
- pRRLsin.PPTshCMV.GFPpre vector
- pCMV-GIN-ZEO gateway vector
- pCMV-GIN-ZEO gateway rc vector
- pCMV-GIN-ZEO 234 gateway
- pLentiPPT-DEST
- p156RRsinPPT.hCMV-GFP.WPRE (LV#5)
- LV#5GR (gateway adapted LV#5)
- pLenti6/BLOCK-IT-DEST vector
- pLP1 (packaging vector)
- pLP2 (packaging vector)
The gene and promoter sequences used are of human and rat origin. We have constructed lentiviral transfer vectors encoding (1) reporter genes (e.g. TNF-luciferase); (2) cDNAs (e.g., human Foxp3, p38) and; (3) shRNAs (p38):

(1) Reporter genes will be used to measure promoter activities of the TNF, IL-6 and Stat-3 genes in primary human immune cells (monocyte/macrophages, T cells). Constructs will contain the promoter(s) and deletion mutants thereof in the presence and absence of the 3' UTR (untranslated region). These experiments will be short-term and result in destruction of target (infected) cells. We will also perform short-term (8-hour) co-culture experiments with adenovirus-infected primary human macrophages (expressing TNF-luciferase reporter genes) and primary human CD4 T cells transduced with Foxp3-expressing lentivirus. Macrophages will be infected with adenovirus for 1 hour and cultured for 24 hours in the absence of virus. Similarly, VD4 T cells will be transduced with lentivirus 14 days prior to co-culture and washed extensively prior to co-culture. The end-points for these experiments will necessitate cell lysis, resulting in destruction of the target (infected) cells. Lysates will be frozen prior to subsequent luciferase activity determination.

(2) Short-term (<4 weeks) transgene expression in human and rat immune cells using lentiviral vectors will be employed to study the role of human genes Foxp3, TREM-1, DAP12, CD38, CD164, transmembrane protein 123 (Tmem123), TLRs, tyrosine kinases, IRF family members, NFKB signaling components (e.g. Myd88), p38 MAP kinase and rat CD16A.

Immortalized human cell lines will be transduced and selected for stable expression of Foxp3. Such cell lines will be tested for replication competency using the p24 ELISA method, and all handling and disposal will be as outlined in the associated GM risk assessment.

(3) Lentiviral vectors expressing shRNA sequences (targeting the same genes as in (2) - above) and embedded in the miR30 backbone will be employed to perform specific gene knockdown in target cell populations.

The above-mentioned gene sequences will be inserted into lentiviral vectors in order to infect human and rat cells of the immune system.

Evaluation of foreseeable effects

None of these gene inserts transfer vector(s) should present a hazard. One of the transgenes, Jund, is a member of the proto-oncogene Jun family however it is an atypical
member as it has a negative effect on cell proliferation and thereby poses little risk (Hernandes et al., 2008; PGM081, section2).

The WPRE sequence located in transfer vectors encodes the potentially oncogenic X-protein sequence. There is a risk of recombination with other viruses and/or latent viral sequences in the target cells although the lentivirus produced is replication incompetent. Routine testing for replication competency of all virus produced will be performed to assess for this possibility and any such virus will be destroyed.

The use of Class II microbiological safety cabinets for II procedures involving the handling of lentivirus vectors will minimize the risk of accidental exposure to workers should a replication competent lentivirus arises during virus production and/or use.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: inactivation by the addition of Microsol3 to a final concentration of 10%, or 1% (final concentration) Trigene/Virkon, as per manufacturers’ instructions. The effectiveness of each reagent is checked on any change in procedure. Inactivated waste will be disposed of via sink into the drains.

Solid waste: plasticware such as plates, culture flasks, are double bagged and either autoclaved at 136º C for 6 minutes under vacuum. The run number is recorded and chart paper traces and printouts kept. The autoclaves are serviced bi-annually and validated yearly by contract using representatives dummy loads in order to check that all areas of the load are reaching the required temperatures. Autoclaved waste then proceeds via the Imperial College clinical waste route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Part 1: PI CID number missing and the GM Centre is: GM165.
1.22: Change last strain to: Murine bone-marrow-derived cells.
1.24: State clearly which of the vectors contain the WPRE elements.
1.25: Third before last paragraph, Typo: 'tehse'
1.3: Please answer the questions in this section
2.1.1.2: Associated hazards: The WPRE element has oncogenic potential and then there is the risk of recombination with other viruses. Although the vectors are replication incompetent.
2.1.1.3: Explain why the answer is 'no'
2.1.1.4: Answer is 'n/a'
2.1.1.5: Answer: not altering existing traits.
2.1.1.6: Answer: None associated.
2.2.1.1, 2.2.1.2 and 2.2.1.3: Explain why the answer is 'none'
2.2.1.4: Answer: None, recombination incompetent
2.3.1.1: Change answer to "Working with handling of viral vectors containing WPRE".
2.3.2.2.b: Change answer to Yes. Also provide details.
2.3.3.2.b: Question is unanswered
2.3.3.3.b: Explain answer
2.3.3.3.d: Leave aerosols to settle first, then disinfect and finally use soapy water.
2.3.3.4.a: Typo: 'tehn'
2.3.3.5, 2.3.3.6 and 2.3.3.7: Questions are unanswered.
2.3.3.8.b: Remove 'Latex gloves'
2.3.3.8.c: Change answer to "No other PPE is used".
2.3.6: Tick box: 2
Part 3: Fill in Tick box.
3.1.1.a: Waste container should be 'sealed' and remove reference to sharps.
3.1.1.b: Change Autoclave cycle details to 136°C for 6 minutes.
Change 'temperature recorder' to 'print out'
Location of backup is: Basement and BSU, in event of a breakdown list the secondary autoclave.
3.2.a: Change answer to, "tubes carried in a rack".
3.31.a: Answer is 'None', delete the answers in rest of the section.
3.32: Answers are: n/a
4: Answer is 'no'
3.5.a: Spillage during transport: disinfect first.
3.5.b: Skin surface contamination: Use microsol wipe, then soapy water.
Medical intervention/prophylaxis: Change answer to section 2.3.5 of the form.
3.7.1: Remove staff that have left and add the CID numbers of those missing.
3.7.2: Question is unanswered: cleaners etc...
Agreed Class 2, Containment Level 2. This project is notifiable to the HSE.

All of the comments of the two committees have been addressed and the requested amendments incorporated into the risk assessments forwarded with this form.

Project Containment

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02/03/2022
Project Ref 165/97.2

Date Ackn’d 08/10/1997
CU2 Project Title Investigation of intracellular signalling in human monocytes

Date Project Ceased 21/07/2011
Class Class 2
Culture VolClass2 Class 2
Consent Granted not applicable

Historical Significant Changes GM165/00.1, GM165/02.1, Transferred to GM553 21/07/2011
Historical Date of Additional Info 14/12/1999, 24/07/2002

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 165/98.1

Date Ackn’d 18/06/1998

CU2 Project Title ADENOVIRUS-MEDIATED GENE THERAPY OF COLLAGEN-INDUCED ARTHRITIS

Class 2

Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022 Page 4186 of 15326
Date Project Ceased
21/07/2011

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements Y

Historical Significant Changes
GM165/02.2 Transferred to GM553 21/07/2011

Historical Date of Additional Info
24/07/2002,

Withdrawn N

Tick if notifying a connected programme of work N

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form N
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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02/03/2022
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(Originally) 09/11/1984

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### Name
UNIVERSITY OF SHEFFIELD

### Name 2

### Department

### Campus Estate or Research Centre

### Building

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### E-mail

### HSE Division
YORKSHIRE AND NORTH EAST

### Comments

### Date at Which Additional Info Submitted
02/03/2022
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02/03/2022 Page 4192 of 15326
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  

Tick if confidential

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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Please enter comments of the GM safety committee on the risk assessment

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Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 168/00.3

Date Ackn'd 12/09/2000

CU2 Project Title IDENTIFICATION OF NOVEL APOPTOSIS INHIBITORS BY EXPRESSION CLONING

Class 2

Non-GMM Consent Granted not applicable

Date Project Ceased
Tick if notifying a connected programme of work

Project notified under transitional arrangements

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 168/01.1

**Class CultureVolClass2 CultureVolumeClass3-4**

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**Non-GMM Consent Granted** not applicable

**Project notified under transitional arrangements** Y

**Withdrawn** N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Project notified under transitional arrangements**: Y

### Historical Significant Changes
- **Historical Date of Additional Info**: Not applicable

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 168/01.11

Date Ackn'd 06/02/2001

CU2 Project Title

THE IDENTIFICATION OF AUTOANTIGENS IN HUMAN DISEASE THROUGH THE USE OF A PHAGE-DISPLAY CDNA LIBRARY CLONING STRATEGY

Date Project Ceased

Class

Consent Granted

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Animal Units

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Large Scale Activities

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Human Clinical Applications

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**Project Ref** 168/01.12

**Date Ackn’ed**

06/02/2001

**CU2 Project Title**

IMMORTALISATION OF HUVE CELLS BY RETROVIRAL TRANSFER OF THE HUMAN TELOMERE REVERSE TRANSCRIPTASE GENE

**Class**

Class 2

**Culture**

VolClass2

**Volume**

Class3-4

**Non-GMM**

not applicable

**Consent Granted**

Project notified under transitional arrangements

**Withdrawn**

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

---

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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### Project Reference: 168/01.13

**Date Ackn'd:** 06/02/2001

**CU2 Project Title:** STUDY OF THE GROWTH HORMONE RECEPTOR TRAFFICKING AND SIGNALLING USING ADENOVIRAL - MEDIATED TRANSFECTION INTO PERMANENT CELL LINES

**Class:** Class 2

**Consent Granted:** not applicable

**Project notified under transitional arrangements:** Y

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#### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

Tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 168/01.14

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<td>ADENOVIRAL TRANSDUCTION OF PRIMARY MACROPHAGES WITH SENSE AND ANTI-SENSE HYPOXIA-INDUCIBLE FACTORS (HIF)</td>
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<td>Class 2</td>
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Non-GMM Consent Granted

Consent Granted: not applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

02/03/2022  Page 4205 of 15326
## Project Additional Information

### Purposes of the contained use

The aim of this project is to determine the role of macrophages in tumour angiogenesis, particularly the role of HIF in the hypoxic induction of the pro-angiogenic activity of macrophages. It is not possible to transfect human macrophages using conventional techniques but these cells have been efficiently transduced with adenovirus.

### Recipient or parental organism

Replication-defective adenovirus will be produced in the human kidney endothelial cell line HEK-293 after conventional transfection. The virus will then be used to transduce primary macrophages which will be co-cultured with tumour cells growing as spheroids.

### Host/vector system

This work will make use of a commercially available adenovirus transduction kit sold by Stratagene. This is an adenovirus type 5 (Ad5) derived vector system comprising the transfer vector, either pShuttle or pShuttle-CMV (the former lacks a promoter which is then inserted along with the gene of interest, the latter includes the CMV promoter to maximise expression of the transduced gene) and a plasmid, pAdEasy-1, which contains the Ad5 viral genome deleted in the E1 and E3 regions. These are recombined in E.Coli to yield the adenoviral vector containing the gene of interest (Group 1, Containment level 1, therefore non-notifiable). The latter is then transfected into the packaging cell line HEK-293, which contains the E1A and E1B Ad5 viral genes, permitting the production of replication-defective adenovirus containing the gene of interest. The recombinant adenovirus will then be used to infect primary macrophages. The laCZ gene has been inserted in the MCS site of the pShuttle-CMV to produce pshuttle-CMV-laCZ and is provided with the AdEasy kit. This construct enables transduction efficiency to be monitored by expression of β-galactosidase.

### Origin & function

The HIF sense and anti-sense oligonucleotides will be produced commercially by TCS

The effect of the expression of these genes will be determined via a variety of functional assays following viral transduction of the primary cells and co-culture with tumour spheroids.

Assays will be performed up to 3 weeks after transduction and then the resultant cells destroyed.

### Evaluation of foreseeable effects

All of the adenoviral vectors to be used are Ad5-derived. Wild-type Ad5 infection may lead to mild upper-respiratory tract infections in children. However, Ad5-derived vectors lack the early region 1 (E1) genes so that replication-competant viral particles can only be produced where these genes are supplied in trans by the complimentary packaging cell line (HEK-293 - see above). The adenoviral vectors are therefore "unlikely to cause human disease" and are assigned to Hazard Group 1 with containment level 1 as the minimum level of containment (ACGM Compendium of Guidance Part 2b).

The vectors supplied with the AdEasy kit also lack E3 genes. This increases the size of the insert that can be introduced into the vector but at the same time also "reduces the likelihood of a GMM causing harm as a consequence of making the virus less able to establish and maintain an infection" (ACGM Compendium of Guidance Annex III).
This is a theoretical possibility that recombination events (between the recombinant virus and the viral sequences in the 293 cells or between the recombinant and wild-type virus) could lead to the occurrence of replication-competent adenovirus. This can be monitored for. HIF is a transcription factor binding to DNA recognition sites known as hypoxia responsive elements (HRE). HIFs are expressed in many cell types and although produced continuously by normoxic cells, they are unstable and rapidly broken down. Under hypoxic conditions the breakdown pathway is inhibited thus upregulating the expression, stability and DNA-binding of HIF. Thus any contamination of the body would be disposed of by the normal breakdown pathway for these proteins. The protein -galactosidase, expressed by the lacZ gene in the control virus, is a widely used reporter with no known deleterious properties.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be treated, by incubation for several hours, with concentrated hypochlorite or Virkon, before disposal to the public sewer. All solid waste will be autoclaved and then disposed of via incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

At a meeting of the Local Genetic Modification Safety Committee on January 23rd 2001, the risk assessment was felt to be suitable and satisfactory to allow this work to proceed as a designated Class 2 project under Level 2 containment.

Project Containment

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Animal Units

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Project Ref 168/01.15
CREATION OF BACTERIAL EXPRESSION CONSTRUCTS, STABLE MAMMALIAN TRANSFECTED CELL LINES FOR THE INVESTIGATION OF THE FUNCTION AND MOLECULAR INTERACTIONS OF PROTEINS IN SIGNAL TRANSDUCTION PATHWAYS

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Investigation of the function of eukaryotic signal transduction components.

Recipient or parental organism
Laboratory E.coli strains BL21 (DE3), DH5a and XL 1 blue.
Defective third generation retrovirus vectors
Mouse, rat, hamster, monkey or human-derived cells in tissue culture.

Host/vector system
Bacteria will be of attenuated laboratory strains of E.coli, primarily BL21(DE3), DH5a and XL1 blue. Plasmids will be derived from the mobilisation-defective pUC vector. All amphotrophic retroviruses will be prepared from third generation packaging lines in which the helper virus lacks packaging signals and the rival genome is divided into at least two non-contiguous components.

Origin & function
The genes of interest will be from mouse, human and other mammalian species, chicken zebrafish or drosophila. Fusion proteins may also be created in some cases with the fluorescent proteins from Coelenterates or with other reporter proteins from insects (such as luciferase) or bacterial chromogenic enzymes.

The natural function of the genetic material will be under investigation, but will fit broadly into the processes of intracellular signalling responses to extracellular signals. Typical molecules of interest will be cell surface receptors, receptor associated protein kinases, small G protein (the ras superfamily), adaptor molecules, scaffolding proteins, non-receptor protein kinases, phosphatases, caspasases and other apoptotic effector proteins and transcription factors.
Mutated genes, either derived from natural sequences or as a result of site-directed mutagenesis, will be expressed in many cases. Some of these mutated genes will encode proteins that have activated or dominant negative functions.
Experimental constructs will be designed to allow biochemical investigation of the functions of the gene products, the raising of anti-sera in appropriate animals for the detection of the endogenous proteins.

### Evaluation of foreseeable effects

#### Health of the Worker

Cytoplasmic and nuclear signalling components are generated within the cytoplasmic phase of the cell. They are not secreted by overexpressing bacteria or cell lines. If cells do rupture and are simultaneously presented (as a result of an accident, with injury, for example) to the tissues of an individual handling the materials, the components will not be available to the normal cellular compartments in which they are functional without passing through the cellular endosomal pathway, where they are likely simply to be degraded. Routine exposure is minimised by good laboratory practice, but if it occurs is likely to result from the inhalation of aerosols containing overexpressing bacteria, cells or supernatants containing retroviruses.

Replication-defective amphotrophic retroviruses will be used for the efficient expression of genes within non-rodent cells, such as human or monkey-derived lines. On infection of the human cells, the defective virus is unable to perform more than a single round of infection, because capsid proteins and reverse transcriptase are not present within the recombinant viral genome, but are provided only by the packaging cell line. In third generation packaging lines, which will be used here, the viral proteins are provided by a segmented retroviral genome that lacks the viral packaging signals. It is thus extremely unlikely that a recombination event could restore either the packaging virus. If it did, though, then it would be able to facilitate the transfer of the defective virus between cells. To ensure that such recombinations are not occurring, amphotrophic packaging cell will be tested monthly while in use for the presence of reverse transcriptase activity.

Bacterial used for the expression of eukaryotic signalling molecules will be of attenuated laboratory strains of E.coli, primarily BL21 (DE3), DH5a and XL1 blue. They are unlikely to colonise the gut in competition with the endogenous flora. All expression systems will rely on inducible promoters and will use non-mobilisable vectors (derived from pUC) and therefore will not be transferred efficiently to endogenous flora. On lysis within the gut, expression products will be exposed to the destructive gut contents and will not be available to act in the cytoplasm where they must be present in order to function. The risks to the worker are therefore extremely low.

#### Environmental Implications

There is no anticipated release either of bacterial agents or retroviruses to the environment. The bacteria are laboratory strains and will be unable to compete in the environment. In the absence of selective pressure, plasmids, which are burdensome to replicate, will tend to be lost. Expression of eukaryotic intracellular proteins by bacteria in the environment will have no foreseeable consequences. The consequences of accidental release would therefore be negligible.

Yields of retroviruses are quantitatively very small, (typically 10,000-100,000 per ml of culture) and quantities of medium used in establishing stable cell lines is typically 10 ml. Retroviruses have a short biological half-life (less than 24 hrs) and are destroyed by drying. the likelihood of the retrovirus reaching an unintended biological target is therefore extremely small. The retrovirus is unable to sustain further cycles of replication after release from the packaging cell line. Should there be accidental release, the implications would be negligible.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not relevant

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None required

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Retrioviral packaging lines will be tested monthly, while in use, for the presence of reverse transcriptase in their cell supernatants according to Pyra et al (1994) Proc Natl. Acad. Sci. (USA) 91, 1544-8.

Retrovirus contaminated waste will be disposed of by mixing medium with sodium hypochlorite at least 1% or will be treated with virusolve, as described by the manufacturers, and disposed of via the drains or will be incinerated. A complete kill is expected.
Bacterial cultures will be treated with sodium hypochlorite solution at >1%. Cultures will be disposed of to the drains after standing for 30 min. A complete kill is expected as this is a standard microbiological practice.

This project was presented to a meeting of the Local Genetic Modification Safety Committee on 23 January 2001. The risk assessment was held to be suitable and satisfactory to allow it to be forwarded to the ACGM as a Class 2 project under level 2 containment.

Project Containment

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Project Ref 168/01.17

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<td>CLONING AND EXPRESSION OF ION CHANNEL PROTEINS IN MAMMALIAN CELLS USING RECOMBINANT VIRAL VECTORS (ADENO AND SINDBIS)</td>
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Tick if notifying a connected programme of work N

Historical Significant Changes

Withdrawn N
Purposes of the contained use

To construct replication deficient, recombinant viral vectors (Adeno-X and Sindbis) containing ion channel genes for gene deliveries into cultured mammalian cells, particularly non-dividing cells, for the study of the properties of the expressed ion channel genes.

Recipient or parental organism

The recipients in this study are different types of mammalian cells (e.g., Human Embryonic Kidney cell lines and Baby Hamster Kidney cell lines) and cultured mammalian cells/tissues (e.g., rat and mouse neural cells, rat and human macrophages).

Host/vector system

Both the Adeno and Sindbis vectors used in this study are replication deficient derivatives available commercially. Both require packaging cell lines to make complete virus particles. The viral particles can undergo only one round of infection.

The Adeno virus vector is available from CLONTECH as linear DNA (Adeno-X). The DNA of this virus has a deletion removing most of the Early region 1 (E1) (which is responsible for cell transformation and tumourigenicity) and E3. The deletion ensures that recombinant adenovirus is not infectious and replication incompetent. The Adeno-X virus is unable to replicate in cells other than the complementing cell line, HEK292, which expresses the proteins required for viral assembly. The replication deficient Adeno vectors have no mechanism for long-term maintenance in the cells.

The Sindbis system used in this study is a replication-deficient derivative of the alphavirus Sindbis commercialised by Invitrogen in a proviral plasmid form, pSinRep5.

pSinRep5 is a plasmid DNA vector used to generate recombinant RNA molecules, by in vitro transcription. The RNA thus produced will be used for transient transfection of a packaging cell line, baby hamster kidney cell line (BHK), commercially available from Invitrogen. The Sindbis structural protein genes have been deleted from the vector to make the virus replication-deficient.

DH-BB (Defective Helper, deleted between BspM 11 and Bam H1) is a plasmid NDA template that contains the genes for the four structural proteins required for packaging of the Sindbis viral genome.

Origin & function

Known ion channel genes will be inserted into the viral genome for transient expression in a target host cell. These genes present no risk to human or animal health or to the environment.

Evaluation of foreseeable effects

The level of exposure of workers to infectious viral particles will be low (no handling of large volumes, no aerosol generation). Neither the recombinant nor the non-recombinant viral particles is likely to have any pathogenicity. In the extremely unlikely event of viral particles infecting a worker through a cut or lesion, the area infected will be very limited as the infection will not spread to the rest of the body and will be without any consequences as the expression of viral and recombinant proteins is transient.

Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment Level 2 required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All culture vessels and objects (mainly disposable plasticware) coming into contact with viral particles will be immediately immersed in fresh 3% Virkon for at least 3 hours and autoclaved at 121 degrees C for 25 minutes. All disposable solid waste will then be packaged for disposal by the University's normal route.

Virkon will be added to all waste culture media to a final concentration of 5%. All liquid waste will then be autoclaved at 121 degrees C for 25 minutes. The liquid waste will then be disposed of down the laboratory sink.

These procedures will result in a 100% kill of cells.

Appropriate disinfectants (Bleach or Virkon) will be used to decontaminate exposed work areas.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

This proposal was presented before a meeting of the Local Genetic Modification Safety Committee on 3 May 2001. The documentation was agreed to represent accurately the work about to be undertaken and the Committee approved the proposal as Class 2.

Project Containment

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Project Ref 168/01.2

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 168/01.3

Date Ackn’ed 06/02/2001

Date Project Ceased

CU2 Project Title EXPRESSION OF INTERLEUKIN-1 HOMOLOGUES IN E.COLI AND MAMMALIAN CELLS

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted not applicable

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref** 168/01.4

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<td>ESTABLISHMENT OF MYELOID CELLS PERMITTING REGULATED GENE EXPRESSION</td>
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Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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If yes, tick to confirm that it is attached to this form N

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Animal Units

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Project Ref 168/01.5

Date Ackn'd 06/02/2001
CU2 Project Title BACTERIAL EXPRESSION OF RECOMBINANT PROTEINS FOR MECHANISTIC
Class 2
CultureVolClass2
CultureVolumeClass3-4

02/03/2022  Page 4217 of 15326
Date Project Ceased

AND STRUCTURAL STUDIES

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
**Project Ref**: 168/01.7

**Date Ackn'd**: 06/02/2001

**CU2 Project Title**: ADENOVIRUS-MEDIATED GENE TRANSFECTION WILL BE USED TO ACHIEVE HIGH LEVELS OF EXPRESSION OF THE POX VIRUS PROTEIN, CYTOKINE RESPONSE MODIFIER A (CRMA).

**Class**: Class 2

**CultureVolClass2**: 

**Consent Granted**: not applicable

**Tick if notifying a connected programme of work**: N

**Project notified under transitional arrangements**: Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

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Project Ref 168/01.8

Date Ackn’d 06/02/2001
CU2 Project Title RETROVIRAL TRANSDUCTION OF PRIMARY AND TISSUE CULTURE CELLS
Class 2
CultureVolClass2 CultureVolumeClass3-4
WITH TISSUE INHIBITORS OF METALLOPROTEINASES AND OTHER ANTI-INFLAMMATORY PROTEINS.

Non-GMM

Consent Granted

not applicable

Tick if notifying a connected programme of work

N

Project notified under transitional arrangements

Y

Withdrawn

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Class 2

Project Ref 168/01.9

Date Ackn’d 06/02/2001

Date Project Ceased

Adenoviral transduction of primary cells with wild-type and mutant tissue inhibitors of metalloproteinases (TIMPs)

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Is an emergency plan required according to regulation 20? N
- If yes, tick to confirm that it is attached to this form N
- Tick to confirm that you have attached a risk assessment to this form N
- Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
### Purposes of the contained use

The aim of the contained use is to identify genes in Aeromonas and Vibrio spp. that are involved in pathogenesis, adherence, motility and LPS biosynthesis. These genes may be involved in the colonisation process of these organisms.

### Recipient or parental organism

- **Escherichia coli K-12**
  - Aeromonas spp. Including (Aeromonas caviae, Aeromonas hydrophila, Aeromonas veronii bv sobria)
  - Vibrio spp. Including (Vibrio parahaemolyticus, Vibrio cholerae)

### Host/vector system

- **Escherichia coli K-12:** CC118 (pir) containing derivatives of the suicide plasmid pKNG101, pUTminiTn5 derivatives.
- **E. coli K-12:** DH5a and XL-1 Blue containing derivatives pBBR1MCS.
- **E. coli K-12:** BL21-DE3 containing derivatives of pET28
E. coli K-12: HB101 (pRK2013)
Aeromonas spp. and Vibrio spp. containing derivatives of pBBR 1MCS, pUTminiTn5 and pKNG101.

**Origin & function**

The genetic material will be obtained from primarily Aeromonas spp. (A. caviae) and Vibrio spp. (V. parahaemolyticus). Initially the whole genomes of the Aeromonas spp. will be represented, as plasmid libraries will be constructed in E. coli (multi-copy). Therefore all gene functions present in Aeromonas will be represented in the library, provided that they can be expressed, which is not always the case.

Homologues of the specific genes identified in Aeromonas, will be isolated from the chromosome of V. cholerae (or other Vibrio spp.) using PCR and information gained from the genome sequence, therefore genomic libraries of these organisms will not be constructed.

**Evaluation of foreseeable effects**

There are no foreseeable harmful effects resulting from the construction of the recombinant E. coli K-12 strains, as they already carry a number of mutations that leave them disabled.

Aeromonas spp. and Vibrio spp. cause a variety of diseases in humans and animals, including fish amphibians and reptiles. Both are considered to belong to ACDP hazard group 2. There is no foreseeable significant increase in harmful effects resulting from the construction of the previously mentioned recombinant Aeromonas spp. or Vibrio spp. in comparison with their wild-type parental strains. There is a possibility that plasmid complemented strains may express certain genes at a higher level than the wild-type. However, as the pathogenicity of these organisms is a complex multi-factorial process requiring many genes, it is extremely unlikely that any clone will have a significant increase in its ability to cause harm or to the survive in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Level 2 work.
(GM E. coli, Aeromonas spp. and Vibrio spp.)
Contaminated liquid waste will be inactivated by immersion in disinfectant (Clearsol 1:100) or sterilised in a validated autoclave. Waste is then removed for incineration.

Contaminated glassware, will be sterilised by pressure in a validated autoclave.
Contaminated consumable waste (plasticware, Petri dishes) in inactivated by pressure in a validated autoclave, or by a validated incineration procedure. A 100% kill is expected in all cases.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
Please enter comments on the GM safety committee on the risk assessment

Comments from the LGMSC were included with the original proposal and this subsequent update was suggested following a visit from the ACGM branch of the HSE, to revise ongoing projects to the new Genetically Modified Organisms (contained use) Regulations 2000.

### Project Containment

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#### Project Ref 168/02.2

- **Date Ackn’ed**: 04/09/2002
- **CU2 Project Title**: THE MODULATION OF PROSTATIC STROMAL CELL PROCESSES BY RETROVIRAL MEDIATED TRANSFECTION: THE PRODUCTION OF STABLE TRANSFECTED PROSTATIC CELL CULTURES.
- **Class**: Class 2
- **CultureVolClass2**: < 1 litre
- **Consent Granted**: not applicable
- **Non-GMM**: N
- **Project notified under transitional arrangements**: N

#### Purposes of the contained use

Specific gene expression in prostatic cell cultures will be modulated using recombinant DNA technology. Initially, cDNAs will be generated as RT-PCR products and cloned. The amplified sequence will then be sub-cloned, either in the sense or anti-sense configuration, into a retroviral expression vector. The retroviral expression constructs will be cloned into a packaging cell line to produce replication incompetent virus particles expressing the gene of interest in either the sense or anti-sense
configuration, under control of the Tet operon. These retroviral particles will then be used to obtain stable transformation of human cell lines expressing the sequence of interest under tetracycline control.

Recipient or parental organism

HOST
RetroPack PT67 retroviral packing cell line (third generation packaging cell line)
The packaging cell line is capable of producing replication incompetent retrovirus (see below for effects) after transfection with the aforementioned plasmids.

Replication incompetent retroviruses
The replication incompetent retroviruses are capable of transfecting a wide range of mammalian cells, inducing the target cells to constitutively express the insert sequence in either the presence, or absence of tetracycline (depending on whether Tet on or Tet off vectors are used). The constitutive expression of cloned sequences could potentially have deleterious effects (see list below)

Human cell lines
Human cell lines that have been infected with replication incompetent retroviruses will constitutively express the cloned sequence, either in the presence, or absence of tetracycline (depending on whether Tet on or Tet off vectors are used). These cells are not able to produce retroviruses. The transfected cells are cell culture dependant, and therefore do not pose a health and safety risk over the untransformed parental cells, with the exception of the theoretical risks associated with recombination.

Host/vector system

The pRev vectors will be transfected into the packaging cell line producing replication incompetent virus particles expression the gene of interest. The pRev plasmid contains only the packaging signal and the packaging cell line contains genes required for non-replicating virus particle formation. These genes are present within the packaging cell line as several independant sequences, reducing the probability of recombination events producing replication proficient virus particles (third generation packaging cell line). These replication incompetent retroviruses will then be used to produce stable transformed human cell lines expressing the sequence of interest.

Origin & function

All expressed sequences will be normal, human cDNA sequences obtained by cloning of human cDNA. Sequences will be expressed in the sense or anti-sense configuration, to either increase expression of, or to abolish endogenous expression of the protein of interest.

Sequences that are likely to render the replication incompetent retroviruses to be replication competent, will not be cloned. In addition, no known transforming oncogene sequences will be used, and no more than one sequence will be transfected into any cell population. The sequences of interest are a heterogeneous group of molecules including growth factors and their receptors, intracellular signalling molecular and proteins involved in the production or degradation of extra-cellular matrix. Lists of sequences that come under these headings, in addition to a small number of miscellaneous sequences are shown below.

LIST OF GENE SEQUENCES TO BE CLONED AND EXPRESSED
Growth factors and receptors, including:
Transforming growth factor (TGF)a
TGFb1-3
Epidermal growth factor (EGF)
Fibroblast growth factor (FGF)1-23
Insulin-like growth factor (IGF)1-2
Connective tissue growth factor (CTGF)
Interleukin-6 (IL-6)
Tumour necrosis factor (TNF)a
Tumour necrosis factor receptor superfamily, member 11 (TNFRSF11) (Osteoprotegerin (OPG))
Colony stimulating factor (CSF)1-2
Bone morphologic protein (BMP)1-9

Cellular signalling proteins, including
Growth factor receptors (see above)
SMA and MAD-related protein (SMAD)1-7
Receptor activator of NK-Kappa-B Ligand; (RANKL)
Androgen receptor (AR)
Estrogen receptor (ESR)1-2
Glucocorticoid receptor (GR)
Retinoid X receptor (RXR) a,b and y
Retinoic acid receptor (RAR) a and B
TNF-related apoptosis-inducing ligand (TRAIL)
TNF-related apoptosis0inducing ligand receptor 1; (TRAILR1)
Apoptosis-related cysteine protease; (CASPASE)1-14

Other proteins, including
Matrix metalloproteinases (MMP)1-26
Tissue inhibitors of metalloproteinases (TIMP)1-4
Plasminogen activators (tPA and uPA)
Plasminogen activator inhibitor (PAI)1-2
A disintegrin and metalloproteinase domain (ADAM) 1-20
A disintegrin-like and metalloproteinase with thrombospondin type 1 motif, (ADAMTS)1-7
Latent transforming growth factor-beta-binding protein (LTBP)1-4

Extracellular matrix proteins, including
Collagen type 1 and IV
Fibronectin

**Evaluation of foreseeable effects**

The replication incompetent retroviruses are capable of infecting a range of mammalian cells, resulting in constitutive expression of the cloned sequence under the control of tetracycline. Transforming oncogene sequences, and viral sequences that will allow production of replication competent viruses will not be expressed. Transient infection with replication incompetent sequences would allow stable transformation of the user. The most likely route of infection would be needle stick injury, or aerosol inhalation. The risks of aerosol inhalation or needle stick injury will be minimised by the use of class II biological safety hoods and by not using any sharp instruments or disposables (syringe needles, scalpels etc.) In both situations, the resulting transformation would be expected to be identical to injectionm or inhalation of naked plasmid DNA. The efficiency of stable transfection via retroviral infection, however, would be substantially higher than with naked DNA. If an infection was to occur, resulting in stable expression of one of the aforementioned sequences, serious effects would not be expected. The expressed sequences are normal gene products. It must be stressed that the modulation of expression of many genes are required for transformation of normal cells to a neoplastic, or malignant phenotype. Therefore the risks of serious effects of infection, if it were to occur, are small.

The use of third generation packaging cell lines ensures that the risk of recombination, producing replication competent retroviruses is a remote and theoretical risk. However, all infected cells will be checked for the presence of replication competent virus in stable transformed cell lines and transfection reactions using a reverse trascriptase activity assay.

The intended genetically modified organisms (human cell lines) are not likely to have any increased health and safety risk associated with modification over the parent cells, other than the theoretical risk of recombination. The cells are dependent on cell culture conditions and modification is therefore unlikely to confer an increased health and
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All potentially contaminated waste will be precept treated, autoclaved and disposed of in sealed waste bins for incineration. Treated waste will be tested for the presence of retroviral activity using a reverse transcriptase activity assay. Combined precept treatment and autoclaving would be expected to achieve 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

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Project Ref 168/02.3

Date Ackn’d 08/10/2002
CU2 Project Title ANALYSIS OF GENE FUNCTION IN THE OPPORTUNISTIC PATHOGEN
Class 2
CultureVolClass2 1-50 litres
CultureVolumeClass3-4
The aim is to learn more about the biology of this fungal pathogen using a genetic approach based on the genome sequence. This follows on from work in progress to sequence the whole genome of this species in the US and the UK. Work currently proposed will focus on genes of secondary metabolism.

Aspergillus fumigatus is a saprophytic filamentous fungus, found commonly in the soil/rhizosphere, with similar metabolic properties to other soil aspergilli such as A nidulans and A niger, growing on organic debris. Its asexual spores (conidia) are amongst the most ubiquitous of airborne fungal spores. Since inhaled spores are dealt with effectively in healthy individuals, its main threat is to immunocompromised hosts. Its prevalence as a pathogen has therefore increased in recent years amongst patients undergoing cancer treatment (especially leukaemia), bone marrow transplants and solid-organ transplants.

Recipient or parental organism

Aspergillus fumigatus is a saprophytic filamentous fungus, found commonly in the soil/rhizosphere, with similar metabolic properties to other soil aspergilli such as A nidulans and A niger, growing on organic debris. Its asexual spores (conidia) are amongst the most ubiquitous of airborne fungal spores. Since inhaled spores are dealt with effectively in healthy individuals, its main threat is to immunocompromised hosts. Its prevalence as a pathogen has therefore increased in recent years amongst patients undergoing cancer treatment (especially leukaemia), bone marrow transplants and solid-organ transplants.

Host/vector system

Host: Aspergillus fumigatus AF293 (wild-type)
Aspergillus fumigatus AF293-1 (uridine-requiring auxotroph)
Vectors: derivatives of pBluescript and pUC19 carrying the pyr-4 gene of Neurospora crassa as a selectable marker to complement the host pyrG mutation, or the hygromycin phosphotransferase gene (hph) of E coli under the control of a fungal promoter as a selectable marker to transform the wild-type strain.

Origin & function

Genetic material will be from extracted Aspergillus fumigatus genomic DNA, or from cosmid libraries of A fumigatus obtained from sequencing centres.

Gene disruption and increased gene expression will be sought in order to analyse gene function in A fumigatus. Initial work will be with genes encoding biosynthetic steps for secondary metabolites. Mutant strains will then be analysed for changes in gene product(s) by small scale fermentation and chemical analysis of culture material. The main aim of the research is to learn more about the biosynthesis of secondary metabolites by filamentous fungi, using A. fumigatus as a model system because of the imminent completion of its genome sequence, giving access to all of its genes.

The preferred route is to look for loss of a compound following disruption of a gene.
Where the gene product is made in very small amounts, as is often the case for secondary metabolites, overexpression of a gene may aid identification of its product.

In some cases, mutant strains with characterized changes in metabolism will be sent to other laboratories for pathogenicity testing, since the possible role of secondary metabolites in pathogenicity is not yet understood.

**Evaluation of foreseeable effects**

Gene disruption/overexpression is likely to result in changes in the amount of secondary metabolite produced by the modified fungal strains.

Secondary metabolites are not usually essential for growth and reproduction of a species, but do include biologically active compounds such as antibiotic and toxins (e.g. gliotoxin, fumitremorgins).

Some of these may play a role during infection, since they may have inhibitory activity against animal cells. Gene disruption is likely to lead to a strain which would still grow, but has lost one of its non-essential functions. Strains with increased expression of a toxin could lead to increased pathogenicity, but since pathogenicity is likely to depend on a combination of many factors, it is unlikely that single change, which could also arise by spontaneous mutation, would have a major effect on pathogenicity or persistence of the modified strain in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

As with unmodified A fumigatus, the main risk for infection or environmental contamination is via airborne conidia. Only dry spores (e.g. on sporulating agar plates) are easily dispersed in the air. Once wetted and converted into conidial suspensions in tween 80/Saline, they are unlikely to become airborne. Harvesting of spores (used for inoculation of liquid and solid media) and inoculation of cultures will be carried out in a Biohazard Cabinet. Outgoing air from the cabinet is filtered, and the cabinet is serviced every 6 months. Prior to servicing, the system is fumigated with formaldehyde (60% formaldehyde in water, overnight fumigation) as recommended by the manufacturers). After routine use for handling spores, the Biohazard Cabinet is swabbed with 20% sodium hypochlorite solution.

Since shaken liquid cultures (mycelial growth) do not conidiate, they do not present the same risk of airborne dispersal. Flask cultures of up to 1 litre culture per flask (up to 1 litre culture per flask) will be grown to extract protein for enzyme assays, and to extract culture supernatant and filtered mycelium to detect metabolites. Mycelium will be harvested by filtration, and disrupted to obtain soluble protein. Culture supernatant and dried mycelium will be used for analysis for metabolites.

After use, plate cultures, mycelium and spent culture medium will be sterilized by autoclaving with the Department of Molecular Biology and Biotechnology. The central autoclave used for sterilization is validated at least every 12 months.

In the event of spillage, the area will be effectively disinfected using sodium hypochlorite (20% solution).

100% kill is obtained by all 3 methods of waste management.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The risk assessment was considered by the local genetic modification safety committee on 22/1/02, who suggested inclusion of further details of environmental risks in the initial risk assessment. This has now been incorporated in the attached risk assessment.

**Project Containment**

<table>
<thead>
<tr>
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**Project Ref** 168/04.1

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<td>19/02/2004</td>
<td>INFECTION OF HUMAN CELLS WITH LABORATORY A2 STRAIN RESPIRATORY SYNCITIAL VIRUS (RSV)</td>
<td>Class 2</td>
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Tick if notifying a connected programme of work N

Withdrawn N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**
Overview or aim of the project: The aim of this project is to determine if the infection of cells by RSV is via the recognition of a specific surface antigen. Previous studies have demonstrated that RSV infects a proportion of cells in vitro. In these studies the fixing and permeabilising of the cells to detect intra cellular RSV antigens modified the detection of other cell surface antigens. Using rg-RSV will allow the sorting of the RSV infected cells by flow cytometry. It would then be possible to stain both the infected and uninfected cells for surface antigens to determine whether a specific phenotype predisposes a subset of cells to RSV infection. This technique can be carried out without disrupting the surface antigens. The identification of a specific antigen required for RSV infection would be a major stepping-stone in the development of RSV treatment and vaccines. The project will require the propagation the laboratory A2 strain and the genetically modified viruses in HeLa cells. RSV encodes 3 transmembrane surface glycoproteins in the viral envelope, G (glycoprotein), F (fusion) and SH (small hydrophobic). It is proposed that at least one of these proteins is essential for RSV infection. Cells will be infected with RSV mutants lacking of these proteins to assess the importance of each for efficient RSV infection. RSV infection will be determined by fix-permeabilising the cells and staining for RSV antigens. Rg-RSV and the deletion mutants will also be used.

The construction of the virus is described in Hallak et al (2000) and a full description of the rescue system is in Collins et al (1995) both papers are attached. The modified virus strains have been developed in the laboratory of Rush-Presbyterian-St Luke's Medical Centre, Illinois. The rg-RSV is the same as the standard A2 RSV laboratory strain, except that it expresses an additional gene (GFP) when it replicates. The rg-RSV and the deletion mutant (described in Techaarpornkul, 2002) are to be kindly donated and no further genetic modifications will be made in our laboratories for the proposed studies.

The infectivity of the virus declines over time so it is necessary to propagate the virus to increase both the infectivity and titre of virus for use in the experiments. The virus will be propagated in HeLa cells. The infected cells will carry no hazards beyond those infected with the laboratory A2 strain of RSV.

HeLa cells: The A2 laboratory RSV, and the modified RSVs will be propaged in HeLa cells. Cells infected with the modified RSV will carry no hazards greater than cells infected with the laboratory A2 strain of RSV. It is expected that titre of infectious virus will be lower than the A2 strain. Cells are unable to survive outside of the tissue culture environment. Good cell culture practice, within a class II laboratory, will be followed when handling the infected cells. Infected HeLa cells will be cultured in a designated experimental incubator.

(Secondary host) human cells: Cells infected with A2 laboratory RSV or modified RSV will carry no hazards greater than cells infected with the laboratory A2 strain of RSV. It is expected that titre of infectious virus will be lower than the A2 strain. Cells are unable to survive outside of the tissue culture environment. Good cell culture practice, within a class II laboratory, will be followed when handling the infected cells. Infected cells will be cultured in a designated experimental incubator. Cells will not be isolated from the researcher for use in these experiments.

The modified RSVs are to be kindly donated by Rush University, Illinois. The rg-RSV is the same as the standard A2 RSV laboratory strain, except it expresses a reporter gene when it replicates. The virus construction and rescue is described briefly below and in detail in Hallak et al, 2000 JViro/ 74:10508-10513, Collins et al., 1995 PNAS 92:11563-11567.

Transfection and Recovery of recombinant RSV: Monolayer cultures of Hep-2 cells were infected with 1 focus-forming unit per cell strain MVA vaccinia virus recombinant expressing T7 RNA polymerase (MVA-17) and transfected with antigenome, N and P plasmids and L and M2 (ORF1) plasmids using LipofectACE.

Preparation of rg-RSV: Full length RSV cDNA clone MP224 used to rescue rgRSV was constructed to contain green fluorescent protein as its first gene by inserting a BstX1 fragment containing the gene start, the NS1 region, the Green Lantern Protein gene and the L gene end.
This BstX1 fragment was generated by transferring the BstX1/BamH1 fragment from the minigenome C41-GFP into another minigenome MP129 to create MP166 and transferring the Xhol/NCOI fragment from MP166 into MP90 a bipartite minigenome containing the GFP flanked by BstXI sites and followed by the luciferase gene, to create MP169. The BstXI fragment from MP169 was moved into the full-length RSV cDNA clone, D46, to generate MP224. MP224 was recovered by transfecting it along with four plasmids expressing the N, P, L and M2-I support proteins into Hep-2 cells transcription from these plasmids was driven by T7 RNA polymerase provided by the recombinant vaccine virus MVA-T7.

Evaluation of foreseeable effects

The laboratory A2 RSV strain is routinely used in RSV studies due to the difficulty in propagating wild type isolates in the laboratory. The virus could potentially infect humans. Infection in adults results in mild-cold-like symptoms including running nose and coughing. The virus will be handled whilst wearing a howie coat and gloves and in a class II cabinet, and cultured and stored with within a contained area using good handling, cell culture and laboratory practice (as described in COSHH assessment). Virus stocks will be contained in a designated area of a -80 degree C freezer.

The addition of the reporter gene on the RSV will not carry hazards greater than the laboratory A2 strain. The deletion mutants will be less hazardous than the A2 strain. The reporter gene does not alter the pathogenicity of the A2 strain. The deletion mutants will have reduced pathogenicity. The reporter gene is unlikely to confer extra pathogenicity to other related organisms.

There is unlikely to be any risk to human health or the environment. The virus does not readily survive outside culture conditions, but can be transferred by aerosola. Good laboratory practice will minimise these risks. The virus is required at high titres for efficient infection. The virus rapidly degrades even at -80 degree C. Beyond passage 7 titres of the modified virus decline.

We will ensure that hazards are contained by implementing good working practice (aseptic techniques) and by regular servicing of the class II safety cabinets.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment for this project will be at level 2. We will ensure that hazards are contained by implementing good working practice (aseptic technique) and by regular testing of the disinfected cultures and by regular servicing of the class II safety cabinets.

Infected and uninfected cells will be disinfected with bleach for 24 hours. The disinfected culture supernatant will be checked for viable virus using the plaque assay. Aliquots of disinfected culture supernatant will be added to cultures of HeLa cells and the formation of viral plaques will be checked by staining with RSV antibody. The containment procedure will be reviewed annually.

We will ensure that hazards are controlled by containment by implementing good working practice (aseptic technique) and regular servicing of the class II safety cabinets.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
A risk assessment for this project was presented to a meeting of the Local Genetic Modification Safety Committee on Tuesday 20 January 2004. It was agreed by the committee that this proposal should be forwarded to the ACGM as a Class 2 project, as soon as a cheque could be raised.

Project Containment

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Project Ref 168/05.1

Date Ackn'd 10/02/2005

Date Project Ceased

Date of Significant Change

Project Additional Information

The aim of this project is to achieve both efficient and stable delivery of lentiviral vectors to vascular cells allowing either over-expression of SERT, IL-1ra and S100A4/Mts 1 or gene knock-down via delivery of short hairpin RNA constructs targeting BMP-RII, SERT, IL-1ra and S100A4/Mts 1 that will induce specific RNA interference and assess their role on signalling pathways and phenotype.
# Parental Micro-organism to propagate Lentiviral DNA

*Escherichia coli STBL3* (invitrogen) are purchased commercially.

# Parental Organisms to propagate replication deficient lentivirus

*Human Embryonic Kidney 293* packaging cells.

# Recipient Organism

*Human and Rodent primary vascular cells.*

---

## Host/vector system

**Hosts:** Human Embryonic Kidney 293 packaging cell line transformed with pCMVSPORT6T.neo plasmid to express the SV40 large T antigen/human and rodent endothelial and smooth muscle cells (primary cells).

**Vector:** pLenti6/V5 Dest (Invitrogen) & pLenti6-CMV/V5 Dest (Custom made by removing the CMV promoter for pLenti6/V5 Dest).

**Over-expression Insert:** human Serotonin transporter (hSERT), human S100A4/Mts1, human Interleukin 1 receptor antagonist (IL-1ra).

**shRNA Insert:** Human SERT, Human Bone morphogenetic protein receptor 2 (BMP-RII) and IL-1ra.

---

## Origin & function

### siRNA

pLenti6/V5 and pLenti6-CMV/V5 produce a replication-incompetent HIV-1 based lentivirus, which can be used to deliver and express the gene/shRNA cassette in mammalian cells.

Lentiviral containing supernatant will be collected from the 293FT (Invitrogen) packaging cells and used to infect both human and rodent primary vascular cells.

**Over-expression Vectors**

- **Over-expression of the full length human serotonin transporter (SERT) cDNA - sequence attached.**
  The function of SERT is to transport the neurotransmitter serotonin into and out of the cell, a mechanism that regulates extracellular levels of serotonin via breakdown by monoamine oxidase following transport. Serotonin transport and signalling has been linked to proliferation and contractility in vascular cells.

- **Over-expression of the full length Interleukin 1 receptor antagonist (IL-1ra) cDNA - sequence attached.**
  The function of IL-1ra is to compete with the pro-inflammatory cytokine IL-1 for the binding of the IL-1 receptor on the cell surface.

- **Over-expression of the full length S100A4/Mts1 cDNA - sequence attached.**
  S100A4/Mts1 is a small calcium binding protein that promotes motility in cancer cells, smooth muscle cells and endothelial cells.

**shRNA Cassettes**

- **Expression of U6-BMP-RIIshRNA to induce specific RNAi-induced knock-down of BMP-RII expression.**
  The bone morphogenetic protein receptor 2 (BMP-RII) signalling pathway has been linked to inhibition of proliferation in pulmonary vascular cells via the SMAD and/or p38 signalling pathways. Knock-down via RNAi should result in a more proliferative phenotype.

- **Expression of U6-SERTshRNA to induce specific RNAi-induced knock-down of SERT expression.**
  The function of SERT is to transport the neurotransmitter serotonin into and out of the cell, a mechanism that regulates extracellular levels of serotonin via breakdown by monoamine oxidase following transport. Serotonin transport and signalling has been linked to proliferation and contractility in vascular cells.

- **Expression of U6-IL-IrashRNA to induce specific RNAi-induced knock-down of IL-1ra expression.**
  The function of IL-1ra is to compete with the pro-inflammatory cytokine IL-1 for the binding of the IL-1 receptor on the cell surface. Knock-down of IL-1ra should allow for
Evaluation of foreseeable effects

Infection of primary human and rodent vascular cells will be relatively short lived, due to the antibiotic selection issue of primary cells. The typical experimental time course will be 3-10 dyas, after which the cells will be destroyed.

The expected effects of these vectors on transduced cells are described above. Although the risk of homologous recombination with wild type virus is low (human vascular cells are purchased from Cascade Biologics and are screened for infectious agents prior to shipping), caution will be observed and therefore handled in a class II Microbiological Safety Cabinet in a dedicated category II suite in the Clinical Sciences Centre.

Likelihood of hazards is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon is used according to manufacturer's instructions (5% w/v) to inactivate liquid waste from viral infections. All glassware/plasticware will be soaked in Virkon. Glassware will then be rinsed and then soaked in bleach. Disposable plasticware and all other waste from the Category II suite is autoclaved before being placed in sealed omnibins and incinerated.

The autoclave is SCS is tested on a 6 month basis with full probe set. These data are displayed in the Category II suite for inspection.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The proposal was presented at the local GMSC at a meeting on 18 January 2005. The local GMSC agreed that this was a Class 2 proposal overall and therefore it would be forwarded to ACGM together with the administration fee for notification/approval.

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### Project Additional Information

**Purposes of the contained use**

The project aims to express RANKL, OPG, TRAIL and related cytokines and their receptors, or components of the Wnt-signalling pathway, and to express anti-sense and si-RNA for these genes in mouse and human bone or tumour cell lines, to assess their effect in models of bone biology, or metastasis to bone, in mice. We intend

* to test whether various human and mouse bone and tumour lines can be infected with retroviral constructs that will lead to the expression of marker molecules such as Aequoria green fluorescent protein (GFP) or E.coli LacZ
* To raise or reduce RANKL, OPG, TRAIL, their receptors, or components of the Wnt-pathway, by transducing their genes, siRNAs or antisense constructs and controls into murine and human bone and tumour cell lines. Modified cells may be grafted into mouse hosts.

We propose to use the pCAGGS self-inactivating HIV-based lentiviral system obtained under licence from St Judes Childrens' Hospital, Memphis, Tennessee, USA (details below), although it may be necessary to employ other lentiviral systems, such as the commercially available HIV-based Virapower system from Invitrogen (see below) in order to achieve our research objectives. Any system used will incorporate the safety features outlined below.

**Recipient or parental organism**

Disabled auxotrophic non-mobilising Escherischia coli K12 strains, or B-derivatives such as BL-21 or DH5-alpha.
Human and rodent primary and established cell lines: 293T (human embryonal kidney) cells (virus packaging cell line), HeLa cells (biological titering) and other mammalian cell lines such as established models of human myeloma (eg NCIH 929, JNJ-3, RPMI 8226, OPM 2 and XG1) murine myeloma (eg 5T2MM and 5T33mm) AND PROSTATE CANCER (EG Incap, pc3), AS WELL AS OTHER BONE AND/OR STROMAL CELLS (EG saos2). It may be necessary to include other established mammalian cell lines in the study in order to achieve certain goals (eg to use TRAIL-sensitive cell lines such as Jurkat in order to investigate rescue of TRAIL-induced apoptosis by endogenous OPG).

**Host/vector system**

**Pcaggs LENTIVIRAL EXPRESSION SYSTEM:**

"Fourth generation" HIV-derived vectors, as follows:

- Gene transfer vector pCL10.1MSCV-IRES-GFP
- Viral packaging vectors: pCAGkGPIR or pCAGkGP3R (Gag/Pol)
- pCAG4-TR2 (Rev/Tat)
- pCAG-VSVG (Vesicular stomatitis virus-G envelope protein pseudotype) or pCAG4-10A1, pCAG4-Amph (both amphotropic envelope protein pseudotype).

Features that prevent generation of live virus, recombination with endogenous retroviruses and viable HIV

**A) pCL10.1 MSCV IRES GFP (gene transfer):**

The 5’ long terminal repeat (LTR) is chimera since the U3 region of the HIV-1 LTR is replaced with a CMV enhancer. A deletion of the U3 region of the 3’ LTR renders it self-inactivating.

A portion of the R region and the U5 region have been removed and replaced with the rabbit B-globin gene polyadenylation site to enhance safety and improve efficiency of vector production.

The Gag-Pol coding sequence has been "inserted" by filling in (and therefore eliminating) the Clal site (nt 830), thus creating a frameshift and termination codon.

**B) Pcaggs (VIRAL PACKAGING):**

This expression plasmid has been used to construct the cassettes expressing the HIV structural and regulatory proteins, as well as various envelope proteins. The accessory proteins tat and rev are expressed together on a separate plasmid from that expressing GAG/GAG-PRO-POL-precursor polyprotein.

The RRE element (included to enhance nuclear to cytoplasmic transfer of gag/pol encoding RNA molecules, and to modulate expression of rev and tat by fostering transport of unspliced transcripts) was incorporated in the rev/tat helper plasmid to ensure that homologous recombination will not generate a plasmid encoding both structural and functional accessory proteins.

**ViraPower lentiviral expression system:**

Features that prevent generation of the live virus, recombination with endogenous retroviruses and viable HIV

The pLenti expression vector contains a deletion in the 3’LTR that does not affect generation of the viral genome in the producer cell line, but results in 'self-inactivation' of the lentivirus after transduction of the target cell. This is a result of the natural duplication of 3’LTR into 5’ position that occurs during integration. Once integrated into the target cell, the lentiviral genome cannot produce packageable viral genome.

Only 3 HIV-1 genes are used, gag, pol, and rev. The absence of the env gene removes the gp160 precursor that is responsible for the lethal macrophage/T cell/neuro-tropism of HIV.

Production of the viruses from the producer cell line requires the transfection of 4 plasmids (three packaging, one the DNA to be packaged) in transient transfection. All four plasmids have been designed, through lack of common sequences, to avoid the possibility of recombination with each other. The plasmids expressing the structural and packaging genes are not packaged with the produced virus, since none of them contain LTRs or the packaging RNA sequence. Thus, replication-competent viruses cannot be produced, nor can the packaging cell line produce retrovirus in any form during routine culture.

Expression of gag and pol has been rendered Rev-dependent, again minimising any possible opportunity for these genes to be transcribed except during the specific
packaging reaction when all the components are present together. The RSV promoter is used in the pLenti expression virus instead of Tat, again minimising HIV genes.

It should be noted that sequences that have caused concern in other systems, such as the Woodchuck Hepatitis virus sequence, are absent from both these systems.

Origin & function

CDBA to be expressed in this study will be cloned, using established reverse transcription - polymerase chain reaction, technology, from tumour, bone or bone-related (e.g. bone marrow stromal) human or rodent cell lines grown in the laboratory. It is intended that they be used to regulate aspects of normal or tumour-related bone turnover in vitro systems, as well as in mouse models of bone biology or disease. For example, OPG expressed by transduced cells is expected to modulate RANKL-induced osteoclastogenesis in an in vitro setting.

Evaluation of foreseeable effects

Environmental assessment:
These replication-incompetent viruses would be inactivated prior to disposal and, given their inability to replicate, they have no significant additional environmental hazard.

Worst case scenarios:
The above protocols should minimise any risk of exposure to personnel using, or in the vicinity of, work using this lentiviral transfection system. Virus particles, virally-transduced cells, and media and supernatants resulting from these systems will never be handled with glassware or hypodermic needles. Thus, the risks of accidental inoculation are remote. The worst scenario would be exposure to a main stock of virus, resulting from centrifuge failure or spillage. Risks of centrifuge failure-associated contamination (the only readily conceivable mechanism likely to generate a substantial aerosol of many infective particles) should be minimised by Good Laboratory Practice (GLP) and the steps outlined in section 12.

The major exposure routes would be cutaneous, mucocutaneous and respiratory. Effective infection of intact skin is unlikely as skin is a very effective barrier and would be minimised by standards of GLP. The viruses may infect mucocutaneous surfaces and respiratory epithelia and microphages with which they come into contact. However, the particles are replication incompetent and have many safety features preventing generation of replication competent viral particles. None of the genes of interest are known oncogenes. Thus, although there is the possibility of limited tissue infection and damage, this would not result in sustained infection or risk of tumour generation or malignant transformation. It might be possible that exposure would generate an immune response to the VSVG or alternative envelope proteins but this would have a protective effect if so. The overall likelihood of serious or long-term harm resulting from experiments using these vectors is extremely small.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Anti-viral disinfectants (virusolve, virkon, bleach) will always be available prior to starting work involving handling of viral particles, in identified containers easily to hand (e.g spraygun).
- All culture will be performed using a Category 2 hood, in a designated Category 2 molecular biology laboratory (DUI5/16 currently; the Committee would be notified before work commenced in any other laboratory). All cells exposed to virus will be handled in the Category 2 hood. After infection, viral supernatant will be removed and the cells washed. Once the media has been changed on at least one further occasion (after which active viral particles should not be present), then functional assays may involve handling transfected cells for brief periods on the bench using good laboratory practice (GLP) but any cells taken out of the laboratory for analysis (e.g flow cytometry) will be fixed or rendered fully non-viable (e.g treated with formaldehyde solutions prior to flow cytometry).
- Where large volumes of supernatants containing, or potentially containing, viral particles require centrifugation, individually sealable buckets will be used in the relevant centrifuge. The bucket and contents will be inspected before opening (in the Category 2 hood). Buckets would be cleaned appropriately using virusolve, virkon or bleach.
after use. Supernatants from virally-transfected cells which had been cultured for more than one media change over several days would not be regarded as being likely to contain significant numbers of active virus, but would be handled according to GLP and neutralised prior to final disposal.

* Hoods will be carefully cleaned after each session in which culture with virus particles takes place and after each session, when cells that have been transfected by virus on a previous occasion are handled.
* Virus particles and supernatants from infected cells will be handled using filtered pipettes and pipette tips.
* All supernatants, plastic ware and cells that have come into contact with virus particles or viral infected cells will be treated with virusolve, virkon or bleach, at concentrations recommended by the manufacturer, for 20-30 minutes prior to discard.
* Standards of GLP will be rigorously enforced (lab coats, gloves, etc).

The committee identified the point of greatest risk in the procedure as the preparation and handling of the stock virus. Aerosolisation of the virus stock was particularly discussed, and it was noted and found appropriate that Dr Buckle would be performing centrifugation inside sealed buckets at all times. Dr Buckle will put up notices to advise other lab users that non-transmissibkle lentiviral particles are being generated in certain labs, and will add notification when the viral stocks are being generated and handled.

The committee asked for assurance, which it received, that the lentiviral system would not be used to transduce oncogenes and it was stressed that the committee needed to give further consideration, perhaps preemptively, to the procedures that would be needed in handling oncogenes in similar systems.

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Project Ref 168/06.1

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<td>13/06/2006</td>
<td>Lentiviral vector-based gene transfer to transduce terminally differntiated cells and</td>
<td>Class 2</td>
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neuronal cells using both in vitro and in vivo models of neurological disorders.

**Project Additional Information**

**Purposes of the contained use**

The purpose of this project is to assess gene therapy approaches to neuroprotection in experimental models of neurological disorders. Neuroprotection will be achieved by using lentiviral vectors expressing neurotrophic factors or anti-oxidant molecules. We also intend to express siRNA or miRNA to silence dominant genes and assess their effect in models of dominantly inherited disorders. Lentiviral vectors will also be used for gene replacement strategies in models of neurological disorders caused by deletion of genes. Lentiviral vectors will also be used for gene replacement strategies in models of neurological disorders caused by deletion of genes. Reporter genes such as green fluorescence proteins (GFP) or E.coli LacZ will be used as controls.

**Recipient or parental organism**

Various non-pathogenic disabled or non-colonisinf E.coli strains or B-derivatives such as BL-21 or DH5-alpha for propagation of plasmid constructs. Immortalized mammalian cell lines, such as HEK293T will be used for viral production. Hela cells and dog osteosarcoma D17 cells will be used for tittering. It may be necessary to include other established mammalian cell lines in the project in order to achieve certain goals. We also plan to use terminally differentiated mammalian cells including neurons (rodent) both in vitro and in vivo. Both in vitro and in vivo studies will be carried out at containment level 2.

**Host/vector system**

We intend to use both primate and non-primate derived replication defective lentiviral vectors. We will be testing two HIV-1-based vectors: (I) the self inactivating (SIN) HIV-based lentiviral system which will be provided by Dr.Nicole Deglon (France); (ii) commercially available HIV-based Virapower™ system from invitrogen. We also propose to use Equine infectious anaemia virus (EIAV)-based lentiviral vectors which will be provided by Oxford BioMedica Ltd (Oxford, UK).

HIV-derived lentiviral vectors:

We propose to use the self inactivating (SIN) HIV-based lentiviral system which will be provided by Dr. Nicole Deglon under MTA from Commissariat a L'Energie Atomique (CEA) CNRS URA2210, Service Hospitalier Frederic Joliot and ImagiGene Program, Orsay Cedex, France, although it may be necessary to employ other lentiviral systems such as the commercially available HIV-based Virapower™ system from Invitrogen in order to achieve our research objectives. Vectors-based on lentiviruses have been refined to a very high safety and efficiency levels. Any system used will incorporate the safety features outlined below.

1. SIN-lentiviral vector system (from Nicole Deglon)
Viral vectors will be generated with a four-plasmid system ("Fourth generation" HIV-based vectors (Zufferey et al. (1997) Nat Biotechnol 17:871-875; Hottinger et al. (2000) J Neurosci 20:5587-5593). The four plasmids are as follows:

- SIN-cPPT-PGK-W, gene transfer vector.
- pCMVΔR-8.92, packaging construct.
- pRSV-Rev, the accessory protein rev plasmid.
- pMD.G, Vesicular stomatitis virus-G envelope protein. It may be necessary to use other envelope proteins such as rabies-G glycoprotein to achieve specific tropism (see below for more details).

Features that prevent generation of live virus, recombination with endogenous retroviruses and viable HIV.

- Production of the viral vectors from the producer cell lines requires the transfection of 4 plasmids (SIN-cPPT-PGK-W, pCMVΔR-8.92, prsv-Rev, pMD.G) in a transient transfection. All four plasmids have been designed through lack of common sequences, to avoid the possibility of recombination with each other. The plasmids expressing the structural and packaging genes are not packaged with the produced virus, since plasmids expressing the structural and packaging genes are not packaged with the produced virus, since none of them contain LTRs or the packaging RNA sequence. Thus, replication-competent viruses cannot be produced, nor can the packaging
- Vector genomes SIN-cPPT-PGK-WPRE:A 400-bp fragment of the U3 region of the 3'LTR was deleted to obtain self-inactivating (SIN) transfer vector. The mouse phosphoglycerate kinase 1 (PGK) promoter was used as internal promoter. This plasmid was further modified by insertion of the posttranscriptional 1 cis-acting regulatory element WHV.
- pRSV-Rev is rev cDNA-expressing plasmid in which the joined second and third exons of HIV-1 rev are under the transcriptional control of the RSV U3 promoter.
- pCMVDR8.92 (packaging construct): The Vpu, Vpr, Nef and Rev coding sequences has been deleted. The accessory protein Rev is expressed on a separate plasmid. Expression of gag/pol is under the control of CMV promoter.

2. Virapower™ lentiviral expression system:

Features that prevent generation of live virus, recombination with endogenous retroviruses and viable HIV

- The plenti expression vector contains a deletion in the 3'LTR that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentiviral vectors after transduction of the target cell. This is a result of the natural duplication of the 3'LTR into 5' position that occurs during integration. Once integrated into the target cell, the lentiviral genome cannot produce packageable viral genome.
- Only 3 HIV-1 genes are used, gag, pol and rev. The absence of the env gene removes the gp 160 precursor that is responsible for the lethal macrophage/T cell/neurotropism of HIV.
- Production of the viral vectors from the producer cell line requires the transfection of 4 plasmids (three packaging, one the DNA to be packaged) in a transient transfection. All four plasmids have been designed, through lack of common sequences, to avoid the possibility of recombination with each other. The plasmids expressing the structural and packaging genes are not packaged with the produced virus, since none of them contain LTRs or the packaging RNA sequence. Thus, replication-competent viruses cannot be produced, nor can the packaging cell line produce retrovirus in any form during routine culture.
- Expression of gag and pol has been rendered Rev-dependant. Again minimising any possible opportunity for these genes to be transcribed except during the specific packaging reaction when all the components are present together.
- The RSV promoter is used in the pLenti expression virus instead of Tat, again minimising HIV genes.
- Sequences that caused concerns in other systems, such as Woodchuck Hepatitis virus sequence are absent from Virapower™ lentiviral system.

EIAV-based lentiviral vector system (generated by Oxford BioMedica Ltd)

The EIAV minimal vector system

Equine infectious anaemia virus (EIAV) is a non-primate lentivirus that causes a self-limiting, lifelong but rarely fatal infection of all Equidae, and is a world-wide disease of...
horses, most prevalent in warmer climates. The aim when designing a EIAV-based lentiviral vector system is three-fold. The first is to minimise the amount of viral sequence in the transfer vector, thus increasing the capacity for transgenes and associated regulatory elements. The third is to express, in the production system, only those viral proteins required for efficient production of transduction competent vector. All three components, vector genome, Gag/Pol and envelope necessary to generate EIAV-base lentiviral system are being optimised by Oxford BioMedica Ltd and the current status is summarised below.

The safety profile of the EIAV vector system

EIAV vector system was modified with safety in mind making use of the strategies used in the MLV vector field to avoid the production of RCR. The most important features for safety are:

- The partition of the components of the vector system in three independent expression cassettes: vector genome, gag/pol and envelope plasmids.
- The minimal sequence homology between the vector, gag/pol and env components;
- The use of human cell lines with low of endogenous retoviral sequences as the basis for producer cell lines.

Some additional features that will reduce the potential for recombinant formation and the potential pathological consequences arising from the presence of such recombinants in the EIAV vector system are: These features provide a high safety margin.

- The use of a heterologous envelope component. Recombination events can not generate wild type EIAV;
- The use of vectors which have SIN or conditional SIN configurations and therefore are almost transcriptionally silent in transduced cells;
- The elimination of accessory proteins from the system. Tat, Rev and S2 can be eliminated from the system without affecting transduction efficiency, however, Rev is maintained in the producer system since it improves titre by increasing cytoplasmic levels of vector RNA. Expression of Rev is from an independent expression cassette, in which rev is ‘coden-optimised’ to minimise the chances of its involvement in recombination reactions.
- Transcription of the vector genome is driven by the human CMV enhancer/promoter fused to the R region of EIAV so that the first base of the transcript is the same as that formed as a result of transcription from the EIAV U3 region. This configuration allows high titres to be obtained in the absence of Tat protein.
- The coden-optimised gag/pol gene (pESYNPG) is now being utilised for construction of EIAV packaging and producer cell lines. The coden-optimisation process increases the safety of the system in two ways. First, gag/pol mRNA is unlikely to be packaged as efficiently as the wild type gag/pol RNA. Secondly, due to the lack of significant homology with the vector in the region of the packaging signal, it is unlikely to be involved in recombination reactions which might result in generation of replication competent retrovirus (RCR), even if it was incorporated into the vector particle. The optimisation process makes the expression of gag/pol Rev-independent, possibly as a result of removing RNA instability sequences.

Vector production methodology

Vector stocks will be generated by transient transfection of human kidney 293T cells plated on 10cm dishes with 3 plasmids (for EIAV vectors) or 4 plasmids (for HIV-based vectors). We will only grow virus producing cells in filter-capped flasks. After transfection for 24-48 h, supernatants will be filtered (0.45μm), aliquoted and stored at -70°C. Concentrated vector preparations will be made by initial low speed centrifugation at 6000 g and 4°C for 16 h followed by ultracentrifugation at 20,000-23,000rpm and 4°C for 90 min. The virus will be resuspended in PBS for 3-4 h, aliquoted and stored at 70°C.

Origin & function

cDNA to be expressed in these studies will be cloned, using established reverse transcription-polymerase chain reaction, technology. Some cDNA plasmids will be received from our collaborators (Oxford BioMedica Ltd, Oxford, UK; Dr. Nicole Deglon, Paris, France; Dr. Arthur Burghes, Ohio State University, USA). It is expected that neurotrophic factor genes (e.g. IGF-1, NT3, GDNF, VEGF) will be used to enhance survival of neuronal cells in vitro systems as well as in vivo models of neurodegenerative diseases. Some genetic material such as siRNA and miRNA expressed transduced cells is expected to suppress a toxic gain in function of genes with dominant mutations.

Evaluation of foreseeable effects

Environmental assessment:
The replication defective viruses would be inactivated prior to disposal and given their inability to replicate, they have no significant additional environmental hazard. The bacteria will also be inactivated (by disinfection) before they leave the laboratory.

Worst case scenarios:

The above protocols should minimise any risk of exposure to personnel using, or in the vicinity of, work using these lentiviral vector systems. The worst scenario would be exposure to a main stock of virus, resulting from the centrifuge failure or spillage. Risks of centrifuge failure-associated contamination (the only readily conceivable mechanism likely to generate a substantial aerosol of many infective particles) should be minimised by Good Laboratory Practice (GLP) and steps outlined in section 12. The major exposure routes would be cutaneous, mucocutaneous and respiratory. Effective infection of intact skin is unlikely as skin is a very effective barrier and would be minimised by standards of GLP. The viruses may infect mucocutaneous surfaces and respiratory epithelia and macrophages with which they come into contact. Again this would be minimised by strict adherence by trained personnel to proper laboratory procedures e.g. use of microbiological safety cabinet class II, masks, eye protection and gloves will be observed. However the particles are replication defective and have many safety features preventing generation of replication competent viral particles. The vectors are replication defective hence spread from an initially infected cell is very unlikely. None of the genes of interest are known oncogenes. Some vectors to be used for this project may contain wild type or truncated forms of woodchuck post-transcriptional regulatory element (WPRE) sequences. Following the recommendation from HSE and the Scientific Advisory Committee on Genetic Modification (SACGM), measures will be taken to minimise the risks when using these sequences:

- Good microbiological practice by using safety cabinet class II
- Adopt procedures that reduce the likelihood of exposure, such as wearing gloves, mask and eye protection, etc
- Animal work which involves direct injection of the vector will be performed with caution and under good laboratory practice. Direct injection of the vector into the brain and spinal cord will be performed using Hamilton syringes fitted with blunt needles. Stereotaxic administrations will be performed using a 5µl Hamilton syringe with a 33-34 gauge blunt-tip needle. The vector solution will be slowly infused at the speed of 0.1-0.2µl/min using infusion pump. Thus, the risks of accidental self injection are remote. However, intramuscular (I.m.) administrations of the vector need to be done using type 2 needle (10°-12° Bevelled, non-coring needle; 33-34 gauge). During i.m. injections, operators will be asked to wear 2 pairs of gloves. We believe that, using 5µl Hamilton syringe, operators could not accidentally inject significant amount of vector if they mishandled the syringe. Based on our previous experience, it is almost impossible for an accident to happen because the 33-34 gauge needles are very thin and could be easily damaged in contact with the skin. Accidents would be minimised by strict adherence by well trained personnel to good laboratory practice. Intramuscular injections will be performed by scientists with extensive experience in the field. Professor Azzouz has successfully used this technique in the past (for more details see Azzouz et al., Nature 429: 413-417 (2004): Ralph et al., Nature Medicine, 11 (4):429-33 (2005)) and will provide training for staff in his team.

N/A

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Anti-viral disinfectants (virusolve, virkon, bleach) will always be available prior to starting work involving handling of viral particles, in identified containers, easily to hand (e.g. spraygun).
- All culture will be performed using a Class 2 hood, in a designed Class 2 laboratory. All cells exposed to the virus will be handled in the category 2 hood. After infection, viral supernatant will be removed and the cells washed. Once the media has been changed on at least one further occasion (after which active viral particles should not be present), then functional assays may involve handling transfected cells for brief periods on the bench using good laboratory practice (GLP) but any cells taken out of the laboratory for analysis (e.g. flow cytometry) will be fixed or rendered fully non-viable (e.g. treated with formaldehyde solutions prior to flow cytometry). Where large volumes of supernatants containing, or potentially containing, viral particles require centrifugation, individually sealable buckets will be used in the relevant centrifuge. The buckets and contents will be inspected before opening (in the Class II hood). Buckets would be cleaned appropriately using virusolve, virkon or bleach.
after use. Supernatants from virtually-transfected cells which had been cultured for more than one media change over several days would not be regarded as being likely to contain significant numbers of active virus, but would be handled according to GLP and neutralised prior to final disposal.

- Hoods will be carefully cleaned after each session in which culture with virus particles takes place and after each session, when cells that have transfected by virus on a previous occasion are handled.
- Viral particles and supernatants from infected cells will be handled using filtered pipettes and pipetted tips.
- All supernatants, plastic ware and cells that have come into contact with virus particles or viral infected cells will be treated with virusolve, virkon or bleach, at concentrations recommended by the manufacturer, for 20-30 minutes prior to discard.
- Standards of GLP will be rigorously enforced (lab coats, eye protection, gloves, etc).
- Other lab personnel will be made aware of the use of lentiviral systems in the laboratory.

Please see annex for handling protocols and contamination management.

Contamination management
1. Where spills occur in contained environments such as Class 2 hoods, neat virusolve, bleach, or virkon will be added and left for 20-30 minutes to neutralise viral particles prior to wiping, cleaning and disposal.
2. If a small contained spill occurs outside of the Class 2 hoods, management will be as above. Any spill outside of Cat 2 hood will be reported to a principal investigator, and assessment made to determine whether avoidable features are present to prevent future accidents.
3. Where aerosolised contamination is suspected to have occurred outside of Class 2 hoods (e.g. as a result of centrifuge failure), the lab will be cleaned immediately to prevent aerosol inhalation. The air conditioning system will replace the air within the room within 10 min and will serve to desiccate and dilute the hazard. A single designated worker will return to the lab after 15 min and apply appropriate disinfectant to liquid traces.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

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02/03/2022  Page 4248 of 15326
The aim of the project is to investigate the role and regulation of genes involved in:

(i) stress sensing and survival (particularly extracytoplasmic function (ECF) sigma factors, their regulators and target genes)
(ii) secretion systems (including components of the secretion machine, their potential substrates and regulators)
(iii) iron acquisition, including regulatory genes
(iv) exopolysaccharide production, including regulatory genes

in the Burkholderia cepacia complex. Some of these genes are likely to play a role in virulence. In addition, we would like to overexpress potential secreted effector proteins from Bcc in E. coli for structure-function analysis.

Recipient or parental organism

Burkholderia cepacia complex (8cc).

The 8cc contains at least 10 closely related environmental saprophytic Gram-negative bacterial species (i.e. 8. cepacia, 8. cenocepacia, 8. multivorans etc), some strains of which can behave as a phytopathogens, while others have plant-protective effects. They can cause opportunistic infections in humans with cystic fibrosis (CF) and chronic granulomatous disease (CGD) Hazard Group 2 biological agent.

(iii) Escherichia coli strains K-I2 and B. Non-pathogenic, limited or no colonisation ability. Hazard Group 1.

Host/vector system

8. cenocepacia strains: 71 5j, J231 5, K58-2, Hill and representative strains from other members of the 8cc, i.e. ATOC 17760.
E. coli strains: MC1061, JM83, DH5cc (hosts for routine cloning).
CC118QLpir) (host for maintaining suicide plasmids).
S17-1, BW19851, 517-i (A.pir), SM1OQ.pir) (hosts for delivering plasmids by conjugation).
8L21 (DE3) (host for overexpression of 8cc proteins).
8cc plasmids: pBBR1MCS and derivatives carrying alternative antibiotic resistance markers (routine cloning vector).
pUCP22, pUCP2ST, pRK415 (routine cloning vectors)
PTZ10, pPR9TT derivatives, pME6O14, pME6O16 (for making iacZ reporter fusions).
pUT derivatives* (containing mini-Tn5 transposons for mutagenesis)
PSHAFT*, pGSStp* pJP56o3* pKNOCK* (for making chromosomal mutants).
F. coli plasmids: pHG165, pUC19, pBluesc np, pACYC184, pLG339, pWSK29 (routine cloning vectors)
pRW50 (for making iacZ reporter fusions)
pET4b, pTrc99A, pGEX-KG (for overproduction of 8cc proteins).
*suicide plasmids (these do not replicate in 8cc. Used as chromosomal integrative vectors or for allelic replacement or to deliver transposons to 8cc).
non-mobilisable broad host-range plasmid.
Further details of strains and plasmids are appended to the accompanying risk assessments.
Note: all plasmids used in 8cc must necessarily also be passaged through E. coli strains, since the cloning steps are carried out using transformable E. coli strains and delivery of the genetic material requires an E. coli donor strain.

Origin & function

(i) 8cc genes involved in sensing and responding to environmental stress (i.e. sigma factors and their regulators, as well as genes that are targets of sigma factors and response regulators that play a role in stress sensing and the stress response). The sigma factor genes are identifiable from the genome sequence, but the regulators and the genes that are targets of each sigma factor are currently unknown.
(ii) 8cc genes involved in protein secretion. Of particular interest is the type VI system, although it is possible that other secretion systems may also be investigated. Genes of interest include those encoding the components of the secretion system, their regulators and candidate substrate (i.e. secreted effector) genes. The genes encoding the different secretion systems are identifiable from the genomic sequence, but the genes encoding the substrates of these pathways (i.e. the secreted effectors) and the regulators are currently unknown.
(iii) 8cc genes involved in iron acquisition and its regulation.
(iv) 8cc genes involved in exopolysaccharide production and its regulation.

Activity 1: Transposon mutagenesis of 8cc. In this approach, a library of mini-Tn5 transposon mutants will be created, in which individual genes within the 8cc genome are insertionally inactivated. Mutants of interest are identified by their phenotype on agar plates (for example, loss of siderophore production, a secretion defect or stress sensitivity).

Activity 2: Subcloning transposon and flanking 8cc genomic DNA into E. coli K12*. Identification of transposon-inactivated genes generated in Activity 1 will be carried out by cloning the transposon and flanking genomic DNA into a plasmid (i.e. pHG165, pUC19 or related plasmids) in E. coli. follow by DNA sequencing.

Activity 3: Targeted inactivation of chromosomal 8cc genes. Genes of interest that are identified by genome sequence analysis will be inactivated to determine the role of the gene. This procedure will involve cloning the 8cc gene into a suicide vector in E. coli, inactivating the gene with an antibiotic or toxic metal resistance cassette, and then transferring the vector to 8cc by conjugation or electroporation. Selection will be imposed for recombinants that have undergone allelic replacement.

Activity 4: Cloning full-length Bcc gene(s) or operon into a broad host-range plasmid and introduction into corresponding mutant (complementation). This confirms that the phenotype of the mutant(s) of interest is due directly to the presence of the transposon (Activity 1) or antibiotic resistance cassette (Activity 3) in the gene of interest, rather than polar effects on downstream transcription. In cases where plasmid expression of a complementing gene turns out to be toxic to the bacterium, the re-introduced gene may be placed under control of a regulatable promoter.

Activity 5: Construction of reporter fusions in 8cc. To monitor the expression of 8cc genes, regulatory regions will be fused to iacZ (or another reporter gene such as gusA, gfp or lux) on a broad host-range, non-self-transmissible plasmid or an integrative (i.e. suicide) vector which will then be introduced into the appropriate host. Where applicable, the regulation of 8cc promoters may also be studied in E. coli K12, either using narrow or broad host-range plasmids.

Activity 6: Overexpression of secreted effector proteins in E. coli. To perform structure-function analysis of proteins secreted by the various secretion systems of 8cc, genes encoding such proteins will be expressed at a high level from a strong inducible promoter contained on an E. coli-specific plasmid. The gene may be fused to a sequence (i.e. hexahistidine or GST tags) that facilitates purification of the overproduced protein.

*As Activity 2 relies on use of GMM5 generated in Activity 1, the risk assessment for Activity 1 will cover this activity.
Evaluation of foreseeable effects

8cc are environmental saprophytic Gram-negative bacteria some of which can behave as phytopathogens, but many species also have plant-protective effects. They can cause opportunistic infections in (compromised) humans with cystic fibrosis or chronic granulomatous disease. They are unlikely to cause an infection in a healthy person. The antibiotics used for selecting plasmids and transposons in these organisms, are not generally used in the treatment of 8. cenocepacia infections. E. coli K-12 and E. coli B are potential colonisers of humans and other animals but are non-pathogenic. Most laboratory strains of C. coli K-12 are not able to colonise humans.

Thus, the acquisition of the antibiotic resistance markers to be used in these activities is very unlikely to constitute a hazard to human health or the environment. Any foreseeable additional properties of these organisms as a result of genetic modification are discussed below.

Activity 1: Transposon mutagenesis. This procedure results in gene inactivation, so it is unlikely that 8cc mutants will be more pathogenic or will be more able to survive in the environment as a result. Mutants will have gained resistance to an antibiotic or, where the iacZ reporter gene is present, gained the ability to hydrolyse lactose. These are not expected to increase the environmental fitness or virulence of the GMM. The plasmid used to deliver the transposon will be a mobilisable suicide vector which cannot be maintained in 8cc.

Activity 2: Subcloning transposon and flanking Dcc genomic DNA into 8. coli K-12. The foreseeable effects on 8cc are the same as described in Activity 1, since the mutants are derived from Activity 1. For the E. coli hosts, this activity will confer resistance to two antibiotics as specified by the plasmid vector and the marker in the cloned transposon. Individual E. coli clones may also express one or more 8cc genes either from a 8cc promoter present on the cloned insert or from a vector promoter present on the plasmid. This could lead to high level expression of 8cc genes in some cases. However, as the laboratory strains of E. coli K-12 employed are non-pathogenic, have a reduced fitness for survival, and do not possess mobilisation functions, there is a negligible risk of harm to humans or the environment.

Activity 3: Targeted inactivation of chromosomal 8cc genes. The initial step will involve the cloning of the 8cc gene of interest into a non-self-transmissible plasmid in E. coli and inactivating it with an antibiotic or metal resistance cassette. The inactivated gene will then be transferred to a suicide vector that is able to replicate only in an E. coli K-12 strain containing the pir gene. Thus, although it is mobilisable, the suicide plasmid will not replicate in any other host. The suicide plasmid containing the inactivated gene will then be transferred to an E. coli delivery strain, which is competent to mobilise the plasmid. In all these steps, there are no adverse foreseeable effects regarding human health or the environment associated with the E. COIL strains for reasons discussed in Activity 2 above. Individual 8cc genes would be extremely unlikely to render the E. coli laboratory strains fit to establish themselves in a human host or to harm a human or the environment.

The plasmid will then be introduced into 8cc, where it fails to replicate. Selection for the antibiotic resistance cassette inserted into the cloned gene allows for the isolation of rare recombinants where the plasmid has integrated into the genomic copy of the 8cc gene by single or double crossover homologous recombination. Double crossovers, resulting in aTlelic replacement and loss of plasmid sequences, will be identified and retained. The resultant strain, containing an insertionally inactivated gene, will be very unlikely to harm healthy individuals and will have acquired resistance to a single antibiotic or toxic metal. However, if a gene encoding a repressor of genes governing iron acquisition or a stress-response, or biosynthesis of a secretion pathway or exopolysaccharide, is inactivated, the GMM may express these genes at an inappropriate time or to a higher level. This is unlikely to make the GMM pathogenic to a healthy individual.

Activity 4: Introduction of individual Sec genes or operons on a broad host range vector into the corresponding mutant (complementation). Apart from the acquisition of the plasmid antibiotic resistance marker, it is unlikely that the phenotype of the recipient would be significantly different to the phenotype of the parent 8cc strain: either complementation will occur or it will not. Although no deliberate attempt is being made to overexpress the gene of interest, it is possible, given the multicopy nature of the system, that in some cases it will be expressed at higher levels than in the wild type strain. However, this is very unlikely to make the GMM more harmful to a healthy individual. Due to the fact that the BHR plasmids to be used are non-self-transmissible (i.e. pBBR1MCS series), they could be mobilised to another organism in the environment if the GM 8cc host naturally acquires a self- transmissible plasmid. However, in most cases, the cloned DNA would be very unlikely to confer a fitness advantage on another bacterium or render it harmful to humans or the environment. In some complementation experiments, the gene under investigation may encode a secreted effector. At present we do not know which genes encode the effectors secreted by the Type VI system or what their functions are (this is one aim of the programme of work to which this notification pertains). To be secreted by the recipient bacterium, the new host would have to express a compatible secretion system. There are unlikely to be any detrimental effects of cloning such genes in E. coli K-12, as the strains used are disabled and cannot colonise. Also, E. coli K-12 does not encode a Type VI secretion system.

Activity 5: Construction of reporter fusions in the 9cc. As only regulatory regions and gene segments, rather than complete gene sequences, are to be cloned into BHR plasmids or suicide plasmids and delivered into wild type or mutant 8cc, there is likely to be a negligible risk associated with this activity. The iac, gus, lux and gfp genes have been used extensively as reporter genes in bacterial systems without deleterious effects. Due to the fact that the BHR plasmids used are mobilisation-defective (i.e.
pPR9TT, pTZ11O), they could be mobilised to another organism in the environment if the GM Bcc host naturally acquires a plasmid containing mobilisation functions. However, the cloned regulatory DNA would be unlikely to confer a fitness advantage on another bacterium or render it a danger to humans or the environment. Integrative (suicide) reporter plasmids cannot be transferred from Bcc to another organism by conjugation as they have integrated into the bacterial genome and cannot replicate. In some cases it may be possible to investigate the regulation of a promoter in E. coli K-12 (for example in the presence of its regulator expressed in tiwis). As the E. coli host will be disabled there are no unforeseen deleterious effects of expressing a regulatory protein from a compatible plasmid.

Activity 6: Overexpression of putative Type VI secreted effector proteins from the Sec in 8. coli. For plasmids which depend upon the phage T7 41 promoter for high level expression of genes in E.coli, the E. coli B host BL21 (DE3) will be used. This strain can be viewed as being equivalent to E.coli K-12 in terms of its ability to behave as a pathogen. In some cases other strong inducible promoters may be used such as the tac promoter. Plasmids used will be derived from the narrow host-range plasmids pBR322 and pUC19 (i.e. pET series, pTrc99A or pGEX series) and will confer ampicillin or kanamycin resistance upon the host. Overproduction of an individual effector protein in BL21 (DL3) is unlikely to render it more pathogenic or increase its fitness for survival in the gut or in the environment, even though such proteins are likely to contribute to the virulence of their natural host. Such proteins require a cognate secretion system for their delivery out of the cell. E. coli K-12 does not encode a Type VI secretion system but it is not yet known whether E. coli BL21 (DE3) possesses a Type VI secretion system. As the identity and mechanism of action of the effectors is currently unknown, it would be prudent to designate the work as a class 2 activity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation not requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMM waste will be inactivated prior to leaving the premises. Small volumes of liquid culture and small disposable items (i.e. plastic tubes, cuvettes and pipette tips) will be inactivated by immersion in disinfectant (5% Virusolve Plus) for a minimum of one hour. This has been validated for wild type B. cenocepacia by mixing 1.0 ml of Virusolve Plus with 1.0 ml of an overnight culture (12 x 1 o cfu/ml) (i.e. final concentration 2.5% Virusolve Plus), leaving foil hour at room temperature, and plating out 0.1 ml dilutions of the mixture on nutrient agar plates. No growth was observed on any of the plates (i.e. > 7.78 log reduction). In an assay run in parallel, Virusolve-treated samples were 'spiked' with untreated bacteria immediately prior to plating. This confirmed that the disinfectant was inactive on agar plates under these experimental conditions (thus validating the assay). In a separate assay, B. cenocepacia colonies were resuspended in broth at 1 5 x 1010 cfu/ml and treated with Virusolve Plus as described above This assay demonstrated complete killing of B. cenocepacia (i.e. > 8.9 log reduction for 8. cenocepacia).

Solid material inactivated by disinfectant will be autoclaved in the same building and then transferred to appropriate containers, whereupon it will be taken to another site by approved contractors for steam pressure treatment. All other items (larger cultures, agar plate cultures! contaminated glassware, larger items of contaminated plasticware) will be inactivated by autoclaving in the same building autoclaves are regularly serviced). This has also been validated by autoclaving an overnight culture of 8. cenocepacia (1.2 x 10 cfu/ml) and plating 0.1 ml of the undiluted culture on nutrient agar plates. This treatment was shown to be 100% effective. Disposable items that have been inactivated by this method will be placed in appropriate waste bags and removed from the site by approved contractors for steam pressure treatment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Recent studies have provided new insight into a way of reprogramming mature cells into a pluripotent embryonic stem cell stage. They show that the over-expression of four or recently even three or two transcription factors, can reprogram mature cells to a more immature cell type, seemingly with the same abilities as human embryonic stem cells (hESC). The ethical concerns connected to the derivation source of hESC could eventually be dismissed, as well as the potentiality of patient specific cells is promising. Compared to standard MLV-based retroviral vectors, which only transduce dividing cells, replication-defective lentiviral vectors (a subgroup of retroviral vectors)
provide an efficient method of gene transduction into both mitotic and post-mitotic primary cells. Additionally they have shown to be specifically effective in hESCs, where they are less silenced than their retroviral counterparts. To study hESCs and the pathways behind the re-programming, we intend to set up this lentiviral expression system in our facilities, able to transfer and appropriately express transcription factors to produce induced pluripotent stem (iPS) cells.

Recipient or parental organism

Bacterial host:
In all experiments the bacterial host strains used are non-pathogenic E. coli B and K12 derivatives: HB101, Stbl3, DG5-a, JM109, XL-1blue (genotypes listed in attached appendix). All are non-colonising and disabled and are therefore unlikely to survive either in the gut or in the environment. We consider assignment of ACDP hazard group1 appropriate.

Mammalian host:
Experimental procedures using virus to introduce novel genetic elements will be performed on human fibroblasts or other primary human cell types, hES cells, hEC cells and their derivatives. Virus particles will be manufactured in Human Kidney 293T cells transformed with SV40 large T antigen. As with all mammalian cells these cell lines are sensitive to culture conditions and have minimal survival potential outside of their normal tissue-culture environment.

Host/vector system

Lentiviral vector System - Overexpression of Transgenes
The majority of viral proteins have been removed from 3rd generation lentiviral transfer vectors; in particular the virulence genes vpr, vif, and nef are not present and are not required for transgene expression. The genes required to produce a packaged, transducible virus are distributed among at least 3 separate vectors. It is unlikely that a replication-enabled lentivirus could be produced, as it is unlikely that the three required recombinations would occur. As a precaution against the minimal risk, however, the work on human cells will be performed at Containment Level II.

In order to produce 3rd generation replication deficient lentiviral vectors, 4 plasmids must be co-transfected into 293 T cells.

We use similar vectors as published (Yu et al. 2007, Science)

1) Transfer vector containing the transgene
   (pSin-EF2-[transgene]-Pur; Transgenes: Lin 28, Nanog, Oct4, Sox2; all from Addgene),
2) Packaging plasmid 1 (gag_polMDLg/pRRE, Addgene),
3) Packaging plasmid 2 (pRSV-Rev, Addgene),
4) Plasmid (VSV-G amphotropic envelope, plasmid pMD2.G, Addgene)

Safety features of 3rd generation lentiviral vectors:

1. Vectors contains a deletion in the 3’LTR that does not affect generation of the viral genome in the producer cell line, but results in 'self-inactivation' of the lentivirus after transduction of the target cell. This is a result of the natural duplication of 3’LTR into 5’ position that occurs during integration. Once integrated into the target cell, the lentiviral genome cannot produce packageable viral genome.
2. Only three HIV-1 genes are used, gag, pol, and rev. the absence of the env gene removes the gp160 precursor that is responsible for the lethal macrophage/T cell/ neuro-tropism of HIV.
3. Production of the viruses from the producer cell line requires the transfection of 4 plasmids (three packaging, once the DNA to be packaged) in a transient transfection. All four plasmids have been designed, through lack of common sequences , to avoid the possibility of recombination with each other. The plasmids expressing the structural and packaging genes are not packaged with the produced virus, since none of them contain LTRs or the packaging RNA sequence. Thus, replication-competent viruses cannot be produced, nor can the packaging cell line produce retrovirus in any form during routine culture.
4. Internal promoters are used to eliminate the need of the HIV-promoter and reduce the risk of recombination events and trans-activation of surrounding genes.
The project requires the use of genes and/or gene fragments sourced either by cloning or from third parties to be overexpressed in human cells.

We want to induce transcription factors shown to induce reprogramming of cells to iPS cells. These transcription factors are Oct4, Nanog, Sox2 and Lin28 (Yu et al. 2007, Science), or Oct4, Sox2 alone with valproic acid (Huangfu et al. 2008, Nat biotech.), which need to be applied in combinations. These human genes are important for pluripotency but are not related to pathogenic phenotyped or known to be oncogenic.

However, one cannot exclude the possibility of tumo-inducing effects, as their function is not well defined. In order to reduce the possibility of accidental exposure of a worker to virus capable of introducing these genes, we propose to carry out the handling of live virus and transduction of EC/ES cells in our designated Class 2 facility.

The established 3rd generation lentiviral vector backbones may contain expression enhancing viral elements such as the WPRE element. These elements are inserted into the cells and may be oncogenic in some mouse models and it is therefore suggests to handle it in a class II environment.

The transfer vectors will possibly include but will not exclusively comprise selection markers such as PuromycinR, BlasticidinR, AmpicillinR or fluorescent proteins such as mCherry, GFP, Venus, Cerulean, mTomato. These proteins are considered non-hazardous owing to their non-mammalian origin. There is no evidence of any oncogenic potential for these proteins thus we consider it highly unlikely that exposure to these proteins would result in harmful effects. However we propose to carry out the handling of live virus and transduction of EC/ES cells in the designated Class 2 facility.

Origin & function

Environmental assessment:
These replication deficient viruses would be inactivated prior to disposal and, given their inability to replicate, they have no significant additional environmental hazard.

Worst case scenario

The above protocols should minimise any risk of exposure to the personnel using or in the vicinity of work using this lentiviral transfection system. Virus particles, virally-transduced cells, and media and supernatants resulting from these systems will never be handled with glassware or hypodermic needles. Thus, the risks of accidental inoculation are remote. The worst scenario would be exposure to a main stock of virus containing one transgene, perhaps resulting from spillage. The major exposure routes would be cutaneous, mucocutaneous and respiratory. Effective infection of intact skin is unlikely as skin is a very effective barrier, and would be minimised by standards of Good Laboratory Practice. The viruses would infect mucocutaneous surfaces and respiratory epithelia and macrophages with which they came into contact. However, the particles are replication incompetent, and have many safety features preventing generation of replication competent viral particles. The genes of interest are not oncogenes. Thus although there might be limited tissue infection and damage, this would not result in sustained infection nor risks of tumour generation or malignant transformation. It might be possible that exposure would generate an immune response to the VSVG protein, but this would have a protective effect if so. The overall likelihood of serious or long-term harm resulting from experiments using these vectors seems extremely small.

Evaluation of foreseeable effects

Not applicable

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

We propose to handle all material in Category 2 culture conditions.
1. Anti-viral disinfectants (virusolve, virkon, bleach) will always be available prior to starting work involving handling of viral particles, in identified containers easily to hand (e.g. spray gun).
2. All culture will be performed using a Cat 2 hood. After infection, viral supernatant will be removed and the cells washed. Once the media has been changed on at least one further occasion (after which active viral particles should not be present), then functional assays may involve handling transfected cells for brief periods on the bench using GLP.
3. Where small volumes of cells potentially containing viable viral particles require centrifugation, tubes will be sealed with parafilm prior to centrifugation.
4. Virus particles will be stored in the -80°C freezer in E229, in cryovials, held inside 50 ml falcon centrifuge tubes or equivalent, in clearly marked packaging.
5. Hoods will be carefully cleaned after each session in which culture with virus particles takes place, and after each session when cells that have been transfected by virus on a previous occasion are handled.
6. Only designated media will be used in cell culture.
7. Virus particles and supernatants from infected cells will be handled using filtered pipettes and pipette tips.
8. No glassware or hypodermic needles will be used at any time with any culture producing or containing virus particles or any supernatant generated from any cell that has ever been exposed to virus particles.
9. All supernatants, plasticware, and cells that have come into contact with virus particles or viral infected cells will be treated with virusolve, virkon, or bleach at appropriate concentrations and for 20-30 minutes prior to discard.
10. Standards of GLP will be rigorously enforced (lab coats, gloves, etc).
11. All personnel using or producing virus will be appropriately registered through the University Health and Safety procedures, listed on the GMAG approval, and taught appropriate safe handling by the principal investigator.

Contamination management

1. Where spills occur in contained environments such as Cat 2 hoods, neat virusolve bleach, or virkon will be added and left for > 10 minutes to neutralise viral particles prior to wiping, cleaning and disposal.
2. If a small contained spill occurs outside of Cat 2 hoods, management will be as above. Any spill outside of the Cat 2 hood will be reported to a principal investigator, and assessment made to determine whether avoidable features are present to prevent future accidents.
3. Where aerosolised contamination is suspected to have occurred outside of Cat 2 hoods, the lab will be cleared immediately to prevent aerosol inhalation. The air conditioning system will replace the air within a room within 10 min and will serve to desiccate and dilute the hazard. A single designated worker will return to the lab after 20 min and apply appropriate disinfectant to liquid traces.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

This has been submitted to and approved by the Local Genetic Modificaton Safety Committee.

Internal GMO project number: GMO 2008_15 (Reviewed January 15, 2009)

Project Containment
The aim of the project is to prepare, isolate, store, and utilise recombinant, replication-deficient adenoviral particles to introduce wild-type or mutant forms of genes encoding either proteins involved in intracellular membrane trafficking or cell cycle checkpoint proteins into a variety of model mammalian cell lines to evaluate their cellular function.

(i) intracellular membrane trafficking involves the continuous sorting of membrane and soluble components between distinct membrane compartments within the cell and which often occurs by the packaging of material into small vesicles carrying specific cargo molecules. This work focuses on the internalisation and processing of clathrin-coated pits as a paradigm of membrane traffic control and seeks to identify components participating in and required for, coated vesicle formation, and progression through the endocytic pathway and to determine how dynamic interactions between these proteins are regulated both temporally and spatially.

Proteins involved in clathrin-mediated endocytosis include liganded receptors such as the transferrin receptor, TIR, coat and ancillary proteins such as clathrin, adaptins and dynamin, and regulators including the small GTPase rab5, GEFs RME-6 and Rabex-5, and the protein kinase AAK1.
(ii) cell-cycle checkpoint pathways in mammalian cells.

This work is concerned with understanding mechanisms of genomic integrity operating at the molecular and cellular level, and determining the consequences when they fail. Chromosomes in eukaryotes control their environment to ensure that genomic integrity is maximised. In normal cells, cell cycle checkpoints serve as a mechanism to monitor chromosomal integrity. In the progression towards tumorigenesis, checkpoint dysfunction is a commonly acquired molecular defect. Conventional cancer therapies, such as cytotoxic chemotherapy and ionising radiation, activate cell cycle checkpoints as part of their mechanism of action and seek to kill cancer cells on the basis of generating sufficient DNA damage to prevent cell replication and promote cell death.

Checkpoint pathways broadly consist of sensors (such as the 9-1-1 complex) which detect breaches in genomic integrity, proximal (such as the inherited breast cancer susceptibility gene, BRCA1, and ATR/ATRIP protein complex,) and distal transducers (such as Chk1 protein kinase, which signal this information through-out the cell, and effectors (such as cdc25 protein phosphatase) which enact cell cycle delay, initiate DNA repair pathways or promote apoptosis.

The broad aim of these projects is to gain a detailed understanding of the cellular mechanisms by utilising adenovirus vectors to constitutively express active or mutated forms of relevant components in cultured mammalian cells thereby manipulating the total cellular activity. Adenovirus vectors are used for protein expression in cells where the transfection efficiency is poor, or where high level or prolonged expression is required.

Recipient or parental organism

1. PER.C6 cells. These well-characterized and authenticated tissue culture cells are derived from human embryonic retinoblasts and have been transformed with the E1a gene of Adenovirus. They thus complement replication-defective adenovirus which lack this gene and are used for the propagation of the otherwise replication-defective virus.
2. A variety of mammalian cell lines such as human or simian lines HepG2, BeWo, HeLa, COS, U2OS, primary culitures (HUVECS) and rodent lines (3T3, L6, CHO). These cells have all been characterised with a history of safe use and are equivalent to ACDP hazard group 1.

Host/vector system

The AdEasy system (Stratagene, USA) includes a shuttle vector (pShuttle) for creating mammalian expression cassettes. Once constructed (through standard restriction enzyme digestion and ligation of the components) the shuttle vector, containing the relevant cDNA sourced from a mammalian expression plasmid background, is a linearized using Pme1 restriction enzyme. Linearised shuttle DNA is co-transformed into the bacterial line, BJ5183 along with the pAdEasy-1 plasmid (contains most of the human adenovirus serotype 5 (Ad5) genome, which is deleted for the genes E1 and E3). The removal of E1 and E3 renders the viruses defective for replication and incapable of producing infectious viral particles in target cells. In the BJ5183 line, the shuttle vector and pAdeasy-1 plasmid undergo homologous recombination. Transformants are selected with standard kanamycin resistance and successful recombination is determined through restriction digestion. Successful recombinants are amplified through transformation and culture in the XL-10 Gold bacterial strain. Purified recombinant adenoviral DNA is digested with Pac 1 (to generate appropriate inverted repeat sequences) and is then transfected into PER.C6 cells.

Recombinant adenovirus is harvested by freeze/thaw lysing of transfected cells. Viral particles are isolated and stored, in screw cap Eppendorf tubes at -80°C, for infection of selected host cells in order to express proteins in these cells.

Origin & function

Genes of human or rodent origin which are known or suspected to participate in mammalian cell cycle checkpoint or membrane trafficking pathways will be used to generate recombinant replication-deficient adenoviral particles capable of infecting a broad range of mammalian model cell lines. The intended function of the GMM is to act as a vehicle for the introduction of wild-type, tagged, dominant-negative or other mutants of genes of interest into cell lines to study their molecular function or to investigate the effects of restoring these genes in cells lines which currently lack them , to investigate their ability to rescue functional irregularities specific to each cell line.

Evaluation of foreseeable effects

The constructs to be used in these studies have not been reported to cause immortalisation or transformation in human cells and in many cases have been used for expression in adenoviral systems in other laboratories with no known harmful effects. Insertion of the constructs will not alter the tissue tropism, or increase the infectivity or pathogenicity of the recipient vector. Scope for recombination with wild type virus is limited and, due to the packaging limits of adenovirus, any such recombinants which might arise would be unviable. Additionally the recombinant adenovirus remains epichromosomal in a human cell, resulting in a minimal chance of insertional
activation/inactivation of host cellular genes. The expression procedure will involve the amplification of adenoviral particles carrying sequences coding for known or suspected checkpoint proteins or membrane trafficking proteins none of which have been shown, or are believed, to be oncogenic. The modified adenovirus proposed is replication-deficient, however in view of the capability of these vectors to infect human cells by aerosol or droplet route, and the high viral titres obtained, the level of containment is Level 2.

In the very unlikely event of a release from the Level 2 Containment facility, the virus might survive in the environment for a similar period of time to the wild-type virus. If the virus were to infect a susceptible host, then expression of the recombinant genes would occur in the infected cells. Since the virus cannot propagate in these circumstances, expression would be limited to the cells initially infected (i.e., once the cell divides, only one daughter cell will contain the adenovirus DNA. Eventually, this would result in loss of the DNA due to natural degradation of the episomal DNA. The effect that expression of the recombinant DNA would have on the cells is expected to be negligible.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The local rules for working in the containment level 2 laboratories will be followed. These practices include the rules for work in a microbiological safety cabinet. Gloves and laboratory coats must be worn at all times. All cuts or abrasions will be covered with waterproof dressings. No sharps of any type (glass Pasteur pipettes, needles, razors) will be present in the vicinity of work when handling viral stocks. Waste will be bagged for autoclaving, and the bags sealed before removing from the designated rooms. Contaminated tips, pipettes etc will be treated with 3% Virkon for at least 60 minutes before draining and disposal into an autoclave bag. Bottles of media used for infection will be isolated from all others and immersed in 3% Virkon before being placed into standard glassware containers. Liquid waste will be treated with 3% Virkon for at least 60 minutes before disposal.

**INCUBATORS**

- Incubators reserved for transduced cultures will be used.

**SPILLAGE**

- Spills will be treated with 3% Virkon. If there is a spillage on the laboratory coat, it will be placed into a biohazard bag and autoclaved.

**AVOIDANCE OF AIRBOURNE TRANSMISSION**

- Procedures will be adopted to avoid the formation of aerosols. Aerosol production will be minimised through the use of microbiological practice and transduced cultures will only be handled within a microbiological safety cabinet (to BS 5726).

**TREATMENT OF VIRUS BEFORE DISPOSAL**

- By autoclaving cultures and plasticware which were in contact with transduced cells and transduced cells the virions will be completely inactivated. Liquid waste will be treated with 3% Virkon for at least 60 mins.

**PROCESSING OF MATERIALS FOR USE IN LOCATIONS OUTSIDE OF THE VIRAL SUITE**

- In the viral suite, media containing virus will be removed from cell cultures and disinfected with 3% Virkon for 60 min. Cells will then be washed extensively (the wash again disinfected) in order to remove viral particles from the cultures. Since the virus used for transduction does not contain a viral genome, and is thus replication-deficient, there will be no viral particles produced by the transduced cells. Thus there is no harm connected to work with cultures previously transduced with these vectors, and these cultures can be handled safely. Containers holding the inactivated virus products will be wiped out 93% Virkon) before removal.

**Monitoring**

- Disinfection: The disinfectant of choice (Virkon) is used in strict accordance with the manufacturer's guidelines.
- Autoclaving: To ensure 100% efficacy, testing of the autoclave is carried out annually by the manufacturer to ensure that the correct temperature and pressure have been reached for the required time. Testing is arranged and test reports are kept by the Departmental Safety Officer.
- Inspectors: Safety Inspections are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are maintained by the Departmental Safety Officer.
Microbiological Safety Cabinets: Serviced and tested on an annual basis. Certificates of conformity are displayed on each cabinet and copies kept by the Departmental Safety Officer.

Negative Pressure Suites: Tested annually by Estates and Buildings to ensure the suite is at negative pressure relative to the immediate surrounding. Testing is arranged and test reports are kept by Departmental Safety Officer.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

This has been seen and approved by the Local Genetic Modification Safety Committee

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 168/09.3

Date Ackn'd 28/04/2009

CU2 Project Title Adenoviral transduction of primary human or murine cells with either, therapeutic genes regulated by tumour specific promoters, and a dominant negative, Inhibitor of B Kinase-ß(IKKß), under the control of the constitutive CMV immediate-early promoter

Class 2 CultureVol Consents

Consent Granted

Date Project Ceased

Withdrew N

Tick if notifying a connected programme of work N

Historical Significant Changes

Project notified under transitional arrangements N

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

To infect primary human or murine cells with an adenovirus encoding a therapeutic gene (such as thymidine kinase), under the control of the prostate specific membrane antigen (PSMA) promoter, a gene that is highly overexpressed in human prostate cancer cells. This is performed in conjunction with the co-transfection of a pCDNA3.1 plasmid encoding the Ad5, E1A region under the control of the HIF-α, hypoxia response element (HRE). This allows the adenovirus, encoding the therapeutic gene, under the control of the PSMA promoter, to undergo a single round of replication in the transduced and transfected primary human or murine cells, but only in areas of hypoxia. When these cells are injected into human prostate tumour bearing mice, these primary cells home to the hypoxic areas within these tumours where they replicate the adenovirus. Subsequent infection of the human tumour cells with the adenovirus initiates expression of the therapeutic gene, only within the tumour cells. Additionally, we would like to use a second adenovirus, where adenoviral E1A gene, from a stereotype Ad2 adenovirus is under the control of the TARP promoter (T-cell receptor, gamma chain alternate reading frame protein) together with the prostate specific antigen (PSA0 and PSMA enhancers (Ad(I/PPT-E1A), allows replication of the adenovirus only in human prostate cancer tissue. This adenovirus is initially transduced into primary human or murine cells, along with the co-transfection of the E1A gene under control of the HRE promoter on the pCDNA3.1 plasmid as described above. This permits delivery of the virus to hypoxic areas of prostate tumours in mice. Here the virus replicates in the primary cells under the control of the HRE and then infects and replicates, under the control of the TARP/PSA/PSMA promoter within the prostate cancer cells only, resulting in tumour cell death. An Ad5, E1/E3 deficient adenovirus encoding a dominant negative version of the 'Inhibitor of kB Kinase-β' (IKK-β DN), under the control of the CMV immediate-early promoter, will be obtained from a collaborator. This virus will be used to transduce primary human and murine cells to inhibit NF-κB defective cells will be studied in vitro or injected into human tumour bearing mice to investigate their affect on tumour biology and progression.

**Recipient or parental organism**

All adenoviruses are serotype Ad5 and carry deletions in the E1/E3 regions, rendering these viruses replication incompetent with the exception of the Ad(I/PPT-E1A) virus that has been reconstituted with the E1A gene from Serotype " human adenovirus under the control of the TARP/PSA/PSMA promoter enhancer regions. The TARP/PSA/PSMA adenovirus is replication competent only in human prostate tumour cells. All murine primary cells are obtained from mice maintained under pathogen free conditions within the animal facility at the University of Sheffield, therefore the risk of these cells harbouring adenoviral infections is minimal. Human primary cells will be obtained from the human blood transfusion service. Consequently, there is a possible risk that these cells might carry adenoviral infection from the general population. However, during the 3-5 day in vitro culture of these cells, the presence of an exogenous adenovirus would result in high levels of cell death, as the virus replicates, as such these cells would not be used for subsequent experimentation. Therefore, there is very low risk of transferring either, transgenic material to wild-type pathogenic strains, conveying replication competence to adenoviruses carrying transgenic material.

**Host/vector system**

HOSTS the pShuttle and pAdEasy vectors will be propagated in mon-pathogenic, disabled or non-colonising Ecoli strains. Per.C6/Adenovirus: There is a low level risk of producing replication competent virus (RCV) by recombination between the adenovirus encoding the therapeutic gene and the E1 viral gene within the packaging cell line. However, unlike HEK293T cells, Per C6 cells contain only the E1 region of the Ad5 genome and virtually no overlapping sequences with the pAdEasy vector. In the unlikely event of RCV, this would immediately infect and kill the primary cell in vitro. Macrophage: Primary human macrophages may also contain wild-type adenoviral sequences that could recombine with the adenoviral vector giving rise to RCV. However, the therapeutic gene would still be under the control of the tumour-specific producer and production of RCV's would rapidly kill the macrophages. In theory, a second recombination event could also lead to deregulation of the toxic gene. Again, however this would rapidly kill the macrophages before they would be used in any further experimentation. Mouse: Adenovirus produced by human macrophages in xenografted tumours might infect murine cells however, this would occur at low level due to the lowered infection rate of murine cells in comparison with human tissue. Therefore, the risk of viral transmission between infected mice is low.
Alphastatin (a-statin): An artificially produced 24 amino acid peptide fragment derived from the N-terminal 24 amino acids of human fibrinogen. This peptide has been shown to inhibit and endothelial cell migration and tubule formation in vitro and reduced tumour growth in vivo and intravascular thrombosis in vivo, in syngeneic murine tumour models. (Staton et al., 2004) a-statin's mechanism of action is not known at present. However, this peptide has not been shown to disrupt the quiescent vasculature.

VirR Thymidine Kinase (vTK): The human analogue of this gene is involved in the production of deoxynucleoside-5'-phosphate required for DNA replication. vTK can be expressed in tumour cells and specifically converts the inactive pro-drugs ganciclovir or acyclovir into their active forms, resulting in the death of infected tumour cells without altering normal cellular DNA replication or affecting uninfected cells. This is often used as a safety measure in gene therapy studies to ensure death of infected cells only. Additionally, the vTK can diffuse and render nearby cells sensitive to ganciclovir and acyclovir, resulting in localised cell death through 'bystander' effect. LIGHT: This is an immunotherapeutic gene and member of the TNF family (tumour necrosis factor superfamily, member 14). This protein functions as a co-stimulatory factor for lymphoid cells as well as triggering the proliferation of T-cells and can also induce the apoptosis of tumour cells. It triggers pro-inflammatory gene expression in endothelial cells and triggers Th1 responses in adaptive immune cells. Bacterial Hemolysin E (HylE): A novel octameric pore forming toxin (Hunt et al., 2008), originally identified in the E.coli-K12 strain and is a virulence determinant. This protein mediates cell death by making the cell membranes permeable to cellular contents. IKKßDN: IKKß is a member of the kinase complex required for the phosphorylation of inhibitor of NFkB (IkB). This phosphorylation step allows the activation of the transcription factor NFkB resulting in inflammatory gene expression in a variety of cell types including endothelial cells and myeloid cells. Sustained activation of NFkB signalling has been shown to mediate pro-tumour functions of macropores in tumours. Introduction of IKKßDN protein would inhibit this pro-tumour program in macropores allowing macropores to promote activation of the adaptive anti-tumour response and mediate cytotoxicity against the tumour cells directly.

Evaluation of foreseeable effects

Alphastatin (a-statin): The consequences of inappropriate expression are limited due to the tumour restricted vascular thrombosis observed in scientific studies. However, there is the possibility of a systemic inhibition to angiogenesis (possible inhibition to female luteal formation during the menstrual cycle) with unknown consequences for wound healing.

VirR Thymidine Kinase (vTK): The unrestricted expression of this gene in areas other than tumours would render these tissues or cells sensitive to acyclovir and ganciclovir treatment, this would be undesirable but on its own would have no detrimental affect without administration of the pro-drugs in vivo.

LIGHT: Unrestricted expression of this gene would have low impact given its role as a co-stimulatory factor for endothelial and T-cells.

Bacterial Hemolysin E (HylE): If the regulation governing the expression of this protein is lost it might result in cell death. However, the level of cell death would depend upon the concentration at which the protein accumulates either locally or systemically.

IKKßDN: The inappropriate expression of this gene would inhibit NFkB signalling in all infected cells, having unknown systemic effects at the organism level. Infected cells might also show a reduced ability to respond to cellular stresses such as hypoxia, ultimately resulting in cell death.

TARP/PSA/PSMA-E1A Adenovirus: In the unlikely event that recombination between this and wild-type virus should occur, a replication competent adenovirus would be
generated that would have the potential to infect human cells. However, as the Ad(1PPT-E1A) virus lacks additional transgenes this would be no more pathogenic than a wild-type Ad5 virus. Use of this virus in nude mice bearing human PC-446C prostate tumours demonstrates complete tumour selectivity (Cheng et al., 2004). Similarly, use of this type of virus in phase-1 human clinical trials has demonstrated a good safety profile and therapeutic effects (DeWesse et al., 2110). Should recombination take place between the Ad(1PPT-E1A) adenoviral replication in cells experiencing hypoxia.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

In vitro culture will be performed in a laminar flow, class II biological safety cabinet. Waste plastics will be disposed of in viral waste bins for autoclaving. Liquid waste will be inactivated for 1hrs using fresh ‘Presept’ solution, with 2500ppm of available chlorine, according to manufacturer’s instructions, before being disposed of down the drain. Where the risk of aerosol production is highest ie during centrifugation, liquids will be contained in sealed buckets, opened only in laminar flow hoods. Human tumour bearing mice, that have been injected with primary murine or human cells, carrying adenovirus, will be maintained under classII animal containment within the animal facility on an isolated air supply, to prevent spread of viral particles to other animals within the facility, in the highly unlikely event that viral particles are generate by the murine hosts. Animal carcasses will be handled and incinerated within the animal facility.

The use of sharps will be kept to a minimum to avoid human contamination however, in order to deliver the primary human or murine cells, infected with adenovirus, the use of syringes is required and represents an unavoidable risk. The use of double gloves may offer limited protection against the risk of infection.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste will be autoclaved in a regularly validated autoclave and disposed of by the Hospital's clinical waste contractor. Liquid waste will be inactivated for at least 1hr using freshly prepared 'Presept' at 2500ppm of available chlorine or 'Virkon' solution at 1% final concentration to give > 5 log killing of bacteria and viruses and then disposed of down drains. Spillages will be treated with 'Virkon' powder, left for 3 minutes, then scraped up and disposed of via the clinical waste bins. Animal carcasses will be treated with 'Virkon' powder, left for 3 minutes, then scraped up and disposed of via the clinical waste bins. Animal carcasses will be handled internally by the animal house facility and incinerated resulting in total inactivation of all animal waste.

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

**Project Containment**

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Identification and characterisation of potential virulence factors of oral pathogenic species: Tannerella forsythia, Porphyromonas gingivalis, Prevotella spp, Streptococcus sanguinis and Streptococcus gordonii

**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to investigate the role and regulation of genes involved in:

1. Biofilm information - including regulatory pathways and extracellular polysaccharide encoding genes. And co-colonisation experiments with Gram-positive oral Streptococcal species.
2. Virulence factor regulation and production: including extracellular proteases, Hemolysin toxin, Bacteriocins
3. Epithelial cell invasion
4. Iron acquisition and its regulation

For Tannerella, Porphyromonas and Prevotella spp., we expect some of these to be essential for virulence.

In addition we aim to clone and overexpress in E. coli selected virulence proteins, from Porphyromonas, and Tannerella, and the bacteriocin nigrescin from Prevotella nigrescens and related Prevotellas spp. For biochemical and structure-function analysis. We also envisage using certain Streptococcal GMMs for co-colonisation biofilm experiments.
Tannerella forsythia is a Gram-negative anaerobic bacterium, which is frequently isolated from patients with periodontal disease. It was formerly known as Bacteroides forsythus and in some literature is named as T. forsythensis. It has extremely fastidious growth requirements and requires N-acetyl neuramic acid supplementation in media to enable growth. It falls into Hazard Group 2 with other Bacteroides spp.


Prevotella spp.: namely P. intermedia and P. nigrescens - Gram-negative, anaerobic black-pigmented bacteria both associated with periodontitis and as commensal organisms. Hazard Group 2.

E.coli K-12 and B strains, Non-pathogenic auxotrophic strains, limited or no-colonisation ability. Hazard Group 1.

Streptococcus sanguinis and Streptococcus gordonii - commensal Gram-positive cocci, primarily in the oral cavity, associated with infective endocarditis and dental infections (pulpitis and periapical disease). Hazard Group 2.

Host/vector system

Tannerella Forsythia ATCC 43037 - type strain, and clinical isolates from the Charles Clifford dental hospital

Porphyromonas gingivalis strains: NCTC 11834, W50 and 381
- ΔrgpA: Δrgpb: Δkgp (containing null mutations and lysine proteases - Prof M Curtis, QMUC London)

Prevotella: P. nigrescens: ATCC 25261
P. intermedia ATCC 25611

Streptococcus spp: S. sanguinis SK36 - ΔsrpA (containing null mutation in a platelet interactive protein)
- S. gordonii DL1 (Challi) - ΔscaA; ΔSspA:SspB: ΔCshA:CshB (containing null mutations in surface adhesins)

E.coli strains: TOP10 (host for routine cloning)
- CC118pir, MC4100pir (for maintaining suicide plasmids)
- BL21, C41, BL21 star (for protein expression)
- S17-1 x BW19851: SM70pir

E. coli plasmids: pBluescript2KS, pUC18, pACYC184, pBBR1MCS-1 (and 2-5) (broad host range, but does not replicate in anaerobic oral strains), pBR329 (routine cloning vectors)

Shuttle vectors: pCp23 (Tc), pCP11(Em), pCP29(Tc) (replicate in E.coli and Flavobacterium species, from Mark McBride University of Wisconsin-Milwaukee), pLYL01 (Tc), pE-COW(Em), pT-COW (tc) (From N. Shoemaker, Illinois) Ply05(Cefoxitin),

Suicide vectors: pVA198 (Em), pLYL03 (Em), pLY001 (tc), pGERM (Em) (From N. Shoemaker, Illinois)

Transposon delivery vectors: pEP4351-Tn4351 delivery plasmid-suicide vector
- phimarEm1 - Himar transposon delivery vector - Em resistance,

Reporter plasmids: lacZ-pGPS123, pRS-series (Simons RW et al., Gene 1987; 53; 85-96),
- lux reporter plasmids: pQF120,
and GFP: pBBR1MCS-GFP-E.coli reporter plasmids
pMJF2 and pMJF3-gus (uidA) reporter vectors (Jeffrey Smith)
pMTT-1,luxAB, reporter vectors
pBAD18, pGEX-4T3 and derivatives, pET15, pTrc99a- E.coli inducible expression plasmids

Origin & function

1. Biofilm formation and virulence factor regulation
It is our aim to characterise the genes up and down regulated during biofilm formation by the oral pathogens Tannerella forsythia and Porphyromonas gingivalis. The choice of genes to investigate has been informed by literature search microarray analysis and from an RT-PCR study of genes putative virulence genes identified from their respective genome sequences. We are also conducting a parallel proteomic study which will identify target proteins/genes involved in this phenomenon. We will generate null mutants of these genes encoding proteins up or down regulated in relation to the biofilm lifestyle. It is expected that this study may identify adhesins, stress proteins, regulators, exopolysaccharide genes and other unforeseen genes. We also intend to attempt to develop a reporter system to assess promoter regulation in vivo using either gfp, lux or gus based systems. It is also our intention to assess the possibility of constructing a transposon mutant library for Tannerella though this has not been attempted before, we have acquired the bacteroides transposon Tn 4351 and a Himar transposon that has been used in Flavobacterium species. We also intend to grow multi-species biofilms using Steptococcus gordonii and Streptococcus sanguinis.

2. Bacterium production
Our previous work identified a putative bacteriocin in Prevotella nigrescens. We intend now to clone and purify the individual genes within this operon to identify the active protein. Since the genome of Prevotella nigrescens has not been sequenced we also intend to use Inverse-PCR to clone flanking DNA from this operon to identify accessory genes for the bacteriocins production. We would then target gene for knockout mutagenesis and complementation studies. Genome searches of related Prevotella spp has identified putative homologous operons which we also intend to clone in to E. coli to assess biochemical activity.

3. Epithelial cell invasion
We have established an efficient means of infecting oral epithelial cells with Porphyromonas gingivalis and have noticed the existence of hyper-invasive strain. We are in the process of assessing the transcriptome of these strains to identify differentially regulated genes that would be targeted for knockdown mutagenesis.

4. Iron acquisition and its regulation
Using similar means to above we will investigate the iron acquisition mechanisms of Tannerella and its regulation.

Activity 1: Transposon mutagenesis: This has never been successfully achieved in T.forsythia but pEP4351 has been used to make Tn libraries in P. gingivalis. We will attempt to introduce the TN4351 or Himar transposons into T.forsythia and P. gingivalis. Mutants of interest will be identified by their phenotype in biofilm assays (microtitre well growth and assessment of adhered cells by crystal violet staining).

Activity 2: Subcloning of Tn flanking DNA If successful, identification of transposon inactivated genes generated in activity 1 will be carried out by cloning the Transposon and flanking genomic DNA into E. coli cloning plasmids such as pBluescriptIIKS in E. coli followed by DNA sequencing.

Activity 3: Cloning of nigrescin operon flanking DNA
This will involve cloning of DNA sequences of unknown sequence that flank the currently known nigABCD operon (see risk assessment). These DNA fragments will be cloned as PCR products into the TOPO-TA cloning kit vector pCR3.1-TOPO before DNA sequencing.

Activity 4: Targeted knockout mutagenesis of oral species AND attempted genetic complementation. This will be performed for selected genes from the genome that may have virulence functions, and of any genes identified from activity 1 and 2. The gene of interest will be an antibiotic resistance cassette that functions in these Bacteroides related species such as ermF or tetQ. We will also attempt to complement mutations by cloning genes of interest in trans into potential shuttle vectors (e.g.pCP23).

Activity 5: Cloning of reporter fusions – To monitor expression of virulence genes in oral species regulatory regions will be fused to lacZ/GFP/gus/lux as applicable on a...
broad-host range non-self-transmissible plasmid or suicide vector that will then be introduced into the appropriate host. Where applicable the regulation of oral bacterial promoters will be studied in E.coli K12 using either narrow or broad-host range plasmids.

**Activity 6:** Overexpression of nigrescin operon genes. The putative nigrescin bacteriocin genes will be cloned into E.coli expression vectors with either His-Tag (pET15b) or GST-tags (pGEX4T3) and overexpressed and purified to assess biochemical activity in vitro.

**Activity 7:** Co-colonisation biofilms- Streptococcus spp and Oral anaerobes. Certain wild-type and mutant Streptococcal spp will be grown in co-culture biofilms with oral anaerobes such as Porphyromonas. The strains used will have been created under risk assessments outlined in this proposal, so by following the considerations outlines therein the co-culture of these organisms will be managed safely.

### Evaluation of foreseeable effects

The oral bacteria T. forsythia, P. gingivalis, P. intermedia and P. nigrescens contribute to the oral disease of periodontitis. This disease has a multifactorial aetiology and acquisition of a single species is not thought to predispose to disease. These organisms are strict anaerobes with fastidious growth requirements – requiring culture on Blood agar or in the presence of Hemin, Menadione and cysteine. Therefore their ability to be transferred to the environment at large is extremely unlikely. While these organisms are associated with periodontal disease this is a multifactorial disease with contributions from the host immune system, risk factors (e.g. smoking, immune status) and from the oral bacterial community as a whole. It is not believed that any one organism is attributable to causing the condition, therefore transfer to humans is not likely to result in a periodontal infection unless other risk factors are present. In addition many of the antibiotic markers that will be transferred to the oral species listed above are unable to function aerobically or in enteric species for example, making their dissemination in the human microflora unlikely. The antibiotic markers will not affect the ability to treat infections since the drugs of choice is metronidazole.

S. sanguinis and S. gordonii are often recovered from cases of infective endocarditis and dental abscesses (periapical infections). Their involvement in infective endocarditis only occurs in at risk patients (i.e. requires the existence of previous damage to cardiac tissue) and for this they need to enter the blood circulation in fairly high numbers. Their involvement in dental abscesses is a part of a multispecies infection that gains access via open dentinal tubules or fractured tooth crowns. Consequently, it is very likely that these organisms would cause disease in healthy subjects.

E. coli K-12 and BL21 strains are potential colonisers of the human gut but are non-pathogenic. Activities 1 and 4 will result in gene inactivation, so it is unlikely that mutants will become more pathogenic or more able to survive in the environment as a result. Mutants will have gained resistance to an antibiotic or a reporter gene trait, but these are unlikely to increase the environmental fitness organisms given their fastidious strictly anaerobic growth requirements. Plasmids used to create these mutations will not replicate in these organisms, so do not pose any potential hazard.

Activities 2, 3, 5, and 6 involve the cloning of DNA from oral species into E. coli strains. Given the non-pathogenic nature of the E. coli strains and non-transmissible nature of the expression and narrow host-range of the cloning vectors used (e.g. pET15, pBAD18, PBBlueScript) the risk to health or of release is negligible. Some BHR plasmids (pBBR series) that are transmissible only when transfer functions are provided in trans by E. coli S-17 strains will be employed, but this inability to self-transmit will avoid their dissemination in the environment. The reporter systems proposed for use here (lux/gfp/lac/gus) have been used extensively elsewhere in bacterial systems without deleterious effects.

Activity 7: involves co-culture of organisms of low-risk to human health between which genetic transfer is very unlikely

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All GM waste will be inactivated before leaving the premises. 
Small volumes of liquid culture and small disposable items (i.e. plastic tubes, cuvettes, pipette tips) will be inactivated by immersion in disinfectant (1% Virkon solution) for a minimum of 24 hours before autoclaving. This procedure coupled with the fact that oral anaerobic bacteria cannot survive for more than an hour in an aerobic environment will ensure safe disposal of GM, material.
Solid material inactivated by immersion in disinfectant will be disposed of in appropriate containers. These are collected by approved contractors who then autoclave them before disposal.
All other items (larger cultures, agar culture plates, glasswares, larger plasticware) will be inactivated by autoclaving in the department of oral pathology (autoclaves are regularly serviced and validated).

Removal of the word “including” from the title of the project.
• On page 4 of the University of Sheffield Risk Assessment form under “Hazards associated with the vector”, also form cu2, page 2 and reference to pBBR1MCS-GFP on page 3 of form cu2- amend to indicate that pBBR1MCS-1 is not E. coli-specific and is a mobilisable BHR plasmid.
• On page 5 of form cu2 amend “5% Virkon solution” to “1%”. Validation for this concentration to be included.
• Clarification required on the Risk Assessment and cu2 forms with regards to the following: “…it is stated that these bacteria are “unlikely to cause infection in healthy individuals”.

The proposal was approved by quorum subject to the above – name removed to arrange a cheque made payable to the Health & Safety Executive and then forward on to name removed for the administration to be passed on to the HSE.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

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Project Ref 168/09.5

Date Ackn’d 25/11/2009

CU2 Project Title Characterisation of the Interactions Between Candida species and host tissues

Class 2 culture

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

### Purposes of the contained use

The aim of the project is to identify the genes and proteins involved in the interaction of Candida species with human leukocytes, endothelial and epithelial cells.

### Recipient or parental organism

Candida species in particular C. albicans and C. glabrata are fungal organisms that are widely distributed and carried commensally in the oral cavity by at least 50% of the healthy population at any given time. These organisms have low pathogenic potential that rarely affect healthy individuals. However, Candida infections can become a potential medical problem when an individual's immune response is significantly impaired and Candida infections are relatively common only in immunocompromised patients. Because of their potential to cause disease in immunocompromised individuals the organisms fall into Hazard Group 2.

### Host/vector system

#### Host

All mutants are derived from Candida albicans and Candida glabrata parent strains. All strains used in the studies outlined below will be provided by our collaborators. See attached table for strains used and relevant references describing their manufacturer.

### Origin & function

1. Adhesion and transmigration of Candida to and through the endothelium

Our aim is to identify the Candida genes and gene products that are involved in the adhesion of Candida to the endothelial cells lining blood vessels. We also plan to
determine the Candida genes and gene products that facilitate the transmigration of Candida across the endothelium. We have access to a panel of Candida mutants that are deficient in adhesion molecules and cell wall molecules that may be potential adhesion molecules (see attached table). We also have the parent strains that these mutants have been developed from and all these are provided by our collaborators (Prof Pete Sudbery, University of Sheffield; Prof. Neil Gow, University of Aberdeen; Dr Lois Hoyer, University of Illinois; Prof. Ken Haynes, Imperial College London; Dr Aaron Mitchell, Carnegie Mellon University, Pittsburgh). We will use these Candida strains to assess their ability to adhere to and transmigrate through monolayers of endothelial cells in in vitro assays. We will use a flow adhesion assay (See fig 1) to measure adherence of Candida to endothelial cell monolayers as this allows adherence to be measured under conditions of flow and shear stress that mimic in vivo conditions. In these assays Candida are flowed across monolayers of endothelial cells grown on microscope slides using an enclosed tubing system and sealed chamber to avoid contamination or aerosol spread. In this system Candida can be re-circulated or removed directly into disinfectant (Vircon).

In addition, the importance of conversion from yeast to hyphal forms of Candida in adherence to the endothelium will be analysed using a C. albicans mutant (SSY50B, available via Dr Steven Saville, University of Texas) in which the conversion from yeast to hyphae is under the control of doxycycline.

2. Adhesion and penetration of Candida to the oral epithelium and leukocytes

We will use some of the wild-type and mutant Candida strains shown in table 1 to analyse the oral epithelial and leukocyte host response to these organisms. Candida will be used to infect monolayers of normal oral keratinocytes, keratinocytes form oral cell lines, leukocyte sub-populations or keratinocytes as part of an in vivo tissue engineered oral mucosal model and the host response analysed using several biological assays.

### Evaluation of foreseeable effects

Candida Sp. Are widely distributed (in the oral cavity, gastrointestinal tract and vagina) are carried commensally by at least 50% of the healthy population at any given time. However, under certain circumstances it is capable of becoming pathogenic leading to oral, vaginal, cutaneous or systemic candidiasis. These infections tend only to occur in immunocompromised individuals. It is a microorganism of low pathogenic potential that rarely affects healthy individuals.

The Candida strains to be used in our studies do not contain inserted genetic material derived from other organisms. These studies will use wild type laboratory strains that have been well characterised or mutated forms of these strains in which genes have been disrupted using short homology regions as previously described (Wilson RB et al. (1999) J. Bacteriology, 181:1868-1874). In all cases the mutated strains which have a 'loss of function' have been shown to be less virulent in their wild-type counterparts which are themselves unlikely to cause disease in healthy individuals. The exception is the SSY50-B strain obtained from Dr Stephen Saville in which yeast to hyphal transformation is under the direct control of doxycycline. The hyphal form (generally considered more pathogenic than the yeast form) can only develop in the presence of doxycycline and is therefore extremely unlikely to cause infection in any individual.

Pathogenicity in Candida is a polygenic trait involving numerous physiological characteristics (e.g. rapid growth, cell wall, morphogenesis, secreted hydrolases, phenotypic switching). Therefore, a reduction in any one of these parameters will attenuate Candida virulence. Furthermore, Candida strains that have been cultured in the laboratory tend to display reduced pathogenicity compared to strains carried commensally by most individuals. It is important to note that although a large number of C. albicans mutants have been analysed, including virulence attributes, metabolic and cell wall genes, there is NO report of a mutation that increases the virulence of this yeast (e.g. Gow et al. (1994) PNAS 91, 6216; Leberer et al. (1996) PNAS 93.13217; Hube et al (1997) Infec. Immun. 65, 3529; Sanglard et al. (1997) Infect. Immun, 65 3539; Navarro-Garcia et al., (2001) FEMS Microbiol. Rev. 25: 245-268). This is unsurprising, since all evidence to date indicates that virulence in C. albicans is a multi-factorial process at the molecular level, with no single virulence factor of over-riding importance. While it is possible that inactivating a repressor might increase virulence this is NOT the case. For example, inactivation of TUP1 or NRG1 (both repressors of hyphal development) decreases virulence by attenuating growth in vivo (Murad et al. (2001) EMBO J. 20, 4742-4752). Therefore, mutations in wild isolates are expected to have a neutral or negative effect upon virulence. Some experiments will be done using C. glabrata. These Candida species are closely related to C. albicans; they generate similar types of infections in humans, but are considered to be less virulent than C. albicans as they do not naturally form hyphae.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GM waste will be inactivated before leaving the premises.

Small volumes of liquid culture and disposable items (e.g. plastic tubes, cuvettes, pipette tips) will be inactivated by immersion in disinfectant (5% Virkon solution) for a minimum of 24 hours before autoclaving.

Solid material inactivated by immersion in disinfectant will be disposed of in appropriate containers. These are collected by approved contractors who then autoclave them before disposal.

All other items larger cultures, agar culture plates, glassware, larger plasticware) will be inactivated by autoclaving in the department of oral Pathology.

Autoclaves are regularly serviced.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

At the Local Genetic Modification Safety Committee meeting that was held on 5 October 2009, this project was approved subject to the following amendment.

Page 5 - 'Likelihood of hazards'- to include a description of the flow assay and to state that the assay is done in an enclosed chamber so there is no risk of contamination or aerosols.

Project Containment

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### Project Additional Information

**Purposes of the contained use**

Bacteria are able to survive and thrive in a wide range of environments. This ability is rooted in altered patterns of gene expression. We propose to use Transposon Mediated Differential Hybridization’ (TMDH) to identify genes that are essential for Salmonella to survive within a particular environment. Briefly, this involves: (i) systemic introduction of large numbers of mutant Salmonella into a specific environments (e.g. aerobic and anaerobic conditions), (ii) isolation and labelling of DNA from Salmonella found in the aerobic and anaerobic cultures (iii) hybridisation of this DNA to a custom-made, genome-wide oligonucleotide array; and (iv) analysis of the differential hybridisation patterns to identify genes that are essential for growth and/or survival in the new condition. The Salmonella mutant library has been created in Cambridge and currently a PhD student in Sheffield is working with the library there. We would like to transfer the library to Sheffield to allow a series of experiments in which the input and output pools can be compared to identify genes that are involved in facilitating Salmonella survival in different environments. This proposal is to characterize the existing library and does not include the creation of new libraries. In follow up experiments we will create specific gene deletions using standard lambda red gene replacement (recombineering) technology to further characterize the phenotypes of mutants identified from screening the TMDH library, and we propose to use plasmid-based complementation experiments and promoter-lacZ fusions to monitor gene expression as part of these characterizations.

**Recipient or parental organism**

The transposon library is of Salmonella Typhimurium SL1344, a mouse-virulent strain. Hazard group 2. Plasmid propagation and molecular biology steps will be done using E. coli K-12 (MG1655, W3110, XL-10 Vblue, DH5alpha, MC1000), non-pathogenic strains with limited or no colonization potential. Hazard group 1.

**Host/vector system**

The plasmid pKD46 will be used in the creation of specific Salmonella Typhimurium mutants for phenotypic characterization following the results of the screening experiments. pKD46 has been widely used to create specific gene replacements in Salmonella. pKD46 has the following features: temperature sensitive replication (repA101ts encodes a protein required for replication that is inactivated at temperatures above 30°C); a conditional replicon (oriR101, requires Pir in trans for plasmid propagation).
replication); encodes lambda Red genes (exo, bet, gam); native terminator (tL3) after exo gene; arabinose-inducible promoter for expression (ParaB); encodes araC for repression of ParaB promoter; Ampicillin resistance. No significant hazards are associated with this vector. We will also use standard vectors (pBR322 and pUC) in complementation experiments. These are widely used commercially available vectors that are non-mobilisable.

For gene expression studies we will make promoter fusions to lacZ in the plasmid pRW50 (Lodge et al., 1992, FEMS Letters 95, 271-276. This is a widely used plasmid with no known hazards.

Origin & function

A library of transposon mutants in Salmonella SL1311 is available at the University of Cambridge. The library was constructed by transposon mutagenesis using a Tn5 transposome (Epicentre Biotechnology) that was modified to include outward facing T7 and SP6 promoters. These promoters allow the generation of RNA in vitro that corresponds to the regions of DNA flanking the site of transposon insertion. The presence of engineered rare restriction sites (Sce-I, Psp-1) can be used to specifically isolate transposon containing DNA fragments. The transposon is locked in because the transposase is not retained, severely limiting the possibility of transfer of antibiotic resistance to a related organism. Therefore, it is unlikely that sequences within the GMM will be transferred to related organisms which will increase the potential hazards associated with the latter.

For the complementation experiments the wild-type gene under the control of its own promoter will be amplified from Salmonella genomic DNA and ligated into pBR322 and/or pUC plasmids. The resulting expression plasmids will be transferred to the appropriate Salmonella mutants by electroporation to test whether the disrupted gene is responsible for the observed phenotype.

Evaluation of foreseeable effects

(a) Mutant library
Salmonella Typhimurium SL1344 is a mouse-virulent strain and can cause gastroenteritis in humans and is thus in Hazard Group 2. The Salmonella Typhimurium mutants in the library are most likely to be debilitated compared to the wild-type because of the gene disruptions created by the transposon. The transposomes that were used to create the library are based on a modified Tn5 that includes T7 and SP6 promoters, rare restriction sites (I-Sce-I and Psp-I) and a neomycin resistance cassette. The transposon is locked in because the transposase is not retained, severely limiting the possibility of transfer of antibiotic resistance to related organisms. Therefore, it is unlikely that sequences within the GMM will be transferred to related organisms in a way that will increase the potential hazards associated with the latter. As stated above it is most likely that the Salmonella Typhimurium mutants will be less harmful than the wild-type because of the associated gene disruptions. Depending on the location of the transposon the following possibilities can be considered: (i) interruption of an essential gene—the Salmonella will not be viable and therefore will not be present in the library; (ii) interruption of a non-essential gene will result in Salmonella that is either unaffected or less viable; (iii) it is highly unlikely that the fitness of Salmonella could be enhanced significantly by a transposon event.

(b) Recombineering and complementation
pKD46 has been widely used to create specific gene replacements in Salmonella. pKD46 has the following features: temperature sensitive replication (repA101ts encodes a protein required for replication that is inactivated at temperatures above 30°C); a condition replicon (oriR101, requires Pir in trans for plasmid replication); encodes Lambda Red genes (exo, bet, gam); native terminator (tL3) after exo gene; arabinose-inducible promoter for expression (ParaB); encodes araC for repression of ParaB promoter; Ampicillin resistance. No significant hazards are associated with this vector. We will also use standard vectors (pBR322 and pUC) in complementation experiments. These are widely used commercially available vectors that are non-mobilisable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The work outlined will be carried out in designated laboratories which meet containment level 2 standards. The work will generate contaminated plasticware (pipette tips, tubes) flasks, agar plates and spent media used in the growth of the bacteria. Bulk liquid waste will be decontaminated using 2% Virkon solution. Glassware will be decontaminated by autoclaving. Solid waste will be disposed of by incineration in line with University procedures which have been approved for disposal of class 1 and 2 GM waste. Hence, all contaminated solid waste (plastics etc.) will be treated with 2% Virkon for decontamination purposes (validated by manufacturer) before autoclaving in designated laboratories within the Department Molecular Biology & Biotechnology. The autoclaves are serviced and tested annually. These measures will ensure effective killing of bacteria and destruction of residual nucleic acids.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Approved no comments

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2  Yes L3 L4 L2 L3 L4 L2</td>
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<td>L4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
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<td>L2 L3 L4 L2 L3 L4 L2</td>
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<td>L4</td>
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Project Ref 168/13.1

Date Ackn’d 17/01/2013

Date Project Ceased

CU2 Project Title An experimental study of the lipophosphoglucan (LPG) independent Leishmania parasite - sand fly mid gut binding mechanism

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2 < 1 Litre

Non-GMM Non-GMM

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Withdrawn N
Project Additional Information

Purposes of the contained use

Measurements of specific binding forces associated with the secondary, lipophosphoglycan (LPG) independent binding mechanism used by Leishmania parasites when in the mid gut of their sand fly hosts. Comparison of results obtained for the wild type, lipophosphoglycan deficient (LPG1) and phosphoglycan deficient (LPG2) parasites will enable analysis of whether observed forces are due to LPG or a different surface molecule on the parasite. NB: flies are not involved in the project.

Recipient or parental organism

Leishmania major and Leishmania mexicana are ACDP Hazard Group 2 pathogens of both humans and other animals. They cause cutaneous Leishmaniasis, which manifests as self limiting ulcerous skin lesions and is naturally transmitted via an intermediate vector (female haematophagous phlebotomine sand flies). Lesions tend to be specific to the area surrounding the site of the bite although diffuse lesions can occur in some cases. They are similar to refractory ulcers and leave burn-like de pigmented atrophic scars once healed. Infections can be treated with agents such as sodium stibogluconate, paromomycin or pentamidine by tropical medicine specialists. These antimicrobials are available through the Department of Communicable Disease, where they have experience of treating these infections.

An infective dose can be anything between hundreds to multiple thousands of metacyclics in typical sand fly bite situations. Infections used for murine trials are typically much larger and established by intra-dermal injection. Although all parasite loads can cause an infection, the severity of the induced pathology is increased with larger doses. In murine models, both a high number and low number challenge caused lesions of different severity but were cleared over a period of several weeks to a couple of months. [N.Kimblin et al., PNAS, 105 29 pp. 10125-10130 (2008)]

Approximately 1 x 10^7 parasites will be in single pellet, and in some cases they will be in metacyclic form. This would therefore be enough to establish a reasonably severe clinical infection should there be an entry point on the skin for any significant proportion of the sample.

Handling of the wild type organisms must be undertaken with due care as direct inoculation could lead to a skin infection (occupational infection). The natural insect vector for the parasites is not present in the UK and so there is no likelihood of ongoing infection and therefore negligible risk to the environment upon accidental release.

Host/vector system

The mutants in this project have been modified by targeted gene replacement or deletion. They are well characterised organisms which have been used for several years to study variations in adhesion properties between wild type parasites and those lacking in certain prolific surface molecules. Details on these modifications are given in the next box.

Origin & function

LPG1 and LPG2 genes were subcloned into BamHI-HindIII-cut pQE30 (Qiagen). Positive clones were subcloned into pBSK+ or pGEM-5Z (Promega). LPG1 and LPG2
vectors had phleomycin and hygromycin resistance genes incorporated in them for positive selection of transfectants. This does not infer any increased virulence to these organisms because (i) they have reduced capacity for survival in both mammals and sand fly vectors (also, no sand flies in UK) and (ii) Phleomycin and hygromycin may act on fly-stages in culture, but are unable to treat leishmaniasis in animals.

Specific mutant information:
Leishmania mexicana LPG1: Homology cloning using a fragment amplified by polymerase chain reaction (PCR) with primers derived from the known sequence of Leishmania donovani was used to isolate the Imexlpg gene. Two rounds of targeted gene replacement were performed by PCR amplification of the 5'-untranslated region (UTR) of Imexlpg1 and amplications of the 3'-UTR of Imexlpg1. This resulted in parasites deficient in LPG but with an up-regulation in proteophosphoglycan surface molecules. Hygromycin phosphotransferase and phleomycin-binding protein containing gene replacement cassettes were transfected into L. mexicana promastigotes. Selection of mutants in culture was achieved by adding hygromycin B and phleomycin to the culture medium. Resistance to other antimicrobials is unaffected. Infection of BALB/c and C57/BL6 mice show that the mutants are at least as virulent as the wild-type strain. [T. Ilg, EMBOJ., 19.9 pp. 1953-1962 (2000)]

Leishmania mexicana LPG2: A spontaneous mutant isolated from an infected mouse and an Imexlpg2 gene deletion mutant. The generated mutant lacks a Golgi guanosine diphosphate-mannose (GDP-Man) transporter; it is deficient in phosphoglycan repeat synthesis and shows downgraded mannoooligosaccharide phosphate cap expression. It was produced through double targeted gene replacement by PCR amplification of the 5'-UTR regions and 3'-UTR regions of Imexpg2. The spontaneous mutant lacks PG repeats but does not show reduced cap production. Hygromycin phosphotransferase and phleomycin-binding protein containing gene replacement cassettes were transfected into the L. mexicana promastigotes. Mutants were selected by adding hygromycin B and phleomycin to the culture medium. Resistance to other antimicrobials is unaffected. The two mutants are indistinguishable from wild type parasites with respect to colonisation of and multiplication inside host cells. They did not show any reduced virulence in BALB/c mice. [T. Ilg, J. Bio. Chem, 276 7, pp. 4988-4997 (2001)]

Leishmania major LPG1: Parasites lacking the LPG1 encoding gene are made through two rounds of targeted gene disruption using a 7-kb HindIII-Smal LPG1::HYG fragment followed by the 6.7-kb BamHI fragment LPG1::PAC. Hygromycin B and puromycin were used to select for mutants but resistance to other anti-infectives is unaltered. The mutant contained normal levels of related glycoconjugates and GPI-anchored proteins but lacked LPG. It still caused infection of BALB/c mice but some reduction in virulence was observed. [G. Späthe, PNAS, 97 16, pp. 9258-9263 (2000)]

Leishmania major LPG2: Parasites lacking both lpg5A and lpg5B genes and which have no LPG or protein-linked PGs due to deletion of the parasite Golgi GDP-Man transporter LPG2. They showed attenuation in early mammalian macrophage infection but, once established, amastigote virulence was maintained in BALB/c mice. They should not have any reduced susceptibility to anti-infectives. [A. Capul, Infection and Immunity, 75 9, pp. 4629-4637 (2007)]

Evaluation of foreseeable effects

Since Balb/c mouse trials have shown that several mutants retain virulence in mammalian hosts (although they do not display enhanced pathogenicity) and there is no reduction in susceptibility to antibiotics used for treatment, the parasites are likely to pose a hazard similar to that of the wild type organisms. The user is at risk when handling the parasites as infection can occur through direct inoculation. There is negligible risk to the environment as the natural insect vector is not present in the U.K. so ongoing disease spread is very unlikely.

All handling will be conducted in a Class II microbiological safety cabinet in a category 2 laboratory and gloves and a lab coat will be worn at all times when handling the pathogens. Good microbiological practice will be followed. The different species and mutants will be handled separately to minimise the risk of cross-contamination. There should not be any additional pathology associated with the mutant strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
Laboratory manipulations, passaging and sample preparation will be carried out in a standard containment level 2 facility and good microbiological practice will be employed. When transferring small live parasite samples to the Department of Physics and Astronomy, the sealed sample holder will be placed within a secondary sealed unbreakable container to prevent accidental spillage in case of dropping or collision with an object. Usage in the AFM suite will be restricted to the AFM with a protective box covering it, and experiments will not be left unattended. The cantilever which has been in contact with the parasites will be disposed of in a contaminated sharps bin after disinfection. The cantilever holder which has been in contact with the imaging media will be disinfected and cleaned after use. All waste will be treated using 70% hypochlorite solution or freshly made up 10% Virusolve solution (the standard killing procedure used by Dr M R's group in the London School of Hygiene and Tropical Medicine) and will be collected, transported back to the Medical School using a yellow bag in a box, and autoclaved. In the event of accidental spillage of live organisms in the Department of Physics and Astronomy, the Medical School spillage procedure will be followed.

As sharps present the main route to infection, they will not be used in the project.

Killing tests mimicking a spill type environment have been carried out for wild type Leishmania mexicana (Sept 2011). The results showed that all spills should be sprayed with 10% Virusolve solution and covered with paper towels. They should be left for at least 5 minutes before being cleared and discarded. This was shown to cause an immediate loss of organism motility (observed using a 20 x magnification optical microscope) and no manipulation after a 72 hour period (i.e. 100% kill). A similar treatment with 70% methylated spirit was only sufficient to cause partial inactivation and reduced multiplication. It is likely that similar kill levels will be observed in tests for the Leishmania major and Leishmania mexicana mutants. Confirmation tests can be conducted once the mutant parasites have been received.

Validated autoclaves in the Medical School will be used to inactivate any remaining material with a 100% kill rate.

Soak contaminated materials overnight in 70% hypochlorite and use 10% Virusolve solution to disinfect surfaces e.g. MSC after use.

Please enter comments on the GM safety committee on the risk assessment

The committee made several suggestions following an initial review of the application. These have since been addressed and approved.

The requests included clarification of the methods used to obtain the mutants, including information about any selective resistance markers, more information about the infective dose and subsequent disease progression, and clarification that sharps and sand flies are not used in the project as they present the main risk of infection and disease spread, respectively. The laboratory numbers in the Department of Physics and Astronomy where the parasites will be imaged using the AFM have also been added to the risk assessment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
The purpose of the work is to elucidate mechanisms by which host cells respond to respiratory consequences and the consequences of these responses. The aim is to improve understanding of the mechanisms of pulmonary infection with the goal of identifying new therapies and preventive measures.

Recipient or parental organism

Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Klebsiella pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa are all human pathogens and all hazard group 2 organisms. Each organism can cause pulmonary infection or invasive disease, including meningitis in the case of S. pneumoniae and N. meningitidis after colonisation of the respiratory tract. P. aeruginosa can also cause opportunistic infections of wounds, including keratitis. Staphylococcus aureus can also cause soft tissue infections. Although vaccination is available for the first three named organisms, it is not effective against group B meningococci the type worked with in the lab and pneumococcal vaccination with polysaccharide is of only moderate efficacy and does not appear to prevent pneumonia. Neither of these vaccines will be routinely offered but future development of more effective vaccines will be monitored and this policy may be modified accordingly. Hib vaccination will be considered for anyone working with Haemophilus influenzae type b on a sustained basis.

All these organisms cause clinical symptoms of fever, respiratory tract illness or invasive disease (including for meningococcal and pneumococcal meningitis). All workers...
will be counselled re potential symptoms and to seek urgent medical advice if they show any symptoms of ill health. All infections are effectively treated with prompt administration of antimicrobials as outlined above. All workers will be advised to contact their GP and to ask for referral to the regional Infectious Disease unit if indicated.

Host/vector system

Inactivated chromosomal genes may or may not contain antibiotic resistance cassettes (e.g. Janus cassettes) but where these are used they will involve antibiotics such as streptomycin, kanamycin, chloramphenicol, lincomycin, macrodiles or tetracyclines which are not the antimicrobials used as the primary treatment of these organisms, which are primarily treated with penicillins, cephalosporins, carbapenams, or where beta-lactam resistant, glycopeptides, lipopeptides or fluoroquinolones. Mutants will be of reduced virulence or will have no alteration of virulence compared to parental strains.

Origin & function

The mutants will have been created by external collaborators using techniques such as insertional mutagenesis or in frame deletions and inactivated chromosomal genes may have been complemented by reinsertion of a copy of the deleted gene, which may have been achieved by use of a plasmid maintained by an antibiotic resistance marker. Confirmation of the genetic mutation and initial analysis of function will have been carried out in the collaborators laboratories. Any potential further manipulation of parental or genetically modified strains will be under the direct guidance of the primary laboratory who have created the strains.

Further details of the genetic modification used to generate the externally acquired GM strains is not included since it is expected these will be covered by the collaborators approvals and the specifics of the modifications will vary with each mutant. We include an appendix of several mutants we anticipate we would like to investigate as part of this study and will update this appendix as further mutants are investigated. This outlines strains, the genes mutated and any antibiotics resistance markers used for the mutant.

Evaluation of foreseeable effects

The GM organisms to be studied will be selected because of their capacity of the wild-type genes to interact with the host innate immune system. As such the consequences of the deletions or of complementation are predictable and will produce strains that are less pathogenic or of comparable pathogenicity to the wild-type strains and will not have altered routes of transmission or host range. The most likely consequence of inadvertent exposure would be colonisation of the skin (for S. aureus) or the respiratory tract for other organisms. Respiratory tract colonisation only occurs for those with underlying lung disease and the same is largely true for H. influenzae, K. pneumoniae and S. aureus. Transient colonisation of S. pneumoniae occurs in 10-70% of the normal population and for N. meningitis in 10-30% of adults so if this were to occur it would replicate a situation that commonly occurs in normal life. If colonisation were to occur there is a small chance of invasive disease. We cannot predict the exact magnitude of this risk but since invasive disease occurs at a frequency of <10/100,000 in adults in the UK that means the risk of invasive disease is <1 in a thousand if it were to occur. As invasive meningococcal disease occurs at a rate of 2-6/100,000 then we can estimate that colonisation is likely to result in invasive disease also in less than 1 case per thousand.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Small volumes (<30ml) of mutants will be propagated and harvested by centrifugation (centrifuge has buckets with lids). All strains will be grown in a sealed incubator shaker. In all cases, spillage's will be safely contained and treated with freshly prepared 1:10 solution of Virusolve Plus and by autoclaving.

Laboratory manipulations with the risk of aerosol generation should be carried out in a microbiological safety cabinet (MSC), for other manipulations a standard containment level 2 facility and the use of good microbiological practice will limit contact with humans and the environment. There is unlikely to be any increased potential hazards arising from the GM bacteria described above as these are likely to be no more fit than the wild-type.
All cultures are inactivated by mixing in fresh 1:10 solutions of Virusolve Plus or solid material is inactivated by autoclaving. Records of bacterial killing in virusolve and maintenance records of autoclave will be used to validate procedures.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Please note this application is to allow investigators work with mutants created by collaborators with appropriate approvals. It will potentially allow the investigators work with a range of mutants of the indicated species but these will all be of less or equivalent virulence to the parental strains.

Approved at the Biosafety Committee 01/10/2012

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
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<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

Animal Units

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

Large Scale Activities

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

Project Ref  168/13.4

Date Ackn'd  06/06/2013

Date Project Ceased

CU2 Project Title

Analysis of cell growth, division and pathogenesis in Gram-positive bacteria

Class  Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM

Consent Granted

Project notified under transitional arrangements [N]

Tick if notifying a connected programme of work [N]
Project Additional Information

Purposes of the contained use

The aim of the project is to identify and characterise genes (and their products) required for cell growth, division and pathogenesis in Gram-positive bacteria. This work will deal with biological processes (such as cell division or cell surface biogenesis) and pathogenesis mechanisms conserved amongst Gram-positive bacteria. For comparison purposes, the project will therefore involve several model organism that will be genetically modified. The work will involve making a large number of genetic constructs to analyse gene function. These will include the use of insertional mutagenesis systems, transposon mutagenesis and replicative plasmids and will involve a wide range of clinical isolates. The model organisms Escherichia coli and Lactococcus lactis will also be used for routine cloning and expression of individual gene products for biochemical and structural characterisation.

The ultimate translational aim is to identify new drug targets and develop new therapeutic strategies to eradicate these pathogens.

Recipient or parental organism

Escherichia coli will be used as a host for routine gene cloning and for the production of recombinant proteins for biochemical and structural characterisation. All E. coli strains used will be derivatives of the K-12 (routinely used for molecular biology applications) and B lineages (routinely used for protein expression). The E. coli strains to be used are considered non-pathogenic (Chart, H et al. Jour. Appl. Microbiol.2000, 89, 1048-1058) and are generally regarded as safe.

Lactococcus lactis will also be used to express recombinant proteins. The strains used are NZ9000 isogenic derivatives (Kuipers OP, de Ruyter PGG, Kleerebezem M, de Vos WM (1998) J Biotechnol, 64: 15-21.) L. lactis is used in the dairy industry and has the GRAS status (Generally Recognised As Safe); it is used as a probiotic.

Some of the Gram positive bacteria used in the context of this application are normally commensals and can be readily isolated from the human normal flora (E. faecalis, E. faecium and all Clostridia spp.) or found in the environment (L. monocytogenes and B. cereus). All the above-mentioned organisms are considered as opportunistic pathogens as they can cause a wide range of infection in exceptional circumstances (e.g. in an immunocompromised host, following antibiotics treatment or during pregnancy for L. monocytogenes) if ingested or in contact with a wound.

S. pneumoniae and D. pyogenes are primary human pathogens, whereas S. suis is a pig pathogen.

The species considered in this application, with the exception of S. pneumoniae, are not readily transmissible via aerosol and the risk of infection to the worker is therefore minimal. The GLP and control measures described below (and in the attached document) will further minimise the risk. In the case of S. pneumoniae, the live organism will be handled exclusively within an appropriate microbiological safety cabinet to minimise the risk of exposure to bacterial aerosols.

The risks associated with each species are detailed below.

E. faecalis and E. faecium are commensal bacteria commonly found in the gastrointestinal and vaginal tracts, and in the oral cavity of Humans and other mammals. They
can cause a wide range of infections in humans including urinary tract infection, peritonitis, bacteraemia and endocarditis, usually associated with perturbation of the gut flora following antibiotic or immunosuppressive therapies. Most *E. faecalis* and *E. faecium* infections can be treated with antibiotics (ampicillin or amoxicillin, eventually in association with gentamycin).

*S. pneumoniae* is a bacterium which can cause several diseases that range in severity from sinusitis and acute otitis media to meningitis, septicaemia, and pneumonia. Rates of carriage are highest in infants and the elderly and much lower in other adults. *S. pneumoniae* are sensitive to several antibiotics (beta-lactams, sulfamides and macrolides). Since *S. pneumoniae* can lead to infections in healthy adults, workers manipulating this particular bacterium will be informed about associated risks and advised to seek early medical attention in the event of a suspected exposure to consider appropriate antibiotic treatment if required.

*S. pyogenes* (Group A Streptococci) are usually found in the respiratory tract, without signs of disease. They can cause a wide variety of clinical manifestations including pharyngitis, scarlet fever (rash), skin (impetigo or cellulitis). Invasive toxigenic infections can result in necrotizing of soft tissues, myositis (inflammation of muscles) and toxic shock syndrome. In most cases, systemic or oral anti-biotherapy (penicillin or clindamycin) efficiently cures *S. pyogenes* infection. However, because *S. pyogenes* can lead to serious infections in healthy adults, workers manipulating this particular bacterium will be informed about associated risks and advised to seek early medical attention in the event of a suspected exposure to consider appropriate antibiotic treatment if required.

*L. monocytogenes* primarily affects older adults, pregnant women, newborns, and adults with weakened immune systems. The manifestations of listeriosis include septicemia, meningitis (or meningoencephalitis), encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion or still birth. The onset of the aforementioned disorders is usually preceded by influenza-like symptoms including persistent fever. Gastrointestinal symptoms such as nausea, vomiting, and diarrhea may occur. Once diagnosed, treatment of listeriosis is not an issue, as the bacterium is sensitive to several class of antibiotics; ampicillin alone or in combination with gentamycin is a treatment of choice.

*S. suis* is an important pathogen of pigs that can occasionally cause zoontic infections in humans. Meningitis is the common manifestation, followed by septicaemia and endocarditis. Most *S. suis* strains respond to treatment with ampicillin and amoxicillin. All *S. suis* derivatives used in the laboratory will be acapsulated derivatives, severely attenuated.

*B. cereus* is a soil bacterium that can cause wound infections, bacteraemia, septicaemia, meningitis, pneumonia, central nervous system infections, endocarditis, pericarditis, respiratory or infections. Some form of infections involving toxigenic strains are associated with nausea, vomiting, and malaise, occasionally with diarrhea. Most strains (if not all) are sensitive to fluoroquinolones and aminosides such as gentamycin.

*C. difficile* can cause antibiotic-associated diarrhoea in humans, which, in a minority of cases, can lead to severe colitis. Advancing age, in combination with underlying health problems and antibiotic therapy, is the greatest risk factor for *C. difficile* infection. There are several effective treatments available, including vancomycin, metronidazole and the *C. difficile*-specific antibiotic fidaxomicin.

*C. perfingens* can cause enteritis in humans and, rarely, gas gangrene following deep wound infections. It causes a variety of veterinary diseases including wound infections and necrotic enteritis. *C. perfingens* enteritis is usually mild and requires no treatment however the organism is susceptible to penicillins, cephalosporins, clindamycin, metrinidazole, and tetracyclines.

*C. sordeillii* causes bloodstream and necrotising soft-tissue infections in animals. In humans, infections (toxic shock syndrome) have been reported in both postpartum and post abortive women and tissue infections have been reported in intravenous drug users. Antibiotic susceptibilities are the same as for *C. perfingens*.

*C. tetani* can cause tetanus in man and in some animals. Infections result from contamination of a deep wound with spores. A highly effective vaccine is available.
C. chauvoei can cause necrotic muscle infections in animals; not known in humans.

C. septicum is an important pathogen of turkeys, in which it causes gangrenous dermatitis. In humans the organism can cause myonecrosis as a consequence of bowel carcinoma and other malignancies. Antibiotic susceptibilities are the same as for C. perfingens.

C. novyi causes a range of gangrenous infections in animals. In humans infection is rare but has been reported in intravenous drug users. The organism is currently being used in clinical trials for the treatment of hypoxic refractory solid tumours.

C. glycolicum causes ulcerative colitis in domestic fowl and can occasionally cause infections in humans (bacteraemia, septic shock, wound infections).

C. histolyticum can cause gas gangrene in humans, but this is now extremely rare.

C. colinum is the causative agent of ulcerative enteritis, a serious disease of the bobwhite quail.

C. sporogenes is an umbrella name given C. botulinum strains which lack the genes encoding the botulinum neurotoxins and is generally regarded as non-pathogenic.

### Host/vector system

We will use broad host-range, non self-transmissible plasmids carrying resistance markers (kanamycin, spectinomycin, neomycin, tetracycline, minocycline, chloramphenicol, streptomycin or erythromycin).

For cloning experiments in E. coli, plasmids carrying various origins of replication will be used, including derivatives of pMB1 p322 and pUC[1,2]), pSC101 (3), p15A (pACYC [4]), colE1 (5) and M13 (6).

L. lactis vectors are pNZ8048 derivatives (7).

For E. faecalis, E. faecium, S. pneumoniae, S. pyogenes, S. suis, L. monocutogenes and B. cereus, derivatives of pWV01 (8) and pam-beta1 (9) replicons will be used.

For clostridia spp. native clostridial replicons will be used, including pBP1, pCB102, pCD6 and pM13 (10).

None of the vectors used will encode resistance to antibiotics used in the treatment of infections caused by these organisms (which are, in any case, rare). None of the vectors used are hazardous and they are very likely to confer any advantage to the host bacterium.

Additional plasmid vectors will also be constructed using standard components such as those described above.

1. We will construct gene "knock-outs" using available genetic tools, e.g. targeted insertional disruption, gene deletion, transposon mutagenesis, etc. These procedures will not introduce foreign DNA into the recipient, other than an antibiotic resistance cassette and non-coding transposon components. The antibiotic resistance markers used will not confer resistance to antimicrobials used in the clinic for outline treatment of infections caused by these microorganisms.

2. We will introduce homologous DNA (same as the host) into wild type and mutant strains, for example, mutant complementation, multi-copy suppression and protein tagging. We will also introduce heterologous DNA (from another related strain or species), for example, trans-complementation with orthologous genes from another species. Introduced genes of interest will include genes encoding cell wall proteins, proteins involved in cell division/wall metabolism, cell surface structure synthesis and envelope biogenesis. None of the inserts will encode proteins constituting a hazard.

3. Wild type and mutant strains will also be modified by the introduction of genes encoding commonly used reporters, e.g. green fluorescent protein (and derivatives), fluorescent flavin mononucleotide-based proteins, luciferase, β-galactosidase, β-glucuronidase etc.

4. At no point will toxin encoding genes, or genes encoding known harmful proteins, be cloned or introduced into any bacterial species.

**Evaluation of foreseeable effects**

**Hazards to the worker:**

The work described will generally involve generating loss-of-function mutants, and complementing such mutants. It is expected that most mutants will have an unaltered or decreased pathogenic potential compared to parental strains. Therefore, there is no foreseeable significant change in the risk associated with the genetic modifications carried out.

No genetic material will be introduced that could be predicted to increase virulence, e.g. bacterial toxins, characterised virulence factors from other species.

It is possible that increasing gene dosage by the introduction of a gene on a plasmid could alter the virulence of a strain. However, maintenance of such a plasmid would be dependent on continued antibiotic selection and the plasmid would be rapidly lost outside of the laboratory.

The risk of transfer of plasmids between species will be negligible. Plasmids used for complementation experiments are able to replicate in a broad range of Gram positive species but are not transferrable in the absence of a helper plasmid carrying mobilisation functions.

Marker genes which confer resistance to antimicrobials currently used against these organisms will not be used.

It is very unlikely that, in the event of exposure, the GMOs generated will harm human health. All of the organisms considered here are capable of causing disease in humans but standard laboratory practice (outlined in the attached document) and the specific control measures outlined in this risk assessment will minimise the risk to the worker. In the event of accidental exposure the worker will be advised to seek immediate medical advice and will be provided with information on strain characterisation, including antibiotic susceptibility. In addition, the GMO's generated are predicted to have an unaltered or a decreased pathogenic potential and will not increase the infection risk.

Some of the organisms described here pose a greater risk to certain individuals or individuals with underlying health conditions. For example, L. monocytogenes is a serious risk to the unborn child. Each worker will undergo a pre-assessment by Occupational Health and will be advised to seek advice should their circumstances change. Where an individual worker is deemed to be at greater risk a specific risk assessment will be prepared in consultation with Occupational Health, the Health and Safety Dept. and the BioSafety committee.

**Hazards to the environment:**

All bacteria to be manipulated are ubiquitous in the environment. Although hazard likelihood is low for all the organisms, the GLP and control measures outlined in the attached risk assessment will further reduce risk. The work will be carried out in designated areas and all waste will be decontaminated prior to disposal.

Standard processes will be designed to avoid/minimise emission, release and spread of HG2 organisms. These include restricting the number of people exposed to the
organisms during routine work and using the minimum practical volumes at a given time. Equipment capable of generating a significant aerosol (e.g. centrifuges) will be operated with appropriate controls.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All contaminated solid waste (agar plates, disposable plasticware, etc) will be placed in yellow bins, autoclaved and disposed via the designated waste contractor.

Liquid cultures will be chemically-inactivated (Virkon 1% or PeraSafe 0.16% for 30 min) or autoclaved prior to disposal down the sink.

All contaminated glassware and reusable plasticware will be decontaminated as above.

Virkon and Perasafe are disinfectants with proven efficacy against Gram positive spp. and are commonly used in both research laboratories and hospital environments. Sporocidal activity has been validated by the manufacturer (see manufacturer's website) and in several articles in the published literature.


The designated waste-disposal autoclaves are serviced and 12-point validated by Consolidated Medical Industries (CMI) twice a year.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment

This project was approved by the BioSafety Committee on the 10/05/2013

**Project Containment**

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Project Ref 168/13.5

Date Ackn’d 22/10/2013

CU2 Project Title Quantifying the extent of colonization of plants by beneficial or pathogenic microbes using isolates that express fluorescent proteins or bioluminescent proteins

Date Project Ceased

Class 2

CultureVol Class 2 < 1 Litre

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To prevent environmental spreading of plant-associated GM microbes

Recipient or parental organism

Phytophthora infestans, Pseudomonas putida KT2440, Pseudomonas syringae pv. tomato DC3000

Host/vector system

The transgenes encoding for fluorescent protein (GFP; Phytophthora infestans and Pseudomonas putida) or bioluminescent protein (Luciferase; Pseudomonas syringae pv. Tomato) are stably inserted into the genome of the organisms.

Origin & function

The GFP-expressing Phytophthora infestans is a pathogenic line that is derived from an isolate that is distributed worldwide, including major Solanum (tomato and potato) growing regions in Europe, North America, and South America. The LuxCDABE-expressing P. syringae pv. Tomato strain is a pathogenic line (DC3000) derived from a North American isolate, but P. syringae pathovars with similar virulence and host range exist worldwide, including Europe and American isolate, but P. syringae pathovars
with similar virulence and host range exist worldwide, including Europe and the UK thus it is already present in the environment. The GFP-expressing P. putida strain is derived from a beneficial plant growth promoting European strain (KT2440). This strain is a non-pathogenic, root-colonising isolate with a relatively wide host range of predominantly cereal plant species. The strain is widely distributed across the world. All three GM strains will be used to assess compatibility with host plants (Arabidopsis, tomato, wheat, or maize) by means of in planta colonization.

All of the above GM organisms have previously been constructed elsewhere, and are described in the following publications:


The GM organisms will be obtained from the following donors:

- GFP-expressing **P. infestans** will be obtained from Dr W, the James Hutton Institute (Dundee, UK).

- GFP-expressing **P. putida** KT2440 will be obtained from Dr N Rothamsted Research (Harpenden, UK).

- Luciferase-expressing **P. syringae pv. Tomato DC3000**, will be obtained from Dr F, John Innes Institute (Norwich, UK).

### Evaluation of foreseeable effects

All experiments will be carried out by trained personnel in the laboratory. Personnel will be required to wear disposable gloves and coloured lab coats, which will be kept within the plant growth facility. The scale of work will vary from a few plants (e.g. 10-20) to one hundred, depending on the nature of the experiment. As for the microscopy analysis of in planta colonization by the microbes, collected material (either leaves of roots) will be sampled to fit within the borders of the microscopy slide, in order to make sure that all colonized material is covered by the fixative (25% glycerol). The fixative immobilises the microbial dispersal and will therefore reduce infectivity of the microbes. As plant material has been detached from the plant both the leaf and associated microbe will die within a day.

#### I. Phytophthora infestans:

As mentioned above, the GFP-expressing line is derived from an isolate that is distributed worldwide, including major Solanum growing regions in Europe, North America, and South America. The host range of this P. infestans strain is primary potato (Solanum tuberosum) and tomato (Solanum lycopersicum). The requested strain expresses the GFP protein from a stable (genomic) GFP gene insertion, which does not alter its virulence, host range or survival. The strain will not be used in greenhouse or field trials.

During the course of all P. infestans experiments, the Oomycete will be kept in its asexual cycle. Asexual spores (chlamydospores) of this microbe germinate to form sporania, which in turn produce infectious zoospores when they come in contact with plant tissues. The pathogen will be cultured from a frozen spore suspension (stored in a locked minus 80 freezer in the C51 lab; Alfred Denny Building). With the exception of quantification of in planta colonization (see below), all experimental procedures will be carried out within the Sir David Read plant growth facility (G-19, Central Annex-Department of Animal and Plant Science). The plant pathogen will be cultured inside an incubator in the G19 growth facility. Agar plugs and/or zoospore suspensions will be prepared in G19. Inoculation will be carried out inside the Conviron growth chambers by applying agar plugs with hyphal material or droplets containing a suspension of zoospores onto leaves, thereby preventing formation of aerosols.

Samples of inoculated leaves will be collected between 1 and 5 days after inoculation and placed onto glass microscopy slides into fixative (25% glycerol), and sealed with...
a cover slip using transparent nail polish and transferred clean into secondary containers. The microscopy slides will be transported from the Central Annex laboratory to the C43 lab in the Alfred Denny Building inside plastic sealed leak-proof secondary boxes containers, where they will be examined further for GFP fluorescence, using an epi-fluorescence microscope.

Escape of *P. infestans* from the growth facility is very low. Escape can only occur via three routes: (a) zoospores (b) chlamydospores (c) infected plant material/colonised agar:

(a) zoospores of *P. infestans*. *P. infestans* zoospores have two flagellae, which allow the zoospore to travel in water on the surface of leaves and in the soil, but will survive outside a watery environment. Plants will be inoculated by placing drops of zoospores onto the leaves. Plants will be watered from below (in a propagator tray) to avoid splashes. The requirements for a suitable host and short viability of germinated zoospores spores make the risk of escape via drainage system low. In addition, when transgenic microorganisms are used in the Conviron chambers, waste water from the cabinet is collected in a special chamber inserted into the drain. The chamber contains an autoclavable filter which will trap any spores. This filter is autoclaved and replaced daily. The water in the chamber is treated with 3% Virkon before disposal.

(b) chlamydospores. Chlamydospores are able to detach and travel in the air. The plant growth chamber is maintained at a negative pressure with respect to the external environment which minimizes the chlamydospore escape. The growth facilities also contain filters on the outlet vents to prevent spores from escaping via this route.

(c) infected plant material/colonized agar.

Plants infected with *P. infestans* can produce chlamydospores to infect other tissues of the same plant or nearby plants. Infected plant material and colonized agar will be contained within the Central Annex of growth facility. Disease symptom scoring will be performed within Conviron growth cabinet. For in planta visualization of *P. infestans*, infected, non-sporulating leaf samples will be collected and fixed onto sealed microscopy slides to prevent escape, as detailed above. Microscopy slides will be transported clean from the Central Annex laboratory to the C43 lab in the Alfred Denny building in plastic leak-proof secondary containers (but slides are sealed anyway). All contaminated (plant) material will be autoclaved and disposed in the Alfred Denny building as detailed under waste disposal.

II. *P. syringae* pv. *Tomato* DC3000.

The requested strain is derived from a North American isolate, but *P. syringae* pathovars with similar virulence and host range exist worldwide, including Europe and the UK thus it is already present in the environment. The luxCDABE operon present in the requested transgenic strain is stably inserted into the bacterial chromosome and does not alter its virulence or host range. This bacterial strain does not produce long-lasting survival structures. The strain will not be used in greenhouse or field trials.

The pathogen will be cultured from a frozen spore suspension (stored in a locked minus 80 freezer in the C51 lab; Alfred Denny Building). With the exception of quantification of in planta colonization, all experimental procedures will be carried out within the Central Annex of Sir David Read plant growth facility (G19). The plant pathogen will be cultured in liquid growth medium inside an orbital shaker that is present in the Central Annex of the growth facility. The O/N culture will be adjusted to the appropriate cell density in the Central Annex laboratory. Inoculation of tomato and/or Arabidopsis will be carried out inside the plant growth chambers of plant growth facility. Inoculations will be carried out by pressure infiltrating of leaves, or dipping the leaves into a bacterial suspension, thereby preventing formation of aerosols. Samples of inoculated leaves will be collected between 1 and 5 days after inoculation and placed into glass microscopy slides into fixative (25% glycerol) and sealed with a cover slide, using transparent nail polish. Microscopy slides will then be transported inside airtight plastic leak-proof secondary container boxes to the C43 lab in the Alfred Denny building, where they will be examined further for bioluminescence, using a CCD camera.

Opportunities for escape of *P. syringae* pv. *Tomato* from the lab or growth facility are very low. Escape can only occur via bacterial cell suspensions or colonized plant material. In vitro cultivation of the pathogen and preparation of the inoculum, will be carried out in the Central Annex laboratory of the growth facility. Colonized plant material will be contained within the growth cabinet during cultivation of inoculated plants. For in planta visualization of *P. syringae* pv. *Tomato*, leaf samples will be collected in the growth chamber, and fixed onto microscopy slides in the Central Annex laboratory, as detailed above. Slides will be transported clean in sealed plastic leak-proof secondary containers from the growth facility to the C43 lab in the Alfred Denny Building. All contaminated (plant) material and agar plates, will be autoclaved as
detailed under waste disposal.

III. P. putida KT2440

As mentioned above, the GFP-expressing strain of this beneficial plant growth promoting bacterium is derived from a non-pathogenic, root-colonising isolate with a relatively wide host range of predominantly cereal plant species. The strain is widely distributed across the world. The GFP transgene does not affect the plant-beneficial traits of the strain, nor does it change its host range. P. putida KT2440 does not produce long-lasting survival structures. The strain will not be used in greenhouse or field trials.

With the exception of quantification of on planta root colonization, all experimental procedures will be carried out in the Central Annex of the Sir David Read plant growth facility. The soil microbe will be cultured in liquid growth medium inside an orbital shaker that is present in the Central Annex laboratory. The O/N culture will be adjusted to the appropriate cell density in the Central Annex laboratory. Soil matrix (sand/peat or sand/vermiculite) will be inoculated with a bacterial suspension prior to planting of seedlings, which will be carried out inside the growth chambers. Root systems will be diluted and plated onto agar plates in the Central Annex laboratory, and kept inside the incubator. After 48h of growth, plates will be analysed for numbers of GFP-fluorescent colonies using a blue-light box that is present in the Central Annex laboratory. Alternatively, root tissues will be collected, placed onto glass microscopy slides into fixative (25% glycerol) and sealed with a cover slip, using transparent nail polish. Slides will be transported clean inside airtight plastic boxes leak-proof secondary containers to the C43 lab in the Alfred Denny building, where they will be examined further for root colonization using an epi-fluorescence microscope.

Opportunities for escape of P. putida KT2440 from the lab or growth facility are very low. Escape can occur via bacterial cell suspension or colonized agar medium/roots/soil. Cell suspensions and agar plates will all be prepared in the Central Annex laboratory of the growth facility. Colonized plant material and contaminated soil will be contained within the growth cabinet during the cultivation of infected plants. Dilution plating of rhizosphere extracts will be carried out in the growth facility. For on planta visualization of P. putida KT2440, root/rhizosphere samples will be collected in the Central Annex laboratory in growth facility, and fixed onto microscopy slides as detailed above. Slides will be transported from the Central Annex to the C43 lab in the Alfred Denny building inside airtight plastic leak-proof secondary containers. All contaminated (plant) material and agar plates will be autoclaved as detailed under waste disposal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is requested or needed for the planned work

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All pots, spoil, plant material will be placed in biohazard bags, sealed and autoclaved before disposal (see next section for more details). Microscopy slides will be placed inside shock bottles prior to autoclaving and subsequently disposed with normal glass waste.

Material contaminated with GMOs is autoclaved at 134°C for 20 minutes. This cycle kills all plant materials and (soil) microorganisms, and has been validated by Bmm Western (the autoclave manufacturer). In all autoclave runs, the temperature is monitored and recorded by a probe placed in the centre of one of the bags being autoclaved. The autoclave is checked and tested by BMM Western every 3 months. Autoclave conditions are automatically recorded and checked and achieved for 3 years. The autoclave is regularly serviced and maintained.

In the unlikely case of a broken/leaking microscopy slide in lab C43, the cohesion of the fixative will prevent spreading of plant-associated microbes in the plant samples. The leak itself will then be treated with 3% virkon, which is insufficient to kill all microbes. Broken slides will be collected in shock bottles and autoclaved as detailed above. Emergency spillage procedure:
In the case of spillage during the in vitro cultivation of the bacterial microbes within the orbital shaker the spillage will be wiped up and the contaminated cleaning materials will be autoclaved. The incubator will be cleaned thoroughly cleaned with 3% virkon.

Is an emergency plan required according to regulation 20?  
Y

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

Project discussed at the UoS Biosafety Committee meeting on the 4th October, Approved by Quorum of 7 members using online committee on 5th October 2013

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**Project Ref** 168/14.1

Date Ackn'd  19/02/2014  
Date Project Ceased

Carbon monoxide (CO) and CO-releasing molecules (CORMs) as potential adjuvants to antibiotics

Class 2  
Consent Granted

Class CultureVol Class 2 Volume Class 3-4

Consent Granted

Tick if notifying a connected programme of work  
N

Project notified under transitional arrangements  
N
**Purposes of the contained use**

In this research, supported by the Leverhume Trust we will (i) test the hypothesis that judicious application of CO and/or CO-RMs, in combination with other antimicrobial agents, especially antibiotics, may be used to combat bacterial pathogens, (ii) understand the interactions between the CO and antimicrobial agents, and (iii) obtain new information on the fundamental mechanisms that underlie the antibacterial activity of CO-RMs. Our working model (the Trojan Horse hypothesis) is that a CO-RM may release CO extracellularly but more likely is transported into a bacterial cell via currently unknown pathways (possible via a membrane importer). In the cell, the CO dissociated from the CO-RM (with formation of iCO-RM, the inactive form). This is followed by reaction of CO with biological targets, notably the haem centres of membrane oxidases. Other targets include membrane transporters, metal homeostasis, intermediary metabolism and transcription factors that regulate gene expression. The fate of CO-RM or iCO-RM in the cell is unknown but the compounds may be re-exported. This work should make a valuable contribution to our understanding of bacterial resistance to CORMs and their potential for acting as adjuvants to antibiotics.

**Recipient or parental organism**

Uropathogenic strains of E. coli (UPECs) are clonally disseminated and cause urinary tract infections (UTI). This project will use strain ST131, a cause of UTI and bloodstream infections in the community, hospitals and long-term care facilities. It belongs to the international O2: H4-ST131 clone. There has been a worldwide increase in UPECs that produce Extended Spectrum Beta Lactamases (ESBLs), which mediate resistance to cephalosporins and monobactams but not cephamycins. This strain is virulent but is widely studied in laboratories worldwide to understand pathogenicity and antibiotic resistance traits.

**Host/vector system**

The genome ST131 and similar bacteria encodes virulence genes commonly associated with UPECs. Some of these factors are encoded by plasmids. The plasmids contain up to 10 antibiotic resistance genes, including genes that mediate resistance to beta-lactams, chloramphenicol, erythromycin and tetracycline. These plasmids will not be manipulated in any way. Lambda-red methods will be used to introduce mutations in the chromosome only in due course (Datensko and Wanner, 2000, PNAS 87, 6640-6645) The only deviation is the use of a gentamycin -resistance cassette not Tn10 or Tn5.

**Origin & function**

The hazards associated with the host organisms are described above. The only alterations proposed would be in genes not thought to affect virulence or antibiotic resistance, but rather in genes whose products are thought to be targets for CORMs, such as transporters and haem proteins. Genes to be targeted will probably decrease fitness of the organism not increase it. Examples are genes involved in solute uptake and efflux, respiratory enzymes, metal homeostasis and intermediary metabolism, especially thiol metabolism. Genes will not be introduced that encode pathogenicity or virulence determinants, that alter susceptibility to the immune system, affect host range or alter the sensitivity of the organisms to prophylactic treatment.

**Evaluation of foreseeable effects**

The risks and potential effects are the same as those of the unmodified strain. Strain ST131 is a cause of UTI and bloodstream infections in the community, hospitals and long-term care facilities.

The work to be carried out or the alterations proposed will not affect transfer to other organism or give rise to harm if transferred to another organism. It is not envisaged that modification will affect the organism's ability to acquire other sequences.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The organism will be contained in the specified areas and its use and storage will be clearly marked. Good Laboratory practice will ensure that the organism is not released. The risk of hazards is low. Laboratory scale cultures (agar plates and liquid cultures up to 1000 ml) will be used. The work outlined will be carried out in the designated laboratory (F13), which has been inspected and meets containment level 2 standards. Workers will wear appropriate coats and gloves. The work will generate contaminated plastic ware (tips, pipettes, small containers), flasks, agar plates and spent media used in the growth of bacteria. Bulk liquid waste will be decontaminated using 2% solutions of Virkon. Glassware will be sterilised by autoclaving. Solid waste will be disposed of in line with the University's procedures which have been approved for disposal of Class 1 and 2 GM waste. All contaminated solid waste (plastic etc) will be treated using 2% solutions of Virkon as recommended by the manufacturer before autoclaving (two are available) in the designated laboratory (F13). Autoclaves are serviced and checked annually. These measures will ensure effective killing and removal of bacteria and any residual nucleic acid material. "Contamination " of workers in this context would be due to inadequate hand washing or poor personal hygiene. The main threat is in causing UTI's via the normal routes. The strain is not an enteric pathogen and will not pose any additional threat over and above normal 'lab' strains in this regard. Showering or drenching a contaminated worker is unlikely to be effective. Workers with UTI-related problems would be expected to consult a doctor whatever the source of infection. To minimise spillage affecting others not using the organism, all materials used for the strain will be labelled, small cultures will be grown in incubators used only for this strain and accidental spills will be treated with Virkon or similar. There is a complete Drizit laboratory spills kit in the corridor a few metres away. Two researchers only will use this strain and they will work in the same bay of the laboratory.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 168/14.2
**Project Additional Information**

**Purposes of the contained use**

To use recombinant vaccinia, an oncolytic virus, to selectively infect mammalian cells in vitro and/or in vivo to assess oncolytic potency in tumour and normal cells and to analyse levels of viral replication, selectivity of infection, oncolytic potency, viral cell viability and mechanisms of tumour cell lysis.

**Recipient or parental organism**

Recombinant oncolytic VACV will be used to infect tumour and normal cells. VACV is a replication competent oncolytic virus that can efficiently infect a wide range of target cells, however viral engineering has rendered some of the rVACV variants tumour selective. The experimental work proposed here involves the addition of VACV particles encoding therapeutic gene sequences to mammalian cell lines (Hazard Group 1 and 2 and Activity Class 1 and 2 cells) in vitro and the direct administration in vivo. Following the transduction of the mammalian cell lines with rVACV, RNA and protein will be extracted from the cells to determine expression levels of viral RNA, target gene RNA or gene product and functional assays will be performed. Following the direct administration of rVACV in vivo, animals will undergo monitoring and tumour measurements (as detailed in the project licence) and/or tissues/cells will be harvested and used in downstream functional assays.

**Host/vector system**

Hosts: The host organism would be normal and cancer cell lines of human and mouse origin. These would include Hazard Group 1 and 2 cell lines and Activity Class 1 and 2 genetically modified cells all of which are covered by appropriate risk assessments.

Vectors: Recombinant attenuated Western Reserve (WR) or Lister strains of Vaccinia are the backbone vectors for carrying the genes and are covered by appropriate risk assessments. Attenuation mechanisms are outlined briefly below and fall under the categories of Disabled and attenuated vectors and Conditionally replicative vectors (outlined
Various permutations of VACV have been created and attenuated for virulence by the deletion of the following genes, alone or in combination:

- **Thymidine Kinase (TK):** TK deletion from VACV engenders enhanced specificity for tumour cells. TK-deleted VACV is highly attenuated in non-dividing cells but is able to replicate robustly in transformed cells.
- **Vaccinia Growth Factor (VGF):** VGF is a viral ortholog of EGF that encourages increased metabolic activity in cells and promotes viral replication and spread. Deletion of VGF from vaccinia virus severely attenuated viral replication in vivo, and increases the LD50 of the virus by over 2 logs. VACV that are deleted for both VGF and TK appear to replicate specifically in tumour cells.
- **B18R:** B18R is a Type I IFN decoy receptor encoded by VACV. Deletion of the B18R gene enables infected cells to generate a stronger type I IFN response in response to VACV infection and protects surrounding nontransformed cells from viral-mediated cell killing by eliciting the antiviral state. Tumour cells often have defects in the type I IFN response, rendering them still susceptible to oncolysis by B18R deleted VACV.

Specific Open reading frames, cDNAs and gene sequences encoding therapeutic gene sequences or therapeutic proteins (e.g. cytokines and antibodies) and/or si/shRNAs or miRNAs (designed to knockdown the expression of cancer causing genes) and reporter genes can be inserted into rVACV. An example of a transgene insert includes (but is not limited to) immune modulating cytokines such as human or mouse granulocyte-macrophage colony-stimulation factor (GM-CSF), a cytokine for stimulating proliferation of haematopoietic stem cells which would augment the anti-tumour and anti-viral immune response.

Guidance on inserts that may have a greater risk of adverse effects on human health can be found in SACGM compendium of guidance, Part 2-2, p37-46. Inserts will generally have a therapeutic effect.

**Origin & function**

Specific Open reading frames, cDNAs and gene sequences encoding therapeutic gene sequences or therapeutic proteins (e.g. cytokines and antibodies) and/or si/shRNAs or miRNAs (designed to knockdown the expression of cancer causing genes) and reporter genes can be inserted into rVACV. An example of a transgene insert includes (but is not limited to) immune modulating cytokines such as human or mouse granulocyte-macrophage colony-stimulation factor (GM-CSF), a cytokine for stimulating proliferation of haematopoietic stem cells which would augment the anti-tumour and anti-viral immune response.

Guidance on inserts that may have a greater risk of adverse effects on human health can be found in SACGM compendium of guidance, Part 2-2, p37-46. Inserts will generally have a therapeutic effect.

**Evaluation of foreseeable effects**

**Potential hazards associated with the recipient host:**

These will vary depending on the cell type used and can be known or unknown. Potential common pathogens might be human pathogens such as Epstein Barr virus or rhinovirus. Primary human samples are screened for the presence of the high risk human pathogens: hepatitis virus and HIV by the supplier. Samples containing such pathogens should not be used.

Note: Hazard Group 2 cells and Activity Class 2 cells present a greater risk than Hazard Group 1 cells, usually due to their potential to harbour human pathogens.

**Potential hazards arising from the gene product:**

The expression of the majority of genes is predicted to generally have little or no adverse effect. However, the precise outcome is difficult to predict. The work outlined in this assessment covers therapeutic transgenes and gene sequences expressed by rVACV. By their very nature these will have a therapeutic effect. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines and expression of a therapeutic transgene will often result in cancer cell death or arrest and render the cells more immunogenic in vivo which would result in greater clearance.

**Potential hazards arising from the combination of gene and host:**

The knockdown of, or increased expression of, the majority of genes is predicted to generally have little or no adverse effect. Gene products will only ever be expressed in cells that are able to support viral replication (tumour cells) and the greater the expression levels, the more likely the cell is permissive to viral replication and will ultimately die due to this property. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines, many of which harbour mutations within these genes or expressing these genes through other delivery vectors (adeno- or lentiviral transduction). Enhanced tumour cell killing is a beneficial additional effect of combination
of host and gene.

Potential for transfer of gene to other hosts:

Vaccinia can infect many cells, although the majority of variants used here are disabled/attenuated and conditionally replicative vectors, i.e. replicate selectively in tumour cells. Normal cells will not support viral replication and the virus will be inactivated. In cancer cells where replication is permissible the virus will be propagated and will result in cell lysis and death and release of virus which will go on to infect other surrounding cells. By their very nature, the replication component virus cells that support viral replication will ultimately die due to viral replication and the level of viral replication will drive the level of the therapeutic transgene which will also enhance immune clearance in vivo or cell killing. Vaccinia replication takes place in the cytosol so there is no chance of stable integration or recombination with the host genome. Additionally many of the variants that will be used are disabled and attenuated vectors.

Poxviruses have a large number of genes, many of which are dispensable for growth in vitro and cause attenuation when disrupted.

Potential for harm to human health in the event of exposure:

Typical characteristics of the poxvirus family include a large dsDNA viral genome (varying from 130 to 300kb in size) which is enclosed in a multi-membrane virion, making them some of the largest known viruses. Replication takes place within the cytoplasm of permissive cells and all the enzymes required to initiate viral gene transcription are carried within the virion. Other general features include the induction of virus containing pustular, epidermal lesions, although the severity of the disease is dependent on the host organism and poxvirus species. (Ref: SACGM, Table 2.10. Host range of poxviruses and the typical symptomatic consequences of infection. *Less common adverse reactions to Vaccinia virus inoculation in humans, p104).

VACV normally has no serious health effects in humans, although it can cause disease of the skin when used as a vaccine vector. Vaccinia virus is usually injected in the dermis where a localised lesion appears (a "take") and then scabs over and heals in about 10-14 days. The vaccination is accompanied by fever, rash, lymphadenopathy, fatigue, myalgia and headaches in some patients. Accidental infection with the virus can occur through contact between the vaccination lesion and broken skin (inadvertent inoculation). Serious complications such as ocular vaccinia, myopericarditis, eczema vaccinatum (a papular, vesicular and pustular rash that is very infectious, 38 cases per million doses), progressive vaccinia (progressive necrosis at the vaccination site, 3 cases per million doses), postvaccinal CNS disease (headache, lethargy, seizures and coma, 12 cases per million doses), foetal malformations and abortion (very rare) sometimes occur after vaccination. Complications are more serious in immunosuppressed individuals and the smallpox vaccine usually causes one death for every million doses.

Contraindications to vaccine are their use in immunocompromised individuals, individuals with certain skin (e.g. eczema) and cardiac diseases and pregnant women. However it must be noted that VACV is a poxvirus that has an extensive safety record and has been used widely as a vaccine vector in vaccination campaign for the eradication of smallpox and is therefore considered to pose minimal risk to human health.

Vaccinia virus may cause disease in situations whereby immunity may be reduced, i.e. during pregnancy, in people with active skin disorders such as eczema or psoriasis, or in immuno-compromised individuals such as those infected with HIV, those individuals who have undergone splenectomy or are on high dose steroid treatment or immune suppressive chemotherapy. It is well documented that vaccinia can be passed to close contacts of vaccine recipients generally with little adverse consequence. Therefore, although an individual with a laboratory-acquired infection is unlikely to receive the virus dose given for vaccination purposes, close contacts, particularly those with contraindications for vaccination, may also be at risk.

The viruses that will be used in this assessment do not cause full blown Vaccinia infection but tend to cause more localised infections such as a pustule or blister (with or without necrosis and scarring) particularly on the hands, mouth or around the eyes. It is possible for one infected area to contaminate and infect another (e.g. finger to eye). A wide
range of photographs of Vaccinia virus infections are available at: www.bt.cdc.gov/training/smallpoxvaccine/reactions/default.htm and by searching for "smallpox vaccine" or Vaccinia virus on the internet. SHE should maintain a list of people who work with Vaccinia and all people that work with Vaccinia will be given a letter for their family doctor to be kept with their general practice notes, to prompt their general practitioner to consider Vaccinia if they develop such a blister or sore.

Potential to disseminate and harm the environment in the event of accidental release:

Poxviruses are highly stable and resistant to dehydration; infectious virus can be stored in dried powder form. Transmission is usually via aerosol or direct contact and infectious virus can survive for protracted periods in dried scab material shed from epidermal lesions. In the event of any release into the environment, genetically modified poxviruses might persist and could be transmitted to other humans or animal species.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All in vivo work will be carried out under Containment Level 2 conditions using rodents. All handling of rVACV agents will be in Class II safety cabinets and all treated animals will be kept in separate red-line isolation cages, which are completely sealed with separate negative pressure air supply and HEPA filters on the inlet and outlet. Our building is a designated building, regulated under the Animal (Scientific Procedures) Act 1986 and is therefore designed to prevent escape and to ensure complete containment of animals.

Only Home Office-approved operatives, trained in safe handling, will be used to undertake in vivo work, to minimise the risk of infection from potentially infectious adventitious agents from the host cell lines.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste is inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04) or where inactivation is not possible as Hazardous Biological waste (EWC 18 01 03). Inactivation will be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

It is well known that Vaccinia and other poxviruses have the capacity to survive for considerable periods in dried material such as detached vaccination scabs, but it is less well appreciated that survival in aqueous solutions can be for several weeks. Live virus can also be isolated from solid surfaces and fabric for as long as two weeks after contamination. For laboratory workers, ingestion, inoculation via needles or sharps, and droplet or aerosol exposure of mucous membranes or broken skin are possible routes of infection. Laboratories working with Vaccinia and other poxviruses should have suitable local rules to control these potential sources of infection, including suitable procedures for decontamination of equipment and surfaces.

It is imperative that strict hygiene is adhered to, including hand-washing after work and the use of an alcohol gel to limit transfer. All door handles and equipment must be wiped down with 20% Trigene Advance as a precautionary measure.

Laboratory coats should be disposable or will require to be washed at a high temperature (at least 60°C) or autoclaved frequently as VV is relatively heat resistant, can survive for long periods on fabrics, and has been shown to transfer from fabrics to other objects by direct contact.

Other information

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 0.02% sodium hypochlorite, 30% isopropanol, 40% ethanol, 0.02% glutaraldehyde, 0.01% benzalkonium chloride, 0.0075% iodine, 30% Sanytex and 0.12% ortho phenylphenol. The virus is resistant to solvent/detergent combinations (TNBP/Triton X-100 and TNBP/ Tween 80) and longer incubation periods (between 10 minutes and 24h depending on the solvent/detergent used) are necessary to
inactivate the virus.

PHYSICAL INACTIVATION: The virus is inactivated by dry heat at 95 ºC for 2 hours. The heat-sensitive fraction of the virus is inactivated by moist heat at 60 ºC while the heat-resistant fraction may take higher temperatures to fully inactivate it. The virus in its aerosol form is also sensitive to UV light (254 nm).

SURVIVAL OUTSIDE HOST: The dried virus can survive up to 39 weeks at 6.7% moisture and 4 ºC.

The Biosafety Committee asked the author to expand on the goals, give an explanation as to why they were doing this work, is it oncolytic, and is it being used for tumour cell lines. The Committee agreed this would be AC2 and therefore require an HSE notification.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Human Clinical Applications

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Project Ref 168/14.3

Date Ackn'd 03/12/2014

CU2 Project Title The physiology and pathogenesis of Neisseria meningitidis

Class

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Non-GMM Consent Granted Yes

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

Investigate the role, function and regulation of *N. meningitidis* (also known as the meningococcus, abbreviated Me) genes involved in:

(i) bacterial physiology, particularly the stringent response, anaerobic metabolism, amongst others

(ii) carbon metabolism, utilisation of lactate, glyceraldehyde, pyruvate, glucose and leucine responsive regulatory protein

(iii) secreted proteins (IgA protease, Gly-1)

(iv) pathogenesis.

In addition we would like to overexpress several of the above classes of proteins from *N. meningitidis* (MC) in *E. coli* for role, structure function studies.

**Recipient or parental organism**

*Neisseria meningitidis* strain MCS8, or strain H44176 both are wild-type serogroup 8 strains. *N. meningitidis* (MC) can be a commensal or serious pathogen of humans with the ability to cause meningitis and septicaemia.

(ii) *Escherichia coli* strains K12 and B. Non-pathogenic or limited colonisation ability. Hazard group 1.

**Host/vector system**

*Neisseria meningitidis* MCS8 or H44176 both are wild-type serogroup B strains. *N. meningitidis* plasmids: pGEMlacZermC neisserial transcriptional fusion vector, pYHS24 an MC ectopic chromosomal integration vector for mutant complementation, both are non-self transmissible and are chromosomal integration vectors in MC.

*E. coli* strains: DH5 alpha, XL-1 blue, JM83 (hosts for routine cloning) BL21 (OE3) or BL21 derivatives for the overproduction of *N. meningitidis* proteins

*E. coli* plasmids: pGEMTeasy, pUC19, pBluescript (routine cloning vectors) pET -derivatives, pGEX-derivatives, pMAL-derivatives (overproduction of *N. meningitidis* proteins)

All plasmids used in MC must be passaged though *E. coli* strains, since cloning steps are carried out using transformable *E. coli* strains. All the MC plasmids have their beta-lactam resistance genes deleted.

**Origin & function**

(i) Me genes involved in bacterial cellular physiology and anaerobic metabolism that help the bacterium respond to environmental stress. The majority of these have been identified from the MeSS genome sequence. Of particular interest are the genes relA and spoT involved in the bacterial stringent response and the genes involved in the response to oxidative and nitrosative stress, anIA, norB, and the genes encoding the regulatory genes narP, narO.
nsR and fnr amongst others.

(ii) Me genes involved in carbon metabolism. Of particular interest are the genes involved in the utilisation, uptake and regulation of pyruvate, lactate, glucose, gluconate and other sugar metabolism. Although amino acid metabolism and regulation may also be investigated (e.g. leucine responsive regulatory protein Lrp).

(iii) Me genes encoding the secreted proteins IgA protease and the potential haemin binding protein GIIY-1 and genes encoding associated proteins.

(iv) Me genes involved in pathogenesis, especially those involved in the interaction of the bacterium or its products with the innate immune system, these will be mainly associated with LPS biosynthesis. capsule. biosynthesis, porin biosynthesis and adhesion such as pili and opacity proteins.

Activity 1. Targeted inactivation of chromosomal MC genes. Genes of interest that have been identified by genome sequence analysis will be inactivated to determine the role of the gene. This procedure will involve spliced overlap extension (SOE) PCR, in which the neisserial gene of interest will be insertionally inactivated by an antibiotic resistance cassette (erythromycin or kanamycin), then the mutated allele on the PCR fragment will be transferred into Me by natural transformation. Selection will be imposed for recombinants that have under-gone allelic replacement. Separately the SOE-PCR fragment will also be cloned into E. coli plasmids and propagated in E. coli to facilitate sequencing to check the correct construction of the SOE PCR fragment. In addition mutant strains in a N. meningitidis MeS8 background that have been created in other national and EU labs using similar techniques will be used, these will be transported to Sheffield, as category A pathogens (UN2814) following packaging instructions P1620.

Activity 2. Cloning of the fun length MC genes into an ectopic chromosomal insertion vector and introduction into the corresponding MC mutant (complementation), where the plasmid can not replicate but rather recombines into the neisserial chromosome. This procedure is employed in order to confirm the phenotype of the mutants of interest generated by activity 1 is due to the insertion of the antibiotic resistance cassette and not down to polar effects on downstream genes.

Activity 3. Construction of reporter fusions in MC. To monitor the expression of the MC genes, regulatory regions will be fused to lacZ in a chromosomal integrative vector that will then be introduced into the appropriate MC host. Where applicable the regulation of MC promoters may also be studied in E. coli K-12 using narrow host range plasmids.

Activity 4. Overexpression of MC proteins in E. coli. To perform, role, structure-function analysis of various Me proteins. Such proteins will be expressed at high level from a strong inducible promoter contained in an E. coli specific plasmid. The genes will be fused to a sequence (i.e. hexahistidine, GST, MBP tags) that facilitates the purification of the overexpressed protein.

Evaluation of foreseeable effects

Neisseria meningitidis aka the meningococcus (MC) is a Gram negative bacterium and is the aetiological agent of meningitis and sepsis syndrome it is a serious pathogen of humans. The antibiotic resistance cassettes used to select the mutants and plasmids in these strains are not generally used to treat N. meningitidis infections (erythromycin and kanamycin). Because beta-lactam antibiotics are used for neisserial treatment any of the vectors used with N. meningitidis will have had their beta-lactam resistance genes deleted, even though the current drug of choice for meningococcal disease is a modern cephalosporin such as ceftazidime, and this antibiotic is known to be resistant to these plasmid encoded beta-lactamases.

E. coli K-12 and E. coli B are potential colonisers of humans and other animals but are non-pathogenic. Most laboratory strains of E. coli K-12 are unable to colonise humans.

Thus the acquisition of the antibiotic resistance markers to be used in these activities are very unlikely to constitute a hazard to human health or the environment.

Any foreseeable additional properties of these organisms as a result of genetic modification are outlined below.

Activity 1. Targeted inactivation of chromosomal MC genes. The initial step will include the amplification of the MC gene of interest and inactivating it through the insertion of an antibiotic resistance cassette using spliced overlap extension.
extension (SOE) PCR. This will generate a non-self-replicating non-mobilisable fragment of DNA that there are no foreseeable effects regarding human health or the environment.

The linear SOE PCR DNA fragment will be introduced into MC by natural transformation where it will be unable to replicate. Selection of the antibiotic resistance cassette inserted into the gene will allow for the isolation of rare recombinants in which homologous recombination has occurred and the mutated allele has replaced the wild-type allele on the MC chromosome. The resultant strain containing the insertionally inactivated gene will be very unlikely to have gained fitness and be more pathogenic than the wild-type strain. The MC mutant strain will have gained resistance to a single antibiotic. These features will be the same for MC strains imported from other national or EU laboratories, these will be transported as category A pathogens (UN2814) following packaging instructions P1620.

Cloning of the inactivated MC gene into a non-self transmissable plasmid in E. coli to facilitate sequence analysis of the construct is not likely to create a foreseeable effect that is hazardous to human health or the environment. The E. coli K-12 are laboratory strains and are non-pathogenic and the Me gene of interest will have been inactivated.

Activity 2. Introduction of individual MC genes in a non-mobilisable chromosomal integration vector. Apart from the acquisition of the plasmid resistance marker, it is unlikely that the phenotype of the recipient would be significantly different from the parental MC strain. Complementation will take place or not. The complementation vector pYHS24 inserts the gene of interest in single copy on the MC chromosome between the genes NMB0103 and NMB0102 on the MC chromosome. There are therefore unlikely to be overexpression issues due to multi-copy or transmission issues to other bacteria. However, the gene of interest will be under the control of the opa promoter which may lead to elevated expression levels compared with the natural gene, but this is unlikely to make the GM strain more pathogenic than the MC wild-type strain. There are unlikely to be any detrimental effects of cloning such genes in E. coli K-12, as these strains are disabled and unable to colonise.

Activity 3. Construction of reporter fusions in MC. Only the regulatory regions and gene segments, rather than the complete structural gene will be cloned into the chromosomal integrative vector and delivered into the wild-type and mutant Me strains. There is likely to be a negligible risk associated with this activity. The lacZ gene has been extensively used as a reporter in bacterial systems. The plasmid pGEMlacZermC' is non-mobilisable and narrow host range and is non-self-transmissable. The cloned DNA would be very unlikely to confer a fitness advantage on Me or other bacteria or result in effects harmful to humans or the environment. In some cases it may be possible to investigate the regulation of a MC promoter in E. coli K-12 (for example in the presence of its regulator in trans). As the E. coli host will be disabled there are no foreseen deleterious effects of expressing a regulatory protein from a compatible plasmid.

Activity 4. Overexpression of MC proteins in E. coli. Plasmids harbouring the phage T7 promoter will be used these depend on the T7 RNA polymerase for high level expression of genes in E. coli, the host E. coli B BL21 (DE3) or similar will be used. The activated expression system is lethal to the host strain. This strain can be viewed as being similar to E. coli K-12 in terms of its ability to behave as a pathogen. In some cases plasmids harbouring other strong promoters may be used such as the lac promoter. Plasmids used will be derived from the narrow host range plasmids (pBR322 and pUC19 (pET series, pGEX, pMAL), and will confer ampicillin or kanamycin resistance upon the E. coli host. Overproduction of an individual Me protein in E. coli BL21 (DE3) is unlikely to render it more pathogenic or increase its survival fitness in the human gut or environment. As the mechanism of action of some of the Me proteins to be expressed is unknown.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NIA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation not requested

02/03/2022
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMM waste will be inactivated prior to leaving the premises.
Small volumes of liquid culture and small disposable items (plastic tubes, cuvettes, pipette tips) will be inactivated by immersion in disinfectant (5% Virusolve Plus) for a minimum of one hour, this has been validated to kill MC.
Solid material inactivated by disinfectant will be autoclaved in the same laboratory and then transferred to the appropriate containers whereupon it will be taken to another site by approved contractors for final disposal.
All other items, (larger cultures, agar plate cultures, contaminated glassware) will be inactivated by autoclaving in the same laboratory (autoclaves are regularly serviced and validated). Items that have been inactivated by this method will be placed in the appropriate waste bags and removed from the site by approved contractors for final disposal.
Only the material specified below will be removed from the containment 3 facility.
Certain material will be brought out of the containment 3 laboratory including ethanol precipitated DNA and RNA extracted and purified from lysed MC cells. These will be brought out in tubes whose exterior surfaces have been wiped in 70% ethanol.
MC protein samples for 50S-PAGE from lysed cells will have been boiled in Laemmli buffer and again transported in tubes wiped with 70% ethanol.
Host and Me cells that have been fixed in 4% paraformaldehyde will be used for microscopy.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

This proposal have been approved by the University of Sheffield Biosafety committee on the 24th November 2014.

Project Containment

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Project Ref 168/15.1
Use of Lentiviral CRISPR or shRNA technology to knockout target genes and assess function

The lentiviral vectors are 3rd generation HIV-based lentiviral vectors, from which the majority of viral proteins have been removed and contain a 3' deletion which results in self-inactivation following transduction. Furthermore, the genes required to produce a packaged, transducible virus are distributed among 3 separate vectors: pMD2.G, pRSV-Rev and pMDLg/pRRE. Therefore it is unlikely that a replication-enabled lentivirus could be produced, given the rare possibility that the required recombination would occur. As a precaution against this minimal risk, however, the work on human cells will be performed at Containment Level II.

In mammalian cell lines, a potential hazard may arise from the introduction of genes into the cells which may enhance the ability of the cell proliferate, or evade host immune surveillance. However, some cell lines already possess proliferative ability due to their cancerous origin, but cannot survive any significant period of time outside of their normal tissue culture environment. Therefore daily precautions will be taken as with any mammalian cell culture work.

The lentiviral vectors are 3rd generation HIV-based lentiviral vector and packaged among 3 separate vectors: pMD2.G, pRSV-Rev and pMDLg/pRRE. As a precaution against the minimal risk of the production of a replication-enabled lentivirus, the work will be performed at Containment Level II. Nucleotide sequences will be used to knockout genes including tumour suppressor genes, proto-oncogenes and genes of unknown function. The potential hazard arising from knockout of such genes could be oncogenic. It is also likely that the target sequence will also target other places in the genome and therefore pose additional hazards of gene editing elsewhere in the genome. We therefore propose to carry out the handling of live virus and transduction of cells in the designated Class 2 facility.
The project requires the use of genes and/or gene fragments sourced either by cloning or obtained from third parties to be overexpressed in human cell lines. The transfer vectors may incorporate one or more genes encoding selection markers, or fluorescence reporter proteins. Examples of these include PuromycinR, BlasticidinR, AmpicillinR or fluorescent proteins such as mCherry, GFP, Venus, Cerulean, mTomato. These proteins are considered non-hazardous owing to their non-mammalian origin. There is no evidence of any oncogenic potential for these proteins thus we consider it highly unlikely that exposure to these proteins would result in harmful effects.

Evaluation of foreseeable effects

A worst-case scenario would be a worker being exposed through a respiratory or dermal route to live lentivirus during titre production such as centrifuge rotor failure. To minimise the risk of exposure all work is to be carried out in a dedicated viral tissue culture room in a class II microbiological safety cabinet. Viral supernatant produced from the 293T cells will not be concentrated and cells transduced with lentivirus will only be removed from class II containment following 4 passages and all cells and viral supernatant for disposal will be treated in a final concentration of 1 % virkon.

Another potential hazardous scenario would be a transduced cell being introduced into a worker through a needlestick injury. However, the human cell lines we intend to use are already proliferative in nature, which are handled routinely in class II containment. Therefore we feel that no special precautions are necessary when handling transduced cells above and beyond the pre-existing requirement for good laboratory practice. Human megakaryocytes and endothelial cells express class I MHC antigens and would most likely illicit rapid immune rejection if accidentally injected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Viral titre will not be concentrated thereby removing the risk of respiratory exposure due to potential centrifuge rotor failure that could occur during ultracentrifugation. Lentiviral supernatant will be inactivated by incubation with Virkon to a final concentration of 1 %, following manufacturers’ instructions (incubation for >30min before disposal). Virkon effectively destroys any virus. Virkon is safe, non-toxic and biodegradable. All other waste, which was in contact with lentiviral supernatant will be disinfected with Virkon to a final concentration of 1 % prior to disposal. This waste is placed in sealed medical waste bins and incinerated. All work will be carried out at containment level II.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Lentiviral supernatants and lentivirally-transduced cells will be inactivated by incubation with Virkon to a final concentration of 1 %, following manufacturers’ instructions (incubation for >30min before disposal). Virkon effectively destroys any virus. Virkon is safe, non-toxic and biodegradable. All other waste, which was in contact with lentiviral supernatant will be disinfected with Virkon to a final concentration of 1% prior to disposal. This waste is placed in sealed medical waste bins and incinerated.
This project proposal was approved by the University of Sheffield Biosafety Committee on 7th May 2015.

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Project Additional Information

#### Purposes of the contained use

Constitutive secretion is a conserved process required for the delivery of newly synthesised proteins and lipids to plasma membrane as well as the exocytosis of extracellular factors such as cytokines, lipoproteins and antibodies. My lab is interested in identifying and characterising the pathways and cellular machinery involved in constitutive secretion. We aim to identify this machinery using a combination of proteomics and functional genomics. The majority of the proteins identified in these screen are likely to be house keeping genes such as (coat proteins, rabs, tethers and SNAREs) so not biologically hazardous or oncogenic. We will use lentivirus to: a) Express cDNAs encoding wild type and mutant proteins required for constitutive secretion in mammalian cells. b) Knock down proteins required for constitutive secretion in mammalian cells using shRNA. c) Knock out proteins required for constitutive secretion in mammalian cells using CRISPR/CAS9.

#### Recipient or parental organism

**Bacterial host**

In all experiments the bacterial host strains used are non-pathogenic E.Coli K12 derivatives: OH5.a, JM109, XI-1blue, HB101 (genotypes listed in attached appendix). All are non-colonising and disabled and are therefore unlikely to survive either in the gut or in the environment. We consider assignment of ACDP hazard group 1 appropriate.

**Mammalian host**

Experimental procedures using virus to introduce novel genetic elements will be performed on primary murine cells and well established cell lines such as HeLa, Caco2 and I.29. Virus particles will be manufactured in Human Kidney 293T cells transformed with SV40 large T antigen. As with all mammalian cells these cell lines are sensitive to culture conditions and have no survival potential outside of their normal tissue-culture environment.

#### Host/vector system

**Lentiviral vector system**

Replication deficient self-inactivating lentivirus particles will be produced by transfection of at least 3 different plasmids. We intend to use 2nd and 3rd generation lentiviral transfer vectors. The majority of viral proteins have been removed; in particular the virulence genes vpr, vif, vpu and nef are not present and are not required for transgene expression.

The vector system consists of: (1) self-inactivating transfer vector containing the expressed DNA sequence (2) Packaging and structural proteins (3) Envelope protein. The genes required to produce a packaged, transducible virus are distributed among at least 3 separate vectors. Thus it is unlikely that a replication-enabled lentivirus could be produced, as it is unlikely that the three required recombinations would occur. However we propose to carry out the handling of live virus and transduction of cells in the designated Class 2 laboratory.

**Transfer vectors**

a) vectors designed to give constitutive gene expression utilising a promoter such as CMV (plenti-Puro, Addgene).

b) vectors designed to constitutively express shRNA utilising promoters such as U6 or H1 (pLKO-Puro, Addgene).

b) vectors designed to constitutively express CRISPR/Cas9 utilising a promoter such as CMV and guide RNA using a RNA pol IIII promoter (lentiCRISPR V2, Addgene).
Packaging plasmids
a) Packaging plasmid 3rd generation (gag_pol_pMDLg/pRRE, Addgene).
   b) Packaging plasmid 3rd generation (pRSV-Rev, Addgene).
   c) Packaging plasmid 2nd generation (psPAX2, Addgene).

Envelope plasmid
a) Amphotropic plasmid, VSV-G (pMD2.G, Addgene).
   b) Ecotropic plasmid, MLV, (pCAG-Eco, Addgene)

Safety features of 2nd and 3rd generation lentiviral vectors
1. Vectors contain a deletion in the 3'LTR that does not affect generation of the viral genome in the producer cell line, but results in 'self-inactivation' of the lentivirus after transduction of the target cell. This is a result of the natural duplication of 3'LTR into 5' position that occurs during integration. Once integrated into the target cell, the lentiviral genome cannot produce packageable viral genome.
2. Virulence HIV genes like vpr, vit, vpu, and nef are not present and are not required for transgene expression. The absence of the env gene removes the gp160 precursor that is responsible for the lethal macrophage/T cell/neurotropism of HIV.
3. Production of the viruses from the producer cell line requires the transfection of at least 3 plasmids (two packaging, one the DNA to be packaged) in a transient transfection. All three plasmids have been designed, through lack of common sequences, to avoid the possibility of recombination with each other. The plasmids expressing the structural and packaging genes are not packaged with the produced virus, since none of them contain LTRs or the packaging RNA sequence. Thus, replication-competent viruses cannot be produced, nor can the packaging cell line produce retrovirus in any form during routine culture.
4. Internal promoters are used to eliminate the need of the HIV-promoter and reduce the risk of recombination events and trans-activation of surrounding genes.
5. We will not concentrate the viral particles.
6. Where possible ecotropic pseudotyped viral particles will be used which are unable to infect humans.

Origin & function
We plan to express cDNAs of wild type and mutant proteins required for constitutive secretion such as coat proteins, rabs, tethers and SNAREs. These proteins are house keeping genes so are not likely to be oncogenic or biologically hazardous. We plan to express shRNA or CRISPR/Cas9 constructs targeting genes involved constitutive secretion such as coat proteins, rabs, tethers and SNAREs. Loss of these proteins is unlikely to confer any known selective advantage to the host. However, we cannot exclude the possibility of tumor-inducing effects, as their functions are not well defined. In order to reduce the possibility of accidental exposure of a worker to virus we propose to carry out the handling of live virus and transduction of cells in our designated Class 2 laboratory.

The 2nd and 3rd generation lentiviral vector backbones may contain expression enhancing viral elements such as the WPRE element which in its wild type form can express the so-called x-protein which may have oncogenic properties. Thus we propose to carry out the handling of live virus and transduction of cells in the designated Class 2 laboratory.
The transfer vectors may also incorporate one or more genes encoding selection markers and fluorescence reporter proteins. Examples of these include 1) PuromycinR, BlasticidinR, AmpicillinR, 2) fluorescent proteins such as mCherry, GFP, Venus, Cerulean, mTomato. These proteins are considered non-hazardous owing to their nonmammalian origin. There is no evidence of any oncogenic potential for these proteins thus we consider it highly unlikely that exposure to these proteins would result in harmful effects. However, we propose to carry out the handling of live virus and transduction of cells in the designated Class 2 laboratory.

**Evaluation of foreseeable effects**

**Environmental assessment**

These replication-incompetent viruses would be inactivated prior to disposal, and given their inability to replicate they have no significant additional environmental hazard.

**Worst-case scenarios**

The above protocols should minimise any risk of exposure to the personnel using or in the vicinity of work using this lentiviral transfection system. Virus particles, virally-transduced cells, and media and supernatants resulting from these systems will never be handled with glassware or hypodermic needles. Thus, the risks of accidental inoculation are remote. The worst scenario would be exposure to a main stock of virus containing one transgene, perhaps resulting from spillage. The major exposure routes would be cutaneous, mucocutaneous, and respiratory. Effective infection of intact skin is unlikely as skin is a very effective barrier, and would be minimised by standards of Good Laboratory Practice. The viruses would infect mucocutaneous surfaces and respiratory epithelia and macrophages with which they came into contact. However, the particles are replication incompetent, and have many safety features preventing generation of replication competent viral particles. The genes of interest are not oncogenes. Thus although there might be limited tissue infection and damage, this would not result in sustained infection or risks of tumour generation or malignant transformation. It might be possible that exposure would generate an immune response to the VSVG protein, but this would have a protective effect if so. The overall likelihood of serious or long-term harm resulting from experiments using these vectors seems extremely small.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

We propose to handle all material in Category 2 culture conditions.

1. Anti-viral disinfectants (Virkon, Trigene, bleach) will always be available prior to starting work involving handling of viral particles, in identified containers easily to hand (e.g. spray gun).
2. All culture will be perfumed within a Class 2 hood, in a designated Class 2 molecular biology lab. All cells exposed to virus will be handled in the Class 2 hood. After infection, viral supernatant will be removed and the cells washed. Once the media has been changed on at least one further occasion (after which active viral particles should not be present), then functional assays may involve handling transfected cells for brief periods on the bench.
3. Where small volumes of cells potentially containing viable viral particles require centrifugation, tubes will be
sealed with parafilm prior to centrifugation.
4. Virus particles will be stored in the -80°C freezer, in cryovials, held inside 50 ml falcon centrifuge tubes or equivalent, in clearly marked packaging.
5. Hoods will be carefully cleaned with 10% solution of Trigene after each session in which culture which virus particles takes place, and after each session when cells that have been transfected by virus on a previous occasion are handled.
6. Only designated media will be used in cell culture.
7. Virus particles and supernatants from infected cells will be handled using filtered pipettes and pipette tips.
8. No glassware or hypodermic needles will be used at any time with any culture producing or containing virus particles, or any supernatant generated from any cell that has ever been exposed to virus particles.
9. All supernatants, plasticware, and cells that have come into contact with virus particles or viral infected cells will be treated with Trigene, Virkon, or bleach at appropriate concentrations and for 20-30 minutes prior to discard.
10. Standards of GLP will be rigorously enforced (lab coats, gloves, etc).
11. All personnel using or producing virus will be appropriately registered through the University Health and Safety procedures, listed on the GMAG approval, and taught appropriate safe handling by the principal investigator.
12. Other lab personnel will be made aware of the use of lentiviral systems in the laboratory.

Contamination management
1. Where spills occur in contained environments such as Class 2 hoods, neat Trigene, bleach, or Virkon will be added and left for >10 minutes to neutralise viral particles prior to wiping, cleaning and disposal.
2. If a small contained spill occurs outside of Class 2 hoods, management will be as above. Any spill outside of the Class 2 hood will be reported to a principal investigator, and assessment made to determine whether avoidable features are present to prevent future accidents.
3. Where aerosolised contamination is suspected to have occurred outside of Class 2 hoods, the lab will be cleared immediately to prevent aerosol inhalation. The air conditioning system will replace the air within a room within 10 min and will serve to desiccate and dilute the hazard. A single designated worker will return to the lab after 20 min and apply appropriate disinfectant to liquid traces.

This project proposal was reviewed and approved by the University of Sheffield Biosafety Committee on the 24th February 2016, with only minor administrative recommendations made to the Proposer.

Project Containment
Project Ref: 168/17.1

Date Ackn'd: 22/02/2017

Investigation of the mammalian host cell biology manipulated by bacterial pathogens Salmonella enterica, enteropathogenic Escherichia coli (EPEC), enterohaemorrhagic E.coli (EHEC) and Shigella species during infection.

Recipient or parental organism:
- Laboratory derivatives of:
  - Wild-type Salmonella Typhimurium (e.g. SL 1344)
  - Auxotrophic for histidine, reducing fitness, and has been used safely in research labs since 1977. Possibly able to cause localised gastroenteritis in humans that resolves itself with 3-7 days or can be managed with antibiotics, though laboratory passage means SL 1344 likely more attenuated.

Project Additional Information:

- Investigate the mammalian host cell biology manipulated by hazard group 2 (HG2) bacterial pathogens Salmonella enterica, enteropathogenic Escherichia coli (EPEC), enterohaemorrhagic E.coli (EHEC) and Shigella species during infection. Recombinant vaccinia virus will also be used to compare the mechanisms by which pathogen exploit host cell biology to establish infections. In particular, the study addresses how bacterial virulence effectors injected and secreted toxins hijack intracellular host cell functions.
- Attenuated Salmonella Typhi (e.g. BR0948/CVO 908-htrA).
The vaccine candidate strain S.Typhi BR0948/CV0908-htrA has been recognised as disabled by the HSE since 1993
and the organism is derogated from CL3 to CL2. The strain carries non-reverting attenuating mutations making it
stably attenuated and fails to colonise the human host and has an excellent safety record with no evidence of human
infection both in the UK and abroad. S.Typhi secretes a cytolethal distending toxin (COT). There is no direct evidence
that the COT of Salmonella Typhi enhances virulence. Rather than inducing overt cytotoxicity and lethality like other
toxins, COT 'cyclomodulins' drive more subtle alterations in cultured mammalian cells by arresting cell cycle
progression. Given the safety record of the BRD948 strain the risk is extremely low.
- Escherichia coli 0157:H7 (henceforth EHEC) EDL933 or TUV93-0 which are both toxin negative for the stx genes
(stx 1 and/or stx 2) encoding Shiga-like STX or Verocytotoxin VT.
The toxin-deleted strains are nevertheless potentially capable of causing gastrointestinal illness, though rarely life
threatening to those in normal health, self limiting, lasting up to 7 days, or readily treatable with antibiotics.
- enteropathogenic Escherichia coli E2348/69 0127:H6 (Levine et al 1978), henceforth EPEC.
EPEC may cause a self-limiting gastrointestinal illness in humans if ingested. At suitable doses (above 1 x 10^6), can
cause self-limiting diarrhoea in a susceptible host, e.g. children, immunocompromised, that is treatable with antibiotics.
- wild-type Shigella flexneri (e.g. serotype 5a strain M90T)
- Shigella species, e.g. Shigella Flexneri M90T, Shigella Sonnei 53G, cause a localised gastroenteritis (Shigellosis)
that resolves itself after several days and is treatable with antibiotics. The pathogens have a low infective dose (10-
100 colony forming units) though the strain M90T is likely attenuated due to lab passage. The risk is very low as it is
managed through the containment and protocols.
- Vaccinia virus (VACV) strain Western Reserve
Commercially available laboratory strain that has been passed in mice and used to already generate vEGPA5L
where the endogenous Vaccinia A5L gene has been replaced with the A5L gene fused to GFP for use as a visual
reporter. VACV can infect humans and was used in hundreds of millions of people as the live vaccine that eradicated
smallpox. A hazard of working with VACV is infection if the skin is pierced, e.g. via a needle or abrasion. The risk of
accidental infection is low and the resulting infection is typically mild, but may cause a rash or fever that is self-limiting
and healed within 2-3 weeks. Infections can be more serious during immunosuppression, pregnancy, or with
eczema/psoriasis sufferers who are precluded from working with VACV.
All the bacterial pathogens in the study use a Type 3 Secretion Sytem (T3SS) to inject virulence effectors into the host
cell (-30-60) that work collectively to establish infections. Thus, no single effector can confer a disease phenotype
when expressed recombinantly from a plasmid, and effectors are considered non-toxic and non-hazardous.
The risks for all pathogens in this form are considered very low and are managed through the containment and protocols.

Host/vector system

<table>
<thead>
<tr>
<th>Source of genetic material</th>
<th>pACYC184 (NEB), and pUC series (e.g. NEB), pBR322 series (e.g. NEB) vectors, and pKD3, pKD4, pKD46, pCP20 plasmid vectors.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin &amp; function</td>
<td>For bacteria, well characterised vector systems with a history of safe use will be used, for example, commercially available non-mobilisable/mobilisation defective plasmids, pUC series, pACYC184, pBR322 series vectors, and pKD3, pKD4, pKD46, pCP20 plasmid vectors. For Vaccinia, the vector pSJH7 or equivalent derivatives of the commercially available vector pUC13 (Hughes et al 1991).</td>
</tr>
</tbody>
</table>

Intended function

High-copy pUC vectors will be used to express GFP or RFP that will act as a reporter to allow direct visualisation of the pathogen during cell infection.

Low-copy pBR322/pACYC184 vectors encoding virulence effectors (e.g. injected via T3SSs) or toxin components (i.e. CDT encoded by S.Typhi) will be used to complement parental null mutant strains and investigate virulence factors inside the target host cell (e.g. manipulation of cell membrane trafficking).

The lambda red gene disruption method will be used to generate specific deletions in virulence genes to generate null mutants. Briefly, antibiotic resistant cassettes will be amplified from pKD3 or pKD4 (maintained in E. coli XL 1 Blue) with primers to allow homologous recombination into the chromosome of the parental target pathogen strain (expressing the lambda Red recombinase from pKD46) and subsequent gene disruption. Antibiotic resistance cassettes will be removed using the FLP-recombinase encoded by pCP20. Mutations will be complemented using expression of the gene on pBR322/pACYC184.

For Vaccinia, the vector pSJH7 or equivalent derivatives of the commercially available vector pUC13 (Hughes et al 1991) that contain the vaccinia late 4b promoter for dominant transient selection of recombinant VACV. pSJH7 is cotransfected into mammalian cell lines along with a second vector containing an attenuated version of the wild type VACV genome (e.g. LIA5L VACV), such that viral replication can only occur following recombination of the two plasmids.

Evaluation of foreseeable effects

In the first bacterial modification high-copy pUC vectors will be used to express GFP or RFP that will act as a visual reporter for bacterial pathogens during cell infection, The modifications are expected to have no effect on the virulence or fitness of any of the recipient pathogens.

The expression of T3SS virulence effectors or the CDT (in the case of S.Typhi BRD948) to complement null mutations in the parental strains involves somewhat higher level expression (~2.5-fold more, Cain et al 2004) that in theory can increase virulence potential, although actual risk is extremely small given that disease relies upon optimal expression levels, and the containment procedures, The likelihood of harm to human health in the event of exposure would be no greater than that with the parental organism and can be considered extremely low, The chance of transfer of the vectors to other organisms in the environment can be considered low given the containment, the use of nonmobilisable vectors, and the use of certain nutritional auxotrophs such as Salmonella strains SL 1344 and BRD948, It is difficult to envisage how any of the genetic manipulations proposed could mediate a harmful phenotype or competitive advantage to the host strains in the environment. The environmental hazards associated with the project are therefore considered no greater than those associated with handling the parental strains, The project involves small scale work for research purposes and all contaminated material will be completely inactivated by autoclaving or proprietary disinfectants prior to disposal, In the highly unlikely event of release into the environment, no risks are envisaged,

The third bacterial modification will involve mutagenesis work via the method of Datsenko and Warner (PNAS 97, 6640-6645, 2000), which will generate attenuated mutant strains that are expected to be less virulent. The excision of antibiotic resistance cassettes will leave an unmarked lesion in a strain of wild type antibiotic susceptibility, Regarding Vaccinia virus, the study plans to use the recombinant vEGFPAS5L strain carrying an A51-GFP gene in place of an endogenous A5L copy for use as a visual reporter. A5L is a core structural virion protein required for VACV morphogenesis and is non-toxic and non-hazardous, The results of the genetic modification reduce the pathogenicity of the parental organism, i.e. evidence indicates vEGFPAS5L is less pathogenic than wild-type VACV (Doceul et al 2010). No advantage in terms of fitness or virulence to the recombinant VACV is expected, or if the A5L-GFP sequence was transferred to a related organism. The risk of accidental infection with vEGFPAS5L is low given the containment and the resulting infection would be expected to typically mild, like the parental host, but may cause a rash or fever that is self-limiting and healed within 2-3 weeks. VACV can infect animals including mice, rabbits and
cattle, and can be transmitted to broken skin, abrasions. Human-to-animal transmission can occur. The risk is extremely low given no animals are to be used in the study, and the containment procedures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

The vaccine candidate strain Salmonella Typhl BRD948/CVD908-htrA has been recognised as disabled by the Health and Safety Executive since 1993 and the organism is derogated from HG3 to HG2. The S.Typhi BRD948 strain carries non-reverting attenuating mutations making it stably attenuated (Tacket et al 1997). The strain fails to colonise the human host and has an excellent safety record with no evidence of human infection both in the UK and abroad and has been proven to be highly attenuated in many scores of human volunteers in phase 2 clinical trials infected with up to 5x1 0 exp 9 colony forming units (Tacket et al1997, Lowe et al 1999, Tacket et al 2000, Tacket & Levine 2007). Genetically attenuated variants of EHEC strains where the Shiga-like (Vero) toxin genes have been deleted will be used in some experiments, but Wild-type EHEC or other verotoxigenic strains will not be used. Non-toxigenic E. coli 0157 lacking the Shiga toxin genes and are designated HG2 pathogens.

For laboratory operations the use of standard Containment Level 2 facilities and good microbiological practice will be sufficient to limit contact with humans and the environment.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Containment protocols that minimise aerosols and spillages during bacteria culture via use of airtight sealed tubes, i.e. falcon tubes, in as small a volume as possible, typically 1 ml.
  - Autoclaving of waste material at 121°C for 60 minutes provides 100% inactivation. The autoclave used for waste is located in the building; all waste to be transported is double bagged in autoclave bags and then sealed in a plastic container. Annual validation of the autoclave by independent means will be performed.
  - For spillages, ethanol is an effective surface disinfectant in an aqueous solution of 70% (v/v) , and ready-to-use proprietary disinfectant, such as Distel spray (formely known as Trigene) which is proven to kill bacteria. Virkon powder can be used directly for larger spillages.
  - Liquid waste will be aspirated into liquid vessels containing proprietary Distel or Virkon proven to disable bacteria/viruses, incubated for at least 30 minutes before disposal of the deactivated waste down the sink.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

This project was approved by the University of Sheffield Biosafety Committee on 15th July 2016, with minor amendments. The committee have also advised that additional health surveillance be provided, if appropriate, for any workers with pre-existing conditions that may increase their risk from infection with vaccinia virus.
We need to increase expression of IL-1B and/or IL-1R1 in cancer cells or remove these molecules from cancer cells using lentiviral transfection methods in order to answer the following research questions:

1. How does IL-1B/IL-1R1 from breast cancer cells affect the spontaneous spread of cancer cells to bone.
2. Establish the importance of direct interactions between IL-1B/IL-1R1 and cancer cells in the spread of early stage disease.
3. Identify how IL-1B produced by cancer cells and IL-1B/IL-1R1 produced by bone each alter the environment in the skeleton that cancer cells live and grow in.
This project uses replication defective lentiviral vectors for the transfer of cDNA sequences of genes of interest into mammalian cells (mouse and human). Lentiviruses are pathogenic to humans, targeting both dividing and non-dividing cells, and can deliver significant amounts of genetic information to the DNA of the host cell. The native envelope of lentiviruses determines its host range. In order to broaden the range of cells that can be infected by laboratory engineered virus the native envelope is substituted for an amphotropic one. These will not be used routinely, but will be used to infect cells that are difficult to infect by other techniques where high levels of transfection efficacy are required.

Recipient/Host(s): Will be standard (disabled) competent E.coli K12, TOPII or B strains for DNA production. We will use standard viral packaging cell lines such as HEK293T for lentivirus production. The packaging cell lines produce replication-incompetent virus and, for increased safety, express the genes needed for virus assembly at two separate sites in the host genome.

Host/vector system

Vector(s): We will use second and third generation lentiviral systems such as the ViraPower system or the pLKO system. These lentiviral systems are based on HIV-1 and will be used to produce infectious viral parcels by co-transfection of 3-plasmids separately expressing the lentiviral vectors, the viral structural components (packaging helper plasmids: pCMV-gag/pol, pSPAX) and envelope protein (pMD2.G, pCMV-VSV-G and others). All viral constructs are replication incompetent, self-inactivating and do not contain Woodchuck Posttranscriptional Regulatory Element).

Normal/expected biological actions of the inserted DNA or transcribed/translated gene product: Evidence from multiple cancer types suggest that knocking out or overexpressing IL1-B and/or IL-1R1 will alter the behaviour of the host cells. Specifically overexpression of IL-1B in non-metastatic prostate cancer cells has been shown to promote bone metastasis whereas knockdown of IL-1B in metastatic cells impaired bone progression (Liu, et al. Cancer Res. 73(11):3297-305). Experimental models of Lewis lung carcinoma and pulmonary adenocarcinoma have shown that local production of IL-1B stimulates tumour growth and metastasis, either through direct proliferative effects or by promoting inflammatory and angiogenic pathways (Saiko, et al. J Immunol. 169(1):469-75; Gemma, et al. Eur J Cancer. 37(12):1554-61). Furthermore, work from our team and others have shown that, blocking IL1-R1 with a receptor antagonist decreased tumour growth, angiogenesis and metastasis from melanoma and breast cancer (Holen, et al. Oncotarget 2016). We are intending to use cell lines transfected to over-express or not express IL-1B/IL-1R1 to establish the function of these molecules in the processes of breast cancer development and bone metastasis.

Technique used to insert vector into host: The vectors will be transfected into mammalian packaging cells using standard, non-viral, transfection techniques including calcium phosphate, electroporation and liposome based methods. The resulting virus containing supernatant will be used to infect the recipient cell lines without further steps involving concentration of the virus containing supernatant.

Origin & function

Once lentiviral vectors have been used to transfer cDNA sequences of genes of interest to mouse and/or human cancer cells these stable cell lines will be used in the laboratory to assess the effects of IL-1B and/or IL-1R1 expression on factors associated with tumourigenicity and metastasis. For all experiments the genetic material will be inserted into immortalised cell lines that have been made from mouse models of human cancer or patient derived xenografts. Cell lines containing transduced to express genes of interest or to not express these genes will be injected into mice to analyse effects of gene expression on metastasis. This work will be carried out with Home Office approval under project licence 70/8964

Evaluation of foreseeable effects

Genetically modified organisms produced are not predicted to pose any risk to human health. The E. Coli used are a non-pathogenic strain and cell lines containing gene inserts pose minimal risk of harm as the DNA is non-transferable once this has become integrated. Although insert of IL-1B is predicted to promote metastasis in cancer and also induces inflammation, these properties will be retained within the cancer cells that have been transduced and these cells pose no additional risk to other metastatic cancer cell lines.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

We have no intention of making GM animals or plants in this project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Use of viral infected cell lines will be at containment level 1 following demonstration that no active virus is present as these cells pose no additional threat above that of parental cells (see hazards arising from alterations in existing pathogenic traits; risk assessment). Presence of active virus will be assessed following exposure of parental cells to conditioned media taken from virally transfected cells. Potential infection of parental cells from active virus in conditioned media will be determined by monitoring reporter gene expression.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All culture will be performed within a Class 2 hood. Antiviral disinfectants (Virkon, virusolve, bleach) will always be available prior to starting work involving handling of viral particles, in identifiable containers easily to hand. Replication incompetent viruses will be inactivated prior to disposal by placing in antiviral agents (described above) for a minimum of >30 minutes before washing down the drains with copious amounts of water.

Hoods will be carefully cleaned with Virkon following handing of bacteria or cells that have been transfected with virus. Virus particles and supernatants from infected cells will be handled using filtered pipettes and pipette tips to prevent contamination of equipment.

All supernatants, plastics and cells that have come into contact with bacteria virus particles or viral transfected cells will be treated with Virkon, virusolve or bleach at appropriate concentrations (as recommended by the manufacturer) for >30 minutes prior to being discarded for incineration.

Where appropriate, autoclaving of potentially contaminated equipment will be performed prior to sending off site for incineration.

Successful autoclaving to ensure deactivation of GMs will be assessed using autoclave indicator tape.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This proposal was reviewed and approved by the University of Sheffield Biosafety Committee on 13 March 2017. Following recommendations from the Committee, the Proposer has confirmed they will use third generation lentiviral vector systems wherever possible for this project work.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tr>
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<td>L3 L4</td>
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02/03/2022
Experimental evolution of bacterial pathogens and their mobile genetic elements

In vitro experimental evolution is an experimental tool for studying the evolutionary dynamics of microbes, such as bacteria. Replicate experimental population of microbes are propagated under controlled laboratory conditions for several 100s or 1000s of generations and the evolution of their phenotypes observed over time. We are interested in how bacterial populations adapt to their abiotic and biotic environment. Genome sequencing is used to determine the mutations targeted by natural selection that underpin the observed evolutionary adaptation. These mutations are then introduced into the ancestral genotype to test their phenotypic effects. Labelled strains of the ancestral genotype allow for these to be distinguished in mixed cultures, allowing for changes in Darwinian fitness to be measured by direct competition. We will use these approaches to understand how opportunistic bacterial pathogens, Escherichia coli and Pseudomonas aeruginosa, adapt to their biotic and abiotic environment. Specifically, we will test how bacterial pathogens adapt following acquisition of mobile genetic elements (e.g. plasmids), and how bacterial pathogens adapt to different microenvironments known to be present within hosts or natural environments.

Recipient or parental organism

Pseudomonas aeruginosa
Escherichia coli

Host/vector system

During this project we will generate the following GMMs:
1) Variants of bacterial strains carrying selectable markers. This allows us to:
   a) select for transconjugants (i.e. bacteria which are carrying the plasmid).
   b) identify differently marked strains when in co-culture, for example, during competition experiments.
2) Variants of bacterial strains in which we have deleted genes that were the target of natural selection during the experimental evolution.
3) Variants of plasmids labelled with derivatives of green fluorescent protein (GFP), enabling their identification using fluorescence.
4) Variants of bacterial strains carrying vectors allowing complementation, e.g. by reintroducing different alleles of a gene into a strain in which that gene has been deleted.
5) Generating these strains will also require us to use standard laboratory E. coli cloning strains such as DH5-alpha and constructs such as pUC18.

Inserted genes:
- aacC1 (encodes gentamicin acetyltransferase-3-1), confers resistance to the antibiotic gentamicin
- strpAB (encodes aminoglycoside 3-phosphotransferase), confers resistance to the antibiotic streptomycin
- aphA1 (encodes aminoglycoside 3-phosphotransferase II), confers resistance to the antibiotic kanamycin
- aadA (encodes aminoglycoside 3-adenylyltransferase), confers resistance to the antibiotic spectinomycin
- gfp and derivatives (encodes green fluorescent protein)
- telAB (and similar), confers tellurite resistance
- lacZ (encodes beta-galactosidase), results in production of blue pigment when grown on X-gal

These inserts encode enzymes that modify antibiotics rendering them non-functional (aacC1, strpAB, aphA1, aadA), that detoxify tellurite (telAB), or that produce fluorescent or chromogenic markers (gfp, lacZ). None of them are likely to pose any direct risk to humans, animals, or plants. The insertion cassettes are designed to encode the gfp variants under the control of the lactose-inducible PA1/04/03 promoter followed by the resistance gene under its own native level promoter. Total expression of inserted proteins is unknown, however as the inserts are generally of low copy number, total amount of protein produced should be similarly low and unlikely to pose any additional risk.

Evaluation of foreseeable effects

P. aeruginosa and E. coli are designated as Class II organisms by ATCC and as Hazard Group 2 by the Advisory Committee on Dangerous Pathogens (ACDP).

E. coli is a commensal bacterium and part of a healthy mammalian gut flora but may also cause opportunistic infections. The project uses environmental and clinical isolates of E. coli. Healthy individuals are not typically at a high risk of infection. Infection may occur where the user is immuno-compromised (e.g. undergoing treatment for cancer or HIV) or via infection of existing wounds or burns. Certain strains of E. coli can cause diarrhoea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses (CDC). Infections of healthy people are readily treatable with antibiotics and most patients recover within 10 days.

P. aeruginosa is an environmental bacterium inhabiting freshwater and soil environments, but also causes opportunistic infections. Healthy individuals are not typically at risk of infection. Infection may occur where the user is immuno-compromised (e.g. undergoing treatment for cancer or HIV, cystic fibrosis patients) or has exposed burns. Infections of healthy people are readily treatable with antibiotics and most patients recover within 10 days.

The addition of resistance markers may increase the risk that infections are harder to treat with antibiotics. However, such risks will be mitigated by selecting resistance gene markers against antibacterials that are not used clinically (e.g. streptomycin, gentamycin).

The inserted genes will be introduced using a mini-Tn7 transposon system or homologous recombination system, both of which introduce the marker into a known location in the genome (confirmed using PCR). Labelling the bacterial chromosome poses little risk of horizontal gene transfer as the delivery vectors are unable to replicate and therefore are rapidly lost. The E. coli cloning strains are highly compromised laboratory strains and are uncompetitive outside of the lab environment.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Chemical disinfection using Virkon or Distel: company testing shows effectively 100% killing of related strains of P. aeruginosa and E. coli.

Autoclaving using standard cycles effects 100% killing. Material contaminated with GMOs is autoclaved at 134 degrees C for 20 minutes. This cycle kills all plant materials and (soil) microorganisms, and has been validated by BMM Western (the autoclave manufacturer). In all autoclave runs, the temperature is monitored and recorded by a probe placed in the centre of one of the bags being autoclaved. The autoclave is checked and tested by BMM Western every 3 months. Autoclave conditions are automatically recorded and checked and archived for 3 years. The autoclave is regularly serviced and maintained.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

This Project proposal was considered and approved by the University of Sheffield Biosafety Committee on 27th June 2018.

**Project Containment**

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**Project Ref** 168/19.3

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02/03/2022
Mycobacteria are a group of pathogens, of which several are important causes of human disease (including M. tuberculosis: the causative agent of TB, M. abscessus: causing deadly infections in cystic fibrosis patients and M. Leprae: the causative agent of leprosy). Immune cells play an important part in both the host’s ability to resist disease, and in the ability of the mycobacteria to avoid the host immune system. Model systems for the study of this disease include the zebrafish larvae, and several important advances in understanding mycobacterial disease have now come from this model (Adams 2011, Clay 2007, Davis 2009, Tobin 2010, Volkman 2010). Mycobacterium marinum (Mm) is a non-tuberculous mycobacterium, and as a human pathogen is an opportunistic pathogen, with no evidence of person to person spread, which occurs commonly in the environment. It is, however, a natural pathogen of fish, causing a wasting illness with disseminated mycobacteria and characterised by the presence of granulomas (Tobin and Ramakrishnan, 2008).

The aim of this research project is to understand how the interaction of mycobacteria with the immune system results in disease, with the aim of developing novel treatment avenues, importantly ones that might circumvent the growing problem of multi-drug resistant infections. To achieve this aim, transgenic fluorescent Mycobacterium marinum will be used to investigate via fluorescent microscopy their interactions with immune cells. These strains will have fluorescent proteins, such as GFP, dsRed and their derivatives inserted using selectable plasmids (with antibiotic resistance genes, such as hygromycin resistance and kanamycin resistance). We will also use genetically modified Mycobacterium marinum with gene knockout. These will be attenuated strains, eg the esx1 mutation which has no type 7 secretion system and will not be more virulent than the wildtype. These mycobacterial strains will be analysed in vitro, and introduced into cell and animal models of infection. In the first instance these will be zebrafish infection models. To demonstrate conservation of cellular mechanisms in mammals, findings from zebrafish will be followed up in vitro, using human/mouse immune cells (primary cells and immortalised cell lines) by infection with Mm in cell culture. Mycobacterium results may be compared to other zebrafish infection models (Hazard Group I and II pathogens only) currently in-use in Sheffield which are covered by existing risk assessments.

Zebrafish infection will be established by injection at early stages (Carvalho et al, 2011), into wildtype or transgenic zebrafish embryos labelling neutrophil, macrophage and other immune markers. At 0-5 days postfertilisation, the larvae will be mounted and imaged on a confocal microscope to acquire qualitative and quantitative information on immune interaction during Mm infection.


Recipient or parental organism

Mycobacterium marinum is an ACDP category II bacterial pathogen. Mycobacterium marinum is a non-tuberculous mycobacterium, and in humans is an opportunistic pathogen, with no evidence of person to person spread, which occurs commonly in the environment. It is, however, a natural pathogen of fish, causing a wasting illness with disseminated mycobacteria and characterised by the presence of granulomas (Tobin and Ramakrishnan, 2008). Mycobacterium marinum M-Strain (aka M-USA), a clinical isolate. Including variants expressing crimson, mCherry and GFP fluorescent reporters. As noted below Mycobacterium marinum does have the potential to cause disease in humans. The GMM would be less likely to cause disease given the burden of carrying the insert without any gain to pathogenicity.

Host/vector system

The vectors carried by the bacteria have no associated hazards. The inserts drive the expression of fluorescent proteins and anti-bacterial resistance. The anti-bacterial resistance inserts are against hygromycin or kanamycin, commonly used laboratory antibiotics which would not be used as a treatment and so no hazard arises from this.

Origin & function

We do not plan to alter any existing pathogenic of Mycobacterium marinum traits ourselves. Attenuated mutant strains would not be made in Sheffield but would be obtained from collaborators in the UK or The Netherlands (or from elsewhere in the EU).

Evaluation of foreseeable effects

Mm has the potential to infect humans, to cause normally superficial infections due to its low tolerance of the high temperatures of mammals. Mm have been described to cause tenosynovitis in the hand and wrist, associated with penetrating injuries in the presence of contaminated water, occasionally progressing to osteomyelitis. More commonly, they cause skin and soft tissue infection following trauma in the presence of contaminated water, most commonly from fish fins, bites or unrelated trauma accompanied by exposure to contaminated water. In temperate climates, infection is commonly related to fishing, fish tank cleaning or in aquarium workers. Due to the lower temperature to which Mm is adapted, infection is usually confined to the skin and extremities. Infection begins as one or more nodules at the site of trauma. Spread to deeper tissues can occur. Treatment is with multiple antibiotics, as with other nontuberculous mycobacteria (Piersimoni, 2008). Infections can be more severe and disseminated in the immunosuppressed, including anti-TNF therapies (Ramos 2010).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For laboratory manipulations, a standard containment level 2 facility and the use of good microbiological practice will
be employed. A Class I or Class II MSC should be used for all manipulations that may generate an aerosol (pipetting, mixing, sonication etc) and sealed rotors or buckets must be used during centrifugation. All contaminated waste will be autoclaved via validated autoclaves or treated with disinfectant (Virusolve Plus, 1:20) before removal from the building. Spillages can be safely contained within the lab and treated with disinfectant (Virusolve Plus, 1:10). 1 part Virusolve Plus per 20 parts water (i.e. 5%) is the recommended concentration. This has been validated in our laboratory for Mycobacterium marinum by mixing 1 ml of 5% Virusolve Plus with an equal volume of an appropriate culture, leaving for up to one hour, then plating serial dilutions of the mixture on suitable plates. Mycobactericidal effect was confirmed by an EN 14348 compatible test, with 4 log10 reduction in growth after 5 minutes.

This project was reviewed and approved by the University of Sheffield Biosafety Committee on 05 June 2019, with only minor recommendations made.

Please enter comments on the GM safety committee on the risk assessment

This project was reviewed and approved by the University of Sheffield Biosafety Committee on 05 June 2019, with only minor recommendations made.

**Project Containment**

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**Project Ref** 168/20.1

**Date Ackn’d** 24/09/2020

**Date Project Ceased**

**CU2 Project Title**

Pseudotyping a second generation defective lentivirus with Spike glycoprotein of SARS-CoV2 (Spike) and mutants thereof for use in Covid19 serology, or with the G protein of Vesicular Stomatitis Virus (VSV-G), as a positive control

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Pseudotyped lentiviruses are required for the safe assay and characterisation of anti-SARS-CoV2 neutralising antibodies in patient serum, without recourse to culturing SARS-CoV2 itself. Mutations of the Spike are included in this application as they will allow us to study the effect of mutation on the neutralising antigenicity of Spike mutants, as they are discovered. Pseudotyping with VSV-G will be required as a positive control for the transduced retroviral construct and as a negative control for neutralisation.

We will use a second-generation HIV-derived system in which some HIV viral components are included on two plasmids, a packaging plasmid and a transfer vector plasmid. A third plasmid will be an expression vector for a heterologous envelope protein, namely either Spike or VSV-G. The significant parental organism is therefore a distant derivative of HIV. Defective virus particles will contain only protein products of the Gag-Pol and Tat genes of HIV and one active LTR RNA sequence. The woodchuck hepatitis virus post-transcriptional response element (WPRE) will be included in the transfer plasmid, to stabilise the transfer plasmid mRNA in the packaging cell line. Expression of the packaging plasmid is driven by a cytomegalovirus (CMV) promoter-enhancer and encodes Gag-Pol, Rev and Tat genes from HIV. The Env gene of HIV, which determines HIV's tropism, is entirely absent from the system. Instead, transduction of lentivirus-like RNA into recipient cells is mediated by Spike or the VSV-G (as a positive control) that is exposed on the external surface of the defective virus particles. The pseudovirus will transduce an RNA segment, derived from the transfer plasmid, for reverse transcription and genomic insertion into the recipient cells. The integrated sequence will include a reporter gene such as a Photinus luciferase or a fluorescent protein gene, under control of a Friend murine leukaemia virus promoter. RNA packaging into defective virus in the helper cell line requires the presence of the packaging signal within the 5' HIV LTR. For a second round of viral replication to occur, the 3' LTR would need to be functional as well because it would be copied into the position of the 5' LTR during replication. The 3' LTR is truncated in the vectors that we will use, which removes the packaging signal sequence and the enhancer sequence. Consequently the incorporated cDNA is self-inactivating for virus replication after integration into the target cell. Virus-like particles will be generated after transient co-transfection of the three plasmids into the packaging cell line, 293T cells. Co-expression of VSV-G or Spike from a co-transfected plasmid will lead to pseudotyping of the defective virus particles. Expression of these exogenous envelope proteins will be driven by CMV promoters. The transfer plasmid and hence the transduced template RNA will encode one or a combination of selectable markers, fluorescent proteins or enzymatic reporter genes, particularly a luciferase. No lentiviral protein-encoding gene will be included in the transfer vector. To yield useful virus-like titres in assays for neutralisation of the defective viral particles by patients' sera, particle production will need to be as high as possible since pseudotyping with coronavirus S-glycoproteins has resulted in less efficient transduction than for particles pseudotyped with VSV-G. For this reason, we intend to use a three plasmid second generation system including pCMVΔR8.91 (Zufferey et al., 1997. Nature Biotech. 15, 871-875) in preference to the available four-plasmid lentiviral systems, which typically produce an order of magnitude fewer transducing particles. An additional potentially hazardous feature ids the WPRE in the transfer plasmid; again, it is incorporated to increase the yield of defective virus particles.
BACTERIA
Plasmids will not contain functional promoters for expression of the mammalian genes in bacteria. Plasmids will be created and manipulated as necessary and will be expanded in the auxotrophic laboratory K12 strain of Escherichia coli, DH5-alpha, which requires only level 1 containment. The bacteria do not survive in the gut or the environment and therefore can represent no significant environmental risk.

PSEUDOTYPED LENTIVIRUS
Level 2 containment will be required for the generation of virus-like particles in immortalised 293T human embryonic kidney (HEK)-derived cells that are stably transformed with large T antigen of Simian Virus 40 (SV40). The vector plasmids contain the SV40 origin of replication and are therefore transiently amplified as episomes after transfection in the presence of the endogenous large T-antigen of 293T cells.

The packaging expression plasmid will be pCMVΔ8.91 or derivatives of it (Zufferey et al.1997, Nature Biotechnology 15, 871-875). Here viral long terminal repeats and the viral genes Env, Vif, Vpr, Vpu and Nef, have been eliminated by deletion. Tat and Rev remain in this plasmid. The packaging sequence (psi) is also deleted from the 5'-end of Gag, so that the packaging RNA itself cannot be packaged. The HIV-LTR promoter has been replaced by the strong, constitutive cytomegalovirus (CMV) promoter-enhancer.

Pseudotyping vectors provide a coat for the defective virus-like particles. Experimentally, we will test whether patient serum can neutralise the entry of virus-like particles into cells as a result of reaction with the pseudotype protein. The second vector is therefore a CMV promoter-enhancer driven expression vector for a humanised coding sequence of the trimeric Spike. A similar vector will be used in control transduction experiments to transduce the VSV-G protein. Neither of these open reading frames can be incorporated into the transduced reporter vector because vectors and ORFs lack packaging sequences.

The transfer vector plasmid will contain the transduced packaged sequence, which will be limited to the following--
An ORF encoding an enzyme based reporter gene such as luciferase, a fluorescent marker protein or a cytotoxic drug-resistance marker. The defective virus particles, produced from the transfected packaging cell line, will transduce the reporter gene's coding sequence into target cells. The transfer sequence is flanked by LTRs and contains the psi and is therefore packaged. HIV Pol protein (reverse transcriptase/RNaseH/integrase) is expressed from the packaging plasmid and associates with viral RNA to allow synthesis of the defective virion cDNA and its incorporation into the target genome.

The transduced construct also contains Woodchuck Hepatitis Virus post-transcriptional regulatory sequence (WPRE) which stabilises the transfer RNA in the packaging cell line and increases particle yield. The WPRE in these vectors encodes a truncation of the small protein (X) which has been identified as oncogenic in mouse liver. The product virus-like particles will be replication incompetent and therefore of no environmental significance, should the virus-like particles escape from containment. The particles are also destroyed by dessication in the environment.

Host/vector system

The recombinant plasmids are either pre-existing constructs or will be derived from the same. All plasmids contain standard antibiotic resistance markers for selection, to allow for plasmid cultivation in E. coli.
Cytomegalovirus promoters are used to drive expression of HIV Gag-Pol, Rev and Tat and (separately) the VSV-G or Spike.
HIV LTR drives expression of the reporter gene and the woodchuck hepatitis virus post-transcriptional response element.
The left HIV LTR contains an HIV-Tat-activated enhancer and the packaging signal (psi) for the transfer plasmid RNA.
Gag is required to form the pseudoviral envelope; Pol is required for the production and integration of the transduced reporter gene. Rev and Tat are required for the expression, in the packaging line, of the LTR-driven RNA sequences that become packaged.
The WPRE element is required to stabilise the transfer plasmid RNA within the packaging cell line, increasing the yield of enveloped reporter gene.
The pseudo-typing Spike glycoprotein is central to the purpose of the process, which is a study of the activity of neutralising, anti-Spike antibodies in convalescent patients' serum. The alternative pseudo-typing envelope gene VSV-G is required as a positive control to assess the production of infectious virus-like particles from the 293T packaging line and as a negative control for the effect of antisera.
Also fundamental to the process, a reporter gene (such as luciferase, a fluorescent protein or a mammalian selectable marker) is required for the quantification of single-round infected cells, allowing the assessment of virus neutralisation.

Evaluation of foreseeable effects

[1] High titre lentiviral transduction of the WPRE has been found to cause tumours in mice. Accidental transduction of the WPRE element into the cells of workers must therefore be considered a potential hazard.
The possibility exists that a series of recombinational events between the transfer vector, the packaging plasmid and an endogenous lentivirus could re-create an infectious lentivirus. However, an endogenous lentivirus would need to provide the Env gene, which determines the tropism of HIV. Gag, Pol, Rev and Tat from the packaging plasmid would need to be incorporated between the LTRs in the transfer plasmid. The defective 3'-LTR would need to be replaced with a functional copy, and Vif, Vpu, Vpr and Nef would need to be introduced from an endogenous lentivirus.

All retroviral vectors present the very small risk, per cell infected of oncogenic gene activation, as a result of the possibility of insertional mutagenesis.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

HIV may only be cultured under level 3 containment but second generation systems, derived from it, such as we are proposing here do not produce detectable virus and are generally created and used under level 2 containment.

We expect to store batches of pseudotyped lentivirus in medium indefinitely at -80°C in screw capped crovials.

To mitigate the potential risk of re-creating replication-competent HIV-like virus, the first batches of defective viruses will be tested as follows and then new batches will be tested at six-monthly intervals and results will be recorded.

Samples of medium will be taken from recipient cells that have been washed approximately 48 hours after infection with the highest multiplicity of virus-like particles. The presence of Pol gene activity will be tested by a standard quantitative method. Positive detection will indicate that cells are producing potentially infectious particles.

If Pol activity is detected, cultures and affected defective virus stocks will be destroyed.

To mitigate the risk of accidental WPRE transduction or oncogenic insertional mutagenesis to the worker, all open handling of transfected helper-cell supernatants, including defective-virus stocks, will be confined entirely to a Class II MSC. All centrifugation of samples containing defective-virus particles will be in screw-capped sealed tubes in a sealed rotor. In the event of suspected breakage, the rotor will be opened after 30 min and after evacuating the laboratory and decontaminated with copious 70% industrial methylated spirit or ethanol. In such an emergency, the operator will wear disposable respirator masks while the rotor is opened and decontaminated.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cell supernatants that contain or might contain defective virus particles will be pooled and inactivated within a Class II MSC by a concentration of at least 5% Virusolve for at least 30 minutes, which has been shown to be effective and is routinely used to destroy HIV. Further validation should not be required. Inactivated liquid waste will be released to the drains.

Plastic waste will be treated with 5% Virusolve for at least 30 minutes before disposal to the contaminated waste stream, which is incinerated.

Bacterial waste from plasmid generation is categorised as Class 1 GM material. Liquid waste will be treated with 1% Virkon or 5% Virusolve. Solid waste, including bacterial plates will be autoclaved in a validated Departmental waste autoclave. Solid autoclave residue is disposed of to the contaminated waste stream and liquid waste to the drains.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project proposal was reviewed and approved by the University of Sheffield Biosafety Committee on 11/08/2020 with no recommendations.
Constitutive secretion is a conserved process required for the delivery of newly synthesised proteins and lipids to plasma membrane as well as the exocytosis of extracellular factors such as cytokines, lipoproteins and antibodies. My lab is interested in identifying and characterising the pathways and cellular machinery involved in constitutive secretion. We aim to identify this machinery using a combination of proteomics and functional genomics. The majority of the proteins identified in these screens are likely to be housekeeping genes such as coat proteins, rabs, tethers and SNAREs so not biologically hazardous or oncogenic.

We will use lentivirus to:

a) Express cDNAs encoding wild type and mutant proteins required for constitutive secretion in mammalian cells.

b) Knock down proteins required for constitutive secretion in mammalian cells using shRNA.

c) Knock out proteins required for constitutive secretion in mammalian cells using CRISPR/CAS9.
Bacterial host
In all experiments the bacterial host strains used are non-pathogenic E.Coli K12 derivatives: DH5-a, JM109, XL-1blue, HB101 (genotypes listed in attached appendix). All are non-colonising and disabled and are therefore unlikely to survive either in the gut or in the environment. We consider assignment of ACDP hazard group1 appropriate.

Mammalian host
Experimental procedures using virus to introduce novel genetic elements will be performed on primary murine cells and well established cell lines such as HeLa, Caco2 and I.29. Virus particles will be manufactured in Human Kidney 293T cells transformed with SV40 large T antigen. As with all mammalian cells these cell lines are sensitive to culture conditions and have no survival potential outside of their normal tissue-culture environment.

Host/vector system

Lentiviral vector system
Replication deficient self-inactivating lentivirus particles will be produced by transfection of at least 3 different plasmids. We intend to use 2nd and 3rd generation lentiviral transfer vectors. The majority of viral proteins have been removed; in particular the virulence genes vpr, vif, vpu and nef are not present and are not required for transgene expression.

The vector system consists of: (1) self-inactivating transfer vector containing the expressed DNA sequence (2) Packaging and structural proteins (3) Envelope protein. The genes required to produce a packaged, transducible virus are distributed among at least 3 separate vectors. Thus it is unlikely that a replication-enabled lentivirus could be produced, as it is unlikely that the three required recombinations would occur. However we propose to carry out the handling of live virus and transduction of cells in the designated Class 2 laboratory.

Transfer vectors
a) vectors designed to give constitutive gene expression utilising a promoter such as CMV (pLenti-Puro, Addgene).

b) vectors designed to constitutively express shRNA utilising promoters such as U6 or H1 (pLKO-Puro, Addgene).

c) vectors designed to constitutively express CRISPR/Cas9 utilising a promoter such as CMV and guide RNA using a RNA pol III promoter (lentiCRISPR V2, Addgene).

Packaging plasmids
a) packaging plasmid 3rd generation (gag_pol_pMDLg/pRRE, Addgene).

b) packaging plasmid 3rd generation (pRSV-Rev, Addgene).

c) packaging plasmid 2nd generation (psPAX2, Addgene).

Envelope plasmid
a) Amphotropic plasmid, VSV-G (pMD2.G, Addgene).
b) Ecotropic plasmid, MLV, (pCAG-Eco, Addgene)

Safety features of 2nd and 3rd generation lentiviral vectors
1. Vectors contains a deletion in the 3'LTR that does not affect generation of the viral genome in the producer cell line, but results in 'self-inactivation' of the lentivirus after transduction of the target cell. This is a result of the natural duplication of 3'LTR into 5' position that occurs during integration. Once integrated into the target cell, the lentiviral genome cannot produce packageable viral genome.

2. Virulence HIV genes like vpr, vif, vpu and nef are not present and are not required for tranogene expression. The absence of the env gene removes the gp160 precursor that is responsible for the lethal macrophage/T cell/neuro-tropism of HIV.

3. Production of the viruses from the producer cell line requires the transfection of at least 3 plasmids (two packaging, one the DNA to be packaged) in a transient transfection. All three plasmids have been designed, through lack of common sequences, to avoid the possibility of recombination with each other. The plasmids expressing the structural and packaging genes are not packaged with the produced virus, since none of them contain LTRs or the packaging RNA sequence. Thus,
replication-competent viruses cannot be produced, nor can the packaging cell line produce retrovirus in any form during routine culture.

4. Internal promoters are used to eliminate the need of the HIV-promoter and reduce the risk of recombination events and trans-activation of surrounding genes.

5. We will not concentrate the viral particles.

6. Where possible ecotropic pseudotyped viral particles will be used which are unable to infect humans.

**Origin & function**

We plan to express cDNAs of wild type and mutant proteins required for constitutive secretion such as coat proteins, rabs, tethers and SNAREs. These proteins are housekeeping genes so are not likely to be oncogenic or biologically hazardous.

We plan to express shRNA or CRIPSR/Cas9 constructs targeting genes involved constitutive secretion such as coat proteins, rabs, tethers and SNAREs. Loss of these proteins is unlikely to confer any known selective advantage to the host. However, we cannot exclude the possibility of tumor-inducing effects, as their functions are not well defined. In order to reduce the possibility of accidental exposure of a worker to virus we propose to carry out the handling of live virus and transduction of cells in our designated Class 2 laboratory.

The 2nd and 3rd generation lentiviral vector backbones may contain expression enhancing viral elements such as the WPRE element which in its wild type form can express the so called x-protein which may have oncogenic properties. Thus we propose to carry out the handling of live virus and transduction of cells in the designated Class 2 laboratory.

The transfer vectors may also incorporate one or more genes encoding selection markers and fluorescence reporter proteins. Examples of these include 1) PuromycinR, BlasticidinR, AmpicillinR, 2) fluorescent proteins such as mCherry, GFP, Venus, Cerulean, mTomato. These proteins are considered non-hazardous owing to their non-mammalian origin. There is no evidence of any oncogenic potential for these proteins thus we consider to highly unlikely that exposure to these proteins would result in harmful effects. However we propose to carry out the handling of live virus and transduction of cells in the designated Class 2 laboratory.

**Evaluation of foreseeable effects**

Environmental assessment
These replication-incompetent viruses would be inactivated prior to disposal, and given their inability to replicate they have no significant additional environmental hazard.

Worst-case scenarios
The above protocols should minimise any risk of exposure to the personnel using or in the vicinity of work using this lentiviral transfection system. Virus particles, virally-transduced cells, and media and supernatants resulting from these systems will never be handled with glassware or hypodermic needles. Thus, the risks of accidental inoculation are remote. The worst scenario would be exposure to a main stock of virus containing one transgene, perhaps resulting from spillage. The major exposure routes would be cutaneous, mucocutaneous, and respiratory. Effective infection of intact skin is unlikely as skin is a very effective barrier, and would be minimised by standards of Good Laboratory Practice. The viruses would infect mucocutaneous surfaces and respiratory epithelia and macrophages with which they came into contact. However, the particles are replication incompetent, and have many safety features preventing generation of replication competent viral particles. The genes of interest are not oncogenes. Thus although there might be limited tissue infection and damage, this would not result in sustained infection nor risks of tumour generation or malignant transformation. It might be possible that exposure would generate an immune response to the VSVG protein, but this would have a protective effect if so. The overall likelihood of serious or long-term harm resulting from experiments using these vectors seems extremely small.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
We propose to handle all material in Category 2 culture conditions.

1. Anti-viral disinfectants (Virkon, Trigene, bleach) will always be available prior to starting work involving handling of viral particles, in identified containers easily to hand (e.g. spray gun).
2. All culture will be performed within a Class 2 hood, in a designated Class 2 molecular biology lab. All cells exposed to virus will be handled in the Class 2 hood. After infection, viral supernatant will be removed and the cells washed. Once the media has been changed on at least one further occasion (after which active viral particles should not be present), then functional assays may involve handling transfected cells for brief periods on the bench.
3. Where small volumes of cells potentially containing viable viral particles require centrifugation, tubes will be sealed with parafilm prior to centrifugation.
4. Virus particles will be stored in the –80°C freezer, in cryovials, held inside 50 ml falcon centrifuge tubes or equivalent, in clearly marked packaging.
5. Hoods will be carefully cleaned with 10% solution of Trigene after each session in which culture with virus particles takes place, and after each session when cells that have been transfected by virus on a previous occasion are handled.
6. Only designated media will be used in cell culture.
7. Virus particles and supernatants from infected cells will be handled using filtered pipettes and pipette tips.
8. No glassware or hypodermic needles will be used at any time with any culture producing or containing virus particles, or any supernatant generated from any cell that has ever been exposed to virus particles.
9. All supernatants, plasticware, and cells that have come into contact with virus particles or viral infected cells will be treated with Trigene, Virkon, or bleach at appropriate concentrations and for 20-30 minutes prior to discard.
10. Standards of GLP will be rigorously enforced (lab coats, gloves, etc).
11. All personnel using or producing virus will be appropriately registered through the University Health and Safety procedures, listed on the GMAG approval, and taught appropriate safe handling by the principal investigator (Peter Andrews).
12. Other lab personnel will be made aware of the use of lentiviral systems in the laboratory.

Contamination management
1. Where spills occur in contained environments such as Class 2 hoods, neat Trigene, bleach, or Virkon will be added and left for >10 minutes to neutralise viral particles prior to wiping, cleaning and disposal.
2. If a small contained spill occurs outside of Class 2 hoods, management will be as above. Any spill outside of the Class 2 hood will be reported to a principal investigator, and assessment made to determine whether avoidable features are present to prevent future accidents.
3. Where aerosolised contamination is suspected to have occurred outside of Class 2 hoods, the lab will be cleared immediately to prevent aerosol inhalation. The air conditioning system will replace the air within a room within 10 min and will serve to desiccate and dilute the hazard. A single designated worker will return to the lab after 20 min and apply appropriate disinfectant to liquid traces.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project proposal was reviewed and approved by the University of Sheffield Biosafety Committee on the 24th February 2016, with only minor administrative recommendations made to the Proposer.
### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L3 L4 L2 L2</td>
<td>L3 L4 L2 L3</td>
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- **Animal Units**
  - L2 L3 L4 L2 L3 L4

- **Large Scale Activities**
  - L2 L3 L4 L2 L3 L4

- **Human Clinical Applications**
  - L2 L3 L4

### Project Ref 168/95.2

- **Date Ackn'd**: 27/02/1995
- **CU2 Project Title**: ANALYSIS OF SPORE GERMINATION OF BACILLUS CEREUS 569
- **Class**: Class 2
- **CultureVolClass2**: not applicable
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 168/95.3

Date Ackn'd 02/03/2022
CONSTRUCTION OF HAEMOPHILUS AND NEISSERIA GENOMIC LIBRARIES IN E COLI

18/04/1995

Date Project Ceased

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

N

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 168/96.1

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Historical Significant Changes

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02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref  168/97.1

Date Ackn'd  25/02/1997

Date Project Ceased

CU2 Project Title

EVALUATION OF IMMUNITY & PROTECTION IN MICE IMMUNISED WITH AMPLICON SYSTEM INFLUENZA VIRUS NUCLEOPROTEIN EXPRESSED IN CELLS WITH DEFECTIVE HERPES SIMPLEX VIRUS

Class 2

Non-GMM Consent Granted

Project notified under transitional arrangements

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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If yes, tick to confirm that it is attached to this form N

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Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 168/98.1

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 168/98.2

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Withdrawn | Tick if notifying a connected programme of work |
| N        | N       |

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

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Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Project Ref 168/98.3

Date Ackn'd 21/12/1998

CU2 Project Title

THE PHYSIOLOGY AND PATHOGENESIS OF NEISSERIA MENINGITIDIS

Class Class 2

CultureVol

Class 2

CultureVol

Class 3-4

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

NOTIFICATION RE SUBMITTED SEE GM168/14.3

Historical Date of Additional Info


Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

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Project Ref 168/99.1

Date Ackn’d 02/12/1999

CU2 Project Title RESPONSES OF NEISSERIA MENINGITIDIS TO NITRIC OXIDE

Class 2
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Please enter comments on the GM safety committee on the risk assessment

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Animal Units

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

<p>| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |</p>
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Campus Estate or Research Centre

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste containing viable GMMs is subjected to sterilisation by autoclaving over a prolonged autoclave cycle. All waste is carried in high sided sealed containers. All procedures are detailed in our codes of safe working practices.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### University of Cambridge
**Address:** Addenbrookes Hospital, Institute of Metabolic Science, Hills Road, Cambridge, CB2 0QQ, England
**Telephone:** 01223 336878, Fax: 01223 330598

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#### Contact Details
**Name:** UNIVERSITY OF CAMBRIDGE
**Name 2:** ADDENBROOKES HOSPITAL
**Department:** INSTITUTE OF METABOLIC SCIENCE - METABOLIC RESEARCH
**Building:**

<table>
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**E-mail**

**HSE Division:** EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted:** 02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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The maximum volume of liquid to be disposed of from a single work session is 2 litres. This is pre-treated with 2% Clearsol (Phenolic) Disinfectant. The pre-treated liquid waste and any bacterial plates are then autoclaved at 126°C for 50 minutes daily as required. The autoclave cycle is verified by a chart recording and a monthly independent check is carried out to ensure the correct functioning of the autoclave.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 170/04.1

Date Ackn'd 08/09/2004

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Lentiviral expression of mutant and wild-type candidate obesity and insulin resistance genes in cell culture. Lentiviral expression of short hairpin RNAs (shRNA) to mediate RNA interference (RNAi) for knock-down genes involved in the development of obesity and insulin resistance.

Class 2

< 1 Litre

Consent Granted Not Applicable

Project notified under transitional arrangements N
The objective of this proposal is to study candidate genes for obesity and insulin resistance by using lentiviral gene transfer technology to express those genes (or mutated genes) in cell lines and primary cell cultures which would otherwise be difficult to transfect. Furthermore, genes involved in the development of obesity and insulin resistance may be knocked-down by RNAi using shRNA expression cassettes integrated into lentiviral vectors. All of the genes we will be studying are involved in energy homeostasis, differentiation, signal transduction or gene expression.

We plan to use lentivirus-based vectors to express genes of interest in both cell lines and primary culture. Likewise, we plan to introduce shRNA expression cassettes with lentivirus vectors into those cells to downregulate the expression of specific genes by RNAi. Rodent cell lines will include CHO (Chinese ovary hamster) cells, FAO hepatocytes, L6 and C2C12 myotubes, and 3T3-L1 pre- and adipocytes. So far, no rodent lentivirus has been identified. Furthermore, these cells are not HIV infected and therefore the replication-deficient lentivirus used are not not able to replicate and no recombination to WT virus can occur. We also plan to express genes of interest or shRNAs in primary cultures of murine, rat or human preadipocytes, differentiated adipocytes, myotubes, hepatocytes or neurons. Primary rodent cells should be of no risk to harbour or produce lentiviruses and therefore recombination and virus production can be excluded after lentiviral gene transfer. Donors of human material will be checked for HIV-infection to reduce the risk and production of infectious virus. In addition, modification of these strategies we may also infect cells with lentiviruses encoding Cre recombinase (LV-Cre). This enzyme mediates recombination of DNA at specific loxP sites. If a DNA fragment is flanked by these loxP sites, it will be excised by Cre-mediated recombination. In this strategy, stable cell lines are established using retrovirally gene transfer using Moloney murine leukemia virus (MMLV) based vectors or lentiviral vectors which contain the GNA encoding the gene of interest, or mutant version of it, downstream of a promoter region with a blocking sequence inter.posing the two. The blocking sequence prevents expression of the gene of interest but is flanked by loxP sites. Once the cells have been cultured, and in most cases differentiated into mature adipocytes or myotubes, infection of the cells with lentiviruses encoding Cre recombinase causes excision of the loxP flanked blocking sequence and consequent induction of expression of the gene of interest. In the absence of target loxP sites the Cre recombinase would be expected to have no effect on cells. The initial retroviral (lentiviral) infection step will be entirely carried out physically separated from the LV-Cre infection. The latter would also be temporally distanced from the former by several cell passages and, typically, a period of culture to terminally de-differentiate the cell type undergoing study. Moreover, the MMLV-based retroviruses used in this case are ecotropic and capable only of infecting rodent cells. The used retroviral and lentiviral vectors are both lacking most of the viral genes and show only limited sequence homology. Therefore no recombination and production of infectious virus is possible in dually infected cells.
lentiviral parts the generation of replication competent virus via homologous recombination can be excluded. Furthermore all of HIV accessory protein genes (vif, vpr, vpu, nef) have been deleted (from all four plasmids) which are associated with possible pathogenesis. Likewise all regulatory genes (tat, rev) are deleted from the transfer vector. Virions containing the vector genome are produced by co-transfecting HEK 293T cells with transfer vector (expressing the gene of interest of the shRNA) together with three helper plasmids expressing the gag/pol genes, the rev gene or a gene encoding a heterologous viral glycoprotein (VSV-G) respectively. The resulting lentiviruses are replication deficient as they lack nearly all viral genes (only minor parts of the gag gene remain which are essential for genome packaging into virions). Furthermore they are mostly self inactivating as they harbour U3 deletions in the 3'-LTR region. Therefore only the gene of interest or the shRNA are expressed from the integrated lentiviral genome. The gene of interest is expressed from the CMV, murine PGK1 promoter or from tissue specific promotors while shRNAs are expressed from either the U6 or the H1 pol III promoter. The transgene expression cassette will be introduced downstream of the rev responsive element (RRE) or into the deleted U3 region in the 3'-LTR. In some cases the post-transcriptional regulatory element from woodchuck hepatitis B virus is introduced downstream of the transgene to enhance its expression. It may be useful to produce bicistronic vectors which express a mammalian gene of interest and a reporter gene (e.g. EGFP) or a gene mediating antibiotic resistance (e.g. puromycin resistance gene) to allow selection of successfully transduced cells. Furthermore it may be necessary to incorporate an inducible expression cassette into the viral genome. For this reason loxP sites may be introduced into the viral genome to take advantage of the Cre/loxP recombination system. Another option may be the use of tetracycline regulated promotors.

Origin & function

1. Wild-type and mutant mammalian genomic DNAs and cDNAs encoding a variety of candidate obesity and insulin resistance genes. These will be cloned either from mammalian mRNA or genomic DNA from mammalian tissue or mammalian-derived cell cultures. Mutations may be introduced into those genes.
2. The reporters GST, EGFP, FLAG, His, Myc, Renilla Luciferase and Firefly Luciferase may be used. These will allow verification of infection rates and quantification of transgene expression levels in cell types used.
3. Short hairpins directed against specific mammalian ORFs will be expressed from the Pol III promoters U6 or H1 and will mediate the specific degradation of the targeted RNA. These loss of function experiments will be carried out to further define gene function.
4. Cre recombinase is derived from bacteriophage P1 and expressed to excise DNA flanked by loxP sites.

Evaluation of foreseeable effects

Expression: Expression of the gene of interest is either driven by the strong PGK-1 or the CMV promotor. Both are active in host cell types. After integration of the reverse transcribed lentiviral genome into the host cell only of the gene of interest will be stably expressed. As genes are most likely involved in metabolism, differentiation, signal transduction or gene expression the probability of toxic or mitogenic effects are very low. But some of the genes or mutants may not be sufficiently characterised and therefore handling will need some caution. The expression of shRNAs is either driven by the U6 or H1 promotor. These Pol III promotors are active in most cell types. By stably expressing those shRNAs the respective target gene will be specifically expressed.

Damage: Replication-defective and self-inactivating lentiviruses are considered unlikely to cause disease for the purposes of Class II classification, although the nature of the inserted gene and of the used glycoprotein for pseudotyping should be considered when assessing potential damage from infection by engineered viruses. As all viral genes are deleted the generated virus is unable to replicate inside any cell. Also cytotoxic or cytopathogenic effects mediated by viral gene products are therefore circumvented.

As mainly self-inactivating (SIN) viruses are used and deleted U3 regions within the 3'-LTR (contains the viral promotor) no downstream gene should transcriptionally activated after integration into the host genome. During the integration process the U3 region is usually copied to the 5'-LTR and drives the transcription of the viral genome. If large parts of the U3 region are deleted the viral promotor is inactive and no transcription is initiated from the 5'-LTR of the provirus. Therefore using SIN vectors the production of infectious virus is further disabled. Only the random integration of the provirus into the host genome of the cell may alter extremely rarely the expression of genes situated near the integration site in individual cells infected.

Viruses pseudotyped with glycoprotein or vesclar stomatitis virus (VSV-G) shop a different host cell tropism and higher stability and may therefore be transmitted not only directly by injection but also by ingestion or via aerosols. However it has been shown that lentiviruses pseudotyped with VSV-G are rapidly inactivated in human serum. Taken the safety measures into further account the risk for humans is very low.

Furthermore these lentiviruses are unable to infect plants and are incapable of replicating in mammalian cells and therefore cannot be transmitted to any animal or human population.

As HIV-1 does not belong to the onco-retroviruses and the genes we are interested in are not recognised oncogenes, work with our virus is not expected to pose a cancer risk. However, the biological activity of mutany genes remain to be characterised. These are likely to differ from the wild-type counterpart. Therefore some degree of caution is required.
will be exercised when handling lentiviruses expressing these.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. All liquid waste will be disinfected/inactivated by treatment with Virkon and autoclaved prior to disposal to drains.
2. All solid waste will be soaked in Virkon, securely contained and afterwards incinerated.
3. Autoclave cycles are calibrated for type and amount of waste. Assumed 100% kill.
4. Disinfection with 70% EtOH will be used to decontaminate exposed work areas (+30 min UV).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

N/A

Project Containment

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Project Ref 170/09.1

Date Ackn’d 01/12/2009

CU2 Project Title Study of regulatory networks involving SECIS binding protein 2 (SBP2) and

Class 2

CultureVolClass2 1-50 Litres

CultureVolumeClass3-4

02/03/2022
Selenium (Se) is an essential trace element which is fundamental to human health. Its biological role is mediated principally by cotranslational incorporation of selenocysteine (Sec) into selenium-containing proteins (25 known human selenoproteins). Selenoproteins represent a diverse group, with functions ranging from metabolism of thyroid hormones (deiodinases, DIOs), removal of cellular reactive oxygen species (ROS) (glutathione peroxidase (GPx) types 1, 2, 3, 4 & 6, thioredoxin reductase (TrxR) types 1, 2, 3), reduction of oxidised methionine (selenoprotein X/MSRB1), transport and delivery of selenium to peripheral tissues (seIP), protein folding and ER stress (Sep-15, SELM), to proteins (SELO, SELV, SEPW, SELK, SELH) whose precise function is unknown. A molecular mechanism that is highly conserved from archaeabacteria to higher eukaryotes mediates the translational incorporation of Sec during selenoprotein synthesis. Interaction of a stem-loop RNA structure (Sec insertion sequence (SECIS) element), usually located in the 3'-UTR of selenoprotein mRNAs, with a multiprotein complex which includes SECIS binding protein 2 (SBP2), leads to Sec incorporation mediated by a specific selenocysteyl-transfer RNA (tRNA[Ser]Sec) at UGA codons. Failure of this mechanism can result in miscoding of the UGA as a stop codon and, depending on its location in the mRNA, the transcript may undergo nonsense mediated decay (NMD). The architecture of SECISBP2 is highly complex, with alternative splicing of the gene to generate multiple transcripts and internal methionine residues capable of directing synthesis of shorter protein isoforms.

Our laboratory (unpublished) and others (Dumitrescu Nature Genetics 2005, Cosmo JCEM 2009) have characterised mutant SBP2 in different patients resulting in a knock down of SBP2 expression. We describe several features in our affected subjects (elevated circulating thyroxine (T4) with low selenium and selenoprotein (SEPP, GPx3) levels, infertility, skeletal myopathy, skin photosensitivity, abnormal immune cell function, enhanced fat mass with preserved insulin sensitivity) that can be attributed to loss of selenoprotein function in specific tissues and have also documented cellular deficiencies of additional members of the selenoproteome whose function is unknown. Non of the patients have been presented with cancer. Our observations define a multisystem disorder, involving the defective biosynthesis of many selenoproteins, which can elucidate their role in human biological processes. The effects of SBP2 inactivation are poorly understood, cell line and animal studies using knockout mouse models has been hindered by lethal phenotype.

With the use of an inducible lentiviral system for introducing shRNA we want to generate stable expressing cells to assess the effect of SBP2 knock down, and/or its splice variants, or knockdown of individual selenoproteins (see appendix) on various physiological processes in different cell types.
themselves present no greater risk, may contain adventitious infectious agents and therefore will be handled at CL2 (risk assessment under COSHH regs).

Host/vector system

The pGIPZ and pTRIPZ lentiviral package systems will be used were described by Kappes et al. 2001; Kappes et al. 2003; Wu et al. 2000; Wu et al. 2001. and developed by Open Biosystems (see appendix). This new generation of lentiviral systems includes a significant number of safety features designed to enhance its biosafety and to minimize its relation to the wild type, human HIV-1 virus. More significantly the reverse transcriptase (RT) and integrase (IN) proteins are split from the native Gag-pol polyprotein and are provided in trans from a separate plasmid producing a novel class of HIV-based vectors. The lentivirus is produced by co-transfecting the packaging plasmid mix (four plasmids for gag, pro, RT, IN, VSV-G, rev and tat expression) and the transfer vector into the TLA-HEK293T cell line (10 cm dish). Particles will be harvested at 48 and 72 hours after transfection and stored at -80°C, spun down in a ultracentrifuge to concentrate or immediately used for transduction of recipient cells (tissue culture with primary human cells and immortalised human and murine cell lines and infection of those cells (6-well dish)).


Origin & function

With the use of an inducible lentiviral system for introducing shRNA we want to generate stable expressing cells to assess the effect of SBP2 knock down, and/or its splice variants, or knockdown of individual selenoproteins (see appendix) on various physiological processes in different cell types.

Our laboratory and others have characterised mutant SBP2 in different patients resulting in a knock down of SBP2 expression. We describe several features in our affected subjects (elevated circulating thyroxine (T4) with low selenium and selenoprotein (SEPP, GPx3) levels, infertility, skeletal myopathy, skin photosensitivity, abnormal immune cell function, enhanced fat mass with preserved insulin sensitivity) that can be attributed to loss of selenoprotein function in specific tissues and have also documented cellular deficiencies of additional members of the selenoproteome whose function is unknown. Our observations define a multisystem disorder, involving the defective biosynthesis of many selenoproteins, which can elucidate their role in human biological processes.

Evaluation of foreseeable effects

The expressed shRNA products will reduce SBP2 or selenoprotein expression and may be expected to affect cellular processes such as growth, differentiation, self-renewal and apoptosis but are not considered to be oncogenic. Defective retroviral/lentiviral in which the helper genes are located on separate blocks of DNA. Such vectors may infect human cells and integrate into the cellular DNA, but when unmodified are considered hazard group 1.

The cells may be transformed/oncogenic; however they are still relatively fastidious and would be incapable of colonising/causing disease; cells could present a genuine risk to corresponding donor, but there will be no opportunity of donors to be so exposed.

The Trans-Lentiviral™ GIPZ Packaging System includes the following key safety features:

- The expression vectors contain a deletion in the 3′ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell.
- The number of genes from HIV-1 that are used in the system has been reduced (i.e. gag, pol, rev, tat and vpr).
- The VSV-G gene from Vesicular Stomatitis Virus is used to pseudotype the vector particles. The HIV-1 envelope has been completely removed from the vector.
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids.
- Although the packaging plasmids allow expression in trans of genes required to produce viral progeny (e.g. gag, pol, rev, tat, env) in the TLA-HEK293T™ producer cell line, none of them contain LTRs or the ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus has been shown to be produced.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture/lab ware will be autoclaved and incinerated. Culture fluids will be treated with 1% Virkon for 0.5 hours, autoclaved and disposed to drains. Recyclable lab ware will be soaked in 1% Virkon for 16 hours. Bench/cabinet surfaces will be wiped down with 10% trigene and 70% ethanol. Spills will be sprinkled with Virkon powder/trigene and vernagel to solidify. Alternatively disinfected liquid waste will be gelled with vernagel in sealed containers labelled with autoclave tape and taken to the autoclave room for transfer to the incinerator. All solid waste will be collected in double autoclave bags, sealed with autoclave tape, labelled with the users name and the room number and will be autoclaved and incinerated.

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The project has been reviewed by the IMS biological and genetic modification safety committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 Yes | L2 | L2
L3 | L3 | L3
L4 | L4 | L4

Animal Units | Large Scale Activities | Human Clinical Applications
---|---|---
L2 | L2 | L2
L3 | L3 | L3
L4 | L4 | L4

Project Ref 170/10.1

Date Ackn'd 23/02/2010

Date Project Ceased

CU2 Project Title Study of cellular responses to protein misfolding in mammalian cells using lentiviral expression systems

Class CultureVolClass2 CultureVolumeClass3-4
---|---|---
Class 2 1-50 Litres

Non-GMM Consent Granted

Non-GMM Consent Granted
Purposes of the contained use

Protein misfolding in cells contributes to important diseases ranging from neurodegenerative disorders, such as Parkinson's Disease, to metabolic diseases such as type II diabetes mellitus. A common theme of these conditions is the accumulation, or the threat of accumulation, of misfolded and unfolded proteins that contribute to attrition of key cellular functions. Cells have an elaborate apparatus for recognizing and responding to the threat of protein misfolding, known as the Unfolded Protein Response (UPR). This apparatus has numerous components that promote fitness in the face of the aforementioned threat. Surprisingly, however, the machinery of the unfolded protein response also has components whose activity, in certain contexts appear to enhance the deleterious effects of misfolding [Ron, 2007 #3066].

Rational therapeutic interventions aimed at the pathophysiology of protein misfolding requires the establishment of experimental systems in which the threat of protein misfolding is imposed and the activity of various components of the response to misfolded proteins (the Unfolded Protein Response) are either inactivated or enhanced. Because the clinically-relevant burden of protein misfolding is experienced disproportionately by terminally-differentiated, non-dividing cells, the tools for constructing the aforementioned experimental system must be geared to manipulating gene expression in non-dividing cells. HIV1-based lentiviruses, modified for safety and gene delivery have emerged as powerful tools for effecting changes in gene expression in terminally-differentiated cultured cells. Therefore, authorization is sought to use such vectors to enforce expression of genes encoding known misfolding-prone proteins in cultured mammalian cells and at the same time alter the cellular response to the misfolded proteins by introducing activated alleles, dominant loss-of-function alleles or shRNA inactivating alleles of genes encoding components of the unfolded protein response. By comparing the response of cells with diverse perturbations in the unfolded protein response to comparable burdens of protein misfolding we can begin to identify components of the unfolded protein response whose activation or inactivation (by pharmacological means) may be of therapeutic benefit in combating diseases of protein misfolding.

Recipient or parental organism

The lentivirus is produced by co-transfecting the packaging plasmid mix and the transfer vector into human HEK293T cells.
The recipient cells will be various murine and human cells in culture.
Both the recipient cells and the producing cells are fastidious and non harmful. They cannot colonise the environment nor cause disease by colonizing human or animal hosts and thus are classified as Hazard Group 1. Cells from human volunteers, which may be used in this project are uncharacterised and although themselves present...
no greater risk, may contain adventitious infectious agents and therefore will be handled at CL2 (risk assessment under COSHH regs).

**Host/vector system**

The pGIPZ and pTRIPZ lentiviral package systems and derivatives thereof like the pLenti6_V5 of invitrogen or the delivery vehicle for Sigma mission shRNA (pLKO.1–Puro) to be used were described by Kappes et al. 2001; Kappes et al. 2003; Wu et al. 2000; Wu et al. 2001 and developed by Open Biosystems. This new generation of lentiviral systems has been used extensively throughout the world and includes important features designed to enhance its biosafety:

The reverse transcriptase (RT) and integrase (IN) genes were split from the Gag-pol polyprotein-encoding gene and are provided in trans from two separate plasmid. The ENV function, provided by the VSV glycoprotein, is introduced in trans by a third plasmid and the only packaging signal is on the transfer plasmid that lacks important HIV genes. Thus, the probability of a series of recombination events that produce a replication-competent virus that can spread in and between hosts is vanishingly small. In the context of this project, the main risk is the consequence of primary infection of humans by high titre viral stocks (see below).

The lentivirus is produced by co-transfecting the packaging plasmid mix (four plasmids for gag, pro, RT, IN, VSV-G, rev and tat expression) and the transfer vector into the HEK293T cells. Particles are harvested over a period of 48 to 72 hours after transfection and are stored at -80°C. If titres are low, the virus can be concentrated by ultracentrifugation. Transduction of recipient cells follows. These will be tissue cultures of primary human or other mammalian cell lines.


**Origin & function**

The shRNA sequences to inactivate genes in the host are derived from the host cell genome. They will be directed to genes that function in protein folding in the endoplasmic reticulum and the response to protein misfolding (examples include: chaperone-encoding genes like Erdj3, enzymes involved in oxidative protein folding like Ero1l and Ero1lb, components of the unfolded protein response like Ern1 or Eif2aK3). These genes are neither oncogenes nor tumor suppressor genes and thus accidental infection of a human host by these viruses is likely to have no greater consequence than infection by an “empty” virus.

To activate components of the unfolded protein response we will use cloned cDNA that will be over-expressed. As the genes in question are neither oncogenes nor tumour suppressor genes and thus accidental infection of a human host by these viruses is likely to have no greater consequence than infection by an “empty” virus.

To promote misfolded protein stress we will express known misfolding-prone mutants versions of secreted proteins, for example, Ins2 with a mutation that prevents proper disulfide bond formation. As the secreted proteins are not oncogenic and in fact harbour mutations that promote endoplasmic reticulum retention the are either inert or enfeeble the targeted cell, the risk of oncogenic transformation by a virus harbouring a misfolding prone protein are diminished compared to the empty virus. A theoretical exception to this principle is a retrovirus encoding a prion-forming protein, which could in an extremely fanciful scenario set up a prion infection in the host. Therefore, proteins with a potential to undergo prionization (e.g. mutant PrPsc) will not be used.
Following is a table of the specific genes:

<table>
<thead>
<tr>
<th>List of mutant genes encoding proteins that misfold in the endoplasmic reticulum and that will be expressed from lentiviral vectors:</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Mouse Ins2C96Y Encodes insulin, a hormone normally present in serum</td>
</tr>
<tr>
<td>b) Human PLAPC121S. Encodes placental alkaline phosphatase, an enzyme normally present in tissue</td>
</tr>
<tr>
<td>c) Human SERPINA1PIZ. Encodes a misfold serum protease inhibitor</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>List of genes encoding proteins that function in the processing of protein folding in endoplasmic reticulum or in the response to the stress of protein misfolding in the endoplasmic reticulum. The expression of these genes will be inhibited by shRNA-containing lentiviral vectors or the genes will be over-expressed by lentiviral vectors:</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Mouse or human Hspa5, Hsp90b1, ERDJ (1-6). Encode chaperones that folds proteins in the ER</td>
</tr>
<tr>
<td>b) Mouse or human PDI1, Erp44, Erp57, Erp72. Encode disulfide isomerases/transfereases than function in oxidative protein folding in the secretory pathway</td>
</tr>
<tr>
<td>c) Mouse or Human Ero1l, Ero1lb, Qsox1, Qsox2. Encode protein disulfide oxidases involved in protein folding</td>
</tr>
<tr>
<td>d) Mouse or human EIF2AK3, ERN1, ATF6. Encode detectors of unfolded protein stress in the endoplasmic reticulum</td>
</tr>
</tbody>
</table>

**Evaluation of foreseeable effects**

RNAi inactivation of most genes involved in protein handling in the endoplasmic reticulum or in the response to misfolded proteins, will enfeeble cells and render them more sensitive to misfolded protein load. Even those genes that encode proteins that are known to reduce the fitness of cells experiencing high levels of misfolded protein stress are not known oncogenes (example CHOP/Ddit3) Thus the risk associated with viruses encoding such genetic elements is predicted to be less than that of empty lentiviruses.

Gain-of-function of unfolded protein response components, which may have a benefit in the special circumstances of severe protein misfolding is unlikely to translate to a survival benefit for accidentally-transduced cells, let alone serve as an oncogenic event. Thus the risk posed by gain-of-function vectors is likewise less than that of empty viruses. Lastly, as noted above, the expression of a misfolding-prone protein is severely enfeebling to cells and part from the theoretical establishment of a new strain of prions the risk posed by such vectors is less than that of the empty ones.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture/lab ware will be autoclaved and incinerated. Culture fluids will be treated with 1% Virkon for 0.5 hours, autoclaved and disposed to drains. Recyclable lab ware will be soaked in 1% Virkon for 16 hours.

Bench/cabinet surfaces will be wiped down with 10% trigene and 70% ethanol. Spills will be sprinkled with Virkon powder/trigene and vernagel to solidify. Alternatively disinfected liquid waste will be gelled with vernagel in sealed containers labelled with autoclave tape and taken to the autoclave room for transfer to the incinerator. All solid waste will be collected in double autoclave bags, sealed with autoclave tape, labelled with the users name and the room
number and will be autoclaved and incinerated.

13. * Is an emergency plan required according to regulation 20?

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project has been reviewed by the IMS biological and genetic modification safety committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units

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Project Ref 170/12.1

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<th>CultureVolumeClass3-4</th>
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<td>Studies on biological clocks and sleep</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<td>Non-GMM</td>
<td>Consent Granted</td>
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Date Project Ceased 24/04/2017

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Project transferred to GM542

Project notified under transitional arrangements N
**Circadian (circa- approximately, -diem day) rhythms are a fundamental property of the life of a cell. When held in temporal isolation, organisms from unicells to humans exhibit behavioural and physiological rhythms that persist with a period of approximately 24 hours. These rhythms are driven by cell-autonomous molecular circadian clocks. They confer selective advantages to organisms by facilitating anticipation, and thereby adaptation to, the alternating day/night cycle. The competitive value of circadian clocks has been demonstrated in prokaryotes and higher plants, whilst disturbance of circadian timing in humans, as seen in rotational shift workers for example, carries significant long-term health costs.**

We have developed novel tools, based on variants of fluorescent proteins and the luciferase enzyme, that allow us to probe the inner workings of the clockwork in more detail than previously possible. Since we now want to study the clockwork in neuronal systems and in primary cells, lentiviral systems are necessary to introduce relevant transgenes into the host cell genome for stable expression, especially in intact organotypic tissue slice cultures. Moreover, we also wish to perturb the clockwork by knocking down the expression of various ‘clock genes’ (and their modifiers such as peroxiredoxin proteins), and lentiviral systems allow this in neuronal and ‘difficult-to-transfect’ cell lines, including primary cells.

HIV1-based lentiviruses, modified for safety, have emerged as powerful tools for effecting changes in gene expression in terminally-differentiated cultured cells such as neurons and organotypic tissue slices. Therefore, we seek authorization to use such vectors to attain our goals in relation to clock research.

**Recipient or parental organism**

The lentivirus is produced by co-transfecting the packaging plasmid mix and the transfer vector into human HEK293FT cells. The recipient cells will be various murine and human cells in culture, including primary cells. Both the recipient cells and the producing cells require specific cell culture conditions to grow and are not harmful. They cannot colonise the environment nor cause disease by colonizing human or animal hosts and thus are classified as ACDP Group 1. Cells from human volunteers, which may be used in this project are uncharacterised and although themselves present no greater risk, may contain adventitious infectious agents and therefore will be handled at Containment Level 2 within the host laboratory.
The ViraPower™ Lentiviral Expression System (Invitrogen), facilitates highly efficient, in vitro or in vivo delivery of a target gene to dividing and non-dividing mammalian cells using a replication-incompetent lentivirus. Similar systems have been developed by multiple companies and use very similar (if not identical) components, e.g. Sigma Mission shRNA pLKO based system. This new generation of lentiviral systems has been used extensively throughout the World and includes important features designed to enhance its biosafety:

- The pLenti expression vector contains a deletion in the 3′ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
- The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).
- The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns et al., 1993; Emi et al., 1991; Yee et al., 1994).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).
- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
- The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.
- Expression of the gag and pol genes from pLP1 has been rendered Rev dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al., 1998).
- A constitutive promoter (RSV promoter) has been placed upstream of the 5′ LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA (Dull et al., 1998).

References


Yu, S. F., Ruden, T. v., Kantoff, P. W., Garber, C., Seiberg, M., Ruther, U., Anderson, W. F., Wagner, E. F., and
activate components of the circadian clock we will use cloned cDNA that will be over-expressed. The genes in question are neither oncogenes nor tumour suppressor genes and thus accidental infection of a human host by these viruses is likely to have no greater consequence than infection by an empty virus. We will also express bioluminescent and fluorescent proteins as 'reporters' of the clock within cells, which are derived from firefly and jelly fish sequences respectively. These have a long history of safe use in various non-replicative lentiviral systems.

A theoretical exception to this principle is a retrovirus encoding a prion-forming protein, which could theoretically set up a prion infection in the host. Therefore, proteins with a potential to undergo prion formation (e.g. mutant PrPSc) will not be used.

The short hairpin RNA (shRNA) sequences used to inactivate genes in the host are derived from the host cell genome and tested in conventional (non-lentiviral) vectors before use to test their function in cell lines. They will be directed to genes that function in the circadian clockwork, usually as transcription factors that switch oscillating genes on/off over the 24 hour day. These genes are neither oncogenes nor tumor suppressor genes and thus accidental infection of a human host by these viruses is likely to have no greater consequence than infection by an empty virus.

List of genes / fusions encoding 'reporter' proteins that will be expressed from lentiviral vectors:

- Basic fluorescent proteins: GFP, RFP, dsRED, CFP, YFP
- Redox-sensitive fluorescent proteins: roGFP1/2, roGFP1/2-Grx1, roGFP1/2-Trx, roGFP1/2-Prx1, roGFP1/2-Prx2, roGFP1/2-GAPDH
- NAD/NADH-sensitive fluorescent proteins: SuperFres
- H2O2-sensitive fluorescent proteins: Hyper, Hyper-2, Hyper2-Grx1, Hyper2-Trx, Hyper2-Prx1, Hyper2-Prx2, Hyper2-GAPDH
- Bioluminescent proteins: Luciferase (Luc2), OxyLUC
- Light-sensitive ion channels: Channelrhodopsin, Halorhodopsin (and variants)
- Light-sensitive expression systems: Lite-On
- Voltage/Calcium sensing fluorescent proteins: Cameleon, Case12, GCAMPS, Arch3, PROPS, VFSP3
- pH-sensitive fluorescent proteins: pHluorin, synapto-pHluorin, pHred

List of genes encoding proteins that function in the circadian clockwork. The expression of these genes will be inhibited by shRNA-containing lentiviral vectors, or the genes will be over-expressed by lentiviral vectors:

- Mouse or human Clock, Bmal1/2. Encode clock-relevant transcription factors.
- Mouse or human peroxiredoxins (Prx1, 2, 3, 4, 5, 6). Encode clock-relevant antioxidant response proteins.
- Mouse or human catalase. Encode clock-relevant antioxidant response proteins.
- Mouse or human superoxide dismutase. Encode clock-relevant antioxidant response proteins.
- Mouse or human thioredoxins (Trx1, 2). Encode clock-relevant antioxidant response proteins.
- Mouse or human glutaredoxin (Grx). Encode clock-relevant antioxidant response proteins.
- Mouse or human glutathione reductases. Encode clock-relevant antioxidant response proteins.

Evaluation of foreseeable effects

Inactivation of most genes involved in circadian clock function by RNA interference (RNAi) will have no significant effect on the cell, and if anything, make them more susceptible to undergo apoptosis. The genes that will be disabled
are not known oncogenes. Thus, the risk associated with viruses encoding such genetic elements is predicted to be
less than that or empty lentiviruses.
Gain-of-function of clock components by overexpression is unlikely to translate to a survival benefit for accidentally-transduced
cells, let alone serve as an oncogenic event. The same is true for bioluminescent and fluorescent reporter
proteins; these have no other recognised function than to produce light. Thus, the risk posed by gain-of-function
vectors is likewise less than that of empty viruses.

8.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture materials and labware will be autoclaved and incinerated which eliminates 100% of the
infectious material. All solid waste will be collected in double autoclave bags, sealed with autoclave tape, labelled
with the users name and the room number and will be autoclaved and incinerated.
Culture fluids will be treated with 2% Virkon for 16 hours (which eliminates 100% of infectious material) and disposed
to drains. Alternatively, disinfected liquid waste will be gelled with Vernagel in sealed containers labelled with
autoclave tape, and taken to the autoclave room for transfer to the incinerator. Spills will be sprinkled with Virkon
powder/Trigene and Vernagel reagent to solidify.
Recyclable labware will be soaked in 1% Virkon for 16 hours (which eliminates 100% of infectious material).
Bench/cabinet surfaces will be wiped down with 10% Trigene and 70% ethanol (which eliminates 100% of infectious
material).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The project has been reviewed by the Institute of Metabolic Science's biological and genetic modification safety
committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the
appropriate conditions.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</table>

02/03/2022
Herpesviruses are ubiquitous pathogens of man and all vertebrates. In humans, herpes simplex virus (HSV) causes oral and genital lesions and occasionally more serious diseases such as keratitis and encephalitis. Following primary infection latent virus resides in sensory neurones and can reactivate from these cells to facilitate recurrent disease/transmission. Murine gammaherpesvirus 68 is a gamma-2 herpesvirus, originally isolated from small free-living rodents in Slovakia. It behaves as a natural pathogen in conventional laboratory mice and has been used extensively as a model for the study of gammaherpesvirus pathogenesis. MHV-68 causes an infectious mononucleosis-like illness but is otherwise avirulent in adult, immunocompromised mice. Spread between mice is rare, even when housed in the same cage; intimate contact is probably required for virus transmission. Infection of humans has not been documented. In mice the virus productively infects epithelial cells and establishes a latent infection in B lymphocytes. The object of this research is to identify metabolic processes that influence the kinetics of virus replication in vitro and viral dissemination in vivo using reporter gene expressing HSV and MHV-68. This will be achieved by infecting mammalian cell lines and both wild type and genetically altered mice. The contribution of individual virus encoded
gene products to such interactions will be assessed using viral mutants with specific gene truncations or deletions. In addition, viral gene products cloned into replication defective retroviral and/or Adenovirus vectors to facilitate the transduction of mammalian cells will be used to probe such interactions further.

### Recipient or parental organism

Parental viruses: HSV 1/2 (HG2 pathogens), MHV68 (HG1 pathogen)
The biological functions of HSV-1, HSV-2 and MHV-68 genes are largely undefined however HSV and MHV-68 do not encode known toxins or proven oncogenes. In common with other well studied and genetically related herpesviruses it is expected that both HSV and MHV-68 will encode gene products including miRNAs that affect multiple cellular pathways/processes.

HSV encodes some 80 different gene products and at least 15 miRNAs involved in various aspects of virus replication, cell modification and immune evasion. The detailed mode of action of these virus encoded products is in many cases poorly understood. A number of gene products are known to be toxic in the expressing cell, for example the HSV-1 UL41 gene shuts off host protein synthesis and overexpression of a number of immediate early gene products prevent cell division (e.g. ICP0).

MHV-68 encodes some 70 different gene products and at least 8 miRNAs involved in various aspects of virus replication, cell modification and immune evasion. The detailed mode of action of these virus encoded products is in many cases poorly understood. A number of gene products are know to be toxic in the expressing cell, for example MHV-68 shuts off host protein synthesis and the virus can induce cell division and proliferation of B cells. In addition, specific gene products are involved in immune evasion, such as the K3 gene product that specifically degrades mouse (but not human) MHC class I, and M3, which is a pan chemokine binding protein.

Standard mammalian cell lines such as NIH 3T3, HEK (human embryonic kidney) 293, MEF (murine embryonic fibroblasts), HeLa and BHK (baby hamster kidney) cells. All mammalian cell lines used have a history of safe use and it is unlikely that they will carry adventitious pathogens.

### Host/vector system

Bacterial artificial chromosomes (BAC): The use of full-length infectious herpesvirus genomes cloned as BAC has significantly improved the process of genetic manipulation of the genomes and is an approach widely used throughout the herpesvirus research community. The risks posed by working with either naked viral DNA or genomes cloned as BACs are low since the specific infectivity of DNA is many orders of magnitude lower than that of fully formed virus particles. Herpesvirus genomic DNA is however capable of infecting human cell lines in culture if introduced in certain ways (e.g. by transfection procedures) and therefore there is a potential risk of infection when working with or handling virus genomes cloned as BACs. The risk is very low because a route of direct delivery of the BAC DNA into cells is necessary for infection and would be highly unlikely during normal laboratory handling. Non mobilizable bacterial genomic vectors to be used: pUC series, HSV cloned as bacterial artificial chromosome in a derivative of pBAC108L (disabled F plasmid), MHV-68 cloned as bacterial artificial chromosome in a derivative of pBAC108L. Bacterial hosts will be multiply disabled E.coli K12 derivatives eg DH5 alpha, DH10B, JC8679 for all bacterial cloning. These derivatives (HG1 pathogens) are disabled and do not compete with normal gut flora.

Adenovirus vectors: The parent vector is an Ad-5 recombinant in which the E1 region is replaced by a lacZ coding cassette. This virus grows only in helper cells (eg 293 cells) that provide the E1 gene product in trans, and is an ACDP hazard group 1 organism. All insertions will be in the E1 region, replacing the lacZ cassette. This effectively eliminates the possibility of generating a replication competent virus carrying the foreign insert by recombination with a wild type virus. Adenoviruses are spread via the respiratory aerosol route and this route represents the major risk of infection.

Retrovirus vectors: Third generation lentivirus vectors lack pol, tat, env, rev and gag genes and can only be packaged...
following complementation with helper plasmids expressing RT, IN, tat, rev together with the VSV G protein. These are expressed from different plasmids so reconstitution of wild type virus is not possible. In addition, the vector genome has a self inactivation LTR to reduce the risk associated with insertional mutagenesis. Host range and virus stability is expanded by incorporation of the VSV G protein.

Herpesvirus vectors: Herpes simplex virus deleted for the gH gene is replication defective and cannot complete a full round of replication. Such vectors can be propagated on gH expressing complementing cell lines that do not result in the generation of detectable levels of replication competent virus since there is no homology between the virus and the gH expression cassette in the CR1 cell line. Following infection of noncomplementing cell lines the virus can only go through a single round of replication and produces non infectious virus progeny. This vector is classified as a class I GMO by the HSE.

Technique used to introduce insert or vector into host:
Infection of mammalian cell lines and mice with recombinant HSV or recombinant MHV-68 containing gene disruptions and/or expressing reporter genes.
Cloned genomic fragments of HSV or MHV-68 will be cloned directly into pUC-based vectors and transformed into E.coli K12 derivatives. HSV or MHV-68 cloned as a bacterial artificial chromosome will be transformed into E.coli K12 derivatives and purified plasmid DNA used to reconstitute virus following transfection of mammalian cell lines. Retroviral and Adenovirus vector mediated transduction of mammalian cell lines with HSV or MHV-68 encoded gene products.

Investigation into the interaction between viral replication and cellular metabolic processes may involve:
1. Genomic cloning of HSV1/2 or MHV68 sequences into plasmid and replication defective viral vectors.
2. Inactivation/modification of virus encoded genes and non-coding RNAs, including alteration of their regulation of expression, using full-length infectious herpesvirus genomes cloned as bacterial artificial chromosomes (BAC).
3. Cloning of reporter genes (e.g. GFP, betagalactosidase, Cre recombinase, luciferase) under latent or lytic cycle promoter control. Each of these proteins has no known biological effects in eukaryotes.
4. The expression of virus and/or cellular gene products in a glycoprotein H (gH) deleted HSV-1 (Class I GMO as agreed with Dr Mark Bale at the HSE) vector. This gH deleted vector is severely disabled and can only be propagated in a gH complementing cell line. The severely disabled nature of such a gH deleted vector makes it very unlikely that it will pose a risk greater that wild type HSV-1 since any cell entering a productive replication cycle will be killed and no infectious progeny will be produced.

Evaluation of foreseeable effects

HSV-1/2 (HG2): Recombinant viruses containing mutations of individual gene products or encoding reporter gene cassettes are most likely to be less virulent than wild type parental virus.
MHV-68 (HG1): recombinant viruses containing mutations of individual gene products or encoding reporter gene cassettes are most likely to be less virulent than wild type parental virus.
Retrovirus vectors: There is a low risk of aerosol transmission and efficient transduction would require breach of mucosal membrane or the skin surface. This would result in transduction of a small number of cells as the virus is defective and cannot spread.
Adenovirus vectors: Deliberate attempts to deliver foreign genes in vivo using adenovirus vectors (in gene therapy experiments) establish that this is an inefficient process. In the event of accidental infection:
1. A small number of cells would express the foreign gene. This might result in death of those particular cells if the gene product was cytotoxic.
2. There is a very small risk that a pre-existing adenovirus infection might result in complementation of a recombinant
vector by wild type virus. Transient growth of the recombinant might then occur. Infected cells would be killed by virus growth or by immune response to infection. There is no expectation that a herpesvirus gene product would exacerbate an adenovirus infection or modify tissue tropism (note the receptor binding proteins of herpesviruses are integral membrane proteins which cannot assemble into and adenovirus virion). Given these factors, the proposed recombinants are of no greater risk than the parental adenovirus vector but given the uncertainty about the functions of herpesvirus gene products, category 2 containment is appropriate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture materials and labware will be autoclaved and incinerated which eliminates 100% of the infectious material. All solid waste will be collected in double autoclave bags, sealed with autoclave tape, labelled with the users name and the room number and will be autoclaved and incinerated. Culture fluids will be treated with 2% Virkon for 16 hours (which eliminates 100% of infectious material) and disposed to drains. Alternatively, disinfected liquid waste will be gelled with Vernagel in sealed containers labelled with autoclave tape, and taken to the autoclave room for transfer to the incinerator. Spills will be sprinkled with Virkon powder/Trigene and Vernagel reagent to solidify. Recyclable labware will be soaked in 1% Virkon for 16 hours (which eliminates 100% of infectious material). Bench/cabinet surfaces will be wiped down with 10% Trigene and 70% ethanol (which eliminates 100% of infectious material).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project has been reviewed by the Institute of Metabolic Science's biological and genetic modification safety committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

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02/03/2022 Page 4366 of 15326
Insulin resistance (IR) refers to reduced ability of insulin to lower blood glucose. In its common form, it is closely associated with obesity, and is causally linked to major pandemic diseases including type 2 diabetes, liver disease, subfertilitf and atherisckerisus. Nevertheless the underlying cellular and molecular basis is poorly un derstood, and this is a major rate limiting step in the development of novel therapies. A highly productive strategy in study of IR has been to focus on patients with rare and extreme disorders of insulin action, whether severe IR, or insulin supersensitivity. The Semple Lab has a large prgramme of genetic study of such affected patients, and using next generation sequencing in collaboration with the Sanger Institute has discovered many single gene variants plausibly linked to the diseases being studied. To verify that these variants do alter gene function it is critical to undertake downstream cellular studies either in model cell lines, or in primary human cells where available.

For the following reaons, the use of the HIV1-based lentiviral vectors described below is highly desirable for these studies:

1. The acute metabolic actions of insulin are exerted on terminally differentiated, non-dividing cells, and so the tools for constructing experimental cellular systems to model in vivo cellular pathology must be geared to manipulatin gene expression in non-dividing cells.

2. The ability to express or knock down genes conditionally is extremely valuable in study of human primary cells, helping to circumvent problems arising from high inter-cell
line variability in some assays. Conditional expression or knockdown or expression in terminal differentiation of primary cells and cell lines before induction, avoiding
problems which are commonly experienced due to adverse effects of overexpression or knockdown on differentiation itself. The pSLIK system described offers the
opportunity to achieve conditional gene expression or knockdown with only one viral infection, which is a major advantage with when working in fragile primary cells with a
finite replicative capacity.

HIV1-based lentiviruses, modified for safety and gene delivery have emerged as powerful tools for effecting changes in gene expression in terminally-differentiated cultured
cells. We propose to use one such vector to allow conditional knockdown or expression of putative pathogenic alleles of genes implicated by human genetic studies in
severe clinical disorders of insulin action, while at the same time, and where necessary, altering known insulin signalling pathways by introducing activated alleles, dominant
loss-of-function alleles or shRNA inactivating alleles of genes known to be involved in insulin signal transduction. By comparing the response of cells with and without
naturally occurring mutation or loss of gene function we can provide evidence that the mutations identified in human genetic studies are indeed disease causing, and can
elucidate their precise role in perturbing insulin action, which will be essential for the development of targeted therapeutic strategies for affected patients.

The lentiviral particles will be used solely in cultured cells. No experiments with lentivirus will be undertaken in animals.

Specifically, the types of studies to be undertaken are as follows:

1. Heterologous expression of naturally occurring mutants known to cause a monogenic disorder of insulin action. In primary cells or cell lines, to create disease models for
screening of pharmacological treatments.
2. Knockdown of expression of genes in which naturally occurring loss-of-function mutation have been identified in association with a disorder of insulin action to provide
evidence for pathogenicity.
3. Overexpression or knockdown of genes functionally involved in insulin action, fat cell differentiation, or other cellular functions being studied, to assess links between
these and the mutations being studied.

The most highly used downstream assays after these manipulations will assess:
1. Effects of insulin on such endpoints as glucose uptake, lipogenesis, lipolysis, gluconeogenesis, apoptosis and cell division.
2. Cell growth at baseline and in response to growth factors
3. Tolerance of DNA damage

Recipient or parental organism

Standard disabled laboratory chemically-competent E. coli derived bacterial strains (DH5alpha, XL-1blue, STBL3) for plasmid propagation. Non-pathogenic to humans and
animals. Cannot survive or establish in the environment.

The lentivirus will be produced by co-transfecting the packaging (pMDLg/pRRE and pRSV-Rev), the pseudotyping (pVSV-G), and the lentiviral transfer plasmids into a
variant of human embryonic kidney 293 cells stably and constitutively expressing the SV40 Large T-antigen (HEK 293T). This is a cell line that has been extensively used in
human or animal host and thus are classified as Hazard Group 1. HEK293T were obtained from ATCC. The packaging pMDLg/pRRE (contains the genes gag, pol, and
RRE) and pRSV-Rev (contains rev), and the pseudotyping pVSV-G plasmids offer maximal biosafety as described in Dull et al. (1) and Klages et al. (2) and were obtained
from the non-profit repository Addgene (www.addgene.com).

The recipient cells will be the following murine/rodent and human cells in culture:

Primary human dermal fibroblasts
Human dermal fibroblasts immortalised using hTERT
Induced pluripotent stem cells and derived cell lines
EBV-transformed human lymphoblastoid cells
Immortalised cell lines:
Human HEK293, HepG2, SGBS
Rodent: 3T3-L1 (preadipocytes and quiescent mature adipocytes), CHO

Both the producer and recipient cells are fastidious and non-harmful. They cannot colonise the environment nor cause disease by colonising human or animal hosts and thus are classified as Hazard Group 1. Only the primary cells from human volunteers and derived cells are uncharacterised, and although they themselves present no greater risk, they may contain adventitious infectious agents, and therefore all work will be undertaken within a Class II microbiological safety cabinet in a Containment Level 2 laboratory (risk assessment under COSHH Regulations). Other cells are well characterised tissue culture cell lines that have been/will be acquired from commercial or non-profit repositories.

The pSLIK Single Lentivector for Knockdown) lentiviral platform system to be used in this project is a HIV-1-based third-generation lentivirus system developed by The Alliance for Cell Signalling (AfCS), Berkeley, CA. The platform was described by Shin et al. (3) and is based in the four-plasmid packaging system originally described by Dull et al. in 1998. We will use the pSLIK system to conditionally express heterologous genes or shRNA sequences for targeted inactivation of host genes in various mammalian and primary human cell lines in culture.

The pSLIK platform is a third-generation lentiviral packaging system. This new generation of packaging system has been extensively used throughout the world and is likely to provide a greater margin of safety than earlier systems(2, 3). Importantly, it includes the following key features engineered for maximal biosafety:
- It uses only a fractional set of HIV genes. All of the four accessory genes (vif, vpr, vpu and nef) which are not crucial for viral growth in vitro but are critical for in vivo replication and pathogenesis have been deleted. Importantly, the two regulatory genes essential for viral replication (tat and rev) have also been deleted from the lentiviral transfer vector.
- The remaining of the virus genome, including rev, has been split onto three separate plasmids.
- The vector's self-inactivating design (SIN) deletes the viral transcriptional promoter and enhancer elements thus further minimising the risk for generating a replication-competent virus (4)
- The HIV-derived constructs, any any recombinant between them, are contingent on trans-complementation and upstream regulatory elements and thus are non-functional outside of the producer cells.
- The virus are pseudotyped with the heterologous fusogenic envelope glycoprotein of the vesicular stomatitis virus (VSV-G, also supplied in a separate plasmid) in place of the native HIV-1 native protein promoter and coding sequence truncated. Furthermore, both the start and stop codons in the naturally occurring x-protein sequence have been deleted.

The VSV G envelope expands the host range and tropism of the vector and confers enhanced virion physical stability compared to lentivirus particles; the modes of transmission are likely to be extended beyond the percutaneous routes of parental lentiviruses to include contact and aerosol routes.

This split-genome, conditional packaging system acts as a built-in biosafety device as it prevents the generation of replication-competent recombinants. The system conserves only three of nine genes of HIV-1 and relies on four separate transcriptional units for the production of transducing viral particles. As the vector particle packages only the genetic information contained in the transfer vector, infection is limited to a single round without spreading. The probability of a series of recombination events that would produce a replication-incompetent in mammalian cells and unable to infect plant cells the risk of transmission to animal populations is effectively null.

In the context of this project, the main risks are those of insertional mutagenesis by the primary vector particles and long term expression of transgenes after vector delivery.

(1) A third generation lentivirus vector with a conditional packaging system" (1998) J. Virol. 72, 8463-8471
(2) A stable cell line for the high-titer production of third generation lentiviral vectors". Mol. Ther. (2000)2, 170-6
The Gene sequences used to for overexpression and knock down studies will be derived from the human or mouse genome. Mutations to be introduced will either have been identified in human genetic studies, or will represent previously studied and well established molecular tools to be used to dissect insulin signalling. Genes to be studied will be those that human studies have implicated in human disorders of insulin action, whether severe insulin resistance or insulin supersensitivity. No oncogenic mutations will be studied, and no tumor suppressor genes are included in the list, thus accidental infection of a human host by these viruses is likely to have no greater consequence than infection by an “empty” virus.

Following is a list of the specific genes to be studied:

Insulin/Phosphatidylinositol-3 kinase/AKT/mTOR signalling pathway

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<thead>
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<th>Gene</th>
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<tbody>
<tr>
<td>INSR*</td>
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<tr>
<td>IGF1R*</td>
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<tr>
<td>IRS1</td>
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<td>IRS2</td>
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<td>PIK3CA*</td>
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DNA damage repair genes known to be mutated in syndromes of severe insulin resistance

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<td>BLM*</td>
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<td>REV3L#</td>
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<tr>
<td>NSMCE2#</td>
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<tr>
<td>POLD1*</td>
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Centrosomal proteins known to be mutated in syndromes of severe insulin resistance

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<th>Gene</th>
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<td>PCNT*</td>
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<td>ALMS1*</td>
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Genes involved in adipocyte differentiation

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<td>BSCL2*</td>
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<td>PPARG*</td>
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<td>CEBPA</td>
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Misc genes known to be mutated in some monogenic severe insulin resistance

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<th>Gene</th>
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<td>LMNA*</td>
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Genes in which plausible pathogenic mutations have recently been discovered in patients with extreme disorders of insulin action

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<td>RHOH1#</td>
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We additionally propose to study further genes, yet to be identified, in which likely pathogenic mutations causing severe disorders of insulin action are identified in ongoing genetic studies.

* Genes in which mutations are known to cause monogenic disorders of insulin action
# Genes recently discovered to harbour mutations plausibly linked to monogenic disorders of insulin action, but so far unpublished.

**Evaluation of foreseeable effects**

**Insulin Signalling Genes**
Given the anti-apoptotic action of insulin on cells, and its critical role in the assimilation of nutrients, expression of loss-of-function alleles or RNAi directed against most genes involved in insulin signalling with enfeebled cells and render them more sensitive to apoptosis. Thus the risk associated with viruses encoding such genetic elements is predicted to be less than that of empty lentiviruses.

Some of the syndromes to be studied are accounted for by gain-of-function alleles in insulin signalling molecules, such as the AKT2 p.Glu17Lys variant that we have reported in patients with severe hypoglucaemia and asymmetric mild overgrowth. Although some insulin signalling molecules are shared by other growth factor signalling pathways, and have been found in cancer, critically, we shall only be studying novel variants that we have discovered occurring naturally in patients with severe metabolic disorders but with no or minimal excess cancer risk across a lifetime.

**DNA damage repair and centrosomal proteins**
A subset of the genes to be studied have role in DNA damage repair or centrosomal function. In all cases modelling of human disease results in cells that show reduced growth and increases sensitivity to apoptosis, rendering them at a selective disadvantage to wild type cells.

**Genes involved in adipocyte differentiation**
Adipocytes are terminally differentiated, non-dividing cells. Several genes involved in programming the differentiation process will be studied. In each case, the consequence of human mutations is known, and is limited to severe metabolic disease due to failed adipose development. There is no reason to suppose that introduction of human lipodystrophy-associated mutations, or knockdown of these genes, will confer any selective advantage on infected cells.

**Genes in which plausible pathogenic mutations have recently been discovered in extreme disorders of insulin action.**
Intensive genetic studies of patients with severe metabolic problems due to impaired or enhanced insulin action continue to be studied. A key aim of future experiments will be to assess whether naturally-occurring mutations identified are likely to cause altered insulin action, and use of the pSLIK lentiviral system to express the mutated genes in primary cells and cell lines will be of enormous value. Although not all the genes and mutations to be identified in these studies are yet known, it is critical to emphasise that 1. Mutations to be studied will have first been identified in patients with metabolic disease, giving no reason to suspect that introduction of these mutations will confer any selective advantage to expressing cells. 2. There is no reason to suppose that any gene products releases from living or dying cells have the potential to exert harmful remote effects on other tissues.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**N/A**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All disposable culture/lab ware will be collected in double autoclave plastic bags, sealed with autoclave tape, labelled with the user's name and the tissue culture room number and will be autoclaved before incineration. 100% kill. All culture fluids will be treated with a solution of 1% Virkon for 30 minutes, autoclaved and disposed to drains. All surfaces (bench/cabinet), tube racks, and pipettors will be wiped with a solution of 10% Trigene and 70% ethanol. Spills will be sprinkled with Virkon powder and gelled with Vernagel. All solid waste will be autoclaved in the laboratory building and then incinerated in the Addenbrooke's Hospital incinerators. Autoclave cycles will be adjusted for type and amount of waste and a systematic record of cycles will be kept on site. Methods give effectively 100% kill
The project has been reviewed by the Institute of Metabolic Science biological and genetic modification Safety Committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2</td>
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- **Animal Units**
  - L2
  - L3
  - L4

- **Large Scale Activities**
  - L2
  - L3
  - L4

- **Human Clinical Applications**
  - L2
  - L3
  - L4

### Project Ref 170/14.2

- **Date Ackn’d**: 19/06/2014
- **CU2 Project Title**: Study of neuronal energy-sensing mechanisms and neurocircuitry implicated in the regulation of energy homeostasis
- **Class**: Class 2
- **Culture Vol**: < 1 Litre
- **Culture Vol Class**: Class 2
- **Non-GMM Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N

### Historical

- **Significant Change ID**: 02/03/2022

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*Please enter comments on the GM safety committee on the risk assessment*

*The project has been reviewed by the Institute of Metabolic Science biological and genetic modification Safety Committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.*
The project aims to understand how metabolic signals (nutrients, hormones) influence the central mechanisms implicated in the regulation of energy homeostasis. More specifically, this research will characterize neuronal metabolic-sensing circuits implicated in the regulation of energy and glucose homeostasis.

The central nervous system is emerging as a unique point of neuroanatomical convergence of multiple signals conveying information about the body’s acute and chronic metabolic status (circulating nutrients and hormones, nutrient-activated afferents, incretins etc...). Specialized neurons have the ability to detect and integrate these signals through poorly characterized intracellular signalling pathways, and engage downstream neurocircuits producing commands to autonomic motor circuits coordinating multiple effectors of energy balance to ensure the maintenance of fuel homeostasis. In addition, recent evidence indicate that metabolic signals may also alter adult neurogenesis in discrete brain areas, which may contribute to the mechanisms through which the metabolic environment regulates central pathways implicated in the control of metabolism.

In this work, we will characterize neuronal metabolic-sensing pathways implicated in the regulation of energy and glucose homeostasis, and decipher the neuronal circuits activated downstream from metabolic sensing neurons to regulate metabolic functions. To do so, we are planning to use tools to 1) alter the expression of candidate metabolic-sensing proteins in discrete neuronal populations in adult mice (including potential adult-born neurons) 2) label neuronal circuits upstream or downstream from nutrient-sensing neurons.

To investigate the role of known or candidate metabolic-sensing pathways in adult neurons and adult-born neurons, we will use lentivectors to express relevant transgenes or shRNA in a cre-dependent manner, and bidirectionally alter the cellular activity of these candidates (overexpression or knockdown) in neurochemically discrete neuronal subpopulations. This will enable us to directly test the role of these pathways in metabolic functions in vivo and gain considerable understanding of the metabolic mechanisms underlying neuronal metabolic sensing.

Lentivectors present several advantages compared to alternative gene delivery strategies. They can infect nondividing cells, and DNA is integrated into the host genome, which enables lineage studies. The immune response in target cells is low. Last, lentiviral backbones enable the insertion of large DNA fragments. The metabolic-sensing pathways of interest include the mTORC1 signalling pathway; proteins whose expression may be up- or downregulated include: TSC1, TSC2, mTORC1, raptor, rictor, Rheb1, p70 S6kinase 1. Following cell-specific modification of the activity of these candidate metabolic-sensing proteins, we will characterize the metabolic consequences using measurements that will include food intake, body weight, adiposity, energy expenditure, glucose tolerance and insulin sensitivity.

To map the network of neuronal connections implicated in metabolic sensing, we will use cre-activable polysynaptic retrograde (PRV Bartha strain) and anterograde (HSV-1 H129) viral tracers that express green or red fluorescent markers in infected neurons. These tools are unique in their ability to travel in a specific direction within the neuronal circuits, which makes them reliable tools to perform neuronal mapping studies. We will use these tracers in transgenic mice expressing CRE in relevant neurochemical subpopulations of the brain and expose these neurons to various metabolic inputs. This will enable us to gain considerable understanding of the organization of the brain circuits implicated in metabolic sensing and the regulation of glucose and energy balance.
amplification. Non-pathogenic to humans and animals. Cannot survive or establish in the environment. Classified as hazard Group 1.

The recipient cells for studies with lentivectors will be the following immortalised cell lines in culture:

- Embryonic Mouse Hypothalamic Cell Lines N41 and N46
- Adult Mouse hypothalamus Cell Line (mHypoA-NPY/GFP)
- Adult Rat hypothalamic GnRH neuronal Cell Line (GT1-7)

Non-pathogenic to humans and animals. Cannot survive or establish in the environment. Classified as hazard Group 1.

The following cell lines will be used for production and titering of high titer tracing viruses HSV1 H129 (initially provided by DR Anderson, California Institute of Technology) and PRV Bartha (initially provided by Dr. Ekstrand, Rockefeller University):

- Adult Cercopithecus aethiops kidney Cell Line (Vero)
- Adult Sus scrofa kidney Cell Line (PK15)

Non-pathogenic to humans and animals. Cannot survive or establish in the environment. Classified as hazard group 1.

Packaging, production and titering of high titer lentiviral particles will be done by a third party (System Bioscience). See "Host/vector system" section below for details on vectors.

Transgenic reporter mice expressing the cre recombinase gene in specific neuronal subpopulations. Mice are transgenic and carry the cre recombinase gene. Not harmful to humans or the environment.

Host/vector system

LENTIVECTORS: Lentiviral based on third-generation packaging replication-incompetent HIV-1: pCDH platform for conditional transgene expression and pSIH1 for conditional shRNA expression

3rd generation lentivectors have been designed with a series of biosafety features that make them replication - incompetent in mammalian cells, and they do not infect plant cells, thus they are very safe to use. Lentivectors have the following biosafety features that make the risk for human health or environmental hazard very low:

1. Self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells through the deletion of the viral transcriptional promoter and enhancer elements, which minimises the risk for generating a replication-competent virus.

2. Efficient Tat-independent production of viral RNA, reducing the number of genes from HIV-1 (less than 30% that are used in this system). Importantly, all of the four accessory genes (vif, vpr, vpu, and nef) which are critical for in vivo replication and pathogenesis have been deleted.

3. The number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev).

4. The corresponding proteins (gag, pol, rev) are expressed from different plasmids that lack packaging signals. The packaging plasmids share no significant homology to any of the expression lentivectors, the pVSV-G expression vector, or any other vector, to prevent generation of recombinant replication-competent virus.

5. None of the HIV-1 genes (gag, pol, rev) are present in the packaged viral genome,as they are expressed from separate plasmids lacking packaging signal. Therefore, the lentiviral particles generated are replication-incompetent.

6. Produced pseudoviral particles will carry only a copy of the expression construct.

7. The virus are pseudotyped with the heterologous fusogenic envelope glycoprotein of the vesicular stomatitis virus (VSV-G, also supplied in a separate plasmid) in place of the native HIV-1 native protein. It was found that lentivirus pseudotyped with VSV-G are rapidly inactivated in human hosts by the human serum complement

However, these vectors integrate the host's genome and therefore could result in insertional mutagenesis through a
series of recombination events (very low risk). The VSV G envelope expands the host range and tropism of the vector and confers enhanced virion physical stability compared to lentivirus particles; the modes or transmission are likely to be extended beyond the percutaneous routes of parental lentiviruses to include contact and aerosol routes. These vectors include the WPRE motif, which increases transduction. For all these reasons, these lentivectors belong to GM class 2.

PRV Bartha strain:

IMPORTANT NOTE: A SAPO LICENSE WILL BE APPLIED FOR AND OBTAINED BEFORE THE START OF THE WORK WITH PRV

PRV is an animal pathogen, lethal in non-native hosts and transmitted via direct mucosal contact. The natural host for PRV is swine, but PRV has also been reported to infect a wide range of vertebrates, which include the cattle, sheep, dogs, cats, goats, chickens, raccoons, possums, rodents, rabbits and guinea pigs. Transmission only occurs when these species are kept in close contact with acutely infected swine. PRV does not infect Humans and is no a health threat to Humans.

The PRV strain we will use (Bartha strain) is a mutated strain with reduced virulence and increased survival times. There is no wild-type PRV present at any stage of preparation.

PRV Bartha causes death after 9 days (220 hours) in an inoculated mouse. PRV Bartha strain is highly neurotropic and the infected hosts suffer from neurological abnormalities. However, the severe pruritus and relatively shorter time to death by infection with wild-type strains of PRV, coupled with the barely detectable infection of the central nervous system, suggests that the fatal outcome of virulent infection is more a result of the host immune system response, or peripheral nervous system injury, rather than the result of fatal viral encephalitis.

There are three known independent mutations contributing to the reduced virulence of our PRV vector (Bartha strain):

Point mutations within UL21, a signal sequence mutation in the UL44 (gC) gene and a 3-kb deletion encompassing US8 (gE), US9 and a large portion of US7 (gI) and US2. Deletion of US8, US7 and US2 also make PRV Bartha completely defective for anterograde transneuronal spread and therefore a highly specific tool for retrograde tracing.

In addition, the PRV Bartha that we will use has been made conditional replication-deficient: an artificial exon has been inserted inside the coding sequence of the thymidine kinase gene, and is flanked with loxP and lox2272 sites (expression controlled with the double-floxed inverted open reading frame system). Therefore, the PRV Bartha strain that we are using replicates only in cells expressing Cre or in cells expressing thymidine kinase (like PK15). These attenuated features enabled PRV-Bartha suitable for tracing studies because they penetrate further into neuronal circuits due to increased host survival time. Classified as class 2 GM and group 2 SAPO pathogen.

HSV-1 H129 strain:

HSV-1 is a human virus, common in the general population. The classic presentation of primary HSV-1 is herpes gingivostomatitis, usually mild (10% of cases can be severe). Reactivation of latent infection results in fever blisters or cold sores, usually on the face and lips which crust and heal within a few days. In rare cases, HSV-1 can lead to meningoencephalitis with high mortality rate if left untreated.

In the context of this work, we are using a modified version of the HSV-1 H129 strain, in which Herpes Thymidine Kinase (HTK), required for replication, has been inactivated and its coding sequence has been replaced with a Cre-dependent loxP-STOP-loxP-tdTomato-2A-TK cassette via homologous recombination using a codon-modified form of HTK to prevent recombination within the coding sequence. Therefore the HSV-1 H129 virus we are using replicates only in cells expressing cre or in cells expressing thymidine kinase (such as Vero cells that will be used to grow more virus). Classified as GM class 2.

Origin & function

We will use lentivectors to express recombinant proteins or shRNA under the control of cre recombinase. The wild type or mutant sequences used for overexpression and knockdown studies will be derived from the rat or mouse.
genome and obtained through a non-profit plasmid depository (addgene) or through collaborators. Genes to be studied include, but are not limited to: TSC1, TSC2, mTORC1, p70 S6kinase 1. These proteins are implicated in cell growth, differentiation and are expected to have little effects on adult neurons. In the tracing studies, the genetic material involved will enable the expression of TK (PRV Bartha) or HTK (HSV-1 H129) replication genes upon cre recombination, as well as reporter proteins (HA), red (Sea anemone) or green fluorescent (jelly fish) proteins in infected neurons that will be used as tracers.

Evaluation of foreseeable effects

All the genetic material to be inserted through the use of lentivectors regulate the activity of signalling pathways associated with cellular metabolic sensing, and is non pathogenic and non hazardous. In addition, expression is limited to cre positive cells and will always be restricted to small neuronal subpopulations within discrete brain areas. Animals infected with our lentivectors will not create risks above those presented by the properties of the starting viral lentivectors. In the case of tracing vectors, non pathogenic and non-infectious reporter proteins (Ha, green or red fluorescent markers) will be expressed, as well as replication genes. None of these genetic modifications are expected to be biotoxic, oncogenic, or increase the risk level of the viral vectors. None of the inserted genetic material is harmful or would express gene products that would be secreted or released upon cell death and cause harm to humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All studies will only be performed by supervised, trained and informed personnel with daily access to SOP and risk assessments. All in vivo viral injections will be performed in line with Level 2 Containment requirements. Viruses will only be handled in a microbiological safety cabinet. Brain viral injections will be performed in a Class I or Class II microbiological safety cabinet. Sharps will not be used with the virus at any point during the surgery and viral vectors will be delivered via cannula after preparatory surgery. Tubes containing unused reagent will be discarded at the end of the experiment as below. Specific disinfection and inactivation methods and waste procedures will be performed as described below. 3rd generation lentivectors do not replicate, stay in the group of cells initially infected (in our experiments, subgroup of neurons expressing Cre in a discrete brain area) and are not excreted. HSV1 is not airborne and is not shed (information obtained through personal communication with the Dr Lynn Enquist, world expert on alpha herpes viruses). Therefore, both of these viruses are contained within infected animals and the risk of contamination through the air, the cage, bedding, saliva or faeces/urine is effectively zero (see below). Therefore we will house these animals in ventilated rooms that are not HEPA-filtered and not a negative pressure, and treat bedding and cages as non contaminated. Infected animals will be maintained in individually ventilated cages, in a locked animal facility with restricted access and isolated from other activities. Suitable protective clothing will be worn (including mask, gloves and overshoes) and animals will always be handled in a class I microbiological safety cabinet. Animals will be sacrificed by fixative perfusion that inactivates the viruses and carcasses will be incinerated. In the event that animals are not fixed, carcasses will be autoclaved on site and incinerated. PRV Bartha studies:

In addition to the containment measures described above, the following specific containment measures will be taken for PRV bartha studies, in accordance with Defra regulations. All in vivo work will be performed in the SAPO designated site under a DEFRA licence with DEFRA CL2 containment measures. All in vivo viral injections of lentivectors will be performed in a class 1 Microbiological Safety Cabinet within a Level 2
Containment Facility. Only trained personnel will have access to infected cages and animals. All staff working with this specific virus will be trained, have daily access to all SOPs related to these reagents, and properly supervised, and not have contact with swine and ruminants for at least 48 hours after working with the PRVBartha virus. In addition, all work will be performed with application of the principles of good microbiological practice and GOSH.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All culture fluids will be treated with 1% Virkon for 30 minutes to 1 hour, autoclaved and disposed to drains. Safety cabinet, bench surfaces and tools will be cleaned with a 10% Virosol solution and 70% ethanol. Spills will be sprinkled with Virkon powder and Vernagel to solidify. All in vitro and surgical solid waste will be collected in double autoclave plastic bags or adequate containers, sealed with autoclave tape, labelled with the user's name and the room number and autoclaved on site before incineration. Surgical tools will be sprayed with virosol and soaked in 70% Ethanol, dedicated the 1 type of virus and not used for other purposes. Carcasses of perfused fixed animals will be double bagged and disposed of by incineration. Carcasses of nonperfused animals will be double bagged, autoclaved and incinerated. Because HSV1 and lentivectors are not shed, all solid animal waste (bedding, faeces etc…) will be treated as non contaminated. For animal experiments with PRV Bartha, cage waste (bedding, faeces etc..) will be autoclaved before disposal by incineration. Carcasses will be fixed and incinirated. IVC cages and water bottles will be autoclaved before re-use and dedicated to PRV experiments.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Ref 170/17.1

**Date Ackn’d** 06/04/2017  
**CU2 Project Title** Study of neuronal energy-sensing mechanisms and neurocircuitry implicated in the regulation of energy homeostasis

**Class** Class 2  
**Culture Vol Class 2** < 1 Litre  
**Culture Volume Class 3-4**

**Project notified under transitional arrangements** Y

**Non-GMM Consent Granted**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Withdrawn** N

**Tick if notifying a connected programme of work** N

## Project Additional Information

**Purposes of the contained use**

Glucose homeostasis. Specifically, this work will characterize neuronal metabolic-sensing pathways implicated in the regulation of energy and glucose homeostasis, and decipher the neuronal circuits activated downstream from metabolic sensing neurons to regulate metabolic functions. We will use lentivectors to alter the activity of metabolic sensing pathways in discrete areas of the central nervous system, and neuronal tracing vectors to map the networks implicated in metabolic sensing.

**LENTIVECTORS**

To investigate the role of known or candidate metabolic-sensing pathways, we will use lentivectors to express relevant transgenes, shRNA and sgRNA/Cas9 to bidirectionally alter the cellular activity of these candidates (overexpression or knockdown) in anatomically and/or neurochemically discrete neuronal populations in vivo or in cell cultures. This will enable us to directly test the role of these pathways in metabolic functions in vivo and gain considerable understanding of the metabolic mechanisms underlying neuronal metabolic sensing. Lentivectors present several advantages compared to alternative gene delivery strategies. They can infect non-dividing cells, and DNA is integrated into the host genome, which enables lineage studies. The immune response in target cells is low, and specific strategies are available to increase tropism. Last, lentiviral backbones enable the insertion of large DNA fragments. The metabolic-sensing pathways of interest include the mTORC1 signalling pathway; proteins whose expression may be up- or down regulated include, for example: TSC1, TSC2, mTORC1, p70 S6 kinase 1, Raptor,
Rheb1, rictor. Following cell-specific modification of the activity of these candidate metabolic-sensing proteins, we will characterize the metabolic consequences using measurements that will include food intake, body weight, adiposity, energy expenditure, glucose tolerance and insulin sensitivity. This work has already been notified to the HSE (19-06-2014, ref GM 170). Here we are introducing new host systems (new immortalized cell lines and primary cultures) and new lentiviral expression platforms, and we propose to perform part of the lentiviral packaging in house.

TRACING VECTORS

To map the network of neuronal connections implicated in metabolic sensing, we will use a cre-activable monosynaptic retrograde viral tracer (RVdG) that expresses fluorescent proteins in infected neurons. This tool is unique in its ability to travel in a specific direction within the neuronal circuits and stop after 1 synapse, which makes them reliable tools to perform neuronal mapping studies. We will use this tracer in transgenic mice expressing CRE in relevant neurochemical subpopulations of the brain and expose these neurons to various metabolic inputs. This will enable us to gain considerable understanding of the organization of the brain circuits implicated in metabolic sensing and the regulation of glucose and energy balance.

The tracing system described here relies on the use of 3 vectors:

1- AAV-FLEX-H2B-GFP-2A-oG, expressing green fluorescent protein (GFP) and the modified rabies Glycoprotein oG in a cre-dependant manner (FLEX cassette).

2- AAV-FLEX-EGFP-TVA, expressing enhanced GFP (eGFP) and the cellular receptor for subgroup A avian leukemia viruses (TVA) in a cre-dependant manner.

3- EnvA pseudotyped Glycoprotein (G)-deleted rabies virus (EnvA+RVdG-dsRed), a modified rabies virus. In EnvA+RVdG-dsRed, the rabies glycoprotein G is deleted, which makes this virus unable to propagate from infected cells. In addition, EnvA pseudotyping makes the virus unable to infect cells unless they express the TVA receptor, not expressed in mammals.

These vectors will be used to characterize neurocircuits implicated in metabolic sensing and label monosynaptic inputs going to a neurochemically-defined subgroup of neurons.

In WT animals, all constructs administered alone or in combination will have no biological effect, as cre recombinase is required for the expression of the inserted genetic material in the AAVs, and EnvA+RVdG-dsRed can not infect mammalian cells and is propagation incompetent.

In transgenic animals expressing cre recombinase, the genetic material inserted in each construct is not expected to have any biological action if vectors are administered separately.

Co-administration of AAV-FLEX-H2B-GFP-2A-G and AAV-FLEX-EGFP-TVA in Cre-expressing animals will induce the expression of TVA receptor and a modified rabies glycoprotein, oG, but is not expected to have any biological effect in the absence of rabies virion.

Biological action will occur only in the presence of the 3 viruses: cre-expressing cells expressing TVA will be infected by the RVdG virus, and if they co-express oG (cells will have to be coinfected with the 2 AAVs), RVdG will spread to cells projecting to the first infected cells in the retrograde manner. In the second-order cells, oG will not be present, and therefore RVdG will not spread further.

We will use the combination of the 3 vectors in animals expressing cre recombinase in small neuronal population of the mouse brain (3000 to 10000 cells will express Cre) and therefore, the number of infected cells will be extremely limited.

Plasmids will be propagated using standard laboratory chemically competent E. coli strains (DH5alpha, STBL3) in rich-medium liquid shaking cultures (200 mL) containing appropriate antibiotics. Plasmid extraction and purification will be achieved using commercial DNA purification mini- and maxi-prep kits (Qiagen).

Packaging and production of lentiviral vector particles: The procedures will be done either in-house or by a third party
Using the established 3rd generation system, the in-house packaging will be done by coexpressing the packaging plasmids pMDLg/pRRE, pRSVREV, and pVSV-G together with the transgene vectors in HEK293T cells. Centrifugations will be performed using sealed buckets. Production and titration of AAVS and EnvA-RGdG virus high titer will be done by a third party (Cal Tech Viral Core, USA).

Transgenic reporter mice expressing the cre recombinase gene in discrete neuronal subpopulations will receive nanoinjections into restricted areas of the brain. Mice are transgenic and carry the cre recombinase gene. Not harmful to humans or the environment.

Host/vector system

LENTIVECTORS:
The lentiviral vectors used are 'pseudotyped' with VSV-G envelope protein meaning they are capable of infecting a wide range of quiescent and non-quiescent cells by an extended range of routes of transmission. They will integrate into the genomes of infected cells leading to long term gene expression and possible insertional mutagenesis. In addition, the vectors utilise the WPRE motif which current advice indicates could be oncogenic and requires handling at CL2.

However, these lentivectors are third generation and have the following biosafety features that make the risk for human health hazard very low:
- self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
- efficient Tat-independent production of viral RNA, reducing the number of genes from HIV-1 (less than 30% of that are used in this system).
- The number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev).
- The corresponding proteins are expressed from different plasmids that lack packaging signals. The packaging plasmids share no significant homology to any of the expression lentivectors, the pVSV-G expression vector, or any other vector, to prevent generation of recombinant replication-competent virus.

Health and Safety

Executive
- None of the HIV-1 genes (gag, pol, rev) are present in the packaged viral genome, as they are expressed from separate plasmids lacking packaging signals. Therefore, the lentiviral particles generated are replication-incompetent.
- Produced pseudoviral particles will carry only a copy of the expression construct.

RABIES

Two vectors will be used in this system: AAV and rabies virus. AAVs are not harmful to humans. Wild type rabies virus is a human pathogen. Here we will use the EnvA-RGdG virus, which we will obtain from the CalTech viral core in the USA. They screen routinely their cultures to ensure the absence of WT rabies virus. EnvA-RGdG has been designed to be unable to infect mammalian cells and is propagation deficient. Due to EnvA pseudotyping, infection requires the presence of TVA receptors, which are absent in mammals. Even in the presence of TVA expressing cells, EnvA-RGdG will not propagate as it will not express the rabies G protein, necessary for propagation.

Origin & function

LENTIVIRUS:
Constructs to be inserted through the use of lentivectors are expected to regulate the activity of signalling pathways associated with cellular metabolic sensing upon expression; products are non-toxic, non-oncogenic and require
production in discrete loci to exert effects on metabolism. Expressed gene might disrupt cellular energy sensing and fluxes, and in some cases lead to impairment of cellular activity, neurotransmitter release and in some cases the death of the infected cell.

The Cas9 in the lentiCRISPR vectors upon expression is non-toxic, non-oncogenic and has negligible non-specific nuclease bioactivity unless co-expressed with targeting sgRNA.

**RABIES**

The inserted genetic material will allow, upon cre recombination, the expression of:

1. TVA, the cellular receptor for subgroup A avian leukosis viruses,
2. oG, a modified rabies glycoprotein, and
3. fluorescent proteins.

These constructs will not cause harm to humans as their expression is conditioned to the presence of cre recombinase. In the event of cre leakage, no pathogenicity is expected to arise from the TVA and oG proteins alone.

**Evaluation of foreseeable effects**

**LENTIVECTORS:**

Health and Safety

Executive

None of the genetic modifications under study are expected to release biotoxic products, or increase the risk level (pathogenicity, oncogenic potential) of the starting viral vectors. The risks for human health are not above the ones associated with the starting vectors. No expressed gene products will be secreted or released upon cell death that would cause harm to humans.

**RABIES**

Animals expressing Cre-recombinase and infected with the 3 vectors will express, in discrete cell subpopulations, the TVA receptor and oG and be exposed to RgdG. Therefore rabies infection and spread will occur. However, because we will use cre-reporter mouse models where only small populations of cells express cre, the second-order infected cells will most likely not express G and therefore virus spread will stop.

In the event of cre leakage, TVA and oG may be expressed in a larger cell population, and in the presence of RgdG, rabies will infect a larger cell population and spread to their first-degree synaptic input. However, only small brain nuclei will be exposed to the viruses as we will perform nano-injections in the discrete regions of the brain parenchyma, and therefore spread of the virus will remain contained within this small brain area and their first-order synaptic input.

Potential generation of propagation competent rabies virus could occur in the event of recombination between the viral RNA and oG RNA produced from the oG AAV virus. However, recombination in (-)RNA viruses has not been documented previously, and experts consulted about this question consider it is impossible. Therefore, the likelihood of propagation competent virus being recovered is virtually nonexistent.

However, to maintain control over the RgdG vector, CL2 control measures including restricted access and safe storage will be put in place.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All studies will only be performed by supervised, trained and informed personnel with daily access to SOP and risk assessments.

LENTIVECTORS.

All in vivo viral injections will be performed in line with Level 2 Containment requirements. Viruses will only be handled in a microbiological safety cabinet. Brain viral injections will be performed in a Class I or Class II microbiological safety cabinet.
cabinet. Sharps will not be used with the virus at any point during the surgery and viral vectors will be delivered via cannula after preparatory surgery. Tubes containing unused reagent will be discarded at the end of the experiment as below. Specific disinfection and inactivation methods and waste procedures will be performed as described below. 3rd generation lentivectors do not replicate, stay in the group of cells initially infected (in our experiments, subgroup of neurons expressing Cre in a discrete brain area) and are not excreted. HSV1 is not airborne and is not shed (information obtained through personal communication with the Dr Lynn Enquist, world expert on alpha herpes viruses). Therefore, both of these viruses are contained within infected animals and the risk of contamination through the air, the cage, bedding, saliva or faeces/urine is effectively zero (see below). Therefore we will house these animals in ventilated rooms that are not HEPA-filtered and not a negative pressure, and treat bedding and cages as non contaminated.

Infected animals will be maintained in individually ventilated cages, in a locked animal facility with restricted access and isolated from other activities. Suitable protective clothing will be worn (including mask, gloves and overshoes) and animals will always be handled in a class I microbiological safety cabinet. Animals will be sacrificed by fixative perfusion that inactivates the viruses and carcasses will be incinerated. In the event that animals are not fixed, carcasses will be autoclaved on site and incinerated.

Rabies:

All in vivo viral injections will be performed in line with Level 2 Containment requirements. Sharps will not be used with the virus at any point during the surgery and viral vectors will be delivered via cannula after preparatory surgery. Tubes containing unused reagent will be discarded at the end of the experiment as below. Specific disinfection and inactivation methods and waste procedures will be performed as described below. Vectors and genetic materials used here will remain contained within the infected animals and the risk of contamination through the air, cage, bedding, food/water, urine and faeces is effectively zero. Therefore we will house these animals in ventilated rooms that are not HEPA-filtered and not a negative pressure, and treat bedding and cages as non contaminated.

Infected animals will be maintained in individually ventilated cages, in a locked animal facility with restricted access and isolated from other activities. Suitable protective clothing will be worn (including mask, gloves and overshoes) and animals will always be handled in a class I microbiological safety cabinet. Animals will be sacrificed by fixative perfusion that inactivates the viruses and carcasses will be incinerated. In the event that animals are not fixed, carcasses will be autoclaved on site and incinerated.

In addition, all work will be performed with application of the principles of good microbiological practice and COSHH.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All culture fluids will be treated with 5% Chemgene for 20 minutes, autoclaved and disposed to drains - 100% kill. Safety cabinet, bench surfaces and tools will be cleaned with a 5% Chemgene solution and 70% ethanol. Effectively 100% kill.

All in vitro and surgical solid waste will be collected in double autoclave plastic bags or adequate containers, sealed with autoclave tape, labelled with the user's name and the room number and autoclaved before incineration. 100% kill Surgical tools will be sprayed with Chemgene and soaked in 70% Ethanol, dedicated the 1 type of virus and not used for other purposes. 100% kill Carcasses will be double bagged and disposed of by incineration. 100% kill Even in the event of cre-leakage, RGDG will not be shed as the infected areas connect to mucous tissues via multiple synapses. All solid animal waste (bedding, faeces etc . . . ) will be treated as non contaminated.
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref 170/21.1**

Date Ackn’d 23/04/2021

CU2 Project Title Knock-down of genes involved in obesity and liver disease using short hairpin RNAs (shRNAs) expressed using an adenovirus

Date Project Ceased 23/04/2021

Class

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Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Project Additional Information

#### Purposes of the contained use

The aim of this work is to:

i) optimise a protocol to knock-down the gene encoding Steroid Receptor Coactivator (SRC)-1 in cells using an shRNA delivered by an adenoviral vector. This experiment will test the optimal multiplicity of infection (MOI), and the number of treatments required to maintain gene knock-down for the duration of the experiment proposed in part ii) (11 days).

ii) we propose to use this protocol to knockdown SRC-1 in 3D co-cultures of primary human hepatocytes, stellate cells and Kupffer cells (this work will be conducted at the premises of a Cambridge-based biotechnology company (CN Bio Innovations Ltd)). In the company’s prior experience, these cells are prone to toxicity when lipid-based transfection reagents such as lipofectamine are used. In their experience, the adenoviral vector serotype 5 has been well tolerated in these cells. We therefore propose to use the same vector backbone for our work.

#### Recipient or parental organism

**Human Hepatic Cell Lines: HepG2, LX-2, THP-1 Cells**

We plan to introduce shRNA expression cassettes with adenoviral vectors into these well characterised (ACDP Hazard Group 1) cells lines to downregulate expression of SRC-1.

#### Host/vector system

Recombinant human adenovirus subtype 5, genetically modified by deletions which remove the E1 and E3 expression cassettes, thereby rendering it non-replicative and reducing the risk of harm to human health.

#### Origin & function

Replication defective viral vectors will be purchased from Vector Biolabs (Pennsylvania, USA), and will contain the following genes:

**SRC-1 shRNA**: Small inhibitory RNA sequence used to inhibit the SRC-1 gene. We will test whether knock-down of SRC-1 can induce accelerated fibrosis in a multicellular liver model.

**eGFP**: This is a constitutively fluorescent protein with an excitation / emission spectrum of 488 / 509 nm. Detection of eGFP in cell types will be indicative of successful viral transduction, and will serve as a control.

#### Evaluation of foreseeable effects

Adenoviral transmission is via the aerosol route - all work will be undertaken in class II biological safety cabinets, thus minimising the risk of aerosol-mediated infection. Further, this vector contains a deletion of the E1 gene, rendering it replication defective in humans, plants and animals, and hence of low risk to human health and environmentally safe.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Once an experiment has been completed, all disposable culture and lab ware will be disposed of in double autoclave plastic bags, which should be duly sealed with autoclave tape, labelled with the user's name and tissue culture room number, autoclaved and subsequently incinerated. All liquid waste will be treated with 10% Chemgene before autoclaving and incineration as above. All surfaces, tube racks and pipettors will be wiped with solution of 10% Chemgene and 70% ethanol.
All solid waste will be autoclaved in the laboratory building and then incinerated in the Addenbrooke's Hospital incinerators. Autoclave cycles will be adjusted to type and amount of waste and a systematic record of cycles will be kept on site. Methods give effectively 100% kill. Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project has been reviewed by the Institute of Metabolic Science's biological and genetic modification safety committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

Project Containment

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Project Ref 170/22.1

Date Ackn'd 07/01/2022

CU2 Project Title In vitro study of the cellular and molecular mechanisms underlying metabolic and neurodegenerative disease

Class 2 Culture Vol Class 2 < 1 L

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Withdrawn N Tick if notifying a connected programme of work Y

Date Project Ceased

02/03/2022
Project Additional Information

Purposes of the contained use

There is evidence that neurodegenerative disease and obesity are epidemiologically linked suggesting shared mechanisms, but the cell types and molecular pathways underlying these connections remain poorly understood. Several of the disease-relevant cell types can be produced in vitro from human pluripotent stem cells (hPSC), but the chemical (e.g. directed) differentiation of these cells frequently produces mixed populations that may contain only a small fraction of the intended cell types. The forced expression of candidate genes, often transcription factors but also including RNA binding proteins, microRNAs (miRNA), and other genetic elements may "forward program" hPSCs and other somatic cell types into the cell type(s) of interest with greater speed, efficacy, purity, and maturity, facilitating downstream studies of cellular function in health and disease by functional and molecular characterisation. These benefits are particularly acute when target cell types are rare, which is true of the extreme diversity of cell types present in the brain. Other cell types of relevance to metabolic and neurodegenerative disease modelling include non-neural cell types that are difficult to produce and study in any other way, and that have no suitable counterpart in animal models. Once produced, the gene expression of target cell populations can be manipulated using gene overexpression, RNA-mediated knockdown techniques, or gene editing techniques such as the CRISPR/Cas9 system, zinc fingers (ZFNs) or transcription activator-like effector nucleases (TALENs). The CRISPR/Cas system consists of two main components: the guide RNA (gRNA) and a Cas protein. Most often, this consists of Cas9 protein from Streptococcus Pyogenes. The gRNA consists of a scaffold domain that associates with Cas9 protein, as well as a unique short oligonucleotide sequence that facilitates binding of the Cas9-gRNA complex to a complementary DNA sequence. The unique RNA sequence and constant scaffold domain can be synthetically fused into a single guide RNA (sgRNA) that can be synthesised in vitro, or expressed off of DNA constructs. If a native Cas9 derivative is used and the targeted DNA sequence contains a protospacer adjacent motif (PAM, which is NGG for Streptococcus Pyogenes Cas9), it will induce a DNA double strand break that can lead to gene knockout (CRISPR KO), which can also be achieved with the use of dual Cas9 nickase enzymes. If an enzymatically dead Cas9 (dCas9) derivative is used and fused to either transcriptionally activating or inhibiting domains, then transcriptional up-regulation (CRISPRa) down-regulation (CRISPRi or CRISPRoff), base editing, epigenetic regulation may occur without introducing DNA double-strand breaks. Gene expression can also be manipulated with small RNA molecules that reduce functional gene expression by either targeting mRNA for degradation or reduce translation, including the RNA interference (RNAi) mediators small interfering RNA (siRNA) or short hairpin RNA (shRNA), and miRNA. The CRISPR/Cas system requires both the sgRNA and Cas9 or dCas9 derivative to be expressed to be functional. These can be expressed from a single DNA or viral vector, or the Cas9 complex can be engineered into a cell line and the sgRNA delivered by transient transfection or a viral vector for greater safety and efficacy. Both approaches will be utilised throughout the project depending on experimental needs, and cell lines stably expressing Cas9 or Cas9 derivatives will be utilised wherever possible. The use of viral vectors throughout these experiments provides several advantages, such as the ability to screen large pools of candidates rapidly and the potential to create stable lines in safe harbour loci. Candidate genes targeted for knockout or knockdown studies in disease relevant cell types will be shortlisted based on the current literature and genetic studies suggesting a role for these genes in the onset and progression of diseases such as obesity and neurodegeneration. For example, genes associated with cilia formation and function (e.g. BBSome proteins, IFT88, Kif3a), cell signalling (KSR2, LEPR, NTRK2, PCKS1, PCKS2), metabolic pathways (HMGCR, HACD2), transcription factors (SREBF2, TBX3) and others have been implicated in obesity phenotypes and will be shortlisted for gene misexpression to study their role in disease. In this project, we aim to 1) produce relevant cell types, test for gain-of-function phenotypes, and generate reporter cell lines using integrating and non-integrating viral vectors and 2) use gene manipulation techniques to elucidate the cellular mechanisms underlying metabolic and/or neurodegenerative disease in relevant cell populations.

Recipient or parental organism

Standard disabled laboratory chemically-competent E. coli-derived bacterial strains (DH5alpha, STBL3, or similar) for plasmid propagation. Non-pathogenic to humans and animals (Hazard Group 1). The lentivirus will be produced by co-transfecting the packaging plasmids (third generation: pMDLg/pRRE and pRSV-rev or second generation: psPAX2), the pseudotyping plasmid (pMD2.G) and the lentiviral expression plasmid into a variant of human embryonic kidney 293 cells stably expressing the SV40 large T antigen (HEK 293FT). This is a cell line that has been extensively used for the optimum generation of lentivirus particles and cannot survive outside of culture or colonies nor cause disease in humans or animal hosts and are therefore considered to be especially disabled (Hazard Group 1). The lentivirus packaging and envelope plasmids
offer maximal biosafety features as described by Dull et al and Kappes et al (1, 2, 3). Sendai virus production will be outsourced to licensed companies such as Charles River Laboratories or equivalent. The recipient cells will be the following murine/rodent cells and human cells in culture: Human induced pluripotent stem cells (hiPSCs) and derived cell lines
•Human embryonic stem cells (hESCs) and derived cell lines
•Primary cell lines (including fibroblasts, astrocytes, and blood-derived cells and others)
•Immortalised cell lines (including HEK293T and others)
Rodent:
•Embryonic fibroblasts (MEF)
•Primary cell lines (including fibroblasts and others)
The various human and/or rodent cell lines used in this project will be only handled within a Class 2 microbiological safety cabinet in a Containment Level 2 laboratory (risk assessment under COSHH regulations for adventitious agents). Immunologically non-self cells cannot survive or establish in humans with normal functional immune systems. Especially disabled (Hazard Group 1).
Other cells are well characterised cell lines that have been/will be acquired from commercial or non-profit repositories.

Host/vector system

Lentivirus System.
The pMD2.G envelope plasmid is used for both 2nd- and 3rd-generation packaging systems and is gift from Didier Trono (Addgene plasmid # 12259 ; http://n2t.net/addgene:12259 ; RRID:Addgene_12259). The psPAX2 2nd generation packaging plasmid is gift from Didier Trono (Addgene plasmid # 12260 ; http://n2t.net/addgene:12260 ; RRID:Addgene_12260). The 3rd generation lentiviral packaging plasmids pRSV-rev (Addgene plasmid # 12253 ; http://n2t.net/addgene:12253 ; RRID:Addgene_12253) and pMDLg/pRRE (Addgene plasmid # 12251 ; http://n2t.net/addgene:12251 ; RRID:Addgene_12251) are both gifts from Didier Trono(1). The pLIX_403 lentiviral platform system is a human immunodeficiency virus type 1 (HIV-1)-based lentivirus system compatible with both second- and third-generation packaging systems and is a gift from David Root (Addgene plasmid # 41395 ; http://n2t.net/addgene:41395 ; RRID:Addgene_41395). The all-in-one lentiCRISPR v2 lentiviral platform is compatible with the same packaging systems and is a gift from Feng Zhang (Addgene plasmid # 52961 ; http://n2t.net/addgene:52961 ; RRID:Addgene_52961) (2). The pKLV-U6gRNA lentiviral platform and related derivatives modified to accommodate two gRNAs is compatible with the same packaging systems and is a gift from Kosuke Yusa (Addgene plasmid # 50946 ; http://n2t.net/addgene:50946 ; RRID:Addgene_50946)(3). These platforms have since been used in multiple studies by different academic laboratories in the UK and other countries. Importantly, it includes the following key features engineered for maximal biosafety (4,5):
-It uses only a fractional set of HIV genes. All of the four accessory genes (vif, vpr, vpu, and nef) which are not crucial for viral growth in vitro but are critical for in vivo replication and pathogenesis have been deleted.Importantly, the two regulatory genes essential for viral replication (tat and rev) have also been deleted from the lentiviral transfer vector.
- The-vector's self-inactivating design (SIN) deletes the viral transcriptional promoter and enhancer elements thus further minimising the risk for generating a replication-competent virus and may reduce the risk of tumorigenesis via promoter insertion (6).
- The HIV-derived constructs, and any recombinant between them, are contingent on trans complementation and upstream regulatory elements and thus are non-functional outside of the producer cells.
- The virus particles are pseudotyped with the heterologous fusigenic envelope glycoprotein of the vesicular stomatitis virus (VSV-G, also supplied in a separate plasmid) in place of the native HIV-1 protein. While this extends the tropism and transmission routes of the vector, it was found that lentivirus pseudotyped with VSV-G are rapidly neutralised by the immune responses triggered by the complement system in humans (7).
- The enhancer of transgene expression derived from the woodchuck post-transcriptional regulatory element (WPRE) and located at the 3' untranslated region of lentivectors has been truncated and does not contain an open-reading frame for the WHV X-protein (in addition to containing only a portion of the X coding sequence, both the naturally-occurring start and termination codons have been mutated). This safety feature is not present in the pKLV-U6gRNA vector, as this vector lacks a WPRE and does not produce a poly-adenylated transcript.
Both second and third generation packaging systems will be used in this project. These systems differ principally in their division of the gag, pol, and rev genes. In...
third-generation systems, rev is on one plasmid while gag and pol are on another. While this nearly eliminates the risk of producing a replication-competent virus, it also reduces the titre and efficiency of the packaging process. In second generation systems, all 3 genes are on a single plasmid, allowing them to be expressed at a near-optimal ratio. This greatly increases the titre and efficiency of packaging, allowing for less virus to be used during transduction. Third generation packaging systems will be used in the first instance; however in cases where using the third generation system negatively impacts the nature of the research due to low efficiencies and low virus titres, the second generation packaging system will be used. While the second-generation system could lead to an increased risk of producing a replication-competent virus, this risk remains very small. This split-genome, conditional packaging system acts as a built-in biosafety device as it prevents the generation of replication-competent recombinants (RCR). The system conserves only three of nine genes of HIV-1 and relies on three or four separate transcriptional units (see above) for the production of transducing viral particles. As the vector particle packages only the genetic information contained in the transfer vector, infection is limited to a single round without spreading. The probability of a series of recombination events that would produce a replication-competent virus that can spread between hosts is negligible. Furthermore, given that these lentiviruses are replication-incompetent in mammalian cells and unable to infect plant cells the risk of spreading is effectively null.


The deltaF-TS Sendai virus system.

These recombinant viruses derive from the Sendai virus which belongs to the Paramyxoviridae family. Sendai virus are enveloped viruses with a nonsegmented negative-strand RNA genome which are not capable of integration in the host genome. DeltaF/TS Sendai viral vectors to be used have had the F gene deleted and encompass specific mutations in the HN and M genes. DeltaF/TS Sendai virus is capable of intracytoplasmic self-replication, leading to the formation of non-transmissible viral-like particles (NTVLP), but is incapable of infecting neighboring cells since progeny lack the F protein, which is one of the endogenous envelope proteins necessary for transmission. The F protein is incorporated in trans during the production of the vector viruses. Importantly, Sendai virus vectors are not likely to generate wild-type virus in a packaging cell line, since homologous recombination between RNA genomes has not been observed in nonsegmented, negative-strand RNA viruses. In addition, the temperature sensitive (TS) HN and M proteins strongly inhibit the production of NTVLP in transduced cells at 37°C. Consequently, DeltaF/TS Sendai can be maintained during several passages in transduced cells without being infectious. Notably, the DeltaF/DS Sendai virus tends to disappear from proliferative cells after several passages since its capacity of replication is limited (1); moreover, previously infected cells that no longer contain the DeltaF/TS Sendai viral genome can be purified using antibody-mediated depletion as described in (1) and the absence of Sendai virus can be validated using PCR to detect the Sendai viral genome or transgene expression. Importantly, this system has been used extensively to generate iPSCs free of genetic modifications and free of viruses (1,2).

DeltaF/TS Sendai virus will be used in the context of reprogramming cell fate and gene overexpression studies. Indeed, such vectors have been proved to be a robust and efficient strategy for reprogramming cells to pluripotency in the literature. The DeltaF/TS Sendai virus vectors will encompass all the coding sequences for the selected transgenes or small RNA molecules used for reprogramming.


### Origin & function

The expression of genetic elements from viruses will be used to 1) alter cell state in order to test for phenotypes related to disease, 2) generate stable reporters, and/or 3) alter cell identity. To ensure the safety of these studies, we will not over-express oncogenes or knock out tumour suppressor genes whose loss is known to cause cancer.

1. To better understand the function of candidate genes in metabolic disease or neurodegenerative disease, we will over-express, knock down, or knock out their function. These genes to be tested have been identified in genomewide association studies (GWAS), or genetic sequencing studies, and/or transcriptional studies of relevant celltypes

02/03/2022

Page 4388 of 15326
and tissues.
2. To generate stable cell lines, genetic elements such as fluorescent reporters will be introduced via lentiviruses into the genomes of hSPCs and other mammalian cell types.
3. To program cell identity, we will use genetic elements that have either been used in the literature, or have been identified to be differentially expressed and/or differentially active in those cell types to ensure cell-type specific differentiation.

**Evaluation of foreseeable effects**

1. Inactivation or over-expression of target genes:
   We expect some selected genes to produce phenotypes relevant to disease, ranging from cell death to minor changes in morphology and/or gene expression, to no detectable effect in control experiment. We do not expect to observe uncontrolled cell growth as we will avoid oncogene overexpression. Therefore, the cells undergoing these genetic perturbations will have a selective disadvantage over wild-type cells.

2. Generation of stable cell lines:
   We do not anticipate biological actions beyond expression of the reporter gene that would render the cells dangerous in any way to human health.

3. Programmed cell identities:
   Typically, cells will be programmed into a post-mitotic state (e.g. neurons) that are incapable of further division. In this case, we would expect to see the loss of mitotic activity and acquisition of neuron-like morphological and transcriptional properties. We will also seek to generate other cell types capable of mitosis such as astrocytes and tanycytes, but as stated above we will avoid working with oncogenes and tumour suppressor genes. These cell types may continue to divide, but will also acquire morphological and transcriptional properties relevant to the target cell population. Reprogramming cells to a pluripotent state will only be performed using non-integrating viruses. Reprogramming is not expected to confer any selective advantage to reprogrammed cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All disposable culture/lab ware will be collected in double autoclave plastic bags, sealed with autoclave tape, labelled with the user's name and the tissue culture room number and will be autoclaved before incineration. 100% kill. All culture fluids will be treated with a solution of 5% Chemgene for 30 minutes, autoclaved and disposed to drains. All surfaces (bench/cabinet), tube racks, and pipettors will be wiped with a solution of 5% Chemgene and 70% ethanol. Spills will be cleaned with 5% Chemgene and 70% ethanol. All solid waste will be autoclaved in the Institute and then incinerated in the Addenbrooke's Hospital incinerators. Autoclave cycles will be adjusted for liquid or solid waste and a systemic record of cycles will be kept on site. Methods give efficiently 100% kill.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

The project will be reviewed by the Institute of Metabolic Science biological and genetic modification Safety Committee, and work will not commence until they are satisfied that the risk assessment is accurate and appropriate.
The nuclear hormone receptor PPARg (peroxisome proliferator-activated receptor gamma) is a ligand inducible transcription factor involved in a number of biological processes, including fat cell differentiation, glucose homeostasis and atherogenesis. A number of PPAR-specific agonists, including naturally occurring fatty acids and prostaglandin J2 and synthetic anti-diabetic thiazolidinediones have been identified and shown to be useful tools for investigating the effects of PPARg stimulation in vivo. For example, thiazolidinediones accelerate differentiation of cultured preadipocytes into adipocytes, activate monocytes and increase leptin secretion from cultured trophoblasts. The effects of PPARg inactivation are poorly understood however. Animal studies using PPARg knockout mouse has been hindered by lethal phenotype and no antagonist ligand have been described. Patients harbouring loss-of-function PPARg mutations exhibit varying degrees of lipodystrophy and insulin resistance. In order to study the function of such mutant PPARg, they will be expressed in mammalian cells using a lentiviral expression system. A major advantage of the lentiviral system is
the possibility to infect most cells from different species.

Recipient or parental organism

A 293 producer cell line will be used to generate the virus particles. Initially, we will analyse the effect of the mutants on the differentiation of murine 3T3-L1 preadipocytes in response to PPARg ligands. Subsequent experiments will address the effects on human preadipocyte and other relevant cells.

Host/vector system

A 293 producer cell line will be transfected with the gene of interest constructs and three plasmids each containing different and necessary proteins for virus formation will result in the generation of the viral particles. These three different plasmids used are CMV 8.9 (gag, pol and tat); pRSVrev (rev); pCMV-VSV-G (VSV-G). Only the pHR'CMV-GFP vector containing the gene of interest contains packaging signals and this will result in lentiviral particles which are replication incompetent and only carry the gene of interest. All cloning and lentiviral vectors lack sequences for prokaryotic expression.

Expression of PPARg and GFP are driven by the CMV promoter which is active in most mammalian cell types. The lentiviral particles are replication incompetent and only carry the gene of interest and GFP.

Origin & function

The human nuclear hormone receptor PPARg is a ligand inducible transcription factor involved in a number of biological processes, including fat cell differentiation, glucose homeostasis and atherogenesis which can be analysed in the appropriate cell lines.

Evaluation of foreseeable effects

Neither GFP nor wild type or mutant PPARg are recognised oncogenes, so work with these lentiviral constructs is not expected to pose a cancer risk.

Expression of PPARg and mutants will lead to differences in gene expression in mammalian cells. This will be evaluated by analysing differences in predipocyte differentiation and gene expression patterns.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

not applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated for 24 hrs with 2% Virkon or Presept tablets before disposal into the laboratory drainage system. Alternatively disinfected waste will be gelled with Vernagel in sealed containers and removed for incineration. Solid waste such as plastics will be double bagged before transfer to a waste autoclave within the building, and then disposed of by incineration on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The project has been reviewed by the CIMR Biological and Genetic Modification Safety Committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

**Project Containment**

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<thead>
<tr>
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**Animal Units**

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**Project Ref** 678/09.3

**Date Ackn'd** 28/07/2014

**CU2 Project Title**

Adenoviral and/or Lentiviral over-expression and siRNA knock-down of reporter constructs and genes to investigate stimulus secretion coupling in enteroendocrine cells in tissue culture

**Class** Class 2

**Culture Volume** Class 2 ≤ 1 Litre

**Consent** Non-GMM Consent Granted

**Project notified under transitional arrangements** N

**Historical Significant Changes**

Transferred from GM678 on 28/07/2014

**Project Additional Information**

Purposes of the contained use

Hormones such as glucagon-like peptide 1 (GLP-1), and glucose-dependent insulino tropic polypeptide (GIP) play key roles in postprandial nutrient homeostasis, but little is...
known of the molecular mechanisms underlying and regulating their release from enteroendocrine cells. Enteroendocrine cells lie scattered throughout the gut epithelium and at least 16 different types, classified by the secreted hormones, have been defined, amounting together to about 1% of the gut epithelium. While they are not readily distinguishable from the surrounding enterocytes (nor from each other) by live cell microscopy, we have recently successfully labeled enteroendocrine cells by expressing fluorescent protein markers under the control of the relevant hormone promoter either delivered as a stable transgene in mice or after adenoviral transfection of mixed gut epithelial cells in primary culture.

We now wish to further investigate the stimulus secretion coupling in these cells using:

(a) fluorescent sensor probes allowing live monitoring of intracellular concentrations of a range of different cell components. Initially we will focus on 2nd messengers such as cAMP and nutrients such as glucose, for which probes based on fusion proteins with fluorescent proteins have been described in recent literature. To achieve the necessary high delivery efficiency for the targeting of a minor cell component in our mixed epithelial cultures, we will initially use adenoviral transfection, which we have used successfully before. Should the lentiviral work (see below) prove more efficient, we will swap the vector system.

(b) Knock-down and/or overexpression of candidate genes. Selection of candidate genes will be guided by expression analysis; having labeled hormone specific subpopulations of enteroendocrine cells with fluorescent marker proteins has enabled us to isolate relatively pure populations of these cells by fluorescent assisted cell sorting. So far we have performed Affymetrix gene expression analysis on glucagon gene expressing L- and pancreatic α-cells, GIP expressing K-cells, and corresponding unlabeled cells from the surrounding tissues (including insulin-expressing pancreatic β-cells), allowing us to identify for example K-cell or L-cell enriched genes. To address their role in enteroendocrine cell physiology, we wish to overexpress and knock-down these genes. As many commercially available shRNA are provided in a lentiviral vector system, we will first investigate if this is suitable to target enteroendocrine cells in culture, using fluorescent marker proteins. As lentiviral vectors have been successfully used to target related endocrine cells, such as pancreatic β-cells, there is a high likelihood for this to succeed. We will then target other genes guided by the expression data from the Affymetrix array results.

(c) While the transgenic approach was successful to identify L- and K-cells in mixed epithelial cultures, we wish to investigate if stimulus secretion mechanisms identified in the murine cultures are preserved in human enteroendocrine cells. To be able to identify the enteroendocrine cells in human cultures we want to express fluorescent proteins under the control of the relevant hormone promoters. To achieve the necessary high delivery efficiency for the targeting of a minor cell component in our mixed epithelial cultures, we will initially use adenoviral transfection, which we have used successfully before in the rodent cultures. Should the lentiviral work (see above) prove more efficient, we will swap the vector system. Once we have demonstrated that we can target human enteroendocrine cells, we will also use constructs developed under (a) and (b) in the human culture system.

Recipient or parental organism

Recipients:

a) E.coli: Strains of bacteria used for initial construction and plasmid amplification are derived from E.coli K12 and are therefore disabled and non-colonising.

b) Mammalian cells: Recipients will be various mammalian cells in culture. Most are moderately well characterised (e.g. HEK293 cell, GLUTag cells) and considered fastidious and non harmful, cannot colonise/cause disease, (ACDP hazard group 1). Primary epithelial cultures established from unscreened human intestinal tissue could harbour infectious agents and should therefore be handled at containment level 2.

Host/vector system

(1) Replication disabled adenovirus vector, based on Ad5. This virus is deleted for essential replication functions (E1A region) and can only replicate in cells complementing this function (HEK 293 cell line). Naturally the virus is transmitted by aerosol to respiratory tissues from where it may infect adenoid tissue. There are no reports of other infections such as enteric/kidney. However as the vector is incapable of completing a single round of replication, no progeny virus can be produced and infection would be (self) limited to any initial cells exposed. The number if cells affected would be related to the amount of virus encountered but effects would not progress to any extent approaching natural disease. The vector is considered HG 1.

(2) Replication disabled lentivirus. Although the starting viruses like HIV themselves are hazard group 3 pathogens, vectors considered for his proposal are all second or third generation (distinguished in the UK by deletion of the tat-gene and replacement of the tat-responsive promoter in the 5’LTR by heterologous alternatives). The theoretical potential for re-emergence of replication competent virus through recombination has been significantly reduced in these vectors, by splitting the replicative, packaging and envelope functions and the desired transgene over ≥ three independent plasmids, therefore requiring multiple recombination events to generate replication competent virus. The emergence of replication competent virus is therefore unlikely and such viruses would be expected to have lost the transgene. In addition, many accessory functions/pathogenicity determinants such as vif have been deleted and they are all self-inactivating, carrying the SIN-deletion in the 3’LTR, including re-activation after integration into the host genome and as the U3 promoter is thereby compromised reducing the risk of transactivation of genes near the integration site.
But, as the constructs all carry other (usually constitutively active (eg CMV)) promoters to drive expression of the transgene this can not be excluded, nor can the inactivation of nearby genes due to integration within these genes be excluded. However, as problems arising from the insertion site have only been reported in studies involving administration of very high viral titers, the likelihood of similar problems in this study is considered low, taking into account the comparatively low viral titers likely to be administered accidentally and the defective replication and self-inactivation of the virus. As some of the commercially available vectors contain the WPRE (woodchuck hepatitis B virus post-transcriptional regulatory element) to boost viral titres and gene expression, and as this element can express an oncogene (part of the X-protein), which has resulted in increased tumor frequencies in mice, these viruses should be considered class 2. The lentiviral particles in this project will be 'pseudotyped' with vesicular stomatitis virus envelope protein G (VSVG); this increases host cell range/tropism and such vectors may represent an aerosol means of transmission in addition to the expected percutaneous risk from lentiviruses. Such viruses are more stable and can be purified to high titre. However, they are rendered sensitive to complement.

Origin & function

The expressed proteins range from fluorescent markers, such as GFP, which are unlikely to be harmful, and fusion proteins of fluorescent proteins with activity retaining protein kinase A catalytic subunit (overexpression of which might alter cell physiology, although the probe has successfully been used to investigate cAMP responses in pancreatic beta-cells, without altering their normal Ca²⁺-homeostasis or stimulant-secretion coupling) to other proteins involved in enteroendocrine physiology. Similarly the knock down targets are genes involved in enteroendocrine physiology, including, but nor restricted to, glucokinase, SGLT-1 and Kir6.2. As overexpression or knock-down of such genes is unlikely to transform infected cells and is not expected to be toxic, the risk to human health or the environment from accidental infection is considered low.

Evaluation of foreseeable effects

As constitutively active promotors are used, virtually every transfected cell will be expected to express the inserted cDNA or shRNA. The expressed proteins range from fluorescent proteins with activity retaining protein kinase A catalytic subunit (overexpression of which might alter cell physiology although the probe has successfully been used to investigate cAMP responses in pancreatic beta-cells, without altering their normal Ca²⁺-homeostasis or stimulant-secretion coupling) to other proteins involved in enteroendocrine physiology. Similarly the knock down infected cells is not expected to be toxic in context of the transfected cells nor after release of proteins from lysed cells the risk to the environment due to accidental infection is considered low. Cells will remain especially disabled and unable to survive outside of laboratory culture.

1) Adenovirus: Adenoviruses are generally species specific; Ad5 infects humans and does not naturally infect other animals (but can experimentally infect cotton tail rats, which are not endogenous in the UK). Therefore there is no risk to other organisms. The virus is replication incompetent therefore could not spread to the wider human population from any infected individual.

2) Lentivirus: VSVG enables these vectors to infect a wide variety of animal cell types including those of different mammalian species. There may be enhanced environmental stability. However the viruses are replication disabled; they cannot produce progeny virus and so cannot spread to the wider human population or other animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable tissue culture plastics will be autoclaved and incinerated. Culture medium will be treated 2% Virkon for 16 h and solidified prior to incineration. Liquid spills will be treated with 2% Virkon (non metallic surfaces) or 10% Trigene (metallic surfaces). Absorbed with paper towels, which will then be autoclaved/incinerated. Benches are cleaned with 10% Trigene.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
This project has been reviewed by the Institute's Biological Genetic Safety. The Committee is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.

### Project Containment

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### Project Ref 678/99.2

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Tick if notifying a connected programme of work: N

Tick if project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

### Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Name

UNIVERSITY OF NOTTINGHAM

Name 2

SCHOOL OF BIOMEDICAL SCIENCES

Department

Campus Estate or Research Centre

CLIFTON BOULEVARD

Road Name

NOTTINGHAM

Town

District

NOTTINGHAMSHIRE

County

Postcode

NG7 2UH

Country

ENGLAND

Tel Number

0115 970 9364

Fax Number

0115 970 9969

E-mail

HSE Division

MIDLANDS

Comments

CLOSED AND MERGED WITH GM 470 ON 18/02/2005

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
Mycology

Transgenic Invertebrates

Transgenic Plants

Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 171/01.1

**CU2 Project Title**

TRANSIENT EXPRESSION OF CONSTITUTIVELY-ACTIVE MUTANTS OF RAS-FAMILY GTPASES AND SRC-FAMILY TYROSINE KINASES IN MAMMALIAN CELLS

**Date Ackn’ed** 22/02/2001

**Date Project Ceased** 18/02/2005

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

GM171/01.1 has transferred to GM470 on 18/02/2005

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 171/01.10

Date Ackn’d 22/02/2001

Date Project Ceased 18/02/2005

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

GM171/01.10 has transferred to GM470 on 18/02/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Project Ref  171/01.11

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<td>HIGH LEVEL EXPRESSION OF HETEROTRIMERIC G PROTEIN SUBUNITS. B-ARK MINIGENE AND G-PROTEIN-COUPLED RECEPTORS IN MAMMALIAN CELLS USING THE SINDBIS VIRUS EXPRESSION SYSTEMS</td>
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Project notified under transitional arrangements

Tick if notifying a connected programme of work

Withdrawn

02/03/2022  Page 4403 of 15326
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 171/01.12

Date Ackn'd 22/02/2001

Date Project Ceased 18/02/2005

CU2 Project Title EXPRESSION IN MAMMALIAN CELLS OF CONSTITUTIVELY ACTIVE FORMS OF THE ALPHA SUBUNITS OF HETEROTRIMERIC G PROTEINS

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes GM171/01.12 Transferred to GM470 on 18/02/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Non-GMM** not applicable

**Consent Granted**

Project notified under transitional arrangements [Y]

**Historical Significant Changes**

GM171/01.13 Transferred to GM470 on 18/02/2005

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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**Project Ref** 171/01.14

**Date Ackn'd** 22/02/2001

**CU2 Project Title** HIGH LEVEL EXPRESSION OF TRANSCRIPTION FACTORS IN EUKARYOTIC CELLS USING ADENO-X OR ADEO-X TET-OFF (CLONTECH) ADENOVIRAL-MEDIATED GENE TRANSFER

**Date Project Ceased** 18/02/2005

**Class** 2

**CultureVolClass2** Consented

**Consent Granted** not applicable

**Project notified under transitional arrangements**  

Tick if notifying a connected programme of work

Withdrawn

02/03/2022  
Page 4408 of 1532
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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**Project Ref** 171/01.15

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**Historical Significant Changes**

GM171/01.15 Transferred to GM470 on 18/02/2005

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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# Project Ref 171/01.16

## Date Ackn'd

22/02/2001

## CU2 Project Title

EXPRESSION OF THE SIGNALLING PROTEINS P110 (P13-KINASE) AND MEKK IN MAMMALIAN CELLS

## Date Project Ceased

18/02/2005

## Class

Class 2

## CultureVolClass2

not applicable

## Consent Granted

Project notified under transitional arrangements

## Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
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Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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**Animal Units**

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Date Project Ceased: 18/02/2005

Withdrawn [N] Tick if notifying a connected programme of work [N]
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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If yes, tick to confirm that it is attached to this form N
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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref: 171/01.18

Date Ackn'd: 22/02/2001

CU2 Project Title: EXPRESSION IN MAMMALLIAN CELLS OF CONSTITUTIVELY-ACTIVE FORMS OF: P13K, RAS, RAF AND PKCI

Date Project Ceased: 18/02/2005

Class: 2

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Historical Significant Changes: GM171/01.18 Transferred to GM470 on 18/02/2005
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Project Ref 171/01.19

Date Ackn'd 22/02/2001

Date Project Ceased 18/02/2005

CU2 Project Title CO-EXPRESSION IN MAMMALIAN CELLS OF APP695 AND UBIQUITIN +1

Class 2

Class CultureVol/mL CultureVolumeClass-4

Consent Granted not applicable

Non-GMM

Project notified under transitional arrangements Y

Historical Significant Changes GM171/01.19 Transferred to GM470 on 18/02/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
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Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Withdrawn N | Tick if notifying a connected programme of work N |
Historical Significant Changes
GM171/01.2 has transferred to GM470 on 18/02/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
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**Project Ref** 171/01.20

- **Date Ackn'd**: 22/02/2001
- **CU2 Project Title**: THE EXPRESSION OF GFP REPORTER CONSTRUCTS AND EUKARYOTIC VECTORS CONTAINING WILD TYPETY, P, E AND DOMINANT NEGATIVE FORMS OF P85 (P13-K SUBUNIT), IRS PROTEINS AND FORKHEAD TRAN-FACT IN MAMM.CELL LINES

- **Date Project Ceased**: 18/02/2005

- **Class**: Class 2
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- GM171/01.20 Transferred to GM470 on 18/02/2005

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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**Tick if notifying a connected programme of work**

**Historical Significant Changes**
GM171/01.21 Transferred to GM470 on 18/02/2005

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<th>Human Clinical Applications</th>
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<td>EXPRESSION OF CONSTITUTIVELY-ACTIVE FORMS OF NOS 1-3, AKT, ERBB2-4, ERA/B, PARP, HIF1A IN MAMMALIAN CELLS</td>
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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 171/01.4

**Date Ackn'd**
22/02/2001

**CU2 Project Title**
CLONAL ANALYSIS OF RODENT CELLS IN ORGAN CULTURE EXPRESSING THE DELTA RECEPTOR TRANSFECTED WITH ECOPROTEIC REPLICACTIONS DEFICIENT RETROVIRUSES

**Class**
Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM**
Consent Granted
not applicable

**Project notified under transitional arrangements**
Y

**Withdrawn**
N

**Tick if notifying a connected programme of work**
N

**Historical Significant Changes**
GM171/01.4 has transferred to GM470 on 18/02/2005

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 171/01.5

Date Ackn'd 22/02/2001
Date Project Ceased 18/02/2005

CU2 Project Title
HIGH LEVEL TRANSIENT EXPRESSION OF RECEPTORS AND G-PROTEINS IN HUMAN CELLS USING ADENO-X OR ADENO-X-TET-OFF (CLONTECH) ADENOVIRAL-MEDIATED GENE TRANSFER

Class 2
Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

GM171/01.5 has transferred to GM470 on 18/02/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

Large Scale Activities

Human Clinical Applications

**Project Ref 171/01.6**

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Historical Significant Changes
GM171/01.6 has transferred to GM470 on 18/02/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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If yes, tick to confirm that it is attached to this form
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Project Containment

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Project Ref 171/01.7

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Historical Significant Changes

GM171/01.7 has transferred to GM470 on 18/02/2005

Project Additional Information


Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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## Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
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Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Large Scale Activities

Human Clinical Applications

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Date Ackn'd  
22/02/2001

Date Project Ceased
18/02/2005

Withdrawn  N  
Tick if notifying a connected programme of work  N  

02/03/2022
GM171/01.9 has transferred to GM470 on 18/02/2005

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Description of waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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Please enter comments on the GM safety committee on the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

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- **Animal Units**
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  - L3
  - L4

- **Large Scale Activities**
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  - L3
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- **Human Clinical Applications**
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  - L3
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**Project Ref** 171/03.1

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- Withdrawn N
- Tick if notifying a connected programme of work N

**Historical Significant Changes**

GM171/03.1 has transferred to GM470 on 18/02/2005

**Project Additional Information**
### Purposes of the contained use

This aim is to express cell signalling molecules in hormone-secreting cell lines, to examine their functional roles in hormone secretion in these cells. The tet-on inducible expression allows for control levels of expression, with activated transcription in the presence of tetracycline (Tc) or analogues.

### Recipient or parental organism

The cell lines used for packaging of the replication deficient retroviral vectors and for the expression of signalling-molecule cDNAs have a history of safe use. Alterations in the properties of the cells used in this work as a result of expression of these cDNAs will be negligible if any. The genetically modified cells are very unlikely to pose more of a hazard than the parental cell lines.

### Host/vector system

Genes of interest will be inserted into the expression vector (pRev-TRE, Clontech). Both this and the pRev-TET-on (Clontech) vector are considered as non-mobilisable. Both these vectors will be propagated in a suitable E. coli strain (e.g. DH5alpha, XL-1 Blue), which are recognised as non-colonising and disabled. The pRevTRE vector and the pRevTET-on plasmid will be independently transfected into the packaging cell line (RetropackTM PT67, Clontech), which produces viral proteins from stably integrated genes. Viral lysates will be used to infect cultured mammalian cell (PC-12, alphaTC, RINm5F, INS-1E), which do not have the ability to infect or transfer the inserted DNA to other cells.

### Origin & function

cDNAs for a number of cell signalling protein molecules, including GTP binding proteins, have been cloned. These will be inserted into the pRev-TRE expression vector (Clontech), into a site not specifically situated to facilitate expression in E. coli. It is highly improbable that the proteins will be expressed within the bacterial cells. The E. coli strains used for propagation are not considered pathogenic to humans or animals and they are expected to have a low probability of survival outside of the laboratory culture environment - they always have auxotrophic requirements which are unlikely to be satisfied outside the laboratory.

The pRevTRE vector and the pRevTET-on plasmid will be independently transfected into the packaging cell line PT67, which allows for the safe, efficient production of high titer, infectious replication-incompetent retrovirus.

Phage produced by the pRevTet-on vector will be used to establish stable Tet-On cell lines expressing the "reverse" tet repressor (rTetR). Once established, the cell line (hormone-secreting) will be infected with the pRevTRE phage containing the inserted gene sequence. Transcription of the gene will only begin upon integration into a stable (r)tTA expressing line and in the presence of Tc or the Tc derivative doxycycline (Dox).

### Evaluation of foreseeable effects

The RetroPack PT67 packaging cell line provides the genes necessary for recombinant virus particle formation: gag (core structural proteins), pol (reverse transcriptase, integrase), and env (coat glycoproteins). The recombinant virus produced by this cell line contain the products of these genes but lack the genes themselves, thus these retroviral particles can infect target cells and transmit the gene of interest but cannot replicate (replication-ioncompetence) within these cells. Retroviral production from subsequently infected cell lines is prevented. All retroviral packaging cells from Clontech have been safety-tested for replication incompetence, and a product analysis certificate accompanies the RetroPack PT67 cells.

Retrovirus such as Moloney murine leukaemia virus (MoMuLV) do not naturally infect human cells, however recombinant virus packaged from the MoMuLV-based vector described here maybe capable of infecting human cells and therefore could have deleterious effect if they were to infect a laboratory worker. The viral supernatants produced could, depending on the gene insert, contain potentially hazardous recombinant virus. Users are advised not to create retroviruses capable of expressing known or potential oncogenes.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All transfections and manipulations of cells will be performed in a Class II biological safety cabinet (identifiable to other users), preventing release of aerosols. Protective clothing, double gloves to be worn at all times.

Because of viral nature of the work, no glassware or sharps will be used, only disposable plasticware. All potential infectious waste (solutions and microbiological plates) will be de-contaminated and autoclaved prior to disposal. Due to the labile lipid-derived nature of membranes of cells etc, the use of alcohol, detergents or bleach will be sufficient for decontamination. In the event of spillage, the area will be effectively disinfected.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee considered that the molecules being expressed using the retroviral vectors pose no significant risk to the people carrying out the procedures or to the environment. However because the ACDP guidelines indicate that the minimum level of containment for the use of retroviral vectors is at level 2 this work has to be carried out at this level.

Project Containment

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Project Ref 171/04.1

Date Ackn’d 27/07/2004

CU2 Project Title Using short hairpin RNA (shRNA) genes and shRNA gene libraries to investigate

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2 ≤ 1 Litre
The aim of this work is to knock down expression of genes by introducing synthetic "genes" (random DNA sequences less than 100bp), encoding shRNA, these are processed by the cell to generate silencing RNAs (siRNAs). The siRNA leads to the degradation of the targeted mRNA and hence knockdown expression of that gene.

shRNA libraries will be used to carry out a genetic screens (when coupled with an intracellular fluorescent reporter system) in tissue cultured cell lines. The shRNA is delivered to the cells by transfection with eukaryotic expression vectors or infection with replication defective adenoviral or lentiviral vectors. This work is directed at identifying components of signal transduction pathways.

Recipient or parental organism

The shRNA library will be constructed in E. coli. The E. coli strains used for propagation are not considered pathogenic to humans or animals and they are expected to have a low probability of survival outside of the laboratory culture environment - they always have auxotrophic requirements which are unlikely to be satisfied outside the laboratory. They are considered to be ACDP group 1. shRNA libraries will also be constructed in commercially-available, replication-deficient adenoviral vectors and replication-defective lentiviral vectors.

The cell lines used for packaging of the replication-deficient retroviral vectors and for the expression of signalling-molecule cDNAs have a history of safe use. Alterations in the properties of the cells used in this work as a result of expression of these cDNAs will be negligible, if any. The genetically modified cells are very unlikely to pose more of a hazard than the parental cell lines.

Host/vector system

The shRNA library will be constructed in E. coli (ACDP group 1 strains). Commercially available eukaryotic expression plasmids with optimal antibiotic selection will be used eg pSilencer (Ambion), pSiren (BD Biosciences), pSiEx(Novagen), psiStrike (Promega). These vectors are mobilisation defective.


Plasmid or virus will be used to infect transfect the cell line of interest (eg HeLa, HEK293, nB2A, IN157, MCF-7, PC12, ES cells, and primary cells such as human macrophage and fibroblasts) (ACDP group 2).
The shRNA genes are small oligonucleotides synthesised in vitro. The shRNA genes (small, random DNA sequences) are cloned downstream of a eukaryotic RNA polymerase III promoter consequently they cannot be expressed in E. coli and not affect their pathogenicity. DNA from the shRNA libraries generated will be used to transfect/infect eukaryotic cells to carry out genetic screens. Using a promoter-specific intracellular fluorescent reporter system in eukaryotic cells, changes in the level of expression from that promoter (detected by a change in fluorescence) will indicate that a component of the signal transduction pathway has been affected by the particular siRNA expressed in that cell.

**Evaluation of foreseeable effects**

The recombinant virus particles are capable of infecting human cells and could have a deleterious effect if delivered to a target tissue. However, as the virus is unable to replicate, only the initially infected cells would be affected, making serious effects very unlikely unless the infection was massive. The packaging cell lines to be used are supplied along with the commercially available vectors and contain the genes necessary for recombinant virus particle formation. Recombinant virus produced in the packaging cell lines contain the gene products provided in trans by the packaging cells, but they lack the genes themselves. The viral particles produced by the packaging cells can infect target cells and transmit the gene of interest but cannot replicate within these cells. Virus production from cells infected subsequently is therefore prevented. (The packaging cell lines provided by commercial suppliers are safety tested for replication incompetence, and a product analysis certificate is provided).

Primary human macrophage and fibroblast cells will be used in some experiments. These will be obtained from donors outside the school and grown to only low passage numbers to avoid any risk of transformation and accidental introduction into the original donor.

The Modified E. coli and viral vectors are not hazardous to the environment and in the event of accidental release from containment are unlikely to survive and propagate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All transfections and manipulations of cells will be performed in a Class II biological safety cabinet (identifiable to other users), preventing release of aerosols. Protective clothing, double gloves to be worn at all times. Because of viral nature of the work, no glassware or sharps will be used, only disposable plastic-ware. All potential infectious waste (solutions and microbiological plates) will be either disinfected [using 2% Virkon] or autoclaved, prior to disposal via drains or incineration as appropriate. Due to the labile lipid-derived nature of membranes of cells etc, the use of alcohol, detergents or bleach will be sufficient for decontamination.

In event of spillage, the spill will be absorbed onto paper towel, the area will be effectively disinfected with Trigene (1:100 dilution) or Virkon (1:100 dilution). Towels to autoclaved prior to disposal.

Disinfectants are effective against the organisms in question. Data on the appropriateness and effective degree of kill for these detergents are provided by Brindle Microbiological Consultants (Trigene) and Antek International; a subsidiary of Du Pont International (Virkon).

The autoclave is subject to calibration and vvalidation by a service company twice yearly.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
The genetic modification safety committee considered that the molecules being expressed and the vectors described post no significant risk to the people carrying out the procedures or to the environment. However because the ACDP guidelines indicate that the minimum level of containment for the use of the viral vectors to be used is at level 2, this work has to be carried out at this level. The GMSC has approved the assessment as CL 2 in respect of the work involving lentiviral vectors, though the genetic modification is unlikely to result in the production of a more harmful GMM than the parental strain.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Name

| Name | MOREDUN RESEARCH INSTITUTE |
| Name 2 | ANIMAL DISEASES RESEARCH ASSOCIATION |

Campus Estate or Research Centre

| Campus Estate or Research Centre | INTERNATIONAL RESEARCH CENTRE |

Building

| Building | PENTLANDS SCIENCE PARK |

Road Name

| Road Name | PENTLANDS SCIENCE PARK |

District

| District | BUSH LOAN |

Town

| Town | BUSH LOAN |

County

| County | EAST LOTHIAN |

Postcode

| Postcode | EH26 0PZ |

Country

| Country | SCOTLAND |

Tel Number

| Tel Number | 0131 445 5111 |

Fax Number

| Fax Number | 0131 445 6111 |

Comments

Date at Which Additional Info Submitted

| Date at Which Additional Info Submitted | 02/03/2022 |

HSE Division

| HSE Division | SCOTLAND |
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**
  - [ ]

- **Give brief details of the genetic modification safety committee**

  - **Laboratory**
  - **Animal Unit**
  - **Growth Room**
  - **Glass House**
  - **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**

- **Tick if confidential** [ ]

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

Project Ref 172/00.2

Date Ackn'd 24/04/2000

CU2 Project Title MOLECULAR & CELL BIOLOGY OF APICOMPLEX PARASITES

Class 2

CultureVol Class 2

Culture Volume Class 3-4

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 172/01.1

Date Ackn’d: 21/02/2001

CU2 Project Title: TO GENETICALLY CHARACTERISE THE BACTERIAL PATHOGENS OF RUMINANTS, MAMMHEMIA HAEMOLYTICA AND PASTEURELLA TREHALOSI

Class: Class 2

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Tick if notifying a connected programme of work: N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022  Page 4446 of 15326
Project Ref 172/01.2

Date Ackn'd 21/02/2001

Date Project Ceased

CU2 Project Title CONNECED PROGRAMME FOR THE CLONING AND EXPRESSION OF RUMINANT CYTOKINES

Class Class 2

CultureVolClass2

CultureVolumeClass3-4

Non-GMM

Consent Granted

not applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 172/01.3

Date Ackn’d  21/02/2001
CU2 Project Title  CONNECTED PROGRAM OF WORK TO STUDY THE PATHOGENESIS OF
Class 2
Class CultureVolClass2 CultureVolumeClass3-4
**Ruminant Gamma Herpesvirus**

Date Project Ceased

Withdrawn: N

Tick if notifying a connected programme of work: N

**Project notified under transitional arrangements:** Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N
### Project Additional Information

**Purposes of the contained use**

Investigation of expression of proteins of medical/veterinary importance in plant viruses such as Tobacco Mosaic Virus (TMV) and Potato Virus X (PVX). TMV will replicate in edible host plants such as spinach, thereby allowing assessment of the chimeric virus particles as edible vaccines.

**Recipient or parental organism**

Tobacco Mosaic Virus (TMV) and Potato Virus X (PVX). See risk assessment for specific details.

**Host/vector system**

Laboratory animals, mice and rats. See risk assessment for specific details.

**Origin & function**

In the first instance, polypeptides of 18 and 41 amino acids from the major outer membrane (MOMP) of Chalmydophila abortus expressed as a fusion with the coat protein of the plant viruses will be tested. Epitopes (peptides) from other pathogens of medical or veterinary importance may also be investigated. See risk assessment for details.

**Evaluation of foreseeable effects**

Wild-type TMV and PVX have inherently high replication error rates. Both TMV and PVX form stable rod-shaped particles in infected tissues and have wide experimental host ranges, including many members of the family Solanceae, predominantly from the genus Nicotiana. Both viruses are mechanically transmissible, with transmission by arthropods, nematodes or fungi not having been reported. PVX is neither seed nor pollen transmitted whereas wild-type TMV can occasionally be transmitted in seeds. TMV and PVX are indigenous to the United Kingdom and are not subject to legislative control; both are Class 1 organisms. Both TMV and PVX are plant viruses that are not harmful to humans or animals and are only pathogenic on their natural plant hosts.

It is not thought that the pathogen derived sequence inserted into the coat protein of the virus will increase the pathogenicity of the recombinant virus in comparison to wild-type. The modified viruses have a lower specific infectivity, replicate to lower levels, move cell-to-cell more slowly and are severely impaired or completely disabled in long distance movement.

See risk assessment for specific details.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

TMV is a member of the Tobamovirus genus. These plant viruses can be killed by 2% solution of Virkon within 1-2 hours. PVX belongs to a different genus (Potexvirus) but is of a similar structure such that virkon affects it in the same way. Recombinant virus will be handled in a Class 2 Safety Cabinet, which will be sprayed with 2% virkon solution after use and left 1-2 hours before re-use. All waste plastics and gloves will be put in double bags, the outside surfaces of which will be sprayed with 2% virkon and left for 1-2 hours prior to removal for autoclaving. All plastics used for immunisation of laboratory animals will be autoclaved as above and needles will be disinfected with 2% virkon before being disposed of into "Sharps" box for incineration. All animal waste and bedding will be disposed of in double bags and sprayed with 2% virkon 1-2 hours prior to incineration. Animals will be killed by terminal anaesthesia and carcasses disposed of in double bags and sprayed with virkon prior to incineration, as before. All surfaces will be sprayed with 2% virkon and any instruments used will be disinfected with 2% virkon 1-2 hours prior to washing and re-sterilisation.
This proposal presents a program of work aimed at testing the feasibility of producing antibodies against proteins of medical/veterinary importance by expressing epitopes from said proteins in an edible form. The proposal indicates that the parental viruses are both considered to be class 1 plant pathogens and the committee considered that the modifications made to each were unlikely to significantly increase the risk of harm either to the environment or to human health. Neither virus is considered to be a human or animal pathogen. However a question was raised over the persistence of the virus (parental or recombinant) in the environment and more details were sought about decontamination procedures. The committee also noted that the Scottish Crop Research Institute (SCRI) who are partners in the project and who produce the recombinant virus handle both recombinant viruses at level 2 containment. The committee agreed that the proposal should be forwarded to the HSE as a "level 2" project.

**Project Containment**

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**Project Ref** 172/02.1

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<td>INFECTION OF LAMBS WITH A NON-REPLICATIVE PSEUDOTYPE RETROVIRUS THAT WILL EXPRESS AN ALKALINE PHOSPHATASE REPORTER GENE AND JAAGSIEKTE RETROVIRUS (JSRV) ENVELOPE PROTEIN (ENV)</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td>not applicable</td>
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<tr>
<th>Date Project Ceased</th>
<th>Non-GMM</th>
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</thead>
<tbody>
<tr>
<td>25/09/2006</td>
<td>N</td>
<td>not applicable</td>
</tr>
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</table>

Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**

The purpose is to model particular aspects of the pathogenesis of JSRV in sheep;  
To determine which cells in the lung express the receptor which allows entry mediated by JSRV Env.  
To determine the effect of expression of JSRV Env alone on infected cells in vivo.

**Recipient or parental organism**

The pseudotyped virus consists of a JSRV envelope and murine leukaemia virus (MLV) gag and pol products encapsidating an RNA pseudo-genome, which encodes an alkaline phosphatase (AP) reporter gene and JSRV env.  This chimaeric virus contains all the components to infect a cell with the appropriate receptor, to integrate and express AP and env under the control of their respective promoters.  However, as no gag, pro or pol genes are present in the packaged RNA this virus is non-replicative and can only act in a single round of infection.  Therefore, so spread to new cells beyond those receiving the "first hit" or between animals is possible.  

The control virus, LAPSN, has a JSRV envelope, MLV gag and pol proteins and encodes expression of AP but lacks the JSRV env gene.  

It is predicted that the JSRV Env will be able to mediate infection of many cell types in the sheep.  
Expression of JSRVEnv can have transforming activity in cells in vitro.  It is not known if this will occur in vivo.

**Host/vector system**

Sheep and lambs.

**Origin & function**

MuLV Gag and pol genes are expressed in the cell line 293-GP (Clontech).  The proteins produced package the pseudo-retroviral genome and provide the enzymes to facilitate infection and integration of the genome.  

The envelope gene expressing the envelope of the pseudotyped virus will determine which cell types express a functional receptor for the virus of origin.  It is provided by an expression plasmid which is transfected into 293-GP cells.  Concomitantly the RNA pseudogenome is provided by co-transfection with plasmid pLAPN-JSLE.  This includes MuLV LTRs, Alakaline phosphatase and JSRV env genes and the appropriate packaging signal.  Detection of AP activity can be used to determine which cells have been infected by this pseudotype virus.  Expression of env will determine whether expression of env has transforming properties in vivo in the specific cells targeted by JSRV.

In the first instance JSRV Env of the same sequence as JSRV21 (Genbank Accession: AF105220) will be used as envelope for the pseudotyped virus and will be expressed from the replication incompetent genome.  Depending on the outcome of these experiments, mutations may be made to define regions important for receptor recognition and or transforming activity.  With this system the two functions of Env can be assessed independently.
### Evaluation of foreseeable effects

The GM virus will consist of RNA packaged by MLV virion and JSRV envelope, which will introduce the genome into the host cell. However, the RNA will encode only Env and alkaline phosphatase and therefore will not be able to replicate. Infection will be a "one hit event" and no further spread within the host or between hosts will be possible.

Cell attachment and entry of the GM virus will be mediated by JSRV Env. Any cell expressing the receptor Hyal-2, including human cells, could therefore potentially be infected. There are likely to be other, post-entry, blocks to JSRV infection of human cells, but this has not been studied. JSRV env will be under the control of the JSRV LTR, as it is in wild type JSRV. This will restrict expression to those cells where the LTR is active, namely type 2 pneumocytes and Clara cells.

This replication defective recombinant retrovirus is therefore likely to be less hazardous than JSRV, and certainly not more hazardous. Wild type JSRV is not considered infectious for humans and no evident occupational risk of working with JSRV infected animals has ever been noted.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
<thead>
<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not applicable</td>
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<tr>
<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
</tr>
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<tbody>
<tr>
<td>KSRV-Env pseudotype viruses retroviruses can be inactivated by more than 99.7% by 50% ethanol solution in as little as 10 second (Coil et al., 2001, J. Virol. 75, 8864-7). Retroviruses can be inactivated (&gt; a 4 log reduction in titre) with 0.5% Virkon within 30 minutes (Manufacturer's handbook). Recombinant virus will be handled in a class 2 safety cabinet which will be sprayed with a 2% Virkon solution and left at least 1h before re-use. All waste plastics and gloves will be put in double bags the outside surface of which will be sprayed with Virkon and left for 1-2 hours prior to removal for autoclaving. All plastics used for immunisation of animals will be autoclaved as above. In the phase 2 stage i.e. the first 2 weeks after inoculation, animal waste and bedding will be disposed of in double bags and sprayed with Virkon prior to incineration as before. All surfaces will be sprayed with Virkon and any instruments used will be disinfected with 2% Virkon 1-2 hours prior to washing and re-sterilisation.</td>
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<table>
<thead>
<tr>
<th>Is an emergency plan required according to regulation 20?</th>
</tr>
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<tbody>
<tr>
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<tr>
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<th>Tick if you are claiming exemption from disclosure for section of the risk assessment</th>
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</table>
(b) 01/08 infection with replication-defective JSRV-based vectors expressing the envelope protein.

Although the parental virus is handled in conventional housing (level 1) this proposal aimed to carry out infection with the chimeric JSRV at containment level 2 in order to minimise the risk, however small, to the animal handlers. Once infected it was proposed to remove animals to containment level 1 after a period of two weeks. Concerns were raised by the committee as to whether or not the chimeric virus although non-replicative could persist in the lungs of infected lambs for any length of time. This would impinge on the proposal to remove the lambs from containment 2 weeks after infection. CC pointed out that the chimeric virus would not be expected to pose any greater risk, and probably a much lesser risk, to human or animal health than the parental JSRV. Nevertheless it was suggested that if possible a pilot experiment should be performed first in order to assess whether or not there was any persistence of the virus. The Committee were happy to allow the proposal to proceed as requiring level 2/level 1 containment, but it was pointed out that clear justification for removal of the lambs to conventional housing would be required.

**Project Containment**

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
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<tr>
<td></td>
<td>L3 L4 L2</td>
<td>L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
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<tr>
<td>Date Ackn'd</td>
<td>21/01/2002</td>
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<tr>
<td>CU2 Project Title</td>
<td>NOVEL REAGENTS FOR THE CONTROL OF HAEMORRHAGIC SEPTICAEMIA</td>
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<td>Class</td>
<td>CultureVolClass2</td>
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<tr>
<td>Class 2</td>
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<td>Consent Granted</td>
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<tr>
<td>Project notified under transitional arrangements</td>
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Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

02/03/2022
### Project Additional Information

#### Purposes of the contained use
- To improve on existing control products

#### Recipient or parental organism
- Pasteurella multocida

#### Host/vector system
- Deletion mutant of Pasteurella multocida

#### Origin & function
- Origin is from naturally occurring outbreak of haemorrhagic septicaemia in Sri Lanka with intention of producing an attenuated strain

#### Evaluation of foreseeable effects
- The strain will be highly attenuated and not expected to have any deleterious effects

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| The containment level to which the animal facility will operate is CL2. All animal rooms to be used will be at negative pressure and conform in all respects to the requirements stated in Table 1c, Schedule 8 for containment at level 2. |

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- N/A

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| All waste generated during these studies will be rendered non-infectious by chemical disinfection, autoclaving or formaldehyde gas sterilisation and disposed of by on-site incineration or via a licenced clinical waste contractor according to the procedures documented and held at the Institute. Chemical disinfection methods will be monitored to ensure that the correct solution strengths are used. Formaldehyde gas generating chemicals and equipment will be calibrated to ensure that the correct concentration of gas is liberated and decontamination chambers and gas cycles will be timed to ensure that surfaces are exposed for the correct period of time. Internal temperature and pressure readings will be taken during sterilisation cycles from all autoclave equipment that alarm in the event of a cycle failure. All non-plastic containing waste will be disposed of by on-site incineration, all plastic waste will be autoclaved on-site prior to disposal via a licenced clinical waste contractor. All waste including infected carcasses, animal excrement and bedding generated during these studies will be placed in plastic bags that, after surface sterilisation with Trigene 1% v/v (Medichem Intl Ltd) in the unit, will be transferred either to the autoclave or to the adjacent incinerator house for burning, as appropriate. All wasted disposal procedures will be recorded using logbooks. Drains in the floor of the animal rooms will be blocked off, liquid waste will be absorbed by sawdust bedding and removed as necessary for incineration. At the end of the experiment after the removal of all solid waste the rooms will be fumigated using formaldehyde prior to washing with high pressure hoses. |

#### Is an emergency plan required according to regulation 20? (N)
- N

#### If yes, tick to confirm that it is attached to this form
- N

#### Tick to confirm that you have attached a risk assessment to this form
- Y
This proposal was deemed to be fairly straightforward. Clarification was sought on the curing of all "non-native" plasmid sequences from the attenuated strain after which approval was given for the proposal to be forwarded to the HSE as requiring Level 2 containment.

**Project Containment**

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<td>Human Clinical Applications</td>
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**Project Ref** 172/02.3

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<td>PROGRAM OF WORK TO IDENTIFY AND CHARACTERISE VIRULENCE DETERMINANTS OF THE OVINE PATHOGEN CORYNEBACTERIUM PSEUDOTUBERCULOSIS</td>
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<td>1-50 litres</td>
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**Historical Significant Changes**

- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**
Purposes of the contained use

Corynebacterium pseudotuberculosis is the causative agent of caseous lymphadenitis (CLA) in sheep and goats, resulting in significant economic losses to sheep and goat industries worldwide. The pathogenesis of C. pseudotuberculosis disease is poorly understood; however, as with all bacterial pathogens, certain proteins produced during infection allow bacteria to evade/combat host immune responses, and persist within the infected host. Proposed is a connected program of work, which will attempt to identify novel C. pseudotuberculosis proteins, and determine their role (if any) as potential virulence factors. A description of the proposed work follows:

Genetic cloning of DNA sequences from C. pseudotuberculosis derived from various sources, including:

i. Polymerase Chain Reactions (PCR)
ii. Libraries of random restriction fragments
iii. DNA flanking the sites of targeted or randomly integrated plasmids

Once cloned, novel DNA sequences will be determined. As novel sequence data becomes available, specific genes (or gene fragments) will be cloned into bacterial expression vectors, for the purpose of obtaining recombinant protein. This will facilitate the study of the proteins’ physical/biochemical characteristics, as well as providing pure antigen in sufficient amounts for ELISA’s, immunisation of rabbits for polyclonal antibody production, and to allow immunisation of sheep prior to subsequent bacterial challenge in vaccine development studies.

A procedure for transforming C. pseudotuberculosis with plasmid DNA will be developed. Temperature-sensitive suicide plasmids, encoding antibiotic resistance markers, will be used to transform cells by electroporation. Transformants will be identified by virtue of their antibiotic resistance at 30°C.

Allele-replacement mutagenesis of C. pseudotuberculosis genes will be performed. Mutant derivatives of putative virulence genes will be constructed in plasmid suicide vectors. Genetic mutations may take the form of deletions, point mutations, or disruptions using antibiotic resistance markers. A non-mobilisable random-insertion suicide plasmid will also be used to create random-insertion mutants of C. pseudotuberculosis. Ultimately, the intention is to create well-defined mutant strains, deficient in particular gene(s) of interest. Subsequent comparison of the mutants with their wild type parent strains will allow the role of the mutant gene(s) in virulence to be studied, both in vitro and in vivo.

Identification of secreted C. pseudotuberculosis proteins, using plasmid-encoded nuclease gene. Random restriction fragments will be cloned as fusions to the nuclease gene. Fragments containing secretional signal sequences will be used to guide the export of the nuclease out of the cell, allowing subsequent detection on selective agar plates. Sequences containing signal sequences will be sequenced.

Identification of inducible promoters of C. pseudotuberculosis. Random restriction fragments will be cloned into plasmids containing promoterless antibiotic resistance genes. Recombinant plasmids containing promoter sequences will be isolated by virtue of conferring antibiotic resistance to the host cell. Replica plating of resistant clones on different media (e.g. high/low pH) will allow identification of promoters induced by specific environmental conditions.

Experimental infection of sheep and/or mice with the C. pseudotuberculosis allele-replacement mutants and their wild type parent strains will be performed.

Recipient or parental organism

Plasmid-containing GMO’s will contain plasmid-encoded antibiotic resistance markers. The majority of cloned sequences will not be expressed in the host strain (E. coli or C. pseudotuberculosis), due absence of complete coding sequences or promoters. Random pGh9:ISS1 insertion mutants will contain chromosomally integrated plasmid (and associated antibiotic resistance). Sequences cloned for protein expression will be propagated in E. coli host strains, where expression is dependent on defined chemical media. It is not anticipated that cloning of any C. pseudotuberculosis sequences in E. coli will result in an increase in virulence (given that all strains are non-pathogenic and auxotrophic). Furthermore, GM C. pseudotuberculosis will likely be less virulent, or of equal virulence, to the naturally occurring, wild type parent strain.

Host/vector system

Host strains for cloning/expression purposes will primarily be non-pathogenic, auxotrophic laboratory E.coli. In some instances proteins expressed from cloned sequences may only be active in the original host, in which case C. pseudotuberculosis will be used as a host strain.

General DNA-cloning/expression vectors (plasmids) will include standard, commercially available, laboratory plasmids, including pPCR-Script and pET-15b, which contain ampicillin resistance determinants. Such plasmids will only be propagated in an E. coli host strain; where expression is dependent on defined chemical media. It is not anticipated that cloning of any C. pseudotuberculosis sequences in E. coli will result in an increase in virulence (given that all strains are non-pathogenic and auxotrophic). Furthermore, GM C. pseudotuberculosis will likely be less virulent, or of equal virulence, to the naturally occurring, wild type parent strain.

To allow mutagenesis of wild type corynebacterial genes, the plasmids pG+host 9 and pGh9:ISS1 will be used. Both plasmids derive from a naturally occurring Lactococcus lactis plasmid, and are unable to replicate at 37°C (in the host cytoplasm). Both can replicate in E.coli and a range of Gram-positive bacteria, and contain the...
Plasmid-containing GMO's will express plasmid-encoded antibiotic resistance markers (ampicillin in E. coli; erythromycin, spectinomycin, and kanamycin in C. pseudotuberculosis). All genes cloned in this work will derive from naturally occurring C. pseudotuberculosis strains, and as such pose no threat in terms of environmental contamination. Genetic material will mainly derive from naturally occurring C. pseudotuberculosis strains. It is the intention to identify virulence factors (genes involved in the ability of the organism to cause disease), and eventually construct defined mutant C. pseudotuberculosis strains, to derive a greater understanding of the mechanisms by which bacterium causes infection. The majority of the work will be performed in vitro, under class 2 containment conditions (such as gene cloning, protein expression, and use of the pFUN, and pMM223 and pMM225 plasmids, construction of mutant strains, and initial characterisation of mutants v's wild type parent strains). In addition to using the pFUN, and pMM plasmids to identify potentially interesting genes, the pGh9:ISS1 plasmid will be used to create random-insertion mutant libraries of C. pseudotuberculosis. This involves integration of the plasmid into the bacterial chromosome, which, in turn results in disruption of gene sequences at the site of insertion. These mutant libraries will be erythromycin resistant at 37°C, due to the chromosomally located resistance gene. Mutants, lacking phenotypically observable traits (such as phospholipase D, secreted nuclease(s), protease(s), etc.) will be identified, and further characterised in vitro. Chromosomal sequences flanking the sites of pGh9:ISS1 insertion will be cloned, sequenced, and used to construct defined mutations. Mutant derivatives of these genes will be created in pG+ host 9, and used to construct stable, mutant strains through a two-step procedure; primarily, the plasmid will be integrated into the C. pseudotuberculosis chromosome homologous recombination. This will result in an intermediate mutant strain, containing a chromosomal copy of the plasmid-borne erythromycin resistance gene (resulting in resistance at 37°C). By selection of a secondary recombination event, stable mutant strains will be obtained as a result of the plasmid exercising from the chromosome, leaving the mutant genes resident in place of the wild type. Such mutants will have lost the erythromycin resistance determinant during excision of the plasmid, and hence pose no threat to the environment. Sequences cloned for protein expression will be propagated in auxotrophic, non-pathogenic E. coli strains. Expressed proteins will be purified to provide antigen for ELISA’s, vaccine formulations, etc.

Evaluation of foreseeable effects

All genes cloned in this work will derive from naturally occurring C. pseudotuberculosis strains, and as such pose no threat in terms of environmental contamination. Genetic material will mainly derive from naturally occurring C. pseudotuberculosis strains. It is the intention to identify virulence factors (genes involved in the ability of the organism to cause disease), and eventually construct defined mutant C. pseudotuberculosis strains, to derive a greater understanding of the mechanisms by which bacterium causes infection. The majority of the work will be performed in vitro, under class 2 containment conditions (such as gene cloning, protein expression, and use of the pFUN, and pMM223 and pMM225 plasmids, construction of mutant strains, and initial characterisation of mutants v's wild type parent strains). In addition to using the pFUN, and pMM plasmids to identify potentially interesting genes, the pGh9:ISS1 plasmid will be used to create random-insertion mutant libraries of C. pseudotuberculosis. This involves integration of the plasmid into the bacterial chromosome, which, in turn results in disruption of gene sequences at the site of insertion. These mutant libraries will be erythromycin resistant at 37°C, due to the chromosomally located resistance gene. Mutants, lacking phenotypically observable traits (such as phospholipase D, secreted nuclease(s), protease(s), etc.) will be identified, and further characterised in vitro. Chromosomal sequences flanking the sites of pGh9:ISS1 insertion will be cloned, sequenced, and used to construct defined mutations. Mutant derivatives of these genes will be created in pG+ host 9, and used to construct stable, mutant strains through a two-step procedure; primarily, the plasmid will be integrated into the C. pseudotuberculosis chromosome homologous recombination. This will result in an intermediate mutant strain, containing a chromosomal copy of the plasmid-borne erythromycin resistance gene (resulting in resistance at 37°C). By selection of a secondary recombination event, stable mutant strains will be obtained as a result of the plasmid exercising from the chromosome, leaving the mutant genes resident in place of the wild type. Such mutants will have lost the erythromycin resistance determinant during excision of the plasmid, and hence pose no threat to the environment. Sequences cloned for protein expression will be propagated in auxotrophic, non-pathogenic E. coli strains. Expressed proteins will be purified to provide antigen for ELISA’s, vaccine formulations, etc.

Origin & function

Pseudomonas aeruginosa will be used as host strains for pFUN constructs. The pMM223 and pMM225 plasmids will be used to identify environmentally inducible promoters of C. pseudotuberculosis. Gene libraries will be constructed, by cloning small fragments directly upstream of a promoterless antibiotic resistance gene (either spectinomycin or kanamycin depending on which vector is used). Subsequently, clones identified as resistant to antibiotic selection under the imposed environmental conditions (e.g. temperature, pH, osmolarity, etc.) will contain inducible promoter sequences, and can be sequenced to allow further characterisation. Both E. coli and C. pseudotuberculosis will be used as host strains for pMM-based recombinant plasmids.

All of the above plasmids proposed for use in this work are non-mobilisable.
proposed containment restrictions, the risk of environmental hazard is considered to be low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All microbiologically contaminated waste generated in this study will be autoclaved prior to disposal (121°C for 15 min), which should result in complete sterilisation. Internal temperature and pressure readings will be taken during sterilisation cycles from all autoclave equipment that alarm in the event of cycle failure. Any animal bedding or carcasses and other non-plastic waste from the clinical studies will be disposed of by on-site incineration, all plastic waste will be autoclaved on-site prior to disposal via a licenced clinical waste contractor.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The committee were satisfied that the risk assessment was appropriate in the most part. However in the parts of the program where unknowns were being expressed it was suggested that unless there was specific evidence to suggest that the expressed product was unlikely to be harmful to humans or the environment then it should be handled at level 2 containment. Specifically in Part 4b if the protein is being expressed is unknown or if it is unlikely to be a virulence determinant then it should be handled at class 2. Also in parts 4e and f there is a chance, albeit small, that a "harmful" protein could be expressed during the initial screening stage and so the committee felt that the screening should be performed at level 2 containment and any candidate clones kept there until it is shown eg. by sequencing, that there are no harmful sequences downstream of the promoter regions or attached to the signal sequences.

**Project Containment**

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TO DETERMINE WHETHER THE MOTIFS/REGIONS OF THE ENVELOPE PROTEIN (ENV) OF JAAGSIEKTE SHEEP RETROVIRUS (JSRV) SHOWN TO BE NECESSARY FOR IN VITRO TRANSFORMATION ARE ALSO IMPORTANT IN VIVO.

Production of virus will take place at the University of California, Irvine, and at University of Athens, Georgia. It will be transported as 1ml aliquots of concentrated tissue culture supernatant in sealed plastic vials in approved packaging for containment of biological hazardous materials. A license has been granted for importation of this material. The work to be carried out at the Moredun Research Institute is the experimental infection of neo-natal lambs with the recombinant JSRV described below.

Expression of JSRV Env has been shown to transform a number of different cell lines. The YXXM motif (amino acids 590-594) in the intracytoplasmic domain of the JSRV Envelope protein has been implicated in transformation by this protein. Substitution of the Y with a D abrogates the transforming activity of this virus in vitro whilst receptor usage and growth kinetics remain similar to wild type JSRV. The purpose is to determine whether this substitution allows in vivo replication without transformation. The result has implications for the use of JSRV Env in vaccination and in retroviral constructs to deliver human gene therapy.

Wild type JSRV (JSRV21) causes lung tumours in infected sheep. JSRV Env (envelope protein) has been shown to have transforming activity in vitro in several cell lines when overexpressed in the context of a Maloney murine leukemia virus vector (Rai et al., 2001, Maeda et al., 2001). This transforming activity was mapped to a Y-X-X-M motif in the intracytoplasmic tail of the Env TM subunit (Palmarini et al., 2001).

A derivative of JSRV21 has been generated called JSRV21 TM-Y590D. In this virus the amino acid tyrosine at 590 of the envelope protein has been changed to aspartic acid by a single nucleotide substitution. It is likely from the in vitro data showing that this Y to D substitution abrogated transformation by Env, JSRV21 TM-Y590D will have reduced or absent transforming activity in vivo compared to JSRV21.
Sheep

**Origin & function**

JSRV virions can be produced in vitro by transient transfection of 293T cells with the plasmid pCMV2JS21 (Palmarini et al., 1999, J.Virol, 73, 6964-6972). These virions have been shown to reproduce ovine pulmonary adenocarcinoma (OPA). In experimentally infected lambs. The plasmid pJSRV21 TM has a single base change which encodes aspartic acid instead of tyrosine at amino acid 590 of Env. Virions will be produced by transient transfection exactly as is done for JSRV21. The only difference between JSRV21 TM and JSRV21 (wild type virus) will be the Y590D substitution. The virus will be produced in the US and shipped to the UK in an appropriate form.

**Evaluation of foreseeable effects**

If the hypothesis is correct infection with JSRV21 TM should produce no symptoms in infected animals, if incorrect then a disease similar to that induced by wild type JSRV is likely. (Note that the mutations are in the intracytoplasmic region of env TM and therefore will not affect the receptor usage/host cell specificity.) This recombinant retrovirus is therefore likely to be less hazardous than JSRV. JSRV is not considered infectious for humans and no occupational risk of working with JSRV infected animals has ever been noted.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We request derogation from full cat2 facilities for the animal experiments; The animals will be housed under category 2 containment conditions except that an autoclave is not available in the same building. We request derogation such that material leaving the containment facility may be bagged, sprayed with 70% ethanol and transferred directly to the incinerator or autoclave as appropriate. We also request derogation from incineration of waste bedding. This is because we believe that the virus, being a retrovirus, is inherently labile and would not be expected to survive for an extended period in the environment. This together with the fact that the virus is not known to be shed either in urine or in faeces would suggest that there is an extremely low likelihood of the straw bedding being significantly contaminated with viable GMOs. Instead we request permission to transfer the straw bedding to a compost heap on a monthly basis. The bedding would then be composted for a minimum of six months. Please note that all other waste generated during the experiment would be inactivated by autoclaving or incineration as appropriate. JSRV21 is a pathogen of sheep, being capable of causing OPA. Experimental data has shown that this in vitro produced virus demonstrates the same phenotype as virus harvested from lung fluid naturally OPA-affected sheep, but is much less efficient at reproducing OPA. In OPA-affected sheep the main risk of transmission comes from those that produce lung fluid as it is well described that this fluid contains large amounts of infectious virus. This fluid is produced when there is significant amount of tumour in these animals. It is very rarely produced in animals experimentally infected with JSRV derived from lung fluid and has not been seen in any of the animals infected with tissue culture derived JSRV. Indeed the animals which have been infected with in vitro derived JSRV mostly had minimal tumour, not visible by gross pathology but detectable histopathologically. Therefore, when OPA is reproduced experimentally but without allowing late stage disease and production of fluid to occur, the risk of transmission of the virus is significantly reduced.

It is assumed that JSRV is transmitted by aerosol. However it is thought that close contact between animals is necessary for transmission to occur. It has been our experience from about 20 years working with the disease that sheep housed in the next stable (ie the other side of a solid wall) do not succumb to OPA. We regularly have field cases of OPA being brought to the institute as a source of lung fluid and tissues for our research. These are housed in similar buildings to the one we propose to use here. Despite the fact that the clinical cases are producing virus, the veterinarians regard as low, the risk of transfer to the other sheep here including those involved in long term experiments.

For the above reasons, we believe that negative air pressure with respect to the outside environment and filtration of air is not necessary for containment of this GMM.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

02/03/2022
Recombinant virus will be handled in a class 2 safety cabinet which will be sprayed with a 2% Virkon solution and left at least 1h before re-use. [Retroviruses can be inactivated (> a 4 log reduction in titre) with 0.5% Virkon within 30 minutes (Manufacturer's handbook)]. All waste plastics and gloves will be put in double bags the outside surface of which will be sprayed with Virkon and left for 1-2 hours prior to removal for autoclaving. All plastics used for immunisation of animals will be autoclaved as above. [JSRV-Env pseudotype viruses retroviruses can be inactivated by more than 99.7% by 50% ethanol solution in as little as 10 seconds (Coil et al., 2001, J. Virol. 75, 8864-7)].

After post-mortem, carcasses will be incinerated. Tissue and blood samples will be handled under cat 2 containment for DNA extraction. Sample remaining at the end of the experiment will be destroyed by incineration.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
The GM safety committee discussed the proposal to infect sheep with the mutated form of the retrovirus JSRV. It was noted that JSRV is not known to be a human pathogen. Many years of experience have shown that there is no known risk to humans of occupational exposure to this virus. There was general agreement that there was good in vitro evidence that the recombinant virus was likely to be potentially less harmful to the environment than the parental or wild type virus, but that since it was a pathogen of sheep then it would need to be handled at containment level 2.

There then followed a debate as to whether or not the containment level 2 room would be required to be kept at negative air pressure with respect to the outside environment or whether or not the air leaving the containment room would have to be HEPA filtered. It was agreed that the virus was likely to be transmitted by aerosol, but that fairly close contact between sheep was required for transmission of the virus. Clinicians and the biological safety officer were content that sheep infected naturally with the wild type virus (and therefore the risk posed by these sheep to other experimental animals we keep on site) could be successfully isolated by housing them in a designated sheep pen known as the "red range". These sheep pens have concrete floors and walls with access doors at two sides, one for experimental workers coming from an anti-room, the other to allow access to the pen for preparing it for animals and for removal of waste and carcasses at the end of the experimental period. On one side of the room the upper part of the wall has "Yorkshire boarding" which essentially has a one inch gap between six inch boards. The opposite wall has louvered panels and windows. Thus the room has a constant air flow through it to comply with Home office regulations. The nearest animals to be experimental group would either be on the other side of solid concrete wall separating the pen from the adjoining one or in a similar pen on the other side of a courtyard. Previous experience has suggested that unaffected animals in adjoining pens or across the courtyard have not succumbed to the disease. It was also pointed out that it was expected that the greatest risk of transmission is likely to be when affected animals have tumour development in the lungs and therefore are producing large quantities of lung fluid which contains the greatest concentration of virus particles. It was noted that animals in the proposed experiment would be monitored for respiratory distress and were likely to be euthanased before there was significant tumour development and therefore fluid production. Experience in the past with experimental infections with wild type virus have generally been halted before tumour could be detected macroscopically and only one experimental animal has ever produced significant quantities of lung fluid. It was also noted that retroviruses are generally labile in the environment and are not suspected to survive for long periods of time outside the animal. Thus separation of animals by relatively small distances was considered generally efficient to separate unaffected animals from the risk. The final consideration taken into account was that it was known that disease caused by JSRV is endemic in the local environment. Taking all the evidence together the committee felt that the risk assessment was such that it did not indicate that the room would be required to be kept at negative pressure or that the extract air required to be HEPA filtered given the balance of probabilities that the virus being used was likely to be less harmful than the wild type virus. However it was pointed out that should the red range pens be used derogation from the HSE would have to be sought for the requirement to have an autoclave in the building housing the pens.

A request for a further derogation was also considered. It was pointed out that due to the labile nature of the virus and the assertion that the virus is not secreted either in the faeces or urine the committee were asked to consider whether or not the straw bedding on the floor of the pens would have to be incinerated or could be composted. The composting procedure would entail removing bedding on a monthly basis and transferring to a compost heap. Composting would be allowed to proceed for a minimum of 6 months. The committee felt that this was a reasonable request and agreed that it could be passed on to the HSE, although it was pointed out that all other waste should be inactivated by normal means.

It was agreed that the project should be notified to the HSE as requiring class 2 containment, without the requirement on air pressure or filtering, but applying for the derogations not to have an autoclave in the building and for the straw bedding to be composted.

### Project Containment

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E. coli 0157:H7 strains that can express verotoxins/shiga-like toxins pose a serious threat to human health and are responsible for about 1000 cases of food-poisoning in the UK each year. As a result of the activity of the phage-encoded toxins gastroenteritis can develop into bloody diarrhoea and may also lead to more systemic effects, particularly damage to blood vessels in the kidney and brain by toxic effects on endothelial cells expressing GB3 (CD77). Ruminants are considered to be the primary carriers of enterohaemorrhagic E. coli (EHEC) 0157:H7 strains and the organism does not cause disease in sheep or cattle. Our work has recently established that EHEC 0157 has a unique tropism for the bovine terminal rectum (final 3-5 cm) and that this is the principle colonisation site in this host. We are now trying to define the bacterial and host factors that drive this specific tropism. Several lines of evidence indicate that one of a number of fimbrial gene clusters, including LPF1/2, may play an important role.

Recipient or parental organism

The parental strain is an E. coli 0157:H7 strain (ZAP198) that was isolated from a human patient and has been cured naturally of the shiga-toxin carrying phage. This has been demonstrated using PCR and toxin test kits. Without the toxin the strain is ACDP 2 rather than ACDP 3. This strain colonises cattle in an identical way to the toxin-positive strains but can be used in level-2 containment facilities.
The parental strain has been genetically modified to delete genes in both the lpf1 and lpf2 operons. These deletions prevent the expression of the ahesins. The deletions were made under the HSE GM Centre number GM215 notification "Analysis of virulence determinant regulation and importance to colonisation and persistance of E. coli 0157 in ruminants and humans" (GM215/01.1; Medical Microbiology, University of Edinburgh). The deleted genes have extensive homology to established chaperone/ usher class fimbrial adhesins in E. coli such as the type 1 fimbriae. The deletion process used several allelic exchange steps in order to delete defined regions of the lpf genes without insertion of any foreign DNA. The genetically modified strains will be used to infect calves to assess effect on colonisation. The same process will also be applied to delete other homologous fimbrial operons in the parental strain which may also be tested in cattle at a later date. These additional chaperone/ usher class adhesins have been called HCP1, 2 and 3. Only the cattle infection experiments will be performed at the Moredun Research Institute.

**Evaluation of foreseeable effects**

There is no reason to suggest that the mutant strains to be used will be any more virulent or competitive in the environment than the parent strain.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Animals will be kept in secure accommodation, which meets the requirements for class 2 containment. All animal handlers will be fully trained. All personnel will wear appropriate personal protection equipment such as rubber boots, gloves and over-suits asx appropriate. All protective clothing will be removed before leaving the animal accommodation and will be disposed of as set out below. Rubber boots will be cleaned with 1% Trigene.

Each experiment is scheduled to last for 4 weeks after which the animals will be euthanased. The carcasses of the animals will be placed in a double layer of strong plastic clinical waste bags, the outside surfaces of which will be decontaminated using 1% Trigene. The carcasses will then be lifted on to a pallet and transferred, by forklift, to the incinerator for immediate incineration. All animal waste, and waste bedding, generated throughout the experiment will be placed in a double layer of plastic clinical waste bags and transferred to the incinerator on a regular basis for incineration. The outside surfaces of the bags will be decontaminated using 1% Trigene before transfer. All sharps generated during the experiment will be placed in a rigid plastic cin-bin, the outside surfaces of which will be decontaminated with 1% Trigene before transfer to the autoclave. After autoclaving the cin-bin will be removed from the PSP site by our licensed clinical waste contractor for incineration. All non-organic waste, ie plastics, rubber gloves etc., will be handled similarly.

All clinical samples generated during the course of the experiment will be placed in appropriate containers for transfer to laboratory areas for further analysis.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
The GMSC agreed that the mutant strains described were unlikely to be any more virulent than the parental strain and agreed that the cattle experiments described could go ahead in level 2 containment. There was some debate as to whether or not all the requirements of level 2 containment had to be applied. The committee unanimously decided that the risk assessment did not require the animal accommodation to be held at negative pressure relative to the pressure of the immediate surroundings, but that all other measures outlined in the tables in Part 3E Annex 1 of the GMO Regulations 2000 Compendium of Guidance should be adhered to.

**Project Containment**

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**Project Ref**  172/04.1

<table>
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<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>30/03/2004</td>
<td>PRODUCTION OF VACCINE AGAINST PASTEURELLA MULTOCIDA</td>
<td>Class 2</td>
<td>≤ 1 litre</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

**Project Additional Information**

**Purposes of the contained use**

To develop a vaccine against bovine pneumonic pasteurellosis caused by the bacterium Pasteurella multocida

**Recipient or parental organism**

02/03/2022

Page 4467 of 15326
**Host/vector system**

Derivative of a naturally occurring P. multocida plasmid engineered to contain a multiple cloning site within a Beta-gal gene, an E. coli origin of replication and an AmpR gene.

**Origin & function**

A naturally occurring gene from Pasteurella multocida

**Evaluation of foreseeable effects**

It is not expected that the gene inserted into the plasmid will be expressed when grown in E. coli, as no regulatory elements will be knowingly cloned into the plasmid although there is the possibility of leaky expression from the Beta-gal promotor. This is not thought to present a problem as E. coli naturally contains an orthologous gene. The intention is to produce a "knockout" strain of Pasteurella multocida lacking the gene in question. This is likely to result in attenuation since removal of the equivalent gene in other Gram-negative bacteria results in attenuation. All plasmid sequences will be cured from the final process organism by withdrawing Amp selection. Therefore the resultant organism should carry no foreign DNA. It is expected that the resultant organism will be less virulent than the parental scale.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste generated during these studies will be rendered non-infectious by autoclaving and disposed of by on-site incineration or via a licensed clinical waste contractor according to the procedures documented and held at the institute. Surface disinfection by 1% "Virkon" or by formaldehyde gas sterilisation. Gas cycles will be timed to ensure that surfaces are exposed for the correct period of time. Internal temperature and pressure readings will be taken during sterilisation cycles from all autoclave equipment that alarm in the event of a cycle failure. All non-plastic containing waste will be disposed of by on-site incineration. All plastic waste will be autoclaved on-site prior to disposal via a licensed clinical waste contractor. All liquid waste will be autoclaved prior to disposal via the drains.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

Y
This proposal involves the removal from a strain of Pasteurella multocida. Although the committee agreed that the proposal was likely to require a level 2 containment, they asked for clarification on several points prior to the submission of the proposal to the HSE. Specifically, clarification is sought as to the specific strain being used for producing the mutan. All relevant information about the strain, in terms of how it was isolated, its associated pathology etc., should be given. Also more information about the suicide shuttle vector should be given as well as a more detailed description of the steps involved in producing the knockout strain. In addition the committee wanted to know if there will be intermediate strains generated with multiple antibiotic resistance genes which are subsequently cured from the final strain. If so what procedures are in place to ensure that the antibiotic resistance genes have been successfully removed from the mutant strain? The committee agreed that the cloning of the Pasteurella multocida gene into pGem-T-Easy could proceed at level 1.

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### Project Ref 172/05.1

**Date Ackn'd**
02/11/2005

**CU2 Project Title**
The development of a vaccine as an aid in the prevention and/or reduction of pneumonic lesions associated with bovine pasteurellosis caused by Mannheimia haemolytica and Pasteurella multocida.

**Class**
Class 2

**CultureVolClass2**
Non-GMM

**CultureVolumeClass3-4**
Consent Granted

**Project notified under transitional arrangements**
Not Applicable

**Withdrawn**
N

**Tick if notifying a connected programme of work**
N

### Project Additional Information

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
### Purposes of the contained use

The intention is that a modified vaccine would be tested in Moredun's experimental pasteurellosis (M. haemolytica) challenge model. The modified vaccine will be shipped to Moredun as a ready to use test material. Calves would be vaccinated with the modified vaccine at 8 weeks of age and challenged at 11 weeks of age. Efficacy will be determined by comparison of clinical findings and lung lesion scores between vaccinated and non-vaccinated calves.

Any modified vaccine remaining at the end of the study will be incinerated or returned to the sponsor.

### Recipient or parental organism

The GM vaccine comprises of two components, the parental organisms of which are:

- **a)** A field isolate of Mannheimia haemolytica originally cultured from bovine pneumonic lung tissue.
- **B)** A field isolate of Pasteurella multocida originally cultured from pneumonic lung tissue. This strain is highly virulent and is capable of replication in the bovine lung.

### Host/vector system

**Not applicable.**

### Origin & function

**a)** The GM vaccine comprises of two components. These are derived from the parental strains indicated above, but have had genetic material coding for major virulence markers removed such that the Mannheimia haemolytica produces a truncated and ineffective leukotoxin and the Pasteurella multocida has a defective capsule.

### Evaluation of foreseeable effects

The host range of this strain of Mannheimia haemolytica, is strictly limited to reminants. The isolates appear to be host specific and neither bovine nor ovine isolates readily cause disease in other host species. In contrast, Pasteurella multocida can be found as a commensal organism in the respiratory and digestive tracts of a wide range of domestic and wild animals. In terms of its potential to spread within the environment, the GM Pasteurella multocida can therefore be regarded as the most hazardous of these GMMs. Pasteurella multocida is classified by the ACDP as hazard group 2, a biological agent that can cause human disease. However, most Pasteurella multocida isolates appear to be specialises for virulence in the host of isolation and so not appear to be able to cause disease in a wide range of species. The parental organism was a field isolate originally cultured from pneumonic bovine lung tissue and it is therefore considered unlikely that the organism can readily spread to, or cause disease in humans. No genetic modifications were made to this strain prior to the specified gene deletion. The gene-deleted organism and therefore presumptively the parent has been shown to be susceptible to a wide range of antibiotics including ampicillin, oxytetracycline and penicillin.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not applicable.**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Schedule 8 of the Contained Use Regulations 2000 requires that for a class 2 activity an autoclave be present in the building. We would like to apply for a derogation with respect to the above requirement pertaining specifically to the inoculation of vaccine in the contained animal facility. The building in which it is proposed to house the animals does not have an autoclave. Thus derogation is sought to transfer disposable materials used throughout the trial (such as syringes, gloves etc.) either to an autoclave such that they can be sterilised prior to disposal or directly to the incinerator. Sharps will be transferred in suitable sharps containers, whereas other material will be double-bagged the outside surfaces of which will be cleaned with 70% ethanol or appropriate chemical disinfectant prior to removal from the containment facility.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All disposable material such as gloves, masks, syringes, protective clothing etc. will be incinerated or autoclaved prior to disposal. Since no shedding of the GMOs is expected straw bedding from the pens will be composted. The composting procedure would entail removing bedding on a weekly basis and transferring to a compost heap. Composting would be allowed to proceed for a minimum of 6 months. The protective clothing will remain within the accommodation until the end of the experiment.
at which point it will be disposed of by incineration. Wellington boots will be cleaned in a Foot and Mouth Disinfectant (FAM - Manufactured by Evans) both prior to entry and exit of the facility.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project involves a vaccine trial using the Moredun model of Pasteurellosis. No GMO will be created at the Moredun, but instead the GMOs concerned will be shipped to the Moredun from the sponsor as a freeze dried preparation. Inoculae will be prepared in a Class II hood prior to transport to the animal facilities in a secure container. Once inside the animal containment rooms syringes will be used to deliver the vaccine subcutaneously. This appears to represent the greatest risk in this particular project, although it is not expected however that either of the strains used will be pathogenic to humans. Nevertheless PPE, particularly masks, must be used to protect individuals during the vaccination process. Once injected it is not expected that the GMOs will be shed by the animals, (the sponsors have evidence to this effect). Although it might be expected that Pasteurellosis in its natural form may be spread by aerosol, the committee felt it was reasonable not to require that the animal accommodation be kept as negative pressure with respect to the outside environment given that there was likely to be no shedding of the GMOs. Similarly given that no GMOs are expected to be shed from the immunized animals the committee were happy that all bedding (together with associated animal waste) can be composted.

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**Project Ref** 172/06.1

**Date Ackn’d** 04/09/2006

**CU2 Project Title** Connected programme of work to investigate the pathogenesis of betaretrovirus-induced tumour of sheep and goats.

**Date Project Ceased**

**Class** 2

**CultureVolumeClass2** 1-50 Litres

**Consent Granted** Not Applicable
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Production of diagnostic tests for infection with jaagsiekte sheep retrovirus (JSRV) and development of vaccines against JSRV/ovine pulmonary adenocarcinoma in sheep.

Recipient or parental organism

B) Cultured mammalian cell lines including sheep cells (Choroid plexus (CP) cells, JS7, JS8), human cells (HEK293, 293T, HeLa, A549), murine cells (NIH3T3) and cells of other species including monkey (Cos-7), rat, hamster (BHK), cow, rabbit and deer cells. Cultured ovine and bovine primary cells including epithelial cells, dendritic cells and peripheral blood mononuclear cells.

Host/vector system
a) bacterial cloning vectors:
   pBluescript II (Stratagene)
   P GEM and derivatives
   other plasmid vectors listed in b), c) d) and e) below.

b) Bacterial protein expression vectors,
   pIVEX2 series (Roche)
   p TrcHis2 series (invitrogen)
   pGEX series (Amersham-Pharmacia)
   pBAD-HIS (Invitrogen)
   pMV10
   Pmal-2c (New England Biolabs)

c) Mammalian expression vectors
   pCI series (Promega)
   PGeneSwitch (inducible system, Invitrogen)
   PcDNA series (Invitrogen) and derivatives pβ-DNA (contains the chicken β-actin promoter instead of CMV-IE promoter)
   pIREShyg3 (Clonetech)
d) Murine leukaemia virus retroviral vectors pBabe-Puro (with pHIT60 MLV Gag-Pol and VSV-G or JSRV Env). HIV and EIAV derived lentiviral vectors.

E) JSRV retroviral vectors: JS-AP-ENV (pCMV-JS-GPP and pCMV2JSRV21ΔCP2).

**Origin & function**

The inserted genes are the protein coding regions and regulatory elements of the exogenous and endogenous strains of JSRV, and of the 2 strains of ENTV. Complete wild type and mutant JSRV proviruses and reporter genes.

**Virus protein coding domains:**

- **Gag** - This is a polyprotein encoding the structural proteins of the viral core. Subdomains of Gag will also be cloned separately, namely MA (matrix protein), PP (phosphoprotein), CA (capsid), NC (nucleocapsid).

- **Pol** - This is a polyprotein encoding the viral enzymes. Subdomains of Pol that will be cloned include DU (deoxyuridine triphosphatase), PR (protease), RT (reverse transcriptase and ribonuclease) and IN (integrase).

- **Env** - This viral polyprotein encodes the structural glycoproteins of the viral envelope. Subdomains of Env that will be cloned separately are SU (Surface protein) and TM (transmembrane protein). SU and TM proteins may also have transforming activity when expressed in mammalian cells.

- **Orf-X** - Predicted viral protein of unknown function.

**Regulatory domains:**

The JSR and ENTV untranslated 5’ and 3’ regions, including the LTRs encode regulatory functions such as transcriptional promoter and enhancer elements packaging signals and splicing signals.

**Molecular clones of replication competent JSRV:**

Currently there are 2 versions of this. The first (pJSRV²¹) contains the complete JSRV provirus. The second is a modified version, denoted pCMV2JS²¹ in which the U3 region of the viral 5’LTR has been substituted with the CMV-IE promoter (derived from pcDNA3 by PCR). These proviruses will be used to transfect mammalian cells in order to produce infectious virions and to evaluate cells for permissive replication. Mutants of these clones will also be constructed and studied.

**Reporter gene sequences:**

- Alkaline phosphatase, enhanced green fluorescent protein, firefly luciferase, β-galactosidase gene sequences.

**Evaluation of foreseeable effects**

The most hazardous GMM in programme will be mammalian cultured cells infected or transfected with wild-type JSRV or derived strains. JSRV is not recognised as a human pathogen and therefore such cells present a hazard to the environment only. Sheep inoculated with wild-type JSRV or mutant JSRV virions present no greater risk than a naturally infected clinical case of OPA.

The greatest risk for human health will be mammalian expression vectors (including viral and plasmid vectors) carrying the JSRV env coding sequences under the control of a eukaryotic promoter. This is because the product of this gene is a putative oncogene. If inoculated into humans, such vectors could result theoretically in expression of Env proteins in the recipient's cells and present a risk of transformation. Evidence from studies in mice suggest that a functional immune system reduces this risk (Wootton et al, (2005), Nature 434:90-7). Retroviral vectors used in this programme will not be replication competent and therefore have no risk of spreading to persons other than those directly handling the vectors.
Bacteria expressing the JSRV Env or TM protein represent the most hazardous groups of bacterial GMMs. However, although the JSRV Env glycoproteins have been implicated in the transformation of type II pneumocytes, such proteins expressed in bacteria will not have post-translational modifications and are therefore unlikely to retain such a function. In addition, these proteins are unlikely to be transforming when applied externally to cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We request derogation from full category 2 facilities for the animal experiments.

The animals will be housed under category 2 containment conditions except that an autoclave is not available in the same building. We request derogation such that material leaving the containment facility may be bagged, sprayed with 70% ethanol and transferred directly to the incinerator. [JSRV-Env pseudotyped retroviruses can be inactivated by more than 99.7% by 50% ethanol solution in as little as 10 seconds (Coli et al., 2001, J.Virol.75, 8864-7).]

JSRV is a pathogen of sheep, causing OPA. Experimental data has shown that virus produced in vitro demonstrates the same phenotype as virus harvested from lung fluid naturally OPA-affected sheep, but is much less efficient at reproducing OPA. In OPA-affected sheep the main risk of transmission comes from those that produce lung fluid as it is well described that this fluid contains large amounts of infectious virus. This fluid is produced when there is significant amount of tumour in these animals. It is very rarely produced in animals experimentally infected with JSRV derived from lung fluid and has not been seen in any of the animals infected with tissue culture derived JSRV. Indeed the animals which have been infected with invitro derived JSRV mostly had minimal tumour, not visible by gross pathology but detectable histopathologically. Therefore, when OPA is reproduced experimentally but without allowing late stage disease and production of fluid to occur, the risk of transmission of the virus is significantly reduced.

It is assumed that JSRV is transmitted by aerosol. However it is thought that close contact between animals is necessary for transmission to occur. It has been our experience from about 20 years working with the disease that sheep housed in the next stable (i.e. the other side of a solid wall) do not succumb to OPA. We regulary have field cases of OPA being brought to the Institute as a source of lung fluid and tissues for our research. These are housed in similar buildings to the one we propose to use here. Despite the fact that the clinical cases are producing virus, the veterinarians regard, the risk of transfer to the other sheep is low. This includes those sheep involved in long-term experiments. From this risk assessment, animal containment rooms will not be held at negative pressure relative to immediate surroundings, In addition, filtration of air is not required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid laboratory waste: Autoclaved in-house

Liquid laboratory waste: Autoclaved in-house or treated with Tri Gene disinfectant and disposed in general drainage. Trigene is quoted as having a 100% kill rate for enveloped viruses (1:100 dilution, for at least 1 hour) [MediChem Website]

Previous analysis has shown that no JSRV RNA can be detected in bedding of inoculated animals. Therefore animal waste and solid bedding will be disposed of by composting.

After post-mortem, animal carcasses will be disposed of by incineration. Tissue retained at post-mortem will be processed and jandled as laboratory waste. Samples remaining at the end of the experiment will be destroyed by incineration.
This connected programme of work is designed to replace all the individual projects previously notified to the committee (and the HSE) over the last 5 years as well as those transferred from the "old" GMO regulations in 2000. The committee felt that the research outlined in the risk assessment was appropriate to be considered as a "Connected Programme" of work. The committee were in overall agreement that the work requiring level 2 containment had been correctly identified and that there were no major exceptions or omissions. Clarification was sought regarding whether the expression of the Env gene in bacterial systems should also be handled at containment level 2. This was not thought necessary as there is no evidence that bacterially produced Env could be oncogenic since it does not have the appropriate post-translational modifications. The committee were also happy that the in vivo work had been adequately assessed and that "conventional" animal housing would be appropriate. Such experiments may require a derogation to be sought and previous communications from the HSE about notification of such experiments checked (see notification GM172/03.1). The committee did not require to see the proposal again and agreed that notification to the HSE could proceed.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<td>Human Clinical Applications</td>
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<td>L2</td>
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Project Ref 172/08.1

Date Ackn'd 05/06/2008
Date Project Ceased 

CU2 Project Title
Connected programme of work to study the pathogenesis of ruminant gamma-herpesviruses.

Class 2
Culture Vol Class 2 < 1 Litre
Consent Granted Not Applicable

Non-GMM

Project notified under transitional arrangements

Tick if notifying a connected programme of work Y
Project Additional Information

Purposes of the contained use

(1) Studies towards elucidating the pathogenesis of ruminant gamma herpesviruses; Characterisation of individual genes and their products, analysis of gene function and the analysis of virus-host receptor interactions.

Recipient or parental organism

The Escherichia coli strains 1M109, DH5u, SIIRE-2 and XL-10 for sequencing and expression studies. Strains 517 pir, DH5ci, 05243, GS500, JM109, DHIOB, DS941, pirSl7 X, GS243, OS500, SWIO2, SW1O5, SW1O6 for BAC cloning. S 17-1 and Electrocomp Genehogs (trfA) E. coli (Invitrogen) for transposon insertion. These are all derivatives of the E. coli K-12 strain and are therefore considered to be disabled or non-colonising hosts. They are considered non-pathogenic to humans and will only survive in the environment for a limited time. Mammalian cell lines: Commonly used laboratory cell lines such as CHO, BHX, HEK293T, COS-1, COS-7, NIH 3T3 and Jurkat T cells will be used for expression of selected genes and for analysis of gene function. In addition bovine turbinate (BT) and embryonic bovine lung (EBL) cell lines will be used for propagation of recombinant viruses, Virus-infected large granular lymphocyte cells lines may also be used for the analysis of gene expression and function. All of these cell lines are considered to be especially disabled hosts and will not survive outside the tissue culture environment.

Host/vector system

a) Bacterial cloning vectors:
   pBluescript II (Stratagene)
   pGEM and derivatives (Promega)
   pCR series cloning vectors (Invitrogen)
   BAC vectors pBe1oBAC, pBluelox and derivatives Other plasmid vectors listed in (b), (c) and (d) below.

b) Bacterial protein expression vectors,
   pIVEX2 series (Roche)
   pIRHis2 series (Invitrogen)
   pGEX series (Amersham-Pharmacia)
   pBAD-HIS (Invitrogen)
   pTrxFus (Invitrogen)

c) Mammalian expression vectors
   pcDNA series and derivatives (Invitrogen)
   pCR series expression vectors (Invitrogen)
The inserted genetic material will include protein coding regions and regulatory elements of ruminant herpesviruses, particularly Ovine herpesvirus-2 (OvHV-2) and Alcelaphine herpesvirus-1 (AlHV-1); genes of particular interest are listed below. Additionally, host genes encoding proteins that may interact with or be regulated by viral gene products will also be studied (e.g. natural killer cell receptors and their ligands). Molecular clones of entire viral genomes will be manipulated in recombinant bacterial artificial chromosome vectors to allow the contributions of specific genes to virus pathogenesis to be studied in vitro and in vivo and to allow the development of attenuated viruses for vaccine development.

Virus genes of interest:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
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<td>Al</td>
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<tr>
<td>A2.0</td>
<td>Leucine zipper protein, transcriptional regulation</td>
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<tr>
<td>0v2.5</td>
<td>IL-b homologue</td>
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<tr>
<td>A3.0</td>
<td>Semaphorin homologue; intracellular signalling</td>
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<tr>
<td>0v2.5</td>
<td>Unknown, possibly secreted</td>
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<tr>
<td>0v3.5</td>
<td>Unknown, possibly secreted</td>
</tr>
<tr>
<td>A4.5</td>
<td>Bcl-2 homologue; cell death regulator</td>
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<tr>
<td>A5.0</td>
<td>GPCR (G-protein coupled receptor); intracellular signalling</td>
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<tr>
<td>A6.0</td>
<td>EBV Bzffhomologue; viral bZIP transactivator</td>
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<td>A7.0</td>
<td>Virus Glycoprotein — EBV BZLF2 homologue (gp42)</td>
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<tr>
<td>A8.0</td>
<td>Virus Glycoproteins — C-type lectin receptor?</td>
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<td>Bcl-2 homologue; cell death regulator</td>
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<td>orf22</td>
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<td>orf25</td>
<td>major capsid protein</td>
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<td>virus glycoprotein</td>
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<td>orf50</td>
<td>R transactivator (lytic cycle)</td>
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<td>Capsid protein</td>
</tr>
<tr>
<td>orf73</td>
<td>Latency associated nuclear antigen homologue</td>
</tr>
<tr>
<td>orf75</td>
<td>FGARAT homologue, marker of lytic cycle</td>
</tr>
</tbody>
</table>

Reporter gene sequences:

- Alkaline phosphatase, greenlyellow/redcyan fluorescent proteins, firefly luciferase, -galactosidase gene sequences.

Epitope tags: FLAG, HA, myc, 6xHis, thioredoxin.

Evaluation of foreseeable effects

The most hazardous GMM in this programme, with respect to both human health and the environment, will be the recombinant vaccinia virus vCB21-R. In a penissive tissue culture host this virus will produce infectious particles that are capable of forming lesions in healthy individuals and causing severe disease in immunosuppressed individuals. Note that all use of this vaccinia virus will be either to produce virus to carry out fusion assays or in the fusion assays themselves, All assays will end-point, so that the virus will not be continuously cultured at any point and growing stocks of virus-infected cells will be kept to a minimum. All work with recombinant vaccinia viruses will be carried out at containment level 2.

Recombinant herpesviruses produced in this programme may replicate to high level in tissue culture and could become environmental contaminants following an accidental release. However, the consequences of such a release are unlikely to be significant. OvHV-b infects most sheep in the UK and superinfection with a recombinant virus is unlikely to be effective. AlHV-1 is restricted to exotic hosts - wildebeest,
hartebeest — and is unlikely to productively infect any host in the UK. Infection of MCF-susceptible hosts by recombinant AIHV-1 or OvHV-2 resulting in MCF would require a significant breach containment, but even in such cases further spread of virus is unlikely as these viruses do not replicate productively in disease- susceptible host species. None of the gene products to be expressed in this programme are likely to have deleterious effects on the environment or on human health either as isolated proteins or expressed in mammalian cells or bacteria. Ruminant gamma-herpesviruses do not appear to have oncogenic activity, unlike primate herpesviruses, and cellular transformation has not been described as a property of any MCF virus gene product. Bacteria expressing conserved herpesvirus antigens such as glycoproteins represent the most hazardous group of bacterial GMJls. Such bacteria might elicit an inappropriate/cross-reactive immune response against human herpesviruses. However, the expressed proteins will not have appropriate post-translational modification and so the likelihood of adverse reaction is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We request derogation from full category 2 facilities for the animal experiments. The animals will be housed under category 2 containment conditions except that an autoclave is not available in the same building. We request derogation such that material leaving the containment facility may be bagged, sprayed with 70% alcohol and transferred directly to the incinerator. Herpesviruses can be inactivated by more than 99.9% in 35% alcohol solution in less than 10 seconds (Croughan & Behbehani, 1988. J. din. Microbiol, 26: 213-215)

It is assumed that MCF is caused by aerosol or contact infection of susceptible species with AIHV-1 or OvHV-2. Studies of MCF in experimental animals carried out here over many years have shown that transfer between susceptible species is negligible. In addition, experimental intranasal infections with OvHV-2 have shown that infection of cattle is much less reliable than that of bison, even with doses of over 10^8 genome equivalents (Taus et al. 2006. Vet. Microbiol, 116: 29-36). This supports our view that the infection of susceptible hosts with MCF viruses constitutes a naturally-contained system. From this risk assessment, animal containment rooms will not be held at negative pressure relative to immediate surroundings. In addition, filtration of air is not required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid laboratory waste: Autoclaved in-house.
Liquid laboratory waste: Autoclaved in-house or treated with Virkon disinfectant (or validated alternative) and disposed in general drainage. Virkon is quoted as having a 100% kill rate for enveloped viruses (5% fmal concentration, for at least 30 minutes) [www.antechh.com]

MCF is a disease with unusual aetiology. The species in which these viruses replicate are not susceptible to disease, while those species that do get MCF do not appear to produce any infectious virus. Therefore horizontal spread of virus between MCF-susceptible animals is unlikely and animal waste and soiled bedding will be disposed of by composting.
After post-mortem, animal carcasses will be disposed of by incineration. Tissue retained at post-mortem will be processed and handled as laboratory waste. Samples remaining at the end of the experiment will be destroyed by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Moredun Institute GMSC agree with the assignation of these studies to category 2 containment. We also support the request for derogation as described above.
**Project Containment**

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**Project Ref** 172/09.1

**Date Ackn'd** 06/10/2009

**CU2 Project Title**
Evaluation of safety in non target species (pig and sheep) of a genetically modified type 1 and type 2 Bovine Virus Diarrhoea Virus vaccine candidate

**Class**

**CultureVol**

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<tbody>
<tr>
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<td>&lt; 1 Litre</td>
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**Consent Granted**

**Non-GMM**

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<tr>
<th>Class</th>
<th>CultureVolume</th>
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<tbody>
<tr>
<td>Class 2</td>
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</table>

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To determine the safety of a modified BVDV type 1 and 2 vaccine in non-target species. Containment level 2 required due to genetically modified nature of the material.

**Recipient or parental organism**

Bovine Virus Diarrhoea Virus type 1 and 2

**Host/vector system**
Origin & function

The attenuated viruses contained in this vaccine product were produced by deletions in the region coding for the polyprotein, no additions have been made to the genome. All the manipulations and production of the infectious viruses were performed in Dr Myers laboratory in Germany (Institut fur Immunologie, Friedrich-Loeffler-Institut d-72001 Tubingen Germany). The viruses contained in the vaccine have previously been worked with at the Institut of Animal Health at Compton where the work was conducted at containment level 2 in naturally ventilated buildings.

The virus mutants exhibit a deletion of the overwhelming part of the genomic region coding for the N-terminal protease NPRO and a deletion of codon 349, which abrogates the Rnase activity of the structural glycoprotein Erns.

No new sequences have been introduced into the mutated viruses. No transfer of wild-type sequences to related micro-organisms have been reported.

No genes are inserted into the BVDV genome, deletions that have been shown to attenuate the virus have been made as detailed in "Meyers G, Ege A, Fetzer C, von Freyburg M, Elbers K, Carr V, prentice H, Charleston B, Schurmann EM: Bovine viral diarrhoea virus: prevention of persistent fetal infection by a combination of two mutations affecting Erns RNase and Npro protease. J Virol. 2007 Apr;81(7):3327-38"

Evaluation of foreseeable effects

BVDV is one of the common causes of infectious abortion in cattle. It is also associated with a wide range of diseases from infertility to pneumonia, diarrhoea and poor growth. BVDV is on of the most important viral causes of disease in cattle in the UK.

BVDV is a member of the family of pestiviruses. Diseases associated with other pestiviruses include classical swine fever and border disease in sheep. Pestiviruses infect cloven hoofed stock only. BVDV has been found in pigs and sheep (indeed many cases of border disease are caused by BVDV not border disease virus).

The organism does not replicate in the environment outside of a host animal and would not be expected to survive for long in the environment and is inactivated by a wide range of disinfectants, including Vikon (1:100 dilution).

In a previous study parenteral injection of the GM BVDV strains in calves produced little or no viraemia. Aerosol transmission has not been assessed with the genetically modified viruses, but lack of transmission of wild type virus has been demonstrated in 2 studies by Niskanen et al. Whereby none of the animals housed with animals during the acute phase of infection with wild type BVDV seroconverted to the agent, even when nose-to-nose contact was forced (Niskanen et al. 2000 & Niskanen et al. 2002) These studies showed that cattle acutely infected with non-cytopathic BVDV are highly inefficient transmitters of the disease.

Approximately 90% of the cattle in the world have evidence of exposure to BVDV, with no reported cases of human disease. Pestivirus has been detected in man, however there is no evidence that the virus will cause disease in humans or even immunosupressed humans, although seroconversion to BVDV has been reported (Gianpaspero M et al, Arch.Virol. 7:53-62). The deletions in the genome of the manipulated viruses are highly unlikely to result in a change in tropism. Thus it is considered that there is no risk to human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We request derogation from full category 2 facilities for the animal experiments.

The animals will be housed under category 2 containment conditions except that an autoclave is not available in the same building. We request derogation such that material leaving the containment facility may be bagged, sprayed with Virkon and transferred directly to the incinerator or autoclave. (see section 12 , below)
Transmission of the GMOs to other animals will be restricted by housing of experimental animals in Tange Box accommodation (Level 2 contained accommodation with natural ventilation). Staff will enter the animal rooms from the laboratory side and prior to entry into animal area, protective suits will be put on, and wellington boots will be changed to those designated for use in each room only. Gloves will be worn when handling animals or material from the room. On completion of the procedures, gloves and other consumable items will be replaced in a yellow bag for incineration. Bags will be placed inside another bag and sealed, then sprayed with Virkon prior to transport to the incineration facilities.

All equipment used to handle virus will be held in Virkon (1:100 dilution) for at least 18 hours prior to incineration or transfer to an autoclave. Sharps will be placed in a burn bin, the sealed burn bin will be incinerated.

At the end of the study the room will be sprayed with a 1:100 solution of Virkon. After 24 hours, the waste will be removed to the midden on site and the rooms will be washed with 1:100 Virkon solution or another suitable disinfectant.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

MRI GMSC reference GM09/07b (revised): "Evaluation of Safety in non-target species (pig and sheep) of a genetically modified type 1 and type 2 BVDV vaccine candidate".

The committee agreed that category 2 containment is appropriate. The containment measures planned for this experiment are suitable and will ensure that the GMM is not transferred to other animals.

Project Containment

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<tr>
<td>L2 Yes</td>
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Project Ref 172/09.2
The majority of Campylobacter species are human pathogens that typically cause self-limiting enterocolitis of varying severity. Many Campylobacters reside in the intestinal tract of various animal species without apparently causing disease and are important zoonotic pathogens through contamination of meat, food products and the environment. Some Campylobacter species exhibit host and/or tissue preference and include C. fetus subsp. Fetus, C. fetus subsp. Venerealis, C. hominis, C. hyointestinalis and C. lari.

In man, Campylobacteriosis is the leading cause of foodborne illness in developed countries and causes much morbidity and mortality in the developing world. Disease is commonly attributed to C. jejuni and C. coli with the former being the commonest bacterial foodborne pathogen both in terms of number of cases of diarrhoeal disease reported via reference laboratories and morbidity related to working days lost and the economic consequences of infection (http://www.hps.scot.nhs.uk/giz/campylobacter.aspx and www.hpa.org.uk). Diarrhoeal disease due to this organism ranges from the acute (the main presentation) to chronic and is typically self-limiting in healthy persons although may be more severe or prolonged in the immunocompromised. Normally disease is managed by fluid replacement and antibiotics are not routinely used therapeutically. Infection can also have significant long-term consequences approximately 1 in 1000 cases subsequently developing Guillian Barre syndrome or Miller Fisher syndrome which are neuropathies associated with particular serotypes of C. jejuni. It is estimated that Campylobacter infection is a priority for DEFRA, Food Standards Agency (FSA) and BBSRC within the UK and EFSA (European Food Standards Agency) across the European Economic Community.

Despite the importance of Campylobacter spp. Including availability of three C. jejuni genome sequences, genomes from four other Campylobacter species (C. lari, C. concisus, C. curvus and C. hominis) factors and mechanisms contributing to disease pathogenesis are comprehended poorly. Improving such understanding is a key step in improving control and reducing the risk posed by these common foodborne pathogens.

We propose to examine the contribution of selected C. jejuni components by use of recombinant proteins, and tagged, reporter and mutant strains. The main focus will be cell surface and secreted components.
The three activities can be summarised as:

I) Cloning of specific PCR- or restriction enzyme-derived fragments from Campylobacter spp. Genomic DNA into plasmid cloning vectors for expression of recombinant proteins.

II) "Tagging" of wild-type strains to generate "WITS" (wild-type isogenic tagged strains) to monitor population dynamics during infection.

III) Transformation, mutation and (where required) complementation of selected genes for which the purpose is to demonstrate the role of gene products.

Recipient or parental organism

C. jejuni is a human pathogen (Hazard Group 2) and all strains are potentially infectious to humans. In the event of infection with C. jejuni, disease is self-limiting (typically recovering in 2-5 days) and does not usually require antibiotic treatment; if treatment were required on clinical grounds, erythromycin or a fluoroquinolone is used. These organisms are handled at containment level 2 and good microbiological practice should ensure negligible risk.

Host/vector system

Generation of mutants, tagged or reporter strains involves use of plasmids and introduction of antibiotic resistance genes, at least as an intermediate step. Plasmids used for tagging and mutagenesis are non-replicating in recipient Campylobacter strains and are only retained by homologous recombination under antibiotic selective pressure; complemented strains and reporter constructs can be generated both by insertion of target into the chromosome or on plasmids such as pMW10 which can replicate. Several resistance genes have been employed in genetic manipulation strategies including CAT (chloramphenicol acetyl transferase), KanR (kanamycin) and TetR (tetracycline). Naturally-acquired resistance to each of these agents has been reported in clinical isolates of Campylobacter and carriage of any of these resistance genes would not affect treatment since erythromycin or a fluoroquinolone are preferred for treatment, and then only in cases where disease is severe or prolonged. Where possible, unmarked mutants (from which the antibiotic resistance gene has been excised) will be produced, although such an approach is not routinely employed with Campylobacter. Genetically manipulated strains are expected to retain the pathogenicity of the parent strains and present no additional infectious hazard.

Origin & function

Genes being targeted compromise mainly surface and secreted (putative and known) proteins as well as key metabolic, regulatory or modifying factors. Mutants and other GMMs generated are expected to be less virulent or of equal virulence to the parental strain. Many preceding studies support this assertion - there are no reports of enhanced infectivity or pathogenicity conferred by mutation of genes in Campylobacter strains. Methods for complementation have recently been developed and are being increasingly used for verification purposes. Complementation with a copy of the wild-type gene returns strains to parental gene complement hence does not increase the hazard through promotion of virulence. Some experimentation may require monitoring of gene expression using reporter constructs and methods for doing so in Campylobacter have been developed.

Evaluation of foreseeable effects

GMMs generated are expected to be less virulent or of equal virulence to the parental strain. Many preceding studies support this assertion - there are no reports of enhanced infectivity or pathogenicity conferred by mutation of genes in Campylobacter strains. Genetic manipulation requires use of antibiotic resistance genes to enable selection - these genes are derived from naturally-occurring bacterial resistance genes. Resistance to each of the possible antibiotics used for selection (chloramphenicol, tetracycline, kanamycin) occurs in clinical environment isolates of Campylobacter species and these differ from drugs of choice for treatment.

A main purpose of this GM is to inactivate genes; hence, if transfer were to occur, any recipient would receive a non-functional gene. Complementation with a copy of the wild-type gene returns strains to parental gene complement hence does not increase the hazard through promotion of virulence. Reporter constructs are based on promoter regions of genes which are non-coding regions with reporter genes such as b-galactosidase (lacZ), green fluorescent protein (GFP) or site specific recombinase none of which are known to provide any advantage to recipients. No novel, harmful phenotype is expected from these manipulations.

Campylobacter spp. Are microaerophilic (i.e. require reduced O2 and increased CO2 levels relative to atmosphere for growth) and cannot replicate aerobically or at ambient temperatures so there will be no replication of the organism in the environment. Any introduced genes are not expected to confer benefit to environmental survival hence the hazard is no greater than the wild-type parent strain.

Containment procedures as outlined for risk reduction for human health should be followed.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Campylobacter species are defined primarily as human pathogens although risk of infection is low in healthy adults, including laboratory personnel. All manipulations of Campylobacter species will be on a small scale, and all handling is carried out in a microbiological safety cabinet at Containment Level 2, therefore exposure to bacteria is minimised. All materials in contact with the organisms are decontaminated by disinfection or autoclaving. All workers will have a copy of the relevant risk assessment, will be appropriately trained and will be expected to work to Good Practice standards. All waste materials and products will be disinfected or autoclaved before disposal.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Moredun research Institute GMSC reviewed the risk assessment "Characterisation of Campylobacter species" (Our ref GM09/12)

The committee agreed that category 2 containment is appropriate for the proposed work. The containment measures proposed are sufficient to ensure that the risk of exposure to the GMMs is extremely low.

Project Containment

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 172/10.1

Date Ackn'd 19/01/2010
CU2 Project Title Genetic modification of a common non-pathogenic parasite of cattle
Class 2
Culture Vol Class 2 < 1 Litre
Class Volume Class 3-4

02/03/2022 Page 4484 of 15326
The majority of cattle harbour a non-pathogenic parasite throughout much of their lives. This parasite is sustained at low-levels without affecting productivity in beef and dairy herds. By utilizing the genetic tractability of these parasites, we aim to develop this parasite as a potential delivery system for antigens or other bioactive molecules in cattle. Our aims are to develop a vaccine-vehicle for the expression of recombinant antigens in cattle.

A common non-pathogenic parasite of cattle.

The organism is a ubiquitous parasite specific to cattle: it cannot infect humans or other animals. Although widespread in cattle herds of Europe and North America, the parasite is non-pathogenic. Indeed, incidence levels of ~80% have been recorded in the absence of clinical signs or effects on productivity with there being extremely few reported incidences of this parasite ever being associated with disease, this being only in severely weakened animals. Reinforcing its non-pathogenic status, the parasite is not listed as a specified animal pathogen by DEFRA. Moreover, there is no evidence that the parasite suppresses the immune response of the host.

The inserted sequences are protein products of two eukaryotic pathogens, Babesia spp. and Dictyocaulus viviparus. These are required to be found in the context of the intact whole organism to generate pathogenicity and disease. There are no anticipated consequences of expression of the selected inserted and expressed genes and protein products.

There are no predicted hazards associated with the plasmid and phage vectors employed. All vectors used are based on commercially available vectors. Vectors are non-mobilisable on the basis of being Bom-, mob-, tra-.

All existing vectors are based, as a background, on the pGEM plasmid series with modifications only occurring by insertion into the pGEM polylinker sites. Vectors developed from these, or related vectors with similar control sequences may also be used as they become available. The list of potential expressed heterologous antigens to be used are:

- Babesia antigen
- Babesia antigen + a parasite surface targeting signal
- Babesia antigen + a parasite secretory signal
Dv ACE1
Dv ACE1+ a parasite surface targeting signal
Dv ACE1+ a parasite secretory signal

Evaluation of foreseeable effects

No hazards to human health are anticipated. The parasite is non-pathogenic in its natural host, cattle. Although widespread in cattle herds of Europe and North America, the parasite is non-pathogenic. Indeed, incidence levels of ~80% have been recorded in the absence of clinical signs or effects on productivity with there being extremely few reported incidences of this parasite ever being associated with disease, this being only in severely weakened animals. Reinforcing its non-pathogenic status, the parasite is not listed as a specified animal pathogen by DEFRA, and pre-screening of a sample of 16 cows at MRI has identified that all cattle already harbour existing infections. The modified parasites to be used in these studies have been engineered to express an antibiotic resistance gene (to allow selection of transformants); however this is not a drug used to treat cattle commonly. The parasite itself can be transmitted only by inoculation of infected blood by biting flies; to exclude access of these vectors to experimental cattle, all animals will be housed in high containment facilities where flies cannot obtain access. The parasites do not form spores and do not produce pathogenic products. They cannot survive outside of blood or tissue culture media and are killed in water.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The parasite is spread by blood-feeding flies. Cattle will be kept under conditions inaccessible to such vectors. The parasite is not shed from experimentally infected cattle. Nonetheless, all cattle waste will be subject to treatment. Firstly, all liquid waste will be autoclaved and all solid waste will be bagged and incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Reference GM09/09: Connected programme of work. "Genetic modification of a common non-pathogenic parasite of cattle".

The committee agreed that the use of the HSU facility to ensure containment of the GMM is entirely appropriate. The Committee approved this programme of work at class 2 containment.

Project Containment

02/03/2022
Purposes of the contained use

Bacterial chondronecrosis with osteomyelitis (BCO) caused by Staphylococcus aureus is the most common cause of lameness in studied flocks of commercial birds and a major economic burden on the global broiler poultry industry. By population genetic analysis, we have discovered that the vast majority of cases of BCO worldwide are caused by a single poultry-specific subtype of S. aureus which has undergone a recent human to poultry host jump followed by genetic adaptation to its new host and wide dissemination (Lowder et al, PNAS 106: 10545-19559, 2009). We have sequenced the complete genome of a representative isolate (ED98) and discovered that several novel mobile genetic elements (MGE) including 2 prophages, 2 plasmids and a pathogenicity island have been acquired since separation from a human progenitor strain. The MGE encode several putative virulence factors not previously identified among human S. aureus strains, including a thiol protease, phospholipase and a novel superantigen. We have examined the distribution of the MGE among a panel of poultry S. aureus isolates and found that they are widely distributed among poultry strains including strains from distinct unrelated clonal lineages. We propose to examine the role of the acquisition of specific MGE in adaptation of S. aureus to poultry in a chicken model of BCO.

All genetically modified S. aureus strains will be generated in the laboratory of Dr. Ross Fitzgerald (Centre of Infectious Diseases, Chancellor's Building, Little France).
Infection challenge experiments of broiler chickens will be carried out at the Moredun Research Institute. Broiler chickens will be infected with S. aureus by intravenous inoculation and by aerosol exposure in isolators. Necropsy, blood and tissue sampling will be carried out in the procedure rooms of the Moredun Research Institute animal facilities. Samples will be brought for follow-up analysis to the Easter Bush Veterinary Research Centre and processed in BSL2 laboratories.

Recipient or parental organism

The project will involve genetic deletion of specified MGE from a wild type poultry clinical isolate of S. aureus in order to test the role of the MGE in the pathogenesis of S. aureus during experimental infection of poultry. In addition, we will introduce the specified MGE into a non-poultry adapted strain of S. aureus of human origin to determine if it confers a survival advantage or contributes to increased virulence in a poultry host.

The wild type S. aureus poultry strains to be modified are strains CIX2 an ED98 or similar strains of the same genotype. The S. aureus strains of human origin include strains MR1, RN4220, SH1000 and strain Newman or other well-characterized strains

Host/vector system

The main plasmid vector used for construction of deletion mutants will be pMAD, an erythromycin resistant strain designed specifically for allele replacement of target genes. By the nature of the allele replacement procedure, the erythromycin marker is used to detect the single cross-over event but is lost after the double cross-over event and so is not maintained in the recipient strain which contains a ‘silent’ mutation. Other vectors routinely used for complementing mutations, or for reporter fusions for tracing infections may be used including pE194, pTS1/2, pCU1, pT181, pSK265, pCL84, pG+Host, members of the PCN shuttle vector series and derivatives of all of these. Additional novel plasmid vectors may be used as they become available.

Origin & function

The inserted genes are associated with several different MGE which have been identified in wild type strains of poultry S. aureus. Many genes encoded in the MGE are of unknown function. However, a description of each MGE and any homology with putative or known virulence factors is provided (see Table 1 of the attached risk assessment form).

Evaluation of foreseeable effects

1) Potential hazards to human health
S. aureus is ACDP hazard group 2. As discussed, the recipient strain will either be of human or poultry origin, each specialised for its own host. Poultry strains have lost the function of several genes and are deleted for several MGE involved in human disease pathogenesis. Accordingly, they are unlikely to be as pathogenic for humans as human-adapted strains, although zoonotic transfer cannot be ruled out. The human strains which have acquired poultry-specific MGE are unlikely to be more pathogenic for humans as the MGE have only been found in association with poultry strains previously and are likely to encode avian-specific functions. The pathogen is usually transmitted through physical contact but infections are opportunistic and usually require a compromised immune system or broken skin for invasive entry. Antibiotics are available for treatment of infection for all strains used in the current study and the antibiotic sensitivity is known for all strains to be employed.

2) Potential hazards to the environment
The recipient microorganism is a commonal strain of poultry which occasionally causes opportunistic infections of poultry. As such the release of wild type or MGE-deleted strains would not represent a novel hazard of potential threat to poultry health. Human strains which have acquired MGE may in theory be more fit for survival in chickens but are not likely to be as well adapted for birds as existing poultry specialized strains which have undergone extensive genetic adaptations to a poultry host. S. aureus is non-pathogenic for plants.

To protect against hazards to human health and the environment all experiments will be carried out under biosafety category class 2 containment. Accordingly, the following protective measures will be taken:

Personal protection:
Personal protection (gloves, face-masks, hair cover and appropriate laboratory clothing) will be used at all times to minimise the risk of infection. In the unlikely case that an accidental laboratory infection occurs affected persons can be treated with antibiotics. For all strains that will be employed in the current study the antibiotic sensitivity is
known. Medical emergency staff can be advised for treatment accordingly. Staff will be made aware that if they feel any adverse effects after working with the chickens they should seek medical attention immediately and inform the medical staff of the organism involved.

Waste management:
All waste and bedding from the infected birds will be bagged and autoclaved or incinerated. Carcasses will be placed in sealed, strong plastic bags which will be surface decontaminated before carriage to the incinerator in plastic bins/trolleys with lids. The carcasses will be placed directly into the incinerator within the bags. Any other material used for the experiments (e.g. scalpels, needles, syringes, gloves, face masks) will be inactivated by autoclaving or incineration.

Disinfection of contaminated surfaces, floors and equipment:
TriGene Laboratory Disinfectant can be used for treatment of Staphylococcus aureus including MRSA and VISA at a dilution of 1:100 for heavy soilage and high-risk areas. A contact time between 10-30 min is indicated to be sufficient for disinfection.

Special containment for aerosol infected birds:
All animals infected by the aerosol route will be maintained in poultry isolators (e.g. BioFlex® B50 Rigid Body Isolator) throughout the experiment to ensure containment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No application for derogation from full containment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste and bedding from infected birds will be bagged and autoclaved or incinerated. Carcasses will be placed in sealed strong plastic bags which will be surface decontaminated before carriage to the incinerator in strong plastic bins/trolleys with lids. The carcasses will be placed directly into the incinerator within the bags. Any other material used for the experiments (e.g. scalpels, needles, syringes, gloves, face masks) will be inactivated by autoclaving or incineration. All equipment used for inactivation of GM material is under regular monitoring and calibration procedures. Records of autoclave/incinerators operations are being produced and kept as required.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Dear A

The MRI GMSC has reviewed your risk assessment entitled “Staphylococcus aureus-host interactions in experimental infection poultry”

The first point raised regards transport of the material between the university and here. Please note that the packaging must comply with UN2814.

Part d) i) Experiment 2. It was noted that the experimental protocol on the proposers project license states: “Birds will be infected 1 day post hatching and maintained in the isolator for a maximum observation period of 3 week. After this period they will be released into biosafety class 2 maintenance rooms and monitored for another 40 days.” However, the GM risk assessment states that “the chickens will be infected and maintained in isolators thus ensuring containment” implying that the birds will remain in isolators throughout.

The committee therefore requests clarification regarding the length of time that the birds will be maintained in the isolators. Justification should be given if they are to be removed into class 2 rooms. The aerosol infection process will obviously contaminate the surfaces of the isolator and also the birds skin and feathers with the bacteria. Information on the likely survival time of S. aureus in this environment should be supplied.

Part d) viii) An additional sentence should be added stating “Staff should be made aware that if they feel any adverse effects after working with the chickens they should seek medical attention immediately and inform the medical staff of the organism involved.”

Part 3.

All aspects of this project are assigned to class 2.

Please make these changes to the risk assessment document and return it.

In addition, a “pathogen fact sheet” as you suggested previously would be very helpful. The document can be put up on the wall for all the staff working with the birds to see.

Yours sincerely

DR C C
Chair,
Moredun Research Institute Genetic Manipulation Safety Committee.

The updated risk assessment and procedures reviewed by the MRI GMSC at a meeting on 28/06/2010. The committee agreed that these are now appropriate for both the laboratory and the in vivo work proposed.

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**Project Containment**

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Animal Units | Large Scale Activities | Human Clinical Applications

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02/03/2022
Project Ref 172/10.3

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<td>Connected programme of work to develop ovine retroviruses as gene delivery vectors</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

### Project Additional Information

**Purposes of the contained use**

Novel viral vectors will be developed from the ovine retroviruses for use as gene delivery vectors for use in vitro and in vivo

**Recipient or parental organism**


b) Cultured mammalian cell lines including sheep cells (Choroid plexus (CP) cells, JS7, JS8), human cells (HEK293, 293T, HeLa, H820), murine cells (NIH3T3) and cells of other species including monkey (Cos-7), rat (208F), hamster (BHK), cow, goat, rabbit, cat and deer. Cultured primary cells including cultured ovine, caprine and bovine epithelial cell, dendritic cells and peripheral blood mononuclear cells.

c) Sheep, mice, cows, mice and rabbits for in vivo studies

**Host/vector system**

a) Bacterial cloning vectors:
   - pBluescript II (Stratagene)
   - pGEM and derivatives
   - pCR2.1
b) Mammalian expression vectors
   - pCI series (Promega)
   - pIREShyg3 (Clontech)
   - pEGFP-C1, pEGFP-N3 (Clontech)
   - pCAGneo (a derivative of pClneo containing the chicken B-actin enhancer-promoter in place of the CMV promoter-enhancer)
   - Other plasmids encoding retroviral vector components listed in (c) and (d)

c) Murine Leukaemia virus retroviral vectors pBabe-Puro (with pHIT60 MLV packaging construct and VSV-G or retroviral Env proteins). HIV lentiviral vectors (pLenti system (Invitrogen)).

(d) Novel retroviral vector systems derived from ovine retroviruses

Origin & function

Retrovirus vectors will be developed based on existing molecular clones of infectious ovine retroviruses using a standard 3-plasmid transient transfection system similar to those in routine use derived from MLV and HIV.

The plasmids used will be:
   (i) Gag-Pol expressing plasmid (packaging construct)
   (ii) Env expression plasmid
   (iii) Vector plasmid

Reporter gene sequences including alkaline phosphatase, enhanced green fluorescent protein (and variants), firefly luciferase, and β-galactosidase will be incorporated to facilitate vector development.

These vectors will be used to study vector (and parent virus) function in cells and tissues from various species, including sheep, goats, cows, rabbits and mice.

Evaluation of foreseeable effects

The most hazardous GMM in this programme will be novel retroviral vectors derived from ovine retroviruses. These new vectors are replication-incompetent and are capable of a single round of infection. We therefore expect them to be at least as safe as using existing and well-characterised retroviral vectors derived from HIV-1 and MLV.

Two general hazards are associated with retroviral vectors: formation of replication-competent retrovirus (RCR) and vector mobilisation. RCR can be generated through recombination events during the transfection step of vector production. Recombination occurs due to regions of sequence homology between the different plasmids used to make the vectors. To minimise this risk, prior to in vivo studies we will design the vectors to eliminate as far as possible any regions of homology, by codon optimisation of the packaging vectors. PCR and infection assays will be used to measure the production of RCR. The hazards to humans associated with a breakdown of containment are small, given that ovine retroviruses are not human pathogens and any RCR generated would be a defective version of these viruses.

Vector mobilisation poses a theoretical hazard to the environment, by rescue of vectors following co-infection with wild-type viruses. However, we will develop self-inactivating vectors for in vivo use thereby minimising the risk. Even if vector mobilisation were to occur, the vectors themselves are replication incompetent and the effects are likely to be negligible in comparison to the effects of the wild-type viruses themselves.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
We request derogation from full category 2 facilities for the animal experiments.

Animals that have been inoculated with retroviral vectors will be housed under category 2 containment conditions except that an autoclave is not available in the same building. We request derogation such that material leaving the containment facility may be bagged, sprayed with 70% ethanol and transferred directly to the incinerator. (Retrovirus vectors can be inactivated by more than 99.7% by 50% ethanol solution in as little as 10 seconds (J Virol. 75:8864-8867). The vectors to be used are replication defective ("one-hit") and unable to spread from the inoculated recipient. Therefore, bedding will be disposed of by composting. Previous analysis using wild-type viruses has shown that viral RNA cannot be detected in bedding of inoculated animals using quantitative real-time PCR (please refer to previous correspondence, notification reference GM172). Animal carcasses will be disposed of by incineration. Tissue taken at post-mortem will be processed and handled as laboratory waste.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid laboratory waste: Autoclaved in-house.

Liquid laboratory waste: Autoclaved in-house or treated with 5-10% TriGene disinfectant and disposed in general drainage. Trigene is quoted as having a 100% kill rate for enveloped viruses (1:100 dilution, for at least 1 hour) [MediChem website]

Animal waste: The vectors to be used are replication defective ("one-hit") and unable to spread from the inoculated recipient. Therefore, bedding will be disposed of by composting. Previous analysis using wild-type viruses has shown that viral RNA cannot be detected in bedding of inoculated animals using quantitative real-time PCR (please refer to previous correspondence, notification reference GM172). Animal carcasses will be disposed of by incineration. Tissue taken at post-mortem will be processed and handled as laboratory waste. Samples remaining at the end of the experiment will be destroyed by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Our ref: GM10/10

Your risk assessment entitled "Connected programme of work to develop ovine retroviruses as gene delivery vectors" was reviewed by members of the MRI GMSC. It was agreed that category 2 containment is appropriate for this work. A notification to HSE is required for cat 2 work therefore form CU2 will need to be completed.

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications
Project Additional Information

Purposes of the contained use
To detect mycoplasmal, microbial and/or extraneous agent contamination in a Sponsor’s Sample. The intention is that the modified virus seeds will be prepared by the Sponsor and shipped to Moredun for testing by the Biosafety Division at Moredun Scientific. The material will be shipped to Moredun as a ready to use test material for sterility, mycoplasma and extraneous agents testing (all according to European Pharmacopoeia). A summary of the work to be undertaken is detailed in the attached Risk Assessment.

Recipient or parental organism
Baculovirus (infectious to insect cells) - Master seed viruses produced on Sf+ insect cells.

Recipient strain is a polyhedrin-negative Autographa californica multiple nuclear polyhedrosis virus (AcMNPV).

In nature, AcMNPV particles are occluded in a polyhedron, a protective matrix consisting predominantly of the protein polyhedrin, which permits survival in the environment and efficient spread to new hosts. The Sponsor’s constructs are polyhedron-negative, limiting potential survival in the environment and spread to insects.

Host/vector system
Baculovirus vector Bac.VD43 (Sample name Bac. VD43 P4 WSV). Polyhedrin-negative.

Origin & function
Bac.VD43 encodes the human LPLS447X gene, expressing a natural variant of human lipoprotein lipase (LPL). Human LPL is a key enzyme in the metabolism of triglyceride-rich lipoproteins. It mediates hydrolysis of triglycerides in chylomicrons and very-low-density lipoproteins, and protects against excessive rises of triglycerides after meals.

**Evaluation of foreseeable effects**

No GMM is being constructed. The activities all involve the use of a previously generated, stable baculovirus vector, Bac.VD43.

Baculoviruses are rod-shaped dsDNA viruses found mainly in insects. Autographa californica multiple nuclear polyhedrosis virus (AcMNPV), is the type species of the nucleopolyhedrovirus genus, and is the most extensively studied and well used baculovirus vector. Baculoviruses are ubiquitous. Insects, and some crustaceans, are the only known natural hosts. Thus, the virus is not considered a hazard to humans or animals.

In nature, AcMNPV particles are occluded in a polyhedron, a protective matrix consisting predominantly of the protein polyhedrin, which permits survival in the environment and efficient spread to new hosts. The Sponsor's recombinant baculovirus is polyhedrin negative, limiting potential survival in the environment and spread to insects.

The construct was generated using a plasmid in which the polyhedrin gene is largely deleted, thus reversion to wild-type is not considered likely. As baculoviruses are insect viruses, without known homologues in humans or animals, it is unlikely that complementation or rescue to a polyhedrin-positive status could occur during the planned work, or following accidental inoculation of an operator. In the event of environmental contamination, recombination with wild-type baculovirus due to dual infection of an insect host, resulting in rescue of the polyhedrin gene, would be expected to result in deletion of the inserted sequence. Thus, no more pathogenic variant would be expected to arise.

The LPL gene is expressed under the control of the cytomegalovirus immediate early promoter. Therefore, following infection of mammalian cells, it is possible that high level expression of this gene could occur. There is no known toxicity associated with expression of this protein. Following accidental exposure of an operator, or infection of mammalian cells, the baculovirus would not replicate and spread. Therefore, within a human/animal host, expression would be expected to be transient, and high and/or sustained systemic expression of LPL is unlikely. Expression of LPL is not expected to alter the host range or pathogenicity of the baculovirus host.

The inserted sequences also include a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), to enhance transgene expression. Woodchuck hepatitis virus (WHV) is a member of the family Hepadnaviridae, of which Human hepatitis B virus is the prototype. The WPRE element overlaps with the coding region for the X protein, and some versions of this element are therefore capable of expressing part of this protein. The precise function of the X protein is uncertain. It is required for efficient infection and replication in vivo, and it has been shown to activate and repress gene transcription, and display anti-apoptotic and pro-apoptotic, and anti-proliferative and proliferative activity, depending on the assay systems used. It has been suggested that the X protein plays a role in the development of liver cancer. Although there is no evidence that the X protein is directly oncogenic, studies have shown that it may act as a cofactor for oncogenesis. Due to the lack of detailed knowledge in this area, it is recommended by the SACGM that constructs containing WPRE elements that could express part of the X protein be classed as GM level 2 or higher.

The pX open reading frame is not mutated in this construct. Therefore, in the absence of data to provide assurance that there is no protein expression from this element, it should be considered possible that part of the X protein could be expressed following infection.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid waste will be soaked in at least 1% Virkon solution for a minimum of 2-3 hours – any plasticware that has been soaked will then be incinerated on site. All solid waste and waste and bedding from the animals will be bagged and autoclaved or incinerated. Animal carcasses will be placed in sealed strong plastic bags and will be...
placed directly into the incinerator within the bags. All carcass and waste bags will be surface decontaminated before carriage to the incinerator in strong plastic clinical waste bins/trolleys with lids. Any other material used for the experiments (e.g. scalpels, needles, syringes, gloves, face masks) will be inactivated by autoclaving or incineration. All equipment used for inactivation of GM material is under regular monitoring and calibration procedures. Records of autoclave/incinerators operations are produced and retained for reference.

The MRI GMSC discussed the risk assessment for this work (our ref GM10/22). The baculovirus construct could potentially express the woodchuck hepatitis virus X protein which may be oncogenic or a co-factor for oncogenesis. Because of this uncertainty the GM should be considered as of potential risk and therefore cat 2 containment should be applied.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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**Animal Units**

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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**Project Ref** 172/11.2

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Date Project Ceased</th>
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<tbody>
<tr>
<td>13/06/2011</td>
<td>Mycoplasma, Sterility and in-vivo Adventitious Agent testing of Genetically Modified Biopharmaceutical and Biological Products Intended for Human or Veterinary use.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Project notified under transitional arrangements</td>
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<tr>
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<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
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</tr>
<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
</tr>
<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>N</td>
</tr>
</tbody>
</table>
### Purposes of the contained use

To detect mycoplasmal, microbial and/or extraneous agent contamination in a sample submitted by a client. The intention is that the GMO will be prepared by the client and shipped to Moredun for testing by the Biosafety Division at Moredun Scientific. The material will be shipped to Moredun as a ready to use test material for sterility, mycoplasma and extraneous agents testing. A summary of the work to be undertaken is detailed in the accompanying Risk Assessment appendix 1. Any modified test material remaining at the end of the study will be incinerated or returned to the sponsor, however it is not envisaged that there will be any material remaining.

### Recipient or parental organism

Examples of host/vector systems which may be submitted for testing are shown in the accompanying Risk Assessment Appendix II.

The majority of recombinant proteins intended for therapeutic use, or for use as vaccines are generated using mammalian or insect cells. Most are expressed using immortalised CHO cells, but other recombinant cell lines based on NS0, BHK 293 and human retinal cells have gained regulatory approval for recombinant protein production. In all instances the recombinant cell lines will be well characterized and suitable for the production of biopharmaceuticals for human or animal use.

### Host/vector system

All of the vectors used for generating the above cell lines will be standard expression vectors designed for stable integration into the host cell genome, based on well-characterised E.coli cloning vectors such as pBR322 pr pUC. Stably integrated vectors would be considered non-mobilisable, unlikely to spread from the host cell and therefore unlikely to increase the risk to the host.

A wide range of virus systems may be used to generate the virus vectors or attenuated viruses submitted for testing, based on hazard group 1 or 2 agents: eg baculovirus, retrovirus, adenovirus, herpesvirus, Vaccinia virus and other pox viruses such as fowlpox, canarypox, modified vaccinia virus (Ankara), Japanese encephalitis virus (all replication incompetent). Lentivirus vectors may be based on hazard group 3 pathogens (eg HIV) but only replication incompetent vectors containing minimal lentivirus genes will be employed and these are considered GM activity Class 2.

### Origin & function

Examples of inserted genes or nucleic acid sequences that may be present are given in the accompanying Risk assessment Appendix III. In most instances the inserted genes will encode therapeutic antibodies, including monoclonal antibodies, vaccine antigens or standard detectable or selectable markers and would not be expected to be toxic.

Vaccine antigens typically represent structural or surface proteins derived from the target microorganism and are usually not biologically active. There may however be some exceptions and some virus proteins may directly contribute to cytopathology in the virus from which they are derived.
Hazards may exist with respect to the risk of GMMs submitted for testing and therefore a cautious approach to classification will be taken. It may not always be possible to predict all the functions or potentially harmful effects of novel therapeutic products or vaccines, especially where clinical data is limited. In instances where there is a level of uncertainty, a worst case scenario will be applied. For example, if the level of gene expression for an insert is unknown, high level constitutive expression will generally be assumed.

Further examples of dealing with potential hazards are shown in the accompanying Risk Assessment Appendix III and IV.

<table>
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<tr>
<th>Evaluation of foreseeable effects</th>
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<tr>
<td>All solid waste will be incinerated on site, all liquid waste will be soaked in 1% Virkon solution for a minimum of 2-3 hours - any plasticware that has been soaked will then be incinerated on site. The solid waste for incineration will be bagged in clinical waste bags then placed into a clinical waste bin for on-site collection. A special uplift will be arranged for carcasses to avoid any risks, and other material will be transported directly from a locked clinical waste bin directly to the PM suite. The PM room attendant will be informed of the material to be uplifted and its content so that he is aware of any risk.</td>
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<table>
<thead>
<tr>
<th>Please enter comments on the GM safety committee on the risk assessment</th>
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</thead>
<tbody>
<tr>
<td>The MRI GMSC approved the connected programme of work where either cat 1 or cat 2 containment will be appropriate depending on the product or cell line submitted by the client for testing. However since the connected programme of work encompasses a range of GMOs the GMSC requested to continue to review individual risk assessments which fall within this connected programme if the product or cell line has already been assigned to cat 2 or if there is any doubt about an assignment of cat 1 at the originating laboratory. (The GM material arriving from clients is always accompanied by a detailed risk assessment and a containment category will have been assigned at their site).</td>
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</table>

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<td><strong>Laboratory Activities</strong></td>
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</table>
**Project Ref** 172/11.3

**Date Ackn’d** 06/12/2011  
**CU2 Project Title** Efficacy of genetically modified Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) vaccine candidates in piglets challenged with a wild type PRRSV

**Class** Class 2  
**CultureVolClass2** Not Applicable  
**CultureVolumeClass3-4** Non-GMM  
**Consent Granted**

**Withdrawn** N  
**Tick if notifying a connected programme of work** N

**Historical Significant Changes**  
**Historical Date of Additional Info**

**Project notified under transitional arrangements** N

---

**Purposes of the contained use**

PRRSV is endemic in the UK and European pig herd and results in considerable economic losses in the sector as well as considerable welfare issues for individual animals on infected properties. In piglets, PRRSV can result in loss of condition, inappetance, an acute and extensive pneumonia (coughing, sneezing, increased respiratory rate), diarrhoea and in some cases lameness may. In breeding sows, there can be a short period of inappetance and some respiratory signs. However, more importantly, there can be reproductive failures with increased still births, abortions and mummification of piglets, as well as increased mortality in piglets that are born up to 30% smaller.

There are a number of PRRSV vaccines currently available for use. However, more effective vaccines will result in considerable savings for the animal health sector as well as an increase in the welfare of pigs. The goal of this project is to determine whether any of the vaccine candidates show any efficacy in the prevention or reduction of clinical disease and virus shedding following an artificial challenge with PRRSV.

The vaccine candidates which will be tested in this project are considered to be the next generation of PRRSV vaccines which if successful, should increase the protection offered in the pig herd against PRRSV.

**Recipient or parental organism**

When compared to the parental strain, the vaccine candidates show attenuation in vitro as reflected by delayed viral growth and/or by increased induction of the immune system. It appears reasonable to expect that these aspects of in vitro attenuation will show corresponding effects of attenuation also in vivo.
### Host/vector system

None

### Origin & function

The parental strain as well as the vaccine candidates based on this strain, originate from a genotype I (EU) PRRSV field strain.

### Evaluation of foreseeable effects

When compared to the parental strain, the vaccine candidates show attenuation in vitro as reflected by delayed viral growth and/or by increased induction of the immune system. It appears reasonable to expect that these aspects of in vitro attenuation will show corresponding effects of attenuation also in vivo.

The attenuated vaccine candidates are considered to be less hazardous that the parental strain, which itself is not considered to be hazardous to humans or the environment. All vaccine candidates have had similar modification/deletion therefore none are considered to be more hazardous.

PRRSV is not considered to be harmful to humans and the genotype 1 strains are not detailed on the DEFRA website as being of particular concern within this country for animals, as the disease is already endemic in the UK. However as not all pigs herds are affected by PRRSV currently, it is not desirable for this modified virus to be released into the environment, especially as the pathogenicity of the GM PRRSV is not as yet ascertained. The modification of the parental strain is not considered to have increased the risk of any infection in humans. In-vitro testing of the GMO vaccine candidates has indicated that the modification has reduced the infectivity of the candidates, which should reduce further any possible concerns.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We request derogation from full category 2 facilities for the animal experiments.

The animals will be housed under category 2 containment conditions except that an autoclave is not available in the same building. We request derogation such that material leaving the containment facility may be bagged, sprayed with 70% ethanol and transferred directly to the incinerator.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Due to the GMO nature of the vaccine candidates, and the potential for spread of the vaccine candidates or the wild type challenge to other pigs on site, it is recommended that a containment level of 2 is applied for this study. The modification of the wild type parental strain will reduce the infectivity to the environment and other pigs on site. However, containment level 2 will be required due to the more virulent nature of the wild type challenge material which will be used to assess the efficacy of the vaccines. The European genotype 1 strains of PRRSV are not detailed on the DEFRA list.

Liquid and solid laboratory waste will be disposed of according to standard Moredun protocols for disposal of infected material. (SOP EMS/PROC/009). Material used in the laboratory will be disinfected under a Class II microbiological cabinet and placed in yellow bags for onsite incineration. Liquid waste (including unused vaccine material) will be disposed of following overnight disinfection with Virkon.

Waste from infected animals will be treated with Virkon and allowed to dry before being disposed of by composting following standard site procedures for disposal of infected animal waste. If conditions are right (about 40°C, pH 7.5) the virus can survive from days to weeks. However, it is very susceptible to adverse conditions, especially drying, and will die off within hours as conditions change from optimum. On the usual pig-associated fomites (plastic, steel, wood, straw, clothing, slurry etc.) at normal environmental temperatures (25-27°C) PRRS virus survives less than a day but it can survive in water for up to 11 days (Benfield et al, 1999a). Thus, normal clean-up procedures with disinfection and drying will kill the virus. The PRRS virus is susceptible to all the commonly used disinfectants including chlorhexidine, formaldehyde, chlorine, iodophors, sodium hydroxide, quaternary ammonium compounds, and the phenolics.
At the end of the study the animals will be transported to the post mortem facility in a sealed vehicle (or sealed containers) where they will be euthanased and then samples will be removed for further analysis. The vehicle used to transport the animals will be cleaned with an appropriate disinfectant (such as 1:100 Virkon or FAM) prior to transport of any other animals. All samples collected will be placed in sealed containers and then placed in sealed bags prior to transport to Moredun Scientific where they will be stored prior to shipment to the Sponsors facility. All personnel will wear appropriate protective clothing which will be incinerated onsite on completion of sampling. All carcasses will be incinerated on site on completion. The post mortem facility will be washed disinfected on completion of work using a suitable disinfectant (as above).

The controls that are proposed will ensure that the GMO’s are contained. No infectious material will be transported off site other than those samples required by the sponsor for further analysis.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

GM11.29  D R (MSL) – Efficacy of PRRSV vaccine candidates

The GMSC reviewed the risk assessment and agreed that the containment measures proposed were sufficient and that a designation of cat 2 was appropriate for this work.

**Project Containment**

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<tr>
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**Project Ref**  172/12.1

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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>13/01/2012</td>
<td>Genetic modification and characterisation of Pasteurella multocida and Mannheimia haemolytica bacteria</td>
<td>Class 2</td>
<td>&lt; 1 Liter</td>
<td>Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>
Tick if notifying a connected programme of work  

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID  

Date of Significant Change  

Project Additional Information

Purposes of the contained use

To develop novel reagents for the control of pneumonia disease caused by these bacteria in bovids

Recipient or parental organism

Pasteurella multocida
Mannheimia haemolytica

Host/vector system

Deletion mutants of Pasteurella multocida or Mannheimia haemolytica

Origin & function

Origin is form naturally occurring outbreaks of disease (pneumonic pasteurellosis in UK calves or haemorrhagic septicaemia in buffalo and cattle in India in the case of Pasteurella multocida or pneumonic pasteurellosis in UK calves in the case of Mannheimia haemolytica) with the intention of producing attenuated strains.

Evaluation of foreseeable effects

The mutated strains will be attenuated and are not expected to have any deleterious effects. Wild-type P. multocida has been reported rarely to cause infections in humans either through dog or cat bites or in immuno-compromised individuals. There have been no reports of human infections with M. haemolytica. Transformation will reduce rather than increase the pathogenic traits of the organism and the planned gene disruption will either have no effect, or decrease the pathogenic capability of the GMM. The risk to humans or the environment is low

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated during these studies will be rendered non-infectious by chemical disinfection or autoclaving and disposed of by on-site incineration or via a licensed clinical waste contractor according to the procedure documented and held at the Institute. Chemical disinfection methods will be monitored to ensure that the correct solution strengths are used. Internal temperature and pressure readings will be taken during sterilisation cycles from all autoclave equipment that alarm in the event of a
All solid laboratory waste will be bagged and surface-disinfected in containment before being taken for sterilisation by autoclaving and disposal. Contaminated liquids will be disinfected by adding Virkon powder and leaving for 2h before disposal via the drains. All infected animals will be killed and, along with bedding and solid excreta, bagged and surface-disinfected in containment and taken directly for incineration. Liquid excreta will be sterilised by heat before disposal to drains. Any plastic waste will be autoclaved on-site prior to disposal via incineration or by licensed clinical waste contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

This proposal was deemed to be fairly straightforward. Clarification was sought on the curing of all "non-native" plasmid sequences from the attenuated strain after which approval was given for the proposal to be forwarded to the HSE as requiring Level 2 containment.

Project Containment

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Project Ref 172/13.1

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<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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</thead>
<tbody>
<tr>
<td>13/08/2013</td>
<td>Connected programme of work to investigate the molecular pathogenesis of veterinary streptococcal pathogens.</td>
<td></td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Non-GMM Consent Granted</td>
<td>N</td>
</tr>
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</table>

Withdrawn N Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**

The purpose of this work is to investigate the mechanisms by which Streptococcus spp. colonise and persist within the host, with particular attention to production diseases of farmed animals. In the course of this work, genetic manipulation of streptococci using recognized methods will be conducted, in order to fulfil the tenets of 'Molecular Koch's Postulates'.

**Recipient or parental organism**

The GMO's to be created will include non-pathogenic Escherichia coli laboratory strains, used as hosts to facilitate the construction of recombinant plasmids for downstream mutagenesis of streptococcal spp. GM E. coli will also be used as means of expressing clone streptococcal genes to facilitate recombinant protein production. All work in E. coli is considered Class 1, and no hazards are anticipated.

GM streptococcal strains will be constructed by targeted allele-replacement mutagenesis of target genes, or by random-insertion mutagenesis to identify novel phenotypes (in vitro). The foreseeable effects of this activity will be varying levels of attenuation of the recipient strains. Extensive in vitro analysis of GM strains will ensure that no strain of enhanced virulence is used for downstream studies.

**Host/vector system**

E. coli strains for routine cloning will be standard, non-pathogenic E. coli K12 derivatives. Furthermore, non-pathogenic E. coli B21- derivatives will be used for production of recombinant streptococcal proteins.

Streptococcal hosts will be naturally-occurring wild-type bacteria isolated from cases of disease, predominantly in farmed animals. The focus of the work is broadly against streptococcal diseases of veterinary importance, and it is anticipated that it may include Streptococcus uberis, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus suis, Streptococcus equi, Streptococcus iniae and Streptococcus parauberis, among others (dependent upon availability of funding for the work).

The vectors used for cloning and expression of streptococcal sequences in E. coli will be commercially-available plasmids, including pTOPO, pPCR-Script, pET-15b and pTrEx.

The vectors to be used for mutagenesis of streptococci will be pG+host 9 and its derivative pGh9:ISS1. pG+host 9 is derived from a naturally-occurring plasmid in non-pathogenic Lactococcus spp. The plasmid has been subjected to hydroxylamine mutagenesis, such that the plasmid-encoded repA gene has been altered. Consequently, the RepA protein, which is responsible for initiating replication of the plasmid at host-cell-division, is unstable above 28°C, meaning that replication of the plasmid within streptococci can be controlled by manipulation of the growth temperature.
pGh9:ISS1 is identical to pG+host 9, with the exception that it contains a cloned sequence corresponding to the Streptococcus agalactiae ISS1 insertion element. This insertion element drives the random integration of the plasmid into the chromosome of host streptococcal cells, allowing the creation of libraries of random mutants which can be screened in vitro to identify phenotypes of interest.

**Origin & function**

The genetic material to be exploited in this work will originate from naturally-occurring wild-type streptococcal species, isolated from cases of clinical disease. The streptococcal genes will encode proteins known (or suspected) to contribute to pathogenesis. The overall purpose of this work is to create modified derivatives of wild-type streptococcal strains which are deficient in the production of specific proteins, so that the contribution of these proteins to virulence can be assessed by comparing wild-type and isogenic mutant derivative strains using defined experimental approaches.

**Evaluation of foreseeable effects**

The streptococcal GM strains to be created in this work will contain altered sequences for individual genes. Following allele-replacement mutagenesis, the sequence of target genes will be altered such that the encoded proteins are either abrogated or reduced in their biological function. These mutants will be otherwise identical to their wild-type parent strains, and will not have antibiotic resistance determinants or other exogenous sequences not normally associated with wild-type streptococcal strains. As such, the expectation is that the GM streptococci will have reduced, or at worst equivalent pathogenicity to the wild-type strains from which they derive.

Similarly, GM strains created by random insertion mutagenesis will be devoid of plasmid 'backbone' sequences (including antibiotic resistance determinant) at the end of the mutagenesis procedure, such that genes will be disrupted by the ISS1 element remaining within the chromosome. This is a naturally-occurring element which does not encode any additional traits. It is highly-unlikely that this procedure will result in strains of increased virulence; rather attenuation is expected as compared to wild-type parent strains, and at worst no difference will be observed. Irrespective, extensive screening of random-insertion mutants will be conducted to ensure that no strains of enhanced virulence are used in downstream studies.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We request derogation from full category 2 facilities for the large animal experiments.

The animals will be housed under category 2 containment conditions except that an autoclave is not available in the same building. We request derogation such that material leaving the containment facility may be bagged, sprayed with 70% ethanol and transferred directly to the incinerator.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Laboratory work: Virkon will be used for all laboratory disinfection (as per the manufacturer's instructions), since this disinfectant is active against a wide range of micro-organisms (including streptococcal species) and is not inhibited by organic materials (as may be present in culture media, clinical samples, etc.) All solid waste material resulting from this work will be autoclaved prior to disposal, while all liquid waste material will be treated with a 3% solution of Virkon for at least 2 hours prior to disposal to drain. Laboratory surfaces and equipment will be disinfected with a 1% solution of Virkon for 10 min prior to rinsing with water.

Animal work: For small animal (rodent) work, all bedding will be double-bagged in nylon bags and swan-neck sealed prior to autoclaving to exit the laboratory. This waste will then be incinerated on-site. For large animal work, the amount of waste is impractical for autoclaving; however, extensive shedding of GMOs is not anticipated. Disinfection of animal facilities and equipment will be conducted by soaking animal bedding and accommodation in the iodophor disinfectant FAM30, which is DEFRA approved for such activities, is active against a wide range of micro-organisms and is not inhibited by the presence of organic materials. Disinfected bedding will be composted, while facilities and equipment will be further high-pressure steam-cleaned. The GM streptococcal strains used for animal work will be extensively-characterised prior to introduction into animals, and will contain 'clean' gene mutations causing the abrogation of or reduction in the biological function of the encoded proteins. There will
be no exogenous DNA sequences present within these GM streptococci which are not naturally associated with wild-type strains. Consequently, these GM strains represent no risk to the environment beyond that associated with naturally-occurring strains, and hence the control measures proposed are equivalent to those used for work involving the Hazard Group 2 wild-type parent strains.

The Moredun GMSC discussed and approved the risk assessment for this connected program if work (Our ref: GM13.24). The committee agreed that the only GMOs derived from wild-type strains that have been shown not to be shed in urine or faeces should be employed in cattle studies. They also agreed that only GM strains that were predicted from in vitro studies to have lower or at least no greater than wild type virulence would be employed in any in vivo studies.

Project Containment

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Project Ref 172/13.2

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<td>Evaluation of safety and immunogenecity of a vectored vaccine in horses</td>
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<tr>
<td></td>
<td>Class 2</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Withdrawn N | Tick if notifying a connected programme of work N
### Project Additional Information

#### Purposes of the contained use

This new vaccine contains the Glycoprotein G from the rabies virus and will be used to immunise horses against rabies. Rabies is 100% fatal in horses and infected horses can theoretically infect humans through bites. A vaccine to immunise horses against the virus would therefore be of significant benefit to both horses, and potentially humans.

#### Recipient or parental organism

The parental strain is Parapoxvirus ovis which has been highly attenuated by passaging 135 times in embryonic lamb kidney cell cultures. The final harvest was plaque purified and cultured 38 times in bovine embryonic lung (EBL) cells. The virus harvest from the 38th pass was used to inoculate Bovine Kidney Cells (BK-KL-3A) and identified as Orf, 38.BEL/1.BK-KL-3A, A11070, 18 April, 1994. This material was then passaged five times on Bovine Kidney Cells (BK-KL-3A) then the virus was adapted to Vero cells. The virus was plaque purified three times by end point dilution. The plaque purified virus was used for the generation of beta galactosidase recombinant parapoxvirus. The beta galactosidase recombinant parapoxvirus was used for the generation of a recombinant parapoxvirus expressing the G gene of the vector virus.

#### Host/vector system

The parapoxvirus strain used as a vector was extensively attenuated by:

- more than 170 passages leading to attenuation by rearrangement of limited terminal regions of the genome which is accompanied by gene deletion (E2L) (Cottone et al., 1998).
- the substitution of the amino acid isoleucine (position 115) in the viral IL-10 (virulence factor) which is described to be very critical for retaining the stimulatory activity of human IL-10 (Rziha et al., 2003).
- deletion of the VEGF-E gene (virulence factor of parapoxviruses) and three further regions of its genome (Rziha et al., 2000).

#### Origin & function

The gene inserted in the parapox vector virus is the rabies virus G gene. Expression is under control of a synthetic early-late poxvirus promoter eIP-1 designed for protein expression in the mammalian cell. No expression will occur outside of the mammalian cell host. The intended function is to elicit a protective immune response against rabies virus infection.

#### Evaluation of foreseeable effects

When compared to the parental strain, the vaccine candidate shows attenuation in vivo and is considered to be less hazardous than the parental strain.
Although the parapoxvirus vector was demonstrated to be safe in permissive and non-permissive animals (see above), there is no data available for humans. However, the risk of infection to humans near the site would be effectively zero, except for the risk of needlestick injury when the animals are injected with the vaccine candidate. In humans, direct contact with another infected person or animal is the normal route of infection with virulent field virus and involves a substantial transfer of infectious virus through a cut or other break in the skin. Due to its high attenuation the vector used in this study is avirulent in the most permissive species, sheep. In addition, horses are a non-permissive species for parapoxviruses which mean that the vector will not replicate and be shed by the host.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**N/A**

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We request derogation from full category 2 facilities for the animal experiments.

The animals will be housed under category 2 containment conditions in except that an autoclave is not available in the same building. We request derogation such that material leaving the containment facility may be bagged, sprayed with 70% ethanol and transferred directly to the incinerator or autoclave.

Due to the more specific handling and housing requirements for horses there are only a few specific areas within the Moredun Group animal accommodation which are capable of housing horses. The accommodation that will be used is a self contained facility with secure access, changing facilities and a double barrier layer (internal pen within the interior of the building) which has sufficient facilities to allow safe handling and maintenance of horses.

Horses are non-permissive hosts for this virus and this will ensure that the GM virus will not be shed by the animals and therefore the bedding and animal waste will not be contaminated. For this reason we request derogation to allow bedding and animal waste to go to the midden for composting without prior treatment.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Sharps** will be stored in sharps bins and disposed of on site by incineration
- Clinical waste will be stored in clinical waste bags and disposed of by incineration.
- Providing derogation is granted, bedding waste will be disposed of by composting as the virus will not be shed by the animals and therefore the bedding will not be contaminated.
- All personnel will wear appropriate protective clothing which will be incinerated onsite on completion of sampling.
- Blood waste (contained in blood tubes) during the study will be disposed of into clinical waste bags which will be disposed of by incineration.
- At the end of the study the animals will be in the cat 2 animal accommodation and incinerated on site. Carcasses will be transported to the incinerator in a covered trailer which will be sprayed down with FAM afterwards.
- All samples collected during the study will be placed in sealed containers and then placed in sealed bags prior to transport to Moredun Scientific where they will be handled and stored under cat 2 containment conditions prior to shipment of the samples required by the sponsor for further analysis, e.g. sera. These should not contain any infectious material but nevertheless the samples will be shipped to the Sponsors facility as a "biological substance category B".

The controls that are proposed will ensure that the GMO is contained.

---

**Is an emergency plan required according to regulation 20?**  
**N**

**If yes, tick to confirm that it is attached to this form**  
**N**

**Tick to confirm that you have attached a risk assessment to this form**  
**Y**
The Moredun GMSC discussed and approved the risk assessment for this project (Our ref: GM13.25).
Given that the vaccine candidate does not replicate in the animal or human host the GMSC felt there was negligible risk from the GMO except at the time of injecting the animals. Even then the risk of harm arising from needlestick exposure to this GMO is very low.
It was discussed whether Category 2 containment measures were more than required but it was agreed that this was justified because the original parapoxvirus from which the attenuated vector virus is derived is classed as BSL2 under SAPO rules.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
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</tr>
<tr>
<td>Animal Units</td>
<td>L2 L3 L4 L2</td>
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<tr>
<td>Large Scale Activities</td>
<td>L3 L4 L3 L4</td>
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</tr>
<tr>
<td>Human Clinical Applications</td>
<td>L2 L3 L4 L2</td>
<td>L2 L3 L4 L2</td>
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</tbody>
</table>

Project Ref 172/14.1

Safety of a Salmonella enteritica serovar enteritidis wild type parent and gene-related mutant when administered to colostrum-deprived piglets

Class 2

< 1 Litre

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N

Project Additional Information

02/03/2022
**Purposes of the contained use**

The objective of this work is to confirm the safety of a mutant Salmonella enterica serovar enteritidis isolate, scheduled for vaccine development, when administered to colostrum-deprived piglets. The vaccine is intended for use in chickens, however, as chickens do not generally present clinical signs following S. enteritidis infection, it is necessary to conduct the testing in a sensitive species. Prior to any additional research work being conducted, it is necessary to confirm the safety of the modified isolate.

<table>
<thead>
<tr>
<th>Recipient or parental organism</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host/vector system</td>
<td>None</td>
</tr>
</tbody>
</table>

**Origin & function**

No genes have been inserted. A total of three genes have been deleted which are involved in the conversion of shikimic acid to chorismic acid, in recombination in the cell and regulation of genes associated with UV damage recovery and DNA repair, resistance to low pH and bile, and flagelin production.

**Evaluation of foreseeable effects**

Although the vaccine candidate has been shown to have attenuating characteristics relative to the originating wild type strain, no assessment has been carried out of its ability to infect or cause disease in humans. It will be assumed for the purposes of this study that human infection is a possibility and biosecurity and control procedures will be utilised to this effect.

Salmonella infection in humans and pigs may result in acute gastroenteritis with sudden onset of abdominal pain, diarrhoea, nausea and vomiting. Salmonella infections usually resolve in 5-7 days and often do not require treatment unless the subject becomes severely dehydrated or the infection spreads from the intestines. In colostrum deprived piglets these symptoms may be more severe and may result in the requirement for intervention by veterinarians.

The genetic modifications encompass the deletion of genes coding for proteins involved in the multiplication, survival or virulence of salmonellae in the host (mammal or chicken). As such it is anticipated that any alteration of existing traits will only give rise either to a deletant with an attenuated phenotype or of equivalent virulence to the parental strain.

The genes are only mobilisable by transducing phage or recombination. In the unlikely event of this happening the receiving strain would lose its ability to produce flagellin and or survive in vivo. This would not be considered as having the potential to increase its virulence.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We request derogation from full category 2 facilities for the animal experiments.

The animals will be housed under category 2 containment conditions except that an autoclave is not available in the same building. We request derogation such that material leaving the containment facility may be bagged, sprayed with an appropriate disinfectant (Anistel 1:100) or fumigated and transferred directly to the incinerator. Anistel is confirmed as effective against a wide range of microorganisms including Salmonella spp.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Salmonella enteritica serovar enteritidis is classified as an ACDP hazard group 2 organism. The proposed deletions will not increase this classification, and may reduce it.
However, as the strains are untested in humans the containment level will be maintained as level 2.

Animals will be housed in specific pathogen free (SPF) rooms within the High Security unit (HSU) at Moredun and waste will be disposed of following the standard waste management procedures within the unit. Solid laboratory waste will be disposed of by incineration following disinfection in Anistel (1/100) overnight. Liquid waste will be disinfected with Anistel (1/100) overnight. Anistel (1/100) has a wide spectrum of activity and is effective against Salmonella spp in both liquid and solid waste at this concentration (see information attached for activity and dilution details from the manufacturer). Dilution for the different materials will be as specified in manufacturers instructions.

The animal phase of the work will be conducted in SPF rooms following the standard containment procedures which should be more than sufficient to provide appropriate level of protection. All personnel entering the animal rooms will be required to wear FFP3 face masks and gloves as well as Tyvek suits and wellington boots which will remain in the rooms. All handling of infected animals will be conducted in the SPF rooms by suitably trained staff. The animals will be inoculated by the oral route using graduated syringes and applicators as appropriate.

All animal waste material produced, including bedding will be bagged and either surface sprayed with a disinfectant overnight (Anistel 1:100) or fumigated then incinerated as per standard procedures. In the laboratory, all material will be handled under a Class II microbiological cabinet with gloves and lab coats being used. Any consumable items that are used will be disinfected in Anistel (1/100) overnight followed by disposal into clinical waste bins which will be incinerated onsite. All animal products (i.e. faeces) will be disposed of in clinical waste bags for incineration.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tr>
<td>L2 Yes L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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Animal Units

| L2 Yes L3 L4 L2 L3 L4 L2 L3 L4 |

Large Scale Activities

| L3 L4 L2 L3 L4 L2 L3 L4 |

Human Clinical Applications

| L3 L4 |
### Project Additional Information

**Purposes of the contained use**

Pleuropneumoniae caused by Actinobacillus pleuropneumoniae (APP) is a severe disease in pigs which has a significant impact on the pig industry. Therefore, a high demand for a low cost and efficacious vaccine against all APP serotypes exists. In this project a number of genetically modified vaccine strains for induction of protection against APP will be tested to confirm whether it induces an immune response and subsequently the vaccines may also be tested to confirm whether it is effective in the prevention or reduction of clinical symptoms / pathology following artificial challenge with Actinobacillus pleuropneumonia.

**Recipient or parental organism**

- **Strain 1:** Salmonella typhimurium SL1344 parental strain (not attenuated) isolated from infected cattle (Hoiseth et al. 1981 Nature). S. typhimurium is a leading cause of human gastroenteritis and is used as a mouse model of human typhoid fever. Strain SL1344 is a histidine auxotroph generated from strain ST4/74 which was originally isolated from the bowel of a calf with Salmonellosis.

- **Strain 2:** Escherichia coli strain 5 (isolated from tonsils of healthy pigs). The strain is not attenuated in any way.

**Host/vector system**

GM vector bacteria were developed from the above S. typhimurium and E. coli strains by genetically modifying them to remove the gene cluster encoding the O-antigen biosynthetic pathway (rfb) so that they do not express endogenous O-antigen.

**Origin & function**

- **Kan resistance gene:** aminoglycoside phosphotransferase
A gene cluster encoding the O-antigen biosynthetic pathway of A. pleuropneumoniae was inserted, replacing the endogenous equivalent in each recipient strain. The intended function is to express the A. pleuropneumoniae specific O-antigen on the surface of the bacterial vector as a potential vaccine against APP.

ApxI, II or III truncated proteins (ApxI/II/III surf.fusion derived from APP).

All Apx toxoids are considered as virulence factors acting in the lung on destroying lung tissue and killing neutrophils and macrophages. These toxins are considered to be a major player in typical lung lesions observed for APP infection. ApxI and ApxII are hemolytic and cytotoxic. ApxIII is strongly cytotoxic to porcine neutrophils and pulmonary alveolar macrophages. However, truncated proteins will be expressed according to the following publications: ApxI truncation: Seah et.al. Nov. 2002 Infection and Immunity p. 6464-6467; ApxII truncation: Kim et.al. 2014 Bioscience, Biotechnology, and Biochemistry (74) p. 1362-1367; ApxIII truncation: Kwang 2004 Vaccine 22 (2004) 1494-1497. The truncated proteins are expected to induce neutralising antibodies but are not expected to exhibit any toxicity. No side effects were reported when used for vaccination in mice.

**Evaluation of foreseeable effects**

The E. coli_5 strain was an isolate from healthy pigs however, as a potential human pathogen, the isolate is characterised as ACDP 2. For pigs this strain was harmless, however a risk for humans cannot be excluded since there are no data or cases described.

Salmonella typhimurium is listed as a human pathogen hazard group 2 in the ACDP list. It is considered that as a maximum, infection of humans may result in similar symptoms to wild type Salmonella typhimurium infection.

The integrated O-antigen biosynthesis genes in the genome do not encode for toxins, potential oncogenic proteins, allergens or growth modulators. These genes are solely present for the synthesis of specific glycans.

The single sugars synthesized get linked to each other and transferred onto Lipid A. This glycan – lipid A complex defines the lipopolysaccharide (LPS).

The integrated Apx toxoids are derived from cytotoxic, haemolytic precursor proteins. The truncation of these are expected to result in loss of toxicity according to the literature however the truncated proteins bound on the surface of vaccine strains have not yet been tested for their toxic potential. One version of the Apx toxoids (ApxII) was tested in its truncated version by injection into pigs in an animal trial performed by Malcisbo. No side effects were observed when pigs received a dose of 400ug protein.

A risk of the transfer of the Kan resistance gene to wild type bacteria via horizontal gene transfer cannot be completely excluded but the likelihood of this is very low as the resistance gene is integrated into the bacterial genome rather than a plasmid. Housing of the animals in contained BSL 2 accommodation will further reduce the risk of any potential transfer to wild type bacteria.

The risk of horizontal gene transfer of the O-antigen biosynthesis cluster of APP2 and the truncated Apx toxoids is the same as described for the Kan resistance gene. The replacement of the E.coli O-antigen biosynthesis gene cluster with the ApxI/II/III truncated proteins is not expected to increase the risks associated with infection by the GMO compared to the host strain. When the ApxII truncated protein purified from the bacteria was injected into pigs at a dose of 400ug, no clinical symptoms were observed. However, while confirmed to not be hazardous to pigs, no additional testing has been carried out to determine whether the encoded truncated Apx toxoids could potentially be harmful for animals or insects.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Laboratory waste (consumables such as pipette tips) will be disinfected for a minimum of 24 hours, in a microbiology class 2 safety hood, using a laboratory disinfectant (Anistel at 1/100 dilution), and then added to clinical waste bags which will be sealed and then incinerated either on site or at an approved off site facility as appropriate. As the material will be incinerated no process testing or monitoring measures will be put in place. It is expected that this method will result in 100% kill of the GM material.

Animal bedding waste will be double bagged in clinical waste bags, in the animal accommodation, which will be surface decontaminated following local protocols (sprayed with Anistel at 1/100 dilution and left overnight in the accommodation), before being incinerated either on site or at an approved off site facility as appropriate. As this material will be incinerated no process testing or monitoring measures will be put in place. It is expected that this method will result in 100% kill of the GM material.

Animal carcasses will be double bagged in clinical waste bags while in the animal accommodation (which will be surface decontaminated as above) before being incinerated either on site or at an approved off site facility as appropriate. As this material will be incinerated no process testing or monitoring measures will be put in place. It is expected that this method will result in 100% kill of the GM material.

The risk assessment entitled “Potential vaccine candidate testing against APP2 infection” (Our ref: GM18.04) was reviewed by the Moredun Research Institute GMSC. It was agreed that CL2 containment is appropriate for this work.

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Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment entitled “Potential vaccine candidate testing against APP2 infection” (Our ref: GM18.04) was reviewed by the Moredun Research Institute GMSC. It was agreed that CL2 containment is appropriate for this work.

02/03/2022
**Project Ref** 172/20.1

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<td>Genetic manipulation of Toxoplasma gondii to determine gene and protein function</td>
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The purpose of this work is to identify and understand proteins of the protozoan parasite Toxoplasma gondii that perform a key role in the parasite's biology, in pursuit of antigens that can be used in the development of a novel vaccine to protect against infection. The removal of specific T. gondii genes for the development of attenuated strains.

**Recipient or parental organism**

Toxoplasma gondii

**Host/vector system**

Mice and sheep

**Origin & function**

Published CRISPR/Cas9 plasmids adapted for use in Toxoplasma gondii will be used in this work (e.g. Brown et al, Bio Protoc, 2018; Guerra et al, PLoS Path, 2018). These plasmids contain the Cas9 gene and sgRNAs (guide RNAs) under the expression of T. gondii promoter sequences. They also contain ampicillin resistance markers for propagation in E. coli. The 20bp sgRNA sites within these plasmids will be modified by PCR to generate sgRNA sites for T. gondii genes-of-interest. Following tranfection in T. gondii, expressed sgRNAs and Cas9 will bind to specifically designated sites in the T. gondii genome and facilitate a double strand break. This work will be performed in T. gondii that have already been modified to lack the ku80 gene, meaning that non-homologous recombination does not take place in these
background strains. Therefore, it will be necessary to co-transfect these strains with a repair template. Repair templates will be generated to facilitate positive selection of genetically modified T. gondii. These repair templates will be designed with 5' and 3' homology arms flanking gene sites targeted for CRISPR/Cas9 DNA breakages. They will contain a sequence encoding genetic tags (e.g. HA, myc, GFP, YFP) or a resistance marker (HXGPRT, providing resistance to mycophenolic acid or pyrimethamine-resistant DHFR, DHFR*). Resistance markers will facilitate positive selection of modified strains when cultured in the presence of mycophenolic acid or pyrimethamine. Because pyrimethamine is the primary treatment for toxoplasmosis, other selectable markers will be considered before the use of the pyrimethamine-resistance DHFR cassette. Where possible, Cas9 plasmids transiently expressing a bleomycin-resistance gene (Guerra et al, PLoS Path, 2018) will be used for strain selection, given the transient nature of resistance in these transfected populations (as opposed to permanent resistance).

**Evaluation of foreseeable effects**

The GMMs generated in this work are not expected to gain virulence. This is because tagging of the genes with commonly used non-hazardous tags should not provide a gain-of-function. Furthermore, the deletion of genes predicted to be essential in T. gondii should result in attenuation of the parasite and complementation of deleted genes is not expected to increase parasite virulence compared to wild-type strains. However, insertion of a resistance markers (e.g. DHFR* cassette) will result in the development of drug-resistant parasites (e.g. pyrimethamine resistance in the case of DHFR* cassette integration). This approach has become an established protocol worldwide in labs implementing CRISPR/Cas9 gene editing in T. gondii (Shen et al, 2014, mBio; Sidik et al, 2016, Cell; Sidik et al, 2018, Nature Protocols). Pyrimethamine is an inhibitor of dihydrofolate reductase. Treatment with this inhibitor blocks the folic acid synthesis pathway and therefore interferes with DNA and RNA synthesis, resulting in parasite death. Parasites containing a DHFR* gene will no longer die in response to pyrimethamine treatment.

Application of Δku80 T. gondii parasites as background strains is now widespread, with many studies documenting their use as a background for more efficient genetic modification of the organism. For example, it is stated in a report by Fox et al (2011, Eukaryotic Cell) that “a Δku80 genetic background enables a higher-throughput functional analysis of the parasite genome to reveal fundamental aspects of parasite biology controlling virulence, pathogenesis, and transmission”. They also show in this study that the number of parasite cysts present in the brains of mice is not significantly different between Pru (wild type) and a Pru Δku80 strain. Huynh and Carruthers (2009, Eukaryot Cell) also directly showed that deletion of the ku80 gene in T. gondii (RH strain) increases transfection efficiency with inserted genes and gene tags, due to an ablation of non-homologous recombination in Δku80 parasites.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

For in vitro and ex vivo work:

Toxoplasma gondii is in biological hazard group 2. All work with the parasite will be performed in a containment level 2 laboratory. Parasite samples will be decontaminated with 1% virkon for a minimum of 60 minutes, liquid waste decanted and discarded down the drain and solid waste subsequently autoclaved. Note, in an in vitro assay to demonstrate effective killing with virkon, no parasites were found to be viable (unable to invade and replicate within host cells) following treatment with 0.1% and 1% virkon for 1 hour. Therefore, treatment with virkon was found to be an effective method for killing T. gondii parasites in vitro (performed by Dr David Smith at Moredun Research Institute, 2020).

For in vivo work:

Any animals infected with GM T. gondii strains generated in this work will be euthanized and destroyed following experimentation and therefore GM strains will not be able to enter the environment. Euthanized mice and sheep will be disposed of by incineration according to section 12 of Moredun Standard operating procedure CLIN/PROC/018:Handling, transfer and Disposal of Waste Containing Hazard Group 2 Pathogens generated within the HSU.

It is also worth noting that non-felid mammalian hosts are "dead end" hosts for T. gondii, in that the parasite cannot be shed from the host and therefore will not enter the environment.
The Moredun Research Institute Genetic Manipulation Safety Committee reviewed the risk assessment and agreed that CL2 is appropriate for this work.

### Project Containment

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<tr>
<td>L2</td>
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<td>L4</td>
</tr>
</tbody>
</table>

- **Animal Units**

| L2 Yes                | L3           | L4           |
| L2                    | L3           | L4           |

### Project Ref

**172/21.1**

- **Assessment of efficacy of genetically modified Campylobacter jejuni vaccine strains in broiler hen model**

**Class**

- Class 2

**CultureVol**

- < 1 Litre

**Class Culture Vol**

- Class Culture Vol

**Class Volume Class**

- Class Volume Class

**Non-GMM**

- Consent Granted

**Date Ackn'd**

- 21/05/2021

**Date Project Ceased**

- 

**Withdrawn**

- No

**Historical Significant Changes**

- 

**Historical Date of Additional Info**

- 

**Significant Change ID**

- 

**Date of Significant Change**

- 02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The objective of this study is to provide information relating to the efficacy of a number of genetically modified Campylobacter jejuni vaccines strains (Salmonella Typhimurium presenting C. jejuni N-glycan on its surface) in the control of Campylobacter infection and shedding in broiler chickens.

Campylobacter species including C. jejuni, are endemic in many chicken flocks in the UK and worldwide (including both layer breeds and broiler breeds). While these bacteria rarely cause clinical disease in the birds, except in cases where the birds are under stress or have preexisting clinical conditions, they are commonly associated with cases of food poisoning in humans through contact with infected eggs and meat, often due to insufficient cooking. A Campylobacter vaccine, capable of reducing bacterial shedding by birds would reduce environmental contamination which would potentially reduce the risk of zoonotic infection.

**Recipient or parental organism**

Salmonella Typhimurium SL1344 parental strain isolated from infected cattle (Hoiseth et al: 1981 Nature); SL1344 is the genetic marked version of the parental strain. Salmonella Typhimurium SL1344 has a natural streptomycin resistancy (encoded on the plasmid pRSF1010).

**Host/vector system**

None

**Origin & function**

**Vaccine 1:**

The following genes of C. jejuni N-glycan biosynthesis cluster were integrated downstream of pagC in a non-coding area of the parental strain (S. Typhimurium SL1344):

- gene: gne  UDP-Glc 4-epimerase
- gene: pglK  ABC-transporter
- gene: pglH  (1→4)-GalNAc transferase
- gene: pglJ  (1→3)-Glc transferase
- gene: pglB  oligosaccharyltransferase
- gene: pglC  Undecaprenyl phosphate bacillosamine 1-phosphate transferase

Tetracycline resistance gene: Tetracycline resistance efflux pump

The natural promoter of the C. jejuni N-glycan biosynthesis cluster was exchanged with the proD promoter (Davis et al. 2010, Nucleic acids research, Vol. 39(3), p1121-1141).

ATPase (apy) encoded in the genome of vaccine 1:

-ATPase gene fused C-terminally with an HIS (histidine) tag is integrated in the non-coding region downstream of the rfaL gene of SL1344

**Vaccine 2:**

The following genes of C. jejuni N-glycan biosynthesis cluster were integrated downstream of pagC in a non-coding area of the parental strain (S. Typhimurium SL1344):

- gene: gne  UDP-Glc 4-epimerase
- gene: pglK  ABC-transporter
- gene: pglH  (1→4)-GalNAc transferase
- gene: pglI  (1→3)-Glc transferase
The integration of the ATPase apy should, based on literature (Proietti et al. 2019 Nature Communications (10)250), increase the recognition of the S. Typhimurium vaccine.
strain in the gut of chicken and increase the rate of clearance of the bacteria from the gut. This hypothesis needs to be verified in the planned animal trials. The deletion of the tetracyclin and streptomycin resistance in vaccine 2 and 3 results in the sensitivity of these strains towards both antibiotics.

A risk of the transfer to wild type bacteria via horizontal gene transfer cannot be completely excluded but the likelihood of this is very low as for all three vaccines, the genes/gene cluster are integrated into the bacterial genome rather than a plasmid. Housing of the animals in contained BSL 2 accommodation within the high security unit will further reduce the risk of any potential transfer to wild type bacteria.

The modifications proposed are expected to result in GMOs of similar potential hazard as the wild-type counterparts. Containment Level 2 accommodation should be enough to minimize the risk to human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory Waste. Laboratory waste, including liquid waste will be disinfected using Anistel at a 1:100 dilution for a minimum of 5 minutes. Laboratory waste will be placed in sealed containers (sealed bottles or clinical waste bags –double bagged) which will be sprayed with 1:100 Anisitel once sealed. Containers will be placed in locked clinical waste bins for removal and disposal by an approved external commercial contractor.

Bird carcasses will be double bagged in yellow clinical waste bags which will be sprayed with 1:100 dilution of Anistel and then placed in sealed plastic containers for disposal by an approved external commercial contractor. As birds don’t produce large volumes of liquid waste, blocking the drains in animal pens and use of absorbent bedding material (sawdust) will be sufficient to absorb any liquid waste. Bedding will be double bagged in yellow clinical waste bags which will be sprayed with 1:100 dilution of Anistel and then placed in sealed plastic containers for disposal by an approved external commercial contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Moredun Research Institute Genetic Manipulation Safety Committee have reviewed your risk assessment “Assessment of efficacy of genetically modified Campylobacter jejuni vaccine strains in a broiler hen model” (Our ref GM21.03) and have agreed with your assessment that the work should be done at containment level 2. Please note that a notification to the HSE is required and the work must not commence until acknowledgement of receipt of the notification by HSE is received.

Project Containment
Project Ref 172/96.1

Date Ackn’d 08/08/1996

CU2 Project Title
ATTEMPS TO RAISE ANTIBODIES AGAINST PROTEINS OF MEDICAL/VETERINARY IMPORTANCE, INITIALLY AN EPITOPE FROM THE C.PSITTACI MOMP GENE

Class 3

Non-GMM
Consent Granted yes

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
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Animal Units

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<tr>
<th>Large Scale Activities</th>
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Project Ref 172/96.2

Date Ackn’d 03/10/1996

CU2 Project Title IDENTIFICATION OF VIRULENCE FACTORS ENCODED BY THE PARAPOX

Class 2
Date Project Ceased

Non-GMM

Consent Granted

not applicable

Tick if notifying a connected programme of work

N

Project notified under transitional arrangements

Y

Withdrawn

N

Historical Significant Changes

GM172/99.1, GM172/00.1,

Historical Date of Additional Info

07/01/1999, 07/03/2000,

Significant Change ID

172/96.2.a

Date of Significant Change

23/04/2009

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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</table>

Project Ref 172/97.1

Date Ackn'd 30/01/1997

Date Project Ceased

VIRULENCE STUDIES WITH KNOCK-OUT MUTANTS OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS

Class CultureVolClass2 CultureVolumeClass3-4

Class 3

Non-GMM Consent Granted yes

Project notified under transitional arrangements

Withdrawn N

Historical Significant Changes GM172/02.4

Historical Date of Additional Info 11/12/2002

Significant Change ID

Date of Significant Change

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref**  172/97.2

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**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**

---

02/03/2022
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref 172/97.3

Date Ackn'd 08/10/1997

CU2 Project Title Genetic manipulation of Pasteurella haemolytica using modified native plasmid DNA

Class 3

CultureVolClass2

CultureVolumeClass3-4

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref 172/trans1

Date Ackn’d 24/12/1993

CU2 Project Title

GENERATION OF MAMMALIAN CELL LINES BY TRANSFORMATION USING RETROVIRAL -MEDIATED TRANSFER OF GROWTH PROMOTING GENES

Class 2

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
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Name

**INSTITUTE OF CANCER RESEARCH**

Name 2

**ROYAL CANCER HOSPITAL**

Campus Estate or Research Centre

Building

Road Name

**15 COTSWOLD ROAD**

District

**BELMONT**

Town

**SUTTON**

County

**SURREY**

Postcode

**SM2 5NG**

Country

**ENGLAND**

Tel Number

0208643 8901

Fax Number

0208 642 9598

E-mail

HSE Division

**EAST AND SOUTH EAST**

Comments

Date at Which Additional Info Submitted

02/03/2022
**Premises Addresses**

<table>
<thead>
<tr>
<th>Date</th>
<th>Name</th>
<th>Department</th>
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<th>Building</th>
<th>Road Name</th>
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<td>21/06/2007</td>
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<td>15 COTSWOLD ROAD</td>
<td>BELMONT</td>
<td>SUTTON</td>
<td>SURREY</td>
<td>SM2 5NG</td>
<td>ENGLAND</td>
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</table>

**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.

---

Project Ref 173/04.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>Culture Vol</th>
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<tr>
<td>08/06/2004</td>
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Date Project Ceased: 21/06/2007

Withdrawn: N

Historical Significant Changes: TRANSFERRED TO GM 80 (21/6/07).

Project Additional Information
### Purposes of the contained use

To prevent exposure of the workers to class 2 GMM

### Recipient or parental organism

Commercially available replication-defective serotype V adenoviral expression system (e.g., Becton Dickson AdenoX system) lacking E1 and E3 genes.

HEK 293 cells which express E1 genes necessary for packaging the virus will be transfected with adenoviral DNA to generate a replication-defective adenovirus.

The virus will be used to infect primary cultures of ovarian cancer and cancer cell lines.

### Host/vector system

Adenovirus as described above.

### Origin & function

Adenovirus as described above.

### Evaluation of foreseeable effects

No foreseeable effects are anticipated with the use of replication-defective adenovirus type V per se. Adenovirus type V is a common respiratory pathogen, and immunity in humans is believed to be lifelong. The parental virus is considered a class 1 agent.

Therefore, any hazard that arises will depend on the nature of the insert. The insert will be shRNA directed to genes identified through micro-array analysis of ovarian cancers and the work to identify these genes is ongoing. A risk assessment will be conducted for each gene which we intend to knockdown, once the identity of the genes is known. However, since the genes identified by the microarray studies are expected to promote a malignant phenotype, interfering with their expression by RNAi is unlikely to promote malignancy. Nevertheless, we may choose to inhibit the expression of genes whose function is not fully understood and for which the effects are not wholly foreseeable.

### Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

Not relevant.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Work will be conducted within a containment level 2 laboratory using a class 2 microbiological safety cabinet. Liquid waste will be treated with chloros at a final concentration of 3% for 12 hours prior to sterilisation by autoclave. Solid waste will be soaked in 10% chloros overnight. Both solid and liquid waste will be collected by the central sterile supplies department and sterilised by autoclave.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y
The risk assessment has been reviewed by the local GMM Committee who have made no additional comments that have not been included in the risk assessment.

Project Containment

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Project Ref 173/07.1

The aim of the project is to use genetically engineered Lentiviruses to produce metastatic models that express a Luciferase marker enzyme and enable the tumours to be visualised in vivo. Cell signalling pathways that are involved in maintaining the metastatic phenotype will be

Class 2  < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

TRANSFERRED TO GM 80 (21/6/07).

purposes of the contained use

To prevent exposure of the workers to Class 2 GMM.
Recipient or parental organism

E.Coli (recA)
DH5 alpha
A commonly used strain with a long history of safe use.

Host/vector system

The following plasmids will be utilised
pFIV-34N
PVSV-G
pSIF1-H1-Puro™shRNA Expression Vector

Origin & function

Our intention is to introduce the luciferase gene into the appropriate established tumour cell lines using an Expression system based upon a Feline Immunodeficiency Virus (FIV) obtained from a commercial source (System Biosciences Inc). The FIV lentiviral expression systems circumvent the possible recombinant issues that normally arise with the use of HIV vectors. We also intend to use the FIV-based short hairpin RNA and double promoter – siRNA Cloning and Expression Vectors that will target selected gene sequences and enable us to produce stable tumour cell lines.

To create functional pseudoviral particles, the lentivector containing the expression construct is co-transfected with the packaging plasmids into the producer cell line. The packaging cells replicate the expression construct and package it into the pseudoviral particles. The pseudoviral particles will then be used to infect (or transduce) target cells and express effector or reporter molecules but cannot replicate within target cells because the viral structural genes are absent and the LTRs are designed to be self-inactivating upon transduction.

Following transduction into the target cells, the expression cassette is reverse transcribed and integrated into the genome of the target cell. After integration, the expression cassette continuously and stably produces high levels of effector or reporter molecules in target cells. Target cells stably expressing the effector molecule will be isolated using the selectable marker contained in the expression vector construct (e.g. puromycin or copGFP). The transduced cells will be assayed for gene breakdown or luciferase production using specific assays, immunoblotting or by flow cytometry. Infected cells will be assayed in vitro and in vivo to determine the effect of knock down or expressed genes on their metastatic potential.

The cell lines that will be transduced are all established human tumour cell lines either derived by the Tumour Biology and Metastasis Team or from ATCC. These will include MDAMB435 (breast), DU145 (prostate), MTSP 12-H (mesothelioma)

Evaluation of foreseeable effects

The feline immunodeficiency virus (FIV) was originally isolated from cat blood. Despite common close exposure of humans to FIV through contact with domestic cats (including bites, scratches, etc), no human infection or disease has ever been associated with FIV.

The lentiviral expression systems are made with a split-genome conditional packaging system which acts as a built in device against the generation of productive recombinants. The number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev), and the corresponding proteins are expressed from different plasmids (for HIV-based packaging plasmids) lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector, to prevent generation of recombinant replication-competent virus. None of the HIV-1 genes (gag, pol, rev) will be present in the packaged viral genome, as they are expressed from packaging plasmids lacking packaging signal – therefore, the lentiviral particles generated are replication-incompetent.

The packaging plasmids are transfected into the 293TN producer Cell Line. In the unlikely event these cells were accidentally injected the risk of pathogenesis is very low due to the recognition of the cells by the immune system as being foreign.
A deletion in the enhancer of the U3 region of 3' LTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**Evaluation of foreseeable events:**

The feline immunodeficiency virus (FIV) was originally isolated from cat blood. Despite common close exposure of humans to FIV through contact with domestic cats (including bites, scratches, etc), no human infection or disease has ever been associated with FIV.

The lentiviral expression system are made with a split-genome conditional packaging system which acts as a built in device against the generation of productive recombinants. The number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev), and the corresponding proteins are expressed from different plasmids (for HIV-based packaging plasmids) lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector, to prevent generation of recombinant replication virus. None of the HIV-1 genes (gag. Pol, rev) will be present in the packaged viral genome, as they are expressed from packaging plasmids lacking packaging signal - therefore, the lentiviral particles generated are replication- incompetent. The packaging plasmids are transfected into the 293TN Producer Cell Line. In the unlikely event these cells were accidentally injected the risk of pathogenesis is very low due to the recognition of the cells by the immune system as being foreign. The infected human cells would be unlikely to be pathogenic due to rapid complement mediated lysis of these cells when recognized by the immune system as of non-self origin. Cells would not be shedding virus as they are replication-defective and therefore do not pose a risk to human health or the environment. In our risk assessment, the modified viruses should not pose a serious risk to the environment. These viruses are extremely unstable, inactivated by detergent, UV light and ethanol, and would not survive outside of the laboratory environment. Animals containing the previously infected human cells are safe to the environment as they would be unlikely to survive outside the laboratory because of their severe immune defect. There is no potential to transfer the GMO to other organisms outside of the experimentally manipulated procedures outlined.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Protective clothing and gloves will be worn at all times. All staff are trained in the good laboratory practise and are fully aware of the Institute local rules for containment level 2 laboratories. Work surfaces, including the inside of microbiological safety cabinets, will be suitably disinfected after use. Centrifugation will only be performed in sealed tubes to prevent aerosols. Sharps will not be used. Liquid waste, such as cell culture medium, which may contain virus or cells, will be rendered safe for disposal by overnight treatment with a QAC based disinfectant (Tresolin K). Plastic pipettes will be soaked in 5% Chlors prior to removal by CSSD. Solid waste will be placed in autoclave bags for collection by CSSD. Material collected by CSSD will be autoclaved on site. The cleaning, disinfecting and disposal procedures will be documented and displayed. The biological spillage procedure will be displayed and HAZ-TAB disinfectants will be available for immediate use. Supernatant will be collected and human tumour and epithelial cells will be exposed to the supernatant in conditions to promote infection. Virus preparation will be certified free of replication competent virus (see below). These cells will then be assayed in vitro and/or injected into animals to measure their oncogenic potential.

Once the lentivirus has infected the cells it should not be able to replicate. In order to test this we will plate NIH3T3 (murine) or ECV304 (Human) cells into 6 well plates (5x10^4/well). Next day we will add 1ml of either fresh or frozen (-70°C) viral supernatant (supernatant 1) (Vector+gene+GFP) plus 2g of polybrene (8mg/ml stock) and incubate overnight. The following day the cells are washed with medium and replaced with fresh medium. After 24 to 48 hours the infectivity will be assessed by assaying the % positive GFP cells. The cells will grow for a further 4 to 6 days (splitting cells if necessary) and overnight supernatant harvested (supernatant 2) from confluent cells. This will allow amplification of the virus.

Plate NIH3T3 or ECV304 cells in a 6 well plate (5x10^4/well) and leave overnight. Add supernatant 2 plus polybrene as before. In addition include as a positive control supernatant 1 plus polybrene. Wash cells next day and add fresh
Question: why are NIH3T3 or EC304 cells being used for the viral activity assay? Are these the cell lines in which studies will be performed?

I thought human tumour cell lines were going to be used for these studies in which case shouldn't these be the cell lines tested for viral activity?

Answer:
Your assumption is correct that no viral particles should be present. This assay is about ensuring that any viral particles there would be detected. I understand that these cell lines are highly sensitive to infection by any virus. DH5 á

It is not clear to me from the proposal what their animal models or the cell lines actually are.

I am only being vague on this issue because we have many animal models and cell lines that we hope to use and I don't wish to be limited by naming just a few.

Initially there will be MDAMB435 (breast), DU145 (prostate), MSTO 12-H (mesothelioma)

The work is categorised as containment level 2 and yet there are not any level 2 labs in the McElwain.

The proposed laboratory (room 019) has been inspected by members of ICR's Health and Safety and has been

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Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Animal Units</th>
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Project Ref 80/transA

Date Ackn'd 02/03/2022
CU2 Project Title
Class
CultureVolClass2
CultureVolumeClass3-4
INFECTION OF MAMMALIAN HAEMATOPOIETIC CULTURES & PRIMARY CELL LINES USING AMPHOTROPIC RECOMBINANT RETROVIRAL PACKAGING STRAINS EXPRESSING GROWTH FACTORS RECEPTORS OR CYTOPLASMIC PROTEINS

Date Project Ceased
21/06/2007

Withdrawn
N

Tick if notifying a connected programme of work
N

Historical Significant Changes
GM80/00.1 - PROJECT TRANSFERRED FROM GM CENTRE 80 (6/6/06)

Historical Date of Additional Info
24/1/94, 17/07/2000

Project notified under transitional arrangements
Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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**Name**

BARTS & THE LONDON SCHOOL OF MEDICINE & DENTISTRY

**Name 2**

CENTRE FOR INFECTIOUS DISEASE

**Campus Estate or Research Centre**

INST OF CELL & MOLECULAR SCIENCE

**Road Name**

4 NEWARK STREET

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

E1 2AT

**Country**

ENGLAND

**Tel Number**

020 7882 5555

**Fax Number**

0207 882 2181

**HSE Division**

LONDON

**Comments**

CLOSED AND MERGED WITH GM774

**Date at Which Additional Info Submitted**

28/11/2001
## Premises Addresses

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## Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee
<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
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<td>Growth Room</td>
<td>Glass House</td>
<td>Large Scale</td>
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</table>

Other (please specify)  

- **Bacteriology**  
- **Parasitology**  
- **Transgenic**  
  - Birds  
  - Animals  
  - Invertebrates  
- **Virology**  
- **Transgenic**  
  - Animals  
  - Fish  
- **Mycology**  
- **Transgenic**  
  - Plants  
  - Other (please specify below)  

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### Project Additional Information

#### Purposes of the contained use

#### Recipient or parental organism

#### Host/vector system

#### Origin & function

#### Evaluation of foreseeable effects

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 174/01.1

Date Ackn'd 25/07/2001

CU2 Project Title CLONING OF POTENTIAL VIRULENCE DETERMINANTS FROM PORPHYROMONAS GINGIVALIS, IN ESCHERICHIA COLI, AND GENERATION OF ISOGENIC MUTANTS

Class 2

CultureVol

Class2 1-50 litres

CultureVolumeClass3-4

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info MERGED WITH GM774
**Project Additional Information**

### Purposes of the contained use

- Molecular cloning and expression, of Porphyromonas gingivalis genes encoding extracellular protease, enzymes involved in protein modifications, lipopolysaccharide biosynthesis, and outer membrane proteins in Escherichia coli.
- Manipulate the above constructs for insertional mutagenesis of genes putatively involved in protease and outer membrane protein expression, and post-translational modification and re-introduce into Porphyromonas gingivalis.

### Host/vector system

**Escherichia coli** K12 derivitives (XL-1 Blue, XL10 Gold, SCS110, SURE, Stratagene), general cloning hosts, will be used. The strains are attenuated, sensitive to bile salts and are unable to survive in the alimentary canal. Porphyromonas gingivalis (see above) will be used for complementation of mutated genes.

E. coli - Plasmids pUC18 - derived (Amp(r), LacZa - pUC18, pUC18notI, pK18) will be the general cloning and manipulating vectors. For expression of poly his-tagged recombinant proteins JFQ- derivitives (commercially known as pQE80, Qiagen) will be used to direct controlled (inducible by Isopropyl-D thiogalactoside, IPTG) expression under ptac or pT5 promoter, respectively.

Porphyromonas gingivalis - pNJR12 (tet(r) Bacteroides/Porphyromonas - E.coli) shuttle plasmid will be used; the plasmid is known to replicate autonomously as a low copy number (<10 copies per bacterium). Alternatively, manipulated genes will be integrated into the genome via the homologous recombination using erythromycin encoding cassette (from pVA2198).

### Origin & function

Chromosomal DNA will originate from Porphyromonas gingivalis and will encode orthologues of: Proteases specific for extracellular arginine - peptide bonds (Lys-gingipain) Proteases specific for extracellular lysine peptide bonds (Arg-gingipain) Outer membrane proteins . Enzymes involved in lipopolysaccharide (LPS) biosynthesis, glycan transferases.

The open reading frames (orfs) will be amplified, cloned and manipulated (by insertion of a specialised macrolide-lincosamide cassette, erm) in E. coli with the main intention of inactivating the corresponding gene in Porphyromonas gingivalis via homologous recombination. These mutants will be assessed, biochemically and genetically, to ascertain their contribution to the metabolism, survival and pathogenicity of Porphyromonas gingivalis.

Some of these orfs will be specially cloned for controlled expression in E. coli for subsequent production of antiserum for studies of protein expression in Porphyromonas gingivalis.

Another set of orfs will be cloned into pNJR12 and introduced into Porphyromonas gingivalis to compliment corresponding mutations to assess interconnecting metabolic pathways.

### Evaluation of foreseeable effects

The genome of Porphyromonas gingivalis W83 has been sequenced by The Institute of Genomic Research (http://www.tigr.org/) and annotated (http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gpg) so only known orfs will be cloned. Protease gene constructs have previously been shown not to yield active enzyme in E.coli. However, expression of active enzymes from some constructs cannot be ruled out. Expression of Porphyromonas gingivalis proteins in E.
coli is unlikely to make the host more pathogenic than Porphyromonas gingivalis itself. Inactivation of Porphyromonas gingivalis genes is expected to lead to metabolic defects that should result in attenuated and poor viability of the bacterium. Complementation of Porphyromonas gingivalis should lead to restoration of normal activities.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All relevant containments are in place to minimise the potential of any risk. All materials (pipette tips, plates, glassware, tissues, gloves, etc) that are contaminated with bacteria or nucleic acids will be thoroughly soaked in 5% Hycolin then autoclaved, or autoclaved directly, and disposed via the College in accordance with departmental containment level 2 safety regulations.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Suggested comments by the local Genetic Modification Safety Committee have been incorporated in the risk assessment.

Project Containment

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<td>L2 Yes</td>
<td>L3 L4 L2 L3</td>
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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 174/01.2

Date Ackn'd 30/11/2001

CU2 Project Title USE OF REVERSE GENETICS TO CREATE AN ATTENUATED INFLUENZA VIRUS

Class 2

CultureVolClass2 < 1 litre
Project Additional Information

### Purposes of the contained use
To produce an attenuated influenza virus that could be used as a live attenuated vaccine against current strains of influenza virus. Current influenza virus vaccines are only about 70% effective, live attenuated virus vaccines like those we are trying to develop may be more effective.

### Recipient or parental organism
The extensively studied PR8 strain of the influenza virus will be used as the recipient influenza strain. The PR8 strain of influenza virus is highly attenuated and does not cause disease in humans. Influenza viruses are classified as ACDP category 2 pathogens.

### Host/vector system
Plasmids containing the cloned genes of influenza virus strain PR8 are being generously donated by Professor Brownlee (Sir William Dunn School of Pathology, University of Oxford). The plasmids are pPoli-PB2-PR8, pPoli-PB1-PR8, pPoli-PA-PR8, pPoli-HA-PR8, pPoli-NP-PR8, pPoli-NA-PR8, pPoli-M-PR8, pPoli-NS-PR8, pPoli-Sapl-Rib, pcDNA-PB1-PR8, pcDNA-PB2-PR8, pcDNA-PA-PR8, pcDNA-NP-PR8. These plasmids are all pUC18 derivatives. These will be transfected into either Vero (African Green Monkey) cells or 293T (Human kidney) cells. Recombinant virus will then be retrieved from these transfected cells (see Fodor E, et al. Rescue of influenza A virus from recombinant DNA, J Virol 1999; 73:9679-9682).

### Origin & function
The introduced genetic material will be the influenza virus neuraminidase and haemagglutinin genes cloned from recent strains of the influenza virus such as A/New Caledonia/11/99. Mutations will be added to these cloned genes to attenuate the virus. Alterations to the neuraminidase gene have been shown to lead to a reduction of virulence (see Solarzano et al. Reduced levels of neuraminidase of influenza A viruses correlate with attenuated phenotypes in mice, J. Gen Virol 2000: 81 Pt 3: 737-742). The recombinant virus will thus contain all the PR8 gene segments except those encoding the neuraminidase and haemagglutinin which will come from a recent strain of the influenza virus. The introduced gene products will be expressed on the surface of the recombinant influenza virus and are intended to act as antigens to provoke a protective immune response to any later challenge by the wild type influenza virus.

### Evaluation of foreseeable effects
The influenza virus normally causes an upper respiratory infection in humans, however, the attenuated PR8 strain is non-pathogenic in humans. The mutations we add to the influenza virus genome are intended to be stable and to attenuate the virus. The haemagglutinin and neuraminidase proteins have been shown to be non-toxic to humans and have been used extensively in vaccines. Vaccines are available to protect against influenza virus infection and there are antiflu drugs such as Tamiflu and.

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**Date Ceased:** 01/09/2009

**Non-GMM Consent Granted:** not applicable

**Historical Significant Changes:** MERGED WITH GM774

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02/03/2022

Page 4549 of 15326
Relenza.

Influenza viruses pose limited environmental risk as they are degraded quickly in the environment (UV, desiccation), the genetic modifications we perform will not change this.

Work with the mutated influenza virus being conducted in Microflow Biological Safety Cabinets in a category 2 GLP laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste produced containing recombinant influenza virus will be treated with Basol a quaternary ammonia compound based detergent. This detergent is known to very rapidly destroy lipid encapsulated viruses such as influenza. To ensure a one hundred per cent kill the waste containing the recombinant virus will be left in the basol for 24 hours. The waste will then be disposed of down the sink. This is a standard technique for disposing of influenza waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Both the parent virus and the neuraminidase and haemagglutinin proteins from recent strains have a history of safe use in humans.

Project Containment

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<td>L3 L4 L2</td>
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<tr>
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<td>L4 L2</td>
<td>L3 L4 L2</td>
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</tbody>
</table>

Project Ref 174/02.1
Project Additional Information

Purposes of the contained use

Molecular cloning of Pseudomonas aeruginosa genes encoding metalloproteases, serine proteases, genes involved in type III secretion systems and genes involved in cobalamin (vitamin B12) biosynthesis in Escherichia coli.

Construction of insertion mutants of the genes mentioned above in Pseudomonas aeruginosa.

Recipient or parental organism

The origin of DNA will be the gram-negative bacterium Pseudomonas aeruginosa.

The genome of P. aeruginosa has been sequenced in a collaboration among the Cystic Fibrosis Foundation, the University of Washington Genome Center and Pathogenesis Corporation (http://www.pseudomonas.com/).

In P. aeruginosa the mutated genes are unlikely to confirm any advantage relative to the wild type strain; complemented strains are expected to restore wild type gene activities.

Host/vector system

Escherichia coli JM109 strain a K12 derivative will be used as a cloning host.

This strain is attenuated, sensitive to bile salts and unable to survive in the gut.

E. coli S17-1 a mobilising strain that carries the transfer function of the plasmid RP4 integrated in its chromosome will be used as the host for the plasmid pEX100T carrying the disrupted P. aeruginosa genes.

The vectors used in this project are:

pUC18, pUC18NotI. Non-mobilisable vectors

pUCP18-Escherichia-Pseudomonas shuttle vector. Non-mobilisable
pEX100T-gene replacement vector, mobilisable. The P. aeruginosa genes cloned in this vector are interrupted by the insertion of a gentamicin resistance cassette.

pUCGm-source of the gentamicin resistance, GM R cassette.

Origin & function

Chromosomal DNA will originate from Pseudomonas aeruginosa and will encode orthologues of:
- Metalloproteases
- Serine proteases
- Genes involved in Pseudomonas aeruginosa type III secretion system
- Genes involved in cobalamin (vitamin B12) biosynthesis

The open reading frames will be amplified, cloned and manipulated (by insertion of a gentamicin resistance cassette) in E. coli with the main intention of inactivating the corresponding gene in P. aeruginosa via homologous recombination. These mutants will be assessed, biochemically and genetically, to ascertain their contribution to the survival and pathogenicity of P. aeruginosa.

The above open reading frames will be cloned into pUCP18 and introduced into P. aeruginosa in order to complement the corresponding mutations.

Evaluation of foreseeable effects

The E. coli JM109 strains carrying the cloned P. aeruginosa genes should not be more hazardous than the P. aeruginosa host. In P. aeruginosa the mutated genes are unlikely to confer any advantage relative to the wild type strain. Complementation of P. aeruginosa should lead to restoration of normal activities.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All relevant containments are in place to minimise the potential of any risk.
All materials (pipette tips, plates, glassware, tissues, gloves) that are contaminated with bacteria or nucleic acid will be thoroughly soaked in 5% Hycolin autoclaved and disposed via the college in accordance with the safety regulations.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Suggested comments by the local Genetic Modification Safety Committee have been incorporated in this and the attached document.
**Project Additional Information**

**Purposes of the contained use**

Molecular cloning of Helicobacter pylori genes encoding proteolytic enzymes of H. pylori identified using a number of bioinformatic approaches.

Construction of insertion mutants of the genes mentioned above in Helicobacter pylori.

**Recipient or parental organism**

a) Escherichia coli DH5a (or similar K12 derivative) (supE44 [lacZYA-argF]U169 (80lacZ M15) hsdR17 (rk-, mk+) recA1 endA1 gyrA96 thi-1) that is recognised as a non-colonisung and disabled. It may be considered equivalent to ACDP category 1. It has limited survivability in the environment. Over expression strain: ER2566


02/03/2022
b) Helicobacter pylori SS1, 26695, J99 and NCTC 11637. All strains carry the Cag pathogenicity island and do not carry any virulence plasmids. 26695 and J99 are the 2 sequenced Helicobacter pylori strains. SS1 is the adapted mouse colonising strain and NCTC 11637 is the adapted Mongolian Gerbil colonising strain. All strains are ACDP category 2 and have very limited survivability in the environment.


Helicobacter pylori: The disruption of a gene with either cat or aphA3 is unlikely to confer any advantage to the wild type strain, apart from the introduction of the antibiotic resistance genes. Neither of these antibiotics have every been and are never likely to be part of treatment regimens for H. pylori infection. If strain cans be complemented, it will restore the Wild-type activities. Further, Large quantities of H. pylori must be ingested along with acid suppressive drugs to allow colonisation.

### Vector System

<table>
<thead>
<tr>
<th>Vector</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUC19</td>
<td>High copy number cloning vector</td>
</tr>
<tr>
<td>pGem</td>
<td>High copy number cloning vector</td>
</tr>
<tr>
<td>pTyb</td>
<td>pTyb is a protein fusion vector with a T7 RNA polymerase expressed off a Lac Promoter</td>
</tr>
<tr>
<td></td>
<td>Overexpression of recombinant proteins will only occur upon addition of IPTG</td>
</tr>
<tr>
<td>pKatA</td>
<td>pGem with katA from H. pylori cloned into lacZ. katA::aphA3 (originating from Campylobacter coli)</td>
</tr>
<tr>
<td>pucCat20</td>
<td>puc19 vector containing cat originating from Campylobacter coli</td>
</tr>
</tbody>
</table>

None of the above vectors are replicated in H. pylori and will be used as suicide vectors.

### Host/vector system

Hosts will be E. coli

Escherichia coli DH5a (or similar K12 derivative) (supE44 (lacZYA-argF)U169 ( 80lacZ M15) hsdR17 (rk-, mk+) recA1 endA1 gyrA96 thi-1 relA1) that is recognised as a non-colonising and disabled. It may be considered equivalent to ACDP category 1. It has limited survivability in the environment. Over expression strain: ER2566

Genotype: F- -fhuA2 [lon] ompT lacZ::T7 gene1 gas sulA11 (mcrC-mrr)114::IS10 R(mcr-73::miniTn10-TetS)2 R(zgb-210::Tn10(TetS) endA1 [dcm].

### Origin & function

All genes identified as potentially encoding proteolytic enzymes of H. pylori will be cloned from the sequenced strain H. pylori 26695. These genes will be cloned into a vector and disrupted using an antibiotic resistance cassette, such as aphA3 or cat encoding kanamycin and chloramphenicol resistance, respectively (originating from Campylobacter coli). Once the genes have been disrupted in the vector, they will be introduced to H. pylori by transformation and incorporated into the genome by allelic exchange. Kanamycin and Chloramphenicol are not currently used and are unlikely to ever be used in therapeutic regimes for the treatment of H. pylori infection.

### Evaluation of foreseeable effects

Creating protease deficient mutants of H. pylori are unlikely to confer any advantage to the organism. It is more likely to compromise the pathogenicity of the bacterium.

Introducing virulence factors into the attenuated derivatives of E. coli K12 is likely to increase the potential virulence of the E. coli. The potential virulence of the E. coli will not exceed that of the Wild-type H. pylori.
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All relevant containments are in place to minimise the potential of any risk.
All materials (pipette tips, plates, glassware and gloves) that are contaminated with bacteria or nucleic acid will be thoroughly soaked in 5% (v:v) hycolin, autoclaved and disposed by incineration via the college in accordance with safety regulations, currently in use for ACDP hazard group 2 organisms.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Suggested comments made by local GM safety committee have been incorporated into this and the risk assessment.

Project Containment

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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 174/02.3

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<th>CultureVolumeClass3-4</th>
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<td>16/08/2002</td>
<td>DETERMINATION OF THE AMOUNT OF BACTERIAL ADHESION TO EUKARYOTIC CELLS IN THE PRESENCE OF OLIGOSACCHARIDES KNOWN TO INHIBIT BACTERIAL ADHESION........</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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<table>
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<tr>
<th>Date Project Ceased</th>
<th>Consent Granted</th>
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<tbody>
<tr>
<td>02/03/2022</td>
<td>not applicable</td>
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</tbody>
</table>
Tick if notifying a connected programme of work

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info
MERGED WITH GM774

Project Additional Information

**Purposes of the contained use**

A marker plasmid will be constructed to allow the monitoring of bacteria either by bioluminescence or fluorescence in the presence of a fluorescent substrate.

**Recipient or parental organism**

Recipient organisms: Escherichia coli K12, Salmonella typhimurium SAL1, Legionella pneumophila NCTC11191, Bacillus cereus NCT2599, Pseudomonas aeruginosa PAK and Burkholderia cepacia NCIMB9091; all are wild type identified laboratory strains. E. coli is ACDP cat. 1 all other organisms are cat. 2.

**Host/vector system**

Plasmid vector (pBBR1MCS4) confers ampicillin resistance and is only mobilisable when the RK2 transfer functions are provided in trans. Plasmid will be modified to also confer chloramphenicol resistance.

**Origin & function**

Firefly luciferase (luc+) from the commercially available plasmid pSR-luc+ or B-galactosidase (lacZ) amplified from the E. coli K12 chromosome will be cloned into the plasmid pBBR1-MCS4. These will allow us to determine the amount of bacteria adhered to eukaryotic cells either by luminescence (luc+) or fluorescent (lacZ).

**Evaluation of foreseeable effects**

Since the plasmid being constructed is only for use as a marker it is not expected to change the virulence properties of the recipients. Therefore a release of the GM organism would result in the same effect as a release of the unmodified organism.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All materials contaminated with GM bacteria will be soaked in 5% (v/v) hycolin (if not directly autoclaved), autoclaved and disposed of by incineration via the college in accordance with the safety regulations currently in use for ACDP hazard group 2 organisms.
Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

none

Project Containment

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<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

Project Ref 174/06.1

Date Ackn'd  22/03/2006
CU2 Project Title  Knockout and over-expression of streptococcal DNA replication and repair genes.
Class  Class 2
CultureVolume  < 1 Litre
Consent Granted  Not Applicable

Non-GMM

Historical Significant Changes
Historical Date of Additional Info  MERGED WITH GM774
Significant Change ID
Date of Significant Change

02/03/2022
**Project Additional Information**

**Purposes of the contained use**
To investigate function of DNA replication, recombination and repair genes, and their role in antibiotic resistance acquisition.

**Recipient or parental organism**
Clinical and laboratory isolates of Streptococcus pneumoniae and other mitis group streptococci.

**Host/vector system**
Genes will either be cloned in E. coli vectors unable to replicate in streptococci in order to mediate incorporation into the pneumococcal/streptococcal genome, or cloned in shuttle vectors such as pVA838 and pVA981 which can replicate in pneumococci/streptococci. In some cases genes will be cloned to allow expression: pMSP3535 allows regulated expression in streptococci.

**Origin & function**

The intention is either to inactivate or to overexpress the genes in streptococci. In some cases genes will be inactivated by insertion of antibiotic resistance cassettes, and reporter genes will be inserted downstream of promoters to assess regulation of expression.

**Evaluation of foreseeable effects**
Modified S. pneumoniae and mitis group streptococci may have an increased mutation rate and some will carry additional antibiotic resistance genes. Hypermutable organisms are generally at a disadvantage due to the accumulation of errors. Clinical isolates known to have a wide range of mutation frequencies are not thought to be associated with increased virulence. There is potential for an increase in mutation to antibiotic resistance. Penicillin is usually the drug of choice for treating infection - penicillin resistance cannot be gained by point mutation, and none of the resistance cassettes to be used confer penicillin resistance.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
All cultures and contaminated materials are autoclaved (effective 100% kill, monitored by regular servicing of autoclave and integrated chart recorder). After autoclaving, disposable solids are removed as clinical waste for incineration, and liquids are disposed of to drain.

**Is an emergency plan required according to regulation 20?**
N

**If yes, tick to confirm that it is attached to this form**
N

**Tick to confirm that you have attached a risk assessment to this form**
Y
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>L3</td>
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</table>

Project Ref 174/06.2

Date Ackn'd 22/03/2006

Date Project Ceased 01/09/2009

Consent Granted Not Applicable

Project notified under transitional arrangements N

Investigation of interactions of enterohaemorrhagic Escherichia coli (EHEC) and enteropathogenic Escherichia coli (EPEC) and cells of the organised lymphoid tissue of the human gut.

purposes of the contained use

Investigation of enterohaemorrhagic Escherichia coli (EHEC) and enteropathogenic Escherichia coli (EPEC) can affect and modulate immune cells of the human gut by injecting proteins via a type III secretion system.
Laboratory derivatives of the E. coli strain EHEC 0157:H7. All strains are Shigatoxin-negative.

Laboratory derivatives of the E. coli strain EPEC 0127:H6. All strains are negative for the EspC enterotoxin.

Host/vector system

The strains to be used carry a plasmid (pFPV25.1) with the gene that encodes for green fluorescent protein (GFP) or the plasmid pCX340 with a gene that encodes with NleD ("Non LEE Encoded effector", an EHEC effector protein) fused to TEM-1 B-lactamase which serves as a reporter protein to directly show that a cell has received a signal via type III secretion.

One EPEC strain carried the pCX340 plasmid with the effector protein (TccP ("Tir-=cytoskeleton coupling protein") fused to TEM-1 B-lactamase which serves as a reporter protein to directly show that a cell has received a signal via type III secretion.

Origin & function

pFPV25.1 expressing the green fluorescent protein (GFP) allows direct visualisation of EHEC and EPEC by fluorescent microscopy.
pCX340 expressing NleD or TccP fused to TEM-1 beta-lactamase serves as a fluorescent-based reporter for cells which have received a signal via type III secretion.

Origin & function

Modification involves introduction of marker genes. These are expected to have no effect on the virulence of the host.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures and contaminated materials are autoclaved (effective 100% kill, monitored by regular servicing of autoclave and integrated chart recorder). After autoclaving, disposable solids are removed as clinical waste for incineration, and liquids are disposed of through the drain.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The comments were that if a member of staff suffers from diarrhea, the PI and the Health and Safety Officer will be notified. A restriction was made on the volume of media in which the organism was cultured (maximum 20 ml).
To make single base changes by site directed mutagenesis in 10 genes involved in replication of the live attenuated Oka Varicella-zoster vaccine virus.

Historical Significant Changes
MERGED WITH GM774

Project Additional Information

Purposes of the contained use
To determine the biological significance of wildtype alleles present in genes involved in replication that occur at significantly lower frequency in the original Oka vaccine preparation but appear to have been selected for in Oka Varicella vaccine viruses that cause rashes.

Recipient or parental organism
Attenuated Oka varicella vaccine virus present in Cosmids and in a BAC.

Host/vector system
Cosmids and BAC are already assembled containing the entire Oka varicella vaccine virus and will be transfected into E. coli to obtain sufficient titres.

**Origin & function**

Genes to be manipulated are involved in either DNA replication, gene expression or are of unknown function but are known to be expressed during latency and may include: Ie10 AND Ie62 (transactivator proteins), Membrane protein (ORF1), Stearoylated membrane protein (ORF 9), glycoprotein C (ORF14), latency expressed protein (ORF21), glycoprotein B (ORF31), ORF39 (unknown function), 50 (unknown function), Origin of replication binding protein (ORF51), Components of DNA helicase/primase complex (ORFs 6, 52, 55) ORF54 (unknown function), Uracil-DNA glycosylase (ORF59).

Mutated cosmids will be transfected into melanoma cells to produce transmissible recombinant virus.

**Evaluation of foreseeable effects**

Recombinant Oka vaccine virus from cosmids or BAC may have increased virulence compared to original Oka vaccine virus but are highly likely to remain attenuated compared to wildtype virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All cultures and contaminated materials are autoclaved (effective 100% kill, monitored by regular servicing of autoclave and integrated chart recorder). After autoclaving, disposable solids are removed as clinical waste for incineration, and liquids are disposed of to drain.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

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</tbody>
</table>
This connected programme of work aims to investigate the neutralisation and tropism properties of different retroviruses. Both ACDP 2 and ACDP 3 retroviruses will be used including HIV 1 and 2, HTLV 1 and 2, SIV and MLV. Retroviral genes will be amplified from infected cell cultures or patient DNA and either full length molecular clones or plasmids containing different retroviral genes will be generated. Transfections into mammalian cell lines will result in either fully infectious or defective virus particles that are not transmissible.

Retroviral full length molecular clones will be propagated in E. coli K12 derivatives and in human cells. E. coli K12 derivatives are disabled and non-colonising. Mammalian cell lines will be used but these will not come from worker. Fully infectious virus will be made following transfection of these plasmids into mammalian cell lines. The foreseeable risk for work with these full length molecular clones is the production of infectious virus. Transfections and all subsequent work with these retroviruses will be done in a CL3 laboratory.

Host cell lines and primary cells including: T-cells, fibroblasts, epithelial cell lines, primary peripheral blood mononuclear cells and macrophages. All cell lines and primary cells will not be from any authorised workers. The bacterial hosts for expression vectors will be disabled E.coli K12 derivatives. The cloning plasmid vectors will include TOPO vectors, pcDNA3.1, pCAGGS, pNL4.3 delta env, pMDG (VSV-G envelope vector) pCNG (packaging plasmids) and pHIT60 (MLV gag/pol vectors). Vaccinia virus.
expressing T7 RNA polymerase: VR2153.

### Origin & function

Retroviral genes amplified from DNA from patients will be cloned into mammalian expression vectors following cotransfection into mammalian cell lines either fully infectious virus will be made of defective, non-transmissible virus capable of only a single round of infection will be made. The intended function is to study the neutralisation and tropism properties of these chimeric infectious or pseudotyped viruses.

The vectors to be used include pCGNCG (Sonoeka et al 1995), PCSGW, pSVIII, pCAGGS, pCR-Blunt II and pCR 2.1. Packaging vectors include p8.3, p8.91. Full length molecular clone vectors include pNL4.3, p89.6 and pHXB2. The envelope deleted plasmid can be pNL4.3 delta env. These retrovirus vector systems are standard laboratory reagents.

The intended function of the plasmid expression vectors, retroviral expression vectors and the general material inserted in them is to change the level of expression of viral and/or host proteins in the cell. This will result in the production of cells and viruses with altered properties.

### Evaluation of foreseeable effects

The bacterial strains used for all plasmid propagations are disabled in key bacterial functions and are unable to survive in the environment or pass genetic material onto host bacterial flora following a breach in containment. Expression of the retroviral full length molecular clones could cause infection if injected intra muscarily in sufficient quantities. These clones will only be grown in a maximum culture volume of 100ml and sharps will be forbidden in this laboratory area in order to minimise the above risk.

PPE in the form of Howie lab coats, goggles and disposable gloves will also be worn. The plasmids used for the generation of pseudotyped retroviruses split the retroviral genome into separate plasmids such that virus production is only initiated when all plasmids are present in a mammalian cell line. These plasmids are always propagated separately to minimise the risk. No mammalian cell culture is present in the CL2 molecular biology laboratory where this work is carried out. The risk of naked DNA is considered to be negligible.

The recipient cell lines and primary cells which are not from person who work in the laboratory are known to be free from human pathogens ACDP2-4 groups. These cells have limited survivability in the environment and have serum and nutrient requirements that would not likely be met outside laboratory tissue culture. Transfection of the full length molecular clones results in fully infectious virus that is capable of infecting a worker. Recombination between the plasmids for the pseudotyped viruses could also result in infectious virus though this is very unlikely. The risk is managed by carrying out transfections into mammalian cells and all subsequent work in a CL3 laboratory. A Code of Practice details all working practices for this laboratory. All staff must be fully trained by an experienced worker and copies of all training records kept. All work with cell cultures is carried out in a Class 1 microbiological safety cabinet which is tested every 6 months. Access to the laboratory is controlled by card. Sharps are forbidden. Vaccinia virus is used to boost the expression of retroviral envelopes. In order to limit the exposure of workers to those only involved in the work directly, it will be handled in the CL3 laboratory where access is controlled and a separate class 1 microbiological safety cabinet and CO2 incubator can be dedicated to this work. Workers with vaccinia virus will be fully apprised of the risks prior to working with the virus as per the attached risk assessment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Containment Level 2:**

Bacterial waste: All GMMs are inactivated in 5% Trigene for at least 16 hours before disposal or autoclaved followed by incineration as per college practice.

**Containment Level 3:** All disposable plasticware is soaked in 5% Trigene in microbiological safety cabinet for at least 16 hours prior to disposal. All plasticware is then placed in double autoclave bags and autoclaved directly out of the CL3 laboratory before being sent for incineration. A temperature of 134°C is maintained for 15 minutes in the autoclave to sufficiently kill all life exposed to this treatment. Two double ended autoclave bags are present in the CL3 laboratory. The efficacy of Trigene has been
tested using a blue spot beta-galactosidase assay for HIV infectivity. No infection could be detected following staining 3 days later.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The GMSC required that laboratory workers would not work with plasmids containing full length viral genomes until containment level 2 training has been completed.

Project Containment

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<td>L3 Yes</td>
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<td>L2</td>
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</tr>
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<td>L2</td>
<td>L3 L4</td>
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</table>

Project Ref 174/08.1

Investigation of neutralising antibodies and anti-viral compounds on hepatitis viruses and the effect of chronic infection on the response to treatment.

Class CultureVol  Class 2 Culture Volume  Class 3-4
Class 3 < 1 Litre 100ml

Non-GMM Consent Granted
Yes

Project notified under transitional arrangements  N

Historical Significant Changes
MERGED WITH GM774

Significant Change ID
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Chronic infection with the hepatitis viruses is an important cause of morbidity and mortality. This connected programme of work aims to investigate the biology of the viruses and potential agents useful in treatment and prevention of infection. Both HBV and HCV will be investigated as will co-infection of HCV and HIV and the effect of HCV infection on HIV virus production and other properties. Viral genes from infectious clones or patient samples transfected into cell lines will result in either fully infectious virus or non-transmissible defective virus particles.

**Recipient or parental organism**

Full-length or partial viral DNA will be cloned into Ecoli. Transfection of full-length RNA or plasmid DNA, or partial length DNA co-transfected with a defective retroviral plasmid construct into mammalian cell lines or primary cells will be used. These will not come from any laboratory worker. The foreseeable risk for work with these full-length molecular clones is the production of infectious virus. Viral RNA will be produced and stored in the CL3 laboratory as will transfections and all subsequent work with these constructs.

**Host/vector system**

Host cell lines and primary cells including: fibroblasts, hepatoma cell lines, and primary cell, T-cells, peripheral blood mononuclear cells and macrophages. All cell lines and primary cells will not be from any authorised workers. The bacterial hosts for expression vectors will be disabled E.coli K12 derivatives. The cloning plasmid vectors will include TOPO vectors, pGEM, pcDNA31, pCAGGS, pNL4.3 delta env, and pSVIII.

**Origin & function**

The intended function of the genetic material is to introduce it into mammalian cells so that it becomes possible to study the neutralisation of the hepatitis viruses and their effect on the functions of the cell. Hepatitis genes will be amplified from infected patients and will be cloned into mammalian expression vectors and following cotransfection into mammalian cells defective, non-transmissible virus capable of only a single round of infection will be made. The vectors to be used include the mammalian expression vectors pSVIII, pCAGGS and pNL 4.3 delta env. Other vectors include pGEM Teasy and the TOPO vectors pCR-Blunt land pCR2.1. Full-length RNA from a plasmid containing cloned JFH1 will be used to transfect mammalian hepatoma cell lines (Wakita et al, 2005). This is known to transfect successfully and produce infectious virus particles. Mutations of this construct or constructs from other genotypes or of mixed genotype will also be used. Further, these HCV expressing cell lines will be infected with HIV and other retroviruses to study the effects on HIV biology. These experiments will result in the production of cells and viruses with altered properties. A well characterised hepatoma cell line which is already transfected with the hepatitis B virus, and produces viral particles into the tissue culture medium, will also be used (Sells et al, 1987)

**Evaluation of foreseeable effects**

The bacterial strains used for all plasmid propagations are disabled in key bacterial functions and are unable to survive in the environment or pass genetic material onto host bacterial flora following a breach in containment. The plasmids used for the generation of pseudotyped retroviruses split the retroviral genome into separate plasmids such that virus production is only initiated when all plasmids are present in a mammalian cell line. These plasmids are always propagated separately in disabled E. coli K12 derivatives to minimize this risk. No mammalian cell culture is present in the CL2 molecular biology laboratory where this work is carried out. The risk of naked DNA is considered to be negligible. Co-transfection of the envelope deleted retroviral plasmid, a plasmid containing a marker gene and the cloned envelope genes into a mammalian cell can result in a single cycle infectious virus which is not transmissible. To mitigate the risk of infection co-transfection and all subsequent work will only be carried out in a CL3 laboratory covered
by a code of practice which details all working practices for this laboratory. All staff must be fully trained by an experienced worker and copies of all training records kept. All work with cell cultures is carried out in a Class 1 microbiological safety cabinet which is tested every 6 months. Access to the laboratory is controlled by card, Sharps are forbidden. Cell lines expressing full length clones of the hepatitis viruses produce small quantities of infectious virus. All such culture and the subsequent experiments will be carried out in a dedicated CL3 laboratory under its code of practice as described above. All work with HIV viruses will be carried out in a CL3 laboratory with a COP detailing all work practices.

[The recipient cell lines and primary cells which are not from persons who work in the laboratory are known to be free from human pathogens ACDP2-4 groups. These cells have limited survivability in the environment and have serum nutrient requirements that would not likely be met outside laboratory tissue culture.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment Level 2:
Bacterial waste: All GMMs are inactivated in 1% Virkon or 5% Trigene for at least 16 hours before disposal. Plates will be autoclaved followed by disposal in the clinical waste as per college practice.

Containment Level 3:
All fluid waste is inactivated in 1% Virkon or 5% Trigene for 16 hours prior to disposal. All disposable plasticware is placed in double autoclave bags and autoclaved within the CL3 laboratory prior to disposal in the clinical waste as per college practice. The performance of the autoclave is tested every 6 months under a service contract. The autoclave is also monitored for each run through its printout and the inclusion of Thermolog chemical integrator strips (Bennett Scientific) to the load.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC were satisfied with the classification as Class 2 or as Class 3 for the risk assessments attached.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 Yes</td>
<td>L4 L2</td>
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02/03/2022
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<tr>
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**Project Ref** 174/98.1

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<td>Class 2</td>
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- Date Project Ceased: 01/09/2009
- Withdrew: N
- Tick if notifying a connected programme of work: N
- Project notified under transitional arrangements: Y

**Historical Significant Changes**

| Historical Date of Additional Info | MERGED WITH GM774 |

**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Large Scale Activities

Human Clinical Applications

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<td>District</td>
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# Premises Conditions

02/03/2022

Page 4571 of 15326
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  

Tick if confidential

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

For activities involving GMMs, describe the waste management measures which will apply to the activity

LIQUID WASTE IS INACTIVATED BY HYPOCHLORITE. SOLID WASTE IS AUTOCLAVED OR TREATED WITH HYPOCHLORITE AND THEN INCINERATED. CLINICAL BIOTECHNOLOGY CENTRE-SOLID WASTE IS AUTOCLAVED
**Project Ref 176/07.1**

<table>
<thead>
<tr>
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<tr>
<td>20/02/2007</td>
<td>Expression of Mouse and Human full-length and truncated recombinant Prion protein (PrP).</td>
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<th>CultureVolumeClass3-4</th>
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Project notified under transitional arrangements N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Production of normal and truncated forms of the mouse and human prion protein within a bacterial expression system. The expressed proteins will be used in the development of diagnostic assays to the prion protein from a range of tissues, in particular blood, brain and lymphoreticular tissue.

**Recipient or parental organism**

E.coli BL21 strain classified as broadly similar to disabled, non-colonising K12 strains under laboratory use providing the insert is unlikely to modify bacterial pathogenicity (ACGM guidance, Newsletter 30, November 2001).

**Host/vector system**

pET vector, mobilisation defective.

**Origin & function**

Mouse and human PrP sequences is derived from host cDNA libraries amplified by PCR. The sequence will encode full-length or truncated normal prion sequence.
Evaluation of foreseeable effects

Recombinant prion protein (PrP) is not, of itself, a toxic or hazardous substance. It is unlikely to be hazardous to the environment. The bacterial strain E.coli BL21 has a history of safe laboratory use and is broadly similar to genetically disabled E.coli strains such as K12 and its variants. It is not able to survive in or colonise a host organism. The plasmid used for cloning and expression is defective in its ability to mobilise.

Expression of PrP in the bacterial host will be into inclusion bodies within the bacterium and will not be in a conformation that is similar to the infectious prion. If, in the unlikely scenario that the GMM containing the PrP construct was able to survive and colonise a host, the expressed PrP is unlikely to be hazardous. However, random pathogenic isoform. For this reason additional containment measures are considered sensible. The work is classified as a class 2 activity. As an additional precaution the work will be carried out in a containment level 3 laboratory. The containment measures taken should minimise any escape of the GMM from the laboratory or accidental exposure of the operator.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Evaluation of foreseeable effects

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This contained use activity will take place in a containment level 3 laboratory and all procedures for treatment of GMO waste will be the same as used for decontamination of pathogenic prion protein. Liquid waste is treated with sodium hypochlorite (minimum concentration of 20,000ppm available chlorine) for at least 1hr then discarded into the laboratory sink and washed into the drain with copious amounts of water. This treatment has been validated in our laboratory and shown to kill all (100%) E.coli cultures under conditions of practical use.

Solid waste us placed into the unsealed autoclave bags and autoclaved at 134°C for 20 minutes. Autoclaved waste is placed into 60L sealable rigid containers and sent for incineration as clinical waste using a licenced external contractor. The autoclave is validated annually and serviced at regular intervals.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

The GMSC has considered the Risk Assessment and the containment measures taken as appropriate for the work undertaken. Notification of this activity will be made to the Local Services Group, covering all employees who work in the centre, before work is started. Minutes of LSG meetings are available to all staff.
Project Ref 176/08.1

Date Ackn'd 15/05/2008

CU2 Project Title Lentiviral vector transduction of mammalian cells for gene expression and/or silencing of red blood cell proteins.

Date Project Ceased

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID 176/08.1a

Date of Significant Change 27/11/2013

Project Additional Information

Purposes of the contained use

To investigate the role of red blood cell adhesion molecules, red cell membrane Band 3 complex proteins, apoptotic pathway proteins, signalling molecules, transcription factors and the role of protein trafficking and internalisation and autophagy of these molecules in human erythropoiesis (red blood cell production).

Recipient or parental organism

1. Lentivirus-based plasmids will be propagated in laboratory strains of the bacterium E.coli. There are no foreseeable effects except that the OMM will be transformed to antibiotic(ampicillin) resistance to facilitate selection. The OMM will not express the target genetic material and, like the parent bacterial strain, will be unable to survive outside the laboratory nor colonise mammalian gut.
2. Lentivirus-based transduction particles will be produced in a cultured, human, embryonic kidney cell line. There are no foreseeable effects - the OMM will not express the target genetic material, will be cultured transiently and will be unable to survive outside the laboratory.
3. Cultured, human, haematopoietic, progenitor cells and cultured, human, haematopoietic cell lines will be transduced using these particles and thus rendered resistant to an antibiotic (puromycin) to facilitate selection. The GMIVI will express the target genetic material but will be unable to survive outside the laboratory.

Host/vector system

1. Lentivirus based plasmid vectors: Transfer vectors - pLKO. 1, Sigma, St Louis, USA or pTRIP transfer vector, Charneau et al., Gene Ther. 2001, 8(3):190-8; packaging vectors (Sigma Mission shRNA or psPAX2, Charneau et al., Gene Ther. 2001, 8(3):190-8) and envelope vector, (pCMV VSV-G, Sigma, St Louis, USA) will be propagated in XL-I blue E. coli cells.
2. Lentivirus-based transduction particles will be produced in HEK293T, a human embryonic kidney epithelial cell line.
3. The ultimate host will be cultured, human, haematopoietic, progenitor cells or cultured, human, haematopoietic cell lines. These cells will be transduced using lentivirus-based transduction particles produced as above.

Origin & function

Genetic materials are small, inhibitory RNA sequences or eDNA sequences for the human genes of the proteins listed below:
1. Green fluorescent protein — a control protein used to determine multiplicity of infection values and to demonstrate effectiveness of transduction or knockdown protocols.
2. Cell adhesion molecules - found on many normal human cells and expressed during erythroid development (e.g. ICAM-4 [CD242]; Lutheran blood group protein [CD239]). Tetraspannins - cell adhesion accessory molecules found on many normal human cells and expressed during erythroid development (e.g. CD81; CD82; CDI5I). Band 3 (CD233 the chloride/bicarbonate exchange transport protein) macrocomplex molecules. Includes some red blood cell restricted molecules such as the blood group active proteins glycophorin A, ICAM-4 and Rhd/CE; Rh associated glycoprotein and other, more widely expressed, proteins such as CD47. Includes associated structural/cytoskeletal proteins such as ankyrin and its isoforms and protein band 4.2 and also cellular proteins involved in the efficient trafficking of Band 3 and associated proteins. The overall function of the complex is gas-exchange between the red cell and tissues.
4. Other well characterised mammalian membrane transport proteins found on red blood cells (including Kidd blood group active protein [urea transporter]; glucose transporter Glut-I and CD98 an amino acid transporter).
5. Red cell membrane proteins that form structural complexes involved in the maintenance of red cell integrity (including glycophorin C and associated cytoskeletal proteins such as protein band 4.1, actin and spectrin).
6. Transcription factors - these control gene expression in a tissue dependent manner and thus direct cell differentiation and function. Examples are well characterised human haematopoietic transcription factors GATA-1, KLF 1 and Spi-1. Haematopoietic transcription factors will be targeted. These molecules are normally expressed during erythroid differentiation but inappropriate or mis-timed expression (such as in this case where expression will be driven from a viral promoter) or knockdown of expression by siRNA may result in arrest of normal differentiation and therefore transformation of the target cell to immortality. For this reason, certain transcription factors such as Spi-1 may be considered as oncogenic or leukaemogenic and their encoding sequences as oncogenes.
7. Intracellular signalling molecules with a role in erythropoiesis. Target molecules include kinases and phosphatases of the Src and MAP pathways.
8. Proteins involved in apoptotic pathways in erythroid cells. Includes pro-apoptotic proteins such as Bak/Bax, BH3-only proteins, caspases, Fas & FasL and associated signalling molecules such as GSK-3. The intended use is to enable the study of loss of function or rescue of function of the targeted proteins within a tissue culture, laboratory model of human red blood cell maturation (erythropoiesis).

Evaluation of foreseeable effects

It is predicted that the GMOs produced in this study will have very similar characteristics to the host cells or organism. E.coli cells used to propagate plasmid vectors will be transformed to antibiotic (ampicillin) resistance but will not express proteins encoded by the inserted genes within the vectors. Human embryonic kidney packaging cells will produce lentivirus-based transduction particles. Transduced, target, haematopoietic cells are predicted to be silenced for expression of the gene target sequence or to over-express the sequence but other characteristics are predicted to remain the same as the host cells. None of the GMOs will be capable of survival outside the exacting conditions of culture used in the laboratory. The system is designed such that virus that is capable of replicating within host cells is not produced.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

02/03/2022
No plants or animals will be used in this study.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Bacterial cultures, petris dishes, tips, etc. contaminated with bacterial strains will be collected in an autoclave bag, an indicator strip will be placed inside so that the indicator “dot” can be seen and autoclaved for 30 minutes at 125°C. Degree of kill 100%. The bag will be inspected and if the indicator strip has changed colour, thus verifying the temperature and duration of heat treatment, the bag will be disposed as clinical waste for incineration by a contractor. The autoclave is serviced annually.
2. Spent tissue culture medium and tissue culture plastic will be treated for 5 minutes with an equal volume of 2% w/v “Virkon” (dipotassium peroxodisulphate, Antec International, Sudbury). Validated by manufacturer to give 100% kill of lentivirus and validated in user’s laboratory to give 100% kill of cultured mammalian cells. Liquid waste will be disposed to drains and waste plastic ware will be disposed as clinical waste for incineration by a contractor.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The project, with an outline risk assessment (part one of attached risk assessment form) was presented to the Institute’s genetic modification safety committee on 8th January 2008. The committee agreed, in the light of advice provided by HSE specialist inspectors during their inspection in July 2007, that this was not a class 1 project and that a more detailed risk assessment and minimum level 2 containment was required. The risk assessment was completed and sent to committee members by email with an invitation to comment. Comments were favourable and one committee member suggested segregation from other work in the laboratory by further containment within a vented tray designed to contain any potential spill or contamination of the exterior of labware. This has now been included in the risk assessment.

The project, its risk assessment and examination by the GMSC and notification to HSE will be communicated to all staff via the local Health and Safety Committee and the “Local Services Group” as required by the legislation.

Project Containment

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GM Centre Number: 177

Data Premises Notified (Originally) 12/04/1985

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Name

UNIVERSITY OF YORK

Name 2

Department

BIOLOGY

Campus Estate or Research Centre

Building

Road Name

District

HESLINGTON

Town

YORK

County

YORKSHIRE

Postcode

YO10 5DD

Country

ENGLAND

Tel Number 01904 432872

Fax Number 01904 432615

E-mail

HSE Division YORKSHIRE AND NORTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
Level 4 (GMMs)

Non-microbial

Other (please specify)  

Tick if confidential

Bacteriology
Parasitology
Transgenic
Birds

Microbiology
Research

Virology
Transgenic
Animals
Transgenic
Fish

Gene Therapy

Mycology
Transgenic
Invertebrates
Transgenic
Plants

Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

Project Ref 177/01.1

Date Ackn'd 30/08/2001

Date Project Ceased 02/03/2022

CU2 Project Title HUMAN IN VITRO MODELS OF CARCINOMA DEVELOPMENT AND PROGRESSION

Class 2
Culture Volume Class 3-4 < 1 litre

Non-GMM Consent Granted not applicable

---
**Project Additional Information**

**Purposes of the contained use**

To use retroviral transduction to introduce genes into normal human epithelial cell lines that will disable the activity of tumour suppressor proteins and provide a model for studying carcinoma development in vitro.

**Recipient or parental organism**

Disabled retrovirus will be produced by transfecting the pLXSN vector engineered to contain a) the HPV 16 E6 gene (pLXSN16E6) (Halbert et al 1991; J Virol 65:473-478) and b) a full-length p53 mutant which acts as negative-dominant inhibitor of wild-type p53 (Gottleib et al, 1994; EMBO J 13:1368-1374) into the PT67 packaging cell line. The retrovirus will be used to infect normal human epithelial cell lines derived from urothelial, breast, colonic, ovarian, prostate and Fallopian tube origins. Following infection, transduced cells will be selected by neomycin resistance and supernatant from the transduced cells will be screened to demonstrate absence of infectious retrovirus. There are no anticipated risks for the environment as all vectors and host strains are incapable of survival outside specialist laboratory conditions. The only perceived risk would be if a research worker were exposed to the infectious virion through an open wound or via a mucosal epithelial surface as this could result in the introduction of genetic material into exposed cells of the research worker. However, this risk is extremely low because of the safety protocols used to limit exposure of the research worker to retrovirus and because gene integration can only occur in proliferating cells. In addition, the retrovirus is disabled and the chance of genetic recombination or complementation to restore infectivity is minimal. This means that following initial infection, the retrovirus is incapable of producing further infective retrovirus and hence cannot lead to widespread or systemic infection.

**Host/vector system**

**Vector**

pLXSN (from Clontech). This vector is derived from Moloney murine leukaemia virus and contains the MoLV 5'LTR and 3'LTR and has a neomycin resistance cassette. A packaging signal and part of the gap gene is downstream from the 5'LTR. This is a replication deficient viral vector that does not produce infectious virus.

**Host**

a) Packaging cell line

Amphotrophic packaging cell line PT67 obtained from Clontech. In this cell line the gag, pol and env viral genes have been introduced separately making it extremely unlikely that an active retrovirus will be produced due to recombination.

b) Normal human epithelial cells

These cells have not been genetically modified to limit their ability to colonise or infect humans and would therefore be categorised as a wild-type host. However, the immunophenotype of human cells from an individual and the inability of the cells to survive outside defined culture conditions means that they may be categorised as a...
disabled host. It is important that normal cell cultures derived from personnel in the laboratory are not used.

**Origin & function**

- a) the HPV16 E6 gene (pLXSN16E6) the product of which binds and target wild type p53 for ubiquitination (Halbert et al 1991; J Virol 65:473-478) and

**Evaluation of foreseeable effects**

Disablement of the functions of the p53 tumour suppressor protein is likely to mimic the early events of malignant transformation of human epithelial cells. Thus, normal cells so treated must be considered premalignant, with enhanced oncogenic potential, posing a similar risk as human tumour-derived cell lines. All cultures involved in this work will be carried out in laminar flow cabinets and culture supernatants will be tested for the presence of infectious virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Packaging cell lines and normal human epithelial cells are unable to survive outside defined culture conditions, meaning that they can be categorised as a disabled host.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Once the genetically modified epithelial cells have been produced and shown not to produce infectious virus they will be classified as Class 1 as they pose no risk to the environment or to human health.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated plasticware will be decontaminated overnight in 2% Virkon and autoclaved before disposal to waste. All liquid waste will be decontaminated with 2% Virkon (final concentration) overnight before disposal to drain.

**Project Containment**

<table>
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<td>L2 Yes</td>
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Initially, this project was overcautiously classified as a Class 3 activity, on the basis that the work involved the use of oncogenes. However GMSC discussion and subsequent comments/advice by HSE Inspectors resulted in a re-classification to Class 2.

It was decided that the hazards associated with this activity would be adequately contained using containment level 2 measures.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick if an emergency plan is required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
### Project Ref: 177/02.1

<table>
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<td>CLONING AND EXPRESSION OF HUMAN PAPILLOMAVIRUS GENES FOR PROTEIN STRUCTURE AND FUNCTIONAL STUDIES</td>
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</table>

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

To study the biology of the proteins encoded by human papillomavirus and to relate the biological properties to the structural properties of the viral proteins and the human cell proteins with which they associated.

**Recipient or parental organism**

Disabled Retrovirus will be produced by transfecting the pLNCX and pLXSN vectors into a packaging cell line (3T3 mouse cell based)

**Host/vector system**

**Hosts**

3 types of host will be used:

1. **E.coli**
   
   (a) E.coli K12 based strains are recognised as non-colonising and disabled, posing very low/negligible risk to both human health and the environment. These will be used for the growth and amplification of all plasmid vectors.
   
   (b) E.coli BL21 and AD494 will be used for protein expression. BL-type strains may represent a potential hazard since they are non K-strains and potentially infective. However there has been no evidence of adverse effects after 11 years of use in this laboratory.
2. Insect - non viable outside the tissue culture laboratory and pose a negligible hazard.
3. Mammalian cells - non viable outside the tissue culture laboratory and pose a negligible hazard.

Vectors:
The proposed vector systems pose a low hazard to humans or the environment. The greatest potential risk would be from transfer of antibiotic resistance rather than expression of the foreign gene.

(a) pET series plasmids (non-mobilisable)
(b) pT7Blue series (non-mobilisable)
(c) pGEM series (non-mobilisable)
(d) baculovirus transfer plasmids pVL-type and pBac series - These baculovirus vectors are deleted for the polyhedrin gene and incapable of survival or replication outside the insect cell in tissue culture. Although capable of infecting human cells these vectors can not replicate in these cells.
(e) Plasmids containing mammalian promoters for transfection into mammalian cell lines and recombination into retroviruses.

Origin & function
This activity will use human papillomavirus (HPV) or bovine papillomavirus (BPV) gene coding for E1, E2 (E4, E5), E6, E7, L1 and L2 PV proteins and human cellular proteins E6 - associated protein (E6-AP), and P53.
PV-encoded proteins are expressed from 8-10 viral genes and are responsible for recruiting the functions of the infected host (mammalian epithelial) cell towards maintenance and replication of the viral genome. The E1 and E2 proteins are responsible for viral replication and transcription and are expressed at low levels in infected cells. E4 and E5 are concerned with facilitating exit of viral particles from cells. L1 and L2 are the virus capsid proteins which coat the viral DNA to form virus particles.

Evaluation of foreseeable effects
Expression of E1, E2, E4, E5, L1 and L2 genes in any system constitutes a low hazard. Expression of E2 (and probably E1) in mammalian cell systems at even moderate levels results in death of the expressing cell and no detectable protein product. Ingestion of purified protein is also unlikely to pose a significant risk since the proteins would be readily digested and unable to enter cells in a functional form. Also, since a large proportion of the human population has had a PV infection, many individuals already possess antibodies to PV proteins.

The PV proteins E6 and E7 pose a greater potential hazard as these proteins are responsible for cellular transformation when expressed in a dysregulated manner in the infected epithelial cells. E6 and E7 genes will be expressed in separate vectors to minimise the potential risk. The risks from the purified protein would be low for the same reasons outlined above.

pLNC X and pLXSN vectors could infect and express foreign genes in human tissue but should pose a low hazard due to the cellular toxicity of the PV genes and their inability to replicate. They are deleted for replication functions and require propagation in specific (non-human) helper cell lines in which complementing genes are expressed from separated cassettes thus minimising the risk. All vectors used to generate the recombinant retroviruses are standard strains, and have been in safe use for more than 9 years.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Contaminated plasticware will be autoclaved before disposal of waste. All liquid waste will be decontaminated with 2% Virkon or presept (2000ppm chlorine) (final
concentration) overnight before disposal to drain.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

For most activities this project is a borderline Class 1/2.  The decision to opt for Class 2 was a result of the use of retrovirus vector combined with potentially very large quantities of biologically active protein for crystallisation studies.

**Project Containment**

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**Project Ref** 177/02.2

**Date Ackn’d** 08/01/2002

**CU2 Project Title** GENERATION AND USE OF RECOMBINANT VIRUSES FOR THE INTRODUCTION OF GENE EXPRESSION CASSETTES INTO MAMMALIAN CELLS

**Class** 2

**CultureVolClass2** 1-50 litres

**Non-GMM** not applicable

**Consent Granted**

**Project notified under transitional arrangements**

**Withdrawn**

**Tick if notifying a connected programme of work**

**Historical Significant Changes**

**Historical Date of Additional Info**
**Project Additional Information**

**Purposes of the contained use**

To assess the relative efficiencies of promoter fragments to target mammalian cells for expression of indicator genes (to assess targeting) and bacterial/viral genes (of no known pathological activity) as prod rug activators.

**Recipient or parental organism**

Replication disabled adenovirus will be produced in cultured human embryonic cell lines (eg 293 cells) and inserted genes expressed in primary human cell cultures. The adenovirus is disabled through deletion of the Ad2 E1 gene and cannot replicate in cells which do not contain a fragment of Ad2 adenovirus (eg 293 cells). Disabled baculoviruses are defective in the polyhedrin gene and other critical genes and can only replicate effectively in cells other than the highly adapted host insect cell lines (eg SF9 and SF21) used in the laboratory. The disabled baculovirus will be produced in these standard insect cell lines and used to infect and express transgenes in primary human cell cultures.

**Host/vector system**

4 types of host will be used:

1. **E.coli - E.coli** K12 derived, recA strain DH5a is recognised as non-colonisung and disabled, posing very low/negligible risk to both human health and the environment.
2. **Insect cells** SF21 and Hi5 (for baculovirus production). Insect cells are very unlikely to become established in a human host following accidental exposure unless the individual is severely immunocompromised.
3. **Mammalian cell line** HEK293 (for adenoviral production). The HEK293 cells were derived from human embryonic kidney cells by transformation with sheared human Ad5 adenoviral DNA. The HEK293 are very unlikely to become established in a human host following accidental exposure unless the individual is severely immunocompromised.
4. **Experimental target cells.** Cultured human cell lines and primary human cells. These are also very unlikely to become established outside of the laboratory environment.

Human cells HEK293, experimental target cells and insect cells are non-viable outside the tissue culture laboratory environment and pose negligible hazard to the environment.

**Vectors:**

1. **Bacterial shuttle vectors**
2. **pUC/AdenX/expresson cassette bacterial plasmids**
3. **parental and recombinant baculovirus DNAs (within the insect cells)**
4. **Recombinant AdenX/expresson cassette adenoviruses**
5. **recombinant baculoviruses**

**Origin & function**

3 types of expression cassette will be cloned into the viral vectors.

1. reporter genes encoding luciferase or the fluorescent proteins EGFP or DsRed2 placed under the control of cloned fragments of promoter sequences from specific human genes (primarily those expressed in the prostate such as PSA, PSMA and PSCA or the minimal CMV promotor as a positive control).
2. Cloned tumour-suppressor and prodrug activating genes (wild type or as fusion constructs with sequences encoding EGFP or DsRed2) under the control of minimal CMV promotor or of cloned fragments of specific human genes as described above.

3. Hybrid GP64 membrane proteins in baculovirus containing growth factor receptor recognition peptides to facilitate attachment of BV to human cells.

### Evaluation of foreseeable effects

**Summary**

Most of the experimental procedures in this activity using adenoviral and baculoviral gene transfer systems constitute a very low risk to humans or the environment. Procedures involving handling of high titres of infectious viral particles constitute a potential risk to humans working within the laboratory, though minimal risk to other personnel or the environment.

**Genes**

Products of the reporter genes EGFP, DsRed2 and luciferase are known to have low toxicity and no transforming activity even when expressed at relatively high levels in human cells. Ingestion of these proteins, expression within a person following exposure to bacterial hosts carrying intermediate constructs of the expression vectors, or expression within a person's cells following exposure to infective (non-replicative) virus, are therefore unlikely to present a significant health risk to humans or the environment.

The risk of insertion, by homologous recombination, of part or all of the expression cassette into the genome of a person accidently exposed to the recombinant virus is considered negligible. However, the most likely outcome of this would be apoptosis of the affected cell, with no discernible effects upon human health.

There is a higher risk associated with the use of tumour suppressor genes. However, tumour suppressor peroteins inhibit cell growth and by their nature suppress rather than activate tumorigenesis. The proteins are not likely to have deleterious effects through ingestion or expression within a person following exposure to bacterial hosts carrying intermediate constructs of the expression vectors. Expression of the native form of tumour-suppressor proteins within a person's cells following accidental exposure to infectious non-replicative virus is likely to cause only growth inhibition of the affected cells, again with no discernible effects upon the health of the affected person.

Greatest hazard would come from infection with virus carrying a dominant-negative mutant form of the tumour-suppressor gene or from disruption of normal tumour-suppressor gene function in infected cells through homologous recombination with expression cassette. Known dominant-negative mutants of tumour suppressor genes will be avoided.

**Vectors**

The bacterial shuttle vectors and the pUC/AdenoX/expression cassette plasmids pose a low hazard to humans or the environment and as they will be used in recA host bacteria, genes from these plasmids are unlikely to be transferred to other bacteria or organisms in the laboratory or the environment.

There is a risk of insertion of part of the adenoviral or baculoviral sequence into the host cell by homologous recombination, causing disruption to normal regulation of the host cell's proliferation or survival mechanisms. However, Baculovirus vectors are deleted for the polyhedrin gene and are incapable of survival or replication outside the insect cells in tissue culture. The AdenoX viruses are double-deletion mutants (E1 and E3) and are thus incapable of replication outside helper cells such as HEK293. The double deletion also considerably reduces any risk of intracellular recombination with naturally occurring adenovirus to form replication competent hybrid virus particles.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Contaminated plasticware will be decontaminated overnight in 2% Virkon and autoclaved before disposal of waste. All liquid waste will be decontaminated with 2% Virkon (final concentration) overnight before disposal to drain.

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**Project Ref** 177/02.3

**Date Ackn'd** 12/12/2002

**CU2 Project Title** USING GENETICALLY MODIFIED PLANT VIRUSES AS VECTORS FOR INDUCING RNA SILENCING OF HOST GENES.

**Class** 2

**CultureVol** Class2 not applicable

**CultureVolume** Class3-4 not applicable

**Non-GMM** Consent Granted not applicable

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**
**Project Additional Information**

**Purposes of the contained use**

To use genetically modified plant viruses, tobacco rattle virus (TRV) and potato virus X (PVX) to induce RNA silencing (see Ratcliff et al, 2001, Plant Journal 25:237-245)

**Recipient or parental organism**

Two plant RNA viruses will be used: tobacco rattle virus (TRV) and potato virus X (PVX). These viruses exist in the UK and are not insect or pollen transmitted. Viral vectors based on these two viruses have been made by the laboratory of Dr David Boulcombe, Sainsbury laboratory, John Innes Centre, Norwich. Essentially the viral vectors are cDNA copies of TRV or PVX with added multiple cloning sites.

**Host/vector system**

The host plant will be Nicotiana benthamiana. Infection will be via Agroinoculation. The viral cDNA clones are carried in Agrobacterium binary vectors and therefore toothpick inoculation will be used to initiate infections. The viruses are not pollen or insect transmissible. Transmission within the containment facility would require mechanical inoculation.

**Origin & function**

Fragments (<300 bp) of plant cDNAs will be inserted into the PVX or TRV vectors. Replicating plant viruses have the capacity to induce RNA silencing of host genes. Therefore when a plant is infected with a virus carrying a particular sequence, the corresponding host gene will be switched off. This is an established technique referred to as virus-induced gene silencing (Boulcombe et al, 1999, Curr. Opin. Plant, Biol. 2:109-113; Ratcliff et al, 2001, Plant Journal 25: 237-245).

For this project, the inserted host fragments will be from genes involved in DNA methylation, chromatin changes and unknown functions. The gene fragments are not designed to produce protein products.

**Evaluation of foreseeable effects**

There is expected to be no danger to human health from the genetically modified plant viruses since these are essentially wild-type viruses that carry RNA sequences of plant genes. The inserted gene fragments are not designed to produce protein products.

The main risk of this work is in the potential to initiate virus infections outside of the containment glasshouse facility. It should be noted that both PVX and TRV are found in the UK and typically produce mild symptoms. The genetically modified viruses will be fully infectious and are expected to retain the same properties as the unmodified parental virus in terms of host range and symptomology. The viruses to be used are not insect or pollen transmissible or seed transmissible in Nicotiana benthamiana. The main route of transmission is mechanical and is therefore should be easier to contain than for viruses that can be spread via a vector.

Any plant host genes that share greater than ~90% sequence identity with the sequences carried by the virus will be switched off by the process of virus-induced gene silencing. Thus in addition to the normal viral symptoms, infected plants may show phenotypes of gene silencing though this would be limited to the plant species from which the gene fragment was derived. The gene silencing phenotypes will depend upon which gene is carried by the virus and could range in severity from no visible change to lethality. It is possible that the modified viruses will be less fit than the parental viruses and so there may be selective pressure for the viruses to lose the inserts. If this occurred then the modified virus would revert to the parental form.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
All level 2 measures will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste material generated will be infected plant material, soil, Agrobacteria-contaminated items such as toothpicks and agar plates, and gloves used to handle plants. All waste material will be autoclaved at 134 degrees C for 15 minutes using a regularly serviced and validated machine. Autoclaving under these conditions is expected to produce 100% kill however the inactivation procedure will be monitored (by assessing the infectivity of autoclaved material) at quarterly intervals.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

It was noted that a class 2 activity classification was justified on the basis that the work involves the use of recombinant plant viruses. It was felt that although the overall risk to the environment was low, full application of containment level 2 control measures would provide satisfactory protection.

Project Containment

Laboratory Activities Glass Houses Growth Rooms

L2 Yes L2 Yes L2
L3 L3 L3
L4 L4 L4

Animal Units

L2 L3 L2
L3 L3
L4 L4

Large Scale Activities

L2 L2 L2
L3 L3 L3
L4 L4 L4

Human Clinical Applications

L2 L2 L2
L3 L3 L3
L4 L4 L4

Project Ref 177/03.1

Date Ackn'd 07/11/2003

CU2 Project Title USsing GENETICALLY MODIFIED PLANT VIRUSES AS VECTORS FOR INDUCING RNA SILENCING OF HOST GENES.

Class 2

CultureVolClass2 < 1 litre

Consent Granted not applicable

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

Experiments using genetically modified plant viruses Tobacco Rattle Virus or Potato Virus X and plants infected with them.

**Recipient or parental organism**

Two plant RNA viruses will be used: the tobacco rattle virus (TRV) and potato virus X (PVX). These viruses exist in the UK and are not transmitted by insects or pollen. Viral vectors based on these two viruses have been constructed in the laboratory of Yale University USA, Sainsbury Laboratory, John Innes Centre, Norwich. The viral vectors consist in cDNA copies of TRV or PVX with multiple cloning sites for the insertion of plant sequences.

**Host/vector system**

The host plants will be species in the genera Nicotiana and Lycopersicon, with a concentration on N. benthamiana and L. esculentum. Infection will be via Agroinoculation. The viral DNA clones are carried in Agrobacterium binary vectors and therefore toothpick inoculation will be used to initiate infections.

**Origin & function**

Genetic material consisting of short plant gene fragments (<300bp) will be inserted into the PVX and TRV vectors. Replicating plant viruses have the capacity to induce RNA silencing in host genes. Any plant host gene sharing more than 90% nucleotide sequence identity with the plant gene fragment could theoretically be switched off by the process of virus-induced gene silencing (VIGs). This methodology is well established and applied widely to identify the function of plant genes - see Baulcombe (1999) Curr Opin Plant Biol 2, 109-13, Ratcliffe et al. (2001) Plant J 25, 237-45, Liu et al. (2002) Plant J 30, 415-29. For this project, the cloned DNA fragments will be chosen to target genes specifically expressed in the epidermis of host plants, in order to identify among them those that are associated with epidermal differentiation processes.

**Evaluation of foreseeable effects**

Hazards arising directly from the inserted gene product. The inserted genetic material will be fragments (<300pb) of plant genes predicted to encode proteins that specifically accumulate in the epidermis. The process of virus-induced gene silencing could theoretically switch off any plant host gene that shares more than 90% identity with the plant gene fragment. However, it must be noted that VIGs has been shown not to be active in all plants, even in those in which TRV and PVX replicate and carry genes of the host plant (unpublished test - Sainsbury Laboratory, Norwich). If the receiving environment was exposed to virus used in this activity. VIGs could only theoretically occur in tomato, since only Lycopersicon and Nicotiana sequences will be used, and Nicotiana species are not grown commercially in the UK. Mutations in epidermal-expressed genes have been shown to result occasionally in loss of plant fitness, usually through lower male fertility and less efficient surface water exchanges - see Chen et al (2003) Plant Cell 15, 1170-85, Todd et al. (1999) Plant J 17, 119-130. Such mutations affect all epidermal tissues, whereas VIGs will cause most potential defects in the epidermis of tissues arising 2 to 3 weeks after inoculation. Silenced plants will at worst have partially defective epidermal function, which is unlikely to have any significant effect on their fitness.

Survivability, hazards from the infectious nucleic acids

Partial plant epidermal gene sequences are not expected to alter viral survivability, since they are essentially additional untranslated genomic RNA in the recombinant virus. No recombinant plant protein product is expected to be produced and no hazard to human health is anticipated.
Likelihood that the GMM could cause harm in the event of the environment being exposed
It is extremely unlikely that the recombinant virus would infect plants in the receiving environment since the virus is transmitted by mechanical inoculation only. In the very unlikely event that infection did occur, the resulting level of harm would also be low - defects on some of the epidermal surfaces of plants containing epidermis-expressed genes sharing the appropriate level of sequence identity.

Fitness of genetically modified viruses
In some cases, inserted plant sequences may cause a reduction of viral fitness due to the additional burden of replicating these sequences. This may increase the selective pressure for the viruses to lose the inserts. We do not expect that additional genomic RNA will increase fitness of the virus.

Hazards arising from the alteration of existing pathogenic traits (e.g. alteration of host range or tissue tropism)
No change in host range or tissue specificity is expected for the recombinant viruses as the inserted sequences are not expected to produce a protein product. Since our research will be limited to epidermis-expressed genes, VIGs may result at worst in defects in epidermal cell morphology or biochemistry, which are not expected to have an effect on viral pathogenicity.

Hazard to humans necessitating additional containment measures
There is no identifiable hazard to humans from these recombinant plant viruses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
<thead>
<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All level 2 measures will be applied.</td>
</tr>
</tbody>
</table>

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste material will include infected plant material, soil, Agrobacterium-contaminated materials such as culture tubes, culture flasks, toothpicks, agar plates and contaminated gloves. All waste will be treated appropriately according to its category. Contaminated plant material and soil will be double-bagged at the source prior to autoclaving. All waste will be autoclaved at 134°C for 15 minutes using a regularly serviced and validated machine. Autoclaving under these conditions is expected to produce 100% kill, although assessing the infectivity of autoclaved materials at quarterly intervals will monitor this inactivation procedure.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Following the debate over the suitability of Containment Level 2 for the first activity that we notified involving VIGs, this further activity (albeit an unrelated activity by a different research group) was reviewed by the GM Safety Committee with interest! The GM safety committee inevitably felt that the theoretical risk was greater compared to the original activity given that the work intends to apply the VIGS technique on Lycopersicon esculentum. However, the committee felt that level 2 containment measures, which aim to minimise release of GM material, were still appropriate for this activity for the following reasons. Firstly, the likelihood of tomato crops becoming infected, in the unlikely event of the environment being exposed, remains very low since the virus is transmitted by mechanical means only. Secondly, the level of harm to any tomato plant that is both infected and displaying VIGs symptoms is also considered low - affected plants displaying only mild symptoms. At worst, the silenced plants are anticipated to have a partially defective epidermal function, which would not significantly impair fitness.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

**Project Ref** 177/04.1

- **CU2 Project Title**: MANIPULATION OF NITROGEN METABOLISM IN BACTERIA.
- **Class**: Class 2
- **Culture Vol Class 2**: < 1 litre
- **Project notified under transitional arrangements**: N
- **Consent Granted**: not applicable

**Project Additional Information**
<table>
<thead>
<tr>
<th><strong>Purposes of the contained use</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Characterisation of the nitrogen metabolic capabilities of pathogenic microbes in order to decipher their functions in bacterial proliferation and pathogenicity.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Recipient or parental organism</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>The project will involve the construction of strains of the pathogenic bacteria Neisseria meningitidis, in which genes conferring the capability to catalyse certain reactions of the nitrogen cycle are disrupted. The modifications will involve 'self-cloning' of N. meningitidis genes back into the host organism. These disruptions will be marked with genes for resistance to antibiotics which are unrelated to clinically important antibiotics. The disruptions will cause pathogenicity of these modified organisms to either remain the same as the wild-type strains, or be reduced.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Host/vector system</strong></th>
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</thead>
<tbody>
<tr>
<td>Hosts for this work will be Neisseria meningitidis (serotype B). No replicative plasmids will be introduced into these strains. PUC type plasmids containing genetic material from the pathogens (genes that in themselves will not confer virulence) will be maintained in E. coli. The scope of this activity may in future be extended to include the analysis of metabolic pathways in other Hazard Group 2 pathogens (possibly, Pseudomonas aeruginosa and Burkholderia cepacia).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Origin &amp; function</strong></th>
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</thead>
<tbody>
<tr>
<td>The relevant genetic material from Neisseria meningitidis encodes enzymes for the metabolism of nitrogen compounds.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Evaluation of foreseeable effects</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Neisseria meningitidis (serotype B) is a ACDP Hazard Group 2 organism, transmissible in aerosols via the respiratory route and capable of causing severe illness and possible death by septicaemia and meningitis. There is currently no effective prophylactic treatment for serotype B. The genetically modified organisms will be equally or less pathogenic than the wild-type parental strain; no additional harmful effects are foreseeable from the intended modifications.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</strong></th>
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<tbody>
<tr>
<td><strong>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</strong></td>
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</table>

<table>
<thead>
<tr>
<th><strong>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid and liquid microbial wastes will be killed by autoclaving, ensuring a 100% kill. Monitoring the effectiveness of the autoclaving will be carried out routinely by conducting viability tests on autoclaved samples and using autoclave test strips. All departmental autoclaves are subjected to an annual inspection test. Waste will be transported to a central departmental autoclave facility in robust, lidded metal containers that will be autoclaved immediately and directly.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Is an emergency plan required according to regulation 20?</strong></th>
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<tbody>
<tr>
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<tr>
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<tr>
<td>N</td>
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</table>

<table>
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<tr>
<th><strong>Tick to confirm that you have attached a risk assessment to this form</strong></th>
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<tbody>
<tr>
<td>Y</td>
</tr>
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</table>

<table>
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<tr>
<th><strong>Tick if you are claiming exemption from disclosure for section of the risk assessment</strong></th>
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<tbody>
<tr>
<td>N</td>
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</tbody>
</table>
Following advice from the HSE, the committee agreed that since there was a possibility that the resulting modified organism could still cause harm, this activity did not satisfy the regulatory definition of self-cloning. The committee agreed that containment measures required for Class 2 activities would provide adequate containment. It was also suggested that, if available, the group leader should consider using a departmental containment level 3 facility for liquid culture manipulations. Although not strictly required for containment of this GM activity, the use of such a facility would offer the advantage of avoiding the use of the shared facility currently used.

Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 Yes | L3 | L4
L2 | L3 | L4
L2 | Yes | L3 | L4

Animal Units | Large Scale Activities | Human Clinical Applications
---|---|---
L2 | L3 | L4
L2 | L3 | L4
L2 | L3 | L4

Project Ref 177/04.2

Date Ackn’d 21/09/2004
CU2 Project Title Identification of genes and processes underlying bacterial biofilm formation. Biofilm formation is when in bacteria adhere to abiotic surfaces, for example to catheters and implants in the body. This can lead to recurring and chronic infection, .........

Class 2 CultureVolume
Class | CultureVolume
---|---
Class 2 | < 1 Litre

Non-GMM Consent Granted Not Applicable
Project notified under transitional arrangements N

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
The project will analyse the biofilm formation process at a molecular level with the goal of identifying genes or processes that can be targeted in the development of new antimicrobial strategies specific for biofilms. This involves constructing mutants, analysing the ability of these mutants to form and disperse from biofilms, and identifying
the underlying reason for their altered properties. In addition, GMM will be constructed that will facilitate analysis of genes expression during biofilm formation using fluorescent proteins (Gfp, Yfp, Cfp).

Recipient or parental organism

Research will be carried out with bacterial species that are normally commensal isolates of the gastro-intestinal tract: Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Enterococcus faecalis. These species however are also causative agents of urinary tract infections, and in many cases have been associated with urinary catheter related infections. They will be mutagenized so that they carry mutations in single genes.

Host/vector system

In addition, GMM will be constructed that will facilitate analysis of genes expression during biofilm formation using fluorescent proteins (Gfp, Yfp, Cfp).

Origin & function

The mutants are made in the parental bacterial species.

In addition, to characterize the genes of interest, they will be cloned and propagated in a E. coli K12 strain (MG155), a non-colonizing bacterial species that is commonly used for this purpose. The cloned genes are not expected to alter this strain with regard to its ability to propagate and survive in the environment.

Evaluation of foreseeable effects

Mutants of the various bacterial species will be selected for their altered ability to adhere to surfaces and to each other, and for altered growth in the environmental micro niche(s) present in a biofilm. Based on the literature, in most cases this will be associated with specifically a decrease in the relevant capacity, which will also negatively affect their ability to colonize. The parental organisms are considered opportunistic pathogens, and this can reasonably be expected to be the same for the GMMs. Thus, they will not pose a health risk for healthy individuals.

The genes clones into E. coli K12 isolates are not expected to alter this strain with regard to its lack of ability to propagate and survive in the environment, since it contains mutations that incapacitate it.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid G waste will be autoclaved on site. A 100% "kill" degree is achieved, which is monitored by periodic viability testing for CFU of waste material. Annual servicing and validation of autoclaves occurs.

GM solid waste and liquids are stored in bins in the laboratory and then taken for autoclaving in another part of the department in sealed, leak proof contains. Treated material is finally disposed of in normal refuse bins for collection by local authorities.
This project was approved as a Class 2 activity, requiring control measures from containment level 2, on the basis that it involved the use of several ACDP hazard group 2 organisms. It was agreed that genetic modification of these organisms did not result in additional hazards to human health or the environment.

### Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
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<tbody>
<tr>
<td>L2 Yes L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
<td>L4 L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
<td>L4 L2 L3 L4</td>
</tr>
</tbody>
</table>

### Project Ref 177/05.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>Date Project Ceased</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>14/04/2005</td>
<td></td>
<td>Cloning of schistosome proteins and characterisation of their functional properties.</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td></td>
<td>Not Applicable</td>
<td></td>
<td>N</td>
</tr>
</tbody>
</table>

### Project Additional Information
## Purposes of the contained use

Expression and manipulation of Schistosoma mansoni proteins with putative immunomodulatory or unknown functions.

## Recipient or parental organism

- **Echerichia coli** strains, eg BL21 or JM109
- **Pichia pastoris** strains, eg X-33 or GS115
- Chinese hamster ovary cells
- Insect cells, eg SF21 or High Five cells

All hosts are non-pathogenic and pose negligible risk to human health.

## Host/vector system

- **E. coli** vectors include pUC, pET, pGEM-T
- **P. pastoris** vector to be used is pPICz
- CHO cell vector to be used is pcDNA4/70
- The Bac-N-Blue baculovirus will be used as a vector with the insect cells

All vectors are either mobilisation defective (eg pET) of non-mobilisable (eg pGEM-T) and are considered to pose a negligible risk to human health.

## Origin & function

The genes to be cloned originate from the human blood fluke *S. mansoni*. The intended function of these gene products is unknown unless significant homology to a protein from another species is detected. It is our hypothesis that some of these proteins may have immunomodulatory function.

## Evaluation of foreseeable effects

GMO constructs will be used for the expression and manipulation of *S. mansoni* proteins. It is anticipated that in most cases the GMOs will be no more hazardous to human health and the environment than the unmodified organisms. This will apply for GMO constructs involving gene inserts of known function eg alkaline phosphatase. However, some of the gene inserts will encode products of unknown function or possibly products with homology to putative immunomodulators. In a minority of cases, it is thought that expressed proteins may, for example, be allergenic. The overexpression of proteins in bulk fermentation cultures (up to 10 litres) and processing of samples with centrifugation and sonication/homogenisation are factors which could increase risk of exposure to proteins with potentially harmful (eg allergenic) properties. As a precautionary measure, it is therefore considered appropriate to apply full Containment Level 2 measures for activities involving the bulk culture of proteins of unknown function, which may have potentially harmful properties.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

## For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

## Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Solid** - Solid waste for disposal will be bagged and autoclaved before disposal. Kill level for these procedures is effectively 100%.
- **Liquid** - Liquid waste will also be chemically treated with Virkon, and autoclaved prior to disposal in designated sinks. Again, the kill level for these procedures is effectively 100%.
Periodic viability checks will be performed after both solid and liquid waste disinfection to validate effectively 100% kill levels. Annual validation of autoclaves are performed.

The possibility of some GMOs expressing proteins with potential immunogenic properties was the key concern of the GM Safety Committee. It agreed that as a precautionary measure, full containment level 2 measures should be applied to the bulk expression of recombinant proteins to control the risk to human health from this potential hazard. The Committee also requested further details on measures to be applied to control the risk of exposure during sample processing using techniques of sonication or centrifugation.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

Animal Units
- L2 L3 L4 L2 L3 L4 L2 L3 L4

Large Scale Activities
- L2 L3 L4 L2 L3 L4 L2 L3 L4

Human Clinical Applications
- L2 L3 L4 L2 L3 L4 L2 L3 L4

**Project Ref 177/05.2**

<table>
<thead>
<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>Culture Vol</th>
<th>Culture Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/10/2005</td>
<td>The use of lentivirus for the introduction of gene expression cassettes into mammalian cells.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Not Applicable</td>
</tr>
</tbody>
</table>

Non-GMM Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Tick if notifying a connected programme of work: N
The purpose of this project is to develop a tool to monitor specific gene activity in cells grown in tissue culture. The approach will be to use a commercially-available system whereby the promoter of the gene of interest is inserted upstream of a fluorescent reporter gene. The lentivirus system has been selected as it will allow highly efficient, stable expression.

Disabled lentivirus will be produced whereby reporter genes encoding luciferase or the fluorescent proteins EGFP or DsRed2 will be placed under the control of cloned fragments of promoter sequences from specific human genes (generally those known to be expressed primarily in the bladder, such as UPK2) or the minimal CMV promoter as a positive control. This will enable us to monitor by fluorescence or luminescence when a particular gene is expressed in the modified cells grown in tissue culture.

An expression cassette (pENTR-5'TOPO) containing promoter of interest and entry vector (pENTR-ORF) containing the reporter gene construct will be amplified in E. coli K12. The cassettes will then recombined using the multisite gateway technology (Invitrogen) into a plasmid containing a disabled lentivirus backbone sequence (pLenti6/R4R2/V5-DEST) in E. coli DB3.1 as host, then propagated in 293FT cells to produce replication-incompetent lentivirus. Tissue cultured mammalian cells (including 293T) are unable to survive outside defined culture conditions, meaning they are categorised as a disabled host. The disabled lentivirus will be used to infect mammalian cells so that expression of the reporter genes (encoding luciferase or the fluorescent proteins EGFP or DsRed2) is regulated from cloned promoter sequence fragments.

EGFP and DsRed2 are derived from Aequoria victoria (EGFP) and Discoma species (DsRed2), respectively. They are proteins commonly used as reporter proteins as they are non-toxic, fluorescent and not expressed by mammalian cells. Luciferase is commonly derived from firefly and renilla and luciferase assays are commonly used to quantitatively assess promoter activity when combined with a foreign promoter or fragment thereof.

We anticipate that fluorescent reporter protein will be expressed in mammalian cells that have active promoter sequence (eg of the correct cell type and differentiation stage). Specific promoter sequences will be selected and cloned on the basis of restricted transcriptional activity to specific cell types and/or stage of differentiation. For example, the uroplakin II gene promoter which is active in terminally-differentiated human urothelial cells. Usually, such promoters would drive expression of gene products required for tissue-specific or differentiated cell function, but in this case they will be cloned upstream of reporter genes in order to monitor activity of the promoter in
The products of the reporter genes EGFP, DsRed2 and luciferase have been shown to have low cytotoxicity and no transforming activity even when expressed at relatively high levels in human cells. Ingestion of proteins, expression within a person following exposure to bacterial hosts carrying intermediate constructs of the expression vectors, or even expression within a person's cells following accidental exposure to infectious (non-replicative) virus, are unlikely to pose a significant health risk. The greatest hazard would come from the unlikely event of insertion, by homologous recombination, of part or all of the expression cassette into the genome of a person accidentally exposed to the recombinant virus. The outcome of such an event cannot be predicted with absolute certainty, although the result of introducing a highly tissue-specific promoter would not be anticipated to be significant and it might be anticipated that such a cell would be eliminated by the host immune system. The proteins are not likely to have deleterious effects through ingestion or expression within a person following exposure to bacterial hosts carrying intermediate constructs of the expression vectors. The highest risk would be from disruption of normal tumour-suppressor gene function in infected cells through homologous recombination with the expression cassette. Risks to the environment are considered negligible: the host cells used are non-viable outside the laboratory and the lentivirus vectors, which are disabled through absence of LTRs in the ViralPower packaging mix, are therefore only expressed in the producer cell and never packaged into virions. The resulting viral particles after mixing with virapower packaging mix are replication-incompetent.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- a) Contaminated plasticware will be decontaminated overnight in 2% Virkon and autoclaved (effectively 100% kill) before disposal to general waste. The autoclave is subject to annual testing and validation using thermocouples, and routine use of chemical indicator tubes.
- B) All liquid waste will be decontaminated with 2% Virkon (final concentration) overnight before disposal to drain.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The committee agreed that this was a CL2 activity based on the current guidance for work involving a 3rd generation replication incompetent Lentiviral expression system. It was felt that the modification would not present further significant hazards to human health or the environment. Indeed, since the GMO is unlikely to cause human disease, the possibility of this activity being graded as Class 1 was considered. However, it was agreed that CL2 should be applied as a precautionary measure, and consistent with recommended containment level for work with these Lentiviral vectors.

#### Project Containment
Purposes of the contained use

Generation and recombinant lentiviruses for overexpression of cDNAs, shRNAs and genomic promoter reporter genes by lentiviral transduction of mammalian cells to identify novel signalling pathways and proteins regulating the activity of connective tissues cells and stem cells and their influence on tissue function in health and disease.

Recipient or parental organism

Replication defective lentiviruses will be produced in cultured human embryonic cell lines (HEK 293 FT) and inserted genetic material (cDNAs, shRNAs or genomic promoter sequences) expressed in mammalian primary cells and cell lines. Virions will be generated by co-transfecting HEK 293 producer cells and plasmid constructs containing the recombinant lentiviral expression cassettes and plasmids supplying helper functions and genes encoding structural and replication proteins in trans (eg gag, pol and rev).

Host/vector system
Host cells to be used include: E. coli K12-derivatives (eg Stb13, INV. F and TOP10), HEK-293 cell line (for lentiviral production) and cultured mammalian cell lines and primary cells (experimental target cells, including material derived from humans). Vectors to be used include: Bacterial sub-cloning vectors (eg pENTRIA, PCR2.1), lentiviral expression cassette vectors (eg pLenti6/V5-DEST, pLenti6/BLOCK-iT - DEST), lentiviral packaging plasmids (eg pLP/VSVDG, pLP1) and resulting recombinant lentiviruses.

3 types of expression cassette will be cloned in the vectors.
1. Reporter genes encoding luciferase, lacZ or fluorescent proteins (eg EGFP, dsRED2) under the control of cloned promoter fragments derived from specific target genes.
2. cDNAs encoding specific genes of interest under the control of the CMV promoter expressed as native wild-type proteins, fluorescent fusion constructs or truncated/mutated variants with constitutively active or dominant negative activity.
3. Small oligonucleotides encoding shRNAs targeting specific genes of interest under the control of the U6 RNA polymerase promoter to induce and determine the effects of gene knockdown.

Genes and promoters of interest include:
A: Receptors, ligands/cytokines and transporters. Receptors, ligands and transporters include those for glutamate (eg NMDAR1, NMDAR2A-D, NMDAR3, GluR1-4, KA1 and 2; GluR5-8 and mCluRs, GLAST, GLT-1, vGLUT1), Wnt (eg Wnt1, Wnt5a, sFRPs, Frizzled, Kremen, LRPs/6, Dickkopf), Notch (Notch 1-4, Jagged), BMP (eg BMP-2) and hedgehog signalling pathways (hedgehog, patched, smoothened). Other receptors, ligands and cytokines with suspected regulatory functions in the control of cell differentiation/function will also be studies (eg RANK, PTH-R, estrogen receptor TGF-, IL-1, IL-6, RANKL, TPO, OPG)

B: Matrix, structural and exocytotic proteins. These proteins include normal constituents of the cell matrix (eg osteocalcin, Type 1 collagen, osteopontin), adhesion molecules (eg APP, neurexins, syndecans, integrins) signalling specific structural proteins (eg receptor interacting proteins; PSD-95, GSKAP, SHANK, Homer) and molecules involved in neurotransmitter release (eg SNAP25, VAMP, syntaxin).

C: Enzymes and transcription Factors. These proteins include those involved in the termination of cholineric signalling (eg AChE, bChE and their C-terminal derivatives), with roles in intracellular signal transduction (eg TIP60, glyceraldehyde - 3-phosphate dehydrogenase, alkaline phosphatase, CaMKII, PKB, eNOS, nNOS presenilins, ADAM10) or enzyme co - regulators (Nct, Pen-2, APH-1). Transcription factors (eg TCF-1, NFE-2, c-FOS, CBFA-1, SMAD4, Gli-1) and repressors (SuiFU, Groucho) activated by cell signalling pathways or that control gene expression in connective tissues will also be studied.

D: Intracellular signalling components. These include molecules required for intracellular signal transduction (eg FRAT/GBP, -Catenin, Dishevelled, Adenomatous Polyposis Coli, Axin/Axil Conductin, GSK-3, Fu, Fe65)

Evaluation of foreseeable effects

Bacterial subcloning vectors, recombinant lentiviral expression cassette vectors and lentiviral packaging plasmids are non-mobilisable and lack essential loci required to mediate DNA transfer from one host to another. Lentiviruses have a history of safe-use and are replication defective, engineered to lack essential genes required for packaging and replicative activity. These are only expressed in the producer cell line and are supplied on separate plasmids which lack regions of homology and LTRs, safeguarding against recombination events that could lead to restoration of these functions in viral progeny. Since packaging and replicative genes are never packaged into the viral genome infective viruses are incapable of undergoing further rounds of replication and can be considered as mobilisable defective. Deletion of the 3' LTR (U3) of the pLenti expression plasmids also results in self inactivation of the virus after transduction into host cells rendering the lentivirus further incapable of producing a packageable viral genome following integration into the host genome. No new replication defective-competent virus can be produced following transduction of host cells. E. coli K12-derivatives used for subcloning (eg Stb13, INV. F and TOP10) are disabled hosts and are non-pathogenic, non-reversional and are unable to colonise the human gut. These strains are unlikely to survive outside the laboratory where they would be subjected to sub-optimal growth conditions (nutrients and temperature) and pose a very low/negligible risk to human health or the environment. HEK-293 (used for virus production) and target mammalian cell lines have a history of safe use, are unlikely to establish outside the laboratory and pose a very low/insignificant risk to human health or the environment. Primary cells are of unknown pathogenicity and will be considered to be pathogenic. Cells are unlikely to colonise the worker except under conditions of severe immunocompromisation.
Lentiviral particles exhibit a wide species tropism and although they could survive for several weeks under non-laboratory conditions, they are replication defective and therefore pose a low-risk to the environment. Some of the cloned gene products are oncogenes or have tumour suppressor activity and although ingestion of these proteins, expression or shRNA gene knockdown within a person following accidental exposure to E. coli or infectious (non-replicative) virus is unlikely, it does constitute a low risk to human health. The greatest hazard is likely to arise from incorporation of cloned gene products/shRNAs into the genome of the worker accidentally exposed to the virus. The most likely outcome of this would be apoptosis of the infected cell or growth retardation, but may include increased risk of cellular transformation for oncogenes. Some oncogenes can act independently which increases the risk posed by the insert, however the overall chances of exposure to workers protected by full biosafety containment level 2 measures (causing oncogenic transformation) is very low, since the virus will be unable to establish in the worker and the virus is unlikely to come into contact with or penetrate the skin, buccal, oesophageal or intestinal mucosa which represent the most likely routes of infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated plasticware (culture dishes, pipette tips, centrifuge tubes etc.) and culture medium used for viral production/transduction will be chemically disinfected by soaking in 1% Virkon solution or 5% Chlorus bleach (5g/L available bleach) for 24 hours. Waste will be double-autoclaved, bagged, clearly marked biohazard and transported to the autoclave in a leak-proof container. Autoclaving is performed on a 20 minute steam sterilisation cycle. The degree of kill achieved is 100% as validated by the manufacturer. Autoclaving efficiency is routinely monitored by thermolog monitoring and dummy loads ensuring that the autoclave is operating for the correct time at an appropriate temperature and pressure. Autoclaves are service annually and validated using thermocouples. Sterilised autoclave waste is placed in a disposal bin for removal to a designated landfill site.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The committee agreed that this was a Class 2 activity based largely on the current guidance for work involving a 3rd generation replication incompetent lentiviral expression system, and also because of the oncogenic nature of some of the inserts. Although it was felt that there was a theoretical possibility of oncogenes integrating into a cell genome of an exposed individual, the risk of this happening and leading to cancer formation was considered low. In addition to the use of physical control measures from CL2, the committee stressed the importance of applying good safety management practices for this activity, and asked the group to consider using alternative facilities from those proposed, which are often busy and shared with several groups.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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02/03/2022
The overall aim of the research programme is to understand the pathogenesis of infectious diseases of major public health importance (e.g. leishmaniasis, pneumoniae), with specific reference to understanding the balance between protective immunity and immunopathology. The results of our research will be of value in the design of new vaccines and therapeutics.

In this GMO project, we will use viral vectors in three main contexts: i) to introduce genes encoding various reporter proteins (luciferase, EGFP, DsRed etc) into mammalian cells in vitro. These "reporter tagged" cells will be used for in vitro experiments and / or be transferred into rodents (by adoptive transfer into normal mice or by bone marrow transplantation) to allow visualisation and tracking of immune responses; ii) to introduce genes encoding potential vaccine antigens / adjuvants into mammalian cells in vitro. These cells will be introduced into rodents by adoptive transfer and / or bone marrow transplantation to enable the efficacy of various vaccination protocols to be assessed; iii) to introduce the genes encoding reporter proteins, vaccine antigens / adjuvants directly as gene therapy in rodents in vivo. The main vectors of choice for these projects will be lentiviruses. This GMO project will utilise pre-constructed lentiviral or adenoviral vectors, obtained either commercially or from other laboratories in the UK or elsewhere.
Non-replicating lentiviruses and adenoviruses will be used to introduce genes into rodent cells, which will then be either studied in vitro or transferred into rodents for further study. In some cases, viral vectors will be directly administered to rodents. The viral vectors will encode for proteins that "tag" cells to allow their detection and with no known influence on function (e.g. fluorescent proteins), or they will encode proteins which may be targets of protective immunity or may help to enhance immunity (i.e. adjuvant molecules).

Host/vector system

The GMO will be constructed in and obtained from the laboratories of our collaborators or commercial suppliers. GMO will normally be obtained as pre-titered viral concentrates.

The lentiviral vectors are disabled by deletion within the 3' LTR which prevents production of packageable virus genomes once the virus has integrated into the target cell. Additionally, the recombinant lentiviral genome contains no viral structural genes so these cannot be expressed in the target cell. The adenoviral constructs are deleted at both the E1 and E3 regions and hence are replicative deficient.

Origin & function

"Reporter" proteins (e.g. EGFP, DsRed, luciferase) will be obtained from commercial sources or other laboratories and are not known to have any activities that are directly toxic or detrimental to the health of humans or animals.

The vaccine "antigens" will encode an antigen from a micro-organism causing human disease e.g. Leishmania. The function of these antigens will be to induce immune responses against the target micro-organism, and they will not be known toxins or other molecules likely to have detrimental effects on human or animal health. The function of the "adjuvant" proteins is to enhance immune responses by manipulating the host immune response. These will be cytokines (e.g. GM-CSF) which are normally made by humans and rodents, and which are often, but not exclusively, are already in clinical use.

Evaluation of foreseeable effects

Adenovirus and lentivirus can infect human cells but all viruses contain multiple deletions are unable to replicate and produce virions except in specialised laboratory host cells. Adenovirus can cause minor respiratory tract disease but because of the mechanism of viral attenuation it cannot cause a pool of replicative virus, so disease in humans would be very minor.

Principle routes for accidental infection with viruses would be needlestick injury or aerosol production.

The expressed reporter gene products and vaccine antigens are not known to have adverse affects on human health, even in the unlikely event that they are expressed for prolonged periods. The antigens have a remote possibility of causing allergic reactions if the individual has been exposed previously and developed specific allergy. Most rodent cytokines to be used in this study are species specific and have no activity on human cells. In cases where species cross-reactivity exists, clinical data suggests that transient exposure of humans to such cytokines would have no derious adverse effects. Long term exposure is unlikely as rodent cells harbouring virus would not survive in humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be adjusted to a final concentration of 10% Trigene for > 24h before being autoclaved at 134°C for 15 minutes at 27psi pressure, the degree of kill is effectively 100%, see nore below.

Solid waste will be placed directly into a stainless steel discard autoclave box, lined with 2 autoclave bags. These boxes will be sealed and labelled before being
autoclaved at 134C for 15 minutes at 27psi pressure, the degree of kill is 100%.

Autoclave validated by the manufacturers initial commissioning, bi-annual service and calibration of waste programme conditions (print out each run kept for with lab
records).
Any glass and other “sharp” material will be disposed of in a “sharps-bin” and taken for incineration.
70% ethanol will be used to clean non-disposable materials and surfaces.
Transfer of experimental material between labs will be in a leak-proof, impact-resistant container.
Lab coats will be autoclaved before laundering.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The committee felt that the viral vectors presented negligible risk to human health and the environment and could potentially be handled at Containment Level 1. Further
detail was requested on the nature of the cytokines and possible effects on human health if exposure occurred. It was concluded that although this activity presented
negligible risk to human health or the environment, Containment Level 2 should be applied as a precautionary measure, and consistent with current guidance and other
activities at York using 3rd generation replication incompetent vectors.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<table>
<thead>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 177/07.1

Date Ackn’d 05/01/2007

CU2 Project Title Identifying and characterizing the molecular basis of pathogenesis and persistence in

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4
This project addresses both the regulation and role of factors in foodborne bacterial pathogens that enable them to persist in animal hosts and be successful as a pathogen in human hosts. Specifically the project aims to elucidate regulatory networks of cell surface factors used in serotyping (O-antigens) but may expand to include factors under the control of phase variation (adhesins for example) that may adversely affect serotyping. The project will also address the biological role of these factors, and how they may affect virulence. These questions will be addressed for Gram negative bacterial pathogens associated with food poisoning. The initial focus is on Salmonella, but the study may expand to include genes from the few Escherichia coli strains (pathogens) that have a similar transmission route and virulence as Salmonella.

Recipient or parental organism

Two types of GMM will be generated for this project, one where E. coli K12 is the recipient, and second where the recipient is the pathogenic isolate Salmonella. Our studies will generate GMM of the Gram negative foodborne bacterial pathogens that mainly cause gastroenteritis, initially of Salmonella. Strains will excluded which code for toxins that render them highly pathogenic and thus resulting in more severe disease, as will those strains that have a high probability to cause systemic disease (Salmonella: serotypes with high probability of causing enteric disease in humans (notably typhus, paratyphoid A and cholera is excluded). Any strains that have been identified as MDR (multidrug resistant) will not be used as recipient. E.Coli pathotypes that may be used as donor of DNA E.coli: enterohaemoragic E coli (EHEC) will be excluded.

Importantly, genes from one pathogenic species or strain will not be expressed in a different pathogenic species or strain, thus limiting the possibility of increased risk.

Host/vector system

Mutations (deletion or insertion) in single genes will be constructed. Genes will be cloned into non-transmissible plasmid vectors. Importantly, genes from one pathogenic species or strain will not be expressed in a different pathogenic species or pathogenic strain, thus limiting the possibility of increased risk.

Origin & function

The genetic material will be derived from Salmonella strains (non-typhoid) and Salmonella phage, and may expand to include genes from E.coli pathotypes that have a similar transmission route and virulence as Salmonella (thus EHEC excluded). The functional groups of genes targeted for mutation will be regulatory proteins, LPS modifying enzymes, adhesions and outer membrane proteins. No toxins will be cloned.
Standard reporter genes will be introduced into the parental strains (including Green fluorescent protein (Gfp) and derivatives from Aequorea victoria and LacZ (beta-galactosidase) from E.coli).

**Evaluation of foreseeable effects**

Based on current state of knowledge, the probability that the mutations will not affect virulence or decrease virulence is high, and the probability that virulence is increased is low. After infections, disease is a multi-factorial process, and bacterial genes must be expressed at the appropriate time and location during infection for the bacterium to cause disease. The worst-case scenario is that the GMM has a higher incidence of progressing from gastroenteritis to systemic disease, but no scenario is known for this to occur as a result of a single mutation or expression of a single gene.

Natural occurring infections of these species are mainly associated with contaminated food or water, and thus with oral ingestion, and mainly cause gastroenteritis. In rare cases infection can lead to systemic disease, mainly in immune suppressed individuals, very young and the elderly. In the laboratory setting the main risks for the GMM are also ingestion and parental infection.

Salmonella: All serovars can cause disease in humans. The broad host range strains to be used in this work mainly cause gastroenteritis, which is a self-limiting disease, and needs to be treated with antibiotics (fluorquinolones are considered optimal). Any strains that have been identified as MDR (multidrug resistant) will not be used as GMM, since this would severely limit treatment options in the very unlikely case systemic infection does occur. The main concern is ingestion and parenteral infection. This applies to the select E.coli strains as well.

The GMMS will have the same risk factors as the recipient strains where the recipient is the pathogenic isolate. Where E.coli K12 is the recipient, the risk will be the same or lower as for the original donor. Thus, the GMMS are considered to present a low to moderate risk to human health and low risk to the environment. All containment level 2 control measured will be applied together with the principles of GMP and good occupational safety and hygiene.

Measures include use of protective clothing (Lab coat), gloves, sterilizing all liquid and solid contaminated waste and use of disinfectants to clean work surfaces.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid and solid GM waste will be autoclaved. GM solid waste and liquids are stored in bins in the laboratory and then taken for autoclaving in the same building in leak proof containers. Solid autoclaved material is finally disposed of in normal refuse bins for collection by local authorities; liquid autoclaved material is disposed of in the sink. The degree of "kill" is effectively 100%.

Autoclave is validated by the manufacturers initial commissioning, bi-annual service and calibration of waste programme conditions (print out of each run kept for with lab records). Periodic viability testing for CFU of waste material.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The committee concluded that the GMOs produced by this activity would not result in organisms that were more harmful than the corresponding unmodified organisms. The assessor was asked to make it clear that there was no possibility of introducing unknown toxin genes into E.coli K12, and emphasise that a safety cabinet would be used for procedures likely to generate aerosols.

**Project Containment**

<table>
<thead>
<tr>
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<th>Glass Houses</th>
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</tr>
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**Project Ref  177/11.1**

<table>
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<tr>
<th>Date Ackn'd 23/05/2011</th>
<th>CU2 Project Title The role of non-coding RNAs in innate antiviral immunity</th>
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<tbody>
<tr>
<td>Date Project Ceased</td>
<td>Class CultureVolClass2 CultureVolumeClass3-4 Class 2 &lt; 1 Litre Non-GMM Consent Granted Project notified under transitional arrangements N</td>
</tr>
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<td>Historical Significant Changes</td>
<td>Withdrawn N Tick if notifying a connected programme of work N</td>
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**Project Additional Information**

The overall aim of the research programme is to understand the mechanisms that govern the innate immune response to pathogens that are associated with infectious diseases of major public health importance. In this GMO project, we will use human herpesviruses and self-inactivating lentiviral vectors to dissect the role of non-coding
**RNAs in immunity.**

Human herpesviruses used in the project will include Kaposi's sarcoma herpesvirus (KSHV), Herpes Simplex virus 1 (HSV-1), and human Cytomegalovirus (CMV). These viruses will be used for in vitro challenge of mammalian cells. The mouse herpesvirus MHV-68 will also be used for similar in vitro experiments. Some of these viruses (such as in the case of KSHV strain used) are engineered to encode for reporter proteins (such as EGFP, luciferase etc). These tags are introduced in the viral genome in such ways so they do not affect viral infectivity or replicator. Self-inactivating lentiviral vectors (pSIN vector, previously used in similar projects elsewhere) will be used for the introduction of human protein-coding genes and non-coding RNAs into primary human cells. These viruses are replication-deficient and virions are not produced from infected cells. Virions are produced through a triple transfection method (lentiviral plasmid + packaging protein plasmid) into a permissive line such HEK293T cells. The separation of the three plasmids ensures that the lentiviral plasmid alone (the fragment of DNA that is integrated in host genome after lentiviral infection) is not capable to replicate. The genes delivered with the lentiviral vectors include non-coding RNAs, signalling proteins, and RNA-binding proteins.

**Recipient or parental organism**

Herpesviruses and self-inactivating third generation lentiviruses will be used to infect mammalian cells (rodent or human, primary, immortalised or transformed. The lentiviruses will encode for fluorescent tags or human genes with a role in immunity. The used herpesviruses are engineered to carry fluorescent or luminescent tags.

**Host/vector system**

Wild-type herpesviruses (Kaposi sarcoma herpesvirus, herpes simplex virus 1, human cytomegalovirus ) engineered to contain reporter cassettes (EGFP, luciferase etc. These reporter genes do not affect virulence of the used viruses.

HIV-based self inactivating lentiviral vectors containing inserts encoding for human genes (non-coding RNAs, signalling proteins, RNA-binding proteins etc). These viruses are incapable of replication due to deletion of their LTR regions as well as structural gene. Individual gene inserts are unlikely to represent any significant hazard.

**Origin & function**

Lentiviruses with tag or gene inserts will be constructed in our laboratory or provided by collaborators. Production of lentiviral particles involves a triple transfection procedure in HEK-293T cells, to achieve one round of replication. The main purpose of using these constructs is to test the role of individual non-coding RNAs and proteins in the regulation of the innate immune response in human primary cells, and identify protective pathways from infection.

The herpesviruses will be grown in our lab or obtained by collaborators. None of the engineered viruses displays increased virulence. The purpose of using theses pathogens is to probe mammalian cells and analyse their anti-pathogen responses.

**Evaluation of foreseeable effects**

Human herpesviruses are used in this project. At the titres used, these viruses are unlikely to cause primary infection of humans. In the extremely unlikely case of injection of a high number of latently infected cells in a human, these cells would be most likely cleared by the immune system.

The lentiviral vectors used contain multiple disabling mutations. None of the host cells or inserted genes could complement or replace the disabled genes. All components for producing lentivirus virions are encoded separately on three different plasmids. The herpesviruses used do not establish permanent infections in vitro and at the doses used are not likely to infect humans. In the extremely unlikely event of injection of the low lentiviral particles preparations used, it is highly unlikely that any cells will become infected and that the gene inserts could cause alone any harm to humans.

The used pathogens and lentiviral products do not have any known toxic or transforming activities in the amounts and forms used. MicroRNAs and RNA-binding proteins have been implicated in disease but the safe usage of low titre, replication deficient lentiviral vectors ensure the practically risk-free use of these genes.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste is sealed in double autoclave bags and subsequently autoclaved (134°C for 15 minutes at 27psi) before disposal. Liquid waste is decontaminated in Virkon or Precept followed by autoclaving before disposal. These procedures lead to 100% killing of used viruses, as monitored by growth curves. Autoclave printouts will be checked after cycles of solid decontamination. Autoclaves are regularly serviced.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The committee noted that work with 3rd generation replicative incompetent, self inactivating Lentiviral vector with inserts unlikely to cause harm, presents negligible risk and could be justified as a Class 1 activity. The possibility of the expression cassette integrating within infected cells and activating harmful genes was raised. However, it was noted that additional biosafety features of the lentiviral vectors reduces this risk. In addition, exposure of worker to levels of virus high enough to cause e.g. tumourigenesis following exposure would be unlikely during normal laboratory based manipulations.

It was agreed that the activity should be assigned to Class 2 on the basis that the work involved the use of Herpes Viruses (Hazard Group 2), capable of causing harm to humans. However, it was noted that the risk of infection from these viruses was low, especially with the application of Containment Level 2 measures and GMP.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Project Ref 177/19.1

Date Ackn’d 01/05/2019

CU2 Project Title Use of reporter genes to characterise plant resistance/susceptibility to fungal and bacterial plant pathogens endemic to the UK

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Consent Granted

Date Project Ceased 02/03/2022
### Project Additional Information

#### Purposes of the contained use

The goal of the research is to understand plant-pathogen interactions to improve plant resistance to biotic stress. Plants will be infected with GM pathogens harbouring reporter genes to follow the dynamics of infections during time as well as under different genetic and environmental conditions. Infections of plant material will be small scale only, typically involving infecting leaf material detached from plants.

#### Recipient or parental organism

The GMM will be mainly necrotrophic fungi such as Botrytis cinerea and Sclerotinia sclerotiorum as well as, possibly, bacteria (e.g. Pseudomonas syringae). All these pathogens are endemic to the UK, and the genetic modifications that they may harbour are reporter genes such as the innocuous Jelly-Fish-Green-Fluorescent-Protein.

#### Host/vector system

Lattuca sativa (lettuce) and Arabidopsis thaliana will be the main plant used for pathogen challenges.

#### Origin & function

The jellyfish Green Fluorescent Protein (GFP) gene will be the main reporter gene to be used. Work could also include the use of other commonly used reporter genes such as human B-glucuronidase (GUS) and firefly luciferase. These genes/proteins are well characterised and have a history of safe use and widely used in bacterial, animal and plants research.

#### Evaluation of foreseeable effects

GMMs to be used present no/negligible risk to human health and a low risk to the environment. The plant pathogens to be used infect a wide range of mono and di-cotyledonous plant species. However, these pathogens are endemic species to the UK (i.e. already present in the wild) and, hence, do not represent a new environmental hazard. The genetic modification with reporter transgenes will not increase the pathogenicity nor host range of the organisms in the event of accidental exposure of the environment. The reporter transgenes themselves are innocuous to both the pathogen and the host plants (as well as to humans). As such Containment Level 2 measures (applied in general laboratory and plant growth facilities) will be appropriate to reduce risks associated with these plant associated GMMs to an acceptable level.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Disposal:
All waste materials are subject to autoclave treatment that should give 100% kill. All autoclave runs are monitored to ensure they achieve the correct temperature, pressure and time. In addition, autoclave cycles are validated by annual independent thermocouple testing to demonstrate that the autoclave holds the specified temperature and pressure for the required period of time.

Solid Waste: plastic waste and infected plant leaf material will be contained within two robust “autoclave bags” and autoclaved (loads exposed to a cycle of 134°C for at least 15 minutes) in the departmental autoclave facilities. Autoclaved solid plastic waste and plant leaf material is transferred to 'tiger bags' and collected by a contractor for deep landfill disposal.

Liquid Waste: waste spore suspension and liquid cultures will be contained in glassware and be autoclaved in the central departmental autoclave facility (liquids exposed to a cycle of 134°C for at least 15 minutes). Autoclaved liquid waste is disposed of to drain with copious amonous of cold water.

Disinfection: Culture/spores spillages will be decontaminated by wiping the contaminated areas with virkon or chloros in accordance with suppliers instructions.

Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

It was questioned why the overall classification of the activity was Class 2 (low risk to human health and the environment) rather than Class 1 (no, negligible risk) given that B. cinerea is a fungal pathogen ubiquitously found in the environment affecting many plants, and commonly used reporter gene labelling (such as GFP) will not alter the hazardous properties of the fungal pathogen. However, it was noted that work involving the use of a GM plant pathogen would normally be Class 2, in line with the GM ‘Compendium of Guidance’ which states that GM activities involving plant pathogens cannot typically be deemed to present ‘no / negligible risk’ (i.e. Class 1) and would require application of some CL2 measures such as an autoclave in the building / restricted access arrangements (i.e. Class 2). It was felt that the application of Containment Level 2 measures for plant associated GMMs would provide appropriate containment. The small scale nature of the activity, typically involving infecting detached leaf material contained in plant growth rooms / cabinets, further reduces the risk associated with the activity.

The need to notify another Class 2 activity involving GFP labelling of microorganisms was also questioned. However, it was noted that whilst the University had previously notified activities involving the use of GFP labelled human pathogens (e.g. P aeruginosa and other similar HG2 organisms) the scope of the notification did not cover GFP labelled plant pathogens.

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L2 L3 L4 L2</td>
<td>L2 Yes L3 L4</td>
</tr>
</tbody>
</table>
Project Additional Information

Purposes of the contained use

These contained uses represent the extension of an existing notified contained use activity (involving Leishmania and Trypanosoma parasite species that are naturally transmitted by direct inoculation) to include parasites (e.g. Trypanosoma cruzi and Toxoplasma gondii) that can be transmitted via mucous membranes or orally (T. cruzi / T. gondii), as well as direct inoculation (T. cruzi).

Specifically, the purpose of the T. cruzi activity is to:

- To study the T. cruzi parasite and T. cruzi-specific immune responses and associated pathology during the course of T. cruzi infection in animal models. To test the efficacy of novel vaccines and drugs for Chagas Disease in animal models of infection
- To culture T. cruzi parasites in vitro in mammalian cell lines for generation of crude antigenic extracts and to study the phenotype of mutant parasites.
- To clone T. cruzi genes into plasmid vectors (engineered for prokaryotic or eukaryotic expression) to express and purify the target proteins for further analyses.
- To target specific T. cruzi genes for disruption (by homologous recombination or CRISPR/Cas9) to generate null, loss-of-function mutants or for over-expression to generate gain-of-function mutants for phenotypic analyses, by the introduction of antibiotic resistance genes into chromosomal sites in the genome of T. cruzi parasites.

The purpose of the T. gondii (and possibly other apicomplexa protozoa) activity is to:
Study the molecular genetics and biochemistry of *T. gondii* and its interaction with the mammalian host. The activity will involve introducing recombinant plasmid DNAs (containing parasite and bacterial sequences) into *Toxoplasma* tachyzoites by nucleofection, and target specific genes for disruption (using CRISPR/Cas9) to generate null, loss-of-function or gain-of-function mutants by the introduction of antibiotic resistance genes into chromosomal sites in the *Toxoplasma* genome. The transgenic parasites produced will be characterised according to nucleic acid and protein content, metabolism, cell cycle, viability and cell structure and infectivity in cultured mammalian cells. Transgenesis will be confined mainly to manipulations within genera i.e. insertion of *Toxoplasma* genes into *Toxoplasma* strains. Intergenera transgenic manipulations and insertion of genes encoding reporter proteins may also be performed.

<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli host:</strong></td>
</tr>
<tr>
<td>Plasmid vectors: pUC based such as the pBS, pSK, pGEX or pEt series.</td>
</tr>
<tr>
<td>Cosmid vectors: pWE15 (Stratagene) or similar</td>
</tr>
<tr>
<td><strong>T. cruzi host:</strong></td>
</tr>
<tr>
<td>Plasmid shuttle vectors: pTEX, pTRIX, pBS or pSK based series including eukaryotic sequences to allow chromosomal integration or expression of introduced genes. Vectors are non-mobilisable and include antibiotic resistance markers and eukaryotic sequences to support chromosomal integration or gene expression.</td>
</tr>
<tr>
<td><strong>T. gondii host:</strong></td>
</tr>
<tr>
<td>Plasmid vectors based on Bluescript plasmid pKS+; pX333 and Bsal plasmids. Vectors are non-mobilisable and include antibiotic resistance markers as well as other reporter genes such as GFP / RFP for fluorescent based selection.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>The sources of the genetic material are bacteria, protozoan kinetoplastid or <em>T. gondii</em> parasites. Target genes for manipulation will be wild type or specifically mutated kinetoplastid / <em>Toxoplasma</em> sequences (fully characterised by DNA sequencing) of known functions (e.g. metabolic, antigenic, structural) or unknown function. None of the genes are predicted to have harmful properties e.g. encode toxins or allergens, increase virulence or change tissue tropism.</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Evaluation of foreseeable effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T. CRUZI ACTIVITY</strong></td>
</tr>
<tr>
<td>The kinetoplastid parasite <em>Trypanosoma cruzi</em> (ACDP Hazard Group 3 human pathogen) is the causative agent of Chagas disease. The human and environmental risks from genetically modified <em>T. cruzi</em> generated by this activity are considered to be no greater than wild type. Gain of function phenotypes may be generated, although it is unlikely that any gain of function mutants will be any more virulent to, or cause different disease tropisms in humans than wild type. Where inter or intra genera gene transfer is performed, no additional risk is foreseeable.</td>
</tr>
</tbody>
</table>
Drug resistance genes will be used as selectable markers to generate loss of function or gain of function mutants. However, these do not provide resistance to commonly used anti T. cruzi drugs.

Risk to the environment as a result of loss of containment is negligible: lab adapted strains to be used can only be maintained on complex media at a narrow temperature range outside of a mammalian host; use of non-mobilisable cloning vectors; susceptible mammals need to be directly inoculated with parasite, and the natural insect vector (the triatomine bug- essential hosts necessary for the lifecycle progression and transmission of the parasite) is absent from the UK environment.

Application of CL3 measures are considered appropriate to provide effective containment for the protection of human health and the environment.

T. GONDII ACTIVITY

Toxoplasma gondii (ACDP Hazard Group 2 human pathogen) is a protozoan parasite responsible for Toxoplasmosis. The human and environmental risks from genetically modified T. gondii generated by this activity are considered no greater than wild type.

No risks are associated with the plasmid vectors. The DHFR encodes resistance to a currently used drug (Pyrimethamine) which is used clinically to treat toxoplasmosis. However, the likelihood of recombination into human cells upon infection is effectively zero. Furthermore, the DHFR is a selection marker used more rarely. In the case it is used, the DHFR encoding gene will be flanked by LoxP sites and the gene will be excised by transient electroporation with Cre recombinase after transgenic parasite selection. This reduces the chances of accidental exposure of the lab personnel to pyrimethamine-resistant parasites.

None of the genes that are ectopically expressed are expected to increase the risks associated with the host strain of T. gondii. Genes do not encode the production of any toxins or allergens, they do not increase virulence or change tropism. Previous studies have generated numerous parasite lines expressing heterologous genes or mutated version of endogenous genes. None of these parasites showed any increased virulence in assays (growth rate in cell culture). Likewise, many other labs in the field use the same system, including for studies in animals and report no increased virulence. On the contrary, it is often observed that parasites are attenuated due to the type of genetic manipulations performed.

Application of CL2 measures are considered appropriate to provide effective containment for the protection of human health and the environment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

T CRUZI ACTIVITY

Full Containment Level 3 measures will be applied for all higher risk T. cruzi work activities (includes T. cruzi culture and sample preparation, and all infected animal related work activities). However, lower risk work activities involving downstream sample analysis of live parasites (includes microscopic imaging, imaging of whole infected animals) will use Containment Level 2 facilities in the 'Cell & Imaging Lab' associated with the Biology 'Technology Facility'.

These facilities do not have the following features typical of CL3: room maintained at negative pressure; room sealable for disinfection; HEPA filtration on extract, an observation window. However, T. cruzi is not transmitted via the airborne route.

Downstream processing of parasite preparations / infected tissue samples / whole (anaesthetised) animals involves the application of routine, well practiced procedures associated with microscopic and imaging systems. Only small volumes (no more than 8mL) of parasite, infected cell preparation will be involved; samples will be double contained during transport in robust, sealed containers, and samples will be maintained, where practicable, in sealed containers or covered (e.g. on the microscope slider holder) during analysis. Once a sample is mounted on a microscope, the incubation chamber will be closed and any additional samples resealed in the transport box. For
some experiments, it may be necessary to remove the lid or add samples to the wells (e.g. drug or reagent addition). Any such manipulations will be kept to a minimum and limited to simple sample addition steps.

Application of a high standard of working practices will be applied at CL2 to maintain effective control of exposure. Use of these CL2 facilities will be supplemented with key working practices also applied for handling T. cruzi samples at CL3, as detailed in a Code of Practice. All individuals directly or indirectly associated with downstream sample analysis at CL2 will receive recorded training on key practices detailed in the CoP. Key practices include the wearing of protective gloves and eye / face protection when handling infective samples; avoiding use of sharps, routine disinfection of work surfaces and equipment following use. In addition, temporary signage (warning of a biological hazard and restriction of access to authorised persons only) will be displayed on the outside of the door of the CL2 room to be used for sample analysis, before work begins. All samples / waste materials will be returned using double containment to CL3 for inactivation.

Movement of infected animals between CL3 containment rooms and multiphoton microscopy room (CL2) for intra-vital imaging will be done in IVC cages, mice will be anesthetised before leaving CL3 BSF rooms, maintained anesthetised throughout the procedure and euthanized after completion of the procedure. Mice will be held in isolated containers at all times while in CL2, all materials and containers will be decontaminated before leaving the room, and carcasses will be taken back to CL3 BSF rooms for disposal.

Cell sorting of samples at CL2 will only be performed with inactivated (using a validated method) sample materials.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid and liquid GM waste will be generated and treated as describe below. All waste will be handled by authorised, trained personnel only.

Liquid GMM waste:
All liquid parasite waste material will be made safe by disinfection (using Distel (diluted 1:10, final volume) a disinfectant validated against Leishmania and Trypanosoma to give 100% kill) before disposal. Spent cultures and vessels will be treated with disinfectant for a minimum of three hours before pouring the liquid down lab disposal sink through a stainless steel sieve to catch any solid matter. The waste bottles are then rinsed and reused. Any caught waste matter is placed in an autoclave bag and treated as solid waste.

Solid GMM waste:
All solid waste material is subject to autoclave treatment (134°C for 15 mins at 27 psi pressure) that should give a 100% kill. All autoclave runs are monitored to ensure they achieve the correct temperature, pressure and time (machine cycle pass/fail run report). In addition, autoclaves are validated by annual indenpentant thermocouple testing to demonstrate that the autoclave holds the specified temperature and pressure for the required period of time. Waste is be collected in metal autoclave bins and either autoclaved directly in the CL3 lab using a double-ended autoclave (T. cruzi solid waste), or securely transferred from the CL2 lab to the autoclave room within the same building (T. gondii solid waste). Autoclaved solid material is transferred to either ‘orange bags’ for ‘alternative treatment’, or ‘tiger bags’ for disposal as non-hazardous offensive waste for collection by a registered waste contractor.

Solid waste from the CL3 animal facility (BSF), (e.g. soiled bedding and food, blue roll, tubes and used gloves) will be inactivated by autoclaving. Animal carcasses / tissues samples will be stored in a freezer located within the containment suite until collected for clinical waste disposal via a registered waste contractor.

All solid waste materials associated with sample analysis of T. cruzi parasite material in the CL2 Technology Facilities will be returned to the CL3 lab using double containment for inactivation using the same waste disposal procedures applied to waste generated in the CL3 lab.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The GMSC agreed that the 2 activities could be covered by a Connected Programme of Work on the basis that T. cruzi and T. gondii are parasites that can both be transmitted via mucous membranes, as well as orally. Both organisms, therefore, require similar working practices (albeit at different Containment Levels) to effectively control risk of exposure. The existing notification for work with Leishmania and Trypanosoma parasites does not cover species that can be transmitted via mucous membranes or orally (T. cruzi and T. gondii). It was also noted that the activities are associated within a larger parasitology research group that use the same two research laboratory buildings. As such, both activities share the same suite of containment facilities, come under the same management system and apply the same waste disposal arrangements.

Generation of gain of function mutants (for example by over-expression of genes) was discussed. It was felt that manipulations would most likely lead to loss of virulence, rather than any gain of function resulting in further harmful properties.

The risk assessment / CoP refers to external validation data confirming the effectiveness of Distell disinfectant against Leishmania / Trypanosoma species. The Committee requested this to be supplemented with internal validation of Distil for T. cruzi cultures. Validation of the method used to inactivate T. cruzi for flow cytometric analysis in the CL2 lab will also requested.

It was concluded that overall this was a Class 3 activity, adequately contained with the application of both Containment Level 2/3 facilities and associated working practices.

### Project Containment

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<td>L2 L3 L4 L2</td>
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<tr>
<td>L3 Yes</td>
<td>L4 L2 L3 L4</td>
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### Animal Units

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<th>Large Scale Activities</th>
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### Project Ref 490/04.2

<table>
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<th>Consent Granted</th>
<th>Non-GMM</th>
<th>Project notified under transitional arrangements</th>
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<tbody>
<tr>
<td>01/10/2012</td>
<td>The interactions between bacteriophages and their bacterial hosts.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Not Applicable</td>
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<td></td>
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<td>Date Project Ceased</td>
<td>Project notified under transitional arrangements</td>
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<td></td>
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</tbody>
</table>
**Project Additional Information**

**Purposes of the contained use**

To date most of our studies in this area have focussed on Streptomyces phages. Thanks to bacterial genomic sequencing we have hypothesised a more general nature to some of our discoveries and we now wish to test some of these theories. We now propose to use several known human pathogens in our research.

**Recipient or parental organism**

Several organisms will be manipulated; Salmonella typhimurium SL 1344 and LT2, Thermobifida fusca, Streptomyces sp. Mycobacterium smegmatis (mc2155), M. avium, M. marinum, M bovis BCG, E. coli K12.

**Host/vector system**

Chromosomal genes in S. typhimurium and in E. coli will be mutated using the lambda red pcr targeting gene replacement system; the red genes will be introduced via pKD20 or pKD46 or similar. Gene replacement in T. fusca and S. coelicolor will rely on homologous recombination via DNA introduced by mating on suicide vectors derived from E. coli ColE1 derived plasmids (eg pSET151). We also intend to use site-specific integrating vectors (eg pSET152). Mutations in the mycobacterial chromosomes will also be generated by homologous recombination with DNA cloned on suicide plasmid vectors or on phasmids. E. coli vectors used will be derived from ColE1, p15A, or defective lambda phage.

**Origin & function**

All the genes we will be studying were discovered through research on host-phage interactions. All the genes when mutated in S. coelicolor give an altered response to phage infection. Some of the genes (the pgl genes) are involved in a phage resistance phenotype in S. coelicolor whilst others (such as pmt1 and ppm1) are required to synthesise the phage receptor. In S. coelicolor the pgl genes involve proteins with the following predicted functions; a protein kinase (pglW), DNA methyltransferase (pgIX), an ATPase (pglY) and a protein of unknown function (pglZ). Homologues of these genes will be knocked out in S. typhimurium, T. fusca and in the genetic elements SXT and CTnR391 (derived from V. cholera and P. rettgeri respectively and transferred by conjugation into E. coli). In each case an antibiotic resistance marker (usually kanamycin, chloramphenicol, apramycin, hygromycin or spectinomycin resistance genes) will replace the pgl gene. The pgl homologues from these organisms will also be cloned into E. coli and Streptomyces. The pmt1 and ppm1 genes appear to be involved in a protein glycosylation pathway, which is common to all the actinomycetes. Pmt1 and ppm1 homologues (and other genes involved in the glycosylation pathway) in mycobacteria will be replaced by antibiotic markers, kanamycin or hygromycin. The pmt1 and ppm1 genes from mycobacteria will also be inserted in E. coli and streptomyces.

**Evaluation of foreseeable effects**

It is unlikely that any of these manipulations described here will result in any increase in the hazardous nature of these organisms. If anything their fitness will be reduced either due to sensitivity to phage infection or due to defects in synthesising key cell wall proteins. Indeed other people's attempts to knockout the pmt1 gene in M. tuberculosis so far have failed. The knockout mutants will contain antibiotic resistance genes but these will no confer resistance to any known therapy for any disease caused by the organisms in question. Cloning of the heterologous glycosylation genes in E. coli K12 strains or in Streptomyces is not likely to confer any degree of pathogenicity on these bacteria. We intend to use E. coli K12 containing the mobilisable SXT and CTnR391 elements which originate from V. cholera and P. rettgeri. These elements are known to encode several antibiotic resistance genes and several genes of unknown function. Given these known properties E. coli containing these
elements will be used under class 2 containment to eliminate any possibility of spread.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The class II safety cabinet will be used for manipulating Mycobacterium avium, M. bovis BCG and Thermobifida fusca at all times. This is because they are known to cause pulmonary disease and there is a clear danger of infection via an airborne route. For S. typhimurium, E. coli, M. smegmatis and M. marinum there is little danger from the airborne route and these organisms will normally be manipulated on the bench with protective clothing and gloves.

The class II safety cabinet will always be used whenever aerosols are expected, such as vigorous mixing or resuspending cells after centrifugation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Trigene kills E. coli, S. typhimurium, Streptomyces sp. T. fusca, M. smegmatis and 5% kills M avium and M Bovis BCG. We expect to get 100% inactivation and validation will be routinely tested. Information on testing of most of these organisms is described at http://www.medichem.co.uk/medical_products.html. Streptomyces sp and Thermobifida fusca are not mentioned in this information but we have performed our own tests on Streptomyces and found 1% Trigene to be sufficient for 100% killing. The concentration of Trigene for killing of T. fusca will be thoroughly tested before GM work begins.

In practice the lab will routinely use 5% Trigene for killing of liquid cultures and for disinfection of contaminated glassware and spills. Disinfection will take place in containers with lids for no less than 24 hours before disposal. Disinfected cultures and glassware will be disposed of down the sink with plenty of water.

Plates will be taped together, placed in a double autoclave bags, sealed and sent for autoclaving on the premises for sterilising.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee accepted the scientific procedures and hazard assessments as reasonable. They requested that only one type of disinfection should be used throughout the laboratories for simplicity sake, which we have implemented by converting to the use of Trigene. The committee also requested a clear statement of when the safety cabinet should be used and when it is safe to work at the bench.

The risk assessment was passed pending these changes. It was decided that the labs where the work will take place would be inspected for compliance with level 2 containment prior to receipt of microbes classified as ACDP hazard group 2.

Project Containment

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</thead>
</table>

02/03/2022  
Page 4622 of 15326
**Project Ref** 8/04.2

**Date Ackn’d** 07/04/2004

**CU2 Project Title**
THE STUDY OF:- 1) GENOME ORGANISATION IN LEISHMANIA AND TRYPANOSOMA BRUCEI, 2) EXPRESSION OF LEISHMANIA AND TRYPANOSOMA BRUCEI GENES, 3) GENETIC MODIFICATION OF LEISHMANIA AND TRYPANOSOMA .....  

**Date Project Ceased**

**Class** Class 3

**Culture Vol Class 2** 2 Litres

**Culture Volume Class 3-4**

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** Yes

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**
Transferred from GM8 on 07/04/2004

**Historical Date of Additional Info**

**Significant Change ID** 8/04.2a

**Date of Significant Change** 25/07/2013

**Project Additional Information**

**Purposes of the contained use**
* to map and sequence target genes in Leishmania species and Trypanosoma brucei, in relation to their chromosomal position and stability between genomes.  
* to clone Leishmania and Trypanosoma brucei genes into plasmid vectors engineered for prokaryotic expression: to express and purify the target proteins for further analyses.  
* to target specific genes for disruption (by homologous recombination) to generate null, loss-of-function of gain-of-function mutants for phenotypic analyses, by the introduction of antibiotic resistance genes into chromosomal sites in the genomes of non-infective Leishmania or T. brucei stages.

**Recipient or parental organism**
- E. coli K12 strains: eg E. coli BL21 (DE3) pLysS  
- Leishmania major MHOM/IL/81 Friedlin  
- Leishmania mexicana MNYC/B762/M379  
- Leishmania donovani MHOM/SD/62/IS-CL2D  
- Leishmania infantum MCAN/es/98/LLM-724

02/03/2022
### Host/vector system

For E. coli host:
- Plasmid vectors: pUC based such as the pBS, pSK, pGEX or pEt series
- Cosmid vectors: pWE15 (Strategene) or similar

For Leishmania/T brucei host:
- Plasmid shuttle vectors: pUC based such as the pBS or pSK series.

### Origin & function

The sources of parasite material (both Leishmania species and Trypanosoma brucei) are collaborating reference centres, both in the UK and abroad, that supply frozen samples in sealed containers for transport into the containment laboratory. Frozen material is stored in the vapour phase of liquid nitrogen until required for in vitro culture.

The genetic material from these parasites will be used:
- in gene mapping studies
- for expression studies to generate recombinant proteins for structural and functional analysis
- to generate null, loss-of-function or gain-of-function Leishmania or T. brucei mutants for phenotypic analyses

### Evaluation of foreseeable effects

Species of Leishmania are causative agents of human Leishmaniasis; the parasite species L. donovani, L. infantum, L. braziliensis are designated hazard group 3 ($ (ACDP categorisation). The remaining species covered by this notification, L. major and L. mexicana, are hazard group 2 (ACDP categorisation), as is Trypanosoma brucei, which is non-infective to man. In susceptible individuals, leishmaniasis is treatable (as detailed in the Code of Practice, June 1992).

The target genes for manipulation will be wild type Leishmania or T. brucei sequences that have been fully characterised by DNA sequencing. The products of these genes are not known to have a role in the progression of human disease but this possibility cannot be discounted for those genes coding for proteins of unknown function. The GMMs are unlikely to express proteins at a level significantly higher than wild type parasites that have not been genetically-modified.

The pathogenicity of Leishmania species and the hazard this imposes to experimental work in the Department of Biological Sciences is already covered by Codes of Practice for cultivation in culture (June 1992) and in animals (January 1993). Genetically-modified Leishmania will be subject to the same safety protocols; the environmental risk is therefore no greater than with unmodified parasites. The life cycle of Leishmania involves transmission of extra-cellular parasite stages between hosts by a sandfly vector, followed by intracellular maintenance within macrophages in the mammalian host. No other organism is known to be at risk from infection. There is no sandfly colony on the South Kensington site of Imperial College, so transmission of the parasite, by inoculation of infective parasites into susceptible mammalian hosts, is impossible.

Transgenic Leishmania will be resistant to one or more antibiotics but this is irrelevant to therapy against human leishmaniasis (for which pentavalent antimonials are the current drugs of choice). Transgenic T. brucei will also be resistant to one or more antibiotics but this is irrelevant as T. brucei is non-infective to man.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Table 1a Measure 2 Laboratory: sealable for fumigation. Justification - Parasites not transmissible through the airborne route.
GM waste is of 2 types: liquid and solid.

Liquid GMM waste: is treated by chemical disinfection, using Chloros diluted 1:10 (final concentration 10,000ppm available chlorine), with contact time > 12 hours. This gives 100% kill, as validated by testing described in the attached risk assessments.

Solid GMM waste: is autoclaved at 134 degrees C for 15 minutes at 27 psi pressure. This gives 100% kill, as validated by testing described in the attached risk assessments.

All clinical waste is sealed in bags and transported on robust trays from the containment facility to the autoclave, via the interlock hatch. This waste is handled by authorised personnel only. After autoclaving, the material is transferred to yellow bags for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The GMSC agreed with the classification and containment measures assigned to this project. Minor modifications were required to be made to the risk assessment form prior to notification to the HSE.

**Project Containment**

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<td><strong>Transitional Premises Class</strong></td>
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<th><strong>Non-GMMs</strong></th>
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Name

NOTTINGHAM TRENT UNIVERSITY

Name 2

SCHOOL SCIENCE AND TECHNOLOGY

Campus Estate or Research Centre

ERASMUS DARWIN BUILDING

Road Name

CLIFTON LANE

Town

NOTTINGHAM

County

NOTTINGHAMSHIRE

Postcode

NG11 8NS

Country

ENGLAND

Tel Number

0115 9418418

Fax Number

0115 9486636

E-mail


HSE Division

MIDLANDS

Comments

GM788 MERGED WITH GM187 AS COMPANY REGISTERED TWICE.

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Chairman, Senior Research Fellow - Life Sciences, Reader in Life Sciences, Chief Technician in Microbiology - Life Sciences, Safety Officer - Life Sciences, Chief Technician - Life Sciences, Senior Lecturer - Life Sciences, Safety Officer - Microbiology, Occupational Health - City Health Centre, Dean of Research - Nottingham Trent University, Student Health Care - Nottingham Trent University, Principal lecturer - Nottingham Trent University, Head of Department - Life Sciences, Post Doctoral Representative - Life Sciences, UNISON Representative, Safety Officer - Nottingham Trent University & Post Graduate Student Representative - Life Sciences.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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The materials are first autoclaved in the department then a registered carrier collects them for incineration.

For activities involving GMMs, describe the waste management measures which will apply to the activity.

The safety committee has approved a format for the assessment, but we are in the process of producing a better revised version.

Please enter comments of the GM safety committee on the risk assessment:

The safety committee has approved a format for the assessment, but we are in the process of producing a better revised version.

**Project Ref 187/05.1**

**CU2 Project Title**

Cloning, mutagenesis and gene expression studies of virulence related genes of Campylobacter jejuni and Campylobacter coli.

**Non-GMM Consent Granted**

Not Applicable

**Consent Granted**

Not Applicable

**Class**

Class 2

**Culture Volume Class 2**

< 1 Litre

**Culture Volume Class 3-4**

Not Applicable

**Project notified under transitional arrangements**

N
Project Additional Information

Purposes of the contained use

Campylobacter jejuni and Campylobacter coli are major human pathogens responsible for the majority of human cases of acute gastroenteritis in the UK. At present it is not possible to distinguish between pathogenic and non-pathogenic strains of Campylobacter as the mechanisms of pathogenicity are still not fully understood. The purpose of this work is to characterise virulence and survival properties of these organisms and to investigate the molecular basis for the variation of virulence (such as toxin expression) that is observed between strains. This work may lead to the identification of novel markers of pathogenicity that will facilitate pathogenic strain identification as well as provide information on the regulation of expression of the cytolethal distending toxin (CDT).

Recipient or parental organism

The resultant GMOs will include Escherichia coli strain TOPO10F', E. coli strain S17-1 ^ pir and other similar disabled strains of E. coli into which genes of Campylobacter origin will be cloned. These E. coli strains are hazard group 1 organisms and auxotrophic, therefore long-term survival outside of the laboratory and in the environment is unlikely. They are not pathogenic to humans and it is unlikely that the GMOs will have any increased virulence or survival capability compared to the wild type strains. Due to the low G+C content of C. jejuni and the differential codon usage between the two organisms it is unlikely that C. jejuni proteins will be efficiently expressed in E. coli.

In addition a range of Campylobacter jejuni and Campylobacter coli strains will be used as hosts for genomic constructs and mutagenesis studies. Campylobacter jejuni and Campylobacter coli are ACDP class 2 organisms. Wild type Campylobacter sp. Is ubiquitous in the environment and can survive in a wide diversity of locations including water, soil, etc. The infectious dose in humans is quite low (500 organisms) and once it has infected the host Campylobacter jejuni can colonise the guts of some animals and birds to high levels. Campylobacter jejuni and coli are pathogenic to humans (enteritis) but usually commensal in most animals and birds. Campylobacters are thought to just survive (persist) rather than multiply to high numbers in the environment and so if escape occurs contamination of the surrounding environment is likely to be low. The genetically modified Campylobacter strains are unlikely to be any more harmful to humans, animals or the environment than the wild type strains.

Host/vector system

Vectors to be used for the CDT expression studies include pMA20T, which is an adaptation of pGU0202, a shuttle vector for use in Escherichia coli and Campylobacter, which contains a Campylobacter-derived kanamycin resistance cassette (aph (3')-III as well as the mob gene and so is mobilisable between strains. The modification of this plasmid in pMA20T includes the insertion of a reporter gene, either the lacZ gene from E. coli MG1655, which encodes b-galactosidase, or gfp, which encodes green fluorescent protein, along the two oligonucleotide linkers to introduce termination sequences and restriction sites for cloning upstream of the reporter gene start codon.

Other vectors include pBR322, a mobilisation defective, general purpose cloning vector, which will not replicate in Campylobacter jejuni and other similar general purpose cloning vectors such as pCR2.1TOPO (Invitrogen), pUC18, pBluescript (Stratagene) and pCRScript (Stratagene) will also be used for the mutagenesis studies as these plasmids will not replicate in Campylobacter and so will act as suicide vectors in this host.

The transposon mutant library was created using an in vitro method of transposition, whereby exogenous transposase enzyme was added to the reaction mixture. This results in a relatively stable insertion as the transposon is unable to transfer in the absence of the transposase enzyme. The "plasmids" that result from the plasmid rescue are mobilisable, but only from a strain of Escherichia coli expressing the pir protein. Even if the plasmid was taken up by another organism it is unlikely, due to the
low G+C content of Campylobacter DNA, that Campylobacter proteins will be expressed in other organisms.

**Origin & function**

CDT promoter regions from Campylobacter jejuni strains will be used in this study. These DNA fragments (up to 1.5 kb in size) will not be expressed themselves but will drive expression of the reporter genes cloned into pMA20T in E. coli.

For gene expression in Campylobacter jejuni small fragments (ca. 300 bp) flanking the cdt promoter from the 5' end of the cdtA gene will be cloned into plasmid pBR322 along with the kanamycin resistance gene from Campylobacter coli, to allow homologous recombination to occur and the insertion of the reporter gene downstream of the cdtA promoter in the genome of Campylobacter jejuni strains, such as 11168, 81116, 81176, 01/35, 01/51, 01/41, AG, DF, 99/224, EX114, 99/419 and EF.

The lacZ reporter gene originates from E. coli MG1655 and the gfp reporter gene is originally from Aequorea victoria.

For the virulence studies genes from Campylobacter jejuni and Campylobacter coli that have similarity to other known virulence factors associated with toxins, invasion, adhesion, fimbrial expression, in vitro survival and other surface exposed proteins will be investigated. In addition any genes identified through the transposon mutagenesis as having a role in invasion or in vitro survival will also be cloned for further characterisation.

**Evaluation of foreseeable effects**

It is unlikely that the GMOs will have any increased virulence or survival capability compared to wild type strains. The Campylobacter jejuni strains will have insertions that are chromosomally located and so transfer of the antibiotic cassettes between strains is thought to be low. For gene expression studies in Escherichia coli, these will be carried out using pMA20T, which is mobilisable. However only putative promoter regions from Campylobacter jejuni will be cloned into this plasmid upstream of the lacZ gene, which encodes B-galactosidase, or the gfp gene, which encodes green fluorescent protein. Overexpression of either of these genes is not known to be harmful to humans, animals or plants. The campylobacter-derived kanamycin resistance gene is used routinely for insertional inactivation in Campylobacter jejuni as this antibiotic is not used for treatment of Campylobacter jejuni infections in humans or animals.

For the transposon mutagenesis the Campylobacter coli chloramphenicol resistance gene is used as a selective marker. This antibiotic is also not used to treat Campylobacter jejuni infections in either humans or animals. The mutants are unlikely to be any more harmful to humans, animals or the environment than the wild type strain, 01/51.

The genetically modified Escherichia coli strain that result from the cloning of various virulence and survival-related genes have the potential of being more harmful than the wild type strain, although this is unlikely. These strains are auxotrophic and so survival outside of the laboratory is unlikely (these Escherichia coli strains will all be handled in a class 2 laboratory). Also due to the low G+C content of Campylobacter jejuni compared with Escherichia coli and the differential codon usage between the two organisms it is unlikely that Campylobacter jejuni proteins will be efficiently expressed in Escherichia coli.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The experiments that will be performed will yield both solid and liquid waste.

Solid waste such as petri dishes, plastic pipettes and other plasticware will be placed in autoclave bags inside designated waste bins. This material will then be autoclaved in a secure containment level 2 laboratory and then incinerated by a local company.
Liquid waste from bacterial cell cultures will be collected in a designated glass container to a maximum volume of 500 ml and autoclaved. Larger volumes (>500 ml) will be split into smaller bottles (no greater than 500 ml) to allow efficient steam transfer. This will achieve 100% kill of the GMOs. Once cool liquid will be disposed of in a designated sink.

Work areas are disinfected with hycolin (a broad spectrum, phenolic disinfectant used at a working concentration of 2% v/v) to prevent dispersal to other areas.

Monitoring of autoclaves, etc, is carried out in accordance with the School of Biomedical and Natural Sciences’ safety policy.

Non-contaminated waste is disposed of separately from contaminated waste and sent for incineration.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The risk assessment that is attached has been reviewed by three members of staff with expertise in genetic manipulation, and revised in the light of comments that were made on it. The revised risk assessment was then approved by all three original reviewers and signed off by Prof. Mark G Darlison, who is Chair of the Genetic Modification (GM) Safety Committee and University Biological Safety Officer.

Project Containment

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Animal Units

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Project Ref 187/05.2

Date Ackn’d 27/09/2005  CU2 Project Title The use of different plasmid vectors and/or viruses encoding either whole, mutated or

Class 2  CultureVolClass2 < 1 Litre  CultureVolumeClass3-4
The purpose of the work is to use known novel tumour antigens to provoke and characterise immune responses in mice and in invitro models. These studies form the basis of preclinical work that may ultimately lead to the development of cancer vaccines. The work falls under the remit of the genetically modified organisms (contained use) regulations 2000 because it involves the manipulation of mutated tumour suppressor genes as well as genes encoding novel tumour associated antigens.

The parental organisms to be used include bacteria (E.coli strains XL1-Blue and TOP10, which are non-colonising, disabled derivatives of E.coli K-12) and well-characterised mammalian cell lines (mouse EL4/A2 lymphoma cells, the hamster fibroblast cell line BHK-21, and human aOs-2 osteosarcoma cells). After insertion of any of the "foreign" DNAs into any of the proposed hosts, none of the resultant genetically modified organisms (GMOs) will have an increased ability to colonise humans, animals or plants. Furthermore, the potential of any of the inserted sequences to be transferred to any other GMO is considered to be effectively zero.

The above parental organisms will be transfected with a number of different vectors. E.coli will be transformed with routine DNA cloning vectors such as pCRO-TOPO, pBluescript II SK +/-, pBR322 (CMV), pBR328, pcDNA3.1/Hygro (+/-) and pSP6. Mammalian cell lines will be transfected with eukaryotic vectors such as pBR322 (CMV), pBR328, pcDNA3.1 Hygro (+/-) and pSP6. In addition, the Disabled Herpes Simplex single cycle (DISC) virus and the Semiliki Forest Virus (SFV), which requires the use of a replicon plasmid and a helper plasmid, will be used.

The DNAs that will be inserted are murine and human truncated, mutated and full-length complementary DNAs (cDNAs) and genes that encode known and potentially novel tumour associated antigens. These cDNAs will derive from human tumour tissues such as prostate, and human-derived cell lines; some of these cDNA sequences will be provided by collaborators.

The DNAs encode proteins that either i.) potential oncogenes; ii.) up-regulated in tumours; or iii.) have not been charctorised. They include sequences encoding mutated p53 and HAGE, and sequences derived from expressed sequence tag (EST) database analysis.

The XL1-Blue and TOP10 E.coli strains are non-colonising, disabled hosts that will have no harmful effect on either human health or safety. The introduction of "foreign"
genetic material, using the vectors mentioned above, will not result in an altered host range and will not increase the ability of the hosts to colonise humans.

The mammalian cell lines do not pose a significant hazard to either human health or safety since they cannot survive outside the laboratory. If accidental penetration were to occur in normal healthy individuals, then these cells would be quickly rejected by the immune system. The introduction of "foreign" genetic material, using the vectors mentioned above, will not result in an altered host range and will not increase the ability of the host to colonise humans. Although some of the DNAs may have oncogenic potential, the proposed level of containment should reduce the risk, to human health and safety, to effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All work with mammalian cells, and the DISC and Semiliki Forest viruses, will be carried out in class II cabinets in containment level 2 laboratories. This will control the risk, to human health and safety, and the possibility of accidental environmental release, to effectively zero. Waste will be disposed of according to the procedures laid down by the school of Biomedical and Natural Sciences' Microbiological Safety Officer, Dr Steve Hammonds (see http://www.ntu.ac.uk/life/sh/safety/).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The experiments that will be performed will yield both solid and liquid waste. Solid waste such as tissue culture flasks, plastic pipettes and other ware will be placed in autoclave bags inside designated waste bins. This material will be autoclaved in a secure CL2 laboratory, and then incinerated by a local company. Glass roller bottles will be soaked overnight in a mixture of 0.5% Presept/1% Teepol. Liquid waste from mammalian cell culture will be collected in a designated glass container, treated overnight with a combination of 0.5% Precept/1% Teepol, and then disposed of via a designated sink. Liquid waste from bacterial cell cultures will be collected in a designated glass container, treated overnight with 0.5% Presept/1% Teepol, and then disposed of via a designated sink. These measures ensure 100% kill. Monitoring of autoclaves, etc, will be carried out in accordance with the School of Biomedical Natural Sciences' safety policy.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The RA that is attached has been reviewed by 3 members of staff with expertise in GM, and revised in the light of recent comments that were made on it. The revised RA was then approved by all 3 original reviewers and signed off by Pro. Mark G. Darlison, who is the Chair of the GMSC and University BSO.

Project Containment

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02/03/2022
The genotypic and phenotypic comparison of Yersinia enterocolitica from humans and animals.  

To investigate the role of various factors as potential virulence or host-specific colonisation factors in Y.enterocolitica.  This will involve mutation and complementation of genes identified via a previous comparative microarray analysis project.

Escherichia coli strain TOP10F' will be used for general cloning and the promoter expression studies as it is easy to use and readily available.  E coli strain S17-1 lambda pir will be used for conjugational transfer of suicide plasmids containing insertionally- inactivated target genes.  A variety of wild-type Y.enterocolitica will be used as hosts for homologous recombination with the introduction of antibiotic resistance markers.

The standard cloning vectors pCR2.1TOPO (Invitrogen) and pACYC184 (New England Biolabs) will be used for cloning and insertional inactivation of genes.  The R6K Ori suicide vector pKNG101 (Kaniga et al. 1991, Gene 109 (1): 137-141) will be used for homologous recombination in the target Y.enterocolitica strains.  Plasmid pMN402 (Scholz et al.2000, Eur J Biochem. 267: 1565-1570) will be used to construct promoter green fluorescent protein (GFP) fusions.

To investigate the role of various factors as potential virulence or host-specific colonisation factors in Y.enterocolitica.  Targeted genes are as yet unknown, though genes
with similarity to other known virulence factors such as toxins, adhesins, fimbriae, as well as regulatory elements are most likely to be investigated. In the majority of cases, the genes will be insertionally inactivated in Y. enterocolitica and the resultant phenotype studied. In some instances, non-coding regions upstream of the gene may be used to study expression of the factors via a reporter gene.

**Evaluation of foreseeable effects**

Wild-type Y. enterocolitica is pathogenic to humans and symptoms range from mild diarrhoea to mesenteric lymphadenitis. The disease is generally self limiting and severe disease is rare. Human infections are primarily due to consumption of contaminated dairy and meat products, in particular porcine food products, as well as contaminated water and occasionally contact with domestic animals. Y. enterocolitica is a commensal of animals. In this project, genes potentially important for colonisation and virulence will be targeted for insertional inactivation. Any resultant contact with animals should result in a reduced colonisation potential. If a genetically modified (GM) organism were to enter the human food chain, expression of virulence factors will be reduced, which in turn should lead to reduced levels of infection. All mutants will be screened for altered pathogenicity via an in vitro cell invasion assay, allowing the rapid identification of any hyper-virulent mutants. In the event that a mutation leads to increased pathogenicity potential, the disinfection and waste management strategies outlined in the attached GM risk assessment should minimise any danger to the environment. Most of the genes to be targeted in this study will be putative colonisation factors, which should decrease adhesion and, therefore, invasion of cells in vitro.

For gene expression studies in Y. enterocolitica, this will be carried out using pMN402, which is mobilisable. However, only putative promoter regions from Y. enterocolitica will be cloned into this plasmid upstream of the gfp gene (which encodes GFP). Overexpression of this reporter gene is not known to be harmful to humans, animals or plants.

The GM E. coli strains that will result from the cloning of various virulence and survival-related genes have the potential of being more harmful than the wild-type strain, although this is unlikely. These strains are auxotrophic and so survival outside of the laboratory is unlikely (note that all of the GM E. coli strains will be handled in a class 2 laboratory).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Laboratory procedures are in place to prevent the escape of GM Y. enterocolitica and/or GM laboratory attenuated E. coli strains. All personnel will be trained in “Good Microbiological Practice” and all work involving GM Y. enterocolitica as well as the GM E. coli strains will be carried out in a Containment Level 2 laboratory with restricted access.

Guidelines for working with hazard group 2 microorganisms are available as a document within the School of Biomedical and Natural Sciences (and a copy is attached to this notification). This document also contains details of how to deal with an accidental release and/or spillage of a hazard group 2 organism and this should, therefore, be read in conjunction with the attached risk assessment.

Howie laboratory coats and gloves will be worn when working with such organisms. Small culture volumes of up to 100ml will be handled on the bench using aseptic techniques. Work areas will be disinfected with hycolin (a broad spectrum phenolic disinfectant used at a working concentration of 2% v/v) to prevent dispersal to other areas.

Autoclaving of waste material will be carried out within the microbiology area.

In the unlikely event of escape, the E. coli organisms will not survive outside of the laboratory. If acquired by humans or animals, these organisms are unlikely to be any more hazardous than wild-type Y. enterocolitica strains already in the environment.
Monitoring of autoclaves, etc, is carried out in accordance with the School of Biomedical and Nature Sciences' safety policy.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment that is attached has been revised by three members of staff with expertise in genetic manipulation, and revised in the light of comments that were made on it. The revised risk assessment was then approved by all three original reviewers and signed off by the chair of the Genetic Modification (GM) Safety Committee and University Biological Safety Officer. This attached Risk Assessment is directly linked to an application previously notified to the Health and Safety Executive, for work on Y.enterococolitica at the Veterinary Laboratories Agency in Surrey.

Project Containment

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Project Ref 187/09.1

Date Ackn'd 04/06/2009

CU2 Project Title Construction of gfp and lux expressing Enterobacteriaceae associated with powdered infant formula

Date Project Ceased

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The primary aim of the project is to determine post-desiccation recovery in powdered infant formula of specified members of the Enterobacteriaceae using lux and gfp expressing strains. A secondary aim is to visualise the tagged organisms inside mammalian tissue culture cells. Banks of random gene fusion/mutants will be created via mating the recipient strain and E. coli SM10-pir pLOFKmgfp or pUT mini-Tn5 Km (transposon donor with promoterless gfp) to give rise to exconjugates. GFP and lux enables quantification of recovery desiccated cells (protein synthesis, and metabolism respectively). While gfp and lux reporter gene expression within these bacteria can allow a better understanding of invasion of host cells. Bacterial transformation with GFP and lux encoding plasmids (pDEST, pBRRMCS and pMN402) will be used to generate tagged organisms without mutagenesis. Bacterial strains expressing gfp or lux simplifies visualization and allows gene expression to be examined in single living cells. The inflammatory nature of Enterobacteriaceae will be examined with a HeLa cell reporter fusing the NFkB promoter to luciferase and galactosidase production to a Rous sarcoma gene promoter.

**Recipient or parental organism**

Enterobacter sakazakii, Ent. Cloacae, Ent hormaechei, Ent. Cancerogenus, Citrobacter freundii, C. koseri, Escherichia coli (containment level 2 and below), Esch. Vulneris, Pantoea agglomerans, Salmonella enterica, Hafnia alvei, Klebsiella pneumoniae and K. oxytoca are all Hazard group 2 bacteria that can be pathogenic to humans, specifically at risk populations such as neonates and immunocompromised individuals. They are colonising, non-disabled organisms that can survive outside the laboratory. All genetic modifications outlined in this proposal are not thought to increase virulence of any strain used in this study.

HeLa57 cells are cervical in origin and are classified as containment level 2 in the ECACC collection database.

**Host/vector system**

E.coli DH5α and E. coli SM10-pir (derivatives of E.coli K12) are hazard group 1 bacteria which are not pathogenic to humans, animals or plants. They cannot survive outside the laboratory but are recognised as 'disabled or non colonising' hosts for genetic manipulation; see the ACGM (2000) Compendium of Guidance. HeLa57A cells carry an NF-KB dependant promoter driving luciferase and a B-galactosidase gene driven by a Rous-Sarcoma promoter (Rodriguez et al 1999) and carrying a Gentamicin selective marker.

pRYluxCDABE contains the pRYluxCDABE genes from the soil bacterium Photorhabdus luminescens. This shuttle vector contains the specific origin of replication sequences for E.coli and a kanamycin cassette for maintenance within the host. Therefore this vector does not integrate or affect E.coli genomic DNA and can only be transformed into competent cells under defined laboratory conditions (Allen and Griffiths, 2001. JFP 64, 2058-62).

pFLOKmgfp- this pUT based plasmid contains the promoterless gfp mut2 gene, isolated from the jellyfish Aequorea victoria, genetically modified to improved fluorescence. The gfp gene and kanamycin resistance selectable marker gene is located on a transposon (Tn10) contained within a suicide vector such that, once the transposition event has occurred the vector is compromised and unable to replicate (Stretton et al. 1998. AEM 64, 2554-9).

pUT mini-Tn5 Km- this pUT based plasmid contains the promoterless gfp mut2 gene, isolated from the jellyfish Aequorea victoria, genetically modified to improve fluorescence. The gfp gene and kanamycin resistance selectable marker gene is located on a transposon (Tn5) contained within a suicide vector such that, once the transposition event has occurred the vector is compromised and unable to replicate (Stretton et al. 1998. AEM 64, 2554-9).

The transposase gene is located outside of the transposable element on the suicide vector. Following transposition the transposase is lost along with the delivery vector.
This increases stability of the mutation and makes transfer of the transposon into other organisms highly unlikely. Random and stable insertions into bacterial chromosomes has been demonstrated previously with this vector (Albertson et al., 1996. FEMS 140. 287-294).

pLUX GFP: this plasmid is based on pDESTR4-R3 (Invitrogen) and contains an ampicillin-resistance marker cassette, a T7 promoter linked to the gfp gene isolated from the jellyfish Aequorea victoria, genetically modified to improve fluorescence, and the luxCDABE operon. This construct has a pBR322 origin of replication and is non mobilisable, limiting the host range.

pBBRMCS lux: this plasmid is based upon broad host range plasmid pBBRMCS carrying kanamycin resistance in addition to luxCDABE. It is of incompatibility group N and could be mobilised by P group plasmids such as pRK2 and has a pBBR1 origin of replication (Lewis et al. 2005. J. Food Eng. 76, 49-52).

pMN402, carries hygromycin resistance, and the GFP+ gene fused to an HSP60 promoter. It is non mobile shuttle vector and has a broad host range to include both gram -positive and negative bacteria. It contains a PAL500 origin of replication and a ColE1 origin of replication (Scholz et al. 2000. Eur. J. Biochem. 267, 1567-1570).

Hela57A cells carry an NF-KB dependant promoter driving luciferase and a B-galactosidase gene driven by Rous sarcoma promoter and carrying a Gentamicin selective marker (Rodriquez et al. 1999. J. Biol. Chem. 274, 9108-9115)

Origin & function

The DNAs encode bacterial luminescence structural and regulatory genes from Ph. Luminescens and green fluorescent protein from the jellyfish A. victoria. The lux and gfp genes are well characterised marker genes. These genes will be biologically active though there is no identified hazard associated with their usage. The reporter genes encode products that are either constitutively expressed in the case of B-galactosidase, or subject to possible high levels of induction in the case of luciferase, neither is associated with any known hazard.

Evaluation of foreseeable effects

The host bacterium E. sakazakii, E. cloacae, and C. koseri are hazard group 2 organisms causing gastroenteritis in humans; E sakazakii and C. koseri can cause meningitis in low birth weight neonates (associated with ingestion of contaminated formula milk). They are colonising, non-disabled organisms that can survive outside the laboratory. However, these organisms are ubiquitous in the environment. There are no recognised hazards associated with GMO's constructed to contain luminescence or green fluorescent protein genes and they are also present naturally in the environment. The resulting genetic modifications are maintained in the host strain under the selective pressure of kanamycin, ampicillin or hygromycin. The addition of pRYluxCDABE and/or gfp is not likely to increase virulence, alter host range, or increase in the ability to colonise humans or animals. As stated above, transference of the antibiotic genes to other organisms is highly unlikely. Neither kanamycin, ampicillin nor hygromycin are first choice antibiotic for Enterobacter or Citrobacter infections. The HeLa cell line is cervical in nature and highly unlikely to survive outside the laboratory. The genetic alterations made in fusing luciferase expression to an NF-KB dependant promoter and fusing B-galactosidase expression to a Rous sarcoma promoter is unlikely yo improve survival inside or outside the laboratory, and transfer of the reporter genes or gentamicin resistance concurrent with these genes is highly unlikely to occur.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The use of the School 'Code of Practice for Microbiology' for category 2 work in containment level 2 laboratories and risk assessment will ensure that the GMO's will not be released into the environment. This will include training of personnel in the use of containment level 2 facilities, use of safety hoods aseptic techniques, the avoidance of aerosol production, laboratory coats to be worn within containment level 2 laboratories, maintenance of negative air pressure within the laboratory, the disinfection of...
working surfaces and equipment (for example, centrifuges), and autoclaving of all bacterial waste will be within the laboratory suite. Both 2% Hycolin, 2% Virkon and Presept will be available for any spillages, and appropriate measures would be taken in the event of spillages. The autoclaving of all waste material will be carried out within the microbiology area. In the unlikely event of escape, the modified GMO's are not likely to be maintained outside of the laboratory (due to the lack of positive selection pressure in addition to the high metabolic demand of ATP production for luciferase activity), in any event, do not represent a greater hazard than the wildtype organism to either humans, animals and plants. Implementation of the above safety measures should control risk of release/exposure to effectively zero.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment that is attached has been reviewed by three members of staff with expertise in genetic manipulation and revised in the light of comments that were made on it. The revised risk assessment was then approved by all three original reviewers and signed off by Prof. S. J. Forsythe who is the Chair of the Genetic Modification (GM) Safety Committee and Dr Matthew Smith, the University Biological Safety Officer.

Project Containment

<table>
<thead>
<tr>
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<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Animal Units: L2 L3 L4 L2 Large Scale Activities: L2 L3 L4 Human Clinical Applications: L2 L3 L4

Project Ref 187/12.1

Date Ackn'd: 27/01/2012
CU2 Project Title: The use of different plasmid vectors and/or viruses encoding telomerase reverse transcriptase to transfet primary cells and generate in house cell lines
Date Project Ceased: 02/03/2022

Class: Class 2
Culture Volume Class 2: < 1 Litre
Non-GMM: Consent Granted
Project notified under transitional arrangements N
The primary aim of work outlined in this document is to use commercially available vectors to immortalise human and murine cell lines. Normal human prostate epithelial cells and even primary prostate cancer cells have a finite lifespan. They undergo approximately 30 population doublings before becoming senescent (3). Immortalisation will enable long term culture and expansion of precious patient material which has otherwise proven difficult to maintain in culture.

Despite extensive work (by others) on the development of human prostate cancer cell lines, the proportion of cell lines that give rise to immortalised prostate cancer cell lines is still disappointingly low (3). Therefore, there are very few primary prostate cancer and normal prostate cancer cell lines which can be studied extensively to further our understanding of prostate cancer biology (4).

In future, we hope to have access to more fresh patient material, such as tissue samples from breast cancer patients. These will also be processed in the same manner, in order to separate different cell populations which will then be characterised and will undoubtedly require immortalisation to enable long-term culture.

The preferred method to immortalise cells is through the expression of the telomerase reverse transcriptase gene (TERT). Several groups have utilised hTERT in a standard procedure to immortalise primary cells and analysis of many TERT-immortalised cell lines has verified that the cells maintain a stable genotype and retain critical phenotypic markers (5).

References

Recipient or parental organism

Human cell Lines
The human cell line 293T, purchased from the ATCC, is free of pathogenic viruses and cannot survive outside its culture media. In addition, in the unlikely event of accidental contamination, the recipient's immune system would quickly reject these cells due to human leukocyte antigen (HLA) mismatch. No immunocompromised individuals will be permitted to conduct work described in this Risk Assessment. Normal human cell lines are considered to be especially disabled hosts (see the ACGM Compendium of Guidance) but they will, nevertheless, be grown in a containment level 2 laboratory.

Primary cells
None of the cells specified below can survive outside of their specified culture media. Furthermore, as stated above, in the unlikely event of accidental contamination, the recipient's immune system would quickly reject these cells due to human leukocyte antigen (HLA) mismatch. As with all clinical samples, there is a risk of hepatitis infections which is a potential hazard to human health therefore these cells are/will be handled very cautiously in a containment level 2 laboratory. With the adoption of good
laboratory practice and the growth of these cells in a containment level 2 laboratory there are minimal risks to human health.

Human Primary Prostate cells
Prior to processing, patient tissue samples are subjected to fungizone treatment and post processing the isolated cells are grown in the presence of penicillin/streptomycin to eliminate any fungal/bacterial contamination. The cells isolated from patient samples are grown as two separate cultures; epithelial and stromal. The epithelial cultures contain a mixture of basal, secretory luminal and neuroendocrine cells. The stromal (connective tissue) cultures contain a mixture of cells including fibroblasts, pericytes, endothelial cells and smooth muscle cells.

Human Primary Cells (other)
In future, we hope to obtain patient samples from other solid malignancies and matched benign tissue. These will also be subjected to fungizone treatment and post-processing the isolated cells will be grown in the presence of penicillin/streptomycin to eliminate any fungal/bacterial contamination. Depending on the tissue type, these will be processed in a similar manner to the prostate tissue samples in order to separate different cell populations for culture prior to immortalisation.

Host/vector system

pUMVC
This is a packaging plasmid for the Moloney murine leukaemia virus (MoMuLV) (a gammaretrovirus) constructs. This plasmid encodes gag and pol, two of the three major proteins encoded within the retroviral genome. Gag is a polyprotein which makes up the retroviral core (capsid). Pol encodes reverse transcriptase, an essential enzyme that carries out the reverse transcription process that take the RNA genome to a double-stranded DNA preintegrate form. The reverse transcriptase gene also encodes an integrase activity and an RNase H activity that functions during genome reverse transcription.

pCMV-VSV-G
This plasmid encodes the third major protein encoded within the retroviral genome; the envelope protein. This protein plays a role in association and entry of a virion into a host cell.

pBABE-neo-hTERT;
This is a mammalian retroviral expression vector encoding human telomerase reverse transcriptase. This plasmid is directly responsible for the immortalisation of the target cells.

The above vectors pose no threat to human health as they are only capable of giving rise to conditionally infectious virus. Hence, the virus produced will only be capable of infecting the host cells once as it is non-replicative.

The use of a three plasmid system to split the genomes of the helper plasmid greatly reduces the risk of replication competence occurring as a result of genetic recombination. The separation of the gag and pol genes from the env gene on separate plasmids greatly decreases the likelihood of replication competent retrovirus (RCR) generation as not only would recombination between the vector plasmid (which would give the packaging sequence) be required, but also between the two split plasmids (6).

References
3) http://www.addgene.org/pgvec1?f=c&identifier=8454&atqx-vsv-g%208454&cmd-findpl
4) http://www.addgene.org/pgvec1?f=c&cmd-findpl&identifier=8449&attag=r&atqx=vsv-%208454
5) http://www.addgene.org/pgvec1?f=c&identifier==1774&atqx-htert%20neo&cmd=findpl
6) Lentiviral vectors. Information and Biosafety considerations
Human telomerase reverse transcriptase is a catalytic subunit of the enzyme telomerase. Telomerase is a ribonucleoprotein polymerase which lengthens telomeres in DNA strands, thereby allowing senescent cells that would otherwise become post mitotic and undergo apoptosis to exceed the Hayflick limit and become potentially immortal, as is often the case with cancerous cells. Deregulation of telomerase expression in somatic cells may be involved in oncogenesis hence hTERT is oncogenic. Following successful transfection and integration of the hTERT gene into the genome of the target cells, the protein will be expressed and biologically active, thus rendering these cells immortal.

Origin & function

As stated, the virus generated through the construction with the three plasmids. (MoMuLV+hTERT) will only be conditionally infectious. i.e. the MoMuLV constructs have been engineered such that they are only capable of infecting cells once (as they are non-replicative). As such, in the unlikely event that they should enter the human body, the inserted DNA would only be produced temporarily and it would trigger an immune response leading to the detection and elimination of the cells producing the protein. Infection of host cells with the virus produced (MoMuLV+hTERT) will render them immortal; i.e. these cells will have acquired the ability to proliferate indefinitely. Immortalisation of host cells will not increase their survival outside the laboratory as they will still be unable to grow in the absence of defined culture conditions. Hence, the immortalised cells will not be more harmful. The expression of telomerase reverse transcriptase will not alter the host range of the host cells neither will it increase their ability to colonise humans, animals or plants. In the unlikely event that the immortalised cells should enter the human body, they would be rejected and eliminated immediately by the immune system which would recognise them as "foreign". The potential for the inserted sequences to be transferred to other GMOs is, with the application of good laboratory practice and the methods of containment described herein, considered to be effectively zero.

Evaluation of foreseeable effects

As stated, the virus generated through the construction with the three plasmids. (MoMuLV+hTERT) will only be conditionally infectious. i.e. the MoMuLV constructs have been engineered such that they are only capable of infecting cells once (as they are non-replicative). As such, in the unlikely event that they should enter the human body, the inserted DNA would only be produced temporarily and it would trigger an immune response leading to the detection and elimination of the cells producing the protein. Infection of host cells with the virus produced (MoMuLV+hTERT) will render them immortal; i.e. these cells will have acquired the ability to proliferate indefinitely. Immortalisation of host cells will not increase their survival outside the laboratory as they will still be unable to grow in the absence of defined culture conditions. Hence, the immortalised cells will not be more harmful. The expression of telomerase reverse transcriptase will not alter the host range of the host cells neither will it increase their ability to colonise humans, animals or plants. In the unlikely event that the immortalised cells should enter the human body, they would be rejected and eliminated immediately by the immune system which would recognise them as "foreign". The potential for the inserted sequences to be transferred to other GMOs is, with the application of good laboratory practice and the methods of containment described herein, considered to be effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The 293T cells, the primary cells and the MoM7uLV viruses (transfected with hTERT) will be cultured in plastic tissue culture flasks (a maximum of ten 50ml flasks at any one time). Plastic flasks and any disposable plastics used during the culture of these cells will be disposed of into a bin for immediate autoclaving followed by incineration. Other material, including glassware and disposable plastic pipettes etc., will be soaked overnight in a mixture of 0.5% Presept®/1% Teepol prior to autoclaving and/or disposal. The cells will be grown in a class II cabinet, with one cabinet dedicated to virus work. Both 0.5% Presept®/1% Teepol and 70% (v/v) ethanol will be available in the cabinet in case of any spillages. This is as recommended by the School Disinfectants Policy. As the cells will not be cultured on a large scale, there will be no risk of any large spillages. Consequently, it is highly unlikely that either cells or viruses would be released into the environment. In the unlikely event of escape, none of the modified GMOs would be able to survive outside of the laboratory. Accidental exposure will be prevented by the use of suitable personal protective equipment (PPE) including gloves, a suitable laboratory coat and, where necessary, a CN P2 v mask which provides protection against viral exposure. General adherence to good laboratory practice will also help to ensure that accidental exposure is avoided. However, in the case of accidental exposure, for example through puncture wounds, a trained first aider will be available to administer first aid. Our collaborators at the M. D. Anderson Cancer Centre (Houston, Texas, USA) have provided us with the information regarding the plasmids and will provide a detailed protocol/standard operating procedure (SOP) and any other information required upon approval of this Genetic Modification Risk Assessment. They have been very cooperative and will continue to provide assistance throughout the duration of this project.
Any new staff will be trained by a member of the laboratory according to the training program for tissue culture activities (developed by Dr E V; see the NTU School of Science training booklet, version 1.2 October 2006) with additional specific training by the named main investigation/supervisory team/laboratory manager involved in this project. Mr S R and Dr M M at the John van Geest Cancer Research Centre and Mr R D at the Anthony Nolan Stem Cell Bank have already used similar systems of production of viral particles in the view of transfecting primary cells as well as cell lines. These persons can provide training to work at a high safety standard.

In order to reduce the potential for mucosal exposure during transduction, those transducing the cells will be required to wear a CN P2 v mask which provides protection against viral exposure. As stated, all procedures involving live, immortalised cells will be conducted in containment level 2 laboratory in a category 2 hood where all culture media/liquids/disposable materials which associate with the cells will be carefully disinfected in 0.5% Presept®/1% Teepol overnight. Furthermore, any materials which contact the cells will also be disinfected with 0.5% Presept®/1% Teepol prior to autoclaving followed by incineration. These disposal and decontamination methods are in adherence to containment level 2 procedures. Therefore, the extremely unlikely release of live hTERT-expressing virus into the culture media would not present a significant hazard since the virus would be destroyed using the disinfection procedures described. All related experiments involving any material from the immortalised cell lines will be concluded in containment level 2 laboratories (there are ample facilities available at the JvGCRC as well as in the interdisciplinary Biomedical Research Centre).

The risk assessment that is attached has been reviewed by three members of staff with expertise in genetic manipulation and revised in the light of comments that were made on it. The revised risk assessment was then approved by all three original reviewers and signed off by Prof S J F who is the Chair of the Genetic Modification (GM) Safety Committee and Dr M S, the University Biological Safety officer.

Please enter comments on the GM safety committee on the risk assessment

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The tumour-associated antigens studied in the John van Geest Cancer Research Centre (JvGCRC) are linked with tumour cell proliferation. Small interfering RNAs (siRNAs) processed from short hairpin RNAs (shRNAs) are a powerful way to mediate gene-specific RNA interference (RNAi) in mammalian cells. Different plasmid vectors and/or viruses encoding shRNA to silence/knockdown tumour-associated antigens will be used to transfect cell lines, produce virus particles and/or inject animals in order to assess the effects of shRNA on tumour cell proliferation both in vitro and in vivo. Also, lentiviral vectors are known to be a very efficient way of gene transfer because of their stable integration in non-dividing and dividing cells and long-term transgene expression.

A number of human cell lines purchased from the ATCC (American Type Culture Collection, USA) and ESTDAB (European Searchable Tumour line Database and Cell Bank, Germany) will be transfected in vitro or in vivo using different plasmid and virus vectors. The human cell lines will include: FM-82, a human melanoma cell line (Originally given by Prof. D. Schadendorff and well described on the ESTDAB website) and PC3, a prostate cancer cell line (Originally purchased from the ATCC) and the prostate cancer cell line OPCT-1 (obtained from Onyvax, Ltd).

Recipient or parental organism

Prokaryotic hosts: Attenuated and non-colonising hosts, such as the disabled E.coli K12 strains XL1-Blue (Stratagene) and TOP10 (Invitrogen), will be used to bulk up DNAs of interest. These bacteria are unable to survive outside of laboratory conditions.

Eukaryotic hosts: Human cell lines, such as FM82 or PC3 cells will be used. These cells are free of pathogenic viruses, and cannot survive outside their culture media. In addition, in the unlikely event of accidental contamination, the recipient's immune system would quickly reject them because of the human leukocyte antigen (HLA) mismatch. No immuno-compromised individual(s) will be permitted to carry out work described in the Risk Assessment. Normal human cell lines are considered to be especially disabled hosts (see the ACGM Compendium of Guidance) but they will, nevertheless, be grown in a containment level 2 laboratory.

Animals: Non-obese diabetic / severe combined immuno-deficient (NOD/SCID) mice will be used. These mice lack B cells, T cells and NK cells, and only survive in a sterile environment. Lentiviral transduction particles, used in a tumour model, are not pathogenic or transmissible, and will not affect these mice. Furthermore, encoded shRNA are under the control of human promoters and will not be expressed in murine cells. NOD/SCID mice will be handled in a containment level 2 laboratory and will be kept in individually ventilated cages. Other immuno-compromised mice may be used in the future.
SureSilencing™ shRNA plasmid for human DDX43 (SA Biosciences, Cat Number: KH11719N): This plasmid is designed to specifically knockdown the expression of DDX43 by RNA interference under stable transfection conditions after performance of the appropriate enrichment or selection procedures. The vector contains the shRNA under control of the U1 promoter and the neomycin resistance gene for the selection of stably transfected cells.

BLOCK-iT™ inducible H1 RNAi entry vector for human T21, primarily, and other potential tumour-associated antigens in the future (Invitrogen, Cat Number: K4920-00 and K4925-00): This vector facilitates tetracycline-regulated expression of a shRNA of interest from an H1/TO RNAi cassette for use in RNAi analysis in mammalian cells. It provides a rapid way to clone double stranded oligonucleotide duplexes encoding a desired shRNA target sequence (e.g.: to target T21) into an entry vector containing an RNA polymerase III-driven expression cassette.

Mission™ shRNA lentiviral transduction particles against DDX43 (Sigma, Cat Number: SHCLNV-NM_018665). The lentiviral transduction particles are produced from a library of sequence-verified lentiviral plasmid vectors for human genes. The libraries consist of sequence-verified shRNAs cloned into the pLKO-puro vector. Unlike murine-based MMLV or MSCV retroviral systems, self-inactivating replication-incompetent lentiviral-based particles will permit efficient infection and integration of the specific shRNA construct into both dividing and non-dividing mammalian cells.

BLOCK-iT™ lentiviral RNAi expression system for human T21, primarily, and other potential tumour-associated antigens in the future (Invitrogen, Cat Number: K4925-00). This expression system facilitates the creation of a replication-incompetent lentivirus that delivers an inducible shRNA of interest to dividing or non-dividing mammalian cells for RNAi analysis.

pGreenFire™ Pathway Reporter Lentivectors Cat. # TR0XX Series (System Biosciences) has a dual reporter system consisting of GFP and Luciferase. These can only be expressed when the relevant transcription factor is present within the cell. This vector will be used to transfect mammalian cell lines in order to report the expression of a particular transcription factor and to sort and study the cell populations according to GFP expression. pGreenZeo lentivectors are third generation HIV lentiviral vectors which are designed to maximize their biosafety features. These HIV-based vectors falls within NIH Biosafety Level 2 criteria because of the possibility of recombination with endogenous viral sequences to form self-replicating virus or of insertional mutagenesis.

Origin & function

The DNA inserted into the cells encodes a shRNA designed to silence tumour-associated antigens such as HAGE or T21 in human tumour cell lines. Each shRNA is or will be inserted downstream of a modified human promoter, respectively, allowing the entire shRNA to be produced. It is believed that shRNA do not have any oncogenic or other harmful properties.

pGreenFire™ Pathway Reporter Lentivectors express copGFP reporter gene followed by the self-cleaving T2A peptide and the firefly luciferase gene under the control of a pathway-specific TRE. Other than GFP and luciferase, no other proteins will be expressed as a result of the transduction with these lentivectors.

Evaluation of foreseeable effects

The potential for the inserted sequences to be transferred to other GMOs is, with the application of good laboratory practice and the methods of containment described herein, considered to effectively zero. However, future users will have to possess a working knowledge of viral and tissue culture techniques, lipid-mediated transfection, and the RNAi pathway.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The different tumour cell lines and the lentiviruses will be cultured either in plastic flasks (a maximum of ten 50ml-flasks at any one time). Plastic flasks will be disposed of into a bin for autoclaving and then incineration. Glass bottles will be soaked overnight in a mixture of 0.5% (w/v) Presept® / 1% (v/v) Teepol. Cells will be grown in a class II cabinet, with one cabinet dedicated to virus work with both Presept® and 70% (v/v) ethanol available in the cabinet in case of any spillages.

Plasmids containing the shRNA of interest will be grown in E.coli in a containment level 2 laboratory. Bacteria will be cultured either on agar plates (typically 15ml volume) or in liquid medium (a maximum of 250ml). Cultures will be stored in 1 ml-volume (under glycerol at -80°C).

Lentiviruses will be injected (either using a syringe or a gene gun) in NOD/SCID mice in a class II cabinet by a trained Personal License Holder. The latter will follow the rules set up by the Home Office Codes of Practice for the housing and care of animals. Needles will be discarded in sharps bin for autoclaving and incineration. Moreover, mice are kept in individually ventilated cages providing an effective barrier protecting animals from airborne contaminants and users from injected replication-incompetent lentiviruses. Mice will be monitored two to three times a week and will be sacrificed if any symptoms arise or when the tumours reach a size of 1cm² according to the Home Office guidelines.

Any spillages will be dealt with as recommended by the School Disinfectants Policy, which is available on the Microbiological Safety website. In addition, the implementation of the Code of Practice for Working with Hazard Group 1 Microbes will ensure that the bacteria and the viruses will not be released into the environment. For this, personnel will be trained in Good Laboratory Practice, aseptic technique, the avoidance of aerosol production, and the disinfection of working surfaces and equipment. All waste will be autoclaved and incinerated. Consequently, it is highly unlikely that either cells or viruses will be released into the environment.

New staff will be trained by a member of the laboratory according to the training programme for tissue culture activities (See the NTU School of Science Training Booklet). An up-to-date list of GM users will be maintained. In the unlikely event of escape, none of the modified GMOs will be able to survive outside of the laboratory.

Accidental exposure, for example through puncture wounds, will be treated by a trained first aider.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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**Project Containment**

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### Project Additional Information

**Purposes of the contained use**

The project aims to generate a transposon mutant library of Helicobacter pylori laboratory strains including 60190 and SS1 to identify genes involved in outer membrane vesicle production and virulence. Additionally, H. pylori mutants lacking key virulence factors including VacA, CagA, CagE, catalase, PPT, GGT will be brought to Nottingham Trent University from collaborator institutions or generated in-house if necessary, and the same virulence factors will be cloned into E. coli for overexpression, mutagenesis and purification.

**Recipient or parental organism**

H. pylori is a recognised human pathogen, but is considered as hazard group 2 because it is unable to persist in the environment for more than a very short time. Adult acquisition is extremely rare, and volunteer studies have shown that large volumes of active culture are needed along with deliberate stomach acid suppression with drugs to cause infection.

**Host/vector system**

Hosts: Escherichia coli DH5α and B834 or closely related strains; these are hazard group 1 strains that are recognised as 'disabled or non-colonising' hosts for genetic manipulation; see the SACGM Compendium of Guidance. E. coli DH5α and B834 are K-12 derived laboratory strains that are non-colonising and disabled, therefore not considered pathogenic to humans, animals or plants. As strains have auxotrophic requirements unlikely to be met outside laboratory culture, the survival of bacteria in the
Vectors: pBR322-derived vectors such as pJET (Fermentas) and pET series plasmids will be used for cloning and expression of H. pylori virulence factor genes in E. coli. For targeted mutagenesis in H. pylori, the target H. pylori genes will be cloned into pBR322-based plasmids then disrupted in vitro with a chloramphenicol or kanamycin resistance marker before transformation back into H. pylori for mutant generation by homologous recombination and marker rescue. These vectors are mobilisation defective and there are no known hazards associated with their use.

For transposon mutagenesis in H. pylori, a transposon based system will be used to introduce random insertion mutations into the H. pylori genome with a chloramphenicol resistance marker. The transposon system will use GPS plasmids and transposase enzyme to mutagenize H. pylori chromosomal DNA in vitro, which will then be transformed back into H. pylori for mutant generation by homologous recombination and chloramphenicol or kanamycin marker rescue. The GPS plasmids are not able to replicate in normal laboratory strains of E. coli and there are no known hazards associated with their use.

The virulence factor genes to be cloned include the VacA and CagA toxins (harmful to human and mammalian cells, CagA may be oncogenic, VacA is immune modulatory). CagE which is part of the Type IV secretion system used to deliver CagA to human cells, catalase and other proteins with diverse effects on human immune cells including GGT and PPT. These proteins will be overexpressed in E. coli and purified in their biologically active forms. The activities of these virulence factors may be harmful to human cells and may become allergenic upon repeated exposure. CagA may have oncogenic effects once inside human cells, but requires delivery into cells via a complex multi-component bacterial secretion system so the risks associated with handling the purified toxin in isolation are considered to be low. All proteins will be produced in small quantities (typically less than 1 mg) under containment level 2 using appropriate PPE (gloves, lab coat) and Good Laboratory Practice including precautions to prevent aerosolisation, to minimise the risk of exposure of workers to the bacterial products.

Origin & function

The virulence factor genes to be cloned include the VacA and CagA toxins (harmful to human and mammalian cells, CagA may be oncogenic, VacA is immune modulatory). CagE which is part of the Type IV secretion system used to deliver CagA to human cells, catalase and other proteins with diverse effects on human immune cells including GGT and PPT. These proteins will be overexpressed in E. coli and purified in their biologically active forms. The activities of these virulence factors may be harmful to human cells and may become allergenic upon repeated exposure. CagA may have oncogenic effects once inside human cells, but requires delivery into cells via a complex multi-component bacterial secretion system so the risks associated with handling the purified toxin in isolation are considered to be low. All proteins will be produced in small quantities (typically less than 1 mg) under containment level 2 using appropriate PPE (gloves, lab coat) and Good Laboratory Practice including precautions to prevent aerosolisation, to minimise the risk of exposure of workers to the bacterial products.

The antibiotic cassettes that we propose to use as selectable markers to facilitate inactivation of genes in H. pylori will confer resistance to kanamycin or chloramphenicol. Neither of these are used in either the treatment or prophylaxis of H. pylori infection.

Evaluation of foreseeable effects

Cloning of H. pylori virulence factor genes into E. coli: Addition of H. pylori virulence factor genes has the potential to increase the pathogenicity of the host E. coli strains, for example via the production of VacA toxin which is harmful to human cells, but overexpression of one virulence factor in isolation would not confer upon the E. coli host strains the ability to infect humans, animals or plants. It is unlikely that any DNA sequence will confer on the E. coli host a significant increase in fitness or pathogenicity due to the multiple disablements present and the multifactorial nature of pathogenicity. Furthermore, expression will be controlled at a low level. The resultant DH5α and B834 strains carrying H. pylori virulence factor genes are not expected to have altered host ranges and will not be able to survive outside of the laboratory. As a precaution, E. coli strains containing H. pylori virulence factor genes will be handled only at containment level 2.

Transposon mutagenesis in H. pylori and targeted mutagenesis to disrupt specific virulence factors: Generation of chloramphenicol or kanamycin resistant H. pylori mutant strains would not affect treatment options, because other antibiotics are used in treatment of H. pylori infections. Introduction of additional antibiotic resistance markers is considered unlikely to affect therapeutic potential in the extremely unlikely event of a laboratory acquired infection.

Since the mutations introduced will cause loss of function of H. pylori genes, most mutants are anticipated to have reduced or unchanged virulence. However, mutation of regulatory genes which may co-ordinately control expression of a number of virulence factors has the potential to generate strains which may express elevated levels of some gene products. Mutation of regulators is considered highly unlikely to result in a strain which will have significantly enhanced virulence given the multifactorial nature of the infection process.

H. pylori is a fastidious, microaerophilic organism which cannot grow in atmospheric oxygen so the potential for transmission and environmental contamination with this organism is very low. We will not be using modified forms of any gene that is not already widely prevalent in the wild-type strain populations, except where a modification is designed to specifically reduce the activity of the encoded product (e.g deletion of protein domains, functional motifs etc.). In the unlikely event that survival of the GMO was sufficient for gene transfer to occur, any such transfer to a wild type microorganism would either not confer a greater virulence attribute than was already prevalent in the
population or would be a redundant duplication of existing genetic material.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The use of "Good Laboratory Practice" and containment level 2 will ensure that the GMOs will not be released into the environment. Measures taken will include the training of personnel in "Good Microbiological Practice", aseptic techniques, the avoidance of aerosol production, etc., the disinfection of working surfaces and equipment (for example, centrifuges), and autoclaving of all bacterial waste. 2% New Hycolin, 2% Virkon and Presept will be available for any spillages, and appropriate measures would be taken to ensure adequate disinfection. The autoclaving of all waste material will be carried out within the containment level 2 laboratory. In the unlikely event of escape, the GMOs will not be able to survive outside of the laboratory and, in any event, will not be hazardous to either humans, animals and plants. Since E. coli DH5α and B834 are non-colonising and H. pylori requires a large infective dose, the risk to humans, animals and plants can be considered to be effectively zero. Hosts and GMOs will be cultured on agar plates (typically 15 ml). Broth cultures will be no larger than 250 ml in volume. Cultures will be stored in 1 ml volumes (under glycerol). Accidental exposure through puncture wounds will be treated by a trained first aider. Implementation of the above safety measures should control the risk of release/exposure to effectively zero.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment that is attached has been reviewed by three members of staff with expertise in genetic manipulation and revised in the light of comments that were made on it. The revised risk assessment was then approved by all three original reviewers and signed off by the Chair of the Genetic Modification (GM) Safety Committee and the University Biological Safety Officer.

Project Containment

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<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Animal Units

Large Scale Activities

Human Clinical Applications
**Project Additional Information**

**Purposes of the contained use**

The broad interest of our laboratory is to determine the changes in the epigenome and cellular signaling that occur in inflammatory diseases and cancer, identify novel targets and investigate therapeutic applications. This project aims to utilise disabled, replication incompetent retroviral and lentiviral systems to deliver genes of interest as cDNA or shRNA against individual genes into mammalian cells for their subsequent analysis in a broad range of cell-based and biochemical assays. Viral mediated methods for transduction of cDNAs into cells provides a very valuable tool for the production of cells stably expressing a gene, short hairpin- or micro-RNA of interest into the host genome.

**Recipient or parental organism**

Cloning strains of bacteria derived from E. coli K12: MACH1, DH5a and Sbtl3, are non-pathogenic, non-hazardous. They do not carry the well-recognized pathogenic mechanisms required by strains of E. coli that cause the majority of enteric infections. These strains are considered to be non-pathogenic and unlikely to survive in host tissues and cause disease. Exposure through eyes and skin may cause irritation with susceptible persons.

**Host/vector system**

Host Cell Lines: Containment level 1 (CL1) human cell lines: NCM356, NCM460 (InCell) immortalised colonic cell lines and HCT116, HT29, CaCo2, SW480, SW620, SW48, LS123, RKO, DLD1 (ATCC) colorectal cancer cell lines, Capan1, PANC1, BxPC3, MiaPaca2, Capan2, CFPAC1, AsPC1, HPAFII (ATCC) pancreatic cancer cell lines, SKHep1, HepG2 (ATCC) liver cancer cell lines. These are established, dissociated mammalian cell lines, no human hazards exist. All cell lines were purchased from ATCC and InCell and are virus-free certified.
Containment level 2 (CL2) SNU475, SNU387, SNU398, SNU449, Hep3B (ATCC) human liver cancer cell lines. These cell lines contain Hepatitis B virus (HBV) as assessed by PCR. Hepatitis is spread through contact with blood and blood products or contaminated hypodermic needles.

Containment level 2 (CL2) HEK293T/17 (ATCC) and AmphoPack-293 (Clontech) cell lines will be used for the production of lentiv- and retro-viruses, and amphotropic viruses, respectively. They give high titres when used to produce viruses and have been widely used for retroviral production, gene expression and protein production. These cell lines contain adenovirus genetic material and HEK293T also contains Simian Virus 40 large T antigen (SV40T). Because these cells do not contain the complete viral genome for the respective viruses the risk of generation of these viruses by these cells is extremely low.


The self-inactivating vector systems or AmphoPack-293 cells will be used to generate replication defective viruses. Genes for replication and structural proteins are absent in the packaged viral genome since genes are supplied by other plasmids in packaging cells. Viral genome contains only the region between the 5’ and 3’ LTR’s. Vectors contain self¬-inactivating 3’ LTR.

Hazards associated with these vectors are summarised as: stable expression of transgenes, insertional mutagenesis and potential for generation of replication competent virus. Replication defective vectors that cannot infect human cells can generally be considered class 1. For replication defective retroviruses and lentiviruses capable of infecting human cells, if the risk assessment demonstrates they are adequately attenuated, it is possible to designate the activity as class 1 :- Factors supporting this classification will include: low risk of generation of RCV (e.g. a third generation packaging system), self -inactivating (SIN) LTR and non-harmful insert. However, contaminated sharps represent a significant hazard, and their use should be excluded for vectors that can infect human cells, if the activity is to be designated class 1.

Origin & function

The inserted sequences are capable of encoding biologically active proteins which may be potentially pathogenic to humans if mutated. The eukaryotic vector systems are optimised for physiological level expression of protein. The DNAs are highly unlikely to alter the pathogenicity or properties of the bacterial host cell. Enforced expression of such sequences in human cell lines, will not affect the malignant properties of the cells. Neither the DNA, encoded protein, bacterial or mammalian transfectants are likely to have any deleterious effect on the environment. The GM microorganisms are incapable of surviving outside of the laboratory environment. Retroviral and Lentiviral particles are very labile, and unstable when exposed to air. In addition, they are very labile at high temperatures and can be disabled effectively with disinfectants (such as Virkon) or 10% bleach solution in water.

Primary Hazards associate with ingestion via the oral route, droplet exposure of the mucous membranes, inhalation (through respiratory droplets), and contact/hand-to-eye transfer. Special hazards: Generalized infections can occur in immunocompromised individuals. Exposure to this agent can cause serious infection in the congenitally immunocompromised, in patients undergoing immunosuppressive treatment for organ and tissue transplants and for cancers, and in human immunodeficiency virus-infected patients.

GM bacteria have a reduced ability to colonise beyond the intestine and reduced persistence compared with pathogenic strains. Laboratory procedures are in place to
prevent the escape of E. coli strains carrying GM plasmids. All personnel will be trained in good microbiological practice and work is carried out in a containment level 2 laboratory with restricted access (CELS206).

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Handling and manipulation of naked DNA will require the wearing of lab coats and disposable gloves. Laboratory coats and nitrile gloves will be worn when working with GM bacteria. Work areas have impermeable surfaces and will be disinfected with freshly prepared Virkon (working concentration of 1% v/v) routinely and after spills to prevent dispersal. Media, cells, and cell lysates will be decontaminated using freshly diluted bleach (final dilution 1:10, minimum 30 minute contact time), or autoclaved before disposal in the sewer, to ensure that the environment is not contaminated with plasmids carrying antibiotic resistance genes or other potentially harmful genes.

All human cell lines have the potential risk of producing unidentified human viral pathogens. Human cell lines, whether designated by the source company as CL1 or CL2 will be cultured in a Class 2 biological safety cabinet using level 2 precautions. Any cell lines infected with the viruses will be handled using level 2 precautions in a laboratory containment level 2 room (CELS205), accessed by card only. All virus work will be performed in bespoke Class 2 microbiological safety cabinet. The specific cabinet will be labelled for virus production and use and increased risk of HepB. All personnel using the bespoke safety cabinet and tissue culture incubator will be immunised against Hepatitis B and will also have their resistance levels checked via an antibody titre test. Users will be experienced in proper pipetting techniques, pipetting down the side of the tube with no forceful expulsion, to reduce aerosol generation. For centrifugation, closed, screw-capped tubes and centrifuge cups with aerosol resistant lids containing O-rings will be used to contain aerosols. Any pipetting will be performed with filter tips, to minimise contamination of instruments, and only plastic pipettes will be used to minimise sharps risk. Pipettes and racks will not leave the biosafety cabinet. Good laboratory practice will include lab coat, eye ware (safety glasses or Face shield), waterproof gloves (nitrile rubber, butyl rubber); designated tissue culture lab coats. Use of double gloves when working with virus supernatants following production in packaging cell lines will reduce the risk of user contamination. Functional, molecular analyses will follow multiple passages of cells transduced with lentiviruses, while cell extracts (RNA, protein) will use reagents that inactivate viruses (Phenol, dithiothreitol). Extensive hand washing will be enforced after working with viruses. All users under this GM will be trained and supervised for these procedures prior to being given clearance for work. Log of personnel involved in virus production and usage, with all information related to the specific procedures and dates will be kept in the lab. 1% Virkon disinfectant and 70% ethanol for workplace and spillage decontamination will be used. Either 1% Virkon or 10% bleach, will be applied before disposal for at least 30 minutes for inactivation of any virus-containing media cultures, pipettes, tips and culture dishes. Microbiological safety cabinet will be in addition treated with UV for 1 hour after the completion of virus-related work. All biological waste will be autoclaved of prior to disposal.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
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</tr>
</tbody>
</table>

**Animal Units**

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
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</tr>
</thead>
<tbody>
<tr>
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**Project Ref** 187/17.1

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<th>CultureVolume</th>
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<td>Characterisation of mobile genetic element stability in pathogenic enterobacteria</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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**Withdrawn** N

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Toxin-antitoxin (TA) modules function on low-copy virulence and resistance plasmids as postsegregational killing systems; they ensure vertical transmission of a plasmid by killing or inhibiting the growth of any plasmid-free daughter cells. My previous work (McVicker & Tang, 2016, Nature Microbiology) has shown that the regulation of a given TA system can itself be adapted to the host environment in a similar way to the virulence genes it helps to maintain. However, the ability of TA systems to successfully
transfer between bacterial hosts and integrate into organisms that already possess their own TA modules (i.e. the ability of TA systems to promote or prevent horizontal transmission) has not been well-studied.

This project proposes two main aims:

1) To investigate the presence of novel or poorly-characterised maintenance elements on the virulence and antimicrobial resistance plasmids/mobile genetic elements of a range of E. coli isolates, to identify new potential targets for therapy and to understand the maintenance of virulence in these highly diverse organisms.

2) To study the contribution of maintenance elements to horizontal gene transfer as opposed to vertical transmission of mobile genetic elements.

These aims will be primarily achieved via the construction of artificial test vectors containing replication, maintenance and/or conjugation machinery from existing pathogens (though lacking the associated virulence genes) and the tagging of “wild” plasmids in existing pathogens with counterselectable markers to study their stability over time in long-term evolution experiments.

In addition to the above, any novel maintenance systems (e.g. TA systems) discovered will be subcloned onto common laboratory vectors and tested for their biological role in various bacteria (typically non-pathogenic E. coli cloning strains and the genes’ native host).

Recipient or parental organism

"Domesticated" laboratory strains of E. coli K-12, e.g. MG1655 and BW25113 derivatives of the Keio collection (Baba et al., 2006). These are non-mobilising, non-pathogenic, hazard group 1 organisms routinely used as model organisms or transduction donors. These strains may survive outside of controlled laboratory conditions but do not pose a significant risk to human health.

Cloning and overexpression strains of E. coli such as DH5α, TOP-10, BL21 and their derivatives. These are non-colonising, non-mobilising, non-pathogenic, hazard group 1 organisms routinely used in the laboratory for maintenance of vectors and expression of proteins for purification. These strains are unlikely to survive outside of controlled laboratory conditions and do not pose a risk to human health.

Verotoxin-negative E. coli O157:H7 (from NTU stocks). This strain is an enteropathogenic form of E. coli that has undergone toxin-disabling mutations to reduce its handling risk. The disabled strain is hazard group 2 and is able to cause self-limiting gastrointestinal infection in humans, though the possibility of more severe haemolytic disease caused by the parent strain has been eliminated. This strain is likely to survive in the environment and may colonise humans via the feco-oral route, but represents a significantly lowered risk compared to its wild-type parent. The pathogen is not airborne. No modifications proposed in this study are expected to increase its virulence or infectious route.

Pathogenic E. coli outbreak isolates obtained from Public Health England and other controlled sources: typically enteroinvasive, enteropathogenic, enteroaggregative or extraintestinal pathogenic E. coli of various serogroups. These organisms typically colonise via the feco-oral route and are not airborne pathogens. All strains are hazard group 2 or lower. In all cases these strains will have been subjected to PCR to confirm absence of known verotoxin genes; many have also been whole-genome sequenced. Nonetheless, strains will often be disease isolates that may cause gastrointestinal infection and diarrhoea with a range of severities (likely to be self-limiting and treatable in healthy human adults), or extraintestinal infection in immunocompromised individuals (unlikely to affect healthy human adults). Some isolates are naturally multi-drug resistant due to pre-existing genes. As disease isolates, these strains are likely to survive well in the environment and represent an acute infection risk in their unmodified form (Dallman et al., 2014; Newitt et al., 2016; Alkeskas et al., 2015). No modifications proposed in this study are expected to increase the virulence or infectious route of these strains.

Host/vector system

Hosts

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Vectors

Laboratory cloning vectors such as pUC19 (Yanisch-Perron et al., 1985) and derivatives. High-copy. Routinely used in the laboratory, non-harmful and non-mobilisable. Antibiotic markers present for selection. Limited host range.

Expression vectors such as pBAD33 (Guzman et al., 1995), pGM101 (McVicker & Tang, 2016) and pET derivatives (Novagen). Low- to medium-copy. Routinely used in the laboratory, non-harmful and non-mobilisable. Antibiotic markers present for selection. Limited host range.

Allelic exchange and recombinering vectors such as pKO3Blue, pKD series, pCP20 and derivatives (Cherepanov & Wackernagel, 1995; Datsenko & Wanner, 2000). Low- to medium-copy. Routinely used in the laboratory, non-harmful and non-mobilisable. Used to enhance genetic manipulation of the host chromosome or large plasmids. Some vectors (e.g. pKD46) promote heightened nonspecific homologous recombination in the host, but do not themselves provide recombination substrates that would cause an increase in virulence of any organism. Many vectors in this category are temperature-sensitive so can be cured as necessary. Antibiotic markers present for selection. Limited host range.

pSTAB vectors (McVicker & Tang, unpublished): de novo constructed “stability test vectors” derived from the replicons of large wild plasmids and containing antibiotic resistance markers for selection. Low-copy, non-mobilisable. Likely to have a limited host range, though this is unknown as the replicons to be used are not characterised. Specifically constructed to study plasmid maintenance elements in a safe and isolated context (separate from the virulence and multidrug resistance genes found on the wild plasmids from which their replicon sequences are cloned).

P1vir bacteriophage (Ikeda & Tomizawa, 1965): a lytic phage capable of transferring genetic material from one species/strain to another, routinely used as the vector for controlled generalised transduction. Bacteriophage cannot infect animals (including humans) or plants. P1vir does not survive well in the environment compared to ubiquitous wild equivalents and cannot undergo lysogeny (dormancy within a bacterial host), so the risk of spreading genetic material to unintended organisms, both within and outside the laboratory, is negligible given good microbiological practice.

Origin & function

In many instances, GM experiments to be carried out will involve the inactivation of existing genes on native virulence/resistance plasmids via the insertion (and subsequent removal or allelic exchange) of a defined antibiotic resistance cassette. The techniques used will include phage transduction and site-directed mutagenesis/recombineering (e.g. Datsenko & Wanner, 2000; Blomfield et al., 1991). The DNA to be introduced in this case will not be hazardous or increase an organism’s virulence, with the exception that antibiotic selection cassettes will necessarily increase the number of antibiotics to which an organism is resistant.

Identified toxin-antitoxin gene pairs may be cloned for analysis, either together or separately. The gene products will be biologically active but non-hazardous to humans. Toxins are generally cytoplasmic bactericidal or bacteriostatic (i.e. “suicidal”) molecules that interfere with processes such as bacterial replication/translation and are not expected to be secreted by the bacterial cell. Toxins are unlikely to have valid human targets and human exposure will be negligible. Antitoxins prevent the above toxic activity and are hazardous to neither the bacterial host nor human workers. No increase in virulence would be expected from the introduction of either of the above DNA sequences; instead, bacterial host fitness is likely to decrease.

Plasmid replicons will be cloned to study them in isolation. These sequences are themselves non-hazardous. Other plasmid maintenance and mobilisation elements (distinct from TA systems described above) may be cloned occasionally in order to study their effects in isolation. These sequences will not encode hazardous products but may modify the stability/transmission of pSTAB test vectors (which are themselves non-hazardous). Mobilisation experiments will only be performed using non-GM plasmids (i.e. the rate of genetic transfer will be identical to the naturally-existing population) or, if using a GM construct, carried out using non-virulence-gene-encoding pSTAB vectors in disabled, non-pathogenic strains. Under no circumstances will virulence/multi-drug resistance plasmids be intentionally/artificially mobilised by the genetic
Despite their tangential relatedness in terms of natural plasmid function, known virulence factors are not the subject of study in this project and will not themselves be intentionally cloned or expressed.

**Evaluation of foreseeable effects**

P1vir bacteriophage transductions will be carried out to move vectors and marked gene mutations between strains under controlled laboratory conditions, observing good microbiological practice. It is unlikely that virulence elements could be accidentally transferred in this manner, as only the desired transductants will survive due to the integration of a selectable marker. P1vir transduction is rare (on the approximate order of 10^-6 events per recipient cell) and therefore the risk of a miscellaneous, unintentional transduction occurring in tandem with an intentional one is negligible.

Where possible, resistance alleles for selection will be introduced into strains that do not already carry broad multidrug resistance, to limit any potential impact on therapy. Furthermore, no modification proposed by this project will directly increase the virulence of any hazard group 2 organism being studied. On the contrary, many modifications made to the hazard group 2 GMOs in question will cause a reduction in virulence/resistance plasmid stability. In the event that such a GMO escapes into the environment, it will be less effective at causing disease than its wild type equivalent. In the event that a plasmid has been made more stable by a modification, the resulting GMO is predicted to be unable to compete effectively with the wild type due to the increased burden of large plasmid replication.

The pathogenic enteric bacteria studied in this project are hazard group 2, able to survive in the environment and cause acute disease in humans. The genetic modifications to be made are not likely to increase any such organism's virulence or ability to survive in the environment.

It is conceivable that virulence plasmid or resistance plasmid stability or transmission rate may be affected by the genetic modifications to be made, though this will not be in excess of naturally-occurring plasmids within the genus. If plasmid stability increases, fitness of host bacteria is likely to decrease, encouraging out-competition of a GM organism by wild type strains outside of controlled laboratory conditions. Non-mobilisable, naturally-occurring virulence and multidrug resistance plasmids will not be intentionally/artificially mobilised by this work.

It is likely that the majority of modifications to be made to wild plasmids, and the majority of genes cloned onto vectors that will be introduced into wild strains, will in fact reduce virulence/resistance by causing defects in native plasmid replication/maintenance. Many vectors that will be transformed or transduced into wild strains will be incompatible with naturally-occurring plasmids (by virtue of a shared replicon) and so their selection will ensure the loss of the native virulence/resistance plasmid – hence, a decrease in infectiveness and potential risk, rather than an increase.

The introduction of antibiotic resistance cassettes in order to construct mutants or assay plasmid stability will necessarily increase the resistance profile of the organisms being studied. This is a known quantity that can be accounted for by good microbiological practice and by careful consideration of existing resistance alleles. Virulence will not be directly affected by these mutations. Indeed, the counterselectable marker to be introduced for plasmid loss assays provides an additional method of killing organisms that contain a GM plasmid (McVicker & Tang, 2016), therefore is more likely to reduce any associated risk rather than increase it.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

GMOs will be handled in rooms rated sufficient for hazard group 2 containment. Staff will be fully trained in the handling of these organisms at this containment level. Precautions to be taken will include good microbiological practice, sufficient PPE (nitrile gloves, Howie lab coats, eye protection where applicable) and the disinfection of contaminated material by chemical agents (e.g. 2% Virkon, able to inactivate enterobacteria and bacteriophage within minutes, or 70% ethanol) and/or autoclaving. Waste disposal and spillages will be handled appropriately using the methods above, according to standard containment level 2 guidelines.
The risk assessment that is attached has been reviewed by three members of staff with expertise in genetic manipulation and revised in the light of comments that were made on it. The revised risk assessment was then approved by all three original reviewers and signed off by the Chair of the Genetic Modification (GM) Safety Committee and the University Biological Safety Officer.

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 187/19.1

Date Ackn’d 22/08/2019

CU2 Project Title Type V secretion in Pseudomonas aeruginosa

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
Pseudomonas aeruginosa is an opportunistic human pathogen and an increasingly urgent problem in healthcare settings. In this project, the secretion of several P. aeruginosa autotransporters or type V secretion systems will be investigated. PlpD is a secreted phospholipase and the prototype of type Vd secretion systems in bacteria. This little-studied subclass of type V secretion appears to combine features of both type Va (classical autotransporters) and type Vb (two-partner) secretion systems. By making truncations, point mutations, domain replacements and fusions with other proteins, the effect on surface localisation of PlpD will be measured both quantitatively and qualitatively. The results will provide information on the regions important for PlpD autotransport, the direction of secretion (N- or C-terminus first), the topology of the proteins, and the requirements in the phospholipase domain for efficient secretion. In addition, the relationship between type Va, type Vb and type Vd secretion systems will be investigated. Specifically, the goal is to produce either an artificial type Vb secretion system from a type Vd secretion system, or vice versa, to functionally demonstrate the evolutionary relationship between the two systems. This will be done by separating the phospholipase domain from the translocation domain. In parallel, an artificial type Vd system will be attempted by fusing the secreted protein (LepA) with the translocator protein (LepB) from the LepAB two-partner secretion system of P. aeruginosa. In addition, various fusions of PlpD, LepA and LepB will be made to see which functions of the systems are needed for efficient secretion. Readout of successful secretion will be either surface display of PlpD or LepA, or presence of these proteins in the culture supernatant. The relationship between type Va secretion and type Vd secretion will be investigated by utilising a type Va-secreted phospholipase of P. aeruginosa, EstA, and performing domain swaps to study the interchangeability of the different domains.

All cloning steps will be performed in E. coli K-12 (DH5<sup>®</sup> or similar), which is a hazard group 1 organism. Expression of plpD (from P. aeruginosa PAO1 with E. coli codon optimisation), lepAB (from P. aeruginosa PAO1, native sequence), estA (from P. aeruginosa PAO1, native sequence) and their derivatives will be performed in the laboratory P. aeruginosa strain PAO1 (hazard level 2). P. aeruginosa is an opportunistic pathogen of humans, animals and plants, but generally does not pose a threat to healthy individuals. For expression, a strain lacking the endogenous plpD gene will preferentially be used (see https://www.frontiersin.org/articles/10.3389/fmicb.2019.00100/full), but the wild-type PAO1 will also be employed for some experiments.

Neither plasmid represents a significant hazard to humans or the environment.
The insert plpD from P. aeruginosa PAO1 codes for an outer membrane phospholipase. The phospholipase is non-toxic and the corresponding knock-out strain is not attenuated in virulence in a Galleria mellonella model (unpublished data), suggesting that the lipase is not a toxin virulence factor of P. aeruginosa. The proteins will be expressed in their entirety, as truncated or point-mutated variants, or as fusion proteins with (non-hazardous) marker proteins.

lepAB encode a two-partner secretion system of P. aeruginosa PAO1, where LepA is a secreted protease and LepB the outer membrane translocator protein. LepA has been reported to induce inflammatory responses and is implicated in virulence. However, in this work, only the TPS domain required for LepA secretion will be expressed, thus excluding the toxic protease domain. LepB will be produced in its entirety, as truncations, and as fusions with EstA, PlpD and LepA.

estA encodes an autotransporter esterase of P. aeruginosa PAO1 that has been implicated in the production of extracellular rhamnolipids, but overproduction of EstA reduces biofilm formation (see https://mic-microbiologyresearch-org.ezproxy.uio.no/content/journal/micro/10.1099/mic.0.037036-0#tab2). However, no direct toxic effects of EstA have been reported. The protein will be produced in its entirety, or parts of it as fusions with LepA, LepB or PlpD.

Evaluation of foreseeable effects

P. aeruginosa is ubiquitous in the environment and is an opportunistic human pathogen that can infect wounds and burns. It is also a significant pathogen of cystic fibrosis patients. However, it rarely causes disease in healthy humans. PlpD does not appear to have a significant effect on the pathogenic potential of P. aeruginosa. LepA, though an established virulence factor, will only be produces as a non-toxic fragment, whereas its partner LepB is not expected to have an effect on virulence apart from the secretion of LepA. EstA has no reported toxicity, and overproduction of this protein has opposite effects on two virulence-related phenotypes. Therefore, EstA overexpression probably does not increase the pathogenic potential of P. aeruginosa. The organism itself is classed as a hazard group 2 organism, and therefore will only be handled in CL2 facilities.

In the unlikely event of escape, the modified GMOs are unlikely to be to represent a greater hazard than the wild-type organism to either humans, animals or plants. All personnel are trained in Good Microbiological Practice and all work is carried out in a containment level 2 laboratory with restricted access. Any future students will receive full safety training for working in a CL2 laboratory and be under supervision of trained staff. Technical staff are already trained in GMO work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The GMOs will only be handled in a CL2 facility with restricted access, and handling the GMOs will make use of safety hoods and aseptic techniques, the avoidance of aerosol production, wearing of laboratory coats and nitrile gloves, maintenance of negative air pressure within the laboratory, the disinfection of working surfaces and equipment (for example, centrifuges) with BioCleanse, and autoclaving of all bacterial waste will be within the laboratory suite. Work areas have impermeable surfaces and are disinfected with new hycolin (a broad spectrum disinfectant used at a working concentration of 2% v/v) or BioCleanse at 1%. Waste disposal and spillages will be handled appropriately using the methods above, according to standard containment level 2 guidelines.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The risk assessment that is attached has been reviewed by three members of staff with expertise in genetic manipulation and revised in the light of comments that were made on it. The revised risk assessment was then approved by all three original reviewers and signed off by the University Biological Safety Officer.

### Project Containment

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### Project Ref 187/20.1

- **Date Ackn'd**: 15/10/2020
- **Date Project Ceased**: 
- **CU2 Project Title**: Generation of TG2 knock down clones using shRNA Lentiviral Particles
- **Class**: Class 2
- **Culture Vol Class**: < 1 Litre
- **Culture Volume Class**: Consented
- **Non-GMM Consent Granted**: Yes
- **Project notified under transitional arrangements**: No
- **Historical Significant Changes**: No

### Project Additional Information
Purposes of the contained use

Transglutaminase 2 (TG2) is a calcium-activated crosslinking enzyme which is involved in a variety of pathologies, including tissue fibrosis, cancer and neurodegeneration. Our research group at NTU has a long-standing expertise in the molecular and cell biology of transglutaminases, which is recognised internationally; our latest work in the area of kidney fibrosis was published in J Amer Soc Nephrol (Scarpellini et al., 2014; Furini et al., 2018). We aim to generate TG2 knock down (KD) clones starting from rat cells (e.g. immortalised kidney epithelial cells NRK52E or primary cells such as neurons and astrocytes) by viral transduction. Specifically, transduction-ready lentiviral particles will be acquired from Santa-Cruz Biotechnology, containing target specific constructs that encode shRNAs designed to knock down TG2 gene expression. Antibiotic selection (puromycin) will be applied to select KD clones post-transduction. The efficiency of TG2-KD will be assessed by either genomic PCR, western blotting and/or TG enzymatic activity assay.

References:


Recipient or parental organism

The resulting GMOs (TG2-KD clones) cannot survive outside the laboratory regulated conditions, and are unable to colonise humans, animals or plants, whereas the inserted sequence is highly unlikely to be transferred to other organisms. The hazard group of transfected rat immortalised and primary cells in vitro can be regarded as hazard group 1 due to the extensive and continuous SPF testing of the source animals and minimal infectious potential of the transfected cells.

Host/vector system

Hosts
NRK52E cell line (ATCC® CRL-1571™) is especially disabled, as it is unable to colonise the worker and contains no known adventitious agents, which are potentially harmful. Accidental injection of NRK52E rat tubular epithelial cells in humans will result in their destruction by the host immune-system, unless the host is severely immune-compromised. NRK52E cell line will not survive outside the laboratory environment.

Primary rat brain cells such as neurons and astrocytes, as well as other primary rat cells isolated from other tissues (e.g. kidney), are not considered hazardous. They will be isolated from specific pathogen free (SPF) animals, which are screened for Adenovirus and lesions associated with Herpes Simplex and Vaccinia. Moreover, rats are not susceptible to Baculovirus or Human Papilloma Virus. Neurons and astrocytes are not capable of infecting humans as a GMO; they are non-colonising and non-pathogenic to humans, animals or plants.

Vectors
TG2 shRNA lentiviral particles (sc-270266-V) consist of a pool of concentrated transduction-ready viral particles, containing 3 target-specific constructs that encode 19-25nt (plus hairpin) shRNA which is designed to knock down TG2 gene expression. Each vial contains 200 μl frozen stock, containing 1.0 x 10^6 infectious units of virus (IFU) in Dulbecco’s Modified Eagle’s Medium with 25 mM HEPES pH 7.3.

Hazards
Lentiviral Particles were developed by the supplier (Santa Cruz Biotechnology) using a 3rd generation packaging system with a single custom expression vector to ensure the particles are replication-incompetent and designed to self-inactivate following transduction (Dull et al., 1998). In summary, the structural and replication genes necessary to produce viral particles are separated onto multiple plasmids for enhanced safety. All wild-type virulence and accessory genes are deleted. A feature of a self-inactivating 3’ long terminal repeat (SIN/LTR) renders the resulting lentiviral particles replication incompetent. Lentiviral particles are packaged in producer 293T cells and, upon co-transfection of the plasmids, all required sequences are available to produce and package a viral particle containing the transgene of interest (in our case, sequences for the synthesis of shRNAs to knock down TG2 gene expression). Only the region between the viral LTRs of the transfer vector is packaged within the viral capsid. In the event recombinants arise, mutations or deletions can be configured within the undesired gene products to render any possible recombinants non-functional (Danos and Mulligan, 1988; Markowitz et al., 1990). In addition, deletion of the 3’ LTR on both packaging constructs further reduces the ability to form functional recombinants.

While the specific details of how these products are packaged is proprietary, this information is not necessary to utilize the Lentiviral Particles in a safe and effective manner. Despite the above safety features, use of HIV-based vectors falls within NIH Biosafety Level 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. Indeed, the company recommends treating these products with Biosafety Level 2 tissue culture facilities.

In our project, we will be using these ready-to-use pre-made viral particles suitable to transfer the shRNA once, to transduce recipient cells. We will not deal with packaging vector and packaging cell line to produce the retroviral particles. These constructs have already been used to successfully transfect rodent primary cells lines (Pinzón et al., 2017).

References:

Origin & function

The shRNAs constructs which will be inserted are designed for gene silencing and do not encode for any protein. Although it is possible that the integrated DNA could be transferred by recombination with hidden viruses, NRK52E is a well-established epithelial cell line, and there are no reports indicating that these cells carry viruses. Similarly, the primary rat cell lines will be isolated from tested SPF animals, therefore free from pathogens. Information about the shRNAs constructs is proprietary.

Evaluation of foreseeable effects

TG2-KD cells will not survive outside the controlled laboratory environment. It is also not possible for them to survive in the human body, as they will be destroyed by the host immune system. KD of TG2 is not hazardous for cells, and TG2-KO mice are viable, without evident deleterious effects (De Laurenzi and Melino, 2001). On the contrary, the reduction of TG2 levels has been shown to be protective on cancer development and fibrosis (Mehta et al., 2010; Scarpellini et al., 2014; Furini et al., 2018), as well as in primary brain cells (Tucholski et al., 2006; Fujita et al., 2006), therefore the procedure should not have risks related to the gene knock-out.
It is unlikely that the resulting GMO will be released in the environment and it will not survive outside the regulated laboratory conditions. Standard practice for handling genetically modified organisms is a further step in delimiting the GMO within the laboratory. Cells will be grown according to the Training Programme For Tissue Culture Activities provided by the University.

All staff involved in this project have already received formal training and induction in cell and tissue culture activities and good laboratory practice by research technicians working in the biosciences department. This involved practice of cell culture (work in sterile conditions, handling of cells and tissue culture materials, cell passaging and freezing/storage), use and maintenance of the tissue culture facility and equipment (centrifuges, laminar flow cabinets, incubators).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Immortalised and primary wild type cells, lentiviral particles and TG2-KD clones will be cultured in a Class 2 biological safety laminar flow cabinet using level 2 precautions, in a laboratory containment level 2 room (Biomed 108), which is located in a department with restricted access (by ID card only), and is protected by a door equipped with a further code lock.

Users will be experienced in proper pipetting techniques, pipetting down the side of the tube with no forceful expulsion, to reduce aerosol generation. For centrifugation, closed, screw-capped tubes and centrifuge cups with aerosol resistant lids containing O-rings will be used to contain aerosols. Any pipetting will be performed with filter tips, to minimise contamination of instruments, and only plastic pipettes will be used to minimise sharps risk. Good laboratory practice will include lab coat, eye-ware (safety glasses or Face shield), waterproof gloves (nitrile rubber, butyl rubber); designated tissue culture lab coats. Use of double gloves when working with lentiviral particles will reduce the risk of user contamination. All personnel accessing the containment level 2 tissue culture room (where human cells are also cultured) will have been immunised against Hepatitis B, and will also have had titres checked to confirm that they responded to this immunisation.

Functional molecular analyses will follow multiple passages of cells transduced with lentiviruses, while cell extracts (DNA, RNA, protein) will use reagents that inactivate viruses (phenol, dithiothreitol). Extensive hand washing will be enforced after working with viruses. Log of personnel involved in virus production and usage, with all information related to the specific procedures and dates will be kept in the lab.

For workplace and spillage decontamination, both 5% CHEMGENE HLD4L disinfectant and 70% ethanol will be used. Either 5% CHEMGENE HLD4L or 10% bleach will be applied before disposal for at least 30 minutes for inactivation of any virus-containing media cultures, pipettes, tips and culture dishes. Laminar flow cabinet will be further treated with UV for 1 hour after completion of virus-related work. All disposable plastic-wear and solid biological wastes will be disposed into bins for autoclaving (lined with an autoclave bag) and autoclaved according to standard operating procedures. Liquid waste will be inactivated using CHEMGENE HLD4L (at a final concentration of 5% v/v) and allowed to disinfect for 24 hours. The same spillage measures will be applied during any live cell imaging or analysis (e.g. with a microscope or other device). If a puncture wound was to occur, medical attention will be provided by first aid trained staff and wounds will be cleansed thoroughly.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The risk assessment that is attached has been reviewed by three members of staff with expertise in genetic manipulation and revised in the light of comments that were made on it. The revised risk assessment was then approved by all three original reviewers and signed off by, the University Biological Safety Officer.

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## Project Ref 187/21.1

**Date Ackn’d**: 28/05/2021

**CU2 Project Title**: Transduction (gain-or loss of function) of primary mouse epidermal keratinocytes and skin stem cells using overexpression and knock-down shRNAs/ORF lentiviral particles

**Class**: Class 2

**CultureVolClass2**: ≤ 1 Litre

**Non-GMM Consent Granted**: Consent Granted

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

## Project Additional Information

**Purposes of the contained use**

Our research group at NTU focuses on the investigation of molecular regulators in skin during development, wound healing, cancer and ageing. We will isolate and characterise mouse skin stem cells (SCs) after gain-or-loss of function of molecular regulators. We hope to enhance our understanding by using shRNA lentiviral particles to either knockout or overexpress our genes and non-coding RNAs (e.g. Long non-coding RNA/microRNAs) of interest in mouse cells (primary mouse epidermal keratinocytes) and FACs-sorted skin-SCs. Our lab has past experience of using lentiviral particles gained at University of Bradford, UK and published in leading scientific...

The protocol will involve isolation of mouse skin cells (primary epidermal keratinocytes) and skin stem cells via Mo-Flo-cell sorter (with the assistance of specialist technician Dr. Stephen Reed, John van Geest Cancer Research Centre, at NTU) and cells will be grown on culture dishes coated with either Matrigel or non-hazardous mouse fibroblasts cells (such as NIH/3T3, ATCC® CRL-1658™) as feeder cells and SCs grown in suitable medium for 2-4 days followed by transduction with viral particles.

Viral particles will be created using the Lentiviral Packaging Kits (Insight Biotechnology, UK; TR30037). The lentiviral transduction particles produced by this kit are replication incompetent. These viral particles will be used to either knock-down, using shRNA, and/or overexpress using ORF (open reading frame) of mouse genes such as Elf5 (for 24-72hrs). This will be followed by either silver or Rhodamine B staining of cells and quantification/analysis for colony forming ability (CF units) and/or, RNA and protein extraction for qPCR and western blot analysis.

Recipient or parental organism

The resulting GMOs (e.g. mouse Elf5 clones) cannot survive outside the laboratory regulated conditions, and are unable to colonise humans, animals or plant cells, whereas the inserted sequence is highly unlikely to be transferred to other organisms. The hazard group of primary cells in vitro can be regarded as hazard group 1 due to the extensive and continuous SPF testing of the source animals and minimal infectious potential of the transfected cells.

The hazard group of transfected HEK293/293T in vitro can be regarded as hazard group 2 that can be pathogenic to humans, specifically at-risk populations such as neonates and immunocompromised individuals. They are disabled/non-colonising, which cannot survive outside the laboratory.

Host/vector system

Hosts

HEK 293/HEK 293T (ATCC® CRL-1573/ATCC® CRL-11268) cell lines are sourced from human embryonic kidney and are epithelial in origin. HEK293/HEK293T are well characterised cells which cannot survive outside the laboratory and are recognised as 'disabled or non-colonising' hosts for genetic manipulation. Accidental injection of HEK293/HEK293T cells in humans will result in their destruction by the host immune-system, unless the host is severely immune-compromised.

Primary mouse epidermal keratinocytes and mouse skin stem cells are not considered hazardous. They will be isolated from specific pathogen free (SPF) animals (mice), which are screened for adenovirus and lesions associated with Herpes Simplex and Vaccinia. As these mice will be acquired from recognised mouses providers (Charles River or Envigo) they will arrive with SPF statement reports. Keratinocytes or skin-SCs are not capable of infecting human cells as a GMO; they are non-colonising and non-pathogenic to humans, animals or plants.

Vectors

We plan to use multiple gene versions of Lenti ORF/shRNA clone plasmids (knock-down, KD or overexpression), which will be sourced from a UK supplier (Insight Biotechnology Ltd, or Cambridge Biosciences, UK). For example, these lenti ORF clone’s expression plasmids (such as, Insight Biotechnology, UK; gene ID: mouse Elf5, cat number: MR203230L4) will be used in combination with the Lentiviral Packaging Kits (Insight Biotechnology, UK; TR30037) to create our ‘in house’ lentiviral particles. Elf5 role has been identified in other epithelial cells (e.g. mammary gland, kidneys) as a regulator of stem/progenitor cells for healthy development (Chakrabarti et al., 2012, Grassmeyer et al., 2017), while no role of Elf5 has been identified in skin cells. This procedure should not have risks related to the gene knock-out or overexpression to humans.

Transcriptional activation of these lenti-shRNA/ORF plasmids leads to the binding of the DNA sequences containing the consensus nucleotide core sequence GGA[AT], resulting in TK promoter activation, which drives expression of downstream gene clone insert (~762 bp). The contents of the Lentiviral Packaging Kit i.e. Packaging Plasmids, (Insight Biotechnology, UK; TR30037) are non-hazardous and cannot survive outside the laboratory environment. *Of note, the lentiviral transduction particles produced by this kit are also replication incompetent.

The structural and replication genes necessary to produce viral particles are separated onto multiple plasmids for enhanced safety. In addition, the third-generation lentiviral vectors described here are considered to be replication-incompetent and self-inactivating vectors, due to the number of essential genes that have been deleted from the third-generation lentiviral packaging system (Gándara et al., 2018).

A feature of a self-inactivating 3' long terminal repeat (SIN/LTR) renders the resulting lentiviral particles replication incompetent. Lentiviral particles are packaged in producer 293/293T cells and, upon co-transfection of the plasmids, all required sequences are available to produce and package a viral particle containing the transgene of
interest. Only the region between the viral LTRs of the transfer vector is packaged within the viral capsid. In the event recombinants arise, mutations or deletions can be configured within the undesired gene products to render any possible recombinants non-functional (Danos and Mulligan, 1988; Markowitz et al., 1990). In addition, deletion of the 3’ LTR on both packaging constructs further reduces the ability to form functional recombinants.

Despite the above safety features, use of HIV-based vectors falls within NIH Biosafety Level 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. Indeed, the company recommends treating these products with Biosafety Level 2 tissue culture facilities, which we will do.

References:

Origin & function

The shRNAs/ORF clones (knock-down, KD or overexpression) constructs which will be inserted are designed for gene silencing and/or expression of the ORF gene with one or more epitope tags or with a fluorescent marker, which are replication-incompetent. Although it is possible that the integrated DNA could be transferred by recombination with hidden viruses.

HEK293/293T cells will need to be cultured in Biosafety Level 2 tissue culture facilities as they contain adenovirus 5 DNA (Ad5) at the left end of their DNA sequence. The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta¬1 subunit and the vitronectin receptor alpha¬v subunit. The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment from nucleotides 1 to 4344 is integrated into chromosome 19 (19q13.2). However, this cell line is not known to cause disease in healthy adult humans.

The primary mouse keratinocytes and skin-SCs will be isolated from tested SPF animals, therefore free from pathogens.

Evaluation of foreseeable effects

The resulting GMOs (e.g. mouse Elf5 clones) cannot survive outside the laboratory regulated conditions, and are unable to colonise humans, animals or plant cells, whereas the inserted sequence is highly unlikely to be transferred to other organisms. The hazard group of primary cells in vitro can be regarded as hazard group 1 due to the extensive and continuous SPF testing of the source animals and minimal infectious potential of the transfected cells. The hazard group of transfected HEK293/293T in vitro can be regarded as hazard group 2 that can be pathogenic to humans, specifically at-risk populations such as neonates and immunocompromised individuals. They are disabled/non-colonising, which cannot survive outside the laboratory.

Elf5-KD or overexpressing cells will not survive outside the controlled laboratory environment. It is also not possible for them to survive in the human body, as they will be destroyed by the host immune system and also the lentiviral transduction particles produced are also replication incompetent and therefore, Elf5-KD or overexpression is not hazardous to cells. Elf5 overexpression has no known effect on mice viability and health, while Elf5-knockout studies have shown no known effects on skin cells in mice (Choi et al., 2008, Grassmeyer et al., 2017), therefore the procedure should not have risks related to the gene knock-out or overexpression.

References:

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

It is highly unlikely that the resulting GMO will be released in the environment but in this scenario, it will not survive outside the regulated laboratory conditions. Standard practice for handling genetically modified organisms is a further step in delimiting the GMO within the laboratory. Cells will be grown according to the Training Programme For Tissue Culture Activities provided by the University and using the guide for Good Manufacturing Practice of lentiviral particles (Gándara et al., 2018). All staff involved in this project have already received formal training and induction in cell and tissue culture activities and good laboratory practice by the lead lab PI (Dr Mohammed Ahmed) and the research technicians working in the biosciences department. This involved practice of cell culture (work in sterile conditions, handling of cells and tissue culture materials, cell passaging and freezing/storage), use and maintenance of the tissue culture facility and equipment (centrifuges, laminar flow cabinets, incubators).

Immortalised and primary wild type cells, lentiviral particles and gene specific overexpression and KD clones will be cultured in a Class 2 biological safety laminar flow cabinet using level 2 precautions, in a laboratory containment level 2 room (Biomed 010), which is located in a department with restricted access (by pin door code). Only authorised user will have access to the code and room. Cell lines will be grown as 10X25ml flasks maximum.

Users will be trained and experienced in proper pipetting techniques, pipetting down the side of the tube with no forceful expulsion, to reduce aerosol generation. For centrifugation, closed, screw-capped tubes and centrifuge cups with aerosol resistant lids containing O-rings will be used to contain aerosols. Any pipetting will be performed with filter tips, to minimise contamination of instruments, and only plastic pipettes will be used to minimise sharps risk. Good laboratory practice will include lab coat, eye-ware (safety glasses or Face shield), waterproof gloves (nitrile rubber, butyl rubber); designated tissue culture lab coats. Use of double gloves when working with lentiviral particles will reduce the risk of user contamination. All personnel accessing the containment level 2 tissue culture room (where human cells are also cultured) will have been immunised against Hepatitis B and will also have had titres checked to confirm that they responded to this immunisation.

Functional molecular analyses will follow multiple passages of cells transduced with lentiviruses, while cell extracts (DNA, RNA, protein) will use reagents that inactivate viruses (phenol, dithiothreitol). Extensive hand washing will be enforced after working with viruses. Log of personnel involved in virus production and usage, with all information related to the specific procedures and dates will be kept in the lab.

For workplace and spillage decontamination, both 5% CHEMGENE HLD4L disinfectant and 70% ethanol will be used. Either 5% CHEMGENE HLD4L or 10% bleach will be applied before disposal for at least 30 minutes for inactivation of any virus-containing media cultures, pipettes, tips and culture dishes. Laminar flow cabinet will be further treated with UV for 1 hour after completion of virus-related work. All disposable plastic-ware and solid biological wastes will be disposed into bins for autoclaving (lined with an autoclave bag) and autoclaved according to standard operating procedures. Liquid waste will be inactivated using CHEMGENE HLD4L (at a final concentration of 5% v/v) and allowed to disinfect for 24 hours. The same spillage measures will be applied during any live cell imaging or analysis (e.g. with a microscope or other device). If a puncture wound was to occur, medical attention will be provided by first aid trained staff and wounds will be cleansed thoroughly.

References:


Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The risk assessment that is attached has been reviewed by three members of staff with expertise in genetic manipulation and revised in the light of comments that were made on it. The revised risk assessment was then approved by all three original reviewers and signed off by Dr Matthew Smith, the University Biological Safety Officer and chair of the GM Safety Committee.

Please enter comments on the GM safety committee on the risk assessment

Tick to confirm that you have attached a risk assessment to this form: Y

Tick if you are claiming exemption from disclosure for section of the risk assessment: N

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Laboratory Activities

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CU2 Project Title

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<td>Elucidating the biology of diabetes and metabolism using viral-mediated modulation of gene expression</td>
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Date Project Ceased

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Project notified under transitional arrangements: N

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

The Interdisciplinary Science and Technology Centre (ISTEC) hosts researchers investigating adverse health related complications arising due to diabetes and metabolic disturbances. The key aims of this work are to determine the impact of modulating specific signalling pathways upon gene expression and cellular activity: factors that may underlie metabolic disease development and progression. The project leader Prof. Mark Christian’s research focuses on investigating key aspects of the biology of brown and white adipocytes. Brown adipocytes serve a unique function to "burn" fat rather than store it, as takes place in white adipocytes. By determining the key gene expression differences between these cell types gives new potential targets to increase energy consumption and promote weight loss. A key property of adipocytes is the storage of fat in lipid droplets. As these organelles are present in all cell types, albeit smaller than in adipocytes he is studying how lipid droplets are regulated in additional cell types including embryonic stem cells. Metabolic disease also causes deleterious pathologies that lead to the manifestation of a number of physical afflictions that impact the quality of life for the individual. These include chronic pain (Dr Hulse) and muscle atrophy (Dr Doig), degenerative conditions that to date lack effective therapeutic intervention. Deficiencies in metabolic capabilities of these tissues result in a decrease in physical attributes through loss of sensory perception of the surroundings and pain, as well as loss in muscle coordination and strength.

The program of work comes under these key aims: 1. Investigating the gene expression patterns during cellular differentiation and adaptation in adipocytes, muscle and neurons. 2. Evaluating signalling pathways that control cellular function in adipocytes, muscle and neurons. 3. Investigating the mechanisms that control lipid droplet dynamics in adipocytes and other cell types. Manipulations with viruses outlined below will be used to generate stable cell lines, over-express genes of interest (including GPR120, CIDEA) and knockout of genes expressing using Crispr/Cas9 gene editing technology. Lentiviral particles will be generated in-house (Virapower expression system) or purchased pre-made.

Recipient or parental organism

Cells that have been transduced with viral vectors which have then been allowed to integrate and the transduction medium changed are assigned to Class 1. The cell lines used are not viable outside the laboratory. Animals are SPF certified by the established provider. Additionally, animals are kept in appropriate housing cages and animal welfare is regularly monitored on campus to ensure animal health. Physical barriers are in place in the animal units such as appropriate caging, door barriers to prevent potential animal movement around the unit and/or outside the facility.

There are no recognised hazards associated with GMOs constructed using the above described DNA inserts. The resulting genetic modifications are maintained in the host strain under the selective pressure. Transference of the antibiotic genes to other organisms is highly unlikely.

Host/vector system

Hosts

Cell culture will be undertaken of cell lines confidently assigned to Hazard Group 1 being: human transformed embryonic kidney cell line (HEK-293), mammary gland cancer (MCF7), human adenocarcinoma cell line (HeLa), transformed primate kidney fibroblast cell line (COS-7), mouse adipocyte cell lines (e.g. 3T3-L1), mouse embryonic stem cell line (NIH3T3), rat adrenal gland pheochromocytoma (PC-12), murine muscle cell line (C2C12), human cardiac and muscle cell line (e.g. AC16, HL1, LCHN2), mouse primary cultures and mouse explants previously generated in-house including IMBAT and IMWAT pre-adipocytes. Primary cell culture (e.g. sensory neurons, cardiac myocytes) will be performed from rodents sourced from UK supplier.

Experimentation involving rodents will be performed upon Home Office authority, inline with the Animals (Scientific Procedures) Act 1986 legislation and approved Project License. Work will be performed in the designated establishment animal unit as setout in the Project license.

Lentiviral vectors are assigned as Class 2 GMMs, according to our current HSE notification. A system such as the Virapower Lentiviral Expression System will be used. The pLenti expression vector contains a deletion in the 3’ LTR (U3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome. The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev). A subclone of HEK-293 (HEK293FT, ThermoFisher Scientific) that allows Lentivirus generation is assigned to CL2. We will generate stable cell lines (pre-adipocyte and myocyte) by transduction with ecotropic
retroviruses for transducing the SV40 Large T antigen. These retroviruses are not capable of infecting human cells and will be used to infect rodent cells. Thus, these viruses are safe for users.

E.coli Strains including K12, XL-1 blue, DH5a, TOP10, BL21, Stbl3. The host organism (E. coli strains listed above) is non-colonising and disabled and can be considered as equivalent to ACDP biological agents hazard group 1. Manipulation of plasmids in E.coli is a Class 1 GM contained use.

Vectors
pLP1: this plasmid contains an ampicillin resistance cassettes and a CMV promoter. This construct is a lentiviral packaging plasmid containing the HIV-1 gag and pol genes. It is non mobilizable and it is assigned to Hazard Group 1.

pLP2: this plasmid contains an ampicillin resistance cassettes and a RSV enhancer/promoter. This construct is a lentiviral packaging plasmid containing the HIV-rev gene. It is non mobilizable and it is assigned to Hazard Group 1.

pLP/VSIG: this plasmid contains an ampicillin resistance cassettes and a CMV promoter. This construct is a lentiviral packaging plasmid for expression of the vesicular stomatitis G glycoprotein. It is non mobilizable and it is assigned to Hazard Group 1.

pLenti6/V5: this plasmid (V49610 – Thermo Fisher) is for lentiviral based expression of a target gene in dividing and non-dividing mammalian cells. The vector has the CMV promoter for driving constitutive expression of the target gene and the blasticidin selection marker for stable selection in mammalian cells. C terminal V5 tag for quick detection. The plasmid is non mobilizable and it is assigned to Hazard Group 1.

Retrovirus:
pLNCX2, pLHCX, pLPCX does not contain the structural genes (gag, pol, and env) necessary for particle formation and replication. Vectors are mobilisation defective, all obtained from Parmjit S Jat (UCL).These plasmid allows the expression of the transcript for Ψ+ extended viral packaging signal and is selectable due to hygromycin resistance gene. This construct produces replication-incompetent virus.

Adeno-associated virus (AAV) is a non-enveloped, single stranded DNA viral vector that has no known links to human illness. It can infect both non-dividing and dividing cells and AAVs are thought to be non-pathogenic to humans as they lack the requisite helper virus to replicate. Under special conditions, AAVs can mobilise but this requires coinfection with a helper virus (e.g. Adenovirus or Herpes simplex virus). These will utilise promoters for constitutive expression including cell specific expression (e.g. synapsin, GFAP, CMV). Most activities with AAVs are low hazard and can take place safely at Containment Level 1.5. Grassmeyer J, Mukherjee M, deRiso J, Hettinger C, Bailey M, Sinha S, et al. Elf5 is a principal cell lineage specific transcription factor in the kidney that contributes to Aqp2 and Avpr2 gene expression. Developmental biology 2017;424(1):77-89.

The genes inserted into plasmids are non-pathogenic and encode transcriptional regulators (of the nuclear receptor superfamily including PPARs, ERRs, ER), transcripational co-regulators (including RIP140, PGC-1a), lipid droplet-associated proteins (CIDEA, CIDEB, CIDEc), signal transduction pathway regulators (RGS7), G protein-coupled receptors (e.g. GPR120), shRNA targeting RGS7 or other genes of interest will be used to knock-down gene expression. These genes, involved in cell signaling and lipid droplet biology, are not predicted to be harmful if over-expressed or if knocked down (e.g. by loss of tumour suppression).

Cas9 or similar emerging systems utilised for Crispr-mediated gene-editing utilising Cas9 protein and a guide RNA to modify gene expression/function. These are utilised with the delivery vectors as outlined. Cas9 protein has demonstrated no known toxicity whilst guide RNA function requires presence of Cas9 protein for function. The Cas9 gene has potential to cause off target effects. These will be minimised by co-expression of single guide RNA strands and the work will be assigned to Hazard Group 2.

Cellular actuators (e.g. Designer Receptors Exclusively Activated by Designer Drugs (DREADD), optogenetics) will allow specific targeted control of cellular activity. These are typically delivered via AAV vector and require exogenous activation via methods including administration of pharmacological agent (e.g. clonzine), eGFP, mCherry and similar utilised as fluorescent markers and gene fusions. These are going to be used to visualise the protein by fluorescence microscopy or to perform
The SV40 T antigen is an immortalising gene and has the potential to extend the proliferative life span of normal somatic cells. This risk is mitigated by using ecotopic retroviral vectors that cannot infect human cells.

### Evaluation of foreseeable effects

Cells that have been transduced with viral vectors which have then been allowed to integrate and the transduction medium changed are assigned to Class 1. The cell lines used are not viable outside the laboratory. Animals are SPF certified by the established provider. Additionally, animals are kept in appropriate housing cages and animal welfare is regularly monitored on campus to ensure animal health. Physical barriers are in place in the animal units such as appropriate caging, door barriers to prevent potential animal movement around the unit and/or outside the facility.

There are no recognised hazards associated with GMOs constructed using the above described DNA inserts. The resulting genetic modifications are maintained in the host strain under the selective pressure. Transference of the antibiotic genes to other organisms is highly unlikely.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not applicable**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**None**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All proposed research activity is outlined to be performed in ISTEC 135/139 and the Biological Support Facility. The containment level 2 laboratories and risk assessment will ensure that the GMOs will not be released into the environment. This will include training of personnel in the use of Containment level 2 facilities, use of safety hoods aseptic techniques, the avoidance of aerosol production, laboratory coats to be worn within containment level 2 laboratories, maintenance of negative air pressure within the laboratory, the disinfection of working surfaces and equipment (for example, centrifuges), and handling and disposal (e.g. autoclaving) of all the waste will be within the laboratory suite.

Waste will be decontaminated immediately or separated by folding over the top of autoclave bags at the end of the session. Solid waste inactivation will be ensured by collecting it in a separate autoclave bag, which will be sealed at the end of each session and immediately autoclaved at 121°C for 20 minutes. Liquid waste will be collected inside the hood in a disposable plastic container and treated with 1% Virkon or 5% Chemgene before disposal down the drains. Any spillage will be wiped with a paper towel containing 2% Virkon or 5% Chemgene. Dispose towels in autoclave bag, wipe contaminated area with ready-use trigger sprays of Chemgene HLD4L, which is effective against retroviruses. Appropriate handling of waste materials and consumables including plastics tips, cell culture flasks will be outlined such as handling of sharps through immediate disposal into designated sharp bins, no needle resheathing.

Environmental impact will be minimal due to containment in the laboratory and decontamination of any waste. Furthermore as this lentivirus is attenuated, it cannot propagate. For the work with E.coli, the genes investigated do not enhance the ability of the E.coli strain to survive or disseminate in the environment or to compete with other bacteria. The genes to be investigated show no pathogenicity towards plants or animal.

All the staff involved in the work will receive a CL2 facility induction followed by procedural training given by the designated trained member. Training records will be retained. Personnel are asked to update the PI if there is a change in their immunosuppression status. Work will be halted and alternative arrangements made if a lab worker is immunocompromised. All personnel will wear appropriate Personal Protective Equipment (PPE) (waterproof aprons, gloves etc) which will be provided and must be worn dealing with a spillage. Under normal lab conditions staff and students will wear lab coats, wear gloves and protective glasses. Hair should be tied back and no open toed shoes allowed. Also all staff and students should wash their hands as they leave the CL2 lab to maintain safety and hygiene.

Cell lines will be handled in lab 135 or 139 (ISTeC) using a class 2 biological safety cabinet, with all waste being treated as detailed above. A separate incubator will be dedicated for cells with virus particles in the supernatant. Virus particles are expressed from 5x10^6 HEK 293T cells in 10ml medium at maximum. All are harvested and either stored or used immediately to transduce host cells. For long term storage at -80°C, virus particles will be stored in screw cap cryovials in a box that is subsequently sealed in a plastic bag to contain the virus in case of freezer failure, storage will be in a dedicated drawer of the -80°C used for Class 2 work only. The autoclaving of all waste material will be carried out within the lab 135 and 139 area (ISTeC). In the unlikely event of escape, the modified GMOs are not likely to be
maintained outside of the laboratory (due to the lack of positive selection), in any event, do not represent a greater hazard than the wild type organism to either humans, animals and plants. Implementation of the above safety measures should control the risk of release/exposure to effectively zero.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

The risk assessment that is attached has been reviewed by three members of staff with expertise in genetic manipulation and revised in the light of comments that were made on it. The revised risk assessment was then approved by all three original reviewers and signed off by Dr Matthew Smith, the University Biological Safety Officer and chair of the GM Safety Committee.

Project Containment

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<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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Animal Units

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<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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Project Ref 788/01.1

Date Ackn'd 04/07/2001  

CU2 Project Title EXPRESSION OF HUMAN TISSUE TRANSGLUTAMINASE IN MAMMALIAN CELLS  

Class 2  

Consent Granted not applicable  

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 litre  

Non-GMM  

Project notified under transitional arrangements
### Project Additional Information

**Purposes of the contained use**

To elucidate the proposed function of tissue transglutaminase (tTG) in the binding to and stabilisation of the extra cellular matrix.

**Recipient or parental organism**

mammalian cell lines including tumour cell lines of murine and human origin.

**Host/vector system**

For expression of tTG in mammalian cells vector systems will include mammalian expression vectors such as pSG5 under which the enzyme is constitutively expressed. For more controlled expression of the enzyme vectors using the tetracycline regulating promoter will be used. The expression vectors will be produced and purified using disabled E. coli eg. JM109.

**Origin & function**

Cell lines and genetic material is available at The Nottingham Trent University, other vectors will be purchased by from commercial sources. Transfected named cell lines and tumour cells will be observed for any phenotype changes especially those in the extra cellular matrix. Tumour cells are often deficient in tissue transglutaminase. Replacing this deficiency by transfection of the enzyme into the cells may have a number of affects on the malignant phenotype. Tumour cells transfected with tissue transglutaminase will be tested for any effects on tumour growth and progression in murine animal models.

**Evaluation of foreseeable effects**

The E.coli hosts to be used are well established disabled bacteria that are unable to colonise the tumour gut. Cultural transfected mammalian cells will not survive outside the tissue culture environment.

Human tumour cells and those transfected with tissue transglutaminase do have the possibility of surviving in the human body if immunologically compatible but only when injected in large quantities eg. $10^6$ (The six should be superscript) cell quantities.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Disposal of infected material will be supervised by staff specifically trained in the requisite techniques.

As Previous - Virkon and Autoclave (Copy from scripts).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

For studies involving the transfection of murine cell lines including murine tumour cell lines these should be undertaken under containment level 1.

For studies involving the transfection of human cell lines including tumour cell lines these should be undertaken under containment level 2.

Using these conditions the committee felt there was no risk to the environment and the risk to human health was minimal.

Project Containment

Laboratory Activities Glass Houses Growth Rooms

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Animal Units Large Scale Activities Human Clinical Applications

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Project Ref 788/01.2

Date Ackn’d 04/07/2001 CU2 Project Title IMMUNO-GENE THERAPY OF CANCER

Date Project Ceased 30/05/2007

Class CultureVolClass2 CultureVolumeClass3-4

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Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**
To enhance anti-tumour immunity and develop strategies for developing cancer vaccines.

**Recipient or parental organism**
Mammalian cell lines, of murine and human origin.

**Host/vector system**
Viral vectors, including disabled adeno and Herpes expression vectors.

**Origin & function**
The vectors have been obtained from commercial sources (either under material transfer agreements or purchased from suppliers). Genes from either antigens (including mini gene sequences encoding immunogenic peptides) and cytokine/immune response genes are incorporated into the vector. Appropriate cell infection leads to gene expression.

**Evaluation of foreseeable effects**
Expression of genes in appropriate cell systems. Viruses are disabled and therefore the progeny are non-infectious. An extensive literature exists on the safety of disables HSV and Adeno viruses. Experiments are designed to evaluate the ability of gene products to activate adaptive and innate immunity to tumours. Expression of genes in the human body is possible, but viruses have limited replicative capacity, and gene products would have a transient effect on the immune system.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
In vivo: use of vectors will be restricted to the appropriate containment facilities (containment 2), and all experiments will conform to Home Office guidelines for the use of animals.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
All waste material will be either inactivated with disinfectant and autoclaving, and animal waste will be prepared for incineration according to appropriate codes of conduct (approved by NTU safety services and the Home Office).
For studies involving the transfection of murine cell lines including murine tumour cell lines these should be undertaken under containment level 1. For studies involving the transfection of human cell lines including tumour cell lines these should be undertaken under containment level 2. Using these conditions the committee felt there was no risk to the environment and the risk to human health was minimal.

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Name

BIBRA INTERNATIONAL LTD

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

WOODMANSTERNE ROAD

Town

CARSHALTON

District

SURREY

County

Postcode

SM5 4DS

Country

ENGLAND

Tel Number

020 8652 1000

Fax Number

020 8661 7029

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

CHANGED FROM TNO BIBRA INTERNATIONAL LTD ON 14/2/2003

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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- Bacteriology
- Parasitology
- Transgenic Birds
- Transgenic Animals
- Transgenic Fish
- Microbiology Research
- Gene Therapy
All material in solid form, plates, tubes etc. possibly contaminated with genetically manipulated organisms is autoclaved and then disposed of by commercial incineration. All liquid waste, maximum culture volume of 4 litres, is treated with chloros and then disposed of via the mains sewage.

The autoclave is regularly serviced and tested for performance. The efficiency of the deactivation is monitored by plating out liquid waste after treatment and by microscopic examination for the presence of bacteria.

<table>
<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 192

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Name

UNIVERSITY OF WESTMINSTER

Name 2

Department

SCHOOL OF BIOSCIENCES

Campus Estate or Research Centre

Building

Road Name

115 NEW CAVENDISH STREET

Town

LONDON

County

GREATER LONDON

Postcode

W1M 8JS

Country

ENGLAND

Tel Number

0207 911 5000

Fax Number

0207 911 5087

E-mail

HSE Division

LONDON

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

- Mycology
- Transgenic Invertebrates
- Transgenic Plants
- Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity
The maximum culture volume to be released at any one time is 500 ml (0.5 litre).

All waste containing GMMs is deactivated by autoclaving at 121 degrees C for 30 minutes. 15 minutes at this temperature is generally accepted as guaranteeing the sterility of autoclave contents but extra time is used at the University of Westminster for any biological waste to further ensure the efficacy of the decontamination process.

The autoclave is tested by the manufacturers (Astell) twice yearly using thermistors enclosed in the load and thermal print outs obtained of its performance. In addition, the autoclave is tested each day that it is used using Browne's tubes to ensure the correct operating temperature is reached. Also, control plates of culture media are incubated to test for their effective sterilisation and decontamination.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Name

UNIVERSITY OF DUNDEE

Name 2

NINEWELLS HOSPITAL

Department

NINEWELLS HOSPITAL AND MEDICAL SCHOOL

Campus Estate or Research Centre

Building

Road Name

District

Town

DUNDEE

County

PERTH AND KINROSS

Postcode

DD1 9SY

Country

SCOTLAND

Tel Number

01382 344104

Fax Number

01382 345501

E-mail

HSE Division

SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee
### Level 1 (GMMs)

### Level 2 (GMMs)

### Level 3 (GMMs)

### Level 4 (GMMs)

### Non-microbial

#### Other (please specify)

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research

- Virology
- Transgenic
- Animals
- Transgenic
- Fish
- Gene Therapy

- Mycology
- Transgenic
- Invertebrates
- Transgenic
- Plants
- Other (please specify below)

### Other(s)

#### For activities involving GMMs, describe the waste management measures which will apply to the activity

- Tick to confirm that you are attaching a summary of the risk assessment
- Tick if you are claiming exemption from disclosure for sections of the risk assessment

#### Please enter comments of the GM safety committee on the risk assessment
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form 

Tick to confirm that you have attached a risk assessment to this form 

Tick if you are claiming exemption from disclosure for section of the risk assessment 

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref  197/00.2

Date Ackn’d  02/08/2000

CU2 Project Title  IMMORTALISATION OF RAT AND HUMAN HEPATOCYTES

Class  Class 2

CultureVolClass2  Class 2

CultureVolumeClass3-4  not applicable

Non-GMM  Consent Granted

Tick if notifying a connected programme of work 

Withdrew  N

Historical Significant Changes

Historical Date of Additional Info
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 197/01.1

INVESTIGATION OF THE MOLECULAR BIOLOGY OF RESPIRATORY SYNCTITIAL VIRUS AND PARAINFLUENZA VIRUS TYPE 3 USING REVERSE GENETICS TECHNIQUES.

Historical Significant Changes

PROJECT CLOSED 2/4/07.
**Project Additional Information**

**Purposes of the contained use**

Human respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV-3) are human pathogens, classed in category 2. The planned modifications are not expected to increase the pathogenicity or fitness of these viruses, or to alter their host range. Therefore, the major hazard associated with this project is the hazard inherent to the recipient PIV-3 and RSV. Containment of these viruses and their mutant derivatives is intended to reduce their risks to human health to effectively zero.

**Recipient or parental organism**

RSV is a class 2 pathogen, it generally causes mild or moderately severe upper respiratory illness, however, it can cause life threatening pneumonia or bronchiolitis in children and chronic cardiac and pulmonary disease, immunocompromised patients and the elderly. PIV-3 is also a class 2 pathogen, it generally causes mild upper respiratory tract illness, but can cause serious lower respiratory tract disease in infants, the elderly, and patients with compromised immune systems. Both viruses are transmitted by direct contact with contaminated surfaces or by large droplet spread. These viruses are unstable and do not persist long in the environment. RSV and PIV-3 are highly prevalent in the human population. 100% of individuals aged 5 years or more have been exposed to them, and reinfection can occur. Both viruses are members of the family Paramyxoviridae, and have genomes consisting of a single strand of negative sense RNA, replicated via a positive-sense intermediate RNA.

**Host/vector system**

The technique of reverse genetics is used to introduce site-specific mutations into virus genomes and is a powerful tool for examining virus biology. In the case of the paramyxoviruses, which have a genome of negative-sense RNA, this involves 'rescuing' virus from DNA copies of virus elements. Reverse genetics systems have recently been established for a number of negative strand RNA viruses, including RSV and PIV-3 (Collins et al, 1995, PNAS USA, 92: 11563-67; Durbin et al, 1997, Virology, 235: 323-32). There are two basic formats of the system: one in which the complete virus genome is used and one using a defective virus genome (minigenome). In the situation in which the complete virus genome is used, plasmids are constructed which encode the complete virus genome RNA and virus polymerase proteins. The plasmids are transfected into cells and expressed by T7 RNA polymerase generated from a recombinant vaccinia virus (modified vaccinia virus Ankara). The virus genome RNA that is expressed is transcribed and replicated by the virus polymerase proteins. This results in synthesis of the full complement of virus proteins and progeny genomes. The genomes are packaged into virus particles and released into the supernatant. This virus is infectious and can be propagated indefinitely.

In the situation in which a defective minigenome is used, a plasmid is constructed which encodes an RNA containing virus cis-acting sequences flanking a reporter gene, or genes, such as chloramphenicol acetyltransferase, luciferase, and green fluorescent protein. The minigenome plasmid is transfected into cells, which are co-infected with homologous virus. The virus polymerase proteins transcribe and replicate the minigenome RNA, resulting in reporter gene expression, and progeny minigenomes are packaged into virus particles. Thus, a mixed population of wild type helper and defective minigenome viruses is produced. The defective minigenome virus can be propagated indefinitely provided wild-type virus is present as a helper.

**Origin & function**

Plasmids that express individual virus proteins, minigenomes, and the complete virus genome were constructed in the laboratories of Drs Peter Collins and Brian Murphy (National Institute of Allergy and Infectious Diseases, National Institutes of Health) using cDNA generated from infectious virus RNAs.

In this study, complete PIV-3 and RSV genomes will be modified by introduction of a reporter gene (or genes) into the virus genome. Reporter gene sequence will be inserted into the RSV or PIV-3 genome, either adjacent to the virus promoter, between virus genes, or within virus genes to create virus-reporter fusion proteins. The reporter genes to be used are commercially available (eg. chloramphenicol acetyltransferase, luciferase, and green fluorescent protein). The purpose of introducing a reporter gene is to allow rapid analysis of virus gene expression using assays for the reporter protein. In addition, because the reporter gene is not essential to virus growth potentially deleterious mutations can be introduced into its flanking cis-acting sequences and assessed.

The intended function of the genetic material in the study is to introduce site-directed mutations (substitutions, insertions, deletions) into the RSV and PIV-3 non-coding sequences and genes to carry out structure-function analysis of cis-acting sequences and virus proteins. Mutations in cis-acting sequences will initially be assessed using the minigenome system. Then selected mutations will be introduced into complete virus genomes to generate mutant infectious virus, as described above. The mutant
viruses will be analysed using standard virological techniques and assays that monitor reporter gene expression.

**Evaluation of foreseeable effects**

The reporter genes to be inserted into the PIV-3 and RSV genomes encode well-characterised proteins that do not interact with either the virus or the host cell e.g., chloramphenicol acetyltransferase, luciferase, and green fluorescent protein, therefore insertion of these genes is not expected to affect virus pathogenicity or host-range. The impact of inserting foreign sequence into the virus genome would be expected to have either no effect on virus biology, or to attenuate the virus due to the increased genome length. Indeed, chloramphenicol acetyltransferase and green fluorescent protein have individually been inserted into the RSV genome previously, and the resulting viruses were slightly attenuated compared to wild-type RSV (Bukreyev et al, 1996, J Virol., 70: 6634-41; Hallak et al, 2000, Virology, 271: 264-75). RSV and PIV-3 will also be modified by introduction of site-directed mutations in the virus genomes, either in cis-acting sequences or virus genes. These mutations are likely to either attenuate the virus, or to have no effect on virus replication. In summary, the modifications to be carried out on RSV and PIV-3 are unlikely to generate viruses with an increased risk to either human health, or the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid and liquid waste will be autoclaved at 121 degrees C for 20 minutes. This will result in effectively 100% kill. The autoclave to be used is validated annually by a Scottish Home and Health official using probes which are inserted into bottles or bags. Each autoclave run is monitored to ensure that the necessary temperature is maintained for the appropriate length of time (a printout showing this information is produced for each run cycle). Contaminated solid waste will be double bagged and placed in dedicated, heavy-duty, leak-proof containers. Liquid waste will be aspirated into Duran bottles (the aspiration system involves a 0.2uM filter between the catch bottle and the pump and a backup Duran bottle to collect any accidental overflow). Terminex will be added to the liquid waste in the Duran bottles to a concentration of 10% as a precautionary measure. Bottles will then be collected in designated leak-proof containers for autoclaving. The waste will be autoclaved promptly following removal from the category 2 facility. Once the waste has been autoclaved it will be disposed of with normal waste.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The Committee accepted information provided by Dr Fearns stating that the most likely mode of transmission of these viruses is by large droplets (unlikely to disperse a large distance) and direct contact. For this reason the Committee agreed that the laboratory, with the additional control measures specified by Dr Fearns, is adequate.

**Project Containment**

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

Lentiviral and oncoretroviral pathogens including human immunodeficiency virus types 1 and 2 (HIV), simian immunodeficiency virus (SIV) and human T cell leukaemia virus type 1 and 2 (HTLV), or their molecular clones will be grown in tissue culture. The virally infected cells will be used for biochemical, genetic and molecular analysis of viral replication. In particular, recombinant viruses will be produced carrying mutations or substitutions in specific genes (including rev, tat, nef and envelope). We will assess the impact of these mutations or substitutiosn on viral entry into cells, viral infectivity, and viral gene expression.

**Recipient or parental organism**

HIV-1, HIV-2, SIV, HTLV-2 and HTLV-1 are ACGM hazard group 3 pathogens that cause, or have the potential to cause, life threatening disease in man. Therefore the appropriate minimum level of containment for infectious replication competent HIV-1, HIV-2 and HTLV-1 is ACGM containment level 3. Principal routes of transmission for wild type and GM viruses will be inoculation by sharps, contact with open wounds and exposure to aerosols containing high concentrations of virus. The recombinant defective viruses will be equally or less pathogenic or "fit" than the parental virus. Retroviruses such as HIV and HTLV are fragile and do not survive for long periods in the environment.

**Host/vector system**
specific viral genes will be modified by incorporation of mutations, or by substitution of viral sequences with heterologous reporter genes. Mutant viral genes will be produced by site-directed mutagenesis using sub-genomic non-infectious viral clones or CDNAs (this type of manipulation is permitted under our existing containment level 1 projects). The site directed mutants (deletions, insertions and substitutions) will be transferred into the full length molecular clones of HIV, SIV or HTLV. For HIV-1, HIV-2, and SIV we will produce recombinant viruses carrying mutations involved in the viral replication cycle; for example, envelope, REV, Tat, Nef and the viral long terminal repeats (LTR). For HTLV-1 and HTLV-2 we will also generate mutations in genes involved in viral replication; including mutations in envelope, Rex, Tax and the LTRs. We will also replace the HIV-1 envelope sequences with those for HTLV-1 env. We will examine the replication kinetics and replicative capacity of the mutant proviral clones.

We will also employ lentiviral vector technology (Kafri et al. 1997. Nature Genet. 17:314-7, Kafri et al. 1999. J Virol. 73:576-84, Poescia et al. 1998. Nature Med. 4:354-357) to transduce "wild type" or mutant gene products involved in the viral replication cycle into cells in tissue culture. We will examine the ability of the transduced gene products to inhibit or stimulate replication of "wild type" infectious HIV or HTLVs in a trans-dominant manner. In particular, we will investigate the effect of viral and/or cellular genes involved in viral entry and replication: for example, we will employ the viral genes listed above, and cellular genes involved in viral entry such as CCR5, CXCR4, CD4, or other host genes involved in viral replication such as CRM1/Exportin.

We will insert the following reporter genes into the HIV, SIV or HTLV genomes: firefly luciferase (luc), green fluorescent protein (GFP), bacterial hygromycin resistance, and the gene for human alkaline phosphatase (HPAP). We will insert these reporter genes into viral sequences required for viral replication such as the nef or env open reading frames of HIV. Recombinant provirus encoding reporter genes in place of envelope will be complemented in trans using plasmid vectors expressing envelope. The virions produced in the envelope complementation system will be competent for a single round of infection. In contrast, reporter genes incorporated at sites such as nef will be competent for infection and replication but will not "fit" than the parental virus. The reporter genes will allow us to readily follow infection and dissemination of virus in culture by simple assay techniques.

Evaluation of foreseeable effects

HIV-1, HIV-2, SIV, HTLV-1 and HTLV-2 are ACGM hazard group 3 pathogens. Therefore the appropriate minimum level of containment for infectious replication competent HIV-1, HIV-2, SIV and HTLV is ACGM containment level 3. Cell lines and primary lymphocytes will be deliberately infected with HIV-1, HIV-2, SIV, HTLV-2 or HTLV-1. The uninfected cell lines may be considered as greatly attenuated microorganisms and present no apparent hazard to laboratory workers or the environment. However, for primary human cells the risk from endogenous agents is high, and most importantly for deliberately infected primary cells and cell lines the hazard is also high. Therefore, all work with deliberately infected cells will be performed at the containment level appropriate for the infectious agent, in this case containment level 3. Infectious proviral clones will be modified by incorporation of mutations within specific viral genes. These mutations will likely reduce the infectivity and pathogenicity of the virus. Some mutations may confer altered infectivity (altered tissue tropism and/or rate of replication) to particular recombinants, however these mutations will not affect the principal route(s) of virus transmission or viral host range. Thus, the recombinant mutant virus will be equally or less pathogenic than the parental virus.

Recombinant lentiviral gene-transfer vectors will be used to transduce cells with wild type and mutant genes that are involved in viral replication. The effect of the transduced genes on infection and replication of HIV-1, HIV-2, SIV, HTLV-1 and HTLV-2 will be assessed. Recombination may occur as a rare event between the lentivector and the infectious wild type virus strains used, however the viral genomes produced through recombination will likely be equally or less fit or pathogenic than the parental wild type virus. Therefore, the containment measures used for infectious replication competent HIV and HTLV strains are also appropriate for containment and use of lentiviral vectors for gene transfer. Importantly, we will substitute the envelope sequences of HIV-1 with those of HTLV-1. Substitution of HIV-1 envelope with env from HTLV-1 will result in expanded tissue tropism for the recombinant HIV and the ability to enter non-human cell lines (Landau 1991. J Virol. 65:162). However, substitution of envelope is unlikely to expand the host range of the recombinant HIV because there are multiple blocks to HIV-1 replication in many non-rimate cell lines. Moreover, transmission of HTLV-1 occurs mainly by cell-to-cell transfer and envelope is primarily responsible for this phenotype (Delamarre, 1997. J Virol. 71:259), consequently HTLV-1 pseudotyped HIV particles exhibit reduced infectivity (Landau 1991. J Virol. 65:162-9.). Therefore, inclusion of HTLV-1 envelope sequences will...
reduce the fitness and attenuate the pathogenicity of the recombinant HIV. The routes of viral infection will not be altered by incorporation of HTLV-1 envelope, and the pathogenicity of the recombinant virus will be equal or less than the parental virus. For the reasons specified, the appropriate containment level for this recombinant virus is ACGM containment level 3.

The reporter genes to be used in this study (luciferase, GFP, HPAP, and bacterial hygromycin resistance) are derived from organisms that are not pathogens of man and the genes have no serious deleterious or toxic effects. The reporter genes are not virulence factors and will not increase the pathogenicity of recombinant HIV, SIV or HTLV. Importantly, the heterologous sequences will disrupt essential viral gene functions by insertional inactivation, resulting in attenuation of the recombinant proviral clones (Deacon et al. 1995. Science. 270:988-91; Lasky et al. 1987. Cell. 50:975-85). Incorporation of reporter genes, or mutant viral genes, will render the recipient virus equally or less pathogenic than the parental virus; indicating that ACGM containment level 3 is appropriate for this work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
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For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

<table>
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<th>All containment measures for class 3 will be applied.</th>
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</table>

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Solid waste: All plastics, gloves, flasks, pipettes, tips etc. will be autoclaved before disposal. Immediately after use pipettes will be immersed in 10% Terminex II or Microsol3 (see below). Subsequently, pipettes and other solid waste will be placed in double autoclave bags, sealed with autoclave tape and placed inside robust plastic leak-proof containers. The waste will then be treated by steam sterilisation in an autoclave. Autoclaved solid waste will be identified as “clinical waste” and processed through the Ninewells Hospital route for disposal of clinical waste (heat treatment followed by landfill).</th>
</tr>
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<tbody>
<tr>
<td>Liquid waste: All liquid waste will be treated by addition of Terminex II or Microsol3 (to 10% Vol./Vol.). Terminex and Microsol3 are detergent based anti-viral agents and, at the recommended concentrations, are effective against HIV, HTLV-1 and HCV (expected degree of kill for these viruses is 99.99%). Tubes, flasks, and plastic bottles containing Terminex-treated media will then be placed in double autoclave bags sealed with autoclave tape, and/or placed in leak-proof plastic containers (as appropriate), and treated by steam sterilisation in an autoclave. Autoclaved liquid waste will be flushed down the sink with copious amounts of water.</td>
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Autoclave: Waste will be autoclaved at 134 degrees C for 5 minutes for solid waste, and 121 degrees C for 15 minutes for liquid waste. The procedure will inactivate 100% of all retroviral particles and cell associated virus. Monitoring of chamber temperature will be carried out with an internal probe during each run. The monitoring procedure will be validated quarterly (to HJM2010) and when a new probe is fitted.

Is an emergency plan required according to regulation 20? | N |

If yes, tick to confirm that it is attached to this form | N |

Tick to confirm that you have attached a risk assessment to this form | Y |

Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment

The Committee agreed that any genetically modified retrovirus created during this work would have broadly similar properties to 'wild type' retroviruses. For this reason Committee accepted the adequacy of the containment measures to be adopted.
THE USE OF ADENOVIRAL AND BACULOVIRAL VECTORS FOR EXPRESSION OF PROTEINS AND RNAi CONSTRUCTS TO MANIPULATE SIGNALLING IN MAMMALIAN CELLS.

The technique will permit the identification of the molecular mechanisms used by insulin to regulate gene expression. Each molecule identified will be assessed further for potential as a therapeutic target for type 2 diabetes.

Replication defective adenovirus and baculovirus will be used. These agents have been modified to prevent their expansion in the absence of helper cells. They cannot reproduce or indeed survive outwith the contained environment. As we will use them to express proteins whose complete function is not yet known the project becomes class 2 categorised.

Recipients or parental organism

Project Additional Information
Host/vector system

The host systems for propagation of the recombinant, replication defective adenovirus are 293 cells (E1 transformed embryonic kidney cells) and 911 cells (E1 transformed human embryonic retinal cells). Cultured mammalian cells are regarded as being unable to colonise the worker provided that their own or a close relatives cells are not cultured (compendium of guidance 2000). They may be considered to be ACDP group 1 for the purposes of the contained use regulations. Baculovirus is propagated within the insect cell lines Sf9. The recombinant, replication defective virus will be used to infect established mammalian cell lines in which the virus is unable to replicate such as: rat hepatoma cells, isolated rat and mouse hepatocytes or neurones.

Origin & function

The following genes will be/have been placed in the replication defective adenovirus vector (see below for details of tene function): AGC protein kinases (active and dominant negative, DN), mTOR (active and DN), MEKK (active/DN), GSK3 (active/DN), insulin-regulated transcription factors (FKHR, HNF3, SREBP1c, others as they become known), protein phosphatases (PP1, PP2A), glucokinase, small G-protein Ras (DN), PH domain-containing proteins. In addition, we will insert RNAi constructs to specifically target protein sequences for degradation. Dealing with these insert sequences is necessary to dissect out their physiological function and identify their role in disease.

Mouse, human, rat, rabbit, S.Pombe, Zenopus Leavis, E.coli, S.cerevisiae are in the sources of donated DNA.

Function of inserted genes:
AGC kinases      signalling molecules that modulate hormone sensing
Protein kinase B, S6K, PKC, PDK1, AMP-activated protein kinase

Other kinases
mTOR        signalling molecule that regulates protein translation
MEKK       signalling molecule that regulates gene transcription
GSK3       signalling molecule that regulates multiple actions of insulin

Insulin-regulated transcription factors
FKHR        target genes unknown
HNF3      mediates insulin antagonism of glucocorticoid induced transcription
SREBP1c    regulates expression of tene involved in fatty acid biosynthesis

Protein phosphatases
PP1, PP2A  antoganise the action of protein kinases

Other proteins
glucokinase - rate limiting step in glucose sensing
small G-protein Ras - regulates multiple actions of growth factors.
Other signalling molecules found in the future to play a role in the action of insulin and action.

PH domains
Plekstrin Homology domains are lipid binding modules that confer specific lipid regulation to a large number (over 100) of cellular proteins (including PKB, PDK1, TAPPs, DAPPS).

Evaluation of foreseeable effects

The AdEasy Vector system will be used: adenovirus Ad5 E1E3 or Ad5 E1E3E4 deleted(). The host-vector system to be used does not generate replication efficient viral particles. The system has been modified (E1 region deleted) so as to prevent replication in human cells. Genes will be inserted into the site of the disabling deletion. This safeguards against the generation of replication competent recombinant adenovirus by homologous recombination with wild type virus. The baculovirus is an insect virus.
that requires a very high titre to insert genetic material into mammalian cells. It cannot replicate in mammalian cells. Expression of genetic material which interferes with cell signalling function will not allow the deleted virus to replicate. The presence of the genes indicated in this application will not change the infectivity or enable replication of the viruses. The possible consequences of expression of the inserted genetic material in man (see above) are such that the classification of the recombinant virus should be group II.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Treatment with 70% ethanol has been shown to be an effective disinfectant for adenovirus, but we will also use an anti-viral aerosol (Mikrozid, S+M Rotherham) to clean work areas and equipment, and a viricidal disinfectant (Terminex II, Arrow, Derbyshire) to treat liquid waste.

Solid waste will be autoclaved (expected degree of kill 100%). It will be identified as clinical waste and processed through the Ninewells Hospital route for disposal of clinical waste (heat treatment followed by landfill). Liquid waste will be collected in the presence of the viricidal disinfectant Terminex II and will be autoclaved (expected degree of kill 100%) and then poured down the sink with copious amounts of water.

Monitoring the efficacy of the autoclave will involve completion of test sheets supplied by Scottish Healthcare Supplies (or an equivalent contractor). This requires keeping a record on the test sheet, for the first production cycle of the day, of the temperature inside the load, pressure and sterilisation time. A record of each production cycle will be kept. Weekly testing involves safety checks, vacuum leak rate test and test of automatic controls. Quarterly testing includes thermometric test for small and normal load and safety check, vacuum leak rate test, and test of automatic controls.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

At its meeting on 13 March 2002 Committee agreed that the facilities and procedures are adequate for the level of risk.

Project Containment

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

This project will focus on mechanisms underlying cardiac resistance to stress as well as lung development and function. Uncovering intracellular signalling pathways mediating some of critical functions of the heart and lungs, as it will be done here, may further increase our understanding of heart and lungs and it may lead to novel and more efficient strategies in therapy of cardiovascular and pulmonary diseases.

**Recipient or parental organism**

Cells and tissues in vitro and in vivo will be used as recipients. All these recipients are well characterised such as Vero/293, HBE, CFBE, CHO, BHK cell lines, cardiomyocytes, rodent lung and heart tissue and others. All cells/tissues that we might use are well established for these purposes and have been routinely used in many laboratories over many years.

**Host/vector system**

Only commercially available disabled and well characterised viral vectors with a history of safe use such as pADEasy-1 (Stratagene) will be used. These viral vector systems can only propagate in special packing cell lines: in normal cells they can not replicate. Inserts will be cloned into the site of disablement and recombinant events leading to replication competent viruses are extremely unlikely given their design. Introduction of genes of interest into these vectors is very unlikely to alter the survivability, tissue tropism/host range, infectivity or alter virus effects upon other organisms and potential route of transmission. These vectors have been in routine and safe use in many laboratories over many years.
Origin & function

Genes that will be inserted into viral vectors encode proteins involved in intracellular signalling in heart and lungs such as lactate dehydrogenase, creatine kinase, diadenosine tetraphosphate hydrolase and others. In addition, mutant forms of these proteins will be also used when dominant-negative strategy is required, i.e. when it is intended to block a specific signalling pathway. All these genes will be cloned into disabled commercially available viral vectors (as described above) and they have not been reported to cause immortalisation or transformation of cells. In theory, some of the constructs might alter cell determination and cell growth. However, it should be pointed out that similar constructs have been used in other laboratories without any harmful effects.

Evaluation of foreseeable effects

The viral vectors used may infect a wide range of mammalian cells. In the case of retroviral vectors they can integrate into the host cell genome and stable express the gene of interest. It is extremely unlikely that adenoviral vectors will integrate into the host cell genome so expression of the recombinant genes will only occur in cells initially infected. Eventually it is likely that recombinant genes will be lost through natural degradation of episomal DNA in infected cells.

All the genes we plan to clone into disabled viral vectors encode intracellular proteins involved in normal signal transduction/cell regulation processes in lungs and heart. None of the gene products is known to have any effect on the infectivity, pathogenicity or replication of viral-based expression systems and similar constructs have been used without harmful effects in numerous other laboratories. It is highly unlikely that they could have any effect on the host range or tropism of the virus, since they do not encode proteins that are cell receptors or extracellular matrix proteins etc. The viral receptors are so specific that different types/strains of viruses use different receptors so that possibility of affecting the tropism or infectivity through expression of any recombinant protein is extremely low.

Some of proteins being expressed have the potential to interfere with intracellular pathways, and hence alter cell determination and growth. However, with the following key controls the residual risk is negligible:

1. Replication defective viral vectors are used to ensure proliferation occurs only in special packaging cell lines.
2. Containment Level 2 is applied to prevent infection of the worker or release into the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste: autoclave then dispose of as controlled waste

Liquid waste: Add Terminex 10%, autoclave and then dispose of to drains.

Sharps waste: collect in an autoclavable sharps-safe container, autoclave then dispose of as clinical waste.

Reusable labware: autoclave if possible, otherwise disinfect with 1% Virkon overnight, rinse out to drains then place in normal wash-up stream. There must be no significant volume of liquid waste present, only trace contamination.

Chemically contaminated GM waste: disinfect with Virkon then treat as purely toxic chemical waste. There must be experimental evidence to support the efficacy of Virkon under the specific conditions of use.

Radioactive GM waste: Disinfect with Virkon then treat as purely radioactive waste. There must be experimental evidence to support the efficacy of Virkon under the specific conditions of use.
Committee reviewed this work on 12 May 2005 and agreed that facilities and procedures are appropriate.

Please enter comments on the GM safety committee on the risk assessment

Committee reviewed this work on 12 May 2005 and agreed that facilities and procedures are appropriate.

**Project Containment**

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**Project Ref** 197/07.1

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<th>Project notified under transitional arrangements</th>
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Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

To develop new therapies for clinically significant conditions.

**Recipient or parental organism**

A range of systems based upon well characterised micro-organisms given ethical approval for human use by Gene Therapy Advisory Committee will be used to deliver genetic material to patients. All these micro-organisms will be either disabled or attenuated variants of pathogens. They will include delivery systems derived from adenoviruses, poxviruses, Herpes Simplex Virus, and retroviruses. Other well characterised delivery systems may be used in future as this technology develops. There is no intention to develop new delivery systems under this notification.

**Host/vector system**

These delivery systems will be approved by Medicines and Healthcare Regulatory Authority for use in human patients. They will be manufactured using fully documented procedures with strict quality control measures.

**Origin & function**

At present the functions of genetic material used in gene therapy trials worldwide include: antigen, antisense, cytokines, deficiency, drug resistance, hormone, marker, oncogene regulator, receptor, replication inhibitor, ribozyme, cell suicide, and tumour suppression (Journal of Gene Medicine). It is likely that this technology will continue to be used to express genetic material with physiologically relevant functions.

**Evaluation of foreseeable effects**

Fully characterised disabled or attenuated micro-organisms (delivery system) will be used to introduce genetic material into human cells for therapeutic purposes. In all cases the GMO will be evaluated in a variety of experimental systems before it is introduced into patients.

Many of these delivery systems are derived from commonly occurring pathogens that large sections of the population will have been exposed to, and cleared from their system (e.g. adenovirus, HSV and vaccinia). Thus they will have pre-existing immunity. The genetic material will not restore pathogenicity of the delivery system or create a more virulent form, or alter its morphological properties (e.g. susceptibility to disinfectants, dessication or heat).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste heavily contaminated with GMM (e.g. syringe, vials, dressings) will be autoclaved (expected degree of kill 100%, autoclaved tested to HTM 2010 standard).

Other waste that could possibly be contaminated with GMM will be treated as Clinical Waste (Thermal treatment).

Standard laundry and cleaning regimes will be adopted for clinical areas.
After lengthy discussion and some minor modifications to the protocols the Committee agrees that the measures being taken are sufficient for the level of risk.

Please enter comments on the GM safety committee on the risk assessment

After lengthy discussion and some minor modifications to the protocols the Committee agrees that the measures being taken are sufficient for the level of risk.

Project Containment

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Project Ref 197/08.1

Date Ackn’d 19/03/2008

Project Title

Medical research involving the use of well characterised viral vectors.

Class 2

Culture Vol Class 2 1-50 Litres

Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Project Additional Information

#### Purposes of the contained use

To investigate at a molecular level biologically important pathways.

#### Recipient or parental organism

A variety of well characterised packaging cell lines that provide the genes deleted from viral vectors in trans either by way of the cells genome or by transfecting in complementing plasmids.

Well characterised and authenticated tissue culture cell lines.

Primary cells.

#### Host/vector system

A range of well characterised, commercially available systems as detailed in Section 2.6 to 2.12 SACGM Compendium of Guidance will be used as research tools. Most commonly, adenoviral, retroviral and lentiviral vector systems will be used.

This notification does not include research to develop new viral vector systems or to investigate virus life cycles.

#### Origin & function

Wild type and mutant forms of genes and genetic material that may have harmful effects (as detailed in Section 2.2 SACGM Compendium of Guidance) including known and putative oncogenes, growth factors, cytokines, non-coding regulatory elements, anti-sense constructs, and siRNA.

This notification does not include known TSE proteins.

This notification does not include expression of genes that are likely to alter properties of the viral vector such as its tissue tropism or host range, susceptibility to prophylaxis or survivability in the environment, nor are they likely to overcome disabling mutations.

#### Evaluation of foreseeable effects

Significant risks to human health arise during the generation of cell lines: once established the cell lines are unlikely to cause harm to human health or the environment.

1. **Risks arising from recombination/complementation events**

   The worst case scenario is that a replication competent virus expressing a harmful gene (e.g., oncogene) is generated either during manufacture of the infectious viral vector particle (i.e., between transfer vector and packaging cell line components) or through co-infection of a person with wild-type virus and viral vector. This scenario is unlikely given the number of recombinations events required.

   It is also possible that the transfer vector reverts to a replication competent virus. The level of risk depends upon the pathogenicity of the wild-type virus, and for high risk viruses this possibility is unlikely given the number of recombination events related to the packaging of the transfer vector.

2. **Risks arising from integration of viral vector into person's genome**

   It is possible that random insertion could lead to activation of cellular genes, disruption of important genes (e.g., tumour suppressor) or acquisition of a harmful gene (e.g., oncogene). The worst case is that permanent changes are induced in an infected cell resulting in tumourogenesis.

3. **Risks arising from inserted genetic material**

   Wild-type and mutant forms of genes and genetic material may have harmful effects (as detailed in Section 2.2 SACGM Compendium of Guidance) including known and putative oncogenes, growth factors, cytokines, non-coding regulatory elements, anti-sense constructs, and siRNA.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be autoclaved (expected degree of kill 100%, HTM 20 10 standard), and then placed in the low risk Clinical Waste stream ie heat treatment by landfill.

Liquid waste will be treated with 10% Terminex before autoclaved (expected degree of kill 100%, HTM 20 10 standard) and then put to drain.

Trace contamination reusable labware and equipment will be autoclaved if possible - if this not possible then it will be disinfected with 1% Virkon overnight before being rinsed to drain.

Any waste that cannot be autoclaved (e.g., chemical or radiological contamination) will be disinfected with 1% Virkon overnight before being disposed of as Special or Radiological Waste.

Project Containment

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Project Ref 197/95.1
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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Animal Units: L2, L3, L4

Large Scale Activities: L2, L3, L4

Human Clinical Applications: L2, L3, L4

**Project Ref** 197/99.1

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<th>Class</th>
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Project notified under transitional arrangements Y

Withdrawn  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID 197/05.2

Date of Significant Change 15/11/2005
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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02/03/2022
Project Ref: 197/99.2

Date Ackn’d: 25/05/1999

Date Project Ceased:

Class: Class 2

Culture VolClass: Class 2

Culture Volume Class: 3-4

Non-GMM: not applicable

Consent Granted:

Project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

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Animal Units

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**Name**

UNIVERSITY OF WEST OF SCOTLAND

**Name 2**


**Department**

BIOLOGICAL SCIENCES

**Building**


**Campus Estate or Research Centre**

PAISLEY CAMPUS C BLOCK

**Road Name**

HIGH STREET

**District**


**Town**

PAISLEY

**County**

RENFREWSHIRE

**Postcode**

PA1 2BE

**Country**

SCOTLAND

**Tel Number**

0141 848 3122

**Fax Number**

0141 848 3116

**E-mail**


**HSE Division**

SCOTLAND

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Tick if confidential

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial
Other (please specify)

Tick if confidential

Bacteriology
Parasitology
Transgenic Birds
Microbiology Research
Virology
Transgenic Animals
Transgenic Fish
Gene Therapy
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<th>Transgenic Invertebrates</th>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

---

### Project Ref 200/01.1

**Date Ackn'd** 09/11/2001

**CU2 Project Title** VIRULENCE MECHANISMS OF THE PLANT PATHOGEN ERWINIA EMYLOVORA

**Class** Class 2

**CultureVolClass2** < 1 litre

**Non-GMM Consent Granted** not applicable

**Date Project Ceased** 11/07/2008

**Withdrawn** [ ]

**Tick if notifying a connected programme of work** [ ]

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

### Project Additional Information

02/03/2022
### Purposes of the contained use

The work will involve mutating wildtype Erwinia amylovora strains using a suicide vector system. The mutations will be introduced to known virulence genes or likely virulence gene regulators, and will either insertionally inactivate the gene of interest or create a reporter fusion to monitor expression. Mutation of virulence genes will compromise pathogenicity, allowing the role of the virulence factor to be characterised. The reporter system will create merodiploid strains that posses both an unmutated copy of the gene and the reporter fusion (inactive) copy. The ability to monitor the expression of virulence/regulatory genes will help increase understanding of how transcription of such factors is modulated during the infection process.

### Recipient or parental organism

Erwinia amylovora is a gram-negative bacterium and a plant pathogen, causing the disease fireblight on a range of hosts including apple, pear and non-crop plants such as Pyracantha, Cotoneaster and Cratagus. E. amylovora poses no threat to human health being unable to grow at temperatures above 30 degrees C. Being a plant pathogen work on E. amylovora is covered by Plant Health licence No. GM/139/2001 (copy attached). The parent strain to be used for genetic manipulation is Erwinia amylovora strain CFBP1430 a French isolate. This strain has been used for some years and its virulence on apple seedlings has been extensively studied. CFBP 1430 is significantly less virulent than other wild-type strains in this system eg. The UK isolate strain OT1. Higher inoculum levels are required for induction of disease and symptoms are less severe.

### Host/vector system

The host strain used for introducing suicide vector systems into E. amylovora is the Escherichia coli K12 derivative SM10 pir. K12 derivatives are auxotrophic mutants and are non-colonising, disabled and unlikely to persist in the environment. SM10 pir is able to mobilise certain plasmids into host strains. The suicide vector systems use a series of mobilisable plasmid vectors containing the conditional oriR6K origin of replication, which can only replicate in host strains expressing the pir gene product. Mobilisation is directed from either an R2K or RP4 origin of transfer. These vectors are commonly used for mutagenesis of Gram-negative bacteria.

### Origin & function

The genetic material will be derived from E. amylovora. Genes will be isolated from either a cosmid library or by PCR amplification from E. amylovora chromosomal DNA. Genes of interest include known virulence genes such as members of the hrp gene cluster used to produce the Type III secretion system, the ams genes involved in biosynthesis of the polysaccharide amylovoran, and the dsp (disease specific) genes. In addition, certain transcriptional regulators postulated to be involved in regulation of virulence factor expression, may be used. These include the csrA gene involved in induction of carbon storage genes. The E. coli lacZ and GUS genes encoding B-galactosidase and B-glucuronidase respectively will be used to analyse transcriptional expression of E. amylovora genes. Both genes/gene products have been extensively characterised and are non-hazardous. These properties have led to their widespread use in cloning vectors and reporter systems.

Antibiotic resistance genes used for insertional inactivation and as markers on suicide vectors will be taken from commonly used E. coli plasmid/cloning cectors and will not encode resistance to antimicrobials in clinical use.

### Evaluation of foreseeable effects

Erwinia amylovora is unable to grow above 30 degrees C and so any GM strains represent no hazard to human health. Being a plant pathogen E. amylovora GM strains represent a potential hazard to host plants. However, for the development of disease symptoms extended periods of warm temperatures are required (above 25 degrees C). There is little likelihood of successful infection occurring in Scotland. Erwinia amylovora does not persist in the environment outside of the host plant and does not form spores. The proposed genetic modification will either result in insertional inactivation of known virulence factor/virulence regulator genes or in the generation of transcriptional reporter fusions.

Inactivation of genes will involve introducing a suicide vector, containing the virulence gene disrupted by the insertion of an antibiotic resistance gene cassette, into E. amylovora. The antibiotic resistance genes used will be against antibiotics not used clinically. As the suicide vector cannot replicate in E. amylovora it will insert onto the chromosome by recombining with homologous virulence gene sequence. A second cross-over event will delete the wild type copy of the gene and vector DNA sequence, leaving only the insertional inactivated gene on the chromosome. The resulting strain will be defective in the production of a virulence factor, which will reduce pathogenicity and thereby prevent infection of host plants. Insertional inactivation of regulatory genes may also compromise virulence. In addition, inactivation of regulatory genes such as csrA is likely to affect other physiological characteristics. In the case of csrA carbon storage will be affected, this will compromise the ability of the resulting
GMM to persist in the environment. As all vector DNA is removed during the second recombination event it is unlikely that any DNA possessing mobilisation function will persist in the GM strains. There is, therefore, minimal potential for GMM DNA to transmit to other organisms. Reporter fusion strains will possess virulence or regulator genes linked to the E. coli gene encoding either B-glucuronidase or B-galactosidase. The suicide vector used for these constructs will contain the promoter and part of the coding sequence of the gene of interest linked to the reporter gene. The resulting vector will be introduced by a single recombination event onto the E. amylovora chromosome. This will generate a merodiploid strain with both a wild type gene copy and a fragment of the gene of interest linked to the reporter. These strains are likely to retain virulence. The gene fragment linked to the reporter will produce a truncated, inactive protein. Both reporter genes have been fully characterised and are non-hazardous to humans and unlikely to influence the stability of the resulting strains in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
<thead>
<tr>
<th>Description</th>
<th>Application</th>
</tr>
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<tr>
<td>Solid waste will be treated by autoclaving at 121 degrees C, 15 psi for 15 minutes. Waste is subsequently incinerated. Autoclaves are serviced annually, are calibrated and have internal thermostats. To validate inactivation of GMM indicator strips are included in each run. Small volumes of waste are inactivated within the laboratory (C229). Larger volumes (contained within autoclave bags) are carried in leak-proof containers to be inactivated, immediately, in the larger autoclave (C325). Full instructions for correct inactivation of E. amylovora/GM waste are displayed in the laboratory and on both autoclaves. Large volumes of liquid waste, up to 100 ml, will be inactivated by autoclaving in the original vessels. Small volumes eg less than 10 mls of culture supernatant will be inactivated in 10% dettol. Infected plant material and soil will be autoclaved and plant growth trays will be immersed overnight in 10% dettol.</td>
<td></td>
</tr>
</tbody>
</table>

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |

Please enter comments on the GM safety committee on the risk assessment

Following a detailed presentation, the GMSC of the University of Paisley has approved this work to be carried out at Containment Level 2.

Project Containment

<table>
<thead>
<tr>
<th>Activity Type</th>
<th>Level 2</th>
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<tr>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

The work will involve the isolation of recombinant plasmid (pUK42) from microbial cultures and the stable/transient transfection of this into mammalian cells. Cells expressing SV40 oncogenes (potential immortalised cells) will be isolated and characterised. Clones with the desired phenotype will be used for further studies. The expression and of immunological proteins and the behaviour of the cells in in vitro immunology experiments will be investigated.

**Recipient or parental organism**

The recombinant plasmid will be grown up in the E. coli strain DH5α which is recognised as being non-colonising and disabled and is not considered pathogenic to humans or animals (Woodcock et al. Nucl. Acid Res. 17, 3469-3478, 1998). The primate and non-primate primary cultures and cell lines to be used are free of any deleterious virus and are very unlikely to be able to survive without specialised media. Primary cultures of cells need specialist media to grow and often establishing the cells in culture is very difficult. It is very unlikely that these cells will be able to survive out with the specialist culture media and facilities used.

**Host/vector system**

The recombinant plasmid pUK42 is based on the plasmid vector pAT153 and contains the SV40 large T and small T antigen sequences, an ampicillin resistance gene and a neomycin resistance gene (FitzGerald et al. in Vitro Cell Dev. Biol. 30, 236-242, 1993). The recombinant plasmid will be grown up in the E. coli strain DH5α which is recognised as being non-colonising and disabled and is not considered pathogenic to humans or animals (Woodcock et al. Nucl. Acid Res. 17, 3469-3478, 1998). The SV40 large T and small t antigen gene products are well documented as being oncogenic, albeit weakly when compared to transforming oncogenes such as ras and
In pUK42, the SV40 sequences are under control of the endogenous promoter which supports strong transcription in a variety of cell types. The SV40 origin of replication is not present in pUK42 and so no self-replicating virus can be generated.

Origin & function

pUK42 is a plasmid derived from the vector pAT153 and which contains sequences from the SV40 virus (genes for small t and large t antigens) whose products are known to be oncogenic. No genetic manipulation of pUK42 will take place in the work herein notified; rather, genes of the plasmid will be expressed in primate and non-primate cells. Selected clones of transformed cells will then be used in experiments to investigate the biology of certain cell types.

Evaluation of foreseeable effects

Neither neomycin nor ampicillin are used clinically and thus the use of genes whose products confer resistance to these antibiotics in this work should not pose any health risk.

The immortalisation of cells donated by workers will not be permitted so as to limit the possibilities of the immortalised cells colonising individuals.

SV40 antigens and neomycin resistance protein would not be expressed in bacterial cells, so bacterial cultures would not be a source of these proteins. Exposure to SV40 large T and/or small t antigen proteins could conceivably result in oncogenesis. The following procedures are to be used to minimise this risk:

* mammalian cells to be cultured in class II cell culture cabinets on a small scale only in a specialist facility with locked doors.
* gloves and lab coats would be worn at all times
* sharps not be used during this work
* cells to be disposed of by autoclaving following overnight soaking of all plastic ware in a bactericidal and viracidal solution.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be treated by autoclaving at 121 degrees C, 15psi for 15 minutes. Waste is subsequently incinerated. Autoclaves are serviced annually, are calibrated and have internal thermostats. To validate inactivation of GMM indicator strips are included in each run. Small volumes of waste are inactivated within the laboratory (C229). Large volumes (containing within autoclave bags) are carried in leak-proof containers to be inactivated, immediately, in the large autoclave (C325). Full instructions for correct inactivation of GM waste are displayed in the laboratory and on both autoclaves. Large volumes of liquid waste, up to 100ml, will be inactivated by autoclaving in the original vessels. Small volumes eg less than 10 mls of culture supernatant will be first inactivated in 10% dettol.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Following a detailed presentation, the GMSC of the University of Paisley has approved this work to be carried out at Containment Level 2.
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### Project Ref 200/15.1

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### Project Additional Information

**Purposes of the contained use**

To study:
- cellular response to herpesvirus infection (HSV-1, HSV-2, CMV, VZV, EBV, HHV-6, HHV-7, KSHV)
- cellular senescence and ageing
- cellular mechanisms of postherpetic neuralgia
- cellular mechanisms of arthritis
- cellular mechanisms of cancer progression

**Recipient or parental organism**
Various widely used and established human and mouse cell lines (e.g. HFF, U2OS, Hepatocytes, iPSC, hESC, human neurons, IMR90s, SW1353) will be obtained from reputable sources e.g. ATCC, ECACC etc., so information as to whether they have been screened for HIV, Hep B, Hep C and EBV will be available. As a result they are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory. The cells will therefore not exert any foreseeable effects on human health. All primary cell lines will be routinely tested for mycoplasma.

To identify viral genes involved in cellular resistance to herpesviruses: mutant variants of human herpesviruses will be generated using BAC mutagenesis (Bacterial Artificial Chromosome) to insert marker or viral genes into the viral genome, delete viral genes from the genome and mutate viral genes within the viral genome by homologous recombination in E. coli. BACs are then transfected into mammalian cells to obtain infectious virus.

**Host/vector system**

**Host:** E.coli, cultured cell lines  

**vector:**  
- 2nd and 3rd generation lentiviruses or the CRISPR/Cas9 system (the need for contained use for CRISPR/Cas9 stems from the use of a lentiviral based delivery system of the components which target the genes).  
- A replication-deficient retroviral vector (pBABE-puro) will also be used to deliver the activated oncogenes such as RasV12 to the human fibroblast cell line IMR-90 (ATCC Number CCL-186) in order to induce senescence. This strategy will be utilized in order to investigate mechanisms of the senescence pathway.  
- human herpesviruses and their respective BACs  
- New Lentivectors: We will employ newer generation, commercially available vectors with improved biosafety properties as they become available to further minimise risk to users on an ongoing basis.

**Origin & function**

**Deleted/mutated genes:**  
- cellular genes involved in intrinsic defense to herpesvirus infection (e.g. Daxx, PML, ATRX, Sp100, SUMO, Ubc9 and others)  
- viral genes involved in intrinsic immunity and latency (e.g. HSV-1 ICP0, HSV-1 LAT, EBV-EBNA, KSHV-LANA and others)  
- genes involved in cell senescence (e.g. HIRA, p53, p16 and others)  
- genes involved in mechanisms leading to arthritis and cancer (e.g. p53, p53-like genes, PAR2)

**Inserted genes:**  
- marker genes including EGFP, EYFP, dTomato and others  
- substitution of homologous viral genes (e.g. HSV-1 ICP0 with CMV pp71)  
- senescent regulatory genes and genes associated with pathogenesis of arthritis

**Evaluation of foreseeable effects**

**Lentiviruses**

The lentiviruses used for gene knock down and mutation will be 2nd and 3rd generation lentiviruses, which are replication defective and depend on helper (containing gag, pol, rev) and pseudotyped packaging vectors. Only co-transfection of HEK-293 cells with helper and packaging plasmids leads to infectious progeny virus. Due to lack of helper functions in the target cells, infection with lentivirus does not lead to productive virus infection or virus release. All lentiviruses used are unable to replicate in a human host and contain a 3' self-inactivating (SIN) long terminal repeat.
pBABE-vector with onogenes will be transiently transfected into Phoenix cells, a 293T-derived packaging cell line that contains the Gag, Pol and Env viral proteins required for making active virus. Subsequently, viral supernatants produced by Phoenix cells will be used to infect human fibroblasts (IMR-90). Both the Phoenix packaging cell line and IMR-90 fibroblasts are used extensively in laboratories and have been screened and found to be negative for HIV, Hep B, Hep C and EBV. There is no risk from the infected cells. Cells are obtained from ATCC and have been rigorously characterized.

The "LentiCRISPR" vector systems utilise a replication-incompetent lentiviral vector chosen for expression of CAS9 and guide RNAs (Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells Shalem et al, Science 343, 84-87 2014).

(III) herpesviruses

All human herpesviruses are approved in the human pathogen hazard group 2.

Handling of (non-infectious) virus BACs falls within hazard group 1 and does not pose a threat to human health.

Handling of all infectious virus preparations and infected cells is restricted to within biosafety cabinets, wearing protective clothing at all times and including use of safety goggles where appropriate.

It is not anticipated that the deletions, mutations or insertions of viral genes will alter virus tropism.

Mutations within the herpesvirus genome are not expected to, and do not in general, lead to a gain of function. However, as a precautionary measure, virus growth and replicative ability will be assessed post mutation to ensure no gain of function has occurred.

All human herpesviruses lead to life long infections and careful attention will be given to all safety precautions associated with biosafety level 2 work with human herpesviruses.

HSV-1/-2: Infections can occur through lesions in the skin. Infections of the eye are can lead to herpes keratitis. Safety goggles will be worn at all times.

HCMV: Risk of foetal damage after primary infection during pregnancy. Pregnant women should not handle HCMV, but may continue to work in a lab where HCMV is being handled subject to risk assessment. Although the used lab strains AD169, Towne and Davis were originally live vaccine candidates and their ability to cause disease is expected to be attenuated, they may still cause harm, especially in pregnant women.

VZV: Potential fetal damage. Women who are or may become pregnant, if seronegative, should consider being vaccinated and restricted from working with VZV.

EBV: Low risk of laboratory acquired infection. No reports of laboratory acquired infection can be found in the literature.

HHV-6: No risk for immunocompetent hosts. Is the causative agent of exanthema subitum (roseola). May reactivate during pregnancy or in immunosuppression.

HHV-7: No risk for immunocompetent hosts. Is a constitutive inhabitant of adult saliva. Has been associated with exanthema subitum (roseola).

KSHV: No risk for immunocompetent hosts. Leads to tumour formation in HIV-1 infected hosts.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level II suite, with access restricted to authorized and trained staff. Dedicated protective clothing is to be worn in Containment Level II areas at all times.

The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. Plastic ware will be used throughout and no needles or sharps will be used during virus preparation.

The use of the Class II biological safety cabinet will guard against exposure to aerosols.

All cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using desinfectant before removal from the Containment Level II suite for final disposal.

Solid waste will be double bagged in biohazard bags prior to removal from the Containment Level II suite and then autoclaved before final consignment of waste to the approved contractor.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>Laboratory Activities</strong></td>
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<td>Animal Units</td>
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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Please enter comments on the GM safety committee on the risk assessment

Discussed and approved.
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee
Level 1 (GMMs)  
Level 2 (GMMs)  
Level 3 (GMMs)  
Level 4 (GMMs)  
Non-microbial  

Other (please specify)  

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<td>Other (please specify below)</td>
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</table>

Other(s)  

For activities involving GMMs, describe the waste management measures which will apply to the activity  

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment
**Project Additional Information**

**Purposes of the contained use**

Acquire pil mutants of *Ps.aeruginosa* from collaborators. Compare ability of wild-type and mutant *Ps.aeruginosa* stains to attach and invade cultured cells in vitro. Functional pili are normally required for virulence.

**Recipient or parental organism**

*Pseudomonas aeruginosa* strain PAK

*Ps.aeruginosa* rarely affects healthy individuals; occasionally it causes a mild, self limiting infection. It can cause serious and lethal infections in the immunocompromised. It is a cause of respiratory infection in people with cystic fibrosis. *Ps.aeruginosa* has a high level of natural resistance to many antibiotics so infections can be difficult to treat.

**Host/vector system**

The disrupted pilA gene in plasmid PRIC276 was introduced via conjugation into *Ps.aeruginosa* PAK. It is stabilised by the inclusion of a tetracycline resistance marker.


**Origin & function**

Isogenic pil mutants of *Ps.aeruginosa* PAK. *Ps.aeruginosa* rarely infects healthy individuals.
Evaluation of foreseeable effects

The mutant is not expected to be any more pathogenic than the wild-type organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All specimens, cultures of micro-organisms, materials and equipment used in the microbiology laboratory and protective clothing worn in the laboratory must be rendered free from contamination before re-use, discarding all repair. Whenever possible, contaminated articles must be autoclaved. Discard autoclaves operate at a minimum of 134°C for 30mins, (chart recorded every load) which must be checked at least annually by the use of recorded thermocouples. Autoclaved waste materials are then incinerated. When autoclaving cannot be used, because for example the nature of the material is heat sensitive or items are required for re-use, or complex laboratory equipment requires servicing, chemical disinfection will be used. When chemical disinfection is used, it is important that all surfaces come into contact with the chemical. Because different chemical disinfectants have different ranges of micro-organisms against which they are effective, only (site) approved disinfectants and procedures will be used. When large items of equipment need to be decontaminated, this can be carried out by fumigation with formaldehyde vapour. Sodium hypochlorite based disinfectants ("Chloros") are effective against Ps. aeruginosa and will be used for surface decontamination of items being transported between the microbiological cabinet and incubator/fridge. Hypochlorite solutions will be used to decontaminate small items used in the cabinet. Hypochlorite solutions at these concentrations will be used:

Surface disinfection 1000ppm
Discard jars 2,500 ppm (for a contact time of several hours/overnight)

Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form Y

Please enter comments on the GM safety committee on the risk assessment

The Site GMSC accepted the risk assessment submitted to it, subject to acceptance by the HSE.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>Animal Units</td>
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<td>L3 L4 L2 L3 L4 L2 L3 L4</td>
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</table>
## Project Additional Information

**Purposes of the contained use**

Genes encoding *F. novicida* and *F. tularensis* metabolic enzymes, regulatory genes and possible virulence determinants will be isolated and inactivated by mutation. Mutated genes will be introduced into *F. novicida* by electroporation and cryotransformation using a suicide vector.

**Recipient or parental organism**

*F. novicida*

**Host/vector system**

Target genes will be isolated from *F. novicida* and *F. tularensis* and cloned into laboratory strains of *E. coli* prior to deletion or insertion mutagenesis. These modified genes will be introduced into *F. novicida*.

**Origin & function**

Genes from *F. novicida* and *F. tularensis* will be mutated such that gene function is lost to obtain attenuation.

**Evaluation of foreseeable effects**

The inactivation of key metabolic enzymes, regulatory proteins or virulence determinants, should result in reduced virulence of *F. novicida*.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All specimens, cultures of micro-organisms, materials and equipment used in the microbiology laboratory and protective clothing worn in the laboratory will be rendered free from contamination before re-use, discarding or repair. Whenever possible, contaminated items will be autoclaved. Discard autoclaves operate at a minimum of 134C for 30 minutes, (chart recorded every load) which will be checked at least annually by the use of recorded thermocouples. Autoclaved waste materials are then incinerated.

When autoclaving cannot be used, because for example the nature of the material is heat sensitive or items are required for re-use, or complex laboratory equipment requires servicing, chemical disinfection will be used. When chemical disinfection is used, it is important that all surfaces come into contact with the chemical. Because different chemical disinfectants have different ranges of micro-organisms against which they are effective, only (Site) approved disinfectants and procedures will be used.

Where large items of equipment need to be decontaminated, this can be carried out by fumigation with formaldehyde vapour. Sodium hypochlorite based disinfectants ("Chloros") are effective against F.novicida and will be used for surface decontamination.

Hypochlorite solutions at these concentrations will be used:
* Surface disinfection - 1000ppm
* Discard jars - 2,500ppm (for a contact time of several hours/overnight)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The Site GMSC accepted the risk safety committee submitted to it, subject to acceptance by the HSE.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Human Clinical Applications</th>
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<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
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Project Ref 202/03.3

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
CLONING AND EXPRESSION OF MARKER GENES, PROMOTERS AND VACCINE CANDIDATE GENES IN STRAINS OF SALMONELLA ENTERICA SEROVAR TYPHIMURIUM AND SALMONELLA ENTERICA SEROVAR TYPHI.

Various vaccine candidate genes (e.g., protective antigen from B. anthracis, SOD gene from Francisella tularensis) and some promoters will be cloned into attenuated strains of S. enterica typhimurium and S. enterica typhi (purA and aroA) for vaccine study.

Some marker genes (e.g., firefly luciferase), and will be cloned into S. typhimurium LT2 for bacterial growth studies.

Recipient or parental organism

- S. enterica serovar typhimurium LT2: rpos mutation
- S. enterica serovar typhimurium SL2361 (aroA auxotroph derived from SL 1344 by imprecise transposon mutagenesis)
- S. enterica serovar typhimurium LB5010 (galE)
- S. enterica serovar typhimurium MT168 (purA auxotroph)
- S. enterica serovar typhi CVD908-htrA
- S. enterica serovar typhi BRD1116
- S. enterica serovar typhi Ty21a

Host/vector system

Initially, genes encoding foreign antigens will be cloned into laboratory cloning strains of E. coli (e.g., JM101, JM109, XLI-Blue), prior to transfer into Salmonella. The expression vectors used will be either non-mobilisable vectors (e.g., pUC series-based plasmids, pLG339-based plasmids, pBlueScript phagemids) or mobilisation-defective vectors (e.g., pBR322-based plasmids). Suicide vectors used for integration of foreign genes into the chromosome of the bacteria may be mobilisation-defective in E. coli (e.g., pHSG422) or self-mobilisable in E. coli pir (e.g., pHSG422) but will not be retained in Salmonella. The vectors may contain ampicillin, kanamycin or chloramphenicol resistance genes for selection.

Origin & function

Vaccine candidate studies in vitro and in vivo.
The various strains of S. enterica serovar typhimurium and S. enterica serovar typhi will contain, and in some cases will express, vaccine candidate genes and their products.

GMOs resulting from this notification will be based on attenuated strains of S. enterica serovar typhimurium and S. enterica serovar typhi which are of low pathogenicity for humans. Strains such as LT2, however, retain their ability to infect tissue culture cells. These strains have long histories of safe use in the laboratory.

The GMOs would have no effects on plants or bio-processes, although they may affect humans or other animals. They tend to be pathogenic for mice and humans at high doses. This risk of infection is considered to be very low. The organism will be handled in an ACGM level II laboratory, and inactivated before disposal. Accidental release under these conditions is unlikely.

Proteins being expressed from the GMOs are potential vaccine candidates. They have no known toxic effects, indeed PA is a component of the present human vaccine.

Wild-type salmonellae can survive for up to 70 days in the soil. The survival of the GMO is not likely to increase this time period. Transfer of genetic modification to other organisms is unlikely.

It is unlikely that the GMO will be fitter as a result of these modifications.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All specimens, cultures of micro-organisms, materials and equipment in the microbiology laboratory and protective clothing worn in the laboratory will be rendered free from contamination before re-use, discarding or repair. Whenever possible, contaminated items will be autoclaved. Discard autoclaves operate at a minimum of 134 degrees C for 30 mins, (chart recorded every load) which will be checked at least annually by the use of recorded thermocouples. Autoclaved waste materials are then incinerated. When autoclaving cannot be used, because for example the nature of the material is heat sensitive or items are required for re-use, or complex laboratory equipment requires servicing, chemical disinfection will be used. When chemical disinfection is used, it is important that all surfaces come into contact with the chemical. Because different chemical disinfectants have different ranges of micro-organisms against which they are effective, only (Site) approved disinfectants and procedures will be used. Where large items of equipment need to be decontaminated, this can be carried out by fumigation with formaldehyde vapour. Sodium hypochlorite based disinfectants ("Chloros") are effective against Salmonella spp. and will be used for surface decontamination of items being transported between the microbiological cabinet and incubator/fridge. Hypochlorite solutions will be used to decontaminate small items used in the cabinet. Hypochlorite solutions at these concentrations will be used:

* Surface disinfection 1000 ppm
* Discard jars 2,5000 ppm (for a contact time of several hours/overnight)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The site GMSC accepted the risk assessment submitted to it, subject to acceptance by the HSE.

Project Containment

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Large Scale Activities  
Human Clinical Applications

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Tick if notifying a connected programme of work Y

Project Ref 202/03.4

Date Ackn'd | CU2 Project Title | Class | Culture Vol | Class Culture Volume |
-------------|-------------------|-------|-------------|----------------------|
17/07/2003 | THE GENERATION OF ATTENUATED MUTANTS OF YERSINIA PSEUDOTUBERCULOSIS AND THEIR EVALUATION AS POTENTIAL ATTENUATED VACCINE CANDIDATES AGAINST YERSINIA PSEUDOTUBERCULOSIS AND YERSINIA PESTIS. | Class 2 | < 1 litre | |

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Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Genes encoding Yersinia pseudotuberculosis metabolic enzymes, surface proteins, regulatory genes and possible virulence determinants will be isolated and inactivated by mutation. The effects of these mutations will be assessed in an appropriate animal model. Site directed mutagenesis of the organism performed using transposon
mutagenesis, based on Tn5 will also be evaluated.

Host/vector system

Target genes will be isolated from Yersinia pseudotuberculosis will be cloned into laboratory strains of E. coli (lambda pir strains such as Escherichia coli cc118  pir, or equivalent), prior to deletion or insertion mutagenesis. These modified genes will be introduced back into Yersinia pseudotuberculosis. Tn5 is carried by pUTminiTn5Km2 in E. coli SY327. E.coli cc118 has no known ability to infect immunologically competent humans, or plants or insects. Although not well characterised, it has a history of safe use in the laboratory and is regarded as non-pathogenic.

Origin & function

Genes from Yersinia pseudotuberculosis will be mutated such that gene function is lost to obtain attenuation.

Evaluation of foreseeable effects

The inactivation of key metabolic enzymes, regulatory proteins or virulence determinants, should result in reduced virulence of Yersinia pseudotuberculosis.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All specimens, cultures of micro-organisms, materials and equipment used in the microbiology laboratory and protective clothing worn in the laboratory will be rendered free from contamination before re-use, discarding or repair. Whenever possible, contaminated items will be autoclaved. Discard autoclaves operate at a minimum of 134 degrees C for 30 mins, (chart recorded every load) which will be checked at least annually by the use of recorded thermocouples. Autoclaved waste materials are then incinerated. When autoclaving cannot be used, because for example the nature of the material is heat sensitive or items are required for re-use, or complex laboratory equipment requires servicing, chemical disinfection will be used. When chemical disinfection is used, it is important that all surfaces come into contact with the chemical. Because different chemical disinfectants have different ranges of micro-organisms against which they are effective, only (site) approved disinfectants and procedures will be used. Where large items of equipment need to be decontaminated, this can be carried out by fumigation with formaldehyde vapour. Sodium hypochlorite based disinfectants ("Chloros") are effective against Yersinia pseudotuberculosis and will be used for surface decontamination.

Hypochlorite solutions at these concentrations will be used:

* Surface disinfection 1000 ppm
* Discard jars 2500 ppm (for a contact time of several hours/overnight)
The site GMSC accepted the risk assessment submitted to it, subject to acceptance by the HSE.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<td>L2 Yes</td>
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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref 202/03.6**

- **Date Ackn’d**: 04/12/2003
- **CU2 Project Title**: THE IMPORTATION OF PLASMID CURED STRAINS OF BACILLUS ANTHRACIS AND THE GENETIC MODIFICATION OF THESE STRAINS IN ORDER TO ISOLATE MINOR VIRULENCE DETERMINANTS
- **Class**: Class 2
- **CultureVolClass2**: 1-50 litres
- **Non-GMM**: not applicable
- **Consent Granted**: Project notified under transitional arrangements

- **Withdrawn**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
**Project Additional Information**

**Purposes of the contained use**

Genes encoding the virulence determinants and potentially protective sub-units and possible virulence determinants will be isolated and inactivated by mutation. The effects of these mutations will be assessed in an appropriate animal model. Site directed mutagenesis of the organism will be performed using transposon mutagenesis or allelic replacement in a plasmid cured strain of B. anthracis. Further cloning on identified genes into E. coli strains will occur.

**Recipient or parental organism**

Bacillus anthracis has been studied for many years and the two major virulence determinants have been identified. A tripartite toxin is encoded by plasmid pXO1 and capsule is encoded by pXO2 (Mock M and Fouet A Annu Rev Microbiol. 2001;55:647-71.). Removal of one or both of these virulence determinants considerably reduces the risk to human health. For example vaccine strains of B. anthracis e.g. ST1 are non-capsulated variants which have lost pXO2. All genetic manipulations will be carried out in a plasmid cured strain of B. anthracis such as UM23CL2. These strains of B. anthracis are considered suitable for use in ACGM II laboratories and have a safe history of use. We aim to use the plasmid cured strains to investigate minor virulence determinants of B.anthracis by mutation. We will target genes which are implicated in virulence of other bacterial pathogens, with reference to the recently sequenced genome of Anthracis (Reed T et al Nature Vol 423 2003 pp81-86).

**Host/vector system**

Target genes will be isolated from B.anthracis will be cloned into laboratory strains of E.coli (lambda pir strains such as Escherichia coli cc 118 pir, or equivalent), prior to deletion or insertion mutagenesis. These modified genes will be introduced back to B.anthracis. Alternatively transposons harboured on suicide plasmids will be used to randomly mutate the genome (Ivins B et al Infect. Immun. Vol 56 (1) 1988 pp 176-191).

**Origin & function**

Genes from B. anthracis will be mutated such that gene function is lost to obtain a measurable phenotype.

**Evaluation of foreseeable effects**

The inactivation of key metabolic enzymes regulatory proteins or virulence determinants, should result in reduced virulence of B. anthracis.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

B. anthracis is an ACDP level III bacterium. Fully virulent strains can cause disease in humans which if untreated may be fatal. Particular risk is associated with inhalational disease which is difficult to treat and fatal if treatment is delayed. We aim to use plasmid cured strains which have reduced virulence at ACGM II. All genetic manipulation will occur in this background.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Specimens, cultures of micro-organisms, materials and equipment used in the microbiology laboratory and protective clothing worn in the laboratory will be rendered free from contamination before re-use, discarding or repair. Whenever possible, contaminated items will be autoclaved. Discard autoclaves operate at a minimum of 134 degrees C for 30 mins (chart recorded every load) which will be checked at least annually by the use of recorded thermocouples. Autoclaved waste materials are then incinerated. When autoclaving cannot be used, because for example the nature of the material is heat sensitive or items are required for re-use, or complex laboratory equipment requires servicing, chemical disinfection will be used. When chemical disinfection is used, it is important that all surfaces come into contact with the chemical. Because different chemical disinfectants have different ranges of micro-organisms against which they are effective, only (Site) approved disinfectants and procedures will be used. Where large items of equipment need to be decontaminated, this can be carried out by fumigation with formaldehyde vapour. Sodium hypochlorite based
disinfectants ("Chloros") are effective against B. anthracis and will be used for surface contamination. Hypochlorite solutions at these concentrations will be used:
- Surface disinfection 100 ppm
- Discard jars 10,000 ppm (for a contact time of several hours / overnight)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Site GMSC accepted the risk assessment submitted to it, subject to acceptance by the HSE.

Project Containment

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<tr>
<td>Animal Units</td>
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Project Ref 202/04.1

Date Ackn'd 21/05/2004

CU2 Project Title Cloning, Expression and Purification of Listeriolysin O (LLO) from Listeria monocytogenes in Escherichia coli

Date Project Ceased

Class 2

CultureVolClass 1-50 Litres

Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work Y

Withdrawn N

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Listeriolysin is a cholesterol requiring cytolsin produced by Listeria monocytogenes. During Listeria infection LLO allows bacteria to escape from the phagolysosome.
Listeriolysin is a phagosome specific cytolsin which has a pronounced acidic pH optimum (Glomski et al., 2002). It also contains a PEST-like sequence which targets it for phosphorylation and/or degradation in the host cytosol (Decatur & Portnoy, 2000).
The gene encoding LLO from L. monocytogenes will be cloned, in frame, downstream of a strong promoter such as T7/tac or equivalent, so as to allow expression as a fusion protein in E.coli. The Llo gene will be expressed in E. coli and the resulting protein will be purified by column chromatography.
This protein will form a key component of the intracellular delivery project which is aimed at generating appropriate immune responses to vaccine subunits.

Recipient or parental organism

E. coli K-12 derivatives such as TOP10F'/DH5α or equivalent
E. coli B strain BL21 (and DE3 component are lysogens) of the bacteriophage DE3 and carry a chromosomal fragment containing the lacI gene, the lacUV5 promoter and the gene for T7 RNA polymerase. The pLysS plasmid encodes the T7 lusozyme used for stringent control of T7 RNA polymerase expression in DE3 host strains.
Commercially available expression vectors will be used (e.g. pGEX vectors (Amersham Biosciences) or equivalent). Vectors have a pUC/pBR322 origin of replication or equivalent, and a strong promoter induced via IPTG for expression. Plasmid encoded antibiotic resistance is to ampicillin. Genes cloned downstream of the strong promoter are expressed as GST/histidine fusion proteins. Vectors allow expression of fusion proteins with fusions at either the N or C terminus.

Host/vector system

E. coli K-12 derivatives such as TOP10F'/DH5α or equivalent
E. coli B strain BL21 (and DE3 component are lysogens) of the bacteriophage DE3 and carry a chromosomal fragment containing the lacI gene, the lacUV5 promoter and the gene for T7 RNA polymerase. The pLysS plasmid encodes the T7 lusozyme used for stringent control of T7 RNA polymerase expression in DE3 host strains.
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Origin & function

LLO gene from L. monocytogenes

Evaluation of foreseeable effects

The DNA will be expressed under the control of an IPTG inducible promoter. However it is unlikely that the structural genes will be expressed to any significant level in the host without the addition of IPTG to induce gene expression.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All specimens, cultures of micro-organisms, materials and equipment used in the microbiology laboratory and protective clothing worn in the laboratory will be rendered free from contamination before re-use, discarding or repair. Whenever possible, contaminated items will be autoclaved. Discard autoclaves operate at a minimum of 134°C, (chart recorded every load) which will be checked at least annually by the use of recorded thermocouples. Autoclaved waste materials are then incinerated. When autoclaving cannot be used, because for example the nature of the material is heat sensitive or items are required for re-use, or complex laboratory equipment requires servicing, chemical disinfection will be used. When chemical disinfection is used, it is important that all surfaces come into contact with the chemical. Because different chemical disinfectants have different ranges of micro-organisms against which they are effective, only (site) approved disinfectants and procedures will be used. Where large items of equipment need to be decontaminated, this can be carried out by fumigation with formaldehyde vapour. Sodium hypochlorite based disinfectants ("Chloros") will be used for surface decontamination of items being transported between the microbiological cabinet and incubator/fridge. Hypochlorite solutions will be used to decontaminate small items used in the cabinet. Hypochlorite solutions at these concentrations will be used:
- Surface disinfection - 1000ppm
- Discard jars - 2,500 ppm (for a contact time of several hours/overnight).

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The site GMSC accepted the risk assessment submitted to it, subject to acceptance by the HSE.

Project Containment

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<th>Human Clinical Applications</th>
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<td>L2 L3 L4 L2</td>
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Project Ref 202/04.2

Date Ackn'd CU2 Project Title Class CultureVol Class2 CultureVolumeClass3-4
03/11/2004 The genetic modification of Burkholder thailandensis, expression of recombinant Class 2 1-50 Litres
We aim to utilise Burkholderia thailandensis as a genetic model to allow identification of gene functions by mutational analysis. B. thailandensis is a non-pathogenic soil saphrophyte which occurs naturally in the environment and is isolated widely from soil and water in North-Eastern Thailand (Brett et al., 1997, Wuthiekanun et al., 1996, Sonthayanon et al., 2002). B. thailandensis is genetically similar to B. pseudomallei and B. mallei but lacks at least one pathogenicity island that is present in those species. B. thailandensis has a similar biochemical profile to B. pseudomallei but can be distinguished by substrate utilisation particularly L-arabinose assimilation. B. thailandensis strains can assimilate L-arabinose, are avirulent in experimental animals and are only found in the environment. All clinical isolates collected have been B. pseudomallei with one exception which is thought to have occurred by direct inoculation of a large amount of environment debris.

Target genes isolated from B. thailandensis will be cloned into laboratory strains of E. coli (lambda pir strains such as Escherichia coli cc118 pir, or equivalent), prior to deletion or insertion mutagenesis. Modified genes will be introduced back into B. thailandensis. Alternatively transposons harboured on suicide plasmids will be used to randomly mutate the genome. Suitability of plasmid DNA is limited by the use of appropriate selective markers. Those suitable for use with the organism are kanamycin, chloramphenicol and tetracycline. Non-antibiotic resistance markers might also be used (e.g. Sucrose sensitivity). Complementation of inactivated genes with DNA derived from the parent strain will be carried out.

In addition recombinant production of putative vaccine antigens will be carried out using suitable expression systems (E.g. pTRC99A).

Genes from B. thailandensis will be mutated such that gene function is lost to obtain a measurable phenotype. Function will be verified by complementation.

Genes amplified by PCR will be utilised in E. coli expression systems to produce recombinant vaccine antigens.

The inactivation of key metabolic enzymes, regulatory proteins or virulence determinants, should result in measurable phenotypes. Recombinant production of vaccine antigens will result in products for use as potential vaccine components.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

B. thailandensis and any resulting GMMs will be handled under ACDP/ACGM level II containment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All specimens, cultures of micro-organisms, materials and equipment used in the microbiology laboratory and protective clothing worn in the laboratory will be rendered free from contamination before re-use, discarding or repair. Whenever possible, contaminated items will bw autoclaved by validated means. Discard autoclaves will be checked at least annually by the use of recorded thermocouples. Autoclaved waste materials are then incinerated. When autoclaving cannot be used, because for example the nature of the material is heat sensitive or items are required for re-use, or complex laboratory equipment requires servicing, chemical disinfection will be used. When chemical disinfectant is used, it is important that all surfaces come into contact with the chemical. Because different approved disinfectants and procedures will be used. Where large items of equipment need to be decontaminated, this can be carried out by fumigation with formaldehyde vapour. Sodium hypochlorite based disinfectants ("Chloros") Hypochlorite solutions at these concentrations will be used:

- Surface disinfection: 1000 ppm
- Discard jars: 2,5000 ppm (for a contact time of several hours/overnight)

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

The Site GMSC accepted the risk assessment submitted to it, subject to acceptance by the HSE.

Project Containment

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Project Ref 202/06.2

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
Construction of gene knock-out mutants of Burkholderia cepacia complex bacteria by insertion of fluorescent reporters for use in investigations of natural genetic transformation events.

**Recipient or parental organism**

- E. coli JM109 (Promega)
- Burkholderia cepacia ATCC 25416, 17759, 9091, C2970, C3159, C1963, LMG C1964
- Burkholderia cenocepacia LMG 18863, LMG J2956, C1394 J415, C2836,
- Burkholderia multivorans LMG 13010, ATCC 17616, 7897, 7732
- Burkholderia stabilis LMG 14294, LMG 14086, 7639, 8088, 300, ERL 347, LMG 3172, LMG 3173, J1743, J493
- Burkholderia vietnamiensis LMG 10929, LMG 18836, 159, 638, 549, LMG C3175, ERL 126, C1704, J2963, C2978

All members of the Burkholderia cepacia complex have been implicated in infection of Cystic Fibrosis patients. However, B. multivorans (genomovar II) and B. cenocepacia (genomovar III) predominate. These organisms are not a major cause of disease in healthy individuals. B. cepacia was first isolated as the causative agent of soft rot in onions.

**Host/vector system**

- Vectors to be employed:
  - pTurboGFP-B: Commercially available prokaryotic expression vector from Cambridge Biosciences. Encodes Green Fluorescent Protein and ampicillin resistance. GFP is under the control of a T5 promoter/lac operator. Will be used as a source of GFP coding DNA.
  - pBR322: Commercially available vector. Encodes ampicillin and tetracycline resistance.
pJQ200: Suicide vector available from LGC. Only replicates in members of the enterobacteriacea. Encodes sacB and gentamicin resistance.


Hosts:
E. coli JM109 and members of the Burkholderia cepacia complex listed above (the latter will only be transformed with pJQ200). Stable plasmid inheritance in Burkholderia cepacia complex is not the desired outcome of these experiments.

Origin & function
Green Flourescent Protein under control of T5 promoter/lac operator and Fire-fly Luciferase under control of trp-lac fusion promoter. This genetic material will be used to create gene knock-out cassettes targeting oprC and zmpA of the Burkholderia cepacia complex.

Evaluation of foreseeable effects
Maximising expression of these flourescent reporters in Burkholderia cepacia complex will not be undertaken. The desired outcome is the knock-out of oprC, which codes for an antibiotic efflux pump protein and zmpA which codes for a protease in members of the Burkholderia cepacia complex. The oprC knock-out will result in an increased sensitivity to chloramphenicol and ciprofloxacin. The zmpA knock-out will result in reduced protease activity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All specimens, cultures of micro-organisms, materials and equipment used in the microbiology laboratory and protective clothing worn in the laboratory will be rendered free from contamination before re-use, discarding or repair. Whenever possible, contaminated items will be autoclaved. Discard autoclaves operate at a minimum of 134C for 30 mins, (chart recorded every load) which must be checked at least annually by the use of recorded thermocouples. Autoclaved waste materials are then incinerated. When autoclaving cannot be used, because for example the nature of the material is heat sensitive or items are required for re-use, or complex laboratory requires servicing, chemical disinfection will be used. When chemical disinfection is used, it is important that all surfaces come into contact with the chemical. Because different chemical disinfectants have different ranges of micro-organisms against which they are effective, only (Site) approved disinfectants and procedures will be used. Where large items of equipment need to be decontaminated, this can be carried out by fumigation with formaldehyde vapour. Sodium hypochlorite based disinfectants ("Chloros") are effective against Burkholderia spp and E.coli spp and will be used for surface decontamination of items being transported between the microbiological cabinet and incubator / fridge. Hypochlorite solutions will be used to decontaminate small items used in the cabinet.

Hypochlorite solutions at these concentrations will be used:
Surface disinfection 1000 ppm
Discard jars 2,500 ppm (for a contact time of several hours / overnight)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
The Site GMSC accepted the risk assessment submitted to it, subject to acceptance by the HSE.

## Project Containment

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

## Project Ref 202/13.1

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<td>08/05/2013</td>
<td>Generation of mutants of Francisella tularensis subsp. holarctica live vaccine strain (LVS)</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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- **Date Project Ceased**: 
- **Withdrawn**: N

## Project Additional Information

**Purposes of the contained use**

Generation of Francisella tularensis subspecies holarctica Live Vaccine Strain (LVS) isogenic mutants: LVS genes including those encoding metabolic enzymes, regulatory
genes, virulence determinants, surface antigens, protein export systems, post-translational modification systems and genes involved in survival will be inactivated by mutation. Mutated genes will be introduced into LVS by electroporation or other suitable DNA delivery methods.

Generation of transposon mutants of LVS: LVS will be transformed with transposons such as Tn5, Tn7 or Himar1 and their modified derivatives, by electroporation or other suitable DNA delivery methods. Transposon mutants will be selected by phenotype.

Confirmation of essential genes: genes predicted to be essential for growth will be assessed by modified isogenic mutagenesis approaches, such as complementation with a second copy of the gene prior to inactivation, or placing the target gene under the control of a heterologous promoter allowing repression or induction of expression.

Altered expression of genes: genes may be constitutively expressed or over-expressed to induce attenuation as a result of deregulation of expression or attenuation as a result of a phenotype detrimental to bacterial survival. Only genes where aberrant expression is predicted to be attenuating will be expressed in this way. Classical virulence facotrs will be excluded.

Some attenuated mutants will also have coding regions from antigens known to increase protectivity in other organisms introduced into them prior to being assessed as potential vaccines.

Pseudogenes in LVS, but intact in fully virulent subspecies tularensis and holarctica strains, predicted to be anti-virulence loci may be restored to assess the impact on virulence.

Site directed mutagenesis of the organism may be generated to characterise selected genes of interest.

Mutants will be characterised in vitro and in vivo. Suitable in vivo models may include mammals, such as mice, or alternative lower order species such as Galleria.

Recombinant LVS, both wild type and mutants, expressing proteins such as GFP or luciferase, will be generated. This will facilitate procedures such as confocal microscopy of infected cells and whole body in vivo imaging of infection. Other recombinants expressing tagged proteins (e.g. FLAG_tags, His-tags) will allow the purification of selected fusion proteins, for example to investigate post-translational modifications or protein-protein interactions.

Complementation of mutants: Mutants which have at least one gene disrupted may be complemented. Reintroduction of the gene or genes mutated on a plasmid will allow check for complementation of any missing phenotype that occurred upon deletion. The purpose of this will be to prove that any phenotype found as a result of the deletion is due to the gene(s). Complementation of mutants has been reported using a stable plasmid and cognate promoter or heterologous promoters, but new tools may need to be developed for this organism.

Specialist equipment (particularly equipment invovled in intravital imaging) may be housed at containment level 3 and require use of wild type or mutant LVS at containment level 3. In this incidence, strains will remain within containment level 3 and may only be removed, to a lower containment level facility, if inactivated by an appropriate method (e.g. formalin fixation or heat-killing) and sterility checked.

**Recipient or parental organism**

Francisella tularensis subsp. Holarctica live vaccine strain (LVS)

Escherichia coli (lab strains such as K12 or B derivatives)

**Host/vector system**

Vectors suitable for use with LVS have been reported and will form the basis of tools to manipulate the organism. Mutational studies will utilise suicide vectors such as pSMP75, pMP590 and pDM4. These vectors typically include a multiple cloning site, origin of replication, transfer genes and a selective marker but are unable to replicate
in LVS. Some suicide plasmids may contain transposons which are capable of random insertion into the LVS genome (e.g. Tn5 carried out on pUTminiTn5Km2). Recombinant expression of proteins may be achieved by chromosomal integration or on plasmids which are able to replicate within LVS. These vectors may include pKK202, pEDL, pCT, pFNLT and pFNLT10 based plasmids. Some have a strong promoter upstream of the multiple cloning sites to allow expression of the cloned genes. Additionally some plasmids such as may include protein purification tags such as glutathione-s-transferase, FLAG and 6xHis. Other vectors may be developed for use with LVS after approval by the local GMSC.

Complementation or expression plasmids used would likely be similar to or part of the pFNLT10 or pFNLT plasmid series (derived from pTOPO/FNL10). Plasmids would be maintained in the cell using an antibiotic resistance gene e.g. chloramphenicol or kanamycin. The gene(s) being investigated would be under the control of an inducible or native promoter.

Selection of recombinant strains will typically be done using antibiotics such as kanamycin, chloramphenicol, tetracycline and ampicillin. Plasmids contain up to two antibiotic resistance genes. Combinations of antibiotics that are recommended for treatment of tularemia will not be used (specifically not tetracycline in combination with chloramphenicol). Alternative selective markers such as sucrose selection or lethal genes (e.g. DNA gyrase) may be used where conventional selection is not appropriate.

Genes will be amplified by PCR from LVS genomic DNA before cloning into appropriate vectors. This genetic material will be used to create mutagenesis or complementation constructs. Expression constructs may be expressed in recombinant E. coli for protein purification.

Heterologous genetic material from other organisms will include labels e.g. GFP, luciferase, and tags e.g. FLAG tags.

Genes of interest will include metabolic enzymes, regulatory proteins, virulence determinants and genes involved in environmental survival.

Evaluation of foreseeable effects

Each genetic modification procedure is individually assessed by the site GMSC. The predicted effect on virulence will be challenged to ensure that, as far as is reasonably practicable, there is not expected to be a rise in virulence compared to that of wild-type LVS. Similarly each genetic modification is assessed by the site GMSC to ensure that there is not expected to be an increase in environmental survival or impact on the environment of the genetically modified strain compared to the wild-type.

In general, it is considered highly unlikely that the inactivation of any given gene will result in an increase in virulence or any alteration to environmental impact. Indeed, it is likely that inactivation of genes will result in strains less able to harm humans, animals or the environment. Production of recombinant proteins in LVS for the purposes of complementation is considered to be highly unlikely to result in an increase in virulence or environmental impact beyond restoration to wild-type levels. Similarly, production of recombinant proteins for the purposes of vaccine production is unlikely to result in any increase in virulence or environmental impact due to the nature of the proteins being produced. As mentioned above, each genetic modification is assessed by the GMSC to ensure that this is the case.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All specimens, cultures of micro-organisms, materials and equipment used in the microbiology laboratory and protective clothing worn in the laboratory will be rendered free from contamination before re-use, discarding or repair.

Whenever possible, contaminated items will be autoclaved. Discard autoclaves operate at a minimum of 134°C for 30 mins, (chart recorded every load) which will be
validated at least annually by the use of 12 point recorded thermocouples. Autoclaved waste materials are then incinerated. When autoclaving cannot be used, because for example the nature of the material is heat sensitive or items are required for re-use, or complex laboratory equipment requires servicing, chemical disinfection will be used.

When chemical disinfection is used, it is important that all surfaces come into contact with the chemical. Because different chemical disinfectants have different ranges of micro-organisms against which they are effective, only (Site) approved disinfectants and procedures will be used. Where large items of equipment need to be decontaminated, this will be carried out by fumigation with formaldehyde vapour. Sodium hypochlorite based disinfectants ("Chloros") are effective against Ft subsp. Novicida and LVS and will be used for surface decontamination of items being transported between the microbiological safety cabinets and incubators or refrigerators/freezers. Hypochlorite solutions will be used to decontaminate small items used in the cabinet.

Hypochlorite solutions at the following concentrations will be used:
- Surface disinfection > 1000 ppm
- Discard jars >10,000 ppm (for a contact time of several hours/overnight)

Please enter comments on the GM safety committee on the risk assessment

All individual activities which fall into this notification will be submitted to the site GMSC. The GMSC will review each assessment and approves activities as suitable and sufficient as appropriate. The GMSC considers the environmental aspects of the activity as well as the human health and safety aspects.

Project Containment

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Project Ref 202/13.2

Date Ackn'd 19/08/2013  CU2 Project Title Generation of mutants of ACDP level 2 alphaviruses

Class CultureVol Class 2 CultureVolumeClass 3-4

Class 2 < 1 Litre

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Project Additional Information

**Purposes of the contained use**

The aim of this work is to generate mutants of ACDP hazard group 2 alphaviruses including Semliki Forest virus (SFV) and Sindbis virus (SIN). Mutants may be generated using either the whole virus or cDNA copies of the viral genome. Mutants generated using plasmids encoding cDNA copies of the genome will be maintained in laboratory strains of E. coli. Virus will be produced from the cDNA genome by linearising the plasmid (by incubation with a compatible restriction enzyme) and reacting with T7 RNA polymerase. The RNA genome will be transfected into eukaryotic cells (by electroporation or commercially available chemical transfection reagents).

**Generation of mutants**

Generation of ACDP hazard group 2 alphavirus isogenic mutants: viral genes including those encoding structural proteins as well as enzymes, regulatory genes and virulence determinants will be inactivated by mutation (either site-directed or random mutagenesis).

Altered expression of genes: genes may be constitutively expressed or over-expressed to induce attenuation as a result of deregulation of expression or attenuation as a result of a phenotype detrimental to viral survival or replication. Only genes where aberrant expression is predicted to be attenuating will be expressed in this way. Classical virulence factors will be excluded.

Mutants will be characterised in vitro and in vivo. Suitable in vivo models may include mammals, such as mice, or alternative lower order species such as Galleria mellonella.

**Complementation of mutants**

Complementation of mutants: Mutants which have at least one gene disrupted may be complemented. Reintroduction of the gene or genes mutated on a plasmid will allow checks for complementation of any missing phenotype that occurred upon deletion. The purpose of this will be to prove that any phenotype found as a result of the deletion is due to the gene(s) deleted and not to polar effects on other genes and will also provide further insights as to the role of the gene(s).

**Expressing foreign genes**

Recombinant alphaviruses, both wild type and mutants, expressing fluorescent or bioluminescent proteins such as GFP or luciferase, will be generated. This will facilitate...
procedures such as confocal microscopy of infected cells and whole body in vivo imaging infection. Other recombinants expressing tagged proteins (e.g. FLAG-tags, His-tags) will allow the purification of selected fusion proteins, for example to investigate post-translational modifications or protein-protein interactions.

Specialist equipment (particularly equipment involved in intravital imaging) may be housed at containment level 3 and require use of wild type or mutant (hazard group or GM class) alphaviruses at containment level 3. In this incidence, strains will remain within containment level 3 and may only be removed, to a lower containment level facility, if inactivated by an appropriate method (e.g. formalin fixation or heat-killing) and sterility checked. If equipment for intravital imaging becomes available at containment level 2 work will be conducted within this facility.

Recipient or parental organism

ACDP group 2 alphaviruses including Sindbis and Semliki Forest Virus

Eserchhia coli (lab strains such as K12 or B derivatives).

Host/vector system

Plasmids suitable for use with SFV and SIN have been reported and will form the basis of tools to manipulate the viruses. Plasmids encoding cDNA genomes of SFV or SIN will be cloned into laboratory strains of E. coli prior to mutagenesis. Mutated plasmids will then be used to create genetically modified SFV or SIN. The vectors described for SFV and SIN are derived from pBR322-based vector and consist of the complete genomic sequence for the virus represented as cDNA and a unique restriction site for the linearisation of the plasmid prior to in vitro transcription of infectious genomic RNA replicas. Other vectors may be developed for use with either SFV or SIN after approval by the local GMSC.

Plasmids to be used in studies with SFV may include; pVA7, pSFV4 and pCMV-SFV4. In pCMV-SFV4 the SFV infection clone DNA (icDNA) is under the control of the CMV promoter. Plasmids to be used in studies with SIN may include; pTR339, pToto series and pTRSB which encode a full-length icDNA of SIN. SIN and SFV cDNA encodes non-structural genes (nsp1, nsp2, nsp3 and nsp4) and structural genes (capsid protein C, envelope proteins; E3, E2, E1 and 6K peptide). SFV and SIN non-structural and structural genes encode whole virus. Non-structural genes encode for proteins involved in RNA genome replication and structural genes encode for proteins that package the genome in a viral particle.

Selection of recombinant strains will typically be done using antibiotics. The plasmids described above confer resistance to ampicillin. Other vectors may be developed which confer resistance to other antibiotics after approval by the local GMSC.

Origin & function

Plasmids encoding cDNA genomes of SFV or SIN will be cloned into laboratory strains of E. coli prior to mutagenesis. This genetic material will be used to create mutagenesis or complementation constructs. Expression constructs may be expressed in recombinant E. coli for protein purification.

Heterologous genetic material from other organisms will include labels e.g. GFP, luciferase, and tags e.g. FLAG tags.

Genes of interest will include structural proteins, regulatory proteins and virulence determinants.

Evaluation of foreseeable effects

Each genetic modification procedure is individually assessed by the site GMSC. The predicted effect on virulence will be challenged to ensure that, as far as is reasonably practicable, there is not an expected increase in virulence compared to that of wild-type SFV and SIN. Similarly each genetic modification is assessed by the site GMSC to ensure that there is not an expected increase in environmental survival and are, therefore, determined to be a class 3 activity, the work will either (i) not be carried out or (ii) a significant change will be submitted. No work (with the proposed genetic modification) will be conducted until the significant change has been accepted.

Introduction of mutations (including silent mutations) into the SFV or SIN genome may have no effect or they may increase or decrease infectivity and pathogenicity due to preferred codon usage of the host. However, the aim of this work is not to purposefully derive SFV or SIN with increased pathogenicity or infectivity. For this reason, silent mutations (that change the nucleotide sequence and not the protein sequence) will be chosen that do not increase the level of codon optimisation for mammalian cells.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All specimens, cultures of micro-organisms, materials and equipment used in the microbiology laboratory and protective clothing worn in the laboratory will be rendered free from contamination before re-use, discarding or repair.

Whenever possible, contaminated items will be autoclaved. Discard autoclaves operate at a minimum of 134° for 30 mins, (chart recorded every load) which will be validated at least annually by the use of 12 point recorded thermocouples. Autoclaved waste materials are then incinerated. When autoclaving cannot be used, because for example the nature of the material is heat sensitive or items are required for re-use, or complex laboratory equipment requires servicing, chemical disinfection will be used.

When chemical disinfection is used, it is important that all surfaces come into contact with the chemical. Because different chemical disinfectants have different ranges of micro-organisms against which they are effective, only (Site) approved disinfectants have different ranges of micro-organisms against which they are effective, only (Site) approved disinfectants and procedures will be used. Where large items of equipment need to be decontaminated, this will be carried out by fumigation with formaldehyde vapour. Virus suspensions and virus-infected cells can also be chemically inactivated by 5% (w/v) Virkon solution. Sodium hypochlorite based disinfectants ("Chloros") are effective against SFV and SIN and will be used for surface decontamination of items being transported between the microbiological cabinets and incubators or refrigerators/freezers. Hypochlorite solutions will be used to decontaminate small items used in the cabinet.

Hypochlorite solutions at the following concentrations will be used:

- Surface disinfection > 1000 ppm
- Discard jars > 10,000 ppm (for a contact time of several hours/overnight)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All individual activities which fall into this notification will be submitted to the site GMSC. The GMSC will review each assessment and approves activities as suitable and sufficient as appropriate. The GMSC considers the environmental aspects of the activity as well as the human health and safety aspects.

Project Containment
The production and use of pseudotyped lentiviral vectors

Date Ackn'd: 03/02/2021

CU2 Project Title: The production and use of pseudotyped lentiviral vectors

Class: Class 2

Non-GMM Consent Granted

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Lentiviral vectors will be used in vitro to mimic the entry of a range of hazardous viruses in a high-throughput manner. This will allow assessment of virus tropism as well as medical countermeasure screening.

Production of pseudotyped lentiviral vectors (Class 1 and Class 2 activities)

Production of second-generation, lentiviral vectors will be carried out by co-transfection of mammalian cells with three separate plasmids (Class 2 activity). These plasmids will be stored and propagated within standard laboratory E. coli cells (Class 1 activity). These plasmids encode a mini-genome (encoding a reporter gene), the HIV-1 structural and replication enzymes, and the donor glycoprotein respectively.

Use of pseudotyped lentiviral vectors (Class 2 activity before removal of virus, Class 1 after)

A range of mammalian cells will be transduced with the vector and then integration and expression of the reporter gene measured. As the hazard is posed by the input lentivirus vector, after removal of residual input lentivirus vector the transduced mammalian cells will be considered Class 1.
Recipient or parental organism

- Lentiviral vectors - based on HIV-1
- E. coli cells
- Mammalian cells

Host/vector system

- The mini-genome, HIV-1 structural and replication enzymes, and donor glycoprotein will be cloned into standard cloning plasmids, such as those based on pcDNA3.1 (driven by a CMV promoter) or a pUC19. These plasmids will contain either an ampicillin or kanamycin resistance marker.

Origin & function

- All genetic material will be synthesised de novo by commercial companies, obtained from collaborators, or cloned form pre-existing constructs.

- pCSFLW or equivalent: plasmid that produces the vector minigenome. The mini-genome consists of the HIV-1 LTR (with a deletion in the U3 region), the HIV-1 packaging signal and a reporter gene (such as GFP/luciferase) that is under control of an internal constitutive promoter (such as but not limited to the SFFV promoter). This mini-genome production is under control of the 5’ LTR of the vector minigenome. Function - provide mini-genome to be incorporated into lentivirus vector;

- pCMVΔR8.9 or equivalent: plasmid expresses the GAG and POL polyproteins under control of the CMV promoter; contains an ampicillin resistance gene. Function - provide HIV-1 structural proteins to form the capsid of the lentivirus vector;

- pCMV-ENV-X or equivalent: plasmid expresses the glycoprotein of choice (X) under control of the CMV promoter; contains either a kanamycin or ampicillin resistance gene. Function - provide the donor glycoprotein to pseudotype the vector.

Evaluation of foreseeable effects

- Genetic material in E. coli cells:
  None - presence of plasmids in bacterial cells will not result in transgene expression due to lack of bacterial promoters. Therefore no HIV, reporter or pseudotyping proteins are produced. In addition, all E. coli are laboratory strains which have multiple auxotrophic markers and are unlikely to survive in the environment; presence of the genetic material would create a metabolic burden.

- Genetic material in mammalian cells:
  Presence of all three plasmids will result in production of replication-deficient lentiviral pseudoviruses. Generation of a replication competent virus (RCV) would require multiple highly specific recombination events between three plasmids that do not contain homologous retroviral sequences, including repair of the deletion in the 3’ LTR. The risk of this is effectively zero. Mammalian cells cannot survive in the environment as they require a nutrient-rich growth medium, an optimal temperature and a narrow pH range. They are also susceptible to drying and UV exposure.

- Pseudovirus:
  Presence of pseudotyped glycoprotein would allow the pseudovirus to enter any cell that the glycoprotein donor virus would be able to infect. Whilst the pseudovirus is replication-deficient and the transgene is considered non-harmful, integration of the pseudovirus’ minigenome into the recipient mammalian cell genome has the potential to disrupt host genes or disregulate the expression of host genes. Retroviruses are enveloped viruses that are highly susceptible to dehydration and are rapidly inactivated outside the host. The pseudotyped glycoprotein could allow infection of an animal species if the glycoprotein was able to bind to the appropriate receptors, however the pseudovirus is completely replication deficient and any infection would be a dead-end event.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All specimens, cultures of micro-organisms, materials and equipment used in the microbiology laboratory and protective clothing worn in the laboratory will be rendered free from contamination before re-use, discarding or repair.

For ACGM class 1 and 2 activities, material will be inactivated with disinfectants such as sodium hypochlorite (10,000 ppm, disinfection upon contact), virkon (5%, disinfection upon contact) or ethanol (70%, 30 minutes contact time) before being autoclaved.

Where large items of equipment need to be decontaminated, disinfection will be carried out by fumigation with a suitable, validated disinfectant vapour (e.g. formaldehyde, VHP).

Make-safe autoclave cycles operate at a minimum of 134°C for 30 minutes, (chart recorded every load) which are validated 6 monthly by the use of 12 point recorded thermocouples. Autoclave cycle print outs are verified by staff before waste is removed from the autoclave. Autoclaved waste materials are then incinerated on-site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All individual activities which fall into this notification will be submitted to the site GMSC. The GMSC will review each assessment and approves activities as suitable and sufficient as appropriate. The GMSC considers the environmental aspects of the activity as well as the human health and safety aspects.

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Animal Units

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Project Ref 202/93.5

Date Ackn'd 02/03/2022
CO-EXPRESSION OF VACCINE ANTIGENS IN VACCINIA VIRUS

Date Project Ceased: 23/11/1993

Class 2

Non-GMM Consent Granted: not applicable

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements: Y

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**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 202/98.2

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
## Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

### For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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**Is an emergency plan required according to regulation 20?**

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
Project Ref 202/98.3

Date Ackn'd 10/05/1999

CU2 Project Title IDENTIFICATION AND CHARACTERISATION OF TREHALOSE BIOSYNTHESIS GENES IN SALMONELLA TYPHIMURIUM

Class 2

CultureVolClass2 Class 2 CultureVolumeClass3-4

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Name**

UK HEALTH SECURITY AGENCY (UKHSA)

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

61 COLINDALE AVENUE

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

NW9 5HT

**Country**

ENGLAND

**Tel Number**

0208 200 4400

**Fax Number**

02083583003

**E-mail**

**HSE Division**

LONDON

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: [ ]

- **Give brief details of the genetic modification safety committee**

- **Level 1 (GMMs)**

- **Level 2 (GMMs)**: Yes

- **Level 3 (GMMs)**

- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**: Tick if confidential [ ]
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 203/01.1

Date Ackn'd 24/01/2001

CU2 Project Title STUDIES ON INFLUENZA A HAEMAGGLUTININ (HA) AND MATRIX (M) PROTEINS

Class Culture Vol

Class 2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Historical Significant Changes
**Project Additional Information**

**Purposes of the contained use**

The work is designed to increase our understanding of the effects of variant influenza A proteins on virus growth and human immunity. Some of the GMMs may also be used to develop diagnostic tests.

**Recipient or parental organism**

The influenza A proteins will be expressed either after transfection into MDCK/Vero cells or other mammalian cells, or incorporated into recombinant influenza A virus and used to infect mammalian cell lines. The transfected cells will not pose any foreseeable environmental or human health hazard. The recombinant viruses will have pathogenicity equal to that of laboratory strains handled under ACDP 2 conditions.

**Host/vector system**

The host system used to manipulate the influenza A sequences will be the E.coli strain TOP10. The vector will be one of the non-mobilisable vectors pc DNA3 or p Pol Isap I Rib (based on PUC18). Additional plasmids will be transfected into mammalian cells pHMG-NP, pHMG-PB1, pHMG-PB2, pHMG-PA which carry genes for NP, PB1, PB2 and PA proteins, under the control of the route HMG (Hydroxymethylglutory1 - coenzyme A conductase) promoter.

**Origin & function**

The genetic material to be cloned will be from human and avian influenza A isolates grown in mammalian tissue culture or eggs. Both the HA and M protein genes will be cloned in separate experiments. The M gene constructs will be used to produce a recombinant virus containing the amantadine resistance associated M gene in a helper virus background. The growth characteristics of the recombinant virus will be studied. The HA gene constructs will be used primarily to produce HA containing cell lines. HA expressing mammalian cells will be used for development of an assay for the detection of antibody to avian HA.

**Evaluation of foreseeable effects**

The GMOs are:
1. E.coli host strains carrying HA or M genes for influenza A. These organisms can be handled safely under category 1 conditions. There are no foreseeable effects.
2. Mammalian cell lines carrying and expressing influenza A genes. These cell lines can be handled safely under category 1 conditions. There are no foreseeable affects.
3. Recombinant influenza virus strains with M genes from parental amantadine resistant virus. These virus constructs can be handled safely under category 2 conditions which are equivalent to those used to handle the parental virus. All laboratory workers involved are vaccinated against influenza and monitored by swabbing in the event of a flu like illness. The recombinant virus would be equivalent to current laboratory strains in virulence.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Autoclaving to kill (100%) of all discarded cultures and contaminated materials.
Level 2 was considered by the Committee to be appropriate for the work proposed. The Committee was concerned about the nature of the recombinant viruses. On consideration it was decided that they would be equivalent to laboratory strains that carry an amantadine resistance conferring gene. Such strains do not pose a significantly greater risk than amantadine sensitive strains, and do occur in nature spontaneously.

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 203/03.1

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<td>CHARACTERISATION OF GENES RESPONSIBLE FOR ANTIBIOTIC RESISTANCE USING CLINICAL BACTERIAL ISOLATES AS HOSTS FOR GENETICALLY-MODIFIED MATERIAL</td>
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<td></td>
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## Project Additional Information

### Purposes of the contained use

The work is designed to increase our understanding of the genes responsible for antibiotic resistance in bacterial pathogens. Some of the genes characterised in these studies may be used to develop reference tests to enable screening and epidemiological studies. Under this programme, we will also study genes involved in DNA mismatch repair, which potentially play a role in the emergence of mutational antibiotic resistance.

### Recipient or parental organism

In these experiments, (i) genes associated with antibiotic resistance will be cloned into antibiotic-susceptible, laboratory-adapted strains of the bacterial genera under investigation, or (kii) gene fragments will be used to ‘knockout’ genes of interest, including DNA mismatch repair genes, from clinical isolates of these genera.

The final GMOs will be considered to have pathogenicity equal to that of the original clinical isolates handled under ACDP 2 conditions. They do not pose any foreseeable environmental or human health hazard; the constructs will be less antibiotic resistant than many current clinical isolates.

### Host/vector system

1. pSUP, pKNOCK and pG+host suicide vectors with E. coli hosts, DH5α, pir, S17-1 pir, S17-1 pir, VE7108 for the site-directed inactivation of genes contributing to antibiotic resistance, or involved in DNA mismatch repair.
2. pBC SK+ for shotgun cloning of Acinetobacter DNA into laboratory-adapted Acinetobacter calcoaceticus strain TRP E27
3. Ready-to-use EZ::TN Transposomes (Epicentre) for random mutagenesis of clinical isolates of Acinetobacter
4. The pAT18/19/28 series of vectors for cloning and transfer of eg recA in enterococci.

### Origin & function

The material to be cloned will be derived from clinical bacterial isolates. This DNA will be ligated into appropriate vectors (see above and attached proposals), and will either be transformed directly into clinical isolates of interest, or, in the case of the directed ‘knockout’ studies, will be cloned and manipulated initially in E. coli strains (as detailed in the attached proposals), before being introduced into the clinical isolates of interest.

The characteristics of the final GMO (derived from the clinical isolate) will be investigated to determine the contribution of the cloned gene to the antibiotic resistance phenotype of the strain.

### Evaluation of foreseeable effects

E. coli host strains with the cloned genes/gene fragments. These organisms can be safely handled under category 1 conditions. There are no foreseeable effects.

Recombinant Acinetobacter clinical isolates carrying cloned genes associated with antibiotic resistance, or fragments thereof. These constructs can be safely handled under category 2 conditions, which are equivalent to those used to handle the parental clinical isolate. All laboratory workers are trained in the manipulation of potentially pathogenic bacteria. The virulence of the recombinant strains is considered to be equivalent to, or lower than, that of the parental clinical isolate.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Autoclaving to kill (100%) of all discarded cultures and contaminated materials.

Agreed that local GMSC will be consulted and new risk assessments completed should this work extend to genera other than Acinetobacter and Enterococcus.

Committee discussed the resulting GMs, but could see no reason why they should be more pathogenic than the original host isolates.

Discussed the possibility that Tn-mutagenesis in Acinetobacter could result in resistance genes on cassettes. As many resistance genes occur as cassettes in integrons in gram-negative species, this was not thought to pose greater risk than clinical isolates.

**Project Containment**

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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**Project Ref** 203/04.2

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<td>10/05/2004</td>
<td>Study of cellular response to measles virus with SSPE derived genes</td>
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<td>Non-GMM</td>
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</table>
**Project Additional Information**

**Purposes of the contained use**

This work is designed to increase our understanding of measles virus (MV) characteristics in the persistent MV infection, subacute sclerosing panencephalitis (SSPE). Comparing host cell responses to wild type and SSPE derived measles viruses may provide clues how MV can evade the host defence systems and the mechanism of SSPE. This knowledge may lead to possible treatments of the infection and prevention of SSPE.

**Recipient or parental organism**

- Cloning of PCR amplified measles virus (MV) genes M and F from acute and subacute sclerosing panencephalitis (SSPE) clinical samples into pCR 2.1-TOPO and/or pGEM T-Easy vectors systems.
- Addition of FLAG tag at the 5’-end of cloned gene by site directed mutagenesis.
- Transfer of gene fragment from the cloning vector into p(+)MV (a pBluescript KS(+) vector containing the entire MV anti-genome (Radecke F. et al, 1995, EMBO 14(23), p5773)) to replace the original gene.
- Co-transfection of 293-3-46 mammalian cells (293 human embryonic kidney cell line stably expressing MV N and P proteins and T7 RNA polymerase) with p(+)MV and a pTM1 including MV L gene.
- Rescue of viable virus expressing the gene isolated from clinical material.
- Comparison of biological activities of original and mutated viruses in cell culture.

**Host/vector system**

Vectors/host: The chemically competent host E. coli TOP10 (F mcrA (mrr - hsdRMS - mcrBC) 80 lacZ M15 lacX75 recA1 and deoR araD139 (ara - leu) 7697 ga/U ga/K rpsL (Str) endA1 nupG) is a disabled commercial strain, developed for safe convenient cloning. This strain carries a very low hazard due to its low potential to colonise animal/human hosts and its poor ability to survive outside the laboratory.

Plasmid pCR 2.1-TOPO (Invitrogen): a construct containing colE1 ori, f1 ori, kanamycin resistance, ampicillin resistance, lacZ, T7 promoter cloning site. It is a mobilisation defective product developed for safe cloning.

pGEM-T Easy (Promega): pUC ori, f1 ori, lacZ, ampicillin resistance.

P(+)MV: pBluescript KS(+) (Stratagene) containing MV anti-genome under T7 promoter control, pUC ori, f1 ori, lacZ, ampicillin resistance.

PEMC-La: pTM1 (Moss et al, 1990, Nature 348, p91) containing MV L polymerase gene controlled by the encephalomyocarditis virus internal ribosome entry site, pUC ori, f1 ori, ampicillin resistance.

**Origin & function**

The material to be cloned will be derived from clinical samples. This DNA will be amplified by PCR and cloned into an appropriate vector (see above). These genes will then be integrated in a MV reverse genetics rescue system to rescue whole virus.
The characteristics of the final GMO will be investigated to determine the contribution of the cloned gene on host cell response to the virus.

**Evaluation of foreseeable effects**

The host/vector system utilises E. coli and the plasmids pCR 2.1, pGEM, p(+)MV. The host is disabled and the vectors are non-mobilisable. No measles protein should be expressed in this procedure. This suggests that the containment level for working with host/vector should be ACGM 1.

Unmodified MV is a Hazard Group 2 pathogen for humans. SSPE derived tissues are handled in a class II microbiological cabinet in a level 2 laboratory. The modified virus is expected to behave like SS:PE derived virus and should therefore be handled under similar safety containment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Autoclaving to kill (100%) of all discarded cultures and contaminated materials. The autoclaves are validated and have thermocouple tests on every run.

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<tr>
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<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
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Please enter comments on the GM safety committee on the risk assessment

Committee discussed the resulting GMs, but could see no reason why they should be more pathogenic than the original host isolates.

**Project Containment**

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<tr>
<td>Animal Units</td>
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**Project Ref** 203/16.1
A Human Immunodeficiency Virus (HIV) retroviral vector system has been adapted to allow the cloning and study of viral genes from HIV-1.

### Project Additional Information

**Purposes of the contained use**

To generate recombinant HIV-1 pseudoviruses for use in the phenotypic and genotypic characterization of viral genes from HIV-1. Study areas include, but are not limited to, investigation of drug resistance and viral replication fitness. Additionally, the pseudoviruses will also be used as reference material for the evaluation and validation of next generation nucleotide sequencing assays.

**Recipient or parental organism**

Lentivirus parental organism: Human Immunodeficiency virus-1 (HIV-1), classified as ACDP hazard group 3. HIV-1 genes of interest or parts thereof will be derived from laboratory-adapted viruses, molecular clones or clinical samples. Plasmids are maintained in K12-derived multiply-disabled E.coli strains HB101, TOP10F, STBL and DH5a. All these strains have multiple functiona l deletions which minimise recombination events (eg. recA1) and render them auxotrophic and unable to survive in the environment (eg. LeuB, ara14, proA2, etc.). The absence of frameshifting, splicing and glycosylation machinery in these bacterial hosts coupled with the absence of prokaryotic promoter sequences in the plasmids precludes expression of HIV-1 proteins. Well-characterised HG1 human cell lines including SupT1, H9, MT2, MT4, and 293T.

**Host/vector system**

The system is based on retroviral vectors developed for gene therapy in humans, where safety is paramount, and physically separates the genetic elements of HIV over two or more expression vectors. This minimises homologous sequences and introduces gene inactivation mutations to decrease the chances for recombination. The pseudoviruses produced are replication incompetent and only capable of a single infectious event. Multiple recombination events between non-homologous regions and/or multiple mutation events would have to occur to create a replication competent virus which is considered to be impossible.
Co-transfection of two or three plasmids provides HIV-1 proteins in trans. The genomic packaging signal is present on RNA transcripts with a combination of a reporter gene and variable fractions of a deleted HIV-1 genome (often highly deleted). HIV-1 surface glycoprotein is not present in pseudovirus virion particles, due to replacement by that of vesicular stomatitis virus (VSV-G).

Plasmids used in this study are detailed below. The two-plasmid system uses plasmids 2 and 4, whereas the three-plasmid system uses plasmids 1-3:

1. p8.91 or p8.9NSX (and derivatives of) encoding HIV-1 Gag-POL, Tat and Rev but with virulence genes vif, vpu and nef deleted, as well as truncation of env (on ly the regions needed for the tat and rev genes are maintained).
2. pMDG encoding the vesicular stomatitis virus glycoprotein (VSV-G) as an envelope protein. For the study of HIV-1 entry and co-receptor usage, the HIV-1 env is expressed from an appropriate plasmid in place of VSV-G.
3. The HIV-1 packaging signal is provided on an RNA that also contains a reporter gene under the control of the SFFV promoter and HIV-1 Long Terminal Repeat regions. This RNA is transcribed from one of the following closely related plasmids: pHR-SIN-CSGW (encoding GFP), pCSGW-YFP (encoding YFP), or pCSFLW (encoding firefly luciferase).
4. pNluc or pHl(WT] and derivatives encoding all HIV-1 proteins except for the virulence factor Nef and receptorbinding Env. In the former, Nef has been replaced by the firefly luciferase reporter gene.

The plasmids are transfected into eukaryotic transformed human cell lines as listed above. Upon transfection, pseudovirus particles will be produced and secreted into the tissue culture medium.

**Origin & function**

Non-vector-derived genetic material will originate from HIV-1 isolates obtained from clinical samples and/or laboratory strains. These will be cloned into the appropriate vector for expression in one of the recombinant pseudovirus systems detailed above, with target genes replacing exactly their plasmid homologues. The genetic material under study may be further subjected to site-directed mutagenesis to investigate the functional and phenotypic effects of specific point mutations.

**Evaluation of foreseeable effects**

Final recombinant virus: Owing to multiple deletions in the packaged genetic material, the recombinant pseudoviruses produced are capable of only a single cycle of infection. This is sufficient for use in a phenotypic assay where reporter gene expression can be quantified or used as reference material for validation or quality assurance of next generation sequencing assays.

Exposure of a lab worker could lead to integration of the packaged genome and localised expression of the reporter gene. The hazards associated with retroviral vectors are through the risk from accidental infection causing insertional mutagenesis and/or over-expression of cellular genes by random integration. The main risk is to the laboratory personnel directly handling the retroviral vectors. However, the replication-defective nature of the HIV vectors ensures that even in the case of a breach of containment and accidental infection, no virus can spread within an individual or from individual to individual.

The final recombinant virus is not a risk to the environment as the replication-defective HIV vectors are extremely susceptible to desiccation and will only survive a few hours at room temperature.

The non-vector-derived material comprises wild-type HIV sequences or those from drug-resistant isolates and will be cloned from HIV molecular clones, near full-length clones, laboratory HIV strains or HIV infected patient material. Insertion of genes from drug-resistant HIV poses no more danger than the original retroviral vector since the drug resistance genes are not packaged into the vector and no viral replication can occur.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Although HIV is classified as an ACDP level 3 biological agent, it poses limited risk of infection to laboratory workers as it normally cannot be transmitted by the airborne route. Therefore the production of retroviral vectors and subsequent work with live recombinant virus (e.g. single cycle phenotypic assay work) is to be carried out at containment level 2 within a Class II microbiological safety cabinet to minimise both human and environmental contact. Disabling mutations and splitting of the HIV genome into 2 or more plasmid vectors that result in a recombinant pseudovirus capable of only a single round of replication means the viruses are not pathogenic when tested in mice and a minimum of 3 recombination events and/or mutations are necessary to produce replication competent, recombinant virus.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste (g loves, plastic ware, etc.) are autoclaved on-site (100% kill). Autoclaves are monitored regularly for performance via thermocouples and records kept on site. Each autoclave discard run is monitored for satisfactory time/temperature levels. Soiled glassware and liquid waste is disinfected with chlorine at final concentration of 2,500ppm (prepared from sodium dichlorocyanurate tablets) and left for a minimum of 16 hours prior to disposal via drain. Work surfaces are decontaminated before and after use by using chlorine-based disinfectants at a minimum concentration of 1,000ppm.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Construction and characterization of Bacterial Artificial Chromosomes (BACs) containing Herpes Simplex Virus (HSV) Full-length genomes

The bacterial artificial chromosome (BAC) vector system will be used for the cloning, manipulation and maintenance of full-length herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2) genomes in E. Coli. The HSV BAC vectors will be used to generate recombinant HSV viruses for use in the characterization of genotype-to-phenotype correlates of viral genes or specific genetic changes from HSV clinical samples or molecular clones. We will use BAC vectors to clone, manipulate and maintain full-length HSV genomes that will then be expressed in eukaryotic cells to generate recombinant viruses. The recombinant viruses will be used to validate the phenotype associated with specific genetic changes identified in clinical isolates such as antiviral resistance. This information will be incorporated into a genotype-to-phenotype interpretation system for evaluation of HSV drug resistance from sequencing data.

HSV-1 and HSV-2, belong to the sub family Alphaherpesviridae in the family Herpesviridae. They are 120-300 nm in diameter and consist of a linear, double stranded DNA genome (152 Kb for HSV-1 and 155 Kb for HSV-2) enclosed within an icosahedral capsid, surrounded by a phospholipid rich envelope. HSV-1 and HSV-2 cause oralabial or anogenital infections with -70% and -20% seroprevalence in the UK population, respectively. Primary infection is selflimiting and is followed by latent infection in neural ganglia from where it can reactivate upon immunosuppression. HSV is classified as ACDP hazard group 2.
1. A BAC transfer plasmid vector will be constructed by introducing homology regions containing the HSV UL3 and UL4 genes into the commercially available BAC vector pBeloBAC11. Intergenic regions e.g. between HSV UL3 and UL4 genes are recommended for insertion of BAC genes which avoids interference of HSV promoter or polyadenylation sequences [Nagel, C.-H., Pohlmann, A. & Sodeik, B. in Herpes Simplex Virus Methods Protoc. (Diefenbach, R. & Fraefel, C.) (Humana Press, 2014)]. In addition, it has been demonstrated that insertion of foreign genes in the intergenic region between HSV UL3 and UL4 has no effect on virus growth in cell culture [Morimoto et al., 2009 Microbial ImmunolS3(3): 155-161].

2. HSV vectors generated by the introduction of a BAC origin of replication into an HSV full-length genome by homologous recombination in eukaryotic host cells. Circular viral genome intermediates which form as part of the HSV nuclear replication cycle will be harvested from the host cells by a modified Hirt method [Arad, U. Biotechniques 24, 760-2 (1998)] and used to transform E. coli.

The plasmid vectors will be maintained in OH10B E. coli cells. This strain is K-12-derived, and has multiple functional deletions which minimize recombination events (e.g. recA 1) and render the cells auxotrophic; and unable to survive in the environment (e.g. LeuB, ara-14, etc.).

The plasmid vectors will be transfected into African green monkey Vero cells or human fibroblast MRC-5 cells to generate recombinant vectors or virus particles.

Sequencing of full-length plasmid, vectors and recombinant viruses will be carried out after each cloning or mutagenesis procedure to confirm the presence of the introduced changes and absence of any undesired changes or foreign BAC genes.

### Origin & function

Specific frame-shift, point mutations or full-length HSV UL23 and UL30 genes or parts thereof derived from laboratory-adapted viruses, molecular clones or clinical samples will be introduced into wild-type molecular clones of HSV-1 and HSV-2 using site directed mutagenesis or by using different cloning techniques. These will then be assessed for their effect on antiviral drug resistance using a cell culture-based phenotypic drug susceptibility assay. The SACs origin of replication and associated genes will be introduced into the HSV genome to allow propagation of HSV vector as a single copy within E. coli cells, and facilitating cloning and stable maintenance of the large HSV genome.

### Evaluation of foreseeable effects

The recombinant HSV viruses that will be generated by the HSV SACs vector system will be constructed to contain no foreign genetic material and will only express genes from naturally occurring HSV viruses. The genetic changes introduced will be nucleotide substitutions that have been identified in clinical isolates to be associated with antiviral resistance. Only changes detected in a single clinical isolate will be introduced simultaneously, therefore it is highly unlikely that novel multi-drug resistance changes would be created by homologous recombination in the mammalian cells or E. coli. Thus, the main safety concerns with regards to using this system can be divided into two areas:

1. The potential for infection of laboratory worker with the recombinant HSV viruses
2. The potential for the insertion of foreign SACs sequences into the human genome

The potential for infection with recombinant HSV virus will be no greater than that for HSV isolates from clinical samples used for phenotypic drug resistance testing. The recombinant viruses will be expressing HSV genomes from laboratory strains or clinical samples and thus pose no greater risk. Appropriate risk assessments and safety measures are already in use for HSV phenotypic drug resistance testing.

The potential for insertion of foreign SACs sequences into the human genome will be eliminated by deleting the SACs sequences from the HSV vectors prior to the generation of recombinant viruses. Furthermore, the viral DNA produced by the HSV vectors persists inside the mammalian cell nucleus as an episomal element eliminating the
possibility of random integration into the host chromo.some.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be inactivated with chlorine diSanfectant followed by disposal through drain. All solid waste (gloves, plastic ware, etc) is sent for autoclaving on site (100% kill). Autoclaves are monitored regularly for performance via thermocouples and records kept on site. Each autoclave discard run is monitored for satisfactory time/temperature levels. Soiled glassware and liquid waste is disinfected with 0.5% hypochlorite or 5,000 ppm (prepared from sodium dichlorocyanurate tablets) and left for a minimum of 16 hours prior to disposal via drain. Work surfaces are decontaminated before and after use by using chlorine-based disinfectants at a minimum concentration of 5,000ppm followed by rinsing with water.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The committee had no objection to the risk assessment that had been undertaken for this GMSC proposal number R01017-538

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</table>
The objective of our work with GMM influenza virus is to increase understanding of immune responses to influenza viruses and influenza vaccines, and of antiviral susceptibility. One way of doing this is to measure antibodies to the different viral proteins including viral surface haemagglutinin (HA) and Neuraminidase (NA) protein. We know that the immune system makes protective antibody to, both proteins, which can be measured in functional assays, such as neutralisation, and HA and NA inhibition assays. These assays require live or native virus so that only functional antibody is measured (making recombinant protein unsuitable for the purpose). This is potentially problematic when the virus under investigation is not a seasonal virus - i.e. the laboratory personnel and the population at large are naïve/not immune to this – and results in the need to create safe viruses through genetic engineering, which are highly attenuated (crippled for replication in humans).

In order to make sure that we are measuring only antibody to the protein target(s) of interest (and not other viral proteins), we need to work with constructs where we can be sure that there are no confounding antibody Interactions. As part of the design of these, we edit which viral proteins are expressed on the virus particle surface to give the best chance of a definitive result. The resulting viruses contain a seasonal virus backbone (internal proteins) (usually A1Puerto Rico/8f1934; = PR8) for safety, expressing the HA and/or NA proteins from the virus of interest. Because of the central role of these surface proteins for virus infectivity; spread and host immunity, they are also important targets for antiviral drugs. We frequently need to investigate how these work and whether viruses develop resistance, which can also be assessed using the aforementioned recombinant influenza viruses.

Recipient or parental organism

The primary organism for GM work within our laboratory is "PR8", a former influenza vaccine strain: A1Puerto Rico/8f1934; = PR8".
Rico/8f1934(H1N1). PR8 is a highly attenuated Influenza strain, which is no longer infectious in humans.

Reassortants derived from PR8 have been used routinely for production of inactivated influenza vaccines for the past 30 years. Use of PR8 as the backbone of GM influenza viruses provides the first line in biological contained use, since any virus generated using PR8 internal genes will be replication attenuated in humans and animals.


To achieve our research and public health objectives, we wish to make use of genetically modified influenza viruses generated in two ways:

1) GM influenza viruses generated by collaborating laboratories.

1.A) Candidate vaccine strains to mitigate potential pandemic influenza virus threats are frequently created and shared through the Global Influenza Surveillance and Response System (GISRS): a network of laboratories experienced in working with influenza viruses. Viruses are shared under the WHO pandemic influenza preparedness (=PIP) framework and recommended by WHO as candidate vaccine strains. These viruses are generated by one of the designated PIP laboratories, de-pathogenized by specific removal of pathogenic sequences, safety tested and distributed through this WHO Framework. GM viruses created for this purpose use a PR8 backbone (6 internal genes) with the HA and NA from the novel, potential pandemic influenza viruses.

We have attached a list of viruses of interest which are part of the pre-existing notification for work with H5N1 viruses designated as CL2. To these H5N1 viruses we wish to add H5NX viruses and H7NX viruses (Table 1). Further viruses will be obtained, always subject to the same biological adaptations for contained use described above, in addition to the physical and chemical containment used within our laboratory.

1.B) We intend to utilise viruses generated at NIBSC specifically for experiments characterising the antibody response to the NA protein. This work requires a GM influenza virus expressing a non-human HA (to prevent anti-HA antibody interference in the assay). All of these reassortant viruses (6:1:1) obtained from NIBSC have been generated by reverse genetics with the HA gene derived from AJequine/Prague/56, the NA gene from different strains (Table 2) and the 6 Internal genes from AJPuerto Rico/8/34 (H1 N1) and are designated for use at CL2 by NIBSC. Their sequence will not be manipulated from the original aliquot.

2) GM influenza viruses generated in our laboratory not using the PR8 backbone.

2.A) The prototype influenza AJH1N1 2009 virus AJEngiand/195/2009 isolated during the first pandemic wave has been used to create a genetic backbone for reverse genetic studies. Since this virus (and therefore all gene segments) is in wide circulation in the community, there is widespread natural immunity. This strain is antigenically similar to the vaccine strain.

Modifications to the HA and NA expressed on the backbone of the AJEngiand/196/2009 strain will be to evaluate receptor binding properties, and the effect of antiviral resistance mutations on virus replication. The final GMMs will have the same pathogenicity and virulence as clinical Isolates of influenza A/JH1 N1 2009 virus. No HA or NA genes from non-seasonal viruses will be utilised on this backbone.

2.B) Cold adapted Ann Arbor backbone virus (either influenza AlAnn Arbour/B/60 or BIAnn Arbour/1/66) are the basis of the Live Attenuated Influenza Virus Vaccines (LAIV) in use in the UK since 2013. These cold adapted strains will contribute the internal influenza virus genes with the viral surface glycoproteins; the haemagglutinin and neuraminidase genes inserted from currently circulating strains, in accordance with the annual WHO recommendation. While Ann Arbor backbone-LAIV produced by AstraZeneca is licensed in North America and Europe respectively, Leningrad backbone-LAIV is produced In Russia (Ultravac®) and India (Nasovac-S®) and used primarily in those two countries. We may use those Leningrad backbone-LAIV strains if we can't get hold of the Ann Arbor backbones (i.e. in case of IP issues) or if we need to clarify differences in efficacy between products.
The host/vector system utilises E.coli as the host and the plasmid pRecBF-BsmBI as the vector. The host is disabled and the vector is non-mobilisable. There will be no attempt to maximise the expression of DNA, biologically active molecules may be expressed but are unlikely to have a deleterious effect. Containment level for working with host/vector will be containment level 2.

The host is a chemically competent E.coli strain TOP10F’ (F’lacIq, Tn10(TetR)) mcrA (mrr-hsdRMS-mcrBC) d40 lacZI(M15II,lacX74 deoR recA1 araD139 A(ara-leu)7687 galU galK rpsL(StrR) endA1 nupG) or similar. The vector (pRecBF-BsmBI) is based on the pUC18 plasmid, which is a non-mobilisable vector.

Origin & function

The inserted genes are those for the Haemagglutinin (HA) and/or Neuraminidase (NA), which are inserted into a backbone of seasonal influenza virus (current circulating, or with attenuating features). Haemagglutinin is a receptor binding protein, which attaches to cell surface receptors and mediates cell fusion. In high pathogenic viruses, this contains a polybasic cleavage site (=virulence determinant). Genetic engineering to remove this site renders the previously high pathogenic phenotype of the protein to low pathogenicity.

Source and type of HA we want to use:

We want to work with HA's from human influenza strains, found on viruses which circulate seasonally; for which vaccines are available and widely used and to which the population is generally immune (ie. H1 and H3). These HA's recognise and bind human upper airway epithelial cell receptors.

We also want to use avian and swine HA which are derived from viruses that could cause a pandemic, (because the population is not immune to these), as these get identified and suitable viruses get distributed by the PIP framework.

We want to use HA derived from NequineJPrague/56(H7N7) for use in NAI assays. While Human influenza viruses preferentially bind to a2,6-linked sialic acid receptors, found in the human respiratory tract, the HA from H7 equine influenza virus has been shown to have a preference for binding to a2,3-linked sialic acid, and differ from other viruses by binding to the N-glycolyl form of sialic acid (NeuSGc) which is the major sialic acid species found in the horse epithelium, but entirely absent from human tissue (and birds). This effectively results in a species barrier for the resulting GMM.


The Neuraminidase is a receptor destroying enzyme (also called sialidase) and cleaves glycan residues from cell surfaces to free budding virus particles which are trapped via their HA on the cell surface receptors as they emerge from the infected cell surface.

Source and type of NA we want to use:

The NAs are either from previous or current seasonal influenza strains of derived from avian and swine influenza; please see table 1 and 2.

Evaluation of foreseeable effects

All GMM covered in this proposal have similar hazard profiles and belong to ACDP2.

GMM with non human HA or NA

PRB is the only parent organisms that will be used as the generic back bone for insertion of any non human HA or NA. It is expected (and has also been shown for rgH5N1 and rg H7N7; rg=Reverse Genetic generated viruses) that these GM viruses are attenuated compared to the wildtype donor virus due to the presence of the internal genes from the laboratory strain PR8, either when grown in vi tro in eggs, or in vivo in infections of chickens or humans.

PRe belongs to previously circulating seasonal influenza A(H1 N1) and was isolated in 1934. It is highly attenuated (it...
is non-infectious in humans) and has been widely used in the field and is commonly used for vaccine reassortment -
the products of which are regularly part of commercial vaccine products with no indication of safety concern due to
this or parts of this strain.

Due to the circulation history of H1N1(s) strains during the periods 1917-1957, 1977-2009, and 2009 to the current
day (H1N1 pdm09), the majority of the population is not immunologically naive to H1N1 viruses.

Viruses are created by cloning the genes for the parent Haemagglutinin and/or Neuraminidase into the PR8
backbone, thereby removing any pathogenicity marker coded in internal genes (e.g. specific amino acid substitutions
in polymerase genes and NS1). No further manipulation of these internal genes is intended.

Pathogenicity in the context of influenza is categorised as high or low and refers to pathogenicity in poultry. It can be
predicted by looking for molecular markers which are mainly on the Haemagglutinin (and on proteins of the viral
backbone such as M1, M2, PA and PB1). The viruses described here carry the HA from seasonal, avian/swine and
equine influenza (Alequine/Prague/S6) on the virus surface. The function of the HA is receptor binding and fusion with
the host cell - this protein determines, which cell species a virus can infect. While the viral backbone determines
attenuation as explained above, the change in HA from H1 to an alternative HA, changes the host range and tissue
tropism from that of AIPuerto Rico/8/1934 (seasonal, with preference to bind a2,6-linked sialic acid receptors) to that
of the alternative HA (e.g. for Alequine/Prague/S6 virus => binding a2,3-linked sialic acid (N-glycolyl form of sialic acid
( Neu5Gc) *, which is not found on human tissue and is also not found in birds thus restricting the potential for spread
to species other than the horse).

The Neuramindases enzymatic function, should not lead to harm - Sialidases are currently trialed in human Phase II
trials as prophylactic treatment to prevent influenza infection (by removing cell surface receptors and thus blocking
virus entry). Both proteins, HA and NA are the main component of subunit vaccines, which make up the majority of
modern trivalent/quadrivalent influenza vaccines. Furthermore, DNA vaccines expressing these genes have been
trialed in animals - no adverse effect.

If handled within the code of practice and VRD H&S regulation, these gene products should not cause direct harm to
the staff, environment or wider public .

... A. S., G. C. Schild, and J. W. Craig. 1975. Trials in man with live recombinants made from AIPR8/34 (HO N1) and


There is a low but theoretical risk of recombination between the GMM and currently circulating strains (by means of
cross-contamination during work or dual infection of staff), potentially leading to viruses capable of infecting humans,
but not recognised by the immune system from being antigenically different to seasonal strains.

=> The main control measure being SEPARATION from other influenza strains and is achieved by exclusively
working in a specifically designated laboratory and a specified waste disposal route as described below.

Reassortment in a person (dual infection with wt virus and GM strain) is unlikely due the ‘design features’ of the GM
viruses (use of HAs that do not bind to human upper airway epithelial receptors; use of an attenuated backbone that
does not replicate in humans) and further reduced by vaccination policy (relative protection from infection with
seasonal influenza), the acquired immunity of healthy adults (from previous exposure to seasonal influenza viruses)
and exclusion of individuals who are Immunocompromised or pregnant from live virus work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None, containment at Level 2 with enhanced control of waste disposal.
SEPARATION: All work with GMM influenza virus is carried out in a designated laboratory (room) reserved for GM work only, within the Respiratory Virus Unit. Different GMM influenza viruses are not used at the same time in the laboratory, or are, separated further, by physical containment i.e. use of different microbiological safety cabinets.

Designated protective clothing
Laboratory coat and disposable gloves will be worn at all times in the lab. The lab coats to be worn by staff in this laboratory are green colour to differentiate the room purpose (white disposable lab coats are available for engineers and visitors). They will not be worn outside the specifically designated GMM laboratory. For disposal, used gloves will be put in yellow bags for incineration of clinical waste and the bag sealed and labelled with room number and date prior to transfer to wash-up for disposal. If gloves are known to be contaminated with influenza GM viruses, disposal is via a disposal jar for autoclave sterilisation. For cleaning, coats are autoclaved as enhanced CL2 waste before going to the laundry as for CL2 coats.

Waste disposal
Non-hazardous waste: Materials which have not been in contact with infective material are placed into black plastic bags, held in the waste bins in the Laboratory, which are removed when full and sealed before placing the bags in the appropriate trolley in the wash-up room and discard via contracted waste collector (offsite).

Uncontaminated plastic from the laboratory can also be recycled via the recycling bins provided in the lab.

Uncontaminated cardboard for recycling can be placed in the appropriate trolley in the wash-up room for collection by porters and discard via contracted recycling partner (off site);

Solid waste with infectious potential; e.g. bench and cabinet waste, and laboratory coats, must be autoclaved using specific sealable autoclave bins if those items had been used for work with reverse genetics constructs with nonseasonal HA and NA. For this, any item is enclosed in the specific autoclave-able bin liners, before being placed in the bin, the bin is labelled correctly and transported to the central autoclave facility by the lab staff, following Standard Operating Procedure (0-061) - the designated route for the waste trolley is stated in this document and has to be followed, while staff also witness the disposal and keep hard copies of the paperwork as evidence for correct waste disposal. Once autoclaved, the waste is discarded via contracted waste collector (offsite) by incineration.

Solid waste from work with all other RG viruses (e.g. constructs with seasonal HA and NA) is treated like wildtype seasonal Influenza as re-assortment with circulating strains is not a risk and is disposed via the route for clinical Category 2 waste. For this, any loose items not collected in a disposal jar (e.g. 96-well plates) are enclosed in the specific autoclave-able bin liners before being placed in the bin. Once autoclaved, the waste is discarded via contracted waste collector (offsite).

Green Lab coats are always autoclaved prior to washing - independent of type of work done.

Liquid waste with infectious potential: is either autoclaved (filled in Shott bottles) or treated with chlorine disinfectant at a concentration of 2500ppm (SOP V5708). Liquid waste is labelled with the date of discard and operator initials. Disinfected waste is transported in a sealed container to a room with a fume cupboard, where it is discarded in the sink (inside the fume cupboard). followed by flushing of the sink with copious water. A Chlorine Disinfection Validation Sheet (VWI33.01) is updated to allow tracking of the process.

Surface decontamination is achieved using chlorine disinfectant at 1000ppm. or Virkon ® for equipment sensitive to chlorine.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The committee had no objection to the risk assessment that had been undertaken for this GMSC proposal number RD0817-536.

Please enter comments on the GM safety committee on the risk assessment

The committee had no objection to the risk assessment that had been undertaken for this GMSC proposal number RD0817-536.

Project Containment

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Project Ref 203/18.2

Date Ackn'd: 20/04/2018

CU2 Project Title: To Determine the Role of Key Genes in Antimicrobial Resistance in Neisseria gonorrhoeae

Class: Class 2

Culture Volume: < 1 L

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Antimicrobial resistance in Neisseria gonorrhoeae is an area of increasing concern with therapeutic options becoming limited. NCTC has a panel of WHO N. gonorrhoeae control strains which have closed genomic sequences and well defined phenotypic and genotypic antimicrobial resistance profiles (Unemo et al., 2016). Mass spectrometry-based quantitative proteomic analysis of this set of strains revealed that in addition to the previously described genotypic markers (23S rRNA mutations and. the mtrCDE efflux pump operon) the macB protein was reproducibly upregulated in WHOV which is a strain which exhibits high level resistance to Azithromycin. The macB gene, according to its E. coli homologue, is an efflux pump for macrolides. However, its role in macrolide resistance in N. gonorrhoeae has not yet been described and further work is required to differentiate association from cause and effect.

Other studies that have examined macrolide resistance isolates of N. gonorrhoeae have concluded that the mechanisms of resistance are unknown in - 30% of resistant isolates examined (Grad et al., 2016). It is currently accepted in the literature that all the mechanisms of macrolide resistance have not been fully described despite NGS data being available for many strains and therefore it is likely that differences in protein expression levels may play a role.

A method to inactivate macB in N. gonorrhoeae through gene homologous recombination is proposed. The method is intended to both (i) disrupt the macB gene and (ii) insert a spectinomycin resistance selective marker from which mutants can be selected for because the transformation efficiency in N. gonorrhoeae is very low (Duffin, 2010). This method has been previously been successfully applied to disrupt a related gene (macA) in drug resistant isolates of N. gonorrhoeae in other studies and therefore is the preferred selection method of choice as there is a precedent for this being a successful, ethical and scientifically acceptable manipulation technique (Rouquette-Loughlin et al., 2005; Golparian, 2014).

References


Recipient or parental organism

Neisseria gonorrhoeae WHOV (NCTC13B1 B).

Host/vector system

A linear synthetic sequence containing macB flanking regions with a spectinomycin resistance expression cassette (aadA gene with promoter). The aadA gene encodes for an enzyme which modifies the spectinomycin molecule rendering the GMO resistant to spectinomycin.
This project is designed to inactivate the macB gene in the N. gonorrhoeae WHOV strain by gene homologous recombination. A linear synthetic sequence containing macB flanking regions with a spectinomycin resistance expression cassette (aadA gene with promoter) will be introduced into WHOV. The linear DNA sequence will be made to order and purchased through DNA synthesis service. The spectinomycin resistant selective marker is the aadA gene which encodes for an enzyme which modifies the spectinomycin molecule rendering the GMO resistant to spectinomycin. The spectinomycin resistance cassette will be synthesised and flanked with macB fragments on both sides (Figure 1). The transformants will be selected by their resistance to spectinomycin.

This approach has been successfully applied to disrupt similar genes (macA gene) in other drug resistant strains of N. gonorrhoeae in previous studies so there is a precedent in place for this method being successfully applied to resistant gonococcal strains.

Evaluation of foreseeable effects

This work will result in a strain of N. gonorrhoeae WHOV with a (i) non-functional macB gene (which may or may not have an effect on the strain's susceptibility to azithromycin) and (ii) contains an additional resistance to spectinomycin. The manipulated strain of WHOV will remain fully sensitive to the first line recommended treatment for N. gonorrhoeae ceftriaxone.

N. gonorrhoeae is an obligate sexually transmitted human pathogen which is transmitted via close and prolonged contact with mucosal surfaces. N. gonorrhoeae does not live outside of its natural host for long periods of time and it is very difficult to contract within a laboratory setting. Laboratory acquired infections of N. gonorrhoeae remain extremely rare. However isolated cases have been reported and eyes are the main route of infection for laboratory acquired infections. The WHOV strain (including an additional spectinomycin resistance marker) will present a very negligible risk to human health within the laboratory setting when the routine lab control measures (described in risk assessment) are implemented.

Origin & function

This work will result in a strain of N. gonorrhoeae WHOV with a (i) non-functional macB gene (which may or may not have an effect on the strain's susceptibility to azithromycin) and (ii) contains an additional resistance to spectinomycin. The manipulated strain of WHOV will remain fully sensitive to the first line recommended treatment for N. gonorrhoeae ceftriaxone.

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Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable all work will be conducted at ACDP2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All infected waste will be placed into Dispo-jars or autoclave bags (agar plates) which are then placed into autoclave bins and transferred to the facilities management department (FMD) for autoclaving at 1210C for 15mins followed by off-site incineration (BRD0026/03-1S; 00870S-1 4). All waste will be managed and transported between labs and the autoclave facility using the service provided by the FMD in accordance with SOP (0060).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The PHE GMO Committee have requested staff performing the work make a declaration to the Occupational Health Department that they do not have a history of penicillin allergy. In addition it was requested that the GMO should be destroyed when all scientific investigations on the GMO have been completed.

### Project Containment

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

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<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

Bacteriology | Yes | Parasitology | Yes | Transgenic Birds | Yes | Microbiology Research | Yes |
Virology | Yes | Transgenic Animals | Yes | Transgenic Fish | Yes | Gene Therapy | Yes |
Mycology | Yes | Transgenic Invertebrates | Yes | Transgenic Plants | Yes | Other (please specify below) | |

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste will be inactivated either by specified disinfection procedures or by autoclaving, prior to final disposal. Final disposal may be by various routes including to the non-hazardous waste stream (black bag/dustbin) or the clinical waste stream for microwaving or incineration. The clinical waste contractor has not made notifications to take waste containing genetically modified organisms, consequently all waste will be inactivated prior to consignment to the contractor.

Tick to confirm that you are attaching a summary of the risk assessment
All risk assessments will be reviewed by the local GM safety committees to confirm:
- proper and valid assessments have been made of the risks to human health and safety and to the environment;
- satisfactory decisions about the appropriate containment and control measures have been made; and
- the approach to risk assessment is in accordance with the guidance provided by ACGM taking into account the parameters detailed in the Regulations.
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref  12/00.2

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
**Project Additional Information**

**Purposes of the contained use**

The Open Biosystems library contains over 70000 individual E. Coli glycerol stocks containin the pGIPZ lentiviral vector or pSM2 retroviral vector. Each stock contains a unique hairpin sequence within the vector designed to target and reduce the expression of the majority of genes within the human genomes via RNA interference (RNAi).

The project will involve lentiviral or retroviral knockdown vector maintenance, storage and use of Open Biosystems shRNA library, including lentiviral mammalian expression plasmids, as well as distribution of clones as bacterial stocks DNA plasmids and or lentivirus to members within University College London for use in various projects.

Specifically, these constructs will be used to identify the key functional regulators of normal and tumour-derived neural stem cell lines.

**Recipient or parental organism**

Cell lines: a panel of mouse and human cell lines derived from mouse or human foetal nervous system and/or adult tissues or tumour biopsies.

Primary cell cultures: derived from mouse or human foetal nervous system and/or adult brain tissues or tumour biopsies.

Established cell lines: 293T (for viral preparations)

Bacterial E.coli strains: HB101, XL1-blue, TOP10, Prime Plus, JM109, BL21, DH5alpha, SURE, KS1000, DE3

**Host/vector system**

Lentiviral vectors: commercial TZV vector from Tranzyme and derivatives (including pGIPZ).


Retroviral vectors: commercial retroviral hairpin pSM (pSHAG-MAGIC-2) vector from Open Biosystems.

The viral vectors are lentiviral vectors. The vectors contain the CMV (Pol ll) promoter to drive expression of the hairpin cassette or gene sequence. The lentiviral vector does not contain the appropriate accessory elements to form a functioning viral particle. Therefore it poses no threat to health or the environment. Packaging and envelope proteins are contained on two additional separate plasmids which are required in combination to produce viral particles. This separation of genetic elements essential for full viral functioning provides an addition safety measure. There shRNA cassettes are designed to target specific genes and will result in their knockdown.

**Origin & function**

The RNAi hairpins are sequences that have been derived de novo and are not from an organism. They have been designed as short sequences with both a sense and antisense and loop sequence. This results in formation of a RNA hairpin following transcription which in turn will trigger the appropriate RNA interference response (RNAi). The accessory protein derive from the HIV-1 virus.

**Evaluation of foreseeable effects**

The hairpin sequence will be transcribed following delivery into the host cells. This hairpin structure will trigger a normal cellular response pathway that results in the target sequence being suppressed in its translation or stability - this is known as the RNA interference pathway. The lentivirus and retrovirus can only infect mammalian cells. The target sequence of the shRNA is designed specifically for human sequences. There is a small risk that 'off target' sequences might be hit, including other mammalian species.

As the sequences are designed to target human genes there is the possibility that if the vector was delivered by accidental transfer to human cells it would result in gene...
knockdown. If the resulting downregulated gene was a gene such as tumour suppressor this might result in aberrant regulation of cell proliferation pathways. However, the virus is replication incompetent and therefore would not spread beyond the initial cell targeted. Genetically normal human cells which received this vector would undergo a process of programmed cell death. Physical insertion of the vector into the host genome might also disrupt critical gene functioning. However, the likelihood of this is extremely low, and similarly cells would trigger an endogenous cell death pathway. Thus, the ability of the virus to be accidentally transferred to human cells is extremely low, there is no possibility for the virus to spread and even if inserted the cells would be removed rapidly as part of their normal cellular response.

If virus is introduced into the body by injection, ingestion or through a wound the virus could potentially enter cells and integrate into the genome. Although the virus is replication incompetent (does not carry the packaging protein) its physical insertion into cellular DNA could result in an oncogenic mutation. This would be a very unlikely event and if so only a very small number of cells would be affected. It would not impact on the pathogenic outcome of an exogenous viral infection. The packaging viral components are from HIV-1 virus. The full length HIV-1 virus is harmful to humans, however the packaging vector used does not contain full length HIV-1 molecule. Many components of HIV-1 that are critical for HIV-1 infection but not required for viral packaging have been removed. So the virus is attenuated and replication incompetent and will not make new viral progeny.

The most dangerous GMM is the lentiviral and retroviral preparations of viral particles. The most dangerous step is the collection, concentration and use of retrovirus/lentivirus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All work will be carried out in level 2 containment facilities within Paul O’Gorman Building. Stably modified human cancer cell lines will ne maintained using level 2 containment facilities.

All staff involved in the project will be made aware of the associated risks described here and will be required to have appropriate safety training prior to beginning work on the project.

Workers will be required to wear lab coats as well as latex or nitrile gloves whilst working on all procedures. Any open wounds will be covered with a bandage also.

All solid waste and any spills will be treated with Virkon and/or trigene advance, both of which are certified to kill 100% of virus, bacteria and cells.

After treatment, all solid waste will be double-bagged, autoclaved, placed in yellow biohazard safety bags and furhter treated by UCL waste services.

All viral supernatant will be stored in double containment and transferred within the laboratory within doubly sealed containers.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

2-5% Trigene advance or Virkon (v/v) will be used to treat liquid waste (18 hrs) and also used to decontaminate an spills and for disinfection of any reusable bottles or other equipment and laboratory materials used. Liquid waste is subsequently poured down the sink after treatment with Virkon. Work surfaces will be wiped down with 5% Trigene advance and 70% ethanol after use. Virkon and Trigene advance are certified by the PHLS to provide 100% viridal and GMO kill under these conditions be denaturing and inactivating viral and cellular proteins (Degree of kills: 100%). Trigene advance and Virkon will be used with its lifespan to ensure required kill is achieved.

Solid waste will be double-bagged in biological waste bags, sealed and autoclaved 132C for 15 min by trained staff then bagged in yellow biohazard bags and removed by UCL waste services. Autoclave runs are regularly validated by waste management staff.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
The project was approved by the GMSC, subject to minor changes, as an Activity Class 2 project.

Project Containment

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Project Ref 18/03.2

MOLECULAR ANALYSIS OF BACTERIAL INTRACELLULAR PROLIFERATION

Class 2

< 1 L

Consent Granted

Not Applicable

Project notified under transitional arrangements

Historical Significant Changes

TRANSFERRED FROM GM 18.

Project Additional Information
Purposes of the contained use

Fundamental research aimed at understanding the molecular and cellular mechanisms of intracellular survival using bacterial models. Our principal model organisms are Listeria monocytogenes and Rhodococcus equi. Our experimental approach involves the inactivation of selected genes of these bacteria by standard laboratory protocols and the analysis of the effect of the gene-disabling mutations on the bacterial intracellular proliferation capacity in vitro, on virulence in vivo, and on the response of the whole bacterial genome as assessed by DNA chip and proteomic technologies. The ultimate goal of this research is to identify targets for new vaccines, antimicrobial therapies and diagnostic tools to combat infections due to intracellular parasites.

Recipient or parental organism

The research will involve the generation of four types of GMMs.

1. Derivatives of L. monocytogenes and R. equi in which selected genes have been "surgically" inactivated to deduce their function by phenotypic comparison with the wild-type parental strain. Gene inactivation will usually be carried out by in-frame deletion, a technique that does not leave any trace of foreign DNA in the bacterial genome. With this technique with GMMs only differ from their parental organism in that they lack a DNA fragment corresponding to the deleted gene(s).

2. Gene-inactivated derivatives of L. monocytogenes and R. equi in which a wild-type copy of the gene under study has been reintroduced to confirm that the observed phenotype is actually due to this gene(s) and not to a secondary, spontaneous mutation affecting other unrelated loci.

3. Non-pathogenic cloning hosts carrying sequences of our model organisms (L. monocytogenes or R. equi), required for the construction of the gene-deletion derivatives described in (1) or for gene function studies. As recipient organisms we will use the following non-pathogenic bacteria:
   * Escherichia coli K-12 or B disabled derivatives (strains DH5a, HB101, C600, HB101, XL1-Blue, etc)
   * Bacillus subtilis
   * Lactococcus lactis
   * Listeria innocua, a non-pathogenic Listeria sp which lacks all known listerial virulence genes
   * A well-characterised derivative of L. monocytogenes ( prfA, picA, hly, plcB, actA) which was irreversibly rendered totally non-pathogenic by deletion of multiple essential virulence genes

4. Miscellaneous GMMs. The following GM bacteria may be incorporated as controls in some of our experiments:
   * Salmonella typhimurium SL1344sifA, a derivative of the laboratory strain SL1344 lacking SifA, a protein required for the maintenance of the vacuole in which Salmonella replicates intracellularly
   * Shigella flexneri laboratory strain SC560, rendered non-pathogenic due to an icsA-disabling mutation which impedes host tissue colonisation
   * Yersinia enterocolitica WA-314 attenuated derivatives due to disabling mutations in the sodA (superoxide dismutase) and irp (invasion associated protein), virulence genes.

Host/vector system

For routine gene cloning we will use non-mobilisable or mobilisation-defective plasmid vectors, such as pUC series-based vectors, pBR322 or Bluescript II for E. coli, and pE194 derivatives for gram-positive bacteria (B. subtilis, L. lactis or Listeria spp). To produce gene-disabling mutations we will use thermosensitive derivatives of pE194 in Listeria and pUC derivatives in R. equi. For inactivation of the uhpT gene in Salmonella typhimurium SL1344sifA and Shigella flexneri SC560 (see below) we may use the "suicide" vector pGP704. This vector can only replicate in, and be mobilised from, strain SM10Lpir, a host E. coli C600 derivative engineered to contain the RP4 mobilisation and R6K replication functions integrated in the chromosome. Once conjugally transferred from this host strain, plasmid pGP704 cannot replicate and be mobilised further.

Origin & function

From Listeria monocytogenes.
In the context of a major EU-funded collaborative initiative in functional genomics of Listeria spp., our group will be allocated a number of L. monocytogenes genes to
inactivate them by in-frame deletion. A complete description of the 2,853 genes of the L. monocytogenes genome and their predicted products is publicly available at http://genolist.pasteur.fr/Listilist. All the gene-disabled derivatives contributed by the collaborating European partners will then be systematically analysed in the different laboratories by a number of approaches, including DNA chip technology and proteomics. Our research will particularly focus on the following Listeria genes.

- prfA, encoding a regulatory factory homologous to CAP/Crp from E. coli
- actA, encoding a surface protein involved in actin-based motility
- plcA, plcB and smcL, encoding phospholipases active on phosphatidylylcholine and sphingomyelin, respectively
- hpt, encoding a sugar phosphate transporter homologous to the E. coli Uhp T permease
- ptsH, encoding a putative glucose-specific enzyme II permease component of a phosphoenolpyruvate sugar phosphotransferase system (PTS)
- bvrABC, encoding a PTS permease system specific for beta-glucoside sugars
- agl, encoding a two-component regulatory system
- luxS, encoding a putative quorum-sensing signalling system.

From R. equi

Our work will focus on choE and choD genes, encoding cholesterol oxidases that are widespread among saprophyte soil dwelling actinomycetes (eg Streptomyces spp). We believe that these enzymes may contribute to the capacity of R. equi to survive within eukaryotic host cells, whether they are mammalian microphages or soil bacteriovorous protists.

From S. typhimurium and S. flexneri

Our work will focus on the uhpT gene, encoding a glucose phosphate transporter identical to that present in E. coli K-12 and widely distributed among bacteria. We believe that this transporter may play a role in the uptake of sugar phosphates in vivo when bacteria are within host cells.

Evaluation of foreseeable effects

Hazard to human health

L. monocytogenes and R. equi, in which we are going to produce gene-disabling mutations, live as saprophotes in the soil and are widespread in nature. Occasionally, they can cause opportunistic infections in humans and animals and are thus classified in hazard group 2 in the Approved List of Biological Agents. Clinical infections occur only rarely, require a predisposing underlying condition and, presumably also, a high exposure. Both L. monocytogenes and R. equi are considered to have low infectivity and virulence. Under the designation L. monocytogenes we also include the closely related species L. ivanovii (formerly known as L. monocytogenes serovar 5), which is less virulent and has a narrower host spectrum than L. monocytogenes (L. ivanovii is not recognised as human pathogen). The gene *knock-out* derivatives of these bacteria will have the same low virulence as the parent strain if the deleted loci do not play a role in infection, or a lower virulence if the deleted genes play a role in infection. The *reconstituted* mutants, in which the deleted gene is reintroduced back for confirmation that the target gene is responsible for the observed phenotype, will have at most the same virulence as the parent strain. All these GMMs will therefore be handled under containment level 2.

The heterologous bacterial hosts that will be used as recipients of intermediate constructs for deletion mutant construction or to study gene function by complementation (E. coli K-12 or B disabled derivatives, B subtilis, L. lactis or L. innocua) are not considered pathogenic for humans or animals (hazard group 1). The attenuated L. monocytogenes (prfA, plcA, hly, plcB, actA) derivative lacks several critical virulence genes and consequently is entirely avirulent and non-pathogenic. The multiple gene deletions in this L. monocytogenes derivate guarantee that it is stably attenuated, there is no real possibility of reversion, and thus is a totally safe host strain.

Thanks to our participation in the Listeria sequencing projects we have precise information on the putative activities of the products encoded by L. monocytogenes. None of the L. monocytogenes genes code for known dangerous toxins, highly aggressive virulence factors or harmful products which can act directly to cause damage, allergy, oncogenesis or growth modulation. In the case where they do contribute to virulence, their role in infection is always subtle and they need to act alongside a number of other virulence and regulatory factors with which they have co-evolved in the source organisms. Therefore, the risk for one of the above heterologous non-pathogenic host organisms gaining virulence by introduction of L. monocytogenes sequences is negligible. Although in principle they might be handled under containment level 1, as a precaution and because they will be handled in parallel with the other bacteria, they will effectively be worked under containment level 2.
The miscellaneous bacteria that we may incorporate in our experiments all have disabling mutations that attenuate their virulence. S. typhimurium SL1344sifA, lacking the protein SifA required for the maintenance of the vacuole in which Salmonella replicates intracellularly, shows impaired replication in macrophages. The S. flexneri icsA derivative SC560 is virtually non-pathogenic (in fact one of its derivatives, strain SC602, is used at the institut Pasteur as the basis for the development of a vaccine against Shigella infection in humans). Yersinia enterocolitica WA-314, with disabling mutations in the virulence-associated sodA and irp genes, has impaired capacity to survive in vivo in animal tissues and is also used as the basis for a live oral vaccine. Nevertheless, these bacteria can have some residual virulence and therefore will be handled under the containment level required by their corresponding wild-type parental strains, which are classified in hazard group 2 (ie level 2).

The cholesterol oxidase genes choE and choD from R. equi, which are widespread among non-pathogenic actinomycetes (eg Streptomyces spp., Brevibacterium sterolicum), and the uhpT gene from S. typhimurium, encoding a sugar phosphate transporter also widely distributed among bacteria, are not expected to confer any virulence property to a non-pathogenic heterologous host.

The resistance genes used as selection markers in the cloning vectors have been approved for use at containment level 1 and confer resistance to "old" antibiotics for which resistance in clinical isolates is already widespread (and therefore are no longer used in clinical practice).

Hazards to the environment
None of the bacteria we will work with are exotic to the UK, are pathogenic to plants, or cause notifiable diseases under specific surveillance by DEFRA. They are already ubiquitous in the environment and are frequently carried by healthy animals. In some cases they may cause opportunistic infections in these animals.

The gene-disabling mutations introduced in the bacteria (in most cases by in-frame deletion, which does not leave any trace of foreign DNA) should normally lead to attenuation in vivo. Although these mutants may retain some of the original virulence of the parent strains, they will complete less well within an animal host and will be cleared from the infected tissues sooner than the wild type. The risk of pathogenicity is therefore reduced and less important than with the corresponding parent strain. The loss of a trait or genetic sequence that has been selected for through evolution is likely to reduce the fitness of the gene-deleted derivative to establish and survive in nature. Consequently, the gene-disabled derivatives, if accidentally released to the environment, are not expected to displace any indigenous population.

Listeria or R. equi bacteria in which the original phenotype has been restored by introduction of the wild-type allele in a plasmid vector always tend to lose these constructs because the vector is not naturally adapted to these host bacteria and represents a physiological burden (being thus rapidly lost in the absence of selective marker pressure). The non-pathogenic cloning hosts containing sequences from Listeria or R. equi are not expected to become established or be hazardous to the environment because the plasmid vectors will not be mobilised and the foreign genes, which are derived from ubiquitous soil dwelling bacteria, are already present in nature as a result of natural decay processes.

The likelihood of accidental environmental release of the GMMs is very low or negligible given the measures that will be applied to control any risk to human health and safety (level 2 containment). If release was to occur, the consequences can also be graded as very low or negligible. Therefore, the environmental risk can be judged as effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The GMM-contaminated waste will consist of broth/agar cultures, plastic labware and glassware. All this material will be inactivated in the building by autoclaving at 121 degrees C for 20 min (100% kill) prior to disposal or recycling. Carcasses of experimentally contaminated animals will be incinerated on site (100% kill). Non-contaminated general waste generated in the containment level 2 facilities and thus potentially exposed to the GMMs will be incinerated.
Discussed at Committee meeting of 19 February 2003 - extra information required re target genes and comment re pregnant workers suggested.

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022

Page 4799 of 15326
INVESTIGATION OF THE PATHOGENESIS OF THE HUMAN ALPHAHERPESVIRUS.

Project Additional Information

Purposes of the contained use
Biomedical research

Recipient or parental organism
Herpes simplex virus; Varicella zoster virus.

Host/vector system
Recombinant herpesviruses (Herpes simplex virus and varicella zoster virus) and replication defective derivatives.

Origin & function
The recombinant viruses will be based on established laboratory strains of HSV and VZV. Deleted viruses may contain marker genes derived from bacteria or the jelly fish green fluorescent protein. Viruses derived from the use of Bacterial artificial chromosome (BAC) technology may contain a bacterial origin of replication in addition to any marker genes or deletions. The HSV-DISC virus contains murine GMCSF that is only 43% similar to Human GMCSF.
The recombinant viruses would be expected to be of less or unaltered pathogenicity as the parental wild type viruses which are ACDP category 2 pathogens.

Use of HerpesBAc to generate modified HSV/VZV virus in tissue culture. In order to generate infectious virus the construct containing the herpesvirus genome as a bacterial artificial chromosome will be transfected into eukaryotic tissue culture cells. This will give rise to a genetically modified thymidine kinase minus (TK) virus. By comparison with other herpesviruses TK strains are normally attenuated HSV and VSZ are both ACDP category 2 pathogens therefore this portion of the work will be carried out in a Class II safety cabinet under Category 2 containment. All subsequent growth of modified virus will also be carried out under category 2 containment.

Generation of recombinant HSV by homologous recombination. Recombinant HSV can be generated following homologous recombination in eukaryotic cells between HSV sequences contained in an appropriate plasmid and superinfecting virus. This is the classical method by which recombinant HSV has been constructed. To select for recombinant virus a selectable marker under the control of an appropriate promoter will be used. Cells will be transfected with these constructs and subsequently infected with HSV. Mutant virus that arises by homologous recombination will be selected for by plaque purification in the presence of appropriate selection. Alternatively genes of interest will be inserted into the virus thymidine kinase gene so allowing selection using antiviral drugs such as Acyclovir.

As this work will generate mutated HSV, an ACDP Category 2 pathogen work based on manipulation of wild type HSV will be carried out under Containment level 2.

It is proposed however to primarily base these recombinants on a glycoprotein H deleted (gH) DISC (defective, infectious single cycle) virus which is replication defective and will only produce infectious virions when cultured on helper cell lines expressing gH. Similar studies to those outlined above will be carried out.

We will obtain from other workers a DISC virus that expresses murine GMCSF.

The DISC virus is replication defective and so cannot be transmitted from host to host. As these constructs are non-replicating the likelihood of infection of humans is low and the likelihood of transmission is effectively zero. The virus will cause a lytic infection at the site of injection and so some localised damage will occur. The insertion of genes (especially immunomodulatory genes) into the DISC virus may slightly increase the likelihood of adverse effects following infection. Therefore this work should be carried out under Containment level 2.

VZV cosmid. We will obtain from colleagues a series of 4 overlapping cosmids that span the entire VZV genome. These are non-mobilisable plasmids and no VZV genes are expressed in bacteria. The risk to humans of these cosmids is low/effectively zero. Mutation of virus sequences contained within these cosmids can be achieved following homologous recombination in bacteria using constructs outlined in section 1. No virus genes will be expressed in bacteria. Growth and manipulation of these cosmids can be carried out under Containment level 1.

To generate infectious VZV the complete set of cosmids are transfected into eukaryotic cells where low efficiency homologous recombination occurs resulting in the formation of full length infectious VZV genomes. The aim is to produce VZV deleted in specific genes for phenotypic analysis. Since these mutated viruses will be replication competent this work will be carried out under Containment level 2.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All spent culture media or other liquids, contaminated glassware, gloves microcentrifuge tubes, micro-pipette tips and tissue culture plastics are rendered safe by autoclaving prior to disposal as general waste. Pipettes (including Pasteurs) are completely immersed for a least 18 hours in Haztabs (Guest Medical) solution (Haztabs)
Solution = 1 tablet/litre of water = 2,500 ppm Chlorine) and then disposed of as clinical waste.

The autoclave is fitted with a thermocouple linked to a chart recorder to monitor the effective completion of the sterilization cycle. SOPs stipulate that no material should be removed from the autoclave without first checking that the cycle was completed. The degree of kill for autoclavng is effectively 100%.

Several studies have reported reductions in viral titre in excess of 99% following short (maximum 2hrs minimum 2mins) exposure to 2,500ppm Chlorine. These studies were carried out on both enveloped and non-enveloped viruses, on dried material, blood and virus solutions. The exposure of contaminated material for in excess 18hrs would be expected to exceed these levels.


Narang and Codd (1983) Action of commonly used disinfectants against enteroviruses J. Hospital Infection 4, 209-212 (100% kill).


This RA covers an extended project aimed at investigating herpesvirus pathogenesis. The initial bacterial cloning work and growth of the cosmid clones was considered straightforward and Containment Level 1. The manipulations of the BACs containing herpesvirus sequences was discussed and it was decided that the arguments laid out in the RA were acceptable and that the possibility of infection arising from ingestion of bacteria carrying these BACs was effectively zero: Containment Level 1. The work with recombinant virus was clearly Containment Level 2. The work with the amplicon was discussed and as the vector is non-replicating and will contain no HSV structural genes the risk to type operator and the environment is low/effectively zero: Containment Level 1. The work with the DISC virus was discussed. It was decided that although these vectors only undergo a single round of replication with no production of infectious virus the fact that they have the potential to establish a latent infection and are expressing an immunomodulatory gene should mean that they are handled at Containment Level 2.

The appropriate notifications to HSE must be completed.

Please enter comments on the GM safety committee on the risk assessment

This RA covers an extended project aimed at investigating herpesvirus pathogenesis. The initial bacterial cloning work and growth of the cosmid clones was considered straightforward and Containment Level 1. The manipulations of the BACs containing herpesvirus sequences was discussed and it was decided that the arguments laid out in the RA were acceptable and that the possibility of infection arising from ingestion of bacteria carrying these BACs was effectively zero: Containment Level 1. The work with recombinant virus was clearly Containment Level 2. The work with the amplicon was discussed and as the vector is non-replicating and will contain no HSV structural genes the risk to type operator and the environment is low/effectively zero: Containment Level 1. The work with the DISC virus was discussed. It was decided that although these vectors only undergo a single round of replication with no production of infectious virus the fact that they have the potential to establish a latent infection and are expressing an immunomodulatory gene should mean that they are handled at Containment Level 2.

The appropriate notifications to HSE must be completed.

Project Containment

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Project Ref 207/02.1

GENOTYPIC AND PHENOTYPIC VARIATION IN HUMAN IMMUNODEFICIENCY VIRUS: RELEVANCE TO TROPISM IN VIVO

Purposes of the contained use

Entry of human immunodeficiency virus (HIV) into human cells usually requires the presence of both CD4 and a member of the seven transmembrane G-protein coupled receptor (GPCR) superfamily on the target cell. Chemokine receptors are the most common GPCRs to be used for viral entry, with CCR5 and CXCR5 appearing to be most significant in vivo. Several other chemokine receptors can however also be used as entry cofactors but their role during the course of natural infection has yet to be fully defined. HIV displays a high rate of mutation and evolution and after primary infection this genetic variation allows the virus to expand its cellular tropism to infect new cell and tissue types. Mutations in the envelope (env) gene of the virus allowing utilisation of different receptors for cellular entry appears to be a key step in this adaptive process and as such can compound the severity of the disease process.

It would therefore be useful to investigate at a molecular level the genetic and phenotypic viral determinants that confer differential tropism. To this end we propose to amplify by polymerase chain reaction (PCR) and clone HIV-1 env genes derived from a range of cell and tissue types into a eukaryotic expression vector or into a HIV proviral backbone. For pseudotype construction, these clones will then be co-transfected into eukaryotic cells in culture, with a vector containing a standard HIV proviral backbone with a non-functional env gene and green fluorescent protein (GFP) replacing the nef gene. Alternatively, a recombinant, replication competent virus will be expressed from a recombinant proviral sequence into which the PCR-derived env sequences has been inserted. Pseudotyped or infectious virus secreted in the culture medium can then be used to attempt infection of either primary cell types or cells stably expressing CD4 and/or one of the chemokine receptors. If infection is successful then both structural proteins and GFP in the viral backbone will be expressed thus allowing relative entry efficiency to be determined either by direct visualisation under fluorescence or by indirect detection of viral antigens.

Recipient or parental organism
Host/vector system

Bacteria (E. coli K12 derivatives: JM 109 (promega) and Top 10F’ (Invitrogen) will be used solely for plasmid DNA amplification, not for protein expression. Vectors used will be pGEMT easy (Promega), pGL3 (Promega), pEGFP-C1 (Clonetech), pCR3.1 (Invitrogen), and pSVIII.

Origin & function

pEGFP-C1 and pGL3 will be amplified in E. coli then transfected, unmodified, using CaPO4 or electroporation into eukaryotic cells (293 T or C8166) as controls for transformation efficiency. Various viral genes and gene fragments will be amplified from clinical specimens and cloned into pGEMT easy or pCR3.1 for DNA sequencing and in the case of pCR3.1 also for transfection into eukaryotic cells (293T, C8166). The pSVIII constructs contain either one of a variety of HIV-1 envelope genes. Env sequences will be directionally ligated into the proviral backbone pNL43 2KpnI as previously described (Dittmar et al. Virus Genes 23: 281-290; 2001), and directly transfected into 293T cells for recombinant virus expression. Alternatively, amplified env sequences will be ligated into the expression vectors pSVIII or pCR3.1, and co-transfected with pNL43 envGFP to generate pseudotypes (He at al., Nature 385: 645-649; 1997). Replication competent or pseudotyped virus will tested against a range of CD4 and co-receptor expressing cell types (U87, NP2, GHOST) or primary cells.

Evaluation of foreseeable effects

The clones will be used both for direct DNA sequencing of inserts and expression in eukaryotic cells to produce virus capable of in vitro replication. The proposed experiment can be separated into two basic sections:

(1) Amplification of DNA in bacteria. Constructs are non-mobilisable plasmids in a disabled host. After bacterial transformation the genetically modified host is unlikely to survive outside the culture conditions. Maintenance of plasmids within bacteria requires specific concentrations of ampicillin the culture media and other conditions unlikely to be replicated in nature. Additionally the presence of the large NL4.3 envGFP plasmid results in a severe reduction in bacterial fitness even when compared to the standard disabled strains, further reducing the likelihood of bacterial spread outside of the culture conditions. The individual plasmid DNA extracted from these bacteria does not contain all the relevant sequences to produce functional virus. The expression of virus requires the mixing of two plasmids in appropriate proportions before careful eukaryotic transfection and can only produce low levels of viral particles capable of a single cell infection and no virus spread. The genetically modified bacteria and extracted DNA therefore represents low/no risk to human health.

(2) Transfection into Eukaryotic cells. The DNA will be transfected into 293 T cells using either calcium phosphate or electroporation. All steps associated with transfection and subsequent infectivity assays with pseudotyped and replication competent HIV-1 will be carried out at containment level III. In the event that DNA will be electroporated into 293T or other mammalian cells, this will be done in an adjacent laboratory which houses the apparatus, but immediately transferred to the containment level III laboratory following transfection. The relatively long replication cycle of HIV-1 between infection and generation of infectious virus (>18 hours) makes the electroporation procedure non-biohazardous; transfer to the containment level III laboratory will in all cases be carried out within 15 minutes of electroporation. Generation of infectious virus will in all cases, produce HIV that is identical to its native configuration; no attempt will be made to modify transcription, envelope proteins etc. We can therefore predict that containment level III facilities designed for handling natural isolates of HIV-1 will be suitable for HIV-1 generation by the above procedures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All spent culute media or other liquids, contaminated glassware, gloves microcentrifuge tubes, micro-pipette tips and tissue culture plastics are rendered safe by autoclaving prior to disposal as general waste. Pipettes (including Pasteurs) are completely immersed for at least 18 hours in Haztabs (Guest Medical) solution (Haztabs
Solution = 1 tablet/litre of water = 2,500 ppm Chlorine) and then disposed of as clinical waste.

The autoclave is fitted with a thermocouple linked to a chart recorder to monitor the effective completion of the sterilisation cycle. SOPs stipulate that no material should be removed from the autoclave without first checking that the cycle was completed. The degree of kill for autoclaving is effectively 100%.

Several studies have reported reductions in viral titre in excess of 99% following short (maximum 2 hrs minimum 2 mins) exposure to 2,500ppm Chlorine. These studies were carried out on both enveloped and non-enveloped viruses, on dried material, blood and virus solutions. The exposure of contaminated material for in excess 18 hrs would be expected to exceed these levels.


Narang and Codd (1983) Action of commonly used disinfectants against enteroviruses J Hospital Infection 4, 209-212 (100% kill)


This is straightforward project that aims to investigate the co-receptor usage of different strains/isolates of HIV. The approach taken is to use PCR to amplify the envelope gene from clinical isolates of HIV and to either 1) generate defective pseudotype HIV strains carrying the expressed env protein gene on the surface of the virus, but without the env gene being carried in the encapsidated genome. Or 2) to generate replication competent HIV carrying the cloned env gene. In the first case the virus generated will be replication defective and so should pose little or no risk to the population or environment. In the second case the virus being recreated is derived from and so similar to those existing in nature and so poses no additional risk above that of wild type HIV. In both cases the use of Category III containment is appropriate. The use of Category 1 containment for the initial cloning steps in bacterial is also appropriate.

Project Containment

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INVESTIGATING THE PATHOGENESIS AND IMMUNE CONTROL OF EPSTEIN BARR VIRUS (EBV) RELATED TUMOURS. TO INVESTIGATE THE INTERACTION BETWEEN LYMPHOCYTE SUBCLASS (CD4/CD8/NK) AND EBV INFECTED CELL LINES.

Biomedical research.

The recombinant viruses will be based on established laboratory strains of vaccinia, (thymidine kinase gene deletion) (western reserve strain) and synthesised by Alan Rickinson at the Institute of Cancer Research, Birmingham University. The EBV sequences were positioned downstream of the vaccinia P7.5 early late promoter.

Recombinant modified vaccinia virus with individual Epstein Barr Virus genes expressed.

LMP-2a
LMP-1
LP
EBNA 2
EBNA 3A
EBNA 3B
EBNA 3C
EBNA 1
MA 9210
Plasmids containing EBV-LMP 1 and 2 genes
The induced genes are to be expressed to (1) act as antigens to stimulate the proliferation of EBV specific lymphocytes, and (2) to allow recognition by lymphocyte effector cells. All EBV coding sequences used to generate the vaccinia virus recombinants were made of B95.8 virus.

**Evaluation of foreseeable effects**

The recombinant viruses would be expected to be of less or unaltered pathogenicity as the parental wild type viruses which are ACDP category 2 pathogens.

Culture of modified Vaccinia virus with Epstein Barr Virus (EBV) constructs.
The recombinant modified vaccinia virus with Epstein Barr Virus inserts were obtained from colleagues at Birmingham University. The vaccinia virus has a deleted thymidine kinase gene and is therefore attenuated compared to wild-type virus. The recombinant virus being approximately tenfold less virulent in mice than wild type virus (Buller et al 1985). Small stocks of vaccinia virus containing EBV constructs will be grown up and stored in the John Hughes Bennett Laboratory at the Western General Hospital where health and safety procedures are in place. Both the Vaccinia Virus and EBV are category 2 pathogens and therefore this portion of the work will be carried out in a Class II safety cabinet under Category 2 containment.

**INFECTION OF HUMAN CELL LINES WITH MODIFIED VACCINIA VIRUS WITH EBV CONSTRUCTS**

EBV negative cell lines will be infected with recombinant vaccinia in a small volume of PBS (0.5ml) in a 10 cm petri dish, the maximum number of cells to be infected with be approximately 10 x 10 (to the power of 6), at a multiplicity of infection 10:1. The cells will be incubated overnight and will normally be used in a chromium 51 release cytotoxicity assay. Both the Vaccinia Virus and EBV are category 2 pathogens and therefore this portion of the work will be carried out in a Class II safety cabinet under Category 2 containment.

**CHROMIUM RELEASE ASSAY**
The chromium release assay is in routine use and all radiological health and safety procedures are in place. In order to carry out this assay we will need to transfer small aliquots of infected cells (less than 10 x 10 (to the power of 6)) into the designated radioactive room and associated tissue culture room routinely used for this work. All cells will be placed in sealed plastic tubes in a sealed plastic box to transfer between rooms. Radioactive waste will be soaked in 1% virkon for at least 30 minutes, monitored for radioactivity and disposed of either down the sink designated for radioactive waste (liquid) or in the appropriate radioactive bins (solid). As the final stage of the experiment requires measurement in the gamma counter, 1% Virkon will be added to the tubes, prior to disposal in the radioactive waste.

**PROLIFERATION ASSAY**
If we require to do proliferation assays, we will decontaminate the cells with 1% virkon for at least 30 minutes prior to use of the Tomtec for counting tritiated thymidine release.

**Reference**
Buller RM, Smith GL, Cremer K, Notkins AL, Moss B

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All spent culture media or other liquids, contaminated glassware, microcentrifuge tubes, micro-pipette tips and tissue culture plastics are rendered safe by soaking in 1% virkon for at least 30 minutes prior to autoclaving to as general waste. All gloves and tissues used will be autoclaved.
The autoclave is fitted with a thermocouple linked to a chart recorder to monitor the effective completion of the sterilization cycle. SOPs stipulate that no material should be removed from the autoclave without first checking that the cycle was completed. The degree of kill for autoclaving is effectively 100%.

Radioactive waste will be soaked in 1% virkon for at least 30 minutes, monitored for radioactivity and disposed of either down the sink designated for radioactive waste (liquid) or in the appropriate radioactive bins (solid). As the final stage of the experiment requires measurement in the gamma counter, 1% Virkon will be added to the tubes, prior to disposal in the radioactive waste.

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The expression of potentially transforming genes was discussed and it was agreed that the lytic nature of the Vaccinia infection made the risk of transformation following infection negligible. The committee requested a comment re any immunomodulatory function of the expressed genes be made. This was done. The use of 30 minutes incubation in a 1% (final concentration) solution of Virkon as a means of disinfection of the small volumes of infected and radioactive samples was deemed satisfactory. (see www.antechh.com)

It was accepted that it was appropriate to carry out the work under category 2 containment.

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**Project Containment**

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### Project Additional Information

**Purpose of the contained use**
Biomedical research.

**Recipient or parental organism**
Murine (MCMV) and human (HCMV) cytomegalovirus, adenovirus, vaccinia virus, herpes simplex type 1.

**Host/vector system**
Recombinant herpesviruses (murine and human cytomegalovirus, herpes simplex type I) and replication defective derivatives.

Adenovirus
Vaccinia virus.

**Origin & function**
The recombinant viruses will be based on established laboratory strains of MCMV, HCMV, HSV I adenovirus and vaccinia. Deleted viruses may contain marker genes derived from bacteria, the jellyfish green fluorescent protein (GFP) or the bacterial lacZ gene. Viruses derived from the use of Bacterial Artificial Chromosome (BAC) technology may contain a bacterial origin of replication in addition to any marker genes or deletions.

**Evaluation of foreseeable effects**
The recombinant viruses would be expected to be of less or unaltered pathogenicity as the parental wild type viruses which are ACDP category 2 pathogens in the case of HCMV, HSV I, adenovirus or vaccinia, or category 1 in the case of MCMV.

Generation of recombinant CMV by homologous recombination. Recombinant CMV is generated following homologous recombination in bacterial cells between CMV sequences contained in an appropriate plasmid and the entire CMV genome contained in a BAC. The CMVBAC DNA is predicted to be infectious and therefore could theoretically be taken up by and infect a host cell if released intact from a killed bacterium in the gut. However, the efficiency by which virus DNA produces an infection following electroporation into permissive eukaryotic cells is low, and the likelihood of BAC DNA being released intact and then being taken up by a cell to produce virus is therefore low/effectively zero. HCMV (but not MCMV) to infect human cells and therefore any infection that did arise could be theoretically give rise to infectious virions. The consequence of infection via this route is however likely to be low as there is little evidence that HCMV is an enteric pathogen and it is unlikely that any released virus would survive. Therefore this portion of the work will be carried out under Category 1 containment.

Use of CMVBAC to generate modified HCMV/MCMV virus in tissue culture. In order to generate infectious virus the construct containing the HCMV or MCMV genome is a
bacterial artificial chromosome (BAC) will be transfected into eukaryotic tissue culture cells. This will give rise to genetically modified viruses. With the exception of MCMV, all viruses are ACDP category 2 pathogens; therefore this portion of the work will be carried out in a Class II safety cabinet under Category 2 containment. All subsequent growth of modified virus will also be carried out under category 2 containment.

Recombinant herpes simplex virus I. The recombinant HSV I strains containing the lac Z or GFP fusion products were obtained from the University of California, Irvine, USA. All work done with the infectious virus will be under Category 2 containment while work on viral nucleic acids will be done under Containment level 1.

Recombinant adenovirus. CMV regulatory sequences of interest are first cloned into the pShuttle-GFP vector. These are then co-transfected with the adenoviral backbone plasmid pAdEasy-1 (deleted for the oncogenic proteins E1 and E3) into bacteria to generate recombinants. Since no virus genes will be expressed in bacteria, these manipulations will be done under Category I containment. Thereafter the Adenovirus/CMV.reporter gene recombinant plasmids are transfected into the adenovirus packaging cell line 293, and the resulting recombinant adenoviruses are used for in vivo and in vitro studies in order to determine the tissue tropism of the wild-type or modified CMV regulatory sequences by measuring reporter gene activity. All this work will be carried out under Containment level 2.

Vaccinia virus. The mutant vaccinia virus contains the bacterial lacZ gene which is unlikely to enhance viral virulence or pathogenicity compared to its wild type counterpart.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Tissue culture plastics and all contaminated liquid waste such as spent culture media or blood products will be autoclaved prior to disposal as general waste. Individual waste bags will be tagged and detailed records will be kept as to their origin and time of sterilization. The autoclave is fitted with a thermocouple linked to a chart recorder to monitor the effective completion of the sterilisation cycle. SOPs stipulate that no material should be removed from the autoclave without first checking that the cycle was completed. The degree of kill for autoclaving is effectively 100%. Dry waste such as pipette tips and eppendorf tubes will be bagged for incineration as clinical waste. Similar tagging for identification of the bag contents will apply.

Recyclable dry waste such as glassware or contaminated equipment will also be rendered safe prior to washing Depending on the material, methods of decontamination may include autoclaving or soaking in a commercial detergent such as Virkon for at least 18 hrs. Disposable dry waste such as gloves, microcentrifuge tubes, micro-pipette tips, agarose gels and tissue towels are incinerated as clinical waste. Pipettes (including Pasteurs) are completely immersed for at least 18 hours in Haztabs (Guest Medical) solution (Haztabs Solution = 1 tablet/litre of water = 2,500 ppm Chlorine) and then disposed of as clinical waste.

Several studies have reported reductions in viral titre in excess of 99% following short (maximum 2 hrs minimum 2 mins) exposure to 2,500ppm Chlorine. These studies were carried out on both enveloped and non-enveloped viruses, on dried material, blood and virus solutions. The exposure of contaminated material for in excess 18 hrs would be expected to exceed these levels.

Narang and Codd (1983) Action of commonly used disinfectants against enteroviruses J. Hospital Infection 4, 209-212 (100% kill)
Tyler et al (1990) Virucidal activity of disinfectants; studies with poliovirus, J Hospital Infection 15, 339-345 (100% kill at 5 mins).

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form
This proposal is concerned with the use of recombinant human and murine cytomegalovirus and also the use of Adenovirus vectors. The proposal was well presented and had considered the relevant points. The work proposed is straightforward and none of the proposed constructs are likely to increase the pathogenicity of the GMOs. It was agreed that the proposal be approved by the GMSC at containment level 2. The PI will be informed that the HSE must be informed via a CU2 prior to initiating this work.

**Project Containment**

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**Project Ref** 207/04.1

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<td>03/03/2004</td>
<td>NEUROPATHOGENESIS OF RNA VIRUSES</td>
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**Historical Significant Changes**

- **Historical Date of Additional Info**: 05/08/2008
- **Significant Change ID**: 207/04.1a
- **Date of Significant Change**: 05/08/2008

**Tick if notifying a connected programme of work**: N
### Project Additional Information

#### Purposes of the contained use

The objective of this project is to enhance fundamental understanding of the neuropathogenesis of RNA viruses by determining genetic loci in viral genomes which determine phenotypic properties of neurotropic viruses.

#### Recipient or parental organism

Semliki Forest Virus, Bunyamwera Virus and Theiler's Virus.

#### Host/vector system

cDNA derived, Semliki Forest Virus, Bunyamwera Virus, Theiler's Virus.

#### Origin & function

Specific gene loci will be targeted in Semliki Forest Virus, Bunyamwera Virus, Theiler's Virus. These sequences may be swapped between different natural isolates that have varying phenotypes or may be mutated or deleted. Any transfer of genetic material will only be between different strains of each virus, there will be no gene transfer between the different species.

Also standard marker and reporter genes such as bacteriophage cre recombinase, fluorescent proteins (eg EGFP) and luciferase.

#### Evaluation of foreseeable effects

Most of the genetic changes that will be introduced into the viruses can be expected to attenuate fitness. However in some cases changes in the sequences of avirulence virus cDNAs which change the sequence to that of a virulent virus cDNA would be expected to increase virulence but the changes are such that it would be only to a level seen in wild type isolates. These engineered recombinant viruses would only have sequences found in natural virus isolates, they are therefore considered highly unlikely to have increased virulence compared to that of wild type isolates.

The viral envelope glycoproteins and capsid proteins which are the major determinants of host range and cell tropism will not be changed in these studies; host range and cell tropism are therefore unlikely to be altered. The changes are not expected to significantly alter susceptibility to host defence mechanisms. There are no vaccines or treatments available for these viruses and so effects of the modifications on these aspects are not applicable.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge to drains.

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Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), dispose via clinical waste stream for microwaving or via the industrial (black bag) waste stream for landfill.

Degree of kill:

Autoclaving - effectively 100% kill (annual validation)
Incineration and microwaving - not applicable, all waste is autoclaved prior to disposal by incineration or microwaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

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Animal Units

| L2 Yes L3 L4 L2 L3 L4 L2 |

Large Scale Activities

| L3 L4 L2 L3 L4 L2 |

Human Clinical Applications

| L3 L4 L2 |

Project Ref 207/04.2

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<tr>
<td>22/03/2004</td>
<td>STUDIES ON THE PHYSIOLOGY AND PROPERTIES OF BACTERIA OF THE GENERA CAMPYLOBACTER AND ARCOBACTER</td>
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<table>
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<th>Class</th>
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<tbody>
<tr>
<td>not applicable</td>
<td></td>
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</table>
The study of the physiology and properties of bacteria of the genera Campylobacter and Arcobacter in order to study their ability to survive in the environment and their responses to physical and chemical stresses. The role of specific cell envelope proteins and their conservation in different strains is also under study. These studies are aimed at improving food hygiene measures and developing new detection methods.

Various strains of Campylobacter jejuni, Campylobacter coli, other Campylobacter spp., Arcobacter butzleri and non-pathogenic Arcobacter species.

Plasmids for transposon mutagenesis, shuttle vector plasmids.

Mutants will be made (knockout, complementation and targeted) mainly to study genes involved in lipopolysaccharide or capsular polysaccharide biosynthesis, cell envelope proteins stress responses and housekeeping genes. Any inserted genes are indigenous to Campylobacter and Arcobacter species.

Standard marker genes like GFP and lacZ and antibiotic resistance (e.g. kanamycin and chloramphenicol) genes will be used.

No adverse effects compared to wild-type bacteria - gene function for the target gene will be eliminated in knockout and transposon mutants and either impaired or wild-type in SDM mutants. Harmful changes leading to either increased infectivity in humans or disease in animals is extremely unlikely.

No applicable

None

Except where implicitly stated otherwise in the risk assessment, all materials (culture vessels, media plates, glassware etc.) are disposed of by autoclaving before disposal. Solids (e.g. plasticware such as pipettes, flasks, tubes etc. and agar plates) - autoclaving using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125)
degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge of any excess liquids to drains, disposal of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclaving using a make safe cycle as specified in BS 2646, Part 3, 1993 (eigher 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

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Spillages - treatment with 1% (v/v) hypochlorite for small spills for 10 min. Paper towels used to absorb liquid are autoclaved before disposal. Hypochlorite is an extremely strong disinfectant for bacteria achieving a 5-log kill at 0.0005% (AISE).

Degree of kill:
Autoclaving - effectively 100% kill (annual validation)
Microwaving or Incineration - not applicable, all waste is autoclaved prior to disposal by microwaving or incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

### Project Containment

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**Project Ref** 207/04.4

**Date Ackn'd** 04/06/2004  
**CU2 Project Title** Role of the tumour suppressor APC (Adenomatous polyposis coli) in medulloblastoma  
**Class** Class 2  
**CultureVolClass2** < 1 Litre
Medulloblastoma is the most common type of brain tumour in children. It is thought that the tumour suppressor gene Adenomatous polyposis coli (APC, a component of the Wnt signalling pathway) can contribute to the development of medulloblastoma. The hypothesis that loss of the tumour suppressor gene APC in mice leads to the development of medulloblastoma (a tumour of the cerebellum) will be tested.

Recipient or parental organism

Mice genetically modified to confer susceptibility to Avian Leukosis Virus (ALV) will be infected with ALV vectors.

Host/vector system

ALV vectors will be propagated in chick embryo fibroblasts then used to deliver genetic material to mice. The work poses no foreseeable risk to human health and safety and appropriate containment procedures will be put in place to prevent environmental release. ALV is a retrovirus that causes leukosis in susceptible chickens. It is not able to infect mammalian cells.

Origin & function

Virus vector expresses cre recombinase from bacteriophage P1, this catalyses recombination between short inverted repeat sequences place in the mouse genome, thereby mediating gene deletion. The experimental mice have been genetically modified not only to express the ALV receptor in the developing cerebellum so enabling tissue specific infection by the vector, but also the APC gene has been floxed so that it is deleted by the cre recombinase on infection by the vector. The vector also expresses green fluorescent protein as an inert marker.

Evaluation of foreseeable effects

No risks to human health and safety have been identified and risks to the environment are comparable to those of the wild type virus with no additional risks arising from the genetic modification. The ALV vector is only able to infect the experimental mice because these have been genetically modified to express tv-a, the ALV receptor, in neural precursor cells in the developing CNS. Similarly, in the experimental mice the APC gene is only deleted because it has been floxed by genetically modifying the mice. Were the ALV vectors to infect a chicken there would be no action by the cre recombinase on genes such as APC. The recombinant virus is therefore considered unlikely to pose any additional risks to the environment when compared to the wildtype parent.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) discharge to drains.

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Degree of kill:
Autoclaving - effectively 100% kill (annual validation).
Microwaving or Incineration - not applicable, all waste is autoclaved prior to disposal by microwaving or incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

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</table>
## Project Additional Information

### Purposes of the contained use
To investigate the mechanisms by which different bacteria cause damage to the lungs alveolar wall. The role of potential virulence factors, such as bacterial adhesive proteins and toxins, will be investigated using mutant strains in which specific genes are deleted or over-expressed on plasmids.

### Recipient or parental organism
Staphylococcus aureus, Streptococcus pneumoniae, and Pseudomonas aeruginosa.

### Host/vector system
Bacteria with plasmid vector.

### Origin & function
Potential virulence genes deleted from bacteria genome and complemented mutant carrying deleted gene on single or multi-copy plasmid.

### Evaluation of foreseeable effects
Deleted mutants are likely to be less harmful than wild-type strains. Complemented mutants carrying deleted gene on single or multi-copy plasmid are likely to be no more harmful than the wild-type strains.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

Large Scale Activities

Human Clinical Applications
Project Additional Information

The aim of this project is to express protein-coding genes from helminth parasites such as Brugia malayi, Schistoma mansoni and Toxocara canis in the protozoal organisms Leishmania major and L. mexicana. The purpose of the work is to investigate genes that modulate or interfere with host immune responses to parasites.

Helminths are multicellular worm parasites which infect more than 1,000 million people worldwide. Their extraordinary prevalence reflects relatively low morbidity and the ability to accommodate to the host immune system. Pathology is generally due to over-zealous reactivity of the immune response to parasite products, and the prevailing paradigm is that helminths release a spectrum of active counter-inflammatory agents which dampen both immunity and immunopathology.

The helminth genes of interest are those thought to be responsible in altering the host immune response, allowing the nematode parasites to establish a chronic infection, and/or blocking the inflammatory process which cause pathology. The project will also include genes from model parasites which are not themselves infective to humans. Some of the genes are homologues of normal mammalian proteins (such as cytokines, which are soluble messagers of the immune system), while others come from gene families which are associated with the infectivity process but for which a defined function is not yet known.

In the proposed work, helminth genes will be engineered into Leishmania. These are well-characterised intracellular protozoal parasites of macrophages, which can be cultivated in vitro, and which provide a well-documented model for immunity to infection.

Recipient or parental organism

Protozoal parasites Leishmania major and Leishmania mexicana.
Host/vector system

Leishmania major, Leishmania mexicana and the shuttle plasmid pSSU.

Origin & function

The criterion for selecting a helminth gene for study under this project will be if it appears possible that the gene in question functions within the host, rather than within the parasite, by interacting with the mammalian immune system or related physiological networks such as inflammation. In addition, control proteins, including marker/reporter products from a range of species (such as jellyfish Green fluorescent proteins and derivatives, or avian ovalbumin), housekeeping proteins from parasites (as negative controls), and inserts from related but non-parasitic species such as Caenorhabditis elegans (which are unlikely to have evolved immune modulatory function) will be tested. Finally, some inserts will be truncated forms (domain-deletions) or site-directed mutants (in which active sites have been annulled).

Evaluation of foreseeable effects

The purpose of this work is to investigate genes that modulate or interfere with host immune responses to parasites. Whilst some genes may result in either no change or an improved or more effective immune response in clearing any infections, it is expected that one or more of the test genes will depress the host immune response to Leishmania, and thereby delay lesion healing. Tropism of different species has not been attributed to differential responses of the host immune system. Thus, immune suppression is likely to prolong survival in vivo, but not to alter the preferred site of infection or host range.

As with the wild-type organism, in the event of accidental infection, onward transmission to other individuals or spread in the environment cannot occur without the sandfly vector, which is absent from the UK. Infection can be treated with antimonial therapy, and the proposed genetic manipulation will not alter susceptibility of the parasite to such therapy.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Degree of kill:

Autoclaving - effectively 100% kill (annual validation)

Microwaving or Incineration - not applicable, all waste is autoclaved prior to disposal by microwaving or incineration.
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

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**Project Ref** 207/04.7

**Date Ackn'd** 29/11/2004

**CU2 Project Title** Environmental responses of bacterial pathogens of animals and man.

**Class** Class 2

**CultureVolClass2** < 1 Litre

**Consent Granted** Not Applicable

**Non-GMM**

**Project notified under transitional arrangements** No

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**
Purposes of the contained use

To explore mechanisms by which a number of defined pathogens of animals and man respond to and adapt to changes in their environment. The principal processes which will be examined will be microbial responses to incubation at low temperature (refrigeration), to oxidising agents (e.g., hydrogen peroxide which is found in sanitizers), interaction with surfaces (e.g., biofilm formation and exopolysaccharide (slime) production, as occurs during colonisation of artificial joints and implants). Fuller understanding of these adaptive processes may result in improved abilities to reduce infection or to enhance animal or human health. The study will focus on Salmonella (non-typhoidal), Campylobacter jejuni, Staphylococcus and Pseudomonas aeruginosa and will use these as comparative model organisms for exploring the similarities and differences in adaptive stress responses.

Recipient or parental organism

The programme is centred on a strategy of gene disruption, restoration and characterisation of function, therefore the recipients are normally the natural bacterial hosts or genetic derivatives of them. Highly related genes or gene fragments from other organisms may also be examined to allow study of how the gene differences convey subtle differences in function.

Salmonella (non-typhoidal), Campylobacter jejuni, Staphylococcus and Pseudomonas aeruginosa. Where cloning DNA fragments is undertaken as the principle purpose, this will use well-studied, debilitated or non-pathogenic hosts, such as E. coli or B. subtilis or attenuated strains of Salmonella.

Host/vector system

Bacterial hosts with standard, well-described, and commonly, commercially available plasmids. Where mobilisable plasmids are used, they will lack the ability for conjugal plasmid synthesis and self-transmission.

Origin & function

Most of the work involves complementation by returning normal bacterial genes or their fragments or specific recombinant derivatives (homologous genes) to the organism from which it was derived although homologues or gene fragments may also be transferred from other organisms where subtle differences in function are of interest. Genes which allow bacteria to recognise and respond to changes in their chemical and physical environments will be studied. The main responses which will be examined are adaptation to low temperature, oxidative stress and the influence of changes in growth phases where biofilm formation and exopolysaccharide production may occur.

Standard antibiotic resistance markers for ampicillin, chloramphenicol, kanamycin and erythromycin and well-characterised, non-harmful markers such as gfp, lux, luc, lacZ and xylE.

Evaluation of foreseeable effects

The general strategy for analysis will be one of gene disruption (disruption of function) and complementation (restoration of function). It is therefore not anticipated that the genetic manipulations of the organisms being studied will substantially enhance their pathogenicity, tissue tropism or host range, or introduce significant additional hazards when compared with those of the parental organisms or those occurring in the natural environment.

Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excreta liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatments or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via chemical waste stream for microwave treatment.

Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose of carcasses via clinical waste stream for incineration and bedding via clinical waste stream for microwave treatment or via the industrial (black bag) waste stream for landfill.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation)
Microwaving or Incineration - not applicable, all waste is autoclaved prior to disposal by microwaving or incineration.

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

Project Containment

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<thead>
<tr>
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<th>Growth Rooms</th>
</tr>
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Project Additional Information

Purposes of the contained use

Bacteroides spp. Are bacteria found in large numbers of the normal healthy human intestine. These bacteria are important for the correct development of the immune system and also for liberating many of the nutrients we consume. The methods by which Bacteroides survives and competes with other bacteria in the gut are not fully understood, but production of variable components on the bacterial cell surface are likely to help, perhaps by allowing it to avoid host immunity. If Bacteroides escapes from the gut into areas of the body in which bacteria are not normally found, for example following bowel surgery, they can cause infection. By studying virulence mechanisms and genes of unknown function further, we hope it will be possible to understand how these unusual bacteria provide benefits to the human host and will also identify potential targets against which new antibiotics can be developed for the times when these bacteria cause infection.

Recipient or parental organism

Bacteroides fragilis; Bacteroides thetaiotaomicron; Bacteroides vulgatus and other Bacteroides spp. Bacteroides spp. Are members of the normal resident microbiota of the human and opportunist rather than aggressive pathogens. Infections caused by Bacteroides spp. Generally occur following abdominal surgery or events that cause perforation of the bowel, eg ruptured appendix.

Host/vector system

Bacterial hosts with plasmid suicide and shuttle vectors.

Origin & function

Genes of interest are those involved in virulence, particularly the regulation of polysaccharide biosynthesis, DNA recombination and associated processes. Insertion and deletion mutants will be made. Antibiotic resistance markers eg, erythromycin resistance, inserted into the genome of any Bacteroides spp. Will not be a threat to human health because these antibiotics are not used therapeutically to treat Bacteroides infections. Transposon mutagenesis is a naturally occurring process and all the species
that we are interested in harbour transposable elements. Any disruption or alteration of gene function as a result of a transposon insertion is therefore already likely to have occurred in the natural environment. Mutations will be complemented, DNA for complementing mutations will be derived from Bacteroides spp.

**Evaluation of foreseeable effects**

The genetic modifications that will be carried out are limited to gene deletion and complementation. This is expected to result in GMOs that are likely to be either less fit or comparable to the wild type equivalent. It is considered unlikely that the changes would increase pathogenicity, alter tissue tropism or host range, or alter susceptibility to human defence mechanisms. Bacteroides spp. Are killed by oxygen at atmospheric levels and therefore will not survive in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via clinical waste stream for microwave treatment.

**Degree of kill:**

Autoclaving - effectively 100% (annual validation)

Microwaving or incineration - not applicable, all waste is autoclaved prior to disposal by microwaving or incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

**Project Containment**
Attaching and effacing (AE) bacteria are a group of naturally occurring non-invasive mammalian intestinal flora, including Citrobacter rodentium and some strains of Escherichia coli, that carry and express a set of genes that together comprise the Locus for Enteric Effacement (LEE). Normal activities of these genes produce proteins allowing the bacteria to attach tightly to gut epithelium after an initial superficial adherence, causing changes to the host cell structure.

The biological processes of AE bacteria during acute infection of hosts are currently being studied in many labs around the world and much has already been elucidated, using in vitro models of infecting cell lines and some studies with human volunteers. Most of the information gained relates to the immediate cellular consequences of AE lesions of host cells, or the incidence of acute diarrhoea, and little is known about any longer term, sub-clinical interaction of the bacteria and host epithelia.

Enteropathogenic (EPEC) strains of E. coli are an example of the AE group of bacteria and are the primary focus of this project. On the basis of strongly suggestive data of the long-term tumourigenic consequences of AE lesions from bacterial attachment to gut epithelia, and the incidence of detectable EPEC in the general population as a risk factor, we are interested in the cellular processes that may link EPEC infection to tumour formation.
Recipient or parental organism

Well characterised strains of enteropathogenic (EPEC) strains of Escherichia coli. Citrobacter rodentium.

Host/vector system

Bacterial host with various standard plasmid cloning vectors.

Origin & function

All strains have been designed and made to delete or inactivate one or more specific genes of the Locus for Enteric Effacement (LEE), important for the attachment and effacement process. Genes are inactivated as a result of insertion of recombinant gene segments with antibiotic-selectable markers.

Evaluation of foreseeable effects

The mutant strains are each defective in one of the proteins encoded by genes of the LEE pathogenicity island and are to be used as specific negative controls to dissect out of the genes/proteins of interest. Inactivation of each of these proteins has been reported to partially (greatly or minimally) reduce the virulence/pathogenicity of the strain. Since these deletion mutants are bacteria that lack proteins allowing fully effective attachment capability to their natural human host, it is not expected that any gain of tissue or species tropism could occur, nor has any such effect been documented so far.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g., plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

Liquids (e.g., samples, culture supernatants, tissue culture media) - treat with Presept tablet, autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g., needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via clinical waste stream for microwave treatment.

Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose of carcasses via clinical waste stream for incineration and bedding via clinical waste stream for microwave treatment or via the industrial (black bag) waste stream for landfill.

Degree of kill:

Autoclaving - effectively 100% kill (annual validation)

Microwaving or Incineration - not applicable, all waste is autoclaved prior to disposal by microwaving or incineration.
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

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Project Ref 207/06.2

Date Ackn’d 13/03/2006
Date Project Ceased 16/12/2015

The contribution of RNA secondary structure to the replication of mammalian positive-stranded RNA viruses

Class 3 CultureVolClass2 up to 100ml CultureVolumeClass3-4

Non-GMM Consent Granted Yes

Project notified under transitional arrangements N

Historical Significant Changes
Transferred to GM553 17/12/2015

Significant Change ID

02/03/2022
### Project Additional Information

#### Purposes of the contained use

To carry out functional investigations of predicted RNA secondary structures in positive-stranded RNA viruses. For the investigations planned, infectious clones from hepatitis C virus (HCV), Theiler's murine encephalitis virus (TMEV), human enterovirus (Coxsackie A21 CVA21) and other non-polio human enteroviruses will be manipulated to investigate a range of potential replication elements and other larger scale RNA structures associated with virus persistence. All manipulations are designed to modify RNA structure without altering the coding capacity of the region altered. All changes will therefore be made at synonymous sites in codons so that the proteins produced by the modified viruses are unchanged. The replication ability of viruses with modified RNA structure will be investigated in cell culture, and where appropriate, in a mouse model (TMEV).

#### Recipient or parental organism

**Bacterial**

Disabled E. coli K12-derived strains for cloning mutated DNA copies of the viruses used for RNA structure investigations. All bacterial strains are unlikely to be harmful to man or the environment.

**Mammalian**

A variety of standard continuous and primary cell lines will be used for virus expression. (3TC, Huh7 and a range of other human hepatoma-derived cell lines, Vero cells and other continuous or primary fibroblast cell lines).

#### Host/vector system

Infectious clones of TMEV, human enteroviruses and HCV.

#### Origin & function

Transfection of in vitro-generated RNA into mammalian cells allows the full replicative cycle of TMEV, other non-poliovirus enteroviruses and HCV to be initiated. These cell lines will therefore produce infectious virus, whose replication abilities can be compared to those of wild-type viruses.

#### Evaluation of foreseeable effects

All bacterial strains are disabled and therefore unlikely to be harmful to man or the environment. All mammalian cell lines for virus expression are in standard use and are known to be non-harmful for human health or safety.

RNA transcribed from clones generates potentially infectious virus particles when transfected into the appropriate mammalian cell line, which are capable of being further propagated in cell culture and on passage into animals. However, none of the mutations introduced to investigate the role of RNA structure on virus replication allow protein coding of the viruses, and no steps lead to the production of chimaeric viruses, the insertion of foreign genes in the viral genomes. Furthermore, none of the mutants are likely to alter virus tropism or host range, nor increase infectivity, virulence or transmissibility. Since all mammalian expression uses transfected viral RNA sequences, no antibiotic resistance genes or gene products will be introduced into mammalian cells. We therefore anticipate that the mutant viruses will generally show impaired replication ability or at most, restored fitness to wild type levels.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc). All solids exposed to potentially infectious TMEV, CVA21 and other non-polio enteroviruses at containment level 2 will be immersed in 3% Trigene for >12 hours or autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) and then disposed of via the clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill. Used agar plates will be autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes). Any excess liquids will be discharged to drains, and solid then disposed of via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill. All HCV-exposed solid materials in the containment level 3 laboratory will be autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes). Any excess liquids will be discharged to drains, and solid then disposed of via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - All culture fluids and other liquids containing potentially infectious TMEV, CVA21 and other non-polio enteroviruses at containment level 2 will be mixed with 3% Trigene for >12 hours or autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) and then disposed of through the drain. HCV culture fluids, and other liquid waste originating from work on HCV at containment level 3 will be mixed with 5% Trigene for >12 hours or autoclaved using a make safe cycle as specified in BS2646 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) and then discharged to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - These will be autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), disposed via clinical waste stream for microwave treatment.

Animal bedding and carcasses - These will be autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes). Carcases will be disposed of via clinical waste stream for incineration and bedding via clinical waste stream for microwave treatment or via the industrial (black bag) waste stream for landfill.

Degree of kill:
Autoclaving - effectively 100% kill of HCV.
Disinfectant - Trigene is used for disinfection and has proven efficacy (> 5 log reduction in infectivity) against highly resistant non-enveloped viruses such as enteroviruses, and also flaviviruses (Sources: MediChem, Abbott Analytical)
Microwaving or Incineration - not applicable, all waste is autoclaved or chemically disinfected prior to disposal by microwaving or incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.
A complex molecular and biochemical interplay exists between virus and host during infection, involving extensive interaction of viral and cellular proteins. Understanding the nature of these virus-host interactions is important for deeper understanding of the establishment and pathogenesis of viral disease.

A key feature of herpesviruses is their ability to produce persistent and latent infection, a mechanism for evading the host immune response. The main aim of this research is to better understand and define how herpesviruses interact with the host cell of the molecular and immunological level during the establishment of acute infection. Our primary interest is definition of the interactions between virus and host which follow infection with cytomegalovirus (CMV), a betaherpesvirus. We will make extensive use of genetically modified forms of human and mouse CMV to examine establishment of infection, replication and host response to the virus. We are particularly interested
In the role of the macrophage as a primary host cell for the virus since it operates as a key regulator of the immune response.

**Recipient or parental organism**

Human and mouse cytomegaloviruses (betaherpes viruses).  
Other viruses to be used for control purposes - typically herpes simplex, vaccinia and adenovirus.

**Host/vector system**

N/A

**Origin & function**

The HCMV and MCMV genomes will be modified by large scale deletions and defined point mutations to target genes involved in the viral replication cycle, particularly regulators of infection, and those which interact with the cellular immune response.

Viruses will contain various standard reporter genes for tracking and assay purposes - green fluorescent protein (GFP) from jellyfish, LacZ from E. coli, UPRT from T. gondii.

**Evaluation of foreseeable effects**

Human and mouse cytomegaloviruses

It is expected that the majority of viral mutations under study would generate a negative effect on virus replication since they are expected to lead to loss of function in the genes targeted. Therefore such alterations are unlikely to generate viruses with increased infectivity, virulence or pathogenicity and will most likely result in decreased infectivity and virulence. In the event of accidental inoculation of these viruses, they would therefore be expected to have either a wild type or attenuated level of infectivity and pathogenesis. It is unlikely that any of the CMV genetic modifications proposed in this project will lead to changes in tropism or the possibility of crossing the species barrier. A group of drugs (ganciclovir and related compounds) are used to control human CMV. The viruses used in this study would be expected to show wild type levels of sensitivity to these compounds. The commonly used genetic markers would not be expected to increase the infection capabilities of the viruses in any way.

Other control viruses

To provide more specific controls for certain techniques, certain wild type viruses carrying standard non-harmful marker and reporter genes markers such as GFP will be used. These modifications will not lead to enhanced replication or pathogenesis of these viruses, any changes in tissue tropism or host specificity, alteration in susceptibility to human defence mechanisms or any adverse effects resulting from inability to treat disease or offer effective prophylaxis where this might be available.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solids (eg plasticware such as pipettes, flasks, tubes etc) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes, or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - liquids will be treated with 1% Virkon for 2 hrs. to inactivate virus prior to autoclaving. Autoclaving will be carried out using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes, or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.
Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes, or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via clinical waste stream for incineration or microwave treatment.

Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes, or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via clinical waste stream for incineration or animal bedding may after autoclaving also be disposed of via the clinical waste stream for microwaving or via the industrial (black bag) waste stream for landfill.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation)
Incineration and microwaving - not applicable, all waste is autoclaved prior to disposal by incineration or microwaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

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**Project Ref** 207/06.4

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<td>Transient expression of foreign cDNAs from tobacco mosaic virus (TMV) and potato</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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The development of systems for the verification, identification and understanding of cellular and subcellular mechanisms involved in viral replication and viral:host interactions by studying transient expression in Nicotiana species of foreign cDNAs from tobacco mosaic virus (TMV) and potato virus X (PVX) based vectors.

Recipient or parental organism
Tobacco Mosaic Virus (TMV) hybrid U1/U5 vectors.
Potato virus X (PVX) genome (strain UK3) and PVX deletion mutants altered in movement functions.

Host/vector system
Host species.
Nicotiana tabacum (tobacco), Nicotiana benthamiana (closely related to cultivated tabacco and an important model host to study plant disease interactions) and other Nicotiana species.

Vector system
The methodology for expression of the TMV or PVX viral vectors in this project is either via agroinoculation (in which case standard cloning and plasmid construction will be employed using well-characterised methods with a history of safe use) or by direct inoculation of virion-like particles into the plant.

Origin & function
Arabidopsis thaliana cDNAs, Nicotiana tabacum cDNAs, N benthamiana cDNAs, Celery (Apium graveolens) cDNAs,
Standard well characterised fluorescent reporter genes such as GFP, dsRed and similar,
Plant viral movement proteins such as eg cauliflower mosaic virus 3a movement protein, patato mop-top virus triple gene block protein 2 and 3 genes, tobacco mosaic virus gene-shuffled movement protein.
The genetic material listed above is to be used for the microscopic identification in plants of the subcellular targeting address or expressed cDNA-GFP (green fluorescent protein) fusions, the identification of proteins that have the ability to move cell-to-cell or enable other proteins to do so and the identification of protein-protein and protein-RNA interactions in vivo. Additionally, this genetic material will be employed in confocal microscopy studies on viral RNA movement in and between plant cells.

**Evaluation of foreseeable effects**

TMV forms very stable rod-shaped particles in infection tissues. It is not seed or pollen transmissible, or transmitted by arthropods, nematodes or fungi-and is readily mechanically transmissible. In comparison to wild-type, virus-hybrid U1/U5 vectors and the recombinants that arise in planta from them are attenuated and restricted in host range and thus, due to the absence of suitable hosts or transmission means in the UK environment, are highly unlikely to have any adverse effects on plants, animals, humans or environment.

PVX is only transmissible by direct contact between infected plants or indirectly through mechanical transmission. PVX is not transmitted by aphids, and its host range is limited to solanaceous plants. It is proposed to use genome (strain UK3) and PVX deletion mutants altered in movement functions and thus less fit than those already in the environment. In comparison to wild-type, virus-hybrid vectors and the recombinants that arise in plants from them are attenuated and any escape in this form due to the mechanisms of transmission that would need to be involved, would pose a negligible risk to plants, animals, humans or environment.

The fluorescent proteins have been expressed in a wide range of experimental systems and there is no known toxicity or allergenicity risk associated with them. The plant cDNAs and viral movement proteins that will be expressed from the plant viral vectors occur in nature, thus novel toxicological effects are predicted. There is no known risk from the Plant viruses to humans and in all previous studies with TMV-based vectors the pathogenicity of these has been reduced in comparison to the progenitor virus.

The effect of over-expression of known and unknown plant cDNAs on vector accumulation cannot be predicted, however, in all previous studies with TMV-based vectors the pathogenicity of these has been reduced in comparison to the progenitor virus. The inclusion of plant derived sequences in a plant virus-based vector gives rise to the possibility of gene silencing. Depending on the source of the cDNA inserts silencing rates of between 1% and 10% have been observed on N. benthamiana, (which is not indigenous to the UK), based on novel phenotypic symptoms. Induction of solencing with endogenous gene sequences or transgene sequences results in reduced viral titre and infections are attenuated in comparison to wild-type virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

* All experimental solutions, material or consumables used to grow or manipulate bacteria/vectors will be autoclaved before disposal.
* All modified plant material is to be kept within the containment area, after which it is disposed of by autoclaving.
* All compost used within the glasshouse/growth rooms will be autoclaved before disposal, andy wash-off from plants will be collected and treated with appropriate chemicals before disposal.

Autoclave procedure - use a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration of microwave treatment or via the industrial (black bag) waste stream for landfill.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation using 12 point thermocouple of worst case loads) microwaving or Incineration - not applicable, ass waste is autoclaved prior to disposal by microwaving or incineration.
Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref  207/06.5**

**Date Ackn’d**  20/07/2006

**CU2 Project Title**

Search for novel drug and vaccine targets in Leishmania major, L. mexicana and L. donovani

**Class**

Class 3

**CultureVolClass2**

1-50 Litres

**CultureVolumeClass3-4**

Non-GMM Consent Granted Yes

Project notified under transitional arrangements  N

**With withdrawn**  N

Tick if notifying a connected programme of work  N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**
Leishmania spp. affect 12 million people worldwide causing a spectrum of diseases from cutaneous to visceral disease. The infections can be cured with drugs such as pentavalent antimonials, miltefosine, amphotericin B but the treatment is often not available or prohibitively expensive for people in developing countries. Therefore, there is a need for novel, cheap intervention strategies, e.g. new drugs or a vaccine. The protozoan parasites are transmitted by a blood sucking insect vector and the geographic distribution of the disease reflects the vector species distribution. The parasites replicate within phagocytes such as macrophages. We study the proteins expressed by the intracellular form of different species, as these potentially lead the way for the development of novel therapies or a vaccine. Our approach is based on the biochemical analysis of the parasite proteome whereby we have already identified nearly 500 proteins, of these a fraction is specifically or predominantly expressed in phagocytes and these include enzymes that may be potential targets for novel drugs and abundant parasite-specific proteins that are likely vaccine candidates. Thus, our strategy is to determine the relevance of the aforementioned candidate enzymes or vaccine candidates for the parasite’s fitness in vitro and in vivo by creating and characterizing parasites where the respective genes were knocked out, in order to select the most promising drug or vaccine targets.
ACDP guidance detailed above, various control measures specified at Containment level 3 are not considered necessary for this work and are proposed to be omitted. Consent is also sought for derogation of some additional measures, notably the laboratory to contain all its own equipment and inactivation of GMMs within the laboratory suite. Justification for not applying full containment level 3 measures is as follows:- Leishmania spp. including L. donovani are unable to form cryptic forms and are very sensitive to dehydration. In addition they depend on nucleic acid precursors for growth that are added to the rich media in which they are grown or provided to these intracellular parasites by host cells. As a consequence they cannot survive in normal media. They are also sensitive to hypoosmolarity and for example easily lyse in water.

- All Leishmania spp. are not per se invasive and require their bloodsucking sandfly vector for transmission and percutaneous penetration as a condition for successful infection.
- The parasites do not represent any hazard of infection through aerosol transfer. Aerosols are tiny droplets of liquid. These dry out within minutes, under the laboratory conditions of constant air flow and room temperature. Once dehydrated, parasites or parasite-infected host cells would not be viable. In relation to the individual control measures to be omitted from both laboratory and animal facilities: 1. Isolation: A dedicated room will be used for the laboratory work, however this will be used for all the work with parasites detailed in this risk assessment (i.e. in classes 2 & 3) and also for other similar parasite work (e.g. with P. falciparum). The room will at all times be operated to containment level 3 working practices and have a documented Code of Practice. The animal facility is separate from the other laboratories in the building being fully contained within a locked laboratory, with access restricted to authorised personnel. 2. Laboratory sealable for fumigation: There is no foreseeable need to fumigate the laboratory. In the event of a major spillage, the bulk of material would be soaked up on absorbent paper towels then autoclaved, killing any parasites. The affected area would be treated with appropriate concentrations of a suitable disinfectant. Any aerosols created that were not cleaned in this initial effort would dry out, killing both host cell and parasite. The area of the laboratory would be left isolated for a period to ensure effective drying. Effective parasite transmission is only possible via direct inoculation of viable parasites.
- 3. Negative pressure, HEPA filtered extract, microbiological safety cabinet or specified measures to control aerosol dissemination: The organism presents no hazard of infection by the airborne route. Class II microbiological safety cabinets are in use in the dedicated laboratory, they are necessary for sterile culture of the organism; they are not required for operator safety. Activities involve small scale, standard laboratory techniques with no aerosol generating procedures. 4. Autoclave and some other equipment not within the laboratory: Although the laboratory contains most of the necessary equipment for the work, some activities use specialist equipment that cannot reasonably be accommodated within the dedicated facility. In all cases a safe system of transport involving secondary containment will be used, any equipment used cleaned and disinfected immediately on completion of the work and any contaminated items either returned to the dedicated laboratory or removed for disposal as waste. The following activities will be undertaken outside the dedicated laboratory: (i) Fluorescence microscopy: a fluorescent microscope is located within the same building. (ii) Fluorescence activated cell sorting and analyses (FACS): a laboratory holding all FACS equipment is located within the building and operated according to a documented code of practice to accommodate this work. (ii) Storage: transfected parasite stocks are frozen and stored at -80°C or in liquid nitrogen within the same building. The freezers are locked and accessed only by authorised personnel. (iii) Autoclaving: autoclaves validated for disposal of waste are present in the same building and within the animal facility.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g. plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill. Liquids (e.g. in waste fluid containers) For the disposal of liquid waste in waste fluid containers, Presept is used at the concentration of 2500 ppm (1 x 2.5g tablet in 560 ml) in accordance with the manufacturers instructions for waste fluid jars. The disinfected liquid waste containers should stand for at least 6 hours to allow adequate exposure of all liquid waste to the Presept before discharge to drains. Alternatively, autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), then discharge liquids to drains. Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge via clinical waste stream for microwave treatment. Animal bedding and carcasses – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via clinical waste stream for incineration or bedding may also be disposed via clinical waste stream for microwave treatment or via the industrial (black bag) waste stream for landfill. Degree of kill: Chemical disinfection (Precept = Dichloroisocyanurate) – Precept has been validated for laboratory use against vegetative bacteria, fungi, viruses and bacterial spores by the manufacturer. Especially the latter are extremely resistant and difficult to kill. Leishmania are eukaryotic cells and do not possess equivalents of protective coats such as fungal cell wall that constitutes a diffusion barrier for chemical disinfectants and cannot form resistant structures such as spores. Dichloroisocyanurates, such as Precept, damage and inactivate organic molecules, e.g. proteins, by halogenation in an indiscriminate way. Thus, their effectiveness is dictated by the amount of organic material present in a solution. The medium in which parasites are grown contains less than 10% of the organic material present in whole blood for which a concentration of 10,000 ppm of Precept has been validated by the manufacturer and found to effectively kill spores as well as very difficult to inactivate viruses. A dosage of 2,500 ppm will be used for disinfection of waste fluids to
halogenate and inactivate the organic material present. Autoclaving - effectively 100% kill (annual validation) Microwaving or Incineration – not applicable, all waste is autoclaved prior to disposal by microwaving or incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

Project Containment

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Animal Units

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</table>

Project Ref 207/06.7

Investigation of herpes virus interactions with the host cell nucleus that involve nuclear transport, gene expression, RNA metabolism, and the nuclear envelope.

Date Ackn’d 23/08/2006

CU2 Project Title

Investigation of herpes virus interactions with the host cell nucleus that involve nuclear transport, gene expression, RNA metabolism, and the nuclear envelope.

Date Project Ceased

Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N
Historical Date of Additional Info

Significant Change ID

Date of Significant Change

### Project Additional Information

**Purposes of the contained use**

Herpes viruses are large double-stranded DNA viruses that are present in a wide variety of organisms, including both vertebrates and invertebrates. More than 130 herpes viruses infecting a range of species (only eight found in humans) have been identified to date, and these are classified into three subgroups, α-, β- and γ-herpesvirinae, based upon their genome sequence and biological characteristics. Individual human herpesviruses (HHVs) are highly prevalent with incidence ranging from one third to >95% of all tested being seropositive in the human population. At least one of the two herpes simplex viruses (HSV-1 and 2) infects up to 90% of the human population, typically causing mucocutaneous ulcers at multiple sites including recurrent oral-labial infections. In its atypical infections HSV is the leading cause of sporadic viral encephalitis and viral-induced blindness in developed countries. Despite the availability of several antiviral agents, herpesvirus infections (such as HSV-1, 2, and HHV-8) remain a serious problem. Although much is known about most stages of the viral life cycle and the HSV genome was sequenced in 1988 (even the latest herpesvirus identified to infect humans, HHV-8 discovered in 1994, was sequenced in 1996), because of the large genome size (~200 Kb) encoding bidirectionally from both strands not all genes are fully characterised. Some of the less detailed characterized stages of the viral life cycle are the interaction of viral proteins with the nuclear transport machinery and transport of viral mRNA and proteins across the nucleus, regulation of gene expression of various kinetic classes of viral genes, and the interaction of viral proteins with the nuclear membrane prior to and during assembly of virions in the nucleus. Genetically modified strains of herpesviruses along with the wild type virus will be used to investigate regulation of gene expression by herpesviral proteins and to study herpesvirus interactions with the host cell, mainly with cellular nuclear pore complex proteins and nuclear envelope proteins. The modified viruses used for this studies may include HSV-1, HSV-2, HHV-3, 4, 5, 6, -7 and -8 as many reagents are available for these viruses and they provide members of the all alpha, beta and gamma-type herpesvirinae as examples for investigation.

**Recipient or parental organism**

Human herpes viruses - herpes simplex virus types 1 and 2 and human herpesviruses- 3, 4, 5, 6, 7 and 8.

**Host/vector system**

N/A

**Origin & function**

The two major categories of human herpesviral genes of interest are - A) viral regulatory genes such as genes from the immediate early class for e.g. ICP27 of HSV or ORF57 of HHV-8 and their homologues; and B) viral tegument and capsid genes for e.g. UL31 and UL34 tegument genes of HSV and their homologues. Viral genes of interest will be either: (a) deleted (i.e. complete null mutant or partial deletion of the genes) to characterize the importance and function of the gene under study, or(b) deleted and provided either as wild-type or mutant in trans (ie by infection in complementary cell lines that express the gene of interest) so as to help in studying its function.

02/03/2022
and kinetics of regulation (its expression can be more exquisitely controlled in trans), (c) mutated (point or substitution or temperature sensitive mutations of the genes) to
dissect the function or functional interactions of the gene under study, or (d) replaced in the virus with their wild-type homologues from other HHVs. If the gene of interest is
essential then the ability of the homologues to provide wild type function would indicate a high degree of conservation, or (e) replaced in the virus with a mutated (point or
substitution or revertant mutations) or chimeric HHV homologue under its endogenous promoter to dissect its functional regions and the complimentary nature and
conserved function of the homologue gene, or (f) fused with epitope tagged versions (e.g. spectral variants of fluorescent proteins such as GFP, RFP, YFP, CFP, or the
recent FLASH tags) for direct live analysis of their localization and interactions within infected cells, or (g) fused with epitope tags (e.g. HA-tag, Histidine tag, TAP tag,
streptavidin tag) for convenient purification of viral proteins and their partners during stages of infection. We could also utilize a herpesvirus mutant that fuses i and ii, having
a non-essential herpesvirus gene deletion (such as a tk- strain) replaced with a marker/reporter gene that is also deleted or mutated in a gene of interest (e.g. ICP27) in
order to accurately quantify the effect of mutating the gene of interest on viral growth. Cellular nuclear envelope proteins that exhibit aberrant localization or accumulation
during wild-type herpesvirus infection will subsequently be tested for interaction with human herpesviral capsid proteins. In order to achieve easy i) localisation and ii)
purification, [eg to monitor gene expression and assess the efficiency of transfection] marker/reporter genes will be introduced in viral genome as inserts- in form of fusions,
and various epitope tags, with the gene of interest. Commonly used marker/reporter gene examples used in this proposal are well characterized and non-harmful.

Evaluation of foreseeable effects

The genetically modified viruses produced in this work are not expected to have increased virulence, pathogenicity, extended tissue tropism or host range or altered
susceptibility to host defence mechanisms when compared to wild type strains and in many cases viruses will be attenuated. Insertion of foreign genes at the thymidine
kinase locus results in viruses that are resistant to the antiviral agent acyclovir, but inactivation of the thymidine kinase also prevents replication in neurons and thus strongly
attenuates the virus progeny. However, in the event of infection with a thymidine kinase negative virus, foscarnet can be used therapeutically.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc) - disinfect with either 10,000 ppm sodium hypochlorite (eg Clorox) or 1% Virkon for at least 12 hours, or autoclave
using a make safe cycle. Liquids (eg samples, culture supernatants, tissue culture media) – disinfect with either 10,000 ppm sodium hypochlorite (eg Clorox) or 1% Virkon
for at least 12 hours, or autoclave using a make safe cycle. Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle. Autoclave
procedure: Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or
134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids as appropriate either via clinical waste stream for incineration or heat treatment
or via the industrial (black bag) waste stream for landfill. Degree of kill: Disinfection – the concentrations and contact times used for disinfection have been shown to result in
> 5 log reduction in virus titres (sodium hypochlorite - Croughan and Behbehani 1988 J. Clinical Microbiol 26, 213-215, Virkon - manufacturers data). Autoclaving -
effectively 100% kill (annual validation) Heat treatment or Incineration via clinical waste stream – not applicable, all waste is disinfected or autoclaved prior to disposal by
heat treatment or incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
### Project Containment

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

### Project Ref 207/06.8

<table>
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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<th>Project notified under transitional arrangements</th>
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<td>05/01/2007</td>
<td>Investigation of Plasmodium falciparum genes involved in adhesion to human host cells.</td>
<td>Class 3</td>
<td>Up to 200ml</td>
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</table>

- **Non-GMM Consent Granted**

- **Tick if notifying a connected programme of work**

### Project Additional Information

**Purposes of the contained use**

The pathology of malaria is caused by the adhesion of parasitized erythrocytes to human host cells (endothelial cells in the microvasculature, uninfected erythrocytes and platelets in the blood stream and syncytiotrophoblast cells in the placenta), which leads to obstruction to blood flow and impaired oxygenation of tissues. Parasitized erythrocytes also bind to immunoglobulin (Ig) M natural antibodies present in normal human plasma, which may play a role in allowing the parasite to avoid clearance by the...
host’s immune system. Previous work has identified a family of genes that play a crucial role in the adhesion of infected erythrocytes to host cells. The main purpose of this project is to understand the mechanisms of adhesion of infected erythrocytes infected with the malaria parasite Plasmodium falciparum to human cells in the clinical symptoms of malaria. We aim to investigate the role of putative parasite adhesion proteins in determining the binding of infected erythrocytes to human cells such as uninfected erythrocytes and platelets. We plan to modify or disrupt the parasite adhesion genes in order to analyse their precise role in the parasite’s adhesion capacity.

Recipient or parental organism

Plasmodium falciparum, the causative organism of the commonest form of human malaria.

Host/vector system

A variety of plasmid vectors that have been designed for Plasmodium falciparum transfection and genetic modification will be used.

Origin & function

We are studying P. falciparum genes that play a role in the adhesion of infected erythrocytes to human host cells. The work will focus on the var multi-gene family that encodes the infected erythrocyte variant surface proteins PFEMP1. Domains of PFEMP1 mediate adhesion to human cells such as erythrocytes, platelets and endothelial cells. The plasmid vectors include drug resistance genes that allow selection of transfected parasite under drug pressure. The drugs used for selection are WR99210, ganciclovir, cytosine deaminase and blasticidin. In addition, well characterised non-harmful tags or markers (such as a myc-tag or green fluorescent protein) will be incorporated into the above vectors to enable us to localise expressed/modified protein.

Evaluation of foreseeable effects

The experiments that we aim to perform are not expected to increase the virulence of the genetically modified P. falciparum parasites above the range found in unmodified P. falciparum because all P. falciparum strains have the capacity to display the adhesion phenotypes eg binding to erythrocytes and platelets. Experiments in which putative adhesion-mediating genes are disrupted or mutated are predicted to reduce the virulence of the genetically modified parasite strain. The genetic modifications we plan to carry out will not affect any genes controlling the invasion, host range or transmission potential of the parasite. The transfection process requires the use of vectors containing selectable markers such as WR99210 (an antifolate drug), ganciclovir (an antiviral), 5-fluorocytosine (an antifungal agent) and blasticidin (an antibiotic). As antifolates are frequently used antimalarials, the genetic modification decreases the susceptibility of the parasites to these chemotherapeutic agents. However, parasites resistant to antifolates are very common in nature and the single drug treatment with antifolates is never used chemotherapeutically. Blasticidin, ganciclovir and 5-fluorocytosine are not antimalarials and therefore their use poses no additional risks to induce resistance against currently used antimalarials.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

In accordance with ACDP guidance, various control measures specified at Containment level 3 are not considered necessary for this work with Plasmodium falciparum and are proposed to be omitted. Consent is also sought for derogation of some additional measures, notably the laboratory to contain all its own equipment. Justification for not applying full containment level 3 measures is as follows:- Plasmodium spp. including P. falciparum are unable to form cryptic forms and are very sensitive to dehydration. In addition they depend on nucleic acid precursors for growth that are added to the rich media in which they are grown or provided to these intracellular parasites by host cells. As a consequence they cannot survive in normal media. They are also sensitive to hypoosmolality and for example easily lyse in water. - Plasmodium spp. are not per se invasive and require their bloodsucking mosquito vector for transmission and percutaneous penetration as a condition for successful infection. - The parasites do not represent any hazard of infection through aerosol transfer. Aerosols are tiny droplets of liquid. These dry out within minutes, under the laboratory conditions of constant air flow and room temperature. Once dehydrated, parasites or parasite-infected host cells would not be viable. In relation to the individual control measures to be omitted from laboratory facilities: 1. Laboratory sealable for fumigation: There is no foreseeable need to fumigate the laboratory. In the event of a major spillage, the bulk of material would be soaked up on absorbent paper towels then autoclaved, killing any parasites. The affected area would be treated with appropriate concentrations of a suitable disinfectant. Any aerosols created that were not cleaned in this initial effort would dry out, killing both host cell and parasite. The area of the laboratory would be left isolated for a period to ensure effective drying. Effective parasite transmission is only possible via direct inoculation of
viable parasites. 2. Negative pressure, HEPA filtered extract, microbiological safety cabinet or specified measures to control aerosol dissemination: The organism presents no hazard of infection by the airborne route. Class II microbiological safety cabinets are in use in the dedicated laboratory, they are necessary for sterile culture of the organism; they are not required for operator safety. Activities involve small scale, standard laboratory techniques with no aerosol generating procedures. 3. Some other equipment not within the laboratory: Although the laboratory contains most of the necessary equipment for the work, some activities use specialist equipment that cannot reasonably be accommodated within the dedicated facility. In all cases a safe system of transport involving secondary containment will be used, any equipment used cleaned and disinfected immediately on completion of the work and any contaminated items either returned to the dedicated laboratory or removed for disposal as waste. The following activities will be undertaken outside the dedicated laboratory:i) Fluorescence microscopy: a fluorescent microscope is located within the same building.ii) Fluorescence activated cell sorting and analyses (FACS): a laboratory holding all FACS equipment is located within the same building and operated according to a documented code of practice to accommodate this work. Whilst this work is in progress access to the FACS facility is restricted to authorised workers trained to CL3 standards and all infectious materials are removed on completion and the area cleaned and disinfected prior to return to normal use. iii) Storage: transfected parasite stocks are frozen and stored at –80οC or in liquid nitrogen within the same building. The freezers are locked and accessed only by authorised personnel.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Most of the material that has come into contact with parasites will be soaked for 24 hours in 2% Virkon. Thereafter, or for any items not disinfected first, the following waste disposal procedures will be used:Solids (eg plastic ware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125οC for at least 15 minutes or 126-130οC for at least 10 minutes or 134-138οC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill. Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125οC for at least 15 minutes or 126-130οC for at least 10 minutes or 134-138οC for at least 3 minutes), discharge to drains. Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125οC for at least 15 minutes or 126-130οC for at least 10 minutes or 134-138οC for at least 3 minutes), dispose via clinical waste stream for heat treatment. Spills are treated with powdered Virkon (for larger volumes) or 2% Virkon (smaller volumes) and aerosols contained with paper towels. Degree of kill: Autoclaving - effectively 100% kill (annual validation) Disinfection – not applicable, all waste is subsequently autoclaved prior to disposal Heat treatment or Incineration – not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

Project Containment

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Bacterial infection is commonly associated with tissue injury. The injury may be produced by bacterial virulence factors, by inflammatory mediators generated in an-exuberant host response, or by both. Dissection of the relative contributuion to tissue injury of bacterial and host factors is hampered by the inability to study bacterial pathogens in "real time" during the course of an infection.

This unsatisfactory position has been superseded by advances in bioluminescence imaging combined with the use of genetically modified micro-organisms expressing luminescent/flourescent marker genes. These tools provide unique opportunities to study the dynamics of infection and will be used here to study the temporal and spatial dynamics of bacterial infection.

**Recipient or parental organism**

Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae

**Host/vector system**

Bacterial hosts with standard transforming plasmid vectors

**Origin & function**

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02/03/2022

Page 4846 of 15326
Well characterised bioluminescent or fluorescent reporter genes such as luciferase (lux) from the bacterium Photorhabdus luminescens and green fluorescent protein (GFP) from the jellyfish Aequoria victoria.

**Evaluation of foreseeable effects**

The incorporation of well characterised, non-harmful reporter genes is not expected to affect virulence, tissue tropism, host range, susceptibility to host defence mechanisms, or susceptibility to available antibiotics. The modified organisms may be expected to have slightly reduced fitness. No antibiotic resistance markers will be introduced.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 C for at least 15 minutes or 126-130 C for at least 10 minutes or 134-138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 C for at least 15 minutes or 126-130 C for at least 10 minutes or 134-138C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels ) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 C for at least 15 minutes or 126-130 C for at least 10 minutes or 134-138C for at least 3 minutes), dispose via clinical waste stream for heat treatment.

Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 C for at least 15 minutes or 126-130 C for at least 10 minutes or 134-138C for at least 3 minutes), dispose of carcasses via clinical waste stream for incineration and bedding via clinical waste stream for heat treatment or via industrial (black bag) waste stream for landfill.

Degree of Kill: Autoclaving - effectively 100% kill (annual validation using 12 point thermocouple of worst case loads). Heat treatment or Incineration - not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

**Is an emergency plan required according to regulation 20?**  N

**If yes, tick to confirm that it is attached to this form**  N

**Tick to confirm that you have attached a risk assessment to this form**  Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety to the environment.

**Project Containment**
Project Ref 207/07.2

Date Ackn'd 11/07/2007

CU2 Project Title Investigation of factors influencing virulence, survival and antimicrobial resistance of the bacterial pathogens infecting the cystic fibrosis lung.

Class Class 2
Culture Volume Class 2 1-50 Litres

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Investigation of bacterial pathogens responsible for opportunistic infection of the cystic fibrosis lung. In particular:
1) Investigate the phenotype associated with putative virulence factors and antimicrobial resistance determinants through gene knockout and complementation studies
2) Assess the importance of individual virulence factors in the ability to establish infection of the mouse lung and/or persistence within human/murine cell lines.

Recipient or parental organism

Burkholderia cepacia complex, Pseudomonas aeruginosa, Ralstonia species

Host/vector system

Standard transforming plasmids / cloning vectors

Origin & function
The genetic material involved will fall into one of two categories:

1) Natural bacterial gene sequences
Putative virulence factors and antibiotic resistance determinants from bacterial species ubiquitous in the environment.

2) Bioluminescent / fluorescent markers
Photorhabdus luminescens luciferase (lux) operon, which has bioluminescent functions allowing gene expression to be monitored by detection of photonic energy (using a charge coupled device); green fluorescent protein (GFP) gene derived from the jellyfish Aequoria victoria, allowing gene expression to be monitored by detection of fluorescence.

Evaluation of foreseeable effects
The GMMOs are likely to display either (a) comparable properties to the wildtype recipient organism, or (b) an attenuated phenotype due to diminished virulence and/or reduced levels of antibiotic resistance. Consequently, harmful properties associated with the GMMOs are comparable to those of the wildtype organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

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Degree of kill:
Autoclaving - effectively 100% kill (annual validation using 12 point thermocouple of worst case loads)
Heat treatment or Incineration – not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

### Project Containment

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### Project Ref 207/07.3

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**Project Additional Information**
### Purposes of the contained use

Multi-drug resistance develops due to the exposure of bacteria to either low or high levels of antibiotics. High-level multi-drug resistance generally arises through the selection of mutations in specific regulators that control antibiotic resistance mechanisms such as efflux pumps. While the physical mechanisms of multi-drug resistance in clinical isolates of K. pneumoniae, Salmonella spp. and Enterobacter spp. have been studied extensively, the role of transcriptional regulators in the control of these mechanisms and other functions has not.

The primary aim of this project is to understand the regulation of ramA, a key regulator in Klebsiella spp, and related regulators so that mutations leading to multi-drug resistance can be identified. Moreover the work will aim to determine what other factors, other than antibiotic resistance mechanisms, are controlled by this family of regulators.

### Recipient or parental organism

| Klebsiella spp., Salmonella spp. and Enterobacter spp. |
| Disabled strains of Escherichia coli |

### Host/vector system

Bacterial hosts with variety of plasmid vectors that will include pUC vector series, pET expression vector series, pGEM, pBAD, pACYC series, pBR322 and its derivatives.

### Origin & function

Deletion and complementation of regulatory genes, their regulators and associated genes, for example TetR family, ramA and regulators of ramA, and homologues from Klebsiella spp., Salmonella spp. and Enterobacter spp.

Heterologous gene expression of Salmonella spp. or Klebsiella pneumoniae gene(s) above into Klebsiella spp, Salmonella spp and/or Enterobacter.

Genomic libraries.

Well characterised reporter genes such as β-galactosidase, luxAB genes (luciferase) or fluorescent protein reporter genes.

Antibiotic resistance and selection markers

### Evaluation of foreseeable effects

The insertion of antibiotic resistance cassettes to disrupt specific genes would confer resistance to that antibiotic, however none will be inserted that are used therapeutically for the bacteria involved. Random transposon mutagenesis would have a similar effect. Introduction of reporter genes will confer the phenotype of a single reporter such as β-lactamase activity to the host strain. Disruption of specific genes in Klebsiella, Salmonella and Enterobacter spp. will prevent expression of those genes. Depending on the function of the gene this will most likely result (a) reduction in virulence or (b) disabled strain. If a regulatory gene is disrupted it could lead to the up and down regulation of an array of genes under its control. Any effects that might increase pathogenicity are expected to remain within the normal range of wild type populations. Complementation of the mutant gene with the wild type gene should result in the restoration of the parental phenotype. Heterologous gene expression is also expected to result in strains with variations comparable to those seen in wild type populations. No effects on host range or tissue tropism are expected.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste.
Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via clinical waste stream.

Degree of kill:

Autoclaving - effectively 100% kill (annual validation using 12 point thermocouple of worst case loads). All waste is autoclaved prior to disposal.

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Project Ref 207/07.4

Date Ackn'd 06/09/2007  
CU2 Project Title Investigations into the pathogenesis of poxviruses.

Class 2  
CultureVolClass2 1-50 Litres  
CultureVolumeClass3-4
**Project Additional Information**

**Purposes of the contained use**

To investigate how Vaccinia virus (VACV) and Cowpox virus (CPXV) replicate and interact with the host’s immune system, leading to a better understanding of VACV genes that affect virulence. This work requires genetic manipulation of VACV and CPXV including the deletion and modification of genes to investigate their role in viral virulence.

**Recipient or parental organism**

- Vaccinia virus
- Cowpox virus

**Host/vector system**

Viral host with plasmid vector

**Origin & function**

Genes likely to affect the host’s response to viral infection will be deleted or mutated. In particular, genes that may be involved in cytoskeletal rearrangement or the modulation of the ubiquitin / proteasome system. Mutations involve minor alterations to amino acid sequence to identify functional domains. Standard non-harmful marker genes (such as E. coli gpt, β-gal, luc or enhanced fluorescent proteins) and epitope tags (such as Flag, c-myc, His or HA) will also be inserted.

**Evaluation of foreseeable effects**

The genetically modified viruses produced in this work are not expected to have increased virulence, pathogenicity, extended tissue tropism or host range or altered susceptibility to host defence mechanisms when compared to wild type strains and in many cases viruses will be attenuated.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

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Degree of kill:
Autoclaving - effectively 100% kill (annual validation using 12 point thermocouple of worst case loads)
Heat treatment or Incineration – not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

Following primary infection, human herpesviruses (HV) establish a life-long latent infection in their hosts. Infection is controlled by the host immune system, mainly by cellular immunity. Loss of immune control of human HV infection, as may occur during inherited, acquired or iatrogenic immunosuppression, results in clinical disease. Some of the ensuing disorders may prove fatal as is the case with Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) in immunosuppressed organ transplant recipients. Research in our laboratory aims to explore the tumorigenic potential of EBV proteins, and to use the knowledge gained to devise novel (antibody- and/or T cell-based) immunotherapeutic strategies to target such EBV proteins expressed by EBV-associated cancers.

Analysis of viral gene function is the key to understanding the transforming, growth and transcriptional regimens undertaken by viruses. However, only so much can be achieved by analysis of these genes in isolation from their parent virus. The generation of bacterial artificial chromosomes (BAC5) containing entire HV genomes allows the use of powerful bacterial cloning strategies to introduce precise modifications into the virus. These modified HV BACs can then be introduced into eukaryotic cells, where the full impact of the modification can be assessed at all stages of the virus lifecycle. Using such techniques, the objective of the current project is to investigate the tumorigenic potential of EBV-derived proteins.

**Recipient or parental organism**

Epstein Barr virus

**Host/vector system**

Bacterial artificial chromosomes containing modified EBV genomes

**Origin & function**

Bacterial artificial chromosomes containing modified EBV genomes
The nature of modifications to be introduced to the EBV genome using BACs involve i) deletion (or loss of function by site-directed mutagenesis) of the latent gene products; ii) reinsertion of deleted genes; iii) inclusion of biomarkers or tags (for example, GFP, luciferase, FLAG, nyc, 6xHis) to enable tracking or visualisation of constructs and antibody detection of viral proteins, well-characterized promoters CMV IEp or SV4Op will be employed to drive expression of such genes; iv) inclusion of the oestrogen receptor gene (or its derivatives) to permit conditional (oestrogen-driven) expression of an EBV gene under study.

**Evaluation of foreseeable effects**

The proposed modifications are not expected to improve recombinant virus viability, or to increase its pathogenicity, compared to wild-type virus. These modifications will involve deleting all (or part) of the latent virus gene(s), or mutating specific motifs within those gene(s) in a manner that incurs loss of protein function. Therefore, we expect the planned modifications not to offer any survival advantage to the engineered virus, and, thus, they are defined as deleterious or neutral in terms of recombinant virus fitness/virulence. None of the proposed work will alter virus tissue tropism or host range because none of the work entails changing the virus receptor binding protein. We do not expect any of the proposed modifications to alter virus susceptibility to normal humoral (antibody) defence mechanisms. However, since latent virus antigens will be knocked out in some constructs the CTL immune response to any infection may be affected. However, those same proteins are crucial for B cell transformation in vitro and, thus, the mutant virus is expected to be much less able to drive B cell proliferation than its wild type counterpart. Taken together, therefore, it seems very unlikely that the mutants present a heightened risk to human health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| Solids (eg, plasticware such as pipettes, flasks, tubes etc) | autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill. |
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| Animal bedding and carcasses | autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for incineration or heat treatment. |
| Degree of kill: | **Autoclaving - effectively 100% kill (annual validation)** |
| Incineration and heat treatment - not applicable, all waste is autoclaved prior to disposal by incineration or microwaving. |

**Is an emergency plan required according to regulation 20?**

- [ ] Yes
- [x] No

If yes, tick to confirm that it is attached to this form

- [ ] Yes
- [ ] No

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Yes
- [ ] No
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM to control the risks to human health and safety and to the environment.

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**Project Ref 207/08.2**

**Date Ackn'd:** 14/10/2008

**CU2 Project Title:** INVESTIGATING FACTORS INVOLVED IN VIRULENCE AND ANTIBIOTIC RESISTANCE IN ACINETOBACTER SPECIES

**Class CultureVolClass2 CultureVolumeClass3-4**

Class 2 1-50 Litres

**Non-GMM Consent Granted**

Not Applicable

**Project notified under transitional arrangements**

N

**Project Additional Information**

**Purposes of the contained use**

Acinetobacter species are important hospital-acquired pathogens that colonise and infect immunocompromised hosts such as patients within intensive care units.
Infections can be difficult to treat due to many strains of Acinetobacter being resistant to commonly used antibiotics. This ability to acquire resistance in a hospital environment such as intensive care units containing immuno-compromised patients has led to the emergence of Acinetobacter species as an increasingly important global health problem. However, relatively little is known about the mechanisms relating to virulence, pathogenicity and antibiotic resistance in these species that have allowed them to become established opportunistic hospital pathogens. This study aims to investigate the features of Acinetobacter species that have led to their success as opportunistic hospital-acquired pathogens. A variety of factors may be studied, but the study will initially focus on antibiotic resistance, particularly on carbapenem resistance in Acinetobacter baumannii.

Recipient or parental organism

Acinetobacter species

Host/vector system

Bacterial host/plasmid vector

Origin & function

Natural bacterial genetic sequences that encode antibiotic resistance determinants and putative virulence factors originating from members of the Acinetobacter genus. Also inducible promoter sequences able to express genes under specific defined laboratory conditions. Suicide vectors will contain a fragment of a target gene or genetic element to enable homologous recombination, resulting in insertional inactivation of that gene or disruption of the element. Complementation vectors will encode a full coding sequence of a target gene, or an entire genetic element, in order to re-introduce a functional copy of the gene or complete copy of the element that was previously inactivated or disrupted with a suicide vector. Complementation vectors will only be used to restore expression of the target gene, returning the isolate back to its original wildtype phenotype. Site-directed mutagenesis of a target gene or genetic element may be performed and the resulting mutated sequence cloned into a complementation vector for expression within a recipient isolate.

Evaluation of foreseeable effects

The GMMOs resulting from this study are likely to have either comparable properties to the wildtype recipient isolates, or to have an attenuated phenotype due to reduced virulence and/or reduced antibiotic resistance. As such, the risk associated with the OMMOs is considered to be the same as those posed by the wildtype recipient organisms. Host range, tissue tropism and susceptibility to host defence mechanisms are not expected to be changed. No modifications will involve antibiotics used therapeutically for treatment of Acinetobacter infections.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g. plasticware such as pipettes, flash, tubes etc and agar plates) — autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill. Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains. Sharps (in sharps bins e.g. needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for heat treatment.

Degree of kill:
Autoclaving — effectively 100% kill (annual validation using 12 point thermocouple of worst case loads). Heat treatment or incineration — not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM to control the risks to human health and safety and to the environment.

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Project Ref 207/08.3

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<td></td>
<td>Not Applicable</td>
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### Project Additional Information

#### Purposes of the contained use

We wish to investigate several key questions about the evolution of social behaviour using the opportunistic pathogen *Pseudomonas aeruginosa* and go on to use our understanding of social evolution theory to understand observations of behaviour in bacterial infections. Specifically, we will investigate the extent to which social behaviour of bacteria effects their ability to infect hosts and cause disease. The social traits we focus on are the production of the iron-scavenging molecule pyoverdine and cell-to-cell communication systems. We will measure the extent to which clinical strains isolated from the sputa of cystic fibrosis patients exhibit these and other social behaviours.

#### Recipient or parental organism

- **Pseudomonas aeruginosa**

#### Host/vector system

- **Bacterial host/plasmid vector**

#### Origin & function

**Inserted genetic material:**
- standard non-harmful reporter genes such as green fluorescent protein (GFP) gene derived from the jellyfish *Aequorea Victoria* and luciferase a gene derived from the firefly *Photinus pyralis*.
- Muc A gene which suppresses the production of alginate in wild type *P. aeruginosa* strains; insertion of this gene restores phenotype of mutant clinical strains to wild type phenotype.
- antibiotic resistance genes for selection purposes.

**Gene deletions:**
- Siderophore (pyoverdine and pyochelin) knockouts are no longer able to chelate bound iron. The ability to chelate iron is crucial to bacterial metabolism and the ability to colonise host tissue where iron is withheld by powerful chelators such as haemoglobin or transferring. The fitness and virulence of these strains is therefore, significantly reduced relative to the wild type in monoculture.
- Quorum sensing knockouts no longer have functioning QS systems. Bacteria with knockouts of these regulatory genes are less able to sense their bacterial neighbours and are disabled with respect to biofilm production, swarming motility and production of secreted virulence factors. As a result, strains that have QS systems disrupted have significantly reduced virulence relative to wild type in monoculture.

**Evaluation of foreseeable effects**

The OMMOs with lux or gfp reporter genes inserted are likely to display comparable properties to wild type organisms.

The GMMOs with social functions disabled by gene deletions or disruptions are either likely to have or have been demonstrated to have reduced fitness and virulence relative to wild type strains. Tissue tropism and host range are not likely to be affected.

Consequently, harmful properties associated with the GMMOs are at the most comparable to those of the wild type organism.

Antibiotic resistance genes will not be ones that are used therapeutically for *P. aeruginosa*.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates and including lettuce leaves, warm oth pupae and C. elegans) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) — autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for heat treatment.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation using 12 point thennocouple of worst case loads)
Heat treatment or Incineration — not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM to control the risks to human health and safety and to the environment.

Project Containment

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<td>Large Scale Activities L3 L4 L2</td>
<td>Human Clinical Applications L3 L4</td>
</tr>
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</table>

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The generation, refinement and characterisation of induced pluripotent stem cells from mammalian cell lines.

Purposes of the contained use

Revolutionary new opportunities for research into and treatment of diseases will be provided by emerging methods for isolation of pluripotent cells from patients. In groundbreaking recent research by Shinya Yamanaka and colleagues it was shown that the introduction of just four specific transcription factors into clut cells induced a small proportion of those cells to acquire many of the characteristics of embryonic stem cells (Takahashi and Yamanaka, 2006), so called ‘nuclear reprogramming’. These cells were named induced pluripotent or “iPS” cells.

To date, reprogramming of human fibroblasts to an iPS status has been achieved by use of retro/lentiviruses, with subsequent multiple random integration events, while introducing the genes of interest. Although viral gene delivery is efficient, there are associated risks with these systems which include ncogenesis and mutagenesis. Therefore, for the future use of iPS cells in clinical and research situations, more refined methodologies of reprogramming are required. This will necessitate a basic understanding of the mechanisms involved in reprogramming including monitoring changes in gene expression which occur during reprogramming and the identification of other potential factors (genes) that can promote cellular programming to an IPS state.

The aim of this project is to establish the IPS technology within the University of Edinburgh. On establishment of this technology within Edinburgh University a number of reprogramming schemes will be engaged including the generation of specific human disease models. Understanding the mechanism of nuclear programming using the above system, we will use this technology to identify other factors that can affect the ability to reprogram to an iPS state.

Recipient or parental organism

Mammalian cells.

Host/vector system

Mammalian cells/retroviral, lentiviral and Herpes virus based vectors.
Origin & function

Origin of material is mammalian and the intended use is to generate induced pluripotent stem cells (iPS). Gene inserts include Sox2, Klf4, Oct4 and c-Myc, these are all involved in regulation of pluripotency and self renewal.

Evaluation of foreseeable effects

Mammalian cells made immortal or reprogrammed to a "pluripotent state" pose minimal risk of harm since any oncogenes present are integrated. The DNA sequences contained in viral vectors are both oncogenic and non-oncogenic and have the potential to cause significant harm because they are in an efficient gene delivery system. The viral vectors to be used are replication defective and therefore in the event of accidental exposure there would be no potential for onward transmission to other persons. However, if a laboratory worker was exposed whilst the vector would not replicate there is the potential for gene inserts to cause harm to the individual, possibly including tumour formation. However this is extremely unlikely as oncogenes such as c-Myc are not individually oncogenic and indeed will drive normal cells to apoptosis as secondary mutatons are required to form tumours. Additional controls will be used to minimise any such risks.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 dgr C for at least 15 minutes or 126-130 dgr C for at least 10 minutes or 134-138 dgr C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - either autoclave using a make-safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 dgr C for at least 15 minutes or 126-130 dgr C for at least 10 minutes or 134-138 dgr C for at least 3 minutes), discharge to drains or disinfect within 1% Virkon solution for at least 1 hour then discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 dgr C for at least 15 minutes or 126-130 dgr C for at least 10 minutes or 134-138 dgr C for at least 3 minutes), dispose via clinical waste system for heat treatment.

Degree of kill:

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Autoclaving - effectively 100% kill (annual validation using 12 point thermocouple of worst case loads). Disinfection - a 1% solution of Virkon disinfectant with a contact time of at least 10 minutes gives a >4.25 log reduction of HIV-1 (data supplied by manufacturer). Heat treatment or Incineration - not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM to control the risks to human health and safety and to the environment.

**Project Containment**

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<td>L2</td>
<td>L3 L4 L2</td>
<td>L3 L4</td>
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</table>

- **Animal Units**
  - L2
  - L3
  - L4

- **Large Scale Activities**
  - L2
  - L3
  - L4

- **Human Clinical Applications**
  - L2
  - L3
  - L4

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**Project Ref** 207/08.5

- **Date Ackn’ed** 31/10/2008
- **CU2 Project Title** Investigation of the mechanisms of tissue fibrosis and regeneration.

- **Class** Class 2
- **CultureVolClass2** 1-50 Litres

- **Non-GMM** Not Applicable
- **Consent Granted**

- **Withdrawn** N
- **Tick if notifying a connected programme of work** Y

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Tissue injury results in a process of healing which constitutes the laying down of scar and tissue regeneration in varying degrees. Many human organ diseases result in the generation of excess scar formation (fibrosis) and inadequate organ regeneration. We therefore aim to investigate these two linked processes in a variety of models and human diseases. To do this we propose to investigate the role of key cell populations and signalling molecules in controlling stem cell behaviour in the context of directed...
models of tissue inflammation and injury.

In order to address the role of Wnt proteins in controlling regeneration in the liver we propose to manipulate the Wnt pathway by delivering inhibitors of the Wnt signalling pathway. Proteins will be delivered via adenoviral vectors due to the efficacy of adenoviral delivery to the liver and inability of adenovirus to integrate genetic material into the host.

We propose to perform a series of experiments to define the role of alveolar and bone marrow derived macrophages in lung fibrosis. Lung fibrosis will be induced by the administration of adenoviruses expressing recombinant molecules that stimulate the production of collagen by myofibroblasts.

### Recipient or parental organism

N/A. Class 2 GMO is replication defective adenovirus as detailed below under vector.

### Host/vector system

Adeonviral vectors are generated in the well-characterised human embryonal kidney cell line 293. The vectors are based on the Adeno5 genome and are deleted in E1 and E3 regions.

### Origin & function

Inhibitors of the Wnt signalling pathway such as Dickkpok (Dkk), Wnt inhibitory factor (WIF) and soluble Frizzled protein (sFz).

Profibrotic molecules such as transforming growth factor beta (AdTGFbeta), connective tissue growth factor (AdCTGF), and members of the interleukin family.

Control genes encoding non-harmful proteins such as murine IgG2Fc.

Reporter genes encoding non-harmful proteins that allow detection of transfected cells.

### Evaluation of foreseeable effects

Recombinant adenoviruses are replication defective and unable to produce infectious viral particles in mammalian cells that do not express E1 genes. In the event of exposure to the recombinant adenoviral, vectors there would be transient expression of the inserted gene. Control and marker genes are not expected to have any harmful effects. The effect of the other active transgenes will be variable and depend on the route of exposure.

Accidental local injection of vectors containing Wnt inhibitors would be expected to be transient and result in a down regulation of cell proliferation locally. Effects of iv administration of Wnt inhibitors would be most marked in the liver but these are expected to be dose related and measures have been taken to not produce quantities of virus likely to cause problems. Exposure to aerosols would be expected to be transient and non-significant.

Accidental exposure to aerosolised fibrosis inducing transgenes may result in delivery of their products to the respiratory tract where transient expression of the transgene could cause fibrosis. However, effects are dose related and the risk of exposure has been minimised and the viral particle number carefully controlled to prevent significant side effects if accidental aerosol inoculation to humans were to occur. Accidental exposure via injection of fibrosis inducing transgenes is unlikely as sharps are not required for the instillation of the vector. As for liver above the predominant site of delivery following accidental injection would be either local, or hepatic if accidentally injected intravascularly. Once again, the expected effects would be transient and non-significant.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g. plasticware such as pipettes, flasks etc and agar plates) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 °C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 °C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) – autoclave using a make safe cycle as specified in BS2646, Part 3 1993 (either 121-125 °C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), dispose via clinical waste stream for heat treatment.

Animal bedding and carcasses – autoclave using a make safe cycle as specified in BS2646, Part 3 1993 (either 121-125 °C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), dispose of carcasses via clinical waste stream for incineration and bedding via clinical waste stream for heat treatment or via industrial (black bag) waste stream for landfill.

Degree of Kill:
Autoclaving – effectively 100% kill (annual validation using 12 point thermocouple of worst case loads) Heat treatment or incineration – not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM to control the risks to human health and safety and to the environment.

Project Containment

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<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
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</table>
### Project Additional Information

**Purposes of the contained use**

This research project will make a major contribution to the understanding of the fundamental mechanisms of both the human and animal TSEs. It is also the aim to study basic mechanisms of neuronal development, homeostasis and neurodegeneration. Our models are used to understand TSE disease mechanisms from the molecular level through to the whole animal and thus develop novel approaches for eradicating disease. The project spans veterinary and medical aspects of TSE disease and addresses problems relating to both animal and human health. This research will allow us to develop a systems biology approach to the study of TSEs and our expectation is that this will allow us to develop a more predictive approach to TSE infection. Our TSE models are unique when compared with other neurodegenerative disease models in that the precise timing of events is known. This provides an important opportunity to examine the very early events of neurodegeneration and it will be through studying such events that the most effective diagnostics and therapeutics will emerge for these diseases.

There are several basic questions that will be addressed through this project, which cover three areas of research. First is the question of the nature of the agent. What is the molecular connection between PrPSc protein, PrP amyloid and TSE infectivity? Second is the question of the role of protein modifications of PrP in TSEs. How does post-translational modification affect the biochemistry of PrP and change TSE susceptibility or pathology? The third area is the genetics of the prion gene family and their association with the onset and phenotypes of TSE disease. How are changes in prion gene sequences influencing TSE susceptibility?

The scientific goal is to understand the underlying molecular mechanisms that link PrP and other prion-family genes to infectivity and to TSE susceptibility.

**Recipient or parental organism**

- Disabled Escherichia coli
- Mammalian cell lines
- Transgenic animals

**Host/vector system**

<table>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>Class 2</td>
<td>1-50 Litres</td>
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</table>

**Non-GMM Consent Granted**

Not Applicable
Bacterial and cell line hosts with plasmid vectors
Animals will be modified by homologous gene replacement or by multicopy random gene integration.
No viral vectors will be used with PrP gene inserts.

Origin & function
Gene inserts are human and animal PrP proteins.
For expression in E. coli the proteins will mainly correspond to wild type or normal polymorphism-carrying mature length proteins. However, truncated proteins and proteins expressed as fusions to other ORFs will be made, thus allowing periplasmic expression, epitope tagging and to provide purification/solubilisation handles.
A wide range of normal, mutant and variant forms of PrPs will be expressed in cell lines and animals. The latter two include PrP proteins with altered posttranslational modifications, those with amino acid changes homologous to human disease-linked mutation and those with amino acid changes associated with human prion diseases.
Other prion-family genes such as shadoo will also be used.

Evaluation of foreseeable effects
There is no evidence for any risk to human health and safety or to the environment from normal PrP proteins and most studies suggest these have a neuroprotective role. Humans and animals with transmissible spongiform encephalopathies (TSEs) which are fatal, degenerative disorders of the central nervous system show deposition of mutated forms of the PrP (prion) protein in their brains. These mutated forms of the PrP protein are pathogenic and have been shown to be transmissible. Several variations (polymorphisms and mutations) in PrP proteins have been linked to these infectious proteins which are called prions. Based on previous findings there is no reason to suggest that any of the recombinant proteins used in this work will be any more harmful than naturally occurring variants and risk assessments have been made on the predictive properties of the PrP proteins by comparisons to naturally occurring disease forms. Further it has already been shown that many PrP proteins expressed in recombinant systems are non-functional and non-infectious. Whilst considerable knowledge has been gained in recent years about prion infectivity and host susceptibility, many questions remain unanswered. Since this work is aimed at better understanding these factors a precautionary approach has been taken in assigning rigorous containment and control measures to these activities.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Animals will be held at animal containment level 2 in a facility with enhanced security and control measures for work with TSEs. These measures will also minimise potential for escape to the environment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All bacterial cultures are treated with 20,000 ppm chloros for at least an hour prior to disposal or autoclaved at 134C for 1 hour (Guidance from the ACDP' TSE Working Group) These methods kill E coli bacteria. These treatments are also reliable methods of inactivating TSE agents to undetectable levels. All nucleic acid preparations would be considered to be non-infectious in terms of TSE infectivity due to the use of phenol or guanidinium in the preparative procedures which inactivate protein (Rowher RG (1991) Curr Topics Microbiol Immunol 172: 195-232). Any protein preparations would not leave the building unless treated as above. Bacterial culture plates, ES cell plates, mammalian cell culture plates and flasks and mouse carcasses are autoclaved at 134C for 1 hour prior to disposal. Our standard inactivation procedures reduce TSE infectivity to undetectable levels and containment and decontamination procedures are monitored for compliance. A QA system to cover all activities from experiments to waste disposal is in operation. Waste leaves the building as clinical waste for incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM to control the risks to human health and safety and to the environment.

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### Project Ref 207/08.7

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<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>04/12/2008</td>
<td>VIRAL-BASED STRATEGIES TO INVESTIGATE CHROMATIN-ASSOCIATED PROCESSES AND REGULATORS OF TUMOUR PROGRESSION.</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Not Applicable</td>
<td>Y</td>
<td>N</td>
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<th>Tick if notifying a connected programme of work</th>
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### Project Additional Information
**Purposes of the contained use**

The research undertaken at the Edinburgh Cancer Research Centre aims to understand how chromatin-associated cellular processes and genes that are deregulated in cancer contribute to tumorigenesis and how this can be exploited to design more effective therapeutics. The ability to both down regulate or over express key proteins involved in tumorigenesis forms an integral part of our research and the aim of this project is to develop replication-defective lentiviral vectors for the transfer of siRNA sequences and cDNAs to proteins of interest into mammalian cells.

We plan to use lentiviral-based vectors to introduce short hairpin RNAs into cells to ablate the function of key cellular components of cell-cell, cell-matrix adhesions and other proteins involved in regulation of these adhesions. We will also target proteins which have been shown to enable cells to metastasise to specific target organs and chromatin-associated proteins involved in epigenetic regulation or structural chromatin proteins. Conversely we will introduce cDNAs to these components (either wild type proteins or mutant proteins in which key regulatory elements have been altered).

Together these approaches will allow us to determine the contribution of individual proteins to the target cell behaviour such as growth, motility and ability to metastasise both in vitro and in vivo and to investigate the function of epigenetic pathways to regulate gene transcription and factors responsible for modulating chromatin structure. In some cases we will use RNAi libraries to identify novel proteins involved in regulation of cellular processes.

**Recipient or parental organism**

Well characterised mammalian cell lines.

**Host/vector system**

Cell lines /commercially available third and fourth generation lentiviral systems

**Origin & function**

i) standard reporter genes such as green fluorescent protein
ii) drug selection markers eg puromycin, neomycin, blasticidin resistance genes and
iii) the cDNA or shRNA insert of interest.

The inserts of interest target a number of different classes of human genes. Firstly, those that alter the adhesive properties of cells. Secondly, those that target proteins which have been shown to enable cells to metastasise to specific target organs. The third class of genes are those that encode chromatin-associated proteins.

**Evaluation of foreseeable effects**

Many of the genes that we are targeting for knock-down or over-expression will alter the behaviour of the host cells and allow us to determine the contribution of individual proteins to the target cell behaviour such as growth, motility and ability to metastasise. In the majority of cases these will not be harmful to human health. However, we will over-express oncogenes and knock-down tumour suppressor genes which have potential to cause harm to human health by altering the tumorigenic potential of infected cells. Therefore with these inserts in these efficient lentiviral vectors which can infect humans, specific control measures are needed. To minimise the potential for tumorigenesis insertion of multiple oncogenes or knock-down of multiple tumour suppressors into the same virus will not be carried out. It is now widely accepted that 4 to 5 genetic changes are required for the transformation of normal cells (Hanahan & Weinberg, Cell:100, 57-70, 2000) so it is unlikely that when introduced alone these will induce a malignant phenotype. Secondly, use of appropriate physical containment and inactivation processes including use of microbiological safety cabinet and gloves will be used to prevent human exposure to the viruses. The viral vectors to be used are replication defective and therefore in the event of accidental exposure there would be no potential for onward transmission to other persons. However for the individual who was exposed, whilst the vector would not replicate there is potential for some of the gene inserts to cause significant harm possibly including tumour formation. Assignment to containment level 3 is not considered necessary but additional controls to minimise the risk are warranted. Access to the tissue culture facility will be restricted to trained personnel and only be undertaken at a time when others not associated with the work are not present thus limiting the numbers of people to potential exposure.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) – either autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains or disinfect with 1% Virkon for at least 2 hours and discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via clinical waste stream for heat treatment.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation using 12 point thermocouple of worst case loads).
Disinfection - A 1% solution of Virkon disinfectant with a contact time of at least 20 mins gives a >99.999% kill of all viruses (data supplied by manufacturer).
Heat treatment or Incineration – not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]  

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM to control the risks to human health and safety and to the environment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L2 L4</td>
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<td>L3</td>
<td>L4 L2 L3 L2</td>
<td>L4 L3</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>

02/03/2022
Investigation of changes in gene expression and Chromatin structure in artificially generated cancer cell lines

In the human body a small number of cells usually undergo changes that give rise to cancer. Some of the initial events take months and even years and by the time tumours become detectable the cells have undergone a number of changes. Isolation and studies of very early human cancer lesions is technically challenging. Inactivation of genes that are crucial for the control of cell proliferation and survival is widely accepted as one of the major hallmarks of cancer. Gene silencing often occurs by a mechanism which involves chemical modification (methylation) of DNA at gene promoter regions. In order to study the very early events of gene silencing at the onset of tumorigenic transformation we would like to establish human cell lines that express a defined set of factors: human telomerase enzyme to maintain the ends of the chromosomes intact and two cooperating oncogenes – SV40 large T antigen and constitutively active forms of Ras. Artificially generated cancer cell lines will be used for in vitro analyses of DNA, RNA and protein as well as for studies in vivo that monitor the capacity of the transformed cells to form tumours in immunosuppressed mice. We expect that these studies will produce valuable information on the early events in the formation of human cancers which cannot be easily addressed in any other way for ethical and practical considerations.

Disabled Escherichia coli and mammalian cells – no potential harmful effects identified.

Host/vector system

Cell lines/Retroviral vectors. Latter are self inactivating and replication-deficient.
The genetic material originated from viral oncogenes and mutant human oncoproteins which transform human cells and cause formation of tumours.

Evaluation of foreseeable effects

Retroviral particles are capable of infecting humans and animals but only a combination of several of the retroviruses carrying human oncogenes and telomerase enzyme is capable of producing oncogenic transformation. It is considered unlikely that tumorigenesis would result following exposure to a retroviral vector containing just one of the gene inserts, however since these are known oncogenes and the amphotropic vectors are pseudotyped, it is considered appropriate to apply certain control measures on a precautionary basis and assign the work to containment level 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Type of Waste</th>
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<tbody>
<tr>
<td>Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates)</td>
<td>Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.</td>
</tr>
<tr>
<td>Liquids (eg samples, culture supernatants, tissue culture media)</td>
<td>Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.</td>
</tr>
<tr>
<td>Sharps (in sharps bin, eg needles, syringes, scalpels)</td>
<td>Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for heat treatment.</td>
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Degree of kill:

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<tbody>
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<td>Effectively 100% kill (annual validation using 12 point thermocouple of worst case loads)</td>
</tr>
<tr>
<td>Heat treatment or Incineration</td>
<td>Not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM to control the risks to human health and safety and to the environment.
# Project Additional Information

**Purposes of the contained use**

The work to be undertaken forms part of an integrated programme of research on human and avian influenza involving researchers from the Interdisciplinary Centre for Human and Avian Influenza virus Research (ICHAIR) and colleagues from the Roslin Institute. ICHAIR (funded by the Scottish Funding Council and the participating University partners) is a collaboration between researchers in The University of Edinburgh, The University of St Andrews and The University of Glasgow.

Recombinant influenza viruses will be used in order to determine the role of specific virus genes in pathogenesis. The viruses constructed will be based on the widely used laboratory strains A/WSN/33 (H1N1), A/Scotland/20/74 (H3N2) (both mouse adapted); A/PR/8/34 (H1N1) and A/Udorn/72 (H3N2) viruses.

---

Project notified under transitional arrangements: N

Date Ackn'd: 03/11/2009

CU2 Project Title: The pathogenesis of Influenza A virus

Class: Class 2

Culture Vol: < 1 Litre

Non-GMM: Consent Granted

Tick if notifying a connected programme of work: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Recipient or parental organism

02/03/2022
Low pathogenic influenza virus strains

Host/vector system
Mammalian established cell lines and primary cell cultures. Recombinant influenza virus strains.

Origin & function
Influenza virus genes involved in virus replication and virus: host interactions.

Evaluation of foreseeable effects
The experiments proposed involve gene deletion, mutation or replacement of specific genes with the corresponding gene from one of the other strains listed all of which are either of low pathogenicity. The influenza virus strains used in this part of the project are classified by ACDP as Hazard Group 2. Since none of the proposed modifications is expected to make the virus more hazardous than the wild-type parent, the work will be assigned to Containment Level 2.

A/WSN/33 (h1N1) and A/Scotland/20/74 are mouse adapted (the other strains do not infect rodents) and have the potential to infect and cause disease in wild rodents. No natural infection of rodents with influenza virus has been documented so no gene transfer to/recombination with wild type murine influenza is possible. Mouse to mouse transmission of murine adapted influenza virus is inefficient and requires close contact between animals. The mouse work will be carried out in the Category 2 containment facilities that include rodent traps on doors and drains to prevent accidental release and there is minimal likelihood of release of virus from these studies.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solids (eg, plasticware such as pipettes, flasks, tubes etc) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125º for at least 15 minutes or 126-130ºC for at least 10 minute or 134-138ºC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg, samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125º C for at least 15 minutes or 126-130ºC for at least 10 minutes or 134-138ºC for at least 3 minutes), discharge to drains.

Sharps (in sharps bin - eg, needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125º C for at least 15 minutes or 126-130ºC for at least 10 minutes or 134-138ºC for at least 3 minutes), dispose to drains.

Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125º C for at least 15 minutes or 126-130ºC for at least 10 minutes or 134-138ºC for at least 3 minutes), dispose via clinical waste stream for incineration or heat treatment.

Degree of kill.
Autoclaving - effectively 100% kill (annual validation) Incineration and heat treatment - not applicable, all waste is autoclaved prior to disposal by incineration or microwaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

### Project Containment

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### Project Ref 207/09.2

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<td>Investigation of Plasmodium falciparum genes involved in invasion of erythrocytes</td>
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### Project Additional Information

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### Purposes of the contained use

The pathology of malaria is caused by the invasion of parasites into human red blood cells and subsequent rupture of these infected cells, releasing new parasites and parasite debris which leads to fever and immune pathology in the human host. Parasites bind to the surface of red blood cells and invade by mechanisms which are not clearly understood. Previous work has identified a number of genes that play a crucial role in the invasion of erythrocytes by the malaria parasite Plasmodium falciparum. We aim to investigate the role of putative parasite invasion proteins in determining the binding to human erythrocytes. We plan to modify or disrupt the parasite invasion genes in order to analyse their precise role in the parasite's invasion capacity.

### Recipient or parental organism

Plasmodium falciparum, the pathogen responsible for the most common and most virulent form of malaria.

### Host/vector system

A variety of plasmid vectors that have been designed for Plasmodium falciparum transfection and genetic modification will be used.

### Origin & function

We are studying P. falciparum genes that play a role in the invasion of erythrocytes. The work will focus on the msp and msp related genes that encode parasite merozite surface proteins. Domains of these MSPs mediate invasion of human erythrocytes. The plasmid vectors include drug resistance genes that allow selection of transfected parasite under drug pressure. The drugs used for selection are WR99210, ganciclovir, cytosine deaminase and blasticidin. In addition, well characterised non-harmful tags or markers (such as a myc-tag or green fluorescent protein) will be incorporated into the above vectors to enable us to localise the expressed/modified protein.

### Evaluation of foreseeable effects

The experiments that we aim to perform are not expected to increase the virulence of the genetically modified P. falciparum parasites above the range found in unmodified p. falciparum because all P. falciparum strains have the capacity to invade erythrocytes. Experiments in which putative invasion-mediating genes are disrupted or mutated are predicted to reduce the virulence of the genetically modified parasite strain. The genetic modifications we plan to carry out will not affect any genes controlling the host range or transmission potential of the parasite.

The transfection process requires the use of vectors containing selectable markers such as WR99210 (an antifolate drug), ganciclovir (an antiviral), 5-fluorocytosine (an antifungal agent) and blasticidin (an antibiotic). As antifolates are frequently used antimalarials, the genetic modification decreases the susceptibility of the parasites to these chemotherapeutic agents. However, parasites resistant to antifolates are very common in nature and the single drug treatment with antifolates is never used chemotherapeutically. Blasticidin, ganciclovir and 5-fluorocytosine are not antimalarials and therefore their use poses no additional risks to induce resistance against currently used antimalarials.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Containment level 3 with derogations (as described and justified below) will be applied with Good Microbiological Practice and Good Occupational Health and Safety.

ACDP guidance on work with P. falciparum:

The Schedule to the certificate of exemption accompanying the Approved List specifies a number of parasites, including P. falciparum, for which full Containment level 3 need not be used. ACDP guidance states that work with such parasites does not generally require an inward flow of air to the laboratory or the use of a microbiological safety cabinet as none of these agents is normally infectious by the airborne route. ACDP recommends that for working with these agents, in research, a separate room...
should be used or a designated area in a larger laboratory. ACDP’s guidance also notes that the laboratory need not be sealable for fumigation.

In accordance with the ACDP guidance detailed above, various control measures specified at Containment level 3 are not considered necessary for this work and are proposed to be omitted. Consent is also sought for derogation of some additional measures, notably the laboratory to contain all its own equipment.

Justification for not applying full containment level 3 measures is as follows:

- Plasmodium spp. including P. falciparum are unable to form cryptic forms and are very sensitive to dehydration. In addition they depend on nucleic acid precursors for growth that are added to the rich media in which they are grown or provided to these intracellular parasites by host cells. As a consequence they cannot survive in normal media. They are also sensitive to hypoosmolarity and for example easily lyse in water.

- Plasmodium spp. are not per se invasive and require their bloodsucking mosquito vector for transmission and percutaneous penetration as a condition for successful infection.

- The parasites do not represent any hazard of infection through aerosol transfer. Aerosols are tiny droplets of liquid. These dry out within minutes, under the laboratory conditions of constant air flow and room temperature. Once dehydrated, parasites or parasite-infected host cells would not be viable.

In relation to the individual control measures to be omitted from laboratory facilities:

1. Laboratory sealable for fumigation:
   There is no foreseeable need to fumigate the laboratory. In the event of a major spillage, the bulk of material would be soaked up on absorbent paper towels then autoclaved, killing any parasites. The affected area would be treated with appropriate concentrations of a suitable disinfectant. Any aerosols created that were not cleaned in this initial effort would dry out, killing both host cell and parasite. The area of the laboratory would be left isolated for a period to ensure effective drying. Effective parasite transmission is only possible via direct inoculation of viable parasites.

2. Negative pressure, HEPA filtered extract, microbiological safety cabinet or specified measures to control aerosol dissemination:
   The organism presents no hazard of infection by the airborne route. Class II microbiological safety cabinets are in use in the dedicated laboratory, they are necessary for sterile culture of the organism; they are not required for operator safety. Activities involve small scale, standard laboratory techniques with no aerosol generating procedures.

3. Some other equipment not within the laboratory:
   Although the laboratory contains most of the necessary equipment for the work, some activities use specialist equipment that cannot reasonably be accommodated within the dedicated facility. In all cases a safe system of transport involving secondary containment will be used, any equipment used cleaned and disinfected immediately on completion of the work and any contaminated items either returned to the dedicated laboratory or removed for disposal as waste. The following activities will be undertaken outside the dedicated laboratory:

   i) Confocal fluorescence microscopy: a fluorescent microscope is located within the same building.
   ii) Fluorescence activated cell sorting and analyses (FACS): a laboratory holding all FACS equipment is located within the same building and operated according to a documented code of practice to accommodate this work. Whilst this work is in progress, access to the FACS facility is restricted to authorised workers trained to CL3 standards and all infectious materials are removed on completion and the area cleaned and disinfected prior to return to normal use.
   iii) Storage: transfected parasite stocks are frozen and stored at −80°C or in liquid nitrogen within the same building. The freezers are locked and accessed only by authorised personnel.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Most of the material that has come into contact with parasites will be soaked for 24 hours in 2% Virkon. Thereafter, or for any items not disinfected first, the following waste
disposal procedures will be used.

Solids (e.g. plastic ware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for heat treatment.

Spills are treated with powdered Virkon (for larger volumes) or 2% Virkon (smaller volumes) and aerosols contained with paper towels.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation)
Disinfection - not applicable, all waste is subsequently autoclaved prior to disposal
Heat treatment or Incineration - not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

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**Project Containment**

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<th>Glass Houses</th>
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</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 Yes L3 L4 L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
</tr>
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<td></td>
<td>L3 L4 L2</td>
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</table>
The pathogen used in these experiments is the porcine reproductive and respiratory syndrome virus (PRRSV). The overall aim of this project is to define the viral and host molecular determinants responsible for the growth and pathogenesis outcome in infected cells.

PRRSV has a 15kb-compact genome where only minimal alterations are possible without affecting virus viability. To overcome these issues the GFP gene in pSD01-08-GFP will be replaced with a short 6-aa Flash tag (Invitrogen). The Flash epitope will also be tested by tagging structural and nonstructural proteins in the PRRSV genome using pSD01-08-GFP and/or the pFL12 backbone. The growth of the newly formed recombinant cloned will be monitored in vitro. Recombinant viral clones that show stability and growth in vitro will be used to investigate the genetics of host innate immune response to PRRSV.

Established host cell lines:
- PK-15: porcine kidney
- 3D4/31: Porcine moncytic cell
- MARC-145: monkey kidney cell*
- Primary Porcine Monocyte/Macrophage cells.

*Marc-145 cell line is derived from the green monkey kidney cells (Kim et al, Arch Virol, 133 p477). This line is commonly used to amplify PRRSV and generate attenuated live vaccines.

Current vector systems:
Vectors:
Genotype 1: The pSD01-08-GFP vector is based on pACYC177 plasmid, containing a hybrid promoter composed of the cytomegalovirus (CMV) or a T7 promoter.
Genotype 2: The pFL12 vector is based on pBR322 backbone containing a T7 promoter.
Classical attenuated live vaccine: Most of these vaccine originate from genotype2 strains
-Prime-Pac (PP) (accession number DQ779791), Kwon et al, vaccine, 24, p7071
-INGELVAC ATP (EF532801)
-RespPRRS (AF159149, allende et al, Arch Virol. 145, p1149Allende)

Origin & function

pFL12 and pSD01-08-GFP infectious clones encode viable PRRSV particles that infect susceptible clones cells without changing cell tropism when compared with natural PRRSV strains. While FL12 virus can only be detected by conventional immuno-detection methods, SD01-08-GFP virus is readily detected by flow cytometry and confocal microscopy (Fanf et al, Journal of virology, 80, p11447). For some of the recombinant strains that will be generated during this project we will replace the GFP with a FlAsH tag useful for labelling and live tracking of viral particles. The FlAsH tag is a short (6 residues) peptide tag commercially available (Invitrogen). Flash will be fused to nsp2 and then to other structural and non structural proteins and used in reverse genetics plasmid-based system to produce new infectious viral particles carrying the Flash tagged viral particles. Cell-permeable FlAsH reagent binds with high affinity to the FlAsH tag and stains the cells when excited at 508 nm wavelength. Emission at 528 nm can be detected using standard fluorescence microscopy settings, allowing identification of infected cells.

Evaluation of foreseeable effects

There is no evidence that PRRSV infects human cells. PRRS is endemic to many countries including UK/Scotland and is not a notifiable disease. The recombinants are based on a vaccine strain which is non-pathogenic. The experiments proposed involve gene deletion, mutation or replacement of specific genes with the corresponding gene from attenuated live vaccine strains listed above. As a first instance we intend to use the PP vaccine. None of the currently proposed modifications is expected to make the virus more hazardous to the environment i.e. animal health than the wild-type parent strains. If genes from other (non-listed) strains are introduced, the potential for hazards to environment of these recombinants will be considered by the local GM safety committee, with reference to the HSE if thought appropriate.

Recombinant PRRS viruses have the potential to infect pigs only as they retain all properties of the wild type parental vaccine strain. Recombination between different strains of PRRSV has been documented in vitro and in vivo (Murtaugh et al. Journal of Swine Health and Production, 2002, 10:15) and if it were to occur the acquisition by a pathogenic strain of the tagged PRRSV genes is exceedingly unlikely to increase the virulence of such a strain. Therefore the consequence of spread to a pig population is negligible.

The likelihood of infection of pigs is extremely low/effectively zero as no pigs exist within the close vicinity, less than 300m, of the research facility at The Roslin Institute. Tissue culture work will produce small amounts of liquid and no procedures which produce aerosols are to be used. Microscopy will be carried out only on fixed tissue. All waste will be autoclaved or burned in accordance with The Roslin Institute regulations (see appendix). Cat2 researchers involved with PRRSV in-vitro experiments will be quarantined for up to 48 hours before being allowed to visit any pig farms. The risk of spread of the recombinant viruses to the wild population is minimal/effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

WASTE DISPOSAL PROCEDURES

Solids (eg, plasticware such as pipettes, flasks, tubes etc) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.
Liquids (e.g., samples, cultures, supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes, discharge to drains.

Sharps (in sharps bin - e.g., needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for incineration or heat treatment.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation)
Incineration and heat treatment - not applicable, all waste is autoclaved prior to disposal by incineration or microwaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Animal Units

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Project Ref 207/10.3

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Consent Granted
**Project Additional Information**

**Purposes of the contained use**

RSV expressing red and green fluorescent protein will be used to visualise RSV replication in vitro (Recipient cells will include both human and mouse cell lines and primary cultures. The principal cell type infected will be epithelium, but will also include cells of the immune system such as macrophage and dendritic cells.) and in vivo (both Wild Type (WT) and genetically modified strains of mouse will be infected).

**Recipient or parental organism**

Wild type RSV cDNA (ACDP CLASSIFICATION 2)

**Host/vector system**

We propose to use RSV virus that has been modified to express either a red fluorescence protein (rrRSV) or a green fluorescent protein (rgRSV). Both vectors were generated in the laboratory of Dr M P. Reference the method of their construction can be found at Hallak et al, J. Virolgy p10508-10513 2000 & Guerrero-Plata et al, Am J Respir Cell Mol Biol, p320-329 2006) and references therin. It is also described briefly in a section below.

The fluorescent protein gene was inserted via a series of minigenome steps into a position before the first gene in a full-length cDNA copy of the RSV genome along with the proper gene start and gene end signals. In this construct, the fluorescent protein gene flanked by RSV gene-start and gene-stop signals was inserted at the boundary between the leader region and the NS1 gene. The recombinant RSV virus thus expressed one additional mRNA, namely either green or red fluorescent protein, compared with wild-type RSV. This plasmid was transfected into Hep-2 cells along with four other plasmids encoding the N, P, L, and M2-1 proteins to support RSV transcription and replication. The cells were also infected with vaccinia virus, MVA-T7, to provide T7 RNA polymerase to drive transcription from each of the plasmids. The resulting virus was then amplified via passage in Hep2 cells then stored as frozen aliquots from which we will be supplied.

**Origin & function**

The vector modification of the WT RSV cDNA was performed at another institution by Dr M P (Hallak et al, J. Virolgy p10508-10513 2000).

The inserted gene products encode fluorescent proteins and do not confer any selective, infectious or propagating advantage on the virus.

**Evaluation of foreseeable effects**

Given that RSV is a commonly acquired respiratory virus and that the modified virus has no selective advantage over WT. There are no foreseeable effects on human health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

WASTE MANAGEMENT MEASURES

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - Treat with precept bleach tablet solution overnight, UV exposure for 20 minutes. Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - treat with precept bleach tablet solution overnight. Discharge to drains.

Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose of carcasses via clinical waste stream for heat treatment or via the industrial (black bag) waste stream for landfill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned

Project Containment

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<tr>
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Project Ref 207/10.4

Date Ackn'd 02/03/2022

CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4
Ticks carry a number of infections of importance in medicine and veterinary medicine. Little is known about how tick cells and ticks respond to microbial infections, how microbes persist in ticks for long periods of time and whether responses to one microbe affect responses or the outcome of infection to another microbe. This project will use a genetically modified tick-borne virus, Langat virus (LGTV) and a genetically modified tick-borne bacterium, Borrelia burgdorferi to investigate the responses of tick cells and ticks to infection, how one infection affects another and how and where these infections persist within ticks.

For LGTV: Disabled, commercially available E. coli strains and eukaryotic cell lines.

Recipient or parental organism
For LGTV: Disabled, commercially available E. coli strains and eukaryotic cell lines.

Host/vector system
LGTV sequence with changes in the replicase genes or insertion of reporter gene sequences in standard bacterial plasmids containing antibiotic resistance genes.

Non-human eukaryotic cells infected with LGTV, LGTV mutants or LGTV containing inserted reporter genes.

Plasmid JAH2 derived from the pBLS590 B. burgdorferi - E. coli shuttle vector. pJAH2 contains the gene for kanamycin resistance and a reporter gene driven by a bacterial 'erp' promoter.

Origin & function
LGTV containing a reporter gene and B. burgdorferi containing a reporter gene will be used to monitor infection or co-infection of eukaryotic cells, arthropods and rodents

Evaluation of foreseeable effects
The LGTV structural proteins will not be changed; there should thus be no change to the tropism or host range of this virus. LGTV is not associated with human disease and has been used as a vaccine for other tick-borne encephalitis viruses. LGTV is not known to be present in the UK environment. It is not known whether it could be naturally sustained here if released, however release from our laboratories would be very unlikely to result in infection of ticks (naturally infected by blood meal) or vertebrates (naturally infected by bite). LGTV is not on the SAPO list. Borrelia causes borreliosis (Lyme disease) in humans. Laboratory acquired human infections have not
been reported. Infection can be treated with antibiotics. This bacterium is present in the UK environment. The GM bacterium would have no selective advantage for survival and establishment in the environment and if released from our laboratories would be very unlikely to infect ticks or vertebrates (natural route of infection is by bite). No harmful properties have been attributed to the inserted reporter genes.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste disposal procedures and protocols for biological material are in place in the Virology Towerblock, Summerhall Square. All biological material is inactivated by autoclaving or incubation with chlorine (as appropriate) before being disposed of through established waste disposal channels.

Solids (e.g. plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or disinfect for >16 hrs, discharge any excess liquids to drains, dispose of solids via clinical waste stream for microwave treatment or via the industrial (black bag) waste stream for landfill.

Arthropod-derived material such as the remains of dissected ticks will be placed in a container with a strong solution of disinfectant. This container will be periodically sieved out and the solid wastes disposed of by autoclaving.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or disinfect for >16 hrs, discharge to drains. Sharps (in sharp bin, e.g. needles, syringes, scalpels) - dispose via clinical waste stream for microwave treatment.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

**Project Containment**

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<td>L4</td>
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</table>

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

To understand the mechanisms of chromatin regulation in *C. albicans*.

The specific goals are to characterise:

1. The pathways responsible for centromere establishments and maintenance. Genes important for centromere integrity will be mutated and the null mutants analyzed for their ability to maintain centromere function.

2. Regulation of gene expression by the RNAi machinery. Sequence of Small Non-coding RNAs in cells carrying mutations in the RNAi component Ago.


Recipient or parental organism

---

**Historical Significant Changes**

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Withdrawn

Tick if notifying a connected programme of work

Project notified under transitional arrangements
C. albicans is a human pathogen in ACDP Hazard Group 2. C. albicans causes superficial infections of mucosal epithelia (thrush) (Odds [1988] Candida and Candidosis, Balliere Tindall). C. albicans can also cause systemic infections which can be fatal, but these only occur in severely immunocompromised patients. To keep things in perspective, it is important to realise that C. albicans is widely distributed. C. albicans is carried commensally at least 60% of the healthy population and most Candida infections arise as an overgrowth of the strains resident in the normal microflora. It is a micro-organism of low pathogenic potential that rarely affects healthy individuals. It only becomes a potential medical problem when an individual's immune responses are significantly impaired. Furthermore, as with most pathogenic micro-organisms, the virulence of C. albicans strains is likely to become attenuated rather than enhanced after prolonged laboratory culture.

The C. albicans strains CA14 (uta13), CAI8 (ura3, ade2), RM1000 (his1, ura3) and BWP17 (arg4, his1, ura3) are the standard hosts for DNA transformation world-wide. These auxotrophies make C. albicans avirulent (Leberer et al. [1996] PNAS, 93, 13217). Transformation with URA3 plasmids partially restores the virulence of CAI4, but this restoration is not complete because the genes neighbouring RA3 remain inactivated. This is enough to attenuate virulence partially (but not completely).

Furthermore, C. albicans strains that have been cultured in the laboratory tend to display reduced pathogenicity compared to strains carried commensally by most individuals.

**Host/vector system**

We will always use non-mobilisable E. coli and C. albicans vectors, in detail:

- Non-mobilizable E. coli vectors (including pUC18/19, pBluescript, pGEM-T; lac-based expression plasmids such as pET vectors).

Specific non-mobilizable C. albicans vectors (low copy replicating and integrating vectors [YPB1, Clp10, Clp20, Clp30]; low copy replicating and integrating vectors for ectopic expression [YPB-ADHpt, pACT1]; integrating expression vectors regulated by methionine [MET3 promoter], maltose [MAL2 promoter], glucose [PCK1 promoter], or doxycycline [tet ON promoter and tet OFF promoter].

**Origin & function**

Pathogenicity in C. albicans is a polygenic trait involving numerous physiological characteristics (e.g. rapid growth cell wall, morphogenesis, secreted hydrolases, phenotypic switching). Therefore, (i) no single C. albicans gene (or combination of genes) will turn E. coli into a pathogen, (ii) a reduction in any one of these parameters can attenuate C. albicans virulence, and (iii) an increase in any one of these parameters is highly unlikely to increase C. albicans virulence.

In addition, the C. albicans transformations will perform inactivate a specific target gene to create a null mutant. These transformations will be performed in multiple disabled C. albicans strains (ura3, ade2, arg4, or his1) to ensure that all transformants will always carry a disabling mutation, thereby rendering them completely avirulent. No null mutations have been described that increase the virulence of C. albicans.

In some experiments, we will express specific genes in C. albicans ectopically using a MET3, MAL or text promoter. In these experiments, the expression level is not maximised. Their ectopic expression levels will be <1% of total cell protein. The ectopic expression of most specific genes is unlikely to affect virulence at all, and in some cases it will be expected to reduce C. albicans virulence by adversely affecting growth. The key point is that the probability of increasing virulence by ectopic expression of a single gene is very low, because pathogenicity is complex and polygenic trait requiring a high level of fitness of the C. albicans cell (Odds [1994] ASM News, 60, 313). Indeed, there is evidence that ectopic expression of factors involved in virulence traits does not increase virulence (Bailey [1997] PhD thesis, University of Aberdeen; Leng [1998] PhD thesis, University of Aberdeen; Rodaki et al. [2006]).

We are planning to generate ectopically express genes involved in:
1. Centromere assembly and propagation
2. RNAI
3. Histone modifications

In all cases, we will express tagged versions (EGFP, FLAG or HA) of genes to be able to examine to localization of specific proteins. The addition of the tag will not effect the function of the gene.
Evaluation of foreseeable effects

Our experiments will be performed on disabled or partially disabled C. albicans strains and the mutations that we will generate will most likely render them completely avirulent. Likewise, the ectopic expression of most specific genes is unlikely to affect virulence at all, and in some cases it will be expected to reduce C. albicans virulence by adversely affecting growth.

Candida species have no effective mechanism for horizontal gene transfer that might allow dissemination of resistance traits.

The likelihood of natural gene transfer to other species is essentially zero. Furthermore, despite the recent discovery of sex in C. albicans, the risk of transfer between C. albicans strains is very low. This is because C. albicans strains mate very inefficiently in vivo [Hull et al., (2000) Science 289, 307-310], probably because the specific growth forms that mate most efficiently aren’t maintained at 37°C [Miller & Johnson (2002) Cell 110, 293-302]. In addition, C albicans must be homozygous at the mating type locus for them to be competent for mating, and the vast majority of clinical isolates are heterozygous at the mating type locus.

The "Likelihood of Hazard" is medium, since we are handling a fungal pathogen and it is not possible to exclude accidents entirely, even though the laboratory of our collaborator has not reported a single accident with a Candida species in over 15years. However, the Candida species of interest are widely distributed, with most individuals already harbouring wild type C. albicans strains. No mobilisable plasmids will be used, and in most experiments, disabled hosts will be used.

The "Consequence of Hazard" is low, since serious infections only occur in individuals with severely compromised immune systems.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

WASTE DISPOSAL PROCEDURES

Solids (e.g. plastic-ware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste stream for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors), 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

SPILLAGES

Particular care should be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that
involves broken glass) unless they are aware of the potential risks and trained in safe working practices.

If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Project Ref 207/11.5

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<td>Use of lentivirus vectors to immortalize and investigate stromal cell lines from human haematopoietic embryonic tissue</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
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</table>

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
### Project Additional Information

**Purposes of the contained use**

To immortalize stromal cell lines from human hematopoietic embryonic tissues

**Recipient or parental organism**

293T cells, human primary cells from the AGM region, yolk sac, foetal liver, umbilical vessels, placenta.

**Host/vector system**

Lenti-hTert-GFP ready to use particles will be used. hTert is driven by CMV promoter. GFP promoter is driven by a separate EF1a promoter. No antibiotic resistance gene is included in this vector. Viral particles that are replication incompetent and only carry the gene of interest are produced; therefore, no other viral species are produced.

**Origin & function**

Tert (telomerase reverse transcriptase) is an enzyme that adds DNA sequence repeats (TTAGGG) to the ends of DNA strands in the telomere region. Without this, primary cells normally senesce and die.

Fluorescent reporter genes will be used in some instances to mark infected cells.

**Evaluation of foreseeable effects**

Not applicable

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatant, tissue culture media - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plasticware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be
autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

Spillages

Particular care should be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that involves broken glass) unless they are aware of the potential risks and trained in safe working practices. If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste stream.

Emergency procedures

In case of emergency the research worker will be seen by trained First Aider. In the event of any accident where exposure to potentially infectious material may have occurred, The Occupational Health Unit (508192) should be informed immediately or if not available alternatively contact the nearest NHS Accident and Emergency Department/Minor Injuries Unit. As with any accident the incident must be reported to the Institute Safety Officer, and a report entered into the Accident Book.
Torque teno virus (TTV, previously also known as transfusion transmitted virus) was first isolated from a patient with cryptogenic hepatitis (Nishizawa et al, 1997 Biochem, Biophys, Res, Commun, ) and is implicated in other forms of hepatitis. However, TTV and the related anelloviruses, Torque teno mini virus (TMLV or TTMV) and Torque teno midi virus (TTMDV), are widespread in the human population and despite over a decade of investigation, their role in pulmonary fibrosis, systemic lupus erythematosus and even cancer although whether the increased detection rates of viral loads of TTV in these studies were causative or an effect of immunosuppression and/ or co-infection is still unknown.

Using degenerate TTV primers, TTV-like viruses have been identified in tissue from wild wood mice (Apodemis sylvaticus) collected in the UK. Subsequent work has shown that related viruses are also present in other wild rodent populations in the UK including bank voles (Myodes glareolus) and field voles (Microtus agrestis) but there has not as yet been any related species identified in house mice (Mus musculus), the species commonly used in the laboratory. The aim of this work is to establish a rodent model for pathogenesis of TTV using clonal and recombinant (traceable marker containing) rodent viruses in order to provide an understanding of its role in disease.

Recipient or parental organism

Rodent TTV strains generated from wild rodents caught in the UK.

Host/vector system

The plasmid vectors to be used for cloning rodent TTV genetic material contain an ampicillin resistance gene but are non-mobilisable or mobilisation deficient and are unlikely to be transmitted to wild-type bacterial strains.

Origin & function

Full-length DNA from will rodent TTV will be used which will only produce infectious virus under laboratory conditions. The other genetic material involved for producing tagged virus has all been used in numerous previous studies with no adverse effects observed.

Evaluation of foreseeable effects

Wild-type rodent TTV strains are not only normally present but highly abundant in wild rodent populations with the exception of Mus musculus. Despite this high prevalence, no evidence has been found of any adverse effect of their presence. Any clonal viruses would be produced from rodents caught in the UK and would therefore
be identical to the currently circulating viruses. The insertion of DNA encoding traceable protein tags into the coding sequences should not significantly alter the function of the TTV proteins. If any effect is seen, it is likely to have adverse effects only to the fitness of the virus itself and is highly unlikely to enhance the pathogenicity or host range of the resulting recombinant virus.

In the unlikely event of human infection from recombinant rodent TTV, the pathogenic consequences in healthy individuals would be expected to be negligible at most as no clear reproducible pathology has been demonstrated in studied cases (including humans, pigs, and rodents) which are viraemic for their host-appropriate viruses or in the cases of deliberate cross-species laboratory infections.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, part 3, 1993 (either 121-125oc for at least 15 mins or 134-138oc for at least 10 mins), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment.

Liquids (eg smaples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, part 3, 1993 (either 121-125oc for at least 15 mins or 134-138oc for at least 10 mins), discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalperls) - autoclave using a make safe cycle as specified in BS 2646, part 3, 1993 (either 121-125oc for at least 15 mins or 134oc-138oc for at least 10 mins), dispose via clinical waste stream for microwave treatment.

Animal bedding and carcasses- autoclave using a make safe cycle as specified in BS 2646, part 3, 1993 (either 121-125oc for at least 15 mins or 134oc-138oc for at least 10 mins), dispose of carcasses and bedding via clinical waste stream for incineration. If larger animals are used autoclaving may not be feasible and an alternative validated method would be used to inactivate the genetically modified micro-organisms. The detail of any such alternative requirement would be notified to HSE as and when necessary.

Degree of kill

Autoclaving. Effectively 100% kill, the efficiency of all autoclaves and sterilising regimes will be monitored using the Porspore2 validation system.

134-138oc for at least 10 mins is the normal cycle for autoclaves within the CSU

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment
There are two aspects to this project. The first aspect regards the investigation of the functional role of herpesvirus miRNAs. The second aspect involves using endogenous cellular miRNAs to investigate the tissue tropism and pathogenesis of herpsviruses.

The viral vectors in this case are the CMV and disabled lentiviral vectors. The potential risks have been detailed above. The herpesBAC DNA is predicted to be infectious and therefore could theoretically be taken up by and infect a host cell if released from a killed bacterium in the gut. The efficiency by which virus DNA produces an infection following electroporation into permissive cells is low, the likelihood of BAC DNA being released intact and then being taken up by a cell to produce virus is therefore low/effectively zero. The BAC is not mobilized in bacteria.
Human cytomegalovirus is a human infectious pathogen. It is in endemic in all populations reaching levels of infection of between 40-100% depending on geographical and socio-economic group. Infection occurs primarily via close contact spread from bodily secretions such as: oropharyngeal, sperm, milk, tears, faeces and blood. It is though that the majority of infection occurs in children and young adults via oral or respiratory routes. During this phase the parotoid gland, lungs and liver are primary sites of infection. Primary infection in the normal host frequently results in a mononucleosis and lymphocytosis. A transient suppression of cellular immunity may be evident following infection. In the normal host, CMV induced symptoms are generally self-resolving. HCMV is classified as a hazard group 2 agent on the Approved List of biological agents.

It is unclear if CCMV or RhCMV infects humans. CMV viruses normally display strict species specificity, however CCMV in particular, is closely related to HCNV and can replicate efficiently in human fibroblast cell lines. As such both viruses will be treated as hazard group 2 agents with the same considerations as HCMV.

MCMV does not infect humans and therefore presents few risks to humans. Lentiviral vectors are disabled and are only able to replicate in helper cell lines. They are well established reagent and not considered to pose any health risk.

**Origin & function**

Components of the RNAi machinery are constitutively expressed in all cells therefore expression of tagged versions of these components are unlikely to increase pathogenesis of the virus. Reporter constructs such as GFP have been extensively used and deemed as safe. Cellular cDNAs identified as miRNA targets will be tagged and cloned into disabled lentiviral vectors. Because these vectors cannot replicate outside of helper cells the risk of any cDNA inserted is minimal.

**Evaluation of foreseeable effects**

Insertion of tagged components of the RNAi machinery or reporter constructs are unlikely to increase the pathogenicity or associated risk with any of the microorganisms. Because Lentiviral vectors cannot replicate outside of helper cells the associated risk is minimal. However work with these vectors will be carried out in category 2 conditions. The bacteria are laboratory adapted multiple autotrophic strains and therefore pose little to no health or environmental risks.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- **Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates)** - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 134-138°C for at least 10 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave.

- **Liquids (eg samples, culture supernatants, issue culture media)** - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 134-138°C for at least 10 minutes), discharge to drains.

- **Sharps (in sharps bin, eg needles, syringes, scalpels)** - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 134-138°C for at least 10 minutes), dispose via clinical waste stream for microwave treatment.

- **Animal bedding and carcasses** - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 134-138°C for at least 10 minutes), dispose of carcasses and bedding via clinical waste stream for incineration. If larger animals are used autoclaving may not be feasible and an alternative validated method would be used to inactivate the genetically modified micro-organisms. The detail of any such alternative requirement would be notified to HSE as and when necessary.
Degree of kill:
Autoclaving: Effectively 100% kill, the efficiency of all autoclaves and sterilising regimes will be monitored using Prospore2 validation system.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

<table>
<thead>
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Project Ref 207/12.1

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<tr>
<td>04/01/2012</td>
<td>Molecular, ecological and evolutionary dynamics of bacterial virulence factors: implications for pathogenicity and control</td>
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<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements N
**Project Additional Information**

### Purposes of the contained use

The aims of this project are to develop and test novel theory regarding the evolution of virulence in opportunistic pathogens. Much of the experimental work will focus on the opportunistic pathogen *Pseudomonas aeruginosa* (hazard group 2 in the Approved List of Biological Agents).

### Recipient or parental organism

The recipient organism is the opportunistic pathogen *Pseudomonas aeruginosa* which is able to colonise and pathogenise humans that are compromised such as sufferers of Cystic Fibrosis and Chronic Obstructive Pulmonary Disease, however it is abundant in the environment, in the soil and waterways and does not cause disease in healthy individuals.

### Host/vector system

Vectors will be standard cloning vectors such as pBluescript II and standard suicide vectors such as pRIC380. These will sometimes be carried by non-pathogenic strains of *E. coli* as the donor strain in conjugation.

### Origin & function

To study gene expression, we will utilise reporter systems based on the luxCDABE operon which is synthetic but was discovered in *Photorhabdus luminescens*. We will also utilise gene interruption and gene deletion protocols where standard antibacterial resistance markers such as GmR and CbR conferring resistance to Gentamycin and Carbenicillin respectively. We will only use resistance markers to 'old' antibiotics outside of clinical use.

### Evaluation of foreseeable effects

In every case our genetic modifications are predicted to reduce the fitness of the parental organism in the environment or reduce virulence in a clinical setting. Though the resulting organisms will pose no extra threat to researchers or the environment, the impact will be reduced to zero via standard class II containment procedures and adhesion to standard laboratory health and safety practices.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**WASTE DISPOSAL PROCEDURES**

- **Solids** (e.g. plastic-ware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

- **Liquids** (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646 Part 3, 1993 (either 121-125°C for at least 15
minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste stream for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

SPILLAGES

Particular care should be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that involves broken glass) unless they are aware of the potential risks and trained in safe working practices.

If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste stream.

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 207/12.2

Date Ackn'd 02/03/2022  CU2 Project Title
Imaging and manipulation and analysis of fungi expressing recombinant fluorescent and luminescent proteins in vitro

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<tr>
<th>Date Project Ceased</th>
<th>11/05/2014</th>
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</table>

**Project Additional Information**

### Purposes of the contained use
To image analyse and manipulate fungi expressing recombinant fluorescent and luminescent proteins in vitro

### Recipient or parental organism
A range of containment level I and II fungi and disabled E. coli K12. Some of the fungi are pathogens of plants and humans.

### Host/vector system
Standard host/vector systems used for yeast and filamentous fungi

### Origin & function
Green fluorescent protein, other fluorescent proteins and the luminescent protein aequorin derived from Aequoria victoria and other marine organisms. These proteins will be used to label fungal proteins and organelles, and measure intracellular calcium, in fungal cells.

### Evaluation of foreseeable effects
There is no possibility for increasing the pathogenicity of the pathogenic organisms with the experiments performed on them in the proposed work. The other non-pathogenic fungi to be used are model organisms that are considered generally safe to work with. The genetic material to be inserted into the recipient fungi and E. coli are not involved in pathogenesis and are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of the cloning host or normal human defence mechanisms. Gene transfer is possible but unlikely to be hazardous

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g. plastic-ware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (bloack bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

SPILLAGES

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If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste stream.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned

Project Containment

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</table>

Animal Units Large Scale Activities Human Clinical Applications

02/03/2022 Page 4901 of 15326
Establishing in vitro model of Rett syndrome using human LUHMES cells

Rett Syndrome is a neurological disorder caused by mutation in the MeCP2 gene encoding protein, which is a competent chromatin. The neurological spectrum of Rett Syndrome suggests that this protein plays an important role in the nervous system. Despite many years of research on Rett Syndrome and MeCP2, our knowledge about the molecular and cellular basis of this disorder is still incomplete.

Limitations of in vivo studies such as the accumulation of indirect effects have impeded understanding of direct consequences of the loss of MeCP2. Thus, it is important to develop simplified models of Rett Syndrome to create the "disease in a dish". This can be achieved for instance by differentiating human neuronal progenitors, which carry various MeCP2 mutations, into a homogenous neuronal population.

We will establish neuronal cultures derived from immortalized human mesencephalic cell line (LUHMES cells). We would like to introduce a plant-based protein degradation system into the genome of LUHMES cells using adeno-associated viruses. Also stably silence the expression of endogenous MeCP2 using shRNA and at the same time introduce modified exogenous MeCP2 using lentiviruses.

In order to fulfill objectives mentioned above we have chosen to use adeno-associated viral vectors and lentiviral vectors because they have negligible probability of inducing insertional mutagenesis and immunogenicity problem. They are also very well suited for transduction of post-mitotic neurons.
In order to fulfil objectives mentioned above we have chosen to use adeno-associated viral vectors and lentiviral vectors because they have negligible probability of inducing insertional mutagenesis and immunogenicity problem. They are also very well suited for transduction of post-mitotic neurons.

**Origin & function**

MeCP2 gene encodes a protein which is a component of chromatin.

**Evaluation of foreseeable effects**

To understand the direct consequences of the loss of MeCP2 encoded protein and how it relates to Rett Syndrome.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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<th>Solids (e.g. plastic-ware such as pipettes, flasks, tubes etc and agar plates)</th>
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<td>Sharps (in sharps bin, e.g. needles, syringes, scalpels)</td>
<td>dispose via clinical waste stream for heat treatment.</td>
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Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

**SPILLAGES**

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If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste stream.

---

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

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**Project Ref** 207/12.4

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**Project Additional Information**

**Purposes of the contained use**

To study the pathogenesis of Semliki Forest virus (SFV) in vitro and in vivo. To define determinants of virus phenotypic properties including, ability to stimulate interferon and to activate cellular genes, ability to trigger apoptosis, ability to cross the blood-brain barrier and ability to produce neurological disease.

**Recipient or parental organism**
Semliki Forest VirusS (sfv). Strains to be used include SFV4, A7(74) and L10.

**Host/vector system**

Well-characterised and established or primary (eg haematopoietic lineage, fibroblast) vertebrate and invertebrate cell lines. Laboratory mice.

**Origin & function**

The marker/receptor genes and the inserted sequences that will be used include:

(i) standard fluorescent markers, for example green fluorescent protein (from the jellyfish Aequorea victoria);

(ii) biochemical markers including enzymes such as renilla (Rennila reniformis) or firefly (Phontinus pyralis) luciferase;

(iii) regulatory (miRNA piwiRNA) RNA recognition elements;

(iv) inhibitors/regulators of insect or plant innate immune responses or cellular pathways. Proteins which interfere with immune responses in vertebrates will not be used in studies generating infectious virus.

(v) Sequences encoding for SFV proteins will be swapped between virus strains.

**Evaluation of foreseeable effects**

Transfer of virus genetic material will only be between strains of SFV with the exception of plant or invertebrate virus sequences known to encode regulators of plant or invertebrate cell functions (eg p19,B2) which have no known function or deleterious effect in vertebrates. The only other foreign genes that will be inserted are known non-harmful marker or reporter genes or RNA recognition elements.

None of these changes would be predicted to increase pathogenicity. Natural viruses have been selected to be best fit and these changes, which generally increase the size of the genome and most likely to attenuate fitness and pathogenicity.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solids (eg plasticware such as pipettes, flasks tubes etc) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for incineration or microwave treatment.
Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for incineration or animal bedding may after autoclaving also be disposed of via the clinical waste stream for microwaving or via the industrial (black bag) waste stream for landfill.

Degree of kill:
Autoclaving - effectively 100% kill
Incineration and microwaving - not applicable, all waste is autoclaved prior to disposal by incineration or microwaving

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned

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Human Clinical Applications

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Project Ref 207/13.1

CU2 Project Title

Role of telomerase and telomeres in cell transdifferentiation processes

Class

Class 2

Culture Volume

< 1 Litre

Non-GMM

Consent Granted

Project notified under transitional arrangements
The aim of the project is to evaluate the role of telomerase and telomere length in cell transdifferentiation processes (the biological event where a differentiated cell differentiates into another cell type without passing through a staminal state).

In particular, we want to transfect both wild type and telomerase negative mouse embryonic fibroblasts (MEFs) possessing different telomere length with lentiviral vectors coding for genes inducing transdifferentiation from fibroblasts to other cell kinds (neurons and muscular cells at the beginning). We aim to determine if different telomere lengths (or telomerase status) affects the ability to undergo transdifferentiation.

Viral vectors we would like to use are the following:
A) Standard commercially available vectors like Clontech Plvx-Tet-On Advanced. Catalog # 632162
E) Addgene plasmid 8455: pCMV-dR8.2 dvpr (packaging plasmid for Lentiviral constructs).

Insert code for normal mammalian genes and standard marker genes.
MyoD (MGI:97275)
Ascl1 (MGI:96919)
Brn2 (MGI:101895)
Myt1l (MGI:1100511)
These genes are involved in mammalian neural differentiation processes (Ascl1, Brn2, and Myt1) and myoblast differentiation (MyoD). Therefore, the expectation is that they will induce the recipient cells to adopt a phenotype more similar to neurons.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solids (e.g. plastic-ware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121 - 125°C for at least 15 minutes or 126 - 130°C for at least 10 minutes or 134 - 138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

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Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures

**SPILLAGES**

Particular care should be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that involves broken glass) unless they are aware of the potential risks and trained in safe working practices.

If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.
Project Additional Information

Purposes of the contained use

A combination of microarray and CAGE data has been analysed and used to define a list of cell cycle genes expressed in neonatal human dermal fibroblasts (NHDFs). The analysis also resulted in the identification of c100 putative novel cell cycle genes. In order to study the cellular localization of the products of these genes and to provide evidence of involvement in the cell cycle, proteins will be fused to eGFP at either the N- or C- terminal and expressed in B+NHDF, HEK-293 ad HEK293T cells.

Recipient or parental organism

NHDF, HEK-293 and HEKs93T cells (Human origin)
### Host/vector system

| E.coli (K12 derived non-colonizing strain)/pDONR223, pcDNA DEST47, pcDNA DEST54 (Invitrogen) |

### Origin & function

| cDNA derived from Human mRNA |

### Evaluation of foreseeable effects

| No sequences known to be oncogenic or tumorigenic will be cloned or expressed in NHDF cells or HEK293 cells. However as c100 unknown genes are being studied, there is a very small risk of cloning and expressing an oncogenic or tumorigenic sequence. The risk of naked DNA entering cells of laboratory workers by electroporation-based methodology or lipid based transfection is negligible. Taking into account the characteristics of the manipulated genes, the vectors employed and the recipient cells as described above, none of the genetically modified micro-organisms or cultured cells used in this work present a risk to human health. |

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| Not applicable |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| Not applicable |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 5 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment.  
Liquid (eg samples supernatants, tissue culture media) - either autoclave using a make safe cycle as specified in BS2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 5 minutes or 134-138°C for at least 3 minutes), discharge to drains or disinfect with 1% Virkon solution for at least 1 hour then discharge to drains.  
Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 5 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for heat treatment. |

### Is an emergency plan required according to regulation 20?  
| N |

### If yes, tick to confirm that it is attached to this form

| N |

### Tick to confirm that you have attached a risk assessment to this form

| Y |

### Tick if you are claiming exemption from disclosure for section of the risk assessment

| N |

---

**Please enter comments on the GM safety committee on the risk assessment**

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.
Cells cultured in vitro can be used for many studies of basic molecular and cell biology as well as for diagnostic purposes. When considering cultured cells as replacements for animals in research it should be remembered that the use of primary cells does not reduce the use of animals; animals are either killed to gain access to the cells of interest or the cells are harvested from accessible sites, some of which cause limited distress (e.g. blood sampling), but others which are more traumatic (e.g. broncho-alveolar lavage on a living animal). Primary cell lines have significant limitations, including heterogeneity, limited capacity to divide in culture and the early onset of senescence. Immortalised cell lines have a number of advantages: unlimited supplies of characterised cells with the same genotype can be used by multiple researchers over prolonged periods of time. The purpose of the contained use for this project is to generate immortalized pig macrophage cell lines and demonstrate their potential for the study of host-pathogen interactions. The generation and evaluation of the cell lines will be addressed through four specific interdependent objectives:
1. To generate a replication defective MMLV-based recombinant vector which encodes the human telomerase reverse transcriptase (Htert).
2. To generate immortalised macrophage cells lines from a range of pig genotypes
3. To determine whether immortalised macrophage cells respond similarly to resident lung macrophages upon challenge with viral pathogens with a macrophage tropism

Recipient or parental organism

Recipient organisms are E. coli, HEK293T cells and primary macrophages

Host/vector system

Host and vector system combination will be as follows: i, E.coli and cloned Htert into the pFBNeo vector to generate the pFB-Htert-Neo recombinant vector, pVPack-VSV-G and the gag-pol vector pVpack-GP; ii, HEK293T cells for the packaging of MMLV and iii) Primary macrophages transduced the replication-defective MML-Htert

Origin & function

The telomerase reverse transcriptase (tert) has a human origin (Htert). The biological function of Htert is to cause immortalization of the transduced macrophages.

Evaluation of foreseeable effects

The biological function of inserted genetic material, the telomerase gene (Htert), will cause the immortalization of the transduced macrophages. Copy number and level of expression are unpredictable. It is possible that depending on the region of insertion into the pig DNA and the conversion to immortalized cells, recombinant cells will potentially harbour new functional characteristic including production of cytokines, allergens or other molecules. As part of the normal procedure set for this experiment we intend to assess the emergence of any new properties of the recombinant immortalized cells. We will screen for cytokines using available ELISA test and RT-PCR assays and compare the results to normal non-immortalised primary macrophages. New recombinant immortalized macrophages that will harbour excessive alteration in its biological response, such as to enhance cytokine production, will be discarded as described in sections below on additional control measures.

Considering the potential remodelling genetic activity of Htert, the likelihood of reactivating/complementing PERVs is not negligible and need to be considered in this work to reduce risk of transmission to human or other cells. Finally it is possible that the inserted genetic material may be oncogenic. Indeed remodelling genetic activity of the Htert inserted genetic material may be oncogenic. Telomerase activity has been found in almost all human tumours but not in adjacent normal cells (Kim, N.Wet al (1994) Science, 266, 2011-2015). The most prominent hypothesis is that maintenance of telomere stability is required for the long-term proliferation of tumours. Thus, escape from cellular senescence and becoming immortal by activating telomerase, or an alternative mechanism to maintain telomeres (Bryan, T.M.(1995) EMBO J., 14, 4240-4248). Constitutes an additional step in oncogenesis that most tumours require for their ongoing proliferation. To reduce the risk associated with the use of oncogenic recombinant cells, work will be carried out in Class II Microbiological Safety Cabinet (MBSC) in line with CL2 facility controls.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste must be inactivated by validated means before pouring fown the drains or leaving the site for incineration. Inactivation may be achieved by autoclaving or disinfection.

1- Autoclaving

This is the preferred means of inactivation and is mandatory for all waste generated in CL2 facilities. In other areas it must be used for waste that cannot be effectively inactivated by disinfection.

Such items may include: Agar plates, cell/bacterial pellets, small tubes, plastic loops, tips and gloves.

Autoclaving procedure: Where autoclaving is used, a cycle of 121°C for 45 minutes, with full steam penetration to the centre of the load, has been shown to be sufficient to
render most materials sterile. Validation in this case will rely on the annual testing of the autoclave to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time. On subsequent runs verification that the correct conditions were reached can be obtained through the use of, for example, chart recorders or appropriate indicators autoclaved with the load.

Bags and Containers: Proprietary autoclave bags should be used. These should not be filled more than 3/4 full in order to allow the necks to be closed with coded ties/tags. Some means of identifying the source of the waste must be shown [lab number/operator]. Bags should be transported to the autoclave in robust leak-proof containers.

2-Disinfection

This generally refers to the use of chemical agents to destroy the potential infectivity of a material. Effective disinfection is dependent upon: i) activity; ii) concentration and iii) length of contact.

Disinfection is not as effective as steam sterilisation in destroying viable organisms, nor is it easily monitored.

Disinfection is a suitable means of inactivation for:

- Reusable items that are heat sensitive
- Liquid cultures of, and equipment used in association with Group 1 organisms
- At the end of the work for decontamination of surfaces and equipment that cannot be autoclaved.

The choice of disinfectant:

Before choosing a disinfectant it is important to refer to the manufacturers data to ensure that the disinfectant will be effective against the organism(s) in question and to determine the recommended concentrations and contact times. The aim of the disinfectant is to reduce the titre by at least 5 logs and that large amounts of protein can interfere with chemical inactivation.

Disinfectants currently in use in the lab 2.073: Haz-tab, Ethanol and Distel (www.tristel.com) however (this current work on immortalized macrophage will exclusively use Virkon (www.antechh.co)m see document 5a, b and c in appendix section) to increase the effectiveness of the disinfection of retroviruses and bacteria and potential PERVs.

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**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

**Please enter comments on the GM safety committee on the risk assessment**  

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

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**Project Containment**

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The overall aim of this project is to identify molecular and cellular factors that govern the development and function of the immune system in the mouse. To this end, we will be following the immune response and the development of immunological memory in vivo following infection of mice with Listeria monocytogenes (PMID 21349093).

Listeria monocytogenes is an opportunistic pathogen that gives rise to extremely rare cases of food poisoning. However, in the elderly, in neonates and among immunocompromised individuals it can cause severe disease, e.g. meningoencephalitis or septicemia. Listeria monocytogenes also poses an additional risk to pregnant women and their unborn child as it actively travels through cells and, therefore, can be transmitted from a mother to the fetus transplacentally and may result in abortion or still birth. Mainly we will use L. monocytogenes that carries a deletion of the gene ActA, aimed to make the bacteria less harmful (attenuated) and specifically to reduce its ability to spread intracellularly, which decreased its ability to transmit to the fetus 1000-fold (PMID 1286064, PMID 17118980). The greatest risk when working with L. monocytogenes is infection via pricking of the skin by contaminated sharp objects. Therefore, it is advisable to use cut-resistant gloves during injection of the bacterial suspension into mice. L. monocytogenes will be handled at containment Level 2. Pregnant women to be advised not to carry out this work due to the possible risks. A separate risk assessment to be completed if they still wish to participate in this research.

The chicken ovalbumin (OVA-albumin) expression cassette carries resistance to the antibiotic erythromycin to allow selection of L. monocytogenes containing the expression cassette. However, the standard treatment of listeriosis includes ampicillin and an aminoglycoside drug and the Listeria monocytogenes we would use is sensitive to the standard treatment antibiotics (PMID 10471548). There are naturally occurring erythromycin-resistant Listeria strains in the UK, but these strains are sensitive to other relevant antibiotics (PMID 10471548). Containment Level 2 precautions will be used when handling bacteria and all potentially infectious waste will be decontaminated before disposal.
The chicken ovalbumin gene is non-pathogenic and it has been introduced into L. monocytogenes and the ActA disabled variants of the bacteria to facilitate monitoring of immune responses to the bacteria. The OVA-albumin expression cassette also carries resistance to the antibiotic erythromycin to allow selection of L. monocytogenes containing the expression cassette. However the standard treatment of listeriosis includes ampicillin and an aminoglycoside drug and the Listeria monocytogenes we would use is sensitive to the standard treatment antibiotics (PMI 10471548).

**Origin & function**

L. monocytogenes are widely distributed in the environment, are not exotic to the UK, are not pathogenic to plants, and none cause notifiable diseases under specific surveillance by DEFRA. The ActA deleted attenuated L. monocytogenes strain is less harmful than naturally occurring Listeria strains, since its dissemination in an infected person is impaired. As there is the possibility for natural spread of L. monocytogenes to other animals within the animal unit, appropriate containment measures will be used to prevent unplanned infection. This normally involves filtered cage tops and use of a quarantine room or use of an isolator in a shared room, to minimise the risk of dust-mediated transmission. In practice, this particular risk is extremely low, as a high infectious dose is required for this organism. Listeria is an intracellular pathogen that is destroyed by innate immune cells that rapidly clear primary and secondary infection. There is a very low likelihood of shedding and cross-infection between mice. Listeria interacts with the adherens junction protein E-cadherin to transverse the intestinal epithelium, but the efficiency of invasion is greatly reduced in mice compared to humans due to a single amino acid substitution in E-cadherin (PMID 21349093). Gloves will be worn where appropriate with particular attention to procedures to minimise any risk of sharps injury. Within the animal units, mice are held in cages inside individually ventilated cages and generally within a quarantined room. Catch trays are used for any injection or manipulation procedures and before starting this work we will clean surfaces with 70% ethanol and after finishing the surfaces are to be decontaminated with Virkon. No special immunisation is required for work with these organisms and good microbiological practice/good hygiene is normally adequate forms of protection. The likelihood of accidental environmental release of the GMMs is very low or negligible, given the measures that will be applied to control any risk to human health and safety (level 2 containment). If release was to occur, the consequences can also be graded as very low or negligible. Therefore, the environmental risk can be judged as effectively zero.

**Evaluation of foreseeable effects**

L. monocytogenes are widely distributed in the environment, are not exotic to the UK, are not pathogenic to plants, and none cause notifiable diseases under specific surveillance by DEFRA. The ActA deleted attenuated L. monocytogenes strain is less harmful than naturally occurring Listeria strains, since its dissemination in an infected person is impaired. As there is the possibility for natural spread of L. monocytogenes to other animals within the animal unit, appropriate containment measures will be used to prevent unplanned infection. This normally involves filtered cage tops and use of a quarantine room or use of an isolator in a shared room, to minimise the risk of dust-mediated transmission. In practice, this particular risk is extremely low, as a high infectious dose is required for this organism. Listeria is an intracellular pathogen that is destroyed by innate immune cells that rapidly clear primary and secondary infection. There is a very low likelihood of shedding and cross-infection between mice. Listeria interacts with the adherens junction protein E-cadherin to transverse the intestinal epithelium, but the efficiency of invasion is greatly reduced in mice compared to humans due to a single amino acid substitution in E-cadherin (PMID 21349093). Gloves will be worn where appropriate with particular attention to procedures to minimise any risk of sharps injury. Within the animal units, mice are held in cages inside individually ventilated cages and generally within a quarantined room. Catch trays are used for any injection or manipulation procedures and before starting this work we will clean surfaces with 70% ethanol and after finishing the surfaces are to be decontaminated with Virkon. No special immunisation is required for work with these organisms and good microbiological practice/good hygiene is normally adequate forms of protection. The likelihood of accidental environmental release of the GMMs is very low or negligible, given the measures that will be applied to control any risk to human health and safety (level 2 containment). If release was to occur, the consequences can also be graded as very low or negligible. Therefore, the environmental risk can be judged as effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

WASTE DISPOSAL PROCEDURES

Solids e.g. plastic-ware such as pipettes, flasks, tubes etc., and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste stream for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1.000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.
Particular care should be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that involves broken glass) unless they are aware of the potential risks and trained in safe working practices.

If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste stream.

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Animal Units

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Project Ref 207/14.4

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Withdrawn N Tick if notifying a connected programme of work Y
### Project Additional Information

**Purposes of the contained use**

This project will utilise a range of viruses modified to express reporter genes as tools to better understand and define how host cellular pathways and pathogen interactions at the molecular and protein level influence the establishment and pathogenesis of viral infection, and provide insight into potential targets for novel anti-viral therapeutics.

**Recipient or parental organism**

In this connected programme a range of different viruses will be used including Adenovirus, HSV-1 and HSV-2, VZV, CMV, EBV, KSHV, MDV, mHV-68, Influenza A virus, RSV, FMDV, SFV and Buyamwera virus.

**Host/vector system**

Several different vector systems including BACs, MVA, adeno- and lentiviral vectors as well as plasmids will be used. For FMDV, a non-infectious replicon system will be used.

**Origin & function**

Several different standard, well-characterised genetic marker genes, such as the jellyfish green fluorescent protein (GFP) and/or other fluorescent proteins (CFP, YFP, DsRed), and firefly (or Renilla, Gussia) luciferase as well as selection markers such as antibiotic resistance genes with no harmful physiological or pharmacological properties will be used.

**Evaluation of foreseeable effects**

All recombinant infectious pathogens used in this study will not be modified with respect to their determinants of tropism i.e. viral glycoproteins. The GM pathogens used and generated within this study will at most be equal to wild-type with respect to transmissibility, virulence, pathogenicity and tropism, an equal containment level is appropriate. In all cases, taking into account the control measures assigned above, the overall risks to the environment form the genetically modified micro-organisms produced in this work is effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable
WASTE DISPOSAL PROCEDURES

All pathogen waste is decontaminated with TriGene (Hayman Medichem). TriGene is a halogenated tertiary amine detergent, a category of detergents proven to be virucidal against enveloped and non-enveloped viruses including HIV, yet with minimum toxicity to man. TriGene is thus a suitable detergent for disinfection of liquid media and plastics used for culture and use of all infectious agents described herein. Furthermore, Virkon powder, a broad spectrum disinfectant active against all known virus families, is used in the event of spillages of infectious material.

Autoclaving is by using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121°-125°C for at least 15 minutes or 126°-130°C for at least 10 minutes or 134°-138°C for at least 3 minutes). This procedure has a degree of kill of 100%. The autoclave undergoes an annual twelve-point validity check to ensure sterilisation effectiveness.

Class II microbiological safety cabinets are tested every 6 months, as recommended by HSE/Scottish Executive guidelines.

Solids (e.g. plasticware such as pipettes, flasks, tubes, etc)
All used tissue culture plastics, pipettes, micro-pipette tips, micro-centrifuge tubes, cryovials, plastic haemocytometers and other plastics are rendered safe by soaking overnight in a solution of 1.25% TriGene (or equivalent). Liquid is drained down the disposal sink within the Containment Level 2 tissue culture laboratory and decontaminated plastics disposed of via the clinical waste stream for incineration or microwave treatment. Both solid and liquid waste will be autoclaved after infected material has been exposed to 1.25% TriGene to ensure 100% kill of all viruses. Non-contaminated, non-laboratory waste, such as empty media bottles, is disposed of via the clinical waste stream for microwave treatment. Non-contaminated, non-laboratory waste (plastic/cardboard wrappings etc) is disposed with general waste for collection by cleaning staff.

Liquids (e.g. samples, culture supernatants, tissue culture media)
All used tissue culture media and other liquids are treated with 1.25% Trigene (or equivalent) for a minimum of 2 hours to inactivate virus prior to discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels, glass slides/cover-slips)
All sharps used are disposed directly into a sharps bin, then autoclaved using a make safe cycle as specified above prior to disposal via the clinical waste stream for incineration or microwave treatment.

Spill management
Liquid spills will be bordered and covered with Virkon powder for 30 mins until all liquid is absorbed and liquid is decontaminated before mopping up with plenty of water.

Additional waste disposal procedures for use of FMDV replicon.

All liquid and waste will be discarded directly into 1.25% Trigene (or equivalent) within the Class II cabinet and inactivated by overnight treatment prior to autoclaving.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

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Withdrawn N

Tick if notifying a connected programme of work N

**Project Additional Information**

**Purposes of the contained use**

*Pseudomonas aeruginosa* is a bacterium that is well known for quorum sensing: individual bacteria produce chemical signals that are detected by other bacteria. The aim of this project is to monitor the growth of colonies of *P. aeruginosa* cells, tracking their individual growth rates (ie fitness) and the spatial structure of the resulting microcolonies, using constitutively fluorescent strains. The induction of various gene expression responses (eg quorum sensing) will be tracked with fluorescent reporters. To achieve this we may need to use mutant strains in which one or more gene is deleted as well as reporter strains in which genes encoding fluorescence, luminescence or...
other standard reporter proteins have been incorporated.

Recipient or parental organism

Pseudomonas aeuginosa: we will use the wild-type laboratory strain PA01 and gene-inactivated derivatives, as well as clinical isolates from the sputum of healthy individuals or cystic fibrosis patients, and natural isolates from soil.

Host/vector system

The vectors will be non-hazardous, widely used and/or commercially available standard plasmid or phage vector systems, some of which confer resistance to standard antibiotics such as kanamycin, ampicillin, chloramphenicol. Where mobilisable (i.e. capable of being mobilised if in the presence of a vector which expresses the components needed for conjugal pilus synthesis and assembly) plasmids are used, they will only be used with a debilitated host or in a host which does not contain the necessary and appropriate set of genes for conjugation of the plasmid.

Origin & function

Inserted DNA will usually encode reporter genes such as green fluorescent protein, luciferase or beta-galactosidase.

We may also make mutants in key quorum sensing genes such as lasl, lasR, rhl, rhlR, pqsA, pqsH, pqsE, pqsR and other genes encoding extracellular products such as EPS and siderophores.

Some of the genetic modifications will result in resistance to antibiotic markers.

Evaluation of foreseeable effects

We do not expect the insertion of reporter genes into the host to alter the disease causing properties of the organism in any way.

Concerning the mutations in key quorum sensing genes, and gene encoding other extracellular products, all of these mutations are expected to reduce the number and amount of exported products such as enzymes, toxins and polymers. Since these products are associated with virulence, our mutations are predicted to decrease virulence. All of the mutants we propose to make have been made previously in other labs and none has been reported to increase virulence.

Although some of our modifications will result in resistance to antibiotic markers, we see no danger in the routine acquisition of resistance to laboratory antibiotics such as Tetracycline, Gentamycin, Chloramphenicol and Ampicillin with no clinical use. Ceftazidime and tobramycin are the mainstay of anti-pseudomonas treatment but ciprofloxacin and meropenem are also occasionally used. There is no expectation that cross resistance between laboratory markers and clinical antibiotics will occur. In addition the carriage of such markers often bears a fitness cost to the organism since its persistence requires the presence of the antibiotic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

WASTE DISPOSAL PROCEDURES

Solids (e.g. plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.
Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for microwave treatment.

Degree of kill:
- Autoclaving - effectively 100% kill (annual validation)
- Microwaving or incineration - not applicable, all waste is autoclaved prior to disposal by microwaving or incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Project Ref 207/14.6

Date Ackn'd 25/09/2014
CU2 Project Title Viral transduction of mammalian cells in culture
Class 2
CultureVolClass2 < 1 Litre
Non-GMM Consent Granted

Date Project Ceased 02/03/2022
For many researchers, the method of choice for genetic alteration in mammalian cells is through viral transduction. In this regard, retroviral and lentiviral particles are particularly commonly-used vectors, with adenovirus and adeno-associated virus (AAV) also in use. None of these vectors are wild-type virus, nor are they replication competent. Therefore, in many cases they will be categorised as class 1, but in some cases they are class 2 and hence fall under this risk assessment. Guidance for classification of research at the IGMM has been obtained from the SACGM Compendium of Guidance.

The mammalian cells that we transduce in tissue culture are predominantly of very low risk. The majority are mammalian cell lines (e.g. HeLa, HEK293) that are well established, well characterised and available from commercial sources such as ECACC or ATCC. Primary mammalian cells are also used as recipient cells. Where these are from patients, they may also fall under separate COSHH risk assessments for ACDP Hazard Group 2 organisms.

The four viral vectors covered under this assessment are retroviruses, lentiviruses, adenovirus and AAV. Each vector is dealt with separately. Retroviruses & Lentiviruses insert into the host chromatin and, as such, are effective in promoting long-term expression of transgenes. In addition, a successful infection carries the risk of insertional mutagenesis. For potentially harmful inserts (e.g. oncogenes etc.), long-term expression of transgenes. In addition, a successful infection carries the risk of insertional mutagenesis. For potentially harmful inserts (e.g. oncogenes etc.), long term expression in a human subject could give rise to pathological outcomes and are considered class 2.

Adenovirus: because of the risk of escape of replication-competent virus, all adenoviruses are considered class 2. Recombinant AAV can integrate into the host genome, although the lack of the rep gene in the transfer vector renders this a very low frequency occurrence. Extensive studies (including clinical trials) have failed to observe insertional mutagenesis and so this poses negligible risk in a laboratory setting. However, long term episomal expression of inserts is often observed. Thus where inserts are of a potentially hazardous nature (e.g. oncogenes, cytokines, biologically active molecules etc.) these vectors are considered class 2.

In all cases the inserted genetic material is designed to have biological properties that could pose a risk to human health and safety. Likely factors to be introduced include oncogenes, sequences that lead to inactivation of tumour suppressor genes, gens involved in innate immunity and sequences that modify basic biology in many ways. For all of the viral vectors described below, expression of oncogenes, cytokines or other molecules that could have detrimental effects on cell or organismal function will be considered class 2. Inserts of unknown (and therefore potentially hazardous) function will be considered class 2.

Mammalian cell lines are highly sensitive to their environmental requirements and survive for very short periods outside the well-controlled tissue culture environment. The risks that these cells pose to the environment is negligible. And with the risks to human health, the inserts would have similarly detrimental effects if introduced into other
mammalian hosts. Some of the vectors used can infect multiple species of mammals and so a hypothetical route for environmental harm does exist. However, the control measures in place in the CL2 laboratory (including proper waste disposal) reduce that risk to a negligible level. The vectors in use are capable of infecting other mammalian species. An important safety feature of the viruses is that they are rendered replication-incompetent and this greatly limits their risk of causing environmental harm. In conjunction with the control measures in place in the CL2 laboratory, these vectors pose negligible risk to the environment. The transduced mammalian cells pose no more risk to the environment than their untransduced counterparts. In all cases, these cells present minimal risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

WASTE DISPOSAL PROCEDURES

Solids (e.g. plasticware such as pipettes, flasks, tubes etc and agar plates) - double-bagged and placed in a protective container in case of leaks. Then autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or disinfect with Virkon (1%w/v) for 30 mins, discharge any excess liquids to drains, dispose of solids via clinical waste stream for heat treatment or via the industrial (black bag) waste stream for landfill - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or disinfect with Virkon (1%w/v) for 30 mins,

Liquids (e.g. samples, culture supernatants, tissue culture media) -

Is an emergency plan required according to regulation 20?  
Y  
If yes, tick to confirm that it is attached to this form  
N  
Tick to confirm that you have attached a risk assessment to this form  
Y  
Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned

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**Project Ref** 207/15.1

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<td>Utilizing wild-type and recombinant RNA viruses to study the antiviral defence mechanism of plants</td>
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**Historical Significant Changes**

- **Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Wild type and recombinant plant RNA viruses will be used to study antiviral defence mechanisms in Arabidopsis thaliana, Nicotiana benthamiana, Solanum tuberosum and Brassica napus. In the process live virus particles of Potato virus X (PVX), Potato virus Y (Pvy), Turnip mosaic virus (TuMV), Tobacco rattle virus (TRY) and Cucumber mosaic virus (CMV) will be re-assembled in planta from cDNA and may be capable of spreading systemically. The viruses are non-pathogenic to humans or animals and do not present a health risk.

**Recipient or parental organism**

Standard marker genes such as GFP will be cloned into viral cDNAs to allow virus movement to be followed. In addition recombinant viruses will be engineered to carry an or part of the 35S promoter in order to investigate sequence requirements of virus-induced transcriptional gene silencing. Neither the expression of wild-type viruses in binary vectors, the insertion of GFP sequences or the use of the 35S promoter have been reported to increase the virulence of plant viruses. As such, these approaches do not pose additional risk compared to the use of viruses as they are found in nature.

**Host/vector system**
The methodology for the expression of the viral vectors will be by agroinoculation into the host plant (in which standard cloning and plasmid construction will be employed using well-characterised methods with a history of safe use) or by direct inoculation of virus particles into the plant.

**Origin & function**

GFP-tagged clones will result in expression of fluorescence of virus particles in vivo, enabling virus movement to be monitored. Replication of recombinant viruses will trigger the antiviral gene-silencing pathway in tobacco, resulting in virus specific sANAs and sequence-specific viral degradation. Virus-specific sRNAs may direct DNA methylation in the nucleus, thus silencing of the constitutive expression of the GFP reporter gene in N. benthamiana 16c will be monitored.

**Evaluation of foreseeable effects**

Neither the expression of wild-type viruses in binary vectors nor the insertion of GFP sequences have been reported to increase virulence. The well described phenomenon of virus induced gene silencing will occur in planta in all cases (Baulcombe 0.1999) and will ameliorate viral load and virulence. Standard marker genes such as GFP tags are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host. Resulting GMOs carry no additional hazards compared with the endemic pathogens already present in the environment. Any transfer of genetic material to other organisms would be of minimal hazard. GMOs would not survive outside laboratory conditions. The risk to plants, animals, humans or environment is negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Growth of Agrobacterium constructs containing virus sequences will be in maximum 100 ml volumes and infiltrated into leaves by a 1 ml syringe-no aerosols will be created. An experimental solutions, materials or consumables used to grow or handle viruses will be autoclaved before disposal. Lab coats and disposable gloves will be worn for the handling of infected plants to prevent mechanical transmission. Transport of plants between growth facilities will be inside sealed plastic containers. In addition to the physical barrier that a glasshouse/growth rooms provides between cultured transgenics and the environment, the glasshouse/growth rooms are equipped with a filtered air system, which will prevent any pollen escape from the facility. Those plants that are allowed to flower for seed production will be bagged at the time of flowering. All plants will be grown using the systemic insecticide, Intercept 60WP, as a soil drench. This has been shown to be highly effective at controlling aphids under greenhouse conditions. All compost used within the glasshouse/growth rooms will be autoclaved before disposal, any wash-off from plants will be collected and treated with appropriate chemicals before disposal. All staff must follow the SOPs for the relevant areas, either the Rutherford Controlled Environment Rooms or the Transgenic Glasshouse. Copies of the SOPs are prominently displayed in the areas, and attached to this RA. Good Occupational Safely and Hygiene will be applied.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g. plasticware such as pipettes, flasks, tubes etc. and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1 300C for at least 10 minutes or
134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste stream for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plasticware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon: 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

**SPILLAGES**

Particular care should be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that involves broken glass) unless they are aware of the potential risks and trained in safe working practices.

If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste stream.

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**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

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**Project Containment**

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02/03/2022
The purpose of our research is to determine the functioning and regulation of immune responses to ensure tissue homeostasis and immunity. So have we for instance shown that the Epidermal Growth Factor (EGF) receptor plays a central role for the functioning of the immune system and for the homeostasis of inflamed tissues. The quality of EGF-R induced signals is dependent on the type of ligand that binds to the receptor. Different EGF-R ligands are expressed under distinct inflammatory conditions and we argue that the state of inflammation is linked via the EGF-R directly to the phenotype of local cell populations. Several pathogens have recognized this specific link and have targeted it as a means of immune escape. To address these different aspects we would like to use in vivo infection models. The chosen pathogens appear particularly suited for such a study, since they have become standard infection models for immunologists, the immune responses upon infection have been studied extensively, and the pathogen-specific immune responses can be quantified in very precise ways. In order to facilitate our research, these pathogens are often gene-deficient for specific genes (attenuated) or express model antigens (such as for instance different forms of ovalbumin) or express fluorescence or enzymatic reporter genes.

The recipient or parental organism

The used pathogen can infect human beings and each of these pathogens could potentially cause severe infections: Listeria monocytogenes is an opportunistic pathogen that gives rise to extremely rare cases of food poisoning. However, in the elderly, in neonates and among immune-compromised individuals it can cause severe disease, e.g., meningoencephalitis or septicemia. Listeria monocytogenes also poses an additional risk to pregnant women and their unborn child as it actively travels through cells and, therefore, can be transmitted from a mother to the foetus.
Transplacentally and may result in abortion or stillbirth. Thus, the spread of L. monocytogenes away from our working places has to be restricted as good as somehow possible and all work with L. monocytogenes will be handled with Good Microbiological Practice and Good Occupational Safety and Hygiene at containment Level 2. Nevertheless, the greatest risk when working with L. monocytogenes is infection via pricking of the skin by contaminated sharp objects. Therefore, it is advisable to use cut-resistant gloves during injection of the bacterial suspension into mice. Upon accidental infection, simple treatment with antibiotics, such as Ampicillin, will rapidly clear the bacterium from the infected person. Pregnant women are advised not to carry out this work due to the possible risks. A separate risk assessment to be completed if they still wish to participate in this research.

Vaccinia virus (VACV) VACV is the live vaccine used to eradicate smallpox and is the prototype orthopoxvirus. During its wide usage as a vaccine, it became apparent that some complications in a small fraction of humans can occur. Possible clinical signs in humans include: Locally: 3-4d post inoculation: papule IS-6d: pustule 18-9d: well-formed pustule / 12d+: scab 117-21 d: scar. Mild systemic illness can accompany the localised reaction, including lever and malaise. Therefore, all work with VACV will be handled with Good Microbiological Practice and Good Occupational Safety and Hygiene at containment Level 2. Vaccination is possible and highly protective. Pregnant women or immune-compromised individuals (e.g. the elderly, those infected with HIV or those taking immune-suppressive drugs such as systemic corticosteroids, alkylating agents or antimetabolites) are advised not to carry out this work due to the possible risks. VACV may also cause complications in people with active skin disorders such as significant eczema or psoriasis. Therefore also individuals with these types of disorders are advised not to carry out this work due to the possible risks. A separate risk assessment to be completed if they still wish to participate in this research.

Influenza virus: Influenza infections can lead to high fever and can be lethal. Nevertheless, the viruses being handled are closely related to currently circulating human viruses and mouse attenuated Influenza strains have lost the capacity of infecting uninjected lillermals and transmission to humans is highly unlikely. Thus, taken together, the used strains have no realistic potential to develop into pandemic strains. Further can be expected that all individuals have naturally been exposed to Influenza viruses that confer protection against the experimentally used strains.

Vaccination with formalin inactivated virus particles is highly effective and transmits full protection. Pregnant women or immune-compromised individuals (e.g. the elderly, those infected with HIV or those taking immune-suppressive drugs such as systemic corticosteroids, alkylating agents or antimetabolites) are advised not to carry out this work due to the possible risks. A separate risk assessment to be completed if they still wish to participate in this research.

The official guidelines for work using Influenza viruses: http://www.hse.gov.uk/biosafety/diseases/acdpflu.pdf has been studied extensively and all our statements are in accordance with these guidelines.

No new recombinant organisms will be created, but existing GM will be imported and used. Typical examples of vector transmissions into the genome of the pathogens are described in the publications stated in the accompanying risk assessment for the individual pathogens.

In brief, for most recombinant Listeria strains (exception for Lm-E1 strains) stable insertion into the bacterial genome was based on transfection with shuttle vectors such as pH5-LV, pAT29, pLSV16 and similar vectors.

Introduction of vectors into viral genomes was normally achieved by co-transfection into virus-infected cells and thus by reverse genetics. Recombinant viruses were then selected by either clonal selection or plaque assays in the presence of selection markers, such as Mycophenolic Acid, xanthine and hypoxanthine.

Vaccinia: pSJH7, that contains the E. coli guanine phosphoribosyl transferase (Ecogp) gene linked to a VACV promoter, flanked by DNA fragments of the viral gene of interest, as well as similar vectors

Influenza: pT3-WSN, pM-PR8-NA-2A- based and similar vectors pH5-LV or pLSV16.
In most cases (please see note 2), the used vectors will have already been deleted in those pathogen strains we receive. Of the remaining parts of the vectors, no harmful properties can be expected.

Of note 1: The different bacterial strains carry antibiotic selection markers such as for Erythromycin (Lm-OVA), Kanamycin (Lm-gp33) or Spectinomycin and Chloramphenicol (Lm-E1). Nevertheless, none of the bacterial strains are resistant for Ampicillin, which is normally used to treat Listeria-infected patients.

Of note 2: The Lm-E1 strains still contain the pAT29 vector that confers Spectinomycin-resistance.

Origin & function
The recombinantly expressed antigens are:
(i) Typical immunological model antigens, which include (but are not restricted to) ovalbumin, hen egg lysozyme or other recombinant forms of model antigens.
(ii) Reporter genes, which include (but are not restricted to) enzymatic reporters, such as ~galactosidase, luciferase, or fluorescent reporter proteins, which include (but are not restricted to) GFP, YFP and derivatives of these.
(iii) Other well-characterised, non-harmful marker genes may also be used.

Evaluation of foreseeable effects
The used pathogens are infectious for humans and can cause disease even in healthy humans. The performed gene manipulations can be expected to induce no greater risk than the wild type strains they were derived from. In most situations, these gene modifications lead to an attenuation of the resulting pathogen. No harmful properties can be expected from the inserted genetic material and its products.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

WASTE DISPOSAL PROCEDURES
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Sharps (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste stream for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant 1%

Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presepl may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

SPILLAGES
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Is an emergency plan required according to regulation 20?  
- Yes

If yes, tick to confirm that it is attached to this form  
- Yes

Tick to confirm that you have attached a risk assessment to this form  
- Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment  
- No

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

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Project notified under transitional arrangements  
- No
This work aims to understand how the genetic information stored on DNA and packaged into chromosomes is transmitted to the next generation. To understand how the molecular mechanisms segregating chromosomes are conserved in evolution, we need to compare these processes in different species. To complement our current work using Saccharomyces cerevisiae and Schizosaccharomyces pombe, we will characterize chromosome segregation in Candida spp. Candida spp. have a similar genome organisation to Saccharomyces cerevisiae, though some chromosomal features are more similar to those of Schizosaccharomyces pombe. Combining work on Candida spp. will allow us to understand how the chromosome segregation process has diverged in these species.

To do this work, we will tag the endogenous proteins with an epitope tag which will allow us to use antibodies to immuno-precipitate the protein, find its binding sites on chromosomes and determine its subcellular localization. We will delete genes and test their function on chromosome segregation processes.

Recipient or parental organism

(Candida spp. as obtained from the National Yeast Resource Centre (Disabled, partly disabled or wild-type).
C. albicans is a human pathogen in ACDP Hazard Group 2. C. albicans causes superficial infections of mucosal epithelia (thrush) (Odds [1988] Candida and Candidosis, Balliere Tindall). C. albicans can also cause systemic infections which can be fatal, but these only occur in severely immunocompromised patients. C. albicans is carried commensally at least 60% of the healthy population and most Candida infections arise as an overgrowth of the strains resident in the normal microflora. It is a micro-organism of low pathogenic potential that rarely affects healthy individuals. It only becomes a potential medical problem when an individual's immune responses are significantly impaired. Furthermore, as with most pathogenic micro-organisms, the virulence of C. albicans strains is likely to become attenuated rather than enhanced after prolonged laboratory culture.
The C. albicans strains CAI4 (ura3), CAI8 (ura3, ade2), RMT000 (his1, ura3) and BWP17 (arg4, hist, ura3) are the standard hosts for DNA transformation world-wide. These auxotrophies make C. albicans avirulent (Leberer et al. (1996) PNAS, 93, 13217). Transformation with URA3 plasmids partially restores the virulence of CAI4, but this restoration is not complete because the genes neighbouring URA3 remain inactivated. This is enough to attenuate virulence partially (but not completely). Furthermore, C. albicans strains that have been cultured in the laboratory tend to display reduced pathogenicity compared to strains carried commensally by most individuals.
C. glabrata is a haploid yeast which is non-dimorphic (takes only one non-filamenleous form). It is considered part of the normal flora of healthy individuals and has generally been considered non-pathogenic. Recently, however, it has been recognised as a human pathogen in immunocompromised individuals [Bolotin-Fukuhara and Fairhead, 2014].
As for C. albicans, we will use auxotrophic laboratory strains to reduce their pathogenicity [Schwarzmuller et al., 2014].

In most experiments, disabled hosts will be used although a small number of experiments may require the use of nondisabled
hosts.

(ii) Standard disabled E.coli strains

**Host/vector system**

(i) Specific non-mobilisable C. albicans vectors (low copy replicating and integrating vectors (YPB1, Clp1 0, Clp20, Clp30); low copy replicating and integrating vectors for ectopic expression [YPB-ADHpt, pACT1 j; integrating expression vectors regulated by methionine (MET3 promoter), maltose [MAL2 promoter], glucose [PCK1 promoter], or doxycycline [tet ON promoter and tet OFF promoter]
(ii) Non-mobilisable E. coli vectors (including pUC18/19, pBluescripl, pGEM-T; lac-based expression plasmids such as pET vectors).

**Origin & function**

This work will target cell division processes including cell cycle control and chromosome segregation control. Pathogenicity in C. albicans is a polygenic trait involving numerous physiological characteristics (e.g. rapid growth, cell wall, morphogenesis, secreted hydrolases, phenotypic switching). Therefore, (i) no single C. albicans gene (or combination of genes) will turn E. coli into a pathogen, (ii) a reduction in anyone of these parameters can attenuate C. albicans virulence, and (iii) an increase in anyone of these parameters is highly unlikely to increase C. albicans virulence.

The Candida spp. transformations we will perform will inactivate a specific target gene to create a null mutant. These transformations will be performed in multiple disabled Candida spp. strains (e.g. combinations of trpl, ura3, ade2, arg4, or his l ) to ensure that all transformants are avirulent. In none of the cases we will be studying is this likely to increase virulence, instead transformants will always carry a disabling mutation, thereby rendering them completely avirulent. No null mutations have been described that increase the virulence of Candida spp.

In some experiments, we will express specific genes in C. albicans ectopically using a MET3, MAL or tet promoter. In these experiments, the expression level is not maximised. Their ectopic expression levels will be <1 % of total cell protein. The ectopic expression of most specific genes is unlikely to affect virulence at all, and in some cases it will be expected to reduce C. albicans virulence by adversely affecting growth. The key point is that the probability of increasing virulence by ectopic expression of a single gene is very low, because pathogenicity is complex and polygenic trait requiring a high level of fitness of the C. albicans cell (Odds (1994] ASM News, 60, 313). Indeed, there is evidence that ectopic expression of factors involved in virulence traits does not increase virulence (Bailey (1 997) PhD thesis, University of Aberdeen; Leng [1999] PhD thesis, University of Aberdeen; Rodaki et al. [2006]).

**Evaluation of foreseeable effects**

Our experiments will be performed on disabled or partially disabled Candida strains and the mutations that we will generate will most likely render them completely avirulent. Likewise, the ectopic expression of most specific genes is unlikely to affect virulence at all, and in some cases it will be expected to reduce C. albicans virulence by adversely affecting growth.

Candida species have no effective mechanism for horizontal gene transfer that might allow dissemination of resistance traits. The likelihood of natural gene transfer to other species is essentially zero. Furthermore, despite the recent discovery of sex in C. albicans, the risk of transfer between C. albicans strains is very low. This is because C. albicans strains mate very inefficiently in vivo (Hull et al., (2000) Science 289, 307-310), probably because the specific growth forms that mate most efficiently aren't maintained at 37°C (Miller & Johnson (2002) Cell 110, 293-302). In addition, C. albicans must be homozygous at the mating type locus for them to be competent for mating, and the vast majority of clinical isolates are heterozygous at the mating type locus.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

WASTE
Health and Safety
Executive
Solids (e.g. plastic-ware such as pipettes, flasks, tubes etc. and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste stream for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

SPILLAGES
Particular care should be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that involves broken glass) unless they are aware of the potential risks and trained in safe working practices.

If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste stream.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.
This work will involve the culturing of trypanosomes (Trypanosoma brucei) in vitro, generation of transfected parasite lines that express fluorescent proteins, and infection of mice with trypanosomes (either derived from culture or in mouse blood) via intraperitoneal, intravenous, subcutaneous or intradermal needle inoculation.

The aims are to:

a) Generate and maintain trypanosome cell lines that stably express fluorescent proteins at a level enabling visualisation in vivo.

b) Amplify trypanosome population in mice from tissue culture or cryostabilated infected mouse blood in order to...
generate a population of viable parasites adapted to in vivo conditions (Le. tissue culture parasites have adapted to in vitro medium, and cryostabilated parasites are variably viable), for the purposes of extracting parasites in order to initiate viable infections in further mice.

c) Analyse the infection dynamics of trypanosome infections, in particular the interaction between parasites and host immune cells at the inoculation site (s kin) and draining lymph nodes.

Recipient or parental organism

T. brucei STI8247

For T. brucei, while some subspecies are human infective, the strain to be used in this laboratory, T. brucei ST18247, has been confirmed by collaborators on multiple occasions to be non-human infective (by assays including incubation with human serum and recombinant serum lytic components, e.g. Capewell et al, 2011, PLoS NTD, 5, e1287). It is extremely unlikely that introduction of transgenes for fluorescent proteins will alter this phenotype.

This strain will, if inoculated into the bloodstream, initiate infections and potentially cause disease in non-primate mammalian species. T. brucei is a SAPO 2 class organism. Trypanosomes will only survive within mammalian (nonhuman or non-primate) hosts or relevant vectors (tsetse flies, which are not endemic to the UK, and some mechanical vectors such as Tabanid flies) - they rapidly die when not in these contexts, and therefore represent minimal environmental risk. If inoculated into susceptible mammalian hosts by mechanical transmission (i.e. insect vector or syringe) the parasites may cause infection and disease, but if these transmission routes are controlled then the risk of this is minimal. T. brucei is an animal pathogen exotic to the UK and is a SAPO-listed organism, and SAPO regulations will therefore pertain to all trypanosome or trypanosome-derived waste.

Host/vector system

The expression construct backbone is T. brucei sequence (comprises PCR products of target genes, plus flanking regulatory sequences, as well as a selectable marker - puromycin resistance gene - these are initially ligated into bacterial plasmids for amplification in bacteria, then the plasmid is extracted, linearised and relevant sequences isolated by restriction enzyme), which enables integration of sequence into the target locus by homologous recombination (trypanosomes will not maintain plasmids or similar extrachromosomal DNA constructs).

Origin & function

The proteins expressed by the transgenic proteins (GFP and mCherry) are fluorescent reporter genes that have been widely used in multiple organisms. In the lines used here, the proteins are expressed in their native forms and are simply used to enable visualisation of the trypanosomes (i.e. are not fused to any other protein whose function or expression level may influence other phenotypes). There is no evidence from other systems that the expression of these proteins in this form alters pathogenicity, and it must be considered extremely improbable that this manipulation will influence the phenotype of human infectivity. Puromycin resistance is equally unlikely to impact on human infectivity.

Evaluation of foreseeable effects

The pathogenicity of the transgenic parasites in the mouse model is unlikely to be affected by the genes inserted, and particularly so with regards to human infectivity. The transgenic parasites will represent minimal potential environmental hazard to human or animal health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g., plasticware such as pipettes, flasks, etc. and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Liquids (e.g., samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g., needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Degree of kill:

Autoclaving: effectively 100% kill. The efficiency of all autoclaves and sterilising regimes will be monitored using the Prospore 2 validation system.

Autoclaves are serviced regularly and undergo thermometric 12-point validation on an annual basis.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
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<td>Animal Units</td>
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<td>Human Clinical Applications</td>
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02/03/2022  Page 4936 of 15326
## Project Additional Information

### Purposes of the contained use

To understand the molecular mechanisms underpinning genome regulation by RNA interference and chromatin modification in Cryptococcus neoformans.

**Objectives:**
1. Identify proteins involved in mediating and regulating RNA interference and chromatin modification
2. Characterise the molecular functions of these proteins
3. Determine the biological functions of these pathways in genome regulation

We will employ a combination of genetic, molecular and biochemical approaches. DNA from C. neoformans will be expressed from episomal plasmid vectors in C. neoformans, S. pombe, S. cerevisiae and E. coli to allow further analysis of expressed proteins. C. neoformans genes of interest will be disrupted by insertion of specific selectable markers such as bacterial drug resistance cassettes (e.g. the nat1 gene from Streptomyces noursei, conferring resistance to nourseothricin, or the Hph gene from Klebsiella pneumonia, conferring resistance to hygromycin B). Some manipulations will involve the introduction of specific DNA elements from other sources e.g. Aequorea victoria green fluorescent protein (GFP), in order to tag proteins of interest to permit either affinity purification or visualisation.

### Recipient or parental organism

Cryptococcus neoformans var neoformans and Cryptococcus neoformans var gattii (wild-type or auxotrophic mutants) have been assigned to ACDP hazard group 2. They are opportunistic pathogens that can infect humans causing...
diseases including meningoencephalitis and pulmonary cryptococcosis, predominantly in immunocompromised individuals. Spores or desiccated yeast cells enter the respiratory tract by inhalation. C. neoformans var neoformans and var gattii are also potential allergens. C. neoformans var neoformans and var gattii are found naturally in the environment, in particular in soil, decaying vegetation and avian guano, and can infect various domestic and wild animals via spore inhalation.

Saccharomyces cerevisiae and Schizosaccharomyces pombe (yeast strains are multiple auxotrophs and are unlikely to survive outside of the culture media) have been assigned to ACDP hazard group 1.

Escherichia coli K12 or 8 derivatives (disabled) which cannot colonise the human gut

E. coli, S. pombe and S. cerevisiae strains to be used have no harmful effects to plants or animals

Host/vector system

Vectors are based on standard plasmids that are well-characterised and non-hazardous. The plasmid vectors that will be used are based on the bacterial pBS, pSr and pUC plasmids and their derivatives, many with the addition of sequences required for replication and selection in the host. These include pET and related vectors for expression in E. coli, pGAD/pGBK yeast-2-hybrid vectors for S. cerevisiae, the pRep series of vectors and derivatives for expression in S. pombe, and pCnTel and derivatives for expression in C. neoformans, as well as other fungal related plasmids known to drive expression in Cryptococci.

Origin & function

Inserts including genes involved in RNAI and chromatin modification and standard selection markers are not expected to have any harmful physiological or pharmacological properties or to affect infectivity or pathogenicity of the recipient host (RNAI and chromatin modification genes are not thought to be involved in pathogenicity, and drug resistance cassettes used do not confer resistance to drugs used in treatment of C. neoformans infection).

Evaluation of foreseeable effects

Resulting GMMOs are not expected to pose any additional risks to human health and safety compared to the unmodified recipients. The resulting GMMOs pose no additional risks compared to those already present in the environment. The risk of transfer of genetic material to other organisms is very low and would be of minimal hazard.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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### Project Ref 207/16.1

- **Date Ackn'd**: 08/01/2016
- **CU2 Project Title**: Characterisation of cellular and immunological responses to Listeria monocytogenes infections
- **Class**: Class 2
- **Culture Volume Class 2**: 1-50 Litres
- **Non-GMM Consent Granted**: Consent Granted
- **Date Project Ceased**: (blank)
- **Project notified under transitional arrangements**: N

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Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [Y] [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N] [ ]

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.
Our aim is to gain a better understanding of molecular mechanisms that allow Listeria monocytogenes (Lm) to invade and to replicate in eukaryotic host cells. Standard cell culture systems are used to analyse how Lm gains entry into different types of eukaryotic cells (e.g. macrophages, epithelial cells), how the bacterium evades the innate immune response and how it replicates in the cytosol and spreads to neighbouring cells. For these analyses we will make use of Lm strains that carry stably integrated fluorescent or bioluminescent marker genes in their chromosome. The project will also make use of mutant Lm strains in which known virulence factors for cellular invasion/replication/cell spreading are inactivated by genetic modification. In vivo infection challenge experiments in mice (wild type and transgenic mouse strains) will analyse the virulence capacity of different Lm strains, mechanisms of host cell tropism and immunological host responses that are involved in pathogen host defence. The objective of this work is to improve our understanding of protective host responses, of pathogenesis mechanisms associated with listeriosis, and to develop vaccines that optimise immunological memory responses in the host. To investigate if identified host defence responses are specific for Lm, infection experiments will be carried out with other bacterial pathogens (e.g. wild type Staphylococci (GM207 05.2, GMt 0/07) and Streptococci (BAI 0414) to compare experimental outcomes. This project will also use eukaryotic expression systems to express reporter tagged (e.g. Flag, GFP) host cDNAs in mammalian cell lines and primary cells. It is not intended to generate new immortalised cell lines in the project. Expression systems will be also used to ablate the function of cellular host genes (e.g. expression of siRNAs or CRISPR/Cas9 constructs). The objective of these experiments is to characterise the function of host genes in innate pathogen defence and/or induction and maintenance of inflammatory responses.

### Project Additional Information

#### Purposes of the contained use

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### Recipient or parental organism

(i) Listeria monocytogenes (WT, gene inactivated derivatives, expressing fluorescent or bioluminescent reporter genes). Listeria monocytogenes (Lm) is widely distributed in the environment where it can be isolated from multiple sources such as human and animal faeces, soil, water, plants and food. The vast majority of human Listeria infections are foodborne in which the pathogen is transmitted to humans through consumption of contaminated food. A rare cutaneous form of listeriosis can occur as a result of a zoonotic transmission of Lm from an infected animal into human skin lesions (concerns certain occupational groups such as veterinarians and farm workers). Cutaneous listeriosis presents clinically with development of papular or pustular lesions in infected skin areas that can be easily treated with antibiotics.

Foodborne listeriosis is a rare but life-threatening disease if certain high-risk groups of patients get infected. Such vulnerable individuals have often predisposing conditions which make them immunocompromised and susceptible to invasive listeriosis. Examples of immunocompromised, high-risk patients are individuals infected with HIV, cancer patients on chemotherapy, patients with chronic diseases affecting immune functions (e.g. hepatitis, chronic lymphocytic leukemia), and the elderly. Severe invasive listeriosis is often associated with sepsis (bacteraemia).
meningitis, and meningoencephalitis. Foodborne Lm infections in healthy persons are usually mild and self-limiting and may cause febrile gastroenteritis with no other complications. Symptoms of febrile listerial gastroenteritis may include diarrhoea, fever, headache and myalgia.

Another specific risk group of Lm infections are pregnant women. Due the capability of the bacteria to pass the blood-placenta barrier, listerial infections may cause foetal loss (spontaneous abortion) or neonatal bacteremia and meningitis in new born babies.

Health and Safety

Executive

The approximate infective (oral) dose of L. monocytogenes is estimated to be 10^7 to 10^10 colony forming units (CFU) in healthy persons, and 10^5 to 10^7 CFU in individuals of high-risk groups (PMID: 19812983; PMID: 8988887). Listeria monocytogenes is an ACDP hazard group 2 organism and will be handled under containment level 2 conditions as specified in an associated BA1 risk assessment (Roslin Institute BA1 0215). This project will mostly make use of already established genetically modified Listeria monocytogenes (Lm) strains. The biological characteristics of these Lm GMO are outlined below. These GMO strains have been widely used in the Listeria research field and are all well characterized (see references for the specific Lm strains listed below). In rare cases, we will genetically modify Lm strains to integrate fluorescent protein reporter genes (e.g. derivatives of green or red fluorescent proteins (GFP, RFP)) into the bacterial genome. Reporter genes will be inserted into well-defined loci in the listerial genome. It is not expected that such genetic modification will increase the pathogen virulence in any way. These strains will be used for bioimaging and analysis of infection phenotypes in cell cultures and experimentally infected mouse strains.

Established Lm strains with genetic modifications and biological effects of introduced mutations:

- Listeria monocytogenes Xen32, lux operon tagged derivative of the parental strain Lm 10403s. This strain is bioluminescent and attenuated in mouse infection models (PMID: 23856386). It expresses the harmless bacterial luciferase and biosynthetic enzymes for synthesis of luciferase substrates from Photobacterium luminescens (PMID: 18370150).

- Listeria monocytogenes EGO-lux EGD::pPL2luxP helB, bioluminescent strain, lux operon tagged derivative of the parental strain Lm EGO. Translational fusion of the listeriolysin promoter hylA with the lux operon (luxABCDE) from Photobacterium luminescens. Constitutive expression of high levels of luminescence, no other phenotype (PMID: 17351089).

- Murinised Listeria monocytogenes strains of parental Lm strains EGOe, 10403s, and Xen32 expressing a modified internalin A gene (InIA) with two amino acid substitutions (Ser92Asn and Tyr369Ser) that allow enhanced binding of InIA to the murine E-cadherin protein. These strains show efficient crossing of the murine intestinal barrier after oral inoculation of mice (PMID: 17540170, PMID: 23617550, PMID: 23856386).

- Listeria monocytogenes rLM-Ova expressing the harmless chicken ovalbumin (OVA) under the listeriolysin 0 (LLO) promoter hylA. This strain will be used for characterisation of T cell responses against Lm in mouse infection experiments (PMID: 11207297).

- Listeria monocytogenes .1.actA, this strain carries a deletion in the actA gene, a virulence factor needed for host actin polymerisation and spreading of Listeria to neighbouring cells. The LactA mutant is heavily attenuated in virulence in mouse infection models (PMID: 1739966, PMID: 23382675).

- Listeria monocytogenes .1.hylA, this strain is deficient of listeriolysin 0 (LLO), a pore-forming toxin needed for bacterial escape from the host phagosome. The .1.hylA mutant demonstrates drastically reduced virulence in mouse infection models (PMID: 250936, PMID: 3138189).

- Listeria monocytogenes strain L028 .1.mdrT, the L028 strain is a commonly studied laboratory strain of Lm which...
carries a naturally occurred mutation which leads to overexpression of the cycI-ic-di-adenosine monophosphate (c-diAMP)-secreting efflux pump MdrT (PMID: 22290148). This upregulation of the MdrT efflux pump leads to induction of strong type I interferon responses in murine macrophages. For the project we will use for control experiments a L028 Lm strain with an intragenic deletion within the mdrT gene. This mutant is unable to induce c-di-AMP stimulated type I interferon responses in mice (PMID: 24970844).

Listeria monocytogenes that will be used in this project is widely distributed in the environment, is not exotic to the UK, is not pathogenic to plants, and does not cause notifiable diseases under specific surveillance by DEFRA. Listeria monocytogenes is a zoonotic pathogen and may cause infections in ruminants and companion animals. These bacteria are ubiquitous in the environment and spontaneous infections in animals occur rarely. The pathogenic mechanisms of L. monocytogenes for animals are the same as for humans and therefore all the conclusions reached in the above sections in relation to the possible hazards to human health are also valid for their potential animal hosts.

The loss-of-function mutations described above for GM L. monocytogenes strains are in most cases in-frame deletions, which do not leave any trace of foreign DNA in the listerial genome. Mouse infection challenge experiments have demonstrated that all of the above described GM listerial strains are strongly attenuated in virulence in vivo. They will compete less well within any animal host and will be cleared from the infected tissues sooner than the wild type. The risk of pathogenicity is therefore reduced when compared with the corresponding wild type parental strain. Consequently, in an unlikely scenario that these gene-disabled GMM are accidental released into the environment, they are not expected to displace any indigenous wild type L. monocytogenes due to a reduction in fitness.

(ii) Escherichia coli

Escherichia coli K-12 and B strains are used for regular cloning experiments in the laboratory. They are disabled, unlikely to survive out with laboratory conditions and with no known capacity to cause human disease. These strains will be used for the cloning of eukaryotic host genes as specified above. E. coli K12 or B strains harbouring fragments or full-length eukaryotic genes will not pose any risk on human health as oncogenes or proto-oncogenes will not be propagated. In rare occasions we will use E. coli laboratory strains to clone targeting vectors for the insertion of fluorescent reporters (e.g. GFP, RFP) or antibiotic selection markers into the listerial genome. These selection markers I reporter genes will not affect the virulence of the host bacterium and therefore will not have any harmful effects on human health. E. coli K-12 and B strains are assigned to ACDP hazard group 1.

(iii) Mammalian cell lines:
Human: HeLa, THP1, HEK 293, A549, CaC02, HaCaT, Cos7
Rodent: J774, RAW 264, CT-26

(iv) Primary murine cells from SPF mice:
Mouse embryonic fibroblasts (MEFs) and Bone marrow-derived macrophages (BMDM)

The likelihood that eukaryotic genes with potential immune modulatory functions will be expressed with eukaryotic expression systems is low. Nevertheless, all such experiments will be carried out under CL2 containment conditions which will prevent environmental release. In the extreme unlikely event that environmental release should occur, the risk to human health can be regarded as very low or effectively zero.

The likelihood of accidental environmental release of the GMMs is very low or negligible, given the measures that will be applied to control any risk to human health and safety (Level 2 containment). In the extreme unlikely event that environmental release would occur, the environmental risk can be regarded as very low or effectively zero.

Host/vector system

The cloning vectors will include many different types that have been developed for molecular biological purposes of propagation in E. coli or Listeria including: pAUL-A Tn4001 luxABCDE, pPL2luxPhelp, pUC series-based vectors, pBR322, pTOPO, and pBluescript II and derivatives of all of these.
Typical vectors used for expression or targeting in mammalian cells include pIRES2EGFP, pCDNA3.1, pDONR-207, pENTR-11, pYFP-DEST, pCFP-DEST, pTH2-Halo Tag, pCMV-Tag1, pHA, pEGFP-N1, pSPCas9 or derivatives of these. Additional novel plasmid vectors may be used as they become available.

All these vectors are non-mobilisable or mobilisation-deficient. The resistance genes used in these vectors have been approved for use at containment level 1 and confer resistance to gold ~ antibiotics for which resistance in clinical isolates is already widespread (and therefore are no longer used in clinical practice). The selection markers I reporter genes to be used are unlikely to cause any harm to human health.

Insertion of antibiotic resistance cassettes in order to disrupt specific genes will confer resistance to that antibiotic. The antibiotic profile of the new generated GM L. monocytogenes strain will be known so that in the unlikely eventuality of an accidental listerial infection caused by a particular GM strain appropriate treatment can be advised. Fluorescent reporter genes might be integrated into the listerial genome in order to make the bacteria amenable to bio imaging. The various reporter genes are commonly used (e.g. red or green fluorescent proteins) and are well characterised and non-harmful. Therefore, the virulence of the new GM listerial strain should not be affected.

Therefore such modified organisms are not expected to represent an increased risk to human health when compared to the wild type equivalent.

Eukaryotic host genes that will be cloned in E. coli laboratory strains or targeted or expressed in mammalian cell lines will not cause additional risk to human health. Examples of such eukaryotic host genes are genes encoding pathogen pattern-recognition receptors, signal transduction proteins, or genes induced or suppressed by cytokines (e.g. tumour necrosis factor a, interferon ~)

Typical examples of such genes are:
- Toll-like pattern recognition receptors
- NLR/Nod-like inflammasome components
- AIM2-like inflammasome receptors
- RNA-helicases
- NF-KB and IRF3/7 signalling components
- JmjC domain containing dioxygenases
- STING pathway DNA-sensing molecules
- Interferon stimulated genes (ISGs) or Interferon-induced genes (IFIS))

Sources of these eukaryotic genes may be genomic DNA or cDNA from different mammalian (e.g. human, mouse, swine, cattle) or avian species (e.g. chicken). We do not anticipate that these potentially immune modulatory genes create a risk to health if introduced and expressed in a human host. We also do not anticipate resulting function of such genes to be toxic or allergenic. Nevertheless, any biomaterial generated from such eukaryotic expression experiments will be treated as potentially hazardous. Experiments will be carried out in CL2 containment laboratories and the generated waste will be appropriately inactivated.

The genetic donor material that will be used has no hazardous potential. This project will not clone or propagate vectors with listerial DNA that may encode bacterial virulence factors. All used inserts will be non-pathogenic and will not be able to cause adverse effects in an unlikely scenario of natural transfer to other organisms.

All antibiotic resistance markers introduced are specific for antibiotics which are not in routine use for the treatment of listerial infections. The main antibiotics used for the treatment of listerial infections are ampicillin, amoxicillin and an aminoglycoside drug (usually gentamicin) and combinations of these drugs (PMID: 24822197). So in the unlikely eventuality of escape and infections occurring the normal treatment protocols would be still effective. Erythromycin resistant listerial strains exist in nature. So a very unlikely escape of the L. monocytogenes rLM-Ova strain from containment level 2 conditions would therefore not introduce a novel phenotype into the environment.
The L. monocytogenes gene knockout derivatives, in which one or more genes are inactivated, will normally be attenuated and less fit to spread in vivo in the event of an accidental infection. However, if the targeted genes play none- or only a minor role in virulence, the deletion mutants will retain the virulence of the parental wild-type strain. Therefore, all of these deletion mutants will need to be handled under the same containment level as the parental strain (i.e. containment level 2).

For example, the attenuated L. monocytogenes GMO strains (6.actA, 6. hly) are mutated derivatives which lack critical virulence genes and have lost their host-colon ising properties. They can be considered to be non-pathogenic but will be nevertheless handled under containment level 2 conditions. The introduced mutations are intragenic deletions with no foreign donor DNA left in the listerial genome.

L. monocytogenes rLM-Ova expresses and secretes the non-pathogenic chicken ovalbumin (OVA). The OVA-albumin expression cassette also carries resistance to the antibiotic erythromycin to allow selection of L. monocytogenes harbouring the expression cassette. However, the standard treatment of listeriosis includes ampicillin and an aminoglycoside drug and the listeria monocytogenes we would use is sensitive to the standard treatment antibiotics (PMID 10471548). There are naturally occurring erythromycin-resistant listeria strains in the UK, but these strains are sensitive to other relevant antibiotics (PMID 10471548).

L. monocytogenes harbouring fluorescent or luminescent reporter genes normally carry no other mutation and will likely retain their wild type level of virulence. These strains will therefore also be handled under the same containment level as the parental strain (i.e. containment level 2).

All GMO strains of L. monocytogenes that will be used in the project or will be used for genetic modification are well-characterised strains of known genotype and antibiotic sensitivity profile.

Escherichia coli K-12 and B strains containing prokaryotic or eukaryotic cloning vectors with the above described inserts are classified under ACDP hazard group 1. Nevertheless, these will be used in containment level 2 laboratories (2nd floor, East Wing, Roslin Institute). Very rarely, we might clone listerial virulence factors in E. coli K12 or B strains to repair or trans-complement previously generated listerial mutations. In such cases we will use vectors (e.g. pE194 derivatives) for Listeria which are non-mobilisable in BSL-2 containment.

Mammalian cell lines transfected with the above expression or targeting vectors are classified ACDP hazard group 1. These cell lines may be also infected with other ACDP hazard group 2 pathogens to characterise host defence responses. Therefore, the cell culture work will be carried out under containment level 2 conditions. Cell lines will or are obtained from certified repositories (e.g. ATCC, DSMZ) which have standardized quality assurance schemes for their products in place (e.g. cell lines are mycoplasma free and tested negative for human pathogenic viruses).

We may also transiently transfect primary cells (e.g. mouse embryonic fibroblasts (MEFs), or bone marrow-derived macrophages (BMDM) from mutant or wildtype mice with expression reporter constructs to complement genetic deficiencies in pattern recognition pathways or to ablate the functions of such genes with siRNA constructs or oligonucleotides. These experiments are classified as ACDP hazard group 1. If such cells are subjected subsequently to infection experiments with class 2 pathogens such as Listeria monocytogenes wildtype or mutant strains they are classified as ACDP hazard group 2. Primary cells will obtained from mouse strains maintained in a specific-pathogenfree (SPF) barrier facility (Roslin BRF). These mice undergo regular FELASA health screens and will not process an additional hazard risk on these experiments that will be carried out in class 2 laboratories (2nd floor, East Wing, Roslin Institute).

The GM listerial strains used in this project will most likely not represent an increased risk to animal health compared to wild type strains should they be released into the environment. Taking into account the control measures assigned for this work the overall risks to the environment from the GMMs produced in this work is effectively zero.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Pipette tips, spreaders, loops must be discarded in the "Dispo safe" jars and inactivated by autoclaving.
Liquid cultures and used bottles I flasks I tubes must be inactivated by first using a disinfectant (e.g. Virkon) and then subsequently autoclaved.
Plates must be taped and discarded in an autoclave bag prior to autoclaving. Autoclave bags should have the group name and date before disposal. They should be loosely sealed with cable ties.
Autoclave bags should be taken to the Roslin central service facility when full. Loosely seal the top with tape and stick a label with lab number on the bag.
Carcasses (dead mice) are inactivated by autoclaving on a make safe cycle (120°-125° C, 15 min) and afterwards incinerated.
Specifics for autoclaving contaminated waste:
Solids (e.g. plasticware such a pipettes, flask, tubes, etc. and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.
Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121·125°C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), discharge to drains.
Sharps (in sharps bin, e.g. needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121·125°C for at least 15 minutes or 126-130 °C for at least 10 minutes or 134-138 °C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.
Animal bedding and carcasses - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126·130 °C for at least 10 minutes or 134·138 °C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment
**Project Ref** 207/16.2

**Date Ackn’d**
20/05/2016

**CU2 Project Title**
Investigations into the pathogenesis of poxviruses

**Class**
Class 2

**CultureVol**
1-50 Litres

**Consent Granted**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**
To investigate how Vaccinia virus (VACV) and Cowpox virus (CPXV) replicate and interact with the host's immune system, leading to a better understanding of the genes that affect their virulence. This work requires genetic manipulation of VACV and CPXV including the deletion and modification of genes to investigate their role in viral virulence.

**Recipient or parental organism**
(i) VACV and CPXV are both ACDP HG2 micro-organisms, capable of causing disease in healthy humans. VACV strains differ in virulence. We will be using low virulence strains (Copenhagen, Lister, MVA) when the experimental aims of the study allow, however it will be necessary to use a higher virulence strain Western Reserve (WR) when
characterising the effect of immunomodulatory proteins.

Poxviruses can be transmitted via droplet, aerosols and direct contact. VACV and other poxviruses have the capacity to survive for considerable periods in dried material such as spills of virus solution. Live VACV can be isolated from solid surfaces and fabric for as long as two weeks after contamination. Infection with wildtype VACV or CPXV is usually mild and localised. Treatment for accidental VACV inoculation is anti-Vaccinia virus immunoglobulin. Cidofovir is effective against poxvirus infection but is not currently licenced for this use. Adverse effects following exposure to VACV are estimated to occur in approximately 1 in 1000 people. A laboratory workers who will be handling VACV must register with occupational health and discuss potential health issues.

VACV is known to be capable experimentally of infecting a wide range of species, however no self-perpetuating cycle of infection in any species has been identified in the UK.

Poxviruses are known to survive in the environment for extended periods of time and there is evidence of wildlife reservoirs of CPXV in the UK and VACV in South America. Cowpox virus has been reported in Great Britain and mainland Europe only. The virus reservoirs in Great Britain are believed to be wild rodents such as bank voles (Clethrionomys glareolus), field voles (Microtus agrestis), wood mice (Apodemus sylvaticus) and house mice (Mus musculus). There have been no reports of disease in wild rodent populations due to CPXV infection. Poxviruses are resilient organisms capable of surviving hostile environmental conditions. Release of VACV into the environment would represent a low risk, CPXV a moderate risk.

(ii) Human cell lines and CV-I monkey kidney cell line

Host/vector system

The Transfer plasmids used in virus construction pose no hazard to human health and safety or the environment.

Origin & function

Marker genes which are not harmful will be expressed in Vaccinia virus and Cowpox virus and include E. coli guanine phosphoribosyl transferase, β-galactosidase, luciferase and enhanced fluorescent proteins.

Genes will be deleted from VACV and CPXV that are predicted to encode virulence factors including: Kelch repeat proteins; Ankyrin repeat proteins; VACV A42R (a profilin-like molecule); VACV FSL (a major membrane protein which is truncated in some attenuated strains of the virus and may therefore be a virulence factor); VACV A47L (encodes CD8+ T cell epitopes); CPXV A59R (predicted to encode a protein with a guanylate kinase-like motif, and could be involved in junctional adhesion of cells) and CPXV M5R (may affect the energy metabolism of an infected cell).

VACV or CPXV proteins will be tagged with epitopes including Flag, c-myc, His or HA.

Evaluation of foreseeable effects

The expression of marker genes encoding products such as fluorescent proteins, luciferase proteins, or E. coli guanine phosphoribosyl transferase by VACV or CPXV has never been reported to alter the tissue tropism or host range or the susceptibility of these viruses to the human defence system, and, if anything, decreases the virulence of the organisms. In some instances, the insertion of the marker gene is designed to disrupt a VACV virulence gene, resulting in significantly decreased fitness of the viruses.

Placing epitope tags on the proteins and expressing them in VACV or CPXV is unlikely to increase risk.

The genes that will be deleted or mutated in VACV and CPXV are predicted virulence factors. Deleting them is expected to make the virus more likely to be destroyed by the host, and therefore have lower virulence and cause less severe disease. On the basis of previous work and by studying the predicted role of the genes to be targeted, it is believed to be highly unlikely that the viral gene deletions planned in this project will result in a more virulent virus, and are much more likely to result in attenuation. It is theoretically possible that a particular mutation could enhance the
function of the protein, causing a greater suppression of host immunity and increasing the virulence of the virus. There is no precedent for this in poxvirus research, and we believe it to be very unlikely to occur. We will not mutate proteins to make them similar to proteins found in viruses of higher virulence. If evidence of increased virus virulence is seen in the murine models of disease, further risk assessments will be carried out.

Deleting multiple immunomodulatory genes from VACV or CPXV will make the virus more susceptible to the host killing mechanisms and therefore less virulent. Other combinations such as employing both a tag and mutation to a VACV gene, or tagging one gene and deleting another, will be very unlikely to increase the virulence of the virus. VACV and CPXV both have very wide tissue tropism and are capable of infecting a wide range of cell culture lines and species including humans. Given the known properties of the genes targeted in this project, there is no reason to expect any effects on host range or tissue tropism.

Given the known properties of the genes targeted in this project, there will be no alteration to the virus epitopes or the DNA replication machinery therefore no change in the efficacy of treatment is expected.

The modifications proposed for VACV and CPXV will not result in additional hazards to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) - autoclave at 121°C for at least 15 minutes, discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - chemical disinfection then to drain for liquid waste. The disinfectant Virkon™ will be used, as the standard poxvirus disinfectant. Virkon contains 50% w/w potassium peroxomonosulfate, 5% sulfamic acid, 15% sodium alkylbenzene sulfonate. A 10% w/v solution will be made fresh on the day of use and diluted a maximum 1:4 in liquid waste (final concentration 2.5%). Minimum contact time 30 minutes. Alternatively liquids may be autoclaved at 121°C for at least 15 minutes before discharge to drains.

Animal bedding and carcasses - autoclave at 121°C for at least 15 minutes discharge to drains. dispose of carcasses via clinical waste stream for incineration and bedding via clinical waste stream for heat treatment or via the industrial (black bag) waste stream for landfill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

## Project Containment

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## Project Ref 207/16.3

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## Project Additional Information

**Purposes of the contained use**

To understand the genetic and molecular network controlling the timing of DNA replication in mammalian cells using genetically modified mouse embryonic stem cells or primary mouse embryonic fibroblasts. The expression of candidate genes will be knocked down using short interfering RNAs, delivered as lentiviral particles. In addition, we are interested in understanding the impact of some of the DNA replication factors or nuclear structural components on the...
replication timing. We will therefore express some of them in tagged form to test interaction with Rif1 or to verify their subcellular localisation in Rif1 deficient cells.

**Recipient or parental organism**

(i) Commercially available E.coli disabled strains such as STBL3 or TOP10 which cannot colonise the human intestine.

(ii) pLKO.1 is a commercially used replication incompetent lentivirus compatible with third generation systems. The probability of generating replication-competent virus through recombination is negligible. The system has also removed virulence genes that are not necessary for shRNA packaging. These features combined have improved biosafety and handling. There are no known incidents of third generation systems producing replication competent virus. The virus can cause harm if gains access to the body as it inserts stably in the genome. However, no secondary effect (e.g., immortalisation or transformation) has ever been detected as a consequence of replication incompetent lentiviral insertion in human cells. pLKO.1 poses no threat to the environment, even in the case of accidental release.

(iii) Transgenic and wild type mouse embryonic stem cells or primary mouse embryonic fibroblasts which pose no threat to human health & safety.

**Host/vector system**

pLKO1 is a commonly used, commercially-available replication incompetent lentivirus compatible with third generation systems. The four plasmid system consists of: the packaging vectors, encoding for Gag/Pol and Rev separately; the vesicular stomatitis virus G-protein envelope vector; and the shANA transfer vector. Resulting particles are replication-incompetent and deletion in the U3 portion of the 3’ LTA eliminates the promoter-enhancer region, further negating the possibility of viral replication. The system has also removed virulence genes which are not necessary for shANA packaging. These features combined have improved biosafety and handling. The probability of generating replication-competent virus through recombination is negligible. There are no known incidents of third generation systems producing replication competent virus. The virus can cause harm if gains access to the body as it inserts stably in the genome. However, no secondary effect (e.g., immortalisation or transformation) has ever been detected as a consequence of replication incompetent lentiviral insertion in human cells. pLKO.1 poses no threat to the environment, even in the case of accidental release.

**Origin & function**

For the lentiviral pLKO.1, the inserts are various shRNA directed against against mouse Rif1 or mouse genes involved in DNA replication or nuclear organisation. The shRNAs would not be able to target the human homologues as even for highly conserved proteins the nucleotide sequence diverges sufficiently to impair recognition by the shANA. In addition, in the majority of the cases, should the shRNA be effective, this would cause severe growth disadvantage to cells.

**Evaluation of foreseeable effects**

(i) Recombinant microorganisms generated from disabled E.coli strains such as STBL3 or TOP10 will pose no threat to human health & safety or the environment.

(ii) The pLKO 1-shRNA lentiviruses are replication incompetent. The probability of generating replication-competent virus through recombination is negligible. The system has also removed virulence genes that are not necessary for shRNA packaging. These features combined have improved biosafety and handling. There are no known incidents of third generation systems producing replication competent virus. The virus can cause harm if gains access to the body as it inserts stably in the genome. However, no secondary effect (e.g., immortalisation or transformation) has ever been detected as a consequence of replication incompetent lentiviral
insertion in human cells, pLKO1 lentiviruses cannot spread in the environment even in the case of accidental release. Even if these viruses did recombine with wild type viruses, the inserted genes are either deleterious for cell growth (shRNAs) or innocuous.

(iii) Mouse cell lines resulting from lentiviral infection pose no threat to human health & safely or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection is performed as follows: Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment either with 1*10 Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Alternatively 10% Sodium hypochlorite may be used; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

Physically clean surfaces disinfected with 70% ethanol.

The autoclave settings for both solid and liquid waste are as follows:
1. Plastic discard (e.g. plastic-ware such as pipettes, flasks, tubes etc.) - the standard autoclave setting is 134°C for a holding time of 5 minutes.
2. Liquid discard (e.g. samples, culture supernatants, tissue culture media) - the standard autoclave setting is 121°C for a holding time of 15 minutes.
3. Sharps waste in sharps containers (e.g. needles, syringes, scalpel blades) - disposed via the clinical waste stream for heat treatment via the University approved contractor.

Is an emergency plan required according to regulation 20? 

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment
The use of disabled rabies virus expressing Green Fluorescent Protein to label oligodendrocytes in the mouse brain

The disabled rabies virus can infect oligodendrocytes but cannot spread to infect other cells. An oligodendrocyte infected by the virus will be highlighted by the expression of Green Fluorescent Protein, so that the number and position of each cell processes can be traced, and the contacts made with other cells identified.

Recipient or parental organism

The disabled rabies virus, described in the following section, will be injected into mouse brain, or cultured with mouse brain slices or in vitro cultured oligodendrocytes. The virus does not integrate into the host cell genome and so the recipient animals/tissues/cells will not be genetically modified.

Host/vector system

We will be using an attenuated version of the rabies virus produced at the National Institute of Natural Sciences, Okazaki Japan:
HEP-ΔG-GFP
HEP - Strain 01 rabies virus HEP-flury
ΔG - Deletion of the G gene from the rabies genome
GFP - Green fluorescent protein

Origin & function

The HEP-flury strain of rabies virus has been modified by the deletion of the gene encoding the G protein, which is essential for viral packaging. The gene has been replaced with the insertion of the gene for the Green Fluorescent Protein from Aequorea victoria. The insertion of GFP has no effect on the pathogenicity or toxicity of the virus.

Evaluation of foreseeable effects

Due to the deletion of an essential coat protein gene the virus cannot transmit from cell to cell therefore the normal disease progression of the rabies virus cannot occur. Mice subjected to injection of the attenuated virus will have a small localised population of neurons and oligodendrocytes take up the virus. The virus will be unable to spread to other cells. The localised containment has been found to cause no cell death or adverse effects over the time period that the animal will be infected. The mice will therefore suffer no adverse effects due to the virus and will remain completely non-infectious to other organisms including humans.

In the unlikely case of human exposure. If an individual is accidentally exposed, peripheral nerves may take up the virus. This cannot spread following infection of these cells due to the disabled nature of virus yet may cause cell death in the infected population. The ultimate effect of this will depend on the number and nature of the nerves affected. Symptoms could include varying degrees of muscle weakness and loss of sensitivity. Although this is an unlikely event, as a vaccine exists for the rabies virus we will enforce that all individuals involved in viral work are vaccinated against the rabies virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The virus will be handled all containment level 2. Animals once injected will be housed in isolators until sacrifice.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Rabies virus itself is a Hazard Group 3 pathogen classified under SAPO. The deletion of the G gene in the virus prevents the normal packaging and export of virus particles and renders the virus replication defective and unable to be transmitted further. The deleted virus is thus effectively disabled or attenuated and we would argue should be classified at a lower Hazard Group, capable of adequate containment at level 2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For disposal, any solutions or materials that contain, or have come into contact with viral particles will be decontaminated by soaking in Virkon (10%) and then subjected 10 autoclaving. Autoclaving (132 degrees celsius, 15 minutes holding time) will give 100% kill at this temperature and time. Surfaces will be sprayed with Virkon 10% solution after use. The carcasses of the animals which will receive injections of deltaG-Rabies Virus will be autoclaved prior to incineration.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

### Project Containment

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**Project Ref** 207/16.5

**Date Ackn'd** 11/08/2016

**CU2 Project Title** The expression of normal cellular prion protein in prokaryotic and eukaryotic hosts to provide substrates for subsequent cell-free prion protein conversion assays

**Class** Class 2

**Culture** Consent Granted

**Consent Granted** Non-GMM

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
We wish to prepare normal cellular prion protein (PrPC) in the form of purified recombinant PrPC expressed in E.coli and in the form of cell extracts containing PrPC from eukaryotic cells genetically engineered to over-express the protein. These will then be used as substrates for seeded prion protein conversion assays.

Recipient or parental organism

The recipient eukaryotic organism is the HEK 293 cell line. It is a commercially available, well-characterised cell line established from primary embryonic human kidney cells transformed with sheared human adenovirus type 5 DNA. The 293-F cell line is a variant of the 293 cell line that has been adapted to grow in suspension culture. The recipient prokaryotic organism used to prepare recombinant PrPC is E.coli BL21 Rosetta strain and the DHS alpha strain.

Host/vector system

For expression in HEK 293-F cells the ptRESne03 vector has been used. piRESne03 is a mammalian expression vector that contains an expression cassette including a multiple cloning site (for the gene of interest) and the gene conferring resistance to neomycin. The cassette is driven by the cytomegalovirus (CMV) major immediate early promoter/enhancer.

For recombinant PrP expression and purification from E.coli, the commonly used pET and pUC vectors are used.

Origin & function

The inserted genetic material is the coding sequence of the prion protein gene (PANP in humans and Prnp in other animals). The gene sequence inserted into the piRESneo3 expression encodes the full length human prion protein amino acid sequence. Thus, transfected 293-F cell lines will over-express (-10 fold) normal cellular prion protein (PrPC). The pET vector is used to express hamster and other mammalian PrPC in E. coli. The pEX vector is a pUC derivative that is used for maintaining the plasmid.

The normal form of human PrPC is expressed in most mammalian cell and tissue types with the highest expression levels found in the brain. The exact role of PrPC is not known, however, it is believed to be involved in signal transduction, cell adhesion and neuronal stress resistance and survival. Prion diseases arise through the conversion of this normal cellular protein to a misfolded and aggregated form. The products of PrPC expression in GM prokaryotes (purified recombinant PrPC) and eukaryotes (cell extracts containing PrPC) will subsequently be used in seeded prion protein conversion assays that mimic this process.

Evaluation of foreseeable effects

293-F is a well characterised, commercially available cell line, which should be 01 no risk to human health and would be incapable of surviving within an accidentally inoculated immunocompetent host. The possibility of contamination with adventitious human viruses or other cell lines is thought to be negligible. Mycoplasma contamination cannot be excluded, but this will be tested for. E.coli BL21 and DH5 alpha are commercial strains that cannot survive in the wild, but carry one antibiotic resistance gene, which could be transferred to other bacteria. Both are disabled and do not colonise the human gut.

It is conceivable that PRNP could mutate or PrPC could spontaneously misfold, leading to the production of pathogenic forms of PrPC associated with prion disease. This potential risk is highlighted because of the severity of the potential consequences even though there is no good evidence to suggest that such spontaneous events occur in cultured cells or E. coli. It is also possible that 293-F cells could be accidentally infected by human or bovine prions in
cultur media but this risk is considered remote. At normal physiological levels human PrPC is not considered a risk to human health. However, Section 34, Page 45 of Part 2 of The SACGM Compendium of Guidance states that GMMs expressing normal prion proteins should be handled at Containment Level 2 as they may be pathogenic at high levels and may also become mutated to forms associated with other human prion diseases in GMM vectors. No constructs will be used in either eukaryotic or prokaryotic cells encoding disease-associated PrP gene (PRNP) mutations.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated from the culture of the transfected 293-F cell line and E. coli will be treated as potentially infectious and decontaminated according local SOPs in line with SACGM Compendium of Guidance to consider the prion risk. Specifically, all bacterial and eukaryotic cell culture waste and consumables will be decontaminated using 2N NaOH for a minimum of 1 hr prior to disposal. This is one of the ACOP prescribed methods for decontamination of prion infectivity and is also sufficient for inactivation of host prokaryotic and eukaryotic cells, and for hydrolysis of nucleic acids. Liquids will be absorbed and contained, and along with similarly decontaminated consumables, join the laboratory clinical (yellow bag) waste stream for subsequent incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
The generation of induced pluripotent stem cells, induced trophoblast stem cells and refinement of the new reprogramming technology using lentivirus delivery of transcription factors

Converting human cells such as skin to other specialised cells such as neurons, blood, heart, liver and pancreas will make it possible to regenerate any damaged or diseased tissue and develop personalised therapies. It is now possible to reprogram biopsied human cells to become stem cells in the laboratory. These stem cells are pluripotent, which means they can potentially give rise to all cell types of the body. However, the current reprogramming technology uses genes called transcription factors (TFs) that are highly inefficient in reprogramming and often linked to oncogenesis, making this technology quite unreliable and risky to use for therapeutic purposes. Thus, we urgently need novel and safe methods to effectively generate cells with the quantity and the quality needed for regenerative therapy.

Strikingly, the TFs; Oct4, Sox2, Klf4 and c-Myc (OSKM) can reprogram fully differentiated cells into a pluripotent state (iPSCs), while Gala3, Eomes, Tafap2c, and c-Myc (GETM) can reprogram differentiated cells to a trophoblast stem cells (iTSCs). We still have extremely poor mechanistic understanding of how TFs operate and impose cell-type specific reprogramming activity. We aim to use this reprogramming technology to address fundamental questions in developmental and stem cells biology. Importantly, we aim to elucidate the molecular features of TFs that impart reprogramming. By defining the minimal domains of TFs that explain reprogramming, we will eliminate the unnecessary risks associated with superfluous domains. This will enable us to engineer new proteins that can deliver efficient, safe and reliable reprogramming.

In our lab we will generate iPSCs and iTSCs with lentiviral delivery of TFs to fibroblasts. Using a Dox-inducible
system, the ectopic expression of TFs will be tightly controlled during the reprogramming process. This will allow us to map the temporal interactions of these reprogramming factors with both the genome and the proteome. Other delivery systems such as plasmid transfection and retrovirus infection are strongly silenced during reprogramming (30 days process) and lack control over a sustained ectopic expression TFs. Therefore, it is essential to use a doxycycline-inducible lentivirus system to deliver and control the expression of TFs during reprogramming.

Chromatin, proteins and RNA will be isolated from infected cells in a time-wise fashion, but not earlier than 5 days post infection, surpassing the 24 hr half-life of lentviruses. All of recipient cells, including the infected fibroblasts are non-pathogenic and cannot survive outside cell culture conditions. There is no risk of virus transmission because the ViraSafe Lentiviral Packaging System (3rd generation) used is replication defective. In our lentivirus delivery system, all the genes are under the control of the Tet-ON regulatory element, requiring the presence of the reverse tetracycline transactivator M2rtTA (a separate gene) and doxycycline (tetracycline antibiotics) for induction. The three separate elements of this system will minimize any inadvertent risk associated with using oncogenic factors. These genes are inserted within two LoxP sites, allowing the complete removal the integrated insert from the genome upon Cre-recombinase treatment.

Recipient or parental organism

E.coli (Stbl3) will be used for cloning and propagation of lentivirus vectors. All packaged genes are under the control of promoters that are not-active in bacteria and the three plasmids needed for viral propagation in mammalian lines will be generated independently in bacterial systems. Thus, there will be no gene expression and no production of virus in E.coli.

293T cells will be used for packaging viral particles. Harvested viruses are highly infectious. Therefore, cultures will be destroyed accordingly after viral harvest using sodium hypochlorite. This cell line is non-viable outside of laboratory conditions.

All recipient cells, including the infected fibroblasts are non-pathogenic and cannot survive outside cell culture conditions. There is no risk of virus transmission because the viral vectors are replication defective.

All recipient cells will not express the inserted lentiviral genes, because they are under the control of the Tet-ON regulatory element requiring the presence of rtM2TA and tetracycline antibiotics. The gene encoding rtM2TA is inserted in a separate vector and will be packaged as a separate lentivirus. Hence, the recipient cells have to be infected with rtM2TA lentivirus plus another lentivirus carrying the gene under the Tet-ON control elements and they have to be treated with more than 1 ug/ml of doxycycline in order to induce the ectopic expression of the lentiviral inserted genes. This will reduce to inadvertently induce the expression of oncogenic factors. The virally integrated genes are bordered by LoxP sites and can be removed by Cre recombinase if required.

Host/vector system

FUW-TetO-LoxP vectors for protein expression: Vectors with cloned coding sequences are on FUW-TetO-LoxP backbone that are replication-incompetent lentiviral vectors. All genes cloned in this vector are expressed under the control of Tet-On promoter, requiring the presence of both rtM2TA (separate gene) and doxycycline (externally supplemented). The vector also contain LoxP sites upstream the LTR integration region, allowing the removal of the inserted gene from the host genome post-infection if required.

The ViraSafe lentivirus packaging system: (pCMV-VSV-G, pRSV-REV, pCgpV) encode for the VSV-g envelope protein and Rev, Gag and Pol packaging proteins. This will convert the Tet-ON vectors to lentiviral particles for subsequent infection with and that gives it broad host range and high transduction efficiency. The viral particles produced contain no HIV coding sequences and are replication incompetent. This system has no inserted sequence under the control of a bacterial promoter. This system improves the packaging plasmid 10 increase performance and reduce the likelihood of recombination between vector components.
All vectors including packaging vectors are separate, allowing flexibility to optimize the vector ratio for maximal lentivirus production and minimize the risk of producing live viral particles from a single plasmid. The lentivirus vectors are replication incompetent, thus reducing the risks of generation of replication competent virus.

Origin & function

mCherry, EGFP are fluorescent proteins used as controls. These proteins are considered harmless and don't pose any risk.

The transcription factors: OCI4, Sox2, Klf4, c-Myc, Gata3, Eames, Tlap2c directly regulate gene expression of target genes. Some of these factors such as c-Myc are known to oncogenic and thus considered as harmful. The reprogramming activity of these transcription factors require the combinatorial ectopic expression of three or more factors together. Each factor will be packaged in a separate virus to reduce the risk of their combinatorial reprogramming activity.

Evaluation of foreseeable effects

293T Lenliviral packaging cells after viral vector transfection are potentially harmful. Produced virus is highly infectious to mammalian cells including human cells. Careful handling of the virus will be carried out in the specified hood and incubator at containment level II. NO sharps or glass will be used during the work phase involving virus. The hood and incubator will be cleaned with 1% Virkon every time after the viral work. Waste management of plastic-ware, culture medium, is described in waste disposal section.

The viral vectors are replication defective and therefore no potential for onward transmission from infected target cells. Inserted genes in the lentivirus will be under the control on a TET-ON system and will not be expressed in the absence of rtM2TA and doxycycline in mammalian systems. The inserted genes are bordered by LTR and Lox? sites, which will integrate into the host genome and can be reversibly removed by Cre-recombinase.

Half-life of the virus particles are about 24 hours in the culture condition. Culture plates which have had contact with active virus particles will not be taken out of the specified tissue culture room before 48 hours and not before being washed more than 3 times with sterile PBS.

The infected cells that had direct contact with live virus particles will be cultured for at least 5 days and passaged at least once, prior to ectopic gene induction with doxycycline. Chromatin, protein and RNA will be purified from cells that have cultured for at least 7 days and washed more than 5 times. No viral particles can be reassembled from the purified chromatin, DNA/RNA or proteins.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All users working with virus must have taken the University GM Safety Course and be working under an approved GM
risk assessment. For each project the TC manager must be informed prior to the start of TC work. Use Disposable lab coat and do not use the same lab coat for other techniques. Disposable lab coats must be worn when working with high titre virus stocks and for the first 7 to 10 days after transduction. They should be autoclaved before disposal via the orange bag route. No glass or sharps should be used when working with virus. All solid and liquid waste should be decontaminated/disposed of by the user at the end of every TC session. For Chemical decontamination of solid waste, a soaking bucket is used which must be labelled with name and the date. Then the waste is disposed 24 hours later. Solid waste disposal:

Stripettes

Soak for 24 hours in 1% Virkon, then red incinerator box.

Plastic ware such as flasks, tubes and agar plates:

Soak in 1% w/v Virkon, or 1,000 ppm Precept (four 0.5g tablets into one litre of water) for 24 hours before discharging any excess liquid via drains. Following chemical decontamination, place all plastic ware and agar plates into the autoclave orange bags within the tin autoclave drums, tie orange bag and place in the white plastic bin - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-13BoC for at least 3 minutes).

Liquid waste disposal
samples, culture supernatants, tissue culture media

Any liquid potentially containing virus should be collected in an autoclavable screw top plastic bottle (stored under the sink). It should NEVER come into contact with the vacuum system. The liquid waste should be chemically disinfected by soaking in 1 % w/v Virkon, or 1,000ppm Precept (four 0.5g tablets into 1 litre of liquid) for 24 hours before discharging via the drains. The container must be labelled with name, group and date.

1% Virkon solution

Virkon Water

1ag Virkon

Sag Virkon (Sachet)

109 Virkon (2x sachet)

1 litre
5 litre
10 litre

Note: Virkon solutions are stable for 7 days and should be discarded after this time or if colour fades.

1,000 ppm Precept:

Four 0.5g tablets into one litre of water

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

**Project Ref 207/16.7**

- Date Ackn'd: 20/10/2016
- CU2 Project Title: Investigation of infectious laryngotracheitis virus of chickens
- Class: Class 2
- Culture Vol: ≤ 1 Litre
- Consent Granted
- Non-GMM

**Project Additional Information**

- Purposes of the contained use: To determine the role of specific virus genes in virus replication.
- Recipient or parental organism: Infectious Laryngotracheitis Virus which causes a respiratory disease in chickens.
<table>
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<th>Host/vector system</th>
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<td>Infectious laryngotracheitis virus in which specific genes have been deleted or replaced with a gene expressing a fluorescent protein.</td>
</tr>
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<td>Virus genes will be deleted. As tropism is primarily determined by interaction of virus glycoproteins with specific avian cellular receptors deletion of genes will not change the tropism of the virus and so recombinant viruses will not infect humans. Green Fluorescent Protein, is commonly used as a reporter tag, which exhibits bright green fluorescence when it is expressed in cells. It has no toxic, allergenic, immunomodulatory or oncogenic properties.</td>
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<th>Evaluation of foreseeable effects</th>
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<td>The recombinant viruses are species (Avian)-specific and will not infect humans. We will not delete/modify genes involved in receptor binding and so will not alter tropism. ILTV is endemic in the UK and the recombinant viruses are likely to exhibit either reduced or unchanged virulence compared to wild type.</td>
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<td>Solids (eg, plasticware such as pipettes, flasks, tubes etc) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains. Autoclaved solids are sent for microwave treatment and subsequent disposal.</td>
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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment
**Project Ref** 207/17.1

**Date Ackn'd** 05/01/2017

**CU2 Project Title** Generation of recombinant Bovine Herpesvirus -1 (infectious bovine rhinotracheitis virus)

**Class** Class 2

**CultureVol** < 1 Litre

**CultureVolumeClass** Class 3-4

**Type** Non-GMM

**Consent Granted** Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To determine the role of specific virus genes in virus replication.

**Recipient or parental organism**

Bovine Herpesvirus -1 which causes a respiratory disease in cattle.

**Host/vector system**

Bovine Herpesvirus-1 in which specific genes have been deleted or replaced with a gene expressing a fluorescent protein.
Virus genes will be deleted. As tropism is primarily determined by interaction of virus glycoproteins with specific bovine cellular receptors deletion of genes will not change the tropism of the virus and so recombinant viruses will not infect humans. Green Fluorescent Protein, and its derivatives, is commonly used as a reporter tag, which exhibits bright green fluorescence when it is expressed in cells. It has no toxic, allergenic, immunomodulatory or oncogenic properties.

**Evaluation of foreseeable effects**

The recombinant viruses are species (Bovine)-specific and will not infect humans. We will not delete/modify genes involved in receptor binding and so will not alter tropism. BoHV-1 is endemic in the UK and the recombinant viruses are likely to exhibit either reduced or unchanged virulence compared to wild type.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg, plasticware such as pipettes, flasks, tubes etc) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains. Autoclaved solids are sent for microwave treatment and subsequent disposal.

Liquids (eg, samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

These procedures will be reviewed when new waste management guidelines are published by the local H&S team.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
Haematopoietic stem cells (HSCs) have the ability to produce every blood cell type of an individual and are therefore of enormous clinical potential for cell replacement therapies. Despite this long-standing interest in HSCs and the employment of bone marrow transplants in the clinic for decades, robust methods for the expansion of HSCs in culture and their derivation from pluripotent stem cells are still lacking. A lot can be learned about the basic mechanisms of HSC production, expansion and differentiation from the study of how these cells are generated and disseminated during development. Furthermore, knowledge of these early processes can shed light on the mechanism of cancer development, as this often involves the re-activation of developmental pathways. The purpose of our work is therefore to identify the mechanisms that regulate HSC generation, expansion, differentiation and migration during development and how some of these early blood cells can give rise to paediatric leukaemias. The results of our studies will help improve current protocols for HSC expansion and generation from pluripotent stem cells and may highlight novel treatment strategies in paediatric leukaemia.
Host/vector system

293T cells will be transfected with the 3rd generation lentiviral vector (miRZIP, Lenti-miR MicroRNA Precursor Clone, pLL3.7, miR-Selection-Fir-Ctx LentiVector, pWPI and pRRL_AF4-MLL_2AdT-1i) or the 2nd generation retroviral vector (MDH1-PGK-EGFP, pMSCVhygro and pMSCV-IRESCFP).

The cells will also be co-transfected with the standard packaging vectors

Lentiviral Vector
- pMD2.G plasmid (encoding the vesicular stomatitis virus glycoprotein)
- psPAX2 (encoding gag-pol and Rev)
- or
- pMD2.G plasmid (encoding the vesicular stomatitis virus glycoprotein)
- pRSV-REV (encoding Rev)
- pMDLg/pRRE (encoding gag-pol)

Retroviral Vector
- pECo (encoding ecotropic envelope protein)

The lentiviral vector based system (miRZIP, Lenti-miR MicroRNA Precursor Clone, pLL3.7, miR-Selection-Fir-Ctx LentiVector, pWPI and pRRL_2AdT-II) will be used to package vectors that allow the inhibition or overexpression of potential oncogenes and tumour suppressor genes. These viral particles will be used on mouse cell lines, primary mouse cells, human-derived immortalized cell lines and primary human cells.

The retroviral vector based system (MDH1-PGK-EGFP, pMSCVhygro or pMSCV-IRESCFP) will be used to package vectors that allow the overexpression of microRNAs or leukaemia fusion genes that could act as oncogenes or tumour suppressor genes. This system can only infect mouse cells.

Origin & function

The viral vectors may contain cDNAs of candidate haematopoietic regulators that we wish to overexpress in target cells to assess how this affects haematopoiesis. Potential regulators could also include miRNAs we wish to overexpress. The vectors may also include knockdown constructs for potential haematopoietic regulator genes or miRNAs. In addition, we also have reporter vectors containing regulatory DNA elements that will express a resistance gene or a fluorescent marker when bound by a transcription factor or miRNA.

As our lab also works on leukaemia, we may wish to use viral constructs for the overexpression or knockdown of leukaemic oncogenes.

The cDNAs, miRNAs and oncogenes may be of human or mouse origin that were cloned in-house, obtained from a commercial source or from a collaborator.

Evaluation of foreseeable effects

All of the host cell lines are non-pathogenic and cannot survive outside cell culture conditions or without recipient mice. No harmful effects are expected.

None of the inserted genetic material is likely to cause any harm to humans. No adverse effects are likely. They are not toxic or immunogenic. The risks of recombination-related problems are minimal.

Our retroviruses can only infect rodent cells and are replication-incompetent.

Our lentiviruses can infect mammals including humans. However, lentiviral particles generated by this system are replication incompetent and all work will be carried out at containment level 2. The genes subject to this study are known oncogenes (e.g. leukaemia fusion genes) or potential oncogenes (e.g. microRNAs). Some of them are potential tumour suppressor genes (e.g. microRNA). Taken together, like in any other lentiviral work, it is possible that knockdown/overexpression of the genes of interest might be harmful to humans in the event of an accidental
exposure, thus all these procedures will be done in the specified hood under the stringent control.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid and liquid waste is treated with virkon for several waste before disposal in tissue culture waste that is then incinerated. 100% kill is achieved with this method.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Animal Units  Large Scale Activities  Human Clinical Applications

| L2 | L3 L4 L2 | L3 L4 L2 | L3 L4 |

Project Ref  207/17.3

Date Ackn'd  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4

29/03/2017  The impact of co-infection on the evolution of drug resistance in Pseudomonas  Class 2  < 1 litre
Purposes of the contained use

Experiments designed to assess competitive ability and antibiotic resistance. *P. aeruginosa* (Laboratory strain, non GMO) will be cultured in isolation and co-cultured with the other, genetically modified (in terms of motility and social signalling), strains of *P. aeruginosa*, and their resistance to common laboratory antibiotics assessed.

Recipient or parental organism

*Pseudomonas aeruginosa* WT laboratory strains will be the target for genetic modifications. We will use the WT and gene-inactivated derivatives.

Host/vector system

Commercially available mobilisation defective cloning vector pBluescript II for *E. coli* and *P. aeruginosa*. pRIC380 suicide vector and derivatives used to mutate lasI. As this is mobilisation defective there is no risk of spread to other organisms should the vector get into the environment.

Origin & function

Mini-CTX and mini-CTX lux derivatives will be used for the integration of exogenous DNA (*P. aeruginosa* promotors) into the chromosome of *P. aeruginosa* with *E. coli* S17-1 as a donor strain. Lastly the plasmids pSB401, pSB403, pSB404 and pSB1075 comprising reporter systems to detect *P. aeruginosa* signal molecules will be hosted by S17-1. By altering the ability of the *P. aeruginosa* strains to respond to signals, we expect them to differ in the way that they respond to antibiotics. These are stable genetic modifications and as such do not pose a risk of spread to other organisms.

Evaluation of foreseeable effects

As our vectors are mobilisation defective there is no risk of spread to other organisms. In addition to this, we will use mutant strains of *Pseudomonas aeruginosa* in genes such as lasR, rhlI, pqsA, pqsE and pqsR which are stable modifications resulting in the organisms to have reduced or negated ability to respond to signal molecules as well as reducing their mobility. These changes mean that in the environment the genetic changes have a very low risk of spread to other organisms and the modified organisms have reduced fitness and competitive ability with wild strains which will greatly diminish survival in an environment outside of the laboratory.

As regards human health and safety our gene modifications are not expected to alter the disease causing properties of the organism and have been shown not to increase virulence in a laboratory setting. The introduction of the new genetic material to these will not increase the pathogenicity of *P. aeruginosa* and thus does not pose an
increased risk to humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

School of Biological Sciences waste inactivation and disposal will be followed.

There will be no use of sharps.

1% final concentration Virkon disinfectant will be used for cleaning. 2% final concentration for spillages with an overnight contact time. Procedures followed will be those identified in the School waste guidance documentation.

Plastic discard (e.g. plasticware such as pipettes, flasks, tubes etc.) - the standard autoclave setting of 134 deg. C for a pulsed holding time of 5 minutes will be used.

Liquid discard (e.g. liquid culture and supernatants) - the standard autoclave setting is 121 deg. C for a pulsed holding time of 15 minutes.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation)
Disinfection — all spillage waste is subsequently autoclaved prior to disposal

Solid waste disposed via University contracted competent waste contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

<table>
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<tr>
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<th>Growth Rooms</th>
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02/03/2022  Page 4969 of 15326
Genome-wide perturbation using lentiviral delivery of CRISPR/Cas9 systems leading to loss or gain of gene expression in mammalian or avian cells

Recipient or parental organism
Replication defective lentiviruses expressing Cas9; guide RNAs targeting host cell genes and selectable markers. Cell lines and primary cells of human and animal source.

Host/vector system
A lentivirus construct containing gene editing components (CRISPR/Cas9, guide RNA and selection marker) will be packaged in and harvested from HEK cells.

Origin & function
CRISPR is a prokaryotic defence response to foreign DNA. Cas9 is an bacterial endonuclease which is targeted to a specific sequence of DNA by short guide RNAs (sgRNA). The Cas9 complex induces double stranded breaks in targeted DNA providing a defence mechanism against the original pathogen. Host animal cells will be transduced with a lentivirus(es) expressing Cas9 and sgRNA(s) to perform editing/deletion in specific regions of the genome in order to determine the function of particular genes. The lentiviral vector will not be able to replicate within the host.
Evaluation of foreseeable effects

The use of a genome-wide CRISPR/Cas9 knockout library will result in some growth perturbation effects in a proportion of the host cells used in vitro. This presents a potential oncogenic risk to the user. For this reason, and in particular, due to the relative similarity between the porcine, mouse and human genomes, appropriate care at the standard practice of Containment Level (CL) 2 will be adhered to during all handling of this utility to prevent contact between the lentiviral particles with skin of the operator/researcher. Only screw cap eppendorfs will be used. No sharps will be used. Waste media will be removed and any remaining retroviral particles inactivated by treatment with Distel® a broad spectrum virucidal disinfectant, in a biosafety cabinet to avoid aerosol generation, at a 1:10 dilution overnight at a designated place. All waste bottles will be clearly labelled with date and content.

None of the bacterial strains or lentivirus are able to colonise hosts both due to the extremely low probability that they would survive outside of the special culture environments in which they are designed to propagate. 293-T viral packaging cells do not survive out of tissue culture conditions and pose no threat to the environment. Transduced cells will not survive out of tissue culture conditions and pose no threat to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g. plasticare such as pipettes, flasks, tubes etc) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125C for at least 15 minutes or 126 - 130C for at least 10 minutes or 134 - 138C for at least 3 minutes), discharge any excess liquids to drains. Autoclaved solids are sent for microwave treatment and subsequent disposal.

Liquids (e.g. samples, culture supernatants, tissue culture media) are treated in a sealed container with 10% Distel overnight and discharged to drains. These procedures will be reviewed when new waste management guidelines are published by the local H&S team.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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## Project Additional Information

**Purposes of the contained use**

To produce lentiviral particles containing trypanosome cDNAs that can be used for use in the production of transgenic mice.

**Recipient or parental organism**

Plasmids will be constructed and propagated in XL-10 gold ultracompetent cells [TetrD(mcrA)183D(mcrCB-hsdSMRmrr) 173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac Hte [F proAB lacQZDM15 Tn10(TetR) Amy Camr] Plasmids will be transfected into Human Embryonic Kidney (HEK)293T cells to produce replication defective lentiviral particles (second generation) pseudotyped with VSV-G. Lentiviral particles will be injected into the perivitelline space of mouse zygotes (single cell embryos) which will then be transferred to pseudopregnant female recipients.

**Host/vector system**

Host cells will be XL-10 gold ultracompetent cells [TetrD(mcrA)183D(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac Hte [F proAB lacQZDM15 Tn10(TetR) Amy Camr], Human Embryonic Kidney (HEK)293T cells and mouse cells (zygotes). The cargo of the lentiviral vector particles will integrate into the genome of the mouse.
zygotes, and offspring born will be transgenic in all tissues. The replication defective nature of the lentiviral vector system means that the genetic elements integrate stably into the mouse genome and will not be mobilised further.

**Origin & function**

Lentiviral vectors will contain a liver specific promoter driving a reverse tet transactivator and a tet response element driving expression of the trypanosome cDNA. In the absence of tetracycline (or a derivative such as doxycycline) it is anticipated that the transcriptional units will be silent. In most cell types, even in the presence of doxycycline, it is anticipated the transcriptional units will be silent. In liver cells it is anticipated that the reverse tet transactivator protein will be expressed, but in the absence of doxycycline this will be unable to bind to the tet response element that drives the trypanosome cDNAs. Only in liver cells and in the presence of doxycycline should the trypanosome cDNAs be expressed. The cDNAs expressed are not likely to increase pathogenesis associated with trypanosome infection.

**Evaluation of foreseeable effects**

Lentiviral particles will be able to invade a mammalian cell, reverse transcribe their RNA cargo and integrate this into the genome of the host cell. This genetic element cannot be remobilised. There is a risk to human health and safety. The VSV-G pseudotype means that lentiviral particles can invade human cells via cuts in the skin or across mucous membranes. Integration into the genome of human cells could result in insertional mutagenesis (eg upregulation or disruption of normal gene expression). Precautions are in place to minimise the risks associated with this material, including appropriate PPE, minimal use of sharps (absent other than for zygote injection) and the use of biosafety cabinets. Lentiviral particles are not environmentally robust, and degrade rapidly in dry conditions. All waste from the production and injection process will be disposed as stipulated in the appropriate risk assessments to minimise further any risk to the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

GM mice will be housed in a contained mouse facility.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Lentivirus-containing waste liquids will be aspirated into a container containing Distel® though a closed aspiration system. After 24h of inactivation the container may be changed and the liquid autoclaved prior to disposal. Surface disinfection will be with 1-10% Distel. 70% EtOH is not sufficient to inactivate HIV and thus lentiviruses. All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping. Each run will be monitored by both Browne TST indicator test stripes and continuous chart/digital recording of the temperature / time profile (CSU) or by digital recording only (BRF).

**Is an emergency plan required according to regulation 20?**  

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If yes, tick to confirm that it is attached to this form

| N |

Tick to confirm that you have attached a risk assessment to this form

| Y |
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

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**Project Ref** 207/18.2

- **Date Ack'n'd**: 26/01/2018
- **CU2 Project Title**: Host responses to Respiratory viruses
- **Class**: Class 2
- **Culture Vol**: ≤ 1 Litre
- **Non-GMM Consent Granted**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

Tick if notifying a connected programme of work N

**Project Additional Information**
Purposes of the contained use

This project focuses on building a base of knowledge around how viruses interact with host cells in order to develop a new type of therapy where, instead of directly targeting the virus, the host cell becomes less hospitable for virus infection. Specifically, this project will identify the host ribonucleic acid (RNA) molecules that are used by respiratory viruses during their life cycles. This is a largely unexplored research area, mainly because it has been very difficult to directly identify the microscopic host RNA-virus RNA interactions that occur within an infected cell. This project will make use of a unique method established at the University of Edinburgh for isolating the protein (Argonaute) that binds to the virus RNAs-host RNA complexes and then carrying out molecular reactions to directly ligate and sequence the RNAs as a way of identifying the specific interactions that occur.

One complication of this technique is that large quantities of largely homogenous infected samples are required to gain enough power for next generation sequencing (NGS) statistical analysis. In normal infection it is difficult to determine the exact number of cells within a flask that are infected at any given time, and the viral load contained within these infected cells. We propose to use Respiratory Syncytial Virus (RSV) and Human Parainfluenza Virus (PIV3) expressing green fluorescent proteins to circumvent these restrictions. Infected cells can be identified in realtime using a fluorescent microscope, and fluorescence intensity should directly correlate with viable viral load per cell. Use of these genetically modified viruses will therefore increase both the accuracy and analytical value of our experiments, substantially advancing our understanding of host responses to infection.

Recipient or parental organism

- WT RSV cDNA (ACDP Classification 2)
- WT PIV3 cDNA (ACDP Classification 2)

Host/vector system

We propose to use RSV virus that has been already modified to express a green fluorescence protein (RSV-GFP) and PIV3 virus (PIV-GFP) that has been already modified to express a GFP. The details of these viruses and their previous construction are published (below) and no further modifications will be made.

For WT RSV cDNA: Fragment of Minigenome C41 into MP129 to create MP169 (then moved to full length RSV cDNA). Methods described in detail in Hallak et al, J.Virology p10508-10513 2000


Origin & function

We propose to use RSV virus that has been already modified to express a green fluorescence protein (RSV-GFP). Reference of the method of construction can be found at Hallak et al, J.Virology p10508-10513 2000 and Guerrero-Plata et al, Am J Respir Cell Mol Biol, p320-329 2006 and references therein. We also propose to use PIV3 virus that has been modified to express a green fluorescence protein (PIV3-GFP). Reference of the method of construction can be found in Roth et al, Antiviral Res, 12-21, 2009.

Evaluation of foreseeable effects

The inserted gene product encodes green fluorescent proteins and do not confer any selective, infectious or propagating advantage on the viruses. Therefore modified viruses and/or the genetically inserted gene product do not represent any increased risk to human health and safety over that of WT RSV or PIV3.

The modified RSV and PIV3 are WT with the exception that they express a fluorescent protein. This protein does not alter cellular or species tropism and confers no selective, proliferative or competitive advantage over WT strains as
ascertained by the producers of this virus, constructed as detailed in the journal articles referenced previously in this form. In the event of exposure of an individual to the modified virus, the response would not be anticipated to be any different to that of WT RSV or PIV3, to which individuals are exposed throughout their lifetime. It is extremely unlikely that this modified RSV or PIV3 will present any increased environmental risk over and above that of WT. The virus does not produce any significantly harmful toxins or proteins. Given that this virus has at most equal proliferative capacity, but more likely less than WT, it is extremely unlikely that in the event of public exposure this virus would result in displacement of WT strains. RSV and PIV3 are not viruses prone to recombination, mutation or alterations in either tropism or increased virulence thus in the unlikely event of infection of primary cells harbouring other pathogenic factors, accidental generation of a more pathogenic RSV or PIV3 is extremely unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All liquids and plastics containing or potentially contacting virus are treated in 5% (final concentration) Teknon Biocleanse Biocidal cleaner solution for inactivation in the microbiological safety cabinet. All liquids containing or potentially contacting virus are treated in 5% (final concentration) Teknon Biocleanse Biocidal cleaner overnight in plastic containers and disposed to drain. All plastics and reagents which contain potentially infectious virus are autoclaved using standard setting of 134 deg.C for a holding time of 5 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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02/03/2022
Project Additional Information

Purposes of the contained use

This risk assessment is intended to cover the packaging of HIV1-based lentiviral vectors containing shRNA against p53, Rb, or CDKN2A and an expression unit of the catalytic subunit of the human telomerase protein (hTERT) and subsequent transduction of target cells in vitro. Any transgene inserted into the lentiviral backbone that is known to act as an oncogene requires an additional risk assessment. These three transgene elements are known to be able to contribute to oncogenicity.

Lentiviral vectors represent a sophisticated gene delivery method that allows integration of genetic material into the genome of both dividing and non-dividing target cells. The system is composed of a number of plasmids derived from human immunodeficiency virus. Co-transfection of HEK293T cells with these plasmids results in the secretion of viral particles into the culture supernatant. Lentiviral particles can be concentrated from culture supernatant to titres of approximately 10^8-9 infectious particles per ml, when assayed on human cells. The transfer vector can carry 7-8 kb of foreign DNA and maintain stable expression once integrated into the target genome. Importantly, several safety features have been included in this system to minimise the potential of replication competent lentivirus (RCL) production, or subsequent mobilisation of the integrated transgene from the genome of the host cell. Importantly, due to the reduction of the lentiviral genome to a bare minimum for incorporation of transgenes into the target cell and expression of other factors needed for the assembly of lentiviral particles in the producer cell only, resulting in incorporation of proteins but not genomic information, these lentiviral particles are unable to form new viral particles in the target cells and are therefore unable to spread.

Recipient or parental organism

Primary cells held on site in the Roslin Institute or isolated from live animals, obtained commercially, or from collaborations. Cells may include but are not limited to lung and...
Intestinal epithelial cells, monocytes and macrophages of different origin

Primary cells from farm or companion animals will be transduced with the lentiviruses generated to immortalize them. Primary cells, which have the potential to carry unknown infectious agents and will only be used at containment 2.

Host/vector system

2nd Generation lentiviral replication system using a three plasmid vector system to generate the single-round infectious lentiviral particles used for transduction of target cells.

The second generation lentiviral system has a low risk of recombination with endogenous retroviruses due to the division of viral elements among three different plasmids. The packaging and envelope plasmids express viral proteins, which are packaged as such into the lentiviral pseudoparticle. These proteins cannot be newly synthesized in the target cell therefore the lentiviral pseudoparticle is single-round infectious.

Transgenes encoded by the lentiviruses packaged under this risk assessment may be oncogenes, and as such have the potential to have significant negative impact if accidentally transduction of the user occurs. However, all work will be carried out in a class 2 biological safety cabinet by trained users wearing appropriate PPE. Sharps (glass wear, scalps or needles) will not be used in the TC suite where lentivirus is produced or handled. Taken together the likelihood of accidental transduction of the user is considered extremely low.

The frequency of RCL generation using lentivirus vectors is considered very low and this fact, in conjunction with the ‘self-inactivating’ feature following transduction, suggests that the potential for shedding of RCL from animals is very low. Indeed, RCL have to date not been reported in association with lentiviral packaging systems (Pauwels et al 2009, Current Gene Therapy 9:459-474).

Lentivirus vectors have been shown to achieve efficient and safe transduction of the murine brain, muscle, and liver and have been used to create transgenic pigs without observable adverse side effects in viable offspring (Roslin). Indeed recent mapping studies of lentiviral vector integration sites seem to indicate that these vectors have less tendency to target dangerous transcription start sites than vectors based on MoMLV – suggesting that these vectors are much less prone to inducing insertional oncogenesis. The potential for oncogenesis as a result of insertional mutagenesis is therefore considered very low.

Origin & function

hTERT and shRNAs targeting livestock proteins TP53, Rb and INK4A will be integrated into the primary cells for immortalisation.

One of the reasons somatic cells enter a senescent state is the shortening of the telomere repeat sequence at the end of eukaryotic chromosomes. TERT (or hTERT in humans) is the catalytic subunit of the telomerase reverse transcriptase enzyme (TER). This protein is inactive in most somatic cells, whereas they still express hTERC and TP1, two telomerase-associated proteins that help to form the TER. Thus, exogenous expression of hTERT restores active TER activity in differentiated cells and has been shown to be able to extend the proliferative cell cycle, thereby immortalizing, certain primary cell types. Immortalization of cells using hTERT was also shown to change the epigenetic programming of DNA methylation and gene expression.

shRNAs targeting the tumor suppressor genes TP53, Rb, or INK4A will further enhance the proliferation and division of primary cells. Whenever possible the shRNA target sequences will be designed in an area unique to the (livestock) animal species as to not target the human gene.

Evaluation of foreseeable effects

The uncharacterized, immortalized cell lines derived from primary cells may still contain unknown pathogens and continued use at containment level 2 is advised until thorough characterization is conducted.

Genetically modified cells are not a hazard to human health. Cell lines or primary cells will not survive outside laboratory conditions and carry no additional hazards compared with those already present in the environment.

The transgenes integrated into the primary cells, once turned to immortalized cell lines, are not expected to have any harmful effects on humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Liquid waste:** Liquid viral waste will be inactivated by disinfection (HazTab for 24 hours). Liquid bacterial culture waste will be inactivated by autoclaving.

- **Plastic consumables:** Rinse with 1% Distel, contain in autoclavable bag, followed by autoclaving.

- **Re-usable Glassware:** 1% Distel with a contact time of 30min or 1% Virkon with a contact time of 1h.
  For metal parts an incubation with Distel is recommended due to the corrosive nature of Virkon.

- For autoclave procedures Browne sterilizer control tubes will be included in the load as a means of routine monitoring of treatment temperatures and time (at least 121°C maintained for 15 min). In the event that the Browne sterilizer control tubes fail to change to a green colour following an autoclave cycle then the load will be autoclaved again using an alternative machine with temperature and time monitored in the same way (eg SAH S17).

The autoclave will be subject to an annual 12 point thermocouple test on a waste cycle.

The efficiency of Distel or Virkon have been measured by independent labs contracted by the manufacturer's and were shown to have efficacy against the viruses specified (HIV). Further disinfectant’s HIV inactivation ability specified in Table 1e.

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**Large Scale Activities**

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**Human Clinical Applications**

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The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

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Tick if you are claiming exemption from disclosure for section of the risk assessment

- **Risk assessment**
  - [ ] Yes
  - [ ] No

Tick to confirm that you have attached a risk assessment to this form

- **Risk assessment**
  - [ ] Yes
  - [ ] No

Tick if you are claiming exemption from disclosure for section of the risk assessment

- **Risk assessment**
  - [ ] Yes
  - [ ] No

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02/03/2022
Cellular regulation of and by the Hippo Pathway

The Hippo pathway has within the past two decades gained great interest as an integrator of cellular homeostasis, where it works as a nexus for diverse cellular signaling. Understanding the regulatory mechanisms of the Hippo pathway has therefore become a major focus in especially regenerative medicine. The chief biological output of the Hippo pathway centers on Yes-associated protein (YAP) and transcriptional coactivator with a PDZ-binding domain (TAZ), which are the prime mediators of the Hippo pathway, when they are active they shuttle to the nucleus and binds to and activate their cognate transcription factors.

As YAP/TAZ is deregulated in many human pathologies including a range of cancers an in depth analysis of precisely how the pathway is regulated and exactly what causes dysregulation of this pathway in human pathologies

Recipient or parental organism
- Standard lab strains of disabled E. coli (e.g. DH5alpha, BL21)
- Multiple human cell types, including cells with regenerative potential and mouse cells grown in tissue culture
- Zebrafish

Host/vector system
- Cloning vectors, siRNA, shRNA & standard expression vectors for mammalian transfection and 3rd generation virus based delivery. The virus are replication defective.

Origin & function
- We use many different targets for overexpression or knockdown. These include genes that regulate dynamic actin rearrangements, cell-cell and cell-substratum adhesion and cell migration. The genes that we introduce into cells are kinases, scaffolding proteins and transcription co-activators and transcription factors. In addition, we use probes and biosensors to identify the cellular localisation of the proteins of interest.
None of the generated cell lines will pose any risk to the environment or human health as they will not survive outside the cell incubator.

Evaluation of foreseeable effects

The E. coli (DH5alpha, BL21) that we use for propagation of DNA are disabled and do not pose any risk under normal laboratory conditions where they are handled with good laboratory practice.

Our primary mammalian target cell lines do not pose any risk nor would we expect them to survive outside their tissue culture environment. None of the generated cell lines therefore pose any risk to the environment or human health. We use a safe replication deficient virus system with immobilised vectors that also ensures these are of no risk to the environment or human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or disinfect e.g. with Virkon or Trigene (1% w/v) for 30 mins or 250mg/l of NaDCC (Has-Tabs, 250mg/l gives at least 140 ppm of chlorine) for >8hrs, discharge any excess liquids to drains, dispose of solids via clinical waste stream for heat treatment or via the industrial (black bag) waste stream for landfill.

 Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or disinfect with Virkon (1% w/v) for 30 mins or 250mg/l of NaDCC (Has-Tabs, 250mg/l gives at least 140 ppm of chlorine) for >8 hrs, discharge to drains.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms
### Project Ref 207/18.5

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#### Project Additional Information

**Purposes of the contained use**

Test the phenotype effect of Legionella spp. traits identified by whole genome sequence screening using genetic mutants.

**Recipient or parental organism**

Legionella pneumophila (Hazard group 2), Legionella longbeachae or other Legionella spp.  
Escherichia coli (Hazard group 1)

**Host/vector system**

Will include different types that have been developed for molecular biological purposes of E. coli and Legionella spp including pET expression vector series, pUC19, pGEM, pMAD, pE194, pTS1/2, pCu1, pT181, pSK265, pCL84, pIMAY, pKOR1, members of the pCN shuttle vector and derivatives, pMBB207, pBC-KS, pWM1007. Tn mutant libraries may be made using the vector pSRS_CM1 marked by either chloramphenicol or erythromycin resistance.
determinants. Additional novel plasmid vectors may be used as they become available.

**Origin & function**

Insertion of naturally occurring gene variants including those predicted to be non-functional due to mis and non-sense mutations. Gene variants are sourced from other strains from the same species. In order to study the effect of the presence of different variants of a target gene, strains which either lack the gene entirely or encode a variant of a gene will be replaced or complemented.

Expression of proteins such as putative virulence factors or proteins indirectly involved in pathogenesis using expression vectors (e.g. *E. coli*) for controlled expression, purification and tagging.

Natural variants or variants generated using site-directed mutagenesis of target genes will be expressed using *E. coli* vectors. The protein products will be tested to determine the effect on bacteria, host cells (e.g. macrophages) or combinations of the above.

Gene variants generated using site-directed mutagenesis where amino acid residues predicted to be in an active site are replaced with other amino acids with a different chemical property or charge.

Other inserted genes as fluorescent proteins from jellyfish and corals as well as β-galactosidase and β-lactamase genes originated from *Escherichia coli* will be used as standard reporter genes and for construction of gene fusions.

**Evaluation of foreseeable effects**

The GM microorganism is predicted to have the same capacity as other naturally occurring variants found in the environment and will be able to survive and persist if the a suitable amoebal host is present. There is no evidence that genetic determinants associated with human infection confers any advantage to survive in the environment.

Insertion of antibiotic resistant cassettes in order to select for plasmid positive strains will confer resistance to that antibiotic. However, the antibiotic resistance introduced to *Legionella* strains (to chloramphenicol, kanamycin or ampicillin) will not affect the normal treatment protocol.

Inserted gene products will be derived from existing strains of *Legionella*, and may be modified by site-directed mutagenesis. The examples of genes that will be targeted include putative virulence factors. The products of these genes are not predicted to have any inherent toxicity, carcinogenic or allergenic properties. These genes may slightly alter various phenotypes of *Legionella*, including virulence determinants. However, it is not predicted to the increase the pathogenic potential of the modified strain above that of naturally occurring strains.

For example, the introduction of an O-actylase gene is predicted to modify the LPS structure on the cell wall of the strain and this specific phenotype has also been shown to be over-represented in strains linked to human infections.

Reporter genes are non-harmful and fused genes for expression analysis will be only introduced to strains which already harbour the genes so the virulence of the host bacterium should not be affected.

The are no predicted hazards arising from the alteration of any pathogenic traits.

In some bacterial pathogens reports of ‘anti-virulence’ genes exist, whereby mutation of the gene enhances virulence. However, such loci are rare, as evidenced by screening of random mutant libraries using methods such as Tn-seq, which indicates that a very small proportion of random mutants screened in animal models are enriched relative to the inoculum following infection. Even where increased fitness of such mutants in vivo appears to exist, it does not automatically equate to increased pathology. The risks posed by engineered mutants is balanced by the risk of naturally-occurring mutation and risks are mitigated by the proposed containment measures.

The transfer of sequences could alter phenotypes in related microorganisms. However, these variants are already naturally occurring and this transfer can also occur in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Disinfection** - All the working surfaces as well as all surfaces in case of a spill should be treated using a 1:10 solution of concentrate Distel. Distel is intended for the decontamination of surfaces of medical devices and is CE marked as Class IIa in accordance with the European Medical Device Directive 93/42/EEC and the 2007/47/EC amendments thereto. This disinfectant is manufactured by Tristel Solutions Ltd under approval by Lloyds Register Quality Assurance to the standards of BS EN ISO9001:2000 and BS EN ISO13485:2003 for the manufacture of disinfectant and sterilising solutions for medical, dental and veterinary use. A 1:50 solution was proven effective against Legionella pneumophila in 1996 by Bridle Microbiological Consultants (BS6471).

- **Autoclaving** - All contaminated materials will be inactivated by autoclaving (100% kill) as specified in BS 2646, Part 3, 1993 at 121°C for at least 15 minutes prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored using chemical indicators (e.g., Browne TST indicator test strips).

**Waste disposal**
- **Solids** (group C - e.g., plasticware such as pipettes, flasks, tubes, etc. and agar plates) - are autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 at 121°C for at least 15 minutes. Any excess liquids are discharged to drains; solids are disposed via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.
- **Liquids** (e.g., samples, culture supernatants, culture media) – are contained in a secure, sealable and robust vessel as a flask. Autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 at 121°C for at least 15 minutes and discharge to drains.
- **Sharps** (e.g., needles, scalpels) – Discarded into rigid “Sharpsafe” containers. The rigid lid should be snapped in place when first constructed and when ¾ full, the flap should be loosely adhered to the lid with autoclave tape. It should then be safely transported to the waste collection point, labelled with the location and date, and sent for autoclave treatment. The sharps container will then be sealed (by clicking the flap in place) and it will be sent for incineration. Autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 at 121°C for at least 15 minutes.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

**Please enter comments on the GM safety committee on the risk assessment**

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

02/03/2022
Project Containment

Laboratory Activities
L2  Yes  L3  L4  L2
Glass Houses
L2  L3  L4
Growth Rooms
L2  L3  L4

Animal Units
L2  L3  L4

Large Scale Activities
L2  L3  L4

Human Clinical Applications
L2  L3  L4

Project Ref 207/18.6

Date Ackn'd 04/07/2018
CU2 Project Title Genetic manipulation of Trypanosoma congolense and Trypanosoma vivax

Date Project Ceased

Class 2
CultureVol
Class 3-4
< 1 Litre

Non-GMM
Consent Granted  Not Applicable

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Our aim is to understand the control of the life cycle of African trypanosomes at the molecular and cytological level. African trypanosomes are major pathogens in sub Saharan Africa, affecting up to 60 million people in 36 countries. They are transmitted by tsetse flies and produce a fatal wasting disease in humans and cattle. Most studies to date have focussed on Trypanosoma brucei spp. that have the potential to infect humans and animals, but this parasite is not the most important cause of livestock disease. Instead, two other species, Trypanosoma congolense and Trypanosoma vivax, are most prevalent and pathogenic.

We have identified a number of potential molecular regulators of the Trypanosoma brucei life cycle. However, the life cycle of Trypanosoma congolense and Trypanosoma vivax is different to Trypanosoma brucei and so might be their underlying molecular regulation. By analysis of their molecular expression in wild type trypanosomes and by perturbation of this in genetically modified parasites our objective is to understand how the molecular control of parasite transmission for
Trypanosoma congolense and Trypanosoma vivax operates. Our long term goal is to identify and exploit strategies that will allow parasite transmission to be disrupted, thereby preventing disease spread.

Recipient or parental organism

Trypanosoma congolense and Trypanosoma vivax are not infectious to humans and cause Nagana in domestic cattle and game animals in sub-Saharan Africa. They are classified as hazard group 2 by SAPO; they are not included with ACDP hazard groups, being non-infective to humans.

Procyclic (insect culture forms) of Trypanosoma congolense and Trypanosoma vivax are not infective to mammals. Trypanosomes do not produce toxins and do not form spores. Parasites do not survive outside of tissue culture or blood. They can only be transmitted by tsetse flies, though T. vivax is capable of mechanical transmission (see below). Infection by ingestion or inhalation is not a viable route of infection; parasites are only infective via the percutaneous route.

We expect most genetic manipulation of the parasite to reduce fitness and/or virulence: our experiments to date indicate perturbed expression of the molecules under study in our laboratory reduce virulence though other may affect development or increase virulence for example if they lose density dependent growth control.

Host/vector system

Constructs for transfection into trypanosomes are derivatives of commercially available, non mobilisable (i.e. Bom-, mob-, tra-) plasmids: pGEM series and pBluescript II. Inserted genes are not under bacterial expression control; they will only be effectively expressed in the parasite. In each case gene expression in the parasite is under tetracycline regulated control: thus, in the absence of tetracycline, gene expression is effectively silenced. Hence the vector and insert in combination present negligible risk.

Origin & function

Origins: T. congolense, T. vivax, T. b. brucei Lister 427; T. b. brucei TREU 927/4; T. b. brucei AnTat 1.1; T. b. evansi AnTat 3/3; T. cruzi (ACDP 3); Leishmania spp. (ACDP 2/3*).

Intended functions:
• Genes that are syntenic or predicted orthologues of genes already identified in Trypanosoma brucei as being involved in parasite differentiation (i.e. density dependent growth control in the mammalian bloodstream or differentiation upon stimulation to transform form bloodstream to tsetse midgut forms)
• Genes differentially expressed or with differential activity in different developmental stages of Trypanosoma congolense or Trypanosoma vivax.
• We will also express orthologous genes from Trypanosoma brucei in Trypanosoma congolense or Trypanosoma vivax. Other kinetoplastid orthologues may also be used (from T. cruzi, Leishmania spp. )

Where these genes or processes have been manipulated to date they have in each case either generated no consequence for the parasite, or have resulted in disruption of the parasite morphology, cell cycle or capacity for developmental processes.

We are also seeking molecules that interact with the above proteins or influence their function or specificity. In some experiments we will use genes from other kinetoplastids (for example Trypanosoma brucei) to functionally complement mutations in the above genes. These experiments will assess evolutionary conservation of the genes under study. Genes responsible for Trypanosoma brucei human infectivity have been identified and experimentally characterised: we will not introduce these genes into Trypanosoma congolense or Trypanosoma vivax.

Evaluation of foreseeable effects

T. congolense, T. vivax, T. b. brucei Lister 427; T. b. brucei TREU 927/4; T. b. brucei AnTat 1.1; T. b. evansi AnTat 3/3; T. cruzi (ACDP 3); Leishmania spp. (ACDP 2/3*).

We are investigating genes involved in life cycle differentiation events. Our experiments will express transgenic proteins, or mutant forms of these proteins or induce the ablation of the transcripts for endogenous trypanosome genes by RNA interference. These perturbations of normal processes in the cell are likely to have no consequence for pathogenicity and, moreover, may decrease parasite fitness though virulence may be increased if gene silencing affects density-dependent growth control. As indicated above, to date manipulation of the genes involved in the processes we study in Trypanosoma brucei have generated cell cycle arrest, lethality, morphological change and
Inappropriate developmental events. Manipulation of the genes under study will also not increase host range (the wild type parasites themselves are already infective to a wide range of mammals), and are very unlikely to confer infectivity of the trypanosomes to humans (the genes linked to human infectivity are known and experimentally well characterised).

Drugs used for selection of transgenic parasites differ from those used for clinical treatment of the disease. Trypanosomes produce no toxins or pathogenic products.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste from the CL3 laboratory in Ashworth 2, room 4.13, is transported to the autoclave in room 4.57 in the CL3 suite in Ashworth 3 in a locked yellow wheelie bin and autoclaved prior to removal for incineration. A robust leak-proof container is used for both storage and transport of waste for autoclaving. Most of the material that has come into contact with trypanosomes will be soaked for 24 hours in 2% Virkon. Thereafter, or for any items not disinfected first, the following waste disposal procedures will be used:

**Solids (e.g. plastic ware such as pipettes, flasks, tubes etc. and agar plates)** - autoclave using a make-safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

**Liquids (e.g. samples, culture supernatants, tissue culture media)** – autoclave using a make-safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

**Sharps (in sharps bin, e.g. needles, syringes, scalpels)** - autoclave using a make-safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for microwave treatment.

**Animal bedding and carcasses** – autoclave using a make-safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose of carcasses via clinical waste stream for incineration and bedding via clinical waste stream for microwave treatment or via the industrial (black bag) waste stream for landfill.

Spills are contained with paper towels and treated with powdered Virkon (for larger volumes) or 2% Virkon (smaller volumes) and aerosols.

Degree of kill:

**Treatment with Virkon** - adding 1 ml of 1% Virkon to 10 ml of 1 x 106 bloodstream form T. brucei cells for at least 10 seconds effectively kills 100% of cells as no recovery is observed two weeks after treatment (Prof. Mark Field, personal communication).

**Autoclaving** - effectively 100% kill (annual validation).

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.
Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Laboratory Activities Glass Houses Growth Rooms

Project Ref 207/18.7

Understanding the changes in mRNA processing that allow the pathogenic fungus Cryptococcus neoformans to survive stress and initiate host infection

Date Ackn'd 23/08/2018

CU2 Project Title

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Aim 1: To understand how sequences specify mRNA regulation in different growth states including lab models of infection by creating designed libraries of chimeric mRNAs composed of various relevant C. neoformans 5’ and 3’ regulatory sequences and mutations thereof, surrounding fluorescent reporter genes. Transform them into C. neoformans by genomic integration, quantify individual chimeric mRNAs and their protein products.

Aim 2: To understand how mRNA and protein sequences affect localization, expression, and function of native Cryptococcus proteins that regulate mRNA processing by creating recombinant proteins in C. neoformans, especially native proteins fused to epitope tags or fluorescent proteins, and mutants of RNA binding proteins, DNA binding proteins, and other proteins that regulate their binding. To test the effect of mutations on mRNA/protein localization, and expression, and downstream mRNA processing.
and cell growth.

**Recipient or parental organism**

Laboratory isolates of Cryptococcus spp., including C. neoformans strains H99, KN99a, and JEC21 (Hazard Group 2); these are Amphotericin B susceptible.

**Host/vector system**

Disabled laboratory strains of E. coli. Non-hazardous standard E.coli plasmids containing replication origins (ColE1) and selectable drug markers (Amp, Kan).

**Origin & function**

 Origins: restriction fragments or PCR amplicons from C. neoformans DNA, either using auxotrophic/antibiotic markers such as NAT /GEO for nourseothricin/G418 resistance, respectively (Chun & Madhani, Meth. Enzym., 2010; Hua et al., Clin Diagn Lab Immunol., 2000) or CRISPR/Cas9 for selection-free transformation (Fan & Lin, Genetics, 2018).

 Vectors are non-mobilisable.

 Intended functions:
 Libraries of chimeric mRNAs composed of various relevant C. neoformans 5’ and 3’ regulatory sequences and mutations thereof, surrounding fluorescent reporter genes to understand how sequences specify mRNA regulation in different growth states, including lab models of infection.

 Native proteins fused to epitope tags or fluorescent proteins, and mutants of RNA binding proteins, DNA binding proteins, and other proteins that regulate their binding and mutations thereof to test the effect of mutations on mRNA/protein localization, expression, and downstream mRNA processing and cell growth.

 Normal/expected function is fluorescence or regulation of gene expression. No known toxins or allergens will be produced. Inserts are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. Gene transfer is possible but unlikely to be hazardous. None of the proposed manipulations will introduce genes for which there is any evidence of additional harmful effects to immuno-competent humans.

**Evaluation of foreseeable effects**

None of the proposed manipulations will introduce genes for which there is any evidence of additional harmful effects to immuno-competent humans. Most deletion mutants reduce the viability and/or virulence, and deletions of less than 3% of genes increase the virulence of C. neoformans in animal models (Liu OW, et al. 2008. Cell 135: 174–188.). We plan to work only with any genes whose deletion decreases, or does not impact, virulence. The effect of GMOs on immuno-compromised individuals is expected to be similar to that of C. neoformans occurring naturally in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Broth cultures, agar plates, absorbent disposable paper towels and infected plastic-ware such as pipette tips will be sterilised by autoclaving, at a minimum of 121ºC for 50 mins, validated by monitoring of internal chamber temperature. This method is widely acknowledged to guarantee complete killing of fungal cultures and spores. Wastes from autoclaving will be disposed of to the drains (liquids) or via the clinical waste stream.

Sharps (in sharps bin, eg needles, syringes, scalpels) – any necessary sharps will be disposed of in sharps containers, which are disposed of via the clinical waste stream for heat treatment via a university approved contractor.
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 L3 L4 L2 L3</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units L2 L3 L4 L2</td>
<td>Large Scale Activities L3 L4</td>
<td>Human Clinical Applications L2 L3 L4</td>
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**Project Ref** 207/19.1

- **Date Ackn'd**: 09/01/2019
- **CU2 Project Title**: Modulation of innate host defence by pathogenic bacteria
- **Class**: Class 2
- **Culture Volume Class 2**: < 1 Litre
- **Consent Granted**: Non-GMM

**Historical Significant Changes**

- **Historical Date of Additional Info**: 02/03/2022
- **Significant Change ID**: 
- **Date of Significant Change**: 
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Project notified under transitional arrangements**: N
### Project Additional Information

**Purposes of the contained use**

The purpose of the work is to elucidate mechanisms by which host cells respond to bacterial infection and the consequences of these responses. The aim is to improve understanding of the mechanisms used by the innate immune system to combat bacterial infections with the goal of identifying new therapies and preventive measures.

**Recipient or parental organism**

The following Class II organisms are included in this notification: Streptococcus pneumoniae, Haemophilus influenzae, Acinetobacter baumannii, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis and S. mitis.

All organisms are hazard group 2 pathogens.

Streptococcus pneumoniae, Staphylococcus aureus, Streptococcus mitis, Enterococcus faecalis, Haemophilus influenzae, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa are all human pathogens.

Each organism can cause pulmonary infection or invasive disease, S. pneumoniae can also cause meningitis after colonisation of the respiratory tract. However with the exception of S. aureus and some H. influenzae, S. pneumoniae and E. coli strains these pathogens are predominantly opportunistic infections of sick patients in a hospital setting or with immune compromise.

S. pneumoniae can cause pneumonia or invasive diseases including meningitis. However, most infections are in people with medical illnesses or at the extremes of age. Preventive vaccines are available which prevent invasive disease.

Pneumococcal vaccination will be offered to all members of staff irrespective of the primary pathogen they work with.

Staphylococcus aureus can cause soft tissue infections. It colonises the skin of approximately one third of healthy individuals. It may also cause invasive disease typically spread by access to the blood, usually in hospitalised patients with cannulae or other instrumentation but sometimes in healthy patients in the community. Illness is characterised by signs of soft tissue infection or patients with invasive disease have fever and chills. There is currently no vaccine but effective antimicrobials are available against the strains we will use.

Haemophilus influenzae type b (Hib) vaccination will be considered for anyone working with on a sustained basis with Hib which can cause pneumonia and invasive disease. We however work primarily with non-typable H. influenzae which typically cause upper respiratory infections or bronchitis in patients with chronic lung disease and for which there is currently no vaccine. It is however an opportunistic infection and unlikely to cause disease in healthy patients.

All the other pathogens are primarily opportunistic infections, meaning they are unlikely to cause infection in healthy staff. *P. aeruginosa* can cause opportunistic infections of wounds, including keratitis. Lung disease would only be expected in people who are immunocompromised or with chronic lung disease. *S. mitis* is part of the normal oral flora and causes rare cases of aspiration pneumonia or invasive disease typically associated with cannulae or prosthetic materials or with poor dentition. *E. faecalis, K. pneumoniae* and *E. coli* are part of the normal bowel flora, these causes abdominal or urinary tract infections following surgical procedures or instrumentation. Pneumonia is described but very rare with enterococci and for the other organisms typically occurs in immunocompromised patients or those on a ventilator. *Acinetobacter baumanii* is an environmental micro-organism that can colonise sick patients typically in intensive care settings. It is an opportunistic infection and like most of the Gram negative bacteria listed is typically selected by use of broad spectrum antimicrobials. Infections include ventilator associated pneumonia. Infections would not be expected in healthy staff but if after investigation of febrile symptoms they were identified they would be treated with antimicrobials.

*S. aureus, S. pneumoniae* and *H. influenzae* can cause clinical symptoms of fever, respiratory tract illness or invasive
disease (signs of fever and chills). All workers will be informed about potential symptoms and to seek urgent medical
advice if they show any symptoms of ill health. All infections are effectively treated with prompt administration of
antimicrobials as outlined above. All workers will be advised to contact their GP and to ask for referral to their local
NHS admissions unit for assessment if indicated. Staff will be offered pneumococcal vaccination to prevent any risk of
S. pneumoniae meningitis and Hib vaccination if working with Hib. The other pathogens as primarily opportunist
infections are unlikely to cause any infections in healthy staff but any staff with chronic medical conditions will be asked
to have a health assessment with occupational health to assess if they should work with the pathogen. All staff will be
asked to report any health symptoms promptly to their GP or University health services promptly as above.

Host/vector system

The mutants have been created by external collaborators using techniques such as insertional mutagenesis or in
frame deletions and inactivated chromosomal genes may have been complemented by reinsertion of a copy of the
deleted gene, which may have been achieved by use of a plasmid maintained by an antibiotic resistance marker.
Inactivated chromosomal genes may or may not contain antibiotic resistance cassettes (e.g. Janus cassettes) but
where these are used they will involve antibiotics such as streptomycin, kanamycin, chloramphenicol, lincomycin,
macrolides or tetracyclines which are not the antimicrobials used as the primary treatment of these organisms, which
are primarily treated with penicillins, cephalosporins, carbapenems, or where beta-lactam resistant, glycopeptides,
lipopeptides or fluoroquinolones.
Confirmation of the genetic mutation and initial analysis of function will have been carried out in the collaborators
laboratories. Any potential further manipulation of parental or genetically modified strains will be under the direct
guidance of the primary laboratory who have created the strains.
Mutants will be of reduced virulence or will have no alteration of virulence compared to parental strains.

Origin & function

The mutants will have been created by external collaborators using techniques such as insertional mutagenesis or in
frame deletions and inactivated chromosomal genes may have been complemented by reinsertion of a copy of the
deleted gene, which may have been achieved by use of a plasmid maintained by an antibiotic resistance marker. Any
potential further manipulation of parental or genetically modified strains will be under the direct guidance of the
primary laboratory who have created the strains.
We include an appendix of several mutants we anticipate we would like to investigate as part of this study and will
update this appendix as further mutants are investigated. This outlines strains, the genes mutated and any antibiotics
resistance markers used for the mutant.
Transposon mutant libraries of strains such as S. aureus or S. pneumoniae may be accessed from collaborators or
genetic transformants of clinical S. pneumoniae on a standard laboratory strain background (TIGR4) generated.
In addition, the mutants will include deletion mutants, point mutations or complementation mutants of genes involved
in bacterial adherence, resistance to host defence strategies or cell death. And will potentially include genes in
involved in the production of capsules, cell wall constituents, surface expressed proteins, toxins, enzymes,
transporters, metabolic pathways, resistance to reactive species, proteases or peptides, iron acquisition or global
regulators (where deletion of the regulator is not anticipated to increase virulence).
In all cases, the mutants will be deemed to have no enhancement of virulence compared to the parental strain or to
the clinical strain in the case of mutant screens involving transformation. In the vast majority of cases, it is anticipated
that the GMMOs will essentially be attenuated versions of the parent strain. Whilst it is possible that insertional
inactivation of a particular gene within a locus may result in elevated virulence, if that gene (or downstream genes
within the locus) plays a role in repression of virulence factors, it is considered unlikely any such effect would raise the
containment level required above that for a hazard group 2 pathogen.
Insertional inactivation of putative virulence genes and antibiotic resistance determinants is likely to render the GMMO less virulent and/or less resistant to antimicrobials. In theory, transposon mutant screens could alter susceptibility to killing in a phagocyte but usually with associated fitness cost that will alter survival in the environment. Mutants will be of reduced virulence or will have no alteration of virulence compared to parental strains. As such the consequences of the deletions or of complementation are predictable and will produce strains that are less pathogenic or of comparable pathogenicity to the wild-type strains and will not have altered routes of transmission or host range. The most likely consequence of inadvertent exposure would be colonisation of the skin (for S. aureus), the respiratory tract (for S. pneumoniae), oral cavity (S. mitis) or gut (E. coli, K. pneumoniae or E. faecalis). However, these laboratory strains would be less likely to outcompete the individuals own strains. Other bacteria studied would be unlikely to colonise immunocompetent hosts only those with lung disease or immunocompromised. Respiratory tract colonisation for agents other than S. pneumoniae only occurs for those with underlying lung disease or in hospital and receiving broad spectrum antimicrobials and the same is largely true for H. influenzae, K. pneumoniae and S. aureus. Transient colonisation of S. pneumoniae occurs in 10-70% of the normal population, so if this were to occur it would replicate a situation that commonly occurs in normal life. If colonisation were to occur there is a small chance of invasive disease. We cannot predict the exact magnitude of this risk but since invasive disease occurs at a frequency of <1/100,000 in adults in the UK that means the risk of invasive disease is <1 in a thousand if it were to occur for S. pneumoniae. Prevalence data for S. aureus bacteraemia is also <1/1000 and rates of invasive disease with the other pathogens listed range from negligible (<1/1,000,000 to 1-10/100,000) all making these highly unlikely events.

Evaluation of foreseeable effects

Insertional inactivation of putative virulence genes and antibiotic resistance determinants is likely to render the GMMO less virulent and/or less resistant to antimicrobials. In theory, transposon mutant screens could alter susceptibility to killing in a phagocyte but usually with associated fitness cost that will alter survival in the environment. Mutants will be of reduced virulence or will have no alteration of virulence compared to parental strains. As such the consequences of the deletions or of complementation are predictable and will produce strains that are less pathogenic or of comparable pathogenicity to the wild-type strains and will not have altered routes of transmission or host range. The most likely consequence of inadvertent exposure would be colonisation of the skin (for S. aureus), the respiratory tract (for S. pneumoniae), oral cavity (S. mitis) or gut (E. coli, K. pneumoniae or E. faecalis). However, these laboratory strains would be less likely to outcompete the individuals own strains. Other bacteria studied would be unlikely to colonise immunocompetent hosts only those with lung disease or immunocompromised. Respiratory tract colonisation for agents other than S. pneumoniae only occurs for those with underlying lung disease or in hospital and receiving broad spectrum antimicrobials and the same is largely true for H. influenzae, K. pneumoniae and S. aureus. Transient colonisation of S. pneumoniae occurs in 10-70% of the normal population, so if this were to occur it would replicate a situation that commonly occurs in normal life. If colonisation were to occur there is a small chance of invasive disease. We cannot predict the exact magnitude of this risk but since invasive disease occurs at a frequency of <1/100,000 in adults in the UK that means the risk of invasive disease is <1 in a thousand if it were to occur for S. pneumoniae. Prevalence data for S. aureus bacteraemia is also <1/1000 and rates of invasive disease with the other pathogens listed range from negligible (<1/1,000,000 to 1-10/100,000) all making these highly unlikely events.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All bacterial cultures from plates are placed in the metal buckets for autoclaving and autoclaved on a decontamination cycle to render them safe and ready for discarding. Material will be inactivated by autoclaving (100% kill) at 121°C for 20 minutes prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile. Contaminated pipettes, pipette tips, spreaders, bacteria infected liquid etc. must be soaked for at least 12 hours in 1% virkon solution to decontaminate, prior to safe disposal. Any bacterial spills will be decontaminated with a 1% virkon solution for two hours before placing in yellow bins for incineration. Animal bedding, waste from animals and carcasses that have been infected with GMMO organisms will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2  Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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<td>Animal Units</td>
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<tr>
<td>Large Scale Activities</td>
<td>L2 L3 L4 L2 L3 L4</td>
<td></td>
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<td>Human Clinical Applications</td>
<td>L2 L3 L4</td>
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### Project Ref 207/19.10

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<td>17/10/2019</td>
<td>Examining the molecular biology of rotaviruses by targeted modification of the virus genome</td>
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#### Project notified under transitional arrangements

N

#### Historical Significant Changes

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<tbody>
<tr>
<td>207/19.10a</td>
<td>14/05/2020</td>
</tr>
</tbody>
</table>
Purposes of the contained use

We will examine how rotaviruses interact with the host cells they infect by comparing wildtype virus replication kinetics with the replication kinetics of viruses which have been modified in specific regions of the virus genome. Modification of the virus genome can be done either to alter the nucleotide sequence of the virus (i.e. synonymously) or the protein sequence (i.e. non synonymously). By identifying regions of the virus genome key for interaction between virus and host, we can identify new therapeutic candidates and vaccine targets.

Recipient or parental organism

Mammalian culture cells or organoid systems will be infected with rotavirus.

Host/vector system

Rotaviruses infect a range of organisms including man, monkeys, bovine species and chickens, and a range of strains specific to different hosts will be used.

Origin & function

The rotavirus genome comprises 11 segments of double stranded RNA. To artificially synthesise a rotavirus which we have the ability to alter the genetic sequence of, we encode the virus genome on 11 discrete plasmids, each corresponding to one segment of the virus genome, with each segment under the control of the T7 RNA polymerase promoter. All 11 plasmids are then co-transfected into BSR-T7 cells which have been engineered to express the T7 RNA polymerase, and so viral RNAs and proteins are produced. This ultimately yields the production of de novo virus particles.

Artificial synthesis of rotaviruses using this approach enables us to modify the sequence of the virus genome. To modify the sequence of the virus, the viral genome sequence encoded on the plasmid is modified. These modifications will be used to make modified viruses which we can characterise in comparison with unmodified virus.

Evaluation of foreseeable effects

Our lab is interested in investigating virus-host interactions, including how the virus interacts with the innate immune response. We are therefore interested in modifying regions of the virus genome that we hypothesise are important for downregulation/ control of the innate immune response. The impact of this would be that the virus would replicate less well.

We are also interested in identifying accessory proteins produced by the virus, and confirmation of their production by mutagenesis to remove them. Viruses encode accessory proteins to assist their replication, so modifications to this effect will again cause a fitness loss to the virus.

We are also interested in how dinucleotide composition impacts virus replication, and to investigate this we will modify the frequency of dinucleotides of interest (usually CpG) by synonymous mutation. Addition of CpGs has been shown to be detrimental to virus fitness for a range of virus (Gaunt et. al., eLife, 2016; Atkinson et. al., NAR, 2014 among others). Removing CpGs from a virus genome may cause a modest increase in fitness for echovirus 7 (Atkinson et. al., NAR 2014) but we were not able to reproduce this for influenza A virus (unpublished data). Extensive recoding of a virus genome is unlikely to cause an improved replication phenotype, due to the likelihood of inadvertent loss of packaging signals, accessory protein open reading frames etc.; therefore, there may be a small increase in virus fitness but this is not considered likely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

RV-containing waste liquids will be aspirated into a container containing Distel® though a closed aspiration system. After 24h of inactivation the container may be changed and the liquid discarded down the sink. Surface disinfection will be with 2% Distel and 70% EtOH. Plastic waste will be soaked in 2% Distel overnight prior to being discarded through the autoclave waste stream. Distel is effective against bacteria, viruses, fungi, mycobacteria and denatures RNA and DNA (https://www.scientificlabs.co.uk/product/TR1366#tab-1). Distel is recommended for use at 1:100 dilution for normal laboratory applications.
(https://www.tristel.com/tristel-products/distel-for-labs) and so 2% is conservative. Distel has demonstrated efficacy against rotaviruses at a 1:100 dilution (https://mixmed.myshopify.com/pages/distel).

All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored using chemical indicators (eg Browne TST indicator test strips).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

### Project Containment

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**Animal Units**

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**Large Scale Activities**

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**Human Clinical Applications**

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### Project Ref 207/19.11

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<tr>
<td>20/12/2019</td>
<td>Gene regulation and innate immune evasion by pathogenic streptococci</td>
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<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Withdrawn Y

Tick if notifying a connected programme of work N

02/03/2022
### Project Additional Information

#### Purposes of the contained use

Define function of bacterial genes and proteins involved in pathogenesis.

#### Recipient or parental organism

- **Streptococcus pyogenes** (group A Streptococcus)
- **Streptococcus agalactiae** (group B Streptococcus)
- **Streptococcus dysgalactiae** (inc. subsp. equisimilis)
- **Streptococcus spp.**
- **Lactococcus lactis**

#### Host/vector system

Plasmids facilitating:
1. Streptococcal protein expression in streptococcal species and Lactococcus lactis
2. Streptococcal gene knockout or replacement (including mutant libraries)
3. Recombinant protein production
4. Protein interaction studies

#### Origin & function

Genetic material involved will originate from streptococcal or other bacterial species.

Functions include: antibiotic resistance markers, reporter genes, gene fusions, gene complementation, modifications to promoter sequences or amino acid residues, expression of streptococcal genes in heterologous species, deletion or functional inactivation of streptococcal genes, expression of naturally occurring alleles.

#### Evaluation of foreseeable effects

Some of the species used are human pathogens (S. pyogenes, S. agalactiae and S. dysgalactiae subsp. equisimilis), whereas Streptococcal spp. are largely pathogens of animals, which can cause humans disease.

The GMOs should be no more hazardous than the parent strains from which they were derived, indeed in the vast majority of cases strains should be less virulent. Any excess risk is related to (a) the introduction of additional antimicrobial resistance genes that may impede the efficacy of antimicrobials if required, and (b) the inadvertent but
rare genetic modification that results in unforeseen increase in virulence, for example in a regulatory gene. Hazards from antimicrobial resistance are minimised by use of antimicrobial markers that are not in clinical use.

While the production of strains more virulent than wild type is unlikely to occur, it is a possibility. Mutations in regulatory genes can occur spontaneously in nature, and are selected for during invasive infection; as such the risk of such mutations arising in nature is possibly greater than the risk that they occur in the laboratory. However, if a more virulent strain is produced, work with this modified agent will stop until the hazards and risks have been considered. While very unlikely that any additional control measures will be necessary, if required, these will be implemented, the assessment be updated and the findings be discussed with the local GM biological safety officer.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not relevant

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be autoclaved via the biohazard waste route followed by alternative treatment or incineration by our waste contractor. Liquids which have been autoclaved will be discharged to sink.

Disinfection
Please give details of disinfectant(s), method and validation including concentration of disinfectant and contact time (eg supplier's instructions or local validation).
Tristel at a 1:100 dilution with contact time of 30 secs – 5 minutes (manufacturer's guidelines for working with Streptococcal species)
https://www.tristel.com/tristel-products/all-tristel-products

Autoclaving
Please give details of autoclave method and validation.
All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored using chemical indicators (eg Browne TST indicator test strips).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment
This project will test the feasibility of utilizing the eukaryotic protist Toxoplasma gondii as a vector for therapeutic protein delivery to the brain. The idea relies on harnessing T. gondii’s features as a brain parasite to enable protein replacement therapy. Using a novel method for driving constitutive secretion of heterologous proteins by T. gondii, we will (1) generate transgenic T. gondii lines that express and secrete the mammalian MeCP2 protein and (2) test this GMMO as a protein delivery system in rodent models of the neurological disorder Rett Syndrome (RTT).
**Host/vector system**

The DNA vector inserted into *T. gondii* is a *T. gondii* expression plasmid (non-viral), introduced by electroporation and integrated into the *T. gondii* genome following drug selection. The plasmid contains a selectable cassette including the gene HXGPRT (complementing a mutation in the parental line). This vector does not pose any hazards. The lab strains used in this study, corresponding to the "type II" lineage of *T. gondii*, have particularly low virulence compared to other natural strains of *T. gondii*.

**Origin & function**

Lab generated plasmid DNA encodes for fusion gene comprised of the endogenous *T. gondii* gene GRA16, fused to murine MECP2. The expression of the insert is controlled by the endogenous *T. gondii* GRA16 promoter and GRA2 3'UTR. The murine MECP2 sequence is codon optimized according to *T. gondii* endogenous codon usage to facilitate optimal protein folding and expression. Infected mice will receive *T. gondii*-derived MeCP2 protein in amounts that are not considered to be deleterious.

**Evaluation of foreseeable effects**

(1) None of the genetic modifications conducted are related to any of the existing pathogenic traits of *T. gondii*. Furthermore, in vitro, the altered *T. gondii* did not exhibit elevated virulence, resistance or growth rate, compared to the unaltered line. (2) On introduced into mice, the risks associated with the *T. gondii* infection are considered low as detailed in the accompanying RA. There is no expected novel or cumulative hazard in *T. gondii* infection of Mec2-null mice compared to wild-type animals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

(1) Before injection, *T. gondii* will be grown in incubators in a CL-2 *T. gondii* lab. (2) All infection procedures will be conducted in the infection section of the Roslin rodent unit which is a secure unit.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

NA

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

(1+2) Plastic waste is neutralized using Distel or similar quaternary ammonium disinfectant (5% w/v solution) for 24 hours, then autoclaved. (2) Sharps will disposed of immediately into a sharps bin and thenautoclaved according to standard practice for sharps. Other items will be autoclaved and then disposed of along with other waste from the Bioresearch & Veterinary Services (BVS). Animal carcasses will be disposed of by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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</tr>
</thead>
</table>

02/03/2022
Project Additional Information

Purposes of the contained use

Human embryonic stem cells (H9) will be differentiated to hepatocyte-like cells, and will either knockdown or overexpress the ten-eleven translocase 1 (TET1) and 2 (TET2) enzymes, using a replication-deficient lentiviral vector. The purpose of this experiment is to interrogate the roles that TET1 and TET2 play in human fatty liver disease, by modelling fatty liver in vitro. The lentivirus will either carry a sequence to overexpress the gene of interest, or shRNA sequences to silence it. Cells will be differentiated until day 15, and then infected for 24 hours. After a further 24 hours, cells will be treated with a cocktail of lactate, pyruvate, and octanoate, to induce a fatty liver-like phenotype in vitro.

Recipient or parental organism

Human embryonic stem cell line (H9) and human embryonic kidney cell line (HEK293)

Host/vector system

The inserts coding either the human TET1 or TET2 catalytic domain, full length human TET1 or TET2 will be cloned into a pSFFV backbone (a gift to the lab). This will then be transfected into HEK293 cells alongside two packaging plasmids: pMDG and pCMVint. This will generate viruses that express genes of interest under a CMV promoter.
For the shRNA sequences, pLKO.1 plasmids carrying shRNA sequences targeting TET1 or TET2 will be purchased from Sigma-Aldrich (Catalogue # SHCLNG-NM_030625). These will be transfected into HEK293 cells alongside two packaging plasmids: psPAX2 and pCMV-VSV-G. This will generate viruses that express genes of interest under a CMV promoter.

Origin & function

We will be using the lentiviral vector to either overexpress the catalytic domain or full length sequence of human TET1 and TET2. Both genes are involved in regulation of gene expression through alteration of DNA methylation patterns. Where TET1 or TET2 are absent, a third isoform, TET3, can provide some compensation for loss of function. We will additionally use lentiviral vectors carrying shRNA sequences to knockdown TET1 and TET2 in vitro.

Evaluation of foreseeable effects

The likelihood of harm to human health is low. The role of Tet1 and Tet2 is to oxidise methylated cytosine, to 5-hydroxymethylcytosine, which is thought to be a stable epigenetic mark. Whilst TET1 and TET2 have been identified as potential oncogenes, and associated with the development of glioblastoma, it is not believed that their impact on genome methylation alone is sufficient to drive changes in gene expression.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable lab coats must be worn when working with high titre virus stocks and during the first 7 to 10 days after transduction. They must be autoclaved before final disposal by incineration via the yellow bag route.

All solid and liquid waste must be decontaminated/disposed of by the user at the end of every TC session.
Surfaces will be sprayed with Virkon 10% solution after use. Surfaces within the class II cabinet should not be sprayed with Virkon 10% solution as it corrodes the metal, use 1% Distal Trigene instead.

Solid waste disposal:
Stripettes
Autoclave stripettes before disposal. Double bag in clear plastic autoclave bags in a metal bucket for autoclaving then place in a yellow bag for disposal by incineration.

Plastic ware such as flasks, tubes and agar plates:
Soak in 1%w/v Virkon, or 1,000 ppm Precept (four 0.5g tablets into one litre of water) for 24 hours before discharging any excess liquid via drains. Following chemical decontamination, place all plastic ware and agar plates into the yellow bags within the tin autoclave drums, tie bag and place in the white plastic bin - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes).
After autoclaving, yellow bags are disposed of by incineration.

Liquid waste disposal
samples, culture supernatants, tissue culture media
Any liquid potentially containing virus should be collected in an autoclavable screw top plastic bottle (stored under the sink). It should NEVER come into contact with the vacuum system. The liquid waste should be chemically disinfected by soaking in 1% w/v Virkon, or 1,000 ppm Precept (four 0.5g tablets into 1 litre of liquid) for 24 hours before discharging via the drains. The container must be labelled with name, group and date.
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref 207/19.5**

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<td>11/07/2019</td>
<td>The expression of cellular proteins associated with neurodegeneration, in particular alpha-synuclein, in prokaryotic hosts to provide substrates for subsequent protein conversion assays</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Non-GMM Consent Granted</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
The purpose of the contained use is to produce recombinant human alpha-synuclein protein to act as a substrate for a newly developed protein aggregation assay called Real-Time Quaking Induced Conversion assay (RT-QuIC). RT-QuIC has been demonstrated to be a very sensitive and specific diagnostic test for Parkinson's Disease and Lewy body dementia.

For recombinant α-syn protein expression and purification, the host will be the E. coli BL21 Rosetta strain. Plasmid maintenance stocks will utilise the E. coli DH5 alpha strain. These are functionally disabled strains that cannot survive in the environment and do not colonise the human gut.

The vectors used will be pET and pUC type vectors such pET24a or pRSET. These vectors are non-transmissible and do not pose a risk to human health or to the environment.

The DNA for making WT human α-syn was cloned by RT-PCR from a cDNA library by Dr Gillian Cooper at the National Institute for Biological Standards and Control (NIBSC). Future genetic inserts will be cloned by RT-PCR from a commercial cDNA library by e.g. EuroFins or GeneArt. The intended use is to express and maintain recombinant protein expression in E.coli.

E. coli BL21 Rosetta strain and the E. coli DH5 alpha strain are functionally disabled strains that cannot survive in the environment and do not colonise the human gut, and as such pose no risk to human health or the environment.

The human a-synuclein recombinant protein is a monomeric protein which has been shown to be non-hazardous using both cellular toxicity assays involving SHSY-5Y cells and using inoculation studies involving mice.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All contaminated materials will be inactivated by autoclaving (100% kill) at 134oC for 30 minutes prior to disposal of waste by incineration. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The genetic modification safety committee has reviewed and approved the risk assessment and agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

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**Project Ref** 207/19.6

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<td>Pseudotype lentivirus system for expressing avian viral glycoproteins to study viral:host interactions</td>
<td>Class 2</td>
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Non-GMM

- Project notified under transitional arrangements

**Project Additional Information**
Purposes of the contained use

This project is involved in the development and establishment of a pseudotype lentivirus expression system to permit investigations of the role of viral surface glycoproteins in mediating interactions with avian hosts. It will generate pseudotype particles, which are chimeric “viruses” consisting of a surrogate virus core surrounded by a lipid envelope with the surface glycoproteins of another virus displayed on the outside of the viral particle.

By removing the genetic element of the virus being studied and replacing it with a suitable reporter, viruses, especially high-pathogenic, can be studied in this safer, single cycle system. The comparative safety of pseudotype viruses circumvents the need for restrictive, expensive, and widely unavailable high-category biosafety facilities, increasing access to research groups interested in highly pathogenic viruses. This pseudotype virus expression system will be utilised for studying avian viruses including avian influenza virus (AIV), avian infectious bronchitis (IBV) and newcastle disease (NDV), to aid vaccine development projects against these avian viruses.

Briefly, pseudotype viruses are generated via co-transfection of “producer” cells with plasmids expressing: (1) the envelope protein of a virus of interest, (2) proteins of a “core” virus, often a retrovirus, and (3) a reporter transgene. The envelope protein will enable the virus to target cells permissive to the virus of interest. During cell transduction, the reporter transgene becomes integrated into the genome of the cell (via lentivirus vector components) and is expressed, permitting quantification.

Recipient or parental organism

Laboratory adapted E. coli such as E. coli HST08 (Stellar competent cells, Takara) and JM109 (Promega) will be used for cloning and plasmid amplification. These recipient non-pathogenic E. coli used for cloning and production of the expression vectors will be laboratory adapted E. coli K-12 derivative strains so survival outwith the defined culture conditions is unlikely and they cannot survive in the human digestive tract or produce toxins. The expression vectors transformed into these E. coli strains are not designed to express in E. coli.

Packaging cells used to produce non-replicating viral particles or recipient cells to be infected with the pseudotype viruses will be established cell lines; human embryonic kidney 293T/17 (HEK293T/17), Madin-darby Canine Kidney (MDCK) and Vero cells and do not pose a significant threat to human health. Titration and serum neutralisation assays using pseudotype viruses will be conducted using cell lines, including HEK 293T/17, MDCKs and Vero cells. [Hazard group 1 for cells, Hazard group 2 for lentiviral particles].

The tropism of the pseudotype particles generated will be altered from that of the parent vector, and will be dependent on the specific glycoprotein expressed by the helper plasmid. The pseudotype particles are replication deficient and will not be able to enter the target cell type without expression of the glycoprotein under investigation or without some transfection mechanism. Using glycoproteins from avian influenza, IBV and NDV will alter, and likely reduce, the number of cell types that the lentiviral particles can enter.

Host/vector system

Host organisms:
Plasmids will be propagated in E. coli JM109, K-12 (Promega) or HST08 (Stellar competent cells, Takara). [Hazard group 2]
Lentiviral particles will be produced by co-transfection of 3 or 4 plasmids into human embryonic kidney (HEK) 293T/17 cells. [Hazard group 1 for cells, Hazard group 2 for lentiviral particles]
Titration and serum neutralisation assays using pseudotype viruses will be conducted using cell lines, including HEK 293T/17, MDCKs and Vero cells. [Hazard group 1 for cells, Hazard group 2 for lentiviral particles]

Vector systems (plasmids):
pl.18 or pcAGGIIs (expression vectors used for gene of interest) - the cDNA construct for the avain glycoprotein under investigation will be inserted upstream of a human cytomeglovirus (CMV) promoter.
pCSFLW (firefly luciferase or GFP lentiviral vector) - reporter construct which is a modified HIV-1 clone containing the packaging elements and a firefly luciferase reporter or a green fluorescent protein reporter. This is a self-inactivating vector, with a safety component incorporated through a deletion in the 3’ long-terminal repeat (LTR), this is
Transferred into the 5' LTR after one round of reverse transcription and abolishes transcription of the full-length virus after it has incorporated into a host cell. This renders the resulting lentiviral particles replication incompetent and will significantly reduce expression from the viral promoter after integration of the reporter into the genome.

pCAGGs-TMPRSS2 or pCAGGs-HAT (protease plasmids)

p8.91 (gag/pol lentiviral vector) - a modified HIV-1 clone, which lacks the retroviral Psi packaging element sequence in addition to the removal of virulence and accessory genes (env, vif, nef, vpu, and vpr genes), which are not required for viral packaging. It has a CMV promoter in lieu of long-terminal repeat (LTR)-based promotion, meaning that p8.91 will provide the necessary genes for the production of the core only.

**Origin & function**

Avian virus glycoprotein sequences will be cDNAs, selected originally from the sequence data of the avian virus under investigation. These cDNAs will be purchased as GeneART DNA fragments, they will be codon optimised and additionally synthesised with the appropriate restriction enzyme sites and kozac sequences added to ensure efficient ligation into expression plasmids and subsequent translation.

Pseudotype virus particles will be produced in HEK 293T/17 cells, a human cell line initially transformed with the adenovirus early region, also expressing the SV40 T-antigen. Virus particles are produced by the co-transfection of a 2nd generation (disabled) lentivirus vector and helper plasmids. The helper plasmids will contain avian viral glycoproteins (p1.18 or pCAGGs), proteases (pCAGGs) and firefly luciferase (pCSFLW). Using glycoproteins from avian viruses will alter, and likely reduce, the number of cell types that the lentiviruses can enter.

The lentiviral vectors are introduced to the HEK293T packaging cells by transfection to produce pseudotype viruses in the cell culture supernatant, these recipient cells will be transduced with the replication incompetent lentivirus particles for 24 hours at 37°C with the chemical transfection agent, Polyethylenimine (PEI). Lentiviral particles are generated in the packaging cells and are released into the growth media. Specifically in the experimental work proposed for AIV, endogenous recombinant neuraminidase (derived from Clostridium perfringens) will be added to the transfection culture after 24 hours to enable budding of viral particles from the cell surface. The growth media and supernatant containing viral particles will be collected after 72 hours of incubation at 37°C, filtered, aliquoted into 1ml aliquots in screw-top Eppendorf tubes and stored in a designated freezer box at -80°C in a locked room.

The pseudotype particle produced will be titrated in HEK293T cells to determine the relative luciferase units (i.e. viral titre) and then subsequently screened in serological assays (serum neutralisation and haemagglutinin inhibition assays) to assess cross-reactivity against avian serum, specific for the virus and the surface glycoprotein under investigation. Additional assays will be performed to assess the role of the viral surface glycoproteins in facilitating interactions with host cells receptors and permitting entry into avian host cells.

**Evaluation of foreseeable effects**

This pseudotype expression system to be used here is a three plasmid based system, with the components necessary for virus production split across multiple plasmids: (1) transfer vector containing transgene e.g. GFP or Firefly luciferase, (2) packaging vector and (3) envelope vector, which decreases the risk of any recombination events. Several safety features have been included in this system to minimise the potential of replication competent lentivirus (RCL) production, or subsequent mobilisation of the integrated transgene form the genome of the host cell. This is a 2nd generation lentiviral replication system displaying low risk of recombination with endogenous retroviruses due to the division of synthetic viral elements (i.e. coding sequences have been significantly altered from the original) and the vectors are self-inactivating.

Lentiviral vectors are disabled and are only able to replicate in helper cell lines. They are a well established reagent and are not considered to pose any significant health risk. The pseudotype virus to be used here will be derived from a HIV-1 clone. It will be a second generation, replication incompetent system. The majority of viral genes required for viral replication have been removed. Others (Gag, Pol and Rev) will be supplied in trans. A deletion in the 3' long-terminal repeat (LTR) allows transcription following transfection of HEK 293T cells but following reverse transcription results in a non-functional 5'LTR in transduced cells and as a result are self-inactivating vectors.

The lentiviruses will be capable of infecting human cells with the consequent insertion of the vector genome into chromosomal DNA but that will be the end of the life-cycle as the virus is replication deficient, self-inactivating, 2nd generation vector which is not carrying genetic material harmful to humans (no human oncogenes or tumour
suppressor genes). The main hazard is the possibility of oncogene activation at the site of integration and the self-inactivating lentiviruses are considered less likely to activate cellular genes near insertion sites than conventional gamma-retroviral vectors. A further potential hazard is the generation of replication-competent virus but the use of self-inactivating vectors makes this highly unlikely.

Accidental inoculation via application to cuts or via needle stick could result in the transduction of human tissue. Due to this risk, sharps will not be used during production, concentration or assays involving lentiviral pseudotype viruses. To avoid unnecessary exposure. All broken skin (cuts, dermatitis, weeping wounds etc.) will be covered with a waterproof dressing and appropriate PPE of lab coat and gloves will be worn at all times. Operator gloves will cover the cuffs of the lab coat whilst working with lentiviral particles.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g. plastic ware, pipette tips, flasks, tubes etc), which have been in contact with lentivirus plasmids or pseudotype particles will be disposed of into a designated container inside the biosafety cabinet. This container will be sealed prior to removal from the cabinet and subsequently autoclaved according to the procedure as stated below.

Lentivirus containing waste liquids will be aspirated into a container containing 10% Distel, a broad spectrum virucidal disinfectant, in a biosafety cabinet through a closed aspiration system to avoid aerosol generation. Waste media will be treated with Distel at a 1:10 dilution for 24 hours at a designated place to inactivate any remaining lentiviral particles and autoclaved prior to disposal. All waste bottles will be clearly labelled with date and content and autoclaved afterwards according to the procedure as stated below. Surface disinfection of the biosafety cabinet will be conducted with 1-10% Distel.

Autoclaving:
All contaminated materials will be inactivated by autoclaving (100% kill) at 134°C with a hold time of 10 minutes prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves are validated at least by annually by thermocouple mapping and each autoclave run will be monitored using chemical indicators (eg Browne TST indicator test strips).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment
Project Additional Information

Purposes of the contained use

Our model of infection employs adult pregnant mice to study ascending vaginal infection caused by Escherichia coli K1. We aim to use this model of infection combined with a genetically modified bioluminescent strain of E. coli O18:K1 (A192PP) to track the course the infection using an IVIS bioluminescence imager. Strain A192 (DSM. No. 10719) (Achtman, et al., 1983) was used to generate this strain. A bioluminescent derivative of E. coli A192PP has been generated by introduction of the luxCDABE operon from the nematode symbiont Photorhabdus luminescens, as performed by collaborators. The luxCDABE operon is supplied through mini-Tn5 mutagenesis using the construct pUTm.

Recipient or parental organism


ACDP hazard group: 2

Host/vector system
pUTmini-Tn5

- Plasmid can only be maintained in lambda pir+ donor strains (e.g. E. coli S17-1 lambda pir).
- Plasmid can be transferred by conjugation to other bacterial strains (pir-) and acts as a suicide vector.
- Transposon is disarmed, so forms stable integrations within recipient chromosome and cannot re-mobilise. (Winson, et al., 1998) The mini-Tn5 is also transcriptionally isolated: a in frame stop codon (TAG) was introduced 18 codons upstream of the luxC ATG.

Origin & function

The inserted transposon is the luxCDABE: lux operon from the nematode symbiont Photobdus luminescens ATCC29999 (Hb strain) (Winson, et al., 1998). With kanamycin resistance cassette (Km2). The operon provides the mechanics for bioluminescence emission from the recipient strain. Recipient strains are resistant to kanamycin (kanR) by acquisition of mini-Tn5 luxCDABE Km2.

Evaluation of foreseeable effects

The inserted transposon is the luxCDABE: lux operon from the nematode symbiont Photobdus luminescens ATCC29999 (Hb strain) (Winson, et al., 1998). With kanamycin resistance cassette (Km2). The operon provides the mechanics for bioluminescence emission from the recipient strain. Recipient strains are resistant to kanamycin (kanR) by acquisition of mini-Tn5 luxCDABE Km2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- Animal housing at BVS LF2 Animal Facility has individually ventilated cages (IVCs).
- Appropriate personal protective equipment (PPE) will be worn at all times for work with animals; including P2 face masks, disposable gloves, overalls, shoe covers and mob caps.
- Infection of pregnant mice will be done in downdraft hoods in SU548 to enable mice to be anaesthetised during bacterial instillation. However, only the trained operator will be in room SU548 when any work with this bacteria is undertaken.
- Infected mice will be housed in individual IVC cages and will not be touched by BVS staff after infection with this bacteria.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

70% ethanol will be used to disinfect all surfaces used in microbiology culture lab (W1.34) and animal facility (LF2). Ethanol has been show to kill E.coli following 10 seconds exposure at all concentrations between 40-100% (Tilley et al, J Bacteriol, 1926).

1% Virkon will be used for cleaning all instruments and spraying – in of materials transported into LF2.

1% Trigene will be used on bench surfaces prior to 70% ethanol as above.

W1.34 Waste:
Disposal route following standard QMRI waste disposal guidelines:
Solids (eg plasticware such as pipettes, flasks, tubes etc) – Soak in Virkon for 12-24h. Discharge any excess liquid to drains, dispose of solids via the yellow bag with yellow tag heat treatment waste stream.
Liquids (e.g., samples, culture supernatants, tissue culture media) – disinfect with Virkon for 1-24 hrs, discharge to drains.
Spills – soak in Virkon or Precept solution for at least 10 min, then soak up with absorbent towel. Discard material for incineration.

Animal Facility
- Autoclave waste will be collected by LF2 staff and will be sterilised by autoclaving for a cycle of 131°C for 30 mins.
- Autoclaved material will then be disposed of by identified trained LF2 staff.
- Mice carcasses will be incinerated and disposed of by LF2 support staff.

Sharps will be treated before use using 70% (v/v) ethanol and disposed of after use in supplied sharps-bins for incineration. Surgical scissors and other surgical instruments (e.g., tweezers) will be autoclaved after use (a designated set of surgical instruments will be used for these experiments). All staff receive training in use of sharps as part of their HO PLH training.
- Contaminated sharps will be placed in sharps bins and will be incinerated. Surgical scissors and other surgical instruments will be cleaned with 70% IMS then soaked in Virkon for at least 10 mins, then wiped with 70% IMS before autoclaving.

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Is an emergency plan required according to regulation 20? [ ]
If yes, tick to confirm that it is attached to this form [ ]
Tick to confirm that you have attached a risk assessment to this form [ ]
Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

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Project Containment

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Project Ref 207/19.8

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Page 5011 of 15326

02/03/2022
The purpose of this project is to interrogate normal and defective fusion mechanisms using in ovo transient transgenics and gene-edited models of candidate fusion genes in the avian eye and additional avian embryonic contexts.

The avian replication-competent retroviral vector system (RCAS: Replication-Competent ASLV long terminal repeat (LTR) with a Splice acceptor virus) will be used to deliver genes of interest to cultured cells to generate loss of function (gene deletions) and gain of function (gene over-expression) genetic tools. These tools will then be used in further cell culture and chicken embryos to test specific experimental hypotheses for understanding mechanisms of epithelial fusion.

**Recipient or parental organism**

E.Coli K12 DH5a for plasmid amplification
Genotype: F- Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17(rk-, mk+) phoA supE44 thi-1 gyrA96 relA1 λ-
E. coli K-12 derivatives including, DH5a are recognised as non-colonising and disabled. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. The RCAS constructs have pBR322 origin of replication for low copy number amplification in competent bacteria, and an ampicillin resistance gene for selection. They are non-mobilisable and the cloned genes are under control of eukaryotic promotors with no known activity in bacterial hosts. Replication of the construct will not confer fitness to the host nor result in any change in pathogenicity or host range. Therefore, the likelihood of expression is effectively zero and the risk to humans and/or the environment is negligible.

UMNSAH/DF-1 for producing viral titres
UMNSAH/DF-1 is an established spontaneously transformed and characterised embryonic chicken fibroblast cell line. The avian source of this immortalised cell line poses low risk to humans due to the genetic relationship (widely divergent) of the cell line to humans. Transfected RCAS plasmid constructs containing genes of interest (which may be of chicken or human origin) will drive production of virus within the host cell line for harvesting and injection into embryonic chicken tissues. The envelope gene from Avian Sarcoma and Leukemia Viruses (ASLV; the family of which RSV belongs) maintains avian-specific host range, therefore RCAS vectors will not replicate in mammalian cells and risk to humans is effectively zero.

UMNSAH/DF-1 are expected to have limited survivability in the environment and have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. We do not anticipate increased survivability in cells expressing the virus. Therefore the risk to the environment is low.

Chicken Embryonic tissues for study
Wild-type Hy-Line fertile chicken eggs and eggs from stable transgenic lines (e.g. memGFP) are obtained from The Greenwood Building (Roslin Institute). Specific
pathogen free (SPF) status eggs are obtained from The Bumstead Building (Roslin Institute). All eggs are incubated at 37°C until the required stage of development reached. No infected embryos will be incubated to develop past embryonic day 10. RCAS Virus will be transduced to embryos and will drive expression of the cloned genes in infected cells. Infected embryos will usually not be allowed to develop beyond 10 days of incubation and will never be allowed to hatch. Chicken embryos can only survive within the egg along with constant incubation at 37°C. It is unlikely that these conditions would be met outside the laboratory. We do not anticipate increased survivability in the embryo when expressing the virus or the genes contained within the vector, therefore the risk to the environment is low.

Host/vector system

The RCAS (Replication-Competent ASLV long terminal repeat (LTR) with a Splice acceptor) vectors are simple, replication-competent avian retroviruses derived from the avian Rous Sarcoma Virus (RSV), (previously described and is commercially available: Hughes et al., J Virol 1987 Oct; 61(10):3004-3012; https://www.addgene.org/search/advanced/?q=RCAS). This virus has been characterised and used extensively (see Morgan and Fekete, 1996. Methods in Cell Biol. 51, 185).

Like RSV, the RCAS viral DNA genome comprises genes, gag, pol and env which code assembly of new viral particles. In RCAS, the src gene, a proto-oncogene, has been deleted and replaced with a ClaI restriction enzyme site for convenient insertion of genes of experimental interest. The splice acceptor of src is retained in the virus to ensure processing of the inserted gene’s mature messenger RNA prior to translation and protein expression. Long Terminal Repeats (LTR) allow insertion of viral genetic material into the host genome, and their promoters further enhance expression.

This retrovirus is replication competent but only infects avian species, and not mammals, by direct injection or transfection. Very high viral titres are required to infect significant amounts of tissue in chick embryos and embryos from pathogen free chickens are most susceptible. The src gene that makes this virus oncogenic in chickens has been removed and replaced with a restriction site so that a transgene can be easily inserted here. Any potentially harmful effects will depend on the gene or antisense construct inserted into RCAS.

As with wild-type viruses, RCAS vectors carry out all aspects of the viral life cycle as well as encode an additional transgene. Once within the host cytoplasm, RCAS can cross the nuclear membrane and integrate in the host-genome where it then acts as a cellular gene under dogmatic control of the host. New viral particles bud from the host membrane and go on to infect neighbouring cells. Thus, once the virus is transduced to host tissues, the transgene can be spread from cell to cell within the embryo. This delivery system was engineered to reduce the number of rounds of viral replication required to produce the viral titre necessary for use, and therefore gives a stable vector with reduced opportunity for rearrangement.

The RCAS virus only replicates in appropriately chosen susceptible avian cells (usually chicken or quail, although others can be used). This work will utilise A or B envelope subgroup RCAS (A, B) and maintains avian-specific host range, therefore this RCAS vector will not infect or replicate in mammalian cells. Normally, mammalian cells lack functional receptors for any of the envelope proteins of the RSV virus and therefore the virus cannot enter mammalian cells. To overcome this barrier, mammalian cells would have to present functional RSV envelope protein receptors on the cell surface. In some cases, particular RCAS vectors will infect mammalian cells, but will not replicate. This capability involves mis-expression of cloned functional receptors in mammalian cells and has only ever been documented in the laboratory (Bates, Young and Varmus, 1993).

Further we will never attempt to express RSV envelope protein receptors in mammalian cells, nor will we generate RCAS vectors that use the envelope proteins found in mammalian retroviruses.

Origin & function

The RCAS system will artificially express novel genes of interest (that may be chicken sequences or gene sequences from other vertebrates including mouse, human or fish) in cell cultures and in vivo embryonic tissues to understand gene function during developmental tissue fusion and to identify functionally-essential protein-protein interactions. We will tag candidates with well-described epitopes (e.g. FLAG, V5, or Myc) or with fluorescent proteins (e.g. green fluorescent protein, Citrine). These tags are required to visualise and specifically isolate the gene products from infected cells during experimental analyses. We will compare the following controls to the wild-type: a truncated variant that contains only the signal peptide fused to GFP, which can be secreted but has no function; GFP-only; and empty vectors. This is to test the specific requirement for extracellular interactions between these proteins and their cognate receptors (both novel and known).
The genes that will be investigated will include genes encoding growth factors, transcription factors, phosphatases, enzymes, receptors, and other genes of so far unknown function.

**Evaluation of foreseeable effects**

**Risks to humans**
Effectively zero. The viral delivery system cannot infect humans and therefore the likely hood of harm is effectively zero. It is believed that RCAS does not cause human disease and it has a history of safe use.

**Risks to the environment**
Strains of chickens differ in their susceptibility to viruses with different envelope subgroups. Most chicken strains purchased from commercial vendors are typically resistant to viruses with E envelope subgroup, while the viruses we will be using most often have envelope subtypes A and B (Morgan & Fekete, 1996, Methods in Cell Biology 51, 185-214).

Direct injection of high experimental viral titres are required to infect embryos and generate efficient expression of inserted genes. In the case of accidental release of virus from level 2 containment facility, there would not be enough virus required to infect avian species in the environment. Furthermore, a wild avian would have to be susceptible to viruses with envelope proteins A or B.

The src gene that makes this virus oncogenic in chickens has been removed. However, integration of the viral genome assembly is effectively random and therefore insertion could occur near a proto-oncogene within the host genome. Integration is not specific or dependent upon proto-oncogenes and therefore the chance of insertion near one is low. This work specifically uses embryos that will be terminated prior to embryonic day 10 and we will never allow infected embryos to hatch.

We believe a combination of these reduces the risk to organisms in the natural environment to effectively zero upon accidental release.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Disinfection**
After use, surface disinfection will be with 1% Distel with contact time of 30mins.

Liquid waste will be collected in polythene lidded containers and treated for 24 hours with 1% Distel solution. After the disinfection period, inactivated solutions are disposed to drains with copious amounts of cold water.

Reusable surgical tools will be treated in 1% Distel for 30 minutes prior to being bagged and autoclaved.
According to manufacturer’s guidelines Distel used at 1% concentration for the recommended time is as effective as using 1.4% formaldehyde to deactivate retroviruses and results in total inactivation. Used at this concentration, Distel is considered by the manufacturer to be non-hazardous to humans (https://www.tristel.com/tristel-products/distel-for-labs).

**Autoclaving**
Solid waste will be collected in double autoclave waste bags immediately after work, sealed with a tie and subjected to autoclaving under approved conditions. 100% kill is
achieved with this procedure. All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware and surgical tools. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile or using chemical indicators (e.g. Browne TST indicator test strips).

Other
Extracts will be made from tissue culture cells transduced by retrovirus vectors for biochemical and cell biological procedures (e.g. microscopy). The simple act of infection inactivates much of the input virus as it becomes uncoated upon entry into the cell. When making cell extracts, residual virus will be inactivated by detergent treatment (i.e. 1% SDS or 0.5% NP40). The effectiveness of the detergent treatments will be validated by plating transduced cell extracts on indicator cells and selecting for epitope tag expression. For microscopy techniques, the transduced cells will be fixed with 4% paraformaldehyde; retroviruses can also be effectively treated with formaldehyde solutions. There will be little or no packaged virus in these infected cells, and any remaining virus should be inactivated by these treatments.

Waste disposal routes
Waste from eggs or embryo tissue infected with RCAS virus will be broken then bagged with absorbent material in a sealed clinical waste bag, along with plasticware contaminated during use of infected eggs or embryo tissue. These sealed bags will be deposited into the anatomical bins in the cold room and then collected and autoclaved to inactivate any RCAS virus vector present prior to incineration as clinical waste. Non-infected and transgenic egg waste will be treated in the same manner in accordance with local procedures.

Solid cell culture waste or plasticware that has been infected or contaminated with RCAS virus will also be sealed in a clinical waste bag, deposited into the anatomical bins in the cold room, collected for inactivation by autoclave prior to incineration as clinical waste.

Liquid waste will be collected in polythene lidded containers and treated for 24 hours with 1% Distel solutions. After the disinfection period, inactivated solutions are disposed to drains with copious amounts of cold water.

General tissue culture waste that has not been contaminated with RCAS virus will be disposed of by the biohazardous waste stream which includes inactivation by autoclave prior to onward processing.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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L2  Yes

02/03/2022
Project Ref: 207/19.9

Date Ackn'd: 04/10/2019

CU2 Project Title:
Development of genetic tools for newly isolated Clostridium species

Class: Class 2
Culture Volume: 1-50 Litres

Non-GMM Consent Granted
Project notified under transitional arrangements

Withdrawn: N
Tick if notifying a connected programme of work: N

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Increased butyric acid and hydrogen production, as well as lab domestication to increase efficiency of growth and biological understandings

Recipient or parental organism
Clostridia isolates: C. butyricum (type strain Rowett) and two newly isolated strains, one is most closely related to the aforementioned C. butyricum, and the other is most closely related to C. beijerinckii. E. coli (CL1, K12 and B derivatives)

Host/vector system
DNA from the Clostridia sp. and reintroduced will result in increased expression of genes involved in the butyrate pathway involved in the production of has (H2) and

Origin & function
DNA taken from the Clostridia sp. and reintroduced will result in increased expression of genes involved in the butyrate pathway involved in the production of has (H2) and

02/03/2022
volatile fatty acids (such as butyric acid).

DNA from Aquorea victoria (jelly fish), Vibrio vulcanifcus, Bascillus subtilis and Pseudomonas putida (all CL1) will endow the Clostridia and E. coli with fluorescence to act as reporters to facilitate ease of manipulation.

The vector systems themselves will endow the Clostridia (and E. coli) with antibiotic (chloramphenicol, thiamphenicol, erythromycin, spectinomycin, tetracycline) resistance for counter-selective purposes.

**Evaluation of foreseeable effects**

None of the introduced genetic material is expected to increase the pathogenicity or survival outside of a lab environment of the recipient organisms, or pose a threat the outside environment should accidental release occur. Furthermore as the Clostridia sp. in question is strictly anaerobic and cannot survive in air its likelihood of escape is further lessened.

The antibiotic resistance (chloramphenicol, thiamphenicol, erythromycin, spectinomycin, tetracycline) acquired by the recipient organisms are also not on the list of primary antibiotics used to treat infections of Clostridia sp. or other anaerobes.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Cultures and contaminated liquid waste containing Clostridia sp. are to killed using PRESEPT (5 g/l, 0.5% w/v) and left for 16 hours. Surfaces are also to be cleaned with this solution as well as 70% ethanol for. Resulting in near 100% killing of cells as well as >5 log10 reduction in spore number (as reported in the literature for Clostridia sp.).

To test this the biocidal ability of the aforementioned treatments, cultures can be incubated with either PRESEPT and ethanol for specific lengths of time, and colony forming units measured indicating the viability of cells after treatment. This will indicate if this is an effective method of killing.

Disposal of killed cultures, petri dishes and contaminated plastics are to be held (separate from CL1 waste) for autoclaving at either 134°C (and held for 5 mins, for plastic waste, and disposed via approved contractor) or 121°C (and held for 15 minutes, for liquid waste, and disposed to drain). Resulting in 100% kill of bacteria and spores.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
Viral uptake in adult and hESC-derived cardiac cell types will be studied using a 2nd generation lentiviral vector pseudotyped either with either the SARS-CoV-2 S protein or vesicular stomatitis virus G (VSV-G). Pseudotyped lentiviral particles will contain the EGFP gene to allow viral internalisation in each cell type to be assessed by flow cytometry. Analysis of reporter EGFP activity following exposure to SARS-CoV-2 S pseudotyped lentiviral particles will provide a safe system to study SARS-CoV-2 cellular uptake. Bald lentiviral particles will be used as a negative control.

Viral replication will then be studied using a human coronavirus 229E GFP mutant (gifted from Professor Juergen Haas). This GFP expressing HCoV-229E virus will permit viral replication to be quantified by monitoring GFP fluorescence. The use of this replication competent virus will provide critical insight into the ability of various types of
Cellular uptake of pseudotyped lentivirus and replication of HCoV-229E will be assessed in both adult and hESC derived cardiac cells. Adult cell types will include human cardiac and pulmonary endothelial cells, cardiac pericytes, and blood outgrowth endothelial cells (from both patients with coronary artery disease and matched healthy subjects).

**Recipient or parental organism**

Viral uptake in adult and hESC-derived cardiac cell types will be studied using a 2nd generation lentiviral vector pseudotyped either with either the SARS-CoV-2 S protein or vesicular stomatitis virus G (VSV-G). Pseudotyped lentiviral particles will contain the EGFP gene to allow viral internalisation in each cell type to be assessed by flow cytometry. Analysis of reporter EGFP activity following exposure to SARS-CoV-2 S pseudotyped lentiviral particles will provide a safe system to study SARS-CoV-2 cellular uptake. Bald lentiviral particles will be used as a negative control.

Viral replication will then be studied using a human coronavirus 229E GFP mutant (gifted from Professor Juergen Haas). This GFP expressing HCoV-229E virus will permit viral replication to be quantified by monitoring GFP fluorescence. The use of this replication competent virus will provide critical insight into the ability of various types of adult and stem cell derived cardiac cells to sustain coronavirus replication.

Cellular uptake of pseudotyped lentivirus and replication of HCoV-229E will be assessed in both adult and hESC derived cardiac cells. Adult cell types will include human cardiac and pulmonary endothelial cells, cardiac pericytes, and blood outgrowth endothelial cells (from both patients with coronary artery disease and matched healthy subjects).

**Host/vector system**

Please see next box.

**Origin & function**

**SARS-CoV-2 S pseudotyped lentivirus:**

The self-inactivating lentiviral vector will be generated using a second generation lentiviral (LV) packaging system. Vectors generated will be pseudotyped to have either the SARS-CoV-2 or Vesicular stomatitis virus (VSV-G) envelope. A bald lentiviral vector lacking the envelope protein needed for host cell entry will also be used as a negative control. The final recombinant lentiviral genome will contain a self-inactivating 3' LTR, a central polypurine tract (cPPT), the EGFP gene (under control of the elongation factor 1 alpha (EF1a) promoter), and woodchuck hepatitis virus postranscriptional regulatory element (WPRE).

Host cells capable of internalising lentiviral particles will be genetically modified by the integration of the proviral DNA into the host genome, permitting the constitutive expression of EGFP under control of the EF1a promoter. The transduction efficiency of lentiviral vectors pseudotyped with SARS-CoV-2 S will be compared to vectors pseudotyped with VSV-G. This will be done by comparing EGFP activity in exposed host cells.

Virus tropism will likely be altered in the SARS-CoV-2 S pseudotyped lentiviral vector. SARS-CoV-2 S pseudotyped virus has recently been reported to be dependent on the cellular expression of ACE2 (Hoffman et al, 2020, Cell 181, 271–280). ACE2 is known to be highly expressed in multiple tissues including the lungs, kidney, heart, and vasculature (Patel et al, Circulation Research. 2016; 118:1313–1326). Bald lentiviral particles will be used as a negative control. The transduction capability of these bald lentiviral vectors in all host cells will be nullified owing to their lack of envelope protein, as shown by Ou et al (Nature Communications. 2020; 11:620).

**Recombinant HCoV-229E:**

This 229E mutant was originally derived by Cervantes-Barragan et al (mBio, 2010, 1:e00171-10). Recombinant HCoV-229E cDNA was produced by recombining vaccinia
virus containing full-length HCoV-229E cDNA with the pHC0V-GP-EGFP plasmid containing the P-glycoprotein-EGFP (GP-EGFP) fusion protein. This recombination event resulted in the replacement of HCoV-229E accessory gene 4 (located in between the genes encoding the spike and envelope proteins) with the gene encoding the GP-EGFP fusion protein. The recombinant HCoV-229E genome will therefore contain all the components found in the wild type HCoV-229E virus except accessory gene 4. This will allow for coronavirus replication to be monitored in infected cells by monitoring GFP fluorescence.

HCoV-229E is known to utilise the human aminopeptidase N (hAPN) as its receptor on human cells (Yeager et al., Nature, 1992, 1357:420-422). hAPN is known to be expressed in numerous cell types including macrophages, stromal cells, smooth muscle cells, endothelium, and epithelium (Dixon et al., J Clin Pathol. 1994, 47:43-47). Virus tropism and host range of the recombinant virus are not expected to be altered compared to WT HCoV-229E.

Evaluation of foreseeable effects

SARS-CoV-2 S pseudotyped lentivirus:
Second generation lentiviral vector(s) will be used. Expression of Rev in trans allows the production of high-titre HIV-derived vector stocks from a packaging construct also containing Gag, Tat, and Pol. This design makes the expression of the packaging functions conditional on complementation available only in producer cells. The gene delivery system, which conserves only four of the nine genes of HIV-1 relies on three separate transcriptional units for the production of transducing particles, offering significant advantages for its biosafety. If, an exposure to humans occurred, the lentivirus is significantly disabled and replication competent free. Even if that person had a pre-existing HIV infection, the chances of recombination are extremely small. Once produced through the packaging system, the recombinant vector is stable.

Given SARS-CoV-2 S pseudotyped lentivirus particles do not contain the gene encoding the SARS-CoV-2 S protein, the chance of generating a novel HCoV-229E genome containing the SARS-CoV-2S gene is effectively zero. To further reduce the probability of a generating a novel recombinant virus, experiments using these two viruses will be timed to not overlap with each other. This reduces the chance of a cell being co-infected with both viruses to effectively zero.

Recombinant HCoV-229E:
HCoV 229E infection causes a range of symptoms and severity dependent on the context of infection. HCoV-229E has been associated with transient and self-limiting febrile and upper respiratory tract infection in immunocompetent adult patients. There is no evidence that this is associated with significant negative outcomes in the short or long term. In children HCoV-229E has a similar pathogenicity. There are cases of patients with detectable coronavirus in respiratory samples presenting with meningitis, however no causative link has been found (Dominguez et al., J Med Virol, 2009, 81:1597-60). In severely immunocompromised patients there are case reports of pneumonia, including fatal cases (Pene et al., Clin Infect Dis, 2003, 37:929-932; Gerna et al., J Med Virol, 2006, 78:938-949). HCoV 229E has been identified in hospitalised Chinese children with pneumonia and asthma (though the immune status is not mentioned) (Zeng et al, Eur J Clin Microbiol Infect Dis, 2018, 37:363-369); in children with bronchiolitis and laryngitis (Brini et al., PLoS One, 2017, 12:e0188325) and SARI (Suresha et al., J Med Virol, 2016, 88:163-5). HCoV 229E has also been identified in the blood of a child presenting with suspected arboviral infection (Bonny et al., Genome Announc. 2017 22;5.pii: e01313-17) and as a co-infection with OC43 in the respiratory tract of a child presenting with acute flaccid paralysis (Turgay et al., J Pediatr Neurosci, 2015 10:280-1).There is no effective vaccine available for HCoV-229E.Vertical transmission from mother to foetus has been suggested in a small-scale study in which 3/6 newborns from HCoV-229E positive mothers also tested positive for the virus (Gagneur et al., Eur J Clin Microbiol Infect Dis, 2008, 27:863-866). Larger scale studies are required to investigate the vertical transmission and risks of HCoV-229E during pregnancy.

Clinical transmission is thought to be through inhalation of fomites. There are no reports of HCoV-229E infection through ingestion, however this has been considered likely for other coronaviridae and should be considered a risk (Jasper et al., Clin. Microbiol. Rev, 2015, 28:465-522).

It is deemed highly unlikely that the recombinant HCoV-229E could revert to wild type HCoV-229E. However, this cannot be completely ruled out if infected individual also currently infected with wild type HCoV-229E. Similarly, the possibility a cell being co-infected with SARS-CoV-2 S pseudotyped lentivirus and recombinant HCoV-229E generating a novel recombinant virus is extremely low but cannot be completely excluded.

N/A
No derogations requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection: All solid and liquid waste will be decontaminated/disposed of by the user at the end of every TC session. Surfaces will be sprayed 1% Trigene followed by 70% IMS after use.

Solid waste disposal:
- Stripettes. Rendered safe by autoclaving, then placed in a yellow bag with a yellow tag for disposal by incineration.
- Plastic ware such as flasks and tubes: Soak in 1%w/v Virkon, or 1,000 ppm Precept (four 0.5g tablets into one litre of water) for 24 hours before discharging any excess liquid via drains. Following chemical decontamination, place all plastic ware into the yellow bags within the tin autoclave drums, tie bag and place in the white plastic bin.
  After autoclaving, yellow bags with yellow tags are disposed of by incineration.

Liquid waste disposal
Any liquid potentially containing virus should be collected in an autoclavable screw top plastic bottle (stored under the sink). The liquid waste should be chemically disinfected by soaking in 1% w/v Virkon, or 1,00ppm Precept (four 0.5g tablets into 1 litre of liquid) for 24 hours before discharging via the drains.

Autoclaving details
All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C 15 minutes prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tr>
<td>L2 Yes</td>
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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
Experiments will be designed to assess competitive fitness, population dynamics and evolution of antibiotic resistance in Pseudomonas aeruginosa (hazard group 2). Strains will be cultured in isolation and co-cultured at varying densities in the presence and absence of antibiotics. Co-culturing may also be carried out with hazard group 1 E. coli strains. Population dynamics and strain composition will be quantified using standard methods such as plating, optical density and fluorescence plate reads, and flow cytometry.

Recipient or parental organism
Pseudomonas aeruginosa PA01 and PA14 laboratory strains

Host/vector system
Standard laboratory cloning vectors, such as pUC18-mini-Tn7 type suicide delivery vector and pTNS1/2 helper plasmid, carried by non-pathogenic E. coli cloning hosts

Origin & function
Fluorescent reporter genes:
- GFP originates from the jellyfish Aequorea victoria. Variants (e.g. YFP, CFP) were generated from GFP by mutations.
- DsRed originates from the cnidarian Discosoma spp. Variants (e.g. mKate, mCherry) were generated from DsRed by mutations.
- The intended function of these genes is to label bacterial strains in a manner that is detectable by devices using fluorescence (plate readers, flow cytometry, microscopy) and thus assess strain composition in bacterial populations.
Natural plasmids carrying antibiotic resistance genes:
- Plasmids such as Rms149, PAMBL1, PAMBL2 were originally isolated from clinical strains of Pseudomonas aeruginosa.
- The intended function of these plasmids is to confer resistance to specific antibiotics in the laboratory strains into which they are introduced.

**Evaluation of foreseeable effects**

We do not expect our genetic manipulations to increase the infectivity or pathogenicity of the bacteria. The introduced plasmids confer resistance to certain antibiotics in clinical usage for treatment of serious P. aeruginosa infections (particularly ceftazidime, meropenem); however, other antibiotic treatment options remain available. Serious infections are rare in healthy individuals, and individuals at elevated risk of serious infection will not be permitted to carry out this work. Infection risk will be further mitigated by strict containment and control measures.

Pseudomonas aeruginosa is widespread in freshwater and soil. In the unlikely event of release to the environment, we expect our genetically modified strains to be at a competitive disadvantage, as carriage of fluorescent reporter genes and introduced plasmids bears a fitness cost. The introduced plasmids are not expected to spread to other bacterial hosts, since these plasmids lack their own conjugative machinery and our bacterial strains do not carry other plasmids providing this machinery for mobilisation.

**Evaluation of foreseeable effects**

We do not expect our genetic manipulations to increase the infectivity or pathogenicity of the bacteria. The introduced plasmids confer resistance to certain antibiotics in clinical usage for treatment of serious P. aeruginosa infections (particularly ceftazidime, meropenem); however, other antibiotic treatment options remain available. Serious infections are rare in healthy individuals, and individuals at elevated risk of serious infection will not be permitted to carry out this work. Infection risk will be further mitigated by strict containment and control measures.

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**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

University of Edinburgh School of Biological Sciences waste inactivation and disposal procedures will be followed.

- All solid and liquid waste material will be autoclaved for sterilisation.
- Consumables (mainly plasticware) - autoclave (121-125 deg. C for at least 15 minutes), discharge any excess liquids to drains, dispose of solids via the industrial waste stream.
- Liquids (e.g. bacterial cultures) - autoclave (121-125 deg. C for at least 15 minutes), discharge to drains.
- Agar plates - autoclave (121-125 deg. C for at least 15 minutes), discharge any excess liquids to drains, dispose of solids via the industrial waste stream.

Degree of kill by autoclaving is effectively 100%. Autoclaves are maintained, serviced and inspected as per the requirements of the Pressure Systems Safety Regulations 2000 and the relevant parts of the British Standard BS 2646. See University guidance Section 9.8.2


Surface decontamination in the laboratory will use 1% Virkon disinfectant or 70% v/v ethanol. Spillages will be inactivated by powdered Virkon disinfectant and mopped up with paper towels. Solid waste is subsequently disposed as per above for autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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<tr>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 207/20.3

Date Ackn'd 14/08/2020

CU2 Project Title

Generation of a BAC transgenic mouse line overexpressing a human protein that will make them susceptible to infection with a human-tropic virus

Class 2

Consent Granted

Project notified under transitional arrangements

Project Additional Information

Purposes of the contained use

The virus that we are interested in infects humans with high efficiency, but infects mice poorly. The cellular receptor for the virus is known. Mice overexpressing the human receptor from a constitutive promoter have been made elsewhere and are readily infectable. The expression pattern of the receptor in these mice is unlikely to approximate...
the spatio-temporal pattern in humans, and the existing mice are not ideal models of the disease. We aim to create a better murine model of the human disease. To do this we will insert a bacterial artificial chromosome (BAC) containing the human gene that encodes this receptor, together with appropriate regulatory elements, into a safe harbour location in the mouse genome such that the receptor is expressed in mice with a similar spatio-temporal profile to that observed in humans. This will make the mice susceptible to the virus and allow us to model disease state and treatment options.

Recipient or parental organism

C57BL/6 x CBA F1 hybrid mice

Host/vector system

BAC clone encoding the human receptor for the virus

Origin & function

BAC clone has been acquired from BACPAC Resources. We intend to integrate this BAC into the mouse genome, such that the encoded gene will be expressed in the resulting mice. This will make them susceptible to infection with our virus of interest.

Evaluation of foreseeable effects

It is anticipated that mice with BAC integrated into their genome will express the human receptor in such a way that they are both readily infectable with our virus of interest and be useful for modelling disease state and treatment options. While we do not intend to carry out infection studies on these mice within our animal facility, there is a small risk that these mice could become infected by exposure to infected animal technicians, and subsequently transmit the infection to other staff.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Mice expressing the human receptor for the virus will be housed in a secure rodent barrier facility requiring swipe card / pin access. The mice will be contained in IVCs, and cage changes will be performed within a class II biological safety cabinet. Animal technicians handling the mice will wear suitable PPE including boots, surgical scrubs, disposable nitrile gloves, N95 fluid resistant face mask and face shield. The number of individual animal technicians responsible for care of these mice will be kept to a minumum.

These measures are intended to minimise the risk of exposing the mice to the virus and subsequent onward transmission to additional animal technicians. A risk assessment (GM Ani 0520) has been performed.

Mice will not be intentionally exposed to the virus within our facility. Future experiments involving infection of the mice with the virus will be performed at CL3 by a separate collaborating organisation(s) under their risk assessments and notifications and not by the University of Edinburgh.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Both before and after cage changes the interior surfaces of the biological safety cabinet will be swabbed with a 1% virkon solution.

All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile.

All carcasses and tissue waste will be disposed of by incineration (effectively 100% kill) according to the protocols set up within our animal facility. Ear clippings taken for genotyping will be disposed of as clinical waste and incinerated (effectively 100% kill).
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Project Ref 207/20.5

Date Ackn'd 23/09/2020

CU2 Project Title A genome-wide loss-of-fitness screen for establishment of infection in Trypanosoma brucei and T. congolense

Class 2 CultureVolClass2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

African trypanosome infections of cattle represent a major restriction on economic development in some of the poorest regions across the globe, and new tools are needed to combat this constraint to cattle rearing in Africa. The primary purpose of this project is identification of the species-specific molecules whose disruption is detrimental to the parasite in the host and/or is key to interactions with the host immune system. This will be achieved through genome-wide RNA interference (RNAi) screens for loss-of-function associated with protein ablation during parasite survival during infections. The aim of a genome-wide RNAi approach is to generate a population of genetically distinct parasites, each containing an individual DNA insert to silence a specific gene upon induction with tetracycline, on a scale sufficient that the number of individual clones provide coverage to effectively knock down the expression of every gene in the trypanosome genome. This library of parasites is then injected into an animal and silencing induced, thereby enabling the testing of several thousand gene silencing events at the same time. If parasite survival in the host is impaired due to specific gene silencing, then fragments of that gene will be reduced/lost from the parasite population after induction of RNAi – detected through Next Generation DNA sequencing (NGS) of the pathogens present in the peripheral blood following induction. This gene will thus be identified as essential and targeted for subsequent development of novel drug therapies and vaccinology.

Recipient or parental organism

The organisms to be used are Trypanosoma brucei and Trypanosoma congolense. Trypanosomes are single-celled protozoan parasites, that are transmitted from host to host by arthropod vectors (tsetse flies) - the trypanosomes have been modified such that each individual cell contains an individual DNA insert that will silence a specific gene upon induction with tetracycline - sufficiently large trypanosome populations therefore provide the ability to silence every gene in the genome individually ("genome-wide library"). T. brucei and T. congolense can infect a variety of mammalian hosts (including livestock species) if inoculated by an arthropod vector or syringe, but cannot infect humans. The insect vectors required for cyclical transmission of African trypanosomes (tsetse flies) are not present in the UK, but trypanosomes can be transmitted inefficiently through mechanical transmission by biting flies (e.g. Tabanid or Stomoxys flies). Trypanosomes do not perform genetic exchange in culture or in the mammalian host. Here we aim to use a genome-wide library approach in which we can analyse the fitness of individual genes during infection to identify genes that are essential for trypanosome infection and establishment in the mammalian host.

Host/vector system

T. congolense and T. brucei have been modified such that each cell contains a single introduced segment of genetic material ("construct") that comprises genes and transcription regulatory elements that enable targeted expression of a segment of trypanosome gene, a tetracycline repressor that prevents activation of that gene expression unless tetracycline is present, and antibiotic resistance genes to enable selective growth in vitro of genetically modified parasites.

Bovine hosts (non-genetically modified) will be sourced either from the University of Edinburgh herd, or approved suppliers, and cattle will be quarantined for one week prior to parasite infection, and maintained in CL2 vector proof facilities (I Block, LARIF or Marshall Building).

Origin & function

For knockdown of genes by RNAi library, DNA fragments have been isolated from trypanosome genomic DNA by direct fragmentation and cloning. All gene fragments have been inserted into the species from which they are derived (i.e. T. congolense or T. brucei) using a DNA construct (elements defined below). The approach taken is designed to specifically identify genes whose reduction of expression results in loss of fitness; therefore, no genes are anticipated to increase pathogenicity, virulence, host range or transmission.

Construct contents: T7 RNA polymerase is derived from a bacteriophage; tetracycline repressor, Puromycin acteyltransferase, neomycin resistance gene and bleomycin resistant gene are bacterial-derived. This construct is stably integrated into the trypanosome genome and not mobile or transmissible between trypanosomes thereafter. These elements enable the expression of gene-specific double stranded RNA in a tetracycline incible fashion - this double stranded RNA then results in reduction in the target gene expression due to the cellular process of RNA interference.
Evaluation of foreseeable effects

The strains of T. b. brucei and T. congolense to be used are not infective to humans. Therefore, the risk to human health is effectively zero. There are no predicted hazards associated with the plasmid and phage vectors employed to generate the GM lines, as vector DNA was removed before insertion of exogenous genes into the genome of T. brucei and T. congolense.

This project will involve production of mutants (gene knockdown in their endogenous species) that will be either phenotypically neutral or detrimental to parasite growth. No modifications proposed are anticipated to increase pathogenicity, virulence, host range or transmission. Moreover, trypanosomes have a complex life cycle with several essential differentiated forms and it is highly unlikely that the transfer of even cryptic genes would result in increased virulence. The antibiotic resistance cassettes to be used are already widely used and have no intrinsic harmful activity. The parasites themselves do not produce toxic products or immune effectors that are linked to human pathogenicity. T. congolense and the strain of T. brucei to be used are infective to a wide range of mammalian species (with the exception of humans, great apes and some species of baboons). However, the range of potential host species or virulence is very unlikely to be changed due to the genetic modification approach being taken.

The risks of transfer to related microorganisms are negligible. While there is a trypanosome species endemic to the UK (Trypanosoma theileri) that circulates in cattle, this is a very distantly related organism, and there is no evidence or expectation that exchange of genetic material could occur between these species. Moreover, genetic exchange in trypanosomes only occurs in their cyclical tsetse fly vectors, in the case of African trypanosomes these do not occur in the UK. Additionally, all of the modifications made during this project are anticipated to be at most functionally equivalent to the wild type and would therefore confer no selective advantage to a related organism. To negate environmental transmission, between animal facility and the laboratory, infected samples will be transported under triple containment.

Trypanosomes will only survive within mammalian hosts or relevant vectors (T. brucei and T. congolense both have a developmental cycle in tsetse fly vectors – not endemic to the UK – and can survive short periods in the proboscis of mechanical vectors, e.g. Tabanid or Stomoxys flies) – they rapidly die when not in these contexts, and therefore represent minimal environmental risk. Trypanosomes are blood-borne parasites, but are not excreted into the environment in secreted/excreted bodily fluids (e.g. sweat, urine, faeces), and require transfer from animal to animal via either infected blood or infected insect vector. If inoculated into susceptible mammalian hosts by mechanical transmission (i.e. insect vector or syringe) the parasites may cause infection and consequently disease.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation is requested for the following measure for the reasons provided herein:

1 – Autoclave in building

For category 2 work conducted in the Marshall Building (Dryden farm) or I Block.

Autoclaves being operated by staff routinely handling GMO/SAPO waste are not available at these locations. Secure containment of GMO/SAPO waste is achieved by transportation of waste generated at these locations to the main Roslin Institute Building where the bulk of GMO/SAPO waste is generated and its management through autoclaving is routinely performed. The distance of transfer of material from these locations to the Roslin Institute Building is short and will be conducted according to the conditions described in the risk assessment (i.e. performed by a trained and competent person in double containment, leak proof boxes clearly labelled as containing GMO/SAPO waste and accompanied with a spill kit). Where possible waste will be inactivated by chemical means (or freezing) prior to transportation to the Roslin Institute Building for autoclaving.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste associated with handling trypanosomes will be disposed of in accordance with SAPO regulations, i.e. disposed of in identified autoclave bags and the waste autoclaved and incinerated. Any infected liquid (e.g. culture medium or blood) will be mixed in >10x volume of 2% Virkon, and disposed of in the sinks in East Wing Level 2 laboratories after at least 1 hour incubation.

At the termination of experiments and to mitigate any risk of transmission by vectors, ruminant hosts will be disposed of by humane euthanasia followed by dismemberment if necessary, double bagged in sealed heavy duty plastic bags, stored at -20°C for a period of 7 days (to inactivate pathogens – the GM modifications will not impact upon the effectiveness of low temperature as a lethal measure against trypanosomes) before being disposed of as a Category 1 animal byproduct by a licensed contractor. The containment area is then subjected to thorough cleaning. All solid waste is collected into clinical waste bags, sealed and sent for incineration. The area is then swept and...
the debris placed in sealed clinical waste bags for incineration. The area is then treated with a proprietary disinfectant before washing down with a steam cleaner and being allowed to dry.

Disinfection: Any infected liquid (e.g. culture medium or blood) will be mixed in >10x volume of 2% Virkon or similar validated disinfectant, and disposed of in the sinks in East Wing Level 2 laboratories after at least 1 hour incubation. Laboratory surfaces will be disinfected by wiping with either 2% Virkon or similar validated disinfectant or by use of 70% ethanol; materials will be disposed of through laboratory waste disposal routes. After animal experiments are completed, surfaces (walls and floors) in the pen containment facility (Marshall Building, I Block or LARIF) will be cleaned using an appropriate validated disinfectant.

Autoclaving: All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. In the case of contaminated materials from Marshall Building, I Block or LARIF (e.g. sharps, disposable gloves), these will be transported to the Roslin Institute building under triple containment for disposal. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tbody>
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<td>L2</td>
<td>L3</td>
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<tr>
<td>L2</td>
<td>Yes</td>
<td>L3</td>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
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<tbody>
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<td>Yes</td>
<td>L3</td>
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**Project Ref** 207/20.6

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>16/10/2020</td>
<td>Recombinant production of intrinsically disordered alpha synuclein protein variants in</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
</tr>
</tbody>
</table>
**Project Additional Information**

**Purposes of the contained use**

The protein variants produced will be used for subsequent biophysical and biochemical analysis including, but not limited to, mass spectrometry, super-resolution microscopy, circular dichroism, and fluorescence microplate assays.

**Recipient or parental organism**

The recipient organisms are standard BL21 and DH5α laboratory strains of E.coli. Both E.coli strains to be used are attenuated in their ability to survive outside of laboratory conditions and are non-pathogenic. These are functionally disabled strains that cannot survive in the environment and do not colonise the human gut.

**Host/vector system**

The vector system used will be a commercially-available pET based plasmid, a non-hazardous carrier of genetic material. The vectors are used to express and maintain recombinant proteins in E. coli and were obtained from Invitrogen. They contain a gene which confers resistance against beta-lactam antibiotics upon the host bacterium.

**Origin & function**

Inserted gene products are human proteins and variants (single point mutations, splice variants etc). Estimated expression levels result in 2-10 mg quantities of recombinant protein produced. Each of the gene inserts used will produce recombinant α-syn in its monomeric form1 at a concentration of 1-5 mg/ml. The pathological and toxic conformers of α-syn are considered to be oligomers, protofibrils and fibrils1,2, whilst the native monomeric form of α-syn is considered to be non-toxic1. Recombinant α-syn exists predominantly as stable unfolded monomers1. The formation of oligomers and fibrils from monomeric α-syn requires specialised procedures involving prolonged shaking at 37°C and high-powered sonication3, and does not occur spontaneously. Evidence for the lack of toxicity of monomeric recombinant α-syn comes from studies using cellular toxicity assays using SHSY-5Y cells4 and inoculation studies using mice.5 The addition of recombinant α-syn to SHSY-5Y cells4 did not result in cell death as measured by the MTS colorimetric assay. Masuda-Suzukake et al injected mice intra-cerebrally with monomeric human recombinant α-syn and found that none of the mice had pathological symptoms or evidence of neuropathology related to α-syn.5 Therefore, there is evidence at both cellular level and using mouse transmission studies that monomeric recombinant α-syn is not associated with disease or toxicity. As such, there is no evidence that monomeric recombinant α-syn poses a hazard to human health.

2. Lashuel HA, Overk CR, Oueslati A and Masliah E. The many faces of α-synuclein: from structure and toxicity to therapeutic target. Nat Rev Neurosci 2013, 14(1) 38–49
Evaluation of foreseeable effects

Due to the attenuation of the E.coli strains used, they have been shown to have limited colonisation ability, therefore making them highly unlikely to colonise healthy individuals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection
Liquid waste contaminated with GMOs expressing alpha-synuclein constructs is disinfected using Virkon. For decontamination of liquid cultures, a 2% Virkon solution should be applied in a 1:1 dilution with the liquid for at least 1 hour prior to disposal.

Autoclaving
All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile and/or using chemical indicators (eg Browne TST indicator test strips).

Solid waste
All solid waste contaminated with either GMOs expressing alpha-synuclein constructs or purified alpha-synuclein proteins is disposed of via the contaminated laboratory waste route. They must be autoclaved (see details above) prior to disposal.

Liquid waste
Liquid waste contaminated with GMOs expressing alpha-synuclein constructs is disinfected using Virkon. For decontamination of liquid cultures, a 2% Virkon solution should be applied in a 1:1 dilution with the liquid for at least 1 hour prior to disposal.

Liquid waste contaminated with purified alpha-synuclein protein will be collected via a designated liquid waste stream. This will then be autoclaved using the above described procedure to ensure deactivation of the protein prior to disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

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**Project Ref 207/20.7**

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>12/11/2020</td>
<td>Therapeutic Role of Stem Cells in Orthopaedic Infections</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>

**Project Additional Information**

Purposes of the contained use

The XEN36 strain of Staphylococcus aureus will be used to track infection in an animal model of fracture repair
<table>
<thead>
<tr>
<th><strong>Recipient or parental organism</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 49525 is a clinical isolate from a bacteraemia patient. <em>Staphylococcus aureus</em> is a usual member of the microbiota of the body and is of low risk to the environment, but in compromised individuals Infection can lead to boils, impetigo, food poisoning, cellulitis and toxic shock syndrome.</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th><strong>Host/vector system</strong></th>
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<tbody>
<tr>
<td>Xenogen 36 contains a native plasmid which is a vector for the bioluminescent properties it possesses. The recombinant gene confers bioluminescent properties, but otherwise does not alter the traits of <em>Staphylococcus aureus</em>. Risk to the environment is low as virulence is unaffected, and the stable recombinant gene is largely propagated by vertical gene transfer to their progeny with no other bacteria involved in experimental procedure.</td>
</tr>
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</table>

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<tr>
<th><strong>Origin &amp; function</strong></th>
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<tbody>
<tr>
<td>The bioluminescent <em>S. aureus</em> strain Xen36 possesses a gram positive optimised luxABCDE operon modified from the bacterial insect pathogen <em>Photorhabdus luminescens</em> at a single integration site in a stable native bacterial plasmid that is maintained in all progeny conferring bioluminescent properties, allowing monitoring of growth in culture.</td>
</tr>
</tbody>
</table>

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<tr>
<th><strong>Evaluation of foreseeable effects</strong></th>
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</thead>
<tbody>
<tr>
<td>The recombinant gene confers bioluminescent properties, but otherwise does not alter the traits of <em>Staphylococcus aureus</em>. Risk to the environment is low as virulence and resistance to drugs or other materials is unaffected, and the native plasmid is transferred largely by vertical transfer to their progeny.</td>
</tr>
</tbody>
</table>

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<tr>
<th><strong>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal facilities are Containment Level 2. Post-surgery and bacterial inoculation animals are housed separately within the animal facility, and are handled with appropriate PPE to protect the user and eliminate possible transfer between animals. However, the GM animal is a low risk to both human health and the environment.</td>
</tr>
</tbody>
</table>

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<th><strong>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</strong></th>
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<tbody>
<tr>
<td>All contaminated materials will be inactivated by autoclaving(100% kill) at 121 or 134 degrees C for 20 minutes prior to disposal of waste or cleaning and recycling of reusable laboratory equipment. Autoclaves are validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature/time profile. Following autoclaving waste is disposed of as per University of Edinburgh regulations for waste routes.</td>
</tr>
<tr>
<td>Disinfection of non-autoclavable items/equipment is done using 1% Virkon for 30 minutes.</td>
</tr>
<tr>
<td>Sharps will be disposed of in a 'sharps' bin and follows the University of Edinburgh regulations for waste routes.</td>
</tr>
</tbody>
</table>

**Is an emergency plan required according to regulation 20?** N

**Tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

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<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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</tbody>
</table>

**Project Ref** 207/21.1

Investigating bacterial host-pathogen relationships in three-dimensional tissue culture models

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>26/02/2021</td>
<td>Investigating bacterial host-pathogen relationships in three-dimensional tissue culture models</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>

Project notified under transitional arrangements

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The overarching goal of this project is to study bacterial host-pathogen interactions by identifying novel bacterial virulence factors in a range of respiratory pathogens using genetic screens and characterizing the mechanisms through which these virulence factors interact with host cells using three-dimensional models of the human lungs. Candidate genes predicted to be important for infection and dissemination will be disrupted through transposon insertion and site-directed mutagenesis. The resulting mutants will be characterized in three-dimensional tissue culture models that allow us to observe bacterial dissemination. Mutants that show a dissemination phenotype will
be complemented to confirm that the phenotype is related to the gene of interest. If initial characterization suggests that the bacterial genes directly interact with host factors or have host modulatory functions, those genes may be expressed in eukaryotic cells to confirm these interactions. In some cases, fluorescent or luminescent cassettes will be transformed into the bacterial strains so that they can be visualized by microscopy.

Recipient or parental organism

1) Pseudomonas aeruginosa (Wild type – strains PA01, PA14, PA ATCC2783) is an opportunistic human pathogen that is capable of causing infection in immunocompromised individuals including urinary tract infections, dermatitis and soft tissue infections, and respiratory infections. However, P. aeruginosa is unlikely to cause disease in healthy adults, and when such infections do occur they are generally mild and treatable (Hancock and Speert, Drug Resist Update. 2000 Aug;3(4):247-255).

2) Staphylococcus aureus (Wild-type- strains SA5003, SH1000, MRSA254, MSSA476) is primarily a commensal bacterium in humans that is commonly present on the skin and mucous membranes of up to 50% of healthy individuals without causing disease. However, S. aureus can also be an opportunistic pathogen most commonly associated with nosocomial infections of wounded skin and soft tissues as well as respiratory and gastrointestinal infections and septicaemia. (Lakhundi and Zhang, Clin Microbiol Rev. 2018 Oct; 31(4): e00020-18).

3) Mycobacterium smegmatis (Wild-type strain Mc2155) is a non-pathogenic strain of mycobacteria commonly found in soil, marine water, and freshwater. It does not cause disease in humans or animals.

4) Mycobacterium marinum (Wild-type strain Mc2155) is an opportunistic pathogen capable of infecting freshwater and marine fish, resulting in chronic progressive disease with significant morbidity and mortality. It is also capable of producing granulomatous lesions in other animals. However, due to strict temperature sensitivity M. marinum does not cause disseminated disease in immunocompetent individuals and there is no evidence of transmission from person to person. (Hashish et al., Vet Q. 2018; 38(1): 35–46).

5) Mycobacterium bovis BCG (disabled vaccine strain) is an attenuated strain of mycobacteria that has been used as a human vaccine against Mycobacterium tuberculosis since 1921. It has a long history of safe use in humans and a wide range of other animals including livestock. In rare cases of immunocompromised individuals, such as those who are HIV+ or have inherited immune disorders, vaccination with BCG has resulted in infections ranging from cutaneous lesions to disseminated disease, but the majority of these infections are self-limiting. (Murphy et al., Tuberculosis (Edinb). 2008 Jul;88(4):344-57).

6) Mycobacterium tuberculosis ΔRD1, ΔpanCD (Disabled laboratory strains mc26030 and mc27000) are attenuated strains of M. tuberculosis from which two distinct virulence regions responsible for disease have been disrupted. Mycobacterium tuberculosis is a hazard group 3 biological agent, but these strains are sufficiently attenuated and so highly unlikely to revert to higher pathogenicity that we believe it can be safely reclassified as hazard group 2. We therefore wish to reclassify this strain to HG2 for the following reasons based on the following evidence of greatly its reduced pathogenicity. The ΔRD1 attenuating mutation is the deletion of the RD1 virulence locus which contains the ESX Type VII secretion system. This is the same mutation responsible for loss of virulence in the BCG vaccine and deletion of this locus alone has been shown to attenuate M. tuberculosis (Lewis et al., Infect Dis. 2003 Jan 1;187(1):117-23). In addition, ΔpanCD deletion results in complete loss of the panCD locus which encodes two genes required for the synthesis of pantothenate, which alone also attenuates M. tuberculosis (Sambandamurthy et al., Nat Med. 2002 Oct;8(10):1171-4) to make reversion to wild-type and makes growth of this strain dependent on media supplementation with pantothenate. Deletion of two distinct loci further attenuates the strains and reduces the possibility of reversion to wild-type as either deletion is attenuating. This strain is highly attenuated in vivo in all animal models tested and is unable to cause disease in mouse, guinea pig, calf, and non-human primate models even at high dose. There have been no reported human infections with this strain, and it has been approved for use in Biosafety Level 2 laboratories in the USA (Sambandamurthy et al., Vaccine. 2006 Sep 11;24(37-39):6309-20; Waters et al., Vaccine. 2007 Nov 7;25(45):7832-40; Larsen et al., Vaccine. 2009 Jul 23;27(34):4709-17.) Strain mc27000 is a direct derivative of mc26030 from with the antibiotic resistance cassette has been removed resulting in an unmarked strain (Ojha et al., Mol Micobiol. 2008 May 5; 69(1) 164-74).

7) Non-pathogenic strains of E. coli K12 (disabled laboratory strains DH5- , BL21, HB101, XL1-Blue) are naturally occurring commensal bacteria that do not express virulence genes are not capable of causing disease in humans even at extremely high doses (Smith HW, Nature 1975 Jun 5;255(5508):500-2).
8) Burkholderia thailandensis (wild-type strain E264) is an environmental bacterium present in the soil and water of tropical regions including Southeast Asia and Northern Australia. It is considered non-pathogenic and does not cause disease in healthy individuals, but is capable of causing infection in mouse models (Morici et al., Microb Pathog. 2010 Jan; 48(1): 9–17.)

9) Eukaryotic cell lines may be classed as recipients if selected to stably maintain eukaryotic expression vectors by antibiotic-mediated selection for chromosomal integration. Cell lines may include A549, EaHy926, THP-1, J774, RAW264, L929 or derivatives thereof. Other similar cell lines may be used as they become available

**Host/vector system**

Give brief details of Vector(s):

1) Plasmids for gene expression in bacteria (non-integrating) will include those of the pMB-, pUC-, pACYC-, pBAD-, pBR-, pGEM-, pGEX-, pEXT-, pSET-, pBluescript-, pEC-, pLAFR, pMLBAD-, and pET- series, as well as derivatives of these plasmids that contain the origins of replication pMB1, ColE1, p15A, pSC1010, R100. pUC, FII, incW, pBRR1, RK2, oriV, or incP and standard antibiotic markers (Amp, Cm, Kan, etc.)

2) Integrating plasmids for gene expression in mycobacteria will include those of the pYUB- and pMH- series and related plasmids derived from these series containing the same mycobacteria and E. coli origins of replication. These vectors are suicide plasmids containing an integrase from the L5 bacteriophage, allowing a single copy to integrate specifically into the attP site of mycobacteria

3) Plasmids for targeted mutagenesis will include those of the pDM-, pEX-, pDONR-, pCDNA- and pKD- series and related suicide vectors produced using the same methodology expressing λRed recombinase or counter selection markers for homologous recombination to facilitate gene deletion.

4) Bacteriophage vectors including the phAE- shuttle plasmids for mycobacteria will be used for targeted mutagenesis. These phage are only capable of replicating within specific strains of bacteria and are not capable of infecting human or animal cells

5) Plasmids for the delivery of mobile elements for random mutagenesis will typically possess a λpir-dependent origin and the mobilisation region from plasmid RP4 permitting their delivery only from λpir-expressing E. coli expressing the RP4 conjugation machinery. These include the pUT-series for plasmids harbouring mini-Tn5 derivatives. Similar replicons will be used to transfer cloned and mutated fragments to recipient genomes by homologous recombination and may possess markers for positive-selection (e.g. sacBR) and antibiotic resistance.

**Origin & function**

1) Fluorescent and luminescent cassettes genes encode proteins that emit light for the purpose of visualization on a microscope or plate reader. These include the LuxABCDE bacterial luciferase locus and fluorescent proteins such as GFP and its derivatives (e.g. YFP, CFP) and dsRed and its derivatives (e.g. mCherry, tdTomato). Antibiotic resistance markers (e.g. gentamicin, chloramphenicol) are carried on the cloning vectors for the purpose of selecting transformant.

2) Random and targeted mutagenesis of bacterial genes by transposable elements will typically result in the loss of function of the gene in which the mutation occurred. Complementation of the native allele by homologous recombination, expression on a plasmid, or insertion on an integrating plasmid will be performed to restore function to the mutated strain. For these experiments, the predicted function of the majority of these genes will be bacterial dissemination or adhesion and invasion of host cells, though other related function may also be included in these studies. The inserted DNA will typically contain an antibiotic resistance cassette conferring reduced susceptibility to the relevant antibiotic.

**Evaluation of foreseeable effects**

1) Fluorescent and luminescent proteins are well characterized, have no known toxicity and are not predicted to cause pathogenicity. The antibiotic resistance markers carried on the cloning vectors confer reduced susceptibility to the relevant antibiotic (e.g. gentamicin, chloramphenicol).

2) In the majority of instances random and targeted mutagenesis of bacterial genes is expected to result in negligible effects on the overall fitness of the mutant strains

02/03/2022
during an animal infection. On rare occasions, mutations may enhance fitness in vivo or alter the tissue- or host-tropism of the recipient, which could potentially result in increased replication within some cell lines or increased growth within certain tissues in vivo. It is also possible that mutations will not alter the function of the gene which would result in a wild-type strain with no impact on virulence. Insertion of foreign DNA may have unanticipated polar effects on upstream or downstream genes. Complementation of the native allele is expected to restore function to the mutated strain. Depending on the expression system used this may result in partial or complete complementation of any defect present, and in some instances may enhance the magnitude of gene expression, or alter the timing of such, relative to that present in nature. The antibiotic resistance markers that will be introduced will confer reduced susceptibility to the relevant antibiotic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be disposed of by heat inactivation via autoclave, 100% kill. Protocols for each of waste that may be generated are as follows:

1. Consumables (e.g. plastic-ware such as pipettes, flasks, tubes etc.) - autoclave at 121-1250C for a minimum of 15 minutes. Disposed via industrial (orange bag) waste route through the University approved contractor.
2. Liquid discard (e.g. samples, culture supernatants, tissue culture media) --autoclave setting at 121-1250C for a minimum of 15 minutes. Disposed to drain.
3. Agar plates - autoclave (121-1250C for at least 15 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (orange bag) waste stream for landfill
4. Sharps waste in sharps containers (e.g. needles, scalpel blades) --disposed via the clinical waste stream for heat treatment via the University approved contractor.
5. Cytotoxic waste, solid and sharps must be segregated separately into purple lidded containers for disposal - disposed via the University approved contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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<td>L3 L4</td>
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</table>

Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Endothelin is a potent vasoconstrictor. We seek to produce a cohort of pigs with an inducible endothelin transgene such that we can study vascular dementia associated with hypertension. To do this we will microinject a lentiviral vector encoding the transgene construct into pig zygotes. The lentiviral vector is non-mobilisable. Once integrated into the DNA of the zygote it becomes a permanent feature of the genome. Once born the transgenic pigs will not pose a higher risk to human health than non-transgenic pigs.

**Recipient or parental organism**

Pig

**Host/vector system**

Second generation lentiviral vector

**Origin & function**

We will use a second generation lentiviral vector encoding the pig endothelin 1 cDNA under the control of a tet-response element to produce transgenic pigs. This will be microinjected into the perivitelline space of pig zygotes, infect the cell and integrate into the genomic DNA.

The lentiviral vector system which we will use has several safety features included to minimise the potential of replication competent lentivirus (RCL) production, or
subsequent mobilisation of the integrated transgene from the genome of the host cell. There is a very low risk of recombination with endogenous retroviruses due to the division of synthetic viral elements (ie coding sequences have been significantly altered from the original) among three different plasmids. The packaging and envelope plasmids (psPAX2 and pVSVG respectively) express viral accessory proteins in the HEK293 packaging cell line such that the desired transgene elements, inserted between the lentiviral LTRs, are packaged into the lentiviral pseudoparticle. The endogenous HIV-1 envelope glycoprotein has been replaced with VSV-G; while this increases the tropism of viral particles, it is also toxic to cells that express it. As the psPAX2 and pVSVG plasmids do not contain the lentiviral packaging signal sequence they are not packaged into the lentiviral pseudoparticle. This feature means that the elements required for producing lentiviral pseudo particles are only present in the transfected HEK293 packaging cells and not in cells that are subsequently transduced.

2nd generation lentiviral vectors are termed “self-inactivating” by virtue of a deletion in the 3’ LTR. This deletion does not affect the transcription and packaging of the recombinant viral genome in the producer cell line, but following reverse transcription results in a non-functional 5’ LTR in the integrated genome. This feature minimises the risk of mobilisation of the integrated construct.

As a consequence the resultant particles are single-round infectious. I am not aware of any literature reporting RCL or mobilisation of the integrated transgene from 2nd generation lentiviral systems. The relevant genetic elements encoded within the lentiviral vector will be the pig endothelin 1 cDNA under the control of a tet-response element. Downstream of this there will be a ubiquitous promoter (hUBc) driving expression of the reverse-tet transactivator protein, with an IRES followed by a neomycin resistance marker gene. In the absence of doxycycline the endothelin transgene is silent. In the presence of doxycycline the conformation of the reverse-tet transactivator is altered such that it is able to bind to the tet-response element, driving expression of the endothelin transgene.

Evaluation of foreseeable effects

The transgenic pigs will not have altered toxicity, allergenicity or behaviour. We anticipate that induced expression of the pig endothelin transgene will result in a hypertensive phenotype. We do not anticipate that induction of hypertension will alter the behaviour of the pig such that it is of greater risk to humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Animals will be housed in secure pens which are 1.5m in height as required by the HO regulations. The pens are contained within a locked facility with access controlled by the facility manager and limited to authorised personnel only. There will always be a minimum of 2 barriers between the animal and the external environment. Animals will be cared for by experienced staff wearing appropriate PPE. All staff involved in the care of these animals will be aware that they are genetically modified. Animals will be segregated by sex at weaning and maintained in single sex groups throughout life, other than to facilitate planned breeding.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Following schedule 1 culling the GM organism can be considered as inactivated and will be disposed of by either incineration or rendering.

Disinfection

All laboratory surfaces that come into contact with tissues/cells from these animals will be wiped with a 1% Virkon solution. Culture plates and pipettes will be soaked in a 1% Virkon solution overnight before disposal.

Autoclaving

All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored using digital read outs.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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**Project Ref** 207/21.12

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<td>17/12/2021</td>
<td>Genome-wide CRISPR screening in non-human mammalian cancer cells</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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- **Non-GMM Consent Granted**
- **Project notified under transitional arrangements** N

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**Project Additional Information**
Purposes of the contained use

To identify essential genes involved in the proliferation, survival or migration of non-human mammalian cancer cell lines in vitro.

Recipient or parental organism

Cell lines derived from dogs, cats and horses.

Host/vector system

Vector: 2nd generation lentiviral vector. This is a replication-incompetent HIV-derived vector composed of three separate plasmids (psPAX2, pMD2.G and LentiCRISPRv2), with additional safety features including self-inactivation of LTR sequences.

Host: cancer cell lines and control fibroblast cell lines derived from dogs, cats and horses; HEK293T cells and closely-related Lentiviral Production Cells (commercially available human embryonic kidney-derived cell lines) for lentiviral production and titration.

Origin & function

The genetic material introduced into the cells comprises the nuclease enzyme spCas9 (originally derived from Streptococcus pyogenes), or a catalytically inactivated version, under an EF-1a core promoter, together with a synthetic sgRNA scaffold and a pool of sgRNA sequences targeted at the host genome, under a U6 promoter, and a puromycin or blasticidin resistance gene to allow antibiotic selection of the targeted cells. The sgRNA protospacer sequences will be synthesised de novo, while the other genetic elements are from the following 3 plasmids: LentiCRISPR-v2 (Addgene plasmid # 52961), lentSAMv2 (Addgene #75112) or Lenti-(BB) -EF1a-KRAB-dCas9-P2A-BlastR (Addgene #118154). The intended function of this CRISPR-Cas9 system is to target each gene in the target genome causing either 'knockout' of the gene by nuclease activity with imperfect non-homologous end-joining repair, leading to introduction of frame-shift mutations, or to increase or inhibit transcriptional activity.

Evaluation of foreseeable effects

Lentiviral vector:
This will have the ability to infect any mammalian cell line in vitro, resulting in expression of the Cas9 enzyme and sgRNA construct, or to infect humans or other animals if exposure occurs via mucous membranes, skin wounds or blood inoculation, but not via intact skin. The virus is replication-incompetent and self-inactivating, so sustained infection or spread of infection is not possible. Expression of the CRISPR/Cas9 constructs would lead to disruption of target gene sequences or expression in cells derived from the target species, with a lower chance of effects in any non-target species such as humans (depending on sequence homology). There is a small risk of oncogenesis after exposure via a clinically significant route, either via insertional mutagenesis or via disruption of oncogenes / tumour suppressor genes.

Genetically modified target host cells:
The genetically modified host cells will comprise a mixed pool of cells in which the function of each gene in the genome is expected to be disrupted individually in a subset of the cells. These cells are expected to exhibit a range of growth characteristics in vitro. All the cells are non-infectious themselves and will not harbour any pathogens dangerous to humans, and the genetic modifications involved will not lead to production of a harmful product. The genetic modifications introduced are not likely to lead to immune evasion or to increase the risk to humans in any way.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

02/03/2022
Liquid waste will be inactivated overnight using 1% Distel, a broad spectrum virucidal disinfection, or tetraclosene sodium (to give a final concentration of 1,000 - 2,500 ppm chlorine), to inactivate any remaining lentiviral particles prior to disposal.

All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored using chemical indicators (e.g. Browne TST indicator test strips).

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

<table>
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<th>Laboratory Activities</th>
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Animal Units  

Large Scale Activities  

Human Clinical Applications

Project Ref 207/21.13

Class  

CultureVolClass2  

CultureVolumeClass3-4

Non-GMM  

Consent Granted

Project notified under transitional arrangements  

N
**Project Additional Information**

**Purposes of the contained use**

To generate human liver cell lines from healthy and diseased human liver for in vitro experiments, with a view to minimize reliance upon rodent models, and to accurately model human liver pathology.

**Recipient or parental organism**

Primary human liver cells, from healthy individuals, and those with liver pathology.

**Host/vector system**

An MuLV (Murine Leukaemia Virus) retroviral vector system will be used to deliver SV40 Large T antigen and TERT, which are genetic inserts commonly used for cell immortalisation.

**Origin & function**

Retroviruses for delivery of Large T antigen and TERT will be generated by TransIT mediated transfection of HEK cells, in OptiMEM media. The plasmids used will be: pUMVC (packaging), pMD2.G (pseudotyping), pBABE-Zeo SV40-LT (transfer plasmid for Large T antigen), pBABE-hygro-hTERT (transfer plasmid for TERT).

SV40 Large T antigen and TERT are genetic inserts commonly used for cell immortalisation. The transformation/immortalization by SV40 LT involves the following mechanism(s): (1) activation of E2F-mediated transcription through binding with Rb-E2F complex and (2) inhibition of p53, by blocking p53-dependent transcription activation and p53-independent growth-arrest. These two mechanisms lead to overcome growth arrest, prevent apoptosis and result in cellular proliferation. Another method used to immortalise primary cell lines is overexpression of telomerase reverse transcriptase (TERT), the catalytic subunit of the telomerase enzyme. The telomerase reverse transcriptase (TERT) protein is inactive in most somatic cells, causing the length of telomeres to shorten with age, leading to senescence. Expression of hTERT prevents the telomere from being truncated during cell division, thus cells are able to avoid replicative senescence.

**Evaluation of foreseeable effects**

Since the genes required for the γ-retroviral packaging and transduction are not encoded by the transfer plasmid, but instead are provided in trans by the other plasmids, the γ-retrovirus product can transduce target cells, but the transduced target cell does not produce additional virus.

Due to the broad tissue tropism provided by VSVG, the final amphotropic, MLV retroviral vectors will be able to infect all mitotic mammalian cells, but will be replication incompetent, unless recombination events occur (with endogenous or exogenous retroviruses, or with packaging components).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Disinfection Virkon RelyOn or 70% ethanol will be used for disinfection. Virkon RelyOn will be used at a final concentration of 1% with a contact time of 10 minutes. 70% ethanol will be used with a contact time of 30 seconds. Surfaces (incl bench tops, floors, small pieces of equipment, Gilsons, samples tubes etc) will be disinfected using a 1% Virkon RelyOn solution with a minimum contact time of 10 minutes or 70% ethanol with a minimum contact time of 30 seconds.

All liquid waste in the liquid waste collection bucket will be mixed with a 2% Virkon RelyOn solution in a 1:1 ratio and left for 10 mins following the conclusion of the experiment. After 10 mins, the mixed liquid waste can be discarded down the laboratory sink.

Solid will be chemically inactivated using Virkon RelyOn as described above prior to autoclaving, given the (low) oncogenic risk posed by the virus. Following chemical inactivation, solid waste will be double bagged in autoclave bags, necks sealed with autoclave tape, clearly labelled and autoclaved at 121-124°C for a minimum of 15 minutes prior to disposal of waste in a yellow bag with yellow tag, via an external contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Project Ref 207/21.14

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<td>22/12/2021</td>
<td>Generation of mitochondrial cybrid cells using fibroblasts from spinal muscular atrophy</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>
**Project Additional Information**

**Purposes of the contained use**

To generate retroviral particles for stable transduction of a fibroblast cell line to enable the subsequent generation of mitochondria-depleted cells.

**Recipient or parental organism**

Human fibroblast cells (although any cell type/species could be used).

**Host/vector system**

Phoenix amphotropic packaging cells will be transfected with a retroviral vector on a LZRS backbone. This host/vector system separates the transfer plasmid from the envelope and packaging proteins to ensure that no single plasmid contains all the necessary components necessary to produce viral particles. Therefore, retroviral particles can only be generated following transfection of the YFP-Parkin transfer plasmid into the Phoenix packaging cell line. The viral particles produced can only infect target cells once and will not produce any new viral particles after the initial infection.

**Origin & function**

Phoenix amphotropic packaging cell lines are derived from human embryonic kidney (HEK) 293T cells. These cells contain:

- A temperature-sensitive T antigen that enables the amplification of vectors containing the SV40 ori and thereby considerably increases the expression levels obtained with transient transfection
- A construct capable of producing the gag-pol packaging element that is under control of a RSV promoter. This construct also contains an IRES-CD8 surface marker downstream of the reading frame of the gag-pol construct to monitor gag-pol production
- A construct for the codification of the envelope protein for ecotropic and amphotropic viruses, which is under control of a CMV promoter

YFP-Parkin retroviral vector has a LZRS vector backbone combined to DNA sequences for eYFP (for immunofluorescence detection) and Parkin (to induce mitochondrial depletion). Parkin is a ubiquitin ligase protein that mediates the clearance of damaged mitochondria upon mitochondrial stress.

**Evaluation of foreseeable effects**

No single plasmid contains all the components necessary to produce viral particles, which can only infect target cells once and will not produce any new viral particles after the initial infection. Following transfection of the YFP-Parkin transfer plasmid into Phoenix packaging cells, YFP-Parkin retroviral particles will be collected from the media.
supernatant and used to transduce a cell line of interest. YFP-Parkin will be stably integrated into the host genome, and upon mitochondrial stress, will mediate clearance of damaged mitochondria from the cells.

In the event of accidental exposure, it is unlikely that the GM virus would cause harm to human health because the viral vector on its own is replication incompetent and would be of minimal risk. In addition, the genetic information contained within the retroviral particles is insufficient to generate new infectious virus.

The transfer plasmid can infect cells from all commonly used mammalian species but again is unlikely to spread because the plasmid is replication incompetent. The expression of each construct in the packaging cell line is under the regulation of different promoters to minimize recombination potential and the generation of recombined viral products.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Tubes containing the virus will be disposed of using Virkon immersion for 24 hours to denature the virus prior to safe disposal and steam decontamination (autoclaving). All contaminated materials (mostly tissue culture plasticware) will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile OR using chemical indicators (eg Browne TST indicator test strips). All waste will be decontaminated before disposal in yellow bags with yellow tags.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications
## Project Additional Information

### Purposes of the contained use
To use hESC/IPSC reporter lines to enhance our understanding of the haematopoietic differentiation process and ultimately increase efficiency of HPC/HSC production.

### Recipient or parental organism
Genes will be introduced into (i) human packaging cell line 293T, (ii) human embryonic and induced pluripotent stem cells (ESC and iPSC), (iii) human umbilical cord blood cells, and (iv) mouse non-haematopoietic cells.

### Host/vector system
Third generation amphotropically packaged HIV-1 derived lentiviruses; pLenti, pSico and FUGW derived expression vectors (Addgene).

### Origin & function
Reporter constructs will contain fluorescent markers eGFP or Venus, derived from the jellyfish Aequorea victoria or tdTomato or mCherry, derived from Discosoma sp sea anemone. Expression of these will be driven by human haematopoietic lineage promoters including those of the ID1, GLI1, WNT, RUNX1, VE-CADHERIN, CD43 genes; expression of the markers will report on differentiation at key points in the haematopoietic lineage. The inserted DNA will be reporting the activity of the promoter regions of specific genes only and should not have any effect on the transcribed/translated gene products of those genes.

For reprogramming constructs, a range of genes (including transcription factors or secreted factors) thought to play a role in differentiation of haematopoietic stem cells will...
be inserted. It is intended that combinations of these factors will be expressed to test for the ability to reprogram recipient cells into haematopoietic stem cells. For expression of factors for reprogramming, these will be under tight control of doxycycline inducible promoter system where expression is reliant upon the addition of doxycycline to derepress.

Evaluation of foreseeable effects

The third generation lentiviral vectors used contain a number of safety features that render them replication incompetent and once transduced the human cell lines will pose a negligible risk of transmission. Some vectors contain the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) to enhance both virus titre and expression of introduced genes. This element can enhance the yield of expressed protein product and may increase the associated risk. However the WPRE is capable of expressing part of the X protein of WHV, and may exhibit oncogenic properties. Injection of lentivirus with the WPRE into mice caused liver tumours. This suggests that the vector backbone may be inherently oncogenic, before it is manipulated to express genetic material and SACGM (2004) recommended this requires containment level 2. The expressed marker, eGFP, is not harmful per se and is not expected to change the behaviour of the cells into which it is introduced. It is not expected to change the host range or infectivity of the vectors used to introduce it into the target cells.

293FT, embryonic /IPS and mouse cells are not infectious and will not colonise a human subject and will not survive outside tissue culture facilities. Genes important for haematopoietic differentiation and genes identified in embryonic HSCs and precursors from gene-expression analysis (specific genes to be decided) but may include oncogenes are on the whole considered low risk. The transduced stem cells and their differentiated progeny cannot assemble the infectious particles. Virally encoded transgenes may be expressed in higher than normal levels because of the presence of the WPRE sequence. For expression of oncogenes if identified in expression screens of stem cells or their precursors then the risk would be thought be low in most cases as the expression is regulated by drugs (eg doxycycline). If the virus were to be introduced into human tissue it may be possible that expression of a normal gene product or oncogene could be produced at levels above that normally found and hence may present a very low but real hazard.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection

All solid and liquid waste must be decontaminated/disposed of by the user at the end of every TC session.

Surfaces will be sprayed 1% Trigene followed by 70% IMS after use. Any spills outside of the hood (on any hard surface, eg. benchtop, incubator or floor) must be wiped down immediately with 1% Trigene/Chemgene then sprayed with 70% ethanol as above.

Solid waste will autoclaved after every session (as described above) before final disposal by incineration via the yellow bag route. Any liquid potentially containing virus should be collected in an autoclavable screw top plastic bottle (stored under the sink). It should NEVER come into contact with the vacuum system. The liquid waste should be chemically disinfected by soaking in 1% w/v Virkon, or 1,00ppm Precept (four 0.5g tablets into 1 litre of liquid) for 24 hours before discharging via the drains. The container must be labelled with name, group and date.

Autoclaving

All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware.

Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile.
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Animal Units

- Large Scale Activities
- Human Clinical Applications

Project Ref 207/21.3

Understanding the changes in gene expression that allow pathogenic fungi Candida spp to survive stress and adapt to hosts

Date Ackn'd 18/06/2021

CU2 Project Title

Consent Granted

Class 2

1-50 Litres

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Page 5049 of 15326
**Project Additional Information**

**Purposes of the contained use**

Increased understanding of pathogen biology to inform future treatment of candidiasis and other conditions caused by these organisms.

**Recipient or parental organism**

This work will use pathogenic Candida species (principally Candida glabrata and Candida albicans, and specifically not Candida auris). Strains include SC5314, CAI4, RM1000, BWP17 and SC5314 (C. albicans) and BG2 and CBS138 (C. glabrata).

These organisms are commonly present as part of human commensal flora but are opportunistic pathogens causing mucocutaneous infection of several body systems, most commonly the mouth, oesophagus and vagina. Risk factors include pregnancy, the use of intrauterine devices and oral contraceptives as well as antibiotic use and diabetes. Systemic disease only occurs in severely immunocompromised individuals. Infection by inhalation of aerosols is not known to occur.

Standard non-pathogenic laboratory Eschericia coli strains (K12 background) will also be used.

**Host/vector system**

**E coli:** pMB1-based non-conjugative plasmids. Standard ampicillin and kanamycin resistance genes, standard fluorescent protein-encoding genes (GFP, RFP etc.) adapted from ACDP 1 invertebrates. Regulatory sequences from Candida species and related yeasts, engineered inducible promoters, engineered tags (His, FLAG, etc). Standard antibiotic resistance markers (Amp/Kan) will be used.

**Candida:** Vectors for CRISPR Cas9 technology, solo system: pV1093 or pV1200. Duet System: pV1025 and pV1090 (Vyas et al. [2015] Sci Adv. 1, e1500248). Genomic integrations using PCR-amplified or restriction digested DNA. No mobilisable vectors involved. Antifungal markers Nat/Geo may be used.

**Origin & function**

Fluorescent or other standard marker proteins derived from non-pathogenic invertebrates (GFP, mScarlet). Regulatory sequences from Candida spp and related yeasts as well as engineered inducible promoters. Engineered tags (FLAG, His etc.). Selectable resistance markers for yeasts and bacteria (Kan, amp, Nat, Geo). Sequences required for CRISP/Cas9 engineering (adapted from S. pyogenes and Candida-derived guide RNA sequences).

**Evaluation of foreseeable effects**

None of the proposed manipulations will introduce genes for which there is any evidence of additional harmful effects to humans or the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid waste and solid materials that have been in contact with microorganisms will be sterilised by autoclaving before disposal by the approved local routes.
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Project Ref 207/21.4

Date Ackn'd 09/07/2021

Date Project Ceased

CU2 Project Title Pigs with disrupted CDKL5

Class

Culture

Volume

Class2

Class3-4

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

CDKL5 Deficiency Disorder (CDD) in humans is a neurodevelopmental encephalopathy characterised by profound impairments, which include autism spectrum disorder (ASD), intellectual disability (ID), epilepsy, movement disorders and autonomic dysfunction. Rodent models of the disease do not develop the full range of phenotype observed in humans. We will produce pigs with altered CDKL5 in order to discover how the disease will manifest in animals with larger brains.

**Recipient or parental organism**

Pig

**Host/vector system**

CRISPR/Cas9 modification of the endogenous pig CDKL5 gene

**Origin & function**

CDKL5 Deficiency Disorder (CDD) in humans is caused by de novo genetic mutations in the cyclin dependent kinase-like (CDKL5) gene. It is a neurodevelopmental encephalopathy characterised by profound impairments, which include autism spectrum disorder (ASD), intellectual disability (ID), epilepsy, movement disorders and autonomic dysfunction. It is possible that modification of the pig CDKL5 gene will result in animals with altered temperament and/or development of seizures. These changes, if observed, may result in the animals being more aggressive.

**Evaluation of foreseeable effects**

Animals will be housed in secure pens which are 1.5m in height as required by the HO regulations. The pens are contained within a locked facility with access controlled by the facility manager and limited to authorised personnel only. There will always be a minimum of 2 barriers between the animal and the external environment. Animals will be cared for by experienced staff. All staff involved in the care of these animals will be aware of the potential for altered behaviours associated with the genetic modification.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Animals will be housed in secure pens which are 1.5m in height as required by the HO regulations. The pens are contained within a locked facility with access controlled by the facility manager and limited to authorised personnel only. There will always be a minimum of 2 barriers between the animal and the external environment. Animals will be cared for by experienced staff. All staff involved in the care of these animals will be aware of the potential for altered behaviours associated with the genetic modification.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Following euthanasia the GM animal is completely inactivated, and then all carcasses will either be incinerated or rendered.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

02/03/2022
The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

<table>
<thead>
<tr>
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<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
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<td>L3</td>
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**Project Ref 207/21.5**

- Date Ackn'd: 05/08/2021
- CU2 Project Title: Development of an induced ovine pulmonary adenocarcinoma model with both replication competent and replication deficient Jaagsiekte Sheep Retrovirus
- Class: Class 2
- CultureVolClass2: < 1 Litre
- Consent Granted

**Project Additional Information**

**Purposes of the contained use**

Ovine pulmonary adenocarcinoma (OPA) is an infectious lung tumour of sheep caused by the betaretrovirus, jaagsiekte sheep retrovirus (JSRV). OPA is widespread throughout the world and causes significant economic and animal welfare issues. The mechanism of pathogenesis in OPA is not fully understood but it has been established that the JSRV Env glycoproteins are capable of transforming fibroblasts and epithelial cell lines in vitro and therefore act as viral oncogenes. In addition, delivery of Env-coding regions to the lungs of mice and sheep in vivo, using viral vectors, results in the growth of lung tumours histologically similar to those present in...
One difficulty in OPA research is that there is no permissive culture system for propagating JSRV in vitro. An important advance in JSRV research was the development of an infectious molecular clone that generates infectious virus when transfected into 293T cells. This in vitro-derived virus causes OPA when inoculated intra-tracheally into young lambs. Importantly, this molecular clone (pCMV2JS21) can be modified to produce mutant viruses, which can be used in vivo to investigate specific aspects of JSRV biology. One derivative of pCMV2JS21 is a replication-defective virus encoding only the JSRV Env protein.

This project will utilise infectious JSRV derived from pCMV2JS21, which although derived using recombinant DNA methods is identical to naturally occurring wild-type virus is still considered a GM virus. In addition, we will use a replication-defective JSRV vector derived from pCMV2JS21. This vector is able to infect cells in a single round of infection and subsequently express the JRV Env protein, but it is unable to spread further.

In terms of lung cancer research, OPA has significant similarities in histological appearance, activation of oncogenic signalling pathways and transcriptional profiles to certain types of human lung adenocarcinomas. These findings have resulted in OPA being proposed as an excellent model to study human lung cancer.

The overriding aims of this study is to understand the infectious process of JSRV. This knowledge is essential for:
1. Developing OPA control methods which can be used in eradication programmes or assurance schemes.
2. Developing an OPA model which can be used in translational studies for human lung cancer research.

Genetically modified replication-defective JSRV (RD-JSRV) and JSRV will be produced at Moredun Research Institute (MRI), covered by their own risk assessments (work to produce the vectors at MRI has been notified to HSE (GM172/06.1)). Virus will be transferred to the Large Animal Research and Imaging Facility (University of Edinburgh) for use in this study.

Using 3–12-month-old sheep, the viruses produced at MRI will be delivered via a bronchoscope into a subsegmental bronchus. Tumour development will be monitored over a 9-month period using thoracic ultrasound and CT imaging. Blood, nasal swabs and exhaled breath condensate will also be collected during this time. The CT scan data will be used to generate 3D images indicating the size and spatial location of tumours. The concentration of JSRV/RD-JSRV in each breath condensate and nasal swab sample will be estimated as copy number/volume by RT-qPCR. RD-JSRV should be undetectable as it cannot replicate.

**Recipient or parental organism**

Wild type JSRV particles contain 2 copies of single-stranded positive sense RNA. Its genome of approximately 7,460 nucleotides contains 4 genes encoding viral proteins. These 4 genes are: gag (encoding the matrix, capsid and nucleocapsid proteins); pro (encoding aspartic protease); pol (encoding reverse transcriptase and integrase enzymes) and env (encoding surface and transmembrane envelope glycoproteins). An additional open reading frame, known as orfX, which overlaps with the pol gene, has also been identified; however, it is not required for in vitro cellular transformation or in vivo oncogenesis. Interestingly, JSRV-induced neoplastic transformation is mediated by the viral Env glycoprotein, although the mechanisms underlying this process are not completely understood. The transforming activity of Env was first shown in vitro using rodent fibroblasts, with subsequent in vivo experiments showing that the administration of viral vectors expressing Env to the lungs of mice and sheep results in adenocarcinoma formation. Env localisation at the plasma membrane may enable it to interact with other molecules such as protein kinases, leading to the activation of downstream pathways that promote cellular proliferation and survival. The Ras-MEK-ERK and PI3K-AKT-mTOR pathways are commonly activated in OPA tumours; others may include EGFR, RON-HYAL2 and heat shock proteins. Following pathway activation, it is likely that further mutations are required for tumours to develop, such as telomerase activation, the activation of other cellular oncogenes or the inactivation of tumour-suppressor genes.

**Host/vector system**

RD-JSRV, produced by co-transfection of 293T cells with 2 plasmids. The first is a vector plasmid (CJ-Env), derived from the infectious molecular clone pCMV2JS21. This vector has a deletion that removes most of the Gag and Pol coding regions, rendering the virus replication-defective. The second is a packaging plasmid that encodes the JSRV Gag-Pol coding regions only. Transfection of cells with these 2 plasmids produces vector particles capable of a single round of infection but that in the recipient cell express only JSRV Env – see next section.
Replication competent JSRV (wild type): pCMV2JS21 has the U3 region of the viral 5’ LTR substituted with the CMV-IE promoter (derived from pcDNA3 by PCR). These proviruses will be used to transfect mammalian cells (293T) in order to produce infectious virions.

Molecular clones of replication competent JSRV:

Replication competent JSRV (wild type): pCMV2JS21 has the U3 region of the viral 5’ LTR substituted with the CMV-IE promoter (derived from pcDNA3 by PCR). These proviruses will be used to transfect mammalian cells (293T) in order to produce infectious virions.

Molecular clones of replication-deficient JSRV:

The vector derives from the infectious molecular clone pCMV2JS21 and is produced by transfecting cells with two plasmids.

The vector plasmid, pCJ-Env, is a modified form of pCMV2JS21 in which the majority of the regions encoding the JSRV Gag and Pol protein have been deleted. This plasmid can therefore express only the JSRV Env protein and cannot direct the production of viral particles. This plasmid also retains elements of the JSRV genome including LTR regions and the packaging signal.

Production of viral-like particles is mediated by co-transfecting 293T cells with pCAG-JS-GP-CTE2X, a helper packaging plasmid that expresses the JSRV Gag, Pro and Pol proteins that form the JSRV capsid. This plasmid also contains two copies of the Mason Pfizer monkey virus constitutive transport element (CTE) for optimal export of unspliced mRNA and a poly A signal derived from the bovine growth hormone. This plasmid does not contain the JSRV packaging signal.

Co-transfection of these 2 constructs into 293T cells will result in the production of vector particles (RD-JSRV) capable of delivering the pCJ-Env genome into target cells in a single round of infection but incapable of replicating further. Homologous recombination to produce a replication competent retrovirus is not possible since there are no sequences in common. Therefore, spread to new cells beyond those receiving the “first hit” or between animals is not possible. Since the packaging signal (Ψ) is present, there remains a theoretical risk that subsequent (inadvertent) infection of an animal carrying the CJ-Env transgene with wild type JSRV could mobilise this vector. However, such mobilisation would not have any increased hazard above that of wild-type JSRV infection, since the env gene is encoded by wild type JSRV. In addition, the risk of cross-infection of experimental animals is negligible due to animal containment procedures.

Expression of JSRV Env in the RD-JSRV vector is controlled by the JSRV LTR thereby restricting expression to type-II pneumocytes and club cells. This means that the risk associated with this vector is no more than wild type JSRV (indeed, less so because RD-JSRV is replication-defective).

Evaluation of foreseeable effects

Hazards of wild-type JSRV to human health
Low risk. Although human lung cells have cellular receptors (HYAL2) to allow virus entry there has been no reported cases of human JSRV-induced disease and JSRV is not considered a human pathogen.

Hazards of RD-JSRV to human health

(i) Hazards associated with the recipient microorganism (e.g. bacterial host or viral vector)
Low risk. RD-JSRV is a disabled version of the wild-type JSRV. It carries the same oncogene as wild-type JSRV and utilises the same determinants (receptor and promoter (LTR)) are the same as the wild-type virus. It has the same envelope proteins and LTRs as wild type JSRV and therefore have the same tropism as wild type virus. Therefore, these vectors represent even less of a risk to human health than JSRV virions. Retroviral vectors used in this programme will not be replication competent and therefore have no risk of spreading to persons other than those directly handling the vectors.

There are no factors specific to this work that increase the risk to any one group. However, severely immunosuppressed workers should not handle transformed human cell lines and pregnant women should avoid working with sheep due to the risk that the sheep harbour other zoonotic agents.

Hazards to the environment.
Low risk. Wild type JSRV causes ovine pulmonary adenocarcinoma (OPA) in sheep and transmission is principally via the respiratory route. OPA is endemic in the UK and given the scale of the experiments it is extremely unlikely that a breach of containment would lead to a measurable increase in the incidence or prevalence of OPA. In addition, experience with infectious lung fluid from sheep with OPA, and with virus produced in vitro, indicates that the amount of virus released from sheep with experimental OPA is less than that used in the experimental inoculum (i.e., we get back less than we put in).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**Hazards of wild-type JSRV to human health**

Low risk. Although human lung cells have cellular receptors (HYAL2) to allow virus entry there has been no reported cases of human JSRV-induced disease and JSRV is not considered a human pathogen.

**Hazards of RD-JSRV to human health**

(i) Hazards associated with the recipient microorganism (e.g. bacterial host or viral vector)

Low risk. RD-JSRV is a disabled version of the wild-type JSRV. It carries the same oncogene as wild-type JSRV and utilises the same determinants (receptor and promoter (LTR)) are the same as the wild-type virus. It has the same envelope proteins and LTRs as wild type JSRV and therefore have the same tropism as wild type virus. Therefore, these vectors represent even less of a risk to human health than JSRV virions. Retroviral vectors used in this programme will not be replication competent and therefore have no risk of spreading to persons other than those directly handling the vectors.

There are no factors specific to this work that increase the risk to any one group. However, severely immunosuppressed workers should not handle transformed human cell lines and pregnant women should avoid working near the risk that the sheep harbour other zoonotic agents.

**Hazards to the environment.**

Low risk. Wild type JSRV causes ovine pulmonary adenocarcinoma (OPA) in sheep and transmission is principally via the respiratory route. OPA is endemic in the UK and given the scale of the experiments it is extremely unlikely that a breach of containment would lead to a measurable increase in the incidence or prevalence of OPA. In addition, experience with infectious lung fluid from sheep with OPA, and with virus produced in vitro, indicates that the amount of virus released from sheep with experimental OPA is less than that used in the experimental inoculum (i.e., we get back less than we put in).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Disinfection**

Anistel (1%) has been validated for use against other enveloped viruses including HIV-1 and hepatitis B virus virus and will be used according to the manufacturer’s instructions; 1:200 with a minimal contact time for hard surfaces of 5 minutes.

Virkon® broad spectrum virucidal disinfectant used according to the manufacturer’s instructions. This disinfectant has proven virucidal efficacy against adenoviridae, parvoviridae, retroviridae and paramyxoviridae. 1% Solution will be prepared as follows: add 10 gm of powder to a litre of warm water and stir until dissolved. Once made up Virkon remains active for 5-7 days. A dye is included with Virkon which is pink in its oxidised form but becomes colourless in its reduced form – indicating that the solution should be replaced. The required minimal contact time for hard surfaces is 10 minutes.

**Autoclaving**

All contaminated materials will be inactivated by autoclaving (100% kill) at 121-134°C with a holding time of at least 15 minutes, prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile. Each run will be monitored using chemical indicators (eg Browne TST indicator test strips).

Solid laboratory waste: Autoclaved in-house.
Liquid laboratory waste: Autoclaved in-house or treated with Anistel or Virkon disinfectant and disposed in general drainage.

Animal waste: Bedding will be disposed of by composting. Previous analysis has shown that no JSRV RNA can be detected in bedding of inoculated animals using quantitative real-time PCR.

Animal carcasses will be double bagged and disposed of by rendering. Tissue retained at post-mortem will be processed and handled as laboratory waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

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Animal Units

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<td>L3 L4</td>
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Project Ref 207/21.6

Date Ackn'd: 06/08/2021

CU2 Project Title: Causes of neurodegeneration in Alzheimer’s disease, ageing, and related degenerative disorders

Date Project Ceased: 02/03/2022

Class: Class 2

Culture Vol Class 2: < 1 Litre

Consent Granted: Non-GMM

Project notified under transitional arrangements N
### Project Additional Information

**Purposes of the contained use**

In this project, we will use tissue from animal models of Alzheimer's and related neurodegenerative diseases to study the causes of brain degeneration and to test candidate therapeutic pathways. We will test the hypothesis that overexpression of a subunit of the ECM protein, Laminin, can ameliorate toxicity caused by amyloid beta which leads to loss of the connections between brain cells and eventual cell death.

**Recipient or parental organism**

Cultured mouse organotypic brain slice cultures from non genetically altered (wild type) and GM mouse pups. Example GM lines to be used include: APP/PS1, C3Ko. Hazard group 1. GM animal tissue used will pose no threat to human health and can be worked with under category 1.

**Host/vector system**

Lentiviruses will be transduced into mouse organotypic brain slice cultures. (Lentiviral vectors: VSV-G pseudotyped third-generation lentivirus). Lentiviral vectors are derived from HIV, which is a member of the retrovirus family. Wildtype lentivirus has a plus-strand linear RNA genome.

A lentiviral vector is first constructed as a plasmid in E. coli. It is then transfected into packaging cells along with several helper plasmids. Inside the packaging cells, vector DNA located between the two long terminal repeats (LTRs) is transcribed into RNA, and viral proteins expressed by the helper plasmids further package the RNA into virus. Live virus is then released into the supernatant, which can be used to infect target cells directly or after concentration.

When the virus is added to target cells, the RNA cargo is shuttled into cells where it is reverse transcribed into DNA and randomly integrated into the host genome. Any gene(s) that were placed in-between the two LTRs during vector cloning are permanently inserted into host DNA alongside the rest of viral genome. By design, lentiviral vectors lack the genes required for viral packaging and transduction (these genes are instead carried by helper plasmids used during virus packaging). As a result, virus produced from lentiviral vectors has the important safety feature of being replication incompetent (meaning that they can transduce target cells but cannot replicate in them).

**Origin & function**

The Lamb1 gene from the mouse genome will be expressed in lentivirus to cause production of the laminin beta 1 protein. This is an extracellular matrix glycoprotein. When expressed in genetically modified Drosophila, this protein protects cells from toxicity of amyloid beta, one of the key pathologies in Alzheimer's disease. Here we will test whether this also occurs in mammalian models (mouse brain slices in culture). To identify transduced cells, the virus will also express green fluorescent protein (originally derived from the jellyfish Aequorea victoria). As a control, another lentivirus will be used expressing the fluorescent protein mCherry (originally derived from a modified dsRed protein expressed in Discosoma sea anemones).

**Evaluation of foreseeable effects**

By design, lentiviral vectors lack the genes required for viral packaging and transduction (these genes are instead carried by helper plasmids used during virus packaging).
As a result, virus produced from lentiviral vectors has the important safety feature of being replication incompetent (meaning that they can transduce target cells but cannot replicate in them).

Whilst there is no known oncogenicity of the Lamb1 protein, a literature search suggested there may be a link with Lamb1 overexpression and cancer. To mitigate this small risk, the viral titre used will be very low, the virus used will be replication incompetent, and appropriate PPE/ safety measures will be taken.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

No sharps will be used other than glass coverslips (which need be used as we have no viable alternative).

Any broken glass which is likely to feature a viable GM component will be disposed of in a sharps bin and autoclaved with a regimen consistent with deactivation of GMO’s before the container is sealed and tagged for uplift.

Any broken glass with no hazardous considerations will be disposed of in a regular Haveshaps bin. All users will be made aware of potential for injury due to broken glass and disposal routes.

All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile.

We will decontaminate all waste before disposal: yellow bag with yellow tag to be used.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

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**Project Additional Information**

**Purposes of the contained use**

Gain understanding on how fungal pathogens adapt and thrive under different stress conditions with the aim to inform future treatment of candidiasis and other conditions caused by these organisms.

**Recipient or parental organism**

This work will use pathogenic Candida species (principally Candida albicans and Candida glabrata). Strains include: SC5314, CAI4, CAI8, RM1000, BWP17, SN78 (Candida albicans) and BG2 and CBS138 (Candida glabrata) and ambient, non-GMO, strains of Candida albicans and Candida glabrata (NHS provided). All strains are HG2.

The above organisms are commonly present as part of human commensal flora but are opportunistic pathogens causing mucocutaneous infection of several body systems, most commonly the mouth, oesophagus and vagina. Risk factors include pregnancy, the use of intrauterine devices and oral contraceptives as well as antibiotic use and diabetes. Systemic disease only occurs in severely immunocompromised individuals. Infection by inhalation of aerosols is not known to occur.

Standard non-pathogenic laboratory Eschericia coli strains (K12 background) and standard non-pathogenic Saccharomyces cerevisiae strains, with a history of safe use (W303, S288C, SK1) will also be used as part of this work. These strains are HG1.
### Host/vector system

1. Specific non-mobilisable Candida spp vectors (low copy replicating and integrating vectors [YPB1, Clp10, Clp20, Clp30]; low copy replicating and integrating vectors for ectopic expression [YPB-ADHpt, pACT1]; integrating expression vectors regulated by methionine [MET3 promoter], maltose [MAL2 promoter], glucose [PCK1 promoter], or doxycycline [tet ON/OFF promoter]; CRISPR Cas9 technology. Solo System: pV1093 (Amp) or pV1200 (NAT1+Amp). Duet System: pV1025 (NAT1+Amp) and pV1090 (Amp).

2. pRS vectors, YIPlac (integration), YCPlac (centromere based), YEPlac (2-micron), PCR tagging vectors (epitope tagging).

3. Non-mobilisable E. coli vectors (pUC-derived vectors, such as: pBluescript, pGEM-T, and bacterial expression vectors: pET vectors, pGEX, pQE).

### Origin & function

Fluorescent or other standard marker proteins derived from non-pathogenic invertebrates (GFP, YFP, tdTomato). Regulatory sequences from Candida spp and related yeasts as well as engineered inducible promoters. Engineered tags (FLAG, His, etc.). Selectable resistance markers for yeasts and bacteria (Kan, Amp, Nat, Geo). Sequences required for CRISP/Cas9 engineering (adapted from S. pyogenes and Candida-derived guide RNA sequences)

### Evaluation of foreseeable effects

None of the proposed manipulations will introduce genes for which there is any evidence of additional harmful effects to humans or the environment. No known toxins or allergens will be produced as a result of insertion in E. coli, Candida albicans, Candida glabrata or S. cerevisiae and inserts will not affect the pathogenicity of any host.

Briefly, insert code for normal or mutant forms of yeast genes involved in cell cycle control, chromosome segregation or regulation of metabolism, often with short epitope tags added to enable immunological detection of expressed proteins, or GFP tags for localisation studies. These yeast genes are not expressed to have harmful physiological or pharmacological properties, or to affect pathogenicity of the cloning host or human defence mechanisms. Some examples of genes to be targeted are those of the Kinetochore complex (CHL4, MCM21, CTF3) and the cohesin complex (MCD1, SCC2) in order to generate null mutants or conditional knockouts. Deletion of those genes will render the strains less virulent than their isogenic strains. No null mutations have been described that increase the virulence of C. albicans or C. glabrata.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste and solid materials that have been in contact with microorganisms will be sterilised by autoclaving before disposal by the approved local routes.

Is an emergency plan required according to regulation 20?  

| N |

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y
### Project Containment

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**Animal Units**

| L2 | L3 | L4 | L2 | L3 | L4 |

**Large Scale Activities**

| L2 | L3 | L4 | L2 | L3 | L4 |

**Human Clinical Applications**

| L2 | L3 | L4 | L2 | L3 | L4 |

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### Project Ref

**Project Ref** 207/21.8

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**Non-GMM Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

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### Historical Significant Changes

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

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### Project Additional Information

**Purposes of the contained use**

To trace and image the axons and cell bodies of neurons that provide input to layer 1 cells in the entorhinal cortex. This will inform us of the nature of the information that layer 1 cells are processing and help set up future experiments that will establish how these inputs contribute roles of the entorhinal cortex in spatial cognition and memory.

**Recipient or parental organism**

NDNF-iRES-Cre mouse line (Strain: C57BL/6JCrj) [Jackson Lab Stock No: 030757].

Neuron-derived neurotrophic factor (NDNF) is a specific molecular marker for neurons in layer 1. By driving expression of Cre recombinase from the NDNF1 promoter is is possible to target a significant portion of the entorhinal layer 1 population.
Host/vector system

EnvA-pseudotyped B19G-deleted rabies virus: pSADB19dG-mCherry
Helper Vector: pAAV-Syn-FLEX-nGToG-WPRE3

Origin & function

The helper vector is an AAV vector that mediates Cre-dependent expression of the TVA receptor, B19oG and nuclear localised green fluorescent protein. Because in the NDNF-IRES-Cre line the expression of Cre is restricted to layer 1 neurons, the gene products encoded by the AAV will be expressed only in these neurons.

SAD-B19 is a genetically engineered rabies virus that has had the B19G glycoprotein removed and has been pseudotyped with the envelope protein EnvA of the Avian Sarcoma and Leukosis virus. As a result, it cannot infect mammalian cells unless they have been modified to express the TVA receptor. Once inside the layer 1 neurons that have already been infected with the helper vector, SAD-B19 can replicate and label layer 1 cells (first-order) and their presynaptic neurons (second-order). It can not spread any further as the second order cells will lack the TVA receptor and B19oG protein.

Evaluation of foreseeable effects

SAD-B19 has been shown to be extremely stable in vivo and has been used in numerous studies without evidence of its reversion to wildtype. It is not expected to be harmful to the host organism or cause any gross phenotypic or behavioural abnormalities.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All NDNF mice will be housed in standard cages in groups of up to 4 before and after surgery. They will be kept at all times within the animal research unit in the Hugh Robson Building, which is a containment level 2 area. All staff have been trained and assessed in handling laboratory mice. Researchers have been trained and assessed by a Named Veterinary Surgeon to perform surgery safely, ensuring the trainee is competent when handling and transporting the animals.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

SAD-B19 will be disposed in sharps bins with a yellow bin, purple lid and yellow tag (for cytotoxic/static waste sharps). For animals injected with the virus, they will be sacrificed 3-4 weeks after surgery by exsanguination during a paraformaldehyde (PFA) perfusion procedure, they will then be decapitated and the brain will be extracted and kept in a PFA solution. The anatomical waste will be placed into an autoclave bag for sterilisation. Following autoclaving the bag will be transferred to yellow clinical waste bags with red tags for disposal via our clinical waste contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

02/03/2022

Page 5063 of 15326
The recently emerged pathogenic SARS-CoV-2 virus continues to cause a major human pandemic. Animal models which accurately reflect the human disease would be of great value for pathogenesis studies and antiviral testing. Current animal models developed for SARS include small rodent models, such as mice engineered to express the human receptor for this virus, hACE2. Although these models demonstrate a disease state following challenge, they do not accurately reflect human disease, with mortality resulting from significant infections in the brain. Furthermore, the scale and gross anatomy of rodents is very different from humans. In contrast, the anatomy of pigs is more similar, with pig lungs equivalent to human lungs in size and structure. A transgenic pig expressing hACE2 will represent an invaluable model for Covid-19 research.

We aim to use a lentiviral vector encoding the hACE2 cDNA under the control of a cytokeratin promoter to produce transgenic pigs. The lentiviral vector will be injected into the perivitelline space of pig zygotes, which will then be transferred to recipients. Additional zygotes may be cultured to blastocyst stage for in vitro analysis. Piglets will be born and genotyped (GMAni0621). Tissues will be collected and cell cultures established at Roslin (covered in the class 2 risk assessment GM 0421). These will be assessed for infectivity by SARS-CoV-2 in the Roslin Institute Building labs under a separate risk assessment. This RA covers the transgenic pigs. It does not cover any

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**Project Ref** 207/21.9

**CU2 Project Title**

Pigs with human ACE2 transgene

**Date Ackn'd** 29/09/2021

**Class** Class 2

**Culture Vol** < 1 Litre

**Non-GMM** Consent Granted

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**Project Additional Information**

**Purposes of the contained use**

The recently emerged pathogenic SARS-CoV-2 virus continues to cause a major human pandemic. Animal models which accurately reflect the human disease would be of great value for pathogenesis studies and antiviral testing. Current animal models developed for SARS include small rodent models, such as mice engineered to express the human receptor for this virus, hACE2. Although these models demonstrate a disease state following challenge, they do not accurately reflect human disease, with mortality resulting from significant infections in the brain. Furthermore, the scale and gross anatomy of rodents is very different from humans. In contrast, the anatomy of pigs is more similar, with pig lungs equivalent to human lungs in size and structure. A transgenic pig expressing hACE2 will represent an invaluable model for Covid-19 research.

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Work involving infection with SARS-CoV-2. Any work using SARS-CoV-2 to infect cells or pigs will be performed in a CL3 laboratory under a separate risk assessment.

Recipient or parental organism

pig

Host/vector system

Second generation lentivirus expressing human ACE2

Origin & function

We will use a second generation lentiviral vector encoding the hACE2 cDNA under the control of a human cytokeratin 18 promoter to produce transgenic pigs. This will be microinjected into the perivitelline space of pig zygotes, infect the cell and integrate into the genomic DNA.

The lentiviral vector system which we will use has several safety features included to minimise the potential of replication competent lentivirus (RCL) production, or subsequent mobilisation of the integrated transgene from the genome of the host cell. There is a very low risk of recombination with endogenous retroviruses due to the division of synthetic viral elements (i.e. coding sequences have been significantly altered from the original) among three different plasmids. The packaging and envelope plasmids (psPAX2 and pVSVG respectively) express viral accessory proteins in the HEK293 packaging cell line such that the desired transgene elements, inserted between the lentiviral LTRs, are packaged into the lentiviral pseudoparticle. The endogenous HIV-1 envelope glycoprotein has been replaced with VSV-G; while this increases the tropism of viral particles, it is also toxic to cells that express it. As the psPAX2 and pVSVG plasmids do not contain the lentiviral packaging signal sequence they are not packaged into the lentiviral pseudoparticle. This feature means that the elements required for producing lentiviral pseudo particles are only present in the transfected HEK293 packaging cells and not in cells that are subsequently transduced.

2nd generation lentiviral vectors are termed “self-inactivating” by virtue of a deletion in the 3’ LTR. This deletion does not affect the transcription and packaging of the recombinant viral genome in the producer cell line, but following reverse transcription results in a non-functional 5’ LTR in the integrated genome. This feature minimises the risk of mobilisation of the integrated construct.

As a consequence the resultant particles are single-round infectious. I am not aware of any literature reporting RCL or mobilisation of the integrated transgene from 2nd generation lentiviral systems. The relevant genetic elements encoded within the lentiviral vector will be the human ACE2 cDNA under the control of the human cytokeratin 18 promoter. It is intended this promoter/transgene combination will enrich and ideally restrict transgene expression to epithelial cells, such that epithelial cells of the respiratory tract of the transgenic pigs will express hACE2. No mammalian selection cassette is present within the integrated transgene.

Evaluation of foreseeable effects

The transgenic pigs will not have altered toxicity, allergenicity or behaviour. Through expression of the human ACE2 transgene it is anticipated that they will be more readily infected by the SARS-CoV-2 virus, and if infected are likely to shed virus. If the transgenic pig were to become infected with SARS-CoV-2 there is a possibility that it could become sick.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Animals will be housed in secure pens which are 1.5m in height as required by the HO regulations. The pens are contained within a locked facility with access controlled by the facility manager and limited to authorised personnel only. There will always be a minimum of 2 barriers between the animal and the external environment. Animals will be cared for by experienced staff wearing appropriate PPE. All staff involved in the care of these animals will be aware of the potential for altered pathogenicity associated with the genetic modification. Animals will be segregated by sex at weaning and maintained in single sex groups throughout life, other than to facilitate planned breeding.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Following schedule 1 culling the GM organism can be considered as inactivated and will be disposed of by either incineration or rendering.

02/03/2022
Disinfection
All laboratory surfaces that come into contact with tissues/cells from these animals will be wiped with a 1% Virkon solution. Culture plates and pipettes will be soaked in a 1% Virkon solution overnight before disposal.

Autoclaving
All contaminated materials will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored using digital read outs.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 207/22.1

Date Ackn'd  25/02/2022

CU2 Project Title
Deciphering the role of secreted proteins in Toxoplasma gondii infection

Class  Class 2
CultureVol Class 2  < 1 Litre
CultureVol Class 3-4

Non-GMM  Consent Granted

Project notified under transitional arrangements  N
This work will investigate parasite proteins for their role in establishing chronic infections. By analysis of their expression in wild type Toxoplasma and by perturbation in genetically modified parasites our objective is to understand how they function in formation of the chronic cyst and parasite survival.

This work will include several arms of genetic manipulation:
1. Generating pools of parasites where each parasite has a different gene disrupted by CRISPR/Cas9. Using this method, 100s of genes can be targeted in the same parasite flask and the important genes are identified by determining which parasites grow more slowly or are unable to form a healthy cyst.
2. Targeting of individual genes of interest by generating conditional or complete gene knockout, and adding tags for localisation/biochemical purposes.
3. Inserting fluorescent or bioluminescent proteins to be able to follow parasite differentiation of infection patterns.

**Recipient or parental organism**

Toxoplasma gondii

**Host/vector system**

All vectors used are standard non-transmissible bacterial plasmids, with drug selection markers for use in bacteria and Toxoplasma. They include promoters to control expression of parasite genes of interest, or alternatively are intended to disrupt parasite genes by inserting selection markers in place of the gene.

**Origin & function**

We will be investigating novel and identified genes proposed to be involved in Toxoplasma differentiation and survival as chronic stage parasites. These genes are all of Toxoplasma gondii origin.

Additionally, fluorescent/bioluminescent proteins will be expressed for tracking parasite growth or differentiation.

Origin: Aequorea Victoria, Lampyridae

Cas9 from Streptomyces pyogenes will be used for generating mutations in parasite genes.

**Evaluation of foreseeable effects**

Alteration of parasite virulence

We are targeting genes involved in establishing Toxoplasma chronic infection. Evidence to date indicates that perturbation of these molecules in genetically modified parasites will either not alter or reduce viability. Parasites failing to form chronic cysts do not show increased virulence in mice (Waldman et al 2020, Cell 180 (2)), while parasites lacking GRA2 or GRA6 are less viable in mouse infection (Mercier et al 1998, Infect Immun 66(9)).

Some of the genetically modified Toxoplasma strains may be more virulent in mice or alter the nature of the observed response in tissue culture cells as compared to their
parent strains. Increased virulence in the mouse model could result from expressing a parasite-secreted protein in a strain that doesn't normally express it or from the disruption of a secreted protein. For example, deletion of the parasite protein GRA15 which normally dampens the immune response to the parasite, leads to a more virulent parasite (Rosowski et al 2011, J Exp Med 208 (1)). However, none of the genetically modified Toxoplasma strains will ever be more virulent than any other wild-type strain available, which vary in their expression of these secreted molecules.

This is an unusual outcome to be aware of and reduced virulence or no change is expected when altering expression of these target genes.

Alteration of drug resistance
The use of drug selection markers is required to select for the integration of DNA vectors in to the Toxoplasma genome. Wherever possible, this work will use selection markers that do not impact treatment of a Toxoplasma infection (eg. Using HXGPRT or CAT genes). When essential, the DHFR selection marker may be used (which is widely used in the research field). In this case the parasites will be resistant to standard drug treatment and potential infections will need to be treated with Atovaquone + sulfadiazine instead of the Pyrimethamine (+sulfadiazine).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
GM Toxoplasma will be used to infect wild type mice. Mice infections (intraperitoneal or oral routes) and mouse dissections will be done in a Class II MSC, within a barred CL2 designated room in a contained animal facility. For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Toxoplasma tachyzoites and bradyzoites are susceptible to killing by chlorine-based disinfectants, 70% ethanol and autoclaving (Spickler, Anna Rovid. 2017. Toxoplasmosis. Retrieved from http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php.). Evidence of killing of Toxoplasma by Virkon treatment is included in Appendix 4 of the risk assessment (provided by Dr David Smith, Moredun Research Institute).

Animal bedding and carcasses – autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 C for at least 15 minutes or 126-130 C for at least 10 minutes or 134-138 C for at least 3 minutes). Thereafter, carcasses will be disposed of via clinical waste stream for incineration and bedding via clinical waste stream for microwave treatment.

Waste residues from cell culture or from bacterial cloning which cannot be autoclaved are inactivated by treatment with Virkon. Additionally, syringes, blunt needles, tubes etc that are contaminated with Toxoplasma culture will be disinfected in Virkon overnight before being placed in bins for autoclaving. Infected cell culture supernatants may be aspirated under vacuum into a sealed flask containing Virkon in quantities sufficient to attain 2% final concentration. Contaminated sharp needles will go directly in to a sharps bin, labelled as CL2, and autoclaved for decontamination and disposed of via the clinical waste stream for heat treatment via the University approved contractor. This applies to sharps from tissue culture and the animal unit.

1. Virkon
   1. 1% solution for:
      • Plasticware (pipettes, pastettes) with surface contamination - soak for a minimum of 2 hours fully immersed
      • Treatment of minor contamination (minimum 10 min contact time) and surface disinfection (benches and floors)
   2. 2% final concentration (overnight contact) for disinfection of liquid cultures and supernatants
   3. Powder or granules will be used to both absorb the liquid and disinfect a spillage or use with liquid waste. For spillages, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon to the liquid (minimum contact time 10 minutes) before mopping up with paper towels, wiping the area with 1% Virkon and disposing of all solid waste via the clinical (orange bag) waste stream.
Alcohols 70% v/v or Distel spray will be used for routine wiping down of Class II MSC.

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

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Project Ref 207/95.1

Date Ackn’d 15/03/1995

CU2 Project Title DELETION OF MURINE GAMMA HERPES VIRUS GENES

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4 not applicable

Non-GMM Consent Granted

Tick if notifying a connected programme of work N

Withdrawn N

Project notified under transitional arrangements Y
This work will use pathogenic Candida species (principally Candida albicans and Candida glabrata). Strains include: SC5314, CAI4, CAI8, RM1000, BWP17, SN78 (Candida albicans) and BG2 and CBS138 (Candida glabrata) and ambient, non-GMO, strains of Candida albicans and Candida glabrata (NHS provided). All strains are HG2.

The above organisms are commonly present as part of human commensal flora but are opportunistic pathogens causing mucocutaneous infection of several body systems, most commonly the mouth, oesophagus and vagina. Risk factors include pregnancy, the use of intrauterine devices and oral contraceptives as well as antibiotic use and diabetes. Systemic disease only occurs in severely immunocompromised individuals. Infection by inhalation of aerosols is not known to occur.

Standard non-pathogenic laboratory Eschericia coli strains (K12 background) and standard non-pathogenic Saccharomyces cerevisiae strains, with a history of safe use (W303, S288C, SK1) will also be used as part of this work. These strains are HG1.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
Please enter comments on the GM safety committee on the risk assessment

Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment

### Project Containment

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### Project Ref 207/95.2

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Tick if notifying a connected programme of work

### Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Research into reproduction and reproductive processes in vitro and in vivo using amphotropic retrovirus and lentivirus, adenovirus, human herpes simplex virus type 1 and human cytomegalovirus.

Recipient or parental organism
Bacteria: E. coli K12 (disabled)
Primary cells: derived from humans, mouse, rat and sheep
Immortalised cell-lines: HEK293 cells and derivatives BHK, HeLa, Vero cell lines (for HSV-1) HFFF2 cell lines (for HCMV)
Animal models: Sheep, marmoset, rat, and mice.

Host/vector system
Retrovirus: gag/pol = vector 1; Env = vector 2;
Delivery and cloning vector either VSV-G or MoMLV = vector 3
Lentivirus: gag/pol = vector 1 (containing WPRE); Rev = vector 2
Delivery and cloning vector VSV-G = vector 3
Adenovirus: shuttle vector containing gene of interest and adenovirus backbone

Origin & function

Genetic material will either be synthetic (PCR amplification product) or derived by cloning of cDNA or genomic material from a reproductively relevant tissue. Thus these genes will be targeted to specialised endocrine regulated tissues to assume full effect. A wide spectrum of material is being investigated, including known oncogenes e.g. c-myc and growth factors e.g. activin subunits

Some HSV-1 and HCMV modified virus will encode beta-galactosidase and green fluorescent protein as reporter proteins to monitor expression of viral genes in infected cultured cells. These sequences and their products are not associated with any health and safety risk.

Functions being investigated include; apoptosis, cell growth, cell differentiation, cellular transformation and cell communication focussing on reproductive processes, virus host interaction focusing on viral lytic cycle and post-translational regulation.

Evaluation of foreseeable effects

E. coli K12 and cell-lines are non-harmful and are unable to survive unless held in lab conditions.

Once packaged the virus particles (adeno, lent, HSV and CMV) will be capable of infecting a number of different species, including human. The GM viruses will be less fit than wild type virus, thus production of wild type virus either deliberately (HSV type 1, see below) or by recombination of the E1a gene from the HEK293 cell genome into the adenoviral genome will produce fitter virus. Stocks of adenovirus will not be repeatedly passaged to counteract the production of wild type, which is likely to occur 1 in 1x10e10.

Adverse effects for retroviruses have been reported from gene therapy trials where large numbers of virus (1x10e11) have been specifically infected into cells, selected for genomic integration using antibiotics then the proviral carrying cells injected into the immune compromised recipient. These individuals were initially immune-compromised, so it is unlikely this would happen in healthy individuals even if exposed to high titres (1x10e9/ml) since the virus would have to be injected directly into the blood stream and then viral integration would have to be specifically selected for.

If viral infection does occur then adverse effects are likely to be low. The viruses self-inactivate once integrated into the genome, and cannot re-activate due to deletion of the U3 in the 3'LTR. Thus even if infected with wild-type virus, eg an individual was carrying a latent HIV infection then the provirus cannot mobilise and propagate the vector or the inserted genetic material. Although activation of genes adjacent to the integration site is possible as discussed above, this cannot be propagated into a clonal population and instead the infected cells are likely to slough off in the respiratory tract and eye. Percutaneous injection of virus is possible, but this would require injection of large amounts of high titre virus. Since it is anticipated that viral titres will not exceed 1x10e9 per ml then the likelihood of large scale integration and oncogenic activation of cellular genes is low. Insertion of oncogenes into these viral vectors poses an additional risk since expression of these will be driven from constitutively active promoters. Adenovirus infection is most likely to occur by inhalation but again in immune competent individuals this is unlikely to generate a persistent infection, instead cells will be sloughed off.

All activities required for propagation of virus will be done wearing gloves and the production of aerosols will be minimised wherever possible and use of sharps and glassware will be avoided or minimised wherever possible. When producing high titer virus preparations eye protection will be worn.

HSV-1 is a common human pathogen for which infection occurs by direct contact with skin or eyes. Worldwide, greater than 40% of the population is seropositive for HSV-1. Accidental infection in the laboratory can occur by splashes of infected material to a mucosal surface or by viral entry through broken skin. Except in immuno-compromised individuals, HSV-1 infection is limited to epithelial cells, in the order of several million, at the infection site, resulting in the appearance of fluid filled vesicles (cold sores) and to the sensory ganglia that innervate the site. Infection of sensory ganglia leads to a life-long latent infection which is asymptomatic. Reactivation, usually triggered by cellular stress, can occur, whereby the virus travels from the ganglia to the site of primary infection and again productively infects several million cells.

Herpes infections of the eye are associated with keratoconjunctivitis and repeated recurrences can lead to scarring of the cornea. In very rare cases, approximately one in a million, herpes infection can lead to encephalitis, which if untreated, has a mortality of greater than 70% and most survivors are neurologically impaired. Herpetic whitlow is an infection caused by herpes simplex virus that affects health care workers. The source of infection is generally thought to be damaged cuticle or skin exposed to HSV infected secretions. This results in a short period of symptomatic infection and the establishment of latency. In the laboratory single acute dose exposure by accidental percutaneous injury could result in herpetic whitlow. Antiviral agents for HSV-1 are acyclovir (Zovirax), valacyclovir and famcyclovir.

HCMV is common in the population (in excess of 40% seropositive worldwide). Infection after birth is usually asymptomatic but sometimes a syndrome resembling infectious mononucleosis or glandular fever is caused by the virus. After initial infection the virus establishes a lifelong asymptomatic latent infection primarily in peripheral blood leukocytes. HCMV infection can lead to serious illness or death in immuno-compromised individuals and transplacental transmission during pregnancy can lead to severe neurological damage or death of the foetus. Infection in the laboratory could occur by exposure of cutaneous or mucosal surfaces to infected material or exposure
by percutaneous injury. The antiviral agent gancyclovir reduces or interrupts viral replication in vivo. All activities involving viral stocks and infected cell culture will be carried out in a biological safety cabinet whilst wearing gloves and avoiding the use of sharps and glassware. Genetically modified HSV and HCMV viruses, depending on the nature of the modified gene and the severity of the mutation, will have either wild type or reduced infectivity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All GM modified animals are housed in purpose built barrier containment facilities. These facilities are kept locked and are kept secure by restricted digital swipe card access. In addition these key facilities are restricted for general access by non-essential personnel.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM WASTE DISPOSAL

Apart from following the normal waste disposal procedures currently in practice within the building, the following rules must be observed:

(a) All solid and sealed liquid waste contaminated with GM material [eg. plastic pipettes, plates, small volumes of sealed liquid cultures, gloves, tips etc.] should be placed in a metal autoclave container lined with either a clear or blue plastic bag. The autoclave is regularly tested, and disposal of GM material via autoclaving, in a tested autoclave, meets the requirements of the GMO (2000) legislation. Autoclaving effectively gives 100% degree of kill and is validated annually. Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment, or disinfect with 10,000ppm available chlorine (Presept tablet: sodium dichloroisocyanurate, or Virkon at an equivalent concentration) for a minimum of 30 minutes in line with guidelines previously provided by the manufacturer. Disinfection - the concentrations and contact times used for disinfection have been shown to result in > 5 log reduction in virus titre (sodium hypochlorite - Croughan and Behbehani 1988 J. Clinical Microbiol 26, 213-215, Virkon - manufacturers data) and a 1% solution of Virkon disinfectant with a contact time of at least 20 mins gives a >99.999% kill of all viruses (data supplied by manufacturer). Discharge any resulting liquids to drains, dispose of solids via clinical waste stream for heat treatment. After autoclaving all waste MUST be placed in an ORANGE bag, and disposed of via the normal clinical waste stream.

(b) Glassware must be decontaminated by soaking in 1% w/v Virkon or 1000ppm Presept [four 0.5g tablets per one liter or four 2.5g tablets per five litres] for 1 hour before being rinsed and placed in the glasswash bucket provided for subsequent incorporation into the general washing process. Uncontaminated broken glassware should be disposed of in the glass waste containers supplied. Empty gas canisters should only be placed in the labeled bin. GM contaminated broken glassware and sharps should be placed in a sharps bin (eg needles, syringes, scalpels etc) and then autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via clinical waste stream for heat treatment.

(c) Large volume, contaminated liquids must be rendered safe by the addition of Presept tablets to a final concentration of [1000ppm], which is 10,000ppm available chlorine (Presept tablet: sodium dichloroisocyanurate, or Virkon at an equivalent concentration) for a minimum of 30 minutes in line with guidelines previously provided by the manufacturer, then discharged to drains. Liquids (eg samples, culture supernatants, tissue culture media etc) can also be autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
**Project Containment**

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**Project Ref**  28/03.1

<table>
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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>14/10/2013</td>
<td>USE OF HIV-DERIVED LENTIVIRUS VECTORS TO EXPRESS GENES IN CULTURED CELLS, ORGAN CULTURE AND MOUSE TRANSGENESIS.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Studies in cancer genetics and developmental genetics, that are an integral component of the concerted research programme on cancer and development genetics that is underway here at the Medical Research Council Human Genetics Unit.

These lentivirus vectors are infective to non-dividing human cells and are integrative to the genome therein, but are non-replicative, ie, self-inactivating, and so infected cells would not produce viral particles and hence pose no risk.

There is a risk of oncogene activation in infected cells due to the insertion, but the risk is low, and the risk of consequent tumourigenesis very low, as changes to several genes are required for these events to occur.

A third generation packaging system after Dull et al (1998) J. Virol 72 8463-8471 will be used, which requires genes from three different vectors for packaging into infectious particles. These vectors designed so as not to be packaged into viral particles and not recombine with each other or with the lentivirus.

These vectors transiently provide at high titre the viral functions required for packaging of the lentiviral construct.

The lentivirus vector to be used initially is LentiLox 3.7. This infects non-dividing human cells under the above circumstances, is stably integrative, but is non-replicative, ie, is self-inactivating. Vector expresses no viral functions once transduced. Thus, target cells can be infected with viral particles made in the packaging line but to infectious particles can then be made by the infected target cells. Wildtype virus does not infect mice and is rare in humans, so recombination with wildtype virus very unlikely. Airborne particles, if produced, would be at very low concentration and unstable.

We wish to include other similar lentivirus vectors to be developed in the future which have the same properties as outlined above (or properties giving lower risk) within the scope of this application.

We intend to use the lentiviral system to express the following classes of gene. Construct-specific risks will be covered in (future) GM Risk Assessments, and experiments only permitted if the GM Safety Committee here categorises the proposed work as Hazard Group 2 (or 1).

i) Expression of siRNAs against genes involved in development and tumorigenesis. siRNAs (short inhibitory RNAs) are short dsRNA molecules which can give sequence-specific knockdown of the expression of endogenous target genes, a process also referred to as RNAi (RNA interference). siRNAs will be expressed, if possible, as short hairpin RNA molecules, or otherwise as pDECAP constructs which express long dsRNA hairpins which are endogenously converted to functional siRNAs. Target genes will mainly be murine, so whenever possible the target sequences will be murine-specific. If this is not possible, such as when the target is human cells in culture, if practicable then the expression of the siRNA will be regulated by an inducible system (ie tetracyclin inducible) to further limit the potential risk of siRNA expression in humans. Examples of genes we intend to study are genes involved in testsis and kidney development and tumorigenesis, such as WT1 and its downstream target genes. Lentiviral siRNA vectors will be used to infect cell culture (murine or human), organ culture (murine) and in the generation of transgenic mice.

ii) Reporter genes, like lacZ, GFP, or its derivates. Promoters responding to endogenous cellular signals involved in tumorigenesis or development, or tissue-specific
promoters, will be cloned in front of these reporter genes and used to monitor the signalling pathways involved. These inserts pose no risk to human health. Reporter constructs will be used in cell culture (murine or human), organ culture (murine) or transgenesis (murine).

After surveying the promoter activity with reporter genes we will use these promoters to drive expression of developmental genes. These genes are predicted to affect early developmental stages and we propose to drive both the active genes and siRNAs. Genes that will be studied for example are Gli3, dHAND, Shh, and members of the Tbx, Nkx, Bmp and Fgf families.

iii) Expression of Cre or FLP recombinases. Cre and FLP are used in combination with conditional mouse models in which part of a gene is placed between loxP or FRT sites. Expression of Cre/FLP will result in deletion of the sequence between the loxP/FRT site and inactivation of the gene. By expressing the recombinases in an inducible or tissue-specific manner full control of inactivation of the target gene is possible. Expression of Cre or FOP poses no risk for human health. Lentiviral expression systems will be used in cell culture, organ culture and transgenesis, all murine.

iv) Expression of accessory genes involved in different tetracyclin responsive expression systems, like rtTA, tTA and Tet-R. These genes regulate the expression of genes the promoters of which have been extended with tet-binding elements, and the expression of the genes will be activated by the presence or the absence (depending on the system) of tetracyclin or its analogue doxycyclin. Expression of rtTA, tTA or Tet-R poses no risks to human health. Lentiviral expression of these genes will be used in cell culture, organ culture and transgenesis.

Evaluation of foreseeable effects

**RISK TO HUMAN HEALTH ARISES DUE TO:**
- Lentivirus vector infective to non-dividing human cells
- Lentivirus vector integrative
- Lentivirus vector at high titre
  - thence potential for insertional mutagenesis of body surface cells by the lentiviral vector and thence potential for oncogene activation in infected cells.

**RISK TO HUMAN HEALTH AMELIORATED AS:**
- Third generation packaging system to be used - requires three different vectors which cannot recombine with each other or the lentivirus.
  - Integration is stable.
  - Lentivirus vector is non-replicative, ie, self-inactivating - no viral function expressed once transduced
  - Tumourigenesis very unlikely
  - Wildtype virus does not infect mice and is rare in humans - recombination thus very unlikely
  - Airborne particles, if produced, would be at very low concentration and unstable
  - siRNA whenever possible will be highly specific to murine genes
  - Knockdown of the siRNA targeted genes in an adult human would not cause transformation
  - Targeted murine genes under developmental-specific and tissue-specific enhancer and although it is a very unlikely possibility, tests will nonetheless be done to ensure that the lentivirus does not become replicative by recombination

**APPROXIMATE EVALUATION OF ABOVE RISK (APPLYING BS8800:1996) TO HUMAN HEALTH:**
- Estimated likelihood = highly unlikely
- Potential severity of harm = extremely harmful
- Risk = moderate and thus control = Containment level 2 or rationalising.

According to the ACGM Compendium of Guidance (Part 2B, Annex III, paragraph 26) HIV-derived lentivirus (such as this) should be Hazard Group 3, unless there is justification for a lower classification. The vector here requires three different vectors for packaging (ie a 'third generation system') and is self-inactivating/non-replicative. Further, the probability for oncogene activation in infected cells is anyway low, and for consequent tumourigenesis is very low. We thus consider there to be sufficient justification for classification of this proposed work as Hazard Group 2, rather than 3.
AND RISK TO THE ENVIRONMENT:
Negligible - transduced cells/animals not infective (see above), all changes somatic (see Section 8), and all virus, cells and tissues/animals destroyed (see Section 12).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Any live-birth transduced mice will be entirely reared, housed and killed within our Transgenic Mouse Facility. This is a secure 'barrier' unit, and as such escape of mice or entry of exogenous rodents is almost impossible. Such animals anyway pose negligible risk to human health or to the environment, as they could not be infective with the lentivirus vector (is self-inactivating/non-replicative).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Complete immersion in 1% Virkon for at least two hours will be used to destroy all cultures and sterilise all items. Virkon is a potent peroxygen disinfectant that will give 100% kill of all agents used, the lentivirus in particular. Virkon is known to give 100% kill of the closely related HIV (US Environmental Protection Agency). Liquid waste will then be disposed to drains. Solid waste is then routinely autoclaved and then sent for commercial incineration. Larger tissue samples/whole animals (ie mice) are routinely disposed by commercial incineration (there is no viral risk from such items anyway).

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

Four GM Risk Assessments attached. Relevant comments include the following.

Committee noted this proposed use at high titre of an integrative retrovirus that could infect non-dividing human cells. According to the ACGM Compendium of Guidance (Part 2N, Annex III, paragraph 26) this should be HG3, unless there is justification for a lower classification. It was further noted that the virus requires three different vectors for packaging (ie a 'third generation system') and is self-inactivating/non-replicative. However, the former must be considered in the context of its infectivity once packaged. Committee were mindful of the potential, albeit very low probaility, for oncogene activation consequent upon infection of and integration into the genome of a worker's cells. Nonetheless, the non-replicative nature of the construct was agreed as sufficient justification for classification of this proposed work as HG2 rather than HG3. The proposed (section 15) HG1 is not sufficient.

Committee requires, as a condition of this approval, that packaging lines are assayed by plaque formation colony assay or equivalent to ensure that the viral vector has not become replicative by recombination (although it is accepted that this is unlikely, it was considered that the high titres used might possibly increase the overall likelihood). This assay must be performed initially, and then repeated after three months of actual project work, and then after every six months of actual project work thereafter. And the acceptable result is to be zero replicative viral particles.
Project Ref 28/98.2

Date Ackn'd 14/10/2013

CU2 Project Title ASSESSING DELIVERY OF BACTERIA TO MAMMALIAN EPITHELIAL SYSTEMS

Class 2

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes TRANSFERRED TO GM 207 ON CLOSURE OF GM28

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 326/01.1

Date Ackn’d 30/06/2004  CU2 Project Title ANALYSIS OF NEURONAL TRANSGENE EXPRESSION AND OF NEURONAL

Class 2  CultureVolClass2 < 1 Litre  CultureVolumeClass3-4

02/03/2022  Page 5081 of 15326
Date Project Ceased: 17/02/2004

VIABILITY

Non-GMM

Consent Granted: Not Applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: GM326/01.1 TRANSFERRED TO GM207 AS OF 30/06/2004

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 326/03.1

Date Ackn’ed: 17/02/2004

CU2 Project Title: TO DEFINE THE ABILITY OF ADENOVIRAL OR ADENO-ASSOCIATED VIRAL (AAV) GENE TRANSFER OF CANDIDATE GENES TO REDUCE BRAIN DAMAGE

Class: Class 2

CultureVolClass2: < 1 Litre

Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Historical Significant Changes: GM326/03.1 transferred from GM 326 as of 30/06/2004
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to assess the ability of a number of biological active genes to reduce brain damage.

**Recipient or parental organism**

These adenoviral or adeno-associated viral vectors will be produced and characterised in Department of Medicine and Therapeutics, University of Glasgow (HSE centre number GM 397). All adenovectors to be used in this study and E1-deleted first generation adenoviral vectors based on the pJM17 system (McGrory, W. J. Bautista, D. S. and Graham, F. L.: A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. This renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. However, all the inserted genes are biologically active. None of the transgenes are proto-oncogenes. All transgenes will be under the control of the CMV promoter. There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use.

AAV vectors have received increasing attention for molecular interventions in vivo and for gene therapy applications due to their low level of immunogenicity in vivo and their ability to integrate into the genome, thus producing sustained expression of transgenes for prolonged periods of time. They also have the ability to infect both dividing and non-dividing cells.

AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Dept of Medicine and Therapeutics, University of Glasgow. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced.

**Host/vector system**

The adenoviral vectors are generated by homologous recombination between pJM17 and shuttle vectors containing transgene expression cassette and flanking E1 sequences. Following homologous recombination in helper 293 cells (which express the helper E1 function in trans, replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses.

AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette.
cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al High-titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Dept of Medicine and Therapeutics, University of Glasgow. AAV vectors are replication defective.

**Origin & function**

The adenoviral vectors DNAs are standard and originated from the laboratory of Dr Graham (McGrory, W. J., Bautista, D. S. and Graham, F.L.: A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vitro and in vivo to generate high-level gene expression in all cells transduced by the adenovirus. The AAV system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All transgenes will be constructed from full length cDNAs obtained from other research institutes and verified in our own laboratory prior to subcloning into the relevant vectors.

**Evaluation of foreseeable effects**

There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome.

AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wild type virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes, or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced. Consequence of environmental exposure - ‘effectively zero’.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures as per HSE containment level 2 for both production of recombinant adenoviruses and AAV in the laboratory and animal experimentation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory-based experiments: during the production of replication-defective adenoviruses, all solid waste (plastics etc.) are autoclaved prior to disposal. All liquid waste from tissue culture is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. The use of sharps is avoided.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y
Passed with amendments by local GMSC 25th September 2002.

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 362/00.1

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Withdrawn Y

Tick if notifying a connected programme of work N

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Project Additional Information

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

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Project Containment

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<th>Laboratory Activities</th>
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Animal Units  Large Scale Activities  Human Clinical Applications

02/03/2022  Page 5087 of 15326
Purposes of the contained use

The purpose of the contained use is to assess whether polymorphism of the human PrP gene are controlling susceptibility and resistance to prion diseases, also known as Transmissible Spongiform Encephalopathies of TSEs. The variants in question have altered amino acid codons and have been found in individuals affected by TSEs but it remains to be proven that there is a direct causal link between the codon change and the TSE disease. The details of the study are confidential in order to protect intellectual property and a successful project may result in a patent application so commercial confidentiality is also claimed. Disclosure would adversely affect the competitive position of the Institute for Animal Health in a highly competitive area of research. Exemption from disclosure is allowed by the Environmental Information Regulations 1992 as amended in 1998 and the relevant exemption is intellectual property protection and commercial confidentiality.

Recipient or parental organism

For the laboratory cloning steps, a section of the mouse PrP gene cloned in to a pBluescript vector will be subjected to in vitro mutagenesis to introduce the required nucleotide changes. The newly created sequences will be cloned into plasmid pBluescript. All constructs will be maintained in disabled E coli bacterial cultures. Details of the constructs and cloning steps are claimed as confidential. Exemption from disclosure is allowed by the Environmental Information Regulations 1992 as amended in 1998 and the relevant exemption is intellectual property protection and commercial confidentiality.

Host/vector system

For the laboratory cloning steps, the plasmid will be pBluescript II KS+. The cloning procedures will be carried out in Stratagene XL disabled bacterial hosts.

Origin & function

For the laboratory cloning steps, the plasmid will be pBluescript II KS+. The cloning procedures will be carried out in Stratagene XL disabled bacterial hosts.
The origins of the genetic material involved are cloned sections of the mouse PrP gene already available in this laboratory from other projects. The intended function of the mutagenised cloned sequences is to make gene targetted transgenic animals expressing a single copy of the transgene under the control of the endogenous PrP gene promoter. The details of this project are claimed to be confidential exemption from disclosure is allowed by the Environmental Information Regulations 1992 as amended in 1998 the relevant exemption is intellectual property protection and commercial confidentiality.

**Evaluation of foreseeable effects**

The GMMs and human health:

Hazard identification

It would be theoretically possible for a laboratory worker to swallow the Ecoli containing human disease associated amino acid codons within the mouse PrP gene. The plasmid pBluescript II is not a prokaryotic expression vector and in combination with the fact that the inserted mouse PrP gene fragment is of large size it is very unlikely that any PrP protein is expressed in these Ecoli and therefore for this altered PrP protein to appear in the gut. If despite this assessment altered PrP protein would be produced and released into the gut, the hazard would be very low as it has been shown that recombinant PrP made in Ecoli is not infectious (Scott et al 1988 Protein Engineering 2: 69-76) Those who believe TSEs (or prion diseases) are caused by an infectious protein put this down to misfolding and non-glycosylation of the Ecoli expressed protein however recombinant PrP expressed in mouse cells (Caughey et al 1988 PNAS 85: 4657) or monkey COS cells (Scott, et al, idid) or converted in vitro to protease resistant form (Hill et al 1999 J Gen Virol 80: 11-14) are also not infectious.

Capacity to survive in the human gut.

The bacteria to be used are recombination deficient so would not be expected to transfer plasmids to other bacteria or to be able to compete successfully with wild-type bacteria.

Assessment of likelihood - low.

There are extensive measures in force at the Institute for Animal Health in Edinburgh to protect staff from any infection with TSEs. All TSEs are treated in the same way in order to make sure procedures are remembered and followed diligently. CJD and BSE in addition, are handled at containment level 3 at the moment.

The minimal containment level within the building is level 2. Some closed, locked and access restricted rooms are used for work with CJD and BSE. These are higher containment than level 2 without being full level 3.

Briefly: No eating or drinking is allowed in laboratory areas

No mouth pipetting is allowed

Protective clothing (lab coats, aprons if necessary) is worn all the time

Disposable gloves are worn, eye protection is worn

Any procedures which could generate aerosols are carried out in safety cabinets

Visors are used if splashing is possible

Closed systems are used for mixing, homogenisation, centrifuging etc

Plastic disposables are used wherever possible in preference to sharps

Waste is disposed of by treating with 20,000 ppm chloros or autoclaving at 134C for 1 hour and followed by removal in hermetically sealed waste bins for incineration

Dedicated rooms are used for procedures involving CJD and BSE infectivity.

Records are kept of all accidents.

A Quality Assurance system is being set up with IAH to closely monitor all procedures, experiments and safety controls.

Assessment of consequence - High

Although expected to be unlikely, if any infectivity is generated by Ecoli containing human disease associated amino acid codons in the mouse PrP gene, this would not be expected to infect humans because of the safety measures in place. However, the consequences might potentially be grave if it did.

Estimation of risk - low

The measures in place to protect human health and safety are well above level 2 containment standards.
The GMMs and Environmental Risk

Hazard Identification

The Ecoli used to replicate the plasmid constructs containing human disease associated amino acid codons within the mouse PrP gene inserts could be discharged from the labs through the drains or in lab waste and affect animals and people in the environment. The plasmid pBluescript II is not a prokaryotic expression vector and in combination with the fact that the inserted mouse PrP gene fragment is of large size it is very unlikely that any PrP protein is expressed in these Ecoli and therefore for this altered PrP protein to appear in the environment. If the altered mouse genes were to be expressed in the Ecoli and this could enter and colonise the gut of animals or people, it might be that the altered mouse PrP protein would be expressed in the gut as well. However, this worst case scenario is highly unlikely as the Ecoli strain used are highly disabled and unable to compete with wild type bacteria. In addition recombinant PrP made in Ecoli is not infectious (Scott et al 1988 Protein Engineering 2: 69-76) Those who believe TSEs (or prion diseases) are caused by an infectious protein put this down to misfolding and non-glycosylation of the Ecoli expressed protein however recombinant PrP expressed in mouse cells (Caughey et al 1988 PNAS 85: 4657) or monkey COS cells (Scott, et al ibid) or converted in vitro to protease resistant form (Hill et al 1999 J Gen Virol 80: 11-14) are also not infectious.

More details are presented with the risk assessment.

Capacity to survive in the environment.
The Ecoli strain used is recombination deficient and should not be able to transfer plasmids to other bacteria, nor would it be expected to survive in competition with other wild type bacteria. If, however unlikely, an infectious agent was to be generated in the Ecoli cells, it may persist in the environment for long periods.

Assessment of likelihood - low

Escape of Ecoli containing human disease associated amino acid codons in the mouse PrP gene is considered highly unlikely. All bacterial cultures are treated with 20,000 ppm chloros prior to disposal or autoclaved at 134C for 1 hour. These treatments are the reliable methods of inactivating TSE agents. All nucleic acid preparations would be considered to be non-infectious due to the use of phenol or guanidinium in the preparative procedures. Any protein preparations would not leave the building unless treated as above to inactivate any infectivity. Waste then leaves the building in hermetically sealed, tamper proof clinical waste bins for incineration.

Assessment of consequence - Low

Human TSE diseases (CJD and GSS) are very rare - worldwide incidence less than 1 in a million new cases per year. There is no evidence of contagious spread of TSEs in humans and no evidence of maternal transmission. Any bacteria which found their way out of the labs would be dead because of standard decontamination procedures. Any protein remaining within them would be at low levels as high expressing promoters would not have been used.

Estimation of risk - low

The standard measures in place will protect environmental, human and animal health and safety.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GM animals:
Institute for Animal Health, Edinburgh is housed in a building with restricted and controlled access at the entry doors (computerised key locking system). The SPF barrier animal house is highly secure with restricted and controlled access at the entry doors. Doors to the animal house are locked with entry only via computer controlled unlocking or number coded locks. Containment of animals is routinely very high for the reason of prevention of contamination of other experiments and animals are housed in a range of facilities all with at least level 2 containment. The mouse is regarded as a closed system with regard to prion diseases but dissections are performed in safety cabinets. For the present study, animal tissues would be prepared and analysed in closed locked dedicated laboratories with higher containment than level 2 (but not full level 3). Disposal of carcases: autoclaving at 134 C for 1 hour then removal for incineration in hermetically sealed bins. Our standard inactivation procedures reduce TSE infectivity to undetectable levels and containment and decontamination procedures are monitored for compliance. IAH is at the moment in the process of setting up a QA system to cover all activities from experiments to waste disposal.

GM ES cells:
Embryonic stem (ES) cell culture is performed in a dedicated locked laboratory with access restricted to essential personnel. Work is performed using disposable plastic equipment wherever possible. All liquids are treated with 20,000 ppm chloros. Tissue culture flasks, plates and falcon tubes (15 ml or 50 ml) which have been in contact...
with ES cells (targeted or untargeted) are sealed with parafilm and placed in waste bags which are removed from the tissue culture suite by a designated operator and autoclaved at 134°C for 1 hour. The waste is then removed for incineration in hermetically sealed. Our standard inactivation procedures reduce TSE infectivity to undetectable levels and containment and decontamination procedures are monitored for compliance. IAH is at the moment in the process of setting up a QA system to cover all activities from experiments to waste disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is claimed for the cloning steps of the project using GMMs.

It is not clear to us where on this form to ask for Derogation for GMOs which are not micro-organisms, so we do so here and in the confidential section 17 which contains the details of the reasoning and the experimental design. Derogation to Class 2 containment is requested for the embryonic stem cell stage of the project and the transgenic mice. Confidentiality is claimed for the details of this request. Exemption from disclosure is allowed by the Environmental Information Regulations 1992 as amended in 1998 and the relevant exemptions are intellectual property protection and commercial confidentiality.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All bacterial cultures are treated with 20,000 ppm chloros prior to disposal or autoclaved at 134°C for 1 hour. These methods kill E. coli bacteria. These treatments are also reliable methods of inactivating TSE agents to undetectable levels. All nucleic acid preparations would be considered to be non-infectious in terms of TSE infectivity due to the use of phenol or guanidinium in the preparative procedures which inactivate protein (Rowher RG (1991) Current Topics in Microbiology and Immunology 172: 195-232). Any protein preparations would not leave the building unless treated as above to inactivate any infectivity. Bacterial culture plates, ES cell plates and mouse carcasses are autoclaved at 134°C for 1 hour prior to disposal. Waste then leaves the building in hermetically sealed, tamper proof clinical waste bins for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This proposal was discussed in detail during 2 separate meetings of the GM safety committee and the proposers provided relevant additional information as requested. The committee members were concerned about various procedures to ensure they were sufficiently contained. The committee members were happy that all safety measures were in place for other projects already. The local NPU Safety officer is part of the GM committee. Staff are entitled to join a union and are represented by appropriate safety officers. Full details of the GM committee deliberations are presented in the confidential sections of this form. Confidentiality is claimed on the grounds of intellectual property protection and commercial confidentiality. These exemptions are allowed under the Environmental Information Regulations 1992 as amended in 1998.

Project Containment

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**Project Ref** 384/94.1

**Date Ackn’ed** 04/06/2008

**CU2 Project Title**

THE PRODUCTION AND USE OF MICE CARRYING PrP PRO-LEU "102" MUTATION

**Date Project Ceased** 19/11/2008

**Class**

Class 2

**CultureVolClass2**

Non-GMM

**CultureVolumeClass3-4**

Consent Granted

Not Applicable

Project notified under transitional arrangements Y

**Withdrawn** N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

SINGLE NOTIFICATION FOR ALL

GM384 = GM207/08.6

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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### Project Ref 384/94.2

**Date Ackn'd** 04/06/2008

**CU2 Project Title**

THE PRODUCTION OF MICE CARRYING MUTATIONS IN THE PrP GENE WHICH WILL PREVENT GLYCOSYLATION OF THE PrP PROTEIN

**Date Project Ceased** 19/11/2008

**Class** Class 2

**CultureVolClass2** Class 2

**CultureVolumeClass3-4** Not Applicable

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** Y

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form
Please enter comments on the GM safety committee on the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

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Project Ref 384/97.1

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Date Project Ceased 19/11/2008

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

SINGLE NOTIFICATION FOR ALL GM384 = GM207/08.6

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref: 384/97.2

Date Ackn'd: 04/06/2008

CU2 Project Title: DEVELOPMENT OF MOUSE MODELS FOR THE STUDY OF BSE

Date Project Ceased: 19/11/2008

Class: Class 2

CultureVolClass2: Class 2

CultureVolumeClass3-4: Not Applicable

Non-GMM: Not Applicable

Consent Granted: Yes

Project notified under transitional arrangements: Yes

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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If yes, tick to confirm that it is attached to this form [N]  
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Tick if you are claiming exemption from disclosure for section of the risk assessment [N]  

Please enter comments on the GM safety committee on the risk assessment

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 384/trans1

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Historical Significant Changes

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Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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### Project Ref: 384/trans2

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<tr>
<td>CU2 Project Title</td>
<td>CHALLENGE OF MICE WITH SINC RELATED PRP GENE ALTERATIONS</td>
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<tr>
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- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: Y

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tr>
<td>L2 L3 L4 L2</td>
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**Project Ref** 384/trans3

**Date Ackn'd** 04/06/2008

**Date Project Ceased** 19/11/2008

**Withdrawn** [N]

**CU2 Project Title** EXPRESSION OF RUMINANT PRP GENES IN TRANSGENIC MICE

**Class** Class 2

**Consent Granted** Not Applicable

**Project notified under transitional arrangements** [Y]

**Tick if notifying a connected programme of work** [N]
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>Human Clinical Applications</td>
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Project Ref 384/trans4

ANALYSIS OF PUTATIVE SCRAPIE ASSOCIATED NUCLEIC ACIDS

Date Ackn'd 04/06/2008

CU2 Project Title

ANALYSIS OF PUTATIVE SCRAPIE ASSOCIATED NUCLEIC ACIDS

Class 2

Consent Granted Not Applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work N

Historical Significant Changes

SINGLE NOTIFICATION FOR ALL
GM384 = GM207/08.6

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Project Containment**

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</table>
The study of oligodendrocyte differentiation, and the oligo-axonal interactions involved in myelination, so as to develop new strategies to enhance repair in Multiple Sclerosis. This project aims to induce the expression of adhesion molecules involved in multiple phases of oligodendrocyte development in myelinating oligodendrocytes. A second objective is to inhibit the expression of candidate genes hypothesized to regulate axo-glial adhesion and myelination using short hairpin RNA’s. The use of viral expression is necessary for these investigations, because myelination occurs over multiple weeks, and the duration of gene expression or repression required is greater than what can be attained using conventional transfection methods.

**Recipient or parental organism**

DH5alpha E.coli (disabled) and other common disabled E.coli K12 strains. LinX or equivalent packaging cell line for retrovirus, and HEK293 FT or equivalent for lentivirus. Cells to be infected are rodent oligodendrocyte and other precursors, neurons, neurospheres or oligospheres.

**Host/vector system**

Bacterial cloning vectors (eg. pUC based vectors). Retroviruses including pSM2 and LMP (Open Biosystems), lentiviruses including pLenti6.2v5 and ViralPower Packaging mix (a mixture of pLP1, pLP2 and pLP/VSVG plasmids; Invitrogen).
The objective of our studies is the knock-down of gene expression by the use of virally expressed small interfering RNA’s, and/or disruption of protein function by virally expressed dominant negative isoforms, resulting in the inhibition of adhesion signalling pathways implicated in myelination, leading to alterations of myelin-forming capacity or the generation of myelin-forming cells.

DNA clones for expression (encoding wild-type or dominant negative forms of adhesion and related signalling molecules) will be obtained from collaborators, or generated by standard molecular biology techniques from DNA libraries held within the laboratory. Short-hairpin RNA sequences will be obtained from Open Biosystems Inc. Or will be designed using software such as "shRNA Retriever" from RNAi Central and then synthesised commercially and inserted into the vectors described above.

**Evaluation of foreseeable effects**

is will cause serious harm to the researcher is very low as the decrease in gene expression could not spread beyond the small number of cells directly exposed to the virus, either within the individual or to other individuals. Similarly, the ectopic expression of wild-type or inactive forms of oligodendrocyte proteins such as integrins may likewise alter the function of cells that are infected. The risk this poses will be minimised in two ways. First, the great majority of proteins expressed or knock-down to study myelination will have no effect on cell proliferation - an entirely different cellular process. In those cases were proteins implicated in myelination have been shown to have secondary effects on proliferation, the constructs used will be designed to express proteins that inhibit or prevent cell proliferation. Second, the very small number of cells affected by any contact of a researcher with the (replication-incompetent) viruses would limit the consequences of such an accident.

c) The vector (LMP) is derived from a mouse stem cell retrovirus (MSCV) vector, and while replication competent, cannot be packaged without viral gag, pol, and env genes, which it does not contain. pLenti contains a deletion in the 3’ long terminal repeat (LTR) that results in self-inactivation of lentivirus after transduction of the target cell, resulting in the virus being replication-incompetent. In both cases, once integrated into the transduced cell, the virus cannot longer be packaged.

The potentially harmful effects to the environment are effectively zero because

a) Genetic material will not have any outside target mammalian cells, and in the case of the small interfering RNA's will be species-specific.

b) The donor micro-organisms are disabled and will therefore not cause environmental harm by transfer to other organisms.

c) The vectors are replication-incompetence that will prevent dissemination or transfer to other organisms.

d) The resulting genetically modified micro-organism are extremely susceptible to dessication and will only survive a few hours at room temperature. Taken with the non-oncogenic nature of the genetic sequences to be used, the specificity of the vectors for mammalian hosts and their replication incompetence the resulting GMM poses no effective risk to the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste will be decontaminated by chlorine bleach and solid waste by autoclaving.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |
Email received, Dept. of Pathology ACGM dated May 16th 2007.
"The Dept. of Pathology ACGM has approved your risk assessment entitled:“The use of lentiviral and retroviral expression vectors to influence gene expression and cell signalling in myeliating oligodendrocytes” as a class 2 project. Moreover the committee had no substantive comments so the risk assessment can be used as is”.

Project Containment

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Animal Units

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Project Ref 541/01.58

Date Ackn’ed 01/04/2004

CU2 Project Title

GENETIC MANIPULATION OF TRYPANOSOMA BRUCEI SPP. INCLUDING T.B. RHODESIENSE, T.BRUCEI, T.GAMIENSE

Date Project Ceased

Class 3

CultureVolClass2

up to 2 Litres

Consent Granted yes

Non-GMM

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Transferred from GM541 on 01/04/2004

Historical Date of Additional Info

17/09/2007

Significant Change ID 541/01.58a

Date of Significant Change

17/09/2007

Project Additional Information

Purposes of the contained use

Identified proteins enriched in particular stages of the trypanosome life cycle will be expressed or their genes manipulated in cultured insect or bloodstream forms of the parasite. This will allow investigation of the development of Trypanosoma brucei spp. through its life cycle at the cellular and molecular level. Our aim is to improve understanding of the function and regulation of these molecules, which it is expected will be clarified by these experiments. This may identify strategies to control the
Transmission of these parasites in Africa.

Recipient or parental organism

GMO: Cultured insect or bloodstream forms of the parasite. Insect forms are non-pathogenic, non-transmissible. Bloodstream forms require tsetse fly transmission. These are only indigenous in Africa. No tsetse fly colonies are maintained at the University of Manchester. Trypanosome do not produce toxins and we expect most genetic manipulation of the parasite to reduce fitness and therefore virulence.

Host/vector system

Parental organism: Trypanosoma brucei spp. blood and insect forms
Vector system: pGEM or pBluescript based bacterial shuttle vectors engineered to enable parasite gene expression or modulation in the parent organism using a tetracycline regulated inducible expression system. These are non mobilisable shuttle vectors. Specific vectors are pET series, pGem, pGemT-easy, pLew100, pZJM, pBluescript II, p2T7, pHD541 and pHD430

Origin & function

1. T. brucei spp. genes or proteins displaying specificity, enrichment or implicated in the involvement in particular life cycle stages of the parasite. Their function is expected to be in progression of the parasite through its developmental cycle and, potentially, parasite metabolism.
2. Reporter gene expression to allow selection of transgenic parasites or detection of the expression pattern or location of those proteins described in (1). This will include genes for resistance to antibiotics used for parasite selection and green fluorescent protein gene fusions for protein localisation.

Evaluation of foreseeable effects

Where gene expression of particular molecules implicated in life cycle regulation are expressed we anticipate differentiation phenotypes to be observed. Specifically we will use markers for each life cycle stage to detect reduced ability to initiate differentiation, enhanced differentiation rates of failure in the programme of differentiation events at particular points. Transgenic parasites harbouring regulatory molecules with specific mutations to enable the isolation of interacting partner molecules (for example substrate-trapping mutants) or which generate altered differentiation phenotype will be used for protein isolation or examined for altered pattern of gene/protein expression. In each case transgenic bloodstream and insect form parasites will be used and differentiation between these stages examined. However, it is not possible to differentiate parasites from the insect form back to the bloodstream form in the absence of the tsetse fly vector. Therefore the life cycle cannot be completed in the laboratory. These genes will be expressed using a tetracycline inducible expression system. Therefore, in the absence of tetracycline the genes are effectively silenced. Antibiotic resistance genes used for selection of transgenic parasites are not those used for clinical treatment of the parasite and will otherwise have no foreseeable effect. Reporter genes (e.g. green fluorescent protein) are for localisation and assay of expression pattern for particular parasite genes or sequences. There is no foreseeable consequence of this.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

T. brucei spp. does not produce spores. They are rapidly killed outside of the culture vessel or blood by desiccation osmotic shock (washing with water, detergents) and cannot penetrate unbroken skin. Parasite genes are expressed or perturbed using a tetracycline inducible expression system and will be effectively silenced outwith the culture vessel. Tsetse flies are required for transmission and completion of the life cycle; these are indigenous only in sub Saharan Africa. Thus the parasites, whilst mammal infective as the bloodstream forms are effectively deficient in mobilisation capacity. The wild type bloodstream form parasite is mammal infective and virulent. It is highly unlikely that genetic modification will enhance this; rather gene manipulation is likely to reduce virulence and transmissibility.

We request derogation from Containment class III to containment class II for specific containment measures.

CONTAINMENT MEASURE:
1. Isolation. The laboratory is separate from other laboratories in the building being fully contained within a locked laboratory, with access restricted to authorised lab personnel.

2. Laboratory sealable for fumigation. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSSH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for sealability for fumigation.

4. Entry via airlock. Trypanosomes can only be transmitted by blood-blood contact or by skin puncture. They cannot survive in air and do not form spores. We request derogation as risk assessment does not indicate a requirement for this measure.

5. Negative pressure. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSSH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for airflow.

6. Filtration of extract and input air. Trypanosomes do not form spores and cannot undergo airborne transmission. Handling of bloodstream form parasites is most commonly as in vitro cultured forms. These are opened only in a microbiological safety cabinet with HEPA filtration. We request derogation to level 2.

7. Microbiological safety cabinet. Trypanosomes do not form spores and cannot undergo airborne transmission. Handling of bloodstream form parasites is most commonly as in vitro cultured forms although purification of parasites from blood is not practical in a safety cabinet. However there is no generation of aerosols. The laboratory is self contained providing containment. We request derogation to level 2.

8. Autoclave in the laboratory suite. There is a central sterilisation facility in the Stopform building, but no autoclave facility within the laboratory. However, all parasites, and material that has come into contact with parasites is rendered non-infective by soaking in 2% Virkon for 12h prior to transport to the autoclave facility. Transport is in sealed containers. Therefore we request derogation to level 2.

11. Shower. Trypanosomes are only transmitted by blood-blood contact or by inoculation. Skin penetration of the naked parasite is not possible. Parasites cannot survive in the external environment. Therefore we request derogation to level 2.

REQUEST FOR DEROGATION OF ANIMAL FACILITIES
The School of Biological Sciences BSU operates as a first class animal containment facility. Laboratory rodents infected with Trypanosoma brucei spp. will be contained in accordance with the regulations defined for Containment level III. However we request derogation to containment level II for specific regulations:

1. Isolation. The BSU is separate from other laboratories in the building being fully contained within a locked laboratory, with access restricted to authorised BSU personnel.

2. Laboratory sealable for fumigation. The trypanosime cell line used has a contained use regulation level of 3 but the containment recommended by the COSSH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for sealability for fumigation because trypanosomes do not produce spores, cannot be transmitted in an airborne form, or by aerosol. Blood:blood contact is required.

4. Entry via airlock. Trypanosomes can only be transmitted by blood-blood contact or by skin puncture. They cannot survive in air and do not form spores. We request derogation as risk assessment does not indicate a requirement for this measure.

5. Negative pressure. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSSH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for airflow.

6. Filtration of air. Trypanosomes do not form spores and cannot undergo airborne transmission. Transmission can occur only by blood-blood contact or inoculation. Standard operating procedures minimise risk of aerosol. We request derogation to containment level II with respect to HEPA filtration of the animal enclosure.

7. Microbiological safety cabinet. Trypanosomes do not form spores and cannot undergo airborne transmission. Operating procedures limit the possibility of aerosol. The BSU is self contained providing containment. We request derogation to level 2.

11. Shower. Trypanosomes are only transmitted by blood-blood contact or by inoculation. Skin penetration of the naked parasite is not possible. Parasites cannot survive in the external environment. Therefore we request derogation to level 2.

14. Control of disease vectors. Trypanosomes can only complete their life cycle in the tsetse fly. These are not indigenous in the UK, or outside sub Saharan Africa. There are no tsetse flies maintained at the University of Manchester.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

A laboratory scale project employing good microbiological practice and good occupational safety and hygiene.
Disenfection with 2% aqueous Virkon solution according to University Policy and Guidance document. Material that has come into contact with parasites is inactivated by soaking in hypochlorite (1:500) for 24h.

Liquid waste and solid waste (other than sharps and animal waste) is sterilised in an autoclave maintained and validated by the manufacturer's service organisation for the destruction of clinical waste prior to transfer by a licensed operator to a commercial incinerator site authorised to treat GM waste. Sharps and animal waste are transferred directly to the incinerator for destruction.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The University GM committee, at a meeting held on 29.4.97, endorsed the decision of the local GM committee (dated 16.4.97) and the two outside experts from other local committees that this project requires Containment Level 2 facilities that are available in laboratories 2.681 and 2.682 of the Stopford Building. No special problems were identified that could not be contained by Good Microbiological Practice. It was noted that Group II organisms were involved and that HSE approval was required before work could start.

Project Containment

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Project Ref 630/01.1

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<td>USE OF RAAV (RECOMBINANT ADENO ASSOCIATED VIRUS) TO INFECT MAMMALIAN CELLS IN CULTURE</td>
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Non-GMM

Consent Granted  Not Applicable

02/03/2022
Withdrawn  N  Tick if notifying a connected programme of work  N

Historical Significant Changes  
Historical Date of Additional Info  
Significant Change ID  
Date of Significant Change  

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?  N**

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

16/03/2001
### Project Containment

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**Laboratory Activities**
- Animal Units
- Large Scale Activities
- Human Clinical Applications

### Project Ref 686/01.1

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<td>AN INVESTIGATION INTO THE ROLE OF DNA METHYLATION IN MURINE EMBRYOTIC STEM CELLS VIA INDUCIBLE EXPRESSION OF RELEVANT GENES</td>
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- Non-GMM: Consent Granted
- Project notified under transitional arrangements

### Project Additional Information

**Purposes of the contained use**
- Introduction of methyl transferase genes, via retrouiral infection, into murine es cells.
### Recipient or parental organism
Murine embryonic stem cells, E14 line.

### Host/vector system
**DH5α**: Host

Non mobilisable plasmids, pLNCX-Derives: vector.

Replication - incompetent retrouirus production in PT67 cells.

### Origin & function
Genetic material derived from non-mobilisable pLNCX plasmids and containing commonly used reporter and marker genes together with one of the following three genes.

1. De Novo Methyltransferase 3A.
2. De Novo Methyltransferase 3B.
3. Methyl Binding Domain 2B.

The vectors enable study of these gene functions in cell culture.

### Evaluation of foreseeable effects
The genetic elements and vector system are well characterised and frequently employed. The proximal aim is to generate replication. Incompetent viral particles for the purpose of infection of murine ES cells and the study of the phenotypic effects of the above-named genes. Since the viral particles will be incapable of autonomous propagation (due to the absence of structural genes) and since the plasmids themselves are non-mobilisable, there are no significant foreseeable environmental or health concerns arising from this experiment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
**Effective containment and control measures:**

1. Non-mobilisable plasmids.
2. Replication - incompetent viral particles.
3. Use of a Level 2 containment facility.
4. Use of a Level 2 tissue culture hood.
5. Chemical and heat inactivation of waste material.
6. Confirmation of inactivation using appropriate methods.
7. Autoclaving of all equipment before removal from facility.
9. Regular inspection of facility and hood.
10. Regular confirmation of satisfactory autoclaving.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management measures:

1. Pipette aspiration of biological cell culture material.
2. Chemical and heat neutralisation of cells and viral particles.
3. Chemical and heat neutralisation of bacterial cultures.
4. Confirmation of 100% neutralisation by demonstration of absolute sterility post treatment.
5. Monitoring of methods using Browne’s steriliser tubes.
6. Removal from facility in appropriate labelled bags for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Discussed fully.
Satisfactory risk assessment.
Recorded in safety committee minutes.

Project Containment

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02/03/2022 Page 5116 of 15326
Project Ref 686/01.2

Date Ackn'd 17/02/2004

CU2 Project Title
ADOPTIVE IMMUNOTHERAPY FOR CANCER USING VACCINIA VIRUS AND RNA-CONTAINING LIQUID VESICLE VECTORS FOR INDUCING IMMUNITY TO EPSTEIN-BARR VIRUS INFECTED CELLS

Date Project Ceased 30/06/2005

Consent Granted
Not Applicable

Class 2
CultureVolumeClass3-4
< 1 Litre

Non-GMM
Project notified under transitional arrangements

Historical Significant Changes
Transferred from GM686 on 17/02/2004

Project Additional Information

Purposes of the contained use
Introduction of Epsien-Barr virus genes into human antigen presenting cells using Vaccinia virus as a vector.

Recipient or parental organism
Recombinant Vaccinia viruses are thymidine kinase negative and consequently less virulent than wild type Vaccinia.
Manipulated human antigen presenting cells are not known or thought to be any risk to the operator of the environment.

Host/vector system
DH 5a Host
Vectors will be non-mobilisable plasmids.
Vaccinia virus will be propagated in TK-143 cells.
All Epstein-Barr virus genes are derived from the phototype B95.8 strain of EBD. The gene products will be used to stimulate an immune response against cells latently infected with EBV, as with some tumour cells.

Evaluation of foreseeable effects

EBV is carried out by nearly all individuals in any community, therefore, the effect of any exposure to a single EBV gene product is likely to be effective zero. Vaccinia virus has been used on a wide scale to immunise people. Vaccinia virus Recombinant strains carrying Rabies Virus genes have also been used in non laboratory settings, notably in Belgium where virus infected meat was used to immunise foxes in the wild.

Plasmid vectors are of the non-mobilisable type. Thus, these should be incapable of passing genetic material to wild-type bacteria.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultural aspirates pass into concentrated Presept. Aerosols will be contained with an 'in line' 0.22mu filter. All solid and liquid waste will be autoclaved after chemical inactivation. All bacterial cultures will be chemically treated and its effectiveness monitored by regualr culturing to test for viability. The autoclave is regularly monitored by use of Browne's tubes and a temperature recorder. Removal of waste after treatment to the clinical waste stream.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

The Oncology Genetic Modification Safety Committee discussed the Risk Assessment and several minor amendments were requested. It was accepted that the amended Risk Assessment be approved by a senior committee member. The Risk Assessment was approved on the basis of this.

Project Containment

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02/03/2022
Project Ref 686/99.3

Date Ackn'd 17/02/2004

Date Project Ceased 30/06/2005

CU2 Project Title
Development of gene delivery viral & non-viral methods for the GM of normal & neoplastic haematological cell type, lab development novel approaches to cellular therapy

Class CultureVolClass2 CultureVolumeClass3-4
Class 2

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Transferred from GM686 on 17/02/2004

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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02/03/2022
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| Tel Number          | 01403 272827     |
| Fax Number          | 01403 323253     |

#### HSE Division

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#### Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Transgenic Fish</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum culture released at one time is 5 litres.

All liquid waste is autoclaved prior to disposal via the drain. All solid waste is autoclaved and then incinerated off site by an external contractor.

The autoclaves are routinely serviced and calibrated (every 6 months or year). Thermographic measurement vials are added weekly to check the required temperature is being reached.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 208/07.1

Date Ackn'd 15/02/2007

CU2 Project Title Development of a idiopathic pulmonary fibrosis (IPF) model to facilitate the design of more effective medical treatments to treat IPF in humans.

Class 2

Culture Vol Class 2 1-50 Litres

Non-GMM Consent Granted Not Applicable

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info
**Project Additional Information**

**Purposes of the contained use**
The contained use aims to develop a model for the human disease idiopathic pulmonary fibrosis to facilitate the design of more effective medical treatments.

**Recipient or parental organism**
A variety of routinely used cell lines and experimental models will be used.

**Host/vector system**
Recombinant replication deficient adenoviruses [AdTGF-beta-1 & AdTGF-beta-1 223/225].
- AdTGF-beta-1 expresses inactive porcine TGF-beta-1.
- AdTGF-beta-1 223/225 contains a mutation of cysteine to serine at positions 223 & 225, rendering the expressed TGF-beta-1 biologically active.
- Both vectors contain a deletion in the E1 region which makes them replication deficient.

**Origin & function**
**Origin of material**
Material is cDNA of the full length porcine TGF-beta-1, isolated from the Okayama and Berg cloning vector and plasmid pPK9A, by digestion with restriction endonucleases KpN (TGF-beta-1) and BamH1 (TGF-beta-1 223/225).
Fragments subcloned into shuttle plasmid pACCMV.PLP.ASR(+) which contains the left end of the human adenovirus type 5 genome (0-17 mU) and a human cytomegalovirus promoter, a multicloning site and an SV40 polyadenylation signal inserted into the E1 region. After spontaneous homologous recombination between the plasmids, recombinant replication-deficient adenoviruses AdTGF-beta-1 & AdTGF-beta-1 223/225 were rescued.

**Intended function of genetic material**
- AdTGF-beta-1 expresses inactive porcine TGF-beta-1
- AdTGF-beta-1 223/225 contains a mutation of cysteine to serine at positions 223 & 225, rendering the expressed TGF-beta-1 biologically active.

**Evaluation of foreseeable effects**
**Vector**
The adenovirus vectors used are replication deficient which limits the chance of survival and transmission. Expression in the lining of the respiratory epithelium declines with time and is limited to ~ 2 months (part 2B-Annex III, HSE compendium of guidance).

**Inserted sequences**
Inserted gene is only recognised by specific receptor. Binding of TGFB1 to receptor in lung triggers events leading to fibrosis. Induction of fibrosis would require large quantities of adenovirus to be delivered to lung and potentially repeat inoculations.

**Control measures in place**
Minimise the risk of development of fibrosis in laboratory workers.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
A variety of engineering and operational controls appropriate to containment level 2 activities will be implemented to ensure effective containment and control of the proposed activities involving genetically modified organisms.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogations applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste (wherever possible) will be sterilised on site by autoclaving at 121°C for at least 20 mins in autoclave that are regularly maintained and UKAS validated. Degree of kill is regarded at 100%. Each autoclave load is monitored with suitable indicators (e.g. autoclave tape, indicator tubes).

All sterilised solid waste will then be disposed by off-site incineration. Asterilised liquid waste will be disposed of via drain.

If necessary small quantities of liquid waste will be disinfected with commercially available, efficacious disinfectants e.g. Virkon. All use will be as directed by the manufacturers guidelines.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

NHRC biological safety committee (includes responsibility for genetic modification) recommended that the risk assessment be modified to restrict the amplification cycles in 293 cells to < 10 for approval at category 1.

Designation as a class 2 activity was considered appropriate for associated work activities.

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Autoclaving at 131 deg C for 50 minutes in a machine that is 12 point tested annually. Subsequent runs are verified by the use of autoclave tape or Browne’s tubes processed with the load. The killing protocol is displayed by the machine at all times.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 212**

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**Name**

**EAST MALLING RESEARCH**

**Name 2**

**Department**

**PLANT BREEDING & BIOTECHNOLOGY**

**Campus Estate or Research Centre**

**Building**

**Road Name**

**NEW ROAD**

**District**

**EAST MALLING**

**Town**

**WEST MALLING**

**County**

**KENT**

**Postcode**

**ME19 6BJ**

**Country**

**ENGLAND**

**Tel Number**

**01732 843833**

**Fax Number**

**01732 521 557**

**E-mail**

**HSE Division**

**EAST AND SOUTH EAST**

**Comments**

Company changed its name from Horticulture Research International to East Malling Research on 30/03/2004

**Date at Which Additional Info Submitted**

02/03/2022

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<td>TN25 5AH</td>
<td>ENGLAND</td>
<td>N</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
All media and material in contact with GM material, as well as GM cultures, are autoclaved prior to disposal. Small volumes of media are sterilised by bleach.

GM plants are grown in growth cabinets and containment glasshouses. GM plants are bagged and autoclaved. Soil used with GM plants is bagged and either autoclaved or steam heat treated according to a Standard Operating Procedure.

Validation of deactivation is carried out using indicator tape and temperature gauges of the equipment. Autoclaves and steaming equipment are regularly inspected.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Please enter comments of the GM safety committee on the risk assessment
### Project Additional Information

#### Purposes of the contained use

Plants will be inoculated with modified ACMV DNA. The modified ACMV lacks a viral coat protein.

#### Recipient or parental organism

Test plants (Nicotiana benthamiana) will be inoculated with modified ACMV DNA. The modified ACMV lacks a viral coat protein and thus is unable to form a viral particle and thus will not be insect transmitted. ACMV symptoms on his host, Manihot esculenta, a non-UK crop, are mild. Its insect vector Bemisia tabaci is not found in the UK. No genes which are toxic or allergic to humans or animals will be cloned in ACMV. Hazard arising from these experiments is negligible and the risk effectively zero.

#### Host/vector system

- **Host**: Nicotiana benthamiana
- **Vector**: ACMV genome A based constructs in piC19H

#### Origin & function

Plasmid with ACMV-specific sequences obtained from Dr J Stanley, John Innes Centre, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK.

Constructs will be used to express foreign gene sequences as inoculated plants.

#### Evaluation of foreseeable effects

ACMV with or without foreign gene insertion is unlikely to be harmful to humans or animals. The modified ACMV genome on inoculated plants is likely to be less fit than wild type.

ACMV is not transmitted by seed or pollen. Its natural host Manihot esculenta is not grown in the UK and its insect vector Bemisia tabaci is not found in the UK.

ACMV clones lack the coat protein gene, so it cannot be insect trasmitted. Genes cloned in the vector are not toxic or allergenic to humans or animals.

The likelihood of hazards arising is negligible.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- Inoculation of host plants will take place in a secure, closed growth room. Access to the growth room will be restricted to authorised personnel.
- A limited number of plants (<50) will be grown in compost in pots on waterproof trays. A limited amount of water will be used in watering. Routinely, there will be no run-off. If run-off occurs, it will be contained.
- The growth room is insect free.
- Transport of waste material will be in secure closed containers and autoclaved.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

all material in contact with GM materials, including GM cultures, plants and growth media will be placed in identified containers and autoclaved prior to disposal. The autoclave is routinely serviced and checked for temperature and pressure.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The application and attached risk assessment have been discussed by the site Genetic Modification Safety Committee. It was agreed that, subject to HSE approval, the work could proceed under the conditions described.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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<td>L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
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Project Ref 212/01.2

Date Ackn’d 03/08/2001

CU2 Project Title

THE USE OF TOBACCO MOSAIC VIRUS (TMV) AS A TOOL FOR THE EXPRESSION OF FOREIGN GENES IN PLANTS

Date Project Ceased

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

Plants will be inoculated with modified TMV RNA containing differing plant genes.

**Recipient or parental organism**

Test plants (*Nicotiana benthamiana*) will be inoculated with modified TMV RNA. TMV with or without modification is unlikely to be harmful to humans or animals. TMV is present in the UK. Disease symptoms caused are moderate and vary in differing hosts such as *Nicotiana tabacum*. TMV is not transmitted by insects or pollen. TMV is transmitted by mechanical inoculation, grafting contact between plants and occasionally seeds (via the testa). No genes that are toxic or allergenic to humans or animals will be cloned in TMV. Hazard arising from the experiments is negligible and risk effectively zero.

**Host/vector system**

*Host: Nicotiana benthamiana*  
*Vector: TMV cDNA with a duplicated coat protein promoter clone in pUC19*

**Origin & function**

Plasmid with TMV-specific sequences obtained from Dr W O Dawson, University of Florida, Lake Alfred, FLA 33850, USA.  
Constructs will be used to express foreign gene sequences as inoculated plants.

**Evaluation of foreseeable effects**

TMV with or without a foreign gene insertion is unlikely to be harmful to humans or animals. The modified TMV genome inoculated on test plants is likely to be less fit than wild type.  
TMV is not transmitted by insects or pollen. It is mechanically transmitted and occasionally (via the tests) seed transmitted.  
Genes clones in the TMV vector will not be toxic or allergenic to humans or animals.  
The likelihood of hazards arising from this work is negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Inoculation of host plants will take place in a secure, closed growth room. Access to the growth room will be restricted to authorised personnel.  
A limited number of plants (<50) will be grown in compost in pots on waterproof trays. A limited amount of water will be used in watering. Routinely, there will be no run-off. If run-off occurs, it will be contained.
The growth room is insect free.

Transport of waste material will be in secure closed containers and autoclaved.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material in contact with GM materials, including GM cultures, plants and growth media will be placed in identified containers and autoclaved prior to disposal. The autoclave is routinely serviced and checked for temperature and pressure.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

This application and attached risk assessment have been discussed by the site Genetic Modification Safety Committee. It was agreed that, subject to HSE approval, the work could proceed under the conditions described.

Project Containment

<table>
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<th>Large Scale Activities</th>
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Project Ref  212/01.3

Date Ackn'd  03/08/2001  CU2 Project Title  GENE EXPRESSION IN PLANTS USING POTATO VIRUS X (PVX) AS AN  Class  Class 2  Culture Vol Class 2  <1 litre  Culture Volume Class 3-4
Project Additional Information

**Purposes of the contained use**

Plants will be inoculated with modified PVX RNA containing differing test genes.

**Recipient or parental organism**

Text plants (Nicotiana benthamiana) will be inoculated with modified PVX RNA. PVX with or without modification is unlikely to be harmful to humans or animals. PVX is present in the UK, but is often not considered a pathogen. PVX is only mechanically transmissible. No genes which are toxic or allergenic to humans or animals will be cloned in PVX. Hazard arising from these experiments is negligible and the risk effectively zero.

**Host/vector system**

Host: Nicotiana benthamiana.
Vector: pP2C2S bacterial plasmid containing a PVX genome with duplicated viral coat protein promoter.

**Origin & function**

Plasmid with PVX-specific sequences obtained from Dr D Baulcombe, John Innes Centre, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK.

Constructs will be used to express foreign gene sequences as inoculated plants.

**Evaluation of foreseeable effects**

PVX with or without foreign gene insertion is unlikely to be harmful to humans or animals. The modified PVX genome on inoculated plants is likely to be less fit than wild type.

PVX is not transmitted by seed or pollen. It is only transmitted by mechanical inoculation.

Genes cloned in the vector are not toxic or allergenic to humans or animals.

The likelihood of hazards arising is negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Inoculation of host plants will take place in a secure, closed growth room. Access to the growth room will be restricted to authorised personnel.

A limited number of plants (<50) will be grown in compost in pots on waterproof trays. A limited amount of water will be used in watering. Routinely, there will be no run-off. If run-off occurs, it will be contained.

The growth room is insect free.

Transport of waste material will be in secure closed containers and autoclaved.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material in contact with GM materials, including GM cultures, plants and growth media will be placed in identified containers and autoclaved prior to disposal. The autoclave is routinely serviced and checked for temperature and pressure.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

This application and attached risk assessment have been discussed by the site Genetic Modification Safety Committee. It was agreed that, subject to HSE approval, the work could proceed under the conditions described.

Project Containment

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Project Ref 212/02.1
**Project Additional Information**

Purposes of the contained use

The aim of the proposed experiment is to test the ability of cloned MBV to replicate in transfected protoplasts of Agaricus bisporus and Coprinus cinereas in vitro.

Recipient or parental organism

Protoplasts of Agaricus bisporus or Coprinus cinereas will be transfected in vitro by plasmid DNA containing MBV or cDNA. MBV is unlikely to be transfected using virions or viral RNA. Experiments will be carried out in vitro. It is unlikely that MBV cDNA will infect wild mushrooms outside tissue culture facilities.

Host/vector system

Host: Agaricus bisporus and Coprinus cinereas

Vector: MBV cDNA in pVC19

Origin & function

Plasmid with MBV-specific sequence (MBV variant tester) MBV with or without foreign gene insertion is unlikely to be harmful to humans or animals. The virus is unlikely to be transmitted by mechanical inoculation of virions or RNA. Experiments will be carried out in vitro using protoplasts unlikely to survive in the environment. Likelihood of hazards arising from this work is negligible.

Evaluation of foreseeable effects

Inoculation of protoplasts will take place in vitro in a contained laboratory.

All material arising from the experiments will be placed in secure containers and autoclaved.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material in contact with GM materials, including GM cultures, plants and growth media will be placed in identified containers and autoclaved prior to disposal. The autoclave is routinely serviced and checked for temperature and pressure.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This application and attached risk assessment have been discussed by the site Genetic Modification Committee. It is agreed that, subject to HSE approval, the work could proceed under the conditions described.

**Project Containment**

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Animal Units

| L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 |

**Project Ref** 212/05.1

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<td>27/01/2005</td>
<td>Nicotiana benthamiana and Nicotiana tabacum plants will be infected with full-length GM viral transcripts, designed to express the human endostatin peptide.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Not Applicable</td>
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Date Project Ceased 02/03/2022
The purpose of the project is to use the plant viruses, Potato virus X (PVX) and Tobacco mosaic virus (TMV), as tools for the expression of foreign genes in plants, with the aim to investigate potential of the PVX and TMV vectors as tools for foreign protein production.

Both PVX and TMV are endogenous viral pathogens and neither is considered to have any serious consequences on infected plants. Both viruses are transmitted in nature by mechanical means, generally plant-plant contact and neither virus is known to be transmitted by arthropod or nematode vectors. In the unlikely event of release of the modified virus to the environment a rapid selection for "wild-type" virus is observed and recombinant viral progeny lacking foreign genetic sequences rapidly arise and dominate quickly dominate the viral population.

The modified PVX and TMV cDNA's with a duplicated viral coat protein subgenomic promoter is cloned in plasmid pBluescript KS (M13-) for propagation in suitable Escherichia voli K12 strains. Nucleic acid sequences of interest will be inserted into the vector using standard molecular biology procedures. Gene expression will be under the control of the coat protein promoter and the expression level will not exceed that of the viral coat protein.

The gene encoding the human endostatin protein will be inserted into cDNA copies of the PVX and TMV viral genomes in such a way that infection of plants with invitro synthesized viral RNA transcripts will permit the infection of host plants and production of recombinant endostatin. Endostatin is a protein that acts as a negative regulator of angiogenesis and therefore has a potentially important role as a therapeutic agent to prevent rumour growth and metastasis.

Evaluation of foreseeable effects

Endostatin is normally produced in humans and animals (eg during wound healing processes) and therefore neither endostatin-coding sequences nor endostatin are considered to pose any health threat to humans and animals. It is unlikely that introduction of the endostatin gene into the PVX or TMV genome will alter the routes of viral vector transmission.

The modified PVXs or TMVs are likely to be less fit than wild type. It is unlikely that expression of human endostatin, a protein that is not normally expressed in plants, will make PVX or TMV pollen or seed transmissible. It is also very unlikely that the modified PVX or TMV will become a vector transmissible (eg by insect, mite, fungal or nematode vectors) and that the host range of the modified PVX will be altered. Therefore, the genetically modified PVX or TMV will not be any more harmful than its parent.
Inoculation of plant material will be performed in a Unigro GroDome. The construction method means that the facility is virtually airtight. The compartments can be negatively pressurised (to approx 40 pascals) to ensure that insects or pollen do not escape and an air exhaust bag filter will be used. The method of construction also means that the steel superstructure and polycarbonate dome can withstand winds up to 120mph and resist impact from flying objects produced by such winds.

Close adherence to standard operating procedures, training of staff, and rigorous implementation of containment measures should protect the environment. Furthermore, the isolated location of the growth room inside a building in the middle of a very large field of perennial fruit crops (which PVX or TMV is not able to infect), should prevent the modified viruses from escaping to infect any susceptible natural host plants.

Gene expression vectors based on PVX and TMV have been used for well over a decade in the UK under containment conditions that are inferior to those of the GroDome without any evidence of escape to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Infection of whole plants with modified PVX will be undertaken in a compartment of the Unigro Grodome Containment Facility. This facility is designed to meet containment level II and is therefore suitable for the proposed research. Access to the facilities (growth room) will be restricted to authorised personnel only.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In the laboratory all media in contact with GM materials as well as GM cultures and plants will be placed in identified containers and autoclaved prior to disposal.

Infected plants will be grown in compost in pots in waterproof trays in a containment growth room inside a building. Only a limited amount of water will be used daily for watering plants. There will be no run-off water. Accidentally contaminated water will be contained.

Plant material and other solid waste will be transported from the Unigro GroDome compartment in secure closed containers, for autoclaving and disposal.

Following recommendation from Defra Plant Health Division, liquid waste and condensate from the cooling coils will be collected, treated with Virkon to give a final concentration of 1% (or another suitable disinfectant at an appropriate concentration) and then autoclaved.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC was convened to discuss this notification, and agreed recommendations have been incorporated into the section above.
Use of pseudomonas syringae pv moresprunourn to deliver Pseudomonas effector genes into plant cells

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
The project will explore the genetic basis of host specificity by using strains that cause bacterial canker on Prunus species. Comparative genomics will be used to compare the pathogenicity factors of these strains to try and find genes important for infecting Prunus species and closely related strains isolated from other plant species.

Recipient or parental organism
Wildtype strains of Pseudomonas syringae/savastanoi (these species names are used interchangeably) from the pathovars morsprunorum, syringae, avellanae, aquilegia, tomato and phaseolicola. These strains were isolated from diseased cherry, plum, hazelnut, aquilegia, tomato and bean plants. All Pseudomonas moresprunorum strains derive from the UK and are only known to cause disease on cherry and plum. Another strain, isolated from Aquilegia vulgaris, will also be used, again isolated from the UK.

Host/vector system
Strains of E.coli (principally DH5a and S17) will be used to store vectors and used for conjugation of the vector into Pseudomonas.
Gene candidates possibly involved in host specificity including secondary metabolite biosynthesis genes and effectors will be cloned and transformed into E. coli and then moved into different strains of Pseudomonas in order to elucidate their functions and if they are targeted by plant resistance. Knockout strains in which some of these genes are deleted may also be produced to further determine function. Cloning will be primarily focused on finding avirulence genes (genes that reduce pathogenicity) so will involve moving candidate avirulence genes from non-pathogens into pathogens to see if they effect virulence. Knock-outs will also focus on reducing virulence. Therefore, GM strains are likely to have reduced virulence on their host, rather than an expanded host range.

### Evaluation of foreseeable effects

The genes involved will function in pathogenicity of the isolate for example effector genes which suppress the plants immune system or secondary metabolites that kill plant cells. In the highly unlikely event of establishment or dissemination, the microbes will likely have reduced virulence compared to wild-type clones and therefore be evolutionarily unfit and unlikely to persist. Were they to do so they would contribute to the already high levels of bacterial canker that are currently present in the wider environments, due to the susceptibility of most cultivated cherry material.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The inserted genetic material will include secondary metabolite biosynthetic genes and effectors from the pathogen. They will be expressed in their active form. The inserted material will never intentionally leave the laboratory due to the use of containment protocols, restricted access protocols and the use of licensed, contained rooms. In the highly unlikely event of establishment or dissemination, the microbes will likely have reduced virulence compared to wild-type clones and therefore be evolutionarily unfit and unlikely to persist. Were they to do so they would contribute to the already high levels of bacterial canker that are currently present in the wider environments, due to the susceptibility of most cultivated cherry material. It is unlikely that transmission would be altered, as this is primarily through mechanical wounding and tissue localisation is unlikely to be altered in a detrimental way (to the plant) due to the ability of the pathogen to already infect multiple major tissue types.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material in contact with GM materials including GM cultures, plants and growth media will be placed in identified containers and autoclaved prior to disposal. The autoclave is routinely checked for temperature and pressure.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

### Project Containment

The committee approved this GMRA and subject to HSE approval this work can proceed.
## Project Ref
212/16.2

<table>
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<tr>
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<td>Gene expression using strawberry mild yellow edge virus</td>
<td>Class 2</td>
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**Project notified under transitional arrangements**

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

### Project Additional Information

#### Purposes of the contained use

The purpose of the project is to use the plant virus, Strawberry Mild Yellow Edge Virus (SMYEV), a relative of the potexvirus Potato virus X (PVX), as a tool for the expression of foreign genes in plants, with the aim to investigate potential of the SMYEV vector as a tool for protein production for the purposes of screening pathogen-derived avirulence genes.

#### Recipient or parental organism

The host of the virus is disarmed Agrobacterium tumefaciens which will then be infected into Fragaria x ananassa.

#### Host/vector system

The SMYEV is the vector system. SMYEV is a potexvirus and is present in the UK. The disease symptoms caused are mild, and SMYEV is often considered not to be a plant pathogen.
The genes to be expressed in using SMYEV will be reporter genes to show that the system works including β-glucuronidase (GUS), a protein which is not normally expressed in plants. When the expression system is working effectively genes involved in disease resistance including effector genes and resistance genes will be expressed using the viral system.

Evaluation of foreseeable effects

The modified SMYEVs are likely to be less fit than wild type. Evidence from Tobacco Mosaic Virus has shown that potexviruses usually lose their transgenic component during the natural infection process and therefore will have reverted to wild type after several passages (see Virology 172:285-292 1989). However, the chimeras may have novel properties, which could alter pathogenicity. It is unlikely that expression of a reporter gene, a protein which is not normally expressed in plants, will make SMYEV (or other potexvirus) pollen or seed transmissible. In future, when using genes from pathogens and genes involved in disease resistance and signalling process it is also unlikely that these genes will increase the fitness of the virus for the same reasons. Potexviruses have small genomes and no virus has been found with a >6.5kb genome. Therefore it is highly unlikely that a transgenic protein could be sustained in the viral genome. It is also very unlikely that the modified SMYEV will became a vector transmissible from plant to vector (e.g. by insect, mite, fungal or nematode vectors) and that the host range of the modified SMYEV will be altered. Therefore, the genetically modified SMYEV will not be any more harmful than its parent.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Although SMYEV is not considered to be a serious pathogen, protection of the environment is still the primary concern. Close adherence to standard operating procedures, training of staff, rigorous implementation of containment measures will protect the environment. Furthermore, the isolated location of the growth room inside a building (Bioassay Basement B) will prevent the modified viruses from escaping to infect any susceptible natural host plants. The growth room is free from insects, so transmission via this route is unlikely. Although SMYEV is transmitted insect vectors, it cannot be acquired from infected plants. Therefore cautious insect control is in place in the growth room, and the facilities will afford secure containment. Transport of waste from the facilities will be in secure closed containers. Compliance with the requirements set down by DEFRA for the parental virus will provide satisfactory environmental protection. The likelihood of hazards arising is therefore negligible and the risk is effectively zero.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material in contact with GM materials including GM cultures, plants and growth media will be placed in identified containers and autoclaved prior to disposal. The autoclave is routinely checked for temperature and pressure.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
This risk assessment is aimed at using a strawberry virus as a tool for expressing genes in planta. This risk assessment was considered to be of negligible risk to human health and low risk to the environment. The containment measures will effectively reduce the risk to zero. As the research is using a plant pathogen the overall classification of the GM material has to be Class 2 and the HSE informed.

The committee approved this GMRA and subject to HSE approval this work can proceed.

### Project Containment

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- **Animal Units**
  - L2 | L3 | L4 | L2 | L3 | L4

- **Large Scale Activities**
  - L2 | L3 | L4 | L2 | L3 | L4

- **Human Clinical Applications**

### Project Ref 212/16.3

- **Date Ackn’d**: 17/03/2016
- **CU2 Project Title**: Investigating the pathogenicity of fungal vascular wilts *Verticillium* sp and *Fusarium* sp
- **Class Culture Vol**: Class 2 | ≤ 1 Litre
- **Consent Granted**: Non-GMM | Consent Granted

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

### Historical Significant Changes
- **Historical Date of Additional Info**: 02/03/2022

### Project Additional Information

- **Purposes of the contained use**: The fungal vascular plant pathogens *Verticillium dahliae* and *Fusarium oxysporum* have an extremely broad host range, and they are widely known for causing wilt. They are soil-borne pathogens, which can live in the soil for long periods of time making the control methods very challenging. The aim of the study is to characterize the
mechanisms of infection, revealing the effector gene repertoire and its influence on pathogenicity. Therefore, effector genes will be inserted and deleted from the fungal organisms to study their function. This will be done by cloning the constructs into E.coli and then transferring the vectors into Agrobacterium tumefaciens which will be used to transform the fungal species.

In addition, circadian clock studies will be performed towards assessing how pathogenicity is affected by the circadian rhythm. This will be carried out in Verticillium dahliae, by knocking out putative clock genes and adding promoter reporters (Luciferase) and fluorescent proteins (e.g. Green Fluorescent Protein).

**Recipient or parental organism**

Verticillium sp (causative of verticillium wilt)
Fusarium sp (causative of fusarium wilt)

**Host/vector system**

E.coli (principally DH5a)
Agrobacterium tumefaciens disarmed (EHA105; GV3101)

**Origin & function**

The cloned genes from Verticillium sp or Fusarium sp are involved in pathogenicity (effector genes which suppress the plants immune system). For knockouts, the inserted DNA will be 500-1000bp upstream and downstream of genes of interest. FP and reporter genes will allow the fungi to fluoresce under a microscope or luminesce the presence of a substrate.

**Evaluation of foreseeable effects**

Some strains of Verticillium dahlia and Fusarium oxysporum are present in the environment. Although care must be taken to avoid their release. Some isolates come from abroad, and they present a risk for the environment since they could be significant if released. Therefore, these strains are to be kept at containment level 2 and are treated as licensed pathogens.

Expression of the effectors genes from pathogens: could increase the fitness of the pathogens. Expression of the reporter genes: will not cause any harm for the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Transformed organisms with effector genes could have altered pathogenicity on plant species and they could cause an increase of virulence. Appropriate containment of these pathogens will be taken (containment level 2).

Transformed organisms with fluorescent proteins, reporter genes or gene knockouts will be no more hazardous than the untransformed fungi.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

none requested

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All material in contact with GM materials including GM cultures, plants and growth media will be placed in identified containers and autoclaved prior to disposal. The autoclave is routinely checked for temperature and pressure.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N
The risk assessment investigating the pathogenicity of fungal vascular wilts Verticillium sp and Fusarium sp was considered to be of negligible risk to human health and low risk to the environment. The containment measures will effectively reduce the risk to zero. As the research is using a plant pathogen the overall classification of the GM material has to be Class 2 and the HSE informed. The committee approved this GMRA and subject to HSE approval this work can proceed.

Project Containment

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<tr>
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Tick to confirm that you have attached a risk assessment to this form: Y
Tick if you are claiming exemption from disclosure for section of the risk assessment: N
### GM Centre Number: 213

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**Name**

ALBUMEDIX LTD

**Name 2**

R&D

**Campus Estate or Research Centre**

CASTLE COURT

**Road Name**

59 CASTLE BOULEVARD

**District**

NOTTINGHAM

**Town**

NOTTINGHAM

**County**

NOTTINGHAMSHIRE

**Postcode**

NG7 1FD

**Country**

ENGLAND

**Tel Number**

0115 955 3355

**Fax Number**

0115 955 1266

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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<th>Date Premises Closed</th>
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<td>NOTTINGHAM SHIRE</td>
<td>NG7 1FD</td>
<td>ENGLAND</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
Mycology

Transgenic Invertebrates

Transgenic Plants

Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

The waste produced is up to a batch maximum of 10 Lt of liquid containing Genetic Manipulated Materials. This waste material are ALL autoclaved at 121 degrees C.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 214

Data Premises Notified (Originally) 14/07/1986

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Name
ROYAL HOLLOWAY & BEDFORD NEW COLLEGE

Name 2
UNIVERSITY OF LONDON

Department
SCHOOL OF BIOLOGICAL SCIENCES

Campus Estate or Research Centre

Building

Road Name
EGHAM HILL

District

Town
EGHAM

County
SURREY

Postcode
TW20 OEX

Country
ENGLAND

Tel Number 01784 443545

Fax Number 01784 434326

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted
02/03/2022
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|                        |                  |            |        | EGHAM HILL | EGHAM | SURREY | TW20 0EX | ENGLAND | N |

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory
Animal Unit
Growth Room
Glass House
Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

Bacteriology
Parasitology
Transgenic Birds
Transgenic Animals
Transgenic Fish
Microbiology Research
Gene Therapy

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 214/01.1

Date Ackn'd 19/02/2001

CU2 Project Title SIGNALLING MOLECULES INVOLVED IN CANCER

Date Project Ceased 25/09/2007

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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### Project Additional Information

**Purposes of the contained use**

Genetically modified Giardia will be produced to study the evolution of the mitochondrion, an energy-generating cellular organelle central to the origins of all extant eukaryotic organisms. The project will also investigate the physiological role of mitochondrially-derived genes and their encoded proteins.

**Recipient or parental organism**

Wild-type Giardia intestinalis, pathogenic.

**Host/vector system**

Non-mobilisable, pUC-derived plasmid vectors will be used to introduce DNA into Giardia by electroporation. Transfectants will be selected on the basis of their acquired marker genes. Acquired episomal or chromosomal foreign DNA is lost from Giardia in the absence of selection.

**Origin & function**

The genetic manipulation of Giardia will entail the introduction of well studied genes with no known harmful or toxic properties to human health or to the environment. Homologous Giardia genes encoding mitochondrial functions (eg cpn60, hsp70) as well as standard reporter genes (eg firefly luciferase, B-galactosidase, neomycin resistance) will be expressed in Giardia. The source of DNA will be the laboratory strain WB (ATCC # 30957) and conventional strains of Escherichia coli.
**Evaluation of foreseeable effects**

Members of the genus *Giardia* parasites a wide variety of vertebrates, including humans, and cause diarrheal disease worldwide, particularly in children. *G. intestinalis* is transmitted in nature as waterborne infective cysts, it is a non-invasive pathogen and secretes no known toxin. About half of infected people are asymptomatic and the infection frequently resolves spontaneously. Under standard laboratory conditions, *Giardia* is cultured as the non-infective trophozoite form. Cultured trophozoites are sensitive to desiccation, detergents, and changes in temperature, pH and osmolarity, and do not survive passage through the stomach. However, infective cysts can be experimentally generated in culture and these may be environmentally transmitted. The most likely route of transmission to people in the laboratory is by oral ingestion. This possibility will be minimised by the use of automatic pro-pipettes and disposable gloves. Further, efficient anti-giardial drugs exist for the treatment of giardiasis (Wolfe MS. 1992. Giardiasis. Clin Microbiol Rev 5, 93-100). The proteins to be expressed are not involved in pathogenicity, so it is unlikely that GMMs will be more harmful to humans than either the host or wild-type *Giardia*. All culture work will be carried out in a class II microbiology safety cabinet. Access to the culturing facility will be restricted.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures and contaminated waste will be treated with 5% chloros and autoclaved prior to disposal as conventional solid/liquid waste. No GMOs are viable after such treatment.

**Project Containment**

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<tr>
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<tr>
<td>Human Clinical Applications</td>
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Project Additional Information

Purposes of the contained use

The project forms one component of a multi-site Medical Research Council (MRC) funded Co-operative Group (CoG) whose remit is the "Rational Development of an HIV/AIDS Vaccine". Other components of the (CoG) are based at Kings College London, Imperial College School of Medicine, St George's Medical School, University of Oxford and University of Reading. While drug complex drug therapies are available to stabilise HIV/AIDS, it is widely accepted that development of effective vaccines is to tackle populations, such as in sub-Saharan Africa where incidence of HIV infection is very high and growing. Studies at RHUL will involve production of genetically modified bacteria, cultured mammalian cells, and replication-defective adenoviral vectors (rAds) which contain partial DNA sequences of the HIV genome. In addition parallel studies will be conducted on the simian immunodeficiency virus (SIV) an organism which infects certain classes of primates but not humans. However, no intact infectious HIV or SIV will be handled. The GMMs will be used in experiments to configure, develop and optimise rAd-based vaccines for preventative and therapeutic immunisation against HIV. It is worth noting that a 1st generation of rAd-based HIV vaccines are already in human clinical trial. The HIV/SIV genomes contains some 10 genes and a range of control sequences, all of which are required to constitute an infective and pathogenic viral particles. In the work at RHUL, individual HIV/SIV gene sequences, or combinations of several sequences be cloned and manipulated in bacteria, and then introduced into rAds: so-called 1st generation [E1/E3-deleted] and 2nd generation [E1/E3/E2 (terminal protein and viral DNA polymerase)-deleted]) rAds of serotypes 5, 11 and 35 will be constructed. These rAd GMM will be able to transduce a range of mammalian cell types (including human) and will then be tested for effective gene delivery and effective production of the putative immunogenically-protective HIV/SIV protein components in a range of cultured human and mouse cells.

Recipient or parental organism

Cultured Escherichia coli K12 derivatives will be genetically modified.
Cultured human and murine cells will be genetically modified.
Adenovirus will be genetically modified (in all cases in replication-defective format).

Host/vector system
Hosts are described above. Non-mobilisable plasmids vectors are derived from pBR/pUC plasmids, and from M13 bacteriophage. Replication-defective adenovirus vectors will be produced and propagated in HEK-293 and 911 cells.

Origin & function

HIV/SIV genetic elements will be derived from cloned fragments of HIV Clade B and C, and SIVmacJ5 isolates produced elsewhere and imported into the containment lab in plasmid form. No intact infectious HIV or SIV genetic isolates or molecular clones will be handled.

The HIV/SIV genomes contain some 10 genes and a range of control sequences, all of which are required to constitute an infective and pathogenic viral particles. In the work at RHUL, individual HIV/SIV gene sequences, or combinations of several sequences be cloned and manipulated.

Evaluation of foreseeable effects

Summary of major risks identified:

(i) GM adenoviral vectors expressing the HIV/SIV nef gene were considered to present the highest level of risk to human health.

(ii) Naturally occurring fully-infectious adenoviruses are mild pathogens designated at level 2 by the Advisory Committee on Dangerous Pathogens (ACDP). Adenovirus infection in humans can cause mild influenza-like illness, gastro-intestinal upsets and/or conjunctivitis, and generally pass without referral to a GP. Some 80-90% of the human population has already been infected/encountered adenovirus and are protectively immunised as a result.

(iii) GM adenoviral vectors produced in this project will be highly attenuated and replication-defective. This means they can infect human cells and thus provide an immune stimulation but cannot replicate and spread.

(iv) GM adenoviral vectors will be genetically-modified to carry several HIV/SIV genes. No infectious HIV/SIV virus is involved. However one product of these genes (the so-called nef gene) has been shown to be toxic when present at high levels in special experimental circumstances ie. widely overproduced in transgenic animals. Thus one of the GMMs being created in the project must be considered as potentially harmful to human health and thus classified as a Group II GMM as defined by HSE.

(v) Volume of production in the project will be limited to low-level, non-industrial, research scale of activity ie. Type A as defined by HSE.

(vi) Potential risks may thus exist of exposure of lab workers or members of the public to GMMs should a breach of contained use occur. Specific operational risks include (i) ingestion, inhalation or topical contact with GMM aerosols, (ii) needlestick injury with GMM contaminated sharps, (iii) inadequate waste transport and/or disinfection procedures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection Procedures and Waste Management.

(i) Chemical disinfectants will be validated under working conditions (eg. high protein concentrations in culture media) against the GMMs being handled either in practice or via manufacturer's specification. Disinfectants will be solutions of hypochlorite (5000 ppm chlorine) or Virkon (2%) or ethanol (70%).

(ii) Autoclave inactivation of GMMs (121 degrees C, 22psi, >30 minutes) will each time be validated by internal thermolog (sensitivity-strip) procedures and outcome
logged. Two thermocouple-certified large autoclaves are available, in case one should malfunction. Bagged waste will be transported to this room in a designated large, wheeled bin with a closely fitting lid, that is labelled 'GMM waste'.

(iii) GM waste containers will be clearly marked until disinfection/inactivation is complete.

(iv) Liquid waste will be discharged to sewer after disinfection by a validated procedure.

(v) Solid waste will be discharged to the domestic refuse after disinfection and/or autoclaving by a validated procedure.

Extract from the Minutes:

10.12 Report on risk assessment submitted
(NB: Consideration of this RA was chaired by JT as Deputy BSO)

a) Section 3, 3.3: Revise re emphasise hazard of nef gene

b) Section 5, 5.1: Noted that adenovirus is a level 2 ACDP pathogen but that replication-defective adenoviral vectors carry much lower if any intrinsic risk.

c) Section 5, 5.11: This paragraph was included in error and should be deleted. No animal vaccination will occur at RHUL. (Now deleted in attached RA)

d) Section 6, 6.4: This paragraph was included in error and should be revised. No animal vaccination will occur at RHUL. This paragraph to be deleted. (Now revised in attached RA).

e) Consider production of an SOP document to minimise the risk of inadvertent sero-conversion.

Recommendation: Provided changes satisfy the Deputy BSO, passed at containment Level 2 for GMMs. (NB: This Project requires CU2 notification to HSE).

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Project Ref 214/11.1

02/03/2022
Project Additional Information

Purposes of the contained use

This programme of work is designed to develop and evaluate gene and cell therapy models and strategies to potentially treat inherited and acquired diseases including severe combined immunodeficiency, spinal muscular atrophy, stroke, Parkinson disease and others. A range of genes will be manipulated in prokaryotic (plasmid and phage) and eukaryotic (plasmid and replication-defective viral) gene transfer vectors, and these vectors used to transfer genes into target cells for the purpose of modelling disease and assessing potential therapeutic benefit.

Recipient or parental organism

BACTERIA
K12 derived E. coli strains such as: TOP10, SURE, JM109, DH5 and other such disabled E.coli strains.

REPLICATION-DEFICIENT VIRAL VECTORS
Retroviral vectors derived from Moloney murine leukaemia virus.
Lentiviral vectors typically derived from Human Immunodeficiency virus-1 (HIV-1).
Adeno-associated virus vectors (typically derived from AAV-2).
Adenovirus vectors (typically derived from AdV-5).

RECIPIENT MAMMALIAN CELL LINES
Laboratory cell lines like human embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, BJ foreskin fibroblasts (ATCC-CRL-2522).
Established cell lines like NIH-3T3 fibroblasts, HT1080, HeLa and HEK293 cell variants, mouse ES cells, L-cells, embryonic carcinoma (e.g. Ntera 2), C6 glioma and N2A neuroblastoma cells, myoblasts (e.g. C2), rat PC12 pheochromocytoma, African green monkey COS7, lymphoid, mieloid and other standard laboratory cell lines.

RECIPIENT MAMMALIAN PRIMARY CELLS
Such as rodent motor neurons, neurons, glial cells, microglia, myoblasts, haematopoietic cells, fibroblasts and other primary cells.
Primary human cells, mostly fibroblasts from skin biopsies, but also those obtained from other tissues.
CLONING VECTORS
Non-mobilisable vector systems e.g. lambda gt and charon-based bacteriophage vectors and pUC/M13mp-based plasmid, filamentous phage or phagemid vectors, unable to replicate outside bacterial cells. For cloning and prokaryotic expression of recombinant DNA sequences, disabled E. coli K12 derived bacterial host strains (e.g. TOP10, SURE, JM109, DH5, etc) will be used.

MAMMALIAN EXPRESSION PLASMIDS
Such as pSV2, pCMV or pVAX plasmid families, or similar. Typically used on cell lines like HEK293T, HeLa, HT1080, L cells or similar. Transfer plasmids used for the production of viral vectors (please see below) also contain mammalian cell expression cassettes.

RETROVIRAL VECTOR SYSTEM (MOLONEY MURINE LEUKAEMIA VIRUS-BASED)
Transfer plasmids: such as pWZL, pBabe, pMIG families or similar.
Packaging plasmids: pGagPol(M57), pEnv(K73-Eco) ecotropic envelope, or similar.
Producer cells: HEK293 variants, or similar.
Packaging cells: Cell Biolabs' HEK293-based Platinum Retroviral Packaging Cell Line E (PlatE, ecotropic), PlatA (amphotropic) or similar. Miller's NIH 3T3 murine fibroblast-based PT cell series (amphotropic), or similar.
Characteristics: In the plasmid form able to replicate only in bacterial cells and, if plasmid backbone contains SV40 ori/enhancer, transiently in T-antigen-expressing cells like HEK293T. In the disabled retroviral vector form unable to replicate in the absence of helper virus, or in non-murine cells when using ecotropic envelope. The vectors are sensitive to inactivation by human complement. These systems are attenuated by the removal of the pol and env genes and partial deletion and repression of gag translation. Self-inactivating (SIN) mutations in the 3' LTR considerably decrease the risk of insertional mutagenesis. Absence of gag, pol and env sequences in the vector, and the use of split function packaging systems (either three-plasmid transient transfection or split-function packaging cells), make extremely unlikely the generation of replication competent retrovirus through recombination events.

LENTIVIRAL VECTOR SYSTEM (HIV-1-BASED, PERHAPS OTHERS FROM NON-HUMAN HOSTS)
Transfer plasmids: such as pHR, pRRL and pCCL families or similar.
Producer cells: HEK293 variants, or similar.
Characteristics: In the plasmid form able to replicate only in bacterial cells and, if plasmid backbone contains SV40 ori/enhancer, transiently in T-antigen-expressing cells like HEK293T. HIV-1 vectors will be generated by transient co-transfection of three (second-generation) or four (third-generation) plasmids into the producer cells. These standard lentiviral vectors are sensitive to human complement. These vectors will be replication defective (pol, env and partial gag deletion). Second-generation packaging plasmids are also vpr, vif, nef and vpu-deleted; third-generation plasmids are additionally tat-deleted. Lentivirus vectors will also have an additional 3'LTR self-inactivating (SIN) deletion, resulting in the loss of promoter/enhancer functions in the 5'LTR in the transduced target cells. The absence of packaging signal, psi, in the plasmids encoding the structural or accessory proteins will prevent the packaging of these sequences into infectious virions. The generation of a replication competent lentivirus will require multiple recombination events, making this extremely unlikely. Integration-deficient lentivector variants are mutated in the catalytic active site of integrase, preventing significant chromosomal integration of provirus and leading to the formation of increased levels of episomal vector circles; this results in highly reduced risk of insertional mutagenesis.

ADENO-ASSOCIATED VIRUS (AAV) VECTOR SYSTEM (TYPICALLY BASED ON AAV-2 GENOME)
Transfer plasmids: such as pAAV and pscAAV families, or similar.
Packaging plasmids: second-generation pAdDeltaF6 and p5E18-VD2/9, or similar.
Producer cells: HEK293 variants, or similar.
Characteristics: In the plasmid form able to replicate only in bacterial cells and, if plasmid backbone contains SV40 ori/enhancer, transiently in T-antigen-expressing cells like HEK293T. This second-generation AAV-2 genome-based production system does not require helper adenovirus. It is based on transient transfection of three plasmids
in HEK293T cells and effectively prevents adventitious appearance of replication-competent derivatives during vector production. Resulting vector is replication-defective. Use of different vector serotypes may alter vector tropism. We will use several serotypes, including AAV9 capsid protein, known to cross the blood-brain-barrier in at least young mammals.

ADENOVIRUS VECTOR SYSTEM (TYPICALLY BASED ON Ad5)
Transfer plasmids: Shuttle plasmid pDeltaE1sp1A, or similar.
Packaging plasmids: pBHG10 (deltaE1, delta psi, deltaE3), or similar.
Producer cells: HEK293 variants, or similar.
Characteristics: In the plasmid form able to replicate only in bacterial cells and, if plasmid backbone contains SV40 ori/enhancer, transiently in T-antigen-expressing cells like HEK293T. These will be replication defective vectors with deletions in the E1 and E3 region. Vectors may also have additional deletions in the E2 and/or E4 regions of the viral genome, or be “gutted”. E1 is the primary early gene transcription factor triggering the replicative gene expression cascade of other early genes, which then leads ultimately to expression of so-called late, or structural genes of the virus. Vectors are produced in E1-transcomplementing 293 cells. Over 90% of individuals are seropositive for Ad5.

PRODUCER AND RECIPIENT CELLS FOR VIRAL VECTORS
Human and other mammalian tissue culture cell lines or primary cells, as described in previous section, producing disabled retroviral, lentiviral, AAV or adenoviral vectors or transduced with them. These cells are unable to colonize humans, unable to survive outside specialised tissue culture conditions and show no evidence of innate ability to secrete transmissible agents. Producer or transduced cell cultures may contain viral vectors resulting from production process or remaining from transduction procedure, with their corresponding potential hazards.

VECTORS
Prokaryotic cloning and expression vectors.
Eukaryotic expression plasmids containing eukaryotic promoters and termination signals (of viral and mammalian origin).
Plasmids encoding retroviral, lentiviral, AAV and adenoviral vectors.
Retroviral, lentiviral, AAV and adenoviral vectors, including HIV-based vectors.

SOURCE OF INSERTS
Human and other mammal, mainly rodent (e.g. BDNF, GDNF, etc.), bacterial (beta-galactosidase, selectable markers, non-toxic C-terminal domain of tetanus toxin or TTC) or Jelly Fish, Aequorea victoria (GFP, CFP, YFP, RFP), or similar.
Non-hazardous inserts may include reporter genes, selectable genes, gene correction (by homologous recombination) cassettes, cDNAs encoding survival of motor neuron (SMN), its paralogs and other non-toxic genes, or similar.
Potentially hazardous inserts have the following functions:

TRANSGENES ENCODING MEGANUCLEASES, ZINC-FINGER NUCLEASES, SITE-SPECIFIC INTEGRASES, TRANSPOSASES AND OTHER DNA-CUTTING AGENTS.
Meganucleases are DNA endonucleases with recognition sites of at least 12-bp. They determine the homing mechanism used by a range of mobile genetic elements. The archetypical enzyme is I-SceI, and a range of artificial variants have been produced. Zinc-finger nucleases (ZFNs) are chimeric enzymes designed to have DNA endonuclease activity on similarly large target sites. ZFNs result from the fusion of zinc-finger domains (endowing site-specific DNA binding) and a non-specific endonuclease domain from a restriction enzyme. Meganucleases and ZFNs are in practice naturally or artificially designed to recognise and cut at rare DNA target sites. Similar effects could be expected from other enzymes or agents able to effect low-level DNA cutting. These DNA-cutting transgenes will be used mostly in the context of mammalian expression plasmids, lentiviral and adenoviral vectors.

NATURAL AND CHIMERIC NEUROTROPHINS, GFLs, CYTOKINES AND OTHER REGULATORS.
Expression of complementary cDNA genes encoding a variety of natural neurotrophins (e.g. brain-derived growth factor, BDNF), GDNF family ligands (GFLs, e.g. glial cell line-derived neurotrophic factor, GDNF), cytokines (e.g. cardiotrophin-1) and other regulatory proteins has shown therapeutic potential in a variety of disease models, including nervous system disorders. Additionally, these proteins can be endowed with retrograde and trans-synaptic transport properties by fusion with the non-toxic C-terminal domain of tetanus toxin (TTC), which may enhance their therapeutic potential. We will mostly use these transgenes in mammalian expression plasmids, lentiviral and AAV vectors.

**TRANSGENES AFFECTING DEVELOPMENTAL AND DIFFERENTIATION PATHWAYS, AND ONCOGENES.**

Human, mouse and perhaps other induced pluripotent stem (iPS) cells with properties similar to embryonic stem (ES) cells can be generated from skin fibroblasts and other cells by co-expression of various combinations of transgenes (for instance OCT4, SOX2, NANOG and LIN28; or OCT3/4, SOX2, KLF4 and c-MYC). These ES and iPS cells can subsequently be induced to differentiate into lineages of interest by transgene expression and/or drug treatment. cDNA sequences originating from humans or other eukaryotes will be used for this purpose. As well as "wild type" sequences, we may include sequences that are known to be mutated (either by deliberate genetic manipulation or by use of cDNA derived from cell lines harbouring known mutations). We will use a range of such cDNAs with the main goals of deriving iPS cells, inducing specific lineage differentiation of ES and iPS cells, or inducing immortalisation (overexpressing TERT) to facilitate tissue culture, mostly using retroviral and lentiviral vectors.

**SMALL INHIBITORY RNAs (siRNAs).**

Small inhibitory RNAs (siRNAs) are double-stranded RNA segments complementary to endogenous mRNA sequences which result in their specific degradation or inhibition. Synthetic siRNAs and gene transfer vectors encoding a variety of siRNAs to mRNAs of interest will be constructed. These siRNA vectors will be transferred into mammalian cells to inhibit gene expression. Target genes may include p53, PARP1, CYCD1 and others, mostly using lentiviral vectors for cassette delivery.

**PROMOTERS AND OTHER REGULATORY AND/OR STRUCTURAL SEQUENCES.**

Genomic sequences originating from humans or other eukaryotes and viruses will be cloned into the plasmid and viral vectors. These sequences are regulatory or structural in nature and include locus control regions (LCRs), chromatin opening elements (e.g. UCOE), insulators, promoters, scaffold/matrix attachment regions (S/MARs), post-transcriptional regulatory elements and others. As well as "wild-type" sequences, we may include sequences that are known to be mutated (either by deliberate genetic manipulation or by use of DNAs derived from cell lines harbouring known mutations). With the exceptions noted below, these elements will be used in mammalian expression plasmids, retroviral, lentiviral, AAV and adenoviral vectors.

Many lentiviral (and perhaps some retroviral) vectors used in the study will contain wild-type or mutated versions of an enhancer of gene expression derived from woodchuck hepatitis virus (WHV) called the woodchuck post-transcriptional regulatory element, WPRE. The wild-type element is capable of expressing part of the X protein from WHV. The truncated or full-length hepadna virus X-proteins may have oncogenic properties. We will treat this as a potential risk in accordance with the recommendations of HSE SACGM. However, it should be noted that HIV vectors containing wild-type WPRE have an excellent safety profile in a tumour-prone animal model and have been extensively used in animal experiments world-wide.

S/MAR sequences can lead to episomal maintenance of circular double-stranded DNA molecules by endowing them with replication and segregation capabilities in mammalian cells. S/MARs are being explored in the context of mammalian expression vectors to study their efficiency to induce such episomal maintenance. Successful episomal maintenance would imply replication of the episome. We are exploring S/MARs in the context of mammalian expression plasmids, lentiviral and AAV vectors.

**Evaluation of foreseeable effects**

**ACCIDENTAL CONTAMINATION OF WORKERS IN THE LABORATORY**

LIKELIHOOD: LOW; CONSEQUENCES: MODEST; OVERALL RISK: MEDIUM/LOW.

Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. Viral vectors are replication-deficient, and those with integration capability are human complement-sensitive with the standard viral envelopes. Established and primary cells are unable to colonize immunocompetent human hosts and show no evidence of innate ability to secrete transmissible agents.

Possible effects of accidental exposure of laboratory workers

The main hazard in the project is deemed to be the potential transduction of a laboratory worker with an integrating vector (retroviral or lentiviral) encoding a pro-oncogenic
transgene or element (i.e. growth factor, oncogene and the like). The route of exposure could be accidental injection, ingestion, inhalation, skin lesions or mucosal surfaces. Biological barriers and class 2 measures are in place to minimise the likelihood of such an event. In any case, as all viral vectors are replication-defective and retrograde transport and blood-brain barrier crossing are inefficient processes, transduction would essentially be limited to the cells directly exposed to the vector, most likely to be differentiated cells lacking extensive proliferative capacity. The likelihood of the integrating vector additionally causing insertion mutagenesis (disruption or over-activation of endogenous gene at integration site) is extremely low at the doses that could be received by accidental exposure. Crucially, given that tumorigenesis is a multi-step process thought to require several genetic alterations, accidental exposure to a single hazardous vector would not be expected to cause cancer but at the most to lead to a cancer-prone state in the affected cells.

**ACCIDENTAL ESCAPE INTO THE ENVIRONMENT**
**LIKELIHOOD:** NEGLIGIBLE; **CONSEQUENCES:** NEGLIGIBLE; **OVERALL RISK:** EFFECTIVELY ZERO.
Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining; all manipulations with bacteria take place at containment level 1. All viral vectors are replication-defective, and manipulations with viral vectors, their packaging or producer cells, as well as their primary and established cell targets will take place at containment level 2. Retroviral and lentiviral vectors are sensitive to desiccation and have a half life of only 5-8 hours under optimum laboratory conditions. AAV and adenoviral vectors are relatively stable and could potentially survive for protracted periods in the environment, but they are non-replicating and essentially non-integrating. Cells are unable to survive outside the laboratory environment. Procedures are in place to deal with accidental spillage. All waste material is dealt with as described under section 12. The laboratories are kept secured when not in use.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity.  (*Measures & Justification*)

The handling of primary human cells and viral vectors that contain potentially hazardous sequences will be restricted to containment level 2. However, vectors that do not encode such potentially hazardous elements, or cells genetically modified by such vectors, may be used at containment level 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste – All contaminated waste is autoclaved in a dedicated Departmental waste autoclave used at 121° C, 22 psi, for >30 minutes (100% kill). Temperature validation provided by means of an internal thermolog. The autoclave is serviced every six months and documentary evidence of correct function stored on site. The resultant sterile waste is then placed in the College’s general waste disposal system.

Liquid Waste – All liquid waste is inactivated with solutions of either hypochlorite (5000 ppm chlorine), Virkon (2%) or ethanol (70%) for at least 1 hour after which it is disposed down the designated sinks.

**Is an emergency plan required according to regulation 20?**  N

**If yes, tick to confirm that it is attached to this form**  N

**Tick to confirm that you have attached a risk assessment to this form**  Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  N

**Please enter comments on the GM safety committee on the risk assessment**
The risk assessment was assessed by our Genetic Modification and Biological Safety Group and the group agreed that this was Class 2 work. Following advice from several members of the group, the risk assessment underwent minor modification and was then approved, subject to receipt of CU2 form by HSE.
The laboratory is developing mucosal vaccines to *c. difficile* (see www.cdvax.org). As part of this work it is necessary to identify suitable antigens from *C. difficile* that are suitable for use in vaccine formulation. This, in turn requires analysis of genes in *C. difficile* by insertional inactivation and the study of mutants. To achieve this targeted insertional mutation is the most efficient method and requires the construction of GMMs.

The parental organism is *Clostridium difficile*, a Gram-positive bacterium. This microorganism is a defined as hazard.
group 2 in the ACDP list of pathogens. This bacterium is strictly anaerobic and short exposure to air will kill it. However, it can form spores which are dormant, heat resistant, desiccation resistant and easily dispersed in air. This organism can produce infection in animals and humans. This almost always occurs in the elderly and those in hospital and who have been treated with antibiotics which precipitates the germination of spores, outgrowth and production of a potentially fatal infection. Control of disease is mediated by antibiotic therapy. C. difficile is unable to produce infection in healthy people who are not under antibiotic therapy but a risk for dispersion is possible. In animals the disease is not controlled and in livestock which are being treated with antibiotics incidents of C. difficile gastroenteritis is common but no control measures are in place.

Host/vector system

The parental strain used for genetic manipulation is derived from the strain referred to as 630. Strain 630 is an 012 ribotype and was isolated from an infected patient. It is used by the research community as a laboratory strain for genetic manipulation. The strain is resistant to erythromycin but for cloning a erythromycin sensitive strain is used (630Derm) that has the natural erm gene deleted. For genetic manipulation the intended purpose is to delete genes in C. difficile, that is, to create knockout mutations. To achieve this appropriate plasmids are constructed using the pMLT8000 (obtained from Pro N. Minton (Nottingham) and then introduced from E. coli to C. difficile by conjugation. Introduced DNA is recombined into the C. difficile chromosome by a double crossover recombination with positive selection using erythromycin resistance. As a double crossover occurs the integration is stable and cannot excise, moreover, no autonomously replicating plasmids are introduced.

Origin & function

To delete DNA of C. difficile it is necessary to construct a plasmid (derived from the pMLT8000 plasmid series) that carry segments of C. difficile DNA flanking an antibiotic resistant gene (typically erytrhomycin resistance). When introduced into C. difficile this DNA recognises self DNA, a recombination event occurs and in the process DNA is deleted from the host genome. There are various ways in which this can occur but in every case no heterologous DNA is introduced other that the ermR gene and segments of non-functional plasmid DNA. Thus, the resulting host genome will not express foreign proteins and, when compared to the host strain will be devoid of one or more C. difficile genes and therefore potentially less 'fit' than its parent.

Evaluation of foreseeable effects

a) accidental escape into the environment
Potential risk when transporting or moving live cultures.
The risk will be minimised by all work being conducted in a level 2 containment facility with robust hygiene and air sterilisation (Medixair UV units). Spills will be contained by Virkon sterilisation and disposal of waste in double sealed bags and autoclaving. When samples are moved to the animal facility sealed chambers (snap lid boxes) will be used and moved on trolleys.

b) bodily contact or ingestion
Low risk since users will always wear appropriate microbiological coats and gloves at all times. The risk of ingestion would be essentially zero since all pipetting uses battery operated pipettors or manual pipettman within an anerobic chamber. Should exposure occur the user will wash hands with appropriate antibacterial soaps. No user will be allowed in the unit who is on antimicrobial treatment or has been (including 2 weeks post termination of treatment).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

nla
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work with bacteria will be conducted in a class 2 designated lab in the Sch Bioi Sciences (rm 4-32).
Solid waste will be double bagged and sealed then autcolaved (121 degreesC 30min at 15 psi). This is sufficient to kill all bacteria.
Liquid waste will be either sterilised by autcolaving (as above) in sealed containers or inactivated using immersion in Virkon (minimum 1 h) which is sufficient to kill spores of C. difficile.
Laboratory spills where they occur will be disinfected using Virkon spray and wipes. Wipes are then bagged and autoclaved as solid waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

GMBSG were satisfied with the risk assessment and assignment to class 2. subject to HSE CU2 approval. Only minor amendments were required. approved by the Biological Safety Officer, chairman of the GMBSG.

Project Containment

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#### Name

UNIVERSITY OF EDINBURGH

#### Department

MEDICAL MICROBIOLOGY

#### Campus Estate or Research Centre

MEDICAL SCHOOL TEVIOT PLACE

#### Road Name

MEDICAL SCHOOL TEVIOT PLACE

#### Town

EDINBURGH

#### District

EAST LOTHIAN

#### Country

SCOTLAND

#### Tel Number

0131 650 3170

#### Fax Number

0131 650 6531

#### E-mail

health.safety@ed.ac.uk

#### Comments

GM215 MERGED WITH GM207 AS OF 17/02/2004

#### Date at Which Additional Info Submitted

17/02/2004
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Animal Unit</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 216

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Name

GLAXO SMITH KLINE RESEARCH & DEVELOPMENT LIMITED

Name 2

Department

Campus Estate or Research Centre

Road Name
THE FRYTHE

District

Town
WELLWYN

County
HERTFORDSHIRE

Postcode
AL6 9AR

Country
ENGLAND

Tel Number
01438 782000

Fax Number
01438 782570

E-mail

HSE Division
EAST AND SOUTH EAST

Comments
CLOSED ON 11/10/2002, WORK TRANSFERED TO GM588 ON 11/10/2002

Date at Which Additional Info Submitted
02/08/2002
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Please enter comments of the GM safety committee on the risk assessment
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LUDWIG INSTITUTE FOR CANCER RESEARCH

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Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Additional Information

Project Ref: 217/03.1

Date Ackn'd: 03/01/2003

CU2 Project Title: USE OF LENTIVIRUS VECTORS TO STUDY CELL GROWTH CONTROLS

Class: Class 2

Culture Vol: < 1 litre

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: N

Historical Significant Changes: Project transferred to GM77

Project Additional Information

02/03/2022
**Purposes of the contained use**

Deregulation of cell controls affecting proliferation and apoptosis is a sine qua non of cancer development. This programme of work will use lentivirus vectors to aid analyses of the functions of cellular and viral genes involved in cell cycle regulation and cell survival. The lentivirus vector system will enable the efficient transduction of human and murine cells grown in tissue culture with either cDNAs encoding cellular and viral genes or cassettes encoding short interfering RNAs (siRNA).

**Recipient or parental organism**

The lentivirus vectors to be used in this programme of work are severely disabled derivatives of human immunodeficiency virus type 1 (HIV-1). A number of genes required for HIV-1 replication and virulence have been deleted from the lentivirus sequences contained in the vector. The virus vectors are propagated as plasmids in E. coli strains. Packaged virus able to infect human, mouse and other animal species are produced by cotransfection of the lentivirus vectors with other plasmids which provide proteins required for packaging in trans. Although the packaged lentivirus is able to transduce efficiently genes cloned in the lentivirus vector plasmid, the virus is totally unable to replicate in these cells. No infectious virus can therefore be produced.

**Host/vector system**

The standard vectors to be used are based on deleted/mutated HIV-1 genomes, pseudotyped with a vesicular stomatitis virus (VSV) envelope (VSV-G) allowing infection of a broad range of cell types. In some cases these recombinant HIV-1 will encode a green fluorescent protein (GFP) from a bicistronic transcript allowing the identification of infected cells by fluorescence microscopy or flow cytometry. In other cases the vector will encode a gene for drug resistance (e.g., Neomycin or Hygromycin). The delivery systems to be used involve three separate plasmids that are transiently cotransfected into the human 293T cell line to produce pseudotyped, packaged viruses. One plasmid encodes the HIV-1 derived vector, one encodes the VSV-G and one provides functions in trans for packaging. Two very similar systems will be used which only differ in the details of generating infectious virus:

**SYSTEM A PLASMIDS**

1. pAG131 - the HIV-IRES-GFP expression construct from which env, vif, vpr, vpu genes have been deleted and the tat gene is inactivated by inverting its open reading frame. These deletions and inversion make the resulting recombinant virus completely incapable of replication in the absence of helper proteins encoded by additional plasmids. The AG131 plasmid still encodes HIV-1 Gag and Pol and will include the cDNA of interest or encode the siRNA.
2. pME-VSV-G - this is a plasmid encoding the VSV-G envelope protein for pseudo-typing the lentivirus.

**SYSTEM B PLASMIDS**

1. pAB286 or pHOX-GFP or pHOX with GFP replaced by the siRNA cassette from pSUPER plus target sequence [see Brummelkamp et al. (2002) Science 196, 550-553.] or a cDNA. These plasmids all encode self-inactivating (SIN) lentivirus with a 400-nucleotide deletion in the 3' LTR. This deletion abolishes all LTR promoter activity and therefore the vectors are incapable of replication. The HIV-1 Vpr, Vif, Vpu, Nef and Tat genes are also deleted in the vector.
2. pRD1274 (pMD.G). This plasmid encodes the VSV-G envelope for pseudotyping the lentivirus.

**Origin & function**

The cDNAs carried by the lentivirus vectors will comprise two classes: (1) cellular and viral genes encoding proteins that promote cell proliferation and/or decrease apoptosis - such genes are generally, but not exclusively, oncogenes and (2) cellular and viral genes encoding proteins that inhibit cell proliferation and/or promote apoptosis - such genes are generally, but not exclusively, tumour suppressor genes. The siRNA cassettes will also comprise two classes: (3) siRNAs directed against cellular and viral genes encoding proteins that promote cell proliferation and/or decrease apoptosis and (4) siRNAs directed against cellular and viral genes encoding proteins that inhibit cell proliferation and/or promote apoptosis. These are described in more detail below:

(1) Lentiviruses carrying cellular and viral cDNAs encoding proteins that promote cell proliferation and/or decrease apoptosis.
Only single growth-promoting (for example EBNA-2, EBNA-3C, LMP1, RUNX1, RUNX3, Id2 and B-myb) and anti-apoptotic genes (for example Bcl-2) will be transduced in this programme of work. As cancer development is a multistep process, transduction with such genes will not transform the infected cells. This programme of work will not include viral oncogenes which have multiple oncogenic activities such as the SV40 T antigen, nor will it include clusters of genes such as HPV16 E6-E7. Genes encoding toxins will not be included in this work.

(2) Lentiviruses carrying cellular and viral cDNAs encoding proteins that inhibit cell proliferation and/or promote apoptosis.

These genes, which include tumour suppressor genes (for example p53, ASPP, RB, P130) and pro-apoptotic genes (for example Bax, Bik and Bad), are expected to inhibit cell growth.

(3) Lentiviruses encoding siRNAs directed against cellular and viral genes encoding proteins that promote cell proliferation and/or decrease apoptosis.

Short interfering RNAs (siRNA) are designed to ablate expression of a specific gene target. Ablation of oncogene expression, for example, would in many instances inhibit cell cycling or promote cell death. Expression of this class of siRNA (for example siRNAs directed against EBNA-2, EBNA-3C, LMP1, RUNXI, RUNX-3, Id2, iASPP, mdm2, RAS and B-myb), would be equivalent to that outlined in (2) above. That is, cell growth is likely to be inhibited. It is noted that siRNA sequence specificity is very precise, and generally there is insufficient homology between the mRNA target sequences of mouse and human genes for cross-species interference to occur. For example, siRNAs directed against mouse genes are unlikely to affect growth of human cells.

(4) Lentiviruses encoding siRNAs directed against cellular and viral genes encoding proteins that inhibit cell proliferation and/or promote apoptosis.

Expression of siRNAs which ablate tumour suppressor gene function has the potential to promote cell growth. Lentiviruses expressing siRNAs directed against, for example, p53, ASPP, RB and p130 may predispose cells to further oncogenic events, but would not by themselves transform the cell. This programme of work will be restricted to expression of a single siRNA directed against this class of genes, or the expression of multiple siRNAs which affect the same regulatory pathway. Examples of the latter include the p53 pathway (eg ablation of p53 or ASPP would be expected to have similar consequences) and the RB pathway (eg ablation of RB and p16 INK4a is likely to be equivalent).

Evaluation of foreseeable effects

Lentivirus vectors are designed to infect efficiently both actively proliferating and resting cells of many different species and lineages. The two major foreseeable effects are accidental transduction of laboratory personnel and animal species such as mice. In this respect, it should be noted that these vectors are replication-defective and the risk is therefore limited to cells accidentally transduced with the packaged vector. The consequences for animal species are therefore negligible, since even in the extremely unlikely event that mice were exposed to the packaged vector, the virus could not propagate.

Exposure of laboratory personnel to packaged vectors containing growth promoting genes such as oncogenes or anti-apoptotic genes (see (1) above) could result in some growth advantage of transduced cells. Similarly, transduction with lentiviruses encoding siRNAs directed to growth inhibitory genes such as tumour suppressor genes and pro-apoptotic genes (see (4) above) could also result in a growth advantage. It should be noted, however, that the single genes targeted in these studies can not by themselves result in cell transformation, even in highly responsive rodent cells. Targeting of combinations of genes with the potential to cause cell transformation has been specifically excluded from this programme of work. It should also be noted that with the scale of production to be used, it is extremely unlikely that accidental transduction will occur in stem cells at risk of carcinogenic development.

Accidental exposure of laboratory personnel with packaged vectors carrying genes that inhibit cell proliferation and/or are pro-apoptotic (see (2) above) is very unlikely to have any consequence. Similarly, transduction with lentiviruses encoding siRNAs directed to growth promoting genes such as oncogenes and anti-apoptotic genes (see (3) above) would only inhibit the growth of the cells infected. In principle, transduction of stem cells could inhibit their growth and prevent them participating in tissue maintenance. In practice, it is unfeasible that sufficient stem cells would be affected in this way to have any consequences for human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be collected in double autoclave waste bags and subjected to autoclaving under approved conditions. 100% kill is achieved with this procedure.

Liquid waste will be collected in polythene contains and treated for 24 hours with 1% Chloros. This achieves 100% kill.

Small laboratory spills will be treated with 70% ethanol; high concentrations of alcohol effectively kill enveloped lentiviruses (van Engelenburg et al., 2002, J. Hosp. Infect. 51:121-125). Larger spills will be cleaned with 1% Chloros. Contaminated paper towels are treated as solid waste.

Extracts will be made from tissue culture cells for biochemical and cell biological procedures (eg microscopy). The simple act of infection inactivates much of the input virus as it becomes uncoated upon entry into the cell. When making cell extracts, residual virus will be inactivated by detergent treatment (ie 1% SDS or 0.5% NP40). Studies have shown that HIV is very sensitive to inactivation even with non-ionic detergents (Ukkonen et al., 1988 Eur. J Clin. Microbiol. Infect. Dis. 7:518-523). The effectiveness of the detergent treatments will be validated by plating transduced cell extracts on indicator cells and selecting for antibiotic resistance carried by the vector. For flow cytometry and microscopy techniques, the transduced cells will be fixed with 70% ethanol or 4% formaldehyde or 4% paraformaldehyde. These treatments achieve 100% kill.

It was unanimously agreed that Containment Level 2 was appropriate in view of the slight theoretical risk to human health of tranasducing genes designed to alter cellular growth controls. It was noted that other groups working with lentivirus vectors had also designated their work as a Class 2 Activity.

Project Containment

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<th>Growth Rooms</th>
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<td>L2 L3 L4</td>
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### Project Additional Information

#### Purposes of the contained use
The generation of bacterial artificial chromosomes (BAC) containing entire herpesvirus genomes allows the use of powerful bacterial cloning strategies to introduce precise modifications into the virus. These modified herpesvirus BACs can be transfected into mammalian cells to study the impact of these mutations in the context of the entire genome. By stimulating the lytic cycle, infectious virus can also be recovered from these transfected cells.

#### Recipient or parental organism
Sequences comprising the Epstein-Bar virus (EBV) and herpesvirus saimiri (HSV) A11-S4 genomes will be inserted into BAC vectors, which will then be cloned and propagated in attenuated E. coli strains DH5 and DH10. Modified BAC constructs will be transfected into mammalian cell lines to generate virus stocks. It is envisaged that HEK 293 cells will be used initially to generate EBV stocks. Replication-defective EBV will be propagated in a 293 line containing an integrated full-length EBV genome to provide replication factors in trans. HSV will initially be propagated in either owl monkey kidney cells or marmoset embryonic fibroblasts, in which the virus is spontaneously lytic. In principle, infectious virus can be generated in any mammalian cell line if the replication cycle is stimulated, and other lines may be used to propagate virus as they become available.

#### Host/vector system
BAC vectors are F-factor-based plasmids which are conjugated-deficient, and are therefore extremely unlikely to transfer to bacteria in the environment. The HVS A11-S4 genome has already been inserted into a BAC vector at the University of Leeds (White et al., J. Gen. Virol., in press). This HSV strain is attenuated and lacks the stpA gene required for oncogenic transformation. Details of the proposed HVS modifications are given in the accompanying risk assessment. EBV sequences to be cloned include the entire wt sequence and derivatives with modifications of immortalisation genes, insertion of non-hazardous marker genes and viral genes as specified in GM217/99.2.
The nature of modifications to be introduced to the EBV genome using BACs are broadly the same as those reflected in previously approved protocol GM217/99.2, namely the deletion or alteration of immortalising genes, the insertion of non-hazardous marker genes or sequences and the construction of viruses that conditionally express defined viral genes. Because modifying herpesvirus BACs does not require full viral function, we will also modify or delete viral genes or structural DNA regions involved in virus latency, replication and packaging, and in the co-ordination of the viral transcription programs. The aim of our work with HVS is to identify the viral genes and DNA motifs involved in the latent and lytic replication and packaging functions of HVS, and to develop the virus as a gene delivery system. This will be achieved by 1) deletion (and subsequent complementation) of genes involved in these processes. 2) in situ modification of these genes (eg fusion to GFP or other tags; mutation of putative domains) to dissect their function. 3) Addition of marker genes (eg antibiotic resistance, luciferase) driven by various promoters to identify differences in gene expression between integrated and episomal expression systems. 4) Tagging the viral episome and arrays of Tet operator repeats to allow the visualisation of the episome in live cells. 5) Cloning human and mouse genetic loci to study expression and regulation of mammalian genes in their natural context. Such work will initially comprise analysis of MHC and immunoglobulin gene loci, but may be extended to other gene loci, however, this work will exclude cloning of oncogenic or other potentially harmful genes.

Evaluation of foreseeable effects

Propagation of BAC-transformed attenuated E. coli strains is not expected to have any adverse effect. The BAC plasmids would be exceedingly difficult to transfer to wt E. coli and other bacterial species, and in any case viral genes would not be expected to be expressed in these bacteria. Generation of virus in transfected mammalian cell lines will give rise to recombinant virus that potentially will be able to replicate in certain primate species, including man. Both EBV and HVS are gammaherpesviruses, which have a restricted host range and grow slowly in vivo. Pathogenesis associated with A strains of HVS have not been observed in man, and the A11-S4 strain to be used has been further attenuated by removal of the transforming stpA gene. EBV is normally transmitted through exchange of saliva, and it is therefore unlikely that the virus would be transmissible under Containment Level 2 conditions. As outlined above, in the event that the recombinant EBV or HVS viruses to be used in this project were accidentally transmitted to laboratory workers, the pathogenicity of the viruses would be no greater, indeed probably less, than the wt viruses.

Release of the recombinant viruses into the environment would have no adverse effect, as both infect only primates.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Extracts will be made from tissue culture cells for biochemical and cell biological procedures (eg microscopy). The simple act of infection inactivates much of the input virus as it becomes uncoated upon entry into the cell. Any residual virus will be inactivated by detergent treatment (ie 1% SDS, 0.5% NP40) when extracts are made. The effectiveness of detergent treatments will be validated by plating transduced cell extracts on indicator cells and staining for virus plaques or viral antigens. For flow cytometry or microscopy purposes, virus-transduced cells will be fixed with 70% ethanol, 4% formaldehyde or 4% paraformaldehyde. These treatments achieve 100% kill.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
It was unanimously agreed that work on manipulating EBV and HVS sequences in BAC vectors in E. coli could proceed at Containment Level 1. Generation of recombinant EBV and HVS viruses by transfection of mammalian cells will be a Class 2 activity. Similarly, infection of mammalian cells in culture with the recombinant viruses will be carried out at Containment Level 2.

**Project Containment**

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**Project Ref** 217/93.1

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**Historical Significant Changes**

GM217/97.1, GM217/99.1, GM217/99.2,

**Historical Date of Additional Info**

17/12/1996, 11/02/1999, 13/10/1999,
Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Name

| OXFORD BROOKES UNIVERSITY |

Name 2

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Campus Estate or Research Centre

| GIPSEY LANE CAMPUS |

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Comments

Date at Which Additional Info Submitted

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<td>OX3 0BP</td>
<td>ENGLAND</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td></td>
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<td>Level 2 (GMMs)</td>
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Tick if confidential

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<th>Parasitology</th>
<th>Transgenic</th>
<th>Microbiology</th>
<th>Research</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
<td></td>
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</tbody>
</table>
Disinfection by immersion
Disinfection by immersion in either hypochlorite solution (2,500 ppm available chlorine by dissolving Haz-Tabs in water) or Verkon (1%) is utilised for overnight treatment of glassware. Hypochlorite or Verkon is also utilised to disinfect suspensions infected with GMMs by incubation overnight. Disinfected liquid is then discarded in the sluice. It is unlikely that more than 5 litres of disinfected sample will be released at any one time.

Disinfection procedures are validated and monitored by a trained member of technical staff using the "in Use" test (Collins, 1983) which assesses survival.

Autoclaving to Destroy
GMM contaminated glassware is autoclaved using the Labclave on the glassware autoclave to destroy cycle at 121 degrees C for 35 minutes. GMM contaminated plastic disposable consumables including all tubes, petridishes, microtitre plates, tips, ependorfs, cuvettes and pipettes are sealed in autoclavable bio-hazard bags and stored in lined bins in a separate waste disposal room (T120) for autoclaving. These are then autoclaved using the Labclave on the Plastic discard autoclave to destroy cycle at 134 degrees C for 35 minutes. Laboratory coats are also autoclaved before being sent to the laundry.

The autoclave to destroy cycles are validated and monitored by a trained member of technical staff using spore suspensions (Sterikon-Bioindicators, Merck) and testing for survival.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 220/02.1

Date Ackn’d 08/11/2002

Date Project Ceased 02/03/2022

CU2 Project Title DEFINING THE AUTOGRAPHA CALIFORNICA NUCLEOPOLYHEDROVIRUS (ACMNPV) GENES IMPORTANT IN BACULOVIRUS TRAFFICKING WITHIN INSECT CELLS, VIRUS TRANSMISSION BETWEEN CELLS AND PERSISTENCE WITHIN INSECT CELLS

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 litre

Non-GMM Consent Granted not applicable
Project Additional Information

Purposes of the contained use

To contain the viruses and virus-infected cells and larvae to be used in the above project.

Recipient or parental organism

For insect cells and larvae the parental organism will be Autographa californica nucleopolyhedrovirus (AcMNPV) C6 strain (Possee, 1986)* containing an intact polyhedrin gene. This is a wild-type baculovirus found in the natural environment. As the accompanying risk assessment indicates, baculoviruses have a long and safe history of use in many countries as a natural biological control agent (Entwistle and Evans, 1985). The AcMNPV virus has also a long and safe history of use as an expression vector for the production of recombinant proteins in insect cells (King and Possee, 1992)*.

* See risk assessment.

Host/vector system

Vector: For insect cells and larvae the vector will be Autographa californica nucleopolyhedrovirus (AcMNPV) C6 strain (Possee, 1986)* containing an intact polyhedrin gene.

Host: The host organisms for these studies are either insect cell lines maintained in vitro: Spodoptera frugiperda Sf9 and Sf21, Trichoplusia ni (TnHi5, Tn368) or laboratory-reared insect larvae (Trichoplusia ni) maintained on simi-synthetic artificial diet (King and Possee, 1992)*.

* See risk assessment.

Origin & function

Origins and functions of the modified viruses.

In this project we intend to make recombinant AcMNPV in which selected AcMNPV genes (ie self genes) are replaced by modified versions. These modified versions will be either (a) tagged with a fluorescent marker protein (eg green fluorescent protein) to make it possible to trace virus infection within cells and larvae or (b) subjected to partial or complete deletions to test gene function in the virus replication cycle in cells or in larvae. [Note: The modified genes will be made by standard cloning techniques in disabled E. coli host strains and are therefore classified as Risk Assessment Level 1. Note: This risk assessment has been approved as Level 1 by OBU ACGM and is, therefore, not part of this submission]. Within the AcMNPV, the normal virus gene will be replaced by the modified gene by homologous recombination in insect cells in culture (a process that is used routinely to make recombinant baculovirus expression vectors; King and Possee, 1992)*.
Details of genes to be tagged or modified
The fluorescent protein tags to be added to the C- or N-terminus of the virus genes are commercially available (green, red and yellow fluorescent protein gene constructs are all from Clontech Ltd), and have been used extensively by us and others in similar studies using polyhedrin-negative baculovirus vectors (Grasela et al., 2000; unpublished data). These studies have shown that the tagged virus proteins behave in the same manner as the unmodified protein. The His (variety of commercial sources) and Flag (Sigma Ltd) tags are also commercially available and are routinely used in many studies to identify and purify the tagged protein from a background of non-tagged proteins.

The virus genes that we intend to modify in one or more of the ways described above include: polyhedrin, p10, chinatinase, p35, IAP1, IAP2, gp64, cathepsin, capsid polyhedron envelope protein, ORF 1629, GP37, GP64, EGT, all late expression factors (lefs), viral capsid associated protein (vp78), actin-rearrangement inducing factor (Arif-1), nuclear matrix associated phosphoprotein (pp31), ODV-E66, GP41, viral capsid associated protein (p95), p26, p74 (Ayres et al., 1994).

* see risk assessment.

Evaluation of foreseeable effects

With respect to human health:
The recipient organism for the AcMNPV vector will either be established insect cell lines maintained in laboratory culture or an established laboratory culture of T. ni larvae which are maintained in a contained facility. The insect cells are not capable of surviving outside of the laboratory culture conditions and pose no known threat to human health (the cells contain no known adventitious agents nor are they known to have transforming properties; even if they were found to contain these agents/properties it is highly unlikely that they would be infectious to human cells) (King and Possee, 1992). The larvae are insects found in the natural environment and pose no known threat to human health (ie do not carry human diseases).

Baculoviruses have a long history of safe use in the environment as natural bio-control agents (Entwistle and Evans, 1985; Carter, 1989). They are highly specific for insect species and although they have recently been shown to be capable of entering human cells by non-specific means under artificially imposed laboratory conditions, there is no virus gene expression and virus replication does not occur. The viruses are found in the natural environment and many studies have shown that they pose no threat to human or other animal health (Entwistle and Evans, 1985). The modified AcMNPV used in this project contains only modified virus genes (ie self genes) that may be deleted, partially deleted or tagged with auto-fluorescent proteins or small peptides. We do not expect or predict that the modifications we intend to make to the specified virus genes will modify baculovirus host-range in respect to hazards to human health.

All genes to be cloned are insect virus in origin. The genes may be tagged with fluorescent protein sequences derived from other sources (these have an established history of safe use as reporter proteins, eg Jelly-fish green fluorescent protein and its colour derivatives). Expression of any genes is strictly limited to insect cell systems because the promoters to be used (natural baculovirus promoter for the specified gene) are insect cell specific (reviewed in King and Possee, 1992; Miller, 1997; Grasela et al., 2000). There is therefore no anticipated or expected harm to human health from the GMMs.

To the environment
ACMNPV contains a polyhedrin gene that encodes a stable protein matrix (polyhedra) that enables the virus to survive in the environment (Granados & Federici, 1986). The GMM will contain a polyhedrin gene and, therefore, has the potential to survive in the environment. However, containment measures (see below) will be used to prevent the GMM being exposed to the environment.

The AcMNPV is specific for a few species of lepidopteran insect and does not infect other animals or humans (see comment above about risk to human health). Therefore the only animals at risk from the GMM are a limited number of species of lepidopteran insects (Huber, 1986; Cory et al., 1994)*. The modifications made to the virus are modifications to virus (self) genes (deletions, partial deletions, tagging with fluorescent proteins or peptides). These modifications are not expected nor predicted to alter host range or virus replication (other than to inhibit replication).

If the GMM (modified virus or larvae infected with modified virus) is released to the environment (Oxford Brookes University Gipsy Lane Campus), it is highly unlikely that it will encounter other baculoviruses/susceptible insects to permit the transfer of genetic material, but it is theoretically possible. The risk to the environment will be reduced by effective control measures, which are listed below.
In assessing our protocols and methods we have identified the following as foreseeable risks in allowing the possible and accidental release of virus or virus-infected cells or larvae:

1. Release of virus through human transfer (hands, clothing)
2. Release of virus through the drains via liquid waste
3. Release of virus through waste disposal of solid waste
4. Release of virus-infected larvae by escaping caterpillars.

We have put into operation the following procedures and regulations to reduce the risk of accidental release of virus or virus-infected larvae as follows:

1. Release of virus through human transfer (hands, clothing)
   All operations with Category 2 recombinant viruses will be carried out in either T110 or T111. All researchers will wear designated laboratory coats, which are autoclaved after use. Coats will be collected in designated bio-hazard bags and transferred to the autoclave for sterilising prior to being sent for routine cleaning. Hands must be washed before leaving the laboratory and sinks with hands-free operated ('magic') taps are located at the exit points of both laboratories.

   All researchers are issued with standard laboratory procedures, which include the washing of hands when leaving the laboratory and the wearing of laboratory coats. All new staff are given an induction programme by the Laboratory Manager for the Insect Virus Research Group.

   The autoclave (Boxer 400/400V (Vacuum)) was new in 2001 and is maintained on a regular service and maintenance contract, which includes annual validation tests to ensure that the cycles we use for the sterilisation of all waste and laboratory coats meets HSE guidelines.

2. Release of virus through the drains via liquid waste.
   Liquid waste containing recombinant virus is generated after infection of insect cells in culture with recombinant virus. All procedures that generate virus-contaminated liquid waste (cell culture medium, reagents, solutions) will be carried out in a designated Class II Safety Hood. All liquid waste will be collected in screw-top glass bottles or tissue-culture growth vessels inside the Class II Hood. The bottles of liquid waste will be transferred to stainless steel autoclave bins and the waste will be autoclaved prior to disposal into the drains. Any sterilised glassware will be recycled using our normal laboratory washing-up procedures.

   The autoclave was new in 2001 and is maintained on a regular service and maintenance contract (with Lab 3), which includes annual validation of our destruct cycles to ensure that the cycles we use for the sterilisation of all liquid waste meets HSE guidelines (121 degrees C for 15 min in the centre of the maximum load). The Class II Safety Hood (UniMAT-BS) complies with BS 5726 and is services and tested twice yearly in line with HSE guidelines.

3. Release of virus through waste disposal of solid waste
   Solid waste containing recombinant virus is generated after infection of insect cells in culture with recombinant virus and after infection of insect larvae. All materials used in the handling of infectious virus are disposable (culture flasks, pipettes, dishes, insect diet pots). All waste is put into bio-hazard bags and these are sealed prior to autoclaving on the solid waste destruct cycle (121 degrees C in the centre of the maximum load). This cycle is validated once per year by Lab 3 as part of their service and maintenance contract.

   The autoclave (Boxer 400/400V (vacuum)) is also insurance inspected on an annual basis (Zurich Insurance). Each individual destruct cycle is monitored by a chart print out of the steam, time and temperature.

4. Release of virus-infected larvae by escaping caterpillars.

   Infected larvae will be maintained in sealed 'polypots', placed in trays (1 larva per pot) and incubated in T111 or a lockable warm incubator room. All larvae transfers will be
carried out on trays with benchkote surrounded by layers of sticky tape to prevent escape of larvae accidentally dropped between culture box and polypot. All polypots are inspected everyday as part of the experimental protocol and the chances of larval escape from the polypots is virtually nil. However, the trays in which the polypots are stored will be lined with benchkote and edged with sticky tape to prevent any escapes from the tray.

All polypots (inc. larvae), benchkote etc. will be sterilised by autoclaving (see above) prior to disposal.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| Not applicable. |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| None |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

#### Management of liquid waste

Liquid waste containing recombinant virus is generated after infection of insect cells in culture with recombinant virus. All procedures that generate virus-contaminated liquid waste (cell culture medium, reagents, solutions) will be carried out in a designated Class II Safety Hood. All liquid waste will be collected in screw-top glass bottles or tissue-culture growth vessels inside the Class II Hood. The bottles of liquid waste will be transferred to stainless steel autoclave bins and the waste will be autoclaved prior to disposal into the drains. Any sterilised glassware will be recycled using our normal laboratory washing-up procedures.

The autoclave (Boxer 400/400 vacuum) was new in 2001 and is maintained on a regular service and maintenance contract (with Lab 3), which includes annual validation of our destruct cycles to ensure that the cycles we use for the sterilisation of all liquid waste meets HSE guidelines (121 degrees C for 15 min in the centre of the maximum load). The Class II Safety Hood (UniMAT-BS) complies with BS 5726 and is services and tested twice yearly in line with HSE guidelines.

#### Management of solid waste

Solid waste containing recombinant virus is generated after infection of insect cells in culture with recombinant virus and after infection of insect larvae. All materials used in the handling of infectious virus are disposable (culture flasks, pipettes, dishes, insect diet pots). All waste is put into bio-hazard bags and these are sealed prior to autoclaving on the solid waste destruct cycle (121 degrees C in the centre of the maximum load). This cycle is validated once per year by Lab 3 as part of their service and maintenance contract.

Laboratory coats are also autoclaved prior to washing.

The autoclave (Boxer 400/400V (vacuum)) is also insurance inspected on an annual basis (Zurich Insurance). Each individual destruct cycle is monitored by a chart print out of the steam, time and temperature.

#### Management of (potential) escaping caterpillars

Infected larvae will be maintained in sealed 'polypots', placed in trays (1 larva per pot) and incubated in T111 or a lockable warm incubator room. All larvae transfers will be carried out on trays with benchkote surrounded by layers of sticky tape to prevent escape of larvae accidentally dropped between culture box and polypot. All polypots are inspected everyday as part of the experimental protocol and the chances of larval escape from the polypots is virtually nil. However, the trays in which the polypots are stored will be lined with benchkote and edged with sticky tape to prevent any escapes from the tray. All polypots (including larvae), benchkote etc. will be sterilised by autoclaving (see above) prior to disposal.

The expected degree of kill that the inactivation procedures will achieve.

It is expected that the destruct cycles described above, for both solid and liquid waste, will achieve 100% kill.
The local ACGM committee met on October 31st 2002 to discuss the proposal. The question of whether an emergency plan was necessary was discussed and the committee concluded that it was not. No foreseeable accident would affect the health and safety of persons outside the premises since no accident could be foreseen that could result in release to the environment because all work is contained within the designated laboratory areas and no GM material leaves these areas. The criteria for containment level 2 were each considered. Records of staff training will be put in place for this activity.

The committee was informed that the proposal had been discussed in some detail with HSE when a representative visited Brookes in May 2002 and that they had visited the facilities and been satisfied with the facilities and the containment procedures which will be in place for this project. The committee was further informed that a draft of the risk assessment for this project had been sent to HSE and that they had been satisfied with the risk assessment and the final level of containment. He made two minor points concerning the presentation of the risk assessment, both of which had been incorporated into the final version presented to the committee.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
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<td>L2 Yes L3 L4</td>
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Project Ref 220/17.1

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<td>Understanding the relationship between the shape of Leishmania mexicana and Leishmania major and their pathogenicity</td>
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<tr>
<td>02/03/2022</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements
### Purposes of the contained use

Research to understand how the shape of Leishmania cells is formed. This work will include culturing the cells and manipulation of their gene expression using standard deletion and overexpression techniques. Outcome of genetic manipulation will be assessed by microscopy to identify changes in the cell shape.

### Recipient or parental organism

- Leishmania mexicana
- Leishmania major

### Host/vector system

1. a series of vectors to provide constitutive expression of transgenes

   References:

2. a series of vectors are designed to provide templates for the PCR generation of amplicons for the introduction of genetic tags or knocking out of specific genes either by direct electroporation or in conjunction with CRISPR/Cas9 system.

   References:

### Origin & function

The main focus is on cytoskeletal genes that are likely to be important in constructing cell shape, motility and cell division. Usually these genes will be tagged to provide epitope or other marking of the expressed proteins. Portions of genes and flanking sequences will be PCR amplified from Leishmania mexicana and Leishmania major genomic DNA and inserted into plasmids for the generation of knock out and tagging vectors.

### Evaluation of foreseeable effects

**Risk to human health**

i) Hazards associated with the recipient organism

Leishmania mexicana and Leishmania major are listed by ACDP as hazard category 2 pathogens. The organisms do not form spores, are rapidly killed outside the culture vessel (or blood) by desiccation, osmotic shock or treatment with 0.2 % Trigene or 2% Virkon solution for 1 hour and the vector required for transmission and completion of the life
cycle is not naturally present in the UK. The organisms cannot penetrate unbroken skin. Genetic modification will likely reduce pathogenicity and most transformations will result in lethality. The organisms will only be maintained in culture form in a category 2 laboratory using a class II microbiological safety cabinet. The laboratory is restricted by swipe card access to authorised personnel only. Dedicated laboratory coats must be worn at all times and gloves must be worn whilst handling Leishmania cultures. Gloves and lab coats must be removed prior to exit.

ii) Hazards related to the vector system
None expected.

iii) Hazards related to the donor/insert sequence
None expected.

v) What is the overall likelihood that, in the event of exposure, the GMO would cause harm to human health?
Low

Risk to the environment
i) What is the capacity for the GMO to survive, establish or disseminate with and/or displace other organisms?
Leishmania mexicana and L. major is cultured in controlled conditions. The organism does not form spores, is rapidly killed outside the culture vessel (or blood) by desiccation, osmotic shock or treatment with 0.2 % Trigene or 2% Virkon solution for 1 hour and the vector required for transmission and completion of the life cycle is not naturally present in the UK.

ii) What is the ability to cause harm to animals?
L. mexicana and L. major - cultured in controlled Cat 2 conditions to prevent this and the vector required for transmission and completion of the life cycle is not naturally present in the UK. The organism cannot penetrate unbroken skin.

iii) What is the ability to cause harm to plants?
No interaction of GMO is expected.

iv) What is its ability to cause harm to micro-organisms?
No interaction of GMO is expected.

v) What is the potential for transfer of genetic material between the GMO and other organisms?
None, as vectors are non-mobilisable.

vi) What is the overall assessment of risk to the environment?
effectively zero

This work has been assessed and passed by the ACGM committee at Oxford Brookes University.

Attached are the full standard operating procedures (SOPs) and are outlined below.

Receipt and transportation of Leishmania mexicana and L. major will be carried out following appropriate regulations. See attached SOP L 1 for more details.

Culturing of the organism carried out in a Cat 2 designated laboratory at Oxford Brookes University in a Class II tissue hood all cultures are disposed of in 2% virkon overnight before autoclaving in an autoclave that is situated in the building. See attached SOP L02 and L03 for more details.

Storage of Leishmania mexicana and L. major will be in liquid nitrogen, which is stored in a secure room that only authorised personnel have access to. See attached SOP L04 for more details.

The culture laboratory and tissue culture hoods will be routinely. See attached SOP LOS for more details.

The waste generated during the culture of Leishmania mexicana and L. major will be treated with 2% virkon overnight before autoclaving. The autoclaves are located in the same building as the tissue culture facilities. See attached SOP L06 for more details.
A set of emergency procedures have been drawn up in case of spillages, accidents involving the organism and breaches of security. Please see attached SOP L07 for more details.
A series of general rules for the culture laboratory have been written, which everyone will adhere to and in doing so reduce the risks involved in culturing this organism. Please see attached SOP LOa for more details.
A set of SOPs have been drawn up for the intended experiments using Leishmania mexicana and L. major. Please see attached SOPs LOg - L 15.
Work involving Leishmania mexicana and L. major will only to be carried out by fully trained staff who will be trained by the supervisor who has 10 years experience culturing this organism.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| n/a |

| n/a |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Liquid waste in volumes greater than 100ml must be placed in the 1 litre Duran bottle in labelled “waste”. Virkon powder or 20m l concentrated Trigene should be added to the waste to give a final concentration of 2% or 0.2% respectively and the bottle should be autoclaved prior to disposal down the sink.
2. Close the lids of contaminated sealable plastic-ware (e.g. tissue culture flasks) tightly within the microbiological safety cabinet and place in the autoclave bag in bucket.
   The contents of non-sealable plastic-ware, eg tissue culture plates, should have 2% virkon or 0.2% Trigene added to each well and these should be then left to soak overnight in the designated area. The contents should be emptied the next day into the white waste pots and the empty plates should be discarded into the buckets.
3. Waste from the aspirator will remain in the liquid trap overnight, prior to disposal in a duran bottle (such as that detailed in point 1 above) containing sufficient concentrated trigene or virkon powder that the final concentration is 0.2% or 2% respectively. The bottle should be autoclaved prior to disposal down the sink.
4. Once metal buckets are full, fold the internal bag over loosely and put on the lid.
5. Contaminated stripettes should be immersed in a vessel containing 2% Virkon or 0.2% Trigene and left overnight, prior to placing in the metal buckets.
6. Small items such as Gilson tips and microfuge tubes are decontaminated by immersing in the white tubs containing 2% Virkon or 0.2% Trigene overnight prior to autoclaving. Syringe bodies are placed in a sharps bin.
7. Waste is autoclaved at 134°C for a minimum of 3 minutes in an annually validated autoclave in the Oxford Brookes University sterilisation unit, prior to disposal via a contracted waste disposal company.
   A 100% degree of kill is expected from these measures.
   See attached SOP L06 for more details

### Is an emergency plan required according to regulation 20?

| N |

### If yes, tick to confirm that it is attached to this form

| N |

### Tick to confirm that you have attached a risk assessment to this form

| Y |

### Tick if you are claiming exemption from disclosure for section of the risk assessment

| N |
Comments from the ACGM committee meeting held on 21 June 2017.
The committee is satisfied that there are the infrastructure and expertise to carry out this work and maintain the required containment measures that are already in place at Oxford Brookes University. The CATII work will take place in a suite of laboratories that are already designated for CAT II work and the staff have shown the correct level of training to carry out this work. The GMO part of the work is using very well established and well characterised procedures and we are satisfied that all the risks have been adequately dealt with and containment is organised correctly.

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**Project Ref** 220/17.2

**Date Ackn'd** 15/12/2017

**CU2 Project Title**
Transient expression (up to 48 hours) of genes in primary human islet cells under the control of a CMV promoter following recombinant baculovirus transduction. The genes to be expressed are reporter genes (lacZ and green fluorescent protein) as well as SOD, Bcl2 and XIAP

**Date Project Ceased**

**Class** Class 2

**CultureVolClass2** < 1 Litre

**Non-GMM** Consent Granted

**Consent Granted** Not Applicable

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

To conduct in vitro expression tests to evaluate the possibility of protecting islet cells from damage during transplantation by expressing protective genes using a baculovirus vector.

Recipient or parental organism

Autographa californica multinucleopolyhedrovirus (AcMNPV), an insect baculovirus

Host/vector system

The vector is a recombinant baculovirus expression vector (based on AcMNPV) in which the natural polyhedrin gene promoter has been replaced with the cytomegalovirus early gene promoter to enable expression of recombinant proteins in mammalian cells (termed BacMam). The vector has a number of gene deletions that mean it is unlikely to survive in the environment; the vector cannot produce polyhedrin and so cannot protect virus particles within polyhedra.

The host cells are human primary islet cells isolated from donated pancreas under Research Ethics Approval at the University of Oxford and held at Oxford Brookes under Research Ethics approval for maximum 48 hour incubation.

Origin & function

Manganese superoxide dismutase 2 (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. It out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. As such, it is an important antioxidant defense in nearly all cells exposed to oxygen. Over-expression of SOD2 has been shown to significantly extend the lifespan of flies and mice.

B-cell lymphoma 2 (Bcl-2) is a member of a family of anti-apoptotic proteins that govern mitochondrial outer membrane permeabilization (MOMP). It is linked to a number of cancers through decreased apoptosis and is involved in resistance to conventional cancer treatment. However, over-expression of Bcl-2 in normal breast cells has not been shown to induce tumor formation.

XIAP - X-linked inhibitor of apoptosis gene encodes a protein that belongs to a family of apoptotic suppressor proteins inhibiting apoptosis, as well as inhibiting caspases involved in cell death. Overexpression of XIAP was shown to prevent beta-cell apoptosis in islet cell transplantation and protect islet cells in mice, enabling the acceptance of graft.

IkBalpha is a cellular protein that acts as a natural inhibitor of Nuclear factor kappa B (NF-KB) by binding and retaining it in the cytoplasm. After exposure to certain cytokines like TNFa and IL-1 b, IkBa is rapidly phosphorylated and targeted for ubiquitin-mediated degradation, which then releases NF-KB to travel to nucleus where it induces proinflammatory genes. However, a mutant version of IkBa that is non-phosphorylatable and non-degradable was shown to be promising in protecting human islet cells in vitro, which can potentially facilitate successful islet transplantation.

SOD-2 and Bcl-2 will be PCR-amplified from commercial plasmids (GeneArt®, Life Technologies Ltd), XIAP, IKBalpha and IKBalpha mutant will be outsourced for gene synthesis by Genewiz. Once the target genes are obtained, they will be inserted into baculovirus BacMam vectors for the transduction of mammalian cells.

These genes have all been over-expressed and published in the literature. The other genes to be expressed are reporter genes (EGFP, LacZ), which also have a history of safe use.

Evaluation of foreseeable effects

The modifications made to the insect viruses are not predicted to change host range or pathogenicity in either the vector system, insect or mammalian cells. The viruses are modified to delete genes that allow them to survive and infect insects in the environment.
The primary human islet cell samples are screened for pathogens as they are cells harvested for human transplantation and only if deemed insufficient in quality or quantity are they released for research purposes. These samples will be used for limited experiments at OBU (transduction with BacMam viruses up to 48 hours) and will not be sub-cultured. All handling of cells will be performed under category 2 level containment controls, with the samples posing no greater risk than other mammalian cells used within the laboratories.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Sterilisation is by chemical disinfectants (freshly made 1 % Virkon, DuPont) and autoclaving.

Liquid waste containing recombinant virus is generated after infection of insect or mammalian cells in culture with recombinant virus. All procedures that generate virus-contaminated liquid waste (cell culture medium, reagents, solutions) will be carried out in a designated Class II Safety Hood. All liquid waste will be discarded to 1 % Virkon solution inside the Class II Hood. The bottles of liquid waste will be left for at least one hour prior to disposal into the drains. Liquid waste can also be collected in glass screw top bottles and autoclaved prior to disposal to drains. Any sterilised glassware will be recycled using our normal laboratory washing-up procedures.

Solid waste generated after infection of insect or mammalian cells in culture with recombinant virus. All materials used in the handling of infectious virus are disposable (culture flasks, pipettes, dishes). All waste is put into biohazard bags and these are sealed prior to autoclaving on the solid waste destruct cycle (at least 121°C in all parts of the maximum load for 15 min). This cycle is validated once per year by (LSS Ltd) as part of their service and maintenance contract. The autoclave is also insurance inspected on an annual basis (Ashdale Engineering). Each individual destruct cycle is monitored by a chart print-out of the steam, time and temperature.

A 100% degree of kill is expected from both of these measures.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The ACGM committee noted that whilst recombinant BacMam GMO were normally classified for use at Containment level 1, the combination with primary human islet cells was assessed at containment category 2.

Project Containment
<table>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: [ ]

Give brief details of the genetic modification safety committee:

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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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| Other (please specify) | | | | Tick if confidential: [ ]

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 221/00.2

Date Ackn'd 15/02/2001
CU2 Project Title DEVELOPMENT OF A SAFE AND EFFICACIOUS VACCINE AGAINST ANIMAL BRUCELLOSIS THAT IS HARMLESS TO HUMANS
Date Project Ceased

Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes
historical Date of Additional Info
Significant Change ID
Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
# Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 221/01.2

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### Project Additional Information

**Purposes of the contained use**

The construction of PrP vectors.

**Recipient or parental organism**

E.coli JM109 cells are the recipients of modified plasmid vectors containing recombinant Prnp gene sequences.

**Host/vector system**

The host is the E. coli strain JM109, which is non-colonising and non-pathogenic to humans. The vector, which is non-mobilisable, is a modified Bluescript II plasmid containing a large portion of the mouse Prnp gene locus.

**Origin & function**

The plasmid vector contains a large portion of the gene locus encoding the mouse Prnp gene. Modifications are made to the DNA sequence of the plasmid vector so that Prnp gene inserts derived from sheep, cattle and kudu are incorporated. The recombinant plasmid vectors are propagated in E.coli cells. Appropriate DNA sequences are prepared for microinjection procedures.

**Evaluation of foreseeable effects**

The recombinant plasmid vector is non-mobilisable, which means that the potential for transfer of genetic material between the GMO and other organisms is unlikely to occur. The host E.coli cells are non-colonising and non-pathogenic to humans, and are therefore not likely to represent a serious hazard to the operator. In addition, the E.coli strain used is auxotrophic, thus its capacity for survival and dissemination in an open environment is limited and is not likely to be significant. Furthermore, as procedures are carried out in a GM-designated level 2 containment laboratory, with the addition of negative air pressure and HEPA filtration of extract air, there is not likely to be a threat of contamination to the outside environment. Waste materials produced in the containment laboratory, which may be contaminated with E.coli or recombinant E.coli organisms, are sealed in two layers of autoclave bags and sealed again within autoclave tins before leaving the containment laboratory for autoclaving and incineration.

The risk to an operator of accidental self-injection of biological material from this project is always present but is likely to represent an unlikely or remote possibility. During handling procedures, protective latex gloves are worn by operators in order to eliminate skin contact with biological materials. As the vast majority of the work involves the use of plasticware, including plastic tips for manipulation of biological materials, accidental self-injection of material is not expected. In the event of accidental ingestion of
biological materials, and in particular transgene DNA, by an operator, it is probably unlikely that the latter would passively recombine with human DNA. However, the actual likelihood of this occurring is unknown. It should be noted that the modified PrP genes under study in this project do not contain polymorphisms analogous to those in human disease, and are very closely related to naturally occurring animals PrPs.

If the mouse PrP plasmid vector, i.e. foreign PrP, was expressed in an operator e.g. following accidental exposure, the consequence of hazard could be severe. However, it is also possible that the consequence may not be severe. This is because, and it should be emphasised that, expression of foreign PrP protein would not itself represent the prion agent. Thus, expression of foreign PrP per se may not lead necessarily to prion pathology or to infectious prion disease.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

NB  Negative air pressure is incidental to the location of the work and is not essential for this project.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Disposable equipment: autoclaved, then incinerated.
- Solid culture plates: autoclaved, then incinerated.
- Culture supernatants: treated with chloros overnight, then disposed.
- Reusable equipment: autoclaved, washed then recycled.
- Plasticware containing chemicals such as phenol or flammable liquids (e.g. ethanol incinerated.)

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by our local GM Safety Committee.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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02/03/2022
# Project Additional Information

**Purposes of the contained use**

Identification of virulence and colonisation factors of campylobacter jejuni.

**Recipient or parental organism**

**Host/vector system**

The host Campylobacter strains to be used are the laboratory adapted strains 81116 and 11168, and other wild type C. jejuni strains from veterinary, environmental and clinical sources. Laboratory adapted E.coli strains (DH5a, HB101, TOPO10F') will be used for cloning and expression studies, all of which are auxotrophic and non-pathogenic. Vectors reported for use in Campylobacter will be used, such as the shuttle vector pUOA18 (Bleumink-Plyum et al., 1999. Microbiology 145: 2145-2151) that replicates in both E.coli and Campylobacter, and suicide vectors that only replicate in E.coli and are also used routinely as standard cloning vectors e.g. pBluescript (Stratagene), pCRScript (Stratagene), pUC18, pCRTOPO (Invitrogen). The vector used in the cosmid library is pLA2917 (Fry et al., 1998. Microbiology 144: 2049-2061) and the transposon delivery plasmid is pOTHM (Golden et al., 2000. Infect. Immun. 68: 5450-5453).

**Origin & function**

All genetic material will be from Campylobacter jejuni strains 81116, 11168 and other strains from human and poultry environments. DNA to be targeted will be that which has similarity to other known virulence factors such as invasins, adhesins, fimbrial genes, genes for colonisation factors or genes that encode proteins which might be...
surface exposed. The exact function of the genetic material is as yet unknown, however this will be investigated in this project by creating mutations in the targeted genes and testing these mutants in tissue culture assay systems and animal models. It is intended that by identifying virulence/colonisation associated genes it might be possible to use them to distinguish between pathogenic and non-pathogenic campylobacters (e.g. by PCR detection). Also an understanding of the mechanisms of pathogenesis and colonisation is essential in order to develop control strategies to reduce the numbers of campylobacters in the human food chain.

Evaluation of foreseeable effects

Wild type C. jejuni is pathogenic to humans however it is a commensal in most animals and birds. C. jejuni is a foodborne zoonotic organism and human infection usually arises from the consumption of contaminated meat, particularly poultry meat, however other sources including pets, water and milk have also been implicated. All GMMs that result during this project will have either a reduced ability to colonise animals and/or birds, or a reduced virulence in humans due to the insertion of antibiotic cassettes into the genes responsible for colonisation and/or virulence in humans. If a GMC jejuni were to escape from the laboratory, it might survive in the environment for a short time, particularly in a moist environment. It is, however, unlikely to grow due to its fastidious growth requirements and the fact that it is thermophilic (optimum growth temperature of 42°C). Should the GMM come into contact with animals or birds, it may be able to colonise the gut asymptomatically, but it may not be able to displace the already established gut flora. There is no evidence to suggest that DNA transfer occurs between strains of C. jejuni in vivo and so it is unlikely that transfer of the antibiotic cassette which is chromosomally located will get transferred to other organisms. If the organism does get into the human food chain it is unlikely to cause serious illness at those genes important in virulence will have been targets for insertional inactivation. Laboratory attenuated GM E. coli strains that are used for cloning purposes are likely to survive for only a short time in the environment as they are auxotrophic. If they do carry C. jejuni genes, these genes are unlikely to be expressed at very high levels, if at all, due to the differences in DNA content and codon usage between the two genera.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste is autoclaved prior to incineration. All re-usable equipment is autoclaved, washed then recycled. Disposal equipment is autoclaved prior to incineration.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

All GM project risk assessments are approved by the local GM Safety Committee prior to work commencing. This assessment has been approved.

Project Containment
**Project Additional Information**

**Purposes of the contained use**

(98NPO2EAV) Generation and selection of candidate virulent/non-virulent biological clones of equine arteritis virus (EAV).

(01/01) Generation of an infectious clone of bovine viral diarrhoea virus.

**Recipient or parental organism**

Equine Arteritis Virus (Bucyrus Velogenic Strain) taken from pleura fluid isolate of an EAV infected horse in the USA in 1971 (William McCollum's Lab. USA)

EAV is an arterivirus in the family Togaviridae and is a positive strand RNA virus.

**Host/vector system**

Experimental vector system E.coli.

**Origin & function**

The genetic material is a chimaera of EAV which involves the insertion of a small region of the ORF-5 (taken from the Velogenic Bucyrus strain of EAV) into the Leiden
strains (a much less virulent strain of EAV) to give a chimaera. This chimaera should function as a more virulent form of EAV.

**Evaluation of foreseeable effects**

This new Chimaera should function as an infectious clone of EAV and may be as virulent/pathogenic as the original Velogenic Bucyrus strain of EAV in the USA. This clone should produce a more severe form of the disease in the horse than the Leiden strain which only causes a mild form of the disease. The horses are expected to make an effective immune response and make a full recovery.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste - autoclaved prior to incineration.
Liquid waste - autoclaved prior to disposal.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been approved by the local GM Safety Committee.

**Project Containment**

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**Animal Units**

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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NOVEL VACCINE FOR INFLUENZA BASED ON DNA

Date Ackn'd: 02/08/2001
Date Project Ceased: 

Class: Class 2
CultuereVolClass2: 1-50 litres
CultuereVolumeClass3-4: 

Non-GMM: not applicable
Consent Granted: 

Project notified under transitional arrangements: Y

Withdrew: N
Tick if notifying a connected programme of work: Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**
To develop a novel vaccine based on DNA for the protection against swine influenza.

**Recipient or parental organism**
Selected influenza viruses

**Host/vector system**
Host: competent rec A, end A E.coli strains INVXF and DH5X. COS7 (African green monkey) cells and PEK (porcine embryo kidney) cells.
Vector: PCR2.1 prokaryotic vector.
    pcDNA 3.1 eukaryotic expression vector (Invitrogen).

**Origin & function**
Original - ‘avian-line’ swine H1N1 influenza virus.

Intended function - Expression of the viral proteins in mammalian cells.

**Evaluation of foreseeable effects**
As only two out of a required eight genes will be used, there will be no adverse biological effects. However, the genes used are, singularly capable of initiating an immune response in the host.

Through vaccination of pigs with the construct it is proposed that viral proteins will be expressed in the pig cells, which will be immunogenic.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste and equipment are autoclaved prior to incineration or cleaning or discard.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All risk assessments are approved by the local GM safety committee before work is allowed to commence.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 221/03.1

Date Ackn’d 02/07/2003

CU2 Project Title

THE INVESTIGATION OF THE MOLECULAR BASIS OF ANTIGENIC VARIATION AND IMMUNOREACTIVITY OF THE GENE PRODUCTS OF BORRELIA SPP.

Date Project Ceased

Class 2

CultureVolClass2 1-50 litres

CultureVolumeClass3-4

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

Cloning of genes and construction of sub-genomic library.

**Recipient or parental organism**

Nucleic acid origin: DNA from relapsing fever Borrelia (B. recurrentis and B. duttonii) outer membrane protein genes derived from PCR.

Outer membrane genes enable antigenic variation involved in immune evasion in their host.

**Host/vector system**

The host organism is Escherichia coli. Commercially available disabled hosts will be used including recombination deficient (recA) or endonuclease A (endA) deficient E. coli K12 derived strains (such as TG-1, XL1-Blue MRF; HB101; TOP10; TOP10F; DH5; JM109, SG13009[pREP4] and M15[pREP4]).

Prokaryotic vectors will have the following features: a bacterial promotor (eg T7) to direct expression of inserted genes. Where genes are inducible, the responsible genes for induction will also be in the plasmid. An antibiotic resistance gene to permit selection of transformed bacteria will also be present in the vector. Sequences encoding fusion tags such as GST or 6-His may be included in the vector to assist in purification of expression proteins. Vectors meeting these requirements include pBluescript; pGEM; pUC; pRSET; pTrcHis, pGEX6T, pQE-30 series; pQE40; pET, pGEX, pcDNA3.1 and pcDNA4.

**Origin & function**

Origin: DNA from relapsing fever Borrelia (B. recurrentis & B. duttonii) outer membrane protein genes derived from PCR.

Intended function: Outer membrane genes enable antigenic variation involved in immune evasion in their host.

**Evaluation of foreseeable effects**

Other researchers have reported difficulties in gaining expression of borrelial outer membrane proteins which can be lethal to host organisms. Consequently, we would predict that resulting GMOs will be less likely to survive outside their growth medium or outside the laboratory.

Dunn et al. Outer surface protein A (OspA) from the Lyme disease spirochete, Borrelia burgdorferi: high level expression and purification of a soluble recombinant form of OspA. Protein Expr Purif. 1990 Nov; 1(2): 159-68.


As the work will involve use of libraries that contain as yet un-characterised genes, it is not possible to predict the effect that these inserts will have on the host. For this reason, manipulations of live material will be done in a class 1 biological safety cabinet located in the containment level 2 laboratory.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid contaminated waste will be placed in autoclave bags within autoclave tins. The bags will not be taped or tied to allow sufficient penetration of steam. Appropriate checks of autoclave efficiency will be made. The cycle conditions will be 121 degrees C, 15 pounds per square inch for 15 minutes. Once autoclaved, waste will be sent for incineration. Procedure for collection and disposal documented in CBU143 and autoclave operation in CBU135.

Liquid waste will be placed into Duran bottles (not to exceed half full), placed in autoclave tins with loose lids and autoclaved as above. Alternatively, this will be disinfected with 5% fresh solutions of either clearsol, lysol, chloros or virkon and left overnight before disposal.

General rubbish will be collected and sent for incineration.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by our local GM Safety Committee.

Project Containment

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Project Ref  221/04.1

Date Ackn'd  02/03/2022

CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4
THE GENOTYPIC AND PHENOTYPIC COMPARISON OF VIRULENT YERSINIA ENTEROCOLITICA FROM HUMANS AND ANIMALS.

Purposes of the contained use

To identify genotypic markers of virulence and human pathogenicity in Yersinia enterocolitica. This will involve mutation and complementation of potential, host-specific colinsation factors identified via an ongoing comparative microarray project.

Recipient or parental organism

Laboratory strains of E. coli such as DH5α, S17.pir will be used for cloning purposes as they are easy to use, readily available, and well defined. S17.pir may be used as a donor strain to perform conjugational transfer of suicide vectors. A variety of wild type Yersinia enterocolitica strains identified as harbouring candidate genes will be used as hosts for knockout mutations if they permit uptake of DNA and recombination events.

Host/vector system

Various standard cloning vectors such as pUC18, pACYC184, pBAD18. Other suicide vectors for allelic exchange such as pPERFORM, pKNG101 (Woodward, M. J. et al 2003. JMM. 299-308), pB307 (Roe, A. J. et al 2003. Infect & Immun. 5900-5909), or pKOBEG (Derbise A et al 2003. FEMS Immun & Med Micro. 113-1156). All the above vectors have been reported in the literature, are easy to use, and readily available.

Origin & function

Wild type Yersinia enterocolitica strains from human and animal environments will provide the genetic material involved. Specific genes are as yet unknown, though genes with similarity to other known virulence factors such as toxins, adhesins, fimbriae, as well as regulatory elements are most likely to be investigated. The intended function of the genetic material is to investigate the role of these factors as potential virulence or host specific colonisation factors in Y. enterocolitica. In the majority of cases the genes will be insertionally inactivated in Y. enterocolitica and the resultant phenotype studied. In some instances non-coding regions upstream of the gene may be used to study expression of the factors via a reporter gene.

Evaluation of foreseeable effects

Wild type Y. enterocolitica is present in the environment, principally as a coloniser of pigs, but also colonises sheep and cattle, as well as domesticated wildlife such as dogs. The organism can also survive in water, soil etc. The infectious dose is relatively high, and whilst Y. enterocolitica is pathogenic to humans. It colonises animals asymptotically. Genetically modified Y. enterocolitica strains will be assumed to have a reduced ability to colonise animals due to mutations in potential...
colonisation/virulence factors. Safety procedures observed in the laboratory should make it unlikely for GM organisms to enter the food chain. Also, classically Y. enterocolitica is divided into 3 distinct families. Biotype 1b strains are considered the major human pathogens, whilst biotype 2-4 strains are considered as opportunistic human pathogens, and biotype 1a strains are classified as non-pathogenic. The cloning to be performed during this project will exclusively involve Biotypes 1a, and 2-4, with the possible exception of one Biotype 1b strain which is non-invasive in in vitro tests.

The E. coli strains that are used for cloning are all auxotrophic and so long term survival in the environment is unlikely. The plasmids used are non-mobilisable, and are readily lost from organisms in the absence of antibiotic selection pressure, with the exception of pKNG101 which requires the .pir protein present in E. coli S17 in order to replicate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For solid contaminated materials, all contaminated waste is placed in autoclave bags inside autoclave tins and the lid secured with tape on 2 opposing sides, though not fastened, to allow steam entry, before the tin leaves the laboratory. Waste tins are autoclaved (134 degrees C for 1 hour) prior to incineration.

Liquid waste is collected in glass bottles and placed in an autoclave tine, in sub-500ml volumes. Waste tins are then autoclaved (134 degrees C for 1 hour) prior to incineration.

All general uncontaminated waste is disposed of separately from contaminated waste and disposed of by incineration.

Thorough disinfection procedures are carried out in the event of any spillages, using 2.5% lysol or 70% ethanol. Laboratory floors and surrounding corridors are disinfected regularly. All contaminated waste is autoclaved and no contaminated waste is disposed of in the drainage system without sterilisation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project has been approved by the VLA GM local safety committee.

Project Containment

| Laboratory Activities | Glass Houses | Growth Rooms |

02/03/2022
Can detection and typing of brucellosis in animals be further improved?

Purposes of the contained use

An "ORFeome" of Brucella genes has been created, consisting of open reading frames (ORF) of the Brucella melitensis 16M and Brucella suis 1330 genomes. A total of 3091 ORFs were cloned into Gateway™ pDONR 201™. This project intends to manipulate selected clones from the ORFeome for the generation of Gateway™ system/E.coli based organisms capable of expression of the encoded Brucella specific recombinant proteins, for use in diagnosis.

Recipient or parental organism

E.coli K12 derivative strain: E.g.: JM109, TOP10, TOP10F', DH5a.

Host/vector system

A number of Gateway™ destination vectors may be used in this project. Each vector will contain a T7 promoter element and a choice of N-terminal fusion tags. Examples of such vectors include pDES™ 15 (N terminal Glutathione S Transferase Tag), pDES™ 17 (N terminal Histidine Tag), pDES™ 14 (without the tag)- All vectors are provided by Invitrogen (www.invitrogen.com)
The ORFs to be used in this project will be obtained from the Brucella ORFeome. The host organisms of the ORFeome are E.coli strain DH5 a-T1rMAX containing the Gateway TM pDNOR 201 TM vector carrying individual ORFs for the entire genome complement of the Brucella melitensis 16M and Brucella suis 1330 strains. These GMMs cannot express the encoded ORFs. They are considered GM class 1 organisms following risk assessment approval by the VLA GMLSC. In this project the ORFs (of Brucella spp., origin) will be transferred to a Gateway TM vector and alternate host cell which is capable of expression of the inserted ORF, under controlled conditions. The expression capable GMMs will be used to generate recombinant proteins that will be used in research projects to develop novel diagnostic tests.

Evaluation of foreseeable effects

The host organisms of the original ORFeome are considered GM class 1 organisms following risk assessment approval by the VLA GMLSC. The original ORFeome consists of a library of E.coli strain DH5 a-T1r MAX containing a single ORF derived from Brucella spp., in the Gateway TM p DNOR 201 tm VECTOR. These GMMs are not capable of expression. Each GMM to be created in this project will also contain a single Brucella ORF, but in a Gateway expression vector system. The E.coli host strains selected for this project are disabled strains requiring defined culture conditions for propagation and survival. The vectors selected (Gateway TM pDES TM) contain the necessary promoters and regulatory sequences for expression of the inserted ORF, therefore these organisms are capable of generating Brucella specific proteins. Since some of the ORFs that could be used may have putatively assigned functions relating to invasion or adhesion, or be of unknown function, it is possible, although unlikely, that the expression of these genes by the E.coli host strain could confer elements of an invasive or adhesive Brucella specific phenotype to the cells. It is more likely that the proteins will not have any biological activity in the E.coli and that the presence of fusion tags will abrogate any activity, or that induced expression of the Brucella protein will be toxic to the host cells and cause their death. Overall the presence of a plasmid bearing and expressing a single Brucella gene is extremely unlikely to significantly alter the virulence/pathogenicity of these attenuated host cells. The genome sequence of Brucella melitensis (DelVecchio, V.G., et al., 2002) was used to generate the original host ORFeome and this data indicates that there are no classical virulence factors or any toxins encoded in the Brucella organism and therefore these are not present in the ORFeome. The underlying mechanism of Brucella virulence is not fully elucidated but seems largely related to the ability of the organism to establish a persistent replicative niche within the acidified phagosome of the host macrophage. This ability to survive in the macrophage is not fully elucidated but it is known to be complex and multifactorial (S. Kohler et al, 2003). For this reason it is very unlikely that the presence and expression of a single ORF can transfer the virulence or pathogenicity of the Brucella to any E.coli host. However, as the exact function of the ORFs to be selected are unknown at this point, we cannot rule out the possibility of the production of a protein that could alter the host strain E.coli phenotype. For this reason we propose that these organisms are designed as GM class 2 pathogens.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Lab waste, which has had contact with GM organisms is processed through incineration. General practice in this laboratory involves the manipulation of all bacterial cultures (Hazard Group 2 and below) in the class II microbiological safety cabinet. Solid waste (Agar plates/Absorbent paper/plastics etc) are all disposed of through autoclaving (15 minutes, 121 degrees C and 15 lbs in -2 pressure), followed by incineration. Liquid cultures/spent culture media samples are sterilised through O/N disinfectant treatment in 5% hycolin. Treated liquids can be safely discarded down the sink with copious amounts of water.

General safe working practices, including restricted laboratory access and good cleaning/sterilisation procedures will prevent the organism from entering the environment. No additional special measures are necessary.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form
Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Historical Significant Changes

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Project Reference

221/04.4

Date Ackn’d

31/08/2004

Date Project Ceased

CU2 Project Title

Bacillus spore probiotics

Class

Class 2

Culture Vol

≤ 1 Litre

Consent Granted

Not Applicable

Project notified under transitional arrangements

N

Tick if notifying a connected programme of work

N

Purposes of the contained use

To generate live recombinant B. subtilis vaccine candidates, which will be able to illicit a protective immune response in poultry against C. perfringens mediated necrotic
enteritis, when administered orally.

Recipient or parental organism

*bacillus subtilis* PY79.

Host/vector system

Plasmid based suicide vectors with regions of homology to the Bacillus subtilis chromosome.

Origin & function

All genetic material will be derived from either the Bacillus subtilis genome or the Clostridium perfringens genome.

It is intended that GM Bacillus subtilis strains will be able to illicit a protective immune response in poultry against necrotic enteritis.

Evaluation of foreseeable effects

No adverse effects are envisaged. Despite the fact that both Bacillus subtilis and C. perfringens are environmentally ubiquitous organisms, all work will be carried out by trained personnel, in line with safety regulations for Hazard Group 2 pathogens.

GM Bacillus subtilis strains will not be modified to express any substance which is known to be harmful to any organism or the environment. Furthermore, the proposed GM Bacillus subtilis strains will have either one or two native chromosomal genes knocked-out. Although these genes are non-essential to survival, their disruption is likely to attenuate the proposed GM Bacillus subtilis strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All laboratory waste (solid or liquid) will be autoclaved, then incinerated, in line with current risk assessments (VLA Weybridge). Autoclaving is for 60 minutes at 134 degrees C and 16 psi (NB: Autoclaves have been validated for efficacy against Bacillus subtilis spores - validation report of 01/10/02).

Where autoclaving is not possible, waste will be contained and incinerated immediately. Surface etc. which can not be autoclaved or incinerated will be disinfected with Virkon S (or other suitable bactericidal disinfectant) after use.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA GM Local Safety Committee.
### Project Additional Information

**Purposes of the contained use**

Insert DNA capable of expressing antisense RNA to target genes will be amplified and cloned into a vector capable of stable replication within *Mycoplasma*. Expression of antisense RNA will be initiated from an inducible vector promoter allowing controlled regulation of target genes.

**Recipient or parental organism**

*Mycoplasma* species including *Mycoplasma mycoides* subspecies *mycoides* small colony type and *Mycoplasma bovis*, *M. c. capripneumoniae*, *M. agalactiae*, *M. c. capricolum*, *M. mycoides* subspecies *mycoides* large colony type, *M. gallisepticum*. 
Host/vector system

pBlueScript (+) (stratagene)
pIVT-1 (University of Alabama)

Origin & function

M. m. m. SC oriC DNA generated through PCR amplification using previously published primers. Antisense RNA generated through PCR amplification of appropriate Mycoplasma genomic sequences. OriC and antisense RNA generated through PCR amplification of Mycoplasma specific DNA sequences. The expression of antisense RNA sequences allowing downregulation of specific genes. Antisense RNA molecules function by hybridising to complementary RNA transcripts. Downregulation occurs through (I) inhibition of translation, (ii) Rapid degradation of mRNA by duplex specific Rnases or (iii) Inhibition by premature termination of transcription. Technology can be rapidly implemented and since gene function is not knocked out it eliminates the production of lethal mutants allowing the study of essential genes.

Evaluation of foreseeable effects

The production of antisense RNA will be induced under laboratory conditions. In addition although gene expression will be reduced it will not be eliminated so should not significantly affect the pathogenicity of these organisms. We will essentially be creating exotic strains and will therefore follow procedures of containment set out in ACDP and SAPO guidelines.

Evaluation of foreseeable effects

Not applicable

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All laboratory waste, which has had contact with the live GM organisms created in this project will be generated in a Class 2 cabinet. All waste is sealed within containers whilst in the cabinet. Containers will be treated with 70% ethanol and allowed to stand in the safety cabinet, for a minimum contact time of 20 minutes and evaporation of ethanol, prior to removal from the cabinet. All waste is to be autoclaved (121 degrees C for 15 minutes) followed by incineration.

All laboratory waste, which has had contact with the live GM organisms created in this project will be generated in a Class 2 cabinet. All waste is sealed within containers whilst in the cabinet. Containers will be treated with 70% ethanol and allowed to stand in the safety cabinet, for a minimum contact time of 20 minutes and evaporation of ethanol, prior to removal from the cabinet. All waste is to be autoclaved (121 degrees C for 15 minutes) followed by incineration.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA GM Local Safety Committee.

Project Containment

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02/03/2022
The majority of genes involved in cell wall synthesis in mycobacteria are unknown. One of the reasons for this is that conventional methods of making targeted gene knock-outs are extremely difficult in mycobacteria due to the lack of efficient homologous recombination in this organism. A more efficient method for obtaining cell wall mutants of mycobacteria is to perform transposon mutagenesis, whereby transposons insert randomly into the chromosome, then to screen these mutants for alteration in cell surface structures using appropriate antibodies.

Mycobacterium bovis BCG, an attenuated strain of mycobacteria derived from virulent M.bovis and can be handled at Containment Level 2. It is the vaccine strain for tuberculosis.

Recipient or parental organism

Mycobacterium bovis BCG, an attenuated strain of mycobacteria derived from virulent M.bovis and can be handled at Containment Level 2. It is the vaccine strain for tuberculosis.

Host/vector system

EZ::Tn system (Epicentre). An EZ::TN Transposome is the complex formed by incubating an EZ::TZ Transposon with EZ::TN™Transposase in the absence of Mg2+. EZ::TN Transposomes are stable so they can be introduced into cells by electroporation. Once in the cell, the EZ::TN Transposome is activated by Mg2+ and the EZ::TN
Transposon can then randomly insert into the host's genomic DNA. This system has previously been shown to work successfully in M. bovis BCG (Stewart, G. et al., poster presentation at "Tuberculosis: Integrating Host and Pathogen Biology", Whistler, Canada, April 2-7, 2005).

Origin & function

The EZ::TN system will be used to deliver the hygromycin resistance gene from Streptomyces hygroscopicus as a selectable marker. The hygromycin resistance gene is ~1.4kb in size and can be expressed in M. tuberculosis complex organisms to confer resistance to hygromycin. The aim of using this vector::insert combination is to randomly disrupt genes in the chromosome of M. bovis BCG to generate mutants which can be selected on the basis of hygromycin resistance. The mutant bank will then be screened for recombinants with defects in cell wall synthesis.

Evaluation of foreseeable effects

Inactivation of genes in BCG will not lead to an increase in its virulence for humans. Recent work (Mol Microbiol. 2002 Nov;46(3):709-17; Proc Natl Acad Sci 2003 Oct 14;100(21):12420-5) has shown that the deletion of the RD1 locus from BCG was one of the key reasons for the attenuation of the vaccine strain during its derivation. As there are no examples of horizontal transfer of DNA in the M. tuberculosis complex, BCG cannot regain this locus; this explains why BCG has never reverted to virulence and is safe to use in humans. A subset of mutants are anticipated to be cell wall mutants, which is the focus of this project. Modification of cell wall components of virulent mycobacteria has been shown to attenuate the recombinants (Nature. 2004 Sep 2;431(7004):84-7); there are no examples of modification of cell wall components leading to increased virulence of the recombinant. Trained workers, minimising the risk to personnel or accidental environment release, will carry out the manipulations described in this Risk Assessment in a dedicated containment level 2 laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste is transported to an autoclave in a closed metal tin. Decontamination is conducted by autoclaving for 1 hour at 135°C after which waste is put into bags for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA GM Local Safety Committee.

Project Containment
Project Ref 221/05.2

Date Ackn’d 28/02/2005

CU2 Project Title THE USE OF COMPARATIVE GENOMICS TO ADVANCE THE UNDERSTANDING AND DIAGNOSIS OF IMPORTANT MYCOPLASMA DISEASES

Class 2 CultureVol Class 2 < 1 Litre

Consent Granted Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

This project aims to identify and express proteins for use in diagnostic assays. Having identified suitable targets, using transposons mutagenesis, those genes will be clones into E. coli vectors for protein expression analysis.

Recipient or parental organism

Mycoplasma species including Mycoplasma mycoides subspecies mycoides small colony type and Mycoplasma bovis, M. c. capripneumoniae, M. agalactiae, M. c. capricolum, M. mycoides subspecies mycoides large colony type, M. gallisepticum will be subjected to Tn mutagenesis to identify cloning targets. Laboratory manipulation of these organisms is governed by ACDP and SAPO guidelines. Cloning and expression studies will be carried out in E. coli hosts: E. coli K-12 derivative strains (eg. JM109, CDJ64/D14, NovaBlue (DE3)), E. coli B derivative strain BL21 (DE3), developed at Brookhaven National Laboratories and E.coli XL10 gold.

Host/vector system

Suicide plasmids containing Tn4001 or Tn916. Cloning and expression studies will be carried out using E. coli vectors including: pG8PLO, pAff8c, (Swedish Institute collaboration), pGEM-T easy and pGEM-T (Promega), plasmids of the pET system (Stratagene).
Tn916 is a class V transposon originally detected within S. faecalis. It was the first element of this group to be discovered and has been extensively studied. Tn4001 is a composite class 1 transposon originally detected within the plasmid pSK1. Following Tn mutagenesis target Mycoplasma DNA will be PCR amplified. The identity of specific inserts are unknown at this stage but are likely to include surface proteins suitable for use in diagnostic assays. Introduction of Tn to mycoplasma genomic DNA causing disruption of open reading frames. Introduction of target DNA to E. coli to carry out expression analysis and produce Mycoplasma proteins for use in diagnostic assays.

**Evaluation of foreseeable effects**

Tn mutagenesis will cause loss of gene expression through disruption of genomic DNA. This may result in the expression of alternative pathogenicity factors within cells. Genetic manipulation within the laboratory will result in the production of exotic strains. All work will be carried out in class 2 containment according to SAPO guidelines and restrictions already in place. Tetracycline resistance will be conferred within GM strains to enable the selection of transformants. Since these organisms are not human pathogens this will not compromise treatment using this antibiotic. Should these organisms be released from laboratory containment the likelihood that they will survive is low. Mycoplasma are host dependant microorganisms unable to synthesise many essential molecules. They are under selective pressure to maintain a reduced genome size. In addition they lack a cell wall making them vulnerable to lysis. The E. coli hosts for expression studies are disabled strains, unable to survive in the environment and requiring complex media and growth conditions. DNA within expression vectors will be under the control of vector promoters. E. coli expression strains BL21 (DE3) and NovaBlue (DE3) have been engineered to contain a genomic insert capable of expressing T7 polymerase. This gene is under the control of a lac promoter and expression is induced on addition of IPTG. In the presence of T7 polymerase expression is induced from T7 promoter sequences within the transformed vector (pAff8c or pET plasmid). This leads to expression of insert DNA. Expression of vector DNA inserts within the suppressor strain CDJ64/D14 from pG8PLO is under the control of a lac promoter and is directly induced by addition of IPTG. It is unlikely that these fragments will be expressed within these strains outside laboratory conditions. It is very unlikely that the level of expression observed within the laboratory will be transferred to wild type hosts in the environment. Only single open reading frames will be transferred to host cells where they will be expressed in isolation from other mycoplasma proteins. It is extremely unlikely that the expression of one mycoplasma protein within a disabled E. coli host strain would be capable of altering virulence and, since mycoplasma lack a cell wall, it is unlikely that these proteins would be surface expressed within the host. Mycoplasma species used in this study are covered by SAPO and as a result of this live mycoplasma and modified E. coli will be maintained and manipulated under class 2 containment conditions.

**Origin & function**

**Evaluation of foreseeable effects**

Tn mutagenesis will cause loss of gene expression through disruption of genomic DNA. This may result in the expression of alternative pathogenicity factors within cells. Genetic manipulation within the laboratory will result in the production of exotic strains. All work will be carried out in class 2 containment according to SAPO guidelines and restrictions already in place. Tetracycline resistance will be conferred within GM strains to enable the selection of transformants. Since these organisms are not human pathogens this will not compromise treatment using this antibiotic. Should these organisms be released from laboratory containment the likelihood that they will survive is low. Mycoplasma are host dependant microorganisms unable to synthesise many essential molecules. They are under selective pressure to maintain a reduced genome size. In addition they lack a cell wall making them vulnerable to lysis. The E. coli hosts for expression studies are disabled strains, unable to survive in the environment and requiring complex media and growth conditions. DNA within expression vectors will be under the control of vector promoters. E. coli expression strains BL21 (DE3) and NovaBlue (DE3) have been engineered to contain a genomic insert capable of expressing T7 polymerase. This gene is under the control of a lac promoter and expression is induced on addition of IPTG. In the presence of T7 polymerase expression is induced from T7 promoter sequences within the transformed vector (pAff8c or pET plasmid). This leads to expression of insert DNA. Expression of vector DNA inserts within the suppressor strain CDJ64/D14 from pG8PLO is under the control of a lac promoter and is directly induced by addition of IPTG. It is unlikely that these fragments will be expressed within these strains outside laboratory conditions. It is very unlikely that the level of expression observed within the laboratory will be transferred to wild type hosts in the environment. Only single open reading frames will be transferred to host cells where they will be expressed in isolation from other mycoplasma proteins. It is extremely unlikely that the expression of one mycoplasma protein within a disabled E. coli host strain would be capable of altering virulence and, since mycoplasma lack a cell wall, it is unlikely that these proteins would be surface expressed within the host. Mycoplasma species used in this study are covered by SAPO and as a result of this live mycoplasma and modified E. coli will be maintained and manipulated under class 2 containment conditions.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All laboratory waste, which has had contact with the live GM organisms created in this project will be generated in a Class 2 cabinet. All waste is sealed within containers whilst in the cabinet. Containers will be treated with 70% ethanol and allowed to stand in the safety cabinet, for a minimum contact time of 20 minutes and evaporation of ethanol, prior to removal from the cabinet. All waste is to be autoclaved (121 degrees C for 15 minutes) followed by incineration.

**Is an emergency plan required according to regulation 20?**

N

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA GM Local Safety Committee.

Project Containment

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Animal Units

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Project Ref 221/05.3

Date Ackn'd: 26/08/2005
CU2 Project Title: CONSTRUCTION OF AND EFFICACY OF AN AROA VACCINE AGAINST SALMONELLA ENTERICA NEWPORT

Class: Class 2
CultureVolClass: < 1 Litre
Consent Granted: Not Applicable

Historical Significant Changes
Withdrawn: N

Project Additional Information

Purposes of the contained use
Construction of aroA vaccine Salmonella enterica Newport and characterisation in in vitro and in vivo models.

Recipient or parental organism
Laboratory strains of E. coli such as DH5a, S17pir. A characterised S. Newport strain confirmed as fully antibiotic sensitive will be used for mutagenesis studies.
Host/vector system

Standard cloning vector pCR 2.1 TOPO. Suicide vector for allelic exchange will be pKNG101.

Origin & function

A Salmonella Newport fully sensitive cattle isolate will be used. The aroA gene and the genes immediately upstream (unknown function) and downstream (serC) will be amplified, allowing deletion of the aroA gene. To allow construction of the region without the aroA gene, resulting in an auxotrophic mutant unable to survive in the absence of supplemented aromatic amino acids.

Evaluation of foreseeable effects

The use of an aroA mutant in vaccine strain construction has been well documented, and benefits from the fact that the GMO is highly auxotrophic, and rapidly dies in the absence of supplemented aromatic amino acids. Due to this the threat to the environment from the GMO is negligible. Due to the emergence of S. Newport as a zoonotic agent we have opted for an isolate sensitive to antibiotics, and have opted for an unmarked mutant, ensuring minimal impact if the organism was released. The E. coli strains that are used for cloning are well auxotrophic and so long term survival in the environment is unlikely. The plasmids used are non-mobilisable, and are readily lost from organisms in the absence of antibiotic selection pressure, with the exception of pKNG101 which requires the lpir protein present in E. coli S17 in order to replicate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste - disposal is by autoclaving or incineration in line with current risk assessments. Autoclaving is for 60 minutes at 134 degrees C after which solid waste is put into bags for incineration. All bedding from animals and carcasses from post-mortems will be double bagged and then disinfected with FarmFluid S and immediately incinerated. The post-mortem facility will be disinfected with FarmFluid S and then fumigated after each post-mortem.

Liquid waste - disposal is by autoclaving in line with current risk assessments. Autoclaving is for 60 minutes at 134 degrees C after which waste is incinerated. Liquid waste from animal excrement will be soaked up with shavings, treated with FarmFluid S and then incinerated. Any liquid waste draining from animal rooms will be disinfected with FarmFluid S and heat treated in holding tanks.

General rubbish - all general rubbish will be double bagged and disposed of by incineration in line with current risk assessments.

Thorough disinfection procedures are carried out in the event of any spillages, using 2.5% lysol, FarmFluid S or 70% ethanol. Laboratory floors and surrounding corridors are disinfected regularly. All contaminated waste is autoclaved and no contaminated waste is disposed of in the drainage system without sterilisation. Formaldehyde fumigation together with Virkon and FarmFluid S disinfection will be used in all animal accommodation. All waste will be double bagged disinfected and immediately incinerated. Environmental monitoring will be carried out routinely.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y
Please enter comments on the GM safety committee on the risk assessment

Agreed.

**Project Containment**

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**Project Ref 221/05.4**

Date Ackn'd

05/09/2005

CU2 Project Title

Cloning of Coxiella burnetii genes for sequence analysis and production of improved diagnostic methods.

Date Project Ceased


Consent Granted

Not Applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

N

**Project Additional Information**

Purposes of the contained use

* Generation of targets by PCR from Coxiella burnetii genomic DNA.
* Insertion of the PCR amplicon into separate constructs enabling downstream sequencing.
Expression of the bacterial protein in-vitro using inducible E. coli cultures to produce potentially useful diagnostic antigens.

**Recipient or parental organism**

The host organism is Escherichia coli. Commercially available disabled hosts will be used including recombination deficient (recA) or endonuclease A (endA) deficient E. coli K12 derived strains (such as TG-1; XL1-Blue MRF; HB101; TOP10; TOP10F; DH5.; JM109, SG13009[pREP4] and M15[pREP4]. The host organism, E. coli, is not considered harmful (ACDP class 1) and the strains used will be disabled reducing their ability to survive within the environment. No adverse effects are anticipated from expression of these genes in E. coli as expression of any single Coxiella gene, intragenic spacer or portion of a gene in the E. coli host is extremely unlikely to alter the virulence of the host strain.

**Host/vector system**

Prokaryotic vectors will have the following features: a bacterial promoter (eg T7) to direct expression of inserted genes. Vectors meeting these requirements include pBluescript; pGEM; pUC; pRSET; pTrcHis, pGEX6T, pQE-30 series; pQE40; PET and pGEX. The host organisms are disabled with reduced ability to survive outside the laboratory. The host organisms are not pathogens of plants or animals. The genetic inserts are linear DNA and the vectors are not mobile.

**Origin & function**

DNA will originate from Coxiella burnetii genomic preparations being derived from PCR. Various genes, intragenic spacers or other targets with likely value for detection and typing Coxiella burnetii will be selected from in silico analysis of genome data and will be amplified by PCR. Cloning will be used to obtain material for sequencing studies where it has been possible to produce sufficient template using PCR alone. Additionally, expression of antigens of potential diagnostic value consequently alleviating the need to utilise virulent CL3 pathogens for this purpose.

**Evaluation of foreseeable effects**

The host organism, E. coli is not considered harmful (ACDP class 1) and the strains used will be disabled reducing their ability to survive within the environment. No adverse effects are anticipated from expression of these genes in E. coli as expression of any single Coxiella gene, intragenic spacer or portion of a gene in the E. coli host is extremely unlikely to alter the virulence of the host strain. Although the detailed pathogenesis of these microbes is not yet fully elucidated, it is known that this is a complicated, multi-factorial process involving multiplication of C. burnetii within the acidified phagolysosome, and likely to be dependent on multiple gene products. Consequently, isolated genes or portions of genes, are unlikely to confer pathogenic properties to their recipient hosts. As the work may involve use of as yet un-characterised genes, it is not possible to predict the effect that these inserts will have on the host. For this reason, manipulations of live material will be done in a class 1 biological safety cabinet.

The strains used (E. coli) are viewed as safe and are well characterised for DNA production. The host organisms are disabled with reduced ability to survive outside the laboratory. The host organisms are not pathogens of plants or animals. The genetic inserts are linear DNA and the vectors are not mobile. If the organisms were able survive, expression of the Coxiella genes can only occur from the prokaryotic expression constructs (pRSET, pTrcHis, and pGEX6T), under certain inducible conditions. It is extremely unlikely that these conditions would be recreated outside a laboratory environment. Furthermore, Coxiella burnetii is considered endemic to Europe and consequently already occurs naturally and therefore already exist in the environment. Given the low likelihood of release beyond the confines of the laboratory together with the reduced ability to survive within the environment and lack of pathogenicity in animals or plants and the lack of vector genetic mobility, the overall risk is considered to be low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste: Solid contaminated waste will be placed in autoclave bags within autoclave tins. The bags will not be taped or tied to allow sufficient penetration of steam. Appropriate checks of autoclave efficiency will be made. The cycle conditions will be 121 degrees C, 15 pounds per square inch for 15 minutes. Once autoclaved, waste will be sent for incineration.

Liquid waste: Liquid waste will be placed into Duran bottles (not to exceed half full), placed in autoclave tins with loose lids and autoclaved as above. General rubbish: General rubbish will be collected and sent for incineration.

Is an emergency plan required according to regulation 20?   N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA GM Local Safety Committee.

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Project Ref  221/06.1

Date Ackn’d  15/03/2006

Date Project Ceased

Class 2

Consent Granted  Not Applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

An investigation of induced expression of the PrP gene and do novo PrPSc generation in cell lines as a model for sporadic BSE and scrapie

Class CultureVol

< 1 Litre

CU2 Project Title

Consent Granted

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

In this project PrP genes (open reading frame and regulatory regions) will be amplified by PCR. The PrP inserts will then be sub-cloned into different mammalian expression vectors. Transfected mammalian cell lines will be exposed to certain types of stress, including oxidative stress, in order to try to induce the pathogenic conformation of PrP. In addition, attempts will be made to infect cells with the BSE and scrapie agent whilst under stress to assess whether they become more or less susceptible to infection.

Recipient or parental organism

The recipient organism for expression vectors will be mammalian cell lines including; Bovine kidney (MBDK), Rabbit kidney (RK13), Sheep choroid plexus (SCP), Rat pheochromocytoma (PC12), Mouse neuroblastoma (N2a), Mouse differentiated neuronal (GT1). Increased transient or stable PrP expression is expected, this is not expected to be pathogenic, however if expressions levels are very high this is a possibility, as seen in PrP over-expressing transgenic mice.

Host/vector system

Expression vector systems include Clontech pLP-IRESneo with CMV promoter giving constitutive expression, Clontech pTet-On or pTet-Off with tetracycline responsive promoter allowing inducible expression, or Clontech pd2EGFP promoterless vector where the PrP promoter can be inserted to drive the expression of a green fluorescent protein (GFP) reporter protein.

Origin & function

The PrP open reading frame (ORF) is contained in a single exon (exon 3) and is approximately 900 bps long. It codes for a GPI anchored protein of ~ 250 amino acids. PrP is normally expressed in many mammalian cells but primarily in the peripheral and central nervous system. The PrP locus is approximately 20Kb in length depending of the species, promoter activity has been reported upstream of exon 1 and within intron 1.

Vector inserts will include PrP ORF (of naturally occurring genotypes) from mammals such as bovine, ovine, mouse, deer, kudu, dog and cat species derived from PCR amplification of genomic DNA. This will allow the study of how PrP genotype and species variation affects susceptibility to TSE infectivity or cellular stress. Alternatively, regulatory segments of the PrP gene such as the promoter or untranslated region (UTR), which may include exon 1 and 2, from mammalian species will be amplified from genomic DNA using PCR. The regulatory regions will be used to drive the expression of a reporter protein so that variation of expression can be assessed under different conditions.

Evaluation of foreseeable effects

The natural function of the PrP protein has not yet been fully determined although it is thought to be implicated in copper transport (Paul & Harris 1998) and may have some superoxide dismutase activity (Brown et al 1999). High level expression of PrP in mammalian cell lines has not been reported to cause spontaneous disease although
there are not many examples available. High level PrP expression in transgenic mice has been reported to lead to an increase in susceptibility to TSE and a reduction in incubation times. Only PrP of naturally occurring, non-human genotypes will be used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be placed in autoclave bags then in tine sealed with tape and placed in autoclave bags before being taken to building 105. All GM cells will be autoclaved for 30 mins at 136 degrees C, 2.2 bar. All TSE infected GM cells will be autoclaved for 30 mins at 136 degrees C, 2.2 bar with the exception of strains infected with 301V mouse BSE which will be autoclaved for 1 hour at 136 degrees C 2.2 bar. All sterile waste will then be incinerated.

All liquid waste will be prepared and autoclaved as above prior to disposal down drains.

General waste is autoclaved as above prior to disposal by incineration.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA GM local Safety Committee.

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Project Ref    221/07.2
Development of novel TB vaccines protecting humans or animals is a major research priority. Defra has been funding research into bovine TB vaccines for cattle for almost a decade, and one objective is to determine protective efficacies in cattle of vaccines developed during the human TB vaccine development effort. In particular, we are contracted by Defra to test the human TB vaccines that have entered, or about to enter human clinical trials. The recombinant Mycobacterium bovis BCG aureC::Hly::Hyg (VPM1002) is one such vaccine with human clinical trials scheduled to begin in Germany in late 2007. This vaccine has in small animal models provided superior protection against M. tuberculosis than wild type BCG (Grode et al 2005 JCI 115(9)2472-9). Our objective is to vaccinate cattle with BCG and VPM1002, and to compare protection against M. bovis challenge using well-established protocols (e.g. Vordermeier et al, Inf. Immun 70:3026-32, 2002).

Mycobacterium bovis BCG

BCG is an attenuated strain of mycobacteria derived from virulent M. bovis and is the only TB vaccine in routine use in humans, and is also part of the most novel TB vaccine strategies. The recombinant version subject to this assessment is further attenuated and has therefore been down-graded from P2 to P1 in Germany, by the German authorities.

Host/vector system

The plasmid VEP::Hly::Hyg is integrated in the bacterial chromosome. The chromosomal shuttle vector pMV306hyg0Hly was used which employs the L5 mycobacteriophage attachment site for integration.

Origin & function

The tuberculosis vaccine Mycobacterium bovis bacilli Calmette-Guerin (BCG) was modified to express the membrane-perforating listeriolysin (hly) of Listeria monocytogenes and the system used to deliver the hygromycin resistance gene came from Streptomyces hygroscopicus as a selectable marker.
The aim of using this vector is to express listeriolysin to disrupt the phagosomal membrane to release mycobacterial antigens into the cytosol for better induction of the immune response towards Th1. The plasmid has been integrated into the bacterial chromosome of M. bovis BCG that then can be selected based on Hygromycin resistance.

**Evaluation of foreseeable effects**

Inactivation of genes in BCG like the ure-C deletion in VPM1002, will not lead to an increase in its virulence for humans. As there are no examples of horizontal transfer of DNA in the M. tuberculosis complex, BCG cannot regain genes; this explains why BCG has never reverted to virulence and is safe to use in humans.

Construction of the VPM1002 clone will not lead to an increase in its virulence in its virulence in humans. Recent work (grode et al 2005 JCI 115(9)2472-9) has shown that the inserted hly gene showed reduced virulence in immune compromised SCID mice. Current toxicology studies with SCID and IFN-y k.o mice underlined the first results and show improved or equal safety profile compared to BCG (Grode et al, personal communications). Trained workers, minimising the risk to personnel or accidental environmental release, will carry out the manipulations described in this Risk Assessment in a dedicated containment level 2 laboratory.

It is extremely unlikely that the mutants of BCG will be released from the laboratory since the bacilli will be stored, analysed and dispensed in a Containment Level 2 laboratory by trained workers. Furthermore, the inoculi will then be transported in secure containers to the CL2 cattle accommodation where the vaccinations will take place. Once inoculated (subcutaneously), the vaccinated animals will serve essentially as primary containment as due to the vaccination route (subcutaneously), which is likely to maintain bacteria within the lymphatic system, makes excretion into environment highly unlikely. Syringes, needles, unused inoculi etc. used for the inoculations will be disposed of by incineration as will be the animals at the end of the experiment. Bacilli to be dispensed in class 1 cabinet.

No data exists on the ability of BCG to persist in the environment. However, the pathogen Mycobacterium bovis, from which BCG was derived, can survive in soil for up to 6 months and on pasture for at least 49 days (Maddock (1933). J Hyg 33:103-17). It is possible that due to its attenuation, BCG is further disabled in its capacity to survive outside of a host organism. However since this has not been established, for the purposes of this assessment it shall be assumed that BCG has the same survival characteristics as parental M. bovis. Environmental species of contrast, long-term persistence of M. bovis requires frequent passage through an animal host. The genetic lesions present in BCG render the organism unable to persist or cause disease in immunocompetent animal hosts (including humans) so it is unlikely that BCG would be able to establish, disseminate or displace other environmental organisms to a significant extent. The greatest hazard associated with exposure of animals to BCG is that cattle infected with BCG become positive for the tuberculin skin test. Although BCG does not persist in cattle and causes no disease, animals that become tuberculin positive through exposure to BCG would be deemed tuberculous by the current TB screening measures in GB and would be slaughtered as a result (please note that cattle held at VLA for experimental purposes are not subject to this routine TB surveillance). The greatest hazard is therefore an economical one for cattle kept on potential farms surrounding the VLA site. However, there are now commercial cattle holdings in close contact to the to the CL2 and CL3 cattle accommodation at VLA Weybridge, which likely hound not result in faecal, aerosol or nasal BCG shedding necessary for such an event to occur. The materials post-mortemed and incinerated, thus preventing any exposure of the environment due to BCG persisting in lymph nodes, or other internal organs. Animals will be housed in Bldg. 268 prior to post-mortem and therefore do not have to be transported to the PM room (270). Pm room to be decontaminated as per instructions for PM. M. bovis infected cows (BACC0001/01).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work is conducted in containment level 2 laboratories or animal accommodation by highly skilled personnel. All manipulations of recombinant BCG are performed within a safety cabinet (class 1), with personnel wearing suitable protective clothing. Unused inoculi and consumables used to vaccinate cows (e.g. syringes) will be incinerated at the end of experiment following housing in Building 268 and PME in Bldg. 270. As these buildings are interconnected also with the incinerator building, no transport of animals is necessary. Bldg 270 to be decontaminated as described in BAAC0001/01.

Laboratory waste is transported to an autoclave within building in a closed metal tin. Decontamination is conducted by autoclaving for 1 hour at 135 degrees after which
waste is put into bags for incineration. Animals will be euthanized and incinerated at the end of the experiment. Waste occurring during the inoculations (needles/ syringes) to be placed in sharps containers, sealed, double-bagged and incinerated.

Laboratory waste is transported to an autoclave within the building in a closed metal tin. Decontamination is performed by autoclaving for 1 hour at 135 degrees after which waste is put into bags for incineration. Waste during the inoculations (left-over inoculi) to be double-bagged and incinerated.

Waste is transported to an autoclave within the building in a closed metal tin. Decontamination is performed by autoclaving for 1 hour at 135 degrees after which waste is put into bags for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref** 221/07.3

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<th>CultureVolumeClass3-4</th>
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<td>28/12/2007</td>
<td>Construction of a Salmonella Typhimurium biosensor strain and the use of GFP tagged wild type Salmonella Typhimurium strains in in vitro assays.</td>
<td>Class 2</td>
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Date Project Ceased

02/03/2022

Project notified under transitional arrangements N
Probiotics are defined as ‘live micro organisms which when administered in adequate amounts confer a health benefit to the host’ (Nomoto, 2005). Probiotics are typically lactic acid producing bacteria, such as Lactobacillus Bifidobacterium and Enterococci. As a result of anaerobic metabolism probiotics bacteria can produce volatile fatty acids (VFA’s), such as acetic, butyric, propionic and valeric acid. Recent studies have illustrated that butyric acid can down regulate the Salmonella pathogenicity island (SPI-1) by activation of the bacterial adaptive response (BarA) I Salmonella invasion regulator (SirA) pathway (Lawhorn et al., 2002; Teplitski et al., 2003). This system senses the VFA environment of the gut and directly regulates Salmonella invasion (Van Immerseel et al., 2006). In response to short chain fatty acids (SCFA) the Salmonella two component regulator system BarAlSirA, directly effects the expression of the SPI-1 transcriptional regulator, Hyper Invasive Locus A (hilA). HilA is directly linked to the invasive potential of Salmonella. The carbon storage regulators are regulatory RNA’s (csrB/C) and the protein (csrA), which are involved in the Salmonella signal transduction pathway and have been linked to hilA expression (Lawhorn et al., 2002; Teplitski et al., 2003). Studies proposed here aim to deduce the effect of VFA’s on promoters (hilA, barA, sirA, csrA, csr8 and csrC) in the Salmonella BarAlSirA pathway. In order to monitor gene expression, reporter fusion mutants will be constructed in the BarAlSirA pathway and therefore used to create a ‘biosensor Salmonella strain’ that can be utilised to probe various environmental conditions that may reduce the invasion of Salmonella into epithelial cells by directly affecting genes in the BarAlSirA pathway. Quantification of green fluorescent protein (GFP) expression from BarAlSirA pathway reporter fusion mutants will be measured by constructing a GFP transcriptional fusion with the relevant promoter. Expression of GFP under various environmental conditions will be undertaken to elucidate the invasive potential of Salmonella test strains under differing conditions. In addition, studies will be extended to GFP tagging of wild-type Salmonella strains for subsequent analysis by confocal microscopy. The tagging will be undertaken with the aid of plasmids (pFPV25.1 or pGP704K) donated by collaborators. Once electroporated into Salmonella strains, these plasmids express GFP under the control of the ribosomal subunit promoter rpsM (RPSM ribosomal subunit) and therefore produce constitutive (constant) fluorescent signal, which is essential for imaging by confocal microscopy. Salmonella strains transfected with rpsM-gfp fusion constructs or other promoter fluorescent protein fusions, will be evaluated by in vitro assays only. These assays will consist of adhesion and invasion tissue culture assays and fermentation batch culture systems.

Recipient or parental organism

Method 1. E. coli K12 DH5α/INVctF’ are standard bacterial cloning hosts and enable the replication of gene constructs in the cloning vector plasmids. E. coli K12 is a non-pathogenic E. coli strain which can be cultured quickly and easily in the lab, however, is unable to survive readily in the environment.

Method 2. The plasmids pKD4 and pKD46 are not cloning plasmids, but either act as host for the kanamycin resistance gene (pKD4) or facilitate recombination (pKD46) as recommended by Datsenko & Wanner (2000).

GFP tagging of wildtype Salmonella isolates. E. coli K12 DH5cz will be used as the cloning host and for amplification and storage of the plasmid pFPV25.1 and R-plasmid containing the rpsM-gfp fusion. The reason for use is as stated above for method 1 with the addition that E. coli K12 DH5ct can accept plasmids containing GFP fusion constructs for use in Salmonella, without any unforeseen perturbations of growth or cellular phenotype.

Host/vector system

02/03/2022
Method 1. Standard commercially available high copy number cloning vectors such as pUC18 or pCR2.1 (Invitrogen). pPERFORM series of suicide vectors - non-replicating in A. pir Salmonella enterica strains.
Method 2. Only the plasmid pKD4 and pKD46 as either a template or facilitator plasmid are used.
GFP tagging of wildtype Salmonella isolates. Only the plasmid pFPV25.1 and R-plasmid containing the rpsM:gfp fusion will be used.

Origin & function
Salmonella enterica and plasmids as follows: green fluorescent protein (gfp) on pWM1007 :AF292556, yellow (yfp) on pWM1008 AF292558, cyan fluorescent protein (cfp) on pWM1009: AF292559 (Miller et al., 2000).
The first rpsM-gfp fusion was constructed by making an rpsM:gfp fusion which was inserted into pGP704K (oriR6K- mob-aph) Vazquez-Torres (1999).
The second plasmid was constructed by the addition of an rpsM-gfp fusion which was introduced into the plasmid Schierack et al (2005) Salmonella enterica promoters, hilA barA, sirA, csrA, csrB and csrC. Salmonella Typhimurium with rpsMgfp/pGP704 inserted into an R-plasmid and pFPV25.1 which is a high copy number derivative which is under the control of the promoter rpsM.
Chromosomal complementation of the hilA promoter, with a hilA-gfp fusion. The purpose of this fusion is to elucidate the effect of various probiotic, prebiotic and symbiotic combinations on the invasiveness of Salmonella Typhimurium SL1 344 in a batch culture fermentation system by measuring the GFP signal intensity from the different promoter fluorescent protein fusions. The mutants will also be used in adhesion and invasion assays on the porcine jejunal cell line IPEC-J2. Where the effects of probiotics and prebiotics can be linked to the effect on hilA expression, the in vitro tissue culture assays. This can be quantified by confocal microscopy and automated fluorescent microtitre plate readers. Subsequently, further fluorescent reporters or fusion proteins could be constructed using the PCR SOEing method already indicated. These would consist of barA, sirA or csrA/B/C promoter-fluorescent protein fusions. The purpose of these reporter gene fusions would be to further elucidate how the probiotics are affecting the BarNSirA pathway.
GFP tagging of wildtype Salmonella isolates. Electroporation of pFPV25.1 or the R-plasmid containing the rpsM:gfp fusion, will enable transformants used in tissue culture aminoglycoside protection assays to be visualized using confocal microscopy. This will reduce the need for the use of antibodies for this work.

Evaluation of foreseeable effects
All experiments involving wild-type and GMO organisms will be conducted in line with safety regulations relevant dealing with the Hazard Group 2 pathogens (refer to risk assessment 8A311 culture).
The hilA-gfp transcriptional fusion will be used to assess different probiotics, prebiotics and various volatile fatty acids for their inhibitory effect on hilA transcription. This will manifest by altered gfp intensity for the Salmonella hilA promoter gfp transcriptional fusion. This transcriptional fusion is chromosomal and is expected to be be extremely stable, which should limit any horizontal transfer of the transcriptional fusions.
The inactivation of putative virulence determinants is unlikely to increase the virulence of test isolates because virulence is a multifactorial process involving many genes. The addition of a hilA promoter gfp fusion is unlikely effect virulence. E. coil K12 is a standard cloning host and has successfully and safely been used in molecular biology for a number of years. In addition E. coil K12 is unable to survive in the environment for long periods time.
The rpsM-gfp fusion is intended to produce a stable GFP marked Salmonella strain which can be quantified in batch culture or tissue culture studies. This plasmid is medium copy number and has been used in multiple publications. Enumeration of Salmonella Typhimurium in batch culture is essential due to the lack of suitable fluorescent in situ hybridization probes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All studies will be performed at Containment Level 2. Thorough disinfection procedures will be carried out in the event of any spillages, using 10% Virkon S or 70% ethanol. 10% Virkon S will be used in the event of large spillages (>10ml), whereas 70% ethanol will be used for small surface spillages <10 ml. Laboratory floors and surrounding corridors are disinfected regularly. Environmental sampling of laboratories and surrounding areas is carried out routinely. All contaminated waste is autoclaved. No contaminated waste is disposed of in the drainage system without prior sterilisation.

Below is a table which describes the waste disposal procedures which will be used for different types of waste, which will be generated during this project.

Solid contaminated materials (cultures, disposables, Disposal by autoclaving in line with current risk etc) assessments. Autoclaving is carried out in the same building (a requirement of GM Class II) for one hour at 134°C. All solid waste is subsequently incinerated on site.

Liquid waste (broth cultures, supernatants, etc) Disposal by autoclaving in line with current risk assessments. Autoclaving is carried out in the building (a requirement of GM Class II) for one hour at 134°C after which liquid waste is tipped down the sink.

General rubbish Disposal by incineration on site in line with current risk assessments.

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<th>Time (hr)</th>
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<td>Liquid Waste</td>
<td>Autoclaving in line with current risk assessments.</td>
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<td>General Rubbish</td>
<td>Incineration on site in line with current risk</td>
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This project assessment has been approved by the VLA GM Local Safety Committee

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

**Project Containment**

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**Animal Units**

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**Project Ref 221/08.1**

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<td>Development and analysis of lyssavirus pseudotype viruses using retroviral vectors.</td>
<td>Class 2</td>
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The objective of this project is to develop an alternative serology assay for assessing neutralising antibody responses to vaccination against or infection with lyssaviruses (e.g. rabies virus). This approach would take advantage of pseudotyped virus technology where glycoproteins from one virus are expressed on the surface of a non-infectious retrovirus particles. These can then be used as a surrogate target in a neutralisation assays without the need for category 3 containment.

The project will be conducted in collaboration with the Department of Immunology and Molecular Pathology, University College London (UCL), who have cloned G-protein cDNA sequences from the Rabies virus (Challenge Virus Standard-II, CVS-I), European bat lyssavirus type-I and -2 (EBLV-I and -2) (at VLA under GM RA 06-02) and expressed (at UCL) as a HIV based pseudotype in human epithelial cells.

The virus particles are generated by co-transfection of a cell-line with three plasmids that encode the target glycoprotein, the retrovirus structural genes and a reporter gene (e.g. green fluorescent protein, GFP) flanked by long-terminal repeats that mediate integration into the target cell DNA). The resulting vector genomes do not code for viral structural genes and viral proteins are provided in trans from the first two plasmids. These vectors are therefore replication incompetent. Details of the constructs, demonstration that they do not give rise to replication competent viruses and lack of pathogenicity in a murine model have been published (Zufferey et al. 1997). The viral supernatants will be used to infect HIV-I susceptible human cells, which will then be checked rigorously for the absence of replication competent helper virus using assays for HIV-I gag protein and reverse transcriptase.

This risk assessment only covers the work planned to take place at the VLA i.e. currently restricted to HIV and MLV based vectors with lyssavirus glycoprotein genes (representatives from all available genotypes) or the glycoprotein of VSV (non-lyssavirus rhabdovirus used as control). The recovered pseudotypes will be assessed for their usefulness in serum neutralisation assays to replace the current virus neutralisation assays performed under high containment (ACDPIII, SAPO4).

Recipient or parental organism

Cell lines: HEK 293T, NP2, BHK-21, TE671, N2A
Bacterial cells e.g. E. coli- strain TOP 10 or similar for lyssavirus plasmid construction (under RA 06/02)

Host/vector system

The human immunodeficiency virus type I (HIV-I) gag-pol construct pCMV-A8.91 (Zufferey at at, 1997) and green fluorescent protein (GFP) reporter construct pCSGW (pHR_SINcPPT-SGW; (Demaison et at, 2002) or firefly luciferase reporter construct (pCSLW, where the luciferase gene has been cloned into pCSGW in place of GFP. Murine leukaemia virus (MLV) gag-pol construct pCMVi (Towers at at, 2000) and GFP reporter construct pCNCG (a LNXC plasmid (CLONTECH) encoding enhanced GFP with CMV driving expression of the RNA) or firefly luciferase
**Origin & function**

Origins: PCR amplicons of the glycoprotein genes of lyssaviruses and VSV

Intended Function: The recovered pseudotypes will be assessed for their usefulness in serum neutralisation assays to replace the current lyssavirus neutralisation assays performed under high containment (ACDPIII, SAPO4). Pseudotypes have three major advantages over live virus neutralisation tests: (1) they can be handled in low biohazard level laboratories; (2) the use of reporter genes such as GFP or β-galactosidase will allow the assay to be undertaken at low cost in laboratories worldwide; (3) each assay requires <10μl of serum. The implementation of robust pseudotype microassays will improve our understanding of the protective humoral immunity current rabies vaccines confer against emerging lyssaviruses, and will be applicable to surveillance studies thus helping control the spread of rabies.

**Evaluation of foreseeable effects**

The MLV and HIV based vectors are molecular clones which when expressed as a pseudotype virus particle are capable of binding and entering a cell. Once within the cell the particle nucleic acid, consisting of the reporter gene only, integrates in to the target cell DNA allowing expression of the reporter. However, the vector genomes do not code for viral structural genes and viral proteins are provided in trans from at least two separate plasmids during particle production. These vectors are therefore replication incompetent and incapable of producing progeny.

Pseudotyped particles are non-pathogenic in mice so would not be expected to be so in humans should accidental exposure occur. The major risk associated with retrovirus vector production and subsequent transduction is the occurrence of replication competent, recombinant viruses (RCR). Although a minimum of 3 recombination events are necessary to produce RCR in these systems, this possibility cannot be totally excluded. While RCR derived from MLV based vectors are unlikely to be more harmful than a wild type virus which is not known to infect humans, RCR derived from HIV vectors may cause harm for humans (HIV is ACDP Hazard Group 3).

The recovery and use of these engineered pseudotype viruses will thus be restricted to GM containment level 2 (Bldg 64, Room 44E) and infected tissue culture cells will be handled in the Class II safety cabinet.

Risk to the environment: Low.

Laboratory measures are in place to reduce the risk of release of any GMO from the designated category II laboratory in which all work will be carried out. Protective clothing (laboratory coats, disposable gloves and overshoes) will be worn by staff working with GMO’s. All waste will be autoclaved prior to incineration. Infected cell culture will be handled in the Class II Safety Cabinet.

Overall Risk: Low

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Solid waste:** will be autoclaved (134°C, 1 hour) prior to disposal by incineration.
- **Liquid waste:** will be autoclaved (134°C, 1 hour) prior to incineration. Liquid waste from live virus manipulations will be discarded into 1% Virkon within the Class II cabinet prior to autoclaving. General rubbish: will be treated as solid waste and autoclaved (134°C, 1 hour) prior to incineration.
- **Work:** will be carried out in dedicated category II laboratories. Antibiotics used in selection of recombinant plasmids will be limited. All material incorporating antibiotics (i.e. culture agar) will be autoclaved and incinerated.
- **Operators:** performing recombinant virus handling and manipulations will adhere to the strict procedures and risk assessments necessary for all live virus work within a Class II safety cabinet. Wearing of laboratory specific PPE (lab coat, gloves, overshoes) will be required at all times. Whilst this work is being undertaken, only rabies-vaccinated staff will be permitted to enter the laboratory.
- **The viral supernatants:** will be used to infect HIV-1 susceptible human cells, which will then be checked rigorously for the absence of replication competent helper virus using assays for HIV-1 gag protein and reverse transcriptase (expertise at UCL).
This project assessment has been approved by the VLA GM Local Safety Committee

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA GM Local Safety Committee

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**Project Ref 221/09.1**

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<td>20/01/2009</td>
<td>Production of Knockout mutants to characterise the function of putative ABC amino acid transporters and SdiA regulated genes in Salmonella Enterididis</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

In order to understand the role of amino acid transporter genes and sdiA related genes I metabolism, pathogenicity and epidemicity of Salmonella Enteritidis, genes will be activated individually and the characteristics of wild type and mutant strains will be compared.

Recipient or parental organism

Salmonella enterica strains with inactivated target genes (amino acid transporter genes and SdiA regulated genes) are unlikely to be more virulent than wild type strains. K12 strains harbouring single target genes which frequently are a single component of an operon are unlikely to increase the virulence of K12 strains.

All wild type strains to be used have already been typed by molecular methods to ensure traceability.

Host/vector system

Host: 1. E.coli K12 (DH5α or INVαF, S17-1λ pir)
2. The final recipient organism: Salmonella Enteritidis strains

Vector: 1. Standard high copy number cloning vectors such as pUC18 or pCR2 (Invitrogen). PERFORM series of suicide vectors -non-replicating in λpir Salmonella enterica strains.
2. Plasmid -pKD4 (Datsenko and Wanner, 2000) will be used as PCR template to create replacements of gene with kan cassette. Plasmid pKD46 (Datsenko and Wanner, 2000) expresses λ red system - y β and exo genes under the control of the P arab promoter. Contains ampicillin® marker. This plasmid contains the red system, whereby Gam inhibits hosts RecBCD exonuclease V so that Bet and Exo gain access to DNA ends and promote homologous recombination.

Origin & function

Origins: Salmonella Enterica

Intended functions of the genetic material involved: Chromosomal insertional inactivation of genes of interest: amino acid transporter genes SEN1417-1419, SdiA regulated genes srgC and rck. The purpose of these genetic modifications is to characterise these amino acid transporter genes and sdiA related genes functions and understand the role of these genes in metabolism, pathogenicity and epidemicity of Salmonella Enteritidis. The characteristics of wild type and mutant strains will be compared. Studies such as in vitro phenotype studies (phenotype array, Biolog), biofilm formation, survival within chicken models and tissue culture models will be conducted.

Evaluation of foreseeable effects

Salmonella enterica strains with inactivated target genes (amino acid transporter genes and SdiA regulated genes) are unlikely to be more virulent than wild type strains. K12 strains harbouring single target genes which frequently are a single component of an operon are unlikely to increase the virulence of K12 strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid contaminated materials (cultures, disposables, etc): Disposal by autoclaving or incineration in line with current risk assessments. Autoclaving is for one hour at
134 °C at 32 psi.

Liquid Waste (broth cultures, supernatants, etc) : Disposal by autoclaving in line with current risk assessments.

Autoclaving is for one hour at 134 °C ar 32 psi after which liquid waste is tipped down the sink.

General rubbish: Disposal by incineration in line with current risk assessments.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA Local Safety Committee

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tr>
<td>Animal Units</td>
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<td>L2 Yes L3 L4</td>
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**Project Ref** 221/09.2

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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<th>CultureVolumeClass3-4</th>
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<td>Cloning and expression of recombinant Listeriolysin-O from Listeria monocytogenes</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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</table>

Project notified under transitional arrangements
### Project Additional Information

**Purposes of the contained use**

- Insertion of the LLO protein coding amplification into pTriEx-1.1 Neo
- Propagation of the recombinant bacterial plasmid. The host is a general cloning host. It is transformable using standard methods.
- Expression of the proteins in a bacterial host under inducible T7 RNA polymerase.
- Assessment of protein expression in bovine cells.

**Recipient or parental organism**

- E. coli DH5α which is a derivative of E. coli K-12 (Cloning Host).
- E. coli BL21 or Rosett (DE3) (Bacterial Expression Hosts)
- Bovine cells (primary dendritic and skin fibroblasts)

**Host/vector system**

- pTriEx-1.1 Neo. (Novagen)

**Origin & function**

The complete open reading frame encoding LLO will be supplied in the pBlusscript plasmid from collaborators in the USA. The LLO insert will be subcloned into pTriEx-1.1 Neo. A his-tagged protein will be expressed and purified from E. coli using Nickel affinity chromatography. The biological activity of the recombinant protein will be confirmed using a sheep erythrocyte lysis bioassay. The protein will then be used in a series of in vitro experiments to assess whether LLO facilitates the cytosolic delivery of antigen to the cytosol of bovine cells resulting in enhanced MHC class I antigen presentation.

To assess the biological effects of endogenously synthesised LLO in bovine cells, cells will be transiently transfected with pTriEx-1.1 Neo expressing LLO.

**Evaluation of foreseeable effects**

The LLO protein has the ability to form pores in the membrane of the phagolysosome of mammalian cells, allowing release of the phagosomal contents into the cytosol of the host cell. LLO also has lytic activity on red blood cells. Whether recombinant E. coli expressing LLO will exhibit enhanced pathogenicity is unknown. However, given that E. coli is not able to grow intracytoplasmically and LLO is only active at acidic pH (optimal pH 5.5) it may be speculated that it is unlikely to do so. In vitro experiments showed that macrophages efficiently phagocytosed and killed recombinant E. coli expressing LLO, and that the LLO was functional and still allowed cytosolic delivery of antigen.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid cultures will be autoclaved for 45 minutes at 134 degs, 31.9 psi (2.2bar) pressure, prior to disposal by incineration.
Liquid waste will be autoclaved for 45 minutes at 134 degs C, 31.9psi (2.2bar) pressure prior to disposal down the drain.
Alternatively liquid waste will be treated overnight with a minimum of 1 part sodium hypochlorite (5-16% available chlorine) to 100 parts of waste liquid before disposal down the drain.
General waste will be autoclaved for 45 minutes at 134 degs C, 31.9psi (2.2bar) prior to incineration.
All methods are designed to kill 100% of bacteria

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA GM Local Safety Committee

Project Containment

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Animal Units

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<th>Large Scale Activities</th>
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<td>L2 L3 L4</td>
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Project Ref 221/09.3

Date Ackn'd 07/07/2009
CU2 Project Title Production of a gfp mutant in Salmonella species
Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 Litre
Non-GMM Consent Granted
The aim of this work is to generate a reference control strain of Salmonella enterica that contains a green fluorescent gene (gfp) in the chromosome that is constitutively expressed. The strain will be used as a positive control to improve the validation of microbiological tests and media for isolation of Salmonella and avoid the reporting of false positive results that arise due to cross contamination. The marked strain can be identified either by monitoring expression of the GFP in the presence of UV light (a commercially available plate reader) or by PCR based methods targeting the gfp gene.

The GFP mutants will be constructed by one of two methods, or possibly be both methods, and these two methods are outlined briefly below:

**Method 1**
The plasmid incorporates a transposon which is used to carry the gfp gene into the chromosome of the recipient bacteria.

* Perform filter mating of suitable Salmonella recipient strains with the donor strain (E.coli S17-1 λ pir) that has the suicide plasmid containing the gfp gene and transposon.
* Select for transconjugants that are kanamycin resistant but ampicillin sensitive (indicating presence of the gfp gene but loss of the suicide plasmid pAG408 –Noah et al., 2005).

**Method 2** (Datsenko & Wanner, 2000)

* PCR gene of interest from recipient (Salmonella) using primers with homology to gene of interest and to region of plasmid pkD4.
* Incorporate gfp gene into modified pkD4.
* Electroporate helper plasmid pkD46 into the recipient bacteria of choice to aid homologous recombination.
* Electroporate the gfp:kan construct into the recipient containing pkD46 and screen for kanamycin resistance which will indicate that homologous recombination has occurred.

**Recipient or parental organism**
Recipient organism is a strain of Salmonella enterica.

**Host/vector system**

**Method 1:**

Host- E.coli K12 (DH5α or INVαF, S17-pir)
Vector - gfp gene which is located on a suicide vector pAG408 (Surez et al., 2007 and Murphy et al., 2007)
Method 2:

Host - Final recipient Salmonella strain

Vectors

a) Plasmid pKD4 Plasmid can only be maintained in E.coli BW25141 (1633) Contains ampicillin® and kanamycin® marker

b) Helper plasmid pKD46.

Maps of both the above 2 plasmid can be found in the paper by Datsenko and Wanner (2000)

Origin & function

The gfp gene originates from the jellyfish Aequorea Victoria.

For method 1 - the gfp gene and kanamycin cassette are located on suicide vector pAG408 which is carried on chromosome of E.coli K12. A map of the plasmid pAG408 can be found in the paper by Suarez et al. (1997).

For method 2 - the gfp gene with its own promoter will be purchased from a suitable company such as Invitrogen. Plasmid pKD4 will also be used as described earlier. Plasmid pKD46, although used, will be lost when the constructs are grown at 37° C.

The intended function of the genetic material is to produce a traceable bioluminescent Salmonella strain outlined in more detail earlier.

Evaluation of foreseeable effects

All experiments involving these organisms will be conducted in line with safety regulations relevant for dealing with the Hazard Group 2 pathogens and this will limit any risks.

The suicide plasmid donor strain for method 1 (E.coli K12) contains a small plasmid unlikely to increase the virulence of the K12 strain. In addition E.coli K12 is unable to survive in the environment for long periods of time.

The gfp gene itself poses no increased risk of infection as the gfp gene does not confer pathogenicity.

Method 1 will insert the gene in an unknown area and may alter gene function. The area into which the gene has inserted will be monitored using appropriate genetic tests such as PCR, sequencing, southern hybridisation or signature tag mutagenesis to check that a virulence gene could not be upregulated.

Method 2 will disrupt gene function. There is no evidence from chicken studies when the gfp gene was inserted into the marRAB locus of Salmonella Typhimurium that the gfp gene in Salmonella strains affected pathogenicity (Randall et al., 2001). Other studies have also shown that disruption of the ompX or ompW genes does not increase the ability of Salmonella to adhere to and invade tissue culture cells (unpublished observations) and aroA mutants are attenuated (Trebichavsky et al, 2006).

The stability of modified strains will be evaluated by serial passage in broth for 10 days and previous studies have shown such mutants to be stable (Randall et al., 2001, Suarez et al, 1997). The phenotype of modified strains will be evaluated by phenotype micro-array (BIOLOG) and growth characteristics will be performed for parent and mutant strains in rich and minimal media.

Field strains exposed to UV light in checking for the presence of the gfp gene may subsequently be stored for further analysis. However, brief exposure to light is not envisaged to produce mutations in these strains and UV based bacterial identification tests based on fluorescent substrates is in regular use in commercial labs.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All studies will be performed at containment level 2 in line with relevant safety guidelines and risk assessments (FES7/1-Culture, Handle and Disposal, FES7/3-Disinfection, FES7/5-Centrifuges, FES7/85-Use of Safety Cabinets). Disinfectant used to treat small spills of any bacterial culture is 70% ethanol, disinfectant to treat larger spills is Interkokask.

Laboratory floors and surrounding corridors are disinfected weekly alternatively with the disinfectants Tegador and TASKI San to eliminate any selection of resistance by using only one disinfectant.

Environmental sampling of laboratories and surrounding areas is carried out monthly. No contaminated waste is disposed of in the drainage system without prior sterilisation/autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

This project assessment has been approved by the VLA GM Local Safety Committee.

### Project Containment

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### Project Ref 221/09.4

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# Project Additional Information

## Purposes of the contained use

We propose to create a mutant library in Mycoplasma bovis (M. bovis) to study such processes as cell adhesion and biofilm formation.

## Recipient or parental organism

We will transform field strains of M. bovis with a suicide plasmid, part of which will integrate into the mycoplasma chromosome. The transposable element that is integrated into the chromosome carries a gene for gentamicin resistance as a selective marker but doesn't carry the transposase gene, thus preventing the re-excision of the element and therefore making stable mutants.

The strains chosen for this study will be good biofilm formers and the mutants will be screened for biofilm formation. Any mutant that does not produce a biofilm or produces a biofilm significantly better than the wild type will be selected and the position of the transposon within the chromosome defined. This will help us to understand the process of biofilm formation in mycoplasmas.

## Host/vector system

The hosts are: (1) E.coli K12 derivative strain, for example TOP10 cells. (2) Mycoplasma bovis, field strain that is not naturally gentamicin resistant, from SEB5 culture collection.

The Vector is: pMT85 - A suicide plasmid with a gentamicin resistance selective marker and transposon 4001.

## Origin & function

Plasmid originally from Staphylococcus. The plasmid also contains Transposon Tn4001 is a composite class 1 transposon originally detected in the Staphylococcus aureus plasmid pSK1 (Mahairas et al, 1989). The tranposase gene has been altered by site directed mutagenesis so that a TGG (Trp) codon was changed to a TGA (stop) codon. This means the gene is prematurely terminated in Eubacteria (such as E.coli) and results in a truncated protein which has no activity (Zimmerman et al, 2005).


(1) The E.coli host strains selected for this project are disabled strains requiring culture conditions for efficient propagation and survival. The presence of the pMT85 plasmid will confer gentamicin resistance to the host cell but will have no effect on the virulence of these bacteria. These would normally be designated as class 1 organisms.

(2) Transformation of M. bovis with the plasmid and its integration into the mycoplasma chromosome is more likely to give a disadvantage to the cell in comparison to the wild type. The integration of the transposable element and the inactivation of genes is unlikely to increase the virulence or persistence of the bacteria.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be autoclaved (134º, 1 hour) prior to disposal by incineration.
All liquid waste will be autoclaved (134ºC, 1 hour) prior to incineration.
General waste will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

This project assessment has been approved by the VLA GM Local Safety Committee

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02/03/2022
The objective of this project is to generate Classical Swine Fever Virus (CSFV) pseudotyped viral particles representative of different CSFV genotypes. CSFV can only be propagated under Defra Containment Level 3 (SAPO III containment), thereby limiting the types of experiments that can be conducted. Pseudotype virus technology allows the generation if non-infectious reporter virus particles that incorporate the surface coat proteins of a virus of interest (such as CSFV) under the direction of non-infectious retrovirus genetic elements that only require GM Class 2 containment.

Our studies will involve use of CSFV pseudotype particles in reporter assays to quantitatively measure rates of cellular uptake of different viral strains, to investigate which primary pig cells are permissive for CSFV and to screen for compounds or antibodies able to inhibit CSFV uptake. Usefulness of CSFV pseudotypes for replacing wild-type virus in serum neutralisation assays will also be assessed.

Recipient or parental organism

Pseudotype particles will be produced by transfection of pig or human tissue culture cell lines with three separate plasmids, one to express CSFV coat proteins, the second to provide retroviral proteins required for virion assembly and a third plasmid to allow expression of packageable RNA encoding a reporter gene such as green fluorescent protein (GFP), luciferase or β-gal.

A theoretical risk associated with pseudotype virus production is the occurrence of replication competent, recombinant (RCR) viruses. A minimum of 3 recombination events to place five genetic elements in the correct spatial orientation would be necessary to produce RCR. This possibility, however improbable with a three plasmid system, cannot be totally excluded. Nevertheless, risks to laboratory workers and animals are minimal as all accessory proteins required for viruses to be infectious are deleted from the system so in the improbable event of recombination occurring, any RCR produced would be non-infectious and would not produce progeny.

Pseudotype particles will be used to non-productively infect pig cell lines and primary cells.
Cell specificity is conferred by the surface coat protein, which, in this project will be derived from CSFV - a virus which is restricted to the porcine host. Once within a cell, the nucleic acid contained in the pseudotyped particle, encoding the reporter gene only, integrates into the target cell DNA allowing expression of the reporter and the process is arrested at this stage. The reporter nucleic acid does not encode viral structural genes and viral proteins are provided in trans from at least two separate plasmid to enable particle production and pseudotyped particles are therefore replication incompetent, non-infectious and do not generate progeny particles.

**Host/vector system**

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<tr>
<th>Category</th>
<th>Description</th>
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<tbody>
<tr>
<td>Cell lines available at V:A that will be used:</td>
<td></td>
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<tr>
<td>Human: HEK 293T (HEK-293T; originally referred to as 293/tsAJ609 neo)</td>
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<tr>
<td>Pig: Porcine cell lines such as pig kidney PK15 and SK6, and the pig aortic endothelial AOC cell line.</td>
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**Origin & function**

PCR amplicons of coat protein coding sequences from different CSFV strains will be cloned into pCAGGS (Riken Center for Developmental Biology).

Pseudotype virus particles will be generated by co-transfection of three plasmids:

1. The coat protein-expressing plasmid described above.
2. A plasmid supplying proteins to allow pseudotype particle assembly. The murine leukemia virus (MLV)-based gag-pol core protein construct, CMVi or the human immunodeficiency virus (HIV)-based plasmid, pCMV-Δ8.91, will be used. These plasmids are safe to use as they contain minimal viral genetic elements and all viral accessory proteins are deleted.
3. A reporter plasmid: Reporter constructs for use with pCMVi are pCNCG (a LNCX plasmid (CLONTECH) encoding GFP or pMLV-luc firefly luciferase reporter construct. Reporter constructs for use with pCMV-Δ8.91 are GFP reporter construct pCSGW (pHR_SINcPPT-SGW); or reporter constructs pCSGW-L, -RL or -β-gal where the luciferase, renilla luciferase or B-gal genes respectively have been cloned into pCSGW in place of GFP.

Human embryonic kidney 293T cells will be used to test protein expression and may be used for production of Pseudotyped particles as they are amenable to transfection and very efficient at expressing exogenous proteins. Porcine cell lines will be used for work with pseudotype particles as they will contain CSFV coat surface proteins and CSFV is restricted to porcine hosts.

**Evaluation of foreseeable effects**

There are minimal foreseeable effects from working with the three-plasmid system as the reporter plasmid does not encode any viral structural genes and viral proteins required for pseudotype production are provided in trans from two separate plasmids. As stated above, a theoretical risk associated with pseudotype virus production is the occurrence of replication competent, recombinant (RCR) viruses. A minimum of 3 recombination events to place five genetic elements in the correct spatial orientation would be necessary to produce RCR and it is highly unlikely that this could occur.

Work with the resulting pseudotype particles has few associated risks as these particles lack the accessory proteins needed for formation of infectious virus and are therefore replication incompetent and non-infectious.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste generated by this project will be completely inactivated by autoclaving (134°C, 1 hour) prior to disposal by incineration.

Liquid waste will be completely inactivated by discard into 1% Virkon prior to autoclaving (134°C, 1 hour) and incineration.

General rubbish will be treated as solid waste and autoclaved (134°C, 1 hour) prior to incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA GM local safety committee

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Project Ref 221/09.6

Date Ackn’d 01/09/2009
CU2 Project Title
Assessment of risk associated with handling (storage and culture) of genetically modified ACDP Hazard Group 1 and 2 bacteria sent from other institutions

Date Project Ceased 02/03/2022

Class 2
CultureVolClass2 < 1 Literature
CultureVolumeClass3-4
Non-GMM Consent Granted
Project Additional Information

Purposes of the contained use

The Phengenix program launched by FES offers the expertise in genotyping and phenotyping of bacterial and eukaryotic cells to the wider research and commercial community. The technologies are applicable to wild type and genetically modified organisms, this risk assessment therefore covers the handling, storage and culture of ACDP Hazard Group 1 and 2 bacteria at the VLA.

Recipient or parental organism

Constructs of genetically modified bacterial species will be supplied by collaborative institute. NO Modifications to be carried out at VLA.

There are several different methods employed to create mutants in a bacteria. The one employed will depend on the purpose of the studies and species of bacteria. Creation of mutants typically relies on the introduction of mutated non-replicating DNA (e.g. in suicide plasmids, transposons, bacteriophages or PCR product), which may or may not be integrated into the chromosome. Where complete ablation of gene function is required, the mutated DNA may contain a copy of the target gene disrupted by a selectable marker e.g. antibiotic resistance genes. However, with modern approaches to mutagenesis, the wild type allele is often replaced with a mutant allele without leaving any antibiotic resistance gene in the cell.

Host/vector system

This application will include the use of any ACDP Hazard Group 1 or 2 bacterium. The bacterium chosen will depend on the nature of the primary studies undertaken at the collaborating institute. Typically bacteria are chosen due to interests of the groups involved e.g. pathogenesis, biochemistry, epidemiology etc. A variety of vectors are available for use in different bacterial species. These are usually non-mobilisable or mobilisation-defective vectors from commercial or academic sources.

Origin & function

DNA sequences are usually obtained from the organism of interest by PCR methods. These sequences may include promoter sequences, encoding and/or non-coding sequences. Mutations are often selected for by the replacement of sequence of interest with a selectable marker (e.g. antibiotic resistance cassette) or reporter gene (e.g. beta-galactosidase, luciferase or fluorescent protein).

The purpose of manipulation of genetic material is to characterise and understand the effect of DNA sequences on the metabolism, pathogenicity and/or epidemiology of the organism of interest. Comparisons will be made with the activities of the progenitor strains to assign DNA sequence function. The studies offered at the VLA will be in vitro phenotypic characterisation to include central metabolism, effects of stress and metabolic effectors.

Evaluation of foreseeable effects

All experiments involving these organisms will be conducted in line with safety regulations relevant for dealing with ACDP I and II bacterial species (refer to risk assessment FES7/1 and FES7/127).
Bacterial strains in which DNA sequences have been manipulated are unlikely to be more virulent than the wild type strains. The GMO is likely to be less virulent than its pathogenic progenitor and can therefore be handled at the ACDP category relevant to wild type. Nevertheless, the specific risk assessment for the organism to be studied will be obtained from the collaborator and any additional risk (if any) will be carefully considered before the work commences at the VLA.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposal:
Solid contaminated materials: Following acceptance of phenotyoping results by customer, wild type and GMO stocks will be destroyed according to local procedures. Disposal by autoclaving (in building 4 room 3 or 36) and incineration in line with current risk assessments (FES7/4) - One hour and 10 minutes at 134°C. All solid waste is subsequently incinerated onsite. Liquid waste: Disposal by autoclaving and incineration in line with current risk assessments (FES7/4) - One hour and 10 minutes at 134°C after which liquid waste is tipped down the sink. General Rubbish: Disposal by incineration on site in line with local procedures.

Special measures necessary to protect the environment: A restricted access laboratory has been assigned in which all studies will be carried out. All live culture work will be prepred in class II safety cabinet that will be regularly fumigated (FES7/75 & FES7/85). All studies will be conducted in line with relevant safety guidelines and risk assessments (FES7/1-Culture, Handle and Disposal, FES7/3-Disinfection, FES7/5-Centrifuges, FES7/85-Use of Safety Cabinets). Disinfectant used to treat small spills of any bacterial culture is 70% ethanol, disinfectant to treat larger spills is Interkokask. Laboratory floors and surrounding corridors are disinfected weekly alternating between 2 disinfectants to avoid any selection of resistance by using only one disinfectant.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the VLA GM Local Safety Committee

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
Project Additional Information

Purposes of the contained use

Protein misfolding cyclic amplification (PMCA) is used in research and diagnosis of animal transmissible spongiform encephalopathies (TSE’s), such as BSE in cattle and Scrapie in sheep. PMCA is a technique akin to PCR in that it amplifies very low levels of product, in this case and the abnormal (disease associated prion protein (PrPSc), to detectable levels. To achieve this, the test sample is mixed with a high abundance of normal (cellular) prion protein (PrPC) and treated with cycles of sonication and incubation over a period lasting for several days/weeks. At regular intervals (currently daily) the PMCA mixture is replenished with fresh PrPC for further conversion/amplification. Currently PrPC is supplied as a tissue homogenate derived from ovine brains, however, there are inherent limitations using this as a source of PrPC. The tissues have to be taken within 30 minutes of post mortem and processed within 45 minutes for the tissue to be suitable for the support of amplification and even then this is not guaranteed. In addition, the ethical issue of using animal derived tissues for what we hope to become an 'off- the-shelf' diagnostic test is questionable (a single sheep brain will support 400-500 tests).

Recent advances in rodent PMCA technology using recombinant PrP (rPrP) (Atarashi and others 2007) is the first step towards a brain free substrate approach. There are a number of advantages for the transfer of this technique to detection of sheep scrapie. Recombinant PrP of different genotypes can be manufactured in milligram quantities. This line of approach would also facilitate the control of the conversion/amplification conditions.

The aim of the project is to determine if recombinant PrPC can be used as a replacement for brain derived PrPC in the PMCA technology.

We will acquire from two research establishments (IDEXX Laboratories and ADAS UK), E.coli strains that have been transformed through the insertion of the PrP gene.
The transformed E.coli will be grown up and recombinant protein expression induced. After induction cells will be lysed and protein extracted from inclusion bodies. Extracted protein will be purified and refolded as required for use in PMCA experimentation.

Recipient or parental organism

The E.coli host strains selected for this project are disabled strains requiring precise culture conditions for efficient propagation and survival. These would normally be designated as class 1 organisms (see SACGM Guidance notes). The vectors selected contain the necessary promoters and regulatory sequences for expression of the inserted gene, therefore these organisms are capable of generating recombinant proteins. The vectors themselves are considered to be non-mobilisable. The foreseeable effects of this GMM relate largely to the 'insert', in terms of the intrinsic properties of the protein of interest and the effect such a protein would have on the virulence and pathogenicity of the host strain.

The production of the prion protein by the modified E.coli host is not expected to alter the pathogenicity or virulence of the E.coli host. The E.coli host is a K12 or B strain derivative, and such is considered disabled. Production of the prion protein is not expected to alter the ability of this E.coli host strain to survive in the environment or become invasive. The vectors used for delivery of the gene of interest to the E.coli host and to drive expression of the protein are non-mobilisable, and thus gene transfer is dependent upon accidental encounter with organisms that can complement the lack of mobilisation function. This is considered unlikely as measures to prevent the release of the organism from the lab are in place.

Host/vector system

The following laboratory adapted E.coli strains have been used:

E.coli K12 derived strain Novablue(DE3)™, E.coli B derived strain BL21 (DE3)™, E.coli Rosetta strain™.

The E.coli K12 hosts allow stable propagation of the recombinant plasmids and, depending upon the vector, inducible expression of the recombinant protein. These E.coli are RecA, EndA deficient and considered 'disabled'. They are amongst the most frequently used bacterial host and are considered to have a good safety profile (see SACGM Guidance notes). These organisms do not possess or express known virulence factors (Muhldorfer and others 2006, Muhldorfer and Hacker 2004, Kuhnert and others 1997 & Bachmann - Strain derivatives and mapping 1996)

E.coli Novablue DE3 strain is a K12 derivative.

E.coli B derivatives are EndA deficient but have intact RecA function. Nevertheless, these strains are recognised as non-pathogenic. (see SACGM Guidance notes, Muhldorfer and others 2006, Muhldorfer & Hacker 2004, Kuhnert and others 1997 & Bachmann - Strain derivatives and mapping 1996).

E.coli Rosetta™ host strains are Tuner™ derivatives designed to enhance the expression of eukaryotic proteins that contain codons rarely used in E.coli (see web based data from vector suppliers - Invitrogen, Sigma Aldrich, Promega & GE life sciences) Tuner™ strains are lacZY deletion mutants of the BL21 (B derived) strain.

Vector

The following expression vectors have been used:

pET-41a and pET-22b(+), both of which are commercially available from Novagen.

pET vectors have a modified pUC origin and are therefore lacking in the Bom and Nic sequences necessary for mobilisation. pUC series and derivatives are non-mobilisable.

Origin & function

The nucleic acid insert will compromise amino acid residues 23-231 of the prion protein gene encoding naturally occurring sheep genotypes and Syrian hamster PrP.

GM recombinant plasmids containing the nucleic acid inserts will be used to produce recombinant proteins in E.coli. Production of the transformed E.coli will be done for us
by external research establishments.

**Evaluation of foreseeable effects**

Very low risk.

The vectors used are impaired in their ability to pass on genetic material to other bacteria. Furthermore, if gene transfer to environmental bacteria was to occur it is unlikely to have any notable effects as the foreign gene is not known to encode proteins that promote any selective advantage or contribute to virulence and pathogenicity.

Release of the cloned genes/plasmids into the environment will have little impact. The vector/plasmid is non-mobilisable and non-conjugative and genetic transfer to other organisms requires the presence of specific helper plasmids under specific conditions (Lebaron and others 1997). This event is considered unlikely. Furthermore, plasmid DNA released into the environment will be naturally degraded by ubiquitous nucleases.

In the unlikely event that transformed E.coli is released to the environment where appropriate conditions exist for its survival and growth, gene transfer to other host species, prokaryotic and eukaryotic is very unlikely.

Many research groups have tried to develop infectious rPrP (Mehlhorn and others 1996 and Hill and others 1999) and been unsuccessful. One group has been able to develop infectious rPrP (Legname and others 2004) but only under strict unusual conditions involving a short 55 amino acid peptide with a mutation at residue 101 (p101L) treated with 3M urea at 37°C before intracerebral inoculation. The risk of our rPrP becoming infectious on release to the environment is extremely low. The genetic sequence we intend to insert into the E.coli does not contain the P101L mutation. It would also be very unlikely to be released in an environment where urea is present at a concentration of 3M and at a temperature of 37°C. The resultant GMM is considered equivalent to ACDP hazard group 2 due to the unknown expression levels of recombinant ovine and hamster PrP, which could be potentially toxic and/or pathogenic at higher concentrations.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Derogation is required for the siting of the autoclave used for this project. Due to the high temperature required for destruction of Prion proteins an alternative autoclave to that within the building is required. This will involve transporting the waste from the building to another no more than 50 metres away across a level (no steps) car park.

Appropriate documentation to support this derogation is provided with this application.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All work with the GMM will be carried out within containment level 3 with derogation (no negative pressure, no requirement for fumigation) laboratory dedicated for work involving TSE's. All work involving GMM's except for incubation of E.coli in growth media will be carried out in a dedicated class 1 safety cabinet up to the point where the E.coli have been lysed using a Bugbuster. Incubation of E.coli in growth media will be carried out in large capped bottles within an integrated shaking incubator with lid. Reusable labware will be treated with either 2M sodium chloride or sodium hypochlorite (20,000ppm free chlorine) to kill any E.coli and deactivate any abnormal prion protein, before removal from the laboratory for cleaning. The safety cabinet will be cleaned with ethanol on a daily basis and with sodium hypochlorite weekly.

Solid waste (Agar plates, absorbent paper, plastics etc) will be disposed of through autoclaving (134-137°C for 30 minutes) followed by incineration. The higher temperatures used for autoclaving are required for deactivating abnormal prion protein. (Transmissible spongiform encephalopathy agents: safe working and the prevention of infection: Publication of revised guidance. June 2003. Department of Health).

Large volume liquid waste cannot be autoclaved using existing equipment used for autoclaving TSE waste. Alternative measures for handling large volume TSE waste have been devised and have been in use for many years. Small volume GMM waste can be absorbed onto tissue paper and treated as for solid waste. Large volume GMM waste (50mls plus) produced in these experiments will be mixed with equal volumes of sodium hypochlorite (40,000ppm free chlorine) for one hour to kill any organisms and deactivate any aggregated PrP, then disposed of by incineration. See attached SOP for waste transport procedures.

Contaminated or potentially contaminated rubbish should be placed in black plastic sacks and disposed of by incineration using general waste management.
Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 221/10.2

Storage, use and culture of luminescent (lux) ACDP hazard group 2 bacteria

Class 2 Culture Volume Class 3-4

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use
To identify the sites of colonisation for Leptospira interrogans and Campylobacter jejuni within the insect model; Galleria mellonella

Recipient or parental organism
1) Leptospira interrogans L495 and E. coli DH5alpha
2) Campylobacter jejuni ATCC 33291 and E. coli DH5alpha

Host/vector system
1) L. interrogans: pAL615 (contains lux genes, pflgB promoter)
2) C. jejuni: pSB377 (contains lux genes), pRY107 (shuttle vector) and pflaA (ATCC 33291).

Origin & function
Photorhabdus luminescens (formerly called Xenorhabdus luminescens) is a Gammaproteobacteria belonging to the family Enterobacteriaceae. It is a bioluminescent bacteria found in terrestrial environments.

• Lux CDABE reporter system - commonly used reporter gene system (Murray et al., 2010; Allen and Griffiths., 2001)
• flaA σ28 promoter - From the test strain ATCC 33291 (Allen and Griffiths, 2001)
• flgB promoter from Borrelia burgdorferi (provided by M. Picardou; pasteur Institute (Murray et al., 2010))

Automatically distinct internal structures of the Galleria larvae were successfully identified through the use of histopathology in a previous project SC0243. However, histopathology failed to identify the colonisation sites within the Galleria. A LUX tagged Campylobacter and Leptospira will provide traceable luminescent bacteria to study tissue tropisms and elucidate sites of colonisation using an In Vitro imaging System (IVIS).

Evaluation of foreseeable effects
No adverse effects are envisaged. All experiments involving these organisms will be conducted in CL2 laboratories in line with safety regulations relevant for dealing with hazard group 2 pathogens (refer to risk assessment FES7/1). The lux operon from Photorhabdus luminescens (formerly called Xenorhabdus luminescens) poses no increased risk of infection as the lux genes do not confer pathogenicity; the genes product's function is to emit light which is not known to be harmful to any organism or the environment (Van Dyk and Rosson, 1998). The luxCDABE fusion has been stably inserted into the chromosome and therefore is unlikely to be lost (Murray er al., 2010; Allen and Griffiths, 2001).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Disposal of bacterial waste and/or infected Galleria will be destroyed using local procedures (autoclaving and incineration). Live, infected Galleria will be frozen at -20°C for at least 16 hours before being autoclaved. Waste and frozen Galleria will be disposed of by autoclaving (Building 4; room 2, Stewart Stockman; room SE.110) and incineration in line with current risk assessments (FES7.4); 70 minutes at 134°C and 15 psi. All solid waste is subsequently incinerated on site.
All organisms are ACDP hazard group two and all manipulations will be carried out in our collaborative institutes and do not require any further modifications at VLA. However, wild type strains will be used as well as the genetically modified strains.

All studies will be carried out in a Class II safety cabinet (FES7/85) as required and always within a containment level two laboratory. All studies will be conducted in line with departmental risk assessments (FES7/1, FES7/3, FES7/5, and FES7/85) and the relevant safety guidelines. Small spills (<20ml) of bacterial culture are treated with 70% ethanol; larger spills (>20 mls) Interkokask or Superkill. Laboratory floors and the surrounding corridors are disinfected weekly with Tegador and TASKI (weekly rota applied) to eliminate any selection of resistance by using one disinfectant. All bacterial waste will be disposed of by autoclaving (134°C, 15 psi for 70 minutes) before incineration.

Transfer of any live ACDP hazard group two cultures will occur in a sealed vessel e.g. a bio bottle or any other container with a sealable lid (Refer to risk assessment FES7/133).

**Project Containment**

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**Project Ref** 221/11.1

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<td>20/09/2011</td>
<td>Expression of retroviral gag protein in cell lines already expressing exogenous PrPc</td>
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<td>&lt; 1 Litre</td>
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02/03/2022
The work aims to express the gag gene from Maedi-visna virus in cell lines already expressing ovine and bovine PrPC. There is growing evidence that co-infection of animals with lentivirus enhances the detection of scrapie in sheep (Salazar et al., 2010), and co-infection has also been shown to increase infecton in cell models (LeBlanc et al., 2006). More recently the co-expression of just gag protein from HIV has been shown to increase sensitivity up to 100x in a cell model of Chronic Wasting Disease (CWD) (Bian et al., 2010).

We have previously generated a series of cell lines based on the rabbit kidney cell line, RK13, constitutively expressing either ovine or bovine PrPC and have shown permissiveness to infection with a subset (7 out of 30) of scrapie field cases (Neale et al., manuscript in preparation). We now aim to co-express the gag protein from Maedivisna virus in these cell lines, as well as the individual proteins that comprise the gag protein - the gag protein once expressedin the cell is cleaved by proteolytic action into three main proteins - matrix, capsid and nucleocapsid. It is proposed that the co-expression of the gag gene from Maedi visna virus in cell lines currently permissive to scrapie infection will increase their sensitivity. Additionally it is hoped that expression of the gag gene, or its component parts, in cells expressin bovine PrP will render them susceptible to infection with BSE. By expressing the 3 major components of the Maedi visna virus gag gene separately it may be possible to elucidate whether one of these componenets is required for increased sensitivity or whether it is a co-ordinated effetct of all the products of the gag gene.

Recipient or parental organism

1. Laboratory adapted E. coli strains:
   - e.g. E.coli K12 derivative strains, e.g. JM109 (Promega), TOP10, TOP10 F'; DH5α (Invitrogen)
   - e.g. ccdB Survival 2 T1R strain (Invitrogen, Cat# A10460).

2. Mammalian cell lines based on the RK13 cell line constitutively expressing ovine or bovine PrPC (covered by GMRA 02/07)
   - The E.coli K12 hosts allows stable propagation of the recombinant plasmids. These E. coli are RecA and EndA deficient and considered 'disabled'. They are amongst the most frequently used bacterial host and are considered to have a good safety profile (anon, 2007). These organisms do not possess or express known virulence facotrs (Muhidorfer et. al., 1996; I Muhldorfer and J. Hacker, 1994; Kuhnert et. al., 1997; Bachmann 1996).
   - The ccdB Survival 2 T1R strain (Invitrogen) is used as it is resistant to the toxic effects of the ccdB gene and may be used to propagate and maintain vectors containing the ccdB gene (e.g. pcDNA-DEST40). It is RecA and EndA deficient. (www.invitrogen.com).

The RK13 cell lines expressing ovine PrPC have been shown to be permissive to scrapie infection (Neale et al., manuscript in preparation), the addition of the gag protein...
expression will hopefully increase sensitivity towards scrapie and BSE inocula with low infectious titres.

Host/vector system

The vector will be the commercially available:

pCI (Promega) + linear puromycin selection (Clontech), pIRES-puro3 (clontech) or pBI-CMV1 (Clontech)

The PrPC expressing cell lines have been generated using either a neomycin or hygromycin antibiotic selection (Neale et al., manuscript in preparation), therefore a different antibiotic selection is required in this case puromycin.

The pCI vector does not contain any mammalian antibiotic selection (Promega Technical Bulletin #TB206) and has to be co-transfected with either a linear antibiotic selection marker or a second plasmid containing antibiotic selection, in this case the linear puromycin selection marker from Clontech will be used.

If the pCI expression vectors do not establish stable expressing cell lines, pIRES-puro3 contains the internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV), which permits the translation of two open reading frames from one messenger RNA. After selection with puromycin, nearly all surviving colonies will stably express the gene of interest, thus decreasing the need to screen large numbers of colonies to find functional clones. The expression cassette of pIRESpuro3 contains the human cytomegalovirus (CMV) major immediate early promoter/enhancer followed by a multiple cloning site (MCS) that precedes stop codons in all three reading frames, a synthetic intron known to enhance the stability of the mRNA, the ECMV IRES followed by the puromycin gene, and the polyadenylation signal from SV40 (Clontech Protocol no. PT3646-5, version no. PR8532553).

If there is a requirement to express two of the components of the gag gene in cell line at one time a bi-directional expression vector such as pBI-CMV1 (Clontech) will be employed. The pBI bidirectional vectors are designed to constitutively express two proteins of interest at similar levels (unlike IRES vectors, in which the expression of the proteins that are upstream and downstream of the IRES sequence varies). In the pBI bidirectional vectors, expression is driven by constitutively active human cytomegalovirus promoters (PminCMV). The gene(s) of interest must contain an initiation codon and a stop codon (Clontech Protocol no. PT4440-5).

Origin & function

The gag gene from Maedi-visna virus. No handling of Maedi-visna will take part at the AHVLA - expression vectors for the gag polyprotein (pN3-GAG) will be acquired from Department of Veterinary Medicine, University of Cambridge. The Matrix, Core, and nucleocapsid constituents of the gag gene will be cloned into the appropriate expression vectors as described above.

The gag gene is being supplied in the pN3 vector (Reina et al 2008 Vaccine 26, 4494). The gag gene is from the EV1 strain of Maedi visna virus (GenBank:S51392.1 bases 314-1848). The full length gene will be PCR amplified with appropriate restriction enzyme overhangs to be cloned into the pCI and pIRES-puro3 vectors.

The individual sequences for the Matrix (nucleotide 506-937), Core (nucleotide 938-1594) and Nucleocapsid (nucleotide 1595-1849) (nucleotide positions from Sargan et al., 1991) constituents will also be PCR amplified with the insertion of peptide tags for ease of identification once expressed in the mammalian cell lines (Matrix-Myc tag, Core-HA tag, Nucleocapsid-VSV-G tag).

Additional sequence added

Myc tag - 5' GAACAAAAACTTATTTCTGAAGAAGATCTG
HA tag - 5' TACCCATACGACGTCCCAGACTACGCT
VSV-G tag - 5' TACACTGATATCGAAATGAACCGCCTGGGTAAG

Expression vectors will be produced with the peptide tags on either the N or C terminal (2 expression vectors for each component).

Evolution of foreseeable effects

Published evidence suggests that the co-infection of animals or cells with Maedi visna virus increases the sensitivity of the organism to scrapie infection (Salazar et al.,...
Further to this expression of the gag gene from HIV increases the sensitivity of cell lines to CWD (a prion disease infecting cervids) (Bian et al., 2010). It is proposed that the co-expression of the gaga gene from Maedi visna virus in cell lines currently permissive to scrapie infection will increase their sensitivity. Additionally it is hoped that expression of the gag gene in cells expressing bovine PrP will render them susceptible to infection with BSE. By expressing the 3 major components of the Maedi visna virus gag gene separately it may be possible to elucidate whether just one of these components is required for increased sensitivity or whether it is a co-ordinated effect of all the products of the gag gene.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste generated in R66, B64 will be autoclaved at 136°C, for a minimum of 1 hour prior to incineration.

Solid waste generated in R26, B63, which may include TSE contaminated waste, will be placed in autoclave bags, loosely sealed then placed in autoclave tins sealed with tape and taken to B150 for autoclaving for 30 mins at 136°C, 2.2 bar. Use of autoclave in building other than where work carried out agreed with HSE (letter 26 September 2006 Ref. GM221). This autoclaved waste is then incinerated.

Liquid waste generated in R66, B63 will be autoclaved 136°C for a minimum of 1 hour and then incinerated if contained in single use plasticware or disposed of to drains if containers are recycled, maximum volume 200ml per bottle.

Liquid waste generated in R26, B63 is placed in a glass bottle in a solution of Chloros (final concentration 20%) and left in the safety cabinet overnight (minimum 16 hours). This waste is then transferred to a dedicated liquid waste plastic container. When the container is at 50% capacity it is sealed, placed in a heavy duty black sack and placed in the dedicated liquid waste pick-up bin located in the locked gas cylinder storage cage at the side entrance to B107. It is collected from this point and incinerated.

**Is an emergency plan required according to regulation 20?**  

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This project has been approved by the AHVLA GMBA Committee

**Project Containment**
Purposes of the contained use

Salmonella enterica is a zoonotic enteric pathogen of warm blooded animals that is a major cause of food poisoning and typhoid fever in the developing world. Subspecies enterica serovars are frequently isolated from livestock production in the UK. Some serovars are isolated from a particular subset of livestock species. S. Mbandaka an emerging serovar, is commonly isolated from cattle, chickens and soya based animal feed. This distribution is very different to that of S. Derby which is predominantly isolated from pigs and turkeys (AHVLA2010). These trends have been maintained over decades and between continents. Thus this would suggest that the mechanisms of persistence and colonisation are pathogen specific and may be an attribute of the interaction between the host and pathogen. It is the aim of this study to better understand the bacterial mechanisms that maintain these distributions.

Comparative functional genomics and phenotypic analysis has shown that S. Mbandaka possesses various adaptations for persistence in the environment, including biofilm formation at ambient temperatures, adhesion to abiotic surfaces and the ability to utilise a wider range of metabolites. Whereas, S. Derby of porcine origin has been shown to be highly invasive and have a higher level of antibiotic resistance and the ability to produce mannose binding type 1 fimbriae at 37°C.

Furthermore, a new genomic island has recently been discovered at the AHVLA in S. Derby, Salmonella Pathogenicity Islands 23 (SPI-23), comprising of 42 open reading frames (ORFs) of which 16 are unique to S. Derby amongst S. enterica SPI-23 contains several type IV pilin genes and four potential effector proteins. In addition five genes are absent from Salmonella pathogenicity island 1 (SPI-1) of S. Derby, one of which has been identified as a novel type three secretion system effector protein.
The studies outlined in this RA aim to further characterise SPI-23 and the unique SPI-1 region through knock-in and knock-out mutants in isolates of both serovars. Introduction and removal of DNA at specific locations will be achieved through allelic exchange (Datsenko and Wanner 2000) using GeneBridges technology. The phenotypic effects of these mutations will be studied in in vitro assays including porcine jejunum (IPECs-J2) cell culture and in vitro organ cell culture (IVOC) to assess the contribution of SPI-23 and SPI-1 to pathogenesis. Expression analysis in the knock-in mutants will also be tested by reverse transcription quantitative real time PCR, to compare mutant expression levels with wild-type isolates containing SPI-23.

Recipient of the plasmid constructs and hosts of targeted mutagenesis are wild-type Salmonella enterica serovars Derby D1 and D2 and Mbandaka M1 and M2. E. coli K-12 derivatives will be used as hosts for plasmid constructs containing SPI-1 and SPI-23.

TOPO TA cloning dual promoter kit (Invitrogen) will be used to produce a non-mobilisable plasmid containing all SPI-23 genes under the control of a T7 and SP6 promoter. The plasmid contains kanamycin and lacZ blue/white screening systems, to select for and maintain transformed isolates.

Through bioinformatical analyses it is believed that SPI-23 is a putative pathogenicity island, with type 4 pili, used in adhesion and type three secretion system effector proteins used during invasion and intracellular survival. In the same study a region of SPI-1 has been identified as containing a putative type three effector protein (Hayward et al., unpublished).

Evaluation of foreseeable effects

Knock-out mutants of SPI-1 and SPI-23 will most likely be less pathogenic compared to wild-type, unmodified Salmonella enterica. Whereas SPI-23 knock-in mutants of S. Mbandaka will be potentially more pathogenic than unmodified environmental isolates. SPI-23 naturally occurs in S.Derby isolates and will be introduced into avirulent isolates of S. Derby D4, D6, D8 lacking the island. The SPI-1 region from S. Mbandaka being introduced into S. Derby is naturally occurring in the closely related non-typhoidal (ACDP category 2) serovar S. Cholerausuis (Chiu et al., 2005). All experiments involving these organisms will be conducted in line with safety regulations relevant for dealing with ACDP hazard group 2 bacterial species (refer to risk assessment BAC2/1 and BAC2/127). All individuals entering the Containment level 2 laboratory where the strains are stored must wear laboratory coats and must wash hands with anti-bacterial soap before exiting the laboratory. Laboratory benches are sprayed down with 70% ethanol after each use. To avoid aerosol production, air conditioning is shut off before isolates are handled in a class II microbiological safety cabinet (MSC).

Disposal by autoclaving in line with current risk assessments (BAC2/4). Autoclaving is carried out in the same building (a requirement of GM Class II) for one hour at 134°C (30 PSI). All solid waste is subsequently incinerated on site.
Disposal by autoclaving in line with current risk assessments (BAC2/4). Autoclaving is carried out in the building (a requirement of GM Class II) for one hour at 134°C (30 PSI) after which liquid waste is tipped down the sink.

Disposal by incineration on site in line with current risk assessments (BAC2/4)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the AHVLA Biological Safety Committee

**Project Containment**

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<th>Growth Rooms</th>
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Animal Units

- L2 L3 L4 L2 L3 L4

Large Scale Activities

- L2 L3 L4 L2 L3 L4

Human Clinical Applications

- L2 L3 L4 L2 L3 L4

**Project Ref** 221/13.1

**Date Ackn’d** 21/02/2013

**CU2 Project Title** Schmallenberg virus (German strain) pathogenesis in vivo using a reverse genetics rescued virus

**Class** Class 2

**CultureVol Class 2** < 1 Litre

**CultureVolume Class 3-4**

- Non-GMM Consent Granted

- Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

02/03/2022 Page 5272 of 15326
### Project Additional Information

#### Purposes of the contained use

The goal of the study using a rescued recombinant virus, offered from St Andrews University, to assess the pathogenicity of a viral clone compared to the genetically heterogeneous material currently used for in vivo experiments in sheep.

#### Recipient or parental organism

The parental virus is a bovine field isolate of SBV from Germany that was rescued at the Univ of St Andrews as described recently (Elliott, R. Bakqori, G. van Knippenberg, I., Koudriakova, E., Li, P. McLees, A. Shi, X and Szemiel, A. M. Establishment of a reverse genetic system for Schmallenberg virus, a newly emerged orthobunyavirus in Europe. J Gen Virol Published ahead of print December 19, 2012, doi:10.1099/vir.0.049981-0). Briefly the rescued virus is genetically identical to the parental strain used.

#### Host/vector system

Due to the fact that we will obtain the rescued virus from collaborators, there is no host/vector system to be used in this study at AHVLA.

#### Origin & function

The genetic material used is a full-length virus. One segment of the genome encodes the nucleocapsid protein, one the viral polymerase and one the glycoproteins.

#### Evaluation of foreseeable effects

The hypothesis is that individual variants of the rescued virus will be equally or less pathogenic than the mix of quasi-species generated by in vivo passage of the wild type only. Based on its genetic identity with the parental strain we presume that it will behave similarly, if not identical to the wildtype virus.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n.a

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The virus has not been classified as SAP or ACDP. Based on current knowledge and failed attempts to demonstrate Abs in humans (Ducomble T, Wilking H, Stark, Takla A, Askar M, Schaade L, et al. Lack of evidence for Schmallenberg virus infection in highly exposed persons, Germany, 2012. Emerg Infect Dis. 2012 Aug. http://dx.doi.org/10.3201/eid1808.120533)SBV remains ACDP unclassified. CL2 has been proposed for GM work with such non-zoonotic Orthobunyaviruses before. However, we will utilise negative pressure, sealable containment facilities with insectocutors, to minimise any vector (midge) associated risks.
Due to the identity of the recombinant virus with its parental strain, we will treat material possibly containing the GMO identically. In brief, solid waste such as animal tissues, animal waste and bedding is collected and ultimately incinerated. Bagged waste will be decontaminated with FAM30 (1:240) or 1% Virkon (contact time 30 minutes) before transfer to tins and subsequent autoclaving (134°C for a minimum of 30min at 30 psi.) or incineration. Liquid effluent from animal facilities will be subjected to heat treatment (effluent is treated by heating to a minimum of 80° C for two hours before release). Smaller volumes from the laboratory will be autoclaved as described above.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the AHVLA Biological Safety Committee

Project Containment

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Project Ref 221/13.2

Date Ackn'd 31/05/2013

CU2 Project Title Recombinant knockout Mycobacterium bovis BCG

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

Mycobacterium bovis is the causative agent of bovine tuberculosis in cattle. Our aim is to vaccinate groups of cattle with laboratory developed genetically modified knockout strains of Mycobacterium BCG and to evaluate whether they are more protective than unmodified BCG against M. bovis challenge. In these strains, the inactivation of the gene will have been achieved either by allelic exchange using homologous recombination or by transposon mutagenesis.

The construction of the Mycobacterium bovis BCG knockout strains will not be done at AHVLA. The strains to be used will be supplied ready for injection by our collaborators.

#### Recipient or parental organism

The recipient of the BCG knockout strain is the cow.

#### Host/vector system

The host is the cow and the vector is a Mycobacterium bovis BCG knockout strain.

#### Origin & function

For knockout strains generated by homologous recombination, mycobacterial genomic DNA such as that prepared from BCG, M. bovis or M. tuberculosis are used as templates for the generation of PCR products. For those generated by transposon mutagenesis, the transposon is delivered into the chromosome of BCG by the temperature sensitive, kanamycin resistance expressing phage MycoMarT7.

The BCG knockout strain will be introduced into cows to induce an immune response that protects them against challenge by virulent M. bovis.

#### Evaluation of foreseeable effects

BCG is an attenuated strain that has been used as a live vaccine against TB in humans and cattle for over 80 years. It has been shown to be safe both in immunocompetent humans and cattle and produces no detrimental side effects. BCG can, however, cause a disseminated disease ('BCG-osis'), which is often fatal, in severely immunocompromised humans, such as those infected with HIV. As a precaution, pregnant women must not be allowed to work with BCG, although no harmful effects have been observed on the foetus where vaccination has been given.

The original M. bovis BCG was first used in 1921 and was derived from virulent M. bovis by serial passages on potato skins. Today, several substrains exist (e.g. BCG Danish, Tokyo, Pasteur) that differ in regions of genomic deletions, antigen expression levels, immunogenicity, and protective efficacy. A single 9.5 kb deletion, known as RD1, common to all substrains and is thought to be primarily responsible for their attenuation. The possibility of reversion of BCG to a virulent phenotype is considered unlikely as the RD1 mutation is a large multi gene deletion and the organism shows very low rates of lateral gene transfer. In addition, complementation of BCG with a clone containing the RD1 region does not fully restore virulence to that of the parent M. bovis, implying that other recombination events are required for restoration of full virulence. There are no recorded instances where BCG has undergone reversion to virulence.
The BCG knockout strains that will be tested are expected to have the same or lower level of virulence than that of non-recombinant BCG. There are no recorded instances where deletion of a gene(s) in BCG has led to an increase in virulence.

It is intended to vaccinate cattle (DBT R23). No detrimental effects are foreseeable in the vaccinated animals as the BCG knockout strains are predicted to have the same or lower levels of virulence than non-recombinant BCG. It is however expected and intended that the knock out strain may give a greater level of protection than BCG, or if protection is equal or lower than wild-type BCG that it will impart other beneficial effects such as the avoidance of skin test reactivity which would be of major benefit as it would allow continuation of tuberculin-based test and slaughter policies alongside vaccination. Vaccine efficacy will be tested by the application of appropriate immunological and microbiological techniques. Again, neither the immune responses generated nor the sampling schedule are expected to have detrimental effects on the animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The CL2 large animal accommodation, such as buildings 417, 418 or 419 at Coomnlands farm have good ventilation and a barrier control system to prevent access to rodents and other small animals. Access to the building is controlled through a swipe card system.

When applied via the subcutaneous route, our data demonstrated that cattle did not shed BCG (please see appendix 1 on the accompanying GMRA) Once inoculated, the vaccinated animals will restrict bacteria within their own lymphatic system; this makes the chance of excretion into the environment negligible (see RA 23). However, to provide for the unlikely event that during the inoculation procedure BCG would be released all solid waste including PPE waste (disposable overall, boots, gloves, face masks, needles, syringes unused inocula etc) will be bagged and incinerated immediately after inoculation as will be bedding during the first 24 h post-vaccination. Waste generated during the 24 h post vaccination period, will be classed as low level waste and disposed according to local ASU SOPs. At the end of the experiment, the animals will be incinerated.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste:
Laboratory waste is contained within a closed metal tin and transported to an autoclave with the aid of a trolley. The service lift must be used to transport waste from the CL2 laboratory located on the ground floor of building 7 to the autoclave located on the first floor. Decontamination is conducted by autoclaving for 1 hour at 135°C after which waste is put into bags for incineration.

Animal accomodation: Needles and syringes, to be disposed of into sharps container, which is then placed into plastic bag, which is then decontaminated with FAM30 (1:20), left for at least 1h and then removed for incineration.

Liquid waste:
Laboratory waste is contained within a closed metal tin and transported to an autoclave with the aid of a trolley. The service lift must be used to transport waste from the CL2 laboratory located on the ground floor of building 7 to the autoclave located on the first floor. Decontamination is performed by autoclaving for 1 hour at 135°C after which waste is put into bags for incineration. Waste occcurring during the inoculations (left-over inoculi) to be double-bagged and incinerated.

Liquid waste in animal contaminated material, see above). Liquid waste after vaccination: see section 4.2.

General waste:
Laboratory waste is transported to an autoclave within the building in a closed metal tin. Decontamination is performed by autoclaving for 1 hour at 135°C after which waste is put into bags for incineration.

Waste associated with animal work: place into appropriate plastic bag, spray/dunk into FAM30 solution (1:20) and after at least 1 hour contact time remove for incineration.
This project assessment has been approved by the AHVLA Biological Safety Committee

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

Laboratory Activities

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<th>Glass Houses</th>
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Animal Units

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Project Ref 221/13.3

Date Ackn'd 13/06/2013

Date Project Ceased

CU2 Project Title

Cloning and expression of plasmid-borne genes for expression studies investigating RNA interference by antisense oligonucleotides

Class 2

Consent Granted

Class Culture Volume Class 2 Culture Volume Class 3-4

< 1 Litre

Non-GMM

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Tick if notifying a connected programme of work

Project notified under transitional arrangements

02/03/2022
### Project Additional Information

#### Purposes of the contained use

Synthetic antisense oligonucleotides (SAO) will be generated and used to silence the expression of target genes in the first instance a beta-lactamase. These experiments will be conducted in either bacteria (the GMO) transformed with a recombinant plasmid or in a cell free expression system using this recombinant plasmid.

#### Recipient or parental organism

The parental organism will be an E. coli bearing a plasmidic CTX-M beta-lactamase gene. The parental strain will have been isolated from veterinary sources (e.g. cattle) from field studies of a UK farm.

#### Host/vector system

A range of hosts may be used including:

1. E. coli K12, DH5α
2. AS19 (a derivative of strain B)
3. BL21
4. BK21 (DE3)
5. E. coliDH10b (F-mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 end A1 araD139 Δ(ara, leu)7697 galU galK λ-rpsL nupG
6. Commercially available strains such as One Shot ® TOP10 (Invitrogen)

Vector:
Commercially available expression/cloning vectors such as pUC11, pUC18/19, pMQAK or similar will be used.

#### Origin & function

PCR products amplified from E. coli strains bearing beta-lactamase plasmids which will include the sequence of the gene (CTX-M beta lactamase) of interest as well as the untranslated region immediately upstream of the start codon (5'UTR) and in some instances the promoter of the target gene may also be included.

A short sequence ~ 200bp from around 50bp upstream of the CTX-M gene and a luciferase or GFP reporter gene will either be commercially synthesised or amplified by PCR and inserted into a suitable plasmid vector.

#### Evaluation of foreseeable effects

Synthetic Antisence Oligonucleotides (SAOs ) will be directed towards the gene of interest (CTX-M) and associated sequence, and will be only homologous with target genes, thus reducing the risk of off-target effects in both bacterial cells and humans. The SAOs are comprised of a nucleic acid base with a modified backbone structure which is resistant to immediate degradation. The safety of SAO use in humans has been demonstrated, they will eventually undergo enzymatic degradation. Presence of bacterial containing CTX-M genes are common in farm animals and as such if plasmid containing a CTX-M gene were released into the environment it would not pose a health risk above that which already exists. Bacteria containing the CTX-M gene alone are treatable with non-penicillin antibiotics.

All experiments using these organisms will be conducted in CL2 laboratories, in line with safety regulations for ACDP Hazard group 2 organisms. Appropriate PPE (Lab coats and gloves) will be worn at all times. A class II safety cabinet will be used for all aerosol generating activities the risk of aerosol spread.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposal of bacterial waste will be inactivated using local procedures (autoclaving and incineration). Waste will be disposed of by autoclaving (70 minutes at 134° and 15 psi. Building 4; room 2) and incineration in line with current risk assessments (BAC2/4); All solid waste is subsequently incinerated on site.

Disposal of liquid waste is by autoclaving and incineration in line with current risk assessments (BAC2/4); 70 minutes at 134°C, 15 psi. Autoclaving using dry cycle for up to 100 ml volumes of liquid cycle for 500ml volumes (validated by probe). Liquid waste will be inactivated with an equal volume of Virkon S (1% w/v; contct time > 30 min) prior to autoclaving.

**Project Containment**

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**Animal Units**

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**Project Ref** 221/13.4

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<td>29/08/2013</td>
<td>Application of reverse genetics technologies to study effective vaccination in pigs and ferrets and the molecular markers for pathogenesis, host tropisms, transmission and antigenicity in mammalian and avian influenza A viruses</td>
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Date Project Ceased 02/03/2022

This project assessment has been approved by the AHVLA Biological Safety Committee.
**Purposes of the contained use**

We propose to use reverse genetics techniques to manipulate the influenza genome to create novel viruses altered specifically in one or more genes. This will also include whole gene replacements. Subsequent in vitro & in vivo infections will demonstrate the effect of specified genetic changes on viral phenotype, eg pathogenicity and host adaptation. The effectiveness of vaccine construct generated by reverse genetics will also be studied in pigs and ferrets.

**Recipient or parental organism**

Influenza A virus

**Host/vector system**

**Host:**
1. E. coli K12 derivative strains will be used for the transformation and amplification of plasmid DNA. Examples are DH5 alpha derived (e.g. NEB 5 alpha or similar) or DH10 beta derived (e.g. TOP10 or similar) or similar strains.
2. Human 293 T cell line, other eukaryotic cell cultures eg MDCK, BHK, Vero, chick embryo fibroblasts (CEFs), chick embryo liver (CEL) cells and swine cell cultures (eg NPTr, NSK).
3. Nine to eleven day old embryonated fowls’ eggs.
4. The recovered viruses will be used to infect mammalian, including pigs and ferrets and possibly avian hosts, including chickens, turkeys and ducks for in vivo studies. This will also include ex vivo organ explants (e.g. trachea, bronchi, lung, turbinates)

**Vector:**
1. pCR-XL-TOPO (Invitrogen).
2. Poll vectors (derivatives of the pcDNA3 vector, non-mobilisable, Invitrogen) and Helper plasmids (with CMV driven promoter to express the 4 polymerase proteins and NP from the A/Victoria/75 H3N2 virus, Invitrogen) - 12 plasmids system (Howard et al., 2007).
3. pHW2006; pHW2000 (with CMV driven promoter) - eight plasmids system (Hoffmann et al., 2000b)

**Origin & function**

All genetic material will be derived from influenza A virus strains. The genetic material will be used to generate infectious viral progeny by reverse genetics. The progeny viruses will then be studied for altered phenotype in in vivo and in vitro models.

All eight full-length (890-2341bp) genes of the influenza genome:
1. Polymerase basic 2 (PB2) (Internal protein)
2. Polymerase basic 1 (PB2) (Internal protein)
3. Polymerase acidic (PA) (Internal protein)
4. Haemagglutinin (HA) (Surface protein)
5. Nucleoprotein (NP) (Internal protein)
6. Neuraminidase (NA) (Surface protein)
7. Matrix (M) (Internal protein)
8. Non-structural gene (NS) (Internal protein)

Viruses will be generated where whole genes or point mutations will be derived from Influenza A virus strains such as:

1. Seasonal Human Influenza isolates - ACDP2/SAPO unclassified
   - H1N1 pandemic
   - H3N2 subtype

2. Ancestral Human Influenza Isolate - ACDP2/SAPO unclassified
   - A/Puerto Rico/8/34 (PR8) H1N1 [commonly used as the virus backbone in RG (reverse genetics) derived vaccines]
   - Genes of the polymerase complex from A/Victoria/75 (human H3N2 isolate) (to provide helper function in the 12 plasmid reverse genetics system)

3. Swine Influenza isolates
   a) Endemic Strains - ACDP2/SAPO unclassified - Avian-like H1N1 subtype
      - H1N1 pandemic
      - Classical H1N1 subtype
      - H1N2 subtype
      - H3N2 subtype
   b) Natural /wild type reassortant strains - ACDP2 or advisory 3/SAPO unclassified
      - A(H1N1)pdm/09 reassortant viruses e.g. A(H1N1)pdm/09 & H1N2, A(H1N1)pdm/09 & H1N2 TRIG, A(H1N1)pdm/09 & avian-like H1N1 A(H1N1)pdm/09 & H3N2 and A(H1N1)pdm/09 & H3N2 TRIG or equivalent viruses.

In some cases, viruses generated by reverse genetics procedures will be supplied to the AHVLA by collaborators. These are likely to include:
- H9N2 & A(H1N1)pdm/09 reassortant viruses e.g. Imperial College London, NIMR, HPA Colindale, NISBC.
- A(H1N1)pdm/09 & H3N2 reassortant viruses ACDP2/SAPO unclassified Kansas State University (Qiao et al., 2012)
- A(H1N1)pdm/09 & H3N2 reassortant viruses ACDP2/SAPO unclassified e.g. Imperial College London, NIMR, HPA Colindale, NISBC,
- PR8 internal with different HA-NA (e.g. H1, H2, H3, H5*, H7*, H9, N1, N2, N3, N7, N9) combinations-routine vaccine candidates. These viruses, and the plasmids encoding the genes of these viruses, exist in many CL2 labs worldwide e.g. UK - Imperial College London, NIMR, HPA Colindale, NIBSC - GMO2/3.
  * Including viruses where the multi-basic cleavage site has been removed to render them equivalent to low pathogenicity avian influenza viruses.

**Evaluation of foreseeable effects**

The reverse genetics system will be used to produce a range of infectious mutants of diverse pathogenicities which will result in viruses that may have altered (i.e. increased, equivalent or reduced) virulence. The mutations to be introduced using the system include those observed in viruses isolated from the field and known to affect infectivity and/or pathogenesis in mammals including humans. Therefore the production of these mutants will allow further understanding on the role of mutations essential for promoting infection and transmission in livestock and humans, the latter using the pig and the ferret as human model of disease. The generation of re-assortant viruses including genes from different species will be representative of potential field re-assortant viruses [e.g. A(H1N1)pdm/09], promoting a better understanding on the role of the pig as a potential mixing vessel and the ability of the virus to cross the species barrier. There is no intention to produce avian and mammalian influenza chimeric viruses similar to those produced and published from the labs of Kawoaka (Imai et al., 2012) and Fouchier (Herfst et al., 2012).

No genetic sequences of SAPO4 influenza viruses will be used to generate organisms by reverse genetics.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

1. All waste, solid contaminated materials, cultures & disposables produced in the designated SAPO unclassified/ACDP2 laboratory is sealed in autoclave bags, decontaminated by spraying with 1% Virkon S (contact time of 10 min), contained in an autoclave tin and autoclaved at 134°C for 60 minutes before incineration.

2. All manipulations with GMOs viruses that are also classified as advisory ACDP3 viruses will be done in ACDP3/SAPO3 or SAPO 4 facilities that have regular inspections by the HSE. Waste disposal from these facilities is strictly controlled in accordance with SAPO the main features are as follows:
   a) Effluent is sterilised by a procedure known to kill the pathogen, heat treatment for 95°C for 2 hours, and this procedure is confirmed as having operated satisfactorily before the effluent is discharged to the public sewer. This includes all liquid waste from the building eg from sinks, toilets, showers.
   b) All biological waste material is sterilised at 134°C for 60 minutes before removal from the containment facility through a double ended autoclave. The functioning of autoclaves is monitored to ensure that time/temperature cycles are completed. The load is then taken immediately to an incinerator on site.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

The project assessment has been approved by the AHVLA Biological Safety Cabinet.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### Project Ref 221/13.5

Date Ackn'd 02/03/2022  
CU2 Project Title  
Class  
CultureVolClass2  
CultureVolumeClass3-4
The aim of this work is to prepare DNA libraries and screen them for expressed functions (e.g. antibiotic resistance or production of antimicrobial compounds).

The libraries will be prepared from DNA extracts of the total microbial content (the metagenome) of various sample types, e.g. faeces, and saliva, that may be of human and animal origin; soil, water and other environmental sites. The resulting clones will therefore contain different fragments of the metagenomic DNA and represent the microorganisms present in the sample. There is a very remote chance of animal or human DNA being cloned into the vectors but any such clones will become apparent upon sequencing, following functional screens. However, it is important to stress that the eukaryotic protein have a low probability of being expressed in bacterial systems due to differences in codon usage and expression.

The library created in this manner will be employed in activity-based screens. The library will be transformed into a heterologous host, e.g. commercially available E. coli cells (e.g. EPI300; Epicentre) or a Gram-positive bacteria of ACDP hazard group 1 or 2 e.g. Streptomyces lividans (Courtois et al, 2003) or Bacillus subtilis (Kaneko et al, 2005) or Straphylococcus aureus (Williams et al, 2006) provided by collaborators e.g. University College London. The transformants will then be cultured under selective pressure to screen for a desired biological activity (phenotype). For example, libraries can be screened for the ability to grow in the presence of antibiotics or the ability to inhibit the growth of an indicator organism. Clones with the desired phenotypes will be recovered, propagated and the insert DNA sequenced to determine the underlying genetic mechanism responsible for antibiotic resistance or inhibition.

The advantage of this activity-based screening approach is that functional genes can be recovered without prior knowledge of their sequence. Consequently, the technique can enable the identification of new genes and it has been successfully employed to identify a range of novel enzymes (for a review see Ferrer et al., 2009), including antibiotic resistance genes (Sommer et al., 2009). Drawbacks of this approach are that it requires that the cloned genes are expressed and the gene products active in the heterologous host, and factors such as codon usage, promoter sequence and interactions with other proteins can all influence the recovery of clones.

Recipient or parental organism

Laboratory adapted E. coli strains:
Commercially available E. coli K12 derivative strains such as EPI300, JM109, TOP10, or DH5a (available from e.g. Epicentrel, Promega and Invitrogen). These strains are routinely used in relevant laboratories at the AHVLA.
Gram-positive bacteria (ACDP hazard group 1 or 2): e.g. Streptomyces lividans, Bacillus subtilis or Staphylococcus aureus.

Both gram-negative and -positive strains will be employed as heterologous hosts, to enable activity-based screening in these two main bacterial divisions. The E. coli K12 hosts allows stable propagation of the recombinant plasmids and, depending upon the vector, inducible expression of cloned genes. K12 derived E. coli are recA, endA deficient and considered 'disabled' (i.e. non-pathogen). They are amongst frequently used bacterial host and are considered to have a good safety profile (anonymous, 2007). These organisms do not possess or express known virulence factors (Muhldorfer et al., 1996; Muhldorfer and Hacker, 1994; Kuhnert et al., 1997’ Bachmann et al., 1996). The E. coli strains used in this work have a long history of safe use and can be safely handled at containment level 1 (Commander, 2008; Anonymous, 2008).

EPI300 cells (Epicentre) will be used as the copy-number of clones with an oriV origin of replication is under the tight control of an inducible promoter linked to the trfA gene, making them ideal for use with the cloning vector pCC1BAC (see3.2.3 below)

EPI300 genotype: F- mcrA Δ(mrr-hsdRMS-mcrBC) Ф80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara, leu)7697 galU galK l- rpsL (StrT) nupG trfA dhfr.
Gram-positive heterologous hosts will be strains of Streptomyces lividans, Bacillus subtilis or Staphylococcus aureus (Courtois et al, 2003; Kaneko et al, 2005; Williams et al, 2006). These will be provided by collaborators e.g. University College London. The Gram-positive strains were derived from the John Innes Culture Collection or are characterised laboratory strains that have been previously published (Kaneko et al, 2005; Williams et al, 2006). They are well characterised, ADP hazard group 1 or 2 (ibid).

Libraries will be created in a several different vectors according to experimental needs, specific examples include:

- pCC1BAC (Epicentre); chloramphenicol resistance selectable marker (see Epicentre link in references).
- pUC18 (many suppliers, e.g. Thermoscientific); ampicillin resistance selectable marker. (see Thermoscientific link in references).
- pUC19 (many suppliers, e.g Thermoscientific); ampicillin resistance selectable marker. (see Thermoscientific link in references).
- pZE21 MCS 1 vector (Expessys); kanamycin resistance selectable marker. (see Expressys link in references).
- pBEST310 (Kaneko et al, 2005; laboratory collection); neomycin resistance selectable marker. (see publication details in references).
- pLEW6832 (Williams et al, 2006; laboratory collection); ampicillin, streptomycin selectable marker. (see publication details in references).

Other vectors may be employed if experimental need dictates. These will be selected from the list of non-mobilisable or mobilisation defective plasmid vetors given in the guidance documents "The generation and use of GM E. coli (K12 or B strain derivatives) for the purpose of recombinant protein production" that is maintained by the SHaW unit on livelink (http://vla43/index/business/safety-intro/safety-gmos.htm).

Origin & function

The nucleic acid insert used for library preparation will be derived from the metagenome (i.e. the total DNA content of a microbial population) of various sample types, e.g. faeces, saliva, and soil. Samples from both humans and animals may be employed in this study. All samples, before usage will be assessed for their metadata i.e. any accompanying information on the health of animal, farm etc. to ensure low risk/hazard samples are used.

Nucleic acid extracts will not be prepared form samples that have been demonstrated to contain ACDP hazard group 3 or 4 organisms.

The cloned genetic material will be used in activity-based screens in which a selection for phenotypes of interest (see section 2.1) will be undertaken. Clones with the desired phenotypes will be recovered, propagated and the insert DNA sequenced to determine the underlying genetic determinant responsible.

The E. coli heterologous hosts selected for this project are disabled strains requiring precise culture conditions for efficient propagation and survival. Thes E. coli are normally designated as containment level 1 organisms (Anonymous, 2007; Commander, 2008; Anonymous, 2008)

Evaluation of foreseeable effects

The Gram-positive heterologous hosts selected for this project are designated as containment level 1 or 2 organisms (Courtois et al, 2003, Kaneko et al, 2005, Williams et al, 2006))
The vectors selected for this work are considered to be non-mobilisable. pZE21 MCS 1 and pBEST310/pLEW6932 contain the necessary promoters and regulatory sequences for expression of the inserted nucleic acid, therefore in these organisms the transcript may be translated to produce a functional polypeptide. The transformation of the vectors employed, when they do not contain an insert DNA sequence, into the heterologous hosts used in this study is not considered to change the host cell ACDP hazard group, as they do not alter the virulence and/or pathogenicity of the host cells. Therefore E. coli transformed with an empty vector will remain at ACDP hazard group 1 and the Gram-positive heterologous hosts (B. subtilis, Saureus, Slividans) will remain at ACDP hazard group 1 or 2. As with all DNA libraries, the exact nature of the nucleic acid insert of each clone cannot be defined at the start of the experiment. Only clones that successfully pass through the function-based screening will be retained for further study. Any clone studied extensively will be characterised by sequencing of the cloned DNA. It should be noted that the creation of DNA libraries is a standard molecular biological technique (Sambrook and Russell, 2006) that has been undertaken safely for many decades. To minimise the potential for inadvertently creating pathogenic clones, no metagenomes from samples known to contain ACDP hazard group 3 or 4 organisms will be used in this work. Additionally, any clone revealed to contain high risk pathogenic gene post sequencing (e.g. the shiga toxin gene) will be transferred to CL3 facilities, in accordance with local procedures (see RA BA2/001).

The GM organisms produced in the work covered by this risk assessment are considered to be ACDP hazard group 2 and GM class 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Solid contaminated materials: cultures, disposables, etc: Disposal by autoclaving (in an autoclave bag in a sealed tin) for one hour and 10 minutes at 134°C (Building 4, Room 2 or 36; Building 63 Room 21) and incineration in line with current risk assessments (BAC2/4). All solid waste is bagged and subsequently incinerated on site.

2. Liquid waste: broth cultures, supernatants, etc: Disposal by autoclaving (in an autoclave bag or container/duran in a sealed tin) for one hour and 10 minutes at 134°C (Building 4, Room 2 or 36) after which liquid waste is tipped down the sink, in line with current risk assessment (BAC2/004).

3. General rubbish: Disposal by bagging and subsequent incineration on site in line with local procedures

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the AHVLA Biological Safety Committee

Project Containment

02/03/2022
Continued TB vaccine development: Improving BCG and developing non-sensitising vaccines for cattle

**Project Additional Information**

**Purposes of the contained use**

Development of novel tuberculosis (TB) vaccines protecting humans or animals is a major research priority. Defra has been funding research into bovine TB (Mycobacterium bovis) vaccines for cattle for almost a decade. These include objectives to: develop vaccination strategies that improve the duration of immunity of the BCG vaccine; identify biomarkers of vaccine induced protection; and to screen novel vaccine candidated. As a valuable tool for this means an established mouse model is also utilised to directly inform the cattle model. The full remit of work proposed here is currently funded through Defra ROAME-projects, such as SE3266.

Towards this central goal of vaccine development, one fundamental aspect is understanding the mechanisms of protection induced by the only human TB vaccine BCG, so that an improved vaccine or vaccine strategy can be developed. Here we propose the use of recombinant strains of BCG as a valuable tool to facilitate this.

In mice, our recent data showed that the live BCG vaccine bacilli persist, at a low level and without causing any clinical disease, for at least 16 months (Kaveh, et al., submitted). Following antibiotic abbreviation of these persisting BCG we have observed two distinct mechanism of immunity. One that is independent of, and one dependent on, the presence of persistent BCG. We hypothesis that the first is due to the induction of long term immunological memory by as yet undetermined CD4 T cells; and the second is due to the constant priming of medium term CD4 effector memory T cells which we are currently characterising. One approach to further analyse immunological memory in the absence of persisting BCG is through the use of metabolically limited (auxotrophic) recombinant BCG Knock-Out (KO) strains that, unlike the...
parental strain, are unable to survive in vivo. Another approach is in the identification of the rare T cells that convey long term immunity. Present at very low frequencies, they are typically beyond the limits of detection of conventional analyses. Through the use of adoptively transferred cloned donor T cells all specific for a defined innocuous foreign protein (e.g. Chicken Ovalbumin, OVA), these cells can be accurately tracked throughout an immune response in a normal recipient mouse immunised with a recombinant BCG Knock-in (KI) strain expressing this protein. Other potential areas of investigation, may track the trafficking of BCG bacilli in the vaccinated host, for instance through the use of recombinant BCG KI strains expressing fluorescent proteins (e.g. GFP). Some strains may also represent promising vaccine candidates and require screening in the mouse model.

The recombinant strains have or will be sourced from external laboratories through collaborations. These will be stored and prepared for immunisation in Bovine TB Research Containment level 2 (CL2) laboratory. Mice will be immunised and housed in high security animal accommodation, cared for by Animal Services staff (ASU), and maintained at CL2. Tissue samples will be transferred to the Bovine TB research CL2 laboratory for immunological or bacteriological analysis.

Recipient or parental organism

The recipient organisms is Mycobacterium bovis bacille Calmette-Guerin (BCG) The construction of recombinant BCG strains will not be done at AHVLA. The strains to be used will only be supplied by our collaborations in UK, EU and North American institutions ready for in vitro culture and immunisation. Only strains that have already been evaluated by external researchers will be used who must supply confirmation that their hazard group is no greater than HG2. No strains that have a known or likely increase in virulence will be used.

Host/vector system

Laboratory mice (e.g. strains BALB/c and C57BL/6) will be immunised with these BCG strains at AHVLA. Cells from these mice will also be stimulated in vitro with these BCG strains. The mouse is a well-established model for the study of bovine TB. Mouse experiments can be carried out faster, in larger numbers and in a cheaper fashion than in the target species. They also allow for a more in depth analysis due to the extensive reagents, methodology and ease of manipulation. For instance, providing the ability to undertake adoptive transfer and analysis of T cells of defined antigen specificity to study immune memory development. Thus, experiments in mice will inform those in the target species, cattle.

Origin & function

BCG Knock-out (KO) strains

Individual targeted genes will be inactivated in these strains to either:

i) Prevent in vivo metabolism, for example by creating auxotrophs that can only grow with the addition on an exogenous source of a specific nutrient only available in vitro.

ii) Alter the presentation of BCG antigens to the immune system in order to induce a superior protective immune response.

BCG Knock-in (KI) strains

The addition of nucleic acid to allow expression of proteins/peptides that will be limited to harmless additions that do not increase the virulence of the organism. For instance:

i) Innocuous proteins foreign to Mycobacteria used only to evaluate a normal BCG induced immune response by providing the ability to track a distinguishable specific response against this defined surrogate BCG antigen. E.g. Ovalbumin from Chicken, which contains an antigenic peptide epitope which is well characterised and used in mouse immunology studies.

ii) Fluorescent proteins/probes that allow the BCG bacilli to be tracked in vivo. E.g. green fluorescent protein GFP is a fluorescent protein from the jelly fish Aequorea Victoria and is used extensively in research to allow detection of cells.

Recombinant strains may also contain the addition of an antibiotic resistance cassette to allow The in vivo selection of successful recombinants only. These will be limited to
Kanamycin or Hygromycin, which are not used for anti-mycobacterial therapy in humans.

**Evaluation of foreseeable effects**

BCG is an attenuated strain that has been used as a live vaccine against humans and cattle for over 80 years. It has been shown to be safe both in immunocompetent humans, cattle and mice, producing no detrimental side effects. BCG can, however, cause a potentially fatal disseminated disease ('BCG-osis') in severely immunocompromised humans, such as those infected with HIV. The original BCG was first used in 1921 and was derived from virulent M. bovis by serial passages on potato skins. Today, several substrains exist (e.g. BCG Danish, Tokyo, Pasteur) that differ in regions of genomic deletions, antigen expression levels, immunogenicity, and protective efficacy. A single 9.5 kb deletion, known as RD1, is common to all substrains and is thought to be primarily responsible for their attenuation. The possibility of reversion of BCG to a virulent phenotype is considered unlikely as the RD1 mutation is a large multi gene deletion and the organism shows very low rates of lateral gene transfer. In addition, complementation of BCG with a clone containing the RD1 region does not fully restore virulence to that of the parent M. bovis, implying that other recombination events are required for restoration of full virulence. There are no recorded instances where BCG has undergone reversion to virulence.

The recombinant BCG strains that are proposed here are expected to have the same or a lower level of virulence than that of a non-recombinant BCG. There are no recorded instances where deletion of a gene(s) in BCG has led to an increase in virulence. All the proposed alterations to BCG are unlikely to increase the risk of a subsequent hazardous mutation.

Mice vaccinated with recombinant KO or KI BCG will induce no foreseeable detrimental effects. Mice are monitored twice daily as part of their standard husbandry and so any unexpected effects that could be detrimental to the mice, would be observed quickly and the animals euthanised. Auxotrophic KO BCG strains will induce a self-limiting infection on immunisation, as opposed to wild type BCG which has been shown to persist for at least 16 months. This will be confirmed by bacterial culture on first evaluation. Immune responses, evaluated as the main aim of all these studies, will also help identify any potential increase in virulence.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste is placed directly into an autoclavable bag (liquids first in a sealed bottle) and then closed inside an autoclave bin/tin and transferred by trolley to an autoclave located in the building. Waste is autoclaved on a cycle that has been shown to maintain a minimum of 121°C for 1 hour. All autoclaved waste is incinerated as standard for the site. The autoclaves are temperature profiled and calibrated every 6 months to ensure cycle criteria are maintained.

After working with the strains or immunised animals, surfaces are disinfected with 70% ethanol, as internally validated removal of items from the lab/animal room

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the AHVLA Biological Safety Committee
Project Containment

Laboratory Activities

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<thead>
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<th>Culture Vol.</th>
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Glass Houses

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Growth Rooms

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Project Ref 221/15.1

Date Ackn'd

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<td>Evaluation of Immunogenecity against Newcastle disease virus of a double recombinant Herpesvirus of turkeys (HVT) expressing the Fusion Gene from Newcastle disease virus (NDV) and the VP2 Gene from Infectious Bursal Disease Virus (IBDV) in chickens</td>
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Date Project Ceased

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Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To determine the efficacy/duration of immunity in pre-vaccinated chickens of the recombinant herpevirus of turkey after challenge with NDV.

Recipient or parental organism

Young chickens.

Host/vector system
Herpesvirus of turkeys (strain FC-126).

**Origin & function**

The F gene of a non-pathogenic strain of NDV (lentogenic vaccine strain Clone 30) and the VP2 gene of IBDV (strain F52/70) 
The fusion (F) protein in NOV is involved in attachment and entry into host cells and is the major antigen responsible for immunity against NOV infection.

VP2 protein is a major structural protein and the major host protective immunogen of IBDV, containing epitopes responsible for the induction of neutralising antibodies.

**Evaluation of foreseeable effects**

It is recognised that the insertion of foreign genetic material coding for proteins associated with the attachment and entry of virus to host cells into HVT may result in novel pathogenic properties. As NOV is associated with mild conjunctivitis and influenza-like symptoms in man it may therefore possible that the presence of the F protein in the virus envelope could result in transmission to man. HVT however is typically cell associated and, although small quantities of infectious cell-free HVT has been detected in the feather dust of chickens vaccinated with HVT, it is considered unlikely that human infection is possible, although it cannot be categorically discounted.

The VP2 protein does not have features like a signal sequence or transmembrane anchor region such as F from NDV or most of the glycoproteins from herpesvirus and is therefore not expected to be exposed on the cell surface. The risk of hazards arising from alteration of the existing traits of the HVT vector is considered low.

There have never been reports of the recombination of HVT with other related herpesviruses of poultry, e.g. virulent if. (serotype 1) MOV or serotype 2, and the possibility of recombination with a virus capable of infecting humans is therefore considered extremely small. Genetic transfer to other organisms in the environment has never been described for herpesviruses, and is therefore considered to be unlikely.

Chicken to chicken spread has been reported with the parental HVT, however the Sponsor's experience with the recombinant viruses is that chicken to chicken transmission does not occur, although chicken to turkey and turkey spread has been seen. Virus replication (in terms of viraemia and organ isolation) is found in chickens but this is predominantly cell-associated virus, mature cell-free virus is only shed in extremely small quantities from the feather follicles. No adverse reactions have been seen with recombinant vi-uses expressing either the F protein or VP2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not Applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Chickens will be vaccinated with the GMM as 18-day-old embryos at the Sponsor's premises (premises number: GM391) and transported to APHA in transport boxes with integral dust filtration. Transport of birds will be under the control of the Sponsor, by a licensed carrier with adherence to all relevant legislation and animal welfare
considerations. This approach has been approved by the Sponsor's own local GM Committee.
Animal waste. Transport boxes, animal carcasses and bedding will be double bagged, and placed in a steri-bin which will be surface disinfected (1% Virkon S for 10 Minutes) prior to being autoclaved and incinerated. Laboratory waste. Sharps associated with the challenge of birds will be placed in a designated sharps box which will be surface disinfected (1% Virkon S for 10 Minutes) and subsequently autoclaved prior to incineration. Autoclave cycle P4 (134°C for 30 mins) as specified in local SOP ASU202 will achieve a 100% kill of the GMM. Any animal liquid waste will be discharged into the effluent treatment plant where it will be heated and held at a temperature of 96°C for two hours, cooled to 38°C and then discharged to the main drainage system.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the APHA Biological safety Committee.

**Project Containment**

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**Project Ref** 221/18.1

Date Ackn'd 09/08/2018

Date Project Ceased

CU2 Project Title

Use of recombinant bovine herpesvirus4 vectored TB vaccine

Class

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Non-GMM Consent Granted

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

To determine the immunogenicity and/or protective efficacy against bovine tuberculosis (bTB) of recombinant herpesvirus-vectored vaccines expressing mycobacterial antigens in a cattle model of bTB.

**Recipient or parental organism**

Bovine. The cow is the target species for bTB and therefore represents the ideal model for the testing of vaccines against the disease.

**Host/vector system**

BoHV-4. Recombinant BoHV-4 has been shown to be immunogenic and proposed as a vector for veterinary vaccines.

**Origin & function**

Synthetically produced mycobacterial DNA sequences from BCG, M. bovis or M. tuberculosis.

- **Rv0287 (TB9.8):** Esat-6 like protein. May be involved in virulence. Component of esx-3 secretion system. Well recognised by T cells from M. bovis infected cattle. It has been part of previous subunit vaccine that provided protection for vaccinated cattle when used in conjunction with adenovirus-vectored vaccine (Dean et al., 2014).
- **Rv0288 (TB10.4):** Esat-6 like protein. May be involved in virulence, but frequent vaccine target antigen. This antigen is well recognized by bovine T cells post-infection and has been demonstrated to protect in murine models against M. tuberculosis and M. bovis challenge. Component of esx-3 secretion system. It has been part of previous subunit vaccine that provided protection for vaccinated cattle when adenovirus-vectored (Dean et al., 2014).
- **Rv1733:** Unknown function, recognized by T cells from M. bovis infected cattle.
- **Rv2389:** Promotes resuscitation and growth of dormant, non-growing bacteria. Also recognized by T cells from M. bovis infected cattle.
- **Rv3620:** Putative ESAT-6-like protein, recognized by bovine T cells post- M. bovis infection.
- **Rv3804 (Ag85A):** Involved in cell wall mycolation. Required for biogenesis of trehalose dimycolate necessary for maintaining cell wall integrity. Demonstrated as protective antigen in TB animal models, and cattle (e.g. delivered as adenovirally-vectored subunit to boost BCG-primed calves).

**Evaluation of foreseeable effects**

Recombinant protein(s) will be expressed in mammalian hosts.
Due to gene targeted deletion of ORF73, the BoHV-4 vector is unable to establish latency and can only infect animals transiently. In addition to this characteristic, the inserted bTB genes have no known toxic effects. Vaccinated animals as the viruses are non-persistent in vivo. It is however expected and intended that the inserted mycobacterial proteins will be expressed and an antigen-specific immune response generated (i.e. the virus will serve as vaccine delivery system). Vaccine efficacy will be tested by the application of appropriate immunological and microbiological techniques. Again, neither the immune responses generated nor the sampling schedule are expected to have detrimental effects on the animals. A clinical monitoring system will be applied by the animal care and veterinary staff involved in the experiments to detect unforeseen adverse effects (including change in behaviour/demeanour, loss of appetite, increased temperature). The monitoring protocol will be also discussed with the involved staff at the pre-start meetings to be held prior to the start of any experiment.

The risk of infection in humans is minimal based on in vitro analysis of replication in human cell lines (Gillet et al., 2004). The use of GFP-marked wild-type BoHV-4 to screen 21 different human cell types showed that human lymphoid and myeloid cells are not susceptible to infection, whereas epithelial-derived cells are susceptible to virus entry (range: 72% to 7%), but only poorly permissive for replication (range: 3% to 0.1% based on viral gene expression). No human cases of BoHV-4 infection or disease have been reported to date and, as such, no detrimental effects on exposed humans are foreseeable. Exposure monitoring by e.g. serological testing of animal carers etc. is therefore not necessary. Although BoHV4-TB1 is regarded as safe, immunocompromised individuals shall not handle this vector or be exposed to it via vaccinated cattle.

The risk to rodents as possible transmitters of virus in the environment is also minimal based on in vivo experimental infection studies in mice and rats. Following in vivo inoculation of mice with Luc-expressing BoHV-4, the virus was shown to home specifically to the liver with persistent expression of Luc in only the liver and no other organ. Viral DNA was not detected in any organ other than liver following at 2 days post-infection (tested kidney, spleen, lung, heart, blood and lymph nodes). No clinical symptoms and no replicating virus were detected in liver at 7 day post-infection (Franceschi et al., 2014). The inoculation of mice with BoHV-4 vectors expressing pathogen target antigens, from blue tongue virus or monkeypox, was not associated with any overt clinical signs, detrimental effect or pathology (Franceschi et al., 2011, Franceschi et al., 2015). In adult rats inoculated with GFP-labeled BoHV-4, expression of GFP was only detected in liver endothelial cells, but not parenchymal cells. No infectious virus was recovered from blood, spleen or liver confirming the lack of replication in vivo (Donofrio et al., 2006).

Solid Waste:
From laboratory: Place in plastic autoclave bag, double bag in second autoclave bag, spray with 70% ethanol, 10 minutes contact time, then autoclave and incinerate.
Self-life of the 70% ethanol is 1 week.
Autoclave conditions: minimum 15 PSI at 121°C, 30 min.
From animal facilities waste: All solid waste including bedding and PPE must be discarded into a plastic bag which is then decontaminated with FAM30 (1:20) for at least 1 h and removed for incineration. Needles and syringes must be
disposed of into sharps container before bagging and processing as before.

Liquid waste:
From laboratory: No cultures performed. Therefore, liquid laboratory waste will be virus stocks or diluted vaccine preparations. Place containers (plastic tubes or cryovials) into plastic bag inside class II MSC, place bag into second bag, spray outside with 70 % Ethanol (leave for 10 minutes), then remove from cabinet for autoclaving and incineration.

Autoclave conditions: minimum 15 PSI at 121°C, 30 min. Volumes to be autoclaved will be less than 10 mL.

From animal facilities: Liquid waste in animal accommodation occurring during vaccinations will be contained in syringes and disposed as part of solid contaminated material (see section 5.1). To prevent liquid animal waste, contaminating the drains, during the first 14 days after inoculation drains will be blocked and liquid discarded by adsorption into solids (straw or bedding) that will be disposed of as described in section 5.1. At the end of the experiment, animal accommodation will be cleaned with FAM30 (1 :20 diluted), contact time 1 h, including drain covers before drains can be opened again. Water troughs will be drained on straw/bedding and this disposed as solid waste.

General rubbish:
Waste associated with animal work: Place into appropriate plastic bag, spray/dunk into FAM30 solution (1 :20) and after at least 1 hour contact time remove for incineration.

This project assessment has been approved by the APHA Biological Safety Committee.

Please enter comments on the GM safety committee on the risk assessment

This project assessment has been approved by the APHA Biological Safety Committee.

Project Containment

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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Project Ref 221/19.1
Cattle challenge with a transposon mutagenised Mycobacterium bovis library

This is a collaborative research project under the EradbTB funding scheme that includes three institutions: Royal Veterinary College (RVC), London School of Health and Tropical Medicine (LSHTM) and Animal & Plant Health Agency (APHA). SK (RVC) is the overall PI of the project.

The overall goal of this project is to identify the bacterial genetic determinants for survival of Mycobacterium bovis (M. bovis) in cattle in vivo. In order to do this we will use Transposon Directed Insertion Sequencing (TraDIS methodology). This approach, which has been used successfully in other bacteria (i.e. Escherichia coli (Eckert et al., 2011). The M. bovis TraDIS library will be screened in vivo in order to select clones for subsequent in vivo confirmation of in vivo essentiality. A selected number of M. bovis mutants will be constructed through homologous recombination and then validated for their essentiality in cattle in vivo.

This work will allow us to identify the genetic requirements for survival of M. bovis in one of its natural hosts thereby increasing our understanding of the physiological adaptations of M. bovis to the bovine host. Additionally, identification of the genes that are essential for survival in the bovine host will provide an unbiased starting point for the rationale design of new and improved vaccines.

Methodology. The library will be received from colleagues at the RVC and tissues and unused library aliquots will be sent to our colleagues licenced facilities at RVC and LSHTM.

In order to measure gene fitness and to define genes essential for survival in the bovine host, we will challenge cattle with this transposon library by using infection protocols developed and established at the APHA (SOP - BAC0337; all SOP and safety documents referred to in this document are listed at the end of the reference section under their own number). Holstein-Friesian cattle that are 4-6 months old will be infected via the endo-tracheal route; this route results in a lesion distribution that largely reflects the natural infection. The infective dose per animal currently used at APHA in the established protocols is 10,000 CFU, but to allow for screening of as many clones as possible per individual animal, we may use up to 70,000 cfu; the library currently holds 70,000 mutants and therefore by using 70,000
mutants we would be expect that each mutant in the library would be represented in the inoculum; similarly, given that 
each gene is expected to be represented by 16 differently inserted mutants, this would ensure adequate 
representation of each mutated gene in every inoculated cow. It has been shown that infection with 3.2x10^4 CFU 
induces similar levels of pathology to lower infectious doses, with the minimal infective dose (the smallest dose that 
establishes disease reproducibly) being 10 CFU (Dean, 2005).

In phase I, twenty-four animals will be infected with up to 70,000 CFU/animal. Eight weeks after challenge, five 
head/thoracic lymph nodes will be collected at post-mortem examination to recover the surviving clones. Due to size 
constraints, it will not be possible to process whole lungs, therefore, at post-mortem lung pathology will be evaluated 
and samples from lung lesions collected. Lymph nodes and lung samples will be macerated and stored in aliquots for 
shipment to the RVC and/or LSHTM for culturing on modified 7H11 plates for evaluation of clone recovery. All 
cultured clones (CFUs) will be collected and input and output mutant pools will be sequenced by WGS and compared. 
Following the above challenge experiment with an M. bovis transposon library, in Phase II we will validate a set of 
mutants for their essentiality in vivo. We will choose 5-10 genes that would have been predicted to be essential in the 
host from the previous experiment to take forward for validation and infect 6 animals at up to 70,000 CFU/animal with 
a mini-library containing equal amounts of up to 21 strains (2x 10 constructed mutants + M. bovis AF2122/97 
reference strain) - each mutant and the wild type M. bovis AF2122/97 strain will be present at an infectious dose of up 
to 3300 CFU. The challenge and recovery will be performed under the same conditions as described above. 
Both the M. bovis transposon library used in Phase I and the set of M. bovis mutants used in Phase II will be 
generated at the RVC. Also, as culturing of M. bovis from tissue samples will be performed at the RVC and/or 
LSHTM, the GM work carried out at APHA will be restricted to the preparation of inocula in the CL3 laboratories; the 
two animal challenge experiments will be carried out in the animal and post-mortem facilities; the maceration of tissue 
will be carried out in the APHA CL3 laboratories (more details below).

Recipient or parental organism

Construction of the Mycobacterium bovis knockout library will be performed at Royal Veterinary College by our 
colleagues there. No genetic manipulation will take place at APHA. The wild type strain in which the library was 
constructed is Mycobacterium bovis AF2122/97. The knock out library will be used to infect cattle at APHA’s ACDP 3 
and SAPO 3 containment facility, building 268 and tissues will be evaluated in building 270.

Host/vector system

The host for the genetic insertions is Mycobacterium bovis AF2122/97. The library will then be used for infection of 
Cattle - Holstein-Friesian male calves (4-6 months of age). 
Phasmid/phage delivery system:
TraDIS - The delivery of the Himar1 transposon in mycobacteria is mediated by a phasmid system called 
cpMycoMarT7.
Plasmid systems for homologous recombination:
Vectors for use in mycobacteria are based on pAL5000.

Origin & function

TraDIS: 
The <>MycoMarT7 phasmid contains the C9 Himar1 transposase, and the MycoMarT7 transposon on the 
temperature-sensitive phasmid <A>AE87. The MycoMarT7 transposon contains a kanamycin resistance gene and two 
outward facing T7 promoters. The <>MycoMarT7 phasmid was obtained from Dr Graham Stewart from the University 
of Surrey with permission from Dr Eric Rubin from Harvard School of Public Health.
Homologous recombination:
The pAL5000 based plasmid will be obtained from Addgene via Graham Hatful; this plasmid contains the origin for replication in mycobacteria (oriM). We also use shuttle vectors that are able to replicate in both E. coli (contain colEl) and mycobacteria (oriM). We use integrative vectors in mycobacteria that are based on the L5 integration system. This system (originally identified in a mycobacteriophage) allows stable integration of the plasmid at a single specific site in the bacterial genome. This system has been used for over 20 years and allows the stable insertion of genes (for complementation) and reporters (gfp, rfp, lacZ). None of the vectors encode the capacity for conjugation.

Evaluation of foreseeable effects

Evaluations related to potential mutagenesis in this study:
Despite the fact that globally a large number of human TB cases are caused by M. bovis, several lines of evidence suggest that M. bovis and phylogenetically related strains do not exhibit the same virulence and transmissibility for the human host as M. tuberculosis sensu-stricto. A recent report (Gonzalo-Asensio, 2014) showed that three mutations affecting the two-component virulence regulation system PhoPR in M. bovis likely account for this discrepancy. Genetic transfer of these mutations into the human TB agent, Mycobacterium tuberculosis, resulted in down regulation of the PhoP regulon, with loss of biologically active lipids, reduced secretion of the 6-kDa early antigenic target (ESAT-6), and lower virulence. The same authors also observed that insertion of an IS6110 element upstream of the phoPR locus may completely revert the M. bovis phoPR-associated fitness loss, which is the case for an exceptional M. bovis human outbreak strain from Spain. The latter observation was seemingly a rare event but this report suggests that M. bovis, in general, may be less transmissible and/or virulent than M. tuberculosis but that evidently small chromosomal changes (SNP or insertion/deletion) could result in a pathogen with similar phenotypic characteristics as M. tuberculosis. However, the smaller chromosomal size of M. bovis (compared to M. tuberculosis; Garnier, 2003) and rare horizontal gene transfer (Namouchi, 2014) suggest that it is unlikely that M. bovis would have the ability to become more pathogenic than M. tuberculosis. Thus, although rare, it is possible that such a mutant could be generated in any M. bovis transposon library. However, M. tuberculosis, like M. bovis, is an ACDP level 3 pathogens and therefore, in the unlikely event that such a mutant were present in the library, it would not result in development an organism which would be more virulent than M. tuberculosis and the level of containment would still be ACDP containment level 3.

Insertion of a Himar1 transposon into the M. bovis genome will potentially disrupt the function of the gene or operon where it has been inserted. Such disruption is unlikely to generate a pathogenic phenotype, as a deletion is more likely to result in attenuation. Nevertheless, there has been a case where gene deletion has marginally increased the virulence of a laboratory strain, as assessed by the survival of SCID mice (ten Bokum, 2008). However, the virulence increase was only observed in severely immunocompromised mice and is only marginal compared to strains circulating clinically. Similarly, shedding will not be expected to be different than that of the wildtype. Therefore, as mentioned above, the general postulation suggests that mutants generated by TraDIS or constructed by homologous recombination are unlikely to result in a pathogen with greater virulence or shedding potential than M. bovis, which is an ACDP containment level 3 pathogen.

Similarly, targeted deletion of mycobacterial genes is more likely to be detrimental to the environmental survival of M. bovis rather than improve it. Therefore, the risks to the environment caused by gene deletion and complementation are not greater than the wild-type organism. To our knowledge, there have been no reported cases of gene deletion causing an increase in virulence for M. bovis.

It should also be pointed out that all first-line drugs against the disease agents causing TB are targeting genes essential for survival in vivo. Therefore, an insertion of Himar1 transposon in such gene would likely not generate a viable mutant. For that reason, it is highly unlikely that TraDIS would generate a drug resistant mutant that can compromise patient treatment if, despite effectively negligible risk, infection would occur.

Evaluations related to vectors/markers: The Himar1 transposon does not code for any pathogenicity determinants and
so is not expected to increase the pathogenicity of the host organism. Himar1 encodes kanamycin resistance for purposes of selection. Kanamycin is not used clinically, therefore in the extremely unlikely event of accidental infection, treatment regimens will not be complicated.

The plasmid vectors used here for genetic modifications in mycobacteria are non-mobilisable. Therefore the risk of transfer of DNA from mycobacteria into other bacteria - or from HG1 to HG2-3 - is negligible. The kanamycin resistance on these plasmids is not used in therapy and have a history of safe use in the laboratory. One plasmid utilises the phage L5 integrase system and sequences stably integrate into the genome. This does not alter the virulence of the bacteria and integration is stable.

It is expected that the random insertion of the transposon for the manufacture of the mutant library will not generate increased virulence or survival or shedding over and above that of the wild type M. bovis; furthermore the control measures and processes in place at CL3 laboratory and animal facilities (HEPA out, negative air pressure gradient, use of MSCs, PPE, disinfectant, waste treatment and disposal, storage of material) will reduce the risk of exposure or release of the GMM to a minimum.

There is no intention to introduce into the environment.

The risks of infecting cattle with virulent mycobacteria and the post-mortem of cattle infected with virulent mycobacteria have been assessed in documents BAAC 2001/06 and BAAC 2001/01, respectively.

The risk of release during transport is low.

The risk of release of the pathogen during transport from RVC to APHA and of infected tissues from APHA to RVC will not be different to equivalent transport of M. bovis wild-type strain. M. bovis is a Category B pathogen and packaging will be done in accordance with the current ADR (class 6.2 packaging) regulations for the Carriage of Dangerous Goods by Road and in accordance with HSE guidelines (RA.MI008 and SOP BAC0349). The risk of release from laboratory or during transit between CL3 facilities on site will not be different compared to equivalent work using an M. bovis wild-type strain. Infectious material will only leave the CL3 facilities in primary container which is packed in double plastic bags, which themselves will be within a sealed approved container (BioJar). This outer container will be sprayed with 70% ethanol before exiting the CL3 facility, as described in SOP BAC 0349.

Within the scope of work at APHA in this study, no heat-inactivation of M. bovis isolates will be performed. Any sample containing M. bovis pathogens and that will leave the containment facilities at APHA will either be shipped as dangerous goods to our collaborators (the RVC or the LSHTM) or will be destroyed.

The risk of release from the laboratory is low. A potential risk for release of mycobacteria to the environment occurs when inocula are prepared for infection of cattle or when tissues are recovered from animals and macerated for recovery of M. bovis transposon mutant. However, all manipulations of live organisms will be undertaken in ACDP3 facilities inside a class 1 microbiological safety cabinets (MSC). Inocula for infection will be prepared following SOP BAC0340 and tissue homogenization according to SOP BAC0346. Release of pathogens from these dedicated laboratories is prevented through engineering controls (HEPA filtration of exhaust air, negative pressure gradients) as well as procedures applied by fully trained and authorised staff as described in SOP BAC0371.

The risk of release from animal accommodation is considered low. All animal work will take place at APHA's ACDP3 animal facility. The facility is run under negative pressure, the air is extracted through HEPA filters and is connected to a backup generator in case of power failures. Staff working in this facility with infected animals will be wearing PPE and RPE as described in SOPs ASU 295. In addition, disinfection procedures to decontaminate all waste and equipment leaving the facility have been established (SOPs ASU305 and ASU234), please see below, waste disposal section. Access to building 268 is controlled by the building officer and is restricted to authorised staff that have been trained and signed off against ASUF453 (SOP ASU 238; listed below).

The endobronchial infection is described in SOP BAC0337. Following inoculation, the syringe and the cannula will be placed directly into a waste bag; the bag will be sealed and placed with the animal waste for incineration. The
Endoscope will be disinfected prior to removal from the facility using Perastal, an approved disinfectant which does not damage the endoscope. The computer used for visualizing the travel of the endoscope through the animal will be wiped with tissues soaked in Trigene and double bagged, sprayed with 70% ethanol and transported to ACDP3 laboratory where it will be fumigated inside an MSC., following SOP BAC0347. At the end of the cattle study, the animal rooms will be disinfected by formaldehyde fumigation performed by suitably trained staff (SOP ASU 241). Post-mortem examination will take place in building 270, which is under negative pressure relative to the outside environment. The air is extracted through HEPA filters. Building 270 is connected to a backup generator in case of power failures. The areas for post-mortem examination work on a laminar flow principle with air being drawn to the back of the bay to the main air handling unit extracts. The highest bID-risk during post-mortem analysis is at the bench when examining tissues for lesions of BTB, which involves the cutting of tissues. Therefore, staff undertaking PM procedures will wear PPE and RPE are essential as indicated in SOP PTH0356. Samples will be released according to SOP PTH0339 for transport to ACDP3 laboratory facilities for further processing. Following the PM procedure the facility will be disinfected as indicated in SOP ASU 023.

Assessment: Low risk
The likelihood of survival outside of laboratory containment is low. Members of the Mycobacterium tuberculosis complex, including M. bovis, are not free living in the environment. Hence they are only able to grow and multiply in an animal if released in the environment. It is therefore highly improbable that tubercle bacilli could out-compete saprophytic bacteria that are adapted to survival in the environment. If M. bovis were released, it would simply persist in soil or water. M. bovis can survive in soil for up to 6 months and on pasture for at least 49 days (Maddock, 1933). However, it is not known if and how infective these bacilli would be and if they could be presented in the form necessary for infection. Moreover, surface deposited bacilli accessible to UV radiation would be rapidly killed. And given the environment that they would be released into as part of this procedure, they will be most likely accessible to UV light (due to the concrete or bitumen surfaces surrounding these buildings). The consequences of bacilli surviving and being potentially infective will be discussed in the next paragraph.

It is unlikely that a transposon gene-deleted M. bovis would survive outside the laboratory longer than a wild-type M. bovis strain, which contains a full complete genome. The consequences of release form the laboratory are high. Exposure of cattle to M. bovis, a bacterial pathogen already present in UK cattle and wildlife hosts, could lead to infection. Humans are also susceptible to infection, as well as other susceptible domestic and wildlife mammals such as cats, dogs or badgers. However, to cause infection, the bacilli need to be present in the appropriate format and/or dosage. Whilst aerosol infection (most common route associated with tuberculosis) requires a very small infective dose (1 to several CFU), generation of aerosols and their ingestion is unlikely due to the low expected bacillary shedding and loads generated. The oral infection dose is by far higher (10,000+ CFU) and it is unlikely that such high bacillary counts would be released. This would also require the direct ingestion through the oral route of the bacilli, which is unlikely. Further, there are no susceptible free-living experimental animals (e.g. cattle, goats, sheep, pigs) in close proximity of the CL3 laboratories in the Stewart Stockman Building to ingest/inhale tubercle bacilli and thus the risk of infection of such animals is effectively zero. Infection risk to humans outside the containment building is also low as the generation of aerosols is unlikely at any stage and unlikely to be of sufficient density and quantity to infect passing staff. Tuberculosis in humans is treatable.

The transposon and Kanamycin resistance gene inserted into the chromosome of M. bovis pose no additional risk in this respect since the drug resistance marker the bacilli contain have been chosen because they are not used in human medicine.

The risk associated with M. bovis without controls is high but on applying the safety measures detailed in the
associated SOPs, the risk of exposure/release from containment is low.

Special measures.
Recombinant mycobacteria will be handled at CL3 at all times; in the CL3 laboratory suite TB-101 or the SLSD suite of building 7 for preparation of inocula for infection and for maceration of tissues; building 268 for animal infection, and building 270 for post-mortem examination.

These buildings comply with the appropriate control measures that will prevent the release of dangerous or genetically modified microorganisms into the environment. These include filtration of air exhaust through HEPA filters in all the facilities in which the organism will be handled; in the case of the laboratory, the organism will be handled only inside Class 1 microbiological safety cabinets, which will protect personnel in the laboratory and provide double filtration between the organism and the environment. In the case of laboratory waste, both liquid and solid waste material will be inactivated by autoclaving at 1360°C for 1 hr prior to incineration; in the case of the animal accommodation, solid waste and liquid waste that had been adsorbed into solid waste (such as animal urine) will be incinerated, waste that reaches the drain will be heat treated for 2 hrs at 960°C prior to release into the normal effluent; in the case of all the post-mortem facility, solid waste will be incinerated and any liquid waste will be inactivated with a 5% solution of FAM30 for 1 hr, which is the DEFRA approved disinfectant with the approved contact time for inactivation of Mycobacterium bovis.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste from ACDP3 laboratory facility. The vectors used and the knock-out mutants of M. bovis included in these experiments are expected to be equally susceptible to heat and autoclaving as any wild-type strain of M. bovis. Therefore, all waste produced in the laboratory CL3 facilities in this experiment, as well as used PPE, will be autoclaved as per validated protocols (for wild-type M. bovis) prior to disposal. In short, waste will be transported to an autoclave in a closed metal tin. Decontamination will be performed by autoclaving for 1 hour at 136°C after which waste will be put into bags for incineration. Waste disposal generated in the animal facility unit will be undertaken according to ASU234 in 500 litre bunded, lockable metal bins with red lids and biohazard signs attached. Waste will be taken to the incinerator for disposal through burning as described in SOP ASU 234. Waste generated during the post-mortem procedure, including clinical waste and carcass, will be loaded into bins double lined with black bags. The plastic bags will be sealed securely with tape and the outside will be sprayed with 5% FAM30. Disposal will then be through incineration as per PTH 0358.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
The risk assessment GMRA 2018/03 - Cattle challenge with a transposon mutagenised Mycobacterium library has been reviewed (July 2018) and approved by APHA Biological Safety Committee.

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### Project Ref 221/19.2

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- Non-GMM
- Consent Granted

- Project notified under transitional arrangements

### Project Additional Information

**Purposes of the contained use**

Nipah virus (NiV) poses a significant epidemic threat due to its broad host range and widespread distribution of Pteropus spp. bats which provide a natural reservoir. Human infection can occur following the exposure to infected
pigs, where NiV causes a respiratory and neurological syndrome. Direct pig-to-human transmission was responsible for the first and most devastating NiV outbreaks in Malaysia and Singapore (1998-99), with nearly 300 human cases and over 100 fatalities. Despite the importance of NiV as an emerging disease, no vaccines are currently approved for human or livestock use. We propose that an inexpensive, safe and efficacious vaccine could be developed for use in pigs to protect against NiV infection and transmission, therefore reducing the risk to public health as well as the developing pig industries and small holder farmers in south and southeast Asia.

Previously, a canarypox vaccine vector (ALVAC strain) expressing the NiV glycoprotein (G) or fusion protein (F) (ALVAC-NiV G and ALVAC-NiV F) protected pigs against experimental NiV challenge (Weingartl et al., 2006). High titres of NiV neutralising antibodies were induced with the ALVAC-NiV G vaccine, while despite the low levels of neutralising antibodies induced by the ALVAC-NiV F; all vaccinated pigs were protected against virulent NiV challenge. Despite these encouraging results and the continued threat posed by NiV, no vaccine candidate has progressed towards market. In this project we are systematically analysing the immunogenicity of NiV vaccine candidates including both recombinant protein subunit and viral vectored vaccines. Providing data to support their further evaluation in efficacy studies being conducted by partner institutions. Groups of pigs will be immunised one to two times with the NiV vaccine candidates via the intramuscular route and bled at weekly intervals to determine vaccine-induced immune responses.

This application specifically addresses the use of recombinant bovine herpesvirus type 4 (BoHV-4)-based vectors delivering NiV G and/or NiV F to immunise pigs. Several biological characteristics of BHV-4 makes it an attractive vaccine delivery vector. These include: no pathogenicity, no oncogenicity as the BHV-4 genome is maintained in an episomal state in dividing cells without chromosomal integration, the capability of to accommodate large amounts of foreign genetic material and ability to maintain transgene expression in both undifferentiated and differentiated cells. Multiple recent studies indicate the potential utility for BoHV-4 for development as a vaccine vector platform, with an ability to induce immune responses against heterologous target antigens (Donofrio et al., 2006, Donofrio et al., 2007 and Donofrio et al. 2008).

Recipient or parental organism

Bovine herpes virus 4 (BoHV-4) is a gamma herpesvirus generally associated with subclinical infections in cattle. A BoHV-4 genome was cloned as a bacterial artificial chromosome (BAC) to create a vector platform that is readily engineerable. The BoHV-4 vector has been demonstrated to be suitable to deliver heterologous antigens and induce potent immune responses in a range of species including pigs (Donofrio et al., 2011). The construction of infectious BoHV-4-derived BAC clones in Escherichia coli, followed by progeny virion reconstitution via transfection into permissive eukaryotic cells, has been successfully pursued as a means to rapidly modify the viral genome and adapt it to specific needs. Expression cassettes are inserted into the TK locus of BoHV-4 genome and optimized with different promoters, such as CMV, EF1a and PGK, as well as the woodchuck hepatitis virus post-transcriptional regulatory element to increase transcript half-life. In this specific case, NiV G and NiV F genes were placed under the transcriptional control of a CMV promoter.

Host/vector system

BoHV-4-based vector expressing NiV G and/or NiV F will be used to immunise pigs.

Origin & function

The Pirbright Institute will receive cells infected with recombinant BoHV-4-based vector expressing NiV G or NiV F from The Department of Medical-Veterinary Science of Parma University (Italy). These recombinant viral vector infected cells will be used to immunise pigs at APHA-Weybridge.
Nucleic acid insert contains Nipah Virus Malaysian strain glycoproteins G and F. NiV Malaysian Strain GenBank accession number HMS4S086.

Intended function of the genetic material is to express antigen and induce NiV antigen-specific immune responses in vaccinated pigs. The induction of NiV specific neutralising antibodies and T-cell will be assessed longitudinally in blood samples and in mucosal and associated lymphoid tissues collected post-mortem.

Evaluation of foreseeable effects

The recombinant BoHV-4 vectors will infect cells within the immunised pig which will lead to antigen expression and the induction of NiV specific immune responses. Infection is expected to be restricted to the inoculation site and systemic spread and virus shedding will not occur. There is the possibility that the recombinant NiV G and/or F protein will be embedded in the envelope of the BoHV-4 virion but this is unlikely to alter the cellular tropism, since BoHV-4 entry occurs by fusion of the mature virion envelope with host cell membranes on a wide variety of cell types. There is a low risk of the release of the viral vectors into the environment from the animal accommodation as BoHV-4 vectors will undergo limited replication localised to the inoculation site in immunised pigs. The recombinant viruses will not be shed from vaccinated animals nor will virus be present in blood and tissue samples subsequently collected from immunised animals. There is no evidence of lesions forming at the inoculation site that could provide a source of virus shedding and transmission.

The likelihood of survival of recombinant BoHV-4 vectors outside of laboratory containment is low. As a member of the enveloped Herpesviridae, recombinant BoHV-4 vectors are expected to possess low environmental stability and direct contact between cattle is typically necessary for transmission of BoHV-4. BoHV-4 can be inactivated by lipid solvents such as ether and chloroform, and by physical and chemical agents; it is heat labile (SO°C, 30 min), trypsin sensitive, inactivated at pH 3, and is not stabilized by magnesium chloride. A comprehensive WHO report on herpesvirus survival in the environment concluded that, depending on ambient temperature, pH, and type of animal waste, time in days required for a 90% virus titer reduction varies widely, ranging from less than 1 week for herpesvirus to more than 6 months for rotavirus. Virus inactivation was faster in liquid manure, a mixture of urine and water (pH> 8.0), than in semiliquid wastes that consisted of mixtures of faeces, urine, water, and bedding materials (pH < 8.0) (Sobsey and Meschke, 2003). Bovine herpesvirus was reduced by 1 log10 within 2.5 days (Pesaro et al., 1995). This reduced survival in the environment is not likely to be affected by the expressed NiV genes, therefore the likelihood of the recombinant BoHV-4 vectors surviving in the environment is low.

The risk of infection of humans with recombinant BoHV-4 vectors is effectively zero. There is no reports of BoHV-4 infecting humans naturally. Sera from naive humans, in contrast to sera from other mammalian species, efficiently neutralises BoHV-4 infectivity. The mechanism of this neutralization is complement-dependent and mediated by natural antibodies against the Gala1-3Gal131-4GlcNAc-R epitope acquired on the viral envelope during replication in bovine, swine and ovine cells, which all express Gala1-3Gal131-4GlcNAc-R. Thus human natural antibodies raised against Gala1-3Gal offer an innate immune mechanism that prevents cross-species transmission of gammaherpesviruses to humans (Machiels et al., 2007). In vitro analysis of BoHV-4 replication in human cell lines showed that human lymphoid and myeloid cells are not susceptible to infection, whereas epithelial-derived cells are susceptible to virus entry (range: 72% to 7%), but only poorly permissive for replication (range: 3% to 0.1% based on viral gene expression) (Gillet et al., 2004). Exposure monitoring by e.g. serological testing of animal carers etc. is therefore not necessary. Although BoHV-4 is regarded as safe, immunocompromised individuals shall not handle the vectors or to be potentially exposed to it when immunizing pigs.

Accidental release of the recombinant BoHV-4 vectors could result in infection of animals in the environment. Expression of NiV G or F protein is highly unlikely to alter host range and/or tissue susceptibility of BoHV-4 vectors. However in the unlikely event of this occurring in most animal species e.g. rodents, horses, goats, sheep and pigs, the recombinant vectors are incapable of spreading. The exceptions are the natural host, cattle, and the laboratory host, rabbits, in which the recombinant BoHV-4 vectors could establish a persistent infection and could be transmitted to
animals with close contact. Accidental infection of cattle or rabbits with recombinant BoHV-4 vectors is not expected
to cause disease. The vectors would induce NiV and/or F specific antibody responses that could result in false
positive results in NiV serological diagnostic assays. However NiV outbreaks have never been described outside of
sporadic outbreaks in livestock and humans in south and southeast Asia and the likelihood of ever screening UK
cattle or rabbit populations for seroprevalence is extremely low. In the unlikely event of homologous recombination of
the recombinant BoHV-4 vector (GMM) with a wild-type herpesvirus (e.g. transfer of the NiV gene), the resulting
recombinant virus will not represent any greater hazard or risk than the original GMM vaccine virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid contaminated materials:
Health and Safety
Executive
Laboratory waste: Place in plastic autoclave bag, double bag in second autoclave bag, spray with 70% ethanol, 10
minutes contact time, then autoclave and incinerate.
Self-life of the 70% ethanol is 1 week.
Autoclave conditions: minimum 15 PSI at 121°C, 30 min.
Animal facilities waste: All solid waste including bedding and PPE must be discarded into a plastic bag which is then
decontaminated with FAM 30 (1 :20) for at least 1 h and removed for incineration. Needles and syringes must be
disposed of into sharps container before bagging and processing as before.
Post-mortems will be performed at days 40-42 of the study. There is no increased risk post-mortem other than those
associated with non-GM post-mortems as no infectious virus will be present in the tissues at this time-point postimmunisation.
Pigs will be transported to the post-mortem room by ASU staff (outlined in ASU SOP 058: Transportation of Animals and General Use of ASU Vehicles. The use of the post mortem room is outlined in SOP
ASU 23 Use and Maintenance - Building 270 and the carcasses will be disposed of by incineration directly from the
post-mortem room (PTH SOP 0358: Waste disposal to cremator from Building 270 containment envelope).

Liquid waste:
Laboratory waste: No cultures performed. Therefore, liquid laboratory waste will be virus-infected SMADSC stocks or
diluted vaccine preparations. Place containers (plastic tubes or cryovials) into plastic bag inside class II MSC, place
bag into second bag, spray outside with 70 % Ethanol (leave for 10 minutes), then remove from cabinet for
autoclaving and incineration.
Autoclave conditions: minimum 15 PSI at 121°C, 30 min. Volumes to be autoclaved will be less than 10 mL.
Animal facilities waste: Liquid waste in animal accommodation occurring during vaccinations will be contained in
syringes and disposed as part of solid contaminated material (see section 5.1). To prevent liquid animal waste,
contaminating the drains, during the first 14 days after inoculation drains will be blocked and liquid discarded by
adsorption into solids (straw or bedding) that will be disposed of as described in section 5.1. At the end of the
experiment, animal accommodation will be cleaned with FAM 30 (1 :20 diluted), contact time 1 h, including drain
covers before drains can be opened again. Water troughs will be drained on straw/bedding and this disposed as solid
waste.
The risk assessment 'Vaccination of pigs with recombinant bovine herpesvirus-4 vectors expressing Nipah virus glycoproteins' was discussed in September 2018 meeting and have been approved by APHA Biological Safety Committee.

### Project Containment

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<th>Human Clinical Applications</th>
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### Project Ref 221/19.3

- **Date Ackn'd**: 13/09/2019
- **Date Project Ceased**: 221/19.3
- **CU2 Project Title**: Recombinant prion production and purification to enable development of RT-Quic technology.
- **Class**: Class 2
- **CultureVolumeClass3-4**: ≤ 1 L
- **Non-GMM Consent Granted**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: No
## Project Additional Information

### Purposes of the contained use

Production of non-infectious recombinant prion protein (rPrP) as a reagent for use in Real Time Quaking Induced Conversion (RT-QuIC) assays. These assays detect prion seeding activity for rapid, discriminatory and ante-mortem diagnostic tests of transmissible spongiform encephalopathies (TSEs).

### Recipient or parental organism

Recombinant PrP plasmids donated by Dr. Byron Caughey (Rocky Mountains Laboratory, Colorado, USA) will be propagated and expressed in 2 different commercially available E.coli strains specifically developed for these applications.

1. TOP1 0 E. coli strain (One Shot® TOP1 0 Chemically Competent Cells, Invitrogen) will be used for propagation of rPrP plasmids.
2. Rosetta 2(DE3) E. coli strain (Rosetta™ 2(DE3) Singles™ Chemically Competent Cells, Novagen) will be used for rPrP synthesis.

### Host/vector system

The host is E.coli, gram-negative bacteria. Commercially available strains will be used.

Two types of vector will be used for the expression of the rPrP of interest. They both come from a commercial source (Novagen).

1. The pET24 vector has been used for the insertion of bank vole rPrP DNA sequence (codons 23-230), and sheep VRQ rPrP DNA sequence (codons 25-234). It has been designed for expression from bacterial translation signals carried with in a cloned insert. It therefore lacks the ribosome binding site and ATG start codon present on the pET translation vectors.
2. The pET41 vector has been used for the insertion of sheep ARQ PrP (codons 25-234). The pET41 vector has been designed for cloning and high-level expression of peptide sequences fused with the 220 aa GST-Tag protein.

### Origin & function


   This sequence corresponds to the natural prion protein as encoded by the bank vole genome, devoid of the signal peptide (residues 1-22) and the C-terminal amino acids that are cleaved off in post-translational processing in the natural host. The bank vole natural prion protein is not pathogenic unless made so by in vivo or targeted templating reactions, neither of which are used in the planned activities at APHA. As the sequence encodes a single mammalian protein that does not self-replicate, it is incapable of self-propagation.


   These sequences correspond to two of the allotypes (136/154/171 V/R/Q resp NR/Q) of the natural prion protein as encoded by the sheep genome, devoid of the signal peptide (residues 1-24) and the C-terminal amino acids that are cleaved off in post-translational processing in the natural host. The sheep natural prion protein is not pathogenic unless made so by in vivo or targeted templating reactions, neither of which are used in the planned activities. As the
sequence encodes a single mammalian protein that does not self-replicate, it is incapable of self-propagation. The genetic material is to be used for production of non-pathogenic rPrP with amino acid sequences corresponding to either bank vole or sheep natural prion proteins. The non-pathogenicity of the produced rPrP will be tested by western blot with and without proteinase K. This test will confirm that our protocol lead to the production of the rPrPc and not the rPrPSC. As described in the project summary, the rPrP produced will be used as a core component of the RTQuIC diagnostic test for TSEs, which is to be developed and implemented in a TSE containment facility at APHA Weybridge.

Evaluation of foreseeable effects

Foreseeable effects on lab workers following incidents such as spillage and accidental exposure by inhalation are considered negligible as the components in the proposed procedure are non-colonizing and non-pathogenic. Exposure of the environment to any of the components used in the procedure as a result of spillage, will not to result in harm to organisms in the environment, as none of the components have colonising ability, nor are they pathogenic or mobilisable:

1) TOP10 and Rosetta 2(DE3) E.coli cells are respectively derived from the strains K-12 and BL21, neither of which produce toxins, are pathogenic or have colonising capability.
2) The pET vectors is based on the pBR322 vector and as such are mobilisation defective.
3) The pRARE2 plasmid displaying the chloramphenicol resistance gene, a component of the Rosetta 2(DE3) E.coli cells, is non-mobilisable, there is no risk of transfer of antibiotic resistance to the environment.

No evidence exists that would indicate a risk that these host and vector properties are liable to change or mutation that would alter these properties.

In summary, none of the components form a risk to operators or environment.
As no TSE agent is present and rPrP is non-pathogenic, decontamination measures for TSE agents will not be required. As mentioned previously (section 3.3.3), the non-pathogenicity of the produced rPrP will be check by western blot, with and without proteinase K.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid contaminated waste and glassware to be recycled to the Media department (empty culture bottles) will be surface-decontaminated by repeated spraying with 70 % ethanol to ensure a contact time of 10 min, within the MSC prior to removal (Validation Document VI013 - Validation of 70% Ethanol for the inactivation of HG3 & HG2 Enterobacteriaceae; VTEC Biohazard Unit Working Procedures V11 ; Approval comms for Validation; BTF846). Waste and glassware will then be placed inside autoclave tins which are clamped shut for transport and surface-decontaminated by spraying with 70 % ethanol , 10 min contact time(VI013). Tins must be labelled using autoclave tape with description of waste type and room number, and only then allowed to be transported to the autoclave (using a dedicated trolley for this task) and take through the autoclaving process by support staff (SOP CBU0143 - Collection and disposal of contaminated material).

The autoclave tins with solid waste will be autoclaved using the "Discard Cycle" (holding temperature 134°C for 60 min) which has been validated previously (March 2013) for solid waste followed by incineration on-site (SOP CBU0135 - Autoclave Operation), except for media glassware which will be cleaned and returned to the Media.
Liquid waste including broth cultures and supernatants will be collected into 500 mL red-top pots (GosselinTM Square High Density Polyethylene Bottles (Corning LC500-11)) inside an MSC to which 7.5 g Lab-Sorb will be added to solidify the waste to a gel. The pots will be closed and surface-decontaminated by spraying with 70 % ethanol, 10 min contact time (VI013) within the MSC prior to removal. They will then be placed inside autoclave tins which are clamped shut for transport and surface-decontaminated by spraying with 70 % ethanol, 10 min contact time (VI013). Tins will then be labelled using autoclave tape with description of waste and room number and only then allowed to be transported using a trolley to the autoclave and take through the autoclaving process by support staff (SOP CBU0143 - Collection and disposal of contaminated material). The autoclave tins with solidified liquid waste will then be autoclaved (SOP CBU0135 - Autoclave Operation) using the “Discard Cycle” (holding temperature 134 °C for 60 min). As the previous validation procedure for eradicating bacterial contamination from solidified liquid waste was for volumes up to 100 mL, an additional validation for the 250 mL solidified waste volumes using non-transformed version of the Rosetta 2(OE3) E. coli cells (prior transformation) has been carried out (April 2019). Waste will be subsequently disposed of for incineration on-site (SOP CBU0143 - Collection and disposal of contaminated material).

This risk assessment was presented to and discussed in the APHA Biological Safety Committee meeting in May 2019. The project assessment has been approved by the committee.
**Project Additional Information**

**Purposes of the contained use**

To generate cultures of animal cells that can replicate indefinitely under laboratory conditions. This reduces the need to cull animals to harvest cells, and also standardises the cell culture for better reproducibility of results.

**Recipient or parental organism**

Potentially, tissue derived from any animal.

**Host/vector system**

A pantropic replicant-incompetent retroviral vector based on Moloney murine leukemia virus.

**Origin & function**

SV40 is a large T antigen of simian virus origin, it is widely used in cell immortalisation. It is also available as a combination of large T and small T antigen. hTERT is the catalytic subunit of human telomerase. It is another commonly used immortalisation gene, but it doesn't transform the cells to the same extent as SV40. Both nucleic acid inserts would allow for cell cultures to propagate indefinitely.

**Evaluation of foreseeable effects**

The retroviral vector is replication incompetent. Therefore, once used to transfect the target animal cells, they cannot reproduce themselves or leave the cell to further infect other cells. They are a single use delivery mechanism.
The genes are incorporated randomly into the host genome. This means the experiment will not always be successful. Most cells will die and can be disposed of via standard APHA waste methods. If successful, an immortalised cell line will proliferate, which can then be stored cryogenically for future use.

If staff were accidently injected with the oncogene SV40, there is the potential for this to cause the growth of tumours in the individual, but would not lead to contagious disease (Barbanti-Brodano et al., 2004). This risk is mitigated to LOW by never using sharps during the process, as sharps are not necessary to perform any of the stages. Transfected packaging cells and their supernatant are always handled in a MSC and with the operator using standard PPE (double gloves and disposable arm sleeves). This is to protect the packaging cell line from undesirable bacterial contamination, but also adds an extra layer of biosecurity which helps reduce any accidental release of the viral vector to the environment.

Cells will routinely be produced only from healthy animals. However, as a precautionary step cell lines generated from non-SPF sources may be screened for adventitious agents post-transduction.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste associated with transfection/transduction work is autoclaved at a minimum of 121°C for 30 minutes before incineration on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment was presented and discussed at September 2019 meeting. It was agreed that transfection/transduction of the cells should be GM class 2 activity, but handling of resulting immortalised cell lines falls onto GM1 class.

Project Containment

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The overall goal of this project is to define the role of the Mycobacterium bovis protein MPB70 in the generation of pathology during infection in cattle. Mycobacterium bovis is an ACDP Hazard Group 3 pathogen. MPB70 is a protein which is constitutively expressed at a high level and secreted by M. bovis. However, in the human pathogen Mycobacterium tuberculosis MPB70 is expressed at lower levels, suggesting a role for MPB70 in host tropism. The NMR structure of MPB70 was determined in 2003 and revealed a FAS1 (Fasciclin-like) domain (Carr et al., 2003). FAS1 domains are present in extracellular proteins and are implicated in cell-to-cell, cell-to-extracellular matrix and extracellular matrix structure adhesion (Park et al., 2009; Seifert, 2018). This suggested a role for MPB70 in cellular aggregation, an initial step in granuloma formation. This hypothesis was explored further to reveal that MPB70 plays a role in driving bovine macrophages to form multinucleated cells, MNC, in vitro (Queval et al., 2021). This work also revealed that M. tuberculosis did not lead to MNC formation from bovine macrophages in vitro, nor did an M. bovis mutant lacking MPB70. Finally, from previous work, it was noted that M. tuberculosis infections of cattle led to much lower numbers of MNCs in granulomas as compared to M. bovis (Villarreal-Ramos et al, 2018; Queval et al, 2021). However, it is not known if this latter observation is due to lower levels of MPB70 production by M. tuberculosis.
M. bovis AF2122/97, which is a GB isolate that was the first M. bovis strain to be genome sequenced (Gamier et al, 2003). It is used worldwide as a well characterised M. bovis strain. No construction of the Mycobacterium bovis knockout strains will be performed at APHA. The M. bovis AF2122/97 wild type, M. bovis AF2122/97 delta mpb70 mutant and M. bovis AF2122/97 delta mpb70(pEW70c2) complemented mutant strains to be used will be supplied by our UCD collaborator at the time of infection in the form of culture stocks ready for dilution and preparation of the inocula. No genetic manipulation of the mutants will be carried out at APHA.

Host/vector system

Mycobacterium bovis AF2122/97; wild type, mutant and complemented mutant will be used to infect cattle (Holstein-Frisian calves 6-8 months of age).

The phAE159 phagemid system was used for homologous recombination. The pEW70c2 plasmid in the M. bovis AF2122/97 delta mpb70(pEW70c2) is derived from pMD31 which contains ColE1 and pAL5000 origins of replication. All vectors are non-mobilizable and non-conjugative.

The DNA insert used to generate the allelic exchange substrate for the M. bovis AF2122/97 mpb70 mutant is the gene encoding hygromycin resistance, originally obtained from Streptomyces hygroscopicus (Garbe et al, 1994). The DNA insert in the pEW70c2 plasmid is the wild type M. bovis AF2122/97 mpb70 gene under the control of its native promoter.

The allelic exchange substrate was used to exchange the chromosomal copy of the wild type mpb70 gene for the disrupted mpb70 gene with a hygromycin selectable marker.

The pEW70c2 plasmid restores the expression of mpb70 in the M. bovis AF2122/97 delta mpb70 mutant back to wild type levels.

Origin & function

Evaluations related to potential mutagenesis in this study:

Recently published data (Queval et al, 2021) indicates that the deletion of mpb70 from M. bovis reduces the ability of the mutant to drive multinucleated cell (MNC) formation from bovine macrophages in vitro. Hence our hypothesis is that this in vitro phenotype of reduced MNC formation will be reproduced during in vivo infection of cattle, representing the reduced virulence of the mutant, and evident after histopathological examination. Furthermore, previous work has suggested a link between BCG vaccine strains that are high producers of MPB70 and osteitis as a complication of BCG vaccination with these strains; conversely, BCG strains that produce low levels of MPB70 have lower rates of osteitis (Wiker, 2008). The complemented mutant containing the mpb70 gene has already been shown to confer wild type expression of mpb70 back to the mutant and to restore the formation of MNC in in vitro. Our expectation is that animals infected with the complemented mpb70 mutant will resemble infection with wild type M. bovis in terms of MNC formation. There is no evidence or expectation that the mutant or complemented mutant have increased virulence as compared to the wild type. Verification of the complemented mutant has maintained the pEW70c2 plasmid will be performed by plating macerated tissue samples on 7H11 agar plates containing Kanamycin. If required, this will also be confirmed by PCR at UCD, using heat killed bacterial pellets shipped from APHA.

In terms of environmental survival of the mutant or complemented mutant, deletion of mycobacterial genes is more likely to be detrimental to the environmental survival of M. bovis rather than improve it. Therefore, the risks to the environment caused by gene deletion and complementation are not greater than the wild-type organism.

Evaluations related to vectors/markers:

The hygromycin marker used for construction of the allelic exchange substrate for inactivation of the mpb70 coding sequence is from Streptomyces hygroscopicus, a soil dwelling actinobacteria. The marker is integrated onto the bacterial chromosomal and as such non-mobilisable.

The pEW70c2 plasmid vector used here for complementation is non-mobilisable. Therefore, the risk of transfer of
DNA from mycobacteria into other bacteria is negligible. Kanamycin and hygromycin are not used in human TB therapy and have a history of safe use in the laboratory. In the extremely unlikely event of accidental infection with the recombinant bacilli, treatment regimens will not be complicated.

Furthermore, the control measures and processes in place at CL3 laboratory and animal facilities (HEPA out, negative air pressure gradient, use of microbiological safety cabinets (MSCs), Personal Protective Equipment (PPE) and Respiratory Protective Equipment (RPE), disinfectant, waste treatment and disposal, storage of material) will reduce the risk of exposure of staff working with the bacteria or release of the genetically modified microorganism to a minimum. As stated in BAAC 2001/06, all staff working with M. bovis must be enrolled with the APHA TB Occupational Health Surveillance Programme. The established Tuberculosis Health Surveillance Protocol must be followed. All staff must have received a BCG vaccination or have evidence of a BCG scar.

**Evaluation of foreseeable effects**

Evaluations related to potential mutagenesis in this study:

Recently published data (Queval et al, 2021) indicates that the deletion of mpb70 from M. bovis reduces the ability of the mutant to drive multinucleated cell (MNC) formation from bovine macrophages in vitro. Hence our hypothesis is that this in vitro phenotype of reduced MNC formation will be reproduced during in vivo infection of cattle, representing the reduced virulence of the mutant, and evident after histopathological examination. Furthermore, previous work has suggested a link between BCG vaccine strains that are high producers of MPB70 and osteitis as a complication of BCG vaccination with these strains; conversely, BCG strains that produce low levels of MPB70 have lower rates of osteitis (Wiker, 2008). The complemented mutant containing the mpb70 gene has already been shown to confer wild type expression of mpb70 back to the mutant and to restore the formation of MNC in in vitro. Our expectation is that animals infected with the complemented mpb70 mutant will resemble infection with wild type M. bovis in terms of MNC formation. There is no evidence or expectation that the mutant or complemented mutant have increased virulence as compared to the wild type. Verification that the complemented mutant has maintained the pEW70c2 plasmid will be performed by plating macerated tissue samples on 7H11 agar plates containing Kanamycin. If required, this will also be confirmed by PCR at UCD, using heat killed bacterial pellets shipped from APHA.

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### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

not applicable

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

#### Solid waste:
Waste from CL3 facilities.
The vector used and the knock-out mutant of M. bovis included in these experiments are expected to be equally susceptible to heat and autoclaving as any wild-type strain of M. bovis. Therefore, all solid waste produced in the CL3 facilities in this experiment, as well as used PPE, will be autoclaved as per validated protocols (for wild-type M. bovis) prior to disposal (SOP BAC0358). In short, waste will be transported to an autoclave in a closed metal tin. Decontamination will be performed by autoclaving for 1 hour at 136°C after which waste will be put into bags for incineration.

Waste disposal generated in the animal facility
This will be undertaken according to ASU234 in bunded, lockable metal bins with red lids and biohazard signs attached. Waste will be taken to the incinerator for disposal through burning as described in SOP ASU 234. Disinfection and removal of equipment (endoscope etc.) will follow the procedure described in SOP BAC0337. Waste generated during the post-mortem procedure, including clinical waste and carcass, will be loaded into bins double lined with black bags. The plastic bags will be sealed securely with cable ties and the outside will be sprayed with 5% FAM30. Disposal will then be through incineration as per PTH 0358 in close interaction with the incinerator staff.

#### Liquid waste:
Waste from the CL3 facilities
Liquid waste is treated as per validated protocols (for wild-type M. bovis) prior to disposal (SOP BAC0358). Liquid waste from the laboratory facility is contained first within a robust, leak proof bottle. For removal from the MSC, the bottle is sprayed with 70% ethanol and placed inside a polyethylene bag; this bag is in turn sprayed with 70% ethanol prior to being taken out of the MSC and placed inside an autoclave tin. The tin is closed and autoclaved at 136°C for 1 hr, after which, the contents of the tin are placed in appropriate containers for incineration.

Waste disposal generated in the animal facility
Liquid waste from animal rooms will be processed as described in ASU305; briefly, liquid waste from animal rooms will drain to the effluent treatment plant in which waste will be heated to 96°C for 2 hrs before being released into the public drains. Liquid waste could also be absorbed into solid waste, for instance straw in which case liquid waste will be disposed through incineration, as solid waste.

Liquid waste from the PM room will be disposed in accordance with SOP PTH 0358.

#### General rubbish:
All rubbish will be solid or liquid and hence dealt with as described above.

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**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N
Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 221/21.2

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Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
**Purposes of the contained use**

Recombinant expression of mycoplasma protein in E. coli to evaluate specific immunogenic proteins, with the aim of determining the suitability as candidate antigens able to offer improved serological tests for mycoplasma disease in host animals.

**Recipient or parental organism**

*Escherichia coli* B derivative BL21 (DE3) for protein expression

- *Escherichia coli* K-12 derivative (DH5a) for DNA cloning and plasmid manipulation

Both *E. coli* strains are regarded as ACDP HG 1 (Health and Safety Executive, 2007). These *E. coli* strains will be purchased as commercial products from New England BioLabs in a competent form to enable heat-shock transformation.

The *E. coli* hosts for expression studies are disabled strains, unable to survive in the environment and require complex media and growth conditions, and are considered ACDP HG1. These *E. coli* strains will be purchased as commercial products from New England BioLabs in a competent form to enable heat-shock transformation.

Host/vector system

- *Escherichia coli* BL21 (DE3) or DH5a will be the host.

The vector systems are listed below:

- **pGEM vector system** (Promega) for cloning and plasmid manipulations. These plasmids have an origin of replication enabling plasmid replication within *E. coli* cells, there are multiple cloning sites to enable DNA cloning via restriction enzyme digestion and DNA ligation, and they confer ampicillin resistance to transformed *E. coli*.

- **Plasmids of the pET system**, (Merck), pGS system (Genscript), or pGEX system (Genscript) for protein expression work. These plasmids confer either kanamycin or ampicillin resistance to transformed *E. coli*, recombinant protein is expression under control of the T7 promoter (pET, pGS) or tac promoter (pGEX), and lac operator, and they also enable fusion of purification affinity tags, glutathione S tranferase, polyhistidine and S-peptide tag.

**Origin & function**

The gene sequence or gene fragment encoding immunogenic protein(s) will be synthesised commercially to mutate TGA (encoded as tryptophan in mycoplasmas) to TGG to enable protein expression in the GM *E. coli*, from the following *Mycoplasma* species:

- **SAPO2**
  - *M. mycoides* subspecies *capri*
  - *M. capricolum* subspecies *capricolum*
  - *M. capricolum* subspecies *capripneumoniae*
  - *M. agalactiae*
  - *M. putrefaciens* (considered SAPO2 for laboratory work)
SAPO2 & Schedule 5

- M. mycoides subspecies mycoides

No SAPO2 mycoplasmas will be genetically modified under this notification. Mycoplasmas lack typical "virulence" genes such as toxins, cytolysins, and invasins. Instead and candidate genes conferring immunogenic functions appear to be membrane-associated lipoproteins with roles in adhesion and immune evasion. The target DNA cloned in a pET plasmid vector will be introduced into E. coli to carry out protein expression analysis and produce mycoplasma proteins/peptides for use in diagnostic assays.

Cloning of one mycoplasma gene with this type of function is extremely unlikely to generate a virulent E. coli with toxigenic properties, especially as the E. coli recipient strains (BL21 (DE3) or K-12 derivatives (DH5a)) are lab adapted, avirulent, and lack expression of genes mediating long-term colonisation of animal host(s).

We anticipate mycoplasma proteins with immunogenic properties applicable to serological diagnostic assays, will be lipoproteins expressed at the mycoplasma cell-surface with adhesion functionality to cells of the host animal (sheep and goat) cells. It is unlikely that the chosen mycoplasma protein(s) will confer virulence or toxigenic properties in recipient E. coli when produced recombinantly as mycoplasmas do not harbour typical bacterial virulence genes or, with a single exception, toxins. Only the human pathogen Mycoplasma pneumoniae (not used in this project) has been found to produce the community-acquired respiratory distress syndrome toxin (CARDS). Candidate genes encoding a hypothetical protein of unknown function will be assessed in terms of structural homology to known bacterial protein toxins using online search databases (blastP, Uniprot). Any candidate proteins with homology to known toxins will be further investigated and assessment of risks associated with planned protein expression will be undertaken to determine whether protein expression can be safely carried out. Every effort will be made to eliminate selection of mycoplasma genes encoding proteins with any kind of theoretical toxigenic function.

The E. coli hosts for expression studies are disabled strains, unable to survive in the environment and require complex media and growth conditions, and are considered ACDP HG1. DNA within expression vectors will be under the control of vector promoters, and the proposed cloning and protein expression vectors do not carry mobility genes or mating pair formation complex genes enabling conjugation, and thus may be viewed as nonmobilisable. As such, the conference of antimicrobial resistance to ampicillin or kanamycin via transformation with these plasmids will be limited to the recipient E. coli strains DH5a and BL21 (DE3); the chance of horizontal transfer of these antimicrobial resistance genes from recipient E. coli is low.

E. coli expression strains BL21(DE3) have been engineered to contain a genomic insert capable of expressing T7 polymerase. This gene is under the control of a lac promoter and expression is induced on addition of IPTG. In the presence of T7 polymerase, DNA expression is induced from T7 promoter sequences within the constructed vector (pET plasmid). This leads to expression of the inserted mycoplasma DNA. It is unlikely that these fragments will be expressed within these strains outside laboratory conditions. In addition, it is very unlikely that the level of expression observed within the laboratory will be induced from these vectors should they be accidentally transferred to wild type hosts in the environment.

It is extremely unlikely that the expression of one mycoplasma protein within a disabled E. coli host strain would result in a virulent genetically modified organism (GMO), since BL21 strain is ACDP 1 and candidate proteins will not confer E. coli with virulence or toxigenic properties. In addition, signal peptides within recombinant protein(s) will be removed at the gene synthesis stage to prevent transportation to the cell-surface in the GM E. coli, and thus removing the risk of GM E. coli acquiring additional functions such as adhesion. Furthermore, risks associated with recombinant protein function may be reduced by production of truncated proteins/peptides rather than full length proteins. Where appropriate, truncated protein/peptides with superior immunogenic properties will be favoured over native full-length proteins, and as such, may reduce/remove recombinant protein/peptide function through loss of tertiary conformation or regions critical for function. Risks of these GMOs to the operators and environment will be controlled and minimised.

Evaluation of foreseeable effects

We anticipate mycoplasma proteins with immunogenic properties applicable to serological diagnostic assays, will be lipoproteins expressed at the mycoplasma cell-surface with adhesion functionality to cells of the host animal (sheep and goat) cells. It is unlikely that the chosen mycoplasma protein(s) will confer virulence or toxigenic properties in recipient E. coli when produced recombinantly as mycoplasmas do not harbour typical bacterial virulence genes or, with a single exception, toxins. Only the human pathogen Mycoplasma pneumoniae (not used in this project) has been found to produce the community-acquired respiratory distress syndrome toxin (CARDS). Candidate genes encoding a hypothetical protein of unknown function will be assessed in terms of structural homology to known bacterial protein toxins using online search databases (blastP, Uniprot). Any candidate proteins with homology to known toxins will be further investigated and assessment of risks associated with planned protein expression will be undertaken to determine whether protein expression can be safely carried out. Every effort will be made to eliminate selection of mycoplasma genes encoding proteins with any kind of theoretical toxigenic function.

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under containment level 2 laboratory conditions, with restricted access to the laboratories where the work is
undertaken, only competent staff will undertake the work, personal protective equipment (including lab coat and
gloves) will be worn at all times, all liquid culturing will be conducted in a class II MSC to reduce inhalation of
aerosolised culture, with opening of centrifugation containers also performed insight a class II MSC. The design of the
DNA cloning and recombinant protein work takes into consideration measures to reduce risk to operators, and this
includes using disabled ACDP HG1 E. coli strains and non-mobilisation plasmids (deficient in conjugation encoding
genes). Live GM E. coli waste generated during this work will never enter the drainage system, it will be inactivated
with heat prior to disposal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All laboratory waste, which has had contact with the live GM E. coli created in this project will be sealed within sweet
jar containers until point of inactivation (BACO243, BAC5-RA016). All waste containers will be sprayed with 70%
ethanol for a contact time of 10 minutes prior to removal from MSC (validation reference: VI013), and will be
transferred to autoclave tins prior to being autoclaved on a cycle of 134°C for 1 hour sterilisation followed by
incineration (validation reference: VHI005).

Liquid waste generated in this work includes:
• GM E. coli culture (maximum of 2 x 400 mL per experiment)
• Culture supernatant (maximum of 2 x 400 mL per experiment)
• GM E. coli cell pellet lysate

Cultures will be contained in conical flasks or bottles with screw cap lids during shaking incubation. Small volumes of
liquid waste, up to 5 mL, will be placed inside sweet jar containers inside an MSC, and sprayed with 70% ethanol for
10 minutes contact time (validation reference: VI013) prior to removal from the MSC. The sweet jar will be placed
inside an autoclave tin and the contents inactivated on a cycle of 134°C for 1 hour (Validation of discard cycle in
autoclave, room 2, building 4, 2013), followed by incineration. Volumes of waste culture/ supernatant larger 40 mL but
less than 100 mL will be inactivated by autoclaving and then disposed of into the drainage system (autoclave
validation March 2013). Volumes of liquid waste >100 mL but 5250 mL will be transferred into 500 mL red-top pots
(GosselinTM Square High Density Polyethylene Bottles (Corning LC500-11)), and inside an MSC 7.5 g Lab-Sorb (or
appropriate weight to solidify volume of waste <250 mL) will be added to solidify the waste to a gel. The pots will be
closed and surface-decontaminated by spraying with 70 % ethanol, 10 min contact time) prior to removal from the
MSC. The pots will be placed inside autoclave tins prior to being autoclaved on a cycle of 134°C for 1 hour to
inactivate any viable GM E. coli (validation number VHI005 & autoclave validation April 2019), followed by
incineration.

Should a spillage occur inside/outside the MSC, procedures outlined in CBUO105 will be followed.
All general laboratory waste will be generated in a class II MSC and may include include:
• Paper towel
• Plastic packaging
• Paper
• Gloves (non-contaminated)

All general waste will be autoclaved on a cycle of 134°C for 1 hour sterilisation followed by incineration.
Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form Y

Tick to confirm that it is attached to this form N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<th>Growth Rooms</th>
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**Project Ref** 221/95.1a

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<td>DEVELOPMENT OF M.BOVIS VACCINES AND COMPANION DIAGNOSTIC TEST - GENERATION OF VACCINE CANDIDATES</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Ref: 221/95.1b

**Date Ackn'd**: 31/08/1995

**Project Title**: DEVELOPMENT OF M.BOVIS VACCINES AND COMPANION DIAGNOSTIC TEST - TESTING OF VACCINE CANDIDATES FOR BOVINE TUBERCULOSIS USING A LOW-DOSE AEROSOL CHALLENGE GUINEA PIG MODEL

**Class**: Class 3

**Culture Class**: CultureVolClass2 - Class 2

**Consent Granted**: yes

**Project notified under transitional arrangements**: Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref: 221/95.1c

Date Ackn’d: 22/02/2001

CU2 Project Title: GENERATION OF VACCINE CANDIDATES

Class: Class 2

CultureVolClass2

CultureVolumeClass3-4

02/03/2022
Date Project Ceased
29/09/2015

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Withdrawn

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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**Animal Units**

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**Project Ref** 221/95.1d

Date Ackn’dd: 22/02/2001

CU2 Project Title: TESTING TB VACCINES IN CATTLE: INTRODUCTION OF LUCIFERASE REPORTER GENE INTO M.BOVIS BCG

Class: Class 2

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 221/95.1e

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**CU2 Project Title**

DEVELOPMENT OF BADGER VACCINES: BCG AS A VEHICLE FOR DNA VACCINES

**Historical Significant Changes**

Tick if notifying a connected programme of work: N

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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If yes, tick to confirm that it is attached to this form N

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Large Scale Activities

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Human Clinical Applications

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Project Ref 221/95.2

Date Ackn'd 22/09/1995
CU2 Project Title PATHOGENESIS OF SALMONELLA SPECIES
Class 2
CultureVolClass2
CultureVolumeClass3-4 Class 2

02/03/2022
**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
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<tr>
<td>Non-GMMs</td>
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**Name**

SYNGENTA LIMITED

**Department**

AGROCHEMICALS

**Campus Estate or Research Centre**

JEALOTTS HILL RESEARCH STATION

**Road Name**


**District**


**Town**

BRACKNELL

**County**

BERKSHIRE

**Postcode**

RG42 6EY

**Country**

ENGLAND

**Tel Number**

01344 424 701

**Fax Number**

01344 455629

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

<table>
<thead>
<tr>
<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
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<tr>
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<td>ZENECA AGROCHEMIC ALS LTD</td>
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<td>BERKSHIRE</td>
<td>RG42 6E</td>
<td>ENGLAND</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
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Tick if confidential

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<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tbody>
<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>
Mycology

Transgenic Invertebrates

Transgenic Plants

Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 222/01.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<tr>
<td>16/08/2001</td>
<td>THE USE OF GM ASHYA GOSSYPII, TO INVESTIGATE THE MODE OF ACTION OF FUNGICIDES</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td>not applicable</td>
<td>Project notified under transitional arrangements</td>
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<table>
<thead>
<tr>
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<td>07/08/2007</td>
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</table>

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

GM Ashbya gossypii, either over expressing or not expressing (gene 'knockout' strains) certain Ashbya gossypii genes, will be used to identify potential pesticidal products. The GM Ashbya gossypii will be imported from the USA under a DEFRA licence (currently being applied for). All work will be carried out in the laboratory.

Recipient or parental organism

Ashbya gossypii is a minor plant pathogen of cotton and not pathogenic to humans. It naturally secretes riboflavin and is grown commercially to produce this vitamin. The over expression or deletion of specific genes is not expected to enhance its pathogenicity. It is not present in the UK so will be handled according to DEFRA licence requirements.

Host/vector system

The vector system used in the genetic modification of the Ashbya is a derivative of the pYES2.1-E vector from invitrogen. The genetic modification will be done in Syngenta labs in the USA and the GMMs imported into the UK for research use.

Origin & function

GMMs over expressing Ashbya genes from Ashbya promoters will be generated together with 'knockout' strains that are not expressing certain genes. The individual clones will be characterised in the USA and used in the UK for identification of potential pesticidal compounds.

Evaluation of foreseeable effects

The GM Ashbya will only be grown in contained facilities, in culture. It will not be used to infect plants. Therefore there are expected to be no environmental or health consequences from the proposed work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be used.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection of work surfaces etc. - Klercide disinfectant will be used to clean work surfaces. It is effective against non-GM Ashbya in culture both on new growth and on established cultures. The genetic modification is not expected to alter this.

Waste management - All waste from work with GM Ashbya will be double bagged within the laboratory for incineration at Jealott's Hill. Any liquid waste will be autoclaved in a facility within the building before being disposed of to foul drain. Information from the Local Rules on autoclaving and incineration on site are attached to this form.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

The Jealott's Hill GMSC reviewed this programme of work at a meeting on the 12th July. It is recommended that the work proceed after required notification to the HSE.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<td>L2 L3 L4 L2 L4 L2 L3 L4</td>
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<td>Human Clinical Applications</td>
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<td>L2 L3 L4</td>
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**Project Ref** 222/01.2

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<th>CultureVol Class</th>
<th>Consent Granted</th>
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</tr>
</thead>
<tbody>
<tr>
<td>22/11/2001</td>
<td>USE OF GM PLANT VIRAL SEQUENCES AS TRANSIENT GENE EXPRESSION VECTORS IN PLANT CELLS AND TISSUES AND GM VIRAL SEQUENCES TO BE CONSTRUCTED ELSEWHERE AND USED AT THE NOTIFIED FACILITY.</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td>not applicable</td>
<td>N</td>
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</table>

<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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<tbody>
<tr>
<td>22/12/2003</td>
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</table>

**Project Additional Information**

**Purposes of the contained use**

To develop a viral vector that can be used to amplify expression of foreign genes in targeted tissues of plants. All proposed work to be done in plant tissue culture.

**Recipient or parental organism**

The plant viral vectors will be used in E. coli, A. tumefaciens, plant cells and plants.
Host/vector system

The GM plant viral vectors will be constructed elsewhere (not in the UK) and used at the notified premises.

Origin & function

A few plant viral sequences from one type of plant viruses will be used and DEFRA licences will be obtained prior to use. Two types of viral vectors will be constructed elsewhere and tested in plant cells/tissues at Jealott's Hill:

1. Gene Replacement Construct: Viral vectors will be used with the essential genes (e.g. coat protein gene) and/or other ORFs replaced by another gene. Initially common reporter genes (eg GUS) will be used with various promoters. If the desired expression levels are achieved then effect genes of interest will replace the reporter genes.

2. Gene Insertion Construct: Viral vectors will be used with reporter genes inserted in any intergenic region of the viral genome. However, the starting vector will have had genes necessary for viral gene expression and regulation, efficient virus replication, host range and symptom development deleted. If the desired expression levels are achieved then effect genes of interest will replace the reporter genes.

Evaluation of foreseeable effects

Human health considerations:
The viruses are plant viruses and do not pose any risk to human health. Any effect genes expressed by the viral sequences will not alter the risk; prior to use, sequences will be screened for homology to known toxins/potential allergens.

Environmental considerations:
The wild type viruses have specific host plants and specific insect vectors of transmission. The insects required for transmission between plants do not occur naturally in the UK and the GM viral sequences will only be used in the contained environment with any DEFRA licence restrictions applied. Thus the liklihood of a specific insect being able to transfer the virus outside of the containment facility is negligible. In addition to this, all the viral vectors have had some or all of the essential viral genes replaced, deleted or interrupted and will not be able to spread from the inoculated site even if the required insect was present. The vector will stay within the plant cells and tissues, replicating and expressing the genes of interest leading to a localised presence of the protein.

Note that there is no intention to use wild type virus or viral particles, only double stranded viral sequences, cloned in the pUC plasmid or Agrobacterium binary vectors will be used as controls.

Conclusion: Given that the planned work is in the contained environment, essential viral sequences for infection and dissemination in the environment are missing and that transmission of the viruses requires specific non-indigenous host insects, the risk to the environment from this work can be considered to be negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Plant cells and tissues will be used in tissue culture and contained growth chambers. Only authorised researchers can access the tissue culture laband the contained growth chamber.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied. DEFRA licence requirements will be met.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will end up in the site incinerator and all liquid waste will be autoclaved prior to disposal to drain. Solid waste will be collected within the laboratory, double bagged and sealed before transfer to our secure waste compound for incineration. Information on incineration and autoclaving is attached to this form - both are expected to give effectively 100% kill of any GMOs.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The Jealott's Hill GMSC has reviewed the risk assessment. The committee accepted the original risk assessment with some changes. These changes have been agreed by the GMSC and incorporated into the document submitted to the HSE.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tbody>
<tr>
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<td>L4</td>
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<table>
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<th>Human Clinical Applications</th>
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</thead>
<tbody>
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Project Ref  222/02.1

Date Ackn'd  21/02/2002

Date Project Ceased  14/07/2009

Withdrawn  N

Consent Granted  not applicable

Tick if notifying a connected programme of work  N

Project notified under transitional arrangements  N

Project Additional Information

USE OF AGROBACTERIUM RHIZOGENS ON DICOTELYDONs TO GENERATE HAIRy ROOTS

Class 2

Consent Granted

< 1 litre

Class Culture Vol Class 2

Volume Class 3-4

Not applicable

Project notified under transitional arrangements

02/03/2022
Purposes of the contained use

The objective of the work is to develop a rapid assay to test nematocidal genes for efficacy against nematodes in plant roots. Hairy roots will be generated using \textit{Agrobacterium} rhizogenes containing genes coding for potentially nematocidal proteins. The GM hairy roots containing the genes of interest will then be subject to analysis.

Recipient or parental organism

The assay requires the use of \textit{Agrobacterium} rhizogenes. This micro-organism is a plant pathogen and is covered by an existing licence issued by DEFRA (PHL 32F/3787).

Host/vector system

Binary vectory will be constructed in E coli K12 and then transferred to armed \textit{A} rhizogenes for plant transformation. The plant material will be tomato, tobacco, potato and soybean.

Origin & function

Potentially nematocidal proteins have been identified from fungal material (not plant or human pathogenic material). The identification was achieved by challenging nematodes with various extracts from the original organism. The proteins were isolated and identified. The genes encoding these proteins have been cloned and this project is to develop a rapid assay to gauge the effectiveness of these genes in a plant tissue system. Plans will be transformed with \textit{A} rhizogenes to generate hairy roots. Nematode bioassays will then be done on the hairy root material to determine which genes should be developed further. The further development will not involve \textit{A} rhizogenes; plant transformation to generate plantlets will be done using disarmed \textit{A} tumefaciens. In addition to the nematocidal genes, reporter genes will be used, for example GUS and red fluorescent protein (from reef coral, \textit{Discosoma} sp.).

Evaluation of foreseeable effects

Human health considerations.
\textit{A} rhizogenes is a plant pathogen and does not infect humans. Therefore no adverse effects are expected from its use. All sequences of genes to be used and expressed in the hairy root system will be put through a database to identify potential toxins/allergens prior to use. Any genes identified as potentially allergenic/toxic will not be progressed further.

Environmental considerations:
\textit{A} rhizogenes is a gram-negative, soil-borne, motile plant pathogenic bacteria and infection with this bacterium causes hairy root disease. In nature, hairy root disease has been observed on a limited number of plant species (eg apple, cucumber and melon). Therefore the natural host range is restricted, as is the economic and agricultural impact, although outbreaks have occurred in the UK. In contrast, artificial inoculation in the laboratory has been successful on a wide number of plant species and is expected to be successful on essentially all angiosperms and gymnosperms. The wild type bacteria naturally infect plants at wound sites and transfer part of their large plasmid, Ri, to the chromosomal DNA of the plant cell. Reproduction is via cellular division only - the bacteria are non-sporulating. Therefore there is no risk from spore dispersal in air. Infection in nature usually occurs at wounds, for example graft sites, and spreads via contaminated garden/farm tools. Aseptic techniques will be used for all manipulations to prevent cross contamination and spread of \textit{A} rhizogenes.

\textit{A} rhizogenes is susceptible to a wide range of antibiotics and, after transformation, an antibiotic will be used to clear the hairy roots of micro-organisms prior to being used for the nematode bioassay. The genes of interest will be screened on databases and will not be used if homology is found to genes involved in the induction of the hairy root phenotype. Similarly, if nematocidal genes of interest are isolated from species that are pathogenic to plants then DEFRA advice will be sought before their introduction into the hairy root system.

Conclusions:
The planned work does not pose a risk to human health. \textit{A} rhizogenes is a plant pathogen and can infect plant species grown in the UK. It can be spread via contaminated soil, water or media. The proposed work will all be done in vitro using aseptic techniques. In addition, DEFRA licence conditions will be adhered to. Thus the risk to the environment from this work is considered to be negligible.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Plant material, ie hairy roots, will be kept in containment level 2 facilities and the conditions in the DEFRA licence will be adhered to.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied. DEFRA licence requirements will be met.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will end up in the site incinerator and all liquid waste will be autoclaved prior to disposal to drain. Solid waste will be collected within the laboratory, double bagged and sealed before transfer to our secure waste compound for incineration. Information on incineration and autoclaving is attached to this form - both are expected to give effectively 100% kill of any GMOs.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Jealott's Hill GMSC has reviewed and accepted the risk assessment.

Project Containment

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 222/03.1

Date Ackn'd 03/01/2003

CU2 Project Title THE USE OF GENETICALLY MODIFIED PHYTOPHTORA INFESTANS IN IN VITRO

Class 2

CultureVolClass2 < 1 litre

CultureVolumeClass3-4
The overall objective of this work is to investigate the mode of action of novel fungicides by using genetically modified Phytophthora infestans.

The GM P infestans strains fall into 3 groups:

1. P infestans genetically modified to over express specific P infestans genes. These strains will be used to identify potential inhibitors of those genes.
2. P infestans genetically modified such that certain genes are down regulated by a gene silencing mechanism. These strains will be used for helping to determine the mode of action of novel fungicides as well as helping to identify whether a gene is responsible for sensitivity or resistance to a particular fungicide.
3. P. infestans genetically modified to express the B-glucuronidase or fluorescent proteins fused to P. infestans proteins. These strains enable the P infestans protein to be visualised on application of a fungicide. This will help to determine the mode of action of novel fungicides.

P. infestans is an indigenous pathogen of Solanaceous plants, including potato and tomato and is found commonly throughout the UK. The pathogen overwinters as mycelium in infected plant material (eg potato tubers left in the ground after harvesting). On infection of young plants the pathogen spreads to the aerial parts where it produces sporangia on sporangiophores. These sporangia can become airborne or rain dispersed. The sporangia are large and heavy and are only viable for a short period of time (if up to 2 weeks) and in order for the disease to spread they must come into contact with a susceptible host. When the sporangia land on wet potato leaves or stems they then germinate and cause new infections. When sporangia land in water they produce zoospores that only survive for a few hours without a suitable host.

CDNA libraries of P infestans sequences in E. coli will be imported from Syngenta laboratories in the USA. The sequences of genes of interest are known and will be used to screen the libraries. These sequences will then be cloned into the vectors for transformation into P infestans.

P infestans is genetically modified via zoospore or protoplast transformation and the transgenes are stably integrated into the genome. GM strains will be imported from the Netherlands and the USA. In addition, the methodology for transforming P infestans will be implemented on site.

The vectors used will be non-mobilisable E. coli vectors, including pUC18/19, pBlueScript, pGEMT and genetin and hygromycin resistance vectors for co-transformation in P infestans under the control of promoters from Phytophthora species.
1. P infestans genetically modified to over express specific P infestans genes. P infestans genes will be inserted into the above vectors under promoters isolated from Phytopthora species.

2. P infestans genetically modified such that it expresses inverted repeats or antisense copies of certain genes leading to silencing of the native gene(s). Since Phytophthora species are diploid organisms and homologous integration of plasmids has not yet been demonstrated, a gene silencing strategy is the best alternative to inhibit gene expression in this plant pathogenic oomycete. The vectors described above will be used with P infestans sequences inserted in antisense orientation.

3. P infestans genetically modified to express B-glucuronidase and fluorescent proteins fused to P infestans proteins. The genes expressing fluorescent proteins have been isolated from various marine reef anemones and corals and the B-glucuronidase gene has been isolated from E. coli. They are commonly used reporter genes enabling visualisation of proteins. The gene fusion will be inserted into the vectors described above.

Origin & function

1. P infestans genetically modified to over express specific P infestans genes.
Origin of material: P infestans plus commonly used marker genes.
Function: The intention is that the over expression of certain genes will lead to an increased amount of the corresponding protein being present in the cells. The strains will be used to identify potential (chemical) inhibitors of the over expressed genes.

3. P infestans genetically modified to express B-glucuronidase and fluorescent proteins fused to P infestans proteins.
Origin of material: P infestans, various marine reef anemones and corals (eg Zozenthus sp., Anemonia majano and Anemonia sulcata) and E. coli.
Function: The fluorescent proteins and GUS are commonly used reporter genes enabling visualisation of proteins. Fusion with P infestans proteins will enable visualisation of proteins when the GM P infestans is challenged with a potential fungicide.

The sequences of P infestans that will be regulated will be those essential for growth, or whose gene products show high affinity to small molecules (fungicides), or that have been identified by classical mutagenesis as being required for the method of action of the chemical which is being investigated.

2. P infestans genetically modified such that it expresses inverted repeats or antisense copies of certain genes leading to silencing of the native gene(s).
Origin of material: P infestans plus commonly used marker genes.
Function: The intention is that the antisense sequences from P infestans will silence the native genes. These strains will be used for helping to determine the mode of action of novel fungicides as well as helping to identify whether a gene is responsible for sensitivity or resistance to a particular fungicide.

Evaluation of foreseeable effects

Risks to human health:
The comycete P. infestans is an indigenous plant pathogen in this country. The pathogen is unable to colonise or infect even immunocompromised humans. However, all introduced protein sequences will be screened for potential allergenicity/toxicity prior to the strains being grown. Any information that shows a cause for concern will be included in the COSHH assessment and the control measures revised.

Risks to the environment:
There are several scenarios that may affect the pathogenicity of the GM P infestans. For example, if a gene involved in the infection process is turned off then a plant may not recognise that it has been infected; this could lead to an increased host range for the pathogen. Conversely, if a gene required for infection is turned off then the pathogen will be less virulent. There are equally valid scenarios for strains containing genes that have been over expressed. For strains expressing the fluorescent protein it is unlikely that pathogenicity will be increased, as the GMM will be putting extra resource into the production of the extra protein.

The above scenarios rely on the GMMs coming into contact with a suitable host plant. Therefore the implementation of appropriate control measures (containment level 2 plus DEFRA requirements) to reduce the likelihood of this happening (ie the opportunity for infection of potential hosts by the GMMs) means that the overall risk of this work to the environment can be considered to be low.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be autoclaved prior to disposal to drain. Solid material will be bagged up and incinerated on site. These procedures will give essentially 100% kill of viable material.

Decontamination of work surfaces will be done using Klercide 5TM. In a kill test this disinfectant has been shown to be effective against non GM P infestans.

Is an emergency plan required according to regulation 20?  

Y

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The GMSC has reviewed this document and, subject to some changes that have been incorporated into the final documentation, has approved the programme of work.

Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<td>L2</td>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Project Ref 222/03.2

Date Ackn'd 20/11/2003

CU2 Project Title THE USE OF GENETICALLY MODIFIED MYCOSPHAERELLA GRAMINICOLA (M. graminicola) in the control of cereal diseases

Class 2

CultureVolClass2 < 1 litre

CultureVolumeClass3-4

02/03/2022

Page 5341 of 15326
The overall objective of this work is to investigate the mode of action of novel fungicides by using genetically modified M. graminicola. The strains will be generated on site and/or imported from the EU, Switzerland the the USA.

The GM M graminicola that we wish to use falls into 3 types:

1. M graminicola genetically modified to over express M graminicola genes. These strains will be used in the characterization of potential inhibitors of those genes.
2. M graminicola genetically modified such that certain genes are down regulated by disruption, mutation or replacement with a marker gene. These strains will be used to determine the mode of action of novel fungicides as well as helping to identify whether a gene is responsible for sensitivity or resistance to a particular fungicide.
3. M graminicola genetically modified to express the B-glucuronidase or fluorescent proteins or fluorescent proteins fused to M. graminicola proteins. These strains enable the M. graminicola protein to be visualised and on application of a fungicide will support the determination and characterisation of the mode of action of novel fungicides.
M. graminicola genetically modified to over express M. graminicola genes. Initially we wish to insert fusion proteins of a B tubulin from M. graminicola with fluorescent proteins. However, included in the scope of this project are other M. graminicola genes yet to be identified. These genes will be identified in E. coli and prior to transferring to M. graminicola the sequences will be screened for homology to known avirulence or virulence genes. Any positives will not be used, as there is no intention to deliberately generate GM M. graminicola that could be of harm to the environment. These strains will be used in the characterisation of potential inhibitors of those genes.

M. graminicola genetically modified such that certain genes are down regulated by disruption, mutation or replacement with a marker gene. These strains will be used to determine the mode of action of novel fungicides as well as helping to identify whether a gene is responsible for sensitivity or resistance to a particular fungicide.

M. graminicola genetically modified to express the B-glucuronidase or fluorescent proteins or fluorescent proteins fused to M. graminicola proteins. These strains enable the M. graminicola protein to be visualised on application of a fungicide and will support the determination and characterisation of the mode of action of novel fungicides. Initially the a and b tubulin genes will be used. However, as described above, other M. graminicola genes are included in the scope of the project.

Evaluation of foreseeable effects

M. graminicola is not known to cause harm in humans and is not recognised as a human pathogen. Given the planned genetic modifications, it is highly unlikely that the GM M. graminicola that we intend to generate will alter this. Note that all new proteins expressed in M. graminicola will be checked for homology to known toxins and allergens prior to use.

There are two main scenarios by which GM M. graminicola could cause harm to the environment. Both of these scenarios require the GMM to escape from containment. We will be using ACGM Containment Level 2 in addition to DEFRA licence requirements. Yeast like cells or mycelium (which are not airborne) will be used and, assuming that they survived the transfer from containment to outside and found a suitable host plant, they would have to be particularly virulent/pathogenic to survive and establish in the well managed situations at Jealott's Hill (ie within our glasshouses or grounds). We therefore consider the likelihood of escape as very low.

* Transfer of genetic material to WR M. graminicola or other related micro-organisms outside of containment leading to increased pathogenicity/virulence and environmental harm. In order for this to happen the GMM would have to be in the right stage of the growth cycle, meet a compatible micro-organism and locate a suitable host plant. Genetic transfer can only occur between the sexual forms of M. Graminicola and we will be using the yeast like cells and/or mycelium. In addition different mating types would have to meet on a suitable host plant. Therefore together with the very low likelihood of escape and survival we consider the likelihood of the GMM transferring genetic material outside of containment as very low.

* Establishment of GM M graminicola outside of containment, wither in the Jealott's Hill glasshouses or in the surrounding grounds. This requires the escape of a GMM with increased pathogenicity/virulence and for that GMM to reach a suitable host. Given that Jealott's Hill is a highly managed environment and that we employ experienced plant pathologists we believe that the likelihood of the GMM escaping containment and establishing itself in the environs of Jealott's Hill can be considered to be very low. However, in the highly unlikely situation of this happening we will know about it.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be autoclaved prior to disposal to drain. Solid material will be double bagged and incinerated on site. These procedures will give essentially 100% kill of viable material.

Decontamination of work surfaces will be done using Klercide 5TM. In a kill test this disinfectant has been shown to be effective against non GM M. graminicola.
The GMSC considered the risk assessment at a meeting on the 13th November. The project was approved as submitted.

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered the risk assessment at a meeting on the 13th November. The project was approved as submitted.

Project Containment

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Animal Units

L2 L3 L4 L2 L3 L4 L3 L4

Large Scale Activities

L2 L3 L4 L2 L3 L4 L2 L3 L4

Human Clinical Applications

L2 L3 L4 L2 L3 L4 L2 L3 L4

Project Ref 222/05.1

Use of genetically modified plant pathogen to help identify novel compounds with fungicidal activity.

Date Ackn’d 22/08/2005

CU2 Project Title

Class 2

Consent Granted

< 1 Litre

Non-GMM Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Use of genetically modified plant pathogen to help identify novel compounds with fungicidal activity.

Recipient or parental organism

M. graminicola is the causal agent of Septoria Tritici leaf blotch of wheat. It is an indigenous pathogen and is present throughout the UK. The disease is characterised by the development of irregular chlorotic lesions that become necrotic in time. The penetration frequency of germinated conidia on wheat leaves is in general low (Rohel et al., Mol Plant Microbe Interact, 2001, 14:156-163). Inside the necrotic lesions the asexual pycnidia and/or the sexual pseudothecia develop. The teleomorphic pseudothecia can be found throughout the growing season and M. graminicola can complete a sexual cycle in the field within five weeks of infection (Hunter et al., Plant Pathol, 1999, 48:51-57). This implies that spread of the disease does not only depend on splash dispersal of asexual pycnidia spores but is also caused by a constant supply of ascospores (Shaw and Royle, Plant Pathol, 1989, 38:35-43). Recent studies revealed that M. graminicola populations are highly diverse and suggested that sexual reproduction has a major influence on the gene structure of M. graminicola populations (McDonald 1999). The sexual pseudothecia is considered to be largely responsible for the oversummering of the disease in regions where crop rotation is a common agricultural practice.

Origin & function

M graminicola is not known to cause harm in humans and is not recognised as a human pathogen. It is highly unlikely that the GM M. graminicola that we intend to use will alter this status.

There are two main scenarios by which GM M. graminicola could cause harm to the environment. Both of these scenarios require the GMM to escape from containment. We will be using ACGM Containment Level 2 in addition to DEFRA licence requirements. Yeast like cells, asexual conidia or mycelium will be used for experimental purposes. Asexual pycnidia and pycnidiospores may be produced on detached leaf pieces. However pycnidiospores are released in a mucilaginous cirrus and are not air-dispersed. Assuming that they survived the transfer from containment to outside and found a suitable host plant, they would have to be particularly virulent/pathogenic to survive and infect in the absence of an extended dew period and establish in the well managed situations at Jealott's Hill (ie within our glasshouses or grounds). We therefore consider the likelihood of escape as very low.

Evaluation of foreseeable effects

M graminicola is not known to cause harm in humans and is not recognised as a human pathogen. It is highly unlikely that the GM M. graminicola that we intend to use will alter this status.

There are two main scenarios by which GM M. graminicola could cause harm to the environment. Both of these scenarios require the GMM to escape from containment. We will be using ACGM Containment Level 2 in addition to DEFRA licence requirements. Yeast like cells, asexual conidia or mycelium will be used for experimental purposes. Asexual pycnidia and pycnidiospores may be produced on detached leaf pieces. However pycnidiospores are released in a mucilaginous cirrus and are not air-dispersed. Assuming that they survived the transfer from containment to outside and found a suitable host plant, they would have to be particularly virulent/pathogenic to survive and infect in the absence of an extended dew period and establish in the well managed situations at Jealott's Hill (ie within our glasshouses or grounds). We therefore consider the likelihood of escape as very low.

* Transfer of genetic material to WT M. graminicola or other related micro-organisms outside of containment leading to increased pathogenicity/virulence and environmental harm. In order for this to happen the GMM would have to be in the right stage of the growth cycle, meet a compatible micro-organism and locate a suitable host plant. Genetic transfer can only occur between the sexual forms of M. graminicola and we will be using the yeast like cells and/or mycelium. In addition different mating types would have to meet on a suitable host plant. Therefore together with the very low likelihood of escape and survival we consider the likelihood of the GMM transferring genetic material outside of containment as very low.

* Establishment of GM M graminicola outside of containment, either in the Jealott's Hill glasshouses or in the surrounding grounds. This requires the escape of a GMM with increased pathogenicity/virulence and for that GMM to reach a suitable host. Given that Jealott's Hill is a highly managed environment and that we employ experienced plant pathologists we believe that the likelihood of the GMM escaping containment and establishing itself in the environs of Jealott's Hill can be considered to be very low.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full ACGM Containment Level 2 under the 2000 regulations will be applied. Note that waste will be inactivated outside of the building so derogation will be needed under the proposed revised regulations.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be autoclaved prior to disposal to drain. Solid material will be double bagged and incinerated on site. These procedures will give essentially 100% kill of viable material. Decontamination of work surfaces will be done using Klercide 5TM. In a kill test this disinfectant has been shown to be effective against non GM M. graminicola.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

On 14th July 2005 the GMSC reviewed and accepted the risk assessment without any technical changes.

Project Containment

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Project Ref 222/07.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

28/11/2007 Development of a protein expression system using genetically modified Class 2 < 1 litre
The objective of this work is to generate genetically modified Mycosphaerella graminicola (lvi graminicola; anamorph Septoria Irifid), which can be used as a protein expression system. Ultimately proteins produced mM graminicola will be used in assays to help investigate the activity and mode of action of novel fungicides. This proposal aims to use the NS1 protein from a variety of influenza A and B virus strains to block the gene silencing machinery of the recipient cells, thus providing an enhancement of heterologous protein expression above current levels.

M. graminicola is the causal agent of Septoria tritici leaf blotch of wheat. It is an indigenous pathogen and is present throughout the UK. The disease is characterized by the development of irregular chlorotic lesions that become necrotic. In time. Under field conditions lv! graminicola over winters as mycelia or pycnidia in infected plants, seeds or field debris. The primary source of inoculate is asexual conidia These are released from the pycnidia by rain, dew or irrigation and splash dispersed to infect leaves. The conidia germinate on the leaf surface, infect the leaves and grow intercellularly within the leaf During this lime necrotic lesions aprar. The fungus produces pycnidia which release conidia and the disease cycle continues (Agrios 1997, Plant Pathology, 4 edition). The penetration frequency of germinated conidia on wheat leaves is in general low (Rohel et al., Mol Plant Microbe Interact, 2001, 14:156-163). During its lifecycle sexual pseudothecia are also produced. These can be found throughout the growing season and M graminicola can complete a sexual cycle in the field within five weeks of infection (Hunter et al., Plant Pathol, 1999, 48:51-57). This implies that spread of the disease is dependant on both the splash dispersal asexual pycnidiaspores and also a constant supply of sexual ascospores (Shaw and Royle, Plant Pathol, 1989, 38:35-43). In laboratory conditions, i.e. in vitro culture, or when infecting plants in the glasshouse or controlled environment conditions, lv! graminicola is not able to undergo sexual reproduction.

Initially, well characterised strains of M graminicola originally imported from the EU will be used. However, ultimately this project may necessitate the use of lab strains imported from Switzerland and the USA. Work with non UK strains will be carried out under an existing Plant Health Licence issued by DEFRA.

M. graminicola will be genetically modified using the Agrobacterium mediated transformation method as reported by Zwiers and De Waard (Cun Genet 2001,39:388-393). Agrobacterium tumefaciens (LBA4404, LBA1 126, GV3 101, LBA1 100, EHA105 and pAG L-l), disarmed by deletion of the phytohormone genes from the Ti plasmid, will be used. A. tumefaciens vectors pGreenFl, pNOV2114, pCGN based and pBINI9 based will be used.
The work will focus on generating 2 groups of M. graminicola, both groups containing sequences derived from influenza NS1 genes with the function of these sequences being assessed by the expression of model proteins, for example, fluorescent proteins or β-galactosidase. NS1 proteins have previously been shown to enhance the expression of GFP in plants by counteracting a host RNA silencing response (Bucher E et al J Gen Virol. 2004 Apr, 85 983-91). The main focus of the work will be the generation of M. graminicola expressing whole NS1 genes from several strains of influenza virus, both human and avian. The NS1 protein has been implicated in counteracting the diverse range of host systems within cells involved with limiting infection by the parent virus. The protein does not function outside the confines of the host cell and is just one of the 11-12 proteins and only one of the 8 RNA components that make up an influenza virus. Therefore the proposed use of NS1 will not allow the reconstitution an influenza virion.

In addition to using whole NS1 genes, M. graminicola will be generated using NS 1 sequences altered to optimise their ability enhance protein expression in the host. For example, using combinations of point mutations, transferring domains between different NS1 proteins or a combination of both techniques.

When generating both sets of GM M. graminicola, genes encoding model proteins will be co-transfected into the cells thus allowing assessment of the effectiveness of the various NS1 containing constructs.

### Evaluation of foreseeable effects

**Environmental considerations:**

It is unlikely that modification of At. ruminwola to express the viral antagonists of gene silencing will alter pathogenicity, host range or tissue tropism. Preliminary work done at Imperial College investigated the effect of a panel of NS1 genes on both homologous and heterologous protein products in transient transformation of mammalian cells. The NS1 was able to up-regulate GFP protein production in mammalian cells, but this did not affect the levels of Vinculin — a typical host protein produced in the cell. This data suggests that NS1 will enhance the expression of heterologous proteins, but not the host’s own proteins in a eukaryotic system. Although this mechanism in mammals is poorly understood, applicable work in plants (Bucher et al. 2004) suggests this enhancement is brought about by the protection of the over expressed coding mRNA for the foreign gene through the NS 1 RNA binding domain, which prevents recmitment to the RNA silencing machinery, which would cause a specific RNA specific silencing action to occur. Therefore the presence of NS1 should not affect native At. ruminicola protein expression and therefore the resulting pathogenicity of the recipient, as these host genes are not nonnally a target for the endogenous RNA silencing apparatus.

In addition, for strains expressing the fluorescent protein it is unlikely that pathogenicity will be increased, as the GM M. graminicola will be putting extra resources into the production of the additional protein. In this work, it is probable that should the NS 1 successfully enhance the production of the chosen marker protein, this will further weaken the M. graminicola.

There is the potential that the NS 1, in antagonizing gene silencing will bring about an alteration in the control of transposons which are controlled by RNAi gene silencing mechanisms in eukaryotic cells. These mechanisms exist to control and limit the rate of gene transfers, It is therefore possible that transposon activity may increase in the presence of NS 1, however this is more likely to have a detrimental affect on the M. graminicola genome as genes are very likely to become disrupted. It would be highly unlikely that a favorable gene constellation will arise from the decrease in RNA silencing abilities.

Genetic transfer to other micro-organisms.

In order for genetic material to be transferred to related micro-organisms the GM At graminicola must be capable of transferring genetic material. In practice this would require At ruminicola to escape from containment into the environment; come into contact with a suitable host, meet a compatible recipient micro-organism and be in the right stage of the growth cycle for the transfer to occur.

Genetic transfer can only occur between the sexual forms of At graminicola. It has a heterothallic, bipolar mating system (Kema et al, Curr Genet, 1996, 30:25 1-258) which means that a sexual cross can only happen if two At graminicola isolates with different mating types meet. Asexual yeast like cells and mycelial forms will be used for this project. At graminicola cannot undergo sexual crosses in the laboratory or when infecting plants grown in the glasshouse or controlled environment conditions.

GM M. graminicola will be grown at ACGM containment level 2 with any extra containment required under the Plant Health Licence covering the host organism. At graminicola will be grown in vitro on agar media in path dishes kept in an airtight plastic container, in liquid media in flasks contained within a growth cabinet or inoculated onto leaf pieces on agar in petri dishes. All plates will be lightly taped shut to prevent loss of the sample should the plate be dropped. All of the work with GM At graminicola will be carried out in a Class 2 microbiological safety cabinet (MSC) that has a HEPA filter on the extract air, in the labs specified on the SOP for the handling of plant pathogens licensed by DEFRA.

From the above it can therefore be assumed that the likelihood of escape of GM At graminicola capable of further infection is very low. However, if it did escape from...
Containment it would have to reach a suitable host in order to establish itself. The closest plants would be either in our own glasshouse or in surrounding fields belonging to Syngenta. Assuming that the yeast-like cells or mycelium survived the transfer outside of containment, the GM At graminicola would have to be particularly virulent/pathogenic in order to establish itself in these well-managed situations.

Therefore, given the control measures and reasons above the likelihood of the GM At graminicola escaping and thus being able to transfer genetic material to related micro-organisms can be considered to be very low. In addition, the likelihood of generating a more pathogenic At graminicola is considered to be low.

Human health considerations: At graminicola is an indigenous plant pathogen in this country. The pathogen is not known to colonise or infect even immuno-compromised humans and therefore poses very little risk to human health. In eukaryotic plant cells, the NS 1 protein acts to sequester double stranded RNA, caused by heterologous gene expression, which would normally be recruited by the IRNA silencing complexes in the cell to generate a specific response to these foreign genes and silence them. (Bucher et al. 2004). It is unlikely that this protein will have an effect on host genes. The protein will not be secreted by the At graminicola and would therefore need to cross the cell wall, effect an entry to the body, and then enter a cell in order for it to act upon exogenous genes. Containment within the At graminicola cell walls and working within microbial safety cabinets when generating aerosols will deny a route of entry to the body.

The NS1 gene has been expressed transiently by several groups in mammalian cell culture, and also been expressed and purified for crystallographic study, with no suggestion or reports of an increased level of toxicity or allergenicity.

The NS1 protein on its own is not infectious and constitutes just one component of the 11-12 necessary components to make an influenza virus. Therefore there is no chance of reconstituting a live virus from this work.

Given the information above and the containment described for environmental protection the risk to human health from carrying out this work is expected to be low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full ACOM Containment Level 2 ll be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste containing experimental material will be double bagged within the laboratory and placed within bins for collection and transfer to the site autoclave facility. Waste will then be autoclaved with the sterilisation phase being at 122°C for 30 minutes and a pressure of 2150mBar. This has been validated locally with a variety of plant pathogens giving 100% kill.

Liquid waste will be collected and autoclaved at 134°C for 20 minutes in an autoclave within the building in which it is generated.

Klercide disinfectant spray will be used for decontamination of work surfaces. It has shown to be effective against actively growing M graminicola.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
The Use of Genetically modified Phytophthora infestans to visualise the effects of fungicides on target genes

Genes native to Phytophthora infestans will be tagged with a gene for green fluorescent protein and then inserted into Phytophthora infestans. The genes to be tagged will be fungicide targets and this process will enable the visualisation of the effects of the fungicides on the resultant GMM in real time and in vivo. The initial work will involve tagging the genes for tubulin, the products of which form part of the cytoskeleton in oomycetes. If this method of visualisation is successful then the same strategy may be adopted for other fungicide gene targets in Phytophthora infestans. The transformation of Phytophthora infestans will be done at the Scottish Crop Research Institute in Dundee with constructs cloned in E. coli K12 provided by Syngenta. The transformants will then be transferred to Syngenta for the application of fungicides and subsequent visualization of the fluorescent protein. If the project is successful then Syngenta may develop the capability of transformation in house. This Notification only covers work at Syngenta.
**Recipient or parental organism**

P. infestans is an indigenous pathogen of Solanaceous plants, including potato and tomato, and is found commonly throughout the UK. The pathogen overwinters as mycelium in infected plant material (e.g., potato tubers left in the ground after harvesting). On infection of young plants the pathogen spreads to the aerial parts where it produces sporangi on sporangiophores. These sporangia can become airborne or rain dispersed. The sporangia are large and heavy and are only viable for a short period of time (up to 2 weeks) and in order for the disease to spread they must come into contact with a susceptible host. When the sporangia land on wet potato leaves or stems they then germinate and cause new infections. When sporangia land in water they produce zoospores that only survive for a few hours without a suitable host.

**Host/vector system**

Vectors will then be non-mobilisable E. coli vectors (including pTOR containing a kanamycin resistance gene for selection of transformants). The vector containing the gene of interest and the green fluorescent protein will be transformed into Phytophthora infestans using naked DNA and a polyethylene glycol/calcium chloride transformation procedure.

**Origin & function**

**Genes of interest;** these will be P infestans genes thought to be the targets for fungicides. Initial work will be done with tubulin genes. Genes will be screened against databases of known avirulence and virulence genes prior to use.

Green fluorescent protein: from Aequoprea coerulescens, this will be tagged to the gene of interest to enable visualisation of that protein when the GM P infestans is challenged with the fungicide.

Both the gene of interest and AcGFP will have the ham34 promoter and ham34 terminator from Bremia lactucae.

The nptll gene from Streptomyces kanamycticus will be used in the vectors to enable selection of transformants.

**Evaluation of foreseeable effects**

**Risks to human health:**

P. infestans is an indigenous plant pathogen in this country. The pathogen is unable to colonise or infect even immunocompromised humans. However, all introduced protein sequences will be screened for potential allergenicity/toxicity prior to the strains being grown. Any information that shows a cause for concern will be included in the COSHH assessment and the control measures revised.

**Risks to the environment:**

It is unlikely that the pathogenicity of GM P infestans will be greater than the parental organism due to the resource needed to produce the GFP. However, all genes of interest will be screened against databases of known avirulence and virulence genes prior to use. There is no intention to deliberately generating GMMs with increased pathogenicity.

In order for genetic material to be transferred to related mico-organisms the GMM must be capable of transferring genetic material, able to escape from containment, come into contact with a suitable host, meet a compatible recipient micro-organism and be in the right stage of the growth cycle for the transfer to occur.

Genetic transfer can only occur between the sexual forms of P infestans. A sexual cross can only happen if two isolates with different mating types meet. There is largely thought to be only one mating type present in the UK (mating type A1), although there are very rare cases of the A2 mating type occurring. In the unlikely event that the GMM escaped the chances of meeting a suitable mating type with which a sexual cross and genetic transfer could occur is therefore low.

All work will be carried out in a Class 2 Microbiological Safety Cabinet and cultures will be double contained when being transported. In addition a disinfectant which has been validated against P infestans will be used to clean work areaas. Together with the waste procedures below the likelihood of any escapes into the environment is...
considered to be low. Thus the overall risk of the work is considered to be low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be double bagged in autoclave bags and placed in purple wheelie bins specifically for waste which must be inactivated prior to leaving site. This includes all materials licensed by DEFRA and any Class 2 GMM waste. These bins are transferred to the Waste Management Compound and autoclaved at Syngenta. Autoclaved waste is then sent off site for incineration. Liquid waste is autoclaved within the same building in which it is generated prior to disposal to drain.

Decontamination of work surfaces will be done using Klercide 5TM which has been validated against P infestans

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The GMSC has reviewed the risk assessment and, subject to some changes that have been incorporated into the final document, has approved the programme of work

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<th>Glass Houses</th>
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Large Scale Activities

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Human Clinical Applications

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Project Ref 222/13.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
To generate GM plant pathogenic micro-organisms in order to investigate the role of individual genes in the mode of action of novel research compounds with potential fungicidal activity.

A genetic change is often responsible for conferring resistance to a fungicide. In this work we will generate defined genetic change resulting in single amino acid changes in a gene product, or, deletion, disruption, over-expression or silencing of a gene in three plant pathogenic species: Magnaporthe grisea, Fusarium graminearum and Phytophthora capsici. The genetically modified plant pathogens will be assessed for growth in the presence of known fungicidal compounds and novel non-commercially available research compounds. In addition the pathogens will be cultured for extraction of DNA, protein or other extracts for biochemical and molecular biology assays. Information from these tests will help deduce the mode of biological action of such compounds and ultimately contribute to the development of novel fungicidal compounds.

The GM plant pathogens produced will only be used for research purposes in contained laboratory experiments for this project. Transformation of the plant pathogens will take place at Syngenta, Jealotts Hill. However, we will also be importing plant pathogens similarly transformed from Switzerland, EU Member States or the USA. Both sets of GM plant pathogens will be subject to the same growth analytical experiments.

M. grisea causes blast disease of rice and wheat and the wheat blast disease is currently distributed only in tropical potential host plants and none of the proposed strains for use is known to infect wheat. The pathogen is able to overwinter in fields on crop debris. The inoculum is asexual conidia and sexual ascospores.

F. graminearum causes Fusarium ear blight of wheat, barley, maize, rye and oats. This disease is found throughout the UK. In the field the fungus survives over the winter on contaminated host debris and grains. The inoculum is asexual conidia and sexual ascospores which are distributed by wind and rain-splash and infect part of the flowers glumes and ears.

P. capsici causes rot on many tissues of a variety of host plants including tomato, green beans, peppers, pumpkins, melon, cucumber, and is found throughout Europe. The inoculum is sporangia or water-borne motile zoospores. The resting spores are a product of sexual mating.

Vectors for M. grisea and F. graminearum will be non-mobilisable E. coli TOPO-based and pUC-based cloning vectors. These are commonly used and commercially available, usually supplied by Invitrogen. Transformation will be done using a zoospore electroporation method.

Recipient or parental organism

Three plant pathogenic species will be used: Magnaporthe grisea, Fusarium graminearum and Phytophthora capsici. The unmodified strains originate form inside and outside of the UK. Strains originating from outside of the UK will be imported and used under Plant Health Licenses issued by DEFRA.

M. grisea causes blast disease of rice and wheat and the wheat blast disease is currently distributed only in tropical potential host plants and none of the proposed strains for use is known to infect wheat. The pathogen is able to overwinter in fields on crop debris. The inoculum is asexual conidia and sexual ascospores.

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P. capsici causes rot on many tissues of a variety of host plants including tomato, green beans, peppers, pumpkins, melon, cucumber, and is found throughout Europe. The inoculum is sporangia or water-borne motile zoospores. The resting spores are a product of sexual mating.
The pathogens will be transformed to modify genes identified as potentially conferring resistance to a research compound. Short sections of DNA with sequence similarity to a fungal or oomycete DNA locus of interest can be inserted into cells. The DNA of the chromosome is replaced by the DNA we put into the cell. In this way it is possible to create defined changes such as one or more of the following: replacement of sequences, deletion of sequences, insertion of antibiotic selectable markers or other elements such as promoters.

This method will also include introduction of a gene sequence containing point mutations that later result in amino acid changes when the DNA is translated into a protein. This approach can be used to test the genetic source of resistance to a research compound, either by inserting changes that will induce resistance, or by inserting changes that will remove resistance in an already resistant isolate.

**Evaluation of foreseeable effects**

**Risks to human health:**
The plant pathogens described are not listed in ACDP hazard groups 2, 3, or 4. There is no evidence that they can mate with human or animal pathogens.

*F. graminearum* is found throughout the UK, and although in rare cases some *Fusarium* species been reported to be capable of infecting immunocompromised humans, *F. graminearum* is not known to infect even immunocompromised humans and is therefore not believed to be a risk to human health.

*M. grisea* is not known to infect even immunocompromised humans and therefore is not believed to be of risk to human health.

The proposed genetic modifications are not expected to change any of the above information regarding risks to human health. However, as a precautionary measure introduced gene sequences will be screened for homology to known toxins and allergens. Any information that is a cause for concern will be included in the COSHH assessment and the control measures revised.

**Risks to the environment**

The proposed work does not involve deliberately trying to increase pathogenicity or host range of the plant pathogens. No fungicide targets to date have been associated with increased pathogenicity or host range, but are either core biochemical or essential structural components of the cell. While it is highly unlikely that the genetic modifications might alter the pathogenicity or host range, all modified genes will be screened against the current published data to try and identify any pathogenicity and host range issues. No genetic modification will proceed where there is published reason to believe that this will increase either virulence or host range of the plant pathogen.

In order for genetic material to be transferred to related micro-organisms the GMM must be capable of transferring genetic material, able to escape from containment, come into contact with a suitable host, meet a compatible recipient micro-organism and be in the right stage of the growth cycle for the transfer to occur.

The worst case scenario is that a research compound tested within this work is developed into a commercially available agrochemical and a GM plant pathogen made resistant to that agrochemical or mode of action is released. However, for this scenario to occur, the GM plant pathogen must first escape from the class 2 control measures. The class 2 control measures already in place have a strong history of containing fungal microorganisms, and are considered sufficient for the control of the plant pathogens listed in this risk assessment. Thus GM plant pathogens with resistance to the research compounds are unlikely to be released to the environment.

In the unlikely event that a release were to occur, the GM plant pathogen would have to survive dispersion of the distances necessary to reach a host plant.

- *F. graminearum* is dispersed by air-borne spores and if it escaped it would have to reach cereal-growing areas to come in contact with other *F. graminearum*. The timing of the infectious period is very small and would have to coincide with cereal anthesis, although overwintering on crop debris is also possible (Brown, Urban et al. 2010)
- *M. grisea* is not found in the UK, and therefore if it does escape, mating is highly unlikely. The spores of this plant pathogen would have to reach a cereal that it could infect. This plant pathogen occurs as host-limited forms, meaning some strains can only grow on some of the potential host plant species. The strains used at Jealott's Hill would have to reach rice or barley.
- In the case of *P. capsici*, the spores are water-borne, and therefore very limited in the distance they could travel. The motile zoospores are only mobile for a number of hours before becoming sessile and encysting. The resting spores are viable for a period of months, but have very low mobility (large and sessile).

Furthermore, the strains of the plant pathogens listed here are highly susceptible to and are controlled by currently employed agricultural crop protection chemicals. Even if
the GMMs were to escape control measures, preferential survival of the GMMs in the environment is highly unlikely. Therefore we consider the risk of GM plant pathogens resistant to commercially available fungicides being released into the environment and having an environmental impact to be very low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be double bagged in autoclave bags and placed in purple wheelie bins specifically for waste which must be inactivated prior to leaving site. This includes all materials licensed by DEFRA and any Class 2 GMM waste. These bins are transferred to the Waste Management Compound and autoclaved at Syngenta. Autoclaved waste is then sent off site for incineration. Liquid waste is autoclaved within the same building in which it is generated prior to disposal to drain.

Decontamination of work surfaces will be done using Klercide 5TM which has been validated against the pathogens listed in this notification.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC has reviewed the risk assessment and, subject to some changes that have been incorporated into the final document has approved the programme of work.

Project Containment

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Animal Units

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<th>Human Clinical Applications</th>
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<td>L2 L3 L4 L2</td>
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Project Ref 222/14.1

02/03/2022
The purpose of the proposed work is to establish a generic protein expression system in Botrytis cinerea for proteins of interest to the development of new fungicides. Expression of proteins from plant pathogens such as Botrytis is not reliable in heterologous systems so a system in the native organism using a native promoter will be used. Proteins of interest will be tagged, for example, with a histidine tag at the N or C terminus to allow easier purification of the target protein by exploiting the metal affinity nature of the histidine residues. This expression system will also be used to heterologously express fungal genes in Botrytis, for example, those form obligate pathogens i.e. that require a plant host in order to grow. Expression of tagged genes from other fungi in Botrytis will facilitate protein purification of these otherwise intractible target proteins. The GM Botrytis will be generated in Syngenta labs in Switzerland and imported for use in Syngenta labs in the UK.

Recipient or parental organism
The original strain of Botrytis cinerea is from Germany and so import and use will be done under Plant Health licences issued by DEFRA. Botrytis cinerea is a fungal pathogen of many plant species although the one used for this work (B05.10) is a laboratory strain so is unlikely to be identical to ones in the field.

Host/vector system
Botrytis cinerea will be genetically modified in Syngenta labs in Switzerland and imported to Syngenta labs in the UK for use. It will be transformed with specific PCR products of a construct containing the genes of interest. The constructs will contain a gene encoding the fungal protein of interest and its genomic flanking sequences. The sequence will be modified to include a tag such as 6-12 histidine residues to aid protein purification and a hygromycin resistance cassette to enable selection of the transformants.

Origin & function
Proteins which are potential fungicide targets will be identified and PCR constructs made of the relevant target gene sequences with the addition of a tag (e.g. histidine tag to allow easier purification of target protein by exploiting the metal affinity nature of the histidine residues) and a hygromycin resistance gene to enable selection of transformants on hygromycin. These GM strains will be used at Syngenta, UK to produce the protein of interest. The protein will be used to set up biochemical assays to aid the discovery of new fungicides and to optimise their intrinsic activity.

02/03/2022
**Evaluation of foreseeable effects**

Risks to human health: Botrytis cinera is a plant pathogen and is not known to colonise or infect humans. The genetic modification is not expected to alter this. The genes of interest, histidine tag and hygromycin resistance are not expected to cause harm in humans. Hygromycin is an antibiotic and not used for human treatment so even if there was a potential for spread of the resistance gene it would have a very limited impact in the UK. Thus the risk to human health from the proposed work is expected to be very low.

Risks to the environment: The most significant part of the Botrytis lifecycle for the proposed work is the release of conidia which will be present in the in vitro cultures. All manipulation of Botrytis cultures will be done in Class 2 microbiological safety cabinets and all cultures will be double contained when transported between facilities. Therefore the likelihood of escape from containment of a replicative body is highly unlikely. In the unlikely event of laboratory Botrytis strain is likely to be lower than that of other fungal pathogens in the environment and so it is unlikely to compete successfully and thrive. In addition, wild type Botrytis cinerea is present in the UK environment and is currently controlled by commercially available fungicides. The effectiveness of these fungicides on the GM strains is unlikely to be affected by the proposed modifications as these are in genes unrelated to the mechanism of action of these commercial fungicides. The surrounding land to the laboratories is farmed commercially by Syngenta and any fungal outbreaks are controlled by commercial fungicides. Thus the risks to the environment form the proposed strains of GM Botrytis cinerea are expected to be very low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None sought

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste from the proposed work will be destroyed as per Plant Health Licence requirements issued by DEFRA:

- Solid waste - solid waste will be collected locally and double bagged in autoclave bags. The waste will be placed in wheelie bins specifically for Plant health Licensed and Class 2 GMM waste and transported to our Waste Management facility for autoclaving prior to sending off site for incineration.
- Liquid waste will be collected locally and autoclaved within the building prior to disposal to foul drain.
- Equipment that has come into contact with Botrytis will be washed with 70% ethanol and centrifuge pots soaked in 1% Virkon overnight. Klercide will be used to decontaminate and clean work surfaces. All of these have been validated internally to be effective against Botrytis.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

The Jealotts Hill Biosafety Committee (GMSC) considered the risk assessment for the proposed work on 24th July 2014 and supported the project and its Notification to the Hse.
**Project Additional Information**

**Purposes of the contained use**

Recipient or parental organism

Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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GM Centre Number: 223

Data Premises Notified (Originally) 14/11/1986

Transferred from 1992 Regs? Y

Transitional Premises Class 3

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

UNIVERSITY OF GLASGOW CENTRE FOR VIRUS RESEARCH

Department

INSTITUTE OF INFECTION, IMMUNITY & INFLAMMATION

Campus Estate or Research Centre

COLLEGE OF MEDICAL, VETERINARY & LI

Building

GARSCUBE ESTATE

District

BEARSDEN

Town

GLASGOW

County

RENFREWSHIRE

Postcode

G61 1QH

Country

SCOTLAND

Tel Number 0141 330 5771

Fax Number 0141 330 5602

E-mail

HSE Division SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)       Tick if confidential

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<td>Plants</td>
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Please enter comments of the GM safety committee on the risk assessment

**Project Ref:** 223/01.1

<table>
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Withdrawn: N

Tick if notifying a connected programme of work: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

** Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 223/01.10

Date Ackn'd 04/06/2001  
CU2 Project Title IDENTIFICATION OF MOLECULAR DETERMINANTS OF VIRULENCE IN FELINE  
Class 2  
Culture Vol Class 2 < 1 litre  
Culture Volume Class 3-4
# Project Additional Information

## Purposes of the contained use

Chimaeric molecular clones of FIV will be generated to enable the molecular determinants of virulence in FIV infection to be defined.

## Recipient or parental organism

FIV is a member of the lentivirus subfamily of the Retroviridae and is a widespread pathogen of the domestic cat. Similar to other lentiviruses, FIV is a host specific, infecting only cats. The virus is transmitted naturally between cats via biting. For further information on the molecular clones, see section 17.

## Host/vector system

**Bacterial hosts** will be derivatives of E coli K-12 (eg XL-1 blue, Top10, Dh5a) and are considered disabled.

The plasmids are derived from pBR328 and are considered non-mobilisable.

## Origin & function

pG8-Mya is a clone of FIV-Glasgow-8 derived from an infected feline T cell genomic library. The FIV-GL8 clone will be genetically modified to introduce mutations representing nucleic acid sequences found in non-virulent isolates in order to identify molecular determinants of virulence that alter in vitro tropism.

## Evaluation of foreseeable effects

The virus is highly labile, does not persist in the environment and is readily inactivated by disinfection. For further information, see section 17.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

## For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Any material coming into contact with GMM material is contained within a sealed container until it is autoclaved in the Department of Veterinary Pathology. Further information, see Section 17.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All materials handled at containment level 2 will be disinfected by steeping in Presept as per manufacturer's instructions.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Project regarded as Class 2

Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

| L2 L3 L4 L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 |

Project Ref 223/01.11

Date Ackn'd 23/11/2001

Date Project Ceased

CU2 Project Title USE OF FELV ISOLATES GENERATED FROM MOLECULAR CLONES IN VITRO.

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
### Purposes of the contained use

Molecular cloned isolates of feline leukaemia virus are grown to generate viral stocks for use in the diagnostic laboratory and for general laboratory use.

### Recipient or parental organism

Recipients are BHK cells which have been infected with FeLV-A, B and C. Persistently infected cell lines were recovered.

### Host/vector system

Molecular clones of FeLV-A, B and C are integrated in separate BHK-derived cell lines. The cells are persistently infected and produce no visible cytopathogenic effect.

### Origin & function

FeLV-producing cell lines have been used routinely as control cultures for samples requiring subgroup identification.

### Evaluation of foreseeable effects

No risk has been identified. FeLV is not a human pathogen. Thus, there is neither virological nor serological evidence that human beings can be infected with the virus, or that contact with persistently infected cats is a risk factor in human disease (reviewed in Loar AS (1987) The zoonotic potential of feline leukemia virus. Veterinary Clinics of North America, Small Animal Practice. 17:105-115); Butera ST et al. (2000) Survey of veterinary conference attendees for evidence of zoonotic infection by feline retroviruses. Journal of the American Veterinary Medical Association. 217:1475-1479).

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cultures are inactivated by immersion overnight in Presept solution and/or by placing the plates directly into an autoclave bag and autoclaving.

### Risk assessment passed.

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**Date of Significant Change**

**Project Additional Information**

**Recipient or parental organism**

Recipients are BHK cells which have been infected with FeLV-A, B and C. Persistently infected cell lines were recovered.

**Host/vector system**

Molecular clones of FeLV-A, B and C are integrated in separate BHK-derived cell lines. The cells are persistently infected and produce no visible cytopathogenic effect.

**Origin & function**

FeLV-producing cell lines have been used routinely as control cultures for samples requiring subgroup identification.

**Evaluation of foreseeable effects**

No risk has been identified. FeLV is not a human pathogen. Thus, there is neither virological nor serological evidence that human beings can be infected with the virus, or that contact with persistently infected cats is a risk factor in human disease (reviewed in Loar AS (1987) The zoonotic potential of feline leukemia virus. Veterinary Clinics of North America, Small Animal Practice. 17:105-115); Butera ST et al. (2000) Survey of veterinary conference attendees for evidence of zoonotic infection by feline retroviruses. Journal of the American Veterinary Medical Association. 217:1475-1479).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cultures are inactivated by immersion overnight in Presept solution and/or by placing the plates directly into an autoclave bag and autoclaving.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

Y

**Please enter comments on the GM safety committee on the risk assessment**

Risk assessment passed.
**Project Containment**

Laboratory Activities  | Glass Houses  | Growth Rooms
--- | --- | ---
L2 Yes | L2 | L2
L3 | L3 | L3
L4 | L4 | L4

Animal Units  | Large Scale Activities  | Human Clinical Applications
--- | --- | ---
L2 | L2 | L2
L3 | L3 | L3
L4 | L4 | L4

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**Project Ref** 223/01.12

Date Ack'n'd  | CU2 Project Title
--- | ---
23/11/2001 | USE OF FELINE IMMUNODEFICIENCY VIRUS (FIV) MOLECULAR CLONES

Date Project Ceased

Class | CultureVolClass2 | CultureVolumeClass3-4
--- | --- | ---
Class 2 | < 1 litre | 

Non-GMM | Consent Granted
--- | ---
| not applicable |

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

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**Project Additional Information**

Purposes of the contained use

Infectious molecular clones of FIV will be compared.

Recipient or parental organism

The infectious molecular clones were derived from naturally occurring field isolates of FIV, a widespread pathogen of the domestic cat. For further information on the molecular clones, see Section 17.

Host/vector system
Bacterial hosts will be derivatives of E. coli K-12 and are considered disabled. The vectors pGEM-7Zf+ (FIV-PET f14) and pBR328 (FIV-GL8 414) are considered non-mobilisable.

Origin & function

The molecular clones were derived from infected feline T cell genomic libraries. The clones will be genetically modified to introduce mutations representing nucleic acid sequences found in non-virulent isolates in order to identify molecular determinants of virulence that alter in vitro tropism.

Evaluation of foreseeable effects

The virus is highly labile, does not persist in the environment and is readily inactivated by disinfection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Any material coming into contact with GMM material is contained within a sealed container until it is autoclaved in the Department of Veterinary Pathology.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials handled at containment level 2 will be disinfected by steeping in Presept as per manufacturer's instructions.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

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<td>Human Clinical Applications</td>
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The purpose of this project is to investigate how Campylobacter jejuni induces inflammation and causes diarrhoea.

Background
Campylobacter jejuni is a microaerophilic, Gram negative, flagellate spiral bacterium. It is the leading cause of bacterial food borne diarrhoeal disease throughout the world (1,2). In addition, infection with C. jejuni is frequently associated with a rare but potentially serious form of neuromuscular paralysis known as Guillain-Barre syndrome (3,4). Human infection is usually acquired by the consumption of contaminated food (especially poultry) or water (5,6,7). The clinical spectrum of enteric disease due to C. jejuni and C. coli ranges from severe inflammatory diarrhoea to a generally mild non-inflammatory (watery) diarrhoea (8,9). The former is the most common clinical presentation in patients from industrialised nations, whilst the latter is the usual pattern seen in developing nations (10). The clinical picture may be explained by frequency of exposure to Campylobacter in an individuals immediate environment (10,11). Ketley (12) has speculated that host status modulates disease presentation. Thus primary disease (no immunity) leads to inflammatory diarrhoea by bacterial invasion of the intestinal lamina propria, generation of an inflammatory response and tissue damage due to host mediators and unknown bacterial molecules. Partial immunity would arrest some of the inflammation associated with primary infection resulting in milder disease.

The induction of inflammation by C. jejuni in different host species. It is unknown, taking the two extreme examples, why humans react with a brisk inflammatory response to C. jejuni infection with associated disease symptoms, yet poultry treat the organism as a commensal. We will measure activation of Nf-kB, a central mediator of host inflammation by functional assays, and chemokine and cytokine release from tissue culture cells and primary intestinal tissue from various animal hosts infected in vitro with C. jejuni, to ask fundamental questions as to how tissues from different animal species respond to infection with Campylobacter. Using wild type C. jejuni NCTC 11168 and derivatives with mutations in genes encoding cell surface and secreted proteins, will enable us to define the host inflammatory mediators induces (or not) in intestinal tissue from different animal species. We will use mutants in which genes encoding biosynthesis or modification of surface structures, together with genes encoding putative toxins, are inactivated. Specifically we wish to examine mutants in genes involved in: 1) Capsule biosynthesis (kpsM) 2) LOS biosynthesis genes (waaF, wla deletion) 3) sialylation of LOS by N-acetyl neuraminic acid (neuB1) and sialylation of flagella (neuB2 and 3) 4) Genes for glycosylation of LOS and flagella (wlaC, wlaD,
5) Phospholipase A (pldA) 6) CLDT (cytolethal distending toxin cld) and 7) Putative haemolysin. (hylA). Julian Ketley's group at the University of Leicester and Brendan Wrens group at the London School of Hygiene have constructed a number of mutants in the above cell surface and secreted molecules of C. jejuni that are implicated in host cell interaction and induction of the host inflammatory response. In collaboration with the groups mentioned we will investigate the role of the genes listed above by using these mutants to infect cell lines and primary tissue biopsies from man and various animal species in order to assess their ability to bind to cells and tissues and elicit host inflammatory cytokine and chemokine production. By directly comparing tissue from animal hosts that give different types of clinical response upon infection, we aim to define host molecules up or down regulated by infection, and bacterial factors inducing these host responses. We can only then begin to understand why different host species respond in different ways to these bacteria in terms of initiation of inflammation at the mucosal surface. We intend to characterise wild type C. jejuni mutants in surface structures and secreted proteins to investigate a specific aspect of host/bacteria interaction namely comparative host inflammatory profiles in response to C. jejuni.

PLANTS OF INVESTIGATION
The following experimental procedures will be performed:
(1) Infection of viable primary tissue by C. jejuni.
(2) NF-kB activation - Nuclear extracts and electromobility shift assay.
(3) Secretion of host cells chemokines/cytokines upon infection.
(4) Cell adhesion, invasion and transcytosis assays.
(5) Whole blood assay for determination of cytokine responses.

Conclusions
These experiments should help define the host inflammatory molecules activated upon infection and which bacterial molecules are implicated in tissue inflammation, critical steps in the disease process. We intend to define the basic biology of host/pathogen interaction of C. jejuni with cells from the gastrointestinal tract of different host species by using defined mutants to dissect these processes.

References
Mutants of Campylobacter jejuni have been generated by inverse PCT mutagenesis in the Departments of Genetics, University of Leicester and Department of Infectious and Tropical Diseases, London School of Tropical Medicine and Hygiene. C. jejuni chromosomal DNA is digested with a restriction enzyme and the products allowed to circularise by self ligation. Primers with a unique restriction site amplify the fragments by inverse PCR and the products are cloned and characterised. Cloned products are digested with the unique enzyme, ligated with an antibiotic cassette (kanamycin) and the ligation mix is transformed into C. jejuni by natural transformation or electroporation.

**Host/vector system**

The origin of the genetic material is from a wild type clinical isolate of Campylobacter jejuni. The function of this material is to use it to make defined mutations in genes thought to be involved in virulence and disease causation in man.

**Evaluation of foreseeable effects**

Campylobacter infections are acquired by ingestion and it is assumed that the wild type strain used in these experiments is fully virulent for man. Thus is may cause clinical features if ingested. In our experiments we aim to make mutants of this organism which will be a virulent for man. Thus the foreseeable effects of these mutants are that they will not cause any clinical manifestations in man.

C. jejuni cannot replicate aerobically so there will be no replication of the organism in the environment. Estimation of risk of disease to man or danger to the environment for genetically modified mutants of C. jejuni is very low and for those shown to be attenuated in disease models is effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The risk of laboratory acquired infection will be minimised by the following procedures:-

1) Good laboratory practice (containment level 2)
2) Disinfection
3) Use of safety cabinets for some procedures
4) Autoclaving and incineration

All practices conform to containment level 2 (Collins, C.H. Safety in Clinical and Biomedical Laboratories, Chapman Hall) for work with pathogens in hazard group 2.

To avoid ingestion of Salmonella after working with them hand washing will break the hand to mouth route of transmission. Cultures will be transported in plate racks and leak proof metal boxes.

Chemical disinfectants are used in bench discard and pipette jars, for treating surfaces that become contaminated by spillage and for disinfecting equipment. For standard decontamination of glassware and plastic ware items are immersed in a bleach solution of 412ppm of available chlorine for at least 12 hours (prepared using Presept tablets). All glass and plastic ware is subsequently autoclaved. For killing bacteria in culture suspension, Presept tablets or a clear phenolic are added to give a solution of 2500ppm of available chlorine or 1% phenolic solution and this is left for at least 2 hours before autoclaving. All bacteria grown on solid media are autoclaved before disposal.

For accidents and spillages the affected area should be immediately flooded with a Hibitaine solution (1:1000). The area should be left for 30 minutes and then mopped with disposal towels and these disposed of by autoclaving. Broken glass should be lifted by forceps or pan and brush. If hands become contaminated they should be washed with Hibiscrub.
The decontamination protocols described have been validated in our laboratory using the relevant bacterial strains. Disinfectant effectiveness is tested by the 'in use' test.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Passed as class 2 with amendments 3/01.

Project Containment

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Project Ref 223/01.6

Date Ackn'd 17/04/2001
CU2 Project Title SIGNATURE TAGGED TRANSPOSON MUTAGENESIS OF SALMONELLA ENTERITIDIS
Date Project Ceased
Class 2
CultureVol < 1 litre
Class CultureVolumeClass
Non-GMM not applicable
Consent Granted
Project notified under transitional arrangements N
Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Human food borne infection due to Salmonella enteritidis continues to be a serious problem in many countries. The organism is predominantly acquired from ingestion of undercooked poultry and eating food containing raw shell eggs. Salmonella enteritidis infection is endemic in poultry flocks in producing countries but the mechanisms of colonisation and the bacterial factors required for survival within the chicken have not been characterised. Knowledge of these factors might enable prophylactic or novel therapeutic measures to be developed to eradicate this organism from the food chain. The technique of Signature Tagged Transposon Mutagenesis (STM) applied to human and animal bacterial pathogens has identified bacterial genes essential for virulence and survival within the host. We intend to use this powerful genetic technique for the identification of genes required for survival of S. enteritidis within its chicken host, characterise the function of these genes and investigate the potential of utilising these genes and their products to develop live or sub unit vaccines to eradicate this organism from infected birds and flocks. This technique will also enable us to understand the basic biology of bacterial/host interaction, with the ultimate goal of safer food for consumers and less food borne human disease.

Background to the Proposal

Human foodborne salmonella infections continue to be a major international health problem, both in terms of morbidity and economic costs. Over 2000 food poisoning serotypes of Salmonella exist and the prevalence of individual serotypes constantly changes. The main reservoirs for human infections are poultry, cattle, sheep and pigs. The recent patterns of human infections internationally have been dominated by Salmonella enteritidis since the middle 1980s and it is still a significant public health problem. Shell eggs and poultry meat are major sources of S. enteritidis, in particular S. enteritidis phage type (PT) 4. This phage type is capable of invading chicken tissues and persisting in the oviduct, and is therefore more likely than others to contaminate eggs internally while they are being formed. Almost nothing is known about the genetic basis of colonisation and infection of poultry by Salmonella species in general and S. enteritidis in particular. The aim of this project is to address this lack of knowledge using the novel technique of Signature Tagged Mutagenesis (STM). STM is a means of identifying bacterial virulence genes that are essential for infection, survival and proliferation within a specific animal. The great advantage of STM is that by utilising tagged mutants, avirulent strains are detected directly. STM has been used successfully for revealing hereto unknown virulence genes that are important in S. typhimurium infection of mice, and also for virulence gene identification in other human and animal pathogens. STM is most powerful when the genome sequence of the target or closely related organisms are available, which facilitates the identification and further characterisation of the genes inactivated by STM. The genomes of Salmonella enteritidis, S. typhimurium and S. typhi have or are in the process of being sequenced.

Measures to either limit or prevent the infection of chickens with S. enteritidis have clear human public health benefits. In order to safeguard the health of the public and protect consumers, salmonella colonisation has to be controlled in farm livestock. The information obtained from this project could be applied to hasten the elimination of S. enteritidis from the food chain. For example, strains with mutations in the genes identified by STM may have utility as live vaccines or alternatively, the gene products may encode protective immunogens which could be included in subunit vaccines for poultry use. These measures may ultimately lead to a direct decrease in human foodborne salmonellosis.

Aims and Objectives

The objectives of this proposal are to identify the genes that are important for S. enteritidis to survive and causes disease in poultry using STM. Microbial genome databases will be interrogated to determine the identity (if known) and possible function of the genes that are shown to be important in vivo in chickens by STM. The role of the different genes will be analysed in vitro using a variety of assays, (eg is the gene involved in attachment to, invasion of or survival and replication within eucaryotic cells?). We will test the ability of mutants of S. enteritidis generated during this project to immunise chickens against S. enteritidis.

Plan of Investigation
STM is a negative selection method for virulence gene identification. Bacterial genes are mutated by the insertion of transposons carrying a unique DNA sequence tag. Animals are infected with a bank of bacteria containing transposon inserts and after a few days organisms are harvested from the internal organs of the animal. The pool of bacteria recovered from the tissue of the animal is compared with the inoculum by hybridisation, to determine which tags (mutant strains) are absent in the output pool. These bacteria have mutations in genes that contribute to survival in the animal. It is then possible to identify the genes which were absent in the bacteria recovered from the animals because the tag present in the transposon acts as a unique marker for that strain.

Generation of Mutant Bank

Signature tagged mutagenesis (STM) was described by Hensel et al for S. typhimurium and identified new virulence determinants for systemic disease in this organism, some of which are being exploited for live vaccine development. We will follow this powerful proven methodology for generation of our mutant bank in Salmonella enteritidis. A transposon (on plasmid pUTminiTn5Km2) will be used for random insertional mutagenesis of the genome of Salmonella enteritidis. Each transposon contains a different DNA sequence tag which allows mutants to be differentiated from each other. The tags comprise 40bp variable central regions flanked by invariant arms of 20 bp, which allow the amplification and labelling of the central portions by the polymerase chain reaction (PCR). The semi-random format by which tags were designed ensures that the same sequence tag should only occur very rarely and therefore the chance of encountering the same tag twice n a pool of 96 different mutants is effectively nil. PCR amplification of the central regions using radiolabelled 2'deoxyctydine 5'-triphosphate (dCTP) produces probes with ten times more radiolabel in the central regions than in each arm. The incorporation of a unique restriction site at the junction of the central regions and the arms allows the arms to be separated after labelling, generating specific DNA probes comprising the central region. A nucleotide bias used in the central regions also avoids the presence of sites for those restriction enzymes used for ligation before hybridisation analysis and for subsequent cloning of virulence genes. Tags will be ligated into pUTminiTn5Km2 and transformed into E. coli CC118. Transformants are pooled and DNA extracted to transform E. coli S-17lamda-pir. Transformants of E. coli S-17lamda-pir are mated on filters with a nalidixic acid resistant wild type Salmonella enteritidis. After overnight incubation bacteria are recovered from filters by incubation in L-broth containing nalidixic acid and kanamycin and then plated onto L-agar with these antibiotics. Exconjugants are screened on Macnoney agar and LA containing ampicillin. Ampicillin sensitive colonies are assembled into 96 well microtiter dishes, which are used to prepare replica DNA colony blots. We intend to screen the mutant bank in a chicken colonisation model in collaboration with Dr Paul Barrow at Compton and in an egg model to assess genes important for survival in hens eggs. This model will assess the ability of the introduced tagged pool to survive within the chicken alimentary tract the primary site of bird colonisation with these organisms. We may also later screen the bank in a systemic infection model. We also wish to screen our mutant bank for survival in hens eggs and the bacterial factors important in persistance in this environment.

Characterisation of Identified Mutants

Mutants identified will be analysed to determine the DNA sequence of the region immediately flanking the mutation. DNA from prospective mutants is digested with a number of restriction enzymes. The enzymes are chosen because they cut once in the polylinker of pUTmini-Tn5Km2. Digests are run on agarose gels, and transferred to Hybond membranes for Southern hybridisation using the kanamycin resistance gene of the transposon as a probe. Enzymes which give fragments of 3-5kb are used to digest DNA, fragments gel purified and ligated into pUC18. Ligation reactions are used to transform competent E. coli to kanamycin resistance. These inserts are sequenced and should represent part of the sequence of a gene of interest. The observed sequence will be used to probe the salmonella and other bacterial genome databases. This will identify the function of the disrupted gene (if known) which will facilitate further phenotypic analysis of the mutant strains. Identified mutants will then be investigated as to their biological role in the disease process by in vitro and in vivo techniques.

References


The mutants generated by these techniques are all expected to be less virulent or of equal virulence to the parental Salmonella strain. The clinical symptoms of a human swallowing a virulent salmonella include diarrhoea, vomiting, fever and abdominal pain. Illness is generally self limiting in any individual with severe infections requiring antibiotic treatment. In the rare event that a mutant may be more virulent than the wild type parent standard laboratory practice for the containment of class II pathogens are more than adequate to contain the risk (see below). Mating experiments will be performed with laboratory strains of E. coli.

Recipient or parental organism

The origin of the genetic material is from a wild type clinical isolate of Salmonella enteritidis. The function of this material is to use it to make random mutations in chromosomal DNA in order to identify genes thought to be involved in virulence and disease causation in man.

Evaluation of foreseeable effects

Salmonella infections are acquired by ingestion and it is assumed that the wild type strain of Salmonella enteritidis used in these experiments is fully virulent for man. Thus is may cause the clinical features of salmonellosis if ingested. In our experiments we aim to make mutants of this organism which will be avirulent for man and in the model of chicken colonisation. Thus the foreseeable effects of these mutants are that they will not cause any clinical manifestations in man.

Estimation of risk of disease to man or danger to the environment for genetically modified mutants of S. enteritidis is very low and for those shown to be attenuated in disease models is effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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2) Disinfection
3) Use of safety cabinets for some procedures
4) Autoclaving and incineration

All practices conform to containment level 2 (Collins, C. H. Safety in Clinical and Biomedical Laboratories, Chapman Hall) for work with pathogens in hazard group 2.

To avoid ingestion of Salmonella after working with them, hand washing will break the hand to mouth route of transmission. Cultures will be transported in plate racks and leak proof metal boxes.

Chemical disinfectants are used in bench discard and pipette jars, for treating surfaces that become contaminated by spillage and for disinfecting equipment. For standard decontamination of glassware and plastic ware items are immersed in a bleach solution of 412ppm of available chlorine for at least 12 hours (prepared using Presept tablets). All glass and plastic ware is subsequently autoclaved. For killing bacteria in culture suspension, Presept tablets or a clear phenolic are added to give a solution of 2500ppm of available chlorine or 1% phenolic solution and this is left for at least 2 hours before autoclaving. All bacteria grown on solid media are autoclaved before disposal.

For accidents and spillages, the affected area should be immediately flooded with a Hibitaine solution (1:1000). The area should be left for 30 minutes and then mopped with disposable towels and these disposed of by autoclaving. Broken glass should be lifted by forceps or pan and brush. If hands become contaminated they should be washed with Hibiscrub.

The decontamination protocols described have been validated in our laboratory using the relevant bacterial strains. Disinfectant effectiveness is tested by the 'in use' test.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

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Please enter comments on the GM safety committee on the risk assessment

Passed as class 2 with amendments 3/01.

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02/03/2022
Project Ref 223/01.7

CU2 Project Title
CONSTRUCTION AND USE OF RETROVIRAL VECTORS EXPRESSING RUNX FAMILY MEMBERS IN PRIMARY AND IMMORTALIZED MURINE CELLS

Date Ackn’d
17/04/2001

Date Project Ceased
18/03/2020

Class
Class 2

CultureVol
< 1 litre

CultureVol

Non-GMM
Not applicable

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes
GM 223/04.1

Historical Date of Additional Info
05/02/2004

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
The use is intended to identify changes in gene expression caused by overexpression of Runx family members and so elucidate the oncogenic mechanism of these genes.

Recipient or parental organism
Moloney murine leukaemia virus is lethal in mice - neonatal infection results in death by thymic lymphoma in 3-4 months. By contrast, the recombinant virus stocks will be replication defective and so a systemic infection is highly unlikely, even if the virus were to escape into the environment and infect susceptible mice. Because the virus is replication defective, the likelihood of spread of the virus to other animals is greatly reduced.

Host/vector system
The constructs will be made in pBabe puro, and packaged in the Phoenix-Eco packaging cell line (Garry Nolan, Stanford University, Stanford CA) so that no replication-competent viruses will be produced. The Phoenix -eco packaging cells are based on 293T cells which have separate gag/pol and env genes so that a combination of unlikely recombination events are required for the production of helper virus (2). The packaging cell lines will be monitored to ensure that no recombinants are produced as the result of infection with other viruses present in the same incubator.

The monitoring will take the form of infection of the packaging cell lines LXSN-EGFP virus, a green fluorescent protein expressing virus which is both defective and based on MoMLV (3). Virus stocks from the packaging cell line infected with LXSN-EGFP virus will be applied to human cells and scanned by fluorescence microscopy to ensure that none of the recombinant viruses have been pseudotyped to form particles which might infect human cells.

Origin & function
Runx2 has been identified as a MYC collaborating oncogene (6) by "retroviral tagging" - the process of cloning proviral integration sites in thymic lymphomas and...
subsequent analysis of these loci for transcribed sequences affected by the insertion of the powerful enhancer elements of the proviral LTR nearby (4). The importance of the deregulated expression of this gene was apparent from the high frequency of integrations at til-1, the integration site near Runx2. Recent work has confirmed the strong collaborative effect between Runx2 and MYC in the development of thymic lymphoma - mice made doubly transgenic for Runx2 and MYC develop tumors much faster and neonatal infection of Runx2 transgenic mice leads to a very high frequency of integrations at c-myc or N-myc (1).

Runx2 is a highly conserved transcription factor (it isa mouse homologue of the Drosophila segmentation gene (runt)) and forms part of a gene family in the mouse along with the Runx1 and Runx3. One of the human homologues of these genes is AML-1, a gene important in acute myeloid leukaemia, as shown by frequent rearrangement of this locus (5). Having produced an animal model for this disease state, we intend to further investigate the molecular mechanisms affected by Runx2 and other family members in primary and immortalised murine cells. Initial results suggest that the normal cell death programs are impaired in the presence of high levels of Runx2. In order to investigate these mechanisms, we propose to make retroviral vectors based on the well known pBabe vectors and use these to make high titre, helper free virus stocks for studies on Runx family members in MEFs and other mouse cells.

Evaluation of foreseeable effects

MoMLV is not included in the ADCP hazard group because it does not infect humans, so delivery of Runx family members to the workers is highly unlikely given that an ectropic packaging cell line will be used. The helper genes in the packaging cell line are separated into two blocks so that recombination to a replication competent virus is highly unlikely. Even in the event of this actually happening and the virus escaping into the environment, the virus tropism will ensure that only mice will be infected. The only possible outcome would be that oncogenesis might be more likely in the infected mice if all the unlikely events took place.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The construction and growth of plasmids containing the Runx family members will be carried out such that no attempt will be made to express the genes in these vectors. The genes will be cloned into a site in the vector (pBabe puro) which contains no promoter elements to drive bacterial expression. The growth of the plasmids in appropriate E.coli K12-derived hosts will only serve to produce enough of the plasmid for later use and so could be carried out at Level 1 containment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All containment tissue culture flasks and centrifuge tubes will be autoclaved whilst culture medium will be treated with VIRKON to inactivate virus.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Project regarded as class 2
Project Ref 223/01.8

Date Ackn'd 11/05/2001
CU2 Project Title TO DEFINE THE EFFECT OF ADENO VIRAL OR ADENO-ASSOCIATED VIRAL (AAV) GENE TRANSFER OF CANDIDATE PROTECTIVE GENES ON LESION FORMATION AND SIZE IN THE APOE KNOCKOUT MOUSE.

Class CultureVol
Class2 CultureVolume
Class3-4

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info

Project Additional Information

Purposes of the contained use

The aim of the project is to assess the ability of a number of biological active genes to attenuate atherosclerotic lesion formation in the apoE-/- knockout mouse. ApoE-/- mice develop substantial peripheral lesions. Candidate biologically active genes will be delivered injected systematically through the femoral vein using standard first generation adenoviral vectors or AAV vectors

Recipient or parental organism

All adenovectors to be used in this study are E1-deleted first generation adenoviral vectors based on the pJM17 system (McGrory, W.J., Bautista, D.S. and Graham, F.L.: A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. This renders the adenoviruses replication defective. However with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propogate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent
unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. However, all the inserted genes are biologically active. None of the transgenes are proto-oncogenes. All transgenes will be under the control of the CMV promoter. There will be a minimal risk of harmful effect of these genes in the organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell that most expression vectors as they will definitely not replicate or integrate in the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use. AAV vectors have received increasing attention for molecular interventions in vivo and for gene therapy applications due to their low level of immunogenicity in vivo and their ability to integrate into the genome, thus producing sustained expression of transgenes for prolonged periods of time. They also have the ability to infect dividing and non-dividing cells. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al. High-tier rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of these sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced.

Host/vector system

The adenoviral vectors are generated by homologous recombination between pJM17 and shuttle vectors containing transgene expression cassette and flanking E1 sequences. Following homologous recombination in helper 293 cells which express the helper E1 function in trans, replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al. High-tier rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow. AAV vectors are replication defective.

Origin & function

The adenoviral vector DNAs are standard and originated from the laboratory of Dr. Frank Graham (McGrory, W.J., Bautista, D.S. and Graham, F.L.: A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vitro and in vivo to generate high-level gene expression in all cells transduced by the adenovirus. The AAV system is published (Zhang, X, de Alwesh, M., et al. High-tier rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All transgenes will be constructed from full length cDNAs obtained from other research institutes and verified in our own laboratory prior to subcloning into the relevant vectors.

Evaluation of foreseeable effects

There will be a minimum risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definately not replicate or
integrate into the host genome. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild-type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of these sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are likely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced. Consequences of environmental exposure - ‘effectively zero’

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures as per HSE containment level 2 for both production of recombinant adenoviruses in the laboratory and animal experimentation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory-based experiments: during the production of replication-defective adenoviruses, all solid waste (plastics etc.) are autoclaved prior to disposal. All liquid waste from tissue culture is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. The use of sharps is avoided.

Animal Experiments: All instruments used in the preparation of animals for GM work will be sterilised by autoclaving. Solutions exposed to the viable GMOs will be disinfected with chlorine-based disinfectant. All plastic ware will be autoclaved prior to disposal. Animals receiving the GMO will be housed in separate cages during the procedure-kill time period. All animal carcasses will be disposed of by incineration. Animal bedding will be autoclaved prior to disposal and cages disinfected prior to being re-used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Passed with amendments by local GMSC

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>
| L2 Yes | L2 | L2
| L3 | L3 | L3 |
| L4 | L4 | L4 |

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L2</td>
<td>L2</td>
</tr>
<tr>
<td>L3</td>
<td>L3</td>
<td>L3</td>
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<tr>
<td>L4</td>
<td>L4</td>
<td>L4</td>
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</tbody>
</table>

02/03/2022
The aim of the project is to assess the ability of a number of biological active genes to attenuate the extent of brain damage and to reduce infarct size induced by middle cerebral artery occlusion (MCAO; a model of stroke) in the brains of normotensive (Winstar Kyoto) and stroke sensitive rats (stroke-prone spontaneously hypertensive rats). Candidate biologically active genes will be delivered prior to (up to 1 week prior) MCAO to allow gene expression to occur. The genes will be delivered using standard replication defective first generation adenoviral vectors via a systemic route (infusion of adenovirus through the femoral vein), intracranially (direct stereotactic injection into the brain) or through lateral ventricle infusion into the cerebrospinal fluid.

All adenovectors to be used in this study are E1-deleted first generation adenoviral vectors based on the pJM17 system (McGrory, W.J., Bautista, D.S. and Graham, F.L.: A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. This renders the adenoviruses replication defective. However with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competant adenovirus (RCA) and will be negative. All stocks will also be purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. However, all the inserted genes are biologically active. None of the transgenes are proto-oncogenes. All transgenes will be under the control of the CMV promoter. There will be a minimal risk of harmful effect of these genes in the organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell that most expression vectors as they will definately not replicate or integrate in the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use.
### Host/vector system

The adenoviral vectors are generated by homologous recombination between pjM17 and shuttle vectors containing transgene expression cassette and flanking E1 sequences. Following homologous recombination in helper 293 cells which express the helper E1 function in trans, replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses.

### Origin & function

The adenoviral vector DNAs are standard and originated from the laboratory of Dr. Frank Graham (McGrory, W.J., Bautista, D.S. and Graham, F.L.: A simple technique for the rescue of early region 1 mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vitro and in vivo to generate high-level gene expression in all cells transduced by the adenovirus. All genes, with the exception of the viral crmA gene (caspase inhibitor), are full-length human cDNA sequences. The adenoviruses have been generated in our own laboratories, provided by international research groups or purchased from the University of Iowa Gene Transfer Core. We are testing these candidate genes, when overexpressed using the adenoviral vector system, to inhibit cell death induced by middle cerebral artery occlusion in rats.

### Evaluation of foreseeable effects

There will be a minimum risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome.

Consequence of environmental exposure - "effectively zero".

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures as per HSE containment level 2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory-based experiments: during the production of replication-defective adenoviruses, all solid waste (plastics etc.) are autoclaved prior to disposal. All liquid waste from tissue culture is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. The use of sharps is avoided.

Animal Experiments: All instruments used in the preparation of animals for GM work will be sterilised by autoclaving. Solutions exposed to the viable GMOs will be disinfected with chlorine-based disinfectant. All plastic ware will be autoclaved prior to disposal. Animals receiving the GMO will be housed in separate cages during the procedure-kill time period. All animal carcasses will be disposed of by incineration. Animal bedding will be autoclaved prior to disposal and cages disinfected prior to being re-used.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
Passed with amendments by local GMSC prior to November 15th 2000.

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L3 L4</td>
</tr>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
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</tbody>
</table>

### Animal Units
- L2 Yes
- L3 L4 L2

### Large Scale Activities
- L3 L4 L2
- L3 L4 L2

### Human Clinical Applications
- L2 L3 L4
- L2 L3 L4

## Project Ref 223/03.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>Date Project Ceased</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
<th>Consent Granted</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/09/2003</td>
<td>18/03/2020</td>
<td>ASSESSMENT OF BRAIN DAMAGE INCLUDING STROKE OF HERPES SIMPLEX VIRUS (HSV) MEDIATED TRANSFER OF A CELL CYCLE PROTEIN INTO BRAINS OF RODENTS</td>
<td>Class 2</td>
<td>CultureVol</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

- < 1 litre
- Class 2
- CultureVol
- Class 3-4
- Not applicable
- Project notified under transitional arrangements

## Project Additional Information

### Purposes of the contained use
To determine if overexpression of the cell cycle protein results in a reduction in infact size following stroke and other models of brain injury.

### Recipient or parental organism
Mice and rats.
### Host/vector system

In vitro - introduction into the central nervous system (CNS) of mice and rats. The vector used in this study is an attenuate form of the Herpes Simplex Virus.

### Origin & function

The gene of interest is a cell cycle protein which is activated upon cellular stress such as ischaemia. It has been shown that expression of this protein is upregulated following cerebral ischaemia and it is thought that the role of this protein may be in influencing cell survival. Therefore overexpression of the cell cycle protein may salvage brain tissue following cerebral ischaemia and provide a novel therapeutic target for the treatment of stroke.

### Evaluation of foreseeable effects

When using human herpes viruses as vectors, consideration should be given to the nature and expression level of the novel gene insert. The HSV virus is an attenuated form and is selectively replication competent, only replicating in dividing cells, therefore the virus will be unable to replicate in the adult CNS. We do not expect that insertion of the genes of interest will affect the growth property of HSV.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

HSV vectors will be stored in a double container in a locked freezer. Vectors will be transported between labs in double containment. Lab coats, gloves and glasses will be worn when handling viral vectors. Any contaminated plastic or glassware will be autoclaved, any liquid waste will be placed in Virkon overnight before being disposed of down the sink. A separate Hamilton syringe will be used for injecting vectors, which will be cleaned with 1% Virkon followed by absolute alcohol. All surgical instruments will be cleaned in 1% Virkon followed by absolute alcohol.

Recovery animals will be placed in cages which will be clearly marked to inform biological service staff of the details of the viral vector. A notice will be placed on the animal room to notify staff of the presence of animals treated with viral vectors. Terminal animals will be killed by perfusion fixation with 2% paraformaldehyde in phosphate buffer and the animal carcasses will be incinerated. Animal cages will be autoclaved.

Cage tops will be securely fastened when animals are being transported between Biological Services and WSI labs which are contained within the same building.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All equipment will be sterilised in Virkon followed by 70% Alcohol. This will completely deactivate any virus and is a method, which is commonly practised in virology. All deactivated waste can then be disposed of down the sink with copious amounts of water. Animals, which have been infected with virus, will be perfusion fixed in paraformaldehyde which will deactivate the virus. Animal carcasses will be incinerated.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

02/03/2022
Risk assessment has been approved by the GM committee.

## Project Containment

### Laboratory Activities
- **L2**: Yes
- **L3**: L4
- **L2**: L3
- **L2**: L4

### Glass Houses
- **L2**: Yes
- **L3**: L4
- **L2**: L3
- **L2**: L4

### Growth Rooms
- **L2**: Yes
- **L3**: L4
- **L2**: L3
- **L2**: L4

### Animal Units
- **L2**: Yes
- **L3**: L4
- **L2**: L3
- **L2**: L4

### Large Scale Activities
- **L2**: L3
- **L2**: L4
- **L2**: L3
- **L2**: L4

### Human Clinical Applications
- **L2**: L3
- **L2**: L4

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## Project Ref 223/04.2

### Date Ackn'd
- **03/12/2004**

### CU2 Project Title
- Molecular biology of JSRV and comparative studies with other Beta- and Gammaretroviruses.

### Class
- **Class 2**

### Consent Granted
- Not Applicable

### Project notified under transitional arrangements
- N

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## Project Additional Information

### Purposes of the contained use

JSRV is the cause of ovine pulmonary adenocarcinoma, a naturally occurring lung cancer in sheep. We wish to study this and similar viruses in terms of viral production in cells and their transformation, mutant virus in cell envelope entry, and viral interference mechanisms using transfection assays.

### Recipient or parental organism

All our worked is carried out using standard laboratory disabled strains such as K12 (DH5a) - it is highly unlikely that the pathogenicity will be altered by the manipulations.
described. There will be no attempt to express any of the viruses or viral genes or oncogenes described in this application in bacteria and the vectors do not even carry an appropriate promoter for bacterial expression. Thus no harmful effect to human health is foreseeable.

**Host/vector system**

GMOs described in this application are cloned in commonly used plasmids such as pBlueScript, pCDNA3 etc.

GMOs used:
- JSRV (Jaagsiekte sheep retrovirus)
- EnJSRVs (endogenous retroviruses related to JSRV)
- M-MLV (Moloney - ecotropic-murine leukaemia virus)
- MMTV (Mouse mammary tumour virus)
- Mason-Pfizer monkey virus (MPMV)
- Replication-incompetent retrovirus vectors

**Origin & function**

The procedures concerning the use of GMOs in our laboratory are (a) Virus production; (b) Transformation Assays; © Entry assays; (d) Transfections; (e) General DNA manipulations.

The JSRV DNA constructs used in our laboratory are derived from the plasmid pJSRV21(7), a full-length infectious molecular clone of a JSRV UK isolate. JSRV does not propagate in tissue culture, likely because the viral LTR are expressed specifically in type II pneumocytes (8). Type II pneumocytes are very difficult to isolate in vitro and lose their phenotype in vitro quickly, consequently no in vitro system for the propagation of JSRV has been obtained so far. (a) Viral production is achieved by transient transfection of 293T cells with pCMV2JS21, a derivative of pJSRV21 whose expression is driven by the cytomegalovirus (CMV) immediate early promoter. The CMV promoter, which replaces the proximal U3 region in the JSRV LTR is lost upon transcription. Thus, transfection of pCMV2JS21 in 293T cells induces production of wild type JSRV (eg the CMV promoter is not part of the genome of the produced virions) (7).

293T is a human cell line derived from HEK 293 cells (ATCC cat # CRL 1573). The 293 cell line was derived by transformation of primary cultures of human embryonic kidney (HEK) cells with sheared adenovirus (Ad5) DNA. 293T cells are further 'transformed' by the SV40 T antigen. 293/293T cells are probably the most widely used cell lines in research laboratories as they are easily transfectable by plasmid DNA.

(b) Transformation assays are performed generally on chicken, mouse or rat primary fibroblasts or immortalised cell lines (such as NIH-3T3, RK3E, 208F etc) by transfecting an expression plasmids for the JSRV Env (pCMV3JS21 GP). We also use a variety of derivatives of pCMV3JS21 GP carrying mutations that negatively affect the transformation ability of the JSRV Env or are fused with a tag epitope such as FLAG, HA or Myc. For comparative purposes chimeric envelopes formed by portions of the JSRV Envelope and the ecotropic Moloney murine leukemia virus (M-MLV) or Mouse mammary tumour virus (MMTV) are also employed. These chimeric envelopes have the aim to dissociate the oncogenic potential of the JSRV Env by the use of its natural cellular receptor (Hyaluronidase-2, Hyal-2). To this end the chimeric envelopes are formed by the receptor binding domain (RBD) of murine retroviruses and the remaining portion of the Env protein is derived from JSRV. Further details can be found in the following reference (9).

C) Entry assays. Viral pseudotypes will be used to test the ability of the JSRV Env mutants to mediate cell entry. Cells used for this purpose are in general sheep cell lines such as SCP, sheep choroids plexus, deer lung cells such as OHHI.Lu or mouse cells expressing the Hyal-2 receptor (3T3-Hyal2). Pseudotypes are formed by transfecting Env-expression plasmids (based on pBlueScript) into 293GP-AP or 293GP-Luc (Clontech). 293GP-AP and 293GP-luc express Gag and Pol (eg the core structural proteins) of murine leukemia virus (MLV) and a MLV-based vector expressing alkaline phosphatase (293GP-AP) or luciferase (293GP-Luc) (for control purposes pseudotypes formed with the M-MLV Env or MMTV Env are also used).

The retroviral pseudotypes described above are replication-incompetent and de facto have to be considered emphytropic retroviral vectors considering that the JSRVEnmv can mediate entry into human cells. Viral pseudotypes will be handled in BSL-2 conditions and great care will be taken into reducing the formation of aerosol and avoid the use of glassware/needles.
The generation of replication competent virus in this system is highly unlikely given the fact that the system uses three separate plasmids to express retroviral particles. In addition these assays last only 48-72 hours, further reducing the chances of selection and enrichment of a replication competent recombinant.

(d) Transfections. Studies to investigate interference mechanisms between enJSRVs and JSRV are carried out in 293, 293T and HeLa cells by transient transfection with pCMV2JS21 (and derivatives) and pCMV2enJS56A1 (and derivatives). Western blotting analysis serves to determine viral particle production (in the supernatant) and viral expression (in cell lysates). We also perform confocal microscopy in HeLa cells transiently transfected with the constructs described above. For comparative purposes analogous studies will be performed with (full length) murine retroviruses, such as the ecotropic Moloney murine leukemia virus (M-MLV), mouse mammary tumour virus (MMTV), Mason-Pfizer Monkey virus (MPMV). In all these cases 293T or HeLa cells will be transfected as above and assessed for viral particle production, expression and/or analyzed by confocal microscopy.

In JSRV/enJS56A1 the determinants of viral particle formation and interference map in the amino terminal region of Gag (the main polyprotein forming the retroviral capsid). We have derived a variety of JSRV/enJS56A1 mutants and chimeras to precisely map the defect possessed by enJS56A1 (6). For comparative purposes MPMV and M-MLV mutants and chimeras will be derived to determine whether amino acids substitutions in the amino terminal Gag can induce a defective phenotype similarly to enJS56A1.

(e) General DNA manipulations. All the infectious molecular clones described above are inserted in common commercially-derived vectors such as pBlueScript that do not have appropriate enhancers or promoter elements for bacterial expression. The host microorganisms are the commonly used DH5α, disabled bacteria derived from E. coli K12 strain. No attempt will be made to express full length viruses in bacterial cells.

Evaluation of foreseeable effects

Viruses used in this study: General comments. Oncogenic retroviruses are highly species-specific and interspecies transmission is avoided by a variety of blocks acting at entry (via the interaction with specific receptors) and post-entry levels (10, 11).

A. JSRV (JSRV21, infectious molecular clone). JSRV is the aetiological agent of ovine pulmonary adenocarcinoma (OPA). (OPA is a naturally occurring infectious disease of sheep that is enzootic in the UK. JSRV appears to be strictly species-specific. The disease is not described in other ruminants such as cows and deer. Even in goats (OPA is difficult to reproduce experimentally where it presents a much longer incubation period than sheep and lesions appear localised. (12).

As mentioned above, there is no convenient tissue culture system for the propagation of JSRV. Viral production is achieved by transient transfection of 293T cells with pCMV2JS21, a JSRV infectious molecular clone. 293T cells are human cells and as all human cells lack a functional α-1,3 galactosyltransferase that is instead present in most mammalian cells. Thus, mammalian cells and not human cells express Gal[α 1-3] Gal terminal carbohydrates and it has been shown that animal enveloped viruses (carrying Gal[α 1-3] Gal) can be inactivated by human complement (13, 14). Production of JSRV particles in human cells might bypass this barrier. However, it needs to be noted that a variety of blocks at various levels have evolved to block interspecies transmission of retroviruses. For example, interspecies transmission of JSRV does not occur between sheep and other animal species where the Gal(α1-3) Gal-induced complement inactivation would not occur.

Access: Jaagsiekte sheep retrovirus can theoretically enter human cells as the human Hyaluronidase-2 can function as a receptor for JSRV (15) but there is no indication of whether JSRV could be able to successfully establish infections in these cells. Unfortunately no tissue culture system is available to propagate JSRV in vitro. It is important to underline that a variety of retroviruses (eg amphotropic murine leukaemia virus, Feline leukaemia virus B) can utilise receptors expressed in human cells (16-18) but there is no indication available that they can actually establish infection in humans and certainly no human disease has ever been associated with these viruses.

Expression: No data is available on the possible expression of JSRV in human cells for the lack of a suitable cell culture system for the propagation of this virus. However, reporter assays using the JSRV long terminal repeats (where the viral promoter and enhancer are located) failed to show any significant activity in human lung-cancer derived cell lines (8). Damage. There are no reports in the literature of a higher incidence of lung adenocarcinoma in sheep farmers, veterinarians, sheep handlers etc. Despite OPA being recognised as a disease entity for more than a century (19) By PCR and RT-PCR no evidence of JSRV has been found in human bronchoalveolar carcinomas and adenocarcinomas (20-22). JSRV is thus unlikely to represent any hazard for humans.
The JSRV Env induces transformation of immortalised cells in vitro (4) and can be compared essentially to an oncoprotein. It is necessary a combination of more than one oncogene to transform human primary cells. For example, JSRV has been reported to transform a human-derived bronchial epithelial cell line (BEAS-2B) but this cell line is derived from cells that have been immortalised by the SV40 T antigen (23).

We also have JSRV mutants with critical amino acid substitutions that abrogate their transforming activity in vitro or their capacity to form viral particles. These mutants do not represent for human health a higher risk than wild type JSRV.

B - enJSRVs. The enJSRVs clones used are replication-defective and consequently do not pose any harm for human health. Also enJS66A1 mutants/chimeras where the ability to produce viral particles has been restored are unlikely to pose any risk to humans considering that: a) they possess a frame shift at the end of the pool gene (5); b) the enJSRVs Env does not induce transformation of rodent fibroblasts (3); c) their LTR (Long terminal repeat where viral enhancers and promoters are located) are relatively weak with respect to JSRV (5).

C - MMTV and M-MLV. MMTV and ecotropic M-MLV are mouse retroviruses and do not pose and harm for human health. Also gag mutants/chimeras for both viruses will not change the tropism of these viruses which is determined by the Env gene.

D - MPMV is one of the 5 serotypes of simian type D retroviruses (now classified as Betaretroviruses) (SRVs, simian retroviruses). A study conducted in the 70s show that about 25% of all macaques had antibody reacting to MPMV core antigen in US research primate centres (24). Access: MPMV can infect in vitro human cells. However, extensive serological surveys in the 1970s and 1980s of humans (including primate centres animals handlers) have shown no proof of SRV infection (25). A relatively minor report has showed Abs against betaretroviral Gag in he4althy African from Guinea but no follow up study has been published since then. It is reassuring that MPMV has been extensively studied for 30 years in many laboratories around the world (eg 26-43) and there is no indication of its transmission to humans. Expression and Damage: The pathogenicity in its natural host is not completely clear. Experimental transmission of MPMV in the early 1970s into infant rhesus monkeys induced an immunodeficiency-like disease (26, 27). In nature, saliva is the major source of infection and the most likely route of natural transmission is by percutaneous inoculation by biting and scratching (44, 45).

In conclusion although the potential for disease potential is low we will treat MPMV as a potential infectious agent (see Section 3 'Application of containment procedures').

E. Replication-incompetent retroviral vectors. Both standard ecotropic and more rarely amphotropic replication-incompetent retroviral vectors will be used in this laboratory to verify the efficacy of the envelope/envelope mutants constructed (see 'entry assays' in 'overview of procedures used') or to conveniently introduce genes (including oncogenes) in cell lines. Vectors used are replication defective and are used in a helper-free system. Viral vectors are produced by using either stable 'packaging' cell lines or transient transfection methods but in all cases viruses are produced by expression of viral proteins with three different plasmids and consequently chances of recombination are virtually eliminated. In addition the procedures employed are only for short term experiments (2-3 days) further reducing the chances of recombination. Ecotropic vectors cannot enter human cells while amphotropic vectors have this ability although they cannot spread.

Cells expressing replication competent viruses (that could work as unwanted virus 'helpers' for our vectors) and cells expressing the above mentioned replication incompetent viruses will be grown in separate incubators. In addition, biosafety cabinets, pipette aids, media stocks will be designated for the use of replication competent viruses to prevent cross-contamination. Access: Vectors carrying oncogenes are those that require more attention from a biosafety point of view. Despite being replication incompetent they could be able to enter human cells although spreading will not occur. Effective access to human cells can likely occur with needle-stick injuries. Our procedure minimise this risk as we do not use needles and glassware with material containing live viruses/vectors. In addition we produce only small amount of vector necessary for small scale infection in tissue culture experiments.

Workers will be using waterproof gloves, lab coats and eye protection. Expression and Damage: Amphotropic vectors carrying oncogenes can theoretically be expressed in human cells. In general the combination of more than one 'oncogene' is necessary to induce cell transformation in vivo. Most oncogenes expressed in normal cells lead to a senescence-like phenotype (ie non-proliferative) but could theoretically render cells more susceptible to transformation. All our procedures involving viruses/vectors avoid the use of needles and glass pipettes to minimize the risk of needle stick injuries which are the main source of accidental retrovirus infection. In addition all workers use lab coats and plastic gloves and eye protectors.

Cells expressing replication competent viruses and cells expressing replication incompetent vectors will be grown in separate incubators. In addition, biosafety cabinets will be designated for the use of replication competent viruses and designated pipette aids and designated media stocks will also help to prevent cross-contaminations.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

With the exception of DNA manipulations (performed at Level 1) we will work at Level 2 facilities with all the GMOs in the laboratory. Despite the hazard to human health being low we will treat all the GMOs and viruses in the laboratory as potential infectious agents and we will employ the same procedures for all viruses and vectors used to eliminate any possible confusion.

Handling and disposal of GMOs and contaminated material will be conducted as defined by the containment level(s) specified above, in compliance with the Faculty Safety Policy and the Local Code of Practice for Handling of Genetically Modified Microorganisms waste media. Spillages and contaminated plastic/glass ware will be disinfected with recommended inactivating agent (virkon, 2% solution) and material subsequently autoclaved. All solid waste is autoclaved. Both treatment are proven to completely inactivate retroviruses.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Passed with amendments by local GMSC and amendments added for this submission on 16th September.

Project Containment

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<thead>
<tr>
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<th>Glass Houses</th>
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Project Ref 223/07.1

Date Ackn’d 03/10/2007
CU2 Project Title Elucidating biological activities of the Herpes simplex virus type 1 ICP27 protein.
Class 2
Culture Class 2 ≤ 1 Litre
**Project Additional Information**

**Purposes of the contained use**
- To grow wild type herpes simplex virus type 1 (HSV-1).
- To grow viruses mutant in the ICP27 gene.
- To grow viruses mutant in the UL13 gene
- To express ICP27 protein in E.coli

**Recipient or parental organism**
- E.coli
- HeLa, Vero, Baby Hamster Kidney cells in cell culture

**Host/vector system**
- E.coli/pGEX
- Mammalian cells in culture/HSV-1 wild type and ICP27 or UL13 mutant virus

**Origin & function**
There are two strains of wild type HSV-1, the KOS virus that was cloned in Chicago and the 17+ virus that was cloned in Glasgow. There are very few genetic differences between these. Both viruses wil be used. The ICP27 gene is a negative regulator of cellular gene expression and a positive regulator of virus gene expression. We possess a bank of viruses with contain deletions or mutation in the ICP27 gene. The mutant viruses are all in the public domain.
- HSV-1 genome with the ICP27 gene deleted
- HSV-1 genome with the ICP27 gene disrupted with a lac-Z gene insertion
- HSV-1 genome with various point mutations and deletions of the ICP27 gene: - deleu, d1-2, d3-4, (all replication deficient 100-fold reduction in progeny) d4-5 (replication defective), d5-6, d6-7 (both replication competent) M11, M15, M16, n263R, and n406R (replication defective)
- HSV-1 genome expressing a EYFP-ICP27 fusion protein

**References**
We also wish to examine the role of the virus protein kinase UL13 in modulating the effects of ICP27 and we possess a virus with the UL13 gene deleted.

The intended functions are to infect cells in culture and to determine differences in efficiency of virus protein expression between the wild type and mutant viruses. For ICP27 this will help map the domain(s) of the ICP27 protein involved in regulating gene expression. The UL13 mutant virus will help discover whether the virus phosphorylates cellular gene expression proteins to modify their functions.

**Evaluation of foreseeable effects**

The risk for human health is small. HSV-1 is present in the environment. Wild type HSV-1 causes cold sores and a high percentage of the human population are chronically infected. Virus contact with broken skin is necessary for infection of naive individuals. Cold sores could develop but these rarely arise in the immunocompetent healthy individual. Apart from d5-6, d6-7, both replication competent, IPC27-mutant viruses are either severely disabled for growth or unable to replicate: 27lacZ (virus null for ICP27; replication incompetent), dleu, d1-2, d3-4, (all replication deficient: 100-fold reduction in progeny) d4-5 (replication incompetent), M11, M15, M16, n263R, and n406R (replication defective). All of these have some defect in replication or gene expression. UL13 mutant viruses are also severely compromised for growth. HSV-1 cannot infect animals other than humans.

E.coli is a bacterium found in the environment and does not cause disease.

ICP27 protein is non-toxic, very labile in the environment and unable to cross cell membranes so has a very low probability of causing deleterious effects upon production in bacterial culture.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Virus material**

Cell culture flasks and vials containing virus stocks will be filled with 1% Virkon and immersed overnight. Flasks will be emptied and autoclaved before disposal. Any spills will be flooded with 1% Virkon and mopped up with tissue and tissues will be bagged and autoclaved before disposal. 1% Virkon gives 100% kill.

**Bacterial material**

Culture flasks and vials will be filled with 1% Virkon and immersed overnight. Flasks will be emptied and autoclaved before disposal. Any spills will be flooded with 70% ethanol and mopped up with tissue. Tissues will be bagged and autoclaved before disposal. These procedures give 100% kill.

All workers will wear gloves during all procedures. Gloves will be bagged and autoclaved after use.

Any transportation of virus stocks will be in a sealed plastic box.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Comments from the GM committee included "please increase background information on herpesvirus infections". This was incorporated into the last risk assessment submitted and the project was approved on the 24 March 2007.

Project Containment

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Project Ref 223/13.1

Date Ack'n'd: 12/11/2013

CU2 Project Title: Primate lentivirus molecular biology, cell tropism and Lentivirus-host interactions

Class: Class 3

CultureVolClass2: < 1 Litre

CultureVolumeClass3-4: < 1 Litre

Non-GMM Consent Granted: Yes

Project notified under transitional arrangements: N

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID: 223/13.1a

Date of Significant Change: 19/01/2016

Project Additional Information

Purposes of the contained use:
Primate lentiviruses are a taxonomic group of complex retroviruses that infect more than 40 species of primates
including humans [1, 2]. HIV-1 (Human Immunodeficiency Virus) is a primate lentivirus that emerged in human populations in the early 20th century [3] following multiple transmissions of Simian Immunodeficiency Viruses (SIVs) from chimpanzees [4]. The less successful human pathogen, HIV-2, emerged in humans following multiple transmissions of a lentivirus from Sooty Mangabeys (SIVsmm) [5]. HIV-1 has infected over 50 million people and has caused an AIDS (Acquired Immunodeficiency Syndrome) pandemic that has consumed more than 25 million lives [6, 7]. Interestingly, primate lentiviruses might have ancient origins [8, 9] and many SIVs are not pathogenic in their natural hosts [10]. This is the case for SIVagm which does not seem to cause disease in infected African Green Monkeys [11], although a paucity of data exists considering natural SIV infections of that natural host [12]. The main HIV-1 animal model is SIVmac, a virus transmitted to rhesus macaques from Sooty Mangabeys. Like HIV-1, this virus is highly pathogenic in the normal host, rhesus macaques, possibly due to its recent emergence in rhesus macaques following unintentional experimental transmission from Sooty Mangabeys [13].

Primate lentiviruses are enveloped viruses that have the basic gag (group specific antigen) pol (polymerase) and env (envelope) structure shared by all retroviruses. Primate lentiviruses have a diploid RNA genome whose expression is regulated by tat and rev. Following infection of a target cell, the retroviral genome is reverse transcribed into DNA, translocated to the nucleus and integrated into host DNA [14, 15]. Lentiviruses also encode a number of ‘accessory’ genes that are dispensable for replication in vitro but whose expression is important for pathogenesis in vivo. These genes are mainly involved in downregulating viral receptors following infection in addition to antagonizing innate and intrinsic immune defences. Understanding exactly how different lentiviruses neutralize species-specific blocks to replication is of huge importance in understanding the emergence of new lentiviruses in human populations and how these viruses replicate within the hostile host [16].

This project will investigate the molecular biology and virus-host interactions of primate lentiviruses, focusing on (but not limited to) HIV-1. In addition the related HIV-2, SIVmac, SIVagm, SIVgor and SIVcpz viruses will also be analysed.

1) Primate lentivirus molecular clones, and GM variants will be examined for their ability to replicate/infect/assemble infectious particles in the presence of human and rhesus ISG libraries (and other bovine and avian ISG-libraries currently under construction). Parental clones and GM molecular clones can then be examined in the presence of different ISGs [17, 18].

2) Primate lentivirus molecular clones, and mutants will be examined for their ability to replicate/infect/assemble infectious particles in cell lines/primary cells from different vertebrate hosts in the presence/absence of interferon or in the presence/absence of exogenous antiviral/dependency factors. The specific aims are to characterise the speciesspecific antiviral activities of these cells and also to characterise/overcome other blocks to replication in non-natural species. Parental clones and GM molecular clones can then be examined in cell lines/primary cells from different species in the presence/absence of interferons. The ability of the parental virus and these GM viruses to induce interferons and other inflammatory cytokines will also be considered.

3) Generation of ‘unbiased’ mutagenized libraries to quantify the ‘genetic robustness’ of HIV-1. We have recently completed such an analysis of the CA region of HIV-1 Gag and we will interrogate the robustness of other HIV-1 ORFs in a similar fashion. Mutant libraries will not only be used to investigate the mutant phenotypes but also to investigate the influence of particular viral sequences on permissivity/sensitivity to restriction factors (for example the mutagenized CA library could be used to investigate the dependency of HIV-1 on TNP03 for infection or the ability to evade isoforms of rhesus TRIM5alpha proteins which usually restrict HIV-1 replication. The ability of the parental virus and these GM viruses to induce interferons and other inflammatory cytokines will also be considered.

4) Characterisation of the biological properties of viral ORFs/elements with particular regard to the evasion or antagonism of host antiretroviral defences (including but not limited to APOBECs, TRIMs, Tetherin, SAMHD1 and MX
proteins). Because of the unknown nature of this research (we don't yet know which viral sequences will require modification in 12 months time) the responses in this form are deliberately general to allow us to respond rapidly and appropriately to novel observations. Our work will however always follow the generic workflows highlighted in the attached risk assessments and these are briefly described below.

Class 3 activities:

Workflow A - Reporter modifications. Reporter genes (such as EGFP or luciferase) or epitope tags will be inserted into the proviral molecular clones. These modifications will either be inserted in frame, replace existing ORFs (such as EGFP replacing Nef) or will be combined with elements (such as viral IRESs or the FMDV 2A translational skip) to facilitate expression.

Workflow B - Introducing coding or non-coding mutations via site-directed mutagenesis or by low fidelity PCR

Workflow C - Introducing deletions. These can be either physical deletions of viral sequence or 'functional deletions' where start codons are removed and additional stop codons inserted.

Workflow D - Chimeric viruses. Making targeted viral chimaeras where a single viral ORF/non-coding element is either inserted into the viral genome or used to replace the orthologous/functionally analogous ORF/element.

Workflow E - Examine sequences in isolation. From time to time it will be necessary to exclude most of the viral genome and consider the influence of a single ORF/element on eukaryotic cells. In all cases this work will be limited to single ORFs and short sequence elements. Viral accessory genes will be the most likely desired targets.

Workflow F - Purification to examine biological properties/identify interaction partners. Because of the nature of scientific research it is impossible to provide an exhaustive list of the viral sequences that might be studied in this way. However the most likely scenarios involve over expression of enzymes or structural proteins to purify them and investigate their biological properties. In this workflow, purification from prokaryotic cells or eukaryotic cells might be desired. In all cases this work will be limited to single ORFs or short nucleic acid elements.


9. Barklis E, Mulligan RC, Jaenisch R: Chromosomal position or virus mutation permits retrovirus expression in


Molecular clones to be used will be primate lentiviruses:
- Human Immunodeficiency Virus-1 (HIV-1)
- Human Immunodeficiency Virus-2 (HIV-2)
- Simian Immunodeficiency Virus of Macaques (SIVmac)
- Simian Immunodeficiency Virus of African Green Monkeys (SIVagm)
- Simian Immunodeficiency Virus of Chimpanzees (SIVcpz)
- Simian Immunodeficiency Virus of Gorillas (SIVgor)

Recipient or parental organism

Prokaryotic hosts will be handled at containment level 1 and eukaryotic hosts at containment level 3 (workflows A-D) and containment level 2 (workflows E-F).

Plasmid vectors:
Viral sequences (including full length clones or partial sequences of HIV-1 or other retroviruses) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Systems for prokaryotic gene expression are under control of an inducible prokaryotic promoter. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV or SV40), eukaryotic (e.g. actin promoter) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin.
Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human cells.

Hosts:
Prokaryotic hosts:
Disabled, commercially available K12 derived E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. The risk to the environment is therefore effectively zero.

Eukaryotic hosts:
Vertebrate cell lines of various origins (typically human, non-human primate, mouse, hamster, bovine, bat, chicken etc.) and stably modified derivatives such as TZM-bl indicator cells (derived from HeLa cells).

**Origin & function**

In prokaryotic cells, only selectable (e.g. antibiotic resistance) genes will be translated; prokaryotic cells may also be used for protein expression and subsequent purification.

Virus sequences will be under the control of a promoter that will only generate transcripts in eukaryotic cells or in in vitro transcription reactions.

In eukaryotic cells, the following RNAs will be produced which can be translated into the following gene products:

- **Retroviral RNA and proteins:** By inserting material from other retroviruses into primate lentiviruses we hope to sensitise/desensitise these viruses to inhibition by certain restriction factors or overcome specific replication blocks in certain cell types. For example adding vif from SIVmac desensitises HIV-1 to APOBEC3 inhibition in macaques.
- **Reporter genes:** Reporter genes of prokaryotic or eukaryotic origin (e.g. luciferase, fluorescent proteins etc.). These will be inserted into viruses to facilitate easy monitoring of viral replication (such as replacing nef with EGFP).
- **Host genes/host non-coding sequences:** By inserting this material into primate lentiviruses we hope to sensitise/desensitise these viruses to inhibition by certain restriction factors or overcome specific replication blocks in certain cell types. For example inserting a cellular promoter in the lentiviral LTR could alter dependency on host factors required for tat-dependent transcription.
- **Sequence changes (deletions/insertions/site directed mutagenesis) in viral sequences:** Mutation or deletion will target disruption of protein functions or non-coding viral sequences. This will allow us to make attenuated viruses sensitive to restriction factors (such as HIV-1 lacking Vpu which is more sensitive to tetherin). This will also allow us to study the genetic robustness of lentiviruses and also make modifications to sensitise/desensitise lentiviruses to host restriction or dependency factors. Modifications could also facilitate new interactions (such as modifying HIV-1 Gag to facilitate incorporation of Vpx, expressed in trans, into HIV-1 virions) or introduce epitope tags to monitor viral protein expression.

Techniques used to introduce vectors into cells:

- **Prokaryotic cells:** Introduction of DNA into E. coli will be by heat shock/chemical transformation or by electroporation. These techniques have been extensively described and are widely used; they rely on getting DNA very close to the bacterial membrane and introducing the genetic material through transient pores or membrane fusion.

- **Eukaryotic cells:** Cells will be transfected using transfection reagents such as PEI or by electroporation. Supernatant containing infectious particles can then be used to infect permissive cell lines.

**Evaluation of foreseeable effects**

Foreseeable effects/risk assessment for human health and safety (also see Risk Assessments):

- **Plasmid vectors:** Viral sequences (including full length clones or partial sequences of HIV-1 or other retroviruses) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA/proviral form. Plasmid vectors are based on
bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Transcription of even complete proviral DNA will result in no infectious particles since retroviral gene expression is heavily dependent on splicing and virion assembly cannot proceed in prokaryotic cells. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV or SV40), eukaryotic (e.g. actin promoter, pathway inducible promoter such as STAT, interferon etc.) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human cells. None of the genes used in these studies are known oncogenes. The hazard of expressing ‘foreign’ genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the foreign gene product and is discussed in more detail below.

HIV-1:
HIV-1 is the aetiological agent of acquired immunodeficiency virus (AIDS) [1-3]. The classification into ACDP hazard group 3 indicates current expert opinion that containment level 3 precautions are considered adequate for controlling the risks associated with working with HIV-1. Changes that will be introduced into the virus sequences will be targeted at disrupting or changing specific functions of viral RNA or viral proteins that will usually attenuate replicative capacity; such changes are highly unlikely to increase virulence (viral load is associated with progression to AIDS) as they modify virus sequences, or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication (indeed modified viruses are often impaired in vitro and in vivo [1-5]). Despite the low probability that genetic modifications will increase the pathogenesis or risk of infection of a given agent, the ACDP hazard group 3 status of the unmodified primate lentiviruses means modified viruses are still potentially hazardous particularly through sharps injuries/ultracentrifuge accidents [6]. It is also possible that modifications under workflows C or D could potentially influence the cell-tropism of HIV-1 in vivo. For example, insertion of SIVmac vpx into HIV-1 (workflow D) could conceivably better arm this virus to infect macrophages and dendritic cells [7] (although this remains to be demonstrated in vivo). Similarly the rate of viral dissemination to different tissues would likely be reduced from HIV-1 viruses not encoding Vpu [8] (workflow C). It is difficult to predict exactly what changes to cell tropism could occur in vivo but they are unlikely to be substantial. This is because all the primate lentiviruses mentioned in this RA infect very similar cell subsets during a course of infection in vivo (CD4+ve T-cells as the major target for infection with limited tropism for macrophages and dendritic cells. Although infection of many other cell subsets has been documented, such as astrocytes, these do not represent a major target for infection). It is therefore unlikely that an agent more pathogenic than HIV-1 (amongst the most pathogenic primate lentiviruses) could be generated in this way.

HIV-2:
HIV-2 is far less pathogenic than HIV-1 but can cause AIDS-like illnesses in humans. The classification into ACDP hazard group 3 indicates current expert opinion that containment level 3 precautions are considered adequate for controlling the risks associated with working with HIV-2. Changes that will be introduced into the virus sequences will be targeted at disrupting or changing specific functions of viral RNA or viral proteins and can be usually expected to attenuate viral replicative capacity; such changes are highly unlikely to increase virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication [9-11]. Despite the low probability genetic modifications will increase the pathogenesis or risk of infection from genetically modified viruses, the ACDP hazard group 3 status of the unmodified virus means modified viruses are still hazardous particularly through sharps injuries/ultracentrifuge accidents. Similarly to HIV-1 it is possible that viruses generated as part of workflows C and D could have altered tropism in vivo. The same reasoning suggests that it is very unlikely that modifying HIV-2 in the manner described in this RA could
make this agent higher risk than HIV-1.

Nonhuman primate (NHP) lentiviruses:
NHP lentiviruses (such as SIVmac, SIVagm SIVcpz and SIVgor). Primate lentiviruses (from NHPs) are only rarely capable of infecting humans and causing disease. Their classification into ACDP hazard group 3 indicates current expert opinion that containment level 3 precautions are considered adequate for controlling the risks associated with working with SIVs. Changes that will be introduced into the virus sequences will be targeted at disrupting or changing specific functions of virus RNA or virus proteins and can usually be expected to reduce replicative capacity; such changes are highly unlikely to increase virulence as they modify viral sequences, or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication. Although extremely rarely able to productively infect humans, cells of human origin are already permissive for many NHP lentiviruses in vitro. Modifications to investigate cellular tropism are therefore unlikely to increase the risk of human infection [12-16]. However it remains possible that introducing sequences from HIV-1/HIV-2 into NHP lentiviruses could make them more infectious/pathogenic to human hosts and humans have been infected with HIV-1 through sharps injuries/ultracentrifuge accidents [6]. However, even when done deliberately to shift tropism, genetic modification has not yet resulted in the generation of lentiviruses that are pathogenic in their new host [5, 17] an important milestone in developing HIV-1 animal models. Whilst inserting material from human immunodeficiency viruses into NHP lentiviruses could make them more infectious or pathogenic to human hosts, this statement should be considered in light of the fact that NHP lentiviruses rarely infect humans and very rarely cause disease. It is extremely unlikely that NHP lentiviruses could be modified in the manner described in this RA to generate higher risk agents than HIV-1.

Hosts
The host/recipient organism:
Prokaryotic organisms: All strains will be disabled, commercially available E. coli K12 derivatives classified as ‘especially disabled hosts’ by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.
Eukaryotic cells:
Cell lines to be used would not survive inside the immunocompetent human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA or infection will confer no growth or survival advantage in or outside the laboratory to these cell lines.
Eukaryotic primary cells would not survive inside the human body (immune rejection) Addition of lentiviral infection will confer no significant growth or survival advantage in or outside the laboratory to these cell lines.
The inserted/donated/mutated genetic material:
Workflow A - Reporter modifications. Reporter genes: Reporter genes of prokaryotic or eukaryotic origin (e.g. luciferase, fluorescent proteins etc.). No harmful properties have been attributed to these proteins. There would be no altered physiology expected if any of these genes were expressed; EGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Photinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other known biological function. As such these modifications do not themselves present a hazard. However viral clones with reporter modifications are inherently hazardous as virus derived from the parental clones are classified as ACDP hazard group 3 modified viruses present a substantial hazard. Accidental infection of HIV-1 has occurred in laboratory/hospital settings through needle/sharps injury and ultracentrifuge accidents [6] and modified viruses could present a similar hazard.
Workflow B -Introduction of coding/noncoding mutations by site directed mutagenesis or low fidelity PCR: The vast
majority of coding mutations have a substantial negative influence on viral replicative fitness (Rihn et al., 2013) [18-20]. Replicative fitness and viral loads are related viral properties and for HIV-1 high viral loads and viral set points are strongly correlated with a rapid progression to AIDS. However, despite most modifications attenuating viral replication, some will be neutral and considering the classification of parental viruses into ACDP hazard group 3, these viruses could present a similar risk to HIV-1. Accidental infection of HIV-1 has occurred in laboratory/hospital settings through needle/sharp injury and ultracentrifuge accidents [6].

Workflow C - Deletion of accessory genes/noncoding elements. Although viruses lacking accessory genes/noncoding elements are often less infectious/less pathogenic, considering the classification of parental viruses into ACDP hazard group 3, these viruses could present a similar risk to HIV-1. Accidental infection of HIV-1 has occurred in laboratory/hospital settings through needle/sharp injury and ultracentrifuge accidents [6].

Workflow C - Deletion of essential viral genes. Although viruses lacking essential genes are severely attenuated, depending on the type of modification/experiment phenotypic reversion is a possibility. Considering the classification of parental viruses into ACDP hazard group 3, these revertant viruses could present a similar risk to HIV-1. Accidental infection of HIV-1 has occurred in laboratory/hospital settings through needle/sharp injury and ultracentrifuge accidents [6].

Workflow D - Viral gene/noncoding element replacement/insertion. Whilst chimeric viruses will usually be attenuated, it is formally possible that introducing genetic information from human immunodeficiency viruses into NHP lentiviruses could result in a modified NHP lentivirus more competent to infect and cause disease to the human host. In this regard modified NHP lentiviruses are potentially as hazardous as HIV-1 (classified into ACDP hazard group 3). Accidental infection of HIV-1 has occurred in laboratory/hospital settings through needle/sharp injury and ultracentrifuge accidents [6] and it is possible modified chimeric lentiviruses could infect humans.

Workflow D - Chimeric LTRs. Whilst chimeric viruses will usually be attenuated, considering the classification of parental viruses into ACDP hazard group 3, these viruses could present a similar risk to HIV-1. Accidental infection of HIV-1 has occurred in laboratory/hospital settings through needle/sharp injury and ultracentrifuge accidents [6].

Workflows E and F - Extremely low risk. No possibility of generating infectious lentiviruses. Plasmid DNA could contain potential oncogenes. This DNA could lead to transient expression of oncogenes if injected. However transfection in vivo is extremely inefficient so the oncogenic potential of these plasmids in humans would be extremely low. It is unlikely that lab strain prokaryotic cells transformed to contain plasmids encoding single viral ORFs/elements pose a significant hazard to humans. It is possible that certain viral ORFs or elements could provide a growth advantage to cells in culture. However any modest advantage would not overcome immune rejection and these cells would be unable to colonise a human host. It is theoretically possible that endogenous retroviruses could be reactivated through complementation of single ORFs. Most endogenous retroviruses are multiply attenuated and functional endogenous retroviral ORFs are usually present elsewhere in the genome making increased risk from single ORF/element transfection unlikely.

Control measures – assign provisional containment level:

i) consequence/severity of effects: High (possible infection with a virus which is fatal unless treated).

ii) likelihood of effects being realised: Low-Extremely low (infection via occupational exposure to HIV-1 is extremely rare).

iii) overall risk: Low

GM bacteria transformed with plasmids containing viral sequences, reporter genes, other eukaryotic/prokaryotic genes or non-coding sequences for the purpose of preparing DNA (workflows A-F) or purifying protein (workflow F) - Containment Level 1.

Eukaryotic cells containing hazard group 3 modified primate lentiviral sequences (capable of forming infectious virus, or capable of undergoing recombination or minor mutations to form infectious virus - workflows A-D) – Containment
Level 3.

- Containment level 3 and the relevant controls required in the SACGM compendium of guidance together with the additional controls as defined in the attached risk assessment will be used for this work. These controls are detailed in the containment level 3 laboratory Code of Practice and Standard operating procedures. Relevant control measures include, no use of sharps, no use of ultracentrifuges, appropriate personal protective equipment, appropriate use of microbiological safety cabinets and rigorous inactivation/disposal procedures. In addition a specific post exposure prophylaxis (PEP) SOP is in place in case of accidental exposure to HIVs/NHP lentiviruses.

- Eukaryotic cells transfected with plasmids encoding viral ORFs/elements - containment level 2 (workflows E and F).

- Containment level 2 and the relevant controls required in the SACGM compendium of guidance with Good Microbiological Practice and Good Occupational Safety and Hygiene together with the additional controls as defined in the attached risk assessment will be used for this work. Relevant control measures include, appropriate personal protective equipment, appropriate use of microbiological safety cabinets and appropriate inactivation/disposal procedures.

Foreseeable effect/risk assessment for the environment:

HIV-1: Already endemic in the UK and not on any SAPO list. Changes that will be introduced into these virus sequences will be targeted at disrupting or changing specific functions of viral RNA or viral proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences, such as reporter genes, which confer no advantage to virus replication. The modified viruses will not have any inserted foreign genes other than reporter genes (which confer no selective advantage and are not known to be toxic), or sequences from other retroviruses or (usually disruptive) changes in viral genes or non-coding sequences. Changes in viral pathogenesis are therefore unlikely other than as described above under the human health and safety section. In the unlikely event that viral tropism is altered through genetic modification, the decreased fitness of these modified viruses makes them extremely unlikely to pose a risk to the environment. It is difficult to imagine a scenario where modified HIV-1 poses more of a risk to the environment than wild-type endemic HIV-1.

HIV-2: Already present in the UK and not on any SAPO list. Changes that will be introduced into these virus sequences will be targeted at disrupting or changing specific functions of viral RNA or viral proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences, such as reporter genes, which confer no advantage to virus replication. The modified viruses will not have any inserted foreign genes other than reporter genes (which confer no selective advantage and are not known to be toxic), or sequences from other retroviruses or (usually disruptive) changes in viral genes or non-coding sequences. Changes in viral pathogenesis are therefore unlikely other than as described above under the human health and safety section. In the unlikely event that viral tropism is altered through genetic modification, the decreased fitness of these modified viruses makes them extremely unlikely to pose a risk to the environment. It is difficult to imagine a scenario where modified HIV-2 poses a risk to the environment.

NHP lentiviruses: These viruses are not endemic in the UK due to an absence of suitable wild populations of natural hosts (although infections have been documented in captivity), and do not appear on any SAPO lists. These viruses are not transmitted by aerosol and require direct fluid contact with permissive mucosa for transmission to occur underlining the low risk these entities pose to the environment. Changes that will be introduced into these virus sequences will be targeted at disrupting or changing specific functions of viral RNA or viral proteins and can overwhelmingly be expected to attenuate virus fitness; such changes are highly unlikely to increase virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences, such as reporter genes, which confer no advantage to virus replication. However, although unlikely, some modifications made as part
of workflow D could potentially make NHP lentiviruses more infectious/pathogenic to the human host. If an individual was infected, the virus was able to persist in the human host and was transmissible between humans (3 extremely unlikely properties). These modified viruses could persist in theory persist in the environment.

Hosts:
Prokaryotic organisms: Disabled, commercially available K12 derived E. coli strains. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. Acquiring antibiotic resistance (from the plasmid vector) or additional sequences (virus or marker gene sequences) will not give these strains any survival advantage in the environment. The risk to the environment is therefore effectively zero.

Eukaryotic cells: Cell lines to be used are not viable outside the laboratory and thus pose no threat to the environment. Addition of plasmid vectors or virus will confer negligible growth or survival advantage in the environment.

In summary
i) consequence/severity of effects: Moderate
ii) likelihood of effects being realised: Extremely Low
iii) overall risk: Low

GM bacteria transformed with plasmids containing viral sequences, reporter genes, other eukaryotic/prokaryotic genes or non-coding sequences for the purpose of preparing DNA (workflows A-F) or purifying protein (workflow F) -
Containment Level 1.

Eukaryotic cells containing hazard group 3 modified primate lentiviral sequences (capable of forming infectious virus, or capable of undergoing recombination or minor mutations to form infectious virus - workflows A-D) – Containment Level 3.
- Containment level 3 and the relevant controls required in the SACGM compendium of guidance together with the additional controls as defined in the attached risk assessment will be used for this work. These controls are detailed in the containment level 3 laboratory Code of Practice and Standard operating procedures. Relevant control measures include appropriate personal protective equipment, appropriate use of microbiological safety cabinets and rigorous inactivation/disposal procedures.

Eukaryotic cells transfected with plasmids encoding viral ORFs/elements - containment level 2 (workflows E and F).
- Containment level 2 and the relevant controls required in the SACGM compendium of guidance with Good Microbiological Practice and Good Occupational Safety and Hygiene together with the additional controls as defined in the attached risk assessment will be used for this work. Relevant control measures include, appropriate personal protective equipment, appropriate use of microbiological safety cabinets and appropriate inactivation/disposal procedures.

BIBLIOGRAPHY
6. Do AN, Ciesielski CA, Metler RP, Hammett TA, Li J, Fleming PL: Occupationally acquired human immunodeficiency virus (HIV) infection: national case surveillance data during 20 years of the HIV epidemic in the
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**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Disinfect Lentivirus contaminated material immediately.

Disposable solids (e.g. plasticware such as flasks, tubes, pipette tips etc.)- soak in 1% Virkon (w/v) for a minimum of 12 hours. Transfer solid contents to clear autoclave bags and autoclave at 121°C for a minimum of 20 minutes prior to final disposal by incineration, remaining liquid to be discharged to drain.
Other solids (agar plates, gloves etc.)- placed in a marked box lined with a clear autoclave bag, and disposed of by autoclaving using a make safe cycle of 121°C for at least 20 minutes. Gloves used for lentiviral work should be disinfected with >70% alcohol/ >1% virkon before putting in receptacle for autoclaving. Liquids (e.g. samples, tissue culture media)- add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours followed by autoclaving and then discharge to drains. Liquids (e.g E. coli bacterial growth medium) sterilise with chloros for a minimum of 12 hrs, and then discharge to drains.

Degree of kill:
Chemical Sterilization by Virkon- effectively 100% kill.
Chemical Sterilization with >70% alcohol - effectively 100% kill.
Autoclaving - effectively 100% kill (every run checked and recorded).
Chemical Sterilization by Chloros- effectively 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<tr>
<td>L2 Yes</td>
<td>L3 Yes</td>
<td>L4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
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Project Ref 223/13.2

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<td>12/11/2013</td>
<td>Influenza virus pathogenesis, host range and virus/host interactions</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
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</tr>
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</table>
The overall aim of our research is to elucidate the molecular and evolutionary determinants of host range, replication and pathogenicity of influenza viruses. This project involves work on several areas including, epidemiology, mathematical modelling of virus evolution, structural biology, and studies on virus-host interactions and molecular pathogenesis. It is the last aspect i.e. virus-host interactions and molecular pathogenesis with which this application is concerned. Within the CVR, the group of Pablo Murcia will generate the tools to produce recombinant influenza viruses and these viruses will form the basis of the work covered in this application. The work proposed will involve both in vitro, ex vivo infections. Embryonated chicken eggs will also be used as a system for growing some virus stocks.

### Recipient or parental organism

**Parental viruses (non-GMOS from which GMOS would be derived)**

- A/equine/Miami/1963 (H3N8)
- A/equine/Fontainebleau/1979 (H3N8)
- A/equine/Argentina/1995 (H3N8)
- A/equine/Newmarket/1993 (H3N8)
- A/equine/South Africa/2003 (H3N8)
- A/equine/Ohio/2003 (H3N8)
- A/canine/New York/2008 (H3N8)
- A/equine/Mongolia/2009 (H3N8)
- A/wild bird/Mongolia/2009 (H3N8)
- A/wild bird/Mongolia/2010 (H3N8)
- A/wild bird/Mongolia/2011 (H3N8)
- A/swine/England/495/2006 (H1N1)
- A/WSN/33 (H1N1)
- A/Puerto Rico/8/34 (H1N1)
- A/California/04/09 (H1N1)
- A/Brisbane/10/07 (H3N2)

**Recipient organisms:**
Equine influenza virus (EIV) H3N8 (genus Influenzavirus A), and derived recombinant viruses.
Canine influenza virus (CIV) H3N8 (genus Influenzavirus A), and derived recombinant viruses.
Human influenza virus H3N2 and H1N1 (genus Influenzavirus A), and derived recombinant viruses.
Other containment level 2 influenza viruses may be considered as long as they are unrelated to those capable of causing infection of humans.
Reporter systems mimicking Influenza virus replication: Minigenomes of the viruses enumerated above.

**Host/vector system**

**Plasmid vectors:**
Viral sequences (full length clones of Influenza viruses; partial sequences of these and other influenza viruses) and other genes (cellular, viral, reporter) are available in plasmid-encoded cDNA form. RNAs can be transcribed from bacterial promoters (e.g., T7 or SP6 by in vitro transcription or plasmid-expressed RNA polymerase in cells) or eukaryotic promoters (CMV, SV40, cellular promoters, baculovirus promoters etc.). Plasmids for expression of influenza virus sequences (virus rescue or minigenome) typically contain an RNA polymerase I promoter and a hepatitis delta virus ribozyme or a mouse RNA polymerase I terminator. Plasmids contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin) and sometimes a selectable eukaryotic drug resistance marker such as neomycin or puromycin resistance. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

**Hosts:**

**Prokaryotic hosts:**
Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste should prevent release of viable organisms. The risk to the environment is therefore effectively zero.

**Eukaryotic hosts:**
Vertebrate cell lines of various origins (canine, equine, avian, hamster, bovine, human, etc.), primary cells and explants of various origins (equine and canine).

**Origin & function**

The genetic material to be used in this project will be originated from three sources:
1- Field isolates propagated in embryonated eggs or cell culture.
2- Plasmids containing viral genetic material provided by other researchers.
3- Chemically synthesised plasmids.

The intended functions of the genetic material involved is the production, purification and characterisation of influenza viruses, as well as the production of minigenomes. The viruses produced will be grown in cells and/or embryonated chicken eggs and further used to infect animal and/or human cell lines as well as canine and equine tissue explants and primary cells. To determine the phenotype of infection of a given virus, readouts will include: the capacity for virus replication in tissue culture cells or explants, the ability of the viruses to induce cytopathic effect or histological changes, and the susceptibility of infected cells to apoptosis. If tracheal explants are infected, we will also test for changes in functional attributes such as ciliary beating.

The minigenome systems will be used in vitro. Cells will be transfected with five plasmids encoding the viral polymerase genes and a reporter gene. After transfection cells will be lysed and the activity of the reporter gene (i.e., luciferase activity or fluorescence) will be measured.

**Evaluation of foreseeable effects**

**Foreseeable effects to humans:**

Viruses.
1- Equine and canine influenza viruses have never been associated with human disease in nature. The same applies to the other animal influenza viruses proposed in this
2- Human influenza is typically a mild and self-limiting infection, acquired generally by the respiratory route. Severity depends on the strain of virus and host but in general tends to fall in a spectrum between asymptomatic to ~ 1 week of fever and malaise in otherwise healthy people. Severe illness can occur but is rare, occurring in less than 1% of cases. This level of risk applies to most wild type human H1N1 and H3N2 viruses and to some swine viruses. The laboratory adapted PR8 and WSN strains of H1N1 human influenza are known to be avirulent in man from many volunteer challenge studies as well as a long (> 70 years) history of safe use in the lab. Even seasonal influenza can have potentially serious consequences for anyone who is pregnant, immunosuppressed, asthmatic, or has other respiratory/underlying chronic diseases. Vaccines are available for the circulating human H1N1 and H3N2 strains and the majority of the population has at least some prior immunity to these viruses. The current human influenza vaccine is recommended for all the members of Pablo Murcia’s laboratory handling influenza. Those at risk of severe consequences of influenza (e.g. pregnant women) will be separately notified of the risks of these pathogens immediately after they identify themselves to their PI and occupational health. Individual risk assessments will be carried out governing these specific cases.

3- Reassortant viruses: Reassortants between CIV strains, EIV strains, and between CIV and EIV viruses will not affect their host range. Because CIV is a direct descendant of EIV, the chances that reassortants of these two viruses can infect humans is negligible. Reassortment between either EIV or CIV with human influenza viruses will be done in such a way to avoid the generation of novel antigenic subtypes with the potential to infect humans (such as H1N8, or H3N1 for example).

4- Influenza A virus minigenomes: Minigenomes pose no risk to human health, including those derived from pathogenic influenza A viruses. They cannot propagate on their own. Even if packaged by co-expression of glycoproteins and thus used to infect cells, their own transcriptional activity would be minimal. If packaged into virions, the associated risk is that of the virus itself as reporter genes in minigenomes are not reported to be toxic.

The inserted/donated genetic material.

1- Viral RNA and proteins: Most individually expressed viral proteins are unlikely to have harmful effects in eukaryotic cells, however some could perturb normal cellular metabolism, predispose or protect against cell death or render cells more or less susceptible to other infections if overexpressed or silenced. It is very unlikely that Influenza A virus RNA or proteins would have any harmful toxin-like effect outside cells. Only antigenomic RNA containing an influenza A virus sequence can be translated or give rise to infectious virus (if all necessary genomic information is provided) in eukaryotic cells. Viral non-coding RNA sequences do not encode biologically active molecules that are likely to induce physiological effects in humans on their own. None of the influenza virus gene products are known to be secreted cytotoxins.

2- Reporter genes: no harmful properties have been attributed to reporter genes of prokaryotic or eukaryotic origin (eg luciferase, fluorescent proteins etc.). There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria, a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Photinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function.

3- Sequence changes in viral sequences: Mutation or deletion will target disruption of structural and non-structural protein functions or non-coding viral sequences and are likely to have no deleterious effects on viral replication, thus not increasing risk or hazards to human health associated by viruses genetically modified in such a way.

Foreseeable effects to the environment:

Viruses

1- Equine influenza virus (EIV) has been endemic in the UK for over 40 years and is not on SAPO or ACDP lists. There is a vaccine available for EIV, although most horses have natural immunity due to vaccination or natural infections.

2- Canine influenza virus (CIV) is not thought to be endemic in the UK, and it is not on the SAPO list. In fact, it is not highly transmissible in the only country in which is endemic (USA). Transmission is common only in highly dense dog populations such as those found in animal shelters. Experimental infection results in mild respiratory...
disease. Serological evidence of infection in British foxhounds has been shown, although no outbreaks have been associated with it. Given the low transmissibility and low pathogenicity of CIV, the chance of accidentally infecting dogs initiating a transmission cycle sustained by local dogs is negligible.

3- Human influenza viruses: Currently circulating human influenza A viruses (pdm H1N1, seasonal H1N1, and H3N2) are globally distributed, there are available vaccines, and the majority of the population has at least some prior immunity to these viruses. Antivirals such as oseltamivir and zanamivir are also available. Currently circulating human H1N1 virus can infect swine and is endemic in this species. There are no reports of animal disease caused by human influenza viruses (with the exception of swine).

4- Reassortant viruses: Reassortants between CIV strains, EIV strains, and between CIV and EIV viruses will not affect their host range and thus will have the same risk as the parental viruses if not lower. As described above, reassortment between either EIV or CIV with human influenza viruses will be done in such a way to avoid the generation of novel antigenic subtypes to which human and animal populations have no prior immunity.

5- Minigenomes do not pose any risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable solids (eg plasticware such as flasks, tubes, pipette tips etc.): soak in 1% Virkon (w/v) for a minimum of 12 hours. Transfer solid contents to clear autoclave bags and autoclave at 121°C for a minimum of 20 minutes prior to final disposal by district council to land fill, remaining liquid to be discharged to drain.

Other solids (agar plates, gloves etc.): placed in a marked box lined with a clear autoclave bag, and disposed of by autoclaving using a make safe cycle of 121°C for at least 20 minutes. Seal contaminated plates (with for example bacterial GMOs) before placing in bag, to avoid lid falling off. Gloves used for influenza virus work should be disinfected with alcohol before putting in containers.

Eggs- after harvesting virus within the safety cabinet, eggs are to be placed in small autoclavable red bags containing paper towel to absorb any fluid coming from the egg. These bags are to be placed in secondary autoclavable bags and autoclaved prior to disposal as regular waste.

Liquids (eg. samples, E. coli culture media, tissue culture media): add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Sharps (eg needles, syringes, scalpels): decontaminate in 1% Virkon overnight then place in sharps bin for incineration.

Degree of kill:
Chemical Sterilization by Virkon: effectively 100% kill.
Autoclaving: effectively 100% kill (annual validation).

Is an emergency plan required according to regulation 20?

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
project containment

Laboratory Activities  Glass Houses  Growth Rooms

L2  L3  L4  L2  L3  L4  L2  L3  L4

Animal Units  Large Scale Activities  Human Clinical Applications

L2  L3  L4  L2  L3  L4  L2  L3  L4

project ref  223/15.1

date ackn'd  09/02/2015
cu2 project title  genetic modification of mosquitoes: studies on physiology and virus-host interactions

class  culturevolclass2  culturevolumeclass3-4

class none  < 1 litre

non-gmm  consent granted

project notified under transitional arrangements  N

withdrewn  N

tick if notifying a connected programme of work  N

historical significant changes

historical date of additional info

significant change id

date of significant change

project additional information

purposes of the contained use

Arboviruses are transmitted to susceptible vertebrates by arthropod vectors such as mosquitoes. Most arboviruses belong to the Flaviviridae, Bunyaviridae and Togaviridae families of RNA viruses. The interactions between arboviruses and their vectors are highly complex. Arboviruses first come into contact with the mosquito midgut following blood-meal, and must infect arthropod tissues, replicate and spread to the salivary glands from where they can be transmitted to vertebrates via saliva after bite. Through these stages,
arboviruses rely on host factors to support replication but are also confronted to the immune system of the host, summarised in [1-3]. Research on antiviral immune responses in vector arthropods has only in recent times seen some progress yet this area of research is crucial in understanding this virus/host interaction and develop novel antiviral control strategies targeting the vector. It is now clear, mainly from research on arbovirus-infected mosquitoes, that vectors do not passively replicate arboviruses but that immune responses are activated. These can result in activation of immune signalling pathways (STAT, Toll etc.), humoral responses in the arthropod hemocoel and most crucially RNA interference (RNAi) responses. It is not known how immune signalling pathways are activated following infection or how exactly they would mediate antiviral activities, and extracellular responses such as phenoloxidase activities also appear to be involved. The RNAi responses are better understood and form a major antiviral defence. In particular the exogenous RNAi and piRNA pathways are increasingly well studied, and some of the proteins such as Ago, Dicer and Piwi identified. In addition, mostly yet unidentified arthropod host proteins (by extrapolation from work on vertebrates [4]) are likely to contribute to virus entry, replication, exit and potentially spread.

Sequencing of the the genome of several vector species such as Aedes aegypti, Culex quinquefasciatus as well as Anopheles gambiae (https://www.vectorbase.org/) and novel genetic modification techniques (sometimes following adaptation from Drosophila melanogaster) allow the study of gene function in vivo. Among genetic modification techniques, site specific endonucleases such as TALENs, CRISPR-based gene knock out/knock in systems as well generation of mosquito lines with tissue specific transgenic promoters (see [5-8] and references therein) have advanced considerably. These techniques are all based on injection of mosquito embryos with either expression constructs and/or other nucleic acids that mediate recombination, insertion/deletion etc into the host genome. This allows completely new insights into mosquito gene function, in the context of viral infection and beyond. Moreover, transient gene silencing techniques based on RNA interference can be used to study gene function. This approach is based on treating mosquitoes (mainly through injection) with dsRNA against a target gene, inducing a RNAi response and target degradation [9-11].

In this project, we aim to further analyse mosquito gene functions by:

1/ Mosquito transgenics engineeing by targeted deletion or insertion of sequences using molecular biological methods by use of recombination/insertion based methods (PhiC31 integrase, Retrotransposons, TALENs, CRISPR technology etc.).
   * Deletions will be genes or non-coding sequences involved in mosquito immunity or physiology and in virus-host interactions.
   * Insertions will include:
     - Non-coding sequences such as viral sequences or promoter/regulatory regions of genes that are expressed in a tissue specific manner, or non-specifically in several/all tissues.
     - Reporter genes (luciferase, fluorescent proteins etc.) to follow physiological processes and to identify transformants as well as follow genetic inheritance.
     - Genes potentially conferring resistance to arbovirus, interfering with arbovirus replication, or genes linked to mosquito processes that are linked to virus replication (including viral genes) or physiological processes.
     - Inverse repeat constructions (or similarly aimed sequences) of mosquito genes to knock down their expression.

The vectors/nucleic acids used will be modified forms of phiC31 integrase or Cas9 that are supplied in the initial creation of lines by micro-injection of embryos. These exogenous integrase/nucleases are lost during the first generation and so the inserted/modified DNA sequences are effectively immobilised in the subsequently established transgenic lines.

Progeny of adults surviving the injection procedure are screened by fluorescence microscopy to identify stable transgenic lines which are then maintained and assayed for relevant phenotypes. These will include changes in arboviral susceptibility/immunity and mosquito fitness and physiology, as well as expression of reporter genes,

2/ Targeted transient silencing of mosquito genes (immune genes, genes involved in virus-host interaction or general physiology) by dsRNA injection and subsequent RNAi induction. Adults surviving to the injection will be assessed for relevant phenotypes like arboviral resistance/susceptibility, mosquito fitness and physiology.

Mosquito species considered here are:
Ae. aegypti (including laboratory strains such as Liverpool red eye, Higgs white eye etc), or related species such as Ae. albopictus, Ae. vexans from tropical or temperate regions.
An. gambiae (including laboratory strains such as Kisumu etc) and related species such as funestus, arabiensis etc from tropical or temperate regions.

As a general rule, we consider as laboratory strains mosquitoes bred and maintained under standard conditions in a containment level 2 laboratory setting.

Origin & function
In the context of this proposal, the following nucleic acid sequences will be introduced into mosquitoes (embryos, adults).

- Plasmids or cDNA constructs containing sequences for: 1) mosquito proteins involved in immune responses, virus-host interactions, physiological processes; 2) viral proteins; 3) reporter genes or other markers genes (luciferases, fluorescent proteins); 4) enzymes and proteins mediating recombination into and out of the mosquito genome; 5) sequences that can be transcribed into RNA (including hairpins to induce gene silencing).

- Double-stranded RNAs: short siRNAs (approximately 14-30 nucleotides) or longer dsRNAs (approximately 30-600 basepairs) targeting cellular or viral RNA for
degradation, or control dsRNAs/siRNAs.

- ssDNA or ssRNA for example for use with the CRISPR system.

Techniques used to introduce nucleic acids into mosquitoes: microinjection into mosquito body cavity or embryos, or introduction into midgut via feeding.

Evaluation of foreseeable effects

Foreseeable effects/risk assessment for human health and safety:

Plasmid vectors and cDNAs:
Mosquito, or viral sequences, protein or coding or non-coding are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Systems for eukaryotic RNA or protein expression are under the control of promoters of eukaryotic (eg actin promoter, tissue specific or pathway inducible) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters may be transcribed in human cells. None of the genes used in these studies are known oncogenes. The hazard of expressing ‘foreign’ genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the gene product.

Recipient organisms:

Prokaryotic organisms: All strains will be disabled, commercially available E. coli derivatives classified as ‘especially disabled hosts’ by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Mosquitoes (also see paragraph 8, below): Mosquitoes of the species indicated above are obtained from colonies in the UK or abroad. Precautions will be taken to minimise the risk of bites or escapes in strict compliance with standard operating procedures (attached separately). Measures to prevent human bites include storage of arthropods in sealed containers; work in designated rooms with restricted access; wearing of gloves and cooling or CO2 or chemical treatment of arthropods to slow their movement and facilitate handling according to standard operating procedures for CL2 (see attachment). Samples (material from dead arthropods) will be transported to our CL2 laboratory according to standard regulations for transport of samples.

Mosquitoes act as vectors to transmit disease between reservoirs and new hosts. Some GMs will reduce the vectorial capacity of the insect, however some modifications may increase the prevalence and intensity of a virus infection in the mosquito. The risk of a mosquito becoming accidentally infected with an arbovirus following escape is very low. Virus stocks are kept in freezers and infected cells within containers (cell culture flasks etc) and incubators, making it physically impossible for the mosquito to become infected. Arboviruses are enveloped viruses, and if incubated in decontaminating solution such as Virkon these are usually inactivated rapidly; moreover the mosquitoes are unlikely to survive a feed on solutions containing decontaminating agents.

The hazard of short lived escapees being a biting pest is high, but the consequences of the modification alone are negligible and similar to native mosquitoes and other hematophagous insects especially as there are no known mosquito-borne arboviruses present in Scotland. As with non GM uninfected mosquitoes, there is a biting hazard of uninfected GM mosquitoes accidentally released into the insectary. GM modifications are highly unlikely to add to the risk as and mentioned above, the risk of mosquitoes becoming accidentally infected is negligible: the consequence of this biting hazard is effectively zero and is no different to non GM mosquitoes. Precautions will be taken to minimise the risk of bites or escapes with the use of UV light traps traps where adult mosquitoes are manipulated and in strict compliance with Standard Operating Procedures (attached separately). The room housing the GM mosquitoes as well as non GM mosquitoes has a double door/gate system, with a badge access to authorized persons only. Dissection as well as injection of mosquitoes (for germline transformation or transient RNAi), and with nucleic acids will take place in specified rooms in strict compliance with standard operating procedures (attached separately). Decontamination procedures are detailed further in the SOP (attached separately).

This has been detailed further below.
Risk to human health depends on the nature of the genetic material inserted or deleted:

Most individually expressed or silenced viral or cellular proteins (for example immunity proteins, including regulators such as activators or inhibitors) or nucleic acid sequences (for example, silencing inducers targeting virus or host proteins, other small RNAs such as miRNA precursors etc.) in transgenic mosquitoes, or dsRNA injected into mosquitoes to degrade target sequences are unlikely to have direct harmful effects in eukaryotic cells, however some could perturb normal arthropod metabolism. It is very unlikely that any such RNA or proteins would have any harmful toxin-like effect outside cells. Transgenic mosquitoes as such are unlikely to pose any additional risks to human health. This analysis excludes transgenic mosquitoes expressing reporter genes of prokaryotic or eukaryotic origin (eg luciferase, fluorescent proteins etc.). No harmful properties have been attributed to these proteins. There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Photinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function. These genes are improbable in our experience to affect mosquito physiology and virus replication.

Foreseeable effect/risk assessment for the environment:

Hosts:

Prokaryotic organisms: Disabled, commercially available E. coli strains. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. Acquiring antibiotic resistance (from the plasmid vector) or additional sequences (virus or marker gene sequences) will not give these strains any survival advantage in the environment. The risk to the environment is therefore effectively zero.

Mosquitoes: Some anopheline and aedine mosquitoes are distributed throughout Britain, or parts of Britain. The aedine and anopheline species we intend to use are more suited to tropical and temperate climates however and are not likely to interbreed with any local species. They are not likely to survive or breed outside the laboratory regardless of genetic modification. We estimate that outside the CL2 facility in the surrounding building and facilities, escaped mosquitoes could survive for one to several days but the lack of humidity and water sources is likely to reduce any chances of survival. Outside the building their chances of survival are low, given the relatively cold climate in the surrounding areas throughout the year and we would also not expect mosquitoes to survive for longer than one or several days.

In summary:
* The hazard of short lived escapees being a biting pest is high, but the consequences are negligible and similar to native mosquitoes and other hematophagous insects. GM modification would not increase this potential and would most likely decrease it and thus the risk is effectively zero.
* There are no known sexually compatible species in the UK for anopheline and aedine and so potential for gene spread is virtually non-existant. GM modification would not increase this potential.
* Biologically active products produced by the GM mosquitoes will be in such small amounts that any direct risk is effectively zero.
* GM mosquitoes have been shown to be genetically stable and loss of GM would be extremely unlikely to increase any potential hazards over the reversion to wild type.
* None of the GMs are expected to change the behaviour of the mosquito and make them more aggressive biters.
* Some GM in the mosquito genome may influence ability to transmit and competence for a given arbovirus. However there infectious material is stored in closed containment and thus not accessible for mosquitoes.
* Risk of escape is minimised by manipulating adults in a closed environment (CL2 insectary). Rooms where adult mosquitoes are manipulated also contain UV light traps. Moreover, sinks are either covered up or contain filters to avoid any eggs escaping through the drains.
* GM mosquitoes will have a GFP genetic markers that can be used to distinguish GM strains and others mosquitoes either visually (GFP expression). Strain genetic background can also be checked by PCR in order to control different GM lines. This will be carried out at regular intervals as part of strain housekeeping.
i) consequence/severity of effects; aedine/anopheline GM mosquitoes: low.

ii) likelihood of effects being realised: Low

iii) overall risk: Medium

All with Good Microbiological Practice and Good Occupational Safety and Hygiene as outlined in Standard Operating Procedure.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Maintenance of GM mosquitoes, or injection with dsRNA will take place in a dedicated insectary at Containment Level 2 (CL2). A standard operating procedure (SOP) as described below has also been added separately. RNA and proteins or other cellular material will be isolated from crushed mosquitoes where sample isolation is required. Live mosquitoes may be monitored by microscopy. GM mosquitoes will be maintained and studied inside closed containers. In addition wingless mosquitoes may be used where removal from containment is required.

THIS EXCLUDES MICROINJECTION OF MOSQUITO EMBRYOS WITH NUCLEIC ACIDS OR PROTEINS AS THESE ARE IMMOBILE AND POSE NO RISK.

MOSQUITO CONTROL MEASURES APPLY FOLLOWING INCUBATION OF EMBRYOS FOR HATCHING.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (pipettes tips, gloves etc.)- place in container (lined with sealed bag), where necessary (see SOP) place in freezer overnight to kill mosquitoes, and dispose of by
autoclaving using a make safe cycle of 121-123°C for 30 minutes.

Arthropod-derived material such as the remains of mosquitoes will be placed in a sealed container (lined with clear bag), where necessary (see SOP) placed in a freezer overnight to kill mosquitoes, and disposed of by autoclaving using a make safe cycle of 121-123°C for 30 minutes.

Liquids (eg. samples) – dispose of by autoclaving using a make safe cycle of either 121-123°C for 30 minutes.

Degree of kill: Autoclaving - effectively 100% kill (annual validation).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4 L2</td>
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**Animal Units**

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**Project Ref** 223/15.2

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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>29/01/2015</td>
<td>Use of viral pseudotypes to investigate viral immunity and pathogenesis</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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</tbody>
</table>
Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID 223/15.2a
Date of Significant Change 23/06/2020

Project Additional Information

Purposes of the contained use

The measurement of humoral immunity (virus neutralising antibodies, VNA), viral cell tropism (e.g. receptor usage), cellular immunity (e.g. the introduction of viral proteins into target cells for ELISpot) and pathogenicity (e.g. the measurement of restriction factor activity in target cells).

Recipient or parental organism

Multiply disabled E.coli host strains

Host/vector system

Retroviral, rhabdoviral and adenoviral vectors prepared in HEK293/293T cells. The choice of system to be used for the generation of viral pseudotypes for the measurement of virus neutralising antibodies/viral entry is somewhat empirical. Thus, while a retrovirus-based system is preferred for lentiviral Env bearing pseudotypes, a rhabdoviral-based system works better for morbilliviral envelope glycoproteins. It is standard practice to compare different vector systems for the most suitable combination of packaging construct, transfer vector and Env expression vector. Because the biology of envelope incorporation is incompletely understood, our list of vector systems employed includes several variants of pseudotyping systems, thus increasing the likelihood that pseudotyping will be successful.

Origin & function

Evaluation of foreseeable effects

The nature of the envelope glycoprotein expressed on the surface of the pseudoparticles has little effect on the hazard associated with the pseudotypes, the primary difference is in the cell tropism, stability and titre of the pseudotypes produced. Thus murine leukaemia virus (MLV) particles bearing a vesicular stomatitis virus G protein (VSV-G) have a broader tropism (infect cells from many species) and are more stable than MLV particles bearing an ecotropic MLV envelope glycoprotein (will only infect murine cells). Similarly, rhabdovirus pseudotypes appear to be the most stable and efficient means of expressing morbillivirus glycoproteins. They have the highest titre and have the highest likelihood of introducing the foreign gene into recipient cells. As the pseudotypes are designed to express green or red fluorescent proteins (GFP or DSRed), luciferase (luc) or an enzyme such as β-galactosidase (lacZ), the risks to the experimenter and associated laboratory workers are very low. In essence, the aim of pseudotype based projects is to study viral biology and immunology without the requirement for handling replication competent virus. The pseudotyping system is, in essence, a protein-wrapped delivery sytem for a marker gene. While the origin of the vectors alters the efficiency of gene uptake into the target cells, the foreseeable risks are very similar and extremely low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All experiments involving the generation of, or infection with replication defective retroviral, rhabdoviral or adenoviral vectors encoding marker genes such as EGFP, RFP, DSRed or luc will be performed in Class II microbiological safety cabinets in the Retrovirus Research Laboratory. All liquid wastes are discarded into Virkon solution while

02/03/2022
solid wastes (cell culture plasticware etc.) are autoclaved. There is no foreseeable risk of environmental damage involved in these studies.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid and solid wastes will be incubated overnight with Virkon >1000 ppm chlorine prior to disposal. Following overnight incubation in Virkon, all cell culture plasticware will be autoclaved prior to ultimate disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 223/16.1

Date Ackn'd 21/01/2016

CU2 Project Title Bunyavirus reassortment and tropism

Class 3

CultureVolClass2 < 1 Litre

Consent Granted Yes

Date Project Ceased

CU2 Project Class CultureVol Class3-4

Page 5418 of 15326
The three segmented genome of bunyaviruses (L, M, S) allows these viruses to enhance their genetic potential by reassortment eg a re-shuffling of segments between strains of the same virus species or between closely related species to give raise to new types of bunyaviruses, which can for example influence tropism and virus host interactions by placing M segment encoded glycoproteins into a new genetic background. In this project reassortment between orthobunyaviruses and the consequences thereof, will be investigated. We will also investigate properties of the M segment eg sequence properties and properties of M segment proteins/glycoproteins by swapping sequences and/or domains between orthobunyavirus M segments.

Reassortant orthobunyaviruses, or orthobunyaviruses with mutated M segment sequences (affecting non coding or coding sequences such glycoproteins, non-structural proteins as described below) produced from hazard group 2/ACDP2 bunyaviruses. Use of these viruses and derived reverse genetics systems is notified as described above. This includes the viruses Bunyamwera (BUNV), Schmallenberg (SBV), Akabane (AKAV), Kairi (KRIV), Bwamba (BWV) and Cache Valley (CVV).

Recipient or parental organism

Reassortant orthobunyaviruses, or orthobunyaviruses with mutated M segment sequences (affecting non coding or coding sequences such glycoproteins, non-structural proteins as described below) produced from hazard group 2/ACDP2 bunyaviruses. Use of these viruses and derived reverse genetics systems is notified as described above. This includes the viruses Bunyamwera (BUNV), Schmallenberg (SBV), Akabane (AKAV), Kairi (KRIV), Bwamba (BWV) and Cache Valley (CVV).

Host/vector system

Prokaryotic hosts:
Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste should prevent release of viable organisms. The risk to the environment is therefore effectively zero.

Eukaryotic hosts:
Vertebrate cell lines of various origins (typically human, mouse, hamster, bovine etc.) and invertebrate cell lines of various origins (mosquito, culicoides, tick etc.). The vertebrate BSR-T7/5 cell line (derived from BSR, a clone of BHK) (Buchholz et al., 1999) express T7 RNA polymerase and will be used for virus rescue (infectious virus cDNA clones under control of a T7 promoter), as may similar cell lines expressing T7 RNA polymerase. 

Expression systems/cloning systems:
Plasmid vectors:
Viral sequences (full length cDNA clones of viral genomes or genome segments) are cloned into plasmids for genetic manipulation and propagation. RNAs can be transcribed from plasmids through bacteriophage promoters (ie. T7 or SP6 by in vitro transcription or plasmid-expressed RNA polymerase in cells) or other systems that are not usually transcriptionally functional on their own in eukaryotic cells. Plasmids contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin). The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

Origin & function

Origin of genetic material: hazard group 2 orthobunyaviruses Bunyamwera (BUNV), Schmallenberg (SBV), Akabane (AKAV), Kairi (KRIV), Bwamba (BWV) and Cache Valley (CVV). Reverse genetics systems and cDNA clones for these viruses are available and serve as source.

02/03/2022
Description of activities:
These experiments will only be carried out with the hazard group 2 viruses Bunyamwera (BUNV), Schmallenberg (SBV), Akabane (AKAV), Kairi (KRIV), Bwamba (BWV) and Cache Valley (CVV).

The following experiments will be carried out:
- Reassortment between orthobunyaviruses either by co-infection or by virus rescue techniques.
- Mutations in M segment sequences: shuffling/exchange of M sequences (for example glycoprotein sequences/domains) between orthobunyaviruses.

This includes production of such viruses which also carry attenuating mutations in the L or S segments, such as viruses in which the virulence factors such as NSs have been removed or viruses with tagged L proteins.

We expect such viruses to mirror the pathogenicity of the parent hazard group 2 viruses, but examples such as Ngari virus show that this may not be the case and precautionary principle applies in experiments that potentially alter the tropism and properties of the resulting viruses.

Risk associated with these novel orthobunyaviruses:
Bunyaviruses with potentially altered host or tissue tropism could potentially be infectious through aerosol, in addition to arthropod bite (natural route of transmission) or accidental inoculation. Changes in glycoprotein sequences can potentially affect tissue tropism and this could potentially include immune cells or other cells relevant in fighting orthobunyavirus infections, or tissues that are not usually targets but which usually control these viruses in humans, or tissues not usually affected by orthobunyaviruses. These may for example include epithelial cells in blood vessels, but also developing foetus etc. Thus, there might be an increased risk to human health from viruses described above unless they carry attenuating mutations such as NSs deletion. Moreover it cannot be excluded that aerosol transmission can take place with such altered orthobunyaviruses.

Risks to the environment:
Reassortment and changes in glycoprotein or other M coding sequences (sequence exchanges) can potentially lead to changes in virulence and pathogenicity. Again precautionary principle applies also with regards to risk to the environment, although vector specificity of reassortants or glycoprotein mutants produced as described, is likely to be similar to that of the parent virus. AKV: this virus infects animals and is closely related to SBV and also transmitted by midges and mosquitoes. BWV: again this pathogen, known to cause mild infections in humans in parts of Africa, can be transmitted by a variety of mosquitoes and is known to circulate across parts of Africa (East, West, Central). KRIV: this virus causes infections in animals and is transmitted by mosquitoes. CVV: infects humans and animals and is transmitted by various mosquitoes. In our laboratories, the risk of accidental infection of insects by ingestion of contaminated material is low and the likelihood of virus released from our laboratory infecting local vectors is effectively zero (see disinfection and waste disposal procedures). As for Rift Valley fever virus for example, advice will be given to lab personnel that they should not be in contact with susceptible animals or visit farms/zoos where they could potentially come into contact with susceptible species for a period of 48 hours following work with the viruses above. A form to this effect will be signed by staff working on these viruses. As stated above, in our laboratories, the risk of accidental infection of insects by ingestion of contaminated material is low and the likelihood of virus released from our laboratory infecting local vectors is effectively zero (see disinfection and waste disposal procedures). The risk to the environment is effectively zero.

Changes in host range:
It is possible, going by current understanding of orthobunyaviruses that changes as those described above (reassortant viruses or viruses with swapped M segment sequences or domains obtained from related viruses) show altered host range compared to parent viruses. M segment are likely determinants of host range, though possibly not the only ones. Therefore such a possibility needs to be taken into account.

Changes in tropism:
Again it is possible, going by current understanding of orthobunyaviruses that changes as those described above (reassortant viruses or viruses with swapped M segment sequences or domains obtained from related viruses) are different from parent viruses and show altered tissue tropism. M segment are likely determinants of host range as well as tissue tropism, though again possibly not the only ones. Therefore such a possibility needs to be taken into account.

Alteration of physical and chemical properties of virions:
This is highly unlikely; the M segment proteins of all orthobunyaviruses are likely to behave very similarly with regards to physical and chemical properties, and exchanging
these is unlikely to alter these.

Reversion to wild type:
Reassortant viruses are highly unlikely revert to parent virus, given the number of mutations required to achieve this. The same conclusions is true for mutations in the M segment, as only point mutations are likely to revert. In any case, the parent viruses are classed as hazard group 2 viruses and as such reversion would again attenuate the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The policy of the laboratory is to assume that materials are "infectious" unless they are specifically known to be otherwise.

All liquid and solid waste is to be decontaminated chemically using >1% Virkon solution before sterilisation by autoclaving. Barrycidal is used for routine disinfection as a spray. When handling protein rich liquid, Virkon powder should be added to give a final concentration of not less than 1%.

(a) Wastes from within the Microbiological Safety Cabinets

Paper waste within the hood shall be sprayed with disinfectant and placed in an autoclave bag within a metal bin for autoclaving. Solids which can be submerged will be soaked overnight in disinfectant (Virkon), disposable plastic pipettes will be soaked overnight in pans containing a disinfectant bath (Virkon), and subsequently autoclaved. Fluids containing potentially infectious will be soaked overnight with disinfectant (Virkon) in large sealed plastic bottles (opened during autoclaving) that are sterilised by autoclaving.

Sharps which arise inadvertently or glass slides /coverslips will be placed in metal bins or plastic beakers of disinfectant and labelled clearly and ultimately autoclaved.

(b) Wastes from outside the Microbiological Safety Cabinets

Other waste generated within the room including soiled protective clothing will be sterilised by autoclaving.

Autoclaving conditions:
Dry waste (porous dry load cycle):
This type of waste will be autoclaved with a chamber control temperature of 123°C and a sterilisation temperature of 121°C for 40 minutes. In order to ensure efficient ingress of steam into mixed solid waste materials (e.g. plastics, gowns, gloves, paper*), and to encourage drying of the load, autoclave programming includes vacuum purge cycles.

*Cardboard may release gases during heating that will prevent efficient sterilisation. To ensure efficient sterilisation, cardboard should be autoclaved separately from other dry materials.

Liquid waste:
This type of waste will be autoclaved with a chamber control temperature of 123°C and a sterilisation temperature of 121°C for 40 minutes. Loads containing liquids will be autoclaved separately from solid wastes. The cycle will run in absence of vacuum and has a fixed cooling period at the end of the cycle.
Degree of kill: Chemical Sterilization by Virkon/Barrycidal- effectively 100% kill. Autoclaving - effectively 100% kill (annual validation).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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Animal Units

Animal Units

Large Scale Activities

Large Scale Activities

Human Clinical Applications

Human Clinical Applications

Project Ref 223/16.2

Date Ackn'd 01/09/2016

CU2 Project Title Innate immunity and host species barriers to viral infections

Class Class 3

CultureVolClass2 ≤ 1 Litre

CultureVolumeClass3-4 ≤ 1 Litre

Non-GMM Consent Granted Yes

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
**Project Additional Information**

**Purposes of the contained use**

The objective of this project is to identify important host and viral determinants of cross-species transmission. We will investigate the ability of interferon stimulated genes (ISGs) of a variety of animal species to restrict replication of a range of important human and animal DNA and RNA viruses of HG 2 and 3. This will allow us to (i) define patterns of antiviral restriction by “homologous” and “heterologous” ISGs; (ii) to assess the risk of future cross-species transmission events and (iii) to identify novel antiviral genes.

**Recipient or parental organism**

DNA and RNA viruses of Hazard Group 2 and 3 will be grown from pre-existing stocks or rescued by reverse genetics that express fluorescent markers like GFP, RFP or luciferase or other markers like HA, V5, iLov. Viruses lacking structural and non-structural proteins will also be grown from pre-existing stocks or rescued by reverse genetics to produce viral like particles, attenuated mutants or replication incompetent viruses. We expect these modifications not to alter the replication, transmission or tropism of the resulting modified viruses. If any the modifications would result in attenuated viruses.

**Host/vector system**

Viruses: DNA and RNA viruses of hazardous groups 2 and 3 modified to express tag markers like GFP and luciferase among others and point mutant and deletion viruses to generate viral like particles, attenuated mutants or replication incompetent viruses.

Prokaryotic hosts:

Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids.

Eukaryotic hosts:

Vertebrate cell lines of various origins (typically human, mouse, hamster, bovine etc.) and invertebrate cell lines of various origins (mosquito, culicoides, tick etc.).

**Origin & function**

Viruses will either be obtained from original stocks or by reverse genetics techniques using nucleic acids.

**Evaluation of foreseeable effects**

Description of activities:

The project consists of two stages, both involving the use of pathogens of Hazard Group (HG) 2 and 3. Essentially the same experimental protocols will be used but the
containment level used will be defined according to the virus HG.

Stage 1: ISGs will be overexpressed in target cell lines by means os transduction with lentiviruses. 48 hours post transduction the transduced cells will be infected with GFP or luciferase tagged DNA or RNA viruses of HG 2 and HG3. At specific times post infection cells will be fixed and viral replication estimated by quantification of GFP/luciferase signal by FACs.

Stage 2: In stage 1 we will identify ISGs that impaired viral replication. This information will be used in stage 2 to understand the molecular mechanisms of viral restriction for a group of viruses of interest (HG 2 and 3). This will include overexpression of specific wild type and mutant ISGs and assessing replication of wild type and mutant viruses as in stage 1.

Because of the unknown nature of the antiviral candidates, viral sensitivity determinants and possible viral countermeasures (we don't yet know which viruses will need to be tested in 12 months time) this risk assessment is deliberately general to allow us to respond rapidly and appropriately to novel observations.

Risk associated with these novel viruses:
Viruses will be rescued by reverse genetics to express fluorescent markers like GFP, RFP or luciferase or other markers like HA, V5, iLov. Viruses lacking structural and non-structural proteins will also be rescued by reverse genetics to produce viral like particles, attenuated mutants or replication incompetent viruses. We expect these modifications not to alter the replication, transmission or tropism of the resulting modified viruses. If any the modifications would result in attenuated viruses.

Risks to the environment:
Reassortment and changes in glycoprotein or other M coding sequences (sequence exchanges) can potentially lead to changes in virulence and pathogenicity. Again precautionary principle applies also with regards to risk to the environment, although vector specificity of reassortants or glycoprotein mutants produced as described, is likely to be similar to that of the parent virus. For viruses of livestock advice will be given to lab personnel that they should not be in contact with susceptible animals or visit farms/zoos where they could potentially come into contact with susceptible species for a period of 48 hours following work with the viruses above. A form to this effect will be signed by staff working on these viruses. As stated above, in our laboratories, the risk of accidental infection of insects by ingestion of contaminated material is low and the likelihood of virus released from our laboratory infecting local vectors is effectively zero (see disinfection and waste disposal procedures). The risk to the environment is effectively zero.

Changes in host range and tropism:
Due to the size of the foreing markers to be introduced it is unlikely that changes in tropism and host range occur. In any case we expect the modified viruses to have reduced infectivity and narrower host ranges and tropism.

Reversion to wild type:
Reassortant viruses are highly unlikely revert to parent virus, given the number of mutations required to achieve this.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

HG 2 viruses will be handled in CL2 facilities under the following rules:
Disposable solids (e.g., plasticware such as flasks, tubes, pipette tips etc.)- soak in 1% Virkon (w/v) for a minimum of 12 hours. Transfer solid contents to clear autoclave bags and autoclave at 121°C for a minimum of 20 minutes prior to final disposal by district council to landfill, remaining liquid to be discharged to drain.

Other solids (agar plates, gloves etc.)- placed in a marked box lined with a clear autoclave bag, and disposed of by autoclaving using a make safe cycle of 121°C for at least 20 minutes. Seal contaminated plates (with for example bacterial GMOs) before placing in bag, to avoid lid falling off. Gloves used for bunyavirus work should be disinfected with alcohol before putting in containers.

Liquids (e.g., samples, E. coli culture media, tissue culture media)- add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

HG 3 viruses will be handled in CL3 facilities under the following rules:

The policy of the laboratory is to assume that materials are "infectious" unless they are specifically known to be otherwise.

All liquid and solid waste is to be decontaminated chemically using >1% Virkon solution before sterilisation by autoclaving. Barrycidal is used for routine disinfection as a spray. When handling protein rich liquid, Virkon powder should be added to give a final concentration of not less than 1%.

(a) Wastes from within the Microbiological Safety Cabinets

Paper waste within the hood shall be sprayed with disinfectant and placed in an autoclave bag within a metal bin for autoclaving. Solids which can be submerged will be soaked overnight in disinfectant (Virkon), disposable plastic pipettes will be soaked overnight in pans containing a disinfectant bath (Virkon), and subsequently autoclaved. Fluids containing potentially infectious will be soaked overnight with disinfectant (Virkon) in large sealed plastic bottles (opened during autoclaving) that are sterilised by autoclaving.

Sharps which arise inadvertently or glass slides/coverslips will be placed in metal bins or plastic beakers of disinfectant and labelled clearly and ultimately autoclaved.

(b) Wastes from outside the Microbiological Safety Cabinets

Other waste generated within the room including soiled protective clothing will be sterilised by autoclaving.

Autoclaving conditions:

Dry waste (porous dry load cycle):
This type of waste will be autoclaved with a chamber control temperature of 123°C and a sterilisation temperature of 121°C for 40 minutes. In order to ensure efficient ingress of steam into mixed solid waste materials (e.g. plastics, gowns, gloves, paper*), and to encourage drying of the load, autoclave programming includes vacuum purge cycles.

*Cardboard may release gases during heating that will prevent efficient sterilisation. To ensure efficient sterilisation, cardboard should be autoclaved separately from other dry materials.

Liquid waste:
This type of waste will be autoclaved with a chamber control temperature of 123°C and a sterilisation temperature of 121°C for 40 minutes. Loads containing liquids will be autoclaved separately from solid wastes. The cycle will run in absence of vacuum and has a fixed cooling period at the end of the cycle.

Degree of kill:
Chemical Sterilization by Virkon/Barrycidal- effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).
The GM committee asked for more detail on the Hazard Group 2 and 3 virus families that would or could potentially be used in the study to allow a more suitable and sufficient risk assessment to be made. This was added to the risk assessment. The aim of Stage 1 of the project was to overexpress ISGs in target cell lines by means of transduction with lentiviruses. There was a connected program of work already notified to HSE (GM223/13.1) which details these lentiviral vector systems, and following comments from the committee this was added to the risk assessment for clarity. More detail was requested on the probability of reversion to the wildtype from tagged versions of the parental viruses and this section of the RA was extended to clarify this.

**Project Containment**

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**Animal Units**

- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

**Project Ref** 223/19.1

- **Date Ackn'd**: 23/01/2019
- **CU2 Project Title**: Use of virus like particles (VLPs) and replicons to understand fundamental aspects of virus biology
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Non-GMM Consent Granted**: Not Applicable

**Date Project Ceased**

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Project notified under transitional arrangements**: N
The objective of this project is to study fundamental aspects of virology and virus-host interactions using VLPs and replicon systems designed to be safely handled at containment level 2. These systems are unable to generate infectious progeny outside of a laboratory as they require one or more essential components to be supplied in trans. We propose to use VLP and replicon systems derived from viruses that normally require containment level 3 or 4 under COSHH Regulations or SAPO.

The research will fall into 3 broad categories:

1. Immunity - We will use VLP and replicon systems to carry out in vitro research to examine how host cells from different species attempt to inhibit and respond to divergent pathogens. Moreover, VLPs provide a valuable way to conduct serology/neutralization studies at containment level 2.

2. Molecular virology - We will use VLP and replicon systems to carry out in vitro research to examine how viral proteins work and what host factors they depend upon to work and whether host or viral components can be chemically inhibited.

3. Structural biology/biochemistry - We will use VLP and replicon systems to determine the structures and biophysical properties of viral proteins and characterise their activities in vitro.

VLP and replicon systems can replicate their genome within an infected/transfected cell and they are (somewhat confusingly) referred to as replication incompetent, replication defective or single cycle because they are unable to sustain more than a single round of infection (without additional component(s) being supplied in trans) because not everything required is encoded by the viral modified/mini genome(s).

The risk assessment is deliberately general to allow us to respond rapidly and appropriately to novel observations/novel available systems. The use of VLP and replicon systems will broaden the study of important pathogens that normally require handling in higher containment (and are therefore oft understudied). However, we will not use a new system without first submitting our intent to the local GM safety committee and considering whether it might constitute a significant change to this risk assessment. In our initial application we have included various iterations of the VLP/replicon approach including some severe human/animal viral pathogens designed to encompass a variety of current approaches and give a flavour for the scope of this work.
VLPs and replicon/minireplicon systems for the viruses requiring containment level 3 or higher under COSHH Regulations or Specified Animal Pathogens Order, including:
- Rabies VLPs
- Vesicular stomatitis virus VLPs
- Marburg VLPs
- Ebola VLPs
- Nipah virus replicons
- Hendra virus replicons
- Tick-borne encephalitis VLPs
- West Nile VLPs
- Dengue VLPs
- Influenza A replicons

In general, replicon/minireplicon systems can be distinguished from VLP systems because replicons lack one or more structural genes and thus do not have a virus like particle component (and are often confined to the transfected/modified cell). In contrast, VLPs must have a cell free stage where the defective/mini genome is packaged into a virus like particle that can then be used to infect new cells (some systems require components to be added in trans to the target cell to detect infection). Crucially, VLP systems cannot be propagated because VLPs do not contain all the genetic material required for sustainable propagation. In each case, the system described is already being handled at containment level 2 in the UK or at the equivalent containment level in the EU/USA.

Host/vector system

The plasmid components come in a range of plasmid DNA vectors. The details of the plasmid vector should not increase the inherent risk of any VLP/replicon systems. Viral components might also be delivered in trans using retroviral or lentiviral systems (such as engineered cell lines expressing Rabies glycoproteins). This will not alter the risks associated with the VLP/replicon systems. Eukaryotic cells will also be modified to express genes not of viral origin (such as interferon stimulated genes or restriction factors). This will usually be achieved using standard plasmid transfection or retroviral/lentiviral vectors. Similarly, eukaryotic cells might be depleted of host genes by knockout or knockdown (plasmid/retro or lentiviral based systems). This will not influence the risk of the VLP/replicon systems.

Origin & function

VLPs and replicons will be generated by transfection/infection of cell lines of various origins with cDNA clones and vectors expressing modified viral proteins and genomes or through infection of cells with VLPs. Due to the lack of expression of viral structural and/or replication genes in the modified genomes contained in the VLPs, VLPs are unable to generate infectious progeny upon infection of unmodified target cells. The proposed modifications in the components of existing VLP and replicon systems (point mutations, deletions, insertions of tags) are unlikely to increase the chance of recombination that could lead to viable progeny. Mutant VLPs will include point mutants, frame deletions, analogous or orthologous gene swaps within members of the same viral family, addition/replacement of reporter genes (Le. luciferase, GFP, CAT, RFP etc.) and tags (Le. GFP, RFP, HA, V5, etc).

Evaluation of foreseeable effects

The proposed modifications (point mutants, frame deletions, analogous or orthologous gene swaps within members of the same viral family, addition/replacement of reporter genes and tags) should not increase the inherent risk of the
VLPs/replicons.

Risks to human health:
In the unlikely event of human exposure, the VLP and replicon systems will be unable to produce infectious progeny to sustain a full cycle of replication and thus the likelihood of harm is minimal/negligible.

Prokaryotic organisms: All strains will be disabled, commercially available E. coli K12 derivatives classified as 'especially disabled hosts' by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic cells:
Cell lines to be used would not survive inside the immunocompetent human body (immune rejection) and are not known to carry intact harmful pathogens. Addition of DNA or RNA or infection will confer no growth or survival advantage in or outside the laboratory to these cell lines.

Eukaryotic primary cells would not survive inside the human body (immune rejection).

Risks of reversion to wild-type/propagation competence:
The VLP/replicon systems are only able to undergo a single round of infection and as such are no longer viruses or pathogens. The foreseeable effects therefore surround the possibility of unintentional generation of viruses derived from the VLP/replicon system (reversion). The major ways this could occur include 1) Recombination occurring in vitro, and 2) Unintentional exposure of a virus-infected cell (either in vitro or in vivo) to a VLP/replicon system. Foreseeable effects for both will be consecutively discussed.

1) Recombination in vitro. The systems have been designed to minimise the risk of recombination. Homologous sequences between the minigenomes and the transcomplementing ORFs have been removed. In many cases, the compartmentalised nature of minigenome replication minimises the risk of recombination as VLP/minigenome RNAs are spatially separated from cellular RNAs. In many systems, unintentional reversion in culture has been specifically investigated and not observed.

2) Unintentional transfection or infection of virus-infected cells in vitro is unlikely to result in reversion. We do not possess the viral counterparts for most of the VLP/replicon systems at our centre (if we do they are stored and used in separate containment facilities) so homologous sequences are therefore unlikely to be present. In the case of Influenza A Virus replicons, reassortance is unlikely as full genome segments will not be used. Thus, there would be minimal consequences arising from unintentional coinfection in vitro.

Unintentional exposure of a virus-infected cell (either in vitro or in vivo) to a VLP/replicon system. Unintentional exposure of humans (already infected with a virus and working at our centre) would not be predicted to result in reversion. Similarly, virus-infected animals in the environment exposed to VLPs would be unlikely to cause reversion. Most similar viruses are not endemic within the UK, homologous sequences are unlikely to be present and because the VLPs can only undergo a single round of infection, it is unlikely that a single cell will be exposed to VLP and endemic virus. In the case of Influenza A Virus replicons, reassortance is unlikely as full genome segments will not be used. Thus, these systems will have minimal consequences following accidental exposure/release to the environment.

Risks to the environment
In our laboratories, the risk of accidental infection of insects by ingestion of contaminated material is low and the likelihood of virus released from our laboratory infecting local vectors or animals in the local environment is effectively zero (see decontamination and waste disposal procedures below). Even in the complete absence of disinfection the replication defective nature of these systems means the risk to the environment is extremely low.

Changes in the viral glycoproteins, including point mutations, deletions, insertions or the use of glycoproteins from related viruses could potentially change the host range of these VLPs. However, given that we will never restore viral genes usually supplied in trans into the viral genomes, the risk to the natural hosts and the level of containment required to handle them remains the same as the parental VLPs as they are unable to sustain more than a single round of infection.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All the systems used in this study are missing at least one essential component that must be supplied in trans to generate infectious progeny. Outside of the laboratory, they are unable to sustain an infection. They are therefore unable to cause severe human disease or be a serious hazard to employees; they cannot be considered as disease-producing organisms and are unlikely to spread from the laboratory and/or to the community. Hence, we seek permission to handle all VLP and replicon systems described in this proposal at containment level 2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable solids (e.g. plasticware such as flasks, tubes, pipette tips etc.) - soak in at least 1 % w/v Virkon solution (final concentration) overnight. Transfer solid contents to clear autoclave bags and autoclave at 135°C for 5 minutes. Other solids (agar plates, gloves etc.) - placed in a marked box lined with a clear autoclave bag, and autoclaved at 135°C for 5 minutes. Liquids (eg. samples, tissue culture media) - add Virkon to final concentration of at least 1 % (w/v) overnight.

Degree of kill:
Chemical Sterilization by Virkon - effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).

Following overnight decontamination in Virkon all liquid waste is to be disposed to drains. Autoclaved solid waste is to be disposed by district council to landfill.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GM Safety Committee asked for more information regarding the risks associated with the presence of replicationcompetent endogenous retroviruses when transducing cells of mammalian origin. The risk assessment was amended to clarify this point. The GMSC was satisfied that containment and control measures were suitable for this project and that class 2 classification was appropriate.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022
Project Additional Information

Purposes of the contained use

The project aims to identify genes and investigate processes involved in the molecular genetics and biochemistry of parasitic protozoa (Trypanosoma and Leishmania spp.) and their interaction with their hosts. Key areas of interest are identifying genes and investigating the mechanisms involved in important phenotypes such as sequestration, drug resistance, virulence, pathogenesis and infectivity. The main approach will be to examine the differences between parasite strains to determine genes involved in these phenotypes.

Recipient or parental organism

Organisms to be used in this study
(a) Trypanosoma spp are capable of infecting a range of mammals, including man, cattle and rodents. The predominant Trypanosoma species used in this study will be Trypanosoma brucei brucei and Trypanosoma brucei gambiense. We will occasionally use other subspecies including (but not limited to) Trypanosoma brucei rhodesiense and Trypanosoma congolense.
(b) Leishmania spp. are capable of infecting a range of mammals including man, dogs and rodents. The predominant Leishmania species used in this study is Leishmania tarentolae, isolated from the Moorish Gecko Tarentola maurianica. This is non-pathogenic to mammals. We may occasionally use Leishmania mexicana, L. infantum, and L. major.
Examined by RNA interference (RNAi). Currently, this is only possible in T. brucei, where several vectors have been designed for expression of double stranded RNA to result in the generation of a strain that is more virulent, has a wider host range or additional tissue tropism than the donor strain. Functions of specific genes will be examined by RNA interference (RNAi). Several vectors have been designed for expression of double stranded RNA corresponding to the target gene using tetracycline induction of the bacterial T7 phage RNA polymerase. Plasmids of choice include the p2Ti (LaCount, D.J., Bruse, S., Hill, K.L., and Donelson, J.E. (2000). Mol. Biochem. Parasitol. 111, 67-76.) and pZJP (Wang, Z., Morris, J.C., Drew, M.E., and Englund, P.T. (2000). J. Biol. Chem. 275, 40174-40179.) series and the T. brucei, pH vectors (Bieber, S., Wirtz, E., Lorenz, P., and Clayton, C.E. (1997). Mol. Biochem. Parasitol. 85, 99-112). These vectors have low to medium levels of trans gene expression.

1. Gene disruption. Generation of mutant parasites by insertional mutagenesis (Insertion of selectable markers by homologous recombination). The types of positive selectable markers that will be used include drug resistance genes of prokaryotic origin, such as neo, hyg, pur, ble, bia or sat. These drug resistance genes do not confer resistance to drugs commonly used to treat trypanosomosis or Leishmania infections in man or animals.

2. Homologous gene expression. The expression of homologous genes in trypanosomes or Leishmania will be achieved from episomal expression vectors or via integration into the genome. These vectors provide low to medium levels of gene expression.

3. Heterologous gene expression. The expression of heterologous genes in trypanosomes or Leishmania will be achieved from episomal expression vectors or via integration into the genome. The commercially available eukaryotic Lexsy gene expression system produces large amounts of correctly post-translationally modified proteins. We may occasionally conduct these experiments to test for cross species complementation. In particular, we wish to test if genes from trypanosomes are able to fulfill the same function in Leishmania (and vice versa). This is of some importance in drug design, as we wish to be able to design inhibitors against drug targets that might have therapeutic value in both related organisms. Thus, genes that are essential in both organisms and that are able to function in each species are good targets for the development of a generic anti-trypanosomatid drug.


5. Inducible expression of endogenous or foreign genes. Again, this relies upon tetracycline induction of the target gene via the bacterial T7 phage RNA polymerase. In T. brucei, the pHD vectors (Bieber, S., Wirtz, E., Lorenz, P., and Clayton, C.E. (1997). Mol. Biochem. Parasitol. 85, 99-112) are examples for inducible expression in both bloodstream and procyclic stage cells. These vectors have medium to high levels of trans gene expression.

6. Gene product localisation will be examined by creating translational fusions of the genes and epitopes to which antisera are commercially available (for example HA, BB2, MYC, TAP, FLAG), or that provide autofluorescence (any of the colour variants of aequorin; GFP, EFP, YFP etc.). These vectors have low to medium levels of expression.

7. Non-trypanosomatid genes for example, encoding reporter genes (e.g. GFP and luciferase) will be expressed from various chromosomal loci to provide measures of expression. These vectors have low to medium levels of trans gene expression.

Host/vector system

Prokaryotic hosts include disabled, commercially available E. coli strains such as E.coli K12 or BL21 derivatives.

Plasmids containing DNA derived from the host; coding and non-coding nucleic acid sequences; promoters and enhancers. These also contain a ‘selectable marker’, usually a drug resistance marker e.g. puromycin, hygromycin and neomycin resistance. Plasmids may also contain material of prokaryotic or bacteriophage origin engineered to deliver e.g. dsRNA (e.g. tet-repressor and T7 polymerase). Reporter genes such as GFP, mCherry and luciferase. Plasmids also contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin).

Mice, both wild type and genetically modified (e.g. genes involved in immune response, etc.) infected with wild-type and GM Trypanosoma or Leishmania.

Tsetse flies (wild-type only) will be infected with wild-type or GM trypanosomes.

We will also perform transmigration assays using endothelial cells of mammalian origin, including genetically modified endothelial cells.

Origin & function

Trypanosoma and Leishmania spp.; lab strains bred and maintained in our facilities (or obtained from collaborators/companies); GM strains already produced in the University of Glasgow (approved class 3 activity GM37/99.1).

The following types of genetic modification for Trypanosoma and Leishmania spp are envisaged:

1. Gene disruption. Generation of mutant parasites by insertional mutagenesis (Insertion of selectable markers by homologous recombination). The types of positive selectable markers that will be used include drug resistance genes of prokaryotic origin, such as neo, hyg, pur, ble, bia or sat. These drug resistance genes do not confer resistance to drugs commonly used to treat trypanosomosis or Leishmania infections in man or animals.

2. Homologous gene expression. The expression of homologous genes in trypanosomes or Leishmania will be achieved from episomal expression vectors or via integration into the genome. These vectors provide low to medium levels of gene expression.

3. Heterologous gene expression. The expression of heterologous genes in trypanosomes or Leishmania will be achieved from episomal expression vectors or via integration into the genome. The commercially available eukaryotic Lexsy gene expression system produces large amounts of correctly post-translationally modified proteins. We may occasionally conduct these experiments to test for cross species complementation. In particular, we wish to test if genes from trypanosomes are able to fulfill the same function in Leishmania (and vice versa). This is of some importance in drug design, as we wish to be able to design inhibitors against drug targets that might have therapeutic value in both related organisms. Thus, genes that are essential in both organisms and that are able to function in each species are good targets for the development of a generic anti-trypanosomatid drug.


5. Inducible expression of endogenous or foreign genes. Again, this relies upon tetracycline induction of the target gene via the bacterial T7 phage RNA polymerase. In T. brucei, the pHD vectors (Bieber, S., Wirtz, E., Lorenz, P., and Clayton, C.E. (1997). Mol. Biochem. Parasitol. 85, 99-112) are examples for inducible expression in both bloodstream and procyclic stage cells. These vectors have medium to high levels of trans gene expression.

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7. Non-trypanosomatid genes for example, encoding reporter genes (e.g. GFP and luciferase) will be expressed from various chromosomal loci to provide measures of expression. These vectors have low to medium levels of trans gene expression.

Reporter genes, such as GFP, antibiotic resistance genes and luciferase. Parasite candidate genes for desired phenotypes will be replaced by antibiotic resistance markers. Candidate genes may be taken from naturally occurring virulent strains and inserted into less virulent naturally occurring strains to examine traits such as infectivity, drug resistance and virulence, e.g. genes for cysteine peptidases, genes for trypanosome cell surface proteins and transporters.

It is envisaged that genes modified in this project will lead to either no change in virulence or a loss of virulence. None of the genes to be modified in this project are thought to result in the generation of a strain that is more virulent, has a wider host range or additional tissue tropism than the donor strain. Functions of specific genes will be examined by RNA interference (RNAi). Currently, this is only possible in T. brucei, where several vectors have been designed for expression of double stranded RNA.

Evaluation of foreseeable effects

It is considered likely that gene disruptions will be detrimental to parasite survival or will have no phenotypic difference from wild type cells. The transformed trypanosomatids expressing foreign genes, or with trypanosomatid genes modified to be linked to epitope tags, are highly unlikely to have phenotypic differences from wild type cells, or from trypanosomatids with gene knockouts. Add-back experiments where genes of interest are knocked out and subsequently replaced will be unlikely to have a higher pathogenic phenotype than naturally occurring virulent parasite strains.

Hazards arising from the alteration of any existing pathogenic traits:
It is possible that disruption of Trypanosoma or Leishmania genes will alter the pathogenicity of the parasites. It is envisaged however that genes modified in this project will lead to either no change in virulence or a loss of virulence (hence the desire to create gene-specific deletion mutants for use as e.g. attenuated live vaccines). None of the experiments are envisaged to increase the hazard beyond the inherent hazard in working with naturally occurring parasites. Genetically modified parasites will be tested in vivo models (mice, tsetse flies). Procedures for maintenance of animals infected with genetically modified trypanosomatids are covered by the Home Office inspectorate; Animals (Scientific Procedures) Act 1986.

Potential hazards of sequences within the GMM being transferred to related micro-organisms:
The potential for GM parasites to transfer the genetically altered material to related organisms is extremely low as for both trypanosomes and leishmania genetic exchange only occurs in the insect vector between compatible strains.

Risk to human health:

Procyclic form trypanosomes are considered non-infective to mammals. The standard operating procedures and containment processes will be followed to ensure that the likelihood of a health hazard is negligible.

Bloodstream form trypanosomes are, however, potentially harmful. An important feature of safe handling of bloodstream stage trypanosomes is that there is no record of transmission of these parasites by the aerosol route; neither can these organisms penetrate unbroken skin or mucous membranes. The only possible route of infection is through direct inoculation, a hazard which is negligible due to strict standard operating procedures involving the use of either blunt needles and/or the use of needle proof gloves.

For promastigote Leishmania, there is no record of transmission of these parasites by the aerosol route; neither can these organisms penetrate unbroken skin or mucous membranes. However, it is possible that Leishmania It is considered likely that gene disruptions will be detrimental to parasite survival or will have no phenotypic difference from wild type cells.

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For promastigote Leishmania, there is no record of transmission of these parasites by the aerosol route; neither can these organisms penetrate unbroken skin or mucous membranes. However, it is possible that Leishmania amastigotes can be transmitted via aerosols and so any work with amastigotes will be handled in a class II microbiological safety cabinet. All work handling Leishmania will be carried out in containment level 2 facilities.

Environmental risks:
Capacity of the GMM to survive, establish, disseminate with and/or displace other organisms:
The chance of escape into the environment is negligible as the genetically modified parasites will be contained within either CL2 or CL3 (T. b. rhodesiense) facilities and handled under COSHH Standard Operating Procedures.
The parasites are unable to survive outside the mammalian body or vector and so do not pose an environmental risk.
Infected mice will be housed in containment facilities in dedicated SAPO rooms. As this is a vector borne infection and the vectors are not native in the UK the possibility of the establishment in the environment is negligible/effectively zero.
Thus, the possibility of escaped infected tsetse vectors establishing in the UK is not possible, as the flies cannot survive for prolonged time periods in our climate.

Ability to cause harm to animals:
The host preferences of each trypanosome species may differ, but Trypanosoma congolense, T. vivax and T. brucei have a wide host range among mammals. Leishmania spp can infect canids, especially dogs. The chance of escape into the environment is however negligible as the genetically modified parasites will be contained within either CL2 or CL3 (T. b. rhodesiense) facilities and handled under COSHH Standard Operating Procedures. The parasites are unable to survive outside the mammalian body or vector and so do not pose an environmental risk. Infected mice will be housed in containment facilities in dedicated SAPO rooms. As these are vector borne infections and the vectors are not native this country the possibility of passing this infection to the vector and then to another animal is negligible.

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Tsetse flies will be housed in dedicated containment facilities (in a separate building from mice) and caged before infection. The possibility of escaped infected tsetse vectors establishing in the UK is not possible as the flies cannot survive in our climate and so do not pose a risk to establishing within the UK.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Infected mice will be housed in containment facilities in dedicated rooms. As this is a vector borne infection and the vectors are not native this country the possibility of passing this infection to the vector and then to another animal is negligible.
Tsetse flies will be housed in dedicated containment facilities (in a separate building from mice) and caged before infection. The possibility of escaped infected tsetse vectors establishing in the UK is not possible as the flies cannot survive in our climate and so do not pose a risk to establishing within the UK.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Work with T. b. rhodesiense will be conducted at containment level 3 with less than the minimum containment conditions (see below) normally required for that containment level under the COSHH Regulations following HSE guidance in the ACDP Approved List of Biological Agents (Pages 8-9 and 30-31).
• There is no requirement for the laboratory to be sealed and fumigated.
• There is no requirement for negative pressure or HEPA filtering of exhaust air before discharge (no risk of infectious aerosols being generated).
• Within the restricted access laboratories, the workplace and equipment may be shared with research activities performed with HG2 Trypanosoma spp. In this case, all work with T. b. rhodesiense will be clearly labelled and separated either physically or temporally from other work in the laboratory wherever possible.
• While observation windows are present within the double inner containment rooms of the tsetse suite, there is no window in the outer “airlock” access doors. A procedure of agreed check-ins will be used to monitor staff while using the tsetse suite.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All liquid and solid waste is to be decontaminated chemically before sterilisation by autoclaving* 3% Chemgene (Trigene/Distel), 3% chloros, 5% Bleach or 1% Virkon will be used for disinfection.
Leishmania and Trypanosoma cultures and infected blood are disposed of into containers containing 3% Chemgene (Trigene/Distel), 3% chloros, 5% Bleach or 1% Virkon, which instantly kills these parasites (bloodstream forms of T. brucei are killed at a concentration of 0.1% within 20s)
• The surfaces of the microbiological safety cabinet and laboratory benches will be disinfected after any activity.
• Contaminated glassware, general apparatus and safety clothing should be decontaminated by immersion in chloros, Chemgene (Trigene/Distel) Bleach or Virkon.
Potentially contaminated lab coats must be autoclaved.

- Specialised equipment should be decontaminated by wiping with tissues soaked in chloros, Chemgene (Trigene/Distel) Bleach or Virkon.
- All potentially contaminated waste materials must be made safe before disposal by immersion in chloros, Chemgene (Trigene/Distel) Bleach or Virkon.
- If a spillage of viable organisms occurs, the contaminated areas must immediately be swabbed with industrial methylated spirit (IMS), chloros, Chemgene (Trigene/Distel) Bleach or Virkon. All accidents or potentially dangerous occurrences must be reported immediately to the Head of Laboratory or local safety Officer.
- Disinfected waste should be drained and transferred to the designated autoclave bags which are sealed when full and autoclaved.

**Autoclaving**

- All infected waste will be autoclaved before disposal. All frozen waste must be brought up to ambient temperature prior to autoclaving.
- On removal from the autoclave, the bags are placed in the clinical waste container which, when full, is sent for incineration by the designated company.
- Uninfected tsetse flies should be killed by freezing prior to disposal by autoclaving. Infected flies should be kept frozen overnight, brought back to room temperature and handled as animal by-product (ABP) waste, and sent for incineration as described.
- All waste bedding and waste material (gloves etc.) and carcasses will be autoclaved and disposed of by incineration undertaken by a commercial waste specialist company. If the autoclave does not meet the required specification at any time, bedding will be stored at –20°C for >48hrs and then disposed of by the same company as hazardous waste and incinerated.

Contaminated syringes and needles should be disposed of in the dedicated sharps bin which, when full, should be sent for incineration by the designated company. Autoclave conditions: Inactivated by autoclaving under conditions that maintain 121°C for at least 30 min with full steam penetration.

All autoclaved waste is to be incinerated


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The GM Safety Committee recommended that minimum containment level required for each parasite (under COSHH Regulations or SAPO) should be clearly stated in the risk assessment.

Leishmania amastigotes present a risk of infection through aerosols; the GMSC asked for the risk assessment to clearly state that all work involving these forms must be carried out in a class II microbiological safety cabinet.

Additional information was requested with regards to the nature of work conducted on animal models and the question of genetic exchanges between parasite strains in animals was raised.

All these points were addressed in the final version of the risk assessment; the GMSC considered that appropriate containment and control measures had been assigned and approved the document for work to be carried out at CL2, except for T. b. rhodesiense which will be handled at CL3 with less than the minimum containment conditions.

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**Project Containment**

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</table>

02/03/2022

Page 5435 of 15326
African trypanosomiasis is a disease caused by the parasites of the genus Trypanosoma. These parasites affect both humans and animals with significant social and economic impact. Although the disease has been recognised for centuries, many aspects regarding the pathogenesis of the disease, and the complex host-pathogen interactions dictating disease outcome, remain unknown. We will use a wide range of immortalised and primary cell lines of human and animal origin: i) to dissect the pathways involved in the crosstalk between African trypanosomes and their vertebrate host, and ii) to explore the pathological basis of infection by African trypanosomes, including neuroinflammation, skin inflammation, and kidney disease, amongst others. These studies will include, but not limited to one or more of the following procedures:

1. Culture of primary cells of human or other animal origin for in vitro studies
2. Culture of immortalised cell lines of human or other animal origin for in vitro studies
3. Genetic manipulation (knockout, knockdown, in situ genome tagging, overexpression) of primary and immortalised cell lines of human and animal origin to study to function of genes of interest in the context of pathogenicity, immune response, and host-pathogen interactions
4. Co-culture of cell lines of human or other animal origin with African trypanosomes. This work will be carried out under the approved class 3 activity GM223/19.2.
5. Generation of 3D culture systems (e.g., spheroids, assembloids) derived from primary and/or immortalised cell lines of human and/or other animal origin
**Recipient or parental organism**

| Cell lines of human or other mammalian origin |

**Host/vector system**

<table>
<thead>
<tr>
<th>Plasmids, viral vectors (e.g. lentivirus, baculovirus, adenovirus), siRNAs, CRISPR/Cas9 system, ribonucleoprotein complexes. Some examples (not exhaustive) are listed below, but others can also be employed:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• pSecTag series of plasmids from Invitrogen (<a href="http://www.yrgene.com/documents/vector/psectag.pdf">http://www.yrgene.com/documents/vector/psectag.pdf</a>)</td>
</tr>
<tr>
<td>• pEGFP and related plasmids (TaKaRa-Clontech, now replaced with pAcGFP1 (<a href="http://www.clontech.com/US/Products/Fluorescent_Proteins_and_Reporters/Fluorescent_Proteins_by_Name/AcGFP1_Fluorescent_Protein">http://www.clontech.com/US/Products/Fluorescent_Proteins_and_Reporters/Fluorescent_Proteins_by_Name/AcGFP1_Fluorescent_Protein</a>)</td>
</tr>
<tr>
<td>• pUC-derived constructs</td>
</tr>
</tbody>
</table>

**Origin & function**

Genetically modified human and mammalian cells will be produced in this project. These cells (e.g. HEK293, hCMEC/D3 just to mention a few examples) will be purchased from commercial suppliers. The cell lines might be considered “wild type”, if they do not have any genetic modifications beyond immortalisation, or “modified” if they harbour genetic modifications beyond immortalisation with SV40 (e.g. constitutive expression of fluorescent proteins). The wild type cell lines will be used to introduce genetic modifications when required. They cannot survive outside of culture media and have a history of safe use. We will also use primary cell lines. These cells will be engineered to create gene deletions, in situ genome tagging, gene knockdown library, overexpression.

Parent lines will be purchased from commercial sources (both immortalised or primary cell lines) which are screened for bloodborne viruses (e.g. HIV, HBC) and/or mycoplasma. Mammalian (non-human) primary cell lines (e.g. from mice) will be derived from animals housed in animal facilities, which are routinely screened for viruses and/or other pathogens.

The intended purpose is to test the function of selected genes in mammalian systems in vitro. The genes to be selected for modification will be dictated by transcriptomic analysis, whole-genome association studies, or related. Genes will be amplified in house or purchased commercially. We will introduce genetic modifications in a homotypic manner (e.g. human genes in human cell lines, for example).

**Evaluation of foreseeable effects**

The genetically modified mammalian cell lines cannot survive outside of culture media and have a history of safe use.

Hazard associated with the vector systems

Plasmids to be used shall be from commercial sources. They will be maintained in the host cell by antibiotic pressure, in the absence of which the plasmid is lost. None of these sequences present any hazard to humans or the environment. The final genetically modified cells will contain stably integrated DNA copies of the lentiviral vectors encoding the transgene expression cassettes. The cells will be free of lentivirus. As a result, they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside the laboratory culture. In addition, they are replication-defective which means that the vector cannot multiply on its own unless supplied with certain proteins in trans. CRISPR-Cas9 system does not encode any protein that could promote replication.

Nevertheless, it will be important to prevent human exposure or environmental release of the replication defective lentiviruses which are used to transiently effect gene transfer. The worse-case scenario in case of infection is integration at a site that disrupts expression of a gene necessary for tumour suppression. Risk against this will be reduced through the use of appropriate physical containment and inactivation processes.

The plasmid vector contains antibiotic resistance genes, which if transferred to a micro-organism in the environment might confer resistance on a species able to infect humans or animals. However, these antibiotic resistance genes are already widespread in the environment and so this is not considered to be a significant hazard. This risk is addressed by rigorous waste disposal procedures.

Hazard arising from inserted gene products

We anticipate that most of the genes to be modified will be related to immune response, host-pathogen interactions, and any other that might be of relevance based on genetic screenings, transcriptomics analysis or genome-wide association studies. The resulting GMMs themselves would not be predicted to be any more harmful to human health than the parental wild-type strains, as there are no genes to be expressed that constitute toxins or pathogenic determinants. The risk of the modified cell lines
To humans is negligible. The transfer of genetic material (including antibiotic resistance genes) into related species in the environment is unlikely due to use of non-mobilizable plasmids. None of the genetic modification is likely to confer a survival advantage in the environment. The GMM cannot survive outside laboratory conditions and so hazard to the environment is minimal.

The proposed use of CRISPR/Cas9 is intended to study the effects of knocking out the host genes in response to infection with African trypanosomes, so would be very unlikely to increase the level of harm, compared to the unmodified strain.

There is no potential for harm to human or animal health or the environment using non-viral vectors.

The mammalian cells would not survive in genetically non-identical humans, causing rapid rejection and will not be expressing toxic or harmful products. All primary cells for these experiments will be obtained from laboratory animals or commercially from serologically tested donors (e.g., HUVECS) and will present no significant risk to human health.

The lentiviral vectors used are multi-attenuated meaning they are devoid of all potentially pathogenic virus encoded functions. In addition, they are replication-defective, which means that the vector cannot multiply on its own unless supplied with certain proteins in trans. CRISPR-Cas9 system does not encode any protein that could promote replication outside of transfected cells and under regulated conditions (e.g., antibiotic selection, induction with tetracycline, etc.)

Due to the restricted host range of the constructs, their lack of ability to survive outside laboratory conditions, restrictions on access to the facility and the risk control and disinfection/disposal measures described in this assessment, we conclude that the risk to human health or the environment by the proposed program is effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste inactivation:
All GMM liquid waste will be inactivated using a validated disinfection method (eg 1% virkon, 5% chemgene or similar).
All solid waste, including waste destined for incineration, will be inactivated by autoclaving prior to disposal (100% kill).
Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.
Waste disposal:
After disinfection all liquid waste will be flushed down the sink with a large amount of water.
Solid waste which was in contact with trypanosomes will be incinerated after autoclaving. Solid waste which was not in contact with trypanosomes will be disposed of by district council to landfill.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
The GM Safety Committee asked for more detailed information on waste inactivation and disposal. The risk assessment was amended accordingly. The containment level required for this work was discussed, including for the experiments involving trypanosomes; conclusion was reached that CL2 was appropriate for the generation and maintenance of GM mammalian cells as described in the risk assessment and the RA was amended to clarify this point. The GMSC was satisfied that containment and control measures were suitable for this project and that class 2 designation was appropriate.

**Project Containment**

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**Project Ref** 223/22.1

**Date Ackn’d** 24/02/2022

**CU2 Project Title** Investigating viral membrane fusion mechanisms using non-replicating and surrogate systems

**Class** Class 2

**CultureVol** Class 2 Culture < 1 Litre

**Consent Granted** Non-GMM Consent Granted

**Project notified under transitional arrangements** N

**Withdrewn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The entry of all enveloped viruses requires the action of virally-encoded membrane fusion proteins that are presented on the surface of virus particles. These proteins are critical determinants of viral transmission, cellular/species tropism and, consequently, pathogenesis. Understanding the molecular mechanisms of viral attachment, receptor
engagement and membrane fusion requires a structure-to-function investigation of viral fusion proteins. The membrane fusion machinery is also a key target of antibody-mediated immunity and a full understanding of virus entry also requires consideration of immune interactions.

We aim to investigate the mechanisms of viral membrane fusion/immune interactions using wild type and mutant viral proteins presented in the context of three non-replicating/surrogate systems: i) retroviral pseudoviruses, bearing the protein(s) of interest and encoding a reporter gene ii) exogenously expressed viral proteins in the context of eukaryotic cells iii) soluble proteins purified to high concentrations. In each case, the viral protein is presented in a context that does not permit viral replication and poses little/no risk to human health or the environment.

In the first instance we will use these systems to investigate the following groups of viruses: coronaviruses, hepaciviruses, pegiviruses, pestiviruses, hepadnaviruses, rhabdoviruses, retroviruses and influenza. Other viral species/genus may also be considered for investigation upon assessment by the local GM safety committee. Nonetheless, we will ensure that the proteins of interest have no known intrinsic pathogenic/oncogenic potential and the principal biological risks will be those associated with the general use of retroviral vectors.

**Recipient or parental organism**

We are investigating the entry mechanisms of enveloped viruses including human pathogens (e.g. hepatitis C, coronaviruses). However, we have selected surrogate experimental systems that do not support the generation of any replication competent viruses and allow us to investigate viral proteins in isolation. We will also investigate the host factors that facilitate virus entry and, therefore, will manipulate human/animal proteins using similar systems. Importantly, we will not generate GMOs carrying proteins of known intrinsic pathogenic/oncogenic potential.

**Host/vector system**

We will use three surrogate experiment systems incorporating different host/vector combinations.

i) Retroviral pseudovirus.

This is a very commonly used approach in which the viral fusion protein(s) of interest are presented on the surface of pseudovirus particles comprised of replication incompetent retroviral packaging components based on either HIV (e.g. 8.91 or NL4.3LUC) or MLV (e.g. pCIG), and a reporter gene encoded by a self inactivating transfer plasmid (Sanders 2002; Temperton et al. 2005; Kim et al. 2001; Hsu et al. 2003). The resultant pseudovirus particles follow the authentic entry pathway of the protein(s) under investigation and can be easily assayed through detection of the reporter gene product, but are incapable of replication. Being highly tractable and safe, this approach is the industry standard for the study of membrane fusion proteins from pathogenic viruses without substantive risk to human health or the environment.

ii) Exogenous protein expression in eukaryotic cells.

When over expressed in cells viral fusion proteins typically accumulate within intracellular compartments or at the cell surface, where they are amenable to functional and immunological characterisation. For example, in vitro fusion assays rely on cell surface expressed viral proteins that are activated to allow membrane fusion between adjacent cells. Alternatively, we will use the cells as a simple source of protein for antigenic evaluation by approaches such as ELISA or immunofluorescence. We will also introduce exogenous host proteins that act as receptors and entry factors for the viruses under investigation. In all cases genes encoding the proteins of interest are introduced by transfection with expression plasmids or through transduction with self-inactivating lentiviral vectors.

iii) Expression and purification of soluble proteins.

Viral membrane fusion proteins are anchored to the virus particle/host cell through transmembrane domains. However, if engineered to remove these regions and to direct efficient secretion, the proteins of interest will accumulate in extracellular medium of transfected cells, where they are available for affinity purification. Alternatively, soluble proteins can be expressed in prokaryotic systems and liberated by cellular disruption. The resultant high purity proteins are then suitable for various assays such as cellular binding activity, biophysical analysis or structural characterisation. Our investigations may also require the same strategy to produce high purity recombinant antibodies that target proteins of interest or soluble versions of host receptor proteins. In all cases genes encoding the proteins of interest are introduced by transfection/ transformation with expression plasmids or through transduction with self-inactivating lentiviral vectors.

Generation of pseudovirus and expression of exogenous proteins will occur in standard eukaryotic cell lines, such as those available through ATCC or similar archives, for examples HEK 293T, CHO or HeLa. Soluble proteins may also be expressed in standard disabled bacterial strains.

For all of the above systems the components originate from DNA plasmids that are generated and propagated in standard disabled bacterial strains (e.g. D5a). The following types of plasmids will be used:

- Retroviral packaging plasmids (e.g. HIV, 8.91 or MLV, pCIG (Naldini et al. 1996; Bock et al. 2000)).
- Transfer plasmids encoding reporter genes (e.g. GFP, CSGW or Luciferase, CSFLW (Demaison et al. 2002)).
- Viral membrane fusion protein expression plasmids. The protein(s) of interest will be encoded in standard expression plasmids (e.g. pCDNA3 or pD607) or
self-inactivating lentiviral transfer plasmids (e.g. pDUAL (Escors et al. 2008; Zufferey et al. 1998)) for introduction in to eukaryotic cells. Similarly, for prokaryotic expression, we will use standard bacterial expression plasmids (e.g. pNIC28-Bsa4).

- Antibody expression plasmids. Recombinant antibodies relevant to our investigation will be encoded on CMV expression plasmids (e.g. Oxgene OGS527) for introduction in to mammalian cells.

- Host receptor protein expression plasmids. Recombinant host-encoded receptor proteins relevant to our investigation will be encoded in standard CMV expression plasmids (e.g. pCDNA3 or pD607) or lentiviral transfer plasmids (e.g. pDUAL) for introduction in to eukaryotic cells, and for prokaryotic expression we will use standard bacterial expression plasmids (e.g. pNIC28-Bsa4).

Genetic manipulation of viral membrane fusion proteins, antibodies and host receptor proteins will be used to test hypotheses using the above experimental systems. These manipulations will be limited to those necessary to investigate the function and immune interactions of the viral membrane fusion proteins. We will make no substantive modifications to the background plasmids or viral vector systems.

References


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMM waste will be treated in line with local institutional practices:

- Liquids (e.g. culture media), viral cultures and cells are inactivated in 1% w/v (final concentration) Virkon solution overnight, then discharged to drain.
- Disposable solids that have been in contact with GM material (e.g. flasks, tubes, pipette tips, etc.) are soaked in 1% w/v (final concentration) Virkon solution overnight, then solid contents are transferred to clear autoclave bags for autoclaving (134°C for 5min) while remaining liquid is discharged to drain.
- Other solids (e.g. agar plates, gloves, etc.) are autoclaved at 134°C for 5min.
- Autoclaved waste is disposed by district council to land fill.

Degree of kill:
- Chemical inactivation by Virkon – effectively 100% kill
- Autoclaving – effectively 100% kill (annual validation)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC requested additional information on the nature of the plasmids used for prokaryotic systems and methods used to generate them; the risk assessment was amended accordingly.

The GMSC was satisfied that the containment and control measures were suitable for this project and that class 2 designation was appropriate.

Project Containment

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02/03/2022
**Project Ref** 223/trans1

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Non-GMM

Consent Granted

yes

Tick if notifying a connected programme of work

N

Project notified under transitional arrangements

Y

Historical Significant Changes

GM223/98.1,

Historical Date of Additional Info

01/01/1998

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref  223/trans1a

Date Ackn'd: 07/07/1994

Date Project Ceased

CU2 Project Title: MEASUREMENT OF CELLULAR IMMUNE RESPONSE TO FELINE IMMUNODEFICIENCY VIRUS AND FELINE LEUKAEMIA VIRUS

Class: Class 2

CultureVolClass2: Class 2

CultureVolumeClass3-4

Non-GMM

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

02/03/2022
Project Ref 223/trans2

Date Ackn’d

CU2 Project Title

Molecular characterisation of cellular receptors for FIV

Date Project Ceased

Class

CultureVolClass2

Class 2

CultureVolumeClass3-4

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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<td>Human Clinical Applications</td>
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</table>

Project Ref 223/trans2a

Date Ackn'd 28/06/1994

CU2 Project Title MUTAGENESIS OF SALMONELLA STRESS RESPONSE GENES

Class CultureVolClass2 CultureVolumeClass3-4

Class 2
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Please enter comments on the GM safety committee on the risk assessment

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**Project Ref 223/trans3**

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<tr>
<td>28/06/1994</td>
<td>MUTAGENESIS OF BORDETELLA SPECIES</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref** 223/trans4

**Date Ackn’ed** 28/06/1994

**CU2 Project Title** PRODUCTION OF EQUINE HERPES 1 AND 4 VACCINE/VECTORS

**Class** Class 2

**Culture Volume Class**
- Culture Vol Class 2
- Culture Vol Class 3-4

**Non-GMM** not applicable

**Consent Granted**

- Project notified under transitional arrangements

**Withdrawn**

- N

**Tick if notifying a connected programme of work**

- N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
**Evaluation of foreseeable effects**

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

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Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 25/99.3

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<td>22/07/1999</td>
<td>PRODUCTION OF RETROVIRUSES ENCODING PAPILLOMA PROTEINS E2, E5,</td>
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Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
## Project Containment

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### Project Ref 26/01.1

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<tr>
<td>26/08/2021</td>
<td>GENETIC MODIFICATION OF ALPHAVIRUSES</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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### Historical Significant Changes

- Date of Additional Info
- Significant Change ID
- Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

The aim of the activities is to utilise SFV for expressing a variety of foreign proteins that could be of either viral or cellular origin. The foreign proteins could be derived from viruses that are human pathogens such as hepatitis C virus (HCV) or respiratory syncitial virus (RSV). The purpose is to use SFV to express high levels of the proteins in mammalian cells. This permits the study of the properties and functions of individual components of a particular virus in the absence of all other viral components.

**Recipient or parental organism**

The vectors used for introduction of foreign sequences into SFV are derived from the SFV viral genome. SFV is a well studied virus which does have a wide natural host range (including birds, rodents and anthropod vectors) but this does not include man.

It is intended eventually to use Sindbis virus also.

**Host/vector system**

Host system(s):
- Bacterial: Disabled E. coli K12-derived strains such as DH5.
- Mammalian: a range of tissue culture cell lines of mammalian origin.

Vector system(s):
- Mobilisation-defective plasmids pGEM1, pSFV1 and its derivatives. SFV-based expression in animal cells requires in vitro synthesis of recombinant RNA from pSFV1-derived and pSFV3 plasmids.

**Origin & function**

Hepatitis C virus (HCV), GBV-B and respiratory syncytial virus (RSV)

The system will be used for studying proteins encoded by these viruses.

**Evaluation of foreseeable effects**

Semliki Forest Virus is being used to study proteins from other viruses in this programme and evaluation of effects has to take into account both it and the other virus species involved. HCV is a natural human pathogen that causes chronic infection. The only documented route of infection is by contact with infected blood. The natural host for GBV-B is unknown, although there are literature reports that it is infectious in tamarins. It is not known whether humans are susceptible to infection. From studies elsewhere on infection of chimpanzees by HCV and GBV-B, infection is only successful using the entire genome. Generation of full-length genomic RNA of HCV in the SFV system is not possible. Furthermore, in the SFV system, there will be no combination of vectors introduced into cells that permits generation of full-length HCV and GBV-B genomes by any recombination mechanism. Hence, infectious HCV cannot be produced. It is not expected that HCV and GBV-B proteins will be toxic to man. Although wild-type SFV can infect animals, the system produces disabled virus that will give only a single round of infection. The most significant consequence of infection by direct inoculation may be an immune response to SFV proteins and those inserted into the vector. There is a possible risk of infection by either aerosols or direct inoculation. Therefore, any SFV particles produced by the vector system will be handled in a safety cabinet and no sharps will be used.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
DISINFECTION POLICY
The three methods for disinfection are as follows:
1. Chloros - 1% v/v
2. Virkon - 1% w/v
3. Autoclaving

All of the above methods can be used for herpes, adeno, vaccinia, bunya, respiratory syncitial, measles, chandipura and baculo viruses. Material derived from other viruses must be disinfected by autoclaving and possibly incineration as described in the relevant risk assessment form.

The methods are also suitable for E.coli.

Chloros (outwith the laboratory)
For bulk overnight steeping of glassware
Laboratories will be supplied daily with a trolley on which are large round vats for heavy glassware and smaller rectangular vats for light glassware. The appropriate volumes of stock Chloros is pre-dispersed in accompanying plastic bottles.
N.B. With the exception of empty pipette cans and chloros containers, no other items are permitted on this trolley.

Virkon (within the laboratory)
For bulk liquids (e.g. discarded tissue culture media)
Virkon powder* should be added to give a 1% w/v final concentration and the container mixed thoroughly.

For large containers (e.g. burrers, large and medium tissue culture flasks)
Treat with 1% w/v Virkon, either by adding powder and filling with water as above, or by dilution of a fresh concentrate. Stock solutions of Virkon must be dated: they retain efficacy for 1 week. Care should be taken to resuspend any pelleted or clumped material and to completely fill the container, swabbing the threads and then inverting closed flasks and burrers to ensure the neck is treated.

With both of the above methods the treated containers must be soaked for a minimum of 1 hour before disposing liquid down the sink, glassware to normal wash-up and plasticware to normal solid waste (black bags). It is recommended that materials being disinfected are clearly marked. For large glass conical flasks (greater than 1 litre capacity) These should be filled with 1% w/v Virkon and placed on the designated trolleys located on the first and second floors. The washroom staff will empty these trolleys first thing in the morning. These trolleys are for the disposal of large flasks only - other material must not be left on them. For surfaces, equipment and instruments Surfaces should be swabbed with 1% w/v Virkon (less than one week old). Equipment and instruments can be similarly disinfected but extreme caution should be exercised with electrical equipment. If in doubt see the Technical Services Manager.

*While Virkon is regarded as an extremely safe compound, care should be taken when dispensing dry powder in bulk. A protective face mask could be used to prevent inhalation.

Autoclaving  For small vessels (e.g. petri dishes and small tissue culture flasks) These should be placed in the biological waste discard boxes (lined with clear bags) in each lab. For higher risk organisms (e.g. clinical isolates, hepatitis, HIV) These should be placed in the biological waste discard boxes (lined with biohazard bags) which will be autoclaved prior to incineration of the contents.

For contaminated material (e.g. burrers with fungi or bacteria) These should not be opened but transported and loaded, at the allotted time, into the "dirty" autoclave in the washroom for inclusion in the liquid discard cycle. All lids must be loosened at the autoclave when the material is loaded. In the event that the "dirty" autoclave is still running the previous cycle, being serviced or out of order, all discarded boxes or other contaminated materials must be returned to their lab of origin. They MUST NOT be left at the autoclave or given to the washroom staff. For other methods - Any other method or disinfecting agent (e.g. sensitivity reactions) must be treated as a separate experimental procedure with it's own evaluation of efficacy and risk assessment form. General Aerosols Benchworkers should be wary of all techniques and manipulations which give rise to aerosols when using hazardous materials e.g. filling used burrers contaminated with viruses. Where practicable these procedures should be performed in an appropriate safety cabinet. Biological materials  It is recommended that all vessels used with biological materials be disinfected e.g. burrers used for non infected cell growth be treated with Virkon before discarding to normal waste. WASTE DISPOSAL The disposal of waste chemicals and solvent can often cost more than the original material. It is important, therefore, when orders are placed for chemicals that only sufficient is ordered for your immediate use. Waste disposal must be considered as integral to all laboratory procedures. These note summarise the principles of waste disposal organisation and describe the approved System of Work for discarding labware. The guidance principle is that scrupulous care must be taken to protect the interest of cleaners, other staff, the public and the environment.

Waste-bag colour code and disposal routes  BLACK = Non-hazardous; cleaners may remove  RED = These are for material requiring the attention of specialist waste disposal operators. Cleaner's can request assistance from specialist staff when the bag becomes full. It is the individual users' responsibility to ensure that the contents of the bag are disposed of as envisaged. The bags must be sealed securely to prevent escape of noxious substances, including aerosols.
disposal companies and are for compounds (e.g. ethidium bromide) identified in risk assessment forms. See "Waste Chemicals" below. YELLOW = These will not be available to laboratory workers. They are for incineration of autoclaved clinical waste. TRANSPARENT/BIOHAZARD = Microbiological waste for autoclaving. These must be transported in the metal disposal boxes and placed in the "dirty" autoclave in the washroom at the appropriate times. General Waste General waste in the laboratory may be put into the normal disposal containers. However, it is important that broken glass and other hazardous waste is kept separate. Waste solvents Solvent waste is collected in metal drums at the back of the spirit store. Potential hazards exist in mixing different solvents and there are separate drums for each of 3 categories of waste: 1) Flammable, e.g. ether, methanol, acetone. 2) Non-flammable, e.g. DMSO, phenol, chloroform. 3) Oils, e.g. waste from centrifuges, pumps, etc.

The material and amount disposed of in each category must be entered into the appropriate log book provided stating your name and date. Consult The Technical Services Manager if you are in any doubt as to which category your waste belongs. Make sure that there is adequate room left in the drum before starting. There should always be an empty drum below the one in use. If you finish a drum, inform The Technical Services Manager. Absorption granules for soaking up any solvent spillage are available in the solvent store.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

From Divisional Safety Committee minute of 22/09/2000

5 Genetic modification proposal: Analysis of GBV-B structural proteins
In discussion there emerged more concern with the introduction of a new virus to the institute than with the proposed GM aspects. It was not known whether the virus is human or tamarin originally. The chief interest was as a model for HCV infection and, in this case, its structural proteins.

5.1 RME noted that there was one report in the literature of VSV glycoproteins being expressed in Semliki Forest Virus.

5.2 There was some concern with the statement "not envisage..... will produce infectious virus" However, part 10 did address concerns.

5.3 It was important to check with the ACDP on the classification GBV. (see 4.2 above)

5.4 The question has to be answered whether it extended previously agreed work, and hence whether a new notification to the HSE was needed.

5.5 It was recommended that the reference to "Experience of expression with HCV....." be moved from part 10 to part 7. Subject to these constraints the proposal was accepted. From the Virology Safety Committee minutes of 07/02/2001

24 Use of Semliki Forest Virus to study RSV proteins

It was noted that the original use of Semliki Forest virus has not constituted genetic modification. A further proposal was now considered to introduce GM of this virus and Notification of a new Activity was indicated. The present proposal represented an additional GM use of SFV.

24.1 The autoclaving of all waste was queried: the normal regime should be adequate (AW to check). Also, the ACGM Guidance Notes provided a model for the environmental risks. (RT to discuss with RS).

24.2 Subject to these changes the proposal was accepted at class 2.

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02/03/2022
**Project Ref** 26/01.2

**Date Ackn’ed** 26/08/2021  
**CU2 Project Title** GENETIC MODIFICATION OF CALICIVIRUSES

**Class** Class 2  
**CultureVolClass2** < 1 Litre

**Non-GMM Consent Granted** Consent Granted

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Study of the structure and function of Norwalk-like viruses, and related viruses of public health significance.

**Recipient or parental organism**

Human small round structured virus (SRSV)  
Feline calicivirus (FCV)

**Host/vector system**

**Hos system(s)**

1. **Prokaryotic**
   
   Plasmid propagation - E.coli TG2, JM101, DH5a, MC1061  
   Expression in E.coli - E. coli BL21, BL21 (DE3)

2. **Eukaryotic**
Yeast protein expression system - Pichia pastoris
Insect cell lines - Spodoptera frugiperda
Mammalian cell lines - MRC5, HeLa, Ohio HeLa, 293, BSC-1, VERO, RD, CHO, BHK

Vector system(s)
1. Prokaryotic
   - Plasmid propagation - pBR322, pUC, PGEM, pJM1, pEMBL, PACYC184, pBLUESCRIPT
   - Protein expression - pGEMEX, pET, pUC119

3. Eukaryotic
   - Protein expression in yeast - pPIC9, pPIC3
   - Protein expression in insect cells - Autographa californica baculovirus
   - Protein expression in mammalian cells - pcDNA3, pREP, pTM1 & pTM3 (vaccinia expression), vTF7-3 (vaccinia T7 system), MVA-T7, fowlpox T7, non-cytopathic Sindbis virus replicons Virus expression in eukaryotic cells

Origin & function
FCV full genome and cloned segments of SRSV will be used to study the structure and function of individual proteins and the replication of the virus.

Evaluation of foreseeable effects
The predicted characteristics of the proteins indicate that there will be no toxic effect on humans or the environment. (Those produced in other laboratories have been used to raise antibodies and had no toxic effect).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

DISINFECTION POLICY
The three methods for disinfection are as follows:-

1. Chloros - 1% v/v
2. Virkon - 1% w/v
3. Autoclaving

All of the above methods can be used for herpes, adeno, vaccinia, bunya, respiratory syncitial, measles, c handipura and baculo viruses. Material derived from other viruses must be disinfected by autoclaving and possibly incineration as described in the relevant risk assessment form.

The methods are also suitable for E.coli

02/03/2022
Chloros (outwith the laboratory)

For bulk overnight steeping of glassware

Laboratories will be supplied daily with a trolley on which are large round vats for heavy glassware and smaller rectangular vats for light glassware. The appropriate volumes of stock Chloros is pre-dispensed in accompanying plastic bottles.

NB  With the exception of empty pipette cans and chloros containers, no other items are permitted on this trolley.

Virkon (within the laboratory)

For bulk liquids (eg. discharded tissue culture media)

Virkon powder *should be added to give a 1% w/v final concentration and the container mixed thoroughly.

For large containers (eg. burrers, large and medium tissue culture flasks)

Treat with 1% w/v Virkon, either by adding powder and filling with water as above, or by dilution of a fresh concentrate. Stock solutions of Virkon must be dated: they retain efficacy for 1 week. Care should be taken to resuspend any pelleted or clumped material and to completely fill the container, swabbing the threads and then inverting closed flasks and burrers to ensure the neck is treated.

With both of the above methods the treated containers must be soaked for a minimum of 1 hour before disposing liquid down the sink, glassware to normal wash-up and plasticware to normal solid waste (black bags) - laboratory personnel must remove any large bulk waste to cleansing department bins. It is recommended that materials being disinfected are clearly marked.

For large glass conical flasks (greater than 1 litre capacity)

These should be filled with 1% w/v Virkon and placed on the designated trolleys located on the first and second floors. The washroom staff will empty these trolleys first thing in the morning. These trolleys are for the disposal of large flasks only - other material must not be left on them.

For surfaces, equipment and instruments

Surfaces should be swabbed with 1% Virkon (less than one week old). Equipment and instruments can be similarly disinfected but extreme caution should be exercised with electrical equipment. If in doubt see the Technical Services Manager.

*While Virkon is regarded as an extremely safe compound, care should be taken when dispensing dry powder in bulk. A protective face mask could be used to prevent inhalation.

Autoclaving

For small vessels (eg petri dishes and small tissue culture flasks)
These should be placed in the biological waste discard boxes (lined with clear bags) in each lab.

For higher risk organisms (e.g., clinical isolates, hepatitis, HIV)

These should be placed in the biological waste discard boxes (lined with biohazard bags) which will be autoclaved prior to incineration of the contents.

For contaminated material (e.g., burrers with fungi or bacteria)

These should not be opened but transported and loaded, at the allotted time, into the "dirty" autoclave in the washroom for inclusion in liquid discard cycle. All lids must be loosened at the autoclave when the material is loaded.

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Other methods

Any other methods or disinfecting agent (e.g., stericol, gluteraldehyde, SDS) required for scientific or personal reasons (e.g., sensitivity reactions) must be treated as a separate experimental procedure with its own evaluation of efficacy and risk assessment form.

General

Aerosols

Benchworkers should be wary of all techniques and manipulations which give rise to aerosols when using hazardous materials eg. filling used burrers contaminated with viruses. Where practicable these procedures should be performed in an appropriate safety cabinet.

Biological materials

It is recommended that all vessels used with biological materials be disinfected eg. burrers used for non infected cell growth be treated with Virkon before discarding to normal waste.

WASTE DISPOSAL

The disposal of waste chemicals and solvent can often cost more than the original material. It is important, therefore, when orders are placed for chemicals that only sufficient is ordered for your immediate use. Waste disposal must be considered as integral to all laboratory procedures. These notes summarise the principles of waste disposal organization and describe the approved System of Work for discarding labware.

The guiding principle is that scrupulous care must be taken to protect the interests of cleaners, other staff, the public and the environment.

Waste-bag colour code and disposal routes

BLACK = Non-hazardous; cleaners may remove
RED = These are for material requiring the attention of specialist waste disposal companies and are for compounds (e.g., ethidium bromide) identified in risk assessment
forms. See "Waste Chemicals" below.

YELLOW = These will not be available to laboratory workers. They are for incineration of autoclaved clinical waste.

TRANSPARENT/BIOHAZARD = Microbiological waste for autoclaving. These must be transported in the metal disposal boxes and placed in the "dirty" autoclave in the washroom at the appropriate times.

General waste

General waste in the laboratory may be put into the normal disposal containers. However, it is important that broken glass and other hazardous waste is kept separate.

Waste solvents

Solvent waste is collected in metal drums at the back of the spirit store. Potential hazards exist in mixing different solvents and there are separate drums for each of three categories of waste:

1. Flammable, eg ether, methanol, acetone.
2. Non-flammable, eg DMSO, phenol, chloroform.
3. Oils, eg waste from centrifuges, pumps, etc.

The material and amount disposed of in each category must be entered into the appropriate log book provided stating your name and date. Consult The Technical Service Manager if you are in any doubt as to which category your waste belongs. Make sure that there is adequate room left in the drum before starting. There should always be an empty drum below the one in use. If you finish a drum, inform The Technical Services Manager. Absorption granules for soaking up any solvent spillage are available in the solvent store.

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<td>risk assessment</td>
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Please enter comments on the GM safety committee on the risk assessment
From the Divisional Safety Committee minute of 03/05/2000


   RME spoke to the proposal which, if accepted, would represent a new programme of work requiring notification to the HSE.

9.1 Caliciviruses caused human gastrointestinal disease typified by an acute but usually shortlived episode of severe diarrhoea and vomiting. Currently they were classified at ACDP level 2.

9.2 Growth in tissue culture could not yet be achieved and there was no testing of Virkon effectiveness. It was noted that were a tissue culture system to become available this would materially alter the safety implications (though not a genetic modification issue in itself).

9.3 The project would involve recovery of unmodified virus from cDNA clones. For future modification of clones a further proposal would be brought to the Committee.

9.4 The Committee accepted the proposal. A submission to the ACGM would follow.

### Project Containment

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<thead>
<tr>
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<td>L2 L3 L4 L2 L3 L4</td>
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<td>Human Clinical Applications</td>
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### Project Ref 26/01.3

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<tr>
<th>Date Ackn'd</th>
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<th>Date Project Ceased</th>
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<th>CultureVol</th>
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<tr>
<td>26/08/2021</td>
<td>GENETIC MODIFICATION OF PARAMYXOVIRUSES</td>
<td></td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Consent Granted</td>
</tr>
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</table>

- Project notified under transitional arrangements
- Non-GMM Consent Granted
- Tick if notifying a connected programme of work

02/03/2022
Project Additional Information

Purposes of the contained use

To study respiratory syncytial virus and related viruses of public health significance.

Recipient or parental organism

Respiratory Syncytial Virus (RSV)

Host/vector system

**Host Systems**

1) Propagation of viral cDNA. Standard laboratory hosts.
   - JM109
   - DH5
   - Late6 (a derivative of SURE, developed for maintenance of large, unstable plasmids such as the RSV antigenome).

2) Expression in mammalian cell-lines i.e. production of infectious virus particles. Established cell lines.
   - HepC
   - BHK
   - Cos-7

**Vector systems**

i) Propagation of viral cDNA
   - pGEM - (Promega)
   - pCITE - (Novagen)

Origin & function

Isolates of RSV
RSV is relatively common in the human population. The experiments are designed to study the functions and interactions of the proteins encoded by the RSV genome. It is expected that engineered virus will be no more hazardous than the wild type. Eventually it is hoped to identify viral proteins which are potential targets for antiviral drugs.

Evaluation of foreseeable effects

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Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

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From Divisional Safety Committee minutes 13/09/2000

10. Genetic modification proposal: Genetic manipulation of respiratory syncytial virus

It was intended to use a commercially available system (from Aviron) which did not need M2. Although the proposer would start using vaccinia strain T7 he would move away from this to a fowlpox or sindbis virus system.

10.01 Fundamentally it would be an RSV rescue system and as such represented a new programme of work. On this basis it was agreed by the Committee at level 1 or (vaccinia) level 2.

10.02 This would be submitted to the HSE as a paramyxovirus programme.

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**Project Ref** 26/01.4

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<th>CultureVolumeClass3-4</th>
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<td>GENETIC MODIFICATION OF EUKARYOTIC CELLS</td>
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Tick if notifying a connected programme of work

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? (If yes, tick to confirm that it is attached to this form)

Tick to confirm that you have attached a risk assessment to this form
Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

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Project Ref  26/01.5

Date Ackn'd  26/08/2021

CU2 Project Title  GENETIC MODIFICATION OF BACULOVIRUSES

Class  Class 2

CultureVolClass2  Non-GMM

CultureVolumeClass3-4  Consent Granted

Project notified under transitional arrangements  Y

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022 Page 5471 of 15326
## Project Additional Information

### Purposes of the contained use
To study the proteins of hepatitis C virus.

### Recipient or parental organism
BVDV

### Host/vector system

**Host system(s):**
- Bacterial: Disabled E. coli K12-derived strains such as DH5
- Mammalian: Human hepatocyte derived cell lines (eg Huh-7, HepG2 etc), BHK, MDBK

**Vector system(s):**
- Mobilisation defective plasmids.

### Origin & function
HCV genomic cDNA sequences expressing viral proteins will be used for structure and function analysis.
If successful, the system will generate chimeric pseudotype BVDV particles carrying HCV structural proteins which may change its host and tissue tropism. Furthermore, the pseudotype particles are likely to encapsidate replication competent chimeric cDNA genome carrying HCV structural genes. Therefore, these particles could infect cells and replicate if injected into humans. It is virtually impossible to predict the outcome of accidental infection of humans with the chimeric particles and hence the categorization of the proposed work as class 3 activities.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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The methods are also suitable for E. coli.

Chloros (outwith the laboratory)

For bulk overnight steeping of glassware.

Laboratories will be supplied daily with a trolley on which are large round vats for heavy glassware and smaller rectangular vats for light glassware. The appropriate volumes of stock Chloros is pre-dispensed in accompanying plastic bottles.

NB With the exception of empty pipette cans and chloros containers, no other items are permitted on this trolley.

Virkon (within the laboratory)

For bulk liquids (eg discarded tissue culture media)

Virkon powder * should be added to give a 1% w/v final concentration and the container mixed thoroughly.

For large containers (eg burrlers, large and medium tissue culture flasks)

Treat with 1% w/v Virkon, either by adding powder and filing with water as above, or by dilution of a fresh concentrate. Stock solutions of Virkon must be dated: they retain efficacy for 1 week. Care should be taken to resuspend any pelleted or clumped material and to completely fill the container, swapping the threads and then inverting.
closed flasks and burrfers to ensure the neck is treated.

With both of the above methods the treated containers must be soaked for a minimum of 1 hour before disposing liquid down the sink, glassware to normal wash-up and plasticware to normal solid waste (black bags) - laboratory personnel must remove any large bulk waste to cleansing department bins. It is recommended that materials being disinfected are clearly marked.

For large glass conical flasks (greater than 1 litre capacity)

These should be filled with 1% w/v Virkon and placed on the designated trolleys located on the first and second floors. The washroom staff will empty these trolleys first thing in the morning. These trolleys are for the disposal of large flasks only - other material must not be left on them.

For surfaces, equipment and instruments.

Surfaces should be swabbed with 1 w/v Virkon (less than one week old). Equipment and instruments can be similarly disinfected but extreme caution should be exercised with electrical equipment. If in doubt see the Technical Services Manager.

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Solvent waste is collected in metal drums at the back of the spirit store. Potential hazards exist in mixing different solvents and there are separate drums for each of three categories of waste:

1. Flammable, eg ether, methanol, acetone.
2. Non-flammable, eg DMSO, phenol, chloroform.
3. Oils, eg waste from centrifuges, pumps, etc.

The material and amount disposed of in each category must be entered into the appropriate log book provided stating your name and date. Consult the Technical Services Manager if you are in any doubt as to which category your waste belongs. Make sure that there is adequate room left in the drum before starting. There should always be an empty drum below the one in use. If you finish a drum, inform the Technical Services Manager. Absorption granules for soaking up any solvent spillage are available in the solvent store.
From the local Committee minute of 01/05/2002:


It was noted that all relevant staff were CL3 trained.

6.1 In discussion no major problems were identified but it was noted that, with BVDV being modified, HSE approval of a new GM Activity would be required.

6.2 It was noted that RME and others had used a similar HCV protease-dependent system.

6.3 Some textual revisions were required, to strengthen the proposal. The introduction should state that BVDV is not a Specified Animal Pathogen. Also, a few more papers on existing recombinants should be cited since there are more recent papers on recombination in vitro (RME to advise). Section 6 should mention that some recombinants will have both BVDV glycoproteins and HCV proteins.

6.4 Subject to these changes, the proposal was agreed for presentation to the HSE as a new class 3 notification.

Project Containment

<table>
<thead>
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<td>L2 Yes</td>
<td>L3 Yes</td>
<td>L4 L2 L3 L4</td>
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</table>

Animal Units

| L2 | L3 | L4 | L2 |

Large Scale Activities

| L3 | L4 | L2 |

Human Clinical Applications

| L3 | L4 | L2 |

Project Ref 26/04.1

26/08/2021 USE OF DISABLED ADENOVIRUS VECTORS DESIGNED FOR EXPRESSION OF

Class 2 CultureVolClass2 CultureVolumeClass3-4

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<th>Class</th>
<th>CultureVolClass2</th>
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This project covers the broad area of the use of disabled adenovirus vectors for the expression of cloned genes of interest in mammalian cells. Several such vector systems are now available, some commercially, and while they vary in detail, they have in common the lack of the essential E1A gene, and therefore they can be propagated only in complementing cell lines. This genetic modification proposal is intended to register the use of adenovirus vectors for gene expression in the Division of Virology. The risk assessment for each such project will be considered on an individual basis as such proposals are made, with specific reference to the vector system and cloned inserts being used. These future proposals will be considered as an amendment to this parent proposal.

Recipient or parental organism

Complementing cell lines 293 and 911, which express the adenovirus E1A protein, will be used to propagate stocks of the recombinant adenoviruses.

Host/vector system

The viruses will be used for experimentation in standard mammalian cultured cell lines such as Hep2, human foetal fibroblasts and Vero cells. The virus vectors are those commercially supplied by BD Biosciences (for full information see http://wwwbdbiosciences.com/clontech/techinfo/manuals/index.shtml). These are rountinely used in many laboratories around the world and are accepted as Category 2 pathogens.

The adenovirus vectors to be used are of two types. Firstly those in which constitutive expression of the cloned genes is under the control of the human cytomegalovirus immediate-early promoter region. Secondly, a tri-partite inducible expression system, in which expression of the cloned genes is controlled by an inducible tetracycline responsive promoter, and the tetR-VP16 fusion activator protein provided in trans by a second adenovirus vector (Ad.CMV-rtTA). Thus the recombinant proteins will be expressed only in cells treated with tetracycline derivatives and co-infected with both adenoviruses.

Origin & function

The initial list of the viruses to be utilised is:-
Ad.CMV-null: Adenovirus vector with HCMV IE promoter and no insert.
Ad.TRE-null: Adenovirus vector with Tet-responsive promoter and no insert.
Ad.CMV-GFP: Adenovirus vector with HCMV promoter and EGFP insert.
Ad.TRE-GFP: Adenovirus vector with Tet-responsive promoter and EGFP insert.
Ad.CMV-PMLwt: Adenovirus vector with HCMV promoter and wt PML insert.
Ad.CMV-rtTA: Adenovirus vector expressing the doxycycline-inducible transactivator of the TRE promoter.
Ad.TRE-PMLwt: Adenovirus vector with Tet-responsive promoter and wt PML insert.
Ad.TRE-ICPO: Adenovirus vector with Tet-responsive promoter and ICPO insert.
Ad.TRE-n212: Adenovirus vector with Tet-responsive promoter and ICPO insert truncated at residues 212.

Abbreviations:
HCMV  Human cytomegalovirus.
TRE  Tetracycline responsive element.
GFT  enhanced green fluorescent protein
PML  Promyelotic leukaemia protein.
ICPO  Infected cell protein O of HSV-1
HSV-1  herpes simplex virus type 1

Evaluation of foreseeable effects

The adenoviruses to be used are defective for the essential E1A gene products and they are also deleted for the E3 region, so they are unable to replicate and spread in normal human cells. However, the inserted genes are designed to be expressed in mammalian cells, and therefore the project is designated as Level 2. Increased expression of the PML proteins is very unlikely to be harmful since PML is expressed in a wide range of cell types, and high level expression of PML is a normal consequence of interferon treatment of cells. ICPO is very unlikely to be more harmful to man when expressed in the tet inducible vector virus pair than when expressed during the course of a normal HSV-1 infection. Detailed safety guidance concerning these adenovirus vectors, and the protocols approved for their use in the National Institutes of Health, Bethesda USA, is available at: http://www.bdbiosciences.com/clontech/techinfo/manuals/index.shtml

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Extract from the Divisional Safety Handbook.

DISINFECTION POLICY

The three methods for disinfection are as follows:
1. Chloros - 1% v/v
2. Virkon - 1% w/v
3. Autoclaving

All of the above methods can be used for herpes, adeno, vaccinia, bunya, respiratory syncitial, measles, chandipura and bacula viruses. Material derived from other viruses must be disinfected by autoclaving and possibly inceration as described in the relevant risk assessment form.

The methods are also suitable for E. coli.

Chloros (outwith the laboratory)
For bulk overnight steeping of glassware
Laboratories will be supplied daily with a trolley on which are large round vats for heavy glassware and smaller rectangular vats for light glassware. The appropriate volumes of stock Chloros is pre-dispensed in accompanying plastic bottles.

NB With the exception of empty pipette cans and chloros containers, no other items are permitted on this trolley.

Virkon (within the laboratory)

For bulk liquids (eg discarded tissue culture media)
Virkon powder *should be added to give a 1% w/v final concentration and the container mixed thoroughly.

For large containers (eg burriers, large and medium tissue culture flasks)
Treat with 1% w/v Virkon, either by adding powder and filling with water as above, or by dilution of a fresh concentrate. Stock solutions of Virkon must be dated: they retain efficacy for 1 week. Care should be taken to resuspend and pellet or clumped material and to completely fill the container, swabbing the threads and then inverting closed flasks and burriers to ensure the neck is treated.

With both of the above methods the treated containers must be soaked for a minimum of 1 hour before disposing liquid down the sink, glassware to normal wash-up and plasticware to normal solid waste (black bags) - laboratory personnel must remove any large bulk waste to cleansing department bins. It is recommended that materials being disinfected are clearly marked.

For large glass conical flasks (greater than 1 litre capacity)
These should be filled with 1% w/v Virkon and placed on the designated trolleys located on the first and second floors. The washroom staff will empty these trolleys first thing in the morning. These trolleys are for the disposal of large flasks only - other material must not be left on them.

For surfaces, equipment and instruments
Surfaces should be swabbed with 1% w/v Virkon (less than one week old). Equipment and instruments can be similarly disinfected but extreme caution should be exercised with electrical equipment. If in doubt see the Technical Services Manager.

* While Virkon is regarded as an extremely safe compound, care should be taken when dispensing dry powder in bulk. A protective face mask could be used to prevent inhalation.

Autoclaving
For small vessels (eg petri dishes and small tissue culture flasks)
These should be placed in the biological waste discard boxes (lined with clear bags) in each lab.

For higher risk organisms (eg clinical isolates, hepatitis, HIV)
These should be placed in the biological waste discard boxes (lined with biohazard bags) which will be autoclaved prior to incineration of the contents.

For contaminated material (eg burriers with fungi or bacteria)
These should not be opened but transported and loaded, at the allotted time, into the 'dirty' autoclave in the washroom for inclusion in liquid discard cycle. All lids must be loosened at the autoclave when the material is loaded.

In the event that the 'dirty' autoclave is still running the previous cycle, being services or out of order, all discard boxes or other contaminated materials must be returned to
their lab of origin. They must not be left at the autoclave or given to the washroom staff.

Other methods
Any other method or disinfecting agent (eg stericol, gluteraldehyde, SDS) required for scientific or personal reasons (eg sensitivity reactions) must be treated as a separate experimental procedure with its own evaluation of efficacy and risk assessment form.

General

Aerosols
Benchworkers should be wary of all techniques and manipulations which give rise to aerosols when using hazardous materials eg filling used burriers contaminated with viruses. Where practicable these procedures should be performed in an appropriate safety cabinet.

Biological materials.
It is recommended that all vessels used with biological materials be disinfected eg burriers used for non infected cell growth be treated with Virkon before discarding to normal waste.

WASTE DISPOSAL
The disposal of waste chemicals and solvent can often cost more than the original material. It is important, therefore, when orders are placed for chemicals that only sufficient is ordered for your immediate use. Waste disposal must be considered as integral to all laboratory procedures. These notes summarise the principles of waste disposal organisation and describe the approved System of Work for discarding labware.

The guiding principle is that scrupulous care must be taken to protect the interests of cleaners, other staff, the public and the environment.

Waste-bag colour code and disposal routes.

BLACK = Non-hazardous; cleaners may remove.
RED = These are for material requiring the attention of specialist waste disposal companies and are for compounds (eg ethidium bromide) identified in risk assessment forms. See "Waste Chemicals" below.
YELLOW = These will not be available to laboratory workers. They are for incineration of autoclaved clinical waste.
TRANSPARENT/BIOHAZARD = Microbiological waste for autoclaving. These must be transported in the metal disposal boxes and placed in the "dirty" autoclave in the washroom at the appropriate times.

General waste
General waste in the laboratory must be put into the normal disposal containers. However, it is important that broken glass and other hazardous waste is kept separate.

Waste solvents
Solvent waste is collected in metal drums at the back of the spirit store. Potential hazards exist in mixing different solvents and there are separate drums for each of three categories of waste.

1. Flammable, eg ether, methanol, acetone.
2. Non-flammable, eg DMSO, phenol, chloroform.
3. Oils, eg waste from centrifuges, pumps etc.
The material and amount disposed of in each category must be entered into the appropriate log book provided stating your name and date. Consult the Technical Services Manager if you are in any doubt as to which category your waste belongs. Make sure that there is adequate room left in the drum before starting. There should always be an empty drum below the one in use. If you finish a drum, inform the Technical Services Manager. Absorption granules for soaking up any solvent spillage are available in the solvent store.

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

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Project Ref 26/11.1
Arboviruses are transmitted to susceptible vertebrates by arthropod vectors such as mosquitoes or ticks. Most arboviruses belong to the Flaviviridae, Bunyaviridae and Togaviridae families of RNA viruses. While arboviruses can cause disease in vertebrates ranging from febrile illness to more severe complications, depending on virus and/or host- as well as cell death in infected cultured cells, this appears not to be the case in the arthropods that transmit them. In fact, arbovirus-infected arthropods display few or no signs of disease (Fragkoudis et al., 2009), and this effect can be mimicked in arthropod cell culture.

Infection of mosquito or tick cells usually begins with an acute phase with high titre virus production followed by a persistent infection which, once established can last the life time of the arthropod cell culture. The biological reasons behind persistence are poorly understood and are believed to be linked to innate immune responses of the arthropod host. Research on antiviral immune responses in vector arthropods has only in recent times seen some progress yet this area of research is crucial in understanding this virus/host interaction and develop novel antiviral control strategies targeting the vector. It is now clear, mainly from research on arbovirus-infected mosquitoes, that vectors do not passively replicate arboviruses but that immune responses are activated. These can result in activation of immune signalling pathways (STAT, Toll etc.), humoral responses in the arthropod hemocoel and most crucially RNA interference (RNAi) responses. It is not known how immune signalling pathways are activated following infection or how exactly they would mediate antiviral activities, and extracellular responses such as phenoloxidase activities also appear to be involved. The RNAi responses are better understood and form a major antiviral defence. Viral replication induces production of double stranded RNA which serves as a substrate for dicer-type nuclease activities to produce virus-induced small interfering RNAs (also called viRNAs in the context of viral infection) which are integrated into the RNA-induced silencing complex (RISC). The RISC presumably mediates antiviral activities by finding viral RNAs through complementarity with viRNAs and then mediating nucleolytic cleavage of those viral RNAs. Although some steps in this antiviral pathway are known, mechanisms, triggers etc. are poorly understood. In addition, arthropod cells might differentially regulate subsets of their own small RNA repertoire following infection by arboviruses.

In this project, we aim to further analyze:

- The induction, regulation and mechanisms of antiviral signalling pathways such as STAT, TOLL, IMD etc. in arthropod cells such as mosquito and tick cells, as well as mosquitoes and tick organ cultures. This project will look at protein functions and signalling regulation within these pathways.
- The induction, regulation and mechanisms of humoral, extracellular antiviral responses. We will analyse viral triggers and key components that lead to activation of extracellular responses. This project will be carried out in arthropod cells such as those derived from mosquitoes and ticks, but also live mosquitoes, and tick organ culture.
To study virus replication in arthropod cells, we will use a cDNA copy of the LGTV genome (a positive-stranded RNA of approx. 11 kb encoding a polyprotein with structural sequences followed by non-structural sequences) available, allowing manipulation of the genome (Rumantsyev et al., 2006). This plasmid has been engineered or will be engineered to delete sequences (for example for replicon production), mutate specific sequences or insert genes such as reporter genes (for example fluorescent molecules such as YFP, eGFP or various types of luciferase), insect immune response inhibitors or drug resistance genes (to facilitate selection of cells harbouring replicons). Genetic changes that will be introduced into the virus sequences (point mutations, deletions, insertions) will be targeted at disrupting specific functions of virus RNA or virus proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virulence. The viral envelope glycoprotein and capsid protein sequences which are the major determinants of host range and cell tropism will not be changed beyond the addition of marker genes such as GFP (as fusion or cleavable inserts into this coding region); host range and cell tropism are therefore unlikely to be altered. Changes to viral sequences will target disruption of viral protein functions or sequences.

LGTV can be directly used to infect cells. cDNA plasmids with LGTV sequences will be amplified using standard disabled laboratory strains of bacteria (usually strain BD1528 which is routinely used for flavivirus cDNAs). Plasmids or transcribed RNA will be transfected into eukaryotic cells. Depending upon the construct, in eukaryotic cells this may result in replication of virus RNA and production of new virions. Virus or virus replicon particles generated from these plasmids will be used to infect eukaryotic cell and tissue cultures, to investigate virus replication in arthropod cells such as tick cells or tick organ cultures (derived from ticks). The use of LGTV expressing a reporter gene such as GFP or luciferase will allow accurate, rapid, efficient, localised and repetitive monitoring of infection of cells and tissues which is important for immunity studies.

In addition to the use of LGTV, we plan to carry out comparative studies between LGTV replicons and replicons of other arthropod-borne flaviviruses such as tick-borne encephalitis or dengue virus; those replicons are not covered by Schedule 5 and no sequences for complete schedule 5 virus genomes (e sequences encoding missing structural genes that are required to complement the full set of viral proteins to produce infectious virion) are stored in-house. Those replicon RNAs will not be packaged; only RNA will be introduced into cells to study replication; replicons will not be mixed in the same cells (either by co-transfection/infection or potential superinfection by spreading virus) with sequences of related viruses from within the same genus to avoid recombination events during co-infection (recombination can only occur when viruses replicate in the same cell). Like LGTV-derived replicons, other flavivirus replicons pose no risk to safety to human and animal health as they cannot spread and transfected cells will be eliminated by cell death or the immune system. Experiments with these replicons will be carried out to study host innate immune responses (RNAi, immune signalling) and/or virus-host cell interactions (protein interactions, replication complex localization in cells etc.). These experiments will complement studies carried out in other laboratories.
2. As a model for Togaviridae (genus Alphavirus) infection of arthropod cells, we will study infections by Semliki Forest virus (SFV). SFV is naturally found in central and Southern Africa and is transmitted between animals and birds by mosquitoes (mainly Ae. aegypti and africanus). SFV can infect man and is classified as a hazard group 2 pathogen by the ACDP. The alphavirus genome consists of a single strand of positive sense RNA of around 12 kb. This RNA codes for two open reading frames. The first codes for the non-structural viral replicase proteins (nsP1, 2, 3 & 4), the second for the viral structural proteins (C, E1, E2 & E3). Expression of the replicase proteins is under the control of a promoter (P) in the 5’ non-coding region of the virus. Expression of the structural proteins is under the control of a subgenomic promoter (SP) which is activated later in infection and leads to a high level of expression from this second open reading frame.

There are several strains of SFV which have been derived from natural isolates, these include prototype SFV4, L10, A7 and A7(74). Each of these strains has been cloned and sequenced and is available as a series of plasmids which can be amplified in standard bacterial systems. In vitro transcription of the plasmid cDNA can give rise to an RNA which when transfected into cells can give rise to infectious virus; alternatively RNA is transcribed from transfected plasmid under control of eukaryotic promoters such as the CMV promoter. The SFV cDNAs can be engineered to give rise to virus mutants or engineered viruses. The prototype strain SFV4 forms the basis of the SFV vector system which is widely used for eukaryotic gene expression (Smerdou and Liljestrom, 1999). SFV natural isolates, cDNA derived viruses and mutant viruses derived from these cDNA clones have been used extensively by us and others. In mice, different strains show different pathogenicity and these are likely to be due to sequence differences. We will produce and use genetically engineered SFV viruses mutated in viral proteins or sequences expressing additional genes (reporter genes such as luciferase or fluorescent proteins, insect immune response antagonists etc.) as part of the non-structural open reading frame, the structural or from duplicated subgenomic promoters (Siu et al., 2011; Attarzadeh-Yazdi et al., 2009). If the coding sequence for a protein such as a reporter gene would be inserted into the viral structural polyprotein-coding sequence, in the resultant hybrid polyprotein the added protein would be separated from the structural proteins by protease cleavage sites. No changes to the sequence of the structural proteins would ensue so that there would be no changes to virus tropism resulting from changes to the structural proteins. Introduction of additional sequences into SFV is likely to provide no change or to decrease virulence. Changes to viral sequences will target disruption of viral protein functions or sequences.

In addition to infection with SFV, it is possible to use virus-derived replicons. In replicons, the structural genes are replaced by a polylinker which allows insertion of foreign sequences. The system exploits the wide cell tropism of SFV, but has no potential for virulence since genes for the viral structural proteins are deleted. A helper system is also available whereby recombinant RNA can be packaged into infectious virus-replicon particles (VRP); this system is deemed extremely safe (Smerdou and Liljestrom, 1999). Packaging of genomic RNA into new virions is absolutely dependent on a packaging sequence in the replicase gene. Packaged VRPs are made by using cells which also express the viral structural genes (usually by cotransfection of structural gene-encoding mRNA transcripts). In the absence of genes encoding the viral structural proteins in replicons, new infectious virus particles capable of initiating a propagating infection cannot be regenerated in VRP-infected cells; VRPs are thus also referred to as ‘suicide particles’. Replicons can be used to express reporter genes or other genes of interest such as insect RNAi inhibitors.

SFV and engineered variants will be used to infect eukaryotic cell and tissue cultures, as well as arthropods such as mosquitoes. The use of genetically modified marker viruses will allow monitoring of infected cells and tissues, determination of the spread of infection and dissection of specific tissues / areas or even specific cells for further analysis. Again these properties are very useful to study immune responses in arthropod cells and tissues.

In addition to the use of SFV we plan to carry out comparative studies with other arthropod-borne alphavirus replicons such as those derived from chikungunya virus; those replicons are not covered by schedule 5 and no sequences for the complete genome (ie sequences encoding structural genes required to complement the viral protein set to give infectious virions) will be stored in house. Those replicons will not be packaged into VRPs, only RNA will be introduced into cells to study replication; replicons will not be mixed with related viruses from within the same genus in the same cell (either by co-infection/transfection, or potential superinfection by spreading virus) to avoid recombination events during co-infection. Like SFV-derived replicons, other alphavirus-derived replicons pose no risk to safety or human health as they cannot spread and transfected cells will be eliminated by cell death or the immune system. Experiments with these replicons will be carried out to study host innate immune responses (RNAi, immune signalling) and/or virus-host cell interactions (protein interactions, replication complex localization in cells etc.). These experiments will complement studies carried out with LGTV and allow us to verify how broadly relevant some findings (those that do not rely on formation of infectious virus or/and virus spread) obtained with a model virus are to other members of the family.
out with SFV and allow us to verify how broadly relevant some findings (those that do not rely on formation of infectious virus or/and virus spread) obtained with a model virus are to other members of the family.

References:


Recipient or parental organism

Arboviruses and derived replicons used will be of the Flaviviridae and Togaviridae (genus Alphavirus) virus families.

Togaviridae:
Semliki Forest virus prototype (SFV4), and strains such A7 and A7(74).
Semliki Forest virus-derived replicons and virus-replicon particles.
Other arthropod-borne alphavirus replicons (for example derived chikungunya virus).

Flaviviridae:
Langat virus (strain TP21, the most commonly used lab strain, or others).
Langat virus-derived replicons.
Other arthropod-borne flavivirus replicons (for example derived from tick-borne encephalitis virus and dengue virus).

Host/vector system

All organisms will be used at containment level 2, which is routine practice.
Plasmid vectors:
Viral sequences (including full length clones or partial sequences of SFV or LGTV) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. RNAs can be transcribed through bacterial promoters (ie. T7 or SP6) or eukaryotic promoters (CMV, SV40 etc.) Plasmids contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin) and sometimes a selectable eukaryotic drug resistance marker such as neomycin or puromycin resistance. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

Hosts:

Prokaryotic hosts:
Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste should prevent release of viable organisms. The risk to the environment is therefore effectively zero.

Eukaryotic hosts:
Vertebrate cell lines of various origins (typically mouse, hamster, etc.) and invertebrate cell lines of various origins (mosquito, tick etc.). Mosquitoes, mainly strains of Ae. aegypti (such as the laboratory strain Liverpool red eye). Tick organ cultures from dissected ticks.

Origin & function

In prokaryotic cells, only selectable (eg antibiotic resistance) genes will be translated.

Virus sequences will be under the control of a promoter that will only generate transcripts in eukaryotic cells or in vitro transcription reactions.

In eukaryotic cells, RNA will be translated into the following gene products:

- Arboviral proteins and RNAs (SFV and LGTV, or replicons of related arboviruses): Proteins involved in replication and transcription of viral genetic material and virus structural proteins. Some of these virus proteins will interact with host-cell components and may affect host cell responses to infection; these interactions are not currently known. If all virus sequences are translated new virions may be generated; replicons are non-propagative viral RNAs (capable of replication). Partial RNAs do not give rise to propagating infectious material but can display biological activities (ie. RNAi inhibitory, expression of some viral proteins for example for packaging of replicons).

- Non-arboviral genes: Genes of prokaryotic (eg CAT) or eukaryotic (eg. reporter genes such as luciferases or fluorescent proteins such as GFP; inhibitors of insect immunity ie. RNAi inhibitors, signalling inhibitors, melanization inhibitors etc.) origin. No proteins known to interfere with vertebrate immune responses will be cloned into full length SFV or LGTV. The recombinant foreign proteins to be expressed provide no significant increase in the hazard to human health; none are toxic or likely to produce disease in the quantities that could be produced by accidental exposure to these systems. Non translated RNA sequences have no known toxic effects and pose no risk to human health.

Techniques used to introduce insert or vectors into cells:

Prokaryotic cells: Introduction of DNA into E. coli will be by heat shock/chemical transformation. These techniques have been extensively described and are widely used; they rely on getting DNA very close to the bacterial membrane and introducing the genetic material through pores or membrane fusion.

Eukaryotic cells: Mammalian cells will be transfected using reagents such a lipofectamine or by electroporation. Virus genomes will be introduced into cultured cells or tick organ cultures by infection with virus or virus replicon particles (VRP), using transfection reagents or by electroporation.
Note: location and nature of where experiments are to be carried out:

GM 26 (CVR Church Street):
- Preparation, characterisation and genetic manipulation of viruses and replicons.
- Infection of cell lines and organ cultures, preparation of lysates, extracts and fixed tissues for downstream analysis.

GM223 (CVR Garscube Estate):
- Infection of Ae. aegypti mosquitoes with Semliki Forest virus (SFV) (wild type or genetically modified) or VRPs by bloodmeal.
- Isolation of SFV, or nucleic acids or proteins from Ae. aegypti mosquitoes for downstream analysis (for example real time PCR for quantification of gene expression, reporter gene assays etc.).
- Fixation of mosquito tissues for downstream analysis (immunofluorescence, fluorescent reporter gene expression).

Evaluation of foreseeable effects

Foreseeable effects/risk assessment for human health and safety (also see Risk Assessments):

Plasmid vectors:
Viral sequences (including full length clones or partial sequences of SFV or LGTV) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Transcription of even complete viral cDNA (SFV or LGTV) will result in no infectious RNA since the promoters are not active in prokaryotic hosts. Systems for prokaryotic gene expression are under control of an inducible prokaryotic promoter. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV or SV40), eukaryotic (eg actin promoter, pathway inducible promoter such as STAT) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human cells. None of the genes used in these studies are oncogenes. The hazard of expressing ‘foreign’ genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the foreign gene product and is discussed in more detail below.

SFV:
In Africa, natural infections have been associated with mild febrile illness (Mathiot et al., 1990). Various laboratory strains of SFV including prototype-SFV4, L10, A7 and A7(74) have been used extensively in many laboratories and even (in the past) for student practical classes. There is one report of laboratory based human disease. Death of a laboratory worker in Germany was associated with infection by the Osterrieth strain of SFV (Willems et al., 1979). This (Osterrieth) strain of SFV is no longer used experimentally. Whilst the above case report is noted, it dates back over 30 years and is difficult to reconcile with the extensive use of other strains of SFV with no reported adverse effects over a 50 year period. This case report makes the unpublished observation that antibodies against SFV can be demonstrated in the serum of many laboratory personnel working with it. It is generally considered that the individual who died was probably immunosuppressed and therefore highly susceptible to infection. Anecdotal reports and reported laboratory incidents indicate no human clinical signs after accidental inoculation with various laboratory strains of SFV. The classification into ACDP hazard group 2 indicates current expert opinion that containment level 2 precautions are considered adequate for controlling the risks associated with working with SFV. The viral envelope glycoproteins and capsid protein sequences are the major determinants of host range and cell tropism and will not be changed beyond the addition of marker genes such as GFP (as fusion or cleavable inserts into this coding region) into the structural open reading frame; host range and cell tropism are therefore unlikely to be altered. SFV is classified as ACDP hazard group 2. Personnel working with this virus are expected to inform occupational health should they become pregnant or immunosuppressed.

LGTV:
Under normal conditions, LGTV is only very rarely pathogenic to humans; live virus has been used in vaccine trials in humans (Dubov et al., 1962). Development of this vaccine was stopped in the 1980s because of rare (1 in 20,000) occurrences of post-vaccinal neurological disease (Rumyantsev et al., 2006). Genetic changes that will be introduced into the virus sequences will be targeted at disrupting specific functions of virus RNA or virus proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virus virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences such as
reporter genes which confer no advantage to virus replication. As for SFV, the viral envelope glycoproteins and capsid protein sequences which are the major determinants of host range and cell tropism will not be changed beyond the addition of marker genes as fusion or cleavable inserts into the region coding the structural proteins; host range and cell tropism are therefore unlikely to be altered. LGTV is not listed on the ACDP list but should be classified as hazard group 2 (current use in the UK and internationally, see http://www.healthsafe.uab.edu/pages/biosafety/biosafetymanual.pdf). Personnel virus working with this virus are expected to inform occupational health should they become pregnant or immunosuppressed.

The host/recipient organism:
Prokaryotic organisms: All strains will be disabled, commercially available E. coli derivatives classified as 'especially disabled hosts' by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic cells and tick organ cultures:
Cell lines to be used would not survive inside the human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA will confer no growth or survival advantage in or outside the laboratory to cell lines of vertebrate or arthropod origin. The tick organ cultures are derived from dissected ticks and are immobile culture systems. As with the cell lines they could not survive in humans. Organ cultures are generally derived from ticks sourced from 'pathogen-free' colonies; ticks from other sources (for example 'field ticks') could carry other pathogens and will not be used in these studies.

Mosquitoes (also see paragraph 8, below): These will be sourced only from 'pathogen-free' laboratory colonies (provided by Prof. Eileen Devaney, University of Glasgow). Precautions will be taken to minimise the risk of bites or escapes in strict compliance with standard operating procedures (attached separately). Work with virus replicons and VRPs will be done under the same procedures and containment as work with infectious virus (wild type or genetically modified). Measures to prevent human infection include storage of infected arthropods in sealed containers; work in designated rooms with restricted access; wearing of gloves and cooling of arthropods to slow their movement and facilitate handling. Samples (dead arthropods or material from these) will be transported to our CL2 laboratory according to standard regulations.

The inserted/donated genetic material:
Viral RNA and proteins, cellular proteins: Most individually expressed viral or cellular proteins (for example arthropod immune response inhibitors) are unlikely to have harmful effects in eukaryotic cells, however some could perturb normal arthropod cellular metabolism, predispose or protect against cell death or render cells more or less susceptible to other infections. It is very unlikely that SFV or LGTV (and other alphavirus, flavivirus or insect virus) RNA or proteins would have any harmful toxin-like effect outside cells. RNA containing the complete SFV or LGTV sequence can give rise to infectious virus in eukaryotic cells; alphavirus and flavivirus replicon RNAs are non-propagative and pose no harm (individual infected cells are likely to die or be eliminated by the immune system). Viral non-coding RNA sequences are likely to have minimal effects on virus replication, especially outside arthropod cell systems, and do not encode biologically active molecules that are likely to induce physiological effects in humans. As described under (9), no proteins or sequence which could interfere with immune responses in vertebrates will be cloned into viral genomes. Reporter genes: Reporter genes of prokaryotic or eukaryotic origin (eg luciferase, fluorescent proteins etc.). No harmful properties have been attributed to these proteins. There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Photinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function. Other non coding RNA sequences: Any effects these elements have on gene expression are likely to be minimal. They may affect gene function or virus replication in individual cells but are unlikely to lead to whole body physiological effects as they do not code for the production of secreted bioactive molecules, and are unlikely to have any biological effects outside arthropod cell systems.

Sequence changes in viral sequences:
Mutation or deletion will target disruption of non-structural protein functions or non-coding viral sequences and are likely to have no or deleterious effects on viral replication, thus not increasing risk or hazards to human health associated by viruses genetically modified in such a way.

Control measures – assign provisional containment level:
GM bacteria transfected with plasmids containing viral sequences - Containment Level 1.
Eukaryotic cells (including tick organ cultures) containing alphavirus or flavivirus sequences unable to form complete infectious virus – Containment Level 1.
Eukaryotic cells (including tick organ cultures) containing full length SFV/LGTV sequences (capable of forming infectious virus, or capable of undergoing recombination or minor mutations to form infectious virus) – Containment Level 2.
Arthropods containing viral sequences unable to form complete infectious virus – Arthropod Containment Level 2.
Arthropods containing full length viral sequences (capable of forming infectious virus, or capable of undergoing recombination or minor mutations to form infectious virus) – Arthropod Containment Level 2.
All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. For work with mosquitoes, wear a white laboratory overall with elastic cuffs, gloves and a mosquito net that covers the head. A microbiological safety cabinet will be used where appropriate.

Foreseeable effect/risk assessment for the environment:

SFV: This virus is not endemic in the UK. It is not on the SAPO list. It is not clear whether suitable vertebrate hosts and insect vectors are present to establish an infection in this country. SFV can infect vertebrates (such as rodents) and certain biting insects. It is unclear whether suitable hosts are present. Transmission to vertebrates is by inoculation and to insect is by feeding on blood of an infected vertebrate. SFV is naturally transmitted by mosquitoes such as Aedes aegypti and Aedes africanus. Given the absence of these specific mosquitoes in Scotland and low pathogenicity of strains, the chance of infected laboratory mosquitoes (unlikely to survive for any extended length of time in the local environment) initiating a transmission cycle sustained by local insects is negligible. The modified viruses or replicons or replicons of other arthropod-borne alphaviruses will not have any inserted foreign genes other than non-harmful genes or sequences, or disruptive changes in non-structural genes or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. In the absence of a suitable host or vector these viruses would not survive in the environment and the risk is effectively zero.

LGTV: This virus is not endemic to the UK. It is not on the SAPO list. LGTV is naturally transmitted by ticks; it was originally isolated from Ixodes granulatus ticks in SE Asia. The competence of UK ticks for this virus is unknown. A related virus, Louping-III virus, is found in ticks throughout the British Isles. Given the location of our laboratories, tick populations are likely to be very low in the immediate vicinity. Ticks generally acquire infection from a blood meal on vertebrates and as ticks are excluded from our laboratories, and viruses stored in closed containers the chance of accidental tick infection is negligible. Modified viruses or replicons or replicons of other arthropod-borne flaviruses do not contain anything other than non-harmful genes or sequences, or disruptive changes in non-structural genes or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. The likelihood of virus released from our laboratory infecting a local tick is effectively zero (see disinfection and waste disposal procedures).

Hosts:

Prokaryotic organisms: Disabled, commercially available E. coli strains. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. Acquiring antibiotic resistance (from the plasmid vector) or additional sequences (virus or marker gene sequences) will not give these strains any survival advantage in the environment. The risk to the environment is therefore effectively zero.

Eukaryotic cells: Cell lines (and tick organ cultures) to be used are not viable outside the laboratory and thus pose no threat to the environment. Addition of plasmid vectors or virus will confer no growth or survival advantage in the environment.

Mosquitoes: Arthropods will be contained as described above under ‘risk to human health’ (and as detailed below, section 8). If arthropods carrying infectious material were to escape from our facilities they would have no survival advantage in the environment but could transmit the infection by bite. Whether this would initiate a natural sustainable cycle of SFV infection in Scotland is unlikely given the generally low mosquito activity. Given that escaped uninfected arthropods and natural arthropods will be
rare and that virus samples are in sealed containers and generally used in microbiological safety cabinets, the chances of arthropods becoming infected in the laboratory outside containment are virtually zero.

In summary

i) consequence/severity of effects: Low

ii) likelihood of effects being realised: Negligible

iii) overall risk: Effectively zero

Final classification: GM bacteria transfected with plasmids containing viral or other eukaryotic sequences (unable to cause disease, minimal hazard to health and environment) - Containment Level 1. Eukaryotic cells (including tick organ cultures) containing viral sequences unable to form complete infectious virus – Containment Level 1. Eukaryotic cells (including tick organ cultures) containing full length SFV or LGTV sequences (capable of forming infectious virus, or capable undergoing recombination or mutation to form infectious virus) – Containment Level 2. Arthropods containing viral sequences (full length, partial) – Arthropod Containment Level 2.

All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. For work with mosquitoes, wear a white laboratory overall with elastic cuffs, gloves and a mosquito net that covers the head. As a routine measure all work will be carried out Containment level 2. A microbiological safety cabinet will be used where appropriate.

References:

Infection of mosquitoes will take place in a dedicated insectary at arthropod containment level 2 (AcCL2). A standard operating procedure (SOP) has been added separately.

Whole mosquitoes will be injected or fed (membrane feeding or capillary tube) with liquid (including blood) containing virus derived material, infectious virus, or a mixture of these. Viruses, RNA and proteins will be isolated from crushed mosquitoes. Virus infected live mosquitoes may be monitored by microscopy. Arthropods will be studied inside closed containers. In addition wingless mosquitoes may be used (wings will be removed from cold immobilised mosquitoes inside containment – glove box; see below.

ArCL2 is suitable for arthropods infected with hazard group 2 pathogens (human or animal) or Class 2 GMOs eg Semliki Forest virus, Langat virus etc.

Containment:

• The ArCL2 room will be arthropod-proof. Entrance door into insectary separated from surrounding facilities by additional door.
• door to facility will have clear signage indicating ‘arthropod containment level 2, no unauthorized entry’;
• any ventilation inlets and outlets of room and incubators will be screened with gauze or similar of pore size small enough to trap the smallest arthropod life cycle stage;
• windows (if present) and doors will be sealed;
• sinks (if present) will have an adequate arthropod-proof trap or be sealed;
• measures will be taken to enable escapees to be easily detected and recaptured or destroyed (white/clear walls);
• solid and liquid waste will be autoclaved (some invertebrates are not killed by chemical disinfectants or fumigants);
• an insecticidal spray will be available for use in an emergency (ONLY) (use of insecticides may render the room unfit for invertebrates for a long period, if not permanently; non-residual type insecticides should be chosen; insecticides do not have an immediate “knock-down” effect on ticks, but could cause long-term damage);
• infected flying insects will be kept in primary and secondary containment (glove box, incubator);
• primary containers for mosquitoes will be either (i) solid material sealed with a tight-fitting (tape secured with an elastic band) net lid or, (ii) a sleeved netting cage;
• secondary containment for mosquitoes will be either: (i) ‘arthropod-proof’ incubator, transparent or with transparent inner door, (ii) large and robust transparent sealed plastic bag with pin-prick air holes, (iii) transparent glove box, (iv) transparent Perspex box sealed (tape or elastic band) with a net cover;
• experimental containers (both primary and secondary) will be labelled to indicate the date, workers initials, arthropod species, number of arthropods and the nature of any infectious agent; the number of arthropods in each standard primary container will be kept to a minimum and for flying arthropods will not exceed numbers that can be easily counted (ie. 20)
• numbers will be adjusted as arthropods are removed (or to record escapees); each arthropod will be accounted for as the work proceeds through to final fixation or disposal;
• all experiments will also be recorded in a log book (species, worker initial, date, number of arthropods, nature of infectious agent);
• flying or crawling arthropods will be handled on white trays to detect escapees;
• infected arthropods will be killed before they are taken out of the containment facility;
• operating protocols designed to minimise escape and bites will be adhered to.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM26 (CVR Church Street)

Solids from cell culture (eg plasticware such as flasks, tubes, pipettes tips etc.) - soak in 1% Virkon (w/v) for a minimum of 12 hours. Dispose of as normal solid waste (black bag).

Other solids (agar plates, gloves etc.) placed in a metal disposal box (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 121oC for 30 minutes.

Arthropod-derived material such as the remains of mosquitoes or tick organ cultures will be placed in a metal disposal box (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 121oC for 30 minutes.

Liquids (eg samples, culture supernatants, tissue culture media) – add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Liquids (E. coli culture medium)- add Virkon to final concentration of 1% (w/v) for a minimum of 12 h, then discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave by using a make safe cycle of 121oC for minutes.

Degree of kill:
Chemical Sterilization by Virkon- effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).

GM223 (CVR Garscube Estate):

Solids (pipettes tips, gloves etc.)- place in container (lined with sealed bag) and dispose of by autoclaving using a make safe cycle of 121-123oC for 30 minutes.

Arthropod-derived material such as the remains of mosquitoes will be placed in a sealed container (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 121-123oC for 30 minutes.

Liquids (eg. samples) – dispose of by autoclaving using a make safe cycle of either 121-123oC for 30 minutes.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation).

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If yes, tick to confirm that it is attached to this form

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<tr>
<th><strong>Tick to confirm that you have attached a risk assessment to this form</strong></th>
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</table>

Tick if you are claiming exemption from disclosure for section of the risk assessment

| **Tick if you are claiming exemption from disclosure for section of the risk assessment** | N |

**Please enter comments on the GM safety committee on the risk assessment**
1. GM 26 committee comments (approval date 25/08/11; see below):

The CVR Church Street GM subcommittee met on 22/08/11 to consider the proposal:
"Arthropod host cell/arbovirus interactions and immune responses induced by virus replication."

Subcommittee members:
R E
J M
A P
F R

The subcommittee members agreed recommendations for modifications to the following topics:

- In Section 12 (Describe the waste management measures etc) of the HSE Notification and Section 16 (Proposed experiments etc, sub-heading Waste Disposal) of the Risk Assessment:
  - Some of the autoclaving cycles specified were not available on the autoclave at the CVR Church Street site.
  - Procedures appropriate for clinical waste, incineration and microwaving were either not in use at the CVR Church Street site or would be expensive to implement. They were considered to be unnecessary for this proposal.
  - The procedure specified placing arthropod derived material into a sealed container for autoclaving. However, autoclaving requires containers be open to allow entry of steam.
  - No procedures for disinfection of contaminated material were described. All material is to be rendered safe by autoclaving, which would entail contaminated material being stored and transported to the autoclaves. This was considered an unacceptable risk, especially for liquid waste, and requires an effective disinfection procedure to be put in place.
  - The subcommittee decided that the relevant entries under section 12 in the HSE Notification and section 16 of the Risk Assessment should be replaced with the existing waste disposal and disinfection policies employed at the CVR Church Street site. These are available on the local intranet. Before this can be approved, validation must be obtained regarding the effectiveness of Virkon for inactivating LGTV and SFV.

- In Section 6 (Purpose of the contained use), reference is made to subgenomic replicons of other arboviruses (eg chikungunya and dengue) that are not otherwise included in this proposal. A full description of the work to be carried out with these agents, the nature of the hazards and the precautions required should be included in both the HSE Notification and Risk Assessment.

Clarification is required for section 8 (Containment and control measures for GMOs that are not micro-organisms; page 11) of the HSE Notification and for section 11, sub-heading Hosts (page 8) of the Risk Assessment concerning the transport of arthropods. The latter states that; "Samples (dead or non-mobile arthropods (our italics) or material from these) will be transported to our CL2 laboratory according to standard regulations." The subcommittee agreed that no living infected arthropods would be allowed in the CVR Church Street site and that they should only be handled in an insectary.
The reference to; “the city centre location of our laboratories” on page 10 of the Risk Assessment is not appropriate for the CVR Garscude site and should be removed.

Under section 7, sub-heading Control measures (page 8) of the HSE Notification, the use of appropriate PPE (not just gloves) should be specified.

An estimate of virus quantities (eg pfu/ml) should be provided in addition to the culture volumes in the HSE Notification, section 9 (Maximum culture volumes per experiment; page 11) and the RA section 16 (Culture volumes; page 14).

The requested changes were made and on 25/08/11 the revised documents were circulated to and approved by the subcommittee members.

2. GM223 committee comments (approval date 20.10.2011, see below):

"The risk assessment was clear but clarification on local mosquito activity was required; more detail on any full length viruses being held, and careful consideration of any possible recombination events was deemed essential. Risks to immune-suppressed personnel needed to be mentioned.

These points have been addressed in form CU2 and Risk Assessment, and on the 20.10.2011 the revised documents were approved.

Dr. G on behalf of the committee."

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Project Containment

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<th>Growth Rooms</th>
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Project Ref 26/12.1

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<td>Bunyavirus molecular biology and virus/host interactions</td>
<td>Class 2</td>
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02/03/2022
The Bunyaviridae is one of the largest taxonomic arbovirus groups (over 300 named isolates). Viruses within the Bunyaviridae are classified into five genera: Orthobunyavirus, Hantavirus, Nairovirus, Phlebovirus and Tospovirus following molecular organization and serotyping [1]. Within a genus, viruses show similar patterns in the sizes of their genome segments and structural proteins, and whether or not non structural proteins are encoded. Bunyaviruses are important examples of emerging viruses, including pathogens of humans and/or animals [2,3]. The bunyavirus genome is a tri-segmented single-stranded RNA genome of negative (or ambisense) polarity that encodes four structural proteins. The genome segments are called L (large), M (medium) and S (small). The L RNA encodes the L protein, the M RNA segment Gn and Gc, and the S RNA the N protein. In addition, some viruses encode non-structural proteins called NSm (M segment) and NSs (S segment). The segments are encapsidated by the nucleocapsid (N) protein and interact with the viral RNA dependent RNA polymerase, the L protein, to form ribonucleoprotein complexes (RNP) known as nucleocapsids. During replication, a positive-stranded antigenome is produced which serves as the template for de novo production of negative-stranded genomes. The polymerase L also transcribes shorter mRNAs which direct synthesis of bunyavirus proteins; interestingly, caps are “snatched” from cellular mRNAs. The bunyavirus terminal 3’ and 5’ sequences are largely complementary and interact to give the genome a characteristic pan-handle structure; these terminal sequences (and other sequence elements adjacent to the protein-coding regions) contain elements regulating transcription and replication of the bunyavirus genome. The RNPs are contained within a lipid envelope into which the two viral glycoproteins Gn and Gc are inserted. Replication occurs in the cytoplasm of infected cells, and viruses mature by budding at Golgi membranes (reviewed in [4]).

Most bunyaviruses are transmitted by arthropod vectors such as mosquitoes or ticks. Infection of arthropod cells is usually not cytopathic while vertebrate cells die following infection. Understanding the molecular basis for the different outcomes of infection in these cells of different origin is of fundamental importance, and may help in the development of new control measures for arboviral diseases. This is likely to imply immune responses (which differ in both hosts) and virus host interactions [5,6]. Research on antiviral immune responses in arthropod vectors has focused on positive-strand RNA viruses. Arthropod vectors activate immune responses upon infection, i.e. immune signalling pathways, humoral responses and most crucially RNA interference (RNAi) responses. Viral replication induces production of double stranded RNA, the RNAi-inducing substrate cleaved by dicer-type nuclease activities to produce virus-induced small interfering RNAs (also called viRNAs in the context of viral infection) which are integrated into the RNA-induced silencing complex (RISC). The RISC mediates nucleolytic cleavage of complementary viral RNAs. In addition, arthropod cells might differentially regulate subsets of their own small RNA repertoire following infection by arboviruses. In vertebrates, it is clear the interferon response is a key factor in limiting bunyavirus replication and this process, as well as bunyavirus antagonism of the interferon (as well as other host antiviral responses such as PKR) is an area which has seen important progress although important questions remain [6].

This project will investigate bunyavirus molecular biology and virus/host interactions, including structure-function analyses of viral proteins and the role of signals within viral RNAs, the interactions of bunyaviruses with host cell components, as well as the role of immune responses in controlling bunyavirus replication.
Two orthobunyaviruses will be at the core of this project. Bunyamwera virus (BUNV) is the prototype virus of the family [7]. Originally isolated in Uganda from infected mosquitoes, most of our understanding of bunyavirus biology is due to studies on this model bunyavirus. We will also study Schmallenberg virus (SBV), which was recently isolated from cattle in Germany and leads to disease (fever, diarrhoea, malformations, abortion) in cattle and sheep [8,9]. Non-hazardous minigenome systems (see below) of bunyaviruses of higher containment levels such as Rift Valley fever virus (RVFV) [10] may also be studied.

A reverse genetics systems that allows manipulation of the BUNV genome is available [11,12]. Cultured cells constitutively expressing bacteriophage T7 RNA polymerase are transfected with a mixture of 3 plasmids, each containing a full-length cDNA copy of one of the bunyavirus genome segments under control of T7 promoter and terminator sequences, and a hepatitis delta ribozyme sequence. Intracellular transcription produces exact copy RNA transcripts that act as viral mRNAs and templates for viral RNA synthesis that result in production of infectious virus. The system was initially established with BUNV and was subsequently adopted by other bunyaviruses such as the orthobunyavirus LaCrosse [13,14]. A reverse genetics system allows us to introduce mutations into the orthobunyavirus genome, as well as to introduce new sequences (such as reporter genes) or change or delete sequences [15,16,17]. Similarly, minigenome systems can be constructed in which only the coding sequences between the terminal orthobunyavirus sequences (either genomic or antigenomic) are replaced with a reporter gene such as Renilla luciferase (RLuc) or eGFP. A minigenome will be transcribed if N and L proteins are co-expressed from expression plasmids, which is useful to study the role of sequences and proteins in replication and in packaging as minigenome RNPs can be introduced into virions following superinfection with BUNV for example [18,19] and potentially also virus replicon particles (VRPs) by co-expression of glycoproteins. Minigenomes are on their own non-propagating and are used to mimik virus replication, and thus are a safe alternative to study for example replication of high-containment bunyaviruses.

In this project, we aim to work on the following topics as listed below. Eukaryotic host systems will be cells of vertebrate or invertebrate origin.

1) Development and use of reverse genetics systems for BUNV and SBV. Such a system is already in use for BUNV, and the SBV system will be designed along the same principles as the BUNV system. Similar systems for other bunyaviruses of the same hazard group may be considered for comparative studies.

Reverse genetics systems will be used to:
- Introduce mutations into the bunyavirus sequences. These can target coding or non-coding sequences although amino acid sequences of the glycoproteins will not be changed, to avoid alterations of host cell tropism.
- Introduce deletions and insertions into bunyavirus sequences, targeting coding or non-coding sequences. Deletions can for example target parts of non-coding regions, insertions can be reporter genes, bunyavirus genes may be completely replaced with other sequences.
- Exchange sequences between bunyavirus segments and within segments. Sequences from within bunyavirus segments will be transferred to other segments or transferred to other regions of the same segment.

Recombinant and wild type bunyaviruses will be used to:
- Study viral growth, gene expression, replication and packaging mechanisms in host cells.
- Study interactions with the host cell (replication, interaction with host cell factors, immunity).

2) Development and use of bunyavirus minigenome systems
- A BUNV minigenome is already in use and similar system will be developed for SBV. Minigenome systems will be used (as read-outs) in studies on protein functions (cellular, viral), sequence function, segment packaging (packaging by glycoproteins co-expression, or following co-infection) and complementation studies (ie. BUNV N in SBV minigenome).
- Minigenome systems of others bunyaviruses will be developed or obtained for studies on replication and packaging (Rift Valley fever virus etc).

3) Functional studies and expression of bunyavirus proteins.
- Manipulation of protein sequences by introduction of point mutations, deletions, insertions, fusion to other sequences such as reporter genes, tags etc.
- Expression of bunyavirus protein to study interactions with host cell components and cellular localisation.
- Expression of bunyavirus proteins by prokaryotic expression systems, for example for protein purification.
- Expression of bunyavirus proteins by eukaryotic expression systems (plasmid, retroviral expression), for example to produce packaging cell lines, cell lines expressing virus-like particles, or cell lines to study protein function.
- Expression of bunyavirus proteins by eukaryotic expression systems (plasmid, retroviral expression), to produce packaging cell lines for bunyavirus sequences (segments or minigenomes; production of single round infectious particles) and cell lines expressing virus-like particles.
- Silencing of bunyavirus gene expression or host genes involved in bunyavirus replication by RNA interference.

4) Studies on host immunity.
- Interactions of bunyaviruses (wild type, recombinant, minigenome) with vertebrate host responses, and counteraction of host responses (for example by NSs proteins) will be investigated. Immune signalling/antiviral responses (including activation mechanisms), and viral interference therewith, will be analysed. This will involve studies of extra- and intra-cellular host molecules. Role of immunity in host cell tropism (infections of cells from different species) will also be investigated. These studies will also use reporter genes inducible upon activation of a given pathway. Vertebrate-origin cell culture models are available.
- Interactions of bunyaviruses (wild type, recombinant, minigenome) with arthropod host responses, and counteraction of host responses will be analysed. Studies of the RNA interference response (host proteins regulating this mechanisms, identification and role of cellular and viral small RNAs, role of bunyavirus sequences in countering host responses) as well the role of immune signalling pathways and antiviral responses (including activation mechanisms) will be investigated. This will involve extra- and intra-cellular pathways (for example extracellular melanisation pathways). These studies will also use reporter genes inducible upon activation of a given pathway. Arthropod-origin cell culture models are available.
- The roles of bunyavirus RNAs in activating or inhibiting vertebrate or arthropod immune responses will be investigated (expressed in viral systems such as Semliki Forest virus or plasmid-based expression).

References:
Viruses and derived replicons to be used will be of the Bunyaviridae family:
- Schmallenberg virus (SBV) (Orthobunyavirus).
- Bunyamwera virus (BUNV) (Orthobunyavirus).
- Recombinant viruses derived from BUNV and SBV.
- Minigenome systems derived from BUNV, SBV, and other bunyaviruses (Rift Valley fever virus etc.).

Other containment level 2 bunyaviruses (Guaroa virus, Maguari virus, Toscana and Sandfly fever phleboviruses etc.) may be considered as required.

No infectious containment level 3 or 4 viruses will be used.

Host/vector system

All organisms will be used at containment level 2, which is routine practice.

Plasmid vectors:
Viral sequences (full length clones of BUNV or SBV; partial sequences of these and other bunyaviruses) and other genes (cellular, viral, reporter) are available in plasmid-encoded cDNA form. RNAs can be transcribed from bacterial promoters (ie. T7 or SP6 by in vitro transcription or plasmid-expressed RNA polymerase in cells) or eukaryotic promoters (CMV, SV40, cellular promoter, baculovirus promoters etc.). Plasmids for expression of bunyavirus sequences (virus rescue or minigenome) contain a T7 RNA polymerase promoter, T7 terminator and a hepatitis delta-derived ribozyme sequence. Plasmids contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin) and sometimes a selectable eukaryotic drug resistance marker such as neomycin or puromycin resistance. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

Lentiviral expression vectors: only at GM Centre 26; use is covered in CVR GM form 41 and amendments.

Hosts:

Prokaryotic hosts:
Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste should prevent release of viable organisms. The risk to the environment is therefore effectively zero.
Eukaryotic hosts:
Vertebrate cell lines of various origins (typically human, mouse, hamster, bovine etc.) and invertebrate cell lines of various origins (mosquito, culicoides, tick etc.). The
vertebrate BSR-T7/5 cell line (derived from BSR, a clone of BHK) (Buchholz et al., 1999) expresses T7 RNA polymerase and will be used for minigenome assays and virus
rescue, as may similar cell line expressing T7 RNA polymerase.

In prokaryotic cells, only selectable (eg antibiotic resistance) genes will be translated; prokaryotic cells may also be used for protein expression and subsequent purification.

Virus sequences will be under the control of a promoter that will only generate transcripts in eukaryotic cells or in vitro transcription reactions.

In eukaryotic cells, the following RNAs will be produced which may be translated into the following gene products:

- Bunyavirus proteins and RNAs (BUNV and SBV, or minigenomes): Proteins involved in replication and transcription of viral genetic material and virus structural
proteins. Some of these virus proteins will interact with host-cell components and may affect host cell responses to infection; few of these interactions are currently known.
If all virus sequences are translated new virions may be generated; minigenomes are non-propagative viral RNAs (capable of replication). Partial RNAs do not give rise to
propagating infectious material but may display biological activities (ie. RNAi inhibitory).

- Non-bunyavirus genes or sequences: Genes of prokaryotic (bacteriophage polymerases) or eukaryotic (eg. reporter genes such as luciferases or fluorescent proteins
such as GFP; inhibitors of arthropod innate immunity, vertebrate or arthropod host genes etc.) origin. Reporter gene sequences will also be present in bunyavirus
minigenome RNA. The recombinant foreign proteins to be expressed provide no significant increase in the hazard to human health; none are toxic or likely to produce
disease in the quantities that could be produced by accidental exposure to these systems. Non translated RNA sequences (including T7 terminator and hepatitis delta
ribozyme in plasmids used for virus rescue or minigenomes) have no known toxic effects and pose no risk to human health.

Techniques used to introduce insert or vectors into cells:

Prokaryotic cells: Introduction of DNA into E. coli will be by heat shock/chemical transformation. These techniques have been extensively described and are widely used;
they rely on getting DNA very close to the bacterial membrane and introducing the genetic material through pores or membrane fusion.
Eukaryotic cells: Cells will be transfected using transfection reagents such a lipofectamine or by electroporation. Virus genomes or sequences will be introduced into
cultured cells by infection with virus or virus replicon particles (VRP). Lentiviruses will be packaged and used to infect target cells.

Note- Nature of experiments are to be carried out at Church Street and Garscube Estate:

GM 26 (CVR Church Street):
- Preparation, characterisation and genetic manipulation of bunyaviruses and bunyavirus minigenomes.
- Protein expression and purification (prokaryotic cells), protein expression and purification (eukaryotic cells including lentiviral expression), protein interaction and
modification studies.
- Infection/transfection of cell lines, preparation of lysates for downstream applications (including sequencing).
- Reporter gene assays, immunofluorescence studies.

GM223 (CVR Garscube Estate):
- As above; exception: separate GM notification for lentiviral/retroviral expression required.
Foreseeable effects/risk assessment for human health and safety (also see Risk Assessments):

Plasmid vectors:
Viral sequences (including full length clones or partial sequences of SBV or BUNV or other bunyaviruses) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Transcription of even complete viral cDNA (SBV or BUNV) will result in no infectious RNA since the promoters are not active in prokaryotic hosts. Systems for prokaryotic gene expression are under control of an inducible prokaryotic promoter. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV or SV40), eukaryotic (eg actin promoter, pathway inducible promoter such as STAT, interferon etc.) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human cells. None of the genes used in these studies are oncogenes. The hazard of expressing ‘foreign’ genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the foreign gene product and is discussed in more detail below.

BUNV:
Infection with this virus can cause a febrile illness with headache, arthralgias, rash and infrequent central nervous system involvement (Gonzalez and Georges, 1988). The classification into ACDP hazard group 2 indicates current expert opinion that containment level 2 precautions are considered adequate for controlling the risks associated with working with BUNV. The viral glycoprotein amino acid sequences are the major determinants of host range and cell tropism and will not be changed beyond the addition of marker genes as fusion or cleavable markers into the structural open reading frame. Genetic changes that will be introduced into the virus sequences will be targeted at disrupting or changing specific functions of virus RNA or virus proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication.

SBV:
This an animal pathogen, and not likely to be a risk to human health by current guidelines (http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=795). Genetic changes that will be introduced into the virus sequences will be targeted at disrupting or changing specific functions of virus RNA or virus proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virus virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication. As for BUNV, the viral glycoprotein amino acid sequences which are the major determinants of host range and cell tropism will not be changed beyond the addition of marker genes as fusion or cleavable markers. SBV is not listed on the ACDP or SAPO list but is recommended to be classified as hazard group animal pathogen 2 (see attached recommendation). We propose to treat SBV like BUNV in terms of risk for human infections and health unless other regulation is in place for a particular pathogen. In addition, personnel working with SBV should not be in contact with susceptible animals or visit farms/zoos where they could potentially come into contact with susceptible species for a period of 48 hours. A form outlining that they have understood their responsibility with regards to minimising the risks to the environment associated with this virus will be signed by personnel working on SBV.

Personnel working with these viruses are expected to inform occupational health should they become pregnant or immunosuppressed, and if planning pregnancy inform the PI so that precautionary measures can be introduced following advice from occupational health.

Bunyavirus minigenomes:
Minigenomes pose no risk to human health, including those derived from pathogenic bunyaviruses such as Rift Valley fever virus. They cannot propagate on their own, replication requires co-expression of L and N proteins. Even if packaged by co-expression of glycoproteins and thus used to infect cells, their own transcriptional activity would be minimal. If packaged into virions, the associated risk is that of the virus itself as reporter genes in minigenomes are not reported to be toxic.

Note 1: Should material such as minigenomes or other sequences be obtained for bunyaviruses listed under schedule 5 regulations, no additional sequences that could potentially be used to assemble the full length genome of a schedule 5 pathogen will be stored in locations that do not have required licenses.
Note 2: No reassortment experiments between BUNV, SBV or closely related orthobunyaviruses will be carried out as class 2 activity, to eliminate the possibility of creating viruses with enhanced pathogenicity such as Ngari (Gerrard et al., 2004). No work with two or more viruses of different bunyavirus species will be carried out in parallel in the same microbiological safety cabinet. Microbiological safety cabinets are decontaminated between experiments (UV and/or alcohol). The risk of accidental co-infection is effectively zero.

The host/recipient organism:
Prokaryotic organisms: All strains will be disabled, commercially available E. coli derivatives classified as ‘especially disabled hosts’ by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic cells:
Cell lines to be used would not survive inside the human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA or infection will confer no growth or survival advantage in or outside the laboratory to cell lines of vertebrate or arthropod origin.

The inserted/donated genetic material:
Viral RNA and proteins, cellular proteins: Most such bunyavirus-expressed viral (such as inhibitors of insect immunity; where tested in vivo this has led to more rapid death of arthropod vectors) or cellular proteins are unlikely to have harmful effects in eukaryotic cells, however some could perturb normal cellular metabolism, predispose or protect against cell death or render cells more or less susceptible to other infections if overexpressed or silenced. It is very unlikely that BUNV or SBV (and hazard group 2 bunyaviruses) RNA or proteins would have any harmful toxin-like effect outside cells. Only antigenomic RNA containing a bunyavirus sequence can be translated or give rise to infectious virus (if all necessary genomic information is provided) in eukaryotic cells; minigenome RNAs are non propagative and pose no harm (individual cells replicating minigenome are likely to die or be eliminated by the immune system). Viral non-coding RNA sequences are important in bunyavirus replication but do not encode biologically active molecules that are likely to induce physiological effects in humans on their own.

No proteins that are known to interfere with immune or host responses in vertebrates will be cloned into bunyavirus genomes for the purpose of virus rescue under containment level 2 conditions, to avoid producing potentially highly pathogenic bunyaviruses by adding a more potent host response antagonist that those already present in bunyavirus genomes.

Reporters: Reporter genes of prokaryotic or eukaryotic origin (eg luciferase, fluorescent proteins etc.). No harmful properties have been attributed to these proteins. There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Photinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function. Introduction of these reporter genes is unlikely to increase the risk associated with these viruses.

Other non-coding RNA sequences (siRNAs, dsRNAs, other RNAs): Any effects these elements have on overall gene expression are likely to be minimal. They may affect gene function or virus replication in individual cells but are unlikely to lead to whole body physiological effects as they do not code for the production of secreted bioactive molecules.

Other sequence changes in viral sequences:
Mutation or deletion will target disruption of non-structural protein functions or non-coding viral sequences and are likely to have no or deleterious effects on viral replication, thus not increasing risk of hazards to human health associated by viruses genetically modified in such a way. No changes will be introduced that will change the amino acid sequence of the glycoproteins Gn and Gc (with the exception of in-frame fusion to reporter genes) so viral tropism is not affected.
Summary:
Genetic changes that will be introduced into the BUNV or SBV sequences as described above can be expected to attenuate virus fitness (with the exception of insect immunity inhibitors, but this does not concern human health); such changes are highly unlikely to increase virus virulence as they modify virus sequences (which are optimized for replication), or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication or have no effects outside the infected cell. Therefore we do not expect increased risk or hazards associated with viruses modified in such a way.

Control measures – assign provisional containment level:
GM bacteria transformed with plasmids containing viral sequences, reporter genes, other eukaryotic/prokaryotic genes or non-coding sequences  - Containment Level 1. Eukaryotic cells containing bunyaviral sequences unable to form complete infectious virus, or plasmids expressing reporter genes, other eukaryotic/prokaryotic genes or non-coding sequences – Containment Level 1. Eukaryotic cells containing hazard group 2 bunyavirus sequences (capable of forming infectious virus, or capable of undergoing recombination or minor mutations to form infectious virus) – Containment Level 2.

All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. A microbiological safety cabinet will be used where appropriate (see below). Work with infectious bunyaviruses should take place in a microbiological safety cabinet. Spray gloves with alcohol when working outside the hood or before disposing.

Foreseeable effect/risk assessment for the environment:
BUNV: This virus is not endemic in the UK, and it is not on the SAPO list. It is not clear whether suitable vertebrate hosts and insect vectors are present to establish an infection in this country. BUNV can infect vertebrates (such as rodents) and certain biting insects. Transmission to vertebrates is by inoculation and to insect is by feeding on blood of an infected vertebrate. BUNV was isolated from mosquitoes in Uganda. Given the absence of these specific mosquitoes in Scotland and low pathogenicity of virus strains, the chance of accidentally infected arthropods initiating a transmission cycle sustained by local insects is negligible. The modified viruses will not have any inserted foreign genes other than non-harmful genes or sequences (with exception of inserted insect immunity inhibitors, which may lead to rapid death of infected arthropods), or (usually disruptive) changes in viral genes (with the exception of glycoproteins, whose amino acid sequences will not be changed) or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. In the absence of a suitable host or vector these viruses would not survive in the environment and the risk is effectively zero. Arthropods are kept out of labs and the risk of accidental infection of insects by ingestion of contaminated material is also effectively zero for BUNV.

SBV: This virus has now been detected in the UK, and infections are most likely the result of midges carried from mainland Europe to the UK (Veterinary Record, News & Reports- see reference below). However other blood-feeding arthropods cannot be ruled out and vector competence studies are required. It is not yet on SAPO or ACDP lists and the recommendation (including for infection of insects) from Scottish Government (Rural and Environmental directorate) is to handle this pathogen as a category 2 animal pathogen (see attached recommendation). We propose, until further notification, to work with SBV at containment level 2 similarly to BUNV. The modified viruses do not contain anything other than non-harmful genes or sequences (with the exception of inserted insect immunity inhibitors, which may lead to rapid death of infected arthropods), or disruptive changes in genes (with the exception of glycoproteins, whose amino acid sequences will not be changed) or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. If SBV was to escape the lab and infect local midges (or possibly other arthropods), there is a significant risk it could initiate a transmission cycle although this virus might arrive here by natural means (competence of local midges for SBV is not known but there is a significant risk they may be able to act as vectors). However arthropods are kept out of labs and the risk of infection of arthropods by ingestion of contaminated material is low and the likelihood of virus released from our laboratory infecting local vectors is effectively zero (see disinfection and waste disposal procedures). In addition, advice will be given to lab personnel that they should not be in contact with susceptible animals or visit farms/zoo where they could potentially come into contact with susceptible species for a period of 48 hours following work with SBV. A form outlining that they have understood their responsibility with regards to minimising the risks to the environment associated with this virus will be signed by personnel working on SBV.
Note: No proteins which could interfere with immune or host responses in vertebrates will be cloned into bunyavirus genomes for the purpose of virus rescue under containment level 2 conditions, to avoid producing potentially highly pathogenic bunyaviruses by adding a more potent host response antagonist that those already present in bunyavirus genomes.

We propose to use containment group 2 bunyaviruses (see point 7) similar to BUNV unless other regulations are in place for a particular pathogen.

Minigenomes: These systems derived from BUNV, SBV or other bunyaviruses are non-propagative and even if packaged, pose no or no additional hazard.

Hosts:

Prokaryotic organisms: Disabled, commercially available E. coli strains. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. Acquiring antibiotic resistance (from the plasmid vector) or additional sequences (virus or marker gene sequences) will not give these strains any survival advantage in the environment. The risk to the environment is therefore effectively zero.

Eukaryotic cells: Cell lines to be used are not viable outside the laboratory and thus pose no threat to the environment. Addition of plasmid vectors or virus or minigenomes will confer no growth or survival advantage in the environment.

In summary

i) consequence/severity of effects: Severe

ii) likelihood of effects being realised: Low

iii) overall risk: Low

Final classification: GM bacteria transformed with plasmids containing viral sequences, reporter genes, other eukaryotic/prokaryotic genes or non-coding sequences (unable to cause disease, minimal hazard to health and environment) - Containment Level 1.

Eukaryotic cells containing bunyaviral sequences unable to form complete infectious virus, or plasmids expressing reporter genes, other eukaryotic/prokaryotic genes or non-coding sequences – Containment Level 1.

Eukaryotic cells containing bunyavirus sequences capable of forming infectious virus, or capable undergoing recombination or mutation to form infectious virus – Containment Level 2.

All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. As a routine measure all work will be carried out at Containment level 2. A microbiological safety cabinet will be used where appropriate. Work with infectious bunyaviruses should take place in a microbiological safety cabinet. This additional layer of security also is to prevent accidental contamination of any arthropods (thus reducing risk to the environment). Spray gloves with alcohol when working outside the hood or before disposing. Traps to catch arthropods will be present in labs were work with live infectious bunyaviruses is carried out.

References:
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfect bunyavirus-contaminated material immediately.

GM26 (CVR Church Street)

Solids from cell culture (e.g. plasticware such as flasks, tubes, pipette tips etc.) - soak in 1% Virkon (w/v) for a minimum of 12 hours. Dispose of as normal solid waste (black bag).

Other solids (agar plates, gloves etc) - placed in a metal disposal box (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 121°C for 30 minutes. Gloves used for bunyavirus work should be disinfected with alcohol before putting in containers.

Liquids (e.g. samples, culture supernatants, tissue culture media) – add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Liquids (E. coli culture medium) - add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - autoclave by using a make safe cycle of 121°C for 30 minutes.

Degree of kill:
Chemical Sterilization by Virkon - effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).

GM223 (CVR Garscube Estate):

Disposable solids (e.g. plasticware such as flasks, tubes, pipette tips etc.) - soak in 1% Virkon (w/v) for a minimum of 12 hours. Transfer solid contents to clear autoclave bags and autoclave at 121°C for a minimum of 20 minutes prior to final disposal by district council to landfill, remaining liquid to be discharged to drain.

Other solids (agar plates, gloves etc.) - placed in a marked box lined with a clear autoclave bag, and disposed of by autoclaving using a make safe cycle of 121°C for at least 20 minutes. Seal contaminated plates (with for example bacterial GMOs) before placing in bag, to avoid lid falling off. Gloves used for bunyavirus work should be disinfected with alcohol before putting in containers.

Liquids (e.g. samples, bacterial culture media, tissue culture media) - add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - autoclave by using a make safe cycle of 121°C for at least 20 minutes, then incinerated.

Degree of kill:
Chemical Sterilization by Virkon - effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).
1. GM 26 committee comments:

The CVR Church Street GM subcommittee met on 22/08/11 to consider the proposal: "Bunyavirus molecular biology and virus/host interactions."

Present:
*********** personal details removed

The following topics were discussed:

The committee members had no concerns regarding the overall aims of the proposed experiments. Overall the work with Bunyamwera virus (BUNV) was approved for handling under normal class 2 conditions. Questions were raised over some aspects of the work with Schmallenberg virus (SBV). Additional questions on risk to the environment and containment were also discussed.

This points have been addressed in form CU2 and the Risk assessment, and on the 15.03.2012 the documents were approved.

On behalf of the CVR Church Street subcommittee, Dr. P

2. GM223 committee comments:

"The risk assessment was clear and comprehensive but a number of clarifications in particular on risk to the environment by SBV (vectors, accidental contamination of local arthropods) were required.

These points have been addressed in form CU2 and Risk Assessment, and on the 15.03.2012 the revised documents were approved.

Dr. G on behalf of the committee."

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
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</table>
Establishment of a reverse genetics system for drosophila C virus

Drosophila C virus (DCV) belongs to the genus Cripavirus now classed within the Dicistroviridae family (previously Picornaviridae) within the order Picornavirales. It is a single stranded, positive-stranded RNA virus with a genome length of approximately 9300 bases. Unlike picornaviruses, the genome encodes two open reading frames (ORF) which encode (towards the 5’ end) an open ORF coding for non-structural proteins and (towards the 3’ end) an ORF coding structural proteins. The non-structural ORF encodes proteins involved in virus replication (including the RNA interference inhibitor 1A), while the structural ORF encodes viral envelope proteins. Both open reading frames are translated via separate internal ribosome entry sites in the genome. A Vpg protein is attached to the 5’ end the 3’ end contains a poly A sequence. These viruses are non-enveloped (1).

DCV has a worldwide distribution (including UK) and is also found in laboratory strains of Drosophila melanogaster and cultured cells (D. Obbard, University of Edinburgh, personal communication); it can also infect a variety of drosophila species (6). Infection leads to more rapid development, females are heavier and lay more eggs. Infection occurs by ingestion and infection is not pathogenic, although this may vary between flies. Interestingly, infection by injection leads to rapid death of flies (4). DCV is not a SAPO or ACDP pathogen; it does not infect vertebrate cells.

The genetic and molecular tools available for D. melanogaster make it a very useful model to study virus-host interactions and virus pathogenesis in an easily grown and genetically manipulated host. DCV has been particularly used in studies on antiviral immune signalling and RNA interference responses in drosophila (7); this research has led the insect virology field for many years. The DCV 1A protein acts as a viral suppressor of RNA interference (10), yet the virology of these virus/drosophila interactions is overall poorly understood in contrast to the extensive knowledge we have of drosophila genes and physiology. DCV is also inhibited by endosymbiotic Wolbachia bacteria in drosophila (9). This is similar other RNA viruses such as arboviruses in mosquitoes (which may be a useful control measure in public health strategies) yet it is not known...
how inhibition by the endosymbiont is mediated and powerful drosophila genetics may be required to solve this question and improve the uses of Wolbachia endosymbions (5).

Despite the strength of D. melanogaster genetics, a major drawback of drosophila immunity research is that no reverse genetics system is available to manipulate and study the genome of DCV. This project aims to develop an infectious DCV clone to apply reverse genetics technology to this virus. A dicistrovirus infectious clone has so far only been described for Rhopalosiphum padi dicistrovirus (RhPV) (2, 8). This clone is not very efficient and one of the major drawbacks is that the length of the 3’ poly A tail is not known for these viruses; it is likely to be similar to picornaviruses and of 20-60 nucleotides in length. However the RhPV infectious clone has no such sequence, bar a very short internal poly A stretch. This may be one of the reasons why the RhPV infectious clone is relatively weak, and possibly efforts to make a DCV infectious clone so far may have failed due to missing 3’ poly A tails.

The prototype DCV strain is isolate EB, but sequences of other isolates are available.

We propose to:
- Propagate the cloned DCV sequence. The DCV genome will be cloned into a plasmid backbone such as pUC-type or other vector. Transcription of the viral cDNA into RNA will be under control of a bacteriophage RNA polymerase promoter (T7, SP6 or similar) or eukaryotic promoter, and RNA introduced into insect cells or infectious clone cDNA transfected into insect cells for virus rescue. Where required, ribozyme-encoding sequences (hammerhead ribozyme, hepatitis delta ribozyme or similar) will be added to the genome ends (5’ and/or 3’ ends) to generate correct termini in the transcribed RNA as has been described previously (3).

- Manipulate the cloned DCV genome by molecular biological methods. This will be achieved by insertion of sequences (reporter genes such as luciferase or fluorescent proteins, drug resistance genes, other eukaryotic genes, non-coding sequences), deletion of sequences (point deletions, deletion of larger sequences) or point mutations within the DCV sequence. No changes will be made to sequences encoding the structural proteins, to avoid changes in viral tropism. Alterations to viral sequences such as those described above usually result in a fitness decrease. Modifications of the viral genome sequence will also include adding of poly A tails either by in vitro poly-adenylation or adding a cloned poly A sequence to the viral cDNA. Chimeras between strains of DCV (exchange of protein-coding sequences or non-coding sequences) will be created to study the role of strain-specific differences on replication, immunity and pathogenesis.

- If infectious DCV can be successfully rescued, we will delete the structural open reading frame 2 (or parts of it) to create non-propagating replicons and a packaging system (co-expression of DCV structural proteins in replicon-transfected cells) to obtain virus replicon particles.

- Individual DCV sequences (coding or non-coding) will be cloned into eukaryotic or prokaryotic vectors (plasmid or Semliki Forest virus or derived replicon) for molecular biological manipulation and/or expression purposes. Manipulated sequences may be re-introduced into the DCV infectious clone.

Studies to be undertaken with a DCV infectious clone and/or derived replicons:
- Immune responses of insect cells against DCV infection. We will study RNA interference and other mechanisms (immune signalling) that control DCV replication and viral interference therewith.
- Replication and polyprotein processing in insect cells. We will analyse sequences and mechanisms involved in these processes, as well as cellular factors involved in promoting or inhibiting DCV replication and spread.
- Virus entry, replication compartment formation and exit from insect cells. The tools for DCV manipulation (in particular viruses encoding reporter genes and replicons/virus replicon particles) developed in this project will allow to track these processes more efficiently (timing of events, determination of virus production etc.).
- Above studies will also be carried out in Wolbachia-infected insect cells to further study the mechanisms by which this micro-organism inhibits RNA virus replication.
- No experiments in vertebrate cells are planned.
Recipient or parental organism

Drosophila C virus. The infectious clone will be based on the sequence of strain EB; other strains or sequences from other strains may also be cloned.

Host/vector system

All organisms will be used at containment level 2, which is routine practice throughout the Church Street CVR site.

Plasmid vectors:
Viral sequences (including full length clones or partial sequences of DCV) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. RNAs can be transcribed through bacterial promoters (ie. T7 or SP6) or eukaryotic promoters. Plasmids contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin) and sometimes a selectable eukaryotic drug resistance marker such as neomycin or puromycin resistance. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

Hosts:

Prokaryotic hosts:
Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste should prevent release of viable organisms. The risk to the environment is therefore effectively zero.

Eukaryotic hosts: Invertebrate cell lines of various origins (drosophila, mosquito, tick etc.). These will be used to grow virus or express/silence individual proteins or sequences. Cells grown in standard media such as L-15 or Schneiders and are maintained in cooled cell culture incubators.

Origin & function

In prokaryotic cells, only selectable (eg antibiotic resistance) genes will be translated.

Virus sequences will be under the control of a promoter that will only generate transcripts in eukaryotic cells or in vitro transcription reactions.

In eukaryotic cells, RNA will be translated into the following gene products:

• Viral proteins and RNAs: Proteins involved in replication and transcription of viral genetic material and virus structural proteins. Some of these virus proteins will interact with host-cell components and may affect host cell responses to infection (for example 1A RNAi suppressor). If all virus sequences are translated new virions may be generated; replicons are non-propagative viral RNAs (capable of replication). Partial RNAs do not give rise to propagating infectious material but can display biological activities (ie. RNAi inhibitory, expression of some viral proteins for example for packaging of replicons).

• Non-viral genes: Genes of prokaryotic (eg CAT) or eukaryotic (eg. reporter genes such as luciferases or fluorescent proteins such as GFP; inhibitors of insect immunity ie. RNAi inhibitors, signalling inhibitors, melanization inhibitors etc.) origin. The recombinant foreign proteins to be expressed provide no significant increase in the hazard to human health; none are toxic or likely to produce disease in the quantities that could be produced by accidental exposure to these systems. Non-translated RNA sequences have no known toxic effects and pose no risk to human health.

Techniques used to introduce insert or vectors into cells:

Prokaryotic cells: Introduction of DNA into E. coli will be by heat shock/chemical transformation. These techniques have been extensively described and are widely used; they rely on getting DNA very close to the bacterial membrane and introducing the genetic material through pores or membrane fusion.

Eukaryotic cells: Cells will be transfected using reagents such a lipofectamine or by electroporation. Virus genomes will be introduced into cultured cells by infection with
virus or virus replicon particles (VRP), using transfection reagents or by electroporation.

**Evaluation of foreseeable effects**

Foreseeable effects/risk assessment for human health and safety (also see Risk Assessments):

Plasmid vectors:
Viral sequences (including full length clones or partial sequences of DCV) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Transcription of even complete viral cDNA will result in no infectious RNA since viral elements are not active in prokaryotic hosts. Systems for prokaryotic gene expression are under control of an inducible prokaryotic promoter. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV, baculovirus-derived IE promoter), eukaryotic (eg actin promoter, pathway inducible promoter such as STAT) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human cells. None of the genes used in these studies are oncogenes. The hazard of expressing ‘foreign’ genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the foreign gene product and is discussed in more detail below.

DCV:
This virus is not known to infect or replicate in human cells or other cells of vertebrate origin. It is not a ACDP pathogen. Work with this virus or recombinant DCV does not pose a risk to human health. Replicons are non-propagating even within virus-replicon particles and pose no risk.

The host/recipient organism:
Prokaryotic organisms: All strains will be disabled, commercially available E. coli derivatives classified as ‘especially disabled hosts’ by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic cells:
Insect cell lines to be used would not survive inside the human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA will confer no growth or survival advantage in or outside the laboratory to these cell lines.

The inserted/donated genetic material:
Viral RNA and proteins, cellular proteins: Most individually expressed viral or cellular proteins (for example arthropod immune response inhibitors) are unlikely to have harmful effects in eukaryotic cells, however some could perturb normal arthropod cellular metabolism, predispose or protect against cell death or render cells more or less susceptible to other infections. It is very unlikely that DCV RNA or proteins would have any harmful toxin-like effect outside cells. RNA containing the complete DCV sequence can give rise to infectious virus in insect cells; replicon RNAs are non-propagating and pose no risk. Viral non-coding RNA sequences are likely to have minimal effects outside arthropod cell systems, and do not encode biologically active molecules that are likely to induce physiological effects in humans.

Reporter genes: Reporter genes of prokaryotic or eukaryotic origin (eg luciferase, fluorescent proteins etc.). No harmful properties have been attributed to these proteins. There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Phontinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function.

Other non coding RNA sequences: Any effects these elements have on gene expression are likely to be minimal. They may affect gene function or virus replication in individual cells but are unlikely to lead to whole body physiological effects as they do not code for the production of secreted bioactive molecules, and are unlikely to have any biological effects outside arthropod cell systems.

Sequence changes in viral sequences:
Mutation or deletion will target disruption of non-structural protein functions or non-coding viral sequences and are likely to have no or deleterious effects on viral replication, thus not increasing risk or hazards to human health associated by viruses genetically modified in such a way. Deletions in the structural genes to produce replicons will result in non-propagating virus-derived RNAs that pose no risk, even if packaged into virus replicon particles. Expression of sequences by Semliki Forest virus and derived replicons is covered under GM26/CVR_AK_1.

Control measures – assign provisional containment level:
GM bacteria transfected with plasmids containing viral or other eukaryotic sequences - Containment Level 1.
Eukaryotic cells containing DCV sequences unable to form complete infectious virus – Containment Level 1.
Eukaryotic cells containing full length DCV sequences (capable of forming infectious virus, or capable of undergoing recombination or minor mutations to form infectious virus) – Containment Level 2.

All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. A microbiological safety cabinet will be used where appropriate.

Foreseeable effect/risk assessment for the environment:

DCV: This virus is not on the SAPO/ACDP lists. It can infect drosophila by ingestion; recombinant virus is likely to be less fit than wild type but may may be able to infect drosophilid species and other insects. Virus is kept within containment and no accidental infections of arthropods can take place. Replicons are non-propagating even within virus replicon particles and pose no risk. Arthropods and food are kept away from the laboratories and waste sterilized before disposal (see below) and it is therefore unlikely that any lab-acquired infection and/or escape can take place.

Hosts:
Prokaryotic organisms: Disabled, commercially available E. coli strains. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. Acquiring antibiotic resistance (from the plasmid vector) or additional sequences (virus or marker gene sequences) will not give these strains any survival advantage in the environment. The risk to the environment is therefore effectively zero.

Eukaryotic cells: Cell lines to be used are not viable outside the laboratory and thus pose no threat to the environment. Addition of plasmid vectors or virus will confer no growth or survival advantage in the environment.

In summary

i) consequence/severity of effects: Low

ii) likelihood of effects being realised: Low

iii) overall risk: Low
Final classification: GM bacteria transfected with plasmids containing viral or other eukaryotic sequences (unable to cause disease, minimal hazard to health and environment) - Containment Level 1.
Eukaryotic cells containing DCV sequences unable to form complete infectious virus – Containment Level 1.
Eukaryotic cells containing DCV sequences (capable of forming infectious virus, or capable undergoing recombination or mutation to form infectious virus) – Containment Level 2.

All with Good Microbiological Practice and Good Occupational Safety and Hygiene. Wear laboratory coat and gloves. As a routine measure all work will be carried out Containment level 2. A microbiological safety cabinet will be used where appropriate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Solids from cell culture (eg plasticware such as flasks, tubes, pipettes tips etc.) - soak in 1% Virkon (w/v) for a minimum of 12 hours. Dispose of as normal solid waste (black bag).
| Other solids (agar plates, gloves etc. ) placed in a metal disposal box (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 121oC for 30 minutes.
| Liquids (eg samples, culture supernatants, tissue culture media) – add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.
| Liquids (E. coli culture medium)- add Virkon to final concentration of 1% (w/v) for a minimum of 12 h, then discharge to drains.
| Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave by using a make safe cycle of 121oC for minutes.

Degree of kill:
Chemical Sterilization by Virkon- effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
1. GM 26 committee comments (approval date 22/02/12; see below):

The CVR Church Street GM subcommittee met on 31/01/12 to consider the proposal:
"Establishment of a reverse genetics system for drosophila C virus"

Subcommittee members:
R E
J M
J M
A P
F R (convener)

As Drosophila C virus only infects arthropods, it is not considered hazardous. Therefore, no objections were raised against the proposed work. However, clarification was requested for the following points:

How will the virus be propagated? The culture conditions should be described.

The Risk Assessment contains the statement "DCV: This virus is not a ACDP pathogen. It is not known to infect or replicate in human cells, and there is no risk associated with using this virus." (page 7, Vectors and GMOs). In the GM application the same section (page 5, point 7) states "DCV: This virus is not able to infect or replicate in human cells or other cells of vertebrate origin." These statements should be made to be consistent and accurate.

More clarity is required over the potential for infection of, and expression in, mammalian cells. This is particularly relevant in light of the subsequent discussion over the need for Notification (see below), which would only be required if there is a threat to human health or the environment. For example, it is proposed to clone genes under the CMV (and possibly other mammalian) promoter. Is this promoter active in insect cells and if not, why is it being used?

Also, SFV can infect mammalian cells so its use as a vector is potentially harmful. In the Notification (page 3) it is stated that "Individual DCV sequences (coding or non-coding) will be cloned into eukaryotic or prokaryotic vectors (plasmid or Semliki Forest virus or derived replicon) for molecular biological manipulation and/or expression purposes." In the Risk Assessment the sentence reads: (plasmid or Semliki Forest virus replicon or derived replicon). These statements should be made to be consistent and accurate.

Although only experiments in insect cells are listed under "Detailed descriptions and description/use of genetic modification:" (page 2-4 of Notification) a positive statement that no experiments in mammalian cells are planned should be inserted.

On page 2 of the Notification it is proposed to clone the DCV genome into a plasmid backbone. However, the section on Host/vector system (page 4) contains the statement that "Viral sequences (including full length clones or partial sequences of DCV) and other genes (cellular, viral or reporter) are available in plasmid-encoded
The discussion on this proposal raised the question of whether a Notification is required in this case. DCV is an insect pathogen that appears to pose no risk to human health and would have low or negligible environmental consequences if it escaped from the laboratory. It therefore resembles baculovirus, which is classified at containment level 1 except when specifically intended as a vector for gene expression in mammalian cells or when encoding viral functions that could themselves transmit to mammalian cells. Page 9-10 of the Risk Assessment Form includes the statement: “If the answer to questions 11 and 12 is negligible, you may believe that you have sufficient information at this stage to classify the project to class 1, as defined in the Contained Use Regulations 2000. In order to do this you should be confident that in the event of a total breach of containment the GMO would be of no or negligible risk to human health or the environment.” The committee therefore requested that the reason for suggesting that work involving DCV is classified as containment level 2 should be further evaluated and the results of such an evaluation be conveyed back to the subcommittee. If a Notification is deemed unnecessary a Risk Assessment would still be required for GM work.

As part of the same discussion it was suggested that to save effort and expense, planned future work on Arboviruses should be assessed in terms of the likely need for further applications for Notification. If more potential applications are identified consideration should be given to the possibility of combining the proposals into a single Notification covering as many viruses as possible.

Response from A K to GM Committee comments (06.02.2012):

**Project Containment**

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**Project Ref** 26/13.1

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<td>Influenza virus pathogenesis, host range and virus/host interactions</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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Project notified under transitional arrangements N
The Orthomyxoviridae is defined by viruses that have a negative-sense, single stranded and segmented RNA genome. Viruses within the Orthomyxoviridae are classified into five genera: Influenza viruses A, B, C, Thogotovirus and Isavirus. Within a genus, viruses show similar patterns in the sizes of their genome segments as well as structural and non-structural proteins. Influenza A viruses are typical examples of emerging viruses, including pathogens of humans and/or animals (5). Influenza B virus appears predominantly restricted to humans but it has also been isolated from seals; and influenza C virus infects humans and pigs. Types A and B are the cause of seasonal annual epidemics of acute respiratory disease among humans, and type A viruses have caused occasional pandemics – worldwide epidemics caused by a virus having new antigenic surface proteins among an antigenically naive population. Influenza A virus infections can cause a severe and debilitating febrile illness that can lead to fatal pneumonia and increase the risks associated with secondary bacterial chest infections, particularly in the very young, chronically ill, immunocompromised or elderly.

The influenza A virus genome is composed of eight segments of single-stranded RNA of negative polarity that encodes for up to 14 proteins. The genome segments are called PB2 (polymerase basic), PB1, PA (polymerase acidic), HA (haemagglutinin), NP (nucleoprotein), NA (neuraminidase), M (matrix) and NS (non-structural). Influenza A viruses are sub-classified according to the antigenic characteristics of the surface glycoproteins, the haemagglutinin (H) and neuraminidase (N). There are 17 H and 10 N subtypes known to date. The four major pandemics of the last hundred years were caused by H1N1 (1918 [Spanish flu], and 2009 [swine flu]), H2N2 (1957, Asian flu) and H3N2 (1968, Hong Kong flu) viruses. Currently, influenza A virus subtypes H1N1 and H3N2, and influenza B virus, are co-circulating in the human population. Avian viruses (such as H5N1, H7N7, and H7N9 viruses) have also caused human infections, and epizootics of highly pathogenic H5N1 and H7N9 avian viruses in several Asian countries since 1997 have raised the spectre of a new pandemic.

During replication, a positive-stranded antigenome is produced which serves as the template for de novo production of negative-stranded genomes. The influenza A virus terminal 3’ and 5’ sequences are highly conserved and largely complementary, and interact to give the genome a characteristic pan-handle structure. The RNPs are contained within a lipid envelope into which the viral glycoproteins (HA, NA and M2) are inserted. Replication occurs in the nucleus of infected cells.

Most influenza viruses are transmitted by direct and indirect contact. Some influenza viruses are transmitted through aerosol droplets. Cytopathic effect (CPE) depends on both viruses and host cells. For example, pandemic H1N1 virus causes CPE in Madin-Darby canine cells whereas most equine influenza viruses do not. At the same time, some equine influenza viruses cause CPE in dog tracheal explants whereas others do not. Understanding the molecular basis for the different outcomes of infection in these cells and tissues of different origin is of fundamental importance, and may help in the development of new control measures for influenza emergence. This is likely to imply immune responses (which differ in both hosts) and virus-host interactions.

Although much is known about the function(s) of individual influenza virus proteins, much more remains to be learned about the factors involved in host–pathogen interactions that affect host range and disease outcome. The overall aim of our research is to elucidate the molecular and evolutionary determinants of host range, replication and pathogenicity of influenza viruses.

The work to be undertaken forms part of a comprehensive programme of viral emergence and molecular pathogenesis, involving researchers from different groups within the Centre for Virus Research (CVR). It involves work on several areas including, epidemiology, mathematical modelling of virus evolution, structural biology, and studies...
on virus-host interactions and molecular pathogenesis. It is the last aspect i.e. virus-host interactions and molecular pathogenesis with which this GM risk assessment is concerned. Within the CVR, the groups of Pablo Murcia and Benjamin Hale will generate the tools to produce recombinant influenza viruses and these viruses will form the basis of the work covered in this risk assessment. The work proposed will involve both in vitro, ex vivo, and in vivo infections. Embryonated chicken eggs will also be used as a system for growing some virus stocks.

Many Hazard Group 2 (HG2) influenza viruses will be at the centre of this project: equine influenza virus (EIV), canine influenza virus (CIV), low pathogenic avian influenza viruses (AIV), and human influenza viruses (H1N1, H3N2). Other viruses which will be used include laboratory-adapted and mouse-adapted strains, as well as currently circulating influenza viruses and influenza viruses that infect other animal species (e.g. swine). We will also generate viruses that contain segments from highly-pathogenic avian influenza A viruses in the background of an attenuated laboratory strain. These viruses will lack a major virulence determinant from highly-pathogenic viruses and will also be engineered such that they do not possess the antigenic novelty associated with pandemic-potential of these strains. Non-hazardous minigenome systems (see below) of influenza viruses will also be studied.

Reverse genetics systems that allow manipulation of the influenza virus genome are readily available (2). Cultured cells are transfected with a mixture of 8 to 12 plasmids, each containing a full-length cDNA copy of one of the influenza virus genome segments under control of a Pol I promoter and terminator sequences, and a hepatitis delta ribozyme sequence. Intracellular transcription produces exact copy RNA transcripts that act as viral mRNAs and templates for viral RNA synthesis that result in production of infectious virus. Similarly, minigenome systems can be constructed in which only the coding sequences between the terminal influenza A virus sequences (either genomic or antigenomic) are replaced with a reporter gene such as Renilla luciferase (RLuc) or eGFP. A minigenome will be transcribed if the polymerase and nucleoprotein genes are co-expressed from expression plasmids, which is useful to study the role of sequences and proteins in replication and also in packaging as minigenome. Minigenomes are on their own non-propagating and are used to mimic virus replication.

1- In vitro and ex vivo infections to study influenza virus replication, host range and pathogenesis.
We will establish and characterise influenza virus infection in cell lines of various origins (human, canine, equine, etc), primary cultures derived mainly from humans, horses and dogs, as well as canine and equine tracheal explants. Primary cell cultures and explants from other animal species might also be employed.

For the canine/equine project, we will mainly be using H3N8 viruses derived from canine and equine strains (for example A/canine/New York/2008 and A/equine/Ohio/2003). Other equine and canine viruses of the same lineage will also be cloned and rescued by reverse genetics. All equine and canine influenza viruses belong to the ACDP hazard group 2. It should be noted that no equine or canine influenza virus has been associated with human disease.

We will also generate by reverse genetics the following human influenza viruses: mouse-adapted A/WSN/33 (H1N1), A/PR/8/34 (H1N1) and the non-mouse adapted 2009 H1N1 pandemic strains (e.g. A/England/195/2009, or A/California/04/2009) as well as other human seasonal strains (e.g. A/New York/312/2001 (H1N1) and A/Victoria/3/75 (H3N2)) as appropriate. All human influenza viruses used will belong to ACDP hazard group 2 and will be predicted to be antigenically similar to components of the seasonal trivalent or quadrivalent vaccine. The viruses will therefore be used at CL2.

Reverse genetics will also be used to construct recombinant viruses with mixed gene segments enabling identification of virulence determinants or identification of host factors that impact specific strains. The ability to perform site directed mutagenesis to produce viruses containing targeted mutations in specific genes will allow further characterization of the role of specific proteins. Readouts will include the capacity for virus replication in tissue culture cells, or explants, as well as ability of the viruses to induce histological changes and the susceptibility of infected cells to apoptosis. In addition, we will examine a number of changes in functional attributes following virus infection, such as ciliary beating if appropriate.

2- Viral genetics of host restriction, adaptation, and interactions with host-cell functions. The contribution that individual virus genes (focusing on the ‘internal’ or non-glycoprotein genes) make to defining host range and pathogenicity will be probed by creating reassortants between the strains of influenza described above in section (1). For the highly-pathogenic avian influenza A viruses, we will not generate nor seek to possess complete sets of reverse genetics clones, and will only use a selection of individual internal segments to generate viruses in the background of attenuated laboratory-adapted influenza viruses (such as WSN or PR8 strains). In this regard, it is
co-transfected into a sub-confluent monolayer of co-cultivated HEK293T and MDCK (Madin-Darby canine kidney) cells. Subsequent amplifications of the virus will be

We will ensure that reassortants between CIV strains, EIV strains, and between CIV and EIV viruses will not affect any immunological protection as they are all phylogenetically very similar (CIV is a direct descendant of EIV). Reassortants between the H3N8 avian-like strains and PR8 is unlikely to result in any increased risk as use of PR8 internal genes together with novel surface glycoproteins is standardised in traditional influenza virus vaccine production worldwide (even with highly virulent influenza viruses such as H5N1), and has been approved by the World Health Organization (6).

Virus reassortments will be generated by reverse genetics that contain select internal segments from highly pathogenic viruses with the surface glycoproteins (HA and NA) from lab-adapted strains (e.g. PR8 or WSN). This will ensure that the viruses are likely to be antigenically similar to the currently used human vaccine, and sensitive to clinically approved NA inhibitors such as oseltamivir. Reassortants will be limited to the ‘internal’ genes of the highly pathogenic virus and are therefore expected to retain the basic tropism of the parental strain, as well as any immunological protection. In the case of using the PR8 and WSN backgrounds, this also means that attenuated characteristics are likely to be maintained. The HA gene of H5N1 viruses is a primary pathogenicity determinant and deletion of its polybasic cleavage site severely attenuates the virus in mammalian and avian models of infection. In addition, the classification of HPAI influenza involves the nature of the haemagglutinin – H5 or H7 subtypes with a polybasic cleavage site expected to produce systemic spread in infected poultry. Current ACDP guidelines state that H5 or H7 viruses modified such that they do not possess a polybasic cleavage site in HA can be handled under CL2 conditions. By only working with the ‘internal’ genes of these strains, we will further reduce any risk as not only will the polybasic cleavage site be removed, but there will be no novel HA (such as H5 or H7) to which humans have never been exposed to previously. The reassortants are unlikely to fall under SAPO regulations as no H5 or H7 HA with a polybasic cleavage site will be used. This will be clarified, and appropriate SAPO licences sought, prior to any work commencing. Use of lab-adapted HA and NA genes (from WSN and PR8) will accentuate any attenuation in humans (e.g. Clements et al., J Clin Micro, 1992, 30 655-62, and Clements et al., J Clin Micro, 1989, 27, 219-22) and is likely to provide cross-protection from H1N1-containing vaccines such as the current seasonal vaccine (the 2009 H1N1 pandemic vaccine (still used in current trivalent vaccines) provides protection against “old” H1N1 viruses between the years 1918 and 1947 (Medina et al., Nature Communications, 2010, Manicassamy et al., PLoSPathogens 2010). PR8 was isolated in 1934, whilst WSN was isolated in 1933.

We will use viruses generated by ourselves and also plan to use recombinant viruses based on A/equine/Ohio/2003, A/canine/New York/2008 (both H3N8), A/WSN/33 or A/PR/8/34 (both H1N1) and A/California/04/2009. In some cases we will add fluorescent tags for labelling and live tracking of viral particles. One possible approach will be to use a FlAsH currently being developed at Institut Pasteur. The FlAsH tag (Invitrogen) is a short (6 residues) peptide tag that has been fused to influenza gene NP and used in a reverse genetics plasmid-based system to produce infectious viral particles carrying the tagged NP protein. Cell-permeable FlAsH reagent, binding with high affinity to the FlAsH tag, is used to stain the cells, and can be excited at 508 nm wavelength. Emission at 528 nm can be detected using standard fluorescence microscopy settings, allowing identification of infected cells. Alternatively, we will use the similar technology SNAP tag, or the autofluorescent GFP/RFP protein domains. Luciferase genes may also be incorporated into recombinant viruses.

In order to compare host range determinants of equine and canine viruses, we will use reverse genetics to construct mutant versions of A/canine/New York/2008 and A/equine/Ohio/2003. The dose of the inoculum will vary, although for most of our experiments we infect explants with a very small dose (200 pfu).

As a reverse genetics system is not currently available for some canine and equine viruses, such a system will be constructed. We will clone various CIV and EIV sequences into an 8-plasmid system widely used for influenza reverse genetics. Genomic segments corresponding to genes coding for proteins HA, NA, M, NS, PB1, PB2, PA and NP including the 3’ and 5’ non-coding regions will be PCR-amplified and cloned into pHW2000 vector in which the cloning site is flanked by the Poll promoter and hepatitis delta ribosome sequences. If necessary, sequences corresponding to PB1, PB2, PA and NP genomic segments will be cloned into pHMG, where protein expression will be under the control of a mouse hydroxymethylglutaryl-coenzyme A reductase (HMG) promoter.

The method to be used for the production of recombinant EIV and CIV by reverse genetics is adapted from previously described procedures (3). The 8-12 plasmids will be co-transfected into a sub-confluent monolayer of co-cultivated HEK293T and MDCK (Madin-Darby canine kidney) cells. Subsequent amplifications of the virus will be
performed on MDCK cells or embryonated chicken eggs.

Reassortant and wild type influenza viruses will be used to:
- Study viral growth, gene expression, replication and pathogenesis in host cells and tissues.
- Study interactions with the host cell (replication, interaction with host cell factors, immunity).

3- Viral genetics using influenza virus minigenome systems
- Minigenome systems for a broad range of influenza A viruses are already in use (1, 4). We will use minigenome systems that have been generated by others. If required, we will derive existing minigenome systems by cloning the segments of interest of the viruses under study (e.g. EIV, CIV, etc). Minigenome systems will be used (as read-outs) in studies on protein functions (cellular, viral), sequence function, segment packaging (packaging by glycoproteins co-expression, or following co-infection) and complementation studies (i.e. CIV PB2 in EIV viral polymerase complex).

4- Functional studies and expression of influenza virus proteins.
- Manipulation of protein sequences by introduction of point mutations, deletions, insertions, fusion to others sequences such as reporter genes, tags etc.
- Expression of influenza virus proteins to study interactions with host cell components and cellular localisation.
- Expression of influenza virus proteins by prokaryotic expression systems, for example for protein purification.
- Expression of influenza virus proteins by eukaryotic expression systems (plasmid, retroviral expression), for example to produce packaging cell lines, cell lines expressing virus-like particles, or cell lines to study protein function.
- Expression of influenza virus proteins by eukaryotic expression systems (plasmid, retroviral expression), to produce packaging cell lines for influenza virus sequences (segments or minigenomes; production of single round infectious particles) and cell lines expressing virus-like particles.

5- Studies on host immunity.
- Interactions of influenza viruses (wild type, reassortants, minigenome) with host responses, and counteraction of host responses will be investigated. Immune signalling/antiviral responses (including activation mechanisms), and viral interference therewith, will be analysed. This will involve transcriptomic studies as well as studies of extra- and intra-cellular host molecules (e.g. proteins). Role of immunity in host cell tropism (infections of cells from different species) will also be investigated. These studies will also use reporter genes inducible upon activation of a given pathway. Cell culture models are available.

References:
A/California/04/09 (pdmH1N1)
A/Netherlands/602/09 (pdmH1N1)
A/Mexico/4108/09 (pdmH1N1)
A/Brisbane/59/07 (H1N1)
A/Brisbane/10/07 (H3N2)
A/Wyoming/3/03 (H3N2)
A/Moscow/10/99 (H3N2)
A/Panama/2007/99 (H3N2)
A/Texas/36/91 (H1N1)
A/Puerto Rico/8/34 (H1N1) [PR8]
A/WSN/33 (H1N1) [WSN]
A/equine/Miami/1963 (H3N8)
A/equine/Fontainebleau/1979 (H3N8)
A/equine/Argentina/1995 (H3N8)
A/equine/Newmarket/1993 (H3N8)
A/equine/South Africa/2003 (H3N8)
A/equine/Ohio/2003 (H3N8)
A/canine/New York/2008 (H3N8)
A/equine/Mongolia/2009 (H3N8)
A/wild bird/Mongolia/2009 (H3N8)
A/wild bird/Mongolia/2010 (H3N8)
A/wild bird/Mongolia/2011 (H3N8)
A/swine/England/495/2006 (H1N1)

B/Yamagata/88

Note: that all of the above viruses are HG2, and will therefore be handled under CL2 conditions.

A/Vietnam/1203/2004 (HPAI H5N1), or related [only the internal segments of this virus will be used]
A/Shanghai/1/2013 (H7N9), or related [only the internal segments of this virus will be used]

The above 2 virus types are HG3, but we will only use genetically modified versions (as detailed below in section 11) which are modified to remove major pathogenicity determinants and the antigenic novelty associated with strains of pandemic-potential. The justifications are reasoned in section 11, and such modified viruses have been assessed locally to be HG2 pathogens, which will be handled under CL2 conditions.

Recipient organisms:

Equine influenza virus H3N8 (genus Influenzavirus A), and derived recombinant viruses.
Canine influenza virus H3N8 (genus Influenzavirus A), and derived recombinant viruses.
Human influenza virus H3N2 and H1N1 (genus Influenzavirus A), and derived recombinant viruses (e.g. see above).

Other containment level 2 influenza viruses (e.g. avian viruses previously characterised as exhibiting low pathogenicity, or swine viruses, etc.) may be considered as long as they are unrelated to those capable of causing infection of humans.
At no point will viruses be produced that have surface antigens with a multibasic cleavage site in H5 or other livestock influenza virus HA proteins. It should also be noted that the equine/canine project and the human/avian projects will be performed by different investigators at different sites. We will therefore physically separate work to minimise the likelihood of reassortment between different viruses. We do not exclude possible future work on viruses expressing H5N1 antigens at CL2 as per current ACDP guidelines (e.g. low-pathogenic H5N1 viruses engineered to lack the polybasic cleavage site: “HALo”). However, such work will only be done after a separate notification to the local safety committee and relevant addendum to our local Risk Assessment.

Human influenza B virus (e.g. B/Yamagata/16/88), and derived recombinant viruses.

Reporter systems mimicking Influenza virus replication:
Minigenomes of CIV, EIV, and other influenza A and B viruses (for example swine influenza virus).

Host/vector system

Plasmid vectors:
Viral sequences (full length clones of Influenza viruses; partial sequences of these and other influenza viruses) and other genes (cellular, viral, reporter) are available in plasmid-encoded cDNA form. RNAs can be transcribed from bacterial promoters (e.g. T7 or SP6 by in vitro transcription or plasmid-expressed RNA polymerase in cells) or eukaryotic promoters (CMV, SV40, cellular promoters, baculovirus promoters etc.). Plasmids for expression of influenza virus sequences (virus rescue or minigenome) typically contain an RNA polymerase I promoter and a hepatitis delta virus ribozyme or a mouse RNA polymerase I terminator. Plasmids contain an origin of replication followed by a prokaryotic selectable marker (usually antibiotic resistance against ampicillin or kanamycin) and sometimes a selectable eukaryotic drug resistance marker such as neomycin or puromycin resistance. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

Hosts:
Prokaryotic hosts:
Disabled, commercially available E. coli strains such as Sure, JM109, XL-strains, DH-strains, Rosetta etc. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste should prevent release of viable organisms. The risk to the environment is therefore effectively zero.

Eukaryotic hosts:
Vertebrate cell lines of various origins (typically human, mouse, avian, hamster, bovine, canine, equine, etc.), primary cells and explants of various origins (typically human, swine, equine and canine).

In prokaryotic cells, only selectable (eg antibiotic resistance) genes will be translated; prokaryotic cells may also be used for protein expression and subsequent purification.

Virus sequences will be under the control of a promoter that will only generate transcripts in eukaryotic cells or in vitro transcription reactions.

In eukaryotic cells, the following RNAs will be produced which may be translated into the following gene products:

1-Influenza viruses and RNAs (or minigenomes): Proteins involved in replication and transcription of viral genetic material and virus structural proteins. Some of these virus proteins will interact with host-cell components and may affect host cell responses to infection; few of these interactions are currently known. If all virus sequences are translated new virions may be generated; minigenomes are non-propagative viral RNAs (capable of replication). Partial RNAs do not give rise to propagating infectious material.

2-Non-influenza virus genes or sequences: Genes of prokaryotic (bacteriophage polymerases) or eukaryotic (eg. reporter genes such as luciferases or fluorescent proteins such as GFP; plant or arthropod immunity modulators) origin. Reporter gene sequences will also be present in influenza minigenome RNA. The recombinant foreign proteins to be expressed provide no significant increase in the hazard to human health; none are toxic or likely to produce disease in the quantities that could be produced by accidental exposure to these systems. Non translated RNA sequences (including T7 terminator and hepatitis delta ribozyme in plasmids used for virus rescue or rescue of other RNA viruses).
minigenomes) have no known toxic effects and pose no risk to human health.

Techniques used to introduce insert or vectors into cells:

1- Prokaryotic cells: Introduction of DNA into E. coli will be by heat shock/chemical transformation/electroporation. These techniques have been extensively described and are widely used; they rely on getting DNA very close to the bacterial wall and introducing the genetic material through pores or membrane fusion.

2- Eukaryotic cells: Cells will be transfected using transfection reagents such as lipofectamine or by electroporation. Virus genomes or sequences will be introduced into cultured cells by infection with virus or virus replicon particles (VRP).

**Origin & function**

The genetic material will originate from the parental organisms:

- A/California/04/09 (pdmH1N1)
- A/Netherlands/602/09 (pdmH1N1)
- A/Mexico/4108/09 (pdmH1N1)
- A/Brisbane/59/07 (H1N1)
- A/Brisbane/10/07 (H3N2)
- A/Wyoming/3/03 (H3N2)
- A/Moscow/10/99 (H3N2)
- A/Panama/2007/99 (H3N2)
- A/Texas/36/91 (H1N1)
- A/Puerto Rico/8/34 (H1N1) [PR8]
- A/WSN/33 (H1N1) [WSN]
- A/equine/Miami/1963 (H3N8)
- A/equine/Fontainebleau/1979 (H3N8)
- A/equine/Argentina/1995 (H3N8)
- A/equine/Newmarket/1993 (H3N8)
- A/equine/South Africa/2003 (H3N8)
- A/equine/Ohio/2003 (H3N8)
- A/canine/New York/2008 (H3N8)
- A/equine/Mongolia/2009 (H3N8)
- A/wild bird/Mongolia/2009 (H3N8)
- A/wild bird/Mongolia/2010 (H3N8)
- A/wild bird/Mongolia/2011 (H3N8)
- A/swine/England/495/2006 (H1N1)
- B/Yamagata/88

- A/Vietnam/1203/2004 (HPAI H5N1), or related [we will only use the internal segments from these viruses]
- A/Shanghai/1/2013 (H7N9), or related [we will only use the internal segments from these viruses]

Alternatively, viral sequences of these and related viruses (similar strains and same ACDP classification) can be synthesised. We do not exclude possible future work on viruses expressing H5N1 surface antigens at CL2 as per current ACDP guidelines (e.g. low-pathogenic H5N1 viruses engineered to lack the polybasic cleavage site: "HALo"). However, such work will only be done after a separate notification to the local safety committee and relevant addendum to our local Risk Assessment.
The intended functions of the genetic material involved is the production, purification and characterisation of influenza viruses, as well as the production and use of minigenomes. In turn, the viruses produced will be used to infect human or animal cell tissues and cells to determine the cellular processes involved in pathogenesis and replication through virological and molecular biological methods.

Evaluation of foreseeable effects

Plasmid vectors: Viral sequences (including full length clones or partial sequences of influenza A virus or other influenza viruses) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers. By themselves these vectors present no risk to human health. Vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Systems for prokaryotic gene expression are under control of an inducible prokaryotic promoter. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV or SV40), eukaryotic (eg actin promoter, pathway inducible promoter such as STAT, interferon etc.) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human cells. None of the genes used in these studies are oncogenes. The hazard of expressing ‘foreign’ genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the foreign gene product and is discussed in more detail below.

Viruses: Influenza is typically a mild and self-limiting infection, acquired generally by the respiratory route. Severity depends on the strain of virus and host but in general tends to fall in a spectrum between asymptomatic to ~ 1 week of fever and malaise in otherwise healthy people. Severe illness can occur but is rare, occurring in less than 1% of cases (see below). This level of risk applies to most wild type human H1N1 and H3N2 viruses and the porcine viruses. Equine H7N7, H3N8 and canine H3N8 viruses have not been associated with significant illness in humans. In addition, the laboratory adapted PR8 and WSN strains of H1N1 human influenza are known to be avirulent in man from many volunteer challenge studies as well as a long (> 70 years) history of safe use in the lab. The GM viruses containing internal segments from H5N1 or H7N9, but with the surface glycoproteins from WSN or PR8 would retain antigenic properties of the lab-adapted PR8/WSN viruses, which are likely covered by the current human influenza vaccine, and which have not been associated with disease despite their wide use worldwide for decades.

Even seasonal influenza can have potentially serious consequences for anyone who is pregnant, immunosuppressed, asthmatic, or has other respiratory/underlying chronic diseases. Vaccines are available for the circulating human H1N1 and H3N2 strains and the majority of the population has at least some prior immunity to these viruses. All laboratory workers and staff within the department are recommended to receive the annual human influenza vaccine, and all workers using shared resources (even if not directly involved in the work) are made aware of the risk assessment for influenza virus work. Those at risk of severe consequences of influenza (e.g. pregnant women) will be separately notified of the risks of these pathogens immediately after they identify themselves to their PI and occupational health. Individual risk assessments will be carried out governing these specific cases.

The antivirals oseltamivir, zanamivir and amantidine are also available for treatment or prophylaxis if needed. We have procedures for staff to seek medical advice and prophylactic treatment following any possible significant accidental exposure events. Note, we will not handle any known antiviral-resistant strains of influenza virus in the same location as other viruses with different antigenic profiles in order to limit the possibility of accidental reassortment generating an anti-viral resistant virus with new antigenic properties.

Vaccines are also available for horses/dogs against EIV and CIV. Workers with these viruses will be advised to not have contact with either horses or dogs for 48h after last handling the viruses.

Personnel working with these viruses are expected to inform occupational health should they become pregnant or immunosuppressed, and inform the PI so that precautionary measures can be introduced following advice from occupational health. We also have a policy whereby those with flu-like symptoms do not come to work, do not handle influenza viruses experimentally, and inform their PI immediately. This policy will further limit any possible accidental reassortment between laboratory viruses and circulating human viruses, should a worker accidentally expose themselves to a laboratory virus whilst simultaneously infected with a circulating virus. The local PI (Pablo Murcia or Benjamin Hale) will take responsibility for enforcing this, and will similarly assume responsibility for the safety and compliance of any visiting workers.

Influenza A virus minigenomes:

Minigenomes pose no risk to human health, including those derived from pathogenic influenza A viruses. They cannot propagate on their own. Even if packaged by
co-expression of glycoproteins and thus used to infect cells, their own transcriptional activity would be minimal. If packaged into virions, the associated risk is that of the virus itself as reporter genes in minigenomes are not reported to be toxic.

Note: No reassortment experiments involving the glycoproteins of human and animal influenza A viruses (for example an influenza A virus carrying a human H1 and an equine N8) will be performed, to eliminate the possibility of creating viruses for which humans or animals have no prior immunity. Furthermore, we aim to physically separate work on different influenza viruses such that accidental reassortment to generate a more pathogenic strain, or an antigenically novel strain with new properties, cannot occur from our work (see details in Risk Assessment).

Hosts

The host/recipient organism: Prokaryotic organisms: All strains will be disabled, commercially available E. coli derivatives classified as ‘especially disabled hosts’ by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic cells: Cell lines and tissues to be used would not survive inside the human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA or infection will confer no growth or survival advantage in or outside the laboratory to cell lines and tissues.

What hazards do the inserted genetic material or other genetic modification pose?

The inserted/donated genetic material:

Viral RNA and proteins, cellular proteins: Most individually expressed viral or cellular proteins are unlikely to have harmful effects in eukaryotic cells, however some could perturb normal cellular metabolism, predispose or protect against cell death or render cells more or less susceptible to other infections if overexpressed or silenced. It is very unlikely that Influenza A virus RNA or proteins would have any harmful toxin-like effect outside cells. Only antigenomic RNA containing an influenza A virus sequence can be translated or give rise to infectious virus (if all necessary genomic information is provided) in eukaryotic cells; minigenome RNAs are non propagative and pose no harm (individual cells replicating minigenome are likely to die or be eliminated by the immune system). Viral non-coding RNA sequences are important in influenza virus replication but do not encode biologically active molecules that are likely to induce physiological effects in humans on their own. None of the influenza virus gene products are known to be secreted cytotoxins.

Reporter genes: Reporter genes of prokaryotic or eukaryotic origin (eg luciferase, fluorescent proteins etc.). No harmful properties have been attributed to these proteins. There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Photinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function.

Sequence changes in viral sequences:

Mutation or deletion will target disruption of structural and non-structural protein functions or non-coding viral sequences and are likely to have no or deleterious effects on viral replication, thus not increasing risk or hazards to human health associated by viruses genetically modified in such a way.

Foreseeable effects to the environment:

Canine influenza virus: This virus is not thought to be endemic in the UK, and it is not on the SAPO list. In fact, it is not highly transmissible in the only country in which is endemic (USA). Transmission is common only in highly dense dog populations such as those found in animal shelters. CIV was first isolated from greyhounds in USA. Experimental infection results in mild respiratory disease. Serological evidence of infection in foxhounds in the UK has been shown, although no outbreaks have been associated with it. Given the low transmissibility and low pathogenicity of CIV, the chance of accidentally infecting dogs initiating a transmission cycle sustained by local
dogs is negligible. Reassortants carrying gene segments from EIV will likely display lower fitness and will pose minimal risks to dogs, if any. The modified viruses will not have any inserted foreign genes other than non-harmful genes or sequences, or (usually disruptive) changes in viral genes or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. In the absence of a suitable host these viruses would not survive in the environment and the risk is effectively zero. Dogs are kept out of labs and the risk of accidental infection of dogs by contact of contaminated material is minimal for CIV. However, as some members of staff bring their dogs to work there is a potential risk of infection of those animals if they access the laboratories.

Equine influenza virus: This virus has been endemic in the UK for over 40 years and infections are most likely the result of horse movement and inadequate vaccination schedules. EIV is not on SAPO or ACDP lists. There is a vaccine available for EIV, although most horses have natural immunity due to vaccination or natural infections. The modified viruses do not contain anything other than non-harmful genes or sequences, or disruptive changes in viral genes or non-coding sequences; changes in virus pathogenesis are therefore unlikely other than as described above under the human health and safety section. If EIV was to escape the lab and infect local horses, there is minimal risk it could initiate a transmission cycle. Horses are kept out of labs and the risk of accidental infection of horses by contact of contaminated material is minimal. The closest horses to the lab are kept at the Weipers Centre and up to date vaccination against EIV is a requirement for admission.

Avian-like equine influenza virus (H3N8): This virus is highly pathogenic in horses (20% mortality) and there is no cross reactivity between classical EIV and avian-like EIV. Reassortants of this virus will be done using PR8 as a backbone (HA and NA of avian like EIV + the six internal genes of PR8), so the resultant viruses will be highly attenuated. No other reassortant viruses including the surface glycoproteins (HA and NA) of avian like EIV will be generated. Reassortants carrying the internal genes of avian-like EIV and the external genes of classical EIV will have a minimum risk to initiate a transmission cycle as most horses in the UK have immunity against the surface glycoproteins of classical EIV. It is important to note that PR8 has been used as a backbone to generate vaccines against highly pathogenic H5N1 viruses and thus is very safe to use. If these reassortants were to escape they would have very low fitness due to the attenuation conferred by the PR8 genetic background.

Human influenza viruses: Currently circulating human influenza A viruses (pdm H1N1, seasonal H1N1, and H3N2) are globally distributed, there are available vaccines, and the majority of the population has at least some prior immunity to these viruses. The antivirals oseltamivir and zanamivir are also available for treatment or prophylaxis if needed.

Viruses constructed will be based on the widely used laboratory strains A/WSN/33 (H1N1), A/PR/8/34 (H1N1) (all mouse adapted and known [WSN, PR8] to be attenuated in man) or more recent low pathogenicity human strains to which most people will have substantial amounts of pre-existing immunological protection: A/Udorn/72 or A/Victoria/3/75 (H3N2) viruses, the current human influenza strains A/England/195/2009, A/California/04/2009 (both 2009 H1N1 pandemic viruses) and the A/New York/312/2001 strain (‘seasonal’ H1N1). Such antigenic and attenuated properties will be maintained in viruses bearing the surface glycoproteins of WSN/PR8 and the internal segments of H5N1 or H7N9 viruses.

The experiments proposed involve gene deletion, mutation or replacement of specific genes with the corresponding gene from one of the other strains listed. For reassortants between the human-derived or laboratory adapted viruses, this is not predicted to create viruses with novel tropism or pathogenicity as they either already share similar characteristics (the recent human isolates) or are highly attenuated in humans (PR8, WSN). In the case of reassortants incorporating genes from animal strains of influenza, we also do not expect any change in tropism because the human or laboratory adapted HA and NA genes will be retained. In the case of reassortants incorporating genes of the avian-like equine influenza virus, the six internal genes will be derived from PR8, thus attenuating the virus significantly.

We propose to use containment level 2 for all other HG2 influenza A viruses (as specified on page 2) similar to CIV and EIV unless other regulations are in place for a particular pathogen.

Minigenomes: These systems derived from other influenza A viruses are non-propagative and even if packaged, pose no or no additional hazard.
Prokaryotic organisms: Disabled, commercially available E. coli strains. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids. Good microbiological practice and sterilisation of all contaminated waste will prevent release of viable organisms. Acquiring antibiotic resistance (from the plasmid vector) or additional sequences (virus or marker gene sequences) will not give these strains any survival advantage in the environment. The risk to the environment is therefore effectively zero.

Eukaryotic cells: Cell lines to be used are not viable outside the laboratory and thus pose no threat to the environment. Addition of plasmid vectors or virus or minigenomes will confer no growth or survival advantage in the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We propose to generate influenza viruses bearing the 6 internal gene segments from H5N1 or H7N9, with the 2 surface glycoproteins of laboratory-adapted H1N1 viruses WSN or PR8. Currently, both H5N1 and H7N9 viruses should be handled at CL3 according to ACDP guidelines.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Disinfect influenza virus-contaminated material immediately.**

**GM26 (CVR Church Street)**

- Solids from cell culture (eg plasticware such as flasks, tubes, pipette tips etc.) - submerged soak in a minimum of 1% Virkon (w/v) for at least 12 hours. Rinse solids then go to landfill. Virkon is added to the plasticware prior to removal from the MSCII.

- Other solids (agar plates, gloves etc.) placed in a sealed container (lined with clear bag) and disposed of by autoclaving using a make safe cycle of 121°C for 30 minutes. Gloves used for influenza virus work should be disinfected with alcohol before putting in containers prior to autoclaving.

- Liquids (eg. samples, culture supernatants, tissue culture media) – add Virkon to final minimum concentration of 1% (w/v) for at least 12 hours, then discharge to drains. Virkon is added to the liquid waste prior to removal from the MSCII.

- Liquids (E. coli culture medium) - add Virkon to final minimum concentration of 1% (w/v) for at least 12 hours, then discharge to drains.

- Sharps (e.g. needles/syringes, scalpels) - decontaminate in 1% Virkon overnight, then place in sharps bin for incineration.

- Spills of infectious liquid - immediately inactivated with powdered Virkon (rather than liquid spray, to minimise aerosol generation - large spill), or absorbed into paper towels soaked in Virkon (minimum 1% - small spill). Soaked paper towels then autoclaved. Surface wiped down with Virkon soaked paper towels and then disinfected with alcohol prior to drying.

**Degree of kill:**

- Chemical Sterilization by Virkon- effectively 100% kill.
- Autoclaving - effectively 100% kill (annual validation).

**GM223 (CVR Garscube Estate):**

- Disposable solids (eg plasticware such as flasks, tubes, pipette tips etc.) - soak in 1% Virkon (w/v) for a minimum of 12 hours. Transfer solid contents to clear autoclave.
bags and autoclave at 121°C for a minimum of 20 minutes prior to final disposal by district council to landfill, remaining liquid to be discharged to drain.

Other solids (agar plates, gloves etc.)- placed in a marked box lined with a clear autoclave bag, and disposed of by autoclaving using a make safe cycle of 121°C for at least 20 minutes. Seal contaminated plates (with for example bacterial GMOs) before placing in bag, to avoid lid falling off. Gloves used for influenza virus work should be disinfected with alcohol before putting in containers.

Liquids (eg. samples, E. coli culture media, tissue culture media)- add Virkon to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.

Sharps (eg needles, syringes, scalpels) - decontaminate in 1% Virkon overnight then place in sharps bin for incineration.

Degree of kill:
Chemical Sterilization by Virkon- effectively 100% kill.
Autoclaving - effectively 100% kill (annual validation).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
1. GM 26 committee comments:

The CVR Church Street GM subcommittee met on 29/04/2013 to consider the proposal:
“Influenza virus pathogenesis, host range and virus/host interactions”

Present:
Roger Everett
John McLauchlan
Joyce Mitchell
Arvind Patel
Emma Thomsen
Frazer Rixon (convener)

The subcommittee agreed the following recommendations.

Vaccination
The existing recommendation is that anyone working with influenza in Church Street should be vaccinated. This requirement for vaccination is not clearly stated in the RA. For example, on pages 16, 20 and 22 of the RA it is stated that vaccines are available against the human influenza but there is no mention that vaccination is advised. In addition, the potential seriousness of infection is downplayed. Thus, on pages 11, 13, 17 and 22 infection is described as mild, or typically mild and self-limiting. This takes no account of the potentially serious consequences for anyone who is pregnant, immunosuppressed, asthmatic, or has other respiratory diseases. We suggest that text is inserted into the forms at appropriate places stating that people working with influenza should be vaccinated and that anyone with a flu-like illness should not report to work or go to A&E, but should inform Occupational Health. In addition, vulnerable groups should be identified in the documents and warned of the potential risk.

Risk
The subcommittee considered that the greatest risk of generating unforeseen viruses through reassortment was posed by workers who were already infected with a circulating strain. Therefore, it is particularly important that the guidelines should make it clear that people suspected to be infected should not work on this project and these guidelines must be enforced. As the proposed work covers two separate sites (Church Street and Garscube), a local PI must assume responsibility for the safety and compliance of any visiting workers at Church Street and this must be clearly stated.

Scope
Although the proposal states (p11 of RA) that “equine, canine, and low pathogenicity avian viruses are unlikely to be used at this (Church Street) site”, the subcommittee did not wish to rule out such use in future. Therefore, the above statement should be removed and the text modified so as not to preclude use of these viruses at Church Street.

Disinfection
Under Waste Disposal (RA page 20) it should be made clear that Virkon is added to infectious liquid waste before it is removed from the safety cabinet.

Text
A number of matters were identified relating to the layout and wording of the RA:
The Church Street site (GM26) is designated as both a primary location (RA page 9) and a secondary location (page 11) for proposed laboratory work. This is ambiguous
and needs to be clarified.
Change BSL 2 laboratory to containment level 2 laboratory (RA page 9).
Church Street policy at present is that oseltamivir-resistant flu can only be handled in room 310. This should be specified.
References to Virkon treatment should make it clear that the final concentration must be ≥1%.
Spills of infectious or contaminated liquids should be inactivated with Virkon before being cleaned up (RA page 15).
Remove the reference to visual inspection of the safety cabinet by the PI (RA page 17).
List COSHH 237 under point 15 (RA page 19).

Following discussion between the co-applicant Ben Hale and Frazer Rixon, a second meeting of the CVR Church Street GM subcommittee was called for 27/05/2013, to discuss further the handling of reassortants containing genome segments from high pathogenicity avian influenza strains.

Present:
John McLauchlan
Joyce Mitchell
Arvind Patel
Emma Thomsen
Frazer Rixon convener.

Invited: Ben Hale

Apologies: Roger Everet

The subcommittee agreed the following additional recommendations:

Background information on the polybasic site and its importance for pathogenicity should be included in the proposal and it should contain a clear statement that viruses containing a polybasic site would not be generated or used.

The proposal could include mention of low-pathogenic H5N1 viruses engineered to lack the polybasic cleavage site: "HALo" but should contain a clear statement that work with such viruses would require prior consideration and approval by the local safety committee.

Methods to prevent accidental reassortment of genome segments resulting in viruses with potentially novel antigenic properties should be specified.
The requested changes were made and on 23/06/13 the revised documents were circulated to and approved by the subcommittee members on 25/06/13.

2. GM223 committee comments 13/05/13

The comments of GM 223 were that this was a detailed risk assessment with a lot of evidence, justification of arguments and references. The recommendations were primarily in agreement with those of GM 26 as detailed above and the PIs were notified accordingly. Clarification on any SAPO licence requirements was requested. Careful consideration should be given to the potential for reassortment of genome segments and this should be reflected in the laboratory practices. All workers using the designated facilities should be made aware of all pathogens and GMOs being handled in the areas. All solid waste generated from this project will be autoclaved and it is recommended that Virkon treated liquid waste also be autoclaved. Where this is not possible then references/documentation should be provided to support Virkon disinfection as a validated means alone for inactivation of the liquid waste generated.

GM 223 approved the revised documents on 24/06/13

### Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2</td>
<td>L3</td>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### Project Ref 26/18.1

- **Date Ackn'd**: 26/08/2021
- **CU2 Project Title**: Investigation of hepatitis B virus and hepatitis D virus infection and replication in cultured cells
- **Class**: Class 2
- **Culture Vol Class 2**: < 1 Litre
- **Class Culture Vol Class 3-4**: < 1 Litre
- **Non-GMM**: N
- **Consent Granted**: Yes
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**: Project transferred from GM26
The objective of this project is to investigate the interaction of hepatitis B virus (HBV) and hepatitis D virus (HDV) with host cells with a view to understanding the roles of viral and host factors in virus infection and replication, and their contribution to virus-associated pathogenesis. We will first establish in vitro cell culture systems to generate infectious virus using well-characterized prototype and patient-derived replication-competent viral genomic DNA. Viruses generated thus will be then used to infect different target cell lines to study, at the mechanistic level, the functions of the relevant viral and host components (e.g. proteins and/or nucleic acids) and the impact of their interactions on virus pathogenesis. It is hoped that the results obtained will inform development of novel anti-viral therapeutic avenues and may lead to bats as a new animal model for HDV.

Description of activities:

HBV is the prototypic member of the family Hepadnaviridae, which includes avian, primate and rodent hepatitis viruses. All hepadnaviruses share the property of restricted host range, a strong, but not exclusive, tropism for hepatocytes, and the ability to cause liver damage. HBV is a major human pathogen causing a chronic inflammatory liver disease often leading to the development of hepatocellular carcinoma (HCC). The clinical significance of HBV is easily demonstrated by the fact that more than 250 million people worldwide are chronically infected. HBV has a relaxed circular partially double-stranded DNA genome of approximately 3.2 kb. Following infection, the HBV genome is transported to the nucleus and converted to covalently closed circular (ccc) DNA which serves as a template for transcription of viral RNAs. Following transport of the pre-genomic RNA and translation in the cytoplasm, selective encapsidation of the RNA into the nucleocapsid ensues, along with encapsidation of RNA polymerase. In the immature capsid, the 3.5 kb mRNA is reverse-transcribed, to yield minus strand DNA. The RNA is degraded and the DNA strand is replicated, producing the second, shorter DNA strand. Nucleocapsids containing DNA genomes then acquire their outer envelope, probably by budding into the endoplasmic reticulum (ER) in areas where viral surface antigens (HBsAg) are inserted. The resulting particles are then transported from the cell by normal pathways of vesicular transport. There are 3 forms of HBsAg (called large, medium and small or L-HBsAg, M-HBsAg and S-HBsAg, respectively) which all share the same C-terminal (i.e. S-HBsAg), with the M-HBsAg being extended at its N-terminus by 55 amino acid (aa) preS2 segment and L-HBsAg additionally containing an N-terminal 108 to 119 aa preS1 domain.

HDV belongs to the genus Deltavirus causes chronic hepatitis D. The virion carries a circular single-stranded negative-sense RNA genome of ~1.7 kb in length. The viral replicative intermediate of positive-sense polarity encodes the delta antigen (HDAg) that is not packaged into the virion. HDV requires HBV as a helper virus in the form of its envelope proteins (i.e. HBsAg) to form infectious virus particles. Co-infection with HDV is associated with more severe pathological complications (e.g. increased risk of liver failure in acute infections and development of liver cancer in chronic infections) compared to HBV alone. HDV RNA replication occurs in the nucleus and the viral ribonucleoprotein is transported to the Golgi where it is enveloped by HBsAg. The secretion of the assembled virion is thought to occur via Golgi. Co-expression of HDV genome with HBsAg results in secretion of HDV particles into cell medium. This medium can be used as a source of HDV to infect susceptible naïve cells in which the virus initiates a single-cycle infection; i.e the virus delivers its replication-competent genome, but is unable to produce progeny virions as the cells lack HBsAg. While HDV has historically been considered a virus of humans, recent metagenomic sequence data obtained here at the MRC – University of Glasgow Centre for Virus Research (CVR) has discovered RNA which appears to represent an analogous virus (HDVb) circulating among wild bats (Desmodus rotundus) in South America (Bergner et al., unpublished data). Little is known of HDVb, but genomic similarities to HDV suggest it is likely to have a similar biology (i.e., narrow host range and requirement for a helper virus such as bat hepadnavirus). Ongoing sequencing studies here at the CVR aim to identify the analogous bat-associated HDVb genome using metagenomic sequencing and PCR. On account of the presence of HBsAg on HBV and HDV virions, both viruses share the same cell entry pathway although their genomes replicate differently. Cell entry is mediated by initial attachment of the virus to the cell surface heparin sulfate proteoglycans (HSPGs) followed by a specific interaction with the hepatocyte-specific sodium taurocholate co-transporting polypeptide (NTCP) (Yan et al., 2012 eLife1,e00049). The virus interaction with HSPG is mediated by the N-terminal preS1 domain of L-HBsAg and the antigenic loop of the S-HBsAg, while the interaction with NTCP is solely mediated by preS1. It is likely that additional as yet unidentified host factors are also required for virus entry into target cells.

The proposed project consists of 6 major components:

1. Generation of infectious viruses: We will use the well-characterized tetracycline-inducible HBV expressing cell line, Hep38.7-Tet (Ogura et al., Biochemical and Biophysical Research Communications 452 (2014), 315–321), to generate HBV for use in work described here. Additionally, we will also transfect mammalian cells (including hepatocytes) with replication-competent HBV DNA derived from well-characterised isolates or patients infected with different viral genotypes and isolates. This is expected to initiate a fully productive virus life cycle in transfected cells. Evidence of virus infection and replication will be monitored by the detection of viral RNA (by...
In vitro evolution of HBV and HDV: To reveal viral determinants of sensitivity/resistance to antiviral factors and reveal potential resistance pathways to antiviral molecules replication in cultured hepatocytes or other permissive target cells (vertebrate origin).

We will be co-transfected together with replication-competent HBV DNA and the yield of HBV will be quantified. In all instances, the methods described in step 1 above will be

Transduction with lentiviral vectors encoding each ISG prior to infection with HBV representing different genotypes and isolates. Alternatively, ISG-encoding vectors

Screen the ability of hundreds of individual ISGs to inhibit viral replication can be considered. Briefly, hepatocytes, including Hep38.7-Tet (or other permissive target cells)

Organoids derived from the human hepatocyte cell lines that stably express the virus entry receptor NTCP. The specificity and the mechanisms of viral entry will be explored further using a panel of in-house generated antibodies to preS1, the N-terminal domain of the L-HBsAg that plays a role in viral entry via its interaction with NTCP. Together, these experiments will allow us to measure and study the mechanics of virus entry and resistance to drugs, and to evaluate vaccine escape.

Infection of cells with clinical HBV isolates: Sera collected from HBV-infected individuals will be used as an inoculum for infection of appropriate cell lines. Initially all patients will be recruited in UK and only samples tested negative for cytomegalovirus and HIV will be used. Samples from overseas and/or from patients screened positive for HIV might be used at a later stage of the project. In this case, samples will be screened for the infectious agents most likely to be present in the sera (depending on their origin). Samples containing adventitious agents will be excluded and any import into the CVR will be subject to the local Safety group approval. Evidence of infection will be monitored as described in step 1 above.

Investigating the effects of interferon-stimulated genes (ISGs) in HBV and HDV infection: Type I interferons (IFNs) suppress the replication of HBV in vitro and in vivo. However, the success of IFN therapy is variable and influenced by the HBV genotype. Despite this, relatively little is known about which host ISGs inhibit HBV and why some genotypes respond better to IFN therapy. We have developed libraries of ISGs from humans, rhesus macaques and cows as well as diverse ISGs from diverse vertebrate species (Schoggins et al., 2011, Nature 472(7344):481-485; Kane et al., 2016 Cell Host Microbe 20(3):392-405). These libraries are arrayed so that in a single screen the ability of hundreds of individual ISGs to inhibit viral replication can be considered. Briefly, hepatocytes, including Hep38.7-Tet (or other permissive target cells) will be transduced with lentiviral vectors encoding each ISG prior to infection with HBV representing different genotypes and isolates. Alternatively, ISG-encoding vectors will be co-transfected together with replication-competent HBV DNA and the yield of HBV will be quantified. In all instances, the methods described in step 1 above will be used to quantify the level of virus infection/gene expression and/or replication in order to identify inhibitory ISGs. Similarly we are interested in which ISGs inhibit the replication of HDV and whether such inhibition is direct or a consequence of HBV inhibition (indirect). Thus, we will examine the ability of different ISGs to inhibit HDV replication in cultured hepatocytes or other permissive target cells (vertebrate origin).

In vitro evolution of HBV and HDV: To reveal viral determinants of sensitivity/resistance to antiviral factors and reveal potential resistance pathways to antiviral molecules or antiviral candidates we will propagate HBV (and HBV and HDV) in the presence of antiviral factors (genes from various species), candidate antiviral molecules, approved antivirals, antiviral cytokines or experimental drugs. The methods described in step 1 and sequencing approaches will be used to monitor changes in the viral genome(s) selected during the passage experiments.
We will use a genetically engineered stable cell line, Hep38.7-Tet, conditionally replicating and secreting HBV particles as source of virus in our studies. Additionally, we will generate HBV and HDV by reverse genetics following transfection with patient-derived infectious genomic DNA into hepatocyte cell lines. Where necessary, appropriate genetic modifications will be introduced into the relevant part of the viral genomic cDNA to generate genetically modified (GM) viruses of interest. These modifications may include specific mutation of amino acids that are functionally and structurally important in virus infection/replication, for reactivity to antibodies of interest, and for conferring antiviral resistance (the latter will be based on those observed in circulating strains, or those identified in our studies on ‘in vitro evolution of HBV and HDV’ as described in point 6, of Section 6). Additionally, we will also attempt to generate viruses expressing reporter genes or epitope tags (including but not limited to GFP, RFP and Luciferase). The modifications are not expected to alter host tropism.

Host/vector system

Viruses: Different WT and GM derivatives of HBV and HDV, including a bat-associated subtype of HDV (HDVb).

Bacterial: Disabled E. col K12-derived strains such as DH5α. These strains can only survive in a controlled laboratory environment and will be used for propagation of plasmids.

Mammalian: Various vertebrate hepatocyte and non-hepatocyte stable cell lines.

Vector systems: Non-mobilisable pUC-derived plasmids, pBluescript, pCDNA and lentiviral vectors

Origin & function

Virus will mainly be obtained from a HBV producer cell line, Hep38.7-Tet, which will be obtained from collaborators in Japan. This cell line is well-characterised by them (Ogura et al., Biochemical and Biophysical Research Communications 452 (2014), 315-321) and is widely used by different groups. It will be imported from Japan as ‘UN2814 Category A infectious Substance affecting humans’ and will be handled in our Containment Level 3 (CL3) facilities. Additionally, we will produce both HBV and HDV by reverse genetics using patient-derived viral genomic nucleic acids for HBV and HDV and bat-derived viral genomic nucleic acids for HDVb. We also plan to attempt to directly propagate viruses derived from clinical samples (serum/plasma) following inoculation into cultured cells.

GM cell lines that ectopically express host proteins of interest (e.g. the virus receptor NTCP, viral dependency or restriction factors) will be generated. In some cases cell lines lacking host factor(s) identified as of interest in the study will be generated either through transient knock-down or permanent gene knock-out technologies. These GM cell lines will allow assessment of the functions of the relevant viral and/or host factors on virus infection, replication and pathogenesis.

Evaluation of foreseeable effects

Risk associated with HBV and HDV generated in this project: Viruses will be rescued by reverse genetics. There are no plans to carry out extensive mutational analyses of the viral genomes - mutations introduced will be mainly based on sequences circulating in patients including those conferring resistance to drugs. Additionally, in order to understand and delineate at molecular level aspects of viral life cycle (e.g. entry, replication, assembly) we may create specific amino acid changes in viral or host proteins of interest, or insert reporter genes or epitope tags (including but not limited to GFP, RFP and Luciferase). However, we expect these modifications to not adversely alter the replication, transmission or tropism of the viruses. If anything, the modifications would result in attenuated viruses.

Risks associated with GM cell lines: As mentioned above, the HBV producer cell line, Hep38.7-Tet, will be used as one source of virus. This cell line will always be handled under our CL3 facilities: the Richard M. Elliott Biosafety Laboratories (REBL) and/or MacRobert Blood-Borne Virus (BBV) laboratory (see section 11 below). All work will be strictly performed as per the criteria laid out in the REBL and BBV CL3 laboratories Codes of Practice and Risk Assessment associated with this Notification (see disinfection and waste disposal procedures). Risk of accidental infection to personnel working in the CL3 laboratory is very low, and the likelihood of virus being released from the laboratory is effectively nil.

Risks to the environment: Both HBV and HDV are Hazard Group 3 pathogens. In accordance with this classification all work involving the generation and handling of these viruses proposed will be performed in our REBL or MacRobert BBV CL3 facilities as described above (see section 11 below). HDVb is currently unclassified with respect to ADCP Hazard Group, and has never been found or suspected to infect humans or species other than bats. Nevertheless, as a precaution, potentially infectious HDVb will be handled only in our CL3 facilities. All work is strictly performed as per the criteria laid out in the REBL and BBV CL3 laboratories Codes of Practice and Risk Assessment associated with this Notification (see disinfection and waste disposal procedures). Risk of accidental infection to personnel working in the CL3 laboratory is very low, and the likelihood of virus being released from the laboratory is effectively nil. Therefore, the risk to the environment is effectively zero.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We have recently been granted derogation under Regulation 19 of the Genetically Modified (Contained Use) Regulations 2014 to dispense with sealability for the MacRobert BBV CL3 laboratory in relation to work with wild-type and genetically modified Hepatitis C virus (HCV) and Human Immunodeficiency virus (HIV) - notified activities GM26/99.3a and GM223/13.1b, respectively.

Similarly to HCV and HIV, HBV and HDV are blood-borne viruses, they are not infectious through the aerosolised/airborne route. Although aerosolised infection by highly concentrated HBV or HDV stocks remains a theoretical transmissible route, no case has been documented. Moreover, both viruses are sensitive to common chemical disinfectants such as 70% Industrial Methylated Spirit (IMS), Virkon and Distel. Little is known oh HDVb, but genomic similarities to HDV suggest it is likely to have a similar biology (i.e., narrow host range and a requirement for a helper virus such as bat hepadnavirus). Considering the transmission routes of HBV and HDV and the scope of work we intend to do (as detailed in this form and the attached risk assessment), we consider that the risk of using highly toxic fumigants (such as formaldehyde or hydrogen peroxide) surpasses the risk of contamination by or release of the viruses and we propose to dispense with fumigation for room disinfection. Instead, we propose to use chemical surface disinfectants for regular and emergency disinfection procedures, as detailed in the attached risk assessment.

Hence, we are seeking derogation from full containment level 3 measures and propose to handle HBV and HDV/HDVb in the MacRobert BBV derogated CL3 laboratory in the same way as already consented by HSE for HCV and HIV: uncontained infectious material will only be handled in microbiological safety cabinets (MSC); no concentration of virus by ultracentrifugation will be permitted and propagation of virus will be limited to no more than 10 to the 6 genome copies/ml. No laboratory-acquired infection of HBV or HDV via aerosols have ever been reported in this concentration range. Any work requiring higher titres of HBV or HDV will necessitate concentration of the virus by ultracentrifugation or other means and will be carried out in the REBL CL3 Suite, which operates under more stringent safety procedures and is suitable for room fumigation for regular and emergency disinfection. See associated risk assessment for fully detailed containment measures, inactivation and waste disposal procedures.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment Level 2 areas:

Liquid waste: add Virkon to final concentration of 1% (w/v) overnight, then discharge to drains.

Disposable solids (eg plasticware such as flasks, tubes, pipette tips etc.): soak in 1% Virkon (w/v) overnight. Transfer solid contents to autoclave bags and autoclave at 121°C for a minimum of 15 minutes prior to final disposal by district council to landfill, remaining liquid to be discharged to drain.

Other solids (agar plates, gloves etc.): place in a marked box lined with a clear autoclave bag, and autoclave at 121°C for at least 15 minutes. Seal contaminated plates before placing in bag, to avoid lid falling off. Gloves should be disinfected with alcohol before putting in containers.

Degree of kill:

Chemical sterilisation by Virkon - effectively 100% kill

Autoclaving - effectively 100% kill (annual validation)

MacRobert BBV derogated CL3 laboratory:

Liquid waste: decontaminate in Virkon 1% w/v (final concentration) overnight. When handling protein rich liquid, Virkon powder should be added to give a final concentration of no less than 1% w/v. After inactivation, discharge liquids to drain.

Solid waste from within the MSC: discard paper waste within the MSC into a metal bin lined with autoclave bag for autoclaving. Cell culture plastics which can be submerged must be soaked overnight in 1% w/v Virkon prior to autoclaving. Flasks, once liquid has been removed, can be rinsed with 1% w/v Virkon, capped, surface-decontaminated with 70% IMS and disposed of into the metal bin outside the MSC for autoclaving. Disposable plastic pipettes must be submerged overnight in 1% w/v Virkon prior to autoclaving. Gloves must be sprayed with 70% IMS prior to being discarded into an autoclave bin.

Sharps which arise inadvertently or glass slides/cover slips must be placed into clearly labelled sharps bins containing enough 1% w/v Virkon solution to cover all material and autoclaved.

Solid waste from outside the MSC: other waste generated within the laboratory, including soiled lab coats/gowns must be placed directly into disposable bags for autoclaving.

All solid waste is to be autoclaved at 121°C for 15 minutes prior to being sent for incineration.
Degree of kill:
Chemical sterilisation by Virkon - effectively 100% kill
Chemical sterilisation by 70% IMS - effectively 100% kill
Autoclaving - effectively 100% kill (annual validation)

The Richard M. Elliott Biosafety Laboratories (REBL) CL3 Suite:
Liquid waste: decontaminate in Virkon 1% w/v (final concentration) overnight. When handling protein rich liquid, Virkon powder should be added to give a final concentration of no less than 1% w/v. After chemical inactivation, liquid waste must be either autoclaved at 121°C for 15 min or disposed through ACTINI effluent treatment plant (135°C for 2 minutes).
Solid waste from within the MSC: spray paper waste with 1% w/v Virkon within the MSC, then with 70% IMS outside the MSC prior to autoclaving. Solid which can be submerged must be soaked overnight in 1% Virkon w/v (final concentration) prior to autoclaving. Solids which cannot be submerged must be filled with or soaked in Virkon 1% w/v (final concentration) for 10 minutes, wiped down with 1% w/v Virkon within the MSC then with 70% IMS outside the MSC prior to autoclaving. Sharps which arise inadvertently or glass slides/cover-slips must be placed into clearly labelled sharps bins containing enough Virkon solution to cover all material and autoclaved.
Solid waste from outside the MSC: other waste generated within the laboratory, including soiled protective clothing, must be sterilised by autoclaving. Solid waste is to be autoclaved at 134°C for 5 minutes, fabrics are to be autoclaved at 134°C for 3.5 minutes.
Following ACTINI treatment or autoclaving, liquid waste is disposed to drain. After autoclaving solid waste is sent for incineration.
Degree of kill:
Chemical sterilisation by Virkon - effectively 100% kill
Autoclaving - effectively 100% (annual validation)

The local GM Committee asked for more detail on the origin of the human clinical samples and the nature of biological agents likely to be present in the sera. This section of the risk assessment was extended to clarify this point. Assurance was sought that in vitro propagation of viruses in the presence of antiviral factors would not give rise to 'super-resistant' viruses and the risk assessment was amended to consider this eventuality. More information was requested on the oncogenic properties of HDV; the risk assessment was extended to clarify this point.

The GM Committee was satisfied that containment and control measures were suitable for this project. The Committee was equally satisfied by the justification for derogation from full containment level 3 and by the boundaries set up for the work to be carried out in the derogated CL3 facility.

Project Containment

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Project Ref** 26/94.1

**Date Ackn’ed**

26/08/2021

**CU2 Project Title**

PRODUCTION OF BUNYAVIRUSES CONTAINING GENOME SEGMENTS DERIVED FROM CLONED CDNA

**Class**

Class 2

**CultureVolClass2**

Class 2

**CultureVolumeClass3-4**


**Non-GMM Consent Granted**


**Project notified under transitional arrangements**

Y

**Withdrawn**

N

Tick if notifying a connected programme of work  

N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**


**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  26/99.3

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Withdrawn  N

Tick if notifying a connected programme of work  N
Historical Significant Changes: Project transferred from GM26

Historical Date of Additional Info:

Significant Change ID: 26/99.3a

Date of Significant Change: 26/07/2017

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 26/trans1

Date Ackn'd: 26/08/2021
CU2 Project Title: GENETIC MODIFICATION OF HERPESVIRUS

Class: Class 2
CultureVol: Non-GMM
ClassVolume: Consent Granted

Project notified under transitional arrangements

Withdrawn: No

Tick if notifying a connected programme of work: No
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: TRANSFERRED FROM GM 26 (1/11/07)
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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If yes, tick to confirm that it is attached to this form  

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**Project Ref**  26/trans3

<table>
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<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>26/08/2021</td>
<td>GENETIC MODIFICATION OF VACCINIA VIRUS AND OTHER CLASS 2 POXVIRUSES</td>
<td>Class 2</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
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</table>

Withdrawn  

Tick if notifying a connected programme of work  

Project notified under transitional arrangements  

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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**Project Ref** 317/06.1

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<td>09/04/2013</td>
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Withdrawn N

Historical Significant Changes

Transferred from GM317 on 09/04/2013

Historical Date of Additional Info

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<td>21/07/2021</td>
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**Project Additional Information**

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Purposes of the contained use

The purpose of this project is to understand the molecular biology of bunyavirus replication, including structure-function analysis of viral proteins, the role of cis-acting signals within vRNA and the interactions of bunyaviruses with host cells.

Recipient or parental organism

E. coli K-12 derivatives (e.g. DH5a), E. coli B strains (e.g. BI-21), S. cerevisiae strains AH109 and Y187 (from Clontech) Mammalian cell lines (e.g. vero, BHK, Hep2, mRC-5, 293, HeLa), insect cell lines (e.g. Aedes albopictus C6/36, S. frugiperda).

Host/vector system

pUC series, pBluescript, pET series vectors for E. coli. pGBK7 and pGADTT7 for yeast cells. pcDNA and non-cytopathic Sinbis virus replicons pSinRep for mammalian cells. pIB/v5-His for insect cell lines. pBac vectors for generating recombinant baculovirus systems. Autographa californica nuclear polyhedrosis (AcNPV) derivatives such as AcPAK6 for insect cell lines. Expression in Vaccinia virus vectors pTF7-5 and pSC11 for constructing recombinant vaccinia viruses. Vaccinia virus vTF-7 and MVA-T7. Lentivirus vector pLenti-IRES-puro.

Origin & function

Bunyaviral genes:- N-nucleocapsid (binds viral RNA), L-viral RNA dependent RNA polymerase, Gn-viral glycoprotein (receptor binding, cell fusion), Gc- viral glycoprotein (Receptor binding, cell fusion), NSs - non-structural protein (interferon antagonist; blocks RNA polymerase II transcription), NSm - non-structural protein (Localises to Golgi- unknown function).

Cellular genes encoding proteins that interact with bunyavirus proteins:- HeLa genome library for screening by yeast-2-hybrid assay. Specific genes:- Med8 (a component of the Mediator complex involved in the regulation of RNA polymerase II mediated transcription), ARC-32 (mosquitto cell homologue of Med8), other individual genes that encode proteins which interact with bunyavirus proteins once they have been indentified by the yeast-2-hybrid screening.

Evaluation of foreseeable effects

E. coli

The K-12 cell lines are known to be non-pathogenic (see ACGM Compendium of Guidance (ISBN: 0-7176-1763-7). The E. coli cell lines have been less well characterised than the K-12 strains. BL-21 cells are less well characterised than K-12 strains but have a long history of safe use. A recent publication has shown that BL-21 cells is non-pathogenic ans unlikely to survive in certain host animal systems (Chart, H. et al J. Applied Microbiology Vol 89, pages 1048-1058 (2000)). It is thus considered that BL-21 cell are non-pathogenic, though care will be taken when handling this cell line.

Baculovirus System:

The Baculoviruses system have a long history of safe use. The system has been used extensively to infect insect cells with foreign genes and there has been no published evidence that viable recombinant virus has been produced in these cells.

Yeast Cells

Yeast cells are considered to be especially disabled host cells and it is not considered that any of the hybrid proteins expressed will alter the biological properties of the host.

Vaccinia vectors:

Vaccinia virus can infect humans and is classified as an ACDP category 2 pathogen. None of the inserted genes to be expressed is likely to significantly alter the pathogenicity of the vaccinia vector. Recombinant genes have been targeted in the viral thymidine kinase gene and such recombinants are considered to be attenuated relative to the parent. Therefore containment level 2 which is used to contain the parent virus is appropriate for handling the recombinant virus. The avoidance of sharps will reduce the major route of infection which is subcutaneous inoculation.
Disabled Lentivirus
Disabled lentivirus vectors are widely used for expressing foreign genes under level 2 containment. Inoculation of the virus into man could result in integration of the viral genome and expression of the foreign gene. Avoidance of sharps will minimise this route of infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
All work with the GMOs will be performed in Category 2 containment facilities as defined in the ACGM Compendium of Guidance (ISBN: 0-7176-1763-7). Appropriate operating procedures will be implemented to ensure only authorised, appropriately trained, personnel can carry out this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All plastic waste from experiments will be autoclaved at 120°C for 30 minutes at 15 p.s.i.
All media and liquids will be treated with Virkon solution before disposal as per the manufacturer's instructions.
Spillages will be dealt with by soaking the area in neat Virkon.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The genetic modification project notification has been studied by the Chemical and Biological Hazards Sub-Committee and has been provisionally ratified the project as a category 2 project as defined by the GMO (Contained Use) Regulations 2000.

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2  Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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</table>
## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

Large Scale Activities

Human Clinical Applications

**Project Ref 613/96.1**

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Historical Significant Changes

Historical Date of Additional Info

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment
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**Project Ref** 613/97.2

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**Historical Significant Changes**
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? ☐

If yes, tick to confirm that it is attached to this form ☐

Tick to confirm that you have attached a risk assessment to this form ☐

Tick if you are claiming exemption from disclosure for section of the risk assessment ☐

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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**Name**

UNIVERSITY OF LIVERPOOL

**Department**

IMMUNOLOGY

**Campus Estate or Research Centre**

3RD FLOOR DUNCAN BUILDING

**Road Name**

DAULBY STREET

**Town**

LIVERPOOL

**County**

MERSEYSIDE

**Postcode**

L69 3GA

**Country**

ENGLAND

**Tel Number**

0151 706 4364

**Fax Number**

0151 706 5814

**HSE Division**

NORTH WEST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

[ ]

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Other (please specify) [ ]

Tick if confidential

[ ]

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Virology | Transgenic | Transgenic | Gene Therapy |
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<tbody>
<tr>
<td>Animals</td>
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<td>Fish</td>
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Maximum volume used 2 litres in 400 ml aliquots.

Waste disposal

1. Contained transfer to sterilising autoclave validated and audited by indicator, and thermocouple testing.
2. Liquid waste or culture fluids. Treated with Virkom or similar proprietary sterilising fluid treated as directed by the manufacturers.

### For activities involving GMMs, describe the waste management measures which will apply to the activity

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<th>Transgenic Plants</th>
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**Tick to confirm that you are attaching a summary of the risk assessment**

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

Please enter comments of the GM safety committee on the risk assessment

02/03/2022
GM Centre Number: 226

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Name

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Name 2

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Campus Estate or Research Centre

<table>
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Road Name

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County

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HSE Division

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Comments

Date at Which Additional Info Submitted

| 02/03/2022 |
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Level 4 (GMMs)</td>
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<tr>
<td>Other (please specify)</td>
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</tbody>
</table>

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

Culture volume: 50 mls maximum.

Deactivation:

a) liquid (spent broth):
   i) volumes of up to 5 mls: treatment with Precept tablets or hycolin for a minimum of 24 h or alternatively by autoclaving.
   ii) volumes of > 5 mls - 50 mls: sterilisation by autoclaving or via incineration.

b) solid (Petridishes, Eppendorf tubes etc.)
   i) transfer into bags or boxes followed by incineration, or by
   ii) autoclaving and transfer into boxes.

Monitoring and deactivation:
Complete inactivation of live cells undertaken within the Department is regularly checked by withdrawing aliquots from disinfected or autoclaved material and plating out on LB agar plates to analyse for microbial growth. No microbial growth has been detected.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 226/12.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>08/02/2012</td>
<td>Molecular analysis of population variation and virulence behaviour of Neisseria spp., Haemophilus influenzae, Helicobacter pylori, and Escherichia coli</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Non-GMM  Consent Granted</td>
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</tbody>
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Date Project Ceased 02/03/2022
Project Additional Information

Purposes of the contained use
To understand the fundamental determinants of virulence and the determination of bacterial physiology and regulatory behaviours relevant to the ways in which bacteria establish and coordinate benign and pathogenic interactions with each other and their hosts, as a framework for the development of novel diagnostic and therapeutic interventions, including vaccine development.

Recipient or parental organism
The species and strains used will be naturally occurring strains of N. meningitidis, N. gonorrhoeae, E. coli, H. influenzae, and H. pylori. Wherever possible / normally these will be fully sequenced strains, where the manipulations are performed in highly determined genetic backgrounds. In addition, where the use of such strains does not compromise the experimental goals, parent strains of N. meningitidis will be partially attenuated ‘capsule null’ strains. Other studies will be performed in laboratory (Class 1) strains of E. coli, where possible derived from sequenced strain K12.

Host/vector system
For the naturally transformable species (N. meningitidis, N. gonorrhoeae, H. influenzae, and H. pylori), DNA transformation / chromosomal insertion/deletion mutants will be generated by natural transformation. For E. coli, plasmid constructs will be used for extra-chromosomal modification, and suicide vectors for targeted chromosomal insertion / deletions.

Origin & function
The origins of the material used will be primarily from other naturally occurring strains, the only other genes will be widely used selectable markers and reporter genes. No marker or reporter will be used that is not widely used and known not to influence virulence. No selectable agent used for routine clinical treatment or prophylaxis will be used. The purpose of the experiments is primarily to explore the contributions of naturally occurring genes and gene combinations to virulence, in terms of gene complement and expression. Thus, the studied functions and genes are endogenous, and are primarily studied in a way that addresses naturally occurring functions, interactions, and evolutionary processes. The functions include core metabolic functions that affect niche adaptation, and virulence factors that directly influence the interaction of bacteria with each other, with the host, and within the host-associated microenvironmental conditions.

Evaluation of foreseeable effects
a) Risk assessment for human health and safety
The unmodified pathogenic species included within this proposal are ACDP hazard group 2 microorganisms. In each instance the donor and recipient species are the same except during gene construction / manipulations in disabled E. coli. Therefore, no new or additional potential risks will be created through the movement of any candidate virulence gene to a new potentially pathogenic species background.
N. meningitidis is the principal cause of bacterial meningitis and is a cause of serious septicaemia. It is normally a harmless human commensal. N. gonorrhoeae is the cause of gonorrhoeae, and more rarely conjunctivitis, pharyngitis, rectal infection and rarely disseminated infection.

H. pylori is a cause of infective gastritis and peptic ulcer disease, that if left untreated can pr-dispose to gastric cancer and, very rarely, lymphoma.

E. coli is a cause of urinary tract infections and occasionally infectious diarrhoea, although it normally exists as a harmless human commensal organism.

Haemophilus influenzae is a cause of respiratory infections, meningitis, and otitis media. The strain used is predominantly Rd, which is a capsule-deficient attenuated strain. The experiments using this species are limited to reporter gene systems that report phase variation, and these do not enhance or alter any virulence property.

The mutations that are to be introduced are each likely to result in a reduction rather than an increase in fitness and / or virulence. The majority of mutations will either fix a gene ON that is normally present within the species and where the removed variability of the gene confers fitness, or will ablate expression of genes resulting in the loss of a normal function. Antibiotic markers will be used that are not normally the primary therapeutic agents. The reporter genes and counter-selectable markers will be either neutral or lead to a reduction in bacterial fitness. Gene transfer in these species does not necessitate the use of vectors that will increase the subsequent movement of altered genes, and the populations are broadly panmictic, and thus combinations of genes created in the laboratory experiment will be occurring naturally, and when not selected / naturally present will be predominately selected against due to reduced fitness. There will be no introduction of virulence genes from one pathogenic species to another. For these reasons none of these manipulations will effect the host range, enhance or broaden tissue tropism, or decrease susceptibility to host defence mechanisms.

It is a characteristic of our work that we study the phenotypic and physiological consequences of naturally occurring behavioural determinants. We do this primarily in panmictic populations in which these elements are free-moving within and between the bacterial populations, and thus the phenotypes generated will be naturally occurring. Thus, in our experiments we will not create phenotypes or strains that are more virulent than those that can and do arise naturally.

After consideration of all factors, the assessment of risk to human health indicates no greater hazard than are likely with the ‘wild-type’ unmodified strains. Therefore all work with these microorganisms will be undertaken at the ACGM Containment Level 2 with the use of safety cabinets as appropriate.

Assignment of provisional containment level: Containment level 2 with the use of a microbiological safety cabinet for the manipulation of N. meningitidis in suspension / whenever aerosols could be created, or within a Category 3 facility. All procedures will be conducted with good microbiological practice and good occupational safety and hygiene.

Additional precautions for working with N. meningitidis:
N. meningitidis represents a greater hazard than the other pathogens included within this programme of research, particularly when in suspension and there is a potential for the generation of aerosols. As a result a microbiological safety cabinet will be used for all manipulations involving suspensions of the organism. Meningococcal work in the laboratory focuses upon serogroup B strains. Wherever possible a strain that has been attenuated by inactivating the siaD gene will be used. This gene is required for the formation of the capsule, which is largely responsible for the organisms’ capacity to evade the immune response and cause invasive disease such as septicaemia and meningitis. Vaccines are not available to the serogroup B meningococci, but vaccines will be offered, where appropriate, depending on the availability of vaccines for the strains that are being handled.

b) Nature of the work to be undertaken:
 Constructs for altering the expression and phenotypes of the pathogens will be prepared in disabled E. coli strains. These will then be transformed into the experimental
pathogen strains. The phenotypes of the altered strains will be determined using standard methods including direct studies of the cells by gel electrophoresis, and investigation of their altered behaviours under different environmental conditions, in cell-culture model systems and animal models as appropriate.

The experiments proposed use small-scale cultures. Up to 200ml maximum culture volumes will normally be used for disabled E. coli. Up to 1L will be used for protein preparation / purification. Work on the hazard group 2 organisms will be predominantly conducted on solid media. Liquid culture volumes will not exceed 200 ml.

No non-standard laboratory operations will be used. Animal containment Level 2 will be used for in vivo work with the use of a microbiological safety cabinet where appropriate.

c) Risk assessment for environmental harm

For the reasons described in the above section on risks to human health and safety, and in the ‘Overview of the project’ the modified strains are likely to be generally reduced in fitness compared to the wild-type organisms. Therefore, in each instance, the risks to the environment of the genetically modified organisms and their capacity to survive, establish and disseminate is likely to be equivalent to, or usually lower than that of the ‘wild-type’ strain. Mutants will be phenotypically and genetically stable and the potential for transfer of genetic material between GMOs and other organisms is negligible. The risk of transfer to humans is medium but the likelihood of a hazard being manifested is negligible because of the containment measures that will be employed and because the resistance markers that will be used are not primary therapeutic agents used against these organisms. E. coli has a broad host range while H. pylori, whilst there are some animal hosts, is primarily a human adapted species. In the cases of the pathogenic Neisseria species, these have no environmental or secondary host reservoirs so their route of dissemination is limited by their species specificity for man. Therefore, for each of these species, the overall risk is effectively zero and no additional containment or control measures are considered necessary to protect the environment other than those described to protect human health.

Containment level 2 will be applied for all laboratory work and animal containment Level 2 for in vivo work. A microbiological safety cabinet will be used for the manipulation of N. meningitidis in suspension / whenever aerosols could be created. All procedures will be conducted with Good Microbiological Practice and Good Occupational Safety and Hygiene. The containment and control measures that will be applied for handling the recombinant bacteria are indicated on the attached tables.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.**

**Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.**

**Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.**

**Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.**
Degree of kill

Autoclaving: effectively 100% kill (annual validation).
Incineration: effectively 100% kill (annual validation).

Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick if confirming that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

The risk assessment is sufficient and appropriate to the work. Both human health and environmental issues are addressed appropriately. The facilities required are available at Brunel.

Please enter comments on the GM safety committee on the risk assessment

The risk assessment is sufficient and appropriate to the work. Both human health and environmental issues are addressed appropriately. The facilities required are available at Brunel.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<table>
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<th>226/12.2</th>
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Molecular analysis of virulence factors of Candida albicans

Class | Culture Vol | Class-2 Culture Volume
|------|-------------|------------------------|
Class 2 | < 1 Litre

Non-GMM Consent Granted

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

02/03/2022
### Project Additional Information

#### Purposes of the contained use

<table>
<thead>
<tr>
<th>Purposes of the contained use</th>
</tr>
</thead>
<tbody>
<tr>
<td>To understand the fundamental determinants of virulence and the determination of fungal physiology and regulatory behaviours relevant to the ways in which C. albicans establishes and coordinate benign and pathogenic interactions with their hosts, as a framework for the development of novel diagnostic and therapeutic interventions</td>
</tr>
</tbody>
</table>

#### Recipient or parental organism

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>The strains used will be naturally occurring strains of C. albicans. These will be fully sequenced strains, where the manipulations are performed in highly determined genetic backgrounds</td>
</tr>
</tbody>
</table>

#### Host/vector system

<table>
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<tr>
<td>DNA transformation, chromosomal insertion/deletion mutants will be generated by transformation</td>
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</table>

#### Origin & function

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
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<tbody>
<tr>
<td>The origins of the material used will be primarily from naturally occurring C. albicans strains. The only other genes will be widely used selectable markers and reporter genes. No marker or reporter will be used that is not widely used and known not to influence virulence. No selectable agent used for routine clinical treatment or prophylaxis will be used. The purpose of the experiments is primarily to explore the contributions of naturally occurring genes and gene combinations to virulence, in terms of gene complement and expression. Thus, the studied functions and genes are endogenous, and are primarily studied in a way that addressess naturally occurring functions, interactions, and evolutionary processes. The functions include filamentation and virulence factors that influence the interaction of C. albicans with the host</td>
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#### Evaluation of foreseeable effects

<table>
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<tbody>
<tr>
<td>a) Risk assessment for human health and safety</td>
</tr>
<tr>
<td>The unmodified opportunistic pathogenic C. albicans included within this proposal is an ACDP hazard group 2 microorganism. In each instance the donor and recipient species are the same except during gene construction/manipulations in E. coli. Therefore, no new or additional potential risks will be created through the movement of any candidate virulence gene to a new potentially pathogenic species background.</td>
</tr>
<tr>
<td>C. albicans is the most common cause of candidates which encompasses infections that range from superficial to systemic diseases. It is normally a harmless human commensal and candidasis is often observed in immunocompromised individuals.</td>
</tr>
<tr>
<td>E.coli is a cause of urinary tract infections and occasionally infectious diarrhoea, although it normally exists as a harmless human commensal organism. Only disabled E. coli strains will be used.</td>
</tr>
</tbody>
</table>
The mutations that are to be introduced are each likely to result in a reduction rather than an increase in fitness and/or virulence. The majority of mutations will either delete or over-express genes.

Antibiotic markers will be used that are not normally the primary therapeutic agents. The reporter genes and selectable markers will be either neutral or lead to a reduction in fungal fitness. Gene transfer in these species does not necessitate the use of vectors that will increase the subsequent movement of altered genes.

After consideration of all factors, the assessment of risk to human health indicates no greater hazard than are likely with the 'wild-type' unmodified strains. Therefore, all work with these microorganisms will be undertaken at the AGCM Containment Level 2. There is a potential for generation of aerosols when working with C. albicans in suspension. Therefore, a microbiological safety cabinet will be used for all manipulations involving suspensions of C. albicans. All procedures will be conducted with good microbiological practice and good occupational safety and hygiene.

b) Nature of the work to be undertaken:

Constructs for altering the expression and phenotypes of the pathogens will be prepared in disabled E. coli strains. These will then be transformed into the experimental pathogen strains. The phenotypes of the altered strains will be determined using standard methods including direct studies of the cells by gel electrophoresis, and investigation of their altered behaviours under different environmental conditions.

The experiments proposed use small-scale cultures. Up to 100 ml maximum culture volumes will normally be used for disabled E. coli. Work on C. albicans will either be on solid media or in liquid culture no exceeding 100 ml.

No non-standard laboratory operations will be used.

c) Risk assessment for environmental harm

For the reasons described in the above section on risks to human health and safety, and in the 'Overview of the project' the modified strains are likely to be generally reduced in fitness compared to the wild-type organisms. Therefore, in each instance, the risks to the environment of the genetically modified organisms and their capacity to survive, establish and disseminate is likely to be equivalent to, or usually lower than that of the 'wild-type' strain. Mutants will be phenotypically and genetically stable and the potential for transfer of genetic material between GMOs and other organisms is negligible. The risk of transfer to humans is medium but the likelihood of a hazard being manifested is negligible because of the containment measures that will be employed and because the resistance markers that will be used are not primary therapeutic agents used against these organisms.

Containment level 2 will be applied for all laboratory work. A microbiological safety cabinet will be used for the manipulation of C. albicans in suspension/whenever aerosols could be created. All procedures will be conducted with Good Microbiological Practice and Good Occupational Safety and Hygiene.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes). Discharge any excess liquids to drains, solids will be collected and incinerated.
Liquids (eg samples, culture supernatants) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes, discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, solids will be collected and incinerated.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill

Autoclaving: effectively 100% kill (annual validation by the manufacturer). Furthermore, the project leader will determine cell survival rate following autoclaving every 4 months.

Incineration: effectively 100% kill (annual validation).

**Project Containment**

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Please enter comments on the GM safety committee on the risk assessment

Dr H - delivery of a presentation on the genetic modification of the human fungal pathogen C. albicans was well received by members of the Biological and Genetic Modification Safety Committee (BGMSC). The project is aimed at understanding virulence and how fungal infections become established.

The chair concluded by advising Dr H to produce a simplified risk assessment for submission to the Health & Safety Executive following discussions with Dr A S and to proceed if no other comments had come through from other Committee members.

<table>
<thead>
<tr>
<th>Is an emergency plan required according to regulation 20?</th>
<th>N</th>
</tr>
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<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
<td>N</td>
</tr>
<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
</tr>
<tr>
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02/03/2022
Molecular mechanisms of T cell mediated immune responses

The establishment of robust T cell immune response with memory function is critical for the development of novel vaccines for infections and cancers. Early growth response 2 (EGR2) has been shown to promote peripheral naïve T-cell proliferation and differentiation under infection condition, however, its role in generating and maintaining immunological memory during immune responses is still not clear. We would like to establish two chronic viral infection models for the mouse experiment to study the role of Egr2 in immunological memory during chronic viral infections.

Both models are provided very unique tools to study the role of Egr2 in immune response in viral infections, (1) recombinant replication deficient adenovirus - Classified as GM01 through risk assessment and(2) rMCMV - Classified as GM02 through risk Assessment.

Viruses that persist in their host have evolved different strategies to evade clearance by the immune system. In humans HIV leads to a persistent infection due to a high replication and mutation rate escaping the host's antibody and cytotoxic T lymphocyte (CTL) response and due to infection and destruction of CD4+ T cells further weakening the antiviral immune response. Cytomegaloviruses (CMV) persist in their host after CTL mediated control of the primary infection in a state of latency; they are not accessible to the immune system because no viral particles are produced. During states of immunosuppression (HIV, drugs, etc.) productive CMV replication and symptomatic CMV disease occurs frequently. It is not clear what the requirements are to maintain efficient CTL responses during persistent viral infection. Particularly, we want to address the question whether the maintenance of efficient CTL responses during a persistent viral infection is dependent on CD4+ T helper cells.

Recipients of MCMV are provided by collaborators from Oxford University, who originally received them from Munich (Germany) and are not generated in the UK.

They introduced the whole MCMV genome as a BAC into E.coli and transfected those with the respective sequences using standard techniques.
Purified, transfected BAC DNA was introduced into fibroblasts that start to produce MCMV particles within 5 days (For details: Messerle et al., PNAS 1997, 26:14759-63 and Wagner et al., J Virol. 1999, 73:7056-60)

**Origin & Function**

Mouse cytomegalovirus produces a spontaneous, persistent, subclinical infection in wild mice with a prevalence of greater than 65%. Infection of laboratory mice is thought to be uncommon. The susceptibility of mice to experimental infection varies with age, dose, route, virus strain and host genotype.

Mouse CMV is infectious only for mice, but it can replicate in cell cultures from several species including mouse, hamster, rabbit, sheep and primates. MCMV has been shown to infect human cells in vitro, but infection leads to an abortive cycle and is not thought to induce any disease.

Even though Mouse cytomegalovirus (MCMV) is a persistent infection in mice, there are no described epitopes of wild type MCMV presented by H-2Db (mouse Major Histocompatibility Complex). We will use recombinant MCMV (rMCMV), with inserts consisting of epitopes from Influenza A and lymphocytic choriomeningiti virus (LCMV) which are well-characterised and known to associate with H-2Db. Therefore cells infected with rMCMV will present known peptides with H-2Db to T cells. Mice deficient or depleted of CD4+ T cells will be infected with rMCMV experimentally. Thereafter we will follow their CTL response on a single cell level with tetramer complexes of H-2Db-peptide, and functionally with ELISpot and 51 Cr-release assays.

**Evaluation of foreseeable effects**

- **a) Risk Assessment for Human Health and Safety**

  The recipient microorganism, Mouse CMV (ACDP hazard group 1) does not infect humans (abonive replication in human cell lines), it doesn't produce any toxins, and there are no associated allergies known.

  The organism will not be produced at Brunel, but will be received from collaborators at Oxford University.

  Genetic modification of this organism will provide no replication advantage compared to wild type MCMV is expected; there will be no change in host range, pathogenicity or virulence; no infection of humans and no expected adverse effects from transfer of genetic material.

  **b) Risk Assessment for Environmental Harm**

  While viral persistence in the external environment would be likely, as mentioned above genetic modification of this organism will provide no replication advantage compared to wild type MCMV; there will be no change in host range, pathogenicity or virulence; no infection of humans and no expected adverse effects from transfer of genetic material. Infection of wild mice (prevalence of wild type MCMV infection in wild mice is 65-90%) would most likely lead to an asymptomatic infection.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All of our mouse work is undertaken in a secure, Home Office approve animal facility on site at Brunel University. This includes compliance with the “Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes”.

Work will be undertaken at containment level 2 within our secure animal facility.

MCMV transmission requires direct contact with contaminated excretion/secretion (although cross—contamination between cages has so far not been reported). Throughout the experiment the animals will be housed in ventilated cages (IVCs) all animal handling, including injections will be undertaken in Microbiological safety cabinets.

In order to minimise:

- spread of MCMY within the animal house,
- release of MCMY into the environment,
- and potential exposure of workers to MCMY

the following will be applied:

- All work involving animals to be undertaken at Animal Containment Level 2;
- laboratory work to be undertaken at Containment Level 2;
- gloves to be worn when handling any potentially infected materials, including animals;
- a microbiological safety cabinet to be used to minimise any aerosol dissemination of MCMY;
- access to areas where the MCMY is handled to be restricted to authorised personnel
- MCMV infected mice to be kept in filter top cages or individually ventilated cages; and
- sentinel mice will be kept in the same room and tested serologically for MCMY infection every 3 months.

Note: Risk assessment indicates there are no significant risk to human health and safety. The control measures specified are primarily to minimise risks to the environment.

These measures should reduce all risks to the environment and human health to zero.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| Not Applicable |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Consumables (mainly plasticware eg pipettes, flasks, tubes)**
- autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

**Liquids (eg samples, culture supernatants, tissue culture media)**
- autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge to drains.

**Sharps (eg needles, syringes, scalpels)**
- dispose of in sharps containers via clinical waste stream for incineration.

**Animal bedding**
- either autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), OR dispose via clinical waste stream for incineration.

**Animal carcasses**
- dispose via clinical waste stream for incineration.

**Degree of kill**
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
Please see below with regards to your BGMSC application:

- Recombinant replication deficient adenovirus
  
  This particular strain has been attenuated by the removal of the E1/E3 genes. In general, in accordance with the Contained Use Regulations 2014 this strain can be classified as class GM 1 and doesn’t require any notification to the HSE. The virus cannot replicate in cells other than 293A cells.

- Recombinant murine cytomegalovirus (rMCMV)
  
  This particular strain does not infect humans, only mice, but could be transmitted between mice in containment, and in the wild. The wild type Murine Cytomegalovirus (MCMV) has been classified as ACDP Hazard Group 1 (reflecting human risk). Risk assessments indicate that there are no significant risks to human health and safety, but due to the fact the this particular strain could survive in the wild a GM2 classification should be placed upon it.

The rMCMV portion of the application will need to be notified to the HSE, and work should not commence until that notification has been acknowledged.

I am happy to discuss this further with you before proceeding, in case anything has been misunderstood. I will be out of office between the 9th and 13th of April, but will return to work on the 16th.

Kind Regards

D

Health, Safety and Environmental Officer (Science and Environment)
Human Tissue Risk and Compliance Officer
Radiation Protection Officer
Brunel University London
Health, Safety And Environment Team & Governance, Information and Legal Office (GILO)
Located in Sports Centre, Room 101
Brunel University London, Uxbridge, UB8 3PH, United Kingdom
T +44(0)1895 274000 | F +44 (0)1895 232806
www.brunel.ac.uk
Acinetobacter baumannii and Pseudomonas aeruginosa are opportunistic pathogens capable of infecting immunocompromised individuals. They represent excellent models to study infection and antimicrobial resistance in Gram-negative bacteria. The purpose of the use of these organisms will be to identify, evaluate and define key mechanisms involved in allowing these bacteria establish infection and display resistance to antibiotic therapy.

**Recipient or parental organism**

Escherichia coli (class 1):

E. coli K12 and derivatives will be used as a workhorse for cloning and as a recipient for gene overexpression and purification constructs. Most commonly used strains will be TG1, HB101, C600, DH5alpha, OP50, CC118, XL1blue, TOP10F, Rosetta, Shuffle, CE1224, DHM1, LEMO21, BL21, SM10,S17.1, Omnimax, Tuner, BL21 (DE3) and BL21 (DE3) pLysS. These strains have a widespread and long history of safe use.

Acinetobacter baumannii (class 2, opportunistic pathogen)
Most well studied and characterised laboratory strains will be used, this includes ATCC 19606, ATCC 17904, AB5075, European Clones I-III. Strains isolated from patients will also be collected and used in the course of the program. A collection of Tn T26 mutants will also be used (Gallagher et al., 2015, http://www.gs.washington.edu/labs/manoil/baumannii.htm) or other transposon mutant libraries may be used.

Pseudomonas aeruginosa (class 2, opportunistic pathogen):
Laboratory strains that have been extensively studied and characterised will be used, these include: PAO1, PA14, PAK, CHA, PA103, PA7, X13273, TB, SG17. A collection of Tn mutants (MAR2xT7) in the PA14 strain will be used (Liberati et al., PNAS, 2006, http://pa14.mgh.harvard.edu/cgi-bin/pa14/home.cgi) or other transposon libraries may be used.

Host/vector system

- pGEM-T: use in generating Acinetobacter allelic exchange constructs, AmpR f1 Ori.
- pKE229: use in generating Acinetobacter allelic exchange constructs, sacB oriR6K.
- pWH1266 (ATCC® 7792™) – constructed from pBR322, pWH1277, ampR, tetR.
- pLOF/Km: Suicide plasmid harbouring mini-Tn10Km; AmpR KmR.
- pWH1266: E. coli-Acinetobacter shuttle plasmid; AmpR TetR.
- pVK100: Cosmid cloning vector; KmR TetR.
- pME8032: TetR complementation vector.
- pTrc200 StrR, SpcR, pVS1 origin, lacql, trc promoter expression vector.
- pACYC184 and derivatives: ChiR, TetR.
- pMo130-TelR pMo130 plasmid and derivatives: Suicide plasmid, xylE+, sacB+, KmR contains 3.26 kb XmaI-digested telluriteresistance cassette from pwFRT-TelR.
- pEX100 and derivatives: SucR. Only replicate in E. coli lmbdapiir strain CC118
- pEX18Tc Suicide vector containing sacB, TcR.
- pSFS2A Containing kanR cassette, an FRT site, FLP1, and CaSAT1 as a SAT1 flipper
- pET22b/28c/28a and derivatives: ColE1 replicon, AmpR and KanR and capable of expressing Histidine N or C-terminal or duel tag.
- pET41a 3C protease, KmR, His GST Glutathion S-transferase) tag
- pSP56 ApR, GmR; the source of GmR cassette
- pQE30Xa and derivatives: Tag histidine (Qiagen). AmpR.
- pEGFP and derivatives: engineering of gfp fusion. AmpR.
- pGEX and derivatives: Tag GST (Glutathion S-transferase). AmpR.
- pBADmycHisA, B, C and derivatives: expression from an arabinose promoter, tag histidine and Myc. AmpR.
- pYZ4 and derivatives: KanR, Construction of phoA fusion.
- GATEWAY plasmids: InvitroGen. System including the entry vector pDONOR and the destination vectors (pDEST derivatives) for recloning and expressing tagged genes.
- pBAD and derivatives: arabinose promoter. AmpR.
- pTrc200 StrR, SpcR, pVS1 origin, lacql, trc promoter expression vector.
- pT7-1 to pT7-6 and derivatives: T7 promoter. AmpR. To be used together with pGP1-2 which carries the gene encoding RNA polymerase from T7 phage. KmR.
- pBAV1K-t5-gfp GFP expressing plasmid for use in Acinetobacter
- pEXT20 General cloning plasmid; AmpR
- pBAVMCS: pBAV1K-t5-gfp with GFP gene removed, E. coli–Acinetobacter shuttle plasmid; KanR
- pBAVMCS-tet pBAVMCS with tetracycline gene from pWH1266 inserted at SacI site
- pUT18c and pKT25 and derivatives: pUT18c, pUT18c, pUT18c+1 and pUT18c+2 (pUC19 derivative), AmpR, contains 3’ region of the gene encoding adenylate cyclase (cyaA), pKT25 (pSU40 derivative), KanR, contains 5’ region of cyaA (Houot et al., 2012).
- pMP190– Broad-host-range, low-copy-number promoter probe vector; IncQ replicon, lacZ CmrTra-
- pBS1479 and derivatives: AmpR. Tandem Affinity Purification (TAP). The TAP-Tag contains a domain CBP (Calmodulin Binding Protein) and a domain Protein A (Affinity for IgG).
- pLAFR1, pLAFR3 and derivatives : IncP, TetR. Cosmid with low copy number.
- pBK-CMV and its derivatives Dual eukaryotic and prokaryotic expression vector. KanR NeoR
- pBRRMC1-5 and derivatives : ChlR (1), TetR (3), KanR (2) AmpR (4), GentR (5).
- pUCP18/19 and derivatives : AmpR.
- p2lux- CmR Integrated lux vector (37-42C) not integrated at 30C
- pRK2073 Used as a helper in plasmid conjugation; Tpr
- pHp45 and derivatives : Interposons carrying resistances : KanR, TetR, HgR and ChlR.
- pSUP102 and derivatives : Tn5 –B10 (KanR), -B11 et -B12 (GentR), -B13 (TetR), -B20 (KanR), -B21 (TetR), -B22 (GentR), -B30 (TetR), -B40 (KanR) et –B41 (TetR).
- pUT-Tn5 and derivatives: TetR.
- pBK-miniTn7 and gfp derivatives. pUC19 based vectors with various resistance for delivery of gfp on the chromosome.
- pML1 (GmR) and pLR27 (KmR) and pALMAR3 (TnR) for Tn5 delivery on the chromosome.
- Mini-CTX1, Mini-CTX_luxCDABE , Mini-Tn5-luxCDABE and Mini-CTX2 (TetR, pMB1 origin, CTX integrase) for engineering lacZ transcriptional fusion on the chromosome.
- pFLP2 for excision of CTX backbone from the chromosom (AmpR, sacB).
- pMo130 Suicide vector for allelic exchange in Acinetobacter; ColE1 ori, RK2 oriT, xyIE, sacB, KmR
- pMH487; pUCP22Not-RNase III-gfp(mut3)-T0-T1, AmpR Gmr
- pcdArA:gfp; pUCP22Not-PcdrARBSII-gfp(mut3)-T0-T1, AmpR Gmr, second messenger reporter construct
- pMH489; pUCP22Not-RNase III-gfp(MV)-T0-T1, AmpR Gmr
- pcdArA:gfp(MV); pUCP22Not-PcdrARBSII-gfp(MV)-T0-T1, AmpR Gmr, Second messenger reporter construct
- pSB401: C6-C8 HSL lux-based reporter , TetR
- pSB1142: C10-C14 HSL lux-based reporter. TetR
- pSB536:C4-HSL lux-based reporter, AmpR

Origin & function

Because of the nature of this project, we could potentially need to use any of the annotated genes available from the genome sequence of the University of Washington laboratory strain of Acinetobacter baumannii (http://tools.uwgenomics.org/cgi-bin/pgat_acinetobacter/elementlist.cgi?id=AB5075UW) or orthologues of these genes found in other strains of Acinetobacter baumannii. A similar approach will be required for Pseudomonas aeruginosa where genes from the lab adapted strains PAO1 and PA14 strains (http://pseudomonas.com), as well as homologous of these genes found in other strains.

Evaluation of foreseeable effects

Host range, tissue tropism and susceptibility to host defense mechanisms are not expected to be altered significantly by any of the genetic modifications expected to be carried out in this project. The GMMs generated in this project are likely to have comparable properties to the wild type recipient isolates, or to have an attenuated phenotype due to reduced fitness and/or reduced antibiotic resistance profile. Some transfer of non-toxic genes between P. aeruginosa and A. baumannii will take place to establish conserved regulatory networks, however the genes in questions are well defined and not expected to significantly alter acute virulence. As such, the risk associated with the GMMs is considered to be the same as those posed by the wild type organisms. The vast majority of all gene insertions will be associated with complementation of previously generated mutants, in which case the hazards will be no more than wild type strains, as they will have reverted to the full wild type genetic cohort. Any fusion constructs or tagging experiments will only involve the insertion of innocuous reporter DNA sequences (His, GST, GFP) none of which will increase the

02/03/2022 Page 5569 of 15326
virulence of recipient strains. All protein expression experiments will take place in an E.coli K12 background. The vast majority of potential proteins that may be overexpressed will be non-toxic enzymes. In the rare instances where a toxin may be expressed for purification, the toxin will have anti-bacterial activity rather than any anti-eukaryotic activity. The probability of this research leading to the need to clone a bacterial toxin with anti-eukaryotic activity is small. Some transfer of non-toxic genes between P. aeruginosa and A. baumannii will take place to establish conserved regulatory networks, however the genes in question are well defined and not expected to significantly alter virulence. GMM could alter the expression of genes associated with motility, biofilm formation, secretion systems, antibiotic resistance and quorum sensing which may affect virulence. Data from the published literature highlights that artificial alteration in expression of these genes comes at a fitness cost and while certain virulence traits may be increased, the overall fecundity of the GMM will likely be reduced as a result. The probability of creating a mutant strain with a genuine increased virulence profile is diminishingly small as these mutants should have already arisen in the global bacterial population. As most of the strains to be used in this project are laboratory adapted strains infection in healthy individuals is not a particular concern. In addition to this no procedure in the laboratory will require direct contact with any of the potential pathogens. In addition, personal protective equipment (PPE) including lab coat and gloves will be worn when working with these strains to further minimize the risk of direct contact with the organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

There will not be any anticipated derogation from full containment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Local waste streams are currently in place to deal with all GMM waste.

Solid Waste: All contaminated solid waste in placed in autoclave bags which are subsequently sealed and autoclaved on standard sterilisation cycles (121-135°C for at least 10 minutes).

Liquid waste: All liquid waste will be treated wil either 1% virkon or PRESEPT effervescent disinfecting tablets (2.5g) for a minimum of 6 hours before disposed of down the laboratory sink.

Sharps: Disposed of in designated sharps bins prior to sterilisation via autoclaving.

Surface: Post working with GMMs all surface will be decontaminated with 70% EtOH, virkon or Presept.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Dr M's project was discussed and reviewed at the Biological and Genetic Modification Safety Committee at Brunel University. The risk assessments and procedures were deemed appropriate for the work planned.

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<table>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
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DH
GM Centre Number: 227

| Data Premises Notified       | 16/03/1987          | Transferred from 1992 Regs? | Y |
| Data Premises Closed         | 29/09/2016          | Transitional Premises Class | 2 |
| Emergency Plan Required?     |                     | Non-GMMs                   | Y |
| Withdrawn                    |                     |                             | N |

Name
ROWETT INSTITUTE OF NUTRITION AND HEALTH, UNIVERSITY OF ABERDEEN

Name 2
Nutrition Division

Campus Estate or Research Centre
UNIVERSITY OF ABERDEEN

Building
UNIVERSITY OFFICE

Road Name
KING'S COLLEGE

Town
ABERDEEN

County
ABERDEENSHIRE

Postcode
AB24 3FX

Country
SCOTLAND

Tel Number
01224 712751

Fax Number
01 224 715349

E-mail

Comments
Premises closed and merged with GM490

Date at Which Additional Info Submitted
02/03/2022
## Premises Addresses

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<tr>
<th>Date</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
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<th>Town</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Tick if confidential</td>
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Bacteriology | Parasitology | Transgenic | Microbiology | Research |
|             |              | Birds      |             |         |
Virology
Mycology
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 227/01.1

Date Ackn'd 21/02/2001
CU2 Project Title ANALYSIS OF NOVEL MOBILE DNA ELEMENTS FROM GUT BACTERIA

Date Project Ceased 29/09/2016

Withdrawn N

Historical Significant Changes This Centre merged with GM490 Project transferred to GM490

Tick if notifying a connected programme of work N

Consent Granted not applicable

Project notified under transitional arrangements Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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02/03/2022
### Project Ref 227/01.2

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<td>21/02/2001</td>
<td>GENE TRANSFER AND HETEROLOGOUS GENE EXPRESSION IN GUT BACTERIA</td>
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**Date Project Ceased:** 29/09/2016

**Class:** Class 2

**CultureVolClass2:**

**CultureVolumeClass3-4:**

**Non-GMM Consent Granted:** not applicable

**Project notified under transitional arrangements:** Y

**Withdrawn:** N

**Tick if notifying a connected programme of work:** N

**Historical Significant Changes:**

<table>
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<th>This Centre merged with GM490 Project transferred to GM490</th>
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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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Animal Units
- L2
- L3
- L4

Large Scale Activities
- L2
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- L4

Human Clinical Applications
- L2
- L3
- L4

Project Ref 227/95.1

Date Ackn'd 14/03/1995

CU2 Project Title PREVENTION OF SALMONELLOSIS IN HUMANS AND FARM ANIMALS BY

Class CultureVolClass2 CultureVolumeClass3-4

Class 2
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Project Containment

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Animal Units

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Project Ref 227/96.1

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<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
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<td>TRACKING OF GENETICALLY MARKED STRAINS OF GUT BACTERIA IN STIMULATED RUMEN CONDITIONS</td>
<td>Class 2</td>
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Historical Significant Changes

- This Centre merged with GM490 Project transferred to GM490

Tick if notifying a connected programme of work: N

Tick if you are claiming exemption from disclosure for section of the risk assessment: N

Is an emergency plan required according to regulation 20?: N

If yes, tick to confirm that it is attached to this form: N

Tick to confirm that you have attached a risk assessment to this form: 

Tick if you are claiming exemption from disclosure for section of the risk assessment: N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
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02/03/2022
Project Ref  227/99.1

Date Ackn'd  07/07/1999
Date Project Ceased  29/09/2016

CU2 Project Title  FATE OF GREEN FLUORESCENT PROTEIN LABELLED SALMONELLA ENTERIDITIS IN THE RAT INTESTINE

Class  Class 2
Consent Granted  not applicable

Tick if notifying a connected programme of work  No

Historical Significant Changes  This Centre merged with GM490 Project transferred to GM490

Project notified under transitional arrangements  Yes

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<td>L4</td>
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Animal Units

<table>
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<tr>
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Large Scale Activities

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<th>Glass Houses</th>
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<tbody>
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Human Clinical Applications

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
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<td>L2</td>
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</table>

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
**GM Centre Number: 229**

<table>
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**Name**

CANCER RESEARCH UK

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

2 REDMAN PLACE

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

E20 1JQ

**Country**

ENGLAND

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<td>E-mail</td>
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<td>LONDON</td>
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**Comments**

COMPANY WAS CALLED IMPERIAL CANCER RESEARCH FUND (ICRF) UNTIL 4/2/2002

**Date at Which Additional Info Submitted**

29/01/2002 18/02/2020
### Premises Addresses

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<td>HERTFORDSHIRE</td>
<td>EN6 3LD</td>
<td>ENGLAND</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
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<thead>
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<th>Virology</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
### GM Centre Number: 230

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#### Name

**KING'S COLLEGE LONDON**

#### Name 2

**CENTRE FOR NEUROSCIENCE RESEARCH**

#### Campus Estate or Research Centre

**GUYS HOSPITAL CAMPUS**

#### Building

**HODGKIN BUILDING**

#### Road Name

#### District

**LONDON**

#### Town

**LONDON**

#### County

**GREATER LONDON**

#### Postcode

**SE1 1UL**

#### Country

**ENGLAND**

#### Tel Number

0207 848 6172

#### Fax Number

0207 848 6569

#### E-mail

HSE Division: **LONDON**

#### Comments

GM230 MERGED WITH GM295 ON 8/9/2003

#### Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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<td>SE1 1UL</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
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<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
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<th>Level 4 (GMMs)</th>
<th>Non-microbial</th>
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Laboratory | Animal Unit | Growth Room | Glass House | Large Scale |

- Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research | Transgenic Animals | Transgenic Fish | Gene Therapy |

02/03/2022
For these activities, the maximum culture volume that could be released at one time would be 2 litres. Established methods are used to inactivate waste. Liquid bacterial waste is inactivated by recognised chemical methods: overnight treatment in freshly-made chlorine-releasing solutions or bleach (1000 ppm available chlorine). For monitoring, samples are placed in a neutralising broth and subjected to liquid and agar plate culture. Solid waste, for example culture flasks and petri dishes, are inactivated by heat sterilisation, and the waste is incinerated. The autoclave used for sterilisation is regularly maintained and Brown's tubes are used to monitor each batch of waste. In each laboratory carrying out GM work, a log book is kept for the monitoring procedures.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 232

<table>
<thead>
<tr>
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<td>Non-GMMs</td>
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<td>Withdrawn</td>
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#### Name
- UNIVERSITY OF NOTTINGHAM

#### Name 2
- BIOSCIENCES GM CENTRE

#### Department
- SCHOOL OF BIOSCIENCES

#### Campus Estate or Research Centre
- SUTTON BONINGTON CAMPUS

#### Road Name
- 

#### Building
- 

#### Town
- LOUGHBOROUGH

#### County
- LEICESTERSHIRE

#### Postcode
- LE12 5RD

#### Country
- ENGLAND

#### Tel Number
- 0115 951 6001

#### Fax Number
- 0115 951 6350

#### E-mail
- www.nottingham.ac.uk/schbiol

#### HSE Division
- MIDLANDS

#### Comments
- GM232 closed and merged with GM470 on 18/02/2005

### Date at Which Additional Info Submitted
- 12/07/2002

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial
Other (please specify)  
Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 232/00.7

<table>
<thead>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<td>EXPRESSION OF NON-TOXIC PROTEINS IN MAMMALIAN CELLS USING RETROVIRUS AND ADENOVIRUS</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Large Scale Activities</th>
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### Project Ref 232/00.8

**Date Ackn'd** | **CU2 Project Title** | **Class** | **CultureVolClass2** | **Consent Granted** | **Project notified under transitional arrangements**
---|---|---|---|---|---
15/02/2001 | EXPRESSION OF PPAR, GABA, AHR, GLUTamate AND GROWTH HORMone RECEPTORS, CYTOCHROME P450 GENES, DELTA - LATROINSECTOTOXIN AND ASSOCIATED GENES, SUCH AS CYTOCHROME B5, P450 REDUCTASE, HSP90, AIP. | Class 2 | CultureVolClass3-4 | not applicable | Y

**Date Project Ceased** | 15/11/2002

**Withdrawn** | N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<th>Laboratory Activities</th>
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Project Ref   232/00.9

Date Ackn'd       CU2 Project Title   Class   CultureVolClass2   CultureVolumeClass3-4

02/03/2022
EXPRESSION OF RECEPTOR GENES AND REPORTER GENES IN MAMMALIAN CELLS

Date Project Ceased: 15/11/2002

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
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**Project Ref** 232/03.1

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<td>PHYSIOLOGY AND PATHOGENICITY OF CAMPYLOBACTER GENE PRODUCTS</td>
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**Historical Significant Changes**

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### Project Additional Information

#### Purposes of the contained use

The aim of the project is to evaluate the role of specific Campylobacter genes associated with the physiology and pathogenicity of the bacterium in vivo. To do this gene sequences and their promoters will be reintegrated into the bacterial genome in order to knock-out or alter their function. Campylobacter genes for which we can ascribe a role will be manipulated in an attempt to express them in laboratory strains of E. coli to study the structure and function of the proteins they encode.

#### Recipient or parental organism

<table>
<thead>
<tr>
<th>Host</th>
<th>Vector</th>
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<tbody>
<tr>
<td>E. coli Top10 cells (Invitrogen)</td>
<td>pCR2.1 Topo plasmid (Invitrogen)</td>
</tr>
<tr>
<td>E. coli BL21(DE3) (Novagen)</td>
<td>Approved vectors based on colE1 replicating plasmids (eg pUC18, pBS and pBC)</td>
</tr>
<tr>
<td>Campylobacter jejuni (ACDP 2)</td>
<td>pET series of expression plasmid vectors (Novagen)</td>
</tr>
<tr>
<td>Campylobacter coli (ACDP 2)</td>
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</tr>
<tr>
<td>Campylobacter lari (ACDP 2)</td>
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<tr>
<td>Campylobacter upsaliensis (ACDP 2)</td>
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</tbody>
</table>

All the recipient Campylobacter species to be used in this study are wild type ACDP group 2 organisms. No multiply-antibiotic resistant isolates will be used which would preclude therapy with standard antibiotic regimes should infection occur.

#### Host/vector system

- **Genomic DNAs of the following:**
  - Campylobacter jejuni (ACDP 2)
  - Campylobacter coli (ACDP 2)
  - Campylobacter lari (ACDP 2)
  - Campylobacter upsaliensis (ACDP 2)
  - Campylobacter bacteriophage DNA

The target gene sequences are expected to encode bacterial cell-surface associated proteins or be responsible for the biosynthesis of surface associated components (e.g., lipopolysaccharide, capsular polysaccharide, lipid and glycosylation modifications of proteins). Gene functions may also include products responsible for intracellular DNA modifications.

#### Evaluation of foreseeable effects

Campylobacter enteritis is the most common form of bacterial food poisoning in the world. Campylobacter infection occurs through ingestion (fecal oral route) and results in a self-limiting diarrhoeal disease. A few rare post-infection cases have been associated with the later development of a paralysing autoimmune disease (Gillian Barre syndrome). There is no specific risk of transmission by aerosol known for these organisms. Campylobacter jejuni is known to encode a cytolethal distending toxin.
However, the toxin contains multiple sub-units all of which would have to be co-expressed. The host Campylobacter species are initially wild type and have the potential to cause human disease. It is therefore proposed that all experiments will be performed under containment conditions ACDP 2. The resulting GMMs are unlikely to pose any further risk to human health as they will contain DNAs of similar origin and that the ACGM containment level be 2, consistent with the host and donor DNAs.

Birds carry thermophilic Campylobacters in their gut without any known detrimental affect, such that most wild birds and domestic poultry are colonised. It is possible that the modified Campylobacters could survive in avian species if they were to be released into the environment. Therefore it is proposed that all experiments will be performed under containment conditions ACGM 2.

When expressing the Campylobacter genes in E. coli it is possible that some of these genes could increase their fitness and/or human or animal colonisation potential. However, the auxotrophically crippled organisms (E. coli K-12 ACDP 1) will still retain limited survivability in the environment. The majority of genes are not known to be toxic, and when expressed in isolation form the remaining Campylobacter host genes are unlikely to produce any deleterious effects over the parental organism. Owing to the potential for increased expression levels in E. coli the containment level for this work is proposed to be ACGM level 2 consistent with the ACDP level of the donor organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Local Incineration: solid wastes are incinerated at 1000 degrees C for 360 minutes until ash (100% kill). These are discarded as solid waste.

Autoclave: Liquid waste is autoclaved at 121 degrees C for 30 minutes until to achieve 100% kill (internal temp and pressure monitors) and discarded to the drains once cool. These measures are coupled with condesate thermocouples and thermologue test strips.

Chemical Treatment: 2% Virkon is used to remove contamination from solid surfaces. This concentration will kill the bacterial rapidly as recommended by the supplier. After 15 minutes contact the disinfectant is diluted and the liquid waste discarded to drain.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project approved at Containment level 2 by GM Safety Committee, which includes representatives of all University employees. The project details were assessed by expert members of the committee and the decision was reported to the general safety committee.

Project Containment
### Project Additional Information

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? \(\text{N}\)

If yes, tick to confirm that it is attached to this form \(\text{N}\)

Tick to confirm that you have attached a risk assessment to this form \(\text{N}\)

Tick if you are claiming exemption from disclosure for section of the risk assessment \(\text{N}\)

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
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<tr>
<td>L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
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<td>L3 L4 L2</td>
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</table>

**Project Ref** 232/trans2

Date Ackn'd 13/02/1994  
CU2 Project Title DEVELOPMENT OF RECOMBINANT PHAGES  
Class Class 2  
CultureVolClass2  
CultureVolumeClass3-4
Date Project Ceased: 18/02/2005

Non-GMM Consent Granted: not applicable

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

Withdrawn: N

Historical Significant Changes: GM232/00.2, GM232/trans2 transferred to GM470 on 18/02/2005

Historical Date of Additional Info: 22/09/2000, 18/02/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
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<tbody>
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## Project Ref 232/trans3

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<th>Class</th>
<th>CultureVolClass2</th>
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<td>22/09/2000</td>
<td>USE OF BIOLUMINESCENCE FOR PHYSIOLOGICAL STUDIES OF BACTERIA</td>
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<th>Non-GMM Consent Granted</th>
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Withdrawn N

Historical Significant Changes
GM232/00.3, GM232/trans3 transferred to GM470 on 18/02/2005

Historical Date of Additional Info
22/09/2000, 18/02/2005
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
Project Ref 232/trans4

Characterisation of the biochemistry and genetics of environmental gene regulation in Staphylococcus

Date Ackn'd: 22/09/2000
Date Project Ceased: 18/02/2005

Withdrawn: N

Non-GMM Consent Granted: not applicable

Historical Date of Additional Info: 22/09/2000, 18/02/2005

Project notified under transitional arrangements: Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<td>Animal Units</td>
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**Project Ref** 232/trans5

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<td>22/09/2000</td>
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Historical Significant Changes
GM232/00.5, GM232/transS transferred to GM470 on 18/02/2005

Historical Date of Additional Info
22/09/2000, 18/02/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
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<th>Laboratory Activities</th>
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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref:** 232/trans6

- **CU2 Project Title:** DEVELOPMENT OF BIOLUMINESCIENT AND FLUORESCENT DERIVATIVES OF PSEUDOMonas spp. FOR STUDIES OF GENE REGULATION

- **Class:** Class 2
- **CultureVolClass2:** not applicable
- **CultureVolumeClass3-4:**

- **Non-GMM Consent Granted:** not applicable
- **Project notified under transitional arrangements:** Y

- **Historical Significant Changes:** GM232/00.6, GM232/trans6 transferred to GM470 on 18/02/2005
- **Historical Date of Additional Info:** 22/09/2000, 18/02/2005
- **Significant Change ID:**
- **Date of Significant Change:**

**Date Ackn’d:** 22/09/2000

**Date Project Ceased:** 18/02/2005

- **Withdrawn:** N
- **Tick if notifying a connected programme of work:** N

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022  
Page 5608 of 15326
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Name

**WYE COLLEGE**

Name 2

**UNIVERSITY OF LONDON**

Department

**BIOLOGICAL SCIENCES**

Campus Estate or Research Centre

Building

Road Name

District

**ASHFORD**

**KENT**

**TN25 5AH**

**ENGLAND**

Tel Number | 01233 812401 |
Fax Number  | 01233 813140 |

E-mail

HSE Division | EAST AND SOUTH EAST |

Comments

Date at Which Additional Info Submitted

**02/03/2022**
## Premises Addresses

<table>
<thead>
<tr>
<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
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<th>Town</th>
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<td>KENT</td>
<td>TN25 5A</td>
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## Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
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- Tick if confidential

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tbody>
<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>
All small-scale cultures eg. petri dish plates, liquid cultures (max volume 200 ml) are disposed of by autoclaving at 121 degrees C (15 psi) in the centre of the load for 30 minutes. Efficiency of autoclaving is tested in each treatment using heat sensitive tape, periodically by plating out from treated cultures, and annually by manufacturer's inspection.

Transgenic plant material, all growing media and posts are disposed of by incineration. All GMO plant material is no longer viable when it leaves the site for incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 234/01.1

**Date Ackn'd** 17/08/2001

**CU2 Project Title** CLONING GENES FOR PATHOGENICITY FROM THE PLANT PATHOGEN PSEUDOMONAS SYRINGAE

**Date Project Ceased** 25/06/2008

**Consent Granted**

- Class: Class 2
- CultureVolClass2: < 1 litre
- CultureVolumeClass3-4: not applicable

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**
## Project Additional Information

### Purposes of the contained use

Increasing basic understanding of the mechanisms underlying bacterial pathogenicity.

### Recipient or parental organism

Genes cloned from one pathovar of *Pseudomonas syringae* (parent) will be transferred into *E. coli* DH5a and into other pathovars of *Pseudomonas syringae*.

### Host/vector system

Vectors used will be broad host range plasmids such as pLAFR3 and CoIE1 replicon based pBluescript.

### Origin & function

Genes transfer will be used to identify genes for avirulence and virulence by function, using altered pathogenicity as the test. Following detection of activity in cosmid clones (up to 30 Kb) sub-cloning will allow functional open reading frames to be analysed. Proteins will be expressed from vector promoters.

### Evaluation of foreseeable effects

The GMOs are not considered a significant risk to human health. Release of the GM *Pseudomonas syringae* strain with altered virulence is unlikely to result in the infection of plants. The bacteria are spread by seed and through rainsplash onto infected leaves. They are not soil-borne pathogens. Nevertheless the *Pseudomonas syringae* strains will not be specifically disabled.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Plants infected with GMOs will be maintained in designated growth rooms as specified in the MAFF (now DEFRA), Plant Health Licence, no PHL30A/37554/2001).

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Autoclaving at 121°C for 30 mins to destroy broth and petri cultures and infected plant material (leaves and pods).
2. Soaking in a presept bath 140ppm chlorine, overnight to decontaminate glassware and plasticware.
3. Incineration of contaminated soil and pots organised by "Whiterose" No GMOs should survive.

### Is an emergency plan required according to regulation 20?

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
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</thead>
<tbody>
<tr>
<td>Is an emergency plan required according to regulation 20?</td>
<td>N</td>
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</table>

### If yes, tick to confirm that it is attached to this form

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<tr>
<th>Question</th>
<th>Answer</th>
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<tbody>
<tr>
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### Tick to confirm that you have attached a risk assessment to this form

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<th>Answer</th>
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<tbody>
<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
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### Tick if you are claiming exemption from disclosure for section of the risk assessment

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
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<tbody>
<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>N</td>
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</table>
Although the hazards posed by the work were not assessed to require greater than containment level 1 for human health, the use of plant pathogens necessitates notification of Class 2 activity.

### Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2</td>
<td>L2</td>
<td>L2 Yes</td>
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| 0207 703 5796 |

**E-mail**

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**Comments**

| GM 235, 295 & 543 CLOSED & MERGED WITH GM386 |

**Date at Which Additional Info Submitted**

<p>| 02/03/2022 |</p>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  
Tick if confidential

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<td>Invertebrates</td>
<td>Plants</td>
<td>specify below)</td>
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Please enter comments of the GM safety committee on the risk assessment

Project Ref 235/07.1

Date Ackn'd 03/12/2007

Date Project Ceased 04/03/2014

CU2 Project Title Analysis of proteins involved in neurodegenerative diseases using viral vector delivery to the nervous system.

Class 2

Culture Vol

Class 2

1-50 Litres

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes GM 235 MERGED WITH GM 386 & PROJECT TRANSFERRED TO GM 3

Project Additional Information

Purposes of the contained use

The studies proposed are based on the use of recombinant viral vectors for gene delivery in vitro in primary neuronal cultures to investigate the pathogenic mechanisms involved in neurodegenerative disorders, including Alzheimer’s disease and amyotrophic lateral sclerosis (ALS). Two viral delivery systems will be used: lentiviral vectors and adeno-associated viral vectors to over-express proteins encoded by genes mutated in familial forms of AD and ALS or protein accumulating in affected neurons, as well as modifying enzymes and interacting partners and to silence the expression of the above proteins using RNA interference.

Recipient or parental organism

E. coli - K12 or B derivatives (e.g. SURE 2, Stratagene and DB3.1 ccdB gene tolerant cells). Human HEK 293T cells for packaging, NIH3T3 cells, HT1 080 cells or equivalent for virus titration. Experiments will be conducted in nonneuronal (e.g. COS-7 and CHO cells) of neuronal (e.g SH-SY5Y and N2a neuroblastoma cell lines as well as in primary rat or mouse neuronal cultures (cortical neurons, motor neurons, dorsal root ganglion neurons, Purkinje cells), including neurons derived from transgenic mice.

Host/vector system
The inserted genetic material will be cloned into the plasmids: pAMICBA-pl-WPRE-BGH for adeno-associated virus construction or into the self-inactivating lentiviral plasmids: pLenti-DEST1TOPO or pLentiLox3.7. Genes encoding the components required for packaging the viral genome are separated onto three plasmids for the lentiviral vectors (pLP1, pLP2, pLPNSVG). The lentiviral plasmids do not contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus.

**Origin & function**

Full-length and truncated cDNA (Homo sapiens and rodents) or cloned genomic fragments encoding the protein known to be mutated or accumulating and aggregating in neurodegenerative diseases as well as sequences designed to silence these proteins by RNA interference. Some examples are listed below.
- Tau, a neuronal microtubule associated protein that stabilises axonal microtubules. Mutations in tau cause a form of fronto-temporal degeneration.
- Sodium calcium exchanger, NCX2, Sodium calcium exchanger, calpain cleaved, NCX3
- Kinases: GSK3alpha and beta, Casein kinase 1, Cdk5 and its activators, CPRK family
- ALS2/alsin, a protein that contains several domains characteristic of GTP exchange factors. Mutations of the ALS2 gene are linked to juvenile familial forms of amyotrophic lateral sclerosis (ALS).
- VAPB (vesicle associated protein B —mutated in familial ALS) and its interacting partners.
- Standard reporter genes (e.g. GFP, Luciferase, -galactosidase) will also be already present or cloned into these vectors.
- Superoxide dismutase 1 (SOD1) mutations in which cause familial ALS

**Evaluation of foreseeable effects**

Transformed E.coli: No significant hazards have been identified. Insertion of the foreign sequences into E coli is not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the host or normal human defence mechanisms. The resulting GMM’s are not expected to carry any additional risks compared to that of the un-modified recipients. Virally transfected HEK293FTIT cells could pose a risk to human health associated with the production of recombinant infectious lentiviral particles. However, as they are self-inactivating replication deficient vectors the risk is only from the insertion of the genetic material, described above. None of the genetic inserts are likely to cause significant effect on an individual as their potential toxic effect is limited to neurons have no known oncogenic properties.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware e.g. pipettes, flasks, tubes) — soaked in hypochlorite solution before autoclaving using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-l2SoC for at least 15 minutes or 126-l3OoC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids placed in a clinical waste bag for incineration by external contractors.

Liquids (e.g. samples, culture supernatants, tissue culture media) — Disinfectant - Trigene left overnight, then dispose of to drain with running water

Safety cabinet - will be washed down with Trigene solution after use.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least :15 minutes or 126-13OoC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids in a clinical waste bag for incineration by external contractors.

Sharps (e.g. needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill

**Autoclaving, effectively 100% kill (annual validation)**
Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection with Trigene, used according to manufacturers instructions under standard conditions, manufacturers validation (e.g. 99.998 % kill).

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The assessment was reviewed by the Institute of Psychiatry Biological and GM Safety Committee in their September 2007 meeting. No concerns were raised and it was approved for notification to HSE.

Project Containment

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Animal Units

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Project Ref 235/08.1

Date Ackn'd 25/11/2008
CU2 Project Title Functional evaluation of genes relevant to psychiatric disorders, neurodegeneration, neurological disease and neural development by virus-mediated gene delivery to neural cells.

Date Project Ceased 04/03/2014

Class 2
CultureVol 1-50 Litres

Non-GMM Consented Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes GM 235 MERGED WITH GM 386 & PROJECT TRANSFERRED TO GM 3

Tick if notifying a connected programme of work N

02/03/2022
Project Additional Information

**Purposes of the contained use**

The project involves the construction of replication incompetent viruses encoding DNA sequences enabling siRNA-mediated gene silencing or gene overexpression, with the aim of studying the role of given candidate genes in psychiatric disorders, neurodegeneration, neurological disease and neutral development. Three viral delivery systems will be used: lentiviral vectors, adenoviral vectors and adeno-associated viral vectors. These will be used to investigate the function of gene products thought to be involved in development and pathophysiology in vitro in primary neuronal cultures. (see 17).

**Recipient or parental organism**

E14, HM2 stem cells; PC12 pheochromocytoma, NG108-15, SH, SYSY, Neuro2A, HD7/7, HD7/109, HD109/109 neuroblastoma; NS5, MHP36 neutral stem cells, HEK, HeLa, HT1080 endothelia, 3T3, COS-7, CHO, MEF fibroblasts, CG4, O2A glioma. E.coli - K12 or B derivatives. Primary murine neutral cells.

**Host/vector system**

The inserted genetic material will be cloned into the plasmids: pAAV-IREShrGFP for adeno-associated virus construction or into the self-inactivating lentiviral plasmids: LeDo-G pSiEW or pLK. Genes encoding the components required for packaging the viral genome are separated into three plasmids for the lentiviral vectors (pLP1, pLP2, pLPVS VG or pLKO.1, PIKO.1-CMV-GFP, PCMV-VSV-G). The lentiviral plasmids do not contain any regions of homology with each other to prevent undesirable.

**Origin & function**

Full-length and truncated cDNA (Homo sapiens and rodents) or cloned genomic fragments encoding the protein known to be mutated or deregulated in psychiatric disorders, neurodegeneration, neurological disease and neutral development as well as sequences designed to silence these proteins by RNA interference.

**Evaluation of foreseeable effects**

Transferred E.coli: No significant hazards have been identified. Insertion of the gene sequences into E.coli is not expected to result in harmful physiological properties or to affect the pathogenicity of the host or normal human defence mechanisms. The resulting GMMs are not expected to carry any additional risks compared to that of the un-modified recipients.

Vira ly transfected HEK293T cells could pose a risk to human health associated with the production of recombinant infectious lentiviral particles. However, as they are self-inactivating replication deficient vectors the risk is only from the insertion of the genetic material, described above. None of the genetic inserts are likely to cause significant effect on an individual as their potential toxic effect is limited to neurons and have no known oncogenic properties.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials that have been in contact with genetically modified material will be sterilized. Consumable (mainly plasticware e.g. pipettes, flasks, tubes) will be soaked in hypochlorite solution before autoclaving using a make safe cycle (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or plates will be autoclaved.
at 121 degrees C for at least 15 minutes before removal form the building in double bags for incineration, thereby ensuring 100% kill.
To achieve 100% kill of liquid material, waste will be treated with Virkon 1% (w/v) or Trigene (2% w/v) and incubated overnight in accordance with manufacturer's recommendations before disposal down a sink. We have opted not to autoclave liquid waste in light of concerns about delays to autoclave availability for this purpose, coupled with the undesirability of autoclaving bleach-treated waste.

Sharps (eg needles, syringes, scalpels) will be autoclaved disposed of as for solid waste.
All animal carcasses will be incinerated.
Safety cabinet will be washed down with Trigene solution after use.
Autoclaves are serviced and validated annually by the manufacturer or an approved contractor

Degree of Kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incineration)
Chemical disinfectant with Trigene, used according to manufacturers instructions under standard conditions, manufacturers validation (eg 99.998% kill).

The IOP GMSC (GM235) discussed the project and approved the projects with minor amendments.

Please enter comments on the GM safety committee on the risk assessment

The IOP GMSC (GM235) discussed the project and approved the projects with minor ammendments.

Project Containment

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Project Ref 235/09.1

Date Ackn'd 03/06/2009

CU2 Project Title Reprogramming of human somatic cells into induced pluripotent stem cells for

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre
The project involves the construction of replication incompetent viruses containing DNA sequences that encode four human transcription factors. These transcription factors will be introduced into primary human somatic cells with the objective of reprogramming them into induced pluripotent stem cells (iPSC). The resulting iPSC lines will be subjected to various differentiation protocols to convert them into desired somatic cell lineages. Of particular interest will be iPSC lines made from primary cells obtained from patients suffering from a range of monogenic and polygenic diseases. The research is expected to shed light on the pathophysiology of early human disease development as well as providing cellular reagents for drug screening and discovery. Two viral delivery systems will be used: lentiviral and retroviral vectors. The transcription factors in the lentiviral vectors will be under the control of doxycycline-inducible promoters.

Recipient or parental organism

Human embryonic kidney packaging cells for making infective lentivirus or retro-viruses. A variety of human primary somatic cell cultures of which skin fibroblasts will make up the most frequent use

Host/vector system

Lenti-x Expression System (Clontech) In this lentiviral system, the final vector [assembled through independent transformation of packaging cells (HEK293T cells) with three plasmids] is a VSV-G pseudotyped, replication incompetent retrovirus derived from HIV-1. The virus does not encode Tat and the inserted transcription factor (Oct4 or Sox2 or Klf4 or c-myc) is under the control of doxycycline. The presence of added doxycycline will be needed for activation of the inserted transcription factors. LVX-Tight-Puro Vector from Clonetech is need to mediate the doxycycline control.

pCMV-VSVG are all retroviruses containing the inserted DNAs indicated. In order to assemble infectious retroviruses, these DNAs and two other plasmids are introduced into human 293T packaging cell lines to create a VSV-G pseudotyped, replication incompetent retrovirus derived from the Moloney Murine Leukemia Virus.

All assembled viruses can infect human and mouse cells. The pMX and pLVX retroviral constructs have ampicillin resistance for bacterial selection. pLVX also has puromycin resistance for mammalian cell line selection

HEK293T cells will be used for packaging

Origin & function
Full length human cDNAs encoding the transcription factors Oct 4, Sox 2, Klf 4, c-myc, and Sall 4 have been selected. The first four of these will be used to reprogram cells. Sall4 has been included because there are rumours that it alone can reprogramme cells. However this technology is moving very fast and it is eminently possible that fewer and different human gene inserts will be required in the future. New and even safer vector delivery systems are being developed and we would like to suggest that HSE permission, if granted, will also cover new vector systems and unspecified human sequences that can reprogram human somatic cells efficiently (subject of course to the caveat that any changes will receive the approval of the local risk assessment committee and will be notified to HSE).

Evaluation of foreseeable effects

Virally transfected HEK293T cells could pose a risk to human health associated with the production of recombinant infectious lentiviral particles. However, as they are self-inactivating replication deficient vectors the risk is only from the insertion of the genetic material, described above. Special note should be made regarding the use of the c-myc insert. C-myc is a known oncogene however in iPSC generation it greatly improves reprogramming efficiency. Since this goes down considerably with age of donor, we think it essential to include in the protocol.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Uncontaminated and contaminated waste will be segregated. Uncontaminated waste will be put in yellow bags for disposal and subsequent incineration. All materials that have been contaminated with genetically modified material will be inactivated by autoclave or chemical sterilisation, prior to disposal via clinical waste route and incineration.

i) All sharps, disposable plastic pipettes and tips contaminated with GM material will be put into Sharpsafe bins, clearly labelled as “GM WASTE”. These will be autoclaved at 121°C for at least 20 minutes before disposal and subsequent incineration. Contaminated sharps will be treated with 2% Trigene for at least 30 minutes before discarding into the sharp safe bin to prevent the build-up of contaminated material.

ii) Plasticware (e.g. tissue culture flasks, petri dishes, tubes) and other solid waste (e.g. gloves, paper towels, solid media) contaminated with GM material will be collected in a clear autoclave bag and autoclaved at 121°C for at least 20 minutes at the end of each day. The waste will then be placed in yellow bags for disposal and subsequent incineration.

iii) Liquid waste contaminated with GM material will be treated with Trigene for at least 30 minutes. This will be done by adding the waste to an appropriate volume of the neat disinfection solution and making up to a final concentration of 2%.

iv) Any reusable containers contaminated with GM material will be left to soak in 2% Trigene for at least 30 minutes before washing. This will be done by addition of the disinfection solution directly into the container or by immersion of the contaminated equipment in a larger vessel containing the disinfection solution.

Degree of kill:
Autoclave waste cycle at 121 for 20 minutes. 100% kill confirmed by annual validation.
Chemical disinfection with Trigene, used according to manufacturers instructions under standard conditions, manufacturers validation (e.g. 99.998 % kill).
Incineration, effectively 100% kill (licensed incinerator).
The Committee had minor comments which have been covered in the signed risk assessment form.

Please enter comments on the GM safety committee on the risk assessment.

Tick if you are claiming exemption from disclosure for section of the risk assessment.

Tick to confirm that you have attached a risk assessment to this form.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
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<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref 235/93.1**

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Withdrawn N

Historical Significant Changes

GM 235 MERGED WITH GM 386 & PROJECT TRANSFERRED TO GM 3

Tick if notifying a connected programme of work N

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

i) All sharps, disposable plastic pipettes and tips contaminated with GM material will be put into Sharpsafe bins, clearly labelled as “GM WASTE”. These will be autoclaved at 121°C for at least 20 minutes before disposal and subsequent incineration. Contaminated sharps will be treated with 2% Trigene for at least 30 minutes before discarding into the sharpsafe bin to prevent the build-up of contaminated material.

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iii) Liquid waste contaminated with GM material will be treated with Trigene for at least 30 minutes. This will be done by adding the waste to an appropriate volume of the neat disinfection solution and making up to a final concentration of 2%

iv) Any reusable containers contaminated with GM material will be left to soak in 2% Trigene for at least 30 minutes before washing. This will be done by addition of the disinfection solution directly into the container or by immersion of the contaminated equipment in a larger vessel containing the disinfection solution.

Degree of kill: Autoclave waste cycle at 121 for 20 minutes. 100% kill confirmed by annual validation.

02/03/2022
Chemical disinfection with Trigene, used according to manufacturers instructions under standard conditions, manufacturers validation (e.g. 99.998 % kill).
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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**GM Centre Number:** 237

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<th>Transitional Premises Class</th>
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<th>Non-GMMs</th>
<th>Withdrawn</th>
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**Name**

LUDWIG INSTITUTE FOR CANCER RESEARCH

**Name 2**

ROYAL FREE & UNIVERSITY COLLEGE LONDON

**Campus Estate or Research Centre**

**Building**

**Road Name**

91 RIDING HOUSE STREET

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

W1W 7BS

**Country**

ENGLAND

**Tel Number**

0207 878 4010

**Fax Number**

0207 878 4040

**HSE Division**

LONDON

**Comments**

**Date at Which Additional Info Submitted**

08/01/2004

02/03/2022
### Premises Addresses

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<tr>
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<td></td>
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<td>GREATER LONDON</td>
<td>W1W 7BS</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.

<table>
<thead>
<tr>
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</table>

**Project Additional Information**
Purposes of the contained use

To generate and use adenoviral particles.

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 237/01.3

Date Ackn'd 10/10/2001

CU2 Project Title
CONSTRUCTION AND USE OF DEFECTIVE HELPER FREE AMPHOTROPIC RETROVIRUSES FOR THE CONDITIONAL IMMORTALISATION OF PRIMARY HUMAN CELLS

Class 3

Consent Granted yes

Non-GMM

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To generate stable producer cell lines that produce high titre replication defective amphotropic retroviruses which can be used for the immortalisation of freshly isolated primary human cells.

Recipient or parental organism
E.coli JS4
PA371 mouse amphotropic producer cells
TE671 - FLY A and RD human amphotropic producer cells
Human cultures transduced singly or with combinations of viruses for producing immortal cell lines

Host/vector system
JS4 - recA derivative of E. coli MC1061 - pBabeNeo/hygro/puromycin, pZipNeoSV(X)1, pLHCX, pLNCX
PA317 - pZipNeoSV(X)1 - retroviral vector
TEFLY A and RD cells - pBabeNeo/hygro, pZipNeoSV(X)1, pLHCX, pLNCX - retroviral vectors

Date Ackn'd
10/10/2001

CU2 Project Title
CONSTRUCTION AND USE OF DEFECTIVE HELPER FREE AMPHOTROPIC RETROVIRUSES FOR THE CONDITIONAL IMMORTALISATION OF PRIMARY HUMAN CELLS

Class 3

Consent Granted yes

Non-GMM

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To generate stable producer cell lines that produce high titre replication defective amphotropic retroviruses which can be used for the immortalisation of freshly isolated primary human cells.

Recipient or parental organism
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PA371 mouse amphotropic producer cells
TE671 - FLY A and RD human amphotropic producer cells
Human cultures transduced singly or with combinations of viruses for producing immortal cell lines

Host/vector system
JS4 - recA derivative of E. coli MC1061 - pBabeNeo/hygro/puromycin, pZipNeoSV(X)1, pLHCX, pLNCX
PA317 - pZipNeoSV(X)1 - retroviral vector
TEFLY A and RD cells - pBabeNeo/hygro, pZipNeoSV(X)1, pLHCX, pLNCX - retroviral vectors

02/03/2022
Primary human fibroblasts, epithelial and endothelial cells - pBabeNeo/hygro/puromycin, pZipNeoSV(X)1, pLHCX, pLNCX- retroviral vectors.

Origin & function

This project aims to develop amphotropic viruses for transducing various immortalising genes into primary human somatic cells.

The immortalising genes are derived from the Papovaviruses SV40 and Polyoma, Human Papilloma virus 16/18, a human cellular gene induced by the Human Papilloma virus 16/18 E6 protein, a fragment of the human p53 gene and the catalytic subunit of the human telomerase gene.

SV40 large T antigen is an immortalising gene that has the potential to extend the lifespan of human cells. It can inactivate the p53 and RB (Retinoblastoma protein family) pathways.

Polyoma large T antigen is also an immortalising gene that can inactivate the RB pathway.

HPV E6 protein is an immortalising gene derived from the Human Papilloma virus 16/18 that inactivates p53, can induce telomerase in human somatic cells, induce the IITF gene and can extend lifespan of primary human cells.

The IITF gene can induce telomerase activity in human cells and thus has the potential to be an immortalising gene.

The catalytic subunit of telomerase can be used to reconstitute telomerase activity in human somatic cells that are null for telomerase and this maintain their telomeres. Maintenance of telomeres is a critical step in the immortalisation of primary human somatic cells.

Evaluation of foreseeable effects

These viruses either singly or in combination with telomere maintenance as induced by E6, IITF or hTERT have the potential to induce immortalisation of human cells. Therefore these viruses do have the potential to induce immortalisation if the operator is exposed to them by inoculation. Thus safety procedures have been put in place so that the risk of a worker infecting their own cells with the retroviruses is extremely low. Moreover since the viruses produced are defective and helper free they should not be able to spread by themselves from the site of infection.

Cultures of human cells after transduction should produce immortal human cell lines which can be propagated indefinitely and should not produce any infectious virus because the viruses that are being prepared are defective and helper free. Such cells should be rejected by the immune system if accidentally inoculated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The virus producer cells and the viruses will be handled and maintained in a level 3 containment facility to ensure there is no accidental release or exposure.

The transduction and selection of the primary human cells will be carried out in the level 3 containment facility. They will then be tested to determine whether they are producing retroviruses by using the culture medium for transduction of control cells. Once they have shown to be negative for virus production, they will then be transferred to a level 2 containment facility for routine culture and subcloning. They will be tested every 3 months to ensure that they remain negative for virus production.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Non requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be treated with chloros for >24 hours at >10% (10,000 ppm). (Validated experimentally 100% kill) Inactivated liquid waste will be disposed of in dedicated drains leading directly to sewer waste.
The committee feels that even though this is initially a level 2 project, the potential hazards involved in constructing and using viruses that can infect human cells and are transducing gene(s) that have the potential to immortalise human cells warrants the extra containment provided by a level 3 facility.

### Project Containment

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**Animal Units**
- L2
- L3
- L4

**Large Scale Activities**
- L2
- L3
- L4

**Human Clinical Applications**
- L2
- L3
- L4

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**Project Ref** 237/03.1

**Date Ackn’ed** 08/07/2003  
**CU2 Project Title** USE OF LENTIVIRAL VECTOR SYSTEMS FOR IN VITRO TRANSDUCTION OF NON-DIVIDING MOUSE CELLS  
**Class** Class 3  
**CultureVolClass2** >200mls  
**Consent Granted** yes  
**Date Project Ceased**  
**Withdrawn** N  
**Tick if notifying a connected programme of work** N  
**Project notified under transitional arrangements** N  
**Historical Significant Changes**  
**Historical Date of Additional Info**  
**Significant Change ID**
### Purposes of the contained use

To generate high titre pseudotyped lentiviral vector systems containing genes that can be used for the immortalisation of non-dividing primary cells derived from P1 3-kinase gene-targeted mice.

### Recipient or parental organism

**E. coli JS4-rec A-derived from E.coli MC 1061.** This is disabled E. coli

293T kidney cells - produced from human embryonic 293 kidney cells by transformation with SV40T. (Studies on in vitro transformation by DNA and DNA fragments of human adenoviruses and simian virus 40. Graham, FL; Abrahams, PJ; Mulder, C; Heijneker, HL; Warnaar, SO; De Vries, FA; Fiers, W; Van Der Eb, AJ. Cold Spring Harb Symp Quant Biol. 1975; 39 Pt 1: 637-50). These cells have a history of safe use for the production of lentiviruses.

Mouse embryonic fibroblasts (MEFs).

### Host/vector system

**E.coli JS4/p8.91, pVSV-G, pHR, pRETR0-SUPER, pHR containing lacZ, SV40T, p85 alpha, p85beta, p55gamma, p110alpha, p110beta, p110delta, pRETRO-SUPER containing a p53-specific short hairpin oligonucleotide.**

293T will be transduced with the 3 plasmids pVSV-G, p8.91 and pHR into which is inserted lacZ, SV40T, p85alpha, p85beta, p55gamma, p110alpha, p110dbeta, p110delta or pRETRO-SUPER into which is inserted a p53-specific short hairpin oligonucleotide to produce the lentivirus.

Mouse embryonic fibroblasts will be transduced with the VSV-G pseudotyped minimal lentiviral vector containing lacZ, SV40T, p85alpha, p85beta, p55gamma, p110alpha, p110beta, p110delta, a p53-specific short hairpin oligonucleotide.

### Origin & function

**Minimal HIV1-based lentiviral vectors tranducing genes corresponding to the reporter enzyme beta-galactosidase (lacZ), wild type SV40T-antigen (U19), the regulatory subunits of the enzyme P13-kinase (p85 alpha, p85beta, p55gamma), the catalytic subunits of P130-kinase (p110alpha, p110beta, p110delta)**

LacZ is an E.coli gene for beta-galactosidase, which is used as a reporter of localised expression. There is no hazard associated with the expression of this gene.

SV40 large T antigen is an immortalising gene derived from the Papovavirus SV40 that has the potential to extend the lifespan of human cells. It can inactivate the p53 and RB pathways.

p53-specific short hairpin oligonucleotide. Expression of this sequence in mouse cells causes suppression of p53 expression (by RNA interference) that results in extended life span-immortalisation of mouse cells.

p85alpha, p85beta, p55gamma, regulatory subunits for P1-3 kinase (P13K). They form heterodimers and stabilise the p110 catalytic subunits. There are no indications that the full length P13K regulatory subunits constitute a potential hazard.

p110alpha, p110beta, p110delta in native or constitutively active (membrane targeted) form (CAAX form). They will be used to "rescue" the proliferative defect caused by inactivation of the respective genes in targeted mouse cells. The wild-type P13K catalytic subunits could be considered as proto-oncogenes that become oncogenic for
Evaluation of foreseeable effects

The E. coli cells are non-colonising and disabled (recA-) and the inserted sequences are driven by eukaryotic specific promoters (eg CMV). Therefore there is no risk from this part of the work.

The 293T cells will produce a lentivirus containing the inserted genes. The production of a lentivirus increases the risk compared to a simple retrovirus as these can enter non-dividing cells. Also pseudotyping the lentivirus with VSV-G increases the risk as this enables the virus to bind to multiple cell types. However, the virus is replication defective and like all retroviruses these lentiviral particles have a limited survival capacity (half-life of 2-4 hours). The main risk to the worker as for HIV1 is from needlestick injury and by operating in a sharps-free environment and using a class II hood in a level 3 environment the risk to the worker is very low.

Primary mouse cells once tested for absence of replication competent virus have no risk.

The main hazard is from the inserts which are potentially immortalised oncogenes. These can immortalise, but not transform, human cells and therefore, in principle create a pre-neoplastic clone in vivo in a worker exposed to them. However, it has been shown (Lundberg et al Oncogene 2002; 21: 4577-4586) that further genetic modifications such as introduction of mutant ras is necessary for full malignant transformation of cells expressing SV40 large T plus telomerase. Also, p53 inactivation alone is not sufficient to mediate stable reversion of senescence in primary human fibroblasts (Shay et al., 1991, Exp. Cell Res., 196:33-39). Finally, oncogenicity of membrane targeted forms of the p1 3-kinase has only been demonstrated in chicken cells, and published evidence shows that a similar phenomenon does not occur in mammalian cells.

Also the virus prepared with the inserts is replication defective and therefore cannot be self-propagating, further reducing the risk from these inserts, but in any event naturally occurring lentiviruses are not transmitted via aerosols.

The main risk comes from needlestick injury or via an open wound or from an aerosol of these high titre viruses. To minimise these risks the work is carried out in a class II laminar flow cabinet in a level 3 room and working in a sharps-free environment. Any wounds will be covered and gloves and laboratory coats will be worn at all times. Aerosol is minimised by not using a vacuum for aspiration of medium.

Therefore although the consequences of the hazard of transfer of the sequence to the worker could be severe, resulting in the generation of a pre-neoplastic cone(s) in the worker, with the application of the described control measures the risk of the foreseeable effects is extremely low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The transfection of plasmids in 293T cells and transduction of virus to human primary cells and cell lines will be carried out in a class II hood in a level 3 containment facility. The recipient mouse cells will be tested to determine whether they are producing lentivirus by taking culture medium from the transduced cells and placing on control cells, and testing for gene transfer. Once they have been shown to be negative for virus production, they will then be transferred to a level 1 containment facility for routine culture and subcloning.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be treated with chloros for >24 hours at >10% (10,000ppm). (Validated experimentally 100% kill) inactivated liquid waste will be disposed of in dedicated drains leading directly to sewer waste.

All solid waste will be soaked in chloros for >24 hours at 5% (5,000ppm), followed by autoclaving for 15 minutes at 120 C, followed by incineration. (100% kill).
All waste inactivation procedures will be carried out within the level 3 containment facility.

Psuedotyping with pVSV-G and insertion of SV40 large T antigen and other potential oncogenes raises this project to level 3. This work will be conducted in the UCL Jules Thorn Category 3 laboratory that is under the control of the Dept of Surgery local rules must be followed and Ludwig staff who use this facility must provide evidence of training for work in Category 3 environments to the LICR Biological Safety Officer.

Please enter comments on the GM safety committee on the risk assessment

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Project Containment

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Project Ref 237/04.1

Date Ackn'd 07/10/2004

CU2 Project Title Use of viral vectors to study growth control of cells in tissue culture.

Class 2

Culture Vol Class 2 < 1 Litre

Consent Granted Not Applicable

Non-GMM

Project notified under transitional arrangements N

Historical Significant Changes N

Tick if notifying a connected programme of work N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that it has been attached to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form Y
**Project Additional Information**

**Purposes of the contained use**

This programme of work will use amphotropic retrovirus and adenovirus vectors to help study the functions of cellular and viral genes that affect the regulation of cell proliferation and cell survival.

**Recipient or parental organism**

Cell lines, such as QBI-HEK293A, which complement the E1A and E1B defect in adenovirus vectors, will be used to package the adenovirus. Once introduced into a complementing cell line that provides the E1 products in trans, adenovirus replication is possible.

Amphotropic or polytropic cell lines such as AmphoPack-293 or retroPack PT67 (BD Biosciences/Clontech) will be transfected with retrovirus, to produce virus capable of infecting human cells. Highly transfectable cell lines such as HEK293T and NIH3T3 cells will be used to package retroviruses.

Mammalian cells including U20s, Saos2, B cell lines will be used to transduce the recombinant viruses.

Cell lines are free of adventitious agents, and have a history of safe usage in the laboratory, equivalent to level 1.

**Host/vector system**

Adenoviruses (commercially available Adenovator or AdEasy or similar vectors that are commercially available). Adenoviruses can cause disease in humans, however, recombinant adenoviruses generated have their E1 and E3 region deleted. Such deletions prevent the recombinant viruses from replicating. The recombinant virus will be replication incompetent and non-pathogenic.

Retrovirus vectors based on non-human animal viruses such as Moloney murine leukaemia virus (Mo-MuLV), such as pMSCVpuro.

**Origin & function**

The following inserted genes cannot alter the pathogenicity of the recipient. The inserted genes to not encode for a pathogen or pathogenic determinant. As cancer development is a multistep process, transduction with such genes will not transform the infected cells.

* Cellular and viral genes that promote cell proliferation and/or decrease apoptosis - such genes are generally, but not exclusively oncogenes. This programme of work will not include viral oncogenes that have multiple oncogenic activities such as SV40 T antigen, nor will it include clusters of oncogenes such as HPV16 E6-E7. Genes encoding toxins will not be included in this work.

* Single growth-promoting (for example ras, E2F1, myc, E1A, mdmd2) and anti-apoptotic genes (for example iASPP, Bcl-XL).

* Tumour suppressor genes (for example p53, ASPP, RB, p130) and pro-apoptotic genes (for example Bax, Bik and Bad).

* SiRNAs: The SiRNA genes used in this work will be directed against regulators of cell growth and survival (e.g. oncogenes, tumour suppressor genes and pro-
anti-apoptotic genes). SiRNA's directed to tumour suppressor genes would be expected to promote cell growth, while those directed to oncogenes would be expected to inhibit cell growth. However, it is extremely unlikely that ablation of a single gene, as proposed in this project, would have major effects on cell growth and/or survival.

**Evaluation of foreseeable effects**

Adenovirus and amphotropic vectors are designed to infect efficiently cells of many different species and lineages. The two major foreseeable effects are accidental transduction of laboratory personnel and animal species such as mice. These vectors are replication defective and the risk is therefore limited to cells accidentally transduced with the packaged vector. The consequences for animal species are therefore negligible, since even in the extremely unlikely event that mice were exposed to the packaged vector, the virus could not propagate. Replication-competent retroviruses can be generated at low frequencies in packaging cell lines, albeit it seems likely that acquisition of viral replication functions by the vector would be at the expense of the inserted transgene. The use of these vectors will be restricted to transduction into cells in tissue culture, and the risk that infectious virus will be transmitted to animal species is minimal under Containment Level 2 conditions proposed for this project.

Retroviruses can cause pathogenicity, e.g. FeLV in cats. However, only severely deleted vectors will be used and recombination to generate WT virus will be impossible with the non-feline packaging systems used. MuLV is pathogenic in mice. Again the vector is severely deleted. Although there is a risk that low titres of replication competent MuLV can be generated during packaging, these will be insufficient to cause disease. In any event, mice have plentiful endogenous retroviruses which potentially are replication competent.

Exposure of laboratory personnel to packaged vectors containing growth promoting genes such as oncogenes or anti-apoptotic genes could result in some growth advantage of transduced cells. Similarly, transduction with retroviruses encoding siRNAs directed to growth inhibitory genes such as tumour suppressor genes and pro-apoptotic genes could also result in a growth advantage. The single genes targeted in these studies cannot by themselves result in cell transformation, even in highly responsive rodent cells. Targeting of combinations of genes with the potential to cause cell transformation has been specifically excluded from this programme of work. It should also be noted that with the scale of production to be used, it is extremely unlikely that accidental transduction will occur at stem cells at risk of carcinogenic development. Because of the very slight risk involved, it is proposed to carry out this activity under Containment Level 2 conditions.

Accidental exposure of laboratory personnel with packaged vectors carrying genes that inhibit cell proliferation and/or are pro-apoptotic is very unlikely to have any consequences. Similarly, transduction with retrovirus vectors encoding siRNAs directed to growth promoting genes such as oncogenes and anti-apoptotic genes would only inhibit the growth of the cells infected. In principle, transduction of stem cells could inhibit their growth and prevent them from participating in tissue maintenance. In practice, it is unfeasable that sufficient stem cells could be affected in this way to have any consequences for human health. Because of the very slight risk involved, it is proposed to carry out this activity under Containment Level 2 conditions.

It is noted that insertion of certain viral oncogenes (such as HPV16 E6 and E7 and some EBV genes) into E1a/E1B-deficient adenovirus vectors can complement their growth defect. Experimental observations show that rescue is incomplete (Steinwaerder et al., Mol Ther. 4:211-216, 2001) and replication of the recombinant is significantly less than that of wild-type virus. The recombinant virus would therefore be debilitated in its ability to spread in an infected human host. The risk associated with such recombinants is therefore no greater than that of the wild-type virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**Containment level 2**

In order to reduce the risk of infection of laboratory workers to an absolute minimum, this work will be carried out in an ACGM Containment Level 2 facility. All staff engaged in such work will wear protective clothing including gloves and goggles.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid waste will be treated with chloros for >24 hours at >10% (10,000 ppm). Validated experimentally 100% kill). Inactivated liquid waste will be disposed of in
dedicated drains leading directly to sewer waste.

All solid waste will be soaked in chloros for >24 hours at 5% (5,000 ppm), followed by autoclaving for 15 minutes at 120 degrees C followed by incineration. (100% kill).

All waste inactivation procedures will be carried out within the level 2 containment facility.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

This is a category 2 project because genes that express oncogenes or promote cell proliferation are being used.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tr>
<td>L2 Yes</td>
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Project Ref 237/04.2

Date Ackn’d  07/10/2004

Date Project Ceased

Withdrawn  N

CU2 Project Title

Use of lentiviral vectors to study cell growth controls.

Class 2

Consent Granted

Non-GMM  Not Applicable

Project notified under transitional arrangements  N

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2  < 1 Litre

Tick if notifying a connected programme of work  N
Project Additional Information

Purposes of the contained use

This programme of work will involve the use of lentivirus vectors to aid analyses of the functions of cellular and viral genes involved in cell cycle regulation and cell survival.

Recipient or parental organism

Human 293 cells.

Host/vector system

Lentivirus vectors (non-replicating): Three separate plasmids will be transiently cotransfected into 293 cells to produce pseudotyped, packaged viruses. One plasmid will encode for the HIV-1 derived vector, one encodes the VSV-G and one provides functions in trans for packaging. Vectors are based on deleted/mutated HIV-1 genomes, pseudotyped with a vesicular stomatitis virus (VSV) envelope (VSV-G).

Origin & function

Vectors are based on deleted/mutated HIV-1 genomes, pseudotyped with a vesicular stomatitis virus (VSV) envelope (VSV-G). Lentivirus vectors provide an efficient means to transduce oncogenes and anti-apoptotic genes into human cells. The vectors can stably integrate into host cell DNA for sustained long-term expression of the cDNA transgene or siRNA. In these studies the cDNAs will encode for, and the siRNAs will be directed against, cellular or viral genes encoding proteins that promote cell proliferation and/or decrease apoptosis, or inhibit cell proliferation and/or promote apoptosis.

Evaluation of foreseeable effects

The pseudotyped recombinant viruses have the ability to infect most cells with subsequent expression of the foreign gene or siRNA. Therefore all progeny cells will express the foreign DNA, but no new HIV-1 virions can be produced from the infected cell because the vectors (which carry deletions and/or mutations) are replication-defective.

There is a finite theoretical risk that if the virus infects a laboratory worker (eg by spillage in the eye) the transgene or siRNA will be expressed in the infected cells. Since the vectors cannot replicate to infect new cells, this significantly reduces the risk. There is also a theoretical risk of a worker being infected and expressing HIV Gag and Pol proteins (from the pAG132 vector or derivatives). However, since the recombinant virus cannot replicate, the number of expressing cells will be very small and the amount of protein expressed very restricted, the likelihood of an individual sero-converting is considered to be vanishingly small.

There is a theoretical risk of recombination with wild-type HIV-1 or HIV-2. However, there is no report of this ever happening. In any case, recombination restoring all HIV genes - which is necessary to produce a replication-competent virus - would delete the transgene, converting the recombinant virus to wild-type.
The recombinant viruses can only be packaged by cotransfecting the three plasmid components of the vector system into 293T cells. Transfected cells producing the recombinant virus must be maintained in tissue culture and cannot survive outside the laboratory. Although theoretically the lentiviruses would be able to infect other animal species such as rodents, the likelihood of this occurring accidentally is negligible. In the event that this did occur, the recombinant virus would be unable to propagate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Containment level 2

In order to reduce the risk of infection of laboratory workers to an absolute minimum, all work will be carried out in a tissue culture safety cabinet in an ACGM Containment Level 2 facility. All laboratory staff engaged in such work will wear protective clothing including gloves and goggles, and no sharps (glass or needles) will be used. Aerosol contamination will be minimised by confining the virus to closed containers during mixing and agitation procedures. Containers will only be opened in the safety cabinet.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid waste will be treated with chloros for >24 hours at >10% (10,000 ppm). (Validated experimentally 100% kill). Inactivated liquid waste will be disposed of in dedicated drains leading directly to sewer waste.

All solid waste will be soaked in chloros for >24 hours at 5% (5,000ppm), followed by autoclaving for 15 minutes at 120 degrees C followed by incineration. (100% kill).

All waste inactivation procedures will be carried out within the level 2 containment facility.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

With a severely disabled virus, this would normally be a category 1 project but the use of inserts that promote cell proliferation raise it to category 2. In addition, the Category 2 containment laboratory on the third floor (where the work will take place) will operate as a sharps-free environment (no needles or glassware of any kind allowed in the facility).

**Project Containment**

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Name

UNIVERSITY OF CENTRAL LANCASHIRE

Name 2

Campus Estate or Research Centre

Building

MAUDLAND BUILDING

Road Name

CORPORATION STREET

Town

PRESTON

County

LANCASHIRE

Postcode

PR1 2HE

Country

ENGLAND

Tel Number

01772 893500

Fax Number

01772 892929

E-mail

HSE Division

NORTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)  

Tick if confidential  

For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum volume of culture is 12 litres.

All waste is autoclaved at 121 degrees C before disposal. The duration of the period of autoclaving is dependent on the volume or quantity of waste. Large volumes of waste eg. 12 L are autoclaved at 121 degrees C for 60 minutes. The autoclave used for large volumes is 'temperature logged' and aliquots of the liquid effluent will be plated out onto agar medium to check for viable organisms before discharge to the drains.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 245**

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**Comments**

PREMISES MERGED WITH GM56 ABERYSTWYTH UNIVERSITY

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

**Level 1 (GMMs)**

**Level 2 (GMMs)**

**Level 3 (GMMs)**

**Level 4 (GMMs)**

**Non-microbial**

**Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
The maximum volume of culture that could be released at any time is 1 litre. All GMMOs and non-GMMOs are autoclaved at 15 psi for 40 minutes in dedicated autoclaves. All autoclaves are checked using thermocouples every 6 months.

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
**Project Additional Information**

**Purposes of the contained use**
To improve understanding of plant metabolism, especially in legumes, by altering gene expression.

**Recipient or parental organism**
Lotus and Trifolium species
Agrobacterium rhizogenes strain C58C1 LBA9402
[Plant pathogen. Class 2. Use requires Plant Health license from DEFRA]

**Host/vector system**
Disabled E. coli/pUC/pIC/p Bluescript/pBin19 derivatives used to transform Agrobacterium rhizogenes
Agrobacterium rhizogenes is used to transform plants genetically eg Lotus and Trifolium.

**Origin & function**
The Genetically Modified Micro-organism Agrobacterium rhizogenes acts as a plant vector and the modifications do not involve manipulating its plant pathogenicity.

Genes isolated from a range of bacterial and plant sources will be introduced into A. rhizogenes, which will then be used to transform plants. None of the inserted genes will be expressed in A. rhizogenes. The inserted sequences include selectable marker genes, reporter genes and known genes or non-characterised plant sequences inserted in sense and antisense orientations that will potentially alter secondary metabolism and symbiotic interactions of the plants transformed with the sequences concerned. Inserted sequences will include
a) selectable markers for antibiotic resistance - kanamycin and hygromycin (from bacteria) - and herbicide tolerance - phosphophinothricin (PPT, bacteria).
b) reporter genes - glucuronidase (bacteria) and green fluorescent protein (jellyfish).
c) secondary metabolism genes from flavonoid pathway - chalcone synthase (plant), dihydroflavonol reductase (plant)
d) symbiotic genes - early and late nodulins, calcium binding protein (plant)
e) sequences isolated from plants, but whose role in the biochemical pathway is unknown, and is being determined.

**Evaluation of foreseeable effects**
Genetically Modified Microorganisms. A. rhizogenes:

**Risk to human health**

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The inserted bacterial and plant genes have no foreseeable effect on human health.

**Risk to Environment**

Agrobacterium rhizogenes is a soil-borne plant pathogen causing hairy root disease. Current information indicates that its pathogenicity has little economic impact on crop production. A rhizogenes is used as a plant vector and the modifications do not involve manipulating its plant pathogenicity. The inserted sense and antisense genes - antibiotic or reporter genes, known genes or non-characterised sequences related to metabolism in plants - are not expressed in the bacterium and are themselves unlikely to alter the pathogenicity of the bacterium.
While A. rhizogenes has the potential for a minor impact on the environment as a plant pathogen, the inserted genes are unlikely to increase that potential. In the unlikely event that genetically transformed A. rhizogenes were to escape and effect indigenous plants, the resultant hairy root tissue would contain and could express any of the inserted genes. However, any expression would be restricted to the hairy roots themselves and these hairy roots are unlikely to regenerate into new plants. Plant regeneration from roots usually requires very specific, controlled culture conditions, and then only occurs in a limited number of species.

The inserted gene would not be expected to confer any significant advantage on the hairy roots unless other unlikely conditions were also met. For example, hairy root tissue transformed with the PPT herbicide tolerance gene (as the result of accidental escape of A. rhizogenes) might be at an advantage if it came into contact with the herbicide. However, this herbicide is not used routinely in British agriculture and, normally, roots die without a shoot system. In a worse case scenario, these plants might be unusual and be able to regenerate shoots directly from the herbicide tolerant hairy roots.

The containment measures adopted will prevent escape of genetically manipulated A. rhizogenes. All bacterial and plant/bacterial work will be done in Class 2 safety cabinets. The bacterium will be maintained in sealed units, cultures will be contained within the laboratory and kept in growth rooms. A warning notice will be displayed next to bacterial cultures and plant cultures potentially containing A rhizogenes as agreed by DEFRA. Plant material will be rigorously tested for sterility to ensure that A. rhizogenes is removed prior to transfer to soil or equivalent in Plant Containment Level A growth rooms and glasshouses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The resulting GM plants will be grown under established facilities under agreed procedures for Containment Level A, in a locked, insect-proof compartment with no free drainage. All transgenic material will be free of Agrobacterium species before transfer to soil.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste is collected in labelled autoclave bags and autoclaved at 121 degrees C, 40 minutes, 15 psi
Autoclave is tested annually using a thermocouple and whenever autoclave is re-commissioned after maintenance work.
Autoclaved waste collected and disposed of by the local authority.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The local Genetic Modification Safety Committee (GMSC) considers that the risk assessment meets current regulations even though the comments on the original risk assessment were made under the regulations enforced in 1993. The agreed procedures state that all manipulations using A. rhizogenes will be done in a Class 2 hood.

The GMSC has now reclassified this activity as recommended by HSE as a Class 2 activity under the current regulations.
## Project Containment

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### Large Scale Activities

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**Name**

UNIVERSITY OF PLYMOUTH

**Name 2**

**Department**

**Campus Estate or Research Centre**

DRAKE CIRCUS

**Road Name**

**District**

**Town**

PLYMOUTH

**County**

DEVON

**Postcode**

PL4 8AA

**Country**

ENGLAND

**Tel Number**

01752 232950

**Fax Number**

01752 232970

**E-mail**

**HSE Division**

WALES AND SOUTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: [ ]
- **Give brief details of the genetic modification safety committee**

<table>
<thead>
<tr>
<th></th>
<th>Laboratory</th>
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<th>Growth Room</th>
<th>Glass House</th>
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<td>[ ] Tick if confidential</td>
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</table>
Waste is autoclaved to inactivate GMMs. A cycle of 121 degrees C at 15 psi for 30 mins is used to inactivate GMMs.

An in-load probe is used to monitor temperature in addition to the normal chamber temperature probe. To monitor the cycle, the loads are marked with autoclave indicator tape and each load carries a temperature indication tube. In addition, there is a chart recorder for load temperature throughout the cycle. Each run is logged.

Spore strips are included in a load on a monthly basis, to verify the autoclaves ability to inactivate microbes.

The autoclave is checked for performance and serviced at least on an annual basis and every 14 months for a pressure check.

The maximum culture volume of GMMs in any one vessel, which could potentially be released at any one time, would be no more than 400 ml. Please note, however, before autoclaving, this volume of culture would be aliquoted into several containers to ensure adequate heat penetration.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
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GM Centre Number: 250

Data Premises Notified (Originally) 15/11/1992

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Emergency Plan Required? Y

Transferred from 1992 Regs? Y

Non-GMMs Y

Withdrawn N
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### Premises Conditions

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Give brief details of the genetic modification safety committee

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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 250/00.1

**Date Ackn'd** 03/11/2000

**Date Project Ceased** 04/05/2018

**CU2 Project Title** ASSESSMENT OF PATHOGENICITY OF BOTRYIS CINEREA DISRUPTION MUTANTS ON FRENCH BEAN (PHASEOLUS VULGARIS), ARABIDOPSIS THALIANA AND CAPSICUM ANNUUM.

**Class** Class 2

**CultureVolumeClass2**

**Consent Granted** not applicable

**Project notified under transitional arrangements**
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 250/00.2

Date Ackn'd: 03/11/2000
CU2 Project Title: INVESTIGATION OF THE RESISTANCE-BREAKING CHARACTER OF RASPBERRY BUSHY DWARF VIRUS (RBDV) ISOLATES USING REVERSE GENETICS

Class: Class 2
CultureVolClass2: CultureVolumeClass3-4
Non-GMM: Consent Granted: not applicable

Tick if notifying a connected programme of work: N
Project notified under transitional arrangements: Y

Historical Significant Changes

Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 250/00.3

Date Ackn'd 03/11/2000

Date Project Ceased 04/05/2018

CU2 Project Title STUDIES ON VIRAL MOVEMENT IN BARLEY USING A PLANT VIRUS THAT EXPRESSES GREEN FLOURESCENT PROTEIN

Class Non-GMM

Culture Vol Class 2

Culture Vol Class 3-4

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Withdrawn  

Tick if notifying a connected programme of work  

Historical Significant Changes  

Historical Date of Additional Info
**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Is an emergency plan required according to regulation 20? [N]
- If yes, tick to confirm that it is attached to this form [N]
- Tick to confirm that you have attached a risk assessment to this form [N]
- Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
### Project Additional Information

**Purposes of the contained use**

A previously described cDNA for a vector based on tobacco-mosaic virus strains U1 and U5 will be genetically engineered to express various carrier proteins with peptide fusions. Plants belonging to the genus Nicotiana will be infected with in vitro transcripts produced from the genetically engineered cDNAs. Levels of in planta protein accumulation and stability of the genetically engineered viruses will be assessed. If adequate levels of recombinant proteins accumulate in planta, methods for purification of the recombinant proteins will be developed.

**Recipient or parental organism**

TMV is indigenous to the UK and the TMV based vector has been previously notified to HSE (GM 250/98.3). Although TMV is a major cause of disease in tobacco, this crop is not grown commercially in the UK. Extensive prior work has shown that GMOs derived from the recombinant TMV-based vector and in planta recombinants that arise for them are less pathogenic to plants than natural TMV (Rabindran S, Dawson WO Assessment of recombinants that arise from the use of the TMV-based transient...
### Host/vector system

- **Host:** Noctiana species
- **Vector:** Recombinant virus based on TMV

### Origin & function

The following proteins will be tested as carrier proteins for peptide immunogens expressed by the recombinant virus in plants:
- Mammalian immunoglobins (IgGs)
- Non-catalytic cholera toxin B-subunit (CTB)
- Non-catalytic E.coli heat labile toxin B-subunit (ETEC)
- VP1 structural protein of foot-and-mouth disease virus
- 3D polymerase protein of foot-and-mouth disease virus

### Evaluation of foreseeable effects

The viral proteins produced by the recombinant plant virus vector occur in nature and have no known risks to human health. Of the proteins that will be tested as carrier proteins IgGs, CTB, ETEC and VP1 have previously been tested as immunogens in mammals without adverse effect, and the 3D protein is present within currently used foot-and-mouth disease virus vaccines. These proteins are thus expected to present no risk to human health. Due to the genetic instability of the TMV-based vectors the sequences of foreign genes are rapidly lost and would not persist in the event of escape of the GMOs into the environment. Extensive prior experiments indicate the GMOs themselves do not present a threat to the environment, because of their genetic instability and the poor competitive fitness of such GMOs carrying foreign gene inserts relative to wild-type virus occurring in nature. The recombinants that will arise in planta from the GMOs will be fitter that the GMOs, however prior experimentation has shown that the recombinants arising in planta are still less pathogenic than wild-type TMV that occurs in nature. Therefore although the GMOs and recombinants have the capability to cause disease this is of little consequence as natural virus is more pathogenic and tobacco is not grown commercially in the UK.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Glasshouse benches resistant to acids etc. derogation applied for on basis that benches are made of wire mesh for ease of cleaning. Receptacles to prevent any run off will be included.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Infected plants and associated materials (soil, plants, gloves) will be autoclaved using a protocol validated to give 100% kill. Plant extracts prepared in the laboratory and material derived from them will be autoclaved using a protocol validated to give a 100% kill. Contaminated surfaces will be disinfected with a proprietary virucide validated to give a 100% kill.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
Previous work has suggested that TMV constructs are very useable and inserts are likely to be lost within 2 passages. All the carrier proteins except FMDV 3D have been expressed previously in transgenic plants, so risks to human health are not predicted. The 3D protein is present in some, though not all foot-and-mouth disease vaccines, but has not been previously expressed in plants. However, there is no risk to the environment due to the likely instability of the construct.

### Project Containment

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### Project Ref 250/01.2

- **Date Ackn’d:** 01/11/2001
- **CU2 Project Title:** DEVELOPMENT OF PLANT VIRUS-BASED VECTORS FOR GENE SILENCING STUDIES IN MONOCOTYLEDONOUS HOSTS
- **Class:** Class 2
- **CultureVol:** < 1 litre
- **Class CultureVol:** Class 2
- **VolumeClass:** < 1 litre
- **Non-GMM:** Consent Granted
- **Project notified under transitional arrangements:** N

### Project Additional Information

**Purposes of the contained use:** The purpose of the project is the development of plant virus-based vectors and inoculation techniques for analysis of monocotyledonous plant gene function through silencing. Plant viral cDNAs, under the control of bacteriophage promoters for in vitro transcription, will be genetically modified through the insertion of genes for easily
detectable reporter proteins to monitor virus movement. The genetically modified viral cDNAs will be further placed under the control of plant promoter sequences to test biolistic and Agrobacterium-mediated inoculation of specific target tissues. The ability of the genetically modified viruses to induce silencing will be tested through the insertion of plant derived cDNA sequences.

**Recipient or parental organism**

- Barley stripe mosaic virus (BSMV), US isolate.
- Foxtail mosaic virus (FoMV), US isolate.
- Soil borne wheat mosaic virus (SBWMV), Japanese isolate.

**Host/vector system**

- **Host:** Escherichia coli strains XL1-Blue and DH5a.
  **Vector:** pUC-based plasmids containing plant viral cDNAs under the control of bacteriophage or plant promoters.

- **Host:** Agrobacterium tumefaciens strains LBA4404 and AGL-1.
  **Vector:** binary plasmids, ie. pBIN19 and pGREEN, containing plant viral cDNAs under the control of plant promoters.

- **Host:** Hordeum volgare
  **Vector:** Recombinant plant viruses based on BSMV, FoMV and SBWMV.

**Origin & function**

- Green fluorescent protein (GFP) from Aequorea sp., reporter protein.
- Red fluorescent protein (RFP) from Discosoma sp., reporter protein.
- B-glucuronidase (GUS) from Escherichia coli; reporter protein.
- Hordeum vulgare cDNAs, candidate genes for silencing.

**Evaluation of foreseeable effects**

The viral proteins produced by the recombinant viruses occur in nature and have no known risks to human health. The reporter proteins GFP, RFP and GUS have been expressed in a wide range of experimental systems and do not present a risk to human health. All three viruses to be used are non-indigenous plant pathogens and thus subject to legislative control. FoMV does not cause economically important disease and its natural hosts are not indigenous to the UK. SBWMV and BSMV are a cause of disease in wheat and barley. In order to provide robust biological containment portions of the coat protein genes of BSMV and SBWMV will be deleted to render them non-transmissible by mechanical and fungal routes respectively. The genetic modification required to disable their natural transmission routes will result in increased symptom severity on inoculated plants. Previous insertion of reporter protein genes into plant viral genomes has resulted in the creation of attenuated viruses and no mechanism for enhanced pathogenicity can be foreseen. The introduction of plant derived sequences into plant viral genomes and the induction of host gene silencing can affect the symptomatology of infections. However, previous experiments with vectors based on RNA plant viruses have shown introduced sequences to be very rapidly lost through in planta recombination events (for review of genetic instability in genetically modified RNA plant viruses see: Lacomme et al (2001) Plant Viruses as Gene Expression Vectors, in: Ring CJA and Blair, ED (Eds) Genetically Engineered Viruses: Development and Applications, BIOS Scientific Publishers, Oxford). Therefore, the genetically modified plant viruses are considered to present less risk to the environment than the natural progenitor viruses.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Infected plants and associated materials (soil, plants, gloves) will be autoclaved using a protocol validated to give 100% kill. Plant extracts prepared in the laboratory and material derived from them will be autoclaved using a protocol validated to give 100% kill. Contaminated surfaces will be disinfected with a proprietary virucide validated to give 100% kill.

The committee discussed the following points:

The risk of whether silencing of genes could result in more severe symptoms was raised, but the committee were informed that introduced sequences are generally rapidly lost. DNA-based inocula were stated to pose no greater risk than DNA constructs for in vitro transcription. For agrobacterium inoculation of barley stripe mosaic and wheat soil borne mosaic viruses, mixed inoculums, with the genomes separated between 2 or 3 plasmids would be used to reduce risk in the event of an escape. The risk of escape would be greater for the monopartite foxtail mosaic virus, but there would be less effect to the environment. It was asked if any risk was foreseen to any UK horticultural crops, but confirmed not to be the case. The strict emphasis of pollen containment was reiterated by the committee.

Please enter comments on the GM safety committee on the risk assessment

The committee discussed the following points:

The risk of whether silencing of genes could result in more severe symptoms was raised, but the committee were informed that introduced sequences are generally rapidly lost. DNA-based inocula were stated to pose no greater risk than DNA constructs for in vitro transcription. For agrobacterium inoculation of barley stripe mosaic and wheat soil borne mosaic viruses, mixed inoculums, with the genomes separated between 2 or 3 plasmids would be used to reduce risk in the event of an escape. The risk of escape would be greater for the monopartite foxtail mosaic virus, but there would be less effect to the environment. It was asked if any risk was foreseen to any UK horticultural crops, but confirmed not to be the case. The strict emphasis of pollen containment was reiterated by the committee.

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Project Ref 250/02.2

Date Ackn’d 17/09/2002

CU2 Project Title AGROINOCULATION OF SOLANUM SPECIES WITH RECOMBINANT PVX CONSTRUCTS IN A STUDY OF RESISTANCE/AVIRULENCE MECHANISMS

Class 3

CultureVolumeClass3-4 less than 1 litre

Non-GMM Consent Granted yes
The aim of this proposed research are to characterise functional roles for Phytophthora Exported Proteins (PEXs) in triggering different forms of resistance within potato. The experimental system proposed is the Agroinoculation of expressed sequence tags encoding proteins with a putative signal peptide into young potato plants and the assessment and RNA analysis of the resultant plant material.

**Recipient or parental organism**

Potato Virus X
A full-length cDNA clone of the potexviurus (pGR106), potato virus X (PVX), will be supplied by our collaborators. Individual cDNAs of unknown function (expressed sequence tags encoding proteins with a putative signal peptide) from Phytophthora infestans, Peronospora parasitica or P. sojae will be inserted into the PVX backbone in which their expression will be controlled by the duplicated PVX coat protein promoter.

**Host/vector system**

The viral genome will be cloned into Agrobacterium tumefaciens T-DNA and in planta synthesis of infectious recombinant PVX transcripts will be driven by the cauliflower mosaic virus (CaMV) 35S promotor. These transcripts will subsequently initiate the formation of virus particles that infect the tissue surrounding the inoculation site.

Plant species to be used initially will include later blight-resistant and susceptible accessions of Solanum papita, S. chacoense, S. verrucosum, and S. demissum. They would be expressed in a range of S. tuberosum differentials containing the full set of R. genes from S. demissum, singly and in combinations, to seek triggers of race-specific resistance to P. infestans. They would also be expressed in progeny derived from S. tuberosum cv. Stirling to seek the triggers of high-level field resistance to P. infestans. In addition, the GMO will also be inoculated onto Nicotiana benthamiana.

**Origin & function**

From an extensive set of P. infestans expressed sequence tags, our collaborators have identified many genes possessing predicted signal peptides, termed PEX (Phytophthora exported proteins). These genes have been individually cloned into a potato virus X (PVX) - Agrobacterium vector for expression in plants. These workers have then used these recombinant PVX constructs for inoculation of Nicotians sp. in a study of non-host resistance/avirulence mechanisms.

We can obtain these recombinant PVX constructs and propose to use them for Agroinoculation of Solanum sp. in a study of resistance/avirulence mechanisms at the species, and also cultivar level. If the P. infestans gene to be expressed from PVX in planta encodes an avirulence gene, we may expect to see localised necrosis (HR) at the point of inoculation, or systemic necrosis if the virus is able to spread through the plant. This response would be confined to one or few host plant genotypes; other host plant genotypes would show no visible response. Expression of putative pathogenicity factors in plants may be more difficult to detect or interpret. For instance, suppression of defence responses by the expressed gene may only be detectable by inoculation and infection with an incompatible P. infestans isolate, and subsequent host defence gene expression analysis. However, in many cases, the recombinant PVX will not lead to any change in the host plant, except for that caused by PVX.
Determination of both pathogenicity factor and avirulence genes from *P. infestans* will provide us with novel targets for both chemical and biotechnological control of late blight. It also will allow us to identify genetic components of more complex host resistance phenotypes such as field resistance to late blight, which can then be used in potato breeding programmes.

In our experiments, only very young plants will be used for inoculation, and inoculated plants will be kept for a maximum of 28 days. Therefore, spread of the virus through pollen dispersal will therefore not occur, as host plants will not be kept in the experimental system for sufficient time for flowering to occur, therefore seed will also not be collected.

Leaf samples will be taken for gene expression analysis. Samples will be immediately frozen in the glasshouse area and transferred in sealed, easily decontaminated containers to SCRI laboratories for RNA extraction. Potato tubers will be destroyed after experiments are completed. Plants will be destroyed by autoclaving after experiments have been completed. All material will be placed into sealable autoclavable receptacles within the glasshouse compartment and transported to the autoclave sited within the facility for destruction.

Plants infected with the GMO may be challenged with pathogenic *P. infestans*, or other non-pathogenic *Phytophthora* sp., to determine if any change to host resistance/susceptibility has occurred due to the expression of the GMO. These additional experiments would take a further 7 days, taking the total potential length of the experiments to 35 days. All material, with the exception of leaf samples retained for RNA analysis, would be destroyed by autoclaving at the conclusion of the experimental work.

**Evaluation of foreseeable effects**

**Risk to human health**

* Wild type PVX
PVX is not known as a hazard to human health and there is no evidence to suggest that it could be.

* Recombinant PVX
The foreign genes to be expressed in the recombinant PVX (the GMO) are of unknown function or biological activity. Transgenes will encode protein products from oomycete plant pathogens such as *P. infestans* (potato late blight), *P. sojae* (soybean root rot), or *Pe. parasitica* (downy mildew of Arabidopsis thaliana). The majority of transgenes to be tested will originate from *P. infestans*.

As the work will be strictly contained and no tubers will be produced from the experimental plants there will be no risk from consumption of novel potato material. Reports exist that consumption of blighted potatoes leads to birth defects of the nervous system, but these have not been proven, and have been largely refuted by Borman and Cryer (1990). Direct testing of *P. infestans* extracts on chick embryos has determined that there was no evidence for toxicity (Wu and Salunke 1978) and that any birth defects are caused by glycoalkaloids present in the potato tuber (Jelinek et al., 1976). No toxicology studies have been carried out on the related species *P. sojae* or *Pe. parasitica*, and there are no records to implicate either organism in human disease. In the extremely unlikely event that any of the expressed gene products are determined in other work elsewhere to be toxic to humans from other experiments, experiments with these genes will cease immediately.

* A. tumefaciens
Agrobacterium tumefaciens occurs naturally in soil and is not known to be a pathogen of humans. It has been used safely as a vector for transgene delivery into plants for many years. It is not expected to pose any threat to human health under the conditions to be used for experimentation. Generic risk assessments for manipulation of A. tumefaciens and agroinoculation are available at SCRI.

* Host plant species
None of the host plants (Solanum sp. or nicotiana sp.) to be used in our experimental system pose a threat to human health under the conditions to be used for experimentation.
* P. infestans
P. infestans occurs as an introduced pathogen on potato crops in the United Kingdom. It is not known as a human pathogen. It is not expected to pose any threat to human health under the conditions to be used for experimentation.

Risk to the environment

* Wild type PVX
Wild type PVX is transmissible by direct contact between infected plants or indirectly through mechanical transmission. PVX is not transmitted by aphids, and its host range is mostly limited to solanaceous plants. At worst, wild type PVX can cause yield losses in potato of up to 15%. In our experiments, the conditions for transmission of the virus will be prevented. Handling of infected plants will be minimised, disposable gloves will be worn for all handling of infected plants, and all materials that come in contact with the infected plants will be placed into sealable autoclavable receptacles within the glasshouse compartment and transported to the autoclave sited within the facility for destruction.

* A. tumefaciens
For agroinoculation of PVX into potato plants, it is highly unlikely that the A. tumefaciens strain would escape. Containment measures include: leaves are inoculated and subsequent watering of plants is from below so no water run off can occur from the leaves, any A. tumefaciens remaining on the leaf surface will be exposed to dessication, and A. tumefaciens does not spread beyond the site of initial inoculation. The glasshouse floor will be sealed, and the drains blocked off. In the highly unlikely event of flooding in the area the run-off will be routed into a holding tank and treated with an oxidising agent prior to release by manually-operated valves to mains drainage. As stated in the risk assessment, gloves will be worn at all times when inoculations are being performed and these will be autoclaved after use and before disposal. In addition, all soil, infected plant material, and any excess water in containment trays will be autoclaved at the conclusion of the experimental work.

* Recombinant PVX
The above actions are expected to prevent the escape of live A. tumefaciens carrying recombinant PVX. In the highly unlikely event of live A. tumefaciens carrying recombinant PVX escaping the containment measures in place, it is unknown whether the recombinant PVX construct could be transferred to other rhizosphere organisms, and hence into new host plants. Although numerous plant viruses can be spread by rhizosphere organisms, such as fungi and nematodes, PVX is not known to spread in this way. It is unlikely that the presence of the PVX constructs would confer any novel phenotype upon A. tumefaciens. This is due to the fact that the PVX constructs should be actively transcribed into infectious virus in the bacterial cell, being under the control of the CaMV35S promoter element, and the transgene itself is under control of the PVX coat protein promoter, both of which are transcribed only in planta. The combination of all of the above factors render the risk of escape of recombinant PVX via the A. tumefaciens route as negligible.

A further containment measure that could be applied to prevent the spread of recombinant PVX into potato fields, in the event of escape from the containment facility, would be to carry out the agroinoculations outside the usual potato growing season, such as the period September to April. Obviously we would wish to carry on these experiments year round but are willing to concede that it may be necessary to restrict the time for recombinant PVX research.

* Modifications to the host range of the GMO
For the majority of transgenes tested, there will be no effect on the infectivity, disease development, or host range of the GMO. However, the GMO may express genes that either cause more intense disease symptoms (necrosis) or suppress the host plant defence responses, thus leading to more rapid disease development and more intense disease symptoms. It is also possible that the expression of transgenes from the GMO may also alter the host range of the virus, leading to subsequent strong selection for the GMO in the unlikely event of escape.

However, in case of any viral escape, recombinant viruses often spontaneously delete the transgene from the viral genome (for example Chapman et al., 1992). This may occur due to viral packaging constraints being ‘overstretched’ with the insertion of the transgene.
In the highly unlikely event of any escape of the GMO, it should be noted that most major varieties of potato grown in the vicinity of SCRI, and throughout Scotland possess field immunity to PVX (for example varieties Maris Piper, Cara, King Edward, Pentland Dell, Pentland Javelin, Pentland Squire).

* P. infestans

Most potato varieties grown in Scotland are however susceptible to late blight, caused by P. infestans. Control of late blight is typically by frequent treatment of crops with fungicides active against P. infestans. It is very unlikely then, that infection of potato crops by escaped recombinant PVX would have any effect on the level or severity of late blight in those crops.

**Laboratory manipulation and analysis of the GMOs** will be performed in a laboratory equipped to containment level 2.

**Experiments on the GMO** will be carried out in the AN building at SCRI, a designated containment glasshouse facility designed to prevent any dissemination of the GMO. The facility is insect and vermin proof. Access is restricted to authorised personnel and the facility is equipped with two 'through the wall' autoclaves. To prevent escape of mechanically transmissible PVX, all handling of infected plants will be carried out with gloved hands and gloves disposed of appropriately. Designated laboratory coats are provided for work in the containment glasshouse and these coats are not removed from the containment facility unless in autoclave bags for sterilisation. Furthermore, any material that might be contaminated by PVX (plant material, pots, soil, labels, gloves) will be placed into sealable autoclavable receptacles within the glasshouse compartment and transported to the autoclave sited within the facility for destruction via accredited autoclave cycles.

The cubicle and benches (for which we apply for derogation as they are of wire-mesh) to be used for testing of the GMO will be cleaned and disinfected prior to commencement, and after completion, of experimental work. Initial analysis of the GMOs will be performed on no more than 50 plants at one time. However, larger numbers of plants will be required if the GMOs are to be screened across progeny derived from S. tuberosum cv. Stirling. In this instance, no more than 200 plants would be required. Escape of live A. tumefaciens will be prevented through the employment of procedures currently used at SCRI for agroinoculation, for which a generic risk assessment exists at SCRI (attached). SOPS include restriction of access to plants and watering only by licensed staff, control of water run-off through the use of trays, and watering from beneath. The glasshouse floor will be sealed, and the drains blocked off. In the highly unlikely event of flooding in the area the run-off will be routed via a default setting into a holding tank and treated with an oxidising agent prior to release by manually-operated valves to mains drainage.

Records of all glasshouse experiments will be maintained for inspection, including all details of plant disposal.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Glasshouse benches resistant to acids etc., derogation applied for on basis that benches are made of wire mesh for ease of cleaning. Receptacles to prevent any run off will be included. The glasshouse floor will be sealed, and the drains blocked off. In the highly unlikely event of flooding in the area the run-off will be routed via a default setting into a holding tank and treated with an oxidising agent prior to release by manually-operated valves to mains drainage.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Infected plants and associated materials (soil, plants, gloves) will be autoclaved using a protocol validated to give 100% kill. Plant extracts prepared in the laboratory and material derived from them will be autoclaved using a protocol validated to give 100% kill. Contaminated surfaces will be disinfected with a proprietary virucide validated to give 100% kill. All material from glasshouse will be bagged within the designated containment cubicle, prior to transportation in closed receptacles to autoclaves within the glasshouse.

**Is an emergency plan required according to regulation 20?**  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

02/03/2022
This is a revision of our original proposal to HSE..

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Tick if notifying a connected programme of work N

### Project Additional Information

P. infestans is a hemibiotrophic comycete pathogen that predominantly infects the foliage of potato plants. Related oomycete pathogens such as P. sojae and
Peronospora parasitica have very different life styles to P. infestans; P. sojae is a hemibiotrophic pathogen of soybean that infects predominantly through the roots, and Pe. parasitica is a strictly biotrophic pathogen that infects Arabidopsis thaliana foliage.

From an extensive set of P. infestans expressed sequence tags, our collaborators have identified many genes possessing predicted signal peptides, termed PEXs (Phytophthora exported proteins). In addition, an extensive targeted gene discovery programme is underway at the Scottish Crop Research Institute (SCRI) aimed at identifying genes involved in the lifecycle stages of P. infestans prior to, and during, infection of potato. In the instance of P. infestans pathogenicity and avirulence genes, these will be genes that are either (or both) strongly up-regulated during infection of potato, or the P. infestans homologues of genes implicated in pathogenesis or avirulence of other plant pathogens. Many of the genes to be tested will have no homology to genes of known function. Our aim is to determine the phenotypic effects of constitutive expression, and/or silencing, of the discovered genes on specific pathogen cell types, pathogenicity, and avirulence. Our gene discovery programme also includes comparative analyses with the pathogens mentioned above, especially Pe. parasitica.

We aim to express avirulence genes from these related pathogens in P. infestans. The question we will address with this research is whether genes involved in avirulence in the named organisms have any role or effect in the P. infestans/potato interaction.

Determination of both pathogenicity factors and avirulence genes in P. infestans will provide us with novel targets for both chemical and biotechnological control of late blight. It also will allow us to identify genetic components of more complex host resistance phenotypes such as field resistance to late blight, which can then be used in potato breeding programmes.

Recipient or parental organism

Isolates of the wild-type oomycete plant pathogen P. infestans will originate from the SCRI Phytophthora culture collection. Details of constructs to be used for transformation of P. infestans are attached (Figure 1). Promoter and terminator sequences (hsp70, ham34) originate from regulatory genes in the lettuce downy mildew pathogen, Bremia lactucae. In vector pSam, the regulation of P. infestans gene elements will be tested. It would be used to drive expression of the green fluorescent protein gene (gfp) for cellular localisation of gene expression during all life cycle stages, including infection of potato. In vector pTor, inserted genes will be constitutively expressed under control of the ham34 promoter. Both transformation vectors contain the antibiotic resistance gene neomycin phosphotransferase (nptII) under control of the same B. lactucae promoters mentioned above.

We aim to express and silence genes involved in both pathogenicity and avirulence in P. infestans. In addition, we also aim to express in P. infestans, genes involved in avirulence originating from P. sojae (a soybean pathogen) and Peronospora parasitica (Arabidopsis downy mildew) to determine whether they will function as non-race-specific (and thus more durable) avirulence genes in the potato-P. infestans interaction. The precise sequence identity of each of the genes to be tested is not known, as the gene discovery phase of the research is still in progress. In the instance of P. infestans pathogenicity genes, these will be genes that are either (or both) strongly up-regulated during infection of potato, or the P. infestans homologues of genes implicated in pathogenesis of other plant pathogens. Many of the genes to be tested will have no homology to genes of known function. For P. infestans avirulence genes, these are being identified by map based cloning, or by a candidate gene approach. It is therefore not yet known what these genes will encode.

For studies into heterologous gene expression and function we aim to express genes in P. infestans from related plant pathogens P. sojae, and Pe. parasitica. The question we will address with this research is whether genes involved in avirulence in the named organisms have any role or effect in the P. infestans/potato interaction. The transgenic P. infestans from these experiments will have either the same phenotypes listed above, or will invoke a general, non-host response in all potato varieties, or no change from the wild type will be observed. Since the transgenes will originate from related pathogens (for which potato is a non-host plant), it is highly likely that functionally related genes are already present in P. infestans.

Host/vector system

Numerous methods for the transformation of P. infestans are available such as transfection of protoplasts, electroporation of zoospores, Agrobacterium tumefaciens transformation or microprojectile bombardment of germinating sporangia. We will use the latter technique initially as it has the potential to be the highest throughput. If microprojectile bombardment is unsuccessful, we propose using the other methods listed.
The GMMs would be tested for pathogenicity against Solanum tuberosum cvs Bintje and Stirling, as well as a range of S. tuberosum differentials containing the full set of R genes from S. demissum, singly and in combinations, to seek triggers of race-specific resistance to P. infestans. The GMMs will also be tested against key species and accessions from the Commonwealth Potato Collection (housed at SCRI) to seek triggers of race-non-specific resistance. Species to be used initially will include late blight-resistant and susceptible accessions of Solanum papita, S. shacoense, S. verrucosum, and S. demissum. They would also be tested against progeny derived from S. tuberosum cv. Stirling to seek the triggers of high-level field resistance to P. infestans.

Constitutive expression of candidate avirulence genes isolated from P. infestans will result in the tested transgenic P. infestans becoming avirulent (or incompatible) with potato varieties carrying specific matching resistance genes. In this case, the host range of the transgenic P. infestans is expected to be reduced from the wild type (with the wild type originally being virulent, or compatible, on the resistance genes tested). For a P. infestans avirulence gene to be expressed, we may expect to see localised necrosis (HR) at the point of inoculation. This response would be confined to one or few host plant genotypes (carrying the cognate R gene); virulence/avirulence specificity towards other host plant genotypes should remain unchanged.

We will also silence avirulence genes from P. infestans. The outcome from these experiments would be transgenic P. infestans that have gained virulence towards specific, cognate resistance genes. In this case, it should be noted that wild type isolates collected locally also display the same virulence/avirulence traits towards the same potato resistance genes. That is, the genes to be tested, and their phenotypic variants, occur naturally in local P. infestans populations.

A further experiment we propose is that of heterologous gene expression and function. We propose to express genes in P. infestans from related plant pathogens P. sojae, and Pe. parasitica. The question we will address with this research is whether genes involved in avirulence in the named organisms have any role or effect in the P. infestans/potato interaction. The transgenic P. infestans from these experiments will have either the same phenotypes listed above, or will invoke a general, non-host response in all potato varieties, or no change from the wild type will be observed. Since the transgenes will originate from related pathogens (for which potato is a non-host plant), it is highly likely that functionally related genes are already present in P. infestans. Therefore, it is unlikely that introduction of the heterologous genes will have any effect on the host range of the transgenic P. infestans. Indeed, the opposite effect of restricting the host range may be more likely, as the host plant may recognise the foreign gene product and initiate a resistance response.

Origin & function

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Evaluation of foreseeable effects

Risk to human health

P. infestans occurs as an introduced pathogen on potato crops in the United Kingdom. It, and other Phytophthora species are not known as human pathogens. No Phytophthora species is expected to pose any threat to human health under the conditions to be used for experimentation.

The foreign genes to be expressed in the recombinant P. infestans (the GMM) are of unknown function or biological activity. Transgenes will encode protein products from oomycete plant pathogens such as P. infestans (potato late blight), P. sojae (soybean root rot), or Pe. parasitica (downy mildew of arabidopsis thaliana). The majority of transgenes to be tested will originate from P. infestans. Reports exist that consumption of blighted potatoes leads to birth defects of the nervous system, but these have not been proven, and have been largely refuted by Borman and Cryer (1990). Direct testing of P. infestans extracts on chick embryos has determined that there was no evidence of toxicity (Wu and Salunke 1978) and that any birth defects are caused by glycoalkaloids present in the potato tuber (Jelinek et al., 1976). No toxicology studies have been carried out on the related species P. sojae or Pe. parasitica, and there are no records to implicate either organism in human disease. In the extremely unlikely event that any of the expressed gene products are determined to be toxic to humans, experiments with these genes will cease immediately.

None of the host plants (Solanum sp.) to be used in our experimental system pose a threat to human health under the conditions to be used for experimentation.

Risk to environment

P. infestans is naturally transmissible by wind or splash dispersal of sporangia from infected leaves. P. infestans may also spread in saturated soils by dispersal of motile zoospores from heavily infected plants, or plant debris in soil. P. infestans exists as two mating types, designated A1 and A2. Mating between A1 and A2 types results in the formation of oospores, which are thick-walled resting spores that may persist for many years in soil. Most potato varieties grown in Scotland are susceptible to late blight, caused by P. infestans. Control of late blight is typically by frequent treatment of crops with fungicides active against P. infestans (oomaticides such as metalaxyl).
For the majority of transgenes tested, there will be no effect on the infectivity, disease development, or host range of the GMM. In the unlikely case of any GMM escape, little is known about the stability of transgenes in P. infestans. However, it has been demonstrated that transgenes in P. infestans may become silenced (Judelson and Shittaker 1995; van West et al., 1999b) and that the silenced state is persistent and transferable. For instance, an escaped transgenic P. infestans silenced for pathogenicity factors would either simply not spread, or would transfer the silenced pathogenicity state to wild type. P. infestans, rendering it less fit.

If the transgene confers a change in the aggressiveness or host range of the GMM, then there may be some selection for the retention and maintenance of the transgene. Again, this is an unlikely situation since pathogenicity in P. infestans is a complex process involving the up and downregulation of many genes, and the constitutive expression of only one of these genes is not likely to have a large impact on the overall aggressiveness of the GMM. Silencing of pathogenicity genes is more likely to yield useful information regarding the phenotypic effects of the gene(s) under study.

Should a GMM silenced for a pathogenicity gene escape, the possibility of its DNA being transferred to another environmental and non-pathogenic organism, or another pathogen, is remote. Pathogenesis in P. infestans, and oomycetes generally, is complex and probably employs many mechanisms differing to those known in the true fungi and bacteria. It is unlikely therefore, that the horizontal transfer of a single P. infestans gene to a fungus or bacterium would confer novel pathogenicity or host range. The only known organism that P. infestans can intercross with is P. mirabilis, and this plant pathogen is not known in the United Kingdom.

Regarding the proposed experiments on heterologous gene expression and function, we wish to express genes in P. infestans from related plant pathogens P. sojae, and Pe. prasitica. The question we will address with this research is whether genes involved in avirulence in the named organisms have any role or effect in the P. infestans/potato interaction. The transgenic P. infestans from these experiments will have either the same phenotypes listed above, or will invoke a general, non-host response in all potato varieties, or no change from the wild type will be observed. Since the transgenes will originate from related pathogens (for which potato is a non-host plant), it is highly likely that functionally related genes are already present in P. infestans. Therefore, it is unlikely that introduction of the heterologous genes will have any effect on the host range of the transgenic P. infestans. Indeed, the opposite effect of restricting the host range may be more likely, as the host plant may recognise the foreign gene product and initiate a resistance response.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

In our proposed experiments, we will only use one mating type of P. infestans in any one experiment, to prevent the formation of any persistent propagules (oospores). Furthermore, the strains of P. infestans to be used in our experiments will be fully sensitive to the oomycicide metalaxyl.

P. infestans cultures will be maintained in sealed agar plates and the risk of escape from these vessels is minimal. It is standard practice in our laboratories to handle P. infestans cultures in a biohazard cabinet to prevent escape of the pathogen, and to protect the cultures from contamination. We propose to maintain and manipulate the GMMs in the same manner.

In our preliminary experiments, the conditions for transmission of P. infestans will be avoided where possible. For instance, whole plants grown in pots will not be used, whole plants (when used) will be in the form of micropropagated material in flasks under sterile conditions within laboratories. The preparation of inoculum in minimum quantities required for the experiment will use conditions to minimise/prevent the formation of aerosols, plants or detached leaves will be drop inoculated to prevent aerosols, the handling of infected plants will be minimised, disposable gloves will be worn for all handling of infected plants, and all materials that come in contact with the infected plants will be destroyed by autoclaving using validated procedures. Initial analysis of the GMMs will be performed on no more than 50 detached leaves at one time. Inoculated detached plant leaves in sealed containers, and whole plants in sterile flasks will also only be opened within the biohazard cabinet.

By using detached leaves in sealed containers and whole plants in flasks for inoculation experiments, the preliminary experiments will be entirely lab-based and contained. Records of all experiments will be maintained for inspection, including all details of plant disposal.

However, larger numbers of leaves will be required if the GMMs are to be screened across progeny derived from S. tuberosum cv. Stirling. In this instance, no more than 200 leaves would be required. Escape of live P. infestans can be prevented through the employment of procedures currently used at SCRI, and mostly through restriction of access to plants. Experiments on the GMM will be carried out in a designated containment glasshouse facility within the High Containment corridor of AN facility at SCRI.
To prevent any dissemination of the GMM. The facility is insect and vermin proof. Access is restricted to authorised personnel and the facility is equipped with a ‘through the wall’ autoclave. Designated laboratory coats are provided for work in the containment glasshouse and these coats are not removed from the containment facility. Further, any material that might be contaminated by the GMM (plant material, gloves, plasticware, flasks) will be placed into bags within the designated area, sealed and autoclaved prior to disposal. The cubicle and benches to be used for testing of the GMM (See part II below) will be cleaned and disinfected prior to commencement, and after completion, of experimental work. The floors of this area are sealed and in the highly unlikely event of flooding the drains are also capped and the default setting on the system would direct run-off to a holding tank where appropriate treatment would be applied to any release into mains drainage.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation is requested for Containment Measure 3 on the basis that the benches in the containment area of the glasshouse are made of wire mesh for ease of cleaning and to enable temperatures to be maintained consistently throughout the facility. Fully waterproof receptacles to prevent any run off from plant pots are routinely included in the SOPS for the Containment Facility. The floors of this area are also sealed and the drain capped. In the highly unlikely event of flooding the drains have an additional cap below the surface of the floor and, in the even less likely scenario of effluent getting past this, the default setting on the system would direct run-off to a holding tank where appropriate treatment would be applied prior to any release into mains drainage.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material used in the laboratory manipulations (plates, tips, gloves) will be placed into autoclave bags in the lab prior to disposal in the validated autoclave in this laboratory. All culture media (less than 1 litre per experiment) will be inactivated, autoclaved and disposed of appropriately. All spillages will be dealt with by standard laboratory hypochlorite or alcohol-based agents which have been shown to be effective against the pathogen.

Initial analysis of the GMMs will be performed on no more than 50 detached leaves at one time, and this work will be done within the laboratory area. All leaves will then be autoclaved and receptacles autoclaved also or treated with bleach.

For glasshouse work, in this instance, no more than 200 leaves would be required. Any material that might be contaminated by the GMM (plant material, gloves, plasticware, flasks) will be placed into bags within the designated area, sealed and autoclaved prior to disposal. The cubicle and benches to be used for testing of the GMM will be cleaned and disinfected prior to commencement, and after completion, of experimental work. Receptacles to prevent any run off will be included routinely and treated with tepid solution between experiments. The floors of this area are sealed and in the highly unlikely event of flooding the drains are also capped and the default setting on the system would direct run-off to a holding tank where appropriate treatment would be applied prior to any release into mains drainage.

Records of all experiments will be maintained for inspection, including all details of plant disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Procedural practices must be strictly adhered to when handling GM Zoospores during inoculation to minimise the risk of aerosols. Although two forms of mating types (A1 and A2) exist for P. infestans, only one may be used in any one experiment to prevent the formulation of persistent oosporex. The committee recommend specialist training for glasshouse staff and the provision of an automatic irrigation system for this area to minimise entry.
The preliminary purpose of the project is the development of plant virus-based vectors and inoculation techniques for the analysis of plant gene function through silencing.

Stage 1 comprises plant viral cDNAs, under the control of bacteriophage promoters for in vitro transcription, which will be genetically modified through the insertion of genes for easily detectable reporter proteins to monitor virus movement. The genetically modified viral cDNAs will be further placed under the control of plant promoter sequences to test biolistic and Agrobacterium-mediated inoculation of specific target tissues. In the first instance VIGS enhancement directed against endogenous plant genes such as Phytoene desaturase (PDS) and transgenes such as green fluorescent protein (GFP) will be assessed by the comparison of viral based vectors carrying single gene cDNA inserts or the equivalent tandem inverted-repeat corresponding to the target sequences.
In the second stage of our programme, the ability of the genetically modified viruses to induce silencing will be tested through the insertion of plant derived cDNA sequences.

In order to trigger VIGS in dicots, non-coding short cDNA fragments in either antisense orientation and/or inverted-repeats will be cloned into the PVX and PLRV genome. The target cDNA are described in Section "Origins and intended functions of the genetic material involved".

For monocots selected cDNAs to be expressed as functional or non-functional proteins in barley are also listed in the appropriate section.

In conjunction with this programme is an investigation of the potential of virally expressed RNA secondary structure folding as dsRNA hairpins in their ability to enhance silencing of target genes once expressed from virus-derived expression vectors on their corresponding hosts.

The eventual objective of the research is to develop a more robust system to silence endogenous genes in roots, leaf and developing/germinating seed.

### Recipient or parental organism

<table>
<thead>
<tr>
<th>Organism/Vector</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley Stripe Mosaic Virus (BSMV), US ISOLATE</td>
<td></td>
</tr>
<tr>
<td>Foxtail Mosaic Virus (fOMV), US isolate</td>
<td></td>
</tr>
<tr>
<td>Soil Borne Wheat Mosaic Virus (SBWMV), Japanese isolate</td>
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</tr>
<tr>
<td>Maize streak virus (MSV), Nigerian isolate, strain Ns, cloned into pBIN19 binary vector</td>
<td></td>
</tr>
<tr>
<td>Wheat dwarf virus (WDV), barley isolate, cloned into pBIN19 binary vector</td>
<td></td>
</tr>
<tr>
<td>Tobacco Mosaic Virus (TMV) vector based on strains U1 and U5 has been genetically engineered to express inserted cDNA fragments from a duplication of coat protein (CP) primoter.</td>
<td></td>
</tr>
<tr>
<td>Tobacco Rattle Virus (TRV)-derived expression vector (isolate PpK20) deleted from the RNA2-2b/2c ORF, hence no longer nematode transmissible, will be used for expression of inserted cDNA fragments from a duplication of CP promoter introduced into RNA2</td>
<td></td>
</tr>
<tr>
<td>Potato Virus X (PVX) strain Uk3 and PVX strain UK3 cloned into pGREENOOO binary vector</td>
<td></td>
</tr>
<tr>
<td>Potato leaf roll virus (PLRV), Canadian isolate, cloned into pGREENOOO binary vector</td>
<td></td>
</tr>
</tbody>
</table>

### Host/vector system

<table>
<thead>
<tr>
<th>Host/vector</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli strains XL1-Blue and DH5a</td>
<td>pUC-based plasmids containing plant viral cDNAs under the control of bacteriophage or plant promoters</td>
</tr>
<tr>
<td>Agrobacterium tumefaciens strains LBA4404 and AGL-1</td>
<td>binary plasmids, ie pBIN19 and pGREEN contaioning plant viral cDNAs under the control of plant promoters</td>
</tr>
<tr>
<td>Hordeum vulgare</td>
<td>recombinant plant viruses based on BSMV, FoMV, SBWMV, MSV, WDV</td>
</tr>
<tr>
<td>Solanaceous species</td>
<td>recombinant plant viruses based on TMV, TRV, PVX and PLRV</td>
</tr>
</tbody>
</table>

### Origin & function

**STAGE 1**

In order to trigger VIGS, corresponding homologous short cDNA fragments of (ranging from 40 up to 200 nucleotides) of the corresponding target plabnt gene will be...
introduced into the viral genome. These are most likely to be non-coding.

The targets are:

- Green fluorescent protein (GFP) from Aequorea sp., reporter protein.
- Red fluorescent protein (RFP) from Discosoma sp., reporter protein
- uidA, B-glucuronidase (GUS) from Escherichia coli, reporter protein
- phytoene desaturase (PDS)
- Genes involved in the biosynthesis of the anthocyanins: C1, b-Peru and A1 (dihydroflavonol-4-reductase gene)

To monitor silencing in vegetative and seed tissues, GFP fluorescence, GUS activity, photobleaching (as a consequence of PDS knock-out in foliar tissues), accumulation of red anthocyanins pigments in bombarded embryos or endosperm cells (following in situ transient expression of C1 and bPeru maize genes encoding transcriptional factors required for anthocyanins biosynthesis, Schweizer et al, 2000) will be evaluated according to which vector combination is being used.

STAGE 2

2.1 VIGS in dicots (solanum species and Nicotainae): (PVX mediated)

2.1.1 Target cDNAs related to disease resistance.

In order to trigger VIGS, non-coding short cDNA fragments in either antisense orientation and/or inverted-repeats will be cloned into PVX and PLRV genome. The target cDNAs are all related to disease resistance.

1. SGT1: A component of the ubiquitin ligase machinery that is involved in disease resistance in plants. Silencing of this gene in N. benthamiana inhibits the HR and thus it acts as a positive control in this experiment. Ref. Pear JR et al., (2002). Ubiquitin ligase-associated protein SGT1 is required for host and non-host disease resistance in plants. (PNAS 99:10865-10869.

2. Cathepsin B: The potato cathepsin B gene was identified as up-regulated specifically in R gene-mediated resistance to Phytophthora infestans (unpublished results) and, as such, is a candidate for having a role in the HR. Cathepsin B in mammalian systems has a regulatory role in apoptosis, a form of programmed cell death. REf: Guicciardi ME, et al., (2001) Cathepsin B knockout mice are resistant to tumor necrosis factor-alpha-mediated hepatocyte apoptosis and liver injury: implications for therapeutic applications. Am. J. Pathol. 159:2045-2054.

3. Adr1: The Adr1 gene (Activated Disease RESistance 1) was isolated from Arabidopsis in a gain-of-function screen for up-regulation of genes that increase resistance to a range of pathogens (unpublished). It is an NBS-LRR protein similar to R gene encoded plant proteins and is believed to coordinate pathway convergence from a range of R gene products.

4. St-WRKY1: The St-WRKY1 gene was isolated from potato and is up-regulated specifically during the early stages of R gene-mediated resistance to P. infestans (unpublished results and ref below). It encodes a transcription factor that binds to 'W' boxes found in the promoters of plant defence-associated genes. REf: Dellagi A, et al., (2000). A potato gene encoding a WRKY-like transcription factor is inducted in susceptible interactions with Erwinia carotovora subspp. atroseptica and Phytophthora infestans and is co-regulated with class 1 endo-chitinase expression. Mol Plant-Microbe Interact 13:1092-1101.

5. Oxysterol binding protein (St-OBP1): The OBP1 gene was identified as up-regulated specifically in R gene-mediated resistance to Phytophthora infestans (unpublished results) and, as such, is a candidate for having a role in the HR. OBPs have been found to play a role in apoptosis in mammalian systems.

No toxicity has been reported as a result of expression or knock-out of the abovementioned genes. The abovementioned genes are involved in specific resistance response (gene-for-gene) to plant-fungus interaction hence should not provide beneficial advantage to the recombinant virus vector.

2.1.2 to investigate the function of potato genes involved in carotenoid metabolism and the potato tuber life-cycle.

Selected cDNAs to be expressed in antisense orientation in potato (S.tuberosum and S.phureja) using the previously described PVX vector.

2.1.2A) carotenoid metabolism:

cDNA fragments cloned from S. phureja tuber encoding lycopene -cyclase (AF321537), lycopene -cyclase (97% identity with X86452 from tomato), -carotene hydroxylase (95% identity with Y14809 from tomato), zeaxanthin epoxidase (96% identity with Z83835 from tomato) and 9-cis epoxycarotenoid dioxygenase (AJ276244).

The genes we are studying are naturally occurring and functional in potato. The enzyme activities encoded by these genes form part of the carotenoid biosynthetic pathway. By modifying their expression level we hope to obtain information that may help us to understand how the carotenoid biosynthetic pathway is regulated. All these genes are well characterised in other plants. For some, stable antisense experiments have been carried out (eg zeaxanthin epoxidase in potato (Romer et al., 2002), -carotene hydroxylase in Arabidopsis (Rissler and Pogson 2001) or mutants have been studied (lycopene -cyclase in Arabidopsis, Pogson et al., 1996). There are no reports that we are aware of, that changes in carotenoid content result in increased or decreased susceptibility to plant pathogens or confer on the plant a selective advantage.

2.1.2 B) Partial cDNA clones isolated from a potato tuber meristem library:

<table>
<thead>
<tr>
<th>GENE</th>
<th>BEST BLAST X (n)</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>dor3ra24</td>
<td>Soybean choline kinase</td>
<td>1.00E-13</td>
</tr>
<tr>
<td>dor3rk12</td>
<td>Tobacco alpha-tubulin</td>
<td>2.00E-24</td>
</tr>
<tr>
<td>dor5sp6a23</td>
<td>Arabidopsis auxin response factor 6</td>
<td>2.00E-06</td>
</tr>
<tr>
<td>dor3rl16</td>
<td>Arabidopsis auxin response factor 1</td>
<td>5.00E-24</td>
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<tr>
<td>dor4ro10</td>
<td>Tobacco aquaporin</td>
<td>1.00E-13</td>
</tr>
<tr>
<td>dor4sd6</td>
<td>Rice argonaute protein</td>
<td>6.00E-57</td>
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<tr>
<td>dor5sp6b11</td>
<td>Tobacco arginine decarboxylase</td>
<td>5.00E-36</td>
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<tr>
<td>dor3rd15</td>
<td>Tobacco translation initiation factor</td>
<td>1.00E-27</td>
</tr>
<tr>
<td>dor3rd16</td>
<td>Arabidopsis leaf argonaute</td>
<td>4.00E-07</td>
</tr>
<tr>
<td>dor3r19</td>
<td>Potato starch phosphorylase</td>
<td>2.00E-14</td>
</tr>
<tr>
<td>dor3r12</td>
<td>Arabidopsis ATPase</td>
<td>8.00E-19</td>
</tr>
<tr>
<td>dor3rk16</td>
<td>Arabidopsis transcription factor BTF3</td>
<td>1.00E-11</td>
</tr>
<tr>
<td>dor4M6</td>
<td>Arabidopsis caseine kinase</td>
<td>8.00E-16</td>
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<tr>
<td>dor4sc11</td>
<td>Potato pyruvate kinase</td>
<td>9.00E-51</td>
</tr>
<tr>
<td>dor5sp6d15</td>
<td>Tomato MAP kinase</td>
<td>8.00E-06</td>
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<tr>
<td>dor5sp6f11</td>
<td>Tomato transcription factor JERF1</td>
<td>9.00E-41</td>
</tr>
<tr>
<td>dor6sp6b3</td>
<td>Tomato S-adenosyl methionine synthase 3</td>
<td>2.00E-41</td>
</tr>
<tr>
<td>dor6sp6b3</td>
<td>Arabidopsis cAMP dependent protein kinase 6.00E-12</td>
<td></td>
</tr>
<tr>
<td>dor3rj3</td>
<td>Tobacco dormancy protein</td>
<td>5.00E-34</td>
</tr>
</tbody>
</table>
We wish to investigate the function of these cDNAs in aspects of the potato tuber life-cycle by down-regulating their expression levels in an in vitro tuberisation system. We expect these genes to exert a regulatory function during tuberisation, dormancy and sprouting and wish to assess the in vitro system as a means to rapidly screen this type of candidate gene. The precise function of the encoded gene products is not known: several show sequence similarity with transcription factors, kinases, phosphatases or other activities that we suspect may be critical to the process such as tubulin genes, genes involved in polyamine biosynthesis (arginine decarboxylase) or channel formation (aquaporin).

We propose to test meristem genes solely in an in vitro system which has a high degree of containment.

We are unaware of any literature which suggests that this manipulation will confer a selective advantage on the plants subjected to VIGS however, for most, no data is available currently.

2.2 - VIGS in monocots (BSMV):

2.2.1): Oleosin, calesosin and steroleosin genes.

Based on the sequence homology to Arabidopsis, rice and sesame we have identified and isolated the following genes from the barley EST and BAC libraries:

* Five oleosin genes: ole1a, ole1b, ole1c, ole1b, ole2;
* Three coleosin genes: cal1, cal2 and cal3
* Two steroleosin genes: ster1, ster2.

The genes we are studying are naturally occurring and functional in the plant (barley) of our interest. By modifying their expression level and/or by expression of the parts of the genes as a GFP fusion protein we hope to obtain information, which may help us to understand the functions of these gene.

Oleosins were identified as a major protein constituent of the plant seed lipid bodies, while calesosins (calcium-binding protein) and steroleosins (sterol=binding dehydrogenase) comprised the minor fraction. The roles of these proteins have been associated with lipid body biogenesis, stabilization, trafficking and mobilization in desiccation tolerant seeds. But, tissue expression patterns, organisation of the genes encoding them and lipid profiles of different tissue types accumulating these proteins indicate that they are in fact multi-functional proteins playing important yet unknown roles in the whole plant development.

The BSMV vector system will be used to deliver untranslatable sequences of oleosins, calesosins and steroleosins into the barley leaves for silencing purposes. The barley leaf areas where the genes of interest are silenced will be determined by the real-time RT-PCR. The sub-cellular structures of the silenced leaves will be subjected to morphological and functional analysis using specific staining procedures (fluorescently labeled phallotoxins, lectins, DIOC6 and antibodies). Expected results are identification of the specific phenotypes associated with dynamics of cyto-skeleton, Golgi and ER.

In addition to their biological interest oleosins have recently been used in a biotechnological context as vehicles for overexpression in transgenic plants of a variety of fusion proteins.

2.2.2 Endogenous barley genes involved in resistance pathways to powdery mildew

The introduction of cDNA sequences into the viral genomes is hoped to induce silencing of endogenous barley genes involved in resistance pathways to powdery mildew. Inverted repeats of cDNA fragments up to 60 nucleotide will be cloned into BSMV genome. The targets cDNAs are Rar1, Mlo and cDNAs homologues to receptor-like kinase and oxysterol binding protein.
1. Rar1 encodes a 25.5-kDa zinc-binding protein interacting indirectly with ubiquitin ligase complex (Azevedo et al., 2002) required for multiple race-specific resistance to powdery mildew. Rar1 knockout should result in a shift from incompatible to compatible interaction of Blumeria graminis fsp hordei 983 on barley cv. Pallas (Mla13-near isogenic line11) variety and fungal growth.

2. Mlo encodes a 52-kDa-transmembranar protein representing a novel calmodulin-binding protein (Kim MC et al., 2002), acting most likely as a negative regulator of Blumeria graminis resistance. Mlo knockout should result in plant cell death control perturbations in the absence of pathogens leading to spontaneous necrosis (Devoto et al., 1999) and a shift from compatible to incompatible interaction of Blumeria graminis fsp hordei Tyne3 on barley cv. Pallas (Mla13- near isogenic line11).

3. cDNAs homologues to receptor-line kinase.

4. Oxysterol binding protein have been isolated in the context of the previously mentioned interaction and are rapidly up regulated during the incompatible interaction. They share homologies to respectively a receptor-like kinase and leucine-rich repeat protein (accession number DBJ: BAB03629.1) encoded by the rice disease resistance gene Xa21 (Song et al., 1995) probably involved in cell surface recognition of a pathogen ligand and subsequent activation of an intracellular defense response and an oxysterol-binding-ortein (accession number GB: AAL32781.1) probably regulating lipid and cholesterol metabolism recently considered as candidates in in vivo cytotoxicity (Colles SIM et al., 2001). If such genes play a predominant role in the outcome of the interaction, therefore either a shift from incompatible to compatible interaction or downstream alteration of plant defense response to challenge with the abovementioned Blumeria strains should be expected.

Once the VIGS system has been characterized in term of timing and localisation of knock-out of the targeted gene, leaf segments will be detached for monitoring fungal growth on petri dishes under contained laboratory conditions in order to minimize the manipulation of whole BSMV infected plants in glasshouse or growth rooms.

**Evaluation of foreseeable effects**

**VIRUSES:**

FoMV does not cause economically important disease, and its natural hosts are not indigenous to the UK.

SBWMV and BSMV are a cause of disease in wheat and barley. In order to provide robust biological containment, sections of the coat protein genes of BSMV and SBWMV will be deleted to render them non-transmissible by mechanical and fungal routes respectively. The genetic modification required to disable their natural transmission routes will result in increased symptom severity on inoculated plants.

TMVs natural host, tobacco, is not grown in Scotland, so the GMOs do not present a risk to the wider environment. The GMOs could present a risk to other plants grown in the institute, though no more than the wild-type virus. Therefore measures will be taken to minimize spread of the GMOs through mechanical transmission and dispersal of pollen or seed.

TRV-based vectors will be derived from RNA2-2b/2c deletion mutants, making them non-transmissible via nematode, and are not likely to have increased seed-transmissibility. Measures will be taken to minimize spread of the GMOs through mechanical transmission and dispersal of pollen or seed.

Potato Leaf Roll Virus (PLRV) and PLRV deletion mutants altered in movement and replication functions will be cloned into pGreenII binary vector driven by a 35S-promoter (Nurkiyanova et al., 2000. Tagging PLRV with the jellyfish green fluorescent protein gene. J. Gen. Virol. 81, 617-626) for Agrobacterium-based infection of Solanaceae. Risk assessments have already been prepared for PLRV and are covered by Plant Health Licences (Licence GM/183/2003, licence GM/183/2003).

Potato Virus X (PVX) genome (strain UK3) and PVX deletion mutants altered in movement functions will be cloned into binary-based (pGREENOOO) PVX-expression vector engineered from PVX strain UK3 driven by a 35S-promoter for Agrobacterium-based infection of in vitro grown potato (cross-reference: SEERAD licence GM 180/2003).
Maize streak virus (MSV), is a plant DNA virus classified in the Mastrevirus genus of the Geminiviridae family. MSV is a non-indigenous virus. No vectors (Leafhopper Cicadulina spp) have been identified in the UK. All known hosts are in the Gramineae, among which many species can be infected. It is not transmitted by mechanical inoculation nor by contact between plants, by seed or by pollen. The insertion of the sequence of interest into the Small Intergenic Region (SIR) using Asnl restriction site should generate constructs effective in replication and allow limited cell-to-cell movement (Shen and Hohn. 1995. Vectors based on maize streak virus can replicate to high copy numbers in maize plants. J. Gen. Virol. 76, 965-969). Maize streak virus clones exist as monomeric copies of the wild type genome in M13 and pUC based vectors, multimeric copies are present in T-DNA binary vectors (eg pBIN19) and pUC. Similar constructs exist for WDV. The binary vector constructs are used for agroinoculation. The pUC based constructs are used for microprojectile bombardment of tissue cultures.

Wheat dwarf virus (WDV) is a plant DNA virus classified in the Mastrevirus genus of the Geminiviridae family. WDV is present in Northern Europe and Scandinavia. Leafhoppers that could be vectors (Psamotettix spp) are not reported in the UK. Triticum aestivum (wheat) and Hordeum vulgare (barley) react differently to infection with the common and barley strains. The barley strain causes dwarfing, poor heads and yellowing in barley, but which is resistant to wheat strains. It is not transmitted by mechanical inoculation; nor transmitted by contact between plants, by seed or pollen. Such a vector has to be developed based on a barley isolate (in collaboration with Dr. M Boulton, JIC, Norwich, UK, WDV barley isolate obtained from Dr B Hohn, Friedrich Miescher Institute, Basel, Switzerland) and previous WDV-based vector development (Gooding P S et al., 1999. Plant cell-directed control of virion sense gene expression in wheat dwarf virus. Nucl. Acids Res. 27, 1709-1718).

These two geminiviruses (MSV, WDV) are maintained in the laboratory, as DNA clones in pUC-based vectors in E. coli, or binary vectors (pBIN19) in Agrobacterium.

General Comments:-
* The previous insertion of reporter protein genes into plant viral genomes has resulted in the creation of attenuated viruses and no mechanism for enhanced pathogenicity can be foreseen.
* The introduction of plant derived sequences into plant viral genomes and the induction of host gene silencing can affect the symptomatology of infections. However, previous experiments with vectors based on RNA plant viruses have shown introduced sequences to be very rapidly lost through in planta recombination events (for review of genetic instability in genetically modified RNA plant viruses see: Lacomme et al., (2001) Plant Viruses as Gene Expression Vectors, in: Ring CJA and Blair, ED (Eds) Genetically Engineered Viruses: Development and Applications, BIOS Scientific Publishers, Oxford).
* Therefore, the genetically modified plant viruses are considered to present less risk to the environment than the natural progenitor viruses.
* For Stage 1 work we propose containment levels appropriate to that of the wild-type virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**GLASSHOUSE**

Stage 1 will be classified in two forms. Firstly, using vectors that, in some cases are well-characterized and expressing well-characterised genes which can be classified as no more than Containment Level 2. Secondly, in widening the spectrum of vectors to be used some cannot yet be described as well-characterised but will be expressing well-characterised genes, however, these will be subject, according to target, to Containment Level 3, until sufficient evidence is accumulated to re-classify them.

Stage 2 will use only well-characterised vectors, but in some cases to express less well-characterised genes. Thus this would constitute Containment level 3 especially in the case of genes involved in plant pathogen interactions, until sufficient evidence is accumulated to re-classify them.

Plants shall be grown in a designated greenhouse compartment or growth chamber, with access restricted to named personnel only. The plants shall be kept clearly labelled and physically separate from other plants. ALL PLANTS WILL BE BAGGED TO REDUCE POLLEN DISPERSAL.

To prevent escape of the mechanically transmissible GMMOs handling of infected plants will be performed with gloved hands. Designated laboratory coats are provided for work in the containment glasshouse and these coats are not removed from the containment facility unless decontaminated first. As a precautionary measure plants will not be allowed to flower. Further, any material that might be contaminated by the GMMOs (plant material, plant pots, soil, labels, gloves) will be autoclaved prior to disposal.

Escape of live Agrobacterium tumefaciens can be prevented through the employment of procedures currently used at SCRI for agroinoculation, for which a generic risk assessment exists at SCRI. That is, restriction of access to plants and watering only by licensed staff, control of water run-off through the use of trays, and watering from beneath. Records of all glasshouse experiments will be maintained for inspection, including all details of plant disposal.
On termination of experiments all plant material, soil and pots shall be transferred to autoclavable bags, sealed and autoclaved.

Any material removed from glasshouse (for experimental analysis, etc) to be carried in sealed container/plastic bags. All analysis to be performed in designated laboratory areas. Contaminated materials to be disposed of following autoclaving or sterilisation. Surfaces and floors in glasshouse compartment shall be cleaned after the experiments to eliminate any plant debris.

LABORATORIES

Laboratories are equipped to the requirements of containment level 2. Autoclaves are available within the building, obviating the need for further transportation. All contaminated materials will be autoclaved or treated with a proprietary virucide. Compliance with level 2 containment measures provides satisfactory environmental protection.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Request for derogation from Schedule 8, Part II, Table 1a(3) - Equipment - surfaces impervious to water etc...

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Infected plants and associated materials (soil, plants, gloves) will be autoclaved using a protocol validated to give 100% kill. Plant extracts prepared in the laboratory and material derived from them will be autoclaved using a protocol validated to give 100% kill. Contaminated surfaces will be disinfected with a proprietary virucide validated to give 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee discussed the following points:

1. The risk of whether silencing of genes could result in more severe symptoms was raised, but the Committee were informed that introduced sequences are generally rapidly lost. DNA-based inocular were stated to pose no greater risk than DNA constructs for in vitro transcription.

2. For Agrobacterium inoculation of barley stripe mosaic and wheat soil borne mosaic viruses, mixed inoculums, with the genomes separated between 2 or 3 plasmids would be used to reduce risk in the event of an escape.

3. The risk of escape would be greater for the monopartite foxtail mosaic virus, but there would be less effect to the environment.

4. It was asked if any risk was foreseen to any UK horticultural crops, but confirmed not to be the case.

5. The strict emphasis of pollen containment was re-iterated by the committee.

Project Containment
**Project Additional Information**

**Purposes of the contained use**

Experiments will be performed to express, from tobacco mosaic virus-based vectors (30B and 30B. CP)cDNA, libraries as fusions to GFP or mRFP for determination of the subcellular address of the expressed plant proteins. The work has three main themes:

* Libraries for subcellular addressing of expressed cDNA-GDP fusions
* Libraries to identify plant proteins involved in RNA splicing
* Libraries to identify protein - protein interactions in plants

Viral libraries will be screened through lesion-based assays on non-transgenic and transgenic plants by means of the observation of fluorescent reporter proteins and microscopic analysis. Library members of interest will be isolated and re-screened after passage; inserts will be recovered through RT-PCR and, after nucleotide sequence determination, reinserted into viral vectors for phenotype confirmation.
Recipient or parental organism

Tabacco Mosaic Virus

30B, hybrid vector based on TMV strains U1 and U5 (Shivprasad et al., 1999).

30B CP, a derivative of 30B in which the U5 derived sequences have been replaced with the 3’ untranslated region of strain U1.

Host/vector system

- Arabidopsis thaliana cDNAs
- Nicotiana tabacum cDNAs
- N. benthamiana cDNAs
- Gene for green fluorescent protein
- Gene for monomeric red fluorescent protein (Campbell et al., 2002)
- Gene for circularly permuted enhanced yellow fluorescent protein (Baird et al., 1999)
- Hordeum vulgare CaM3 gene (Link & Zielinski, 1989)
- Hordeum vulgare Mlo gene (Blueshges et al., 1997)
- Potato mop-top virus triple gene block protein 2 and 3 (TGBp2 and TGBp3, 13kDa and 21kDa) genes (Cowan et al., 2002)
- N. plumbaginifolia RNA-binding protein 45 (Lorkovic et al., 2000)
- N. plumbaginifolia uracil-binding protein 1 (Lambermon et al., 2000)

Origin & function

1. Libraries for subcellular addressing of expressed cDNA-GFP fusions

Experiments will be performed to express cDNA libraries as fusions to GFP or mRFP for determination of the subcellular address of the expressed plant proteins. The vector 30B has been described previously. This vector is a hybrid of tobacco mosaic virus (TMV) strains U1 and U5, and has been genetically engineered to express foreign proteins from a duplicated subgenomic promoter. Derivatives of 30B (30B.GFP & 30B.mRFP) will first be produced in which the initiating methionine codon or the stop codon of the gene encoding the fluorescent protein is replaced with a polylinker sequence. Subsequently cDNA will be produced primarily from Arabidopsis thaliana, but also possibly from N. tabacum and N. benthamiana, will be cloned directionally into these polylinkers to produce vectors capable of expressing plant cDNAs as fusions to the amino- or carboxy-termini of the fluorescent proteins. Populations of viral vectors containing the cDNA libraries will be inoculated after reassembly with purified TMV coat protein to non-transgenic N. benthamiana or N. tabacum, less than forty eight plants per experiment, or transgenic N. benthamiana or N. tabacum 35SS::30K (TMV) plants (CB lines 7-11), in order to enhance infectivity. As each fluorescent infection focus is initiated by a single clone from the population the subcellular distribution of the fluorescent protein in individual lesions on inoculated leaves will be determined, within three weeks of plant inoculations, by confocal laser scanning microscopy (CLSM). Infection foci showing subcellular distributions of interest will be passaged to N. benthamiana or N. tabacum to confirm the phenotypes by CLSM of the fluorescent lesions on the inoculated leaves. Subsequently RNA will be extracted and used in RT-PCR to recover the portions of the genetically modified viral genomes that encompass the plant derived sequences. The RT-PCR products will be cloned into 30B.GFP or 30B.mRFP to recheck the phenotypes of CLSM of fluorescent lesions on inoculated plant leaves.

2. Libraries to identify proteins involved in splicing.

We wish to develop a high-throughput viral-based screen for identification of genes involved in splicing. To do this transgenic N. tabacum plants will be used that carry an m-gfp5-ER transgene (Haseloff, 1999) with an internal mini-exon (Simpson et al., 2002) under the control of a 35S promoter. Enhancement of alternative splicing will be monitored through the visual observation of increased fluorescence resulting from increased GFP expression and the observation of GFP expression. To test the system candidate genes UBP1, RBP45 and PTB, which have previously been shown to affect splicing efficiency, will be cloned into 30B CP. The constructs produced will be inoculated onto the transgenic tobacco plants and enhancements in GFP expression sought. If this proves successful, N. tabacum cDNA libraries will be cloned into the
same vector and libraries inoculated to the same transgenic plants. Any infection foci showing enhanced fluorescence will be used for RNA extractions. The extracted RNA will be used for RT-PCR and the amplification products cloned prior to nucleotide sequence determination. Inserts of interest may be cloned back into viral vectors to confirm observed enhancements in alternate splicing.

3. Libraries to identify protein-protein interactions in plants.

We wish to develop a high-throughput viral based screen for identification of interacting protein pairs in plants. To do this, use will be made of a circularly permuted form of enhanced yellow fluorescent protein (cp-EYFP) whose level of fluorescence is dependent on the interaction between protein pairs fused to its carboxy- and amino-termini. To test the feasibility of this system genes encoding known protein pairs will be fused to the 5’ and 3’ ends of cp-eyfp, and the gene fusion inserted into 30B. CP. The engineered viruses will be inoculated to the leaves of non-transgenic N. benthamiana or N. tabacum plants or transgenic N. benthamiana or N. tabacum 35S::30K(TMV) plants (CB lines 7-11), in order to enhance infectivity. Protein interactions will be monitored through visual observation of fluorescence, microscopy and fluorimetry of plant extracts. Test pairs will include H. vulgare CaM and Mlo, and Mlo mutants encoding amino acid substitutions within the CaM-binding peptide. This protein pair have been previously shown to interact in vitro and in vivo (Kim et al., 2002A) and the barley MLO protein is involved in pathogen and stress responses (Piffanelli et al., 2002; Kim et al., 2002b). The other pair of proteins that will be tested are TGBp2 and TGBp3 of PMTV, which have previously been shown to interact in a yeast two-hybrid system (Cowan et al., 2002). These two viral movement proteins have previously been expressed independently as GFP fusions from the 30B vector without enhancing viral pathogenicity. The two proteins act in concert with TGBp1 to traffic viral RNA. If the aforementioned experiments prove successful it is would be out intention to create vectors containing genes encoding fusions between selected bait proteins, cp-EYFP and libraries of plant proteins. Interacting pairs would be identified through the inoculation of viral libraries to plants, isolation of infection foci showing enhanced fluorescence and recovery of the expressed target sequences through RT-PCR and cloning of the amplified products.

**Evaluation of foreseeable effects**

**Tobacco Mosaic Virus**

TMV forms very stable rod-shaped particles in infected tissues. It is readily mechanically transmissible, but not seed or pollen transmissible. Further, TMV is not transmitted by arthropods, nematodes or fungi. TMV has a world-wide distribution, including the UK, and is a cause of disease in tobacco. TMV has a wide experimental host range including many members of the family Solanaceae, predominantly from the genus Nicotiana. In comparison to wild-type virus, hybrid U1/U5 gectors and the recombinants that arise in plants from them are attenuated and restricted in host range.

For Part 1. Such TMV-based vectors have been shown to be competitively unfit in comparison to wild-type TMV and to rapidly undergo recombination events in plants to delete foreign sequences, resulting in the formation of pseudo wild-type viruses that are also unfit in comparison to wild-type TMV (Rabindran, S. and Dawson, W.O., 2001).

For Part 3. Although the combination of the two viral proteins is not expected to enhance the movement characteristics of the TMV-based vector, a possibility exists. However, the use of 30B. CP, which lacks a viral coat protein gene, provides biological containment and renders the GMMOs non-transmissible.

**Inserted gene produce**

The fluorescent proteins have been expressed in a wide range of experimental systems previously and there are no known risks associated with them. The plant cDNAs and a viral movement proteins that will be expressed from the plant viral vectors occur in nature, thus no novel toxicological effects are predicted. However, the levels of expression achieved with a viral vector may be greater than occur naturally. There is a possibility that this may lead directly to increased levels of possibly allergenic proteins (Krebitz, M. et al., 2000) and indirectly to altered levels of metabolites (Kumagai, et al., 1995; Kumagai et al., 1998)

**Alteration of existing pathogenic traits.**

The insertion of the PMTV gene sequences into 30B. CP are, in the absence of TGBp1, not predicted to have any effect on the intracellular movement properties of the TMV-based vector. The effect of over-expression of known and unknown plant cDNAs on vector accumulation cannot be predicted, for instance calmodulin genes are both
up and down regulated during TMV infection (Yamakawa et al., 2001) and many other genes are up or down regulated (Itaya et al., 2002). However, in all previous studies with TMV-based vectors the pathogenicity of these has been reduced in comparison to the progenitor virus.

The inclusion of plant derived sequences in a plant virus-based vector gives rise to the possibility of gene silencing and the insertion of a partial phytoene desaturase (PDS) sequence from Lycopersicon esculentum in a TMV-based vector, in sense of antisense orientations, and magnesium chelatase subunit H sequences from N. benthamiana have been shown to induce gene silencing and symptom alteration in N. benthamiana (Kumagai, M. H. et al., 1995; Biriart, J.-B. et al., 2003). Depending on the source of the cDNA inserts silencing rates of between 1% and 10% have been observed in N. benthamiana, which is not indigenous to the UK, based on novel phenotypic symptoms (Large Scale Biology Corporation, unpublished data). However, attempts to cause VIGS with vectors carrying Nicotiana pds sequences have not resulted in visible VIGS phenotypes on the related species N. excelsior, N. clevelandii, N. excelsior or N. tabacum cultivars (LSBC, unpublished data). Further, the efficacy of TMV-based VIGS in N. benthamiana has been shown to be dependent on the cDNA insert size and the presence of such inserts leads to substantial decreases in viral titre (Hiriart et al., 2003). The inclusion of complete cDNAs from Capsicum annuum, Lycopersicon esculentum, Oryza sativa and Betula pendula in TMV-based vectors had led to protein over-expression rather than gene silencing (Kumagai et al., 1995; Kumagai et al., 1998; Kumagai et al., 2000; Krebitz et al., 2000).

Recently it has been shown that silencing of NbEDS1 in N. benthamiana transgenic for the resistance gene N compromised the N-mediated resistance against TMV (Peart et al., 2002). However, to achieve this effect it was necessary to establish silencing by inoculation of a Tobacco Rattle Virus-based VIGS vector three weeks in advance of TMV challenge and further VIGS of twenty other genes putatively involved in N-mediated resistance did not produce any consistent loss of TMV resistance. In another study prior silencing with TRV-based vectors of resistance-related genes permitted a TMV-based vector to escape N gene mediated resistance (Liu et al., 2002). More recently silencing of genes, the products of which interact with the TMV RNA replicase, has resulted in both decreased and increased viral accumulation, the latter phenotype being mimicked by herbicide treatment (Abbink et al., 2002).

However, as before, silencing was achieved through prior inoculation with a TRV-based vector. Experiments with TMV-based vectors carrying pds sequences have only shown photo-bleaching in non-inoculated leaves and photo-bleaching occurs at least seven days post inoculation, after the appearance of systemic viral infection. Therefore, how silencing of an endogene by a TMV-based vector could alter the pathogenic traits of that vector is not perceived.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**GLASSHOUSE**

Experiments on the GMMOs will be carried out in a level 2 containment glasshouse facility to prevent dissemination of the GMMOs. Individual clones from the population will be represented by single lesions or by single plants for the subset chosen for phenotypic confirmation (<100 at a time) and plants will not be propagated for extended periods (<3 weeks). The glasshouse facility is insect and vermin proof. Access is restricted to authorized personnel and the facility is equipped with a "through-the-wall" autoclave.

To prevent escape of the mechanically transmissible GMMOs handling of infected plants will be performed with gloved hands. Designated laboratory coats are provided for work in the containment glasshouse and these coats are not removed from the containment facility unless decontaminated first. As a precautionary measure plants will not be allowed to flower. Further, any material that might be contaminated by the GMMOs (plant material, plant pots, soil, labels, gloves) will be autoclaved prior to disposal. Where samples need to be transported to the laboratory for analysis this will be done in secure containers.

**LABORATORIES**

Laboratories are equipped to the requirements of containment level 2. Autoclaves are available within the building, obviating the need for further transportation. All contaminated materials will be autoclaved or treated with a proprietary virucide. Compliance with level 2 containment measures provides satisfactory environmental protection.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Infected plants and associated materials (soil, plants, gloves) will be autoclaved using a protocol validated to give 100% kill. Plant extracts prepared in the laboratory and material derived from them will be autoclaved using a protocol validated to give 100% kill. Contaminated surfaces will be disinfected with a proprietary viricide validated to give 100% kill.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

None

Please enter comments on the GM safety committee on the risk assessment

None

Project Containment

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Project Ref 250/04.1

Date Ackn’d 29/12/2004

Date Project Ceased 04/05/2018

CU2 Project Title The insertion of cauliflower mosaic virus into host genomes during natural viral infections.

Class 3

CultureVolClass2 Class

Consent Granted Yes

Project notified under transitional arrangements N
The aims of the project are to study:
1. Whether Cauliflower mosaic virus (CaMV) inserts into plant genomes that are genetically modified (GM) to express genes under the control of the CaMV 35S RNA promoter.
2. The consequences of such insertion events for the use of GM plants and how this compares to the situation in non-GM plants that are infected with CaMV.

Modified versions of CaMV will be prepared to facilitate the analysis. These will be inoculated to GM and non-GM plants of four species, Arabidopsis thaliana, Brassica napus, Nicotiana benthamiana and Nicotiana tabacum, which will be assessed for evidence of CaMV integration and for effects on the stability and expression of the transgene.

Transgenic Arabidopsis plants will also be generated that contain inserted CaMV genomes, thus permitting assessments of the consequences of germline integration, even in the event that few or no CaMV integrants are found to result from CaMV infection. These plants will be studied to assess whether transcription, reverse transcription or episomal disease occurs, in the presence or absence of conditions of stress. The plants will also be used to assess the effect of CaMV integration on a range of vital plant functions.

Wild-type CaMV is found world-wide in temperature regions. Its host range includes species in the families Brassicaceae, Solanaceae and Resedaceae. Natural transmission is by aphid vectors and the virus is also mechanically transmissible, but is not reported to be seed transmissible. CaMV infection of brassicas grown as food crops is widespread and is not associated with any risk to human health.

Modified versions of CaMV will be prepared for use in determining whether CaMV sequences integrate into plant genomes of non-GM and GM host plants of CaMV. The first of these modified CaMV involves replacing CaMV Gene II (encoding a protein required only for the aphid transmission of CaMV) with a smaller gene encoding dihydrofolate reductase (dhfr), derived from the bacterial plasmid R67. The modified virus will therefore no longer be aphid-transmissible. A similar construct made 20 years ago was shown to provide resistance to methotrexate in CaMV-infected plants (Brisson et al., 1984). Our construct differs from the earlier one in including upstream sequences encoding the 20 bp RNA promoter sequence from the phage T7 (T7pol). This would allow the dhfr gene to be expressed in bacteria that contain and express the T7pol. This construct is required to maximise the chances of detecting integration of CaMV sequences into non-GM and GM plants which are hosts of CaMV.

Other modified CaMV involves the strains of CaMV designated H7 and W260. CaMV H7 is a recombinant virus composed of elements from strains D4 and CM1841 (Schoelz and Shepherd, 1988). CaMV W260 is a strain that, unlike other CaMV strains, which show no movement in Nicotiana tabacum, will replicate and move cell- to cell.
cell in N. tabacum, but will not move long distance in this host.

These strains will be modified by insertion of a novel PCR-tag sequence into the Gene VI of CaMV. This sequence will be used to facilitate detection of integration into plant genomes that already contain other CaMV sequences. Insertion of a similar PCR tag has been noted to have no observable effect other than slightly slowing the rate of viral infection (Dr J E Schoelz, personal communication).

Host/vector system

Construction of modified CaMV will be carried out in plasmid vectors in Escherichia coli.

To initiate infection, viral DNA will be excised from the vector at the cloning site and mechanically inoculated to Brassica compestris cv. Just right, which will serve an inoculum source for further studies.

The modified CaMV produced in Stage 1 will be used to inoculate four plant species:

- Arabidopsis thaliana
- Brassica napus
- Nicotiana benthamiana
- Nicotiana tabacum

This will provide an assessment in three different host species and none non-host species, respectively. Inoculation will be to both unmodified and GM plants of each species. To determine whether CaMV infection affects the expression and atability the transgene, the four GM species will be assessed. The GM plants will all express the gene encoding the jellyfish green fluorescent protein (GFP) under the control of the CaMV 35S promoter (Haseloff et al., 1997; Ruiz et al., 1998; Halfhill et al., 2001; Harper & Stewart, 2000).

A determination of whether CaMV integration affects transgene expression and stability or vital plant functions requires having plants in which integration has been shown to occur. If the frequency of integration is quite low, it is conceivable that integration will not be detectable in the germline of the plants inoculated above, but may occur at a significant frequency in the hundreds of thousands to millions of plants present in field situations. Therefore, it is necessary to establish the consequence of germline integration, should such occ ur. To this end, we will generate GM A. thaliana plants that contain inserted CaMV genomes. These plants will allow assessment of the consequences of germline integration; in the absence of detecting such events by infection of non-GM and GM plants.

Origin & function

A. thaliana plants will be transformed with three types of CaMV genomes:

1) a single full-length CaMV genome;
2) two, tandem full-length copies of the CaMV genome;
3) a cDNA copy of the 35S RNA, containing repeated sequences at the ends of the cDNA.

As it is not known in which form the CaMV genome itself could naturally integrate, nor which form is able to permit episomal replication, it is necessary to examine the three most likely forms in which the entire genome of CaMV could integrate. The genome forms will be introduced into commonly used binary plasmids, such as pBIN19 and pGREEN, and transformed into disarmed, non-tumorigenic A. tumefaciens strains (LBA4404 and AGL-1) prior to transformation.

To determine the effects of the integration of the CaMV genome on the expression and stability of a transgene pollen from the above transgenic lines will be used to fertilise transgenic A. thaliana expressing the gene encoding the GFP, under the control of the 35S promoter. The progeny plants will be examined to confirm the presence of both the GFP transgene and the inserted CaMV genome.

Progeny plants derived from these plants will be used for analyses involving examination of the effects of CaMV insertion on the expression and stability of the transgene. This analysis will be done as described above for the A. thaliana, B. napus, N. benthamiana, and N. tabacum plants, transgenic for GFP under the control of the 35S promoter, and infected with CaMV. These data will determine the effects of CaMV integration on the expression and stability of a GFP transgene.
To determine whether CaMV integration affects vital functions, two approaches will be taken. In the first approach, several key parameters will be measured. These include:

I) levels of starch accumulation;
ii) levels of Rubisco accumulation;
iii) plant size
iv) root weight
v) number of seeds set
vi) time to seed set and senescence
vii) germination frequency of seeds.

The GM plants containing integrated CaMV genomes will be compared to non-GM plants, and GM plants expressing the GFP gene. Fifty plants of each will be inoculated and the plants will be incubated for either 30 days (A. thaliana and N. tabacum) or 90 days (B. napus and N. benthamiana). If there is an effect on the level of GFP expression, representative plants will be assessed further to determine whether the effects are due to silencing of the transgene (by examining the level of expression of the transgene mRNA) or modification of the transgene (by examining the expression of the transgene in the next generation). If the latter occurs, then the transgene will be cloned out of representative plants and the sequence analysed, to determine the nature of the modification to the transgene. In addition, the experiments with N. tabacum will assess whether limited (W260) or no (H7) movement of CaMV in the inoculated leaves only will still result in systemic silencing of the 35S RNA promoter-driven transgene. These assays will permit a determination of whether and to what extent infection by CaMV affects expression of a transgene and the stability of the transgene in an infected plant.

To determine whether integration occurs in the somatic tissue, leaves from 10 infected plants of each species will be harvested 30 days after inoculation for A. thaliana, and N. tabacum, and at 30, 60 and 90 days after inoculation for B. napus and N. benthamiana.

To determine whether integration occurs in the germline, seeds from the above infected plants will be collected and germinated, and leaves will be processed as for the leaves from the parent plants. PCR will then be used and a series of primer pairs to determine whether integration can be detected. The nature of the PCR products will be confirmed by cDNA cloning into E. coli and nucleotide sequence determination. In the first instance, primer pairs will be used to determine whether tandem or nearly tandem insertion occurs of two copies of the CaMV genome. In the second instance, a different strategy will be used to detect single copy insertion events.

If the integration occurs with a high frequency, then many of the sector should result in the positive detection of CaMV sequences. If no PCR products are detected by this assay, then it suggests that the incidence is low or nil. To determine whether integration occurs with a very low frequency, DNA will be extracted from the above plants infected with CaMV containing the dhfr gene. This DNA will be cut with restriction enzymes not present in the modified virus and the DNA fragments will be ligated into a plasmid vector and transformed into E. coli expressing the T7pol and spread onto plates containing methotrexate. The only E. coli cells that will grow are those that contain the CaMV sequences with the dhfr gene under the control of the T7pol. The plasmids from E. coli cells that are able to grow under these conditions will be analysed to verify the presence of the CaMV (and dhfr gene) sequences, as well as flanking plant DNA sequences. These combined approaches will determine whether CaMV sequences can integrate into the genome of their hosts and with what frequency.

To assess whether transcription, reverse transcription (RT) or episomal disease occurs in CaMV-integrated plants, both the progeny of plants containing CaMV inserted into the germline following infection (if they occur), as well as the transgenic A. thaliana plants transformed with CaMV genomes will be analysed. These analyses will enable us to determine whether integration of the CaMV genome leads to episomal expression and disease, or transcription and RT of the integrated CaMV DNA.

Specific changes in environmental conditions may result in transcription, RT, or episomal expression and disease from the integrated CaMV genomes. The specific environmental conditions that will include pathogen attack. In the last case, the plants will be infected by Cucumber mosaic virus (CMV), which can infect any of the above species, does not have a DNA phase to its replication, and has no relationship to CaMV. Moreover, CMV has a well-characterised suppressor of gene silencing, and gene
silencing may be important to prevent expression of the integrated CaMV genome. Infection by CMV will itself induce symptoms, as may any of the other environmental effects. Thus, the environmentally altered and CMV-infected plants would have to be examined for expression of CaMV RNAs and DNAs as described above. These analyses will be made at various times during the life of the plant and should establish whether environmental conditions can affect the propensity of the integrated sequences to become expressed or produce replicating virus.

Evaluation of foreseeable effects

Wild-type CaMV is found worldwide in temperate regions. Natural transmissions is by aphid vectors and the virus is also mechanically transmissible, but is not reported to be seed transmissible. CaMV infection of brassicas grown as food crops is widespread and is not associated with any risk to human health.

To determine whether integration of CaMV affects expression and stability of the transgene, GM plants that show integration into the germline will be assessed for effects on GFP expression and stability, as described above. Similarly, the progeny of such plants also will be assessed for effects on GFP expression and stability. Plants with low somatic integration frequencies will be examined, but the effects would be expected to be negligible to non-detectable. Plants with high levels of somatic integration would be expected to show some effects.

The previous insertion of reporter genes into plant viral genomes has resulted in the creation of attenuated viruses and no mechanism for enhanced pathogenicity can be foreseen.

Assessment of the possible properties of the GM CaMV, the GM host plants and the possibility and likelihood that they could cause harm to the environment.

Stage 1. MODIFIED VIRUSES. These will be kept in either the laboratory or in a contained glasshouse and have little-to-no risk of escape. The H7 and dhfr-expressing variants of CaMV are not transmissible by aphids, the natural vector of CaMV and thus these viruses cannot be transmitted to other plants. Gene II encodes the protein needed for aphid transmission and has been replaced by the dhfr gene in that construct, while H7, which contains Gene VI from strain D4 (Schoelz et al., 1986) is derived from CaMV strain 1841 and contains Gene II of strain CM1841, which was shown to be not transmissible by aphids (Woolsten et al., 1987).

In addition, CaMV is already endemic to the UK environment, found in both crops as well as cruciferous weeds (Raybould et al., 1999; Thurston et al., 2001; Pallet et al., 2002).

Stage 2. INOCULATED PLANTS. The W260 variant will be used only for the experiments involving examination of integration into N. tabacum vs. N. benthamiana in the inoculated leaves, in the first 30 days after inoculation and thus will have little opportunities for escape. The effects of escape of virus from the plants are the same as from the laboratory which has already been covered above. The escape of seeds from transgenic plants in which the virus has been integrated into the germline is considered unlikely, since (a) we have never seen such examples of escaped seeds growing near the glasshouse, (b) except for B. napus, the others are not crop plants grown in Scotland, and B. napus seeds are too large to be wind-borne; and (c) it is unlikely that we will see many plants with natural integration into the germline. The effects of such escape, if it could occur, on the release of CaMV into the environment should also be negligible, as specified above.

Stage 3. TRANSFORMED PLANTS. Only arabidopsis thaliana plants will be transformed, and these will be transformed with CaMV-dhfr, which does not have the aphid transmission gene. A. thaliana is not a crop plant, but an annual weed. These plants have a short life cycle and are not considered a reservoir for insects or viruses for spread to crop plants. If some plants escaped and propagated virus, the virus would not be transmissible to other plants for the reasons given above. Moreover, if the escaped plants crossed with wildtype ecotypes of A. thaliana, these plants would not have a selective advantage, but most likely would become infected from episomal replication of the integrated CaMV sequence, be less competitive than the wild ecotypes, and be eliminated in competition situations.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GLASSHOUSE

Plants shall be grown in a designated Containment Level 3 greenhouse compartment or growth chamber, with access restricted to named personnel only. The plants will be clearly labelled and physically separated from other plants. All plants will be bagged to reduce pollen dispersal and a strict pollen and seed management regime will be implemented. The seeds harvested in Stage 2 and 3 will be clearly labelled and stored in a locked cabinet within the containment area. For handling pollen during the
pollination stage full body disposable overalls will be worn.

To prevent escape of the mechanically transmissible GMMOs, handling of infected plants will be performed with gloved hands. Designated laboratory coats are provided for work in the containment glasshouse and these coats are not removed from the containment facility unless decontaminated first. Any material that might be contaminated by the GMMOs (plant material, plant pots, soil, labels, gloves) will be autoclaved prior to disposal.

Records of all glasshouse experiments will be maintained for inspection, including all details of plant disposal. On termination of experiments all plant material, soil and pots shall be transferred to autoclavable bags, sealed and autoclaved.

Any material removed from the glasshouse (for experimental analysis, etc) to be carried in sealed containers/plastic bags. All analysis to be performed in designated laboratory areas. Contaminated materials to be disposed of following autoclaving or sterilisation.

Surfaces and floors in glasshouse compartment shall be cleaned after the experiments to eliminate any plant debris.

LABORATORIES
Laboratories are equipped to the requirements of containment level 2. Autoclaves are available within the building, obviating the need for further transportation. All contaminated materials will be autoclaved or treated with a proprietary virucide. Compliance with level 2 containment measures provides satisfactory environmental protection.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Schedule 8. Part II, Table 1a(3)
For C3 glasshouse containment we apply for derogation for the requirement of impervious bench surfaces, and request that our alternative which is the use of impervious trays for control of run-off water, in conjunction with sealed drains be considered.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Infected plants and associated materials (soil, plants, gloves) will be autoclaved using a protocol validated to give 100% kill. Plant extracts prepared in the laboratory and material derived from them will be autoclaved using a protocol validated to give 100% kill. Contaminated surfaces will be disinfected with a proprietary virucide validated to give 100% kill.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

The committee discussed the following points:
1. The level of containment for the pollen work was discussed at length and a strict pollen and seed management was deemed necessary.
2. The BSO further questioned the number of plants needed, but were assured by the proposer that such numbers were required for experimental purposes.

Project Containment
**Project Ref** 250/05.1

**Date Ackn'd** 14/10/2005

**CU2 Project Title** Use of TRV and TMV vectors for expression of pesticidal proteins.

**Class** Class 2

**Culture Vol** < 1 Litre

**Non-GMM Consent Granted** Not Applicable

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To test the utility of TRV- and TMV-based vectors for the expression of fluorescent proteins, nematicidal, fungicidal/oomyceticidal or aphidical proteins in plants. The aim of the project is (1) to test a TMV-based system for aphid (Myzus persicae MP1) uptake of virally expressed fluorescent proteins, (2) to evaluate the expression of selected fungicidal/aphidical peptides by a TRV-based vector, and (3) test their efficacy in protecting plants from pest attack.

**Recipient or parental organism**

Recipients. K20.RNA2.A2b. A previously described vector based on the PpK20 strain of tabacco rattle virus (TRV) (MacFarlane and Popovich) will be modified to express nematicidal or fungicidal/oomyceticidal proteins. The vectors produced will lack the 2b gene, which is required for nematode transmission (MacFarlane, 2003) 30B. A hybrid vector based on TMV strains U1 and U5 (Shivprasad et al., 1999) will be modified to express aphidical proteins.

**Host/vector system**
Lycopersicon esculentum for expression of fungicidal/oomyceticidal proteins.

Nocotiana benthamiana for expression of fluorescent reporter proteins or candidate insecticidal proteins.

Arabidopsis, tomato and potato plants for expression of nematicidal proteins.

**Origin & function**

Temporin A is a small cationic antimicrobial peptide originating from the European red frog (Rana temporaria) and is a non-cysteine-containing, linear, alpha helical peptide that has both antibacterial and antifungal activity and will be tested for fungicidal activity.

The mannose-specific lectin protein from Allium sativum (garlic) has known anti-microbial and anti-insect activity and plants infected with virus expressing the lectin are expected to be more resistant to insects.

Chicken egg white cystatin is a protein inhibitor of cysteine proteinases and it is expected that it will affect nematode life-cycle making plants expressing it more resistant to nematode infection.

Lepista nuda mannanase is a protein from the edible fungus Agaricus nudus with high homology to a mannanase of the cultivated mushroom Agaricus bisporus, which is a ligninolytic leaf-litter degrader and is also expected to affect nematode life-cycle.

A fifteen amino acid peptide identified in phage display assays that binds to and exerts an adverse effect in vitro on Globodera rostochiensis (V. Blok, unpublished data) also expected to affect nematode life-cycle.

GFP: green fluorescent protein from Aequorea victoria, and mRFP: monomeric red fluorescent protein from Discosoma sp. Both used as marker genes.

**Evaluation of foreseeable effects**

TRV is indigenous to the UK and has a broad experimental host range including Solanaceae, Cruciferae and Leguminosae. TRV is mechanically transmissible and seed transmissible (from 1% to 40% respectively for Capsella pastoris and Viola arvensis), but is not transmissible by inter plant contact and requires a specific nematode (Paratrichodorus pachydemus) for transmission between plants. Prior experiments have shown TRV-based expression vectors to be attenuated relative to the progenitor virus and that foreign inserts are lost through recombination.

TRV-based vectors have been shown to be efficient at silencing endogenous plant genes in various Solanaceous species (Ryu et al., 2004), however this is not pertinent as plant genes will not be introduced.

TMV forms very stable rod-shaped particles in infected tissues. It is readily mechanically transmissible, but not seed or pollen transmissible. Further, TMV is not transmitted by arthropods, nematodes or fungi. TMV has a world-wide distribution, including the UK, and is a cause of disease in tobacco. TMV has a wide experimental host range including many members of the family Solanaceae, predominantly from the genus Nicotiana. The vector 30B has been described previously. This vector is a hybrid of TMV strains U1 and U5, and has been genetically engineered to express foreign proteins from a duplicated subgenomic promoter. Such TMV-based vectors have been shown to be attenuated, restricted in host range and competitively unfit in comparison to wild-type TMV and to rapidly undergo recombination events in planta to delete foreign sequences, resulting in the formation of pseudo wild-type viruses that are also unfit in comparison to wild-type TMV. TMV-based vectors are poor inducers of silencing and have only been shown to do this in the non-indigenous species N. benthamiana.

Viral expression of temporin A may affect the microfauna of the infected leaf surface.

The mannose-specific lectin is expressed naturally in plants and is unlikely to be cytotoxic to plants and there is no known effect on human health. No expected effect on TRV pathogenicity is expected from the nematicidal proteins or peptide which may increase nematode fitness and alter their life-cycle.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For aphid challenges it is proposed to transport infected plants in sealed containers to a lockable controlled environment room in an insectary where the infected plants will be placed with aphids in aphid-proof Perspex boxes. These boxes are water impervious and a watering system allows external watering of the plants. Any analyses will be performed in Class 2 laboratories. At the end of experiments any material that might be contaminated by the GMOs (plant material, plant pots, soil, matting) will be autoclaved in a Class 3 facility prior to disposal or treated with a proprietary virucide, ie propagator and insect boxes.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any material that might be contaminated by the GMOs (infected plant material, plant pots, soil, labels, gloves) will be autoclaved prior to disposal. Propagator and insect boxes will be treated with a proprietary virucide.

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Note was made of the point that no silencing of plant genes was likely given that the expressed genes were of non-plant origin nor that it was likely that any effect on virus pathogenicity or transmissibility would occur. Note was also made of the requirement for a derogation for experiments to be carried out in the insectary facility.

### Project Containment

<table>
<thead>
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### Project Ref 250/07.1

**Date Ackn'd:** 10/01/2007

**CU2 Project Title:** Use of Potato Mop-Top Virus (PMTV) (Swedish and Scottish isolates) full-length clones to study virus movement, host specificity and transmission.

**Class:** Class 3

**CultureVol:** < 1 Litre

**Non-GMM Consent Granted:** Yes

**Withdrawn:** No

**Tick if notifying a connected programme of work:** No

**Project notified under transitional arrangements:** No
**Project Additional Information**

**Purposes of the contained use**

With the help of the fluorescently tagged constructs the cell-to-cell movement of PMTV will be studied, and the localisation and function of the viral proteins will be elucidated. Constructs with mutations in the triple gene block proteins and in the coat protein (CP) readthrough protein will also be used to pinpoint important domains in these proteins.

Another area of study will be the acquisition and transmission of PMTV by Spongospora subterranea. The acquisition and transmission of PMTV and the interaction between different S. subterranea "strains" identified by sequence variation and/or aggressiveness traits in various host systems, will be studied by infecting plants with both S. subterranea and PMTV under controlled environmental (pot test or nutrients cycling system) conditions.

**Recipient or parental organism**

Recipient: Potato Mop-Top Virus (PMTV) Swedish and Scottish isolate full-length clones. PMTV is a plant pathogen infecting potato. The virus is transmitted by Spongospora subterranea in soil and water and is poorly manually transmissible to potato but can infect experimental hosts this way. Tagging of the virus proteins with fluorescent proteins is not expected to alter its characteristics, transmission or pathogenicity. Mutations in the triple gene block proteins are expected to affect the cell-to-cell movement of the virus and possibly its distribution in the host plant. These experiments do not involve Spongospora subterranea and the virus will not be transmissible in soil or water. Mutations in the coat protein/readthrough protein are expected to affect the interactions with Spongospora subterranea. Experiments involving Spongospora subterranea will result in the GMM becoming water-borne.

**Host/vector system**

Nicotiana benthamiana, N. tabacum, Lycopersicon esculentum and Solanum tuberosum for GMM growth, cell-to-cell movement and vector transmission experiments.

Spongospora subterranea will also be used as the virus vector in experiments studying the virus acquisition and transmission processes.

**Origin & function**

DNA encoding fluorescent proteins such as GFP or RFP or other fluorescent proteins will be introduced into a cDNA clone of PMTV RNA 1, 2 or 3 as fusions to virus genes. Transcript RNA will be synthesized from the cDNA and mechanically inoculated to the test plants (N. benthamiana, N. tabacum). The vectors are based on previously described full-length clones of PMTV (Sacenko et al., 2003 Journal of General Virology 84, 1001-1005). The final constructs will contain the gene for a fluorescent protein introduced to different parts of the full-length clones in order to localise fluorescent protein-viral gene product fusions in the infected plants, monitor virus advance and transmission and the infection process.
PMTV (Swedish isolate) is not indigenous to the UK and differs from the indigenous Scottish PMTV in the sequence of the coat protein-read through domain. The Swedish isolate also has a 109 nucleotide extension at the 3'end of the read-through domain. The read-through domain has been implicated in the transmission by the Spongospora vector, but the function of the 3'end extension in the Swedish isolate is not known. Constructs containing mutations in the read through protein will be used to study its function in acquisition and transmission by the vector.

Mutations in the triple gene block proteins are expected to inhibit cell-to-cell movement of the virus. Mutant versions of the GMM that are characterised as defective in cell-to-cell movement will be inoculated to transgenic plants expressing the cognate triple gene block protein, either native or fused to a fluorescent protein to determine if the defect in cell-to-cell movement can be complemented in this way.

Evaluation of foreseeable effects

Addition of fluorescent proteins to the virus is not expected to change the properties of the virus. Mutations in the triple gene block proteins are expected to inhibit cell-to-cell movement of the virus. Mutations in the read through protein are expected to abolish vector transmission. The modifications are unlikely to increase the pathogenicity or alter the host range of the virus. The genetic stability of the constructs containing foreign genes is predicted to be low and modified viruses are likely to replicate within infected plant cells, but lose inserted sequence during propagation and passage leading to reversion to wild type or near wild type as experienced with other virus-derived vectors, e.g. Ziegler et al., 2000 (Molecular Breeding 6, 327-335). The RNA transcript from the Swedish isolate full length clones cannot be mechanically transmitted to Solanum tuberosum (unpublished results, E Savenkov). Experiments that involve inoculation of only the virus to host plants will be carried out at containment level 2.

Spongospora Subterranwa, the vector for PMTV, is a plasmodiophorid organism, able to infect roots of a wide range of plant genera. However, infection of non-Solanum host species has only been seen to produce zoosporangial inoculum, which is relatively short-lived (a few hours at most). Long-lived sporeballs which would be a threat to subsequent potato or tomato crops produced in these hosts. In potato and tomato infections, there is a possibility that PMTV infected sporeballs could be produced, and infected natural hosts would be therefore destroyed at an early stage of infection before sporeball formation to eliminate this risk. S.subterranea is water and soil-borne and will not be transmitted by aerial means. The GMM is associated with S.subterranea is a water-borne pathogen and water run-off will be prevented; consequently these experiments will be carried out at containment level 3.

PMTV is a plant virus and not known to be harmful to humans. Human consumption of potatoes from PMTV or S.subterranea infected plants is not known to be harmful. There are no known harmful effects of GFP or RFP or other fluorescent proteins either. The virus constructs are not likely to be pathogenic or harmful to humans.

The GMO constructs will replicate and form particles. The probability of displacement of other isolates of PMTV, in particular the indigenous Scottish isolate is very small as the GMO will not have any competitive advantage, and in the case of mutations to the readthrough or triple gene block proteins will be at a disadvantage.

The GMO is not known to be pathogenic to animals, only to host plants. There is no known toxicity of the viral products or of the expressed foreign genes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any material that might be contaminated by the GMM (infected plant material, plant pots, soil, labels, gloves) will be autoclaved prior to disposal and this will give a 100% kill. The autoclaves used have been validated for the destruction of tobacco mosaic virus (TMV) which forms much more stable particles than PMTV does. Conditions are used that result in no detectable TMV being recovered from plant material after autoclaving as assessed by inoculation to sensitive local and systemic host plants.
The GM Safety Committee noted that the work involved a non-disabled plant pathogen and that experiments that involved the fungus host would result in the GMM becoming water-transmissible albeit for a relatively short period of time as the viruliferous zoospores are short-lived. Experiments involving the fungus will be carried out at containment level 3 to prevent run-off water from plants. Experiments looking at cell-to-cell movement that do not involve the fungus host can be carried out at containment level 2 as the virus alone is not spread in run-off water.

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**Project Ref** 250/07.2

**Date Ackn’d** 05/11/2007

**CU2 Project Title** Pectobacterium atrosepticum as a delivery vector in screening for effector function in secreted proteins from Pectobacterium atrosepticum and Phytophthora infestans.

**Class** Class 3  
**Culture Vol Class 2** < 1 Litre  
**Culture Vol Class 3-4** < 1 Litre

**Non-GMM Consent Granted** Yes

**Date Project Ceased**

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**
Pectobacterium atrosepticum (syn. Erwinia carotovora ssp. atroseptica) causes blackleg disease on potato, and disease is restricted to that host. P. atrosepticum is indigenous to the UK. The genome of P. atrosepticum has recently been sequenced and this has revealed that numerous factors such as pectolytic enzymes and phytotoxic compounds, are required for full pathogenicity in this organism. Also pathogenicity determinants (effector proteins) such as DspE are secreted by a type III secretion system (T3 SS) and some effector proteins in plant pathogens may suppress host defence responses such as the hypersensitive response. The ability of plant pathogens to actively target components of host defence responses can in part define the host range of the pathogen.

Host range in plant pathogens is also mediated by avirulence gene products. The expression of these genes by the pathogen is recognised by the host plant. A resistance response (the hypersensitive response (HR), a form of programmed cell death) is initiated by the plant, and the infection is contained. The pathogen is termed incompatible with that host, Absence of the avirulence gene products will lead to compatibility, and development of disease. Avirulence genes can operate at the host species level, as well as the variety, or cultivar level. At the cultivar level, each avirulence gene is matched by a cognate resistance gene, the so-called gene-for-gene interaction, For instance, potato resistance gene R3a is matched by Phytophthora infestans avirulence gene allele Avr3a. Interaction of P. infestans carrying Avr3a with a potato carrying R3a results in recognition of infection, and a resistance response; the pathogen is incompatible. Compatibility, or successful infection, results where the pathogen carries avr3a (virulence allele) and is not recognised by the host plant carrying R3a.

The discovery of a functional T3 SS in P. atrosepticum, a frill genome sequence for potato pathogens P. atrosepticum and P. infestans, and defined pathogenicity deficient mutants for P. atrosepticum provides an opportunity to use P. atrosepticum to study the delivery and effect of putative pathogenicity factors from both organisms in host (potato) and non-host (Nicotiana benthamiana) plants. The genes to be tested will be those that are either (or both) strongly up-regulated during infection of potato, or the homologs of genes implicated in pathogenesis or avirulence of other plant pathogens. Many of the genes to be tested will have no homology to genes of known function. Our aim is to determine the effect of delivery into host plant cells on the host ability to respond either in terms of non-host or innate immunity, or resistance gene mediated response (hypersensitive response). Determination of both pathogenicity factors and avirulence genes in P. atrosepticum and P. infestans will provide us with novel targets for both chemical and biotechnological control of potato diseases. It also will allow us to identify genetic components of more complex host resistance phenotypes such as field resistance, which can then be used in potato breeding programmes.

The question we will address with this research is whether genes involved in avirulence or pathogenicity in the named organisms have any role or effect in plant/pathogen interactions. The GMMs from these experiments will either invoke a resistance response in N. benthamiana or potato, or no change from the wild type will be observed.

Recipient or parental organism

Isolate SCRI-1043 of P. atrosepticum represents the wild type genetic background to be used in these experiments. Isolate SCRI—1043 was originally isolated from potato in Scotland. The GMMs from these experiments will either invoke a resistance response in N. benthamiana or potato, or no change from the wild type will be observed. Since the transgenes will originate from a pathogen that is itself restricted to potato hosts, it is unlikely that introduction of the heterologous genes will have any effect on the host range of the GMM. Indeed, the opposite effect of restricting the host range may be more likely, as the host plant may recognise the gene product as an avirulence protein and initiate a resistance response.

P. atrosepticum occurs as an endemic pathogen on potato crops in the United Kingdom. Although an enterobacterium closely related to Escherichia coil, P. atrosepticum has never been recorded as a human or animal pathogen. P. atrosepticum is not expected to pose any threat to human health under the conditions to be used for experimentation. P. infestans occurs as an introduced pathogen on potato crops in the United Kingdom. It and other Phytophthora species are not known as human pathogens. P. infestans is not expected to pose any threat to human health under the conditions to be used for experimentation.

For the majority of transgenes tested, there will be no effect on the infectivity, disease development, or host range of the 0MM. In the unlikely case of any 0MM escape, little is known about the stability of transgenes in P. atrosepticum. However, the transgenes are located on non-integrative plasmids that are unlikely to persist for long periods of
time without ampicillin selection in P. atrosepticum. Since the potato pathogen P. atrosepticum is to be used, and the transgenes to be tested originate from a pathogen restricted to potato hosts, it is highly unlikely that the host range of the GMM would be extended. An emerging consensus from the study of many bacterial, fungal and oomycete plant pathogens (including P. atrosepticum and P. infestans) is that pathogenicity is a complex process involving the action of many genes; the addition of only one of these genes is not likely to have a large impact on the overall aggressiveness of the GMM. In bacteria, where horizontal gene transfer is well documented as is considered to have had a role in changes in pathogenicity, this has often been due to the transfer of entire operons (clusters of several genes) such as for toxin biosynthesis or Typelli secretion. Should a GMM expressing a pathogenicity gene escape, the possibility of its DNA being transferred to another environmental and non-pathogenic organism, or another pathogen, is remote. In addition to the plasmid harbouring the effector transgene having a high probability of loss from P. atrosepticum, pathogenesis in many pathogens is typically complex and probably employs many mechanisms. It is unlikely therefore, that the horizontal transfer of a single P. infestans gene to a fungus or bacterium would confer novel pathogenicity upon a non-pathogen or increase host range of an existing pathogen.

Host/vector system

Vectors are based on the commonly used non-integrative pOEM series of plasmids, which are also functional in P. atrosepticum; the plasmid vectors therefore contain an ampicillin resistance gene. The T3SS effector DspE from P. atrosepticum is an orthologue of AvrE from Pseudomonas syringae and shows the bias towards high serine (20%), low aspartate (2%) in the first 50 amino acids of T355-translocated effectors in P. syringae. The promoter region, containing the hrp box for HrpL-dependent induction, along with the first 100 amino acid encoding region of dspE, will be fused to P. infestans effector genes, minus the signal peptide-encoding sequences. Potato resistance genes will be expressed from A. tumefaciens strain AGLO.

Origin & function

We aim to express P. infestans candidate pathogenicity and avirulence genes from P. atrosepticum in different assays. The precise sequence identity of each of the genes to be tested is not known, as the bioinformatics analyses of the P. infestans genome sequence for candidate genes are still in progress. In the instance of P. infestans pathogenicity genes, these will be genes that are either (or both) strongly up-regulated during infection of potato, or the P. infestans homologues of genes implicated in pathogenesis of other plant pathogens. Many of the genes to be tested will have no homology to genes of known function, Initial experiments will use Avr — R gene pair of Avr3a and R3a, both of which have been isolated. Overexpression of the Avr3a gene in stable transformants of P. infestans has not led to any increase in the aggressiveness of that organism. For defined or candidate avirulence genes from P. infestans where the cognate resistance gene from potato has been cloned, the transgenic P. atrosepticum will be coinfiltated into plant leaves with A. tumefaciens strain AGLO expressing the cognate resistance gene. Delivery of both components (AVR and R) is expected to trigger the hypersensitive response. These experiments will be carried out in either potato or N. benthamiana. For candidate effector genes, transgenic P. atrosepticum will be infiltrated first into N. benthamiana leaves followed later by infiltration with wild type Erwinia amylovora, for which AL. ben/hamiana is a non-host. Where a candidate effector is acting to suppress defence responses, the HR triggered by B. amyllovora will be inhibited.

Evaluation of foreseeable effects

The GMMs from these experiments will either invoke a resistance response in N. benthamiana or potato, or no change from the wild type will be observed. Since the transgenes will originate from a pathogen that is itself restricted to potato hosts, it is unlikely that introduction of the heterologous genes will have any effect on the host range of the GMM. Indeed, the opposite effect of restricting the host range may be more likely, as the host plant may recognise the gene product as an avirulence protein and initiate a resistance response. For defined or candidate avirulence genes from P. infestans where the cognate resistance gene from potato has been cloned, the transgenic P. atrosepticum will be coinfiltated into plant leaves with A. tumefaciens strain AGLO expressing the cognate resistance gene. Delivery of both components (AVR and R) is expected to trigger the hypersensitive response. Expression of candidate avirulence genes isolated from P. infestans will result in the GMM becoming avirulent (or incompatible) with potato varieties carrying specific matching resistance genes. In this case, the host range of the GMM is expected to be reduced from the wild type (with the wild type originally being virulent, or compatible, on the resistance genes tested). For a P. infestans avirulence gene to be expressed, we may expect to see localised necrosis (HR) at the point of inoculation. This response would be confined to one or few host plant genotypes (carrying the cognate R gene); specificity towards other host plant genotypes should remain unchanged.

Risk to human health
P. atrosepticum occurs as an endemic pathogen on potato crops in the United Kingdom. Although an enterobacterium closely related to Escherichia coli, P. atrosepticum has never been recorded as a human or animal pathogen. P. atrosepticum is not expected to pose any threat to human health under the conditions to be used for experimentation. P. infestans occurs as an introduced pathogen on potato crops in the United Kingdom. It and other Phytophthora species are not known as human pathogens, P. infestans is not expected to pose any threat to human health under the conditions to be used for experimentation. A. tumefaciens occurs naturally in soil and is not known to be a pathogen of humans. Disauned A. tumefaciens strains have been used safely as a vector for transgene delivery into plants for many years. It is not expected to pose any threat to human health under the conditions to be used for experimentation.

The foreign genes to be expressed in the 0MM are of unknown function or biological activity. Transgenes will encode protein products from P. atrosepticum or P. infestans (potato late blight). No toxicology studies have been carried out on P. infestans or P. atrosepticum, and there are no records to implicate either organism in human disease. In the extremely unlikely event that any of the expressed gene products are determined to be toxic to humans, experiments with these genes will cease immediately.

Risk to the environment
P. atrosepticum is spread in saturated soils by dispersal from heavily infected plants, or plant debris in soil. P. atrosepticum can also persist on roots of alternate host plants such as Brassica sp. Almost all potato varieties grown in the UK are susceptible to P. atrosepticum. There is no effective chemical control against P. atrosepticum. It is simple to maintain P. atrosepticum in sealed agar plates and the risk of escape from these vessels is minimal. It is standard practice in our laboratories to handle P. atrosepticum field isolate cultures in a class 2 biohazard cabinet to prevent escape of the pathogen, and to protect the cultures from contamination. We propose to maintain and manipulate the GMMs in the same manner.

In our experiments, the conditions for transmission of P. atrosepticum will be avoided where possible. For instance, preparation of inoculum will use good microbiological practice, small plants will be inoculated by infiltration to prevent aerosols, inoculated plants will be kept in sealed propagator boxes, handling of infected plants will be minimised, disposable gloves will be worn for all handling of infected plants, and all materials that come in contact with the infected plants or cultures will be destroyed by autoclaving.

For the majority of transgenes tested, there will be no effect on the infectivity, disease development, or host range of the 0MM. In the unlikely case of any 0MM escape, little is known about the stability of transgenes in P. atrosepticum. However, the transgenes are located on non-integrative plasmids that are unlikely to persist for long periods of time without ampicillin selection in P. atrosepticum. Since the potato pathogen P. atrosepticum is to be used, and the transgenes to be tested originate from a pathogen restricted to potato hosts, it is highly unlikely that the host range of the 0MM would be extended. An emerging consensus from the study of many bacterial, fungal and oomycete plant pathogens (including P. atrosepticum and P. infestans) is that pathogenicity is a complex process involving the action of many genes; the addition of only one of these genes is not likely to have a large impact on the overall aggressiveness of the GMM. In bacteria, where horizontal gene transfer is well documented as is considered to have had a role in changes in pathogenicity, this has often been due to the transfer of entire operons (clusters of several genes) such as for toxin biosynthesis or TypeIII secretion. Should a GMM expressing a pathogenicity gene escape, the possibility of its DNA being transferred to another environmental and non-pathogenic organism, or another pathogen, is remote. In addition to the plasmid harbouring the effector transgene having a high probability of loss from P. atrosepticum, pathogenesis in many pathogens is typically complex and probably employs many mechanisms. It is unlikely therefore, that the horizontal transfer of a single P. infestans gene to a fungus or bacterium would confer novel pathogenicity upon a non-pathogen or increase host range of an existing pathogen.

As an additional containment measure, staff carrying out these experiments will not visit potato fields or SCM potato glasshouses (other than the containment glasshouse) on the day of experiments involving the 0MM.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Glasshouse benches resistant to acids etc: derogation applied for on the basis that the benches in the containment area are made of wire mesh for ease of cleaning and to enable temperatures to be maintained consistently throughout the facility. Fully waterproof receptacles to prevent any run off water from plant pots are included routinely in the SOPs for the facility. By using small plants (less than 20 cm height) in sealed containers, there will be no water run off. By keeping all inoculated plant material in sealed vessels, there is no possibility of water ingress as a result of flooding. The floors of this area are also sealed and the drain capped. In the highly unlikely event of flooding...
the drains have an additional cap beneath the floor and if this should fail the default setting on the drainage system would direct the effluent to a holding tank where chemical disinfection could take place before release to mains drainage.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any material including flasks or plates used for growth of cultures, inoculated plant material plant pots, compost, labels, gloves etc. that might be contaminated by the GMOs will be autoclaved before disposal. A 5 log reduction in the number of microorganisms will result and will be validated by plating out of a liquid culture before and after autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This was an amended version of a proposal that had been submitted previously (December 2006) suggesting a lower containment level and which had been rejected by the committee. The committee was now satisfied with the containment measures suggested and are now happy for this work to proceed. Institute Safety Office indicated that dedicated freezers should be made available for this work and that material should not be stored beside other GM material,

Project Containment

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Project Ref 250/07.3

Date Ackn’d 05/11/2007

CU2 Project Title Study of interactions between plants and bacteria pathogenic for humans.

Date Project Ceased 02/03/2022

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted Not Applicable
To investigate the interactions that occur between plants and human pathogens: pathogenic Escherichia coil (including nonverotoxigenic (non-VTEC) serotypes 0157, 026 and 0111 and uropathogenic strain CFT073), Salmonella enterica serovar Typhimurium and Kiebsielia pneumoniae. The work will make use of comparative genomic analyses based on the genome sequence of Pectobacterium atrosepticum and these pathogens to analyse gene orthologs between the different pathogens that may be involved in association and survival on plants. Genes of interest will be analysed by (i) mutation in the wild type background of K coil, S. Typhimurium or K pneumoniae and complementation (plasmid-based or single copy via alleleic exchange); (ii) cloning into plasmid-based vectors and over-expression in a negative control background; (iii) fusion to reporter genes (both plasmid-based and single copy chromosomal fusions).

The bacterial strains in the proposal are pathogenic to humans and are all defined by the HSE as Category 2 classification. NonVTEC K coil strains are attenuated by deletion of the shiga-toxin genes and are derived from the well-characterised sequenced isolates EDL933 and Sakai, or from field isolates. Loss of the verotoxigenic genotype and phenotype has been confirmed by PCR and/or vero cell assay in the source laboratories (Centre for Infectious Diseases, the University of Edinburgh) E. coil CFT073, S. Typhimurium (e.g. strains SL1344 and LT2) and K pneumoniae (e.g. Kp 342 and ATCC 700721) are all wild type strains that do not require attenuation for Category 2 classification. K coil K-12 (Category 1 classification) will also be used as an intermediate strain for cloning DNA of interest into plasmids. It will also be used as a control organism in experimental work, as it represents a negative control, Genes targeted for manipulation will be those potentially involved in survival on plants and not those associated with pathogenesis on either plants or humans, Any risks associated with pathogenesis on either of these hosts will thus be extremely unlikely. The manipulations and modifications will focus on deletions of virulence determinants, and comparison to wild type parent strains. None of the GMMs will carry insertions of ‘foreign’ virulence factors, i.e. from one species to another. If foreign DNA is inserted it will be in the form of reporters (such as LacZ, GnsA or fluorescent proteins), antibiotic marker genes (listed above). As these bacteria are not pathogenic on plants, there is no risk to plants associated with spread to the wider environment. An increased risk to humans is unlikely.

Manipulation requires the use of both transposons (mTn5, delivered on the plasmid pUT APR), and plasmids. All manipulations will be carried out under antibiotic selection, Removing selective pressure results in dilution of the plasmid by bacterial growth. The plasmids will be transferrable to enteric bacteria. Lysogenic bacteriophage specific to E. coil (P1) or S. Typhimurium (P22) will be used to mobilize marked (with antibiotics) mutations or reporters into wild type backgrounds.

Genes and regulatory DNA possibly involved in adherence to, and persistence within, plant tissue from K coil strains EDL933, CFT073 and Sakai, or from field isolates, or from S Typhimurium (e.g. strains SL1344 and LT2) and K pneumoniae (e.g. Kp 342 and ATCC 700721) will be manipulated by mutagenesis, complementation or
Evaluation of foreseeable effects

Genes targeted for manipulation will be those potentially involved in survival on plants and not those associated with pathogenesis on either plants or humans. Any risks associated with pathogenesis on either of these hosts will thus be extremely unlikely. Modifications which result in mutagenesis of a particular gene will reduce that characteristic of the GMM. Complementation of mutants will restore phenotypes to wild type levels. Manipulation of the factors will not alter the normal route of transmission of E. coli or S Typhimurium GMM to the human host. Although manipulation requires the use of antibiotics, the wild type susceptibility to antibiotic treatment will not be affected as the antibiotics used in treatment have superseded those used in manipulations by several generations- For the majority of enteric infections, fluoroquinolones are effective. Furthermore, this work will not use strains that carry multi-thug resistance, such as S. Typhimurium DTIO4. In particular, specific guidelines (HPA) for E. coli 0157 indicate that there is no convincing evidence to suggest that antimicrobial agents alter the natural history of VTEC infection or the duration of faecal excretion of the organism. Small-scale studies have investigated co-trimoxazole, aminoglycosides, or beta-lactam antibiotics rather than the fluoroquinolones. The work in this proposal will only make use of verotoxin-negative strains, which means that even if sub-inhibitory concentrations of antibiotics were administered, it will not lead to lysis and release of verotoxin associated with wild type strains of VTEC.

No DNA transfer between pathogens will take place but, as is commonly carried out, F. coil K-12 will be used to transfer genetic material for mutagenesis etc. Any inserted DNA sequences will not increase the virulence of the GMM over that of wild type. The manipulations should not affect the survival of the GMM over that of wild type, Manipulation requires the use of both transposons (mTn5, delivered on the plasmid pUT APR), and plasmids. All manipulations will be carried out under antibiotic selection. Removing selective pressure results in dilution of the plasmid by bacterial growth. The plasmids will be transferable to enteric bacteria. Lysogenic bacteriophage specific to F. coil (P1) or S Typhimurium (P22) will be used to mobilize marked (with antibiotics) mutations or reporters into wild type backgrounds. The likelihood of the 0MM establishing an infection in a human host is no greater than the wild type. The probability of this happening is no greater than if the work focused on wild type bacteria. The manipulations listed above in (2) will not affect pathogenicity, infectivity, toxicity, virulence, allergenicity, colonization, parasitism, symbiosis or competition over the wild type and should not render the 0MM detrimental for natural flora and fauna or alter the capacity of the GMM to cause harm to the environment. None of the manipulations (deletions or insertion of reporter cassettes) will confer a selective advantage, if either transferred to enteric bacteria already in the environment or if the GMM were released into the environment. Chromosomal mutations (random or defined) and insertion of reporter systems into the chromosome will be stable. Even if the 0MM were to revert to wild type, it poses no greater risk than the parent wild type strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any material including flasks or plates used for growth of cultures, inoculated plant material plant pots, compost, labels, gloves etc. that might be contaminated by the GMOs will be autoclaved before disposal. A 5 log reduction in the number of microorganisms will result and will be validated by plating out of a liquid culture before and after autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The minutes of the GM safety committee that discussed the project are below:

- (BSO) took the opportunity to discuss this submission with the HSE inspectors on their recent visit and they seemed satisfied with the Risk Assessment and the Category 2 classification.

(Institute Safety Officer) had contacted the Biological Safety Officer at the Moredun Research Institute regarding this submission and she was happy with the RA although made a few suggestions regarding SOPs (These have been incorporated into the risk assessment).

i.e. Dedicated lab coats or gowns will be required and kept in the cat 2 lab, indicated that these labcoats will be of a different (style to identify them from "ordinary" labcoats.

Staff should be trained and written records of staff training will be required. pass training records to These documents must identify individuals by name.  -

SOPs for daily work as well as for dealing with accidental spillages should be available in the lab.

(Deputy BSO) emphasized the need for care when handling human pathogens. A dedicated laboratory should be identified for this work. All lab users should be aware of the Category 2 work being carried out in the laboratory. A list of authorized users should be posted on the designated laboratory door.

needs to notify HSE and should liaise with him regarding this matter.

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### Project Ref 250/08.1

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<th>CultureVolumeClass3-4</th>
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<th>Historical Significant Changes</th>
<th>Historical Date of Additional Info</th>
<th>Significant Change ID</th>
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### Project Additional Information

#### Purposes of the contained use

Plant pathogens (bacteria, oomycete or fungi) will be transformed with vectors directing the expression of marine invertebrate-derived fluorescent proteins with a β-barrel structure (either native or fused to proteins derived from the pathogen to be transformed) and localisation of the pathogen or pathogen-derived protein in plants infected with the pathogen will be determined by fluorescence microscopy or confocal microscopy. Recombinant viruses will also be generated containing fluorescent protein genes and similar localisation studies performed on plants infected with single viruses.

#### Recipient or parental organism

**Viruses**

Infectious clones of plant pathogenic viruses will be used to either make infectious transcripts in vitro, or infectious clones in agrobacteria binary vectors will be applied by agroinoculation. Viruses will include:

1. Aphid transmissible viruses: Potato virus A, Potato virus Y, Potato leafroll virus, Black raspberry necrosis virus, Cucumber mosaic virus, Cauliflower mosaic virus.
2. Nematode transmitted viruses: Tomato black ring virus, Tobacco ringspot virus, Tomato ringspot virus, Tobacco rattle virus
3. Mite-transmitted viruses: Raspberry green leaf blotch virus, Blackcurrant reversion virus
4. Manually-transmitted viruses: Tobacco mosaic virus, Potato virus X, , Barley stripe mosaic virus, Turnip vein chlorosis virus, Tomato bushy stunt virus, Groundnut rosette virus, Pea enation mosaic virus 2
5. pollen transmitted virus: Raspberry bushy dwarf virus
6. beetle transmitted virus: Rubus Chlorotic mottle virus
7. Oomycete transmitted virus: Potato mop-top virus

The viruses are pathogens of plants and cause a variety of disease symptoms in plants. Addition of fluorescent proteins is not expected to affect their pathogenicity, host range or mode of transmission. Containment level 2 will normally be applied except where it is suspected that either pollen-transmission or water-borne transmission of a virus via a vector can occur in which cases containment level 3 will apply in the glasshouse.

**Fungi and Oomycetes**

Fungi will include the cereal pathogens: Rhynchosporium secalis, Ramularia collo-cygni, Phaeosphaeria nodorum, Mycosphaerella graminicola, Magnaporthe grisea, Oculimacula yallundae, Pyrenophora/Drechslera, Gaeumannomyces graminis, Bipolaris sorokiniana, Claviceps purpurea, Fusarium spp, Pyrenophora tricirepentis, Pyrenophora graminea, Pseudoseptoria stomaticola, Ustilago spp and Rhizoctonia solani.

Oomycetes will include the potato-infecting Phytophthora infestans, Spongospora subterranea and Phytophthora erythroseptica; soft fruit-infecting Phytophthora fragariae and Phytophthora idaei; Arabidopsis thaliana-infecting Phytophthora brassicae and Hyaloperonospora arabidopsis (syn. H. parasitica); woody host-infecting Phytophthora ramorum, Phytophthora alni and Phytophthora kernoviae; soybean-infecting Phytophthora sojae and broad host range Phytophthora andina, Phytophthora capsici, Phytophthora cinnamomi, Phytophthora palmivora, Phytophthora cryptogea, Phytophthora cactorum, Phytophthora cambivora, Phytophthora dechleri, Phytophthora nicotianae, Phytophthora megasperma, Aphanomyces eutiches, Pythium ultimum and Pythium oligandrum.

These cause a variety of symptoms on host plants including: leaf and tuber blight, root, stem and fruit rot, stem canker and downy mildew. Pythium oligandrum is plant associated and also infects and lyases fungi and other oomycetes. Addition of fluorescent proteins is not expected to affect their pathogenicity, host range or mode of transmission. Containment level 2 will apply in the glasshouse except were water-borne transmission is suspected in which cases containment level 3 will apply.
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iii. Mite-transmitted viruses: Raspberry green leaf blotch virus, Blackcurrant reversion virus.


v. pollen transmitted virus: Raspberry bushy dwarf virus.

vi. beetle transmitted virus: Rubus Chlorotic mottle virus.


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Bacteria

These will include the following plant pathogenic bacteria:

i. Pectobacteria atroseptica which is endemic in Scotland and causes disease only in potato, but may survive on plants including other crops without disease symptoms. It is transmitted via contaminated vegetative material.

ii. Pectobacteria carotovora which is limited to potato in Scotland but has wider host range in warmer countries. It is transmitted via contaminated vegetative material. It lives in soil as a saprophyte and is endemic in Scotland.
iii. Dickeya species (D. dadantii, D. dianthicola, D. chrysanthemi, D. zeae, D. paradisiaca and D. dieffenbachiae) cause disease on different plants including some crops in warmer countries. D. dianthicola and possibly other species are present on potato in Europe. They are transmitted via contaminated vegetative material but there is some evidence that the Dickeya species are water-borne in transmission at least in some instances.

iv. Pantoea ananatis is a pathogen of eucalyptus in South Africa but can also cause disease on maize and onion.

v. Erwinia amylovora, causes disease of apples and pears.

vi. Streptomyces scabies infects potatoes and other root crops including beets, radish, turnip, carrot and parsnips. It survives in soil and infected plant material and is transmitted as spores

vii Pseudomonas aeruginosa

vii Burkholderia cepacia

Containment level 2 will apply in the glasshouse except for the Dickeya species where there is evidence of water-borne transmission and for which containment level 3 will apply in the glasshouse.

Host/vector system

The transformation vectors for fungi, bacteria and oomycetes are based on non-integrative pGEM bacterial plasmids or transposons (mTn5, delivered on the plasmid pUT ApR).

Origin & function

Genes encoding fluorescent proteins with a β-barrel structure from marine invertebrates such as green fluorescent protein gene (GFP), yellow fluorescent protein (YFP), red fluorescent protein (mRFP or dsRED), or tandem dimer tomato fluorescent protein (tdTomato) will be added to the plant pathogens either in their native form or fused to other genes of the pathogen. This will allow localisation of the pathogen (or pathogen-derived protein) by detection of fluorescence under ultra-violet light.

For fungi and oomycetes promotor and terminator sequences originate from the Aspergillus nidulans glyceraldehyde-3-phosphate dehydrogenase gene. The vectors also contain the antibiotic resistance gene hygromycin phosphotransferase (hpt) under control of the same promotor mentioned above to allow for selection in fungi and the ampicillin resistance gene to allow propagation of the plasmid in the bacterium Escherichia coli.

Evaluation of foreseeable effects

There is a long history of the safe use of genes encoding marine invertebrate-derived fluorescent proteins with a β-barrel structure such as green fluorescent protein gene (GFP), yellow fluorescent protein (YFP), red fluorescent protein (mRFP or dsRED), or tandem dimer tomato fluorescent protein (tdTomato) either in their native form or fused to other proteins. We expect to see that, following transformation, transformants will become fluorescent under specific light wavelengths. No increase in aggressiveness is expected, as these fluorescent tags have been used extensively in other virus, fungal, oomycete, and bacterial plant pathogens without increasing the pathogenic capabilities of the pathogens. Indeed, the opposite effect of reduced aggressiveness is more likely as constitutive overexpression of a heterologous gene is likely to place a metabolic burden on the pathogens. It is also common for plant pathogens kept in culture (as would be the GMMs) to become attenuated in virulence or lose the ability to produce spores (where appropriate). It is not expected that addition of such a gene will affect the pathogenicity, host range or aggressiveness of a pathogen, nor is it expected that plants infected with pathogens expressing these genes will behave any differently from those infected with unmodified pathogens.

Risk to human health

Most of the pathogens are restricted to plants and present little hazard to human health. Two of the bacteria (Pseudomonas aeruginosa and Burkholderia cepacia) opportunistically infect humans and are in ACDP group 2.
Addition of genes encoding fluorescent proteins is unlikely to make the resulting GMMs any more harmful than the parental pathogens. Infection of the host plants with pathogens should present no greater risk than plants infected with unmodified pathogens. Expression of fluorescent proteins will not affect the ability of the plants to survive or diseminate, instead infection with the GMMs should decrease the fitness of the plants. Expression of fluorescent proteins should not increase the multiplication of the pathogens and infected plants should not become greater reservoirs for pathogens. Spread of the genes encoding fluorescent proteins to other organisms will cause them to fluoresce under ultra-violet light but no other adverse or beneficial effects are expected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Glasshouse benches resistant to acids etc: derogation applied for on the basis that the benches in the containment area are made of wire mesh for ease of cleaning and to enable temperatures to be maintained consistently throughout the facility. Fully waterproof receptacles to prevent any run off water from plant pots are included routinely in the SOPs for the facility. The floors of this area are also sealed and the drains capped. In the highly unlikely event of flooding the drains have an additional cap beneath the floor and if this should fail the default setting on the drainage system would direct the effluent to a holding tank where chemical disinfection could take place before release to mains drainage.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any material including flasks or plates used for growth of cultures, inoculated plant material plant pots, compost, labels, gloves etc. that might be contaminated by the GMOs will be autoclaved before disposal. A 5 log reduction in the number of microorganisms will result and will be validated by plating out of a suspension of GMM before and after autoclaving. For viruses autoclaves have been validated for killing of tobacco mosaic virus by inoculation of autoclaved infected plant material onto sensitive local and systemic host plants and no infection of test plants was observed.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The committee noted that the purpose of the risk assessment was to determine the localisation of a number of different plant pathogens. An increase in the number of people expressing an interest applying such approaches had been seen and this risk assessment was to allow a single application to be made for consent to carry out the work. An email enquiry to HSE had indicated that inclusion of a number of different pathogens as a linked programme of work should be acceptable.

Project Containment

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The role of protein transport in the pathogenicity of Streptomyces scabies

Genes of S. scabies known or thought to be involved in secretion of bacterial proteins have been replaced by targeted mutagenesis by a collaborator at the University of Dundee. We now wish to obtain these strains and investigate whether the protein secretion systems that have been deleted contribute to the virulence of S. scabies by performing basic plant virulence assays on Arabidopsis, potato and radish plants.

One protein secretion system that we know contributes greatly to virulence is the Tat system. We wish to carry out confocal laser scanning microscopy (CLSM) to look at how plant roots are infected by wild-type and tat mutant strains. To this end, constructs have been introduced into the bacteria that express green-fluorescent protein to label the bacteria so that they may be visualised. The pathogen-infected plant material will be transported to the microscopy suite in a sealed container and the microscope cleaned with 70% ethanol after use.

We also wish to investigate the role of individual proteins that are secreted by the Tat system. Knockout mutants of these will be constructed and assessed in virulence assays. Those found to contribute to pathogenicity will be fluorescently labelled and their localisation in the plant roots visualised using CLSM to give an indication of their plant cell target and helping to elucidate the role of the Tat system in the disease process.

Similar work is proposed for a second secretion system, the Esx system. This has not been examined previously in S. scabies although plays a role in pathogenesis of other Gram-positive bacteria, including Mycobacterium tuberculosis and Staphylococcus aureus.
Streptomyces scabies, an opportunistic pathogen causing common scab of potato and other tuber crops.

**Host/vector system**

S. scabies cosmid library constructed in SuperCos pSET152 derived plasmids that integrate into the chromosome at the phage phiC31 site. Both must integrate into the chromosome in order to be maintained as they cannot replicate in Streptomyces (suicide vectors).

**Origin & function**

n-frame gene deletions are marked with antibiotic resistance genes. It is expected that these will prevent secretion of a sub-set of bacterial proteins and possibly affect virulence of S. scabies. Constitutive expression of green-fluorescent protein allows for visualisation of strains during microscopic examination.

**Evaluation of foreseeable effects**

Mutations in the Tat system of protein secretion cause attenuation in virulence of S. scabies. The effects of other mutations are uncertain, although they are also expected to lead to a reduction in virulence. This is yet to be ascertained.

S. scabies is an opportunistic plant pathogen that causes scab on root vegetables. Scab causing Streptomyces sp are already endemic in the UK and do not cause economically significant damage on crops other than potatoes. S.scabies is potentially transmissible in run-off water. No reports of adverse effects of S. scabies on human health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Glasshouse benches resistant to acids etc: derogation applied for on the basis that the benches in the containment area are made of wire mesh for ease of cleaning and to enable temperatures to be maintained consistently throughout the facility. Fully waterproof receptacles to prevent any run off water from plant pots are included routinely in the SOPs for the facility. The floors of this area are also sealed and the drains capped. In the highly unlikely event of flooding the drains have an additional cap beneath the floor and if this should fail the default setting on the drainage system would direct the effluent to a holding tank where chemical disinfection could take place before release to mains drainage.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Any material including flasks or plates used for growth of cultures, inoculated plant material plant pots, compost, labels, gloves etc. that might be contaminated by the GMOs will be autoclaved before disposal. A 5 log reduction in the number of microorganisms will result and will be validated by plating out of a suspension of GMM before and after autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
This work requires glasshouse Containment Level 3 as this is potentially a water-borne pathogen, and will require notification to the HSE. For work in the microscopy laboratory, as with other work on potentially water-borne pathogens, safe transport and disinfection of all equipment in contact with infected material will be required. The proposal was approved subject to those amendments.

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### Project Ref 250/10.2a

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### Project Additional Information

- Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 250/11.1

Functional characterisation of Rhynchosporium secalis and Ramularia collo-cygni pathogenicity genes through targeted gene disruption, tagging with fluorescent proteins and transient overexpression of secreted proteins (putative effectors) in the leaves of cereals seedlings.

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Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID 250/11.1a

Date of Significant Change 17/11/2016

Project Additional Information

Purposes of the contained use

Rhynchosporium secalis and Ramularia collo-cygni are important fungal pathogens of barley in the UK. Leaf scald (R. secalis) is classified as the most damaging disease of barley and is a major reason for fungicide use. The severity of epidemics varies between seasons, regions and crops. These diseases are accredited for losses to farmers in yield and quality estimated at £6.7M in 2000, despite fungicide applications to 95 % of crops.

R. secalis grows symptomlessly under the cuticle, especially where walls of adjacent cells are joined before producing new conidia and finally, visual symptoms. R. secalis may also complete its infection cycle asymptptomatically. To invade and grow within the host plant, it must produce a range of secreted effector proteins to suppress host innate immunity triggered by pathogen associated molecular patterns (PAMPs) such as chitin. Populations of R. secalis can change rapidly, defeating new barley resistance genes after just several seasons of widespread commercial use.

Infection by Ramularia can result in a yield decrease of 0.6 tonnes per hectare and has been shown to cause yield losses of 20 %.

Despite the damage that R. secalis and R. collo-cygni inflict on barley crops, knowledge of their pathogenicity factors is almost non-existent. The challenge is therefore to gain a greater understanding of novel and essential pathogenicity determinants, as these represent good targets for host plant recognition to protect itself. Some pathogenicity determinants essential for the core biology of the pathogen during infection may also represent efficient fungicide targets.

One of the most revealing strategies to elucidate gene function is to remove its activity through mutagenesis, knockout, or gene silencing. R. secalis and R. collo-cygni, like most fungal plant pathogens, are haploid which allows the use of knockouts. R. secalis and R. collo-cygni transformation has already been used at SCRI to successfully introduce genes encoding fluorescent proteins.
Selected R. secalis and R. collo-cygni gene sequences will be prioritized for functional analysis through targeted gene disruption by bioinformatic analyses such as predicted location (e.g., secreted or membrane bound), Pfam domains, or knowledge of structure (e.g., C-rich). RERAD funded R. secalis genome and interaction transcriptome sequencing will provide further information about the extent of pathogenicity gene families as well as a subset of genes expressed during barley colonization by R. secalis. The R. secalis genome sequence will allow identification of candidate pathogenicity factors (including effectors) specific to R. secalis. It also provided the R. secalis sequence of KU70 gene allowing creation of KU70 deletion transformant with increased targeted gene replacement frequency.

The aim of this project is functional characterisation of R. secalis and R. collo-cygni pathogenicity factors including their effect on pathogenicity through targeted gene knockouts, complementation, localisation using fluorescent protein tagging, and identification of functions for R. secalis or R. collo-cygni secreted proteins during barley pathogenesis and/or resistance response.

Recipient or parental organism

Endemic isolates of the wild-type fungal plant pathogens R. secalis and R. collo-cygni will originate from the SCRI culture collection.

For cloning Barley stripe mosaic virus (BSMV) genomes and R. secalis genes we will only use disabled E. coli strains e.g., XL1-Blue, DH5α, SURE, etc. and disarmed A. tumefaciens strains e.g., C58C1 (pCH32), GV3101 (pMP90), etc. These microorganisms are unlikely to persist in the human lungs or the gut, or indeed survive outside culture medium.

The ND18 strain of BSMV that will be used in this study is not indigenous to the UK and thus is subject to legislative (plant health) control. We have a license to move and keep this pathogen. BSMV is not known as a hazard to human health and nor is there any reason to suppose that it could be unless specifically modified to express toxic proteins or proteins that produce toxic products. BSMV has no known arthropod, fungal, or nematode vectors. The main route of natural spread of BSMV seems to be by plant to plant contact. The only natural hosts of BSMV known are barley and wheat. Wild oats (Avena fatua) can also be infected occasionally. BSMV infection is already widespread but does not cause serious agronomic problems even in susceptible cultivars. BSMV is also known to be transmitted through seed to 90% of the offsprings depending on the virus strain. This virus is also pollen-borne and can infect pollinated plants. However, this is not a major problem in barley and wheat as both are self-pollinated within the closed floret. To eliminate this potential hazard due to accidental release of recombinant BSMV the experimental plants will be discarded before they reach the flowering stage.

Host/vector system

The transformation vectors are based on non-integrative bacterial plasmids containing different antibiotic resistance genes. pCAMBgf must contains the antibiotic resistance gene hygromycin phosphotransferase (hpt) under control of the Aspergillus nidulans tryptophan synthase C (TrpC) promoter for constitutive expression together with the SGFP gene under the control of the Pyrenophora tritici-repentis TOXA gene promoter. pAB8-1 contains the bleomycin resistance-encoding gene (ble) from Streptoalloteichus hindustanus, under the control of promoter (gpdA) from Aspergillus nidutans genes; pFBT009 contains nat1 cassette conferring resistance to nourseothricin.

Origin & function

The inserted DNA will contain an antibiotic resistance gene: hygromycin phosphotransferase under control of the Aspergillus nidulans tryptophan synthase C (TrpC) promoter, bleomycin resistance-encoding gene (ble) from Streptoalloteichus hindustanus, under the control of promoter (gpdA) from Aspergillus nidutans genes, or nat1 cassette conferring resistance to nourseothricin for selection of transformants, and a marine invertebrate fluorescent protein gene (e.g., GFP), for localisation of viable hyphae and other fungal structures during infection of barley, flanked by the partial sequences of R. secalis gene to be disrupted.

In case of complementation or transient overexpression the inserted DNA will contain the endogenous R. secalis gene, which might restore R. secalis pathogenicity to that of wild type, and an antibiotic resistance gene. In case of fluorescent proteins tagging the inserted DNA will contain the endogenous R. secalis gene with fluorescent protein sequence at its C or N terminus and an antibiotic resistance gene.

The function of R. secalis genes coding for secreted proteins, which we propose to investigate during this project are unknown but are most likely play roles in the
communication events between R. secalis and barley leaves. As R. secalis is a pathogen of barley we do not anticipate much activity towards other plant species, including wheat. In fact, BSMV virus has a much broader host range than the fungus. The possibility exists that the fungal proteins under study might trigger the death of barley leaves due to them functioning as host-specific or non-host-specific effectors or toxins. As yet none of these proteins are currently known to be plant toxins. As the fungus from which these proteins originate is a barley pathogen, should any toxins or defence suppressors be identified they are likely to function primarily against barley and will not impact upon other plants nor any animals. The worst case scenario might be that barley leaves inoculated with the viral transgene might display a trailing necrosis response. All the genes to be tested originate from this fungus primarily infecting barley and therefore no change in host specificity of the virus is anticipated. Neither virus nor fungus are airborne, therefore there is no risk of breach of containment through airborne particles. All the genes are predicted to function at the host-pathogen interface (in communication events) and none have anticipated enzymatic activities, as they possess no known functional domains (e.g. for enzymes or transporters etc.), and will not influence sensitivity to antifungal compounds.

**Evaluation of foreseeable effects**

**Risk to human health**

None anticipated.

R. secalis and R. collo-cygni occur as endemic pathogens on barley crops in the UK. These and other fungal pathogens of cereal species are not known as primary human pathogens. Neither fungal species is expected to pose any threat to human health under the conditions to be used for experimentation.

BSMV is also exclusively a plant pathogen. There are no reports of the virus, or reasons to believe the fungal genes under study, pose any risk to humans or animals.

The precise sequence identity of each of the fluorescent protein genes to be used is known. These genes and their encoded proteins have been expressed in a wide variety of organisms, including fungal, oomycete and bacterial plant pathogens, with no adverse effect on human health.

Transformation of fungal material is not expected to pose any risk to human health under the conditions to be used for experimentation. Transformation of protoplasts and Agrobacterium-mediated transformation are common laboratory method of gene delivery in fungal/oomycete genetics and have been used for many years (including at SCRI) without adverse effect to human health.

**Risk to the environment**

R. secalis is naturally transmissible by splash dispersal of spores from infected leaves; spores are produced within a mucilage matrix. To reduce this risk the R. secalis inoculated plants will never be watered from the top. Fungal conidia are not airborne, so there is no risk of airborne breach of containment during these studies. It is also possible that R. secalis and R. collo-cygni may be spread by infected seed, but the role of this form of transmission is not fully understood. These experiments will not involve plants which are flowering so no seed borne issues will occur. All plants used in the experiments will be destroyed before the flowering stage.

Most barley varieties grown in Scotland are susceptible to R. secalis and R. collo-cygni. Control is typically by frequent treatment of crops with fungicides. Furthermore, the strains of R. secalis and R. collo-cygni to be used in our experiments will be fully sensitive to common fungicides.

It is simple to maintain R. secalis and R. collo-cygni cultures in sealed agar plates and the risk of escape from these vessels is minimal. It is standard practice in our laboratories to handle fungal cultures in a Class II biohazard cabinet to prevent escape of the pathogen, and to protect the cultures from contamination. We propose to maintain and manipulate the GMMs in the same manner. Inoculated detached plant leaves in sealed containers will also only be opened within the Class II biohazard cabinet. Containers holding infected leaves from whole glasshouse grown plants will also only be opened inside the Class II biohazard cabinet or containment level 2 laboratory.

In our experiments, the conditions for transmission of R. secalis and R. collo-cygni will be avoided where possible. For instance, preparation of inoculum will use conditions to minimise/prevent formation of aerosols, plants or detached leaves will be drop inoculated to prevent aerosols, handling of infected plants will be minimised, infected plants will be watered from below; disposable gloves will be worn for all handling of infected plants, and all materials that come in contact with the infected plants will be destroyed by autoclaving.
It is not expected, or likely, that expression of gene encoding fluorescent proteins would alter the host range of R. secalis. Disruption of R. secalis pathogenicity genes will make it less or non-pathogenic. We expect to see that, following transformation, transformants will become fluorescent under specific light wavelengths. No increase in aggressiveness is expected, as these fluorescent tags have been used extensively in other fungal, oomycete, and bacterial plant pathogens without increasing the pathogenic capabilities of the pathogens. Indeed, the opposite effect of reduced aggressiveness is more likely as constitutive overexpression of a heterologous gene is likely to place a metabolic burden on the pathogens. It is also common for plant pathogens kept in culture (as would be the GMMs) to become attenuated in virulence or lose the ability to produce spores.

Complementation with the plasmid caring the disrupted gene can restore R. secalis pathogenicity to wild type level. Should a GMM expressing a pathogenicity gene escape, the possibility of its DNA being transferred to another environmental and non-pathogenic organism, or another pathogen, is remote. Pathogenesis in many pathogens is typically complex and probably employs many mechanisms. It is unlikely therefore, that the horizontal transfer of a single gene to a fungus or bacterium would confer novel pathogenicity upon a non-pathogen or increase host range of an existing pathogen.

To minimize risk of transmission, glasshouse work will be carried out under containment level 3, since the work involves barley seedlings infected with a GM fungal pathogen which produces spores on the leaf surface, and little is known about potential water-borne spread.

It has been previously shown (Pogue et al., 2002, Annu. Rev. Phytopathol. 40: 45-74; Bruun-Rasmussen et al., 2007, Mol. Plant Microbe Interact. 20: 1323-1331) that sequences inserted into BSMV vector are unstable as the virus replicates. That means passaging the recombinant virus more than once to another plant usually results in either complete loss of the heterologous sequence or its significant shortening. Therefore, accidental release of recombinant BSMV may cause disease on the initially infected plants. However, due to instability of the insert the hazard would be eliminated in any secondarily infected plants and the virus would be no more severe than wild type virus. BSMV infection is already widespread but does not cause serious agronomic problems even in susceptible cultivars (Jackson & Lane, 1981, Handbook of Plant Virus Infections and Comparative Diagnosis, ed. E Kurstak, pp. 565-625, Amsterdam: Elsevier).

Recombination of wild-type and genetically modified BSMV is possible, though the resulting recombinants are likely to be less virulent than the wild-type viruses. Recombination between BSMV and genomes of the other known Hordeiviruses, Anthoxanthum latent blanching virus (ALBV), Lychnis ringspot virus (LRSV), and Poa semilatent virus (PSLV), is theoretically possible. However, ALBV, LRSV and PSLV, are rare and they are not known to cause diseases of cultivated cereal crops. Therefore, a risk of gene transfer from BSMV to other Hordeiviruses is negligible.

The fungal strain to be used in these experiments is non-transgenic and already possesses all the genes this project aims to study. It very unlikely that this fungus would acquire genes from the virus as there is no known interaction between the two organisms. In the very unlikely scenario that the fungus were to acquire the viral encoded transgene as a second copy it might become more aggressive towards barley if the gene were to encode a strong virulence factor. Therefore no fungal material would be re-isolated alive from infected leaves.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Glasshouse benches resistant to acids etc: derogation applied for on the basis that the benches in the containment area are made of wire mesh for ease of cleaning and to enable temperatures to be maintained consistently throughout the facility. Fully waterproof receptacles to prevent any run off water from plant pots are included routinely in the SOPS for the facility. The floors of this area are also sealed and the drain capped. In the highly unlikely event of flooding the drains have an additional cap beneath the floor and if this should fail the default setting on the drainage system would direct the effluent to a holding tank where chemical disinfection could take place before release to mains drainage.

Containment Level 3 facilities sealable for fumigation: derogation applied for on the basis that fumigation for plant pathogens is not routine. Glasshouse cubicles within the facility will be disinfected by power-washing with a mixture of
Hortisept and Verritex. Hortisept is a horticultural disinectant that is effective against most fungi, viruses and bacteria, and which has been formulated for use in glasshouses and polytunnels. Verritex is a horticultural cleaning fluid that is used in conjunction with Hortisept to facilitate the removal of biofilms from glasshouse surfaces.

Containment level 3 facilities - HEPA filters: derogation applied for on the basis that R. secalis and R. collo-cygni are regarded as splash transmitted rather than aerosol transmitted. Spores are produced in a mucilage matrix and the outlet fans in the glasshouse cubicles are fitted with Primary Grade Panel Filters G4 which should retain these if they were to be dispersed.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any material including flasks or plates used for growth of cultures, inoculated plant material, plant pots, compost, labels, gloves etc. that might be contaminated by the GMOs will be autoclaved before disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee expressed concern that there may be a possibility of sporulation and questioned if the plasmids used are mobilisable. The proposer will be requested to clarify this on the Risk Assessment. The committee agreed that there was little potential hazard posed by expression of the fluorescent proteins in the pathogens. It was noted that the pathogens are spread on leaf surfaces by splash inoculation. Little is known about the possibility of water-borne transmission; roots of plants do become infected but it is not known if this happens directly or by spread of pathogen through the plant. It is possible that the pathogen could survive for short periods of time in run-off water but it is not known if it could be infective by that route. Questions were also raised about containment of fungus spores on the surface of plants grown to maturity in the glasshouse and it was felt that plants should be maintained in a cubicle under negative pressure. Containment level 3 was recommended for growth of plants to maturity due to the uncertainties over possible water-borne spread of the pathogens and to contain fungal spores present on the surfaces of plants.

Project Containment

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Project Ref 250/11.2
We are interested in whether specific host plant genes can influence susceptibility to disease. Consequently we intend to infect Nicotiana benthamiana transgenic plants which either over-express or have compromised expression of these genes with a variety of Pseudomonas strains. Pseudomonas syringae pv. Tomato DC3000 (Pst DC3000) is unable to replicate in Nicotiana benthamiana as it encodes HopQ1-1, a protein which activates hypersensitive cell death defence mechanisms in this plant. Subsequent deletion of HopQ1-1 in this P. syringae strain renders it virulent as the plant no longer mounts the same level of antimicrobial defence (however the plant may still initiate a basal defence mechanism which may slightly lower bacterial titres in planta). Pst genes such as HopAA1-2 and HopG1 play similar roles in plant defence modulation to HopQ1-1.

We would like to use Pst DC3000 and mutant strains in these Hop genes to identify the plant pathways which are important in hypersensitive and basal defence mechanisms. Furthermore, we would like to test the applicability of P. syringae mutants (e.g. HopQ1-1 and others) to act as 'effector – detector vectors'. These mutants could, for example, be used to express other pathogen effectors (e.g. Phytophthora infestans or Potato Cyst Nematode- PCN) in Solanaceae such as potato, tomato and Nicotiana species to study their effects on virulence and/or avirulence.

Candidate pathogen effectors will be selected based on transcription profiles and those specifically induced during infection will be prioritised. Furthermore, effectors that contain matches to Pfam domains or have shown to interact with important virulence targets will also be included.

In parallel, we also aim to exploit the genetic resource of Arabidopsis, in order to identify key genes involved in these defence pathways. Pst DC3000 is able to replicate and cause disease in Arabidopsis, however Pst DC3000 expressing AVRBS induces a plant defence response which limits infection. We aim to use several "on-site" Arabidopsis mutants and transgenics which have altered defence responses in order to gain a better understanding of the pathways involved in mediating resistance to Pseudomonas.
Origin & function

AVRB, an avirulence protein derived from pathovar glycinea (Staskawicz, B., et al, 1987, Journal of Bacteriology). Candidate pathogen effectors from P. infestans and potato cyst nematode selected based on transcription profiles and induced during infection including effectors that contain matches to Pfam domains or have shown to interact with important virulence targets.

Evaluation of foreseeable effects

Pseudomonas syringae pv. tomato DC3000 is not pathogenic to mammals but does cause bacterial speck diseases in tomato and Arabidopsis. The bacterium exists as an epiphyte and invades appropriate plant tissues via stomata or wounding. HopQ1-1 and HopAA1-2, HopG1 mutants of Pst have an increased host range that include Nicotiana benthamiana does not affect the disease state in tomato or Arabidopsis. It is currently unknown if other Solanaceae including potato and Nicotiana spp. are also infected by these mutant Pst lines but we would like to establish this.

Expression of AVRB in Pst DC3000 induces a necrotic response in several hosts, most notably Arabidopsis. This response severely restricts host range of this pathogen and, as such, is less hazardous to the environment.

No effects on human health are foreseen.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Glasshouse benches resistant to acids etc: derogation applied for on the basis that the benches in the containment area are made of wire mesh for ease of cleaning and to enable temperatures to be maintained consistently throughout the facility. Fully waterproof receptacles to prevent any run off water from plant pots are included routinely in the SOPs for the facility. The floors of this area are also sealed and the drain capped. In the highly unlikely event of flooding the drains have an additional cap beneath the floor and if this should fail the default setting on the drainage system would direct the effluent to a holding tank where chemical disinfection could take place before release to mains drainage.

Containment Level 3 facilities - sealable for fumigation: derogation applied for on the basis that fumigation for plant pathogens is not routine. Glasshouse cubicles within the facility will be disinfected by power-washing with a mixture of Hortisept and Verritek. Hortisept is a horticultural disinfectant that is effective against most fungi, viruses and bacteria, and which has been formulated for use in glasshouses and polytunnels. Verritek is a horticultural cleaning fluid that is used in conjunction with Hortisept to facilitate the removal of biofilms from glasshouse surfaces.

Containment level 3 facilities - HEPA filters: derogation applied for on the basis that P. syringae is seed- or splash-transmitted in conditions such as heavy rain or hail which cause crop damage, rather than aerosol transmitted.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any material including flasks or plates used for growth of cultures, inoculated plant material plant pots, compost, labels, gloves etc. that might be contaminated by the GMOs will be autoclaved before disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
This work may increase pathogenicity and the host range therefore glasshouse work should be done at containment level 3.

Labwork can be done at level 2 and some plant work (detached leaf assays, etc) could be done in the laboratory in sealed containers at CL2.

**Project Containment**

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**Project Ref** 250/13.1a

**Date Ackn'd** 30/09/2013

**CU2 Project Title** Derogations from the Contained Use requirements affecting several notifications

**Class** Consent Granted

**Non-GMM Consent Granted**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick if you confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Investigation of the function of plant proteins targeted by plant pathogen effectors through Tobacco Rattle Virus induced silencing

Purposes of the contained use

To promote disease many plant pathogens deliver sets of effectors inside their hosts. These effectors interact with host target proteins to modify their function to the benefit of the pathogen. We have identified plant proteins that putatively interact with Phytophthora infestans (potato late blight), Phytophthora capsici and Potato Cyst Nematode (PCN) effectors from Solanum tuberosum, Solanum lycopersicum and Nicotiana benthamiana. We aim to silence plant targets of pathogen effectors in N. benthamiana to examine their role in disease susceptibility and plant immune signalling.

Small, untranslatable, cDNA fragments (150-350bp), corresponding to selected host plant genes, will be cloned in anti-sense orientation in to a Tobacco Rattle Virus (TRV) – based vector to induce silencing in plants. The small fragments will be from N. benthamiana orthologues of genes encoding proteins known to interact with pathogen effectors or other proteins known to be involved in plant immunity. Host effector targets under examination for the role in plant immunity may also play a role in a range of plant processes.

Silencing of one target of the P. infestans effector Avr3a, the defence –associated E3 ligase CMPG1, has been shown to decrease the cell death triggered by some plant receptors and make N. benthamiana less susceptible to P. infestans. The effector Pi02860 interacts with two potato proteins, a 14-3-3 protein involved in protein stability and another E3 ligase (NRL). As with CMPG1, silencing of NRL has been shown to decrease the susceptibility of N. benthamiana to P. infestans. In contrast silencing of another E3 ligase, NbPUB17, which has a role in plant defence, has been shown to enhance P. infestans leaf colonization. Thus silencing of such effector targets can both decrease and increase plant susceptibility to the pathogen that produces the effector.
The effector PiAvr3a is known to interact with thirteen potato proteins including components of the plant exocyst pathway (Sec3 and Sec5) and the hypothetical protein Kipi30. PexRD2 is an RXLR effector that interacts with potato MAPKKK ε, a positive regulator of cell death. Pi17316 interacts with a different MAP3K, known as VIK1 (VH1-interacting kinase), which has role in vascular development in Arabidopsis. These and other effector targets will be silenced to investigate their function and the extent of their roles in cell death and defence.

Recipient or parental organism

Tobacco Rattle Virus occurs widely, including in the UK. It can infect more than 400 plant species and can cause diseases of crop plants including potato, tomato, sugar beet, tobacco and ornamentals. TRV causes spraing in potato, which makes tubers unmarketable for human consumption. Many common weed species are hosts of TRV and act as a reservoir of the virus in the field. TRV is spread naturally by root-feeding nematodes, but can be spread mechanically, by pollen and by seed. The genome consists of two positive-sense RNA molecules. The larger RNA 1, which is capable of autonomous replication, encodes genes required for replication and cell-to-cell movement. The smaller RNA 2 encodes the capsid protein required for transmission and other non-structural genes such as 2b, which is involved in nematode transmission. RNA 2 by itself is not infectious. A genetically modified UK strain will be used.

Host/vector system

The plant species Nicotiana benthamiana will be used as a host for silencing experiments. This species originates from tropical / sub-tropical regions of Australia. It is not found in the UK and is expected to be incapable of establishing in the external environment. Like its relative tobacco it contains toxic alkaloid compounds, e.g. nicotine, and is not consumed.

Agrobacterium tumefasciens-delivered, binary vectors containing TRV genome components have been used extensively over the last fifteen years to induce gene silencing in various TRV host plant species for basic plant science studies. Agrobacterium strains containing binary vectors with kanamycin selectable markers will be used in the proposed studies. The T-DNA portions of these binaries will contain a wild-type RNA1 cDNA or modified RNA2 cDNAs under the control of Cauliflower Mosaic Virus 35S promoters. The modified RNA2 forms will lack the non-structural genes involved in the natural nematode transmission mechanism and hence will be partially disabled. The TRV-based system that will be used in these studies has been shown to be incapable of establishing infections and silencing in potato, which is bred at the institute and grown commercially widely in the surrounding area.

Origin & function

Sequence fragments from Nicotiana benthamiana genes will be inserted in anti-sense orientation in to the RNA 2 component of the TRV-based system to induce silencing of endogenous N. benthamiana genes. The fragments will not be expressed in planta. The choice of genes to be silenced will be based on whether the proteins encoded by them, or orthologues from other plant species, interact with effector proteins from the above mentioned plant pathogens. The specific sequence choice will be guided by efficacy of silencing and avoidance of cross-silencing of other N. benthamiana genes and genes in other plant species. The genes that will be silenced are expected to be involved in plant immunity and responses to pathogens, but may also be involved in other plant processes. Publications in the literature show that silencing of such genes can result in both increased and decreased susceptibility of N. benthamiana to pathogen attack.

Evaluation of foreseeable effects

The disabled E. coli strains, disarmed Agrobacterium tumefasciens strains and Tobacco Rattle Virus that will be used in these studies are not pathogenic to humans and thus the risk to human health is minimal. Instead the greater foreseeable risk is to the environment. The GMOs are expected to induce silencing in N. benthamiana making it more or less susceptible to pathogen attack, however this species is not present in the external environment or of any economic importance. The most easily foreseeable detrimental effect is that the genetically modified TRV form could transiently induce silencing in other Solanaceous species with adverse consequences on pathogen susceptibility. Aside from potato, which is not infected or silenced by the TRV vector to be used in these studies, such species, e.g. tomato, are disperse in the external environment and of little economic importance lowering the risk. In the event of escape the genetically modified form of TRV would be as a competitive disadvantage relative to wild-type forms already present in the external environment as it is partially disabled through the removal of the genes involved in the natural nematode transmission route. However, there is a need to minimize dissemination to the external environment. Transmission by nematodes, as in nature, is precluded by the disablement and control of pathogens in the glasshouse; mechanical transmission of the virus will be controlled by use of gloves and dedicated labcoats; escape of pollen and seed will be controlled by prevention of flowering, though there are no plants in the external environment to receive pollen and this species is not adapted to the Scottish climate. In an imaginary scenario a nemtode transmissible form of the GMO might be reconstituted through recombination with wild-type viurs in the external...
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

As in previous notifications a derogation is requested for containment measure 3 in glasshouses, i.e. impervious and easily cleaned benching. The glasshouses at the institute have metal mesh benching to allow air circulation and facilitate temperature control. Instead, water-impervious, easily-disinfected, polypropylene trays / boxes will be used to allow disinfection and minimize run-off. Further, experiments will be carried out in glasshouse compartments in which the drains have been sealed. In addition, when the drains from these compartments are opened the water is collected in a holding tank which could be chemically disinfected prior to discharge to mains drainage.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Material contaminated with the GMMs (e.g. cultures, culture vessels, gloves, plasticware, plants, plant pots and compost) will be routinely autoclaved to give 100% kill. An exception to this is the plastic trays used in the glasshouse, which will be treated with 2% Virkon overnight to give a greater than six log reduction in viable Agrobacterium and also inactivate Tobacco Rattle Virus. In addition to thermo-metric testing of autoclaves, inactivation of the relatively thermo-sensitive E. coli, Agrobacterium tumefaciens and TRV will be monitored by the inclusion of Class 5 steam chemical integrator strips (121°C, 15 minutes) in all autoclave waste loads. Inactivated cultures will be discarded to mains drains and autoclaved plasticware etc sent for incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
In this submission the use of TRV-based vectors for studies of genes involved in resistance responses was proposed. The form of the vector used means that the GM virus is not transmissible by nematodes as found in nature. However, it is still mechanically transmissible, and transmissible by pollen and seed. The GMSC thought that silencing of endogenous N. benthamiana genes involved in resistance presented a negligible hazard for human health. The use of Agrobacterium for delivery gave rise to another possible escape route. However, escape down the drains was unlikely to bring the GMO in to contact with plants and the fact that the two components of the viral genome were separated between two bacterial strains made it extremely unlikely that an infection would be established in the external environment by this route. If the GM virus were to escape in to the external environment it might infect a wide range of plant species. The sequences inserted to induce silencing are derived from N. benthamiana. While the GM virus can efficiently induce silencing in this highly susceptible host, published data shows this vector would not be capable of inducing silencing in the field in the economically important crop of potatoes. The GMSC thought silencing in others members of the Solanaceae, e.g. tomato, could be possible in the event of escape and suggested that sequences used for silencing should be checked against tomato and potato genome databases to ascertain the likelihood of this. The GMSC recognized that removal of the genes involved in nematode transmissibility provided a level of biological containment and that such transmissibility was unlikely to be re-acquired in the event of an escape without the loss of the sequences responsible for inducing silencing. The GMSC’s opinion was that the GM viruses would be less consequential disease causing agents than the progenitor virus and expected inserted sequences to be lost during virus propagation. The BSO informed the GMSC that the use of such a TRV-based vector had previously been notified to the HSE as part of a wide-ranging Class 3 application and though some genes involved in resistance had been specified in this application there was not such a wide range and TRV had not been specified. Containment Level 2 is automatically required for work with GM pathogens and it was thought a new notification to the HSE should be made to cover this work. To control the risks to the environment the GMSC thought that the Containment Level 2 measures were suitable to reduce the risks to the environment to negligible with the additional control measure of plants being disbudded to prevent seed set and precluding this route as a means of escape. The GMSC provisionally approved the proposal at Containment Level 2. However, the GMSC thought that the proposal should be re-written to more lucidly present the case, there being excessive repetition of facts in multiple locations. The inclusion of a more appropriate reference for TRV vector instability was suggested. It was noted that work should not start until the HSE have been notified and acknowledged receipt of the notification. A revised version of the risk assessment was submitted to the GMSC and approved.

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### Project Ref 250/16.2

**Date Ackn'd**

21/10/2016

**CU2 Project Title**

Unravelling the contribution of the Agrobacterium tumefaciens Type 6 secretion system to microbiota assembly and function in planta.

**Class**

Class 2

**CultureVolClass2**

< 1 Litre

**CultureVolumeClass3-4**

Non-GMM

Consent Granted

Project notified under transitional arrangements N

02/03/2022
Purposes of the contained use

The overall aim of this project is to define the biological role of the Type 6 secretion system (T6SS) in the recruitment and functioning of the bacterial communities thriving at the root-soil interface, designated the rhizosphere microbiota. The T6SS is used widely throughout gram-negative bacterial species in injecting effector proteins and virulence factors (such as proteins, toxins, or enzymes) from across the interior (cytoplasm or cytosol) of a bacterial cell into a target cell. In Agrobacterium, the T6SS confers a competitive advantage over other bacteria during the colonisation of plant habitats (e.g., leaves; [Ma et al., 2014 Cell Host Microbe, 16:94]. In this project we will use available Agrobacterium tumefaciens strains impaired in the functioning of the T6SS and we will combine them with other non-pathogenic taxa to generate bacterial consortia of known composition. These consortia will be used to inoculate barley seedlings grown in autoclaved soil and monitor their impact on plant performance (e.g., by determining seedling dry weight fluctuations on inoculated specimens) and microbiota formation (e.g., by using next-generation sequencing approaches and colony count).

Recipient or parental organism

Agrobacterium tumefaciens is a widespread, including in the UK, gram-negative, non-spore forming, motile, rod-shaped, soil bacterium. It is the causal agent of crown gall disease in over 140 species of eudicots. Economically important disease is restricted to a limited number of horticultural species like perennial fruit, nut, ornamental and vine crops. Barley is a non-host for this pathogen and studies indicated that A. tumefacies can be retrieved from the rhizosphere microbiota of ‘symptomless’ barley plants. The strain that will be used originates from the USA and as a non-indigenous plant pathogen is held under licence from Scottish Government.

Host/vector system

pJQ200SK. This vector was used to induce mutations in components of the T6SS. This is a suicide vector permitting gene replacement and mobilization into a wide range of Gram negative bacteria (Quandt and Hynes, 1993, Gene 127:15). These mutants were constructed at Academia Sinica, Taipei, Taiwan, by creating markerless in-frame deletion mutant instead of inserting any antibiotics resistance gene by double homologous recombination in all the tssL deletion mutants. Therefore, backbone plasmid sequences will not be left on the mutant strain (Prof Erh-Min Lai, Academia Sinica, Taipei, Taiwan, personal communication).

Origin & function

The DNA inserted during the construction of the GMOs caused a site directed deletion in certain components of the T6SS. In the final constructs that have been obtained from the external source there is no residual foreign sequence, merely a deletion of native sequence and this has been confirmed by sequencing.

Evaluation of foreseeable effects

The resulting GM A. tumefaciens is not disarmed and still has the potential to be pathogenic to plants. Unmodified Agrobacterium tumefasciens is commonly occurring in the external environment. Although the GMO still has the potential to be pathogenic to plants, it is impaired in a biological function that is required to successfully compete with other bacteria in soil. Therefore, their persistence in the natural environment is likely reduced (Ma et al., 2014 Cell Host Microbe, 16:94). The genetic modifications made are deletions in the bacterial chromosome and thus are expected to be stable. Although, crop species that the GMOs could cause disease in are not grown commercially in the vicinity, the strain of Agrobacterium tumefaciens used to generate them is not indigenous and held under licence. Therefore, there is a need to control dissemination of the progenitor organism and its GM derivatives to the environment. Possible routes of escape are known and managed.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

It is intended that experiments will be carried out in plant growth chambers, plant growthrooms and possibly in glasshouses. These are not fitted with impervious, easily-cleaned benching, e.g. the glasshouses at the institute have metal mesh benching to allow air circulation and facilitate temperature control. Therefore, as in previous notifications a derogation is requested for containment measure 3, i.e. impervious and easily cleaned benching. Instead plant pots will be placed in water-impervious, easily-disinfected, polypropylene trays / boxes to minimize run-off and allow disinfection. In the case of growth cabinets, any water escaping from these trays will be collected for inactivation. In the case of growth rooms, the trays will be placed on steel mesh above drip trays so that any escaping water can be inactivated and the growth room drains will be sealed. If glasshouse compartments are used the plastic trays will be placed on absorbent matting that can be autoclaved at the end of experiments. Further, experiments would be carried out in glasshouse compartments in which the drains have been sealed. In addition, when the drains from these compartments are opened the water is collected in a holding tank which could be chemically disinfected prior to discharge to mains drainage.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Material contaminated with the GMOs (e.g. cultures, culture vessels, gloves, plasticware, plants, plant pots and compost) will be routinely autoclaved to give 100% kill. An exception to this is the plastic trays used in the glasshouse, which will be treated with 2% Virkon overnight to give a greater than six log reduction in viable Agrobacterium. In addition to thermo-metric testing of autoclaves, inactivation of the relatively thermo-sensitive Agrobacterium tumefasciens will be monitored by the inclusion of Class 5 steam chemical integrator strips (121°C, 15 minutes) in all autoclave waste loads. Inactivated cultures will be discarded to mains drains and autoclaved plasticware etc sent for incineration. Equipment for the preparation of root specimens for downstream analysis will be autoclaved or decontaminated in Virkon before being re-used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The type 4 secretion system is intact in the Agrobacteria and thus they have the capacity to be pathogenic. However, the literature indicates that mutations in the type 6 secretion system make Agrobacterium competitively less fit relative to other microbiota in the rhizosphere. This will be tested under controlled conditions with a non-host plant, barley. The method used to generate the GM bacteria means there is no residual foreign inserted sequence, only deletions of native sequence. Experiments will be carried out in a dedicated growth cabinet that is being purchased. The GMSC expected that the GM bacteria would be less fit than the progenitor, non-disarmed organism, but this was an exotic, pathogenic strain requiring a Plant Health Licence. It was thought that run-off could be easily controlled in a plant growth cabinet to avoid exposure of the environment to this non-indigenous strain. Agrobacterium is not pathogenic to humans so the hazard to human health is negligible and instead the greater hazard is to the environment. The GMSC thought that Containment Level 1 measures in the laboratory and Containment Level 2 measures for the plant growth cabinet were appropriate to control the hazard to the environment. It was thought that the risk assessment should be amended to indicate that run-off will be collected for inactivation and that the cabinet will be disinfected with Virkon after use. As this is novel work a notification will have to be submitted to the HSE and work cannot start until they have acknowledged receipt of this. Should indicate in risk assessment that a MSC is not required and request a derogation from requirement for solid benching as not included in cabinet.

Project Containment
# Project Ref: 250/20.1

**Date Ackn'd**: 25/03/2020

**CU2 Project Title**: Isolation of antibiotic resistance genes using green-florescent protein (GFP)-expressing Pseudomonas putida and Escherichia coli

**Class**: Class 2

**CultureVol**: < 1 Litre

**CultureVolumeClass**: Class 2

**Non-GMM Consent Granted**: Yes

**Project notified under transitional arrangements**: No

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

## Project Additional Information

**Purposes of the contained use**

The purpose of the research is to screen soil and in-stream water samples from rural catchment areas for the presence of antibiotic resistance genes in bacteria from within these environments and subsequently characterise these genes using selection (growth) in the presence of a range of antibiotics and PCR. Genes which are present on mobile genetic elements, such as those conferring antibiotic resistance, can spread from one species of bacteria to other, unrelated bacteria using natural horizontal gene transfer mechanisms, such as conjugation. This can result in the recipient strain acquiring new traits such as resistance to antibiotics. Only isolates expressing the GFP and showing antibiotic resistances will be characterised.

**Recipient or parental organism**

Non-pathogenic GM strains of Pseudomonas putida and E.coli engineered to express GFP will be used: P. putida
strain KT2442 (GFP+, kanR, rifR) and Escherichia coli strain CV601 (GFP+, kanR, rifR). Strain KT2442 is a spontaneous rifampicin resistant mutant of P. putida strain KT2440, which was originally an environmental isolate, that promotes plant growth and is used as a biocontrol agent for plant pathogens. It is not considered pathogenic for humans (ATCC 47054, BSL1) and was the first Gram-negative soil bacterium to be certified as a safety strain by the Recombinant DNA Advisory Committee. E. coli CV601 is a disabled (Thr-, Leu-, Thi-) E.coli K-12 derivative which does not possess the toxin gene(s) and is auxotrophic.

### Host/vector system

The intention is to allow bi-parental mating on 2-5 cm filters using natural process such as conjugation to transfer natural genetic elements, from environmental soil/water bacteria to the GM, GFP-expressing recipient strains. The genes for GFP and antibiotic resistance (kanamycin, rifampicin) are chromosomal and are not transferred.

### Origin & function

The origin of the (new) inserted genetic material is from the natural bacterial flora present within the soil/water environment. Selection will be for a range of antibiotic resistances on growth media and expression of GFP.

### Evaluation of foreseeable effects

The GM recipient strains present a minimal hazard to human health or the environment. Transformed derivatives of the GMMs that have gained additional antibiotic resistance genes may grow on media containing the antibiotics and will express GFP. The acquisition of additional antibiotic resistance genes is not predicted to affect the pathogenicity of the recipient, GM strains and would impose a genetic load, but could reduce treatability. In theory, other genes present on mobile elements, such as toxins, might also be transferred to the recipients. The acquisition of toxin genes is not predicted to make the recipient GM strains pathogenic, but could increase the hazard they present if toxins are expressed. However, toxin genes are normally chromosomal and the likelihood of co-acquisition of plasmids carrying toxin genes along with plasmids carrying antibiotic resistance genes is considered very low as this has not been noted in previous research. Thus, this is not an expected hazard, but in theory could occur.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogations required

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid culture and solid waste will be autoclaved (123°C/60 minutes) to give 100% kill prior to disposal. Autoclaved liquid waste will be disposed to drain. After autoclaving solid waste will be sent for commercial incineration. Monitoring of the effectiveness of the autoclaving of the liquid waste will be done by subculturing and Class 5 steam chemical integrator strips (123°C, 60 minutes) will be included in all autoclave waste loads to monitor autoclave processes.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form
The GMSC noted that Containment Level 2 measures were required on account of microbiological work with environmental samples, rather than on account of the genetic modifications. The GMSC did not perceive a risk to human health or the environment from the genetically modified recipient strains and thought they should be classified as Class 1. The GMSC noted that only the environmental bacteria with the antibiotic resistances selected for would be propagated to the level of single colonies and only trans-conjugants derived from the HG1 GMMs would be propagated beyond this. The GMSC thought that if the HG1 GMMs acquired toxin genes that this would not convert them into pathogens, but would increase the hazard. The GMSC thought that the probability of obtaining transconjugants with toxin genes was probably very low, but could not be excluded. The GMSC thought that, although it might be argued that the proposed work could be classified as Class 1, as Containment Level 2 was required for work with the environmental, non-GM, bacteria the proposed work should as a precaution be classified as Class 2 and notified to the HSE.

Project Containment

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<th>Growth Rooms</th>
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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 250/22.1

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Withdrawn N Tick if notifying a connected programme of work N

Historical Significant Changes
The purpose of the contained use is to test the utility of the Tobacco Rattle Virus (TRV) - based system described in a paper by Ellison et al. (Multiplexed heritable gene editing using RNA viruses and mobile single guide RNAs. Nature Plants 6, 620–624 (2020). https://doi.org/10.1038/s41477-020-0670-y) for Virus Induced Gene Editing (VIGE) in Nicotiana benthamiana and Solanum tuberosum plants carrying a Cas9 nuclease transgene with the purpose of generating CRISPR/Cas9 genome edited progeny plants. Initial studies will be aimed at editing phytoene desaturase (PDS) genes, commonly used as a visible reporter, in N. benthamiana and potato. If these experiments are successful the objective would be to extend the use of the TRV-based system to edit other genes of interest through the use of transgenic plants of these species as a research tool for analysis of plant gene functions. If successful this system could allow faster, targeted, precise knock-out or modification of plant genes of interest than is currently possible.

The plant species Nicotiana benthamiana and Solanum tuberosum are not native to the United Kingdom. The former originates from drier sub-tropical/tropical regions and is probably incapable of establishing in the UK. It is a species widely used in experimental plant research. Potato is a commercial crop widely grown in the UK. Its cultivation is achieved through planting of tubers rather than through the use of true seed. It is unlikely to be able of establishing outside cultivation. Both species contain toxic compounds, but toxic parts of the plants are not consumed.

Tobacco Rattle Virus is distributed around the world and is present in the UK. It can infect more than 400 plant species and can cause diseases of crop plants that include potato, tomato, beets, tobacco and ornamentals. TRV causes spraying in potato, which makes tubers unmarketable for human consumption. TRV is spread naturally by root-feeding nematodes, but can be spread mechanically and by seed. The genome consists of two positive-sense RNA molecules. The larger RNA 1, which is capable of autonomous replication, encodes genes required for replication and cell-to-cell movement. The smaller RNA 2 encodes the capsid protein required for transmission and other non-structural genes such as 2b, which is required for nematode transmission. RNA 2 by itself is not infectious. Plant virus vectors based on TRV have been used extensively over the last 25 years as tools in plant research, in particular in gene silencing studies.

N. benthamiana and S. tuberosum plants carrying a Cas9 nuclease transgene will be produced by stable transformation using disarmed Agrobacterium tumefasciens. Transformations will be accomplished with a standard binary vector in which the Cas9 gene will be under the control of Cauliflower Mosaic Virus (CaMV) promoter and Nopaline Synthase (NOS) terminator sequences, with antibiotic resistance genes for kanamycin selection in bacteria and plants.
Plants infections by the genetically modified TRV-based vector will be initiated by agro-infiltration with disarmed Agrobacterium tumefaciens transformed with binary vectors containing segments of the viral genome under the control of CaMV and NOS promoter and terminator sequences for transient transcription in plant cells. These binaries will also include antibiotic resistance genes for selection in bacteria, but not markers that can be selected for in plants. The two components of the viral genome, RNA 1 and RNA 2, will be provided by separate binary vectors transformed in to separate A. tumefaciens bacteria that will be mixed prior to agro-infiltration of plant leaf tissue.

**Origin & function**

The cDNA for the RNA 2 of TRV RNA 2 has previously been modified by removal of the 2b and 2c genes, the former of which is required for nematode transmission, to disable the virus in nematode transmission. Further, it has been previously modified to include a subgenomic promoter sequence from a related tobravirus to direct transcription of inserted sequences in planta.

In this case a synthetic single guide RNA (sgRNA) will be placed under the control of the subgenomic promoter to direct gene editing in Cas9 transgenic plants. The sgRNA comprises a short, 20 nucleotide, guide RNA that directs the Cas9 nuclease to the specific target for gene editing and a tracrRNA that provides a scaffold for binding of the Cas9 nuclease. The short guide RNA will in the first instance be specific for PDS genes.

The new TRV-based vectors also include plant derived sequences to increase the efficiency of gene editing by the virus in Cas9 transgenic plants. The plant derived sequences are from the Arabidopsis thaliana Flowering Locus T (FT) open reading frame or a tRNA for isoleucine sequence. FT is a phloem mobile signal molecule that promotes the transition to flowering, while the isoleucine tRNAs has a central role in protein synthesis. Both of these sequences have been identified as phloem mobile sequences.

**Evaluation of foreseeable effects**

The introduction of the Cas9 gene into N. benthamiana and S. tuberosum is not of any perceived benefit to the transgenic plants and will not make them weedier. The Cas9 protein is not in itself toxic or allergenic. Hence, the transgenic plants are not perceived to prevent a greater risk to human health or the environment than the progenitor plants.

The inclusion of the single guide RNA sequence in the TRV-based vector will lead to gene editing of the target gene, but the length of the plant gene specific sequence is such that it should not lead to gene silencing. Thus this sequence should only have an effect on transgenic plants. Editing of the PDS gene is expected to be detrimental to the host transgenic plant causing bleaching and possibly dwarfing. In the case of PDS gene edited progeny seed are not expected to give viable plants. Virus induced gene editing of other target genes would be likewise limited to Cas9 transgenic plants, but depending on the target might result in progeny plants that are fitter than the progenitor wild-type plant and such larger GMOs would need to be separately risk assessed.

The inclusion of the A. thaliana FT sequence in the TRV-based vector was reported not to cause silencing in N. benthamiana, but its inclusion might be expected to result in silencing of FT in host plants in the Brassicaceae. This would delay floral development and be detrimental to infected / silenced plants.

The inclusion of the FT and tRNA mobile sequence elements in the TRV-based vector has been shown to enhance genome editing in Cas9 transgenic plants. However, the inclusion of these elements has also been shown to enhance systemic spread / accumulation of the virus vector over the progenitor vector. This change could increase the mechanical transmissibility of the new virus vector and it is not known how it compares with natural, wild-type virus. However, the new virus vector is still disabled in nematode transmission, the most important natural route of transmission, due to the deletion of the 2b gene. A recombination mechanism by which the 2b gene and nematode transmissibility could be reacquired without loss of the foreign, introduced sequence is not apparent. Previously TRV-based vectors have been found to be unstable with introduced sequences being lost through recombination. Whether this will be true for the mobile RNA sequence elements is not known, as their retention may have a selective advantage for the virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The larger GMOs, i.e. the N. benthamiana and S. tuberosum plants with Cas9 transgenes and any derived progeny, will be used in combination with the Class 2 GMMs. Therefore, the same containment measures required for containment of the GMMs will be used for containment of the LGMOs.
As in previous notifications a derogation is requested for containment measure 3 in glasshouses, i.e. impervious and easily cleaned benching. The glasshouses at the institute have metal mesh benching to allow air circulation and facilitate temperature control. Instead water-impervious, easily-disinfected, polypropylene trays / boxes will be used to allow disinfection and minimize run-off. These trays will be placed on mesh benches that have been covered with plastic sheet and absorbent matting that can be autoclaved after use. Further, experiments will be carried out in glasshouse compartments in which the drain covers have been sealed. In addition, when the drains from these compartments are opened the water is collected in a holding tank which could be chemically disinfected prior to discharge to mains drainage.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Material contaminated with the GMMs and LGMOs (e.g. cultures, culture vessels, gloves, plasticware, plant pots, compost and plants) will be routinely autoclaved to give 100% kill. An exception to this is the plastic trays used in the glasshouse, which will be treated with 2% Virkon overnight to give a greater than six log reduction in viable Agrobacterium and also inactivate Tobacco Rattle Virus. In addition to thermo-metric testing of autoclaves, inactivation of the relatively thermo-sensitive E. coli, Agrobacterium tumefasciens and TRV will be monitored by the inclusion of Class 5 steam chemical integrator strips (121°C, 15 minutes) in all autoclave waste loads. Inactivated cultures will be discarded to mains drains and autoclaved plasticware etc sent for incineration.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The GMSC did not perceive a risk to human health in the proposed experiments. The GMSC thought that, while the use of TRV to deliver sgRNAs for gene editing was novel and differed significantly from the use of TRV for VIGS, this was little risk to the environment as gene editing could only occur in transgenic plants carrying a Cas 9 transgene. While the inclusion of A. thaliana FT sequences had not affected flowering in N. benthamiana its inclusion in the vectors might induce VIGS and have detrimental effects on flowering in other host species. That the inclusion of the mobile plant RNA sequences in the new TRV based vectors increased systemic spread / accumulation over the progenitor vector was of concern to the GMSC as this was an increase in virulence that made the vector more mechanically transmissible. It was not clear how the virulence of the modified vector compared to wild-type virus found in the external environment, but the GMSC did not see that this change could restore nematode transmissibility to the disabled vector. The GMSC thought that considering earlier publications it was quite unlikely, but not impossible, that the new TRV-based vectors would be able to infect the economically important potato crop. The GMSC thought that escape should be prevented to preclude the possibility of possible environmental harm. The establishment of infections in the environment through water-borne escape of Agrobacterium was considered highly unlikely due to the separation of the two viral genome components between different bacteria, but possible glasshouse run-off to drains needed to be controlled. The GMSC thought that the proposed containment measures were suitable to control the possible risks to the environment and approved the proposal at Class 2. Because of the novelty of the work and perceived possible increase in virulence the GMSC thought this had to be notified to the HSE.

The GMSC indicated that the sponsor needed to clarify what laboratory would be used to do the work as those in AF were not suitable and would need to import viral clones and possibly GM seed under a Plant Health License.

Project Containment
Project Ref 250/94.1

Date Ackn'd 30/08/1994

Date Project Ceased 04/05/2018

CU2 Project Title Elucidation of the factors regulating colonisation of soil aggregates.

Class 2

Non-GMM not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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**Project Ref**  250/95.1

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 250/96.1

**CU2 Project Title**

**ANALYSIS OF POTATO VIRUS X (PVX) FOR PRODUCTION OF ANTIGENIC PEPTIDES IN PLANTS AND DELIMITATION OF PVX VECTOR FOR PROTEIN PRODUCTION IN PLANTS**

**Date Ackn’d** 08/05/1996

**Class** Class 2

**Culture Vol Class 2**

**Consent Granted** not applicable

**Project notified under transitional arrangements** Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
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### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Project Ref 250/98.2

Date Ackn’d 08/12/2000

CU2 Project Title TRANSFORMATION OF PLANT PATHOGENIC AND SOIL BOURNE BACTERIA

Class 2

CultureVolClass2 < 1 litre

CultureVolumeClass3-4
WITH GFP FROM A VICTORIA AND USE OF TRANSFORMANTS TO STUDY INFECTIONS IN PLANTS AND POPULATION DYNAMICS AND TRANSPORT IN SOILS

Date Project Ceased
04/05/2018

Tick if notifying a connected programme of work
N

Project notified under transitional arrangements
Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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 Withdrawn N

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022  
Page 5748 of 15326
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**Name**

NATIONAL INSTITUTE FOR BIOLOGICAL STANDARDS & CONTROL

**Campus Estate or Research Centre**

**Name 2**

**Department**

**Road Name**

BLANCHE LANE

**District**

SOUTH MIMMS

**Town**

POTTERS BAR

**County**

HERTFORDSHIRE

**Postcode**

EN6 3QG

**Country**

ENGLAND

**Tel Number**

01707 641000

**Fax Number**

01707 646730

**E-mail**

LONDON

**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Significant Change

251/05.3
251/05.5
251/05.4

Date of Additional Information (significant change only)

23/11/2005
23/11/2005
23/11/2005

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<td>310 CAMBRIDGE SCIENCE PARK</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The established safety committee of the company will also take on the role of GM safety. Meetings are currently held once a month.
The GMSC subgroup will consist of: Chief Operations Officer (Chairman), Quality, Health and Safety and Environment Officer, Biological Safety Officer and research scientist, Safety Officer and research scientist (with relevant experience), Facilities Manager.

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Level 1 (GMMs)  Yes

Level 2 (GMMs)
**For activities involving GMMs, describe the waste management measures which will apply to the activity**

The GMM will only be cultured on a small scale (10-200ml maximum). Work areas will be disinfected after every use with Virkon. Any spill will be treated with disinfection (Virkon) according to manufacturers’ instructions immediately after spillage. All waste, solid and liquid, will be autoclaved at 121 degrees C, 15 psi for 20 minutes and then disposed by normal routes. The normal functioning of the autoclave will be monitored to estimate the “degree of kill” on a regular basis (sterilisation indicator strips/temperature recorder). External contractors (SW Scientific) service the autoclave annually.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All members of the GMSC have seen the risk assessment and agree with the provisional classification (Class 1)
**CONSTRUCTION OF ISOGENIC MENINGOCOCCAL STRAIN PANELS**

**Date Ackn'd:** 24/05/2000

<table>
<thead>
<tr>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<td>CONSTRUCTION OF ISOGENIC MENINGOCOCCAL STRAIN PANELS</td>
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**Non-GMM Consent Granted:** not applicable

**Project notified under transitional arrangements:** Y

**Withdrawn:** N

**Tick if notifying a connected programme of work:** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 251/00.2

Date Ackn'd: 21/07/2000
CU2 Project Title: EXPRESSION OF HIV/SIV PROTEINS USING RECOMBINANT VACCINIA VIRUSES CONTAINING HIV/SIV SUBGENOMIC FRAGMENTS

Class: Class 2
CultureVolClass2: not applicable
CultureVolumeClass3-4: not applicable

Non-GMM: not applicable
Consent Granted: not applicable

Withdrawn: N
Tick if notifying a connected programme of work: N
Project notified under transitional arrangements: Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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02/03/2022
Project Ref 251/00.3

Date Ackn'd 20/02/2001

CU2 Project Title SITE-DIRECTED MUTAGENESIS OF CLONED POLIOVIRUS CDNA

Date Project Ceased

Class 2

Culture Volume

Class Culture Vol Class 2 Culture Volume Class 3-4

< 1 litre

Non-GMM not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 251/01.1

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<td>19/04/2001</td>
<td>ATTENUATED SALMONELLA TYPHIMURIUM TRANSFECTED WITH DNA</td>
<td>Class 2</td>
<td>1-50 litres</td>
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PLASMIDS CONTAINING MYCOBACTERIAL AND CYTOKINE GENES

The aim of this project is to clone a number of DNA constructs expressing mycobacterial antigens and cytokine genes into attenuated Salmonella typhimurium for use in immunogenicity studies. The constructs have already been used in other related studies within the laboratory. Initially the mycobacterial antigens to be tested will be ESAT-6, 65Kda antigen and MPT-83; cytokine genes of interest will initially be Interleukin 12 (IL-12) and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF). This study may be expanded in the future as other DNA constructs become available.

Bacteria to be used will be the attenuated (aro) strain of Salmonella typhimurium. This bacteria has a defined mutation in the prechorismate biosynthetic pathway and is therefore unable to generate aromatic amino acids. This strain has been used extensively as an experimental tool to express and deliver host proteins.

The host will be Salmonella typhimurium as described above. The genetic material is documented below.

The genetic material used in this study will be DNA constructs containing genes for cytokines and mycobacterial antigens. These constructs have been generated for use in DNA vaccination protocols. Initially the cytokines of interest will be GM-CSF and IL-12 and the mycobacterial antigens will be ESAT-6, 65Kda antigen and MPT-83. These constructs have been previous used in other related studies in the laboratory in other protocols and will be used in immunogenicity studies within the laboratory.

Due to its defined mutation, the bacteria pose little risk to humans (due to its limited survival time in the mammalian host) or to the environment.

The mycobacterial antigens cloned into a DNA vaccine construct have no biological activity, are neither enzymes or toxins and do not change the behaviour of the bacteria.

The use of cytokines is assumed to be safe due to the inability of the bacterial to survive in a mammalian host.
The DNA vectors to be used in this study are considered to be non-mobilisable and gene transfer is therefore highly unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full level 2 containment will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be disposed of by autoclaving followed by incineration. Validation and control of waste management will be held on file at NIBSC. Hycolin will be used to disinfect surfaces.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered that the risk assessment and the level of containment were appropriate for the work.

Project Containment

<table>
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Project Ref 251/01.2

Date Ackn'd 19/04/2001
CU2 Project Title REVERSE GENETICS OF MUMPS VIRUS
Class 2, CultureVolClass2 < 1 litre
CultureVolumeClass3-4
Project Additional Information

**Purposes of the contained use**

The main objective of the project is to produce mumps mutant viruses using the rescue virus approach for confirming the structure-function correlation, proposed previously, to understand the mechanisms of virulence, attenuation, persistence and pathogenicity. It is also vital to study mumps mutants so that strict quality control measures can be implemented effectively to some brands of mumps vaccines that otherwise do not have satisfactory safety and efficacy records.

**Recipient or parental organism**

Continuous cell lines of primate sources eg HeLa, Vero, Hep-2, 293 cells, etc.

**Host/vector system**

E. coli K-12 strains, mumps recombinant plasmids, pCITE type, containing genomic or sub-genomic length DNA fragments under T7 RNA polymerase promoter, a disabled vaccinia virus vector (MVA-T7), cell lines of primate sources eg HeLa, Vero, Hep-2, 293 cells, etc.

**Origin & function**

The genetic materials that will be manipulated in the proposed study are originated from the disabled vaccinia virus vector (MVA-T7) and mumps virus vaccine strain. The intend function of MVA-T7 vector system is to supply T7 RNA polymerase enzyme, in vitro, for the transcription of gene inserts encoding for the synthesis of nucleocapsid, phospho and the large proteins of mumps virus and additionally to provide copies of the full length RNA template for in vitro encapsidation and replication. The overall objective of the genetic manipulations that will be carried out under this project is to produce and supply essential components of the virion in vitro for the production of mutant viruses with known genetic finger prints.

**Evaluation of foreseeable effects**

A full risk assessment has been carried out for the proposed project of reverse genetics of mumps virus (copy enclosed). The areas covered under the risk assessment are: human and environmental hazards associated with the recipients micro-organism, hazards arising directly from the insert gene product and the vector system(s), hazards arising from the alteration of existing pathogenic traits including host range, tissue tropism and the likelihood of causing harm to human health in case of accidental exposure.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Class 2 containment applied throughout.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated plastics and culture fluids, etc, will be disinfected overnight in 10% Chloros, the solid waste then will be autoclaved and incinerated while the liquid waste will be discarded in a designated sink. Validation and details of control waste management is held of file at NIBSC.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The GSNC considered that the risk assessment and the proposed containment measures were appropriate for the work.

**Project Containment**

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**Project Ref** 251/01.3

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Date Project Ceased

02/03/2022
The aim of this project is to introduce a plasmid for the direct expression of green fluorescence protein (GFP) into Bordetella pertussis that will facilitate the study of phagocytosis and related events particularly relevant to investigate the role of adenylate cyclase toxin (ACT) in pertussis infection and immunogenicity. The labelling of live Bordetella pertussis with GFP permits the bacteria to be easily imaged and quantified and will enable us to measure phagocytosis by techniques such as spectroscopy, microscopy or FACS. Further, GFP expressing B. pertussis can be used to study bacterial colonisation in vivo.

The bacteria to be used in this study will be the wild type challenge strain Bordetella pertussis 18323. Bordetella pertussis is a containment level 2 pathogen. It is an obligate human pathogen that colonises the respiratory tract and therefore is handle in a class II EPC. This strain of Bordetella has been used extensively within the laboratory and related risk assessments for the preparation and handling of B. pertussis are well defined and included in different SOPs.

The host will be the strain Bordetella pertussis 18323 as described above. The genetic material is documented below.

GFP is a well characterised protein from the jellyfish Aequorea Victoria that produces a fluorescent product when expressed in prokaryotic or eukaryotic cells. GFP has been used as a genetic reporter system offering advantages over some other reporters and also as a fluorescent marker with potential use to study bacterial pathogenicity. Different GFP expression vectors have been constructed with such purposes suitable for different bacterial species. The here proposed plasmid constructs for the direct high expression of GFP from a constitutive B pertussis promoter, have been already used in Bordetella pertussis by others for similar studies. Moreover, the genetic modifications proposed have been reported not to mask the activity of adenylate cyclase toxin, or affect bacterial growth or other gene expression, in contrast with other labelling methods. Hence, its interest to study host-parasite interactions. The plasmids also carry an antibiotic resistance marker for easy screening of the colonies.

Neither the protein nor the genes to be expressed have associated specific hazards.

We intend to introduce plasmid constructs for the expression of GFP into B. pertussis to study phagocytosis and related events in pertussis infection and immunogenicity. The GFP is expressed as a cytoplasmatic protein and has no other known function than to emit green fluorescence when irradiated with light of the appropriate wavelength, thus it is very unlikely to change the behaviour and pathogenicity of the bacteria. No increased risk to human health has been described in the large number of publications available to date. Therefore we regard the risk of infection with this modified strain as no greater than the wild-type Bordetella strain.

B. pertussis appears to be adapted exclusively to the human respiratory tract and has no other known environmental or animal reservoirs. The modified GFP expressing B.
pertussis is very unlikely to change this. Thus, neither the parental strain nor the GMO would represent a hazard to the environment. The environment risk can be judged as in the same level as other class 2 biological agents.

The GFP expressing vectors to be used in this study will need to be introduced in B. pertussis challenge strain by electroporation. The very unlikely event of the plasmid transferring between bacteria unintentionally, it is improbable that it would increase the virulence of the recipient micro-organism due to the mentioned characteristics of the GFP vector.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full level 2 containment will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be disposed of by autoclaving followed by incineration. Chemical disinfection with chloros-derivates will be used for FACS waste decontamination before disposal. Validation and control of waste management will be held on file at NIBSC. Hycolin will be used to disinfect surfaces.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered that the risk assessment and the level of containment were appropriate for the work.

Project Containment

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</table>

02/03/2022
The aim of this project is to use different Bordetella pertussis mutant, which have been genetically modified to block the expression of specific virulence factors, to study the effect of antigenic variation on immunity to B. pertussis. This will permit the study the role of individual antigens in pertussis infection and immunogenicity, through the use of isogenic strains that only differ from each other in their ability to express particular antigens.

The bacteria to be used in this study will be the wild type strains Bordetella pertussis Tohoma and Wellcome 28. These strains are used for studies of B. pertussis infection. B. pertussis is an obligate human pathogen that colonises the respiratory tract. It is a containment level 2 pathogen and is handled in a class II MSC to reduce the risk of laboratory acquired infection from aerosols. These strains of Bordetella has been used extensively within the laboratory and relevant risk assessments for the preparation and handling of B. pertussis are attached. The details of their construction are described in references 5-17 in the Risk Assessment (attached).

The host will be the strain Bordetella pertussis Tohoma and Wellcome 28 as described above.

The Bordetella pertussis strains to be used are deletion mutants that fail to express of one or more of the following virulence factors: pertussis toxin, pertactin, filamentous haemagglutinin, adenylate cyclase, haemolysin, fims virulence gene promoter and LPS. These strains are gifts from other laboratories. They are deletion mutants devoid of one or more of the virulence factors. The genetic modifications carried out lead to reduced expression of the virulence factors. None of the strains have associated specific hazards.
We intend to use these strains to study the role of individual antigen in pertussis infection and immunogenicity. As the strains lack one or more virulence factors, it is very unlikely that they represent an increase risk to human. Therefore we regard the risk of infection with these modified strains as no greater than the wild-type Bordetella strain. B pertussis appears to be adapted exclusively to the human respiratory tract and has no other known environmental or animal reservoirs. There is no reason to expect the modified B. pertussis strains to pose an increased risk to the environment or other animal species.

Evaluation of foreseeable effects

We intend to use these strains to study the role of individual antigen in pertussis infection and immunogenicity. As the strains lack one or more virulence factors, it is very unlikely that they represent an increase risk to human. Therefore we regard the risk of infection with these modified strains as no greater than the wild-type Bordetella strain. B pertussis appears to be adapted exclusively to the human respiratory tract and has no other known environmental or animal reservoirs. There is no reason to expect the modified B. pertussis strains to pose an increased risk to the environment or other animal species.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full level 2 containment will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be disposed of by autoclaving followed by incineration. Validation and control of waste management will be held on file at NIBSC. Hycolin will be used to disinfect surfaces.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered that the risk assessment and the level of containment were appropriate for the work.

Project Containment

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

This project involves the development and use of genetically modified viral vectors based on Retroviruses (MLV), Lentiviruses (HIV-1), Adenoviruses (AdV), Adeno-associated viruses (AAV) and Herpes simplex viruses (HSV). This research is aimed towards the identification of reference reagents and biological assays to standardise viral vectors for proposed use in clinical trials for the gene therapy of human diseases. This work is an important activity that will contribute to the quality, efficacy and safety assessments of gene transfer products.

**Recipient or parental organism**

Cells of primate sources, eg 293, TE671, HUVEC

**Host/vector system**

Attenuated MLV vector (MLV-A), Lentiviral vector (SIN), AdV and AAV vectors containing a reporter gene, eg eGFP, laz or puro under CMV promoter.

**Origin & function**

The genetic materials that will be manipulated in the proposed study are originated from attenuated HIV-1, AdV, AAV, MLV and HSV vectors. All the vectors have been modified considerably from their parent viruses. The intended function of vector system is to supply transient expression of viral proteins and cis element for the delivery and expression of the reporter genes, eg eGFP, Laz and puro. The cytokine genes, eg TNFa, IFNy, VEGF and EGFP, will be used as expression markers for the determination of vector efficiency. The overall objective of the genetic manipulations carried out under this project is to assess the safety and efficacy of the viral vectors.

**Evaluation of foreseeable effects**

A full risk assessment has been carried out for the proposed project of the development and use of viral vectors for gene therapy. The areas covered under the risk
assessment are: human and environmental hazards associated with the recipient's micro-organism, hazards arising directly from the insert gene product and the vector systems, hazards arising from the alteration of existing pathogenic traits including host range, tissue tropism and the likelihood of causing harm to human health in case of accidental exposure. In summary, all the vectors used in this project are disabled and pose no detectable hazard to human. The parental viruses will NOT be used at anytime. The GMM will be manipulated at small scales within the containment level 2 facilities. The GMM would NOT survive and become established in the environment and in any case it would not be a hazard to human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Class 2 containment applied throughout.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated plastics and culture fluids etc will be disinfected overnight in 10% Chloros, the solid waste then will be autoclaved and incinerated while the liquid waste will be discarded in a designated sink. Validation and details of control waste management is held on file at NIBSC.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered that the risk assessment and the proposed containment measures were appropriate for the work.

Project Containment

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Project Ref 251/03.1

Date Ackn'd 02/03/2022
Although the GMM will not be cultured at NIBSC, the GMM will be used for immunisation. Risk assessment of the GMM has assigned class 2 containment and hence the immunisation will be carried out in class 2 containment facility.

The parental MVA vaccinia virus has been administered to over 120,000 humans for the purposes of smallpox prophylaxis. It has restricted replication in man.

There are 5 major deletions in the genome of the recombinant MVA virus, rending it replication deficient in humans. No specific receptor is required for infection. The mechanism involves pH-independent fusion with host cell plasma membrane.

The inserted genes are of two types, the first are short DNA fragments derived from the novel translocation between bcr and abi on the philadelphia chromosome, which is only present in people with CML and a T cell epitope from tetanus toxoid. These genes are cloned in open reading frame linked by three amino acid residues. In normal healthy individuals these target antigens are not present and are therefore immunogenic. Active immunity against these targets provides no health risk. The DNA fragments are not oncogenic.

The second type of tene insert is the extracellular domains of a platelet glycoprotein (GPVI). GPVI is expressed on platelets and megakaryocytes in humans and is one of the collagen receptors required for platelet activation. Humans are tolerant to GPVI, and the likelihood of breaking tolerance after exposure is low. A small minority of people when exposed to self antigen in the context of MVA-HumanGPVI could produce symptons of autoimmune thrombocytenia (AI), which is a treatable condition. A high dose antigen is requires in order to break tolerance. The amount of mMVA-human GPVI transferred by accidental needle stick is unlikely to be above this threshold.

Well characterised vaccinia vectors, free from harmful sequences, as in the case of rMVA, will nto increase the stability of the GMM in the environment and will not transfer any resistance amarkers to microorganisms not known to acquire them naturally. rMVA proposed for this project is a highly attenuated strain. It can infect but has restricted...
replication in humans and can only replicate in chicken egg fibroblasts. The characteristics of the recombinant vaccinia in terms of host range and pathogenicity are unlikely to be enhanced compared to the parental virus. The protein products of the inserted genes will not be incorporated into progeny vaccinia virus particles. The infectivity and immune response to rMVA is unaffected from that of the wild type. There is no risk associated with recombination of separate vaccinia viruses. The replication incompetence is confirmed both prior to and following mofigivsyon og yhr bitud. Yhr tômBS id erll vhstsvyrtidfr sfn gtr gtom hstngul drwumvrd snf id trplvsysjon frgivirny in humsn. Rcpodutr yo tômBS yhrtrgtr or podrd rcyrtrmrly loe tidk yo humsn hrrlyh. Yhr rggrvyd og yhr hmryiv indtrty on humsn hrrlyh snf yhr mbitionmmry eill br minimal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The rMVA will be used in containment level 2 laboratories. Transmission, results from exposure of skin, especially damaged skin or via the respiratory route will be minimised by the wearing of gloves and gowns. The amount of waste arising from immunisation will be less than 500 uL per experiment and will be disposed of by incineration. Spillages will be disinfected according to the laboratory rules. This involves soaking of materials and equipment in freshly made 1% (w/v) Virkon or 2.5% chloros overnight. Work surfaces will be wiped with 1% (w/v) Virkon or 70% (v/v) ethanol. These measures will reduce the likelihood of all hazards to negligible levels. The overall risk therefore is minimal with the proposed containment and control measures.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered that the risk assessment and the proposed containment measures were appropriate for the work.

Project Containment

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02/03/2022
The aim of this genetic modification work is to introduce plasmids for the direct expression of GFP and or LUC into S. pneumoniae so that S. pneumoniae cells expressing GFP and or LUC can be used in a project to standardise an opsonophagocytic assay used to study the efficacy of pneumococcal vaccines. Bacteria expressing both GFP and LUC will facilitate the study of the process of phagocytosis by differentiated immune cells and the standardisation of the assay. 

The bacterial strains used in this study will be Streptococcus pneumoniae are containment level 2 pathogens. They will therefore be handled according to the containment level 2 code of practice. The strains used will have capsular polysaccharides (serotypes) that are used as vaccine components and will have been genetically characterised by multi locus sequence typing (MLST). Pneumococcal isolates that have been responsible for causing mucosal or invasive infections may be used. Pneumococci can be carried asymptomatically in the nasopharynx.

The host will be the S. pneumoniae strains as described above. The genetic material is detailed below.

The green fluorescent protein originally produced by the jelly fish Aequorea victoria and luciferase originally from the firefly Photius pyralis have both been extensively used to study a) the localisation of bacterial cells and b) gene expression in eukaryotic cells and tissues. Both GFP and LUC genes will be purchased as elements of commercially available plasmid vectors. When excited by the appropriate wavelength of light GFP fluoresces without the need for additional cofactors or substrates whereas LUC requires adenosine tri phosphate (ATP). The gfp and or luc genes will be cloned into a shuttle plasmid vector, either pSET1, pSET2 or pSET3, all of which are capable of replicating in both Escherichia coli and Streptococcus pneumoniae. The host bacterial strain used for the construction of the recombinant plasmid vectors is
Escherichia coli JM101. The pSET GFP and/or LUC constructs will subsequently be transformed into the S. pneumoniae strains detailed above. Once transformed these pneumococcal strains will express GFP and or LUC as well as resistance to spectinomycin. The fluorescence or bioluminescence of S. pneumoniae may be monitored using various techniques (e.g., microscopy, spectroscopy, flow cytometry) during the process of opsonophagocytosis.

**Evaluation of foreseeable effects**

GFP and LUC are both expressed as cytoplasmic proteins and have no other known function other than to fluoresce when irradiated with light of the appropriate wavelength or luminesce respectively. It is very unlikely to change the behaviour, the pathogenicity, or the virulence of the bacteria. No increased risk to human health has been described in the large numbers of publications available to date. Therefore the risk of infection with this modified strain is considered to be no greater than with wild-type Streptococcus pneumoniae strains.

**Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full level 2 containment will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste will be disposed of by autoclaving followed by incineration.
Chemical disinfection with phenolic-based disinfectant will be used for spills.
Validation and control of waste management will be held on file at NIBSC.
The appropriate disinfectant or 70% ethanol will be used to disinfect surfaces.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

The GMSC considered that the risk assessment and proposed level of containment were appropriate for the work.

**Project Containment**

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Animal Units  
Large Scale Activities  
Human Clinical Applications
**Project Additional Information**

**Purposes of the contained use**

- Evaluation of efficiency and safety aspects in the use of lentiviral vectors for therapeutic gene delivery
- Lentiviral vectors are potentially infectious for the operator thus containment level 2 is required.

**Recipient or parental organism**

1. E coli K12 derivatives will be used to propagate plasmids containing genes required to produce a lentiviral vector.
2. Eukaryotic cell line 293T will be used as recipient cells in transfection of the plasmids.
3. Hela cells will be used for the transduction of lentiviral vectors.

**Host/vector system**

1. Plasmids have a pBR322 backbone - ie pUC-based vectors. The details of these plasmids have been published and the vectors are widely used in research.
2. The lentiviral vector produced is based on human immunodeficiency virus (HIV-) sequences

**Origin & function**

Four plasmids are involved:

- a. HIV-1 gag, pro, pol and RRE sequences under the control of a cytomegalovirus (CMV) promoter
- b. HIV-1 rev sequence under control of Rous sarcoma virus (RSV) promoter
c. RSV promoter, HIV-1 U5 sequences, HIV-1 packaging signal, partial gag, RRE, transgene under control of non HIV-1 promoter, HIV-1 U5. This is a self inactivating vector as the HIV-1 U3 regions are attenuated.
d. Vesicular stomatitis virus G envelope under control of CMV promoter.

Transient transfection of these plasmids will result in the components being combined to produce a lentiviral vector. Many of the sequences are HIV-1 derived allowing the vector to access mitotically inactive cells. Formation of replication-competent retrovirus is prevented due to the absence of a retroviral envelope. The spread of genes across many plasmids minimises the chance of recombination events, as does the presence of a self-inactivating (SIN) vector limiting the lentiviral vector to a single round of infection. The VSV-G envelope broadens the cell tropism of the vector. All the HIV-1 accessory genes not essential for viral replication have been eliminated.

Evaluation of foreseeable effects

Genetically disabled E. coli K12 variants (such as JM109) are unlikely to survive outside of a laboratory environment due to their auxotrophic requirements for thymidine. Furthermore, recombinant plasmids are derived from poorly mobilizable vectors. The GMMs themselves are not hazardous to the environment. Neither the cloned genes nor their products are hazardous to plants found in the environment. It is unlikely that the GMM will be taken up and the recombinant protein expressed by animal cells unless injected. It is unlikely that the plasmids will produce proteins outside of a cell as the promoters require cellular transcription factors for their activation: each plasmid is designed to be active in mammalian cells thereby producing the protein encoded. The combination of plasmids does not harbour HIV-1 proteins which can result in infectious wild type virus. The transgene, EGFP is not an enzyme nor is it known to be toxic and thus is unlikely to alter the behaviour of recipient cells. The VSV-G envelope gene is capable of mediating endocytosis thereby entering a cell. The Rev protein controls RNA nuclear export in wild type HIV-1. The gag, pro and pol genes encode the capsid, protease and both the reverse transcriptase and integrase enzymes of HIV-1, respectively. These proteins are not reported to cause any adverse effect on their own. Transient expression of the viral and non-viral proteins from the plasmids should they be taken up by cells could theoretically occur. This is an unlikely event through routes other than direct injection. Moreover, the design of each plasmid incorporates safeguards to prevent establishment of viral infection unless all of the packaging vector units are present in a cell.

It is unlikely that the 293T cells sequences will recombine with regions of the vector sequences to produce a novel virus as the lentiviral vector is severely attenuated and limited to one round of replication. The lentiviral vectors themselves are not hazardous to the environment. Neither the cloned genes nor their products are hazardous to plants found in the environment. It is unlikely that the GMM will be taken up by animal cells unless directly injected. Virus production is highly unlikely even in the event that all four plasmids are taken up by a cell: replication pseudotyped envelope will allow entry of the GMM into many cell types the vector is replication deficient and self-inactivating.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be used.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. All equipment and materials associated with bacterial cultures will be disinfected in 10% Microsol3 (Anachem) prior to disposal via autoclaving, or re-use in the laboratory. Using Microsol3 at a 1:50 dilution results in a reduction of bacterial cells of >4.5 log (Gram positive and Gram negative bacteria).
2. All cell culture waste will be disinfected in 2.5% (v/v) Chloros (giving 2,500 ppm available CL) overnight prior to discard via the drain with copious amounts of water. All disposable materials and equipment are disposed of by soaking in 2.5% (v/v) Chloros overnight by autoclaving. The effective use of Chloros as a disinfectant is established against viruses (enveloped, non-enveloped, bacteria, bacterial spores, organic matter, TSE).
The genetic modification safety committee considered that the risk assessment and the proposed containment measures were appropriate for the work.

**Project Containment**

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**Project Ref** 251/05.2

- **Date Ackn'd**: 19/05/2005
- **CU2 Project Title**: Cloning and over-expression of prion protein

**Class** | **CultureVolClass2** | **CultureVolumeClass3-4**
---|---|---
Class 3 | 6 x 1ltr |  

**Non-GMM Consent Granted** | **Yes**

**Project notified under transitional arrangements** | **N**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Is an emergency plan required according to regulation 20?** | **N**

**If yes, tick to confirm that it is attached to this form** | **N**

**Tick to confirm that you have attached a risk assessment to this form** | **Y**

**Tick if you are claiming exemption from disclosure for section of the risk assessment** | **N**

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee considered that the risk assessment and the proposed containment measures were appropriate for the work.
**Project Additional Information**

**Purposes of the contained use**

Transmissible spongiform encephalopathies are a group of neurodegenerative disease that occur through a change in conformation of the prion protein (PrP). The normal form of the protein, PrPc, is converted from a soluble to an insoluble from that acquires resistance to digestion with proteinase K (PrP Sc).

Currently diagnosis of TSEs relies on post-morten examination of neural tissue. Levels of human TSE agent can only be determined by titration in mice. There is a need to find an alternative methods diagnosing and measuring TSE content, the use of cell lines that are susceptible to infection by human TSEs would provide an invaluable tool for achieving both goals.

Recombinant prion protein (PrP) expressed in bacteria will be used to standardise diagnostic assays. Cell lines over-expressing PrP will be evaluated for their ability to amplify the infectious agent varient Creutzfeldt-Jakob Disease (vCJD) in standard reference materials.

Production of recombinant normal prion protein has been assigned to containment level 2. Generation of a lentivirus vector expressing the normal human prion protein will be carried out in a laboratory approved for work with human TSEs (Containment level 3 with derogation).

**Recipient or parental organism**

1. E coli K12 derivatives will be used to propagate plasmids containing the prion protein open reading frame.
2. E coli BL21 cells will be used for expression of the prion protein in bacteria.
3. Eukaryotic cell lines 293T and RK13 will be used in transient expression experiments.
4. S2 cells will be used for stable expression in insect cells.
5. Human cell lines expressing endogenous retroviral elements (HERVs, eg Tera 1, a teratocarcinoma cell line) will be used for the expression of the prion protein from a lentivirus vector.

**Host/vector system**

1. All plasmids are based on either a pUC or pBR322 backbone and are widely used in research.

**Origin & function**

The plasmids involved in this connected program are as follows:
1. Prion protein (human, bovine, mouse, hamster, ovine and deer from normal tissue or cell lines) under the control of the T7 promoter controlled by the laci operator sequence
2. Normal prion protein under the control of the CMV promoter
3. Normal prion protein under the control of the metallothionein promoter (in insect cells)
4. Lentivirus expression of prion protein is under the control of CMV promoter
5. Normal human or bovine prion protein expressed from endogenous promoters but with multiple integrations, increasing overall levels of expression, in mice.

Prion protein expressed in bacteria will be purified and used as a standard reference material. Purified protein will be refolded to produce the scrapie-associated conformer (PrP Sc), refolding experiments will be carried out in the NIBSC prion laboratory which is containment level 3 with derogation.

Stable cell lines over-expressing PrP will be evaluated for their ability to replicate PrP SC. Tagged versions of PrP will allow the production of nascent PrP SC to be evaluated.

Prion protein expressed in insect cell culture will be affinity purified.

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02/03/2022
Cell lines expressing human prion protein from a lentivirus cassette will be evaluated for their ability to replicate/amplify PrP Sc from human tissue homogenates (sporadic and variant CJD).

### Evaluation of foreseeable effects

Genetically disabled E. coli K12 variants (DH5a, INVFa, BL21) are unlikely to survive outside a laboratory environment due to their auxotrophic requirements for thymidine. Plasmids used for cloning and expression have only a limited ability to mobilise. The GMMs are not hazardous to the environment. There is no evidence that cloned PrP or recombinant protein is hazardous to the environment. Recombinant protein expression would only be possible after injection. Promoters used for expression of prion protein would not function outside the cell.

Purified protein would only be handled in a containment level 2 setting. Attempts to re-fold recombinant prion protein will be carried out in the prion unit laboratory which has been designated as containment level 3 with derogation. There is no evidence that suggests that recombinant prion protein even when re-folded has any ability to induce disease. However, the re-folded protein will be de-contaminated as if it were potentially infectious.

Cell lines which stably over-express the prion protein will be maintained in containment 2 laboratory and attempts to infect cell lines with PrP SC will be carried out in the prion unit laboratory.

Lentivirus stocks will be produced in the main prion unit laboratory, all viral stocks will be treated as for infectious virus (media will be treated with 2,500 ppm hypochlorite) all plastic ware used during preparation and amplification of stocks will be submersed in chloros prior to disposal as clinical waste (incineration, as per prion unit COP).

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be used for over-expression of prion protein in bacterial cultures and for the maintenance of cells (mammalian and insect) stably over-expressing prion protein.

Re-folding of bacterial expressed recombinant proteins will be carried out in the prion unit laboratory which has been assigned as a containment level 3 laboratory with derogation.

Lentivirus vectors overexpressing normal prion proteins will be packaged and amplified in the prion unit laboratory which has been classified as containment level 3 with derogation (not sealable therefore fumigation of laboratory can not be undertaken).

Attempts to infect cells over-expressing prion protein will also be carried out in the prion unit laboratory.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. All equipment and media used for bacteria cultures will be decontaminated using 2% microsol overnight. Flasks etc will be autoclaved before further use in the laboratory. Microsol at a concentration of 2% results in the reduction of bacterial cells of >>>>>>>.5.4log (Gram positive and Gram negative bacteria).

2. Re-folded recombinant proteins will be treated with 2N (NaOH prior to disposal by incineration (in compliance with prion unit code of practice).

3. Cell culture waste will be disinfected in 2.5% (v/v) chloros (giving 2,500 ppm available C1) overnight before discard via the drain with copious amounts of water. All disposable plastics will be disposed of after autoclaving. Plastics used during prion infectivity experiments will be decontaminated overnight in 2N NaOH before disposal by incineration (in compliance with prion unit code of practice). Chloros and 2N NaOH have been approved as suitable agents for decontamination of TSEs.
The GMSC considered that the risk assessment and the proposed containment measures were appropriate for the work.

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Project Ref 251/06.1

Date Ackn'd 19/04/2006

Transfer of commercially-produced lentiviral vectors, encoding short hairpin RNAs (ShRNAs) against the calcium-sensing receptor, into mammalian cells to induce knockdown of the gene product by RNA interference (RNAi)

Class 2

Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

The extracellular calcium-sensing receptor (CaR) has been shown to be expressed on the insulin-secreting B-cells within the pancreas, although its function has yet to be described. In an effort to knock down the expression of this receptor in pancreatic B-cells, we will utilise commercially available transduction-ready lentiviral particles (Mission shRNA lentiviral transduction particles) to transduce mammalian cells with specific shRNA constructs and generate cell lines with stable expression of these shRNAs, to facilitate knock down of the CaR gene by RNA interference.

Recipient or parental organism

There is no propagation of lentiviral vectors as the lentiviral particles are commercially available and ready for transduction into mammalian cell lines. The recipient cell lines are well established (MIN6 - mouse insulinoma, HEK293 - human embryo kidney cell line) and are tissue culture adapted so will not survive outside of laboratory culture. They are not considered pathogenic to humans and animals. Once efficient knock down by RNAi has been shown in the HEK293 cells, the human shRNA constructs will be used to transduce primary human islet beta cells, dispersed from human islets of Langerhans (King's College Hospital, Islet transplantation Unit). These islets are screened for HIV and Hapatitis prior to release.

Host/vector system

The lentiviral particles are produced from a pLKO.1 puromycin vector which is a widely used vector in research. The vector contains the 5' long terminal repeat (LTR), SIN/LTR (3' LTR), and Psi Packaging Signal of HIV-1 and these permit viral packaging using a 2-plasmid lentiviral packaging system. Using this multiplasmid approach, resulting viral particles are replication incompetent and cannot be propagated. Also, a deletion in the U3 region of the 3' LTR (SIN/LTR) does not affect generation of the viral genome during packaging, but results in loss of the transcriptional capacity of the viral LTR once transferred to target cells. The vector also contains the puromycin resistance gene for selection in mammalian cells.

Origin & function

s is the aim of this work to utilise commercially available transduction-ready lentiviral particles (Mission shRNA lentiviral transduction particles, Sigma) to transduce mammalian cells with specific shRNA constructs and generate cell lines with stable expression of these shRNAs to facilitate knock down of the CaR gene. Once these cell lines have been established, the role of the CaR in pancreatic B-cells will be evaluated by analysing the function of the cells. These functional tests include measurements of insulin secretion by RIA, measurements of intracellular calcium flux, proliferation and apoptosis assays and analyses of gene expression.

Evaluation of foreseeable effects

Once stably transduced, the cells will express the shRNA which is not toxic and non-mobilisable because of the self-inactivating feature of the lentiviral vector, meaning the target cell cannot produce and release new lentiviral particles. Although possible, the chances of incorporation of the vector into the genome in a position that alters the expression of neighbouring genes such as oncogenes are extremely remote. Although the pseudotyped envelope will allow entry of the GMM into many cell types, the vector is replication deficient and self-inactivating, therefore the chances of the vector entering the human genome and reducing the expression of the CaR are extremely remote. Should this occur, the predicted effect of exposure is difficult to assess but is unlikely to cause harm to human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be soaked in 10% (v/v) Chloros (giving 2,5000 ppm available CI) overnight and discarded in a designated sink with copious amounts of water in

02/03/2022
accordance with guidelines for disposal of enveloped virus. As a result of the location of the work all disposable materials and equipment are disposed of by autoclaving and incineration after soaking overnight in 10% (v/v) Chloros (giving 2,5000 ppm available CI). Plastic labware is used. Validation and details of control waste management is held on file at NIBSC.

Any spillages are managed in accordance with the COP for Laboratory 5088. Briefly, liquid spills are decontaminated with absorbent towels soaked with 10% v/v hyrcolin. Contaminated surface areas are wiped with 10 (v/v) Chloros (see above) for 30-60 minutes. Solid waste materials are autoclaved before being disposed.

Cuts, splashes, etc of contaminated material onto exposed skin is washed with copious amounts of soap and water, if appropriate a wound is made to bleed freely. The area is covered, a dressing applied. Eye injuries are washed with copious amounts of water. A copy of emergency procedures for cuts and splashes is displayed in the laboratory.

The GMSC considered that the risk assessment and the proposed containment measures were appropriate for the work.

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered that the risk assessment and the proposed containment measures were appropriate for the work.

**Project Containment**

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<tr>
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**Project Ref** 251/07.1

<table>
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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<tr>
<td>01/08/2007</td>
<td>The handling and propagation of Doxycycline dependent HIV and SIV.</td>
<td>Class 3</td>
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**Project Additional Information**

**Purposes of the contained use**

We shall receive Human Immunodeficiency Virus and Simian Immunodeficiency Virus from the laboratory (University of Amsterdam) that have been genetically engineered so that replication of these viruses is regulated by the Tet-On regulatory system. We shall propagate these viruses and investigate the impact on cells of infection with these viruses in the presence and absence of the antibiotic doxycycline.

**Recipient or parental organism**

Human Immunodeficiency Virus or Simian Immunodeficiency Virus

**Host/vector system**

The parental micro-organism HIV or SIV will be propagated and handled by infecting cell lines such as Sup T1, C8166 or CEMx174 cells or primary peripheral blood mono nuclear cells.

**Origin & function**

The parental micro-organisms are lab adapted HIV (e.g strain HIV-1 Lai) or SIV (e.g SVmac239) that have been molecularly cloned and so amendable to genetic manipulation.

The Tet-On system is derived from E.coli K12 tet operon for the Tet and the inducible Tet R gene regions and the eukaryotic transactivator derived from the C terminal portion of VP16 derived from Herpes Simplex virus.

The constructs have been prepared and expressed by the group of University of Amsterdam and demonstrated that the expression of the progeny viruses are dependent upon the presence of doxycycline in tissue culture medium.

**Evaluation of foreseeable effects**

The propagation and handling of doxycycline dependent HIV and SIV may result in 3 outcomes:

1) The continued maintenance of genetic information so that the expression of recombinant viruses remain doxycycline dependent.
2) The modification and selection of recombinant viruses that cannot express in the presence or absence of doxycycline (ie replication incompetent viruses)
3) The modification and selection of recombinant viruses for which expression is no longer regulated excessively by the presence of doxycycline (ie revertant viruses)

In no situation is it anticipated that the virus will alter tropism since the genetic changes do not alter the env gene which is the principal determinant of receptor binding and cell entry by the virus.

In all cases recombinant viruses remain an enveloped retrovirus that will remain effectively controlled under bio-containment conditions appropriate for the parental virus.
and susceptible to the same range of disinfectants and inactivation procedures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

It is possible that the future development of the connected programme will include the genetic manipulation of full length infectious molecular clones of doxycycline dependent HIV or SIV, with the purpose of introducing changes designed to alter the level of dependence of the virus on doxycycline for virus replication. A risk assessment has not been submitted with this application, however, it is likely that such work would be proposed to be performed under the same bio-containment level conditions as those used to handle full length recombinant infectious clones of HIV and SIV inserted in plasmids and propagated in E.coli K12. In principle, this is bio-containment level 2 with additional restrictions on restricted entry to the laboratory and the exclusion of glass and sharps from the laboratory area.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All laboratory waste derived from tissue culture will be soaked in disinfectant (10% v/v Chloros or 10% Microsol3) overnight. Liquid waste will be discarded into the drains and solid waste will be autoclaved prior to disposal. All disinfectants used have proven efficacy against enveloped viruses and the heat labile nature of retroviruses is well documented.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

**Project Containment**

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**Project Ref** 251/07.2

The GMSC considered that the risk assessment and the proposed containment measures were appropriate for the work.
Molecular mechanisms of the formation, composition and structure of Neisseria meningitidis outer membrane vesicles (OMV) and the refinement of serological analysis of OMV vaccines.

Nelsseria meningitidis is a class 2 pathogen. It only colonises humans and has no environmental reservoir. It is usually carried asymptomatically in the nasopharynx but occasionally invades the subepithelial tissue when it may proceed to cause septicaemia and/or meningitis.

Recipient or parental organism

Neisseria meningitidis is a class 2 pathogen. It only colonises humans and has no environmental reservoir. It is usually carried asymptomatically in the nasopharynx but occasionally invades the subepithelial tissue when it may proceed to cause septicaemia and/or meningitis.

Host/vector system

The DNA to be transformed into N. meningitidis will utilise a plasmid shuffle vector (pMidg) able to replicate in both N. meningitidis and Escherichia coil. This vector also has the N. meningitidis Ncr strong promoter to drive expression of the cloned insert. In addition the vector also contains the kanamycin resistance gene for the selection of transformed colonies when grown on media containing the antibiotic kanamycin (C.A. O'Dwyer., Infect Immun. 2004 Nov;72(1 I ):651 1-8). In addition the Escherichia coil plasmid vector (pUCI 9) will be used, it is envisaged that this plasmid is solely used for in construction of additional DNA sequences and none of this plasmid will be transformed into N. meningitidis.

Origin & function

The pMidg plasmid will also harbour an operon isolated from the E. coil strain TopI0. In E. Coli these proteins form a complex which spans the peptidoglycan layer and effectively joins the Inner and outer membrane. This has the net effect of stabilising the outer membrane. In addition, sequences of the N. meningitidis porA gene and promoter will be amplified to provide regions for homologous recombination with the wild type sequence. Synthesised oligonucleotides will be annealed and used. A copy of the kanamycin resistance gene for the selection of transformed colonies when grown on media containing the antibiotic kanamycin will also be introduced.

Evaluation of foreseeable effects

It is hoped that after transformation the N. Meningitidis strain will express fully functional genes of the operon from the plasmid vector. The subsequent production of
proteins following their correct formation and localisation would lead to the linking of the inner membrane, peptidoglycan and the outer membrane as seen in E. coli. This protein complex would provide additional stabilisation of the outer membrane and inhibit the formation of naturally produced or detergent induced OMV. The implications of the proposed alterations for meningococcal virulence are unknown; however, it is unlikely that they will increase virulence as the proposed mutants are likely to be less fit for survival in vivo because of alterations to the bacterial cell surface. Neisseria meningitidis is naturally competent and it is highly likely it will have encountered the operon before as it is present in most gram negative bacterial species. The fact that it is not already present in the genome suggests that there is no advantage to the organism from expressing these genes. If the expression of the operon results in reduced vesicle production, one would expect a consequent reduction in the release of lipo-oligosaccharide and hence a reduction in inflammation, which is ultimately responsible for the pathology of meningococcal disease. In addition vesicle production, a known virulence factor in other bacterial species, would also be reduced.

N. meningitidis PorA expression is naturally variable due to the presence of a poly-G track found between the -10 and -35 sequences in the porA promoter. In the wild-type meningococcus this variation occurs through replication slippage as the polymerase crosses the poly-G region. This will be modified to generate spacer sequences of different lengths. It is hoped that after recombination of this sequence into the wild type porA promoter that the different spacers will create a range of gene expression and a set of strains, differing only in the level of the PorA protein in their outer membrane. The pathogenic properties of the bacterium will probably be unaltered in the constructs. The maximum expression of the altered promoters is expected to be no more than that of the wild type promoter, while reduced or lack of expression likely to have a deleterious effect on the growth. All the equivalent variants in promoter spacer length have been isolated from natural populations. Removing the variability in PorA expression, may well eliminate a mechanism by which N. meningitidis evades the human immune response to the highly immunogenic PorA protein.

The likelihood of all GMM's causing harm to human health following exposure is no more than for the wild-type pathogen. Finally, the organism will be resistant to kanamycin, an antibiotic that is not used to treat meningococcal infections but this resistance will only be sustained while selection is maintained.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

no derogation requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All Cultures of transformed N. meningitidis are sterilized by autoclaving prior to disposal. Autoclaving is carried out at a minimum of 134°C for a minimum of 30 minutes. Prior to autoclaving additional controls for the safe disposal of cultures are also employed. Petri dish lids are taped to the base to avoid any dispersal of bacteria. Broth cultures are first disinfected by adding Microsol 3 to a final concentration of 10% and left for 30 minutes before being autoclaved. Once autoclaved the liquid waste is deemed harmless disposed of down the sink. All plate cultures and disposable plastic is sent off site for incineration after autoclaving.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered that the risk assessment and the proposed containment measures were appropriate for the work

Project Containment

02/03/2022
### Project Ref 251/08.1

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Project notified under transitional arrangements: **N**

#### Project Additional Information

**Purposes of the contained use**

The GMO is being considered for Phase I clinical trial as an investigational medicinal product and so specific dissemination issues arising from the genetic modifications have been identified in preclinical investigations. Specifically, more information on aspects of GMO release from individuals vaccinated with the GMO has been requested with specific information on the potential for shedding of the GMO. There is access to veterinary clinical sample material from cows vaccinated with the rBCG that may assist in clarifying these issues.

**Recipient or parental organism**

The organism (BCG) is the vaccine strain and has been administered to millions of people (neonates, children, young adults, adults and the aged) worldwide for over 80 years. However BCG is Classified as Level II for the purposes of culture and laboratory handling. Laboratory adapted strain of M. bovis originally isolated from bovine origin in late 1800’s.
Another organism used in the project is Escherichia coli (Category I) only for the purpose of cloning and amplification of the plasmid.

**Host/vector system**

The GMO is a recombinant form of BCG. BCG (Prague Strain) was modified to create ureC(KO) hly+ rBCG, which contains the Listeria monocytogenes protein listeriolysin 0 (Hly), which allows escape from the intracellular organelle, the phagosome - and is deficient in urease C, a molecule that has a role in pH neutralization of the phagosome. Deletion of the gene encoding urease C via insertion of a Hygromycin cassette has ensured an acidic pH in the phagosome, thereby promoting the activity of Hly. The strategy is designed to facilitate antigen presentation through phagosome escape and engagement of MHCII antigen presentation. The gene coding for Listeria monocytogenes protein listeriolysin 0 (Hly) has a highly focussed functionality and can act in only very limited circumstances — see (a). It has no local or systemic implications. The urease C knockout is carried out by a gene disruption mechanism using a Hygromycin cassette located with the Hly gene driven by the native mycobacterial promoter for Antigen 85.

**Origin & function**

Hygromycin is not in general use in clinical practice and therefore the HygR characteristic has no significant implications.

**Evaluation of foreseeable effects**

The gene coding for Listeria monocytogenes protein listeriolysin 0 (Hly) has a highly focussed functionality and can act in only very limited circumstances — see (a)- It has no local or systemic implications. The urease C knockout is carried out by a gene disruption mechanism using a Hygromycin cassette located with the Hly gene driven by the native mycobacterial promoter for Antigen 85. Hygromycin is not in general use in clinical practice and therefore the HygR characteristic has no significant implications. Preclinical investigations have demonstrated that the GMO is MORE attenuated (safer) than the parent strain currently used worldwide in human vaccination programmes. (See attached publication — Fig 4 page 2475). The gene coding for Listeria monocytogenes protein listeriolysin 0 (Hly) has a highly focussed functionality and can act in only very limited circumstances — see (a). It has no local or systemic implications. The urease C knockout is carried out by a gene disruption mechanism using a Hygromycin cassette located with the Hly gene driven by the native mycobacterial promoter for Antigen 85. Hygromycin is not in general use in clinical practice and therefore the HygR characteristic has no significant implications.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard disinfection protocols for BCG and E. coli will suffice (these are already in place), autoclave and incineration where specified by laboratory practice.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
The GMSC considered that the risk assessment and the proposed containment measures were appropriate for the work.

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**Project Ref** 251/09.1

**CU2 Project Title**

Viral based vector delivery systems for efficient transduction and stable gene expression in primary cell lines and adult and human embryonic stem cell lines.

**Class**

Class 2

**CultureVol**

< 1 Litre

**Non-GMM Consent Granted**

Project notified under transitional arrangements

**Historical Significant Changes**

Withdrawn

**Project Additional Information**

**Purposes of the contained use**

Retrovirus and baculovirus based viral vector delivery systems will be used for efficient transduction and stable gene expression in primary cell lines and adult and embryonic stem cell lines.

Banking and characterisation of induced pluripotent stem cells, embryonic stem cell lines, adult stem cell lines and human and mouse feeders created and modified using...
Retrovirus and baculovirus based viral vector delivery systems.

Recipient or parental organism

Viral vectors: There is a pathogenicity associated with the unmodified viruses, for example:
In the case of Murine Moloney Leukaemia Virus, leukaemia in the mouse. This virus has a tropism for mice.
HIV/AIDS in humans in the case of lentivirus, based on the HIV retrovirus.
Baculovirus has a tropism for insect cells and shrimp. It does not pose a hazard to humans since it does not replicate in mammalian cells, and is also inactivated by human complement.

Cell lines: Established cell lines will be used for lentivirus and retrovirus production (e.g. 293FT; Platinum-E cells; ΔΕ cells). 293FT cell line is derived from the 293F cell line and stably expresses the SV40 large T antigen from the plasmid pCMVSPORT6Tag.neo. 293F cell line is a fast growing variant of the transformed human embryonic kidney (293) line. Platinum-E (based on the 293 cell line) and ΔΕ (based on the NIH 3T3 cell line) were generated using constructs for the expression of the retroviral structural proteins (gag, pol and env). A number of cell lines will be transduced with the viral vectors produced in these projects, including, but not limited to, human foetal lung fibroblasts (e.g. MRC5 and IMR90 (ATCC)) and hES cell lines (e.g. SHEF3 (UKSCB)).

Host/vector system

Retrovirus vectors are a traditional delivery system for stable gene expression which will be utilised in this project. Specifically, the pMXs2 and the pBabe vectors will be used in conjunction with Platinum-Ecotropic (Plat-E) packaging cells (pMXs vector, Cell Biolabs Inc.) or ΔΕ packaging cells3 (pBabe vector). These retrovirus vectors are based on the Murine Molony Leukemia Virus (MMLV), and contain the 5’ and 3’ LTR’s plus the ψ packaging signal and a multiple cloning site. The gag genes are disrupted in order to generate vectors free of gag and gag-fusion proteins. Replication-incompetent viruses are produced in the packaging cell lines, which contain packaging constructs for gag, pol and env on two separate plasmids, and can efficiently deliver the gene of interest to the cell to provide stable, long-term expression of the target gene. The system used is a third generation lentiviral system, which includes a number of safety features to enhance its biosafety and minimize its relation to the wild-type HIV-1 virus:
• The pLenti vector contains a deletion in the 3’LTR that results in “self-inactivation” of the lentivirus after transduction of the target cell line.
• Multi-plasmid approach: structural components required for packaging the viral genome are separated onto four plasmids. The plasmids have been engineered so as to not contain any regions of homology with each other to prevent undesirable recombination events that may lead to generation of a replication-competent virus.
• The packaging plasmids allow in trans expression of gag, pol, rev and env proteins for viral progeny to be produced in the 293FT packaging cell line. None of the plasmids contain LTRs or the ψ packaging sequence, therefore none of the HIV-1 structural genes are present in the packaged viral genome, and cannot be expressed in the transduced target cell line. The lentiviral particles produced are replication-incompetent and only carry the gene of interest.
• Gag and pol expression from the pLP1 plasmid has been rendered Rev-dependent by the addition of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev.

Baculovirus can infect and replicate in insect cells lines, and has also been shown to effectively transduce (but not replicate in) a variety of both dividing and non-dividing mammalian cells. While expression of viral genes does not take place, baculovirus-based vectors are used for transient expression of genes drive by promoters/enhancers that are normally functional in mammalian cells. Baculovirus vectors will contain the genes required for IPS cell generation (e.g. transcription factors Oct4,uso4, cMyc, Sox2, IN28 and Nanog). In addition to the production of IPS cells, baculoviral vectors may also be engineered to deliver transgenes to both IPS and hES cells for further study into the mechanisms of pluripotency/differentiation and the role of methylation in these processes.
With the exception of one gene, the lentivirus/retrovirus/baculovirus particles will contain non-toxic transgenes (e.g. transcription factors Oct4, Klf4 and Sox2, shRNA or reporter genes) and therefore have no associated pathology, and are not overtly oncogenic in humans, mice or insects. One retrovirus and one baculovirus vector used in this study will contain the oncogene, c-Myc.

Evaluation of foreseeable effects

The inserted genes do not alter pathogenicity or tropism. The lentivirus and retrovirus particles are designed to enter a range of cell types and deliver transgenes for integration into the host cell's genome. The cells will then express these genes, of which the majority are non-toxic, and therefore do not alter pathogenicity or tropism. The viruses are replication incompetent, and therefore extremely unlikely to be able to produce progeny. In addition, the chances of the vector incorporating into the genome in a position that alters the expression of neighbouring genes (such as oncogenes) are extremely remote.

As the engineered lentivirus and retrovirus are replication defective and restricted to a single round of infection in the target cells, it is highly unlikely that the transgenes could be transferred to a related microorganism. Lentivirus vectors can enter a number of cell types, and should this occur the predicted effect of exposure is difficult to assess, although the transgenes are non-toxic, and unlikely to cause harm to human health. The vectors are also replication deficient and viral replication in the host is therefore extremely remote. The retrovirus vectors are ecotropic, and therefore cannot infect human cells.

The baculoviral vectors are replication defective, so it is highly unlikely that the viral particles will be able to transfer the genetically modified sequences to another microorganism. With the exception of one vector that contains c-myc, the majority of vectors will contain non-toxic transgenes. Although the genetic insert is under a mammalian promoter and the predicted effect of exposure is therefore difficult to assess, the vectors are replication deficient, and baculovirus is rapidly inactivated by human complement.

Origin & function

The inserted genes do not alter pathogenicity or tropism. The lentivirus and retrovirus particles are designed to enter a range of cell types and deliver transgenes for integration into the host cell's genome. The cells will then express these genes, of which the majority are non-toxic, and therefore do not alter pathogenicity or tropism. The viruses are replication incompetent, and therefore extremely unlikely to be able to produce progeny. In addition, the chances of the vector incorporating into the genome in a position that alters the expression of neighbouring genes (such as oncogenes) are extremely remote.

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Evaluation of foreseeable effects

The inserted genes do not alter pathogenicity or tropism. The lentivirus and retrovirus particles are designed to enter a range of cell types and deliver transgenes for integration into the host cell's genome. The cells will then express these genes, of which the majority are non-toxic, and therefore do not alter pathogenicity or tropism. The viruses are replication incompetent, and therefore extremely unlikely to be able to produce progeny. In addition, the chances of the vector incorporating into the genome in a position that alters the expression of neighbouring genes (such as oncogenes) are extremely remote.

As the engineered lentivirus and retrovirus are replication defective and restricted to a single round of infection in the target cells, it is highly unlikely that the transgenes could be transferred to a related microorganism. Lentivirus vectors can enter a number of cell types, and should this occur the predicted effect of exposure is difficult to assess, although the transgenes are non-toxic, and unlikely to cause harm to human health. The vectors are also replication deficient and viral replication in the host is therefore extremely remote. The retrovirus vectors are ecotropic, and therefore cannot infect human cells.

The baculoviral vectors are replication defective, so it is highly unlikely that the viral particles will be able to transfer the genetically modified sequences to another microorganism. With the exception of one vector that contains c-myc, the majority of vectors will contain non-toxic transgenes. Although the genetic insert is under a mammalian promoter and the predicted effect of exposure is therefore difficult to assess, the vectors are replication deficient, and baculovirus is rapidly inactivated by human complement.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation from full containment requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be soaked in 10% (v/v) Chloros (giving 2500ppm available Cl) or Microsol 3+ (10% v/v) overnight and discarded in a designated sink (Biotherapeutics laboratory room 5038 or UK Stem Cell Bank) with copious amounts of water in accordance with guidelines for disposal of enveloped virus. All disposable materials and equipment are disposed of by autoclaving and incineration after soaking overnight in 10% v/v Chloros or Microsol 3+ (10% v/v). Plastic labware is used. Validation and details of control waste management is held on file at NIBSC.

All spillages are managed in accordance with the COP for UKSCB, CBI clean room and Biotherapeutics 5038/5030 laboratories. Briefly, liquid spills are decontaminated with absorbent towels soaked with 10% v/v Chloros or Microsol 3+. Contaminated surface areas are wiped with 10% v/v chloros or Microsol 3+ for 30-60 minutes. Solid waste materials are autoclaved before being disposed according to NIBSC safety practices.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GMSC considered that the risk assessment and the proposed containment measures were appropriate for the project.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
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<td>L2</td>
<td>L3</td>
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<tr>
<td>L2</td>
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### Project Ref 251/11.1

**Date Ackn'd**: 25/02/2011  
**CU2 Project Title**: Use of recombinant Attenuated Measles Virus construct to investigate immunogenicity

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<th>Class</th>
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<th>CultureVolumeClass3-4</th>
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**Non-GMM Consent Granted**: Yes

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

1. Evaluation immunogenicity of attenuated measles virus vectors.
2. The constructs are potentially infectious for the operator and thus containment level 3 is required.

**Recipient or parental organism**
CD4 T cells harbouring the vector will be handled locally.

**Host/vector system**

The vector will be assembled in a collaborating laboratory and is designed to allow expression of recombinant genes. It can be handled at Containment Level 2.

**Origin & function**

The vector backbone is the measles virus and contains recombinant proteins.

This vector will enable the immunogenicity of this vector to be evaluated.

**Evaluation of foreseeable effects**

Generally, there is no specific guidance for replicative vectors like recombinant measles virus. The closest guidance could come from gene therapy (GT) vectors and relates to quality aspects (cell lines used, seed characterization) and safety aspects (preclinical safety, integration, persistence).

Infection of humans with the cloned sequences to be used is not known to cause disease and the measles vector will not integrate into host DNA.

The GMM is unlikely to present a greater risk to the environment than does the current childhood attenuated measles vaccine, but because the vector may have altered tropism the effects of infection in humans is unknown. Hence, the vector will be used under containment level 3 to minimise release to the environment.

The vector is unlikely to survive outside of a laboratory environment as it does not replicate extracellularly.

The vector and its insert are unlikely to be taken up and the recombinant protein expressed by animal cells unless injected. It is unlikely that the vector will produce proteins outside of a cell. The GMM itself is not hazardous to the environment nor is the cloned gene or its product hazardous to wild animals or plants found in the UK.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In the unlikely event that virus be spilled, the spill will be disinfected by soaking in a 2.5% (v/v) Chloros solution (giving 2500 ppm available QAC) overnight prior to discard via the drain with copious amounts of water. All disposable materials and equipment are disposed of by soaking as above and then discarded via autoclaving. The effective use of Chloros as a disinfectant against viruses (enveloped and non-enveloped) and organic matter is established.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
The genetic modification safety committee considered that the risk assessment and the proposed containment measures were appropriate for the work.

## Project Containment

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### Animal Units
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- L3
- L4

### Large Scale Activities
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- L3
- L4

### Human Clinical Applications
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- L3
- L4

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## Project Ref 251/14.1

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<td>Assessment of the use of a GM Salmonella typhimurium strain C5.507</td>
<td>Class 2</td>
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</table>

- Non-GMM Consent Granted
- Project notified under transitional arrangements
- Withdrawn

**Project Additional Information**

**Purposes of the contained use**

Evaluation of immunogenicity of candidate Typhoid conjugate Vaccines

**Recipient or parental organism**

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02/03/2022 Page 5791 of 15326
```
Host/vector system
The vector will be assembled in a collaborating laboratory and is designed to allow expression of recombinant genes. It will be handled at Containment Level 2.

Origin & function

S. typhimurium C5.507 is a genetically modified strain derived from S. typhimurium C5 and expresses the Vipolsaccharide (ViPS) capsule from S.Typhi. S. typhimurium C5.507 was isolated from Vi-positive S. typhimurium C5 recipients following conjugation experiments with S. typhi at Institut Pasteur (Paris, France). Strain C5.507 harbours the Salmonella Pathogenicity Island (SPI)-7 of S.typhi, which contains the via genes responsible for expression of the Vi capsule and a kanamycin resistance gene (Hale et al., Vaccine 2006;24:4312-20). The vector will be obtained from a collaborator.

Evaluation of foreseeable effects

The pathogenicity island SPI-7 carries a kanamycin resistance gene and consists mainly of genes involved in regulation Vi expression. Toxin genes have not been identified and the plasmid therefore has no intrinsic toxin activity. The capsule does not add to the virulence of the GM: compound to the parent strain C5 the virulence of C5.507 was not increased in Balb/c mice (Hale et al. 2006). As these are hyper susceptible mice it is reasonable to assume that virulence of the GMM has not increased.

The organism is not indicated as a human pathogen and the expression of the Vi PS is unlikely to change its host range.

The pathogenicity island SPI-7 of C5.507 carries a kanamycin resistance gene and the island is believed to be stable despite the presence of characteristics of a potentially mobile element. The gene content of SPI points to an origin as enzymes are present besides genes that regulate the capsular Vi PS expression. Toxin or toxin-like genes have not been identified. SPIs related to SPI-7 have been found in S.enteric serovar Paratyphi C and S. enterica serovar Dublin (both human pathogens). Soil bacteria are thought to be a natural reservoir for SPI-7 or its close relatives (Pickard et al. J Bacteriol 2003; 185:5055-65). Thus transmission of the plasmid/SPI between bacteria may well be occurring in the soil under natural conditions. The transfer of SPI-7 to C5 was successful in the presence of kanamycin. Because kanamycin levels required for a successful transfer are unlikely to occur in the environment, stable introduction of SPI-7 in new transformants is unlikely and hence expression of the genes and establishments of new Vi+ strains or species is bound to be limited under natural circumstances.

The parent strain is not a human pathogen and its virulence in the natural host is also limited. Transmission by the oral route is required and human disease (if any) is associated with food poisoning and only if sufficient numbers will have to be ingested and stomach acidity will have to be by-passed. In addition staff who will work with relative high levels of live organisms have been vaccinated with Vi PS and will thus be immune to infection with the GMM. Aerosol transmission is an unlikely route for Salmonella Infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is sought

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Methods for disinfecting and dealing with spillages (in accordance with laboratory COP). Benches and equipment will be sprayed with Virkon (DuPont) prior to commencing work and after completion of each piece of work. Virkon is a multi-purpose disinfectant effective in cleaning up hazardous spills, disinfecting surfaces and soaking equipment. It has a wide spectrum of activity against viruses, some fungi, and other bacteria. Virkon will be added to all liquid waste which will be discarded via the laboratory sink with copious amounts of water. If spillages occur, Virkon will be sprayed onto the area and any remaining liquid will be soaked up again with paper towels.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The genetic modification safety committee considered that the risk assessment and the proposed containment measures were appropriate for the work. The Minutes of the committee meeting reflect this decision. A report of this Committee's business is reported to the main Health and Safety Committee, membership of which includes management and scientific representatives from across the Institute and Trade Union representative.

**Project Containment**

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**Project Ref** 251/14.2

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<td>31/07/2014</td>
<td>Evaluation of recombinant and attenuated vaccine candidates for tuberculosis</td>
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Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y
### Project Additional Information

#### Purposes of the contained use

Evaluation of candidate vaccines for tuberculosis. The Connected Programme currently covers two Class 2 activities, both of which are included in this Notification, as well as an overview of the Programme of work.

#### Recipient or parental organism

Candidate vaccines will have been created and safety tested in collaborating laboratories and will be handled at Containment Level 2.

#### Host/vector system

The current licensed vaccines for prevention of tuberculosis (TB) is BCG vaccine. Due to its variability in efficacy in many different developing countries, new vaccines are actively under development. Many vaccine candidates for TB are in clinical studies, including live, attenuated Mycobacterium tuberculosis (Mtb) candidates. They are recombinant Mtb preparations, attenuated by genetically modification; and they are tested (by collaborators) with safety either the same or better than BCG vaccine. Thus they have been classified as ACGM2 organisms. There are many different strategies to attenuate the Mtb by various genetic modifications.

One of the examples is MTBVAC which is recombinant of MT103, which is a clinical isolate of Mtb and is naturally urease deficient and sensitive to the frontline anti-TB drugs used in the clinic. This live, attenuated MTBVAC is constructed to contain two independent non-reverting deletions in the virulence genes phoP and fadD26 [1-3]. It does not contain antibiotic resistance markers, making this strain fully sensitive to front line anti-TB drugs on current market for treatment.

The recombinant BCG preparations are live, attenuated and genetically modified (either by insertion or selection) mycobacteria. There are various strategies for generating rBCG strains in order to improve the safety (e.g. the non-replicating candidates targeting for immunocompromised individuals) and/or enhance protections in M. tuberculosis challenge systems. One of the strategy is designed to facilitate antigen presentation through phagosome escape and engagement of MHC-1 antigen presentation, such as the ureC(KO) hly+ rBCG, which contains the Listeria monocytogenes protein listeriolysin O (Hly), which allows escape from the intracellular organelle (phagosome), and is deficient in urease C, a molecule that has a role in pH neutralization of the phagosome. Another example is the BCG zmp1 mutant showing increased colocalization with late endosomal markers, suppresses inflammasome activation exhibits facilitated antigen presentation and enhanced immunogenicity of mycobacterial antigens in immunized hosts. Alternatively, these rBCGs can also be expressed and secretes the important T-cell antigens, such as ESAT-6 and CFP-10 which are found in Mtb but not in wildtype BCG[4] or proteins related to latent TB.

The proposed project is to evaluate the quality and potency of various attenuated Mtb preparations as vaccine candidates for TB using microbiology, immunology and molecular biology techniques, such as cultural (CFU), flow cytometry, ELISPOT assays and PCR.

#### Evaluation of foreseeable effects

All new TB candidate vaccines are tested for safety (by collaborators) with safety data either the same or better than wildtype BCG: and are classified as ACGM2 organisms prior to shipment to NIBSC. The proposed project is to evaluate the quality and potency of various rBCG and attenuated Mtb preparations as vaccine candidates for TB.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is sought
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The method is identical to that used for BCG vaccine. Diluted alcohol (70%) or Surfanios/Microsol (5%) will be used for spills and for the clean up of areas after use. All waste will be disinfected prior to being autoclaved before disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee considered that the risk assessment and the proposed containment measures were appropriate for the work. The Minutes of the committee meeting reflect this decision. A report of this Committee's business is reported to the main Health and Safety Committee, membership of which includes management and scientific representatives from across the Institute and Trade Union representatives

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Animal Units

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Project Ref 251/16.1

Date Ackn'd

CU2 Project Title

Study of biology and invasion of Plasmodium falciparum

Class CultureVolClass2 CultureVolumeClass3-4

Class 3 < 1 Litre

Non-GMM Consent Granted

Yes

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

02/03/2022
Project Additional Information

Purposes of the contained use

1. The evaluation of biology and invasion properties of genetically modified P. falciparum
2. There is a low risk of the GM parasites being infectious for the operator, given the absence of the required vector
the only route of infection is via needlestick or similar penetrative injury. However, growth of the parasites to possible
high titres and use alongside wild type parasites directs the work to CL3.

Recipient or parental organism

Plasmodium falciparum (a variety of well characterised laboratory strains and lor recently derived clinical isolates
may be used).
E.coli will be used to construct and screen plasmids, and amplify them to produce quantities required for transfection.
E. coli and Pichia pastoris will also be used to generate recombinant proteins required for in vitro cellular or
biochemical work related to Plasmodium invasion.

Host/vector system

Vectors widely used to transform Plasmodium species may carry either the resistance marker human DHFR mutated
to encode resistance to WR9921 0 or Blasticidin S deaminase gene from Aspergillus terreus to confer resistance to
Blasticidin. These do not affect resistance to anti malarials chloroquine, mefloquine, artemisinin or doxycycline which
can be used (or their derivatives) to treat malarial infections.
Vectors (e.g. pGEX4T, pET28b) for E. coli or (pDUAL, pFASTBACK-HT) S. frugiperda transformation are standard
commerically available vectors encoding resistance to ampicillin or kanamycin for agar plate based selection. They
encode protein tags (such as HIS or GST) for fusion protein expression and purification.
In addition, amplification or synthesis of the genes of interest will be performed for cloning into the appropriate vectors
for propagation.
E.coli will be used to construct and screen plasmids, and amplify them to produce quantities required for transfection.
E. coli and Pichia pastoris will also be used to generate recombinant proteins required for in vitro cellular or
biochemical work related to Plasmodium invasion.

Origin & function
The backbone is P. falciparum

**Evaluation of foreseeable effects**

Parasites may be genetically manipulated in order to:
- i) Alter gene expression,
- ii) Alter translation i.e. protein knockdown,
- iii) Transiently express constructs,
- iv) Enable episomal expression of gene of interest (for complementation or overexpression),
- v) Reporter gene insertion for tagging of gene or protein (eg. fluorescence),
- vi) Gene knockout.

As result of these manipulations the ability to invade and grow in specific red blood cells may be altered. For example, overexpression of genes that are required for invasion could potentially lead to parasites with a better ability to infect selected human red blood cells in vitro. However, it is not expected to significantly increase risk as the proposed genetic modification will not be expected to change the routes of accidental infection (penetrative injury with infected blood) and in conjunction with existing control strategies this remains a very low likelihood event. The modifications are not expected to alter the response to the likely anti-malarial therapy used in the event of a laboratory acquired infection.

Genetic deletion, protein knockdown, and transcriptional inactivation or reduction will most-likely render the parasite less effective at invading host cells and as such will be less pathogenic than the wild type.

The proposed genetic manipulations are not expected to change the susceptibility of the parasite to anti-malaria drugs nor alter parasite biology with exception of the target genes.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None requested

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Inactivation methods currently used for P. falciparum will be applied to genetically modified parasites in accordance with the Code of Practice (B31 & FCS), NIBSC, and HSE regulations. There is no expectation of any change in the efficacy of methods as a result of genetic modification.

Routine inactivation of contaminated materials will be conducted by soaking in a beaker of 10% chloros overnight. The liquid waste can then be disposed of via the sink and the solid waste transferred to autoclave bags for exit via the autoclave route.

**Spillages:**

**Inside the hood**

In the event of a spillage inside the hood, the infectious material must be decontaminated with 70% IMS and mopped up with absorbent material. All absorbent material used to clean up any spillage is disposed of 10% chloros for decontamination overnight. The inside of the safety cabinet must be cleaned thoroughly with 70% Ethanol. Once the spillage is decontaminated a spray cleaner can be used to remove stubborn spots and stains.

**Outside the hood**

All users working in the lab at the time must be immediately made aware of the spillage so that it cannot pose a risk to them. The laboratory manager must be informed immediately. If the spillage is small, it may be diluted with 10% chloros and mopped up with absorbent material and then the area thoroughly cleaned with 70% ethanol. The spillage
must not be left unattended. If a large spillage occurs> 50 mis, the lab must be cleared of users, the laboratory manager informed and sufficient undiluted chloros should be added to inactivate the material. The spillage is contained with absorbent material and left overnight, mopped up and the area cleaned thoroughly with 70% ethanol.

Waste Management:
Routine inactivation of contaminated material is conducted by soaking in a beaker of 10% chlorus overnight. The liquid waste can then be disposed of via the sink and the solid waste transferred to autoclave bags for exit via the autoclave route.
Material for autoclaving is placed in autoclave bags enclosed within autoclave bins; a Browne's tube is placed in a glass universal or bijou in each bin. The bin is autoclaved at 1340 C for 30 minutes on a plastic discard cycle. The autoclave is operated by trained individuals only.
Evidence of effective steam penetration in each autoclave bin is ascertained by checking the Browne's Tube (redgreen colour change). evidence of effective steam penetration the contents of the bin can be disposed of. The autoclaved waste is then be removed and placed into the appropriate bag for disposal (clinical waste is incinerated by third-party contractors).
Laboratory gowns generally do not need to be placed in an autoclave bag within the bin unless known contamination has occurred. If contamination of laboratory gown is known to have occurred then swabbing with absorbent material soaked in 70% IMS will be conducted prior to autoclave.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
The NIBSC Biological Safety sub-Committee reviewed the GM assessment on 19 August 2016. The BSO had made comments prior to this meeting in consultation with the Project Assessor. The Committee was content that the assessment had considered hazards and risks and that the proposed control measures and containment were appropriate for the activity.

Project Containment

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02/03/2022
Project Ref   251/93.1

Date Ackn'd   14/06/1993

CU2 Project Title   INFLUENZA VIRUS GENE RESCUE

Date Project Ceased

Class   CultureVolClass2   CultureVolumeClass3-4

Class 2

Non-GMM   Consent Granted

not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref**  251/93trans

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

*Application for derogation from Schedule 8, Part II, Table 1a (8) - autoclave required in the laboratory suite*

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- **Is an emergency plan required according to regulation 20?** N
- **If yes, tick to confirm that it is attached to this form** N
- **Tick to confirm that you have attached a risk assessment to this form**
- **Tick if you are claiming exemption from disclosure for section of the risk assessment** N

*Please enter comments on the GM safety committee on the risk assessment*
## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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## Project Ref 251/94.4

**Date Ackn’d:** 08/10/1994

**CU2 Project Title:** CONSTRUCTION AND USE OF THE SEMLIKI FOREST VIRUS BASED VECTORS CONTAINING RECOMBINANT RETROVIRAL VECTORS

**Class:** Class 2

**CultureVolClass2:**

**CultureVolumeClass3-4:**

**Non-GMM Consent Granted:** not applicable

**Project notified under transitional arrangements:** Y

**Withdrawn:** N

**Tick if notifying a connected programme of work:** N

## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 251/96.2

Date Ackn'd  02/03/2022  CU2 Project Title
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 251/99.1

Date Ackn’d 16/03/1999

CU2 Project Title SAFETY STUDIES OF MAMMALIAN AND AVIAN ENDOGENOUS RETROVIRUSES

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 251/99.2

Date Ackn'd 26/07/1999

CU2 Project Title
IMMUNOGENICITY OF GENETICALLY MODIFIED HELICOBACTER PYLORI

Class 2

CultureVolClass2

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Project Ref: GM43/01.4

Date Ackn'd: 01/01/2015

CU2 Project Title: EXPRESSION OF INDIVIDUAL HSV GENES IN EUKARYOTIC VIRAL VECTORS

Class: Class 2

CultureVolClass2: 1-50 Litres

CultureVolumeClass3-4:
Date Project Ceased

Historical Significant Changes
Transferred from GM43 on 01/01/2015

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To investigate the functions of herpesvirus gene products in virus replication and pathogenesis.

Recipient or parental organism
Adenovirus 5, Amphotropic retrovirus, Vaccinia virus: coding sequences derived from mammalian alpha or beta herpesviruses.

Herpes simplex virus, Human cytomegalovirus: ‘reporter genes’ (eg LacZ, firefly luciferase, green fluorescent protein).

Host/vector system
Eukaryotic viruses (ACDP group 2)/mammalian cell lines (established, commercially available).

Origin & function
Origins: Sequences will be sub-cloned from pre-existing plasmid clones. In some instances herpes virus sequences will be isolated from purified viral DNA.

Functions: The biological properties of herpes virus genes are considered in the detailed risk assessment. These genes will be expressed in mammalian cell lines (via eukaryotic vectors) to examine the location and trafficking of herpes virus proteins, or to provide helper functions for disabled herpesviruses. ‘Reporter genes’ will be inserted into herpesvirus genomes to inactivate specific virus genes and to identify the resulting disabled or attenuated viruses.

Evaluation of foreseeable effects
The insertion of ‘reporter genes’ into viral genomes is expected to reduce the replication capacity of the recipient virus or to reduce its virulence. No increase in virulence, alteration of tissue tropism or modification of host range is predicted.

Herpesvirus genes act in concert to achieve virus replication. Some of the gene products are known to be cytotoxic but these are not secreted and only the expressing cell is expected to be affected. Mammalian alpha and beta herpes viruses are not oncogenic and transforming genes have not been identified in these viruses. Herpesvirus gene function is, however not fully understood and some herpesvirus genes are known to evade or modify host immune responses. The eukaryotic vectors used are either fully disabled or heavily attenuated. No change in tissue tropism or host range of the vectors expressing herpesvirus genes is foreseen (see detailed risk assessment).

The assessment to human health indicates no greater hazard than those of the wild type unmodified virus.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard validated methods will be used:

Liquids: made 2000ppm available chlorine (5% chloros) and disposed via drains after a minimum of 12 hours.

Mixed disposable waste: Validated autoclave, 121 degrees/30 min, then disposed as domestic waste.

Disposable pipettes: Either a) as for mixed disposable waste or b) immerse in solution of 2000ppm available chlorine (5% chloros) for minimum 12 hours, followed by incineration.

Re-usable materials: Autoclave, 121 degrees/30 min.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

I had forgotten to inform you that the local ACGM committee had reviewed and approved the 6 applications.

Project Containment

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02/03/2022
GM Centre Number: 253

Data Premises Notified (Originally) 05/11/1987

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Name

UNIVERSITY OF CAMBRIDGE

Name 2

Department

PHYSIOLOGY, DEVELOPMENT & NEUROSCIENCE

Campus Estate or Research Centre

Building

Road Name

DOWNING STREET

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB2 3EG

Country

ENGLAND

Tel Number 01223 333899

Fax Number 01223 333840

E-mail

HSE Division EAST AND SOUTH EAST

Comments

GM402 MERGED WITH THIS CENTRE ON 12/1/2006.

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial
Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  
Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Additional Information

#### Purposes of the contained use

Adenovirus vectors (Ad5, E1-E3-) encoding Cre recombinase and/or marker genes will be obtained from a commercial source and used to transduce mouse cells in culture in order to delete specific genes.

#### Recipient or parental organism

Primary cells from lab mice: no risk.

#### Host/vector system

Adenovirus vector is itself of no risk as the essential E1 gene is deleted so it cannot replicate. However, homologous recombination in helper cells during production can recreate a low level of replication-competent adenovirus (Ad5, wild-type except for E3 gene deletion; the transgene would be lost), for which the manufacturer can only specify an upper limit of 1 in 10^4 particles. Adenovirus is a mild human respiratory pathogen.
Cre recombinase (for excising genetically tagged genes); Green Fluorescent Protein; beta-Galactosidase (for labelling cells). All non-hazardous.

**Evaluation of foreseeable effects**

The GMOs are not expected to give any effects on human health nor on the environment. The only risk is from possible low levels of replication-competent wild-type adenovirus, which is a mild human respiratory pathogen. The only risk is from the original vector stock as Ad5 cannot replicate in mouse cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Treatment of liquid and plastic waste with 2% Distel or Virkon for 1hr; surface wipe down with 70% ethanol; UV exposure, waste disposal via autoclave. Effectively 100% kill.

**Project Containment**

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The purpose of the project is to understand the movements and interactions of bacteria and immune cells during infection in a zebrafish model.

Recipient or parental organism

Pseudomonas aeruginosa (PAO1, PA14, PAK) and Pseudomonas alcaligenes. This is an opportunistic category 2 human respiratory pathogen. Immunocompromised individuals, individuals with lung disease (e.g. cystic fibrosis) are susceptible to infection. Otherwise healthy individuals come into contact day-to-day with this pathogen without any problem and disease development. Salmonella enterica serovar Typhimurium (ATCC SL1344, NTCC 12023, SL1027). This is an opportunistic category 2 human pathogen and Gram-negative bacterium that is a common source of food poisoning. Staphylococcus aureus (ATCC SL1344, NTCC 12023, SL1027). This is a gram-positive coccal bacterium. S. aureus bacteria are frequently found in the nose, respiratory tract and on the skin. Although S. aureus is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections and food poisoning.

Host/vector system

Plasmids (non-mobilisable PUC or pBR322 derivatives, pGEM-T), phages (P22, Φ11)

Origin & function

We will use mutants that are well characterised and with similar or attenuated virulence and we will use strains in
which a fluorescent protein has been inserted for visualising the bacteria by confocal microscopy. The expression of a fluorescent protein does not increase the virulence of the bacteria.

**Evaluation of foreseeable effects**

The GMOs are not expected to have increased virulence but will carry all the risks associated with the wild type strains which are hazard group 2 pathogens. Therefore appropriate containment measures will be taken, described in detail in the attached risk assessment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste and cultures will be disinfected with 1% Virkon (or equivalent commercial agent) for several hours (according to the manufacturer Virkon is proven to inactivate all these pathogens and many more within a minimum of 10 minutes). Virkon is preferable over bleach due to the fact that it is much less toxic and safe to handle. It also has a built-in dye indicator of biological activity of the target sample. Solid waste will be collected in appropriate collection bins for biological waste and then will be autoclaved and incinerated. Expected degree of killing is 100%

**Is an emergency plan required according to regulation 20?**  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

**Please enter comments on the GM safety committee on the risk assessment**

The risk assessment has been reviewed by a biological safety committee of the PDN Department and a biological safety committee of the Pathology department. Several experts on these pathogens have provided their input and judged that the proposed containment is appropriate for the work. A room has been provided for this type of containment level (CL2) work in the Department of Physiology, Development and Neuroscience. While awaiting establishment of this room, the work will be temporarily carried out in the Department of Pathology in established CL2 rooms with the supervision of experienced staff.

**Project Containment**

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### Project Additional Information

**Purposes of the contained use**

Our laboratory aims to understand the biology of neurodegenerative diseases such as tauopathy and Huntington's disease. To this end we need to be able to study potential modifier genes for these diseases by knockdown and overexpression. To study genetic modifiers, we must introduce DNA or RNA into the mature tissue. In order to get efficient distribution of DNA or RNA into the CNS and retina, we need to use lentivirus to allow the DNA/RNA to be stably expressed within the tissue. The lentiviral vector (carrying the transgene of interest), integrates into the genome of the cell of the host and is therefore stably expressed for the lifetime of that cell. The virus cannot be shed by the cell and therefore cannot spread or be released and therefore there is no transmission risk to other organisms.

**Recipient or parental organism**

Lentivirus particles will be injected into zebrafish (Danio rerio) larvae, therefore zebrafish larvae are the final recipient. Injected larvae will be culled following injection therefore no progeny will be produced and there will not be any germine integration of the integrated DNA/RNA.
In order to generate lentiviral particles, we will work under GM678/12.2. Mammalian 293T cells will be transfected with a three plasmid system where virus replicative, packaging and envelope functions are split over three plasmids and virus particles will be purified for subsequent delivery into the recipient (zebrafish larvae).

**Origin & function**

cDNA or RNA for genes encoding the following will be delivered by lentivirus:

**Health and Safety Executive**

a) cDNA or guide RNA for genes encoding potential modifiers of neurodegenerative disease and related conditions, including forms of tauopathy, Huntington's disease and other dementias to study their affect on disease progression
b) cDNA or guide RNA for genes encoding potential modifiers of autophagy to study their effect on disease progression
c) markers and reporters derived from fluorescent proteins to label intracellular components and monitor them in vivo

**Evaluation of foreseeable effects**

a) Genetic modifiers of neurodegenerative diseases
The expression of genes involved in neurodegenerative diseases may be cytotoxic to the cells which integrate lentivirus (i.e. neuronal cells in the host zebrafish tissue) as these genes are expected to modify (i.e. ameliorate or exacerbate) late onset neurodegenerative diseases (i.e. host may be a wildtype or a genetically modified zebrafish that has a neurodegenerative phenotype). The spread of the virus is limited to the cells in vicinity to the site of injection. It has been shown in rodents that 1 μL injected volume spreads approximately 1-2 mm in brain (Parr-Brownlie et al., 2015), therefore by extrapolation, we would expect that 100 nL volumes used in our study would typically spread 100 - 200 μm in the host tissue. The recipient zebrafish larvae will be culled within 10 days of lentiviral injection for post-mortem analysis.

b) Autophagy modulators and c) reporters
The action of the expressed proteins or guide RNAs which modulate autophagy is unlikely to be cytotoxic in wildtype cells and the fluorescent reporter constructs are expected to be biologically inert. None of the genes to be used are known oncogenes and are therefore extremely unlikely to foster tumour progression. The expression of any of these genes, even in the long term, in a minor population of cells at the likely sites of exposure is unlikely to be harmful and expression would not spread beyond exposed tissues.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The virus cannot replicate and does not spread from the host cell to other cells. It is not possible for the virus to escape into the environment to affect animals or plants. The recipient zebrafish larvae will be culled at the end of the experiment and will be kept in the containment level 2 room in an environmentally controlled incubator for the duration of the experiment. They will not be returned to the animal facility and will not be used to generate offspring. All equipment will be treated with Virkon after use and solid waste material (e.g. disposable plasticware, gloves) will be incinerated.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None applied for

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All disposable labware (e.g. disposable plasticware, gloves) will be autoclaved and incinerated. Liquid waste will be
treated with 1% virkon. Permanent laboratory equipment (e.g. hood, microscope, incubator, bench surfaces) will be wiped with 70% ethanol at the end of experimental work.

This risk assessment has been reviewed and approved by the GM safety committee for the Department of Physiology, Development and Neuroscience and been approved by the Biological Safety Officer of the School of Biological Science. A suitable room available to carry out this work.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form Y

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)  
Tick if confidential

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
Virology | Transgenic Animals | Transgenic Fish | Gene Therapy
---|---|---|---
Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

The largest volume of culture used will be 250 ml. The work will be confined to Lab 6.42 James Parsons Building, Byrom Street Campus, Liverpool John Moores University. Waste will be bagged and subjected to autoclaving.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

02/03/2022
GM Centre Number: 260

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**Name**

BRITISH AMERICAN TOBACCO

**Name 2**

R & D CAMBRIDGE

**Campus Estate or Research Centre**

210 CAMBRIDGE SCIENCE PARK, MILTON RD

**Road Name**

210 CAMBRIDGE SCIENCE PARK, MILTON RD

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 0WA

**Country**

ENGLAND

**Tel Number**

01223 420284

**Fax Number**

01223 423448

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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The maximum culture volume that could be released at any one time is 10 L.

Waste Disposal

Liquid cultures: 10% hypochlorite is added to give a final concentration of approximately 1% and the solutions are left to stand for a minimum of 2 hrs before disposal. Experiments have shown this is much greater than the time and concentration required to kill the bacteria we use.

Solid waste is placed in sterilin bags and autoclaved. Each bag is numbered and the disposal recorded. Browns tubes in the centre of the autoclave load are used to check for satisfactory autoclave conditions.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
| Data Premises Notified | 21/03/1988 | Transferred from 1992 Regs? | Y | Transitional Premises Class | 1 |
| Data Premises Closed | N | Emergency Plan Required? | | Non-GMMs | N | Withdrawn | N |

**Name**

| BLOND MCINDEE CENTRE FOR MEDICAL RESEARCH |

**Name 2**

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**Campus Estate or Research Centre**

**Road Name**

**Town**

| EAST GRINSTED |

**County**

| WEST SUSSEX |

**Postcode**

| RH19 3DZ |

**Country**

| ENGLAND |

**Tel Number**

| 01342 313088 |

**Fax Number**

| 01342 301701 |

**E-mail**

**HSE Division**

| EAST AND SOUTH EAST |

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify) Tick if confidential

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
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**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
| **Data Premises Notified (Originally)** | **23/03/1988** |
| **Transferred from 1992 Regs?** | **Y** |
| **Transitional Premises Class** | **2** |
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| **Transitional Premises** | **N** |
| **Emergency Plan Required?** | **N** |
| **Non-GMMs** | **N** |
| **Withdrawn** | **N** |

**Name**

**CENTRE FOR REPRODUCTIVE BIOLOGY**

**Name 2**

**MRC HUMAN REPRODUCTIVE SCIENCES UNIT**

**Campus Estate or Research Centre**

**QUEEN'S MEDICAL RESEARCH INSTITUTE**

**Road Name**

**47 LITTLE FRANCE CRESCENT, OLD DALKEITH RD**

**Town**

**EDINBURGH**

**County**

**EAST LOTHIAN**

**Postcode**

**EH16 4TJ**

**Country**

**SCOTLAND**

**Tel Number**

**0131 242 6200**

**Fax Number**

**0131 228 5571**

**E-mail**

**HSE Division**

**SCOTLAND**

**Comments**

**MOVED TO NEW PREMISES ON 8TH JULY 2002 ALL ACTIVITY TRANSFERRED TO GM207 01/03/2011**

**Date at Which Additional Info Submitted**

**15/05/2002**
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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### Level 4 (GMMs)

### Non-microbial

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#### Bacteriology
- Parasitology
- Transgenic
- Birds

#### Virology
- Transgenic
- Animals
- Transgenic
- Fish

#### Mycology
- Transgenic
- Invertebrates
- Transgenic
- Plants

#### Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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DATE PROJECT CEASED

02/03/2006

TRANSGENE EXPRESSION TO PITUITARY LACTOTROPH CELLS

Non-GMM

Consent Granted

Not applicable

Project notified under transitional arrangements

Y

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

PROJECT CLOSED DOWN 2/03/06

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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**Project Ref 263/01.2**

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<th>CultureVolumeClass3-4</th>
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<td>Class 2</td>
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**Historical Significant Changes**

PROJECT DOWNGRADED TO CLASS 1 BY THE NOTIFIER 2/3/06.
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
This risk assessment covers a connected programme of work that encompasses the research of the Centre for Reproductive Biology (MRC Human Reproductive Sciences Unit and University of Edinburgh Department of Reproductive and Developmental Sciences).

Introduction

Reproduction encompasses many physiological and molecular processes. These include transcription, mRNA stability, translation and post-translational control. Second messenger signalling utilising G-protein coupled receptors and hormones. Biosynthesis and secretion of hormones or growth factors from highly specialised endocrine regulated tissues or gametes requires regulated synthesis and release and the interplay of numerous factors.

The aim is to manipulate the genetic material of reproductive tissues, organs and relevant cell-lines to create models that will be used to research reproduction and reproductive processes. Genetic material will be obtained from tissues and organs that impact on reproduction these including; testes, ovary, prostate, pituitary, hypothalamus, uterus and breast. Experiments will investigate what impact removing and adding back genetic information will have on the eventual reproductive phenotype. To achieve this, experiments will be conducted in vitro on cultured cell-lines, and in vivo on specified animal models using genetically modified viruses. These will either be self-inactivating non-replicating viruses that integrate into the host DNA, or attenuated adenoviruses that do not integrate. However, the proposed lentivirus...
Vectors carry a woodchuck post-translational response element (WPRE) as part of the vector backbone and this element is thought to be inherently oncogenic due to the presence of a sequence that expresses the WHV X protein. Injection of lentivirus with the WPRE into mice caused liver tumours. This suggests that the vector backbone is inherently oncogenic, before it is manipulated to express genetic material.

The particular focus of Herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV) will be used to study the mechanisms whereby these viruses interact with the infected host cell with the aim of investigating regulation of translation, which is particularly important in gametes. These studies will focus, in particular, on the regulation of viral messenger RNA translation by the host protein synthesis machinery and the role therein of essential viral regulatory proteins and their interactions with host cell factors. These studies will be carried out by infecting cultured cells with both wild type virus and genetically modified virus in which relevant viral genes have been partially or completely inactivated. Some mutant virus will carry foreign DNA sequences encoding beta-galactosidase and green fluorescent protein (GFP) and its derivatives, either to replace viral genes or as fusions to viral genes as a tool to monitor their expression. Infected cells will subsequently be lysed in detergent, thus inactivating the virus, and processed for biochemical and molecular biological analysis.

### Recipient or parental organism

**Bacteria:** E coli K12 (disabled)

**Primary cells:** derived from humans, mouse, rat and sheep

**Immortalised cell-lines:** HEK293 cells and derivatives BHK, HeLa, Vero cell lines (for HSV-1) HFF2 cell lines (for HCMV)

**Animal models:** Sheep, marmoset, rat, and mice.

### Host/vector system

**Retrovirus:** gag/pol = vector 1; Env = vector 2;

Delivery and cloning vector either VSV-G or MoMLV = vector 3

**Lentivirus:** gag/pol = vector 1 (containing WPRE); Rev = vector 2

Delivery and cloning vector VSV-G = vector 3

**Adenovirus:** shuttle vector containing gene of interest and adenovirus backbone

### Genetic material will either be synthetic (PCR amplification product) or derived by cloning of cDNA or genomic material from a reproductively relevant tissue. Thus these genes will be targeted to specialised endocrine regulated tissues to assume full effect. A wide spectrum of material is being investigated, including known oncogenes e.g. c-myc and growth factors e.g. activin subunits

Some HSV-1 and HCMV modified virus will encode beta-galactosidase and green fluorescent protein as reporter proteins to monitor expression of viral genes in infected cultured cells. These sequences and their products are not associated with any health and safety risk.

### Functions being investigated include; apoptosis, cell growth, cell differentiation, cellular transformation and cell communication focusing on reproductive processes, virus host interaction focusing on viral lytic cycle and post-translational regulation.

### Evaluation of foreseeable effects

E coli K12 and cell-lines are non-harmful and are unable to survive unless held in lab conditions.

Once packaged the virus particles (adeno, lent, HSV and CMV) will be capable of infecting a number of different species, including human. The GM viruses will be less fit than wild type virus, thus production of wild type virus either deliberately (HSV type 1, see below) or by recombination of the E1a gene from the HEK293 cell genome into the adenoviral genome will produce fitter virus. Stocks of adenovirus will not be repeatedly passaged to counteract the production of wild type, which is likely to occur 1 in 1x10e10.

Adverse effects for retroviruses have been reported from gene therapy trials where large numbers of virus (1x10e11) have been specifically infected into cells, selected for genomic integration using antibiotics then the proviral carrying cells injected into the immune compromised recipient. These individuals were initially immune-compromised, so it is unlikely this would happen in healthy individuals even if exposed to high titres (1x10e9/ml) since the virus would have to be injected directly into the blood stream and then viral integration would have to be specifically selected for.

If viral infection does occur then adverse effects are likely to be low. The viruses self-inactivate once integrated into the genome, and cannot re-activate due to deletion of...
the U3 in the 3'LTR. Thus even if infected with wild-type virus, eg an individual was carrying a latent HIV infection then the provirus cannot mobilise and propagate the vector or the inserted genetic material. Although activation of genes adjacent to the integration site is possible as discussed above, this cannot be propagated into a clonal population and instead the infected cells are likely to slough off in the respiratory tract and eye. Percutaneous injection of virus is possible, but this would require injection of large amounts of high titre virus. Since it is anticipated that viral titres will not exceed $1 \times 10^{6}$ per ml then the likelihood of large scale integration and oncogenic activation of cellular genes is low. Insertion of oncogenes into these viral vectors poses an additional risk since expression of these will be driven from constitutively active promoters. Adenovirus infection is most likely to occur by inhalation but again in immune competent individuals this is unlikely to generate a persistent infection, instead cells will be sloughed off.

All activities required for propagation of virus will be done wearing gloves and the production of aerosols will be minimised wherever possible and use of sharps and glassware will be avoided or minimised wherever possible. When producing high titre virus preparations eye protection will be worn. HSV-1 is a common human pathogen for which infection occurs by direct contact with skin or eyes. Worldwide, greater than 40% of the population is seropositive for HSV-1. Accidental infection in the laboratory can occur by splashes of infected material to a mucosal surface or by viral entry through broken skin. Except in immuno-compromised individuals, HSV-1 infection is limited to epithelial cells, in the order of several million, at the infection site, resulting in the appearance of fluid filled vesicles (cold sores) and to the sensory ganglia that innervate the site. Infection of sensory ganglia leads to a life-long latent infection which is asymptomatic. Reactivation, usually triggered by cellular stress, can occur, whereby the virus travels from the ganglia to the site of primary infection and again productively infects several million cells. Herpes infections of the eye are associated with keratoconjunctivitis and repeated recurrences can lead to scarring of the cornea. In very rare cases, approximately one in a million, herpes infection can lead to encephalitis, which if untreated, has a mortality of greater than 70% and most survivors are neurologically impaired. Herpetic whitlow is an infection caused by herpes simplex virus that affects health care workers. The source of infection is generally thought to be damaged cuticle or skin exposed to HSV infected secretions. This results in a short period of symptomatic infection and the establishment of latency. In the laboratory single acute dose exposure by accidental percutaneous injury could result in herpetic whitlow. Antiviral agents for HSV-1 are acyclovir (Zovirax), valacyclovir and famcyclovir.

HCMV is common in the population (in excess of 40% seropositive worldwide). Infection after birth is usually asymptomatic but sometimes a syndrome resembling infectious mononucleosis or glandular fever is caused by the virus. After initial infection the virus establishes a lifelong asymptomatic latent infection primarily in peripheral blood leukocytes. HCMV infection can lead to serious illness or death in immuno-compromised individuals and transplacental transmission during pregnancy can lead to severe neurological damage or death of the foetus. Infection in the laboratory could occur by exposure of cutaneous or mucosal surfaces to infected material or exposure by percutaneous injury. The antiviral agent gancyclovir reduces or interrupts viral replication in vivo. All activities involving viral stocks and infected cell culture will be carried out in a biological safety cabinet whilst wearing gloves and avoiding the use of sharps and glassware. Genetically modified HSV and HCMV viruses, depending on the nature of the modified gene and the severity of the mutation, will have either wild type or reduced infectivity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All GM modified animals are housed in purpose built barrier containment facilities. These facilities are kept locked and are kept secure by restricted digital swipe card access. In addition these key facilities are restricted for general access by non-essential personnel.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM WASTE DISPOSAL

Apart from following the normal waste disposal procedures currently in practice within the building, the following rules must be observed:

(a) All solid and sealed liquid waste contaminated with GM material [eg. plastic pipettes, plates, small volumes of sealed liquid cultures, gloves, tips etc.] should be placed in a metal autoclave container lined with either a clear or blue plastic bag. The autoclave is regularly tested, and disposal of GM material via autoclaving, in a tested autoclave, meets the requirements of the GMO (2000) legislation. Autoclaving effectively gives 100% degree of kill and is validated annually. Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment, or disinfect with 10,000ppm available chlorine (Presept tablet: sodium dichloroisocyanurate, or Virkon at an equivalent concentration) for a minimum of 30 minutes in line with guidelines previously provided by the manufacturer.
Disinfection - the concentrations and contact times used for disinfection have been shown to result in > 5 log reduction in virus titres (sodium hypochlorite - Croughan and Behbehani 1988 J. Clinical Microbiol 26, 213-215, Virkon - manufacturers data) and a 1% solution of Virkon disinfectant with a contact time of at least 20 mins gives a >99.999% kill of all viruses (data supplied by manufacturer). Discharge any resulting liquids to drains, dispose of solids via clinical waste stream for heat treatment. After autoclaving all waste MUST be placed in an ORANGE bag, and disposed of via the normal clinical waste stream.

(b) Glassware must be decontaminated by soaking in 1% w/v Virkon or 1000ppm Presept [four 0.5g tablets per one liter or four 2.5g tablets per five litres] for 1 hour before being rinsed and placed in the glasswash bucket provided for subsequent incorporation into the general washing process. Uncontaminated broken glassware should be disposed of in the glass waste containers supplied. Empty gas canisters should only be placed in the labeled bin. GM contaminated broken glassware and sharps should be placed in a sharps bin (eg needles, syringes, scalpels etc) and then autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via clinical waste stream for heat treatment.

(c) Large volume, contaminated liquids must be rendered safe by the addition of Presept tablets to a final concentration of [1000ppm], which is 10,000ppm available chlorine (Presept tablet: sodium dichloroisocyanurate, or Virkon at an equivalent concentration) for a minimum of 30 minutes in line with guidelines previously provided by the manufacturer, then discharged to drains. Liquids (eg samples, culture supernatants, tissue culture media etc) can also be autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

Please enter comments on the GM safety committee on the risk assessment

Staff representative UofE: asked for additional explanation to be inserted into introduction detailing why lentivirus carrying a woodchuck promoter response element (WPRE) is designated as GM level 2.

Staff representative MRC: Wanted clear listing of recipients/hosts especially primary cell-lines. Asked for clarification on containment of aerosols? Agreed to insert clause stating that protective eyeware will be worn at the bench and that all virus to be used at the bench will be aliquoted into screw cap tubes to demarcate them as GM level 2.

UofE Biological Safety Officer: What measures will be put in place to protect pregnant workers and workers with long-term health problems that may also be immuno-compromised? It was agreed that the existing GM competency form will have clauses inserted to state the risks and workers will be asked to sign these before commencing work.

QMRI Safety advisor: Requested that the waste disposal measures included clear statements on use of sharps and the autoclave cycle used.

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L2 Yes</td>
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### GM Centre Number: 264

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**Date at Which Additional Info Submitted:**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial
Other (please specify) Tick if confidential

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Bacteriology  Parasitology  Transgenic  Microbiology  Research
Virology  Transgenic  Transgenic  Gene Therapy
Animals  Birds  Fish
**Project Ref**  264/97.1

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- **Class 3**
- **Consent Granted**
  - yes

- **Project notified under transitional arrangements**
  - Y

**Withdrawn**
- 

**Historical Significant Changes**
- GM264/00.1,GM264/01.1

**Historical Date of Additional Info**
- 01/03/2000, 06/04/2000,

**Project Additional Information**

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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If yes, tick to confirm that it is attached to this form N

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Please enter comments on the GM safety committee on the risk assessment

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**Name**

**SBA & LONDON HOSPITAL MEDICAL COLLEGE (QMX)**

**Name 2**

**QUEEN MARY & WESTFIELD**

**Campus Estate or Research Centre**

**Road Name**

**WHITECHAPEL ROAD**

**Town**

**LONDON**

**Building**

**5TH FLOOR ALEXANDRA WING**

**District**

**Tel Number** 0207 377 7000

**Fax Number** 0207 377 7636

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<td>Transgenic Birds</td>
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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum culture volume 5 L, treated O/N with chlores/bleach - small sample to culture plate dispose.
Smaller cultures autoclaved 20 minutes at 125 degrees C (plates etc.) and then incinerated.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 266/01.1**

**Date Ackn'd** 07/09/2001

**CU2 Project Title** ASSESSMENT OF VIRAL TRANSFECTION OF CELLS

**Class** Class 2

**Culture Vol** < 1 litre

**Consent Granted** not applicable

**Non-GMM** not applicable

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**
Purposes of the contained use

To judge expression of transgenes from constructed viral vectors in primary and established cell lines.

Recipient or parental organism

Human and rodent primary cells.

Host/vector system

replication deficient adenovirus,
replication deficient strain of equine infectious anaemia virus (lentivirus)

Origin & function

Transcriptional factors and similar markers are involved in ontogeny. When expressed in the correct temporal manner during cellular development they direct differentiation.

EGFP (enhanced Green Fluorescent Protein), and improved variant of GFP which was originally isolated from Aequorea victoria, - a jellyfish. This protein has no biological effect other than fluorescing when illuminated.

Evaluation of foreseeable effects

There are no recorded cases of human or rodent infection by equine infectious anaemia virus (EIAV).

EIA vectors are designed to be replication defective. In EIAV vector system the envelope packaging component is the VSV-G protein, derived vesicular stomatitis virus (a rhabdovirus).

The VSV-G sequence has no homology with the vector genome or gag/pol construct, making formation of a replication competent virus by recombination very unlikely.

Since VSV-G pseudo-typing of EIAV retroviral core particles allows their entry into human cells, the EIA vector systems should be classified as class 2.

The adenovirus family (Adenoviridae, genus Mastadenovirus) is divided into specific subgroups. We shall employ proviral DNA from replication deficient Adenovirus (serotype 5, group DE1/DE3), under the control of the cytomegalovirus -1-E promoter/enhancer (CMV). This group (DE1/DE3) of adenovirus is completely non-pathogenic.

In the adenovirus vector system a packaging cell line expressing the E1 protein in trans is required. Since adenovirus vectors retain their ability to enter human cells, the adenovirus vector system should be classified as class 2.

The systems have been designed to permit entry to human cells. The replication deficiency will ensure no systemic infection could occur in an exposed individual however local change cannot be eliminated in the event of direct contact. This will be controlled by use of gloves and good microbiological practice at containment level 2.

The inserts are not harmful.

Primary human and rodent cells and transformed human cells do not survive well in the environment.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

100% kill by autoclave. The autoclave is maintained on contract and each load is monitored by thermocouple. Charts are inspected for deviation from normal. Aberrant behaviour would initiate investigation and re-autoclaving. At monthly intervals the continuing efficacy of the system is checked with thermalog devices within waste sacks.

When cool, autoclaved culture liquid is discharged via the laboratory drain. Solid waste in sacks is removed from the laboratory area and compacted prior to collection by an approved WasteContractor
The committee are informed that full level 2 containment will be required and this is appropriate for culture of primary human cells. The cells are derived from normal healthy individuals and tested for absence of HIV and HepB/C. The GM alterations do not substantially alter the risk of harm from modified cells.

Meeting 5 September
The main element of risk is with the virus (vectors). EIAV, Equine infectious anaemia is not pathogenic to humans but is level 2 on account of environmental hazard. Adenovirus produces non symptomatic infection in human and is also assigned to level 2. Both viruses have been specifically disabled.

There is no risk of oncogenic hazard on local injection. The transcription factors only have effect in suitable target cell populations. General contact, such as with muscle or skin & other epithelial cells, would have no effect for these cells are already differentiated.

The two assessments are linked for HSE notification for which the current fee is 440. The project in future may include other differentiated target cell types.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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**Animal Units**

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**Project Ref** 266/02.1

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**Non-GMM**

<table>
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<tr>
<td>not applicable</td>
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Project notified under transitional arrangements N
### Project Additional Information

**Purposes of the contained use**
- To generate replication defective adenoviral vectors.
- To use the adenoviral vectors to transduce target cells.
- To assess the expression of transgenes from the constructed adenoviral vectors in primary and established cell lines.

**Recipient or parental organism**
- Bone marrow stromal cells of human origin.
- Primary cardiomyocytes, neurons, bone marrow cells, hepatocytes, kidney cells and fibroblasts of rodent origin.
- Various laboratory cell lines such as HeLa, CHO, HEK293 etc.

**Host/vector system**
- Replication defective adenovirus.

**Origin & function**
- The transcription factors Nkx2.5 and GATA4 are involved in directing the development of stem cells. When expressed in the correct temporal manner during cellular development they direct differentiation into cardiac cells.
- They would not be expected to exert an effect on adult cells (fully differentiated cells).
- The promoter regions of MHC, ApoA2, and the NFB response elements are DNA sequences. These would not be expected to exert an effect on adult cells.
- GFP (and variants that have been developed to improve fluorescence characteristics) has been isolated from a jellyfish. The protein fluoresces when illuminated with light of an appropriate wavelength. The protein has no known adverse biological effects and is therefore not expected to exert an effect on adult cells.
- MAPKAP is a protein involved in a signal transduction pathway. There is no published data to suggest that overexpression of this protein (eg. as under transcriptional control of the CMV major IE promoter) will exert any adverse effects on adult cells.

**Evaluation of foreseeable effects**
- The adenovirus family (Adenoviridae, genus Mastadenovirus) is divided into specific subgroups. We shall employ proviral DNA from replication deficient Adenovirus (serotype 5, group DE1/DE3), under the control of the cytomegalovirus - 1-E promoter/enhancer (CMV). This group (DE1/DE3) of adenovirus is completely non-pathogenic. In the adenovirus vector system a packaging cell line expressing the E1 protein in trans is required. Since adenovirus vectors retain their ability to enter human cells, the adenovirus vector system should be classified as class 2.
- In generating the recombinant adenovirus vectors there is a remote possibility of generating replication competent adenovirus (RCA). This occurs as a result of...
recombination between the E1 expressed in trans from the packaging cell line and the E1 deleted virus. Continued expansion of the recombinant virus increases the incidence of RCA. To avoid the generation of RCA and to limit the occurrence of RCA, viral stocks will be generated from the first expansion virus.

The replication deficient adenovirus system has been designed to permit entry to human cells. The replication deficiency will ensure no systemic infection could occur in an exposed individual however local change cannot be eliminated in the event of direct contact. This will be controlled by use of gloves and good microbiological practice at containment level 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not required.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

100% kill by autoclave. The autoclave is maintained on contract and each load is monitored by thermocouple. Charts are inspected for deviation from normal. Aberrant behaviour would initiate investigation and re-autoclaving. At monthly intervals the continuing efficacy of the system is checked with thermalog devices within waste sacks.

When cool, autoclaved culture liquid is discharged via the laboratory drain. Autoclaved solid waste in sacks is removed from the laboratory area and compacted prior to collection by an approved Waste Contractor.

**Is an emergency plan required according to regulation 20?**

N

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

Y

**Tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

Y

**The experimental protocol will avoid generation of RCA and limit the occurrence of RCA by generating viral stocks from first generation modified virus.**

**Project Containment**

<table>
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<tr>
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</tr>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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02/03/2022
Project Additional Information

Purposes of the contained use

Re-identification of an assay test system component with object of either:
continuing with use of this component in an assay if it a non-bioactive fragment of E7 (in which case the assessment will revert to class 1, low risk without notification requirement) or
if it is a full length oncogenic E7, to use the component for a limited time while the assay is run in parallel with a substitute to ensure continuity/overlap of the data sets.

Recipient or parental organism

E.coli TG1

A disabled host of the K12 family, unable to survive in the human gut. Requires thiamine to grow.

F' traD36 lacLq(lacZ)M15 proA+B/supE (hsdM-mcrB)5 (rk-mk+McrB-

Host/vector system

E. coli TG1 and pGEX-4T-1, (GenBank Accession No. U13853

Origin & function

Data available indicates the genetic material involved, a GST-E7 fusion, was chosen as a positive control in a QA procedure because it was conveniently at hand during development (in a USA lab) 1993.
It is probable that it is an E7 fragment which would be considered unlikely to cause harm. We currently do not have evidence for this.
An alternative (GST fusion) has already been tried in the assay with poor assay results.

E7 is the oncogenic Human Papillomavirus protein.

Evaluation of foreseeable effects
Delivery of whole E7 is unlikely even if it is present. If it did via inoculation there is low probability of transformation at site of injection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
100% kill by autoclave. The autoclave is maintained on contract and each load is monitored by thermocouple. Charts are inspected for deviation from normal. Aberrant behaviour would initiate investigation and re-autoclaving. At monthly intervals the continuing efficacy of the system is checked with thermalog devices within waste sacks.

When cool, autoclaved culture liquid is discharged via the laboratory drain. Autoclaved solid waste in sacks is removed from the laboratory area and compacted prior to collection by an approved Waste Contractor.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
May 02
Project is to grow and establish sequence of this insert. If it is a fragment of E7 then production will continue to use it because it is not harmful to humans or environment. If it is a full E7 then production will use it for a limited time (6 months) while an alternative low risk GST fusion is sourced and proven in the required tests.

/initial comment on circulation to GM Committee
1. As written this assessment should be a containment level 2. Both the Brenner classification and the containment for a potentially oncogenic expressed protein would indicate 2.
2. If the construct is for QC of antiGST antibody, why is this being done on a fusion with a potentially oncogenic protein? Is this just one of a panel of fusion proteins being used? Many other GST fusions with biologically safe proteins must be available. We probably have some being made in one of our current project - let me know if you would like a contact name.

22 July 2002 Committee comments.
The incorporation of the oncogenic papilloma virus E7 protein into the GST fusion (if indeed present - as the purpose of the proposal is to establish whether or not a functional E7 is present) raised the Damage Factor on the Brenner scale, and the GM Class from 1 to 2. This assessment was provisionally approved as GM Class 2 pending a decision as to whether or not to proceed. Alternatives may be acceptable.
Action: MF to advise
Action: CL (Bioassays rep.) to provide budget code for 480 fee to HSE if notification for GM Class 2 process is to proceed.

November 02
Alternative fusion which was tried has given poor results. Notification (to HSE) is now required to enable growth of the original to provide DNA sample for analysis and for use to provide continuity of data.

Project Containment

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**Name**

LIFEARC

**Name 2**

**Department**

**Campus Estate or Research Centre**

STEVENAGE BIOSCIENCE CATALYST (SBC)

**Building**

THE ACCELERATOR BUILDING

**Road Name**

GUNNELS WOOD ROAD

**District**

STEVENAGE

**Town**

**County**

SG1 2FX

**Country**

ENGLAND

**Tel Number**

0208 906 3811

**Fax Number**

0208 906 1395

**E-mail**

**HSE Division**

LONDON

**Comments**

**Date at Which Additional Info Submitted**

15/10/2001
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 267/00.2

Date Ackn'd 20/09/2000

CU2 Project Title THE USE OF RETROVIRAL VECTORS FOR THE EXPRESSION OF ZINC FINGER PROTEINS

Class 2

CultureVolClass2 Consents Granted

Class 3-4

Non-GMM Consent

Consent Granted not applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Animal Units | Large Scale Activities | Human Clinical Applications
---|---|---
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Project Ref  267/02.1

Date Ackn’d | 23/05/2002 | CU2 Project Title
---|---|---
ANALYSIS OF DRUG SENSITIVITY OF HBV POL GENE MUTANTS IN VITRO

Date Project Ceased | 04/05/2007

Class | CultureVol
---|---
Class 3 | Class2 Class3-4

CultureVolume
500 ml volumes

Consent Granted
yes

Project notified under transitional arrangements
N

Withdrawn
N

Tick if notifying a connected programme of work
N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Research into the discovery of novel antiviral agents with activity against drug resistant strains of HBV.

Recipient or parental organism

HBV DNA will be modified by the introduction of mutations in the pol gene and cloned in E. coli. The mutations introduced will be those known to occur in vivo after treatment of HBV positive patients with nucleoside analogues. The mutated DNA will be transfected into hepatoma cell lines and infectious genetically modified HBV produced.
Host/vector system

(i) HBV DNA will be cloned into pBluescript (Stratagene) and amplified in E. Coli JM109;
(ii) Alternatively pCMVHBV (pBR322) will be used.

Origin & function

HBV DNA will be obtained from either (i) human sera; (ii) virions produced by 2.2.15 cells (HepG2 cells transfected with HBV DNA); or (iii) pCMV HBV (obtained from Dr C Seegers, Fox Chase Cancer Centre, USA), Genetically modified HBV DNA will be used to assess the sensitivity of drug resistant HBV to novel antiviral agents.

Evaluation of foreseeable effects

The only foreseeable effect when hepatoma cells are transfected with the genetically modified HBV DNA will be the secretion of infectious HBV with resistance to various drugs used for the treatment of HBV infected individuals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

I am requesting that the following measures need not be applied under Schedule 8 Part II:

Containment Measure 8 - Autoclave, required in laboratory suite

Justification -
* All waste is chemically disinfected with a chlorine disinfectant prior to it leaving the containment area.
* The autoclave used for sterilising the waste produced from the above project is sited in the same corridor and within 2 metres of the laboratory exit.
* The waste is transported in sealed bags within robust boxes.
* The person/s transporting the waste will be a member of the laboratory staff and have been specifically trained for this task. The person/s concerned will be familiar with the Code of Practice and will have been deemed competent by the Head of Division.
* The waste will always be placed straight into the autoclave and the run commenced straightaway.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

* Liquid wastes will be treated with a solution of 2500 parts per million available chlorine. The resultant solution will be left to stand overnight. This is a well recognised and standard method of disposal and approaching 100% kill is expected.
* Solid waste will be autoclaved using a cycle of 130 degrees C for 15 minutes. Again this is a standard method of disposal and 100% kill is expected.
* Solid waste is sent for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The Committee have reviewed this project and endorse the Classification of 3 and approve it for work at Containment level 3.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Animal Units**

**Large Scale Activities**

**Human Clinical Applications**

**Project Ref** 267/10.1

**Date Ackn’ed**

**CU2 Project Title**

The use of virally-transfected mammalian cells to study the cell signalling pathways of inflammatory processes induced by virus infection

**Class**

Class 2

**CultureVol**

Winsor

**ClassVolume**

< 1 Litre

**Consent Granted**

Yes

**Project notified under transitional arrangements**

No

**Withdrawn**

No

**Tick if notifying a connected programme of work**

No

**Project Additional Information**

**Purposes of the contained use**

To understand how manipulation of the host immune response can be used to regulate viral infection for both therapeutic options and or potentially vaccination.

**Recipient or parental organism**

The recipient strains will be A549 human lung adenocarcinoma cell lines that stably express the IFN promotor fused to GFP, classified as class I containment material by ATCC.
Host/vector system

Recombinant parainfluenza type 5 virus, PIV5 (formerly known as simian virus 5 SV5)), a non-segmented negative strand RNA virus (Paramyxovirus family) whose genome encodes eight viral proteins.

Origin & function

This PIV5 is a recombinant virus that lacks the V protein C-terminal specific domain, referred to as PIV5ΔC (He et al., 2002, Virology. 303: 15-32). It is well documented that the V protein plays an essential role in evasion of the host immune response (specifically by blocking the production and subsequent signalling of type I interferons (IFN-α/β)).

The recombinant PIV5ΔC is therefore unable to disrupt the IFN pathway like its wild type counterpart and is unable to target STAT1 for degradation, or prevent nuclear localisation of the key transcription factor IRF3 and thereby prevent the release of IFNβ.

Evaluation of foreseeable effects

PIV5 is known to infect a range of cell types including primary human cells. Indeed, there has been no report of a cell line that is resistant to PIV5 infection. Importantly, PIV5 causes very little cytopathic effect in infected cells. PIV5 also infects most mammals including humans and is not associated with any clinical disease with the exception of canine kennel cough. The recombinant genetically modified version of this wild type virus, PIV5ΔC as mentioned above, has been disabled in its ability to disrupt the host cell's cellular defence, but it cannot be guaranteed that this will have the same safety profile as the wild type PIV5. Based on the known safety profile of the wild type PIV5, there should be no adverse effects on human health or the environment. The Advisory Committee on Dangerous Pathogens, does not list parainfluenza virus type 5, only parainfluenza virus types 1-4 which are classified as containment 2 microorganisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste arising from conditioned tissue culture medium will be deactivated in freshly prepared Chloros solution, 10000ppm for at least 30 minutes, prior to disposal via the drains. Solid waste such as plastic consumables, dishes etc will be disposed in a dedicated double-bagged bin for the collection of waste from containment 2 work only. This will subsequently be autoclaved prior to disposal. There will be no use of sharps in any of the experimental manipulations.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The Committee were pleased that the applicant was taking an over cautious view. They endorsed the risk assessment and approved the project for work at Containment 2

Project Containment

02/03/2022
The use of lentivirus for transfection of hard-to-transfect mammalian cells

Establishment of transfected cells lines for use in validation assays of therapeutic antibodies.

The production of packaged lentivirus will be carried out in the HEK 293FT cell line from Invitrogen and only differs from the widely used HEK 293T cell line by its fast growth. The T refers to the expression of the SV40 large T antigen, which has shown to be beneficial for generating the lentivirus. The produced virus can infect primary cells, but is not capable of reproduction.

The recipient cell line of recombinant genes will primarily be the human NK cell line NK92 (CRL-2407) available from ATCC but it will also be beneficial to use other hard-to-transfect cell lines used in therapeutic antibody characterisation studies.
NK-92 is an interleukin-2 (IL-2) dependent Natural Killer Cell line derived from peripheral blood mononuclear cells from a 50 year old Caucasian male with rapidly progressive non-Hodgkin's lymphoma. The cell line is dependent on the presence of recombinant IL-2 and a dose as low as 10 U/ml is sufficient to maintain proliferation; cells will die within 72 hours in the absence of IL-2. The cell line is cytotoxic to a wide range of malignant cells; it kills both K562 cells and Daudi cells in chromium release assays.

Host/vector system

pLenti vectors contain the following features:

- Rous Sarcoma Virus (RSV) enhancer/promoter for Tat-independent production of viral mRNA in the producer cell line (Dull et al., 1998)
- Modified HIV-1 5’ and 3’ Long Terminal Repeats (LTR) for viral packaging and reverse transcription of the viral mRNA (Dull et al., 1998; Luciw, 1996) Note: The U3 region of the 3’ LTR is deleted (U3) and facilities self-inactivation of the 5’ LTR after transduction to enhance the biosafety of the vector (Dul et al., 1998)
- HIV-1 psi (Ψ) packaging sequence for viral packaging (Luciw, 1996) HIV Rev response element (RRE) for Rev-dependent nuclear export of unspliced viral mRNA (Kjems et al., 1991; Malim et al., 1989)
- Polypurine Tract from HIV (cPPT) for increased viral titer (Park et al., 2001)
- Human cytomegalovirus (CMV immediate early enhancer/promoter for high-level constitutive expression of the gene of interest in a wide range of mammalian cells (Anderson et al., 1989; Boshart et al., 1985; Nelson et al., 1987)

Origin & function

The genes will be intact or fragments of: human kinases, transcription factors and receptor genes and soluble secreted proteins and plasma membrane proteins that when expressed in mammalian cells will alter cell physiology and signalling pathways; and/or are potential therapeutic targets in human disease. Furthermore, genes can be introduced into mammalian cells to re-establish a function that potentially was lost during cell line formation, but otherwise part of the wildtype cell line's natural repertoire - such as introduction of CD16 expression in the NK92 cell line. This creates a functional cell line for antibody-dependent cellular cytoxicity assays for characterisation of therapeutic antibodies.

None of the genes are oncogenes and this work will not produce toxic agents or oncogenes. These genes will change according to the project needs and new drug targets taken on by MRCT. In the future, other genes are also envisaged to used, however these proteins will be limited to those that have the same safety and risk profile as those outlined above. If the latter does not apply, then a new risk assessment will be made.

Evaluation of foreseeable effects

The most potentially hazardous GMO will be the recombinant lentivirus, which can transduce primary human cells. Transmission can occur by penetration of the skin via puncture or absorption through skin lesions and by mucous membrane exposure of the eyes, nose and the mouth. However, the genetically modified recipient cell lines will not be able to produce replicating lentivirus and neither will primary human cells, so any transmission will be limited to the initial site of infection. The virus will not carry oncogenes or toxic genes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work with the lentivirus will be carried out in containment level 2 laminar flow hood. Sealed centrifuge rotors and/or safety cups will be used for centrifugation of samples to minimise the contamination risk. All liquid waste will be treated with 10% bleach or SuperQ for at least 30 min to inactivate the virus. Solid waste such as disposable plastic consumables will be soaked in 10% bleach before autoclaving and being disposed as other autoclaved waste. Surfaces will be cleaned with Super Q which will also inactivate the lentivirus. There will be no use of sharps in any of the experimental manipulations.
The Biological Safety Committee approved the project at Class 2 for work at Containment 2.

Please enter comments on the GM safety committee on the risk assessment.

The Biological Safety Committee approved the project at Class 2 for work at Containment 2.

### Project Containment

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### Project Ref 267/14.1

- **CU2 Project Title:** The Use of Adenovirus for over-expression of recombinant proteins in mammalian cells
- **Class:** Class 2
- **CultureVolClass2:** < 1 Litre
- **CultureVolumeClass3-4:** Non-GMM
- **Consent Granted:** Consent Granted

---

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
Project Additional Information

Purposes of the contained use
The primary aim of the project is to overexpress functionality active mammalian proteins that are required in sufficient quantities for high throughput screening of compound libraries and for monitoring the optimization of chemical inhibitors that have been synthesized for the purposes of drug discovery.

Recipient or parental organism
Replication-disabled adenovirus serotype 5 (ad5).

Host/vector system
Hosts
1. E. coli
2. Human embryonic kidney cell line HEK293
3. Human cervical carcinoma cell line (HeLa cells)

Origin & function
Vector system
pAd/CMV/V5-DEST and pAd/PL DEST (Invitrogen,"ViraPower")

Evaluation of foreseeable effects
PMCA1, PMCA2, PMCA3 and PMCA4: PMCA is a calcium pump located at the plasma membrane. We will use human isoforms of PMCA1, 2, 3 and 4. There is no published data to indicate that PMCAs have any cytotoxic effect. GCaMP2: is a GFP based calcium sensor which will fluoresce when it binds calcium. Luciferase: is an enzyme that emits light originally isolated from firefly Photinus pyralis. It is used as a "reporter" for the activity of any regulatory elements that control its expression. Green fluorescent protein (GFP): is a protein that exhibits bright green fluorescence when exposed to blue light and is originally isolated from the jellyfish Aequorea victoris. It is also used as an "expression reporter". None of the proposed genes are known to be oncogenic. There are no foreseeable effects. With respect to the potential hazard arising from the unlikely accidental transduction of the specific gene of interest to man, the adenovirus does not integrate into the host and cannot replicate. Since the virus is replication-incompetent, the presence of the viral genome and gene of interest is transient and will eventually be diluted out as cell division occurs.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Chlorine-containing disinfectants, such as Chloros (10000ppm) in a concentration of 2500 ppm will be used for complete deactivation of viral proteins. Liquid waste arising from conditioned tissue culture medium or unused virus stock will be deactivated in freshly prepared Chloros solution, 2500ppm for at lest 30 minutes, prior to disposal via the drains. Solid waste such as plastic consumables, dishes etc will be disposed in a dedicated double-bagged bin or the collection of waste from Containment 2 work only. This will be autoclaved prior to disposal.
The Institute Biological Safety Committee have approved this project for work at class 2

Please enter comments on the GM safety committee on the risk assessment

The Institute Biological Safety Committee have approved this project for work at class 2

**Project Containment**

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Animal Units

Large Scale Activities

Human Clinical Applications

**Project Ref 267/15.1**

Date Ackn'd: 23/03/2015

CU2 Project Title: The use of lentiviral vectors to transduce mammalian cell lines with potentially oncogenic gene sequences

Class: Class 2

CultureVolClass2: < 1 Litre

Non-GMM: Consent Granted

Project notified under transitional arrangements: "N"

Withdrawn: "N"

Tick if notifying a connected programme of work: "N"

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

The primary aim of the project is to facilitate the transduction of mammalian cell lines that are very difficult to transfect and obtain stable and high expression with the gene of interest by standard transfection procedures such as lipid-based or electroporation methods. The resulting genetically modified cells lines will be used in functional studies for therapeutic antibody generation.

Recipient or parental organism

The cell line to be used to produce the viral particles is the human HEK293T and the cells to be transduced by them are a variety of immortalized mammalian lines which are not considered hazardous to human health, before or after contact with the virus. OriGene’s overexpression lentiviral vector is a 3rd generation lentiviral vectors with improved biosafety. These safety features are the following:

- The pLenti expression vector is replication deficient as it contains SIN (Self Inactivation), a deletion in the 3’ LTR (6.U3). This SIN deletion does not affect lentiviral packaging, yet results in ~ self-inactivation” after integration into the transduced cell. The integrated lentiviral genome is no longer capable of self-replication.
- The number of genes from HIV-1 that are used in the 3rd generation lenti system has been reduced to three (Le. gag, pol, and rev). TAT, an essential gene for viral replication is eliminated.
- A constitutive promoter (RSV promoter) has been placed upstream of the 5’ LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA.
- The packing plasmids (Gag, pol, rev and VSV-G) supporting lentiviral packaging are separated onto three plasmids; none of them contain LTRs or the 4’ packaging sequence.
- Only the lenti vector for gene overexpression contains the packaging signal that can be packaged into viral particles.
- The VSV-G gene from Vesicular Stomatitis Virus is used as the pseudo-envelope, further reducing the chances of recombination with viral genome.

Despite the safety features discussed, the lentiviruses produced with OriGene’s lenti system can still pose some biohazardous risk since it can transduce primary human cells. It is highly recommend that lentiviral stocks are treated and generated using this system as Biosafety Level 2 (BL-2) organisms and strictly follow all published BL-2 guidelines with proper waste decontamination.

Host/vector system

Host: mammalian cell lines (to express the protein of interest), including HEK 293T that will also be used to produce the viral packaging.

Vectors: - pLenti-C-mGFP, allowing the identification of infected cells by fluorescence microscopy.
- Lenti-vpak packaging plasmids mentioned above.

Origin & function

The genetic material to be expressed will include Human and Murine anaplastic lymphoma receptor tyrosine kinase (ALK) genes that were purchased from OriGene. This receptor plays an important role in the development of the brain and exerts its effects on specific neurons in the nervous system. This gene has been found to be rearranged, mutated, or amplified in a series of tumours including anaplastic large cell lymphomas, neuroblastoma, and non-small cell lung cancer. The chromosomal rearrangements are the most common genetic alterations in this gene, which
result in creation of multiple fusion genes in lumourigenesis, including ALK (chromosome 2)/EML4 (chromosome 2), ALKIRANBP2 (chromosome 2), ALKIATIC (chromosome 2), ALKTFFG (chromosome 3), ALKINPM1 (chromosome 5), ALKISQSTM1 (chromosome 5), ALKIKIF5B (chromosome 10), ALKICLTC (chromosome 17), ALKITPM4 (chromosome 19), and ALKIMSN (chromosome X). Wild type ALK and truncated ALK have not been described as oncogenic and only the full length wild type gene truncations thereof will be used to produce viral particles.

Evaluation of foreseeable effects

The mammalian cell lines are established long term lines and can be regarded as Class 1. The final GMO cell lines will contain stably integrated ONA copies of the lentiviral vectors encoding the gene of interest but will not have infectious viruses, therefore posing no hazard to human health or the environment. There is no risk of the infection spreading to the community via human-human contact. The packaged virus is unstable in the general environment and lentiviruses are rendered replication deficient. In lentivirus, the risk of reversion to the wild type is virtually non-existent as the lentivirus lacks four structural genes that reside on non-homologous plasmids in the packaging line. Furthermore, the experimental vectors lack a wild type 3' LTR that is essential for efficient viral replication. The recombinant genes to be expressed from the viral vectors are potential oncogenes, possibly contributing to excess proliferation and prolonging the life of damaged or mutant cells. In addition, lentiviral transduction leads to the stable integration of the virus into the host cell's genome. Therefore there is a very small but identifiable risk to an infected individual of long term alteration of gene expression. However only cells that come in direct contact with the packaged virus could become infected and, as tumourigenesis is recognised to require multiple events (e.g. further mutations in growth pathways), it is unlikely that a single viral infection will result in the formation of a tumour. Viral vectors are engineered to transfer and integrate specific DNA sequences into the genomes of target cells and are designed to be replication-defective to avoid further spread after the initial transfer even.

The route of transmission to deliver lentiviruses is through airways and eyes. The laboratory worker is thus under risk from aerosols and through sharp injury during handling. Exposure to skin, eyes and lungs would be minimal as gloves and protective clothing are used at all times, no sharps are used and all work will be carried out in a Containment Level 2 laboratory and cellular infections in a Class II Microbiological Safety Cabinet or in closed centrifuge systems. The modified viruses should not pose a serious risk as they are inactivated by detergent, UV light and ethanol. The half-life of the virus is 6-9hrs in tissue culture media at 37 °C, it is unknown how long it would survive in the environment. Any residual virus on tissue culture plastics, solid waste and liquid waste is inactivated by use of appropriate disinfectants. MSCs are located in specified rooms designated as Containment Level 2 laboratories and only competent and trained operators would carry out these procedures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste disposal
All handling of the virus will be carried out in containment level 2. There will be no glassware, blades, needles or sharps used in work involving lentivirus particles. It is advisable to have a container filled with a suitable decontamination liquid in the safety cabinet, with which to
rinse pipettes, pipettes' tips and small plastic containers before disposing of them into the waste container, so to minimize the risk of contamination to the worker when taking them out of the safety cabinet. The method of choice for sterilization is to autoclave the waste material. Used material awaiting sterilization must be stored in a safe and secure manner. Waste must be stored in autoclave bags (blue) and in the robust boxes provided. These boxes must be clearly labelled with the date, room number and Containment 2, and left securely closed on the corridor to be taken to be autoclaved.
Waste for incineration must be placed into either a sharps bin where appropriate, or packed into double yellow bags. Both containers must be labelled with the room number of origin and the date and Containment 2, the bags should be left in an appropriate location where a stores porter can pick them up for incineration. All waste produced must be autoclaved.
Care must be taken to ensure that the bags are not overloaded causing them to split. Similarly care must be taken to ensure that items which are liable protrude through plastic bags e.g. pipettes, are packed properly so that they do not cause a hazard to staff and others.
For general applications the recommended disinfectant is a chlorine based one i.e. Presept. The use of other disinfectants must be justified prior to use.
For routine decontamination.
• Liquid waste inactivated with Chlorine based disinfectant e.g. Presept - 10,000 ppm available changed daily and stock made up weekly.
• Decontamination with Presept 2,500 ppm followed by washing with a suitable detergent.
• For routine washing of benches, MSC, etc., a solution of 1 % Super Q should be used.
Adequate contact time must always be allowed for effective disinfection.
For spillages and accidents
Minor spillages: Presept solution @ 2,500ppm available chlorine

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The Institute Biological Safety Committee has passed this project for work at Containment level 2.

Project Containment

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Animal Units
Large Scale Activities
Human Clinical Applications

02/03/2022
### Project Ref: 267/93.1

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**Historical Significant Changes**

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**Project Additional Information**

**Purposes of the contained use**

- 

**Recipient or parental organism**

- 

**Host/vector system**

- 

**Origin & function**

- 

**Evaluation of foreseeable effects**

- 

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- 

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 267/trans

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Tick if notifying a connected programme of work N
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Additional Information**
### Purposes of the contained use

Generation of HIV-1 infectious virus or virus products from plasmids carrying HIV-1 genome sequences. The clones will undergo genetic modification resulting in, for example, the generation of virus resistant to known HIV inhibitors, or the incorporation of markers. The resultant viruses will be used to develop "in vitro" screens for novel anti-HIV compounds. The virus resistant mutations already exist "in vivo" and are not novel mutations.

### Recipient or parental organism

Plasmids containing HIV-1 genome (potentially full sequence)

### Host/vector system

### Origin & function

The HIV clones obtained through the NIH AIDS reagent Programme Repository or equivalent contributors, will be genetically modified using standard molecular biological techniques. The HIV DNA will be transfected into established human cell lines to generate replication competent virus or express virus products.

### Evaluation of foreseeable effects

The GMO will result in products which are of equal hazard status to, or less hazardous than, the wild type and/or its products. Any modifications which confer resistance to licensed anti-HIV therapeutics are identical to those which occur naturally in treated individuals.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Destrict autoclaving (134 degrees C 30 Cat2, 126 degrees C 50 Cat3) of all waste prior to incineration. All liquid waste to be sealed in plastic containers and placed into designated waste bins. Pipettes, tips, culture plates disinfected in chloros (Cat2) or Cidex (prior to autoclaving).

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

---

**Please enter comments on the GM safety committee on the risk assessment**

---

**Project Containment**

02/03/2022
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GM Centre Number: 271

Data Premises Notified (Originally) 04/08/1988
Transfered from 1992 Regs? Y
Transitional Premises Class

Data Premises Closed 31/01/2007
Transitional Premises Emergency Plan Required? N
Non-GMMs N
Withdrawn N

Name

BIOGEMMA UK LTD

Name 2

Department

Campus Estate or Research Centre

Road Name

200 CAMBRIDGE SCIENCE PARK MILTON RD

Town CAMBRIDGE

County CAMBRIDGESHIRE

Postcode CB4 0GZ

Country ENGLAND

Tel Number 01223 723333

Fax Number 01223 723330

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted 20/08/2001
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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities
- Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

Treatment of contaminated waste is by autoclaving at 121 degrees C, 1.2 bar for a minimum of 15 minutes.

The autoclaves are inspected twice yearly, one being a full function test including a twelve point thermocouple test with each of the appropriate disposal loads.

Regarding volumes for disposal: The largest volume of O/N cultures would be in the region of 720 ml (72 x 10 ml).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### UNIVERSITY OF LIVERPOOL

<table>
<thead>
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## Premises Addresses

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Give brief details of the genetic modification safety committee

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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

- Tick to confirm that you are attaching a summary of the risk assessment
- Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Generate improved Avian Pneumovirus (APV) vaccines by introducing rational changes using infectious clone technology.

Current commercial vaccines have been created randomly by serial passage of field virus isolates in cell culture. The mutations generated appear to be reversible. These vaccines have been shown to lead to disease in commercial poultry, almost certainly as a result of their reversion back to the parent type virus.

The aim of this project is to introduce attenuating mutations using reverse genetics/infectious clone technology. It is anticipated that stable vaccines, incapable of reversion will be generated. This will be by either:
- incorporation of several attenuating motifs (identified from studying existing vaccines and their progenitors)
- deletion of genes demonstrated to attenuate another similar pneumovirus (Respiratory syncytial virus).

Candidate vaccines will be tested in turkeys in the Leahurst Poultry Isolation Unit.

Avian pneumovirus is in common circulation in commercial poultry, game birds and probably other unidentified avian species. In experimental conditions, APV has been shown to infect only a minority or avian species. Infection of a range of birds (turkeys, chickene, ducks, geese, pheasants, guineafowl and pigeons) resulted in virus only being isolated from the first two (Gough 1988). Experimental infection of the most susceptible avian species known (turkeys) causes mild, non-fatal respiratory disease. Infection in chickens is frequently asymptomatic and game birds infected with APV do not show disease. Wild seagulls have also been found to be seropositive for the virus but disease has not been observed. It is thought likely that other wild birds may be infected but no significant studies have been carried out.

Because of its low pathogenicity, APV is considered to be a category 1 organism (though not yet formally classified). Field strains are treated at this level of containment in UK laboratories (IAH Compton, CVL Weybridge, Intervet Cambs. Vet. Path Liverpool University) and similar laboratories abroad.

With APV infection in commercial turkeys farms, the virus can predispose to secondary pathogens and there can be serious economic losses. With commercial chickens farms disease is rarely seen but reduced weight gain and egg laying statistics suggest that a sub clinical infection probably occurs.

APV is a labile enveloped virus which has a short life in the environment outside of an avian host. Turkeys placed in a room vacated of infected birds did not become infected. It can be demonstrated to transmit to other birds in close contact in experimental conditions. Bird to bird contact has been shown to be the most successful method of virus transfer between birds but aerosol transmission by infected respiratory droplets probably also plays a part. The initial 1980's APV outbreak which spread rapidly from East Anglia to Wales, against the prevailing wind suggests that direct contact with personnel and vehicles to be the main mode of transmission. This is supported by the fact that a key hatchery in southern Scotland which has avoided such contact still remains free of the disease, 15 years later.

The virus itself is being attenuated hence no host is involved.

The virus itself is being attenuated hence no host is involved.

Origin: APV field virus.
Function of modified organism: vaccination of turkeys and chickens against disease caused by APV.
Evaluation of foreseeable effects

The initial work will produce a virus of identical sequence to the field strain and it will therefore have exactly the characteristics of that strain and hence present identical risks.

The modified viruses (the candidate vaccines) would either show reduced pathogenicity or remain unaltered. This has proved the case with similar reverse genetics work with other members of the Paramyxoviridae (e.g. RSV, NDV, Rinderpest). Any viable viruses resulting have all shown reduced or unaltered pathogenicity. Tropism stayed the same or become more restricted.

The risks to the environment would be expected to be the same or less than those encountered when making and testing conventional live attenuated APV vaccines and less than when using field strains (as used routinely in all vaccine challenge models). The changes introduced will either remove virulence factors or introduce attenuating mutations (as identified by sequencing existing conventional live vaccines and their progenitors). In contrast, the conventional live vaccines in general use and produced by random mutations, would be likely to introduce larger unknown changes. However, such changes have never been observed.

APV causes an acute respiratory infection and has never been shown to or suspected of causing persistent infections. In experimental conditions, turkeys remain infected for a short period (approximately 1 week) and it appears likely to be of advantage to the virus to produce maximal disease and virus shedding within that period. Rapid generation of high viral titres and maximum virulence are likely to be the infection characteristics most likely to give the virus a selection advantage. Hence, it is likely that current field strains are close to the maximum potential virulence of the organism. Certainly, viruses randomly mutated during conventional vaccine development have never been found to increase virulence - rather, they have become less virulent or virulence has remained unchanged.

It is likely that vaccines are even less fit to survive in the environment than wild-type strains. Conventional vaccine studies employing molecular biological diagnostics have indicated that live vaccines do not persist in the environment. During long term studies on UK turkeys farms, vaccine was only detected after it has been applied to the actual groups of birds, whereas field strains were found to cycle routinely. It is assumed that this reflects the vaccine's reduced fitness compared to better adapted field strains, in general circulation in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

AVP is labile envelope virus which is not stable in the environment outside of a host. Mild agents such as 70% ethanol readily inactive the virus.

LABORATORY
All manipulations and culture work will be contained within laboratory 1.10. Generated viruses may be moved in sealed containers to en-suite freezer facilities for storage.

Manipulations will be performed in purpose installed class 2 cabinet. Areas within the cabinet will be wiped down routinely with Virkon after use.

All infected materials will be transferred to autoclave bags which will be contained within lock top polyethylene bins. The combination will prevent spillage or penetration in the event collision. The combination will be moved to the en-suite autoclave where bags will be transferred and treated.

ISOLATION UNIT
Birds will be vaccinated with the GMO then 3 weeks later challenged with field virus to test vaccine efficacy.
The unit contains 8 poultry rooms. Entry to the unit is via a locked door into a robing area where full overalls, head covering and gloves are put on. Boots are dipped in FAM disinfectant. After robing a central corridor is entered and from here there is access to each poultry room via its own anti room. Exhaust air is from each poultry room is HEPA filtered. Footbaths containing FAM disinfectant for boot dipping are in the corridor and inside the entrance of each anti room. Overalls, head coverings and gloves are discarded into FAM disinfectant after use, while still within the outer secure area.

Rooms and anti rooms will be disinfected by Virkon spay before and after each experiment.

Poultry and litter will be bagged and incinerated after each experiment. Both are almost certainly NOT infectious by the experiment finish but, in any event, persisting virus would be wild type field virus (not the GMO).

Drains within poultry areas are sealed for the duration of the experiment and until disinfection has occurred.

Is an emergency plan required according to regulation 20?  

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If yes, tick to confirm that it is attached to this form

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Tick to confirm that you have attached a risk assessment to this form

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Tick if you are claiming exemption from disclosure for section of the risk assessment

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Please enter comments on the GM safety committee on the risk assessment

The safety committee were in full agreement with the risk assessments.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref 272/01.2**

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02/03/2022  Page 5891 of 15326
Project Additional Information

**Purposes of the contained use**
To investigate putative virulence homologues of FHV with a view to improve the safety and efficacy of vaccines

**Recipient or parental organism**
FHV is a respiratory pathogen of cats not known to infect non-felids.
FHV in which putative virulence homologues have been deleted is expected to show an attenuated phenotype.

**Host/vector system**
FHV/gfp

**Origin & function**
The gfp expression cassette to be inserted has been obtained by PCR from the commercially available vector pEGFP-N1 (Clontech UK Ltd).
GFP is a marker protein that fluoresces green and is being used to allow the identification and selection of recombinant FHVs.

**Evaluation of foreseeable effects**
Recipient micro-organism FHV is not listed in ACDP hazard groups 2, 3, or 4. Any recombination (unlikely) would only result in reversion to wild type phenotype. There are no known hazards associated with the inserted gene product and its expression (personal communication, Clontech, attached). GFP has been widely used as a marker in many infectious systems and no harmful effect has ever been demonstrated following insertion of this gene. Deletions of putative virulence homologues or other alpha-herpesviruses have resulted in attenuation of the virus and as FHV is not infective for humans it is highly unlikely that the GMM would be infective for humans.
No aspect of the proposed work require specific control measures to safeguard human health. FHV only survives up to 24 hours in the environment and is highly susceptible to standard disinfectants. FHV with specific deletions in putative virulence homologues would be expected to be attenuated relative to wild type virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
none applied for
All laboratory waste is placed in autoclave bags and transported to the autoclave in lidded buckets. Waste is autoclaved at a temperature of 121°C for 20 minutes. The autoclave is routinely monitored and maintained according to the guidelines laid down by the insurers. After autoclaving, the waste bags are secured in yellow clinical waste sacks and placed in the refrigerated container designated for such waste awaiting collection by the University contractor, for ultimate incineration.

![Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)](image)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The local genetic modification safety committee has ratified this risk assessment without comment

### Project Containment

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**Name**

*IMPERIAL COLLEGE SCHOOL OF MEDICINE AT CHARING CROSS HOSPITAL*

**Department**

CHARING CROSS HOSPITAL

**Campus Estate or Research Centre**

**Building**

**Road Name**

ST DUNSTANS ROAD

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

W6 8RP

**Country**

ENGLAND

**Tel Number**

0208 846 7038

**Fax Number**

0208 846 7377

**E-mail**

**HSE Division**

LONDON

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Level 4 (GMMs)

Non-microbial

Other (please specify)  

Tick if confidential  

Bacteriology  

Parasitology  

Transgenic  

Microbiology  

Research  

Virology  

Transgenic  

Gene Therapy  

Animals  

Transgenic  

Fish  

Mycology  

Transgenic  

Transgenic  

Other (please  

specify below)  

Invertebrates  

Plants  

Other(s)  

For activities involving GMMs, describe the waste management measures which will apply to the activity  

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment  

---

Project Ref 275/03.1  

Date Ackn'd 04/02/2003  

CU2 Project Title INVESTIGATION OF CELLULAR IMMUNE RESPONSES TO VIRUSES USING VACCINIA VIRUS VECTORS WITH INSERTS FROM (A) HUMAN IMMUNODEFICIENCY VIRUS AND (B) PAPILLOMA VIRUS.  

Class 2  

CultureVolClass2 < 1 litre  

ClassVolumeClass3-4  

Non-GMM  

Consent Granted  

Date Project Ceased 02/03/2022  

Page 5896 of 1532
**Project Additional Information**

**Purposes of the contained use**
To study cellular immune responses in individuals infected with HIV.

**Recipient or parental organism**
Vaccinia virus containing one of the following genes: HIV-1, gag, env, pol, nef; influenza; nucleoprotein, matrix protein, pol; papilloma virus (HPV16 and 18) E6 and E7 fusion protein -- the E6 and E7 proteins have oncogenic properties. Transformation requires E7 to bind to retinoblastoma protein. To remove oncogenic potential the E7 protein is mutated at two amino acid residues to abolish binding to retinoblastoma protein. There is no evidence in the literature that modification of the vaccinia increases its pathogenicity.

**Host/vector system**
Vector: vaccina virus
Host cell: TK 143, a thymidine kinase negative cell line.

**Origin & function**
Inserted genes: HIV-1 gag, env, pol, nef, influenza matrix, nucleoprotein, pol Papilloma virus types 16 and 18 E6 fused to a mutated E7 protein.

The genes will be transfected into human dendritic cells to facilitate antigen presentation to stimulate virus specific cytotoxic T cells.

**Evaluation of foreseeable effects**
Accidental exposure to the vaccinia modified vector may result in the generation of an immune response to the product of the inserted gene. No other clinical outcome would be expected as a result of the modification.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste (plastics): 2% Virkon overnight. The manufacturer specifies that this will give 100% kill. The decontaminated waste will be then autoclaved prior to disposal via the Chelsea and Westminster Hospital waste disposal services.

Liquid waste: via autoclave, 134 degrees centigrade applied including air extraction, heating and steaming for 30 minutes. The manufacturer specifies that this will give 100% kill. Indicator strips to be used to monitor every autoclave run. Regular six monthly servicing and testing of the autoclave by the manufacturer.

The principal investigator to monitor staff to ensure correct disposal routine is followed.

Is an emergency plan required according to regulation 20?  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The committee agreed that a class II classification was appropriate for the work and that the safety precautions outlined were satisfactory.

Project Containment

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<th>Laboratory Activities</th>
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Project Ref 275/04.1

Date Ackn’d 11/06/2004  
CU2 Project Title Immunity to Kaposis Sarcoma Herpes Virus (KSHV)

Class 2  
CultureVolClass2 < 1 Litre  
Consent Granted  
Non-GMM Not Applicable
**Project Additional Information**

**Purposes of the contained use**

To study cellular immune responses to KSHV in healthy immunocompetent individuals and in individuals infected with HIV.

**Recipient or parental organism**

KSHV containing a Green Fluorescence protein (GFP) insert. The modified KSHV genome contains an insert of GFP (expressed by the elongation 1-alpha promoter) and the neo gene (expressed by the RSV promoter). This construct was inserted through homologous recombination between ORF 57 and ORF 59 of the KSHV genome (Gao et al, J. Virol 2001.75: 1378-1386). The recombinant KSHV has been designated as rKSHV:152. There is no evidence that the modification of the KSHV increases virulence or pathogenicity.

**Host/vector system**

Vector: KSHV
Host cells: BCBL-1, a human primary effusion lymphoma cell line containing wild type KSHV.

**Origin & function**

Inserted gene: GFP derived from Jellyfish.

GFP will be used to detect recombinant KSHV infection in a range of mammalian cell types including endothelial cells, fibroblasts, lymphocytes and myeloid cells.

Infected cells will be used as targets for KSHV specific NK cell and T-cell responses.

**Evaluation of foreseeable effects**

KSHV infection does not result in pathogenic effects in healthy individuals. This virus can lead to the establishment of tumors in HIV-1 patients.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste (plastics): 2% virkon overnight. The manufacturer specifies that this will give 100% kill. The contaminated waste will then be autoclaved prior to disposal via...
Chelsea and Westminster Hospital Waste disposal service.

Liquid Waste: via autoclave, 134 degrees centigrade applied including air extraction, heating and steaming for 30 minutes. The manufacturer specifies that this will give 100% kill. Indicator strips to be used to monitor every autoclave run. Regular 6 monthly servicing and testing of the autoclave by the manufacturer.

The principal investigator to monitor the staff to ensure that the correct disposal routine is followed.

Project Containment

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Animal Units

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Please enter comments on the GM safety committee on the risk assessment

The committee agreed that a class II classification was appropriate for the work and that the safety precautions outlined were satisfactory.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

**Level 1 (GMMs)**

**Level 2 (GMMs)**

**Level 3 (GMMs)**

**Level 4 (GMMs)**

**Non-microbial**

**Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
Maximum volume produced at any one time - 500 ml bacterial culture, dozen or so petri dishes containing transformed plant material.

Disposal methods: Liquid culture through autoclaving on cycle designed for decontaminating liquid loads (130 degrees C, 20 minutes), plant material in petri dishes through autoclaving on cycle designed for decontaminating agar plates (130 degrees C, 30 minutes).

Liquid load (sealed glass) discard. Air is removed from the chamber by progressive dilution with steam in a series of negative pulses, followed by a pre-sterilising stage (steam admission) to achieve load temperature of 130 degrees C. The sterilising stage is 130 degrees C for 20 minutes.

Agar plate (plastic) discard. Air is removed from chamber by progressive dilution with steam in a series of negative and positive pulsing, followed by pre-sterilising stage (steam admission) to achieve load temperature of 130 degrees C. The sterilising stage is 130 degrees C for 30 minutes.

In both cycles the timer starts when the temperature controller fed by the load probe senses the sterilising temperature.

Monitoring: Each load is recorded (chart recorder) via a temperature probe.

Validation: Annual testing and certification of the cycles using thermocouples (performed by external service engineers).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
## GM Centre Number: 277

<table>
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### Name

**ELI LILLY & CO LILLY RESEARCH CENTRE**

### Name 2

### Campus Estate or Research Centre

#### Road Name

**SUNNINGHILL ROAD**

#### Town

**WINDLESHAM**

#### County

**SURREY**

#### Postcode

**GU20 6PH**

#### Country

**ENGLAND**

#### Tel Number

**01276 853000**

#### Fax Number

**01276 474390**

### HSE Division

**EAST AND SOUTH EAST**

### Date at Which Additional Info Submitted

**02/03/2022**
### Premises Addresses

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<td>SUNNINGHILL ROAD</td>
<td>WINDLESHAM</td>
<td>Surrey</td>
<td>GU20 6PH</td>
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**  
  - [ ]

- **Give brief details of the genetic modification safety committee**

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**
- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**
  - [ ] Tick if confidential
Solid waste inactivated by autoclaving at 134 degrees C for 15 minutes.

Liquid waste inactivated by autoclaving as above or treatment with appropriate disinfectant eg. Virkon, Hibitane (suitable after testing in-use experiments).

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 277/08.1

Generation and use of lentiviral vectors to investigate the role of proteins associated with various psychiatric and neurological disorders such as Schizophrenia, Alzheimer's Disease and Depression.

Date Ackn'd 12/06/2008

Date Project Ceased 24/08/2020

Withdrawn N

Tick if notifying a connected programme of work N

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 1-50 Litres

Consent Granted Not Applicable

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

The purpose of the study is to use a lentiviral vector system to express reporter genes or overexpress/knockdown specific genes or proteins thought to play a role in psychiatric and neurological disorders, in rodent primary neuronal cultures, cultured cell lines or rodent brain slice preparations.

Recipient or parental organism

HEK 293T cells will be used to propagate the virus
Target cells to be infected include:
Neuronal (e.g. SH-SY5Y, NG1 08-1 5) or non-neuronal (e.g. CHO) Hazard Group 1 cell lines
Rodent primary neuronal cultures and rodent brain slice preparations
Primary human cells will not be used

Host/vector system

Only lentiviral delivery systems that are replication deficient with a history of safe laboratory use will be used. Most commercially available lentiviral packaging system are at least 3rd generation versions and utilise split-genes to separate structural and other components required for packaging onto several plasmids. This reduces the risk of generating replication-competent lentivirus because multiple recombination events are necessary to create a virus that harbours the sequences required for independent replication. Some examples of these plasmids are shown below.

Vectors for packaging genes:
pVSVG, pLPNSVG, pDelta8.9, pLP1 and pLP2, ViraPower packaging mix (Invitrogen), Lenti-X HT Packaging Mix (Clontech)
Vector for inserting genes/shRNA of interest:
pLKO.1 (Sigma), pLenti (Invitrogen), Lenti-XTM Tel-On Advanced and Lenti-X Tet-Off Advanced (Clontech)

Origin & function

Human and rodent cDNAs encoding full-length or partial protein sequences known to be associated with psychiatric and neurological disorders or shRNA to knock down genes of interest associated with these disorders.
For example:
Phosphodiesterases — enzymes that hydrolyse the second messengers cAMP and cGMP thereby regulating the transcription of many genes
Tau I Tau kinases — Tau is a neuronal microtubule-associated protein that stabilises axonal microtubules. Phosphorylation of tau by kinases such as cdk5 and GSK-3f has been associated with Alzheimer’s disease.
The cDNAs may be tagged with reporter genes (e.g. EGFP).
Evaluation of foreseeable effects

The generation of lentivirus containing the gene of interest is the most hazardous part of the process with potential routes of exposure being through broken skin, self-inoculation and mucus membranes. The use of sharps, such as needles, will be prohibited whilst the use of a Class II biological safety cabinet will guard against exposure to aerosols.

The effect of any one of the genetic inserts mentioned above is likely to be minimal and would be confined to the local area of infection due to the replication deficiencies of the virus. However, there would be an increased risk of a recombination event if the individual were to be already infected with a wild-type lentivirus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (e.g. pipettes, flasks): Will be soaked overnight in disinfectant. Excess liquids will then be discharged to drain. The remaining solids will be transported from the laboratory area to the autoclave in sealed metal boxes on a trolley. Following autoclaving, residual waste will be placed in a sealed bin for incineration by external contractors. Liquids (e.g. medium wash-off): Will be treated with disinfectant overnight then disposed of to drain with running water.

Safety cabinets Will be wiped down with 70% ethanol! 1% SDS. The cabinets are serviced and containment checked every 6 months.

Degree of kill:
- Autoclave — effectively 100% kill. Autoclave is serviced and validated every 3 months
- Incineration — effectively 100% kill
- Chemical disinfection will be with 1% Virkon or 10% MicroSol 3Plus following the manufacturers’ instructions — from the manufacturers’ data sheets kill will be effectively 100%. This will be verified experimentally once Class 2 work commences.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was reviewed by the Erl Wood genetic modification safety committee on 8th May 2008. Appropriate amendments were made and the assessment was subsequently approved for submission to the HSE.

Project Containment

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<th>Growth Rooms</th>
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<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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02/03/2022
| Animal Units | | | 
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L2 | L3 | L4 | L2 | | L3 | L4 | L2 | | L3 | L4 | | |
### GM Centre Number: 279

**Data Premises Notified (Originally)**  
17/01/1989

**Transferred from 1992 Regs?**  
Y

**Transitional Premises Class**  
1

**Data Premises Closed**  
16/06/2003

**Transitional Premises Emergency Plan Required?**  
N

**Non-GMMs**  
Y

**Withdrawn**  
N

---

**Name**  
INSTITUTE OF ARABLE CROPS RESEARCH (IACR)

**Name 2**  
UNIVERITY OF BRISTOL

**Department**  
AGRICULTURAL SCIENCES

**Campus Estate or Research Centre**  
LONG ASHTON RESEARCH STATION

**Road Name**

**Town**  
BRISTOL

**County**  
AVON

**Postcode**  
BS41 9AF

**Country**  
ENGLAND

**Tel Number**  
01275 549265

**Fax Number**  
01275 394281

**E-mail**

**HSE Division**  
WALES AND SOUTH WEST

**Comments**  
SITE CLOSED AS OF 6/6/2003

**Date at Which Additional Info Submitted**  
18/06/2003
## Premises Addresses

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## Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee

### Premises Conditions

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<th>Laboratory</th>
<th>Animal Unit</th>
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- Other (please specify)

- Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
**GM Centre Number: 280**

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**Name**

INTERVET UK LTD

**Department**

SEVERAL

**Campus Estate or Research Centre**

**Building**

THE ELMS

**Road Name**

THICKET RD

**District**

HOUGHTON

**Town**

HUNTINGDON

**County**

CAMBRIDGESHIRE

**Postcode**

PE17 2BQ

**Country**

ENGLAND

**Tel Number**

01480 464242

**Fax Number**

01480 461541

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

GM280 Closed and merged with GM391 on 31/03/2005.

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<tr>
<th>Laboratory</th>
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<th>Growth Room</th>
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Non-microbial

Other (please specify)

Tick if confidential

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<th>Microbiology Research</th>
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<tr>
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<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Laboratories.
1. Disabled bacteria
2. Recombinant viruses
3. Transfected cell lines - Disinfected by immersion, in Presept (hypochlorite) solution overnight or by autoclaving.
   Authentication of disinfection efficacy has been established or is under investigation by attempting to culture GMMs or similar preparations from disinfected material.

Maximum culture volumes of any culture a), b) or c) at any given time would be 5000 ml, but usually less than 2000 ml.

Animal waste:
1. Animals infected with GMMs are double-bagged and placed in sealed plastic containers for incineration.
2. Waste - solid faecal matter is scraped into sealable, leakproof container. The outside is sprayed with disinfectant. Disposal of by incineration as above.
4. Animal rooms - after removal of waste material - see point 2 above - rooms steamed as point 3 above and fumigated with formaldehyde after disinfectant wash.

Isolators
Waste to sealable, leakproof containers or via sealed drums to sealed pit then treated with sodium hypochlorite. Thoroughly cleaned/disinfected then formaldehyde fumigation.

Checks.
1. 5 temperature probes in steam steriliser, record temperatures reached during the process.
2. Regular validation checks.
3. Periodic checks to recover microorganisms (pre and post sterilisation process).
4. Tests to ascertain minimum temperature and time necessary to kill 100% of microorganisms under study.

Clinical Waste
1. Annual inspection of facilities of clinical waste disposal contractor.
2. Full records kept of all materials disposed of in this way.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 291

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**Name**

UNIVERSITY OF WALES COLLEGE OF MEDICINE

**Department**

MEDICAL BIOCHEMISTRY

**Campus Estate or Research Centre**

**Road Name**

HEATH PARK

**Town**

CARDIFF

**District**

CEREDIGION

**County**

CEREDIGION

**Postcode**

CF14 4XN

**Country**

WALES

**Tel Number**

029 2074 2951

**Fax Number**

029 2074 5440

**HSE Division**

WALES AND SOUTH WEST

**Comments**

GM291 CLOSED DOWN AND MERGED WITH GM130 ON 26/04/2005.

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

1. **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

2. **Give brief details of the genetic modification safety committee**

   - **Laboratory**
   - **Animal Unit**
   - **Growth Room**
   - **Glass House**
   - **Large Scale**

   - **Level 1 (GMMs)**
   - **Level 2 (GMMs)**
   - **Level 3 (GMMs)**
   - **Level 4 (GMMs)**

   - **Non-microbial**

   - **Other (please specify)**

   - **Tick if confidential**

   - **Bacteriology**
   - **Parasitology**
   - **Transgenic Birds**
   - **Microbiology Research**
   - **Virology**
   - **Transgenic Animals**
   - **Transgenic Fish**
   - **Gene Therapy**

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 291/01.1

Date Ackn'd: 22/02/2001  CU2 Project Title: To clone and sequence the rat and mouse analogues of the human compliment inhibiting protein CD59 and to identify the functionally important domains of human complement inhibiting protein

Date Project Ceased: 26/04/2005

Class: Class 2  Culture Vol Class 2: Class 2  Culture Volume Class 3-4: Class 2

Consent Granted: not applicable

Non-GMM: N

Historical Significant Changes: Project transferred to GM130 on 26/04/2005

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

Project Additional Information:

Historical Date of Additional Info: 22/02/2001

Significant Change ID: 22/02/2001

Date of Significant Change: 22/02/2001

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
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Project Ref 291/01.2

Date Ackn'd 22/02/2001

Date Project Ceased 26/04/2005

CU2 Project Title PRODUCTION OF RECOMBINANT IMMUNOGLOBULIN FUSION PROTEINS (HUMAN, RODENT AND CHICKEN ORIGIN) FUSED TO HUMAN/RODENT/CHICKEN LECTIN PROTEINS (GM291/ACK.C.PG/1)

Class 2

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Historical Significant Changes Project transferred to GM130 on 26/04/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

| L2 L3 L4 | L2 L3 L4 | L2 L3 L4 |

Large Scale Activities

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Human Clinical Applications

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Project Ref 291/01.3

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<tr>
<td>22/02/2001</td>
<td>PRODUCTION OF HUMAN IGGFC FUSION PROTEINS WITH MOUSE AND RAT CD55/CD59 PROTEINS. (GM291/AKC.BPM/7)</td>
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Withdrawn N

Tick if notifying a connected programme of work N
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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**Is an emergency plan required according to regulation 20?**  N

If yes, tick to confirm that it is attached to this form  N
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
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**Project Ref 291/01.4**

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<td>22/02/2001</td>
<td>CHARACTERISATION OF MOUSE DAF (CD55). STRUCTURE / FUNCTION ANALYSIS OF VARIENT FORMS OF THE MOLECULE (GM291/AKC.BPM/6)</td>
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<th>Date Project Ceased</th>
<th>Class</th>
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**Historical Significant Changes**

- Project transferred to GM130 on 26/04/2005

**Project Additional Information**
**Purposes of the contained use**

- Recipient or parental organism

- Host/vector system

- Origin & function

**Evaluation of foreseeable effects**

- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Is an emergency plan required according to regulation 20?**

- If yes, tick to confirm that it is attached to this form

- Tick to confirm that you have attached a risk assessment to this form

- Tick if you are claiming exemption from disclosure for section of the risk assessment

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02/03/2022
## Project Ref 291/01.5

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### Project Additional Information

#### Purposes of the contained use

#### Recipient or parental organism

#### Host/vector system

#### Origin & function

#### Evaluation of foreseeable effects

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Tick if notifying a connected programme of work

Withdrawn

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
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**Project Ref** 291/03.1

- **Date Ackn'd**: 07/07/2003
- **CU2 Project Title**: USE OF RECOMBINANT VACCINIA VIRUSES AS VECTORS TO EXPRESS T CELL ANTIGENS AND OTHER VIRAL PROTEINS
- **Date Project Ceased**: 26/04/2005

**Project notified under transitional arrangements**

- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- Project transferred to GM130 on 26/04/2005

---

**Project Additional Information**

---
Purposes of the contained use

Recombinant vaccinia viruses will be used for several purposes:
1. To immunise mice with recombinant antigens
2. To measure immunity against recombinant antigens.
3. To infect cells in vitro for the purpose of screening immune responses.
The antigens to be used represent non-functional proteins either of viral or mammalian origin.

Laboratory work involving recombinant vaccinia viruses will be carried out at ACGM Containment Level 2 with the use of a microbiological safety cabinet. In laboratory situations, sharps will not be used and workers will wear gloves at all times. In the case where animals will be inoculated with recombinant vaccinia viruses, virus will be delivered via the intraperitoneal, intravenous or subcutaneous routes. In all cases needles will be used. Workers involved in inoculation of animals will be trained to a high level to minimise the risk of needle-stick injuries. The highest inoculum used will be 10 million plaque forming units per mouse. Vaccinia virus infection does not spread within mouse or human communities. The virus does not cause persistent infection since it lyases cells upon infection. Infection with recombinant vaccinia virus may however lead to some blistering or scabbing during the acute phase of infection. Tissues recovered from infected animals will be manipulated within microbiological safety cabinets and animal carcasses will frozen and later disposed of by incineration.

Recipient or parental organism

The recombinant vaccinia viruses to be used have already been constructed through homologous recombination of plasmid DNA (pSC11) into the thymidine kinase gene of vaccinia virus (Western Reserve, WR). Inserted genomes encode for: 1) mouse melanocyte antigens; 2) the glycoprotein of murine leukaemia virus; 3) influenza antigens; 4) antigens from lymphocytic choriomeningitis virus and 5) non-functional murine proteins. A complete list is shown in Appendix 1. These inserted tenes pose no risk.
Indeed, insertion of these genes into vaccinia virus WR, reduces the virulence of the virus 10,000 x (Buller et al. 1985. Nature 317: 813).

Host/vector system

The recombinant vaccinia viruses to be used have already been constructed through homologous recombination of plasmid DNA (pSC11) into the thymidine kinase gene of vaccinia virus (Western Reserve, WR).

Origin & function

Inserted genes encode for: 1) mouse melanocyte antigens; 2) the glycoprotein of murine leukaemia virus; 3) influenza antigens; 4) antigens from lymphocytic choriomeningitis virus and 5) non-functional murine proteins. A complete list is shown in Appendix 1. These inserted tenes pose no risk. Indeed, insertion of these genes into vaccinia virus WR, reduces the virulence of the virus 10,000 x (Buller et al. 1985. Nature 317: 813).

Evaluation of foreseeable effects

Use of recombinant vaccinia viruses within the laboratory will be confined to a class II microbiological safety cabinet. In some situations it will be necessary to infect mice and therefore to transport viruses from the laboratory to the animal house. Vials containing vaccinia viruses will be transported from the laboratory to the animal house within sealed containers. The possibility of spillage from sealed containers is very unlikely. In the event that a spillage does occur, the spillage will be dealt with according to local rules at the University of Wales College of Medicine.

With regards to environmental risks, it is known that there was no colonisation of domestic or wild animals in the UK following widespread administration of vaccinia virus to humans in the smallpox eradication campaign, nor has the recombinant vaccinia virus that has been used to control rabies in part of Belgium and France led to colonisation of wild-species. There is no reason to believe that recombinant vaccinia viruses produced in this work are any more likely to survive and become established in the environment than the wild-type virus. Thus, taking into account the control measures assigned above, the overall risks to the environment from the modified organisms produced in this work are effectively zero. Therefore no additional containment or control measures are considered necessary to protect the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables including plasticware and pipettes will be autoclaved for at least 15 minutes at 121-130 degrees C. Subsequently autoclaved waste will be disposed of by incineration with other clinical waste. Liquid waste will be treated with Hycolin for 30 minutes prior to discharge to drains.

Animal bedding will be autoclaved and animal carcasses will be destroyed by incineration.

Degree of Kill:
Autooclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Additional detail required in environmental assessment - now added. Advice should be sought regarding avoidance of handling virus by persons with sensitive skin conditions, eg eczema. Agree with assessment as Class 2 activity requiring Containment Level 2 facilities.

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Tick if you are claiming exemption from disclosure for section of the risk assessment N
GM Centre Number: 292

Data Premises Notified (Originally) 23/12/1988

Data Premises Closed 26/04/2005

Transferred from 1992 Regs? Y

Transitional Premises

Transitional Premises Class 2

Non-GMMs N

Emergency Plan Required? N

Withdrawn N

Name

UNIVERSITY OF WALES COLLEGE OF MEDICINE

Name 2

Department

HAEMATOLOGY

Campus Estate or Research Centre

Building

Road Name

HEATH PARK

District

Town

CARDIFF

County

CARDIFF

Postcode

CF4 4XN

Country

WALES

Tel Number 029 2074 2903

Fax Number 029 2074 4869

E-mail

HSE Division

WALES AND SOUTH WEST

Comments


Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**
  - [ ]

- **Give brief details of the genetic modification safety committee**

- **Laboratory**

- **Animal Unit**

- **Growth Room**

- **Glass House**

- **Large Scale**

- **Level 1 (GMMs)**

- **Level 2 (GMMs)**

- **Level 3 (GMMs)**

- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**
  - [ ] **Tick if confidential**

- **Bacteriology**

- **Parasitology**

- **Transgenic Birds**

- **Microbiology Research**

- **Virology**

- **Transgenic Animals**

- **Transgenic Fish**

- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 292/01.1**

<table>
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<tr>
<th>Date Ackn'd</th>
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- **Date Project Ceased**: 26/04/2005
- **Non-GMM Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

- **Withdrawn**: N
- **Historical Significant Changes**: Transferred to GM130 on 26/04/2005

---

**Project Additional Information**

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

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Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

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Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Project Ref 292/trans1

### Date Ackn'd
23/02/2001

### CU2 Project Title
THE ROLE OF MOLECULAR ABNORMALITIES ASSOCIATED WITH LEUKARMIA IN SUNVERTING NORMAL HAEMATOPOIETIC DEVELOPMENT

### Date Project Ceased
26/04/2005

### Class
Class 2

### CultureClass
Consent Granted
Not applicable

### Non-GMM

### Project notified under transitional arrangements
Y

### Withdrawn
N

### Tick if notifying a connected programme of work
N

### Historical Significant Changes
Transferred to GM130 on 26/04/2005

### Project Additional Information

#### Purposes of the contained use

#### Recipient or parental organism

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02/03/2022
GM Centre Number: 294

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Name

KINGS COLLEGE LONDON

Name 2

UNIVERSITY OF LONDON (STRAND & WATERLOO CAMPUS)

Department

SCHOOL OF HEALTH AND LIFE SCIENCES

Campus Estate or Research Centre

SCHOOL OF HEALTH AND LIFE SCIENCES

Building

FRANKLIN-WILKINS BUILDING

Road Name

120 STAMFORD ST

District

Town

LONDON

County

GREATER LONDON

Postcode

SE1 8WA

Country

ENGLAND

Tel Number

020 7848 5620

Fax Number

020 7 8485762

E-mail

none

HSE Division

LONDON

Comments

Centre closed and merged with GM295 on 18/01/2006

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Laboratory</th>
<th>Animal Unit</th>
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Other (please specify) [Tick if confidential]

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
- Mycology
- Transgenic Invertebrates
- Transgenic Plants
- Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 295

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#### Name
- **KINGS COLLEGE LONDON**

#### Name 2
- **UNIVERSITY OF LONDON GUYS CAMPUS**

#### Campus Estate or Research Centre
- **GUYS CAMPUS**

#### Road Name
- **GUYS CAMPUS**

#### Town
- **LONDON**

#### County
- **GREATER LONDON**

#### Postcode
- **SE1 9RT**

#### Country
- **ENGLAND**

#### Tel Number
- **0207 836 5454**

#### Fax Number
- **0207 848 2837**

#### E-mail
- **LONDON**

#### HSE Division
- **LONDON**

#### Comments

#### Date at Which Additional Info Submitted
- **02/03/2022**
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Give brief details of the genetic modification safety committee

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**Laboratory**
- Bacteriology
- Virology
- Mycology
- Other(s)

**Animal Unit**
- Parasitology
- Transgenic Animals
- Transgenic Invertebrates

**Growth Room**
- Transgenic Birds
- Transgenic Fish

**Glass House**
- Microbiology Research
- Gene Therapy

**Large Scale**
- Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity
### Project Ref 295/01.1

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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ANALYSIS OF THE MECHANISM OF INTRACELLULAR TRAFFICKING OF PRION PROTEIN

Historical Significant Changes
Project transferred to GM386

Project notified under transitional arrangements

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 295/01.3

Date Ackn'd 15/02/2001

CU2 Project Title IN VITRO STUDIES OF TSE INFECTION USING MOUSE SCRAPIE-DERIVED MODELS

Class Class 2

CultureVolClass2 Non-GMM Consent Granted not applicable

ClassVolumeClass3-4 Project notified under transitional arrangements Y

Date Project Ceased 08/09/2003

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Project transferred to GM386

Historical Date of Additional Info

02/03/2022
**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
**Project Ref** 295/02.1

**CU2 Project Title**
INTRACELLULAR TRAFFICKING OF MUTANT FORMS OF PRION PROTEIN

**Class**
Class 3

**Culture Volume**
Class 2 Culture
Volume: 0.5 litre

**Non-GMM Consent Granted**
yes

**Project notifed under transitional arrangements**
N

**Historical Significant Changes**
Transferred to GM386 on 08/09/2003

**Project Additional Information**

**Purposes of the contained use**

Prion protein is a normal component of many of our cells (especially neurons) that has the unusual ability to change its conformation, either spontaneously or in response to genetic mutation or to prion infectious agent, to an amyloidogenic form that resists proteolysis, accumulates in the brain and appears to be capable by itself of propagating infection. While recent studies have been increasingly successful in replicating elements of this conformational conversion using recombinant prion protein modified in the test tube, no one has produced an infectious agent except within a living cell, suggesting that the full conversion from normal to infectious protein requires the action of some cellular process. The trafficking of prion protein from the cell surface into endosomes is a candidate for such a cellular process since the acidification that occurs in endosomes is used by other proteins (eg diphteria toxin) and viruses (eg 'flu) to change their conformation, enabling the latter types of agent to fuse with the membrane and enter the cell.

Curiously, there is not a strong correlation between the accumulation of this infectious form of protease-resistant prion protein, and neural degeneration. Recently it has...
become apparent that prion protein is capable, during its biosynthesis, of being incorporated into the membrane, not only by its major mechanism (linkage to the luminal surface of the endoplasmic reticulum by a glycosylphosphatidylinositol (GPI) anchor), but also by utilisation of an internal cryptic transmembrane sequence. The proportion of prion protein that is incorporated via this alternative route, which is increased by some mutations that in man predispose to familial CJD, has been found to correlate better with neurotoxicity than has the accumulation of amyloidogenic prion protein in fibrils.

The underlying cause of neurophathology and toxicity in each case is aberrant trafficking of prion protein. Similar faults in protein trafficking are increasingly becoming to be seen to cause other amyloid diseases such as Alzheimer's.

The purpose of the contained use is to enable us to mutate residues (or groups of residues) within normal mouse prion protein to enable us to study particular aspects of the trafficking of this protein, and in particular its translocation into the endoplasmic reticulum and its recycling between the cell surface and endosomal compartments. The mutations will be assessed firstly for their effect on the trafficking of the protein, either in transfected cell lines (N2a cells) or in simplified cell-free systems (microsomal or endosomal preparations); mutations that change trafficking characteristics will then be evaluated for their interaction with delivered mouse scrapie agent (from non-BSE derived strains).

Recipient or parental organism

The parental organism (original source of inserted cDNA was normal (non-infected) mouse brain.

The recipient organisms are:
1. XL2-Blue, derived from XL1-Blue, multiple auxotroph, classified as disabled or non-colonising (ACGM Compendium of Guidance, Part 2A, Annex II, Section 11).
2. N2a mouse neural cell line, maintained without harm or escape in many labs since its derivation in 1969.

Host/vector system

XL2 Blue/pCDNA for construction of expression vectors with mutated PrP
Mouse N2a neural cell line/pCDNA3/4 for expression of above in mammalian cells.

Origin & function

Mouse cDNA encoding normal mouse prion protein was derived originally from mouse brain library. Fragments of it will be subjected to PCR-based directed mutagenesis, which when confirmed by sequence will be incorporated into expression vectors (CDNA 3/4) along with an epitope or fluorescent protein tag (normally inserted after the signal sequence (amino acids 1-22), either immediately before aa 23; Lee et al (2001) J. Neurochem. 79: 79-87) or slightly more distally (after aa 42; Negro et al (2001) Mol. Cell. Neurosci. 17:521-38) where it appears not to perturb prion protein trafficking), for the following purposes:

1. Translocation of prion protein: individual amino acids will be altered to one with an expected functionally neutral character primarily within the signal sequence (amino acids 1-22), or the surrogate transmembrane sequence (aa's 100-136) or the GPI anchor (aa 231 ff). These alterations very the proportion of prion protein that is incorporated into the membrane via its standard means (GPI anchor) compared to its minor variations, as a type 1 or type II transmembrane protein (Kim et al, (2001) J. Biol. Chem 276: 26132-40; Stewart, R. S. et al (2001) Mol Biol. Cell 12: 881-9). These constructs incorporated as cDNA into expression vectors will primarily be transfected into N2a cell lines to allow cell fractionation followed by immunoaffinity purification to determine the chaperone associations of the proteins (Graham, C H Anderton, B A & Morris, R J (2001) submitted for publication) as they traffic between the biosynthetic compartments of the cells. Studies may also be done where these constructs are introduced into primary cultured neurons by microinjection or by chemically-assisted transfection (Ca 2+, Liptofectine etc), to allow comparison with real neurons. In addition, sense RNA may be transcribed using an RNA polymerase, capped and polyadenylated before being used in in vitro translation studies with microsomes (Kim et al (2001) J. Biol Chem 276: 26132-40).

2. Interaction of externally applied prion infectious agent with translocation of mutant prion protein at the endoplasmic reticulum. To determine whether and how exogenously applied prion infectious agent could alter the routing of prion protein as it is made on the ER, mouse scrapie-associated fibrils or whole homogenate of mouse scrapie-infected brain or cells will be added to cells expressing normal or mutant forms of prion protein (from step 1), and the proportion of protein translocated in the
transmembrane forms (and its subsequent fate) determined, with particular attention paid to any change in the chaperone proteins associated. It should be noted that here and in 4 below, long studied mouse strains of scrapie derived originally from sheep (ME7, Chandler) and not from bovine or human sources will be used.

3. Endocytic trafficking of mutant forms or prion protein. We have an existing CL2 approved project (GM295/01.2) for investigating the effect of mutations in the N-terminus (aa's 23-108) of prion protein upon the trafficking of prion protein from the cell surface to intracellular compartments; this application will extend this by using mutations within the C-terminal domain, primarily with the surrogate transmembrane domain and GPI anchor (as in 1 above).

4. Interaction of externally applied prion infectious agent with mutant prion protein. We have an existing CL2 approved project (GM295/01.3) for investigating the interaction of mouse scrapie agent with cells; this application will extend this by studying the interaction with cells bearing mutations in prion protein that affect the endocytic trafficking of this protein (as in 3 above).

**Evaluation of foreseeable effects**

It must be assumed that at least some of the mutations we produce will predispose the mouse prion protein to adopt an infectious or neurotoxic conformation. Regarding the infectious form, the amounts produced will be small, contained, and rigorously denatured by oxidants before disposal for incineration. Moreover, the point mutations involved will in no way break down the species barriers for transmission of the infection to either man or to cattle, so that any escape of the mutation should affect no more than any local mouse populations. Regarding the potential neurotoxicity of the transmembrane forms of prion protein, these are not protease resistant and will be broken down normally by our usual physiological barriers in the gut and skin (and by our containment and denaturation procedures). There is no reason to think there is any species barrier to the neurotoxicity, but equally the barriers to a foreign protein reaching a nerve in our body are formidable.

At the genetic level, in the regions that will be mutated the mouse sequence is either identical or very similar to the human sequence. CL3 level of containment, with accompanying SOPs (minimisation of aerosols, use of sharps etc) will ensure that escape of mutated sequences does not occur, but should, despite these precautions, cDNA encoding a mutant form of prion protein be injected into eg the finger of a researcher, and become translated into mutant protein, all the other prion protein in the body would remain human and non-mutated, so that any production of infectious prion should be restricted to the site of injection.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Given the work involves mutant prion protein, all work will be done under containment level 3 conditions with the additional safeguards appropriate for working with mouse scrapie itself. Of these the most important are those for waste disposal, outlined below.

Liquid waste will be disinfected in 20,000 ppm active chlorine (as sodium hypochlorite) overnight before being washed down the drains with copious water. Solid waste will be double bagged for autoclaving (135 1 degree C, 18 min) prior to incineration.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid wastes: Double bag and autoclave at high vacuum, 135 1 degree C for 18 min (or, if not possible inactivate overnight with 20,000 ppm active chlorine then double bag), dispose by incineration as clinical waste.

Liquid waste: Inactivate with 20,000 ppm active chlorine in the form of sodium hypochlorite overnight, wash down drains with copious water.

For treatment of liquid wastes 20,000 ppm active chlorine is considered to be a completely effective procedure for inactivation of TSE agent; for solid waste, incineration is completely effective, the autoclaving will substantially reduce the titre of any agent (should it be produced) adding an extra level of security during the transport to the incinerator.

Monitoring is only applicable to the autoclave step, where we use Brown tubes within a load, and have a printed readout of each cycle; the autoclave is serviced annually. The ultimate form of the prion protein will be fragmented and highly oxidised carbon derivatives with no biological activity.
The Genetic Modifications Safety Committee of the MRC Centre for Developmental Neurobiology reviewed this application at its meeting of 13 December 2001. The Committee were satisfied that the steps taken to contain and dispose of the mutant mouse prion proteins and the constructs encoding it were sufficient and conformed fully to national guidelines and local needs. The staff involved have been fully consulted in drafting the Risk Assessment, and effective Standard Operating Procedures are in place. The Committee therefore accepted this application.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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### Project Ref 295/02.2

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<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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Date Project Ceased: 08/09/2003

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: Transferred to GM386 on 08/09/2003
**Project Additional Information**

**Purposes of the contained use**

The purpose of the contained use is to enable us to pursue studies of primate lentiviral replication (that is, HIV-1, -2, and SIV) and of murine leukemia virus (MLV) without exposure of personnel or of the external environment to these pathogens. In addition, contained use will enable proper decontamination of the laboratory space and large equipment used for these studies.

HIV is the causative agent of Acquired Immunodeficiency Syndrome (AIDS). HIV-2 and SIV are related viruses that cause less severe disease in human being. MLV is a retrovirus with a tropism for a variety of mammalian cells.

**Recipient or parental organism**

A variety of immortalized cell lines will be utilised for these studies including:

- Human T lymphoid (Jurkat, CEM, SupT1, C8166, HUT78, H9)
- Human fibroblast (HeLa, 293T)
- African green monkey fibroblast (COS)
- Murine fibroblast (3T3, L)
- Murine Lymphoid (EL4, Timi)
- Quail fibroblast (QT6)

**Host/vector system**

HIV-1, -2, SIV or MLV viruses will be passaged in the immortalised cell lines mentioned above.

**Origin & function**

All immortalised cell lines and wildtype viral isolates are obtainable from the AIDS repository at the US National Institute of Health.

The genetic material involved is intended to aid in the investigation of viral protein function and the molecular mechanisms of viral assembly and infection. Generally, by removing regions of the viral genome, we can examine in what ways the virus life cycle is altered and thereby determine the role of the deleted region.

**Evaluation of foreseeable effects**

In comparison to wild type virus, all modified HIVs will have reduced pathogenicity as a consequence of disruption of essential viral sequences. Importantly, all such viruses will be maintained and used under category 3 conditions. All necessary precautions are being undertaken to prevent infection of workers or release from the containment 3 laboratory. Special attention has been paid to minimisation of aerosols and sharps. In the unlikely event of exposure of a worker to the virus, prophylactics and emergency procedures are on hand.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Given the work involves primate lentiviruses, all work will be done under containment level 3 conditions. Of these the most important are those for waste disposal, outlined below.

Liquid waste will be disinfected in 10% active chlorine (as sodium hypochlorite) overnight before being washed down the drains with copious water. Solid waste will be...
double bagged for autoclaving (135 1 degree C, 18 min) prior to incineration

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid wastes: Double bag and autoclave at high vacuum, 135 1 degree C for a minimum of 18 minutes. After autoclaving, waste is bagged in designated yellow bags, offloaded from the site as clinical waste and, ultimately, incinerated.

Liquid waste: Inactivate with 10% active chlorine in the form of sodium hypochlorite overnight, wash down drains with copious amounts of water.

For treatment of liquid wastes 10% active chlorine is considered to be a completely effective procedure for inactivation of retroviruses. For solid waste, autoclaving is completely effective.

Monitoring is only applicable to the autoclave step, where we use Brown tubes within a load, and have a printed readout of each cycle; the autoclave is serviced biannually.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee of the MRC Centre for Developmental Neurobiology reviewed this application at its meeting of December 13th 2001. The Committee were satisfied that the steps taken to contain and dispose of the primate lentiviruses and infected cells and the constructs encoding it were sufficient and conformed fully to national guidelines and local needs. The staff involved have been fully consulted in drafting the Risk Assessment, and effective Standard Operation Procedures are in place. The Committee therefore accepted this application.

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Human Clinical Applications

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<th>L4</th>
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**Project Additional Information**

**Purposes of the contained use**

The purpose of this activity is to study the intracellular signalling mechanisms that mediate the response of cells to growth factors and extracellular matrix molecules, including proliferation and migration, and thus play a role in the development of human cancers and in cellular differentiation.

**Recipient or parental organism**

(i) E. coli

(ii) murine cells (NIH3T3, and the NIH3T3 variants 293T, GP+E86, GP+EnvAm12)

(iii) human primary keratinocytes and carcinoma cell lines

(iv) monkey cells (COS-7)

**Host/vector system**

(i) Host: E. coli DH5a; Vector: pCEV27, pBABEneo/puro plasmid, pMSCVneo, pMSCVhygro, pLib, pRx-bsr, pRcCMV, pC1, pGET-T, pPCR-Script

(ii) Host: GP+E86 murine fibroblasts; Vector: pBABEneo/puro, pMSCVneo, pMSCVhygro, pLib, pRx-bsr plasmid

(iii) Host: GP+envAm12 murine fibroblasts; Vector: pBABEneo/puro, pMSCVneo, pMSCVhygro, pLib, pRx-bsr plasmid

(iv) Host: Murine and human mesenchymal cells (osteoblasts, chondrocytes, fibroblasts, and adipocytes) either as established cell lines or primary cultures, as well as osteoclasts, osteoclast precursors, haematopoetic stem cells, and embryonic stem cells. Vector: pBABEneo/puro plasmid, pMSCVneo, pMSCVhygro, pLib, pRx-bsr virus

(v) Host: NIH3T3 murine fibroblasts; Vector: pBABEneo/puro plasmid

(vi) Host: HNEK, H357, A431, HN12, HN4 & MCF7 human keratinocytes; Vector: pBABEneo/puro virus

(vii) Host: 293T cells; Vector: PINCO (modified from plasmid LZRSpBMN-Z).
Mouse and human cDNAs encoding signalling intermediates, cell cycle control genes, and AP-1 transcription factors, as well as novel cloned target genes, will be obtained by PCR, or excised from existing plasmid vectors, subcloned into viral plasmids, and used to produce amphotropic/ecotropic viruses as a means of expressing them in the target cells (see above). The intended function is to investigate growth factor and extracellular matrix-dependent signalling pathways that regulate cellular differentiation, proliferation and motility, all of which are key aspects of cancer development.

Evaluation of foreseeable effects

The vectors to be used in these studies are replication-defective recombinant retroviruses, and have been well characterised and extensively used (Morgenstern & Land, Nucl Acids Res 18: 3587, 1990; Gasperi et al., Journal of Leukocyte Biology 66: 263-267, 1999). They are non-mobilisable in mammalian cells, and are rapidly inactivated by serum. The cell lines (GP+E86 and GP+envAm12 are third generation packaging cell lines, and have been specifically constructed to prevent recombination events between introduced constructs and endogenous viral structural sequences. Investigators using this system have not observed production of any replication-competent virus (RCV) (Markowitz et al., Virology 167: 400, 1988). The recipient cell lines are free of helper virus, and transduction of these lines with replication-defective viruses will not generate RCV. In spite of these findings, assays to detect RCV will be carried out as a matter of course. Thus, although the vectors will be packaged with ecotropic and amphotropic envelopes, and could conceivably infect rodent (ecotropic) and human cells (amphotropic), such viruses would be incapable of replication. Furthermore, these viruses are unstable at ambient temperatures, with a half-life of around 2 hours. Some of the gene products encoded by cDNAs cloned into these vectors have oncogenic potential. Therefore, the assessment is that work with both ecotropic and amphotropic viruses will be carried out at level 2. The containment level and procedures proposed for the study, together with the inherent safety of the packaging systems, will make the environmental risk effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from bacterial and vertebrate cell cultures will be aspirated and treated with bleach (Haztabs; 1 tablet per litre = 2,500 ppm chlorine) for 12 hours. The bleach will be made freshly on the day of use according to manufacturers instructions. This will result in at least 99.999% kill of bacteria and 100% kill of viral GMMs and cell lines which are extremely liable. In addition, the retroviruses and cell lines have a very short half life at room temperature. Inactivated culture supernatants will be discharged to the drainage system. Solid waste will be double bagged, sealed, and placed within and autoclaved in a closed metal container. This will prevent any accidental release of GMMs into the working environment. The waste will be autoclaved at 136 degrees C for 30 minutes. This will result in complete inactivation of GMMs. Autoclave function will be monitored by annual services, the use of autoclave indicator tape (which measures a temperature of 134 degrees C for 3 minutes) during every run. Autoclaved waste will then be incinerated by White Rose.
The GMSC accepted the risk assessments for these projects.

Class of Activity 2.

### Project Containment

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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### Project Ref 295/02.4

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<td>21/01/2002</td>
<td>GENE EXPRESSION AND SIGNALLING DURING CRANIOFACIAL AND LIMB DEVELOPMENT</td>
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</table>

Non-GMM: not applicable

Consent Granted: not applicable

Project notified under transitional arrangements: N

Tick if notifying a connected programme of work: Y

### Project Additional Information

The purpose of this activity is to characterise the molecular function of regulatory molecules during craniofacial and limb development; how aberrations of these mechanisms may result in malformations and disease; and to understand how we might prevent and/or treat these conditions.

Recipient or parental organism
1. **Host/vector system**

| 2. Host: chick and quail fibroblasts; Vector: RCAS(BP), RCAN retroviruses |
| 3. Host: COS-7 cells; Vector: pcDNA3 |
| 4. Host: murine cartilage (ATDC5) and myogenic cells (sol8 and C2C12); Vector: pJMF2 |
| 5. Host: NIH3T3 murine fibroblasts; Vector: pJMF267. Host: D17 canine fibroblasts; Vector: pCXL |
| 6. Host: Rat B1 Fibroblasts; Vector: LNCX murine retrovirus |

**Origin & function**

Human, chick, zebrafish, Monodelphis, Xenopus and mouse cDNAs for this project have been isolated from cDNA libraries and cloned into the vectors listed above. The intended function is to investigate signalling pathways involved in muscle development and in the development of sensory structures, and to uncover the signalling mechanisms underlying human diseases and syndromes.

**Evaluation of foreseeable effects**

The vectors to be used in these studies are, with the exception of the RCAS(BP) virus, incapable of replication/transmission outside the laboratory.

The vector, RCAS(BP) is an avian specific retrovirus based on Rous sarcoma, from which the host derived oncogene src has been removed. This virus has been characterised and used extensively (see Morgan and Fekete, 1996. Methods in Cell Biol. 51, 185). The retrovirus is unable to infect cells other than avian cells and is only transmitted by direct injection/transfection of avian cells. For infection and growing viral stocks, O line cells which are free of any known ASLV endogenous viruses, eliminates the possibility of recombination between exogenous and cryptic endogenous virus. The retrovirus, pCXL vector, can infect avian, primate and rodent cells. This vector is replication defective. Thus, the infective virus can only be produced in a specialised packaging cell line. Thus once injected or introduced into the host avian cells there is minimal possibility of viral spread within the tissue or outside the laboratory. The expression plasmids are not released from the host tissue culture cells and are used within dedicated containment 2 laboratories. They, therefore, pose no risk to the outside environment.

Exposure and risk to health of the researcher is reduced to negligible levels by (a) performing culture work in a class 2 MSC and (b) injecting the retrovirus or using retrovirally infected cells in a dedicated area of containment 2 laboratory where other workers do not have free access. In addition, before carrying out these procedures all personnel are fully trained. This will reduce risk of accidental injection of either the RCAS(BP) or pCXL virus to the absolute minimum.

For all these cell/retroviral studies, infected cells will be contained within dedicated incubators. All GM manipulated material described above will be destroyed on site. Together with the instability of the viruses at ambient temperature this generates effectively zero risk to the environment.

Cells containing the retroviruses and cell lines transfected with plasmid expression constructs/retroviruses will be cultured in dedicated incubators in a containment level 2 tissue-culture laboratory. The retroviruses, RCAS(BP) and pCXL will also be used within a dedicated area of a level 2 molecular laboratory, again infected cells will be cultured in dedicated incubators. All material will be destroyed on site immediately after use. Liquid waste will be bleached and therefore, the GM material will be destroyed, within the contained tissue culture laboratory. Solid waste will be autoclaved. Transport to the autoclave from the tissue culture and molecular laboratories, will be in double bags within contained metal boxes. The waste will be autoclaved in these boxes to prevent any possible accidental spillage/release. Autoclaved waste will then be placed in yellow bags for incineration by White Rose.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from bacterial and vertebrate cell cultures will be aspirated and treated with bleach ( Haztabs; 1 tablet per litre = 2,5000 ppm chlorine) for 12 hours. The bleach will be made freshly on the day of use according to manufacturers instructions. This will result in at least 99.999% kill of bacteria and 100% kill of viral GMMs and cell lines which are extremely liable. In addition, the retroviruses and cell lines have a very short half life at room temperature. Inactivated culture supernatants will be discharged to the drainage system. Solid waste will be double bagged, sealed, and placed within and autoclaved in a closed metal container. This will prevent any accidental release of GMMs into the working environment. The waste will be autoclaved at 136 degrees C for 30 minutes. This will result in complete inactivation of GMMs. Autoclave function will be monitored by annual services, the use of autoclave indicator tape (which measures a temperature of 134 degrees C for 3 minutes) during every run. Autoclaved waste will then be incinerated by White Rose.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC accepted the risk assessments for these projects.

Class of activity 2.

Project Containment

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Project Ref 295/03.1

Date Ackn'd 10/02/2003

CU2 Project Title ANTIBODY MODULATION OF TB INFECTION

Class 3

CultureVolClass2 75 ml

CultureVolumeClass3-4 75 ml
**Purposes of the contained use**

Mycobacterium tuberculosis (MTb) is a class 3 human pathogen and requires level 3 containment facilities for use. The recombinant MTb expressing luciferase gene would be used to significantly decrease the experimental times for bacterial determination in infected cultures and BCG expressing either luciferase or green fluorescent protein (GFP) would allow additional flexibility in substituting pathogenic MTb in certain types of assays. Luciferase and GFP are biologically harmless genes but as the recipient strain is pathogenic the containment level 3 would be required for the use of recombinant strains as for the wild type MTb. The same applies to Mycobacterium bovis (MB) expressing luciferase gene which would be used to uncover differences in pathogenesis between these two closely related species.

**Recipient or parental organism**

The recipient organism is bacterium Mycobacterium tuberculosis (MTb) or the attenuated strain of Mycobacterium bovis (MB) known as BCG (Bacilliys of Calmette and Guerin). MTb and MB are class 3 organisms and BCG is Class 2

**Host/vector system**

pSMT1 and pSMT3, both non-mobilisable vectors.

**Origin & function**

Luciferase gene is of bacterial origin and its function in the intended recombinant strains of Mycobacteria is to serve as reporter gene for quantitative determination of bacteria. The green fluorescent protein (GFP) is derived from jellyfish and is a fluorescent marker for tracking down the recombinant bacteria during infection of cells.

**Evaluation of foreseeable effects**

The recombinant Mycobacterium tuberculosis and Mycobacterium bovis expressing luciferase and BCG expressing luciferase or GFP are generated several years ago in the laboratory of Douglas Young at Imperial College London (Lab GM77). They have been used since then worldwide and several publications have quoted the use of these strains. Both genes are reporter constructs. Luciferase is an enzyme that converts certain type substrates in to chemiluminescent products and GFP is a fluorescent protein that can be easily identified by fluorimetric techniques such as flow cytometry (FACS). These two genes have been used in numerous experimental systems with no biologically harmful effects every being reported. The use of these two genes would normally require level 1 containment measures but as the recipient strains are class 3 (MTb and Mb) or class 2 (BCG) organisms, it is necessary to apply the class 3 or respectively, class 2 containment measures. There is no indication from the originator or from the others who have used these strains that they are in any way different in terms of virulence from the corresponding wild type strains.
Therefore, the environmental or personal risk from using these strains is no different from that of wild type strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Re: APPLICATION FOR DEROGATION FOR AUTOCLAVE FACILITY FOR CONTAINMENT LEVEL 3 LABORATORY

During a recent inspection by HSE, it was pointed out to us that there had been a change in the regulations defining what constitutes a laboratory suite, and as a result our containment level 3 facility (room 311, floor 28 Guy’s Tower) contravened the new interpretation under the Genetically Modified Organism (Contained Use) Regulations 2000 (CU2000). I therefore am applying for derogation from Schedule 8 of CU2000.

The relevant features, following the guidance attached to Newsletter 30 are:

a) Both the autoclave and the containment facility are located at the far end of the building remote from the main access to the floor. They are joined by a short corridor that is not used by students, non-scientific staff or members of the general public. There are no offices at this end of the building, only laboratories.

b) The infection with Mycobacterium tuberculosis and mycobacterium bovis occurs through respiratory route. The infectious dose has not been firmly established but it is thought that the size of aerosol-born droplets containing bacteria is critically important (i.e. several droplets containing 3-5 bacteria each, if inhaled by an immuno-susceptible person may establish an infection). Only 5% of infected individuals develop the disease.

c) The highest concentration of bacteria ever present in the waste is 10^7/ml which is the remaining stock solution from the experiment (the stock solution is 1 ml, thus the amount of this waste is always less than 1 ml). The unused stock solution is routinely treated with hycolin for several hours prior to disposal. Typical concentration of bacteria in the experimental liquid waste is 10^5/ml or less.

d) The waste is in loosely tied autoclave bags contained in metal autoclave bins.

e) Following the inspection we have purchased autoclave tins with clip-on lids to replace the identical ones without clip-on lids.

f) as in (e) above.

g) The container will be placed on a trolley following an agreed procedure that has been in place for the last 2 years.

h) the removal of waste from the Containment Laboratory does not take place unless it is clear that the autoclave is available for use. After this waste has been loaded into the autoclave low level waste may also be added, but only by the staff loading the level 3 waste.

i) As in (h) above.

j) The container is placed directly in the autoclave and the tray that sits on the trolley is then also placed in the autoclave.

k) The only personnel that move the waste are trained for work at containment level 3.

l) The SOP (revised to include the use of clip-on lids) for use of the Containment facility is attached. This contains an emergency procedure to be followed in the event of a spillage during waste transport.
1. All glassware and plastics must be autoclaved on a cycle to reach 126 degrees C before removal from the laboratory suite. Autoclave bins with properly fitting clip on lids containing the waste in loosely tied autoclave bags must be taken from the CL3 laboratory to the autoclave immediately prior to autoclaving. The following procedures will be follows:
   i) at least two trained staff must be available to carry out these procedures
   ii) the autoclave is room 3245 must be available for use
   iii) one member of staff will act to open doors; one will move the autoclave bins from the CL3 facility onto the transport trolley
   iv) the autoclave bins will be removed from the CL3 facility and placed on a tray on a trolley outside the CL3 laboratory
   v) autoclave bins from the CL3 laboratory will be taken directly to the autoclave and placed in the autoclave
   vi) when all autoclave bins have been loaded into the autoclave, the tray will also be placed in the autoclave and the autoclave cycle will be started.

2. If a spillage of CL3 infectious material occurs during transport of waste, the area will be disinfected by treatment with Presept, and/or by swabbing with disinfectant.
   i) Any contaminated clothing must be removed and any affected skin should be washed with soap and water. In the event of cuts, the First Aid Officer should be contacted.
   ii) Notification of the Biological Safety Officer and the relevant Line Manager must then take place. They will decide if any further action is required.

The waste consisting of disposable plastics and liquid waste in volumes of 100 ml or less in plastic containers will be placed in plastic bags, sealed and transferred to metal bins with clip-on lids. The standard internal operating procedure for removal and autoclaving of waste will then be applied (for details please see above application for derogation of autoclave facility for CL3 room).

The autoclave will operate the cycle at 126 degrees C for 80 min at 2.5 bar. The waste will then be placed in bags and disposed to designated bins.

This procedure is effective for complete killing of Mycobacterium tuberculosis wild type as determined by re-plating of the autoclaved material on microbiological plates. Similar test will be conducted for GM strains of MTb and MB (ie following completion of the cycle the metal been containing the waste will be returned to CL3 room in a reverse of the removal of waste procedure and the waste placed in Microbiological Safety Cabinet. An aliquot of the liquid waste and a swab from the solid waste will be tested for the presence of live bacteria by microbiological methods. Only following the negative result of the test will the remaining waste be removed from the room in a repeat of standard procedure and autoclaved prior disposal. This test-procedure will be repeated each time when the nature or the quantity of the waste significantly differs from the routine waste.

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
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<tbody>
<tr>
<td>Is an emergency plan required according to regulation 20?</td>
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<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
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<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
</tr>
<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>N</td>
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</tbody>
</table>
The proposal was looked at by two members of Safety Committee who were satisfied with risk assessments and proposed risk categorization.

The class of activity is 2/3 as detailed in the KCL GM RA.

**Project Containment**

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref**  295/03.2

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<td>FUNCTIONAL ANALYSIS OF EXOCYTOSIS, ENDOCYTOSIS AND RECYCLING</td>
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<th>Project notified under transitional arrangements</th>
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<td></td>
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<th>Withdrawn</th>
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The aim of this study is to investigate the role of candidate intracellular proteins involved in exocytosis, endocytosis and membrane recycling by expressing them acutely using recombinant Semliki Forest Virus.

**Recipient or parental organism**
The virus to be used is the replication deficient, recombinant Semliki Forest Virus (SFV) as this offers the only viable means for acutely expressing exogenous proteins in populations of post-mitotic cells and established cell lines. SFV is an enveloped positive-strand RNA virus in the alphavirus genus of the Togaviridae family and has been extensively studied in cell biology and virology laboratories. Replication deficiency is achieved by deleting the codons encoding the structural proteins necessary for packaging the RNA from the viral genome (Liljestrom, P. and Garoff, H. (1991) "A new generation of an imal cell expression vectors based on the Semliki Forest virus replicon" Biotechnology 9, 1356-1361 Knight D E (1999) "Secretion from bovine chromaffin cells acutely expressing exogenous proteins using a recombinant Semliki Forest Virus containing an EGFP reporter". Molecular and Cellular Neuroscience 14: 486-505). Certain features of SFV make it unique for use as an expression system. As an RNA virus, it has a broad host range and rapidly synthesize exogenous proteins encoded in its genome. It effectively takes over the host protein synthesis.

Host/vector system

The virus will be used to infect bovine adrenal medullary cells (maintained in culture for about one week after isolation from the adrenal gland) and into established cell lines, such as BHK, HEK, HEP2 and PC12 cells. DH5a bacteria will be used to grow up plasmids necessary for the production of the RNA viral genome. The plasmids to be used are pSFVEGFPsubX, pSFVXiresEGFP to construct the viral genome, pSFV-help1, pSFV-help2 to construct the structural proteins necessary to generate the recombinant virus, and pRK5, pEGFP, pEYFP as subcloning vectors and to construct fusion proteins (Knight D E (1999) "Secretion from bovine chromaffin cells acutely expressing exogenous proteins using a recombinant Semliki Forest Virus containing an EGFP reporter". Molecular and Cellular Neuroscience 14: 486-505). None of the DNA vectors alone are pathogenic or oncogenic. The RNA viral genome derived from pSFVEGFPsubX and pSFVXires EGFP will eventually kill the infected cells as the viral RNA will replicate and take over the cell's endogenous protein synthesis machinery.

Origin & function

The proteins to be expressed are all proteins that are thought to be involved in membrane traffic or are used to detect membrane traffic. They fall into four groups.

a) Well characterised membrane proteins that are used as models for membrane traffic, and include various subunits of ligand gated ion channels (Serotonin and glutamate receptors) and also secretory products (chromogranin and human growth hormone)


c) Small GTP binding proteins including rab 3 and rab 5, and the subunits of the heterotrimeric GTP binding protein.

d) Putative calcium sensors (or fragments thereof) including synaptotagmins, freqeulin and calmodulin.

Evaluation of foreseeable effects

The recombinant Semliki Forest Virus is replication incompetent. The efficiency of viral RNA replication and the rapid exogenous protein synthesis effectively starves an infected cell of its own protein synthesis. The lifetime of an infected cell ranges from less than 1 day for BHK cells to about 3 days for post mitotic chromaffin cells. The proteins to be expressed by the recombinant virus are all proteins that are thought to be involved in membrane traffic or are used to detect membrane traffic. When expressed in isolated bovine adrenal medullary cells, which have been maintained in culture, they might affect the rate and extent of catecholamine secretion and the subsequent uptake and recycling of vesicular membrane.

Expression of secretory markers will allow regulated secretion to be monitored in nominally constitutively secreting cells. There are no foreseeable effects on the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be autoclaved at 121 degrees C for 20 minutes before being disposed of as clinical waste.
All fluid waste will be incubated in 3% Virkon overnight before being disposed of in the sink.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

Please enter comments on the GM safety committee on the risk assessment

The local GMSC approved the risk assessment for this project on 17th September 2003. The class of activity is 2.

Project Containment

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</table>

Project Ref 295/08.1

Date Ackn'd 08/01/2008  

Date Project Ceased 04/03/2014  

Cancer cell motility and imaging.

Class 2  

Consent Granted Not Applicable  

PROJECT TRANSFERRED ON MERGER OF GM295 WITH GM386  

Tick if notifying a connected programme of work  

Y
The key objective is to bridge the gap between Imaging, Cancer Cell Biology and Medicine, particularly in the field of translational cancer research. The aims are summarised as follows:

1. Development of cancer cell imaging facilities which can measure biochemical changes in cancer patient-derived tumour samples, including fresh primary tumour cells.
2. Investigation of the function of specific gene products/protein in a range of mammalian cell lines and primary cells, by gene expression or knockdown.
3. The cells for these gene expression or knockdown studies will include primary tumour cells, primary human T cells, human T-cell leukaemia cells, monocytes and endothelial cells, and primary cells derived from mouse tissues. For these studies, we use plasmid-based expression vectors, retroviruses, adenoviruses and lentiviruses.

Recipient or parental organism

E. coli strains: E.coli JM101, JM109, DH5alpha, DH1OB, DB 3.i, InvcuF+, JS4, XL1-Blue, BL2i(DES), TOPIO. TNV1 10, Kl2 strains DHSu, BL21(DE3) and TOP10

Cells:
- Human breast — MCF7, MDA-MB-231, MDA-MB-435, MDA-MB-361, MDA-MB-468
- Human T-cell — Jurkat, NKL, CCRF-CEM
- Human embryonic kidney — 293T, 293A, 2930P0
- Mouse Fibroblast — PT67
- Rat Breast Carcinoma — MTLn3, MTLn3e
- Human hepatocyte — HepG2
- Human B-cell — 721.221, Raji
- Human NK cell — YT, NK92
- Human Myelogenous Leukaemia — K562
- Primary human tumour cells, T-cell leukaemia cells, primary human T cells and endothelial cells, and primary cells derived from mouse tissues.

Host/vector system

Host:
- B. coli strains: Ecofl JM101, JM109, DH5alpha, DH1OB, DB 3.i, InvcuF+, JS4, XL1-Blue, BL2i(DES), TOPIO, INVI 10, Kl2 strains DHSu, BL21(DE3) and TOP10

Vector names:
- For expression in mammalian cells: pEGEP C 1/2/3 and Ni pECFP-C1. pEYFP-C1 are members of the Clontech "Living Colours" family of vectors pcDNA 3.1+ and pcDNA3J MycHis vectors (Jnvitrogen)
- For expression in E.coli:
  - pGEX vectors (Aniersham)
  - pLPCX, pLHCX (Clontech)
- Lentiviral vectors
  - pLL4.4 (CAG promoter EGFP-MCS)
- Lentiviral packaging plasmids (3rd generation tour plasmid based system)
pCMV-VSVG, pRS V-REV, pMDLg/pRRE, pAR 8.91 (encodes accessory proteins for replication including Gag-Pol from WV-i), pMD.G (encodes VSV-G), pLKOJ-puro (Mission vector), p'HRsincptSkW-eGFP

Adenoviral vectors: pCR220 (Serotype 5 backbone with E1 and E3 regions deleted)

Websites for vectors:
eGFP/eCFP/eYFP vectors: http://www.clontech.com/images/pt/dis_vectors13025jdf
pcDNA3.1 myc/His http://www.invitrogen.com/contentfs vectors/pcdna3 Imychis %20manydf pGEX vectors
http://www4.amershambiosciences.com/pdfs/970004M2-0 1.pdf pLPCX
http://www.c1ontech.com/images/pt/PT3299-5.pcf
pLHCX
pLL4.4

The pLenliLox system was developed in the labs of frank Gertler and Luc VanParis at MIT, Cambridge, USA.
http://wwwnamrecomdoifinder/ 10 I 038/ngl 17

Lentiviral packaging plasmids
http://www.medecine.unige.ch/—salmon/main/pMD2G.jpe
http://www.medecine.unige.ch/—salmon/main/yRSV Rev iDea
http://wwwmedecineazjgech/—salmon/mainpMDLprREjpec
pLKO.1-puro:
http://www.siemaalrich.com/AreaofInterest/Life Science Functional Genomics and RNAlibrary InformationNector
Man Jitml
pCR220:
http://www.patentstorm.us/patentsf629l 21 4-description.html

Host:
Human T-cell line — .Turkati-luman embryonic kidney ccl line —2931, 293A, 293GPG
Mouse Fibroblast cell line — P167
Rat Breast Carcinoma cell line — MTLn3, MTLn3e
Human hepatocyte cell line — HepG2
Human B-cell line—72121I, Raji
Human NK cell line — Yl, NK92, NKL
Human Myelogenous Leukaemia cell line — K562
Primai-y human rumour cells, primary human 1 cells and endothelial cells, and primary cells derived from mouse tissues.

Origin & function

Human, mouse, rat cDNA5 are obtained from existing eDNA libraries.

Lentiviral vectors encoding shRNAs targeting human proteins, obtained from Sigma (Mission library)

The intended function is to investigate the regulation of cancer cell and leukocyte motility

Evaluation of foreseeable effects

Lentivirus:
The pLentilox lentiviral vector system is a so-called 3 generation lentiviral vector that has been extensively
modified to prevent viral replication outside the packaging cells:
- pLentilox has a self-inactivating 3'LTR (TATA box deletion) and large deletions result in absence of env, tat, rev, vpr, vpu, v(f and nef. Therefore, there are no viral gene
products expressed from pLentiox.
- the gag, pot, and coy genes are supplied in trans from separate plasmids only in the packaging cell line.

References for packaging vectors:

References for self-inactivating LTR:

Adenovirus:
- The pCR220 (adenovirus serotype 5 backbone) is a plasmid containing most of the human adenovinis serotype 5 (Ad5) genome, with deletions in the genes E1 and E3. This vector has been developed to infect but not replicate in non-permissive target cells. The E1 gene is provided in the packaging cell line (HEK293 cell line) in trans. Exposure and risk to health of the researchers is reduced to negligible levels by performing the class 2 work in a class 2 microbiological safety cabinet in a dedicated area of a containment 2 laboratory. All personnel are fully trained to reduce the risk of accidental spill of virus to a minimum. For all these cell/retroviral studies, infected cells will be contained within dedicated incubators. All GM manipulated material will be destroyed on site.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from bacterial and vertebrate cell cultures will be aspirated and treated with bleach (Haztabs; 1 tablet per litre 2,500 ppm chlorine) for 12 hours. The bleach will be made freshly on the day of use according to the manufacturers instructions. This will result in at least 99.999% kill of bacteria and 100% kill of viral GMMs and cell lines. In addition the cell lines and retroviruses have a very short half life at room temperature. Inactivated culture supernatants will be discharged to the drainage system. Solid waste will be double bagged, sealed and autoclaved. This will prevent any accidental release of GMMs into the working environment. The waste will be autoclaved at 136°C for 30 minutes. This will result in complete inactivation of GMMs. Autoclave function will be monitored by annual services and the use of autoclave tape (which measures a temperature of 134°C for 3 minutes) in every autoclave run. Autoclaved waste will then be incinerated by White Rose.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Please find attached the GM Proposal and Risk Assessment for the project entitled “Cancer cell and leukocyte motility and imaging”. I am satisfied that they have made the amendment requested b) at its meeting on September 4th, and would be grateful if you could sign as appropriate. Below is the relevant section from the minutes of that meeting.

I From
12006
1/2
2005 cancer cell motility and imaging
1.
I.
Randall Institute introduced the project. had previous received approval at Level 1 for the project, but wished to incorporate work with primary cells using attenuated viral vectors (lenti and adeno), which is categorised as Class 2. This was now a joint submission with
2A, (ii) 4: Answer should be “No”, and subsequent explanation of “Yes” response should be removed.

Approved at CL2 subject to implementation of above amendment, completion of CU2 form, payment to HSE and provision of completed signatures page.

Letter from School Safety Manager and representing the committee:
College Safety Officer.

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Project Ref 295/10.1

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<td>10/08/2010</td>
<td>Investigation of signalling pathways in human myometrial smooth muscle cells and epithelial cells of the reproductive tract</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Date Project Ceased 04/03/2014

Withdrawn N Tick if notifying a connected programme of work N
The overarching goal of this study is to gain greater functional insight into the physiological and pathophysiological regulation of the female reproductive tract during pregnancy. Our work focuses on
i) The control of uterine smooth muscle growth and excitability and ii) the response of reproductive tract to infection and inflammation.
i) Ion channels and related proteins play a major role in regulating uterine excitability and rhythmic contraction.

We are interested in the contribution of potassium channels (alpha and beta subunits) which are hypothesised to promote uterine quiescence in early-mid pregnancy (Aaronson et al., 2006; McCallum et al., 2009; Ohya et al., 2009) and calcium channels (e.g. TrpCs; Dalrymple et al., 2004; 2007) and related proteins (e.g. Stim and Orai, caveolin and flotillin) which can control cell growth, excitability and responses to agonists.

ii) Toll-like receptors and related signaling pathway (e.g. MAPkinases, cytokines etc) on reproductive tract (e.g. cervical and vaginal cells and human myometrium) responses to infection.

In order to investigate their functional roles in regulation of parturition/contraction we intend to introduce a range of molecular tools which include:
1) Over expression and assessment of gene promoter regulation in well known model cell systems (eg HEKs) which may help to assess the role and significance of genes taking part in human pregnancy and parturition
2) Stable shRNA delivery (using commercially available plasmids and viral vector systems) which is a highly effective strategy for permanently disrupting the function of individual genes in order to study their role in signaling pathways in myometrial cells (primary cultures and hTERT HM cell line) and epithelial cells of the reproductive tract (VK2/E6E7cell line)

Recipient or parental organism
- Primary cultured and passage 1-6 pregnant human myometrial smooth muscle cells (HMSM)
- Human myometrial cells immortalised with retroviral hTERT vector (HTER-HM)
- Vaginal epithelial cell line (ATCC CRL-2616 ) (VK2/E6/E7)

Host/vector system
- Lenti-X 293T cells
- HEK 293
The viral vectors which will be used in the study are listed below however we might use other vectors with similar properties without changing the containment level:

Lenti-X pLVX-shRNA1, SMARTvector® 2.0 Lentiviral shRNA Particles, GIPZ Lentiviral shRNAmir

All lentiviral constructs contain all the viral processing elements necessary for the production of replication-incompetent lentivirus and elements responsible for improved viral titer and overall vector function.

Genes encoding the structural and other components required for packaging the viral genome are separated onto mix of plasmids minimizing the threat of recombinant replication competent virus production. Lack of homology between the packaging mix of plasmids and Lenti-vectors also prevents transfer via homologous recombination.

After transduction viruses cannot replicate autonomously in target cells (SIN vectors).

Non-mobilisable (Bom-, Mob-, Tra-) plasmid vectors which may be used in the study:

- pIRES2-EGFP: Expression vector which contains bacterial and mammalian promoter and internal ribosome entry site (IRES) that allows the co-expression of a marker gene and another gene from a single transcript

- pKD: siRNA expression plasmid H1 promoter, Fl ori, , PuC ori, Amp

- pGL3: Basic plasmid which lacks a resident promoter. Encodes firefly luciferase which expression depends on insertion of the promoter sequence. SV40 promoter, Amp, ori, Luc+)

**Origin & function**

Inserts are all human based sequences

Stim (1-2): Stim proteins have emerged as potential candidates underlying store-operated channels (SOCs). STIM1 is a calcium sensor integrated within endoplasmic reticulum. It has been shown to relocate to the plasma membrane on store depletion and activate store-operated calcium entry (SOCE). STIM2 may be a negative regulator of STIM1-induced SOCE and is thought to be involved in the regulation of basal/cytosolic and endoplasmic reticulum Ca2+.

Orai: Orai proteins are thought to form the plasma membrane channel regulated by STIM. There is compelling evidence that Orai1 encodes the Ca2+ selective SOC channel/ICRAC current in a range of non excitable cells, but as SOC currents display distinctly different characteristics in excitable cells, it has yet to be fully established whether Orai channels or STIM can also mediate this more non-selective cation current alone.

KCNQs: KCNQ (Kv7) genes encode a family of K+ channel α-subunits most extensively investigated in cardiomyocytes and the CNS where they contribute to action potential repolarisation and subthreshold excitability respectively. More recently a role for Kv7 channels in smooth muscle has emerged, with functional roles being seen in the vasculature, the GI tract and the uterus.

KCNE(1-5): KCNE subunits modulate KCNQs and other voltage-gated channels in vitro and in vivo

Caveolin(1-2): Caveolins serve a structural role, inducing and maintaining caveolae invaginations and can act as a scaffold proteins to sequester and modulate the activity of many signalling effector and receptor proteins.

Flotillin(1-2): Flotillins are integral membrane proteins and constituents of lipid rafts. Flotillin-1 and -2 were originally discovered in neuronal cells as Reggie-2 and -1, respectively, and they are thought to be involved in signalling transduction and modulation.

Toll-like receptors (2,4): Toll-like receptors (TLRs) are pattern recognition receptors and serve as the first line of defense in host immunity. Current data suggests that TLR2 and TLR4 may play a protective and/or regulatory role in an effort to limit a lethal inflammatory response.
TrpC: Transient receptor 'canonical' channel proteins are proposed to form store-operated (SOCE) and receptor operated (ROCE) calcium channel entry in a variety of tissues. We have shown that several TrpC proteins are gestationally regulated in myometrial tissue and that pregnancy associated stimuli (stretch, cytokines) induce differential expression of TrpC proteins in human myometrial smooth muscle cells.

None of above mentioned genes and their products are potentially hazardous, infectious or oncogenic. Their overexpression will not have any influence on the environment.

**Evaluation of foreseeable effects**

The ability of retroviruses to integrate into the host cell chromosome raises the possibility of insertional mutagenesis and oncogene activation. Both these phenomena are well known in the interactions of certain types of wild-type retroviruses with their hosts.

In order to improve the risk-benefit ratio associated with retroviral-mediated techniques several approaches are currently developed, like the design of self-inactivating (SIN) vectors containing an internal promoter to drive transgene expression instead of the long terminal repeat-containing enhancer of standard retroviral and lentiviral vectors, reduced homology between retroviral vector and packaging components and split-genome conditional packaging systems.

Probability of survival of host cells or viruses in environment is very unlikely and disable genomes of these vectors prevent events of replication and creating new strains of wild type viruses.

Contact with viral particles and infection of cells at the contact site could resist in transient immune response with local swelling and inflammation. This is unlikely to be more than a minor effect. Moreover 3rd and 4th generation lentiviral constructs cannot replicate after infection so no virus could be passed on to other people.

Low to none if experimental work is done with all safety procedures maintained:

1. Personal protective clothing (gloves, lab coats)
2. MSC class 2, mechanical pipetting devices, safety centrifuge caps and rotors, autoclave, sharp containers, clinical waste yellow bags
3. All laboratories have lockable doors and sinks for hand washing and can be easily cleaned and decontaminated

Each researcher completes an individual risk assessment for their work and are aware of potential hazards.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste contaminated with GMOs will be inactivated so as to ensure that any contact between the GMOs and humans or the environment is limited to an extent commensurate with the risks identified in the risk assessment and to provide a high level of protection for humans and the environment.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

02/03/2022
The project was discussed at the Joint GMSC of the Schools of Biomedical and Health Sciences and Dental Institute. The following comment was made:
Approved at Class 2 subject to implementation of minor amendments and provision of completed signatures page to GMSC Secretary.

(Please note this department was formerly under the management of the School of Medicine, GM386, but is still under the overall management of King’s College London.)

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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Project Ref 342/95.1

- Date Ackn'd: 12/02/2003
- CU2 Project Title: EVALUATION OF SYNTHETIC IMMUNOGENS AS NOVEL VACCINES
- Class: Class 2
- Culture Vol Class 2: Class 2
- Culture Volume Class 3-4: not applicable
- Non-GMM: not applicable
- Consent Granted: Project notified under transitional arrangements
- Withdrawn: N
- Tick if notifying a connected programme of work: N

Historical Significant Changes:
Project transferred to GM386 on 8/9/2003

Project Additional Information

Purposes of the contained use:
(i) Propogation of small stocks and recombinant Vaccinia Virus (rVV) and recombinant Adeno Virus (rAd) .
Infection of simian and murine antigen presenting cells in vitro with rVV and rAd which will be used to restimulate in vivo primed cytotoxic T lymphocytes.

51Cr labelling of rVV or rAd infected cells (see (ii)).

Inoculation of rhesus macaques

Recipient or parental organism

(i) Thymidine Kinase deficient vector WR strain vaccinia virus.
(ii) Adenovirus -5. (1.88kb E3 deletion).

Vaccinia virus deleted in the thymidine kinase (TK) gene from Strain WR Adenovirus 5 (1.88kb deletion in early region 3 (E3)).

Host/vector system

The hosts used in propagating recombinant vaccinia virus (or recombinant Adenovirus) are mammalian cell lines which cannot survive in the environment and this can be considered safe. The vector (WR strain of vaccinia virus) is Thymidine Kinase negative and thus attenuated in replication of virulence. Adenovirus 5 is replication competent in mammalian cells, but cannot survive outside the host used in its propagation.

Origin & function

rrVV SIVgag, rVV EBVgpH wild-type Vaccinia (WR strain) small scale propagation, infection of cell lines in vitro for immunological assays.
rrVV SIVgag-pol, rVV SIVgag, rVV SIVenv, rVV SIVnef*, rVV SIVrev, small scale propagation, infection of cell lines in vitro for immunological assays.
raD-5-Luciferase, raD-5-B-galactosidase, small scale propagation, infection of cell lines in vitro for immunological assays.
rrVV SIVgag, rVV SIVenv, rVV SIVnef*, in vivo immunisation of rhesus macaques via the mucosal, Intra-dermal and Intra-muscular routes.

Evaluation of foreseeable effects

Although recombinant vaccinia and recombinant Adeno-5 viruses can persist in a non-replicating form (outside their host) for an unknown length of time, at Containment Level 2 where the material is handled in a Class 2 exhaust ducted Hepa filtered safety cabinet (MAT design), the risk of release to the environment is effectively zero. The products of genes expressed by the vectors used are not known to be toxic, oncogenic or mediate pharmacologic effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid waste inactivated overnight in 10% chloros (14,000 ppm available chlorine). Hypochlorite level checked with starch iodine strips. Solid waste subsequently drained from chloros, autoclaved 121oC x 20 mins (in leakproof containers).
Solid animal waste collected for incineration.
Inactivated liquid waste to mains drainage. Inactivated solid waste for incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form
The committee have reviewed and approved this risk assessment and the facilities where this work will be conducted. The committee is satisfied that the personnel involved with work at Containment Level 2 have received the appropriate training.

**Project Containment**

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Animal Units

- L2 L3 L4 L2 L3 L4 L2 L3 L4

**Project Ref** 386/04.1

**Date Ackn'd** 21/09/2007

**CU2 Project Title**

The use of RNA inhibition to assess gene function in primary cultured human myometrial cells. Myometrial cells will be transfected with plasmid vectors containing gene specific antisense gene fragments to inhibit gene translation.

**Class** Class 2

**CultureVolClass2** < 1 Litre

**CultureVolumeClass3-4**

Non-GMM Consent Granted

- Not Applicable

**Project notified under transitional arrangements** N

**Withdrawn** N

**Historical Significant Changes**

TRANSFERRED FROM GM 386 - 21/9/07 PROJECT TRANSFERRED BA

**Date of Significant Change**

21/09/2007

**Project Additional Information**

**Purposes of the contained use**

The plasmid vectors and inserts to be used require containment level 1 conditions. The host cells to be transfected will be primary cultured human myometrial smooth
muscle cells and passaged human myometrial smooth muscle cells. Primary cultured cells require containment level 2, thus all work will be performed at containment level 2.

Recipient or parental organism

The recipient cells will be primary cultured human myometrial smooth muscle cells and also passaged human myometrial smooth muscle cells.

Host/vector system

The host cells will be primary cultured human myometrial smooth muscle cells and also passaged human myometrial smooth muscle cells. The vectors we plan to use are non-mobilisable plasmid vectors.

Origin & function

The non-mobilisable plasmid vectors will contain small gene fragments that will generate RNAs that will be processed to 23 mers. Inserts will be chemically synthesised and will be homologous to human genes. Transfection of the plasmid will inhibit gene translation and will enable physiological functions to be determined.

Evaluation of foreseeable effects

We plan to transfected myometrial cells with plasmid vectors containing short gene fragments, which will functionally knock out specific genes rather than add genes. Transfected and untransfected human myometrial cells are unable to survive outwith tissue culture facilities, so they will have minimal effect to the environment. Prior to disposal, cells will be inactivated using 50% Virkon. Cells and all culture materials will be incinerated with hospital waste. Cells are unable to colonize laboratory workers. The non-mobilisable plasmid vectors that we plan to use are unable to enter cells without mechanical or chemical manipulation and plasmids are unable to transcribe the inserted gene fragment out with the host cell, there will be minimal risk to the laboratory staff or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Transfected cells and culture medium will be treated with 50% Virkon which will cause cell inactivation. In addition, cells will be unable to remain viable out of tissue culture facilities. Cells and tissue culture materials will be autoclaved in a vacuum cycle at 135 degrees C for 5 minutes and then incinerated with hospital clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The assessment was approved by the local GMSC committee on 23 June 2004 with some minor amendments. The class of activity is 2.

The safety audit of the laboratory where the work will be carried out has also been done.

02/03/2022  Page 5976 of 15326
### Project Containment

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### Project Ref 543/94.1

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### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
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| **Data Premises Notified** | 17/01/1989 |
| **(Originally)** | |
| **Transferred from 1992 Regs?** | Y |
| **Transitional Premises Class** | 1 |
| **Data Premises Closed** | N |
| **Transitional Premises Emergency Plan Required?** | N |
| **Non-GMMs Withdrawn** | N |
| **Withdrawn** | N |

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<td><strong>Department</strong></td>
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<td><strong>Road Name</strong></td>
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<tr>
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<td><strong>County</strong></td>
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<tr>
<td><strong>Postcode</strong></td>
<td>G1 1XW</td>
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<td><strong>Tel Number</strong></td>
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<tr>
<td><strong>Fax Number</strong></td>
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<td><strong>Page 5979 of 15326</strong></td>
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### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs) | | | | |
Level 2 (GMMs) | | | | |
This will consist of autoclaving or in the case of anything designated clinical waste, incineration.

Local departmental rules cover annual servicing of autoclaves and sterility testing either by chemical indicators, B. stearothermophilus spore survival or both.

No untreated waste is allowed to leave the University.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick if confidential

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Purposes of the contained use

The infection of mammalian cells with adenovirus carrying genes which will, in the majority of cases, selectively block the NFkB signalling pathway through the transient expression of dominant negative genes. The use of recombinant adenovirus will allow an investigation of the regulation of inflammatory signalling in mammalian cells that is not possible using other transfection methods because of their low efficiency: normal lipid-based transfection strategies give a 10-40% transfection efficiency in rat aortic smooth muscle cells, adenovirus facilitates a transfection efficiency of 90% or greater.

Recipient or parental organism

Human adenovirus is a double-stranded DNA virus of which there are more than 40 serotypes. These wild-type viruses are all category II biological agents. The adenoviral genome which forms the basis of the constructs here is Ad5 which is responsible for mild respiratory infections in children. The normal route of infection is believed to be through aerosols and the majority of adults are likely to have antibodies to the virus.

The Ad5 adenoviral genome is well characterised, the commercially available Ad5 genome (Adeno-Xtm) used in this study has had large sections of the Early Regions 1 and 3 deleted to make it replication-incompetent. Such replication-incompetent Ad5 viruses are generally considered a category I biological agent (dependent on the inserted genes). The 293 cell line has been transformed to stably express the Ad5 E1 genes essential for virus replication and therefore acts as a packaging cell line for the growth of the virus. Other mammalian cells which are susceptible to infection by the virus are transiently and non-cytopathically infected (cell lysis does not take place). The replication-incompetent adenoviral vector cannot be maintained in tissue (it persists for less than eight weeks in respiratory epithelium). The adenoviral DNA is not incorporated into the host genome, the adenoviral genes are not transcribed and the DNA is not replicated. Adenovirus is not known to naturally infect any other species. These adenoviral constructs are therefore "unlikely to cause human disease" or be a particular hazard in the environment.

Each new recombinant adenovirus is screened for the ability to replicate outside of the permissive cell line eg by looking for plaque development after infection of a non-permissive cell line. If an adenoviral construct is found to be replication-competent it will be destroyed. Humans are the natural reservoir for wild-type adenovirus and there is a possibility of a recombination event occurring between the wild-type adenovirus and the recombinant virus to produce a replication-competent recombinant adenovirus. However there is a very low probability of the recombination event occurring and a negligible risk of a viable virus resulting.
A number of cultured cell types will be infected with adenovirus: eg 293 cells (human embryonic kidney cells); human aortic smooth muscle cells; rat aortic smooth muscle cells; human umbilical vein endothelial cells; human keratinocytes (primary cultures, HaCaT, NCTC 2544); RAW 264.7 macrophages (Murine). It is possible that a primary cell culture may harbour a virus (eg Epstein-Barr) which could trans-complement the E1 deletion in the replication-incompetent, however this is very easily detected, as previously described, by the development of plaques and the rounding up and detaching of cells. Under these circumstances the infected cells would be destroyed by the methods detailed in part 12.

**Host/vector system**

The gene of interest is inserted into pShuttle, a high copy number 4.1 kb shuttle vector containing a multiple cloning site, the human immediate early cytomegalovirus promoter (CMVIE), the bovine growth hormone polyadenylation signal, a kanamycin resistance gene and pUC origin. Plasmids are propagated in standard disabled E.coli (XL1 blue). The gene expression cassette is excised from pShuttle and introduced into the adenoiral genome by an in vitro ligation reaction. The adenosiral genome into which the expression cassette containing the gene of interest is introduced contains an ampicillin resistance gene and pUC origin. The adenosiral genome must be linearised by the Pac I restriction enzyme and transfected, eg by Lipofectamine TM, into 293 cells before infectious (but replication-deficient) virus is produced. A number of flasks of 293 cells are then infected with this virus to produce stocks quantities of the virus. The adenosiral genome is used in the commercially available Adeno-X TM genome (Clontech). No manipulation of wild-type adenovirus is carried out in the lab.

**Origin & function**

Adenoviral constructs containing a human gene will be made in the laboratory, these will include: Inhibitory kappa B (IκB)-a (dominant negative); IκB- (dominant negative); IκB Kinase (IKK)-A (dominant negative); IKK- (dominant negative); IKK-y (dominant negative); NFκB- inducing Kinase (dominant negative); MEKK1/2/3/4 (dominant negative); MKP-2; Green Fluorescent Protein; -galactosidase. These genes are either innocuous markers such as GFP or -galactosidase or genes which would be expected to negatively regulate inflammation or growth of cells. As such the risk to health associated with expression of the genes themselves is low.

**Evaluation of foreseeable effects**

The adenoviral constructs will be expected to inhibit the NFκB signalling pathway at several levels blocking the inflammatory signalling stimulated by compounds such as the bacterial endotoxin component, lipopolysaccharide, and tumour necrosis factor-α. NFκB activation is implicated in a range of diseases such as, atherosclerosis, asthma, arthritis, cachexia, cancer, diabetes, euthyroid sick syndrome, AIDS, inflammatory bowel disease and stroke. Particular emphasis has been placed on inhibition of NFκB signalling as a cancer therapy. NFκB is required for oncogenesis at multiple levels and inhibition of NFκB strongly enhances the apoptotic potential of many forms of chemotherapy. A range of anti-apoptotic genes are activated by NFκB eg c-IAP-1, c-IAP2, XIAP, TRAF1, TRAF2, A1/Bfl-1 and IEX-IL (for review see: Baldwin, A.A. (2000)). The inhibition of NFκB signalling is believed to be responsible for the condition incontinentia pigmenti (a defect in an upstream kinase (IKK-y) has been identified in humans (Smahi et al., 2000)). In mice, knock-outs of NFκB subunits p50 or p52 had no effect but the double-knockout displayed osteopetrosis (Iotsova et al., 1997) and a block in osteoclast differentiation (Franzoso et al., 1997). Mice in which the p65/Re1A subunit was knocked out died at day 16 of development as a result of extensive liver apoptosis (Bel et al., 1995). However the consequences to health of an individual being infected with adenovirus carrying a gene which will inhibit the NFκB pathway is unlikely to be severe since non-steroidal anti-inflammatory drugs and glucocorticoids which both block NFκB activation are used for long term treatment of disease and can be tolerable with minor side-effects. Expression of the genes would also be transient (as described in the first part of section 7) and since adenovirus is not known to naturally infect any other species no negative effects on human health or the environment are foreseen. It is possible that the virus itself will elicit an immune response in an infected individual. The deletion of the E3 region of the Ad5 genome means that there is increased MHC translocation to the cell surface this could mean that an infected individual might have an increased inflammatory response but should also mean that the virus would be cleared more rapidly from the tissue. since the genes involved in this study are selected to inhibit the inflammatory response any influence of the E3 deletion may be countered.


Franzoso, G. et al. (1997) Requirement for NF-kB in osteoclast and B-cell development.. Genes Dev. 11, pp. 3482-3496.


**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste whenever possible is disinfected by immersion in a 2,500 ppm sodium hypochlorite solution overnight. A 30 minute soak is sufficient to kill 100% of the virus. The waste along with gloves and similar items is then bagged for autoclaving (121°C, 15 minutes free steam) before disposal. The autoclave is situated close to the laboratories where the work will take place, it is overhauled and tested annually by a specialist engineer, a chart recorder will allow monitoring of the time and temperature of the autoclave during the programme run. Samples of adenovirus will be autoclaved and then tested for the ability to infect 293 cells as a means of validating the autoclaving process. The liquid waste is also disinfected in a 10,000 ppm sodium hypochlorite solution (final concentration) overnight, before being disposed of down a designated sink. Hypochlorite-treated adenovirus samples will be dialysed to remove the sodium hypochlorite and then tested for the ability to infect 293 cells as a means of validating this disinfection process.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The local GM Safety Committee (GMSC) has considered this risk assessment in an iterative process, based on the procedures outlined in the ‘Compendium’. Comments were obtained from every member of the Committee and a revised version (‘round 2’) of the risk assessment prepared. It was the unanimous view of the Committee that the work proposed fell clearly into Class 2, as defined by the Regulations.

The local GMSC acts as a subcommittee of the overall ‘Statutory Advisory Committee on Safety’, that is the forum of communication between employer and all employees of the University of Strathclyde.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L2</td>
<td>L3</td>
<td>L4</td>
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Project Additional Information

Purposes of the contained use

The research programme aims to improve the knowledge and understanding of the biochemistry, molecular biology and immunology of the parasitic protozoa, Toxoplasma gondii, Acanthamoeba castellani and Leishmania spp (L. major, L. Mexicana, L. donovani and L. infantum). Genetic manipulation of these organisms allows us to investigate parasite molecules involved in metabolism, growth regulation and virulence. Genetically modified micro-organisms may have the potential to be developed as attenuated live vaccines. Genetic modification of the organisms allows us to validate potential drug targets. It is envisaged that the genetic modification would make the organisms less able to survive in vivo.

Recipient or parental organism

Organisms to be used in this study

(a) Toxoplasma gondii is capable of infecting a range of mammals, including man, cattle and rodents. Transmission is through ingestion. Symptoms are normally mild to asymptomatic although congenital transmission can be serious. Infection with the RH strain (virulent) may be serious if left untreated.

(b) Leishmania spp. are capable of infecting a range of mammals including man, dogs and rodents. The predominant Leishmania species used in this study is Leishmania Mexicana, the causative agent of cutaneous leishmaniasis. L. Infantum, and L. donovani which can cause visceral disease) and L. major may also be used on an occasional basis. Transmission is through the bite of a sand fly. Cutaneous leishmaniasis can leave unsightly scars while visceral leishmaniasis can be fatal if left untreated.

(c) Acanthamoeba castellanii are normally free-living protozoa, but have recently been identified opportunistic parasites of humans. They are responsible for Acanthamoeba keratitis most commonly in contact lens wearers and for Granulomatous Amoebic Encephalitis a condition that can develop in the immunocompromised patients (including those with HIV and AIDS).
Toxoplasma, Leishmania mexicana and Leishmania major and Acanthamoeba are classed as ACDP hazard group 2. Both non-genetically modified parasites and
generally modified parasites are handled in accordance with ACDP guidelines. It is considered unlikely that genetically modified T.gondii, Acanthamoeba or Leishmania will
cause harm to humans or the environment above that inherent in the pathogenicity of the wild type parasites themselves.

L. donovani and L. infantum are ACDP hazard group 3. Since transmission requires a bite from an infected female sand fly, handling of L. donovani and L. infantum will be
carried out without all the conditions of Hazard Group 3 organisms as described in the HSE document ‘Biological agents: managing the risks in laboratories and healthcare
premises’ 05/05 Appendix 3.2. Thus the laboratory does not need to be maintained at an air pressure negative to the atmosphere as the agents are not transmissible
through the airborne route, the laboratory does not need to have exhaust air extracted through HEPA filters (although any work carried out that could result in aerosols will
be carried out in Class II microbiological safety cabinet). The laboratory does not need to be resealable as the organisms cannot survive or multiply in the environment and
are extremely easily broken down.

However, although the HG3 organisms in use are derogated and do not need full CL3 containment laboratory facilities it does require certain CL3 management and
procedural measures as specified in Appendix 3.2 of the ACDP guidance document ‘Biological Agents – Managing the Risks in Laboratories and Healthcare Premises’
which will be employed. This document can be viewed at http://www.hse.gov.uk/biosafety/biologicalagents.pdf
Specifically, (1) Segregation of work from any other going on in that particular laboratory where this is shared or there are different projects being undertaken. (2) Need for
records of those working with HG3 organisms. (3) Observation window to view occupants or a means of checking on workers.
The work will be separated from the other work by performing it in a separate room within the containment level 2. Importantly, none of the proposed genetic modifications
are envisaged to be capable of altering the organisms to change this assessment.

Host/vector system

The hosts for the genetic manipulations will be T.gondii, Acanthamoeba and Leishmania major, Leishmania donovani, Leishmania infantum and Leishmania Mexicana.
T.gondii can be propagated in tissue culture using a variety of mammalian cell lines as host cells. The leishmania species and Acanthamoeba can be grown axenically in
tissue culture prior to and following genetic manipulation.
Drug resistance genes of prokaryotic origin, such as neo, hyg, pur, ble, bla, CAT or sat will be used as positive selectable markers in T.gondii, Acanthamoeba and
Leishmania. These drug resistance genes do not confer resistance to drugs commonly used to treat Toxoplasma or Leishmania infections in man or animals. Green
fluorescent protein from jellyfish may also be used as a positive selectable marker.
Negative selectable markers will be (i) Herpes simplex virus thymidine kinase in combination with the drug ganciclovir (ii) Trichomonas vaginalis methionine-gamma-lyase
in combination with the compound trifluromethionine.
In addition, the availability of T.gondii lacking HXGPRT (hypoxanthine-xanthine-guanine phosphoribosyl transferase) allows T.gondii HXGPRT to be used as positive
selectable marker in the presence of mycophenolic acid and as a negative selectable marker in the presence of 6-thioxanthine Donald and Roos (1998) Molec. Biochem.
Parasitol. 91, 295-305. This system has the benefit of not introducing any resistance genes into the T.gondii.

Origin & function

Selection methods for gene knockout and episomal gene expression in T.gondii, Acanthamoeba and Leishmania require plasmid vectors containing prokaryotic antibiotic
selectable markers (eg.neomycin phosphotransferase conferring resistance to the antibiotic G418) or the green fluorescent protein (GFP) from the jelly fish Aequorea
Victoria (including colour shifted mutants) or fire fly luciferase (including enhanced mutants). Intended functions are to either delete or episomally over-express a particular
genome to understand (a ) its function and or location in a cell. (b) its role as a cysteine proteinase. (c) its role in metabolism and potential as a drug target and (d) its role in
infectivity.
DNA encoding genes from the parasites (included mutated versions of these) and in some instances DNA from other organisms (e.g. human, yeast) may be expressed in
the parasites in order to test whether this heterologous DNA is functional in the parasite. (genetic cross species complementation)

Evaluation of foreseeable effects

The overall aim of this work is to gain an understanding of the molecular genetics, biochemistry and immunology of the parasitic protozoa Toxoplasma gondii,
Acanthamoeba and Leishmania spp. and their interaction with the host. This should facilitate drug target identification, validation and design.

Organisms to be genetically modified (1) Toxoplasma gondii (2) Acanthamoeba castellanii (3) Leishmania Mexicana, Leishmania donovani, Leishmania Mexicana and L.major.

There are four major research themes to this project. The foreseeable effects are identical for the 4 species of Leishmania and are thus discussed together. The particular parental strain of Leishmania is probably the most significant factor in determining which of the proposed GMMs are most hazardous (see below).

(a) Understanding how Toxoplasma and Leishmania regulate their cell cycle.
We have isolated a number of genes from Toxoplasma and Leishmania that have homology with genes known to regulate the cell cycle in yeast and higher eukaryotes (eg cyclin-dependent kinases and cyclins). We wish to manipulate these genes in the parasites in order to define their function and in particular to determine if the genes are essential for parasite survival. Essential genes are likely to encode proteins with value as novel drug targets. As these genes are crucial to cell cycle progression in other eukaryotes, it is likely that disruption or modification of them will be detrimental to the parasite, which will therefore be less virulent than wild type. It is also of significant value to test if similar genes from very divergent organisms (such as humans or yeast) are able to function in the same capacity in the parasites (a standard test to determine functional homology between genes of different species). Thus, we wish to express the human and yeast cdc2 genes in Leishmania mutants lacking crk3 gene to test if they are functional homologues. Similar studies will be performed in Toxoplasma gondii. This will provide information on gene function between highly divergent species. The heterologous DNAs to be used for transfection of the parasites do not encode a toxin, oncogenic protein, allergen or other protein with a potentially harmful biological activity.

(b) Cysteine proteinases of Toxoplasma gondii and Leishmania
We have isolated a number of cysteine proteinase genes from Leishmania. We wish to use genetic modification to elucidate their function in the parasites. We have shown that cysteine proteinase deficient mutants of Leishmania (lacking 1 to 19 cysteine proteinase genes) have reduced virulence (Mottrom et al., (1996) PNAS 93,6008-6013). These mutants have potential as attenuated live vaccines. Cysteine proteinase genes, altered by site-directed mutagenesis, will be expressed in Leishmania mutants lacking cysteine proteinase genes, in order to study proteinase expression and processing. The resulting mutants are likely to be less virulent than wild type Leishmania. There is no reason to believe that expressing cysteine proteinase genes in Leishmania will increase the virulence of the mutants or alter their pathogenic characteristics.

Genes to be analysed (Im, Leishmania Mexicana); Cathepsin L/B-like cysteine proteinases: Imcpa. Imcpb, Imcpc, thcp. (2) GPI:protein transamidase Imgpi8, tbgpi8.

(c) Metabolically important enzymes and other potential drug targets in T.gondii, Leishmania and Acanthamoeba castellanii
We have recently isolated a number of genes encoding metabolically important enzymes of T.gondii and Acanthamoeba castellanii. These include enzymes of the shikimate pathway and of type –II fatty acid biosynthesis such as chorismate synthase and FabI. We have shown that inhibitors of certain enzymes are able to restrict the growth of T.gondii and Acanthamoeba in vitro (Roberts et al., (1998) Nature 393:801-805; McLeod et al., (2001) International Journal of Parasitology 31:109-13; Roberts et al., unpublished). Mutant parasites lacking these functional enzymes are likely to be attenuated (as has been noted for a number of bacterial pathogens lacking shikimate pathway enzymes). Genes to be analysed from T.gondii include those encoding all the shikimate pathway enzymes and potential branches from the shikimate pathway; and all enzymes involved in Type II fatty acid biosynthesis. Genes to be analysed from Acanthamoeba include those encoding all the shikimate pathway enzymes and potential branches from the shikimate pathway and the alternative oxidase. Similar studies will be performed in Leishmania.

(d) Establishment of T.gondii in the host cell
T.gondii is an obligate intracellular parasite and as such uses a number of parasite derived molecules to invade and modify the host cell in which it resides. In particular proteins released from the dense granules (GRA), rhoptries (ROP) and micronemes (MIC) assist in this process.
Mutant parasites lacking these proteins are likely to be unaffected, (due to multiple redundancies) or to be adversely affected (attenuated). Genes to be analysed from *T. gondii* include the GRA, ROP and MIC genes.

*Toxoplasma gondii*, *Acanthamoeba* and *Leishmania* major and *Leishmania* Mexicana are classed as ACDP hazard group 2. *Leishmania donvani* and *Leishmania* infantum are hazard Group 3, but can be derogated to handling under containment level 2. The work to be undertaken is a small scale activity. Both non-genetically modified parasites and genetically modified parasites will be handled in accordance with ACDP guidelines. Genetically modified parasites will be tested in in vivo models (mice, guinea pigs, rabbits and cotton rats, occasionally parasites will be tested in mutant or genetically modified mice such as RAG-/- or IFN-gamma-/-). Procedures for maintenance of animals infected with genetically modified parasites are covered by the Home Office Inspectorate; Animals (Scientific Procedures) Act 1986

**Part 1 INHERENT BIOLOGICAL PROPERTIES OF GENETICALLY MODIFIED MICRO-ORGANISM**

For properties of micro-organisms to be used in this study, see above for recipient or parental organisms.

(i) Considerations relating to whether the product of the inserted gene has a biological activity which can act directly to cause harmful effects.

Three types of genetic modification for *T. gondii*, *Acanthamoeba* and *Leishmania* are envisaged.

(a) Gene disruption.

Generation of mutant parasites by insertional mutagensis (Insertion of selectable markers by homologous recombination). It is considered likely that gene disruptions will be detrimental to parasite survival or will have no phenotype difference from wild type cells.

Two types of positive selectable markers will be used. (i) Drug resistance genes of prokaryotic origin, such as neo, hyg, pur, ble, bla, CAT or sat. These drug resistance genes do not confer resistance to drugs commonly used to treat *Toxoplasma* or *Leishmania* infections in man or animals. (ii) Green fluorescent protein from jellyfish.

Two types of negative selectable marker will be used (i) Herpes simplex virus thymidine kinase in combination with the drug ganciclovir (ii) Trichomonas vaginalis methionine-gamma-lyase in combination with the compound trifluoromethionine.

In addition the availability of *T. gondii* lacking HXGPRT (hypoxanthine-xanthine-guanine phosphoribosyl transferase) allows *T. gondii* HXGPRT to be used as a positive selectable marker in the presence of mycophenolic acid as a negative selectable marker in the presence of 6-thioxanthine (Donald and Roos (1998) Molec. Biochem. Parasitol. 91. 295-305). This system has the benefit of not introducing any resistance genes into the *T. gondii*.

(b) Homologous gene expression

The expression of homologous genes in *T. gondii*, *Acanthamoeba* or *Leishmania* will be achieved from episomal expression vectors or via integration into the genome. These vectors have low to medium levels of expression.

(c) Heterologous gene expression

The expression of heterologous genes in *T. gondii*, *Acanthamoeba* or *Leishmania* will be achieved from episomal expression vectors or via integration into the genome. These vectors have low to medium levels of expression. These experiments are designed to test for cross species complementation. It is also of significant value to test if similar genes from very divergent organisms (such as humans or yeast) are able to function in the same capacity in the parasites. Thus, we wish to express the human and yeast cdc2 genes in leishmania mutants lacking the crk3 gene to test if they are functional homologues. This will provide information on gene function between highly divergent species.

Other heterologous genes include reporter genes such as green fluorescent protein (GFP) from the jelly fish *Aequorea victoria* (including mutants of this protein engineered to give greater fluorescence or a different colour of light emission) and luciferase from the firefly *Photinus pyralis* (or mutant versions of this gene from this or other organisms).

The heterologous DNAs to be used for transfection of the parasites do not encode a toxin oncogenic protein, allergen or other protein with a potentially harmful biological activity.

*Leishmania*, *Acanthamoeba* and *T. gondii* risk assessment. As access will be restricted to contained handling in a containment level 2 laboratory and expression will be low to medium levels of non-toxic and modified host genes, risk levels are kept to a minimum.

(ii) Consideration relating to whether the inserted gene encodes a product that might act alongside the existing characteristics of the recipient micro-organism, so as to
endow the GMM with altered pathogenic properties.

It is possible that disruption of T.gondii, Acanthamoeba or Leishmania genes will alter the pathogenicity of the parasites. It is envisaged that genes modified in this project will lead to either no change in virulence or a loss of virulence (hence the desire to create gene-specific deletion mutants for use as attenuated live vaccines). None of the genes to be modified in this project are thought to encode proteins that would be sufficient to alter the pathogenic properties of the organism with respect to tissue tropism or host range. Cysteine proteinases of Leishmania have been identified as virulence factors. We wish to express modified cysteine proteinase genes in Leishmania to investigate the function of the protein in the parasite. Our extensive work (and that of others) suggest that expression of modified cysteine proteinases will not alter the pathogenic properties of the parasites above that of the wild type cell line.

Drug resistance genes of prokaryotic origin, such as neo, hyg, pur, ble, bla, CAT or sat, will be used. These drug resistance genes do not confer resistance to drugs commonly used to treat T.gondii, Acanthamoeba or Leishmania infections in man or animals (the preferred drugs of choice being pyrimethamine and sulphadiazine (for T.gondii), antimonials (for Leishmniaisis) and polyhexamethylene biguanide [PHMB] and chlorhexidine (for Acanthamoeba)). Neomycin has been used in various combinations with other antimicrobials in the treatment of Acanthamoeba keratitis, but is not a first line choice. Consequently, Acanthamoeba genetically modified to carry the Neo selectable marker should be susceptible to standard treatment. Furthermore, in the ubiquitous nature of Acanthamoeba in the environment, the incidence of Acanthamoebiasis is extremely rare and has been documented in those individuals who perform poor contact lens care and in the immunocompromised. Immunocompetent people are at very low risk to infection and this risk can almost, if not completely eliminated by use of safety goggles (rather than safety glasses).

(iii) Consideration of the most hazardous GMM to be created

The most hazardous GMM generated is likely to be Leishmania donovani as these are inherently more infective than Acanthamoeba and less amendable to treatment than Acanthamoeba or Toxoplasma. Most of the proposed genetic modifications are expected to produce attenuated organisms lacking one or more genes. Therefore, the most hazardous is likely to be a GM Leishmania expressing a reporter construct such as GFP that does not decrease the fitness of the organism.

(iv) Considerations relating to whether an inserted sequence, that does not give rise to a harmful phenotype in the recipient micro-organism, could give rise to harm as a result of natural gene transfer to another, possibly related organism.

Leishmania and Acanthamoeba are essentially asexual organisms. Sexual exchange has not been reported for Leishmania or Acanthamoeba. T.gondii can reproduce sexually but this only happens in the domestic cat. Genetic exchange with other organisms has not been reported for T.gondii, Leishmania or Acanthamoeba.

The genetically modified parasites will be contained with ACDP containment level 2 facilities. The chance of escape into the environment is negligible. The details of the appropriate systems of work are held within the appropriate COSHH risk assessments and standard operating procedures.

Part 2 Animal Work

Genetically modified T.gondii and Leishmania will be grown in animals (mice, rats, guinea pigs, rabbits). The University of Strathclyde has an animal facility with ACDP containment level 2 classification. It is not envisaged that genetically modified T.gondii and Leishmania will present any additional environmental hazard than wild type parasites with respect to animal infection. There is no increased risk envisaged in using the genetically modified micro-organisms rather than the parental micro-organisms in genetically modified animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GM modified T.gondii or Leishmania may be inoculated into wild type or KO mice (of various backgrounds eg BALB/c) to analyse their virulence for example RAG deficient and IFN-gamma deficient etc. Guinea pigs, rabbits or rats may also be used to grow parasites. These animals will be housed in a security monitored home office licensed facility in appropriate surroundings at containment level 2. The risk of these reaching the external environment is negligible. All animal tissue will be incinerated (by the University appointed contractors) and spillage of body fluids and surfaces that have been in contact with tissue will be treated with Virkon. All animal handling will involve the use of gloves. All workers should have tetanus vaccination up to date. Bites and scratches with an antiseptic plaster. Toxoplasma and Leishmania cannot be transmitted by bites or scratches.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
We apply for derogation of the handling of *L.*donovani and *L.*infantum to be carried out without all the conditions of hazard group 3 (HG3) organisms as described in the HSE document 'Biological agents: managing the risks in laboratories and healthcare premises' 05/05 Appendix 3.2 Thus the laboratory does not need to be maintained at an air pressure negative to the atmosphere as the agents are not transmissible through the airbourne route, the laboratory does not need to have exhaust air extracted through HEPA filters (although any work carried out that could result in aerosols will be carried out in Class II microbiological safety cabinets). The laboratory does not need to be sealable as the organisms cannot survive or multiply in the environment and are extremely easily broken down. Outside the laboratory the parasites can only be transmitted by the bite of a female sandfly. The work will be separated from the other work by performing it in a separate room within the containment level 2 facility. Importantly, none of the proposed genetic modifications are envisaged to be capable of altering the organisms to change this assessment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All parasite manipulation will be conducted in accordance with ACDP guidelines. Acanthamoeba, *T.*gondii and *Leishmania* promastigotes and amastigotes are handled under containment level 2.

1) Are any of the work procedures likely to generate aerosols? An important feature of safe handling of *Leishmania*, is that there is no record of transmission of these parasites by the aerosol route; neither can these organisms penetrate unbroken skin or mucous membranes. The consequence of this is that the safety measures required are relatively straightforward. *T.*gondii and Acanthamoeba can be transmitted by aerosols and work should be carried out in class II microbiological safety cabinets in containment level 2 conditions.

2) How will you dispose of waste material?

Animal waste will be disposed of through an authorised waste disposal company, provided through the Clinical Waste Service.

Solid waste is stored in small waste bins within the laboratory. The bins have lids and hold inner liners, which are autoclave bags. When the liners are no more than 3/4 full, they are removed from the bin, loosely sealed with autoclave tape (to allow steam penetration during autoclaving), labeled with lab number, operator and date and then transported to the autoclave room on a trolley. The bags are logged into the room and autoclaved on the same day that they are delivered. The bags and the logging procedure designates them 'GM' which results in their inclusion on a run on the validated autoclave with recorder. The record is inspected to ensure that the correct temperature has been reached for sufficient time. The record is kept on file by the autoclave room staff. The autoclave material is disposed of in solid waste by an authorised waste disposal company, provided through the Clinical Waste Service. The routine autoclave regime will be 126 °C for 30 min, which has previously been established to produce a 100% kill (British Pharmacopeia 2005, Vol IV, Appendix XVIII A385-A388).

Liquid waste (e.g cultures in flasks or 'spent broth' wherein the cells have been separated from the culture both by centrifugation) will be sterilised in the culture flasks the same day by addition of Virkon ® to a final concentration of 1% w/v (10% w/v for Acanthamoeba), and left overnight. Virkon-disinfected solutions will be disposed of into the waste water supply. Virkon solutions are stable for up to 7 days ( manufacturer’s data), and thereafter disinfectant solutions will be discarded (the pink colouration fades). Virkon is a peroxygen compound and disinfection requires a contact period of at least 10 min for 1% w/v solution: see http://www.antechh.com/framehet.html.

Contaminated glassware and centrifugation buckets from cultures will be disinfected by complete submersion in 1% w/v (10% w/v for Acanthamoeba), Virkon® overnight (polypropylene and polystyrene centrifugation buckets, steel and glass are not corroded by 1% w/v Virkon). Glassware and buckets will then be rinsed in tap-water and dried.

Small volume spills (up to 100ml) on hard surfaces (floors, work benches, trolleys) will be disinfected by mopping up the spill with absorbent tissue paper and spraying the surface with a 1% w/v (10% w/v for Acanthamoeba) Virkon solution or sprinkling the area with powder Virkon and leaving for at least 20 minutes and drying with paper towel to remove any remaining white deposit. Paper towels will then be bagged for disposal as solid waste (above).

Large volume spills (100ml to 0.5l) on hard surfaces (floors, work benches, trolleys): Absorbent granules will be used to cover the spill, if necessary, contained using absorbent booms. Granules and booms will be bagged for disposal as solid waste (above). The contaminated area will then be disinfected by wiping or spraying the surface with a 1% Virkon solution or sprinkling the area with powder Virkon and leaving for at least 20 minutes and drying with paper towel to remove any remaining white deposit. Paper towels will then be bagged for disposal as solid waste.
3) Does your laboratory avoid sharps?
Yes, where possible. However, where it is necessary to use sharps, the laboratory provides Sharps containers and has guidance for the use and safe disposal after use.

4) Has the disinfectant been validated?
Yes, Virkon kill has been validated in house to kill 100% of the Leishmania and Toxoplasma when used as per manufacturer’s instructions (1% weight/volume). For Acanthamoeba a 10% w/v has been found to be necessary to kill 100% of organisms. The autoclave has also been validated. When genetically modified organisms are created these will also be tested. This is done by incubation in Virkon at different concentrations for different lengths of time and examination by microscopy.

5) Does the nature of the work preclude it being undertaken by any workers who have a serious skin condition?
No. All workers must wear adequate protective clothing, including laboratory coats and disposable gloves, safety glasses or safety goggles (in the case of Acanthamoeba handling)

6) Will workers receive any vaccination?
No vaccinations are currently available for Leishmania, human toxoplasmosis or Acanthamoeba.

7) Is the recipient micro-organism controlled by DEFRA?
No

Assignment of control measures

It is believed that the genetically modified T.gondii, Acanthamoeba and Leishmania provide no significant additional risk over and above the wild type parasites. Non-gentically modified T.gondii, Acanthamoeba and Leishmania are currently handled at Containment Level 2. All genetically modified T.gondii, Acanthamoeba and Leishmania will also be handled in Containment Level 2 facilities only.

Conclusions from risk assessment
1. The work will be undertaken at containment level 2.
2. The work is classified as being Class II under the Contained Use Regulations, 2000.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
Work with Toxoplasma, Leishmania and Acanthamoeba has been performed at Strathclyde University for a number of years. This notification of intention to conduct individual contained use activities will enable the extension of these existing studies to the use of genetically modified forms of these organisms. The University Genetic Modification Safety Committee has reviewed this application in detail, together with a relevant COSHH risk assessment form and a local Genetic Modification risk assessment form. The committee is in agreement that the assessment of risk has been appropriately performed and that the work can proceed at Strathclyde University. Strathclyde University is already registered with HSE as a Class 2 site.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
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### Project Ref 298/08.1

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<td>Class 2</td>
<td>1-50 Litres</td>
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</table>

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<thead>
<tr>
<th>Non-GMM</th>
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### Project Additional Information

#### Purposes of the contained use

The work involves the investigation of metabolism of T. vaginalis in order to identify novel biochemical pathways that might be suitable for therapeutic intervention. The
essentiality of potential drug targets will be analysed by gene knockout (either by gene deletion or RNAi). The cellular localisation and expression of proteins will also be determined using epitope-tagged constructs.

Recipient or parental organism

The parental organism is Trichomonas vaginalis, which is listed in ADCP hazard group 2. The parasite infects the human urogenital tract and is sexually transmitted — there are no other known routes of transmission.

Host/vector system

Vectors for genetic modification of T. vaginalis will be derived from the non-mobilisable E. coli vectors (Bom-/(Nic-), Mob- and Tra-). They will contain well characterized T. vaginalis promoters such as the ap65-1 or SCS promoters to direct expression of homologous and heterologous genes. Vectors for gene deletion will contain a selectable marker gene flanked by sequences homologous to the 5’ and 3’ flanking sequences of the target gene. RNAi methods will use a tetracycline inducible system (Ortizt and Johnson, 2003. Mol. Biochem. Parasitoli. 128, 43-49.

Origin & function

Inserted material: the majority of material cloned and expressed on the plasmids is derived from T. vaginalis. Typically these are genes that will have been deleted in order to study function. The fact that the genes are from the genome strain of the parasite (for which draft genome sequence is available) allows excellent bioinformatic based analyses, which informs the researcher of the likely function of the target gene. Two key areas of interest involve (i) the study of, vaginalis genes involved in central metabolism of the parasite, including cysteine synthase (Westrop et al., 2006. J.Biol Chem. 281, 25062-75) mercaptopyruvate sulfurtransferase and thioredoxin reductase (Coombs et al. 2004. J.Biol Chem. 279, 5249-56) (ii) the study of T. vaginalis genes involved in the biosynthesis of surface molecules (these do not include surface virulence factors). The heterologous genes expressed include the drug resistance genes from prokaryotes used as positive selectable markers for construction of over-expressing cell lines and gene-deletion mutants. The markers used in this study include puromycin resistance (pac), G418 resistance (neo), hygromycin resistance (hyg), bleomycin resistance (ble), ampicillin resistance (bla). Promoter fusions drive known and well characterised reporter genes and epitope tags of synthetic origin. The reporter genes include green fluorescent protein (GFP) from the jelly fish Aequorea victoria and mCherry fluorescent protein, chloramphenicol acetyl transferase (CAT) and b-galactosidase. Development of inducible expression systems for RNAi will require heterologous expression of the Tet-repressor from E. coli and the bacteriophage 17 RNA polymerase. None of the heterologous genes expressed encode a toxin, oncogenic protein, allergen or other protein with a potentially harmful biological activity. The markers used for selection in T. vaginalis or in E. coli do not confer resistance to an antibiotic used in the treatment of T. vaginalis infections.

Evaluation of foreseeable effects

Trichomonas vaginalis is listed in ADCP hazard group 2. The parasite infects the human urogenital tract and is sexually transmitted — there are no other known routes of transmission. Infections are asymptomatic to mild (vaginitis. or urithritis) and are successfully treated with the nitroimidazole drugs, metronidazole and tinidazole. The parasite has no cyst stage and the organism has no capacity for survival in the environment. Humans are its only natural host. The genetic manipulations in this study are not envisioned to lead to over-expression of proteins or other metabolites that are harmful to humans. None of the genes in this study are thought to encode proteins that are sufficient to alter the pathogenic properties of T. vaginalis with respect to host range or tissue tropism. The genetic manipulations planned in this study are intended to lead to either no change in virulence or a loss of virulence. The drug resistance genes used as positive selection markers (such as puromycin acetyl transferase in combination with puromycin) do not confer resistance to drugs used in the treatment of T. vaginalis infections (metronidazole or tinidazole). Genetically modified T. vaginalis strains will be propagated in-vitro as trophozoites in complex defined medium. They are not able to survive in the environment. Sexual exchange of genetic information has not been reported for this ‘organism. Genetic exchange between T. vaginalis and other microorganisms has not been reported. It is envisioned that the genetically modified T. vaginalis strains will be either less hazardous or about the same as the parental organism (ACOP Hazard group 2). The containment level required is ACDP laboratory containment level 2. No additional measures above ACDP laboratory containment level 2 are required.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Conclusion from risk assessment:
(1) The work is classified as GM class 2 under the Contained Use Regulations, 2000.
(2) The work will be carried out under ACDP containment level 2 conditions.

Waste management.
(1) Liquids containing the GM organism will be exposed to an appropriate disinfectant at a known effective concentration. After disinfection the liquid waste is disposed of to the drain. The disinfectant Virkon (http://www.antechh.com/frameset.html) has been validated in house to kill 100% of T.vaginalis following 30 mm incubation at room temperature, at the manufacturer’s recommended concentration (1 % weight/volume). Disinfection procedures will be validated for new GM T.vaginalis strains by incubation of growing cultures of the organism with different concentrations of Virkon for different times and examination by microscopy to determine viability. (2) Solid waste including disposable culture flasks will be autoclaved in a validated autoclave under conditions established to produce 100% kill. The autoclaved waste is disposed of by an authorised waste disposal company, provided by the University’s clinical waste service. (3) Contaminated glassware and centrifugation buckets will be disinfected by complete submersion in 1% (weight/volume) Virkon overnight. Glassware and buckets are then rinsed in tap-water and dried. Small volume spills (up to 100 ml) on hard surfaces (floors, work benches and trolleys) will be disinfected by mopping up the spills with absorbent paper towels and spraying with 1% (weight/volume) Virkon. Paper towels are then bagged for disposal as solid waste (see above). Large volume spills (100 ml to 1000 ml) on hard surfaces: Absorbant granules will be used to cover the spill and bagged for disposal as solid waste. The contaminated surface will be cleaned by sprinkling the contaminated area with Virkon powder, leaving for 20 minutes and drying with a paper towel to remove any remaining white deposit. Paper towels are then bagged for disposal as solid waste.

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms

02/03/2022
Project Additional Information

Purposes of the contained use

PARS are G-protein coupled receptors whose upregulation is observed in a number of disease states including rheumatoid arthritis and multiple sclerosis. The goal of this project is to characterise the organisation and interaction among intracellular Ca2 stores in smooth muscle using novel genetic technique. The lentiviral gene delivery system will be used to transduce the cells with fluorescent5 protein-tagged PARs in various rodent (rat & mouse) and immortalised (human and rodent in origin) cell types including neurones, astrocytes, smooth muscle cells and fibroblasts. The lentiviral gene delivery sytem will be used to transduce the cells with fluorescent protein-tagged PARs (green fluorescent protein).

Recipient or parental organism

E Coli strain
The host is disabled. Ecoli. K12 Auxotrophic strain is non-pathogenic to humans. coli are used only to amplify the viral transfer and packaging plasmids. Genes in these plasmids are not under the control of bacterial promoters and are thus not expressed in the bacteria. Each bacterial strain contains only one plasmid, to prevent recombination of viral genes between plasmids in the bacteria. STSL3 E coli will be used to amplify the packaging and transfer ’ectors (a standard plasmid DNA extraction). The scale of the activity is small, that of any standard lab plasmid amplification. The STBL3
E. coli are recombinase deficient, reducing the risk of unwanted homologous recombinations of long terminal repeats (LTRs). As previously stated, the gene products of the packaging and transfer vectors are not expressed in E. coli due to the absence of bacterial promoters. Human embryonic kidney cell line (HEK293 or HEK293 FT cells)
The host is disabled. HEK29S FT cells (InVitrogen) are a Human Embryonic Kidney cell line optimised for viral transfection. These cells are unable to survive outside tissue culture conditions and are antigenically incompatible if accidentally introduced to humans. These cells will be grown under containment level 2 conditions. The cells are used for the generation of the pseudoviruses and preparations are validated not to contain replication competent particles. Any preparations transduced with the harvested pseudovirus should not make virus since the transducing particles are replication incompetent and self-inactivating. Thus, the appearance of any capsid protein in supernatants from such preparations is indicative of replication competent retrovirus (RCR) and must be discarded. RCR testing is done with an ELISA kit to detect the p24 gag capsid protein in cells transduced with the virus. If the virus is functioning correctly, the gag gene is excluded from the virus, thus transduced cells cannot make new viruses, and consequently the culture medium assay will be negative.

Guinea-pig colonic myocytes
Guinea-pig portal vein myocytes
SO-i0Op1 of concentrated viral stock will be added directly to the culture medium of the myocytes under containment level 2 conditions. From this point on, the myocytes, medium and all cultureware associated shall be treated with level 2 containment and disposed of according to stated procedures (all media changes will be carried out in a class II microbiological safety cabinet, following this, all waste will be disposed of as per virus instructions). Cells will be safely transported to the microscope. Dishes will be sealed with parafilm and placed inside a larger culture dish inside a polystyrene box labelled clearly as virus containing. Virkon solution and blue roll will be made available in case of spillage and the microscope will be decontaminated after use with Virkon.

The myocytes are transduced with the pseudoviral particles. Pyhazards are unchanged. The virus only delivers GEP or plN-KDEL genes and these are self-inactivating and so incapable of being incorporated into new viruses. However, in the unlikely event that myocytes contained the lentivirus, these transgenes could not recombine into new viruses, and even if they possibly could, they would merely deliver an additional GFP or plN-KDEL gene with them which would make no difference to the hazard posed by the virus.

Host/vector system

Packaging Vectors — pRSV-Rev, pMDLg/pRRE and MD2.VSVG
Transfer Vector — pCCL.sin.PPTprom[Insert]*.Wpre
[Insert] refers to EGFP or plN-KDEL (an ER/SR targeting GFP) All the vectors are based on a pUC19 backbone, are defective in transfer to other hosts, are Bom, Tr&Mob and non-mobilisable (see SACGM ‘Compendium of Guidance’ 2007).

Packaging vectors:
pMDLg/pRRE - Genes = gag and pol. Encodes structural proteins (gag) and enzymes (reverse transcriptase, integrase & protease) for viral replication
These are also a rev response element in the encoded RNA (RRE, see later) - CMV promoter
pMD2.VSVG - Gene = VSV-G. Encodes envelope glycoprotein from the vesicular stomatitis virus - CMV promoter
pRSV-Rev - Gene = Rev. Rev interacts with transcribed viral RNAs (via the RRE) to facilitate nuclear export such that translation may take place. Rev is therefore beneficial for the expression of structural proteins to make daughter virions - RSV promoter

Transfer vectors:
pCCL.sinPPTprom.EGFP.Wpre - Gene = EGFP. Expresses EGFP - CMV promoter.

Origin & function

The lentiviral gene delivery system we plan to use is a 4 generation system, specifically designed for gene therapy applications. The origins of the lentiviral delivery system lie in attempts by Naldini and colleagues to engineer replication-defective HIV-1
Since the generation of this 1 generation system successive modifications have been made which enhance its safety and efficacy. This system is in use and is being further developed.

02/03/2022
developed in laboratory at the University of Leicester.
The advantages of the above lentiviral system are multi-fold and include:
1) Sustained expression of transgenes following stable integration into the host genome
2) Transgene expression in non-dividing cells (of special significance to muscle biologists).
3) Packaging and expression of large inserts (at least T5kbp).
4) Broad target cell range.
5) Low inflammatory potential due to lack of replication activity.
The goal of this project is to characterise the organisation of the intracellular Ca2 store, the sarcoplasmic reticulum, in smooth muscle, using a novel genetic technique. The lentiviral gene delivery system will be used to transduce the cells with a store located green fluorescent protein (c3FP). The GFP or p1N-KDEL (an ER/SR targeted enhanced GEP) will be used to target the sarcoplasmic reticulum. The use of the lentiviral gene delivery system is required because of the known difficulties in transfecting differentiated smooth muscle cells.

Evaluation of foreseeable effects
The lentiviral gene delivery system has been developed for gene therapeutic applications and, being replication deficient and self-inactivating has low toxicity. The system, first developed by Naldini and colleagues, has undergone numerous modifications to enhance biosafety and improve transduction efficiency. Therefore, there are no foreseeable difficulties.
In the event of replication competent retrovirus (RCR) generation, all viral stocks will be immediately disposed of with virkon (approved method). However, risk of generating RCRs is minimal. The only way RCRs could be generated is by multiple recombination events which have caused all packaging genes to end up on one plasmid, with packaging signals flanking them. Packaging and transfer vectors will be kept in separate bacterial cultures, so no cross-recombination can occur, and we will only transiently transfect the FT cells. The transfer vector is also self-inactivating; any genes transferred by the virus cannot be incorporated into new viruses as they will lack viral promoters. Therefore, the probability of RCR generation is virtually zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All handling of the host bacterium will take place within a containment level 1 laboratory, using standard microbiological practice which will be sufficient to keep contact with humans and the environment to negligibly low levels.
Solid waste (e.g. agar plates containing bacteria, pipette tips, used gloves etc) is stored in small waste bins within the laboratory. The bins have lids and hold inner liners, which are autoclave bags. After every use and/or when the liners are 3/4 full, they are removed from the bin, and placed inside a second autoclave bag to minimize the risk of spillage from a torn autoclave bag. The outer bag will then be loosely sealed with autoclave tape (to allow steam penetration during autoclaving), labeled with lab number, operator and date and then transported to an autoclave room on a trolley. The bags are logged into the room and autoclaved on the same day that they are delivered. The bags and the logging procedure designates them 'GM', which results in their inclusion on a 'run' on the validated autoclave with recorder. The record is inspected to ensure that the correct temperature has been reached for sufficient time. On that basis the autoclaved material is disposed of in solid waste; removed by authorised waste-disposal specialist companies. The record is kept on file by the autoclave room staff. The routine autoclave regime will be 126°C for 30 mm, which has previously been established to produce a 100% kill (see waste inactivation guidelines regarding autoclaving and chemical disinfection (http://www.mis.strathacuklSecretariat/Publications/local/gmsc/gms-com.html)).
Liquid waste (e.g cultures in flasks or ‘spent media’ wherein the cells have been separated from the culture media by centrifugation) will be sterilised in the culture flasks the same day by addition of Virkon® to a concentration of 1% w/v, and left overnight Virkon-disinfected solutions will be disposed of into the wastewater supply. Virkon 1% solutions are stable for up to 7 days, and thereafter disinfectant solutions will be discarded (the pink colouration fades). (Virkon is a peroxygen compound and disinfection requires a contact period of at least 10 mm for 1% w/v solution: see http://www.relyon.dupont.com/index2.asp) Virkon will be used under the conditions set out by the manufacturer to ensure suitable inactivation. Please see www.relyon.dupont.com
Virally-treated (HEK293 cells, myocytes) and packaging cells (HEK293-ET) will be disposed of following disposal of culture medium by addition of 1% w/v Virkon to the culture vesSEL. The culture media will be drained and disinfected as previously detailed and the empty flask will be disinfected with 1% w/v Virkon. After leaving overnight, liquid waste will be disposed to the wastewater supply. Cultureware will subsequently be disposed as per solid waste.

Contaminated glassware and centrifugation buckets from cultures will be disinfected by complete submersion in 1% w/v Virkon overnight (polypropylene and polystyrene centrifugation buckets, steel and glass are not corroded by 1% w/v Virkon). Glassware and buckets will then be rinsed in tap-water and dried.

The designated Class II Microbiological Safety cabinet and centrifuge will be disinfected by thoroughly wiping down with 1% w/v Virkon solution after every use. Wiping materials (e.g. blue roll) shall be disposed of as per solid waste. The Class II Microbiological Safety cabinet shall also be irradiated for 5 minutes with UV light. This is carried out as an extra precaution.

Virkon is sufficient to inactivate the virus.

Glass microinjector pipettes will be disinfected by complete submersion in 1% w/v Virkon overnight, washed and disposed of in safety bins.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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**Project Ref** 298/08.3
PARs are G-protein coupled receptors whose upregulation is observed in a number of disease states including rheumatoid arthritis and multiple sclerosis. The goal of this project is to determine the physiological and pathophysiological role of PARs in various rodent (rat & mouse) and immortalised (human and rodent in origin) cell types including neurons, astrocytes, smooth muscle cells and fibroblasts. The lentiviral gene delivery system will be used to transduce the cells with fluorescent protein-tagged PARs (green fluorescent protein-PAR-1 (PAR1-GFP), enhanced yellow fluorescent protein (EYFP)-PAR-2 (PAR2-YFP) and enhanced cyan fluorescent protein (ECFP)-PAR-4 (PAR4-CFP) to investigate the consequences of receptor overexpression, EYFP alone as a control or with PAR RNAi to investigate receptor knockdown.

The host is disabled. E.coli K12 Auxotrophic strain is non-pathogenic to humans. E.coli are only used to amplify the viral transfer and packaging plasmids. Genes in these plasmids are not under the control of bacterial promoters and are thus not expressed in the bacteria. Each bacterial strain contains only one plasmid, to prevent recombination of viral genes between plasmids in the bacteria. STBL3 Ecoli will be used to amplify the packaging and transfer vectors (a standard plasmid DNA extraction). The scale of the activity is small, that of any standard lab plasmid amplification. The STBL3 E coli are recombinase deficient, reducing the risk of unwanted homologous recombinations of long terminal repeats (LTRs). As previously stated, the gene products of the packaging and transfer vectors are not expressed in the E.Coli due to the absence of bacterial promoters.

Human embryonic kidney cell line (HEK293 or HEK293 FT cells).

The host is disabled. HEK293 FT cells (Invitrogen) are a Human Embryonic Kidney cell line optimised for viral transfection. These cells are unable to survive outside tissue culture conditions and are antigenically incompatible if accidentally introduced to humans. These cells will be grown under containment level 2 conditions. The cells are used for the generation of the psuedoviral particles and preparations are validated not to contain replication competent particles. Any preparations transduced with the harvested pseudovirus should not make virus since the transducing particles are replication incompetent and self-inactivating. Thus, the appearance of any capsid protein in supernatants from such preparations is indicative of replication competent retrovirus (RCR) and must be discarded. RCR testing is done with an ELISA kit to detect the p24 gag capsid protein in cells transduced with the virus. If the virus is functioning correctly, the gag gene is excluded from the virus, thus transduced cells cannot make new viruses, and consequently the culture medium assay will be negative.
Lentivirus preparation and characterization will be carried out by Dr Kumlesh Dev at University College Cork. Viral replication incompetence/deficiency will be confirmed prior to transfer to the University of Strathclyde.

Primary cell culture: small quantities of 0.5-20.0 ul of concentrated pseudoviral particles (1x 10 (6) - 1x 10(10) muscle cells or fibroblasts) under containment level 2 conditions. The cells are transduced with pseudoviral particles, which are self-inactivating and so incapable of being incorporated into new viruses. The cells, medium and all culture shall be treated with level 2 containment and disposed after Virkon (1%w/v) and/or autoclave decontamination (all media changes will be carried out in a class II Microbiological Safety Cabinet). Prior to any movement of transduced cells from the Containment Level 2 laboratory, the bathing medium will be changed twice in order to reduce the risk to Containment level 1 activity/material. Cells will then be transported in sealed containers to microscopes (SIBS419, SIBS421, SIBS565, SIBS66, Centre for Biophotonics Rm 1&3). Microscopes to be used will be labelled for viral use. Dishes will be sealed with parafilm and placed inside a larger culture dish inside a polystyrene box labelled clearly as virus containing. Virkon solution (1% w/v) and blue roll will be made available in case of spillage and the microscope will be decontaminated after use with 1% w/v Virkon.

Organotypic Slice Culture: Small quantities of 0.5-20.0 ul of concentrated pseudoviral particles (1 x 10 (6) - 1 x 10 (10) TU/ml) will be added directly to the culture medium of organotypic slice cultures under containment level 2 conditions. Alternatively, 0.5 - 20.0 ul concentrated viral stock will be injected into the region of interest of an organotypic slice culture, for example CA1, CA3 or dentate gyrus region of a hippocampal slice culture or molecular or Purkinje cell layer of cerebellar slice culture using an electronically controlled glass microinjector pipette. This injection will require a dissection microscope and will be performed under containment level 2 conditions. Care should be taken when using glass pipettes and appropriate training will be taken prior to use. Should an injury be sustained, e.g. skin puncture, take appropriate action to encourage some bleeding, wash with running water and use antiseptic wipes to clean the injury and report the accident immediately to the Departmental Safety Convenor or the Biological Safety Officer. The organotypic slices are transduced with the pseudoviral particles which are self-inactivating and so incapable of being incorporated into new viruses. From this point on, the cells, medium and all culture shall be treated with level 2 containment and disposed after Virkon and/or autoclave decontamination (all media changes will be carried out in a class II Microbiological Safety Cabinet). Prior to any movement of transduced organotypic cultures from the Containment Level 2 laboratory, the bathing medium will be changed twice in order to reduce the risk to Containment Level 1 activity/material. Organotypic slices will then be transported in sealed containers to microscopes. Microscopes to be used will be labelled for viral use. Dishes will be sealed with parafilm and placed inside a larger culture dish inside a polystyrene box labelled clearly as virus containing. Virkon solution and blue roll will be made available in case of spillage and the microscope will be decontaminated after use with Virkon.

The vector

The system will use the cloning vector pLL4.0 as described by Osinde et al (2008) and the packaging vector from Invitrogen. A list of the individual vectors and references are given below. Also refer to Fig 1 which shows the vectors to be used.

Packaging vectors

ViraPower Packaging Mix: This mix contains an optimized mixture of the three packaging plasmids, pLP1, pLP2 and pLP/VSVG. These plasmids supply the helper functions as well as structural and replication proteins in trans required to produce the lentivirus. For further details please see http://tools.invitrogen.com/contents/sfs/manuals/virapower lentiviral systems man.pdf

Cloning vector:

pLL4.0: the EGFP will be replaced with EYFP, fluorescently tagged PARs or PAR RNAi and the CMV promoter replaced by cell type specific promoters including Phosphoglycerate Kinase (PGK) or synapsin. Please see Rubinson et al (2003) and Osinde et al (2008).

Origin & function

The lentiviral gene delivery system we plan to use is based on that described by Rubinson et al (2003) and Osinde et al (2008).

The advantages of the above lentiviral system are multi-fold and include:
1) Sustained expression of transgenes following stable integration into the host genome
2) Transgene expression in non-dividing cells.
3) Packaging and expression of large inserts (at least 7.5kbp)
4) Broad target cell range.
5) Low inflammatory potential due to lack of replication activity.

The origins of the lentiviral delivery system lie in attempts by Naldini and colleagues to engineer replication-defective HIV-1. Since the generation system successive modifications have been made which enhance its safety and efficacy.

The goal of this project is to determine the physiological and pathophysiological role of PARs in various cell types including neurons, astrocytes, smooth muscle cells and fibroblasts. The lentiviral gene delivery system will be used to transduce the cells with either fluoresceinently tagged PARs, EYFP as a control or RNAi. The use of the lentiviral gene delivery system is required because of the known difficulties in transfecting differentiated cells.


Evaluation of foreseeable effects

The lentiviral gene delivery system has been developed for gene therapeutic applications and, being replication deficient and self-inactivating has low toxicity. The system, first developed by Naldini and colleagues, has undergone numerous modifications to enhance biological safety and improve transduction efficiency. Therefore, there are no foreseeable difficulties.

In these systems the provirus are deleted from all regulatory and accessory genes (tat, rev, vif, gene W and gene Y), most gag/pol sequences, most of the envelope gene and most of the U3 region. Lentiviral vectors can mediate efficient delivery, integration and stable expression of transgenes in dividing as well as non-dividing human cells. Hence the use of a Class 2 facility for the production and purification of the viral particles is of multi-use to our experimental approaches. All materials and equipment will be disinfected after use and biological wastes will be autoclaved.

In the event of replication competent retrovirus (RCR) generation, all viral stocks will be immediately disposed of with Virkon. However, risk of generating RCRs is virtually zero. The only way RCRs could be generated is by multiple recombination events which have caused ALL packaging genes to end up on one plasmid, with packaging signals flanking them, which is highly improbable. Packaging and transfer vectors will be kept in separate bacterial cultures, so no cross-recombination can occur, and we will only transiently transfect the FT cells. The transfer vector is also self-inactivating; any genes transferred by the virus cannot be incorporated into new viruses as they will lack viral promoters. Therefore, the probability of RCR generation is virtually zero.

Direct Lenti virus contact
Counselling may be required when considering the potential hazard of protein X. This will be sought from Occupational Health, University of Strathclyde and/or the NHS.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All handling of the host bacterium will take place within a containment level 1 laboratory, using standard microbiological practice which will be sufficient to keep contact with
Humans and the environment to negligibly low levels.

Solid waste (e.g. agar plates containing bacteria, pipette tips, used gloves etc) is stored in small waste bins within the laboratory. The bins have lids and hold inner liners, which are autoclave bags. After every use and/or when the liners are 3/4 full, they are removed from the bin, and placed inside a second autoclave bag to minimise the risk of spillage from a torn autoclave bag. The outer bag will then be sealed with autoclave tape labelled with lab number, logged into the room. Prior to autoclaving, the seals are loosened (to allow steam penetration during autoclaving) and autoclaved on the same day that they are delivered. The bags and the logging procedure designates them GM which results in their inclusion on a 'run' on the validated autoclave with recorder. The record is inspected to ensure that the correct temperature has been reached for sufficient time. All GM waste is autoclaved as above and removed as Clinical Waste by the University's authorised Waste Contractor. The record is kept on file by the autoclave room staff. The routine autoclave regime will be 126°C for 30 min, which has previously been established to produce a 100% kill.

Liquid waste (eg cultures in flasks or 'spent media' wherein the cells have been separated form the culture media by centrifugation) will be inactivated in the culture flasks the same day by addition of Virkon to a concentration of 1% w/v and left overnight. Virkon-disinfected solutions will be disposed of into the wastewater supply. Virkon 1% w/v solutions are stated to be stable for up to 7 days, and thereafter disinfectant solutions will be discarded (the pink colouration fades). However if discolouration occurs prior to 7 days, new stocks will be prepared (Virkon is a peroxygen compound and disinfection requires a contact period of at least 10 min for 1% w/v solution: see http://www.relyon.dupont.com/index2.asp and part vii below). Virkon will be used under the conditions set out by the manufacturer to ensure suitable inactivation. Please see www.relyon.dupont.com (please note Virkon is also known as ReplyOn MSD).

Virtually-treatment cells, including organotypic slice cultures and HEK293-FT cells, will be disposed of following disposal of culture medium by addition, to a final concentration of 1% w/v, of Virkon to the culture vessel. The culture media will be drained and disinfected as previously detailed and the empty flask will be disinfected with 1% w/v Virkon. After leaving overnight, liquid waste will be disposed to the wastewater supply. Cultureware will subsequently be disposed as per solid waste.

Contaminated glassware and centrifugation buckets from cultures will be disinfected by complete submersion in 1% w/v Virkon overnight (polypropylene and polystyrene centrifugation buckets, steel and glass are not corroded by 1% w/v Virkon). Glassware and buckets will then be rinsed in tap-water and dried.

The designated Class II Microbiological Safety Cabinet and centrifugation will be disinfected by thoroughly wiping down with 1% w/v Virkon solution. Wiping materials (e.g. blue roll) shall be disposed as per solid waste. The Class II Microbiological Safety Cabinet shall also be irradiated for 5 minutes with UV light. This is carried out as an extra precaution. Virkon is sufficient to inactivate the virus.

Glass microinjector pipettes will be disinfected by complete submersion in 1% w/v Virkon overnight, washed and disposed of in glass safety bins.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University Genetic Modification Safety Committee has reviewed this application in detail, together with the local Genetic Modification risk assessment for (attached). The Committee is in agreement that the assessment of risk has been appropriately performed and that work can proceed at Strathclyde University, which is already registered with HSE as a Class 2 site.
# Project Additional Information

**Purposes of the contained use**

To assess the over-expression or knockdown of genes associated with cognition and their signalling partners, in the rat brain. The viral vectors (pseudotyped lentiviral and rAAV) contain modified genomes into which genes/shRNAs of interest have been inserted. The viral vectors will be injected into specific regions of the rat brain. Transduced regions of the rat brain will be analysed by in situ hybridisation, immunofluorescence and western blotting. The rats will also be assessed in behavioural tasks of cognitive performance.

**Recipient or parental organism**

Hooded Lister male rats.
Host/vector system

VSV-G pseudotyped lentiviral particles consist of a vesicular stomatis virus G glycoprotein (VSV-G glycoprotein) envelope, HIV-1 Gag-encoded capsid proteins, HIV-1 Pol-encoded reverse transcriptase and integrase enzymes and two RNA copies of the expression constructs. RAAV particles consist of a capsid of AAV serotype 5/7/8/9, two ITR sequences of AAV serotype 2 and one single stranded DNA copy of the expression constructs.

Origin & function

The genomes of the viral particles do not contain any viral genes but only viral sequences to facilitate nuclear export and packaging of the expression construct into virions (5’ and 3’ LTRs, Psi packaging signal and WPRE for the lentiviral particles or AAV2 ITR and WPRE for the rAAV particles). There have been a few reports of some forms of this element expressing truncated forms of the WHV x protein, which has oncogenic properties. Studies into the association of WPRE and oncogenicity are ongoing. Rat cDNAs and synthetic shRNA sequences will be cloned into the viral vector genome. In addition cDNAs for fluorescent markers, including GFP and eGFP will be included. Upon infection/transduction of host cells, the RNA genome of the lentivirus is reverse transcribed into double stranded DNA which is randomly integrated into the genome of the host cell and the single stranded DNA of the rAAV is used as a template to generate double stranded DNA. This primarily remains episomal but in a small percentage of infections it integrates into the genome of the host cell.

Tranduced cells will therefore express the viral DNA. The cDNA transgenes in the viral genome will result in over-expression of genes associated with cognition, whilst the shRNAs will result in gene expression knockdown of genes associated with cognition. Transduced cells will be observed fluorescently. As the viral genomes do not contain any viral genes there will be no over-expression of viral genes in infected cells and consequently no production of new viral progeny.

Evaluation of foreseeable effects

These viral particles are replication deficient. They therefore perform a "one hit" infection without viral progeny being formed. For this reason, animals which have been injected with this agent should not excrete the viral particles in any form. Thus no special measures need to be adopted for the housing of these animals. The genetic modification is localised to the infected cells (small brain regions). The viral vectors do not carry any viral genes and therefore cannot make new viral progeny in infected cells. Therefore no virus can be shed from infected rats. Upon injection, the virus infects brain cells within 1-2mm of the injection site. These cells will contain the transgenes encoded in the viral genome. There will be no viable virus circulating in the rats and no new viral progeny produced. There is no germline transmission of the genetic modification and so the genetic modification to the brain cannot be inherited.

The routes by which exposure to the viral particles present a risk to health, if control measures are not adopted are as follows: Penetration or absorption through skin or cut in skin, oral self inoculation and accidental parenteral inoculation via needle stab. This may result in an immune response such as transient swelling and inflammation localised to the site of injection or penetration. There may be overexpression of genes in a small number of cells around the injection site. Overexpression of EGFP containing pseudoviral particles is not a biological hazard. Psychosis-related PsyRING genes are not predicted to cause a risk to health of the individual involved.

The viral vectors do not contain the WPRE (Woodchuck Hepatitis Virus Post-transcriptional regulatory element) to facilitate nuclear export of viral mRNA. There have been a few reports of some forms of this element expressing truncated forms of the WHV X protein, which is though to have ongogenic properties. Only the cells infected by the original virus will contain this sequence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Animals will be housed individually following surgical procedures. The room where these animals will be kept will be labelled, as will the individual cages to indicate that viral work has taken place. Animals do not require to be housed in a separate room since the likelihood of animals, other than those who have been injected, coming into contact with viral particles is minimal. Animal house staff will be fully informed of animals that have undergone the procedure.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All plasticware and experimental reagents that come into contact with the viral particles and transduced cells will be soaked in 3% Virkon (final concentration) for 24 hours in a designated category II hood, prior to sealing in two autoclave bags. The waste will then be autoclaved and disposed of via the University's authorised waste disposal contractor. Genetically modified brains will be dissected form the rats and transported from Strathclyde University on dry ice, in sealed containers and taken to the PsyRING laboratories at the University of Glasgow by taxi/public transport by the research scientists involved in this project. Rat carcasses will be stored within the animal facility at Strathclyde University at -20°C and disposed as clinical waste via the University's authorised waste disposal contractor for incineration. The genetically modified rat brains will be sectioned onto glass slides for immunohistochemistry at the University of Glasgow. These sections will be autoclaved and disposed as routine glass waste at the University of Glasgow. Unused brain material will be stored at -20° and disposed as clinical waste via the University's authorised waste disposal contractor for incineration. Disposal of transduced material is covered by Glasgow University GM committee ref:GM37/n.08.2/A001.

The University Genetic Modification Safety Committee has reviewed this application in detail, together with the local Genetic Modification risk assessment form (attached). The Committee is in agreement that the assessment of risk has been appropriately performed and that work can proceed at Strathclyde University, which is already registered with HSE as a Class 2 site.

**Project Containment**

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**Project Ref 298/10.1**

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Non-GMM | Consent Granted
The work will involve disrupting genes that encode for the virulence determinants of selected ACDP category 2 pathogenic bacteria in order to attenuate virulence. As such, we intend to generate GMM's that are actually less virulent than the parent strains in order to study the mechanism of disease by mutagenesis of virulence factors. We will also test the attenuation of virulence using a variety of tissue culture based assays as well as using non mammalian hosts such as Caenorhabditis elegans and Acanthamoeba spp. To assess alterations in host-pathogen interactions without the use of whole animal models. Strains to be examined include:

- Salmonella enterica (non-typhoidal serovars and the attenuated vaccine strain Ty21a) (PH, NT, JY)
- Escherichia coli (including lineage of Shigella sonnie; non-vero/shiga-toxin-producing) (PH, NT, JY)
- Campylobacter jejuni (PH)
- Corynebacterium diphtheriae, Corynebacterium spp (PH)
- Staphylococcus aureus and coagulase-negative staphylococci (PH,NT)
- Mycobacterium marinum, Mycobacterium smefmatis (PH, NT)
- Nocardia farcinica and related Nocardia spp. (PH)
- Burkholderia cepacia (NT)
- Pseudomonas aeruginosa syringae (NT)
- Vibrio vulnificus and related category 2 Vibrio spp., excluding Vibrio cholerae (NT)

A. Cloning of DNA fragments from pathogenic bacteria is non-mobilisable vectors into laboratory strains of E. coli such as DH10B, DH5a, JM110, JM109, MC1000 etc. with a view to sequencing or mutating the fragments.
B. Generating mutants in wild type strains by random transposon mutagenesis using eg. mud-lac and resulting in insertional inactivation, linked in some cases with the creation of transcriptional fusions to reporter genes.
C. Creation of mutants of pathogens by allele replacement using a suicide vector (or insertion duplication vector) e.g pK18mob.
D. Allele replacement using a gene that expresses a marker protein which is highly unlikely to have any biological effect (such as beta-galactosidase, luciferase, green-fluorescent protein, red-fluorescent protein) into a pathogen to monitor gene expression under different environmental conditions.
E. Expression of proteins at a high level in a disabled host bacterium (eg. E. coliBL21 to enable further chemical/biochemical characterisation of the protein.) Proteins will also be expressed in the original pathogenic host organism in order to complement mutations.
F. Expressing in the originating strain of a gene from a pathogenic bacterium running off its own promoter on a shuttle vector (eg pNV19), in order to complement a mutant phenotype, thus confirming the function of that gene.
G. Expression and disruption of natural antibiotic resistance determinants in a host bacterium to enable further characterisation of the resistance mechanism.
### Hosts for activities A-G as listed above.

Activity A - Disabled laboratory strains of *E. coli* and pathogenic bacteria as described above including *Salmonella enterica* (non-typhoidal serovars and the attenuated vaccine strain Ty21a), *Escherichia coli* (including lineage of *Shigella sonnie*; non-vero/shiga-toxin-producing), *Campylobacter jejuni*, *Corynebacterium diphtheriae*, *Corynebacterium smegmatis*, *Nocardia farcinica* and related *nocardia* spp., *Burkholderia cepacia*, *Pseudomonas aeruginosa*, *Pseudomonas syringae*, *Vibrio vulnificus* and related category 2 *Vibrio* spp.

Activities B, C and D - Wild type pathogenic bacteria as described above in section 6.

Activity E - Lab strains of *E. coli* and pathogenic bacteria as described above.

Activity F - Lab strains of *E. coli* and pathogenic bacteria as described above.

Activity G - Lab strains of *E. coli* and pathogenic bacteria as described above.

### Host/vector system

Hosts for activities A-G as listed above.

Activity A - Disabled laboratory strains of *E. coli* including DH5a, DH10B, MC1000, BL21, JM109 and pathogenic bacteria as described above, including *Salmonella enterica* (non-typhoidal serovars and the attenuated vaccine strain Ty21a), *Escherichia coli* (including lineage of *Shigella sonnie*; non-vero/shiga-toxin-producing), *Campylobacter jejuni*, *Corynebacterium diphtheriae*, *Corynebacterium* spp, *Staphylococcus aureus* and coagulase-negative *staphylococci*, *Mycobacterium marinum*, *Mycobacterium smegmatis*, *Nocardia farcinia* and related *nocardia* spp., *Burkholderia cepacia*, *Pseudomonas aeruginosa*, *Pseudomonas syringae*, *Vibrio vulnificus* and related category 2 *Vibrio* spp.

Activities B, C and D - Wild type pathogenic bacteria as described above in section 6.

Activity E - Lab strains of *E. coli* and pathogenic bacteria as described above.

Activity F - Lab strains of *E. coli* and pathogenic bacteria as described above.

Activity G - Lab strains of *E. coli* and pathogenic bacteria as described above.

### Vecotrs for activities A-G as listed above.

Activity A - Any non-mobilisable or mobilisation-defective vector.

Vectors include:


Activities B, C and D - Non-mobilisable or mobilisation defective vectors that can be propagated in safe laboratory strains of *E. coli* strains but not in target species, eg. pUC replicates in safe laboratory strains of *E. coli* but not in *C. jejuni*. Or *Corynebacterium, Nocardia, or mycobacteria*; show temperature sensitive replication in *E. coli* eg pNIL and pGOAL series; recombine with the target gene on the chromosome to cause insertional duplication of the plasmid in *S. pneumoniae*, eg p326(cat)pDL278(spc),pCR2(erm).

pSUP102 and derivatives: Tn5-B10 (KanR0, B11 and B12 (GentR), B13 (tetr), B20 (KanR) Transposon mutagenesis vector (Hobbs et al. PiiS and PiIR, a two component transcriptional regulatory system controlling expression of type 4 fimbriae in Pseudomonas aeruginosa. Molecular Microbiology (1993) vol. 7 (5) pp. 669-82).


pML1 (GmR) and pLR27 (KmR), and pALMAR3 for Tn5 delivery to the chromosome (Runyen Janecky et al. The virulence plasmid-encoded impCAB operon enhances survival and induced mutagenesis in Shigella flexneri after exposure to UV radiation. Infection and Immunity (1999) vol. 67 (3) pp. 1415-23).


pKNG101 and pEX100 and derivatives, host restricted to the attenuated E. coli strain CC118 (Bordi et al. Regulatory RNAs and the HptB/RetS signalling pathways fine-tune Pseudomonas aeruginosa pathogenesis). Molecular Microbiology (2010).


Activity E - Non mobilisable vectors, eg pGEX series, the pET and pCAL vectors, that express the protein, either from strong promoters or from the gene's native promoter in its native form or as part of a his-tagged, calmodulin-binding peptide-tagged or similar fusion to an innocuous protein. pET family vectors include pET21a and pET28, pQE30X (for generating his tagged proteins, Qiagen), pEGFP and derivatives for engineering GFP fusions. pAC or pAN and derivatives: aviTag. AmpR. pBAD vectors and derivatives for use as arabinose inducible expression vectors. GATEWAY plasmids (Invitrogen) for recloning and expressing tagged genes. pT7 and derivatives for overexpression of tagged proteins.

(Kelin et al.; Expression cloning of a cDNA encoding a retinoblastoma-binding protein with E2F-like properties. Cell (1992) vol. 70 (2) pp. 351-64

Studier et al. Use of T7 RNA polymerase to direct expression of cloned genes. Meth Enzymol (1990) vol. 185 pp. 60-89


Activities F and G - Non-mobilisable or mobilisation defective shuttle vectors which carry origins of replication that work in both E. coli and a given other pathogen (eg Corynebacteria, Mycobacteria and Nocardia - pNV18, pK18, pEPR1 and pMP220 in P. aeruginosa and B. cepacia). The vectors used in activities F and G include vectors already listed above associated with activities A-E.


Origin & function

The GMM's generated in this work will be defective in selected virulence determinants such as:
1) Protein secretion systems e.g. PA1663 and PA2359 from P. aeruginosa, that regulate a novel secretion system
2) Metabolic pathways that allow colonisation of other organisms e.g. nitric oxide reductase
3) Stress response systems e.g. nitrosative stress response genes
4) Transcriptional regulators of the above systems e.g. sigma factors such as rpoN. As such, the resulting strains will be less virulent than the wild-type parent strain. We
will also utilise common selective markers from existing systems such as widely used antibiotic resistance markers. Promoter sequences from parent strains will be fused
to genes encoding commonly used enzymatic and fluorescent markers such as GFP and beta-galactosidase. Disease causing ability of wild type and mutated strains will
be assessed using cell based tissue culture assays and non-mammalian infection models such as C. elegans and Acanthamoeba spp.

Well charcterised transposable elements, eg for Salmonella - Mud-lac: For pseudomonas - mini-Tn5, mini-ctx and its derivatives mini-Tn5-luxCDABE, mini-Tn5-gfp,
mini-Tn5-lac: Beta galactosidase, firefly or luminescent vibrios for luciferase, jellyfish for GFP and other fluorescent marker proteins; for Mycobacteria, Corynebacteria and
Nocardia - Tn5 derivatives EZ-Tn (Epicentre), pNV18, pK18 and their derivatives.

Genes overexpressed in E. coli will include transcriptional regulators that control the expression of virulence determinants in the pathogenic strains (e.g. enhancer binding
proteins from P. aeruginosa, that regulate a novel secretion system), although the targets of regulation are not present in E. coli

**Evaluation of foreseeable effects**

Disease causing ability of wild type and mutated strains will be assessed using cell based tissue culture assays and non-mammalian infection models such as C. elegans
and Acanthamoeba spp.

Effects of mutating transcriptional regulators will be assessed using promoter fusion assays by measuring the activity of fused beta-galactosidase, green fluorescent protein
(or its derivatives) or luciferase.

For specific activities:
- Activity A - No risk to human health, this part of the work falls into containment level 1.
- Activities B, C and D - The disruption of putative virulence genes anf their fusion to reporter genes will produce a GMM which will be no more hazardous than the wild type
  (likely to be less hazardous) and which can therefore be handled at the ACDP category relevant to the wild type - i.e. level 2.
- Activity - The disruption of putative virulence genes and their fusion to reporter genes will produce a GMM which will be no more hazardous than the wild type and which
can therefore be handled at the ACDP category relevant to the wild type - i.e. level 2.
- Activity D - The introduction of the gene for the marker protein and disruption of the resident gene will produce a GMM which will be no more hazardous than wild type and
can therefore be handled at the ACDP category relevant to the wild type - ie level 2.
- Activity E - Proteins that will be over-expressed and purified in E. coli will include transcriptional regulators that will be out of their genomic context (ie. They will have no
regulatory target in E. coli).
- Activity F - The shuttle vector simply re-introduces a characteristic that was already present in the wild type and had then been removed by mutagenesis. The procedure will
therefore produce a GMM which will be no more hazardous than the wild type.
- Activity G - No risk to human health

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All workers on level 6 of the SIPBS Hamnett Wing (Class 2 area), including those who are not directly involved, will be informed that GM work involving the organisms listed
above is ongoing.

Contaminated disposable plasticware are collected in autoclave bags for sterilisation after experiments. Other disposable plastics and non-disposable glassware are
soaked in 5% (W/V) fresh (< 48 hours old and still pink in colour) virkon overnight prior to disposal or washing and re-use to autoclave bags for sterilisation. Always clean
the work bench surface after work.
Solid-Solid waste for disposal will be bagged in approved autoclave bags and loosely sealed with autoclave temperature indicator tape and placed in the waste boxes provided by SIPBS and autoclaved at 121°C before disposal. Waste boxes will be labelled with the laboratory of origin and a contact name and telephone number. Waste will be disposed of via the University's approved contractor. Kill level for these procedures is effectively 100%. The autoclave is validated annually.

Liquid - Liquid waste will also be chemically treated with Virkon to a final concentration of 5%, and autoclaved prior to disposal in designated sinks. Virkon solution should be fresh (no more than 48 hours old). Again, the kill level for these procedures is effectively 100%


Small spillage (less than 25 ml), will be dealt as follows. A) on even surface (eg bench top): paper towels are to be used to absorb the spillage and then 5% (W/V) of fresh Virkon solution. Paper towels are collected to autoclave bags for sterilisation prior to disposal. B) Broken culture tubes in a centrifuge: collect broken tubes to autoclave bags, absorb the bacterial culture with paper towels from the rotor and collect paper towels to autoclave bag. Spills in the centrifuge will be cleaned by removal of the rotor, immersion in 1% Virkon for 10 min followed by its washing with soapy water, rinsing and drying. The centrifuge bowl will be cleaned by wiping with Virkon solution soaked paper towels which will be collected to autoclave bags for subsequent sterilisation by autoclaving. Spill granules are available for large spillages (e.g. >50ml). Spill granules should be collected and disposed of by autoclaving in the manner described above. The area should then be cleaned with fresh 5% Virkon solution as described above. Culture volumes are less than 1 litre.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University Genetic Modification Safety Committee has reviewed this application in detail, together with the local Genetic Modification risk assessment forms. The committee is in agreement that the assessment of risk has been appropriately performed and that the work can proceed at Strathclyde University. Stathclyde University is already registered with HSE as a Class 2 site.

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The brain is a complex network, where billions of neurons are highly interconnected. We know very little about how diverse neurons work collectively to process incoming sensory signals and to make appropriate actions with correct decisions. Traditionally, neurophysiological investigations with in vivo electrophysiology have revealed neural correlates of such processes. However, "optogenetic" approaches now allow us to elucidate "casual" relationships between neural activity in the specific circuit and behaviours. The channelrhodopsin-2 (ChR2) and halorhodopsin are such optogenetic actuator proteins, which allow us to manipulate neuronal activity with high temporal resolution. Given this context, this activity aims to investigate the neural mechanisms of information processing at the neural circuit level. By manipulating neural activity with optogenetic tools, effects of manipulated activity on neural transmission and behaviours will be assessed.

Recipient organism is rats.

The AAV vector will be stereotaxically injected in the brain. The virions will infect/transduce nerve cells in the close vicinity (approx 1mm radius of the injection site and will not replicate outwith this site.

Following infection, double stranded DNA is synthesised from the viral single stranded DNA which can either remain in the nucleus (episomal) or in a small number of instances may integrate into the host genome. Due to the injection site in the brain, minimal tropism and lack of viral replication, no germ cells will be infected by the virus.
AAV vectors contain recombinant transgene sequences (see below) flanked by the AAV inverted terminal repeats (ITRs) which consist of only 6% of the wild type AAV genome. The removal of the viral structural genes renders the vector replication-incompetent. Therefore they are unable to establish a productive infection and thus amplify in infected cells. It is a "1 hit" infection in brain cells.

The following plasmids are used to produce the viral particles:

- CaMKlla.ChaR2(H134R)-EYFP
- CaMKlla.ChR2(H134R)-mCherry
- CaMKlla.eNpHR3.0-EYFP
- CaMKlla.ChR2-EYFP
- CAG.ChR2(H134R)-mCherry
- Syn.eNpHR3.0-EYFP
- Syn.ChR2(H134R0-EYFP
- Syn.ChR2(H134R)-mCherry

These plasmids and viral particles are commercially available from the Penn Vector Core at the University of Pennsylvania or the University of North Carolina. The maps of these plasmids are available online at www.addgene.org or www.optogenetics.org

Origin & function

The vector is AAV where one of the following plasmids will be inserted:

- CaMKlla.ChR2(H134R)-EYFP.WPRE
- CaMKlla.ChR2(H134R)-mCherry.WPRE
- CaMKlla.eNpHR3.0-EYFP.WPRE
- CaMKlla.ChR2-EYFP
- CAG.ChR2(H134R)-mCherry.WPRE
- Syn.eNpHR3.0-EYFP.WPRE
- Syn.ChR2(H134R0-EYFP.WPRE
- Syn.ChR2(H134R)-mCherry.WPRE

All AAV vectors are commercially available.

All AAV vectors contain recombinant transgene sequences flanked by the AAV converted terminal repeats (ITRs) which consist of only 6% of the wild type AAV genome. The removal of the viral structural genes renders the vector replication-defective and dependent on adenovirus helper functions. Thus, the viral particles are replication incompetence/deficiency will be performed by the Penn Vector Core at University of Pennsylvania or the University of North Carolina before transfer to University of Strathclyde.

<Promoters>

- CaMKlla promoter: a promoter of alpha CaMKII. Expression under control of this element tends toward to the forebrain, in particular excitatory neurons.
- CAG promoter: a combination of the cytomegalovirus (CMV) early enhancer element and chicken beta-actin promoter. The CAG promoter is frequently used to drive high levels of gene expression in mammalian expression vectors.
- Syn promoter: a promoter of human synapsin 1. Expression under control of this element tends toward to neurons.

<Transgenes>

- ChR2 and ChR2(H134R): Channelrhodopsins are a subfamily of opsins proteins that function as light-gated ion channels. They serve as sensory photoreceptors in unicellular green algae, controlling phototaxis. Expressed in cells of other organisms, they enable the use of light to control intracellular acidity, calcium influx, electric excitability, and other cellular processes. Three channelrhodopsins are currently known and ChR2 is one of them. ChR2 is nonspecification channels. ChR2(H134R) is
introduced the mutation of Histidine 134 to Arginine in order to optimize its performance.

eNpHR3.0 Halorhodopsin (NpHR) is a light-driven ion pump, specific for chloride ions, and found in phylogenetically ancient archaea, halobacteria. Just as ChR2 opens up to the ability to activate excitable cells (such as neurons) with blue light, NpHR opens up the ability to silence excitable cells with brief pulses of yellow light. Enhanced NpHR3.0 is the latest version of engineered NpHR, where the C-terminal trafficking signal from the potassium ion channel Kir2.1 is added to improve the localization to the plasma membrane and inhibitory capacity.

<Reporter>
EYFP: One of derivatives of GFP (green fluorescent protein), which is protein composed of 238 amino acid residues that exhibits bright green fluorescence when exposed to blue light. This enhanced yellow fluorescent protein is a colour mutant GFP.

mCherry: One of the Fruit Fluorescent Proteins, which are mutants derived from mRFP1, monomeric mutant of DsRed, by directed mutagenesis. It has been demonstrated stable expression, perform successfully in numerous fusion applications, and are already well characterised and recognised in the literature.

<Other element>
WPRE: Woodchuck Hepatitis Virus Post-transcription Regulatory Element. The WPRE contains an enhancer element We1, a promoter for the woodchuck hepatitis virus (WHV) X protein, and the sequence for the first 60 amino acids of the X protein. The WPRE therefore has potential promoter activity and the potential to express a 60 amino-acid X protein-derived peptide. It has been suggested that the X protein is pivotal to the generation of liver cancers. It has been suggested that the X protein truncated at the C-terminal may have oncogenic properties if expressed (Tu et al 2001) Cancer Research 61, 7803-7810). However, many studies have suggested that the X promoter is non-functional in the absence of a second enhancer, We2, which is not present in the WPRE, and this has been confirmed in the context of at least a lentiviral vector (Nash et al 2004 J Gene Med 6, 974-983). At present there is no direct evidence that the N-terminal fragment used as a cis element in vectors is oncogenic (Kingsman et al 2005 Gene Therapy 12, 3-4). Thus, although there still remains the possibility that the WPRE might present a negligible risk when handling transfected tissue thereafter if expressed, it is unlikely that the X promoter becomes functional without We2, and that X proteins truncated at the N-terminal have oncogenic properties.

Evaluation of foreseeable effects

In the unlikely event that these GMOs were to escape, they may be able to survive for an unknown period of time and may survive long enough to breed. However, there would be no germline transmission of the genetic modification and thus the genetic modification would not be transmitted to the offspring.

Thus, the GM does not increase the level of risk to the health of the human population and to the environment above that of non-GMO if animals were to escape.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Rats are indigenous to the UK and therefore in the unlikely event that these GMOs were to escape they would probably be able to survive and breed in the environment outside the containment. However, the genetic modification will only be in the brain and not in the germ cells and therefore cannot be transmitted.

Additionally, ChR2 or NpHR expression does not increase the capacity for environmental survival or give the GMO a competitive advantage. The genetic modification to these animals will exist solely in the small number of region specific transduced cells in the brain.

Thus, the GMO does not represent an increased hazard to the environment compared to the parental organisms and consequently presents minimal risk to the environment. Therefore, control measures will be secured animal unit with rodent barriers.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

A the right viral dilution stage, wate and plastic-ware will be exposed to Virkon® to a concentration of 3% w/v, and left overnight. Virkon-disinfected solutions will be
disposed of into the waste water supply. Virkon 3% solutions are stable for up to 7 days, and thereafter disinfectant solutions will be discarded (the pink colouration fades). (Virkon is a peroxigen compound and disinfecton requires a contact period of at least 10 min for 1% w/v solution: see www.anachem.co.uk and part vii below).

Non liquid waste will then be placed into designated autoclave bags. This waste will be double bagged. When the bags are no more than 3/4 full, they are loosely sealed with autoclave tape (to allow steam penetration during autoclaving), labelled with lab number, operator and date and then transported to the autoclave room (BPU RW538) on a trolley. The bags are logged into the room and autoclaved on the same day that they are delivered. The bags and the logging procedure designates them 'GM', which results in their inclusion on a 'run' on the validated autoclave with recorder. The record is inspected to ensure that the correct temperature has been reached for sufficient time. All GM waste is autoclaved as above and removed as Clinical waste by the University's authorized Waste Contractor. The record is kept on file by the autoclave room staff. The routine autoclave regime will be 126°C for 30 min, which has previously been established to produce a 100% kill.

At the injection stage, in the unlikely event of a spillage these areas will be disinfected by wiping the surface with a 23% w/v Virkon solution, leaving for at least 20 minutes and drying with paper towel to remove any remaining white deposit. Paper towel will then be bagged for disposal as solid waste. Any plastic-ware will then be disposed of as described above.

Since the viral particles are replication deficient and will not be shed from the animals, it will not be necessary to dispose of bedding separately and no specific disposal procedure is required.

Cadavers will initially be frozen before being disposed of via the University's Clinical Waste Service to an authorised waste contractor.

**Is an emergency plan required according to regulation 20?**

- **No**

If yes, tick to confirm that it is attached to this form

- **No**

Tick to confirm that you have attached a risk assessment to this form

- **Yes**

Tick if you are claiming exemption from disclosure for section of the risk assessment

- **No**

**Project Containment**

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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The GM committee discussed the risk assessment for the work proposed by Dr S (University of Strathclyde ref GMM43 and GMO74). The committee was of the view that the risks had been appropriately assessed and that the correct measures were in place for the safe use and disposal of all GM materials.
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**Name**

BANGOR UNIVERSITY

**Department**

BIOLOGICAL SCIENCES & OCEAN SCIENCES

**Campus Estate or Research Centre**

**Road Name**

DEINIOL ROAD

**District**


**Town**

BANGOR

**County**

GWYNEDD

**Postcode**

LL57 2UW

**Country**

WALES

**Tel Number**

01248 382323

**Fax Number**

01248 370731

**E-mail**


**HSE Division**

WALES AND SOUTH WEST

**Comments**


**Date at Which Additional Info Submitted**

30/06/2003

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial
- Other (please specify)

Tick if confidential
Bacteriology
Parasitology
Transgenic
Birds
Microbiology
Research
Virology
Transgenic
Animals
Gene Therapy
Mycology
Transgenic
Invertebrates
Other (please
specify below)
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 299/11.1

**Date Ackn’d** 13/01/2011

**CU2 Project Title** The use of a lux-marked non toxigenic strain of Escherichia coli O157:H7 and a lux marked strain of Salmonella enterica serovar typhimurium to study the effect of environmental parameters on the survival and activity of possible pathogens

**Date Project Ceased**

**Class** Class 2

**CultureVolClass2** < 1 Litre

**CultureVolumeClass3-4**

**Class CultureVolClass2 CultureVolumeClass3-4**

**Non-GMM Consent Granted**

**Project notified under transitional arrangements**

**Withdrawn**

Tick if notifying a connected programme of work

**Historical Significant Changes**

**Historical Date of Additional Info**

02/03/2022
E. coli O157:H7 is a toxigenic strain of E. coli and ingestion of the organism may occasionally lead to severe illness including life threatening hemolytic-uremic syndrome. E. coli O157:H7 is a class 3 organism according to the ACDP classification and as such requires level 3 containment for safe use, a requirement that can limit studies on this organism due to the expense of constructing and maintaining such facilities. A naturally occurring strain of E. coli O157:H7, retaining the O157 and H7 phenotypes but which is non-toxigenic, since it lacked the toxin genes stx1 and stx2, was previously chromosomally marked with the lux CDABE cassette using transposon mutagenesis to provide a low-risk substitute to verocytotoxin producing E. coli O157:H7 in environmental survival studies (Ritchie et al 2003). A strain of S. enterica serovar typhimurium containing a plasmid borne lux gene fusion is also available enabling safer and more accurate detection and quantification of this organism (Lewis et al. 2006). The purpose of this project is to use a luminescence assay to determine how a range of environmental parameters effect the survival and activity of the bacteria. This project will not involve the creation of any new GMOs.

The recipient E. coli strain was a naturally occurring isolate of E. coli O157:H7 which was shown via PCR to lack the genes stx1 and stx2 which encode cytotoxins. The recipient Salmonella strain was S. enterica serovar typhimurium DT104 which is a well characterised and common pathogen in humans.

The recipient E. coli strain was mated with with E. coli s17 lambda pir which contained a suicide plasmid harbouring a transposon Tn5 derivative with a luxCDABE cassette. The absence of the suicide plasmid in the transposon mutated E. coli O157:H7 strain was confirmed by an inability to grow on ampicillin (the plasmid conferred ampicillin resistance) and via small scale plasmid DNA preparations.

The recipient S. enterica strain was transformed, via electroporation, with the broad host range plasmid pBBR1MCS-5 in which the lux CDABE genes from Photorhabdus luminescens had been cloned. Gentamycin selection was used to confirm transormation since the plasmid encodes gentamycin resistance.

The genetic material transferred to the E. coli O157:H7 strain included a gene for kanamycin resistance enabling antibiotic selection for successful transposon mutagenesis together with the lux CDABE cassette from Photorhabdus luminescens encoding the ability to produce bioluminescence.

The genetic material transferred to the S. enterica strain included a gene for gentamycin resistance enabling antibiotic selection for successful transformation together with the lux CDABE cassette from Photorhabdus luminescens encoding the ability to produce bioluminescence.

There are no hazards arising directly from the inserted DNA since the genes do not encode the production of any toxins or other virulence factors or extend the host range of the bacteria. In the case of the genetically modified strain of E. coli, the likelihood of the inserted DNA being transferred to related organisms is small and in any case they would not increase the pathogenicity of the recipient organisms. The naturally occurring non-toxigenic strain of E. coli O157:H7 does not pose a risk to the environment and the genetic modification performed has not resulted in any increased potential for environmental damage.

The likelihood of the inserted material in the modified strain of S. enterica being transferred to other organisms is greater since the plasmid containing the cloned DNA has a broad host range and is mobilisable if the RK2 transfer function is provided in trans (Kovach et al 1995). However, even if transfer did occur it would not increase the pathogenicity of the recipient organisms or their ability to cause environmental damage.

It is well-established that the toxin genes stx1 and stx2 play a key role in virulence of entero-hemorrhagic strains of E. coli O157:H7. The lack of stx genes in the naturally occurring non-toxigenic strain of E. coli O157:H7 means that it is a low risk to human health. However, if this bacteria were to acquire the ability to produce toxins via plasmid
Transfer from another organism it has the potential to cause disease since it retains the intimin phenotype and can colonise human guts (Ritchie et al. 2003). In such circumstances the genetic modification of the strain increases the risk to humans marginally since kanamycin would no longer be an effective antibiotic with which to treat any disease. It should be noted, however, that kanamycin is actually very rarely used as a treatment for E. coli O157:H7 infections in any case.

S. enterica serovar Typhimurium DT104 is recognised as a major cause of food poisoning worldwide and is assigned to Hazard Group 2. The wild type strain is characterised by multiple drug resistance and hence the genetic modification does increase marginally the risk to human health since gentamycin would no longer be an effective treatment in case of infection.

References:

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This work will produce a variety of wastes including contaminated pipette tips, agar plates and liquid cultures. Contaminated pipette tips are immediately placed in a solution of sodium hypochlorite (12-14% v/v aqueous solution) and subsequently autoclaved using a cycle including an initial steam purge and kill step of 121 °C, 15 psi for 20 minutes. All petri dishes and liquid wastes are killed via autoclaving as described above. Autoclave tape is routinely used and the efficacy of the kill cycle regularly checked by autoclaving a thick culture (approx 1 E9 colony forming units per ml) and subsequently plating on non selective agar and checking for growth at 37 °C over a 4 day period. All autoclave use is documented and autoclaves are subject to regular inspections and maintenance.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Approved as Class 2

Project Containment

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<th>Growth Rooms</th>
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02/03/2022
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Name

SEKISUI DIAGNOSTICS (UK) LTD

Name 2

Department

Campus Estate or Research Centre

Road Name

LIPHOOK WAY

District

ALLINGTON

Town

MAIDSTONE

County

KENT

Postcode

ME16 0LQ

Country

ENGLAND

Tel Number

01622 608000

Fax Number

01622 607801

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

company changed its name from GENZYME BIOCHEMICALS LIMITED, company changed its name to Sekisui Diagnostics(UK) Ltd

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

Non-microbial
Project Ref 301/01.1

Date Ackn’d 15/02/2001

CU2 Project Title LONG TERM STORAGE OF GMM CULTURES NOT IN USE FOR DEVELOPMENT OR MANUFACTURING

Class Class 2

CultureVolClass2

CultureVolumeClass3-4

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

02/03/2022
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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### Project Additional Information

**Purposes of the contained use**

To minimise the risk of infection from class II cell lines, in accordance with good microbiological practice and the protection of both work and worker. Work carried out at the premises will be limited to research activities.

**Recipient or parental organism**
The cell lines used may include adenovirally transformed lines (e.g., HEK-293) or cells classed as Biosafety level 1 that have been transfected with mammalian expression vectors or via adenovirus. The cell lines themselves are not infectious.

### Host/vector system

The GM cell lines may be virally transformed, adenovirally transfected or stably transfected using expression vectors. Adenovirus has been identified by the ACDP as a Level 2 Biosafety Hazard. However, the risk of transmission to humans in this activity is considered negligible. In some cases the inserted product may be expressed as a membrane bound protein. The antigens under study are not in themselves pathogenic but should be handled according to good microbiological practice.

### Origin & function

Adenovirus has been used to transform some commercially available cell lines, in order to render them immortal for long-term culture. Adenovirus is also as a means of introducing a gene of interest for over-expression, usually at the cell surface, for use as a selection target. The product may occasionally be released into the medium as a soluble protein, or purified from the cell, but will then be captured for use as a selection target or for in vitro characterisation and testing of the antibodies under development.

### Evaluation of foreseeable effects

Biosafety level 2 hazard is introduced to the proposed work by use of adenovirally transformed or transfected cell lines. Other cell lines or products are currently evaluated at level 1. The risk of transmission of adenoviruses from these systems to humans is negligible and the cell and genetic material will not survive in the external environment. Standard level 2 decontamination and containment facilities are in operation. Overall, the foreseeable effects are considered negligible.

### Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cell lines will be cultivated in disposable plasticware at a scale from 50ul to 500ml under aseptic conditions and harvested by centrifugation. All waste resulting from these procedures will be either chemically inactivated or autoclaved prior to disposal. All equipment and materials used within the procedures will also be sterilised by autoclaving for 20 minutes at a temperature of 121 degrees C or chemically disinfected (e.g., Virkon, TriGene, or Proceine40) prior to disposal or re-use. All operations will be performed in laboratory facilities in which spillages can be effectively disinfected so the wider environment is unlikely to become contaminated.

### Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

This project has been approved as a class 2 GM activity on the basis of the attached risk assessment.
## Project Containment

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GM Centre Number: 302

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Name

UNIVERSITY OF WALES COLLEGE OF MEDICINE

Name 2

UNIVERSITY HOSPITAL

Department

MEDICINE

Campus Estate or Research Centre

Building

Road Name

HEATH PARK

town

CARDIFF

District

CEREDIGION

County

Postcode

CF14 4XN

Country

WALES

Tel Number  
029 2074 2903

Fax Number  
029 2074 4869

E-mail

none

HSE Division

WALES AND SOUTH WEST

Comments

GM302 CLOSED DOWN AND MERGED WITH GM130 ON 26/04/2005.

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: [ ]

- Give brief details of the genetic modification safety committee:

  - Laboratory
  - Animal Unit
  - Growth Room
  - Glass House
  - Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

- Other (please specify): [ ] Tick if confidential

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick if you are claiming exemption from disclosure for sections of the risk assessment.

Tick to confirm that you are attaching a summary of the risk assessment.

Please enter comments of the GM safety committee on the risk assessment.

---

**Project Ref** 302/01.1

**Date Ackn’d** 22/02/2001

**CU2 Project Title** EXPRESSION OF INFLUENZA MATRIX PROTEIN BY USING A REPLICATION-DEFICIENT ADENOVIRUS

**Date Project Ceased** 26/04/2005

**Class** Class 2

**CultureVolClass2** not applicable

**CultureVolumeClass3-4** not applicable

**Non-GMM Consent Granted** not applicable

**Consent Granted** not applicable

**Project notified under transitional arrangements** Y

**Withdrawn** N

**Historical Significant Changes** Transferred to GM130 on 26/04/2005

**Historical Date of Additional Info**

**Significant Change ID**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment
### Project Ref 302/01.2

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- **Project notified under transitional arrangements**: Y

#### Historical Significant Changes
- Transferred to GM130 on 26/04/2005

#### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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</thead>
<tbody>
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Project Ref 302/01.3

Date Ackn'd 02/05/2001  CU2 Project Title ADENO VIRAL INFECTION OF CELLS FROM DISEASED TISSUE AS A TOOL TO

Class 2  CultureVolClass2 < 1 litre  CultureVolumeClass3-4

02/03/2022  Page 6035 of 15326
This project aims to use replication deficient adenoviral vectors to investigate intracellular signalling in cell lines and in human cells from diseased synovial tissue. These replication deficient adenoviruses can still infect cells but not replicate within them. Thus, if some person would deliberately or accidentally inhale these viruses or rub them into his eyes, a mild rhinopharyngitis (similar to a common cold) or conjunctivitus (harmless eye inflammation), lasting a few days at most, would ensue. Many people have immunity due to previous exposure, however, as this is a common pathogen. The project will not involve the production of new recombinant adenoviruses on site using vector DNA, but will involve growup of adenoviruses from imported stock using the 293 cell line, and infection of cells.

Host/vector system

This project aims to use replication deficient adenoviral vectors to investigate intracellular signalling in cell lines and in human cells from diseased synovial tissue. These replication deficient adenoviruses can still infect cells but not replicate within them. Thus, if some person would deliberately or accidentally inhale these viruses or rub them into his eyes, a mild rhinopharyngitis (similar to a common cold) or conjunctivitus (harmless eye inflammation), lasting a few days at most, might ensue. There could be no other complications unless a massive dose of virus was deliberately injected intravenously. The project will not involve the production of new recombinant adenoviruses on site using vector DNA, but will involve growup of adenoviruses from imported stock using the 293 cell line, and infection of cells. These imported stocks are all from the Kennedy Institute of Rheumatology and have been carefully monitored to exclude contamination with wild-type adenovirus.

Origin & function

It is primarily planned to use

1) A replication deficient adenovirus with no insert (as a control).
2) A replication deficient adenovirus transferring the beta-galactosidase gene. This is a non-harmful molecule that is useful for determining viral infectibility.
3) A replication deficient adenovirus transferring the green fluorescent protein gene. This is a non-harmful molecule that is useful for determining viral infectibility.
4) A replication deficient adenovirus transferring the gene for I-kappa-B the natural inhibitor of NF-kappaB, an anti-inflammatory molecule.
5) A replication deficient adenovirus transferring the dominant negative form of the I-kappa-B kinase-2, a modification that would also be expected to have an anti-inflammatory effect.
6) A replication deficient adenovirus transferring the dominant negative form of the p38 MAP kinase, a modification that would also be expected to have an anti-inflammatory effect.

All of these have been constructed from plasmids at the Kennedy Institute, London. These molecules are all anti-inflammatory rather than pro-inflammatory, as would be expected if they would be any use as treatment for inflammatory diseases. No adenoviruses with oncogenic genes or sequences will be used. No animal work is planned. The adenoviral work will take place in a category II facility with a class II vented hood designated for adenoviral work. A locked, designated -70 degree virus freezer will be used for virus storage. There is a designated incubator for infected cells.

**Evaluation of foreseeable effects**

All vectors used will be based on adenovirus serotype 5, and will be replication deficient due to disabled in the E1 region by an insert that prevents the packaging of viable particles. The E1 region is replaced with inserted foreign DNA. These replication deficient adenoviruses can still infect cells but not replicate within them. Thus if some person would deliberately or accidentally inhale these viruses or rub them into his eyes, a mild rhinopharyngitis (similar to a common cold) or conjunctivitis (harmless eye inflammation), lasting a few days at most, might ensue, in case the person infected did not, like the majority of the population, already possess antibodies to the wild type virus. All inserted gene products will be anti-inflammatory rather than the opposite. None will have mutagenic or oncogenic effects. They should not cause any excess risk. There will be no alteration of existing pathogenic tracts, except for the replication deficiency mentioned above, which will not cause any hazard but rather the opposite. Since all inserted gene products in this study will be anti-inflammatory rather than the opposite, and none will have mutagenic or oncogenic effects, they should not cause any excess risk compared with an adenovirus without an insert. There is thus a risk of very slight and time limited harm should a non-immune person be exposed to these adenoviral vectors.

It is known that recombinant adenovirus reverts to wild type at a low frequency (10^-7). To monitor this and exclude any risk, cytopathic assays are regularly used every 2-3 months to check for replication-competent virus. Since all inserted gene products in this study will be anti-inflammatory rather than the opposite, and none will have mutagenic or oncogenic effects, they should not cause any excess risk compared with an adenovirus without an insert.

The parental virus is ACDP Hazard Group 2. Since all inserted gene products in this study will be anti-inflammatory rather than the opposite, and none will have mutagenic or oncogenic effects, they should not cause any excess risk compared with an replication deficient adenovirus without an insert. Therefore the provisional containment level is 2.

It is not thought that these replication incompetent adenoviruses would pose a risk to animals or plants in the environment, particularly as good care is taken to protect human health. Nor would there, as these viruses will be kept in a locked freezer, be any risk of exposure in a fire, landslide or flooding.

Since the decision was made to apply containment level 2, the activity class is confirmed as Class 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation is made.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be autoclaved prior to incineration. The laboratory is separated from the rest of the building by means of a lockable door. Culture medium and liquid waste should be disinfected using 10% Microsol, with exposure at least 1h prior to disposal in the sink.
The committee was in principle satisfied with the risk assessment for this project and that the work requires Containment level 2 facilities and is class 2.

Project Containment

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<tr>
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Project Ref 302/01.4

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<td>26/04/2001</td>
<td>GENERATION OF DEFINED DELITIONS IN THE HUMAN CYTOMEGALOVIRUS (HCMV) AND HUMAN HERPESVIRUS-6 (HHV-6) GENOMES WILL BE PERFORMED TO INVESTIGATE GENE FUNCTION</td>
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Tick if notifying a connected programme of work Y

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### Project Additional Information

#### Purposes of the contained use

Generation of defined deletions in the human cytomegalovirus (HCMV) and human herpesvirus-6 (HHV-6) genomes will be performed to investigate gene function. To further analyse betaherpesvirus gene function sub-genomic or cDNA fragments will be subcloned and expressed using a range of prokaryotic and eukaryotic expression vectors.

#### Recipient or parental organism

HCMV and HHV-6 are both ACDP category 2 pathogens that are both predicted to encode a large number of open reading frames (in the order of 200). Currently functional information is available for a relatively small number of their genes. Both viruses are associated with mild disease in healthy individuals but occasionally HCMV causes severe disease in immunocompromised individuals. There is no compelling evidence to link either virus with cancer. Both virus are species-specific with productive replication being effectively restricted to human cells. Deletion of genes would usually be expected to reduce virus pathogenicity. Both viruses are expected or known to encode genes that modify cellular physiology and others that modulate the immune response.

The cloning of such genes in a replication-deficient Ad vector will generate recombinant viruses that are more readily transmitted, will provide high level expression and may induce the expression of protein that have a biological effect both in humans and animals. Adenovirus is a ADCP category 2 pathogens although the deletion of the E1 gene region will make both unable to replicate or promote efficient early phase gene expression. Being replication-deficient the recombinant viruses have limited potential for lateral spread, although this could be facilitated by a co-infection with a wt Ad. Wild type Ad type 5 is capable of productive replication in very few species and is associated with mild upper respiratory tract infection in childhood.

#### Host/vector system

E.coli JM109, TOP10 or XL1-blue. Prokaryotic vectors include pcDNA3.1/ct-GFP-Topo, pBLUETOPO, pUC-based vectors containing the HCMV major IE promoter (pMV100), adenovirus transfer vectors pAL200 (and variants with alternative cloning sites), pJM17 (contains the complete Ad5dl 309 genome), episomal vectors containing the EBV origin of replication and the EBNA1 gene (p220.2).

The recombinant adenoviruses will be propagated in a helper cell line expressing an E1 helper function (eg 293 or 911 cells) and then used to infect target cells in vitro or in vivo for the generation of antibodies.

For the generation the HCMV genome will be used in the first instance. The HCMV strain AD169 and Toledo genomes will be used in preference. Deletion of the HHV-6 genome is planned in future but will be dependent on the development of better systems for growing the virus.

#### Origin & function

HCMV strains AD169 and Toledo are characterised laboratory isolates. The Strain AD169 genome is sequenced and published and there is extensive sequence data available for strain Toledo.

HHV-6 strain Z29 and U1102 have been fully sequenced. We intend also to investigate sequence variation in a range of fresh clinical isolates.

#### Evaluation of foreseeable effects

The expression of individual HCMV or HHV-6 genes in cells transiently or in established lines is of low potential hazard. Both viruses are expected or known to encode genes that modify cellular physiology and others that modulate the immune response. The cloning of such genes in a replication-deficient Ad vector will generate recombinant viruses that are more readily transmitted, will provide high level expression and may induce the expression of protein that have a biological effect both in humans and animals. HCMV encodes 6 gene that modulate class I presentation, two functions that modulate resistance to NK cells and both viruses encode chemokine and chemokine receptor homologues.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solution (tissue culture media or buffers such as PBS) is inactivate by adding to less than equal volume 2500 ppm Sodium Dichloroisocyanurate (Actichlor), after 4h solution can be discarded to mains drainage. Pipettes immersed in 2500 ppm Sodium Dichloroisocyanurate for 4h before transferred to plastic container for autoclaving, all plasticware is autoclaved after exposure to virus. Actichlor is effective against all viruses and the high concentration overcomes high organic content of media. Animals may be inoculated in a contained isolator or if Class I or Class II microbiological safety cabinet. All animal waste is incinerated.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved.

Project Containment

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Project Ref 302/01.5

Date Ackn'd CU2 Project Title
01/06/2001 DEVELOPMENT OF ADENOVIRUS VECTOR SYSTEMS

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 1-50 litres
This notification covers the development of adenovirus vectors designed to facilitate the induction/modulation of specific immune responses by transgenes expression of. Host immune responses to vector components can be proinflammatory and often over-ride or subvert potential biological effects associated with the transgene. Specific mutations in the E2, E3, E4, L5 genes will be incorporated into adenovirus type vectors to limit breakthrough expression from replication-deficient adenovirus vectors. Ad5 vectors utilise CAR as a primary receptor for virus infection. We intend to deliver transgenes to cells that do not express detectable levels of CAR (notably lymphocytes). Using established technology, we intend to modify the fibre gene so that the expressed protein will no longer recognise its native CAR receptor and so that it will recognise specific receptors present on target cells. The modified fibre will not be inserted into an adenovirus genome but expressed as a complementing function in a helper cell line. Many individuals have been exposed to adenovirus type 5 and will also generate strong immune responses on exposure to this vector. Adenovirus type 4 has been used as a live viral vaccine. Fewer individuals have seroconverted to adenovirus type 4 and it can potentially also be used in combination with Ad 5 vectors in prime:boost vaccination protocols. We wish therefore to develop replication-competent and replication-deficient adenovirus type 4 vectors. A range of tumour-associated and virus antigens will be expressed in these vectors.

Preliminary prokaryotic cloning steps are necessary to generate the recombinant adenoviruses. Genetically disabled E.coli K12 strains specifically developed for application in recombinant DNA cloning experiments will used. These strains have limited potential to colonise the gut or survive in the environment. Adenovirus is an ACDP category 2 pathogen. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Adenovirus type 4 infection is also associated primarily with mild, debilitating respiratory tract infections but can occasionally cause an acute respiratory disease (ARD), acute haemorrhagic conjunctivitis or rarely a fatal non-bacterial pneumonia. To protect against infection, a non-attenuated live Ad4 vaccine has been administered to military recruits in North America. This live viral vaccine consists of an enteric-coated tablet containing live, non-attenuated, tissue culture grown Ad4 virus. The enteric coating prevents infection of the respiratory tract and when swallowed virus is only released from the tablet in the stomach where it infects the gut inducing protective immunity but not disease. Over half a million doses of this vaccine has been administered without complication and it has proved to be effective in preventing ARD. It has been proposed that this vaccine and method of administration might be used as a vector to generate an immune response against both Ad4 and the heterologous immunogens. Ad4 recombinants expressing a range of viral genes have been generated and tested in animals and humans with promising results. The Ad4 virus strain used in the vaccine virus will be used as the basis for all Ad4 vectors and recombinants constructed. Deletion of the E1 gene region will render the adenovirus vectors replication-deficient. Replication-deficient Ad4 vectors by their nature have limited potential for lateral spread although this could be facilitated by co-infection with a wt adenovirus. Both wild type Ad4 and Ad7 exhibit a severe host restriction and are capable of productive replication in very few species other than man.
E. coli strains JM109, TOP10 and XL1-blue will be used. Prokaryotic vectors include pcDNA3.1/ct-GFP-Topo, pBLUETOPO, pUC-based vectors containing the HCMV major IE promoter (pMV100), adenovirus transfer vectors pAL200 (and variants with alternative cloning sites). Commercial Ad5 vector systems will be used as the basis for vector development. From Microbix (Canada) pJM17 (contains the complete Ad5d/309 genome) and the Admax TM vector system will be used. From Qbiogene (Hareford, Middx) the Ad5 based AdEasy TM vector system, which involves all recombination events be performed in E.coli, will be used.

Ad4 vectors will be generated de novo from the vaccine strain.

Replication-deficient adenoviruses will be propagated in a helper cell line expressing an E1 helper function (eg 293 or 911 cells) and then used to infect target cells in vitro or in vivo. A 293 cell line expressing E4 will be obtained from a commercial source (Microbix). Additional helper cell lines will be constructed in 293 or 911 cells.

### Host/vector system

A number of human and murine tumour-associated antigens will be cloned.

- **gp100** cloned cDNA normal human and mouse melanocytes [Bakker et al, J Exp Med 179:1005, 1994]
- **Melan A** cloned cDNA normal human and mouse melanocytes [Coulie et al, Immunity 2:167, 1995]
- **BAGE** cloned cDNA melanoma and normal testis [Boel et al, 178:489, 1993]
- **Mage 1** cloned cDNA melanoma and normal testis [Castelli et al, J Cell Physiol 182: 323, 2000]
- **Mage 2** cloned cDNA melanoma and normal testis [Castelli et al, J Cell Physiol 182: 323, 2000]
- **Mage 3** cloned cDNA melanoma and normal testis [Castelli et al, J Cell Physiol 182: 323, 2000]
- **Mage4** cloned cDNA melanoma and normal testis [Castelli et al, J Cell Physiol 182: 323, 2000]
- **NY-ESO-1** cloned cDNA melanoma and normal testis [Chen et al, PNAS 94: 1914, 1997]
- **CT10** cloned cDNA melanoma and normal testis [Gure et al, Int J Cancer 85:726, 2000]
- **SOX** cloned cDNA colon carcinoma and normal testis [Tureci et al, Int J Cancer 77:19, 1998]
- **ZIC** cloned cDNA colon carcinoma and normal testis [Tureci et al, Int J Cancer 77:19, 1998]

Human cytomegalovirus and Human herpes virus 6 are closely related human herpesviruses capable inducing disease in immunocompromised individuals but with no recognised association with cancer. Genes from both these viruses will be cloned into adenovirus vectors for the purpose of generating and measuring immune responses. Venezuelan Equine Encephalitis virus (VEEV) is an Alphavirus that replicates in the cytoplasm of infected cells. The virus particle contains genomic RNA packaged in a capsid consisting of core proteins and an envelope containing the glycoproteins Env1 and Env2. Two other proteins Env3 and 6k may also be found in the virus particle. These two small proteins contain the translocation signal sequence for Env1 and Env2. Being an Alphavirus, the structural proteins are translated from a subgenomic RND as a single polyprotein that is then cleaved by cellular proteases. Env2 contains a major protective epitope. Replication-competent and replication-deficient adenovirus recombinants encoding VEEVEnv3Env2/6K will be generated.

### Origin & function

Some of the tumour-specific antigens are expressed in melanocytes and are involved in the melanin biosynthetic pathway. All such proteins are expressed either in normal testis or in melanocytes. None of these proteins in isolation have any toxic, oncogenic, carcinogenic or allergenic properties since they are all expressed in normal cells. The risk of any autoimmune disorder triggered by an overexpression of these proteins is negligible because no autoimmune effect has been reported in healthy volunteers and melanoma patients with high frequencies of cytotoxic T lymphocyte specific for these proteins. (Van Pel et al., Immunological Review. 145:229, 1995; Coullie et al., J Cancer 50:289, 1992). The immune response specific to colon carcinoma antigens is still ill defined, and very few proteins have been shown to be specifically expressed by colon cancer cells. One of the most promising family of newly characterised colon cancer proteins are the SSX proteins, which are expressed in a high proportion of colon.

The expression of individual HCMV or HHV-6 genes in cells transiently or in established lines is of low potential hazard. Both viruses are expected or known to encode
genes that modify cellular physiology and others that modulate the immune response. The gene product of VEEV E3, E2 and 6k sequences are not known to be toxic, allogenic or oncogenic although E2 is associated with a strong humoral immune response. The cloning of such genes is a replication-deficient Ad vector will not generate recombinant viruses that are more readily transmitted, will provide high level expression and may induce the expression of protein that have a biological effect both in humans and animals.

This work involves standard laboratory protocols and there are no unusual procedures that require additional containment measures. A more detailed risk assessment was performed for the local ACGM safety committee.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solution (tissue culture media or buffers such as PBS) is inactivate by adding to less than equal volume 2500 ppm Sodium Dichloroisocyanurate (Actichlor), after 4h solution can be discarded to mains drainage, Pipettes immersed in 2500 ppm Sodium Dichloroisocyanurate for 4h before transferred to plastic container for autoclaving, all plasticware is autoclaved after exposure to virus. Actichlor is effective against all viruses and the high concentration overcomes high organis content of media.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved, suitable and sufficient Class 2 Containment Level 2

Project Containment

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**Project Ref**: 302/96.4

**Date Ackn'd**: 22/02/2001

**CU2 Project Title**: ANALYSIS OF EPSTEIN BARR VIRUS LATENT GENE FUNCTIONS (I) INSERTION OF VIRAL GENES INTO EUKARYOTIC EXPRESSION VECTORS (II) TRANSFECTION INTO MAMMALIAN CELLS

**Date Project Ceased**: 26/04/2005

**Class**: Class 2

**Culture Vol Class**: 2

**Culture Volume Class**: 3-4

**Non-GMM**: Not applicable

**Consent Granted**: Yes

**Project notified under transitional arrangements**: Yes

**Historical Significant Changes**: Transferred to GM130 on 26/04/2005

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**
- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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Historical Significant Changes

Transferred to GM130 on 26/04/2005

Historical Date of Additional Info

02/03/2022
Significant Change ID
Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
A PHASE IIA, OPEN LABEL TRIAL TO ASSESS THE SAFETY, IMMUNOGENICITY AND EFFICACY OF A PRIME-BOOST STRATEGY OF TA-CIN ADMINISTERED IN ASSOCIATION WITH TA-HPV TO PATIENTS WITH HIGH GRADE ANO-GENITAL
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref  312/95.1

Date Ackn'd  11/04/1995  

CU2 Project Title  STRUCTURE AND FUNCTION STUDIES OF NORMAL AND MUTANT  

Class 2  

Class  CultureVolClass2  CultureVolumeClass3-4  

Class 2  

Class 2
Transferred originally from GM312 to GM302 then transferred from GM302 on 26/04/2005.

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment  

Project Containment  

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Animal Units | Large Scale Activities | Human Clinical Applications |
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Name

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Campus Estate or Research Centre

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Date at Which Additional Info Submitted

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

**Level 1 (GMMs)**

**Level 2 (GMMs)**

**Level 3 (GMMs)**

**Level 4 (GMMs)**

Non-microbial

Other (please specify) Tick if confidential
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 309

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Name

IMPERIAL COLLEGE OF SCIENCE TECHNOLOGY & MEDICINE

Name 2

NATIONAL HEART AND LUNG INSTITUTE

Campus Estate or Research Centre

Building

GUY SCADDING BUILDING

Road Name

DOVEHOUSE STREET

District

Town

LONDON

County

GREATER LONDON

Postcode

SW3 6LY

Country

ENGLAND

Tel Number

0207 351 8278

Fax Number

0207 376 3442

E-mail

HSE Division

LONDON

Comments

GM549 & GM853 MERGED WITH GM309 ON 19/3/2004, Closed & merged with GM8

Date at Which Additional Info Submitted

19/03/2004
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Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Other (please specify) Tick if confidential

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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste (max culture volume 500ml) will be chemically treated with an appropriate disinfectant (Hycolin, Chloros or Virkon) used at the manufacturers specified concentration for the appropriate length of time. Solid waste will be autoclaved.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Project Ref  309/01.1

Date Ackn’d  31/07/2001  CU2 Project Title  USE OF RECOMBINANT REPLICATION-COMPETENT SENDAI VIRUS (SEV) AS A NEW GENE TRANSFER AGENT FOR GENE THERAPY OF INHERITED AND ACQUIRED DISEASES

Date Project Ceased  02/08/2017

Class  Class 2  CultureVolClass2  < 1 litre

Consent Granted  not applicable

Project notified under transitional arrangements  N

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes  Transferred to GM8 on closure of GM309

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recombinant seV will be used to transfer reporter and therapeutic genes to different cell types (in vitro and in vivo) and compared to currently available gene transfer vectors.

Recipient or parental organism

Sendai virus (SeV) is an enveloped virus with a nonsegmented negative-strand RNA genome of 15384 nucleotides and is a member of the family Paramyxoviridae. The SeV genome contains six major genes: nucleoprotein (NP), phosphoprotein (P) and large protein (L) form a ribonucleoprotein (RPN) with the SeV genomic RNA. The matrix protein (M) engages in the assembly of viral particles. Two envelope glycoproteins, hemagglutinin-neuraminidase (HN) and fusion protein (F) mediate the attachment of virions and penetration of RNPs into infected cells. SeV replication is independent of nuclear functions and does not have a DNA phase. Therefore, it does not transform cells by integrating its genetic information into the cellular genome. SeV has been reported to naturally infect rodents (causing upper respiratory tract infections) and has never been reported to infect humans.

Host/vector system
Transgenes are normally inserted immediately before the ORF of the viral 3'-proximal nucleocapsid (NP) protein gene in a full-length SeV CDNA copy. The inserted gene is flanked by "gene start signal" and polyadenilation/stop sequences and recognised by the viral RNA polymerase.

### Origin & function

The Sendai genome derives from the Z-strain. The recombinant virus has been developed by a Japanese biotech company, DNAVEC. The recombinant SeV will carry different transgenes:

1. bacterial transgenes, such as beta-galactosidase or CAT;
2. Eukaryotic tran genes such as luciferase (from Photinus pyralis) and GFP (from jellyfish);
3. Therapeutic genes

### Evaluation of foreseeable effects

1. Recombinant SeV does not have an altered tropism compared to wild type virus. However, insertion of a transgene reduces the replication speed as well as the final virus titre in a way, that is proportional to the size of the transgene, carried. In vivo a remarkable attenuated replication and pathogenicity were generally seen (Sakai et al., FEBS Lett 1999; 456: 221-226).
2. Virus replication and transmission to neighbouring cells has been demonstrated in the host species (rodents). SeV has never been reported to infect humans.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Recombinant Sendai virus is stored at-80C in 1-2 ml vials separately from the biological samples. All experiments are carried out in class II cabinets. After use, cabinets are UV irradiated for at least 30 minutes, Single-use plasticware is used. The solid waste produced is double-bagged and autoclaved (121C/1 atm). For the liquid waste, Hypachlor for > 10 min with chlorine (2500 ppm final concentration) is used. Personnel will wear labcoats, gloves, masks and overshoes.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form  | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | Y |

Please enter comments on the GM safety committee on the risk assessment

The Generic Modification Safety Committee has reviewed the application in great detail. The committee is satisfied that the virus poses no risk to humans and that adequate measures have been taken to ensure that the virus will be wholly contained within the dedicated unit set up specifically for this work.

### Project Containment

02/03/2022
The aim of this project is characterise pseudotyped SIV-based gene transfer agents in the lung. Proof-of-principle for gene therapy for cystic fibrosis (CF) has been established in several clinical trials (1). However, gene transfer is generally inefficient and of short duration. Our group has a long standing interest in CF gene therapy and we have evaluated several non-viral and viral gene transfer agents in pre-clinical studies (2). However, most gene transfer agents are inefficient and express the transgene only very transiently (less than one week). This includes commonly used liposomes, adenoviral vectors and Sendai virus (SeV). Lentiviral vectors, such as SIV have recently been suggested for airway gene transfer, as they transduce non-dividing cells (3). Lentiviral vectors are retroviruses, that have to integrate into the host genome and therefore offer the potential for long lasting gene expression. We have previously assessed generation 3 SIV, pseudotyped with the traditional pleiotropic envelope coat protein, VSV-G airway gene transfer and shown that transfection efficiency is very poor (unpublished data). Here, we want to evaluate an SIV vectors pseudotyped with the F and HN envelope protein from parainfluenza viruses (3). Parainfluenza viruses such as Sendai virus have been shown to transduce airway epithelial cells efficiently. In addition, VSVG-pseudotype lentiviral vectors will be used as controls. The main difference between 2 and 3 generations SIV is that the viral genes gag-pol and rev are on one plasmid for production of 2 generation viruses, but are separated onto two plasmids for the production of 3rd generation viruses, which
further reduced the chance of recombination and production of replication competent viruses.

References:

Recipient or parental organism

The Simian immuno deficiency virus isolated from Africa green monkey (SiVagm) has to the best of our knowledge not been associated with pathology in its natural host or animal models (1). The vector used is described by Nakajima etal, and is a self-inactivating (SIN) vector, because the U3 region of the 3’ long terminal repeat is deleted (2). This region contains viral promoter and enhancer elements. Deletion of the U3 region will prevent mobilisation by replication-competent viruses. SiV vectors pseudotyped with F and HN protein or VSV-G envelope proteins will be produced by DNAVEC, Inc (Japan).

A four plasmid system will be used for production 2nd generation SiV:
Plasmid 1: CMV prom-F protein or (VSVG-protein)
Plasmid 2: CMV prom-HN protein
Plasmid 3: CMV prom-gag-pol-rev
Plasmid 4: Modified LTR-CMV prom-R-U5-Y-cPPT-prom-transfene-WPRE-Du3-R-U5 (as described by Dull T 1998)

F/HN or VSV-G pseudotyped SiV vectors will be used to transduce transformed cells such as C127 and 293T cells. The recombinant virus will carry reporter genes such as B-galactosidase or functional genes, such as the cystic fibrosis transmembrane conductance regulator (CFTR). Some viral constructs may contain the cPPT (central poly purine tract) and the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) to enhance transgene expression.

Using to above construct replication competent SiV vectors have never been generated (DNAVEC Inc, personal communication).


Host/vector system

The SiV vector is derived from a Simian immunodeficiency virus, which is not pathogenic in its natural host. The vector is not replicating and has been further inactivated by removing the 3’ LTR (SIN) vector. There are several reports in the literature stating that people working with primates have been exposed to SiV, but have not developed an immunodeficiency.

Origin & function

The pseudotyped SiV vector is produced by DNAVEC, Inc (Japan).

Evaluation of foreseeable effects

SiV, as all Lentiviruses integrate randomly into the genome of transduced cells, which may be associated with pathogenicity, if integration occurs into an oncogene or tumor suppressor gene. However, this is considered a very unlikely event as only about 3% of the human genome encode genes and only a very small proportion of these are putative oncogenes or tumor suppressors. Most recently a clinical trial using retrovirus-mediated gene transfer into hematopoetic cells of SCID children has reported two cases of Leukemia. However, a large number of humans has been treated with retroviruses and apart from these two cases, no adverse side-effects have been reported and may in the French Scid children be linked to using the gamma c-chain cDNA.

Recombination of SiV (SIN) vectors or other lentiviral vectors hgas, to the best of our knowledge, never occurred in preclinical studies or clinical studies. The risk is effectively zero as the vectors are replication defective and many viral genes have been deleted.
If transduction into the gonads of animals outside the laboratory would occur, the virus would be maintained outside the laboratory. However, careful safety precautions will be in place to prevent this. In addition the virus has a short half-life and is non-replicating.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Chemical disinfection (Hypachlor for >10 min with final chlorine concentration of 2500 ppm, killing capacity: effectively 100%) will be used for all solid GM waste. Solid waste will include plastic labware including pipette tips and tissue culture plates are likely to contain live virus particles. In addition to chemical disinfection all GM waste will also be autoclaved and subsequently be disposed of using clinical waste procedures.

Liquid tissue culture medium will be treated with 1% Virkon, according to manufacturers recommendations, leading to effectively 100% killing.

Is an emergency plan required according to regulation 20?  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

The Imperial College genetic modification committee’s comments included:
1. Clarify containers used for transportation of GMM (Section 10.2)
2. State that lab coats and gloves are worn at all times for any laboratory work (Section 7.1)
3. Discuss health surveillance with occupational health.

All comments were considered and included in the risk assessment.

Project Containment
Purposes of the contained use

Chronic obstructive pulmonary disease (COPO) is an inflammatory disease that results in poorly reversible airflow limitation, with symptoms including chronic cough, shortness of breath and sputum production. Macrophages are inflammatory cells that are essential in the maintenance of lung homeostasis. These cells are highly phagocytic, and are crucial in the clearance of bacterial and cellular debris. Macrophage phagocytosis is impaired in COPO, resulting in bacterial colonisation and increased risk of infection, and there has been increasing incidences of patients with COPO being infected with Aspergillus. Aspergillus Fumigatus may affect macropahge phenotype and function, as this has also been affiliated with worsening symptoms at exacerbation. We plan to investigate uptake of Aspergillus Fumigatus by macrophages derived from human blood and lung samples.

Recipient or parental organism

Aspergillus Fumigatus strain ATCC46645 (ACOP classification 2).

Host/vector system

Aspergillus Fumigatus has been modified to ubiquitously express GFP so as to be suitable for imaging purposes, FACS and phagocytosis experiments.  
1. The eGFP gene (Clontech, Heidelberg, Germany) and the gpdA promoter from A fumigatus were amplified by high fidelity PCR (Roche, Mannheim, Germany) using oligonucleotides GFP-For, GFP-Rev, gpdA Prom-For and gpdAProm-
Rev. The amplification products were cloned into the pOrive cloning vector (Qiagen, Hilden, Germany) and sequenced using external plasmid-derived primers. The fragments were subsequently cloned into the EcoRV site of pBluescript KS+ (Stratagene, Amsterdam, the Netherlands). The hygromycin resistance cassette containing the hygromycin B phosphotransferase gene of E. coli under the control of the gpdA promoter was isolated from plasmid pUCGH pyrG and inserted into the Kpnl site of pBluescript KS+. Both fragments were cloned into pBluescript KS+ in opposite orientation resulting in plasmid pMAF1, that was transformed into A fumigatus protoplasts using a method described previously (Langfelder et al., 1998).

**Origins & function**

GFP label in Aspergillus is fluorescent on the FITC channel.

**Evaluation of foreseeable effects**

Aspergillus fumigatus has been modified to ubiquitously express GFP so as to be suitable for imaging purposes, FACS and phagocytosis experiments. The addition of the GFP gene allows the microbe to become fluorescent. There are no further functional changes to the microbe, and therefore it poses no greater threat to human health compared to the unmodified strain.

To create this GFP labelled strain, a hygromycin resistance cassette was inserted into the plasmid. The strain would therefore be resistant to this antibiotic. This should be noted in case of accidental infection to a human user.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

A Class II Microbiological safety cabinet (MSC) will be used when handling/processing samples. After use, the safety cabinet will be decontaminated using 2% Distel or 2% Virkon. All buckets used in centrifuges will be fitted with lids when spinning samples, and the centrifuge and buckets will be decontaminated post use using 2% Distel.

In case of spillage within the centrifuge, the centrifuge lid will remain closed for 1 hour to allow any aerosol to settle. After 1 hour, the spilled material will be wiped up, area will be sprayed with 2% Distel or 2% Virkon. Distel bottle will be kept near the centrifuge.

Solid Waste: Solid waste will be collected in a biohazard bag inside the Biosafety Cabinet. Pipette tips will be collected in a small Biobin, and the bin closed and deposited into the biohazard bag (in the Biosafety Cabinet) at the end of the work session. Solid waste exposed to A Fumigatus are first disinfected with 2% virkon, prior to disposal in Biobins or biohazard bags. At the end of the work session, the biohazard bag will be closed, sprayed with 70% EtOH, and deposited into a biohazardous waste container. These will be autoclaved, and taken off-site to be incinerated.

Liquid Waste is normally aspirated into a vacuum flask containing 1/10 volume Virkon. The vacuum flask must have a final concentration of at least 10% virkon, for a minimum time of 30 minutes prior to drain disposal. Liquid waste that is not aspirated must be treated with virkon, to a final concentration of 2%, in the hood, allowing a minimum time of 30 minutes to inactivate fungus.
Many thanks for the attached and I note that all changes noted in e-mails below and in the form C have been made to the 8101. However I do have some additional comments for both the CU2 and 8i01. Hope this makes sense. Please send the completed forms back to Biosafety@imperial.ac.uk and we will fill in our bits. Many thanks

CU2 - The must read the same as the Bi01 and vice versa. I have the following comments
Section 5 - This should be the Title of the activity from the Bi01. Lentiviral transfection for overexpression studies to correct defective phagocytosis in human MOM in COP. O. If this title no longer fits the activity please change it in the Bi01 and the Cu2
Section 6 - The highlighted text does not appear to be in the Bi01. Please put this explanation in the Bi01. 1.1 is probably the best place for it.
*and there has been increasing incidences of patients with COP being infected with Aspergillus. Aspergillus Fumigatus may affect macrophage phenotype and function, as this has also been affiliated with worsening symptoms at exacerbation. We plan to investigate uptake of Aspergillus Fumigatus by macrophages derived from human blood and lung samples*
Section 7 -
Recipient - This is different to the text for the recipient strain in 2.15 of the Bi01. Please make it the same. Le. Aspergillus Fumigatus strain ATCC46645 (ACOP classification 2).
Vector - This is different to the text for the recipient strain in 2.16 of the Bi01. Please make it the same. i.e. Aspergillus fumigatus has been modified to ubiquitously express GFP so as to be suitable for imaging purposes, FACS and phagocytosis experiments.
1. The eGFP gene (Clontech, Heidelberg, Germany) and the gpdA promoter from A. fumigatus were amplified by high fidelity PCR (Roche, Mannheim, Germany) using oligonucleotides GFP-For, GFP-Rev, gpdA-Prom-For and gpdAProm-Rev. The amplification products were cloned into the pDrive cloning vector (Qiagen, Hilden, Germany) and sequenced using external plasmid-derived primers. The fragments were subsequence quently cloned into the EcoRV site of pBluescript KS+ (Stratagene, Amsterdam, the Netherlands). The hygromycin resistance cassette containing the hygromycin B phosphotransferase gene of E. coli under the control of the gpdA promoter was isolated from plasmid pUCGH-pyrG and inserted into the KpnI site of pBluescript KS+. Both fragments were cloned into pBluescript KS+ in opposite orientation resulting in plasmid pMAF1, that was transformed into A. fumigatus protoplasts using a method described previously (Langfelder et al., 1998).
Origins and intended functions of the genetic material - This is different to the text for the recipient strain in 2.17 of the Bi01. Please make it the same. Le. GFP label in Aspergillus is fluorescent on the FITC channel.
7. Please add the following text which is in the CU2 to the Bi01 sections 2.18 and 4.20
*Aspergillus fumigatus has been modified to ubiquitously express GFP so as to be suitable for imaging purposes,
FACS and phagocytosis experiments. The addition of the GFP gene allows the microbe to become fluorescent. There are no further functional changes to the microbe, and therefore it poses no greater threat to human health compared to the unmodified strain.

To create this GFP labelled strain, a hygromycin resistance cassette was inserted into the plasmid. The strain would therefore be resistant to this antibiotic. This should be noted in case of accidental infection to a human user.

Section 9.1 This answer is different to the 8i01 section 4.4 please make sure that the volumes are the same.

Section 12 is different to the waste section 7 in the 8i01. Please take the text from the CU2 which is nicely detailed and add it to the 8i01. Please do not refer to an MSC as a "hood" you can use the phrase Microbiological Safety Cabinet or MSC.

Section 15 I will add this once you have modified the above.

Section 16 this is usually the PI.

Section 18 we will complete this.

GM Safety Committee 4th Dec 2015

Dear Professor

Following the discussion of your proposal entitled "Lentiviral transfection for overexpression studies to correct defective phagocytosis in human MOM in COPO" (GMIC-4695), the committee request that the following amendments are made to the proposal prior to approval:

Reviewer 1

Section 2.15; This section asks for recipient species and strains with their ACDP classification, add Aspergillus fumigatus here.

Section 2.16 List the vector that has been used for aspergillus here, what resistance cassette is used? What type of GFP?

The added GM Aspergillus fumigatus (GFP labelled) is a class 2 GMO because the host is HG2. This project was originally a Class 1. This will mean that the project will now require notification to the HSE.

Section 2.17 GFP has a functional property; it's fluorescent ... otherwise it would be pointless expressing it

Section 4.16 GFP is not completely non-functional, you use it because it expresses fluorescent protein

Section 4.17, does the GFP vector have a selective resistance cassette? This might have an impact on treatment if somebody gets exposed to Aspergillus

Section 5.5, Who grows the Aspergillus? In which Jab? under which risk assessment? Is it just being donated or are people from RB campus going to SK campus 10 grow this up? Or just to collect?

Section 5.13 Tick the box for using FACS analysis or Sorting and mention SOP

Section 14+15 Aspergillus work is not mentioned here. GM Aspergillus fumigatus (GFP labelled) is Class 2

Reviewer 2

All looks ok in principle. I believe the room that the work will be taking place in is B148 not B146 at Guy Scadding. Also there is a discrepancy between centrifuge spillage procedures, in the form it correctly states that it will be left for 1 hour to allow aerosols to settle, but in all the SOPs it says 15mins - this is needs to be amended.

Could you please ensure that the risk assessment is revised (add GMIC number) and the amendments above addressed. I will then forward the form onto Occupational Health to complete. The completed HSE CU2 form (attached to this email), and copy of the Purchase Order (for BACs Payment) made payable to the HSE should be sent to me, I will then send it all to the HSE. Please ensure the information given in HSE Form relates to that provided in your risk assessment.

For further information, please see http://www3.imperial.ac.uk/safety/subjectsbiosafety/gmprocedures

Please note that th is work must be notified to the HSE and an acknowledgement receipt received prior to this work commencing.
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Name

**IMPERIAL COLLEGE SCHOOL OF MEDICINE AT QUEEN CHARLOTTES & CHELSEA HOSPITAL**

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Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

<table>
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<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Level 2 (GMMs)</td>
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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
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<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment.
### GM Centre Number: 311

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**Name**

| BRITISH BIOTECH PHARMACEUTICALS LTD |

**Name 2**

**Department**

**Campus Estate or Research Centre**

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**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)

Tick if confidential

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<th>Parasitology</th>
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</tr>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref** 311/01.1

<table>
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Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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311/01.2

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

<table>
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<th>Laboratory Activities</th>
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### Project Ref 311/01.3

- **Date Ackn'd**: 15/02/2001
- **CU2 Project Title**: EXPRESSION OF FUNGAL ORFS
- **Class**: Not applicable
- **Consent Granted**: Not applicable
- **Project notified under transitional arrangements**: Y

Tick if notifying a connected programme of work N

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02/03/2022 Page 6075 of 15326
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 311/01.4

Date Ackn'd 15/02/2001

Date Project Ceased 12/08/2004

CU2 Project Title EXPRESSION OF FUNGAL ORFS IN YEAST

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

Tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
GM Centre Number: 312

Data Premises Notified (Originally) 06/11/1991

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed 26/04/2005

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

UNIVERSITY OF WALES COLLEGE OF MEDICINE

Department

PATHOLOGY

Campus Estate or Research Centre

Building

Road Name

District

HEATH PARK

Town

CARDIFF

County

CEREDIGION

Postcode

CF14 4XN

Country

WALES

Tel Number 029 2074 2903

Fax Number 029 2074 4869

E-mail

HSE Division WALES AND SOUTH WEST

Comments

GM312 CLOSED DOWN AND MERGED WITH GM130 ON 26/04/2005.

Date at Which Additional Info Submitted

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

Virology Transgenic Animals Transgenic Fish Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 312/02.1**

**Date Ackn'd** 28/01/2002

**CU2 Project Title** MODULATION OF SIGNALLING IN LYMPHOCYTES BY VIRAL VECTORS

**Date Project Ceased** 26/04/2005

**Class** Class 2

**CultureVolClass2** < 1 litre

**CultureVolumeClass3-4**

**Non-GMM Consent Granted** not applicable

**Project notified under transitional arrangements** N

**Withdrawn** N

**Historical Significant Changes**

Project transferred to GM130 on 26/04/2005

**Project Additional Information**
Purposes of the contained use

I wish to study the molecules involved in lymphocyte growth. This will take place in B and T-lymphocyte cell lines as well as primary human cells. The molecules chosen include ras, P13K, Jak, and STATs, all of which are activated in response to T-cell receptor or cytokines, the molecules that result in T-cell growth. Human primary lymphocytes are not easily transfected and infection remains the best way to modulate their genotype.

Recipient or parental organism

The GMO that will be generated are retroviruses based on the murine (MuLV) system containing molecules involved in lymphocyte growth and signalling.

Host/vector system

A retroviral vector system has been chosen. DNA corresponding to molecules involved in lymphocyte signalling will be introduced in the pinco retroviral plasmid. This plasmid will be introduced into the Phoenix AM cell line that allows the production of amphotropic retroviruses. Phoenix is a second-generation retrovirus producer line for the generation of helper free ecotropic and amphotropic retroviruses based on the murine (MuLV) system. The lines are based on the 293T cell line. The Phoenix cell line was chosen because it is a well established cell line that has been extensively tested for helper virus production and established as been helper-virus free. The cell line contains two separate plasmids for the generation of the env protein and the gag-pol protein. Both the gag-pol and envelope constructs with non-moloney promoters were used to minimise recombination potential. Different retrovirus cannot be produced from these cells.

Origin & function

The genetic material for the retroviruses are derived from the murine (MuLV) system. The genes that will be introduced into these vectors are mammalian in origin. Each of these have been chosen because of their role in the manipulation of the immune system. While all of these molecules play an important role in growth and/or survival, none have been shown to induce lymphocyte malignancy alone. Wild type, active and inhibitory forms of the molecules will be studied.

Evaluation of foreseeable effects

The retroviruses by themselves do not constitute a risk. DNA corresponding to many different proteins have been described on the attached sheet. Details from each of these gene products have been given. Whenn considering the possible hazard to human health it is appropriate to consider the gene product that has the most potential to cause harm. The virus with the most potential to cause harm is the V12-ras retrovirus. However, this has been used safely in other Departments within the University of Wales College of Medicine. This retrovirus could result in the expression of V12 in human cells. However this would only cause harm if administered in large doess or were to become infectious. However, the amounts of virus generated will be low and there are many features of the retroviral system that proscribe the ability of the retrovirus to become infectious.

Three other important factors:
(1) While V12 is an oncogene, it is unable to transform primary human cells alone. The short half life of the virus minimises the duration of any exposures. None of the other inserts are oncogenic.
(2) The pathogenicity of the retrovirus is unaffected by any of the inserts and there is unlikely to be any transfer of harmful sequences to related viruses.
(3) The viruses generated cannot cause a productive infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste contaminated with GMMs will be treated with freshly prepared 2500 ppm sodium dichloroisocyanurate. This completely destroys the retrovirus.
The Genetic Modification Safety sub-committee found the risk assessment to be suitable and sufficient and agreed that the work was Class 2, requiring Containment Level 2 facilities.

### Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
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**Historical Significant Changes**

Project transferred to GM130 on 26/04/2005
The immune system is controlled by a complex balance between the innate and the adaptive immune responses. IL-10 is associated with shifting the balance of the immune response away from a TH1 and towards a TH2 response. Work in other centres has demonstrated that adenovirus (Ad) vectors encoding the Epstein-Barr virus (EBV) interleukin 10 (IL-10) homologue is effective in suppressing inflammation in animal gene therapy models of autoimmune disease (Apparailly et al 1998 J Immunol. 160, 5213). Recently, a functional human cytomegalovirus IL-10 homologue has also been identified (Kotenko et al 2000 PNAS 97, 1695). We wish to use Ad recombinants encoding human, rat, EBV and HCMV IL-10 in both in vitro and in vivo assays. An Ad vector is required for efficient expression of these molecules. We intend to test the relative efficiency of the various versions of IL-10 on dendritic cell (differentiation and maturation), CD8+ cytotoxic T cells (CTL) and NK cell function in vitro. Major histocompatibility class I molecules play a key role in functional interactions of all these three cell types and are regulated by IL-10. As part of this programme of work, we will use permission to express both classical (HLA-A,C) and non-classical (HLA-E and HLA-G) molecules in replication-deficient Ad vectors. HLA-A,C are highly polymorphic and a number of variant genes may be expressed. In contrast HLA-E and HLA-G are highly conserved but subtle variants do exist and may be expressed.

The capacity of NKX2.5 to modulate myeloid cell function in vitro will be examined in a pilot study. LIR-1 is an inhibitory receptor found on cells of the myeloid lineage, T cells and NK cells that interacts with classical MHC-1 molecules and HCMV MHC-1 homologue gpUL18. We intend examining functional interaction between HCMV gpUL18 and LIR01. This notification covers the limited use of Ad recombinants designed to modulate immunological responses by transgene expression. In particular, we intend testing replication-deficient adenovirus recombinants encoding the EBV and HCMV IL-10 homologues in an established in vivo experimental model of rheumatoid arthritis.

Preliminary prokaryotic cloning steps are necessary to generate the recombinant adenoviruses. Genetically disabled E. coli K12 strains specifically developed for application in recombinant DNA cloning experiments will be used. These strains have limited potential to colonize the gut or survive in the environment.

Adenovirus is an ACDP category 2 pathogen. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Deletion of the E1 gene region will render the adenovirus vectors replication-deficient. Replication-deficient Ad vectors by their nature have limited potential for lateral spread although this could be facilitated by co-infection with a wt adenovirus or gene rescue from the helper cell line.

Commercial Ad5 vector systems will be used as the basis for vector development.

For the construction of replication-deficient adenovirus recombinants:

- The Admax vector system from Microbix, Canada (pDC511, pDC512, pDC515 and pDC516, PBHGfrtDE1,3FLP,pXCXL-based transfer vectors, pJM17) will be used.
- From Qiagen (Hareford, Middx) the Ad5 based AdEasytm vector system (pAdEasy-1, pShuttle, pShuttle-CMV, pShuttleCMV-LacZ) will be used.

Replication-deficient adenoviruses will be propagated in a helper cell line expressing an E1 helper function (e.g. 293 or 911 cells) and then used to infect target cells in vitro or in vivo.

Human, rat, EBV and HCMV IL-10 genes will be used to evaluate their relative capacity to modulate a range of immune responses in vitro and in vivo assays. NKX2.5 is a transcription factor associated with maturation of cardiomyocytes, its effect on myeloid cell maturation will be evaluated.

- HLA-A, HLA-B, HLA-C, HLA-E, HLA-5 and the human cytomegalovirus MHC-1 homologue UL18 as test ligands of LIR-1 and to examine function of IL-10.
LIR-1 is an inhibitory receptor originally identified by high-affinity binding to synthetic gpUL18 and subsequently shown to bind a broad range of MHC-1 molecules.

All DNA are cDNA clones obtained commercially or gifts from collaborators. DNA sequences are known and will be confirmed in adenovirus transfer vectors prior to generation of recombinant virus.

Evaluation of foreseeable effects

It is recognised that there is a potential hazard associated with using Ad recombinants encoding transgenes capable of modulating the immune response IL-10 is a 17-18kDa homodimer that can be secreted by T cells, macrophages and EBV- transformed B cell lines. IL10 functions by binding the IL-10 receptor. IL-10 was first recognized for its ability to inhibit activation and effector function of T cells, monocytes, and macrophages, and is a multifunctional cytokine with diverse effects on most haemopoietic cell types. The principal routine function of IL-10 appears to be to limit and ultimately terminate inflammatory responses. In addition to these activities, IL-10 regulates growth and/or differentiation of B cells, NK cells, cytotoxic and helper T cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells. hIL-10 is active on both mouse and human cells, whereas mIL-10 is effective only on mouse cells. The mature hIL-10 and EBV viral IL-10 (vIL-10) amino acid sequences are 84% identical, and most differences occur in the N-terminal 20 amino acids. We are primarily interested in utilizing and evaluating the EBV IL-10 vireme as a positive control (standard) in a rat model of rheumatoid arthritis. This is a widely used system. to date, there are at least 20 publications by other laboratories describing results of experiments using Ad EBV IL-10 recombinants including demonstration that they are effective in inhibiting the induction of arthritis in experimental animal models. Human and rat IL-10 are expected to have similar or lower efficacy. The cmvIL-10 has only low homology to hIL-10. It is only 27% identical to hIL-10, yet nonetheless binds to and signals via the IL-10R complex. The CMV IL-10 homologue is expected to be less potent than either human or EBV IL-10.

This construct is thus expected to have a biological function following in vivo delivery. There is evidence in mice and humans that elevated IL-10 production usually imposes some limits on the effectiveness of antipathogen immun responses, especially innate immunity and adaptive Th1 responses. This cost if often outweighed by the ability of IL-10 to protect the host from collateral damage by antimicrobial cytokines and effector molecules. IL-10 has been considered an attractive candidate for therapeutic use based on its potent in vitro immunomodulating activities and proven effects in animal models of acute and chronic inflammation, autoimmunity, cancer and infectious disease. Phase I and II clinical trials investigating safety, tolerance, pharmacokinetics, pharmacodynamics, immunological and hematological effects of single or multiple doses of IL-10 administered by intravenous (iv) or subcutaneous (sc) route. IL-10 has been tested in specific patient populations including those with Crohn's disease, RA, psoriasis, and patients suffering from chronic hepatitis C infections. Administration of IL-10 (7 days iv) reduced the Crohn's disease activity index score in patients with steroid-refractory Crohn's disease and showed some clinical benefit in a larger 28-day sc safety and efficacy study in patients with chronic active Crohn's disease. Similarly, a trend towards efficacy and a good safety profile was observed when IL-10 was administered for 28 days to RA patients. Single intravenous (iv) or subcutaneous (sc) doses of IL-10 resulted in transient dose-dependent changes in white blood cell populations, including increases in total white blood cells and neutrophils.

There are a number of theoretical hazards associated with this experiment. Firstly, as the recombinant virus is intended for in vivo use, there is the potential that the virus could spread from animal to animal and hence get into the natural population. However any in vivo injections will be local and given the fact the virus cannot penetrate more than a few layers of cells, the systemic spread of the virus should be negligible. Even in the highly unlikely event of the spread of the virus systemically, the recombinant virus is replication deficient, is not maintained in dividing cells and as such is incapable of horizontal spread throughout a population. Additionally wild-type Ad type 5 is able to undergo productive virus replication in very few non-human cells.

A second potential hazard is the exposure of humans to the recombinant virus. All manipulations of the virus will be performed in a class II biological safety cabinet in the laboratory. In vivo injections of Ad recombinants encoding recombinant genes into animal models will be carried out in an animal isolator, a negative pressure facility (i.e in a class I cabinet) or a class II cabinet. A potential human infection will be a needle stick injury. Use of sharps will be kept to a minimum but are required for animal inoculations. In this event, gene expression from the Ad recombinant should be localised and the potential inhibition of complement localised and transient. In the unlikely scenario that the recombinant inhibitor becomes systemic, for the reasons stated above (inability to replicate without E1 complementation and the loss of the virus in dividing cells) the effect of the complement inhibitor should be transient. Also the effect of the complement inhibitor in humans should be minimal as the dose will have been designed for a rate and should therefore be at too low a level for a significant effect in humans. Horizontal spread of the recombinant virus through the human population is not likely for the same reasons as it is not likely in the rat population.
Classical and non-classical MHC molecules are widely expressed on human cells. The over-expression on non-classical HLA A-C molecules could promote antigen specific or allospecific immune responses and expression of any of the classical or non-classical HLA molecules could suppress NK recognition but should this occur the risk is minimal. LIR-1 (also known as ILT-2) is an inhibitory receptor thus its over-expression in lymphocytes or myeloid cells could suppress the function of that the target cell. The Ad5 vector infects lymphoid and myeloid cells relatively inefficiently. It is extremely unlikely that with the natural biological containment that the gene could be delivered with sufficient efficiency to exert a biological effect in man or animals. NKX2.5 transcription factor (a cell specific expression marker in cardiomyocytes) that will be subcloned into a replication-deficient Ad vector. There is no known transforming activity or toxicity associated with this gene. Use of this construct has been independently approved at ACGM 2 level elsewhere.

These recombinant viruses will be contained using ACGM level 2 procedures as stipulated in the risk assessment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Virus in solution (tissue culture media or buffers such as PBS) is inactivate by adding to less than equal volume 2500 ppm Sodium Dichloroisocyanurate (Actichlor), after 4h solution can be discarded to mains drainage. Pipettes immersed in 2500 ppm Sodium Dichloroisocyanurate for 4h before transferred to plastic container for autoclaving, all plasticware is autoclaved after exposure to virus. Actichlor is effective against all viruses and the high concentration overcomes high organic content of media.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

A minor modification was made to the title for accuracy.

Notification of this project as an extension of a connected programme of work would be necessary.

Containment level 2, class 2 was appropriate

Approved

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
## Project Additional Information

### Purposes of the contained use

The proposed project involves the construction of adenoviral recombinants for the expression of complementary inhibitory molecules in cell lines in vitro and eventually in animal models of disease.

### Recipient or parental organism

Preliminary prokaryotic cloning steps are necessary to generate the recombinant adenoviruses. Genetically disabled E-coli K12 strains JM109 and XL1-blue specifically developed for application in recombinant DNA cloning experiments will be used. These strains have limited potential to colonise the gut or survive in the environment.

Adenovirus is an ACDP category 2 pathogen. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Deletion of the E1 gene region will render the adenovirus vectors replication-deficient. Replication-deficient Ad vectors by their nature have limited potential for lateral spread although this could be facilitated by co-infection with a wt adenovirus or recombination with the E1 gene in the helper cell line.

### Host/vector system

E-coli strains JM109 and XL1-blue will be used. Prokaryotic vectors include pUC-based vectors including the Ad transfer vector pAL119. A commercial Ad5 vector...
systems provided by Prof Frank Graham will be used as the basis for the vector (Microbix, Canada). pJM17 contains the complete Ad5dl309 genome vector system cloned into the pBR322-based vector pBRX. Recombinant viruses will generated following recombination in 193 cells. Prokaryotic cloning experiments are required to manipulate the complement inhibitory genes prior to recombination into the Ad genome. Expression will not be sought in E. coli and no specific hazard has been identified for prokaryotic manipulation. Replication-deficient adenoviruses will be propagated in a helper cell line expressing an E1 helper function (eg 293 or 911 cells) and then used to infect target cells in vitro or in vivo.

**Origin & function**

The molecules of interest include human CD59, DAF (Decay Accelerating Factor), MCP (Membrane Cofactor Protein) and CR1, the rat analogues of DAF, CD59 and MCP and the rat complement inhibitor Crry. It is intended to clone these molecules in two forms: a) the natural form which includes the sequence necessary to anchor the protein to the cell surface and b) with a deletion of the sequence responsible for anchoring the protein to the membrane so that it is secreted from cells in a soluble form. Secreted forms of complement inhibitors do occur naturally. We also intend to perform mutagenesis on the predicted active sites of the complement inhibitors to examine function. Such constructs can be expected to have reduced hazard potential under most circumstances, although a dominant mutation could possibly reduce the efficiency with which complement action is inhibited.

DAF and CR1 have decay accelerating activity of the bimolecular enzyme complexes which constitute the convertase in both the classical and alternative complement activation pathways. They dissociate the C2a or the Bb subunits from the formed convertases and may also prevent the subsequent association of their procatelititic precursors C2 and B with membrane bound C4b or C3b molecules. Hence by accelerating the decay of preformed convertases and inhibiting the formation of new ones, DAF and CR1 can prevent amplification of the C3 activation on the host cell surface. Cr1 also acts as a cofactor for the cleavage of iC3b to C3c and C3d.g (iC3b can bind and activate neutrophils via CR3). CR1 also acts in vivo to mediate phagocytic binding of particles opsonized with C3b and participates in the transport of immune complexes on erythrocytes.

Like CR1, MCP acts as a cofactor for the factor-I cleavages of C3b and C4b and hence prevents the formation of active C3/C5 convertases. Although this inhibits both the classical and alternative pathways of the complement system, MCP is thought to act preferentially on the convertase of the alternative pathway.

CD59 works at the end of the complement pathway by inhibiting the assembly of the MAC (Membrane Attack Complex). It does so by incorporating into the partially formed MAC by binding to C5b8 and interfering with the subsequent binding and polymerization of C9.

The specific mechanism for Crry function is still unclear although it is thought to be a functional homologue for human MCP and/or DAF.

The main function of the complement system is its role in both the adaptive and humoral immune system in killing foreign microorganisms. Other biologically important functions mediated by the complement system include:

1) Low molecular weight anaphylotoxins C3a, C4a, and C5a which promote smooth muscle contraction and increase vascular permeability.

2) Large C4b and C4b fragments involved in the binding to the complement activator and therefore interact with specific receptors to allow efficient clearance of the activating cell of particle including virus.

3) Degradation of the fragments of C3b to iC3b, C4d.g and C3d which are also important in the clearance of the immune aggregates and the triggering of the receptor mediated activities including the regulation of the immune response.

Hence expression of the human (only) complement inhibitory proteins could result in a reduction/inhibition of systemic complement in man and potentially inhibit to varying degrees one, some or all of the above activities leading to an increased susceptibility to infection and the accumulation of immune complex precipitates.

The recombinant virus will be based on an Ad5 dl309 background with an additional deletion in the E1 gene at which site the insert will be inserted. The deletion of the E1
gene make the Ad recombinant replication-deficient and thus replication of the virus requires complementation by a helper function. 293 or 911 cells both carry an integrated copy of the Ad5 E1 gene and hence will be used for the construction and propagation of new recombinant virus. The complement inhibitory genes will be inserted at the site of the E1 deletion.

**Evaluation of foreseeable effects**

Activation of complement is known to be an important factor in numerous diseases including the development and propagation of inflammation of the rheumatoid joint. Efficient inhibition of complement could therefore ameliorate disease. This has been shown previously in the case of rheumatoid arthritis, by systemic and local inhibition of complement using complement inhibitory proteins. This project is an extension of this work but is intended to avoid repeated protein injections by assessing the viability of in situ expression of complement inhibitors using adenoviral vectors.

There are a number of theoretical hazards associated with this experiment. Firstly, as the recombinant virus is intended for in vivo use, there is the potential that the virus could spread from animal to animal and hence get into the natural population. Complement inhibitors exhibit host specificity and can be considered as a significant hazard only in the homologous species. However any in vivo injections will be local and given the fact the virus cannot penetrate more than a few layers of cells, the systemic spread of the virus should be negligible. Even in the highly unlikely event of the spread of the virus systemically, the recombinant virus is replication deficient, is not maintained in dividing cells and as such is incapable of horizontal spread throughout a population. Additionally wildtype Ad type 5 is able to undergo productive virus replication in very few non-human cells.

A second potential hazard is the exposure of humans to the recombinant virus. All manipulations of the virus will be performed in a class 11 biological safety cabinet in the laboratory. In vivo injections of Ad recombinants containing human complement inhibitory proteins into animal models will be carried out in an animal isolator, a negative pressure facility (ie in a class 1 cabinet) or a class 11 cabinet. A potential human infection will be a needle stick injury. Use of sharps will be kept to a minimum but are required for animal inoculations. In this event, gene expression from the Ad recombinant should be localised and the potential inhibition of complement localised and transient. In the unlikely scenario that the recombinant inhibitor becomes systemic, for the reasons stated above (inability to replicate without E1 complementation and the loss of the virus in dividing cells) the effect of the complement inhibitor should be transient. Also the effect of the complement inhibitor in humans should be minimal as the dose will have been designed for a rat and should therefore be at too low a level for a significant effect in humans. Moreover there are a total of 10 known complement regulatory molecules and the transient increase of one or two of these molecules should not significantly imbalance the immune system. Horizontal spread of the recombinant virus through the human population is not likely for the same reasons as it is not likely in the rat population.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solution (tissue culture media or buffers such as PBS) is inactivate by adding to less than equal volume 2500 ppm Sodium Dichloroisocyanurate (Actichlor), after 4h solution can be discarded to mains drainage. Pipettes immersed in 2500 ppm Sodium Dichloroisocyanurate for 4h before transferred to plastic container for autoclaving, all plasticware is autoclaved after exposure to virus. Actichlor is effective against all viruses and the high concentration overcomes high organic content of media.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Please enter comments on the GM safety committee on the risk assessment

Assessment suitable and sufficient. Concern expressed re use of sharps. Questioned - no alternative for inoculating animals.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<th>Human Clinical Applications</th>
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Project Ref 312/02.4

Date Ackn'd
26/1/2002

CU2 Project Title
MODULATION OF LYMPHOCYTE FUNCTION WITH ADENOVIRAL VECTORS

Class
Class 2

Consent Granted
not applicable

Project notified under transitional arrangements N

Historical Date of Additional Info
Project transferred to GM130 on 26/04/2005

Project Additional Information

Purposes of the contained use
I wish to study the molecules involved in lymphocyte growth. This will take place in B and T-lymphocyte cell lines as well as primary human cells. The molecules chosen include P13K, Jak, and STATs and other molecules that modulate cell growth and gene expression. Human primary lymphocytes are not easily transfected and infection
remains the best way to modulate their genotype.

Recipient or parental organism

The GMO that will be generated are adenoviral system containing molecules involved in lymphocyte growth and signalling.

Host/vector system

The adenoviral host vector systems are commonly used and well-characterised. All adenovirus vectors will be replication deficient due to deletion in the E1 gene region; this is a recognised safety feature. Repair of the deletion in the E1 gene region may occur due to homologous recombination with the E1 gene in the helper cell line (an extremely unlikely event) or following co-infection of a cell with a wild type Ad. In the unlikely event of such a recombination, it is likely to result in concomitant deletion of the transgene.

Origin & function

The genetic material for the adenoviral system are derived from the human Ad5 system. The genes that will be introduced into these vectors are mammalian in origin. More information is shown on the attached sheet. Each of these have been chosen because of their role in the manipulation of the immune system and gene expression. While all of these molecules play an important role in growth and/or survival, none have been shown to induce lymphocyte malignancy alone. Wild type, active and inhibitory forms of the molecules will be studied.

Evaluation of foreseeable effects

The adenoviruses by themselves do not constitute a risk. When considering the possible hazard to human health it is appropriate to consider the gene product that has the most potential to cause harm. The virus with the most potential to cause would be the active PKB or dominant negative PML. Both of these proteins have the capacity to enhance cell survival and proliferation. However neither alone is sufficient to cause malignant transformation. Cells of the haematopoetic system are most at risk from these proteins. However, these cells are poorly infectible by adenovirus. Furthermore, the transient nature of expression following adenoviral infection makes the expression unlikely to affect human health.

As the Ad is replication deficient, ie cannot replicate in host cells unless E1 sequences are provided in trans (eg in HEK 293 cells for virus propagation). It is considered highly unlikely that this will happen spontaneously. Furthermore efficient delivery of large number of virus particles would be required to infect cells. Expression using the Ad vector is only transient and is quickly lost in dividing cells as it only integrates DNA at a low frequency. The Ad vector is unlikely to cause any permanent change in cells it infected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste contaminated with GMMs will be treated with freshly prepared 2500ppm sodium dichloroisocyanurate. This completely destroys the adenovirus.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Please enter comments on the GM safety committee on the risk assessment

The GMSC found the risk assessment to be suitable and sufficient.

**Project Containment**

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**Project Ref** 312/02.5

- **Date Ackn’d**: 03/12/2002
- **CU2 Project Title**: PRODUCTION OF REPLICATION DEFICIENT ADENOVIRUS RECOMINANTS ENCODING DOMINENT NEGATIVE AND WILD TYPE MUTANTS OF VARIOUS ISOFORMS OF THE SIGNAL TRANSDUCTION MOLECULE, PROTEIN KINASE C.

- **Class**: Class 2
- **CultureVolClass2**: < 1 litre
- **Non-GMM**: not applicable
- **Consent Granted**: Project notified under transitional arrangements

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

Project transferred to GM130 on 26/04/2005

**Project Additional Information**

**Purposes of the contained use**

The dominant negative and wild-type protein kinase C constructs will be transfected into cells in order to determine the role of isoforms of this enzyme in cell signalling leading to volume regulation, cell proliferation and drug resistance.
Recipient or parental organism

Recipient myocytic cells are only capable of proliferating to a couple of generations before they terminally differentiate, transfection of these cells is therefore unlikely to pose a hazard, however all work with the transfected cells will be conducted in a class 2 laminar flow cabinet and all media and labware treated with Virkon solution.

Breast cancer cell lines such as MCF-7 and our Tamoxifen resistant derivative thereof are able to grow in culture for significant periods of time. Transfection with the virus however would only be transient and the biological hazard therefore negligible. These cell lines do not contain other viral particule which might recombine with the vector beginning introduced, such as has been found in cell such as Hela cells. Again all work will be conducted in a class 2 laminar flow cabinet and all media and labware treated with Virkon before disposal.

Host/vector system

The host vector system is well characterised and available commercially. All adenovirus vectors used will be replication deficient due to deletion in the E1 gene region; This is a recognised safety feature. Repair of the deletion in the E1 gene region may occur due to homologous recombination with the E1 gene in the helper cell line (an extremely unlikely event) following co-infection of a cell with a wild-type adenovirus. Such a recombination event is likely to result in concomitant deletion of the transgene, is extremely unlikely but not impossible. Such recombinations also only usually occur within a subgenus not between subgenus. Adenovirus vectors are associated with extremely efficient in vivo delivery and can be spread by aerosol. This hazard will be contained by restricting manipulation with the virus to biological safety caninets and sealed centrifuge rotors. Adenovirus have no mechanism for cytolysis so in culture most viral particles remain cell-associated, although they can spread to neighbouring cells, leading to plaque formation.

Origin & function

PKC- 'Gutless' tetracycline - regulated binary adenoviral vectors will be supplied  (Harding et al., 1997, J. Neurochem 69 2620-3 and Harding et al., 1998, Nature Biotech 16: 553-5) (Hussain S, Assender JW, Bond M, Wong LF, Murphy D, Newby AC 2002; "Activation of protein kinase-C is essential for cytokine-induced metalloproteinase-1, -3 and 9 secretion from rabbit smooth muscle cells and inhibits proliferation." J. Biol Chem.: 277; 30: p27345-52). The original kinase defective (point mutation within the AT binding site 275 Lys> Trp) dominantnegative mutant was obtained from Dr Moscat, Universidad Autonoma de Madrid.

PKC-a and : Wild-type and kinase defective (point mutations within the AT binding site) dominant negative mutants of PKC-a and PKC- have been obtained from Dr Ohba, Instit of Mol. Oncology, Showa University, Tokyo (Nature 325: 161-66, 1987; Mol Cell Biol 18: 5199-5207, 1998; EMBO 13: 2331-2340, 1994). Rabbit PKC-a cDNA (-4~+2647), mouse PKC- cDNA (-13+2525/genbank:X60304), Dom neg PKC-1 cDNA (-42647 with 1 nucleotide mutation, AAGATCC>ACGATCC: 368 k>r), Dom neg PKC- cDNA (-13+2525 with 4 nucleotide mutation AAGTGTC>GCATGCC: 376 k>a). These will be used to determine the effect of over or non-expression of these signalling molecules on cell proliferation and Tamoxifen resistance in breast cancer and regulatory volume decrease in myocytes.

Evaluation of foreseeable effects

There is a potential hazard associated with using adenovirus recombinants to express transgenes that are overtly transforming or have a strong capacity to primate host cell proliferation. Protein kinase C is a signal transduction molecule associated with cell proliferation in a number of cellular systems. Although not thought to be the driving force behind cell replication, it does appear to act as a reostat, enhancing proliferation/apoptotic responses. The inserted gene product could therefore potentially be harmful, however due to the transient nature of the infection (see below), this risk is thought to be minimal. Dominant negative mutants of protein kinase C have previously been widely used in other laboratories and by the co-applicant. "Activation of protein kinase-C is essential for cytokine-induced metalloproteinase-1, -3 and 9 secretion from rabbit smooth muscle cells and inhibits proliferation". J Biol Chem.: 277 (30): p27345-52).

As the adenovirus is replication-deficient due to E1 and E3 deletions, they can not replicate in host cells unless E1 sequences are provided in trans (eg in HEK 293 cells for virus propogation). It is considered highly unlikely that this will happen spontaneously. Furthermore efficient delivery of large numbers of virus particles will be required to infect target cells, which is unlikely to ahppen, in vivo. Furthermore, since the adenovirus is replication deficient, no persistant long term effects are likely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solutions (such as tissue culture media or buffers such as PBS) will be inactivated by addition of more than an equal volume of sodium Dichloroisocyanate (Actichlor, 25000 ppm). After 4h, the solution will be discharged to main drains. Pipettes will be totally immersed in 2500 ppm sodium dichloroisocyanurate for 4h before transferring to plastic containers for autoclaving. All plasticware will be autoclaved after exposure to the virus. Acticlor is a broad spectrum and rapid acting biocide, effective against all viruses. The high concentrations used here will ensure that the high organic content of the media is overcome and 100% killing should be achieved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Reviewed risk assessment by GM Safety Committee.

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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 312/02.6

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<th>CultureVolumeClass3-4</th>
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<td>17/12/2002</td>
<td>MANIPULATION OF ACTIVITY OF THE INTERFERON SIGNALLING PATHWAY IN CULTURED HUMAN CELLS</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td></td>
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the aim of the project is to manipulate the levels of activity of the interferon signalling pathways in cultured human cells, both normal and immortalised, and potentially in combination with infection with other human retroviral vectors, already covered by HSE notifications in our laboratory, such as that encoding Ras. We have identified the interferon pathway as an important area of investigation due to our previous study of the effect of activated Ras in thyroid cells. Ras confers a limited proliferative potential to thyrocytes. However the cells eventually cease to divide. We have analysed gene expression in this arrested state using DNA microarrays and it appears that the interferon pathway is involved. This project will involve manipulating the interferon response pathways in a background where mutant Ras has already been introduced. Interferons play key roles in mediating antiviral and antigrowth responses and modulating the immune response, particularly in response to viral infection. Interferons are proteins naturally produced by many cells in the body. The interferons are pleiotropic cytokines that are induced in response to virus infection and act in a paracrine fashion to elicit an antiviral state in nearby cells. Binding of interferons to their cell-surface receptors induces a tyrosine kinase signalling cascade that leads to the activation of latent cytoplasmic signal-transducer-and-activator-of-transcription (STAT) factors. Activated STATs then translocate into the nucleus, where they are targeted to conserved promoter-enhancer sites to induce the transcription of interferon-responsive genes, that encode for protein with potent antiviral, growth-inhibitory, anti-tumour and immunomodulatory properties. There are 3 classes of interferon: interferon alpha, beta and gamma. Interferon alpha and beta signal through the Type 1 pathway, and interferon gamma uses the closely-related Type II pathway. We are interested in altering the levels of various members of the interferon signalling pathways, to attempt to modulate signalling of each pathway. We intend to do this by over-expression of various components of the pathways, the use of inhibitory genes, either naturally occurring (e.g. IRF2) or known dominant negative mutant alleles (e.g. of STAT 1). Cells will be studied in culture (e.g. phase contrast microscopy, the response to drug or other treatments), or killed and then subjected to cellular and molecular biology assays, such as immunocytochemistry, Western blotting, and extraction of RNA and DNA for subsequent analysis. We may also during the course of this work generate dominant negative and loss-of-function versions of the genes specified on accompanying risk assessments and below. These will be tested in the same manner as the wild type genes, as described above.
Cytokines: Interferons alpha, beta and gamma
Interferon alpha/beta receptor: Cell-surface receptor for interferon alpha and beta
Interferon gamma receptor: Cell-surface receptor for interferon gamma
Components of interferon signalling pathway: Interferon Regulatory Protein 1 (IRF1); Interferon Regulatory Protein 2 (IRF2); Interferon Regulatory Protein 3 (IRF3); Interferon Regulatory Protein 5 (IRF5); Interferon Regulatory Protein 6 (IRF6); Interferon Regulatory Protein 7 (IRF7); Interferon Regulatory Protein 8 (IRF8, ICSBP); Interferon Regulatory Protein 9 (IRF9, p48); Janus Kinase 1 (JAK1); Janus Kinase 2 (JAK2); TYK2; SHP-2 (tyrosine phosphatase); Cytosolic phospholipase A2 (CPLA2).
Components of interferon signalling pathway and transcription factors: Signal Transducer and Activator of Transcription 1 (STAT1); Signal Transducer and Activator of Transcription 2 (STAT2); Signal Transducer and Activator of Transcription 3 (STAT3); CREB-binding protein (CBP/p300)
Inhibitor of IRF-1: Nucleophosmin (NPM)

Evaluation of foreseeable effects
With regard to the hazards associated with the host/recipient the following have been considered including the pathogenicity of host strain, virulence infectivity and toxin production. In this case recipients and hosts are non pathogenic.
With regard to the hazards rising directly from the inserted gene these are non oncogenic and are downstream members of signalling pathways.
With regard to the hazards arising from the alternation of existing pathogenic tracts there is no increase in infectivity or pathogenicity and no disabling mutation within the recipient can be overcome due to the insertion of the foreign gene. Foreign genes do not encode pathogenicity determinants from a related organism.
Considering whether an inserted sequence, that does not give rise to harmful phenotype in the recipient micro-organism could give rise to harm as a result of natural gene transfer to another possibly related organism this is extremely unlikely to have a harmful effect.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All retroviral work will take place in a dedicated Class II facility. All liquid waste is sterilised at point of use by sodium hypochlorite. All solid waste is sterilised in adjacent autoclave facility. Use of glass and needles will be avoided to reduce the risk of sharps injury.
Autoclave thermocouple tested annually, also daily monitoring of efficacy of autoclave. Discussed determining efficacy of disinfectants with HSE inspector on recent visit. Relying on manufacturer's quality control and the very unstable nature of GMOs involved.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Project passed at meeting at containment level 2. Minor revision requested in order to clarify justification of project. Risk assessment modified accordingly and signed off.
Project Ref 312/03.1

Date Ackn'd 22/01/2003
CU2 Project Title CLONING OF HUMAN CYTOMEGALOVIRUS SEQUENCES

Date Project Ceased 26/04/2005

Class Class 2
CultureVolClass2 1-50 litres

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Project transferred to GM130 on 26/04/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
We intend to perform a systematic investigation of Human cytomegalovirus (HCMV) gene function by the cloning and expression of HCMV genes in cell lines, baculovirus vectors and replication-deficient adenovirus vectors. The vast majority of assays will be performed using cultured cells in vitro. Occasionally, animals will be inoculated with As recombinants primarily to generate antibody specific for the expressed transgene.

Recipient or parental organism
Preliminary prokaryotic cloning steps are necessary to generate the recombinant adenoviruses. Genetically disabled E. coli K12 strains specifically developed for application in recombinant DNA cloning experiments will be used. These strains have limited potential to colonise the gut or survive in the environment. Continuous cell lines expressing HCMV gene is also envisaged Prokaryotic cloning experiments, transient and continuous cell lines will be at ACGM category 1 level.
Adenovirus is an ACDP category 2 pathogen. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Deletion of the E1 gene region will render the adenovirus vectors replication-deficient. Vectors will also contain defined deletions in the non-essential E3 gene region. Replication-deficient Ad vectors by their nature have limited potential for lateral spread although this could be facilitated by co-infection with a wt adenovirus or gene rescue from the helper cell line. The use of more disable Ad vectors, specifically gutless vectors, is envisaged when feasible.

Baculovirus vectors are replication competent in insect cells but replication-deficient in mammalian cells. The vectors lack the non-essential polyhedrin gene.

**Host/vector system**

- E. coli strains DH5α, JM109, BJ583 and XL1-blue will be used. Details of vectors for ACGM1 work is given in the risk assessment.
- Commercial Ad5 vector systems will be used as the basis for vector development.
- For the construction of replication-deficient adenovirus recombinants:
  - The Admax vector system from Microbix, Canada (pDC511, pDC512, pDC515 and pDC516, pBHGFrtDE1,3FLP, pXCXL-based transfer vectors, pJM17) from Qbiogene (Hareford, Middx) the AdEasy vector system (pAdEasy-1, pShuttle, pShuttle-CMV, pShuttle-CMV-LacZ) and a gutted Ad vector as described by Schneider et al (J. Virol. 2002, 76 1600).
- Replication-deficient adenoviruses will be propagated in a helper cell line expressing an E1 helper function (eg 293 or 911 cells) and then used to infect target cells in vitro or in vivo.
- Baculovirus Vectors: Introgen Insect Select and Novagen BacVector System. SF9 cells for virus growth of baculovirus vectors. HCMV genes may be placed under the control of the baculovirus polyhedrin promoter to provide for high level expression in SF9 cells. Genes may also be inserted into the baculovirus vector under the control of a constitutive promoter namely the HCMV major IE promoter. Baculovirus can infect (inefficiently) but not replicate in mammalian cells. Baculovirus vectors thus can thus be used as a replication-deficient gene delivery system in mammalian cells as an alternative to adenovirus vectors.
- Tissue culture cells including primary fibroblasts, U373, THP-1, primary myeloid cells.
- Animals may be inoculated with Ad recombinants, primarily with the intention of generating polyclonal or monoclonal antibodies for the further characterisation of gene products.

**Origin & function**

- Genes will be cloned from any human cytomegalovirus isolate including the laboratory strains AD169, Towne and Toledo. Sequences will also be cloned from clinical isolates. HCMV is a ubiquitous herpesvirus with the largest genome of any characterised human virus (approx 235Kb). It is designated as an ACDP 2 agent. The virus is extremely well-adapted to its host with the vast majority of infections passing unnoticed. Between 50-100% of adults are seropositive depending socio-economic conditions. In healthy individuals primary infection is normally asymptomatic or associated with a mild febrile infection, followed by lifelong persistence (clinically silent) in its host. The virus is thought to establish ‘latency’ in a subset CD34+ myeloid progenitors with reactivation accompanying cellular differentiation. However, HCMV is an occasional cause of heterophile-negative infectious mononucleosis and a major viral vane of congenital malformation. In severely immunocompromised individuals (notably late stage AIDS patients and bone marrow recipients), HCMV disease is associated with morbidity and mortality. HCMV is not believed to cause cancer nor to encode oncogenes. ‘Transforming regions’ of the genome have been defined but have extremely low potency (Macarski and Courcelle, 2001; In Fields Viology, ed; DM Knipe & PM Howley pp2675-2705. Lippencott William & Wilkins, Philadelphia). Although HCMV is known to encodes a number of immunomodulatory genes, there is no compelling evidence that HCMV infections are immunosuppressive.

- The complete sequence of the laboratory-adapted HCMV strain AD169 was determined by Chee et al (1990) and was predicted to encode 208 non-overlapping ORFs of greater than 80 amino acids. However, strain AD169 is known to harbour a number of defects, most spectacularly a 15 kb deletion that has since been sequenced in strain Toledo. Mocarski and Courcelle (2001) have recently updated the analysis of the HCMV genetic content and this was used as a basis for the risk assessment (attached). The complete sequencing of the HCMV clinical isolate Merlin is now being undertaken in the University of Glasgow (A. Davison, personal communication). The HCMV isolate Merlin will be used in the majority of our studies to avoid issues of genetic instability associated with laboratory-adapted strains. However, we also envisage a need to clone DNA fragments and genes from other HCMV laboratory strains and primary isolates to analyse sequence variation. The definition of HCMV ORFs by Mocarski...
and Courcelle (2001) is known not to be definitive. More detailed analysis will identify additional functional ORF and delete spurious ones. HCMV also encodes untranslated transcripts (eg the B2.7 gene). We seek to cover the expression of all HCMV genes including untranslated transcripts. The herpesviruses encode a core set of genes associated with nucleic acid metabolism, DNA replication, capsid assembly and the production of infectious viruses that can be readily predicted from their sequence. A number of functions associated with transcriptional regulation and immune function have been identified, some by homology to cellular proteins. However, clearly a large proportion of the HCMV genome awaits detailed analysis.

There are few antibodies available to HCMV proteins. HCMV ORFs will therefore also be expressed as fusion proteins with GFP/RFP or YFP variants, a 6xHis tag, a streptavidin-binding peptide tag, a tetracysteine peptide tag or an Fc fusion protein to monitor expression, facilitate purification or identify binding ligands.

**Evaluation of foreseeable effects**

Most HCMV genes either have no known biological functions or encode functions that have no predictable detrimental effect when expressed in the proposed vectors. A risk analysis is performed on HCMV gene product with known functions that have the highest potential to cause harm. The virus does not encode any known oncogenes or toxins. HCMV gB is involved in virion attachment to a currently unknown cellular receptor. Recently, microarray experiments and other systems indicated that the binding of gB to its receptor modulates the transcription of a large number of cellular genes including the induction of an interferon-like response (Simmen et al, PNAS 2001; 98; 7140). Recombinant gB thus has the potential to induce transcriptional effects when expressed. Although normally a membrane protein, secreted forms of gB would have the potential to effect cells in trans. An Ad5 recombinant expressing HCMV gB is capable inducing a neutralising immune response (Marshall et al, J. Infect. Dis, 162, 1177). Ad is not enveloped so expression of gB does not have the potential to effect Ad5 tropism. Ad5 recombinants encoding cell-associated or soluble versions of gB have the capacity to transiently modulate expression in trans following in vitro or in vivo administration. There is a potential hazard with Ad-gB recombinant viruses that should gB released from cells it has the potential to modulate transcription in adjacent cells. This effect may be similar to an interferon response, is liable to be detrimental to the cell but should be transient.

IE2 is the predominant regulatory protein in the virus. It is a promiscuous transactivator that interacts with a wide range of cellular factors (including p53 and Rb), may inhibit apoptosis, promotes cell cycle progression and cell cycle arrest in G1/S. By itself IE2 is not transforming, indeed expression of this protein in mammalian vectors has proved problematical as it appears incompatible with long term cell survival, probably due to the block in cell cycle progression. IE2 has some capacity to rescue an Ad E1a deletion but does not rescue a complete E1 deletion. An ADIE2 recombinant has been shown in microarray analysis to increase the levels of RNA molecules that promote cell cycle progression (Song and Stinski, PNAS 99, 2836). Infection with an Ad5 1E2 construct is liable to be inconsistent with the long term survival of the cell (as is a wt Ad5 infection) but would not be expected to result in the release of virus nor cell transformation. HCMV encodes a series of additional regulators of cellular gene expression that appear to be significantly less potent than IE2; including IE1 (weak transactivator; disperses PML-bodies), pUL36 (IE) the virion proteins ppUL82 (pp71), ppUL69, pIRS1 and pTRS1. There is no clear potential for hazard associated with the expression of these molecules. HCMV has become a paradigm for investigating virus modulation of cellular immune systems. A number of immunomodulatory genes have been characterised and it is anticipated that a significant number of uncharacterised HCMV genes will be found to interact with immune systems. The expression of genes capable of modulating immunological r esponses in Ad5 vector systems constitute a potential hazard.

HCMV UL111a is a functional IL-10 homologue. IL-10 used therapeutically to suppress immunological responses yet is well tolerated and in high doses could predispose to infection. Ad recombinant encoding cellular and EBV vIL-10 have been used in animal models to suppress immunopathologies. The CMV homologue is believed to be of lower potency. As a secreted virokine Ad recombinants have a theoretical potential to induce transient, localised suppression of Th1 immune responses. There is a low level potential hazard with Ad-UL111a recombinant viruses that has recently been approved at ACGM2 level in a separate notification from this laboratory. HCMV encodes a number of putative secreted immunomolecules that may be considered to represent a comparable hazard level to the UL111a gene product and a substantial number of additional secreted glycoproteins is predicted by analysis of ORFs and preliminary expression studies. Preliminary data indicates the hypervariable UL146 gene product (vCXC-1) to be an IL-8-like chemokine that induces chemotaxis of neutrophils. UL147 also exhibits cysteine spacing and weak homology to CXC chemokines. Additional uncharacterised chemokine-like ORFs are expected to be functional.

US2, US3, US6 and US11 encode glycoproteins that act on infected cells to downregulated cell surface expression of MHC-1, and thus they can be expected to promote evasion of CD8+ CTLs. The leader sequence from UL40 acts to upregulate cellular HLA-E expression and thus promote protection against attack by CD94/NKG2A+ NK cells. gpUL16 promotes the intracellular retention of ligands for the NK activating ligand NKG2D and this may also impede NK recognition. GpUL18 is expressed on the cell surface where it binds the inhibitory ligand Lir-1 that is present of NK subsets, T cell subsets anad myeloid cells. The functional role of gpUL18 is controversial, it may...
inhibit NK and T cell subsets. Four HCMV genes (UL33, UL78, US27, US28) are predicted to encode 7TM receptors. US28 encodes a functional C-C chemokine G-protein-coupled receptor (GCR) which binds both CC and CX3C chemokines and signals in response to some. Experiment by others using an AdUS28 recombinant infecting vascular smooth muscles cells indicate that US28 expression can direct cells movement (Sgtrebnow et al, Cell 1999; 99, 511). HCMV is predicted to encode additional NK inhibitory mechanisms and additional glycoproteins that may be expected modulate the expression of existing surface glycoproteins, to promote intracellular signalling and/or promote interaction with other cells. All these genes would normally only be expected to functions only in the transgene-expressing cell and thus are of lower potential hazard. The hazard with cell surface inhibitory receptors (eg UL18) may be increased if expressed as soluble forms to a hazard level similar to that of cmvIL-10 (UL111a). There is a potential hazard associated with the expression of biologically active molecules in native and modified (soluble or epitope tagged) forms. The hazard is reduced by the vector being replication-deficient. Although, the Ad vector has the potential to provide for relatively efficient in vivo delivery of these agents, in both animal models and in clinical trials it has been difficult (although possible) to deliver transgenes with Ad vectors efficiently enough to induce therapeutic effects with immunomodulatory cytokines such as IL-2 and IL-10. Hazards associated with using replication-deficient Ad recombinants encoding HCMV immunomodulatory genes are similar to those with human cytokines. This hazard should be controlled by providing ACGM-2 containment.

Baculovirus recombinants have the potential for environmental harm due their capacity to kill insects. Enhanced baculovirus pathogenesis/virulence as a consequence of expressing a novel transgene is therefore to be avoided or controlled. Baculovirus is an enveloped virus and HCMV protein expressed in a baculovirus recombinant have the potential to be incorporated into baculovirus virions. HCMV has an extreme restricted tropism productively infection only certain human cell types. HCMV infection also requires a complex series events involving a number of HCMV glycoproteins acting together (gB, gH, gL, GO). While the HCMV receptor has not been characterised, the complex nature of the HCMV infectious process make it extremely unlikely that the expression of an HCMV gene product would be capable of modifying the tropism of the baculovirus vector. The immune system of insects and humans have diverged. The HCMV immune modulators have evolved to have very precise effects on the human immune system. It is extremely unlikely that an HCMV-encoded immune modulator would be able to mediate a significant function in insects. Soluble chemokines and virokines depend on interactions with a matched receptors. Genes involved in MHC-1 downregulation and NK evasion also depend on highly specific interaction with molecules such as the MHC-1 proteins, TAP or NK ligands that are not likely to have survived evolutionary divergence. Baculoviruses-expressing HCMV genes have an extremely low likelihood of having enhanced virulence in insects. In certain cases HCMV genes may also be inserted into baculovirus under the control of a constitutive promoter for transgene expression to mammalian cells. Specific baculovirus recombinants would then have the potential to express HCMV immune modulators in human cells as with replication-deficient Ad vectors. The potential hazard is lower because Baculoviruses infect human cells relatively inefficiently and following infection there is an absolute barrier to replication. There is extremely low likelihood of enhanced environmental hazard with these constructs, for the same reasons as given above.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Animals are inoculated in a Class 2 safety cabinet. The site of inoculation is disinfected according to agreed protocols before the animals are placed in filter-top cages. It is not anticipated that the virus will remain viable in the animal nor be secreted from the animal. Nethertheless, both the animal and waste are incinerated according to standard procedures in Biomedical Services approved by the Health and Safety Unit.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
The committee queried 'whether or not the environmental Risk Assessment was sufficient for the use of baculovirus'. One has now been appended to the Risk Assessment.

The committee was happy that the containment level and classification of work assigned was appropriate. Class 1 Containment Level 1 for prokaryotic organism and cell lines. Containment level 2 for adenovirus and baculovirus.

### Project Containment

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- **Animal Units**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Large Scale Activities**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Human Clinical Applications**: L2 L3 L4 L2 L3 L4 L2 L3 L4

### Project Ref 312/03.2

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<td>10/02/2003</td>
<td>IDENTIFYING THE FUNCTIONS OF EPSTEIN-BARR VIRUS GENES USING PLASMID VECTORS AND RECOMBINANT VIRUS VECTORS (BACULOVIRUS, VACCINIA VIRUS, AND REPLICATION-DEFICIENT ADENOVIRUS VECTORS)</td>
<td>Class 2</td>
<td>1-50 litres</td>
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- **Project notified under transitional arrangements**: N

### Project Additional Information

- **Withdrawn**: N
- **Project transferred to GM130 on 26/04/2005**
### Purposes of the contained use

We intend to perform a systematic investigation of human Epstein-Barr virus (EBV) gene functions by the cloning and expression of EBV genes in cell lines using baculovirus, vaccinia virus and replication-deficient adenovirus vectors. Previous work by our group has focussed mainly on the limited number of 'latent' viral genes expressed in B lymphoblastoid cell lines growth-transformed by EBV. However, the virus contains about 80-90 genes in total. Most of these are expressed only in cells entering the lytic virus productive cycle. The functions of some lytic cycle genes are quite well characterised (eg the BZLF1 immediate-early gene, encoding a transcriptional activator which initiates a cascade of viral gene expression) while the products and functions of many other genes are uncharacterised. The broad aims of this project are to investigate the mechanisms regulating induction and progress through EBV lytic cycle, and to screen for novel functions of EBV genes, particularly those which modulate immune responses. Experiments involving the use of replication-deficient recombinant adenoviruses may also include the use of a plasmid expression vector for the adenovirus receptor (CAR) to render specific cell lines susceptible to infection. Other components of the work designed to elucidate the mechanisms of EBV immunomodulating genes may also involve expression of components of the antigen processing pathway (eg HLA and TAPs). The vast majority of experiments will be performed on cell lines, but animals may occasionally be inoculated with Ad-recombinants primarily to generate antibodies specific for the transgene.

### Recipient or parental organism

Preliminary prokaryotic cloning steps are necessary to generate the recombinant viruses. Genetically disabled E.coli K12 strains specifically developed for application in recombinant DNA cloning will be used. These strains have limited potential to colonise the gut or survive in the environment. Prokaryotic cloning experiments will be at ACGM category 1 level.

Adenovirus is an ACDP category 2 pathogen. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Deletion of the E1 gene region renders the adenovirus vectors replication-deficient, which limited the potential for lateral spread. Theoretically, lateral spread could be facilitated by co-infection and a wt adenovirus or by gene-rescue from the helper line.

Vaccinia virus is an ACDP category 2 pathogen. Recombinant viruses may retain the pathogenic potential of wild-type vaccinia. The WR strain is a replication-competent lytic virus which has the potential to cause lesions, whereas the MVA strain is non-lytic and has to be generated in chicken embryo fibroblast (CEF) or BHK-21 cells. However, neither WR nor MVA recombinants are compatible with cell survival. Insertion of foreign genes into the thymidine kinase (TK) locus results in a TK phenotype which is attenuated relative to wild-type virus. The MVA strain is already highly attenuated.

Baculovirus vectors are replication competent in some insect cells but are replication-incompetent in mammalian cells. The vectors lack the non-essential polyhedrin gene.

### Host/vector system

- **Prokaryotic hosts:** E. coli strains DH5a, JM109, BJ583 and XL-blue will be used.
- **Adenovirus recombinants:** Commercial Ad5 vector systems will be used. The Admax vector system from Microbix, Canada; the AdEasy vector system from Qbiogene, Hareford, Middx. Additionally, the gutted Ad vector as described by Schneider et al (J. Virol. 2002, 76:1600). Replication-deficient adenoviruses will be propagated in a helper cell line expressing an E1 helper function (eg 293 or 911 cells) and then used to infect target cells in vitro or in vivo.
- **Vaccinia virus recombinubabts:** These will be made in the Western Reserve (WR) or in the Modified Virus Anraka (MVA) vaccinia strains. WR virus will be reaped in TK 143 cells, while MVA will be prepared in CEF or BHK-21 cells.
- **Baculovirus recombinants:** Commercial Insect Select (Invitrogen) and BacVector (Novagen) systems. Sf9 insect cells for growth of baculovirus vectors.
- **Target cells:** Tissue culture cells, including human tumour lymphoid (eg Burkitt's lymphoma B cell lines, and Jurkat T cell line) and epithelial cell lines (eg Hele, 293). Primary lymphoid and fibroblast cells.
- **Animals:** Animals may be inoculated with Ad recombinants, primarily to generate polyclonal or monoclonal antibodies.
### Origin & function

Genes will be cloned from Epstein-Barr virus isolates, including B95.8 and Raji which have been used to generate a complete 'wild-type' sequence (EBV-wt; accession number AJ507799) of 171,823 basepairs with approximately 80-90 known or postulated genes. Epstein-Barr virus is a category 2 ACDP pathogen. It is a lymphotropic/epitheliotropic gamma-herpesvirus that is carried as a life-long persistent infection by more than 90% of adults. Despite the asymptomatic nature of EBV infection, the virus has the potential to transform one of its principal target cells, B lymphocytes, and experimental infection of B cells in vitro leads to the establishment of lymphoblastoid cell line (LCL). These LCL carry EBV predominantly as a 'latent' (ie non-productive) infection. In healthy EBV-positive individuals, the persistent virus infection is effectively controlled by immune mechanisms, particularly cytotoxic T cells (CTLs). Since EBV is a virus with transforming potential, one potential hazard of the vectors to be generated is the possibility that the expressed genes may be oncogenic. The role of EBV genes in cellular transformation is well studied. At least five of the 'latent' genes are known to be absolutely essential for the ability of EBV to transform human B cells. The main function of the EBNA1 protein is maintenance of the EBV episome during cell replication. EBNA2, EBNA3A and 3C are nuclear proteins that activate and/or repress transcription of EBV and cellular genes. LMP1 is a membrane protein that mimics a constitutively active receptor of the CD40/TNF superfamily. Both LMP1 and EBNA3C have been defined as having oncogenic or immortalizing properties in rodent fibroblast assays. However, all attempts to demonstrate conversion of non-malignant human cells to a full malignant phenotype with any single EBV gene have been unsuccessful. While some of this work will necessitate using EBV 'latent' genes in recombinant adenovirus or vaccinia virus expression vectors, the main objective of this project is to identify functions of the lytic cycle genes, most of which are poorly characterised, if at all. The original impetus for this work derived from a wish to identify those genes responsible for down-regulating expression of components of the antigen processing pathway in cells harbouring EBV as a productive, lytic infection. Therefore, it can be deduced that we expect some EBV genes to have immunomodulatory functions. Indeed, one well-characterised gene (BCLF1) is known to encode an IL10 homologue. However, there is no compelling evidence to suggest that EBV infection is immunosuppressive; on the contrary, it elicits potent immune-responses.

In addition to vectors expressing wild type EBV genes and mutated genes, other constructs will be required for this project. The use of adenovirus vectors on B lymphocytes, a natural host for EBV, is hampered by the fact that these cells often express only low levels of adenovirus receptor (CAR). Therefore, a human or porcine CAR-expression plasmid will be used to make stable transfectants of established cell lines to generate lymphocytes that can be efficiently infected with replication-deficient adenovirus vectors. The potential risk of the CAR expression plasmids in this context is minimal.

Some experiments, designed to establish the mechanisms by which EBV genes modulate antigen presentation, will also require the use of expression plasmids or recombinant viruses for components of the immune recognition process (eg human MHC, TAP, NK receptors).

Other experiments will use a number of reporter plasmids with specific transcription factor binding motifs to characterise signalling functions or to indicate activation of lytic cycle. Furthermore, reagents generated from related notified projects (“modulation of lymphocyte function with adenoviral vectors” - Dr Brennan; and “Analysis of Epstein-Barr virus latent gene functions” - Prof Rowe) will be used to assist elements of this project. For generation of new expression plasmids, we will use well characterised constructs, some of which are designed to incorporate 'tags' to allow detection and/or isolation of the expressed proteins. These tag sequences are unlikely to pose any significant additional hazard.

### Evaluation of foreseeable effects

Most EBV genes have no known biological functions or functions that have no predictable detrimental effect when expressed in the proposed vectors. The 'latent' genes associated with the growth-transforming functions of EBV may be considered to constitute the greatest potential hazard when expressed in the proposed vectors. Both LMP1 and EBNA3C have been defined as having oncogenic or immortalising properties in rodent fibroblast assays. However, it is clear that EBV requires the co-operative action of at least 5 genes (EBNA1, EBNA2, EBNA3A, EBNA3C, and LMP1) to immortalize human B cells. Therefore, the hazard posed by accidental expression of these genes is substantially reduced. Furthermore, the adenovirus and vaccinia virus vectors should give only transient expression whereas the potentially pathogenic effects of the latent genes require persistent expression. Finally, all EBV latent genes (with the possible exception of EBNA1) are effectively targeted by cytotoxic T cell immune response. Thus, the potential hazard with regards possible transformation of human cells infected with Ad virus recombinants expressing EBV latent genes is acceptably low. This view is supported by the fact that similar work has already been approved at Birmingham (Rickinson Group) and Liverpool (Blaake group) and is being performed under category 2 containment.

The effects of most EBV lytic cycle genes on cellular gene expression and phenotype is unknown - which is the very reason for undertaking this project. However, none of the lytic cycle associated EBV genes studies have been reported to show oncogenic properties. Indeed two of the immediate-early genes (BZLF1 and BRLF1) have been
expressed by others in Ad virus vectors and have been shown to selectively inhibit growth of EBV-positive tumours (J. Virol., 2002. 76:10951). Unlike many other members
of the herpesvirus family, EBV encodes relatively few genes that are obvious homologues of immunomodulatory molecules. The notable exception is BCRF1 which is
highly homologous to IL-10 and shares functional properties with the human cytokine. IL-10 is used therapeutically to suppress immunological responses, and is well
tolerated in high doses although it may predispose to infection. Ad recombinants encoding cellular and EBV vIL-10 have been used in animal models to suppress
immunopathologies. As a secreted virokine, vIL-10 Ad recombinants have a theoretical potential to induce transient and localised suppression of Th1 immune r
esponses. Therefore, there is a low level potential hazard with Ad-BCRF1 recombinant viruses.

Adenoviruses are ACDP level 2 agents that are naturally infectious to the respiratory tract as aerosols. If accidental inoculation were to occur, the viral DNA would be
introduced into a number of cells giving transient expression of the transgene over a period of a few days to weeks. However, all Ad vectors used will be
replication-deficient due to deletion of the E1 and E3 gene regions; this is a recognised safety feature. Therefore, following accidental inoculation, further spread would not
occur. With time, the genome will be lost from the infected cells, this process being more rapid in proliferating cells. Chromosomal integration of Ad virus sequences is a
rare event (in the region of one integration in 10(4) exponentially growing cells). It is generally agreed that most cells of the respiratory tract epithelium are post-mitotic and
on the differentiation pathway. Therefore, it is our opinion that a considerable intake of virus, much greater than 10(5) pfu, would be required to produce a single integration
event. Working practices are designed to minimise the risk of any human contamination, and the likelihood of accidental infection followed by viral integration is almost
negligible. Repair of the E1 deletion of the Ad vectors could potentially occur in vitro due to homologous recombination with the E1 gene in the helper cell line, or in vivo
following the unlikely event of coinfection of a single cell with recombinant Ad virus and a natural wild-type Ad virus. Such recombination events are likely to result in
concomitant deletion of the transgene in the E1 locus. Generation of a replication-competent Ad carrying the whole or part of the transgene is not impossible, but extremely
unlikely. It is notable that attempts to detect such complementation by dual infection in the cotton rat model gave negative results. The potential hazard of the recombinant
adenoviruses should be adequately controlled by adhering to ACGM-2 containment.

Vaccinia viruses are ACDP category 2 agents which have the ability to a wide range of cells in human and non-human hosts. Vaccinia may cause puustular lesions at the
site of infection, and can cause severe disease in individuals with active skin disorders such as eczema or psoriasis. Acute conjunctivitis may occur after inoculation into
the eye but permanent eye damage is rare. Disseminated vaccinia necrosum can occur in immunosuppressed or immunodeficient individuals. Recombinant viruses may
retain the pathogenic potential of wild-type vaccinia. The WR strain is a replication-competent lytic virus which has the potential to cause lesions, whereas the MVA styrian
is non-lytic and has to be generated in chicken embryo fibroblast (CEF or BHK-21 cells. However, neither WR nor MVA recombinants will be compatible with cell survival.
Therefore only transient expression of the inserted genes will be obtained, and the additional risk posed by the inserted genes will be low. It is not clear whether EBV
glycoproteins would be expressed in the vaccinia virus envelope, but it is not expected that this would alter the tropism of the virus. The potential hazards of the r
ecombinant vaccinia viruses should normally be adequately controlled by adhering to ACGM-2 containment. However, individuals with skin disorders are at greater than
normal risk for working with vaccinia viruses and should seek additional advice.

Genetically modified baculoviruses retain the ability to infect cells of the insect host species but do not efficiently infect human cells. During infection of insect cells,
expression of the gene insert will occur under the control of the Polyhedrin or P10 promoters, but these promoters are not expected to be functional in human cells. It is not
clear whether EBV glycoproteins would be expressed in the baculovirus envelope, but the complexity of the EBV infection process makes it extremely unlikely that insertion
of a single EBV protein would alter the tropism of a recombinant baculovirus. Therefore the nature of the EBV gene insert in recombinant baculovirus itself is not expected
to pose any additional risk to human health or to the environment. A case could be made for using the recombinant baculoviruses at ACGM level 1, but taking a more
cautious view we propose to adhere to ACGM-2 containment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Virus in solution (tissue culture medium or buffers such as PBS) will be inactivated by adding at least an equal volume of 2500 ppm sodium dichloroisocyanurate (Antichlor). After 4 hr, the solution can be discarded to mains drainage and flushed with excess water. Contaminated pipettes are immersed in 2500 ppm sodium dichloroisocyanurate (Antichlor) for 4 hr prior to transferring to a containing for autoclaving. All plasticware is autoclaved after exposure to virus. Actichlor is effective against all viruses and the chosen concentration overcomes the high organic content of tissue culture media.

Animals may be inoculated with recombinant adenoviruses in a Class 2 microbiological safety cabinet. The site of inoculation is disinfected according to agreed protocols before the animals are placed in filter-top cages. It is not anticipated that the virus will remain viable in the animal nor be secreted from the animal. Nevertheless, both the animal and all animal waste is incinerated according to standard procedures in Biomedical Services approved by the Health and Safety Unit.

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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<th>Human Clinical Applications</th>
</tr>
</thead>
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<td>L3</td>
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**Project Containment**

**Project Ref** 312/99.1

**Date Ackn'd** 07/12/1999

**CU2 Project Title** MODIFICATION OF TELOMERE FUNCTION USING AN ADENOVIRUS VECTOR SYSTEM

**Date Project Ceased** 26/04/2005

**Class** Class 2

**CultureVolumeClass3-4** not applicable

**Project notified under transitional arrangements** Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project transferred to GM130 on 26/04/2005
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 312/99.2

Date Ackn'd: 07/12/1999  
CU2 Project Title: MODIFICATION OF TELOMERE FUNCTION USING AN EQUINE INFECTIOUS ANAEMIA VIRUS SYSTEM

Class: Class 2  
CultureVol: Class 2  
CultureVolume: Class 3-4

Non-GMM: Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N  
Tick if notifying a connected programme of work: N

Historical Significant Changes:  
Project transferred to GM130 on 26/04/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
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#### Date Project Ceased

26/04/2005

#### Withdrawn

N

#### Non-GMM

not applicable

#### Consent Granted

Y

#### Project notified under transitional arrangements

Y

#### Historical Significant Changes

Transferred to GM130 on 26/04/2005

#### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Project transferred to GM130 on 26/04/2005
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
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GM Centre Number: 314

Data Premises Notified (Originally) 27/06/1989

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed Y

Transitional Premises Non-GMMs N

Emergency Plan Required? N

Withdrawn N

Name
ROYAL VETERINARY COLLEGE

Name 2
UNIVERSITY OF LONDON

Department
Campus Estate or Research Centre

Building

Road Name
ROYAL COLLEGE STREET

District CAMDEN TOWN

Town LONDON

County GREATER LONDON

Postcode NW1 0TU

Country ENGLAND

Tel Number 0207 468 5000

Fax Number 0207 368 2342

E-mail

HSE Division LONDON

Comments

Date at Which Additional Info Submitted 02/03/2022
Premises Addresses

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<th>Town</th>
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<th>Post-code</th>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)
<table>
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<td>Virology</td>
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<td>Transgenic Fish</td>
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<td>Transgenic Plants</td>
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<td>Other(s)</td>
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<td>For activities involving GMMs, describe the waste management measures which will apply to the activity</td>
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<td>Tick to confirm that you are attaching a summary of the risk assessment</td>
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**Project Ref 314/00.1**

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<td>23/02/2000</td>
<td>IMMUNISATION OF WOODCHUCKS WITH AN INFECTIOUS WOODCHUCK HEPATITUS VIRUS (WHV) CLONE &amp; A RECOMBINANT WHV/HUMAN HEPATITUS B VIRUS S GENE CHIMERA</td>
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Date Project Ceased:  [Date Not Specified]
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**Project notified under transitional arrangements**

### Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Is an emergency plan required according to regulation 20?**

- **Yes**

**Tick to confirm that you have attached a risk assessment to this form**

- **Yes**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Additional Information**

**Purposes of the contained use**

To maintain a specific pathogen free colony of less than 40 mice as a guarantee colony for the National Institute for Biological Standards and Control

Project notified under transitional arrangements: N
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</thead>
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<tr>
<td><strong>Host/vector system</strong></td>
<td>Balb-c's were cross-bred at NIBSC onto ICR mice already transgenic for Trangene 21 polio virus receptor (Tg 21 PVR).</td>
</tr>
</tbody>
</table>
Intended function (at NIBSC, not RVC): to screen human polio vaccines for neurovirulence. |
| **Evaluation of foreseeable effects** | A worst case scenario is that the mice escape, breed with wild mice to give a population of mice with this Mendelian dominant Tg 21 PVR gene and thereby set up a new animal reservoir for polio virus after it has been eradicated in man. However experiments have shown viral transmission between TgPVR mice is poor (WHO Bulletin OMS, Vol 71, 1993 - pages 497-502). Balb-c mice are albino, docile and used to ad-lib water and pelleted food in a controlled temperature cages and so would be unlikely to survive and interbreed to set up a sustainable mouse colony in central London. |
| **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)** | The mice are kept in metal cages in a positive-pressure isolator whose plastic film mice cannot gnaw. The isolator is in a secure room with swipe card in a secure building with computer-controlled entry by encoded swipe card and also card holder's code. There are no drains and the one in the adjacent preparation room has an in-built rodent trap. The animals are attended to by qualified technicians with three or more years experience of isolators. Sick animals are reported to the named veterinary surgeon. Live mice never leave the isolator. |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

---

The waste from the isolator and any culled animals are bagged in the isolator, double bagged in the exit port of the isolator and then autoclaved on site before being tertiary bagged for incineration at Cambridge pet crematorium.

**Is an emergency plan required according to regulation 20?**

- N

**If yes, tick to confirm that it is attached to this form**

- N

**Tick to confirm that you have attached a risk assessment to this form**

- Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- N

---

**Please enter comments on the GM safety committee on the risk assessment**

This was passed without comment in 1998.
Hepatitis C virus (HCV) is a major human pathogen worldwide causing persistent infection, chronic disease and is associated with a high risk of development of hepatocellular carcinoma. Although complete genome sequence and cDNA clones are available for HCV, the testing of potential anti-viral therapies is constrained by the lack of efficient in vitro virus culture systems and the in vivo challenge systems (humans and chimpanzees). These limitations do not apply to the closely related veterinary pestivirus, bovine viral diarrhoea virus (BVDV), for which infectious cDNA clones, in vitro culture systems and large animal challenge systems are well established. To facilitate the in vivo assessment of potential anti-viral therapies for HCV, we propose to investigate the use of BVDV as a model for HCV and further develop a small animal challenge model (neonatal rabbits) for BVDV infection. The challenge of rabbits with BVDV is an issue not within the remit of this application (BVDV is an ACDP category 1 pathogen).

However, to exploit the potential of this system we will also construct recombinant viruses where key components of the HCV genome are substituted into a BVDV infectious cDNA. This will generate chimeric viruses capable of replication in the bovine cell culture system but which are dependent on HCV functions to direct protein expression or replication. These chimeras will eventually be targets for the development of HCV-specific anti-viral therapies. The recombinant plasmids containing these
clones are not infectious (BVDV is an RNA virus, the plasmid clones are DNA). The infectious RNAs for recombinant (chimeric) viruses will either be generated in vitro (in a microfuge tube) using a bacteriophage T7 polymerase promoter or following transfection of the plasmid DNA into a mammalian cell line engineered to express the T7 RNA polymerase. The RNA produced in this way is chemically fragile but is capable of replication if introduced into a suitable host cell and may produce infectious virus.

As this proposal intends to deliberately create novel chimeras between an endemic veterinary virus and a human pathogen, issues of safety and containment need to be carefully addressed, particularly with respect to potential changes in the host range of virulence of any chimeric viruses.

To avoid these hazards the project will introduce only HCV sequences which will not affect the tropism of the recombinant virus, including the 5'-untranslated region (which has no protein coding capacity but directs the initiation of viral protein synthesis) or sequences encoding virus non-structural proteins such as NS3 (serine protease and NS5B (RNA polymerase)). Passive markers for gene expression such as the green fluorescent protein may also be used.

Recipient or parental organism

Bovine viral diarrhoea virus (BVDV) is a worldwide pathogen of artiodacyla (even toed ungulates), primarily cattle, sheep and to a lesser extent, goats and pigs. This pestivirus is endemic in the UK and usually associated with a mild or inapparent clinical illness which results in reduced milk yield and reproductive performance. Natural immunity to BVDV is high (70-90% of animals) and effective vaccines are available.

All work will be based on the highly lab-adapted, cytopathogenic NADL strain of BVDV which is licensed as a live virus vaccine in the USA. Cytopathogenic strains of BVDV are unable to establish persistent in utero infections in cattle.

BVDV is not known to infect humans. At no stage will infectious HCV be used.

Host/vector system

Infectious cDNA clones of the NADL strain of BVDV will be modified to include additional sequences or to replace normal viral sequences. These include: firstly, the HCV 5'-untranslated region (the internal ribosome entry site or IRES initiates protein expression) and later, green fluorescent protein (marker protein) or the HCV non-structural proteins NS5 (RNA polymerase) or NS3 (serine protease). These sequences are not toxic. It is unlikely that these components will have any effect on host range. The basis for this statement is the observation that both virus protein expression and RNA replication occur in non-permissive (BHK) cells transfected with pestivirus RNA; substituting the BVDV sequences with the HCV homologues is unlikely to change this situation. Host range is determined by the interaction of the virus structural proteins with a host specific, cell surface receptor molecule: virus structural proteins limit the susceptible host range. NO ATTEMPT WILL BE MADE TO CHANGE THE VIRAL STRUCTURAL PROTEINS in this project. Infectious cDNA clones or RNA derived from them will be transfected into mammalian cells in culture and the properties of the chimeric viruses generated will be compared with those of the parental BVDV strain. At no stage will infectious HCV be used.

Origin & function

Sequences encoding the green fluorescent protein (GFP) or regions of the hepatitis C virus (HCV) genome will be cloned into the BVDV infectious cDNA clone. The function of GFP will be to provide a passive marker for viral gene expression, facilitating the quick and simple detection of virus growth within cells. The HCV derived sequences will be noncoding genome sequences (the 5'-untranslated region containing the internal ribosome entry site). This non-coding RNA sequence controls the expression of viral protein synthesis or regions encoding viral non-structural proteins such as NS3 (serine protease) and N35B (RNA polymerase). These proteins assist the replication of the viral genome within an infected cell. They do not play a role in virus entry into an uninfected cell. None of these changes are likely to affect the pathogenicity, host range or tropism of the chimeric viruses. Previously published work describes similar viruses (see Frolov, McBride and Rice (1998) RNA 4:1418-1435 and lai VC, Zhong W, Skelton A, Ingravallo P, Vassilev V, Donis, Hong and Lau (2000) J Virol 74:6339-47) and there is no evidence that these chimeric viruses have modified growth characteristics in cell culture (except that some recombinants replicate poorly in vitro).

The eventual aim would be to use the chimeric viruses to test the efficacy of anti-HCV specific anti-viral therapies. This would be done by growth of the virus in cells cultured in vitro and, if these studies were successful, later application may be made to assess the viruses in a laboratory rabbit challenge model.

Evaluation of foreseeable effects

The cDNA plasmid clones present no foreseeable risk (both BVDV and HCV are RNA viruses, the cDNA clones are DNA) as potentially infectious RNA can only be produced under the control of T7 RNA polymerase promoter, either in vitro or in a specially engineered cell line expressing this protein. T7 RNA polymerase is not normally
produced by the E. coli strains that contain these plasmids or the host species potentially susceptible to infection with BVDV.

Previously published data confirms that RNA from cDNA clones of BVDV and CSFV, can be transfected into cells in culture. During transfection the viral RNA is complexed with agents that mediate the transfer of nucleic acid directly into the cell cytoplasm and bypass the normal mechanisms of receptor-mediated cell infection. When cells permissive for normal virus infection were used for transfection of infectious RNA, both viral RNA and proteins were produced and assembled into virus particles capable of extensive cell to cell spread within the culture dish. When cells non-permissive for virus infection were used for transfection of infectious RNA eg BHK cells, viral RNA and proteins were produced. This confirms that these cells are not susceptible to virus infection but can support intracellular replication of the virus (Behrens et al, J Virol 72:2364-2372, 1998). This indicates that host range is limited by the interaction of the virus structural proteins with a host specific, cell surface receptor molecule and that virus structural proteins limit the susceptible host range. NO ATTEMPT WILL BE MADE TO CHANGE THE VIRAL STRUCTURAL PROTEINS in this project. This means that the host range and tissue tropism of the chimeric viruses should be the same as the parental virus - primarily infecting cattle or sheep but not humans.

Infectious virus will be produced either in vitro or in vivo following transcription by T7 RNA polymerase. The RNA produced in this way is chemically fragile but is capable of replication in a suitable host cell (if RNA produced in vitro it is very difficult for it to get into a cell) but if introduced, it may produce infectious virus. This virus will be capable of infecting susceptible bovine cells. However, the parental BVDV strain is the lab-adapted NADL strain which will infect cattle but is not considered to be pathogenic or virulent. The previously published literature on this subject suggests that the modifications proposed are likely to have a neutral effect or to decrease the replicative fitness of the chimeric viruses (Frolov, McBride and Rice RNA 4:1418-1435, 1998). This means that chimeras are unlikely compete efficiently with wild type virus.

The risk of infection in human cells by the BVDV chimeras is unchanged compared to the parental BVDV which is unable to infect human cells, a property that is determined by the virus structural proteins which will be entirely derived from BVDV. The risk of infection of cattle by the BVDV chimeras is unchanged and the available published data suggests that these changes will have a neutral or a detrimental effect on the ability of the virus to replicate in bovine cells (see Frolov, McBride and Rice (1998) RNA 4:1418-1435 and Lai VC, Zhong W, Skelton A, Ingravallo P, Vassilev V, Donis, Hong and Lau (2000) J Virol 74:6339-47). I anticipate that the virulence and pathogenicity of the recombinant virus will not be increased compared to the parental NADL strain and the virus will be unlikely to replicate to high titres in cattle.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be sealed in plastic bags, stored within a robust container before transfer to the autoclave for inactivation prior to disposal by offsite incineration. All liquid waste will be mixed with Virkon to give a final concentration of 1% and stored overnight before the residue is flushed away with excess water. Plastic pipettes/pippette tips will be immersed in Virkon overnight before the liquid is drained off, the plastic put into bags for autoclaving and disposal.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
At a special meeting on July 22 2002 the assessment of this project as class 2 was considered to be correct because BVDV is an animal pathogen and the HCV genes being introduced are non-structural, do not encode toxic proteins or enable BVDV to infect man.

The committee asked Dr Collins to expand on whether the inserted genes enhance the pathogenicity of BVDV. She commented this is a complex issue. The parent virus that is to be modified is the NADL isolate which is a cytopathic BVDV isolate and is used as a live vaccine in the USA. It does not normally establish a persistent or virulent infection. The reason why GM-NADL is being treated as an animal pathogen is that, if it recombined in vivo with another BVDV genome in a cow, it could become capable of infecting calves in-utero and inducing a life-long viraemia in them. The additional inserts will not alter the BVDV vector's ability to cause life-long infections or its tissue tropism because these are determined by the structural genes: the serine proteases, RNA polymerase and ribosomal entry sites are not known to control tissue tropism of BVDV. She commented how the HVC serine protease uses a different substrate to the equivalent protease in BVDV but inserting this gene into BVDV would not alter its growth properties in cell culture (see Lai VC, Zhong W, Skelton A, Ingravallo P, Vassilev V, Donis, Hong and Lau (2000) J Virol 74:6339-47). Similarly adding the ribosomal entry site of HCV to BVDV will have a neutral or detrimental effect as evidenced by the growth in-vitro being 10-1000-fold less than wild type BVDV (see Frolov, McBride and Rice (1998) RNA 4:1418-1435).

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L4</td>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 314/05.1

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<th>CultureVol</th>
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<td>11/07/2005</td>
<td>Construction and analysis of mutants of Actinobacillus pleuropneumoniae and Streptococcus suis.</td>
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<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Class 3-4</td>
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<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tbody>
<tr>
<td>N</td>
<td>Not Applicable</td>
<td>N</td>
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Withdrawn N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**

To prepare and characterise both defined and random transposon mutants of the pig pathogen Actinobacillus pleuropneumoniae in the laboratory.

To prepare and characterise both defined and random transposon mutants of the pig pathogen *Streptococcus suis* in the laboratory.

The project is intended to construct random transposon mutants in order to select those genes which contribute to survival using transposon screen by microarray (TSM) and signature-tagged mutagenesis (STM). Defined mutants of *A. pleuropneumoniae* and *Streptococcus suis* will also be constructed to test the effect of particular mutations.

The purpose of this contained use is to allow ongoing work, already covered by CU2 (Imperial College - *A. pleuropneumoniae* [Ref GM77/02.4; research conducted University of Cambridge - *S. suis* [Ref GM407/97.2] to be taken forward.

**Recipient or parental organism**

*Actinobacillus pleuropneumoniae* is a Gram-negative bacterial pathogen. It is naturally present in pigs throughout the UK in which it causes pig respiratory disease (contagious porcine pleuropneumonia). It is host specific and does not survive for long in the environment outside the respiratory tract of the pig (Nicolet, 1992; Rycroft & Assavacheep - unpublished). Transmission is via direct or close aerosol contact between pigs (Velthuis, 2002). *A. pleuropneumoniae* is harmless to humans. There is no hazards to human health.

*Streptococcus suis* is a Gram-positive bacterial pathogen. It causes meningitis and septicaemia in the pig and is a rare zoonosis with the capability of causing meningitis in humans when introduced through wounds. It is widespread among farm pigs in the UK and is thought to be carried in the upper respiratory tract (Sanford & Higgins, 1992). It survives poorly in the environment, especially in dry conditions (Clifton-Hadley & Enright, 1984).

The GMMs will be mutants of *A. pleuropneumoniae* or *Streptococcus suis*, derived by either insertion of a transposon, or by allelic exchange to replace a specific gene with a mutant form of the gene. Some mutants will be restored to normal function by complementation using the functional gene expressed on a plasmid. Apart from the introduction of marker genes such as Kanamycin resistance, the experiments will be self-cloning, ie inserted DNA will be otherwise derived from the host organism.

Hazards to the environment could only arise if the organisms were able to gain access to the pig population outside the containment facility. This could only happen through carriage outside the facility by humans. Such a possibility will be prevented by the measures outline below.

Such hazard would be very unlikely to lead to disease or harm to pigs which would be materially different from that caused by the natural parental organisms and GM *A. pleuropneumoniae* or *S. suis* would be expected to die out naturally and not harm the environment. GMMs which carry a mutation, or additional genetic load due to a plasmid complementing that mutation, would be less (or not more) fit to survive in the competitive environment of the pig than the wild type.


Actinobacillus pleuropneumoniae 4074 and other strains carrying part or all of one of the following plasmids:

For introduction of mutations:
pUC18 series cloning vectors carrying mutant A. pleuropneumoniae gene. For introduction of mutant A. pleuropneumoniae genes or transposon mini-Tn10 by conjugation (pUC18 carrying mobRP4) from E. coli to A. pleuropneumoniae. Since colE1-based plasmids will not replicate in A. pleuropneumoniae, these are suicide vectors when transferred to this organism.

PBKM1 conjugative plasmid based on pBluescript SK (with mobRP4), multiple cloning site, Kan® and transcriptional fusion of the omA promoter with the sacB gene for sucrose sensitivity [Oswald et al., 1999]. For introduction of mutant A. pleuropneumoniae genes. The plasmid will be lost from the desired mutant organisms by its conferring sensitivity to sucrose.

For introduction of A. pleuropneumoniae genes for complementation:
pMIDG plasmid capable of replication a A. pleuropneumoniae with the sodC promoter of A. pleuropneumoniae for expression of foreign genes. For complementation experiments to express A. pleuropneumoniae genes previously mutated on the chromosome. [Web et al., 2001]

pYG53-series plasmids. These are closely related to the natural Mannheimia haemolytica plasmid pMHSCS1 (accession: NC 002637) which replicates in A. pleuropneumoniae. Confers chloramphenicol resistance. For complementation experiments to express A. pleuropneumoniae genes previously mutated on the chromosome.

Streptococcus suis P1/7 (serotype 2) carrying transposon Tn917 and Tn917-tag.
A bank of Streptococcus suis mutants carrying transposon mutations previously introduced on the vector plasmid pTV408 by electroporation. This is a temperature-sensitive plasmid carrying mutant at the non-permissive temperature (37-42 degrees C). pTV408-tag also carries signature tags in the transposon (unique sequences of DNA that allow the identification of individual mutants amongst mixed pools of mutant bacteria). These have been introduced into the strain to make a mutant bank in earlier work at University of Cambridge.

References


Origin & function

1. Self-cloning: Actinobacillus pleuropneumoniae chromosomal DNA mutated by deletion or insertion and re-introduced by allelic exchange.


3. Transposon mini-Tn10 or similar (for random mutagenesis) in A. pleuropneumoniae.

4. Transposon Tn917 carrying erythromycin resistance in Streptococcus suis.

The aims of the project are to generate mutants of A. pleuropneumoniae and Streptococcus suis by transposon mutagenesis and by defined, site-directed mutagenesis,
using allelic exchange. A selectable marker will be used in most cases: either Kanamycin resistance from mini-Tn10 or from pUC4K. A counter-selectable marker, such as sacB conferring sucrose sensitivity to recognise those mutants which have lost the plasmid vector, will also be used.

Genes of A. pleuropneumoniae or S. suis will also be cloned and transferred to the mutant parent to restore the mutant function. The genes will be transferred using replication-competent vector plasmid such as pYG53 (Lalonde & O'Hanley, 1989), pJFF224 (Frey, 1992) or pMIDG (Webb et al., 2001). This is necessary to demonstrate that lost phenotype can be restored by reintroduction of the gene alone.

References:


Evaluation of foreseeable effects

Actinobacillus pleuropneumoniae is a respiratory pathogen of the pig, causing the widespread disease contagious pleuropneumonia. There are no other known natural hosts and humans are not affected by this organism. The wild-type organism is present on the large majority of normal pig farms throughout the world.

Genetic modification will be the introduction of random or genetically defined mutations. The effect of knocking-out a gene is very unlikely to make this organism more pathogenic than it already is and is extremely unlikely to alter the host range of the pathogen to infect other species. In other cases the strain will be as virulent as the parent. Genetic modification is not expected to alter the survival in the environment, which is already very poor, and mutations will tend to make the organism less fit to survive in a pig host than the wild-type. Introduction of erythromycin, kanamycin or chloramphenicol resistance markers will not confer selective advantage in relation to natural A. pleuropneumoniae since erythromycin, kanamycin or chloramphenicol are not used in therapeutically in the treatment of pigs.

Experiments will also be conducted to complement the mutations by expression of the gene, from a plasmid within the mutant, using a promoter known to be effective in A. pleuropneumoniae. Reversal of the mutation in this way is needed to unequivocally demonstrate the effects of the mutation are due to the mutation alone.

There is relatively small risk through the accidental release of genetically modified S. suis or A. pleuropneumoniae, reaching and surviving in the environment. However, because these organisms are inherently very delicate and are unable to survive outside the body of an animal for very long, we consider survival or spread of the organisms (and their mutants) from the laboratory to be a very unlikely event and therefore of extremely low risk.

Genetic modification will be the introduction of random or genetically defined mutations. The effect of knocking-out a gene is very unlikely to make this organism more pathogenic than it already is and is extremely unlikely to alter the host range of the pathogen to infect other species.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Incineration off-site.

For activities involving GMMs, you will normally need to apply all the measures specified as requirements for the relevant containment level. If, however, your risk assessment indicates that any of those measures are unnecessary, you may ask for permission to omit them. Indicate any such measures with a brief justification that includes reference to the relevant parts of the risk assessment. You CANNOT claim confidentiality for the actual containment measures (unless your intellectual property rights might be affected) BUT the justification may be claimed as confidential. If any claim is made for confidentiality, the confidential information must be included in section 17 together with justification.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste, including plate cultures, will be sealed in plastic bags, stored within a robust container before transfer to the autoclave for inactivation prior to disposal by offsite incineration. All liquid waste will be mixed with Virkon to give a final minimum concentration of 1% and stored overnight, or alternatively boiled before the residue is flushed away with excess water. Plastic pipettes/pipette tips will be immersed in 1% Virkon overnight before the liquid is drained off, the plastic put into bags for autoclaving and disposal.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form
Y

Tick to confirm that you have attached a risk assessment to this form
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment
N

Please enter comments on the GM safety committee on the risk assessment

1. The use of conjugative instead of non-mobilisable plasmids is noted as necessary.
2. The purchase of solid robust containers for carriage of class 2 GMOs from lab to autoclave is necessary.
3. The forms question on safety cabinets is worded badly and their class should be omitted. (All have been addressed and advice acted upon where required).

Project Containment

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Project Ref 314/08.1

Date Ackn'd 17/07/2008

Date Project Ceased

CU2 Project Title Effects of gain-of-function and loss-of-function genetic manipulation of cells and tissues.

Class 2

Consent Granted Not Applicable

Class CultureVolume

Class 2 < 1 Litre

Non-GMM

Consent Granted

Not Applicable
**Withdrawn** N  
**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

## Project Additional Information

**Purposes of the contained use**

To characterise function of regulatory molecules during development of skeletal tissues. Various strategies will be used to achieve 'gain-of and 'loss-of-function' of distinct genes and their effects on regulation of cell behaviour described. Modification in expression will be verified. RCAS(BP) vectors harbouring specific genes will be used to deliver proposed modifiers of cell behaviour and signalling.

**Recipient or parental organism**

i) E. Coli XL1-Blue. H) RCASBP (A and B) retrovirus (helper genes are in 2 separate blocks of DNA to eliminate chances of reversion to replication competence by a single recombination. Although RCAS(BP) has sic oncogene removed, it retains minimal oncogenic potential in chicks, but not mammalian species cram et al 2002; J Virol. 76(9), 4275-4286; Du et al., 2007 PLos Biology, 5(10), e276). These reports provoke the Class 2 categorisation of RCAS (BP) retroviral use in avian cells and tissues in our studies.

**Host/vector system**

i) E. Coli XL1-Blue. This is effectively a pro-virus as it provides only a DNA copy of the viral genome and includes an origin for replication allowing it to act as a plasmid, and ii) Avian cell line (DF1) and avian cells/tissues. DF1 cells will be used to ‘grow-up’ RCAS(BP) viruses containing genes of interest and these will be used to transfect avian cells and tissues in vitro and in ovo. Unlike other viral delivery systems, the RCAS(BP) system does not require the use of a packaging cell as these viruses are replication competent.

**Origin & function**

cDNAs for this project have been isolated and cloned into the vectors listed. The intended function is to investigate signalling pathways involved in development of musculo-skeletal system and to uncover the signalling mechanisms underlying human diseases and syndromes. Vectors will contain genes in either native1 constitutively-active or dominant-negative configuration, and will initially include representatives of: i) Glycosaminoglycan synthesis enzymes and regulatory proteins (uridine diphospho-glucose dehydrogenase, UDPGD); ii) Wingless (Wnt) family members (eg. Wnt-4, and 5a), Wnt-antagonists (eg. Noggin, DKK); iii) Signalling molecules and their receptors (eg. mitogen-activated protein kinase (MAPK and p38) and protein kinase (PKC and family members); iv) Fibroblast Growth Factors (FGF5); v) Bone Morphogenetic Protein (BMP) family members; vi) cyclooxygenases (COX5); vi) Markers of skeletal cell differentiation (eg. connective tissue growth factor (CTGF), growth differentiation-5 (GDF5) and BMP-receptors). The intended function of such vector gene insertion and the purpose of this activity is to characterise the molecular function of regulatory molecules during the development of skeletal cells; and to understand how such ‘gain-of and ‘loss-of-function’ strategies applied in vitro effects the regulation of skeletal cell behaviour described. The RCAS(BP) system is ideal for the delivery of genes of interest to avian cells and tissues and the choice of genes to be delivered is based upon their likely and proposed involvement in the regulation of skeletal cell behaviour. We will examine the effects of these genes, where possible, in their native, constitutively-active or dominant-negative configuration. It is unlikely that these genes present any known risk in their native configuration, and although the risks associated with their constitutively-active or dominant-negative configuration is not known, it is unlikely that they represent any significant oncogenic, cross-species, tissue tropic, toxic and transmission risk.
Chick retroviruses (RCAS(BP)A or B) infect avian but not mammalian (human) cells. With the exception of RCAS(BP) virus, vectors used are incapable of replication/ transmission outside the laboratory. Exposure and health risk to the researcher is negligible by performing all work as Class 2 procedures, where other workers do not have free access. None of the genes we intend to use are known oncogenes and furthermore they will only be manipulated using the RCAS(BP) system that fails to infect mammalian cells. All of the work will be carried out in accordance with Safety Policy and Local rules, and performed in level 2 flow cabinets. All these procedures are in agreement with Local Rules preparation of viruses will take place in appropriately designated area within the lab at the RVC. Excess virus will also be disposed of by decontamination as outlined (Form B, section iv) and all solid matter disposed of by incineration. Although the RCAS(BP) retrovirus has had the src oncogene removed, it appears to retain minimal oncogenic potential in chicks as demonstrated by the studies of Tam et al (2002; J Virol. 76(9), 4275-4286). These authors have shown that a untranslated RNA, bic, in RCAS(BP) is capable of cooperating with c-myc to support infrequent and restricted tissue-specific oncogenicity; 2% of very late stage (l8day old) embryonic chicks receiving 0.1 ml of virus suspension containing 1 Omillion infectious units of virus per ml, injected directly into the chorioallantoic vein, developed lymphoma, but not erythroblastosis, myelocytomatosis, sarcoma or adenocarcinoma. This indicates a very low risk of complications in our studies when using avian cells and tissues. There is also a report indicating limited scope for somatic gene transfer using the RCAS(BP) retrovirus into mice with no obvious deleterious effects apparent due to the inability of RCAS(BP) to proliferate in mammals (Duet al., 2007 PLos Biology, 5(10), e276). There is therefore no risk to mammalian species. Nevertheless, despite the very small insignificant risk associated with RCAS(BP) retroviral use in avian cells and tissues in our studies all work using these viruses will be categorised as Class 2. The work will all be done in a laboratory without any contact with the animal house where non-breeding chickens are only kept on an occasional basis Any disease risk to birds from this project is therefore very small

Evaluation of foreseeable effects

No viable GMO will be produced — see attached Risk Assessment

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

RCAS(BP) retroviral-mediated gene transfer will be carried out in accordance with Safety Policy and Local rules, and performed in level 2 flow cabinets. Separate lab coats and double gloves will be worn. Liquid waste from bacterial and vertebrate cell cultures will be treated using standard, approved disinfection procedures; with freshly prepared VirkonTM in accord with the Manufacturer's instructions for more than 24 hours, and disposed of appropriately. This will result in at least 99.99% kill of bacteria and 100% kill of viral GMMs and cell lines. In addition, the retroviruses and cell lines have a very short half-life at room temperature. All solid waste, including plastics and waste chick materials will be placed in double autoclave bags and placed within an outer robust stainless steel container, sealed with clips. This will be transported by dedicated, trained staff from designated areas to a new autoclave in the same building, autoclaved at 136 degrees centigrade and then treated as clinical waste. and removed from the RVC by a professional transport company for incineration at an off-site crematorium (Cambridge Pet Crematorium). Autoclave function will be monitored by annual services, the use of autoclave indicator tape (which measures a temperature of 134 degrees centigrade for 3 minutes) and checked using ComplyTM ThermologTM Steam Chemical integrator indicator sticks (3M no.2134) during every run. This will result in complete inactivation of GMMs. Preparation of viruses will take place in appropriately designated area within the lab at the RVC. Excess virus will also be disposed of by decontamination as outlined (above). These designated areas all within a single building that is different from that in which any viable, hatched avian species will be kept For this reason as well as those outlined above, the risk to avian health is insignificant.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
To review ass 2 project # 189 with on Effects of gain-of-function and loss-of-function genetic manipulation of cells and tissues. This was an update of a project started in 2003 to include a replication competent avian leukosis virus vector (RCAS) from which the original src gene has been removed. This virus does not replicate in mammals is termed class 2 because of advice on low oncogenicity of every transmissible avian leucosis virus participated in discussion of his project which centered on 3 issues. Firstly, how reasonable is the case for RCAS pathogenicity to animals. The evidence is based on a paper in which intravenous injection of one million infectious units of virus into 18-day-old embryos caused 2% lymphomas within 4 months (Tam et al, J Virol, 76:4275). In the RVC class 2 project most avian embryos will be killed before day 10 of embryonation and none will be allowed to hatch. The work will all be done in a laboratory without any contact with the animal house where non-breeding chickens are only kept occasionally. Disease risk to birds is therefore very small. Secondly, was insertion of the wingless (wnt) gene into RCAS able to increase RCAS oncogenicity for chickens or self-injected humans. The wnt genes being used are known not to be potentially oncogenic, unlike wnt 1 and 3, and so this was not considered to add to tumour risk. Anecdotal evidence was that several cases of RCAS self injection did not result in any lesion, confirming the inability of RCAS to grow in mammalian cells. A third issue, unrelated to RCAS, concerned why waste from zebra fish in which harmless genes were being expressed by disabled virus, needed to be disinfected by Virkon. The committee concluded disinfection was unnecessary as no infectious 0MM could enter the water.
Project Additional Information

Purposes of the contained use

The purpose of the contained use in this project is to make defined deletion mutants of R. equi and to characterise the effects of these mutations on the ability to cause disease in mouse models of infection.

Recipient or parental organism

Both Escherichia coli and Rhodococcus equi will be used in this project. E. coli will be used as a cloning host for the manipulation of R. equi DNA. The strains that will be used are (crippled) laboratory E.coli K12 derived strains DH5alpha and TOP10. These strains are ACDP hazard group 1 and have a history of safe use in molecular biology (Sambrook & Russell, 2001)

R. equi is a Gram positive opportunistic bacterial pathogen that causes bronchopneumonia in foals. It is an ACDP hazard group 2 organism. The route of infection in foals is through the inhalation or ingestion of contaminated soil (Muscatello et al., 2009; Takai, 1997). R. equi infections have been described in humans however, such instances are often associated with an immuno-compromised status and are rare (Esteves et al., 2007). Transmission in humans is thought to be by inhalation or ingestion of R. equi in the environment (Verville et al., 1994). The strain used in this project is a virulent foal isolate and has been widely used for studies of the type proposed here. (De La Pena-Moctezuma et al., 1996; Hondalus & Mosser, 1994; Kelly et al., 2002; Navas et al., 2001; Pei et al., 2006 Pei et al., 2007; Wall et al., 2005). This strain contains a conjugative plasmid (VapA) that codes for a number of virulence factors (Takai et al., 2000).

The GMMs will be E.coli containing R. equi DNA cloned in a non-integrative, self-replicating extra-chromosomal plasmid. This is non-self cloning ie. the inserted DNA is not derived from the host organism. Mutants of R. equi will be derived by allelic exchange to replace a specific gene with a deleted version. Also, complementation of these mutations will be achieved by expressing the wild-type R. equi gene on a self-replicating extra-chromosomal plasmid. These manipulations are self cloning ie. the inserted DNA is derived from the host organism.

References


Host/vector system

pUC18/19 - These are widely used for the cloning and manipulation of genes in E. coli. They are ampicillin resistant and non-conjugative (Sambrook and Russell, 2001). These vectors will not replicate in R. equi and will only be used in E. coli.

pSelAct - This is a suicide vector based on pBlueScript (II) KS. It is able to replicate in E. coli but no R. equi (van der Geize et al., 2008). It will be used for the manipulation of R. equi DNA in E. coli and for the introduction of the deleted version of the gene(s) into the R. equi genome. It is resistant to apramycin and contains cytosine deaminase and uracil phosphoribosy transferase as the counter-selective markers. It contains oriT for conjugation however for conjugation to occur an an accesory plasmid encoding RP4 needs to be present. The conjugative function of the plasmid will not be functional under the conditions of use in this project. This plasmid will be lost from the desired mutant organisms so none of the markers on the vector will be present in the final GMO.

pREV series - These are shuttle vectors that are able to replicate in both E. coli and R. equi. They will be used for the ccomplementation of mutations. They will be used in both E. coli and R. equi. They are non-conjugative and code for apramycin resistant (Mangan et al., 2005)

References


1. Non self-cloning: R. equi DNA will be cloned in pUC18/19 and pSelAct for manipulation in E. coli. R. equi DNA will be extracted from the wild-type strain R. equi 103P+. The pUC series of vectors are commercially available.

2. Self cloning (deletion by allelic exchange): R. equi DNA cloned in pSelAct will be re-introduced into R. equi for the deletion of the genes by allelic exchange. pSelAct will be attained from the R. equi research community.

3. Self cloning (complementation): R. equi DNA cloned into pREV vectors and introduced back into R. equi for the restoration of function of the deleted gene. pREV vectors will be attained from the R. equi research community.

4. Target R. equi genes. This project focuses on genes involved in fatty acid and cholesterol catabolism and associated transcriptional regulators e.g. prpB, prpC, prpD, fadB, choD, choE, hsaD, kstR, and kstR2. The hypothesis is that R. equi uses fatty acids and/or cholesterol for survival in vivo and deletion of these genes will cause attenuation. The rationale for this is that deletion of gene(s) involved in these pathways in the closely related pathogen M. tuberculosis has caused attenuation (Brzostek et al., 2007; Munoz-Elias & McKinney, 2005). It is not possible to give a complete list of the genes that will be deleted as these may change as the project progresses, however, these will be genes involved in the same pathways e.g. fatty acid catabolism, cholesterol catabolism and associated transcriptional regulators.

The aim of this project is to generate deletion mutants of R. equi using allelic exchange. The mutagenesis procedure takes place in two steps. A deleted version of the gene is made in the vector pSel/Act using E. coli as a host for propagation of the vector. This construct is then introduced into R. equi where a single recombination event results in the integration of a deleted version of the gene and the vector sequence into the R. equi genome. The vector is then selected against using the counter-selective markers cytosine deaminase and uracil phosphoribosyl transferase. A double cross over event results in the loss of the integrated vector from the genome and replacement of the wild-type gene with a deleted version.

Complementation experiments involve the re-introduction of the wild-type gene back into R. equi. These experiments will use the pREV vectors and are required to demonstrate that the effects observed are due to gene deletion and are not the result of artefacts.

References


Evaluation of foreseeable effects

The E. coli host strains are non-pathogenic and disabled but the effects of genetically modifying this host strain has to be evaluated. Cloning and manipulation of R. equi genes involved in fatty acid or cholesterol catabolism using e.coli as a host is not expected to alter the pathogenic status or enable the bacterium to survive in the environment. Additionally, these genes will not be expressed in E.coli so the risks presented by this genetic manipulation are minimal. The genetically modified version of E. coli is not expected to be more or less hazardous than the wild-type.

R. equi is a soil organism and hazards could in theory arise if there was a breach in containment. As the route of infection in foals and in man is the inhalation or ingestion of contaminated soil and dust (Takai, 1997; Vervill et al., 1994), if the GMMs were to multiply rapidly within the environment and then foals or humans coming into contact with the contaminated area could be at risk of infection with the GMMs.

However, such hazards are very low as the GM R. equi strains described here are unlikely to be able to survive within the environment or cause disease with a better ability than wild-type. Firstly, the genes that will be deleted are involved in pathways that are used by the bacterium for survival in the soil and in the host therefore if the deletion has any effect, it is expected to result in decreased survival (Hughes & Sulaiman, 1987; Wall et al., 2005, Brzostek et al., 2007, Munoz-Elias and McKinney, 2005).
Secondly, GMMs which carry the complementing plasmid will be expected to be less fit due to additional genetic load. Therefore the GM strains of R. equi are not expected to pose an enhanced threat to human health or the environment in comparison with the wild-type strain.

The deletion mutants of R. equi do not carry any antibiotic resistance markers however, the complementing mutants will be apramycin resistant, therefore the selective advantage of apramycin in the unlikely event of a breach of containment has to be considered. Apramycin is used in animal husbandry however, it is not currently used to treat R. equi infections in foals nor is it used in human medicine. Therefore it is unlikely to confer a selective advantage to the modified strains.

R. equi contains a conjugative plasmid (VapA) encoding for virulence factors, therefore the effects of the genetic manipulation on the mobilisability of the plasmid has to be considered. There is no reason to assume that genetically modifying the genome of R. equi by gene deletion or complementation (as proposed here) will affect the conjugation functions of this plasmid.

The overall assessment of the effects of genetically modifying R. equi as described here is that the modified strains pose no more of a threat to human helath or to the environment than the wild-type.

References


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The RVC does not have facilities suitable for the sterilisation of small animal (mice) carcasses therefore the mice infected with GM R. equi must be collected and incinerated off-site by a licensed GM disposal company. The waste will be bagged and placed in single use plastic containers with lid locks and removed from site for incineration by Peaceful Pets (Norfolk) HSE GM site number 966. Contaminated sharps arising from the in vivo work will also be placed immediately in a sharps bin with lockable lids and removed from site for incineration by the same company.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste generated from the molecular biology laboratory, including plate cultures, will be sealed in plastic bags and stored within a robust stainless steel container before transfer to the auto-clave for inactivation (15 minutes at 121°C) prior to disposal off-site by approved RVC procedures.
All liquid waste generated from the molecular biology laboratory will be disinfected with 5% Trigene overnight before being disposed of down a suitable sink in the category 2 laboratory. 5% Trigene has been tested on concentrated culture (5x108 CFU/ml) and we have seen a complete kill after overnight disinfection (16h).

The waste generated from the in vivo experiments (animal carcasses and bedding) will be bagged and placed in single use plastic containers with lid locks and removed from site for incineration. The cages will be autoclaved prior to washing in an automated cage washing system. Contaminated sharps arising from the in vivo work will also be placed immediately in a sharps bin with a lockable lid and removed from site for incineration.

The waste water from the animal drinking bottles will be disinfected with 5% Trigene before disposal down a suitable sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
1. It is stated that the lab staff will be screened by Occ Health - should the BSU staff listed also be screened (perhaps they are routinely but I think it should be noted)? All staff that come into contact with R. equi, GM R. equi or unsterilised waste will be screened by occupational health before the project commences (SD4200 - Part B, section 4viii).

2. For the in vivo work - what precautions will BSU staff take when feeding and cleaning out the cages of the immunised animals? (or is it covered by COSHH). The cleaning of the cages at the end of the experiment will be done in a containment level 2 hood with personnel wearing PPE - lab coats, gloves, overshoes and a 3M 9322 P2 face mask (SD4200-Part B, Section 4). COSHH assessments will be done when work commences.

3. It is mentioned that the mice are culled at 4 days post challenge - are the cages cleaned in the interim (ie daily etc) to remove faeces - if so I think that the procedure for handling this should be noted. Routine maintenance (cleaning, feeding, watering) will not be required over this time frame (SD4200-Part B, sections 1i and 4i).

4. Will the animals be housed in the same room as other mice (other experiments) - or will they be in a separate room? The animals will be kept in experimental groups in individually ventilated cages (IVC). The air passing out of these cages is HEPA filtered and will minimise the presence of contaminated cage material in the room. The IVC system is housed in a single room. There will be no other experimental mice in the room (SD4200 - Part B, section 1i).

5. It is not clear in these forms what the pREV shuttle vectors are used for. As I understand, pSel/Act is used for the generation of the deletion mutants, why do the pREV vectors need to be used? The pREV vectors need to be used for complementation of the mutation. This is the restoration of function and shows that the effects seen are due to the genetic modification and not artefacts. It is a standard control in these types of experiments.

6. Clarify that RP4 is not being used? The functions of RP4 are not being used. pSel/Act does not code for RP4 functions, however, the RP4 functions are encoded on VapA, a plasmid found in wild-type R. equi 103 P+. The risks associated with this have been addressed (SD4200 - Part B, sections 1iv and 3iv).

7. Also are sharp bins autoclaved before being taken off site - or are these taken by Peaceful Pets too? The sharps will be placed immediately in sharps bins with lockable lids. They will be removed from site for incineration without prior autoclaving (SD4200 - Part B, secton 4ii).

**Project Containment**

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<th>Growth Rooms</th>
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**Project Ref** 314/11.1

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The role of Staphylococcus aureus genetic variation in host-adaptation

Purposes of the contained use

Meticillin-resistant Staphylococcus aureus remains a major problem for human healthcare providers worldwide. During the past eight years, a pig-adapted strain MRSA ST398 has emerged which spreads rapidly amongst pigs but can also infect humans (Voss et al. Emerg Infect Dis 2005; 11: 1965-6). This project will investigate host-specific characteristics and the potential for naturally occurring exchange of genetic material between colonising strains of MRSA ST398 from pigs and people. Genetic modification of test strains is required to differentiate between pig-specific and human-specific strains. The growth and characteristics of GM strains will be compared to those of the wild-type MRSA ST398 during a 6-week colonisation study on gnotobiotic piglets in isolators. Two similar studies with the wild-type MRSA ST398, which had required comprehensive safety measures, have already been conducted by the group within the past 24 months.

Recipient or parental organism

S. aureus and methicillin-resistant S. aureus (MRSA) are class 2 organisms. S. aureus is a coloniser of 25% of healthy humans. MRSA is a coloniser of approximately 2% of patients admitted to hospital in the UK. As a coloniser, S. aureus and MRSA appear to have no detrimental effect on the host. S. aureus is an opportunistic pathogen. The predominant infection caused by S. aureus and MRSA is a skin infection. However, patients that are immunocompromised can be susceptible to S. aureus/MRSA which can cause serious and even fatal infection. The widespread distribution of S. aureus ensures it is one of the most common causes of hospital acquired infection in immunocompromised patients. Antibiotic resistance is a major concern in hospitals as antibiotics are widely used to prevent infection as well as treat it. Infections of humans by the pig-associated strain MRSA ST398 have been reported and differences in clinical presentation or virulence have not been described (Wulf et al. Eur J Clin Microbiol Infect Dis 2011; Epub ahead of print).

Host/vector system

Suicide vectors for S. aureus will be used to inactivate putative virulence genes. An example is the use of pCN50 (Charpentier et al., 2004, Applied & Environmental Microbiology, 70: 6076-85) which contains a temperature selective replication of origin and chloramphenicol resistance gene for S. aureus, and an origin of replication and ampicillin resistance gene for E. coli. The cloned gene in the vector is knocked out by a resistance gene cassette such as erythromycin. The plasmid is transferred to S. aureus, where it undergoes homologous recombination with the intact gene twice (ie. once on each side of the gene of interest, Campbell integration), and this leads to loss of most or all of the sequence encoding the putative virulence gene (for example sraP) and replacement with the deleted version of the gene, and loss of all the suicide vector genes. These steps will be performed by the team of Dr Jodi Lindsay, St George's, University of London under separate CU2 notifications (GM95/02.4 and GM95/00.1).
Inserted sequence(s): Antibiotic resistance marker, usually erythromycin or tetracycline or chloramphenicol. All of these resistance traits are found in naturally occurring isolates of S. aureus and MRSA in our community.

For all handling of the GMOs by co-workers, training and the Codes of Practice and SOPs set by host-institution will be followed (as stated in the attached Memorandum of Understanding between the RVC and SGUL).

Origin & function

The inserted gene sequence will be an antibiotic resistance marker, either erythromycin, tetracycline or chloramphenicol. The sequence poses no known or likely health hazard. All of these resistance genes are found in many naturally occurring staphylococcal isolates, including S. aureus and MRSA, in our community, in the environment, on humans, animals and wildlife. The gene sequence is derived from one of the pig-associated MRSA ST398 isolates. The insertion of a resistance gene will allow differentiation of the test isolates on selective agar supplemented with the relevant antibiotic. Growth of the wild-type will be inhibited by addition of antibiotic to the agar while the GMO strain will grow. This will enable quantification of colony forming units for each strain, allow assessment of growth dynamics and competition between the strains and inform on behaviour of colonising MRSA on intact skin.

At present, MRSA causes about 25% of all S. aureus hospital infection in the UK, and the majority of MRSA isolates are already resistant to erythromycin. Tetracycline and chloramphenicol resistance is found in about 10% of hospital-associated MRSA isolates, although none of these drugs is routinely used to prevent or treat infection. All isolates in the UK are currently susceptible to glycopeptide antibiotics. Overall, most patients are probably in contact with S. aureus, and do not develop infection. However, resistance to these three antimicrobials should not compromise the outcome of treatment in the case of infection.

Evaluation of foreseeable effects

The replacement of a putative virulence factor with an antimicrobial resistance gene is unlikely to alter the existing pathogenic traits of S. aureus. The deletion of potential virulence genes is likely to produce GMO that are less fit than their parent, and unlikely to present a threat. The addition of antibiotic resistance genes may give the GMO a selective advantage. However, such resistances are already found in naturally occurring human isolates. However, all of these resistance genes are found in many naturally occurring staphylococcal isolates, including S. aureus and MRSA, in our community, in the environment, on humans, animals and wildlife. Nevertheless, the unlikely possibility of increased threat associated with deletion mutants is understood and considered.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Off-site incineration. The RVC has no large incineration facility. Pig carcasses will be sealed in clinical waste bags via the isolator ports, transferred into hard plastic clinical waste bins with firmly closing lids and labelled as GMO content before removal from site. They will be collected by a licensed GM disposal company:

i) from the Camden site by Novus, Vetspeed,A505 Main Road, Thriplow Heath, Nr Royston, Hertfordshire, SG8 7RR

ii) from the Hawkshead site by Peaceful Pets, West Rudham, King's Lynn, Norfolk, PE31 8SY

Transport of bacteria from SGUL to RVC will be in packaging according to packaging Instructions 650 for Biological Substances Category B: Organisms will be transported on agar slopes or in 2ml broth vials contained in a leakproof primary receptacle, a leakproof secondary container and with absorbent material between both, sufficient to soak up the total amount of fluid. These will be packed in a rigid out packaging, robust enough to withstand a 1.2m drop and labelled with name, address, telephone number of the RVC supervisor, a hazard diamond label and marked as 'Biological substance Category B'.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The construction of the GMOs and associated waste disposal will be carried out by the team of Dr *, St George’s, University of London, under her HSE notifications GM95/02.4 and GM95/00.1.
The work at RVC will involve 1. growing the bacteria on solid agar or in small volumes (typically 2ml) of liquid broth in a containment level 2 laboratory (in a class 2 microbiological safety cabinet), 2. transfer of GMO vials into three piglet isolators through a sealed port (disinfection with peracetic acid spray), 3. atraumatic inoculation of gnotobiotic piglets within sealed isolation chambers (isolators), 4. sampling of piglets by swabbing of skin and 5. growth and counting of bacteria in the laboratory (RVC risk assessment attached). Appropriate Codes of Practice, SOP and COSHH documents have been prepared for the procedures involved and all involved personnel are made aware of these.

Comprehensive protocols for safe disposal of containment level 2 organisms are already in place at all sites. S. aureus and MRSA are class 2 organisms and two gnotobiotic piglet experiments have already been carried out by the group within the last 24 months with disposal procedures for MRSA safely implemented.

In the laboratory, small plastic waste, including the swabs from piglets, will be immersed in 1% Virkon overnight before draining off the fluid and disposal in clinical waste bags by incineration (off site, Novus/Vetspeed or Peaceful Pets). Larger plastic items (petri dishes) will be autoclaved (121 degrees Celsius for 15 minutes) before disposal by incineration. Small volumes of liquid waste are deactivated in 1% Virkon before disposal. Sharps (glass slides) will be disposed in sharps containers which will be autoclaved and subsequently incinerated. Fluid and sharps containers will be labelled for GMO use.

At the end of the piglet study, needles will be required for euthanasia of the animals. Piglets will be sedated prior to euthanasia with azaperone (Stresnil, recommended dose per kg bodyweight given rectally by plastic pipettes, followed by a 20-minute quiet time in darkness to achieve maximum sedation). This will minimise the risk of accidental needlestick injury and avoid the need for a second person restraining the animal for euthanasia. Needles used for euthanasia will be disposed off intact and unsharpened together with the syringe body in sharps containers within the piglet isolators (sharps containers are autoclaved and included in isolators prior to study begin). Carcasses will be disposed of as described above (Novus/Vetspeed).

Aerosols will only be generated within the isolators. The isolators are designed to prevent micro-organisms entering or leaving the isolators, and animals are handled through gloves. Isolators will be kept in rooms with requirements appropriate for CL2 animal facility rooms for GMM work (SACGM Part 3, page 59). All material that needs to be introduced into the isolators will go through ports, be sprayed with peracetic acid and an at least 30-minute contact time. Material to come out of the isolators will be transferred into bags or containers (kept inside the isolators before commencement of study), then sprayed out through the port as described above and then directly transferred into clinical waste bags for incineration or (swabs) taken to the containment level 2 laboratory for processing. If the isolators fail, our experiment has failed, so there is enormous incentive to ensure no release of organisms.

At the end of the study, piglets will be sedated and euthanased within the isolators, carcasses transferred into sealed containers through the ports and collected by Novus/Vetspeed.

All small isolator equipment will be bagged as clinical waste before off-site incineration. Isolators and faeces contained in the lower part of the isolator are decontaminated by using Virkon sprays and soaks overnight as far as reasonably practical. Swabs off isolator faecal contents will be taken and the process repeated several times until nil bacterial growth is detected before dismantling the isolators. However, it will be assumed that complete sterility may not be achievable due to the high faecal load. Thus, piglet isolators will be dismantled using protective clothing (Ty-Vek boiler suit, gloves, masks, overshoes). Faeces, plastic films and gloves are bagged as clinical waste and collected for incineration. All metal parts of the isolators will be cleaned with detergents in the in the automated cage washer at 80 degrees and disinfected with 1% Virkon solution. Afterwards floors will get cleaned with detergent and disinfected with Virkon 1%.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment
The activity has been reviewed and approved by the local RVC GMO committee. The risk assessment submitted to the SGUL GMSC was reviewed and approved after more explicit information was given on
1) the putative host-specificity genes,
2) waste disposal and
3) the measures in place for the reduction of needlestick injuries.
All comments were considered and addressed. The final version of the risk assessment is included with this form.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### Project Ref 314/12.1

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<tr>
<td>24/10/2012</td>
<td>Construction and analysis of mutants of Mycoplasma species</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
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</table>

Non-GMM

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Purposes of the contained use

Construction of transposon mutants and site-specific mutants of Mycoplasma species. Analysis of these mutants in vitro and in their natural host animal species for function and phenotype in relation to pathogenicity. Complementation of those mutants using a gene expressed for function and phenotype in relation to pathogenicity. Complementation of those mutants using a gene expressed from an OriC-based plasmid. Screening of pools of mutants for selection (in vitro and in vivo) for mutants less-fit to survive in the animal body.

Recipient or parental organism

Mycoplasma species, including Mycoplasma gallisepticum, Mycoplasma cynos and Mycoplasma hyopneumoniae. These are pathogens of chickens, dogs and pigs respectively. Other, similar mycoplasmas are included in this notification.

Host/vector system

pBSC-1 miniHimar (Mariner) transposon carrying puromycin resistance or tetM, delivered to Mycoplasma sp on a suicide vector such as pUC18. OriC-based plasmid derived from the OriC-region of the Mycoplasma to be transformed to Mycoplasma sp in place of natural replicative plasmids.

Origin & function

The origin of the inserted DNA is bacterial plasmid genes such as antibiotic resistance (tetM) together with components of transposable elements such as miniTn4001PsPuro [Algire et al. (2009). New selectable marker for manipulating the simple genomes of Mycoplasma species. Antimicrob Agents Chemother. 53, 4429-4432], and miniHimar [Bouhenni et al. (2005). Identification of genes involved in cytochrome c biogenesis in Shewanella oneidensis, using a modified mariner transposon. Applied & Environmental Microbiology, 71, 4935-4937.] The intended function is to insert the transposon to cause random mutations.

Secondly, OriC DNA is the region of chromosomal replication origin, derived from Mycoplasma species, used to facilitate the construction of plasmids able to replicate in Mycoplasma species. [Lee et al. (2008). Development of a replicable OriC plasmid for Mycoplasma gallisepticum and Mycoplasma imitans, and gene disruption through homologous recombination in M.gallisepticum. Microbiology 154, 2571-2580]. The intended function is to allow complementation of gene function lost through mutation, and also to insert flanking DNA into mycoplasma species to cause allelic replacement mutations.

Evaluation of foreseeable effects

The GMMs will be mutants of Mycoplasma species derived by either random insertion of a transposon, or by allelic exchange to replace a specific gene with a deleted or mutant form of the gene. Some mutants will be restored to normal function by complementation using the functional gene expressed on an OriC-based plasmid. Apart from the introduction of marker genes such as puromycin resistance or tetracycline resistance, and the inverted repeat sequences of the transposable element, the experiments will be self-cloning, ie. Inserted DNA will be otherwise derived from the host organism.

Hazards to the environment could only arise if the organisms were able to gain access to the host animal population outside the containment facility. This could only happen through direct or indirect contact with other animals or by carriage outside the facility by humans. Such a possibility will be prevented by the measures outlined below.

Such hazard would be very unlikely to lead to disease or harm to the host animals into which they were introduced beyond that which would be caused by the natural parental organisms. GMMs which carry a mutation, or additional genetic load due to a plasmid complementing that mutation, would be less (or not more) fit to survive in the competitive environment of the host animal than the wild type.

Forseeable effects on the environment arising from the intended modifications are expected to make the organisms less fit to survive in the environment or their host animal. Mutation in the GMO are not expected to enhance pathogenicity or ability to spread or survive in the environment and no such natural or engineered mutants have been recorded.

Animal mycoplasmas are highly adapted to their own host. Human disease due to these animal pathogens, which are naturally widespread among livestock, has never been recorded and is therefore unforseeable in these mutant mycoplasmas.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste, including cultures will be sealed in plastic bags, stored within a robust container before transfer to the autoclave for inactivation (121°C for 15 minutes) prior to disposal by offsite incineration. All liquid waste will be mixed with Virkon or hypochlorite to give a final minimum concentration of 1% Virkon or 200 ppm hypochlorite and stored overnight, or alternatively boiled before the residue is flushed away with excess water. Plastic pipettes/pipette tips will be immersed in 1% Virkon or 200ppm hypochlorite overnight before the liquid is drained off, the plastic put into bags for autoclaving and disposal.

Animals such as pigs or chickens, infected with GMM, will be killed on the premises. Carcasses will be opened in the post-mortem room for removal and examination of tissues. Samples for culture may be taken from the lungs to the laboratory. Carcass material will be disposed of by sealing in plastic sealable medibins with a weight limit of 25 kg per bin and removal by a licensed disposal company (e.g. Cambridge Pet Crematorium, for incineration. Alternatively, lung tissues may be homogenised, within a Class 1 microbiological safety cabinet within the post-mortem suite. After sampling for laboratory procedures, remaining homogenised tissue will be autoclaved and then destroyed via the general clinical waste disposal. Bedding waste from the Containment level 2 animal facility will be inactivated by soaking with 1% Virkon for at least 12 hours. Liquid waste is prevented from entering the drains and is treated with an excess of 1% Virkon for at least 12 hours before released into the drains.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Form A section 2b. Does not mention replacement of the kanR sequences with TetR which is mentioned in FormB (section 1iii).
Form B section 1 Third paragraph on antimicrobial selection marker should also address the use of TetR which is mentioned later in the form.
Form B Section 3iii - How measures will be put in place to avoid needle-stick injuries when injecting pigs
Form B Sectio 3 iv "waste disposed of according to guidelines" Does this refer to just "animal waste" or also bedding material - What guidelines are being referred to?

Part A states;
"Waste disposal procedures: Disinfection overnight with 1% Trigene. All contaminated culture plates will be autoclaved before disposal. "However Part B states "Solid and liquid cultures and contaminated waste materials will be disposed of from the laboratory by autoclaving followed by incineration. "Suggesting liquid waste will also be autoclaved. I think it needs to be consistent.

Part B 2 states;
"The antimicrobial selection marker on the miniHamar 1 transposon inserted into M. hyopneumoniae confers resistance to kanamycin which is not used to treat any disease in the pig. Other antibiotic resistance elements may be used." - what other antibiotic resistance elements - should these be listed? Could these be a problem?
### Project Ref 314/16.1

**Date Ackn’ed:** 03/02/2016  
**CU2 Project Title:** Production and use of a green fluorescent protein expressing Campylobacter jejuni to investigate the location of bacterial colonisation in the chicken caeca in the presence or absence of Eimeria tenella co-infection

**Class:** Class 2  
**CultureVolClass2:** < 1 Litre  
**CultureVolumeClass3-4:** Non-GMM  
**Consent Granted:**

**Withdrawn:** N  
**Tick if notifying a connected programme of work:** N

**Project notified under transitional arrangements:** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

Use a green-fluorescent protein (GFP) expressing C. jejuni to determine the impact of E. tenella co-infection on bacterial colonisation in the chicken caeca.

**Recipient or parental organism**

Recipient will be Campylobacter jejuni 81-176. This strain has been routinely used within the group for in-vivo trials for more than seven years, first at the Institute for Animal Health and more recently at the Royal Veterinary College, University of London. During this period no pathogenic effects or instability have been detected in chickens.
Host/vector system

The vector is the plasmid pHG101, supporting expression of green fluorescent protein, kindly provided by the University of Saskatchewan under material transfer agreement (MTA). The plasmid contains the following background components: a Campylobacter kanamycin resistance gene, Campylobacter origin of replication (repS), E. coli ColE1 origin of replication, consensus primer (Pc) and a translation initiation region (TIR). The original plasmid also contains a mobility (mob) gene which will be removed by targeted double restriction enzyme digestion and re-ligation prior to use, confirmed by PCR and sequencing.

Origin & function

The plasmid pHG101 was kindly provided by the University of Saskatchewan under MTA. This plasmid was originally constructed using fragments from plasmid AF29255 and plasmid pQSI-25. The Campylobacter origin of replication, ColE1 origin of replication and Campylobacter kanamycin resistance cassette were obtained from plasmid AF29255. The GFP gene was amplified from plasmid pQSI-25. Purified plasmid AF29255 and purified GFP PCR product were digested with enzymes EcoRI and AatII. The two DNA fragments were ligated and transformed into E. coli DH5α, plated out and colonies screened by whole cell PCR. The original plasmid also included a Campylobacter mobility element (mob), although this has been removed by targeted restriction digestion, plasmid purification and re-ligation. Campylobacter jejuni grown overnight from a frozen stock.Growing bacteria will be re-streaked onto a new plate to obtain confluent growth. Plates will be incubated overnight and bacteria harvested with 800 µl electroporation buffer (15% glycerol/9% sucrose) and centrifuged for 1.5 mins at 9000 x g. Supernatant will be decanted and cells resuspended in 800 µl of the same buffer and centrifuged again. Supernatant will be decanted and pellet resuspended in 125 µl of the same buffer and kept on ice. For every 40 µl of cells 1 µg of plasmid DNA (pHG101) will be added and mixed. The mix will be transferred into a pre-chilled electroporation cuvette and cells will be pulsed with 1.25 kV with a constant range of 4-6 mS in a Gene Pulser xcell (BioRad) electroporator (or similar). Immediately 500 µl of SOC media will be added to the cuvette and mixed well. The resultant Campylobacter line will be known as C. jejuni 81-176G. Campylobacter jejuni 81-176G will then be orally dosed to chickens in facilities approved for work with genetically modified Eimeria (at derogated containment level 3) in order to map variation in the colonisation of a green fluorescent protein (GFP) expressing C. jejuni in the chicken caeca in the presence or absence of wild-type E. tenella.

Evaluation of foreseeable effects

Campylobacter jejuni - ACDP Category 2. Campylobacter jejuni can cause gastro-intestinal disease in humans. The vector confers resistance to kanamycin. Nonetheless, the recipient C. jejuni isolate remains susceptible to all antibiotics routinely used to control this pathogen, as such the addition of kanamycin resistance is not expected to incur any additional hazard.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be collected in autoclave bags and placed in solid waste bins for autoclaving (121-125°C for at least 15 minutes). Solid waste is then sent for incineration after autoclaving through the Royal Veterinary College's retained waste disposal supplier.
Liquid waste will either be treated by autoclaving as above or decontaminated using Trigene at a concentration of 1:20 for a minimum of ten minutes prior to drain disposal. Lab coats will be sealed in autoclave bags after use and autoclaved before they go to the laundry. All animal rooms contain dedicated over clothes, boots and disposable gloves which are put on as part of the room entry procedure and removed as part of the room exit procedure. Personal protective equipment will be supplemented with a suitable face mask during periods of C. jejuni infection.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

JD suggested that the project might be split, with the E. coli part involving mob gene deletion could be handled internally as a separate RA. This would simplify your HSE application.

SM clarified that the entire mob region of the plasmid will be removed and that removal will be verified by genetic analysis (e.g., RA digestion, PCR, sequencing).

FD and MA requested that a more detailed description of waste inactivation be inserted, and specify that treated liquid is disposed down the sink.

JD suggested removal of Clostridium perfringens from abstract, to avoid potential confusion.

OS clarified that the GMO pathogen-infected animals are finally culled, double bagged and autoclaved on-site.

Project Containment

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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 314/19.2

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<td>20/06/2019</td>
<td>Identification of carbohydrate-based structures on the surface of Theileria parva</td>
<td>Class 2</td>
<td>Not Applicable</td>
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The overall aim behind the proposed work is to identify carbohydrate-based structures on the surface of Theileria parva sporozoites/schizonts which are involved in the very early stages of infection of host cells and subsequently to genetically interfere with the synthesis/expression of these structures. The objectives are to:

1. Identify an efficient transfection system for parasitized (Theileria parva and Theileria annulata (Tp and Ta, respectively) schizonts and sporozoites) cell lines (bovine leukocytes) testing PHMB, Superfect and Nucleofector with fluorescently tagged oligonucleotides (ODN).
2. Determine suitable cell penetrating peptides (CPP) for efficient transfection of parasitized Theileria schizonts and sporozoites cell lines (bovine leukocytes) using different CPP sequences with fluorescently tagged nucleic acid analogues (PNA and MO).
3. Try to target products in the synthesis process of p104, PIM and previously determined glycans of Theileria parva schizonts with different antisense agents (plasmid DNA I dsRNA).

The genetically modified organisms will be Theileria (T.) annulata and T.parva sporozoites and schizonts.

The plasmid to be used at the beginning is a novel foreign protein expression vector pMotif-EF-1a-100, which was derived from pRNAi-GL (Takara, Japan), a conventional mammalian protein expression vector, and can be propagated in E.coli. This plasmid has been published here (Vet Parasitol. 2015 Mar 15;208(3-4):238-41. doi: 10.1016/j.vetpar.2015.01.013. Epub 2015 Jan 25. Transfection of live, tick derived sporozoites of the protozoan Apicomplexan parasite Theileria parva. De Goeyse I, Jansen F, Madder M, Hayashida K, Berkvens D, Dobbelkaere D, Geyser D.) In this vector, the Original CMV promoter gene was replaced with the elongation factor 1 alpha (EF-1α) gene promoter region of T. parva, as used successfully in transfection of Babesia and Plasmodium(Femandez-Becerra et al., 2003, Suarez and McElwain, 2009). The minus 263 to 169 position of the 5' region of the EF-1α orf (TP01_0726) was amplified from the T. parva Muguga strain genomic DNA. The promoter sequence is preceded by a motif sequence, consisting of three tandem putative transcription factor binding sites of T. parva (Guo and Silva, 2016).
Further, a transmembrane Azami Green sequence, preceded by a secretion signal sequence was integrated to visualize transfected parasites. Azami Green is a green-emitting fluorescent protein from a stony coral, Galaxea, which absorbs light maximally at 492 nm and emits the green light at 505 nm. The protein is stable and fluorescence is brighter in cultured cells than EGFP (Karasawa et al., 2003). This plasmid will initially be used to transiently-transfect purified T. annulata and T. parva sporozoites and schizonts, which will be generated from frozen down stabilates. The optimal vector and technique for transfection will need to be determined. Methods to be tested are polyhexamethylene biguanide (PHMB), Superfect, the 4D-Nucleofector system Lonza and cell penetrating peptides (CPPs) to deliver either plasmid DNA or dsRNA.

Origin & function

PHMB has a solid safety record and can be beneficial in wound care (Chindera et al. 2016). In contrast, as typical for cationic polymers, high concentrations are cell toxic and most commonly used as biocides. Although PHMB seems to be a potent antiparasitidal candidate drug, much lower concentrations could also be used as a delivery vehicle for nucleic acid immunomodulators or plasmids which will be examined here. Cell penetrating peptides are synthetically constrained through various chemical modifications that stabilize a given structural fold with the potential to improve competitive binding to specific targets (Haidar et al., 2018). Both, plasmids and CPPs are non-mobilisable and considered as non-hazardous.

References:

Evaluation of foreseeable effects

Theileria annulata and parva are not viable outside the host cells, and are unable to infect non-ruminant cells. Apart from that the transfection is expected to decrease the infectivity of the parasite. The obligately intracellular Theileria sporozoites and schizonts only survive a very short period [hours] outside the tick salivary glands or cell cultures. They are only able to infect ruminant cells after the transmission via specific ticks [Rhipicephalus appendiculatus for Theileria parva and Hyalomma spp. for Theileria annulata], both of which are currently not present within the UK, or after direct inoculation with their host cells.

Vectors used for this project will be plasmid DNA, cell penetrating peptides (CPPs) and PHMB. Both plasmids and CPPs are non-mobilisable and considered as non-hazardous. PHMB in low concentrations as it will be used for this project are non-toxic but are used for beneficial effects instead (see above).

References:
**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

This project will focus on cell culture work. No larger GMOs will be used.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The work will be carried out in a Biological Safety Category 2 laboratory.

Health and Safety

Executive

Surfaces which suspected to be contaminated with Theileria will be decontaminated with 1 % Virkon. Glass-ware, metals or other rigid solids will be soaked in 1 % Virkon solution for 24 hours, steam treated, autoclaved and then either disposed or washed for re-use.

Contaminated solid waste such as plastic, paper, organic matter or fabric will be steam treated, autoclaved and then disposed as clinical waste.

Theileria sporozoites and schizonts reside obligately intracellular in the cells of their hosts [ticks and ruminants] and are therefore very fragile and vulnerable in the environment as well as under laboratory conditions. The required tick vectors [Rhipicephalus appendiculatus and Hyalomma spp.] are not native in the United Kingdom so that the disease can only be transmitted by injecting sporozoites directly into the blood of cattle.

Contaminated liquid waste such as liquid parasite and/or host cell cultures will be treated by addition of 1 % Virkon (by adding approx. equal volumes of 2%) and left to stand for 24 hours before disposal into the drains.

All Theileria parva and Theileria annulata pathogens are stored frozen in liquid nitrogen.

All the processing of the pathogens for freezing are undertaken in a specified pathogen laboratory.

The frozen specified pathogens are stored in the RVC's Liquid Nitrogen storage facility. Access to this storage facility is restricted.

When Theileria Specified Animal Pathogens are moved from one designated facility to another within the RVC, this will be done using properly labelled UN602 standard containers. The containers are never opened outside the Specified Animal Pathogen handling laboratory or other SAPO designated areas of the RVC.

A record of all stored material is kept, and all additions and removals are promptly entered into the records database.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units
Large Scale Activities
Human Clinical Applications

Laboratory Activities
- Glass Houses
- Growth Rooms

Project Ref 314/19.3

Date Ackn’d 02/08/2019
CU2 Project Title
- Investigation of the role of innate immune receptors in bovine tuberculosis using fluorescently labelled Mycobacterium bovis Bacille Calmette-Guerin (GFP-BCG)

Class 2
CultureVolClass2 < 1 Litre
CultureVolumeClass3-4
Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
The aim of this project is to investigate the role that innate immune receptors, such as toll-like receptors and C-type lectin receptors, play in bovine TB infection. Assays will be performed to measure the uptake of fluorescently labelled M. bovis BCG (GFP-BCG) by monocyte derived macrophages and dendritic cells in the presence and
absence of antibodies designed to block specific innate immune receptors.

Recipient or parental organism

*Mycobacterium bovis Bacille Calmette-Guerin (BCG) (Pasteur Strain).*

Host/vector system

The vaccine strain of *M. bovis* BCG expressing a mutant Green Fluorescent Protein (GFP) was constructed previously by M. Chambers (Imperial College London, UK). No further modification of this organism will occur on site.

Briefly, the pMOD-12 dual promoter expression plasmid [1] was modified for use. The unnecessary mycobacterial origin of replication was removed. The mycobacteriophage L5 integrase gene [2-4] was inserted to create an integrating vector, pMIN12. The Maxygen pBAD-GFP cycle 3 plasmid was used for its optimised synthetic mutant gene of the GFP [5]. This gene was amplified by PCR and ligated into pMIN12 downstream of the heat shock protein 70 (Hsp70) promoter, which also promotes kanamycin resistance. This new plasmid is referred to as pBright GFP.

References:


Origin & function

GFP is a green-emitting fluorescent protein, originally isolated from the jellyfish Aequorea victoria, which acts as a reporter system enabling real-time visualisation of the pathogen by flow cytometry and UV microscopy [1]. The phage L5 integrase gene was used to enable stable integration of the GFP into the host genome. This system was originally identified in a mycobacteriophage [2] and has been used for over 20 years and allows the stable insertion of reporter genes in to mycobacteria.

The plasmid contains an antibiotic resistance gene for kanamycin to enable selection of GFP positive host mycobacteria. This antibiotic is not used in therapy and has a history of safe use in the laboratory.

References:


Evaluation of foreseeable effects

*M. bovis* BCG (Pasteur) is an attenuated vaccine strain for *M. tuberculosis* and although there are isolated incidences of infection following vaccination (0.06 to 1.56 cases per million vaccinations) [1] the risk to human health is low and can be further minimised by the use of recommended working practises.
The environmental risks of M. bovis BCG are low as there are no instances of it infecting animals in a natural setting, and current knowledge suggests that it cannot survive within the protozoan environmental niche found in soil [2]. The plasmid used to generate the GFP-BCG is non-mobilisable and non-hazardous, its function is to generate a green-fluorescent reporter protein, it is unlikely to confer a selective advantage or increase the virulence/pathogenicity of the BCG.

References:

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This project will focus on cell culture work. No larger GMOs will be used.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The work will be carried out in a Biological Safety Category 2 laboratory. Surfaces which are suspected to be contaminated with BCG will be decontaminated with 5% Chemgene HLD. a disinfectant know to be mycobactericidal. Contaminated solid waste such as agar plates will be sterilised by autoclaving at 15 psi, 121 ·C for 15 minutes. The waste will be transferred from the lab to the autoclave in a sealed metal bin. GMO residues in pipette tips, cell culture plates and eppendorff tubes will be sterilised by soaking in a 5% Chemgene HLD disinfectant for at least 16 hours. After sterilisation items will be disposed of as clinical waste. Large 10-50ml cultures, containing high bacterial titres, that need to be disposed of will be sterilised by autoclaving at 15 psi, 121 ·C for 15 minutes. The cultures will be transferred from the lab to the autoclave in a sealed metal bin. Residual bacterial cultures and cell culture supernatants will be sterilised in 5 % Chemgene for at least 16 hrs. Any cells used in uptake assays will be fixed in 4% paraformaldehyde for 30 mins, to kill the BCG internalised by the cells, before being analysing. After analysis they will be disposed of as clinical waste.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N
This is a low risk project. The host is widely used as a vaccine strain, the vector and insert systems are widely used. The RA has correctly identified risks associated with the antimicrobial resistance marker. The waste handling plans are sufficient. The applicants should ensure that workers understand the waste handling procedures and avoid excessive inactivation. All workers on this project must watch the GMO training video.

**Project Containment**

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**Project Ref** 314/95.1

- **Date Ackn’d**: 21/03/1995
- **CU2 Project Title**: ANALYSIS OF HEPATITIS C VIRUS
- **Class**: Class 2
- **Non-GMM**: not applicable
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Project Containment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Activities</td>
</tr>
<tr>
<td>L2</td>
</tr>
<tr>
<td>Animal Units</td>
</tr>
<tr>
<td>L2</td>
</tr>
</tbody>
</table>
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
<td>Animal Units</td>
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</table>

Project Ref  97/08.1

Date Ackn'd  31/03/2011

Date Project Ceased

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Development of Eimeria tenella as a vehicle for the expression of heterologous coding sequences.

Class  Class 2

CultureVolClass2

CultureVolumeClass3-4

Consent Granted  Yes

Project notified under transitional arrangements  N

02/03/2022  Page 6155 of 15326
### Project Additional Information

#### Purposes of the contained use

**Objective:** To transfect Eimeria tenella with constructs designed to express sequences coding for heterologous protozoan, bacterial and viral antigens to facilitate assessment as a vaccine delivery vehicle.

#### Recipient or parental organism

The Eimeria species, including E. tenella, have been rated by the Advisory Committee On Dangerous Pathogens (ACDP) as requiring a containment level of 1. The Eimeria species are completely host-and, in examples, tissue-specific: E. tenella specifically parasitizes the chicken caecae and poses no risk to human health or safety.

#### Host/vector system

The commercially available vector pGEM-Teasy (Promega) will be used throughout these studies, together with a series of vectors developed for the purpose of transfecting Eimeria species parasites (Clark et al 2008. mol Biochem Parasitol 162:77-86) The XL1-Blue MRF Laboratory Escherichia coli strain (deficient in all known restriction systems [(mcrA183, (mcrCB-hsdSMR-mrr)173], endonuclease(endA) and recombination (recA) deficient will be used throughout these studies.

#### Origin & function

DNA templates encoding the Eimeria maxima SAG, Campylobacter jejuni CjaA and Avian influenza hemagglutinin antigens (HA) are already available within the IAH in plasmid stocks (derived by PCR amplification from gDNA or directly synthesised). Thus, there is no requirement to handle either C. Jejunii or the Avian influenza virus. Amplification and manipulation of E. maxima, An ACDP hazard group 1 pathogen, is routine within the IAH. All antigens will be expressed under the control of eimerian stage-specific promoters, limiting antigen production to defined steps of the transfection process which will be controlled as described in section 12.

The primary function of these studies is to generate lines of E. tenella that express heterologous antigens in order to assess the parasite’s potential as a vaccine delivery vehicle. Thus, once created, each line will be assessed for expression of the target antigen (i.e. by RT-PCR and immunolabelling) prior to use to immunise SPF chickens within the IAH experimental facility to monitor correlates of immune protection. Added value will be gained through the phenotypic assessment of all parasite lines generated for divergence from the recipient strain.

#### Evaluation of foreseeable effects

The Eimeria species are antigenically complex micro-organisms and their interaction with the host is mediated by a complex series of proteins expressed in a sequential manner. All three candidate coding sequences described in this application are considered likely to contribute to host/pathogen interaction in their natural context but whilst the application of such non-self proteins might elevate the potential for novel recipient tropism within the chicken, the addition of a single protein to such a complex process is unlikely to have a significant impact. Once cloned, these sequences will be expressed under the control of one or more stage specific promoters, limiting expression to the sporozoite and/or merozoite lifecycle stages.

**I) E. maxima surface antigens (SAGs).** Preliminary unpublished data suggest that SAG proteins expressed by E. tenella mediate parasite/host interaction but not invasion. Single parasite RT-PCR has revealed the concurrent expression of seven distinct SAG proteins. Whilst addition of a single novel SAG gene has the potential to modify the recipient parasite's ability to attach within the host, the possibility of a significant change in tropism is limited.

**II) C. jejuni CjaA antigen.** The C jejuni CjaA antigen has been shown to localise to the inner bacterial membrane where it is thought to function as a component of the ABC transporter system. CjaA acts as a receptor for the amino acids glutamine and cysteine. Thus, whilst CjaA could affect Eimeria/host interaction, expression on the surface of one or more zoite stages of the E.tenella lifecycle via a GPI anchor and not as an internal componant of the cell wall will minimise the influence of any possible effects. Expression of the native CjaA under the control of its own promoter in a Salmonella Typhimurium crp-cya-strain previously yielded no increase in virulence or persistence.

iii) Avian influenza HA's have been shown to play an essential role in mediating host cell entry. Pathogenicity among avian influenza isolates has been linked to the presence of a polybasic amino acid sequence at the HA cleavage site. The HA coding sequences to be used will be obtained by PCR amplification from existing artificially synthesised plasmid constructs which lack a polybasic amino acid sequence at the HA cleavage site. HA antigens have previously been delivered to chickens using viral vectors without severe repercussions (eg fowl pox virus, Newcastle disease virus).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory Eimeria species parasites have never been known to infect humans, thus laboratory handling of all stages of unmodified parasites is appropriately carried out with Hazard Group 1 containment. The transfection process introduces DNA by electroporation into Eimeria sporozoites, a stage of the lifecycle that exhibits severely limited environmental persistence and can only initiate infection following direct cloacal or oral (after bicarbonate gavage) inoculation. Following genetic transformation, the only stage of the parasite that requires additional containment and disposal measures is the oocyst. After recovery, oocysts will be stored in clearly marked, sealed bottles within a designated refrigerator. Bottles of transformed oocysts will be opened only in fumigatable rooms and all manipulations of these oocysts will be carried out in these rooms. Purified sporozoites will be removed to the laboratory for further experiments and these can be safely handled (as outlined above). Doses of oocytes may be removed, double bagged and in sealed containers, and taken to the EAH for infection of animals. All glassware, plasticware, paperware and liquids that contain oocysts, or are potentially contaminated with oocysts, will be fumigated with ammonia before autoclave sterilization.

Experimental Animal House. The passage, handling and disposal of oocysts (the environmentally stable stage of the eimerian lifecycle) will be carefully controlled. Animals are housed in strict isolation and cultured parasites are recovered in rooms which are then sealed for fumigation with ammonia, which destroys oocysts. The EAH facilities used are kept dry throughout all trials to limit oocyst survival and are ammonia fumigated in situ, together with all cages and any waste, at the conclusion of each experiment. All solid waste is removed after fumigation in sealed bags for incineration. Foot baths are removed before each trial commences and water traps are installed. All waste water is removed in sealed containers for autoclaving prior to disposal. Post fumigation all rooms are cleaned and re-fumigated shortly before re-stocking.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment was reviewed by the IAH Compton sites Genetic Modification Safety Committee. It was passed with no problem.

**Project Containment**
<table>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<td>L4</td>
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<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
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<td>L4</td>
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<th>Transitional Premises Class</th>
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<tr>
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<td>Non-GMMs</td>
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<tr>
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<td></td>
<td>Withdrawn</td>
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</table>

**Name**

GLAXO SMITH KLINE RESEARCH & DEVELOPMENT LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

COLDHARBOUR ROAD THE PINNACLES

**District**


<table>
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<th>Town</th>
<th>County</th>
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<th>Country</th>
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<tr>
<td>HARLOW</td>
<td>ESSEX</td>
<td>CM19 5AW</td>
<td>ENGLAND</td>
</tr>
</tbody>
</table>

**Tel Number**

01279 622000

**Fax Number**

01279 622100

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

COMPANY CLOSED & MERGED WITH GM558 ON 11/10/2002

**Date at Which Additional Info Submitted**

02/08/2008
### Premises Addresses

<table>
<thead>
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<th>Date Closed</th>
<th>Name</th>
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<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
<th>Withdrawn</th>
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<td>11/10/2002</td>
<td>GLAXOSMITHK LINE RESEARCH &amp; DEVELOPMENT LIMITED</td>
<td>Research &amp; Development</td>
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<td>CORE TECHNOLOGY FACILITY</td>
<td>LABORATORY G28</td>
<td>MANCHESTER INCUBATOR BUILDING</td>
<td>48 GRAFTON STREET</td>
<td>MANCHESTER</td>
<td>GREATER MANCHESTER</td>
<td>M13 9XX</td>
<td>ENGLAND</td>
<td>N</td>
</tr>
</tbody>
</table>

### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- **Give brief details of the genetic modification safety committee**

  - **Laboratory**
  - **Animal Unit**
  - **Growth Room**
  - **Glass House**
  - **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

  - **Tick if confidential**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 315/01.1**

<table>
<thead>
<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
</tr>
</thead>
<tbody>
<tr>
<td>05/01/2001</td>
<td>EXPRESSION OF MCP-1 IN E.COLI</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td></td>
<td>not applicable</td>
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</tr>
<tr>
<td>11/10/2002</td>
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<td></td>
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<td></td>
<td>Project notified under transitional arrangements</td>
<td>Y</td>
</tr>
</tbody>
</table>

Withdrawn N

Tick if notifying a connected programme of work N
### Purposes of the contained use

Monocyte chemoattractant protein 1 (MCP-1) is a chemokine that predominantly affects the chemotaxis and biological activity of cells of the monocyte/macrophage lineage. It is thus thought to play a key role in the infiltration and activation of these cells in chronic inflammatory conditions, including during their accumulation in atherosclerotic plaques and their formation of disease-characteristic foam cells involved in pathogenic fatty streaks. The evaluation of MCP-1 and its role in disease will require production of the protein in-house.

The MCP-1 cDNA will be inserted into a disabled strain of E.coli (K-12 derivative or LS-21) with the intention of achieving maximum expression. There is literature precedent for E.coli producing fully biologically active MCP-1, and such material is commercially available. Point mutations will be introduced at specific residues to enable modification of activity and to determine those residues which are important to activities of the molecule. The native and modified proteins will be purified by conventional means.

### Recipient or parental organism

Disabled E.coli strains such as the K12 derivative DH5x and BL21 are non-colonising and not pathogenic to humans or animals (ref1). They are unlikely to be able to survive outside the laboratory environment. These organisms fall into ACDP hazard group 1.

**ref 1:** Chart H, Smith HR, La Ragione R, and Woodward MJ (2000) Journal of Applied Microbiology 89 (6) 1048

### Host/vector system

Non-mobilisable vectors based on pUC, which has a history of safe use, will be used in the host organism described above.

### Origin & function

The gene for human MCP-1 and MCP-1 variants will be expressed in E.coli to provide material for evaluation of the protein's role in disease and to seek new treatments for any such diseases.

### Evaluation of foreseeable effects

Biologically active MCP-1 might have deleterious consequences if delivered to a responsive tissue, e.g. in the gastrointestinal tract of a research worker (induction of inflammatory reactions, etc). The work does not involve processes which generate aerosols in anything other than minute qualities, and to protect the work all manipulations with the recombinant organism will be carried out in a class 2 biosafety cabinet. Recombinant E.coli could be delivered to a research worker by ingestion, but the organisms are unable to survive for more than very short periods in the mammalian gut and the amounts that could be delivered are likely to be small and for a short duration. Nevertheless, because MCP-1 might have some deleterious effect on the health of research worker, it is prudent to apply some of the measures of containment level 2(CL2), which will effectively reduce the risks to negligible.

The organisms are unable to survive for a significant time outside the laboratory, so pose no threat to the environment. All waste will be inactivated by validated means (routinely by heat treatment).

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Waste cultures, supernatants, and cell debris of up to 1 litre will be inactivated by autoclaving in validated autoclaves (>120 deg C > for 15 min); larger volumes will be heat-treated in fermenters (>120 deg C for > 15 min); plastic and other solid waste will be autoclaved as described and/or incinerated. |

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No points were raised and it was agreed that the work could commence under conditions of ACGM CL2.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
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<tr>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 315/01.2

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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
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<tr>
<td>24/01/2001</td>
<td>ADENO VIRAL EXPRESSION OF A MAMMALIAN NUCLEAR HORMONE RECEPTOR AND ANALOGUES IN ANIMAL CELLS</td>
<td>Class 2</td>
<td>Class 2</td>
<td>Class 2</td>
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</table>

<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/10/2002</td>
<td>not applicable</td>
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</table>
### Project Additional Information

**Purposes of the contained use**

The T cell receptor Not1 is part of a nuclear hormone receptor superfamily regulating transcription, and including oestrogen, corticosteroids, vitamins A and D. It was identified as a transcriptionally upregulated gene and is highly homologous to a mouse gene which appears to be essential to normal development. A splice variant of Not1, Not1a, behaves as a dominant negative in reporter assays. In order to analyse the function of the receptor it is desirable to overexpress Not1 and its splice variant in a range of cell lines. The use of a viral approach should overcome the inherent problems associated with expression of receptors in many cell types. The operator will be a designated member of staff with appropriate training in viral practice and containment. The work will be carried out in a laboratory approved for adenovirus work.

**Recipient or parental organism**

The recipient host is E1a- and E3- deleted adenovirus (serotype Ad5) which is replication defective. The virus is able to replicate only in specific helper cells and is unable to cause a productive infection in human hosts. Therefore the host is in the lowest ACDP hazard group, Group 1.

**Host/vector system**

Human or mouse Not1 and Not1a, under transcriptional control of a strong promoter such as CMV, will be inserted into the essential E1 region of the adenovirus genome using the ADEasy vector (or similar) system (Stratagene, Ref 1). Therefore any recombination events which resulted in the replacement of this region wild type E1 would also result in the loss of the inserted gene, precluding the formation of replication competent virus expressing a novel product. Recombinant virus will be produced in helper cells such as the human 293 cell line as described (ref 1).

Ref 1: [http://www.stratagene.com/vectors/expression/adeasy.htm](http://www.stratagene.com/vectors/expression/adeasy.htm)

**Origin & function**

The genes were cloned within SmithKline Beecham from proprietary DNA libraries. The recombinant Adenovirus will be used to infect primary neuronal cell cultures or cell lines in order to study the function of Not1 and analogues in cultured cells.

**Evaluation of foreseeable effects**

Although the novel receptor has no known pathological effects, there is a theoretical risk that expression of a nuclear receptor in tissues might cause up- or down-regulation of gene expression, with unknown effects. Because the DNA cannot replicate, it is anticipated that large doses of virus would be required to cause any such effects in infected tissue: the only likely route of infection would be by airborne particles. The work does not involve processes which generate aerosols in anything other than minute quantities, and to protect the work all manipulations with the recombinant organism will be carried out in a class 2 biosafety cabinet. The measures of containment level 2 (CL2) which will be applied will effectively reduce the risks to negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste cultures, supernatants, and cell debris will be inactivated by autoclaving in validated autoclaves (>120 deg C for > 15 min); plastic and other solid waste will be autoclaved as described and/or incinerated.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Committee agreed that a hazard category of CL2 was appropriate because it was anticipated that any adverse effects in operators would be mild, restricted to the exposed site of infection, and of short duration.

The virus is unable to spread to the community.

It was agreed that the work could commence under conditions of ACGM CL2 with use of a Biological safety cabinet for manipulation of cells and virus.

### Project Containment

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<td>L2</td>
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### Project Ref 315/01.3

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<tr>
<td>26/01/2001</td>
<td>EXPRESSION OF INTRACELLULAR PROTEIN KINASES AND ANALOGUES IN ANIMAL CELLS</td>
<td>Class 2</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Date Project Ceased: 02/03/2022
Intracellular protein kinases represent important cascades in the control of cell growth and arrest. We intend to investigate the functions of two closely related kinases which are potential therapeutic targets. Exchange of the amino acids at one or two points of the protein sequence allows forms of the kinases which are either constitutively active or constitutively inactive. These forms will be used as tools to assist in defining the role of the kinases.

The work proposed involves the insertion of the naturally occurring protein kinases and their constitutively active and dominant negative forms into defective adenoviral vectors, with subsequent expression in mammalian cells. The use of a viral approach should overcome the inherent problems associated with expression of receptors in many cell types.

The operator will be a designated member of staff with appropriate training in viral practice and containment and the work will be carried out in a laboratory approved for adenovirus work.

The recipient host is an E1a- and E3- deleted adenovirus (serotype Ad5) which is replication defective. The virus is able to replicate only in specific helper cells and is unable to cause a productive infection in human hosts. Therefore the host is in the lowest ACDP hazard group, Group 1.

Recipient or parental organism
The recipient host is a E1a- and E3- deleted adenovirus (serotype Ad5) which is replication defective. The virus is able to replicate only in specific helper cells and is unable to cause a productive infection in human hosts. Therefore the host is in the lowest ACDP hazard group, Group 1.

Host/vector system
The recombinant genes under a strong promoter such as CMV will be inserted into the essential E1 region of the adenovirus genome using the ADEasy vector (or similar) system (Stratagene, ref 1). Therefore any recombination events which resulted in the replacement of this region with wild type E1 would also result in the loss of the inserted gene, precluding the formation of replication competent virus expressing a novel product.

Recombinant virus will be produced in helper cells such as the 293 cell line as described (ref 1).

Ref 1: http://www.stratagene.com/vectors/expression.adeasy.htm

Origin & function
The genes were isolated from human DNA libraries. The recombinant Adenovirus will be used to infect mammalian cell cultures in order to study the function of the kinases and to determine whether they are suitable therapeutic targets.

Evaluation of foreseeable effects
There is a theoretical risk that expression of a kinase in tissues might cause up- or down-regulation of gene expression, with unknown effects. However, because the DNA cannot replicate, it is anticipated that large doses of virus would be required to cause any such effects in infected tissue; the only likely routes of infection would be by
airborne particles or splashes into the mouth. The work does not involve processes which generate aerosols in anything other than minute quantities, and to protect the work all manipulations with the recombinant organism will be carried out in a class 2 biosafety cabinet. The measures of containment level 2 (CL2) which will be applied will effectively reduce the risks to negligible.

The organisms are unable to survive for a significant time outside the laboratory, so pose no threat to the environment. All waste will be inactivated by validated means (routinely by heat treatment). The Adenoviral vectors are unable to replicate in human hosts.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste cultures, supernatants, and cell debris will be inactivated by autoclaving in validated autoclaves (>120 deg C for > 15 min); plastic and other solid waste will be autoclaved as described and/or incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee agreed that a hazard category of CL2 was appropriate because it is anticipated that any adverse effects in operators would be mild, restricted to the exposed site of infection, and of short duration. The virus is unable to spread to the community.

The proposal was approved at CL2 (local guidelines for recombinant adenovirus applied, including use of a Biological safety cabinet for manipulation of cells and virus).

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
Receptor kinases are an important element in the control of the growth of specific cell lines. Specific protein ligands interact with target receptors on the surface of cells to activate receptor kinases, and transmit the signal to control growth or arrest. GSK has identified a range of receptor kinases, and we now intend to identify their ligands, which are potential therapeutic targets. The objective is to express putative kinase ligands and to test them for activity on the various receptors. This proposal covers expression of ligands and putative ligands for receptor kinases for use in the study of receptor kinase activation. The proposed work will typically involve subcloning of the ligand cDNA for expression in disabled E. coli, mammalian cells, and the insect cell/baculovirus system. Inducible expression systems may be used. Modified cDNAs may be expressed with alterations to modulate activity or to produce combined products (tagged ligands for the purpose of tracking or immobilising the ligand).

Recipient or parental organism

E. coli disabled hosts such as K12 strains (e.g. JM109, DH5a); standard insect cell/baculovirus systems; and mammalian cell cultures such as Cos7, HEK293, CHO, SK-N-MC and other neuroblastoma lines.

Host/vector system

E. coli disabled hosts such as JM109, DH5a using non mobilisable vectors (eg. pGEX1, pGEX2T, pET16, pBluescript); expression in insect cell cultures using standard baculovirus systems such as the pFASTBAC vector; mammalian cell lines such as Cos7, HEK293, CHO, SK-N-MC and other neuroblastoma lines, using vectors with poor expression in E. coli (eg. pcDNA3.1, pECE, pCDN, pcDNA1, pCMV5).
Origin & function
The genes were isolated from human cDNA libraries. The objective is to express putative kinase ligands and to test them for activity.

Evaluation of foreseeable effects
A receptor ligand might have a deleterious effect if delivered to a target issue. Therefore, measures to prevent ingestion or inoculation will be applied, including use of a microbial safety cabinet, wearing of gloves, using procedures which minimise aerosols, and avoiding the use of sharps. The work will be carried out at CL2. The measures of containment level 2 (CL2) which will be applied will effectively reduce the risks to negligible.

The organisms are unable to survive for a significant time outside the laboratory, so pose no threat to the environment. All waste will be inactivated by validated means (heat treatment).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste cultures, supernatants, and cell debris will be inactivated by autoclaving in validated autoclaves (>120 deg C for > 15 min); plastic and other solid waste will be autoclaved as described and/or incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The proposal for receptor kinase ligands was approved at CL2 for E.coli using non-mobilisable vectors only, and approved at CL1 for animal cells.

Review (Dec 2000)
The Committee agreed that a hazard category of CL2 was appropriate for both animal cells and E coli.

Project Containment

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</table>
**Project Additional Information**

**Purposes of the contained use**

Intracellular protein kinases represent important cascades in the control of cell growth and arrest. We intend to investigate the functions of two closely related kinases which are potential therapeutic targets. Exchange of the amino acids at one or two points of the protein sequence allows forms of the kinases which are either constitutively active or constitutively inactive. These forms will be used as tools to assist in defining the role of kinases.

The work proposed involves the insertion of the naturally occurring protein kinases and their constitutively active and dominant negative forms into defective adenoviral vectors, with subsequent expression in mammalian cells. The use of a viral approach should overcome the inherent problems associated with expression of receptors in many cell types.

The operator will be a designated member of staff with appropriate training in viral practice and containment and the work will be carried out in a laboratory approved for adenovirus work.

**Recipient or parental organism**

The recipient host is E1a- and E3- deleted adenovirus (serotype Ad5) which is replication defective. The virus is able to replicate only in specific helper cells and is unable to cause a productive infection in human hosts. Therefore the host is in the lowest ACDP hazard group, Group 1.

**Host/vector system**

The recombinant genes under a strong promoter such as CMV will be inserted into the essential E1 region of the adenovirus genome using the ADEasy vector (or similar).
system (Stratagene, ref 1). Therefore any recombination events which resulted in the replacement of this region with wild type E1 would also result in the loss of the inserted gene, precluding the formation of replication competent virus expressing a novel product. Recombinant virus will be produced in helper cells such as the 293 cell line as described (ref 1).

Ref 1: http://www.stratagene.com/vectors/expression/adeasy.htm

Origin & function

The genes were isolated from human cDNA libraries. The recombinant Adenovirus will be used to infect mammalian cell cultures in order to study the function of the kinases and to determine whether they are suitable therapeutic targets.

Evaluation of foreseeable effects

There is a theoretical risk that expression of a kinase in tissues might cause up- or down-regulation of gene expression, with unknown effects. However, because the DNA cannot replicate, it is anticipated that large doses of virus would be required to cause any such effects in infected tissue; the only likely routes of infection would be by airbourne particles or splashes into the mouth. The work does not involve processes which generate aerosols in anything other than very small quantities, and to protect the work all manipulations with the recombinant organism will be carried out in a class 2 biosafety cabinet. The measures of containment level 2 (CL2) which will be applied will effectively reduce the risks to negligible.

The organisms are unable to survive for a significant time outside the laboratory, so pose no threat to the environment. All waste will be inactivated by validated means (heat treatment).

The Adenoviral vectors are unable to replicate in human hosts.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste cultures, supernatants, and cell debris will be inactivated by autoclaving in validated autoclaves (>120 deg C for > 15 min); plastic and other solid waste will be autoclaved as described and/or incinerated.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
The Committee agreed that a hazard category of CL2 was appropriate because it is anticipated that any adverse effects in operators would be mild, restricted to the exposed site of infection, and of short duration. The virus is unable to spread to the community.

The proposal was approved at CL2 (local guidelines for recombinant adenovirus applied, including use of a Biological safety cabinet for manipulation of cells and virus).

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Project Ref 315/01.6

Date Ackn'd: 20/09/2001

CU2 Project Title: EXPRESSION OF TRANSMEMBRANE RECEPTORS AND THEIR REGULARITY PROTEINS IN MAMMALIAN CELLS USING THE BACULOVIRUS SYSTEM

Class: Class 2

Consent Granted: not applicable

Project notified under transitional arrangements: N

Tick if notifying a connected programme of work: N

In the search for new and effective pharmaceuticals with minimal side effects, GSK intends to express known and novel membrane receptors in mammalian cells in order
To obtain functional data and to determine the specificity of closely related receptors, Regulatory proteins such as accessory G proteins which modulate the function of the receptor, and may be essential for activity, will also be expressed and evaluated. To avoid the generation of large numbers of stable recombinant cell lines, we intend to use a recombinant baculovirus system which will allow transient expression in mammalian cell lines. Baculoviruses are a well studied system for the production of recombinant proteins in insect cells. They are unable to replicate in mammalian cells, and the usual insect-specific promoters are ineffective in mammalian cells. By replacing the standard polyhedrin gene promoter sequence with that of an efficient mammalian promoter (such as the CMV IE promoter/enhancer) baculoviruses are able to express recombinant products in mammalian cells, though they remain incapable of replication in these cells. (Condreay et al 1998). Baculovirus particles containing the product gene under CMV promoter control will be generated in insect cell cultures using standard procedures, and used to transfect mammalian cell lines. The transient gene expression, generally at low levels compared to stable cell lines, can be increased ten-fold or more by addition of a histone deacetylase inhibitor such as sodium butyrate which inhibits the normal silencing of transfected DNA.

Ref: Condreay et al (1998) PNAS 96 127

Recipient or parental organism

Baculovirus CL1
These organisms fall into ACDP hazard group 1

Host/vector system

For generation of recombinant baculovirus, shuttle vectors containing the product gene under CMV promoter control are transfected into insect cells (e.g. Sf-9) using standard procedures such as the Bac to Bac expression system (Life Technologies).

(Recombinant cell lines expressing receptors and G-proteins: CL 1)

Origin & function

GSK proprietary libraries

Evaluation of foreseeable effects

The baculovirus is able to transfect mammalian cells, but is unable to replicate in them. Efficient expression of the gene products under control of the CMV promoter is achieved only by addition of an inhibitor of cellular histone deacetylase. It is possible that inappropriate expression of a transmembrane receptor or regulatory protein could have detrimental effects. However, the only likely routes of infection would be mouth, nose or eyes, and the amounts which might enter by such routes (via aerosols or splashes) would be small (microlitres). In addition, baculoviruses are rapidly inactivated (>99% in 30 minutes) by the complement system of normal human serum (Hofmann and Strauss '98). The work will use standard laboratory and bioreactor methods and does not involve processes which would generate significant quantities of aerosols. Needles (sharps) will not be used. Laboratory operations involving production of baculoviruses and transfection of cell lines will be carried out in a microbiologic al safety caninet, which will be sufficient to reduce the likelihood of infection to effectively zero. Bioreactor operations will be carried out under CL2 conditions as defined in table 2 of the GMO Regulations 2000. Typically, the baculovirus will be used to transfect mammalian cells either in multi-well plates (eg 96-well) for assay, or in bioreactors for preparation of batches of cells. Batches of cells will be collected (centrifugation) and washed under CL2 conditions.

The transient recombinant cell lines expressing receptors generated by this system, including low levels of residual virus, are unlikely to pose a risk to health, and may be handled under conditions of CL1 (eg for analysis). Similar stable cell lines have previously been categorised as CL1.


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste cultures, supernatants, and cell debris of up to 1 litre will be inactivated by autoclaving in validated autoclaves (>120 deg C for > 15 min); larger volumes will be heat-treated in fermenters (>120 deg C for > 15 min); plastic and other solid waste will be autoclaved as described and/or incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The Committee approved the Risk assessment for production of baculovirus and transfection of cell lines at CL2.

**Project Containment**

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<thead>
<tr>
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<th>Large Scale Activities</th>
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**Project Ref**  315/01.7

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<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<td>21/09/2001</td>
<td>UTILISATION OF STREPTOMYCES SPECIES SUCH AS STREPTOMYCES LIVIDANS AS AN EXPRESSION SYSTEM FOR THE PRODUCTION OF CYTOKINES Class 2 &lt; 1 litre</td>
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Historical Significant Changes

Historical Date of Additional Info

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The aim is evaluate and utilise Streptomyces lividans for production of heterologous proteins of biopharmaceutical relevance. Initially murine TNFa will be overexpressed and secreted by Streptomyces lividans to evaluate the conditions required for high level protein production. If successful the work may be extended to use of other Streptomyces species and expression of other cytokines, including novel cytokines. Laboratory work with Streptomyces will be carried out at up to 0.5L volumes. If required, larger volumes of crude product (up to 15L) will be produced from bioreactor cultures.

#### Recipient or parental organism

Streptomyces species such as Streptomyces lividans. These organisms fall into the lowest ACDP hazard group 1.

#### Host/vector system

Non-pathogenic Streptomyces species transfected with non-conjugative plasmids (unable to transfer to other species).

#### Origin & function

GSK proprietary libraries.

#### Evaluation of foreseeable effects

Human and non-human cytokines may have biological activity if delivered to a site of action (eg if ingested or inhaled). The use of Class 2 biological safety cabinets and gloves will reduce the likelihood of harm from laboratory work to effectively zero. For larger scale work, the operation of bioreactors is designed to minimise release through exhaust gases or during sampling of cultures, and the work will be effectively contained at CL2 according to Schedule 8 of the GMO (Contained Use) Regulations 2000.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste cultures, supernatants, and cell debris of up to 0.5 litre will be inactivated by autoclaving in validated autoclaves (>120 deg C for >15 min); larger volumes will be heat-treated in fermenters (>120 deg C for >15 min); plastic and other solid waste will be autoclaved as described and/or incinerated.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y
The Committee approved the risk assessment at CL2.

**Project Containment**

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**Animal Units**

- L2 L3 L4 L2 L3 L4 L2 L3 L4

**Project Ref 315/01.8**

**Date Ackn’d**: 09/11/2001

**CU2 Project Title**: EXPRESSION OF KINASES IN MAMMALIAN CELLS USING THE BACULOVIRUS SYSTEM

**Class**: Class 2

**Culture Vol**: 1-50 litres

**Consent Granted**: not applicable

**Non-GMM**: not applicable

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

In the search for new and effective pharmaceuticals with minimal side effects, GSK intends to express known and novel kinases in mammalian cells in order to obtain functional data and to determine the specificity of closely related kinases. Kinases are a large class of enzymes found in all cell types which activate their target proteins by...
phosphorylation. Frequently, the target molecule itself will be a kinase, which when activated can progress the signal by phosphorylation of the next enzyme in the signal cascade. The kinases may be modified to modulate their activity and to evaluate the mechanism of action, for example by analysing the effect of single amino acid substitutions. To avoid the generation of large numbers of stable recombinant cell lines, we intend to use a recombinant baculovirus system which will allow transient expression in mammalian cell lines.

Baculoviruses are a well studied system for the production of recombinant proteins in insect cells. They are unable to replicate in mammalian cells, and the usual insect-specific promotors are ineffective in mammalian cells. By replacing the standard polyhedrin gene promoter sequence with that of an efficient mammalian promoter (such as the CMV IE promoter/enhancer) baculoviruses are able to express recombinant products in mammalian cells, though they remain incapable of replication in these cells. (Condreay et al 1998). Baculovirus particles containing the product gene under CMV promoter control will be generated in insect cell cultures using standard procedures, and used to transfect mammalian cell lines. The transient gene expression, generally at low levels compared to stable cell lines, can be increased ten-fold or more by addition of a histone deacetylase inhibitor such as sodium butyrate which inhibits the normal silencing of transfected DNA.

Recipient or parental organism

Baculovirus CL1
These organisms fall into ACDP hazard group 1.

Host/vector system

For generation of recombinant baculovirus, shuttle vectors containing the product gene under CMV promoter control are transfected into insect cells (eg. Sf-9) using standard procedures such as the Bac to Bac expression system (Life Technologies).

(Recombinant cell lines expressing kinases: CL1)

Origin & function

Libraries of human DNA from academic collaborators.

Evaluation of foreseeable effects

The baculovirus is able to transfect mammalian cells, but is unable to replicate in them. Efficient expression of the gene products under control of the CMV promoter is achieved only by addition of an inhibitor of cellular histone deacetylase. It is possible that inappropriate expression of a kinase could have detrimental effects. However, the only likely routes of infection would be mouth, nose or eyes, and the amounts which might enter by such routes (via aerosols or splashes) would be small (microlitres). In addition, baculoviruses are rapidly inactivated (>99% in 30 minutes) by the complement system of normal human serum (Hofmann and Strauss '98)*. The work will use standard laboratory and bioreactor methods and does not involve processes which would generate significant quantities of aerosols. Needles (sharps) will not be used. Laboratory operations involving production of baculoviruses and transfection of cell lines will be carried out in a microbiological safety cabinet, which will be sufficient to reduce the likelihood of infection to effectively zero. Bioreactor operations will be carried out under CL2 conditions as defined in table 2 of the GMO Regulations 2000. Typically, the baculovirus will be used to transfect mammalian cells either in multi-well plates (eg. 96-well) for assay, or in bioreactors for preparation of batches of cells. Batches of cells will be collected (centrifugation) and washed under CL2 conditions. The transient recombinant cell lines expressing kinases generated by this system, including low levels of residual virus, are unlikely to pose a risk to health, and may be handled under conditions of CL1 (eg. for analysis). Similar stable cell lines have previously been categorised as CL1.


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Waste culture, supernatants, and cell debris of up to 1 litre will be inactivated by autoclaving in validated autoclaves (>120 deg C for >15 min); larger volumes will be heat-treated in fermenters (>120 deg C for >15 min); plastic and other solid waste will be autoclaved as described and/or incinerated. These treatments will give complete killing of the cells and baculovirus.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The Committee approved the Risk assessment for production of baculovirus and transfection of cell lines at CL2.

**Project Containment**

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**Project Ref 315/01.9**

Date Ackn'd 09/11/2001

Date Project Ceased 11/10/2002

Withdrawn N

Tick if notifying a connected programme of work N

EXPRESSION OF NUCLEAR RECEPTORS IN MAMMALIAN CELLS USING THE BACULOVIRUS SYSTEM

Class 2

Consent Granted not applicable

Project notified under transitional arrangements N
In the search for new and effective pharmaceuticals with minimal side effects, GSK intends to express known and novel nuclear receptors in mammalian cells in order to obtain functional data and to determine the specificity of closely related receptors. Nuclear receptors are ligand regulated transcription factors which mediate their physiological effects by modulating the expression of target genes. The nuclear receptors may be modified to modulate their activity and to evaluate the mechanism of action, for example by analysing the effect of single amino acid substitutions. To avoid the generation of large numbers of stable recombinant cell lines, we intend to use a recombinant baculovirus system which will allow transient expression in mammalian cell lines.

Baculoviruses are a well studied system for the production of recombinant proteins in insect cells. They are unable to replicate in mammalian cells, and the usual insect-specific promoters are ineffective in mammalian cells. By replacing the standard polyhedrin gene promoter sequence with that of an efficient mammalian promoter (such as the CMV IE promoter/enhancer) baculoviruses are able to express recombinant products in mammalian cells, though they remain incapable of replication in these cells. (Condreay et al 1998). Baculovirus particles containing the product gene under CMV promoter control will be generated in insect cell cultures using standard procedures, and used to transfect mammalian cell lines. The transient gene expression, generally at low levels compared to stable cell lines, can be increased ten-fold or more by addition of a histone deacetylase inhibitor such as sodium butyrate which inhibits the normal silencing of transfected DNA.


Recipient or parental organism

Baculovirus CL1.
These organisms fall into ACDP hazard group 1.

Host/vector system

For generation of recombinant baculovirus, shuttle vectors containing the product gene under CMV promoter control are transfected into insect cells (eg Sf-9) using standard procedures such as the Bac to Bac expression system (Life Technologies).

(Recombinant cell lines expressing nuclear receptors: CL 1).

Origin & function

Libraries of human DNA commercially available or from academic collaborators.
The baculovirus is able to transfect mammalian cells, but is unable to replicate in them. Efficient expression of the gene products under control of the CMV promoter is achieved only by addition of an inhibitor of cellular histone deacetylase. It is possible that inappropriate expression of a nuclear receptor could have detrimental effects. However, the only likely routes of infection would be mouth, nose or eyes, and the amounts which might enter by such routes (via aerosols or splashes) would be small (microlitres). In addition, baculoviruses are rapidly inactivated (>99% in 30 minutes) by the complement system of normal human serum (Hofmann and Strauss '98)*. The work will use standard laboratory and bioreactor methods and does not involve processes which would generate significant quantities of aerosols. Needles (sharps) will not be used. Laboratory operations involving production of baculoviruses and transfection of cell lines will be carried out in a microbiological safety cabinet, which will be sufficient to reduce the likelihood of infection to effectively zero. Bioreactor operations will be carried out under CL2 conditions as defined in table 2 of the GMO Regulations 2000. Typically, the baculovirus will be used to transfect mammalian cells either in multi-well plates (eg 96-well) for assay, or in bioreactors for preparation of batches of cells. Batches of cells will be collected (centrifugation) and washed under CL2 conditions.

The transient recombiant cell lines expressing nuclear receptors generated by this system, including low levels of residual virus, are unlikely to pose a risk to health, and may be handled under conditions of CL1 (eg for analysis).


Contains and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| Description of waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate) |
| Waste cultures, supernatants, and cell debris of up to 1 litre will be inactivated by autoclaving in validated autoclaves (>120 deg C for >15 min); larger volumes will be heat-treated in fermenters (>120 deg C for >15 min); plastic and other solid waste will be autoclaved as described and/or incinerated. These treatments will give complete killing of the cells and baculovirus. |

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

The Committee approved the Risk Assessment for production of baculovirus and transfection of cell lines at CL2.

**Project Containment**
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**Comments**

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**Date at Which Additional Info Submitted**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

Bacteriology  Parasitology  Transgenic  Microbiology  Research
             Birds          Birds          Research
Virology    Transgenic  Transgenic  Gene Therapy
             Animals       Fish            
Mycology    Transgenic  Transgenic  Other (please
             Invertebrates  Plants  specify below)
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 13/07.2

Generation and analysis of recombinant hepatitis C virus. This will involve the cloning and modification of cDNA, recovery of virus by transfection of cells in culture and analysis of virus replication.

Class CultureVol
Class 3 1-50 Litres 50ml

Non-GMM Consent Granted Yes

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work Y

Historical Significant Changes
Project transferred from GM 13 06/07/2015

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
The broad aim of this project is to better understand the replication of hepatitis C virus. The relatively compact hepatitis C virus genome encodes all the proteins and
contains the required genetic elements to replicate in a suitable cell line. We are particularly interested in the structure and function of RNA elements that are involved in the replication process. A better understanding of HCV replication may enable novel therapeutic methods to be developed to limit virus infectivity or spread in the infected host.

**Recipient or parental organism**

The parental organism is hepatitis C virus, an ACDP level 3 human pathogen. At the time of application this virus only undergoes a full replication cycle in the human hepatoma Huh7 cell line. Therefore virus can only be recovered from cDNA under controlled laboratory conditions and can only be cultivated in a specific cell culture system. The virus cDNA is contained in plasmids under transcriptional control of a phageT7 promoter. In the absence of exogenously supplied T7 polymerase there can be no transcription and hence subsequent expression of HCV.

**Host/vector system**

The plasmid vector system is derived from pUC. It is therefore non-mobilisable. It is maintained in standard disabled laboratory strains of E. coli (DH5a, JM101 etc.). Virus production will follow in vitro T7-mediated transcription and transfection of Huh7 cells in culture. Progeny virus will be propagated in Huh7 cells. Huh7 cells are a permanent cell line defective in interferon production. They are the only cell culture system in which hepatitis C replication has been reproducibly demonstrated.

**Origin & function**

The parental genetic material is hepatitis C virus. Initially studies will be based on the genotype 2a JFH-1 infectious cDNA and virus recovered from this clone (Wakita et al., Nature Medicine 11, 791-796). Future studies will include additional genotypes as they become available in other laboratories. The JFH-1 system reproducibly produces infectious virus in Huh7 cells that can be passaged onto fresh cells. Mutations will be introduced into the cDNA into regions we suspect are involved in controlling virus replication. In particular we are interested in short stem-loop structures that are located in the core protein-encoding and NS5b polymerase-encoding regions. Mutations will be introduced to disrupt these structures and duplications of the structures will be introduced elsewhere in the genome. None of the changes are in regions of the virus implicated in influencing the primary tropism of the virus - they are not in the E1 or E2 envelope-coding regions. Mutations will include deletions, substitutions and short insertions.

A similar range of mutations will be introduced into a sub-genomic replicon system in which the structural proteins of HCV are removed and replaced with a reporter gene (luciferase) or an antibiotic coding gene (conferring G418 resistance). The sub-genomic replicon is transcribed in vitro using bacteriophage T7, the RNA transfected into Huh7 cells, and replication 'monitored' by an increase in luciferase activity, or the outgrowth over time of neomycin-resistant colonies in cell culture. The sub-genomic replicon cannot transfer from cell to cell and cannot encode infectious virus.

Some of the substitutions to be introduced will be of analogous regions of other genotypes of HCV. More divergent sequences are not included within the scope of this proposal i.e. the sequences are all derived from hepatitis C virus.

Studies from a number of laboratories have shown that disrupting this type of cis-acting replication elements (that are the targets of this project) is either neutral or deleterious to virus replication. There is no reason to suspect that the mutations to be introduced will enhance replication above that of the parental virus, or that the changes will increase or otherwise influence the tropism or pathogenicity of the recovered virus.

**Evaluation of foreseeable effects**

Hepatitis C virus is exclusively a human pathogen. Transmission is almost exclusively parenteral, with some limited evidence for sexual transmission. There is no evidence for aerosol or faecal-oral transmission of the virus. None of the changes proposed - if they yield viable virus at all - are expected (or designed) to alter the cell, tissue or host tropism of the virus, or influence the route by which the virus can be transmitted. The majority of the changes are expected to be deleterious to virus replication, and to reduce or abrogate virus fitness. There is no reason to expect that the planned changes will reduce the immune response to the virus, which clears infection in about one third of cases. Instead, a less fit virus may be more susceptible to immune clearance.

No vaccine is available to prevent HCV infection. Antivirals - currently ribavirin and interferon, with protease inhibitors shortly - are of limited benefit and lead to viral clearance in a proportion of cases.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For bacterial work, all solid waste and glassware is autoclaved at 121oC for at least 15 min. The autoclave is validated by thermocouple test annually. Liquid cultures are chemically decontaminated by treatment with 2500 ppm chlorine bleach for a minimum of 2 hours prior to disposal. Both these treatments give a 100 % kill for standard laboratory strains of E. coli.

Cell culture work not involving recovery or propagation of viable virus e.g. transfection and analysis of hepatitis C virus sub-genomic replicons, will be conducted in a level 2 tissue culture laboratory. All cells will be handled in a class 2 microbiological safety cabinet with HEPA-filtered exhaust. Waste is autoclaved or chemically inactivated prior to disposal. All staff handling replicon-transfected cells are trained.

Cell culture studies involving the attempted recovery and propagation of infectious hepatitis C virus will be conducted in a level 3 laboratory. Virus and infected cells will be handled in class 1 microbiological safety cabinets by personnel who have been specifically trained and approved to work in a level 3 facility. All biological waste is inactivated by treatment with a quarternary ammonium detergent and waste is autoclaved within the facility prior to disposal. Virus storage is in vapour phase, liquid nitrogen, within the level 3 facility.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Minutes of the Genetic Manipulation Safety Committee meeting held on January 19th 2007 relevant to the notification

GMSC 695 Professor D J. E
Cloning and mutagenesis of hepatitis C cDNA
This project aims to dissect the role of structured RNA elements in the replication of hepatitis C virus. The study will involve the manipulation (using standard molecular approaches in E. coli host/vector systems) – by deletion, modification and insertion – of stem-loop structures previously predicted by bioinformatic analysis.
The virus cDNA cannot be replicated, or the proteins expressed in E. coli. Expression requires in vitro transcription by bacteriophage T7 polymerase, transfection into a permissive human hepatoma cell line (only one of which has reproducibly been shown to support replication) in which translation occurs. Translation involves cellular factors absent from bacterial cells. The disabled nature of the host, lack of mobilisation or conjugative features of the vector reduce the possibility of environmental risk to effectively zero.

Environmental Risks: Consequence: negligible; Likelihood: low; Overall: Effectively zero.

GMSC 696 Professor D J.E
Analysis of Hepatitis C virus replication using sub-genomic replicons. The aim of this project is to dissect the role of structured RNA elements in the replication of Hepatitis C virus. Functional analysis of the modified stem-loop structures will be undertaken using a hepatitis C virus sub-genomic replicon system. These consist of a virus cDNA from which the coding region for the core, E1 and E2 structural proteins have been deleted and replaced with an antibiotic (or enzymatic) selection marker. Following transfection of susceptible cells with RNA generated in vitro replication can therefore be monitored by an increase in antibiotic resistant colonies, or enzymatic activity. Due to the absence of the structural proteins the sub-genomic replicons is incapable of making virus particles and cannot be transmitted from cell to cell. Replication only occurs in the Huh7 human hepatoma cell line.

Protein expression depends absolutely upon replication in one particular human hepatoma cell line. The sub-genomic replicons alone are incapable of being transmitted from cell to cell. Virus pseudotypes can be generated by co-infecting a replicon-containing cell with a suitable helper virus. However, replication would be restricted to a single passage. In the absence of exogenously added helper virus Huh7 cells are known not to contain any viruses capable of pseudotyping the sub-genomic replicons, and are not known to contain any adventitious agents. The requirement for Huh7 cells is due to the exquisite sensitivity of the replicon to the presence of interferon – Huh7 cells are defective in interferon production. Therefore, transfer to the environment in extremely unlikely, and if it did occur then the likelihood of the genome replicating would be very small. Finally, in the absence of any cell-to-cell transmission, damage would be restricted to a single recipient cell.

Environmental Risks: Consequence: negligible; Likelihood: negligible; Overall: Effectively zero.

GMSC 697 Professor D J. E
Replication of hepatitis C virus – studies using replicating virus system.
The aim of this project is to analyse the role of conserved RNA structures in the replication of hepatitis C virus. Our in vitro and in silico studies have identified a number of stem-loop structures that we believe are implicated in fundamental aspects of virus replication. We propose to modify these structures by mutagenesis, deletion and substitution analysis and determine the effect upon the replication of the virus.

The following points were noted and discussed by the committee:
The project will involve mutagenesis of pre-existing stem-loop structures with the intention of disrupting the structure without altering the encoded protein. The majority of the changes will therefore involve synonymous substitution. It is also proposed to introduce a second copy of the stem-loop structure at an alternative position within the genome – either in a non-coding region, or at a proteolytically cleaved junction of the polyprotein. In these cases the insert will be a 'self' sequence, derived from the same or closely related genotype of HCV.

Changes are expected to have a deleterious effect upon virus replication. All the changes DJE has already constructed in HCV sub-genomic replicons are either detrimental, or have no detectable effect upon replication. None enhance the aspects of replication we are currently able to quantify (i.e. genome copy number per cell).

Furthermore, all the stem-loop structures of interest are located in the region encoding the non-structural proteins of HCV – specifically the NS5b RNA dependent RNA polymerase. There is no evidence that this protein specifically alters the host range of HCV. The virus only replicates efficiently in Huh7 liver cells in culture due to exquisite sensitivity to the presence of interferon – Huh7 cells cannot produce interferon.

Regarding susceptibility to antiviral drugs where treatable, HCV responds to interferon and ribavirin. There is no reason to expect the substitutions to be made to the polymerase will enhance resistance to either of these treatments.

Proposed categorisation: Hepatitis C virus only infects humans. Parenteral transmission to chimpanzees – but not other non-human primates – has been demonstrated, but the virus is cleared and the infected animals only present with transient symptoms (enhanced ALT levels indicative of liver damage). Virus clearance is by the acquired immune response.

Hepatitis C virus is an ACDP level 3 pathogen. GMSC Decision: Con. Level 3.

Environmental Risks: Consequence: severe; Likelihood: negligible; Overall: effectively zero.

Project Containment

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<th>Growth Rooms</th>
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Animal Units

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Project Ref 26/99.2

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Non-GMM Consent Granted

Project notified under transitional arrangements Y
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**

**If yes, tick to confirm that it is attached to this form**

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref**  317/00.1

**Date Ackn'd**  28/11/2000  
**CU2 Project Title**  HUMAN CELL SYSTEMS FOR INVESTIGATING THE MOLECULAR MECHANISMS FOR RADIATION CARCINOGENESIS  
**Class**  Class 2  
**CultureVolClass2**  Class 2  
**CultureVolumeClass3-4**  Class 2  
**Non-GMM**  Consent Granted  not applicable  
**Tick if notifying a connected programme of work**  N  

**Historical Significant Changes**  
**Historical Date of Additional Info**  
**Significant Change ID**  
**Date of Significant Change**  

**Project Additional Information**  
**Purposes of the contained use**  
**Recipient or parental organism**
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref:** 317/04.1

### Date Ackn'd
10/12/2004

### CU2 Project Title
Analysis of Foot and Mouth Disease (FMDV) Replicon-Derived RNA Replication

### Class
Class 2

### CultureVol
< 1 Litre

### Non-GMM
Not Applicable

### Consent Granted

### Project notified under transitional arrangements
N

### Withdrawn
N

### Tick if notifying a connected programme of work
Y

### Historical Significant Changes

### Historical Date of Additional Info

### Significant Change ID

### Date of Significant Change

### Project Additional Information

#### Purposes of the contained use
The production of transcript RNA from a cDNA 'Replicon' copy of the Foot and Mouth Disease virus (FMDV) genome. The 'Replicon' is a plasmid encoding the FMDV genome with a major deletion within the region encoding the capsid proteins. The RNA will then be transfected into BHK21 cells to analyse the process of FMDV RNA replication in vivo.

#### Recipient or parental organism
The plasmid containing the Replicon will be grown in the bacterial cell line - JM109.

The plasmid will be transcribed in vitro, then replicon-derived transcript RNA will be transfected into Baby Hamster Kidney (BHK) 21 cells.

#### Host/vector system

The FMDV 'Replicon' plasmid DNA was produced by McInerney et al [J. Gen Virol Vol. 81 1699-1702 (2000). The plasmid will be grown and any gene modifications will be performed using pBSKII, pGEM or pcDNA plasmids, all of which are non-mobilisable.

The plasmids will be used to generate Replicon RNA which is non-infectious (as it does not have the FMDV capsid genes to allow packaging). The RNA will then be used to transfect BHK21 cells.

#### Origin & function

The FMDV Replicon will be obtained from the Institute for Animal Health, Pirbright Laboratory, or, constructed at the University of St. Andrews using procedures that are published (McInerney et al., 2000. J. Gen. Virol. 81:1699-1702) without using any form of viable virus.

The JM 109 and BHK 21 cells will be purchased from reputable suppliers.
The function of the genetic material is to analyse FMDV replication processes with the aim of understanding how FMDV replicates within cells. Of particular interest is how the virus proteins alter the flow of membranes within the cell to form vesicular replication complexes. The understanding of this process may provide a means of controlling an outbreak of this virus during the period where animals are vaccinated (emergency 'ring' vaccination), but have no sero-converted.

Evaluation of foreseeable effects

The Replicon can not directly form infectious virus particles as capsid sequences have been deleted preventing the formation of particles to package and transmit the RNA to another susceptible cell.

The biological containment could be breached if the ‘Replicon’ could naturally recombine with another related virus to gain a capsid gene that will allow packaging. Bioinformatic analyses by Dr Ryan has shown that the FMDV sequence is very poorly conserved with any other members of the Picornoviridae. A comparison of sequence similarly amongst genera for which recombination does not occur shows that recombination between replicon-derived FMDV RNA with another member of the Picornoviridae (to form infectious virus particles) is not a realistic risk (details of similarity analyses, probability of recombination are available upon request from Dr Ryan). This question was fully discussed at a meeting of the Genetically Modified Organisms (Contained Use) Scientific Committee (GMO(CU)), and it was agreed this was not a realistic risk.

All plasmids that contain the FMDV capsid gene or related genes will be destroyed before the Replicon will be delivered to the University thus removing the possibility of accidental recombination.

There is a possibility that the Replicon could be stolen and used for malicious purposes. Appropriate security measures will be implemented to stop this occurring.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All work with the replicon will be performed in Category 2 containment facilities as defined in the ACGM Compendium of Guidance (ISBN: 0-717601763-7). Appropriate operating procedures will be implemented to ensure only authorised appropriately trained, personnel can work with the Replicon.

As the Replicon does not have the capsid gene, it cannot package FMDV RNA and thus cannot produce infectious virus particles. Also, the Replicon plasmid does not have any eukaryotic promoters thus the plasmid cannot be expressed in susceptible animals. These features will act as a natural biological containment of the Replicon system. To avoid accidental recombination with plasmids that may have FMDV capsid genes, all plasmids which contain the FMDV capsid gene or similar genes will be destroyed before the arrival of the Replicon to the University isolated RNA from the Replicon is inherently susceptible to hydrolysis, thus if any naked RNA escaped from the containment facilities it would be destroyed very quickly.

All waste generated from the work will be autoclaved before disposal. Any spillages of the 'Replicon' will be treated with neat hypochlorite.

To avoid malicious use of the Replicon, it will be stored within a locked box, itself stored within a locked freezer. Comprehensive records will be kept of the use of the Replicon so that all of the plasmid can be accounted for. The control measures will be regularly reviewed and audits undertaken to ensure the containment and security systems are sufficient.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste from experiments using the Replicon will be autoclaved at 120 degrees C for 145 minutes at 15 psi.

Spillages will be dealt with by soaking the area in neat hypochlorite.

Any spillages of bacterial or eukaryotic cells into the drainage system will be dealt with by putting neat hypochlorite (500ml) being put down the drain, left for 10 minutes prior to the drain being flushed with running water for 30 minutes to ensure appropriate dilution.
At the first meeting of the School of Biology Safety Committee the properties of replicon-derived RNA was discussed with particular reference to containment. Dr Ryan discussed the scientific aspects of using the replicon, the biological properties of the transcript RNA derived from the replicon and presented a bioinformatic analysis of the similarities between different regions of the FMDV genome and those of similar regions of other picornaviruses. This analysis was performed to quantify the risk of recombination between replicon-derived FMDV and vRNA from other picornaviruses. It was suggested that a further bioinformatic analysis should be performed to determine the maximum length of nucleotide identity between FMDV and other viruses should be performed. This was analysed and Dr Ryan presented the data to a second meeting of the School of Biology Safety Committee. It was agreed that the maximum lengths of sequence identity between FMDV and other picornaviruses was too short to present a risk from RNA recombination between FMDV and other members of the family. The meeting was in (unanimous) agreement that the project required only category 1 containment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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</table>

Animal Units

| L2 L3 L4 L2 L3 L4 L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 L2 L3 L4 L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 |

Project Ref 317/05.1

Date Ackn'd 04/07/2005

CU2 Project Title Development of tumour targeting adenoviruses

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work Y

Date Project Ceased

Withdrawn N

02/03/2022
Project Additional Information

Purposes of the contained use

The purpose is to produce recombinant adenoviruses that replicate selectively in tumour cells and can be used to treat cancer.

Recipient or parental organism


Host/vector system

pUC19 and similar cloning vectors. pRS406 and similar S. cervisiae vectors. pNKBAC39 and similar BAC vectors. pGL3 and similar luciferase vectors. pcDNA3 and similar mammalian expression vectors. pBABE and similar oncoretroviral vectors. pRRL and similar lentiviral vectors. pMD2G, pCMV-dR8.74, pMDLg/pRRE, pRSV-Rev and similar packaging plasmids for lentivector vectors. See http://tronolab.com for maps of lentiviral vectors and packaging plasmids.

Origin & function

The genes to be inserted include jellyfish GFP; firefly luciferase; E. coli lacZ, nfsB; phage Cre; S.cerevisiae FCY1; human CTNNB1, LEF1, HIF1A, TP53, PP1R15A, MMP7, and shRNA targeting human DPYD, APC, CBY, TP53 (for information on human gene symbols see http://www.gene.ucl.ac.uk/nomenclature/). The origins of the materials include the American Type Culture Collection (#ATCC- website www.atcc.org) and other standard scientific suppliers (eg Invitrogen - website www.invitrogen.com).

The function of the genetic material is to generate tumour targeting adenoviruses to treat cancer (so-called 'oncolytic viruses'). The viruses replicate selectively in tumour cells. They contain multiple mutations that attenuate them in normal cells. The attenuating mutations include mutation of the viral promoters and deletion of viral genes. Prodrug-activating enzymes expressed by the virus are used to convert harmless prodrugs into active chemotherapeutic agents. This allows targeting of the active drug to cells infected with the virus. Retroviral vectors are used to modify normal cells (eg, expression of an oncogene) or tumour cells (eg, expression of a tumour suppressor gene). These modified cells are used to confirm that the oncolytic vectors have the correct specificity. Tumour targeting peptides are inserted into the capsid of the oncolytic vector to enhance the infection of tumour cells. The tropism and efficacy of the oncolytic viruses are tested in vitro using cell cultures and in vivo using immunodeficient mice with human tumour xenografts.

Evaluation of foreseeable effects

Adenoviral vectors:
Replication-competent wild type adenoviruses cause acute, self-limiting upper respiratory tract infections. The general population has life-long immunity to these viruses as a result of infection in childhood. The genetic modifications of the virus restrict viral gene expression and viral replication to tumour cells. Intravenous injection of similar genetically modified viruses into patients participating in clinical trials has shown that the viruses cause only minor side effects unless the dose exceeds 10^12 viral particles. The risk of accidental exposure to such large amounts of virus is effectively zero when the virus is used under level 2 containment. Detailed further information of adenoviral vectors is given in the ACGM Compendium of Guidance (ISBN: 0-7176-1763-7).

Retroviral vectors:
Pseudotyped retroviral vectors can infect a wide range of cells, leading to integration of the viral genome into the cellular genome and expression of foreign genes. The viruses are replication-defective because essential viral genes are deleted. Expression of oncogenes from the integrated virus increases the risk of malignant transformation of the recipient cell. Actual tumour formation requires infection of the same cell with multiple different viruses, or the use of complex protocols, such as infection ex vivo of a patient's lymphocytes in the context of a clinical gene therapy trial. The risk of these events occurring accidentally is effectively zero under level 2 containment. Detailed further information on retroviral vectors is given in the ACGM Compendium of Guidance.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
All work with the GMOs will be performed in Category 2 containment facilities as defined in the ACGM Compendium of Guidance. Appropriate operating procedures will be implemented to ensure only authorised, appropriately trained, personnel can carry out this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All plastic waste from experiments will be autoclaved at 120 degrees C for 30 minutes at 15psi.
All media and liquids will be treated with Virkon solution before disposal as per the manufacturer's instructions.
Spillages will be dealt with by soaking the area in neat Virkon.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Four members of the GMSC agreed that this should be a class 2 project.
The comments of the full GMSC for the University of St Andrews will be sent to you when they next meet.

Project Containment

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<thead>
<tr>
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</table>

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The purpose of this project is to understand the molecular biology of bunyavirus replication, including structure-function analysis of viral proteins, the role of cis-acting signals within vRNA and the interactions of bunyaviruses with host cells.

**Recipient or parental organism**

E.coli K-12 derivatives (e.g. DH5a), E.coli B strains (e.g. BI-21), S cerevisiae strains AH109 and Y187 (from Clontech) Mammalian cell lines (e.g. vero, BHK, Hep2, mRC-5, 293, HeLa), insect cell lines (e.g. Aedes albopictus C6/36, S. frugiperda).

**Host/vector system**

pUC series, pBluescript, pET series vectors for E.coli. pGBKT7 and pGADDT7 for yeast cells. pcDNA and non-cytopathic Sinbis virus replicons pSinRep for mammalian cells. PIB/V5-His for insect cell lines. pBac vectors for generating recombinant baculovirus systems. Autographa californica nuclear polyhedrosis (AcNPV) derivatives such as AcPAK6 for insect cell lines. Expression in Vaccinia virus vectors pTF7-5 and pSC11 for constructing recombinant vaccinia viruses. Vaccinia virus vTF-7 and MVA-T7. Lentivirus vector pLenti-ires-puro.
**Origin & function**

Bunyaviral genes:
- N-nucleocapsid (binds viral RNA)
- L-viral RNA dependent RNA polymerase
- Gn-viral glycoprotein (receptor binding, cell fusion)
- Gc-viral glycoprotein (Receptor binding, cell fusion)
- NSs - non-structural protein (interferon antagonist; blocks RNA polymerase II transcription)
- NSm - non-structural protein (Localises to Golgi- unknown function)

Cellular genes encoding proteins that interact with bunyavirus proteins:
- HeLa genome library for screening by yeast-2-hybrid assay. Specific genes: Med8 (a component of the Mediator complex involved in the regulation of RNA polymerase II mediated transcription), ARC-32 (mosquitto cell homologue of Med8), other individual genes that encode proteins which interact with bunyavirus proteins once they have been indentified by the yeast-2-hybrid screening.

**Evaluation of foreseeable effects**

**E. coli**

The K-12 cell lines are known to be non-pathogenic (see ACGM Compendium of Guidance (ISBN: 0-7176-1763-7). The E.coli cell lines have been less well characterised than the K-12 strains. BL-21 cells are less well characterised than K-12 strains but have a long history of safe use. A recent publication has shown that BL-21 cells is non-pathogenic ans unlikely to survive in certain host animal systems (Chart, H. et al J. Applied Microbiology Vol 89, pages 1048-1058 (2000)). It is thus considered that BL-21 cell are non-pathogenic, though care will be taken when handling this cell line.

**Baculovirus System:**

The Baculoviruses system have a long history of safe use. The system has been used extensively to infect insect cells with foreign genes and there has been no published evidence that viable recombinant virus has been produced in these cells.

**Yeast Cells**

Yeast cells are considered to be especially disabled host cells and it is not considered that any of the hybrid proteins expressed will alter the biological properties of the host.

**Vaccinia vectors:**

Vaccinia virus can infect humans and is classified as an ACDP category 2 pathogen. None of the inserted genes to be expressed is likely to significantly alter the pathogenicity of the vaccinia vector. Recombinant genes have been targeted in the viral thymidine kinase gene and such recombinants are considered to be attenuated relative to the parent. Therefore containment level 2 which is used to contain the parent virus is appropriate for handling the recombinant virus. The avoidance of sharps will reduce the major route of infection which is subcutaneous inoculation.

**Disabled Lentivirus**

Disabled lentivirus vectors are widely used for expressing foreign genes under level 2 containment. Inoculation of the virus into man could result in integration of the viral genome and expression of the foreign gene. Avoidance of sharps will minimise this route of infection.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All work with the GMOs will be performed in Category 2 containment facilities as defined in the ACGM Compendium of Guidance (ISBN: 0-7176-1763-7). Appropriate operating procedures will be implemented to ensure only authorised, appropriately trained, personnel can carry out this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All plastic waste from experiments will be autoclaved at 120°C for 30 minutes at 15 p.s.i.
All media and liquids will be treated with Virkon solution before disposal as per the manufacturer's instructions. Spillages will be dealt with by soaking the area in neat Virkon.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification project notification has been studied by the Chemical and Biological Hazards Sub-Committee and has been provisionally ratified the project as a category 2 project as defined by the GMO (Contained Use) Regulations 2000.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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</thead>
<tbody>
<tr>
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</table>

Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 317/07.1

Date Ackn'd 29/11/2007

CU2 Project Title Manipulation of brain cholinergic systems in vivo.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work Y

Historical Significant Changes

Withdrawn N

Project notified under transitional arrangements N

02/03/2022 Page 6200 of 15326
Project Additional Information

Purposes of the contained use

Investigate the effect of lentiviral vectors on the production of choline acetyltransferase and beta-2-nicotinic receptors in localized brain regions of the rat brain

Recipient or parental organism

Use of third generation HIV based pTRIP delta U3 lentivirus system

Host/vector system

The lentivirus will be grown in 293T cells at the Pasteur Institute, Paris

Origin & function

The inserted gene is a short hairpin DNA producing an interfering RNA which blocks production of choline acetyltransferase or beta-2-nicotinic receptor subunits.

Evaluation of foreseeable effects

Inhibition of the production of choline acetyltransferase and beta-2-nicotinic receptor subunits (determined by immunohistochemical and receptor binding methodologies)

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Category 2 containment facilities

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Where feasible all items will be autoclaved. Where it is not feasible to autoclave, then items will be treated with 2% Virkon. The manufacturers have produced evidence to validate that Virkon will reduce the titre of virus by at least 10

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]
Please enter comments on the GM safety committee on the risk assessment

It was agreed that this project was a category 2 project

## Project Containment

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<th>Laboratory Activities</th>
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## Project Ref 317/07.2

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<th>CU2 Project Title</th>
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<td>28/12/2007</td>
<td>Development of mammalian cells expressing altered levels of polycomb proteins.</td>
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<th>CultureVolumeClass3-4</th>
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<th>Non-GMM</th>
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<tbody>
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<table>
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<tr>
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<tbody>
<tr>
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</table>

### Project Additional Information

**Purposes of the contained use**

The purpose of the project is to perturb polycomb epigenetic signalling pathways in mammalian cells in order to investigate polycomb biology and explore the role of polycomb proteins in cancer progression

**Recipient or parental organism**

-
Host/vector system

pUC19 and similar cloning vectors. pRS406 and similar S. cervisiae vectors. pNKBAC39 and similar BAG vectors. pGL3 and similar luciferase vectors. pcDNA3 and similar mammalian expression vectors. pBABE and similar oncortroviral vectors. pPRL and similar lentiviral vectors. pMD2G, pCMV-dR8.74, pMDLgplRRE, pRSV-Rev and similar packaging plasmids for lentiviral vectors. See http://ltronolab.com for maps of lentiviral vectors and packaging plasmids.

Origin & function

The genes to be inserted include jellyfish GFP; firefly luciferase; E.coli lacZ, nfsB; phage Cre; S.cerevisiae FCY1; human EZH2, SUZ12, ESRI, SMII, hTERT, MYC, CTNNB1, and shRNA targeting SUZ12, EZH2, DNMT1 (for information on human gene symbols see http://www.gene.ucl.ac.uk/nomenclature/). The origins of the materials include the American Type Culture Collection (ATCG - website www.atcc.org) and other standard scientific suppliers (eg Invitrogen - website www.invitrogen.com).

The function of the genetic material is to generate tumours from normal cells or to suppress tumour cells in order to investigate tumour formation. Retroviral, lentiviral or adenoviral vectors are used to introduce the genetic material into cells (eg, expression of an oncogene) or tumour cells (eg, expression of a tumour suppressor gene). Different contributions of genes to the transformation process will reflect their role in influencing the tumour phenotype in breast cancer.

Evaluation of foreseeable effects

Adenoviral vectors:
Replication-competent wild type adenoviruses cause acute, self-limiting upper respiratory tract infections. The general population has life-long immunity to these viruses as a result of infection in childhood. Expression of oncogenes from replication-defective virus increases the risk of malignant transformation of the recipient cell. Actual tumour formation requires infection of the same cell with multiple different viruses, or the use of complex protocols, such as infection in vivo of a patients lymphocytes in the context of a clinical gene therapy trial. The risk of these events occurring accidentally is effectively zero under level 2 containment. Detailed further information on adenoviral vectors is given in the ACGM Compendium of Guidance (ISBN: 0-7176-1763-7).

Retroviral and lentiviral vectors:
Pseudotyped retroviral and lentiviral vectors can infect a wide range of cells, leading to integration of the viral genome into the cellular genome and expression of foreign genes. The viruses are replication-defective because essential viral genes are deleted. Expression of oncogenes from the integrated virus increases the risk of malignant transformation of the recipient cell. Actual tumour formation requires infection of the same cell with multiple different viruses, or the use of complex protocols, such as infection in vivo of a patients lymphocytes in the context of a clinical gene therapy trial. The risk of these events occurring accidentally is effectively zero under level 2 containment. Detailed further information on retroviral vectors is given in the ACGM Compendium of Guidance (ISBN: 0-7176-1763-7).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All work with the GMO5 will be performed in Category 2 containment facilities as defined in the ACGM Compendium of Guidance (ISBN: 0-7176-1763-7). Appropriate operating procedures will be implemented to ensure only authorised, appropriately trained, personnel can carry out this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All plastic waste from experiments will be autodaved at 120 oC for 30 minutes at 15 p.si. All media and liquids will be treated with Virkon solution before disposal as per the manufacturers instructions. Spillages will be dealt with by soaking the area in neat Virkon.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Paper/07/07/f- Perturbation of epigenetic signalling in mammalian cells - The Sub-Committee discussed two aspects of this project. The first was the use of lentivirus vectors. After significant discussion of this matter it was agreed that the lentivirus system used would a replication deficient system and thus could be performed in category 2 containment facilities. The second area of discussion was the use of adenoviral vector systems. It was also agreed that the adenoviral vector system could also be carried out in category 2 containment facilities. It was agreed that this work should be categorised as a Category 2 Project.

Action - It was agreed that the Secretary would inform the Sub-Committee's decision.

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Project Ref 317/08.2

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Non-GMM Consenent Granted [Yes]

Project notified under transitional arrangements [N]

02/03/2022
### Project Additional Information

**Purposes of the contained use**

To identify lipid biosynthetic genes that will allow the disruption of the growth and transmission of Trypanosomes and other parasites.

**Recipient or parental organism**

Trypanosoma cruzi, Leishmania braziliensis, Leishmania chagasi, Leishmania donovani, Leishmania infantum and Plasmodium falciparum are all categorised by the ACDP as category 3 human pathogens.

All other pathogens which may be used will be category 2 or 1.

**Host/vector system**

(I) List of recipient strain(s),

The host strains used are Trypanosoma brucei brucei. Although distinct isolates may be used for the analysis of strain variability, these do not differ in their potential risk with respect to genetic modification. The particular strains used for our experiments in >95% of cases will be:

- Trypanosoma brucei
- Lister S427
- TREU 92714

The other remaining 5% of the work will utilise the following pathogens:

- Trypanosoma cruzi
- CL Bruner
- Y starin
- Leishmania spp m4or mexicafra
- donovani
- tropica
- braziliensis
- mfnantum
- Plasmodium falciparum
- 010
- 3D7
- Toxoplasma gondii
- RH
- E.coli
- DH5αL

---

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

---

02/03/2022
Various TS and Null mutants of the lipid biosynthetic genes mentioned in this project

If a micro-organism, what other organism(s) (e.g. animals, plants) will the recipient strain infect
Rodent: Mouse (Balbc) and Rat (Wistar)

List of vectors
T.brucei pLew 100 pLew 82 pGEM 5zf p217
p217-177
Leishmania IR-SAT1
T.cruzi pTc INDEX
Plasmodium
pHH1 pH2 pH TK pH PR
Yeast pRS316
E.coli All of the above vectors will be manipulated using E.coli as a shuffle organism for vector construction
TOPO
TOPO pBAD pETSa pET 15b pET 16b pET 19b pET 20b
pET 29b
pET32b
pET duet
pGEX 4.1
pGEX6.1
pLou3 (MBP)

Genes involved in lipid biosynthesis in the protozoan parasites mentioned above will be investigated by forming knockout, RNAi knockdown and overexpression cell lines. The genes in question will also be overexpressed in E.coli for recombinant expression, purification and activity studies. In a few circumstances complementation & TS or null mutants yeast will allow functional complementation studies.

The genes to be studied will be involved in:

a) phospholipid biosynthesis (including uptake of precursors), remodeling and degradation.

b) glycolipid formation i.e. glycosylphosphatidylinositol anchors and N-glycosylation. i.e. dolichol-phosphate-mannose synthase C3DP-man pyrophosphorylase, PNQase F, SPI anchor de-N-acetylase.

c) Sterol biosynthesis

i.e. the enzymes of the mevalonate pathway i.e. IDI-isopentenyl diposphate isomerase and CYP51- 14- demethylase

Evaluation of foreseeable effects

We expect most genetic manipulation of the parasite to reduce fitness and/or virulence: our experiments to date indicate perturbed expression of the molecules under study in our laboratory reduce virulence.
For example, manipulation of the genes studied to date have in each case either generated no consequence for the parasite, or have resulted in disruption of the parasite morphology, cell cycle or capacity for essential developmental processes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All work to be performed in Category 3 containment facilities when they become available in the animal house

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work with parasite pathogens will be carried out in category 3 containment facilities within this University.
Where feasible all items will be autoclaved within a category 3 containment facility.
Where it is not feasible to autoclave, then items will be treated with 1% Virkon. The manufacturers have produced evidence to validate that Virkon will reduce the titre of virus by at least i05

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The relevant section of the last University of St And rows Genetic Modification Safety Committee meeting which discussed submission is given below. This project has also been scrutinised by the Health and Safety Committee for the building where the work will be carried out.

University of St Andrews
Report of the Meeting of the Chemical and Biological Hazards Sub-Committee of the University Health and Safety Committee.

A meeting of the above sub-committee was held on Wednesday 17th December 2007 at 2.15pm in the Lochnagar Room, Mansfield House.

Present: - (University Biological Hazards Adviser) (Convenor)  
(Secretary)
CBHSC/07/07 Ratification of New Genetic Modification Projects

Paper07/07gg - Genetic validation of drugs targets in protozoan parasites - The Sub-Committee discussed the use of ACDP Category 2 protozoans and Category 3 protozoans. They were informed that. toes not intend to grow Plasmodium parasites in an arthropod cycle thus there is very limited risk of the protozoan escaping into the environment.

It was agreed that this project has now been ratified and should be categorised as a Category 3 Project

Action - It was agreed that the Secretary would inform the Sub-Committee’s decisiona

The meeting ended at 4.15 pm
### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
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<td>L3 L4</td>
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<td>Large Scale Activities</td>
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<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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<tr>
<td>Date Ackn’d</td>
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<tr>
<td>CU2 Project Title</td>
<td>Molecular Biology of HIV/SIV and Other Retroviruses</td>
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<td>CultureVol</td>
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<td>CultureVolumeClass3-4</td>
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<td>Consent Granted</td>
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</table>

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

Elucidation of fundamental aspects of retroviral replication has and will suggest novel targets for the development of antiretroviral therapies. It is notable that no approved antiretroviral drugs currently target the Gag protein. Towards this aim current areas of research in this project include, 1. Understanding Gag cleavage sites as molecular targets for antiretroviral drug development, 2. Understanding the role of the envelope gp41 glycoprotein long cytoplasmic tail in lentivirus replication and 3. Understanding the role of host factors in retroviral assembly and release pathways with a particular emphasis on determining factors that facilitate envelope incorporation into the assembling particle.

**Recipient or parental organism**

02/03/2022
Viruses:
- Human immunodeficiency virus type 1 and 2 (HIV-1 and HIV-2)
- Simian immunodeficiency virus (SIV)
- Equine infectious anemia virus (EIAV)
- Feline immunodeficiency virus (FIV)
- Murine leukemia virus (MLV)

Host/vector system

Mammalian cell lines:
- Human carcinoma and fibroblast cell lines: HeLa, TZM, P4-P5 MAGI, Ghost and derivatives.
- Human T lymphoid: Jurkat, CEM, HUT78, HUTCC5, H9, SupT1, MT4, M8166.
- African green monkey fibroblasts: COS, Vero
- Crandall feline kidney cells: CrFK.
- Murine fibroblasts: 3T3, Murine lymphoid: EL4
- Canine Thymus: CF2Th
- Equine dermal cells: ED
- Cell lines from the ATCC, HPACC or the NIH AIDS reagent program and partners at NIBSC.

Human primary cells: Human peripheral blood monocytes (PBMCs) and macrophages. Donated from anonymous health volunteers.

Bacterial strains: E. coli (K12 strains), which have been attenuated to prevent survival in non-laboratory environment: e.g. JM107, Stab12, Stab13, Top10, DH5a BL21.

Details of procedures can be found in the attached risk assessment

Origin & function

Reporter genes: L-glactosidase (e.coli), luciferase (firefly), green fluorescent protein (jelly fish) etc. Selectable marker genes (e.g. puromycin resistance gene).

Host proteins known or hypothesized to be required for retroviral replication:
- CD4, CCR5, CXCR4 - T cell and chemokine receptors used for HIV entry.
- Members of the ESCRT complexes (e.g. Tsg101, VPS4, Alix) - Cellular endosomal sorting machinery, which is hijacked by HIV to promote virus release.
- Ubiquitin - binds to proteins as a post-translational modification that labels proteins for protein sorting or proteosomal degradation pathways, known to promote HIV particle release.
- Tetherin/BST-2 - interferon-induced membrane protein that inhibits HIV virus release.
- TIP47 - Lipid cargo protein, which is thought to promote HIV-1 Env incorporation by acting as a bridging molecule between the MA domain of Gag and the Env gp41 protein.
- Calmodulin - calcium-binding protein hypothesized to be involved in HIV replication, as it is known to bind MA domain of Gag and the Env gp41 protein.
- ADAM10 - disintegrin/metalloproteinase hypothesized to be involved in HIV replication

Please note: Should our appreciation of function or new candidate genes be identified, I would amend this risk assessment accordingly using the extension system.

Evaluation of foreseeable effects

Wild type viruses are -
- HIVs: ACDP hazard group 3,
- SIVs: ACDP hazard group 3;
- EIAV, FIV, MLV: ACDP hazard group 1

Details of the effects of the triple and dual vector systems are given in the risk assessment
Category 3 work will be undertaken in the category 3 containment facilities at the Centre for Biomolecular Sciences, North Haugh, University of St Andrews, St Andrews Fife KY16 9ST. Attached is the Standard Operating Procedures for this laboratory. These will be modified for work with HIV and a copy of the modified SOP will be sent to the HSE Biological Agents Unit.

Other work will be carried out in category 2 containment facilities in the new Medical and Biological Sciences Building at the University and in the Centre for Biomolecular Sciences building.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Category 3 waste will be autoclaved - For Plastic Discard, for all plastics/paper waste, 121°C/15 min/small volume select. - For Fluid Discard, for large volume liquid waste, 121°C/15 min/large volume select.

For Category 3 laboratory: Virkon at 1% will be used as a disinfectant as it has been validated to be effective against retroviruses. Contaminated waste will be soaked in virkon solution. Laboratory workers in the CL3 wear disposable gloves, gowns, foot shields and eye shields all of which are removed prior to leaving the facility. All material will be autoclaved before leaving the CL3 facility. The approved standard operating procedures for the CL3 facility will be strictly adhered to.

For category 2 laboratory: Virkon at 1% will be used as a disinfectant as it has been validated to be effective against retroviruses. Contaminated waste will be soaked in virkon solution. Laboratory workers in the CL2 will wear disposable gloves, eye shields and lab coats. All materials will be autoclaved.

There will be no use of sharps materials for this work.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
See the following comments from members of the Chemical and Biological Hazards Management Group of the University of St Andrews which acts as the genetic Modification Safety Committee for the University:

I wanted to have a word with B before replying to you on this matter. The points that were raised in our building safety meeting about (i) virus particle 'pseudotyping' (VSV-G protein) and (ii) particle inactivation have been addressed and my rating of this project is Category 3.

Convenor- Chemical and Biological Hazards Management Group

Having read the project and discussed it with B I felt that CL3 is appropriate for this project. B has told me that there will have to be a written change in the SOP to accommodate certain procedure die the use of triton X100 to inactivate the virus before it is removed from CL3 thi is to allow the samples to be used in luciferase assays. Dr A has shown referenced that validate the effectiveness of this method.

JN

As I and PR signed it following a safety meeting attended by many BMS Pls, C, P R, R E, A M and T S I think we have covered the bases at this end. There are some issues that need sorting viz SOP but that’s in hand.

BP

There are portions of this project that fall into each of 1, 2 and 3. Clearly the work at the CNMS cat 3 unit is category 3 work. The work at the Medical School labs is category 2 with some DNA work that is category 1 (the Medical School labs are all category 2 rated space). In terms of maximal rating for each location, Medical School labs - category 2, CBMS contained unit - category 3.

PR

I have read through the GM1 for C A’s project. I agree with their assessment - the work with HIV and SIV viruses should be Cat3. The use of Cat 2 with containment seems reasonable for the other viruses.
Having read both this project, I believe the work with viable HIV and SIV viruses must be undertaken in category 3 containment as should work with the animal pathogen Equine infectious Anaemia Virus.

Work with the disable systems that cannot produce viable virus can be undertaken in category 2 facilities. Where there is a change in tropism of the virus or vectors which may allow expression of potentially hazardous genetic material, this work should be carried out in category 3 containment.

Deputy Director of Environmental, Health and Safety Services.

From a CL3 point of view we are content with approval for those aspects of the work to be done at level 3. The SOPs for handling HIV and related viruses are currently being discussed and will be forwarded for Safety Office information soon. C is also preparing their COSSH assessments.

Director of the Category 3 facilities at the University

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**Project Ref** 317/13.1

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<th>CU2 Project Title</th>
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<th>CultureVolumeClass3-4</th>
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<td>21/08/2013</td>
<td>Molecular analysis of arenaviruses</td>
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Project notified under transitional arrangements N

02/03/2022
## Project Additional Information

**Purposes of the contained use**

- Analysis of the methods of replication Arenaviruses and and expression of Arenavirus proteins

**Recipient or parental organism**

- coli K12 strains (eg DH5a), E. coli B strains, Mammalian cell lines (Vero, BHK, HepG2, HeLa, MDCK, A549, BSR-T7, Huh-7 and 1321N1) and Insect cell lines (Spodoptera frugiperda - SF-9)

**Host/vector system**

- pUC based systems, pGEX, pHISTEV, pGEM-T, pSP72, pMAL-c2X and pET, pcDNA, pcAGGS, pHH21, pUC19, pTVT7R, pBac, AcPAk6, Lentivirus vectors pdl-MCS-R-puro, pdl-MCS-R-blast, plKO-MCS-puro, plKO-MCSblast, pKOTetR

**Origin & function**

- Genes whic produce proteins which interact with Arenavirus proteins, proetins which are involved in the caspase dependent and independent apoptosis pathway and genes for proteins involved in cellular - Arenavirus interactions.

**Evaluation of foreseeable effects**

- Possible risk of expression of Arenavirus proteins involved in pathogenesis.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- N/A

- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Where practicable, all waste products will be autoclaved at 121°C for 15 minutes at 15 psi. Where this is not possible, waste will be treated with 1% virkon.

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 317/15.1

- **Date Ackn'd**: 25/02/2015
- **CU2 Project Title**: Phenotyping allelic variation in Plasmodium knowlesi
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **CultureVolumeClass3-4**: Non-GMM
- **Non-GMM Consent Granted**
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

02/03/2022
**Project Additional Information**

**Purposes of the contained use**
Research into the infectivity of Plasmodium knowlesi

**Recipient or parental organism**
Plasmodium knowlesi
K12 E. coli for growing up plasmids

**Host/vector system**
K12 E coli and pHH4 and pMA

**Origin & function**
pknbpaxa and pknbpbxb - Genes related to the infectivity of Plasmodium knowlesi
More details in the attached risk assessment

**Evaluation of foreseeable effects**
There is the potential to affect the infectivity of Plasmodium knowlesi
More details in the attached risk assessment

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
The Plasmodium cells will only be allowed to grow to Merozite stage and thus cannot infect normal insect vectors. All work will be done in microbiological safety cabinets. Accidental infection will be treated with Coartem (from Novartis) which will be administered by a medically qualified person from the School of Medicine.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be treated by autoclaving at 121°C at 15psi for 30 minutes. Liquid waste will be autoclaved at 121°C at 15psi at 30 minutes where possible. Where liquid waste cannot be autoclaved, the liquid waste will be treated by 2-5% Prespt or 2-5% Virkon. All glass ware will then be washed with 5% Decon to ensure that no erythrocytes will survive

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

02/03/2022
From: M R  
Sent: 16 November 2014 14:01  
To: P S  
Subject: RE: GM Projects  

Dear P,  
I have looked at the projects and I assess these projects as;  
Dr J C S (Medicine) - category 2 project  
Best Regards,  
M.  

Prof. M R  
Biomedical Sciences Research Complex (BSRC),  
School of Biology, University of St. Andrews,  
North Haugh, St. Andrews KY16 9ST  
Fife, Scotland, Uk.  

From: BSRC Health and Safety  
Sent: 20 November 2014 13:00  
To: P S  
Subject: Re: Genetic Modification projects  
But yes, category 2 for Janets project.  
Ma.  

From: P R  
Sent: 06 November 2014 13:50  
To: P S  
Subject: RE: Genetic Modification projects  

Dear P.  
J C S  
Class 2 project. Due to not the manipulations which are class 1, but the fact that Plasmodium knowlesi is a listed cat2 non-GM organism, making the whole project class 2.  
P------------------------------------------

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</table>

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N
This work is to investigate the regeneration and plasticity of injured nervous system.

Cell lines will include PC12, RPE cells, iPS cells, rodent primary neuron cells and packaging cell lines HEK293T cells.

Vectors to be used: pcDNA3, pEYFP and similar vectors. Lentiviral vectors will include pMD2.G, pRSV-Rev, pMDLg/pRRE, pLJM, pLKO.1 Details of these vectors can be found in the attached risk assessment.

Origin & function

Neuronal growth promoting molecules including tyrosine kinase receptors (trk), Integrins - Transmembrane receptors that mediate signals between their intracellular matrix. Cell reprogramming factors eg Oct4, Klf4, Sox2, cmyc. Fluorescent proteins and epitope tags eg GFP or V5.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Containment level 2 facility as defined by the SACGM Compendium of Guidance
http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/ See risk assessment for details

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be treated by autoclaving at 121°C at 15psi for 30 minutes. Liquid waste will be treated by autoclaving at 121°C at 15psi for 30 minutes where possible. Where liquid waste cannot be autoclaved, the liquid waste will be treated with 1% Virkon. Surfaces will be wiped down before and after work with 70% ethanol

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 317/15.3

Date Ackn’d 13/08/2015

CU2 Project Title Introduction of light switchable proteins into mammalian cells using viral transduction

Class 2

Culture Volume

Class 2 < 1 Litre
The aim of the project is to introduce a variety of light switchable proteins into mammalian cells in order to gain light mediated control over neural function.

**Recipient or parental organism**
- Prokaryotic cell lines: XL10Gold, DH5a, Stb13 and other disabled E.coli K12 strains
- Eukaryotic cell lines: HeLa, HEK293, SH-SY5Y, hNSCs (Human h9 derived), commercially available human derived iPSC lines, primary rat neurons

**Host/vector system**
- Bacterial vectors - pc-DNA3.1, pGEM-T, tol2-GFP, pDEST32, pMH14, pG and FCK plasmids
- Viral vectors - Adeno Associated Viruses (AAV)

**Origin & function**
- Channelrhodopsins, Archaeorhodopsins, ASAP1, Phytochrome based dimerizing system (PhyB-PIF), Crytochrome based dimerizing system (Cry2-C1B1), CRISPR/dCas9 gene activation repression system, fusion proteins from dCas9 and Cry2/PhyB, common fluorescent markers (e.g. GFP, mCherry, mOrange2, YFP, dTomato).

**Evaluation of foreseeable effects**
- The bacterial systems will have minimal potential effects on human health or the environment. AAV transfected human cells will have minimal effect as the genome is replicative defective requiring trans co-infection with a second helper virus to allow viable virus be generated.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Containment level 2 facilities as defined in the SACGM Compendium of Guidance
(http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be treated by autoclaving at 121°C at 15psi for 30 minutes. Liquid waste will be treated by autoclaving at 121°C at 15psi for 30 minutes where possible. Where liquid waste cannot be autoclaved, the liquid waste will be treated with 1% Virkon. Surfaces will be wiped down before and after work with 70% ethanol.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  317/16.1

Date Ackn’d  08/06/2016  CU2 Project Title  Analysis of herpesvirus-host cell interactions

Class  Class 2  Culture Volume  ≤ 1 Litre
**Project Additional Information**

**Purposes of the contained use**

The purpose of the work is to: 'This project aims to further our understanding of infection and pathogenesis caused by CMV and, for comparison, other herpesviruses. To this end, we are studying virus-host interactions at the molecular and cellular level.'

**Recipient or parental organism**

Disabled Escherichia coli (E. coli) laboratory strains derived from E. coli K12 (e.g. DH10B, GM169, JC8111, JM109, SURE, SURE2); Disabled E. coli K12-derived laboratory strains, i.e. DH10B, DY380, EL11, EL250, EL350, GS243, GS500, GS1783, SW102, and SW105; Disabled E. coli laboratory strains derived from E. coli K12 (e.g. M15[pREP4]) and E. coli B (e.g. BL21 (DE3), BL21-CodonPlus(DE3)-RIL, Rosetta); Established, fully characterised cell lines (e.g. 293T, H1299, HeLa, NIH3T3, MRC-5, U2OS); Primary cell cultures of murine origin; Primary cell cultures of human origin; Infectious, replication-incompetent retroviruses; Recombinant CMVs

**Host/vector system**


Bacterial expression plasmids pBAD series, pET series, pGEX series, pQE series, pT-E1E2S1 (Uchimura)

Mammalian expression plasmids

Oncoretroviral transfer vector plasmids

Lentiviral transfer vector plasmids
These will be used in combination with a second generation packaging system consisting of the HIV-1 gag-pol/rev/tat plasmid psPAX2 (Addgene plasmid #12260) and VSV-G envelope plasmid pMD2.G (Addgene plasmid #12259).

CMV-BACs
- pMBO131-derived mini-F vectors carrying complete, fully functional wild-type genomes of the human CMV strains AD169, FIX/VR1814, Merlin, PAN, Phoebe, Powers, TB40/E, Towne and TR
- pMBO131-derived mini-F vector carrying the complete, fully functional genome of the murine CMV Smith strain

**Origin & function**

**Human and murine cytomegalovirus**

**Evaluation of foreseeable effects**

There is a potential for human CMV to infect workers though not to spread as it is not stable as an aerosol.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

There will be:

- Biological containment in that murine CMV will not be able to spread to humans and Human CMV will not spread to the murine population which could infect the wild population.
- All work will be undertaken in a category 2 containment laboratory. All work with infectious agents will be carried out in Class II microbiological safety cabinets. Details of full containment can be found in the attached risk assessment.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Where practicable, all waste will be autoclaved prior to disposal, at 121°C at 15psi for 30 minutes. Waste which cannot be autoclaved (e.g. large volumes of culture media) will be treated with 5% Virkon or greater for at least 12 hours prior to disposal.

**Is an emergency plan required according to regulation 20?**

**If yes, tick to confirm that it is attached to this form**

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

Please enter comments on the GM safety committee on the risk assessment
The following comments were received as follows:
From: M R  
Sent: 15 December 2015 12:34  
To: P S  
Subject: RE: Dr M N GM Project  
I have carefully read through the application submitted by Dr. N and I assess this application as requiring a category 2 level of containment.  
Best regards,  
M.  
Prof. M R,  
Biomedical Sciences Research Complex (BSRC),  
School of Biology, University of St. Andrews,  
North Haugh, St. Andrews KY16 9ST  
Fife, Scotland, UK.  

From: P R  
Sent: 10 December 2015 12:39  
To: P S  
Subject: RE: Dr M N GM Project  
is a class 2 project.  
P  
from: T S  
Sent: 10 December 2015 13:28  
To: P S  
Subject: Re: Dr M N GM Project  
After careful consideration, I believe this to be a class 2 project  
T  
rom: BSRC Health and Safety  
Sent: 18 December 2015 11:23  
To: P S  
Subject: re: Dr M N GM Project  
Regarding Dr M N GM Project form/application.  
I believe that from the information given and from discussions with Prof R R, that this project should be assigned Category 2.  
Kind regards,  
Dr. M S. A  
Health and Safety Coordinator  
BSRC  
University of St. Andrews  
St. Andrews  
01334 467257

**Project Containment**
Project Additional Information

Purposes of the contained use

The proposed work is therefore to introduce HLA-B27 and HLA-A2 as folding and nonfolding variants into short term cultures (up to 3 days) of normal healthy subject derived human blood DC and macrophages using lenti and adeno viral constructs, and then to kill and lyse the cells for subsequent analysis by techniques such as immunoblotting and SWATH mass spectrometry. This will provide a global picture of protein pathways that are being induced by misfolded HLA-B27. In addition modulation of assembly of MHC class I will be achieved using commercially available lentiviral products with nontumour associated components of the MHC class I assembly and quality control pathway.

Recipient or parental organism
Top10: F-, mcrA, II(mrr-hsdRMS-mcrBC), ct>80lacZM15, IIlacX74, recA 1, araD139, II(araleu)7697, galU, galK, rpsL(strR), endA 1, nupG.


293T Cells: Human Embryonic Kidney T Cells.

AdenoX 293 Cells: derived from HEK-293 (Human Embryonic Kidney) stably infected with Adenovirus serotype 5. Produce viable viral vectors upon transfection with a linearized adenoviral vector or viable seed stock.

If a micro-organism, what other organism(s) (e.g. animals, plants) will the recipient strain infect?

Viral particles will be collected from the AdenoX 293 cells and from the HEK-293T cells and used to infect monocyte-derived DCs (moDCs), CD303+CD45+ pDCs, CD1c+CD141+ mDCs and macrophages.

**Host/vector system**

pMDLg/pRLRE encodes a HIV-1 Gag/Pol open reading frame (Dull et al., 1998).

Sequence: [http://www.addgene.org/12251/](http://www.addgene.org/12251/)

pRSV-Rev encodes a HIV-1 Rev open reading frame (Dull et al., 1998).

Sequence: [http://www.addgene.org/12253/](http://www.addgene.org/12253/)

pMD2.G encodes a VSV-G open reading frame (Dull et al., 1998).

Sequence: [http://www.addgene.org/12259/](http://www.addgene.org/12259/)

pRDproLF encodes an envelope protein from the RD114 feline endogenous retrovirus (Ikeda et al., 2003). See reference for sequence.

Lentivirus vector plasmids:

- pCLLhPGK-B27
- pCLLhPGK-B27.C1 01 S
- pCLLhPGK-A2
- pCLLhPGK-A2.C101S

Based on pCLLhPGK-GFP (Dull et al., 1998)

pAdeno X-CMV:

The pAdenoX-CMV (Linear) Vector is a linearized adenoviral expression vector designed to constitutively express a gene of interest in mammalian cells. The ends of the vector serve as the In-Fusion cloning site, allowing direct and rapid cloning of your gene system of interest. Expression of the gene of interest is driven by the constitutively active human cytomegalovirus immediate early promoter (PCMV IE).
pAdenoX-CMV contains a LlE1/LlE3, replication-deficient, serotype 5 adenovirus genome (Ad5) that is engineered for use in gene delivery and expression studies. The Ad5 genome is flanked by inverted terminal repeats (ITR), which are necessary for the replication of adenoviral DNA. The vector also contains a pUC replication origin and an ampicillin resistance gene (Ampr) for propagation and selection in E. coli.

pLenti based vectors sourced commercially from Origene (http://www.origene.com/destination_vector/PS100064.aspx)

Origin & function

HLA-B27:05, HLA-A2:01 and non-folding mutants of both these, with cysteine at position 101 mutated to serine.

The Human Leukocyte Antigen (HLA) is located on chromosome 6. These genes form the human version of MHC class 1 and are present on the surface of nearly every cell. These genes are highly polymorphic. In humans there are multiple HLA types expressed at once, HLA-A, -B and -C. MHC class one presents proteins found inside cells to immune cells to aid prevention of pathology and infection.

Components of the MHC class I assembly pathway such as MHC class I alleles, accessory and chaperone molecules including tapasin, calreticulin, calnexin, protein disulfide isomerase, Immunoglobulin Binding Protein (BiP). All components are not associated with tumorigenesis.

Evaluation of foreseeable effects

Competent E.coli used are non-pathogenic and therefore are not hazardous to humans. Transformation of these cells by our target genes will not increase their pathogenicity or survivability.

pAdenoX-CMV cannot replicate adenoviral vectors on its own therefore transformation into Stellar Competent cells will not cause production of adenovirus. pAdenoX-CMV is derived from pUC and is therefore non-mobilisable.

AdenoX 293 cells (Clontech): An Adenovirus serotype 5 transformed Human embryonic kidney cell line for the production of Adenoviral stocks. Stocks can be produced either by transfection with a linearized Adenoviral vector or infection with viable Adenoviral seed stock. Hazards could arise if an unintended adenovirus were to infect the cell line. This will be prevented by standard culture procedures in sterile conditions. All waste fluids or unused cells will be treated with 3% Virkon. ACDP hazard group 2.

We do not propose to use any oncogenically associated Adenoviruses.

Plasmids to construct lentiviral vector are third generation and do not produce viral particles on their own. Furthermore when combined into HEK-293T cells they will complementarily produce a fractional lentivirus as only gag, pol and rev are expressed. The particles will be non-replicatable and gag and pol are only expressed upon rev promoting their nuclear export. The only HIV genes present will be gag, rev and pol as tat has been replaced with a strong constitutive promoter. The lentiviral vector is packaged by 4 non-overlapping expression constructs, two expressing HIV proteins and the other two, one expressing the envelope of a different virus and the other containing the transgene flanked by self-inactivating (SIN) LTRs. Moreover, all HIV sequences known to be required for encapsidation and reverse transcription are absent from these constructs, with the exception of the portion of the gag gene that contributes to the stem-loop structure of the HIV-1 packaging motif. With respects to infectious virus particles, the vectors will contain self-inactivating (SIN) LTRs and therefore will be unable to replicate.

Inserted gene produces a protein that requires processing by human cells in order to be functional therefore inserted gene is not hazardous on its own or when transformed into competent bacteria. Only potential hazard from adenoviral or lentiviral vector infecting cells. However the adenovirus does not integrate into the host genome meaning
It cannot transfer between cells.

There is no evidence that HLA-B27 is involved with MS. The Adenoviral vectors do not integrate with the host cell genome. If an Adenovirus accidentally infected a human there should be no occurrence of AS unless the individual is already susceptible. The lentivirus particles can integrate into a host cell genome however they are self-inactivating therefore this would not spread to other cells. If the human cell then expressed HLA-B27 the cell would be destroyed by the immune system.

Viral products and associated products will be handled in a level 2 biological safety hood. Workers will wear double nitrile gloves and laboratory coats when using products.

There is little hazard as the genes to be inserted do not fold correctly and cannot be expressed on bacterial cells. Plasmids used are non-mobilisable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All work will be done in Category 2 Containment facility. All workers will be HLA-B27 negative and will be tested for this (The named people on this application are not HLAB27 positive, and any future named persons will be tested for HLA-B27 expression by simple flow cytometry of blood obtained from finger prick test (approx. 30-50 microlitres only required.). Biological material will be autoclaved at 121°C at 15psi for 30 minutes where practicable. The disinfectants to be used will either be 2mg/ml Precept (NaDCC (sodium dichloroisocyanurate)) and also 3% Virkon (Pentapotassium bis(peroxymonosulfate)).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All biological material will be autoclaved at 121°C at 15psi for 30 minutes where practicable. Where this is not practicable, the following chemical disinfectants will be sued 2mg/ml Precept (NaDCC (sodium dichloroisocyanurate)) or 3% Virkon (Pentapotassium bis(peroxymonosulfate)).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
University of St Andrews
Genetic Modification Project – SP –
The Role of HLA-B27 in Ankylosing Spondylitis
(Correspondence of the Genetic Modification Safety Working Group of the University of St Andrews Chemical and Biological Hazards Management Group)
Introduction
The following is the e-mail correspondence of the above Genetic Modification Safety Working Group regarding Dr Powis application to undertake work on Category 2 Genetically Modified Organisms.
Correspondence
From: Sent: 06 November 2017 14:46
To:
Subject: Re: Notification of a GM project from SP School of Medicine
Dear P
I’ve now had a chance to read this proposal. Apologies for the delay in responding.
Overall I think the classification proposed is probably correct. I have the following Q’s.
What would happen if the worker using the engineered Ad or retrovirus is HLA-B27? Is there a danger of an autoimmune response resulting from inadvertent laboratory infection? I appreciate that many of the protein being expressed are misfolding derivatives. How is it proposed that cells expressing these are eliminated? Presumably through a cellular immune response? My immunology is sufficiently out of date that I’m not sure if T cells would be clonally deleted during development if they recognise ‘self’ (which used to be the model). If that’s the case then perhaps an autoimmune response may not happen. Alternatively, perhaps expressing the altered, misfolding, variant of the protein might be presented by a different HLA and induce a response.
I think some additional clarity on these would be useful.
It’s not clear why they keep Virkon in a glass bottle in their Class 2 hood.
Finally, presumably the Ad5 vector system they are using is somehow attenuated? That being the case the ‘hazard to human health’ may be incorrect. The implication there is that it is not attenuated. However, my reading of the relevant manuals suggests that it is a virus in which the E1 and E3 regions have been deleted – this explains the need to passage them in the Adeno-X 293 cells which provide these function in trans. That being the case the resulting recombinant Ads will not be able to replicate in the cells it infects. This needs better clarification – it’s not clear from what is written that the applicants understand the system they are using.
Regards
D
—
D J E, Professor of Virology
Biomedical Sciences Research Complex
North Haugh, University of St. Andrews
St. Andrews, KY16 9ST
Dear P,

Please find attached the amended GMO notification form and a document in response to the questions raised by Prof. E. Furthermore, the grant for the HSE fee to be charged to is: SMDO-XCA006.

Regards,

FC
Powis Laboratory Postdoctoral Research Assistant

GMO application: The role of HLA-B27 in ankylosing spondylitis

Response to the questions raised by Prof. D. E.

Query: What would happen if the worker using the engineered Ad or retrovirus is HLA-B27? Is there a danger of an autoimmune response resulting from inadvertent laboratory infection? I appreciate that many of the protein being expressed are misfolding derivatives. How is it proposed that cells expressing these are eliminated? Presumably through a cellular immune response? My immunology is sufficiently out of date that I’m not sure if T cells would be clonally deleted during development if they recognise ‘self’ (which used to be the model). If that’s the case then perhaps an autoimmune response may not happen. Alternatively, perhaps expressing the altered, misfolding, variant of the protein might be presented by a different HLA and induce a response.

Response: This is a very good point. The observed data is that MHC class I molecules on normal cells present peptides that inevitably include some fragments derived from MHC class I molecules – since MHC class I molecules are subject to the same misfolding and degradation stresses as essentially every other protein trying to fold in the ER. You are tolerant, as pointed out, by both central tolerance (thymic deletion of autoreactive T cells) and peripheral tolerance (Treg cells and suppressive cytokines etc). If someone was infected with a virus that had a different MHC class I allele, it would be recognised as foreign and CTL, and possibly also NK cells, would kill that infected cell. This is essentially the basis of graft rejection – tissue with foreign MHC is rejected by the host immune system. This covers accidental infection of a worker.
whilst growing or infecting cells.

D does raise the next valid point – what if the person is HLA-B27 positive. The model for AS suggests that it is the increased burden of misfolding that seems unique to HLA-B27 that can push the folding machinery into cell stress and pro-inflammatory cytokine release – so would infection with a B27 virus push susceptible HLA-B27 positive people into AS? I don't think I can absolutely rule out this scenario in theory, however, the clinical observation is that AS requires years to develop, and any expression using our non-replicating virus would be unlikely to last more than a few days in any cell. Further it would likely require a massive dose of virus to infect enough immune cells capable of driving the inflammatory pathway of IL-23 (which is known to be a key driver of AS). These cells are dendritic cells, sparsely distributed around the mucosal surfaces, and it would be again unlikely to ever infect enough cells to drive into AS.

The next point is the number of people who are HLA-B27 positive. This is often stated as 6-8% in UK, but a few years ago for a CSO grant we screened 100 healthy normal volunteers in the med school and working in the Bute Building. We found only 2 positive people. I suspect the St Andrews population – with a high non-UK background typical of a University town accounted for the low percentage. So the chance of being HLA-B27, in St Andrews is lower than normal. Also, based on the 'accepted' 6-8% figure, there are almost 5 million people in the UK with HLA-B27, but the number with AS is around 150,000. So any person with HLA-B27 is at relative low risk of getting AS.

The next issue is what if someone were to accidentally inject the virus infected cells we will make in culture. This is easily resolved, the cells come from outbred normal healthy donors, so express 6 other HLA-A, B and C alleles on them in total. The host immune system would reject these cells based on the incompatibility with these 6 alleles. (To exclude all risk, identical twins will not be allowed to participate).

Perhaps the simplest way to resolve this issue that D correctly raises is to not allow anyone who is HLA-B27 positive to handle virus, or infected cells prior to lysis. Neither of the people on the application are HLA-B27 positive, and we can screen any future person that might be added to this work, using a simple finger-prick and flow cytometry of the white blood cells to determine HLA-B27 expression.

Query: It's not clear why they keep Virkon in a glass bottle in their Class 2 hood.

Response: This was from support information provided by Dr P R, the Virkon is used to flush vacuum aspirator lines and wipe any spills. We can easily replace with a plastic bottle if requested.

Query: Finally, presumably the Ad5 vector system they are using is somehow attenuated? That being the case the 'hazard to human health' may be incorrect. The implication there is that it is not attenuated. However, my reading of the relevant manuals suggests that it is a virus in which the E1 and E3 regions have been deleted – this explains the need to passage them in the Adeno-X 293 cells which provide these function in trans. That being the case the resulting recombinant Ads will not be able to replicate in the cells it infects. This needs better clarification – it’s not clear from what is written that the applicants understand the system they are using.

Response: apologies for the confusion, the Ad5 vector system is attenuated and cannot replicate in infected cells. We will alter the hazard to human health section appropriately.

GMO application: The role of HLA-B27 in ankylosing spondylitis

Response to the questions raised by Prof D E.
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Response: apologies for the confusion, the Ad5 vector system is attenuated and cannot replicate in infected cells. We will alter the hazard to human health section appropriately.

From:
Sent: 20 November 2017 11:12
To:
Subject: Re: Powis Lab GMO Application

It remains a class 2 project.
PR
School of Medicine.

From;
Date: Saturday, 18 November 2017 at 21:15
To:
Cc:
Subject: FW: Powis Lab GMO Application
Dear Colleagues,
Professor E had some concerns about Dr P’s project involving the use of genetically modified organisms. In reply to these concerns, Dr P has re-written the risk assessment and also has attached their reply to these concerns. I would be grateful if you could consider the reply from Dr P. As the risk assessment has been modified I would be grateful if you could consider the modifications to this project and give your considered opinion about what category of project this would be under the genetically modified organisms (contained use) regulations 2014.
Many thanks for your co-operation in this matter.
Best wishes,
PS

FROM: BSRC Health and Safety
SENT: 21 November 2017 12:17
TO:
SUBJECT: Re: Powis Lab GMO Application

Dear P
I would still consider this a Category 2 project. I again defer to the expertise that you, D and T bring to this. As long as Dr. P agrees to Prof. E suggestions, then I think he has all the other safety measures in place.
Regards,
M
MA
Health and Safety Coordinator
BSRC
University of St. Andrews
St. Andrews

DATE: Saturday, 18 November 2017 at 21:14
TO:
SUBJECT: FW: Powis Lab GMO Application

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BSRC
University of St. Andrews
St. Andrews

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BSRC
University of St. Andrews
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Many thanks for your co-operation in this matter.
Best wishes,
PS
From: TS
Sent: 29 November 2017 12:02
To: P
Subject: Re: Notification of a GM project from SP, School of Medicine

Hi P,
seems like all of the concerns have been addressed yes class 2
TS
School of Biology

-------------------------------------------------------------------------------------------------------------------------------------

From: St Mary’s Animal Unit Safety
Sent: 29 November 2017 16:37
To: PS
Subject: RE: Notification of a GM project from PS, School of Medicine

Hi p
My position on this being a category 2 project remains the same.
Regards
DT
School of Psychology and Neuroscience

Project Containment

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Project Ref 317/18.2

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<td>Not Applicable</td>
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Project notified under transitional arrangements N

02/03/2022
Page 6235 of 15326
There are an estimated 46.8 million people worldwide suffering from dementia. Currently, there are no disease modifying treatments for this disease resulting in a $1 trillion global financial burden. The underlying causes and mechanisms involved in Alzheimer's disease (AD) are still relatively unknown involving changes in many genes and in order to develop potential disease modifying drugs, we must investigate these underlying causes more closely and if found would have a conceivable huge global impact. The project aims to introduce genes encoding a variety of proteins involved in Alzheimer's disease into several in vitro cellular assays and manipulate their expression levels to elucidate their role in neuronal activity and metabolic pathways implicated in AD. 17βHSD10/ABAD is known to interact with both Aβ(40 and 42) a hallmark pathology of AD, leading to distortion of the native enzyme structure. In vitro experiments have shown that the interaction between 17βHSD10 and Aβ is cytotoxic with a build-up of reactive oxygen species and toxins leading to mitochondrial dysfunction. Using site-directed mutagenesis the loop-D region of 17βHSD10 was identified as the binding site for Aβ and subsequently a 28-amino acid peptide was synthesised encompassing this region, which was termed the 17βHSD10 decoy peptide. Significantly, inhibition of this interaction was also shown to translate into a cytoprotective effect in cortical neuron cell culture experiments. There is a second approach which also holds merit in treating AD; the direct modulation of 17βHSD10 activity. In vitro experiments with SHSY-5Y cells exposed to the 17βHSD10 inhibitor AG18051, showed a reduction in mitochondrial dysfunction and oxidative stress associated with the interaction between 17βHSD10/Aβ whilst also protecting cells from Aβ mediated cytotoxicity (Lim et al. 2011). Furthermore in 2014, Valasani et al. demonstrated that in cortical mitochondria isolated from mouse brain, 17βHSD10 inhibition was protective against Aβ induced mitochondrial dysfunction, respiratory function and respiration rate (as measured by swelling in response to calcium, cytochrome-c oxidase activity and ATP levels respectively). Proving that inhibiting 17βHSD10 activity is also a viable therapeutic approach for the treatment of AD. To this end lentiviral constructs will be used to create a more AD like environment and used as therapeutic screening platforms against 17βHSD10. Other experiments include investigating the metabolic profile changes in the AD systems and establishing carbon source changes and mechanisms. Each year, genome Wide Association Studies (GWAS) suggest new genes and risk factors that could be involved in AD. Recently, a new gene, FRMD6/Willin was identified as an Alzheimer's disease (AD) risk candidate (Shen et al 2013). Subsequently, FRMD6/Willin was found to be one of the upstream components of the newly emerging Hippo pathway and
can induce the nuclear translocation of a transcriptional co-activator YAP (Angus et al 2012). Preliminary data from the FGM group has shown that FRMD6/Willin is expressed in the mammalian central nervous system (CNS), notably human synaptoneurosome preparations, however its overall function has not been fully investigated in the mammalian CNS. Kibra, a protein found in kidney and brain and Merlin are also reported to be upstream regulators of the Hippo pathway at the same level as Willin (Angus et al 2012).

Importantly, KIBRA has been reported for its role in memory issues (Zhang et al 2014), while genetic mutations within Merlin gene have been linked to schwannomas (Petrilli et al 2016). In addition, YAP has been recently linked to neurite outgrowth (Jia et al 2016) and myelin development (Fernando et al 2016). Also, FRMD6/Willin was reported for its role in peripheral nerve regeneration (Moleirinho et al 2013a). All these findings imply the potential involvement of the Hippo pathway in neuronal behaviour. Furthermore, KIBRA has been extensively reported as a regular for synaptic plasticity in mammalian brain (Tracy et al 2016; Heitz et al 2016), though there is no evidence to directly show KIBRA modulates synaptic plasticity through the activation of Hippo pathway. However, considering that SNPs within Willin gene are AD risk candidates and Willin protein is found in the human synapses, it is of particular importance to investigate its potential role in the CNS. In particular we will establish both overexpression and knockdown gene expression neuronal cell line by using Lentiviruses gene delivery system.

Recipient or parental organism

Typical disabled E. coli strains (e.g. XL10 Gold, DH5alpha, Stbl3, Top10 and other commercially available strains), Standard mammalian cell lines to be used in this project include: HeLa, HT-22, HEK293, SHSY-5Y, A172 and U373 astrocyte cell lines, hNSCs (human H9-derived), commercially available human-derived iPSC lines, primary mouse and rat cortical neurons and primary mouse astrocytes.

Host/vector system

Lentiviruses can transduce both actively proliferating and non-dividing cells. Lentivirus integrate their genetic material into the host cell allowing for stable, longterm expression of the transgene. The lentiviral vectors are based originally on HIV but contain <25% of the HIV genome. They have deletions removing all of the viral genes (gag, pol, env, tat, rev, vif, vpr, vpu and nef). The vector 3’ LTR contains a deletion that inactivates both LTRs in the integrated provirus. Infectious virus particles are produced by transient transfection of the vector plasmid, a gag-pol plasmid and an envelope plasmid into 293T cells. The vectors are replication incompetent and are used under level 2 containment. Replication competent lentiviruses will not be used. For lentiviral vectors the VSV G gene is normally expressed from a plasmid (eg pMD2G) that is transiently transfected into the packaging cell. The viruses produced are replication incompetent because the gag, pol and env genes are not present in the vector genome. The lentiviral vectors are used under level 2 containment and replication competent lentiviruses will not be used. Lentiviral-producing cells will be destroyed at the end of each experiment. For reducing gene expression in cells technology systems such as shorthairpin (sh) RNA targeting gene of interest and shScramble will be cloned into pLKO.1puro lentiviral vector. pSD16 (packaging plasmid) and pSD11 (gag-pol) will be used for viral
packaging. For overexpressing the genes of interest systems such as pLenti6.4/EF1a/gene of interest or the control plasmid pLenti6.4/CMV/V5-MSGW/lacZ will be used for transduction. Viral PowerTM Packing Mix plasmids will be used for viral vector packaging. DNA plasmid will be transfected using chemical transfection (Lipofectamine or other transfection reagents) into packaging cells (HEK293) in order to generate viral particles.

Origin & function

17βHSD10/ ABAD: (or Amyloid-β binding alcohol dehydrogenase) is homotetrameric protein localised to the mitochondrial matrix, with the highest levels of tissue expression (characterised by immunoblotting) found in the brain, liver, heart, kidney and gonads. It is a multi-functional protein with main roles reported to be in fatty acid oxidation and isoleucine degradation. In AD, 17βHSD10 is found to be overexpressed in neurons and inhibiting it's activity has been shown to protect against AD relevant insults.

APP: Amyloid precursor protein, is best known as the precursor molecule whose proteolysis generates beta amyloid (Aβ), a polypeptide containing 37 to 49 amino acid residues, whose amyloid fibrillar form is the primary component of amyloid plaques a hallmark pathology found in the brains of Alzheimer's disease patients.

FRMD6/Willin: a member of the ezrin/radixin/moesin (FERM) domain-containing protein family, (Gunn-Moore et al 2005). Willin is an upstream component of the Hippo pathway which defines a signalling pathway that controls organ size by coordinating the transcription factor YAP (Angus et al 2012). Although most published studies regarding Willin have been conducted on cell lines outside the mammalian CNS, initial studies have shown that Willin along with other components of the Hippo signalling pathway (MST1/2, TAZ and YAP) are present in the adult mouse brain. Additionally, preliminary data from the FGM group has also indicated Willin and KIBRA (another upstream regulator of Hippo pathway) are expressed in human synaptoneurosome preparations of the frontal cortex from postmortem human brain tissue. KIBRA: has been intensively reported as a regulator for synaptic plasticity in mammalian brain (Tracy et al 2016; Heitz et al 2016), though there is no...
evidence to directly show KIBRA modulates synaptic plasticity through the activation of Hippo pathway. However, considering that SNPs within FRMD6/Willin and KIBRA genes are AD risk candidates and FRMD6/Willin protein is found in the human synapses, it is of particular interest to investigate its potential role in the CNS, and its potential role in synaptic plasticity.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable – There will be no use of genetically modified animals in this project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Where practicable, all waste will be autoclaved at a minimum of 121°C at 15 psi for 30 minutes. Where it is not possible to autoclave waste, the waste will be treated with at least 1% Virkon. See risk assessment

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Subject: Re: Notification of a Genetic Modification Project by Professor Gunn-Moore

Dear P,

No unfortunately we did not receive these comments before Christmas, however please find attached the GM form where I have incorporated Prof Evans’ comments. All comments have been addressed except the "virkon in glass”, yes there are other options but that is just how it is done in the Medical Building, we are not based in BSRC.

Best wishes
L
LA
Postdoctoral Research Fellow
University of St Andrews
Professor Frank Gunn- Moore Group
Medical and Biological Sciences Building
Biophotonics Office Room 137
North Haugh
St Andrews
Fife
KY16 9TF

-----------------------------------------------

From: BSRCDIR Director
Sent: 08 December 2017 09:51
To:
Subject: Re: Notification of a Genetic Modification Project by Professor Gunn-Moore

Dear P,

I've read through this application from F and am reasonably satisfied with what is proposed.

The named genes to be expressed and/or shutoff are not known to be oncogenic and their expression or control under the conditions proposed should not be a problem. I am satisfied with the proposed categorisation of the studies.
I have one particular concern and it’s on the scope of the proposal. We, rightly, encourage applications with breadth to minimise trivial amendments as projects change or expand. However, the statement

In this project, any of the genes in the human genome involved in Alzheimer’s disease may be manipulated using lentiviral transduction. Examples of genes to be manipulated are:

In section iv) on page 5 seems a bit of a catch all. I don’t know how many genes this might include, but with a trait as widespread as AD I suspect that GWAS studies could turn up (or have turned up) all sorts of things. At the most extreme this statement gives carte blanche to look at anything. I don’t know how we could both reduce the breadth and allow flexibility … perhaps something about allowing ‘any gene UNLESS there was well-documented risks in terms of toxicity, oncogenesis etc.’.

They make a repeated point about destruction of lenti producer lines, but no mention of storage of the lentiviruses produced. I assume that they will do this – to avoid the need to make them repeatedly – so consider the destruction of the producer lines a bit of an irrelevance. If so, they should probably clarify the conditions of storage.

As a general point, if they’re using commercial expression systems it would help to name the supplier … I think the main expression system they are going to use is from Thermo Fisher. It makes checking these things a bit easier. This applies to all GM proposals, not just this one.

Two final points … why are they storing Virkon in glass in their category 2 hoods? I’m sure there are suitable plastics for this. Perhaps I’m wrong? Secondly, the GM proposal states that all workers with the Lenti’s will be vaccinated against Hep B. I think this should be a recommendation, rather than a requirement. There is risk associated with all vaccination. If the view of the applicant is that they MUST be vaccinated then the wording should probably be changed to something like “Only workers vaccinated etc. will be permitted to work with etc.” though this might have implications for some current staff.

Regards
D

----
Prof. D E
Biomedical Sciences Research Complex
North Haugh
University of St. Andrews
St. Andrews, Fife, KY16 9ST

The University of St. Andrews is a charity registered in Scotland (SC013532)
From: BSRC Health and Safety
Sent: 08 December 2017 10:25
To: 
Subject: Re: Notification of a Genetic Modification Project by Professor G M

Dear P,

I would recommend that Prof G M GM project should be category 2.

As others have mentioned in the past for other projects, if the Virkon in the biological safety cabinet can be put into a plastic bottle, then that may be preferable.

Kind regards,
M

School of Biology
University of St Andrews

From: 
Sent: 06 December 2017 14:29
To: 
Subject: Re: Notification of a Genetic Modification Project by Professor G M

Dear P,

I'm happy to approve this as a class 2 project.

Thanks,
P

Lecturer in Experimental Pathology
Hi P

I have been through it and yes the uses lentiviral particles in this context is fine as a category 2 project.

Cheers

Professor T S
School of Biology
University of St Andrews

---
From: 6 December 2017 14:28
To: 
Subject: Re: Notification of a Genetic Modification Project by Professor G M

Hi P

I have been through it and yes the uses lentiviral particles in this context is fine as a category 2 project.

Cheers

Professor T S
School of Biology
University of St Andrews
Professor G M submitted a Notification of work with GM organisms on the 20th November 2017. To my horror I have realised that I missed this application and have not submitted to this working group for review. This is a wholly unacceptable level of service to Professor Gunn Moore and also to Dr A for which I apologise.

Please find attached the GM project application from Professor G M. I would be grateful if you could categorise this project with respect to the Genetically Modified Organisms (Contained Use) Regulations 2014. Professor G M and Dr A have categorised part of this project which uses lentiviral particles as a category 2 project. I would be grateful if you could especially review this part of the project.

Best wishes,

PS

Environmental, Health and Safety Services,
Bute Building,
Queens Terrace,
St Andrews, Fife, KY16 9TS

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**Project Containment**

<table>
<thead>
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**Project Ref** 317/18.3

**Date Ackn'd** 14/06/2018

**CU2 Project Title** The biology of Zika virus- replication and recombination

**Class** Class 2

**CultureVolClass2** ≤ 1 Litre

**CultureVolumeClass3-4**

**Non-GMM** Consent Granted

**Consent Granted** Not Applicable

**Project notified under transitional arrangements** N

Tick if notifying a connected programme of work N
Project Additional Information

**Purposes of the contained use**

We propose to investigate the replication and evolution of Zika virus using a reverse genetic approach. Infectious cDNAs for the virus have been generated in other laboratories. From these, viral RNA can be transcribed in vitro, transfected into permissive cells and the recovered virus subsequently characterised. Specifically, we are interested in the RNA structures within the virus genome that influence replication and translation. We are additionally interested in how these viruses evolve, particularly in whether the formation of hybrid or recombinant viruses is a replicative or non-replicative process.

**Recipient or parental organism**

Cell lines - Mammalian: HeLa, A549, RD (all human), Vero (nonhuman primate), BHK (hamster). Insect: C6/36 (Aedes Recipient of albopictus) and AF5 (Aedes egypti i: a clonal line derived from parental organism Aag2 cells) and AF319 (a clonal cell line derived from AF5 which have been engineered using CRISPR-Cas to be defective in expression of Dicer2 [Dcr2]). AF5 and AF319 are described fully in Varjak et al., 2017 DOI: 10.1128/mSphere.00144-17.

**Host/vector system**

E coli K12 strains DH5 JM109 HB101 Also pCC1BAC based plasmids will be maintained in E coli EP1300 cells The genotype of these bacterial cells is given in the attached risk assessment Plasmids to be used are pUC, pEMBL, pBluescript or pGEM all of which are commercially available and well characterized These bacterial plasmids will have antibiotic markers for ampicillin, chloramphenicol or Kanamycin Zika virus cDNAs are maintained in low copy numbers bacmids based on pCC1 Bac

The Zika virus cDNA will be modified by insertion of a T7 promoter and ribozymes hammerhead at the 5 prime end and hepatitis delta virus ribozyme at the 3 prime end of the cDNA as appropriate allowing efficient and high-level expression of full-length RNA in vitro RNA will be transfected into suitable
mammalian or insect cells using Lipofectamine 2000 or equivalent, or electroporated, to allow recovery of virus. Targeted CRISPR Cas knockouts of cellular genes such as XRN1 will use the pAcsgRNA/Cas9 plasmid modified by inclusion of a suitable guide RNA. Full details of this vector are available in the risk assessment.

Origin & function

Zika virus has been isolated from naturally infected humans, monkeys and mosquitoes (the latter of the genera Aedes, Anopheles and Mansonia). Antibodies to the virus have been detected in a wide range of species including domestic sheep, goats, horses, cows, ducks, rodents, bats, orangutans, and carabaos (water buffalo) suggesting that the virus is able to replicate in many species. In cell culture the virus replicates in many placental, neuronal, muscle, retinal, testicular, renal and pulmonary human cell lines. Non-human cell lines from primates, pig, rabbit, hamster, chicken and mosquito also support productive Zika virus replication.

Evaluation of foreseeable effects

None of the viral proteins are known or could be predicted to be toxins, oncogenic, allergens or modulators of growth or differentiation. This is based upon extensive analysis of the closely related Dengue virus, West Nile Virus or Yellow Fever viruses. It should be noted that there are live-attenuated Yellow Fever vaccines which are egg-adapted strains containing multiple attenuating mutations. These viruses still encode the full range of virus proteins and are not toxic, oncogenic, allergenic or known to modulate cell growth or differentiation. Zika virus does encode a cellular protease (NS3) which is a multi-functional protein with additional ATP-dependent helicase (RNA) activity. There are potentially cellular targets for this protease - either specific i.e. naturally targeted for proteolysis during the virus replication cycle - or non-specific, due to conservation of the protease cleavage site. Over-expression of the protease in mammalian cells could therefore be cytotoxic though this would be limited to the cell in which the protease was expressed. All protein expression work will be plasmid-based using non-mobilisable standard over-expression plasmids derived from pBR322-type vectors. Expression of Zika virus full-length RNA will be under control of a bacteriophage T7 promoter engineered into the appropriate region of pCC 1-SP6-ZV preceding the Zika virus cDNA (in place of the SP6 promoter already present). The expression of the fulllength RNA will only occur when purified DNA-dependent bacteriophage T7 polymerase (purchased commercially) is used in vitro with purified modified pCC1-SP6-ZV. Expression in the E.
coli host cannot occur in the absence of SP6 or T7 polymerase which are very tightly regulated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All biological material will be autoclaved at 121 oC at 15psi for 30 minutes where reasonably practicable. Where autoclaving is not practicable, 1 % virkon solution will be used for 1 hour to disinfect cultures. A 5% virkon solution will be used to disinfect spillages of concentrated virus infected cultures

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
See Attached Document

Project Containment

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Project Ref 317/19.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4
02/03/2022
Single transcription rate of 16S rRNA by Mycobacterium abscessus under different conditions

The M. abscessus Molecular Bacterial Load Assay (MBLA) uses real-time reverse transcriptase quantitative polymerase chain reaction (RTqPCR) to accurately detect and quantify M. abscessus bacterial load from patient’s sputum samples. The tool targets a variable 16S rRNA region to identify M. abscessus and uses the cycle threshold (Ct) obtained from the experiment alongside a standard curve to calculate an estimated CFU count.

The conversion of 16S rRNA Ct to CFU count is done through the use of a standard curve. This is based on several studies using different mycobacterial species, where rRNA was shown to have a correlation with cell viability. rRNA is shown to have a longer half-life than mRNA but still shorter than DNA, making it ideal as a surrogate for cell viability. It is therefore essential to better understand the rRNA and CFU/ml relationship of M. abscessus. The MBLA always uses the same standard curve regardless of the treatment of the patient. It is important to understand the impact of the currently used antibiotics on rRNA concentration within each cell, as some antibiotics might affect rRNA without having an impact of cell viability.

Moreover, to ensure the standard curve is appropriate to be used to estimate CFU/ml, variability of rRNA concentration within cells under the same condition need to be better understood.

M. abscessus unlike most bacteria has a single copy of 16S rRNA. Due to this I aim to look at the production of 16S rRNA through the use of GFP as a measure of transcription rate. This will allow me to observe the cells at a single level and study the diversity in transcription rate within a given culture. To achieve my aim the cells will be grown normally, as well as being put under stresses such as antibiotics, starvation and acidification.

Recipient or parental organism
Mycobacterium abscessus (NCTC 13031)
E. coli DH5a and E. coli SM10 Lambda pir cells

Host/vector system
pRE107 (backbone pGP704) – sequence can be found at https://www.addgene.org/43829/
sgPal1 (p2Tol-U6-sgPal1-HygR) – sequence can be found at https://www.addgene.org/71483/
sfTq2-C1 (backbone pEGFP-N1 a pUC derivative) – sequence can be found at https://www.addgene.org/117932/
The first gene to be inserted is DeGFP, a destabilized version of GFP (green fluorescence protein). It has a half-life of 2 hours, therefore due to its quick degradation it can correlate to gene transcription rate. Sequence for this gene can be found at https://www.addgene.org/14760/.

The second gene to be inserted is HygR, hygromycin resistance gene. Hygromycin is an FDA approved antibiotic food additive for swine and poultry. It is not used for humans.

The third gene is not going to be inserted into the M. abscessus genome but it is part of the vector that will be electroporated into M. abscessus. AmpR, ampicillin resistance gene (part of vector pRE107).

In the case of an internal control being necessary, two genes will be present in a plasmid electroporated into M. abscessus. One, a super folded version of CFP (cyan fluorescence protein). The second gene is kanamycin resistance gene. Kanamycin is used to treat a range of infections caused by human pathogens (e.g. Serratia marcescens, E. coli).

**Origin & function**

There are no risks with the E. coli recipient organisms as they are E. coli K12 disabled and non-colonizing host bacteria.

M. abscessus is not listed in ACDP, however Public Health England (NCTC) provide M. abscessus strains as ACDP hazard group 2. The countries which have classified M. abscessus have also assigned it Biosafety Level 2 (https://my.absa.org/tiki-index.php?page=Riskgroups&default%5bcontent%5d=ABSCESSUS).

Furthermore, M. abscessus is part of the M. chelonae-abscessus complex and is highly similar to M. chelonae. HSE have assigned M. chelone ACDP hazard group 2.

M. abscessus is ubiquitous in soil and aquatic environments. It is an opportunistic pathogen, which can cause pulmonary disease in vulnerable hosts with underlying structural lung disease, such as cystic fibrosis and bronchiectasis.

It has been implicated in various infections, usually pulmonary usually in the context of severe anatomical or immunological compromise. M. abscessus may be a commensal organisms (non-pathogen) and consequently the presence of M. abscessus in a patient's respiratory sample is not always indicative of M. abscessus pulmonary disease (PD).

Pulmonary disease caused by M. abscessus complex is challenging to treat usually because of the nature of the underlying disease. Although there is no standard treatment, current guidelines suggest the administration of macrolide-based therapy in combination with intravenously administered antimicrobial agents. There is no way to follow the response of patients to antibiotics and the relationship between laboratory tests and patient response is not well understood.

References:
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be treated by autoclaving at 121°C at 15 psi for 30 minutes where practicable. Where autoclaving is not feasible, Virkon at 5% and Tristel made up according to the manufacturers instructions

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

See attached Document- GM Safety Committee Comments - Mycobacterium abscessus

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We work on several degenerative conditions for which the pathological mechanisms are not understood, but are linked by likely defects in cellular RNA metabolism. Spinal Muscular Atrophy (SMA) is an inherited form of motor neurone disease and one of the most common genetic causes of death in childhood. It results from expression of insufficient amounts of the survival of motor neurons protein (SMN). In SMA, death of spinal motor neurons leads to progressive muscular degeneration, which is the most debilitating symptom, but SMA also affects other tissue types. SMN has a number of known and predicted roles in mRNA metabolism. There is increasing evidence that similar molecular pathways may be involved in other neurodegenerative conditions such as myotonic dystrophy type 1 (DM1), which is caused by the accumulation of faulty mRNA in foci in the cell nucleus and amyelotrophic lateral sclerosis (ALS), or classical MND, which has a number of different genetic causes as well as the more common sporadic appearance.

Nuclear bodies: new insights into assembly/dynamics and disease relevance.
Sleeman JE, Trinkle-Mulcahy L.
Curr Opin Cell Biol. 2014 Jun;28:76-83. doi: 10.1016/j.ceb.2014.03.004

mRNP assembly, axonal transport, and local translation in neurodegenerative diseases.
Khalil B, Morderer D, Price PL, Liu F, Rossoll W.

We amplify plasmids in E.coli and use these to over-express tagged versions of endogenous proteins in mammalian cell lines or to reduce expression of endogenous proteins in mammalian cell lines. We use cell culture models of SMA involving reduction of SMN expression by shRNA expression in which to study cellular defects in SMA and cell culture models of DM1 expressing artificial mini-genes to mimic the faulty
RNA foci. We plan to develop cell culture models of some of the genetic types of ALS. We are also
developing techniques to differentiate commercially-obtained cells from patients with neuromuscular
conditions into neural and other cell types for further study.

**Recipient or parental organism**

a) Disabled E.coli laboratory strains:

<table>
<thead>
<tr>
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<th>Relevant Features</th>
</tr>
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<tbody>
<tr>
<td>HiColi (DH5alpha derivative)</td>
<td>F- endA1 glnV44 thy-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(r - m +), λ -</td>
</tr>
<tr>
<td>TOP10 (DH10B derivative)</td>
<td>F- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL(StrR) endA1 λ -</td>
</tr>
<tr>
<td>XL10 gold endA1 glnV44 recA1 thy-1 gyrA96 relA1 lac Hte Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 tetR F'[proAB lacIqZΔM15 Tn10(TetR Amy CmR)]</td>
<td></td>
</tr>
</tbody>
</table>

Occasional use of JM101, DH5alpha, Rosetta

b) Lentiviral vectors pLVX-TetOne-Puro and pLKO-Tet-On

https://www.snapgene.com/resources/plasmid-files/?set=viral_expression_and_packaging_vectors&plasmid=pLVX-TetOne-Puro

https://www.addgene.org/21915/

c) Tissue culture cell lines:

Human: HeLa (and derivatives), SH-SY5Y (and derivatives), lens epithelial cell lines (various), neural cells differentiated from iPS cells in a collaborators lab, commercial myoblast lines, 293T, primary fibroblast and lymphocyte lines from patients with neurodegenerative conditions and non-affected controls from commercial sources. Our usual source for these cells is the Coriell institute, where strict quality control measures are in place

https://www.coriell.org/0/Sections/Support/Global/QCcells.aspx?PgId=409

The cells are screened for bacteria, mycoplasma and HIV.

Mouse: C2C12, embryonic stem cells, NSC34 motor neurone-like cells. All well characterised commercial lines.

**Host/vector system**

Bacterial vectors: pBluescript (for plasmid propagation only, no promoter), pGEX pGEM, pET, series (plasmid propagation only, no induction of expression). pENTR/D-Topo

Eukaryotic expression vectors: pSG5 family, pEGFP family (expression driven by CMV promoter), pJN1 and pJC3 (2A) vectors and derivatives to express multiple proteins simultaneously, pBi-tet vector and derivatives to express two proteins under a Dox inducible promotor.

siRNA vectors to express shRNAs and miRNAs in mammalian cells: pSuper family, pExtra, BLOCK-IT inducible miRNA system (expression driven by eukaryotic promoters).

Lentiviral Vectors for expression of proteins or shRNAs: pLVX-TetOne-Puro and pLKO-Tet-On

None of these are transmissible in bacteria and they do not contain prokaryotic
promoters for the inserted sequences.

**Origin & function**

Coilin: structural protein of Cajal bodies; NEAT1: structural LNC RNA for paraspeckles; Neurochondrin and other neural specific proteins including NeuroD1, Acs11, Myt11, NCDN and Pou3f2 involved in cellular differentiation. DMPK: gene containing expansion in DM1. SMN, ASF, U2AF, U2B\*, U1A, HTRA2beta, SmB, SmD1, SmD3, SmE, SmN-, U2A\*, U2B\*, U1BP1, U11/1248, 35, 20, 25 and 65, MBNL1, CUGBP1, NONO, PSPC1, FUS, SFPQ. All multi-functional RNA binding proteins, mostly constitutive or alternative pre-mRNA splicing factors. GFP- green fluorescent protein to mark transfected cells mCherry- red fluorescent protein CFP- cyan fluorescent protein YFP- Yellow fluorescent protein mini-SOG-(super oxide generator), APEX2 epitope tags detectable by light and electron microscopy PRMTs- protein methyltransferases involved in methylating proteins Cyclophilin B – mitochondrial protein used as a non-nuclear control. Luciferase- used as a control

**Evaluation of foreseeable effects**

See attached risk assessment

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All work will be undertaken in a category 2 containment laboratory at the Biomedical Sciences Research Complex at the University of St Andrews.

Biological material will be autoclaved at 121oC at 15psi for 30 minutes where practicable. The disinfectant used when autoclaving is not practicable will be 5% Virkon (pentapotassium bis peroxymonosulfate)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All material will be autoclaved at 121oC at 15psi for 30 minutes where practicable. Where autoclaving is not practicable, VIRKON will be used as a chemical disinfectant according to the manufacturers instructions.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
Head of the BSRC Building:
SMN protein modifications.
All of the E. coli modifications – whether of SMN or the panoply of other RNA-binding or pre-mRNA splicing proteins – are level 1 activities in my opinion. The host/vector combinations are well-established and will not propagate outside the laboratory. All, whether host or target, are very highly unlikely to be toxins or oncogenes. All of the lentivirus engineering work in E. coli is also a level 1 activity for much the same reasons.
The lentivirus production and use in mammalian cells is proposed as a level 2 activity. I read the paperwork for this system (https://www.takarabio.com/products/gene-function/tet-inducible-expression-systems/tet-on-3g-systems/tet-on-3g-lentiviral) and for pLVX-TetOne-Puro. Expression is doxycycline-dependent and very tightly regulated. The lentivirus particles are packaged into VSV-G envelope proteins (so will have a very broad target host and tissue range) and are based upon HIV-1. These types of vectors are widely regarded as level 2. In my view this is cautious, largely reflecting the insertion and expression of a gene that would

Safety Co-ordinator for the BSRC Building
After consideration, I would agree with E that this project is a category 2 project for the work with lentiviruses thus the project will be category 2.
S after considering this project, I believe it to be a category 2 project.
R
This is a class 2 project due to the use of lentiviral particles.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2 L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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</table>

Project Ref 317/21.1

Date Ackn’d 08/10/2021
CU2 Project Title Characterization of enzymes and biofilms in bacterial ESKAPE pathogens

<table>
<thead>
<tr>
<th>Class CultureVolClass2 CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 2 consent granted</td>
</tr>
</tbody>
</table>

Non-GMM Consent Granted
**Project Additional Information**

**Purposes of the contained use**

By 2050 10 million lives could be claimed a year by drug resistant infections. We must develop new strategies for antimicrobial drugs. Often in infections bacteria form biofilms, requiring concentrations of antibiotics up to 1000 fold higher to be treated. Cyclic dipeptides are molecules produced by organisms in all domains of life, and their function is unknown. They can inhibit bacterial growth and/or biofilm formation, albeit by undetermined mechanisms. The majority of the biological effects caused by cyclic dipeptides are inter-species and in some instances inter-kingdom, mediated host pathogen interactions.

We study enzymes from bacteria and fungi involved in the production of different cyclic dipeptides. We characterise each enzyme biochemically and structurally and determine their substrate scope. We will also use the enzymes to produce libraries of cyclic peptides to be tested for various biological activities.

We are also interested in peptide/protein chemistry more generally, and work with transferases and proteases enzymes that are important for protein quality control and maintenance, as well as response to stress.

Six bacterial species have been identified by the Infectious Disease Society of America (IDSA) as being especially dangerous due to their potential multidrug resistance mechanisms and virulence. They are referred to as ‘ESKAPE’ pathogens, which is an acronym for Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species. Our work is focused on understanding basic biology, virulence and adaptations to stress in ESKAPE pathogens in the context of peptide/protein metabolism. We are mainly focused is P. aeruginosa and S.aureus but this work will set the stage to compare gram positive and gram negative bacteria more broadly in future work.

Cover the name of the strain of micro-organism(s) and/or animals and/or plants should be provided, as well as the name of the wild-type organism from which it is derived and the extent to which it is disabled. - Strains derived from Pseudomonas aeruginosa PA14 - https://www.phe-culturecollections.org.uk/products/bacteria/detail.jsp?collection=nctc&refl&NCTC%2013619

### Recipient or parental organism

1. UCBPP-PA14 (commonly referred to just as PA14) Wild Type
2. Laat deletion — gene PA14_30270
3. Δate deletion — gene PA2618
4. E aat Date double deletion
5. taat + Prha aat integrated at Tn7 recombinase site — contains a rhamnose inducible promoter controlling expression of aat gene.
6. ate + Prha ate integrated at Tn7 recombinase site — contains a rhamnose inducible promoter controlling expression of ate gene.
7. GFP-PA14 — gene for green fluorescent protein constitutively expressed.

Future work will involve additional deletions of genes encoding putative cyclodipeptide synthases and proteases. It will include similar mutations/deletions in other ESKAPE pathogens. More specifically:

7. Derivatives of Staphylococcus aureus NCTC 12232
8. Derivatives of Acinetobacter baumannii NCTC 12156
When handling bacteria, use good laboratory practice and wear appropriate laboratory coats, protective eye wear and gloves. A Hazard Group 2 organism may cause human disease and may be a hazard to laboratory workers, but is unlikely to spread to the community as it is more likely to only infect severely immunocompromised people. Persons must not be immunosuppressed or taking immnosuppressive medication when working with bacterial pathogens. Checks will be made to ensure any staff/students working in the lab are not immunocompromised or taking immnosuppressive medication.

<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutations are in the genome, none in vectors</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclodipeptide synthases, aminocyl-tRNA protein transferases, proteases.</td>
</tr>
<tr>
<td>All DNA to be inserted has been amplified by PCR.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Evaluation of foreseeable effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>All organisms are ACDP hazard groups 2.</td>
</tr>
<tr>
<td>Information obtained from <a href="https://ehs.cornell.edu/research-safety/biosafety-biosecurity/biological-safety-manuals-and-other-documents/bars-other">https://ehs.cornell.edu/research-safety/biosafety-biosecurity/biological-safety-manuals-and-other-documents/bars-other</a></td>
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<tr>
<th>Health Hazards</th>
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</thead>
<tbody>
<tr>
<td>Signs and symptoms of infection may include:</td>
</tr>
<tr>
<td>· Flu-like symptoms (i.e. fever, headache, dehydration, weight loss, lethargy)</td>
</tr>
<tr>
<td>· Cutaneous symptoms (i.e. skin lesions, rash)</td>
</tr>
<tr>
<td>· Gastrointestinal symptoms (i.e. loss of appetite, nausea, vomiting, diarrhea)</td>
</tr>
<tr>
<td>· Respiratory symptoms (i.e. coughing, sneezing)</td>
</tr>
<tr>
<td>· Musculoskeletal symptoms (i.e. joint and muscle pain)</td>
</tr>
<tr>
<td>· Reproductive Health concerns (i.e. abortion, fetal abnormalities)</td>
</tr>
<tr>
<td>Immunizations: None available Prophylaxis*: None available</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Agent Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival Outside Host</td>
</tr>
<tr>
<td>Disinfection</td>
</tr>
<tr>
<td>Pseudomonas can survive for months on dry surfaces and inanimate objects, and are one of the bacteria most frequently isolated from patients with nosocomial infections; growth observed in distilled water can survive up to months with minimal nutrients; humidity can improve persistence 1:10 bleach Dilution</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory Hazards</th>
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<tbody>
<tr>
<td>· High energy-creating activities (centrifugation, sonication, high pressure systems, vortexing, tube cap popping)</td>
</tr>
<tr>
<td>· Handling of sharps (needles, scalpels, microtome blades, broken glass, etc.)</td>
</tr>
<tr>
<td>· Splash/droplet-creating activities (shaking incubators, liquid culturing, mechanical pipetting)</td>
</tr>
<tr>
<td>· Equipment contamination</td>
</tr>
<tr>
<td>· Exposed skin/uncovered wounds</td>
</tr>
</tbody>
</table>

| Signs and symptoms of infection may include: |
| · Cutaneous symptoms (i.e. skin lesions, rash) |
| · Gastrointestinal symptoms (i.e. loss of appetite, nausea, vomiting, diarrhea) |
- Lymphoreticular symptoms (i.e. enlarged internal organs or lymph nodes)

Immunizations: None available  Prophylaxis*: None available

Agent Viability

Survival Outside Host Disinfection

Survives on carcasses and organs (up to 42 days), floors (less than 7 days), glass (46 hours), sunlight (17 hours), UV (7 hours), meat products (60 days), coins (up to 7 days), skin (30 minutes to 38 days) (citation needed).

Depending on colony size, S. aureus can survive on fabrics from days to months 1:10 bleach Dilution

Laboratory Hazards

- High energy-creating activities (centrifugation, sonication, high pressure systems, vortexing, tube cap popping)
- Handling of sharps (needles, scalpels, microtome blades, broken glass, etc.)
- Splash/droplet-creating activities (shaking incubators, liquid culturing, mechanical pipetting)
- Equipment contamination
- Exposed skin/uncovered wounds

Information about Acinetobacter baumanii:

Health Hazards

Signs and symptoms of infection may include:

- Flu-like symptoms (i.e. fever, headache, dehydration, weight loss, lethargy)
- Cutaneous symptoms (i.e. skin lesions, rash)
- Respiratory symptoms (i.e. coughing, sneezing)

Immunizations: None available  Prophylaxis*: None available

Agent Viability

Survival Outside Host Disinfection

A. baumannii can survive in the environment and has been isolated from soils, foods, and inanimate surfaces.

1:10 Bleach Dilution (30+ seconds)

Other disinfectants

Autoclave for 30 minutes

Inactivated by moist heat (15 minutes at 121°C) and dry heat (1 hour at 160-170°C)

Laboratory Hazards

- High energy-creating activities (centrifugation, sonication, high pressure systems, vortexing, tube cap popping)
- Handling of sharps (needles, scalpels, microtome blades, broken glass, etc.)
- Splash/droplet-creating activities (shaking incubators, liquid culturing, mechanical pipetting)
- Equipment contamination
- Exposed skin/uncovered wounds

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Where practicable, items will be autoclaved at 121oC for 30 mins under 15 psi.

Non-autoclavable liquid waste will be treated with Virkon  Virkon for at least 30 minutes. Spillages will be dealt with by applying 5% Virkon to the spoillage and leaving for at

02/03/2022  Page 6257 of 15326
least 1 hour. Autoclaved or 5% Virkon treated solid waste will be sent for incineration using the company Tradebe.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Yes class 2 activity again as with previous E.coli GMO

Project Containment

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<td>L4 L3 L4</td>
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Project Ref 317/22.1

<table>
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<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>19/01/2022</td>
<td>Role of plasma fatty acid and zinc dynamics in platelet functioning. Implications for pathological clotting</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
<td>N</td>
</tr>
</tbody>
</table>

Date Project Ceased

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Zinc is an essential micronutrient that is required for many biological processes including control of coagulation. When platelets become activated, they release zinc, which in turn binds to plasma proteins and cell surface receptors to regulate the clotting. Currently we do not understand how zinc gets into platelets, how it is stored or how these processes may be altered in diseases associated with abnormal clotting. Elevated plasma free fatty acid (FFA) levels are associated with conditions such as cancer, obesity and diabetes, which also increase the risk of developing thrombotic blood clots. Our studies suggest that the high levels of FFA disrupt zinc handling in the blood potentially leading to thrombotic complications. Here we will examine where zinc is stored in platelets and will characterise the zinc transport machinery involved in controlling platelet zinc flux. Finally, we will look at how zinc and elevated plasma FFAs contribute to platelet functioning.

At present we lack a complete picture of which zinc transporters and/or channels contribute to Zn2+ homeostasis in platelets. In humans there are 24 ZnTs/ZIP transporters, with most cell types possessing multiple transporters. We will extract mRNA and synthesise cDNA to perform an RT-PCR expression screen using gene-specific primers to determine which zinc transporters are present. From this, we anticipate around 10-15 transporters being expressed and relative mRNA expression of those identified will be determined using qPCR (relative to RPLPO housekeeping gene). As validation in human platelets, antibodies directed against 5-6 of the most abundant transporters will be sourced. Suitable antibodies will be used to determine relative protein expression of each in Zn2+-treated (20 pM)/untreated platelets. The antibodies will also be used to determine the localisation of specific transporters (in combination with organelle probes) in platelets. Of particular interest is to determine which transporter(s) is responsible for movement of Zn2+ into a-granules. If we can identify a candidate ZnT for this function then we will follow this up by examining Zn2+ accumulation targeted shRNA knockdown and CRISPR/Cas9 knockout of the relevant transporter(s) in human Meg-01 megakaryocyte-like cells.

Platelet calcium dynamics will also be examined in 96-well plate format (in the presence/absence of zinc and FFAs) using an established method that utilises the Fluo 4 Ca2+ probe. Additionally, knockout and knockdown of calcium transports/channels and the effect on calcium dynamics within platelets will be evaluated. Our preliminary data shows MG23/TMEM109 is highly abundant in platelets. To our knowledge this is the only ER-located Ca2+-permeable channel. Therefore, we will block IP3R using the specific inhibitor, xestospongin C to determine flux. Importantly to complement this, we also require to knockdown and/or knockout MG23/TMEM109 in Meg-01 megakaryocyte-like cells using shRNA and CrispR/Cas9, respectively and examine how agonist-mediated Ca2+ dynamics in these cells is affected.

Transfection of Meg-01 and other megakaryocyte-like cells is difficult using nucleofection/electroporation or lipid-based transfection agents. To date, successful transfection of these cells has only been reported through use of lentiviral delivery. For these experiments we will use commercially available pre-prepared lentiviral particles. We will not be propagating lentivirus in the laboratory ourselves.

Cardiomyocytes also have high abundance of MG23/TMEM109. MG23/TMEM109 may play a key role in disease progression mechanisms in heart failure and affect calcium leak from the sarcoplasmic reticulum (a major Ca2+ store within cardiomyocytes). Pathophysiological (>1 nM) levels of cytosolic Zn2+ can also potentiate MG23-channel activity. Therefore calcium and zinc dynamics will be evaluated in cells targeted with shRNA knockdown and CRISPR/Cas9 knockout of the relevant calcium, zinc and MG23/TMEM109 transporter(s).
Recipient or parental organism

(i) List of recipient strain(s)
Cover the name of the strain of micro-organism(s) and/or animals and/or plants should be provided, as well as the name of the wild-type organism from which it is derived and the extent to which it is disabled.

- Standard mammalian cell lines will be used in this project and include:
  - Megakaryocyte/Megakaryoblast and similar cell lines (such as MEG-01 - ATCC: CRL-2021, DAMI - ATCC: CRL-9792, K-562 — ATCC: CCL-243)
  - Cardiomyocyte and similar cell lines (such as H9c2(2-1) — ATCC CRL-1446)
  - HEK293 and derivative cell lines (such as HEK 293T/17 — ATCC CRL-11268)
- Primary Cardiomyocytes and Megakaryocytes/Megakaryoblasts
- Commercially available human-derived iPSC lines

(ii) If a micro-organism, what other organism(s) (e.g. animals, plants) will the recipient strain infect

Retrovirus
VSVG pseudotyped lentiviral vectors can infect cells from many mammalian species, including human and mouse cells. Replication incompetent retroviral vectors will have been used.

Host/vector system

pCRISPR-LvSG03 and similar premade CRISPR lentiviral particles
psi-LVRu6GP and similar premade shRNA lentiviral particles

Disabling mutations:
1. A deletion in the enhancer of the U3 region of 3′LTR which ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
2. The RSV promoter upstream of 5′LTR allows efficient Tat-independent production of viral RNA, reducing the number of viral genes that are used in this system.
3. The commercially generated lentiviral particles will be replication-incompetent. None of the HIV-1 genes (gag, pol, rev) will be present in the packaged viral genome, as they are expressed from packaging plasmids lacking packaging signal. Therefore, the lentiviral particles generated are replication-incompetent.

Origin & function

In this project, any of the genes in the human genome involved in calcium and zinc dynamics maybe manipulated using lentiviral transduction. Examples of genes to be manipulated are:

- Zinc Transporters (ZnT) 1-10. These transporters control the efflux of zinc from the cytoplasm out of the cell and from the cytoplasm into vesicles
- Zrt- and Id-like proteins (ZIP) 1-14. These transporters control the influx of zinc into the cytoplasm from outside the cell and from vesicles.
- Mitsugumin 23 (MG23/TMEM109). A voltage-dependent, cation-conducting channel localized to the sarcoplasmic/endoplasmic reticulum and nuclear membranes in a wide variety of cells.
- Calcium channels and transporters (such as the inositol trisphosphate receptor and sarcoplasmic reticulum Ca2+-ATPase)

Various manipulation techniques using lentiviral transduction will be used. These include:

- shRNA knockdown
- CRISPR/Cas9 knockout

Additionally, genes maybe introduced for cell selection and visualization of infected cells by fluorescence microscopy. Examples of these genes include:
Evaluation of foreseeable effects

Hazards associated with the recipient organism (e.g. bacterial host or viral vector, animal, plant etc)
Factors to consider include whether the recipient microorganism is listed in ACDP hazard groups 2, 3 or 4. Other relevant factors may be the microorganism's mode of transmission, disease symptoms, host range, and tissue tropism as well as an indication as to whether vaccines or chemotherapeutic agents are available. Information should also be provided on any disabling mutations and whether there is any possibility of any disabling mutations being complemented or reverting. If an animal or plant, are these organisms inherently dangerous (e.g. toxic plants, production of allergens etc)
The lentiviral vectors are based on HIV (ACDP 3). Lentiviral vectors contain <25% of the HIV genome. A deletion in the enhancer of the U3 region of 3'LTR which ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
The RSV promoter upstream of 5'LTR allows efficient Tat-independent production of viral RNA, reducing the number of viral genes that are used in this system.
The commercially generated lentiviral particles will be replication-incompetent. None of the HIV-1 genes (gag, pol, rev) will be present in the packaged viral genome, as they are expressed from packaging plasmids lacking packaging signal. Therefore, the lentiviral particles generated are replication-incompetent. The vectors are normally used under level 2 containment (ACGM guidelines 2B-III-26 for lentiviral vectors). Replication competent lentiviruses will not be used.
(ii) Hazards arising directly from the inserted gene product (e.g. cloning of a toxin gene or oncogene)
Consideration should be given to whether the inserted DNA encodes a toxin, an oncogenic protein, an allergen, a modulator of growth or differentiation (hormone or cytokine) or any other protein, which may result in potentially harmful biological activity. Where the function of the inserted gene is unknown, it may help to describe the function of any known homologues. Please note that even a normal human gene may be harmful if overexpressed, especially if the overexpression is in tissues that do not normally express the protein.
eGFP and mCherry have no known harmful effects. The other human genes or shRNAs/CRISPR/Cas9 listed have no immediate toxicity but are potentially oncogenic. Known or suspected oncogenes, or shRNAs inactivating tumour suppressor genes, will not be cloned into replication competent vectors. Expression of oncogenes or inactivation of tumour suppressor genes by RNA interference (expression of shRNA) using retroviral vectors can result in stable transformation of human cells. The best studied case is transformation of human fibroblasts and mammary epithelial cells (Rangarajan, A., Hong, S. J., Gifford, A., and Weinberg, R. A. 2004, Species and cell type-specific requirements for cellular transformation. Cancer Cell 6, 171-183). To achieve quantitative transformation, multiple changes were required, including expression of telomerase, SV40 large T antigen (targeting p53 and Rb), SV40 small T antigen (targeting PP2A), and an oncogenic mutant of ras (targeting Raf, PI3K, and Ras-GEFs). Transformation of human cells is thus difficult, even with the most potent oncogenes expressed from the most efficient gene transfer vectors (amphotropic retroviruses in this case). A laboratory accident in which the same human cell in a laboratory worker was simultaneously or sequentially infected with multiple retroviral vectors is almost inconceivable under level 2 containment save as a malicious act (essentially, deliberate injection of a mixture of viruses). Gene therapy trials have shown that it is a great deal more difficult to stably express a gene in a human individual than was naively expected based on cell culture experiments. Gene therapy for single gene defects like cystic fibrosis, muscular dystrophy or haemophilia has given no successful long term reconstitution of gene expression, despite the use of a wide range of vectors injected in amounts many orders of magnitude greater than could ever occur in a laboratory accident. A critical factor in the failure of gene therapy has been the immune response to the transgene. Unlike clinical vectors, all laboratory vectors contain, in addition to the primary transgene, antibiotic resistance genes which are non-human and
will provoke a strong immune reaction. There is now evidence from gene therapy trials showing what sort of measures are required to produce malignant disease in vivo in humans. Three patients in a French gene therapy trial have developed leukaemia. All were treated ex vivo with a retrovirus expressing a gene required for lymphocyte proliferation. Because of the nature of the disease, these were the only lymphocytes that could divide when reinfused into the patient. There was thus a massive expansion of the transduced cells, coupled with a lack of any immune response to the vector or transgene. This constellation of events could not conceivably arise in any laboratory accident.

(iii) Hazards arising from the alteration of existing traits (e.g. alteration of pathogenicity, host range, tissue tropism, mode of transmission or host immune response)

One factor to consider is whether the inserted gene encodes a pathogenicity determinant, such as an adhesin, a penetration factor or a surface component providing resistance to host defence mechanisms. Another important consideration is whether the inserted gene encodes a surface component, envelope protein or capsid protein that might bind to a different receptor to that used by the recipient microorganism. Consideration should also be given to whether the inserted DNA (or the plasmid sequence) encodes resistance to a drug or antibiotic that might be used for the treatment of a laboratory-acquired infection. If an animal or plant, will the inserted gene affect the tropism of human pathogens, will the modified organism act as a new 'reservoir' for a human pathogen etc.

Genes conferring resistance to puromycin, neomycin and hygromycin are used to select human cells transduced with retroviruses. The presence of antibiotic resistance genes in all commonly used retroviral vectors lessens the risk of retrovirus-infected cells surviving in the human body because the immune system recognizes the transduced cells as foreign and kills them.

(iv) The potential hazards of sequences within the genetically modified organism being transferred to related organisms

Factors to consider include whether widespread dissemination of the inserted gene as a result, for example, of either gene transfer or recombination of the GMM with a wild-type microorganism, would be a matter of concern. If this is the case an important consideration will be whether, in the event of a breach of containment could the genetically modified organism could survive in the environment for long enough for such a gene transfer to take place. Retroviruses are enveloped viruses that are rapidly inactivated in the environment. For these experiments we will use commercially available pre-prepared lentiviral particles. As viral particles will be used, there will be no issues of plasmid recombination. However, infection by the viral particles — even with an "empty vector/ no gene" viral particle - is potentially oncogenic due to genome insertion of the plasmid DNA.

(v) Any other relevant information.

Lentiviral vectors:

Lentiviral vectors are used to stably express foreign genes in cells that are difficult to infect with oncoretroviral vectors, including non-dividing cells and primary human cells. The lentiviral vectors are derived from HIV. HIV is a retrovirus which has the same basic structure as simple oncoretroviruses, with the addition of the accessory genes tat, rev, vif, vpr, vpu and nef. The general approach is similar with lentiviral and oncoretroviral vectors: the vector DNA is transcribed into RNA and packaged into infectious particles in a packaging cell. For these experiments we will use commercially available pre-prepared lentiviral particles. No RNA or DNA for any viral gene is present in the infectious particle. The infectious particles contain viral proteins including the enzymes necessary for reverse transcription and integration, so that the vector can be converted back into DNA and stably integrated into the genome of the target cell after infection. Since lentiviral vectors are derived from HIV, safety was the primary concern during development of the vectors. Maps of the described vectors can be found on https://www.genecopoeia.com/. Some viruses contain two expression cassettes, the first for a transgene (eg GFP under a CMV promoter), the second for a selectable marker (eg the puromycin resistance gene, pac, under an SV40 promoter). The expression cassette may contain a 0111 promoter (U6 or H1), which is used to express short hairpin RNAs (shRNAs) for RNA interference.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Where practicable, all biological waste will be autoclaved at 121°C at 15psi for 30 minutes.
Non-autoclavable waste will be treated with 2% Virkon for at least 1 hour. Spillage will be dealt with by applying 5%
Virkon to the spillage and leaving for 1 hour.
Autoclaved waste or 5% Vorkon treated solid waste will be sent for incineration using the company Tradebe.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Members of the Chemical and Biological Hazards Management Group (which acts as the University Genetic Modifications Safety Committee under the Genetically Modified Organisms (Contained Use) Regulations 2014) have stated:
- YES category 2 project,
- It's a class 2 project in my opinion. Thanks,
I would suggest category 2 for this lentiviral project. Kind regards

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2  Yes</td>
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<table>
<thead>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tbody>
<tr>
<td>L2 L3 L4 L2</td>
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Project Ref 317/transA

02/03/2022
<table>
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<tr>
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<tr>
<td>Purposes of the contained use</td>
</tr>
<tr>
<td>Recipient or parental organism</td>
</tr>
<tr>
<td>Host/vector system</td>
</tr>
<tr>
<td>Origin &amp; function</td>
</tr>
<tr>
<td>Evaluation of foreseeable effects</td>
</tr>
<tr>
<td>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</td>
</tr>
<tr>
<td>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</td>
</tr>
<tr>
<td>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</td>
</tr>
</tbody>
</table>
Is an emergency plan required according to regulation 20?  
N
If yes, tick to confirm that it is attached to this form  
N
Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

## Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units

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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
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## Project Ref 317/transB

<table>
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<td>18/12/2000</td>
<td>PROTEIN DEGRADATION AND TRANSCRIPTIONAL CONTROL</td>
<td>Class 2</td>
<td>CultureVolumeClass3-4</td>
<td>not applicable</td>
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Non-GMM

Project notified under transitional arrangements  
Y

Withdrawn  
N

Historical Significant Changes

TRANSFERRED TO GM 6 (23/2/07).
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<th>Glass Houses</th>
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02/03/2022
Project Ref 317/transC

Date Ackn’d 18/12/2000

CU2 Project Title VACCINE DESIGN

Class 2

CultureVolClass2 Class 2

CultureVolumeClass3-4

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes GM317/95.1,

Historical Date of Additional Info 11/01/1995

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
<td>Animal Units</td>
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Project Ref 317/transD

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<td>PARAMYXOVIRUSES, IMMUNITY AND DISEASE</td>
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02/03/2022  Page 6268 of 15326
Date Project Ceased

Tick if notifying a connected programme of work

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements

Non-GMM

Consent Granted

not applicable

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

### Project Ref 554/99.1

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<th>Culture Volume Class 3-4</th>
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<td>INDUCTION OF CHROMOSOMAL ABNORMALITIES BY HUMAN PAPILLOMAVIRUSES (HPVs)</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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- **Non-GMM**
- **Consent Granted**

- **Project notified under transitional arrangements**

- **Withdrawn**

- **Tick if notifying a connected programme of work**

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
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Data Premises Notified: 04/04/1990
(Originally)

Transferred from 1992 Regs?: Y

Transitional Premises Class: 1

Data Premises Closed: N

Transitional Premises: N

Emergency Plan Required?: N

Non-GMMs: Y

Withdrawn: N

Name

UNIVERSITY OF GLASGOW

Name 2

INSTITUTE OF CARDIOVASCULAR & MEDICAL SCIENCES

Department

Building

Campus Estate or Research Centre

Road Name

300 BALGRAYHILL ROAD

District

BALORNOCK

Town

GLASGOW

County

RENFREWSHIRE

Postcode

G21 3UR

Country

SCOTLAND

Tel Number

0141 211 4000

Fax Number

0141 211 4940

E-mail

HSE Division

SCOTLAND

Comments

FORMERLY GLASGOW ROYAL INFIRMARY

Date at Which Additional Info Submitted

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<th>Date</th>
<th>Premises Closed</th>
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<th>Town</th>
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<th>Country</th>
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<td>Western Infirmary</td>
<td>Immunology, Infection, Inflammation</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Other (please specify)</td>
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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref**  318/05.1

**CU2 Project Title**
This project aims to investigate the mechanisms of action of a group of Pseudomonal toxins secreted by a specialised secretion apparatus.

**Date Ackn'd**  22/02/2005

**Date Project Ceased**

**Class**  Class 2
**CultureVolClass2**  < 1 Litre
**CultureVolumeClass3-4**

**Non-GMM**
Not Applicable

**Consent Granted**

**Tick if notifying a connected programme of work**  N

**Project notified under transitional arrangements**  N

---

02/03/2022  Page 6276 of 15326
### Project Additional Information

#### Purposes of the contained use

These studies will help understand how Pseudomonal aeruginosa causes human disease and thus may allow the development of more effective therapies.

#### Recipient or parental organism

The hosts used are: 1) Pseudomonal aeruginosa. This is a human pathogen, classified therefore as Class II but as outlined above only infects the immunocompromised, patients with cystic fibrosis and those with severe burns. 2) Some constructs will be cloned with the aid of K-12 derived Escherichia coli derivatives. These are metabolically disabled and hence classified Class I. In addition, as they lack type III secretion system, they cannot introduce the cloned Pseudomonal toxins into human cells.

#### Host/vector system

Vectors: We will use pUC based vectors for cloning in E. coli which are mobilisable. We will use as pUC based series of bacterial vectors, termed pUCP, for expression in Pseudomonas; this is driven by the native promoter of the toxins. These vectors cannot readily be transferred to other bacterial species from Pseudomonas.

#### Origin & function

Origin of the genetic material. Genes for the exoS, T, U and Y proteins and their cognate chaperones will be derived by PCR form naturally isolated organisms, or directly from other researchers, again isolated form naturally occurring microbes. Activities of the inserted genetic sequence and of the product. The secreted toxins can produce cell death when introduced intracellularly. They have no oncogenic activity. We will also use antibiotic resistance genes to select for the introduced material in Pseudomonas. These genes are already extremely well spread in naturally occurring Pseudomonads, and thus do not represent an increase in the difficulty of treating Pseudomonal infection.

#### Evaluation of foreseeable effects

Given that the genetic modifications proposed do not alter the intrinsic pathogenicity of this organism, there is no greater risk to human health from the GMMOs than from the naturally occurring microbe. Infection is extremely unlikely to occur in immunocompetent, well individuals exposed to environmental Pseudomonas Aeruginosa. In the laboratory, the application of good laboratory practice reduces this risk even more.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Strict waste management will be implemented. Liquid waste will be decontaminated by treatment with 1% hycolin and left for 15 minutes. This waste will then be disposed of via designated sinks. We have validated that this treatment effectively kills all microbes in the waste. Solid waste will be treated by autoclaving at 123 degrees C for 15 minutes in autoclaves within our building. These autoclaves deal with the clinical waste produced by the hospital microbiology department, and are maintained and validated as providing effective microbicidal action on a regular basis.
The GMSC agreed that this was clearly a Class 2 project. Professor Evans had previously submitted this notification for approval as a class 2 project to GMSC GM31 at Imperial College, London, and also provided the CU2 form and risk assessment from this previous submission. Professor Evans had in effect transferred this project to Glasgow. Notification as a Class 2 Activity to HSE was required.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Project Ref 318/08.1

Date Ackn'd 11/11/2008

CU2 Project Title
Construction and use of retroviral and lentiviral vectors to express normal or mutated proteins and shRNA constructs in primary and immortalized human cells.

Class 2

< 1 Litre

Non-GMM
Not Applicable

Consent Granted

Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
# Project Additional Information

## Purposes of the contained use

The aim of this project is to generate replication-deficient retroviral and lentiviral vectors that express either established/potential oncogens or shRNA constructs from RNA interference libraries. This will enable the expression of these constructs in target cell populations, followed by selection/identification of these cells based on either the target gene or reporter construct. The goal of this project will be to identify factors that result in cellular processes that cause tumourigenesis of target cells, which may ultimately enable a route for therapeutic intervention.

## Recipient or parental organism

Recipient or parental organism:

Human Primary Cells: Embryonic stem cells - H1, HUES9; Mesenchymal stem cells and CD133+ adherent stromal progenitor cells; Mononuclear cells from peripheral blood PBMCs), bone marrow and cord blood - from normal healthy donors and lymphoid, myeloid and stem cells derived from PBMC's; Endothelial cells derived from umbilical and peripheral vessels; Leukaemic cells derived from consented leukaemia patients.

Cell lines: HL-60, K562 (BCR-ABL=), KG1a; HEK 293T (cells for lentiviral production); AAV-HT1080 (Human fibrosarcoma cell line, for viral titration); PT-67; Phoenix - Amphi.

## Host/vector system

Retroviral Vectors: MIEV, MIGR-1, MSCV-1GFP, pSUPER.Retro, pSUPER.retro.GFP. The retroviral vectors used in this set of studies are derived from the Murine stem Cell virus (MSCV). The genes involved in viral replication, gag, pol, and env have been removed from the retroviral genome, thus ensuring that the retroviral vector is unable to replicate and infect surrounding cells.

Lentiviral vectors: pHIV-7-GFP, pCMV-Rev, pCMV-G, pCHGP-2, pCMV-HIV-1, pLVX-AcGFP1-N1 (or-C1). The gene delivery system which conserve only three of the nine genes of HIV-1 relies on four separate transcriptional units for the production of transducing, offering significant advantages for its biosafety.

## Origin & function

### Human PKC-α, -β-δ-ε-ζ-Θ-Ι-λ unmutated, deletion mutants and full length point mutations

### Human VASP and deletion mutants

### Mouse Notch family mutants and Notch-related proteins

### Small G protein Ras, unmutated, deletion mutants and full length point mutations and related proteins

### Raf kinase, unmutated, deletion mutants and full length point mutations and related proteins

### HoxB4-ERT2 tamoxifen-inducible cassette

### AKR1C3, BCR-ABL, AML (RUNx1), SCL, COX2, ABCG2, BMI-1, PGDS

A number of these genes are confirmed proto-oncogenes (eg. Runx1, Ras, BCR-ABL), and more are potential oncogenes.

### siRNA constructs to all of the above inserts, such as:

- pHIV-7-GFP-sh-SP1 (lentiviral vectors expressing short hairpin RNA against Bcr-Abl)
- pHIV-7-GFP-sh-SP2 (lentiviral vectors expressing short hairpin RNA against Bcr-Abl)

These constructs could potentially behave as tumour suppressors.

## Evaluation of foreseeable effects

Generation of Retroviral supernatants from amphotropic cell lines:

Plasmids encoding the retroviral constructs will be transfected into the packaging cell lines Phoenix-Ampho or RetroPak-PT-67 to produce amphotropic retrovirus. Theses helper free cell lines contain gag, pol and env genes encoded by two separate plasmid expression vectors both of which lack the retroviral cis-acting packaging signal to minimise the likelihood of replication competent virus arising through a single recombination event thus minimising the risk of spread to personnel or the environment.
Transfection into the packaging cell line provides these gene products, permitting production of infective virus. At this stage the virus is capable of infecting human cells, however, further production of virus from an infected cell is extremely unlikely, since the virus lacks the gag-pol and env genes. In the packaging cell line these genes are carried in two separate constructs and under the control of non-Moloney promoters, therefore, removing the risk that a single recombination event could result in the virus acquiring the genes necessary to become replication competent. As a result they do not pose a risk to human health or the environment. A risk of minor localised infection does exist when handling the packaged infection virus, and as the inserts may possess a proto-oncogenic effect all steps will be taken to prevent this by using the appropriate level of containment and inactivation procedures - hazard containment 2 is therefore recommended and all work will be carried out in Class II biological safety cabinets.

Lentiviral vectors

Parental wildtype lentiviruses are hazard group 3 biological agents. However, third generation lentiviruses can be used at a lower level (Containment Level 1 or 2) depending on the activity and due to the extreme disablement of the vector system. The inserts are biologically active, and may possess a proto-oncogenic effect. The final GMMs are therefore disabled lentivirus vectors capable of expressing high levels of candidate therapeutic genes. A risk of minor localised infection does exist when handling the packaged viral supernatant, therefore all steps will be taken to prevent this by using the appropriate level of containment and inactivation procedures - hazard containment 2 is therefore recommended and all work will be carried out in Class II biological safety cabinets with appropriate protective handling considerations.

Virally-infected cells will be injected into mice within a category II hood within the Central Research Facility (CRF). Containment and control measures are considered as follows:
- The cells cannot propagate outside the mouse;
- New viral particles cannot be generated from the transduced cells as the viral vectors are replication-competent;
- Host-mouse cells cannot become infected as a result of inoculating the mouse with cells transduced with the vector, as it only delivers the gene of interest and the marker protein GFP and there is no establishment of a pro-virus within these cells, therefore making it extremely unlikely that the left over viral sequences could be rescued by endemic viruses encoded by the host mice leading to cycles of replication;
- Expressed protein may be oncogenic, therefore close attention will be paid to the mice as set out in the home office licence governing this work;
- Exposure to aerosols is minimised by performing all procedures in a category II hood;
- Needlestick injuries will be minimised by training all personnel as required in the home office licence and needles will be used with extreme care and never re-sheathed.

Medical advice will be sought in the unlikely event that a needlestick injury occurs;
- The facility has double doors and animal containment doors to minimise the chance of escape, therefore the chance of animal escaping into the wild is extremely unlikely. However if this were to happen and breeding occurred with a wild type population of mice there would be no transfer of the introduced genetic material as it is not germline expressed;
- Minimal risk to humans from adventitious agents and no environmental hazards envisaged.

If the nature and volumes of the virus to be used and the inserts involved, there will be minimal risk of harmful effect in the unlikely event of human exposure. The likelihood of animals coming into contact with any virus particles is zero. Safe disposal is assured through autoclaving and incineration of all animal carcasses.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

There is a risk of minor localised infection when handling the viral supernatant, therefore all liquid culture waste materials will be incubated overnight in 1x Chloros or Virkon solution before discharging to the sink. All solid waste will be washed with Chloros or Virkon solution before being double bagged in yellow bags and treated as clinical waste (incineration). Contaminated sharps (syringes) will be disposed in sharps bins and treated as clinical waste.

The likelihood of animals coming into contact with any virus particles is zero due to the fact that they will be inoculated with cells that are virally transduced with replication deficient virus. However, safe disposal is assured through autoclaving and incineration of all animal carcasses.
The risk assessment was considered at a meeting of the GM Centre 318 safety committee on September 3rd 2008. The committee consists of experts in genetic modification activities representing all departments within the Faculty. The risk of assessment was approved as a Class 2 project unanimously by the committee.

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**Project Ref 318/12.1**

**Date Ackn'd**

11/04/2012

**Date Project Ceased**

**CU2 Project Title**

Construction and use of gene cassettes to express model antigens and fluorescent proteins in Porphyromonas gingivalis

**Class**

Class 2

**CultureVolClass2**

< 1 Litre

**CultureVolumeClass3-4**

Non-GMM

Consent Granted

**Project notified under transitional arrangements**

N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**
Anaerobic fluorescent protein is a non-toxic fluorescent protein similar to GFP isolated from Aequorea victoria but without the requirement for oxygen to form its fluorophore. It is routinely cloned into microorganisms to provide a means of visual tracking by observed fluorescence. Neither the gene, nor the expressed protein are considered hazardous and will not confer pathogenic properties onto the host organism. Anaerobic fluorescent protein expression by P. gingivalis will allow for fluorescent imaging of bacteria in vitro and in disease models.

OVA peptides and Hen egg lysozyme are non-toxic proteins isolated from Gallus domesticus and are routinely cloned into microorganisms and routinely used as model antigens in immunology. P. gingivalis expressing these proteins will be used to elucidate immune responses to this bacteria.

Evaluation of foreseeable effects

P. gingivalis is a non-motile, obligate anaerobe and a pathogen of both humans and mice where it's proliferation in the oral cavity results in chronic inflammation leading to gum and bone destruction. The organism mediates disease through several virulence factors such as haemolysins, a polysaccharide capsule, Proteases known as gingipains and a variety of other host targeting enzymes. Genetic modification of this bacteria with the suggested inserts will not increase the hazard to human health as the gene products are not expected to increase virulence of the bacterium.

The genetic constructs inserted into the chromosome confer ampicillin resistance to allow for selection of transformants. However, as this is not the antibiotic of choice for eradication of P. gingivalis it doesn't increase the capacity of the organisms to infect or survive within an exposed individual. Moreover, exposure to wild type (wt) P. gingivalis is unlikely to result in infection without prior depletion of the oral microbiota in susceptible individuals and the GMMO will not have an enhanced ability to infect over the wt. The recipient strain is potentially capable of colonizing the oral cavity of humans and animals, however, this is highly unlikely as for successful colonization as the existing oral flora must be first depleted with antibiotics, followed by repeated swabbing of the teeth to introduce the exogenous strain. Our strain poses no additional threat compared to the bacteria wt. The organisms will not be hazardous to the environment and the gene sequences have been inserted into the chromosome of P. gingivalis using double homologous DNA recombination and are non-mobilisable, prohibiting horizontal transfer of the recombinant DNA. The gene products of the genetic material are non-toxic and will not pose any hazard to human health or alter the virulence of the organism. The genetically altered host organism will not be more or less hazardous than the wild type. There will be no change in oncogenicity, pathogenicity or tropism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The organism poses a risk to human health should inoculation and proliferation in the oral cavity occur. However the organism is unlikely to find a microbiological niche.
Within the microbiota of healthy individuals without prior treatment with antibiotics, to deplete the resident bacteria, followed by repeated swabbing with greater than 10^9 bacteria/dose. Repeated exposure with lower numbers of bacteria does not result in infection in predisposed mice and although the potential exists that infection could occur, the risk is low. If an infection were to occur following exposure then treatment with amoxicillin or a similar broad-spectrum antibiotic would eradicate the GMMO. The GMMO is also susceptible to chlorohexidine mouthwashes which could be used to assist in resolution of infection. Staff/students on a current course of antibiotics should take special precaution while working with the microorganism. In the in vivo PD model it is possible that an animal bite may occur and result in exposure to P. gingivalis at the wound. P. gingivalis is not associated with pathology in humans without the oral cavity, however the potential exists that the organism could survive in necrotic tissue and it has been isolated from both infected and uninfected ulcers of the skin but has not been linked with pathology. Oral or topical broad-spectrum antibiotic use would be an appropriate to eradicate the microorganism in this situation. However, P. gingivalis has not been reported as a causative organism in wound infections and although a theoretical risk exists it should be considered very low. Containment and control measures detailed in the generic GM animal risk assessment will be followed, with special considerations as detailed below:

- Infection of animals will be carried out using good aseptic technique to avoid environmental contamination and to reduce the potential for cross infection.
- Needlestick injuries will be minimised by training all personnel as required in the home office licence and needles will be used with extreme care and never re-sheathed. Medical advice will be sought in the unlikely event that a needlestick injury occurs;
- The facility has double doors and animal containment doors to minimise the chance of escape, therefore the chance of animal escaping into the wild is extremely unlikely. In the event of escape, the spread of infection is extremely unlikely.
- Safe disposal is assured through autoclaving and incineration of all animal carcasses.

All work with GM animals within Biological Services facilities at University of Glasgow is performed to Class 2 containment levels.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For small volume spills, hard surfaces will be disinfected by blotting with absorbent tissue paper and spraying the surface with a 1%w/v Virkon solution, leaving for at least 20 minutes and drying with paper towel to remove any remaining white deposit. Paper towels will then be bagged for disposal as solid waste (below). If spillages come into contact with users affected items (gloves, lab coats, other items) will be treated as for solid waste. All spills will be reported to the appropriate departmental safety officer for the location of the spill. Solid waste (e.g. agar plates containing bacteria) is stored in small waste bins within the laboratory. The bins have lids and hold inner liners, which are autoclave bags. When the lines are no more than 3/4 full, they are removed from the bin, loosely sealed with autoclave tape, labelled with lab number, operator and date and then transported to the autoclave room. The bags are logged into the room and autoclaved on the same day that they are delivered. The bags and the logging procedure designates them 'GM', which results in their inclusion on a 'run' on the validated autoclave with recorder. The record is inspected to ensure that the correct temperature has been reached for sufficient time. On that basis the autoclaved material is disposed of in solid waste; removed by authorised waste-disposal specialist companies. The record is kept on file by the autoclave room staff. The routine autoclave regime will be 126°C for 30 min, which has previously been established to produce a 100% kill. Liquid waste from cultures will be sterilised in the culture flasks the same day by addition of Virkon® to a final concentration of 1%w/v, and left overnight, Virkon-disinfected solutions will be disposed of into the waste water supply. Contaminated glassware and centrifugation buckets from cultures will be disinfected by complete submersion in 1% w/v Virkon® overnight. Animal carcasses and bedding will be processed as special waste. Thus, if necessary, will be held at -20°C and marked as 'GM'. Storage in this condition will prevent further bacterial growth prior to autoclave, or immersion in virkon (1% w/v) overnight. Carcasses and bedding will then be disposed of through incineration by an authorised waste disposal company, provided through biological services, or as per the guidelines in Biological Services Generic Animal Appendix. Animal cages will be decontaminated by immersion in virkon (1% w/v)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]
The Risk Assessment (attached) was submitted to the GM Safety Committee Meeting for consideration in December 2011. The RA was considered appropriate. Specifically, there was detailed background to the project and management of activities provided. It was noted that a very specific environmental status is required in the oral cavity to enable establishment of an infection, however the parental microorganism is ACDP Class 2 and since these GMOs were of equivalent virulence it was deemed that Class 2 containment was appropriate. The applicants make specific reference to highlighting issues to staff on the project with respect to being on concurrent antibiotic treatment while working with these GMOs. However, the RA clearly deals with appropriate management and mitigation of risk with all activities planned.

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Project Ref 318/15.1

Date Ackn'd 28/01/2015

Construction and use of genetically modified influenza viruses to express protein or fluorescent tags

Class Culture

Vol Culture

Class 2 < 1 Litre

Project notified under transitional arrangements

Consent Granted

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

The aim of this project is to construct and use genetically modified influenza viruses that will allow us to track the virus following infection or track the immune response that the virus generates. The overall aim of our research is to characterise more fully the immune response to influenza virus immunity by vaccination.

The GM influenza virus will be used to infect GM mice that have been approved for use by the local GM committee: GM Centre- 318 Risk Assessment reference numbers: 318/027, 318/030, 318/34, 318/35, 318/36, 318/42, 318/46, 318/48, 318/60, 318/62, 318/63, 318/64, 318/66, 318/67, 318/76, 318/79

Recipient or parental organism

Virus vectors: Established laboratory strains of influenza A virus will be used, namely: A/WSN/33 (H1N1) [WSN] and A/Puerto Rico/8/34 (H1N1) [PR8]

Host/vector system

Plasmid vectors: Viral sequences (e.g. full-length clones of influenza viruses, partial sequences, or sequences of influenza virus ORFs), as well as sequences of other genes (e.g. common genetic reporters such as RFP or GFP) are available in cDNA form or will be generated by RT-PCR. Sequences will be sub-cloned into appropriate DNA plasmids such that RNAs can be transcribed from bacterial promoters (e.g. T7 or SP6), eukaryotic promoters (e.g. CMV, SV40, cellular Poll promoters, cellular RNA Poll promoters), or viral promoters. Plasmids for expression of influenza virus RNA segment sequences will typically contain an RNA Poll promoter and a hepatitis delta virus ribozyme or a mouse RNA Poll terminator. Plasmids contain an origin of replication and a prokaryotic selectable marker (e.g. drug resistance to neomycin or puromycin).

Prokaryotic host cells: Bacterial strains used to propagate plasmids will all be commercially available disabled E. coli derivatives classified as ‘especially disabled hosts by ACDP’.

Eukaryotic host cells: Vertebrate cell-lines of various host species origin will be used (typically canine, or murine), primary murine cells will also be used.

Mice: either mice bred in our facility or purchased from vendors (e.g. Harlan and Charles River) will be infected with the virus in our animal facility

Origin & function

The WSN and PR8 viruses were originally isolated from humans in 1933 and 1934 respectively. The viruses have been propagated in cell lines (in the majority of cases a canine cell line, MDCK) fo many years. The wildtype WSN and PR8 viruses to be used in these studies was provided by Dr B H, Centre for Virus Research, GU. The WSN-OVA-II will be provided by Prof T, Rochester New York, USA.

The viruses have not been attenuated, they replicate normally or potentially less well than the wild-type virus as the addition of genes to small RNA viruses can affect their replication. The modifications we will make, addition of fluorescent or epitope tags, will not make the virus more virulent.

Evaluation of foreseeable effects

Virus vectors: Established laboratory strains of influenza A virus will be used, namely A/WSN/33 (H1N1) [WSN] and A/Puerto Rico/8/34 (H1N1) [PR8]. These viruses are predicted to be antigenically similar to components of the currently available seasonal human influenza vaccine.

Influenza virus typically infects airway epithelial cells, therefore, the most likely route of transmission is via the formation of an aerosol that is then inhaled by the operator. The virus could also be ingested or enter via a splash to the eyes or mouth. The modifications that will be made will not alter the route of viral transmission nor will they increase the virulence of the virus.

Human influenza is typically a non-serious self-limiting infection in otherwise healthy adults, generally acquired by the respiratory route. Severity of infection depends on the strain of virus and health status of the host, but in general tends to fall within a spectrum of asymptomatic to ~1 week of fever and malaise. The laboratory strains of influenza virus which will be used here (PR8 and WSN), are known to be avirulent in humans from volunteer challenge studies and have a >70 year history of safe laboratory use.
The majority of humans are expected to have at least some prior immunity to circulating human influenza viruses such as H1N1, H3N2, and influenza B virus. Immunity to these strains will provide a degree of protection to other HG2 influenza viruses. To boost any natural immunity, all staff handling influenza viruses are offered the annual seasonal influenza vaccine.

Severe illness due to human influenza viruses can occur but is extremely rare, occurring in less than 1% of cases, and is always associated with underlying health conditions such as pregnancy, Immunosuppression, asthma, or other respiratory/chronic diseases. Anyone who might have compromised resistance to disease for any reason (such as pregnancy or immunosuppression) is required to inform the PI and seek advice from the University Occupational Health Advise regarding the need for additional precautions.

Plasmid vectors: Viral sequences (e.g. full-length segment clones of influenza viruses, partial sequences, or sequences of influenza virus ORFs), as well as sequences of other genes (e.g. common genetic reporters such as RFP or GFP) are available in cDNA form or will be generated by RT-PCR. Sequences will be sub-cloned into appropriate DNA plasmids such that RNAs can be transcribed from bacterial promoters (e.g. T7 or SP6), eukaryotic promoters (e.g. CMV, SV40, cellular PolII promoters, cellular RNA Pol promoters), or viral promoters. Plasmids for expression of influenza virus RNA segment sequences will typically contain an RNA PolII promoter and a hepatitis delta virus ribozyme or a mouse RNA PolII terminator. Plasmids contain an origin or replication and a prokaryotic selectable marker (e.g. antibiotic resistance against ampicillin or kanamycin) and sometimes a selectable eukaryotic marker (e.g. drug resistance to neomycin or puromycin). These strains can only survive in a controlled laboratory environment and good microbiological practice should prevent release of viable organisms. These bacteria will be handled at CL1, which is routine practice. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host.

Prokaryotic host cells: Bacterial strains used to propagate plasmids will all be commercially available disabled E. coli derivatives classified as 'especialy disabled hosts' by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic host cells: Vertebrate cell-lines of various host species origin will be used (typically canine, or murine), primary murine cells will also be used. Cells to be used would not survive inside the human body due to immune rejection. Addition of NA, RNA or infection will confer no growth or survival advantage to cells either inside or outside the laboratory.

Either mice bred in our facility or purchased from vendors (e.g. Harlan and Charles River) will be infected with the virus in our animal facility. Infected mice typically clear the virus in 8-10 days post-infection. Live virus is only found in the lungs of infected animals, therefore lungs represent the only source of potential infection. Live animals are very unlikely to transmit virus to humans as influenza infection in mice does not cause sneezing. Isolated infected lungs can pose an infection risk if cell/tissues are handled such that aerosols are generated.

Were the mice to escape from containment, it is unlikely that they would survive long enough to breed with the native population and transfer of the modified influenza virus would be extremely unlikely (naive mice housed in the same cage as infected animals do not become infected).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated fluids/liquid waste (eg. Virus samples, culture supernatants, tissue culture media) and contaminated disposable plasticware (e.g. flasks, tubes, pipette tips) is collected immediately into a >1% Virkon (w/v) solution within the MSCII and left to inactivate fully for a minimum of 12h. Small plasticware is submerged in the Virkon, whilst large flasks are filled with >1% Virkon and the outside surface is disinfected with 70% ethanol. Decontaminated plasticware is disposed in lab autoclave waste bins. Gloves used fo virus work are disinfected with 70% ethanol before putting in autoclave containers.
Autoclaving
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Other
Infected animals are housed in filter cages. In the majority of cases, infected mice clear the virus in 8-12 days post-infection. In all cases bedding and animal carcasses will be autoclaved and incinerated.

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Project Ref 318/15.2

Date Ackn'd: 14/07/2015

Use of Candida albicans to infect mice, including genetically modified Candida albicans that may express chicken egg ovalbumin protein (C.alb-OVA).
The aim of this project is to use the Candida albicans fungal strain to allow us to analyse the initiation of innate and adaptive immune responses to fungal infections. Infection of cultured cells and mice with C.alb will enable us to determine mechanisms by which the immune system recognises and responds to fungal infections. Experiments with infected mice will include some where the use of CD4+ T-cells that specifically recognise and respond to OVA-protein will allow us to determine the specific mechanisms by which the immune system recognises and responds to fungal antigens. This project proposes to use C.alb and the C.alb-OVA strain to investigate immune mechanisms induced after infection with fungi, including investigating T-cell mediated mechanisms of immune regulation that are dependent on recognition of fungal antigens.

Recipient or parental organism

Candida albicans, including Candida albicans expressing ovalbumin will be used to infect mice.

Host/vector system

C. albicans strains will be obtained from UK collaborator. No further genetic modification will occur in this project.

Origin & function

The C. alb and C.alb-OVA will be obtained from collaborators at the University of Aberdeen, who have extensive experience in experimentation with C.albicans. C.alb-OVA has been genetically manipulated to specifically express the protein ovalbumin (OVA). The intended function of this is to stimulate C.alb-specific immune responses in vitro and in vivo. In some experiments, the immune response will be analysed using OTII (T-cells) cells that specifically recognise OVA. OTII cells will be adoptively transferred into mice alongside C.alb-OVA infection and subsequent C.alb-specific T-cell responses measured.

Evaluation of foreseeable effects

Pathogenicity in C.albicans is a polygenic trait involving numerous physiological characteristics (e.g. rapid growth, cell wall, morphogenesis, secreted hydrolases, phenotypic switching). Therefore, the genetic modification of C.albicans to express OVA protein is highly unlikely to increase C.albicans virulence. Furthermore, this C.albicans strain that has been cultured in other laboratories tends to display reduced pathogenicity compared to strains carried commensally by most individuals. The ectopic expression of OVA will be <1% of total cell protein. The ectopic expression of OVA is unlikely to influence virulence at all, and may in fact be expected to reduce C.albicans virulence by adversely affecting growth. OVA expression will also have no benefit in allowing the organism to survive, establish, disseminate with or displace other organisms. The probability of increasing virulence by ectopic expression of a single gene/protein is very low, because pathogenicity is complex and is a polygenic trait requiring a high level of fitness of the C.albicans cells.

The risk of infection of wild mammals (such as rodents) through accidental release of C.albicans or due to escape of infected animals is low because to succumb to such infections, immunocompetent animals must be injected intravenously with relatively high infective doses (<10^4 CFU/g body weight). Mice infected with C.alb-OVA in this...
study will be sacrificied within one week of infection, preventing any severe adverse effects which may be associated with long term candida infection.

C.albicans is a human pathogen which can cause superficial infections of mucosal epithelia, but will only cause systemic infections in severely immunocompromised individuals. C.albicans is carried commensally by at least 60% of the healthy population and most infections arise as an overgrowth of strains already resident in the normal microflora. It is a microorganism of low pathogenic potential that rarely infects human individuals. it only becomes a medical problem in individuals whose immune responses are significantly impaired. Only small amounts of Candida will be used in these experiments (mostly 10^6, rarely 10^8 Candida cells per animal). A healthy human individual would clear these doses from their bloodstream within minutes, therefore risk of infection from the bite of an infected animal is minimal.

Either mice bred in our facility or purchased from vendors (e.g. Harlan and Charles River) will be infected with C.alb in our animal facility. Infected mice will be sacrificed within one week of infection. Live animals are very unlikely to transit the infection to humans as C.albicans is widely distributed and carried commensally in at least 60% of the healthy population. Most candida infections arise as an overgrowth of the strains resident in the normal microflora. Only small amounts of Candida are used in these experiments (mostly 10^6, rarely up to 10^8 Candida cells per animal). A healthy human individual would clear these doses from their bloodstream within minutes. Lab workers are unlikely to be exposed to the following factors which increase the susceptibility to systemic Candida infections:

1) Use of cytotoxic or strongly immunosuppressive drugs
2) Use of catheters/any intravascular prosthetic device
3) Age (very young or very old individuals are more prone to infection)
4) Serious trauma, particularly when the abdomen is involved
5) Abdominal surgery
6) Immunosuppression
7) Long term use of antibiotics.

Lab workers will be made aware of these risk factors and are asked specifically to make their supervisors (or Occupational Health) aware if they are exposed to the risk factors mentioned above. Potential routes of entry into the body include orally, or via cuts, grazes and lesions in the skin, or via the bite of an infected animal. Appropriate clothing must be worn and a strict code of personal hygiene must be followed. Use of sharps should be carried out carefully, with two people involved in holding and injecting to minimise risk of accidents and animal bites.

The risk of infection of wild animals from accidental release of C.alb-OVA or from infected animals is low as immunocompetent animals must be injected intravenously with relatively high infective doses (<10^4 CFU/g body weight) for successful infection. The ectopic expression of OVA protein does nor result in any phenotypic or genetic instability, and Candida has no effective mechanism for horizontal gene transfer that might allow dissemination of genetically modified traits to other organisms.

I acknowledge that the guidelines in the GM Rodent Generic Appendix are sufficient to safely handle and house mice infected with the proposed genetically modified strain of C.albicans (C.alb-OVA).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Under Home Office regulations mice infected with genetically modified C.albicans (C.alb-OVA) will be subjected to an approved Schedule 1 method of humane killing and disposed of by an approved method.

Disinfection
Disinfection to be carried out using 1% Trigene/Distel TM307 which is known to be effective against fungi.
Autoclaving

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

OR

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using chemical indicators (e.g., Browne TST indicator test strips).

Contaminated sharps must be disposed of in sharps bins reserved for this purpose. Any fungal cultures remaining should be destroyed by autoclaving. Any animal samples from infected animals will be disposed of by autoclaving before. Waste from infection suites will be removed from the room in autoclave bags and autoclaved.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment:

This RA was reviewed by the committee members of GM318 in June 2015, one committee member proposed changes that should be made to the risk assessment with regard to ensuring that animals which received the GM candida albicans were appropriately assessed as GM organisms themselves. The proposer duly edited the submitted RA to incorporate these important aspects. The RA was then approved at containment level class 2.

Project Containment

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Project Ref 318/16.1
The aim of this project is to employ transgenically modified reporter Streptococcus pneumoniae to dissect the pathogenesis of experimental murine meningitis and sepsis, and to investigate the generation of antigen-specific immune responses during infection.

Genetically modified Streptococcus pneumoniae strains have been generated in Liverpool by the Kadioglu group. The proposed studies will allow us to apply in vivo imaging and immunological methods developed in Glasgow to track the migration and interactions of bacteria and immune cells.

Recipient or parental organism

Streptococcus pneumoniae strains, including lab-adapted strain D39 serotype 2, but also other clinically relevant serotypes such as 1, 3, 4, 5, 6B, 19F etc., were labeled with a fluorescence protein markers commonly known as green fluorescent protein (or GFP), and red fluorescent protein (or tagRFP), using previously published procedures (Kadioglu et al. 2001 FEMS Microbiol Lett., 194(1): 105-110, Beilharz et al. 2015 Appl Environ Microbiol 81(20):7244-52). The generation of the required reporter gene constructs and subcloning of the insert into the vector plasmid were carried out by the Kadioglu group at the University of Liverpool, under GM554/14. The constructs were expressed in DH5alpha cells before transformation of Streptococcus pneumoniae.

Host/vector system

Vector plasmid-GFP1: the integration construct is erythromycin resistant. For more detail, see below and in: Kadioglu et al. 2001 FEMS Microbiol Lett., 194(1): 105-110

Vector plasmid pKB01_tagRFP: Expresses tagRFP (codon adapted for Streptococcus pneumoniae); under control of Zinc inducible promoter PZn. Plasmid (chloramphenicol resistant) integrates in S. pneumoniae bgaA locus by double cross-over. For more detail, see below and in: Beilharz et al. 2015 Appl Environ Microbiol 81(20):7244-52.

Mice: either mice bred in our facility or purchased from vendors (e.g. Harlan and Charles River) will be infected with pneumococci in our joint research animal facility.

Project Additional Information

Purposes of the contained use

The aim of this project is to employ transgenically modified reporter Streptococcus pneumoniae to dissect the pathogenesis of experimental murine meningitis and sepsis, and to investigate the generation of antigen-specific immune responses during infection.

Genetically modified Streptococcus pneumoniae strains have been generated in Liverpool by the Kadioglu group. The proposed studies will allow us to apply in vivo imaging and immunological methods developed in Glasgow to track the migration and interactions of bacteria and immune cells.
The plasmid pGFP1 contains gfp in the shuttle vector pVA838. The gene, for the mut 3 variant of GFP, was excised as a BamH1–Sph1 fragment from pKEN2, and ligated into pVA838, previously cut with BamH1 and Sph1, to generate pST1. Expression of gfp is from an uncharacterised pneumococcal promoter, isolated from a library of pneumococcal DNA in pST1. Chromosomal DNA was isolated from pneumococcal clinical isolate CCUG10175 (University of Göteborg Culture Collection). DNA was digested with Sau3a and fragments between 0.2 and 2 kb ligated into dephosphorylated BamH1-digested pST1.

RFP gene was first fused to the Zn2-inducible promoter PZn, resulting in construct pKB01-PZn-RFP. The zinc-inducible promoter was then replaced by the competence-induced promoter PssbB, resulting in plasmid pKB01-PssbB-RFP, which was excised from pLA18 using SphI and EcoRI and introduced into pKB01-RFP plasmids cut with the same restriction enzymes.

To produce large amounts of recombinant plasmids, the library was first electroporated into Escherichia coli DH5a. Plasmid DNA was isolated from all transformants and then the pooled DNA used to transform wild-type pneumococcus lab-adapted strain D39, using competence-stimulating peptide. Pneumococcal transformants were selected on Brain–Heart Infusion agar containing erythromycin (1 mg/ml, GFP label) or chloramphenicol (0.2mg/ml, RFP label).

New transformants, containing new fluorescence reporters (particularly infra red shifted fluorescent proteins with enhanced optical performance in vivo), but with the same characteristics as the GFP/RFP transformants above, may be generated in Liverpool for use in Glasgow.

**Evaluation of foreseeable effects**

*Streptococcus pneumoniae* is a microorganism commonly found in the normal upper respiratory tract flora. The fluorescently-labelled pneumococci will be created on a D39 background, a commonly used non-human pathogenic laboratory strain. The genetically altered host organism will not be more or less hazardous than the wild type. There will be no change in fitness, virulence or tropism.

The antibiotic resistance genes chosen to screen for the recombinant pneumococci are not themselves harmful. In addition, selection during our experiments will require antibiotic levels in excess of 50 µg/ml in order to overcome the inherent resistance of the strains. These are levels in excess of any that would be of therapeutic use. Inserting fluorescent markers does not affect fitness or confer any additional virulence. Previous studies have shown the insert DNA to be stable within prokaryotic expression systems.

The green fluorescent protein gene, originally isolated from the jellyfish *Aequorea Victoria*, is routinely cloned into microorganisms to provide a means of visual tracking by observation of the expressed GFP. Neither the gene, nor the expressed protein, are considered hazardous and will not confer pathogenic properties onto the host organism.

Similarly, RFP is a red fluorescent reporter initially derived from the Sea Anemone *Entacmaea quadricolor*. It is optimised for excitation/emission at longer wavelengths, significantly improving confocal and multiphoton imaging. Neither the gene, nor the expressed protein, are considered hazardous and will not confer pathogenic properties onto the host organism.

Current literature suggests that the human upper respiratory tract is the sole niche of *S. pneumoniae* and, thus, that spread occurs via close contact with an infected individual e.g., saliva and respiratory droplets. Therefore, *S. pneumoniae* is not likely to survive in the event of a breach of laboratory containment. The only hazard that might be relevant in this scenario would be the transfer of antibiotic resistance genes to other bacteria. However, this potentiality is very low. The strain will have no plasmid DNA, hence, for gene transfer to occur a series of very low frequency events would have to take place. The transformation was designed such that fluorescent markers such as GFP or RFP are stably integrated into the chromosome and cannot excise or reform.

Other than those hazards intrinsic to *S. pneumoniae*, there are no foreseeable environmental hazards represented by the genetically modified microorganisms described herein.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Either mice bred in our facility or purchased from vendors (e.g. Harlan and Charles River) will be infected with pneumococci in our animal facility. Naïve mice housed in the...
same cage as infected animals do not become infected. However, as a precautionary measure against the unlikely event of transmission between cages, mice infected with pneumococci will be housed in individually ventilated cages (IVC).

Were the mice to escape from containment, it is unlikely that they would survive long enough to breed with the native population and transfer of the modified pneumococci would be extremely unlikely. Similarly, live animal are extremely unlikely to transmit pneumococci to humans, as pneumococcal infection in mice does not induce any forms of sneezing or coughing.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

NA

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposal of waste materials:

Solid waste is stored in small waste bins within the laboratory. The bins have lids and hold inner liners, which are autoclave bags. When the liners are no more than ¾ full, they are removed from the bin, loosely sealed with autoclave tape (to allow steam penetration during autoclaving), labelled with lab number, operator and date and then transported to the autoclave room on a trolley. The bags are logged into the room and autoclaved on the same day that they are delivered. The bags and the logging procedure designates them 'GM', which results in their inclusion on a ‘run’ on the validated autoclave with recorder. The record is inspected to ensure that the correct temperature has been reached for sufficient time. On that basis the autoclaved material is disposed of in solid waste; removed by authorised waste-disposal specialist companies. The record is kept on file by the autoclave room staff. The routine autoclave regime will be 126°C for 30 min, which has previously been established to produce a 100% kill.

Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Liquid waste will be sterilised the same day by addition of Virkon® to a final concentration of 1% w/v, and left overnight. Virkon-disinfected solutions will be disposed of into the waste water supply. Virkon 1% (w/v) solutions are stable for up to 7 days, and thereafter disinfectant solutions will be discarded (the pink colouration fades). (Virkon is a peroxygen compound and disinfection requires a contact period of at least 10 min for 1% w/v solution: see http://www.antechh.com/frameset.html).

Contaminated glassware and centrifugation buckets from cultures will be disinfected by complete submersion in 1% w/v Virkon® overnight (polypropylene and polystyrene centrifugation buckets, steel and glass are not corroded by 1% w/v Virkon). Glassware and buckets will then be rinsed in tap-water and dried.

Virkon is extensively used and validated for a large range of disinfection procedures, including medical and laboratory procedures, see: http://www.antechh.com/frameset.html.

Animal carcasses and bedding. These will be autoclaved before disposal. Thus, if necessary, waste will be held at -20°C and marked as ‘GM’. Carcasses and bedding will then be disposed of as conventional biological services waste.

Is an emergency plan required according to regulation 20? 

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
The risk assessment was reviewed by GM Centre 318 safety committee in July 2016 and approved after revision. The committee consists of experts in genetic modification activities representing all departments within the Faculty. The risk assessment was approved as a Class 2 project by the committee and was assigned GM reference number GM318/202.

### Project Containment

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**Date Ackn'd**
30/08/2018

**CU2 Project Title**
The construction and evaluation of oncolytic virus vectors as therapy for cancer

**Class**
Class 2

**CultureVol**
< 1 Litre

**CultureVolume**
Class 2

**Consent Granted**

### Project Additional Information

**Purposes of the contained use**
To evaluate the therapeutic potential of selectively replicating ('oncolytic') virus vectors in human cancers, especially ovarian cancer
To modify and amplify wild-type and deletion mutant oncolytic virus vectors already produced in this laboratory or in the lab of other researchers

**Recipient or parental organism**

The recipient cell lines HEK293, A549, JH293 and Vero will be used. All are commercially available cell lines. Human and murine carcinoma cell lines (especially ovarian cancer lines).

**Host/vector system**

Oncolytic adenoviruses (deletion mutants) and wild-type adenoviruses
Oncolytic herpes simplex virus with deletions in the neurovirulence gene ICP34.5

**Origin & function**

Mammalian genes that are deleted or inserted into the viral genome and have been chosen to perform their intended function: either to increase tumour-specific killing by the virus (e.g. tumour suppressor or apoptosis-inducing genes), to inhibit the activity of oncogenic pathways, or to increase the immunogenicity of infected tumour cells. Documentation exists to indicate that this can be achieved safely. No wild-type oncogenes or direct toxins will be inserted.

**Evaluation of foreseeable effects**

The deletions induced in viral vectors are designed to narrow the host range, so that there is reduced replication and killing within normal cells, thus increasing the therapeutic index in ovarian cancer cells. Mechanisms to achieve this will include introduction of specific deletions to restrict replication and the use of tumour and tissue-specific promoters.
Replication-deficient adenoviruses are incapable of replication in cells lacking adenovirus E1 function. Infection of normal human cells as well as malignant cells results in no infectious virion production. The tumour selective mutants can infect normal cells but the ability to replicate is highly attenuated.
The replication competent adenoviruses used will contain mutations within the viral genome that are necessary for replication in normal cells but are complemented by the altered gene expression in cancer cells. All oncolytic herpes simplex viruses used have ICP34.5 deletion, which dramatically reduces neurovirulence in both human and mice.
The deletions occur in regions of the viral genome that are necessary for replication in normal cells and are complemented by the altered gene expression in cancer cells. The deletions induced in viral vectors are designed to narrow the host range, so that there is reduced replication and killing within normal cells, thus increasing the therapeutic index in ovarian cancer cells. Mechanisms to achieve this will include introduction of specific deletions to restrict replication and the use of tumour and tissue-specific promoters.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Full containment level 2 will be used, as set out in the Regulations, including appropriate treatments for bulk, contaminated solid waste.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be used, as set out in the Regulations; i.e. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level II laboratories, with access restricted to authorised staff, including appropriate treatments for bulk, contaminated solid waste.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All viral supernatants and spent medium will be inactivated using 10,000 ppm free chlorine before removal from the Containment Level II laboratories for final disposal. This process will result in 100% inactivation of infectious virus.
Solid waste will be bagged in biohazard bags prior to removal from the Containment level II lab and then placed in boxes which are sealed prior to removal from the CL2.
suites. Boxes remain sealed and are autoclaved before the contents is removed for collection by accredited waste contractors. Autoclaves produce a digital record of load temperature achieved and are validated by annual thermocouple mapping. This process will result in 100% inactivation of infectious virus. Solid animal waste and animal carcasses, including used cages/soiled mouse bedding, will be bagged in biohazard bags prior to removal from this Containment level II virus room and then autoclaved before collection by accredited waste contractors.

This RA was reviewed by the committee members of GM318 in June 2018. The applicant made requested modifications to the risk assessment. The risk assessment (which is enclosed) was then passed by the committee at Class 2 with reference number 318/217.

Project Containment

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Project Ref 318/19.1

Investigating cell migration and immune responses using virus infection and virus-like particles (VLPs)

Date Ackn'd 07/06/2019

Date Project Ceased

Class Culture Vol

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N
## Project Additional Information

### Purposes of the contained use

The aim of this project is to investigate how the immune system responds to a range of viral challenges, focusing on migration of immune cells. This will generate fundamental insights in basic biology, and benefit development of drugs against pathogens relevant for human and animal health.

We will use either virus or virus-like particles (VLPs) depending on the pathogen and research question. VLPs resemble viral particles, but cannot propagate as they do not contain all required genetic material. Therefore they cannot replicate beyond the first round of infection. This makes VLPs a safe alternative to study early events during viral infection. We will study the immune response to 3 different groups of viruses:

1. mosquito-transmitted viruses, which are an increasing threat to human and animal health
2. rabies VLPs, a safe alternative to study early replication and host response to rabies, which kills at least 55,000 people every year
3. polyomavirus, which are harmless in healthy individuals but can cause severe disease in people who are immune compromised, for instance because of co-infection with other viruses, or immunomodulatory treatment such as for multiple sclerosis.

### Recipient or parental organism

We will use a variety of cell lines and primary cultures derived from humans, rodents and arthropods. Wild type or genetically modified mice will be inoculated with the described viruses and VLPs.

### Host/vector system

1. mosquito-transmitted viruses: We will focus on Togaviridae (mostly Semliki Forest Virus), Flaviviridae, and Bunyaviridae. For most of these, plasmids with the full genome, including mutant variants with reporter/immune modulatory inserts are available.
2. Rabies VLPs: Cell lines/plasmids containing the rabies Glycoprotein gene, and separately a plasmid containing the other rabies genes, including mutants with reporter/immune modulatory inserts, which together result in VLPs. If cells are infected with these VLPs, the new viral particles they make lack the glycoprotein which is essential to enter cells.
   As such, these “bald” virus-like particles cannot infect new cells, and are only infectious for a single round of infection.
3. Polyomaviruses: Plasmids containing viruses such as BK and JC virus, which can be used to make virus in permissive cell lines.

### Origin & function

1. Mosquito-transmitted viruses: virus genetic material is derived from viral RNA, possibly altered to investigate impact of the viral genes on the immune response. Genetic markers, for instance encoding fluorescent proteins to trace viral replication, can be inserted in these viral genomes. Additionally, mammalian and/or arthropod genes, or (complementary) microRNA sequences can be inserted to investigate the impact of these on viral replication, dissemination, host immune response and pathology.
2. Rabies VLPs: Viral genomes originate from wild type and lab strains. Additionally genes as described for mosquito-transmitted viruses can be inserted to trace viral replication, or investigate the host immune response.

3. Polyomavirus: Currently no genes can be inserted in these viruses.

Evaluation of foreseeable effects

1. Mosquito-transmitted viruses: Based on extensive use in many labs including ours shows that the proposed safety measures are suitable to protect workers and the environment. Insertion of genetic markers/other additional genes slightly reduces the ability of the viruses to replicate. Some of these viruses can be neurotropic, and we will not change the structural viral genes that may make the viruses more neurotropic in humans. For mouse-infections, mosquito-transmitted viruses are not airborne and as their vectors are not indigenous in the UK, they do not readily transmit between animals in the wild.

2. Rabies Virus-Like Particles: As these VLPs upon infection do not generate infectious virus, this is a very safe alternative to using rabies virus. We have stringent measurements to prevent genetic recombination that could theoretically result in restoration of replication. Importantly, we do not have complete rabies virus which could increase the risk of recombination. Rabies VLPs cannot produce infectious progeny virus and are therefore by nature not transmissible between animals.

3. Polyomaviruses: the human polyomaviruses to be used in this study have very high prevalence in the general population (seroprevalence >50%) and healthy individuals do not experience any clinical signs or symptoms when infected. We will also use a mouse strain which does not infect humans. Polyomaviruses already circulate in feral mice, and lab strains only cause disease in very young or immunodeficient mice. Murine polyomavirus does not replicate in human tissues and thus the risk of contamination is very low. Infection of mice (including GM mice) with the described viruses/VLPs is not expected to result in a survival advantage of mice. The mouse strains we work with do not have a competitive advantage over wild type mice, and are likely to be severely compromised in the wild. None of the genetic alterations in our mice have been associated with improved breeding. Mice infected with these viruses will be kept in designated areas with extra safety measures such as special cages with virus filters to prevent infected animals from spreading GM viruses to other animals.

Our safety measures, which include thorough training of staff, where necessary working in class II biological safety cabinets, wearing PPE such as lab coats and gloves, and suitable disinfection and autoclaving of waste will reduce the likelihood of exposure to an extremely low level.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All experiments will be performed at containment level 2 as described in the Regulations:
- infection and dissection will be performed in designated areas, in case of aerosol-transmissible viruses/VLPs in class II safety cabinets. Live infected mice will be held in special filtered or individually ventilated cages to prevent transmission to other mice or humans.
- access to buildings to be worked in is restricted to authorised staff, who are trained to work with mice.
- suitable waste management is in place, infected material and carcasses will be autoclaved before leaving the properties.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Containment level 2 will be used for all GMM work; virus generation and infection will be carried out in Class II safety cabinets in containment level II laboratories, appropriate waste disposal is established within the building.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus-infected liquid and disposable solid waste will be inactivated using Virkon (minimum 1% final concentration) before removal from Containment Level II laboratories. Solid waste will subsequently be double-bagged in biohazard bags and autoclaved locally (121°C for 30 minutes) before either landfill or if hazardous, uplifted and incinerated by an accredited company.

Solid animal waste and mouse carcasses (including soiled mouse bedding) will be bagged and autoclaved locally before collection by accredited waste contractors. Cages will be autoclaved and washed before reuse.

Degree of virus inactivation:
Chemical Sterilization by Virkon: effectively 100% kill.
Autoclaving: effectively 100% kill. Autoclaves are tested annually, and each run is monitored by digital recording of temperature/time profile.
This GM RA was reviewed in April 2019 by the local GM committee GM 318, upon which the applicant made the requested modifications of the risk assessment in response to comments from the committee. The GMRA was approved by GM318 on the 8th May 2019.

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**Project Ref** 397/01.1

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<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<td>TO DEFINE THE EFFECT OF ADENOVIRAL OR ADENO-ASSOCIATED VIRAL (AAV) GENE TRANSFER OF CANDIDATE GENES ON BLOOD PRESSURE AND ORGAN DAMAGE IN THE STROKE PRONE SPONTANEOUSLY HYPERTENSIVE RAT</td>
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<td>≤ 1 Litre</td>
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**Historical Significant Changes**

PROJECT TRANSFERRED FROM GM 397 (26/10/07)  

**Historical Date of Additional Info**

PROJECT TRANSFERRED FROM GM 397 (26/10/07)
### Project Additional Information

**Purposes of the contained use**

Confidentiality claim - please see section 17.

The aim of the project is to assess the ability of a number of biological active genes to reduce blood pressure, cardiac hypertrophy and other phenotypic changes associated with hypertension in the SHRSP animal model.

**Recipient or parental organism**

All adenovectors to be used in this study are E1-deleted first generation adenoviral vectors based on the pJM17 system (McGorry, WJ Bautista, DS and Graham, FL: A simple technique for the rescue of early region 1 mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. This renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative.

All AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is rendered replication-defective by addition of a gH-herpes vector. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is rendered replication-defective by addition of a gH-herpes vector.

In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. This renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. However, all the inserted genes are biologically active. None of the transgenes are proto-oncogenes. All transgenes will be under the control of the CMV promoter. There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore the natural topism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use. AAV vectors have received increasing attention for molecular interventions in vivo and for gene therapy applications due to their low level of immnogenicity in vivo and their ability to integrate into the genome, thus producing sustained expression of transgenes for prolonged periods of time. They also have the ability to infect both dividing and non-dividing cells.

AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is rendered replication-defective by addition of a gH-herpes vector.

None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced.

**Host/vector system**

The adenoviral vectors are generated by homologous recombination between pJM17 and shuttle vectors containing transgene expression cassette and flanking E1 sequences. Following homologous recombination in helper 293 cells (which express the helper E1 function in trans), replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by go-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus).
adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al. High-tier rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow. AAV vectors are replication defective.

Origin & function
The adenoviral vector DNAs are standard and originated from the laboratory of Dr Frank Graham (McGory, W J Bautista, DS and Graham FL: A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vivo to generate high-level gene expression in all cells transduced by the adenovirus. The AAV system is published (Zhang, X, de Alwesh, M., et al. High titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All transgenes will be constructed from full length cDNAs obtained from other research institutes and verified in our own laboratory prior to subcloning into the relevant vectors.

Evaluation of foreseeable effects
There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced. Consequence of environmental exposure - 'effectively zero'.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures as per HSE containment level 2 for both production of recombinant adenoviruses and AAV in the laboratory and animal experimentation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Laboratory-based experiments: during the production of replication-defective adenoviruses, all solid waste (plastics etc.) are autoclaved prior to disposal. All liquid waste from tissue culture is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. The use of sharps is avoided.

Animal experiments: All instruments used in the preparation of animals for GM work will be sterilised by autoclaving. Solutions exposed to the viable GMOs will be disinfected with chlorine-based disinfectant. All plastic ware will be autoclaved prior to disposal. Animals receiving the GMO will be will be housed in separate cages during the procedure-kill time period. All animal carcasses will be disposed of by incineration. Animal bedding will be autoclaved prior to disposal and cages disinfected prior to being re-used.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Please enter comments on the GM safety committee on the risk assessment
Passed with amendments by local GMSC 1.2.2001.

Project Containment

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Project Ref 397/01.2

Date Ackn'd 26/10/2007
CU2 Project Title
TO DEVELOP AND TEST TROPI SM MODIFIED ADENOVIRAL AND ADENO-ASSOCIATED VIRAL (AAV) VECTORS FOR SELECTIVE AND ENHANCED GENE TRANSFER TO VASCULAR ENDOTHELIAL CELLS IN VITRO AND VIVO
Class 2
CultureVolClass2 1 Litre
CultureVolumeClass3-4
Non-GMM
Consent Granted Not Applicable

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
PROJECT TRANSFERRED FROM GM 397 (26/10/07)

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
The aim of this project is to restrict the tropism of adenoviral and AAV vectors. Currently, both these vector types poorly tranduced vascular cells in vitro and in vivo, but

02/03/2022 Page 6302 of 15326
AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette. Targeting ligands will be incorporated into fiber-deleted vectors as described by von Seggern (Von Seggern DJ, Huang S, Fleck SK, Stevenson SC, Nemerow GR. Adenoviruses vector pseudotyping in fiber-expressing cell lines: Improved transduction of Epstein-Barr virus-transformed B cells. JOURNAL OF VIROLOGY 74: (1) 354-362 JAN 2000). E1 deletion renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use.

AAV vectors have received increasing attention for molecular interventions in vivo and for gene therapy applications due to their low level of immunogenicity in vivo and their ability to integrate into the genome, thus producing sustained expression of transgenes for prolonged periods of time. They also have the ability to infect both dividing and non-dividing cells. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwes, M., et al. High-titer AAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). Targeted AAV vectors will be prepared using published methods are produced by replication-defective AAV vector particles (Giord A, Ried M, Wobus C, Lahm H, Leike K, Kleinschmidt J, Deleage G, Hallek M. Genetic capsid modifications allow efficient re-targeting of adeno-associated virus type 2. Nature Medicine. 1999;5:1052-1056). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced.

In addition, we are restricting the tropism of AAV and Ad vectors not expanding it. There will be no foreseeable deleterious effect of enhanced gene transfer to endothelial cells.

AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette. Targeting ligands will be incorporated into fiber-deleted vectors as described by von Seggern (Von Seggern DJ, Huang S, Fleck SK, Stevenson SC, Nemerow GR. Adenoviruses vector pseudotyping in fiber-expressing cell lines: Improved transduction of Epstein-Barr virus-transformed B cells. JOURNAL OF VIROLOGY 74: (1) 354-362 JAN 2000). E1 deletion renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use. All adenovectors to be used in this study are E1-deleted first generation adenoviral vectors based on the AdEASY system (He, T-C., Zhou, S., de Costa, LT., Kinzler, KW, Vogelstein, B.A simplified system for generating recombinant adenoviruses. PNAS. 95:2509-2514, 1998). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. The adenoviral vectors are generated by recombination in vitro and shuttle vectors containing transgene expression cassette and flanking E1 sequences (He, T-C., Zhou, S., de Costa, LT., Kinzler, KW, Vogelstein, B. A simplified system for generating recombinant adenoviruses. PNAS. 95:2509-2514, 1998). Following transfection into helper 293 cells (which express the helper E1 function in trans, replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses.

In addition, we are restricting the tropism of AAV and Ad vectors not expanding it. There will be no foreseeable deleterious effect of enhanced gene transfer to endothelial cells.
cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. The system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating amplicons and gH- herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Western Infirmary University of Glasgow. AAV vectors are replication defective.

Origin & function

The adenoviral vector DNAs are standard and originated from the laboratory of Dr Bert Vogelstein (He, T-C., Zhou, S., de Costa, L.T., Kinzler, KW, Vovelstein, B. A simplified system for generating recombinant adenoviruses. PNAS. 95:2509-2514, 1998). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vitro and in vivo to generate high-level gene expression in all cells transduced by the adenovirus.


The targeting ligands (small peptides) have been isolated by phage display technology for their ability to bind vascular endothelial cells in vitro and in vivo. These peptides are selected for incorporation into the Ad and AAV vectors for tropism modification of viral vectors.

Evaluation of foreseeable effects

There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome.

AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01 of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced. In addition, we are restricting the tropism of AAV and Ad vectors not expanding it. There will be no foreseeable deleterious effect of enhanced gene transfer to endothelial cells.

Consequence of environmental exposure - ‘effectively zero’.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures as per HSE containment level 2 for both production of recombinant adenoviruses and AAV in the laboratory and animal experimentation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory-based experiments: during the production of replication-defective adenoviruses, all solid waste (plastics etc.) are autoclaved prior to disposal. All liquid waste from tissue culture is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. The use of sharps is avoided.
Animal experiments: All instruments used in the preparation of animals for GM work will be sterilised by autoclaving. Solutions exposed to the viable GMOs will be disinfected with chlorine-based disinfectant. All plastic ware will be autoclaved prior to disposal. Animals receiving the GMO will be housed in separate cages during the procedure-kill time period. All animal carcasses will be disposed of by incineration. Animal bedding will be autoclaved prior to disposal and cages disinfected prior to being re-used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Passed with ameendments by local GMSC 1.2.2001

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 397/94.2

Date Ackn'd 26/10/2007

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes PROJECT TRANSFERRED FROM GM 397 (26/10/07)

Project notified under transitional arrangements Y
Purposes of the contained use

The aim of this project is to restrict the tropism of adenoviral and AAV vectors. Currently, both these vector types poorly transduced vascular cells in vitro and vivo, but are highly permissive for transduction of non-vascular cells. We will use small targeting ligands (peptides) to develop tropism modified adenoviral and AAV vectors and test the ability of these modified viruses to provide cell selective gene transfer in vitro and in vivo. All adenoviral vectors will express reporter genes.

Recipient or parental organism

All adenovectors to be used in this study are E1-deleted first generation adenoviral vectors based on the AdEASY system (He, T-C., Zhou, S., de Costa, T., Kinzler, KW, Vogelstein, B.A. simplified system for generating recombinant adenoviruses. PNAS. 95:2509-2514, 1998). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. Targeting ligands will be incorporated into fiber-deleted vectors as described by von Seggern (Von Seggern DJ, Huang S, Fleck SK, Stevenson SC, Nemerow GR. Adenovirus vector pseudotyping in fiber-expressing cell lines: Improved transduction of Epstein-Barr virus-transformed B cells. JOURNAL OF VIROLOGY 74: (1) 354-362 JAN 2000). E1 deletion renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use. AAV vectors have received increasing attention for molecular interventions in vivo and for gene therapy applications due to their low level of immunogenicity in vivo and their ability to integrate into the genome, thus producing sustained expression of transgenes for prolonged periods of time. They also have the ability to infect both dividing and non-dividing cells. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating ampiclon and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). Targeted AAV vectors will be prepared using published methods are produce replication-defective AAV vector particles (Girod A, Ried M, Wobus C, Lahm H, Leike K, Kleinschmidt J, Deleage G, Hallek M. Genetic capsid modifications allow efficient re-targeting of adeno-associated virus type 2. Nature Medicine, 1999;5 1052-1056). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10^69 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced. In addition, we are restricting the tropism of AAV and Ad vectors not expanding it. There will be no foreseeable deleterious effect of enhanced gene transfer to endothelial cells.
Host/vector system

The adenoviral vectors are generated by recombination in vitro and shuttle vectors containing transgene expression cassette and flanking E1 sequences (He, T-C., Zhou, S., de Costa, LT., Kinzler KW, Vogelstein, B. A simplified system for generating recombinant adenoviruses PNAS. 95:2509-2514, 1998). Following transfection into helper 293 cells (which express the helper E1 function in trans, replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper function necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow, AAV vectors are replication defective.

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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Laboratory-based experiments: during the production of replicatin-defective adenoviruses, all solid waste (plastics etc) are autoclaved prior to disposal. All liquid waste from tissue culture is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. The use of sharps is avoided.
Animal experiments: All instruments used in the preparation of animals for GM work will be sterilised by autoclaving. Solutions exposed to the viable GMOs will be disinfected with chlorine-based disinfectant. All plastic ware will be autoclaved prior to disposal. Animals receiving the GMO will be housed in separate cages during the procedure-kill time period. All animal carcasses will be disposed of by incineration. Animal bedding will be autoclaved prior to disposal and cages disinfected prior to being re-used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Passed with amendments by local GMSC 1.2.2001.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Large Scale Activities

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Name

ASTRAZENECA

Name 2

Department

MOLECULAR BIOLOGY

Campus Estate or Research Centre

Building

Road Name

BAKEWELL ROAD

District

Town

LOUGHBOROUGH

County

LEICESTERSHIRE

Postcode

LE11 5RH

Country

ENGLAND

Tel Number

01509 644000

Fax Number

01509 645555

E-mail

HSE Division

MIDLANDS

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
  - Animal Unit
  - Growth Room
  - Glass House
  - Large Scale

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref:** 323/01.1

<table>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref: 323/01.2

Date Ackn'd: 13/09/2001
Date Project Ceased: 26/11/2010

CU2 Project Title: EXPRESSION OF A WILD TYPE MAPKKK AND ITS DOMINANT NEGATIVES USING AMPHOTROPIC RETROVIRAL MEDIATED GENE TRANSFER

Class: 2
CultureVolClass2: < 1 litre
Non-GMM: not applicable

Project notified under transitional arrangements: N

Project Additional Information

Purposes of the contained use:
To produce the GMOs expressing the proteins for use in screening compounds in the process of drug discovery.

Recipient or parental organism

Cloning host
E.coli K12: DH5a - a disabled host. ACDP Hazard Group 1
Top 10 - a disabled host. ACDP Hazard Group 1
These strains are not considered pathogenic or able to persistently colonise humans or animals. They have limited survivability in the environment. They have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. There is no plasmid mobilisation/transfer genes in these host.

Expression hosts
Mammalian cell lines eg. RAW, HEKs ACDP Hazard Group 2
Human primary cells ACDP Hazard Group 2
Host/vector system

All cloning vectors used will be non-mobilisable or mobilisation defective. These all contain antibiotic resistance genes to allow selection. Resistance to this is common and of limited clinical significance.

Vector system No. 1 Amphotropic Replication Deficient Retroviruses (RDRs) (see Section 17 for information on this system). They all include an antibiotic resistance gene to allow selection.

Origin & function

Origin of clone is from a cDNA library. Intended function is to express the clone in order to determine the effect of over-expressing the wild type protein or its dominant negative mutants on the cell lines transduced.

Evaluation of foreseeable effects

The gene of interest encodes the sequence of a MAP kinase kinase kinase. On expression it is anticipated that the protein will be correctly folded and active. The gene of interest is not a known oncogene.

Expression of the inserts in the recombinant organism are unlikely to affect the pathogenicity of the expression host. Contact of the replication deficient retroviruses (RDRs) containing the wild type MAPK KK gene should be avoided, as transduction of MAPK KK into the cells of a worker could result in an unknown effect.

The RDRs are highly labile and are highly unlikely to transfer this sequence to either humans or other organisms because of the containment measures used.

In the worst-case scenario, a replication competent amphotropic retrovirus capable of expressing functional wild type MAPK KK is produced that productively infects an individual via body fluid contact (eg. sharps injury). The individual therefore would be infected with a potentially damaging retrovirus with unknown effects. The virus would be transmissible by body fluid contact.

The likelihood of this happening is negligible because:

1. The retroviral vectors themselves do not contain any of the functions (gag, pol and env) that are essential for replication. Integration of the vectors results in their inactivation due to a deletion in the 3' LTR. All of these functions must be restored in order to produce a replication competent retrovirus (RCR).

2. Capture events are most likely by homologous recombination with wild type retrovirus. As the homologous regions flank the engineered region of the virus containing the MAPK KK, homologous recombination would result in deletion of the MAPK KK gene.

3. As the tropism of any PCR produced would be dictated by the endogenous retrovirus providing the gag, pol and env functions, homologous recombination with wild type retrovirus would produce a virus which would not pose a significant risk to human health.

4. The RDRs are going to to used to transduce primary human cells and therefore may come into contact with endogenous human retroviruses. However, as the PDRs are based upon a non-primate retrovirus the chance of homologous recombination to produce an RCR is greatly reduced.

5. Endogenous retroviral infection of the transduced cells could provide the functions required to re-package viral genomes in trans. The use of self-inactivating vectors means that following integration of the RDR DNA, the normal retrovirus 5'-LTR promoter is silenced. Expression of the MAPK KK is driven from a promoter that is downstream of the packaging sequence. Hence, all transcripts produced will be incapable of being packaged. There is no evidence to suggest that the deleted 5' LTR has promoter activity.

6. Any RCR produced would be expected to be highly unstable and could be attenuated by contact with surfaces, normal disinfectants and desiccation. The RDRs are not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture supernatant.
on an open wound. The use of sharps will be prohibited, the use of gloves will be mandatory and the generation of aerosols minimised.

A more likely scenario is that a worker contaminates himself or herself with an RDR containing wild type MAPKKK. This may result in an increase in the activation of the signalling MAP kinase pathways in the transformed cells. However, the RDRs are highly unstable, attenuated by desiccation and not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture supernatant on an open wound. The use of sharps will be prohibited, the use of gloves will be mandatory and the generation of aerosols minimised.

The likelihood of harm to human health occurring is considered negligible due to the containment measures used.

As the survival of the vectors in the general environment is poor and given that the work will be carried out at CL2, the risk of animal exposure is therefore very small. Thus, the overall risk to the environment is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are “White Rose Ltd” and have been registered with the HSE. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain. There will be 100% kill.

All liquid waste is inactivated by chemical means using concentrated disinfectants, either Hycolin solution (Coventry Chemicals Limited) for E. coli work or TriGene (MediChem International) for Tissue culture/RDR work to a final concentration of 2% (as recommended by both suppliers). The solutions are left for 24 hours before disposal via the drain together with copious amounts of water.

Hycolin has been validated using the BS:6905/87 as the modified Kelsey Sykes test, the Australian TGA test, MAFF, ES and MIC tests. Hycolin is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species.

TriGene has been validated using BS6471, BS EN 1276, NF 72-150, NF 72-200, 72-180 and is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species.

As a result of treatment no viable GMMs from Liquid or solid waste will remain, there will be 100% kill.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y
Please enter comments on the GM safety committee on the risk assessment

This proposal has been approved as risk class 2. It was finally approved after minor modifications at the GMSC meeting on 27.7.01.

### Project Containment

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### Project Ref 323/01.3

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### Project Additional Information

#### Purposes of the contained use

To produce the GMOs expressing the protein for target validation and for use in screening compounds in the process of drug discovery.

#### Recipient or parental organism
Cloning host. E. coli K12:

DH5a - a disabled host. ACDP Hazard Group 1
Top 10 - a disabled host. ACDP Hazard Group 1
Other E. coli K12 strains. ACDP Hazard Group 1
These strains are not considered pathogenic or able to persistently colonise humans or animals. They have limited survivability in the environment. They have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. There are no plasmid mobilisation/transfer genes in these hosts.

Expression hosts
Mammalian cell lines eg. RAW, HEKs ACDP Hazard Group 2
Human primary cells ACDP Hazard Group 2
Insect cell lines - especially disabled hosts
E. coli K12 strain - a disabled host
S. cerevisiae - an especially disabled host.

Host/vector system
All cloning vectors used will be non-mobilisable or mobilisation defective.
These all contain antibiotic resistance genes to allow selection. Resistance to this is common and of limited clinical significance.

Vector system No. 1 Amphotropic Replication Deficient Retroviruses (RDRs) (See Section 17 for information on this system). They all include an antibiotic resistance gene to allow selection.

Vector system No. 2 Mammalian expression vectors (see Section 17 for information on this system). They all include an antibiotic resistance gene to allow selection.

Vector system No. 3 Baculovirus expression system (see Section 17 for information on this system).

Vector system No. 4 E. coli expression vectors (see Section 17 for information on this system). These vectors are either non-mobilisable or mobilisation defective. They all include an antibiotic resistance gene to allow selection.

Vector system No. 5 Yeast expression vectors (see Section 17 for information on this system). These vectors are non-transferable. They all contain selection markers.

Origin & function
Origin of clone is from a cDNA library. The intention is to express the protein or mutants thereof, in primary cells or cell lines, to determine the function of the protein. Also it is intended to express the protein for the purposes of developing a screening assay.

Evaluation of foreseeable effects
The gene of interest encodes the sequence of a frp and may modulate wnt/frizzled signalling pathways. On expression it is anticipated that the protein will be correctly folded and active. The gene of interest is not a known oncogene, but may potentially be oncogenic.

Expression of the insert in the recombinant organisms is unlikely to affect the pathogenicity of the expression hosts listed above. Contact of the replication deficient retroviruses (RDRs) containing the frp gene should be avoided, as transduction of a frp into the cells of a worker could result in an unknown effect.

The RDRs are highly labile and are highly unlikely to transfer this sequence to either humans or other organisms because of the containment measures used.

In the worst-case scenario, a replication competent amphotropic retrovirus (RCR) capable of expressing functional frp is produced that productively infects an individual via
body fluid contact (eg. sharps injury). The individual therefore would be infected with a potentially damaging retrovirus with unknown effects. The virus would be
transmissible by body fluid contact.

The likelihood of this happening is negligible because:

1. The retroviral vectors themselves do not contain any of the functions (gag, pol and env) that are essential for replication. Integration of the vectors results in their
inactivation due to a deletion in the 3’ LTR. All of these functions must be restored in order to produce a RCR.
2. Capture events are most likely by homologous recombination with wild type retrovirus. As the homologous regions flank the engineered region of the virus containing
the frp, homologous recombination would result in deletion of the frp gene.
3. As the tropism of any RCR produced would be dictated by the endogenous retrovirus providing the gag, pol and env functions, homologous recombination with wild
type retrovirus would produce a virus which would not pose a significant risk to human health.
4. The RDRs are going to be used to transduce primary human cells and therefore may come into contact with endogenous human retroviruses. However, as the RDRs
are based upon a non-primate retrovirus the chance of homologous recombination to produce an RCR is greatly reduced.
5. Endogenous retroviral infection of the transduced cells could provide the functions required to re-package viral genomes in trans. The use of self-inactivating vectors
means that following integration of the RDR DNA, the normal retrovirus 5’- LTR promoter is silenced. Expression of the frp is driven from a promoter that is downstream of
the packaging sequence. Hence, all transcripts produced will be incapable of being packaged. There is no evidence to suggest that the deleted 5’ LTR has promoter
activity.
6. Any RCR produced would be expected to be highly unstable and could not be attenuated by contact with surfaces, normal disinfectants and desiccation. The RDRs are
not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture
supernatant on an open wound. The use of sharps will be prohibited, the use of gloves will be mandatory and the generation of aerosols minimised.

A more likely scenario is that a worker contaminates himself or herself with an RDR containing frp. This may result in an increase/decrease in the activation of the
wnt/frizzled signalling pathways in the transformed cells. However, the RDRs are highly unstable, attenuated by desiccation and not known to be transmissible by aerosol.
Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture supernatant on an open wound. The use of sharps will
be prohibited, the use of gloves will be mandatory and the generation of aerosols minimised.

The likelihood of harm to human health occurring is considered negligible due to the containment measures used.

As the survival of the vectors in the general environment is poor and given that the work will be carried out at CL2, the risk of animal exposure is therefore very small.
Thus, the overall risk to the environment is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are
"White Rose Ltd" and have been registered with the HSE. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and
contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain. There will be 100% kill.

All liquid waste is inactivated by chemical means using concentrated disinfectants, either Hycolin solution (Coventry Chemicals Ltd) for E. coli and yeast work or TriGene
(MediChem International) for Rissue culture/RDR work, to a final concentration of 5% (as recommended by both suppliers) or Virkon for insect cell/baculovirus work to a
final concentration of 1%. The solutions are left for 2 hours before disposal via the drain together with copious amounts of water.

Hycolin has been validated using the BS:6905/87 as the modified Kelsey Sykes test, the Australian TGA test, MAFF, ES and MIC tests. Hycolin is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. Hycolin has also been tested in house under conditions used for decontamination.

TriGene has been validated using BS6471, BS EN 1276, NF 72-150, NF 72-200, 72-180 and is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. Trigene has also been tested in house under conditions used for decontamination.

Virkon has been validated in house under conditions used for decontamination.

As a result of treatment no viable GMMs from liquid or solid waste will remain, there will be 100% kill.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

This proposal has been approved as risk class 2. It was finally approved after minor modification at the GMSC meeting on 30/08/01.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 323/02.1

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02/03/2022  Page 6319 of 15326
**Project Additional Information**

**Purposes of the contained use**
To produce the GMOs expressing the protein for target validation and for use in screening compounds in the process of drug discovery.

**Recipient or parental organism**

**Cloning host**
Packaging cell line for replication deficient adenovirus. This packaging cell line has been designed for use with the adenoviral vectors covered by this proposal. The cells complement for two adenoviral gene cassettes essential for adenovirus replication that have been deleted in the vectors. No sequence overlap exists between the packaging cell line and the adenoviral vectors preventing homologous recombination and making the emergence of replication competent adenovirus extremely unlikely.

**Expression hosts**
Human primary cells ACDP Hazard Group 2. The cells are non-transformed. No adventitious agents are believed present. For COSHH purposes they should be treated as risk class 2. Cells are from an external source; individuals must not culture their own cells.

**Host/vector system**

Vector system: Replication Deficient Adenovirus (RDAs). The adenoviral genome has been divided between two vectors (adapter and helper). The adapter plasmid contains an inverted terminal repeat (containing the origin of replication) and the packaging signal (a series of AT-rich sequences). An essential adenoviral cassette has been deleted and replaced by the gene of interest under the control of a promoter. The helper cosmid contains the remainder of the adenoviral genome with some adenoviral gene cassettes removed. Two of the deleted adenoviral gene cassettes are essential for replication hence the resulting adenoviral is replication deficient.

**Origin & function**

Origin of cline is from a cDNA library. The intention is to express the protein or mutants thereof, in primary cells, to determine the function of the protein.

**Evaluation of foreseeable effects**

The gene of interest encodes the sequence of a Rab protein (wild-type dominant regenerative/constitutively active mutant versions and a Rab fusion). Rab proteins are localised to the cytoplasmic face of organelles and vesicles involved in the biosynthetic/secretory and endocytic pathways in eukaryotic cells and are thought to play a regulatory role in vesicle transport. Most rabs are ubiquitous and each localises to a particular membrane compartment, although some are cell-type or tissue specific. It is anticipated that the protein will be correctly folded and active. The gene of interest is not a known oncogene.
Expression of the insert in the recombinant organisms is unlikely to affect the pathogenicity of the expression hosts listed above. Contact of the replication deficient adenovirus (RDAs) containing the rab gene should be avoided, as transduction of a rab into the cells of a worker could result in an unknown effect. The most likely effect is perturbation of cellular vesicle transport.

The RDAs are transmissible by aerosol and fluid: fluid contact and are highly unlikely to transfer this sequence to either humans or other organisms because of the containment measures used.

In the worst-case scenario, a replication competent adenovirus (RCA) capable of expressing functional rab is produced that productively infects an individual via body fluid contact (eg sharps injury) or aerosol inhalation. The individual therefore would be infected with a potentially damaging adenovirus expressing a rab protein with unknown effects. The virus would be transmissible by body fluid contact.

The likelihood of this happening is negligible because two adenoviral gene cassettes essential for replication have been deleted in the adenoviral vectors. Production of replication-defective adenovirus requires the use of a specialised packaging cell line which complements for these essential adenoviral genes. No sequence overlap exists between the packaging cell line and the adenoviral vectors preventing homologous recombination and making the emergence of replication-competent Adenovirus extremely unlikely. If a member of staff is naturally infected with wild-type adenovirus this may provide an opportunity for complementation of the deleted gene cassettes enabling the generation of RDA in the host. However, recombinant adenovirus can only become replication-competent by exchange of both complete expression cassettes, requiring two homologous recombination events.

A more likely scenario is that a worker contaminates himself or herself with a replication deficient adenovirus (RDA) containing a Rab protein. The likelihood of harm to human health occurring is considered low because:

(1) As with naturally occurring adenovirus, the adenoviral constructs covered by this proposal will elicit a host immune response. This poses a potential hazard if delivered in high quantities. This has been highlighted by a fatality in a gene therapy trial (Science 286 p2244) following the injection of a massive dose of crippled adenovirus (38 trillion virus particles) into the liver. However, the risk to staff is negligible because such large quantities of adenovirus would never be handled, equivalent to over 3L of RDA stock (5-10 million virus particles per microlitre, stored in 0.25 ml alequots).

(2) Replication-deficient adenoviral particles are able to transduce human cells (particularly airway epithelial and gastrointestinal cells). However, these viruses will induce both a humoral and cellular immune response which will lead to targeted elimination of infected cells. This means that rab protein expression will be very short-lived. Hence any potential harmful effects associated with rab protein expression will be transient and will not present a long-term problem.

(3) Transduction is possible through direct fluid-fluid contact and so risk is via entry through open wounds. This presents negligible risk since the use of sharps will be prohibited and as part of standard laboratory practice open wounds will be covered.

(4) Adenivirus is transmissible by aerosol so the highest risk of exposure is via entry through airways and transduction of airway epithelial cells. However, the viral DNA will not be replicated and is not directly integrated into the host genome. Since airway epithelial cells exhibit a high rate of cell turnover any viral DNA will be lost over time and transgene expression will be transient. Following aerosol exposure to RDA it is possible that the viral DNA could be replicated and packaged generating more virions if the host is already infected with naturally occurring adenovirus of the same serotype. However, such virions will be replication-deficient. This could lead to transduction of neighbouring cells and localised spreading of transgene expression. Transduced cells are more likely to be targeted and removed by the host immune response, restricting the spread of RDA. Expression of the rab protein (wild-type or variants) in airway epithelial cells is unlikely to cause harm to the user. This cell type exhibits regulated secretion of lung surfactant. However, there is no evidence that the Rab protein of interest is endogenously expressed, so the absence of relevant affector proteins and target membranes means that this Rab protein is unlikely to participate in vesicle transport in this cell type. However, if low affinity binding between the over-expressed Rab protein and unrelated Rab effectors or target vesicles does occur it is possible that high levels of overexpression could perturb normal vesicle transport in the cell.

Overexpression studies using Rab 11 in HeLa cells provides evidence that Rab overexpression does not effect other transport pathways regulated by other Rab proteins (J.
However, this cannot be ruled out in the case of every Rab protein or cell type so the risk to the user is low following exposure. The overall risk to human health is considered negligible because the likelihood of exposure to aerosols is negligible due to the containment measured used.

Over-expression of a Rab protein in a cell type in which it is endogenously expressed may perturb normal vesicle trafficking. Transduction of cells found circulating in the blood requires fluid:fluid contact via sharps injury or open wounds. This represents negligible risk since sharps are prohibited and open wounds will be covered. Transduction of cells present in the gut requires ingestion of inhalation of virions. However, the risk of exposure leading to transduction is negligible since the vast majority of virions would be inactivated during passage through the stomach. Aerosol exposure may risk transduction of cells located within the lung tissue. However, for this to be possible the RDA would need to breach the epithelial barrier. Moreover, since gene expression will be both transient and localised, accidental exposure is unlikely to have a significant impact on the health of an individual. However, since this cannot be ruled out this presents low risk to the user.

Adenovirus does not directly integrate the transgene into the host genome, however random integration is possible but poses no significant risk since this is a rare event. The risk of potential harm is only significant if the transgene is oncogenic. No Rab proteins are known to be oncogenic. However, one Rab protein (Rab38) is known to be expressed in 80-90% of melanoma, but rarely in nonmelanocytic malignancies (Cancer Research 2000, 60(13)p3584). Rab38 has a unique C-terminus which would allow post-translational farnesylation and palmitoylation, lipid modifications normally occurring in oncogenic ras proteins but not in other rabs. Hence, the Rab protein of interest (a) is not expected to be oncogenic and so random integration poses negligible risk.

There is no evidence that human adenovirus can naturally infect animals, and replication is very limited. However, replication has been shown to occur in the lungs of experimentally infected cotton rats (not indigenous to the UK) administered at high doses. Given that the work will be carried out at CL2, the risk of animal exposure is therefore very small. Thus, the overall risk to the environment is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are "White Rose Ltd" and have been registered with the HSE. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain. There will be 100% kill.

All liquid waste is inactivated by chemical means using the concentrated disinfectant TriGene (MediChem International) to a final concentration of 5% (as recommended by supplier). The solutions are left for 2 hours before disposal via the drain together with copious amounts of water.

Tri-Gene has been validated using BS6471, BS EN 1276, NF 72-150, NF 72-200, 72-180 and is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. TriGene has also been tested in house under conditions used for decontamination.

As a result of treatment no viable GMMs from liquid or solid waste will remain, there will be 100% kill.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
This proposal was approved by the GMSC on 23 November 2001 as risk class 2. It was noted that this proposal was on the borderline between class 1 and class 2. Greatest concern would be if constitutive active Rab protein could have effects in epithelial cells. No evidence that it would, but difficult to exclude entirely. Possibility of some effect on transduced cells in lung epithelial layer impossible to exclude. Will be classified as risk class 2 with a requirement to control aerosols being the most important measure needed to reduce risk.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Conf</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Animal Units
- L2
- L3
- L4

Large Scale Activities
- L2
- L3
- L4

Human Clinical Applications
- L2
- L3
- L4

Project Ref 323/02.2

CLONING AND EXPRESSION OF A WNT PROTEIN USING AMPHOTROPIC RETROVIRAL MEDIATED GENE TRANSFER AND OTHER EXPRESSION METHODS.

Date Ackn'd 11/01/2002

Date Project Ceased 26/11/2010

Class 2

Culture Vol Class 2 < 1 litre

Consent Granted not applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work Y
## Project Additional Information

### Purposes of the contained use

To produce the GMOs expressing the protein for target validation and for use in screening compounds in the process of drug discovery

### Recipient or parental organism

<table>
<thead>
<tr>
<th>Cloning host</th>
<th>E.coli K12</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH5a - a disabled host. ACDP Hazard Group 1</td>
<td></td>
</tr>
<tr>
<td>Top 10 - a disabled host. ACDP Hazard Group 1</td>
<td></td>
</tr>
<tr>
<td>Other E. coli K12 strains. ACDP Hazard Group 1</td>
<td></td>
</tr>
</tbody>
</table>

These strains are not considered pathogenic or able to persistently colonise humans or animals. They have limited survivability in the environment. They have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. There are no plasmid mobilisation/transfer genes in these hosts.

### Expression hosts

- Mammalian cell lines eg RAW, HEKs ACDP Hazard Group 2
- Human primary cells ACDP Hazard Group 2
- Insect cell lines - especially disabled hosts
- E.coli K12 strain - a disabled host
- S. cerevisiae - an especially disabled host

### Host/vector system

All cloning vectors used will be non-mobilisable or mobilisation defective. These all contain antibiotic resistance genes to allow selection. Resistance to this is common and of limited clinical significance.

- **Vector system No 1 Amphotropic Replication Deficient Retroviruses (RDRs).** They all include an antibiotic resistance gene to allow selection.
- **Vector system No. 2 Mammalian expression vectors.** They all include an antibiotic resistance gene to allow selection.
- **Vector system No. 3 Baculovirus expression system.**
- **Vector system No. 4 E.coli expression vectors.** These vectors are either non-mobilisable or mobilisation defective. They all include an antibiotic resistance gene to allow selection.
- **Vector system No. 5 Yeast expression vectors.** These vectors are non-transferable. They all contain selection markers.

### Origin & function

Origin of cline is from a cDNA library. The intention is to express the protein or mutants thereof, in primary cells or cell lines, to determine the function of the protein.

### Evaluation of foreseeable effects

The gene of interest encodes the sequence of a wnt protein and may modulate wnt/frizzled signalling pathways. On expression it is anticipated that in some of the listed expression systems the protein will be correctly folded and active. The gene of interest is not a known oncogene, but may potentially be oncogenic.

Expression of the insert in the recombinant organisms is unlikely to affect the pathogenicity of the expression hosts listed above. Contact of the replication deficient retroviruses (RDRs) containing the wnt gene should be avoided, as transduction of a wnt protein into the cells of a worker could result in an unknown effect.

The RDTs are highly labile and are highly unlikely to transfer this sequence to either humans or other organisms because of the containment measures used.
In the worst-case scenario, a replication competent amphotropic retrovirus (RCR) capable of expressing functional wnt protein is produced that productively infects an individual via body fluid contact (e.g., sharps injury). The individual therefore could be infected with a potentially damaging retrovirus with unknown effects. The virus would be transmissible by body fluid contact.

The likelihood of this happening is negligible because:

1. The retroviral vectors themselves do not contain any of the functions (gag, pol, and env) that are essential for replication. Integration of the vectors results in their inactivation due to a deletion in the 3' LTR. All of these functions must be restored in order to produce a RCR.
2. Capture events are most likely by homologous recombination with wild type retrovirus. As the homologous regions flank the engineered region of the virus containing the wnt gene, homologous recombination would result in deletion of the wnt gene.
3. As the tropism of any RCR produced would be dictated by the endogenous retrovirus providing the gag, pol, and env functions, homologous recombination with wild type retrovirus would produce a virus which would not pose a significant risk to human health.
4. The RDRs are going to be used to transduce primary human cells and therefore may come into contact with endogenous human retroviruses. However, as the RDRs are based upon a non-primate retrovirus the chance of homologous recombination to produce an RCR is greatly reduced.
5. Endogenous retroviral infection of the transduced cells could provide the functions required to re-package viral genomes in trans. The use of self-inactivating vectors means that following integration of the RDR DNA, the normal retrovirus 5' - LTR promoter is silenced. Expression of the wnt protein is driven from a promoter that is downstream of the packaging sequence. Hence, all transcripts produced will be incapable of being packaged. There is no evidence to suggest that the deleted 5' LTR has promoter activity.
6. Any RCR produced would be expected to be highly unstable and could be attenuated by contact with surfaces, normal disinfectants and desiccation. The RDRs are not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture supernatant on an open wound. The use of sharps will be prohibited, the use of gloves will be mandatory and the generation of aerosols minimised.

A more likely scenario is that a worker contaminates himself or herself with an RDR containing the wnt gene. This may result in an increase or a decrease in the activation of the wnt/frizzled signalling pathways in the transformed cells. However, the RDRs are highly unstable, attenuated by desiccation and not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture supernatant on an open wound. The use of sharps will be prohibited, the use of gloves will be mandatory and the generation of aerosols minimised.

The likelihood of harm to human health occurring is considered negligible due to the containment measures used.

As the survival of the vectors in the general environment is poor and given that the work will be carried out at CL2, the risk of animal exposure is therefore very small. Thus, the overall risk to the environment is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are "White Rose Ltd" and have been registered with the HSE. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain. There will be 100% kill.
All liquid waste is inactivated by chemical means using concentrated disinfectants, either Hycolin solution (Coventry Chemicals Limited) for E. coli and yeast work or TriGene (MediChem International) for Tissue culture/RDR work, to a final concentration of 5% (as recommended by both suppliers) or Virkon for insect cell/baculovirus work to a final concentration of 1%. The solutions are left for 2 hours before disposal via the drain together with copious amounts of water.

Hycolin has been validated using the BS:6905/87 as the modified Kelsey Sykes test, the Australian TGA test, MAFF, ES and MIC tests. Hycolin is lethal against all common gram-positive pathogens, all gram-negative pathogens and Mycobacterium species. Hycolin has also been tested in house under conditions used for decontamination.

TriGene has been validated using BS6471, BS EN 1276, NF 72-150, NF 72-200, 72-180 and is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. Trigene has also been tested in house under conditions used for decontamination.

Virkon has been validated in house under conditions used for decontamination.

As a result of treatment no viable GMMs from liquid or solid waste will remain, there will be 100% kill.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

Project Safety

Project Containment

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<td>L4 L3 L4 L2</td>
</tr>
</tbody>
</table>

Animal Units

| L2 L3 L4 L2 | L3 L4 L2 L3 | L4 L3 L4 L2 |

Large Scale Activities

| L2 L3 L4 L2 | L3 L4 L2 L3 | L4 L3 L4 L2 |

Human Clinical Applications

| L2 L3 L4 L2 | L3 L4 L2 L3 | L4 L3 L4 L2 |

Project Ref 323/02.3

Date Ackn'd CU2 Project Title

Class CultureVolClass2 CultureVolumeClass3-4

20/03/2022
**Purposes of the contained use**

To produce the GMOs expressing the protein for target validation and for use in screening compounds in the process of drug discovery.

**Recipient or parental organism**

**Cloning host**
- E. coli K12:
  - DH5a - a disabled host. ACDP Hazard Group 1
  - Top 10 - a disabled host. ACDP Hazard Group 1
  - Other E. coli K12 strains. ACDP Hazard Group 1

These strains are not considered pathogenic or able to persistently colonise humans or animals. They have limited survivability in the environment. They have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. There are no plasmid mobilisation/transfer genes in these hosts.

**Expression hosts**
- Mammalian cell lines eg RAW, HEKs ACDP Hazard Group 2
- Human primary cells ACDP Hazard Group 2
- Insect cell lines - especially disabled hosts
- E.coli K12 strain - a disabled host
- S. cerevisiae - an especially disabled host.

**Host/vector system**

All cloning vectors used will be non-mobilisable or mobilisation defective. These all contain antibiotic resistance genes to allow selection. Resistance to this is common and of limited clinical significance.

Vector system No. 1 Amphotropic Replication Deficient Retroviruses (RDRs). They all include an antibiotic resistance gene to allow selection.
Vector system No. 2 Mammalian expression vectors. They all include an antibiotic resistance gene to allow selection.
Vector system No. 3 Baculovirus expression system.
Vector system No. 4 E. coli expression vectors. These vectors are either non-mobilisable or mobilisation defective. They all include an antibiotic resistance gene to allow selection.
Vector system No. 5 Yeast expression vectors. These vectors are non-transferable. They all contain selection markers.

Origin & function

Origin of cline is from a cDNA library. The intention is to express the protein or mutants thereof, in primary cells or cell lines, to determine the function of the protein.

Evaluation of foreseeable effects

The gene of interest encodes the sequence of a frizzled receptor and may modulate wnt/frizzled signalling pathways. On expression it is anticipated that in some of the listed expression systems the protein will be correctly folded and active. The gene of interest is not a known oncogene, but may potentially be oncogenic.

Expression of the insert in the recombinant organisms is unlikely to affect the pathogenicity of the expression hosts listed above. Contact of the replication deficient retroviruses (RDRs) containing the wnt gene should be avoided, as transduction of a wnt protein into the cells of a worker could result in an unknown effect.

The RDTs are highly labile and are highly unlikely to transfer this sequence to either humans or other organisms because of the containment measures used.

In the worst-case scenario, a replication competent amphotropic retrovirus (RCR) capable of expressing functional wnt protein is produced that productively infects an individual via body fluid contact (eg sharps injury). The individual therefore could be infected with a potentially damaging retrovirus with unknown effects. The virus would be transmissible by body fluid contact.

The likelihood of this happening is negligible because:

1. The retroviral vectors themselves do not contain any of the functions (gat, pol and env) that are essential for replication. Integration of the vectors results in their inactivation due to a deletion in the 3’ LTR. All of these functions must be restored in order to produce a RCR.
2. Capture events are most likely by homologous recombination with wild type retrovirus. As the homologous regions flank the engineered region of the virus containing the wnt gene, homologous recombination would result in deletion of the wnt gene.
3. As the tropism of any RCR produced would be dictated by the endogenous retrovirus providing the gag, pol and env functions, homologous recombination with wild type retrovirus would produce a virus which would not pose a significant risk to human health.
4. The RDRs are going to be used to transduce primary human cells and therefore may come into contact with endogenous human retroviruses. However, as the RDRs are based upon a non-primate retrovirus the chance of homologous recombination to produce an RCR is greatly reduced.
5. Endogenous retroviral infection of the transduced cells could provide the functions required to re-package viral genomes in trans. The use of self-inactivating vectors means that following integration of the RDR DNA, the normal retrovirus 5’ - LTR promoter is silenced. Expression of the wnt protein is driven from a promoter that is downstream of the packaging sequence. Hence, all transcripts produced will be incapable of being packaged. There is no evidence to suggest that the deleted 5’ LTR has promoter activity.
6. Any RCR produced would be expected to be highly unstable and could be attenuated by contact with surfaces, normal disinfectants and desiccation. The RDRs are not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture supernatant on an open wound. The use of sharps will be prohibited, the use of gloves will be mandatory and the generation of aerosols minimised.

A more likely scenario is that a worker contaminates himself or herself with an RDR containing the wnt gene. This may result in an increase or a decrease in the activation of the wnt/frizzled signalling pathways in the transformed cells. However, the RDRs are highly unstable, attenuated by desiccation and not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture supernatant on an open wound. The use of sharps will be prohibited, the use of gloves will be mandatory and the generation of aerosols minimised.
The likelihood of harm to human health occurring is considered negligible due to the containment measures used.

As the survival of the vectors in the general environment is poor and given that the work will be carried out at CL2, the risk of animal exposure is therefore very small. Thus, the overall risk to the environment is negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

None

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are "White Rose Ltd" and have been registered with the HSE. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain. There will be 100% kill.

All liquid waste is inactivated by chemical means using concentrated disinfectants, either Hycolin solution (Coventry Chemicals Limited) for E. coli and yeast work or TriGene (MediChem International) for Tissue culture/RDR work, to a final concentration of 5% (as recommended by both suppliers) or Virkon for insect cell/baculovirus work to a final concentration of 1%. The solutions are left for 2 hours prior to disposal via the drain together with copious amounts of water.

Hycolin has been validated using the BS:6905/87 as the modified Kelsey Sykes test, the Australian TGA test, MAFF, ES and MIC tests. Hycolin is lethal against all common gram-positive pathogens, all gram-negative pathogens and Mycobacterium species. Hycolin has also been tested in house under conditions used for decontamination.

TriGene has been validated using BS6471, BS EN 1276, NF 72-150, NF 72-200, NF 72-180 and is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. Trigene has also been tested in house under conditions used for decontamination.

Virkon has been validated in house under conditions used for decontamination.

As a result of treatment no viable GMMs from liquid or solid waste will remain, there will be 100% kill.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** Y

**Please enter comments on the GM safety committee on the risk assessment**

This proposal has been approved as risk class 2. It was finally approved after minor modification at the GMSC meeting on 21.9.01

02/03/2022 Page 6329 of 15326
Project Ref 323/02.4

Date Ackn'd: 18/01/2002

Date Project Ceased: 26/11/2010

CU2 Project Title: CLONING AND EXPRESSION OF AN INHIBITOR OF APOPTOSIS PROTEIN USING AMPHOTROPIC RETROVIRAL MEDIATED GENE TRANSFER AND OTHER EXPRESSION METHODS.

Class: 2

Consent Granted: not applicable

Project notified under transitional arrangements: N

Purposes of the contained use:

To produce the GMOs expressing the protein for target validation and for use in screening compounds in the process of drug discovery

Recipient or parental organism:

Cloning host - E. coli K12:-
DH5a - a disabled host. ACDP Hazard Group 1
Top 10 - a disabled host. ACDP Hazard Group 1
Other E. coli K12 strains. ACDP Hazard Group 1
These strains are not considered pathogenic or able to persistently colonise humans or animals. They have limited survivability in the environment. They have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. There are no plasmid mobilisation/transfer genes in these hosts.

Expression hosts
- Mammalian cell lines eg RAW, HEKs ACDP Hazard Group 2
- Human primary cells ACDP Hazard Group 2
- Insect cell lines - especially disabled hosts
- E. coli K12 strain - a disabled host
- S. cerevisiae - an especially disabled host

Host/vector system
All cloning vectors used will be non-mobilisable or mobilisation defective. These all contain antibiotic resistance genes to allow selection. Resistance to this is common and of limited clinical significance.

- Vector System No. 1 Amphotropic Replication Deficient Retroviruses (RDRs). They all include an antibiotic resistance gene to allow selection.
- Vector System No. 2 Mammalian expression vectors. They all include an antibiotic resistance gene to allow selection.
- Vector System No. 3 Baculovirus expression system.
- Vector System No. 4 E. coli expression vectors. These vectors are either non-mobilisable or mobilisation defective. They all include an antibiotic resistance gene to allow selection.
- Vector System No. 5 Yeast expression vectors. These vectors are non-transferable. They all contain selection markers.

Origin & function
Origin of cline is from a cDNA library. The intention is to express the protein of mutants thereof, in primary cells or cell lines, to determine the function of the protein.

Evaluation of foreseeable effects
The gene of interest encodes the sequence of an inhibitor of apoptosis. On expression it is anticipated that in some of the listed expression systems the protein will be correctly folded and active. The gene of interest is not a known oncogene, but may potentially be oncogenic.

Expression of the insert in the recombinant organisms is unlikely to affect the pathogenicity of the expression hosts listed above. Contact of the replication deficient retroviruses (RDRs) containing an inhibitor of apoptosis gene should be avoided, as transduction of an inhibitor of apoptosis protein into the cells of a worker could result in an unknown effect.

The RDRs are high labile and are highly unlikely to transfer this sequence to either humans or other organisms because of the containment measures used.

In the worst-case scenario, a replication competent amphotropic retrovirus (RCR) capable of expressing a functional inhibitor of apoptosis protein is produced that productively infects an individual via body fluid contact (eg sharps injury). The individual therefore would be infected with a potentially damaging retrovirus with unknown effects. The virus would be transmissible by body fluid contact.

The likelihood of this happening is negligible because:
1. The retroviral vectors themselves do not contain any of the functions (gag, pol and env) that are essential for replication. Integration of the vectors results in their inactivation due to a deletion of the 3’ LTR. All of these functions must be restored in order to produce a RCR.
2. Capture events are most likely by homologous recombination with wild type retrovirus. As the homologous regions flank the engineered region of the virus containing the inhibitor of apoptosis gene, homologous recombination would result in deletion of the inhibitor of apoptosis gene.
3. As the tropism of any PCR produced would be dictated by the endogenous retrovirus providing the gag, pol and env functions, homologous recombination with wild type retrovirus would produce a virus which would not pose a significant risk to human health.
4. The RDRs are going to be used to transduce primary human cells and therefore may come into contact with endogenous human retroviruses. However, as the RDRs are based upon a non-primate retrovirus the chance of homologous recombination to produce an RCR is greatly reduced.

5. Endogenous retroviral infection of the transduced cells could provide the functions required to re-package viral genomes in trans. The use of self-inactivating vectors means that following integration of the RDR DNA, the normal retrovirus 5' - LTR promoter is silenced. Expression of the inhibitor of apoptosis protein is driven from a promoter that is downstream of the packaging sequence. Hence, all transcripts produced will be incapable of being packaged. There is no evidence to suggest that the deleted 5’ LTR has promoter activity.

6. Any PCR produced would be expected to be highly unstable and could be attenuated by contact with surfaces, normal disinfectants and desiccation. The RDRs are not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture supernatant on an open wound. The use of sharps will be prohibited, the use of gloves will be mandatory and the generation of aerosols minimised.

A more likely scenario is that a worker contaminates himself or herself with an RDR containing the inhibitor of apoptosis gene. This may result in an increase or a decrease in apoptosis signalling pathways in the transformed cells. However, the RDRs are highly unstable, attenuated by desiccation and not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as needle stick injury or spilling culture supernatant on an open wound. The use of sharps will be prohibited, the use of gloves will be mandatory and the generation of aerosols minimised.

The likelihood of harm to human health occurring is considered negligible due to the containment measures used.

As the survival of the vectors in the general environment is poor and given that the work will be carried out at CL2, the risk of animal exposure is therefore very small. Thus, the overall risk to the environment is negligible.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are "White Rose Ltd" and have been registered with the HSE. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain. There will be 100% kill.

All liquid waste is inactivated by chemical means using concentrated disinfectants, either Hycolin solution (Coventry Chemicals Ltd) for E. coli and yeast work or TriGene (MediChem International) for tissue culture/RDR work, to a final concentration of 5% (as recommended by both suppliers) or Virkon for insect cell/baculovirus work to a final concentration of 1%. The solutions are left for 2 hours before disposal via the drain together with copious amounts of water.

Hycolin has been validated using the BS:6905/87 as the modified Kelsey Sykes test, the Australian TGA test, MAFF, ES and MIC tests. Hycolin is lethal against all common gram-positive pathogens, all gram-negative pathogens and mycobacterium species. Hycolin has also been tested in house under conditions used for decontamination.

TriGene has been validated using BS6471, BS EN 1276, NF 72-150, NF 72-200, 72-180 and is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. Trigene has also been tested in house under conditions used for decontamination.

Virkon has been validated in house under conditions used for decontamination.
As a result of treatment no viable GMMs from liquid or solid waste will remain, there will be 100% kill.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

This proposal has been approved as risk class 2. It was finally approved after minor modification at the GMSC meeting on 21.09.01.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Conf L3 L4 L2 L3 L4</td>
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Project Ref 323/02.5

Date Ackn'd 03/09/2002

CU2 Project Title ADENOVIRAL DELIVERY AND EXPRESSION OF A RGS PROTEIN (A) IN HUMAN PRIMARY CELLS

Class 2 CultureVolClass2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Historical Significant Changes

Tick if notifying a connected programme of work Y
**Project Additional Information**

**Purposes of the contained use**
To produce the GMOs expressing the protein for target validation and for use in the process of drug discovery

**Recipient or parental organism**

**CLONING HOST**
Packaging cell line for replication deficient adenovirus. This packaging cell line has been designed for use with the adenoviral vectors covered by this proposal. The cells complement for two adenoviral gene cassettes essential for adenovirus replication that have been deleted in the vectors. No sequence overlap exists between the packaging cell line and the adenoviral vectors preventing homologous recombination and making the emergence of replication competent adenovirus extremely unlikely (see Section 17 for more info on this system)

**EXPRESSION HOSTS**
Human primary cells ACDP Hazard Group (see Section 17 for more info) The cells are non-transformed. No adventitious agents are believed present. For COSHH purposes they should be treated as risk class 2. Cells are from an external source; individuals must not culture their own cells.

**Host/vector system**

**VECTOR SYSTEM:** Replication Deficient Adenovirus (RDAs). The adenoviral genome has been divided between two vectors (adapter and helper). The adapter plasmid contains an inverted terminal repeat (containing the origin of replication) and the packaging signal (a series of AT-rich sequences). An essential adenoviral cassette has been deleted and replaced by the gene of interest under the control of a promoter. The helper cosmid contains the remainder of the adenoviral genome with some adenoviral gene cassettes removed. Two of the deleted adenoviral gene cassettes are essential for replication hence the resulting adenovirus is replication deficient. (see Section 17 for more info on this system)

**Origin & function**
Origin of cline is from a cDNA library. The intention is to express the protein in primary cells, to determine the function of the protein.

**Evaluation of foreseeable effects**
The gene of interest encodes the sequence of an RGS protein. RGS proteins (Regulator of G protein Signalling) strongly modulate the activity of G proteins. Their best known function is to inhibit G protein signalling by accelerating GTP hydrolysis (acting as GTPase activating proteins, GAP) by stabilising the transition state conformation.
of G-alpha, thus turning off G protein signals. Some RGS proteins also possess non-GAP functions. Evidence is emerging that besides G protein inhibition, they can enhance G protein activation by serving as effectors or acting as scaffold proteins to gather other regulatory molecules together. When RGS binds activated Galpha it brings with it other functional units. There are also many examples of regulated RGS expression. RGS levels can inhibit rapid induction following physiological signals. Additionally, some RGS proteins exhibit nuclear localisation when overexpressed, suggesting a role in gene activation. It is anticipated that the protein will be correctly folded and active. The gene of interest is not a known oncogene.

Expression of the insert in the recombinant organisms is unlikely to affect the pathogenicity of the expression hosts listed above. Contact of the replication deficient adenovirus (RDAs) containing the RGS gene should be avoided, as transduction of RGS into the cells of a worker could result in an unknown effect. The most likely effect is perturbation of G-protein signalling.

The RDAs are transmissible by aerosol and fluid:fluid contact and are highly unlikely to transfer this sequence to either humans or other organisms because of the containment measures used.

In the worst-case scenario, a replication competent adenovirus (RCA) capable of expressing functional RGS is produced that productively infects an individual via body fluid contact (eg sharps injury) or aerosol inhalation. The individual therefore would be infected with a potentially damaging adenovirus expressing RGS protein with unknown effects. The virus would be transmissible by body fluid contact.

The likelihood of this happening is negligible because two adenoviral gene cassettes essential for replication have been deleted in the adenoviral vectors. Production of replication-defective adenovirus requires the use of a specialised packaging cell line which complements for these essential adenoviral genes. No sequence overlap exists between the packaging cell line and the adenoviral vectors preventing homologous recombination and making the emergence of replication-competent Adenovirus extremely unlikely. If a member of staff is naturally infected with wild-type adenovirus this may provide an opportunity for complementation of the deleted gene cassettes enabling the generation of RDA in the host. However, recombinant adenovirus can only become replication-competent by exchange of both complete expression cassettes, requiring two homologous recombination events. (see Section 17 for more details).

A more likely scenario is that a worker contaminates himself or herself with a replication deficient adenovirus (RDA) containing RGS protein. The likelihood of harm to human health occurring is considered low because:

(1) As with naturally occurring adenovirus, the adenoviral constructs covered by this proposal will elicit a host immune response. This poses a potential hazard if delivered in high quantities. This has been highlighted by a fatality in a gene therapy trial (Science 286 p2244) following the injection of a massive dose of crippled adenovirus (38 trillion virus particles) into the liver. However, the risk to staff is negligible because such large quantities of adenovirus would never be handled, equivalent to over 3L of RDA stock (5-10 million virus particles per microlitre, stored in 0.25 mL aliquots).

(2) Replication-deficient adenoviral particles are able to transduce human cells (particularly airway epithelial and gastrointestinal cells). However, these viruses will induce both a humoral and cellular immune response which will lead to targeted elimination of infected cells (see Section 17 for more details). This means that protein expression will be very short-lived. Hence any potential harmful effects associated with RGS protein expression will be transient and will not present a long-term problem.

(3) Transduction is possible through direct fluid-fluid contact and so risk is via entry through open wounds. This presents negligible risk since the use of sharps will be prohibited and as part of standard laboratory practice open wounds will be covered.

(4) Adenovirus is transmissible by aerosol so the highest risk of exposure is via entry through airways and transduction of airway epithelial cells. However, the viral DNA will not be replicated and is not directly integrated into the host genome. Since airway epithelial cells exhibit a high rate of cell turnover any viral DNA will be lost over time and transgene expression will be transient. Following aerosol exposure to RDA it is possible that the viral DNA could be replicated and packaged generating more virions if the host is already infected with naturally occurring adenovirus of the same serotype. However, such virions will be replication-deficient. This could lead to transduction of neighbouring cells and localised spreading of transgene expression. Transduced cells are more likely to be targeted and removed by the host immune response, restricting the spread of RDA. Overexpression is expected to attenuate G-protein signalling. However, the overall effect on cell function is unknown. It is also possible that high levels of RGS protein may affect other signalling pathways in epithelial cells normally controlled by other RGS proteins. The overall risk to human health is considered negligible because the likelihood of exposure to aerosols is negligible due to the containment measured used.
Over-expression of an RGS protein in a cell type in which it is endogenously expressed may perturb normal G-protein signalling. Transduction of cells found circulating in the blood requires fluid:fluid contact via sharps injury or open wounds. This represents negligible risk since sharps are prohibited and open wounds will be covered. Transduction of cells present in the gut requires ingestion of inhalation of virions. However, the risk of exposure leading to transduction is negligible since the vast majority of virions would be inactivated during passage through the stomach. Aerosol exposure may risk transduction of cells located within the lung tissue. However, for this to be possible the RDA would need to breach the epithelial barrier. Moreover, since gene expression will be both transient and localised, accidental exposure is unlikely to have a significant impact on the health of an individual. However, since this cannot be ruled out this presents low risk to the user (for more information see Section 17).

Adenovirus does not directly integrate the transgene into the host genome, however random integration is possible but poses no significant risk since this is a rare event. The risk of potential harm is only significant if the transgene is oncogenic. The RGS protein of interest (a) is not expected to be oncogenic and so random integration poses negligible risk.

There is no evidence that human adenovirus can naturally infect animals, and replication is very limited. However, replication has been shown to occur in the lungs of experimentally infected cotton rats (not indigenous to the UK) administered at high doses. Given that the work will be carried out at CL2, the risk of animal exposure is therefore very small. Thus, the overall risk to the environment is negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**None**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**N/A**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are "White Rose Ltd" and have been registered with the HSE. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain. There will be 100% kill.

All liquid waste is inactivated by chemical means using the concentrated disinfectant TriGene (MediChem International) to a final concentration of 5% (as recommended by supplier). The solutions are left for 2 hours before disposal via the drain together with copious amounts of water.

TriGene has been validated using BS6471, BS EN 1276, NF 72-150, NF 72-200, 72-180 and is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. Trigene has also been tested in house under conditions used for decontamination.

As a result of treatment no viable GMMs from liquid or solid waste will remain, there will be 100% kill.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

Y
This proposal was discussed and approved as Risk Class 2 at the GMSC on 26/3/02 subject to minor amendments. These amendments have been included.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L3</td>
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**Project Ref** 323/02.6

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- Project notified under transitional arrangements: N
- Withdrawn: N
- Tick if notifying a connected programme of work: Y

**Historical Significant Changes**

- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

This proposal will cover the use of replication deficient adenoviral (RDA) particles to express the cell surface protein heat stable antigen HSA (mCD24). The purpose of these experiments is to allow selection of virally transduced cells using antibodies against HSA.

**Recipient or parental organism**

CLONING HOST
Packaging cell line for replication deficient adenovirus. This packaging cell line has been designed for use with the adenoviral vectors covered by this proposal. The cells complement for two adenoviral gene cassettes essential for adenovirus replication that have been deleted in the vectors. No sequence overlap exists between the packaging cell line and the adenoviral vectors preventing homologous recombination and making the emergence of replication competent adenovirus extremely unlikely (see Section 17 for more info on this system).

EXPRESSION HOSTS
Human primary cells ACDP Hazard Group (see Section 17 for more info) The cells are non-transformed. No adventitious agents are believed present. For COSHH purposes they should be treated as risk class 2. Cells are from an external source; individuals must not culture their own cells.

Host/vector system

VECTOR SYSTEM: Replication Deficient Adenovirus (RDAs). The adenoviral genome has been divided between two vectors (adapter and helper). The adapter plasmid contains an inverted terminal repeat (containing the origin of replication) and the packaging signal (a series of AT-rich sequences). An essential adenoviral cassette has been deleted and replaced by the gene of interest under the control of a promotor. The helper cosmid contains the remainder of the adenoviral genome with some adenoviral gene cassettes removed. Two of the deleted adenoviral gene cassettes are essential for replication hence the resulting adenoviral is replication deficient. (see Section 17 for more info on this system)

Origin & function

Origin of cline is from a cDNA library. The intention is to express the protein in primary cells, to determine allow selection of transduced cells.

Evaluation of foreseeable effects

HSA. Heat stable antigen (HSA) was cloned originally from mouse (J. Immunology 145 p1952). At the DNA level, it is most homologous to human CD24, although the mature product products demonstrate limited homology. The mature HSA is a peptide of 27 amino acids, heavily glycosylated, and attached to the cell membrane with a GPI anchor. In the mouse, HSA is expressed on mature granulocytes, thymocytes and B-cells. Expression is generally high in precursor cells, and lost as cells terminally differentiate. Cross-linking of HSA leads to an increase in apoptosis in B-cell precursors. Transgenic mice overexpressing HSA have reduced pre-B-lymphocytes (J. Immunology 156 pp479-88). Cross-linking of HSA can promote activation and proliferation of T-cells. HSA can promote cell adhesion, by binding to P-selectin. 2. In many cases a fluorescent protein will be co-expressed with HSA. It is anticipated that the protein(s) will be correctly folded and active. Neither HSA or the fluorescent protein are known oncogenes, or predicted to be oncogenic.

Expression of HSA in the recombinant organisms is unlikely to affect the pathogenicity of the expression hosts listed above. Contact of the replication deficient adenovirus (RDAs) containing the HSA gene should be avoided, as transduction of HSA into the cells of a worker could result in an unknown effect, although since this is a mouse protein with significant differences at the amino acid level to its closest human homologue, it is unlikely to have functional effects in humans.

The RDAs are transmissible by aerosol and fluid:fluid contact and are highly unlikely to transfer this sequence to either humans or other organisms because of the containment measures used.

In the worst-case scenario, a replication competent adenovirus (RCA) capable of expressing functional RGS is produced that productively infects an individual via body fluid contact (eg sharps injury) or aerosol inhalation. The individual therefore would be infected with a potentially damaging adenovirus expressing RGS protein with unknown effects. The virus would be transmissible by body fluid contact.

The likelihood of this happening is negligible because two adenoviral gene cassettes essential for replication have been deleted in the adenoviral vectors. Production of replication-defective adenovirus requires the use of a specialised packaging cell line which complements for these essential adenoviral genes. No sequence overlap exists between the packaging cell line and the adenoviral vectors preventing homologous recombination and making the emergence of replication-competent Adenovirus extremely unlikely. If a member of staff is naturally infected with wild-type adenovirus this may provide an opportunity for complementation of the deleted gene cassettes enabling the generation of RDA in the host. However, recombinant adenovirus can only become replication-competent by exchange of both complete expression cassettes, requiring two homologous recombination events. (see Section 17 for more details).
Amore likely scenario is that a worker contaminates himself or herself with a replication deficient adenovirus (RDA) containing RGS protein. The likelihood of harm to human health occurring is considered low because:

1. As with naturally occurring adenovirus, the adenoviral constructs covered by this proposal will elicit a host immune response. This poses a potential hazard if delivered in high quantities. This has been highlighted by a fatality in a gene therapy trial (Science 286 p2244) following the injection of a massive dose of crippled adenovirus (38 trillion virus particles) into the liver. However, the risk to staff is negligible because such large quantities of adenovirus would never be handled, equivalent to over 3L of RDA stock (5-10 million virus particles per microlitre, stored in 0.25 mL aliquots).

2. Replication-deficient adenoviral particles are able to transduce human cells (particularly airway epithelial and gastrointestinal cells). However, these viruses will induce both a humoral and cellular immune response which will lead to targeted elimination of infected cells (see Section 17 for more details). This means that protein expression will be very short-lived. Hence any potential harmful effects associated with RGS protein expression will be transient and will not present a long-term problem.

3. Transduction is possible through direct fluid-fluid contact and so risk is via entry through open wounds. This presents negligible risk since the use of sharps will be prohibited and as part of standard laboratory practice open wounds will be covered.

4. Adenovirus is transmissible by aerosol so the highest risk of exposure is via entry through airways and transduction of airway epithelial cells. However, the viral DNA will not be replicated and is not directly integrated into the host genome. Since airway epithelial cells exhibit a high rate of cell turnover any viral DNA will be lost over time and transgene expression will be transient. Following aerosol exposure to RDA it is possible that the viral DNA could be replicated and packaged generating more virions if the host is already infected with naturally occurring adenovirus of the same serotype. However, such virions will be replication-deficient. This could lead to transduction of neighbouring cells and localised spreading of transgene expression. Transduced cells are more likely to be targeted and removed by the host immune response, restricting the spread of RDA.

5. Overexpression of HSA in airway epithelial cells represents low risk to the user, since studies in mouse have limited the function of HSA to signalling in pre-B-lymphocytes. These are located in the bone marrow which will not be infected from aerosol exposure. In addition, the amino acid sequence of mouse HSA is very different from its human gene homologue, and so is unlikely to be functional in humans, and will most likely be recognised as foreign and be cleared rapidly by normal immune responses. In addition, the likelihood of exposure to aerosols is insignificant due to the containment measured used, so the danger to humans will be negligible.

There is no evidence that human adenovirus can naturally infect animals, and replication is very limited. However, replication has been shown to occur in the lungs of experimentally infected cotton rats (not indigenous to the UK) administered at high doses. Given that the work will be carried out using replication incompetent virus at CL2, the risk of animal exposure is therefore very small. Thus, the overall risk to the environment is negligible.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are "White Rose Ltd" and have been registered with the HSE. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain. There will be 100% kill.

All liquid waste is inactivated by chemical means using the concentrated disinfectant TriGene (MediChem International) to a final concentration of 5% (as recommended by supplier). The solutions are left for 2 hours before disposal via the drain together with copious amounts of water.
TriGene has been validated using BS6471, BS EN 1276, NF 72-150, NF 72-200, 72-180 and is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. Trigene has also been tested in house under conditions used for decontamination.

As a result of treatment no viable GMMs from liquid or solid waste will remain, there will be 100% kill.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Approved by GMSC on 14.5.02 subject to minor modifications. Modifications have been incorporated.

Project Containment

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<th>Glass Houses</th>
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Project Ref 323/03.1

Date Ackn’d 04/02/2003
CU2 Project Title ADENO VIRAL DELIVERY AND EXPRESSION OF A G PROTEIN-COUPLED RECEPTOR IN HUMAN PRIMARY CELLS AND CELL LINES
Date Project Ceased 26/11/2010
Class 2
CultureVolClass2 < 1 litre
Consent Granted not applicable
Non-GMM
Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

To produce the GMOs expressing the protein for target validation and for use in screening compounds in the process of drug discovery.

**Recipient or parental organism**

**Cloning Host**

Packaging cell line for replication deficient adenovirus. This packaging cell line has been designed for use with the adenoviral vectors covered by this proposal. The cells complement for two adenoviral gene cassettes essential for adenovirus replication that have been deleted in the vectors. No sequence overlap exists between the packaging cell line and the adenoviral vectors preventing homologous recombination and making the emergence of replication competent adenovirus extremely unlikely.

**Expression hosts**

Human primary cells ACDP Hazard Group 2. The cells are non-transformed. No adventitious agents are believed present. For COSHH purposes they should be treated as risk class 2. Cells are from an external source; individuals must not culture their own cells.

**Host/vector system**

Vector system: Replication Deficient Adenovirus (RDAs). The adenoviral genome has been divided between two vectors (adapter and helper). The adapter plasmid contains an inverted terminal repeat (containing the origin of replication) and the packaging signal (a series of AT-rich sequences). An essential adenoviral cassette has been deleted and replaced by the gene of interest under the control of a promoter. The helper cosmid contains the remainder of the adenoviral genome with some adenoviral gene cassettes removed. Two of the deleted adenoviral gene cassettes are essential for replication hence the resulting adenoviral is replication deficient.

**Origin & function**

Origin of cline is by PCR from genomic DNA. The intention is to express the protein or mutants thereof, in parimary cells, to determine the function of the protein.

**Evaluation of foreseeable effects**

The protein of interest is a G protein-coupled (7TM) receptor. Its activating ligand is unknown. The second messenger responses generated by activating the receptor and the effects of these on a cell are not known. Expression of the GPCR mRNA in the human is restricted; Activation of the GPCR is not expected to occur in the absence of the (unknown) ligand. Expression of the GPCR may alter normal cell function in the presence of the activating ligand.

It is anticipated that the protein will be correctly folded and active. The gene of interest is not a known oncogene.

Expression of the insert in the recombinant organism is unlikely to affect the pathogenicity of the expression hosts listed above. Contact of the replication deficient adenovirus (RDAs) containing the GPCR gene should be avoided as transduction of the GPCR into the cells of a worker could result in an unknown effect.

The RDAs are transmissible by aerosol and fluid/fluid contact and are highly unlikely to transfer this sequence to either humans or other organisms because of the
containment measures used.

In the worst-case scenario, a replication competent adenovirus (RCA) capable of expressing functional GPCR is produced that productively infects an individual via body fluid contact (eg sharps injury) or aerosol inhalation. The individual therefore would be infected with a potentially damaging adenovirus expressing the GPCR protein with unknown effects. The virus would be transmissible by body fluid contact.

The likelihood of this happening is negligible because two adenoviral gene cassettes essential for replication have been deleted in the adenoviral vectors. Production of replication-defective adenovirus requires the use of a specialised packaging cell line which complements for these essential adenoviral genes. No sequence overlap exists between the packaging cell line and the adenoviral vectors preventing homologous recombination and making the emergence of replication-competent Adenovirus extremely unlikely. If a member of staff is naturally infected with wild-type adenovirus this may provide an opportunity for complementation of the deleted gene cassettes enabling the generation of RDA in the host. However, recombinant adenovirus can only become replication-competent by exchange of both complete expression cassettes, requiring two homologous recombination events.

A more likely scenario is that a worker contaminates himself or herself with a replication deficient adenovirus (RDA) containing the GPCR protein. The likelihood of harm to human health occurring is considered low because:

1. As with naturally occurring adenovirus, the adenoviral constructs covered by this proposal will elicit a host immune response. This poses a potential hazard if delivered in high quantities. This has been highlighted by a fatality in a gene therapy trial (Science 286 p2244) following the injection of a massive dose of crippled adenovirus (38 trillion virus particles) into a liver. However, the risk to staff is negligible because such large quantities of adenovirus would never be handled.

2. Replication-deficient adenoviral particles are able to transduce human cells (particularly airway epithelial and gastrointestinal cells). However these viruses will induce both a humoral and cellular immune response which will lead to targeted elimination of infected cells. This means that GPCR protein expression will be very short-lived. Hence any potential harmful effects associated with the GPCR protein expression will be transient and will not present a long-term problem.

3. Transduction is possible through direct fluid-fluid contact and so risk is via entry through open wounds. This presents negligible risk since the use of sharps will be prohibited and as part of standard laboratory practice open wounds will be covered.

4. Adenovirus is transmissible by aerosol so the highest risk of exposure is via entry through airways and transduction of airway epithelial cells. However, the viral DNA will not be replicated and is not directly integrated into the host genome. Since airway epithelial cells exhibit a high rate of cell turnover any viral DNA will be lost over time and transgene expression will be transient. Following aerosol exposure to RDA it is possible that the viral DNA could be replicated and packaged generating more virions if the host is already infected with naturally occurring adenovirus of the same serotype. However, such virions will be replication-deficient. This could lead to transduction of neighbouring cells and localised spreading of transgene expression. Transduced cells are more likely to be targeted and removed by the host immune response, restricting the spread of RDA. The effect of expression of the GPCR in airway epithelial cells is unknown. It is considered unlikely to cause harm to the user because the GPCR is not endogenously expressed by this cell, therefore the relevant signalling/effector proteins and ligand may be absent.

5. Transduction of cells present in the gut requires ingestion or inhalation of virions. However, the risk of exposure leading to transduction is negligible since the vast majority of virions would be inactivated during passage through the stomach.

6. Transduction of cells found circulating in the blood requires fluid:fluid contact via sharps injury or open wounds. This represents negligible risk since sharps are prohibited and open wounds will be covered.

7. Adenovirus does not directly integrate the transgene into the host genome, however random integration is possible but poses no significant risk since this is a rare event. The risk of potential harm is only significant if the transgene is oncogenic. The GPCR is not known to be oncogenic.
Aerosol exposure may risk transduction of cells located within the lung tissue. However, for this to be possible the RDA would need to breach the epithelial barrier. Moreover, since gene expression will be both transient and localised, accidental exposure is unlikely to have a significant impact on the health of an individual. However, since this cannot be ruled out this presents low risk to the user.

There is no evidence that human adenivirus can naturally infect animals, and replication is very limited. However, replication has been shown to occur in the lungs of experimentally infected cotton rats (not indigenous to the UK) administered at high doses. Given that the work will be carried out at CL2, the risk of animal exposure is therefore very small. Thus, the overall risk to the environment is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are "White Rose Ltd" and have been registered with the HSE. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain. There will be 100% kill.

All liquid waste is inactivated by chemical means using the concentrated disinfectant TriGene (MediChem International) to a final concentration of 5% (as recommended by supplier). The solutions are left for 2 hours before disposal via the drain together with copious amounts of water.

TriGene has been validated using BS6471, BS EN 1276, NF 72-150, NF 72-200, 72-180 and is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. TriGene has also been tested in house under conditions used for decontamination.

As a result of treatment no viable GMMs from liquid or solid waste will remain, there will be 100% kill.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This proposal was approved by the GMSC on 12.8.02 as risk class 2.

**Project Containment**
Project Ref 323/03.2

Date Ackn'd 14/10/2003
Date Project Ceased 26/11/2010

CU2 Project Title MODULATION OF A TARGET PROTEIN EXPRESSION IN HUMAN CELL-LINES USING A GENE MODULATOR EXPRESSED BY RETROVIRAL VECTORS.

Class 2
CultureVolumeClass2 < 1 litre
Non-GMM not applicable

Project notified under transitional arrangements N

Withdrawn N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
To produce the GMOs expressing modulatory proteins for target validation in the process of drug discovery.

Recipient or parental organism

Cloning host
E. coli K12; Top 10 - a disabled host. ACDP Hazard Group 1. This strain is not considered pathogenic or able to persistently colonise humans or animals. It has limited survivability in the environment. It has auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. There are no plasmid mobilisation/transfer genes in this host.

Expression hosts
Mammalian cell lines ACDP Hazard Group 2. Considered as especially disabled hosts with very limited ability to survive in the environment.
Host/vector system

All cloning vectors used will be non-mobilisable or mobilisation defective. These all contain antibiotic resistance genes to allow selection. Resistance to these are common and of limited clinical significance.

Amphotropic, Replication Deficient Retroviral vectors (RDRs) based on MMLV. They all include an antibiotic resistance gene to allow selection.

Origin & function

Insert DNA will be designed and supplied by an external company. Intended function is to express the encoded protein to modulate the gene of interest in mammalian cells.

Evaluation of foreseeable effects

Encoded protein is expected to specifically modulate the gene of interest. Thus potential effects of expression relate to the gene of interest and are detailed in Section 17.

Expression of the inserts in the recombinant organism is unlikely to affect pathogenicity.

The RDRs are highly labile and are highly unlikely to transfer the insert sequence to either humans or other organisms because of the containment measures used.

In the worst-case scenario, a replication competent amphotropic retrovirus, capable of expressing functional insert protein, is produced that productively infects an individual. The individual therefore would be infected with a potentially damaging retrovirus. The virus would be transmissible by body fluid contact.

The likelihood of this happening is negligible because:

1. The retroviral vectors themselves do not contain any of the functions (gag, pol and env) that are essential for replication. Integration of the vectors results in their inactivation due to a deletion in the 3' LTR. All of these functions must be restored in order to produce a replication competent retrovirus.

2. Capture events are most likely by homologous recombination with wild type retrovirus. As the homologous regions flank the engineered region of the virus, homologous recombination would result in deletion of the insert gene.

3. As the tropism of any replication competent retrovirus produced would be dictated by the endogenous (murine) retrovirus providing the gag, pol and env functions, homologous recombination with wild type (murine) retrovirus would produce a virus which would not pose a significant risk to human health.

4. The RDRs are based upon a murine retrovirus the chance of homologous recombination to produce a replication competent retrovirus is greatly reduced.

5. Endogenous retroviral infection of the transduced cells might provide the functions required to re-package viral genomes in trans. The use of self-inactivating vectors means that following integration of the RDR DNA, the normal retrovirus 5' LTR promoter is silenced. Expression of the insert is driven from a promoter that is downstream of the packaging sequence. Hence, all transcripts produced will be incapable of being packaged. There is no evidence to suggest that the deleted 5' LTR has promoter activity.

5. If a replication competent retrovirus was produced it would be expected to be unstable and could be attenuated by contact with surfaces, normal disinfectants and desiccation. Retroviruses are not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture supernatant on an open wound. The use of sharps will be prohibited, the use of gloves will be mandatory and the generation of aerosols minimized.

A more likely scenario is that a worker contaminates himself or herself with a replication defective retrovirus (RDR) containing the insert gene. This may result in expression of protein in the transduced cells. However, the RDRs are highly unstable, attenuated by desiccation and not known to be transmissible by aerosol. Transmission would therefore have to be through direct fluid/fluid contact such as a needle stick injury or spilling culture supernatant on an open wound. The use of sharps will be prohibited.
the use of gloves will be mandatory and the generation of aerosols minimized.

The likelihood of harm to human health occurring is considered negligible due to the containment measures used.

As the survival of the vectors in the general environment is poor and given that the work will be carried out at CL2, the risk of animal exposure is very small. Thus, the overall risk to the environment is negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

None

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid GMM contaminated waste will be triple contained in biohazard waste bags/containers and suitably labelled and incinerated off site. The contractors who will carry this out are "White Rose Ltd" (registered with the HSE). This solid waste will be comprised mainly of plasticware used in the culturing of the organisms. As a result of treatment no viable GMMs from solid waste will remain. There will be 100% kill.

All liquid waste is inactivated by chemical means using concentrated disinfectants, either Hycolin solution (Coventry Chemicals Ltd) for E. coli work or TriGene (MediChem International) for tissue culture/RDR work, to a final concentration of 2% (as recommended by both suppliers). The solutions are left overnight before disposal via the drain together with copious amounts of water.

Hycolin has been validated using the BS:6905/87 as the modified Kelsey Sykes test, the Australian TGA test, MAFF, ES and MIC tests. Hycolin is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species.

TriGene has been validated using BS6471, BS EN 1276, NF 72-150, it is bactericidal and virucidal and efficiently kills eukaryotic cell lines. Trigene has also been tested in house under conditions used for decontamination. As a result of treatment no viable GMMs from liquid or solid waste will remain, there will be 100% kill.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

Y

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

Y

**Please enter comments on the GM safety committee on the risk assessment**

This proposal was approved by the GMSC on 22/7/03 as risk class 2.

**Project Containment**
### Project Ref 323/04.1

- **Date Ackn'd**: 20/10/2004
- **CU2 Project Title**: Cloning of mammalian pro-forms of two cytokines and the mature cytokines and their expression in E. coli
- **Class**: Class 2
- **Culture Volume Class**: 1 Litre
- **Non-GMM**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

#### Purposes of the contained use

To produce the GMOs expressing the proteins for use in the process of drug discovery

#### Recipient or parental organism

**Cloning hosts**
- E. coli K12 -
- Top 10 - a disabled host. ACDP Hazard Group 1
- Other E. coli K12 strains. ACDP Hazard Group 1

These strains are not considered pathogenic or able to persistently colonise humans or animals. They have limited survivability in the environment. They have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. There are no plasmid mobilisation/transfer genes in these hosts.
Expression hosts
E. coli B834 (DE3) strain - a disabled host. ACDP Hazard Group 1
This strain is an auxotrophic variant of BL21 and is therefore more disabled than BL21. According to HSE guidelines BL21 has considerably less colonisation potential than wild type strains but has greater colonisation potential than K12 strains. BL21 may be considered unlikely to colonise and establish a persistent infection in the gut of a healthy individual.

Host/vector system
All cloning and expression vectors used will be non-mobilisable. These all contain antibiotic resistance genes to allow selection in E. coli. Resistance to these are common and of limited clinical significance.

Cloning vectors:-
Plasmids pCR2-topo & pCR4blunt-topo
E. coli Expression system. Please see section 17 for details of the expression vector.

Origin & function
Origin of the clones will be via PCR amplification from tissue cDNA or a cDNA library. Intended function is to express the clone in order to determine the effect of the ligand on human cell lines and primary cells.

Evaluation of foreseeable effects
The genes of interest encode the sequence of two cytokines. As the genes of interest are both pro-inflammatory cytokines, the abnormal production of either protein or their pro-forms in bacteria and subsequent release is likely to cause a localised inflammation with the other potential side-effects as shown in the clinical trials depending on the amount of cytokine release.

An expression in E. coli it is anticipated that the protein will be correctly folded and active. The gene of interest is not a known oncogene. The DNA constructs would be designed in such a way that none of the cytokine proteins would be secreted from intact viable E. coli ie in the mature cytokine constructs there is no secretary leader sequence and the pro-forms of the cytokine proteins lack the correct and necessary E. coli signal sequences for the protein to be secreted from these cells.

For harm to occur, the E. coli cells expressing the cytokine must be introduced into the body via (a) ingestion or (b) sharps injury. In the first instance (a) the cells introduced are likely to express the cytokine as the subject is likely to have ingested some food containing the inducer of expression. However the E. coli cells need to lyse, releasing the protein. In the process of lysing, the cells that produced the cytokine proteins will be killed. This lysis of E. coli cells could result in the release of varying amounts of cytokine depending on the number of cells ingested. It is known that both cytokines are expressed in E. coli in amounts up to 10-20 mg of active protein per litre. Therefore ingestion of just one ml of induced culture could result in the release of cytokine into the body at concentrations higher than the maximum tolerated dose of a clinical trial. Ingestion is deemed highly unlikely to occur because of the fact that mouth pipetting is not allowed as part of good laboratory practice, gloves will be worn and the production of aerosols minimised. (b) Introduction of the bacteria capable of expressing one of the cytokines via a sharps injury is unlikely to result in harm as the E. coli cells are a non-pathogenic laboratory strain with a long history of safe use. The ability to express the cytokine will not increase their potential to infect a subject and if the cells do get into the blood it is highly unlikely that any extra cytokine protein will be produced as the expression of the proteins is under the control of a very tightly regulated promoter and the inducer will not be present in the blood. Therefore for the cytokine proteins to be released, the cells need to contain the cytokine already before introduction into the body and must lyse releasing the protein. At the site of a sharps injury the subject would respond with a localised inflammation - in the process of healing the wound. The number of cells likely to be introduced into a wound by a sharps injury would be expected to be low. According to the data on phase 1 clinical trials patients dosed sub-cutaneously with either cytokines did not reach the maximum tolerated dose at up to 100ng/kg per patient so to have a severe effect a large number of cells would need to be introduced into the wound. A very severe injury to a worker introducing volumes of culture of 1 ml into a wound is extremely unlikely to occur because of the existing practices in the laboratory - as plasticware rather than glassware is to be used to culture the cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
None
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are "White Rose Ltd" and have been registered with the HSE. This solid waste will be comprised mainly of plastic ware used in the culturing of the organisms and contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain.

All liquid waste is inactivated by applying by chemical means using concentratred disinfectant, Hycolin solution (Coventry Chemicals Limited) to a final concentration of 2% (as recommended by suppliers). The solutions are left for 24 hours before disposal via the drain together with copious amounts of water.

Hycolin has been validated using the BS:6905/87 as the modified Kelsey Sykes test, the Australian TGA test, MAFF, ES and MIC tests. Hycolin is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. Hycolin has been tested in house and shown to cause a 100% kill at working concentrations between 0.25-2%.

As a result of treatment no viable GMMs from liquid or solid waste will remain.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The cytokines described in this assessment will be expressed in an active form in E. coli. It is likely that they will be expressed at levels that could cause harm to a worker if ingested or introduced into a wound in large volumes. This is based on literature descriptions of l.v dosing of the cytokines to human cancer patients where detrimental effects were noticed at relatively low levels of exposure. The maximum tolerated dose for l.v administration was 5ug - a level likely to be present in 1 ml of a bacterial culture. Although it was agreed that to sustain this level of exposure it would require a severe injury to a worker resulting in an open wound, it was felt that the likelihood of this being realised was low rather than negligible. To minimise exposure, the assessment recommends that the wearing of gloves is mandatory and that specific measures are implemented to control aerosol dissemination. These measures take the Containment Level required to 2. In addition, the GMSC recommended that plastic flasks should be used for cultures being grown for expression purposes, as a serious cut from a broken glass flask was viewed as being the most likely source of exposure.

The COSHH risk of handling active cytokines during purification should also be highlighted in the assessment.

The proposal was passed as Risk Class 2 notifiable to the HSE.

Project Containment
<table>
<thead>
<tr>
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<th>Glass Houses</th>
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</tr>
</thead>
<tbody>
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**Project Ref** 323/04.2

**Date Ackn'd** 26/12/2004

**CU2 Project Title** Expression of wild type and dominant negative kinase in mammalian cells using an adenovirus expression system.

**Date Project Ceased** 26/11/2010

**Class** Class 2

**Culture Vol Class** < 1 Litre

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To produce the GMOs expressing the protein for target validation and for use in screening compounds in the process of drug discovery.

**Recipient or parental organism**

**Cloning Host**

Packaging cell line for replication deficient adenovirus. This packaging cell line has been designed for use with the adenoviral vectors covered by this proposal. The cells compliment for two adenoviral gene cassette essential for adenovirus replication that have been deleted in the vectors. No sequence overlap exists between the packaging cell line and the adenovirus vectors preventing homologous recombination and making the emmergence of replication competent adenovirus extremely unlikely.

**Expression Host**

Human primary cells ACDP Hazard Group 2 The cells are non-transformed. No adventitious agents are believed present. For COSHH purposes they should be treated as risk class 2. Cells are from an external source; individuals must not culture their own cells.
Replication Deficient Adenovirus (RDAs). The adenoviral genome has been divided between two vectors (adapter and helper). The adapter plasmid contains and inverted terminal repeat (containing the origin of replication) and the packaging signal (a series of AT-rich sequences). An essential adenoviral cassette has been deleted and replaced by the gene of interest under the control of a promoter. The helper cosmid contains the remainder of the adenoviral genome with some adenoviral gene cassette removed. Two of the deleted adenoviral gene cassettes are essential for replication hence the resulting adenovirus is replication deficient.

Host/vector system

Replication Deficient Adenovirus (RDAs). The adenoviral genome has been divided between two vectors (adapter and helper). The adapter plasmid contains and inverted terminal repeat (containing the origin of replication) and the packaging signal (a series of AT-rich sequences). An essential adenoviral cassette has been deleted and replaced by the gene of interest under the control of a promoter. The helper cosmid contains the remainder of the adenoviral genome with some adenoviral gene cassette removed. Two of the deleted adenoviral gene cassettes are essential for replication hence the resulting adenovirus is replication deficient.

Origin of clone is by PCR from cDNA. The intention is to express the protein or mutants thereof, in primary cells, to determine the function of the protein.

Evaluation of foreseeable effects

The protein of interest is a kinase. It is involved in inactivation of kinase cascades. Over-expression of the dominant negative kinase may EITHER inhibit NF-kB activation, OR have the same ability to activate as wild type kinase. Therefore the over expression of a kinase dominant negative mutant could cause the down regulation potentially leading to increased apoptosis of infected cells exposed to certain stimuli. On the other hand over expression of the dominant negative mutant could cause activation of the same pathways in the cells.

It is anticipated that the protein will be correctly folded and active. The gene of interest is not a known oncogene.

Expression of the insert in the recombinant organisms is unlikely to affect the pathogenicity of the expression hosts listed above. Contact of the replication deficient adenovirus (RDAs) containing the kinase cDNA should be avoided as transduction of the cDNA into the cells of a worker could result in activation of the cells resulting either in a localised inflammation or apoptosis of the transduced cells.

The RDAs are transmissible by aerosol and fluid:fluid contact but are highly unlikely to transfer this sequence to either humans or other organisms because of the containment measures used.

In the worst-case scenario, a replication competent adenovirus (RCA) capable of expressing functional Kinase is produced that productively infects an individual via body fluid contact (e.g. sharps injury) or aerosol inhalation. The individual therefore would be infected with a potentially damaging adenovirus expressing the kinase protein which then causes a localised inflammation. The virus would be transmissible by body fluid contact.

The likelihood of this happening is negligible because two adenoviral gene cassettes essential for replication have been deleted in the adenoviral vectors. Production of replication-defective adenovirus requires the use of a specialised packaging cell line which complements for these essential adenoviral genes. No sequence overlap exists between the packaging cell line and the adenoviral vectors preventing homologous recombination and making the emergence of replication-competent Adenovirus extremely unlikely. If a member of staff is naturally infected this may provide an opportunity for complementation of the deleted gene cassette enabling the generation of RDA in the host. However, recombinant adenovirus can only become replication-competent by exchange of both complete expression cassettes, requiring two homologous recombination events.

A more likely scenario is that a worker contaminates himself or herself with a replication deficient adenovirus (RDA) containing the kinase. The likelihood of harm to human health occurring is considered low because:

1) As with naturally occurring adenovirus, the adenoviral constructs covered by this proposal will elicit a host immune response. This poses a potential hazard if delivered in high quantities. This has been highlighted by a fatality in a gene therapy trial (Science 286 p2244) following the injection of a massive dose of crippled adenovirus (38 trillion virus particles) into the liver. However, the risk to staff is considered negligible because the quantities of particles used in this work will be about 1000x less than this.

2) Replication-deficient adenoviral particles are able to transduce human cells (particularly airway epithelial and gastrointestinal cells). However, these viruses will induce both a humoral and cellular immune response which will lead to targeted elimination of infected cells. This means that kinase protein expression will be very short-lived. Hence any potential harmful effects associated with the kinase protein expression will be transient and will not present a long-term problem.

3) Transduction is possible through direct fluid-fluid contact and so risk is via entry through open wounds. This presents negligible risk since the use of sharps will be prohibited and as part of standard laboratory practice open wounds will be covered.

4) Adenovirus is transmissible by aerosol so the highest risk of exposure is via entry through airways and transduction of airway epithelial cells. However, the viral DNA will not be replicated and is not directly integrated into the host genome. Since airway epithelial cells exhibit a high rate of cell turnover any viral DNA will be lost over time and transgene expression will be transient. Following aerosol exposure to RDA it is possible that the viral DNA could be replicated and packaged generating more virions if the host is already infected with naturally occurring adenovirus of the same serotype. However, such virions will be replication-deficient. This could lead to transduction of neighboring cells and localised spreading of transgene expression. Transduced cells are more likely to be targeted and removed by the host immune response, restricting...
the spread of RDA. The effect of expression of the kinase in airway epithelial cells would be either to cause activation of the cells or apoptosis of the cells.

5) Transduction of cells present in the gut requires ingestion or inhalation of virions. [ ]. However, the risk of exposure leading to transduction is negligible since the vast majority of virions would be inactivated during passage through the stomach.

6) Transduction of cells found circulating in the blood requires fluid:fluid contact via sharps injury or open wounds. This represents negligible risk since sharps are prohibited and open wounds will be covered.

7) Adenovirus does not directly integrate the transgene into the host genome, however random integration is possible but poses no significant risk since this is a rare event. The risk of potential harm is only significant if the transgene is oncogenic. The Kinase is not known to be oncogenic. Aerosol exposure may risk transduction of cells located within the lung tissue. However, for this to be possible the RDA would need to breach the epithelial barrier. Moreover, since gene expression will be both transient and localised, accidental exposure is unlikely to have a significant impact on the health of an individual. However, since this cannot be ruled out this presents low risk to the user.

There is no evidence that human adenovirus can naturally infect animals, and replication is very limited. However, replication has been shown to occur in the lungs of experimentally infected cotton rats (not indigenous to the UK) administered at high doses. Given that the work will be carried out at under class 2 containment, the risk of animal exposure is therefore very small. Thus, the overall risk to the environment is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid GMM contaminated waste will be triple-bagged in biohazard waste bags and suitably labelled and incinerated off site. The contractors who will carry this out are "White Rose Limited" and have been registered with the HSE. This solid waste will be comprised mainly of plasticware used in the culturing of the organisms and contaminated tissues used in decontamination procedures. As a result of treatment no viable GMMs from solid waste will remain.

All liquid waste is inactivated by chemical means using the concentrated disinfectant TriGene (MediChem International) to a final concentration of 5% (as recommended by supplier). The solutions are left for 2 hours before disposal via the drain together with copious amounts of water.

TriGene has been validated using BS6471, BS EN 1276, NF 72-150, NF 72-200, 72-180 and is lethal against all common gram positive pathogens, all gram negative pathogens and mycobacterium species. TriGene has also been tested in house under conditions used for decontamination.

As a result of treatment no viable GMMs from liquid or solid waste will remain.

Procedures and relevant training and safety inspections are in place to ensure that all personnel deal with waste correctly as specified above.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y
This assessment describes the use of adenovirus particles capable of infecting humans. The major risk would come from inhalation and subsequent infection of lung epithelial cells. Expression of kinase or dominant negative isoforms could lead to a localised proinflammatory response. To minimise exposure, the assessment recommends that the wearing of gloves is mandatory, the use of a microbiological safety cabinet/enclosure and that specific measures are implemented to control aerosol dissemination. These measures take the Containment Level required to 2.

## Project Containment

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## Project Ref 323/07.1

<table>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Expressing the green fluorescent protein in Mycobacterium bovis bacillus of Calmette and Guerin (BCG).</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
</tr>
</tbody>
</table>

Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements

## Project Additional Information

### Purposes of the contained use

Mycobacterium bovis BCG engineered to express the Green Fluorescent Protein (GFP) as a reporter protein will be used to determine the feasibility of using fluorescent imaging to monitor cell infection and subsequent bacterial determination.
Mycobacterium bovis bacillus of Calmette and Guerin (BCG) is an ACDP class 2 organism. BCG is an attenuated strain of the pathogenic species M. bovis and since 1948 has been given in a live form intradermally as a vaccine for neonates, children and adults to protect against tuberculosis. The genetic basis for its attenuation is now known. It has three deletions (RD1-RD3) relative to its progenitor M. bovis, and 10 deletions (RD1-RD10) relative to the human pathogen M. tuberculosis, which encompass 119 open reading frames (Behr et al., 1999 Science 28: 1520-1523). It has a history of safe use and it was estimated in 1991 that 2.5 billion people had by then been vaccinated with BCG. It can cause adenitis and disseminated BCG infection (BCGosis) is a recognized but rare consequence of BCG vaccination, and traditionally has been seen in children with severe immune deficiencies. Its frequency is reported as less than 5 per million vaccinees. All these complications are rare and amenable to antibiotic therapy.

Recipient or parental organism

Mycobacterium bovis bacillus of Calmette and Guerin (BCG) is an ACDP class 2 organism. BCG is an attenuated strain of the pathogenic species M. bovis and since 1948 has been given in a live form intradermally as a vaccine for neonates, children and adults to protect against tuberculosis. The genetic basis for its attenuation is now known. It has three deletions (RD1-RD3) relative to its progenitor M. bovis, and 10 deletions (RD1-RD10) relative to the human pathogen M. tuberculosis, which encompass 119 open reading frames (Behr et al., 1999 Science 28: 1520-1523). It has a history of safe use and it was estimated in 1991 that 2.5 billion people had by then been vaccinated with BCG. It can cause adenitis and disseminated BCG infection (BCGosis) is a recognized but rare consequence of BCG vaccination, and traditionally has been seen in children with severe immune deficiencies. Its frequency is reported as less than 5 per million vaccinees. All these complications are rare and amenable to antibiotic therapy.

Host/vector system

Plasmid pSMT3 is an E.coli - Mycobacteria shuttle vector (De Smet et al., 1999 Microbiology 145: 3177-3184). The E.coli origin of replication is from pUC18 while the Mycobacteria origin is a 1.25kb fragment from the Mycobacterium fortuitum var fortuitum cryptic plasmid pAL5000. The plasmid pSMT3 is non-transferrable in both E. coli and Mycobacteria and expresses the selectable hygromycin resistance gene from Streptomyces hygroscopius and GFP under the control of the constitutive hsp60 promoter from Mycobacteria tuberculosis.

The plasmid pHLEG4 is an E.coli - Mycobacteria shuttle vector that expresses an unstable form of GFP(Carroll et al., 2005 Applied and Environmental Microbiology 71(6); 3077-3084 and Blokpoel et al., 2003 J. Microbiol. Methods 54; 203-211). The E.coli origin of replication is from pUC18 while the Mycobacteria origin is from the Mycobacterium fortuitum var fortuitum cryptic plasmid pAL5000. The plasmid pHLEG4 is non-transferrable in both E. coli and Mycobacteria and expresses the selectable kanamycin resistance gene from Tn903 and can express GFP under control of the Tet inducible promoter.

The integrating shuttle cosmid vector pYUB178 (Pascopella et al., 1994 Infect Immun 62:1313-1319 ) contains an E.coli origin of replication derived from pUC19, the L5 attP site, the L5 integrase gene, the kanamycin resistance gene from Tn903, the lambda cos sequence. The plasmid is non-transferable in E.coli and mycobacteria. The L5 mycobacteriophage attachment site (attP) and integrase gene (int) mediate site-specific integration into the attB site of the mycobacterial chromosome. The green fluorescent protein is inserted into the vector under the transcriptional control of the Mycobacteria bovis hsp65 promoter.

Origin & function

The green fluorescent protein is a well characterised reporter gene with no known associated health hazards. The production of BCG with GFP will be at other establishments. BCG engineered in the same way has been produced previously. No changes have been evident when tested for changes in virulence or effects on cytokine production or surface molecule expression by antigen presenting cells in vitro (Luo et al., 1996 Clin Diag Lab Immunol 3: 761-768). Hygromycin and kanamycin have no clinical application in the treatment of tuberculosis and so use of the hygromycin and kanamycin resistance genes will not compromise any treatment. These strains will retain sensitivity to isoniazid and rifampicin, the antibiotics normally used to treat M. bovis infection. BCG as the established vaccine strain for human use is avirulent and no effect on virulence is forseen. The project involves small-scale work for research purposes and all contaminated material will be inactivated by autoclaving prior to disposal. Therefore, the environmental or personal risk from using these strains is no different from that of the parental strain.

Evaluation of foreseeable effects

The green fluorescent protein is a well characterised reporter gene with no known associated health hazards. The production of BCG with GFP will be at other establishments. BCG engineered in the same way has been produced previously. No changes have been evident when tested for changes in virulence or effects on cytokine production or surface molecule expression by antigen presenting cells in vitro (Luo et al., 1996 Clin Diag Lab Immunol 3: 761-768). Hygromycin and kanamycin have no clinical application in the treatment of tuberculosis and so use of the hygromycin and kanamycin resistance genes will not compromise any treatment. These strains will retain sensitivity to isoniazid and rifampicin, the antibiotics normally used to treat M. bovis infection. BCG as the established vaccine strain for human use is avirulent and no effect on virulence is forseen. The project involves small-scale work for research purposes and all contaminated material will be inactivated by autoclaving prior to disposal. Therefore, the environmental or personal risk from using these strains is no different from that of the parental strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Consumables (mainly plasticware e.g. pipettes, tubes, multiwell plates) will be autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes). The autoclave is situated in the same building and in close proximity to the laboratory where the activity will be carried out. Once autoclaved, the waste will be disposed of via the industrial waste stream for incineration. Pre-prepared plastic vials containing 10E8 cfu/ml recombinant M.bovis BCG will be opened in Class II hoods. The vials and all consumables used will be discarded into sharps bins within the hood. At the end of each working day the sharps bin will sealed within the hood and transferred to the autoclave. All 96 well plates will be sealed before transferring to the autoclave. Trigene at 2% will be used as a surface disinfectant. Trigene has been demonstrated by the manufacturer to be an effective disinfectant against M.bovis when used at this concentration for this purpose. Agar plates will be autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), and then disposed of via clinical waste stream for incineration.

Project Containment

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<th>Laboratory Activities</th>
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**Name**

ALUMEDIX LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

MABEL STREET, THE MEADOWS

**District**

**Town**

NOTTINGHAM

**County**

NOTTINGHAMSHIRE

**Postcode**

NG2 3ED

**Country**

ENGLAND

**Tel Number**

0115 955 3355

**Fax Number**

0115 955 3311

**E-mail**

**HSE Division**

MIDLANDS

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**
- **Give brief details of the genetic modification safety committee**

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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- **Non-microbial**

- **Other (please specify)**

- **Tick if confidential**

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The waste produced is 12 - 14 cubic meters of liquid per week containing modified Saccharomyces cerevisiae. This material is heat treated at 80 degrees C for 10 minutes in a kill tank. A sample of each batch is monitored for inactivation. The heating of the kill vessel has been validated by thermocouple.

The resulting slurry is collected by a registered waste organisation by tanker and 50.1 of biocide (Tego2000) is added to help restrain bacteria from living on the dead yeast, the slurry is pressed at the waste collection centre and the yeast is then incinerated.

Each kill cycle is sampled for growth and this information is captured on controlled worksheets and a Laboratory Alert Notices is issued if samples grow.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 325


**Name**

| ALBUMEDIX LTD |

<table>
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**Campus Estate or Research Centre**

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**E-mail**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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</table>
The waste produced is up to a maximum of 200 LT of liquid per week containing modified Saccharomyces cerevisiae. This material is heat treated at 80 degrees C for 10 minutes in a kill tank. A sample of each batch is monitored for inactivation.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 326

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#### Name

**UNIVERSITY OF EDINBURGH**

#### Name 2

**BIOMEDICAL SCIENCES**

#### Campus Estate or Research Centre

**HUGH ROBSON BUILDING**

#### Road Name

**GEORGE SQUARE.**

#### Town

**EDINBURGH**

#### County

**EAST LOTHIAN**

#### Postcode

**EH8 9XD**

#### Country

**SCOTLAND**

#### Tel Number

**0131 650 3721**

#### Fax Number

**0131 650 3711**

#### E-mail

**SCOTLAND**

#### HSE Division

**SCOTLAND**

#### Comments


#### Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

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Give brief details of the genetic modification safety committee

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref  326/01.1

Date Ackn’d  23/02/2001

CU2 Project Title  ANALYSIS OF NEURONAL TRANSGENE EXPRESSION AND OF NEURONAL VIABILITY

Date Project Ceased  30/06/2004

Class  Class 2

CultureVolClass2  < 1 Litre

Consent Granted  not applicable

Project notified under transitional arrangements  Y

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes  GM326/01.1 TRANSFERRED TO GM 207 AS OF 30/06/2004

Historical Date of Additional Info

02/03/2022  Page 6364 of 15326
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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<th>Laboratory Activities</th>
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**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to assess the ability of a number of biological active genes to reduce brain damage.

**Recipient or parental organism**

These adenoviral or adeno-associated viral vectors will be produced and characterised in Department of Medicine and Therapeutics, University of Glasgow (HSE centre number GM 397). All adenovectors to be used in this study and E1-deleted first generation adenoviral vectors based on the pJM17 system (McGorry, W. J. Bautista, D. S. and Graham, F. L.: A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. This renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on cesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. However, all the inserted genes are biologically active. None of the transgenes are proto-oncogenes. All transgenes will be under the control of the CMV promoter. There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use.

AAV vectors have received increasing attention for molecular interventions in vivo and for gene therapy applications due to their low level of immunogenicity in vivo and their ability to integrate into the genome, thus producing sustained expression of transgenes for prolonged periods of time. They also have the ability to infect both dividing and non-dividing cells.

AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Dept of Medicine and Therapeutics, University of Glasgow. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced.

**Host/vector system**

The adenoviral vectors are generated by homologous recombination between pJM17 and shuttle vectors containing transgene expression cassette and flanking E1 sequences. Following homologous recombination in helper 293 cells (which express the helper E1 function in trans, replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses.

AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette.
cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al High-titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Dept of Medicine and Therapeutics, University of Glasgow. AAV vectors are replication defective.

Origin & function

The adenoviral vectors DNAs are standard and originated from the laboratory of Dr Graham (McGrory, W. J., Bautista, D. S. and Graham, F.L.: A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vitro and in vivo to generate high-level gene expression in all cells transduced by the adenovirus. The AAV system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All transgenes will be constructed from full length cDNAs obtained from other research institutes and verified in our own laboratory prior to subcloning into the relevant vectors.

Evaluation of foreseeable effects

There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome.

AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wild type virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes, or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced. Consequence of environmental exposure - 'effectively zero'.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures as per HSE containment level 2 for both production of recombinant adenoviruses and AAV in the laboratory and animal experimentation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory-based experiments: during the production of replication-defective adenoviruses, all solid waste (plastics etc.) are autoclaved prior to disposal. All liquid waste from tissue culture is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. The use of sharps is avoided.

Animal experiments: All instruments used in the preparation of animals for GM work will be sterilised by autoclaving. Solutions exposed to the viable GMOs will be disinfected with chlorine-based disinfectant. All plastic ware will be autoclaved prior to disposal. Animals receiving the GMO will be housed in separate cages during the procedure-kill time period. All animal carcases will be disposed of by incineration. Animal bedding will be autoclaved prior to disposal and cages disinfected prior to being re-used.
Passed with amendments by local GMSC 25th September 2002.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Tick to confirm that you have attached a risk assessment to this form  

Tick to confirm that it is attached to this form  

Is an emergency plan required according to regulation 20?  

<table>
<thead>
<tr>
<th>Project Containment</th>
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<tbody>
<tr>
<td>Laboratory Activities</td>
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<td>Animal Units</td>
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GM Centre Number: 331

Data Premises Notified (Originally) 28/11/1993

Transferred from 1992 Regs? Y

Transitional Premises

Class 2

Data Premises Closed

Emergency Plan Required? N

Transitional Premises

Non-GMMs N

Withdrawn N

Name

PFIZER LTD

Name 2

Department

CENTRAL RESEARCH

Campus Estate or Research Centre

Building

Road Name

RAMSGATE ROAD

District

Town

SANDWICH

County

KENT

Postcode

CT13 9NJ

Country

ENGLAND

Tel Number 01304 616161

Fax Number 01304 652505

E-mail

HSE Division EAST AND SOUTH EAST

Comments

INCLUDES FORMER GM620

Date at Which Additional Info Submitted

02/03/2022

Page 6370 of 15326
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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</table>

02/03/2022
Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)  

Tick if confidential

Bacteriology
Parasitology
Virology
Transgenic Animals
Mycology
Transgenic Invertebrates
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 331/02.1
The aim of the present study is to transfect primary human keratinocytes with HPV11 DNA. Several workers have reported transient, low level episomal replication of viral templates in this system (Mungal et al. (1992) J. Virol 66 p3220-3224; Thomas et al., (2001) J. Virol 75, p7564). These cells will be plated on Methocel and the subsequent replication of HPV genomes will be monitored by PCR and/or Southern blots. The transfected cells will be dosed with small molecular weight compounds to assay for anti-viral activity.

The recipient host cells are commercially available primary keratinocytes isolated from normal human tissue, (Clonetics TM) which have been screened for HIV-1, hepatitis B and hepatitis C. These cells have a finite life span of 15 doublings.

Origin & function

The construct pHV11 is available from the ATCC (number 45151) and represents the entire genome of the low risk HPV11 cloned into the BamH1 site of pBr322. This recombinant DNA is amplified and cut with BamH1 to release the HPV11 DNA. This is recircularised with ligase and used to co-transfect primary keratinocytes with a neomycin selectable marker. The intention is to grow the transfected host cells on methyl cellulose, which induces partial differentiation and therefore provides a semi permissive environment for HPV replication. The episomal HPV DNA will increase in copy number but will not produce viral particles due to lack of capsid protein expression (Ruesch et all, (1998) J. Virol 72 pp5016-5024). The replication is evident for 24-72 hour, after which the cells are lysed and DNA is purified, and then used as a template in PCR studies and/or Southern lots, which quantitate the amount of HPV replication achieved.

Evaluation of foreseeable effects

HPV11 encodes E6 and E7 proteins which interact with p53 and Rb, respectively. However, because HPV11 is a "low risk" virus, these interactions are unlikely to
transform the host cell. Therefore, the transfected cells would retain their limited life span of 15 doublings. Other early genes such as E1, E2 will be expressed which will co-operatively bind to the URR within the HPV genome and sustain episomal replication to approximately 50-100 copies per permissive cell. No late capsid protein will be expressed in this system (see above). The E5 protein will be expressed in the keratinocyte membranes and may enhance mitogenic signaling via the EGF receptor. A common feature of these systems is the integration of HPV DNA into the genome of the host cell, which can occur within a short time frame. This integration would invalidate the assay and therefore the cells would be disposed in 1% Virkon.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation sought for this activity

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk of escape of GMO's to the environment, all contaminated waste streams generated during the course of this work will be decontaminated within the laboratory block. Cultures will be grown within leak proof vessels. Fermenters will be set up in such a way that accidental spillage will be contained. Virkon disinfectant will be added to liquid waste prior to disposal. The efficacy of Virkon disinfectant has been validated by an independent consultancy on behalf of Pfizer Ltd under conditions representative of the in use conditions applied in our biological research laboratories.

Solid waste will be autoclaved prior to incineration.

The only foreseeable release of viable waste from our facility is on the form of aqueous waste that has been inadequately treated, possibly in the event of an accidental spill to a sink. Such waste is emitted via our drainage system to our own Waste Water Treatment Facility (WWTF) before final discharge to the River Stour. Processing via the WWTF is such that the viable concentration will be massively diluted. The retention time within the WWTF is approximately 5 days. The HPV11 transfected keratinocytes have limited survivability in a hostile liquid environment away from its natural host or an enriched laboratory environment and is unlikely to remain viable after this period of time. Additionally the treatment regimen in the WWTF includes a 24hr disinfection step. Waste is held at elevated pH for 24 hours prior to discharge following neutralisation. Thus the risk of viable organisms entering the local environment is effectively zero.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

This assessment has been circulated to the GMSC for review and has received their approval.

Project Containment

| Laboratory Activities | Glass Houses | Growth Rooms |

02/03/2022
Project Additional Information

Purposes of the contained use

The aim of the project is to identify genes required for survival of fungal pathogens which could represent potential targets for the development of new antifungal drugs. Various genes will be deleted with concomitant insertion of a selectable marker. The resulting mutant strains will be assessed for their ability to grow in vitro and then inoculated into rodents to assess their ability to cause infection.

Recipient or parental organism

Parental organisms will be class 2 fungal pathogens, including Candida albicans, Candida glabrata, Candida spp and Aspergillus fumigatus.

Host/vector system

In some cases a non-mobilisable, linearised E. coli plasmid will be used to transfer the gene deletion cassettes. Such plasmids are unable to replicate in fungi. In other cases, no vector will be used - the gene deletion cassette will be transferred as a linear piece of DNA.

Origin & function
The genetic material involved will consist of 4 elements:
1. Small fragments of the 5' and 3' ends of the fungal gene which is intended to be deleted. These will target integration of the deletion cassette to the gene of interest through homologous integration. The purpose of this DNA is to create a deletion of a fungal gene.

2. In some cases, the genetic material will contain a fungal promoter, such as MAL2 or MET3, which is induced by nutritional supplements. The purpose of this is to place a fungal gene of interest under control of a promoter which can be regulated. The promoter is only switched on under specific growth conditions in the laboratory and can be switched off by changing growth conditions. The promoter is switched off under growth conditions in a rodent. In this way, fungal cells can be grown up, then the promoter switched off to evaluate the effects of losing the gene function (similar to deleting the gene).

3. A selectable marker. This will most often be a metabolic gene derived from the fungal host which will complement an auxotrophic mutation in the host strain, eg. URA3, HIS3, LEU2 for Candida sp or pyrG, hisB for Aspergillus. It may also be an antibiotic resistance gene, eg zeocin resistance, or a fluorescent marker gene. The purpose of the marker is to allow selection for fungal cells which have taken up the deletion cassette DNA.

4. In some cases, the genetic material will also include E. coli plasmid sequences. Non-mobilisable vectors will be used. The plasmid will contain an E. coli origin of replication and selectable marker such as ampicillin resistance. Neither of these elements are functional in fungi, therefore the plasmid can not be replicated or transferred and can only be maintained when it integrates into the fungal genome. The E. coli DNA has no purpose in these experiments - it is merely left over from the cloning work to create the deletion cassette.

Evaluation of foreseeable effects

The purpose of the project is to delete, or switch off, genes which are believed to be required for growth and pathogenicity of fungal cells, therefore the expected outcome is that the genetic modification will result in a fungal strain with impaired growth and pathogenicity. In the worst case, the genetic modification may have no effect on these characteristics and we will end up with a strain which has characteristics similar to the host organism. There is no known examples where deletion of a fungal gene has resulted in increased growth or pathogenicity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation sought for this activity.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk of escape of GMOs to the environment, all contaminated waste streams generated during the course of this work will be decontaminated within the laboratory block. Cultures will be grown within leak proof vessels. Virkon disinfectant will be added to liquid waste prior to disposal. The efficacy of Virkon disinfectant has been validated by an independent consultancy on behalf of Pfizer Ltd under conditions representative of the in use conditions applied in our biological research laboratories. Solid waste will be autoclaved prior to incineration. Animal experiments are carried out in a secure unit. Following completion of experiments, the animals are euthanased and the carcasses autoclaved within the unit. Single use overalls are worn in the animal unit and these are autoclaved following use. Disposable hats, gloves, face masks and shoe covers are worn and are incinerated after use.

The only foreseeable release of viable waste from our facility is on the form of aqueous waste that has been inadequately treated, possibly in the event of an accidental spill to a sink. Such waste is emitted via our drainage system to our own Waste Water Treatment Facility (WWTF) before final discharge to the River Stour. Processing via the WWTF is such that the viable concentration will be massively diluted. The retention time within the WWTF is approximately 5 days. Genetically modified Candida species and Aspergillus fumigatus have limited survivability in a hostile liquid environment away from their natural host or an enriched laboratory environment and is unlikely to remain viable after this period of time. Additionally the treatment regimen in the WWTF includes a 24 hr disinfection step. Waste is held at elevated pH for 24 hours prior to discharge following neutralisation. Thus the risk of viable organisms entering the local environment is effectively zero.
The membership of the GMSC approved the risk assessment without further comment or need for clarification.

Project Containment

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<th>Laboratory Activities</th>
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<td>L3 L4 L2 L3 L4</td>
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Project Ref 331/05.3

Date Ackn'd: 29/11/2005
CU2 Project Title: The generation of infectious HCV from HCV plasmids transfected into human hepatoma cells.

Date Project Ceased: 21/12/2011

Class: Class 3
CultureVolClass2: Non-GMM
CultureVolumeClass3-4: not exceed 100ml

Consent Granted: Yes

Project notified under transitional arrangements: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

Generation of infectious HCV will enable the infection of susceptible cells to identify potential targets for anti-HCV therapeutics.

**Recipient or parental organism**

The GMO will have the characteristics of "wild-type" HCV from which it was derived in that it will be fully infectious, can be transmitted only through exposure via infected blood, sexual contact with an HCV carrier or puncture wounds with contaminated material. All work involving HCV will be performed under Category III containment following a code of practice that will define our safe working practices. This will be largely based on our current code of practice written for work involving HIV, which has been performed safely on this site since 2001.

**Host/vector system**

Initially, human hepatoma cell-lines will be used for this work, but as other cell lines or primary cells become identified that are able to support the replication of HCV, then they could be used. Should another cell line be identified as permissive, we would undertake a risk assessment and may potentially include it in future work where the risk assessment concludes that the risk profile is unaltered. The human hepatoma cell line currently identified for this work, is HuH-7 a cell line that is well established and has a history of safe use within Pfizer and worldwide.

**Origin & function**

The origin of the genetic material is from a patient infected with HCV who developed fulminant liver failure. The virus was isolated from his serum and virus sequenced. If the virus can be successfully grown, then work involving other isolates from different geographical locations and different serotypes would also be used. It may also be required to subclone genes of interest from one virus into another to determine the role they play in the viral life cycle and susceptibility to antiviral therapy.

**Evaluation of foreseeable effects**

Infectious HCV is a pathogen capable causing significant human disease. Acute infection may be resolved without further complications in between 30-50% of those exposed. Those who go on to chronic carriage, defined as being HCV RNA positive for >6 months, are at a high risk of significant liver disease, which may take up to 20 years to develop. There are currently no fully effective treatments available for HCV infection and the end stage treatment for HCV liver disease is liver transplantation. There is no evidence for transmission by exposure to infected aerosols, only through the routes detailed above so the likelihood of accidental exposure through release would be minimal. There is no evidence for productive infection of any species other than chimpanzee with human HCV isolates, so that although this is a high risk pathogen for humans, accidental exposure to the environment would have no significant impact.

Our existing code of practice for HIV is being updated to additionally accommodate HCV appropriate practices. All extracted air from these laboratories is HEPA filtered, so that in the event of a spillage of infectious material, there would be no discharge of infected aerosol to the environment. All work with infectious material would take place within class I or class I/III MBSCs. There would be secondary containment of all infectious material for transfer within CL3 labs and during culture periods in incubators etc. Our current disinfection protocols have been shown to work against a range of enveloped viruses similar to HCV. These include the use of Virkon at 2% for treatment of contaminated lab waste such as cell culture ware and cell culture fluid or other infectious liquids. Post disinfection liquid waste is disposed to the onsite Waste Water Treatment Facility (WWTF) where a further cycle of treatment including disinfection takes place prior to discharge. Solid waste from the labs is autoclaved before being sent for incineration on-site. The labs are on a pre-scheduled programme of vapourised hydrogen peroxide fumigation to allow routine shutdown and maintenance. The same fumigation process may be used in the event of spillage of infectious materials outside of a Microbiological Safety Cabinet.

Due to the lack of a reproducible cell culture system for expansion of HCV, information such as the stability of virus outside the body, in cell culture fluid for example, is unknown.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Information to justify derogation.

The derogation sought for this activity, and the information to justify it is the same as that submitted to the HSE and granted previously .. Your ref GM331 dated 24th March 2005.

The controls indicated below ensure that the transfer of (treated) waste to an autoclave that is not ‘en suite’ does not lead to a significant risk of exposure:

Disinfection procedure

* All materials that have been in contact with the virus (plastics and liquid) are immersed in 2% Virkon disinfectant within a functional microbiological safety cabinet for an overnight period of not less than 16 hours. Waste comprises either a) loose lab plastics (plastic tubes, pipettes and microtitre tips) which are fully immersed in approx 2 litres of 2% Virkon, or b) multiwell plates containing assay liquid to which Virkon is added to create a 2% final concentration.

* Post overnight disinfection - liquid waste from a) is discarded to the drainage

* Post overnight disinfection - solid waste ie disinfected lab plastics from a) and multiwell plates containing small volumes of disinfectant are transferred to a bin for autoclaving. See below.

* Disinfected solid waste (see above) is transferred into leakproof stainless steel autoclave bins within the CL3 laboratory.

* Lids are placed on the bins and taped in place with autoclave indicator tape.

* The external surface of each bin is wiped with 70% ethanol within the CL3 laboratory.

* Bins are then taken from the CL3 laboratory into the CL3 Annex (still within the CL3 suite) and placed onto a robust trolley. Nb. Our CL3 Code of Practice requires that open virus is only handled inside the MSCs within the CL3 laboratories and thus risk of contamination or exposure within the annex is extremely limited. When stock virus is transported from the store into the laboratory via the CL3 annex double containment is employed at all times and external surfaces are surface disinfected.

* The wheels of the trolley are sprayed with 70% ethanol before exiting the CL3 suite.

* Before exiting the CL3 suite a member of CL3 staff confirms that the corridor area between the suite and the autoclave is not occupied (this operation will routinely take place at the end of the working day at which time the rest of zone 2 will typically be unoccupied).

* The trolley is then wheeled directly to the autoclave and bins transferred. The robust construction of the trolley, the short and unobstructed distance and the fact that the area is otherwise unoccupied all combine to make the risk of an accident that could result in spillage highly unlikely. In this context it is also important to emphasise that all of the waste contained within the autoclave bins has been disinfected prior to loading into the autoclave bins. The transfer operation is estimated to take approx 2 minutes.

* The autoclave cycle (134 degrees C/15 mins) is initiated.

* A sign is placed on the ‘dirty’ side of the autoclave to indicate that in the unlikely event of cycle failure the autoclave may only be accessed by CL3 suite staff. This is because the autoclave cycle would always ‘fail to safe’ enabling it to be accessed from the ‘dirty’ side only.

* Autoclave waste is removed following successful completion of the cycle and is subsequently sent for on-site incineration by CL3 staff.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste would be treated for a minimum of 12 hours by exposure to at least 1% Virkon before being sent to the onsite WWTF for treatment prior to discharge. The efficiency of this disinfectant protocol has been externally validated on our behalf for numerous organisms including enveloped viruses likely to have a similar profile to HCV. The WWTF process includes a 5-day holding period and a 24-hour disinfection cycle. Any solid waste coming into contact with HCV would also be treated with 2% Virkon for at least 14 hours prior to disposal. All solid waste would be autoclaved prior to incineration in line with the Pfizer site guidelines. In case of a spillage outside of the MBSCs and in the case of access to the containment facility for maintenance procedures, the area would be fumigated with hydrogen peroxide using a validated process carried out by an external contractor under Pfizer supervision. These processes have been shown to kill viruses related to HCV, but have not been definitively shown to kill HCV due to the absence of a reproducible cell culture system.
The risk assessment was discussed at the Genetic Modification Safety Committee (GMSC) meeting on 27th September 2005. A copy of the risk assessment was circulated to the full GMSC membership who gave it their approval.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L3 L4</td>
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Project Ref 331/08.1

Date Ackn’d: 04/09/2008
Date Project Ceased: 06/03/2009

CU2 Project Title: Construction and use of Lentivirus vectors for the expression of heterologous proteins and inhibitory RNA sequences in mammalian cells.

Class: Class 2
Culture Volume: < 1 litre
Consent Granted: Not Applicable

Non-GMM: Yes
Historical Significant Changes: 16/11/2009
Withdrawn: No
Tick if notifying a connected programme of work: Yes
Historical Date of Additional Info: 16/11/2009
Significant Change ID: 331/08.1a
**Project Additional Information**

**Purposes of the contained use**

The lentivirus vector system will be used for the following experimental purposes:

1. To evaluate gene function in normal body cell biology and disease by overexpression or RNAi-mediated knockdown of the relevant gene function in primary cultured cells from normal and diseased tissues.
2. To generate stable cell lines expressing genes of interest to be used in the development of cell-based screening assays and disease-relevant in vitro cell models. These assays will be used to explore gene function and to screen compounds for activity at drug targets.

**Recipient or parental organism**

Recipient organisms will be cultured mammalian cells.

**Host/vector system**

The lentiviral vector systems used for these studies will be third generation replication-defective lentiviral vector systems. Only gag, pol and rev genes from HIV will be employed and these will be supplied in trans to facilitate virus packaging. All other HIV genes have been deleted. Examples of commercially available vectors with these properties include invitrogen's ViraPower (pLenti vectors), and Stigma's pLKO.1 vector systems.

High titre virus stocks will be generated by cotransfecting a 293FT packaging cell line with transfer and packaging vectors.

**Origin & function**

Genes to be overexpressed will be derived from human or other relevant mammalian species (e.g. rodent, dog, non-human primate). Genes will be those considered to be of potential relevance in disease processes and will be overexpressed in cultured cells to determine their functional activity and to enable discovery of novel drugs targeting the gene product. Genes will include those encoding cell receptors, ion channels, enzymes and regulatory proteins from cellular signalling pathways. Genes will be derived from in-house cloning efforts or from academic or commercial sources.

Inhibitory RNA sequences will be targetted to genes considered to be of potential relevance in disease processes. Targetted genes will include those encoding cell receptors, ion channels, enzymes and regulatory proteins from cellular signalling pathways. Inhibitory RNA sequences will be obtained from commercial sources.

**Evaluation of foreseeable effects**

The risk associated with overexpression or knockdown of individual genes will be considered on a case by case basis and reviewed locally by the GM safety committee prior to work being started. Particular consideration will be given to genes that may be oncogenic or immunomodulatory.

VSV-G pseudotyped lentivirus strains will be used and these strains will have the ability to infect human cells and cells from other mammalian species. However, because these strains are replication defective, the virus can only carry out a single round of infection. Structural genes and other components required for packaging the viral genome are separated onto three plasmids and have been engineered so as not to contain any homologous sequences to prevent undesirable recombination events that could lead to the generation of a replication-competent virus. Cells that may contain virus sequences able to mobilise the lentivirus vectors by providing structural genes in trans will be excluded from these experiments.

Provirus insertion into the host genome will occur with high frequency and while this is unlikely to generate a harmful phenotype in the cultured mammalian cell (host cells from workers will not be used), there is a low probability of infection of a worker e.g. through accidental inoculation with packaged virus. Integration and long term expression of the recombinant gene or cDNA encoding inhibitory RNA could occur. There is a theoretical risk of transformation of cells by insertion of oncogenes or by knock-down of tumour suppressor genes. Because of the replication-defective nature of these vectors, this could only occur at low frequency and therefore unlikely to have a detrimental effect as a single event is unlikely to result in cellular transformation. Lentiviruses can also act as insertional mutagens. The
likelihood of this occurring is reduced by the use of a self-inactivating vector which disables the LTR regions in the integrated vector and thus reduces the risk of oncogene activation at the site of insertion. Additionally, because the vector is replication-defective, the level of exposure that might occur following accidental introduction of the virus will be low and thus reduces the probability of virus insertion at a site likely to promote tumourigenesis. However, because these vectors incorporate strong heterologous promoters and because provirus insertion is a feature of retrovirus biology, work with these vectors is assessed as a class 2 activity and the use of appropriate containment is used to minimise exposure.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk of escape of GMO's to the environment, all work will be performed in containment level 2 biology laboratory. Cultures will be grown within leakproof vessels. All contaminated waste streams generated during the course of this work will be decontaminated on site. Virkon disinfectant will be added to liquid waste and left for a defined contact period prior to disposal. Solid waste will be autoclaved and subsequently incinerated prior to disposal. Additional information on disinfection procedures is included in departmental safety manuals. The only foreseeable release of viable waste from our facility is in the form of aqueous waste that has been inadequately treated, possibly in the event of an accidental spill to a sink. Such waste is emitted via our Waste Water Treatment Facility (WWTF) before final discharge to the River Stour. Processing via the WWTF is such that the virus concentration will be massively diluted and will not reach the river Stour for approximately 5 days after leaving the laboratory. The treatment regimen in the WWTF includes a 24hr disinfection step. Thus the risk of viable organisms entering the local environment is effectively zero.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project was discussed with the HSE during a routine inspection by the Biological Agents Unit of the Pfizer facilities in April 2007. Under the new guidance from SACGM the Lentivirus work could be assigned as a class 2 activity. The HSE recommended that a generic notification and risk assessment should be submitted as a broad connected programme of work covering all Lentiviral vector work when the next Lentivirus project was proposed. Individual assessments would then be written at Pfizer for each proposed project but there would be no need to notify HSE of any future projects unless they fell outside the connected programme. A copy of the risk assessment was circulated to the full GMSC membership who gave their approval.

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms

02/03/2022
Use of Staphylococcus aureus strains that have been genetically modified to eliminate or prevent expression of genes contributing to virulence.

The main parental organism is Staphylococcus aureus Newman strain. This strain was derived from a clinical isolate in 1954 and has since been widely used as an experimental laboratory strain. This strain is antibiotic sensitive and does not exhibit a highly pathogenic phenotype. Knock-out of virulence genes in other antibiotic resistant S. aureus parental strains exhibiting a similar level of pathogenicity will be subject to approval by the local GM safety committee.

Defined genetic deletions in virulence genes will be introduced into the genome of the parental strain by transposon mutagenesis or by homologous recombination using plasmids that are otherwise propagated in E.coli laboratory strains.
Genes that are known to be involved in pathogenicity of S. aureus will be disrupted by transposon mutagenesis or excised by homologous recombination. This modification will knock-out the expression of the genes and may introduce an antibiotic resistance (erythromycin or tetracycline) gene for selection of the modified strains. Knock-out of specific genes involved in virulence will be subject to risk assessment and approval by the GM safety committee.

**Evaluation of foreseeable effects**

The knock-out of alk genes under current consideration has been shown to render resulting strains less pathogenic than the isogenic parental strains. Current knock-out constructs harbour gene cassettes that confer erythromycin or tetracycline resistance. The risk of conferring resistance to erythromycin or tetracycline is considered insignificant since neither is the drug of choice for therapy of infection with the S. aureus Newman strain.

The risk associated with the knock-out of further individual genes will be considered on a case-by-case basis and reviewed locally by the GM safety committee prior to work being started. Particular consideration will be given to genes that may be global regulators of virulence and the use of antibiotic markers will be avoided or restricted to those that are not used for therapy of infection with S. aureus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

To minimise the risk of escape of GMO's to the environment, all work will be performed in a containment level 2 biology laboratory. Cultures will be grown within leakproof vessels. All contaminated waste streams generated during the course of this work will be decontaminated on site. Virkon disinfectant will be added to liquid waste and left for a defined contact period prior to disposal. Solid waste will be autoclaved and subsequently incinerated prior to disposal. Additional information on disinfection procedures is included in the departmental safety manuals. The only foreseeable release of viable waste from our facility is in the form of aqueous waste that has been inadequately treated, possible in the event of an accidental spill to a sink. Such waste is emitted via our Waste Water Treatment Facility (WWTF) before final discharge to the River Stour. Processing via the WWTF is such that the bacterial concentration will be massively diluted and will not reach the river Stour for approximately 5 days after leaving the laboratory. The treatment regimen in the WWTF includes a 24 hr disinfection step. Thus, the risk of viable organisms entering the local environment is effectively zero.

**Is an emergency plan required according to regulation 20?**

- [ ] Y

If yes, tick to confirm that it is attached to this form

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] N

**Please enter comments on the GM safety committee on the risk assessment**

A copy of the risk assessment was circulated to the full GMSC membership in July 2008 who gave their full approval without comments or modifications.

**Project Containment**
Project Additional Information

Purposes of the contained use

Parapoxvirus ovis (PPVO) strain D1701 will be used for the following experimental purposes:
1. To provide information on the characteristics of the expressed heterologous proteins.
2. To provide information on optimal growth conditions.
3. To produce pre-GMP master seed material.

Recipient or parental organism

Host/vector system

The parapoxvirus strain D1701-VrV used as a vector was extensively attenuated by more than 170 passages leading to attenuation by rearrangement of limited terminal
regions of the genome which is accompanied by gene deletion (E2L), the substitution of the amino acid isoleucine (position 115) in the viral 1L-10 (virulence factor) which is described to be very critical for retaining the stimulatory activity of human 1L-10 and the deletion of the VEGF-E gene (virulence factor of parapoxviruses and three further regions of its genome.

Origin & function
The vector contain the foreign gene will be infected in different mammalian cell culture systems to investigate its growth. Genes to be expressed will be derived from E.coli (only ßgalactosidase) and relevant mammalian viruses (e.g. cat, dog, pig, cattle, etc). No human virus gene will be expressed. Genes will be those considered to be of potential relevance in disease processess particularly with regard to protection, and will be expressed in cultured cells to determine their functional activity.

Evaluation of foreseeable effects
The primary host of parapoxviruses are ungulates. However, humans (and cats) can be alternate hosts reacting with localised epidermal lesions which heal without scaring. Although the parapoxvirus vector was demonstrated to be safe in permissive (sheep) and non-permissive animals (pigs, dogs), there is no data available for humans. Therefore, the risk for humans using the parapoxvirus (ACDP hazard group 2). For laboratory workers, ingestion, parental inoculation, and droplet or aerosol exposure of mucous membranes or broken skin are possible routes of infection. Work with this virus will be undertaken in a class 2 MSC using asceptic technique to reduce the risk of infection. Sharps will not be used. Infection through a direct contact with infected person or animal involves a substantial transfer of infectious virus through a cut or other break in the skin, which minimizes its ability to spread. Expression is under control of a synthetic early-late poxvirus promoter eLP-1 designed for protein expression in the mammalian cell. No expression will occur outside of the mammalian cell host. Damage should be considered on a case by case basis taking into account all known properties of the expression product or closely related proteins whose properties may be reflected.

In the event that a spillage does occur the quantity of the resultant spill will be such that it can be disinfected and a clean up operation performed within the confines of the laboratory without significant risk of escape to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
To minimise the risk of escape of GMO's to the environment, all work will be performed in containment level 2 biology laboratory. Cultures will be grown within leakproof vessels. All containment waste streams generated during the course of this work will be decontaminated on site. Virkon disinfectant will be added to liquid waste and left for a defined contact period prior to disposal. Solid waste will be autoclaved and subsequently incinerated prior to disposal. The only foreseeable release of viable waste from our facility is in the form of aqueous waste that has been inadequately treated, possibly in the event of an accidental spill in a sink. Such waste is emitted via our Waste Water Treatment Facility (WWTF) before final discharge to the River Stour. Processing via the WWFT is such that the GMO concentration will be massively diluted and will not reach the River Stour for approximately 5 days after leaving the laboratory. The treatment regime in the WWFT includes a 24hr disinfection step. Thus the risk of viable organisms entering the local environment is effectively zero.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
A copy of the risk assessment was circulated to the full GMSC membership who gave their approval.

**Project Containment**

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**Project Ref** 331/10.1

- **Date Ackn’d**: 27/09/2010
- **CU2 Project Title**: Establishing a Hepatitis B virus (HBV) replication assay
- **Class**: Class 3
- **Culture Vol Class 2**: < 1 Litre
- **Culture Volume Class 3-4**: 50ml max
- **Non-GMM**: Consent Granted
- **Consent Granted**: Yes
- **Date Project Ceased**: 21/12/2011
- **Withdrawn**: No
- **Tick if notifying a connected programme of work**: No
- **Project notified under transitional arrangements**: No

**Project Additional Information**
### Purposes of the contained use

This assay will support an exploratory project seeking an antiviral cure for HBV infection

### Recipient or parental organism

Huh7 and HepG2 human liver cell lines

### Host/vector system

The HBV A938 strain (subtype adw2) will be cloned into a variety of commercially available vectors (pGEM7Zf+; pUC19, pCMV Script). This virus strain was chosen because the genome is well characterised and has been used extensively in the literature. The 3221 bp genome has been fully sequenced (Priesler-Adams et al [1993] Nucleic Acid res 21 p2258; Accession number X70185). Variants of HBV sequence to either C, S, P or X genes may be used in the assessment of barriers to drug resistance

### Origin & function

The expected result from the transfection of Huh7 cells with HBV sequences will be the production of HBV titres, including a proportion that will be infectious to humans. Occupational exposure in the laboratory setting would require a puncture wound (likely as a result of a needle-stick injury, injury with other contaminated sharp instruments), or as a result of contamination of the mucous membranes (eyes, nose and mouth transfer or via aerosols). Acute infection of non-immune non-vaccinated adults ranges from asymptomatic or mild disease to rarely fulminant hepatitis. Disease is more severe among adults ranges from >60 years. The fatality rate among non-immune, non-vaccinated infected cases reported to Center for Disease Control (CDC) is 0.5% - 1%. The risk for chronic infection varies according to the age at infection and is greatest among young children. Approximately 90% of infants and 25% -50% of children aged 1-5 years will remain chronically infected with HBV. By contrast, approximately 95% of non-vaccinated adults recover completely from HBV infection and do not become chronically infected (CDC, website).

Once inside a non-immune, non-vaccinated host, HBV is transported in the blood to the liver where it infects liver cells. The incubation period of acute HBV infection is about 75 days but it ranges from 45 to 200 days. The virus spreads efficiently in the liver and causes a spectrum of disease, ranging from acute hepatitis to more chronic liver disease and liver tumours. Asymptomatic infection and illness without jaundice does occur, particularly in the immunocompromised. The likelihood of a non-immune non-vaccinated colleague developing chronic infection is inversely related to age at the time of infection. Viral protein is secreted into the blood and its presence is useful marker of infection (usually HbsAg). Theses chronically infected individuals, who may be totally without symptoms, also present a major risk to non-immune health care workers and others accidentally exposed by, for instance, a needle-stick injury. In addition, the continued presence of viral proteins is associated with progressive liver damage (chronic active hepatitis and cirrhosis) and increased risk of primary liver cancer. Much of the damage to the liver in chronic cases is believed to be as a result of immune responses to the infection.

### Evaluation of foreseeable effects

The expected result from the transfection of Huh7 cells with HBV sequences will be the production of HBV titres, including a proportion that will be infectious to humans. Occupational exposure in the laboratory setting would require a puncture wound (likely as a result of a needle-stick injury, injury with other contaminated sharp instruments), or as a result of contamination of the mucous membranes (eyes, nose and mouth transfer or via aerosols). Acute infection of non-immune non-vaccinated adults ranges from asymptomatic or mild disease to rarely fulminant hepatitis. Disease is more severe among adults ranges from >60 years. The fatality rate among non-immune, non-vaccinated infected cases reported to Center for Disease Control (CDC) is 0.5% - 1%. The risk for chronic infection varies according to the age at infection and is greatest among young children. Approximately 90% of infants and 25% -50% of children aged 1-5 years will remain chronically infected with HBV. By contrast, approximately 95% of non-vaccinated adults recover completely from HBV infection and do not become chronically infected (CDC, website).

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation applied for

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk of escape of GMO's to the environment, all work will be performed in containment level 3 biology laboratory. Cultures will be grown within leak-proof...
vessels. All contaminated waste streams generated during the course of this work will be decontaminated on site. Virkon disinfectant will be added to liquid waste and left for a defined contact period prior to disposal. Solid waste will be autoclaved and subsequently incinerated prior to disposal. Additional information on disinfection procedures is included in departmental safety manuals. The only foreseeable release of viable waste from our facility is in the form of aqueous waste that has been inadequately treated, possibly in the event of an accidental spill to a sink. All water from the CL3 Suite is held in an intercept tank and can be subjected to heat treatment before release if necessary. Such waste is emitted via our Waste Water Treatment Facility (WWTF) before final discharge to the River Stour. Processing via the WWTF is such that the GMO concentration will be massively diluted and will not reach the river Stour for approximately 5 days after leaving the laboratory. The treatment regimen in the WWTF includes a 24hr disinfection step. Thus the risk of viable organisms entering the local environment is effectively zero.

The risk of infection to humans near the Sandwich site would also be effectively zero. In humans, direct contact with another infected person is the normal route of infection and involves a substantial transfer of infectious virus. We note that other non-human primate species can be infected with HBV (gibbon, gorilla and tree shrew (Tupaia belangeri) if injected with high HBV titres. The location of the facilities reduces this xenotropic transmission risk to zero.

The risk assessment for this work has been approved by the local Genetic Modification Safety Committee.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The risk assessment for this work has been approved by the local Genetic Modification Safety Committee

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Project Ref 331/95.3

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Date Project Ceased
14/02/2011

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements
Y

Withdrawn
N

Tick if notifying a connected programme of work
N

Historical Significant Changes
GM331/96.1, GM331/99.2, GM331/00.1,

Historical Date of Additional Info

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 331/95.4

Date Ackn’d 05/09/1995

CU2 Project Title DISRUPTION OF THE URA3 GENE IN CANDIDA ALBICANS AND CANDIDA GLABRATA

Date Project Ceased 14/02/2011

Consent Granted not applicable

Project notified under transitional arrangements Y

Significant Change ID GM331/96.1,GM331/99.2,GM331/00.1,GM331/01.1.

Historical Date of Additional Info 07/07/1996, 17/11/1999, 14/12/2000, 04/01/2001

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Additional Information

Purposes of the contained use
To investigate the ability of HIV pseudotypes expressing different envelope to infect CD4+ lines expressing one or more chemokine receptor family. Infection will be determined by detection of luciferase activity.

Recipient or parental organism
HIV-1 pseudotyped virus (derived from HIV-1 clone pNL4-3) variety of mammalian cell lines.

Host/vector system

Origin & function
pNL4-3 HIV clone (pNL4-3-Lu-R+/E-) and HIV env clones obtained from Gladstone Institute, USA. The DNA clones will be co-transfected into mammalian cell lines to generate pseudotyped virus.
The pseudotype virus lacks a functional envelope gene, it is, therefore, unable to propagate in infected cell lines. As a consequence the pseudotype is considered to be non-pathogenic. The nef gene has been replaced with luciferase as a marker.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Infectious waste will be sealed in plastic containers and disposed as solid waste by autoclaving followed by incineration. Pipettes and pipette tips will be soaked in glutaraldehyde prior to above treatment. Only disposable plasticware will be used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
</tr>
</thead>
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Project Ref 625/99.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Name**

MARIE CURIE CANCER CARE RESEARCH INSTITUTE

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

THE CHART

**District**

**Town**

OXTED

**County**

SURREY

**Postcode**

RH8 OTL

**Country**

ENGLAND

**Tel Number**

01883 722306

**Fax Number**

01883 714375

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

GM CENTRE CLOSED

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
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<td>SURREY</td>
<td>RH8 0TL</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
<th>Level 4 (GMMs)</th>
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<th>Other (please specify)</th>
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<td>Transgenic</td>
<td>Microbiology</td>
<td>Gene Therapy</td>
<td>Tick if confidential</td>
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<td></td>
<td>Birds</td>
<td>Research</td>
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### Project Ref 332/01.1

**CU2 Project Title**
PRODUCTION OF RECOMBINANT HERPESVIRUS EXPRESSING NATIVE PROTEINS LINKED TO A FLUORESCENT MARKER (GREEN FLUORESCENT PROTEIN).

**Date Ackn'd**
15/02/2001

**Date Project Ceased**
31/03/2007

**Class CultureVolClass2 CultureVolumeClass3-4**
Class 2

**Non-GMM Consent Granted**
not applicable

**Project notified under transitional arrangements**
Y

**Historical Significant Changes**
TRANSFERRED TO GM 77 ON 31/3/07.

**Withdrawn**
N

**Tick if notifying a connected programme of work**
N

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<td>L3 L4 L2 L3</td>
<td>L3 L4 L2 L3</td>
</tr>
</tbody>
</table>
### Project Additional Information

**Purposes of the contained use**

Generation of herpes viruses containing natural proteins linked to a marker - Green Fluorescent Protein (GFP). This will allow detection of fusions in living cells and analysis of pathways involved in virus assembly. Use of mutated proteins linked to GFP will further aid understanding of mechanisms involved.

**Recipient or parental organism**

Routinely used mammalian cell lines, e.g. Vero, COS, etc. all are well characterised and authenticated with a history of safe use.

**Host/vector system**

HSV-1 strains, e.g. HSV-1 strain 17, Macro Plaque and tsB7.

**Origin & function**

Native marker genes fused to the marker gene for Green Fluorescent Protein (GFP) which is available commercially. Work involving mutated genes will also be undertaken. GFP has fluorescent activity which enables the location of the fusion protein to be determined. Insertion will be into the natural region of the gene under investigation. Gene sequences of interest are either regulatory, for transcription initiation, or are structural.
The inserts display no potential for a pathogenic phenotype. Expression will be identical to that of native viral genes. The recombinant virus will be identical to the parental strain except for expression of the desired candidate gene linked to GFP.

If the modification were to affect the virus it is most likely to disrupt the function for example, by the recombinant protein being too large to permit viral development. No effect on viral susceptibility to anti viral drugs such as acyclovir is envisaged.

The likelihood of an environmental hazard is negligible as herpes virus particles are labile and inactivated by drying.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste (tissues, gloves, plasticware) is autoclaved at 121°C for 15 minutes (as measured in load) to give effectively 100% kill.

Sharps (needles used in gradient harvest) are disinfected by contact with 1% Virkon overnight prior to disposal into a sharps bin. Manufacturers validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

Waste media is inactivated by addition of Virkon tablets to a final concentration of not less than 1% and left overnight. Manufacturers validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

Pipettes are decontaminated by complete submersion in 1% Virkon overnight. Manufacturers validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

If the use of glassware is unavoidable, this is decontaminated by complete submersion in 1% Virkon overnight. Manufacturers validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM Safety Committee on the risk assessment

The assessment has been approved by the Marie Curie Research Institute Biological Safety Committee for work at containment level 2.

Project Containment
Replication deficient lentiviral constructs encoding shRNAs will be used in the study of microtubule-associated proteins.

Historical Date of Additional Info
transferred to GM 13

Recipient or parental organism
Cell lines with a history of safe usage within the scientific community. Any primary cells used are isolated from sources held in a controlled environment and should therefore be free of pathogens. Any E.coli variants, cultured cells and replication-deficient lentivirus particles produced cannot survive outside the laboratory. Neither the generated virus particles nor the transduced cells are pathogenic to animals or plants.

Host/vector system
Well characterised E.coli K12 derivates, such as TOP1O. Lentiviral particles are designed for use in gene therapies and thus have the potential to infect human cells in vivo, however as none of the HIV-1 structural genes are actually present in the packaged viral genome, they are not able to propagate in vivo.

Lentiviral constructs encoding shRNAs targeted against murine microtubule-associated proteins will be transferred, using lentivirus, to cell lines. shRNAs will be used to bring about RNA interference, silencing the targeted genes and enabling studies on the function of microtubule-associated proteins. Other constructs expressing microtubule-associated proteins with epitope tags or fluorescent proteins will also be produced in order to allow live cell imaging and biochemical experiments.

Origin & function

Lentiviral constructs encoding shRNAs targeted against murine microtubule-associated proteins will be transferred, using lentivirus, to cell lines. shRNAs will be used to bring about RNA interference, silencing the targeted genes and enabling studies on the function of microtubule-associated proteins. Other constructs expressing microtubule-associated proteins with epitope tags or fluorescent proteins will also be produced in order to allow live cell imaging and biochemical experiments.

Evaluation of foreseeable effects

Inserts display no potential for a pathogenic phenotype. The likelihood of an environmental hazard is negligible as none of the particles produced can survive outside the laboratory. Neither the generated virus particles nor the transduced cells are pathogenic to animals or plants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste (tissues, gloves, plasticware) is autoclaved at 121 degrees Centigrade for 15 minutes (as measured in load) to give effectively 100% kill. Waste media is inactivated by addition of Virkon tablets to a final concentration of not less than 1% and left overnight. Manufacturer’s validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes. Pipettes are decontaminated by complete submersion in 1% Virkon overnight, followed by autoclaving at 121 degrees Centigrade for 15 minutes (as measured in load) to give effectively 100% kill; Virkon manufacturer’s validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This assessment has been approved by the Marie Curie Research Institute Biological Safety Committee for work at Containment Level 2.

Project Containment
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<td>L2 L3 L4</td>
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<table>
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<th>Animal Units</th>
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<th>Human Clinical Applications</th>
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<tbody>
<tr>
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**Project Ref** 332/trans1

<table>
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<tbody>
<tr>
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<td>EXPRESSION OF TRANSCRIPTION FACTORS AND ONCOGENIC SEQUENCES IN MAMMALIAN TISSUE CULTURE CELL LINES</td>
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<th>Class</th>
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<tr>
<td></td>
<td>not applicable</td>
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Withdrawn N

Tick if notifying a connected programme of work N

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Project Containment**

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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**Project Ref** 332/trans3

- Date Ackn’d: 07/03/1993
- CU2 Project Title: LONG TERM STORAGE OF CLASS 2 GMMS
- Date Project Ceased: 06/04/2010
- Class: 2
- Culture Vol: Class 2 Culture Volume
- Class Culture Vol: Class 2 Culture Volume Class 3-4
- Non-GMM: Consent Granted: not applicable
- Project notified under transitional arrangements: Y
- Withdrawn: N
- Historical Significant Changes: GM332/94.4, GM332/95.4, GM332/97.1
- Historical Date of Additional Info: 01/02/1994, 19/12/1995, 25/07/1997
- Significant Change ID
- Date of Significant Change

02/03/2022
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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#### Name

**NATURAL HISTORY MUSEUM**

#### Campus Estate or Research Centre

**CROMWELL ROAD**

#### Road Name

**CROMWELL ROAD**

#### District

**CROMWELL ROAD**

#### Town

**LONDON**

#### County

**GREATER LONDON**

#### Postcode

**SW7 5BT**

#### Country

**ENGLAND**

#### Tel Number

**0207 938 9333**

#### Fax Number

**0207 938 8937**

#### E-mail

**HSE Division**

**LONDON**

#### Comments

**Date at Which Additional Info Submitted**

**02/03/2022**
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Animals

Virology Transgenic Birds Microbiology Research

Transgenic Fish Gene Therapy
Maximum culture volume is 500ml supernatent. Liquid waste is deactivated by contact with a solution of 2% Hycolin bactericide for 12-24 hrs in accordance with the manufacturer's instructions. Solid waste material is bagged and autoclaved within 24 hours. The autoclave cycle runs for 30 mins at a minimum of 134°C at a pressure of 2.22 bars which provides a deliberate overkill. Autoclaves are tested annually to validate that the correct temperature and pressure have been reached for the required time. Heat reactant tape is used to provide verification that each load reaches the correct conditions.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
<tr>
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**Name**

DE MONTFORT UNIVERSITY

**Name 2**

**Department**

**Campus Estate or Research Centre**

THE DAVID ATTENBOROUGH LABS

**Building**

**Road Name**

**District**

**Town**

**County**

LEICESTERSHIRE

**Postcode**

LE7 9SU

**Country**

ENGLAND

**Tel Number**

0116 2551 551

**Fax Number**

0116 257 7752

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- [ ] Laboratory
- [ ] Animal Unit
- [ ] Growth Room
- [ ] Glass House
- [ ] Large Scale

#### Level 1 (GMMs)

#### Level 2 (GMMs)

#### Level 3 (GMMs)

#### Level 4 (GMMs)

#### Non-microbial

#### Other (please specify)  

Tick if confidential

- [ ] Bacteriology
- [ ] Parasitology
- [ ] Transgenic Birds
- [ ] Microbiology Research

02/03/2022
Page 6415 of 15326
Waste generation.
Solid waste: approximately 1000 litres per week.
Liquid waste: approximately 15 litres per week.
Both types of waste are autoclaved using regularly inspected apparatus. The solid waste is removed from the premises and incinerated by Rentokil Initial UK Ltd.

Virology
Transgenic Animals
Mycology
Transgenic Invertebrates
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
| Data Premises Notified (Originally) | 28/12/1989 | Data Premises Closed | 29/06/2009 | Emergency Plan Required? | N | Non-GMMs | N | Withdrawn | N | ...
|-----------------------------------|------------|----------------------|------------|---------------------------|---|----------|---|-----------|---| ...
| Name                              | GRAY LABORATORY CANCER RESEARCH TRUST | Name 2 | MOUNT VERNON HOSPITAL | Department | Building | PO BOX 100 | District | Town | NORTHWOOD | County | MIDDLESEX | Postcode | HA2 8LE | Country | ENGLAND | Tel Number | 01923 828611 | Fax Number | 01923 835210 | E-mail | HSE Division | LONDON | Comments | Centre closed and transferred to University of Oxford GM 553 | Date at Which Additional Info Submitted | 02/03/2022 |
**Premises Addresses**

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<td>MIDDLESEX</td>
<td>HA2 8LE</td>
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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**Project Ref** 337/02.1

<table>
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<td>16/10/2002</td>
<td>EFFECT OF ANGIOPOIETIN 1 DELIVERED BY ADENOVIRUS (ANGLADENO)</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**
**Purposes of the contained use**

Determine whether high levels of Ang1, which influences the permeability of blood vessel walls to circulating plasma proteins, affect tumour growth and sensitivity to treatment (combretastatin and other). In detail:

Ang1adeno (constructed elsewhere) will be propagated in human kidney 293 cells to high titer and harvested. Mammalian cells will be infected with this replication-defective adenovirus and assayed for ang1 production and function. Ang1adeno will be administered and effect of treatment (CA4P or other vascular targeting agent) analysed some time later.

**Recipient or parental organism**

Long term established cell lines will be used, which are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive autonomously outside the laboratory. Cell lines to be used include: human HEK293, human HT29, murine CaNT. Since the virus has been constructed elsewhere, there is no need for bacterial amplification. For in vivo transduction adenovirus will be be administered. No infectious virus has every been recovered from transduced animals or humans, even if viral genome was detectable by PCR amplification.

**Host/vector system**

A gutless adenovirus (replication deficient recombinant human adenovirus 5) will be used to deliver angiopoietin 1 (Ang1). This adenovirus is deleted for E1 (transcriptional regulator, required for assembly of infectious particles) and E3 (evasion of host immunity) and can only be propagated in specific, modified cells (human embryo kidney HEK293)). It is therefore unlikely to spread from the transduced cells to humans or any other organisms. However, owing to the nature of production of these viruses, there is a possibility of reversion to wild-type viruses by recombination with viral genes stably expressed within 293 cells, thus producing wild type virus. In the adenovirus used in this project (Adenoquest, Q-Biogene) the transgene (Ang1 has been inserted into the region where E1 has been deleted. That should ensure the loss of the transgene in the event of recombination to generate wild-type, replication efficient virus.

**Origin & function**

The gene to be used is of human origin. Angiopoietin 1 is involved in the maturation phase of angiogenesis. It is a growth factor which acts on tyrosine kinase receptors specifically on endothelial cells. Over-expression of Ang 1 or treatment with exogenous Ang 1 results in vessels which are particularly resistant to leaking. Ang 1 acts as an endothelial cell survival factor preventing apoptosis. In this project Ang1adeno will be used to determine whether high levels of circulating Ang1 affect treatment.

**Evaluation of foreseeable effects**

Overall, the risk posed by this project is low. Mammalian cells (rodent and human) are very low risk as they are unable to survive in a non-histocompatible host. Unmodified Ad5 is associated with mild respiratory infections in children. The majority of the population is likely to have antibodies to the wild type virus, and therefore the immune system would rapidly clear any new potential infection. The virus however is non-enveloped and relatively resistant to desiccation. Unmodified Ad5 is a biological hazard group 2, and the replication deficient Ad5 virus is likely to be less pathogenic. However, due to the nature of the insert, the GMO is potentially more hazardous than the replication-deficient virus. There is no evidence in the literature that Ang1 is oncogenic. Ang1 poses a very low risk to humans and the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste, including liquid waste, will be inactivated by 2x autoclaving (temperature probes and autoclave tape will be used, and printouts per batch kept for validation) and chemical means (Vircon), as appropriate. Waste will be transferred in sealable containers. Solid waste will additionally be incinerated prior to disposal. These measures...
should kill all viable microorganisms.

Class II safety cabinets will be serviced twice a year (containment and functional validation and certification by approved contractors) following formaldehyde fumigation. All contaminated areas, equipment (including cryostats and microscope stages) and plastic waste will be cleaned after each use using Vircon and 70% ethanol or IMS. Containment: all work will be carried out in designated laboratories using good laboratory practice following the Gray Cancer Institute codes of practice (see risk assessment for further details).

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Comments from the GMAG Safety Committee meeting held on 26 September 2002.

After careful consideration of safety aspects and location of the project the committee approved the application.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Conf</td>
<td>L3 L4 L2 L3</td>
<td>L4 L2 L3 L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2 L3 L4 L2 L3</td>
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**Project Ref** 337/02.2

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<td>EXPRESSION OF RHO PROTEINS IN MAMMALIAN HUMAN AND RODENT CELLS USING REPLICATION-DEFECTIVE ADENOVIRAL VECTORS.</td>
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<th>Non-GMM</th>
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Project notified under transitional arrangements
### Project Additional Information

#### Purposes of the contained use

To efficiently express Rho proteins in human and rodent endothelial cells using replication-defective adenoviruses. The aim is to determine whether Rho proteins are involved in the functional response of tumour blood vessels to tubulin binding agents and whether by modulating their expression this response can be altered. This project will involve the expression of Rho proteins using replication-defective adenoviral vectors in normal or tumour-derived human or rodent endothelial cells and the effect of treatment (combretastatin or other vascular targeting/tubulin-binding agent). Subsequently analysed.

Primary viral stocks (supplied by UCL) will be amplified by further rounds of infection in human kidney 293 cells. Virus will be harvested, purified and used to infect mammalian cells.

#### Recipient or parental organism

Human kidney cell line 293 will be used for purification of recombinant adenoviral particules. Primary cell lines as well as long-term established cell lines as well as that have no capacity to survive autonomously outside the laboratory will be infected with the adenoviruses. Cell lines to be used include: human or rodent primary endothelial cells and transformed human endothelial cell lines. No infectious virus has ever been recovered from transduced animals or humans, even if viral genome was detectable by PCR amplification. Since the viruses have been constructed elsewhere, there is no need for bacterial amplification.

#### Host/vector system

Replication deficient recombinant adenovirus strain 5 deleted of regions 1 and 3 (Ad5 1 3) containing cDNAs for Rho, Rac, cdc42, B-galactosidase, or GFP (Wojciak-Stothard et al, J cell Science, 2001, 114:1343-1355). This adenovirus is deleted for E1 (transcriptional regulator, required for assembly of infectious particles) and E3 (evasion of host immunity) and can only be propagated in specific, modified cells (human embryo kidney HEK293). It is therefore unlikely to spread from the transduced cells to humans or any other organisms. However, owing to the nature of production of these viruses, there is a possibility of reversion to wild-type viruses by recombination with viral genes stably expressed within 293 cells, thus producing wild type virus. In the adenoviruses used in this project the transgenes were inserted into the region where E1 has been deleted. That should ensure the loss of the transgene in the event of recombination to generate wild-type, replication efficient virus.

#### Origin & function

Human cDNAs encoding the small GRPases Rho, Rac, Cdc42, B-galactosidase and GFP (supplied by Dr Ridley, see Wojciak-Stothard et al, J cell Science, 2001, 114:1343-1355). The intention is to inhibit or activate specific intracellular signalling pathways by expressing these proteins transiently in cultured mammalian cells. Cells will be subsequently exposed to tubulin binding agents used as anti-vascular agents in the treatment of cancer. B-galactosidase and GFP will be used to identify cells that have been infected.

#### Evaluation of foreseeable effects

Adenoviruses are able to infect mammalian cells. The viruses used here are replication-defective, and therefore will not multiply in mammalian cells or spread between organisms. Safety procedures are in place so that the risk of a worker infecting their own cells with the adenoviruses is extremely low. Although the viruses are replication defective, adenoviral DNA can persist for several weeks in cells, and thus the proteins could potentially damage the cells infected. Infected cells are normally rapidly evaluated.

02/03/2022
cleared by the immune system, as most people have previously been infected with adenoviruses. There is no indication that Rho family proteins would hinder clearance of cells by the immune system. The waste disposal procedures eliminate the possibility of live adenoviral particles escaping from the Category 2 laboratory where they are handled, therefore the risk to the environment is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be deactivated with hypochloride (10,000 ppm active chloride) for at least 6 h and then disposed of down laboratory sinks. Pipette tips, culture dishes and pipettes will be deactivated with hypochloride (2,500 ppm active chloride) for at least 6 h and then autoclaved twice at 138 degrees C for 30 min and then disposed of by incineration. Solid waste will be autoclaved twice as above and then disposed of by incineration. Waste will be transferred in sealable containers. Temperature probes and autoclave tape will be used, and printouts per batch kept for validation. These measures should kill all viable microorganisms.

Class II safety cabinets will be serviced twice a year (containment and functional validation and certification by approved contractors) following formaldehyde fumigation. All contaminated areas, equipment (including cryostats and microscope stages) and plastic waste will be cleaned after each use using Vircon and 70% ethanol or IMS.

Containment: all work will be carried out in designated laboratories using good laboratory practice following the Gray Cancer Institute codes of practice.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Comments from the GMAG Safety Committee meeting held on 26 November 2002. The Committee endorsed the risk assessment and HSE application.

Project Containment

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<td>Human Clinical Applications</td>
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02/03/2022
Project Reference: 337/02.3

Date Ackn'd: 09/12/2002
Date Project Ceased: 29/06/2009

CU2 Project Title: IMMORTALISING AND TRANSFORMING MOUSE EMBRYO FIBROBLASTS USING RETROVIRUS

Class: Class 2
Culture Volume: < 1 litre
Non-GMM Consent: not applicable

Project notified under transitional arrangements: N

Historical Significant Changes

Withdrawn: N

Tick if notifying a connected programme of work: N

Project Additional Information

Purposes of the contained use

We aim to produce immortalised, transformed mouse embryo fibroblasts (MEFs). These MEFs are derived from knock-out, transgenic mice which express only single isoforms of the vascular endothelial growth factor (VEGF: 121, 164 or 188).

In detail:
DNA from plasmid vectors containing the Simian Virus 40 (SV40) large T antigen or activated ras will be prepared from transfected E. coli cultures. The plasmids will then be transfected by non-viral methods (Ca-phosphate or similar) into specific packaging cells (BOSC 23). BOSC cells contain the packaging signals required to produce infectious ecotropic retroviral particles. High titre virus will be harvested from the BOSC cells and used to transduce mouse embryo fibroblasts (MEFs). The MEFs will first be immortalised, using SV40 large T antigen, and subsequently transformed to tumourigenic cell lines using activated ras. The 'fibrosarcoma' cells can then be used to form tumours in mice.

The vectors (pBABE and pLXSN) will be transfected into E. coli Top10 cells and the DNA prepared (Maxi prep). These E. coli are multiple auxotrophs, recombination deficient. The packaging cells, BOSC 23, are a human kidney cell line which has undergone a range of modifications. Human embryonic epithelial kidney cells were transformed and immortalised using sheared human Ad5, producing the cell line 293. These were further modified for high transfectability by addition of a temperature sensitive (ts) SV40 large T antigen, producing cell line 293T/17. In several further steps the retroviral genes gag, pol and env were inserted to produce a helper-free packaging cell line, BOSC 23 (Pear et al., PNAS, 1993). BOSC 23 cells are specifically used as ecotropic (mouse specific) envelope-expressing cells which produce infectious retrovirus at high titer.

The retrovirus will subsequently be used to infect mouse embryo fibroblasts (MEFs), derived from transgenic mice. These primary cells have a limited life span unless they are immortalised. No known risks are attached to their use.

Mammalian cells are very low risk as they are unable to survive in non-histocompatible hosts.

Recipient or parental organism

The vectors (pBABE and pLXSN) will be transfected into E. coli Top10 cells and the DNA prepared (Maxi prep). These E. coli are multiple auxotrophs, recombination deficient. The packaging cells, BOSC 23, are a human kidney cell line which has undergone a range of modifications. Human embryonic epithelial kidney cells were transformed and immortalised using sheared human Ad5, producing the cell line 293. These were further modified for high transfectability by addition of a temperature sensitive (ts) SV40 large T antigen, producing cell line 293T/17. In several further steps the retroviral genes gag, pol and env were inserted to produce a helper-free packaging cell line, BOSC 23 (Pear et al., PNAS, 1993). BOSC 23 cells are specifically used as ecotropic (mouse specific) envelope-expressing cells which produce infectious retrovirus at high titer.

The retrovirus will subsequently be used to infect mouse embryo fibroblasts (MEFs), derived from transgenic mice. These primary cells have a limited life span unless they are immortalised. No known risks are attached to their use.

Mammalian cells are very low risk as they are unable to survive in non-histocompatible hosts.
The retroviral vectors to be used are based on the Moloney murine leukemia virus (MoMuLV), or on both MoMuLV and Moloney murine sarcoma virus (MoMuSV), pBABE and pLXSN, respectively. They are ecotropic, i.e., they will only infect mouse cells due to the virus envelope carried within the BOSC cells. The retroviral vectors (pBabe and PLXSN) have most of the viral structural genes missing, so that these vectors cannot replicate (Morgenstern and Land, NAR, 1990; Clontech). To prepare viral stocks, packaging cells are used which contain the complementary genes in the form of an integrated provirus, but lacking the sequence recognized by the packaging apparatus. Thus, the packaging provirus produces all the proteins required for packaging of viral RNA into infectious virus particles but it cannot package its own RNA. Instead, RNA transcribed from the transfected vectors is packaged into infectious virus particles and released from the cells. The resulting virus stock is termed helper-free because it lacks wild-type replication-competent virus. Production of wild type virus by homologous recombination is largely eliminated by multiple genetic changes of the packaging cells and the vector, and more than two crossover events would be required for generation of replication competent virus (Pear et al., 1993).

The retroviruses to be used here are ecotropic infecting only mouse cells. The risk to humans is therefore very low, but care has to be taken not to endanger the in-house mouse colony. The wild type virus is oncogenic in mice. This risk is greatly reduced because the viruses are replication deficient and can only be propagated in specific packaging cells. Additionally, retroviruses require close contact and dividing cells for transmission, and their survival in the environment is poor.

**Host/vector system**

The SV40 large T-antigen (adenovirus EB1 55k protein, SV40 Tag) causes cell transformation in tissue culture and in animals. Unlike many other oncogene products that must act co-operatively to fully transform cells, the T antigen alone can convert primary cells to tumorigenic cells. SV40 Tag binds and inactivates cellular tumour suppressor gene products, including Rb and p53. It interferes with p53 leading to a reduction in cellular apoptosis and subsequent immortality. SV40Tag cooperates with ras oncogene to transform rat embryo fibroblasts (Cavender et al., J Virology, 1995).

Oncogenes are mutated forms of proto-oncogenes which function to control cell proliferation and differentiation. When proto-oncogenes mutate to become oncogenes the result is excessive cell multiplication leading to malignant transformation. The largest class of oncogenes are signal transducers that act as proteins which transmit signals from a receptor to their cellular target. These include the Ras family. Uptake of GTP activates Ras. Activated Ras phosphorylates the first member of a three-enzyme cascade (MAPK kinase cascade). Activated MAPK has an immediate impact upon a variety of cytoplasmic processes and a later impact upon gene expression, where it phosphorylates a transcription factor increasing expression of its target genes. The cytosolic effects of MAPK are accomplished by phosphorylation of inhibitors (Inh) of translation initiation factors. (TIFs). The phosphorylation changes the affinity of the inhibitor for the TIF, releasing and activating the TIF. The result is increased translation of target mRNAs in the cytosol.

Both SV40Tag and activated ras function as oncogenes able to transform the target cells. Both genes are controlled by strong constitutive promoters.

**Origin & function**

Overall, the risk posed by this project is low. Although the genes used to immortalise the cells are oncogenic, the ecotropic retrovirus is replication deficient and infects only mouse cells. Risk to human health is therefore low. The BOSCs and immortalised MEFs carry a very low risk to human and animal health as they are unable to establish themselves in non-histocompatible hosts. Once the oncogenes have integrated in the MEFs, and no life virus can be demonstrated, cells can be handled safely at level 1.

**Evaluation of foreseeable effects**

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste, including liquid waste, will be inactivated by 2x autoclaving (temperature probes and autoclave tape will be used, and printouts per batch kept for validation) and chemical means (Vircon), as appropriate. Waste will be transferred in sealable containers. Solid waste will additionally be incinerated prior to disposal. These measures should kill all viable microorganisms.

Class II safety cabinets will be serviced twice a year (containment and functional validation and certification by approved contractors) following formaldehyde fumigation. All
Contaminated areas, equipment (including cryostats and microscope stages) and plastic waste will be cleaned after each use using Vircon and 70% ethanol or IMS. Containment: all work will be carried out in designated laboratories using good laboratory practice following the Gray Cancer Institute codes of practice.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Comments from the GMAG Safety Committee meeting held on 26 November 2002. The Committee endorsed the risk assessment and HSE application.

### Project Containment

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<th>Non-GMM</th>
<th>Consent Granted</th>
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<tr>
<td>09/12/2002</td>
<td>CONSTRUCTION OF INDUCIBLE PLASMID SYSTEMS ENCODING ANGIOGENIC GROWTH FACTORS</td>
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Date Project Ceased 29/06/2009
Withdrawn N
Tick if notifying a connected programme of work N
Historical Significant Changes
Historical Date of Additional Info
**Project Additional Information**

**Purposes of the contained use**
We will investigate whether acute modulation of angiopoietin (Ang 1/2) or vascular endothelial growth factor (VEGF) activity in tumours can affect tumour vascular permeability and tumour response to vascular targeting agents (eg combretastatin A4-P). We will produce stably transfected tumour cells using the inducible Ecdysone or Tet-on/Off systems.

**Recipient or parental organism**
Bacterial hosts are multiple auxotrophs unable to survive in humans or the environment; the mammalian cells are long established cell lines (a tissue culture system where the vector does not have the ability to transfer DNA to other cells).

**Host/vector system**
The vectors are specifically designed as inducible systems which require specific inducing agents (Muristerone A, tetracyclin or similar) for gene expression, without inducing agents basal gene expression is very low; they are commercial plasmids (pIND and pVgRXR (Invitrogen); pTRE and pTet-on/pTet-Off (Clontech)) which are designed to express the genes of interest in mammalian cells but not in bacteria; the vectors are non-mobilisable.

**Origin & function**
The growth factor-encoding genes are unlikely to cause any damage to humans or the environment. The properties of the growth factors are well known and documented in the literature. Ang 1 and 2, and VEGF are of human origin and are involved in the maturation phase of angiogenesis. They are growth factors which act on tyrosine kinase receptors specifically on endothelial cells. Although they might give cells a growth advantage, they do not induce tumorigenicity, and therefore pose a low level of risk.

Ang1: Over-expression of Ang1 or treatment with exogenous Ang1 results in vessels which are particularly resistant to leaking. Ang1 acts as an endothelial cell survival factor preventing apoptosis.

Ang2: Ang2 counteracts blood vessel maturation/stability mediated by Ang1. Ang2 induces de-stabilisation of blood vessels during the process of angiogenesis.

VEGF: VEGF induces endothelial cell proliferation, migration and tube development. Both VEGF and Ang1 act as endothelial cell survival factors, preventing apoptosis. Adenoviral delivery of VEGF has been used for coronary artery disease and other cardiovascular diseases in over 14 gene therapy protocols worldwide.

The growth factors are not oncogenic and pose a low risk to humans and the environment.

**Evaluation of foreseeable effects**
Overall, the risk posed by this project is low. The GMO, namely bacteria (auxotrophs) and mammalian cell lines (low risk) are considered low risk. The growth factors are not oncogenic and pose a low risk to humans and the environment. HSE definition: Oncogenes make cell tumorigenic or give growth advantage, but oncogenes that give growth advantage without inducing tumorigenicity are low level of risk.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

---

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste, including liquid waste, will be inactivated by 2x autoclaving (temperature probes and autoclave tape will be used, and printouts per batch kept for validation) and chemical means (Vircon), as appropriate. Waste will be transferred in sealable containers. Solid waste will additionally be incinerated prior to disposal. These measures should kill all viable micro organisms.

Class II safety cabinets will be serviced twice a year (containment and functional validation and certification by approved contractors) following formaldehyde fumigation. All contaminated areas, equipment (including cryostats and microscope stages) and plastic waste will be cleaned after each use using Vircon and 70% ethanol or IMS.

Containment: all work will be carried out in designated laboratories using good laboratory practice following the Gray Cancer Institute codes of practice.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Comments from the GMAG Safety Committee meeting held on 26 November 2002:
The Committee came to the conclusion that this project should be presented to the HSE as a level 2 project, not as a level 1 project as initially envisaged, due to the nature of the growth factors involved.

Project Containment

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<th>Growth Rooms</th>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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02/03/2022
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**Name**

KINGS COLLEGE

**Name 2**

UNIVERSITY OF LONDON

**Department**

IMMUNOBIOLOGY ORAL PATHOLOGY & IMMUNOLOGY

**Campus Estate or Research Centre**

GUY'S CAMPUS

**Building**

FLOOR 3 NEW GUY'S HOUSE

**Road Name**

ST THOMAS STREET

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

SE19RT

**Country**

ENGLAND

**Tel Number**

0207 955 4355

**Fax Number**

0207 955 8894

**E-mail**

**HSE Division**

LONDON

**Comments**

MERGED WITH GM 295 ON 12/2/2003

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Glass House</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 342/95.1

Date Ackn'd: 11/10/1995  
Date Project Ceased: 12/02/2003

**CU2 Project Title**  
EVALUATION OF SYNTHETIC IMMUNOGENS AS NOVEL VACCINES

**Class**  
Class 2

**CultureVol**  
Class 2 Culture Volume Class 3-4

**Non-GMM**  
Consent Granted: not applicable

**Project notified under transitional arrangements**  
Y

**Historical Significant Changes**  
Project transferred to GM386 on 8/9/2003

---

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify) Tick if confidential

Laboratory Animal Unit Growth Room Glass House Large Scale

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
The maximum culture volume that could be released as liquid waste would be in the order of 200 mls. per day, although it would normally be less than this. At present this is treated with a Biocide prior to disposal via drains, but this is about to be changed in favour of autoclaving for 20 minutes at 120 deg. Contaminated plasticware is autoclaved as above prior to collection and subsequent incineration by an accredited waste disposal contractor.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 351

Data Premises Notified (Originally) 14/03/1990

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

ZOOLOGICAL SOCIETY OF LONDON

Name 2

Department

INSTITUTE OF ZOOLOGY

Campus Estate or Research Centre

Building

Road Name

REGENT'S PARK

District

Town

LONDON

County

GREATER LONDON

Postcode

NW1 4RY

Country

ENGLAND

Tel Number 0207 449 6631

Fax Number 0207 586 2870

E-mail

HSE Division LONDON

Comments

Date at Which Additional Info Submitted

02/03/2022

Page 6437 of 15326
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<td>Microbiology Research</td>
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02/03/2022
The maximum volume of deactivated culture waste that will be disposed of at any one time will be 0.5 litre. All liquid and solid contaminated waste will be deactivated through autoclaving. This method of deactivation has been validated for the relevant autoclaves by attempted culture of routinely used strains of E.coli from autoclaved waste - no bacterial growth was observed from the waste. The deactivation method will be monitored through the use of temperature sensitive strips placed in the waste.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 353**

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**Name**

UNIVERSITY OF CAMBRIDGE

**Name 2**

ADDENBROOKES HOSPITAL

**Department**

DEPT OF MEDICINE

**Campus Estate or Research Centre**

**Road Name**

HILLS ROAD

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 2QQ

**Country**

ENGLAND

**Tel Number**

01223 336850

**Fax Number**

01223 336846

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

21/08/2002

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02/03/2022

Page 6440 of 15326
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

| Laboratory | Animal Unit | Growth Room | Glass House | Large Scale |
|------------|-------------|-------------|-------------|-------------|-------------|

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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Project Ref 353/00.1
ANALYSIS OF HUMAN CYTOMEGALOVIRUS SPECIFIC CYTOTOXIC T CELL AND NATURAL KILLER CELLS USING HCMV DELETION MUTANTS

Date Ackn'd: 12/01/2000

Date Project Ceased: 12/01/2000

Class 2

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: GM353/00.1

Historical Date of Additional Info: 12/01/2000

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 353/00.3

Date Ackn'd 12/01/2000

CU2 Project Title

HUMAN CYTOMEGALOVIRUS GENE REGULATION DURING LATENT AND LYTIC INFECTION OF HUMAN CELLS.

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes GM353/00.3,

Historical Date of Additional Info 12/01/2000

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022

Page 6445 of 15326
Project Ref 353/00.6

Date Ackn‘d 04/05/2000

Date Project Ceased

CU2 Project Title EVALUATION OF A VACCINE AGAINST HPV 16 & 18 AS TREATMENT FOR ANO-GENITAL INTRAEPITHELIAL NEOPLASIA

Class 2

CultureClass2 CultureVolumeClass-4

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref 353/10.1

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<td>08/07/2010</td>
<td>Investigation into TB Associated Genes in Host Responses to BCG Infection using a</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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The project involves using BCG-EGFP for visualization of BCG infection of macrophage in culture to analyse cellular gene expression changes after infection. Experiments will include the preparation of BCG-EGFP and subsequent infection of primary human macrophages.

**Recipient or parental organism**

The parental organism is Bacillus Calmette-Guerin (BCG) vaccine strain.

**Host/vector system**

Plasmid pTKmx for generation of BCg-EGFP is a mycobacterium-E.coli shuttle vector that harbors a promoterless xylE reporter gene downstream of a transcriptional terminator. This shuttle introduce enhanced green fluorescent protein gene (egfp) into BCG.

**Origin & function**

The green fluorescent protein (GFP) is protein composed of 238 amino acids (26.9kDa), which exhibits bright green fluorescence when exposed to blue light. GFP was first isolated from the jellyfish Aequorea victoria. GFP has been optimized for brighter fluorescence and higher expression in mammalian cells, thus the modified GFP called enhanced GFP (EGFP).

**Evaluation of foreseeable effects**

BCG vaccination are effectively used for prevention of TB in the world, and complications of BCG vaccination are uncommon. BCG has been confirmed as effective and safe in the control and prevention of TB in human for more than 60 years. Cattle are the natural host of BCG.

EGFP has been a popular marker for both cellular and molecular research for decades and no harmful effects of over-expression have been reported. Therefore, the severity of accidental infection with BCG-EGFP should not exceed that of BCG vaccinations.

BCG-EGFP has been used for research purposes for over 20 years and accidents using BCG-EGFP have not reported. Therefore it is not expected to have any effect on the pathogenic or environmental survival characteristics of the unmodified BCG host.

The members of research team have no contact with cattle or other bovine species.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Culture and application of BCG-EGFP will be carried in full Containment Level 2 laboratories.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All discarded cultures, media and materials for tissue culture/bacterial culture and infection will firstly be treated with Trigene (1:20). Trigene has a wide spectrum of activity against viruses, some fungi, and bacteria. 5% Trigene has been tested specifically on mycobacteria cultures and has been shown to be 100% effective. After Trigene treatment, flasks, plates and tubes etc will then be autoclaved - this is standard operating procedure for the CL2 laboratories in the Department of Medicine. Autoclaves are routinely tested using "spore strips".

Liquid waste will be disposed to the drain. These measures will reduce the likelihood of all hazards to negligible (100% kill). The overall risk is therefore effectively zero with the proposed waste management measures.

Experiments are planned to infect primary macrophage cultures using an EGFP-tagged BCG. Although a hazard group 2 organism, BCG vaccination is effectively used for prevention of TB - complications of BCG vaccination are uncommon. An EGFP-tagged variant would be unlikely to alter the natural pathogenicity/tissue tropism of BCG. The committee agreed with the risk assessment of the work as a Class 2 activity. All work will be carried out in the Department of Medicine's containment level 2 laboratories.
Identifying human genetic variants that control gene expression in macrophages upon Mycobacterium tuberculosis (MTB) infection

Date Project Ceased

19/05/2016

Consent Granted

Yes

Non-GMM Consent Granted

Project notified under transitional arrangements

N

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The project involves using:

- MTB-GFP (green), MTB-mCherry (red) and MTB-Charge3 (red) for visualization of MTB infection of cultured macrophages to monitor phagocytosis and analyse cellular gene expression changes upon to infection. Experiments will include the preparation of MTB-GFP/mCherry/Charge3 and subsequent infection of primary human macrophages.

- MTB-luxAB will be used to monitor survival of bacteria after infection. Experiments will include the preparation of MTB-luxAB and subsequent infection of primary human macrophages.

- MTB transposon mutants will be used in the same manner to study the role of certain genes in the above mentioned procedures.

Recipient or parental organism

The parental organism is Mycobacterium tuberculosis H37RV laboratory strain

Host/vector system

MTB-GFP:
plasmid pATB51

MTB-mCherry:
plasmid pCHERRY3 (Addgene ID 24659)
vector backbone: pSMT3-S (5582 bp, hygromycin resistant), insert: mCherry (1450 bp, synthetic gene)/, promoter: Psmyc, host: DH5alpha
MTB-Charge3: plasmid pCHARGE3 (Addgene ID 24658)
vector backbone: pSMT3-S (5582 bp, hygromycin resistant), promoter: Psmyc, insert: Turbo (1450 bp, synthetic gene), host: DH5alpha

MTB-luxAB:

MTB transposon mutants:

Origin & function

The inserted plasmids have no eukaryotic promoter, they encode a green fluorescent protein (EGFP)/red fluorescent protein (mCherry, Charge3) or luciferase gene.

The green fluorescent protein (EGFP) exhibits bright green fluorescence when exposed to blue light. GFP was first isolated from the jellyfish Aequorea victoria. GFP has been optimized for brighter fluorescence and higher expression in mammalian cells, thus the modified GFP called enhanced GFP (EGFP).

Red fluorescent proteins (mCherry, Charge3) are synthetic genes derived from DsRed, which was isolated from Discosoma striata. They have red-shifted excitation and emission maxima.

luxAB genes were originally subcloned from Vibrio harveyi into vector SMT1 and are expected to generate luciferase, an oxidative enzyme that catalyses light-emitting reactions.

Evaluation of foreseeable effects

The genetic modifications made by the insertion of fluorescent genes do not alter the virulence nor pathogenicity of the MTB H37Rv strain. Therefore, the foreseeable effects remain the same as for the parental strain.

Any gene function abolished or modified in the transposon library could result in a GMM that could have
A) no change in properties
B) reduced ability to grow or cause disease.

In these cases (A and B) the direct hazard of the inserted gene product results in a GMM that is less than or equal to that of the parental wild-type organism.

C) could, hypothetically, have a phenotype altered in favour of increased pathogenicity. For instance:

(i) Deletion of a repressor of virulence could result in bacteria in which genes normally only expressed during a particular phase of infection are switched on permanently. This may at first sight imply an increased hazard, but this is unlikely as the mechanism of pathogenicity in MTB is based on the ability to switch multiple sets of genes on and off at e.g. specific times.

(ii) Deletion of a growth repressor could increase replication; this is highly unlikely as the slow growth rate of mycobacteria is considered to be controlled by e.g. ribosome number. Thus, again, multiple genetic manipulations would be required to influence growth rate. Also, there is no evidence to suggest that increased growth rate would lead to an increase in TS virulence.

(iii) Deletion of a dominant antigen involved in immune recognition could allow the bacteria to avoid recognition and cause more disease. This is, again, highly unlikely as immune recognition is a multiple-antigen dependent event and it is known that TS deletion mutants of dominant antigens have reduced or unaltered virulence. Also, this would be highly unlikely as mycobacteriophages used for Mtb transposon mutant library deliver the transposon of interest in a gene-specific mechanism which allows a single and specific gene deletion for each mycobacteria.
With all this in mind, we believe it acceptable for the MTB transposon mutant library to be treated as hazard group 3 organism, exactly as the wild-type bacterium. In essence, GMM pathogenicity is unlikely to increase, and infectious route highly unlikely to change, therefore there is no increased exposure hazard risk or disease risk. It is also likely that many of these transposon mediated changes arise by natural mutation in the environment and many uncharacterised clinical strains of M.tuberculosis may well have such altered phenotypes.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The present notification does not contain any larger GMO.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Cultures and applications of the MTB strains described in the present notification will be carried in full containment level 3 laboratories, already routinely handling MTB, therefore no derogation is requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

After work, surfaces will be disinfected with 70% ethanol allowing a contact time until complete dry of ethanol, then with 10% Distal for a minimum of 10 minutes. Liquid waste will be discarded into 100% Distal. As the effective concentration of Distal is lower than 10%, any dilution made by addition of liquid waste will not reach a final concentration below 10%. Solid waste will be sterilised by autoclaving in the CL3 autoclave followed by incineration on the Hospital site. All surfaces in the CL3 laboratory will be disinfected with 70% ethanol and then 10% Distal on a weekly basis. Microbiological safety cabinet shall be fumigated prior to a cabinet service/inspection and always after any spills of infectious material. This is standard operating procedure for the CL3 laboratories in the Department of Medicine. These measures will reduce the likelihood of all hazard to negligible (100% kill). The overall risk is therefore effectively zero with the proposed waste management measures.

| Is an emergency plan required according to regulation 20? | Y |
| If yes, tick to confirm that it is attached to this form | Y |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

**Please enter comments on the GM safety committee on the risk assessment**

Experiments are planned to infect monocyte-derived macrophages using a EGFP/mCherry/Charge3/luxAB-tagged and mutant MTB. Although a hazard group 3 organism, the tagged variants would be unlikely to alter the natural pathogenicity/tissue tropism of MTB. The committee agreed with the risk assessment of the work as a Class 3 activity. All work will be carried out in the Department of Medicine's containment level 3 laboratory (Rm 5801). This laboratory is already registered and is routinely using un-modified MTB.

**Project Containment**

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02/03/2022
The aim is to characterise genetic factors expressed by Hazard Group I or 2 (HG1 or HG2) bacterial associated with infection or colonisation of vertebrates. We study multiple HG1 and HG2 bacteria including Salmonella enterica, Citrobacter rodentium (a mouse specific pathogen), Shigella sonnei, Clostridium difficile, Listeria monocytogenes, Klebsiellas, Staphylococci, Pneumococi, Enterococci, Aeromonas spp and Escherichia coli. Any Enterohemorrhagic E. coli (EHEC) used at CL2 do not encode verotoxins. Specific HG2 organisms excluded from this risk assessment are those on the Schedule 5 list of bacteria as well as Vibrio cholerae, Neisseria meningitidis, Bordetella pertussis/parapertussis and Corynebacterium diphtheriae. The allowed bacteria are associated with infections that can be acquired by different routes including oral, respiratory, parenterally through damaged body surfaces or via the urogenital tract. We also study commensal and environmental bacteria that do not normally cause infection. The aim of this work is to better understand the mechanisms by which these bacteria colonise or infect their hosts. We are also interested in how they avoid therapies such as vaccines and antibiotics by acquiring resistance. Our work may lead to the development of new antibiotics or vaccines.

Functional analysis of virulence-associated factors through genetic manipulation requires that the genes encoding them be mutated and the resultant phenotype characterised through various assays. It is also essential to confirm that any reduction in virulence is indeed due to the mutation created and not by so called ‘off target’ effects involving non-targeted genes. Mutagenesis is performed by a variety of techniques that involve introducing mutated DNA back into the targeted host bacteria. Validation is performed by reintroducing the wild type gene on a plasmid/chromosome and confirming restoration of the wild type phenotype in the complemented strain. E.
coli K12 routinely acts as an intermediate host for manipulating cloned DNA prior to reintroduction into the recipient bacteria. Antibiotic resistance genes and other non-toxic biological reporter genes (e.g. LacZ, CAT, GFP, Lux etc.) can be introduced into the recipient during manipulation. The recombinant bacterial products of this work are, however, sensitive to the antibiotics recommended for therapy of human infection.

To characterise bacterial proteins of interest we will overexpress these in calcium competent, non-virulent E. coli K-12 or B (DE3) strains using the pET vector system (transformation and induction by isopropyl β-D-1-thiogalactopyranoside, IPTG), and subsequently purify them for protein-based assays. The proteins will in most cases be fused with affinity tags such as 6HIs or FLAG tags to aid purification.

Individual or blocks of genes will be mutated using a variety of approaches including transposition, transduction, the one step lambda red system, other PCR-based systems, via suicide vectors or using recombinase/PCR approaches. Targeted genes may be amplified by PCR or recovered from chromosomal digests and cloned into existing suicide, expression or other vectors. Initially, the cloned genes may be introduced into a permissive intermediate host - E. coli K12, analysed (e.g. digests and sequencing) and then to their final host - a strain for mutagenesis or protein overexpression. Since we are sometimes working on related enteric pathogens that can share some virulence determinants and mechanisms of infection (e.g. S. enterica, E. coli, Citrobacter) we may introduce plasmids from one source into strains from other named species backgrounds. In some experiments non-toxic reporter genes and antibiotic resistance genes will be introduced into the mutated target gene.

Host bacteria include: -

K-12 and B derived E. coli (for the purposes of this project will be used only under containment level 2 requirements).
Salmonella enterica (all non-hazard Group 3 serovars) including disabled S. Typhi
E. coli (EHEC used at CL2 are verotoxin-negative).
Citrobacter rodentium
Shigella sonnei
Campylobacter spp
Listeria spp
C. difficile
Klebsiella spp.
Aeromonas spp
Staphylococci
Streptococci
Yersinia spp (all non-Hazard Group 3 species – specifically excluding Y. pestis)

Recipient or parental organism

We can use all wild type HG2 organisms as targets for mutagenesis except those mentioned in section 6. These bacteria are capable of causing diseases in humans and are classified below according to the main phenotypic characteristics. K-12 derived E. coli is a common intermediate host. The targeted bacteria include: -

Gram-negative enterics

The bacteria normally live in the intestines of humans and other animals where they can be part of the normal flora. Some are capable of causing enteric diseases (gastroenteritis, diarrhoea, inflammation etc.) and some can spread systemically in compromised hosts. They are normally spread via oral ingestion and not by aerosols.

Salmonella enterica (all non-hazard Group 3 serovars)
E. coli (EHEC used at CL2 are verotoxin-negative).
Citrobacter rodentium
Shigella sonnei
<table>
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<th>Pathogens</th>
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<td>Campylobacter spp</td>
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<td>Yersinia spp (all non-Hazard Group 3 species – specifically excluding Y. pestis)</td>
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**Gram-positive pathogens**

Can live on body surfaces but can form skin and wound infections. Can live in the respiratory tract.

- *Clostridium difficile*
- Enterococci spp
- Staphylococci
- Streptococci
- Pneumococci
- Listeria spp

**Others**

These can cause a variety of infections in humans. Common hospital-acquired bacteria

- Acinetobacter
- Pseudomonads

We use a variety of well characterised plasmid vector systems in addition to the hosts described in the previous section.

**Host/vector system**

We use a variety of well characterised plasmid vector systems in addition to the hosts described in the previous section.

Mob indicates mobility status (Mob + or -).

Tra indicates ability to self-conjugate (+ or -)

BoM is Basis of mobility and cannot be mobilised

The vectors include:

- pCVD442, (AmpR, Mob+, suicide vector can only grow in strains that have pir)
- PWSK29, (AmpR, derived from pBSKII and pGL339)
- pHG75, (AmpR, TcR, Mob-, Tra-, Bom-)
- TopoXL, (KanR, Mob-, Tra-, Bom-)
- pGEMT, (AmpR, Mob-, Tra-, Bom-)
- pUC18/19, (AmpR, Mob-, Tra-, Bom-)
- pACYC184, (CmR, TcR, Mob-, Tra-, Bom-)
- pAT153 and their derivatives (AmpR, TcR, Mob-, Tra-, Bom-)
- pET28a (KanR)
- pET22b (AmpR)

AmpR (Ampicillin resistance), KanR (Kanamycin resistance), CmR (Chloramphenicol resistance), TcR (Tetracycline resistance). None of these antibiotics would be used to
treat clinical disease associated with these pathogens.

Origin & function

Most of the DNA used in manipulations originates from the same bacteria we are targeting (homologous gene replacement). Antibiotic resistance genes and other non-toxic biological reporter genes (LacZ, CAT, GFP, Lux etc.) can be introduced into the recipient during manipulation. The recombinant bacterial products of this work are, however, sensitive to the antibiotics recommended for therapy of human infection. Genes may be introduced in expression vectors into non-hazardous E. coli K-12 or B strains for over-expression and purification of recombinant proteins. The recombinant bacterial products of this work are not likely to be hazardous and the recombinant bacteria will remain sensitive to the antibiotics recommended for therapy of human infection.

Evaluation of foreseeable effects

The mutants are characterised to confirm the nature of the introduced mutation (e.g. by DNA sequencing). They may then be analysed by a variety of phenotypic approaches including growth characteristics, antibiotic sensitivity, infection of mammalian cells, DNA/RNA/protein analysis, metabolomics, imaging etc. Complementation can be attempted to restore the wild type phenotype by introducing a wild type copy of the gene(s) back into the mutant bacteria via a plasmid or by introducing a copy into the genome. Occasionally, we may introduce a non-toxic reporter gene such as one encoding green fluorescent protein or β-galactosidase back into the targeted bacteria to monitor gene expression activity or ‘tag’ the bacteria to facilitate phenotyping.

We ensure that we do not move genes around that can potentially create a microorganism with enhanced virulence. For example, we would not introduce a gene encoding a known toxin into a non-toxic variant. Thus, there is no reason to assume that any of the mutants we generate will result in an enhanced virulence, although this cannot be completely excluded. Potential hazards to humans and the environment are mitigated by good practice, containment and training. In case of pregnancy or other immunocompromising conditions, the group leader is to be informed as early as possible (as outlined in the associated handbooks and SOPs).

In a normal healthy individual infection is a possibility. The infection route can be via mucosal surfaces or perforations in the skin. A number of symptoms can be associated with infections including diarrhoea, fever, general malaise etc. If good practice is adhered to, there is very little risk of individuals or other laboratory workers being infected. Some of these bacteria can infect animals but these are not present in the contained environment where experiments are performed. Care is taken to minimise contamination of surfaces or the formation of aerosols (e.g. via the use of disinfectants, protective clothing and safety cabinets). There is controlled access to the facilities and individual are trained to the appropriate standards.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All culture and application of bacteria will be carried in full Containment Level 2 laboratories.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

We operate a number of comprehensive and proven waste management measures which are conducted according to our local protocol for handling HG2 pathogens. This protocol has been in use for many years and involves the autoclaving of clinical waste material, chemical inactivation of liquid waste followed by autoclaving and careful management of waste movement. The GMM will be used within a containment level 2 laboratory in which all bacterial cultures, glassware etc. is decontaminated prior to disposal. Thus drains, sinks etc. do not pose a mode of transmission to the environment. Air movement is also strictly regulated in the laboratory environment. Solid biological waste is autoclaved using temperatures, cycles and conditions appropriate for the inactivation of biological material. Autoclaving is performed by departmental staff using approved conditions. temperature is 121 C for 30 minutes. Glassware etc. is decontaminated, as described above, prior to being autoclaved.

All disinfectants are used according to the manufactures instructions. We have validated the killing activity on each pathogenic species and look for 100% kill. Validation assays are routinely performed.
Risk assessment was presented to the Department of Medicine's Biological Safety Committee and then to the Department of Medicine's over-arching Safety Committee and authorised as Class 2 activities.

Please enter comments on the GM safety committee on the risk assessment

Risk assessment was presented to the Department of Medicine's Biological Safety Committee and then to the Department of Medicine's over-arching Safety Committee and authorised as Class 2 activities.

### Project Containment

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<td>Large Scale Activities L3 L4</td>
<td>Human Clinical Applications L2 L3 L4</td>
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### Project Ref 353/20.3

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>01/10/2020</td>
<td>In vitro infection of macrophages with Mycobacterium tuberculosis</td>
<td>Class 3</td>
<td>&lt; 1 Litre</td>
<td>Yes</td>
<td>N</td>
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</tbody>
</table>
### Purposes of the contained use

We seek to understand the host & pathogen interactions that occur during tuberculosis (TB), which in humans is caused by Mycobacterium tuberculosis (Mtb). Discoveries made using our zebrafish-Mycobacterium marinum (Mm) model system will be tested for their applicability in the human-Mtb system in vitro.

### Recipient or parental organism

Mycobacterium tuberculosis strains H37Rv. Rifampicin or isoniazid mono-resistant strains may also be used, and also clinical strains (excluding XDR strains) may be used to test results observed in laboratory strains, but none of these will be drug-resistant.

Escherichia coli K12, and a range of mammalian cell lines such as monocyte cell lines THP1 and MonoMac6, and the macrophage cell line J774.2

### Host/vector system

- Mtb-GFP (green), Mtb-mCherry (red) and Mtb-Charge3 (red)/Mtb-luxA and plasmids pATB51, plasmid pATB51, plasmid pCHARGE3, pSMT
- Mtb transposon mutants will also be used - phage: PhiMycoMarT7, insert T7 promoter, transposase gene, E. coli ori

### Origin & function

1. Strains will be made fluorescent by expressing either GFP, mCherry, Charge3, luxAB or other derivatives of these proteins.
2. A Mtb transposon library will be used to knock-out any gene in Mtb, which will then be screened for specific phenotypes. Knocked-out genes will then be complemented by either the identical gene from Mtb or from related Mycobaterial species to see if the wild-type gene activity can be rescued.
3. Mtb strains with deletions in specific genes, or gene fusions with epitope tags of fluorescent proteins, may also be generated using CRISPR/Cas9.

### Evaluation of foreseeable effects

The genetic modifications made by the insertion of fluorescent genes do not alter the virulence nor pathogenicity of the Mtb H37Rv strain. Therefore, the foreseeable effects remain the same as for the parental strain.

Any gene function abolished or modified in the transposon library could result in a GMM that could have:
- A) no change in properties
- B) reduced ability to grow or cause disease.
- C) could, hypothetically, have a phenotype altered in favour of increased pathogenicity. For instance
  - In these cases (A and B) the direct hazard of the inserted gene product results in a GMM that is less than or equal to that of the parental wild-type organism. In the case of (C): (i) Deletion of a repressor of virulence could result in bacteria in which genes normally only expressed during a particular phase of infection are switched on permanently. This may imply an increased hazard, but is unlikely as the mechanism of pathogenicity in Mtb is based on the ability to switch multiple sets of genes on and off at e.g. specific times. (ii) Deletion of a growth repressor could increase replication; this is highly unlikely as the slow growth rate of mycobacteria is considered to be controlled by e.g. ribosome number. And multiple genetic manipulations would be required to influence growth rate. Also, there is no evidence to suggest that increased growth rate would lead to an increase in TS virulence. (iii) Deletion of a dominant antigen involved in immune recognition could allow the bacteria to avoid recognition and cause more disease. This is highly unlikely as immune recognition is a multiple-antigen dependent event and is highly unlikely as immune recognition is a multiple-antigen dependent event and it is known that TS deletion mutants of dominant antigens have reduced or unaltered virulence.
  - In essence, GMM pathogenicity is unlikely to increase, and the route of infection is also highly unlikely to change; therefore there is no increased hazard associated with the GMMs and the risk of disease is unchanged.
Only GM micro-organisms will be produced in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Full Containment Level 3 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All Containment Level 3 waste (solid and liquid) will be autoclaved (134°C for 15 min), before removal for offsite incineration. It is expected that this will achieve 100% kill.

Project 2019/1 is to study the mechanism of infection of Mycobacterium tuberculosis, a hazard group 3 pathogen. Strains of M. tuberculosis to be grown include strain H37Rv, clinical isolates and naturally occurring multi-drug resistance (MDR) strains. Extreme drug resistance (XDR) strains will not be grown. All strains will be characterised for antibiotic resistance/susceptibility to ensure appropriate antibiotics are available should a worker be potentially infected. Strains will be grown on plates and in small scale liquid cultures. In addition to wild type strains, M tuberculosis will be genetically modified: (i) strains will express fluorescent proteins such as GFP, mCherry, LuxAB and their derivatives to allow visualisation by fluorescence light microscopy, (ii) a transposon library will be used to knock-out any potential gene in M. tuberculosis and plasmids will be used to express either identical or analogous genes from Mycobacterial strains to see if the phenotype arising from the knock-out can be complemented, (iii) the CRISPR/Cas9 system will be used to introduce mutations or tags to potentially any gene in M. tuberculosis. None of hgenetic modifications will increase the pathogenicity of M. tuberculosis or its ability to survive in the environment. None of genes expressed are toxic or oncogenic. The project is therefore correctly classified as Activity Class 3 and all handling of M. tuberculosis strains will be performed in a Class I MSC in a Containment Level 3 laboratory.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Project Ref  353/95.1
ANALYSIS OF SMOOTH MUSCLE CELLS EXPRESSING PROTO-ONCOGENES AND ANTI-ONCOGENES (CLASS 2) : GENERATION AND USE OF ADENOVIRAL VECTORS FOR GENE THERAPY

Date Ackn'd: 17/06/1995
Date Project Ceased: 12/01/2000

Class Culture Vol Class 2 Culture Volume Class 3-4
Class 2

Non-GMM Consent Granted: not applicable
Project notified under transitional arrangements: Y

Withdrawn: N
Tick if notifying a connected programme of work: N

Historical Significant Changes: GM353/00.2,
Historical Date of Additional Info: 12/01/2000

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 353/95.1A

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<td>17/06/1995</td>
<td>ANALYSIS OF SMOOTH MUSCLE CELLS EXPRESSING PROTO-ONCOGENES AND ANTI-ONCOGENES (CLASS 3)</td>
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<tr>
<td>yes</td>
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</table>

Tick if notifying a connected programme of work N

Withdrawn N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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</table>

02/03/2022
Project Ref: 353/trans1

Date Ackn'd: 01/02/1992

CU2 Project Title: HIV PACKAGING AND VECTOR STUDIES (CLASS 3)

Class: Class 3

Culture Vol: Class 2

Culture Volume: Class 3-4

Non-GMM: yes

Consent Granted: yes

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes:
GM353/00.4,

Historical Date of Additional Info: 12/01/2000,

Significant Change ID: 

Date of Significant Change: 

Project Additional Information

Purposes of the contained use:

Recipient or parental organism:

Host/vector system:

Origin & function:
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]
If yes, tick to confirm that it is attached to this form [ ]
Tick to confirm that you have attached a risk assessment to this form [ ]
Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 353/trans2

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<tbody>
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<td>26/11/1993</td>
<td>ANALYSIS OF IMMEDIATE EARLY GENES OF HUMAN CYTOMEGALOVIRUS</td>
<td>Class 2</td>
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26/02/2022
Tick if notifying a connected programme of work  N

Project notified under transitional arrangements  Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

Project Ref 43/01.6

Date Ackn’d 07/11/2001

CU2 Project Title CLONING AND EXPRESSION OF GENES OF VIRAL HUMAN MOUSE OR PARASITE ORIGIN AND OTHER RELATED ACTIVITIES

Class 2

Culture Volume Class 2-4 1-50 litres

Non-GMM not applicable

Consent Granted

Tick if notifying a connected programme of work N

Withdrawn N

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022

Page 6466 of 15326
### Purposes of the contained use

1. Identification of viral proteins that block various aspects of the host immune response.
2. Characterisation of the activity of viral and parasite proteins in vitro, in binding assays to several ligands and functional analysis, and comparison with the properties of the human or mouse counterparts.

### Recipient or parental organism

<table>
<thead>
<tr>
<th>Vaccinia virus and amphotropic retroviruses expressing reporter genes (e.g., LacZ, firefly luciferase, green fluorescent protein), selectable genes that confer resistance to specific compounds (Ecogpt, puromycin resistance gene) or the ability to grow in a rabbit cell line (vaccinia virus K1L gene), or coding sequences derived from viruses (poxviruses, herpesviruses), parasites (leishmania, schistosoma), humans or mice.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectromelia viruses expressing (i) reporter genes (e.g., LacZ, firefly luciferase, green fluorescent protein), (ii) selectable genes that confer resistance to specific compounds (Ecogpt, puromycin resistance gene), (iii) gene from poxviruses or herpesviruses or (iv) mouse or human genes.</td>
</tr>
</tbody>
</table>

### Host/vector system

<table>
<thead>
<tr>
<th>Eukaryotic viruses (ACDP group 2).</th>
</tr>
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<tbody>
<tr>
<td>Ectromelia virus (ACDP group 1).</td>
</tr>
<tr>
<td>Mammalian cell lines (established, commercially available) (ACDP group 1).</td>
</tr>
</tbody>
</table>

### Origin & function

**Origin**: Sequences will be amplified by polymerase chain reaction using viral, parasitic or cellular DNA as a template, or will be subcloned from pre-existing plasmid clones.

**Function**: The biological properties of the genes are considered in the detailed risk assessment. These genes will be expressed in mammalian cell lines (via eukaryotic vectors) to examine the location and function of proteins. Reporter and selectable genes will be inserted into viral genomes to inactivate specific viral genes and to identify the resulting recombinant virus.

The biological effects derived from inactivation of genes in poxviruses (vaccinia or ectromelia viruses) or from the replacement of ectromelia virus genes by homologous genes will be determined in vivo in a mouse model of infection.

### Evaluation of foreseeable effects

The insertion or reporter or selectable genes into viral genomes is expected to reduce the replication capacity of the recipient virus or to reduce its virulence. No increase in virulence, alteration of tissue tropism or modification of host range is predicted.

The eukaryotic vectors used (vaccinia, retrovirus) are either fully disabled or heavily attenuated. No change in tissue tropism or host range of the vectors expressing foreign genes is foreseen (see detailed risk assessment). The assessment to human health indicates no greater hazard than those of the wild type unmodified virus.

Whereas vaccinia virus and cowpox virus can replicate in a number of mammalian species, ectromelia virus is restricted to mice. Recombinant poxviruses lacking specific genes are expected to be attenuated and to transmit poorly between individuals. No change in tissue tropism or host range of the vectors expressing foreign genes is foreseen (see detailed risk assessment). The assessment to human health indicates no greater hazard than those of the wild type unmodified virus.
The ectromelia virus mutants lacking immunomodulatory genes are expected to be attenuated. Insertion into ectromelia virus mutants of related genes from other poxviruses or herpesviruses, or mouse/human genes, with similar functions, is unlikely to increase ectromelia virus virulence to levels higher than wild type. However, if an increased virulence is observed, it is unlikely to change the tissue and host tropism of ectromelia virus (highly restricted to mice), and therefore to represent a greater hazard to human health than wild type ectromelia virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| None |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| None |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Standard validated methods will be used: |
| Liquids: made 2000ppm available chlorine (5% chloros) and disposed via drains after a minimum of 12 hours. |
| Mixed disposable waste: Validated autoclave, 121 degrees/30 min, then disposed as domestic waste. |
| Disposable pipettes: Either a) as for mixed disposable waste or b) immerse in solution of 2000ppm available chlorine (5% chloros) for minimum 12 hours, followed by incineration. |
| Re-usable materials: Autoclave, 121 degrees/30 min. |

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

Please enter comments on the GM safety committee on the risk assessment

I had forgotten to inform you that the local ACGM committee had reviewed and approved the 6 applications.

## Project Containment

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02/03/2022
## Project Additional Information

### Purposes of the contained use

1. Understanding the biochemical and molecular basis for the function of receptors for the constant (Fc) region of IgG, FcgammaRs and 2. Dissecting the role of viral gene products in lytic replication, the establishment of latency and immune evasion.

### Recipient or parental organism

The cell lines we wish to transfect are murine B-cells lines and primary human dendritic cells and macrophages. None of these cell types are readily transfectable using standard transfection technology, necessitating the use of lentiviral vectors based on human immunodeficiency virus type 1 (HIV-1).

### Host/vector system

A three plasmid transient transfection system will be used to generate recombinant, replication-defective retroviruses. 293T cells, a human cell line carrying SV40 T antigen will be transfected with (a) An HIV-1 'self inactivating vector' (SIN). (b) An HIV -1 derived retroviral sequence © a plasmid encoding the envelope plasmid derived from vesicular stomatitis virus (VSV) G protein.

### Origin & function

1. Human and mouse cDNAs encoding Fcgamma receptors and their gamma chain subunits.
2. Human and mouse cDNAs encoding the soluble tyrosine kinase lyn.
3. MHV68 cDNA encoding M2 gene products.
4. Mutant forms of the above genes which will include point mutants and deletion mutants of putative functional domains.
5. The reporters His, FLAG, GFP, and lacZ.

Cells transduced with these genes will be used for the study of the effect of wild type and mutant Fcgamma receptors on the response of cells to immune complexes and opsonised bacteria. We hope to examine their role on phagocytosis, endocytosis and intracellular trafficking of immune complexes, cytokine production and antigen presentation using techniques established within this laboratory.

**Evaluation of foreseeable effects**

The genes to be expressed are not known to be harmful and so the classification of the work is determined largely by the nature of the host/vector systems. The lentiviral (HIV) vectors are disabled, self-inactivating (they are unable to propagate following transfection) and multi-component. Hence the risk of generating replication competent viruses (bearing the transgenes) is extremely low.

The lentiviral binding protein has been replaced by VSV G protein which confers potentially a much broader host range/tissue tropism. In response to this, these vectors will be at class 2. Furthermore, the VSV protein pseudotyping represents an aerosol/ingestion opportunity for infection in addition to the percutaneous risk associated with HIV. Therefore the risk assessment indicates the use of a microbiological safety cabinet for operator protection. Although complement sensitive, vectors bearing the VSV glycoprotein are reported to have increased environmental stability emphasizing the importance of paying due regard to principles of GMP/GOSH.

While there is a theoretical potential for animal infection with these virus vectors, all lentivirus work will be carried out in a building where no live animal work is undertaken thus minimizing the risk. Furthermore, as with inadvertent human contact, the viral vectors are self-inactivating and the expressed proteins are not known oncogenes. Thus the consequences of expression in a few cells would be minimal.

The worst case scenario therefore for an exposed individual would be infection of pre-cancerous cell by viral vector expressing immune evasion protein perhaps allowing tumour progression. The most accessible tissue is lungs, but likelihood is small (pre-cancerous tissue would be very much a minority tissue in healthy persons) and virus is always handled in a safety cabinet. This scenario is considered highly unlikely to occur, as all evidence to date suggests that insertion of the M2 protein into other cells would not enhance proliferation or permit immune evasion. Moreover there is no evidence that pre-malignant bronchial epithelium is surveyed by the immune system, and there is no data to suggest that a protein stimulating B cells could enhance immune evasion by epithelial cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be treated for 24 hours with 2% Virkon or Presept tablets, then gelled if necessary with Vernagel in a sealable container. This is double bagged and transferred to a waste autoclave, and then incinerated on site.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
This project has been reviewed by the Institutes Biological and Genetic Modification Safety Committee. This is satisfied that the risks have been properly assessed and the work will be carried out under the appropriate conditions and controls.

**Project Containment**

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**Project Ref** 678/11.1

**Date Ackn'd** 17/09/2019

**CU2 Project Title** Modulation of host immunoreceptors by viruses

**Class** Class 2

**CultureVol** < 1 Litre

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes** Project transferred from GM678

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

For a viral infection to be successful the virus must survive in the presence of the host immune response. Viral survival strategies have evolved to modify or evade these host immune responses including the modification of host immunoreceptors. This project addresses the modification of host immunoreceptors by the human herpesvirus and lentivirus families.
Human herpesviruses are a family of enveloped double-stranded DNA viruses. There are three subfamilies: alphaherpesvirinae [herpes simplex viruses 1 and 2 (HSV-1, 2); varicella zoster virus (VZV)], betaherpesvirinae [human cytomegalovirus (HCMV), human herpesvirus-6 (HHV-6) and human herpesvirus -7 (HHV-7)] and gammaherpesvirinae [Epstein-Barr virus (EBV) and Kaposi's sarcoma herpesvirus (KSHV)]. The herpesviruses are ubiquitous pathogens of all vertebrates, although infections are usually highly species specific. All herpesviruses apart from KSHV and HSV-2 infect the majority of the human population (seroprevalence of HSV-1, VZV, HCMV, HHV-6 and -7, EBV is 50-100% depending on the population studied, whereas HSV-2 approximately 20%, KSHV 1-50% depending on the population studied). One common feature of all herpesvirus infections is lifelong viral persistence after primary infection despite a strong cell-mediated immune response. Each virus can generate a latent infection in certain cell types. In immunocompromised individuals, such as recipients of organ transplants and patients with advanced HIV infection, reactivation of herpesviruses such as VZV, HCMV, EBV and KSHV, has the potential to cause severe disease.

Human immunodeficiency virus (HIV) is the lentivirus that causes AIDS. This condition is characterised by progressive failure of the immune system, which allows opportunistic infections and cancers to thrive. HIV-1 establishes chronic infections, affecting approximately 33 million people worldwide. Despite considerable advances in HIV science, the goal of eradicating HIV- remains elusive.

There is already considerable evidence of immunoreceptor modulation by herpesviruses and lentiviruses; for example, MHC class I is removed from the cell surface by HSV, HCMV, EBV and KSHV as well as HIV. This proposal aims to investigate novel mechanisms and consequences of viral manipulation of host immunoreceptors during infection. We aim to investigate both (a) the role of individual viral genes on host immunoreceptors (herpesviruses and lentiviruses), as well as (b) whole viral infection in both lytic and latent phases (herpesviruses only). (a) We will clone individual viral genes into delivery vectors, and deliver them to relevant cultured host cells. We will then characterise the effects of these modifications on the host cell. (b) We will generate herpesviral and disabled HIV-1 stocks in established human cell lines from viral seed stocks in established human cell lines from viral seed stocks or bacterial artificial chromosomes encoding the viral genome. Clinical isolates and their genetically modified derivatives will be used as well as laboratory adapted strains. In vitro infection of human cell lines and primary cell lines will then be carried out to model both lytic and latent viral infections. We will then characterise the effects of these viral modifications on the host cell. Viruses with deletions of, or tags attached to individual genes will be utilised in order to further characterise cellular effects.

**Recipient or parental organism**

Recipients will be various mammalian cells in culture. Most are well characterised and considered ACDP hazard group 1. Cells from volunteers are uncharacterised and although themselves present no greater risk, may contain adventitious infectious agents and therefore will be handled at CL2 (risk assessment under COSHH regs). Mammalian primary cells or cell lines are especially disabled and unable to survive or propagate outside of laboratory culture. Culture cells expressing the viral genes may be altered in particular surface proteins they express but those changes will not over ride the especially disabled nature of the cells.

The herpesviruses to be studied as detailed above are all ACDP hazard group 2 and will therefore be handled at CL2 within a class II microbiological safety cabinet (MSC). Pregnant or known immunocompromised individuals will not perform any of this work. The modified herpesviruses should be no more pathogenic than wild type. Although there are virus deletion mutants with increased virulence over their parent (for example deletion of A41L in vaccinia virus), to our knowledge, herpesvirus deletion mutants that show increase in virulence have not been described.

Viral gene delivery vectors (retroviruses, lentiviruses) to be used are those typically considered class 1 GMMs, and will all be self-inactivating, and therefore once packaged, are unable to propagate. A three plasmid transient transfection system will be used to generate the recombinant retroviruses and lentiviruses, thereby decreasing the likelihood of recombination within the packaging cell lines to generate replication competent virus. The retroviral vectors are based on the Moloney murine leukaemia virus in which the gag, pol and env genes have been deleted. Gag and pol are supplied in trans on a packaging plasmid. We will generate VSV-G pseudotyped virus in order to deliver the virus particles to human cells. The lentiviral vectors are based on an HIV-1 self-inactivating vector (SIN) containing a deletion in the U3 region of the 3’ LTR of the virus. In addition, the gag, pol, env, nef, vpu and vpr genes have been deleted. Again, a second packaging plasmid encoding gap, pol, rev and tat will be used along with the plasmid encoding VSV-G to make pseudotyped viruses that will enter human cells. A packaging plasmid encoding nef, vif, vpu and vpr may also be used, in order to improve transduction efficiency of primary human cells. While VSV-G increases host range and their tropism and mode of transmission may also be altered from that exhibited by wild-type retroviruses, aerosol risk to the airway epithelium appears limited but the inability to infect the apical cell surface. The AdZ adenoviral vector system will be used to generate recombinant adenoviruses. These vectors carry a deletion of the E1 gene region rendering them replication incompetent.
in non-complementing cell lines. In addition, the E3 region (important for viral pathogenesis and immune evasion) has also been deleted making them less pathogenic in humans. They are those typically classed as class 2 GMMs and remain so as the inserts are placed within the deleted regions. All three types of viral vectors will be harvested and used in a CL2 laboratory.

The molecular clone that encodes the disabled HIV-1 to be used in this project is derived from the HIV-1 backbone and is currently approved for use when working under CL2 conditions in the Department of Medicine at Addenbrooke's Hospital, Cambridge. To increase the safety of the system, the vector components are segregated onto 2 plasmids: an HIV-1 molecular clone with a critical env deletion and a second plasmid expressing the VSV-G envelope protein. None of the individual plasmids is capable of generating infectious viral particles and, to avoid the generation of replication competent virus via homologous recombination, the constructs do not contain overlapping homologous sequences. Since none of the cell lines or vectors carry intact env-encoding sequences, infectious HIV particles cannot be produced.

The viruses and viral delivery vectors we will use are ACDP hazard group 2/class 1 GM. The viruses are deactivated by treatment with trigene, virkon or 70% EtOH and autoclaving. Therefore following the waste procedures outlined below should eliminate risk of spread to the environments. Many of the herpesviruses are also strictly human specific, therefore there is no risk of spread to other animals or plants. The viral vectors are all replication disabled so will be unable to disseminate in the wider animal or human populations.

Modifications will be to add a tag such as a fluorescent protein to viral proteins, or to delete or disrupt viral genes and subsequently restore them. Expression of tags is not expected to be detrimental to human health or the environment. Deletions/disruptions to viral genes are not expected to increase infectivity/pathogenicity. Herpesvirus genes cloned into viral gene delivery vectors may confer an ability to alter immune responses to infected cells. However, the large number of viral immune evasion genes encoded by the human herpesviruses demonstrates the need for a virus to encode more than one immunoavasin in order to effectively evade human immune responses. We will only express a single herpesviral gene in each gene delivery vector. As the vectors are disabled, any accidental infection of the epithelial cells of the researcher would be localised. Transduced cells would not be able to produce viral particles. Due to the high turnover rate of epithelial cells, any localised infection would not be expected to be long lasting.

Pseudotyped env-deleted HIV-1 viral particles are replication deficient, but theoretically able to transduce epithelial cells of the researcher. If this occurred, all HIV-1 proteins except env would be expressed, but no replication competent virus would be formed. This protein expression would not be expected to cause harm, and the Mammalian tissue culture cell lines (eg HFF2, THP-1, KG-1, HeLa, U373, U937, Huh7, HCT116, Jurkat, Daudi, NK-92, NK-L, HL60, 220, T1, 293, Ramos).

Primary human cells (eg monocytes, dendritic cells, T cells, B cells, NK cells).

Human herpesviruses including HSV-1 (eg strains: F, KOS, 17, SC16), HSV-2 (eg strains: 333, HVD), VZV, EBV, HCMV (eg strains: Toledo, Merlin, TB40/E, VR1814/FIX-BAC and laboratory adapted strains AD169 and Towne), HHV-6, HHV-7 and KSHV.

A disabled HIV-1 virus with a critical env deletion will also be used.

Bacteria: Top10, Sure 2, alpha-select, clean genome.

Viral gene delivery vectors including retroviruses, lentiviruses and adenoviruses.

Bacterial artificial chromosomes (BACs) that cover the genomes of the herpesviruses and adenovirus serotype 5 so that insertion/deletions in the viral genome can be generated in vitro prior to the generation of recombinant viruses.

Standard cloning vectors (such as TOPO and pcDNA3) and BAC shuttle vectors will be used to clone viral gene disruptions (such as truncations) and to add inserts/tags onto viral genes or to rescue deletion viruses. In addition, these cloning vectors will be used to express individual viral genes to allow their study in host cells.

The HIV-1 molecular clone with the env deletion will be used for targeted gene disruption in vitro prior to the generation of lentiviral particles.
Inserts will be used to label herpesviral and lentiviral genes to enable their visualisation during viral infection (such as the YFP, GFP or RFP fluorescent tags) or to simplify biochemical analyses of viral genes (such as HA, Strep, Myc and Flag tags). To examine the biological role of individual herpesviral and lentiviral genes, gene deletions/disruptions and subsequent reconstitutions will also be used.

Expected biological action of inserted DNA/RNA or transcribed/translated gene product:
The inserts will be commonly used markers/tags as detailed above. Apart from fluorescence, these tags are biologically inert; their expression should not increase viral virulence or fitness.

Deletion or disruption of viral genes is not expected to increase virulence. For reconstitution of deletion viruses, only the original gene will be re-inserted and therefore no increase in virulence over the original strain is expected. No modifications will be made that are designed to introduce new biological activities into viral proteins. Over-expression of viral genes in viral gene delivery vectors should not pose any risk additional to use of the viruses themselves.

**Evaluation of foreseeable effects**

Pseudotyped particles have previously been classified as class 2.

Bacterial artificial chromosomes and plasmids will be used to generate recombinant virus. These contain antibiotic resistance markers which could in theory present a problem to the user if they become infected with the bacteria in which the plasmids/BACs were grown. However, in practice the bacterial strains we use are highly attenuated and unlikely to cause disease, while the antibiotic resistance markers encoded by these plasmids/BACs (Ampicillin, kanamycin and chloramphenicol) are active against antibiotics which are no longer in general use in clinical practice. So whilst the plasmids/BACs pose no threat in a bacterial culture, once transfected into eukaryotic cells the recombinant DNA can be packaged into infectious virions and transfection will therefore be carried out in a CL2 laboratory and subsequent cultures treated as live virus.

The disabled nature of the bacterial hosts will not be altered by the plasmids or BACs in use - no expression is anticipated and would not in any case be compatible with bacterial systems. The bacterial strains we use are highly disabled and unable to replicate to any extent outside the lab. Never-the-less, appropriate control measures, including disinfection (with virkon) of liquid cultures and autoclaving of bacterial plates, will be taken.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Conventional molecular biology techniques will be used for cloning in a CL1 laboratory. Bacterial artificial chromosomes and viral gene delivery vectors will be transfected into mammalian cell lines in a CL1 laboratory then transferred to the CL2 laboratory within 24 hours of transfection (to allow us to evaluate transfection efficiency with a fluorescence microscope). All manipulation of liquid will be carried out in a class II Microbiological safety cabinet (MSC) to reduce the risk of aerosol dissemination. Tubes for ultracentrifugation of virus for the production of concentrated virus stocks will be loaded in MSC into buckets that will be sealed and transported to the ultracentrifuge in secondary containers. Similarly, once the ultracentrifugation is complete, the tubes will be transported back to the CL2 laboratory and the buckets opened in the MSC to allow the virus to be resuspended and aliquoted. Since there is no -80°C storage facility in our CL2 laboratory, small aliquots of virus will be transported to 5.29 in secondary containment for storage. Fluorometric and microscopic analysis will be conducted on fixed samples, and protein and nucleic acid analysis will be conducted on material which has been lysed in the CL2 facility prior to transport to the laboratory.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

The ultracentrifuges available for concentrating virus preparations are outside of CL2 labs. Virus preparations will be sealed within the centrifuge tubes and buckets within the MSC in CL2 and transported in a shatterproof container. The surfaces of centrifuge tubes and transport containers will be decontaminated with detergent prior to removal from CL2 and only be re-opened once returned to the MSC in CL2. The ‘comments’ section of the ultracentrifuge logbook will be used to warn other users that the centrifuge contains a CL2 organism. Following centrifugation, the buckets will be returned to the MSC in the CL2 for processing. The buckets will then be immersed in detergent in the MSC prior to removal back to 5.14 for cleaning, rinsing and drying.

The -80 degree freezer for storing virus preparations and supernatant is also outside of the CL2 labs. Vessels containing supernatant and concentrated virus preparations will be decontaminated with Trigene and put into a shatterproof container for transport to and storage at -80 in Lab 5.19
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be disinfected with Virkon overnight, gelled using Vernagel, double bagged, sealed and labelled for autoclave then incineration according to the Department CL2 procedures. All solid waste will be double bagged and labelled for autoclave then incineration (pipettes will be disinfected in 1% trigene before removal from MSCs to prevent drips). Work surfaces will be wiped down with trigene and 70% ethanol. Small spillages will be mopped up immediately with tissue and disinfected with 1% trigene or 2% virkon. Large spills will be covered with absorbent paper and then sprayed with 10% trigene or 2% virkon, and then mopped up after 30 minutes. The absorbent material will then be disposed of in the solid autoclave waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project has been reviewed by the Institutes Biological & Genetic Modification Safety Committee. The Committee is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 678/15.1

Mechanisms of protein degradation - using (i) overexpression or depletion of tagged or untagged genes involved in protein breakdown in lentiviral and retroviral systems

Date Ackn'd 16/09/2019

Date Project Ceased

Class

CultureVolClass2 < 1 Litre

Consent Granted

Project notified under transitional arrangements N
All mammalian cells have to control their protein content to remove damaged proteins and regulate cell growth. Any disruption in cellular protein levels can cause uncontrolled cell turnover or the accumulation of misfolded proteins that occur in neurodegenerative conditions. The major mechanisms for controlling intracellular protein levels are through ubiquitination, proteasomal degradation and autophagy. This project aims to address how ubiquitinated proteins are differentially selected and efficiently delivered to the proteasome for degradation. The research encompasses several main themes:

1) How different polyubiquitin chains are decoded.
Specificity in the ubiquitin system is generated by the ability of ubiquitin to form eight different polyubiquitin chain linkages. Each type of ubiquitin linkage must be correctly interpreted to facilitate the desired outcome, and ubiquitin binding proteins (UBPs) provide this critical link between chain recognition and cellular fate. For example, we showed chain-specific UBPs facilitate lysine-48 chains to bind the proteasome but block lysine 63-chains from binding this degradative complex. UBPs for other polyubiquitin chain types were unknown, but we have identified UBPs that selectively recognize lysine-11 polyubiquitin chains, an abundant linkage with a critical role in regulating mitosis. Using biochemical and cell biology approaches we aim to investigate the function and physiological importance of these lysine-11 specific UBPs.

2) How ubiquitin binding proteins regulate protein turnover.
We will use forward genetic screens in near-haploid human cells (KBM7) to identify genes required to regulate the degradation of proteins by the proteasome. By the insertion of random mutations into cells expressing fluorescent proteasome reporters, we can identify genes required for the efficient degradation of these ubiquitinated substrates. Retroviral gene traps and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology will be used in the screens. As ubiquitination is also involved in non-proteasomal degradation, we will examine how ubiquitinated proteins are recruited to autophagy and lysosomal pathways.

3) Regulation of the hypoxia response pathway.
We will use forward genetic screens and proteomics to determine novel genes in the regulation of hypoxia inducible transcription factors (HIFs, a pathway critically regulated by ubiquitination). Genes identified as regulating HIFs will be characterized in cell line based assays and primary cells (macrophages and fibroblasts) for their importance in the hypoxia response. Other oxygen-dependent regulatory mechanisms, for example oxidative phosphorylation and protein hydroxylation, will also be studied. To investigate these aims we will use lentiviruses and retroviruses to:
a) Generate stable mammalian cell lines encoding UBPs, ubiquitin enzymes, genes regulating protein breakdown or oxygen consumption of cells using pHRSIN vectors.
b) Use shRNA with pSIREN vectors to confirm the function of genes identified in the mutagenesis screens.
c) Generate stable knock-out or knock-in (tagged) cell lines of genes identified in the genetic screens using CRISPR technology in lentiviruses encoding the Cas9 nuclease and guide RNAs (sgRNA) (pLKO-TRC).
d) Use stable and tetracyclin-inducible shRNA and sgRNAs. These stable cell lines with be utilised to further characterise the cellular effects of genes required in the regulation of the ubiquitin proteasome system.

Recipient or parental organism

Mammalian tissue culture cell lines (eg HFF2, THP-1, KG-1, HeLa, U373, U937, Huh7, HCT116, Jurkat, Daudi, NK-92, NK-L, HL60, T1, 293, Ramos).
Primary human cells (eg monocytes, dendritic cells, skin fibroblasts, muscle cells, T cells, B cells, NK cells).
Bacteria: Top10, Sure 2, alpha-select, clean genome.

Host/vector system

Viral gene delivery vectors including retroviruses, lentiviruses, and adenoviruses.
The lentivirus and retrovirus plasmids contain the WPRE-X
2nd generation retrovirus and lentivirus packaging systems will be used.

Origin & function

Plasmids and other nucleic acid vectors:
Standard cloning vectors (such as TOPO and pcDNA3) and BAC shuttle vectors will be used to clone gene disruptions (such as truncations) and to add inserts/tags onto genes or to rescue deletions. In addition, these cloning vectors will be used to express individual genes to allow their study in host cells.
Inserts:
Inserts will be used to (i) label genes to enable their visualisation during physiological and biological studies of protein degradation (such as the YFP, GFP or RFP fluorescent tags), (ii) encode proteins involved in the ubiquitin proteasome system, autophagy or the hypoxia response, with no known toxicity or oncogenicity, or (iii) to simplify biochemical analyses of genes of interest (such as HA, Strep, Myc and Flag tags). To examine the biological role of individual genes, gene deletions/disruptions and subsequent reconstitutions in the ubiquitin proteasome pathway, autophagy, lysosomal function and the hypoxia response pathway.

Evaluation of foreseeable effects

The inserts will be commonly used markers/tags as detailed above. Apart
from fluorescence, these tags are biologically inert; their expression should not increase activity of the target genes.
No modifications will be made that are designed to introduce new biological activities into the proteins. Over-expression of genes involved in the ubiquitin proteasome system, autophagy and hypoxia response should not pose any additional risks. Uncharacterised genes will only be introduced into viruses when they have been better characterised using standard mammalian expression vectors and shown to function in the recognition of ubiquitin chains, protein degradation, autophagy or hypoxia regulation, and therefore unlikely to be either toxic or oncogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be disinfected with Virkon overnight, gelled using Vernagel, double bagged and sealed and labelled for autoclave then incineration according to the Department CL2 procedures. All solid waste will be double bagged and labelled for autoclave then incineration (pipettes will be disinfected in 1% Distel before removal from MSCs to prevent drips). Work surfaces will be wiped down with Distel and 70% ethanol. Small spillages will be mopped up immediately with tissue and disinfected with 1% Distel or 2% virkon. Large spills will be covered with adsorbent paper and then sprayed with 10% Distel or 2% virkon, and then mopped up after 30 minutes. The absorbent material will then be disposed of in the autoclave waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

None

Project Containment

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<th>Laboratory Activities</th>
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02/03/2022
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02/03/2022
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**Name**

NOVARTIS INSTITUTE FOR MEDICAL SCIENCES

**Name 2**

SANDOZ INSTITUTE FOR MEDICAL RESEARCH

**Campus Estate or Research Centre**

**Building**

5 GOWER PLACE

**Tel Number**

0207 333 2139

**Fax Number**

0207 387 4116

**E-mail**

**HSE Division**

LONDON

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

WC1E 6BN

**Country**

ENGLAND

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Laboratory</th>
<th>Animal Unit</th>
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The maximum culture volume that could be released at any one time is 10 litres. All waste is deactivated either by adding 1% Virkon or by autoclaving. Autoclave tape that undergoes a change in colour, is used to monitor that the autoclaving process is carried out correctly. Tissue culture waste is regularly inspected to ensure that plastic ware is not disposed of without first being deactivated. Information about how to deactivate and dispose of waste is provided in the departmental safety handbook, the standard operating procedures and an annual safety seminar attended by all new members of the department.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 357/07.1

Role of MEF2 transcription factors in neuronal differentiation and function.

Class 2

< 1 Litre

Consent Granted

Not Applicable
We are interested in understanding the function and regulation of the myocyte enhancer factor-2 (MEF2) family of transcription factors in neurons. The purpose of the contained use is to investigate the role of this transcription factor family by using recombinant Lentiviruses to express reporter genes to monitor MEF2 activity, gain and loss of function mutants of MEF2 proteins, activators and inhibitors of MEF2 factors, and short hairpin RNAs to down-regulate their expression.

Recipient or parental organism

HEK 293T cells, neuronal cel lines, primary neurons, glial cells, and neural progenitor cells isolated from rodent brains in dissociated and slice cultures.

Host/vector system

Recombinant Lentiviral vectors.

Origin & function

The Recombinant Lentiviral will encode proteins that will allow us to investigate the role of the mammalian myocyte enhance factor-2 (MEF2) family of transcription factors in neuronal differentiation and function. The genetic material will encode MEF2 reporter genes, gain and loss of function mutants of MEF2, activators and inhibitors of MEF2, and short hairpin RNAs against transcription factors.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Lentiviruses will be produced in HEK 293T cells by an established 3 plasmid approach in a containment level 2 tissue culture facility with restricted access. The recombinant lentiviruses produced in 293T cells are capable of only a single round of infection and will be used to infect neuronal cell lines, primary neurons, glial cells and neural progenitor cells where there are replication-defective. Infection of cells will done in containment level 2 facilities. Use of sharps will be eliminated in all procedures involving the use of lentiviruses. At the end of the experiment, all cells and waste material that makes contact with virus will be treated with detergent (1% Virkon) for 30 minutes ans autoclaved before disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste material that makes contact with virus will be treated with detergent (1% Virkon) for 30 minutes and autoclaved before disposal. This is expected to kill effectively 100% of lentiviruses.

The risk assessment was reviewed by the Departmental Biological Safety Committee comprising the Departmental Biological Safety Officer, Departmental Safety Officer, the school of biological sciences Safety Officer and 4 other members of staff. The committee was satisfied with the risk assessment and noted that access to the containment facility where the work will be carried out needs to be restricted.

Project Containment

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Project Ref 357/14.1

Identification of novel cancer genes using Crispr/Cas9 screening approaches

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Historical Significant Changes

Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**
- Identification of novel cancer genes using Crispr/Cas9 screening approaches.

**Recipient or parental organism**
- Primary mouse mammary cells from wild-type and transgenic mice, primary human mammary cells and primary naked mole-rat cells.

**Host/vector system**
- pMSCV retroviral expression vector and third generation HIV-1-based self-inactivating and replication disabled lentiviral expression vectors (pKLV, pCDH, pLKO, pGIPZ and pTRIPZ vectors and/or similar)

**Origin & function**
- Lentiviral transduction of cells in vitro to deliver Cas9 and gRNA vectors.

**Evaluation of foreseeable effects**
- Initiating, enhancing or blocking cell death, proliferation or differentiation or transformation.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Virkon (a peroxygen compound) used at a concentration of 1% is known to have a wide range of microbial activity and is bacteriocidal against many human viruses, including HIV. All tissue culture material and disposable plastic ware will be soaked for a minimum of 18-24hrs in a freshly prepared 1% Virkon solution (manufacturers guidelines for 100% kill). The fluid is then disposed down a laboratory sink with excess water. Solid waste is then double bagged in biohazard bags and incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Biological Safety Committee have approved this risk assessment with minor alterations applied.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Y

Project Containment

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**Name**

MONSANTO UK LTD

**Name 2**

**Department**

THE MARIS CENTRE

**Campus Estate or Research Centre**

**Road Name**

45 HAUGHTON ROAD

**District**

TRUMPINGTON

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 2LQ

**Country**

ENGLAND

**Tel Number**

01223 849200

**Fax Number**

01223 844425

**HSE Division**

EAST AND SOUTH EAST

**Comments**


**Date at Which Additional Info Submitted**

01/09/2000
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Other (please specify)

Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.

### Project Ref 358/99.1

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Name**

CANTAB BIOPHARMACEUTICALS LTD

**Campus Estate or Research Centre**

**Road Name**

155 CAMBRIDGE SCIENCE PARK, MILTON RD

**Town**

CAMBRIDGE

**District**

CAMBRIDGESHIRE

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 0GN

**Country**

ENGLAND

**Tel Number**

01223 423413

**Fax Number**

01223 423 458

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

CHANGED IN APRIL 2001 FROM CANTAB PHARMACEUTICALS RESEARCH LTD, CHANGED TO CANTAB BIOPHARMACEUTICALS LTD 22/12/2009

**Date at Which Additional Info Submitted**

07/02/2003
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<td>N</td>
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<td></td>
<td>ANIMAL UNIT</td>
<td>CAMBRIDGE</td>
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<td>HIRE</td>
<td>HIRE</td>
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</tr>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment.

Tick if you are claiming exemption from disclosure for sections of the risk assessment.

Please enter comments of the GM safety committee on the risk assessment.

Project Ref 359/00.1

Date Ackn'd 08/09/2000

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

CONSTRUCTION OF ATTENUATED STRAINS OF NEISSERIA MENINGITIDIS
CONSTRUCTION OF LIVE ATTENUATED VACCINE.

Class 2

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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</tbody>
</table>

Animal Units

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L2</td>
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<td>L4</td>
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<tr>
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Project Ref 359/trans1

Date Ackn'd: 25/06/1990

Date Project Ceased

CU2 Project Title: EXPRESSION OF HUMAN PAPILLOMAVIRUS (HPV) SEQUENCES IN VACCINA VIRUS

Class: Class 2

CultureVolClass2: Class 2

CultureVolumeClass3-4: not applicable

Non-GMM Consent Granted

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L3</td>
<td>L4</td>
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<tr>
<td>L2</td>
<td>L2</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>--------------</td>
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<td>L2</td>
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<td>Emergency Plan Required?</td>
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<td>N</td>
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<td>N</td>
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### Name

**PUBLIC HEALTH ENGLAND**

### Name 2

**MANCHESTER MEDICAL MICROBIOLOGY PARTNERSHIP**

### Campus Estate or Research Centre

**MANCHESTER ROYAL INFIRMARY**

### Road Name

**P O BOX 209, OXFORD ROAD**

### Town

**MANCHESTER**

### County

**GREATER MANCHESTER**

### Postcode

**M13 9WZ**

### Country

**ENGLAND**

### Tel Number

**0161 276 5747**

### Fax Number

**0161276 5744**

### E-mail

**HSE Division**

**NORTH WEST**

### Comments

### Date at Which Additional Info Submitted

**30/09/2004**

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02/03/2022

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Page 6504 of 1532
Premises Addresses

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<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
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<tr>
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<td>HEALTH PROTECTION AGENCY</td>
<td>MANCHESTER LABORATORY</td>
<td>HPA NORTH WEST, MANCHESTER MEDICAL MICROBIOLOGY PA</td>
<td>HEALTH PROTECTION AGENCY NORTH WES</td>
<td>CLINICAL SCIENCES BUILDING</td>
<td>P O BOX 209, OXFORD ROAD</td>
<td>MANCHESTER</td>
<td>CHESHIRE</td>
<td>M13 9WZ</td>
<td>ENGLAND</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
Virology | Transgenic Animals | Transgenic Fish | Gene Therapy
---|---|---|---
Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below)
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref 541/09.2**

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<th>CultureVolumeClass3-4</th>
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<tr>
<td>23/10/2012</td>
<td>munogenic and genetic characterisation of putative vaccine candidate antigens from the recently expanded group B meningococcal ST269 clonal complex</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
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Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

A broadly cross-reactive meningococcal vaccine developed by Novartis Vaccines is currently undergoing phase 3 outer membrane proteins – factor H binding protein (fHBP) fused with a further protein GNA2091), GNA2132 (fused with a further protein GNA1030 and Neisserial adhesion A (NadA). The vaccine also has an outer membrane vesicle component. fHBP is a virulence factor that binds the human complement factor H thereby enhancing bacterial survival in the host. The function of GNA2132 is unknown though it has been implicated in heparin binding, which may also lead to enhanced survival within the host. NadA is avirulence factor involved in host cell adhesion and invasion.

In order to predict the efficacy of the vaccine in England and Wales it is necessary to characterise native case isolates for fHBP, GNA2132 and NadA in terms of a) genetic variation, b) surface expression levels and c) serum bactericidal antibody (SBA) susceptibility to pre and post-vaccination sera – the gold standard assay for predicating Men B vaccine efficacy (there is no reliable animal model for meningococcal disease). To enable the evaluation of surface expression, e.g. by Whole cell ELISA and Immunoblotting, it is necessary to compare wild type native isolates against negative control (gene-knockout) isolates such that e.g. non-specific antibody binding may be accounted for. Similarly, in order to evaluate SBA susceptibility, knockout isolates of a) each gene, b) different combinations of 2 genes and c) all of the genes, will be required to help elucidate the SBA activity against any single antigen. Single and multiple knock out strains have been developed for these antigens, for the purpose outlined above, by Novartis vaccines (Sienna, Italy). It is our intention to obtain these isolates from Novartis for use in the present study.

**Recipient or parental organism**

The knockout strains will be required for comparison with the wild type strains used in the immunoassays and are therefore required to be wild-type in all respects other than that of the gene/s being disabled. The GMM constructs will therefore be treated as wild -type. A deficit of one or more of the antigens - fHBP, GNA2132 and NadA is likely to significantly reduce invasivenesses and the ability in the host.

**Host/vector system**

fHbp, NadA and gna2132 knockout isolates and combination thereof were constructed at Novartis Vaccines, Siena, Italy. Briefly, In order to generate Neisseria meningitides mutant strains lacking the main antigens contained in the vaccine, three knockout plasmids were constructed for the deletion of all or part of the respective antigen-coding gene and replacement by allelic exchange with an antibiotic resistance cassette. Using standard cloning procedures, upstream and downstream flanking regions of the gna2132 and fhbp genes were amplified by PCR from the MC58 genome and cloned on either side of an erythromycin resistance cassette into the pBluescript (Pharmacia) cloning vector, generating the knockout plasmids pBSUDgna2132:Erm and pBSUDfhbp:Erm, respectively. For the generation of the NadA knockout construct, the nmb1994 gene and surrounding upstream and downstream regions were amplified by PCR from the MC58 genome and cloned into the pBluescript cloning vector, and subsequently an internal HinclI fragment at the 5’ end of the coding region was substituted with the erythromycin resistance cassette, generating pBS961:Erm. For generation of the single knockout mutants, the knockout plasmids were linearised and transformed into the wildtype strains. Erythromycin resistant colonies were selected and checked by PCR for correct insertion due to a double homologous recombination event and colonies with correct PCR profile were further analysed by Western Blot for the lack of expression of the respective antigen.

For generation of a single Nm derivative strain lacking the expression of two or three antigens, a stepwise deletion by allelic replacement strategy was used in-which double-knockouts were constructed using Erythromycin and Kanamycin cassettes and triple-knockouts were constructed using Erythromycin, Kanamycin and Chloramphenicol cassettes.

**Origin & function**

The function of the genetic material, i.e. antibiotic resistance genes, is to enable the selection of isolates in which the genes of interest have been successfully removed.

The erythromycin (Erm) cassette contains a Streptococcus pneumoniae gene for rRna adenine N-6-methyltransferase. This protein produces a dimethylation of the adenine residue at position 2058 in 23S rRNA, resulting in reduced affinity between ribosomes and erythromycin resulting in erythromycin resistance.
The Kanamycin (Kan) cassette contains the Campylobacter coli aphA-3 gene. The gene product is an aminophosphotransferase (APH) that inactivates kanamycin by transferring the γ-phosphate of ATP to the hydroxyl group in the 3’ position of the pseudosaccharide.

The chloramphenicol (Cin) resistance cassette contains the Campylobacter coli chloramphenicol acetyltransferase (cat) gene. The enzyme chloramphenicol acetyltransferase covalently links acetyl groups to the chloramphenicol molecule. This in-turn prevents chloramphenicol from binding to the ribosome – its site of action.

The knockout isolates and their respective antibiotic cassettes are listed in the table below:

<table>
<thead>
<tr>
<th>Genes Knocked Out</th>
<th>Strain</th>
<th>Clonal complex</th>
<th>Sequence type</th>
<th>Country</th>
<th>Type</th>
<th>resistance</th>
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</thead>
<tbody>
<tr>
<td>Fhbp</td>
<td>MC58KOfhbp</td>
<td>32</td>
<td>74</td>
<td>UK</td>
<td>B:15:PI.7,16b</td>
<td>Erythromycin</td>
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<tr>
<td></td>
<td>44/76KOfhbp</td>
<td>32</td>
<td>32</td>
<td>Norway</td>
<td>B:15:PI.7,16</td>
<td>Erythromycin</td>
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<tr>
<td></td>
<td>NZ98/254KOfhbp</td>
<td>41/44</td>
<td>42</td>
<td>New Zealand</td>
<td>B:4:PI.4</td>
<td>Erythromycin</td>
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<tr>
<td></td>
<td>MC58KOnadA</td>
<td>32</td>
<td>74</td>
<td>UK</td>
<td>B:15:PI.7,16b</td>
<td>Erythromycin</td>
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<td></td>
<td>5/99KOnadA</td>
<td>8</td>
<td>1349</td>
<td>Norway</td>
<td>B:2b:PI.5,2</td>
<td>Erythromycin</td>
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<tr>
<td></td>
<td>NMBKOnadA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Erythromycin</td>
</tr>
<tr>
<td></td>
<td>MC58KOgna2132</td>
<td>32</td>
<td>74</td>
<td>UK</td>
<td>B:15:PI.7,16b</td>
<td>Erythromycin</td>
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<td>NGH38KOgna2132</td>
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<tr>
<td></td>
<td>MC58KOgna2132KOfhbp</td>
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<td></td>
<td></td>
<td>Erythromycin+Kanamycin</td>
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<tr>
<td></td>
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<td></td>
<td></td>
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<td>Erythromycin+Kanamycin</td>
</tr>
<tr>
<td></td>
<td>NMBKOnadAKOgna2132</td>
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<td>Erythromycin+Kanamycin</td>
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<tr>
<td></td>
<td>44/76KOgna2132</td>
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<td></td>
<td></td>
<td>Erythromycin+Kanamycin</td>
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<tr>
<td></td>
<td>MC58KOgna2132KOfhbpKOnadA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Erythromycin+Kanamycin+Chloramphenicol</td>
</tr>
</tbody>
</table>

Evaluation of foreseeable effects

The pathogenic traits of these strains are likely to be lessened since isolates lacking nadA (that previously harboured the gene) are likely to be less invasive whilst fHbp deficient isolates are less likely to survive within the host due to the loss of a virulence factor shown to be important in this respect. Strains deficient in gna2132 may also be less likely to survive within the host since the GNA2132 antigen is has been shown to bind hepin - also proposed to enhance survival within the host.

The single gene knockout strains were all constructed using Erythromycin resistance cassettes. Double-knockouts were constructed using Erythromycin and Kanamycin cassettes and the triple-knockouts were constructed using erythromycin, kanamycin and chloramphenicol cassettes. These antibiotics do not constitute first line therapeutic/prophylactic antibiotics against meningococci (current guidelines include penicillin, cefotaxime or ceftriaxone for therapy and rifampicin, ciprofloxacin or ceftriaxone to eradicate carriage in close contacts/exposed individuals). The presence of one or more exogenous antibiotic resistance genes may also place a disadvantageous burden on these strains.
Meningococci are obligate human pathogens and survive only within the human nasopharynx.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A - all GMOs will be treated as wild-type in accordance with the level 2 containment stipulated above

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMO waste procedures are the same as those of wild-type meningococci and these are designed to achieve 100% kill with zero viable GMOs remaining after treatment. Contaminated laboratory waste is rendered safe by immersing in trisgene solution within the safety cabinet (liquid waste) or is placed into dry disposal jars for autoclaving in room 2.01 and then removal for incineration. All laboratory bench surfaces are routinely disinfected with 1% trisgene solution after work is completed. Safety cabinets are wiped down with tristel duo and tristel duo impregnated wipes. In the event of a major spillage the room is immediately evacuated after turning on all safety cabinets. The Head or Lead Biomedical Scientist and Safety Officer are informed. When it is deemed safe to clear the now dried spillage, tristel-fuse is used to flood the area. A local Code of Practice for working with meningococci is in use; this is a detailed document covering safety aspects of all cultural activities with live meningococci whether GMO or wild type. It gives details of vaccination requirements, operation of safety cabinets, disinfection and disposal procedures, centrifuge operation and emergency procedures and other topics. All staff are required to read and understand the document before commencing any work with meningococci.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The local GMO committee reviewed the risk assessment and local application form at length and were generally happy with the contents. The committee asked for more detail of working with meningococci to be added; in particular the means of controlling aerosol production and ensuring worker safety training. This detail has been added to the risk assessment.

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
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### Name

UNIVERSITY OF EDINBURGH

### Campus Estate or Research Centre

EASTERBUSH VETERINARY CENTRE

### Town

ROSLIN

### District

SCOTLAND

### Tel Number

0131 650 1000

### Fax Number

0131 650 6289

### Comments

Merged with GM207 on 17/02/2004

### Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

<table>
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<td>PENTLANDS SCIENCE PARK</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
<th>CultureVolume</th>
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</thead>
<tbody>
<tr>
<td>05/01/2000</td>
<td>EXPRESSION OF OVINE CYTOKINES IN ADENOVIRUS</td>
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</table>

**Historical Significant Changes**

Project transferred to GM207 on 17/2/2004

**Consent Granted**

Not applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Withdrawn
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022 Page 6514 of 15326
<table>
<thead>
<tr>
<th>L2</th>
<th>L3</th>
<th>L4</th>
<th>L2</th>
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</thead>
<tbody>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>Non-GMMs</td>
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</tr>
</tbody>
</table>

**Name**

**MRC HAMMERSMITH**

**Name 2**

**IMPERIAL COLLEGE SCHOOL OF MEDICINE**

**Campus Estate or Research Centre**

**Road Name**

**DU CANE ROAD**

**Town**

**LONDON**

**District**

**County**

**GREATER LONDON**

**Postcode**

**W12 0NN**

**Country**

**ENGLAND**

**Tel Number**

**0208 740 3163**

**Fax Number**

**0208 740 3448**

**E-mail**

**HSE Division**

**LONDON**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

<table>
<thead>
<tr>
<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
<th>Withdrawn</th>
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<td>H HOSPITAL</td>
<td>DU CANE ROAD</td>
<td>LONDON</td>
<td>GREATER LONDON</td>
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<td>ENGLAND</td>
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</tbody>
</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<tr>
<td>Level 2 (GMMs)</td>
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<td>Level 3 (GMMs)</td>
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Tick if confidential

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tr>
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<td>Transgenic Fish</td>
<td></td>
<td>Gene Therapy</td>
</tr>
<tr>
<td>Virology</td>
<td></td>
<td></td>
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</tbody>
</table>
Mycology
Transgenic
Invertebrates
Transgenic
Plants
Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 363/01.1

**Date Ackn'd** 11/05/2001

**CU2 Project Title** TO PRODUCE IMMORTALISED HUMAN MUSCLE PRE-CURSOR CELLS.

**Class** Class 2

**CultureVolClass2** < 1 litre

**Non-GMM** Not applicable

**Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**
Purposes of the contained use
To conditionally immortalise human muscle cells in order to obtain long-lived clones that can be expanded in tissue culture and form muscle in-vivo.

Recipient or parental organism
Retrovirus - amphotropic encoding human telomerase and a temperature sensitive mutant of SV40 large T antigen Replicatsin defective. Transduced human muscle cell.

Host/vector system
Primary human muscle cells to be transfected by a recombinant, disabled retrovirus. Inserts - human telomerase and a temperature sensitive SV40 large tumour antigen.

Origin & function
The retroviral supernatent is obtained from the Group below and there is no culture to be done at the CSC of the original packaging cell lines. Ref: O'Hare et al Proc Natl Acad Sci (2001) 98:646-651

Evaluation of foreseeable effects
Immortalisation of the target 1 muscle cells that one incapable of replicating the retrovirus. There is a small potential risk that the operators own cells could become transformed - but this is thought unlikely as the transforming gene is temperature sensitive (33C), and would be operating at opt containment level 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
1 muscle cells will be handled in a Class II Safety Cabinet. The cells will be incubated in a separate CO incubator in a category II laboratory.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste Culture Supernatant and disposable plastics are all autoclaved - giving 100% cell kill. A thermocouple probe is placed in the waste to ensure the correct temperature is reached. A chart recorder, records that the discord cycle passes. The waste then goes into the hospitals clinical waste stream and is tagged and traceable.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
- Sharps are not to be used.
- Retrovirus Supernatant is to be stored in a clearly marked, locked container.
Project Containment

Laboratory Activities
L2 Yes L3 L4 L2 L3 L4
Glass Houses
L2 L3 L4 L2 L3 L4
Growth Rooms
L2 L3 L4 L2 L3 L4

Animal Units
L2 L3 L4 L2 L3 L4
Large Scale Activities
L2 L3 L4 L2 L3 L4
Human Clinical Applications
L2 L3 L4

Project Ref 363/06.1

Date Ackn'd 15/03/2006

CU2 Project Title Studies to inform on the processes of drug resistance

Date Project Ceased

Class 2

Culture Vol Class 2 < 1 Litre

Consent Granted Not Applicable

Non-GMM

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

How epithelial and ribroblast cells are immortalised, transformed and maintain drug resistance and to identify novel gene products involved in cell models of tumourigenesis.

Recipient or parental organism

Mammalian cell lines, all with a history of safe use and not expected tp represent a hazard to human health.

Host/vector system
Helper vectors: pRSREV, pMD2G, pMDL RRE
Lentiviral vectors: pWPTS, pWPXL, pWPI, pLVTHM, pLKO.1 or its derivatives.

Origin & function

Host cells are of human origin and may be primary, transformed or cell cycle-arrested. The inserts will be mammalian and viral cDNAs and shRNAs; regulatory nucleic acid sequences and genes conferring resistance to antibiotics. Some cells will have a very limiting proliferative ability in culture before reaching an irreversible growth arrested state, and these cells are difficult to transfected by standard methods. The use of recombinant retroviral infection as a means of delivering the appropriate DNA into cells is required.

All lentiviral vectors are based on HIV-1 but lack of all viral auxilliary genes, ie vpr, vif, vpu and nef. Second generation helper packaging plasmids will be used to produce replication-incompetent virus. The auxiliary proteins and the VSV-g envelope protein are provided by three separate helper plasmids that contain no regions of homology to the HIV-1-based vectors. Cell lines created by retroviral infection will be maintained by selection with the appropriate antibiotics (either blasticydin, neomycin hygromycin or puromycin). It is important to note that the lentiviral vectors contain the woodchuck post-transcriptionally regulatory element (WPRE). Due to recent concerns about their potential oncogenicity (Gene Therapy AOP 28 Oct 2004), the GTAC has recommended as a precaution to use such vectors at category 2 containment level.

Evaluation of foreseeable effects

The host strains are not pathogenic and inserted gene products encode for wild type, mutated or tagged versions of mammalian and viral cDNAs of genes involved in tumourigenesis and drug resistance. None of the vectors or inserts has mobilisable elements. It is not expected that over-expression of any of the genes to be used would alter existing properties of the cell lines they will be transfected into. None of the cell lines to be used are capable of colonising a healthy individual and we do not expect the proposed manipulations to change this. The mutations will not affect virulence, infectivity or stability and the pathogenicity will not be altered from the wild-type. All the genes are likely to be expressed in humans and their products may be biologically active, therefore, the recombinant gene could cause drug resistance if expressed. Some mammalian cell lines may be made antibiotic resistant, however, since these would be unable to establish themselves outside of the laboratory there is no risk to human health. There is no deliberate intention for the product to be harmful to health. It is highly unlikely they would provoke a toxic or allergic response, or act as a hormone.

There is very limited risk that harmful genes could exist in the cDNA libraries, ie 0.0001%. Stable cell lines expressing different transgenes involved in tumourigenesis will be generated by retroviral transduction and expression of some transgenes and/or suppression others have been proposed to be involved in tumourigenesis (Hannahan and Weinberg, 2000). Genes involved in the acquisition of drug resistance could have potentially oncogenic properties. However it is known to be a multifactorial event and since they will never be used at the same time in transfection or transduction experiments with other potentially transforming genes it is considered that the oncogene cannot act alone to cause tumours. When expressed alone in normal cells many of these oncogenes do not result in transformation but elicit protective programmes such as apoptosis or senescence, thus preventing oncogenesis. The amphotropic viral vectors would be able to infect humans but could not replicate except in the most unlikely event that they recombine with an endogenous retrovirus. Even then it is unlikely that expression of a single oncogene or suppression of a tumour suppressor would cause tumours, as multiple genetic alterations (4-6) are required for inducing tumours. Safeguard mechanisms exist impeding the accumulation of these alterations in the organisms instead of unrestrained proliferation they induce growth arrest or apoptosis. Limited volume non-concentrated cultures, will be made in a Class II cabinet. Risks of aerosol contamination of staff and environment minimised by enclosing the plates and tubes outside of the cabinet. No sharps will be used and the laboratory will have restricted access. Containment Level 2 labels will be posted and personnel trained in emergency procedures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For liquid waste inactivation with Microsol 3 (Anachem Ltd) at dilutions of not less than 1/100, 30 min minimum contact time, as specified by the manufacturer.

02/03/2022
The committee considered the risk assessments valid and appropriately classed as category 2 and requiring level 2 containment. The laboratories are suitable for this.

The committee was fully convinced that Project 57 and 59 are part of a connected programme of work, being carried out by the same PI and his team. The reason for two assessments was to allow him to explain the work more fully. The same PI is collaborating on another project, using the same techniques and materials provided by him. This project is particularly directed at chemotherapy drug resistance in cell models of tumourigenesis. We ask that they all be considered as a connected programme within our institute.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
<td>L3 L4 L2 L3</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2</td>
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### Project Ref 363/06.2

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<td>25/07/2006</td>
<td>Studies to inform on the processes controlling chromatin structure.</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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</table>
**Project Additional Information**

**Purposes of the contained use**

How cell cycle proteins and proteosomes control chromatin structure and their potential role as tumour suppressors/oncogenes in carcinogenesis

**Recipient or parental organism**

**Human Cells, primary, transformed or cell cycle-arrested**

The recipient cell lines will be the following human cells: human embryonic derived epithelial and fibroblast cell, HMECs, NHK, HPrEC, Wi-38, TIG, IMR-90, breast cancer cells (MCF-7, T47-D, ZR-75-1, MDA-MB-231, SK-BR-3), prostate cancer cells (LnCaP, PC-3, DU-145) and other common human cell lines obtained from the ATCC. All of these cells have a history of safe usage and are not expected to represent a hazard to human health.

**Host/vector system**

**Bacterial plasmids and HIV-1 based lentiviruses. Contain WPRE. Especially disabled E.Coli K12 or B derivatives (egDH1, DH5, HB101, JM101, JM105, JM109, TG2, SUREtm, XL1-Blue, BMH-71-18 mutS and ES1301 mutS or derivatives AuxotrophicSaccharomyces cerevisiae mutants for YAC propagation**

**Origin & function**

**Plasmid Vectors:**

PGEM series, pUC series, pAlter, pcDNA3, pTOPO, pCineo, pcDNA3pBUDCE4Yac4 and their derivatives.

**Helper vectors:**

pRSREV, pmMD2G, pMDL RRE

**Lentiviral vectors:**

Pwpts, Pwpxl, pWPI, pLVTHM, pLKO.1 or its derivatives

All lentiviral vectors are based on HIV-1 but lack of all viral auxilliary genes, i.e. vpr, vif, vpu and nef. Second generation helper packaging plasmids will be used to produce replication-incompetent virus. The auxiliary proteins in one hand, and the VSV-g envelope protein in other hand are provided by three separate helper plasmids that contain no regions of homology to the HIV-1 based vectors.

**Mammalian tissue culture cells:** 293T cells will be used to produce replication-incompetent virus. The auxiliary proteins in one hand, and the VSV-g envelope protein in other hand are provided by three separate helper plasmids that contain no regions of homology to the HIV-1 vectors. Three separate recombination events would be required to produce replication-competent virus. The combined choice of the multiple packaging plasmids and the replication defective nature of the vector used will prevent the production of helper/replication competent virus by the transduced cells, reducing the risk to the environment. Human cells expressing different oncogenes will
be generated by infection with lentiviruses. Cell lines created by retroviral infection will be maintained by selection with the appropriate antibiotics (either blasticydin, neomycin hygromycin or puromycin). It is important to note that the lentiviral vectors contain the woodchuck post-transcriptionally regulatory element (WPRE). Due to recent concerns about their potential oncogenicity (Gene Therapy AOP 28 Oct 2004), the GTAC has recommended as a precaution to treat this vector as category 2 containment level.

**Evaluation of foreseeable effects**

Selection of successful transformation into recipient cell lines will result in acquisition of antibiotic resistance. Effects of introduced genetic modifications may result in senescence, apoptosis, cell cycle arrest, inhibition of growth, increased proliferative rate, changes in sensitivity to DNA damaging agents depending on candidate gene introduced. Since all experiments are limited to cultured cell lines only, none of the effects will be passed onto intact organisms/animals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

For liquid waste inactivation with Microsol 3 (Anachem Ltd) at dilutions of not less than 1/100, 30 min minimum contact time, as specified by the manufacturer. The supervisor ensures appropriate training and use of the disinfectant. The disinfectant has been validated by the manufacturer to kill 100% of organisms in use. The validation credentials are checked periodically as a monitor to ensure this disinfectant is still suitable.

Solid waste materials are collected separately from other lab waste and subjected to autoclave decontamination. The cycle is set for discard and it is well established that a circle of 134°C for 15 mins with full steam penetration to the centre of the load is sufficient to kill 100% of retroviruses, bacteria and tissue culture cells. A probe is inserted into the load centre to ensure monitoring of each cycle, which is recorded. The autoclave has a quarterly check and an annual 12-point thermocouple test to ensure efficiency of cycle. Should the autoclave fail a run-checked by the operative using printout and error message - the loads are not released to the clinical waste route until a fully validated run has occurred.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

**Project Containment**

The committee considered the risk assessment valid and appropriately classed as category 2, requiring work at containment level 2. The designated laboratories are suitable and sufficient for these purposes.
Project Ref 363/08.1

Date Ackn'd 22/12/2008

Identification of regulatory components of the AMPK/AMPK-related kinase cascades and downstream pathways using shRNA knock-down and overexpression studies.

Class 2

Consent Granted Not Applicable

Non-GMM

Project notified under transitional arrangements N

Recipient or parental organism

The lentiviral transfer vector, pLKO.1 and retroviral expression vector, pMSCV come in E.coli strain DH5 alpha T 1R. HEK293T cells will be used to packaged these vectors into virus, and the viruses will be used to transduce mammalian cells including HEK293, HEK293T, COS, HeLa, primary mouse hepatocytes, Tcells, IMR90 fibroblasts and stem cells.

Host/vector system

The aim of this project is to screen for components involved in the regulation of and downstream components of AMP-activated protein kinase (AMPK) and AMPK-related protein kinase cascades. During this study, lentivirus and retrovirus will be used to package shRNAs targeting potential proteins involved in regulation of these cascades, and cDNA of components of the pathways. The effect of this knock down or overexpression in cells will be studied with regard to phosphorylation status of AMPK and/or the AMPK-related kinases, and known downstream effects.
Vectors: pLKO.1 vector  
Sigma-Aldrich 'packaging vector'  
Sigma-Aldrich 'envelope vector'  
pMSCV retroviral expression vector (Clontech)  
pCL-10A1 Retrovirus Packaging Vector (Imgenex)

**Origin & function**

HEK293T cell will be co-transfected with lentiviral packaging mix (envelope vector and packaging vector) and shRNAs in the lentiviral transfer vector, pLKO.1. A combination of specific shRNA clones against a single target will be co-transfected together to give a mixed population of shRNA.

To produce retrovirus, HEK293T packaging will be cotransfected with retrovirus packaging vector and retroviral expression vector, pMSCV, containing specific sDNAs for kinases involved in the AMPK/AMPK-related kinase signaling cascades.

In both cases, the HEK293T packaging cells will transcribe this DNA, synthesise viral RNA and proteins, and package a viral particle. These viruses will be used to transduce various types of mammalian cells. The shRNAs target a selection of known proteins and will be used to knock down proteins in the AMPK and AMPK-related kinase signalling pathway either individually or in groups. The retroviral transduction will result in overexpression of AMPK and AMOPK-related kinases, or other components of the signalling pathways.

To produce retrovirus, HEK293T cells will be cotransfected with retrovirus packaging vector and retroviral expression vector, pMSCV, containing specific sDNAs for kinases involved in the AMPK/AMPK-related kinase

**Evaluation of foreseeable effects**

The shRNAs to be used in these experiments come in lentiviral (HIV)-plasmid (pLKO.1) and will be cotransfected into packaging cells (HEK293T) alongside plasmids for packaging proteins (MISSION lentiviral packaging mix, Sigma-Aldrich) to produce lentiviral particles. The lentiviral vector systems have been developed with enhanced safety features. It is a third generation lentiviral vector system, with three plasmids consisting of:

1. The packaging vector, which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions.
2. The vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector, which provides the heterologous envelope for pseudotyping.
3. The shRNA transfer vector, which contains the sequence of interest as well as the cis acting sequences necessary for RNA production and packaging.

The retroviral vector system is a two plasmid system, with a MSCV Retroviral expression vector, derived from the murine stem cell PCMV virus (PCC4-cell-passaged myeloproliferative sarcoma virus), and a Retrovirus packaging vector. The multi plasmid approach increases the safety because viral structural genes have been placed on different genetic units, and multiple recombination events must occur before replication competent virus structural genes have been placed on different genetic units, and multiple recombination events must occur before a replication competent virus would be generated. Furthermore, areas of homology have been minimized to decrease the chance of homologous recombination. Virulence genes have been removed from the viral plasmids. Both the Lentivirus and retrovirus to be produced in this study are replication incompetent, due to the elimination of wild-type enhancers in the long terminal repeat region. The vectors contain a self-inactivating 3'LTR that renders them unable to produce infectious virus when it integrates into the host chromosome.

To minimise the possibility of self inoculation, personal protection measures will be taken, for example needles will not be used when handling biological agents and appropriate personal protection will be used (Lab coat, gloves, safety glasses). To minimise any possible aerosol effects of the viral stocks, appropriately capped centrifuge bottles and screw-capped tubes will be used. Trigene advanced has been shown to completely inactivate lentivirus and retrovirus at a final concentration of 1:100 and so will be used to thoroughly clean everything involved in these experiments.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A
Liquid waste will be treated with Trigene Advance at a final concentration of 1% for 30 minutes, shown by the manufacturer to give a complete deactivation.

Solid GM waste material is collected separately in designated 30 litre yellow bins or autoclavable Sharpsafe bins, clearly labelled as GM waste and with the group name. When operator has agreed to receive it, waste will be brought over to the autoclave for treatment. All routes must be internal, and the GM waste must never be left unattended. Gloves and lab-coats should be worn. In the event of spillage during transport to the autoclave, the area must be isolated and then promptly cleaned with 1% Trigene.

The operator loads the autoclave and runs the plastic discard cycle (134°C for 15 minutes) which has been established sufficient to kill 100% of retroviruses, bacteria and tissue culture cells. The temperature probe is put into the waste of the bin to ensure waste in the centre of the bin is inactivated and monitors every run. Chart paper supply and printouts are checked before and after each run. Should the autoclave fail a run the loads are not realeased to the clinical waste route until a fully validated run has occurred. The run number is recorded and the traces and printouts are kept until the waste will have been finally disposed of as clinical waste. All staff operating the autoclave have been trained in manual handling and are also trained in appropriate spillage procedure. 1% Trigene is the disinfectant to be used in case of inactivated waste being spilled in or out of the autoclave. The autoclave has quarterly checks and an annual 12-point thermocouple test to ensure the efficiency of the cycle.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee considered the risk assessment valid and appropriately classed as category 2 requiring level 2 containment. The laboratories are suitable for this level of work.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<thead>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 363/10.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
To study the plasticity of cells

Purposes of the contained use
To identify epigenetic mechanisms involved in regulating the reprogramming of somatic cells to iPS and to study the effects of knocking down target genes on cell differentiation and reprogramming. Reprogramming will be induced by co-expression of various transcription factors and enzymes in lentiviral vectors. Lentiviral vectors containing a variety of shRNA or DNA target sequences will be used for the knockdown and over expression studies. Lentiviral vectors where chosen because they enable the transduction of primary and non-dividing cells.

Recipient or parental organism
Lentiviral vector constructs are provided commercially as bacterial cultures of E. coli (DH5alpha strain). The LB agar E. coli stab cultures will be sub-cultured on LB agar plates before a bacterial colony is grown in large amounts in LB medium. Vector plasmid DNA will be isolated from the culture using a Qiagen plasmid purification kit. HEK293T cells will be used to package the plasmid vectors into replication incompetent virus. Recipient cells of the lentivirus include various primary mouse somatic cells and cell lines.

Host/vector system
Vectors:
Expression: pLKO.2 containing target shRNA (Thermo Scientific) pLove containing transgene (Addgene)
Packaging: pRSV-Rev, pMDLg/pRRE (Addgene)
Envelope: pND2.G (Addgene)

Origin & function
Delivery of the target gene into mouse somatic cells and cell lines will be achieved using lentiviral systems based on HIV-1. For example, the four pluripotency factors (OSKM) are cloned into the lentiviral vector pLove and the shRNAs that target transcription factors are cloned into the transfer vector pLKO.1 For some of the gene knockdown experiments, a combination of shRNA clones against a single target will be co-transfected together to give a mixed population of shRNA.

In both cases the HEK293T packaging cells will transcribe the DNA, synthesize with viral RNA and proteins, and package a viral particle. These viruses will be used to transfect various types of somatic mouse cells. The pluripotency factors are well described and have been shown, repeatedly, to produce iPS. The shRNA's will target a
selection of transcription factors and enzymes that we believe are involved in cell differentiation and reprogramming.

**Evaluation of foreseeable effects**

The OSKM transcription factors to be used in these experiments have been cloned into the pLove lentiviral vector and will be co-transfected into packaging cells HEK293T’s together with plasmids for the packaging proteins (Addgene) to produce lentiviral particles. For the shRNA’s the target gene is contained within the lentiviral vector pLKO.1 and will be co-transfected into the packaging cells (HEK293T). We are using a third generation lentiviral vector system. The 3rd generaton packaging system offers maximal biosafety but is more cumbersome to use as it involves the transfection of four different plasmids into the 293T producer cells (two packaging plasmids, an envelope plasmid, and the lentiviral vector). For safety purposes the lentiviral vectors pLKO.1 and pLove contain a chimeric 5’LTR in which the HIV promoter has been replaced with RSV (pLKO.1) or CMV (pLove), thus making them TAT-independent. The multi-plasmid approach increases the safety of the system because viral structural genes are contained on different constructs, thus multiple recombination events would have to occur for a replication competent virus to be generated. Moreover, virulence genes have been removed from the plasmid vectors and the lentiviral vectors contain a self-inactivating 3’ LTR that renders them unable to produce infectious virus when it integrates into the host chromosome.

To reduce the possibility of self inoculation needles will not be used and personal protection in the form of lab coat, gloves and safety glasses will be worn. To avoid generating aerosols only screw capped tubes and bottles will be used for processing and storing the viral stocks. The detergent Trigene Advanced has been shown to completely inactivate lentivirus at a final concentration of 1:00 for 30 minutes. Trigene will be used to decontaminate any plasticware used during the experiments and to clean the class II cabinet used to conduct these experiments.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste will be treated with Trigene Advanced at a final concentration of 1% for thirty minutes, manufacturers recommendation to give complete deactivation. All plasticware used and discarded during these experiments will be soaked with Trigene Advanced overnight.

Solid GM waste, after appropriate soaking in Trigene and rising, will be collected in autocalvable sharpsafe bins, clearly labelled as GM waste with the group name. The waste will be autoclaved. The autoclave operator runs the wast on the plastic discard cycle (134C for 15 minutes) because these conditions are established to kill 100% of retroviruses, abcteria and cells. A temperature probe monitors each autoclave run to check that the desired temperature conditions are reached. The temperature during each run is recorded on chart paper. The wste is only released to the clinical waste routed after a fully validated run. All staff operating the autoclave have been trained in manual handling and are also trained in appropriate spillage procedure (use of 1% Trigene as disinfectant if any inactivated waste is spilt in or out of the autoclave).

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
The committee were satisfied with the risk assessment they had no concerns

**Project Containment**

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**Project Ref** 363/10.2

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Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to determine genetic modifiers of cardiocascular traits. The function of candidate genes, identified through proteomic or genetic screens, will be evaluated in vitro using functional genomic approaches prior to progressing to in vivo studies. See attached document for full details

**Recipient or parental organism**

Bacterial cells (for cloning and protein expression) and Mammalian cell lines (for expression of cloned sequences) including: E. coli Top 10, E. coli BJ5183, 293 cells (or
Host/vector system

Shuffle vectors (pShuttle or pAdTrack), Replication deficient Adenovirus (pAdEasy-1, -2) and other relevant commercial vectors from recognised suppliers (e.g. Stratagene, Invitrogen).

Origin & function

We propose to study genes that regulate cardiovascular phenotypes. Over-expression of any gene product has potentially deleterious effects whether oncogenic, apoptotic or affecting other cellular functions. However, to have detrimental effects the abnormal gene needs to be continually expressed or transferable. For the reasons given below this does not apply to in vitro low titre adenoviral work and it is believed that the inserted sequences are not capable of transducing cells. That said, if any particular gene is known to have oncogenic properties, work using adenovirus expressing that gene will be undertaken in a microbiological safety cabinet. Sequences will be expressed in the above mentioned mammalian cells using the Shuttle vectors or adenoviral vectors generated. The gene/protein expressed is not expected to be harmful to environment or operator. Although viral, bacterial and mammalian genes can be expressed in humans, recombinant type V adenovirus mediated-transgene expression is epigenetic, transitory and non-transferable (replication deficient i.e. the virus does not propagate). Therefore if the correct procedures are followed, this procedure is unlikely to be harmful to the environment or operator.

Evaluation of foreseeable effects

As the plasmids specified are not expected to produce biologically harmful products, these experiments should not be harmful to the user or the environment. The plasmids are incapable of propagation outside of laboratory setting. Replication deficient virus was confirmed as E1 deficient by qPCR using a safety protocol developed by Dr Cook. Despite this, care will be taken to reduce possible sources of contamination. Appropriate PPE will be used when handling the reagents (lab coats, gloves etc). Mammalian cell culture will be carried out in a relevant cell culture hood. Screw-capped centrifuge bottles and eppendorfs will be appropriately capped and handled to avoid aerosol. Vessels (e.g. flasks and falcon tubes) will be securely placed in the shaking incubator in secondary containers to prevent spillage of bacterial cultures and potential damage to the vessels themselves. In the event of spillage, the spill will be contained with paper tissues, and the affected area decontaminated with Trigene. In the event of a tissue culture spillage the same procedure will be followed except after decontamination with Trigene, the area will also be cleaned with a 70% ethanol solution as a further step to ensure that the area is sufficiently clean for further tissue culture. All paper waste will be disposed of as GM solid waste.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Solid GM and tissue culture waste will be disposed of as outlined in section 8b of the attached document. Liquid waste will be treated with either Trigene according to manufacturer's instructions, or Virkon (1-3% solution) for a minimum of 8 hours. Virkon is active as long as it retains its pink colouration, which is easily verified visually.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated with Trigene Advance at a final concentration of 1% for 30 minutes, shown by the manufacturer to give complete deactivation. Solid waste material is collected separately in designated 30 litre yellow bins or autoclavable Sharpsafe bins, clearly labelled as GM waste and with the group name. When operator has agreed to receive it, waste will be brought over to the autoclave for treatment. All routes must be internal, and the GM waste must never be left unattended. Gloves and lab-coats should be worn. In the event of a spillage during transport to the autoclave, the area must be isolated and then promptly cleaned with 1% Trigene. The operator loads the autoclave and runs the plastic discard cycle (134C for 15 minutes) which has been established sufficient to kill 100% of retroviruses, bacteria and tissue culture cells. The temperature probe is put into the waste of the bin to ensure waste in the centre of the bin is inactivated and monitors every run. Chart paper supply
and printout are checked before and after each run. Should the autoclave fail a run the loads are not released into the clinical waste route until a fully validated run has occurred. The run number is recorded and the traces and printouts are kept until the waste will have been finally disposed of as clinical waste. All staff operating the autoclave have been trained in manual handling and are also trained in appropriate spillage procedure. 1% Trigene is the disinfectant to be used in case of inactivated waste being spilled in or out of the autoclave. The autoclave has quarterly checks and an annual 12 point thermocouple test to ensure the efficiency of the cycle.

Further details provided in the attached document

Is an emergency plan required according to regulation 20?  
[No]

If yes, tick to confirm that it is attached to this form  
[No]

Tick to confirm that you have attached a risk assessment to this form  
[Yes]

Tick if you are claiming exemption from disclosure for section of the risk assessment  
[No]

Please enter comments on the GM safety committee on the risk assessment

The committee considered the risk assessment valid and appropriately classed as category 2 requiring level 2 containment. The laboratories are suitable for this level of work.

Project Containment

<table>
<thead>
<tr>
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</table>

Animal Units
- L2
- L3
- L4
- L2

Large Scale Activities
- L3
- L4
- L2

Human Clinical Applications
- L3
- L4

Project Ref 363/11.1

Date Ackn'd: 25/08/2011

CU2 Project Title: Hormone signalling and energy homeostasis: defining the central nervous system molecules and circuits through the use of virally-mediated gene expression

Class: Class 2

CultureVolClass2: < 1 Litre

Non-GMM: Consent Granted

Project notified under transitional arrangements: [No]
The purpose of this project is to investigate the signalling mechanisms and neuronal networks involved in the regulation of energy homeostasis and endocrine function. The work carried out under this application will lead to the production of mice in which specific genes are manipulated in the central nervous system using virally mediated gene transfer. Replication deficient lentiviral particles expressing genetically modified constructs (eg intracellular signalling molecules, ion channel proteins, short interfering RNA (siRNA)) will be injected into mouse brains. Insertion of loxP sites into the viral constructs will limit expression to only the neuronal populations that express cre-recombinase (promoter driven in transgenic mice). This approach may overcome developmental issues that arise from traditional transgenic mice models since it can be used to manipulate genes only in the adult. Neuronal specific expression of light-sensitive ion channel proteins (i.e. channel-rhodopsin or halorhodopsin) will be used to link acute neuronal excitability with behavioural output (i.e. energy homeostasis, reward). Together these approaches will permit examination of the brain circuits that regulate appetite and bodyweight.

Expression vectors containing various DNA and siRNA constructs will be sent for commercial packaging (i.e. Stratech Scientific Ltd) to produce lentiviral particles. Delivery of genetic materials (cDNA and siRNA) will be achieved by viral transduction following injection of the lentiviral particles into the brains of mice which may be either wild-type mice or themselves genetically modified (i.e. carrying stable transgenes and/or with targeted deletion of specific genes).

Commercial vector systems will be used to generate the constructs (e.g. pCDH-CMV-MCS (Stratech Scientific Ltd), pLKO.1 (Open Biosystems), pLenti-EF1 (Stanford University, USA). Packaging, expansion and purification of viruses will not be performed in the laboratory but undertaken at a company using proprietary systems (Cell Biolabs; ViraSafe™ packaging system (pCMV-Eco, pRSV-Rev, pCGP-V)). Viruses will be shipped to the laboratory and stored until use.

Expression vectors containing channel-rhodopsin or halorhodopsin (light sensitive proteins derived from alga (Chlamydomonas reinhardtii) and bacteria (Halobacteria), respectively) will be used for optogenetic studies and will be obtained from Stanford University, USA (http://www.stanford.edu/group/diab/optogenetics). Lentiviral expression vectors will be obtained from commercial sources (i.e. Stratech Scientific Ltd; Open Biosystems) and will be used to construct genetically modified proteins (signalling molecules and ion channels derived from mouse DNA) and siRNA (synthesized commercially). Expression of these constructs will be used to manipulate the signalling mechanisms used by neurons to regulate energy homeostasis.

The lentiviral vector system has been developed with enhanced safety features. It is a third generation lentiviral vector system, with three plasmids consisting of 1) the packaging vector, which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions; 2) the vesicular stomatitis virus G-protein envelope vector which provides the heterologous envelope for pseudotyping; and 3) the expression vector, which contains the sequence of interest as well as the cis acting sequences necessary for RNA production and packaging. All three components are required to produce a viral particle that is capable of infecting a host organism. The multi plasmid approach increases the safety because viral structural genes have been placed on different genetic units, and multiple recombination events.
must occur before a replication competent virus would be generated. Furthermore, areas of homology have been minimized to decrease the chance of homologous recombination. Virulence genes have been removed from the viral plasmids. The lentiviral particles that will be produced in this study are replication-competent, due to the elimination of wild-type enhancers in the long terminal repeat region. The vectors contain a self-inactivating 3' LTR that renders them unable to produce infectious virus when it integrates into the host chromosome. Once these viral particles have infected the neurons it is highly unlikely that adjacent cells or other organisms can be infected and therefore the risk to the wider environment is extremely low. The lentiviral vector system will only be used to deliver non-transforming sequences (i.e. not oncogenes).

Moreover, expression of the viral constructs will be cre-recombinase dependent which will remove the possibility that expression is permissible in organisms that do not express cre-recombinase (i.e. animals and plants). Therefore the viral inserts will only be expressed in cre-recombinase transgenic mice used in the proposed studies. It is anticipated that manipulation of these signalling molecules will alter the normal regulation of body weight and food intake. To minimize the possibility of self inoculation of the lentiviral particles, personal protection will be used (lab coat, gloves, safety glasses). To minimize any possible aerosol effects of the viral stocks, appropriately capped centrifuge bottles and screw-capped tubes will be used. Stocks of the lentiviral particles will be opened only in class II containment cabinets to avoid inhalation. Trigene advanced at a final concentration of 1:100 has been shown to completely inactivate lentivirus and will be used to thoroughly clean surfaces and equipment used in the studies.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any liquid waste generated will be treated with Trigene Advance at a final concentration of 1% for 30 minutes, shown by the manufacturer to give complete deactivation.

Solid genetically modified waste material is collected separately in designated 30 litre yellow bins or autoclavable Sharpsafe bins, clearly labelled as genetically modified waste and with the group name. When operator has agreed to receive it, waste will be brought over to the autoclave for treatment. All routes must be internal, and the genetically modified waste must never be left unattended. Gloves and lab-coats should be worn. In the event of a spillage during transport to the autoclave, the area must be isolated and then promptly cleaned with 1% Trigene.

The operator loads the autoclave and runs the plastic discard cycle (134°C for 15 minutes) which has been established sufficient to kill 100% of retroviruses, bacteria and tissue culture cells. The temperature probe is put into the bin to ensure waste in the centre of the bin is inactivated and monitors every run. Chart paper supply and printout are checked before and after each run. Should the autoclave fail a run the loads are not released to the clinical waste route until a fully validated run has occurred. The run number is recorded and the traces and printouts are kept until the waste will have been finally disposed of as clinical waste. All staff operating the autoclave have been trained in manual handling and are also trained in appropriate spillage procedure. 1% Trigene is the disinfectant used in case of inactivated waste being spilled in or out of the autoclave. The autoclave has quarterly checks and an annual 12 point thermocouple test to ensure the efficiency of the cycle.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The committee considered the risk assessment valid and appropriately classed as category 2 requiring level 2 containment. The laboratories are suitable for this level of work.

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### Project Ref 363/12.1

**Use of retroviral vectors to express candidate oncogenes and tumour suppressor genes**

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**Purposes of the contained use**

The role of human and mouse genes will be studied with particular interest in their effects on cells that might cause or prevent cancer or alter the course of treatment of cancer.
Recipient or parental organism

Host/vector system
Transfection of MuLV derived retroviral vectors, lentiviral vectors and packaging plasmids into 293T cells to generate virus containing supernatant and infection of cells with supernatant

Origin & function

Evaluation of foreseeable effects
Precautions must be taken to ensure operators are not exposed to virus particles that could infect their cells since theoretically infected cells may have an increased chance of developing into cancer

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid GM waste (plastic ware) will be autoclaved, Liquid GM waste will be disinfected using Trigene (used at 1:50 dilution consistent with instructions given by the company that produces this product, Medichem International) for a minimum contact time of one hour.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The committee considered the risk assessment valid and appropriately classed as Category 2 requiring level 2 containment. The laboratories are suitable for this level of work

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Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

02/03/2022
Mycology  Transgenic  Transgenic  Other (please
Invertebrates  Plants  specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum culture volume = 100 ml.
All waste is deactivated at 121 degrees C for 30 minutes in a UKAS accredited autoclave.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 366

Data Premises Notified (Originally) 04/01/1991

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed 01/04/2006

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

HANNAH RESEARCH INSTITUTE

Name 2

Department

BIOCHEMISTRY & MOLECULAR BIOLOGY

Campus Estate or Research Centre

Building

Road Name

District

Town AYR

County SOUTH AYRSHIRE

Postcode KA6 5HL

Country SCOTLAND

Tel Number 01292 674000

Fax Number 01292 674003

E-mail

HSE Division SCOTLAND

Comments

Centre closed and ceased all GM activities as of 1/4/2006

Date at Which Additional Info Submitted 02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

1. Solid wastes contaminated with, or likely to be contaminated with GMOs to be autoclaved at 121 degrees C for 40 minutes to achieve a total kill of all microorganisms.
2. Completion and validation of autoclaving to be confirmed by use of suitable indicator tape.
3. Liquid wastes contaminated with, or likely to be contaminated with GMOs to be retained within original culture vessel or sterile-transferred into fresh vessels and subsequently autoclaved, then disposed to waste drainage.
4. Small volumes of liquid waste and small items of solid waste (eg micropipette tips) may be disposed into a reservoir or biocidal solution (eg Virkon or RBS SR80 made-up according to manufacturers' instructions).
5. The largest cultures used are 1 litre in volume.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

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Name

LGC (TEDDINGTON) LTD.

Name 2

LABORATORY OF THE GOVERNMENT CHEMIST

Department

LIFE SCIENCES RESEARCH

Campus Estate or Research Centre

Building

Road Name

QUEENS ROAD

tel Number

0208 943 7000

Fax Number

0208 943 2767

E-mail

HSE Division

LONDON

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

**Level 1 (GMMs)**

**Level 2 (GMMs)**

**Level 3 (GMMs)**

**Level 4 (GMMs)**

**Non-microbial**

**Other (please specify)**

Tick if confidential

**Bacteriology**

**Parasitology**

**Transgenic Birds**

**Microbiology Research**

**Virology**

**Transgenic Animals**

**Transgenic Fish**

**Gene Therapy**
The maximum culture volume that could be released at any one time is 500 ml. Waste is deactivated by autoclaving at a minimum of 121 degrees C for 30 minutes. The effectiveness of the deactivation procedure is monitored by the use of autoclave indicator tape and steam sterilisation indicator strips.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 375

Data Premises Notified (Originally) 26/06/1990
Transferred from 1992 Regs? Y
Transitional Premises Class 1
Data Premises Closed N
Transitional Premises Emergency Plan Required? N
Non-GMMs Y
Withdrawn N

Name
ASTON UNIVERSITY

Name 2

Department
SCHOOL OF LIFE & HEALTH SCIENCES

Campus Estate or Research Centre

Road Name
ASTON TRIANGLE

District

Town
BIRMINGHAM

County
MIDLANDS

Postcode
B4 7ET

Country
ENGLAND

Tel Number
0121 359 3611

Fax Number
0121 359 0733

E-mail

HSE Division
MIDLANDS

Comments

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
Virology
Mycology
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref** 375/11.1

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Date Project Ceased

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

Production of cells by virally mediated transduction that can be stimulated with light using Channel Rhodopsins and monitored using genetically encoded fluorescent calcium proteins. Production of fluorescent cells that can be used to track, the identity of neurons and astrocytes.

**Recipient or parental organism**

| Algae for Channel Rhodopsin |
| Jelly fish for RFP and YFP |
| Anenome for CFP adm mCherry |
| Humans for GFAP, CamKII, ubiquitin and synapsin promoter |
| Bacteria for B-Lactamase resistance |
| Cytomegalovirus for CMV promoter |
| Bovine Rhodopsin β2 adrenergic receptor |
| Bovine Rhodopsin α1 adrenergic receptor |
| Gag-pol and Pro form HIV |
| Chicken Gallus gallus for CHS4 insulator element |
| VSV-G vesicular stomatitis virus from simian virus |
| NTera 2.D1 cell line derived from a human testicular tumour |
| RenCell VM cell line derived from human foetal brain |

**Host/vector system**

E. coli DH5α Genotype (F- Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rk-, mk+) phoA supE44 λ- thi-1 gyrA96 relA1)

The bacterial host cells (DH5α) are an E. coli K12 derivative categorised as disabled and non-colonising. These cells have biological limitations that preclude their survival in the gut, lung and elsewhere in the body. Due to the autotrophic requirements of these cells, this strain has limited survival capacity in the environment, and is therefore unlikely to survive outside of laboratory culture. This strain belongs to biological agents hazard group 1, for which containment level 1 is appropriate.

The Stbl3™ E. coli strain is designed for cloning direct repeats found in lentiviral expression vectors. Genotype:F- mcrB mrr hsdS20 (rB-, mB- ) recA13 supE44 ara-14 galK2 lacY1 proA2 rpsL20 (Strr ) xyl-5 - leu mtl-1.

The Stbl3™ cells are derived from an E. coli K12 strain designated as disabled and non-colonising. The parental strain belongs to biological agents hazard group 1.

293FT cells

Self inactivating Lentivirus vectors will be produced typically in 293FT cells using a split packaging system. 293FT cell are a human cell line initially transformed with the adenovirus early region, also expressing SV40 T-antigen. Virus particles are produced by co-transfection of vector and helper plasmids as mentioned previously. Due to the presence of the SV40 T-antigen it is recommended that this cell line is handled under at least Biosafety level 2.

ReNcell VM

The ReNcell VM immortalised cell line (Millipore) from the ventral mesencephalon is an immortalized human neural progenitor cell line capable of differentiating into cells of neural lineage upon the removal of growth factors from the medium. Originally derived from the ventral mesencephalon region of human foetal brain tissue the line was immortalised by the incorporation of the V-Myc oncogene by retroviral transduction with the Moloney murine leukemia virus. The cell line is certified pathogen free.
The NTERA-2 cl.D1 cell line is a pluripotent human testicular embryonal carcinoma cell line. This clone differentiates along neuroectodermal lineages after exposure to retinoic acid (RA) or hexamethylene bisacetamide (HMBA). The American Type Culture Collection (ATCC) recommends handling the cell line under at least Biosafety level 1.

**Organotypic mouse brain slices**
Brain slices derived from rodents housed within Aston Universities’ biomedical facility are pathogen free. It is therefore recommend that this material is handled under at least Biosafety level 1.

**Lentiviral vectors**
We will use 2nd generation SIN/replication deficient lentiviruses to allow gene transfer of ChR/OptoXR genes under the control of neuronal/astrocytic gene promoters or inducible promoters (e.g. tetracycline responsive promoters) in postmitotic cells. Control lentiviruses may also be generated expressing green fluorescent protein under the same promoters.

These vectors will also contain drug-resistance selectable markers conferring cell resistance to e.g. Zeocin, Blasticidin, hygromycin or neomycin. This risk assessment also covers the preparation of the DNA genomes of these lentiviral vectors as plasmids in E. coli. This work will be carried out under category 2 containment.

**AVV**
GM adenovirus (replication-defective). We will use adenoviral vector (AVV) to allow gene transfer of Case12 constructs to postmitotic cells. This will be carried out under category 2 containment.

Human Embryonic Kidney 293 cells (HEK293) cells. Derived from human embryonic kidney cells. Since HEK cells express a number of adenoviral genes, they can be used to propagate adenoviral vectors in which these genes (typically, E1 and E3) are deleted. American Type Culture Collection (ATCC) recommends handling the cell line under at least Biosafety level 1.

**Origin & function**

- **Algae for Channel Rhodopsin**
- **Jelly fish for GFP, RFP and YFP**
- **Anenome for mCherry and CFP**
- **Humans for GFAP, CamKII and synapsin promoter**
- **Bacteria for β-Lactamase resistance**
- **Cytomegalovirus for CMV promoter**
- **Bovine Rhodopsin β2 adrenergic receptor (OptoXR)**
- **Bovine Rhodopsin α1 adrenergic receptor (OptoXR)**
- **Human Ubiquitin C promoter**
- **Human for Oct4 promoter**
- **Chicken Gallus gallus for CHS4 insulator element**
- **Gag-pol and Pro form HIV**
- **VSV-G vesicular stomatitis virus from simian virus**
- **Case12 calcium indicator based on GFP variants and Calmodulin**
- **Cameleon calcium indicator based on GFP variants and calmodulin**

Channelrhodopsins are a subfamily of opsin proteins that function as light-gated ion channels. They serve as sensory photoreceptors in unicellular green algae, controlling phototaxis, i.e. movement in response to light. Expressed in cells of other organisms, they enable the use of light to control intracellular acidity, calcium influx, electrical...
excitability, and other cellular processes. Three channelrhodopsins are currently known: Channelrhodopsin-1 (ChR1), Channelrhodopsin-2 (ChR2), and Volvox Channelrhodopsin (VChR1). All known Channelrhodopsins are unspecific cation channels, conducting H+, Na+, K+, and Ca2+ ions.

Opto-XR proteins are chimeric fusions of bovine Rhodopsin and adrenergic G-Protein Coupled Receptors allowing optical control of GPCR signalling cascades. Proteins are activated by 500nm light.

Case12
Case12 genetically encoded sensor is based on the circularly permutated fluorescent protein (cpFP) fused to or inserted into calcium sensitive (calmodulin) domain. Such Ca2+ sensors were constructed by fusing calmodulin and its target peptide M13 (fragment of myosin light chain kinase) to cpFP. In the presence of Ca2+, calmodulin binds to the M13 peptide, causing conformational changes in the vicinity of the chromophore and thereby influencing cpFP fluorescence.

Cameleon
Fluorescence resonance energy transfer (FRET)-based Ca2+ indicator. It was created by fusing BFP, calmodulin, calmodulin-binding peptide M13 and EGFP. In the presence of calcium, Ca2+ binds to M13, which enables calmodulin to wrap around the M13 domain. This brings the two GFP-variant proteins closer to each other, which increases FRET efficiency between them.

CHS4
Chicken Hypersensitive Site 4 Insulator element. The CHS4 insulator element was initially identified at the extreme 5’ end of the chicken β-globin locus where it acts as a domain insulator to insulate proximal genes from the effects of locus control regions present in the β-globin domain. The use of the CHS4 domain in recent years has been in the integration of the domain into expression constructs for stable transfection of mammalian cells. Here it can serve two purposes. It may be used to insulate endogenous genes from the effects of plasmid encoded promoter regions (here the region has been used to prevent insertion activation of genes, when virus mediated transduction of cells is performed). The region has also been described as preventing the epigenetic silencing of inserted genes by heterochromatin closing, thus ensuring the stable expression of inserted genes in transfected cells. Incorporation of this element into the construct would be carried out only in vector based transformations, and the element will be inserted at both ends of the construct where it would be expected to prevent the unintended insertion of genomic genes by promoters in the construct.

eGFP
Enhanced Green fluorescent protein EGFP. The nucleic acid sequence encoding EGFP is included for the expression of EGFP in transfected cells. EGFP has a long history of safe use as a fluorescent reporter protein. No attempt will be made to purify the protein, which will only be expressed in the NT2 cells. GFP is unlikely to pose a risk to the operator or environmental organisms as preliminary data on the deliberate administration of GFP to weaned rats suggested that GFP exhibits low allergenicity and is not likely to represent a health risk (Richards et al 2003).

Cyan / mCherry fluorescent protein
Both Cyan fluorescent protein and Cherry fluorescent protein nucleic acid sequences have been derived from anemone fluorescent protein by site directed mutagenesis. As members of the newer generation of fluorescent reporter proteins, little toxicological data exists on the proteins themselves, but both proteins share a great deal of homology with the eGFP protein and have a history of safe use as fluorescent reporters, including their expression in transgenic animals with no detrimental effects.

hUBC Promoter
The Human Ubiquitin C promoter is a DNA promoter sequence of human origin, which drives expression of the Ubiquitin c protein in mammalian cells. When used to drive expression of reporter genes, the promoter would be expected to result in moderate expression of controlled genes in a constitutive manner in most mammalian cells.

Syn1 Promoter
The Hyman Synapsin 1 promoter is a DNA promoter sequence of human origin containing the 21 bp Neuron restrictive silencer element, restricting the expression of genes from this promoter to neural cells or cells expressing RE1 silencing transcription factor. In neuronal cells this promoter would be expected to drive moderate levels of transcription, and hence production of the target protein. Virtually no protein production would be expected in non target cells.

Oct4 Promoter
The Oct4 promoter is the DNA sequence of the human Oct 4 promoter. The promoter directs expression of the Pou domain protein Oct4 in stem cells. Oct4 expression is seen in undifferentiated stem cells until epigenetic modification of the promoter inactivates transcription of Oct4 with the resulting differentiation of the cells. In undifferentiated stem cells it would be expected that this promoter would drive moderate level expression of the downstream fluorescent reporter gene. Upon differentiation of such cells or after transfection into non stem cell lines, no expression of the downstream gene would be expected.

GFAP promoter
The GFAP promoter drives expression of GFAP (glial fibrillary acidic protein) almost exclusively in astrocytes. Expression of fluorescent reporter genes from this promoter would be expected to be limited to astrocytic cells resulting in moderate expression of the reporter protein. Expression in non astrocytic cell lines would not be expected.

Lentivirus vectors derived from HIV will be second and third generation vectors for expression of ChR/OptoXR, e.g. pLenti-CaMKIIa-hChR2(E123T-H134R)-EYFP, pLenti-CaMKIIa-hChR2-EYFP-WPRE (Karl Deisseroth, Stanford University). These are used to produce lentivirus particles by co-transfection with helper constructs psPAX2 and pmd2g, in which gag-pol (third generation vectors) or gag-pol and pro (second generation vectors) and an envelope protein are expressed from separate co-transfected plasmids in 293FT cells (Natacha Klages et al 2000). All vectors are designed to minimize the chance of recombination events that could incorporate the helper functions to generate a replication-competent virus. Only “self-inactivating” (SIN) lentivirus vectors will be used; these have a deletion within the U3 region of the 3'LTR, which removes enhancer and promoter sequences. Upon completion of one life-cycle from the RNA genome of the virus particle, via reverse transcription to integration into chromosomal DNA, the deleted U3 region is copied to the 5'LTR. As a result, the 5'LTR becomes incapable of transcribing full length viral genomes. This further reduces the possibility of oncogenic potential. Lentiviruses will generally be pseudotyped using the VSV-G envelope glycoprotein; this is widely used as it permits efficient transduction of a wide variety of cell types, and makes the virus particles relatively resistant to shear stress, facilitating their concentration by centrifugation if required to increase the titre.

Lentiviruses are generally considered less prone to cellular transformation than conventional MuLV-based retroviral vectors, although a high frequency of liver tumours has been produced in mice following foetal or neonatal virus administration. The precise cause remains debatable; the tumours appeared not to be associated with HIV-based vectors, and one hypothesis was that the WPRE present in some of the vectors may have been capable of expressing a partial “X-protein”, of which at least the full length version is considered oncogenic. Use of SIN vectors theoretically reduces the chance of activating oncogenes at the site of insertion into target cell DNA.

Adenoviruses
Replication-deficient adenovirus vectors are usually based on adenovirus E1-E3 deletion mutants. Deletion of the E1 gene region (both E1A and E1B) removes a region of the genome associated with cellular transformation and generates a vector that is not only replication-deficient but also cannot activate early phase gene expression. Cell lines that will be used to facilitate initial production, amplification, and titering of replication-incompetent adenovirus such as HEK293 cells contain a stably integrated copy of E1 that supplies the E1 proteins (E1a and E1b) in trans required to generate adenovirus.

The infectious nature of human adenovirus will permit spread by aerosol or ingestion. An individual may be also infected by injection (via sharps) or direct contact with existing wounds and mucosal membranes (e.g. eyes). However, the recombinant viruses are replication-defective, and thus their genomes would soon be lost from the vast majority of infected cells. Chromosomal integration of the adenoviral sequences is a rare event, in the region of one integration event per 10^4 exponentially growing infected cells. Although the numbers of cells undergoing replication in the respiratory tract at any one time is not precisely known, it is generally accepted that only a minor fraction of cells in the infected tissue will be infected. No extension of host range expected; the E1 deletion prevents replicative spread of the virus except in specially constructed cell lines that complement the E1 deletion.

The infectious nature of human adenovirus will permit spread by aerosol or ingestion. An individual may be also infected by injection (via sharps) or direct contact with existing wounds and mucosal membranes (e.g. eyes). However, the recombinant viruses are replication-defective, and thus their genomes would soon be lost from the...
The vast majority of infected cells. Chromosomal integration of the adenoviral sequences is a rare event, in the region of one integration event per 10^4 exponentially growing infected cells. Although the numbers of cells undergoing replication in the respiratory tract at any one time is not precisely known, it is generally accepted that only a minor fraction of cells in epithelial tissues of the respiratory tract are dividing and that the majority of the cells in epithelium are post-mitotic, on the differentiation pathway. It is our opinion that a considerable intake of virus would have to occur to produce an integration event in vivo. Even if such an integration event did occur, it is clear that the expression of CR2 would be insufficient to cause malignant change in a human cell. The risks of tumour formation by accidental infection with an adenovirus/CR2 recombinant must be very small. Nevertheless, appropriate precaution should be adopted to minimise the possibility of accidental infection.

**PcDNA3.1**

PcDNA3.1(+) and PcDNA3.1(-) are 5.4 kb vectors derived from pcDNA3 and designed for high-level stable and transient expression in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements: Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells. Multiple cloning sites in the forward (+) and reverse (-) orientations to facilitate cloning. Neomycin resistance gene for selection of stable cell lines. Episomal replication in cells lines that are latently infected with SV40 or that express the SV40 large T antigen.

**pZsGreen1**

pZsGreen1 (Clontech) encodes ZsGreen1, a variant of the Zoanthus sp. green fluorescent protein (ZsGreen1) that has been engineered for brighter fluorescence. The ZsGreen1 coding sequence contains a series of silent base pair changes for optimal expression in mammalian cells. pZsGreen1-DR can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the multiple cloning site (MCS), located upstream of the ZsGreen1-DR coding sequence. Downstream SV40 polyadenylation signals direct proper processing of the 3’ end of the ZsGreen1-DR mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in E. coli, and an f1 origin for single-stranded DNA production. A neomycin-resistance cassette (NeoR) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter upstream of the cassette expresses kanamycin resistance in E. coli. Without the addition of a functional promoter, this vector will not express ZsGreen1.

**pJTI Fast DEST (Invitrogen)**

The pJTI Fast Dest vector contains attR sites compatible with the attP sites in the pDONR vectors, for in vitro recombination of desired DNA inserts. The vector has a pUC origin of replication and also encodes ampicillin resistance under a bacterial promoter for propagation and selection E. coli. The vector is non-mobilisable in the E. coli host strain. The vector also encodes the ccdB gene which interferes with e. coli DNA Gyrase, preventing the growth of the parental vector in E. coli. The vector contains a PhiC31 attB recognition site for targeted integration of the vector into the mammalian genome in the presence of phage PhiC31 integrase. The vector contains no viral origin of replication for propagation in mammalian cells as it is intended to be used only for integration using PhiC31.

The vector also encodes a Hygromycin resistance gene under the control of the herpes simplex virus thymidine kinase promoter for selection of the integrated vector in mammalian cells, expression of which is terminated by the respective HSV Thymidine kinase polyadenylation signals.

**pJTI PhiC31 Int**

The pJTI PhiC31 Int vector encodes PhiC31 integrase from the streptomyces phage under the control of the cytomegalovirus immediate early promoter for high level expression in mammalian cells. The vector has no viral origin of replication for propagation in mammalian cells as it is intended to simply provide a temporal supply of the PhiC31 integrase by transient transfection of target cells. The plasmid has a pUC origin of replication for propagation in E. coli and encodes the ampicillin resistance gene (β lactamase) for selection in E. coli.

Plasmids would not be expected to confer any selective advantage to host cells from cloned elements. However, plasmids do encode antibiotic resistance. The risk of transfer of resistance to organisms in the wider environment would be expected to be low. Plasmid DNA does not contain mobilisation elements and would therefore not be expected to survive for prolong periods outside of lab culture. Bacterial hosts would not be expected to survive outside of lab culture. Thus persistance of plasmid DNA in the outside environment would be limited. Transfer between environmental organisms and the non mobilisable plasmid would be expected to be far less likely.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No genetically modified organisms will be generated in the course of this project. Only organotypic brain slices obtained from mice will be used in this project.
The use of self inactivating lentiviral vectors effectively limits the virus to a single infective cycle thus after several changes of culture media the final drug selected cells lines will not contain infectious viral particles and could be handled on the bench if required, for cell fixing and cell staining protocols. Imaging of virally transformed cells will not take place until at least 4 weeks post drug selection for stable clones by which time no viral particles would be expected to remain. Hence stably transduced cell lines could be handled under class one conditions for imaging purposes. However this derogation only applies to supervised monitoring of the cell lines. In all other aspects such as storage and disposal level 2 containment will be applied as outlined in the risk assessment.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
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<tbody>
<tr>
<td>The production of aerosols is not intended and will be minimised by using capped tubes and flasks in sealed centrifuge buckets. All waste and contaminated glassware and plastics will be autoclaved according to HSE requirements prior to disposal or cleaning and re-use in the 6th floor autoclave. No sharps will be used during the work thus minimising the risk of operator contamination. In the case of accidental spillages of culture media containing cells, the contaminated area can be effectively disinfected with 1% Virkon, and the small volume inherently prevents the spread of contamination to the wider environment.</td>
<td></td>
</tr>
</tbody>
</table>

1% Virkon will be used for most surface decontamination. Disposable plastic tips for micropipettors, may be decontaminated by drawing up Virkon disinfectant, and soaking in Virkon for a minimum of 20 minutes. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above.

All tissue culture will be carried out in a designated laminar flow hood with filters appropriate for cells of category Biosafety level 2. Safety cabinets are tested and maintained on a regular schedule; class 2 cabinets have a KI discus test and maintenance every 6 months, for class I cabinets this is on an annual basis. The tissue culture expression will be on a small scale of less than $10^8$ cells. Levels of protein expression are unlikely to exceed 1mg/liter. All tissue culture waste will be autoclaved. No sharps will be used. The designated incubator for culture of virally infected cells will be equipped for wet heat sterilization.

Warning lights, and chart recorder, indicate whether or not the autoclave run has achieved the appropriate temperature for the required time. The autoclave is run by trained staff, who are instructed to treat waste as still contaminated if the autoclave run was not to specifications. Under such circumstances the waste would be autoclaved again, after rectifying any reason for the malfunction. The autoclave is inspected and serviced on a regular basis.

All liquid waste is treated by autoclaving at 130°C for 30 minutes, before disposal to drains. Disposable solid waste which is or may be contaminated with GMOs is also inactivated by autoclaving at 130°C for 30 minutes, before removal as “clinical waste” by specialist contractors, with final disposal by incineration.

**Expected degree of kill:**

Autoclaving achieves effectively 100% kill of all GMOs.

The manufacturer’s information indicates efficacy of Virkon against a variety of adenoviruses at 1% concentration, and also against a number of strains of E. coli at dilutions ranging from 1% to 0.125%. Colleagues at Birmingham University have demonstrated that 15 minutes exposure to 1% Virkon achieves >4 log10 kill for adenovirus-infected cell pellets, and >6 log10 kill of adenovirus seed stocks. The effectiveness of Virkon in the deactivation of plasmid DNA was tested with plasmids of a similar size to those that will be created in the work outlined above.

Incubation of plasmid DNA in a 1% solution of Virkon for 2 minutes reduced the transformation efficiency of plasmid DNA in transforming E coli cells from $2.8 \times 10^6$ transformants / µg DNA to $4.2 \times 10^4$ transformants / µg DNA. Incubation for 20 minutes in this 1% Virkon solution completely abolished the ability of the DNA to transform cells. There is no reason to suggest that the plasmids described in this work will be less susceptible to chemical inactivation than those tested.

---

**Is an emergency plan required according to regulation 20?**

- Yes
- No

**If yes, tick to confirm that it is attached to this form**

- Yes
- No

**Tick to confirm that you have attached a risk assessment to this form**

- Yes
- No
All Members of the biological safety committee agreed to the proposed work. 
Professor C B (biological safety officer) 
Life and Health Sciences, 
Aston University, 
Birmingham 
B4 7ET, UK.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<td><strong>Animal Units</strong></td>
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### Project Ref 375/12.1

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<td>Analysis of the role of transglutaminases in normal and disease-related cellular physiology</td>
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<td>&lt; 1 Litre</td>
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<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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<td>N</td>
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</table>

### Historical Significant Changes
- Withdrawn: N
- Tick if notifying a connected programme of work: N
### Project Additional Information

#### Purposes of the contained use

| To determine the role of transglutaminase in normal cell processes and in models of disease. |
| To deliver shRNA into a variety of transglutaminase-expressing cell types to reduce expression and examine phenotype. To introduce wild-type and mutant transglutaminase proteins into cells to determine alterations in phenotype. |

#### Recipient or parental organism

| A variety of mammalian cell lines including mouse 3T3 fibroblasts, human dermal fibroblasts, mouse embryonic, ECV304, human osteoblasts, chinese hamster ovary (CHO), HEK 293T, human umbilical vascular endothelial cells (HUVEC), IB3-1 airway epithelial cell line, C38 airway epithelial cell line, Beas-2b, Calu-3, 16-HBE, THP1 monocytes PC12, endothelial cell lines. |

#### Host/vector system

| We will use 2nd and 3rd generation lentiviruses to allow gene transfer of transglutaminase genes under the control of viral promoters or inducible promoters (e.g. tetracycline responsive promoters) in cells. Control lentiviruses may also be generated expressing fluorescent proteins. The following lentiviral vectors will be used: pLenti™6, pLKO.1, pLKO-Tet-On, pLenti-Ill-UbC, pFB-ERV, pCFB-EGSH, pLVX-Tet-On/Off Advanced, pLVX-Tight-Puro, psPAX2, pMD2G, FG12, FIGB & FUGW. Other associated plasmids are as follows: pLP1, pLP2, pLP/VSVG, pcDNA3.1(+). |

#### Origin & function

**Transglutaminases**

are a family of enzymes (EC 2.3.2.13) that catalyze the formation of a covalent bond between a free amine group (e.g., protein- or peptide-bound lysine) and the gamma-carboxamide group of protein- or peptide-bound glutamine. Bonds formed by transglutaminase exhibit high resistance to proteolytic degradation.

Transglutaminase mutants will be functionally inactive mutants, to allow determination of the effects of these mutations on the expression, localisation and function of transglutaminases in normal physiology and disease models. shRNA will be used to reduce transglutaminase expression in cell models.

**TGM1**

Keratinocyte transglutaminase is highly expressed in terminally differentiated epidermal keratinocytes where it is responsible for formation of a stable cross-linked corneocyte layer.

**TGM2**

Tissue transglutaminase is expressed ubiquitously. It plays a role in wound healing, apoptosis, extracellular matrix deposition and stabilisation, cell attachment and survival. It is involved in a variety of pathologies such as celiac disease, tissue fibrosis, cancer, neurodegenerative disorders. As well as its transglutaminase activity, TG2 also has GTPase activity, functioning as a G protein participating in signaling processes. It also acts as a kinase, protein disulfide isomerase and deamidase.

**TGM3**

Epidermal transglutaminase, like TGM1, is involved in cross-linking of proteins in the skin cornified envelope.

**TGM6**

Member of the transglutaminase family which is present in the neurological cells and could be responsible for neurological disorders.

**FXIIIa**

Factor XIIIa is the transglutaminase subunit of Factor XIII. It is responsible for the stabilisation of polymerised fibrin in blood clots.
**eGFP**
Enhanced Green fluorescent protein EGFP. The nucleic acid sequence encoding EGFP is included for the expression of EGFP in transfected cells. EGFP has a long history of safe use as a fluorescent reporter protein. No attempt will be made to purify the protein. GFP is unlikely to pose a risk to the operator or environmental organisms as preliminary data on the deliberate administration of GFP to weaned rats suggested that GFP exhibits low allergenicity and is not likely to represent a health risk (Richards et al 2003).

**Cyan/Yellow fluorescent protein**
Cyan and yellow fluorescent protein nucleic acid sequences have been derived from anemone fluorescent protein by site directed mutagenesis. As members of the newer generation of fluorescent reporter proteins, little toxicological data exists on the proteins themselves, but they share a great deal of homology with the eGFP protein and have a history of safe use as fluorescent reporters, including their expression in transgenic animals with no detrimental effects.

**shRNA**
Short synthetic oligonucleotides based on the sequences for mouse and human TGM1, TGM2, TGM3, TGM6 and FXIIIA.

### Evaluation of foreseeable effects

In cell lines transfected via lentiviral infection, expression of high levels of fluorescent proteins such as EGFP would not be expected to alter the potential pathogenicity of the cells. Expression of EGFP would be expected to be contained within cells, and in comparison to expression of the protein in bacteria or yeast, total protein expression will be at relatively low levels. No attempt to purify the protein will be made. EGFP has been shown to demonstrate low allergenicity, and it’s expression in the cell line would not be expected to in anyway interfere with the normal immune response of an operator, to non operator derived mammalian cells.

With respect to the viral work, minimal hazard would be expected of the viral plasmids in E. coli where generation of infectious viral particles would be impossible.

Expression of the transglutaminase proteins TGM1, TGM2, TGM3, TGM6 and FXIIIA, which are normal cellular products and their functionally-inactive mutants in eukaryotic cells is not expected to alter the cells in such a way that could be hazardous to human health.

The main hazard is associated with the lentiviruses that will be generated in packaging cells. The viruses will be capable of infecting human cells, with the consequent insertion of the vector genome into chromosomal DNA. The main hazard is the possibility of oncogene activation at the site of integration. Consideration of the experiences from human gene therapy clinical trials suggests this risk is low, as any accidental exposure would involve many logs fewer virus particles, nevertheless precautions to avoid exposure are appropriate. A further potential hazard is the generation of replication-competent virus. As described above, use of split packaging systems for retrovirus, and second / third generation SIN vector systems for lentivirus, makes this highly unlikely. The use of self inactivating lentiviral vectors effectively limits the virus to a single infective cycle thus after several changes of culture media the final drug selected cells lines will not contain infectious viral particles and could be handled on the bench if required, for cell fixing and cell staining protocols.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

It is the responsibility of the person undertaking the work with the GMM to check the correct operation of equipment used, on every occasion. Latex gloves, culture vessels, centrifuge tubes etc should be inspected before and during use, to ensure leak-free operation. The work area and equipment should be checked before and after use for any spillages which might contain GMMs, and appropriate remedial action taken if necessary. The work surface must be decontaminated after use.
Project supervisors have a responsibility to ensure that persons in their group are following the specified containment and control measures, and arranging for appropriate training of new workers.

The work will be carried out in specialist laboratories (MB633, MB634 and MB635) with restricted access through a number-coded security lock and double-doors. Work with live virus particles will be contained to a single laboratory (either MB634 or MB635) with no carriage of virus-containing materials between laboratories. Access will only be available to individuals with a working knowledge of tissue culture and required safety procedures. Signage stating the level of work and the necessary caution will be displayed prominently. There is an operator washing/changing facility outside of the inner culture laboratory.

Designated laboratory coats or tissue culture gowns. Any cuts or abrasions should be appropriately protected (e.g. waterproof dressing or protective gloves).

Storage in appropriately labelled, screw-capped plastic tubes, in secondary boxes within freezer in containment lab. Tube contained within an appropriate secondary container able to contain any potential leaks, if transport between labs is required.

The production of aerosols is not intended and will be minimised by using capped tubes and flasks. All waste and contaminated glassware and plastics will be autoclaved according to HSE requirements prior to disposal or cleaning and re-use in the 6th floor autoclave. No sharps will be used during the work thus minimising the risk of operator contamination. In the case of accidental spillages of culture media containing cells, the contaminated area can be effectively disinfected with 1% Virkon, and the small volume inherently prevents the spread of contamination to the wider environment.

1% Virkon used for most surface decontamination. Disposable plastic tips for micropipettors, may be decontaminated by drawing up Virkon disinfectant, and soaking in Virkon for a minimum of 20 minutes. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above.

All tissue culture will be carried out in a designated laminar flow hood with filters appropriate for cells of category Biosafety level 2. Safety cabinets are tested and maintained on a regular schedule; class 2 cabinets have a KI discus test and maintenance every 6 months, for class I cabinets this is on an annual basis. The tissue culture expression will be on a small scale of less than 10^8 cells. Levels of protein expression are unlikely to exceed 1mg/liter. All tissue culture waste will be autoclaved. No sharps will be used. The designated incubator for culture of virally infected cells will be equipped for wet heat sterilization.

Warning lights, and chart recorder, indicate whether or not the autoclave run has achieved the appropriate temperate for the required time. The autoclave is run by trained staff, who are instructed to treat waste as still contaminated if the autoclave run was not to specifications. Under such circumstances the waste would be autoclaved again, after rectifying any reason for the malfunction. The autoclave is inspected and serviced on a regular basis.

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Expected degree of kill:
Autoclaving achieves effectively 100% kill of all GMOs.

The manufacturer’s information indicates efficacy of Virkon against a variety of adenoviruses at 1% concentration, and also against a number of strains of E. coli at dilutions ranging from 1% to 0.125%. Colleagues at Birmingham University have demonstrated that 15 minutes exposure to 1% Virkon achieves >4 log10 kill for adenovirus-infected cell pellets, and >6 log10 kill of adenovirus seed stocks. The effectiveness of Virkon in the deactivation of plasmid DNA was tested with plasmids of a similar size to those that will be created in the work outlined above.

Expected degree of kill:
Incubation of plasmid DNA in a 1% solution of Virkon for 2 minutes reduced the transformation efficiency of plasmid DNA in transforming E coli cells from 280000 transformants / µg DNA to 420000 transformants / µg DNA. Incubation for 20 minutes in this 1% Virkon solution completely abolished the ability of the DNA to transform cells. There is no reason to suggest that the plasmids described in this work will be less susceptible to chemical inactivation than those tested.
Reviewer 1: "as a number of labs are mentioned it may be best to try to limit this 1 or two. If they really do need to transport the virus from their main lab to their tissue culture then they need to spell out how they will do this ie double sealed containers, clean gloves labcoats in different areas etc."

Reviewer 2: "it would be wise to contain the work in one lab, rather than allowing it to proceed in 633, 634 and 635. Equally, I note that the cell line 293FT should be "at least" level 2 containment, which is another reason for careful, localised containment."

Reviewer 3: "As far as I can see the application appears to be in order. The risk assessment/safety precautions/containment seem appropriate. A well established disinfectant (Virkon) with BS activity is in place for waste material and hard surface disinfection."

Reviewer 4: "The only thing I would say is to stipulate where the viral work will be performed – at the moment it is spread over 3 labs in which the project is being conducted but I think it should be narrowed down to one lab if not one Microbiological safety cabinet. It then partially segregates the higher risk work from the other projects and the rest of the work that is being conducted, thereby giving greater control."

Reviewer 5: "this is widely used technique and so the potential hazards are well understood and the control mechanisms are widely used. Thus as far I can tell, this application seems to be in order."

Reviewer 6: "the work should be restricted (where possible) to one laboratory and perhaps in a clearly demarcated area within. If this cannot be avoided and transport between areas is necessary, a stringent procedure needs to be in place."

Applicant's reply: The risk assessment has been modified to make it clear that no transport of virus will occur between laboratories.

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**Project Containment**

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Project Additional Information

Purposes of the contained use

To test the hypothesis that astrocytes and specifically astrocytic gliotransmitter release is essential for synaptic experience-dependent plasticity (EDP) measured in cortical neurones.

2. To determine if, and how, the optical induction of astrocyte activity can be used to manipulate/control plasticity.

Recipient or parental organism

Algae for Channel Rhodopsin
Jelly fish for mCherry and eYFP
Humans for GFAP, CamKII and synapsin promoter
Bacteria for β-Lactamase resistance
Cytomegalovirus for CMV promoter
Bovine Rhodopsin β2 adrenergic receptor
Bovine Rhodopsin α1 adrenergic receptor
Gag-pol and Pro from HIV1
Chicken Gallus gallus for CHS4 insulator element
VSV-G vesicular stomatitis virus from simian virus
Yeast for GAL4 sequence
Woodchuck Hepatitis Virus for WPRE element
Human for eIFα
Human for NFκB p65 protein element
CASE 12 modified eYFP jelly fish
**Host/vector system**

GCaMP modified GFP Jellyfish

**E. coli DH5α Genotype**
**E. Coli Mach1-TR1 Genotype**
**E. Coli Stbl3™ Genotype**
293FT cells
pLenti-CaMKIIa-hChR2(E123T-H134R)-EYFP
pcDNA.1vShis-opto-a1AR-EYFP
pcDNA.1vShis-opto-b2AR-EYFP
pcDNA.1v/hChR2-mCherry
pLenti-CaMKIIa-hChR2-EYFP-WPRE
AAV-sGFAP-Case12
AAV-GFAP-ChR2
pcDNA.1vShis-opto-a1AR-EYFP
pcDNA.1vShis-opto-b2AR-EYFP
pZsGreen1-syn1-ChR2-mCherry
psPAX2
pMD2.G
pLenti 6.4 R4 R2-V5 Dest
PCR-8-Topo
pENTR 5 TOPO
GCaMP1-6

**Origin & function**

Channelrhodopsins are a subfamily of opsin proteins that function as light-gated ion channels. They serve as sensory photoreceptors in unicellular green algae, controlling phototaxis, i.e. movement in response to light. Expressed in cells of other organisms, they enable the use of light to control intracellular acidity, calcium influx, electrical excitability and other cellular processes. Three channelrhodopsins are currently known: Channelrhodopsin-1 (ChR1), Channelrhodopsin-2 (ChR2), and Volvox Channelrhodopsin (VChR1). All known Channelrhodopsins are unspecific cation channels, conducting H+, Na+, K+, and Ca2+ ions.

Opto-XR proteins are chimeric fusions of bovine Rhodopsin and adrenergic G-Protein Coupled Receptors allowing optical control of GPCR signalling cascades. Proteins are activated by 500nm light.

Case12/GCaMP

Case12 and GCaMP are genetically encoded sensors based on the circularly permuted fluorescent protein (cpFP) fused to a calcium sensitive (calmodulin) domain. Such Ca2+ sensors were constructed by fusing calmodulin and its target peptide M13 (fragment of myosin light chain kinase) to cpFP. In the presence of Ca2+, calmodulin binds to the M13 peptide, causing conformational changes in the vicinity of the chromophore and thereby influencing cpFP fluorescence.

eGFP

The nucleic acid sequence encoding Enhanced Green Fluorescent Protein (EGFP) is included for the expression of EGFP in transfected cells. EGFP has a long history of safe use as a fluorescent reporter protein. No attempt will be made to purify the protein, which will only be expressed in the brain cells. GFP is unlikely to pose a risk to the operator or environmental organisms as preliminary data on the deliberate administration of GFP to weaned rats suggested that GFP exhibits low allergenicity and is not likely to represent a health risk (Richards et al 2003).

Cyan / mCherry fluorescent protein

Both Cyan fluorescent protein and Cherry fluorescent protein nucleic acid sequences have been derived from anemone fluorescent protein by site directed mutagenesis. As members of the newer generation of fluorescent reporter proteins, little toxicological data exists on the proteins themselves, but both proteins share a great deal of
homology with the eGFP protein and have a history of safe use as fluorescent reporters, including their expression in transgenic animals with no detrimental effects.

**hUBC Promoter**
The Human Ubiquitin C promoter is a DNA promoter sequence of human origin, which drives expression of the Ubiquitin c protein in mammalian cells. When used to drive expression of reporter genes, the promoter would be expected to result in moderate expression of controlled genes in a constitutive manner in most mammalian cells.

**Syn1 Promoter**
The Hyman Synapsin 1 promoter is a DNA promoter sequence of human origin containing the 21 bp Neuron restrictive silencer element, restricting the expression of genes from this promoter to neural cells or cells expressing the RE1 silencing transcription factor. In neuronal cells this promoter would be expected to drive low to moderate levels of transcription, and hence production of the target protein. Virtually no protein production would be expected in non-target cells.

**GFAP promoter**
The GFAP promoter drives expression of GFAP (glial fibrillary acidic protein) almost exclusively in astrocytes. Expression of fluorescent reporter genes from this promoter would be expected to be limited to astrocytic cells resulting in moderate expression of the reporter protein. Expression in non-astrocytic cell lines would not be expected.

**2Xsyn1 promoter**
This promoter utilises artificial transcriptional activators to enhance transgene expression from the Syn1 promoter. This strategy involves cell-type-specific expression of the strong chimeric activator GAL4p65 consisting of the transactivation domain of nuclear factor-kappa B (NFκB) p65 protein fused to the DNA-binding domain of the GAL4 protein from yeast. GAL4p65 production is limited to neuronal cells as it is under the control of the Syn1 promoter. The Gal4p65 protein binds to multiple Gal4-binding sites placed upstream of the second copy of the Syn1 cell-specific promoter, leading to amplified expression of the transgene (GFP, YFP, CFP, RFP, ChR2). The Gal4 DNA binding domain, is specific to the promoter sequence, thus expression amplification from the p65 protein will be limited to the inserted transgene.

**Somatostatin promoter**
This promoter drives gene expression in neuroendocrine neurons of the periventricular nucleus of the hypothalamus. Expression of genes from this promoter would be expected to be limited to neuroendocrine neurons of the periventricular nucleus of the hypothalamus resulting in moderate expression of the reporter protein.

**WPRE (Woodchuck Posttranscriptional Regulatory Element) from the**
Woodchuck hepatitis virus is placed directly downstream of the gene of interest. It increases the nuclear export of the RNA transcript thus enhancing transgene expression (Mastroyiannopoulos et al., 2005; Zufferey et al., 1998).

cPPT (Polypurine Tract) from the HIV-1 integrase gene increases the copy number of lentivirus integrating into the host genome (Park, 2001) and allows for a two-fold increase in viral titre. WPRE and cPPT together produce at least a fourfold increase in protein expression in most cell types, compared to other vectors that do not contain these elements.

EIFα
Human promoter for lower but more ubiquitous expression in cells and in vivo with lower risk of promoter shut down.

V5 Epitope Tag for detecting the recombinant protein of interest (Southern et al., 1991)

Blasticidin (bsd) resistance gene for selecting stably transduced mammalian cell lines (Kimura et al., 1994)

Chloramphenicol resistance gene (CmR) located between the two attR sites for Counter selection

attR sites Bacteriophage λ-derived DNA recombination sequences that permit recombinational cloning of the gene of interest / miR/sh RNA and promoter of interest from GatewayR entry clones (Landy, 1989).

ccdB gene Produces the ccdB protein which targets E. coli DNA gyrase, preventing DNA replication of bacterial cells thus permitting negative selection of the plasmid

U3/HIV-1 truncated 3' LTR Allows viral packaging but self-inactivates the 5' LTR for biosafety purposes (Dull et al., 1998). The element also contains a polyadenylation signal for transcription termination and polyadenylation of mRNA in transduced cells.

**Evaluation of foreseeable effects**

It would not be expected that expression of the inserted gene would occur in bacterial hosts or cells used for viral production as proteins are under the control of cell specific promoters. Expression will only be expected to occur in neuronal or astrocytic cells where moderate expression of the optogenetic constructs would be expected from the cells in which the virus has been integrated.

Transformed E. coli cells would not be expected to alter the pathogenicity of the original host. The cells would still have limited survival in human or other hosts, as such the acquired resistance to the antibiotic kanamycin should not pose a risk to human health as the cells should be incapable of establishing an infection.

With respect to the Viral work minimal hazard would be expected of the viral plasmids in E. coli where generation of infectious viral particles would be impossible.

The produced ChannelRhodopsin –XFP, Opto-XR, Case12, are unlikely to pose a risk to the host, operator or environmental organisms.

The main hazard is associated with the packaging of lentviruses in cells. The viruses will be capable of infecting human cells, with the consequent insertion of the vector genome into chromosomal DNA. The main hazard is the possibility of oncogene activation at the site of integration. Consideration of the experiences from human gene therapy clinical trials suggests this risk is low, as any accidental exposure would involve many logs fewer virus particles, nevertheless precautions to avoid exposure are appropriate. A further potential hazard is the generation of replication-competent virus. As described above, use of split packaging systems for retrovirus, and second / third generation SIN vector systems for lentivirus, makes this highly unlikely. The use of self-inactivating lentiviral vectors effectively limits the virus to a single infective cycle thus after several changes of culture media/natural clearance in mice/rats, the final drug selected cells lines/mice/rats will not contain infectious viral particles and could be
The majority of individuals appear to undergo primary infection with Ad5 in childhood, and both humoral and cell-mediated immunity combine to prevent further productive infection in the adult. Such immunity will reduce viral entry into cells, and hasten the immune destruction of infected cells, in the event of accidental human contamination with the recombinant virus. Working practices are designed to minimise the risk of human contamination.

The recombinant virus will not be capable of autonomous spread in the event of accidental operator infection. In the unlikely event that an operator were to receive an 'infectious dose' of virus via the respiratory tract, it would only lead to replication of the GM virus if they had a concurrent infection with wild type virus, and the same cells took up both the wild type and GM viruses. It is known that the subgroup C serotypes (includes Ad5) can persist in the adenoids and tonsils following primary infection, but the mechanism by which the virus is maintained is not known. However, the number of virus-containing cells is very low, and most virus remains cell-associated, and can only be detected after prolonged incubation of the tissue in vitro. Thus the risk of double infection with recombinant and wild type virus is considered to be very low. Attempts to detect such complementation by dual infection in the cotton rat model gave negative results. The possibility of co-infection in the gut must also be considered; while it is known that in some instances there is prolonged shedding of subgroup C viruses via the alimentary canal, there is no evidence that there is long term persistence of the virus, so the opportunity for co-infection of a single cell with both wild type and recombinant is considered remote. In conclusion, it seems unlikely that accidental human infection with the replication-defective adenovirus would result in its rescue, either with helper virus or as a replication-competent recombinant; or that human-to-human transfer would take place.

Contents and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

It is the responsibility of the person undertaking the work with the GMM to check the correct operation of equipment used, on every occasion. Nitrile gloves, culture vessels, centrifuge tubes etc should be inspected before and during use, to ensure leak-free operation. The work area and equipment should be checked before and after use for any spillages which might contain GMMs, and appropriate remedial action taken if necessary. The work surface must be decontaminated after use.

Project supervisors have a responsibility to ensure that persons in their group are following the specified containment and control measures, and arranging for appropriate training of new workers.

Designated laboratory coats or tissue culture gowns. Any cuts or abrasions should be appropriately protected (e.g. waterproof dressing or protective gloves).

Storage in appropriately labelled, screw-capped plastic tubes, in secondary boxes within freezer in containment lab. Tube contained within an appropriate secondary container able to contain any potential leaks, if transport between labs is required.

The production of aerosols is not intended and will be minimised by using capped tubes and flasks. All waste and contaminated glassware and plastics will be autoclaved according to HSE requirements prior to disposal or cleaning and re-use in the 6th floor autoclave. No sharps will be used in the majority of this work thus minimising the risk of operator contamination. In the case of accidental spillages of culture media containing cells, the contaminated area can be effectively disinfected with 1% Virkon, and the small volume inherently prevents the spread of contamination to the wider environment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Virkon used for most surface decontamination. Disposable plastic tips for micropipettors, may be decontaminated by drawing up Virkon disinfectant, and soaking in Virkon for a minimum of 20 minutes. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above.

All tissue culture will be carried out in a designated microbiological safety cabinets with filters appropriate for cells of category Biosafety level 2. Safety cabinets are tested and maintained on a regular schedule; safety cabinets have a KI discus test and maintenance every 6 months, for class I cabinets this is on an annual basis. The tissue culture expression will be on a small scale of less than 108 cells. Levels of protein expression are unlikely to exceed 1mg/litre. All tissue culture waste will be autoclaved.
The designated incubator for culture of virally infected cells will be equipped for wet heat sterilization.

Warning lights, and chart recorder, indicate whether or not the autoclave run has achieved the appropriate temperate for the required time. The autoclave is run by trained staff, who are instructed to treat waste as still contaminated if the autoclave run was not to specifications. Under such circumstances the waste would be autoclaved again, after rectifying any reason for the malfunction. The autoclave is inspected and serviced on a regular basis.

All liquid waste is treated by autoclaving at 121°C for 30 minutes, before disposal to drains. Disposable solid waste which is or may be contaminated with GMOs is also inactivated by autoclaving at 121°C for 30 minutes, before removal as “clinical waste” by specialist contractors, with final disposal by incineration.

Expected degree of kill:
Autoclaving achieves effectively 100% kill of all GMOs.
The manufacturer's information indicates efficacy of Virkon against a variety of adenoviruses at 1% concentration, and also against a number of strains of E. coli at dilutions ranging from 1% to 0.125%. Colleagues at Birmingham University have demonstrated that 15 minutes exposure to 1% Virkon achieves >4 log10 kill for adenovirus-infected cell pellets, and >6 log10 kill of adenovirus seed stocks. The effectiveness of Virkon in the deactivation of plasmid DNA was tested with plasmids of a similar size to those that will be created in the work outlined above.

Incubation of plasmid DNA in a 1% solution of Virkon for 2 minutes reduced the transformation efficiency of plasmid DNA in transforming E coli cells from 2.8 x 106 transformants / µg DNA to 4.2 x104 transformants / µg DNA. Incubation for 20 minutes in this 1% Virkon solution completely abolished the ability of the DNA to transform cells. There is no reason to suggest that the plasmids described in this work will be less susceptible to chemical inactivation than those tested.Class II biological safety cabinets will be used for handling of mouse brain slices/cells, in order to maintain sterility for subsequent experiments.

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## Project Additional Information

### Purposes of the contained use

The purpose is to manipulate the genetic material of reproductive and vascular tissues, organs and relevant primary cells and cell-lines to create models that will be used to research normal physiological mechanisms and disease processes. The overall object is to generate the adenoviruses, adeno-associated viruses, lentiviruses and retroviruses carrying genetic material and use these viruses in the cells and animals for the study of reproductive and vascular function and diseases.

### Recipient or parental organism

- Cytomegalovirus for CMV promoter
- Woodchuck Hepatitis Virus for WPRE element
- Human for cystathionine gamma lyase (CSE)
- Human for angiopoietin-1 and -2 (Ang-1, -2)
- Mouse for soluble Flt-1, endoglin (sFlt-1, sEng)
- Human for micro RNA
- Human for VEGF family, LRIG1, HO-1, BVR and eNOS
- Jelly fish for mCherry and eYFP
- Bacteria for β-Lactamase resistance

### Host/vector system

For hosts:
- E. coli DH5α Genotype
- E. Coli Stbl3™ Genotype
- 293FT cells
For Vectors:
(i) Standard cloning vectors for manipulation and propagation in E.coli, such as PCR-8 and pENTR 5 TOPO.
(ii) Standard reporter plasmids and expression plasmids, such as pcDNA3.1.
(iii) Tetracycline inducible system vector, pBl-G.
(iv) Adeno-associated virus Type-2, The pHelper AAV system (Stratagene) employs a series of 3 plasmids: pHelper, pRC and pCMV.
(v) Lentivirus, pCLlsin.PPT.hPGK.GFP.pre, pRSV-Rev and pMDLg/pRRE.
(vi) Retroviruses, two systems, (1) The MFG retrovirus vector uses a series of 3 plasmids pMD.MLV(gag-pol), pMD.G(env) and pMMP. (2) LNCX plasmid.

Origin & function
The VEGF family
The VEGF family (VEGF-A –B –C -D –E and placenta growth factor/PIGF) and their cognate receptors (Flt-1, Flt-4, KDR and neuropilin-1/2) are key mediators of angiogenesis, vascular remodeling and maintenance [Yancopoulos et al., (2000) Nature. 407:242]. VEGFs bind the high-affinity Flt-1, Flt-4 and KDR tyrosine kinase receptors, which are largely restricted to endothelial cells, and their functional activities include increased proliferation, migration, and procoagulant activity [Yancopoulos et al., (2000) Nature. 407:242].

The neuropilins (neuropilin-1/-2) have recently been shown to modulate VEGF receptor signaling in endothelium to enhance the activities of KDR and inhibit the actions of Flt-1, in addition to mediating the effects of VEGF in neurons [Neufeld et al., (2002) Trends Cardiovasc Med. 12:13]. Although the VEGF family members are associated with malignant progression through the promotion of tumor angiogenesis and metastasis, they have not been linked to the primary/initiating events involved in cell transformation.

Angiopoietins and Tie receptor families
The angiopoietins (Ang-1 to -4) act in concert with the VEGFs to co-ordinate vascular proliferation, differentiation and maintenance/integrity [Yancopoulos et al., (2000) Nature. 407:242]. Angiopoietins (the most physiologically significant of which are Ang-1 and Ang-2) bind to the Tie-2 receptor tyrosine kinase. Ang-1 activates Tie-2 leading to endothelial cell migration, sprout formation and survival in vitro; and reduces vessel permeability and promotes angiogenesis, vessel branching and stability in vivo. Whereas Ang-2 antagonises Ang-1/Tie-2 signalling and enhances angiogenesis in the presence of VEGF in vivo. Recently, it has been recognised that Ang-2 may also signal via Tie-2 to enhance endothelial cell survival [Yancopoulos et al., (2000) Nature. 407:242]. Tie-2 is an orphan endothelial receptor tyrosine kinase that has also been shown to promote endothelial cell survival [Kontos et al., (2002) Mol. Cell. Biol. 22:1704]. The Angiopoietins and Tie receptors have not been linked with cell transformation. The up-regulation of Ang-2 and autocrine stimulation of Tie-2 is thought to lead to initial regression of parasitized host vasculature that occurs during the development of some tumours [Holash et al., (1999) Science. 284:1994]. In established rodent tumour models Ang-1 and Ang-2 have been shown to either reduce or enhance angiogenesis/malignant progression.

Heme-oxygenase (HO-1/-2)
Heme oxygenase 1 (HO-1) is a rate-limiting enzyme, which catalyses the heme to the carbon monoxide (CO), iron and biliverdin/bilirubin. HO-1 has the cytoprotective function and can be induced by oxidant, apoptosis and inflammation. In angiogenesis, vascular endothelial growth factor (VEGF) up-regulates the expression of HO-1 in vascular endothelial cells, which promote the angiogenesis process and inhibit the preeclampsia [Gozzelino, et al., (2010) Annu Rev Pharmacol Toxicol. 50:323; Idriss, et al., (2008) J Am Coll Cardiol. 52:971].

Biliverdin Reductase
Biliverdin reductase (BVR) was firstly described as NADH-dependent enzyme that converts biliverdin to bilirubin [Schluchter and Glazer, (1997) J Biol Chem 272: 13562].

Cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS), and 3-mercapto-sulfurtransferase (MST)

CBS and CSE are expressed in rat and human in utero tissues. Mice genetically deficient of H2S producing enzyme CSE leads to age-dependent hypertension, severe hyperhomocysteinemia, and impaired endothelium-dependent vasorelaxation [Yang et al., (2008) science 322:587]. The homozygous (CSE−/−) and heterozygous (CSE+/−) mutant mice were viable, fertile, and indistinguishable from their control wild-type littermates in terms of growth pattern. In addition, cardiac-specific overexpression of CSE (α-MHC-CGL-Tg mouse) significantly limits myocardial ischemia-reperfusion injury, which is associated with an inhibition of myocardial inflammation and a preservation of both mitochondrial structure and function after injury. [Elrod et al. (2007) Proc. Nat. Acad. Sci.: USA 104:15560]. It has been shown that although CBS−/− mice born at normal rate, but they often suffer from growth retardation, normally die within 5 weeks after birth [Watanabe et al., (1995) Proc. Nat. Acad. Sci.: USA 92:1585].

Endothelial Nitric Oxide Synthesis (eNOS)

Nitric oxideNitric oxide synthases (NOSs) are a family of eukaryotic enzymes that catalyze the production of nitric oxide (NO) from L-arginine. NOS is one of the most regulated enzymes in biology. There are three known isoforms, two are constitutive (cNOS) and the third is inducible (iNOS) [Majano et al., (2001) Hepatology; 34: 1218]. Endothelial NOS (eNOS) generates NO in blood vessels and is involved with regulating vascular function [Dudzinski et al., (2006) Annu. Rev. Pharmacol. Toxicol.; 46: 235; Ahmad et al., (2006) Circ. Res.; 99: 715]. eNOS is also associated with plasma membranes surrounding cells and the membranes of Golgi bodies within cells.

Leucine-rich repeats and immunoglobulin-like domains 1(LRIG1)

LRIG1 is a newly discovered integral membrane protein. The first LRIG transcript was discovered in mouse (also called lig-1) [Suzuki et al., (1996) J. Biol. Chem. 271:22522]. Disruption of LRIG1 gene leads to psoriasiform epidermal hyperplasia and LRIG1 inversely correlates with proliferative ability of epidermal keratinocytes. More recently, LRIG1 was showed as a negative attenuator of signaling by receptor tyrosine kinases [Gur et al., (2004) EMBO J. 23:3270] and negatively regulates EGFR, Met receptor, Ret receptor [Sweeney et al., (2004) J. Biol. Chem.279: 47050; Goldoni et al., (2007) Oncogene 26:368; Ledda et al., (2008) J Neurosci. 28:39]. Furthermore, LRIG1 interacts with EGFR via extracellular domain and subsequently causes receptor internalization and degradation. In our lab, LRIG1 has been show to be up regulated around three fold under pre-eclamptic condition (unpublished data).

LacZ

LacZ encodes β-galactosidase (LacZ), an intracellular enzyme that cleaves the disaccharide lactose into glucose and galactose [Fowler, Zabin, (1970) J. Biol. Chem. 245:5032]. β-galactosidase assay is used frequently in genetics, molecular biology for a blue-white screen, and other life sciences.

eGFP

The nucleic acid sequence encoding Enhanced Green Fluorescent Protein (EGFP) is included for the expression of EGFP in transfected cells. EGFP has a long history of safe use as a fluorescent reporter protein. No attempt will be made to purify the protein, which will only be expressed in the target cells. GFP is unlikely to pose a risk to the operator or environmental organisms as preliminary data on the deliberate administration of GFP to weaned rats suggested that GFP exhibits low allergenicity and is not likely to represent a health risk (Richards et al 2003).

mCherry fluorescent protein

Cherry fluorescent protein nucleic acid sequences have been derived from anemone fluorescent protein by site directed mutagenesis. As members of the newer generation of fluorescent reporter proteins, little toxicological data exists on the proteins themselves, but both proteins share a great deal of homology with the eGFP protein and have a history of safe use as fluorescent reporters, including their expression in transgenic animals with no detrimental effects.
Tetracycline inducible expression lentiviral system
TRE element and mini 35S promoter from pBI-G vector will be used to replace the hPGK promoter in pCCLsin.PPT.hPGK.GFP.pre to make a tetracycline inducible lentiviral vector. Tetracycline or Doxycycline is used to induce the target gene expression in this system. The TRE element and mini 35S act as promoter and drive the target gene expression, but have no transcriptional or translational products.

WPRE
(Woodchuck Posttranscriptional Regulatory Element) from the Woodchuck hepatitis virus is placed directly downstream of the gene of interest. It increases the nuclear export of the RNA transcript thus enhancing transgene expression (Mastroyiannopoulos et al., 2005; Zufferey et al., 1998).

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(Polypurine Tract) from the HIV-1 integrase gene increases the copy number of lentivirus integrating into the host genome (Park, 2001) and allows for a two-fold increase in viral titre. WPRE and cPPT together produce at least a four fold increase in protein expression in most cell types, compared to other vectors that do not contain these elements.

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(CmR) located between the two attR sites for Counter selection.

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It would not be expected that expression of the inserted gene would occur in bacterial hosts or cells used for viral production as proteins are under the control of cell specific promoters. Expression will only be expected to occur in cells where moderate expression of the constructs would be expected from the cells in which the virus has been integrated/infected.

Transformed E. coli cells would not be expected to alter the pathogenicity of the original host. The cells would still have limited survival in human or other hosts, as such the acquired resistance to the antibiotic kanamycin should not pose a risk to human health as the cells should be incapable of establishing an infection.

With respect to the viral work minimal hazard would be expected of the viral plasmids in E. coli where generation of infectious viral particles would be impossible.

The produced target genes are unlikely to pose a risk to the host, operator or environmental organisms.

The main hazard is associated with the packaging of viruses in cells. The viruses will be capable of infecting human cells, with the consequent insertion of the vector genome into chromosomal DNA for AAV/lentiviruses. The main hazard is the possibility of oncogene activation at the site of integration. Consideration of the experiences from human gene therapy clinical trials suggests this risk is low, as any accidental exposure would involve many logs fewer virus particles, nevertheless precautions to avoid exposure are appropriate. A further potential hazard is the generation of replication-competent virus. As described above, use of split packaging systems for retrovirus, and second / third generation SIN vector systems for lentivirus, makes this highly unlikely. The use of self-inactivating lentiviral vectors effectively limits the virus to a single infective cycle thus after several changes of culture media/natural clearance in mice/rats, the final drug selected cells lines/mice/rats will not contain infectious
viral particles and could be handled at level 1.

The majority of individuals appear to undergo primary infection with Ad5 in childhood, and both humoral and cell-mediated immunity combine to prevent further productive infection in the adult. Such immunity will reduce viral entry into cells, and hasten the immune destruction of infected cells, in the event of accidental human contamination with the recombinant virus. Working practices are designed to minimise the risk of human contamination.

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Project supervisors have a responsibility to ensure that persons in their group are following the specified containment and control measures, and arranging for appropriate training of new workers.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Virkon used for most surface decontamination. Disposable plastic tips for micropipettors, may be decontaminated by drawing up Virkon disinfectant, and soaking in Virkon for a minimum of 20 minutes. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above.

All tissue culture will be carried out in a designated microbiological safety cabinets with filters appropriate for cells of category Biosafety level 2. Safety cabinets are tested and maintained on a regular schedule; safety cabinets have a KI discus test and maintenance every 6 months, for class I cabinets this is on an annual basis. The tissue culture expression will be on a small scale of less than 1x10e8 cells. Levels of protein expression are unlikely to exceed 1mg/litre. All tissue culture waste will be
autoclaved. The designated incubator for culture of virally infected cells will be equipped for wet heat sterilization.

Warning lights, and chart recorder, indicate whether or not the autoclave run has achieved the appropriate temperate for the required time. The autoclave is run by trained staff, who are instructed to treat waste as still contaminated if the autoclave run was not to specifications. Under such circumstances the waste would be autoclaved again, after rectifying any reason for the malfunction. The autoclave is inspected and serviced on a regular basis.

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Autoclaving achieves effectively 100% kill of all GMOs. The manufacturer’s information indicates efficacy of Virkon against a variety of adenoviruses at 1% concentration, and also against a number of strains of E. coli at dilutions ranging from 1% to 0.125%. We have previously demonstrated that 15 minutes exposure to 1% Virkon achieves >4 log10 kill for adenovirus-infected cell pellets, and >6 log10 kill of adenovirus seed stocks. The effectiveness of Virkon in the deactivation of plasmid DNA was tested with plasmids of a similar size to those that will be created in the work outlined above.

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Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

Increased expression of antibiotic drug targets in the ACGM-category 2 microorganisms *Mycobacterium abscessus* and *Mycobacterium bovis BCG* in order to study the effects on microbial physiology and verify mechanism of action.

**Recipient or parental organism**

*Mycobacterium abscessus NCTC 13031, Mycobacterium bovis BCG Pasteur 1173P2, Mycobacterium tuberculosis H37Rv*

*Mycobacterium abscessus NCTC 13031*: M. abscessus is part of a group of environmental mycobacteria and is found in water soil and dust. It has been known to contaminate medications and products including medical devices. M. abscessus can cause a variety of infections. Healthcare associated infections due to this bacterium are usually of the skin and soft tissues under the skin. It is also a cause of serious lung infections in persons with various chronic lung diseases such as cystic fibrosis. People with open wounds or who receive injections without appropriate skin disinfection may be at risk for infection by M. abscessus. Rarely, individuals with underlying respiratory conditions or impaired immune systems are at risk of lung infection. Transmission of M. abscessus can occur in several ways. Infection with M. abscessus is usually caused by injections of substances contaminated with the bacterium or through invasive medical procedures, employing contaminated equipment or material. Infection can also occur after accidental injury where the wound is contaminated by soil. There is very little risk of transmission from person to person. M. abscessus is an ACDP-Category 2 organism.

*Mycobacterium bovis BCG Pasteur 1173P2*: M. bovis is a major cause of tuberculosis in a range of animal species and man. It was the progenitor of the M. bovis bacillus Calmette-Guerin (BCG) strain used as a vaccine against tuberculosis. BCG was derived by serial passage on slices of potato imbibed with glycerol for 13 years, once the strain was proven to no longer be virulent it was disseminated and different laboratories continues this passaging. This particular strain, M. bovis BCG 1173P2, has been passaged 1,173 times at the Pasteur Institute, Paris France. Different centers have different stocks of BCG, which have continued to change with time, accumulating insertions, deletion and single nucleotide polymorphisms. M. bovis BCG is an ACDP-Category 2 organism.
Mycobacterium tuberculosis H37Rv: M. tuberculosis H37Rv is the most studied strain of tuberculosis in research laboratories. It was first isolated by Dr Edward Baldwin in 1905. The strain came from a 19 year old patient with chronic pulmonary tuberculosis at the Trudeau Sanitorium and was initially names strain H37. Over time it was found to have variable virulence in animal models based on which medium it was grown on. Strains with different virulence were then intentionally produced, with H37Rv denoting R (rough) and v (virulent). The strain was used for many laboratory studies and later became the standard for tuberculosis research, with the complete genome published in 1998. M. tuberculosis is an ACDP-Category 3 organism and as a result, only genetic material from this organism is used in our work as we do not have containment level 3 facilities at Aston University.

The above information was extracted from https://www.cdc.gov/hai/organisms/mycobacterium.html

Host/vector system

Mycobacterium abscessus NCTC 13031, Mycobacterium bovis BCG Pasteur and Escherichia coli (strains BL21 DE3 and TOP10) using the following vectors: pMV261, pVV16, pET28b, pET23a

For Mycobacterium abscessus and Mycobacterium bovis BCG, please see "A. 1. The Donor" information.

Escherichia coli TOP10 and BL21 DE3 strains are standard laboratory non-pathogenic strains for gene cloning and manipulation.

pMV261: A shuttle plasmid capable of replication in E. coli and in mycobacterial species (mycobacterial origin of replication from Mycobacterium fortuitum plasmid pAL5000) using kanamycin resistance for selection. The cloned mycobacterial gene will be under the constitutive mycobacterial hsp60 promoter.

pVV16: Derived from pMV261, pVV16 is a shuttle plasmid capable of replication in E. coli and in mycobacterial species (mycobacterial origin of replication from Mycobacterium fortuitum plasmid pAL5000) using kanamycin resistance for selection. The plasmid contains a hystidine-tag and a signal sequence. The cloned mycobacterial gene will be under the constitutive mycobacterial hsp60 promoter.

pET28b: The pET28a-c vectors carry an N-terminal His tag/Thrombin/T7 and is used extensively for recombinant protein production in E. coli. pET28b carries a kanamycin resistance cassette.

pET23a: The pET23a vector carries an N-terminal His tag/T7 promoter and terminator and is used extensively for recombinant protein production in E. coli. It carries an ampicillin resistance cassette.

Origin & function

Rv2763c (dfrA), Rv2764c (thyA), Rv0268c (blaC), Mab_2875


Rv0268c (blaC): Class a beta-lactamase (BlaC). Hydrolyses beta-lactams to generate corresponding beta-aminoacid

Mab_2875: Class a beta-lactamase (BlaC). Hydrolyses beta-lactams to generate corresponding beta-aminoacid

Evaluation of foreseeable effects

Genes (section 7) encoded in pMV261 and pVV16 will be expressed in M. abscessus and M. bovis BCG in order to assess the change in antibiotic sensitivity phenotype. Mycobacterial genes cloned into pMV261 and pVV16 will be expressed via the hsp60 promoter in the host strains. The promoter is constitutively expressed and thus there
is a 'steady' moderate expression, rather than 'overexpression' usually seen with inducible promoters. Genes encoded in pET vectors will be expressed in E. coli and purified my IMAC.

The proposed work is to investigate how the enhanced expression of certain genes impacts the antimicrobial susceptibility to known antimicrobial compounds. This strategy is widely employed in order to establish the mechanism of action of antimicrobials by reducing sensitivity to specific antimicrobial agents, thus indicating target engagement. This work does not increase the pathogenicity of strains, but does result in reduced sensitivity to defined classes of antibiotic as well as all molecular genetics involving antibiotic resistance markers. This does not pose an enhanced risk to human health as the compounds being investigated are not currently administered treatment. If the vector were to enter the body, the genes would not be expressed. Therefore, the GMOs should not represent an increased risk over the parent organism.

M. abscessus can cause a variety of infections. Healthcare associated infections due to this bacterium are usually of the skin and soft tissues under the skin. It is also a cause of serious lung infections in persons with various chronic lung diseases such as cystic fibrosis. People with open wounds or who receive injections without appropriate skin disinfection may be at risk for infection by M. abscessus. Rarely, individuals with underlying respiratory conditions or impaired immune systems are at risk of lung infection. Transmission of M. abscessus can occur in several ways. Infection with M. abscessus is usually caused by injections of substances contaminated with the bacterium or through invasive medical procedures, employing contaminated equipment or material. Infection can also occur after accidental injury where the wound is contaminated by soil. There is very little risk of transmission from person to person. M. bovis BCG is an attenuated strain of M. bovis that is widely used as a human vaccine strain. Although infections due to M. bovis BCG have occurred, these are predominantly in individuals with severe immunocompromisation and therefore the risks of infection are nominal and not enhanced by the genetic modifications proposed. Immunocompromised individuals are at higher risk of infection and disease and will not be permitted to work in the BSL2 laboratory. Both Mycobacterium abscessus and Mycobacterium bovis BCG are categorised as ACDP-Category 2 organisms.

M. abscessus is an environmental mycobacterial strain, therefore the environmental risk is negligible.

Mycobacterium bovis BCG is an attenuated strain and lacks the necessary virulence machinery to infect healthy hosts (including animals). It is a temperature sensitive organism requiring a host body temperature to grow, therefore the environmental risk is negligible. GMOs will be contained and handled without contact to other microbes, therefore the risk of transfer of genetic material is nominal. Likewise other biologicals employed in this work will be handled with the same level of containment. All material will be disinfected by autoclave once work is completed.

<table>
<thead>
<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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</thead>
<tbody>
<tr>
<td>N/A</td>
</tr>
</tbody>
</table>

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Contaminated tubes, pipette tips, cryovials, loops will be disposed in autoclavable, disposable waste bags and autoclaved. Biocleanse 1:10 dilution will be added to waste liquids (old cultures and culture supernatants) prior to autoclaving. Pipetting and spreading of cultures is likely to generate minimal amounts of aerosols, however as all work with M. abscessus and M. bovis BCG is to be carried out in a Class II cabinet, any minimal amounts of aerosols generated will be contained. No metal or glass-based sharps will be used. Micropipette tips and disposable loops will be disposed of in an appropriate waste bag prior to disinfection by autoclaving. All glycerol stocks will be stored in 2ml cryovials at -80oC inycrovial box. Transportation if required will be done by designated courier services in accordance with conditions of carriage for ACDP Group 2 organisms. The spread of large volume spills will be limited by absorbent materials. The absorbed material will be disposed of in autoclavable waste bags and decontaminated by autoclave. Small spills inside the Class II biosafety cabinet will be disinfected with 1:10 Biocleanse. Disinfection in this manner should prevent the release of genetically modified material. |

02/03/2022
The GMO Risk Assessment for Genetic Manipulation of Mycobacteria sp. was reviewed by Aston University Health and Safety committee and they raised the following questions which were addressed as follows:

1. Will records of training be kept?
   - In this section I have included the sentence “Training and records of training will be completed by Group Leader”.

2. How is this grown. If not at Aston where? How is material obtained.
   - I have included the sentence “Genomic DNA is provided by the BSL-3 tuberculosis laboratory at University of Birmingham. Genomic preparations are screened for absence of viable cells before removal from BSL-3 laboratory.”

3. Should these not be listed before?
   - The host characteristics for E. coli are in the appropriate place as confirmed by Russell Collighan.

4. Clarify the antibiotics used here would not be used in a possible treatment.
   - The text already contained the sentence, “This does not pose an enhanced risk to human health as the compounds being investigated are not currently administered treatment.” For further clarification, I have added, “These are experimental compounds and do not form part of the recommended treatment regime.”

5. Control of exposure? Concerns over shared areas and equipment.
   - I have included the statement, “Equipment that is used for this work is not shared with other groups and the microbiology laboratory (MB327) is a controlled work area with restricted access.”

6. Class of hoods and lab? 2?
   - I have included the statement, “Laboratory MB327 is a BSL-2 laboratory and these organisms will be handled in a class 2 biosafety cabinet.”

7. Will media be decontaminated with Virkon?
   - I have amended the sentence to read, “All material will be disinfected with Biocleanse (as per disposal procedure below), autoclaved and incinerated once work is completed.”

Please enter comments on the GM safety committee on the risk assessment

<table>
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<th>Question</th>
<th>Answer</th>
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<tr>
<td>Is an emergency plan required according to regulation 20?</td>
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<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
<td>N</td>
</tr>
<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
</tr>
<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>N</td>
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</table>
8. Is this proven to kill this strain. Has this been tested in the lab?

- I have added the sentence, "Biocleanse is the recommended disinfectant for Mycobacteria sp. including Mycobacterium tuberculosis ([link to datasheet])."

9. Details. Where stored etc.

- Further clarification on the spill procedure is provided. No specific "spill kit" is required as working volumes are restricted to 100ml and therefore disinfection procedure stated is appropriate (as discussed with Russell Collighan.)

10. Spill kit location

- Please see point 9.

Attached is the corrected .pdf of the GMO Risk Assessment entitled "Cox 2019 GMO Risk Assessment" which was deemed satisfactory by Aston University Health and Safety committee following review.

### Project Containment

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<tr>
<th>Laboratory Activities</th>
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GM Centre Number: 376

Data Premises Notified (Originally) 02/07/1990

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Name

NATIONAL COLLECTIONS OF INDUSTRIAL & MARINE BACTERIA LTD

Name 2

NCIMB

Department

Campus Estate or Research Centre

CRAIBSTONE ESTATE

Building

FERGUSON BUILDING

Road Name

BUCKSBURN

District

Town

ABERDEEN

County

ABERDEENSHIRE

Postcode

AB21 9YA

Country

SCOTLAND

Tel Number 01224 711100

Fax Number 01224 711299

E-mail

HSE Division SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
<tr>
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<th>Animal Unit</th>
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Tick if confidential

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Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment.
### GM Centre Number: 377

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**Name**

KING'S COLLEGE MRC CENTRE FOR DEVELOPMENT NEUROBIOLOGY

**Name 2**

LONDON UNIVERSITY GUYS CAMPUS

**Campus Estate or Research Centre**

**Building**

4TH FLOOR NEW HUNT'S HOUSE

**Road Name**

GUY'S CAMPUS

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

SE11UL

**Country**

ENGLAND

**Tel Number**

0207 955 4574

**Fax Number**

0207 4038883

**E-mail**

**HSE Division**

LONDON

**Comments**

GM377 MERGED WITH GM295 ON 8/9/2003

**Date at Which Additional Info Submitted**

02/03/2022
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- Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

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**GM Centre Number: 382**

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**Name**

UNIVERSITY OF EXETER

**Name 2**

HATHERLY LABORATORIES

**Department**

SCHOOL OF BIOLOGICAL SCIENCES

**Campus Estate or Research Centre**

**Building**

**Road Name**

PRINCE OF WALES ROAD

**District**

**Town**

EXETER

**County**

DEVON

**Postcode**

EX4 4PS

**Country**

ENGLAND

**Tel Number**

01392 264674

**Fax Number**

01392 263700

**E-mail**

**HSE Division**

WALES AND SOUTH WEST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
Virology Transgenic Animals Transgenic Fish Gene Therapy
Mycology Transgenic Invertebrates Transgenic Plants Other (please specify below)
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity
Please enter comments of the GM safety committee on the risk assessment

Project Ref 382/00.1

Date Ackn'd 27/03/2000
Date Project Ceased

CU2 Project Title THE BIOFILM FORMATION OF SALMONELLA ENTERITIDIS UNDER CHILLED CONDITIONS

Class 2
CultureVolClass2
Consent Granted Not Applicable

Non-GMM

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee approved the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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- Animal Units
- Large Scale Activities
- Human Clinical Applications

**Project Ref** 382/03.1

<table>
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<th>CU2 Project Title</th>
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MOLECULAR GENETIC ANALYSIS OF PATHOGENICITY OF THE RICE BLAST FUNGUS MAGNAPORTHE GRISEA

Purposes of the contained use
The project will use molecular genetic analysis to identify genes from M. grisea that are essential for the fungus to cause plant disease. Recombinant strain of M. grisea will be generated where genes have been mutated by targeted gene replacement or by random insertional mutagenesis. The ability of the recombinant M. grisea strains to cause disease will then be tested. The other principal activity will include generation of reporter gene constructs to investigate regulation of genes involved in causing plant disease.

Recipient or parental organism
Magnaporthe grisea strain Guy11 and derivatives will be used as the fungal host strains in all experiments. Guy11 is a rice pathogenic strain of the fungus originally isolated from French Guiana in 1988. The Escherichia coli K12 strains to be used in this work are JM109 (Promega), XL-1Blue (Stratagene), DH5a (GibcoBRL).

Host/vector system
Plasmid vectors used are all pUC or pBluescript derivatives. The fungal transformation vectors utilise the Hygromycin phosphotransferase (Hph) gene from E. coli or the Bialophos resistance gene encoding phosphinothricin acetyl transferase from Streptomyces hygroscopicus as selectable markers. These genes are regulated by the Aspergillus nidulans. TrpC promoter. An allele of the M. grisea acetolactate synthase gene, ILV1, under control of its own promoter is also used as a selectable marker bestowing resistance to sulfonylurea. The vectors are derived from pCB1004, pCB1265 and pCB1532. These are described in Genbank submissions AF013601 and AF013602. The green fluorescent protein-encoding gene (GFP) will be expressed under control of native M. grisea promoters. The vectors used for fungal transformation cannot replicate extrachromosomally in fungi. The insert into host DNA.

Origin & function
The nature of the work to be carried out is to delete or disrupt native M. grisea genes. The only inserted genes are the selectable marker-encoding genes described above. Complementation analysis will introduce the native M. grisea gene into corresponding gene replacement mutants in order to restore the wild type trait.

Evaluation of foreseeable effects
The GMMs to be generated in this project are likely to be attenuated for virulence and in no case are they anticipated to have any plant pathogenic capabilities that exceed those of the wild type M. grisea Guy11 strain. The GMM strains to be generated will not have harmful properties to human health.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste in the form of agar plate cultures and infected rice plants are disposed of by autoclaving at 121 degrees C at 15 psi for 45 minutes under conditions prescribed by the DEFRA licence. Experiments in the laboratory have demonstrated the effectiveness of this procedure for sterilising material. Liquid waste is disposed of by disinfecting in a 1:100 dilution of Virkon for a minimum of 30 minutes. This procedure has also been demonstrated to be effective in preventing survival of M. grisea in the laboratory.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The GMSC noted that M. grisea was a pathogen of rice but not of UK plants: although barley could be infected in the laboratory this required high titres of spores and high humidity; barley was not infected by this fungus in Northern Europe. The risk assessment was confirmed at containment levels 1 (E. coli) and 2 (M. grisea) following specific advice from the GM inspector (GMSC minutes 02/03 and 03.05).

Project Containment

<table>
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<tr>
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Animal Units

| L2 | L3 | L4 |
| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |
| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |
| L2 | L3 | L4 |

Project Ref 382/03.2

Date Ackn’d 31/03/2003

CU2 Project Title IDENTIFICATION OF GENES INVOLVED IN BIOFILM FORMATION OF

Class 2

CultureVolClass2 1-50 litres

CultureVolumeClass3-4
To identify gene promoters of Salmonella which are preferentially induced during biofilm formation, growth, and maturation when compared to planktonic growth.

Recipient or parental organism

Salmonella enterica serovar Typhimurium SL 1344: ACDP Class 2
Escherichia coli K12.

Host/vector system


Plasmid pZEP08 containing the promoter-less gfp gene: in Escherichia coli K12.

Native promoter-gfp fusions integrated at the chromosomal putPA locus in Salmonella enterica serovar Typhimurium SL1344.

Origin & function

The gfp gene from Aequoria victoria encodes green fluorescent protein (GFP) for use as a reporter to monitor promoter function.

pKD46 from Escherichia coli and its bacteriophage: to enable targeted chromosomal integration of linear promoter-gfp fusion DNA in Salmonella enterica serovar Typhimurium SL1344.

Promoter sequences from Salmonella enterica serovar Typhimurium SL1344 will be cloned in Escherichia coli K12 and then reintroduced back into S. Typhimurium as linear gfp fusions.

Evaluation of foreseeable effects

Salmonella is pathogenic to animals and humans, although it causes only limited gastro-intestinal infection in healthy individuals. Introduction of a promoter-gfp fusion is
not expected to enhance the pathogenicity of S. Typhimurium SL1344 because it will contain only a single copy of a gene promoter, not the entire structural gene, and this is upstream of a gene encoding a harmless protein. In addition, these promoters are naturally occurring in this bacterial genome. Plasmid pKD46 confers resistance to ampicillin but its replication is temperature-sensitive and the plasmid will be lost by exposure to 37 degrees C. Introduction of the fusions onto the chromosome results in a marker that is chloramphenicol resistant and kanamycin sensitive. These are not the drugs of choice for treatment of infections in a mammalian host by these infectious agents and thus would present no selective advantage in the event of an accidental release. Likewise, ampicillin is not the choice of antibiotic for the treatment of such infections. Although most infections do not require the use of antibiotics, in the case of severe or chronic cases Ciprofloxacin would be prescribed.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste materials will be disposed of by autoclaved at 121 degrees C for 60 minutes. Experiments in the laboratory have demonstrated the effectiveness of this procedure for sterilising material. Additionally, all equipment used (including benches) will be swabbed daily with disinfectant (1% Virkon). Experiments within this laboratory have demonstrated the effectiveness of these procedures against Salmonellae.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved at Class 2 (GMSC minute 03/08e).

Project Containment

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<tr>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 382/07.1
Construction and Characterisation of Yersinia pseudotuberculosis mutants.

To identify Y. pseudotuberculosis genes and gene products which play roles in disease and which could in the longer term be exploited as targets for antimicrobials or vaccines.

Recipient or parental organism

Yersinia pseudotuberculosis

Origin & function

For mutagenesis, genes will be inactivated by the targeted insertion of plasmid DNA (eg pKNOCK) or the random insertion of DNA (eg Tn5). For allelic replacement mutagenesis a functional copy of the gene will be replaced with an inactivated gene. In all cases antibiotic resistance markers (kanamycin, ampicillin, tetracycline or chloramphenicol resistance) allow the selection of mutants and the levansucrase gene allows the selection of double cross-over mutants. Plasmid pAJD434 encodes trimethoprim resistance and encodes the lambda red recombinase, promoting high efficiency recombination between the PCR product and the target gene. Plasmid pAJD434 is present only during the mutagenesis protocol and is cured before the bacteria are characterised and/or used for virulence studies.

For complementation studies, the inactivated gene is replaced with a functional gene i.e to restore the wild type phenotype. Antibiotic resistance markers (kanamycin, ampicillin, tetracycline or chloramphenicol resistance) allow the selection of complementet mutants. Complemented mutants may express two antibiotic resistance markers.
For example, the marker of the original mutant, and the marker for the complementing plasmid.

Green fluorescent protein (GFP), derivatives of GFP, DsRED 1-E5 or the luxCDABE operon could be expressed in wild type and mutant bacteria to allow the bacteria to be tracked in host cells or host tissues. Antibiotic resistance markers (kanamycin, streptomycin, or chloramphenicol resistance) allow the selection of complemented mutants. The expression of genes of interest could also be monitored by cloning the relevant promoters upstream of reporters such as GFP, derivatives of GFP, DsRED 1-E5 or beta-galactosidase.

Note; Uncomplicated cases of diarrhoea due to Y. pseudotuberculosis usually resolve on their own without antibiotic treatment and with only supportive care (fluids, electrolytes). However, in more severe or complicated infections. Antibiotics such as aminoglycosides, doxycycline, trimethoprim-sulfamethoxazole or fluoroquinolones may be useful. All of the mutants constructed in this study would be susceptible to trimethoprim-sulfamethoxazole and / or fluoroquinolones.

**Evaluation of foreseeable effects**

The most hazardous GMM would be a strain of Y. pseudotuberculosis which is as virulent as the wild type strain but expresses and antibiotic resistance marker(s).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Where possible all waste from the laboratory will be autoclaved using a destruct cycle, at 125oC for at least 30 min. In addition, disposable plastics used during experimental work will be immersed in 10% chloros. Solid waste materials generated during working will be bagged before autoclaving. Liquid waste (unless otherwise indicated, see below) will be made to a final concentration of 10% chloros and stored in glass bottles before autoclaving. When required, animal waste will be treated with 10 % v/v Teknon Biocleanse overnight before autoclaving.

Autoclave loads will be recorded and the autoclave will be serviced and validated annually using thermocouples.

Where it is not possible to autoclave waste (e.g where it contains phenol or organic solvents) bacteria will be chemically inactivated with 10% chloros, 10 % Teknon Biocleanse or in the case of phenol waste by adding 5% hycolin. Particulate materials within 10 % volume of the material will be collected by centrifugation, washed in phosphate buffered saline and plated onto nutrient agar to establish that viable bacteris are not present. Waste material will be disposed of by incineration when free of viable Y. pseudotuberculosis.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee approved this work at containment level 2.
**Project Containment**

Laboratory Activities

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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**Project Ref 382/08.3**

Date Ackn'd: 26/08/2008

Date Project Ceased:

CU2 Project Title: Genetic modification of pathogenic fungi

Class: Class 2

CultureVolClass2: < 1 Litre

Consent Granted: Not Applicable

Non-GMM: Yes

Project notified under transitional arrangements: Yes

Withdrawn: No

Tick if notifying a connected programme of work: Yes

**Project Additional Information**

**Purposes of the contained use**

The purpose of this work is to further our understanding of fungal virulence through the use of Candida albicans and Aspergillus fumigatus as model systems. Through this we would also aim to identify and validate novel therapeutic targets.

**Recipient or parental organism**

The ACDP class C. albicans and A. fumigatus in hazard group 2. C. albicans is a commensal of the normal flora and causes opportunistic infections ranging from superficial mucosal infections to systemic infections in the immunocompromised. A. fumigatus is widely distributed in nature and causes opportunistic infections ranging from allergic aspergillosis to systemic infections in the immunosuppressed.
Vectors for transformation will have been constructed in E. coli or amplified directly by PCR. in all cases the vectors will be integrated into the host's genome via recombination.

**Host/vector system**

Origin & function

The majority of the work proposed involves the manipulation and alteration of existing genes (for example gene deletions or promoter replacements). This will involve the targeted insertion of DNA into specific locations in the genome. The inserted DNA will contain an auxotrophic marker for the selection of transformed cells (e.g. URA3, LEU2 HIS1, ADE2 and ARG4 in C. albicans or pyrG, argB, or lysB in A. fumigatus) or positive selection markers (derived from foreign DNA e.g. nourseothricin resistance in C. albicans or hygromycin, phleomycin or pyrimidine resistance in A. fumigatus). Additional foreign DNA present in the inserted DNA will include: 1. S. typhimurium hisG—present as a direct repeat around the C. albicans URA3 gene for gene deletions and subsequent recycling of the marker. 2. Tetracycline transactivator (a fusion between Tn10 tetR and either viral VP16 activation domain or S. cerevisiae HAP4 activation domain) to regulate expression of genes. 3. Reporter genes such as Beta-galactosidase, luciferase, glucuronidase and GFP. 4. Small epitope tags (e.g. V5, SxHis, HA, FLAG or Myc) to allow protein analysis. 5. The E. coli plasmid backbones (pUC and pBR based) that the insert is contained on will in some cases also become inserted into the genome.

**Evaluation of foreseeable effects**

The genetic modifications proposed would not be expected to increase the virulence of the GMO above that of the parental organism. Therefore the risk to human health can be considered equivalent to that of dealing with the parental organism.

The only potential hazard, in healthy individuals, of handling C. albicans would be from exposure resulting in a mucosal infection. However the risk of this hazard being realised, even if exposure occurred, would be low.

The only potential hazard, in healthy individuals, of handling A. fumigatus would be from inhalation exposure causing a mild allergic reaction. Procedures likely to generate aerosols would be conducted in a class 2 microbiology safety cabinet to prevent exposure. Therefore the risk from the organism would be very low.

All the genetic modifications proposed would be non mobilisable therefore the risk of transfer of sequences to other micro-organisms is remote. The GMO’s produced would be potentially as fit as the parental organism and may therefore be able to persist in the environment if a breach in containment occurred. However, the GMO’s would have no selective advantage, the risk to the environment is therefore very low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid waste is double bagged in autoclave bags and collected daily. Waste is autoclaved for 1 hour at 121 °C. The autoclave is serviced and calibrated every 6 months to ensure continued effectiveness.

Liquid waste is also inactivated by autoclaving or for small volumes (< 100 mL) by chemical treatment. Culture vessels and supernatants are decontaminated with a 1:50 dilution of VIRKON (Antec International) and are left for 16 hours prior to disposal down laboratory sink. Glassware is then rinsed and washed by dishwasher, plastic ware is further autoclaved prior to disposal. This method has been validated to give 100% kill.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

02/03/2022
The committee approved the risk assessment.

**Project Containment**

<table>
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**Project Ref** 382/13.1

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<td>A functional genomic toolkit for the wheat pathogen Mycosphaerella graminicola</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Historical Significant Changes

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<th>Date of Significant Change</th>
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Tick if notifying a connected programme of work

Project notified under transitional arrangements

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**
M. graminicola is an important pathogen of wheat. This project will enable functional genomic analyses of this organism by a number of molecular methods.

Recipient or parental organism

M. graminicola strain IPO323 (wild type, sequenced) and derivatives thereof. IPO323 was originally isolated from the Netherlands in 1981.

Host/vector system

We will use vectors described below, and others that do not differ substantially, but that have not yet been constructed.

Plasmids constructed using the pGEM-T-EASY system and derivatives thereof, e.g. a hygromycin resistance cassette with 3’ and 5’ gene flanking regions for targeted gene deletion (as described in Kershaw and Talbot, Proc Natl Acad Sci U S A. 2009 Sep 15;106(37):15967-72).

A Clp10-like plasmid that allows targeting to a single gene locus (e.g. Bowler et al., 2010 Mol Plant Pathol 11:691)

M. graminicola entry-level clones in the Gateway vector pDONR221 (Invitrogen)

M. graminicola Gateway destination vectors will be constructed based on the functionality of the Lindquist S. cerevisiae Gateway plasmids (Alberti et al., 2007 Yeast 24:913; 16.)

The vectors used for fungal transformation cannot replicate extra chromosomally in fungi.

Origin & function

Gene deletion mutants: Full knockout or knockdown mutants will be constructed. These will be used to assess the function of genes in the pathogenesis of M. graminicola. For gene inactivation mutants, the foreign DNA will encode an antibiotic resistance cassette e.g. hygromycin or an auxotrophic marker such as URA3, HIS3, LEU2, TRP1 and will not confer resistance to the main anti-fungal treatment for the M. graminicola. i.e. azoles or azoxyxstrobins.

Re-integration mutants: As a control for gene deletion experiments, the parental copy of the deleted gene will be re-integrated into the mutant background by combining the gene with a selectable marker and integration targeted to its native locus.

A set of inducible promoters that can be used in M. graminicola will be generated; for example a promoter sequence from the nitrate reductase gene, NIA1, will be used and its ability to drive nitrate-inducible gene expression of a GFP reporter tested. Both small molecule and nutrient-regulated promoters will be used.

Target M. graminicola genes (or truncations thereof) may be modified to encode an N or C terminal epitope tag (such as 6xHis, FLAG, Myc, HA, TAP), to assist in visualisation or purification. Additionally, for live cell imaging the M. graminicola genes may be fused to GFP, YFP, CFP, or dsRedstar or other such tags. The genes will be inserted into the genome at their native locus under the control of their natural promoter, or the TET promoter (or other inducible promoters), or constitutively active loci.

Controlled over-expression strains will be constructed using over-expression plasmids to study genes whose phenotype is of particular interest/ Standard nonmobilisable vectors will be used in all experiments, thus minimising risk of gene transfer. The cloned genes are not likely to increase the level of risk of transfer. They are also unlikely to be harmful to humans or animals.

Evaluation of foreseeable effects

The genetic modifications involved would not be expected to increase the virulence of the GMO above that of the parental organism, nor would they be envisioned to impact on any other trait such as drug resistance.

The risks associated with transmission of genetic material to other organisms is negligible as the vectors used are not able to replicate in M. graminicola. The only possible
route for transmission of DNA would be via recombination with another eukaryote, and this has not been documented.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No GM plants or animals will be produced.

Where *M. graminicola* is used to infect plants, this will be carried out in plastic bags by spraying a suspension of spores with an artist's airbrush. The spores land either on the leaves of the plant or the inside of the plastic bag. The plants are sealed in bags for 48 hours and then incubated for a further 2-4 days in a designated growth chamber. The bags used for spraying plants are to be autoclaved after use. The airbrush is sterilised with ethanol. All plant material, including soil, will be autoclaved after use. Pots are sterilised in Virkon for 30 minutes. The growth chamber is to be routinely disinfected with Virkon.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste in the form of agar plate cultures, broth cultures and infected plants are disposed of by autoclaving at 121 °C at 15 psi for 45 minutes under conditions prescribed by the DEFRA licence. Experiments in the laboratory have demonstrated the effectiveness of this procedure for sterilising material. Virkon treatment has been validated experimentally in the laboratory. Autoclaving has been validated experimentally elsewhere.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The GM safety committee agreed that this work should be carried out at level 2. The assessment at level 2 is considered due to risk to the environment: the organism in question is a significant pathogen of wheat, and the University's DEFRA license for its use mandates that activities should be carried out only in class II cabinets.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
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<td>L2: Yes</td>
<td>L2: L3</td>
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02/03/2022
The use of fluorescent tagged Coxsackievirus B3 (CVB3) virus (encoded in a plasmid) to assess cellular susceptibility to Coxsackievirus B infection

<table>
<thead>
<tr>
<th>Purposes of the contained use</th>
</tr>
</thead>
<tbody>
<tr>
<td>This eGFP tagged CVB3 virus (which is currently encoded in a plasmid) will be utilised for the following analyses:</td>
</tr>
<tr>
<td>1. We are transfecting human pancreatic cell lines with different isoforms of a receptor that mediates CVB-infection. The eGFP-tagged CVB3 virus will be used in these cells to assess the impact of the any alterations we make to the receptor on the infection efficiency. Infection efficiency will be assessed by either western blotting, RT-PCR, flow cytometry or immunofluorescence microscopy.</td>
</tr>
<tr>
<td>2. As part of our groups other projects (STATs; beta cell genes) we are looking at the impact that alterations of anti-viral pathways have on infection efficiencies with CVB virus.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Recipient or parental organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>The pCVB3-eGFP vector has been generated previously (Feuer JCV 2002; 76(9) p 4430-4440). The vector will be transfected into human pancreatic cell lines or HeLa/Hep2 to enable the production of infectious eGFP-tagged CVB3 virus. Virus isolated from these cells will be utilised for further experiments.</td>
</tr>
<tr>
<td>Virus strain: CVB3 H3</td>
</tr>
<tr>
<td>The virus has had an eGFP tag added to the 5’ end, just upstream of its 5’UTR region, this has been shown to reduce the infectivity of the virus compared to the parental CVB3 virus. This virus is still capable of mediating a productive infection (Feuer 2002 JCV).</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Host/vector system</th>
</tr>
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<tbody>
<tr>
<td>pCVB3-eGFP. The vector contained a Ampicillin resistance gene, T3 and T7 promoters. In addition to the vector backbone it has had the full length CVB3 polyprotein linked to eGFP. The polyprotein encodes the viral capsid proteins (VP1-4) and non-structural proteins required to mediate the productive infection of the cell.</td>
</tr>
<tr>
<td>Once this vector has been transfected into pancreatic derived cell lines or HeLa and Hep2 cell lines, the cells will produce and secrete eGFP-tagged CVB3 virus. The supernatant (containing the infectious virus) will be collected and utilised in further infection experiments. This virus will be utilised to monitor and track infection in pancreatic cell lines.</td>
</tr>
</tbody>
</table>
Origin & function

Evaluation of foreseeable effects

The vector itself is not harmful, however the CVB3 it produces is a level 2 pathogen. A specific risk assessment, COSHH form and biosafety manual have been produced for the handling of the virus and this has been approved by our Health and Safety committee.

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for all activities involving known or potentially infectious materials. ALL WORK SHOULD BE CARRIED OUT IN A VIRUS ONLY CLASS II BIOSAFETY CABINET WHICH WILL BE UV IRRADIATED AFTER EACH USAGE. ALL CELL CULTURES INFECTED WITH VIRUS WILL BE MAINTAINED IN A SPECIFIC VIRUS ONLY INCUBATOR. ALL EQUIPMENT UTILISED DURING THE CULTURE OF THESE CELLS WILL BE DECONTAMINATED AFTER EACH USAGE BY UV IRRADIATION OR DECONTAMINATION WITH VIRKON (BOTH OF WHICH EFFICIENTLY NEUTRALISE CVBs)

A DETAILED MANUAL HAS BEEN PRODUCED DESCRIBING THIS AND FULL TRAINING WILL BE GIVEN TO ALL LABORATORY PERSONAL WORKING WITH THE VIRUS.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon has been tested against high titre Poliovirus (a member of the same family of enteroviruses as CVB). Solutions (<5 days old) at a concentration of 1% (w/v) inactivate poliovirus within 5 minutes. All liquid waste will be treated for >15 minutes. Data from Virkon MSDS (attached) and associated references. All spills will be treated as below:

SOP
1. Wear gloves and lab coat when dealing with the spill, if not already wearing them.
2. Cover the spill, including any contaminated equipment, with cloth or paper towel to contain it.
3. Ensure others in the area now of the spill and to avoid the spill area.
4. Working from the outside margins of the spill area, towards the centre, apply sufficient 1% Virkon solution (less than 5 days old) on top of the paper towels to soak them.
5. Leave for 10 minutes to allow the Virkon to inactivate any virus present in the spill.
6. Clear away the materials into clear sealable plastic bags prior to disposal in the nearest yellow biohazardous waste bag, including gloves used for clean-up. If any glass or sharps are involved use a dustpan or piece of stiff cardboard to dispose of these materials into the appropriate containers.
7. Complete the spill record table.

In addition, UV light rapidly inactivates CVB. Ref https://www.ncbi.nlm.nih.gov/pmc/articles/PMC126408/#po=40.4762
The virus only biosafety cabinet is subjected to 20 minutes UV irradiation following all procedures. All pipettes are retained within the hood and are subjected to a virkon wipe down and UV irradiation after each use.

CVB virus are also highly susceptible to heat inactivation - 30 minutes at 60C
Stocks of the pCVB3-eGFP vector will be stored in a specified GM labelled freezer.

Control Measures for the CVB3-eGFP virus produced following transfection:
1. Viral stocks are collected from cultured cells within the Biosafety level 2 cabinet with all the normal control measures in place (detailed in Biosafety manual). For long term storage the virus is stored in liquid nitrogen or at -80C. Any vials containing virus will be carefully decontaminated prior to leaving the biosafety cabinet and will be transported in a second closed container that should not open/ break if dropped.
2. Any virus within infected cells in which RNA or protein is to be collected will be completely neutralised by the lysis buffer. All vials will be decontaminated prior to leaving the hood.
3. Any virus within infected cells in which we will subsequently use for immunofluorescence microscopy of for flow cytometry will have virus neutralised during the fixation step (4% paraformaldehyde).
4. All persistently-infected cell lines will be sub-cultured only within the Biosafety level 2 cabinet according to the CVB biosafety manual and maintained in the virus only incubator (RILD 4.20).

Other considerations:
Centrifugation of material containing virus: Any centrifugation steps will be completed using lided centrifuge containers that are loaded with the vials in the Biosafety level II cabinet and surface decontaminated (spray and wipe down with virkon) prior to removal from the hood. In the event of a breakage within the centrifugation container, it will be transferred to the BSC II prior to being opened for decontamination. Any spills and decontamination of equipment will be dealt with as detailed below and in the Spill Clean-up procedures in the CBV Biosafety Manual.

SOP for disposal of CBV contaminated waste (taken from CBV Biosafety manual)

1. Wear gloves and lab coat when dealing with the waste, if not already wearing them.
2. Active virus will only be worked with in the hood and all waste generated within the hood should be treated in the following manner:
   - Liquid containing virus will be removed using the vacuum pump system within the hood, to the sealed waste container (outside of the hood) that is part of the pump system. The waste container will already contain sufficiently strong Virkon solution to allow for the additional liquid that may be added during any procedure. After finishing procedures, as part of the clean-up, users should swirl the waste bottle to ensure that any splashes within the containers are washed with the Virkon solution. At least 10 mins must elapse since the last additional of waste to the container, prior to disposal of the waste down the sink. This has been shown to be sufficient to neutralise CVB viruses.
   - Solid waste or virus contaminated disposable items should be placed in the sealable bag, within the waste bin, inside of the hood. The bag should be sealed after procedures are completed but not removed from the hood until at least 15 mins later (while hood is left running). Prior to removal from the hood spray the outside of the bag with virkon and place the sealed bag gently into the yellow bag, within a cardboard container, for disposal. Following a clean down with Virkon the hood and pipettes will be UV irradiated for 20 minutes to ensure all virus is neutralised.
3. The yellow bin and cardboard container should be labelled with the user’s details when setting it up initially for virus related waste. It should be placed under the virus use only hood and should also be clearly labelled as being for virus related waste only. The bag should be closed when it is ¾ full, the cardboard container closed and the label completed. Always remember to set up a new yellow bin prior to closing the old one, dispose of the gloves used to close the old bag in the new bin. Gloves should not be used when closing the cardboard container. In accordance with standard instructions within the lab, the sealed container should then be placed by the cage in the level 3 freezer room for disposal through already established procedures.
The GM safety Committee have approved this risk assessment.

Please enter comments on the GM safety committee on the risk assessment

The GM safety Committee have approved this risk assessment.

**Project Containment**

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</table>

**Animal Units**

| L2 | L3 | L4 | L2 | L3 | L4 |

**Large Scale Activities**

| L2 | L3 | L4 | L2 | L3 | L4 |

**Human Clinical Applications**

| L2 | L3 | L4 |

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**Project Ref 382/18.2**

- **Date Ackn'd**: 17/05/2018
- **CU2 Project Title**: The use of lentivirus (encoded in a plasmid) to explore the morphological and molecular consequences of disrupting genes and altering DNA methylation at CpG sites associated with neuropsychiatric disorders
- **Class**: Class 2
- **CultureVol**: Class 2, ≤ 1 Litre
- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

---

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

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**Project Additional Information**

**Purposes of the contained use**

Autism spectrum disorder (ASD) is one of the the most common neurodevelopmental diseases, affecting social interaction, communication, interests and behaviour. We aim to further understanding of the molecules, cells and circuits that underlie ASD affecting behaviour abnormalities. We will use cell line models to examine the morphological and molecular consequences of genetic variants associated with ASD in humans. We will use a new tool that has revolutionised our ability to model genetic
mutations; the CRISPR-Cas9 gene editing system. This system involves stable transfection of plasmids which are designed to generate constructs for expression or knock down of specific genes in neuroblastoma cell lines and adult mouse neurons. Once stably transfected, the cell lines will be analysed for expression efficiency by Western blotting, RT-PCR and flow cytometry. They will also be used in assays to assess for morphological changes using immunofluorescence microscopy.

Along with the genetic component of psychiatric disorders there is also a known epigenetic component in ASD, Alzheimer's Disease, Bipolar Disorder, Depression, Schizophrenia and Suicide. One change, DNA methylation, has been strongly associated to be altered in patients with these disorders. DNA methylation at known CpG sites has been shown to upregulate or downregulate gene expression dependent on methylation status. We will use cell line models to examine morphological and molecular consequences of altering methylation status of CpGs associated with these neuropsychiatric disorders. We will use CRISPR-Cas9 which has been modified to include a deactivated Cas9 (dCas9) linked to DNMT3a enzyme for targeted methylation and TET1 enzyme for targeted demethylation. This system involves stable transfection of plasmids which are designed to generate constructs for targeted methylation and demethylation of specific CpG sites in cell lines. This plasmid system generates lentivirus containing the plasmids and targeting gRNA plasmid to infect cell of interest. Once cell lines have been transfected they will be analysed for expression efficiency using western blotting, RT-PCR and flow cytometry. They will also be used in assays to assess the methylation status and changes in gene expression following altered methylation.

Recipient or parental organism

List of recipient strain(s): HeLa (human), Neuro-2A (mouse), HEK293 and HEK293T (human), SH-SY5Y (human), HPCOA07 (human), CTXOE16 (human), COS-7 (monkey), 3T3 (mouse), PC12 (rat), Human neuroprogenitor (human), Mouse neuroprogenitor (mouse), Mouse primary culture (mouse)

Virus strain: Human immunodeficiency virus 1 (lentivirus)

Host/vector system

There are three vectors (transfer, envelope and packaging) which we will use together to form a lentiviral system. The presence of gRNA would also be required for CRISPR-Cas9 gene editing to take place. All of our contracts are obtained from commercial or academic sources. The plasmids described here have all been generated previously and used in previous research (where applicable, publications are listed). We are not intending to make any additional alterations to the plasmids, aside from the inserted genes (which are all listed below).

List of vector(s):
- pENTR 221 CYFIP1 (Open Biosystem)
- pCDH GFP Gateway (originally made by Eric Wexler) (pCDH vectors have EF-1alpha promoter and puromycin selectable marker)
- pCDH GFP for control (non toxic)
- pCDH GFP-CYFIP1
- pPS-T2A-RFP Gateway (originally made by Eric Wexler) (pPS vectors have hU6 promoter and ampicillin resistance)
- pPS-T2A-RFP-CYFIP1 (originally made by Asami Oguro-Ando)
- pCMVdeltaR8.74 packaging (contains GAG, POL, REV, TAT labeled as "psPAX2 for lent package vector")
- pMD2.G envelope (contains VSVg, labeled as "VSVG for lent package vector")
- pGIPZ GFP1 for siRNA control (Open Biosystem) (pGIPZ vectors have T7 promoter and puromycin resistance)
- pGIPZ non scramble control (Open Biosystem)
- pGIPZ si CYFIP1 pM3-1 (Open Biosystem)

Commercial plasmids:
- pL-CRISPR.EFS.GFP (Addgene plasmid 57818) (EFS promoter, ampicillin selectable marker)
- pL-CRISPR.EFS.RFP (Addgene plasmid 57819) (EFS promoter, ampicillin selectable marker)
- pLenti-C-mGFP (Origene PS100071) (CMV promoter, chloramphenicol selectable marker)
- pgRNA-modified (Addgene plasmid 84477) (U6 promoter, CMV promoter, puromycin selection marker, mCherry selection marker)
- Fuw-dCas9-Dnmt3a (Addgene plasmid 84476) (UbC promoter, dCas9, DNMT3a, ampicillin resistance, truncated 5' LTR driven by CMV promoter)
1. List of function of inserted gene(s):

Vectors containing sufficient cDNA sequence and promoters/enhancers to allow expression of full-length or mutated protein. Ampicillin, kanamycin, puromycin, neomycin cassettes used for resistance/selection.

Amyloid precursor protein (APP)
- Amyloid precursor protein (APP)
- Amyloid precursor protein (APP) domain

Axon guidance receptors and their ligands
- Neurophilin-1
- Neurophilin-2
- Plexins A family
- Class III semaphorins

Calcium-binding proteins
- Parvalbumin
- Neurocalcin-d
- VILIP-1
- Calbindin-1

Contactins
- Contactin1
- Contactin2
- Contactin3
- Contactin4
- Contactin5
- Contactin6

Cytoskeleton proteins
- Actin or actin-binding
  - Actin
  - Drebrin
  - Cofilin
  - Myosin IIB

Microtubule or MT-binding
- EB1
- EB3
Fluorescent proteins- for visualisation of tagged constructs in live and fixed cell preparations
- Green fluorescent protein (GFP)
- Yellow fluorescent protein (YFP)
- Red fluorescent protein (RFP)
- Cyan fluorescent protein (CFP)
- Cherry
- Dendra2
- Kalama
- Ametrine
- Tomato
- DsRed
- Brainbow
- GECO
- Ruby
- Neptune

 Trafficking proteins
- Caveolin
- Clathrin

 Cytoplasmic protein
- FMR1
- CYFIP1

 Transmembrane receptor
- AMPA receptor

 Cell adhesion protein
- CHL1
- NRXN1
- NRXN2
- NRXN3
- NLGN1
- NLGN2
The vectors which individually comprise the lentiviral system are all safe, and only when combined create a level 2 pathogen. A specific risk assessment and COSHH form have been produced for the handling of the virus and this has been approved by the University of Exeter Health and Safety committee.

Containment requirements: Biosafety level 2 practices, containment equipment and facilities for all activities involving known or potentially infectious materials. All work should be carried out in a virus only class II biosafety cabinet which will be UV irradiated after each usage. All cell cultures infected with virus will be maintained in a specific virus only incubator. All equipment utilised during the culture of these cells will be decontaminated after each usage by UV irradiation or decontamination with virkon (both of which efficiently neutralise lentivirus).

The total amount of viral material, even at high titre, will only be several hundred microliters, meaning the exposure to the host will be minimal.

Full training will be given to all laboratory personal working with the virus.

Evaluation of foreseeable effects

<table>
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<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Virkon is a widely used laboratory disinfectant which is known to efficiently neutralise cells. There is no indication that modification of cells, in the manner described in this risk assessment, will make cells more resistant to the cytotoxic effects of virkon. All liquid waste will be treated for >15 minutes. Data from Virkon MSDS (attached) and associated references. All spills will be treated as below:

**SOP**

1. Wear gloves and lab coat when dealing with the spill, if not already wearing them.
2. Cover the spill, including any contaminated equipment, with cloth or paper towel to contain it.
3. Ensure others in the area know of the spill and to avoid the spill area.
4. Working from the outside margins of the spill area, towards the centre, apply sufficient 1% Virkon solution (less than 5 days old) on top of the paper towels to soak them.
5. Leave for 10 minutes to allow the Virkon to inactivate any virus present in the spill.
6. Clear away the materials into clear sealable plastic bags prior to disposal in the nearest yellow biohazardous waste bag, including gloves used for clean-up. If any glass or sharps are involved use a dustpan or piece of stiff cardboard to dispose of these materials into the appropriate containers.
7. Complete the spill record table.

In addition, UV light rapidly inactivates lentivirus. Ref https://www.ncbi.nlm.nih.gov/pmc/articles/PMC126408/#fpo=40.4762
The virus only biosafety cabinet is subjected to 20 minutes UV irradiation following all procedures. All pipettes are retained within the hood and are subjected to a virkon wipe down and UV irradiation after each use.

Lentivirus are also highly susceptible to heat inactivation - 30 minutes at 60C

Control Measures for the lentivirus produced following transfection:
1. Viral stocks are collected from cultured cells within the Biosafety level 2 cabinet with all the normal control measures in place. For long term storage the virus is stored at -80C. Any vials containing virus will be carefully decontaminated prior to leaving the biosafety cabinet and will be transported in a second closed container that should not open/break if dropped.
2. Any virus within infected cells in which RNA or protein is to be collected will be completely neutralised by the lysis buffer. All vials will be decontaminated prior to leaving the hood.
3. Any virus within infected cells in which RNA or protein is to be collected will be completely neutralised by the lysis buffer. All vials will be decontaminated prior to leaving the hood.
4. All persistently-infected cell lines will be sub-cultured only within the Biosafety level 2 cabinet and maintained in the virus only incubator (RILD 4.20).

Other considerations:
Centrifugation of material containing virus: Any centrifugation steps will be completed using lided centrifuge containers that are loaded with the vials in the Biosafety level II cabinet and surface decontaminated (spray and wipe down with virkon) prior to removal from the hood. In the event of a breakage within the centrifugation container, it will be transferred to the BSC II prior to being opened for decontamination. Any spills and decontamination of equipment will be dealt with as detailed below.

SOP for disposal of lentivirus contaminated waste:
1. Wear gloves and lab coat when dealing with the waste, if not already wearing them.
2. Active virus will only be worked with in the hood and all waste generated within the hood should be treated in the following manner: Liquid containing virus will be removed using the vacuum pump system within the hood, to the sealed waste container (outside of the hood) that is part of the pump system. The waste container will already contain sufficiently strong Virkon solution to allow for the additional liquid that may be added during any procedure. After finishing procedures, as part of the clean-up, users should swirl the waste bottle to ensure that any splashes within the containers are washed with the Virkon solution. At least 10 mins must elapse since the last additional of waste to the container, prior to disposal of the waste down the sink. As an interim measure, or if the pump is not working, set up some concentrated virkon solution in a beaker and place this in the hood for liquid waste. If a beaker has to be used ensure that beaker itself is wiped down on removal from the hood, which should be done after the hood has run for the additional 15 mins after completion of work. Solid waste or virus contaminated disposable items should be placed in the sealable bag, within the waste bin, inside of the hood. The bag should be sealed after procedures are completed but not removed from the hook until at least 15 mins later (while hood is left running). Prior to removal from the hood spray the outside of the bag with virkon and place the sealed bag gently into the yellow bag, within a cardboard container, for disposal.
3. The yellow bin and cardboard container should be labelled with the user's details when setting it up initially for virus related waste. It should be placed under the virus use only hood and should also be clearly labelled as being for virus related waste only. The bag should be closed when it is ¾ full, the cardboard container closed and the label completed. Always remember to set up a new yellow bin prior to closing the old one, dispose of the gloves used to close the old bag in the new bin. Gloves should not be used when closing the cardboard container. In accordance with standing instructions within the lab, the sealed container should then be placed by the cage in the level 3 freezer room for disposal through already established procedures.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The GM safety Committee have approved this risk assessment

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

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Animal Units
| L2 | L3 | L4 | L2 |

Large Scale Activities
| L2 | L3 | L4 |

Human Clinical Applications
| L2 | L3 | L4 |

Project Ref 382/19.1

Date Ackn'd
30/09/2019

Date Project Ceased

CU2 Project Title
The Molecular and Cellular biology of Candida species

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn
N

Tick if notifying a connected programme of work
N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
This project covers a range of specific applications with the overall goal of understanding the regulation and mechanisms of yeast and hyphal growth, yeast-hyphae
morphogenesis, hyphal invasiveness, cell wall biosynthesis, antifungal drug sensitivity, mating and reproduction, micronutrient homeostasis, nutrient and stress responses and pathogenicity in Candida species. These species include Candida albicans, C. glabrata, C. dubliniensis, C. tropicalis, C. parapsilosis, C. orthopsilosis, C. metapsilosis, C. krusei, C. guillermondii, C. lusitaniae, C. haemulonii and C. auris. This project also covers experiments related to understanding immunity against C. albicans, particularly the role of mammalian pattern recognition receptors (PRRs) in these immune responses. The specific goals are to characterise:

1. Regulation and roles of chitin, glucan and mannoproteins and cell wall biosynthetic enzymes in cell wall synthesis, cell wall architecture, cell division and pathogenicity.
2. Regulation and roles of morphogenesis, polarized cell growth and tropic responses.
3. Regulation and roles of other virulence attributes such as secreted hydrolases and biofilm formation on medical devices.
4. Role of signalling molecules, transcription factors, and target genes in stress and nutrient adaptation, microbial assimilation and pathogenesis.
5. Regulation of antifungal drug tolerance and resistance, and the influence of antifungal drugs on virulence attributes and pathogenesis (antifungals include classical antifungal drugs, antimicrobial peptides and anti-Candida antibodies).
6. Interactions between Candida, the microbiota and the immune system during gastrointestinal colonisation.
7. Interaction of Candida cells with cells of the human immune system and mammalian cell lines.
8. Roles of PRRs and their downstream signalling pathways in anti-microbial immunity (primarily anti-fungal immunity, although protection against bacteria and viruses is also examined) and in maintaining homeostasis.
9. The influence of sexual and parasexual processes in generating genetic diversity observed between diverse clinical isolates.

During experiments small amounts of live Candida cells (<10^6 cells/sample) are examined by microscopy, FACS or Fluorescent Microscopy. Local safety procedures have been established for the decontamination and/or disposal of samples and equipment. Samples are transported for FACS analysis and for fluorescent microscopy under controlled conditions, secondary containment procedures. Samples for transport must be contained in sealed, leakproof containers as well as in a further outer sealed containers for secondary containment. Some experiments will involve GM Candida cells being cultured with mammalian culture cells in approximately 250ml volumes using locally established category 2 protocols.

**Recipient or parental organism**

Some recipient organisms include ACDP Hazard Group 2 organisms.

Recipient organisms include the following:

2. *Saccharomyces cerevisiae*: Generally we will use multiply disabled, non-pathogenic derivatives of S288C. Some experiments are performed on clinical isolates of S. cerevisiae (these isolates are less pathogenic than C. albicans. They are unable to establish infections in immunocompetent mice).
3. *Candida albicans*: Standard molecular strains carry disabling markers that are often complemented during transformation. They include CAI4 (ura3, ura3/ura3) and derivatives thereof, CAI8 (ura3, ade2, ura3/ura3, ade2/ade2), RM1000 (his1, ura3, ade2/ade2, his1/his1), BWP17 (his1, ura3, arg4), SN78 (ura3, his1, leu2, ura3/ura3, his1/his1, leu2/leu2), SN148 (ura3, arg4, his1, leu2, ura3/ura3, arg4/arg4, his1/his1, leu2/leu2). Clinical isolates of C. albicans are used in some experiments (SC5314).
4. *Candida glabrata*: Recipient strains carry disabling markers such as ade2, his3, ura3, his3, trp1 or lys2. These strains consist of clinical isolates of C. glabrata, that are to be used in some experiments (ATCC2001/CBS138), BG2 (clinical isolate wild type) and derivatives thereof (ura3 KO), 85/038 (clinical isolate wild type), 1184 (clinical isolate wild type), NCPF3605 (clinical isolate wild type), NCPF3309 (clinical isolate wild type).
5. *Candida dubliniensis*: Recipient strains carry disabling markers such as ura3, a non-revertible deletion. Clinical isolates of C. dubliniensis are used in some experiments (Wu284).
7. *Pichia pastoris*: This non-pathogenic yeast is used for deliberate expression of some specific C. albicans proteins. Recipient strains carry disabling auxotrophic markers (e.g. his4, aox1).

**Host/vector system**

Vectors include:

1. Non-mobilisable E. coli vectors (including pUC18/19, pBluescript, pDONR207, pGEM-T, lac-based expression plasmids such as pET vectors).
2. Lambda ZAPII for expression of cDNAs in E. coli.
3. Non-mobilisable S. cerevisiae vectors, often of the pRS series (including multicopy 2mm vectors; single copy YCp replicating vectors; single copy integrating YIp vectors; single copy GAL-based expression vectors).

4. Specific non-mobilisable C. albicans vectors (low copy replicating and integrating vectors [YPB1, Clp10, Clp20, Clp30]; low copy replicating and integrating vectors for ectopic expression [YPB-ADHpt, pACT1]; integrating expression vectors regulated by methionine [MET3 promoter], maltose [MAL2 promoter], glucose [PCK1 promoter], or doxycycline [tet ON promoter and tet OFF promoter]). Some of these vectors are being used in laboratories all over the world.


Specific non-mobilisable P. pastoris expression vectors with AOX1 promoter and terminator [e.g. pPIC9].

The majority of work proposed is for Candida genes that are annotated on the Candida Genome Database (www.candidagenome.org) and include:

- Chitin synthetic genes (e.g. CHS1, CHS2, CHS3, CHS4, CHS5, CHS6, CHS7, CHS8, GFA1, CDA2, BNI4, CHT1, CHT2, CHT3)
- Mannosylation genes (e.g. MNT1-5, MNN2,5,9,10,11, OCH1, HOC1, PMR1, VAN1, MNN1-family: MNN1, MNN 12, MNN 13, MNN 14, MNN 15, MNN 16; MNN2-family: MNN2, MNN21, MNN22, MNN23, MNN5, MNN51, PMR1, CW4H1, ROT2, MNS1, PFT1, PMT2, PMT6, ANP1)
- Hypha-specific genes (e.g. HYR1, HGC1, ALS8, HWP1, ECE1)
- Other cell wall protein genes (about 100 in total).
- Secreted hydrolases and other virulence-associated genes (e.g. SAP1-10, LIP1-10, CHT1-3)
- Transcription factor genes (e.g. TUP1, SSN6, NRG1, RF1G1, SKO1, GCN4, MNS2/4, CAP1, HST1F, HST2F, RPR1, EFG1, EFH1, CPH1, CPH2, CZF1, CRZ1, RLM1, HAC1, CAS5, BCR1, TEC1, RIM101, CSR1, ZCF gene family)
- Signalling genes (e.g. PIK1-2, C2ST20, HST7, CEK1, CPP1, RAS1, CYR1, TPK1, TPK2, CNB1, BMH1, MKC1, HOG1, CNA1, other annotated kinases and phosphatases)
- Calcium signalling genes (e.g. CCH1, MID1, MID2, FIG1, CNA1, CNB1, CRZ1, PMR1, FIG1, YVC1)
- ABC transporters (e.g. CDR1, BENR)
- Stress mitochondrial function and metabolism genes: (e.g. CTA1, TRX1, TRR1, TRX1, GLR1, ENA22, EBP5, PYK1, PCK1, ICL1, FAA21, POX4, POT11)
- Auxotrophic and dominant selectable markers (e.g. URA3, HIS1, ARG4, ADE2, LYS2, SAT1, NAT1)
- Regulated promoters (e.g. MET3, MAL1, MAL2, PCK1, tet), and “constitutive” promoters (e.g. ACT1, ADH1, TDHS3, TEF1)
- Sterol synthesis genes (e.g. ERG1, ERG11)
- Reporter genes (e.g. yEGFP, yEYFP, yEGFP, Renilla luciferase, Gaussia luciferase, SfLacZ, SaLexA, LIFEACT, IRFP)
- DNA replication and repair genes, as well as meiosis-specific factors which may be involved in the evolution of antifungal drug resistance (e.g. REV1, REV7, SPO11, HOP1, RAD51, DLH1)
- Candida genomic, transposon mutagenesis, over-expression and cDNA libraries.
- Orthologues of a number of the above genes from other Candida species such as C. auris, C. parapsilosis, C. guilliermondii, C. lusitaniae, C. krusei
- Cell polarity genes (e.g. CDC24, CDC42, RSR1, BEN1, BNI1, BNR1, AXL2, RAX2, PXL1, BUD5, BUD2)
- Genes encoding proteins involved in fungal cell division, such as septins (e.g. CDC3, CDC10, CDC11, CDC12, SEP7, SPR3, SPR28), proteases (e.g. KEX2), myosin (e.g. MYO1), cyclins (e.g. HGC1) and cyclin-dependent kinases and phosphatases (e.g. CDC28, CDC14)
- Genes encoding metal ion transporters or proteins involved in metal ion acquisition (e.g. ZRT1, ZRT2, ZRT3, ZRC1, PRA1, orf19.5428, orf19.52, orf19.3874, orf19.3769, orf19.3132)
- Genes involved in acylglycerol and sterol ester metabolism (e.g. orf19.6501, DPP1, DPP2, DPP3, orf19.6941, SMP2, orf19.5426, orf19.4699, orf19.4864, ARE2, orf19.2050, orf19.1887)
- Genes involved with polarised growth and hyphal steering (e.g. CDC42, CDC24, BEN1, PXL1, TPK2, CST20, CLA4, FGR29, SST2).

The work will be kept within the boundaries of Cat2.
Candida spp. are a human fungal pathogen in ACDP Hazard Group 2. Candida spp. are commensals of the natural flora of the human body and are microorganisms of very low pathogenic potential and very rarely affect healthy individuals however when an individual is immunocompromised, such as HIV patients or organ transplant recipients, infection can occur, therefore Candida spp. are classed as opportunistic pathogens.

The genetic modifications proposed in this work are not expected to increase the virulence of the genetically modified micro-organism beyond that which is already associated with the parental organism. Also it is not anticipated that this proposed work would have an effect on any other traits of the GMM such as increased drug resistance therefore the increased risk to human health potentially caused by the construction of these GMM's is essentially equivalent to the risk already proposed by the parental organisms, which is classed as ACDP Hazard Group 2.

The majority of work proposed here involves the alteration of already existing genes, such as gene deletions and promoter replacements, with genes being replaced with auxotrophic markers, such as leu2, ura3, his1, ade2, and arg4, or with positive selection markers that is derived from foreign DNA such as nourseothricin resistance. Other foreign DNA will also be incorporated into the genome such as S. typhinerium HisG, tetR-SchAP4AD which is a tetracycline transactivator, or reporter genes, such as b-galactosidase, luciferase, GUS, GFP and its variants. Therefore it is highly unlikely that a hazard will arise as a result of directly inserting these DNA into Candida spp. Ultimately the genes that are being targetted for this proposed work are believed to have important roles in Candida spp. viability and virulence and we do not anticipate that the introduction of auxotrophic markers, an anti-biotic resistance marker, or any other genetic modification, in the work described here, to enhance the ability of Candida spp. to cause disease above that which is already associated with the parental organism or that it may confer resistance to current clinical antifungal treatments. therefore the risk to human health is essentially equivalent to the parental organism.

The proposed GMMs to be constructed in this project are not expected to show an altered hazard to the environment compared to the wild type strain and as Candida is a commensal fungi of the human population, alteration of existing traits is expected to pose no hazard to the environment. Furthermore, the gene products of the plasmids are not toxic or oncogenic. Candida organisms may be able to persist in the environment if a breach in containment occurred, however the integrated genes into the genome will posses no selective advantage compared to the wild type and will not confer resistance to current anti-fungal treatments, thus gene transfer would not be a hazard. Also the vectors used are non-mobilisable or mobilisation defective, thus cannot be transferred to other microorganisms.

**Evaluation of foreseeable effects**

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**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

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**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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<td>Solid Waste: Solid waste, such as pipette tips, eppendorf tubes, agar plates etc, will be disposed of into a sealed autoclave bag prior to being sterilised via autoclaving at 134°C for 40 minutes. Autoclave function is assessed and recorded via chart recorder and the autoclave is calibrated and serviced quarterly to ensure continued effectiveness, as well as being tested via 12 point thermocouple testing performed annually. Once inactivated the waste will then be disposed of into yellow clinical waste bags and ultimately disposed of via the university waste management route.</td>
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<td>Glassware: Contaminated glassware is to be disinfected using a 5% ChemeGene solution before being sterilised in the autoclave at 121°C for 15 minutes.</td>
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<td>Sharps: Sharps, such as syringe needles, scalpal blades, microscope slides etc, are to be disposed of into designated sharps cinbins. Once full the cinbins are sealed and locked before being double bagged into clinical waste bags and incinerated.</td>
</tr>
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Project Containment

Laboratory Activities          Glass Houses          Growth Rooms
L2  Yes                  L2                  L2                  L3                  L3                  L2
L3                  L3                  L3                  L3                  L4                  L3
L4                  L4                  L4                  L4

Animal Units           Large Scale Activities    Human Clinical Applications
L2                  L2                  L2                  L3                  L3                  L3
L3                  L3                  L4                  L4                  L4
L4

Project Ref  382/19.2

Date Ackn'd          CU2 Project Title
27/11/2019          Interspecies interactions and mechanisms of killing of the fungus, Candida albicans, by the bacterium, Pseudomonas aeruginosa

Class          CultureVol          Class2          CultureVolume
Class 2          < 1 Litre

Non-GMM          Consent Granted

Withdrawn          Tick if notifying a connected programme of work
N          N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
**Purpose of the contained use**

Candida albicans and Pseudomonas aeruginosa are opportunistic pathogens that can be co-isolated from the cystic fibrosis lung. They have also been used as a model systems for the study of interspecies communication. Previous work by Dr. B has shown that killing of C. albicans hyphae, but not yeast, by the bacterium can occur through the activity of bacterial secreted factors but is more efficient in the presence of the bacterium (Brand et al, 2008). The goals of the project are to: determine the role of P. aeruginosa quorum-sensing (QS) signalling in the killing of C. albicans; elucidate the mode-of-action in the killing of C. albicans by P. aeruginosa; and characterise inter-species interactions in the context of mixed-species biofilms.

**Recipient or parental organism**

The recipient organism is Pseudomonas aeruginosa. All GM modifications in P. aeruginosa will be carried out by collaborators at research institutes/Universities that specialise in genetic modification of P aeruginosa. The strains used in Exeter will not contain vectors, and no vectors or plasmids will be used or held in Exeter.

**Host/vector system**

The P. aeruginosa GM strains used in these experiments are generated in the well-described wild-type backgrounds PAO1 (Stover et al. 2000) and PA14 (Rahme et al. 1995), two fully sequenced strains.

**Origin & function**


**Evaluation of foreseeable effects**

P. aeruginosa is an environmental bacterium that lives naturally in water, moist soil and swamp conditions. It can become an opportunistic human pathogen in patients with compromised epithelial and immune defences, such as sufferers of cystic fibrosis or those with wounds or burns. P. aeruginosa falls within the ACDP Hazard Group 2. The selectable markers confer resistance to the antibiotics gentamicin or tetracycline, which are not commonly used to treat infection by P. aeruginosa. Nevertheless, these antibiotics will not be used routinely in Exeter, since the insertions are stable in the genome.

The inserted genes (fluorescence markers such as GFP) are not associated with significant risk. The disruption of quorum sensing functions is likely to reduce toxin production and the virulence of P. aeruginosa (Williams and Camara, 2009; Rumbaugh et al. 1999; Rumbaugh et al. 2009). The most hazardous GMM is likely to be the wild type parental P. aeruginosa strains as the genetic modifications involve the inactivation of virulence-related functions such as quorum sensing. These mutations are likely to reduce virulence. A human infection by any of these mutants can be eradicated by antibiotic therapy.

The mutants used will have QS genes chromosomally disrupted, so there is little chance of the natural transfer of the mutated gene to other organisms.

Although we are handling a potential pathogen, the risks associated with this project are estimated to be low.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste: Liquid waste will be inactivated using the autoclave at 121°C for 15 minutes or alternatively will be treated with 5% Chemgene and allowed a contact time of at least 20mins. This is sufficient to achieve >99.99% killing (by plating). Treated liquid waste would then be disposed of down a designated laboratory sink.

Solid Waste: Solid waste, such as pipette tips, eppendorf tubes, agar plates etc, will be disposed of into a sealed autoclave bag prior to being sterilised via autoclaving at 134°C for 40 minutes. Autoclave function is assessed and recorded via chart recorder and the autoclave is calibrated and serviced quarterly to ensure continued effectiveness, as well as being tested via 12 point thermocouple testing performed annually. Once inactivated the waste will then be disposed of into yellow clinical waste bags and ultimately disposed of via the university waste management route.

Glassware: Contaminated glassware is to be disinfected using a 5% Chemgene before being sterilised in the autoclave at 121°C for 15 minutes.

Sharps: The use of sharps will be minimised. These will be disposed of in designated sharps cinbins. Once full the cinbins are sealed and locked before being double bagged into clinical waste bags and incinerated.

Spills: In the event of a spill the contaminated area is flooded with concentrated Chemgene (to 5%, final conc.), mopped up with paper towels, and re-swabbed with 5% Chemgene.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The enclosed GMM application has been accepted by our Exeter GMM Committee, with a view to inspecting the Class 2 Laboratory (Geoffrey Pope 301) once the refurbishment has been completed.

Project Containment

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Dermatophyte infections are the most common fungal infections, at any one given moment affecting up to 20% of the population worldwide. These are superficial infections, some of which can be recurrent infections. Little is known about the underlying pathogenicity mechanisms. Therefore, the overall goal of this study is to investigate the pathogenesis of the dermatophytes *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis* and *Epidermophyton floccosum*. Our specific goals are to examine the regulation and mechanisms of cell wall biosynthesis, host immune responses, and responses to nutrients and stresses.

**Recipient or parental organism**

We will examine dermatophytes isolated from human clinical infections. These include clinical isolates of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis* and *Epidermophyton floccosum*.

**Host/vector system**

*Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis* and *Epidermophyton floccosum*. The *Trichophyton mentagrophytes Ku80 null mutant strain* (TmKu80M9) (Yamada et al., 2009 FEMS Micro Letts) may also be utilised as this strain undergoes increased homologous recombination, increasing the likelihood of obtaining the null mutant of interest. We will use targeted gene disruption to create null mutants, or will overexpress specific genes using PCR generated
disruption cassettes or disruption cassettes cloned in E. coli K12. Transformation vectors will either carry the
Hygromycin B phosphotransferase (hbp) gene, the neomycin phosphotransferase (nptII) gene (Yamada et al., 2008 J.
Dermatol Sci), or the nourseothricin acetyltransferase (NAT) dominant selectable marker (Alshahani et al. 201 O Med
Mycol).

Origin & function

Genes to be investigated initially include cell wall synthesis (chitin, melanin and glucan) genes and genes involved in
stress pathways (Hog1 pathway, etc.). Mutants expressing an iRFP (infra-red fluorescent protein) reporter will also be
created to allow in vivo imaging in experimental models.

Evaluation of foreseeable effects

Dermatophyte infections are extremely common, affecting up to 20% of the population at any one given moment, and
can infect anyone, including healthy individuals. There is evidence that treatment with immunosuppressive drugs may
increase the chances of developing a dermatophyte infection. Lab workers are made aware of this risk factor and are
asked specifically in the Local Regulations to make their supervisors (or Occupational Health) aware if they are
exposed to this risk factor.

Dermatophyte fungal infections can affect the skin on almost any area of the body, such as the scalp, legs, arms, feet
groin, and nails. Symptoms of infection include itchiness, redness, scaling, cracking of the skin, or a ring-shaped rash
may occur. Standard antifungal treatments include oral and/or topical antifungal therapy with azoles or allylamines,
particularly itraconazole and terbinafine.

To date, any stable gene disruptions have either led to reduced growth rates or have had no effect on growth.

Similarly, virulence of the strains has either been reduced or unaffected. Transformation methods (biolistic
transformation, electroporation, Agrobacterium-mediated transformation) have not been demonstrated to alter
virulence in other systems.

All strains that are genetically modified will be assessed for altered susceptibility to antifungal agents (azoles) which
could be used to treat infection.

Horizontal gene transfer is extremely rare in fungi. The mutations will be to dermatophyte chromosomal genes, not
placed on mobile genetic elements.

The dermatophyte species in this study are anthropophilic in nature, living exclusively on humans. Other species are
zoophilic, living on animals, or geophilic, living in soil. The majority of human infections occur through contact with an
infected person or due to exposure to contaminated clothing, towels or bedding. However, humans can also become
infected with zoophilic species through contact with infected animals. The risk of infecting wild animals with
anthropophilic species is extremely low; in fact, experimental models of anthropophilic species infection have proved
virtually impossible to establish due to the low infectivity of human-associated species in rodents and other animals.
Therefore, none of the strains generated in this project are likely to present a hazard to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Glassware: Contaminated glassware is to be disinfected using a 5% Chemgene before being sterilised in the autoclave at 121°C for 15 minutes.

Sharps: The use of sharps will be minimised. These will be disposed of in designated sharps cinbins. Once full the cinbins are sealed and locked before being double bagged into clinical waste bags and incinerated.

Animals and Tissues: All carcases will be autoclaved within the BSU, and all tissues are autoclaved prior to disposal. Our standard procedures for use of Chemgene have been validated to kill 99.99% of dermatophyte species (http://www.starlab.de/download/STARLAB-HLD4L-MicrobiologyTestSummary-v3.pdf)

Autoclaves are validated at least annually by means of biological indicators. In daily use, monitoring is by thermocouple temperature recordings and autoclave stripes included in each load.

On a routine basis, samples from autoclaved waste or Chemgene-treated waste will be plated and assessed for viability.

Once GMMs have been inactivated, normal disposal routes will be followed for liquid and solid waste. Killed waste will be disposed of down the sink drain and inactivated solid waste will be discarded by CFA processing.

Spills: The contaminated area will be flooded with concentrated Chemgene (to a final 5% concentration) and mopped with paper towels, then washed with 5% Chemgene. The contaminated materials will be autoclaved to inactivate any organisms.

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Please enter comments on the GM safety committee on the risk assessment

The enclosed GMM application has been accepted by our Exeter GMM Committee, with a view to inspecting the Class 2 Laboratory (Geoffrey Pope 301) once the refurbishment has been completed.

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Project Containment

02/03/2022
**Project Ref** 382/19.4

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<tr>
<td>27/11/2019</td>
<td>Studies of growth, cell wall synthesis and tropic responses of the fungus, Cryptococcus neoformans</td>
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**Class** | **Culture Vol Class** | **Culture Volume Class** |
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<tr>
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**Non-GMM Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

We are dissecting the molecular mechanisms that underlie specific pathogenesis related phenotypes in the fungus, *Cryptococcus neoformans*: the re-orientation of tip-growing cells in response to external stimuli; changes in cell morphology and capsule formation that occur during infection; and genes that promote resistance to host immune attack.

**Recipient or parental organism**

*Cryptococcus neoformans* (Serotype A) is a human pathogen in ACDP Hazard Group 2. This fungus is ubiquitous in the environment and can commonly be isolated from decaying wood and soil that is contaminated with bird-droppings. The infectious particles are spores and dessicated yeast cells that become airborne when decaying material is disturbed. Particles enter the respiratory tract through inhalation and are carried and cleared asymptomatically by immunocompetent hosts. *C. neoformans* is an opportunistic pathogen and colonisation can lead to the development of Cryptococcal meningitis but ONLY if the host immune system is severely impaired. For example, presentation with this infection is considered a defining condition in the diagnosis of AIDS. No transmission of Cryptococcosis among animals or humans has been recorded (Faggi et al, 1993, Mycoses) and infection of an individual through microbial manipulation of the organism or spores has never been reported, in more than 40 years of research. *C. neoformans* is
a micro-organism of low pathogenic potential in healthy individuals.

**Host/vector system**

Wild type Cryptococcus neoformans strains (H99, KN99 and clinical isolates). Genes are deleted in wild type clinical isolates of C. neoformans (e.g. H99 and KN99 - opposite mating types). H99 is a clinical isolate that has become the reference strain for this Serotype (Toffaletti et al, 1993, J. Bacteriol.). KN99 is a strain that was created through a series of backcrosses between a clinical isolate a strain, 125.91, and H99 to obtain an congenic a strain that is calculated to be 96.9 % similar to H99 (Neilsen et al, 2003, IAI). In murine and rabbit models of Cryptococcal meningitis, KN99 was no more virulent than the clinical isolate, H99. H99 and KN99 will be used as control strains in this study.

Mutants are generated by other laboratories, e.g. Elizabeth Ballou, Carolina Coelho, Joe Heitman and Andrew Alspaugh (Duke University, USA) and Hiten Madhani (UCSF, USA). The genes are disrupted using a PCR based strategy using primers to amplify a 5’ and a 3’ region of homology (Gene-X-Right and Gene-X-left) to the targeted gene from genomic DNA and the selectable marker (NEO, HYG or NAT).

**Origin & function**

Targetted genes are involved in: calcium signalling (e.g. CCH1, MID1, PMR1/ECA1, calcineurin, CNA1, CNB1, CBP1); the biosynthesis of chitin and chitosan (e.g. CHS1-8, CSR1-3); RhoGTPase cell polarity pathways; metabolism (e.g. ICL1, MLC1); and capsule formation, morphogenesis, stress pathways and virulence factors.

**Evaluation of foreseeable effects**

As stated above, Cryptococcus neoformans is an opportunistic pathogen that can cause Cryptococcal meningitis but ONLY if the host immune system is severely impaired (e.g. in AIDS patients). No transmission of Cryptococcosis among animals or humans has been recorded (Faggi et al, 1993, Mycoses). The risk of contracting Cryptococcal meningitis in healthy individuals is negligible. Lab workers are unlikely to be exposed to the following risk factors:

a) use of cytotoxic or strongly immunosuppressive drugs (e.g. systemic corticosteroids),

b) general immunosuppression (e.g. organ transplant recipient, neoplastic disease)

c) AIDS patients

Lab workers are made aware of these risk factors during their initial induction into the lab and are asked specifically in the Local Regulations to make their supervisors (or Occupational Health) aware if they are exposed to any such risk factors.

Symptoms of Cryptococcal infection include headache, nausea, stiff neck and seizures (common in other forms of brain infection). Standard antifungal treatments include Amphotericin B, azoles and echinochandins.

To minimise the likelihood of infection, C. neoformans strains will be maintained as yeast cells on nutrient agar plates. C. neoformans spores are not aerial, but are embedded within a mating interface matrix. There is little chance of their becoming airborne from a mating plate. Additionally, spore production is relatively inefficient within the time periods necessary for spore collection (3-10 days). The chance of spore inhalation or spore dissemination from a mating plate is very low, and the chances of infection in a healthy human are nearly zero. However, to minimize risk of exposure, Any procedure that may result in aerosols will be carried out in a designated Class II safety cabinet.

The perturbation of target genes is most likely to attenuate the inability of the micro-organism to colonise the host. (Fromtling et al. 1982; Chang & Kwon-Chung 1994; Alspaugh et al., 2000; Ballou et al, 2010; Ballou et al., 2013). Also, the use of hygromycin, neomycin and nourseothricin resistance markers does not compromise treatment of infection with the preferred antifungal agents, Amphotericin B and azoles. The likelihood of natural gene transfer to other species is essentially zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
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<tr>
<td>Animals and Tissues: All carcases will be autoclaved, and all tissues are autoclaved prior to disposal. Our standard procedures for the use of Chemgene have been validated experimentally to kill &gt;99.99 % of C. neoformans vegetative cells. Viability tests have been performed on Chemgene -exposed cultures. Autoclaves are validated at least annually by means of biological indicators. In addition, we have validated killing at &gt;99.99 % by plating samples harvested from the centre of autoclaved samples. In daily use, monitoring is by thermocouple temperature recordings and autoclave-indicator tape included in the loads. Bench-top autoclaves in our laboratory are also monitored on a daily basis using autoclave-indicator tape and by checking the external temperature and pressure gauges. Our Laboratory Manager tests these autoclaves on a monthly basis using Browne’s tubes, which are included in the load and change colour on exposure to correct autoclave conditions. Killing by these autoclaves has been validated at &gt;99.99 % by plating from solid and liquid samples taken from the centre of the autoclave. In the event of a spill, the contaminated area is flooded with Chemgene (to 5 % final conc), mopped up with paper towels and reswabbed with 5 % Chemgene. Killing at &gt;99.99 % has been validated experimentally. Contaminated material is autoclaved.</td>
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| Is an emergency plan required according to regulation 20? | N |
|---|
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |
The enclosed GMM application has been accepted by our Exeter GMM Committee, with a view to inspecting the Class 2 Laboratory (Geoffrey Pope 301) once the refurbishment has been completed.

**Project Containment**

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**Project Ref** 382/19.5

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<td>Investigating the role of the Aspergillus cell wall and metabolism in pathogenesis, immune recognition, homeostasis and antifungal drug resistance</td>
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**Date Project Ceased**

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**Project Additional Information**

**Purposes of the contained use**

This application covers a range of specific projects with the overall goal of obtaining an improved understanding of the contributions of specific Aspergillus cell wall components, regulators and metabolic enzymes to the virulence and antifungal drug resistance of this fungus.

The specific goals are to characterise:
1) Molecular host-fungus interactions by analysing PAMPs (pathogen associated molecular patterns) on the Aspergillus cell wall being recognized by PRRs (pathogen recognition receptors) on immune cells (human cells, experimental murine models and alternative experimental infection models as Galleria mellonella wax moth larvae model, and the zebrafish model).

2) PAMPs and PRRs that modulate immune responses.

3) Intracellular signal transduction pathways used by PRRs.

4) Cell wall components of Aspergillus that contribute to its virulence, pathogenesis, drug targets and drug resistance mechanisms.

5) Remodelling of the cell wall structure in response to environmental changes.

6) Regulation of fungal biofilm formation and development.

7) Resistance to, and interaction with, antifungal agents and novel compounds including antimicrobial peptides and monoclonal antibodies.

8) Regulation of cell wall synthesis and architecture.

9) Role of cell wall proteins in cell wall structure and pathogenicity.

10) Function of chitin biosynthetic enzymes in cell wall structure and division.

11) Isolation of fungal cell walls for carbohydrate and proteomics analysis

12) Role of specific O- and N-glycosylating enzymes in cell wall structure and adhesion.

13) Role of specific signalling molecules, transcription factors, and target genes in stress responses, starvation responses, and pathogenicity.

14) Regulation of secreted hydrolytic enzymes and multi-drug transporters.

15) Effects of gene mutation and antifungal compounds on hyphal growth behaviour.

16) Sugar nucleotide biosynthetic pathways contributing to cell wall formation as potential antifungal drug targets.

17) Pathways involved in the generation and detoxification of reactive oxygen and nitrogen species.

Recipient or parental organism

Aspergillus is a human pathogen in ACDP Hazard Group 2. Aspergillus causes three principal diseases following inhalation of the spores: allergic bronchopulmonary aspergillosis (ABPA), pulmonary aspergilloma and invasive aspergillosis. However the clinical manifestations and severity of the disease depends upon the immunologic status of the patient. ABPA is a condition where the fungus causes allergic respiratory symptoms, such as wheezing and coughing, but does not actually invade and destroy tissue. APBA is rarely found in healthy people, and is most common in people with asthma or cystic fibrosis. Pulmonary aspergilloma (or “fungal ball”) usually occurs in people with other forms of lung disease, such as emphysema or tuberculosis. Invasive aspergillosis generally affects people who have severely compromised immune systems, such as people who have had a bone marrow transplant or solid organ transplant, people who are taking high doses of corticosteroids, and people undergoing chemotherapy for cancers, such as leukaemia. Rarely, persons with advanced HIV infection can also acquire the infection. The majority of infections are caused by A. fumigatus, the non-fumigatus Aspergillus species are less frequently encountered as causative pathogens and display a decreased virulence.

To keep things in perspective, it is important to realise that A. fumigatus and the other Aspergillus species are ubiquitous in the environment and are commonly found in soil, on plants, in decaying plant matter, household dust, building materials, and in some foods. Most people breathe in spores of Aspergillus every day and this does not cause harm, as people with healthy immune systems are able to clear this organism without any adverse effects. Thus, Aspergillus species are micro-organisms of low pathogenic potential not affecting healthy individuals and only become a potential medical problem when an individual’s immune responses are impaired. Furthermore, as with most pathogenic micro-organisms, the virulence of Aspergillus strains become attenuated, rather than enhanced, after prolonged laboratory culture.
Therefore, the risk of life-threatening fungal infections with the species described here is negligible in healthy individuals. Lab workers are unlikely to be exposed to the following factors, which increase the susceptibility to infections with Aspergillus:

1. Use of cytotoxic or strongly immunosuppressive drugs (e.g. systemic corticosteroids, chemotherapy for cancer)
2. Patients generally immunosuppressed (e.g. organ transplant recipient, neoplastic disease, severe burns)
3. Pre-existing illnesses that can predispose to infection e.g. Chronic Granulomatous Disorder, Cystic Fibrosis, Asthma.

Lab workers are made aware of these risk factors and are asked specifically in the Local Regulations to make their supervisors (or Occupational Health) aware if they are exposed to any such risk factors. In the event of an infection, standard antifungal treatment includes voriconazole, itraconazole, lipid amphotericin formulations, caspofungin, micafungin, and/or posaconazole.

Host/vector system

We plan to use strains of genetically modified Aspergillus species and clinical isolates which were generated in other Universities. We also plan to disrupt genes in A. fumigatus strain CEA17 (ku80Δ genetic background). This strain is used worldwide for the generation of A. fumigatus mutants as the strain has higher homologous recombination efficiency than wild type strains. Genes are deleted using a positive selectable marker, e.g. hygromycin or pyrithiamine resistance or the pyrG blaster. Alternatively genes may be placed under the regulation of an inducible promoter e.g. A. nidulans alcohol dehydrogenase gene (alcA promoter).

We will not use vectors but will integrate PCR-generated linear DNA cassettes or exploit CRISPR-Cas9 to modify the Aspergillus genome. For most experiments we will be using fungi with mutations which target, either directly or indirectly, the fungal cell wall biosynthetic machinery, signal transduction pathways, metabolism and/or virulence factors. This would include fungi with targeted mutations in genes involved in, for example, metabolism and transcription. Fungi overexpressing fungal or foreign genes, such as GFP, may also be used. Small culture volumes (less than 100ml, in most cases 10 ml) will be used. For in vivo experiments normally up to $10^8$ fungal conidia are administered per animal. Wild type immunologically competent animals are normally resistant to these, and even higher, inocula.

Some of the Aspergillus strains that may be used will be auxotrophic and therefore largely avirulent, but, by necessity (in virulence studies for example) other strains will not be genetically disabled. This includes null mutations in clinical (wild) isolates. It is important to note that a large number of A. fumigatus and non-fumigatus Aspergillus mutants have already been analysed in various laboratories world-wide, but there are NO reports of a mutation that increases virulence in immunocompetent animals. However, a limited number of mutations have been shown to have slight enhancing effects on virulence in studies using severely immunocompromised mice.

In some experiments, we will also use Aspergillus strains ectopically expressing specific genes, such as GFP or luciferase for example. The expression of these genes will have no effect on virulence.

The use of genetically modified strains of Aspergillus species under laboratory conditions represents minimal risk to human health. To date, there have been no reported cases of laboratory-acquired infections using this organism (http://www.phac-aspc.gc.ca/msds-ftss/msds11e-eng.php).

Origin & function

Genes to be modified in Aspergillus may include:

1. Chitin synthetic genes
2. Mannosylation genes
3. Beta-glucan synthetic genes
4. Hyphal-specific genes
1. Cell wall protein genes
2. Cell wall polysaccharide genes
3. Secreted hydrolases and other virulence-associated genes
4. Transcription factors
5. Signalling genes
6. pH-responsiveness genes
7. Calcium signalling genes
8. ABC transporters and efflux pumps
9. Stress and metabolism genes
10. Genes involved in programmed cell death
11. Genes that affect drug resistance
12. Auxotrophic and dominant selectable markers
13. Sterol synthesis and lipid biosynthesis genes
14. Expression of reporter genes (e.g. yEGFP, yEYFP, yEGFP, Renilla luciferase, Gaussia luciferase)
15. Mutant libraries necessary for screening for desired phenotypes.

Few, if any, of the mutations are likely to increase virulence.

Evaluation of foreseeable effects

There is no possibility of genetic exchange between the modified Aspergillus species and human cells.
As described above, there is a possibility of increased virulence for a few of the strains in severely immunocompromised individuals. However, the risk of infection is negligible in immunologically competent laboratory staff.
The likelihood of natural gene transfer to other organisms is essentially zero.
Aspergillus species are saprophytic organisms ubiquitously found in the environment. The risk of infection of wild mammals (such as rodents) through accidental release of laboratory strains of Aspergillus is negligible because to succumb to infection with laboratory strains, the animals need to be immunocompromised and subjected to extremely high infective doses (>10^6 CFU). Therefore, none of the strains used in this project are likely to present a hazard to the environment. Nevertheless, the transport of all organisms between laboratories and facilities as indicated above will be performed in sealed containers.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste: Liquid waste will be inactivated using the autoclave at 121°C for 15 minutes or alternatively will be treated with 5% Chemgene and allowed to have a contact time of at least 20mins. According to the manufacturer's instructions. Treated liquid waste would then be disposed of down a designated laboratory sink.
Solid Waste: Solid waste, such as pipette tips, eppendorf tubes, agar plates etc, will be disposed of into a sealed autoclave bag prior to being sterilised via autoclaving at 134°C for 40 minutes. Autoclave function is assessed and recorded via chart recorder and the autoclave is calibrated and serviced quarterly to ensure continued effectiveness, as well as being tested via 12 point thermocouple testing performed annually. Once inactivated the waste will then be
disposed of into yellow clinical waste bags and ultimately disposed of via the university waste management route.

Glassware: Contaminated glassware is to be disinfected using a 5% Chemgene before being sterilised in the autoclave at 121°C for 15 minutes.

Sharps: Sharps, such as syringe needles, scalpel blades, microscope slides etc, are to be disposed of into designated sharps cinbins. Once full the cinbins are sealed and locked before being double bagged into clinical waste bags and incinerated.

Animals and Tissues: All carcasses will be autoclaved within the BSU, and all tissues are autoclaved prior to disposal. Validation will involve regular testing of the autoclaves by our laboratory using Browne tubes and biannually by independent external assessors to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time.

In the event of a spill, the contaminated area is flooded with Chemgene 5%, mopped up with paper towels, and reswabbed with Chemgene 5% and a final clean with 70% ethanol.

The enclosed GMM application has been accepted by our Exeter GMM Committee, with a view to inspecting the Class 2 Laboratory (Geoffrey Pope 301) once the refurbishment has been completed.

Please enter comments on the GM safety committee on the risk assessment

The enclosed GMM application has been accepted by our Exeter GMM Committee, with a view to inspecting the Class 2 Laboratory (Geoffrey Pope 301) once the refurbishment has been completed.

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Project Ref 382/19.6

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<td>27/11/2019</td>
<td>Characterisation of the C-type lectin receptor, CLECSF8, and other C-type lectins</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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This application covers a range of specific projects with the overall aim to define the ligand and range of mycobacterial species that are recognised by a C-type lectin receptor, and to determine the physiological role of this receptor in vitro and in vivo. In particular, this project will utilise a range of non-pathogenic mycobacterial species including both wild-type and GM Mycobacterium smegmatis and M. bovis BCG.

Recipient organisms include M. bovis BCG strains Tokyo and Pasteur and M. smegmatis mc2155. All mycobacterial strains used in this project are either listed as hazard group 2 (M. bovis BCG) or are not listed (M. smegmatis). M. smegmatis is a non-pathogenic soil organism, but due to its taxonomic grouping it is being treated as a group 2 organism. M. bovis BCG is routinely used as a vaccine against M. tuberculosis in humans.

In some of the experiments, mycobacterial strains will be tested using standard rodent models of infection in wild-type and receptor knockout mice. Small amounts of these strains will be transported to the BSU in sealed containers and administered into animals by intratracheal inoculation in a containment level 2 facility. The animals receiving these organisms will be housed in individually ventilated cages (IVCs). This will be carried out by trained and licensed personnel. BSU staff will not be directly involved in these experiments but are aware of the associated minor risks and local rules will be posted.

In some of the experiments, tissues which may contain small amounts of live GM bacteria are removed from infected animals and transported back to the lab in sealed containers for various experiments including the determination of bacterial burden and histology. Frozen, unfixed sections may be analysed by fluorescence microscopy. These sections would contain very small amounts of bacteria therefore present minimal risk. Local GM regulations will be posted in all locations.

In other experiments small amounts of fixed GM mycobacterial cells will be analysed by flow cytometry (<106 cells per sample). Local GM safety procedures have been established for the decontamination of samples and equipment. Fixed samples are expelled directly into 5% Tri-gene. Local GM regulations will be posted in all locations.
A microbiological safety cabinet will be used when working with M. bovis BCG or whenever aerosols are expected such as when resuspending cells after centrifugation.

M. smegmatis can survive in the environment but the GMMs will be compromised by their genetic modification. Similarly M. bovis BCG will also be compromised and survival is likely to be very short-lived. The severity of contamination of the environment by any of these mycobacterial strains is therefore likely to be low.

The inserted/modified DNA will not encode known harmful gene products. Thus it is unlikely that the GMMs will have any harmful effects on the environment.

Transport of all organisms between laboratories will be performed in sealed containers.

Origin & function

No new GMOs will be generated in Exeter. The nature of the mycobacterial ligand is currently unknown but possible candidates include genes encoding pathways responsible for the production of cell wall components such as lipids, PIM1,2, PIM6, MAME, LAM, man-LAM, TDM, which could be involved in host cell recognition. More commonly, we will be utilising mycobacterial strains expressing fluorescent markers, such as GFP or commonly used markers for studying immunological processes, such as ovalbumin.

Evaluation of foreseeable effects

All mycobacterial strains used in this project are listed as hazard group 2 (M. bovis BCG and M. smegmatis). M. smegmatis is a non-pathogenic soil organism, but due to its taxonomic grouping it is being treated as a group 2 organism. M. bovis BCG is routinely used as a vaccine against M. tuberculosis in humans. No GM organisms will be generated in Exeter.

The risk of life-threatening infections with M. smegmatis and M. bovis BCG is negligible in healthy individuals. However, M. smegmatis has been reported to cause localized skin and soft tissue infections following traumatic injury or cardiac surgery. Immunocompromised and elderly individuals are more susceptible to M. bovis BCG, which can cause pulmonary infections.

In some of the experiments, mycobacterial strains will be tested using standard rodent models of infection in wild-type and receptor knockout mice. Small amounts of these strains will be transported to the BSU in sealed containers and administered into animals by intratracheal inoculation in a containment level 2 facility. The animals receiving these organisms will be housed in individually ventilated cages (IVCs). This will be carried out by trained and licensed personnel. BSU staff will not be directly involved in these experiments but are aware of the associated minor risks and local rules will be posted.

In some of the experiments, tissues which may contain small amounts of live GM bacteria are removed from infected animals and transported back to the lab in sealed containers for various experiments including the determination of bacterial burden and histology. Frozen, unfixed sections may be analysed by fluorescence microscopy. These sections would contain very small amounts of bacteria therefore present minimal risk. Local GM regulations will be posted in all locations.

In other experiments small amounts of fixed GM mycobacterial cells will be analysed by flow cytometry (<10^6 cells per sample). Local GM safety procedures have been established for the decontamination of samples and equipment. Fixed samples are expelled directly into 5% Tri-gene. Local GM regulations will be posted in all locations.

A microbiological safety cabinet will be used when working with M. bovis BCG or whenever aerosols are expected such as when resuspending cells after centrifugation.

M. smegmatis can survive in the environment but the GMMs will be compromised by their genetic modification. Similarly M. bovis BCG will also be compromised and survival is likely to be very short-lived. The severity of contamination of the environment by any of these mycobacterial strains is therefore likely to be low.

The inserted/modified DNA will not encode known harmful gene products. Thus it is unlikely that the GMMs will have
any harmful effects on the environment. Transport of all organisms between laboratories will be performed in sealed containers. It is extremely unlikely that gene deletion and complementation with heterologous genes will alter the virulence of the GMM compared to that of the parent organism. In fact, deletion of cell wall components is most likely to reduce virulence, as has been demonstrated in the literature (Mahon RN et al, 2012, Smith I, 2003). The likelihood of the cloned DNA being transferred by accident to other organisms via natural gene transfer is very low. The vectors used in mycobacteria are self-replicating plasmids that do not contain identifiable mobilisation functions or they are modified bacteriophage. The latter are derived from a temperature sensitive conditionally replicating lytic phage TM4. There could be transfer therefore of the TM4 derivatives to other mycobacteria but this would lead to lysis of the recipient (30°C) or recombination (37°C) and allele exchange which would compromise the recipient. Both scenarios are likely to be rare.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid Waste: Liquid waste will be inactivated using the autoclave at 121°C for 15 minutes or alternatively will be treated with 5% Chemgene and allowed to have a contact time of at least 20mins. According to the manufacturer's instructions. Treated liquid waste would then be disposed of down a designated laboratory sink.

Solid Waste: Solid waste, such as pipette tips, eppendorf tubes, agar plates etc, will be disposed of into a sealed autoclave bag prior to being sterilised via autoclaving at 134°C for 40 minutes. Autoclave function is assessed and recorded via chart recorder and the autoclave is calibrated and serviced quarterely to ensure continued effectiveness, as well as being tested via 12 point thermocouple testing performed annually. Once inactivated the waste will then be disposed of into yellow clinical waste bags and ultimately disposed of via the university waste management route.

Glassware: Contaminated glassware is to be disinfected using a 5% Chemgene before being sterilised in the autoclave at 121°C for 15 minutes.

Sharps: The use of sharps will be minimised. These will be disposed of into designated sharps cinbins. Once full the cinbins are sealed and locked before being double bagged into clinical waste bags and incinerated.

Slides from microscope facilities: Slides are transported to/from microscope rooms in plastic petri dishes with their tops sealed with tape or parafilm, and then disposed of as per Sharps.

Animals and Tissues: All carcasses are autoclaved within the BSU, and remaining tissues are autoclaved prior to disposal.

Cytometry facility: Fixed samples are expelled directly into 5% Chemgene and equipment will be decontaminated with 5% Chemgene.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The enclosed GMM application has been accepted by our Exeter GMM Committee, with a view to inspecting the Class 2 Laboratory (Geoffrey Pope 301) once the refurbishment has been completed.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
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</tbody>
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Liquid Handling

Animal Units

Large Scale Activities

Human Clinical Applications

**Project Ref** 382/19.7

- **Pattern recognition receptors in immunity and homeostasis: transduction of cell lines and primary murine cells using lentiviruses**

- **Date Ackn’d** 27/11/2019

- **Date Project Ceased**

- **Class** Class 2

- **Consent Granted** Non-GMM

- **Non-GMM Consent Granted**

- **Project notified under transitional arrangements** N

**Historical Significant Changes**

- **Historical Date of Additional Info**

- **Significant Change ID**

- **Date of Significant Change**

**Project Additional Information**

- **Purposes of the contained use**
  
  Our primary goal is to define the roles of C-type lectin-like receptors, Natural Killer lectin-like receptors, Toll-like
receptors and other pattern recognition receptors (PRRs) in immunity. We target PRRs to modulate immune
responses, determine the intracellular signal transduction pathways used by PRRs, follow the temporal dynamics of
phagocytosis and phagosome maturation, and examine the involvement of PRRs in maintaining homeostasis.
To achieve these goals we perform experiments where the cellular functions of PRRs are characterised using cells
which are transduced to express these receptors and examine their interaction with various pathogens, other cell
types, and cellular or serum components.
Most of this work is carried out by transfecting and transducing cell lines and primary murine cells (which is under a
separate application). However, we now need to transfet haematopoietic cells and cell lines, such as macrophages,
which are notoriously difficult to transfect using our current methodology. Therefore, to transduce these cells, we wish
to use lentiviruses as these are much more efficient and increasingly used within the field.

Recipient or parental organism

i) Bacterial expression:
The bacterial organisms are E. coli K12 derivatives and are unlikely to survive in the human gut or elsewhere. None of
the genes expressed here would be expressed in these bacterial cells since the genes lack a bacterial promoter. Even
if expression did occur, it would not be anticipated to confer any pathological advantage to the bacterial host.
ii) HEK293 FT (Invitrogen) and 293 LTV (Cell Biolabs) producer cell lines
These cells are used to produce lentiviral vectors.
iii) Transduced cells
We will use the lentiviruses to transduce primary murine cells and cell lines with PRR or phagosome maturation
regulation encoding genes. We will also use lentiviruses to transduce human cell lines with PRR genes. Primary
human cells will NOT be transduced. Transduced cell lines will be screened to identify stably transduced clones,
which will be selected for long term culture.

Lentiviral vectors:
To generate the packaged lentiviruses for cell transduction, three plasmid vectors are required using the Virasafe
system (Cell Biolabs): pRSV-Rev, either pCMV-VSV-G (amphotropic) or pCMV-ecoEnv (ecotropic) and pCgpV.
- pCgpV packaging Vector- carries the coding sequences for HIV-1 gag (viral core proteins) and pol (viral replication
enzymes for replication and integration of lentivirus) genes. This plasmid contains CMV promoter to allow high level
expression of the HIV-1 gag and pol genes in mammalian cells. It also has the Rev response element (RRE) to permit
- Rev-dependent expression of gag and pol genes and a polyA signal for efficient transcription termination and
polyadenylation of mRNA. A codon wobble sequence is included at the start of the gag gene to reduce sequence
homology with lentivirus expression vector sequences.
- pRSV-Rev carries the open reading frame for HIV-1 Rev which encodes Rev, the protein that interacts with the RRE
on pCgpV to induce gag and pol expression and to promote the nuclear export of unspliced viral RNA for packaging
into viral particles. The plasmid also contains RSV promoter sequence, therefore abrogating the need for the viral Tat
protein.
- pCMV-VSV-G- carries the coding sequence for the envelope G glycoprotein from Vesicular Stomatitis virus to allow
production of pseudotyped lentivirus with a broad host range. The CMV promoter and polyadenylation signal allow
expression of the VSV-G gene in mammalian cells and allows efficient transcription, termination, and polyadenylation
of mRNA.
- pCMV-ecoEnv- carries the coding sequence for the envelope protein (gp70) of Murine Leukaemia Virus (MLV)
allowing production of pseudotyped lentivirus only able to infect mouse and rat cells.
For expression of the PRR receptor and phagosome maturation regulation genes we will initially use the System
Biosciences vector pCDH-EF1-MCS-T2A-puro. This vector uses the EF1 promoter for heterologous gene expression
and contains a Multiple Cloning Site, a WPRE element (to enhance stability and translation of the transcript), a SV40
polyadenylation signal (to enable efficient termination of transcription and processing of recombinant transcripts), a hybrid RSV/5'LTR promoter (to provides a high level of expression of the full-length viral transcript in producer 293 cells), the genetic elements necessary for packaging, transducing, and stably integrating the viral expression construct into genomic DNA (cPPT, gag, env, LTRs etc.), a SV40 origin (which would enable stable propagation of the pCDH plasmid in mammalian cells, a pUC origin (for high copy replication and maintenance of the plasmid in E.coli cells), an ampicillin resistance gene (for selection in E.coli cells), a puromycin resistance gene (to enable selection of mammalian cells), and a T2A peptide-enabled dual expression system (to enable protein co-expression). We may also use pCDH-EF1-MCS-IRES-GFP (System Bioscience) which works on similar principals.

We will also utilise lentiviral vectors such as the pSIH-H1-copGFP vector (System Bioscience) to monitor packaging efficiency and any other vectors required for controls. We also include basic pUC, pEGFP (a pUC based plasmid, Clontech), and pCMV6 (pBR322 based plasmid, Origene) which carry the following murine phagosome maturation regulation encoding genes fused to fluorescent tags (e.g. GFP, RFP): wild-type or functional mutants (constitutively active or dominant negative versions) of members of the Rab GTPase family (i.e. Rab2a, Rab5, Rab7 and Rab14) as well as LAMP-1 and V-ATPase. These plasmids are maintained in E. coli, and are non-transmissible. They are specifically designed for expression of fusion proteins (for e.g. the Rabs in mammalian cells, not E. coli, and therefore these E. coli strains carry minimal risk. These plasmids will only be used in transient transfection assays of murine cells and cell lines.

HEK293 FT and 293 LTV producer cell lines will be transfected with pRSV-Rev, either pCMV-VSV-G (amphotropic) or pCMV-ecoEnv (ecotropic) and pCgpV (see above).

The following safety features ensure that these vectors are unable to replicate:
1. The expression vectors contain a deletion in the 3′ LTR (ΔU3) which causes “self-inactivation” of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a packageable viral genome.
2. The minimal number of genes from HIV-1 are used in the packaging system and has been reduced to three (i.e. gag, pol, and rev) and tat is no longer required.
3. The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope protein.
4. The three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line. None of them contain LTRs or the Ψ packaging sequence. This ensures that none of the HIV-1 structural genes are present in the packaged viral genome, and are therefore never expressed in the transduced target cell. No new replication-competent virus can be produced.
5. The lentiviral particles produced in these systems are replication-incompetent and only carry the gene of interest. No other viral species are produced.
6. Expression of the gag and pol genes from pCgpV has been made Rev-dependent by the presence of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev.

Host/vector system

HEK293 FT and 293 LTV producer cell lines will be transfected with pRSV-Rev, either pCMV-VSV-G (amphotropic) or pCMV-ecoEnv (ecotropic) and pCgpV (see above).

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Origin & function

Constructs will contain the full, part or mutated version of the open reading frame of the gene of interest. In the case of phagosome maturation regulators, specifically the members of the Rab GTPase family under study, the mutations to be introduced will result in either constitutively activated or dominant negative versions of these proteins. Other constructs will be chimeras with the aim of expressing receptors with tags (e.g. haemagglutinin or fluorescent proteins), defined ligand binding domains, or defined intracellular signalling domains. Expression of these constructs will be driven by the promoter in the vector. The protein of interest will be expressed either intracellularly or on cellular membranes.
All genes of interest will be:
- human or mouse PRRs or mouse phagosome maturation regulators involved in vesicular trafficking, cell-cell adhesion and phagosome acidification (e.g. C-type lectins: Mannose receptor, Dectin-1, Dectin-2, CLECSF8, CLEC-1, MICL, CLEC-2, CLEC12B, CLEC9A, MINCLE, DCAR, KLR11, KLR12
- Scavenger receptors: Scavenger receptor B1, Scavenger receptor A1, LOX-1, SCARF1, CD36
- Toll like receptors: TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11
- Collectins: SP-A, SP-D, MBL
- Integrins: CR3
- Rab GTPases: Rab2a, Rab5a, Rab7a, Rab14
- Lysosomal-associated membrane glycoproteins: LAMP-1
- Proton transporters: V-ATPase

E. coli

There is unlikely to be any hazard to the environment from these bacterial organisms. The host bacterial organisms are E. coli K12 derivatives and they have a proven safe history of use in routine cloning work.

Lentiviral vectors

Lentiviruses require close contact for their transmission and their survival in the general environment is poor; they are temperature, desiccation, detergent, protein denaturant and pH sensitive. Replication deficient lentiviruses cannot propagate so therefore, after infection of a cell they are incapable of further replication. It will be possible to demonstrate that the lentiviral vectors are replication deficient by plating 5% of the vector stock supernatant into mitotically active indicator cells (e.g. 293FT cells) and monitoring for syncitia formation or by PCR analysis of cells that have been transduced with lentiviral vector stocks using primers specific for pro-viral components, such as VSV-G. The insertion of these genes is not expected to alter the tropism of the lentiviral vector. Lentiviral particles carrying phagosome maturation regulation genes represent an increased risk given the possibility of their acquiring tumorigenic potential. However, the strict following of containment level 2 safety practices and decontamination procedures would secure that no worker becomes in contact with these viral particles and that used surfaces are decontaminated after work has ceased.

Factors such as congenital immunodeficiency and secondary immunodeficiency caused by serious illness or chemotherapy would increase the susceptibility to infection by micro-organisms, including the genetically modified micro-organisms described in this application. In carriers of wildtype HIV there may be a theoretical risk of recombination of the lentiviral vector with wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project, these risks will be made clear to all personnel in the laboratory.

We would not expect any specific symptoms as the result of an infection with the genetically modified microorganisms described in this application since the lentiviral vectors cannot replicate.

Evaluation of foreseeable effects

E. coli

There is unlikely to be any hazard to the environment from these bacterial organisms. The host bacterial organisms are E. coli K12 derivatives and they have a proven safe history of use in routine cloning work.

Lentiviral vectors

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We would not expect any specific symptoms as the result of an infection with the genetically modified microorganisms described in this application since the lentiviral vectors cannot replicate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid Waste: Liquid waste will be inactivated using the autoclave at 121°C for 15 minutes or alternatively will be treated with 5% Chemgene and allowed to have a contact time of at least 20mins. According to the manufacturer's instructions. Treated liquid waste would then be disposed of down a designated laboratory sink.

Solid Waste: Solid waste, such as pipette tips, eppendorf tubes, agar plates etc, will be disposed of into a sealed autoclave bag prior to being sterilised via autoclaving at 134°C for 40 minutes. Autoclave function is assessed and recorded via chart recorder and the autoclave is calibrated and serviced quarterly to ensure continued effectiveness, as well as being tested via 12 point thermocouple testing performed annually. Once inactivated the waste will then be disposed of into yellow clinical waste bags and ultimately disposed of via the university waste management route.

Glassware: Contaminated glassware is to be disinfected using a 5% Chemgene before being sterilised in the autoclave at 121°C for 15 minutes.

Sharps: Sharps will NOT be used for this work.

Gloves and lab coat will be worn, and a microbiological safety cabinet will be required.

Validation will involve regular testing of the autoclaves to demonstrate using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time. All autoclaves will be validated at least biannually. High expectation of 100% kill of GM microorganisms exposed to 121°C for 15 minutes.

Standard procedures for use of Chemgene (5% for high level disinfection, Medimark Scientific) have been validated experimentally for HIV lentiviruses and causes apoptosis of cells and denaturation of RNA and DNA.

Autoclaves are validated at least annually by means of biological indicators. In daily use, monitoring is by thermocouple temperature recordings and autoclave strips included in the loads. Bench-top autoclaves in our laboratory are also tested regularly. We test these autoclaves monthly, and in addition they are examined by external experts twice a year.

Once GMMs have been inactivated, normal routes of disposal are used for liquid and solid waste. Killed liquid waste is discarded down the sink. Inactivated solid waste is discarded by CFA processing.

Spills: The contaminated area is flooded with concentrated Chemgene (to 5%, final conc.), mopped up with paper towels, and re-swabbed with 5% Chemgene. Killing at >99.99% has been validated experimentally for enveloped viruses such as lentiviruses. Contaminated material is autoclaved.

All equipment, including microscopes and cytometers, is decontaminated by wiping and flushing with 5% Chemgene.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The enclosed GMM application has been accepted by our Exeter GMM Committee, with a view to inspecting the Class 2 Laboratory (Geoffrey Pope 301) once the refurbishment has been completed.

Project Containment
We aim to establish a transformation protocol for genetic modification of strains from the Sporothrix species complex, by using gene disruption, CRISPR-Cas9 gene editing and overexpression of small RNAs. This genetic manipulation will allow:
(a) The identification the molecular components responsible for the stimuli elicited in innate immune cells by EVs isolated from Sporothrix complex species;
(b) The dissection of putative virulence factors, such as melanin formation, cell wall structure and pathogen-associated molecular pattern molecules (PAMPs);
(c) The investigation of fitness attributes that are important for pathogenicity, such as stress and nutrient responses and;
(d) The examination of potential drug targets, such as regulators of ergosterol and glucan biosynthesis.

Most experiments involve the modification of wild type Sporothrix isolates. Efficient genetic engineering techniques need to be developed for Sporothrix to facilitate important new insights into fungal virulence mechanisms and identify new targets for more effective antifungal therapies.
and animal sporotrichosis. S. mexicana, S. luriei, S. pallida, and S. chilensis belong to the environmental clade that is related to opportunistic infections in immunocompromised patients (Garcia Carnero et al., 2018).

Opportunistic sporotrichosis infections in humans are generally caused by traumatic inoculation via contaminated plant matter or from scratches and bites from infected animals. For this reason, the use of sharps will be banned for experiments with Sporothrix (with the exception of microscope slides). All lab workers will be made aware of the risks of working with Sporothrix and of potential infection via breaks in the skin or, for immunocompromised individuals, via inhalation. Therefore, researchers with any skin conditions or abrasions will wear personal protection equipment (gloves) when handling Sporothrix.

In immunocompromised patients, sporotrichosis can arise through pulmonary infections via inhalation of spores. For this reason, work with Sporothrix spores will be performed in a class 2 biological safety cabinets in a designated Sporulating Fungus Suite within our Cat 2 laboratory.

The treatment for sporotrichosis requires a long-term administration of antifungal drugs, from 3-12 months depending on the severity of the disease, as well as the infecting species. Itraconazole is the drug of choice in uncomplicated cutaneous manifestation. Alternatively, fluconazole, terbinafine or a combination of drugs can be administrated for resistant isolates. For systemic sporotrichosis, the treatment is performed using amphotericin B and itraconazole (Mahajan, 2014).

### Host/vector system

#### Sporothrix:
Recipient strains will include Sporothrix brasiliensis, Sporothrix schenckii, Sporothrix chilensis, Sporothrix globosa, Sporothrix luriei, Sporothrix mexicana and Sporothrix pallida. S. brasiliensis, S. schenckii, S. globosa and S. luriei are clinical (wild type) isolates obtained from patients in South America and the USA. S. chilensis, S. mexicana and S.pallida are environmental (wild type) isolates from South America.

We will attempt to make ku80 and pyrG mutants to enhance homologous recombination in Sporothrix and to provide an auxotrophic marker for transformation (i.e. uracil auxotrophy). Based on other fungal pathogens, this is likely to render the transformation host avirulent strains (Brand et al., 2004).

Transformation will exploit the Hygromycin B phosphotransferase (hbp) gene, the phleomycin gene (Punt & van den Hondel, 1992), the nourseothricin acetyltransferase (NAT) (Alshahni et al., 2010), and the pyrithiamine resistance gene (Kubodera et al., 2000) as dominant selectable markers. In some experiments we will use the PyrG gene to complement the pyrG mutation (selecting for uracil prototrophy) (d’Enfert, 1996).

Disruption and CRISPR-Cas9 cassettes will be generated by PCR and cloned into Jet or pUC19 containing the selection marker (hygromycin, phleomycin, nourseothricin or pyrithiamine resistance). These constructs will be transformed into Sporothrix by excising them from pJet or pUC19 using specific restriction enzymes. If these strategies don’t work, we will exploit the Agrobacterium-mediated co-transformation toolkit to transform Sporothrix.

#### Aspergillus:
The disabled Aspergillus niger and Aspergillus oryzae strains, ATNT ΔpyrG (Geib & Brock, 2017), OP12 pyrG negative (Geib et al., 2019) and those strains deleted for pabA gene (unpublished data)

E. coli: The disabled E. coli strains, DH5-α (NEB), 10-β (NEB) and Stellar (Takara) will be used for cloning of plasmids containing specific Sporothrix genes and, BL21 Rosetta 2 (DE3) (Novagen) will be used as hosts for the heterologous of those genes. Standard non-mobilisable Ampicillin resistance-based plasmids will be used in E. coli.

#### Agrobacterium
Agrobacterium helper strains, such as AGL1, EHA105, LBA1100, LBA1126 or LBA4404 will be used to mediate the transformation of Sporothrix (Hooykaas et al., 2018). Rifampicin, gentamicin, tetracycline, and carbenicillin resistance genes will be used to select positive clones of Agrobacterium (Hooykaas et al., 2018).

### Origin & function

Target genes will include those involved in cell wall synthesis (e.g. chitin, melanin and glucan synthesis) stress pathways (e.g. Hog1 signalling, Yap1-like transcription factors, cell wall remodelling, etc.), and nutrient responses.

Other genes will be investigated as new hypotheses are generated. The GM Committee will be informed of any significant changes in project direction and will be provided with details of genes that are being targeted for genetic modification.

Sporothrix: Most genes will be deleted or inactivated, not inserted.

Aspergillus: Specific Sporothrix genes will be overexpressed (e.g. melanin biosynthesis, enzymes related to secondary metabolites production, antigenic proteins from Sporothrix)

E. coli: Specific genes will be overexpressed (e.g. melanin biosynthesis, enzymes related to secondary metabolites production, antigenic proteins from Sporothrix).
Agrobacterium: plasmids containing interference RNA or gene deletion constructs for transformation of Sporothrix.

**Evaluation of foreseeable effects**

The Sporothrix GMOs are unlikely to be any more hazardous than their wild type parental strains, since we aim to knock out genes associated with virulence or to overexpress antigenic proteins. However, the Sporothrix species of interest are classified as ACDP Hazard Group 2. Therefore, by definition, they have potential to cause harm, but these infections are not life-threatening in healthy individuals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid Waste: Liquid waste will be inactivated using the autoclave at 121°C for 15 minutes or alternatively will be treated with 5% Chemgene and allowed to have a contact time of at least 20mins. According to the manufacturer's instructions. Treated liquid waste would then be disposed of down a designated laboratory sink.

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Glassware: Contaminated glassware is to be disinfected using a 5% Chemgene before being sterilised in the autoclave at 121°C for 15 minutes.

Sharps: Sharps, such as syringe needles, scalpel blades, will be banned for work with Sporothrix. Microscope slides will be used for some experiments, and these are to be disposed of into designated sharps binbins. Once full the cinbins are sealed and locked before being double bagged into clinical waste bags and incinerated.

Animals and Tissues: All carcases will be autoclaved within the BSU, and all tissues are autoclaved prior to disposal.

Validation will involve regular testing of the autoclaves by our laboratory using Browne tubes and biannually by independent external assessors to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time.

In the event of a spill, the contaminated area is flooded with Chemgene 5%, mopped up with paper towels, and reswabbed with Chemgene 5% and a final clean with 70% ethanol.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The enclosed GMM application has been accepted by our Exeter GMM Committee

**Project Containment**

02/03/2022
Constructing a replication incompetent adenovirus vector capable of expressing putative immunogenic antigens of the fungal pathogen Aspergillus fumigatus

The often-fatal fungal lung disease invasive aspergillosis (IA) has claimed more than 8 million human lives since its emergence ~ 40 years ago. IA-related mortality is unacceptably high (50-90%) and usually results from respiratory failure due to haemorrhage occurring at sites of fungal invasion. Given the life-saving potential of anti-invasion therapies, a high premium has been placed upon identifying effectors of tissue invasion, against which drugs and vaccines might be engineered. However, the quest for causal associations between tissue-invasive phenotypes and secreted fungal products has repeatedly failed, presumably due to likely functional redundancy amongst the effector repertoire and insufficiency of genetic approaches in A. fumigatus with which to drive high throughput analyses of secreted fungal products. Using mouse models of IA, in vitro infections of cultured human lung cells, functional
genomics and a high throughput A. fumigatus gene deletion methodology we have discovered a first-in-field cohort of 42 effectors of tissue invasion (eTINs) which govern lytic death of human lung cells. We will now combine state-of-the-art genetic, single cell imaging and effector-trap approaches with our established methodological workflow to conclusively define the invasive A. fumigatus secretome and to characterise the modes of entry and/or lytic activity of the individual effectors. This work will reveal for the first time the molecular basis of tissue invasive IA pathologies and define the genomic cohort of targets against which anti-invasion therapies and novel vaccines and diagnostics can be designed.

Recombinant Adenovirus vectors have been used as a vaccine delivery mechanism in animal models and clinical trials with excellent efficacy and safety profiles, for example the ChAdOx1 Oxford/AstraZeneca SARS-CoV-2 recombinant spike protein vaccine which is now licenced. Novel vaccine delivery mechanisms such as this have reinvigorated interest in developing the first vaccine against an invasive fungal pathogen. This current project aims to construct a replication incompetent adenovirus vector using the commercially available pAdEasy system (human adenovirus serotype 5) that is capable of expressing putative immunogenic antigens of the fungal pathogen Aspergillus fumigatus. Genes of interest will be selected from the cohort of effectors of tissue invasion identified in previous work performed by the Bignell Lab (summarised above). Putative vaccine candidates will be selected according to the strength of association with tissue invasion, expected immunogenicity, lack of cytotoxicity, and lack of human orthologue. To demonstrate proof of concept, pilot work will use an immunogenic epitope from the Aspergillus fumigatus Aspf1 gene since this has been characterised by the Thornton Lab previously and several materials and reagents exist already. Once work flows are established adenovirus vectors containing putative vaccine candidates will be constructed. Downstream we plan to use these vectors as a vaccine delivery mechanism in murine IA model vaccination studies (not the subject of this GM notification).

The construction of an adenovirus vector involves the following steps:
- Clone gene of interest into the pAdTrack-CMV shuttle vector (this vector contains a GFP reporter as well as the gene of interest, both under regulation of CMV promoter).
- Perform homologous recombination of the shuttle vector with the pAdEasy-1 replication-incompetent adenovirus backbone (E1/E3 deleted) using a non-pathogenic strain of E. coli (BJ5183 cells).
- Transfect the recombinant adenovirus vector into a producer cell line - HEK293 cells or derivatives thereof. This allows the growth of a recombinant adenovirus vector stock.
- Purify and concentrate the recombinant adenovirus vector for use in downstream applications.

All procedures will be performed following specific Standard Operating Procedures (SOPs). Steps involving virus transfection and amplification using HEK293 cells will be performed in tissue culture at containment level 2 using a class 2 biological safety cabinet and appropriate PPE. The cloning platform we will use is a commercial product that is marketed for research purposes. The pAdEasy system plasmids and reagents are available through AddGene (https://www.addgene.org/browse/article/1814/). This system has been used extensively by other groups for similar purposes.

https://www.addgene.org/16399/
https://www.addgene.org/16405/

Recipient or parental organism

Adenovirus is a non-enveloped, non-segmented, linear dsDNA virus approximately 36kb in length of the ACDP Hazard Group 2. There are >60 serotypes separated into groups A-G that are associated with human infections. Adenovirus displays broad species and tissue tropism, gaining entry to a human host via the Coxsackie Adenovirus Receptor (CAR) or CD46 (serogroup B only) and readily infects many epithelial cell lines. It then undergoes a two-stage lytic replication cycle intracellularly. Human adenovirus infections most often result in a mild, self-limiting upper respiratory tract infection in an immunocompetent host. The usual duration of illness is five to seven days, although symptoms may persist for up to two weeks. Bacterial superinfections can occur. Less commonly, human adenovirus infections are associated with gastrointestinal, ophthalmologic, genitourinary, and neurologic diseases. Rarely, fatal infections can occur in immunocompromised hosts. Adenoviruses are of great interest
as vectors for gene or vaccine delivery and a variety of recombinant vectors exist for such purposes. The adenovirus vector delivery system used in the Bignell group is ΔE1/E3 human adenovirus 5 (pAdEasy) – all utilised vectors and resulting virus-containing supernatants are replication incompetent and solely single-cycle infectious clones handled in CATEGORY 2 facilities. Rarely, ΔE1/E3 human adenovirus 5 vectors can recombine with E1 provided by the packaging cell line (HEK293 cells), replacing the transgene insert in the adenovirus vector and thus producing a replication competent virus capable of establishing a productive infection in a human host. Therefore, all adenovirus stocks will be checked for the presence of replication competent virus before long-term storage at -80 °C - if replication competent adenovirus is detected then the stock will be inactivated with chemogen and discarded according to local waste disposal policies (detailed below). Adenovirus is a non-enveloped, non-segmented, linear dsDNA virus approximately 36kb in length of the ACDP Hazard Group 2. There are >60 serotypes separated into groups A-G that are associated with human infections. Adenovirus displays broad species and tissue tropism, gaining entry to a human host via the Coxsackie Adenovirus Receptor (CAR) or CD46 (serogroup B only) and readily infects many epithelial cell lines. It then undergoes a two-stage lytic replication cycle intracellularly. Human adenovirus infections most often result in a mild, self-limiting upper respiratory tract infection in an immunocompetent host. The usual duration of illness is five to seven days, although symptoms may persist for up to two weeks. Bacterial superinfections can occur. Less commonly, human adenovirus infections are associated with gastrointestinal, ophthalmologic, genitourinary, and neurologic diseases. Rarely, fatal infections can occur in immunocompromised hosts. Adenoviruses are of great interest as vectors for gene or vaccine delivery and a variety of recombinant vectors exist for such purposes. The adenovirus vector delivery system used in the Bignell group is ΔE1/E3 human adenovirus 5 (pAdEasy) – all utilised vectors and resulting virus-containing supernatants are replication incompetent and solely single-cycle infectious clones handled in CATEGORY 2 facilities. Rarely, ΔE1/E3 human adenovirus 5 vectors can recombine with E1 provided by the packaging cell line (HEK293 cells), replacing the transgene insert in the adenovirus vector and thus producing a replication competent virus capable of establishing a productive infection in a human host. Therefore, all adenovirus stocks will be checked for the presence of replication competent virus before long-term storage at -80 °C - if replication competent adenovirus is detected then the stock will be inactivated with chemogen and discarded according to local waste disposal policies (detailed below).

Host/vector system

BJ5183 E. coli (AdEasy-1 cells): This non-pathogenic (biosafety level 1) E. coli strain will be used to amplify the plasmid vectors. These competent cells are recombination proficient bacterial cells carrying the pAdEasy-1 plasmid that encodes the Adenovirus-5 genome (E1/E3 deleted). These cells supply the components necessary to execute a recombination event between the pAdEasy-1 vector and an AdEasy® shuttle vector containing the gene of interest, thus generating a recombinant adenovirus genome that contains the gene of interest. They contain streptomycin and ampicillin antibiotic resistance genes for selection.

- Replication incompetent recombinant adenovirus (hAd5 serotype): This ACDP hazard group 2 organism is a first generation adenovirus vector. The E1 (essential for replication and downstream late gene expression) and E3 (role in virus immune evasion) genes are deleted from this construct resulting in replication incompetence. A GFP reporter under the regulation of a CMV promoter is included. A transgene of interest is cloned into the E1 cassette under the regulation of a CMV promoter. The resulting virus, when produced in a producer cell line e.g. HEK293T, is of equivalent hazard to the parental organism (and potentially of lesser hazard given its replication incompetence).

- HEK293T producer cell line: The 293T cell line, originally referred as 293tsA1609neo, is a highly transfectable derivative of human embryonic kidney 293 cells, and contains the SV40 T-antigen. They have been transfected with sheared adenovirus 5 (Ad5) DNA - The Ad5 insert has been cloned and sequenced previously, and it was determined that a colinear segment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). This cell line is able to produce adenovirus E1 in trans, thus complementing the replication incompetent human adenovirus serotype 5 vector. This is a producer cell line used to amplify a stock of replication incompetent adenovirus vector.

- pAdTrack-CMV: Shuttle vector for use in AdEasy System. For expression of transgenes under a CMV promoter when a GFP tracer is desired. Contains a kanamycin antibiotic resistance gene for selection.


Origin & function

Use of the human cytomegalovirus major immediate-early enhancer to drive expression of both GFP and the 'gene of interest' is expected to direct high-level expression in a broad range of cell types. There are no anticipated hazards associated with the expression of GFP. Aspf1 - the Aspergillus fumigatus gene selected for the initial proof-of-concept work in this project - is a fungal ribotoxin. In preliminary data generated by the Bignell lab, the Aspf1 knockout Af293 Aspergillus fumigatus strain is of equivalent cytotoxicity to the wild-type strain. It is not thought to be a virulence factor in invasive aspergillosis. It has been associated with allergic bronchopulmonary aspergillosis; this is a potential hazard to lab staff working with the adenovirus vectors but since the vector is replication incompetent, all procedures will be conducted at
incubated overnight. The following day, small colonies are picked, grown in 2 mLs LB broth containing kanamycin, and plasmid DNA is extracted using a conventional method.

- GFP: Enhanced Green fluorescent protein - constructed by subcloning the gene encoding enhanced GFP from pEGFP-C1 into the shuttle vector by the original authors. This does not affect the virulence or pathogenesis of the parental organism.
- Aspf1: this is a ribotoxin produced by Aspergillus fumigatus. It is an allergen that has been associated with allergic bronchopulmonary aspergillosis. It is not thought to be a major virulence factor in models of invasive aspergillosis. We will clone a specific epitope sequence from this gene into the adenovirus vector - this epitope has previously been shown by the Thornton lab to not elicit an IgE response and reduces cytotoxicity whilst maintaining the capability to elicit a B cell response. This will remove the risk of inducing an allergic response and cytotoxicity.
- Antibiotic resistance genes - kanamycin or ampicillin: these are used for selection of E. coli containing the plasmid of interest during cloning. The E. coli used in this project are non-pathogenic and unable to survive within a human host or in the environment.
- Other putative immunogenic Aspergillus fumigatus genes: Additional effectors of tissue invasion will be selected from the cohort currently being characterised in the Bignell Lab. Following the initial proof-of-concept phase (construction of Aspf1 epitope-containing recombinant adenovirus vector), additional Aspergillus fumigatus genes of interest will be cloned into the adenovirus shuttle vector and used to generate recombinant adenovirus - an update to this GM application will be written at this stage to include the final selected genes of interest and subsequently submitted to the local GMO committee for approval. Putative vaccine candidates will be selected according to the strength of association with tissue invasion, expected immunogenicity, lack of cytotoxicity, and lack of human orthologue. The literature will be reviewed to gather previous in vitro and in vivo data pertaining to selected genes.

**Evaluation of foreseeable effects**

This project will construct replication incompetent adenovirus vectors carrying an Aspergillus fumigatus-derived transgene insert. Possible hazards to human health include:

- direct contact with adenovirus at mucous membranes or broken skin e.g. splash or spillage
- accidental ingestion
- accidental inhalation following aerosolisation
- accidental inoculation of adenovirus by sharps or broken glass
- exposure to any of the above in an immunocompromised host (any potential harm likely augmented relative to an immunocompetent host)
- generation of replication competent adenovirus (rare - any potential harm following exposure via above mechanisms likely increased)

This project will create recombinant human adenovirus serotype 5 vectors containing transgene inserts (putative vaccine candidates) with a GFP reporter. A GM cell line - HEK293T - is used for amplification of the viral stock. During cloning, chemically competent non-pathogenic E. coli (BJ5183) are used for homologous recombination of the shuttle and adenovirus backbone vectors. None of the modifications used during this project are anticipated to increase the risk to human health or the environment relative to the parental organism.

The adenovirus vector delivery system to be used in the Bignell group is ΔE1/E3 human adenovirus 5 (pAdEasy) – all utilised vectors and resulting virus-containing supernatants are replication incompetent and solely single-cycle infectious clones handled in CATEGORY 2 facilities. Replication incompetent adenovirus vaccines based on a Chimpanzee adenovirus (e.g. ChAdOx1 SARS-CoV-2 recombinant spike protein vaccine) are already licenced and have been distributed to hundreds of millions of people globally with excellent safety records. The ΔE1/E3 human adenovirus 5 (pAdEasy) system we propose to use is very similar to this.

A gene of interest – in this case, an immunogenic Aspergillus fumigatus Aspf1 epitope – is cloned into an adenovirus shuttle vector using either restriction enzyme digestion or the GeneArt Seamless Cloning technology. The adenovirus shuttle vector (pAdTrack-CMV) is a plasmid that allows expression of transgenes under a CMV promoter and contains a GFP reporter for downstream applications. This shuttle vector is subsequently verified, linearised, and transformed into AdEasier-1 cells (BJ5183 non-pathogenic E. coli strain that contains a pAdEasy-1 adenovirus ΔE1/E3 backbone vector). The shuttle vector and the adenoviral plasmid have matching left and right homology arms which facilitate homologous recombination of the transgene into the adenoviral plasmid. The resultant transfectant is plated onto LB/kanamycin plates and incubated overnight. The following day, small colonies are picked, grown in 2 mLs LB broth containing kanamycin, and plasmid DNA is extracted using a conventional method.
alkaline lysis method and purified e.g. Qiagen MiniPrep. Recombinant plasmids are verified, restriction enzyme digested and transfected into HEK293 cells using a lipofectamine transfection method. The E1 gene is trans-complemented by HEK293 cells resulting in the growth of an adenovirus vector stock. Virus replication is monitored using GFP expression with a fluorescent microscope. After 7-10 days, cells are scraped off the flask using a rubber policeman and undergo four freeze/thaw cycles using either a dry ice or methanol bath and a 37°C water bath. Supernatants are either stored at -20°C or used to infect naïve HEK293 cells in a second round of stock amplification. A similar process is followed before a third round of amplification. In these rounds of amplification, no more than 2x T75 tissue culture flasks of HEK293 cells will be required. This typically results in a virus titre of 10^7 or 10^8. Presence of recombinant adenovirus vector is confirmed using Western Blot and/or PCR in a susceptible non-producer cell line e.g. A549 (the Bignell group already works with this cell line). If higher virus titres are required, 15-20x T75 tissue culture flasks will be prepared and followed by purification and concentration using the commercial Agilent Ad5 Adenovirus Purification Kit. All procedures will be performed following specific Standard Operating Procedures (SOPs). Steps involving virus transfection and amplification using HEK293 cells will be performed in tissue culture at containment level 2 using a class 2 biological safety cabinet and appropriate PPE. Adenovirus vector stocks will be stored frozen at -80°C within appropriately sealed containers. Any contaminated materials used in the process will be disposed of in sealed plastic bags and inactivated by autoclaving according to MRC CMM SOPs for waste management and the procedures outlined in the waste management section below. Adenoviruses are often purified by ultracentrifugation on caesium chloride gradients. To avoid the use of needles and risks associated with ultracentrifugation, we have identified a commercial adenovirus extraction kit that does not require the use of needles and we will use this kit.

No alterations to existing traits are anticipated for the adenovirus vector or transgene inserts during the procedures for this project. Specifically, we do not anticipate any change in pathogenicity, host range, tissue tropism, or mode of transmission. The adenovirus vector used in this project is ΔE1/E3 - the ΔE1 renders the vector replication incompetent and the ΔE3 renders the vector more immunogenic (E3 is involved in viral immune evasion). The increased immunogenicity may result in an enhanced inflammatory response inside human cells, but this risk is mitigated by the replication incompetence and immunocompetent host.

Human adenoviruses infect a broad range of human cells and therefore gene transfer following accidental ingestion or through direct skin contact is a possibility. The risk of accidental inoculation by needle stick or aerosolization of high titre viral stocks will be reduced by prohibiting the use of sharps, wearing double gloves during viral culture, ensuring no skin is exposed, and only handling stocks and cultures containing viral particles in a Class II safety cabinet. All work surfaces will be decontaminated after work has been completed and immediately following any spillage. Transport of adenovirus-containing material will only be permitted in double sealed containers.

Adenoviruses are non-enveloped DNA viruses, relatively stable and resistant to dehydration. Viruses can survive for protracted periods in aerosols and water. Most adenovirus vectors have been derived from human viruses, which are not thought to be able to replicate efficiently in animal cells. Therefore, it is unlikely that activities with these vectors will represent any significant risk to the environment. Human Ad5 vectors have been shown to enter (but not replicate efficiently in) cells of mouse, rat and canine origin. Therefore, it is unlikely that the GMM will be able to survive or disseminate in the environment.

All work will be conducted in a class 2 biological safety cabinet. Aerosols may be generated by spillages during centrifugation - samples will be processed in buckets with a lid and loaded/unloaded within the class 2 safety cabinet. The use of sharps will not be necessary for the procedures in the work flow described within this application.

Individuals with a congenital or acquired immunodeficiency would be at increased susceptibility to infection with pathogenic micro-organisms, including adenovirus. Individuals known to be immunocompromised will not be allowed to work on this project, these risks will be made clear to all personnel in the laboratory. We would not expect any specific symptoms following infection with the genetically modified micro-organisms described in this application since the adenovirus vectors are replication incompetent. The E. coli BJ5183 cells used in this project are non-pathogenic strains. Vaccinations or health surveillance are not necessary for the work described in this project.

The genetic modifications proposed in this work are not expected to increase the virulence of the genetically modified micro-organism beyond that of the parental organism. Also it is not expected that this work would have an effect on other traits of the GMM such as increased drug resistance therefore the risk to human health posed by the construction of these GMM's is essentially equivalent to those of the parental organism. The most hazardous GMM is the adenovirus vaccine delivery vector.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: To be inactivated by autoclave at 121 degrees Celsius for at least 15 minutes. Alternatively, in cases with lower liquid volumes (<100ml), liquid is to be treated with 5% Chemgene HLD4L and allowed to have a contact time of at least 20mins, according to the manufacturer's instructions.

Solid waste: Solid waste is double sealed in autoclave bags prior to being autoclaved and incinerated.

Glassware: Soaked with 5% Chemgene or autoclaved at a minimum of 121°C for a minimum duration of 15 minutes.

Plasticware: To be sterilised by disinfection and autoclaving and incinerated when necessary.

Sharps: Sharps will not be used during the procedures required for this project.

DECONTAMINATION: 5% Chemgene

VALIDATION: Chemgene disinfectant will be used to inactivate virus-containing liquids – the manufacturer has validated efficacy against adenovirus. We will perform a local validation of Chemgene efficacy against adenovirus using the recommended working concentration with the aim of achieving 100% kill. Validation of autoclaves will involve testing on a monthly basis using Browne tubes and biannually by independent external assessors to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time.
Innate immunity against fungal pathogens: manipulation of genes in murine cells using lentiviruses

We focus on understanding innate immunity against fungal pathogens. Fungal pathogens kill over a million humans each year. Our aim is to investigate contribution of immunometabolic genes support immunity against C. neoformans, and other fungal pathogens. We will target murine immune and metabolic genes to define the contribution of those genes in antifungal immunity. We will examine the consequences of manipulating/engineering, generally through deletion, these genes for immune and metabolic functions and their relationship to intracellular signalling pathways. To do this we will manipulate genes via a Crispr-Cas9-lentiviral approach, primarily in immortalized cell lines, albeit gene-engineering of primary cells may be warranted in certain key experiments.

Disabled E. coli strains will be used to amplify the plasmid vectors. Disabled Lentiviruses that express different sgRNA and Cas9 targeting immune metabolic genes will be generated. HEK293 producer cell lines will be transfected with these Lentiviral constructs. Immortalized murine cell lines and when warranted primary murine cells will be transfected with these disabled viruses.

Recipient or parental organism

1. Bacteria-Stbl3 E.coli- derived from K12.

The natural environment of E. coli is in the gut but these experimental strains are laboratory adapted, being classified as disabled hosts in the ACGM guidelines, and are unlikely to survive in the human gut or elsewhere.
2. Lentivirus:
These are a type of retrovirus, unique because they can infect both dividing and non-dividing cells and are stably integrated into the host genome. This is because their pre-integration complex (or virus shell) encodes a localisation sequence that is recognised by the import machinery of the nucleus, enabling the virus to get through the intact membrane of the nucleus of a target cell. HIV is a lentivirus and is surrounded by a lipid bilayer with membrane proteins protruding from it. One of these proteins is gp120 which binds to CD4 receptor on T helper cells. All retroviruses encode three protein components – gag, pol and env (which necessary for virus replication), but HIV also encodes other regulatory and accessory genes. To enable lentiviruses derived from HIV to transduce a wide range of cell types (rather than just CD4 receptor +ve cells), the lentiviral vector are constructed to express a different coat protein, the envelope protein from vesicular stomatitis virus. Lentiviruses have two strands of RNA in the form of a single-stranded positive sense RNA genome with long terminal repeats (LTRs) at either end. At the 5’ end there is a Psi (Ψ) sequence which is required for packaging of viral RNA into viral capsids. Their RNA must be reverse transcribed from RNA into the first strand of DNA which is catalysed by reverse transcriptase that is encoded by the HIV provirus. The host DNA polymerase then syntheses the second strand to give double stranded DNA. Following reverse transcription, the LTRs then mediate the integration of the double-stranded DNA into the host genome.

The next two stages of the lentiviral life cycle involve the gradual infection of T Helper cells and then the rapid production of infective viral particles that are released into the blood by the host cell lysis to infect other cells. These final stages of the lentiviral cycle will not occur when using REPLICATION DEFICIENT lentiviral vectors.

Lentiviral vectors are produced by co-transfecting a packaging cell line (in this case the HEK293 cell line) with the lentiviral expression construct, the envelope plasmid, and the lentiviral packaging system that contains plasmids carrying genes encoding the necessary structural and replicatory proteins detailed below. Thus components are expressed in trans within the packaging cell line. The packaging system may be 1 or 2 plasmids (2nd or 3rd generation, respectively).

The 2nd generation packaging systems contain gag, pol, rev, tat. The 3rd generation packaging systems contain 2 separate plasmids with rev in a separate plasmid from gag and pol, and without tat (requiring a 3rd generation transfer plasmid, whose replication is tat-independent). This results in increased biosafety but significantly reduced yields and efficiency.

The resulting lentiviral vector does not contain any of the genes required for viral replication within the target cell line. We will make our own lentiviral vectors, or obtain them from commercial source or from collaborators.

3. List of recipient cell line(s):
HEK293 (human) only for lentiviral production purposes. These cells are used to produce lentiviral vectors.
Immortalized BV2, J774.16, RAW264.7 (murine macrophages) and Bone-marrow derived macrophages, microglia (murine primary macrophages) and other primary murine cells. These cells are the transduced experimental cells where we wish to achieve gene engineering.

4. List of vector(s), all available through Adgene:
- pMD2.G (Adgene #12259) envelope (contains VSVg): allow production of pseudotyped lentivirus with a broad host range.
- psPAX2 (Adgene #12260) 2nd generation packaging (contains gag, pol, rev, tat): allows production of functional virus at the stage of HEK293 cells. Will not be packaged into newly produced virus, thus making virions replication incompetent, but infectious.
- pMDLg/pRRE (Adgene #12251) & pRSV-Rev (Addgene#12253): 3rd generation lentiviral packaging system (2 plasmids, with rev separate from gag, pol): will not be packaged into newly produced virus, thus making virions replication incompetent, but infectious.

For simultaneous sgRNA and Cas9 transfer:
- lentiCRISPR v2 (Adgene #52961) (contains insertion site for gRNA and only point of modification, S. pyogenes CRISPR-Cas9, ampicillin and puromycin resistance cassettes); 3rd generation transfer plasmid, woodchuck hepatitis B virus regulatory element (WPRE), “self-inactivating” (SIN) lentiviral vectors.
- pSpCas9(BB)-2A-GFP (PX458) (Adgene #48138) - (contains insertion site for gRNA and only point of modification, S. pyogenes CRISPR-Cas9, -A2-GFP to allow for fluorescent screening of transfected cells, ampicillin and puromycin resistance cassettes); 3rd generation transfer plasmid, WPRE, “self-inactivating” (SIN) lentiviral vectors.
For sequential transfection of Cas9 and sgRNA: This allows increased levels of manipulation or increased efficiency in harder-to-transfect cells by first selection and enrichment of cells expressing high levels of Cas9, (cells may be frozen for long-term storage once selection is finished) and followed by a second lentiviral transfection, which contains the sgRNA.

- Cas9: lentiCas9-Blast (Adgene #52962). 3rd generation transfer plasmid, contains S. pyogenes Cas9 sequence, ampicillin and blasticidin resistance), WPRE, "self-inactivating" (SIN) lentiviral vectors.


Control plasmids:
- pLJM1-EGFP transfer-control: this plasmid serves as a transfection control. GFP expression is induced upon efficient transfection. (expresses GFP, ampicillin and puromycin resistance cassettes).

**Host/vector system**

HEK 293 cells will be transfected with envelope, packaging and transfer plasmids (see above in List of vectors). Murine immortalized cell lines and murine primary cells will be infected with the above produced non-infecting virions.

The following safety features ensure that these vectors are unable to replicate:cons

1) The transfer vectors contain a deletion in the 3' LTR (ΔU3) which causes "self-inactivation" of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a packageable viral genome. This minimizes chances of recombination.

2) The minimal number of genes from HIV-1 are used in the packaging system and has been reduced to four (i.e. gag, pol, rev, tat) in 2nd generation systems and to three (gag, pol, rev) in 3rd generation systems.

3) The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope protein.

4) The packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, and tat only in 2nd generation packaging systems ) in the HEK293 producer cell line, however none of them contain LTRs or the Ψ packaging sequence. This ensures that none of the HIV-1 structural genes are present in the packaged viral genome, and are therefore never expressed in the transduced target cell. No new replication-competent virus can be produced.

5) The lentiviral particles produced in these systems are replication-competent and only carry the gene of interest. No other viral species are produced.

**Origin & function**

Constructs will generate single-guide-RNA (sgRNA) molecule and the Cas9 enzyme in murine cells, resulting in gene engineering, usually resulting in full abolishment of activity of the gene targeted by sgRNA. After functional validation of gene engineering, the generated cells will be studied in immune and metabolic functional assays to understand their contribution to antifungal immunity.

List of murine genes:
Genes to be engineered are mouse immunometabolic genes, which include:
- Metabolic genes: Irg1/Acod1, Ldh1, Cox6b1, Bcl2a1, Ndufa8a2, Pex7, Abcd5
- Antimicrobial genes: iNOS(NOS2), IFNg, Cybb

Other constructs which will be used include reporter constructs, such as GFP, which will be used to measure transduction efficiency, for example. Other controls include a non-targeting sgRNA and transfection with transfer plasmids without sgRNA (empty plasmid).
There is unlikely to be any hazard to the environment from these bacterial organisms. The host bacterial organisms are E. coli K12 derivatives and they have a proven safe history of use in routine cloning work.

Lentiviral vectors
Lentiviruses require close contact for their transmission and their survival in the general environment is poor; they are temperature, desiccation, detergent, protein denaturant and pH sensitive. Replication deficient lentiviruses cannot propagate so therefore, after infection of a cell they are incapable of further replication. It will be possible to demonstrate that the lentiviral vectors are replication deficient by plating 5% of the vector stock supernatant into mitotically active indicator cells (e.g. HEK293 cells) and monitoring for syncitia formation or by PCR analysis of cells that have been transduced with lentiviral vector stocks using primers specific for pro-viral components, such as VSV-G. The insertion of these genes is not expected to alter the tropism of the lentiviral vector. Lentiviral particles carrying sgRNA to Idh1 and WPRE represent an increased risk given the possibility of their acquiring tumorigenic potential. However, the strict following of containment level 2 safety practices, personnel protective equipment (goggles, gloves, disposable sleeves) and decontamination procedures would secure that no worker becomes in contact with these viral particles and that used surfaces are decontaminated after work has ceased.

Factors such as congenital immunodeficiency and secondary immunodeficiency caused by serious illness or chemotherapy would increase the susceptibility to infection by micro-organisms, including the genetically modified micro-organisms described in this application. In carriers of wildtype HIV there may be a theoretical risk of recombination of the lentiviral vector with wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project, these risks will be made clear to all personnel in the laboratory.

We would not expect any specific symptoms as the result of an infection with the genetically modified micro-organisms described in this application since the lentiviral vectors cannot replicate. Whenever possible we will use safer 3rd generation packaging systems.

Factors such as congenital immunodeficiency and secondary immunodeficiency caused by serious illness or chemotherapy would increase the susceptibility to infection by micro-organisms, including the genetically modified micro-organisms described in this application. In carriers of wildtype HIV there may be a theoretical risk of recombination of the lentiviral vector with wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project, these risks will be made clear to all personnel in the laboratory.

We would not expect any specific symptoms as the result of an infection with the genetically modified micro-organisms described in this application since the lentiviral vectors cannot replicate. Whenever possible we will use safer 3rd generation packaging systems.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

To minimise the chance of accidental injection: The use of sharps (e.g. needles and scalpels) will be prohibited when handling cultures of cells or bacterial vectors or plasmids, essentially at any stage of this work.

To minimize generation of aerosols (including if accidental spills occur) for steps containing virions:
- Lentiviral virions will only be handled in a class II safety cabinet to minimise the risk of accidental inhalation or ingestion. Whenever possible we will use safer 3rd generation packaging systems.
- Secondary containment for steps containing virions is required during incubation and transport, as well as use of contained centrifuge rotor during centrifugation, as this minimizes risk of accidental spills. Secondary containment should be sealed before leaving biosafety cabinet and only opened inside biosafety cabinet, unless absolutely necessary for microscopy or other analysis. Media with virions will be preferentially used in flasks with screw-on lids (versus plates) to minimize risk of spillage. Flasks/plates will be sprayed with disinfectant on the outside and placed in secondary containment (transport box).
- Use of dedicated and restricted spaces, Work should only be performed when the room is at low occupancy, by trained competent individuals. Appropriate signage on outer TC door, on flow hood and incubators indicating that live infectious virus is present.
- In addition to laboratory coat and gloves, users will double gloves, use a dedicated laboratory coat or disposable sleeves, goggles, to avoid skin or mucosal contact and deposition on surfaces. All work will be conducted in a Containment Level 2 laboratory.
- Mandatory use of filter pipettes and tips for liquid transfers with immediate disposal of liquids into disinfectant (described in waste disposal), and prohibited pouring of liquids.
- Any equipment (microscopes) should be wiped with disinfectant immediately after use, and used during low occupancy periods.
- Additional personnel training will be established.
To minimize generation of aerosols during all other steps (non-infectious):
Primary containment and use of contained centrifuge rotor during centrifugation. Whenever possible we will use safer 3rd generation packaging systems.
In addition to laboratory coat and gloves, users will double gloves.
Further control measures are: use of dedicated and restricted spaces, use of filter pipettes and tips for liquid transfers. Additional personnel training will be established.

LIQUID WASTE:
5% Chemgene high level disinfectant (as per the manufacturer’s instructions)

GLASSWARE:
Soaked with 5% Chemgene and autoclaved at a minimum of 121°C for a minimum duration of 15 minutes

PLASTICS:
All plasticware (tips and pipettes) must be rinsed, by pipetting in a solution of 5% chemgene prior to placing in the small autoclave bags (or cardboard autoclave boxes). This prevents accidental drips containing live virus, which may leak from the autoclave bags. Plates and Flasks after use and in contact with media which may contain virus will be soaked in chemgene for 20 min before removal from biosafety cabinets. There will be a container with 5% chemgene in biosafety cabinet to allow rinsing of all plasticware as the work proceeds. Effectively anything in contact with virus containing liquid will be inactivated prior to leaving the flow hood and entering the waste stream. Plasticware will then be autoclaved.

DECONTAMINATION:
5% Chemgene for 20 min in biosafety cabinet, followed by 70% ethanol wiping all surfaces.

VALIDATION:
Our procedures for the use of Chemgene have been validated experimentally to kill 100% of virus and cells.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2, L3, L4</td>
<td>L2, L3, L4</td>
<td>L2, L3, L4</td>
</tr>
</tbody>
</table>

02/03/2022
The goal of this work is to characterize the mechanisms through which hypothalamic neurons coordinate their activity to release ACTH-stimulating neuropeptides and regulate the HPA axis. In order to realise this project we will use viral vectors to specifically transduce cells of interest (corticotrophs and hypothalamic neurons, for example). Following transduction we will be able to monitor and/or control the electrical/calcium activity of these cells.

pLenti6-POMC-eYFP-WPRE, pLenti6-POMC-ppANF-eYFP-WPRE, plenti6-POMC-GCaMP6s-WPRE will be transfected (together with packaging plasmids and envelope plasmid) into 293FT HEK cells in order to produce 3rd or 2nd generations lentiviruses.

3rd and 2nd generation lentiviral vectors will be used to transduce:
- AtT-20 cells in order to determine the titre of the virus
- Primary neurons or pituitary cells from wild-type mice/rats.
- Wild-type mice/rats (via stereotaxic injection).
- Primary neurons or pituitary cells from transgenic mice/rats
- Transgenic mice/rats (via stereotaxic injection)
**Host/vector system**

The viral vectors used will be 2nd or 3rd generation lentivirus.

**Origin & function**

The plasmids will be used to introduce genes for:

- Labelling/identifying neurons or pituitary cells (e.g. fluorescent proteins such as GFP and TdTomato)
- Monitoring the activity of neurons or pituitary cells (e.g. genetically-encoded calcium indicators)
- Controlling neurons or pituitary cells (e.g. using opsins such as channelrhodopsin2, archaerhodospin)

**Evaluation of foreseeable effects**

The two main safety concerns surrounding the use of lentivirus are:

- The potential for generation of replication-competent lentivirus.
- The potential for oncogenesis.

The potential for generation of replication-competent lentivirus is addressed by the design of the vectors and by safe laboratory practice. In terms of vector design, 2nd and 3rd generation lentiviral systems separate transfer, envelope, and packaging components of the virus onto different vectors. The transfer vector encodes the gene of interest and contains the sequences that will incorporate into the host cell genome, but cannot produce functional viral particles without the genes encoded in the envelope and packaging vectors. Unless recombination occurs between the packaging, envelope, and transfer vectors, and the resulting construct is packaged into a viral particle, it is not possible for viruses normally produced from these systems to replicate and produce more virus after the initial infection. In this regard, 3rd generation systems are considered even safer than 2nd generation systems because the packaging vector has been divided into two separate plasmids (resulting in a four plasmid system in total).

Additionally, the transfer plasmids pLenti6-POMC-eYFP-WPRE, pLenti6-POMC-ppANF-eYFP-WPRE, and plenti6-POMC-GCaMP6s-WPRE have a deletion in the 3'LTR of the viral genome that is transferred into the 5'LTR after one round of reverse transcription. This deletion abolishes transcription of the full-length virus after it has incorporated into a host cell.

The potential for oncogenesis is largely based on the specific insert contained within the lentiviral transfer vector (dependent upon whether or not it is an oncogene). pLenti6-POMC-eYFP-WPRE, pLenti6-POMC-ppANF-eYFP-WPRE, and plenti6-POMC-GCaMP6s-WPRE do not contain any oncogene and therefore the likelihood for oncogenesis is effectively null.

The original plasmids are designed to transduce cells in a POMC dependant manner, meaning only neurons or pituitary cells that express POMC will in theory express the gene insert. This promoter may be changed in the future in order to target other cell types. Similarly, the sequence of the plasmid might be altered in order to change the gene expressed (e.g. switch the reporter protein eYFP to mCherry). We will not introduce any genes that cause disease or are known to be harmful. After modifying the transfer plasmids and before making viruses, we will sequence the entire plasmid to make sure that the changes will not lead to alteration of pathogenicity, host range, tissue tropism, mode of transmission or host immune response.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Animals will be held in a Class 2 animal facility, however the risk of operator exposure from infected animals that have been inoculated with non-replicating viruses (2nd and 3rd generation lentiviruses) is minimal, as is the potential for virus shedding, and it is therefore appropriate to house these animals in the same manner as non-infected animals.
**SOP for waste management** is as follows:

- Gloves and laboratory coats/gowns will be worn when dealing with waste.
- All virus-containing waste will be treated in the following manner:

  * Virus-containing liquids will be removed using pipettes and placed directly in a container containing 1% Virkon or bleach (4.5-5% sodium hypochlorite). The waste container will contain sufficient Virkon or bleach to allow for additional liquid to be added during the procedure. A period of at least 15 minutes will elapse after the last addition of waste into the waste container, after which the liquid waste will be disposed of in the sink. The sink will be thoroughly rinsed.

  * Smaller virus-contaminated solid waste items will be placed in a container filled with 1% Virkon or bleach for 5 minutes, and then placed in a bucket containing 5% Virkon for at least one hour. The waste will then be disposed of in a biohazardous yellow bin. Larger solid waste may be sprayed with 1% Virkon or bleach, and then placed into a sealed autoclave bag in the biosafety cabinet. The bag will be sprayed with 1% Virkon; then at least 15 minutes later the bag will be taken out to be autoclaved.

  * Sharp waste will be treated with 1% Virkon (as described above for small solid waste) and then disposed of in a biohazardous sharp container.

Virkon has been tested against high titre lentiviruses. A solution (<5 days old) at a concentration of 1 % (v/w) inactivates lentiviruses within 5 minutes. All liquid waste will be treated for >15min.

**SOP for spills** is as follows:

- Wear gloves and lab coat when dealing with the spill.
- Cover the spill, including any contaminated equipment with cloth or paper towel to contain it.
- Ensure others in the area know of the spill and avoid the spill area.
- Working from the outside margins of the spill area towards the centre, apply sufficient 1% Virkon solution on top of the paper towel and soak it.
- Leave for 10 minutes to allow the Virkon to inactivate any virus present in the spill.
- Clear away the material into clear sealable plastic bags prior to disposal in the nearest yellow biohazardous waste bag, including gloves used for clean-up. If any glass or sharps are involved use of dustpan or stiff piece of cardboard to dispose of these materials into the appropriate containers.
- Complete the spill record table.

**Animal waste:**

- All virus-infected carcasses and body parts will be bagged up in clinical waste bags and stored in a freezer; these bags will be disposed of in a clinical waste bin which is collected once per week for incineration.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**

N

02/03/2022
The University of Exeter GM safety Committee have approved this risk assessment.

Please enter comments on the GM safety committee on the risk assessment

The University of Exeter GM safety Committee have approved this risk assessment.

Project Containment

<table>
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Animal Units

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Project Ref 382/22.1

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

We aim to express the f1 filamentous phage protein IV (pIV) in P. aeruginosa, with the aim of increasing the uptake of antibiotics into bacteria.

Recipient or parental organism

P. aeruginosa is an opportunistic human pathogen that can cause a variety of diseases. Most cystic fibrosis patients are chronically colonized with P. aeruginosa and it is the leading cause of death by Gram-negative bacteria. Localized infection following surgery or burns commonly results in a generalized and frequently fatal bacteremia. Urinary tract infections following surgery or burns commonly results in a generalized and frequently fatal bacteremia. Necrotizing P. aeruginosa pneumonia may occur in other patients following the use of contaminated respirators. P. aeruginosa can cause severe corneal infections following eye surgery or injury. It is found in pure culture, especially in children with middle ear infections. It occasionally causes meningitis following lumbar puncture and endocarditis following cardiac surgery. It has been associated with some diarrheal disease episodes. Since the first reported case of P. aeruginosa infection in 1890, the organism has been increasingly associated with bacteremia and currently accounts for 15% of cases of Gram-negative bacteremia. The overall mortality associated with P. aeruginosa bacteremia is about 50%. Some infections (e.g., eye and ear infections) remain localized; others, such as wound and burn infections and infections in leukemia and lymphoma patients, result in sepsis.

P. aeruginosa rarely causes disease in healthy persons. Treatment options for P. aeruginosa infections include quinolones, cephalosporins, tobramycin and polymyxins.

Host/vector system

Escherichia-Pseudomonas shuttle vector pHERD30T (1). pHERD30T contains an araBAD promoter, gentamicin resistance and a multiple cloning site.

Origin & function

To express pIV in P. aeruginosa, we will clone gene IV (gIV) from the E. coli expression plasmid pPMR132 into the Escherichia-Pseudomonas shuttle vector pHERD30T. Plasmids will be introduced to P. aeruginosa (PA14 and PA01) by electroporation, and tested for pIV protein expression using SDS-PAGE and Western blot using an anti-pIV antibody. We will plate cells onto agar containing different antibiotic strips/discs to determine MIC levels. We will also test a mutant of pIV with a Ser to Gly substitution at position 324 (pIV-S324G).


Evaluation of foreseeable effects

Transformed P. aeruginosa will contain antibiotic resistance to gentamycin as detailed above. They will also express the pIV protein, which is controlled. The protein is not a virulence factor or a toxin and there is no reason to believe that it will increase the virulence of the organism. It is more likely that pIV will make the GMO less toxic than the parent as pIV expression causes membrane stress and will potentially allow the uptake of a broader range of antibiotics.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be disposed of according to the General GM waste disposal guidance (GMG-010).

Chemical disinfection should be used routinely for decontamination of discarded liquid cultures, possibly small amounts of solid material (e.g. small sample tubes), surfaces and spillages. GM contaminated tissue culture plates, pipettes etc must either be autoclaved or chemically disinfected by soaking in 1% w/v Virkon, or 1,000 ppm Presept (four 0.5g tablets into one litre of water) for at least two hours before discharging any excess liquid via drains or autoclaved at 121°C at 1.15 bar, for 15 mins.
Autoclave function is assessed and calibrated and serviced annually to ensure continued effectiveness. The outcome is recorded in the log book.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 553/08.5

Date Ackn'd 08/08/2013  
CU2 Project Title Social evolution and virulence of Pseuomonas aeruginosa

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Transferred from GM553 08/04/2013

Historical Date of Additional Info

02/03/2022  
Page 6654 of 15326
### Project Additional Information

#### Purposes of the contained use

Social traits, such as co-ordinated behaviour, anticompetitor toxins and iron-scavenging, can be important for the success of pathogenic bacteria. We are addressing under which conditions these traits are beneficial to the bacteria, and how the expression of these traits affects their pathogenicity in animal (caterpillar) hosts. Mutation rates can affect the evolution of these traits, and we are also investigating this question.

#### Recipient or parental organism

Pseudomoas aeruginosa is a hazard group 2 pathogen which can cause infection of immunocompromised individuals, people with cystic fibrosis, and can potentially establish an infection through burns and cuts. Infections can be fatal if untreated.

#### Host/vector system

All genetically modified strains of Pseudomonas aeruginosa to be used are knockouts. The majority of which are transposon-insert mutants (with associated tetracycline resistance cassettes from Escherichia coli) obtained from external sources.

MutS knockout was made by digesting the wildtype allele, liberating an internal fragment, and then ligating. This non-functional allele, and an associated kanamycin resistance cassette from E. coli, was then inserted back into the wild type background by conjugation from an E. coli donor.

#### Origin & function

- **bacteriocin (pyocin) mutant (pys2 knockout)** - Bacteriocins cause cell lysis of susceptible P. aeruginosa strains.
- **pyoverdin mutant (pvdF knockout)** - Pyoverdin is the primary iron-scavenging siderophore of P. aeruginosa.
- **Quorum sensing (QS) mutants (lasR, rhlR knockouts) and knockouts of genes under the control of QS expression (lasB, rhlA; which encode elastase and rhamnolipid, respectively)** - QS describes changes in gene expression in a wide range of genes (such as those coding for proteases (eg elastase) and surfactants (rhamnolipids)) in response to high concentrations of diffusible signal.
- **Type IV pili mutant (pilA knockout)** - Type IV pili are involved with social motility.
- **mutS knockout** - Results in less efficient DNA repair, and hence an approximately 100-fold higher mutation rate. Strain.

#### Evaluation of foreseeable effects

- Pyoverdin mutants have reduced ability to obtain iron, hence are likely to grow less well, and are expected to be less virulent, in humans. There is no reason to suspect the growth or virulence of the pyocin mutant would be altered in human hosts.
- Quorum sensing mutants do not express a range of traits, including many traits crucial for successful growth of bacteria in vivo, such as proteases (elastase), polysaccharides for biofilm formation and surfactants (rhamnolipid). They will therefore be less virulent.
- The type IV pili mutants have reduced motility and attachment, so would be less efficient at colonising human hosts.
- The mutS mutant typically shows reduced virulence in published in vivo studies using mouse models.
- The antibiotic cassettes are not the antibiotics typically used for treatment of P. aeruginosa, hence they will not increase risks to human health.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Caterpillar carcasses - Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for incineration.

Degree of kill

- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 10% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

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### Project Ref 8/07.1

**Date Ackn'd** 15/03/2012

**Date Project Ceased**

**CU2 Project Title** Molecular Analysis of Fungal Virulence.

**Class** Class 2

**Culture Volume** 1-50 Litres

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

#### Purposes of the contained use

The aim of this project is to define, at a molecular level, the virulence attributes of fungi, in particular Aspergillus fumigatus, Aspergillus nidulans, Aspergillus flavus, Candida albicans and Candida glabrata. We also intend to investigate selected attributes in the model fungus Saccharomyces cerevisiae.

#### Recipient or parental organism

**Escherichia coli:** strains in common laboratory use e.g. DH 5 alpha, XL-20 will be used as cloning tools. These strains, which are generally derivatives of the K12 strain, have a widespread and long history of safe use. They also contain numerous mutations eg thi-1 which render the strains auxotrophic and therefore unlikely to survive outside the laboratory environment.

**Aspergillus nidulans:** there are a huge number of classically produced mutant strains of A.nidulans. These have been constructed in many strain backgrounds and often have been crossed many, many times. These strains have a long history of safe laboratory use and usually contain auxotrophic mutations eg argB that would prevent survival outside the laboratory.

**Aspergillus fumigatus:** Af237,D141 and ATCC46645, Af293 and CEA 10 and auxotrophic derivatives thereof eg pyrG or antibiotic selection-mediated gene disruption derivatives thereof eg hygromycin-resistant clones, will be used to construct knock-out, regulatable and reconstituted strains. These backgrounds have a long and safe
history of use worldwide. Additionally the pyrG auxotrophies would prevent survival outside the host.

Candida albicans: SC5314, CA14 and CA110 and auxotrophic derivatives thereof eg his3/his3, ura3 will be used to construct knock out regulatable and reconstituted strains. These strains have a long and safe history of use worldwide.

Candida glabrata: ATCC2001 and auxotrophic derivatives thereof eg his3, ura3, trp1 will be used to construct knock-out, regulatable and reconstituted strains. These strains have a long and safe history of use worldwide.

Pichia pastoris: GS115 and SMD 1163 and auxotrophic derivatives thereof eg his4 will be used to express selected fungal proteins. These strains have a long and safe history of use worldwide.

Saccharomyces cerevisiae: the majority of laboratory strains are derived from three major wild type lineages S288C, W303 and Σ1278b. Most contain multiple auxotrophies and would not survive outside the laboratory.

### Host/vector system

We will use standard E.coli and S.cerevisiae vectors eg pUC, pBluescript, YE, YI and YC series.
We will use a series of C.glabrata episomal vectors (Kitada et al, Gene 175:105, 1996)
Mobilisable Aspergillus vectors will not be used.

### Origin & function

The majority of inserted genes will be from the same species, eg used to complement a null allele to create a reconstituted strain, and therefore do not constitute the construction of a GMM.
We are investigating fungal virulence and wish to analyse all attributes associated with the ability to cause disease. Current examples of genes under investigation (and the functions that they are known, or predicted to encode) include C. glabrata ACE2 (transcription factor) C.glabrata CTS1 (endo-chitianse) and A.fumigatus PacC (transcription factor). We will also analyse other genes related to virulence in these species.

We will also construct Aspergillus and Candida DNA libraries in S.cerevisiae.
We will also add selected tags eg GFP, TAP, HA, etc to selected genes.
We will use heterologous antibiotics genes eg ampR, bleR, hygR as markers of transformation.

### Evaluation of foreseeable effects

The GMM are disabled and are unlikely to be able to survive in the environment and the modifications proposed (gene inactivation) would not confer a selective advantage even if they were to be transferred to a wild type organism. The likelihood of hazard is therefore considered to be no greater than that of the parental strains.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: Liquid waste (culture media) will be treated by the addition of Virkon to a final concentration of 1% and then left for at least 120 mins. According to manufacturer's data this treatment results in 100% killing of the GMMs. Treated liquid waste will be then disposed of via a designated sink.
Solid waste in plastic bags (tissue culture flasks, plastic pipettes, pipette tips and eppendorf tubes) will be autoclaved at 134°C for 30 mins. This will result in 100% killing of the GMMS. A chart recorder will indicate the successful completion of the autoclave cycle and the autoclave is tested by 12 point thermocouple testing. The waste will then be disposed of by the clinical waste route.

Q 1.3 not really answered. If none of the proposed GMMs are likely to be any more hazardous than the host without an insert, then this should be stated, and a justification given e.g. perhaps there are no known toxins in any of the fungal pathogens being used, or measures will be taken to specifically exclude any toxin genes from being expressed?

Reviewer 2:
2.2.1.1 PI needs to consider the worst case scenario for strains that do not contain auxotrophies.

Reviewer 3:
The hazards need to be identified and then explanations of how the risks are reduced by operational practices mentioned here.
1.21 Paragraph3: give some examples of genes being disrupted and/or replaced. Write explicitly the kind of phenotype assays that will be carried out to test the effects of the genetic modifications.
1.23 Give more details of the plasmids used for transformation of the fungi (Aspergillus sp and Candida sp). Specify the selectable markers used.
2.1.1.2 List the hazards to human health posed by the organisms used. Explain the diseases caused, not just the reasons to think that the risks are mitigated by the auxotrophies.
2.1.1.3 Give examples of the "majority" of genes. List some examples of the remaining "minority". Here is the first mention of complementation of the auxotrophies by the transforming plasmids. This appears to negate some of the mitigating effects described extensively in preceding sections. Therefore clear statements about this must appear in many of the introductory paragraphs.
2.3.2.1 In order to study the function of putative virulence genes, phenotypic assays including infection models require an infectious GMM: this will by definition be a hazard to humans and the environment. Hence this MUST be explicitly stated in the introductory paragraphs to all sections.

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**Name**

WOLFSON WOHL CANCER RESEARCH CENTRE

**Name 2**

UNIVERSITY OF GLASGOW

**Department**

RADIATION ONCOLOGY

**Campus Estate or Research Centre**

GARSCUBE ESTATE

**Building**

SWITCHBACK RD

**Town**

GLASGOW

**County**

RENFREWSHIRE

**Postcode**

G61 1BD

**Country**

SCOTLAND

**Tel Number**

0141 330 4890

**Fax Number**

0141 330 4127

**HSE Division**

SCOTLAND

**Comments**

FORMERLY CRC BEATSON LABORATORIES

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

02/03/2022 Page 6662 of 15326
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Purposes of the contained use

The aim of this project is to generate replication-defective lentiviruses that express marker genes including firefly or renilla luciferases and/or destabilised variants, GFP and related fluorescent proteins or bacterial nitroreductase under the transcriptional control of various human, viral, or synthetic gene regulatory elements including normal or site-mutated variants of the human telomerase hTR and hTERT promoters, the human p16, p 21 and CDK4 promoters and commonly used "constitutive" viral promoters. The constructs will be used to assess basal activity and, through the use of small molecules, siRNA, or transient overexpression of cell signalling genes, regulatory mechanisms of cancer and senescence associated gene promoter by reporter assay or cell ablation studies in a variety of cancer and normal cell types including primary haematopoietic and stem or progenitor cells.

Recipient or parental organism

Recipient cells will include both commonly used normal (untransformed) cell strains such as W138 and MRC5 and common cell lines that are widely used by research laboratories and are not considered to pose any inherent hazard to human health nor have the capacity to survive outside laboratory and do not harbor any adventitious agents. Examples of cell lines to be used include:
Human cancer and vitro immortalised cell lines 5637, A2780, C33A, HCT116, A549, HT29, Colo320, HeLa, Jurkat, K562, MCF7, HEK293, GM847, KMST6. Additional recipient cells include sub-clones of common cell lines such as 293T, A2780-CP70 and HCT116-p53-/- and stably transfected variants such as BJ-hTERT wherein the genetic and epigenetic traits associated with the sub-clone or transgene are not considered to increase the overall inherent hazard to human health of the cell line itself or following transduction with lentivirus. Additional recipients are to include normal or tranformed primary human cells obtained from donors with appropriate consent such as T-lymphocytes, CD34+ haematopoietic progenitors, mesenchymal stem cells and cells derived from ascitic fluid of patients displaying advanced tumours of the peritoneal cavity. Additional recipients are to include primary cells from animals obtained under appropriate license including rat primary neural cells. All such primary cells will be cultured exclusively under category II containment.

**Host/vector system**

Host Bacteria for growth of the plasmids are standard disabled laboratory strains of E.Coli such as DH5a and JM109 or the Invitrogen proprietary strains Stbl£ (F-mcrB mrr hsdS20(rB-, mB-) recA13 sup E44 ara-14 galK2lacY1 proA2 rpsL20(StrR) xyl-5 * leu ntl-1) or TOP10 (F-mcrA *mrr-hsdRMS-mcrBC *80lacZ*M15 *lacX74 recA1 araD139 *(araleu) 7697 galU galK rpsL (StrR) end A1 nupG). * replaces a symbol as symbols can't be shown

The system to be used for lentivirus construction is the Invitrogen ViraPower Promoterless Lentiviral Gateway Expression System (www.invitrogen.co, catalogue number K5910-00). The system combines gateway cloning and lentiviral cloning technologies for construction of a 3rd generation lentivirus pseudotyped with the G Glycoprotein gene from vesicular stomatitis virus. The system allows incorporation of any transgene and gene regulatory element in the lentiviral vector. The system comprises:

1. The pENTR 5'-TOPO® TA Cloning Kit for production of a gateway entry clone containing a gene/promoter of interest> The vecotr contains no sequences necessary for virus production.
2. A promoterless pLenti6/R4R2/V5-DEST destination vector into which the promoter and gene of interest are transferred. This expression plasmid contains elements that allow packaging of the construct into virions.
3. Additional packaging plasmids pLP1, pLP2, and pLP/VSVG. And 293FT packaging cells. The packaging cells will be 293FT which will be used for the initial rescue of the virus from transected DNA and for general virus propagation and will also express the marker genes of interest.

Vector features necessary for viral production:

- **pLenti6/R4R2/V5-DEST:**
  - Rous Sarcoma Virus (RSV) enhancer/promoter for Tat-independent production of viral mRNA in the producer cell line.
  - Modified HIV-1 5' and 3' Long Terminal Repeats (LTR) for viral packaging and reverse transcription of the viral mRNA (Note: The U3 region of the 3' LTR is deleted (*U3) and facilitates self inactivation of the 5' LTR after transduction to enhance the biosafety of the vector.
  - HIV-1 psi (*) packaging sequence for viral packaging.
  - HIV Rev response element (RRE) for Rev-dependant nuclear export of unspliced viral mRNA.
  - HIV gag/pol coding sequences encoding genes required for structure and replication of the virus.
  - HIV Rev response element (RRE) for rev-dependent expression of gag/pol genes.

- **pLP1:**
  - HIV Rev OTF under transcriptional control of the RSV promoter encodes the Rev protein, which interacts with the RRE on pLP1 to induce gag and pol expression, and on the pLenti6/V5 expression vector to promote the nuclear export of the unspliced viral RNA for packaging.

- **pLP2:**
  - HIV Rev OTF under transcriptional control of the RSV promoter encodes the Rev protein, which interacts with the RRE on pLP1 to induce gag and pol expression, and on the pLenti6/V5 expression vector to promote the nuclear export of the unspliced viral RNA for packaging.

- **pLP/VSVG:**
  - VSVG coding sequence under control of the CMV promoter encodes the envelope G glycoprotein from Vesicular Stomatitis Virus to allow production of a pseudotyped virus.

The system comprises numerous biosafety features designed to produce a self-inactivating, replicacation defective lentivirus with a negligible risk of homologous recombination between the different plasmid elements. Risk of mobilisation is therefore negligible.

- A deletion in the 3' LTR (*U3) results in "self-inactivation" of the lentivirus after transduction of the target cell.
- The number of genes from HIV-1 that are used in the system has been reduced to three (gag, pol, and rev).
- The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope.
Genes encoding structural and other components required for packaging are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent recombination events. None of the packaging plasmids contain LTRs of the packaging sequence. Thus, none of the HIV-1 structural genes are present in the packaged viral genome. The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced. Expression of the gag and pol genes is prevented in the absence of Rev.

A constitutive promoter (RSV promoter) has been placed upstream of the 5' LTR in the pLenti6/R4R2/V5-DEST vector to offset the requirement for Tat.

Origin & function

No protein is expected to be expressed in bacterial strains used for plasmid propagation. The marker gene products will be expressed in the transfected cells used for virus propagation and in the target cells. All of the protein coding gene sequences to be used in this study have previously been widely used in similar studies and none are considered to present a significant hazard to human health. Renilla and firefly luciferases derived from commercially available pGL3 or pGL4 (firefly) or phRG orpRL vector families (Promega) encode reporter proteins which catalyse luminescent conversion of the substrates luciferin and coelenterazine.

Nitroreductase (NTR) is an E.Coli derived flavoprotein which catalyses the reduction of a variety of nitroaromatic small molecules such as the nontoxic substrate CB1954, which is converted to a toxix bifunctional alkylating agent in NTR expressing cells. NTR has been used by several laboratories for enzyme/pro-drug gene therapy applications and cell ablation studies and has been safely expressed in human tissues in clinical trials.

Fluorescent proteins such as GFP (green fluorescent protein), or related proteins CFP, RFP, and YFP are used widely as reporter proteins to mark cells in which transgene is expressed on exposure of transfected cells to appropriate wavelengths of light.

The hTR and hTERT promoter sequences direct expression of the encoded transgenes exclusively in cells with telomerase activity such as cancer cells or some stem cells. Senescence associated gene promoters p16 and p21 are expected to limit transgene expression to cells undergoing senescence. Viral promoters such as SV40 or CMV will be used as positive controls and are expected to direct expression of the encoded transgenes in all cells.

Evaluation of foreseeable effects

The final genetically modified cells will contain stably integrated DNA copies of the lentiviral vectors encoding the transgene expression cassettes. The cells will be free of lentivirus. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside the laboratory culture. Nevertheless, it will be important to prevent human exposure or environmental release of the replication defective lentiviruses which are used to transiently effect gene transfer. The worse case scenario in case of infection is at a site that disrupts expression of a gene necessary for tumour suppression. Risk against this will be reduced through the use of appropriate physical containment and inactivation processes. Hazard containment II is therefore recommended and all work will be carried out in Class II biological safety cabinets.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk to human health it will be essential to prevent exposure to retrovirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Class II containment laboratories, with access restricted to authorised staff. The amount of retrovirus or lentivirus handled will be limited to no more than 25ml of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will...
result in 100% inactivation of infectious virus. Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area. Positive clones will be expanded and checked for virus production before removal from the category II laboratory. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells or assay of reverse transcriptase activity. Once these criteria are met the infected target cells will be removed from Class II containment and handled using standard tissue culture processes.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

The proposal was reviewed and approved as Class II at the Safety Committee Meeting on the 13th May 2008

Project Containment

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Project Ref 25/13.1

Date Ackn'd: 29/04/2014  
CU2 Project Title: The construction and evaluation of adenovirus vectors as therapy for cancer

Date Project Ceased: 01/01/2018  
Class: Class 2  
Culture Volume: < 1 Litre  
Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work
Project Additional Information

Purposes of the contained use

The aim of this project is to amplify wild-type and deletion mutant oncolytic adenovirus vectors already produced in the applicant's previous lab; to evaluate their anti-tumour efficacy in in vitro and in vivo models of human malignancy, particularly ovarian cancer.

Adenovirus serotypes Ad5, Ad11 and Ad35 will be used as reference standards for work performed with Ad5 (mainly E1A) deletion mutants.

Replication competent and replication defective mutants will be constructed using the 'Ad-Easy' system to infect permissive human cell lines. These new mutants may encode transgenes that could induce tumour cell killing alone or enhance the anti-tumour properties of replication-competent deletion mutants.

Evaluation of viral efficacy of mutants will be performed on Human and Murine cell lines by a variety of methods.

Viral manufacture of replication defective vectors will be performed in HEK293 cell line to provide the E1 'in-trans'.

Concentration of adenoviral constructs from 750ml final cultures will be via Caesium Chloride centrifugation.

Recipient or parental organism

The recipient cell lines HEK293 or A549 will be used. Both are commercially available cell lines which require specific laboratory culture and as such pose negligible risk to humans.

HEK 293 is rated Biosafety level 2 because of an E1 fragment of adenovirus embedded in it. It is this fragment that is essential to this series of experiments with E1 deletion adenoviral mutants.

Cell line A549 is rated Biosafety level 1.

Host/vector system

One vector system will be oncolytic deletion mutants of Adenovirus. Replication of oncolytic deletion mutants of Adenovirus is attenuated in normal cells/tissue.

Multiple mechanisms for this attenuation exist, depending upon the nature of the deletion. Publications attest to the selectivity of replication –between normal human cells compared to a panel of human malignant cells – see Heise et al Nature Med (2000) 6:1134. In addition, multiple phase I and II trials of oncolytic adenovirus have shown no replication in normal tissues.

Wild-type adenoviral vectors, used as reference standards, are replication competent and could infect humans. However, wild-type adenovirus infection in humans with an intact immune system leads to a mild and self-limiting 'flu-like illness.

Origin & function

Mammalian genes are to be inserted into the adenovirus genome and have been chosen to perform their intended function; either to increase tumour-specific killing by the virus (e.g. tumour suppressor or apoptosis-inducing genes), to inhibit the activity of oncogenic pathways or to increase the immunogenicity of infected tumour cells.
Documentation exists to indicate that this can be achieved safely. No wild-type oncogenes or direct toxins will be inserted.

The virus will be applied to various established human and murine ovarian carcinoma cell lines; obtained from ATCC and other reputable sources and have consequently been screened for bloodborne viruses. In addition, the human cell lines have also all been previously STR verified and are known to be mycoplasma-free.

**Evaluation of foreseeable effects**

The deletions induced in adenovirus vectors are designed to narrow the host range, so that there is reduced replication and killing within normal cells, thus increasing the therapeutic index in ovarian cancer cells. Mechanisms to achieve this will include introduction of specific deletions to restrict replication and the use of tumour and tissue-specific promoters.

During process development for clinical grade manufacture of one of the mutants being used, no recombination between vector and HEK293 sequences was detected (limit of detection 1 in 109 genomes) – thus it is believed that the risk of reversion to wild-type virus following amplification in HEK293 cells is extremely low.

Replication-deficient viruses are incapable of replication in cells lacking adenovirus E1 function. Infection of normal human cells as well as malignant cells results in no infectious virion production.

The replication competent adenoviruses used will contain mutations within the viral genome that attenuates replication in normal cells and tissues. The deletions occur in regions of the viral genome that are necessary for replication in normal cells but are complemented by the altered gene expression in cancer cells. Mutants used can only replicate efficiently in cells with deregulated p53 and pRB pathways respectively.

The tumour selective mutants can infect normal cells but the ability to replicate is highly attenuated.

Wild-type adenoviral vectors are replication competent and could infect humans. Wild-type virus infection in humans with an intact immune system leads to a mild and self-limiting 'flu-like illness.

The normal route of adenovirus infection is via aerosol. The use of correctly trained and supervised staff, ClassII biological safety cabinets, lab coats and gloves, disinfection and autoclaving of waste will reduce the likelihood of exposure to an extremely low level.

The virus will be applied to various established human and murine ovarian carcinoma cell lines. Because ovarian cancer cells do not contain any adenovirus genomic DNA, there is no risk of recombination of deletion mutants to generate wild-type revertant virus. Thus, generation of adenoviruses and infection of ovarian cancer cells is not considered to pose any inherent hazard to human health. The GM viruses do not have the capacity for survival outside of the laboratory. The recipient cells will therefore not have any foreseeable effect on human health.

E.coli strains are highly attenuated laboratory strains and do not present any hazards to humans.

Human adenoviruses do not replicate in murine tissue - this is true of wild-type, replication-restricted oncolytic viruses and replication-defective E1-deleted vectors.

The only replication following injection into in vivo tissue will be in malignant tumour tissue.

All injections of virus into vivo tissue will be undertaken in class II hoods within the specialist unit, thus minimising the risk of an aerosol-mediated infection during the injection process.

Multiple human studies demonstrate that virus shedding after intraperitoneal, intravenous or intratumoural injection is undetectable. Thus, the risk to humans from murine cell line tissue is negligible as the viruses are replication-deficient or tumour specific and the gene products are not considered harmful to health.
Full containment level 2 will be used, as set out in the Regulations, including appropriate treatments for bulk, contaminated solid waste.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be used, as set out in the Regulations; i.e. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level II laboratories, with access restricted to authorised staff. including appropriate treatments for bulk, contaminated solid waste.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All viral supernatants and spent medium will be inactivated using 10,000 ppm free chlorine before removal from the Containment Level II laboratories for final disposal. This process will result in 100% inactivation of infectious virus.

Solid waste will be bagged in biohazard bags prior to removal from the Containment level II lab and then placed in boxes which are sealed prior to removal from the CL2 suites. Boxes remain sealed and are autoclaved before the contents is removed for collection by accredited waste contractors. Autoclaves produce a digital record of load temperature achieved and are validated by annual thermocouple mapping. This process will result in 100% inactivation of infectious virus.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Questions were raised and answered regarding management of containment during centrifugation of virus

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Project Ref 383/02.1

Date Ackn’d 11/06/2002  
CU2 Project Title GENE TRANSFER USING DISABLED ADENOVIRUS VECTORS CONTAINING  
Class 2  
CultureVol Class 2 < 1 litre  
CultureVolumeClass3-4

02/03/2022
The purpose of this study is to develop replication-defective disabled adenovirus vectors for the transfer of enzyme-prodrug systems regulated by telomerase gene promoter sequences. The telomerase gene promoters targets and restricts expression of the cancer therapeutic enzyme-prodrug systems to cancer cells. The telomerase targeted adenovirus vectors will be examined for their potential to express the enzyme-prodrug system in cell lines. The long-term aim is to use adenovirus vectors for the treatment of cancer.

The cells to be used are immortalised human cancer cell lines and human epithelial and haematopoietic cells. Human cells in culture are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive autonomously outside of the laboratory. It is not anticipated that the proposed modification will alter these properties. The final GMO cell lines will therefore not exert any foreseeable effects on either human health or on the environment.

The vectors under development are based on a commercially available replication-defective disabled adenovirus vector system based on adenovirus serotype 5(Ad5). The Adeasy adenovirus vector is deleted for E1 and E3 virus sequences rendering the virus replication defective. The virus is unable to replicate except in a complementing cell lines such as 293 cells (a human embryonic kidney cell line which expresses the left 11% of the Ad5 genome). The replication defective vector can be considered unlikely to cause disease but will be classified in hazard group 2. This combined with the rigorous waste inactivation procedure and inherent fragility of adenoviruses means that there are no foreseeable effects on either human health or on the environment.

The genetic material to be introduced into the cell lines consists of adenovirus vectors containing cancer specific therapeutic genes. It is anticipated that these studies will lead to a better understanding of cancer development and lead to new therapeutic options for cancer treatment.

The replication defective adenovirus vectors carrying the non-toxic inserts of cancer therapeutic genes are unlikely to be hazardous to humans. The possibility of exposure to recombinant viruses generated by recombination with wild type virus needs to be considered but the inserts are non-toxic and so are unlikely to represent an increased hazard over wild type virus. Careful attention will be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation. This combined with the rigorous waste inactivation procedure and inherent fragility of adenoviruses means that there are no foreseeable effects on either human health or on the environment.
human health or on the environment. Level 2 containment will be implemented using biological safety cabinets.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk to human health it will be essential to prevent exposure to adenovirus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Class 2 containment laboratories, with access restricted to authorised staff. The amount of adenovirus handled will be limited to no more than 25 ml of virus stock with the titre range of 10^10 to 10^11 particles/ml. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation unless essential, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Precept tablets before removal from the Class 2 Laboratories for autoclaving and final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from Class 2 laboratories and autoclaved prior to disposal. Cells will be cultured using designated bottles of culture medium that will not be used to passage other cell cultures. Unused medium will not be removed from the Class 2 laboratories without inactivation with Precept tablets. Authorised staff will wear dedicated lab coats in the Class 2 laboratories that are not worn in the general laboratory area.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

The Risk Assessment pertaining to this notification was discussed in detail and approved at a meeting of the Cancer Research UK Beatson Laboratories Safety Committee held on 7th November 2001.

Project Containment

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Animal Units

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Project Ref 383/09.1

Purposes of the contained use

Replication-deficient lentiviral vectors (pFG12 vector) will be used to deliver the activated oncogenes RasV12 and BRAFV600E to primary human cell lines to induce senescence. This strategy will be used to further analyze the senescence pathway.

A replication-deficient retroviral vector (pBABE-puro) will also be used to deliver the activated oncogene RasV12 to the human fibroblast cell line IMR-90 (ATCC Number CCL-186) in order to induce senescence. This strategy will be utilized in order to investigate mechanisms of the senescence pathway.

Recipient or parental organism

The GMM will be transfected into 293FT cells in order to produce virus and the virus will then be applied to primary human cell lines, such as fibroblasts, melanocytes and keratinocytes, which are widely employed in laboratories and have all been screened for HIV, Hep B, Hep C and EBV. There is no risk from the cells infected. Cells are typically obtained from ATCC.

The GMM (pBABE-puroRasV12) will be transiently transfected into Phoenix cells, a 293T-derived packaging cell line that contains the Gag, Pol and Env viral proteins required for making active virus. Subsequently, viral supernatants produced by Phoenix cells will be used to infect human fibroblasts (IMR-90). Both the Phoenix packaging cell line and IMR-90 fibroblasts are used extensively in laboratories and have been screened and found to be negative for HIV, Hep B, Hep C and EBV. There is no risk from the infected cells. Cells are obtained from ATCC and have been rigorously characterized.

Host/vector system

The ViraPower Lentiviral Expression System (Invitrogen) uses a pLenti expression vector that contains a deletion in the 3' LTR that results in "self-inactivation" of the lentivirus after transduction of the target cells. When integrated into the target cell the lentiviral genome is therefore no longer capable of producing packagable viral genome. This third generation vector system is based upon a HIV-1 lentivirus, although it is lacking all HIV-1 genes except gag-pol and env, which are provided in trans by
a transient transfection strategy. Also, the virus produced is to be pseudotyped with VSV-G envelope protein rather than HIV-1. The vector requires three other packaging plasmids that allow expression in trans of proteins required to produce viral progeny (e.g. gag, pol, rev, env) in the 293FT producer cell line. These packaging plasmids separately allow for the expression of gag and pol, a RRE-containing sequence, and the rev protein sequences from HIV-1. None of the packaging plasmids contain LTRs or the Ψ packaging sequence, and therefore, none of the HIV-1 structural genes are actually present in the packaged viral genome so they are never expressed in the transduced target cell. No new replication-competent virus can be produced. (Dull, et al., J. Virology, 8463-8471, 1998).

The pBABE vector system is a retroviral vector construct that has been derived from Moloney murine leukemia virus (MMLV). As with lentiviral constructs, the pBABE retroviral vector also requires three additional packaging plasmids that drive expression in trans of proteins required to produce viral progeny (i.e. gag, pol, rev and env) upon transfection into a producer cell line (Phoenix). The pBABEpuro vector has been designed to avoid homology between the vector and packaging constructs, in order to decrease the chance of homologous recombination in the Phoenix packaging cells.

Origin & function

The Ras and BRAF genes belong to the MAP kinase pathway, which mediates cellular responses to growth signals. When constitutively activated through mutation (RasV12 and BRAFV600E) senescence is conferred through the upregulation of the tumor suppressors p53 and p16INK4a. Activating BRAF mutations are present in up to 80% of melanomas while mutations in the ras gene are very common in adenocarcinomas of the pancreas (90%), the colon (50%), and in myeloid leukemia (30%). Although these gene products are very common in human cancers, additional mutations are required to drive a malignancy. Also, the hazards of the activated oncogene are very minimal due to the inability of the 293FT and Phoenix cells to create a replication-competent virus. In addition to the inserted oncogenes, the carrier plasmid (FG12) contains the woodchuck hepatitis virus posttranscriptional regulatory element (WRE) to increase the level of transcription. Although controversial, WRE may have oncogenic activity but should not pose a hazard due to the safety precautions that are already built in for the activated oncogenes.

Evaluation of foreseeable effects

It is not anticipated that the inserted sequences will alter the host-range or tissue tropism of the virus. Some of the genes we wish to express will oncogenic proteins. Current knowledge indicates that the de-novo transformation process of human cells requires the perturbation of six-pathways (Rangarajan et al, Cancer Cell, 2004 (2):171-83) and therefore manipulation of individual pathways is not expected to confer a serious cancer risk to normal human cells. None of the genes we wish to manipulate encode toxins.

Hence, it is unlikely that replication defective viruses would be effective carcinogens in humans in the short term. Single oncogenes are not generally considered sufficient to convert primary human cells to full malignancy, and replication defective viruses are not capable of dissemination even in a permissive host, however there is potential for harm to human health in the event of infection. It will however, be important to prevent human exposure or environmental release of the replication defective adenoviruses which are used to transiently to effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes.

The possibility of exposure to recombinant viruses generated by recombination with wild type virus needs to be considered and may, although unlikely, cause pathological effects in humans. Careful attention will, therefore, be placed on containment and control measures that minimise aerosol production and opportunity for recombination and complementation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk to human health it will be essential to prevent exposure to virus. Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level 2 laboratories, with access restricted to authorised staff. The amount of virus handled will be limited to no more than 40ml of virus
stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. No needles or sharps will be used during virus preparation, whilst the use of the Class II biological safety cabinet will guard against exposure to aerosol. In addition, cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm chlorine) before removal from the Class II laboratories for final disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class II laboratories and autoclaved prior to final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Class II laboratories that are not worn in the general laboratory area.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The project was approved as Class II by the Safety Committee in December 2008

Project Containment

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Project Ref 383/12.1

Date Ackn'd 16/07/2012

Date Project Ceased

Investigating the role of apoptosis, mitochondria and autophagy in cancer through the retroviral generation of stably expressing cell lines

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CU2 Project Title

02/03/2022  Page 6675 of 15326
### Project Additional Information

#### Purposes of the contained use

Retroviral vectors will be used to produce retrovirus in order to generate a variety of human and murine, normal and cancer cell lines stably expressing the below proteins. These will be used to study apoptosis, autophagy and mitochondria in relation to cancer development and treatment response.

#### Recipient or parental organism

Producer cells: Phoenix Ecotropic and Phoenix Amphotropic packaging cells These were obtained from Dr. Gary Nolan, University of Stanford and are extensively used for retroviral generation.

Target cells: Hela, MCF-7, SVEC 4-10, NIH3T3, L929, NIH SA, SV40 transformed murine embryonic fibroblasts, U20S, HT90, Caco-2, Jurkat, These cell lines are widely used by research laboratories, are well characterized, and have been obtained from either ECACC or ATCC and subsequently been screened for human pathogens including HIV, HBV, HCV and EBV. They do not harbour adventitious agents. They are not considered to pose any inherent hazard to human health nor do they have the capability to survive outside the culturing environment of the laboratory.

#### Host/vector system

Retroviral vectors: pBabe puro, pBabe hygro, pLZRS ires zeo, PMX ires GFP, pRsuper retro

The vectors are again widely used by research laboratories and have been developed to provide the viral package signal, transcription and processing elements, and a target gene. The Packaging cells, into which the vectors are inserted, provide the envelope components.

#### Origin & function

- **Inserts:**
  - **Apoptosis related:** Bcl-XL, Mcl-1, tBid, PUMA, Bax, Bad, Bak, Bfl-1, Mcl-1, Bcl-2, Caspase-3, DD Caspase 3, Caspase-9, Caspase-8, RIP3K, RIP1K, FADD, TNFR-1, Trail, Fas, PGAM5, Drp-1, Cytochrome-c GFP, Smac mCherry, Omi mCherry, caspase activity FRET reporter probe, Parkin, PINK1
  - **Autophagy:** mCherry LC3, eGFP LC3, eGFP mCherry LC3, eGFP p62, mCherry p62, Atg7, Atg5, Atg12, DD Atg5
  - **Mitochondrial:** mito matrix dsRed, mito matrix YFP, mitochondrial outer membrane targeted GFP, mitochondrial intermembrane space targeted Cre recombinase

#### Evaluation of foreseeable effects

Hazards associated with target/recipient cell lines
Phoenix Ecotropic or Phoenix Amphotropic cells are extensively used for retroviral generation. The target cells have been sourced from either ECACC or ATCC and subsequently been screened for human pathogens including HIV, HBV, HCV and EBV. They are widely used within and outside the Institute and are unable to survive outside a laboratory environment. Consequently, the cells should not have any foreseeable effects on human health or the environment.

Hazards associated with the vector system and inserted gene products

pBabe puro/pBabe hygro (Addgene), pLZRS zoe (Addgene), pMX ires GFP (Addgene), pSuper Retro (Addgene) are all replication incompetent retroviral vectors that allow production of retroviruses encoding proteins or shRNA targeting proteins of interest. Stable cell lines will be selected with puromycin (pBabe puro/pSuper retro), zeocin (pLZRS zeo), hygromycin (pBabe hygro) or sorted for GFP expression (PMX ires GFP).

Using the Phoenix cells and the above plasmids, viral progeny are only produced following transfection of the plasmid encoding the gene of interest (because this provides the viral packaging signal in trans). Produced virus are replication incompetent, since they lack various genes essential for viral replication. The genes, including Gag-Pol and Env are provided in trans by the Phoenix packaging cell line.

None of the gene inserts to be used are known oncogenes in themselves. Pro-apoptotic proteins such as Bax and Bak can only kill cells upon massive overexpression, it is not anticipated that retroviral transduction will attain the required levels. Many of the fluorescent fusion proteins are used solely as reporters, with no known toxic or oncogenic properties. None of the gene inserts to be used are expected to alter viral tropism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus generation and infection of target cells will be carried out in Class II biological safety cabinets in the Containment Level II laboratories, with access restricted to authorized and trained staff. The amount of retrovirus handled will be limited to no more than 5ml of virus stock. The most likely routes through which individuals could be exposed to virus are needle-stick injuries or aerosols generated during experimental procedures. Plastic ware will be used throughout and no needles or sharps will be used during virus preparation. The use of the Class II biological safety cabinet will guard against exposure to aerosols.

All cell cultures will be contained within clearly labelled boxes during incubation to prevent accidental spillage of medium. All viral supernatants and spent medium will be inactivated using Presept tablets (10,000 ppm free chlorine) before removal from the Containment Level II laboratories for final disposal.

Solid waste will be double bagged in biohazard bags prior to removal from the Containment Level II laboratory and then autoclaved before final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Cells will be cultured using designated bottles of medium that will not be used to passage other cell cultures. Authorised staff will wear dedicated lab coats in the Containment Level II laboratories that are not worn in the general laboratory area.

Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by infection of appropriate indicator cells and assays of reverse transcriptase activity. Only once these criteria have been met will the infected target cells be removed from Containment Level II laboratory and handled using standard tissue culture procedures.
The project was approved as Class II by the Safety Committee on 12 June 2012.

### Project Containment

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### Project Ref 383/14.1

**CU2 Project Title**

The evaluation of oncolytic herpes simplex virus vectors as therapy for cancer

**Class**

- Class 2

**CultureVolume**

- Class 3-4

- ≤ 1 Litre

**Non-GMM Consent Granted**

- Not Applicable

**Date Ackn’d**

- 18/03/2014

**Date Project Ceased**

- 01/01/2018

**Withdrawn**

- N

**Historical Significant Changes**

- Project Transferred to GM31 on 01/01/2018

**Historical Date of Additional Info**

- 

**Significant Change ID**

- 

**Date of Significant Change**

- 

02/03/2022
# Project Additional Information

## Purposes of the contained use

The aim of this project is to evaluate the therapeutic potential of selectively replicating ('oncolytic') Herpes simplex virus 1 vectors in human cancers, especially ovarian cancer. Specifically, we aim to evaluate the pathways of cell death activated by oncolytic HSV in ovarian cancer and the role of innate and adaptive immune responses in modulating therapeutic efficacy of oncolytic HSV1 vectors.

## Recipient or parental organism

- Human ovarian cancer cells; murine ovarian cancer cells; African green monkey kidney cells

## Host/vector system

- HSV-1

## Origin & function

All vectors will be based upon the vector 1716, which is HSV-1 deleted in the neurovirulence gene ICP34.5. 1716 itself has no inserted viral gene products. There are also derivatives of 1716 encoding GFP, Firefly luciferase and RFP. Further double deletion mutants (eg ICP34.5 and ICP47) with increased selectivity for malignant cells may also be evaluated.

## Evaluation of foreseeable effects

Deletion of ICP34.5 dramatically reduces neurovirulence. Although 1716 remains capable of replicating within malignant cells, replication is dramatically attenuated in normal neuronal tissue, as confirmed in clinical trials of 1716 and other ICP34.5-deleted vectors. Risk to human health is therefore effectively zero. Parental Wild-type HSV1 causes fatal encephalitis in mice. However, previous data show that 1716 is avirulent in mice, including by direct intracranial injection. There is no reason to suspect that 1716 has enhanced environmental survival factors or altered host range compared with HSV-1, which is already present in the environment. Overall risk to organisms other than humans and to the environment is therefore effectively zero.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

To minimise the risk to the laboratory staff all work will be undertaken within dedicated MSC class 2 cabinets within class 2 tissue culture facilities. All materials will be decontaminated in 10,000 ppm chlorine (e.g. Virkon, Chlorsept) before removal from MSC class 2 cabinets and autoclave-mediated destruction. Virus is stored at -80°C in cryovials. Following defrosting on ice, cryovials are not opened until placed within MSC class two cabinets, thus minimising the risk of spillage within the lab. In the case of accidental spillage either within or without the MSC class two cabinet, all materials will be decontaminated with 10,000 pm chlorine.
For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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<th>Disinfection</th>
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<tr>
<td>All materials potentially contaminated with 1716 will be decontaminated in 10,000 ppm chlorine (Chlorsept) before removal from MSC class 2 cabinets.</td>
<td>All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving, using the following regime; gradual pulsing of temperature and pressure to final pressure of 3040mBar and temp of 134°C - 39 mins, sterilisation cycle @ 3040mBar, 134°C - 20min, Pressure venting - 1min drying and pressure vent – 30s, air wash, pressure vent and cooling - 3mins, air Break - 1min, cooling - 11mins (100% kill), prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile. Following disinfection and autoclaving, all waste will be incinerated.</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment

The project was assessed as Class 2 by the GM383 Safety Committee in February 2014.

## Project Containment

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**Project Ref** 383/14.2
Construction and use of retro- and lentiviral vectors to express normal or mutated proteins and shRNA constructs in primary and immortalized murine/human cells (Ref:25/09.1)

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**Project Additional Information**

**Purposes of the contained use**

The project will utilise retro- and lentiviral vectors to deliver shRNA, reporter genes and dominant negative constructs directed against autophagy, metabolism and epigenetic pathways to human and murine cells including leukaemic stem cells and other tumour cell lines.

**Recipient or parental organism**

Murine/human leukaemic cell lines, solid human tumour cell lines, human fibroblast cell lines, murine stem- and progenitor cells and primary human blood cells.

**Host/vector system**

Replication deficient retro-and lentiviral vector system.
Additional vectors to project 25/09.1:
Retroviral vectors: pBabe, pWZL, MIGR-1, pSUPER.Retro.
Lentiviral vectors: pGIPZ, pLKO.1-puro, pLKO.1-GFP, pLenti-CMV.
Packaging plasmids: p-CMV-HIV1 and psPAX (encode for Gag-encoded capsid proteins, Pol-encoded reverse transcriptase and integrase enzymes) and VSV-G (encodes for glycoprotein envelope).

**Origin & function**

Retroviral system: The majority of retroviral vectors have been derived from oncogenic retroviruses, such Moloney murine leukemia virus (MoMLV), that efficiently infect actively dividing cells. The viral env gene, produced by the package cell line, encodes the envelop protein, which determines the viral infectivity range. Transfection into a package cell line, such as HEK239FT (a human embryonic kidney cell line transformed with adenovirus E1a and carrying a temperature sensitive T antigen; Invitrogen) or HEK293T derivatives Phoenix-Eco or Phoenix-Ampho (second-generation retrovirus producer lines for the generation of helper free ecotropic and amphotropic retroviruses; Orbigen Inc.) produces high-titer, replication-incompetent viruses. pBabe, pWZL, MIGR-1 and pSUPER.Retro vector systems contain the bacterial origin of replication, antibiotic-resistant (puromycin, hygromycin or neomycin) gene for the growth of infected cells to select stable cell lines.
Lentiviral system: Lentiviral vectors have become widely used due to their ability to infect non-dividing cells, which gives them an advantage over retroviral vectors for certain applications. pGIPZ (Open biosystems), pLKO.1 (Open biosystems/Sigma) and pLenti-CMV (Addgene) are replication-incompetent lentiviral vectors chosen for expression of shRNAs (pGIPZ/pLKO) or mRNA (pLenti-CMV). This lentiviral system allows for transient and stable transfection of RNA and also production of viral particles using lentiviral packaging cell lines, such as HEK293FT. pGIPZ, pLKO.1-puro and pLenti-CMV contain antibiotic resistance marker (puromycin) and pLKO.1-GFP contains fluorescent (GFP) selectable marker that allows selection of stable cell lines.

As stated above, the project will utilise retro- and lentiviral vectors to deliver shRNA, reporter genes and dominant negative constructs directed against human and murine cells including tumour cell lines.

Evaluation of foreseeable effects

The retro- and lentiviral system includes a number of safety features designed to enhance its biosafety and to minimize its relation to the wild-type virus. The reverse transcriptase and integrase proteins are split from the native Gag-Pol polyprotein structure and are provided 'in trans' on a separate plasmid. Removing the reverse transcriptase and integrase from the packaging construct prevents the viral replication machinery from functioning. This system prevents the generation of recombinant viral particles that possess the required functional Gag-Pol structure for DNA mobilization and the emergence of replication competent virus. The probability of reversion to the wild type is effectively zero.

Although the packaging and envelope (p-CMV-HIV1/psPAX2/pVSV-G) plasmids allow expression in trans of genes required to produce viral progeny (e.g. gag, pol, rev, tat, env) in the HEK293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. This ensures that the retro- and lentiviral vectors are unable to replicate and infect surrounding cells, thus making them safe to use in the laboratory without any risk of viral infection to cells in the environment.

The potential routes of transmission of the virus are known; retro- or lentivirus may possibly be delivered to epithelial cells through accidental inhalation, skin exposure, or oral ingestion; or accidentally to the bloodstream via a wound in the skin. Although the risk of infection to humans is effectively zero, it is important to prevent human exposure or environmental release of the replication defective viruses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Amphotrophic retro- and lentiviruses are capable of infecting a range of mammalian species, however the viruses are replication defective and therefore incapable of causing infectious disease.

All liquid and solid waste will be inactivated by treatment with precept tablets and/or autoclaving prior to disposal to ensure that live virus does not come into contact with the environment. Furthermore, retro- and lentiviruses are fragile and unlikely to survive in the environment for sufficient time to encounter a susceptible host.

Combined with the containment measures to protect human health, these considerations indicate that the risk posed to the environment by the proposed activity is effectively zero.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection
All materials potentially contaminated with retro- or lentivirus will be decontaminated in 10,000ppm chlorine in solution (e.g. Chlorsept) before removal from laminar flow hoods.

Autoclaving
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving, using the following regime; gradual pulsing of temperature and pressure to final pressure of 3040mBar and temp of 134 °C - 39 mins, sterilisation cycle @ 3040mBar, 134 °C - 20min, Pressure venting - 1min drying and pressure vent –
30s. air wash, pressure vent and cooling - 3mins, air Break - 1min, cooling - 11mins (100% kill), prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Following disinfection and autoclaving, all waste will be disposed of as clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project was assessed as Class 2 by the GM383 Safety Committee in April 2014.

**Project Containment**

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**Project Ref** 383/16.1

Date Ackn'd: 27/07/2016

CU2 Project Title: In-depth characterisation of central nervous system leukaemia

Class: Class 2

Culture Volume: Class 3-4

Class Culture Volume: Class 2 < 1 Litre

Non-GMM: Consent Granted

Project notified under transitional arrangements N

Withdrawn: N

Tick if notifying a connected programme of work N
Our group is focused on the unravelling of acute lymphoblastic leukaemia (ALL) survival mechanisms when cancer cells escape primary tumour sites (bone marrow) and reach secondary sites such as the central nervous system (CNS). In order to better understand the underlying mechanisms, we intend to either knock down (KD) or knock out (KO) key genes in fatty acid/lipid synthesis cascade (and associated pathways) in established human leukemic cell lines SEM (DSMZ:ACC-546) or REH(ACC-22) and study the biological consequences of those KDs or KOs on the tumour cell survival.

Constructs will be amplified in non-pathogenic E.Coli (DH5alpha or Stbl3) bacteria strain. Viral particles will be produced in cell lines such as HEK293T (human kidney cells, ATCC® CRL-1573™). Human leukemic cell lines such as SEM (DSMZ:ACC-546) or REH(ACC-22) will be infected with viral particles.

Replication deficient lentiviral vector systems such as: 2nd generation Tet-ON PLKO-1 euro, or later generation vector systems. Packaging plasmids (for production of viral particles) such as: psPAX2 (Addgene#12259) and VsVg (Plasmid #8454 Addgene).

Packaging plasmids (for production of viral particles) such as: psPAX2 (Addgene#12259) and VsVg (Plasmid #8454 Addgene). Plasmids such as Tet-ON PLKO.1-puro-based plasmids subcloned with shRNA or CRISPR construct against SCD1 and/or other genes in related metabolic pathways (PLKO-backbone from Addgene Plasmid #21915).
Evaluation of foreseeable effects

The retro/lentiviral system we are going to use includes a number of safety measures both to increase biosafety and minimise the biological risks to experimenters/third-party humans and the environment. It is replication incompetent. The reverse transcriptase and integrase proteins are split from the original Gag-Pol polyprotein structure and are provided on a separate plasmid, which prevents the viral replication machinery from functioning. This thus prevents the emergence of a fully functional replication competent virus. Although the packaging and envelope (psPAX2, pVsVg) plasmids allow trans expression of genes necessary to the production of viral particles (gag, pol, tat, rev) in the HEK293T producer cell line, none of them contains LTR packaging sequences. That means that none of the structural genes are present in the packaged viral genome and are ever expressed in the transduced target cell. This ensures that retro/lenti viruses are very unlikely to replicate and infect surrounding cells, thus making relatively safe to use in the laboratory without any risk of viral infection to cells in the environment. Retro and lentiviral vectors will encode shRNAs or CRISPR constructs (targeting specific mRNAs involved in fatty acid and lipid synthesis and catabolism, along with related pathways). The final genetically modified cells will contain stably integrated DNA copies of the viral vectors encoding the transgene expression cassette but will be free of virus. As a result they will pose no hazard to human health or the environment, since they are very unlikely to colonise humans or survive outside the laboratory culture. In addition the insert targets proteins with no known oncogenic potential or suspected physiological, pathological and or pharmacological effect. Although the risk of infection to humans is relatively low, it is important to prevent human exposure or environmental release of the replication defective viruses which are used transiently effect gene transfer. The worst case scenario in case of infection is chance integration at a site that disrupts expression of a gene necessary for tumour suppression. However, there is only very slight chance that this could occur.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials will be decontaminated in 10,000 ppm chlorine in solution (E.g. Virkon) before removal from laminar flow hoods autoclave-mediated destruction. Virus may be stored at -80 degrees in cryovials. Following defrosting on ice, cryovials are not opened until placed within a Class II biological safety cabinets, thus minimising the risk of spillage within the lab. In the case of accidental spillage either within or without the Class II biological safety cabinets, all materials will be decontaminated with
10,000 ppm chlorine solution.
Disinfection:
All materials potentially contaminated with lentiviruses will be decontaminated in 10,000 ppm chlorine solution before removal from the laminar hood.

Autoclaving:
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving, using the following regime: gradial pulsing of temperature and pressure to final pressure of 3040mBar and temperature of 134 degrees Celsius: 39 mins, sterilisation cycle at 3040mBar and temperature of 134 degrees Celsius, 20 mins, Pressure venting, 1min drying and pressure vent, 30s air wash pressure vent and cooling, 3mins air break, 1min, cooling 11 min (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of temperature/time profile.

Waste Disposal:
Following disinfection and autoclaving, all waste will be treated as a clinical waste.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This project was assessed as Class 2 by the GM383 Safety Committee in June 2016

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Animal Units</th>
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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<th>CultureVolumeClass3-4</th>
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<td>02/03/2022</td>
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Production of retro- and lenti-viral vectors for transfection of patient derived cell lines (PDCLs) from a pancreatic cancer cohort

Project Additional Information

Purposes of the contained use

Different genetic inserts will be used throughout the project. Some will encode shRNA constructs designed to interfere with the mRNA of genes suspected to facilitate PC progression, while others will contain those genes themselves to be inserted into the host genome. Although these gene inserts may be considered oncogenic, they are primarily likely only to contribute to cancer progression within the context of PC, therefore induction of expression itself is highly unlikely to transform infected tissue. Inserts that code for fluorescent reporter genes such as GFP and RFP will also be used, with each insert containing additional antibiotic selection markers. No viral particle generated will be replication-competent, with very little chance of reversion to replication competency, and genetically modified host cells will be free from virus after initial exposure period, limiting the risks involved in handling culture material.

Genes to be repressed via shRNA or overexpressed via cDNA expression include those known to regulate epigenetic components within the cell, such as SWI/SNF complex member, ARID1A and EZH2, a subunit within the polycomb repressive complex 2 (PRC2) methyltransferase, as well as transcriptional regulators such as HNF1A, NR4A1, FAK.

Recipient or parental organism

Three sets of PC PDCLs, all derived from human tissue and generated at various cancer research centres, will be exposed to viral infection over the course of this project. The majority of cell-lines were established The Kinghorn Cancer Centre (TKCC), Australia, obtained from patient derived xenografts (PDXs). Additional cell-lines involved were generated via the outgrowth method at the Technische Universität Dresden, Germany, as well as derived from tissue samples from patients grown directly on collagen at the Mayo Clinic, USA. All PDCLs involved in the project have been cleared for use within a Containment Level 1 environment.

Host/vector system

The DH5α strain of E. coli will be used to amplify plasmid vectors, and HEK293T cells will be transfected with expression vector + packaging system plasmids to produce non-replicating virus particles from the pLenti-C-mGFP-P2A-Puro expression vector and a 2nd or 3rd generation 2-vector packaging system: pMDLg/pRRE, encoding Gag and Pol, and pRSC-Rev, encoding Rev. PC PDCLs established by external collaborators as previously described will be exposed to virus/viral vectors.

2nd Generation Packaging Vector: psPAX2, encoding Gag, Pol, Rev and Tat viral proteins.

3rd Generation Packaging Vectors: pMDLg/pRRE, encoding Gag and Pol, and pRSC-Rev, encoding Rev.

3rd Generation Transfer Vectors: pLenti-siRNA-GFP, pLenti-puro and pLKO.1 puro, all encoding LTRs (with truncated 5’ LTR), Psi region and custom genomic insert.

The viral vectors involved in this project are comprised of two primary genetic components, a transfer vector and a packaging system. Transfer vectors allow for the production of proviral cDNA strands modified to contain specific genes, while the packaging system contains genes that facilitate the generation of viral particles. 3rd generation vector systems separate packaging elements across two plasmids, thus further limiting the possibility of reversion to replication competency in the event of recombination. Packaging cells that are induced to express both constructs, as well as a viral envelope protein, are therefore able to generate infectious, non-replicating virus particles carrying those genes of interest that will be inserted into the host genome postinfection.

Evaluation of foreseeable effects

The DH5α E. Coli strain is highly attenuated, and does not present hazards to humans. The viral particles produced by the packaging cell line are non-replicative, and have been constructed to prevent any possibility of reversion to wild-type virus. Use of a 3rd generation packaging system splits the genes capable of generating infectious viral particles in trans across separate plasmids to limit the possibility of a reversion event, and packaging plasmids lack any Psi packaging and LTR sequences. This renders the lentivirus particle replication-deficient upon infection of host cells, as the only inserts which are incorporated into the host genome are present in a separate transfer vector. Only a recombination event that transfers all essential packaging genes, as well as the additional VSV-G envelope gene onto the transfer vector could lead to replication-competent virus, which is highly unlikely to occur in 3rd generation system where packaging genes are split across multiple plasmids.

Origin & function

The viral vectors involved in this project are comprised of two primary genetic components, a transfer vector and a packaging system. Transfer vectors allow for the production of proviral cDNA strands modified to contain specific genes, while the packaging system contains genes that facilitate the generation of viral particles. 3rd generation vector systems separate packaging elements across two plasmids, thus further limiting the possibility of reversion to replication competency in the event of recombination. Packaging cells that are induced to express both constructs, as well as a viral envelope protein, are therefore able to generate infectious, non-replicating virus particles carrying those genes of interest that will be inserted into the host genome postinfection.

Initial experiments are planned to assess effects of overexpressing epigenetic modulators, such as ARID1A and EZH2, as well as transcriptional regulators such as HNF1A, NR4A1, FAK. Evidence suggests that these epigenetic/regulatory genes may act as either tumour suppressors or oncogenic drivers in a contextual dependent manner, and thus overexpression alone is highly unlikely to be transformative.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full level 2 containment will be used as set out in the Regulations:
Virus generation and infection of target cells will be carried out in Class 2 biological safety cabinets in Containment Level 2 environments, with access restricted to authorised staff.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All viral work will be carried out within Class II biological safety cabinets in order to maintain sterile tissue culture conditions, which will incidentally reduce the possibility of accidental exposure to and limiting existing routes of transmission of viral particles. All materials used within safety cabinets will be decontaminated via disinfection before removal from the cabinet, thus preventing any chance of viral escape. In the case of any accidental spillages of medium containing virus particles, any potentially infected surfaces and materials will be decontaminated with Virkon solution. All viral supernatants and spent medium containing viral particles will be inactivated using 10,000 ppm free chlorine before removal from Containment Level 2 laboratories for disposal. All contaminated solid materials will be bagged in biohazard bags prior to removal from Containment Level 2 lab and placed in boxes which are sealed prior to removal from the CL2 suites. Boxes will remain sealed until contaminated waste can be inactivated by autoclaving using the following regime: gradual pulsing of temperature and pressure to a final temperature of 134°C and pressure of 3040 mBar for 39 minutes; 20 minute sterilisation cycle at 3040 mBar and 134°C; 30 seconds of pressure venting; 3 minute air wash, pressure vent and cooling; 1 minute air break; 11 minutes cooling (100% kill). This is prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclave activity is validated by annual thermocouple mapping and each run is monitored by digital recording of the temperature/time profile. Once disinfected and autoclaved, waste generated will be disposed of as by accredited waste contractors.
This project was assessed as Class 2 by the GM383 Safety Committee in November 2018 and signed by PI and GMBSO in May 2021.

Please enter comments on the GM safety committee on the risk assessment

This project was assessed as Class 2 by the GM383 Safety Committee in November 2018 and signed by PI and GMBSO in May 2021.

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GM Centre Number: 384

Data Premises Notified (Originally) 24/08/1990
Transferred from 1992 Regs? Y
Transitional Premises Class 2
Data Premises Closed 04/06/2008
Transitional Premises Emergency Plan Required? N
Non-GMMs Y
Withdrawn N

Name
INSTITUTE FOR ANIMAL HEALTH

Name 2
MRC NEUROPATHOGENESIS UNIT

Department
NEUROPATHOGENESIS UNIT

Campus Estate or Research Centre
OGSTON BUILDING

Building

District

Road Name
WEST MAINS RD

Town
EDINBURGH

County
EAST LOTHIAN

Postcode
EH9 3JF

Country
SCOTLAND

Tel Number 0131 667 5204
Fax Number 0131 668 3872

E-mail

HSE Division SCOTLAND

Comments

Date at Which Additional Info Submitted
02/03/2022
## Premises Addresses

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<th>Building</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Other (please specify) Tick if confidential

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<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tbody>
<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment.

Tick if you are claiming exemption from disclosure for sections of the risk assessment.

Please enter comments of the GM safety committee on the risk assessment.

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**Project Ref 384/04.1**

- **Date Ackn'd**: 22/06/2004
- **CU2 Project Title**: Polymorphisms in the human PrP gene and susceptibility to prion diseases
- **Class CultureVolClass2 CultureVolumeClass3-4**: Class 2 < 1 Litre
- **Non-GMM Consent Granted**: Yes
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: No
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: Yes

**Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

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02/03/2022
### Purposes of the contained use

The purpose of the contained use is to assess whether polymorphism of the human PrP gene are controlling susceptibility and resistance to prion diseases, also known as Transmissible Spongiform Encephalopathies of TSEs. The variants in question have altered amino acid codons and have been found in individuals affected by TSEs but it remains to be proven that there is a direct causal link between the codon change and the TSE disease. The details of the study are confidential in order to protect intellectual property and a successful project may result in a patent application so commercial confidentiality is also claimed. Disclosure would adversely affect the competitive position of the Institute for Animal Health in a highly competitive area of research. Exemption from disclosure is allowed by the Environmental Information Regulations 1992 as amended in 1998 and the relevant exemption is intellectual property protection and commercial confidentiality.

### Host/vector system

For the laboratory cloning steps, a section of the mouse PrP gene cloned into pBluescript vector will be subjected to in vitro mutagenesis to introduce the required nucleotide changes. The newly created sequences will be cloned into plasmid pBluescript. All constructs will be maintained in disabled E. coli bacterial cultures. Details of the constructs and cloning steps are claimed as confidential. Exemption from disclosure is allowed by the Environmental Information Regulations 1992 as amended in 1998 and the relevant exemption is intellectual property protection and commercial confidentiality.

### Origin & function

The origins of the genetic material involved are cloned sections of the mouse PrP gene already available in this laboratory from other projects. The intended function of the mutagenised cloned sequences is to make gene targetted transgenic animals expressing a single copy of the transgene under the control of the endogenous PrP gene promoter. The details of this project are claimed to be confidential exemption from disclosure is allowed by the Environmental Information Regulations 1992 as amended in 1998 the relevant exemption is intellectual property protection and commercial confidentiality.

### Evaluation of foreseeable effects

The GMMs and human health:

**Hazard identification**

It would be theoretically possible for a laboratory worker to swallow the Ecoli containing human disease associated amino acid codons within the mouse PrP gene. The plasmid pBluescript II is not a prokaryotic expression vector and in combination with the fact that the inserted mouse PrP gene fragment is of large size it is very unlikely that any PrP protein is expressed in these Ecoli and therefore for this altered PrP protein to appear in the gut. If despite this assessment altered PrP protein would be produced and released into the gut, the hazard would be very low as it has been shown that recombinant PrP made in Ecoli is not infectious (Scott et al 1988 Protein Engineering 2: 69-76) Those who believe TSEs (or prion diseases) are caused by an infectious protein put this down to misfolding and non-glycosylation of the Ecoli expressed protein however recombinant PrP expressed in mouse cells (Caughey et al 1988 PNAS 85: 4657) or monkey COS cells (Scott, et al, idid) or converted in vitro to protease resistant form (Hill et al 1999 J Gen Virol 80: 11-14) are also not infectious.

**Capacity to survive in the human gut.**

The bacteria to be used are recombination deficient so would not be expected to transfer plasmids to other bacteria or to be able to compete successfully with wild-type bacteria.

**Assessment of likelihood - low.**

There are extensive measures in force at the Institute for Animal Health in Edinburgh to protect staff from any infection with TSEs. All TSEs are treated in the same way in order to make sure procedures are remembered and followed diligently. CJD and BSE in addition, are handled at containment level 3 at the moment.

**The minimal containment level within the building is level 2.** Some closed, locked and access restricted rooms are used for work with CJD and BSE. These are higher containment than level 2 without being full level 3.
Briefly: No eating or drinking is allowed in laboratory areas
No mouth pipetting is allowed
Protective clothing (lab coats, aprons if necessary) is worn all the time
Disposable gloves are worn, eye protection is worn
Any procedures which could generate aerosols are carried out in safety cabinets
Visors are used if splashing is possible
Closed systems are used for mixing, homogenisation, centrifuging etc
Plastic disposables are used whenever possible in preference to sharps
Waste is disposed of by treating with 20,000 ppm chloros or autoclaving at 134C for 1 hour and followed by removal in hermetically sealed waste bins for incineration
Dedicated rooms are used for procedures involving CJD and BSE infectivity.
Records are kept of all accidents.
A Quality Assurance system is being set up with IAH to closely monitor all procedures, experiments and safety controls.

Assessment of consequence - High
Although expected to be unlikely, if any infectivity is generated by Ecoli containing human disease associated amino acid codons in the mouse PrP gene, this would not be expected to infect humans because of the safety measures in place. However, the consequences might potentially be grave if it did.

Estimation of risk - low
The measures in place to protect human health and safety are well above level 2 containment standards.

The GMMs and Environmental Risk
Hazard Identification
The Ecoli used to replicate the plasmid constructs containing human disease associated amino acid codons within the mouse PrP gene inserts could be discharged from the labs through the drains or in lab waste and affect animals and people in the environment. The plasmid pBluescript II is not a prokaryotic expression vector and in combination with the fact that the inserted mouse PrP gene fragment is of large size it is very unlikely that any PrP protein is expressed in these Ecoli and therefore for this altered PrP protein to appear in the environment. If the altered mouse genes were to be expressed in the Ecoli and this could enter and colonise the gut of animals or people, it might be that the altered mouse PrP protein would be expressed in the gut as well. However, this worst case scenario is highly unlikely as the Ecoli strain used are highly disabled and unable to compete with wild type bacteria. In addition recombinant PrP made in Ecoli is not infectious (Scott et al 1988 Protein Engineering 2: 69-76) Those who believe TSEs (or prion diseases) are caused by an infectious protein put this doen to misfolding and non-glycosylation of the Ecoli expressed protein however recombinant PrP expressed in mouse cells (Caughey et al 1988 PNAS 85: 4657) or monkey COS cells (Scott, et al ibid) or converted in vitro to protease resistant form (Hill et al 1999 J Gen Virol 80: 11-14) are also not infectious.
More details are presented with the risk assessment.

Capacity to survive in the environment.
The Ecoli strain used is recombination deficient and should not be able to trans fer plasmids to other bacteria, nor would it be expected to survive in competition with other wild type bacteria. If, however unlikely, an infectious agent was to be generated in the Ecoli cells, it may persist in the environment for long periods.

Assessment of likelihood - low
Escape of Ecoli containing human disease associated amino acid codons in the mouse PrP gene is considered highly unlikely. All bacterial cultures are treated with 20,000 ppm chloros prior to disposal or autoclaved at 134C for 1 hour. These treatments are the reliable methods of inactivating TSE agents. All nucleic acid preparations would be considered to be non-infectious due to the use of phenol or guanidinium in the preparative procedures. Any protein preparations would not leave the building unless treated as above to inactivate any infectivity. Waste then leaves the building in hermetically sealed, tamper proof clinical waste bins for incineration.

Assessment of consequence - Low
Human TSE diseases (CJD and GSS) are very rare - worldwide incidence less than 1 in a million new cases per year. There is no evidence of contagious spread of TSEs in humans and no evidence of maternal transmission. Any bacteria which found their way out of the labs would be dead because of standard decontamination procedures. Any protein remaining within them would be at low levels as high expressing promoters would not have been used.

Estimation of risk - low
The standard measures in place will protect environmental, human and animal health and safety.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**GM animals:**
Institute for Animal Health, Edinburgh is housed in a building with restricted and controlled access at the entry doors (computerised key locking system). The SPF barrier animal house is highly secure with restricted and controlled access at the entry doors. Doors to the animal house are locked with entry only via computer controlled unlocking or number coded locks. Containment of animals is routinely very high for the reason of prevention of contamination of other experiments and animals are housed in a range of facilities all with at least level 2 containment. The mouse is regarded as a closed system with regard to prion diseases but dissections are performed in safety cabinets. For the present study, animal tissues would be prepared and analysed in closed locked dedicated laboratories with higher containment than level 2 (but not full level 3). Disposal of carcasses: autoclaving at 134 C for 1 hour then removal for incineration in hermetically sealed bins. Our standard inactivation procedures reduce TSE infectivity to undetectable levels and containment and decontamination procedures are monitored for compliance. IAH is at the moment in the process of setting up a QA system to cover all activities from experiments to waste disposal.

**GM ES cells:**
Embryonic stem (ES) cell culture is performed in a dedicated locked laboratory with access restricted to essential personnel. Work is performed using disposable plastic equipment wherever possible. All liquids are treated with 20,000 ppm chloros. Tissue culture flasks, plates and falcon tubes (15 ml or 50 ml) which have been in contact with ES cells (targeted or untargeted) are sealed with parafilm and placed in waste bags which are removed from the tissue culture suite by a designated operator and autoclaved at 134C for 1 hour. The waste is then removed for incineration in hermetically sealed. Our standard inactivation procedures reduce TSE infectivity to undetectable levels and containment and decontamination procedures are monitored for compliance. IAH is at the moment in the process of setting up a QA system to cover all activities from experiments to waste disposal.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
No derogation is claimed for the cloning steps of the project using GMMs.

It is not clear to us where on this form to ask for Derogation for GMOs which are not micro-organisms, so we do so here and in the confidential section 17 which contains the details of the reasoning and the experimental design. Derogation to Class 2 containment is requested for the embryonic stem cell stage of the project and the transgenic mice. Confidentiality is claimed for the details of this request. Exemption from disclosure is allowed by the Environmental Information Regulations 1992 as amended in 1998 and the relevant exemptions are intellectual property protection and commercial confidentiality.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
All bacterial cultures are treated with 20,000 ppm chloros prior to disposal or autoclaved at 134C for 1 hour. These methods kill E.coli bacteria. These treatments are also reliable methods of inactivating TSE agents to undetectable levels. All nucleic acid preparations would be considered to be non-infectious in terms of TSE infectivity due to the use of phenol or guanidinium in the preparative procedures which inactivate protein (Rowher RG (1991) Current Topics in Microbiology and IMMunology 172: 195-232). Any protein preparations would not leave the building unless treated as above to inactivate any infectivity. Bacterial culture plates, ES cell plates and mouse carcasses are autoclaved at 134C for 1 hour prior to disposal. Waste then leaves the building in hermetically sealed, tamper proof clinical waste bins for incineration.

**Is an emergency plan required according to regulation 20?**

If yes, tick to confirm that it is attached to this form

02/03/2022

Page 6695 of 15326
This proposal was discussed in detail during 2 separate meetings of the GM safety committee and the proposers provided relevant additional information as requested. The committee members were concerned about various procedures to ensure they were sufficiently contained. The committee members were happy that all safety measures were in place for other projects already. The local N PU Safety officer is part of the GM committee. Staff are entitled to join a union and are represented by appropriate safety officers. Full details of the GM committee deliberations are presented in the confidential sections of this form. Confidentiality is claimed on the grounds of intellectual property protection and commercial confidentiality. These exemptions are allowed under the Environmental Information Regulations 1992 as amended in 1998.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 Conf L3 L4 L2 L3 L4</td>
<td>L2 Conf L3 L4</td>
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Project Ref 384/94.1

Date Ackn’d

14/03/1994

CU2 Project Title

THE PRODUCTION AND USE OF MICE CARRYING PrP PRO-LEU "102" MUTATION

Date Project Ceased

04/06/2008

Class

Class 2

Class CultureVolClass2 CultureVolumeClass3-4

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

GM384/96.4, GM384/99.1

Historical Date of Additional Info

30/04/1996, 04/02/1999

Significant Change ID
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment
# Project Ref

**384/94.2**

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**CU2 Project Title**

- **01/07/1994**
- **THE PRODUCTION OF MICE CARRYING MUTATIONS IN THE PrP GENE WHICH WILL PREVENT GLYCOSYLATION OF THE PrP PROTEIN**

**Class**

- **Class 2**

**CultureVolClass2**

- **not applicable**

**CultureVolumeClass3-4**

- **Y**

**Date Project Ceased**

- **04/06/2008**

**Withdrawn**

- **N**

**Tick if notifying a connected programme of work**

- **N**

**Historical Significant Changes**

- **N**

**Historical Date of Additional Info**

- **N**

**Significant Change ID**

- **N**

**Date of Significant Change**

- **N**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

- Class 2

**Host/vector system**

**Origin & function**

02/03/2022
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<thead>
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Animal Units

| L2                    | L3           | L4           |
| L2                    | L3           | L4           |

Large Scale Activities

| L2                    | L3           | L4           |
| L2                    | L3           | L4           |

Human Clinical Applications

| L2                    | L3           | L4           |
| L2                    | L3           | L4           |

Project Ref 384/97.1

Date Ackn’d 01/04/1997  CU2 Project Title DEVELOPMENT OF MOUSE MODELS FOR THE STUDY OF HUMAN  Class 2
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<td>L2 L3 L4 L2 L3 L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 384/97.2

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<td>Class 2</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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02/03/2022
Project Ref 384/97.3

CU2 Project Title
STUDY OF SHEEP PRP GENE VARIANTS IN TRANSGENIC MICE

Class 2

Non-GMM

Consent Granted
not applicable

Project notified under transitional arrangements Y

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
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**Project Ref** 384/trans1

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Date Project Ceased
04/06/2008

Tick if notifying a connected programme of work
N

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements
Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 384/trans2

Date Ackn’d 23/02/2001

CU2 Project Title CHALLENGE OF MICE WITH SINC RELATED PRP GENE ALTERATIONS

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

| Laboratory Activities | Glass Houses | Growth Rooms |

02/03/2022
### Project Ref 384/trans3

<table>
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<th>Date Ackn’d</th>
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**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 384/trans4

Date Ackn’d 23/02/2001
CU2 Project Title ANALYSIS OF PUTATIVE SCRAPIE ASSOCIATED NUCLEIC ACIDS
Class 2
CultureVolClass2
CultureVolumeClass3-4

02/03/2022  Page 6709 of 15326
**Date Project Ceased**

04/06/2008

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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GM Centre Number: 386

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### Premises Addresses

<p>| Date Premises Closed | Name                           | Department                      | Name 2                          | Campus Estate or Research Centre | Building          | Road Name        | District | Town       | County   | Post-code | Country | Withdrawn |
|----------------------|--------------------------------|---------------------------------|---------------------------------|----------------------------------|------------------|-----------------|----------|------------|----------|-----------|---------|-----------|----------|
|                      | KINGS COLLEGE LONDON           | SCHOOL OF MEDICINE             |                                 | DENMARK HILL CAMPUS              | BESSEMER ROAD     | LONDON          | GREATER LONDON | SE5 9PJ   | ENGLAND    | N        |          |         |           |
|                      | KINGS COLLEGE LONDON           | SCHOOL OF MEDICINE             | KINGS COLLEGE HOSPITAL          |                                   | DENMARK HILL      | LONDON          |          | SE5 9RS    | ENGLAND  | N         |         |           |
|                      | KINGS COLLEGE LONDON           | SCHOOL OF MEDICINE AND DENTISTRY | THE RAYNE INSTITUTE             |                                   | 123 COLD HARBOUR LANE | LONDON          |          | SE5 9NU    | ENGLAND  | N         |         |           |
|                      | KINGS COLLEGE LONDON           | KINGS COLLEGE HOSPITAL         | DULWICH                         |                                   | EAST DULWICH GROVE | LONDON          |          | SE22 8QF  | ENGLAND  | N         |         |           |
|                      | KINGS COLLEGE LONDON           | INSTITUTE OF PSYCHIATRY        |                                 |                                   | DENMARK HILL      | LONDON          |          | SE5 8AF    | ENGLAND  | N         |         |           |
|                      | KINGS COLLEGE LONDON           | WATERLOO SITE                  |                                 |                                   | 150 STAMFORD ROAD | LONDON          | GREATER LONDON | SE1 9NN   | ENGLAND    | N        |          |         |           |
|                      | KINGS COLLEGE LONDON           | GUY'S HOSPITAL                 |                                 |                                   | THOMAS GUY HOUSE, GUYS TOWER &amp; NEW GUY'S HOUSE | LONDON BRIDGE |          | SE1 9RT    | ENGLAND  | N         |         |           |</p>
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**Premises Conditions**
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Please enter comments of the GM safety committee on the risk assessment

### Project Ref 235/07.1

<table>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<td>Analysis of proteins involved in neurodegenerative diseases using viral vector delivery to the nervous system.</td>
<td>Class 2</td>
<td>1-50 Litres</td>
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#### Withdrawn

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#### Historical Significant Changes

PROJECT TRANSFERRED ON MERGER OF GM235 WITH GM 386

#### Project Additional Information

**Purposes of the contained use**

The studies proposed are based on the use of recombinant viral vectors for gene delivery in vitro in primary neuronal cultures to investigate the pathogenic mechanisms involved in neurodegenerative disorders, including Alzheimer's disease and amyotrophic lateral sclerosis (ALS). Two viral delivery systems will be used: lentiviral vectors and adeno-associated viral vectors to over-express proteins encoded by genes mutated in familial forms of AD and ALS or protein accumulating in affected neurons, as well as modifying enzymes and interacting partners and to silence the expression of the above proteins using RNA interference.

**Recipient or parental organism**

*E. coli - K12 or B derivatives (e.g. SURE 2, Stratagene and DB3.1 ccdB gene tolerant cells). Human HEK 293T cells for packaging, NIH3T3 cells, HT1 080 cells or equivalent for virus titration. Experiments will be conducted in nonneuronal (e.g. COS-7 and CHO cells) of neuronal (e.g SH-SY5Y and N2a neuroblastoma cell lines as well as in primary rat or mouse neuronal cultures (cortical neurons, motor neurons, dorsal root ganglion neurons, Purkinje cells), including neurons derived from transgenic mice*

**Host/vector system**

The inserted genetic material will be cloned into the plasmids: pAMICBA-pl-WPRE-BGH for adeno-associated virus construction or into the self-inactivating lentiviral plasmids: pLenti-DEST1TOPO or p LentilOx3.7. Genes encoding the components required for packaging the viral genome are separated onto three plasmids for the

02/03/2022
lentiviral vectors (pLP1, pLP2, pLPNSVG). The lentiviral plasmids do not contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus.

**Origin & function**

Full-length and truncated cDNA (Homo sapiens and rodents) or cloned genomic fragments encoding the protein known to be mutated or accumulating and aggregating in neurodegenerative diseases as well as sequences designed to silence these proteins by RNA interference. Some examples are listed below.

- Tau, a neuronal microtubule-associated protein that stabilises axonal microtubules. Mutations in tau cause a form of fronto-temporal degeneration.
- Sodium calcium exchanger, NCX2, Sodium calcium exchanger, calpain cleaved, NCX3
- Kinases: GSK3alpha and beta, Casein kinase 1, CdkS and its activators, CPRK family
- ALS2/alsin, a protein that contains several domains characteristic of GTP exchange factors. Mutations of the ALS2 gene are linked to juvenile familial forms of amyotrophic lateral sclerosis (ALS).
- VAPB (vesicle associated protein B —mutated in familial ALS) and its interacting partners.
- Standard reporter genes (e.g. GFP, Luciferase, β-galactosidase) will also be already present or cloned into these vectors.
- Superoxide dismutase 1 (SOD1) mutations in which cause familial ALS

**Evaluation of foreseeable effects**

Transformed E.coli: No significant hazards have been identified. Insertion of the foreign sequences into E coli is not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the host or normal human defence mechanisms. The resulting GMM’s are not expected to carry any additional risks compared to that of the un-modified recipients.

Virtually transfected HEK293FTIT cells could pose a risk to human health associated with the production of recombinant infectious lentiviral particles. However, as they are self-inactivating replication deficient vectors the risk is only from the insertion of the genetic material, described above. None of the genetic inserts are likely to cause significant effect on an individual as their potential toxic effect is limited to neurons have no known oncogenic properties.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware e.g. pipettes, flasks, tubes) — soaked in hypochlorite solution before autoclaving using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids placed in a clinical waste bag for incineration by external contractors.

Liquids (e.g. samples, culture supernatants, tissue culture media) — Disinfectant - Trigene left overnight, then dispose of to drain with running water

Safety cabinet - will be washed down with Trigene solution after use.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids in a clinical waste bag for incineration by external contractors.

Sharps (e.g. needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical disinfection with Trigene, used according to manufacturers instructions under standard conditions,
manufacturers validation (e.g. 99.998 % kill).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The assessment was reviewed by the Institute of Psychiatry Biological and GM Safety Committee in their September 2007 meeting. No concerns were raised and it was approved for notification to HSE.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L3 L4</td>
<td>L2</td>
</tr>
<tr>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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Project Ref 235/08.1

Date Ackn'd 04/03/2014
CU2 Project Title Functional evaluation of genes relevant to psychiatric disorders, neurodegeneration, neurological disease and neural development by virus-mediated gene delivery to neural cells.
Class Class 2
CultureVolClass2 1-50 Litres
Consent Granted Not Applicable
Non-GMM Project notified under transitional arrangements N

Withdrawn N
Tick if notifying a connected programme of work N
Historical Significant Changes PROJECT TRANSFERRED ON MERGER OF GM235 WITH GM 386
Historical Date of Additional Info

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
The project involves the construction of replication incompetent viruses containing DNA sequences that encode four human transcription factors. These transcription factors will be introduced into primary human somatic cells with the objective of reprogramming them into induced pluripotent stem cells (iPSC). The resulting iPSC lines will be subjected to various differentiation protocols to convert them into desired somatic cell lineages. Of particular interest will be iPSC lines made from primary cells obtained from patients suffering from a range of monogenic and polygenic diseases. The research is expected to shed light on the pathophysiology of early human disease development as well as providing cellular reagents for drug screening and discovery. Two viral delivery systems will be used: lentiviral and retroviral vectors. The transcription factors in the lentiviral vectors will be under the control of doxycycline-inducible promotors.

Recipient or parental organism

Human embryonic kidney packaging cells for making infective lenti- or retro-viruses. A variety of human primary somatic cell cultures of which skin fibroblasts will make up
Host/vector system

Lenti-x Expression System (Clontech) In this lentiviral system, the final vector [assembled through independent transformation of packaging cells (HEK293T cells) with three plasmids] is a VSV-G pseudotyped, replication incompetent retrovirus derived from HIV-1. The virus does not encode Tat and the inserted transcription factor (Oct4 or sox2 or Klf4 or c-myc) is under the control of doxycycline. The presence of added doxycycline will be needed for activation of the inserted transcription factor (Oct4 or sox2 or Klf4 or c-myc). pLVX-Tight-Puro Vector from Clontech is needed to mediate the doxycycline control.

pCMV-VSVG are all retroviruses containing the inserted DNAs indicated. In order to assemble infectious retroviruses, these DNAs and two other plasmids are introduced into human 293T packaging cell lines to create a VSV-G pseudotyped, replication incompetent retrovirus derived from the Moloney Murine Leukemia Virus.

All assembled viruses can infect human and mouse cells. The pMX and pLVX retroviral constructs have ampicillin resistance for bacterial selection. pLVX also has puromycin resistance for mammalian cell line selection.

HEK293T cells will be used for packaging.

Origin & function

Full length human cDNAs encoding the transcription factors Oct4, Sox2, Klf4, c-myc, and Sall4 have been selected. The first four of these will be used to reprogram cells. Sall4 has been included because there are rumours that it alone can reprogramme cells. However this technology is moving very fast and it is eminently possible that fewer and different human gene inserts will be required in the future. New and even safer vector delivery systems are being developed and we would like to suggest that HSE permission, if granted, will also cover new vector systems and unspecified human sequences that can reprogram human somatic cells efficiently (subject of course to the caveat that any changes will receive the approval of the local risk assessment committee and will be notified to HSE).
The Committee had minor comments which have been covered in the signed risk assessment form.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Large Scale Activities

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Human Clinical Applications

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Project Ref 235/93.1

Date Ackn'd: 04/03/2014

CU2 Project Title: INVESTIGATION OF THE BIOLOGICAL PROPERTIES OF PRION PROTEIN & ITS ROLE IN SPONGIFORM ENCEPHALOPATHIES

Class: Class 3

Consent Granted: Yes

Non-GMM: Yes

Withdrawn: N

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

Historical Significant Changes: PROJECT TRANSFERRED ON MERGER OF GM 235 WITH GM386

Project Additional Information

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? ☒

If yes, tick to confirm that it is attached to this form ☒

Tick to confirm that you have attached a risk assessment to this form ☒

Tick if you are claiming exemption from disclosure for section of the risk assessment ☒

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

02/03/2022
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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- **Animal Units**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Large Scale Activities**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Human Clinical Applications**: L2 L3 L4 L2 L3 L4

**Project Ref**: 295/01.3

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</table>

- **Non-GMM**: Consented
- **Consent Granted**: not applicable
- **Projects notified under transitional arrangements**: Yes

**Historical Significant Changes**
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Prion protein is a normal component of many of our cells (especially neurons) that has the unusual ability to change its conformation, either spontaneously or in response to genetic mutation or to prion infectious agent, to an amyloidogenic form that resists proteolysis, accumulates in the brain and appears to be capable by itself of propagating infection. While recent studies have been increasingly successful in replicating elements of this conformational conversion using recombinant prion protein modified in the test tube, no one has produced an infectious agent except within a living cell, suggesting that the full conversion from normal to infectious protein requires the action of some cellular process. The trafficking of prion protein from the cell surface into endosomes is a candidate for such a cellular process since the acidification that occurs in endosomes is used by other proteins (e.g., diphtheria toxin) and viruses (e.g., flu) to change their conformation, enabling the latter types of agent to fuse with the membrane and enter the cell.

Curiously, there is not a strong correlation between the accumulation of this infectious form of protease-resistant prion protein, and neural degeneration. Recently it has become apparent that prion protein is capable, during its biosynthesis, of being incorporated into the membrane, not only by its major mechanism (linkage to the luminal surface of the endoplasmic reticulum by a glycosylphosphatidylinositol (GPI) anchor), but also by utilisation of an internal cryptic transmembrane sequence. The proportion of prion protein that is incorporated via this alternative route, which is increased by some mutations that in man predispose to familial CJD, has been found to correlate better with neurotoxicity than has the accumulation of amyloidogenic prion protein in fibrils.

The underlying cause of neuropathology and toxicity in each case is aberrant trafficking of prion protein. Similar faults in protein trafficking are increasingly becoming to
be seen to cause other amyloid diseases such as Alzheimer's.

The purpose of the contained use is to enable us to mutate residues (or groups of residues) within normal mouse prion protein to enable us to study particular aspects of the trafficking of this protein, and in particular its translocation into the endoplasmic reticulum and its recycling between the cell surface and endosomal compartments. The mutations will be assessed firstly for their effect on the trafficking of the protein, either in transfected cell lines (N2a cells) or in simplified cell-free systems (microsomal or endosomal preparations); mutations that change trafficking characteristics will then be evaluated for their interaction with added mouse scrapie agent (from non-BSE derived strains).

Recipient or parental organism

The parental organism (original source of inserted cDNA was normal (non-infected) mouse brain.

The recipient organisms are:
1. XL2-Blue, derived from XL1-Blue, multiple auxotroph, classified as disabled or non-colonising (ACGM Compendium of Guidance, Part 2A, Annex II, Section 11).
2. N2a mouse neural cell line, maintained without harm or escape in many labs since its derivation in 1969.

Host/vector system

XL2 Blue/pCDNA for construction of expression vectors with mutated PrP
Mouse N2a neural cell line/pCDNA3/4 for expression of above in mammalian cells.

Origin & function

Mouse cDNA encoding normal mouse prion protein was derived originally from mouse brain library. Fragments of it will be subjected to PCR-based directed mutagenesis, which when confirmed by sequence will be incorporated into expression vectors (CDNA 3/4) along with an epitope or fluorescent protein tag (normally inserted after the signal sequence (amino acids 1-22), either immediately before aa 23; Lee et al (2001) J. Neurochem. 79: 79-87) or slightly more distally (after aa 42; Negro et al (2001) Mol. Cell. Neurosci. 17:521-38) where it appears not to perturb prion protein trafficking), for the following purposes:

1. Translocation of prion protein: individual amino acids will be altered to one with an expected functionally neutral character primarily within the signal sequence (amino acids 1-22), or the surrogate transmembrane sequence (aa's 100-136) or the GPI anchor (aa 231 ff). These alterations will be incorporated into cell lines to allow cell fractionation followed by immunoaffinity purification to determine the chaperone associations of the proteins (Graham, C H Anderton, B A & Morris, R J (2001) submitted for publication) as they traffic between the biosynthetic compartments of the cells. Studies may also be done where these constructs are introduced into primary cultured neurons by microinjection or by chemically-assisted transfection (Ca 2+, Liptofectine etc), to allow comparison with real neurons. In addition, sense RNA may be transcribed using an RNA polymerase, capped and polyadenylated before being used in in vitro translation studies with microsomes (Kim et al (2001) J. Biol Chem 276: 26132-40).

2. Interaction of externally applied prion infectious agent with translocation of mutant prion protein at the endoplasmic reticulum. To determine whether and how exogenously applied prion infectious agent could alter the routing of prion protein as it is made on the ER, mouse scrapie-associated fibrils or whole homogenate of mouse scrapie-infected brains will be added to cells expressing normal or mutant forms of prion protein (from step 1), and the proportion of protein translocated in the transmembrane forms (and its subsequent fate) determined, with particular attention paid to any change in the chaperone proteins associated. It should be noted that here and in 4 below, long studied mouse strains of scrapie derived originally from sheep (ME7, Chandler) and not from bovine or human sources will be used.

3. Endocytic trafficking of mutant forms or prion protein. We have an existing CL2 approved project (GM295/01.2) for investigating the effect of mutations in the N-terminus (aa's 23-108) of prion protein upon the trafficking of prion protein from the cell surface to intracellular compartments; this application will extend this by using mutations within the C-terminal domain, primarily with the surrogate transmembrane domain and GPI anchor (as in 1 above).
4. Interaction of externally applied prion infectious agent with mutant prion protein. We have an existing CL2 approved project (GM295/01.3) for investigating the interaction of mouse scrapie agent with cells; this application will extend this by studying the interaction with cells bearing mutations in prion protein that affect the endocytic trafficking of this protein (as in 3 above).

**Evaluation of foreseeable effects**

It must be assumed that at least some of the mutations we produce will predispose the mouse prion protein to adopt an infectious or neurotoxic conformation. Regarding the infectious form, the amounts produced will be small, contained, and rigorously denatured by oxidants before disposal for incineration. Moreover, the point mutations involved will in no way break down the species barriers for transmission of the infection to either man or to cattle, so that any escape of the mutation should affect no more than any local mouse populations. Regarding the potential neurotoxicity of the transmembrane forms of prion protein, these are not protease resistant and will be broken down normally by our usual physiological barriers in the gut and skin (and by our containment and denaturation procedures). There is no reason to think there is any species barrier to the neurotoxicity, but equally the barriers to a foreign protein reaching a nerve in our body are formidable.

At the genetic level, in the regions that will be mutated the mouse sequence is either identical or very similar to the human sequence. CL3 level of containment, with accompanying SOPs (minimisation of aerosols, use of sharps etc) will ensure that escape of mutated sequences does not occur, but should, despite these precautions, cDNA encoding a mutant form of prion protein be injected into eg the finger of a researcher, and become translated into mutant protein, all the other prion protein in the body would remain human and non-mutated, so that any production of infectious prion should be restricted to the site of injection.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Given the work involves mutant prion protein, all work will be done under containment level 3 conditions with the additional safeguards appropriate for working with mouse scrapie itself. Of these the most important are those for waste disposal, outlined below.

Liquid waste will be disinfected in 20,000 ppm active chlorine (as sodium hypochlorite) overnight before being washed down the drains with copious water. Solid waste will be double bagged for autoclaving (135 1 degree C, 18 min) prior to incineration.

For treatment of liquid wastes 20,000 ppm active chlorine is considered to be a completely effective procedure for inactivation of TSE agent; for solid waste, incineration is completely effective, the autoclaving will substantially reduce the titre of any agent (should it be produced) adding an extra level of security during the transport to the incinerator.

Monitoring is only applicable to the autoclave step, where we use Brown tubes within a load, and have a printed readout of each cycle; the autoclave is serviced annually. The ultimate form of the prion protein will be fragmented and highly oxidised carbon derivatives with no biological activity.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N
The Genetic Modifications Safety Committee of the MRC Centre for Developmental Neurobiology reviewed this application at its meeting of 13 December 2001. The Committee were satisfied that the steps taken to contain and dispose of the mutant mouse prion proteins and the constructs encoding it were sufficient and conformed fully to national guidelines and local needs. The staff involved have been fully consulted in drafting the Risk Assessment, and effective Standard Operating Procedures are in place. The Committee therefore accepted this application.

**Project Containment**

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**Project Ref** 295/02.2

- **Date Ackn’d**: 08/09/2003
- **CU2 Project Title**: MOLECULAR STUDIES OF HIV AND SIV REPLICATION
- **Class**: Class 3
- **CultureVolClass2**: Class 3
- **CultureVolumeClass3-4**: 200 ml
- **Non-GMM**: yes
- **Consent Granted**: yes
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: 295/02.2a
- **Date of Significant Change**: 18/06/2018

Tick to confirm that you have attached a risk assessment to this form: Y

Tick if you are claiming exemption from disclosure for section of the risk assessment: N
**Project Additional Information**

**Purposes of the contained use**

The purpose of the contained use is to enable us to pursue studies of primate lentiviral replication (that is, HIV-1, -2, and SIV) and of murine leukemia virus (MLV) without exposure of personnel or of the external environment to these pathogens. In addition, contained use will enable proper decontamination of the laboratory space and large equipment used for these studies.

HIV is the causative agent of Acquired Immunodeficiency Syndrome (AIDS). HIV-2 and SIV are related viruses that cause less severe disease in human beings. MLV is a retrovirus with a tropism for a variety of mammalian cells.

**Recipient or parental organism**

A variety of immortalized cell lines will be utilized for these studies including:

- Human T lymphoid (Jurkat, CEM, SupT1, C8166, HUT78, H9)
- Human fibroblast (HeLa, 293T)
- African green monkey fibroblast (COS)
- Murine fibroblast (3T3, L)
- Murine Lymphoid (EL4, Timi)
- Quail fibroblast (QT6)

**Host/vector system**

HIV-1, -2, SIV or MLV viruses will be passaged in the immortalised cell lines mentioned above.

**Origin & function**

All immortalised cell lines and wildtype viral isolates are obtainable from the AIDS repository at the US National Institute of Health.

The genetic material involved is intended to aid in the investigation of viral protein function and the molecular mechanisms of viral assembly and infection. Generally, by removing regions of the viral genome, we can examine in what ways the virus life cycle is altered and thereby determine the role of the deleted region.

**Evaluation of foreseeable effects**

In comparison to wild type virus, all modified HIVs will have reduced pathogenicity as a consequence of disruption of essential viral sequences. Importantly, all such viruses will be maintained and used under category 3 conditions. All necessary precautions are being undertaken to prevent infection of workers or release from the containment 3 laboratory. Special attention has been paid to minimization of aerosols and sharps. In the unlikely event of exposure of a worker to the virus, prophylactics and emergency procedures are on hand.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Given the work involves primate lentiviruses, all work will be done under containment level 3 conditions. Of these the most important are those for waste disposal, outlined below.

Liquid waste will be disinfected in 10% active chlorine (as sodium hypochlorite) overnight before being washed down the drains with copious water. Solid waste will be double bagged for autoclaving (135 1 degree C, 18 min) prior to incineration.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid wastes: Double bag and autoclave at high vacuum, 135 1 degree C for a minimum of 18 minutes. After autoclaving, waste is bagged in designated yellow bags, offloaded from the site as clinical waste and, ultimately, incinerated.

Liquid waste: Inactivate with 10% active chlorine in the form of sodium hypochlorite overnight, wash down drains with copious amounts of water.

For treatment of liquid wastes 10% active chlorine is considered to be a completely effective procedure for inactivation of retroviruses. For solid waste, autoclaving is completely effective.

Monitoring is only applicable to the autoclave step, where we use Brown tubes within a load, and have a printed readout of each cycle; the autoclave is serviced biannually.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee of the MRC Centre for Developmental Neurobiology reviewed this application at its meeting of December 13th 2001. The Committee were satisfied that the steps taken to contain and dispose of the primate lentiviruses and infected cells and the constructs encoding it were sufficient and conformed fully to national guidelines and local needs. The staff involved have been fully consulted in drafting the Risk Assessment, and effective Standard Operation Procedures are in place. The Committee therefore accepted this application.

Project Containment

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Project Ref  295/02.3

Date Ackn'd  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4

02/03/2022  1532602/03/2022
The purpose of this activity is to study the intracellular signalling mechanisms that mediate the response of cells to growth factors and extracellular matrix molecules, including proliferation and migration, and thus play a role in the development of human cancers and in cellular differentiation.

**Recipient or parental organism**

(i) E. coli
(ii) murine cells (NIH3T3, and the NIH3T3 variants 293T, GP+E86, GP+EnvAm12)
(iii) human primary keratinocytes and carcinoma cell lines
(iv) monkey cells (COS-7)

**Host/vector system**

(i) Host: E.coli DH5a; Vector: pCEV27, pBABEneo/puro plasmid, pMSCVneo, pMSCVhygro, pLib, pRx-bsr, pRcCMV, pC1, pGET-T, pPCR-Script
(ii) Host: GP+E86 murine fibroblasts; Vector: pBABEneo/puro, pMSCVneo, pMSCVhygro, pLib, pRx-bsr plasmid
(iii) Host: GP+EnvAm12 murine fibroblasts; Vector: pBABEneo/puro, pMSCVneo, pMSCVhygro, pLib, pRx-bsr plasmid
(iv) Host: Murine and human mesenchymal cells (osteoblasts, chondrocytes, fibroblasts, and adipocytes) either as established cell lines or primary cultures, as well as osteoclasts, osteoclast precursors, haematopoetic stem cells, and embryonic stem cells. Vector: pBABEneo/puro plasmid, pMSCVneo, pMSCVhygro, pLib, pRx-bsr virus
(v) Host: NIH3T3 murine fibroblasts; Vector: pBABEneo/puro virus
(vi) Host: HNEK, H357, A431, HN12, HN4 & MCF7 human keratinocytes; Vector: pBABEneo/puro virus
(vii) Host: 293T cells; Vector: PINCO (modified from plasmid LZRSpBMN-Z).

**Origin & function**

Mouse and human cDNAs encoding signalling intermediates, cell cycle control genes, and AP-1 transcription factors, as well as novel cloned target genes, will be obtained by PCR, or excised from existing plasmid vectors, subcloned into viral plasmids, and used to produce amphotropic/ecotropic viruses as a means of expressing them in the target cells (see above).

The intended function is to investigate growth factor and extracellular matrix-dependent signalling pathways that regulate cellular differentiation, proliferation and motility, all
of which are key aspects of cancer development.

**Evaluation of foreseeable effects**

The vectors to be used in these studies are replication-defective recombinant retroviruses, and have been well characterised and extensively used (Morgenstern & Land, Nucl Acids Res 18: 3587, 1990; Gasperi et al., Journal of Leukocyte Biology 66: 263-267, 1999). They are non-mobilisable in mammalian cells, and are rapidly inactivated by serum. The cell lines (GP+E86 and GP+envAm12 are third generation packaging cell lines, and have been specifically constructed to prevent recombination events between introduced constructs and endogenous viral structural sequences. Investigators using this system have not observed production of any replication-competent virus (RCV) (Markowitz et al., Virology 167: 400, 1988). The recipient cell lines are free of helper virus, and transduction of these lines with replication-defective viruses will not generate RCV. In spite of these findings, assays to detect RCV will be carried out as a matter of course. Thus, although the vectors will be packaged with ecotropic and amphotropic envelopes, and could conceivably infect rodent (ecotropic) and human cells (amphotropic), such viruses would be incapable of replication. Furthermore, these viruses are unstable at ambient temperatures, with a half-life of around 2 hours. Some of the gene products encoded by cDNAs cloned into these vectors have oncogenic potential. Therefore, the assessment is that work with both ecotropic and amphotropic viruses will be carried out at level 2. The containment level and procedures proposed for the study, together with the inherent safety of the packaging systems, will make the environmental risk effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste from bacterial and vertebrate cell cultures will be aspirated and treated with bleach (Haztabs; 1 tablet per litre = 2,500 ppm chlorine) for 12 hours. The bleach will be made freshly on the day of use according to manufacturers instructions. This will result in at least 99.999% kill of bacteria and 100% kill of viral GMMs and cell lines which are extremely liable. In addition, the retroviruses and cell lines have a very short half life at room temperature. Inactivated culture supernatants will be discharged to the drainage system. Solid waste will be double bagged, sealed, and placed within and autoclaved in a closed metal container. This will prevent any accidental release of GMMs into the working environment. The waste will be autoclaved at 136 degrees C for 30 minutes. This will result in complete inactivation of GMMs. Autoclave function will be monitored by annual services, the use of autoclave indicator tape (which measures a temperature of 134 degrees C for 3 minutes) during every run. Autoclaved waste will then be incinerated by White Rose.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

The GMSC accepted the risk assessments for these projects.

Class of Activity 2.

**Project Containment**
Purposes of the contained use

Mycobacterium tuberculosis (MTb) is a class 3 human pathogen and requires level 3 containment facilities for use. The recombinant MTb expressing luciferase gene would be used to significantly decrease the experimental times for bacterial determination in infected cultures and BCG expressing either luciferase or green fluorescent protein (GFP) would allow additional flexibility in substituting pathogenic MTb in certain types of assays. Luciferase and GFP are biologically harmless genes but as the recipient strain is pathogenic the containment level 3 would be required for the use of recombinant strains as for the wild type MTb. The same applies to Mycobacterium bovis (MB) expressing luciferase gene which would be used to uncover differences in pathogenesis between these two closely related species.

Recipient or parental organism

The recipient organism is bacterium Mycobacterium tuberculosis (MTb) or the attenuated strain of Mycobacterium bovis (MB) known as BCG (Bacillary of Calmette and Guerin). MTb and MB are class 3 organisms and BCG is Class 2.
Host/vector system

pSMT1 and pSMT3, both non-mobilisable vectors.

Origin & function

Luciferase gene is of bacterial origin and its function in the intended recombinant strains of Mycobacteria is to serve as reporter gene for quantitative determination of bacteria. The green fluorescent protein (GFP) is derived from jellyfish and is a fluorescent marker for tracking down the recombinant bacteria during infection of cells.

Evaluation of foreseeable effects

The recombinant Mycobacterium tuberculosis and Mycobacterium bovis expressing luciferase and BCG expressing luciferase or GFP are generated several years ago in the laboratory of Douglas Young at Imperial College London (Lab GM77). They have been used since then worldwide and several publications have quoted the use of these strains. Both genes are reporter constructs. Luciferase is an enzyme that converts certain type substrates into chemiluminescent products and GFP is a fluorescent protein that can be easily identified by fluorimetric techniques such as flow cytometry (FACS). These two genes have been used in numerous experimental systems with no biologically harmful effects ever being reported. The use of these two genes would normally require level 1 containment measures but as the recipient strains are class 3 (MTb and Mb) or class 2 (BCG) organisms, it is necessary to apply the class 3 or respectively, class 2 containment measures. There is no indication from the originator or from the others who have used these strains that they are in any way different in terms of virulence from the corresponding wild type strains. Therefore, the environmental or personal risk from using these strains is no different from that of wild type strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Re: APPLICATION FOR DEROGATION FOR AUTOCLAVE FACILITY FOR CONTAINMENT LEVEL 3 LABORATORY

During a recent inspection by HSE, it was pointed out to us that there had been a change in the regulations defining what constitutes a laboratory suite, and as a result our containment level 3 facility (room 311, floor 28 Guy's Tower) contravened the new interpretation under the Genetically Modified Organism (Contained Use) Regulations 2000 (CU2000). I therefore am applying for derogation from Schedule 8 of CU2000.

The relevant features, following the guidance attached to Newsletter 30 are:

a) Both the autoclave and the containment facility are located at the far end of the building remote from the main access to the floor. They are joined by a short corridor that is not used by students, non-scientific staff or members of the general public. There are no offices at this end of the building, only laboratories.

b) The infection with Mycobacterium tuberculosis and mycobacterium bovis occurs through respiratory route. The infectious dose has not been firmly established but it is thought that the size of aerosol-born droplets containing bacteria is critically important (ie several droplets containing 3-5 bacteria each, if inhaled by an immuno-susceptible person may establish an infection). Only 5% of infected individuals develop the disease.

c) The highest concentration of bacteria is present in the waste is 10^7/ml which is the remaining stock solution from the experiment (the stock solution is 1 ml, thus the amount of this waste is always less than 1 ml). The unused stock solution is cultured with hycolin for several hours prior to disposal. Typical concentration of bacteria in the experimental liquid waste is 10^5/ml or less.

d) The waste is in loosely tied autoclave bags contained in metal autoclave bins.

e) Following the inspection we have purchased autoclave tins with clip-on lids to replace the identical ones without clip-on lids.
f) as in (e) above.

g) The container will be placed on a trolley following an agreed procedure that has been in place for the last 2 years.

h) the removal of waste from the Containment Laboratory does not take place unless it is clear that the autoclave is available for use. After this waste has been loaded into the autoclave low level waste may also be added, but only by the staff loading the level 3 waste.

i) As in (h) above.

j) The container is placed directly in the autoclave and the tray that sits on the trolley is then also placed in the autoclave.

k) The only personnel that move the waste are trained for work at containment level 3.

l) The SOP (revised to include the use of clip-on lids) for use of the Containment facility is attached. This contains an emergency procedure to be followed in the event of a spillage during waste transport.

DECONTAMINATION

1. All glassware and plastics must be autoclaved on a cycle to reach 126 degrees C before removal from the laboratory suite. Autoclave bins with properly fitting clip on lids containing the waste in loosely tied autoclave bags must be taken from the CL3 laboratory to the autoclave immediately prior to autoclaving. The following procedures will be follows:

i) at least two trained staff must be available to carry out these procedures

ii) the autoclave is room 3245 must be available for use

iii) one member of staff will act to open doors; one will move the autoclave tins from the CL3 facility onto the transport trolley

iv) the autoclave tins will be removed from the CL3 facility and placed on a tray on a trolley outside the CL3 laboratory

v) autoclave bins from the CL3 laboratory will be taken directly to the autoclave and placed in the autoclave

vi) when all autoclave bins have been loaded into the autoclave, the tray will also be placed in the autoclave and the autoclave cycle will be started.

2. If a spillage of CL3 infectious material occurs during transport of waste, the area will be disinfected by treatment with Presept, and/or by swabbing with disinfectant.

i) Any contaminated clothing must be removed and any affected skin should be washed with soap and water. In the event of cuts, the First Aid Officer should be contacted.

ii) Notification of the Biological Safety Officer and the relevant Line Manager must then take place. They will decide if any further action is required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The waste consisting of disposable plastics and liquid waste in volumes of 100 ml or less in plastic containers will be placed in plastic bags, sealed and transferred to metal tins with clip-on lids. The standard internal operating procedure for removal and autoclaving of waste will then be applied (for details please see above application for derogation of autoclave facility for CL3 room).

The autoclave will operate the cycle at 126 degrees C for 80 min at 2.5 bar. The waste will then be placed in bags and disposed to designated bins.

This procedure is effective for complete killing of Mycobacterium tuberculosis wild type as determined by re-plating of the autoclaved material on microbiological plates. Similar test will be conducted for GM strains of MTb and MB (ie following completion of the cycle the metal been containing the waste will be returned to CL3 room in a reverse of the removal of waste procedure and the waste placed in Microbiological Safety Cabinet. An aliquot of the liquid waste and a swab from the solid waste will be
tested for the presence of live bacteria by microbiological methods. Only following the negative result of the test will the remaining waste be removed from the room in a repeat of standard procedure and autoclaved prior disposal. This test-procedure will be repeated each time when the nature or the quantity of the waste significantly differs from the routine waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The proposal was looked at by two members of Safety Committee who were satisfied with risk assessments and proposed risk categorization.

The class of activity is 2/3 as detailed in the KCL GM RA.

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</tbody>
</table>

Project Ref 295/08.1

Date Ackn'd 04/03/2014

CU2 Project Title Cancer cell motility and imaging.

Date Project Ceased

Class 2

CultureVolClass2 < 1 Litre

Consent Granted Not Applicable

Class 3-4

Non-GMM

Project notified under transitional arrangements N

Tick if notifying a connected programme of work Y

02/03/2022
Project Additional Information

**Purposes of the contained use**

The key objective is to bridge the gap between Imaging, Cancer Cell Biology and Medicine, particularly in the field of translational cancer research. The aims are summarised as follows:

1. Development of cancer cell imaging facilities which can measure biochemical changes in cancer patient-derived tumour samples, including fresh primary tumour cells.
2. Investigation of the function of specific gene products or proteins in a range of mammalian cell lines and primary cells, by gene expression or knockdown.
3. The cells for these gene expression or knockdown studies will include primary tumour cells, primary human T cells, human T-cell leukaemia cells, monocytes and endothelial cells, and primary cells derived from mouse tissues. For these studies, we use plasmid-based expression vectors, retroviruses, adenoviruses and lentiviruses.

**Recipient or parental organism**

<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli strains:</strong> Ecofl JM101, JM109, DH5alpha, DH1OB, DB 3.1, InvceF', JS4, XL1-Blue, BL2I(DES), TOPIO, TNV1 10, K12 strains DHSu, BL21(DE3) and TOP10</td>
</tr>
<tr>
<td><strong>Human breast</strong> — MCF7, MDA-MB-23 1, MDA-MB-435, MDA-MB-361, MDA-MB -468</td>
</tr>
<tr>
<td><strong>Human T-cell</strong> — Jurkat, NKL, CCRF-CEM</td>
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<tr>
<td><strong>Human embryonic kidney</strong> — 293T, 293A, 2930P0</td>
</tr>
<tr>
<td><strong>Mouse Fibroblast</strong> — PT67</td>
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<tr>
<td><strong>Rat Breast Carcinoma</strong> — MTLn3, MTLn3e</td>
</tr>
<tr>
<td><strong>Human hepatocyte</strong> — HepG2</td>
</tr>
<tr>
<td><strong>Human B-cell</strong> — 721.221, Raji</td>
</tr>
<tr>
<td><strong>Human NK cell</strong> — YT, NK92</td>
</tr>
<tr>
<td><strong>Human Myelogenous Leukaemia</strong> — K562</td>
</tr>
</tbody>
</table>

**Primary human tumour cells, T-cell leukaemia cells, primary human T cells and endothelial cells, and primary cells derived from mouse tissues.**
Vector names:
For expression in mammalian cells: pEGEP C 1/2/3 and Ni pECFP-C 1. pEYFP-C1 are members of the Clontech “Living Colours” family of vectors pcDNA 3.1+ and pcDNA3J MycHis vectors (Invitrogen)
For expression in E.coli:
pGEX vectors (Aniersham)
Rwovir vectors:
pLPCX, pLHCX (Clontech)
Lentiviral vectors
pL4.4 (CAG promoter EGFP-MCS)
Lentiviral packaging plasmids (3rd generation tour plasmid based system)
pCMV-VSVG, pRS V-REV, pMDLg/pRRE, pAR 8.91 (encodes accessory proteins for replication including Gag-Pol from WY-i), pMD.G (encodes VSV-G), pLKOJ-puro (Mission vector). p’HRsincptStKw-eGFP
Adenoviral vectors: pCR220 (Serotype 5 backbone with EI and E3 regions deleted)
Websites for vectors:
eGFP/eCFP/eYFP vectors; http://www.clontech.com/images/pt/dis_vectorsP13025jdf
pcDNA3 .1 myc/His https ://www.invitrogen.com/content/sfs/vectors/pcdna3 .1+pdf
pGEX vectors
http://www.amershambiosciences.com/pdfs/970004M2-0 1 .pdf pLPCX
http://ww.c1ontech.com/images/pt/PT3299-5, pdf
pLHCX
pL4.4
The pLenliLox system was developed in the labs of Frank Gertler and Luc VanParis at MIT, Cambridge, USA.
http://wwwnamrecomdolfinder/ 10 1 038/ngl 17
Lentiviral packaging plasmids
http://www.medecine.unige.cbl —salmon/main/pMD2G.jpe
http://www.medecine.unige.ch/ -sahnon/main/yRSV Rev iDea
http://wwwmedecineazjgech/ —salmon/mainlpMDlpRREjpec
pLKO.1-puro:
http://www.siemaaldrich.com/Areaoflnterest/Life Science Functional Genomics and RNAlibrary InformationNector
Man Jitml
pCR220:
http://www.patentstorm.us/patentsf629l 21 4-description.html
Host:
Human breast cell line — MCF7, MDA-MB-231, MDA-MB-435, MIA-MB-361, MIJA-ME-468
Human T -cell line — .Turkati-luman embryonic kidney ccl line —2931, 293A, 293GPG
Mouse Fibroblast cell line — P167
Rat Breast Carcinoma cell line — MTLn3, MTLn3e
Human hepatocyte cell line — HepG2
Human B-cell line—721211, Raji
Human NK cell line — Y1, NK92, NKL
Human Myelogenous Leukaemia cell line — K562
Primary human tumour cells, primary human 1 cells and endothelial cells, and primary cells derived from mouse tissues.
Human, mouse, rat cDNA are obtained from existing eDNA libraries. Lentiviral vectors encoding shRNAs targeting human proteins, obtained from Sigma (Mission library)

The intended function is to investigate the regulation of cancer cell and leukocyte motility

Evaluation of foreseeable effects

Lentivirus:
The pLentilox lentiviral vector system is a so-called 3 generation lentiviral vector that has been extensively modified to prevent viral replication outside the packaging cells:
- pLentilox has a self-inactivating 3'tLTR (TATA box deletion) and large deletions result in absence of env, tat, rev, vpr, vpu, v(f and nef. Therefore, there are no viral gene products expressed from pLentilox.
- the gag, pot, and coy genes are supplied in trans from separate plasmids only in the packaging cell line.

References for packaging vectors:

References for self-inactivating LTR:

Adenovirus:
- The pCR220 (adenovirus serotype 5 backbone) is a plasmid containing most of the human adenovirus serotype 5 (AdS) genome, with deletions in the genes El and E3. This vector has been developed to infect but not replicate in non-permissive target cells. The El gene is provided in the packaging cell line (HEK293 cell line) in trans.

Exposure and risk to health of the researchers is reduced to negligible levels by performing the class 2 work in a class 2 microbiological safety cabinet in a dedicated area of a containment 2 laboratory. All personnel are fully trained to reduce the risk of accidental spill of virus to a minimum. For all these cell/retroviral studies, infected cells will be contained within dedicated incubators. All GM manipulated material will be destroyed on site.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from bacterial and vertebrate cell cultures will be aspirated and treated with bleach (Haztabs; 1 tablet per litre 2,500 ppm chlorine) for 12 hours. The bleach will be made freshly on the day of use according to the manufacturers instructions. This will result in at least 99.999% kill of bacteria and 100% kill of viral GMMs and cell lines. In addition the cell lines and retroviruses have a very short half life at room temperature. Inactivated culture supernatants will be discharged to the drainage system.

Solid waste will be double bagged, sealed and autoclaved. This will prevent any accidental release of GMMs into the working environment. The waste will be autoclaved at 136 ºC for 30 minutes. This will result in complete inactivation of GMMs. Autoclave function will be monitored by annual services and the use of autoclave tape (which measures a temperature of 134 ºC for 3 minutes) in every autoclave run. Autoclaved waste will then be incinerated by White Rose.

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
Please find attached the GM Proposal and Risk Assessment for - project entitled “Cancer cell and leukocyte motility and imaging”. I am satisfied that they have made the amendment requested b) the at its meeting on September 4th, and would be grateful if you could sign as appropriate. Below is the relevant section from the minutes of that meeting.

Randall Institute

introduced the project. had previous received approval at Level 1 for the project, but wished to incorporate work with primary cells using attenuated viral vectors (lenti and adeno), which is categorised as Class 2. This was now a joint submission with

2A, (ii) 4: Answer should be “No”, and subsequent explanation of “Yes” response should be removed.

Approved at CL2 subject to implementation of above amendment, completion of CU2 form, payment to HSE and provision of completed signatures page.

Letter from

School Safety Manager and representing the committee:

College Safety Officer.


Project Containment

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<thead>
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Animal Units

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Project Ref 295/10.1

Investigation of signalling pathways in human myometrial smooth muscle cells and epithelial cells of the reproductive tract

04/03/2014

Consent Granted

Project notified under transitional arrangements N
The overarching goal of this study is to gain greater functional insight into the physiological and pathophysiological regulation of the female reproductive tract during pregnancy. Our work focuses on:

i) The control of uterine smooth muscle growth and excitability and ii) the response of reproductive tract to infection and inflammation.

i) Ion channels and related proteins play a major role in regulating uterine excitability and rhythmic contraction.

We are interested in the contribution of potassium channels (alpha and beta subunits) which are hypothesised to promote uterine quiescence in early-mid pregnancy (Aaronson et al., 2006; McCallum et al., 2009; Ohya et al., 2009) and calcium channels (e.g. TrpCs; Dalrymple et al., 2004; 2007) and related proteins (e.g. Stim and Orai, caveolin and flotilin) which can control cell growth, excitability and responses to agonists.

ii) Toll-like receptors and related signaling pathway (e.g. MAPkinases, cytokines etc) on reproductive tract (e.g. cervical and vaginal cells and human myometrium) responses to infection.

In order to investigate their functional roles in regulation of parturition/contraction we intend to introduce a range of molecular tools which include:

1) Over expression and assessment of gene promoter regulation in well known model cell systems (eg HEKs) which may help to assess the role and significance of genes taking part in human pregnancy and parturition

2) Stable shRNA delivery (using commercially available plasmids and viral vector systems) which is a highly effective strategy for permanently disrupting the function of individual genes in order to study their role in signaling pathways in myometrial cells (primary cultures and hTERT HM cell line) and epithelial cells of the reproductive tract (VK2/E6E7cell line)

Recipient or parental organism

- Primary cultured and passage 1-6 pregnant human myometrial smooth muscle cells (HMSM)
- Human myometrial cells immortalised with retroviral hTERT vector (HTER-HM)
- Vaginal epithelial cell line (ATCC CRL-2616 ) (VK2/E6/E7)

Host/vector system

Host cells:
- Lenti-X 293T cells
- HEK 293
- E. Coli DH5alpha

The viral vectors which will be used in the study are listed below however we might use other vectors with similar properties without changing the containment level:
Lenti-X pLVX-shRNA1, SMARTvector® 2.0 Lentiviral shRNA Particles, GIPZ Lentiviral shRNAmir

All lentiviral constructs contain all the viral processing elements necessary for the production of replication-incompetent lentivirus and elements responsible for improved viral titer and overall vector function.

Genes encoding the structural and other components required for packaging the viral genome are separated onto mix of plasmids minimizing the threat of recombinant replication competent virus production. Lack of homology between the packaging mix of plasmids and Lenti-vectors also prevents transfer via homologous recombination. After transduction viruses cannot replicate autonomously in target cells (SIN vectors).

Non-mobilisable (Bom-, Mob-, Tra-) plasmid vectors which may be used in the study:

- **pIRES2-EGFP**: Expression vector which contains bacterial and mammalian promoter and internal ribosome entry site (IRES) that allows the co-expression of a marker gene and another gene from a single transcript.
- **pKD**: siRNA expression plasmid H1 promoter, Fl ori., PuC ori., Amp)
- **pGL3**: Basic plasmid which lacks a resident promoter. Encodes firefly luciferase which expression depends on insertion of the promoter sequence. SV40 promoter, Amp, ori, Luc+).

### Origin & function

**Stim (1-2):** Stim proteins have emerged as potential candidates underlying store-operated channels (SOCs). STIM1 is a calcium sensor integrated within endoplasmic reticulum. It has been shown to relocate to the plasma membrane on store depletion and activate store-operated calcium entry (SOCE). STIM2 may be a negative regulator of STIM1-induced SOCE and is thought to be involved in the regulation of basal/cytosolic and endoplasmic reticulum Ca2+.

**Orai**: Orai proteins are thought to form the plasma membrane channel regulated by STIM. There is compelling evidence that Orai1 encodes the Ca2+ selective SOC channel/ICRAC current in a range of non excitable cells, but as SOC currents display distinctly different characteristics in excitable cells, it has yet to be fully established whether Orai1 channels or STIM can also mediate this more non-selective cation current alone.

**KCNQs**: KCNQ (Kv7) genes encode a family of K+ channel α-subunits most extensively investigated in cardiomyocytes and the CNS where they contribute to action potential repolarisation and subthreshold excitability respectively. More recently a role for Kv7 channels in smooth muscle has emerged, with functional roles being seen in the vasculature, the GI tract and the uterus.

**KCNE(1-5)**: KCNE subunits modulate KCNQs and other voltage-gated channels in vitro and in vivo

**Caveolin(1-2)**: Caveolins serve a structural role, inducing and maintaining caveolae invaginations and can act as a scaffold proteins to sequester and modulate the activity of many signalling effector and receptor proteins.

**Flotillin(1-2)**: Flotillins are integral membrane proteins and constituents of lipid rafts. Flotillin-1 and -2 were originally discovered in neuronal cells as Reggie-2 and -1, respectively, and they are thought to be involved in signalling transduction and modulation.

**Toll–like receptors (2,4)**: Toll-like receptors (TLRs) are pattern recognition receptors and serve as the first line of defense in host immunity. Current data suggests that TLR2 and TLR4 may play a protective and/or regulatory role in an effort to limit a lethal inflammatory response.

**TrpC**: Transient receptor ‘canonical’ channel proteins are proposed to form store-operated (SOCE) and receptor operated (ROCE) calcium channel entry in a variety of tissues. We have shown that several TrpC proteins are gestationally regulated in myometrial tissue and that pregnancy associated stimuli (stretch, cytokines) induce differential expression off TrpC proteins in human myometrial smooth muscle cells.
None of the above-mentioned genes and their products are potentially hazardous, infectious, or oncogenic. Their overexpression will not have any influence on the environment.

**Evaluation of foreseeable effects**

The ability of retroviruses to integrate into the host cell chromosome raises the possibility of insertional mutagenesis and oncogene activation. Both these phenomena are well known in the interactions of certain types of wild-type retroviruses with their hosts.

In order to improve the risk-benefit ratio associated with retroviral-mediated techniques several approaches are currently developed, like the design of self-inactivating (SIN) vectors containing an internal promoter to drive transgene expression instead of the long terminal repeat-containing enhancer of standard retroviral and lentiviral vectors, reduced homology between retroviral vector and packaging components and split-genome conditional packaging systems. Probability of survival of host cells or viruses in environment is very unlikely and disable genomes of these vectors prevent events of replication and creating new strains of wild type viruses.

Contact with viral particles and infection of cells at the contact site could resist in transient immune response with local swelling and inflammation. This is unlikely to be more than a minor effect. Moreover 3rd and 4th generation lentiviral construct cannot replicate after infection so no virus could be passed on to other people.

Low to none if experimental work is done with all safety procedures maintained:

1. Personal protective clothing (gloves, lab coats)
2. MSC class 2, mechanical pipetting devices, safety centrifuge caps and rotors, autoclave, sharp containers, clinical waste yellow bags
3. All laboratories have lockable doors and sinks for hand washing and can be easily cleaned and decontaminated

Each researcher completes an individual risk assessment for their work and are aware of potential hazards.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste contaminated with GMOs will be inactivated so as to ensure that any contact between the GMOs and humans or the environment is limited to an extent commensurate with the risks identified in the risk assessment and to provide a high level of protection for humans and the environment.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
The project was discussed at the Joint GMSC of the Schools of Biomedical and Health Sciences and Dental Institute. The following comment was made: Approved at Class 2 subject to implementation of minor amendments and provision of completed signatures page to GMSC Secretary.

(Please note this department was formerly under the management of the School of Medicine, GM386, but is still under the overall management of King’s College London.)

### Project Containment

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<td>L3 L4 L2 L3</td>
<td>L3 L4 L2 L3</td>
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</table>

- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

### Project Ref 342/95.1

#### Date Ackn’d
08/09/2003

#### CU2 Project Title
EVALUATION OF SYNTHETIC IMMUNOGENS AS NOVEL VACCINES

#### Class
Class 2

#### CultureVolClass2
Class 2

#### CultureVolumeClass3-4
Non-GMM

#### Consent Granted
not applicable

#### Project notified under transitional arrangements

#### Withdrawn
N

#### Tick if notifying a connected programme of work
N

#### Historical Significant Changes

#### Historical Date of Additional Info

#### Significant Change ID

#### Date of Significant Change

### Project Additional Information

**Purposes of the contained use**

(i) Propogation of small stocks and recombinant Vaccinia Virus (rVV) and recombinant Adeno Virus (rAd) .

(ii) Infection of simian and murine antigen presenting cells in vitro with rVV and rAd which will be used to restimulate in vivo primed cytotoxic T lymphocytes.
Recipient or parental organism

(i) Thymidine Kinase deficient vector WR strain vaccinia virus.
(ii) Adenovirus -5. (1.88kb E3 deletion).
Vaccinia virus deleted in the thymidine kinase (TK) gene from Strain WR Adenovirus 5 (1.88kb deletion in early region 3 (E3)).

Host/vector system

The hosts used in propagating recombinant vaccinia virus (or recombinant Adenovirus) are mammalian cell lines which cannot survive in the environment and this can be considered safe. The vector (WR strain of vaccinia virus) is Thymidine Kinase negative and thus attenuated in replication of virulence. Adenovirus 5 is replication competent in mammalian cells, but cannot survive outside the host used in its propagation.

Origin & function

rrVV SIVgag, rVV EBVgpH wild-type Vaccinia (WR strain) small scale propagation, infection of cell lines in vitro for immunological assays.
rrVV SIVgag-pol, rVV SIVgag, rVV SIVenv, rVV SIVnef*, rVV SIVrev, small scale propagation, infection of cell lines in vitro for immunological assays.
rAD-5-Luciferase, rAD-5-B-galactosidase, small scale propagation, infection of cell lines in vitro for immunological assays.
rVV SIVgag, rVV SIVenv, rVV SIVnef*, in vivo immunisation of rhesus macaques via the mucosal, Intra-dermal and Intra-muscular routes.

Evaluation of foreseeable effects

Although recombinant vaccinia and recombinant Adeno-5 viruses can persist in a non-replicating form (outside their host) for an unknown length of time, at Containment Level 2 where the material is handled in a Class 2 exhaust ducted Hepa filtered safety cabinet (MAT design), the risk of release to the environment is effectively zero. The products of genes expressed by the vectors used are not known to be toxic, oncogenic or mediate pharmacologic effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid waste inactivated overnight in 10% chloros (14,000 ppm available chlorine). Hypochlorite level checked with starch iodine strips. Solid waste subsequently drained from chloros, autoclaved 121°C x 20 mins (in leakproof containers).
Solid animal waste collected for incineration.
Inactivated liquid waste to mains drainage. Inactivated solid waste for incineration

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The committee have reviewed and approved this risk assessment and the facilities where this work will be conducted. The committee is satisfied that the personnel involved with work at Containment Level 2 have received the appropriate training.

## Project Containment

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### Project Ref 386/01.1

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<td>19/02/2001</td>
<td>EXPRESSION OF RECOMBINANT PROTEINS ASSOCIATED WITH TYPE 1 DIABETED MELLITUS, IN ORDER TO STUDY THE AUTOIMMUNE RESPONSE TO ISLET CELL AUTOANTIGNES</td>
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<th>Non-GMM</th>
<th>Consent Granted</th>
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Tick if notifying a connected programme of work  

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<th>Withdrawn</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>N</td>
<td>Y</td>
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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
THE FUNCTION OF THE PRB PATHWAY IN HUMAN HAEMOPOIETIC CELLS

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 386/01.11

**CU2 Project Title**

SEROLOGICAL IDENTIFICATION OF TUMOUR ASSOCIATED ANTIGENS IN MYELOID LEUKAEMIAS

Date Ackn'd 23/02/2001

Date Project Ceased 13/10/2010

Withdrawn N

Tick if notifying a connected programme of work N

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
### Project Ref 386/01.12

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- **Non-GMM**: not applicable
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

### Project Additional Information

#### Purposes of the contained use

#### Recipient or parental organism

#### Host/vector system

#### Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Project Ref 386/01.13

Date Ackn'd 23/02/2001  
CU2 Project Title M-CSF RESPONSIVE T CELLS ; POTENTIAL ROLE IN IMMUNOTHERAPY OF  
Class 2  
CultureVolClass2  
CultureVolumeClass3-4
CANCER

Date Project Ceased 08/09/2003

Non-GMM Consent Granted not applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Project Ref** 386/01.14

**Date Ackn’d** 15/02/2001

**CU2 Project Title** CONSTRUCTION AND DEVELOPMENT OF NON-VIRAL DNA VECTORS AND GENE CONSTRUCTS FOR GENE TRANSFER TO STOMATIC CELLS IN VITRO

**Class** Class 2

**CultureVolClass2**

**Consent Granted** not applicable

**Project notified under transitional arrangements** Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

02/03/2022
**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022

Page 6759 of 15326
### Project Ref 386/01.15

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Withdrawn: **N**  
Tick if notifying a connected programme of work: **N**

Historical Significant Changes: Project transferred to GM543 on 08/09/2003 PROJECT REOPENED AS TRANSFERRED BACK FROM GM543 ON MERGER WITH GM386

Project notified under transitional arrangements: **Y**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Withdrawn | N |
Tick if notifying a connected programme of work | N |

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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02/03/2022 Page 6764 of 15326
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

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Project Date 22/02/2001
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

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Project Ref 386/01.6

Date Ackn’d 22/02/2001

CU2 Project Title SWITCHING ISLET CELLS INTO CELL CYCLE PROGRESSION

Class Class 2

CultureVolClass2

CultureVolumeClass3-4

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

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Project notified under transitional arrangements **Y**

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

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**Host/vector system**

**Origin & function**
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Project Ref 386/01.8

Date Ackn'd 22/02/2001

CU2 Project Title ANALYSIS, CLONING, MANIPULATION AND EXPRESSION OF MUTANTY RAS

Class 2

CultureVolClass2

CultureVolumeClass3-4
GENES IN MYELOID LEUKAEMIA PRIMARY CELLS AND CELL LINES

Date Project Ceased: 18/01/2006

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>L2 L3 L4</td>
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Project Ref 386/01.9

Date Ackn'd 22/02/2001

CU2 Project Title RETROVIRUS VECTORS FOR GENE THERAPY

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
M-CSF is overproduced by a range of human tumours and contributes to the malignant phenotype by enhancing growth, invasive potential and contributing to an immunosuppressive environment in the vicinity of tumour deposits. In this study, it is proposed to engineer T cells to express the receptor for M-CSF. The underlying hypothesis is that such genetically modified T cells will acquire a growth/functional advantage in proximity to tumour deposits which produce M-CSF. In support of this, we have previously shown that the human M-CSFR is functionally active when expressed in immortalised CD4+ and CD8+ T cell lines. Addition of M-CSF to these cells co-stimulates IL-2 driven growth and activation of these cell lines (HSE centre No. for that project GMR 76-2).

Two questions are posed in the present study. First we wish to evaluate the functional activity of the human M-CSFR when ectopically expressed in primary human T cells. To achieve this, retrovirus-mediated gene transfer will be used to express this molecule in T cells derived from normal healthy donors. In vitro assays will be used to assess the ability of M-CSF to co-stimulate activation, growth and cytotoxic activity of these transduced T cell cultures.

The second phase of the project is designed to address the question of whether M-CSFR+ T cells would prove useful in targeting an in vivo established tumour. To test this, an animal model will be established using the ovalbumin-expressing tumour cell line, E.G7 which will be inoculated by intraperitoneal (IP) injection into syngeneic immunocompetent mice (C57/BL6). Tumour-bearing animals will be treated by IP injection with T cells derived from the OT1 transgenic mouse (which expresses a
transgenic T cell receptor specific for an epitope in Ovalbumin). This treatment is known to cause partial control but not eradication if IP-inoculated E.G7 tumour. Here OT1 T cells will be engineered to express the human M-CSFR (or mock transduced as control) and tumour cells will be transduced with retroviruses encoding for the secreted and membrane-associated isoforms of M-CSF. Using this approach it is hoped to determine whether the co-stimulatory activity of M-MCSF in T cells can be harnessed to improve tumour control in this model.

Recipient or parental organism

Recipient organisms are as listed below (hosts). No additional recipient organisms (eg animals, plants) will be used. Parental organisms are as follows: (i) Prokaryotic - DH5a are ultimately derived from E. coli. (ii) Viral - The replication-defective retroviral vectors for use in this study are all ultimately derived from the Moloney murine leukaemia virus. (iii) Eukaryotic - all fibroblasts cell lines including retrovirus packaging cell lines are ultimately derived from NIH3T3 cells.

Host/vector system

a. VECTORS - All viral vectors to be used have been mutated (by extensive deletion) to make them replication incompetent i.e. they do not produce infective virus though they do generate defective virus-like particles. The viral vectors (detailed below) do not have any inherent pathogenicity and are fully sensitive to human complement.

a. Host range and species specificity: vectors will be packaged by ecotropic (GP+env86) packaging cell lines, which have the capacity to infect murine cells. For retrovirus-infection of primary human T cells, vectors will also be packaged using PG13 (which imparts a Gibbon Ape leukaemia virus pseudotype).

b. Tissue specificity: No attempt will be made to generate tissue specific vectors.

c. Promoters and regulatory elements: Three expression vectors will be used for the purpose of the study (see attached figures for maps): (i) pBabe hygro (Morgenstern & Land (1990) Nucleic Acids Res. 18, 3587); (ii) pBabe puro (Morgenstern & Land (1990) Nucleic Acids Res. 18, 3587); (iii) MFG (Riviere et al (1995) PNAS 92, 6733-7. Promoter usage is as follows:

<table>
<thead>
<tr>
<th>Expression of insert DNA</th>
<th>Expression of selectable marker</th>
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<tbody>
<tr>
<td>pBhygro</td>
<td>retroviral long terminal repeat (LTR)</td>
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<tr>
<td>pBpuro</td>
<td>retroviral LTR</td>
</tr>
<tr>
<td>MFG</td>
<td>retroviral LTR</td>
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</tbody>
</table>

b. Hosts

Eukaryotes

Retroviral packaging cell lines GP+env86, PG13
Primary human T cells (activated with the mitogenic lectin, PHA)
CD8+ T cells derived from the OT1 transgenic mouse (activated with the mitogenic lectin, Con A).
E.G7 (an OVA-transfected variant of the EL4 immortalised murine thymoma cell line)

Prokaryotes

All hosts are derived from the DH5a strain of E.coli.

Origin & function

The inserts to be expressed using the vectors described above are as follows:

A. Using MFG, an insert will be expressed which comprises three elements;
   1. cDNA encoding for the human M-CSFR. This was originally provided by Dr N Dibb (Dept of Obstetrics and Gynaecology, Imperial College, Hammersmith campus). The intention of this study is to generate T cells which are co-stimulated in an M-CSF-dependent manner. Preliminary studies using immortalised T cell lines indicate that the
M-CSFr is functionally active in T cells but only in the presence of ligand (M-CSF).

2. Internal ribosome entry site - derived from Encephalomyocarditis virus. The purpose of this element is to facilitate translation of the subsequent cDNA - purchased from Clontech.

B. Using the pBabe series of vectors, it is planned to express cDNAs encoding for the membrane-associated and secreted isoforms of human M-CSF, PUC-based plasmids containing these cDNAs have been supplied by Dr Kirsten Koths (Chiron). These plasmids will be expressed in the E.G7 tumour cell line in order to model the effects of an M-CSF-producing tumour in vivo. Once this has been achieved, it is planned to treat such mice with T cells engineered to express the human C-CSFr.

Evaluation of foreseeable effects

The principle potential hazard associated with the use of retroviral-mediated gene transfer is the possibility that recombination may occur between the expression vector and the complementary sequences found in the packaging cell line, leading to the generation of replication competent virus (RCR). Generation of RCR is rendered less likely by the use of the GP+env86, and PG13 packaging cell lines, in which at least 3 independent recombination events are required for PCR generation. In addition, the viral vectors for study are fully sensitive to human complement.

As detailed in the preceding section, it is highly unlikely that any of the recombinant retroviral constructs to be generated in this study will prove transforming. M-CSF exerts no effect in the absence of the M-CSFr and vice versa.

It is envisaged that there will be a negligible risk to human health or the environment associated with the use of the prokaryotic or eukaryotic hosts specified since these are unable to colonise human hosts.

In addition, any theoretical risks posed by the above are further reduced by the containment and waste disposal measures to be employed. Bacterial work will take place at containment level 1 while all work with retroviral vectors and T cells will operate at level 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

1. OT1 mice, transgenic for an Ovalbumin-specific T cell receptor, will be used as donors for gene transfer and adoptive immunotherapy. It is proposed that these animals will be housed and manipulated under class 1 conditions.

2. In the present proposal, it is planned to establish retrovirus-transduced tumours in C57/BL6 mice. Furthermore, it is additionally planned to treat these established tumours with retrovirus-transduced OT1-derived T cells. A risk assessment for this protocol is detailed in Section 6 of the accompanying risk assessment form. Although this assessment indicates that the risk associated with establishment of this model is "effectively zero". it is planned to house all animals harbouring retrovirus-transduced cells under class II conditions. Furthermore, all manipulations of such animals (eg IP injections) will be performed under class II conditions.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials that have been in contact with genetically modified material will be sterilised. All solid waste will be autoclaved at 121 degrees C for 90 minutes before removal from the building in double bags for incineration, thereby ensuring 100% kill. To achieve 100% kill of liquid material, waste will be treated with 10,000ppm hypochlorite and incubated overnight (in agreement with manufacturers recommendations) before disposal down a sink. We have opted not to autoclave liquid waste in light of concerns about delays prior to autoclave availability for this purpose, coupled with undesirability of autoclaving bleach-treated waste. Periodic in-house testing will be performed to assess the % kill of prokaryotic and eukaryotic hosts by the overnight bleaching approach as described. All animals carcasses will be incinerated.
Project Containment

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**Project Ref** 386/02.2

- **Date Ackn'd**: 23/01/2002
- **CU2 Project Title**: DEVELOPMENT OF AN INTEGRATING ADENOVIRAL VECTOR
- **Class**: Class 2
- **Culture Vol Class 2**: < 1 litre
- **Non-GMM**: not applicable
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: N

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Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Purposes of the contained use

Adenoviral vectors provide the most efficient vehicles available for gene delivery, but because they integrate very inefficiently, the expression of incorporated transgenes is only transient. The aim of this proposal is to develop a modified adenovirus vector able to integrate into the host cell genome.

The long-term aim of this study is to use disabled adenovirus vectors (generated from Ad5 virus with deletions in the E1 region alone or with deletions in both E1 and E3 regions), for delivery of genes, the product of which will be useful either as cell markers (e.g., β-galactosidase, Green Fluorescence Protein, etc.) or will confer, either directly or indirectly, anti-tumour activity. Genes with anti-tumour activity can be grouped into the following categories:

- Anti-oncogenes such as wild-type p53 and p16;
- Immune stimulatory cytokines such as IL-2, IL-4, IL-6, IL-12, GM-CSF;
- Immune co-stimulatory factors such as B7.1, B7.2, CD40 Ligand;
- Antibodies and single-chain variable fragment of antibodies (scFv) against oncogenes such as c-erbB2, bcr-abl, etc.
- Prodrug convertases conferring greater susceptibility to the cytotoxic activity of prodrugs (e.g., HSV-tk and Gancyclovir).

The ultimate aim of these studies is to use the vectors for genetic modification of human tumour cells with the eventual aim of application of the vectors and modified cells to clinical trials for gene therapy of cancer.

### Recipient or parental organism

**Bacteria** -
Attenuated and mobilisation defective E.coli (K-12) derivatives such as DH5α and XL1 Blue, unable to survive in the absence of specialised growth conditions.

**Adenoviral packaging cells** -
Recombinant adenovirus vectors and plasmids needed for generation of new disabled vectors will be obtained from collaborating groups in the UK and USA. The vector will be propagated in mammalian cell lines with stable integrations of adenovirus genome with specific deletions. The presence of these deletions both in the packaging cells and in the vector will reduce the chances of recombination mediated generation of replication competent vectors. The 293 cell used for Ad vector production contains only the left 11% of AD5 genome, therefore providing E1a and E1b gene products in trans. The vectors will have at least the E1 region deleted, however, replication competent virus can be generated by recombination.

Events resulting in generation of recombinants with the E1 region replaced. However, such recombinants will have lost the inserted gene of the vector as a consequence of the recombination event.

The more recently developed vectors and packaging cells contain additional deletions, further reducing the chance of generation of replication competent vectors. Where possible we shall use these newer vector systems developed either by ourselves or obtained from other workers in the field.

**Established human and mouse tissue culture cell lines.**
Spontaneously immortalized, unable to colonize human hosts, unable to survive outside specialised tissue culture environs.

### Host/vector system

**Cloning vectors** -
pUC series, pBluescript and TOPO based, in bacterial (K-12) hosts such as DH5α and XL1 Blue, unable to replicate in non-bacterial cells.

**Disabled adenoviral vector** -

pJM17 based deleted in E1a and E1b and therefore unable to replicate. The E1a and E1b function are provided in trans by the commonly used packaging cell 293 which contains 5000 bp of the left end of the Ad5 genome. Homologous recombination can result in the release of replication competent adenovirus. This is unlikely to have deleterious effects in immun-competent hosts because of previous exposure and the presence of antibody titres in most humans. Recently developed packaging cell lines such as PerC5 contain no homologous sequences between packaging construct and vector and should in theory prevent the formation of replication competent virus. These types of packaging cells will be used if they become freely available but due to company ownership they are out of reach of most research institutions.

Human and mouse tissue culture cell lines -
Unable to colonise humans, spontaneously immortalised, no evidence of the ability to secrete transmissible agents, unable to supply helper functions to disabled adenoviral vectors.

Origin & function

The genetic material originates from Ad5 and is intended as efficient vector for the introduction of marker genes such as neomycin, puromycin, GFP etc from bacterial and other origin and are used to follow the efficiency of transduction and expression of these genes in established tissue culture cell lines.

Evaluation of foreseeable effects

Waste -
All waste material is dealt with as described under section 12 - This covers the deliberate removal of material from the laboratory environment.

Accidental escape into the environment -
All air is filtered (Hepa) before venting to the environment, the labs are provided with spill kits and SOP's for dealing with accidental spillage. All manipulations with bacteria take place at containment level 1. Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. All manipulations with adenoviral packaging cells, adenoviral vectors and established human tissue culture cell lines take place at containment level 2. Cell lines are unable to survive outside the laboratory environment. White coats and gloves are mandatory.

In the event of escape of disabled adenoviral vectors they are unable to replicate in the absence of helper function, are sensitive to desiccation, and have a short half life. In the event of infection the vast majority of the population have been pre-exposed to Ad5 and have active antibody titres.

Accidental contamination of workers in the suite -
Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. Established cell lines (mouse and human) are unable to colonise human hosts.

Security -
The building has 24 hour security, and swipe card only access, visitors must report to the security and be escorted whilst in the building.

The capacity of the vector to integrate into the genome carries the risk of insertional mutagenesis. However, this risk would be comparable to that of retroviral vectors which do integrate by nature.

Risk assessment
Expressible DNA is deliberately inserted in frame downstream of a strong promoter. Expression Factor 1.
A biologically active substance which might have a deleterious effect if delivered to a target tissue. Damage Factor 10.3
The vector is non-mobilisable and especially disabled. Access Factor 10-12.
Overall risk factor is 10-15

However, in view of the easy "access" of adeno vectors, all handling will be strictly under Containment Level 2.
This is based on advice from HSE and takes into account the fact that work with the wild-type vector requires containment at level 2. However, attention is drawn to the fact that in the presence of genes with immune stimulatory products, particularly in the vectors with E3 deletion, there is increased risk of induction of acute inflammatory responses in case of accidental exposure. However, by the same token, the increased immune stimulatory nature of these vectors will reduce the risk of a chronic infection.
and chronic adverse effects. Therefore despite the replication incompetence of these vectors, and the safe record of use in human trials, they will be handled with strict adherence to containment level 2 requirements and avoidance of aerosol generation, to reduce the risks of accidental respiratory infections.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste -
All contaminated waste is autoclaved in the departmental autoclave that is used only for waste. The moist heat autoclave is used at 121 degrees C for 60 minutes. Temperature validation provided by means of thermocouple probe. The autoclave is serviced annually by Meadowrose Scientific and documentary evidence of correct function stored on site. Under these conditions complete kill of organisms is guaranteed. The resultant sterile waste is double bagged in yellow bags and taken away and incinerated by Medical School contractors (White Rose).

Liquid waste -
All liquid waste is inactivated with 10,000 ppm of Chlorine for greater than 10 hours after which it is disposed of down the designated sink. Under these circumstance complete kill of organisms is guaranteed.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

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AN INVESTIGATION INTO THE MECHANISMS GOVERNING THE EXPRESSION OF HUMAN CD28

**Purposes of the contained use**

To determine the consequences of the expression full length and splice variant cDNA constructs of human and mouse CD28 in established human and mouse tissue culture cell lines.

**Recipient or parental organism**

Bacteria - Attenuated and mobilisation defective E. coli (K-12) derivatives such as DH5 and XL1 Blue, unable to survive in the absence of specialised growth conditions.

Retroviral packaging cells -

GP+E-86, murine fibroblast derived, split helper function, Ecotrophic for mouse, human complement sensitive

PG13, murine fibroblast derived, split helper function, restricted amphotrophic, human complement sensitive

Unable to colonise human hosts, unable to survive in the absence of specialised tissue culture environs

Established human and mouse tissue culture cell lines - NK, T-cell and myeloid cells - Spontaneously immortalised, unable to colonise human hosts, unable to survive outside specialised tissue culture environs.

**Host/vector system**

Cloning vectors - Puc 13 and TOPO, in bacterial (K-12) hosts such as DH5 and XL1 Blue, unable to replicate in nonbacterial cells.

Disabled retroviral expression vector - pBabe. Puro. In the plasmid form - Able to replicate only in bacterial cells and transiently in SV40 transformed cells. In the disabled retroviral vector form unable to replicate in the absence of helper virus. Attenuated by the removal of the pol and env genes and partial deletion and repression of gag translation, compliment sensitive when secreted from mouse derived GP+E86 and PG 13 packaging cells, absence of gag, pol and env sequences reduces recombination.
likelihood with helper virus sequences.

Retroviral packaging cell lines - The human complement sensitive (restricted amphotrophic) PG13 and the Ecotropic GP-E86. Split helper function increase the number of recombination events required to generate replication competent retrovirus. The presence of replication competent retroviral vectors is determined on cells banks by testing the ability of infected target cells to secrete infectious virus. Bank samples are cultured for limited (10) passages before destruction.

Human and mouse tissue culture cell lines - Unable to colonise humans, spontaneously immortalised, no evidence of the ability to secrete transmissible agents, unable to supply helper functions to disabled retroviral vectors.

Origin & function

The genetic material originates as mRNA from human and mouse T-cells. The intended function of the genetic materials is that the full length transcript cDNA and the splice variants should be expressed in human and mouse tissue culture cell lines to allow the determination of the ability of such transcripts to be detected by antibodies directed against CD28. In addition the function of these protein products will be tested for the ability to bind the counter-receptor B7 and activated downstream T cell activation events.

Evaluation of foreseeable effects

Waste - all waste material is dealt with as described under Section 12 - This covers the deliberate removal of material from the laboratory environment.

Accidental escape into the environment - All air is filtered (Hepa) before venting to the environment, the labs are provided with spill kits and SOPs for dealing with accidental spillage. All manipulations with bacteria take place at containment level 1. Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to the gut lining. All manipulations with retroviral packaging cells, retroviral vectors and established human tissue culture cell lines take place at containment level 2. Cell lines are unable to survive outside the laboratory environment. Retroviral vectors are unable to survive in the presence of human complement. White coats and gloves are mandatory.

In the event of escape of disabled retroviral vectors they are unable to replicate in the absence of helper function, are sensitive to desiccation, and have a half life of only 5-8 hours under optimum laboratory conditions. In addition PG13derived vectors are unable to infect recipient permissive cells in the presence of normal complement. GP+E86 derived vector are unable to infect cells other than mouse. In the event of infection the protein product of the cDNA that would be integrated reflects that found in normal tissue and has not been designed for high expression.

Accidental contamination of workers in the suite - Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. Established cell lines (mouse and human) are unable to colonize human hosts. Disabled retroviral vectors are sensitive to human complement and in the case of GP+E86 derived vectors unable to infect humans. In addition the retrovirus is unable to replicate in the absence of halper functions and the protein product of the cDNA that would be integrated reflects that found in the normal human and has not been designed for high expression.

Security - The building has 24 hour security, and swipe card only access, visitors must report to the security and be escorted whilst in the building.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste - all contaminated waste is autoclaved in the departmental autoclave that is used only for waste. The moist heat autoclave is used at 121 degrees C for 20
minutes. Temperature validation provided by means of thermocouple probe. The autoclave is serviced annually by Meadowrose Scientific and documentary evidence of correct function stored on site. Under these conditions complete kill of organisms is guaranteed. The resultant sterile waste is double bagged in yellow bags and taken away and incinerated by Medical School contractors.

Liquid waste - all liquid waste is inactivated with 10,000ppm of Chlorine for greater than 10 hours after which it is disposed of down the designated sink. Under these circumstances complete kill of organisms is guaranteed.

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Project Ref 386/02.4

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Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

T-cells secrete a wide variety of cytokines that mediate their different effector functions. We propose to study one possible mechanism by which T-cells execute their effector function, by knocking out their ability to produce IFN-y. Our strategy to achieve that is by expressing an intracellular antibody (intrabody) inside the endoplasmic reticulum (ER). This intrabody will bind to IFN-y in the ER and hopefully prevent it from being secreted. These cells will be used in our different models and systems which will help us elucidate effector functions of T-cell derived IFN-g.

Recipient or parental organism

Recipient organisms are as listed below (hosts). No additional recipient organisms will be used.

Bacteria - attenuated and mobilisation defective E. coli (K-12) derivatives such as DH5 and XL1 Blue, unable to survive in the absence of specialised growth conditions.

Retroviral packaging cells -
GP+E-86, murine fibroblast derived, split helper function, Ecotrophic for mouse, human complement sensitive
PG13, murine fibroblast derived, split helper function, restricted amphotrophic, human complement sensitive.

Unable to colonise human hosts, unable to survive in the absence of specialised tissue culture environs.

Established human and mouse tissue culture cell lines - NK, T-cell and myeloid cells - Spontaneously immortalised, unable to colonise human hosts, unable to survive outside specialised tissue culture environs.

Host/vector system

Cloning vectors - pAK-100, pUC-18 derivatives, in bacterial (K-12) hosts such as DH5 and XL1 Blue, unable to replicate in nonbacterial cells.

Mammalian expression vectors - pCDNA-3 (Invitrogen) - Able to replicate only in bacterial cells and transiently in SV40 transformed cells.

Disabled retroviral expression vector - pBabe. Puro. In the plasmid form - Able to replicate only in bacterial cells and transiently in SV40 transformed cells. In the disabled retroviral vector form unable to replicate in the absence of helper virus. Attenuated by the removal of the pol and env genes and partial deletion and repression of gag translation, compliment sensitive when secreted from mouse derived GP+E86 and PG13 packaging cells, absence of gag, pol and env sequences reduces recombination likelihood with helper virus sequences.

Retroviral packaging cell lines - The human complement sensitive (restricted amphotrophic) PG13 and the Ecotrophic GP+E86. Split helper function increase the number of recombination events required to generate replication competent retrovirus. The presence of replication competent retroviral vectors is determined on cells banks by
testing the ability of infected target cells to secrete infectious virus. Bank samples are cultured for limited (10) passages before destruction.

Human and mouse tissue culture cell lines. Unable to colonise humans, spontaneously immortalised, no evidence of the ability to secrete transmissible agents, unable to supply helper functions to disabled retroviral vectors.

Origin & function

The genetic material originates as mRNA from mouse hybridoma cell line. The intended function of the genetic materials is that the partial transcript cDNA should be expressed in rat and mouse tissue culture cell lines and primary T-cells. These cells will be tested for their ability to produce IFN-y in the presence of the intrabody. In case down regulation of IFN-y production is observed these cells will be further tested in assays to determine T-cell function.

Evaluation of foreseeable effects

Waste - All waste material is dealt with as described under section 12 - This covers the deliberate removal of material from the laboratory environment.

Accidental escape into the environment - All air is filtered (Hepa) before venting to the environment, the labs are provided with spill kits and SOP’s for dealing with accidental spillage. All manipulations with bacteria take place at containment level 1. Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. All manipulations with retroviral packaging cells, retroviral vectors and established human tissue culture cell lines take place at containment level 2. Cell lines are unable to survive outside the laboratory environment. Retroviral vectors are unable to survive in the presence of human complement. White coats and gloves are mandatory.

In the event of escape of disabled retroviral vectors they are unable to replicate in the absence of helper function, are sensitive to desiccation, and have a half life of only 5-8 hours under optimum laboratory conditions. In addition PG13 derived vectors are unable to infect recipient permissive cells in the presence of normal complement. GP+E86 derived vector are unable to infect cells other than mouse. In the event of infection the protein product of the cDNA that would be integrated reflects that found in normal tissue and has not been designed for high expression.

Accidental contamination of workers in the suite - Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. Established cells lines (mouse and human) are unable to colonize human hosts. Disabled retroviral vectors are sensitive to human complement and in the case of GP+E86 derived vectors unable to infect humans. In addition the retrovirus is unable to replicate in the absence of helper functions and the protein product of the cDNA that would be integrated reflects that found in the normal human and has not been designed for high expression.

Security - The building has 24 hour security, and swipe card only access, visitors must report to the security and be escorted whilst in the building.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste - all contaminated waste is autoclaved in the departmental autoclave that is used only for waste. The moist heat autoclave is used at 121 degrees C for 20 minutes. Temperature validation provided by means of thermocouple probe. The autoclave is serviced annually by Meadowrose Scientific and documentary evidence of correct function stored on site. Under these conditions complete kill of organisms is guaranteed. The resultant sterile waste is double bagged in yellow bags and taken away and incinerated by Medical School contractors.

Liquid Waste - All liquid waste is inactivated with 10,000ppm of Chlorine for greater than 10 hours after which it is disposed of down the designated sink. Under these
circumstance complete kill of organisms is guaranteed.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

The SC of the GMSC approved this project as a Class 2 activity.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 Yes</td>
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Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tbody>
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**Project Ref** 386/02.5

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<tr>
<td>09/04/2002</td>
<td>1. TRANSIENT STIMULATION OF CELL GROWTH BY PROTEIN TRANSDUCTION OF GROWTH STIMULATORY AGENTS. 2. MODIFICATION OF MAMMALIAN CELLS WITH TUMOUR...</td>
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Date Project Ceased  

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<tr>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td></td>
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</tbody>
</table>

Non-GMM

Consent Granted  

| not applicable |

Project notified under transitional arrangements  

| N |

Withdrawn  

| N |

Tick if notifying a connected programme of work  

| Y |

Historical Significant Changes  

Project transferred to GM543 on 08/09/2003 PROJECT REOPENED AS T
Project Additional Information

Purposes of the contained use

To determine the effect of SV40 Large T antigen and tumour-suppressor genes such as Apoptin (VP3), p53, p16, p19ARF and pRB proteins on a range of cells, when added exogenously as fusions with the TAT protein transduction peptide.

Recipient or parental organism

SV40 Large T:
Bacteria - Attenuated and mobilization defective E. coli (K-12) derivatives such as ER 1647, DH5-a and XL1 Blue, unable to survive in the absence of specialised growth conditions.
Human fibroblasts - Detroit 551 cells, non-immortalized, limited replicative life span, unable to colonize human hosts, unable to survive outside specialized tissue culture environs.

Apoptin (VP3), p53, p19ARF and pRB proteins:
Retroviral packaging cells: GP+E86, murine fibroblast derived with split helper function, ecotrophic for mouse, sensitive to human complement. PG13 murine fibroblasts derived, split helper function, restricted amphotropic, sensitive to human complement.
Established and primary mouse and human tissue culture cell lines: epithelial, fibroblasts and myeloid types. These cell lines are spontaneously immortalised, unable to colonise in human hosts, unable to survive outside special tissue culture environments.

Host/vector system

SV40 Large T:
* Cloning vector - pET22b (Novagen Ltd), in bacterial host, unable to replicate in nonbacterial cells. No expression of specific gene products in the absence of exogenously provided T7 polymerase AND isopropyl-thiogalacto-pyranoside (IPTG).

Apoptin (VP3), p53, p16, p19ARF and PRB proteins:
* PBABE and MFG series of retroviral vectors with murine leukaemia virus derived LTR promoter. These vectors are produced by safe packaging cell lines such as GP+E68 and PG13. These vectors have an LTR promoter and infect only rodent cells (GP+E68) or a wide range of cells including human (PG13). No inherent pathogenicity other than low risk of insertional mutagenesis is associated with these viruses.
* Mobilisation defective pUC based vectors with large array of different promoters such as SV40, CMV. Bacterial expression vectors such as pTriEx-1, in disabled bacterial hosts such as E. coli K-12 auxotrophic mutants for propagation.
* Spontaneously immortalised human and mouse tissue culture cell lines, unable to colonise humans. There is no evidence of the ability of these cells to secrete transmissible agents. These cells are unable to supply helper functions to disabled retroviral vectors.

Origin & function

SV40 Large T
* The genetic material originates as mRNA from human and/or mouse cells. The indicated function of the genetic materials is growth stimulatory.

Apoptin (VP3), p53, p16, p19ARF and pRB proteins:
* The genetic material originates as mRNA from human and/or mouse cells. The indicated function of the genetic material is that the full length cDNA products are tumour suppressors, including FHIT, p16/INK4a, p19/ARF, p21/waf1 and p53. These are cloned into mobilisation defective plasmid vectors including bluescript pCDNA3.1, pTRE2, pSVNeo and/or pBABE replication incompetent retroviral vector and will be expressed in human and mouse tissue cancer cell lines to determine their ability to apoptose.
Evaluation of foreseeable effects

Waste - All waste material is dealt with as described under Section 12 - This covers the deliberate removal of material from the laboratory environment.

Accidental escape into the environment;
a) from bacteria: All manipulations with bacteria will take place at containment level 2. Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. In the absence of combination stimulation of induction, expression of the protein in bacteria will be virtually zero. Protein preparations from induced bacteria will be via detergent lysis, to avoid aerosol production.
b) From tissue culture - All air is filtered (Hepa) before venting to the environment, the labs are provided with spill kits and SOPs for dealing with accidental spillage. All manipulations with human tissue culture cell lines take place at containment level 2. Cell lines are unable to survive outside the laboratory environment. White coats and gloves are mandatory. In the event of escape of disabled retroviral vectors, hazard is unlikely because they are unable to replicate in the absence of helper function and are sensitive to dessication and have a half life of only 5-8 hours under optimum laboratory conditions. In addition, PG13 derived vectors are unable to infect recipient permissive cells in the presence of normal complement. GP+E86 derived vectors are unable to infect cells other than mouse. In the event of infection and integration, the protein products of the cDNA being examined in this way (e.g. apoptin(VP3), p53, p16, p19ARF and pRB proteins) do not have deleterious effects in normal animal cells.

Accidental contamination of workers in the suite - Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. The vector DNA is unable to propagate or express in human cells. Established cell lines used are unable to colonize human hosts.

Security - The building has 24 hour security, and swipe card only access, visitors must report to the security and be escorted whilst in the building.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste - all contaminated waste is autoclaved in the departmental autoclave that is used only for waste. The moist heat autoclave is used at 121 degrees C for 40 minutes. Temperature validation provided by means of thermocouple probe. The autoclave is serviced annually by Meadowrose Scientific and documentary evidence of correct function stored on site. Under these conditions complete kill of organisms is guaranteed. The resultant sterile waste is double bagged in yellow bags and taken away and incinerated by Medical School contractors (White Rose).

Liquid Waste - All liquid waste is inactivated with 10,000 ppm of Chlorine for greater than 10 hours, after which it is disposed of down the designated sink. Under these circumstances complete kill of organisms is guaranteed.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The GMSC approved the RA for this project Activity Class 2.

### Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### Project Ref 386/02.6

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<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<td></td>
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<tbody>
<tr>
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</table>

### Project Additional Information

**Purposes of the contained use**

The aim of this project is to inhibit HIV infection of CD4+ T cells by expression of cellular factors which are able to block one or more of the steps required for a productive virus infection.

**Recipient or parental organism**

Bacterial hosts:  E coli, K-12 DH5, XL1 blue, and similar DISABLED bacterial hosts. Unable to survive in the absence of specialised growth conditions.
Retroviral packaging cells: GP+E86, murine fibroblast derived, split helper function, ecotropic for mouse, human complement sensitive, PG13, murine fibroblast derived, split helper function, restricted amphotropic, human complement sensitive, unable to colonise human hosts, unable to survive outside specialised tissue culture conditions. Murine and Human primary and established cell lines: T cell, HeLa, murine fibroblasts - spontaneously immortalized unable to colonise human hosts, unable to survive outside specialised tissue culture conditions.

Cloning vectors: pUC13 and TOPO bacterial plasmids, in bacterial (K-12) hosts, unable to replicate in non-bacterial cells.
Disabled retroviral expression vector: pBabe.puro, in the plasmid from - able to replicate only in bacterial cells and transiently in SV40 transformed cells. In the disabled retroviral vector form, it is unable to replicate in the absence of helper virus. Attenuated by the removal of the pol and env genes and partial deletion and repression of gag translation. It is compliment sensitive when secreted from mouse derived GP+E86 and PG13 packaging cells. Absence of pol, env, and gag sequences reduces the likelihood of recombination with helper virus sequences.
Retroviral packaging cell lines: The human sensitive (restricted amphotropic) PG13 and the ecotropic GP+E86 cells will be employed to package recombinant retrovirus. Split helper function increases the number of recombination events required to generate replication competent retrovirus. The presence of replication competent retroviral vectors is determined on cell banks by testing the ability of infected target cells to secrete infectious virus. Bank samples are cultured for limited (10) passages before destruction.
Murine and Human primary and established tissue culture cell lines: Unable to colonize humans, spontaneously immortalised, no evidence of the ability to secrete transmissible agents, unable to supply helper functions to retroviral vectors.

Host/vector system

Cloning vectors: pUC13 and TOPO bacterial plasmids, in bacterial (K-12) hosts, unable to replicate in non-bacterial cells.

Disabled retroviral expression vector: pBabe.puro, in the plasmid from - able to replicate only in bacterial cells and transiently in SV40 transformed cells. In the disabled retroviral vector form, it is unable to replicate in the absence of helper virus. Attenuated by the removal of the pol and env genes and partial deletion and repression of gag translation. It is compliment sensitive when secreted from mouse derived GP+E86 and PG13 packaging cells. Absence of pol, env, and gag sequences reduces the likelihood of recombination with helper virus sequences.

Retroviral packaging cell lines: The human sensitive (restricted amphotropic) PG13 and the ecotropic GP+E86 cells will be employed to package recombinant retrovirus. Split helper function increases the number of recombination events required to generate replication competent retrovirus. The presence of replication competent retroviral vectors is determined on cell banks by testing the ability of infected target cells to secrete infectious virus. Bank samples are cultured for limited (10) passages before destruction.

Murine and Human primary and established tissue culture cell lines: Unable to colonize humans, spontaneously immortalised, no evidence of the ability to secrete transmissible agents, unable to supply helper functions to retroviral vectors.

Origin & function

The aim of this project is inhibition by expression of host cell and/or viral factors that can block the infection. Therefore the inserts are of mammalian or viral origin and their function in this context will be inhibition of different stages in the viral life cycle.

Evaluation of foreseeable effects

Waste - All waste material is dealt with as described under section 12. This covers the deliberate removal of material from the laboratory environment.
Accidental escape into the environment - all air is filtered (HEPA) before venting to the environment, the labs are provided with spill kits and SOP's for dealing with accidental spillage. All manipulations with bacteria take place at containment level 1. Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to gut lining. All manipulations with retroviral packaging cells, retroviral vectors, and established murine and human tissue culture cell lines take place containment level 2. Cell lines are unable to survive outside the laboratory environment. Retroviral vectors are unable to survive in the presence of human complement. White coats and gloves are mandatory. All work involving HIV will take place at containment level 3. Decontamination and destruction of all cultures and vectors at the end of each culture, without passage of any generated virus from one culture to another. The genetically modified cells will not be used for virus propagation.

In the event of escape of disabled retroviral vectors, they are unable to replicate in the absence of helper function, are sensitive to desiccation, and have a half-life of only 5-8 hours under optimum laboratory conditions. In addition PG13 derived vectors are unable to infect recipient permissive cells in the presence of normal complement. GP+E86 derived vectors are unable to infect cells other than mouse.

Accidental contamination of workers in the suite - Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to gut lining. Established cell lines (mouse and human) are unable to colonise human hosts. Disabled retroviral vectors are sensitive to human complement and in the case of GP+E86 derived vectors unable to infect human hosts. In addition, the retrovirus is unable to replicate in the absence of helper function and the cDNAs expressed do not contain oncogenic sequences nor pathogenic.

Security - The building has 24 hour security, and swipe card only access, visitors must report to the security and be escorted whilst in the building. Containment level 3 laboratory has restricted access to only named persons.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste - all contaminated waste is autoclaved in the departmental autoclave that is used only for waste. The moist heat autoclave is used at 121 degrees C for 20 minutes. Temperature validation provided by means of thermocouple probe. The autoclave is serviced by Meadowrose Scientific and documentary evidence of correct function is stored on site. Under these conditions complete killing of organisms is guaranteed. The resultant sterile waste is double bagged in yellow bags and taken away and incinerated by external contractors (White Rose). The containment 3 facility has an autoclave inside the laboratory.

Liquid waste - All liquid waste is inactivated with 10,000 ppm of Chlorine for greater than 10 hours after which it is disposed of down the designated sink. Under these circumstances complete kill of organisms is guaranteed.

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC approved the RA for this project: Class of activity 3.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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</thead>
<tbody>
<tr>
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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 386/03.1

Date Ackn'd 14/03/2003

CU2 Project Title UTILISATION OF GENE MEDIATED APPROACHES TO DESTROY RESIDUAL

Class 2

Culture Vol Class 2 1-50 litres

Culture Volume Class 3-4
**Project Additional Information**

**Purposes of the contained use**

Studies designed to find effective ways of destroying tumour cells that remain in the body after surgery that are resistant to conventional radiotherapy.

**Recipient or parental organism**

Human, rat and mouse cells maintained in vitro. The overall containment level that will be applied to these studies is 2.

**Host/vector system**

Host: human, murine or rat squamous cells.

Vector systems:

A. Adenovirus and selectively replicating adenovirus.
B. Salmonella species modified to carry either the cytosine deaminase gene or anti-angiogenic peptides.

**Origin & function**

The vectors all induce lysis of rodent and human tumour cells. In the case of the salmonella derived vectors tumour lysis occurs after exposure of the host to the prodrug 5-fluorocytosine. Treatment with vectors carrying anti-angiogenic peptides is designed to prevent outgrowth of blood vessel sprouts. It is anticipated that this treatment will prevent tumour outgrowth and spread.

**Evaluation of foreseeable effects**

Non-replicating adenoviruses or selectively-replicating adenoviruses and Salmonella species are all used with the intent of destroying tumour but not normal cells. There are no foreseeable hazards to human health or the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste. Solid waste will be double-bagged and autoclaved at 121 degrees C for 90 minutes followed by incineration. Sharps are discarded into designated sharps containers for incineration off site by White Rose Environmental.

Surgical instruments. Surgical instruments will be treated with a chlorine-releasing agent (for example HazTabs) and then scrubbed and autoclaved. All our autoclaves are on a service contract and are tested annually to ensure they meet the required specification for temperature and time holding.

Liquid waste. Decontamination with a chlorine-releasing solution at a concentration of greater than 10,000 ppm available chlorine for greater than 10 minutes prior to disposal down a designated sink.

The disinfectant to be used will be a chlorine-releasing cyanurate (Haz Tabs or PreSept), an agent that has good activity against Salmonella species and adenovirus.

The activity of HazTabs against Salmonella was demonstrated by Bloomfield and Miles (Journal of Applied Bacteriology 1979, 46:65-73) using a capacity test which demonstrated a 9log reduction following treatment of a test inoculum of 2.5 x 10 (to the power of 10) cfu/ml of Salmonella typhi. When 20% plasma (equivalent to serum) was included in the test 3,000mg/l of available chlorine was needed to kill Staphylococci. As HazTabs generate 10,000 ppm available chlorine (equivalent to 7,000 mg/l) and the concentration of serum in media will typically be less than 2%, this is a good disinfectant for the work to be carried out and is unlikely to become exhausted (Bloomfield and Uso 1985, Journal of Hospital Infection, 6@20-30).

The efficacy of HazTabs against viruses is shown by studies incorporating dried suspension of Polio virus and demonstrating a 5 log reduction following treatment with 9,200 ppm available chlorine after 1 minute, and at 5 and 10 minutes no virus was recovered, (Tyler R., Alylisse G.A.J., Bradley C. Virucidal activity of disinfectants: Studies with the Poliovirus, Journal of Hospital Infection, 15,339-45).

The work proposed will not generate "high-hazard" waste and we do not propose to test whether organisms can be grown from the waste. However, every care will be taken to ensure that the concentration of chlorine is appropriate and liquid waste is treated for a minimum of 10 minutes to compensate for the presence of serum.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The local GMSC approved this as a class 2 activity.

Project Containment

<table>
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<th>Glass Houses</th>
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02/03/2022
The use of RNA inhibition to assess gene function in primary cultured human myometrial cells. Myometrial cells will be transfected with plasmid vectors containing gene specific antisense gene fragments to inhibit gene translation.

The plasmid vectors and inserts to be used require containment level 1 conditions. The host cells to be transfected will be primary cultured human myometrial smooth muscle cells and passaged human myometrial smooth muscle cells. Primary cultured cells require containment level 2, thus all work will be performed at containment level 2.

The recipient cells will be primary cultured human myometrial smooth muscle cells and also passaged human myometrial smooth muscle cells.

The host cells will be primary cultured human myometrial smooth muscle cells and also passaged human myometrial smooth muscle cells. The vectors we plan to use are non-mobilisable plasmid vectors.
The non-mobilisable plasmid vectors will contain small gene fragments that will generate RNAs that will be processed to 23 mers. Inserts will be chemically synthesised and will be homologous to human genes. Transfection of the plasmid will inhibit gene translation and will enable physiological functions to be determined.

**Evaluation of foreseeable effects**

We plan to transfect myometrial cells with plasmid vectors containing short gene fragments, which will functionally knock out specific genes rather than add genes. Transfected and untransfected human myometrial cells are unable to survive outwith tissue culture facilities, so they will have minimal effect to the environment. Prior to disposal, cells will be inactivated using 50% Virkon. Cells and all culture materials will be incinerated with hospital waste. Cells are unable to colonize laboratory workers. The non-mobilisable plasmid vectors that we plan to use are unable to enter cells without mechanical or chemical manipulation and plasmids are unable to transcribe the inserted gene fragment out with the host cell, there will be minimal risk to the laboratory staff or the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Transfected cells and culture medium will be treated with 50% Virkon which will cause cell inactivation. In addition, cells will be unable to remain viable out of tissue culture facilities. Cells and tissue culture materials will be autoclaved in a vacuum cycle at 135 degrees C for 5 minutes and then incinerated with hospital clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

The assessment was approved by the local GMSC committee on 23 June 2004 with some minor amendments. The class of activity is 2.

The safety audit of the laboratory where the work will be carried out has also been done.

**Project Containment**

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<thead>
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Animal Units Large Scale Activities Human Clinical Applications

02/03/2022 Page 6796 of 15326
Project Ref 386/04.2

Date Ackn'd 29/12/2004

CU2 Project Title NADPH oxidase and redox signalling in cardiovascular pathophysiology.

Date Project Ceased

Class 2 CultureVolClass2 ≤ 1 Litre

Class CultureVolClass2 CultureVolume

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Purposes of the contained use

Lentiviral and adenoviral vectors for use in the transfection of isolated cardiovascular cells and tissues.

Recipient or parental organism

Human and eukaryotic cell lines for viral production and experimental work and prokaryotic cells for vector propagation.

Host/vector system

Lentiviral vectors: pCMV R8.2, pCMV R8.91, pMD.G, pHR'SinctwSV, pLVTHM, etc
Adenoviral vectors: pDC316-p^7m9, pDC316, pShuttle-M1(p47(W193T)), pShuttle-M1, etc
Prokaryotic host cell lines for molecular subcloning: TOP10F', TOP10F, DH5a, XL1-Blue, JM109, DH101, TG1
Eukaryotic host cell lines for testign constructs: COS-7 cells, etc
Eukaryotic host cell lines for producing virus particles: HEK-293 cells
Normal primary culture cells/tissues: primary human or rodent cardiomyocytes, vascular smooth muscle, fibroblasts, endothelial cells and/or aortae or muscle strips.

Lentiviral: Split-genome HIV-1 based lentiviral packaging system to produce replication disabled vectors (generation 2.5; self-inactivating lentiviral system). This includes a plasmid to express lentiviral functional (gag-pol) and/or accessory genes (vpr, vif, rev, tat, ref and nef) driven by a CMV promoter (plasmids pCMV R8.2 and pCMV R8.91 and variants thereof which don't alter the risk factor), attenuated by the removal of the packaging signal, , and the 5' and 3' LTR. The viral genes are prevented from
packaging into the infectious virion and can only apply their functions in trans from the packaging host. A second plasmid encodes a pseudotype envelope protein - Plasmid pMD.G encodes the Vesicular Stomatitis Virus G protein (VSV-G). The use of an envelope pseudotype instead of the HIV envelope minimises the risk of homologous recombination and the generation of wild-type and replication competent t virus. The lentivector will be similar to plasmid (pHR' series) encoding the packageable lentiviral vector to express a heterologous gene of interest transcribed from a strong mammalian promoter such as CMV. These vectors are attenuated by the removal of pol, env and accessory genes and partial deletion and repression of gag translation. All pHR'-derived vectors are self-inactivated by deletion within the U3 region of the 3' LTR, which results in the generation of an inactivated 5' LTR promoter following reverse transcription. Self-inactivated vectors are therefore unable to replicate following integration into target cells even in the presence of helper function, further minimising the risk of generating replication competent virus.

Adenoviral: Adenoviruses differ from lentiviruses in that they remain episomal (do not integrate into the host genome) and therefore are expressed transiently within the infected cells. Replication incompetent adenovirus particles are made via homologous recombination events in HEK 293 cells. The E1 and E3 deletions out of the wild-type adenoviral genome ensures that recombinant adenovirus is not replicative except in HEK 293 cells. The pShuttle system from Clontech is proven safe and efficient and will be the main system used for our adenovirus experiments.

Wherever possible we shall be inheriting vectors containing gene sequences of interest to us via collaboration with other labs. Although the vectors may not be based upon exactly the same parental backbone as presented here, they will be of essentially the same design and will not increase the risk factor.

The cDNA inserted into the vectors described above will be subcloned from gift plasmids or plasmids obtained through the HGMP resource centre. Some cDNAs may be cloned in house using RT-PCR of mRNA isolated from cells or tissues. Antibiotic resistance and other marker genes are from prokaryotic species, green fluorescent protein from Aequorea victoria (jellyfish), transcription factors, enzyme and signalling components are initially cloned from mammalian cells and tissues, including rat, mouse and human. Eukaryotic promoter/enhancer elements from commercially commonly available vectors.

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The project will involve the overexpression of biological factors in cardiovascular cells and tissues in order to elucidate at a molecular level the signalling pathways that mediate pathophysiological effects. Cells such as primary cardiomyocytes, endothelial cells and vascular smooth muscle cells are difficult to transfer efficiently by standard non-viral approaches, thus gene transfer by viral delivery is necessary.

Our overall aim is to study the role of oxidative stress in the pathophysiology of cardiovascular disorders such as left ventricular hypertrophy, heart failure, endothelial dysfunction and atherosclerosis. Oxidative stress acts through (a) direct modification of cellular proteins and membranes; (b) inactivation of the potent signalling molecule nitric oxide (NO); and © modulation of redox-sensitive signal transduction pathways. A family of ROS-generating enzymes, the NADPH oxidases (Noxs), is recognised to be a major source of ROS involved in redox signalling. Work by our group and others has demonstrated an important role for NADPH oxidases in the development of endothelial dysfunction as well as components of the cardiac hypertrophic phenotype. Furthermore, separate Nox isoforms are activated by distinct stimuli and have divergent downstream effects both in the vasculature and the heart. This programme of work will use a combination of ex vivo preparations and cultured cell systems to investigate: (i) the mechanisms of activation of different Nox isoforms in cardiovascular cells; (ii) the relative roles of different Nox isoforms in cardiovascular cells; (iii) the mechanisms of ROS-mediated cardiac contractile dysfunction; (iv) therapeutic approaches to inhibiting NADPH oxidase activity.

Inserts will comprise components of the NADPH oxidase enzyme complex either as wild-type or dominant-negative versions and short hairpin cDNA complexes designed to disrupt the function of the NADPH oxidase complex. Infection of cell lines and the virus particles made with these inserts will be expected to have perturbed physiological responses and/or transcriptional profiles. The production of these altered cells lines will allow pathophysiological experiments to be done testing the function of each of the components of the NADPH oxidase complex. These studies will substantially increase our understanding of the roles of NADPH oxidase in the pathophysiology of left ventricular hypertrophy and endothelial dysfunction, and may provide the basis for the development of novel therapeutic strategies for human disease.

Expression of the cDNA moleculars described above are intended to produce biologically active products. The expressed products will have activity relevant to the experimental system being studied. Thus cDNA encoding NADPH oxidases, will be expected to perturb physiological responses of the eukaryotic cells that involve the
generation of reactive oxygen species (e.g., in redox signalling).

None of the genes or DNA fragments to be used encode a known oncogene. The materials and organisms to be used present little potential hazard since no known pathogens or oncogens are involved. The prokaryotic hosts and eukaryotic cells to be used are unlikely to survive outside of the culture conditions, colonise animals or humans or transfer genetic material to other hosts.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The risk assessment for prokaryotic work indicates that it can safely be carried out at containment level 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste products, such as agar plates, plastics etc. contaminated with GM material will be disposed to autoclave bags (double bagged to prevent penetration) which will be sterilised by autoclaving at 121 degrees for 30 min. Bags of autoclaved waste will be placed in yellow bags for incineration.

Waste cell culture medium from cultures containing GM eukaryotic cells and growth medium from GM prokaryotic cell cultures will be sterilised by adding chlorine release tablets (e.g., Presept) or hypochlorite to provide a concentration of 10,000 ppm chlorine and standing in a Microbiological Safety Cabinet for at least 3h. The sterilised medium will be disposed of down the sink with copious amounts of water.

Sharps including pipette tips will be collected in designated sharps bins, which will then be autoclaved as above and incinerated.

These are standard procedures and are recognised to kill all GM organisms and brownning tubes are used to monitor the "kill"

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The local GMSC approved the Risk Assessment for this project on 08/11/04. The class of activity is 2.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 Yes</td>
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Use of RNA inhibition to assess gene function

The plasmid vectors and inserts to be used require containment level 1 conditions. The host cells to be transfected will be primary cultured and passaged human cells, including T lymphocytes, dendritic cells and airways smooth muscle cells. Primary cultured cells require containment level 2, thus all work will be performed at containment level 2.

The recipient cells will be primary cultured and also passaged human cells detailed above.

The host cells will be primary cultured and also passaged human T lymphocyte cells. The vectors we plan to use are non-mobilisable plasmid vectors.

The non-mobilisable plasmid vectors will contain small gene fragments that will generate RNA’s that will be processed to 23 mers. Inserts will be chemically synthesised and will be homologous to human genes. Transfection of the plasmid will inhibit gene translation and will enable physiological functions to be determined. cDNA’s will be derived by pcr from pre-existing cDNA libraries.
**Evaluation of foreseeable effects**

We plan to transfect cells with plasmid siRNA vectors containing short gene fragments, which will functionally knock out specific genes. The duration of our transfection experiments will be 1-7 days. Secondly, we will overexpress certain cDNA's. Transfected and untransfected human cells are unable to survive out with tissue culture facilities, so they will have minimal effect on the environment. Prior to disposal, cells will be killed using 1% Virkon. Cells and all culture materials will be autoclaved. Cells are unable to colonise laboratory workers. The non-mobilisable plasmid vectors that we plan to use are unable to enter cells without mechanical or chemical manipulation and plasmids are unable to transcribe the inserted gene fragment out of the host cell, there will be minimal risk to the laboratory staff or the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Transfected cells and culture medium will be treated with 1% Virkon which will cause cell death. In addition, cells will be unable to remain viable out of tissue culture facilities. Cells and tissue culture materials will be autoclaved in a vacuum cycle at 121 degrees for 20 minutes and then incinerated with hospital clinical waste.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

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**Project Containment**

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**Animal Units**

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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 386/05.2

Date Ackn'd: 04/03/2005

CU2 Project Title: Regulation of T-cell differentiation & cytokine gene expression

Class: Class 2
CultureVol: Class 2 Culture Volume: < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Tick if notifying a connected programme of work

Project Additional Information

Purposes of the contained use

The plasmid vectors and inserts to be used require containment level 1 conditions. The host cells to be transfected will be primary cultured and passaged human cells, including 293 cells, T lymphocytes, dendritic cells and airways smooth muscle cells. Primary cultured cells and lentiviral transduction requires containment level 2, thus all work will be performed at containment level 2.

Recipient or parental organism

The recipient cells will be primary cultured and also passaged human cells detailed above.

Host/vector system

The host cells will be 293 cells and primary cultured and also passaged human cells detailed above. The vectors we plan to use are a lentivirus system LentiLox. The vector systems used a third generation packaging system and generates self-inactivating lentivirus. Viral particles will be generated by cotransfection into 293 cells.

Origin & function

The lentiviral vectors are pMDLg/pRRE, pMD.G, pRSV-Rev and pLentiLox 3.7 and have been described in detail: D.A. Rubinson et al. Nature Genetics vol. 33 pages 401-406 (2003).

The lentiviral vectors will contain small gene fragments that will generate RNA’s that will be processed to 23 mer duplexes. Inserts will be chemically synthesised and will be homologous to human genes. Transduction of the virus will inhibit gene translation and will enable physiological functions to be determined. cDNA’s will be derived by polymerase chain reaction from pre-existing cDNA libraries.
We plan to transduce cells with lentiviral siRNA vectors containing short gene fragments, which will functionally knock out specific genes. The total duration of these manipulation experiments will be up to 28 days. Transduced human cells are unable to survive tissue culture facilities, so they will have minimal effect to the environment. Prior to disposal, cells will be killed using 1% Virkon. Cells and all culture materials will be autoclaved. Cells are unable to colonize laboratory workers. The lentiviral vectors that we plan to use require cotransfection with three packaging vectors and are unable to enter cells without mechanical or chemical manipulation and plasmids are unable to transcribe the inserted gene fragment out of the host cell, there will be minimal risk to laboratory staff or the environment.

Evaluation of foreseeable effects

N/A

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Transduced cells and culture medium will be treated with 1% Virkon for more than 15 mins which will cause death. In addition, cells will be unable to remain viable out of tissue culture facilities. Cells and tissue culture material will be autoclaved in a vacuum cycle at 121 degrees for 20 mins and then incinerated with hospital clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The assessment was approved by the local GMSC committee on 23rd June 2004, with some minor amendments. The class of activity is 2.

A safety audit of the laboratory where the work will be carried out has also been done.

Project Containment

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Large Scale Activities

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Human Clinical Applications

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Disabled retroviral vectors (including both murine and lentivirus based vectors) provide efficient vehicles for gene transfer and expression in both primary and established mammalian cells.

This project concerns the development of novel retroviral (and lentiviral) vector inserts, constructs, packaging cells and modes of 2 optimised transduction, for the, efficient delivery and expression of single and multiple genes to target cells.

We have previously developed strategies for the use of paramagnetic particle (PMP) for capture and concentration of retroviral and lentiviral vectors, resulting in increased vector titre and/or infectivity by several orders of magnitude. We now wish to continue this work for achievement of even greater vector titres and for targeted infection of specific cell types. These are both still major obstacles in the clinical application of gene therapy. As part of these studies we are developing new retrovirus/lentivirus producing cells and will test new vector concentration and targeting strategies. These vectors will also be used for the expression of immune modulatory factors, in order to induce immunological reactions (primarily cytotoxic T cell mediated responses) against solid tumours and leukaemia.

We have recently been awarded permission by Gene Therapy Advisory Committee (GTAC) of the Department of Health for a E Phase-I clinical study, to assess the effect of vaccination with genetically modified myeloid leukaemic cells that have been engineered to express B7.1 (an immune costimulatory molecule expressed on the surface of antigen presenting cells) and IL-2 (a T cell stimulatory cytokine). This is achieved by Infection of primary and established leukaemic cells with a replication defective, self-inactivating, lentivirus vector encoding B7.1 and IL-2. We are now in discussion with Medicines and Healthcare products Regulatory Agency (MHRA) for the production of clinical grade HIV based lentivirus vector (self inactivating vectors encoding B7.1 and IL-2), in the GMP facility in the Rayne Institute at Denmark Hill (Rayne Cell Therapy Suite).
Bacteria – K12 derived E. coli strains such as: DH5α (F−λ80d/acZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17(rl, Mh) supE44 thi-1 gyrA96 relA1), STBL2 (F−mcrA Δ(mcrBC-hsdRMS-mrr) recA1 endA1 lon gyrA96 thi-1 supE44 relA1 Δ(lac-proAB)), and other such disabled and/or non-colonising E.coli strains that are unable to survive in the absence of specialized growth conditions.

Retroviral packaging cells -
- GP+env E-86, murine fibroblast derived, split helper function, ecotropic for mouse, human complement sensitive.
- GP+env AM12, murine fibroblast derived, split helper function, amphotropic, human complement sensitive.
- PT67 murine fibroblast derived, split helper function, amphotropic, human complement sensitive.
- PG13 murine fibroblast derived, split helper function, restricted amphotropic, human complement sensitive.
- 293 GPG7-VSV-G, human 293 derived, split helper function, xenotropic, human complement sensitive.
- Tally (TELceB6-Ampho, TELceB6-RDII4)- human TE671 derived, split helper function, amphotropic, limited human complement sensitivity.
- FLYA13, FLY-RD114 - Human HT1080 derived, split helper function, amphotropic, minimal human complement sensitivity. Unable to colonize human hosts, unable to survive in the absence of specialized tissue culture environs.
- Phoenix-A and Phoenix-E - human 293 derived, split helper function, amphotropic (A) and ecotropic (E), human complement sensitive.

Lentiviral producer cells -
- STAR-Ampho, STAR-RDII4, 293T and modified derivatives as hosts for transient lentiviral vector production.

Recipient cells
Established human and mouse tissue culture cell lines such as 293T, TE671, HTI080, HeLa, K562, HL-60, NB4, U937, NIH3T3. Primary human cells, such as acute myeloid leukaemia blasts, peripheral blood T-cells, CD34+ myeloid cells, as well as other primary and established mammalian cells.

All cell types to be utilized will be unable to colonize human hosts and unable to survive outside specialized tissue culture environs.

Host/vector system
Cloning vectors - Puc 13 and Pcr 2.1-TOPO, in bacterial (K-12) hosts such as DH5α and XL1 Blue, unable to replicate in nonbacterial cells.

Plasmid eukaryotic expression vectors - such as pSecTag2A, pIRESPuro3

Disabled retroviral expression vector - pWzl.blast, pBabe.puro, pIL2B7.blast, pEGF.blast, pBMN-GFP, pBMN-Z pBabe. ΔLNGFR. In the plasmid form - able to replicate only in bacterial cells and (in some cases) transiently in SV40 transformed cells. In the disabled retroviral vector form unable to replicate in the absence of helper virus. Attenuated by the removal of the pol and env genes and partial deletion and repression of gag translation, compliment sensitivity conferred by packaging cell. Absence of gag, pot and env sequences in the vector, and the use of split function packaging cells, reduces likelihood of recombination-mediated generation of replication competent retrovirus.

Retroviral packaging cell lines -
- GP+env E-86, murine fibroblast derived, split helper function, ecotropic for mouse, human complement sensitive.
- GP+env AM12, murine fibroblast derived, split helper function, amphotropic, human complement sensitive.
- PT67 murine fibroblast derived, split helper function, amphotropic, human complement sensitive.
- PG13 murine fibroblast derived, split helper function, restricted amphotropic, human complement sensitive.
- 293 GPG7-VSV-G, human 293 derived, split helper function, xenotropic, human complement sensitive.
- FLYA13, FLY-RD114 - Human HT1080 derived, split helper function, amphotropic, minimal human complement sensitivity. Unable to colonize human hosts, unable to survive in the absence of specialized tissue culture environs.
- Phoenix-A and Phoenix-E - human 293 derived, split helper function, amphotropic (A) and ecotropic (E), human complement sensitive.
These vectors will be generated in split-genome packaging/producer cells in order to reduce the risk of recombination-mediated acquisition of the deleted genes in the vector. For the retroviral vectors this will be achieved by transfection of at least two plasmids one encoding gag and pol another the env gene. The split helper function increases the number of recombination events required to generate replication competent retrovirus. The absence of replication competent retrovirus is determined by testing the infected target cells for production of infectious virus.

Lentiviral helper plasmids and vectors

Helpers - pCMVΔR8.2, pCMVΔR8.91, pMDLg/pRRE, pRSV-rev, pMDG, pFBSALF,
- Vectors - Replication defective (helper dependent) HIV based lentiviral vectors (e.g. pLenti6N5-GW/lacZ, or vectors with the prefix HR' or RRL' in the list below). Other HIV based lentiviral vector will be both replication defective and self-inactivating due to the removal of a 400 base pair deletion in the U3 region of the 3'LTR (vectors with SIN in the their name).
- Non-exhaustive list of current vectors: pH'CMV/IacZ, pH'R'CMVGFP/SIN, pH'R'PGK/GFPWSIN, pH'R'SINcPPT-SEW, pLenti6N5-GW11acZ, pH'R'SINctwSVGFP, pH'R'SINctwSV71, pH'R'SINctwSV12L271, pH'R'SINctwSV12L2871, pH'R'SINctwSVGFP, pH'R'SINctwSVIL-2/87.1.

In the plasmid form - replication is restricted to bacterial cells and (in some cases - plasmids with SV40 origin of replication) in transiently in SV40 transformed cells. In the disabled vector form unable to replicate in the absence of helper functions.

Lentivirus production will involve the co-transfection of the vector plasmid together with at least two other plasmids encoding gag, pol, env and the accessory genes (vpr, vif, rev, tat and nef). The absence of packaging signal, \( \psi \), in the plasmids encoding the a structural or accessory proteins will prevent the packaging of these sequences into infectious virions, other than by recombination events. The generation of a replication competent lentivirus will require multiple recombination events, making this very unlikely. In any event the self-inactivating vectors, will have a substantially reduced chance of replication in target cells, even in the presence of helper functions, further minimising the risk of generation of replication competent virus.

No retroviral vectors and only some lentivirus vectors (the self inactivating lentivirus vectors) used in the study will contain an N enhancer of gene expression derived from woodchuck hepatitis virus (WHV) called the woodchuck post-transcriptional regulatory c element, WPRE. This element is capable of expressing part of the X protein from WHV. The truncated or full-length hepadna W virus X-proteins may have oncogenic properties. Although this has not been proven, we will treat this as a potential risk in accordance with the recommendations of SACGM.

Human and mouse tissue culture cell lines or primary cells - Unable to colonize humans, spontaneously immortalized, no evidence of the innate ability to secrete transmissible agents, unable to supply helper functions to disabled retroviral vectors.

Origin & function

Vectors: Expression plasmids containing eukaryotic promoters (of viral and eukaryotic origin). Plasmids encoding retroviral and lentivirus vectors, including HIV based lentivirus vectors. Retro- and lentivirus vectors.

Source of Inserts: Human and rodents (e.g. IL-2, B7.1, ALNGFR, etc.), Bacterial (LacZ, Biotin ligase, biotin synthase, blastcidin SR, PuromycinR) or Jelly Fish, Aequorea victoria (gfp).

Function:

Immunomodulatory Factors - The expression of factors such as IL-2 and B7.1 will promotes immune recognition and stimulate a immunological responses against the transduced cells expressing these factors, and also against non-transduced cells with similar antigenic properties. Therefore, a potential hazard is the induction of autoimmune disease, due to enhanced immunological reactions.

Marker genes - Drug resistance genes such as blastcidin SR, PuromycinR, etc., enable the drug mediated selection of the transduced cells, where as marker genes such as LacZ, GFP, ALNGFR, etc., allow the detection of the transduced cells either N by their inherent chomogenicity (GFP) or use of precursor agents converted to chomogenic products (LacZ) or the antibody mediated recognition of the transduced cells (ALNGFR). Other factors such as bacterial biotin synthase and biotin ligase promote the synthesis and transfer of biotin to target proteins, allowing targets for avidin mediated recognition (staining, concentration, etc.) of the biotin labeled proteins or...
viral particles containing such labeled proteins on their outer surface.

**Evaluation of foreseeable effects**

Accidental escape into the environment - EXTREMELY UNLIKELY: All air is filtered (Hepa) before venting to the environment, the labs are provided with spill kits and SOPs for dealing with accidental spillage. All manipulations with bacteria take place at containment level 1. Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. All manipulations with retro- and lenti-iviral vectors, their packaging cells, as well as their primary and established cell targets will take place at containment level 2. Cells are unable to survive outside the laboratory environment. White coats and gloves are mandatory. Disabled retroviral and lentiviral vectors are unable to replicate in the absence of helper function, are sensitive to desiccation, and have a half life of only 5-8 hours under optimum laboratory conditions.

All waste material is dealt with as described under section 12 - This covers the deliberate removal of material from the laboratory environment.

Accidental contamination of workers in the suite - VERY UNLIKELY: Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. Established and primary cells are extremely unlikely to colonize human hosts. Many (but not all) of the retroviral and lentiviral vectors to be used in this project will also have additional sensitivity to human compliment. In addition all retrovirus and lentivirus vectors used in this project have deletions in their genome to make them unable to replicate in the absence of helper functions. The absence of reversion to replication competent is very unlikely with the specific design of the retrovirus and lentivirus vectors used in this project. The lentivirus vectors have additional deletions in their 3’ LTR, making them self-inactivating vectors.

Possible effects of accidental exposure of laboratory workers: In a worse case scenario the vectors could infect the laboratory worker and integrate into the genome of the infected cells. In such cases the eukaryotic gene promoter and enhancer sequences could induce or stimulate expression of nearby genes including those with oncogenic potential. Insertional N mutagenesis could also result in the disruption of activation of tumour suppressor genes, thus increase the risk of oncogenesis. Furthermore, the presence of WPRE sequence present in some of the vectors may have an oncogenic potential. The other potential risk is the induction of immunological responses (stimulation or suppression) due to the expression of the immune regulatory factors. The consequence of this may be the induction of autoimmune or Immune suppression disease. Therefore the vectors to be used in this project will be amongst the most attenuated of the available retro and lentivirus vectors. They will have multiple deletions, will be dependent on the presence of helper functions, and will in addition contain 3’ LTR deletions resulting in the self-inactivation of the lentivirus vector in target cells (infected cells will not be able to produce new virus). All operators will be specifically trained for the safe handling of these vectors, use protective clothing (laboratory coat and gloves), and operate a no-sharps/no-aerosol policy in the containment level 2 laboratories in which the lenti and retroviral vectors are handled.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**NOT APPLICABLE**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

The handling of retro and lentivirus vectors that contain potentially hazardous sequences (such as WPRE) will be restricted to containment level 2. However, vectors that do not encode such potentially hazardous genes, or cells genetically modified by such vectors, may be used at containment level one. This will apply to all such studies including the proposed Phase-1 human clinical trial. These vectors will encode marker genes (e.g. EGFP, ΔNGFR, β-galactosidase, etc), and/or immune regulatory genes (for instance B7.1 alone or in combination with IL-2).

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste - All containment waste is autoclaved in a dedicated Department waste autoclave. The moist heat autoclave is used at 121°C for 30 minutes. Temperature validation provided by means of thermocouple probe. The autoclave is serviced every six months by Rodwell Scientific Instruments and documentary evidence of correct function stored on site. The resultant sterile waste is double bagged in yellow bags and taken away and incinerated by Medical School contractors.

Liquid waste - All liquid waste is inactivated with 10,000ppm of Chlorine for greater than 10 hours after which it is disposed of down the designated sink.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
The local GMSC considered the risk assessment for this project on 17 February 2005 and recommended a number of amendments. The enclosed copy of the risk assessment has been accordingly revised and incorporates these amendments.

**Project Containment**

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- Animal Units
  - L2
  - L3
  - L4

- Large Scale Activities
  - L2
  - L3
  - L4

- Human Clinical Applications
  - L2 Conf
  - L3
  - L4

**Project Ref** 386/05.4

- **Date Ackn'd**: 26/07/2005
- **CU2 Project Title**: Characterisation and exploitation of T cell immunoregulation in transplantation tolerance.
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Non-GMM Consent Granted**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Project Additional Information**
### Purposes of the contained use

This research will involve the generation of galectin-1, transfected primary human T cells, using retroviral transduction methods, to analyse the role of this lectin in T cell immunoregulation. On the basis of the results obtained, new strategies for generating tolerogenic T cells specific to allo- and self-antigens will be investigated that could be exploited in developing strategies for the induction of tolerance.

### Recipient or parental organism

Recipient cells - primary human T cells derived from buffy-coat purified peripheral blood mononuclear cells.

### Host/vector system

Vector: Replication defective retroviral vector constructed using amphotrophic barring packaging cell line.

### Origin & function

**Origin of inserts (genus, species, strain):** mammals - human placenta derived cDNA.

**Functions of insert:** Galectin-1, a lectin protein, also named in literature as B-galactoside-binding protein (B-GBP) is involved in cell cycle arrest and thus the negative proliferation of T cells. The purpose of this project is to determine exactly how galectin-1 mediates its suppressive effect of regulatory T cells and to dissect the molecular mechanism implicated in such an effect.

The specific aim of the project is:

* To generate and characterise panels of human CD4+ T cell clones expressing galectin-1 by performing retroviral transfection of T cells with human and mouse-derived Galectin-1 protein, respectively. Human galectin-1 is inserted in pCR3.1 vector murine galectin-1 is cloned in CDM8 vector. Retroviral constructs will be generated using the Phoenix ecotropic and amphotropic 293 packaging cell line.

### Evaluation of foreseeable effects

Cloned galectin-1 protein is unlikely to alter the pathogenicity of the recipient cells as we neither propose work with oncogenes nor genes derived from pathogens that might cause harm. Moreover, the plasmid and retroviral vectors planned for use and cloning sequences used are known to be devoid of any transposable elements from both literature available and our own experimental work, respectively.

In addition, the vectors used and viral constructs generated will be replication-incompetent with a very short half-life when exposed to environmental conditions different than the specific culture conditions required for maintaining their activity.

All vectors have previously been well used and characterised by several groups. No reports have shown them to be harmful in vitro or in vivo since they were first described. The vectors used will be replication-incompetent with a very short half-life when exposed to environmental conditions different than the specific culture conditions required for maintaining them active.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Non requested - full containment measures of Class II activity will be followed.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste materials, including cultures, will be made safe by autoclaving. Bulk liquid wastes will be disinfected and inactivated using an equivalent volume of 2% Virkon.

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02/03/2022  
Page 6809 of 15326
solution (ie final concentration of Virkon is 1%). Fully deactivated liquid waste is then disposed of by draining down the sink with water.

The laboratory and work area using genetically modified microorganisms has an accidental spill kit available containing undiluted disinfectants for immediate use in case of spillage. Bench tops and equipment will be disinfected on a regular basis in accordance with good laboratory practice procedures.

Solid waste from GMO work will be treated by inactivation by incubating the materials for a minimum period of 30 minutes with 1% Virkon solution, then autoclaved (autoclave cycle: moist heat, 20 minutes at 121 degrees celcius). Inactivated solid material is then disposed of as clinical waste, by incineration at an off-site facility for Guys Hospital.

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**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Animal Units**

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**Project Ref** 386/05.5

**Date Ackn'd** 30/08/2005

**CU2 Project Title** The application of RNA interference technology and overexpression of human genes via transient and stable transfection technology upon primary endothelial cells and endothelial progenitor cells and established cell lines of human origin.

**Class** Class 2

**CultureVolClass2** < 1 Litre

**CultureVolumeClass3-4**

---

**Non-GMM** Consent Granted

**Consent Granted** Not Applicable

**Project notified under transitional arrangements** N
### Purposes of the contained use

The use of primary human cell lines (human endothelial cells and endothelial progenitor cells).

### Recipient or parental organism

Human microvessel endothelial and human endothelial progenitor cells and established cell lines of human and animal origin will be transfected with expression vectors and small interfering RNAs (siRNA's) to develop cells with specific genes belonging to the ADAMs family of disintegrin-metalloproteases targeted for overexpression/downregulation respectively.

### Host/vector system

**pCDNA3**

### Origin & function

Vector inserts were cloned from human carcinoma cell line MDA-MB-468 cDNA library and from canine smooth muscle cell cDNA library and supplied to us by collaborators.

### Evaluation of foreseeable effects

Risk assessment for this activity has placed the likelihood of GMM surviving outside of the tissue culture laboratory environment as zero and has placed an overall risk factor of 10-18 with a likelihood of hazard being effectively zero.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Solid - Autoclave prior to incineration (140 degrees C for 20 min autoclave).**
- **Liquid - >10,000ppm chlorine for >12 hours (10% Chloros; 10% Diversol; 1% Virkon) and disposed in a designated sink in Class 2 laboratory.**
The risk assessment was approved subject to minor amendments by the GMSC 386 on the 4th October 2004

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

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Project Ref 386/06.1

Date Ackn'd 24/04/2006

CU2 Project Title

Characterisation of Directed DNA Methyltransferases

Date Project Ceased

Class 2

Consent Granted

Non-GMM Not Applicable

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use
The analysis of function of gene targeted DNA methyltransferases, designed to methylate specific gene promoters and downregulate their expression in a heritable manner.

Recipient or parental organism
Bacterial: E. coli K-12 (Top10, ER1647) disabled bacterial hosts. Unable to survive in the absence of specialized growth conditions. Unable to survive in the gut.
Modified cell lines and primary cells should not be able to colonize other human or animal hosts and also will not be able to survive outside a class II microbiological safety cabinet.

Host/vector system
Cloning Vectors: pCDNA3.1 mammalian expression vector (standard commercial unmodified vector). Human primary and humane/murine established tissue culture cells: Unable to colonize humans, no evidence of the ability to generate transmissible agents.

Origin & function
The genetic material encodes a protein comprising artificial gene specific zinc finger proteins fused to bacterial cytosine DNA methyltransferases. The purpose of this enzyme is to target methylation to the cellular genome at regions predetermined by the zinc finger recognition specificity.

Evaluation of foreseeable effects
Waste: All solid waste material is dealt with as follows: Solid waste: All contaminated waste is autoclaved in the departmental autoclave that is used only for waste. The moist heat autoclave is used at 121°C for 40 minutes. Temperature validation provided by means of thermocouple probe. The autoclave is serviced by Meadowrose Scientific and documentary evidence of correct function is stored on site. Under these conditions complete killing of organisms is guaranteed. The resultant sterile waste is double bagged in yellow bags and taken away and incinerated by external contractors (White Rose).
Liquid waste: All liquid waste is inactivated with 10,000 ppm of Chlorine for greater than 10 hours after which it is disposed of down the designated sink. Under these circumstances complete killing of organisms is guaranteed.
Accidental escape into the environment: The work will be carried out under level-II containment laboratories, and generation of aerosols will be minimized (use of sealed tubes, handling of open containers in level - Class II Microbiological Safety cabinets, etc.). The labs are provided with spill kits and SOP's for dealing with accidental spillage. The bacterial hosts used in this study will be attenuated for growth in the absence of special growth media, and for the ability to adhere to the lining in the gut. All tissue culture manipulations will take place at containment level 2. Cell lines are unable to survive outside the laboratory environment. White coats and gloves are mandatory.
Accidental contamination of workers in the laboratory: Established and primary cell lines (mouse or human) are unlikely to colonize human hosts. Security: The building has 24 hour security, and swipe card only access, visitors must report to the security and be escorted whilst in the building. Only qualified personnel, or trainees under qualified supervision, have access to the level 2 containment laboratories.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste: All contaminated waste is autoclaved in the departmental autoclave that is used only for waste. The moist heat autoclave is used at 121°C for 40 minutes.
Temperature validation provided by means of thermocouple probe. The autoclave is serviced by Meadowrose Scientific and documentary evidence of correct function is stored on site. Under these conditions complete killing of organisms is guaranteed. The resultant sterile waste is double bagged in yellow bags and taken away and incinerated by external contractors (White Rose).

Liquid waste: All liquid waste is inactivated with 10,000 ppm of Chlorine for greater than 10 hours after which it is disposed of down the deignayed sink. Under these circumstances complete killing of organisms is guaranteed.

This project was initially approved as a class 2 activity by the GMSC in August 2005.

Project Containment

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Project Ref 386/06.2

Date Ackn'd 22/08/2006

CU2 Project Title Studies of changes to the airways and vasculature in asthma.

Class 2

Culture Vol Class 2 < 1 Litre

Non-GMM

Consent Granted Not Applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work N

Withdrawn N
### Project Additional Information

#### Purposes of the contained use

The plasmid vectors and inserts to be used require containment level 1 conditions. The host cells to be transfected will be primary cultured human and rodent smooth muscle cells and small vessels. Primary cultured cells require containment level 2, thus all work will be performed at containment level 2.

#### Recipient or parental organism

The recipient cells will be primary cells from human and mice and rats.

#### Host/vector system

The host cells will be primary cultured human and rodent smooth muscle cells and small vessels. The vectors we plan to use are non-mobilisable plasmid vectors.

#### Origin & function

The non-mobilisable plasmid vectors will contain small gene fragments that will generate RNA's that will be processed to 23 mers. Inserts will be chemically synthesised and will be homologous to human genes. Transfection of the plasmid will inhibit gene translation and will enable physiological functions to be determined. cDNA inserts used to overexpress full length proteins will be derived by rtPCR from human, mouse or rat reverse transcribed RNA pools.

#### Evaluation of foreseeable effects

We plan to transfect primary cultured human and rodent smooth muscle cells and small vessels with plasmid vectors containing short gene fragments, which will functionally knock out specific genes; and cDNA's encoding full length proteins. All cells and vessels in this study are unable to survive outside tissue culture facilities, so they will have minimal effect to the environment. Prior to disposal, cells will be inactivated using a 1% Virkon solution then autoclaved. Cells and all culture materials will be incinerated with hospital waste. Cells are unable to colonize laboratory workers. The non-mobilisable plasmid vectors that we plan to use are unable to enter cells without mechanical or chemical manipulation and plasmids are unable to transcribe the inserted gene fragment outside the host cell, there will be minimal risk to the laboratory staff or the environment.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Transfected cells will be autoclaved at 121°C for 20 mins and then incinerated with hospital clinical waste, culture medium will be treated with 1% Virkon for greater than 15 mins which will cause death of any cells therein. Media will be disposed to drain diluting with copious amounts of water. The cells used will be unable to remain viable out of tissue culture facilities.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

The assessment was approved by the local BSC committee on 2nd February 2006, with some minor amendments. The class of activity is 2. A safety audit of the laboratory where the work will be carried out has also been done.

### Project Containment

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### Project Ref 386/06.3

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<td>Generation and characterization of a human embryonic stem cell model of Huntington's disease.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Withdrawn N  

Tick if notifying a connected programme of work N
Huntington's disease (HD) is a late onset inherited neurodegenerative disease. In order to understand the molecular changes that occur in HD it is essential that cell models of this disease are available. Throughout the research community, many cell models have been established and although these have had many uses, they also all have limitations. In all cases, the HD mutation has been introduced into cells that are immortal, either because they have been transformed or because they have been established from a natural malignancy. Therefore, these cells divide, unlike the neurons that are affected in patient brains. Also these established cell models often contain abnormal numbers of chromosomes (polyploidy) which is a problem if one wishes to study the cellular pathways that have been abnormally affected in the disease.

Therefore we wish to introduce the HD mutation into human embryonic stem cells to produce a much better cell model of HD. These cells will not be immortalized and can be differentiated into any cell type. In the first instance we would choose to differentiate them into neurons and muscle cells.

The recipient cells are well characterized human embryonic stem cell lines (Pickering et al.2003. Reprod Biomed. Online 7.353-364). They can be maintained in a pluripotent non-differentiated state by culturing them in specialized cocktails of growth factors. They can be differentiated into different cell types, by changing the constituents of the culture medium.

The autosomal dominant mutation that causes Huntington's disease is the increase in the length of a CAG repeat in exon 1 from the normal range (CAG) 6-35 to the pathogenic range (CAG) 40-->200

The human embryonic stem cell lines contain two normal copies of the HD gene. We shall replace the CAG repeat in one of the HD genes with a longer repeat that is in the pathogenic range. This will be carried out by homologous recombination. Cell lines will be selected in which the only genetic modification has been a change in the length of this repeat. These cell lines will be characterised and cellular pathways associated with HD will be identified.

Once the cells have been established and characterized, we shall try to improve the HD phenotype by altering the expression of other genes in pathways predicted to modify HD. Potential modifiers will either be overexpressed by introducing the gene of question into the cell line, or their expression will be knocked down by introducing RNAi into the cell line. Modifying genes and RNAi sequences will be introduced by lentiviral infection.

The genomic DNA for homologous recombination at the HD gene locus will be constructed by cloning in bacterial non-expression plasmids. The constructs will be prepared by cloning in the bluescript vector (Stratagene) and propagation will in non-mobilisable/disabled E coli hosts. These manipulations are containment level 1 and there are no foreseeable adverse effects.
Gene sequences and RNAi sequences that have been predicted to modify the phenotype of the HD-embryonic cell line will be cloned into lentiviral vectors prior to introduction into the human HD cells. Either FIV or HIV-1 (Invitrogen) lentiviral vector systems will be used. The lentiviruses will be transiently transfected into 293T cells along with the packaging plasmids that allow production of the infectious particles. The coding sequences of the two packaging constructs cannot undergo recombination with the infectious virion which itself contains less than 5% of original wild type viral sequences. The probability of a replication competent vector being generated via homologous recombination with wild type virus or packaging construct rescue is therefore negligible. This would require multiple homologous recombination events including recombination with either wild type FIV or wild type HIV, which is never introduced in the production process. The lentiviral vector is also self-inactivating due to deletions being present in the 3'LTRs. Cloning into the lentiviruses and production of the infectious particles is containment level 1 and no adverse effects are anticipated.

The human DNA containing a long CAG repeat will be obtained from pre-existing cosmid and plasmid clones. This will be used to replace the CAG repeat in one of the HD genes in the human embryonic stem cells. The DNA containing the final long repeat will be purified away from vector sequences prior to electroporation.

The DNA for homologous recombination will be electroporated into humans ES stem cells. Cells containing recombination events will be identified through selectable markers that confer resistance to various antibiotics. Resistant colonies will be analysed by molecular techniques to ensure that the desired recombination at the HD locus is the only genetic manipulation that has occurred.

The expression level of other genes that have been predicted to modify the HD phenotype of the HD ES cells will be either over expressed by introducing the genes of interest or its expression will be knocked down by introducing RNAi sequences. The gene or the RNAi will be introduced as lentiviruses. The gene sequences will be isolated by RT-PCR from RNA that has been prepared from other non-primary, non-diseased human cell lines (e.g fibroblast or lymphoblastoid). RNAi sequences will be derived from oligonucleotides.

This project will be at containment level 2 because we are manipulating a human primary cell line.

Origin & function

The DNA for homologous recombination will be electroporated into humans ES stem cells. Cells containing recombination events will be identified through selectable markers that confer resistance to various antibiotics. Resistant colonies will be analysed by molecular techniques to ensure that the desired recombination at the HD locus is the only genetic manipulation that has occurred.

Introduction of genes and RNAi by lentiviruses:
The lentiviral vector is replication incompetent and therefore even though it will insert into the host genome of the HD ES cells it will not be able to replicate and become infectious.

The genes that we shall introduce in order to modify the HD phenotype or whose expression will be reduced will be of known function and will not be anticipated to have any adverse effects.

Insertional mutagenesis at the site of lentiviral integration is a possibility. In order to identify phenotypes arising from such events, the phenotypes of at least two independent cell clones will be compared after infection with lentiviruses. If two clones show the same phenotype, this is unlikely to arise from insertional mutagenesis as integration into the same genomic region is extremely unlikely to occur in two separate lines.

The woodchuck hepatitis virus post-transcriptional regulatory element will be present in some of our lentiviral vectors. As the oncogenic potential of this element is currently under scrutiny, vectors containing WPRE will be treated with the assumption that harmful sequences are present as recommended by the guidelines from GTAC and HSE.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Cell Culture:

All cell culture will be performed in a class II microbiological safety cabinet. Appropriate protective clothing (lab coats and latex gloves) will be used. Nitrile gloves and powder free gloves will be available for staff allergic to latex and powder. Pipettes and culture flasks will be disposed of directly into autoclave bags, sealed and autoclaved. Liquid waste will be removed directly into disinfectant. A biological spill kit will be at close hand.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste is first autoclaved at 134°C for 20 min and then sealed in biohazard containers and incinerated.
Liquid waste is disinfected with Vircon at 10g per litre overnight and washed down the laboratory sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

GM386 GMSC approved this as a CL2 activity on 1st June 2006.

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Project Ref 386/07.1

Date Ackn'd 17/08/2007
CU2 Project Title Modelling Gene Therapy Applications in Cell Lines and Primary Human Cells.

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 Litre
Project Additional Information

Purposes of the contained use

The purpose of the contained use is to genetically modify cell lines and primary human somatic and stem cells with plasmid and lentiviral vectors with the aim of assessing efficacy of potential gene therapy applications. This will include the testing of therapeutic genes and the conditional immortalisation of primary human somatic and stem cells.

Recipient or parental organism

Human embryonic human cell line (293T) expressing the SV40 large T antigen will be used as the host for transient transfection with the lentiviral packaging system for the production of infectious lentiviral vector particles. Primary human somatic and stem cells (e.g. neuronal stem cells, pancreatic stem cells, hepatic stem cells, myoblasts, CD34+ haematopoietic stem cells) will be transduced with the lentiviral vectors or transfected with plasmids for functional analysis.

Host/vector system

PLASMID AND LENTIVIRAL VECTOR CONSTRUCTS

HIV-based lentiviral vector: pRRL_c_s3bglod.cadII: This vector has a self-inactivating (SIN-18) backbone. It contains no viral protein encoding genes and includes a short 3’ HBB cloned in reverse orientation to preserve intron and non-coding cis-acting regulatory sequences: central polyuridine tract (cPPT) and packaging domain ().

Origin & function

The protein coding genes within the lentiviral vectors will be either reporter (e.g. EGFP, galactosidase) or potentially therapeutic (e.g. human -globin, dystrophin) genes aof known function. These genes will be placed under the control of a number of either tissue specific (e.g. human beta-globin and dsemin locus control regions, promoters and enhancers) or ubiquitous (e.g human HNRPA2B1/CBX3 dual divergently-transcribing promoter region) transcriptional regulatory elements. All componenets are of known function and based on this are determined to be of negligible health risk.

Evaluation of foreseeable effects

The lentiviral vectors are devoid of their own genome and are self-inactivating. They are therefore incapable of replication and spread beyond the point of intial infection.

The therapeutic genes that will be incorporated into the lentiviral vectors are all of known function and are known not to be pathogenic.

The tissue culture cells that will be used to either generate the lentiviral vectors or to be transduced with the lentiviral vectors or transfected with plasmids are incapable of
survival outside of the laboratory environment. They therefore pose negligible health risks to individuals and environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

We have two methods of treating waste according to the nature of the material: solid waste will be autoclaved at 121C for 30 min, then transferred to yellow bins for incineration. Liquid waste is disinfected overnight with 10,000ppm chlorine (Precept) before disposal via the drainage system. The use of sharps such as needle, metal scalpel blades or glass Pasteur pipettes is prevented by using plastic alternatives. The formation of aerosols is prevented by mixing in capped tubes. Potential aerosol-releasing manipulation will be carried out in biological safety cabinets. When necessary, a formaldehyde bomb is used for fumigation in the microbiological safety cabinets. After an accident such as spills or release, the area will be cleared of personnel and have restricted access. The affected area will then be decontaminated with bleach followed by appropriate disposal of waste. A complete biological spill kit is present.

During work, lab coats, gloves (powdered and non-powdered nitrile), goggles will be worn and work will be performed in specified areas for easier containment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was reviewed by the King's College London Biological Safety Committee (BSC) on the 7th June 2007. The BSC found the risk assessment to be (i) clearly presented, (ii) comprehensive, (iii) precise in detail regarding the nature of the GMO's and genes to be used, (iv) precise in detail of the nature of the work that will be conducted with the GMO's and (v) met the requirements of the local national safety regulations.

The risk assessment was approved pending the completion of minor modifications.

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The goal of this project is the development of a genetic approach to re-target human T-lymphocytes to common solid tumours using chimeric antigen receptor (CAR) technology. In brief, CAR consist of a targeting moiety (most commonly a single chain antibody fragment (scFv) or modified ligand) coupled via a hinge and transmembrane module to an endodomain that elicits T-cell activation and effector function.

Recipient organisms are as listed below (hosts). No additional recipient organisms (eg animals, plants) will be used. Parental organisms are as follows: (i) Prokaryotic — DH5a are ultimately derived from E.coli. (ii) Viral - The replication-defective retroviral vectors for use in this study are all ultimately derived from the Moloney murine leukaemia virus. (iii) Eukaryotic — all fibroblasts cell lines including retrovirus packaging cell lines are ultimately derived from NIH3T3 cells.

Vectors: All viral vectors to be used have been mutated (by extensive deletion) to make them replication incompetent. Thus, viral vectors (detailed below) do not have any inherent pathogenicity and are fully sensitive to complement.

(i) CAR will be targeted to tumour cell surface molecules including prostate-specific membrane antigen (PSMA), carcinoembryonic antigen (CEA), ErbB2 (scFv-based),
MUC1, ErbB4 (ligand based), colony-stimulating factor-I receptor (CSF-I R; ligand based). Signalling domains will consist of CD3z +1- co-stimulatory modules (CD28, TNF receptor family members etc). In some cases two or more CAR will be co-expressed in T-cells in order to deliver complementary signals upon engagement of two tumour antigens.

(ii) Cytokine receptors - to enable the regulated expansion of gene-modified T-cells in a tumour- or drug-regulated fashion. An example of the former is the colony-stimulating factor-I receptor (CSF-I R) which is active when ectopically expressed in T-cells, conferring responsiveness to the tumour-associated cytokine, CSF-I.

(iii) To facilitate detection of gene-modified cells, (enhanced) red or green fluorescent protein may be expressed. In some cases, this may be joined in-frame to other cDNA inserts to engineer a fusion protein.

(iv) Internal ribosome entry sites (IRES) or 2A peptide cleavage systems may be used to enable expression of more than one open reading frame from a single transcript.

(v) Genetic resistance genes will facilitate selection of gene-modified cells (using puromycin, G418, blasticidin, zeocin etc).

(vi) To recreate the environment associated with cancer-associated cytokine overproduction, immortalized tumour cells may be genetically engineered to express tumour-associated cytokines such as CSF-I.

(vii) To eliminate gene-modified cells, suicide genes may be used (eg HSV-TK; caspase 9 fusion genes). (viii) To provide antigen-expressing tumour cells, targets may be expressed in immortalized tumour cells, eg Erb6i-4, CEA, PSMA, CSF-iR, MUC1

(ix) To facilitate imaging, bioluminescent imaging reporters (firefly or renilla luciferase) or PET reporter genes (eg HSV-TK, Na/I symporter) may be expressed. (x) Inactivated (mutated) controls will be generated for all inserts as appropriate.

**Evaluation of foreseeable effects**

The principle potential hazard associated with the use of retroviral-mediated gene transfer is the possibility that recombination may occur between the expression vector used and the complementary sequences found in the packaging cell line, leading to the generation of replication competent virus (RCR). Generation of RCR is rendered less likely by the use of the GP+env86, and PG13 packaging cell lines, in which at least 3 independent recombination events are required for RCR generation. In addition, the viral vectors for study are fully sensitive to human complement. It is envisaged that there will be a negligible risk to human health or the environment associated with the use of the prokaryotic or eukaryotic hosts specified since these are unable to colonize human hosts.

In addition, any theoretical risks posed by the above are further reduced by the containment and waste disposal measures to be employed. Bacterial work will take place at containment level 1 while all work with retroviral vectors and T cells will operate at level 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials that have been in contact with genetically modified material will be sterilised. All solid waste will be autoclaved at 121 degrees C for 90 minutes before removal from the building in double bags for incineration, thereby ensuring 100% kill. To achieve 100% kill of liquid material, waste will be treated with Virkon 1% and incubated overnight (in agreement with manufacturers recommendations) before disposal down a sink. We have opted not to autoclave liquid waste in light of concerns about delays prior to autoclave availability for this purpose, coupled with undesirability of autoclaving bleach-treated waste. Periodic in-house testing will be performed to assess the % kill of prokaryotic and eukaryotic hosts by the overnight bleaching approach as described. All animals carcasses will be incinerated.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

02/03/2022
The risk assessment was approved by the KCL School of Medicine Biological Safety Committee subject to minor amendments on 7-10-2007. All amendments have been made exactly as requested.

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms

L2  Yes  L3  L4  L2  L3  L4  L2  L3  L4

Animal Units  Large Scale Activities  Human Clinical Applications

L2  L3  L4  L2  L3  L4  L2  L3  L4

Project Ref  386/08.2

Date Ackn'd  07/05/2008

CU2 Project Title  Kidney Inflammation and Fibrosis.

Date Project Ceased

Class  CultureVol  Consent Granted

Class 2  < 1 Litre  Not Applicable

Project notified under transitional arrangements  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022  Page 6824 of 15326
Chronic kidney diseases (CKD) are characterized by chronic inflammation and refractory fibrosis. We have 3 major focuses in our research aiming at developing new therapies: (i) Analogues of vitamins A and D; (ii) Ras and Rho; (iii) T-type channels.

1. Vitamin A and D analogues antagonises renal inflammation and fibrosis
The specific hypothesis is that vitamin A and 0 derivatives may have both anti-inflammatory and anti-fibrotic effects in the kidney. The specific aims are to test the above hypothesis by addressing their effects in vitro models of fibrosis, TGF-beta signaling, MAP kinase, AP-1 and NF-kappaB and MKP-1 activities in kidney mesenchymal and epithelial cells. Experiments involving gene modification procedures include: transfection of the cells with a Smad3 activity reporter plasmid, plasmids expressing Smad7, dominant negative mutants of RARs, RXRs, vitamin D receptor, and other nuclear receptors, MAP kinases ERK1/2, MEk5/ERK5, p38 and JNK, siRNAs of RXRalpha, beta, gamma and RARalpha, beta and gamma, vitamin D receptor, MKP-1, etc.

2. Roles for Ras and Rho in inflammation and fibrosis
The specific hypothesis is that Ras and Rho subtypes play different roles in inflammation and fibrosis in the kidney. The specific aims are to test the above hypothesis by addressing their roles in unilateral ureteral obstruction (UUO) and anti-Thyl.1 nephritis rodent models and kidney mesenchymal and epithelial cells. Experiments involving gene modification procedures include: Expression of H, K and N-Ras, Rho A, B, C and E and short hairpin siRNAs for each isoform. Also Ras binding domain of Raf-glutathione-s-transferase fusion protein, Rho binding domain Rhotekin-GST fusion protein and Rac binding domain of PAK-GST fusion protein.

3. Role for the T-type channels in inflammation and fibrosis
The specific hypothesis is that T-type channels play some roles in inflammation and fibrosis in the kidney. Ib pepific aims are to test the above hypothesis by addressing their roles in unilateral ureteral obstruction (UUO) and anti-Thyl.1 nephritis rodent models and in kidney mesenchymal and epithelial cells. Experiments involving gene modification procedures include: Transfection of cells with siRNAs to knockdown 3 T-type Calcium channel isoforms (Cav3.1, Cav3.2 and Cav3.3).

Recipient or parental organism
Identify the species and tissue origins of all the cell lines to be used and specify cell lines being used: Primary human mesangial cells provided by Prof. Lucio-Cazana (Spain), human (Saleem) and mouse (Mundel) podocyte cell lines, NRK49F rat renal fibroblasts, SM43 and FM14 rat mesangial cell lines, wild-type and Smad2 or Smad5 knockout mouse embryonk fibroblasts.

Describe the method(s) of immortalisation: The NRK49F rat renal fibroblasts, SM43 and FM14 rat mesangial cell lines, mouse embryonic fibroblasts are spontaneous cell lines. Human and mouse podocyte cell lines were immortalised by transfecting with a temperature- sensitive SV40-T gene. Establishment of transgenic mice: In some studies, Alb/TGF-betal and Ren-lcrrGF-betal transgenic mice will be used. No further gene modification will be done to these mice.

Host/vector system
1. Type of vector Plasmid, cosmicl, etc): Plasmid
2. Vector name: pcDNA3.1, pSG5, pMEP4, pBPSTRI, pRSh, CDM8, pCEP4, pRE, pmaxGFP, pEF-BOS.
3. Provide a map/www/scientific reference for each vector, has this been done:
   - pMEP4: http://lmcb.asm.org/cgi/reprint/25/4/1475
   - pBPSTRI: http://lmcb.asm.org/cgi/reprint/19/6/3212
   - pRSh: http://www.jbc.org/cgi/reprint/276/1 6/12697
   - CDM8: http://www.jbc.org/cgi/reprint/276/16/12697
   - pCEP4: http://www2.kumc.edu/solab/LabLinks/vectors/pcpep4.htm
   - pmaxGFP: http://www.amaxa.com/fileadmin/groups/marketing/Downloads/Protocols/RNAiOP_sIRNAlastKit. pdf
Origin & function

Genetic materials: Plasmids, siRNAs and antisenses of MKP-1, MKP-ICS, RARs, RXRs, dnRARs, dnRXRs, WT and dn ERK, WT and dn JNK, WT and dn p38, ADH5, RALDHs, Cyp26, ABCAI, AP1-SEAP, NFkappaB-SEAP, RARE-SEAP, wild-type, constitutive active and dominant negative mutants of Rho.

Origins: From academic collaborators or commercial suppliers.

Intended functions: These genetic materials are modulators of signal transduction. They will be used in in vitro studies to explore the roles for the MAP kinase, retinoid, Ras and Rho, AP-1 and NF-kappa B in cell biology.

Evaluation of foreseeable effects

These gene materials will change the expression of specific genes and change the phenotypes of the cells and thus might change the natural course of inflammation and fibrosis. None of these materials is carcinogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No GM plants will be used or created in this project. Well-established transgenic mice will be used but not further modified. The transgenic mice will be brought in from National Institutes of Health, University College London and other American or European collaborators. All the animals will be housed by the King's Biological Services and all procedures will be carried out according to the Animals (Scientific procedures) Act 1986, with appropriate personal and project licences in place.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Treat waste:
Solid: Autoclave and then sent for incineration. The length of sterilisation cycle will be more than 20 mm and the temperature is 121 degree centigrade. Indicator tapes will be used to indicate the wastes have been autoclaved.
(ii) Liquid: 1% Virkon. Contact time should be longer than 60 mm. At UCL, it has been confirmed that exposure longer than 10 mm will reduce micro-organisms to below detectable level, The Virkon-treated liquid will be poured down into sink with copious amounts of water

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project was approved by School of Medicine Biological Safety Committee (GM386) with minor amendments, which have now be made.
## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

Recipient organisms are listed as below (hosts):

### Host/vector system

Host E.coli JM101, JM109, DH5alpha, DH10B, DB 3.1, Invaf, JS4, XL1-Blue, BL21(DES), TOP10, INV110, K12 strains DH5a, BL21(DE3). Such hosts are naturally attenuated, known to be non-virulent and are fully sensitive to a wide range of antibiotics.
Origin & function

Evaluation of foreseeable effects

The vectors don't contain any potentially harmful sequences. They contain non-coding DNA sequences. There is a minimal risk that retroviral or lentiviral plasmid DNA could integrate into human genomic DNA.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials that have been in contact with genetically modified material will be sterilised. All solid waste will be autoclaved at 128 degrees C for 20 minutes before removal from the building in double bags for incineration, thereby ensuring 100% kill. To achieve 100% kill of liquid material, waste will be treated with virkon 1% and incubated overnight (in agreement with manufacturers recommendations) before disposal down a sink. We have opted not to autoclave liquid waste in the light of concerns about delays prior to autoclave availability for this purpose, coupled with undesirability of autoclaving bleach-treated waste. Period in-house testing will be performed to assess the %kill of prokaryotic and eukaryotic hosts by the overnight bleaching as described.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was approved by the KOL School of Medicine Biological Safety Committee subject to minor amendments on 21st August 2008. All amendments have been made exactly as requested.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2</td>
<td>L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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02/03/2022
### Project Additional Information

#### Purposes of the contained use

The aims of the project are to define the key molecules involved in human and murine T-cell activation and differentiation. The understanding of these fundamental processes will allow a rational design of treatment for autoimmune, infection, allergic and neoplastic disease.

#### Recipient or parental organism

Recipient organism will be human or mouse T-cells. Parental organism will also be human or mouse. Parental and recipient will generally be identical.

#### Host/vector system

Genes of interest into PCR2.1 TOPO or Pbluescript in the first instance. Genes then sequenced and transferred to viral vectors such as MSCV-EGFP, PGC-IRES (retroviral vectors), Plentilox 3.7, pMDLg-gRRE (lentiviral vectors) using various packaging/helper plasmids such as pMD.g, pRSV-rev and VSV-G.

#### Origin & function

Genes involved will be various mouse/human genes controlling T-Cell activation and/or differentiation. Alternatively transduced genetic material may be ShRNAs designed specifically to knock down or silence these genes. Genes will originate in human or mouse primary T-Cells and will be cloned and then sub cloned into viral vectors following PCR. Genes of interest will be identified in the first instance by array analysis. ShRNAs are small 18-30 mers and are generated by a commercial company.

#### Evaluation of foreseeable effects

Forseeable results are that the inserted (or knocked down) genes will influence the process of differentiation or activation in the target T-cells. Examination of these cells
with a variety of techniques (ie ICC, RT-PCR) will yield a greater understanding of the molecular mechanisms of these processes. The virus produced during this project is potentially infective to humans if VSVG pseudotyped, however the virus is replication deficient and a multi vector system is used for maximum safety of handling. Further, SOP's are in place to prevent the information of aerosols and limit the use of sharps in the lab which should further cut down on any potential hazards. All procedures are performed in a designated area within a class 2 MSC. The modified target cells are not hazardous and are unable to survive outside lab conditions. All material transduce by virus or used in the production of virus is disposed of via methods listed in section 12.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid: All solid waste autoclaved, 20 minutes 121 degrees C and then transferred to yellow bag clinical waste route for incineration.

Liquid: All liquid waste is treated with a 1% virkon solution for at least 1 hour before being disposed of in a designated sink with copious running water. Autoclaving gives 100% kill rates. This can be tested every cycle and efficacy of the autoclave is routinely tested during servicing which is performed on an annual basis. Virkon also gives an effective 100% kill rate. Documentation is available from suppliers, see risk assessment for further details.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

BSC approved GMRA subject to minor amendments.

Project Containment

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Project Ref 386/09.2
The analysis of the role of the complement regulator CD46 during cell/cell interactions (human T cells and human epithelial cells) using primary cells and cell lines in which CD46 or CD46-interacting proteins have been silenced via siRNA technique or mutated forms of these proteins introduced.

CD46 IN T CELL RESPONSES. We recently found that the activation of the complement regulator CD46 on human CD4+ T cells induces the de novo development of adaptive Tregs that can suppress the proliferation and function of effector T cells in the vicinity. The in vivo induction conditions of complement-induced cTregs, their tissue localization and mode of action as well as their impact on other participants in the immune response are poorly defined. Under this part of the project, we aim to understand the CD46-mediated signals leading to cTreg induction as well as identify a novel CD46 ligand that can induce these signals.

CD46 IN EPITHELIAL CELL LAYER INTEGRITY. E. coli infections are the most common complication after renal transplantation. CD46 expressed on uroepithelial cells functions as a receptor for C3b-opsonised E. coli. As in T cells, CD46 activation also triggers important signalling events in epithelial cells: CD46 signals are central in the uptake of pathogenic E. coli by uroepithelial cells and subsequent promotion of infection. We aim to identify the mechanism by which E. coli uses CD46 to gain cell entry and hypothesize that changes in the cell layer integrity/permeability induced via CD46-mediated signalling events support bacterial invasion.

HUMAN:
A. Primary human CD4+ T cells (isolated from blood of healthy volunteers or blood packs from the National Blood Supply). These cells are non-adherent (suspension) and have not been previously mutated or altered and therefore show the normal proliferation, cytokine secretion and life span behavior of freshly cultured human CD4+ T cells. We anticipate having these cells (without manipulation and after siRNA treatment) in culture for 5-10 days per experiment. More than 90% of the cells will by that time be exhausted and apoptotic/dead; remaining cells will be killed.
B. Jurkat, clone E6-1; human CD4+ T lymphocyte; acute T cell leukemia. The Jurkat suspension cell line was established from the peripheral blood of a 14-year old boy. Clone E6-1 cells produce large amounts of IL-2 after stimulation with phorbol esters and either lectins or monoclonal antibodies against the T3 antigen (both types of stimulants are needed to induce IL-2 production). This is a pseudodiploid human cell line. The modal chromosome number is 46, occurring in 74% with polyploidy at 5.3%. The karyotype is 46,XY,-2,-18,del(2). Cells were obtained from the ATCC, USA, and not further manipulated. As this line is immortalized, we do not expect an effect on cell proliferation/survival.

C. HEK293 (human fetal epithelial cell line, adenovirus 5-transformed). This is an adherent hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2%. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). Cells were obtained from the ATCC, USA, and not further manipulated.

D. A498 (human kidney epithelial cell line, clear cell renal cancer). A498 cells, established from a clear cell renal cell cancer of a 52 years old male patient. Cells were obtained from ATCC, USA, and not further manipulated.

BACTERIA:

A. DH5α (bacteria, E. coli strain, commercially available, generally used for plasmid transformation). This is a well characterised, commercially available E. coli strain that is used for plasmid transformation and cloning purposes. Genotype: F- supE44 hsdR17 recA1 gyrA96 endA1 thi-1 relA1 deoR lambda-. The strain was obtained from Invitrogen and not further manipulated.

B. BL21-CodonPlus-RIL (bacteria, E. coli derivative, commercially available, commonly used for protein expression). BL21-CodonPlus-RIL cells contain extra copies of the argU, ileY, and leuW tRNA genes, which recognize codons that are a problem predominantly in organisms with AT-rich genomes. BL21-CodonPlus-RP competent cells contain extra copies of the tRNA gene argU, which recognizes the AGA and AGG arginine codons, and the tRNA gene proL, which recognizes the proline codon, CCC. These codons appear frequently in GC-rich genomes, such as mammals. Genotype: E. coli B- ompT hsdS(rB– mB–) dcm+ Tetr gal λ(DE3) endA Hte [argU ileY leuW Camr. The strain was obtained from Stratagene and not further manipulated.

C. UT5600(DE3) (bacteria, E. coli K12 derivative used for protein expression). This is a E. coli K12 derivative, commercially available and purchased from New England Biolabs. Genotype: F- ara-14 leuB6 secA6 lacY1 proC14 tsx-67 Δ(ompT-fepC)266 entA403 trpE38 rfdD1 rpsL109 xyl-5 mtl-1 thi-1.

Host/vector system

A. pET-15b vector (host:bacteria). Protein expression vector designed and sold by Novagen. 5708 bp in size; pBR322 ORI; T7 Promoter; His-Tag; MCS; T7 Terminator; lacI gene. The vector was purchased from Novagen and not further modified (except for the insertion of cDNA coding for CCPs1-4 of human CD46 – see below).

B. pcDNA-3.1 vector (host:bacteria, mammalian cells). pcDNA™3.1 vector is commercially available, 5428 bp in size and designed for high-level stable and transient expression in mammalian hosts. The vectors contain the following elements: Human cytomegalovirus immediate-early (CMV) promoter, MCS, neomycin and ampicillin resistance genes, T7 promoter, f1 origin, pUC origin. The vector was purchased from Invitrogen.

C. pcDNA6.2-GFP pcDNA6.2-V5, pcDNA6.2-C-YFP vectors (host: bacteria, mammalian cells). These three vectors are all derived from the same 7500 bp sized protein expression vector developed and sold by Invitrogen. The Vivid Colors™ Fluorescent Protein Gateway® Destination Vectors allow fusion of a protein of interest (here CD46, SPAP, α-E-catenin) to the widely used and well-characterized fluorescent proteins from the jellyfish Aequore (i.e. Green Fluorescence Protein (GFP) and Yellow Fluorescence Protein (YFP) or with a V5 tag to which a number of Abs exist. The vectors contain the following elements: CMV promoter, options to fuse GFP, YFP or V5 to the N- and C-terminus of the selected protein, f1 origin, pUC origin, T7 promoter, ampicillin and blasticidin resistance genes.

Origin & function

cDNAs USED FOR PROTEIN EXPRESSION IN BACTERIA AND/OR MAMMALIAN CELLS:
A. CD46/CCP1-4; CD46/CCP1-4M. This cDNA codes for CCPs 1-4 of the human complement regulator CD46. CD46 is expressed on all cells and prevents complement deposition (opsonins C3b/C4b) on host cells. The cDNA has been derived from a healthy donor. The CD46/CCP1-4M contains a mutation in the C3b/C4b binding site and can therefore not regulate complement anymore. No potential hazards are currently linked to this gene product.

B. SPAK; SPAKM. This cDNA codes for the human serine/threonine kinase SPAK. SPAK is expressed in most cells and activated during cellular stress and regulates MAP kinases. The cDNA has been derived from a healthy donor. The SPAKM cDNA contains a mutation in the kinase site and lacks therefore MAP activation activity. No potential hazards are currently linked to this gene product.

C. α-E-catenin; α-E-cateninM. This cDNA codes for human α-E-catenin. This protein is ubiquitously expressed and involved in tight junction formation and may also function as tumor suppressor gene in epithelial cells. The cDNA has been derived from a healthy donor. The α-E-cateninM mutant contains a mutation in the F-actin binding site. Deficiency/mutations in this protein have been linked to colon cancer.

D. E-cadherin; E-cadherinM. This cDNA codes for human E-cadherin. E-cadherin is preferentially expressed in epithelial cells and involved in tight junction formation. It may also function as tumor suppressor gene in epithelial cells. The cDNA has been derived from a healthy donor. The E-cadherinM mutant contains a mutation in β/α-catenin binding site. Mutations or deficiencies in this protein are linked to colon cancer and metastasis.

siRNAs TO BE TRANFECTED/DELIVERED INTO MAMMALIAN CELLS:

A. siRNA CD46. 22 shRNA targeting human CD46.

B. siRNA SPAK. 22 shRNA targeting human SPAK.

C. siRNA α-E-catenin. 22 shRNA targeting human α-E-catenin.

D. siRNA E-cadherin. 22 shRNA targeting human E-cadherin.

The 4 listed siRNAs (targeting the RNAs of the genes described above) are synthetic RNAs and have all been purchased from AB Biosciences. These siRNAs are delivered via electroporation into target cells and have been shown to reduce target protein expression to about 20-30% compared to non-treated cells. Protein knockdown of all 4 listed proteins has been achieved and published previously by a number of groups including our laboratory. No potential hazards have been linked to these approaches/experiments.

Evaluation of foreseeable effects

FORSEEABLE EFFECT OF GENETIC MANIPULATION ON HOSTS:

Human CD46 and SPAK are ubiquitously expressed proteins. The full-length wild type as well as several mutant forms of these proteins have previously been over-expressed in human primary cells and cell lines by several investigators including the proposer (CK) of this GMO assessment application. These processes did not lead to any reported increase in toxicity, infectivity or other potentially hazardous features of these cells.

E-cadherin and α-E-catenin are proteins involved in tight junction formation and tumor suppression. Thus, the silencing of these proteins in the human HEK293 and A498 epithelial cell lines could potentially lead to higher/uncontrolled proliferation of these cells and pose most likely the most hazardous genetically modified organism within this work. However, these GMO are non-infectious in nature, risk of exposure for humans/environment is minimal and their survival in the environment is unlikely (see below).

The host range of the E. coli bacteria strains used in this work covers technically a variety of mammalian hosts. However, these strains are commercially available; they have been specifically designed for the safe laboratory use and contain no virulence factors. Even in the case of accidental transmission (which should be prevented by protective closing, working at appropriately equipped designated work spaces and waste disposal/disposal of used materials according to the Good Practice Code), the survival/proliferation of the GMO in mammals is unlikely. Also, CD46 has already been expressed in the bacteria strains that are used in this work and no unexpected/negative effects on the bacteria have been observed.
LIKELIHOOD OF HUMAN EXPOSURE AND ENVIRONMENTAL HARM:
The likelihood of human exposure to any of the GMOs listed in this assessment application is minimal. Researchers are all trained in laboratory SOP and COP, will wear protective clothing at all times (laboratory coats, gloves etc.) and all primary cells and cell/bacteria lines are cultured in tightly closed cell culture flasks or spill safe culture dishes. Cultures will only be opened under sterile conditions in a Microbiological Safety Cabinet (MSC). The MSC is a Class II, is on a service contract and was last serviced and KI tested (service and KI test result: pass and pass) on 17/04/08 by 'Environmental Validation Solutions'. Thus, any accidental spillage will be detected immediately and the affected area cleaned/disinfected using the appropriate measures that laid out in the laboratory COP, which is on file with each laboratory member and also displayed on the outside of the MSC. The MSC itself is located in a lockable room only accessible from the laboratory and this room is designated solely for cell culture work carried out by members of the laboratory. Potential aerosols from the E. coli bacteria strains used for sub cloning and protein expression as well as the uropathogenic bacteria will be prevented/kept to minimum by culturing these in closed flasks, handling them generally in leak and air-proof containers (i.e. centrifugation in buckets with a safety lid) and opening containers in MSCs.

In addition, likelihood of human exposure to the GMO is minimal as these GMO are 1. Non-infectious in nature and 2. Have been generated without an infectious agent (virus). In addition, they are unable to survive in the environment for longer than 3-4 hrs. Although the HEK293 cells line was originally generated via adenovirus 5-immortalization, the cells contain only residual virus sequences but are not producing/shedding infectious virus. In addition, none of the inserted/mutated DNA sequences is known to confer toxicity or infectivity.

We are NOT using animals or plants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

We are NOT using animals or plants.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We do not apply for any derogation from full containment for Class 2 activity.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any biologically contaminated waste (i.e. cell/bacteria cultures, glassware [4 liter glass containers are used to grow bacteria cultures needed for tetramer production], materials and media used in cell/bacteria cultures will be treated with the disinfectant Virkon. Liquids: liquids will be treated with a Virkon solution such that 1% of final Virkon concentration is reached and incubated for at least 20 min. Waste fluid can then be poured down the laboratory sink with copious amounts of water. Materials will be fully immersed for at least 30 min in 1-2% Virkon solution, then double-bagged into autoclave bags and autoclaved for am minimum of 40 min at 120 oC using a designated waste autoclave in room G66-050-350. The autoclave is inspected every 6 month by ‘Priorclave’ and the last inspection was 29/08/2008; inspection result: pass. Autoclaved plastic ware will the be double bagged in a Clinical Waste Yellow bag, sealed with red tape and put into the Clinical Waste container in room G68-050-480. These measure reduce the likelihood of exposure to a minimum. The proposed experiments do not involve injections, thus, exposure due to usage of needles or scalpels is unlikely. Nonetheless, a sharps COP is in place and a designated approved container for sharps and for broken glassware are located in the laboratory. Full containers will be deposited in room G68-050-480 and picked up/handled by a professional waste disposal company.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
The project risk assessment was reviewed by the GM Safety Committee on 19th February 2009. The assessment was approved subject to minor amendments. The Committee considered that this GMRA was a model example.

**Project Containment**

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**Project Ref 386/10.1**

- **Date Ackn’d**: 07/01/2010
- **CU2 Project Title**: Analysis of potential oncogenes in transformation of primary cells
- **Date Project Ceased**
- **Class**: Class 2
- **CultureVolClass2**: ≤ 1 Litre
- **Consent Granted**: Non-GMM

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to assess the biological impact of the expression of human oncogenes and in particular those identified in association with specific cytogenetic abnormalities in human leukaemia. We wish to target tissues that closely mirror those affected in the naturally occurring disease. This procedure will involve gene transfer into human derived packaging cells and generation of a replication defective recombinant retrovirus and lentivirus capable of targeting human and murine cells. The recombinant virus will contain specific, potentially oncogenic gene products that are found in association with different subsets of leukaemia. The virus will be used to infect...
human and murine cells in vitro and the targeted cells will be assessed both in in vitro assays and in vivo.

Recipient or parental organism

Primary human cells purified from cord blood, bone marrow and peripheral blood or mouse primary bone marrow, foetal liver cells and embryonic cells. These cells will have a normal genotype or carry well defined genetic abnormalities ie will be immortalised with well defined genetic abnormalities – primary cells transformed with proto-oncogenes.

Human and murine cell lines eg. HL-60, NB4 cells, MV4;11, NIH3T3, commercially available from ATCC and ECACC. These are standard tissue culture cell lines. Human cell lines may be derived from patient material e.g. HL-60 is derived from a patient with acute promyelocytic leukemia. Murine cell lines may be derived from immortalised murine cells e.g. NIH3T3.

Escherichia coli K12 bacteria derivatives, e.g. DH10beta, DH5alpha. All are commercially available from eg Invitrogen or Promega. Disabled, non-colonising and non-pathogenic to humans and animals

DH5alpha: F- φ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17(k-, m+) phoA supE44 thi-1 gyrA96 relA1 λ-

DH10beta: F- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara, leu)7697 galU galK λ- rpsL nupG

Packaging cell lines eg HEK293 and NIH3T3 and their derivatives are commercially available from ATCC. HEK293 are transformed human embryonic kidney cell line. Cell line was derived from fetal kidney embryonic cells transformed with adenovirus 5 DNA. NIH3T3 are derived from mouse embryonic fibroblasts

Host/vector system

pRRL-X Plasmid used for generating lentivirus where X = gene of interest

Used to generate self-inactivating and replication incompetent lentivirus. The pRRL vector is a lentiviral (HIV)-based plasmid. It is safe to use due to its modified features (deletion of a number of accessory genes implicated in the virulence of HIV, minimal genome of the viral particles, non-replicating and self-inactivation features), making it incapable of producing virus once infected into the host cell.

pHR-X Plasmid used to generate lentivirus where X = gene of interest

Used to generate self-inactivating and replication incompetent lentivirus. The pHR vector is a lentiviral (HIV)-based plasmid. It is safe to use due to its modified features, making it incapable of producing virus once infected into the host cell.

pLKO-X Plasmid used to generate lentivirus where x = shRNA against protein of interest

Used to generate self-inactivating and replication incompetent lentivirus. The pLKO.1 vector is a lentiviral (HIV)-based plasmid. It is safe to use due to its modified features (deletion of a number of accessory genes implicated in the virulence of HIV, minimal genome of the viral particles, non-replicating and self-inactivation features), making it incapable of producing virus once infected into the host cell. The human U6 promoter (a pol III promoter) is used to drive expression of the shRNA hairpin.

pMSCV-X Plasmid used to generate retrovirus where X = gene of interest

Used to generate self-inactivating and replication incompetent retrovirus. Derived from the Murine embryonic stem cell virus and LN retrovirus. It is safe to use due to its modified features (deletion of a number of accessory genes) making it incapable of producing virus once transduced into the host cell.

All the above are plasmids which contain viral elements such as LTR and packaging signal and also elements necessary for propagation in E. coli e.g. DH10B. However there are elements missing e.g. gag, pol, env (e.g. VSV-G) which are only provided in trans (packaging plasmids) in the packaging cell line to create a functional, but replication incompetent viral particles. To further enhance biosafety these elements are split to at least 2 different packaging plasmids to minimise the chance of recombination and therefore production of a replication competent virus. Packaging plasmids encode genes (e.g. capsid, reverse transcriptase) essential for generating infectious viral particles.

Origin & function

The genetic material involved originated from chromosomal translocations found in leukaemia patients.

E2A-FP and MLL-FP Aberrant transcription factors and putative proto-oncogenes identified in patients and literature.

Nup 98-FP and FP-RAR Aberrant transcription factors that can potentially immortalise and transform cells.

RXR family Aberrant transcription factor and member of nuclear receptor family.

MN1-FP and AML1-FP Aberrant transcription factors and putative proto-oncogenes.

ZFN's Zinc finger nucleases with or without donor sequences cut dsDNA at predetermined sequence
Fusion of DNA binding protein (zinc finger) and DNA cleaving protein (nuclease)

FP (fusion proteins) are naturally occurring and derivatives of them are used for structure/function analysis.

**Evaluation of foreseeable effects**

The worst possible consequence of exposure to modified virus would be that operator’s cells could be infected by the virus and the virus integrated into the genome. This could theoretically result in insertional mutagenesis which may lead to the disruption of the function of an important gene which may cause cell death or cellular transformation. It may be theoretically possible that the experimental gene of interest or construct of interest encoded by the virus may be expressed. There may be a very small risk that expression of oncogenes may result in tumorigenesis. Viral vectors are engineered to transfer and integrate specific DNA sequences into the genomes of target cells and are designed to be replication-defective to avoid further spread after the initial transfer event. The likelihood of this happening is effectively zero. Exposure to skin, eyes and lungs would be minimal as gloves and protective clothing are used at all times, no sharps are used and all work will be carried out in a Containment Level 2 laboratory and cellular infections in a Class II Microbiological Safety Cabinet. The modified viruses should not pose a serious risk as they are inactivated by detergent, uv light and ethanol. The half life of the virus is 6-9hrs in tissue culture media at 37oC, it is unknown how long it would survive in the environment. They are also replication defective so will not be shed from the modified cells. Any residual virus on tissue culture plastics, solid waste and liquid waste is inactivated by use of appropriate disinfectants. MSCs are located in specified rooms designated as Containment Level 2 laboratories and only competent and trained operators would carry out these procedures. The modified virus is replication deficient and there is an extremely low risk of transfer of genetic material to wild-type organisms.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

All the measures specified as requirements for the containment level 2 procedures will be adhered to.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Disposable plastic contaminated with virus must be sprayed with disinfectant before placing into autoclave bag and autoclaving.
Plastic pipettes and tips are soaked for at least 12 hours in 1% Virkon solution before being sealed in autoclave bag and autoclaved.
All media must be treated with 1% Virkon solution for at least 12 hours.
All viruses and E.coli are stored at -80°C in designated area of freezer in sealed containers. E.coli grown on LB agar plates are sealed and stored in designated area of fridge for maximum of two week and disposed of by autoclaving.
Autoclaves are serviced and tested every 6-months (December 2009). All waste is autoclaved at 121oC for 30minutes.
MSCs are tested every 6-months and an annual operator protection (KI discus) test was performed in November 2009.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
The Project was discussed and approved as a Class 1 activity subject to minor amendments on 3rd December 2009.

Project Containment

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Project Ref 386/10.4

Date Ackn'd 17/08/2010

CU2 Project Title Functional characterisation of disease causing mutations

Class 2

Culture Volume Class 3-4

1-50 Litres

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The major focus of the work is to identify the genes and delineate the biochemical pathways underlying a range of human genetic disorders using both established and novel technologies.

We plan to achieve these goals by carrying out a range of cell-based functional assays. These include the cloning and transfection of wild-type and mutagenised cDNA transcripts (e.g. from whole or partial disease causing genes such as Dymeclin) which may be transiently or stably expressed in cell lines. If necessary, gene regulatory
regions (e.g. promoter regions) will also be cloned in appropriate vectors. siRNAs will be cloned into appropriate vectors and stably expressed in cell lines to knock down expression of disease causing genes.

The majority of this work will be carried out using standard containment level 1 cloning procedures. However a small proportion of this work will involve the use of lentiviral based vectors to create stable knock down cell lines which will be carried out at containment level 2.

**Recipient or parental organism**

**Class 1 work**
- For generation of standard cloning vectors the recipient cells will be attenuated E.coli cell lines.
- For experimentation the recipient cells will be human and mouse cell lines.

**Class 2 work**
- For generation of virus particles the recipient cell line will be HEK293 cells.
- For experimentation the recipient cells will be human cell lines such as HeLa and HeCat

**Host/vector system**

**Class 1 work**
- For class 1 work the cDNA transcripts and / or regulatory regions will be cloned into a range of mammalian expression vectors (e.g. pcDNA) which will be selected by resistance antibiotics - e.g. Ampicillin, Zeocin, Kanamycin, Neomycin, Puromycin and may be designed to result in fusion of the transcript with tags (e.g. flag, myc), fluorescent proteins (e.g. GFP) or result in the expression of markers (e.g. B-Gal or Luciferase).

**Class 2 work**
- This will make use of the lentivirus vectors using 2nd and 3rd generation packaging systems (e.g. GIPZ lentivirus system). New generation Lentiviral Vectors have been modified to reduce cytoxicity and reduce their ability to replicate. Replication and vector functions are carried on separate, multiple plasmids allowing transfections and transductions to be carried out using replication competent virus and replacing coat with VSV-G reduces likely toxicity (although will broaden host cell range).

**Origin & function**

**Class 1 work**
- DNA sequences for cloning will be generated by PCR from human or rodent cDNA and / or genomic DNA Mutagenesis will be carried out using commercially available systems such as Quikchange (stratagene).

**Class 2 work**
- Vectors will be purchased with siRNA insert for each gene cloned, e.g. GIPZ vector systems (open biosystems)

**Intended functions**

**Class 1 work**
- The genetic material will be intended to either over-express or knock down the gene of interest or to study the effect on expression of regulatory regions. Genes of interest have a range of functions depending on the disease system under study e.g. Dymeclin, a peripheral golgi protein of unknown function in which we identified mutations in patients with Dyggve-Melchior-Clausen syndrome and now seek to determine its physiological role in normal and disease systems.

**Class 2 work**
- The lentiviral vector system will be used to knock down expression of genes of interest in order to study the functional consequences of down-regulated expression e.g. CDKAL1, a gene in which we have identified mutations associated with psoriasis.
Potential Hazard to Human Health

Class 1 work
Likelihood of exposure to humans is low.

E. coli - lines are attenuated and non-infectious.

Cell lines - due to immune rejection of non-self tissue, it is highly improbable that accidental exposure would result in survival and replication in normal healthy individuals. Mammalian cells have very stringent requirements for growth and are very susceptible to dehydration and to exposure to ultraviolet radiation. Outside of the animals from which they are derived, growth and survival requirements can only be met by using specialised media, the correct temperature range, optimum pH and an adequate oxygen concentration. These constraints mean that cell lines will pose risk minimal risk to both human and the environment.

Class 2 work

Likelihood of exposure to humans is low.

Work with Lentiviral vectors will be carried out at containment level 2 to protect against exposure. The modifications to the new generation viruses reduce cytoxicity and make it very unlikely that viruses will have the ability to replicate outside experimental conditions.

Potential hazard to the environment

Class 1 work

Organisms are unlikely to survive outside the laboratory.

E. coli - Attenuated strains are auxotrophic for nutrients that will be scarce except in specialised media. These transformants would not be expected to replicate and may not survive in the environment. However, disabled E. coli strains have been shown to persist for several days in the environment. Good microbiological practice will be sufficient to protect both human health and the environment.

Cell lines - Mammalian cells have very stringent requirements for growth and are very susceptible to dehydration and exposure to ultraviolet radiation. Outside of the animals from which they are derived, growth and survival requirements can only be met by using specialised media, the correct temperature range, optimum pH and an adequate oxygen concentration. These constraints mean that cell lines will pose minimal risk to both human health and the environment.

The likelihood of organisms passing GM material to wild type organisms is very low.

E. coli - Non mobilisable plasmids are used to prevent transfer between bacteria. Attenuation means that bacteria are unlikely to infect wild type organisms preventing transfer from occurring and low survivability in the environment for these strains reduces the opportunity for transfer.

Cell lines - Cell lines are unlikely to be able to survive in both the environment and host organisms preventing the opportunity for transfer to wild type organisms. In addition cell lines do not have the capability of genetic transfer without reproduction.

Class 2 work

Lentiviral vectors - Lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. Therefore, the survivability of
lentiviruses is not thought to pose a risk to the environment.

Rapid inactivation of virus means that there is little likelihood of transfer of genetic material to wild type organisms.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste likely to be contaminated with GMOs is collected in autoclave bags and autoclaved prior to disposal. Autoclave cycle: 121 oC 20 minutes. This is above the standard kill time for decontamination of hazardous material. Following decontamination waste is disposed of by incineration.

Autoclaves are destruct tested every 3 months and insured by Sun Alliance.

Liquid waste is treated with Virkon, Precept, or Trigene or other appropriate commercially available biocide according to the manufacturers instructions for 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

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The GM Risk Assessment for this project was discussed by the School of Medicine GMSC on 11/02/2010. The project was approved subject to minor amendments as a Class 2 activity.
Scientific aims: to investigate the molecular mechanisms regulating immunoglobulin heavy chain class switch recombination using primary human B cells isolated from patients undergoing routine tonsillectomies. To investigate this phenomenon certain genes thought to act upon the system will be overexpressed or knocked down and the effect of these manipulations investigated by the isolation of RNA and DNA followed by analysis by sequencing and PCR.

Genes of interest for manipulation include: Transcription factors (e.g. Helios and E-box binding factors), Chromatin remodeling enzymes (e.g. Histone methyl transferases SETDB2, G9a, SUV39H, MLL), factors involved in DNA recombination and repair (e.g. AID, UNG, RPA, PKA, MSH2, XRCC1 polb) and miRNAs (155, 21 and 29). DNA to be cloned for analysis purposes includes: Immunoglobulin heavy chain locus, BCL6, Pax5, myc, globin genes, b-actin.

This project involves the use of E. coli and lentiviral vectors exclusively for the purpose of cloning, sequencing and manipulating recombinant inserts. None of the vectors support the expression of those inserts in E. coli.

A number of previously genetically manipulated mammalian cell lines, which have been obtained from cell culture collections, and primary B cells isolated from the lung, nose, tonsils, and blood are detailed in this application. DNA and RNA is to be extracted from these cells, and either cloned or analysed directly by sequencing and PCR based methods.

In addition this project will undertake to transiently transfect primary human cells with short interfering RNAs and miRNAs to knock down the expression of genes of interest. Long term overexpression of cDNAs, shRNA and miRNA constructs will be carried out by delivery of mammalian expression vectors containing cloned cDNA/shRNA/miRNA by lentiviral mediated delivery mechanisms.

Recipient or parental organism

Escherichia coli: strains in common laboratory use e.g. DH 5 alpha, XL-1 blue, TOP10 will be used as cloning tools. These strains, which are derivatives of the K12 strain,
have a widespread and long history of safe use. They also contain numerous mutations eg thi-1 which render the strains auxotrophic and therefore unlikely to survive outside the laboratory environment.

Well characterised packaging cell lines, transformed human embryonic kidney (HEK) cell lines, will be used for the propagation of the recombinant viral vectors used to overexpress the genes of interest or deliver inhibitory si and shRNAs.

Primary human B cells and well characterised and authenticated tissue culture cell lines, RAMOS, BJAB, BL-2, CL-01 RPMI 8866 and 8226, A20, HeLa will be used as targets for the overexpression or inhibitory vectors. These lines will be transfected with recombinant viruses then analysed in various ways in order to investigate the function of the genes/proteins of interest. All primary human material will be treated as potentially hazardous and appropriate precautions will be taken to prevent infection. The cell lines used are all well characterised with a long history of safe use.

Host/vector system

Host:
Escherichia coli strains e.g. DH 5 alpha, XL-1 blue, TOP10 are derivatives of the K12 strain, recognised as non-colonising and disabled. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture.

HEK packaging cell line and well characterised and authenticated tissue culture cell lines, RAMOS, BJAB, BL-2, CL-01 RPMI 8866 and 8226, A20, HeLa will be used. These cell lines are all well characterised with a long history of safe laboratory use.

Primary human B cells will be isolated from the tonsils of donors undergoing routine tonsillectomies. All primary human material will be treated as potentially hazardous and appropriate precautions will be taken to prevent infection. The cell lines used are all well characterised with a long history of safe use.

Vectors:
pUC based plasmid backbones (PUC 18, PUC 19, pGEM T-easy vectors and pCR2.1-and related TOPO vectors) are used for cloning vectors, these are non-mobilisable.

Lentiviral packaging vectors will be used to generate defective 3rd generation Lentivirus (HIV-1 based). The viral genes used to produce these vectors (gag, pol, rev and VSV-G envelope genes) are contained on discrete plasmids (pMDLg/pRRE, pC1-VSVG CMV-VSVG, pRSV-Rev). Packaging vectors lackLTRs and have no packaging signal, the packaged viral genome has self-inactivating LTRs. This system ensure minimal replication competence and recombination likelihood.

Mammalian expression vectors (pGIPZ, pLentiLox3.7, psPAX2, pMD2.G) Contain LTR or CMV/RSV promoter driving expression of transgene flanked by cis-acting elements necessary for its encapsidation, reverse transcription and integration. Packaged viral genome has self-inactivating LTR. This is the only genetic material transferred to target cells and contains the viral backbone and transgene cassette.

Origin & function

Non-coding gene products for Ig heavy chain fragments will be produced for propagation prior to downstream analysis (PCR & sequencing) – no potential hazards.

Non-coding gene products for a variety of “control gene” fragments will be produced for propagation prior to downstream analysis (PCR & sequencing), gene fragments will include: globin, BCL6, Pax5, myc, b-actin, – no potential hazards.

We will over-express and knock-down a variety of miRNAs in our mammalian cells; this will entail overexpression of the active gene product in a mammalian overexpression system. The function of these miRNAs is currently of unknown and they will therefore be treated as potentially oncogenic.

A number of histone modification enzymes and chromatin remodelers, including: SETDB2, G9a, SuV39H and MLL, will be studied by over-expression of the active gene product in a mammalian overexpression system and si/shRNA mediated knockdown some of these genes have been described as having oncogenic potential, all
constructs will therefore be treated as such.

A number of transcription factors including Helios, E box factors (Eno1, Id2, ARNTL), AP1 factors (ATF3, NFIL3) and NfkB factors (NFkB1/2, TRIB2,3) will be studied by over-expression of the active gene product in a mammalian overexpression system and si/shRNA mediated knockdown. The oncogenic potential of some of these genes is unknown; as such they will all be treated as potentially oncogenic.

A number of involved in the DNA repair/recombination pathways (e.g. AID, UNG, RPA, PKA, MSH2, XRCC1 polb) will be studied by over-expression of the active gene product in a mammalian overexpression system and si/shRNA mediated knockdown. The oncogenic potential of some of these genes is unknown; as such they will be treated as potentially oncogenic.

Evaluation of foreseeable effects

E. coli: - Likelihood of exposure to humans is low, lines are attenuated and non-infectious.

Cell lines - due to immune rejection of non-self tissue, it is highly improbable that accidental exposure would result in survival and replication in normal healthy individuals. Mammalian cells have very stringent requirements for growth and are very susceptible to dehydration and to exposure to ultraviolet radiation. Outside of the animals from which they are derived, growth and survival requirements can only be met by using specialised media, the correct temperature range, optimum pH and an adequate oxygen concentration. These constraints mean that cell lines will pose minimal risk to both human health and the environment.

Primary Human material: B cells will isolated from tonsils obtained from patients undergoing routine tonsillectomies. The use of any human material represents an infection hazard from unknown disease causing agents. All human derived material will be handled in a class II microbiological safety cabinet and standard good laboratory practice followed.

We aim to use lentivirus to transduce cell lines and primary human B cells with plasmids to either over express or inhibit the expression of a number of genes of interest. The risks from accidental infection by lentiviruses are insertional mutagenesis and over/under expression of cellular genes by random integration or uptake of expression vectors. The main risk is to the laboratory personal directly handling the lentivirus. The risk attached to using the replicative defective HIV vectors is minimised because these viruses are extremely susceptible to dessication and will only survive a few hours at room temperature. In addition, the replicative defective nature of the HIV vectors ensures that even in the case of a breach of containment and accidental injection, no virus can spread from individual to individual.

The DNA sequences used are capable of encoding biologically active proteins which have unknown oncogenic potential in humans. Sequences encoding biologically active oncogenic proteins are classified as carcinogens for which containment level 2 is appropriate. Neither the DNA, encoded protein, bacterial or mammalian transfectants are likely to have any deleterious effect on the environment. The GM microorganisms are incapable of surviving outside of the laboratory environment. All laboratory procedures will follow good microbiological practice. GM microorganisms will be manipulated in a Class II Microbiological safety cabinet.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All GMOs will be handled in Class II safety cabinets with air-circulation through double filters within a 'viral work only' laboratory. Work is carried out in such as way so as to minimize entry/exit to and from the room. All reagents used in these procedures are stored within the room. The surfaces of all Tubes/Flasks & centrifuge buckets taken from the room i.e. for centrifugation are wiped with disinfectant prior to removal. Spill kits are present within this lab. All waste materials are autoclaved immediately upon completion of experiments thereby minimizing escape of GMO into the environment. The GM microorganisms used are incapable of surviving outside of the laboratory environment. All laboratory procedures will follow good microbiological practice. Written records of staff training will be obtained and checked before commencement of work by individuals. Handling and manipulation of naked DNA micro-organisms and GMOs will require the wearing of lab coats and disposable gloves. Where possible, sharps will be avoided in all operations. Disposable plasticware will be used throughout. All laboratory procedures will follow good microbiological practice.

Waste handling procedures:
Solid: All waste autoclaved, 20 minutes 121oC (SOP-DRMA2) then to clinical waste and incineration.
Liquid: Treated with HAZTABLES (SOP-DRMA2 - 4.5g Haz-Tabs added to a litre of water will make a solution of 10,000 p.p.m. available chlorine) for greater than 15mins then
disposed via sink with copious amounts of water.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste handling procedures:
Solid: All waste autoclaved, 20 minutes 121°C (SOP-DRMA2) then to clinical waste and incineration. The efficacy of autoclaving procedure will be validated by visual monitoring of internal control "Browne Sterilizer Control Tubes" (Albert Browne Ltd UK) on each occasion.

Liquid: Treated with HAZTABS (SOP-DRMA2 - 4.5g Haz-Tabs added to a litre of water will make a solution of 10,000 p.p.m. available chlorine) for greater than 15mins then disposed via sink with copious amounts of water.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Reviewed by School Of Medicine BSC on the 5th August 2010. Approved CL2 subject to minor amendments, which have now been completed.

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Animal Units

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Project Ref 386/11.1

Date Ackn’d 23/09/2011  CU2 Project Title Study of skin Immunology, Biology and Pathology

Class 2  CultureVolClass2 < 1 litre  CultureVolumeClass3-4

02/03/2022
The objective of this programme is to dissect molecular, immune and genetic pathways of inflammatory skin disorders, such as psoriasis, and tissue malignancies such as melanoma.

The initial phase of our studies on psoriasis and tissue malignancies such as melanoma will employ in vitro disease-relevant models to determine the functional role of disease-associated genes such as HLAC, CD1d in psoriasis, and Fc receptor and tumour-associated antigens (e.g. HMW-MAA pathway) in melanoma. The experiments will focus on the interaction of these genes with their potential receptors at the protein level in vitro and at the whole system level in vivo.

Our in vitro studies will utilise human cells including tumour cells, lymphocytes or other immune cells, which will be isolated from healthy volunteer and/or patient blood, tissues or tumours, and immortalized using retroviral vectors.

The studies involve the use of standard containment level 1 cloning procedures, however, a portion of the studies will involve lentiviral expression into human primary cells and will be carried out at containment level 2.

### Recipient or parental organism

**Containment Level 1 activities**
- For vector generation the recipient cells will be E. coli strains which are unable to colonise.
- For experimentation the recipient cells will be human and murine cell lines.

**Containment Level 2 activities**
- For generation of virus particles the recipient cell line will be HEK293 cells.
- For experimentation the recipient cells will be cell lines such as HaCaT and BAF-3, primary murine and human cells such as hematopoietic stem cells (HSC), lymphocytes and keratinocytes.

### Host/vector system

**Containment Level 1 activities**
- cDNA transcripts and/or regulatory regions will be cloned into a range of mammalian expression vectors (e.g. pcDNA3) which will be selected by resistance to antibiotics.
and may be designed to result in fusion of the transcript with tags (e.g. flag, myc), fluorescent proteins (e.g. GFP) or result in the expression of markers (e.g B-Gal or Luciferase). The generated constructs will be used for transfection of standard murine or human cell lines such as HEK293T, HeLa or HaCaT.

**Containment Level 2 activities**
Will involve the use of lentiviral systems using 2nd and 3rd generation lentiviral vectors that have been modified to reduce cytotoxicity and reduce their ability to replicate. The supply of viral packaging from separate plasmids makes the associated risks of using the vector system low.

### Origin & function

All genetic material are of human or mouse origin, apart from GFP/eGFP (jellyfish Aequorea Victoria) and B-glactosidase, which is of bacterial origin. The genetic material will be used to determine their functional roles in psoriasis and melanoma.

Inserts for conventional expression (e.g in plasmids of the pcDNA3 series):

i) Reporter genes
   - LacZ, GFP, firefly luciferase: Reporter genes; Lac Z: encodes protein beta-galactosidase; GFP; encodes 'Green fluorescent Protein'; firefly luciferase; encodes enzyme luciferase, which catalyzes a reaction with luciferin to produce light; no known potential hazards.

ii) MHC and MHC-like genes.
   - HLA-C: MHC class 1. Capacity to interact with CD8 T cells and innate immune receptors (KIRs) on NK and NKT cells; candidate gene for psoriasis susceptibility; no known potential hazards.

iii) Cytokines and chemokines
   - IL-2: Cytokine instrumental in body's response to microbial infection; produced by antigen-activated T cells; facilitates production of immunoglobulines made by T cells and induces differentiation and proliferation of NK cells; use in treatment of cancer (malignant melanoma; renal cell cancer); no known potential hazards.
   - IL-22: Cytokine involved in innate immune response against viral infection; no known potential hazards.
   - IL-4, IL-10, IL-13: Anti-inflammatory cytokines produced by T-helper 2 (Th2) CD4+ T-cells; no known potential hazards.
   - IL-17A, IL-22: Pro-inflammatory cytokines produced by Th17 cells; no known potential hazards.
   - CXCL16, CX3CL1: Membrane-bound chemokines; can be proteolytically cleaved from cell surface to generate soluble molecules; role in cell adhesion and leukocyte migration; no known potential hazards.
   - TNF, IFN-γ: Pro-inflammatory cytokines; signal via NFκB (TNF) and STAT1 (IFN-γ); TNF: induces apoptosis and inflammation, inhibits tumorigenesis and viral replication; IFN-γ: activates lymphocytes to enhance anti-microbial and anti-tumour effects; no known potential hazards.

iv) Receptors
   - FR-α, FR-β: Genes encoding the folate receptor which binds folic acid (vitamin B9); no known potential hazards
   - KIRs: Killer Immunoglobulin-like receptors (innate immune receptors); expressed on Natural Killer (NK) and Natural Killer (NKT) cells; interact with MHC class I molecules;
interaction can be inhibitory or activating; dysregulation of KIR/class I interaction important in human disease (e.g. viral infections, cancer, autoimmunity); no known potential hazards.

NKG2C, NKG2D: NKG2C: activating receptor expressed by T cells, as well as by natural killer (NK) cells and macrophages; receptor for MHC class I like molecule MICA No known potential hazards; NKG2D: activating receptor expressed by T cells, as well as by natural killer (NK) cells, interacts with HLA-E; no known potential hazards.

FcγR, FcγRII, FcεRI, FcεRII (CD23): Fc Receptors; expressed e.g. on NK cells, macrophages, neutrophils and mast cells; involved in protective function of the immune system: bind to antibodies that are attached to infected cells or invading pathogens; function e.g. in antibody-mediated phagocytosis or antibody-dependent cell-mediated cytotoxicity; no known potential hazards.

IL-2R, IL-4R, IL-10R1, IL10R2, IL-12R, IL-29R, IL-23R: Cytokine receptors; no known potential hazards.

HMW-MAA: High molecular weight- melanoma associated antigen; potential role in tumour cell adhesion, spreading and invasion; no known potential hazards.

NY-ESO-1: Member of the cancer-tests family of tumour antigens; can be expressed in melanoma; elicits humoral immune responses; promising target for cancer vaccination; no known potential hazards.

v) Intracellular signalling molecules and peptides

Bcl-2, Bcl-xl: Bcl-2 abd Bcl-xl are inhibitors of apoptosis: no known potential hazards.

MMPs: Matrix-Metalloproteinases; zink-dependent endopeptidases; capable of degrading extracellular matrix proteins; involved in cleavage of cell surface receptors, role in cell proliferation, migration, differentiation angiogenesis, apoptosis and host defence; no known potential hazards.

LL37 (CAMP): Human cathelicidin; antimicrobial peptide; potent antibacterial and antiviral activity; no known potential hazards.

S100 proteins, e.g. S100A8: S100 calcium binding protein A8; antimicrobial peptide; also involved in regulation of cellular processes such as cell cycle progression and differentiation; no known potential hazards.

Raf, Raf, cdk, ERK, MEK, IRAK, IRAK4, TAK1: Intracellular signalling molecules; no known potential hazards.

SMAD2, SMAD3, SMAD4, SMAD7: TGF-beta signalling proteins; no known potential hazards.

vi) Viruses

TK39: Herpes simplex virus mutant; no known potential hazards.

vii) Telomerase for immortalisation (inserts for retro-/lentiviral expression)

hTERT: Human telomerase reverse transcriptase (hTERT), contained in pBABE-hTERT retroviral vectors; hTERT has immortalization and therefore oncogenic potential.

viii) Inserts for retro-/lentiviral expression

CD1d: See above

HLA-C: See above
Evaluation of foreseeable effects

The likelihood that exposure to GMOs would have consequences to the health of humans is low.

E. coli harbours the potential of causing infection to humans, however, E.coli lines are attenuated and non-infectious, therefore they are unlikely to cause any harm to human health.

Mammalian cell lines may have oncogenic potential, however these have very stringent requirements for growth and are very susceptible to dehydration and to exposure of ultraviolet light radiation. Outside the hosts from which they are derived, growth and survival requirements can only be met using specialised media, the correct temperature range, optimum pH and an adequate oxygen concentration. These contraints mean that the cell line as well as primary cells will pose minimal risk to both human health and environment.

Lentiviral vectors have been modified to reduce cytototoxicity and reduce their ability to replicate. The supply of viral packaging from separate plasmids makes the associated risks of using the vector system low. hTERT has oncogenic potential and so care must be taken to avoid exposure to this gene by working at containment level 2.

Inserts are not known to be hazardous although some may have oncogenic potential as they have a possible role in cell proliferation; therefore all inserts are cloned in non-mobilized vectors.

Lenti and retroviral preparations are checked for replication incompetence prior to use.

Any mammalian cells transduced by the above methods may harbour oncogenic potential, so all procedures will be carried out at containment level 2, and decontamination, autoclaving procedures will minimise any risk of transfer to humans or the environment. Safe working laboratory practices will be followed. Personal protection equipment e.g., lab coat and disposable nitril gloves will be worn.

In order to minimize exposure risk, use of sharps will not be used when there is a reasonable alternative.

The potential hazard to the environment is low as there are stringent containment procedures in place which include aseptic techniques, decontamination and autoclaving procedures. Such procedures will ensure that the risk of transfer of any genetic material to wild-type organisms will be effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The likelihood of exposure to humans is low. There is a regulatory requirement for suitable protective clothing to be worn. Specified disinfection procedures are in place and effective disinfectants are available for use. All waste material containing viable GMO are inactivated by validated means before disposal before final disposal. Refer to Section 12.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste likely to be contaminated with GMO is collected in autoclavable bags, supported in rigid container and autoclaved prior to disposal. Autoclave cycle 121
degrees Celcius for 20 mins. Following decontamination, the waste is incinerated.

Autoclaves are validated annually and insured by Sun Alliance.

Liquid waste is treated with 2% Virkon (final concentration) or Presept (10,0000 pm) or other appropriate commercially available disinfectants may be used according to the manufacturer's instructions for 100% kill.

The GMRA reviewed on 7th April 2011 and approved as suitable and sufficient for Containment level 2 activity by the King's College London School of Medicine Biological Safety Committee.

Project Containment

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Project Ref 386/11.2

Date Ackn'd 05/10/2011

Reprogramming primary human keratinocytes into induced pluripotent stem (iPS) cells

Class 2

CultureVolumeClass3-4

< 1 Litre

Non-GMM

Consent Granted

Project notified under transitional arrangements N
Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Primary culture of human cells
Use of viral systems to deliver reprogramming factors into cells

Recipient or parental organism

Primary human keratinocytes

Host/vector system

GENOME NON-INTEGRATING VIRUSES We will use modified multi-attenuated self-inactivating (SIN) lentiviral vector pHR'SIN, devoid of all accessory and potentially pathogenic genes. The vectors encode either one (Oct4) or more (Klf4, Sox2, c-Myc and LIN28) reprogramming factors as well as either d2eGFP or mCherry. The vectors are synthesized by GeneArt AG, Germany (www.geneart.com).

GENOME INTEGRATING VIRUSES The Stemgent Lentivirus Set, distributed by Sigma Aldrich, which consists of four lentiviruses, each of which is capable of expressing one of the four (LIN28, Oct4, Sox2, Klf4) reprogramming transcription factors when transduced into mammalian cells.

Origin & function

The purpose is reprogramming of adult cells into pluripotent state. All genes that involved in induction of reprogramming (Klf4, Sox2, c-Myc, Lin28, Oct4) are of human origin. c-Myc and Klf4 proteins are reported to demonstrate oncogenic properties.

mCherry and d2eGFP are used to monitor transduction. mCherry is a modified gene from the marine anemone tetrameric Discosoma striata encoding red fluorescent protein. d2eGFP is a modified gene from the jellyfish Aequorea victoria encoding green fluorescent protein.

Evaluation of foreseeable effects

HR'SIN-cPPT-SEW lentiviral vectors will be of SIN configuration and thus replication defective and incapable of spreading.

Stemgent lentiviral vectors:
The platform used by Stemgent eliminates hazards of autonomous replication during the packaging process by combining a disabled viral genome with a unique manufacturing process. Also, gene functions that facilitate the enclosing of the silencing sequence in a viral capsid (e.g., Gag, Pol, Env) are distributed into multiple helper plasmids (which do not contain significant regions of homology) during packaging. This tactic further minimizes the probability of recombination events that might otherwise generate viruses capable of autonomous replication. With these safety measures in place, the Stemgent IPS cell Lentivirus particles are to be employed only in standard Containment Level 2 tissue culture facilities and should be treated with the same level of caution as with any other potentially infectious agent.

LIKELIHOOD OF EXPOSURE: Low. The work is done exclusively in a microbiological safety cabinet (MSC). Moreover, because of working in a CL2 lab, personnel will use labcoats and nitrile gloves at all times. Nitrile gloves are the most protective gloves when compared with vinyl and latex gloves as they have better barrier protection.
properties. Both the gloves and labcoats are disposable. Personal Protective Equipment (PPE) required: nitrile gloves and labcoat. Facemasks are required to protect sterility of the lab. Personal Protective Equipment and facemask to be used at all times. Aseptic technical work to be performed only within a MSC.

CONSEQUENCES OF EXPOSURE: If the worker becomes exposed to virus, the likely route would be a spill or splash onto exposed skin. Even if this were to happen, infection is extremely unlikely due to the protective layer of stratum corneum of epidermis.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid non-sharp material (gloves, cell cultures, plasticware) is disposed into yellow medical waste bags that will be incinerated.

All liquids are treated with 1% Virkon® S solution for 4 h before disposal.

Serological pipettes and pipette tips are placed in doubled thick plastic autoclave transparent bags and autoclaved before being incinerated. The autoclave decontamination cycle lasts a minimum of 45 min at 134°C (the time is increased according to the amount of waste to be treated). The autoclaved waste is then put in a yellow sharp bin, which is closed and tagged. This yellow bin is then disposed through the normal hospital waste stream.

Is an emergency plan required according to regulation 20?  

[ ] N

If yes, tick to confirm that it is attached to this form

[ ] N

Tick to confirm that you have attached a risk assessment to this form

[ ] Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

[ ] N

Please enter comments on the GM safety committee on the risk assessment

The School of Medicine GMSC reviewed the risk assessment on 9th June 2011 and approved it at CL2 subject to minor amendments which have subsequently been completed...

**Project Containment**

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02/03/2022
The objective of this programme is to investigate pathogenic mechanisms important in Huntington's Disease and to validate therapeutic targets by the following approaches:

1) The generation and characterization of cell culture models of HD (including primary cell lines)
2) The manipulation of the levels of genes of interest in cell culture models of HD (including primary cell lines).

The studies involve the use of standard containment level 1 molecular biology techniques using plasmid vectors with human or mouse genomic DNA or cDNA inserts in bacterial hosts and commercially available mammalian cell lines. We will also carry out gene silencing studies (siRNA or miRNA) using adenoviral, adenovirus associated (AAV), lentiviral or retroviral systems in the above hosts.

A proportion of these gene silencing studies, however, will be performed in primary human cell lines derived from HD patients / unaffected controls and will be carried out at containment level 2.
possibility of transduced cell lines colonising laboratory workers. The likelihood of any cell lines, primary or otherwise, existing outside the tissue culture environment is effectively zero. There is no potential for oncogenicity and likelihood of deleterious consequences is effectively zero.

Host/vector system

While all vector systems used are CL1, all experiments involving primary human cell lines will be at CL2. Lentiviral, adenoviral, AAV and retroviral work involving E.coli and commercially available mammalian cell lines, although assessed at CL1, will also be performed under CL2 conditions as an additional precaution. All vector handling (after suitable staff training) will be within a Biosafety cabinet and PPE worn. Efforts will be made to limit the use of needles and other sharps. Solid and liquid waste will be autoclaved prior to disposal, work surfaces regularly decontaminated e.g 10% bleach and disposable gloves regularly changed. Standard Operating Procedures and Risk Assessments will be in place and rigorously adhered to.

Gene silencing in cell lines (siRNA or miRNA) will be carried out using lentiviral, retroviral, adenoviral or adenovirus associated (AAV) vectors. These commercially available vectors are replication incompetent due to deletion of essential genes. The necessary genes are supplied in trans on helper plasmids and cell lines, making the associated risks of using these vectors extremely low as detailed below for each vector system:

Lentiviruses: Lentiviral vectors can transduce human cells, presenting a risk to investigators. This risk will be reduced to a minimum using the precautions outlined in the preceding paragraph. Furthermore, the commercial lentiviral vector is replication incompetent due to large deletions. The coding sequences of the two packaging constructs cannot undergo recombination with the infectious virion which itself contains less than 5% of original wild type viral sequences. The probability of a replication competent vector being generated via homologous recombination with wild type virus or packaging construct rescue is therefore negligible. This would require multiple homologous recombination events including recombination with wild type HIV, which is never introduced in the production process. Therefore, the lentiviral vectors are non-viable and there is no potential for the release of infectious virus from the cell line. Irrespective of the low risk, vectors are tested to ensure that they are not replication competent. Current lentiviral vectors have minimal effect on the mammalian immune system and there is no reason to expect that any of the inserts will increase an immune response.

Adenoviruses: The adenoviral vector plasmid pAdEasy-1, containing most of the human adenovirus serotype 5 (Ad5) genome, is deleted for the genes E1 and E3. The removal of these two viral genes creates space for foreign DNA and eliminates self replication capabilities. Therefore, the adenoviral vectors are non-viable and are there is no potential for the release of infectious virus from the cell line. Irrespective of the low risk, vectors are tested to ensure that they are not replication competent. AdEasy vector is non-insertional: Because the recombinant adenovirus remains epichromosomal in the human host cell, there is only a remote possibility of activation or inactivation of host cell genes resulting from interruption by the transfected gene(s). Current adenoviral vectors have minimal effect on the mammalian immune system and there is no reason to expect that any of the inserts will increase an immune response.

AAVs: Adeno-associated viruses (AAVs) are derived from defective paroviruses, which depend on essential helper functions provided by other viruses, such as adenovirus and herpes virus, for efficient viral replication and propagation. AAV has no etiologic association with any known diseases and has been successfully used to establish efficient and long-term gene expression in vivo in a variety of tissues without significant cellular immune responses or toxicity. The rep and cap genes have been removed from the viral vector that contains AAV-2 ITRs and are supplied in trans on the plasmid pAAV-RC. Remaining adenovirus gene products required for the production of infective AAV particles are supplied on the plasmid pHelper (i.e. E2A, E4, and VA RNA genes) that is co-transfected into cells with human AAV vector DNA. The remaining adenoviral gene product is supplied by the 293 host cells, which stably express the adenovirus E1 gene. Therefore, the AAV vectors are non-viable and there is no potential for the release
of infectious virus from the cell line. Irrespective of the low risk, vectors are tested to ensure that they are not replication competent.

Retroviruses: pLPCX does not contain the structural genes (gag, pol, and env) necessary for particle formation and replication; however, these genes are stably integrated into PT67. Subsequent introduction of pLPCX, containing Ψ+, transcription and processing elements, and the gene of interest produces high-titer, replication-incompetent infectious virus. These retroviral particles can infect target cells and transmit the gene of interest (which is cloned between the viral LTR sequences), but cannot replicate within these cells since the cells lack the viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation. Therefore, the retroviral vectors are non-viable and there is no potential for the release of infectious virus from the cell line. Irrespective of the low risk, vectors are tested to ensure that they are not replication competent. Current retroviral vectors have minimal effect on the mammalian immune system and there is no reason to expect that any of the inserts will increase an immune response.

**Origin & function**

All genetic material is of mouse or human origin, apart from GFP/EGFP (jellyfish Aequorea victoria), luciferase (firefly Photinus pyralis) and B-galactosidase (bacterial origin).

The genetic material will be used to determine its functional role in the pathogenesis of Huntington’s disease, for example:

Human or mouse genomic DNA, for example Huntingtonin (Htt), a protein involved in cell signaling, transport and protection against apoptosis, which in HD, is mutated by a poly-glutamine triplet repeat expansion within the first exon, leading to protein mis-folding and subsequent disruption of protein/protein interactions. Other inserts encode genes which are dysregulated in HD such as Histone Deacetylase 4 (Hdac4), a regulator of gene expression and Heat Shock Factor 1 (Hsf1), a heat stress induced transcription factor. Inserts will come from pre-existing plasmid, cosmids or BAC clones, from mouse tissue or cell lines, or from human cell lines (not primary and not diseased). These genes are not oncogenes and have no oncogenic potential. They do not encode toxins, antagonists of tumour suppressors or other cell cycle genes. We will not prepare large volumes of vectors containing these genes. There is no potential hazard from these genes or their products.

**Evaluation of foreseeable effects**

See section above

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Plasmids: Solid waste is autoclaved within the department, following which it is placed into griff bins and sent for incineration by licensed contractor. Autoclave and cycle time: 134°C, 20 min for 100% kill. The efficiency of autoclaving is monitored using autoclave instrumentation, chemical and biological indicators (B. stearothermophilis). Liquid waste is disinfected with 1% w/v Virkon overnight and washed down the laboratory sink.

Lab coats are worn in the laboratory at all times. Latex gloves are worn for bacteriological work. Nitrile gloves and
powder free gloves are available for staff allergic to latex and powder. SOPs are followed for microbiological and cell culture work as described in the Departmental codes of practice.

The project proposal was reviewed by the GMSC for KCL School of Medicine 4th August 2011. The project was approved subject to minor amendments.

Please enter comments on the GM safety committee on the risk assessment

The project proposal was reviewed by the GMSC for KCL School of Medicine 4th August 2011. The project was approved subject to minor amendments.

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Project Ref 386/12.2

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<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Historical Significant Changes

Withdrawn N  
Tick if notifying a connected programme of work N
### Project Additional Information

**Purposes of the contained use**

The purpose of this project is to study the effect of Retinoic Acid (RA), a metabolite of Vitamin A, on the immune system. Understanding how RA impacts on immunity has profound implications in global health as well as the management of immune related diseases, like Multiple Sclerosis, Rheumatoid arthritis and Lupus.

**Recipient or parental organism**

For experimentation recombinant Listeria monocytogenes (Lm) has previously been engineered by insertion of the gene encoding the 2W1S peptide.

**Host/vector system**

N/A

**Origin & function**

2W1S peptide

The 2W1S peptide is a variant of an I-Ab-binding peptide from the α chain of the murine I-Ed MHCII molecule. This petide will prime 2W1S specific T cells.

**Evaluation of foreseeable effects**

The likelihood that exposure to GMOs would have consequences to the health of humans is low.

In order to minimize exposure risk, use of sharps will not be used when there is a reasonable alternative.

The potential hazard to the environment is low as there are stringent containment procedures in place which include aseptic techniques, decontamination and autoclaving procedures. Such procedures will ensure that the risk of transfer of any genetic material to wild-type organisms will be effectively zero.

Listeria monocytogenes (Lm) ΔactA-2W1S has been attenuated by the targeted deletion of the actA locus (Lm ΔactA-2W1S). The listerial actA gene is required for polymerization of host actin into filaments, which allows cell-cell spread using components of the host cell’s actin cytoskeleton. Thus the Lm ΔactA strain is unable to spread from cell-cell, and displays a three order of magnitude decrease in virulence without interfering with immunogenicity.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The likelihood of exposure to humans is low. There is a regulatory requirement for suitable protective clothing, specifically side fastening lab coats and gloves, to be worn. All activity with Lm restricted to CL2 laboratories and Class II MSCs. Specified disinfection procedures are in place and effective disinfectants are available for use. All waste material containing viable GMO are inactivated by validated means before disposal before final disposal. Refer to Section 12.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste and cell suspensions to be treated with 1% w/v Virkon solution as directed by the manufacturers protocol and then poured to drain. All plastics that have come into contact with GMOs are to be treated with 1% w/v Virkon as directed by the manufacturers protocol and then bagged or placed in sharps bins (pipette tips and serological pipettes) for off-site incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The KCL School of Medicine Biological Safety Committee reviewed this risk assessment on 7th June 2012 and approved it at CL2 subject to minor amendments.

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Project Ref 386/12.3
The remodelling of a variety of pathological vascular lesions such as venous thrombi, atherosclerotic plaques, intimal hyperplasia, aneurysms, other venous diseases and chronic venous ulcers may be characterised by similar processes. Inflammatory cells, neutrophils, lymphocytes & monocytes, invade the lesion and may release transcription factors, chemokines, growth factors and proteases, which orchestrate tissue remodelling. The roles of these molecules are largely undefined. The work outlined in our proposal includes creating vectors for increasing or decreasing the expression of various proteins in models of venous disease. These studies include introducing viral vectors into primary human blood cells.

Recipient will be macrophages isolated from human donors.

The vectors to be used are attenuated adenoviral, adeno-associated viral, retroviral or lentiviral vectors. These will be designed to express wild type proteins, constitutively active or inactive mutants, or to produce siRNA to knock-down native protein levels. We wish to characterise the effects of these alterations on macrophage function in in vitro experiments. The adenoviral vector to be used is serotype 5 with deletions of the E1 and E3 regions. The probability of reversion to wild type is very low, as the deletions are non-contiguous, making rescue by homologous recombination with wild type adenovirus very unlikely. The adenoassociated virus is serotype 2 containing woodchuck hepatitis post-transcriptional regulatory element sequences and is missing rep and cap genes, which are essential for replication and packaging. The retrovirus to be used carries a macrophage-specific promoter, is based on the Maloney murine leukemia virus, and has deletions of essential promoter and enhancer elements in the 3' long terminal repeat, making the vector self-inactivating (SIN). Packaging genes are deleted. The lentivirus is third generation VSV-G pseudotyped and is replication-incompetent.
| Human hypoxia factor 1alpha (HIF1α); wild type and protease-resistant mutant; transcription factor sensitive to low oxygen levels which promotes the formation of blood vessels. |
| Human tissue plasminogen activator (tPA); serine proteinase which converts plasminogen into plasmin, which breaks down fibrin clots. |
| Human urokinase-type plasminogen activator (uPA); serine proteinase which converts plasminogen into plasmin, which then breaks down fibrin clots. |
| Human vascular endothelial growth factor (VEGF); promotes the formation of blood vessels. |
| Human placental growth factor (PLGF); promotes the formation of blood vessels. |
| Human nuclear factor of activated T cells (NFAT); family of transcription factors involved in immune responses. |
| Human forkhead box protein C2 (FOX-C2); transcription factor involved in development of varicose veins. |
| Human GATA transcription factors; involved in regulation of gene expression in haematopoietic cells. |
| Human TIE2 receptor; tyrosine protein kinase which acts as a cell-surface receptor for angiopoietin1, 2 and 4, regulating angiogenesis, endothelial cell survival, proliferation, migration and adhesion. |

**Origin & function**

All of the viral vectors to be used are attenuated and unable to reproduce without laboratory-specific helper cells to provide essential deleted genes in trans. In the intended recipient cells, the vectors are expected to express the specific proteins cloned into the vector, sometimes together with marker proteins such as green fluorescent protein, or to suppress the expression of native proteins. The expression or loss of the various proteins is anticipated to modulate the response of macrophages to various added factors, which will be tested in in vitro experiments. The modification of human macrophages in a contained manner is not likely to present a hazard.

The accidental inoculation of personnel with modified macrophages should also not be a hazard, as the cells should be rapidly cleared by the immune system. Laboratory workers will not be allowed to work with their own modified macrophages.

Exposure of workers to viral vectors should also result in rapid clearance by the immune system. Any vectors which manage to infect cells will be unable to reproduce, so any effect of transgene expression should be minimal. There is a small risk of insertional mutagenesis if retroviral vectors integrate into a workers chromosome; however, this has never been documented in laboratory workers.

**Evaluation of foreseeable effects**

All of the viral vectors to be used are attenuated and unable to reproduce without laboratory-specific helper cells to provide essential deleted genes in trans. In the intended recipient cells, the vectors are expected to express the specific proteins cloned into the vector, sometimes together with marker proteins such as green fluorescent protein, or to suppress the expression of native proteins. The expression or loss of the various proteins is anticipated to modulate the response of macrophages to various added factors, which will be tested in in vitro experiments. The modification of human macrophages in a contained manner is not likely to present a hazard.

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**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

None under the current project

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None requested

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid waste (tips, serological pipettes, tissue culture plastic, vials and other containers) disinfected by immersion in 10 % Microsol3+ overnight. After rinsing with water, plastic waste is disposed of via clinical waste stream (orange bags).

For liquid waste, Microsol3+ is added to 10 % and left overnight. Waste is then flushed down drain with lots of water. Surfaces are wiped down with 10 % Microsol3+.

Microsol3+ is effective against bacteria, fungi and viruses, and overnight incubation at the stated dilution should result in 100 % kill.
The KCL School of Medicine Biological Safety Committee reviewed the assessment on 16th August 2012 and approved it at GM Class 2 with no amendments required.

Please enter comments on the GM safety committee on the risk assessment

The KCL School of Medicine Biological Safety Committee reviewed the assessment on 16th August 2012 and approved it at GM Class 2 with no amendments required.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 386/12.4

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<td>Class 2</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The overall aim of this project is to study immune regulation in humans during steady state (health) and during inflammation, with a focus on rheumatic disease. More specifically, it aims to uncover cellular and molecular mechanisms via which the inflammatory processes is initiated and regulated and how this is altered by therapeutic agents.

The specific objectives of this project are:

1) To generate expression vectors for select candidate genes of interest for transient expression of proteins in mammalian cell lines and primary human cells. The vectors will co-express fluorescence marker proteins such as GFP.

2) To knock down or decrease expression of genes using siRNA/ microRNA approaches in mammalian cell lines and primary cells.

#### Recipient or parental organism

Recipient cells - Human mononuclear cells - primary and cell lines; bacterial cells - E. coli (DH10b strain) for vector propagation.

Source genes - Various human genes (detailed in the sections below), enhanced green fluorescent protein (eGFP) originally from jelly fish, as a marker of exogenous gene expression and specific antibiotic resistances genes (Ampicillin and Kanamycin) for vector propagation in E. coli.

#### Host/vector system

**HOSTS**

- **i. For vector generation**
  - E. coli DH10b - Standard lab strain, allows replication of large vectors at high copy number. Genotype: araD139 Δ ara-leu7697 fhuA lacX74 galK (Φ80 Δ lacZM15) mcrA galU recA1 endA1 nupG rpsL (StrR) Δ(mrr-hsdRMSmcrBC)
  - **ii. For experimentation**
    - Human peripheral blood T cells and monocytes - Isolated from peripheral blood of healthy donors and arthritis patients
    - Human synovial fluid T cells and monocytes - Isolated from synovial fluid collected from the inflamed joints of consenting arthritis patients
    - HeLa cell line - Well established human cervical carcinoma cell line (ATCC #: CCL-2)
    - HEK293T cell line - Human Embryonic Kidney cell line variant of the HEK293 cell line expressing the SV40 large Tantigen (ATCC #: CRL-11268)
    - HL-60 - Human promyelocytic cell line (ATCC #: CCL-240)
    - Jurkat - Human T-cell leukaemia cell line (ATCC #: TIB-152)
    - THP-1 - Human monocytic leukaemia cell line (ATCC #: TIB-202)
    - U937 - Human histiocytic lymphoma monocyte cell line (ATCC #: CRL-1593.2)
    - CEM - Human acute lymphoblastic leukaemia cell line (ATCC #: CCL-119)

**VECTORS**

- pCMV-SPORT6 - Mammalian expression vector with CMV promoter and Ampicillin resistance gene. Commercially available from Invitrogen.

---

Date of Significant Change

Project Additional Information

Purposes of the contained use

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**VECTORS**

- pCMV-SPORT6 - Mammalian expression vector with CMV promoter and Ampicillin resistance gene. Commercially available from Invitrogen.
pCA-IRES-eGFP - Mammalian expression vector with CMV enhancer and chicken beta actin promoter with an IRES GFP reporter and Ampicillin resistance gene; kind gift of Dr L. Carlin, King's College London.

pEGFP-N1 Mammalian expression vector with CMV promoter eGFP reporter and Kanamycin resistance gene; kind gift of G. B, King's College London.

Origin & function

Except for the selection/marker genes (eGFP, ampicillin resistance), the sequences of the genes to be over-expressed are to originate from human cells. All vectors will be introduced into E. coli with the sole purpose of propagating the vector, and not for any expression studies. Expression of the genes/vectors in human cells will be for the sole purpose of studying the features and functions of the protein encoded by the genes in an entirely in vitro system; the vectors will not be introduced into living animals.

Evaluation of foreseeable effects

Genetic sequences to be inserted

Name: Human CADM1 gene
Function & potential hazards: Cell surface expressed adhesion molecule. CADM1 was originally identified as a tumour suppressor gene (TSLC1: Tumour suppressor in Lung Cancer 1), however this project aims to examine its role in chronic inflammatory conditions rather than in cancer. The gene is normally expressed in very rare populations of healthy blood cells (a DC subset, 0.05% of PBMC), and at very, very low levels in healthy monocytes, which is the cell type to be used for the experiments proposed here. Knockdown of this gene is intended to be carried out by transient siRNA transfection only; no stable knockdown experiments will be performed. As per routine practice in the laboratory, no staff member will perform these experiments on cells isolated from themselves, their close relatives or lab members closely involved in these experiments. With these precautions followed, the potential risk from this gene in the experiments proposed will be low.

Name: Human EGLN3 gene
Function & potential hazards: Intracellular protein involved in regulation of hypoxic responses of cells and activation of HIF-1/2 proteins. No known potential hazards expected on over-expression or knock down.

Name: Human IKZF3 gene
Function & potential hazards: A transcription factor involved in lymphocyte development. A member of the Ikaros family of transcription factors, IKZF3 (also known as Aiolos) plays an essential role in regulation of B-cell differentiation, proliferation and maturation. It is involved in regulating BCL2 expression and controlling apoptosis in T cells in an IL2-dependent manner. Aiolos expression may be dysregulated in chronic lymphocytic leukemia (CLL).
Over/underexpression of Aiolos in T/B cell lines could therefore form a potential hazard. As per routine practice in the laboratory, no staff member will perform these experiments on cells isolated from themselves, their close relatives or lab members closely involved in these experiments, and it is expected that only short-term cell culture experiments will be performed. With these precautions followed, the potential risk from this gene in the experiments proposed will be low.

Name: Human CRTAM gene
Function & potential hazards: Cell surface expressed on activated CD8 T cells and NK cells. No known potential hazards expected on over-expression or knock down.

Name: eGFP
Function & potential hazards: Commonly used fluorescent protein originally identified and cloned from jellyfish. No known potential hazards expected on over-expression.

Environment: No foreseeable effects on human health or the environment are anticipated provided that the safety precautions outlined below are followed.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

1. All GM work performed with human cells will be carried out in a certified microbiological safety cabinet, housed in a separate room (tissue culture room) in the main laboratory.
2. All GM materials will be thoroughly disinfected/de-activated prior to disposal as detailed in section 12 below.
3. Access to the laboratory where this work will be carried out is restricted to authorised, trained personnel only, who are allowed to work unsupervised. All users will be required to have read and signed the Laboratory Codes of Practice.
4. Spills - The following procedures will be followed in case of spills
   - Use biological spill kit
   - Allow aerosols to settle for 10 mins prior to clean up
   - De-activate/disinfect using 1% Virkon solution for 1h
   - Absorb the Virkon/spill with spill absorbent mats provided in the spill kit (located in Taams lab bay, window bench)
   - Dispose mats as follows:
     - For lab E.coli strains dispose off in biohazard bins
     - For human contaminated waste dispose mats off in specially marked biohazard bags (special bags for autoclaving human waste)
     - Place warning notice to indicate area of spillage
     - Dispose of plastic waste in biohazard waste bin

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste: All solid GM waste of human origin such as excess cells, or remaining unfixed cells after an experiment, as well as equipment/tubes contaminated with human waste, will be autoclaved (120°C for 20 min) on site first for full (100%) degradation before disposal for incineration with hospital waste.
Liquid waste: All liquid waste of GM human and microbial origin eg. culture supernatants will be decotaminated with Virkon at 10 g/L overnight for full (100%) killing/disinfection and disposed via the laboratory sink.
Sharps: All sharps and pipette tips contaminated with GM human waste will be collected in a yellow sharps bin clearly marked as containing human waste, autoclaved and disposed by incineration with hospital waste.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The project was reviewed by the KCL School of Medicine Biological Safety Committee on 16th August 2012 and approved as Class 2 GM subject to minor amendments.
### Project Containment

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### Project Ref 386/13.1

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<td>Class 2</td>
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### Historical Significant Changes

- **Historical Date of Additional Info**
  - 

### Project Additional Information

#### Purposes of the contained use

- Production of retro and lentiviruses, transduction of cells with these viruses, plasmid amplification in bacteria, yeast 2-hybrid assays, transfection of mammalian cells.

#### Recipient or parental organism

- The E Coli host will be the multiply disabled K12 strain DH10b using a variety of well charaterised bacterial, mammalian and yeast expression vectors such as pCR3.1, pCAGGS, pGBKT7 and pBABE.
- The S cerevisiae (yeast) host will be Y190 using pGADT7 and pGBK7 vectors.
The mammalian recipients will be 293T, HeLa, BJ fibroblast and MCF10A cells and will be transfected with the above expression vectors purified from bacteria.

**Host/vector system**

The E Coli host will be the multiply disabled K12 strain DH10b using a variety of well charaterised bacterial, mammalian and yeast expression vectors such as pCR3.1, pCAGGS, pGBK7 and pBABE.

The S cerevisiae (yeast) host will be Y190 using pGADT7 and pGBK7 vectors

The mammalian host will be 293T cells and will be transfected with the above expresison vectors purified from bacteria.

**Origin & function**

cDNAs encoding numerous genes both wild type, point mutated and truncated forms. For the majority of these cloned inserts there is no evidence to suggest that any of the inserts have harmful effects.

To allow immortalisation of non-transformed cells, cells will be transduced with a plasmid allowing expression of human Telomerase and/or SV40 T-antigen (as for 293T cells described above). Positive controls such as H-Ras G12V will be used, with handling of H-Ras performed under CL2 conditions. No autologous cell lines will be used.

Individual viral genes that are important for virus assembly were provided from well characterised viral vector systems. There is no evidence that any single gene or protein has virulence potential in vitro. In addition, there is no documented risk associated with handling naked viral DNA under standard/controlled CL1 conditions. The lentiviral packaging system comprises some genes from HIV-1 but they do not constitute an infectious HIV-1. the retroviral packaging system comprises some genes from MLV. These inserts are introduced into cell lines under CL2 conditions, but vectors employed will always contain less than two-thirds of a provirus and cannot result in infectious virus production

**Evaluation of foreseeable effects**

All vectors, inserts and hosts described are not considered pathogenic. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture.

The likelihood that any of the bacterial, yeast or eukaryotic cells could survive in a human host and/or transfer the inserted sequences to other organisms is effectively zero. All work with eukaryotic cells will be conducted in a microbiological safety cabinet, reducing the potential for aerosol transmission, and protective gloves, laboratory coat and safety goggles will be used.

In the majority of cases, vectors are simply being propagated using E.coli as the host, their encoded protein products are not expressed. The exception is the expression in yeast or bacterial expression vectors, however, there is no evidence that the expressed proteins presents toxicity.

The retro and lenti-viral vectors employed are multiply disabled and thus replication incompetent. Control measures for dealing with waste are detailed below.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

NA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

NA

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
In all cases, GMOs will be inactivated by autoclaving (121 °C for 60 minutes for solid waste) or Virkon (1% v/v, overnight, for liquid waste, prior to disposal to the drains). Surfaces and instruments will be disinfected using 70% Ethanol. All disposables will be autoclaved prior to incineration. Autoclaves are serviced 6-monthly.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

The Risk assessment was reviewed by KCL’s School of Medicine Biological Safety Committee on 7th June 2012 and approved subject to minor amendments.

Project Containment

<table>
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Project Ref 386/14.1

Date Ackn’d 06/02/2014

CU2 Project Title
Expression of cDNAs or shRNAs affecting cell proliferation in mammalian cells by retroviral (lentiviral) mediated gene transfer

Class 2
CultureVolClass2 < 1 Litre
CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

02/03/2022
Plasmids containing various gene constructs will be first amplified in bacteria and used to transiently transfect established mammalian cell lines. Amphototropic pseudotype lentiviruses will then be produced and used to deliver the gene constructs to other mammalian cells. The observed phenotypic changes in the recipient cells will be studied and recorded using routine molecular and cell biology laboratory techniques.

The recipient cell cultures will be of primary human epidermal cells, oral squamous cell carcinoma cells or established fibroblast cell lines. The expression of gene constructs which modify the regulation of cell differentiation or proliferation in the recipient cells is expected to produce an increase or decrease in cell division and altered differentiation states.

All epidermal cell cultures have been derived from clinical biopsies. The presence of adventitious pathogens would be more likely if cells were sourced from populations known to have an elevated prevalence of HepB, HepC, HIV, HPV or other pathogens. Therefore low risk patients are selected by interview prior to surgery at the time consent is given and those who have received blood transfusions or have a lifestyle likely to suggest a high risk infection with blood borne viruses, are excluded.

Genes encoding proteins involved in cell proliferation and differentiation will be cloned into plasmid vectors and used to transform bacteria. The E. coli host will be the multiply disabled K12 strain DH10b and a variety of well characterised bacterial, mammalian expression vectors such as pDONR221, pLX301 and pBABE will be used. The plasmid components of a lentiviral vector system will be purified from bacteria and used to transfect HEK293T cells resulting in the production of infectious pseudotyped amphotropic lentiviral particles.

The viral packaging plasmid, PsPAX2, contains genes for Gag, Pro, Pol, RRE and Ampicillin resistance. The envelope plasmid pMD2.G encodes VSV-G protein using a CMV promoter and Ampicillin resistance. pTRIPZ-shRNA plasmid is used for Tet-inducible shRNA constructs and also contains, IRES, tRFP, TRE, self-inactivating 3'SIN LTR and Puromycin resistance. pGIPZ-shRNA plasmid is used for constitutively active shRNA constructs and also contains CMV, RRE, tGFP, IRES, WPRE, self-inactivating 3'SIN LTR and Puromycin resistance.

Plasmids containing the genes of interest and viral assembly genes will be transfected into a mouse cell line and cultured for 24 to 48 hours. The virus will be collected in cell culture supernatants and transferred to other cultures of epithelial or fibroblast cells for the transfection process. The genes needed for packaging the construct into viral particles are present on three or more different plasmids that cannot by themselves be packaged into viral particles. This means that cells can be infected with viral particles made in packaging cell lines, but infectious viral particles cannot be made in the target cells. Exceptionally, the presence of adventitious "helper" retrovirus in a host cell could restore replication competence to the system. Although the components of virus production are present as individual genes in multiple vectors the process of stable integration into the host genome can not yet be predicted so the host and viral genes may be positioned to interact in a complimentary way which could cause cell proliferation. Many of the proposed gene constructs under study are themselves proto-oncogenes or components of mitogenic signalling pathways.

The VSV-G enveloped pseudotype particles have a wide host range which includes humans and expression of the cDNA gene constructs under study has the potential to...
give a proliferative growth advantage to the recipient or neighbouring cells, this can occur by the overexpression of modified proto-oncogenes (Myc, β-catenin, Tcf/Lef, MEK1, Ras, Raf, Rac, Rho, cdc42, PI3K) and pluripotency genes (Nanog, Sox2, Klf, Oct4) or by knocking down tumour suppressor genes. The constructs are modified to be constitutively active, inducibly activated, or have a dominant negative effect, e.g. Ephrins, and E-cadherin, components of the hedgehog signalling pathway (Delta, Notch, Sonic Hedgehog, Patched), TGF-signalling pathway (Smads), EGF-signalling pathway (LRIG) and α and β integrin subunits.

Commercially designed shRNA constructs will also be obtained which specifically target areas of interest within the human genome and prevent expression of individual genes. The recipient cells may acquire a growth advantage over normal or control infected cells as a result of subsequent direct or inadvertent modulation of cellular signalling pathways.

Evaluation of foreseeable effects

All vectors, inserts and host cells described are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture.

The likelihood that any of the bacterial or eukaryotic cells could survive in a human host and/or transfer the inserted sequences to other organisms is effectively zero.

All work with eukaryotic cells will be conducted within a restricted laboratory area using a microbiological safety cabinet, reducing the potential for aerosol transmission, and personal protective equipment (gloves, laboratory coat) will be worn during all manipulations.

The lentiviral vectors employed are able to infect humans but are multiply disabled and thus replication incompetent so virus could not spread from cell to cell except in the presence of adventitious 'helper virus'. However accidental exposure resulting in infection and integration into the genome of lentiviral proto-oncogene inserts brings an increased risk due to the long term nature of the expression produced and the risk (albeit very low) of insertional mutagenesis producing tumorigenesis. Great care will therefore be taken to avoid the use of sharps, such as needles and scalpels, in the presence of infectious virus and any pre-existing skin lesions will be kept covered.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be inactivated by exposure to a final concentration of Virkon, 1% w/v solution for a minimum time of 10 minutes, according to the manufacturer’s instructions, prior to disposal to the drains. Surfaces and instruments will be disinfected using 1% Virkon followed by 70% ethanol.

Solid plastic waste will be exposed to Virkon, 2% w/v solution overnight and discarded into clinical waste bags or rigid plastic "Sharps" boxes which will be sealed before removal by a licensed contractor or incineration

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The maternal and fetal disease group has a program of work that focuses on the aetiology of gestational metabolic disease. A particular focus of the research of the group is the mechanisms underlying Intrahepatic Cholestasis of Pregnancy (ICP), a relatively common (affecting 1 in 150 UK pregnancies) liver disease presenting with raised maternal serum bile acids; with potentially serious consequences for the fetus. To understand the molecular aetiology of this disease, a diverse research program is active. This includes genomic studies to identify susceptibility loci (SNP genotyping and sequencing (Sanger and NGS)) building on previous genetic studies. We are also...
performing in vitro and in vivo endocrine signalling experiments to determine the role of reproductive hormones in the disease. This is based on the group’s identification of cross-talk between bile acid and progesterone metabolite signalling pathways in the liver.

In addition the group have recently identified an in-utero “programming” effect of raised bile acids in a mouse model (offspring have a pre-diabetic phenotype) and this is being explored in further experiments, with analysis of the effect of circadian rhythms. Future work will expand from ICP to investigate gestational diabetes and gestational lipid derangements.

Clinical and translational studies (mostly at present at Imperial College London) are focused on the elucidation of mechanisms of adverse pregnancy outcomes and studies of the metabolic profile of women with ICP with an emphasis on analysis of gut-liver signalling (the FGF19 pathway).

Recipient or parental organism

Primary human cells:
- HUH7 cells (human hepatoma cell line - spontaneously immortal)
- HepG2 cells (human hepatome cell line - spontaneously immortal)
- BeQo cells (human coricarcinoma cell line - spontaneously immortal)
- HEK293 (human embryonic kidney cell line - adenviral transformation)
- HEK293T (human embryonic kidney cell line - adenviral transformation)
- HT29 (intestinal adenocarcinoma cells - spontaneously immortal)
- HT116 (intestinal adenocarcinoma cells - spontaneously immortal)
- CaCo 2 cells (intestinal adenocarcinoma cells - spontaneously immortal)
- NCI -H716 (colorectal adenocarcinoma cells - spontaneously immortal)

Host/vector system

E. coli TOP10
pcDNA3.1 (Life Technologies)
pBIND (Promega)
pGEX6P2 (GE Healthcare)
pGL3/4 (Promega)
pENTR1A (Life Technologies)
pAd/CMV/VS-dest from Invitrogen

Origin & function

mouse ppar-alpha - nuclear receptor which modulates cellular differentiation, metabolism and development. No known or expected hazard from expression
mouse ppar-gamma - Nuclear receptor which modulates cellular differentiation, metabolism and development. No known or expected hazard from expression.
mouse lxr-alpha - Nuclear receptor, controls cholesterol metabolism. No known or expected hazard from expression
mouse fxr - Nuclear receptor, sensor for bile acid levels. No known or expected hazard from expression
humsn lxr-alpha - Nuclear receptor, mediates the biological function of retinoids. No known or expected hazard from expression.
GFP - green fluorescent protein. No known or expected hazard from expression
2xLXR RE luc - Synthetic lxr response elements with luciferase gene. No known or expected hazard from expression
h ET-1 promoter sequence - The promoter sequence of endothelin-1 (vasoconstrictor) as cloned into a luciferase-expressing vector. No known or expected hazard from expression.

Evaluation of foreseeable effects

All of the cell lines and vector systems described above are commercial products that have a considerable history of safe use in laboratories around the world. The E. coli strains have a very little ability to survive in humans.

The gene products are not known to carry any specific risks. It is unlikely any significant hazard will occur as protein expression only happens in very small quantities.

All cells are performance assayed and test negative for HIV-1, mycoplasma, hepatitis-B, hepatitis-C, bacteria, yeast and fungi. Cell performance and characterization are
measured after recovery from cryopreservation. Clonetics™ Media are formulated for optimal growth of specific types of normal human cells. Certificates of analysis (COA) for each cell strain are shipped with each order.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be inactivated prior to disposal:
Solid waste will be autoclaved at 121°C for 30 minutes prior to incineration off-site by licenced contractor.
Liquid waste will be treated with Virkon (2% working solution) for 30 minutes then poured to drain.

Is an emergency plan required according to regulation 20?  

No

If yes, tick to confirm that it is attached to this form

No

Tick to confirm that you have attached a risk assessment to this form

Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment

No

Please enter comments on the GM safety committee on the risk assessment

This project was reviewed by King's College London, School of Medicine Biological Safety Committee on 03/10/2013 and approved the project, subject to minor amendments, at Class 2.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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Animal Units

| L2 L3 L4 L2 | L3 L4 L2 L3 | L4 |

Large Scale Activities

| L3 L4 L2 L3 | L4 L2 L3 | |

Human Clinical Applications

| L3 L4 L2 L3 | |

Project Ref 386/14.3

Date Ackn'd CU2 Project Title

Class CultureVol Class2 CultureVolume Class3-4

02/03/2022

Page 6872 of 15326
The research group is interested in the function of a group of proteins that share a common protein domain, namely a zinc-binding motif. The proteins themselves are a diverse group with implication through mutation in leukaemia and breast cancer. The aim of this work is generally two fold:

1) To assess the biological impact of the expression of zinc finger proteins, in particular those identified to be targeted by the translocations in acute promyelocytic leukaemia (APL). We wish to target APL cell lines that closely mirror the naturally occurring disease. This procedure will involve inserting the gene of interest (e.g. SUMO1/2/3, PML-RARA, IRF2BP2-RARA) into a pMIG or pMSCVpuro retroviral vector and transfecting this construct into the Phoenix-Eco packaging cell line. We will use the pGIPZ lentiviral vector for RNAi studies in combination with pCMV-dR8.74 (gag, pol and rev) and pMD2.G (env) to produce lentivirus in 293T. The supernatant from these cells will be used to infect murine and human cell lines and murine primary cells (e.g. MEFs) in vitro.

2) To assess DNA damage repair and ubiquitin ligase activity in stem cells with BRCA1 mutations. The project aims to generate induced pluripotent stem (iPS) cells from a somatic cell line (keratinocytes). For the generation of iPS cells the CytoTune-iPS Sendai Reprogramming Kit (Invitrogen) will be used. Once the iPS cells are obtained, the work will be followed by irradiation of cells, Western blot and immunofluorescence analyses for DNA repair proteins and for ubiquitin ligase activity. The project also involves siRNA silencing of the BRCA1 gene.

Recipient or parental organism

HEK293T: Human embryonic kidney cells, derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted
Human hair keratinocyte cell lines: Somatic cell lines derived from patients with BRCA1 gene mutations
HeLa: Human cervical cancer cell line
COS-7: African Green Monkey SV40-transfected kidney fibroblast cell line
K562: Human immortalised myelogenous leukaemia cell line from a CML patient in blast crisis
NB4: Human acute promyelocytic leukemia cell line cytogenetically characterized by a t(15;17) (q22;q11-12)
translocation encoding the PML-RARA oncogene
NB4-LR2: Human acute promyelocytic leukemia cell line encoding a mutant form of the PML-RARA oncogene, retinoid maturation resistant
U937: Human monocytic cell line obtained from histiocytic lymphoma
U937-PR9: Human monocytic cell line obtained from histiocytic lymphoma with a Zn-inducible promoter which can switch on expression of the PML-RARA oncogene
KG1: Human acute myeloid leukaemia cell line, deficient in human p53
ME-1: Human acute myeloid leukaemia cell line characterized by an inv(16) (p13;q22) rearrangement encoding the CBF/MYH11 oncogene
Kasumi-1: Human acute myeloid leukemia cytogenetically characterized by a t(8;21) (q21;q22) translocation encoding the AML1-ETO oncogene
WI38: Human fibroblast-like fetal lung cell line

Common laboratory E. coli strains (DH5a, GeneHog, TOP10, XL1 Blue)

Mammalian cells
HEK293T cells: Human embryonic kidney cells, derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted
Phoenix-ECO: Derivative of HEK293T, second-generation retrovirus producer cell line for the generation of helperfree ecotropic and amphotropic retroviruses, contains constructs producing gag-pol and env

Non-viral vectors
DR-GFP (M. Jasin, Memorial Sloan-Kettering Cancer Center and Cornell University Graduate School of Medical Sciences, New York)
Vector to measure homologous recombination (HR), consists of direct repeats of mutated GFP genes: a full-length GFP mutated to contain an I-SceI site and a 5' and 3'-truncated GFP. Repair of the I-SceI-generated double-stranded break by HR results in GFP-positive cells; puromycin and ampicillin resistance gene
EJS-GFP (J. Stark, City of Hope Graduate School of Biological Sciences, Duarte, California)
Vector to measure non-homologous end-joining (NHEJ), contains a promoter that is separated from a GFP coding region by a puro gene that is separated by two I-SceI sites. Once double-stranded breaks are generated by I-SceI and the puro gene is excised by NHEJ repair, the promoter is joined to the rest of the expression region, leading to restoration of functional GFP; puromycin and ampicillin resistance gene
pGEMT-easy (Promega)
Linearised vector with a single 3'-terminal thymidine at both ends for ease of cloning, ampicillin resistance gene
pcDNA3.1 (Invitrogen, now Life Technologies)
High level stable and transient expression in mammalian hosts, CMV promoter, episomal replication in cell lines that are latently transfected with SV40 or express SV40 large T-antigen (e.g. COS-1 and COS-7), neomycin resistant
pBluescript (Stratagene, now Agilent)
For cloning and sequencing. Extensive polylinker with 21 unique restriction enzyme sites flanked by T7 and T3 RNA polymerase promoters that can be used to synthesize RNA in vitro, ampicillin resistance gene
pBluescript SK II+ (Stratagene, now Agilent)
For cloning and sequencing. PLac, lacZ, f1 (+) ori, pUC origin, ampicillin resistance gene
pEGFP-C2/N2 (Clontech)
Genes cloned into the multiple cloning site will be expressed as fusion proteins to the C- (C2) or N- (N2) terminus of EGFP (a red-shifted variant of wild-type GFP), SV40 origin of replication for expression in cells expressing SV40 Tantigen and a pUC origin, can make stable mammalian lines, Neomycin and kanamycin resistant, Herpes simplex
virus thymidine kinase allows G418 resistance.

pGL3 (Promega)
Luciferase reporter vector, SV40 promoter upstream of the luciferase gene, Col E1 and F1 origins, ampicillin resistant

pSG5 (Stratagene, now Agilent)
For expression, F1 origin, SV40 early promoter and polyadenylation signal, T7 promoter, β-globin intron II allows splicing of expressed transcripts, ampicillin resistant

pREP4-tk-luc (A. Zelent, Institute of Cancer Research, London)
Luciferase reporter vector, RSV LTV promoter, TK promoter, EBNA-1 gene, pUC origin, OriP, ampicillin resistant for expression in bacteria and hygromycin resistant for mammalian cell selection.

Viral vectors

pMSCVpuro (Clontech)
Retroviral vector, does not include gag, env or pol genes so is unable to replicate, has a CMV promoter, ampicillin resistant for bacterial expression, puromycin resistant for mammalian selection

pMIG (Addgene)
Retroviral vector MSCV 2.2 inserted with the internal ribosomal entry site (IRES)-GFP sequence, does not include gag, env or pol genes so is unable to replicate, has a CMV promoter, ampicillin resistant for bacterial expression, puromycin resistant for mammalian selection

pGIPZ (Thermo Scientific)
Lentiviral vector, tGFP, IRES, shRNA, WPRE, does not include gag, pol or env genes so is unable to replicate, has a CMV promoter, ampicillin resistant for bacterial expression, puromycin resistant for mammalian selection

pCMV-dR8.74 (Addgene)
Packaging vector, ColE1 Origin, GPT, CMV, Gag, Pol, RRE, ampicillin resistance

pVSV-G (Clontech)
Packaging vector, envelope plasmid, ColE1 Origin, CMV, IVS, VSV-G, ampicillin resistance

psPAX2 (Addgene)
Packaging vector, SV40 ori, CAG promoter, cPPT, RRE, HIV_Rev_NES, ampicillin resistance

pMD2.G (Addgene)
Packaging vector, envelope plasmid, CAG enhancer, CMV promoter, CMV2 promoter, Glob_int, VSV-G, pBR322 ori, ampicillin resistance

Sendai Virus Kit (Invitrogen, now Life Technologies)
Contains four SeV-based reprogramming vectors, each capable of expressing one of the four Yamanaka factors (i.e. Oct4, Sox2, Klf4 and c-Myc), 5 genes for viral proteins (starting from 3' end): Nucleocapsid protein, Phosphoprotein, Matrix protein, Hemagglutinin-Neuraminidase and Large protein. The gene encoding the Fusion (F) protein has been deleted, rendering the virus incapable of producing infectious particles from infected cells. In addition, the presence of functional mutations such as temperature sensitivity in the amino acid sequence of several SeV proteins (SeV/TSΔF, SeV/TS12ΔF, and SeV/TS15ΔF) renders the vectors easily removable from transduced cells.

Origin & function

P65: Human, wild-type, transcription factor, NFkB
SUMO1/2/3: Human, wild-type, protein modifier, will be inserted into viral vectors
BRCA1: Human, wild-type and mutant, oncogene involved in breast cancer, siRNA
Ubiquitin: Human and mouse wild type and mutant, protein modifier
OCT3/4: Human, wild-type, transcription factor, marker for undifferentiated cells, in SeV
SOX2: Human, wild-type, transcription factor, pluripotency marker, proto-oncogene, in SeV
KL4: Human, wild-type, transcription factor, pluripotency marker, proto-oncogene, in SeV
C-MYC: Human wild-type transcription factor, proto-oncogene, in SeV
PLZ: Human and mouse, wild type and mutant, can be pro-oncogenic when fused to RARA
PML: Human and mouse, wild type and mutant, can be pro-oncogenic when fused to RARA
RARA: Human, wild-type, transcription factor, fused to various partner genes in APL
PML-RARA: Human, wild-type and mutant, oncogene that can cause APL, will be inserted into viral vectors
PLZ-RARA: Human, wild type, oncogene that can cause APL
IRF2BP2-RARA: Human, wild type and mutant, oncogene that can cause APL, will be inserted into viral vectors
P50-RARA: Murine, mutant transgene, fusion protein that causes permanent dimerisation of retinoic acid receptor alpha (RARA)
elF4E: Murine, eukaryotic translation initiation factor that directs ribosomes to bind to the mRNA cap, also involved in mRNA transport from the nucleus
RFP: Red fluorescent protein
PRH: Human, involved in cell proliferation and differentiation
TRAP1: Human and mouse, wild-type, hsp90-related protein, molecular chaperone involved in cell signalling, protein folding and morphologic evolution
HSP90: Human, wild-type, molecular chaperone involved in cell signalling, protein folding and morphologic evolution
HGF promoter: Human, wild-type and mutant, paracrine cellular growth, motility and morphogenic factor
STAT3: Human, wild-type and mutant, transcription factor, proto-oncogene

Evaluation of foreseeable effects

Hazards to human health
Although the effects are ultimately unknown, the likelihood of transfected or primary cells surviving outside the tissue culture environment is effectively zero. No adventitious agents are known to be secreted from the cell lines used in this project.

The viral vectors used in this project are all replication defective. Therefore, they cannot undergo more than a single round of transduction and establish a productive infection in an individual. Most of the original viral genome of the retroviral/lentiviral vectors has been deleted such that disease-causing genes are absent and the pathogenicity of the vector markedly reduced compared to the parental strains. Infectivity of the resulting virus is only possible via packaging constructs/cell lines. Similarly, SeV vectors used in the Sendai virus kit are no longer capable of producing infectious particles from infected cells because the viral genome lacks the F-gene.

Second and third generation lentiviral vectors usually elicit a relatively low immune response compared to nonintegrating vectors. As well as having minimal effect on the mammalian immune system, there is no evidence to suggest that the gene products or any of the inserts will increase any immune response. Human is not a natural host for the SeV and this virus is not pathogenic to humans.

In case of accidental exposure, the use of retroviral/lentiviral vectors carries a risk of triggering oncogenic events in transduced cells by upregulation of cellular protooncogenes at the site of vector integration (insertional mutagenesis). The genotoxic risk is greatly reduced for the pGIPZ lentivector due to self-inactivating (SIN) 3’ LTR. Additionally, the retroviral vectors containing oncogenic inserts such as leukaemia-associated fusions (i.e. PML-RARA, IRF2BP2-RARA) are more likely to induce tumourigenesis. Other oncogenes (i.e. c-MYC, SOX2, KLF4) will only be delivered via SeV reprogramming vectors. Since SeV does not integrate into the host genome and remains in the cytoplasm there is no risk of insertional mutagenesis. Moreover, the host cell can be cleared of the vectors and reprogramming factor genes by exploiting the cytoplasmic nature of SeV and the functional temperature sensitivity mutations introduced into the key viral proteins. To minimise the likelihood of accidental exposure, only low volumes of viruses will be used and the work will be conducted following all safety rules for working in a Containment Level 2 (CL2) facility.
Hazards to the environment
The likelihood of the GMO surviving in the environment is effectively zero. The likelihood of transfected or primary cells surviving outside the culture environment is low. The viral vectors used in this project have a short half-life outside a host cell and are replication deficient so they would not be able to reproduce. Attenuated E. coli strains are dependent on nutrients supplied in specialized media and therefore do not persist in the environment. The risk of transfer of genetic material to wild-type organisms is effectively zero. The retroviral/lentiviral vectors used in this project are replication incompetent and therefore would not be able to reproduce and infect wild-type organisms. Wild-type SeV is highly contagious in host species (i.e. rodents), but the infection does not persist in immunocompetent animals. In addition, the SeV genome in the kit is missing its F-protein, rendering the virus incapable of producing infectious particles from infected cells

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be soaked in a disinfectant with a proven wide spectrum virucidal, bactericidal and fungicidal activity (1% Virkon, VWR) overnight to achieve greater than 99.999% kill prior to sink disposal. Solid waste, such as tissue culture flasks, tips, etc., will be soaked in 1% Virkon prior to autoclaving. Autoclave bags will be closed when ¾ full and autoclaved at 121°C for 30 minutes then transferred for incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The project was reviewed by the School of Medicine Biological Safety Committee on 11th April 2013 and approved the project at GM Class 2 subject to minor amendments

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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## Project Additional Information

### Purposes of the contained use

Lentiviral vectors pseudotyped with filoviral surface glycoproteins will be used to study the cell biology and immunology of filoviruses.

### Recipient or parental organism

1. Transformed human, primate or murine cell lines cultured by standard procedures.
2. Isolated human primary leukocytes subsets (macrophages, dendritic cells, T cells and B cells) used to assess the host range of the filoviruses and the response of these cells to lentiviral challenge. These cells will be isolated from healthy donors with informed consent.
3. Primary murine leukocyte subsets.

### Host/vector system

**HOSTS:**
1. E. coli DH5a or DH10b bacterial strains will be used as hosts for the propagation of the plasmids by standard methodologies.
2. Transformed human embryonic kidney cell line HEK293T will be used as mammalian cell hosts for the production
3. Yeast (S. cerevisiae) Y190 will be used for yeast-2 and 3-hybrid assays examining interaction of human and individual filovirus proteins.

VECTORS:
- Filoviral glycoprotein pseudotyping of lentiviral or retroviral vectors:
  The cellular biology of Ebola virus entry into a target cells will also be investigated using gammaretrovirus or lentivirus vectors pseudotyped with filoviral glycoproteins. These are regular replication defective MLV or HIV-based vectors produced from 3 plasmid transfections including the genome plasmid containing a reporter gene (eGFP, RFP, Firefly luciferase, Beta-galactosidase), a Gag-Pol packaging plasmid and an Ebolavirus glycoprotein expression vector instead of standardly used VSV-G expression vector. These will be used in experiments when only GP function is being assayed. The only filovirus protein expressed in this system is the glycoprotein. These vectors are well characterized in the literature and are non-hazardous.
- The plasmid vectors expressing genes under contained use:
  All individual filoviral proteins will be expressed from the mammalian expression vector pCR3.1, pCAGGS for transfection in 293T cells, or pGBK7 or pGADT7 for expression in Yeast 2 hybrid assays.

ORIGINS:
Glycoprotein genes cloned into pCAGGS will be used from the Ebolavirus strains Zaire, Sudan, Reston, Tai Forest and Budibungyo and from the related Marburg virus.

Plasmid propagation:
• All plasmids will be propagated in E coli DH5alpha, DH10b or STBL2 bacteria by standard methodologies. Plasmids will be purified by Qiagen kits or commercial equivalents. All bacterial strains are multiply attenuated and pose no risk to handlers.

Production of lentiviral vectors pseudotyped with filoviral envelopes:
• HEK293T cells will be transiently transfected with a lentiviral vector genome encoding a reporter gene (mainly eGFP or mCherry fluorescent proteins) along with a standard HIV-1-based vector packaging plasmid supplying the lentiviral structural proteins and enzymes, and expression vectors for various strains of filoviral GPs (see below). 48h post transfection, lentivectors will be harvested and target cells challenged, with read-out being fluorescent protein expression by FACS.

Yeast assays:
Yeast (S. cerevisiae):
Yeast are used for yeast-2-hybrid and yeast-3-hybrid assays to test for protein:protein interactions between selected EBOV proteins and human proteins. Plasmid expression vectors containing biosynthetic selectable markers and encoding genes of interest fused to GAL4 activation or DNA binding domains are transformed into exponentially growing Y190 cultures. Selected transformants are then tested for beta-galactosidase expression.
Evaluation of foreseeable effects

Retroviral/lentiviral vectors:
MLV-based oncoretroviral vectors and HIV-based lentiviral vectors. HIV naturally infects human cells whereas MLV infects cells of many mammalian species. Our work will use retroviral/lentiviral vectors pseudotyped with the G proteins of VSV, EBOV or MARV. VSV-G can mediate entry into any mammalian cell type. EBOV naturally infects myeloid cells and endothelial cells, and retroviral/lentiviral pseudotypes can infect a subset of human and primate cell lines. With respect to the laboratory research activities outlined here, transmission of could occur through the exposure of unprotected and broken skin to liquids containing VLPs or viral vectors, e.g. tissue culture supernatants from living cells producing with viral vectors or VLPs.

Most Hazardous component:
The most hazardous biological agents are the VSV-G pseudotyped lentiviral vectors as they have the ability to transduce an integrated vector genome into most mammalian cells with varying efficiencies. However all viral vectors used are replication incompetent. There is no evidence that any single gene or protein has virulence potential when expressed in bacteria. Finally, there is no documented risk associated with routine handling of naked filoviral or retroviral DNA under CL1/2 conditions.

Consequences of exposure to any of the above:
Bacteria/Yeast and mammalian cell lines: All bacteria, yeast and cell lines have demanding requirements for maintaining cell viability, which will not be met in the general environment. The E coli strains used for propagation are multiply attenuated and pose no hazard to users. There is no posibility of generating filoviral or lentiviral particles in bacterial strains propagating the plasmids.

Retrovirus: MLV-based oncoretroviral vectors and HIV-based lentiviral vectors. Our work often uses retroviral vector virions pseudotyped with the G protein of VSV, and therefore able to infect cells of many species. There is a potential risk of insertional mutagenesis, but as the vectors are replication-defective, this is a low risk. There are no documented cases of harmful consequences of accidental exposure to the MLV- based or HIV-based vectors discussed herein.

Exposure to skin, eyes, and lungs are minimal as gloves and protective clothing are used all the time, no sharp are used and all viral vector work is performed in a class 2 MSC.

Tissue culture plastics, solid waste and liquid waste is inactivated by use of 10% P3-Steril. It should be noted that when 10% P3-Steril is added to a tissue culture monolayer, the cells are completely lysed and therefore it will decontaminate all viruses. All solid waste is subsequently autoclaved and incinerated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Tissue culture plastics, solid waste and liquid waste is inactivated by use of 10% P3-Steril (a quaternary ammonium compound). It should be noted that when 10% P3-Steril is added to a tissue culture monolayer, the cells are completely lysed and therefore it will decontaminate all enveloped viruses and viral like particles. All solid waste is subsequently autoclaved and incinerated. Inactivated liquid waste will be disposed of via the drainage. All bacterial culture waste is inactivated with 2% Virkon, which completely kills all viable E coli, before drainage disposal.
The project was reviewed by the KCL School of Medicine Biological Safety Committee on 4th December 2014 and was approved subject to minor amendments.

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<table>
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Project Ref 386/16.1

Date Ackn'd 31/08/2016

CU2 Project Title Molecular Interactons of flaviviridae viruses with the host cell

Date Project Ceased

Class 3

Culture Vol Class 2 < 1 Litre

Culture Volume Class 3-4 < 1 Litre

Non-GMM Consent Granted Yes

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
The Catanese lab studies the molecular cell biology of Flaviviridae. This family of positive-sense, single-stranded RNA viruses includes hepatitis C virus (HCV), dengue virus (DENV); Zika virus (ZIKV); West Nile virus (WNV), Japanese encephalitis virus (JEV) and Yellow fever virus (YFV) among others. Their genome is translated into a single polyprotein that is subsequently cleaved into 10 proteins: 3 structural proteins (the capsid protein and two envelope glycoproteins), and 7 non-structural proteins (see figure 1 in appendix A).

Flaviviridae viruses produce a wide range of diseases including fever, arthralgia, rash, liver failure, haemorrhagic fever and encephalitis.

Initial research will primarily focus on HCV and ZIKV. At a later stage, we intend to expand these studies to include WNV, JEV, DENV and YFV. Through collaborations, we began comparative studies with other RNA positive-strand viruses, including the Alphaviruses Sindbis virus (SINV) and Semliki Forest virus (SFV), which led to discovering conserved exploitation strategies of cellular factors with HCV. We therefore would like to include SINV and SFV as part of our model systems.

Our goal is to gain insights into the subversion strategies devised by these viruses (i.e. cytoskeleton rearrangements, endomembrane remodeling during virus replication/assembly, vesicle trafficking and cell-to-cell spread) that promote successful viral dissemination. Furthermore, we will study the functions of virion-associated host proteins to investigate how these cellular factors contribute (either positively or negatively) to the propagation of infection. These studies will provide insights into the exploitation of physiological cellular processes by this related group of viruses. The research team will address these questions using a multidisciplinary experimental strategy that includes molecular genetics, cell biology, biochemistry and structural biology.

This risk assessment covers all work performed in our lab under CL2 and CL3 conditions, and therefore relates to procedures involving the introduction (and expression) of viral genomes into permissive cells. This includes work with replication-competent viruses. Both immortalized cell lines and primary human cells are used for this work. CL 2 will be used to study individual viral proteins of the Flaviviridae members (i.e. HCV, DENV, ZIKV), which are not infectious, and full length ZIKV, SFV and SINV. CL 3 will be used for experiments with full length infectious HCV.

Experiments outside the CL2 and CL3 facilities will be conducted on fixed (4% paraformaldehyde (PFA) or 100% methanol) cells/tissues/supernatants that are no longer infectious.

**Recipient or parental organism**

Our goal is to generate key tools and experimental systems to study the biology of Flaviviridae infection in cell culture and compare it to other RNA positive-strand viruses (SFV and SINV). Specifically:

Full length HCV, ZIKV, SFV and SINV genomes will be generated by in vitro transcription of molecular cDNA clones and electroporation/transfection of permissive cells.

Virus-containing supernatants will be inoculated onto naïve cells to propagate infection in cell culture. We will produce soluble Flaviviridae envelope proteins, pseudoparticles and subgenomic replicons. None of these system is infectious and can be safely used in CL2 laboratories. These constructs will be either generated by molecular cloning or synthesised by Origene and will be used to test host and cell permissiveness of various isolates, to understand aspects of the molecular biology of viral replication, and the potential impact of viral genetic changes on pathogenicity.

**Host/vector system**

Constructs encoding for viral genomes or individual viral proteins will be amplified in bacteria and expressed in permissive eukariotic cells (i.e. hepatocytes or hepatoma cells for HCV; skin, neuronal, placental cells for ZIKV; BHK, Hela, 293T and hepatoma cells among others for SINV and SFV).

**Origin & function**

1. Production of soluble Flaviviridae envelope glycoproteins.
   1.1. Soluble forms of viral envelope glycoproteins will be produced in 293T cells by transfecting constructs lacking the transmembrane domains, leading to their secretion. This approach was used successfully for several viruses of the Flaviviridae family, including HCV and DENV (8, 9).
   1.2. Tagged versions (6x-His, HA, FLAG) will be created to facilitate protein purification, and to enable biochemical studies to search for Flaviviridae cellular receptors/interacting partners.
1.3. These proteins will be used in cell culture to determine if they can compete with infectious particles and inhibit virus infection.


2.1. ZIKV pseudoparticles (ZIKVpp) and HCV pseudoparticles (HCVpp) will be produced as done previously with other Flaviviridae (10). 293T cells will be co-transfected with expression vectors encoding: (i) viral envelope proteins, (ii) gag-pol proteins of either murine leukemia virus (MLV) or human immunodeficiency virus (HIV) and (iii) a retroviral ge-name bearing a reporter gene such as luciferase or green fluorescent protein to allow quantitative measure of viral en-try into target cells.

2.2. This experimental system will help define the host-range and cellular tropism of ZIKV entry. We will screen the permissiveness of neuronal, placenta-derived, blood brain barrier, testis and prostate cells to ZIKVpp infection, using either flow cytometry or luciferase production as readout. Pseudoparticles constitute a very safe and versatile system to express any variant glycoproteins that may be identified in severe cases of ZIKV disease to study whether specific genetic alterations lead to changes in virus host and/or tissue tropism.

2.3. Using pseudoparticles, we will be able to express patient-derived viral glycoproteins to address the neutralization properties of virus-specific antibodies present in patient sera, and to identify cross-neutralizing epitopes.

3. Generation of Flaviviridae sub-genomic replicons

A sub-genomic replicon is a non-infectious, self-replicating RNA construct containing the viral non structural (NS) genes, where the regions encoding for the structural proteins have been deleted or replaced by foreign genes (11). As a result, no infectious particles can be produced with this system, making for a very safe cell culture experimental model.

3.1. ZIKV and HCV sub-genomic replicons will be constructed to define the minimal set of NS genes required for virus replication in a range of cells. Reporter (i.e. luciferase, GFP) or antibiotic resistance genes will replace the structural region of the genome, enabling rapid screening and quantification of virus replication and the generation of cell lines stably replicating ZIKV and HCV, respectively.

3.2. Cells from different hosts (i.e. mosquito, monkey, human) and tissues (including skin, placenta, brain and testis) will be screened for permissiveness to ZIKV replication upon transfection of in vitro-transcribed viral replicons. We also address how interferon treatment and expression of interferon-stimulated genes (ISGs) impact on ZIKV and HCV replication, using ISGs libraries available at KCL.

3.3. We will introduce a range of genetic variations found in the NS regions from different ZIKV and HCV isolates into sub-genomic replicons to determine the relative replication ability of geographically distinct strains/genotypes. It was recently shown that the current ZIKV Asian lineage spread is associated with significant NS1 codon usage adaptation to resemble more closely that of human genes, which could promote ZIKV replication and increase viral titers (12). Similarly, ZIKV isolated from a fetus with microcephaly revealed two amino acid substitutions in NS1 and NS4B (2). It is unclear whether these represent adaptations of the virus to enhance replication in the brain. We assess these muta-tions in our systems to determine the impact of these genetic changes on ZIKV replication and tropism.

3.4. We will study the ultrastructure of ZIKV- and HCV-infected tissues by transmission electron microscopy to gain a better understanding of the sites of viral replication and the resultant tissue damage. This aim will be applied to in vitro replication studies and biopsies obtained from the current epidemic.

4. Virus-host interaction studies with full length HCV (CL3), ZIKV, SFV and SINV (CL2) genomes. These will include down regulation (with siRNA/shRNA) and over-expression studies of cellular factors to determine their role on the propagation of HCV, ZIKV, SFV and SINV infections. The expression of both cellular and viral proteins will be as-sessed by biochemistry and flow citometry or visualised by fluorescence and electron microscopy.

Evaluation of foreseeable effects

Cell culture infectious HCV (HCVcc) is a HG3 virus capable of establishing chronic infections and causing progressive liver disease. However, it is possible to cure infected individuals with a combined regime of interferon and ribavirin, furthermore, a range of new anti-virals are dramatically improving treatment efficacy (>90% cure rate).

The original HCV clones were derived from human patients, specifically GenBank accession # AB237837.1 and derivatives thereof, originally described in Lindenbach, Science, 2005,309, p623-6.

HCVcc is transmitted exclusively by percutaneous inoculation and is not capable of transmission by air or ingestion. In a research setting, the primary risk comes from contaminated sharps or, potentially, eye-splash exposure. However, these risks are all but eliminated under containment level 3 (CL3). Gloves, eye shields and protective clothing are used all the time, no sharp are used and all viral work is performed in a class II MSC.

Modification of HCVcc clones by introduction of specific mutations and protein tags/fluorescent proteins that allow us to visualise and quantify viral infection is not expected to increase infectivity.

The ZIKV clones used in these studies are from either the Asian or African lineages and are described in Haddow et al., PLoS Negl Trop Dis. 2012;6(2):e1477. doi: 10.1371/journal.pntd.0001477.

Zika virus (ZIKV) is a HG2 mosquito-borne flavivirus distributed throughout much of Africa and Asia and recently spreading through the Americas. Infection with the virus
may cause acute febrile illness that clinically resembles dengue fever. It is thought to be transmitted by percutaneous inoculation. Possible consequences of ZIKV infection include: fever, rash, joint pain, or conjunctivitis, muscle pain and headache. SFV is spread mainly by mosquito bites. Both Wild-type SFV and fluorescently-tagged viral genomes (i.e. SFV_nsp3/GFP) will be used in these studies. SFV can cause infection but there is only one recorded incident of pathogenesis.

The prototype of the Alphaviruses, SINV has a broad host range, shuttling between an insect vector to a vertebrate host for its transmission cycle (Hernandez et al., Curr Protoc Microbiol. 2005 Oct;Chapter 15:Unit 15B.1. doi 10.1002/9780471729259.mc15b01s00). As a result, in tissue culture, SINV can replicate in several cells of both vertebrate and invertebrate origin.

Wild-type SINV utilized in these studies was generated from plasmid pToto1101 (Rice, C. M., et al. (1987) J. Virol. 61, 3809 –3819). Virus expressing GFP as an in-frame fusion within nsp3 was described previously (Liang, Z et al. (2005) GeneTher. Mol.Biol.9,317–324) and is here designated nsp3-GFP Sindbis. SINV can infect humans, producing a rash-arthritis syndrome. However, these Alphaviruses need an insect vector for human transmission and cannot spread directly from human to human.

The major risks to be considered for research with HIV-1 based lentivirus vectors are the potential for generation of replication-competent lentivirus (RCL). This risk has been removed with the later generation lentivirus vector systems that use a heterologous coat protein (in our case VSV-G) in place of the native HIV-1 envelope protein, thus reducing the risk of RCL generation. To improve safety, transfer vectors are all replication incompetent and in some cases additionally contain a deletion in the 3’LTR, rendering the virus “self-inactivating” (SIN) after integration.

The pseudotyping env gene is carried on a separate plasmid to other viral components/sequences and cannot be packaged, limiting infection by the pseudotyped virus to a single round only.

ZIKV, SFV, SINV and pseudotyped retroviral particles carrying VSV Env glycoprotein can infect an expanded number of cell types and cells from additional species. The most probable route of exposure for laboratory research activities involving these viruses would be dermal via sharps (needle-sticks), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure of the eyes, nose, and mouth. Another route would be inhalation via aerosols depending on the use of equipment such as centrifuges or vortex mixers. Potential insertional mutagenesis through the use of lentiviral vector systems.

Viral sequences encoding specific proteins may be sub-cloned into other plasmids to allow production of virus proteins in isolation, however these will pose no risk to human health.

There are no identified risks to the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General procedures for working in the Department are outlined in the Departmental Code of Practice (CL1, CL2 & CL3). All work is covered by Departmental Standard Operating Procedures, which should be followed in conjunction with the relevant COSHH risk assessments and this Biological Risk Assessment. All staff must have a written training record

Solid: all solid waste will be soaked overnight in 20% P3-Steril (final concentration), a quaternary ammonium compound, and autoclaved (moist heat for 60 min at 121 oC). All solids will be place in yellow bags for subsequent incineration by licenced contractor.

Liquid: all liquid waste will be treated with 20% P3-Steril (for at least 1h) and then put down the sink and rinsed with copious quantities of water.

We have demonstrated effective virus inactivation after 1h incubation. In house validation of HCV inactivation by P3-Steril was successfully conducted and proved complete removal of infectivity from the sample (see detailed protocol and results in appendix of CoBRA file).

Similar testing with 20% P3-Steril will be conducted on SINV, SFV and ZIKV once these virus are on site to ascertain complete inactivation

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The project was reviewed by the Faculty of Life Sciences and Medicine Biological Safety Committee on 28th April 2016 and approved it as a Class 3 GM activity subject to a number of amendments.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Project Ref 386/17.1

Investigation of the combination of D-LAK antimicrobial peptides and isoniazid and/or rifampicin to overcome drug resistant tuberculosis

Date Ackn’d 24/11/2017

Date Project Ceased

Class 2 CultureVolClass2 < 1 Litre

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
### Purposes of the contained use

To study the antimicrobial activities of anti-TB formulations, *Mycobacterium smegmatis* (Harzard Group 1) and severely attenuated *Mtb* (HG 2) strains will be used in in vitro studies. Monocyte cell line (ATCC TIB 202™) will be differentiated into macrophages using phorbol 12-myristate 13-acetate (PMA) for the study of intracellular antimicrobial activity. NMR and HR-MAS will be exploited to elucidate mechanism of actions of D-LAK peptides.

### Recipient or parental organism

Fully drug sensitive laboratory *Mycobacterium tuberculosis* strain H37Rv

### Host/vector system

N/A

### Origin & function

Genetic modification resulted in four double auxotrophs with deletions in designated positions in the genome involving Leucin, pantothenate, lysine and RD1 protein as well as the introduction of hygromycin resistance cassette.

| Bleupan ΔleuD ΔpanCD hygR | mc2 6020 ΔlysA ΔpanCD hygR |
| mc2 6030 ΔRD1 ΔpanCD hygR | mc2 7000 ΔRD1 ΔpanCD unmarked |
| lysA, leuD and panCD involve in the biosynthesis of lysine, leucine and pantothenate respectively. Corresponding deletions in strains lead to impairment of protein synthesis affecting normal metabolism and respiration. The *M. tuberculosis* ΔleuD ΔpanCD double auxotroph has two independent, attenuating, non-revertible mutations, which delete the essential leuD and panCD loci in *M. tuberculosis*. The resulting strain is highly attenuated in vivo and auxotrophic for both leucine and pantothenate (i.e. unable to grow without leucine and pantothenate supplemented growth medium). |
| Region of difference 1 (RD1) is the main genetic modification involved in the attenuation of the vaccine strain BCG. Deletion of RD1 completely removes 7 genes (Rv3872–Rv3878) and truncates 2 others (Rv3871 and Rv3879c). In which two of them encode the strong immunogenic antigens and virulence factors—the early secreted antigenic target (ESAT-6) and the culture filtrate protein (CFP-10). Studies have demonstrated RD1 is required for full virulence of *Mtbc*. (The Journal of Infectious Diseases (2003) 187:117–23, Scientific Reports (2015) 5:17078-91) |
| Hygromycin resistance (hygR) cassette is introduced as a selectable marker of strains. |
| Strains have previously been notified under GM Notifications GM 77/94.4 and GM 77/98.2. |

### Evaluation of foreseeable effects

There is no evidence that the severely attenuated strains are hazardous to human in contained use. Studies have also shown that the strains are effectively cleared by immunocomprised SCID or interferon gamma knock-out mice.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

There will be no larger GMOs in this study.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

For sample preparation of NMR metabolomic studies, the *M. tuberculosis* strain will be fixed by 2% formaldehyde for...
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid GMM culture will be treated with 10% Surfanios, mycobactericidal agent based on amine and quaternary ammonium compounds, overnight before pouring to drain with plenty of running water. Containers will be dispatched as orange clinical waste for incineration by licensed contractor. Solid GMM contained waste such as plates and tips will be placed in autoclave bags for autoclaving at 121°C for at least 15 minutes. The autoclave is located on a different floor in the same building.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The project was reviewed by the Former GM295 Biological Safety Committee (now incorporated into GM386) on 01/11/2016. It was approved subject to minor changes.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 386/18.1

Date Ackn'd 09/02/2018

CU2 Project Title Understanding the mechanisms of EBV (Epstein-Barr virus) induced immune responses in human T lymphocytes

Class Class 2

CultureVolClass2 < 1 Litre

Consent Granted Not Applicable
We propose to investigate the effects of EBV on T cell functionality with a focus on the IL-12 pathway in human lymphocytes by use of a GM strain of EBV (delta-mir) and comparing it to WT strains. Wild type strains of EBV can suppress the release of IL-12 by the infected cells and thereby reduce the effector functions of CD4+ and CD8+ T-cells. While CD4 T cells require IL-12 for induction of the T helper 1 phenotype, which is vital in combatting viral infections, in CD8 T cells IL-12 functions as an important third signal cytokine, allowing full activation and cytotoxic functions as well as the development of T cell memory, again playing a vital role in anti-viral immunity. The suppression of IL-12 production by WT EBV is mediated via viral microRNA expression, whereas the delta-mir GM strain has been modified to produce no functional MicroRNAs and is therefore unable to interfere with IL-12 production in infected cells in addition to also showing loss of other immune suppressive mechanisms (like the down-modulation of HLA molecules).

**EBV Delta-mir**
Modified strain of EBV that has modifications to the BART and BHRF1 genes such that the virus produces no mature MicroRNAs. Wild type EBV produces over 40 different MicroRNAs.

**Host/vector system**
EBV Delta-mir BAC is transfected into HEK-239T cells via standard transfection methods (Calcium phosphate/lipofectamine or similar) and transfection efficiency assessed by GFP expression. Transfected cells can be selected for with hygromycin drug treatment. Viral stocks are subsequently generated by further transfection of vectors containing BZLF1, BRLF1 and BALF4 to initiate viral lytic cycle. WT EBV stocks to be generated via a similar approach. Viral stocks subsequently used to infect human CD19+ve cells for use in CD4/CD8 co-culture experiments. All vectors (EBV BACS as well as accessory vectors) are initially cultured in transformed bacteria under drug (ampicillin) selection.

**Origin & function**
Modifications to EBV present in DElta-Mir construct are modifications to the BART and BHRF1 genes such that Delta-Mir EBV produces no mature MicroRNAs. Genetic modifications in this case is a loss of function.
EBV BAC constructs (both WT and Delta-mir) contain GFP and Hygromycin resistance genes. Hygromycin resistance allows drug selection of transfected 293T cells when generating viral stocks and GFP allows flow cytometric assessment of transfection efficiency and also infection efficiency when using viral stocks. Both Wt and Delta-Mir have ampicillin resistance genes to allow selection in bacteria. Accessory vectors used when generating viral stocks contain BZLF1, BRLF1 and BALF4 genes. BZLF1 and BRLF1 genes trigger the EBV lytic cycle and are required to generate appreciable viral levels (293T cells transfected solely with EBV BAC produce little or no virus). BALF4 encodes the GP110 precursor an envelope protein that enhances the subsequent viral stocks ability to infect human cells. All vectors also have ampicillin resistance genes to allow selection in bacteria.

**Evaluation of foreseeable effects**

The GM EBV virus (Delta-Mir) will have no functional MicroRNAs whereas the WT EBV has over 40. The individual functions of these Mirs is unknown but they are thought to have a variety of functions in regulating viral and host cellular genes. These functions would include a number of pathways that aid the virus in establishing a latent infection, such as inhibiting apoptosis of the host cell, inhibiting expression of viral genes (thus avoiding immune response) as well as inhibiting the II-12 pathway.

We would envisage that the GM Delta-Mir EBV virus would be less able to regulate the immune response of CD4/CD8 cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid GM waste (plates, flasks etc) is disposed of into clear waste bags and then autoclaved at 121°C for 20 minutes. After inactivation by autoclave waste is disposed of in yellow waste bags in waste room. Waste bags from this point are collected by external contractor and ultimately incinerated. Serological pipettes are placed in a 7L yellow Biobin, when full this bin is loosely sealed (i.e. flap is folded over) and then autoclaved as above before disposal as above. Pipette tips are placed in a 1L yellow Biobin and loosely sealed when full before being autoclaved and disposed of as above. Biobin autoclaving , both for serological pipettes and pipette tips has been validated as effective using temperature indicators. Autoclave for waste disposal is serviced and maintained on a 6 monthly basis and function will be independently checked with temperature indicators during the proposed experimental work. Liquid waste (cell culture media, viral stocks) is treated with liquid Distel disinfectant (to a concentration of 1% Distel) and left for at least 1 hour before disposal down designated laboratory sink. Distel is EN14476 tested for this purpose (greater than 4-log reduction in virus with 1 hour contact at 1/100 dilution) and is also tested for Bacteriacidal and fungicidal activity.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The project was reviewed by the King's College London Biological Safety Committee for the Faculty of Life Science and Medicine on 5th October 2017.
The project was approved as a Class 2 GM activity requiring CL2 subject to minor amendments.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 386/18.3

Molecular cloning, expression in Escherichia coli, and insertional mutagenesis of genes putatively involved in protease expression, post-translational modification, macromolecular biosynthesis and virulence in Porphyromonas and related organisms

Date Ackn'd 05/10/2018

CU2 Project Title

Molecular cloning, expression in Escherichia coli, and insertional mutagenesis of genes putatively involved in protease expression, post-translational modification, macromolecular biosynthesis and virulence in Porphyromonas and related organisms

Class 2

< 1 Litre

Non-GMM

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
## Project Additional Information

### Purposes of the contained use

Manipulation of Porphyromonas / Bacteroides DNA for expression in Escherichia coli and mutagenesis of Porphyromonas / Bacteroides genes, by homologous recombination.

### Recipient or parental organism

Recipient species: E. coli XL-1 Blue MRF', E. coli SURE , SCS110, E. coli XL-10 Gold and derivatives will be used. These strains are K12-derived, are attenuated and unable to survive in the gut - sensitivity to bile salts. Donor species: Porphyromonas gingivalis and Bacteroides sp. These organisms are sensitive to oxygen and requires specialised anaerobic conditions and media for growth and propagation.

### Host/vector system

- **E. coli** - pUC18not, pUC18/19-derivatives, JFQ301 (derivative of commercial pQE80, Qiagen)
- **P. gingivalis**- E.coli derived plasmids do not replicate in P. gingivalis:
  - Gel-purified fragments, from vectors propagated in E. coli will be used for electroporation. pNJR12 is autonomously replicating, low copy number shuttle plasmid, will be used to complement mutations in P. gingivalis.

### Origin & function

Intended genes are from Porphyromonas / Bacteroides and encode:
- Protease specific for arginine-peptide bonds
- Protease specific for lysine-peptide bonds
- Putative outer membrane proteins / biomolecular translocation
- Lipopolysaccharide (LPS) biosynthesis
- Glycan transferases and capsular polysaccharide biosynthesis

### Evaluation of foreseeable effects

E. coli – The scale is limited to < 1 litre and K12 strains are unable to survive in the gut. Cloned proteases are known not to retain enzymatic activities. In most cases, incomplete (internal fragments) and promoter-less P. gingivalis genes will be cloned, with no intention of expressing the gene product. When expression is necessary (e.g. to use products for antibody generation), the products are unlikely to have the post-translational processing and the level of maturation that is required in P. gingivalis. Thus, GMO is unlikely to be more harmful than P. gingivalis itself.

P. gingivalis - Mutated genes are unlikely to confer any advantage relative to the wild type strain; some of these genes are expected to be critical to the organism’s ability to grow and survive. There are no reports of mutated genes leading to improved virulence in the mouse subcutaneous model. Similarly, complemented strains are expected to restore wild type activities. The scale will be confined to < 500ml.

Both systems are unlikely to be detrimental to health.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NOT APPLICABLE

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All relevant containments are in place to minimise the potential of significant risk. All materials that come in contact with DNA and or bacteria will be decontaminated either by thoroughly cleaning with 1% (v/v) ChemGen (Starlabs) or by autoclaving 136 °C for 55 minutes, and disposed of via incineration as yellow clinical waste by licenced contractor.

Spent media / culture will be autoclaved at 136 °C for 55 minutes or (liquid media only) treated with ChemGen (1% (v/v) final concentration) overnight. Autoclaved solid media will be disposed of via incineration as yellow clinical waste by licences contractor. Autoclaved or disinfected liquid media will be poured to drain with copious quantities of water.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The project was reviewed by the Joint IoPPN/Dental Institute Biological Safety Committee on April 22nd 2018. It was approved with minor amendments.

Project Containment

- **Laboratory Activities**
  - L2
  - L3
  - L4

- **Glass Houses**
  - L2
  - L3
  - L4

- **Growth Rooms**
  - L2
  - L3
  - L4

- **Animal Units**
  - L2
  - L3
  - L4

- **Large Scale Activities**
  - L2
  - L3
  - L4

- **Human Clinical Applications**
  - L2
  - L3
  - L4

**Project Ref** 386/19.1

- **Date Ackn’d** 22/11/2019
- **CU2 Project Title** Molecular Biology of Influenza A Virus (IAV) replication
- **Class** Class 2
- **CultureVolClass2** < 1 Litre
- **CultureVolumeClass3-4**
**Date Project Ceased**

Non-GMM Consent Granted

**Tick if notifying a connected programme of work**

N

Project notified under transitional arrangements

**Withdrawn**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

To identify the molecular and cellular processes that regulate the early steps of IAV infection, including the activities of interferon (IFN) regulated genes and investigate the molecular pathogenesis of Influenza A Virus using cultured cell models

**Recipient or parental organism**

**Bacteria - Attenuated and mobilization defective E. coli (DH5α, and DH10β), unable to survive in the absence of specialised growth factors, and unabe to colonize humans.**

Mammalian cell lines (such as MDCK, HEK 293T, HeLa, A549, U87-MG):
- MDCK cells will be infected with IAV for production of IAV stocks
- HEK 293T cells will be transfections with multi-component (non-replicative) systems based on the gamma-retroviral MLV or lentiviral HIV to produce viral vectors coding for single cellular genes or indicator genes.
- HeLa, A549 or U87-MG cells will be modified with viral vectors to express single cellular genes or indicator genes

**Retroviral vectors:** MIGR1 (gamma-retroviral expression vector), pRRL and pHR (lentiviral expression vectors)
- Single cellular genes or indicator genes will be inserted into retroviral expression plasmids for the generation of vectors

**IAV A/Victoria/3/75 (H3N2 strain) - Isolated clinical specimen in Australia, in 1975**

**Host/vector system**

To grow plasmids - E coli strains (DH5α, and DH10β).

To grow virus stock - MDCK (IAV) and HEK293T cells (retroviral vectors).

**IAV A/Victoria/3/75 NanoLuc reporter virus - Engineered influenza reporter virus where genome segment 3 expresses a polymerase acidic protein–2A–NanoLuc fusion protein**

**Tran et al, J.Virol (2013) 87, 13321.**

**MIGR1- MIGR1 (a retroviral expression vector for use in eukaryotic cells; refer to Pear et al., Blood vol 92, pp 3780. Non self-inactivating (Non-SIN). Amp resistance gene.**

**GFP expressing vector.**


**Can be used to insert single cellular genes cDNAs.**

**pHR (SIN-dE SFFV-Ctrl-P2A-SBP-dLNGFR-WPRE)- A retrovirus vector derived from HIV. Refer to PLoS One. 2014 Oct 31;9(10):e111437. doi:**

**10.1371/journal.pone.0111437. eCollection 2014. Self-inactivating (SIN). Amp resistance gene. Can be used to insert single cellular genes cDNAs.**

**pEasiLV (CG149 real)- A doxycycline-inducible lentiviral vector for expressing cDNAs, refer to Goujon, Nature vol 502, pp 559-562. Self-inactivating (SIN).**

pLenti-SIREN- A modified pCSGW containing the U6-promoter driven cassette for shRNA hairpin expression from pRetro-SIREN.

pLenti-CRISPR-Lentiviral vector encoding CRISPR guide RNA and the Cas9 nuclease for engineering specific gene knockouts. Encodes either Puromycin resistance of GFP

pCMVR8.91- pCAGGS encoding Mo-MLV Gag and Pol proteins for packaging retroviral vectors, such as pMIGR.

pCAGGS-MLVgagpol- pCAGGS encoding Mo-MLV Gag and Pol proteins for packaging retroviral vectors, such as pMIGR.

pMDG.2- Expression vector encoding the VSV-G envelope glycoprotein for pseudotyping retroviral and lentiviral vectors.

Origin & function

Segments of HIV, MLV or VSV- HIV (e.g. gag, pol, rev, vpr, vif, vpu and nef) or MLV (gag and pol) genes; will be used for the preparation of retrovirus or lentivirus vector stocks for gene transduction into mammalian cells.

VSV env is carried on a separate expression plasmid that is transiently transfected into cells producing retrovirus or lentivirus cores for the purpose of particle pseudotyping.

cDNAs corresponding to mammalian genes - Different mammalian genes (e.g., MX1, NUP214, other interferon-inducible genes, etc) will be analyzed to determine their biological function or effects on IAV replication.

cDNAs corresponding to non-mammalian genes - Marker/ reporter/ indicator genes are used widely (e.g., chloramphenicol acetyl transferase [CAT], β-galactosidase, luciferase, or naturally fluorescent proteins [GFP, YFP, etc.]).

Evaluation of foreseeable effects

There is no evidence that any of the above genes or proteins have virulence potential in vitro when expressed in mammalian cells. In addition, there is no documented risk associated with handling naked HIV DNA under standard/controlled CL1 conditions.

Inserts derived from retroviruses are introduced transiently into cell lines under CL1 conditions. They always contain less than two-thirds of a provirus and they cannot result in replicating infectious retroviruses. They will produce a viral vector that only has the capacity for a single-round infection.

The indicator virus A/Victoria/3/75 NanoLuc is a live virus able to infect humans, causing influenza, that additionally expresses the NanoLuc reporter protein upon infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This project will not use larger GMOs, only cultured cell systems.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

NOT APPLICABLE

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1) Solid: All solid waste will be rinsed in P3 Steril (10% in water; recommended and validated germicide for enveloped viruses and mammalian cell lines – (Refer to 1) Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008, (CDC), 2) Conform to Regulation (EC) No 1907/2006 (REACH), and 3) Webber et al, 20, 12, Infection control and Hospital Epidemiology 2015), left overnight and then autoclaved (moist heat for 25 min at 1210C). The autoclave is serviced twice a year by Getinge. All autoclaved solids will be placed in yellow bags for subsequent incineration by KCL contractor.

2) Liquid: All liquid waste will be treated with P3 Steril (final concentration 10%), put down the sink and rinsed with copious quantities of water.

Spillage procedure: Wear laboratory coat and nitrile gloves. Use prepared solution P3 Steril (10% in water). If a spill occurs in a centrifuge, leave for 30 min for aerosols to settle. Cover spill with 10% P3 Steril solution starting from the outer edges of the spill. If any glass, needle or other sharp material is present, use a dustpan and brush or forceps to collect them; place in Sharps bin (yellow lid). Wipe up P3 Steril inactivated liquids with paper towels. Rinse surface with 10% P3 Steril and then water, wipe up with more paper towels. Place towels in yellow waste bag. All re-usable contaminated equipment (e.g. centrifuge following containment failure, the forceps or dustpan and brush used in the clean-up) should be carefully cleaned with 10% P3 Steril and then with water. Report all significant spills via KCL reporting system, AIRS.
The project was reviewed by the Faculty of Life Sciences and Medicine Biological Safety Committee on 7th February 2019 and approved it as a Class 2 GM activity subject to a number amendments.

**Project Containment**

- **Laboratory Activities**
  - L2: Yes
  - L3
  - L4

- **Glass Houses**
  - L2
  - L3
  - L4

- **Growth Rooms**
  - L2
  - L3
  - L4

**Project Ref** 386/20.1

- **Date Ack'n'd**: 13/05/2020
- **CU2 Project Title**: Characterization of neutralizing antibody responses in COVID19 patients
- **Class**: Class 2
- **CultureVolClass2**: Class 2
- **CultureVolumeClass3-4**: < 1 Litre

**Historical Significant Changes**

- **Historical Date of Additional Info**: 02/03/2022
# Project Additional Information

## Purposes of the contained use

To study neutralizing antibody responses in COVID-19 infected and recovered individuals. We aim to identify patients that have developed a robust neutralizing antibody response and use single B cell technologies to isolate the monoclonal antibodies (mAbs) with neutralizing activity that can be investigated as immunotherapeutics and be used to guide future vaccine design strategies. This work will also help identify recovered patients whose sera could be used for treatment of COVID-19 patients in ICU.

## Recipient or parental organism

| Bacteria - Attenuated and mobilization defective E. coli (STBL2, and DH10β), unable to survive in the absence of specialised growth factors, and unable to colonize humans. |
| Cells (including HEK-293T, HeLa, HeLa/TZM-bl, Vero-E6, A549, Caco2 and HEK-293F) |
| - HEK 293T cells will be transfected with multi-component (non-replicative) systems based on the gamma-retroviral MLV, lentiviral HIV or VSV-deltaG to produce SARS-CoV-2 pseudotyped virus for use in neutralization assays. |
| - HeLa, A549 or Vero-E6, and Caco2 cells will be used as reporter cell lines for neutralization assays |
| - HEK 293F cells will be used for antibody and glycoprotein expression. |

## Host/vector system

| To grow plasmids - E coli strains (STBL2 and DH10β). |
| To grow virus stock - HEK293T cells (retroviral vectors). |

## Origin & function

| Segments of HIV, MLV or VSV- HIV (e.g. gag, pol, rev, vpr, vif, vpu and nef) or MLV (gag and pol) genes; will be used for the preparation of retrovirus or lentivirus vector stocks for gene transduction into mammalian cells. |
| Viral surface glycoproteins include; SARS-CoV, SARS-CoV-2, MERS, VSV and will be used for the purpose of pseudotyping. |
| Rescued heavy and light chain variable genes will be cloned into IgG expression vectors containing the human heavy and light chain constant regions. |

## Evaluation of foreseeable effects

| There is no evidence that any of the above genes or proteins have virulence potential in vitro when expressed in mammalian cells. In addition, there is no documented risk associated with handling naked HIV DNA under standard/controlled CL1 conditions. Inserts derived from retroviruses are introduced transiently into cell lines under CL1 conditions. They always contain less than two-thirds of a provirus and they cannot result in replicating infectious retroviruses. They will produce a viral vector that only has the capacity for a single-round infection. |

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This project will not use larger GMOs, only cultured cell systems. Contained

## For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

1) Solid: All solid waste will be rinsed in P3 Steril (10% in water; recommended and validated germicide for enveloped viruses and mammalian cell lines – (Refer to 1)
Infection control and Hospital Epidemiology 2015), left overnight and then autoclaved (moist heat for 25 min at 121°C). The autoclave is serviced twice a year by Getinge.
All autoclaved solids will be placed in yellow bags for subsequent incineration by KCL contractor.

2) Liquid: All liquid waste will be treated with P3 Steril (final concentration 20%), put down the sink and rinsed with copious quantities of water.
Spillage procedure: Wear laboratory coat and nitrile gloves. Use prepared solution P3 Steril (10% in water). If a spill occurs in a centrifuge, leave for 30 min for aerosols to
settle. Cover spill with 10% P3 Steril solution starting from the outer edges of the spill. If any glass, needle or other sharp material is present, use a dustpan and brush or
forceps to collect them; place in Sharps bin (yellow lid). Wipe up P3 Steril inactivated liquids with paper towels. Rinse surface with 10% P3 Steril and then water, wipe up
with more paper towels. Place towels in yellow waste bag. All re-usable contaminated equipment (e.g. centrifuge following containment failure, the forceps or dustpan and
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Project Containment

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Project Ref 386/20.3

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<thead>
<tr>
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<tr>
<td>06/11/2020</td>
<td>A longitudinal, cohort study to understand clonal haemopoiesis and immune modulation in the healthy ageing population and in myeloid neoplasms and bone marrow failure</td>
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<th>Date Project Ceased</th>
<th>Class</th>
<th>CultureVolClass2</th>
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<td>02/03/2022</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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The aim of our program is to understand the pathogenesis of myeloid neoplasms and leukemias using multiple approaches using primary patient tissues as well as patient derived cell-lines and cells derived from haematologically normal patients.

1. The first part of the project will study the genomics, transcriptomics and proteomics of the human primary cells. We will be studying the stem cells/progenitor like cell compartments isolated from blood or bone marrow aspirates of patients using in vitro assays.

2. The second part of the project will assess the biological impact of the expression of human oncogenes or other ‘mutated genes’, particularly those genes identified in association with myeloid neoplasms and leukaemias.

This procedure will involve either transfection of plasmids or generation of a replication defective recombinant lentivirus capable of targeting human cells to make genetic modifications. Genetic modifications will include gene editing using CRISPR-Cas systems, gene over-expression or shRNA knockdown of potential oncogenes. Plasmids will be generated using competent E. coli. Infective viral particles will be generated using HEK-293T ‘producer’ cells in vitro. Transfection of target cells (with plasmid) or infection (with lentiviral particles) will be carried out in vitro. The effects of modified target cells will be assessed using various in vitro assays, including cell proliferation and colony assays, flow cytometry and microscopy.

3. The third part of this project will use vector constructs with gene combinations for the creation of induced pluripotent stem cells (iPSCs).

Recipient or parental organism

E. coli: commercially-sourced, attenuated and mobilization defective E. coli (listed below), unable to survive in the absence of specialised growth factors, and unable to colonize humans.

- TOP10
- DH10B-T1
- BL21DES
- STBL3
- DH5alpha

Mammalian cell lines which may be genetically modified are listed below:

- NIH3T3
- Mouse embryonic fibroblast cell line
- U937
- Human Histiocyota cell line
- K562
- Human Myelogenous leukaemia cell line
- NB4
- Human Promyelocyte cell line
HL60
Human Promyelocyte cell line
MCF7
Human Breast cancer cell line
Hela
Human Cervical adenocarcinoma cell line
THP-1
Human acute monocytic leukaemia
ML-2
Human acute myelomonocytic leukaemia
MONO-MAC-6
Human acute monocytic leukaemia
KARPAS-45
Human T cell leukaemia
KASUMI-1
Human acute myeloid leukaemia
REH
Human B cell leukaemia
MV4-11
Human acute myeloid leukaemia
RS4;11
Human B cell precursor leukaemia
HB1119
Human B cell precursor leukaemia
SEM
Human B cell precursor leukaemia
hOLM-13
Human acute myeloid leukaemia
SKO1
Human acute myeloid leukaemia
KG1
Human acute myeloid leukaemia
Ramos (RA 1)
Burkitt's lymphoma
RAJI
Human Burkitt lymphoma cell line
OCI-AML3
Human acute myeloid leukaemia cell line
TF-1
Human acute erythroleukaemia cell line
MOLM-13
Human acute myeloid leukaemia cell line

Cell lines used to produce viral particles: HEK293T, PG13 (Murine NIH-3T3-derived)

Host/vector system
Viable cells might be transduced with known or possibly oncogenic genes/sequences by transfection, lentiviral vector infection.

Viable cells: i) primary haematopoietic cells derived from donor bone marrow or blood
ii) human leukaemia cell lines: see list above; viral and plasmid producing cell types: see list above.

Viral and non-viral plasmids used are detailed below, including their target cells.

Non-viral Plasmids:
- pcDNA3.1+/Thermo Fisher
- E coli and eukaryotic cells
- Original TA cloning vector pCR2.1
- E coli TOP10
- pGEX4t-1 Sigma
- Bacteria
- pET22b pET30a Novagen
- Bacteria
- pGEM3 Promega
- Bacteria
- pSecTag2A Invitrogen
- Bacteria, and mammalian cells
- pIRESPuro3 Takara
- Bacteria, and mammalian cells
- pIRESHyg3 Takara
- Bacteria, and mammalian cells
- pEC-RP
- Bacteria, and mammalian cells
- pGL3-Control Promega
- Bacteria, and mammalian cells
- pCOX
- Bacteria, and mammalian cells
- pIDO
- Bacteria, and mammalian cells
- pOct4/KLF4/SOX2/CMYC
- Bacteria, and mammalian cells
- pOct4/SOX2
- Bacteria, and mammalian cells

‘Helper’ Plasmids:
- pMDLg/pRRE Didier Trono
- Bacteria, and mammalian cells
- pRSV-rev Didier Trono
- Bacteria, and mammalian cells
- pMDG Didier Trono
- Bacteria, and mammalian cells
- pFBSALF
- Bacteria, and mammalian cells

Lentiviral Vector Plasmids:
Origin & function

Viral origin genetic material:
Segments of HIV, MLV or VSV- HIV (e.g. gag, pol, rev, vpr, vif, vpu and nef) or MLV (gag and pol) genes; will be used for the preparation of retrovirus or lentivirus vector stocks for gene transduction into mammalian cells.
VSV env is carried on a separate expression plasmid that is transiently transfected into cells producing retrovirus or lentivirus cores for the purpose of particle pseudotyping.
Human origin genetic material is intended for the study of the involvement of these sequences in the induction/progression of haematological malignancies. These include oligonucleotides and cDNAs derived from human DNA sequences.
Marker/reporter/indicator genes are used widely (e.g., chloramphenicol acetyl transferase [CAT], β-galactosidase, luciferase, or naturally fluorescent proteins [GFP, YFP, etc.]).

Evaluation of foreseeable effects

7. Characteristics of the GMO(s) including the evaluation of foreseeable effects (cont'd)
   1. There is a risk that the researcher may inadvertently become infected with viral particles used to deliver genetic modifications. However, viruses used in these experiments are self-inactivating and replication incompetent once they infect the target cell and so their effects would be limited. Researchers will be suitably trained to handle viruses safely, and waste contaminated with viruses will be disposed of safely (see later section).
   2. There is a theoretical risk that a researcher may inadvertently contaminate themselves internally with genetically modified primary cells carrying oncogenic mutations. The risk that any experimental cells could be inadvertently administered parenterally (e.g. into the bloodstream) is small due to use of appropriate PPE and safe disposal of sharps. Furthermore, any experimental cells will be recognised as 'foreign' by a competent immune system and eliminated. To our knowledge, there have been no documented cases of researchers acquiring malignant disease through this route.
Exposure to skin, eyes and lungs would be minimal as gloves and protective clothing are used at all the times. All viral as well as human tissue experiments are performed in a Microbiological Safety Cabinet. Tissue culture plastics, solid waste and liquid waste is inactivated by use of appropriate disinfectants. Microbiological Safety Cabinets are located in specified rooms designated as CL2 laboratories and only competent and trained operators are allowed to perform these procedures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable plastic, plastic pipettes and tips contaminated with virus or human cells are soaked for at least 12 hours in 1% Virkon before placing into autoclave bag and autoclaving.

Autoclave 134°C, 5 minutes. 121°C, 20 minutes this will kill off 100% of any viable organism. Autoclave inspected and tested on a yearly basis.

Final disposal via incineration by an external licensed contractor.

All media must be treated with 1% Virkon solution for 12 hours to render inactive and then flushed into the drain with lots of water.

Is an emergency plan required according to regulation 20? \( \text{N} \)

If yes, tick to confirm that it is attached to this form \( \text{N} \)

Tick to confirm that you have attached a risk assessment to this form \( \text{Y} \)

Tick if you are claiming exemption from disclosure for section of the risk assessment \( \text{N} \)

Please enter comments on the GM safety committee on the risk assessment

The project was reviewed by the Faculty of Life Sciences and Medicine Biological Safety Committee on 25th August 2020 and approved it as a Class 2 GM activity. The approved CoBRA is appended to this application for reference.

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Project Ref 386/21.1

Date Ackn'd 21/01/2021

CU2 Project Title Studies of Erythropoiesis, Globin Gene Regulation and red cell disorders

Class 2

CultureVolClass2 < 1 Litre

Consent Granted

Date Project Ceased

02/03/2022

Page 6902 of 1532
### Project Additional Information

**Purposes of the contained use**

Blood samples will be collected by qualified clinicians and nurses from haematologically normal and sickle cell disease patients (Kings College Hospital) under NHS guidelines. Samples will be transported to the Rayne Institute where they will be processed and stored as either cell-pellets or viable cells. Requested samples will be transported on dry ice to the lab. Samples will be lysed in a Class II microbiological safety cabinet for preparation of DNA, RNA or protein for downstream applications such as protein analysis, PCR, Nextgen Sequencing, single cell sequencing etc. DNA, RNA and/or protein will be isolated from cell pellets which have been stored in liquid nitrogen and are therefore not viable. Viable cells will be prepared in dedicated Class II microbiological safety cabinet by staining with antibodies for FACS analysis, FACS Sorting, Cytof analysis or cytokine profiling of blood or cell supernatant.

Animal work will be performed at the Denmark Hill KCL BSU where primary haematopoietic cells from mice will be isolated mice and used for experiments to establish an in vitro model of erythropoiesis. Viable cells may be used for downstream applications as described above.

**Aims:**

The aim of our research program is to understand the development of erythropoiesis, globin gene regulation and the pathogenesis of red cell disorders via multiple approaches using primary patient tissues as well as patient derived cell-lines, established immortalised human cell-lines, mouse models and cells derived from haematologically normal patients. Using human or mouse erythroid cell lines or human and mouse primary haematopoietic stem and progenitor cells, we investigate how under/over-expression or mutation of our genes of interest influence the propagation, maturation and function (including globin gene expression) of erythroid cells. The experimental readouts of our work include data on transcription factor binding, chromatin looping, gene expression (mRNA and protein levels), red cell membrane physiology, growth and differentiation behaviour.

Our experimental pipelines include (CRISPR/Cas9) gene editing enabling us to decipher gene and protein function as well as the influence of human mutation and common human genetic variability on red blood cell formation and function. Our work is aimed at understanding human diseases affecting red blood cells, such as sickle cell disease and at identifying targets for novel therapeutic approaches.

**Recipient or parental organism**

Viable cells derived from blood from haematologically normal and sickle cell disease patients.

**Host/vector system**

Viable cells might be transduced with known or possibly oncogenic genes/sequences by transfection, retroviral or lentiviral vector transduction.

**Origin & function**

Genetic material is derived from human and murine sequences and is intended to study the involvement of these sequences in the induction/progression of haematological
and erythroid physiological development and disease.

Evaluation of foreseeable effects

The most hazardous biological agent most likely to impair human health will be from adventitious agents contaminating the primary human cells samples, and the most likely of these will be patient samples at presentation. Primary human cells (whether GM or otherwise) have the added possibility of contamination with adventitious agents. Human blood and bone marrow cells are treated as if it were infectious to lab workers. In addition, all the staff members are offered a Hepatitis B vaccination as a precautionary measure.

The most hazardous genetically modified constituent would be the MYB and BCL11A constructs regarding their oncogene status and modified virus (e.g. lentivirus pseudotyped with VSV-G) carrying putative oncogene(s). There is no reason to believe that the GMO may be more dangerous to man and the environment than the recipient strain. Any new gene target to be used is not expected to have oncogene activity exceeding that of MYB. Over-expression of both proto-oncogenes, BCL11A and MYB, have been found to increase malignant behaviour of human tumour and leukaemia cells (references, e.g., Jiang BY et al. Mol Cancer 2013 and Zhao L et al. Oncogene 2014).

The risk to researchers of developing cancer from our procedures is non-existent because modified viruses are replication incompetent and disabled/self-inactivating once target cells are infected.

Lentiviral vectors have become standard reagents for the genetic manipulation of mammalian cells. Viral vectors are engineered to transfer and integrate specific DNA sequences into the genomes of target cells and are designed to be replication-defective to avoid further spread after the initial transfer event. Any infected cells would normally be expected to be targeted by the immune system in all healthy individual(s) with a competent immune system such as any researcher in our lab. There have been no reported incidences of infection resulting in insertional mutagenesis or infection resulting in expression of vector insert resulting in unwanted cell proliferation.

CRISPR/Cas9 gene editing will use single-stranded DNA and ribonucleoprotein complexes to modify genomic sequences via nucleofection. They are not viral vectors, so the potential for integration into the operator's cells and result in an inherited modification is non-existent.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable plastic, plastic pipettes and tips contaminated with virus or human cells are soaked for at least 12 hours in 1% Virkon before placing into autoclave bags and autoclaving.

Autoclaving is for 134°C, 5 minutes, 121°C, 20 minutes. The autoclave is inspected and tested on a yearly basis.

Final disposal via incineration by an external licensed contractor.

All media must be treated with 1% Virkon solution for 12 hours and then flushed into the drain with lots of water.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
**Project Additional Information**

**Purposes of the contained use**

The Malim lab investigates the molecular pathogenesis of human viruses using cultured cell models of infection. Broadly speaking, the group employs a multi-disciplinary strategy that includes molecular genetics, cell biology, biochemistry, structural biology, genomics, bioinformatics, and cohort-based analyses. These will be extended by applying them to induced pluripotent stem cell (iPSC) lines, which possess intrinsic/natural genetic variation and can be exploited as models to define proteins and networks that regulate the HIV-1 life cycle.

Primary peripheral blood mononuclear cells (PBMCs) obtained from consenting healthy individuals will be obtained via the Infectious Diseases Biobank, and used to generate iPSC cell lines. To achieve this, they will use the CytoTune-iPS 2.0 Sendai Reprogramming kit from ThermoFisher, which is composed of three Sendai virus (SeV)-derived vectors expressing Klf4, Oct3/4, Sox2 and c-Myc. These well-established vectors are based on Sendai virus (a mouse parainfluenza virus type I from the
paramyxoviridae family) that has had the gene that encodes the fusion protein (F) deleted. These vectors cannot replicate or produce virus particles, and the genome sequence of iPSCs is not changed by this process.

Recipient or parental organism

Primary cells (PBMCs) isolated from blood of consented healthy adult donors. Donor bloods will be obtained through the King’s College London Infectious Diseases Biobank.

Host/vector system

Sendai vectors (ThermoFisher): These vectors contain transgenes that express hOCT3/4, hSOX2, hKlf4, and hc-myc. The vectors are based on a modified, non-transmissible form of Sendai virus, which has the gene encoding for the fusion protein deleted.

Origin & function

The genetic sequences inserted in the Sendai vectors are hOCT3/4, hSOX2, hKlf4, and hc-myc. These transcription factors can reprogramme human cells into a pluripotent state. These genes include the oncogene hc-myc.

Evaluation of foreseeable effects

The Sendai vectors and PBMCs are both handled under CL2 conditions. Sendai vectors cannot replicate, but can alter the expression of genes in exposed cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This project will not use larger GMOs, only cultured cell systems.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1) Solid: All solid waste will be rinsed in P3 Steril (10% in water; recommended and validated germicide for enveloped viruses and mammalian cell lines – (Refer to 1) Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008, (CDC), 2) Conform to Regulation (EC) No 1907/2006 (REACH), and 3) Webber et al, 20, 12, Infection control and Hospital Epidemiology 2015), left overnight and then autoclaved (moist heat for 25 min at 121°C). The autoclave is serviced twice a year by Getinge. 10% P3 Steril has been shown by our group to inactivate several viruses including HIV-1 and SARS-CoV-2. All autoclaved solids will be placed in yellow bags for subsequent incineration by KCL contractor.

2) Liquid: All liquid waste will be treated with P3 Steril (final concentration 10%), discarded down the sink and rinsed with copious quantities of water. Spillage procedure: Wear laboratory coat and nitrile gloves. Use prepared solution P3 Steril (10% in water). If a spill occurs in a centrifuge, leave for 30 min for aerosols to settle. Cover spill with 10% P3 Steril solution starting from the outer edges of the spill. If any glass, needle or other sharp material is present, use a dustpan and brush or forceps to collect them; place in Sharps bin (yellow lid). Wipe up P3 Steril inactivated liquids with paper towels. Rinse surface with 10% P3 Steril and then water, wipe up with more paper towels. Place towels in yellow waste bag. All re-usable contaminated equipment (e.g. centrifuge following containment failure, the forceps or dustpan and brush used in the clean-up) should be carefully cleaned with 10% P3 Steril and then with water. Report all significant spills via KCL reporting system, AIRS.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The project was reviewed by the Faculty of Life Sciences and Medicine Biological Safety Committee on April 2020 and approved it as a Class 2 GM activity subject to a number of amendments.

**Project Containment**

<table>
<thead>
<tr>
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<th>Growth Rooms</th>
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**Project Ref** 386/21.3

**Date Ackn'd** 05/05/2021

**CU2 Project Title** Characterisation of interferon mediated block to Hantavirus infection

**Class** 2

**Culture Volume** ≤ 1 Litre

**Non-GMM Consent Granted**

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To identify interferon stimulated genes (ISGs) that decrease Hantavirus replication in cell culture. This will be done by knocking down individual ISGs in cells before treating...
them with interferon alpha, infecting them with Puumala virus (PUUV) or Tula virus (TULV) and assessing viral replication using focus forming unit assays, quantitative polymerase chain reaction (qPCR) and western blotting. The results will help explain the variation in severity of these infections in people.

Recipient or parental organism

Bacteria - Attenuated and mobilization defective E. coli (STBL2, and DH10β), unable to survive in the absence of specialised growth factors, and unable to colonize humans.

Cells (including HEK-293T, Vero-E6 and A549)
- HEK 293T cells will be transfected with multi-component (non-replicative) systems based on the gamma-retrovirus MLV or lentivirus HIV in order to generate viral vectors to knock down genes in other cell lines.
- A549 or Vero-E6 cells will be used for infection assays with PUUV and TULV.

Host/vector system

To grow plasmids - E. coli strains (STBL2 and DH10β).
To grow virus stock - HEK293T cells (retroviral vectors).

Origin & function

Segments of HIV (e.g. gag, pol, rev, vpr, vif, vpu and nef) or MLV (gag and pol) genes; will be used for the preparation of retrovirus or lentivirus vector stocks for gene transduction into mammalian cells. VSV env is carried on a separate expression plasmid that is transiently transfected into cells producing retrovirus cores for the purpose of particle pseudotyping.

Different mammalian genes (e.g., MX1, NUP214, other interferon-inducible genes, etc) will be cloned into the above expression vectors and used to determine their biological function or effects on PUUV and TULV replication.

Evaluation of foreseeable effects

There is no evidence that any of the above genes or proteins have virulence potential in vitro when expressed in mammalian cells. In addition, there is no documented risk associated with handling naked HIV DNA under standard/controlled CL1 conditions.

Inserts derived from retroviruses are introduced transiently into cell lines under CL1 conditions. They always contain less than two-thirds of a provirus and they cannot result in replicating infectious retroviruses. They will produce a viral vector that only has the capacity for a single-round infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This project will not use larger GMOs, only cultured cell systems.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1) Solid: All solid waste will be rinsed in P3 Steril (10% in water; recommended and validated germicide for enveloped viruses and mammalian cell lines – (Refer to 1) Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008, (CDC), 2) Conform to Regulation (EC) No 1907/2006 (REACH), and 3) Webber et al, 20, 12,
Infection control and Hospital Epidemiology 2015), left overnight and then autoclaved (moist heat for 25 min at 121°C). The autoclave is serviced twice a year by Getinge. All autoclaved solids will be placed in yellow bags for subsequent incineration by KCL contractor.
2) Liquid: All liquid waste will be treated with P3 Steril (final concentration 20%), put down the sink and rinsed with copious quantities of water.
Spillage procedure: Wear laboratory coat and nitrite gloves. Use prepared solution P3 Steril (10% in water). If a spill occurs in a centrifuge, leave for 30 min for aerosols to settle. Cover spill with 10% P3 Steril solution starting from the outer edges of the spill. If any glass, needle or other sharp material is present, use a dustpan and brush or forceps to collect them; place in Sharps bin (yellow lid). Wipe up P3 Steril inactivated liquids with paper towels. Rinse surface with 10% P3 Steril and then water, wipe up
with more paper towels. Place towels in yellow waste bag. All re-usable contaminated equipment (e.g. centrifuge following containment failure, the forceps or dustpan and brush used in the clean-up) should be carefully cleaned with 10% P3 Steril and then with water. Report all significant spills via KCL reporting system, AIRS

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment

The project was reviewed by the Faculty of Life Sciences and Medicine Biological Safety Committee on 1st October 2020 and approved it as a Class 2 GM activity subject to a number amendments.

Project Containment

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Project Ref 386/21.4

Date Ackn'd 30/09/2021  

CU2 Project Title Molecular mechanisms of tissue repair and scar formation  

Class 2  

CultureVolClass2 < 1 Litre  

Consent Granted  

Non-GMM  

Project notified under transitional arrangements  

N

Tick if notifying a connected programme of work  

N

Historical Significant Changes
### Project Additional Information

#### Purposes of the contained use

Our overarching aim is to understand the cell and molecular mechanisms underlying scarring. We have recently discovered a unique extracellular matrix composition and architecture in keloids and are now questioning the functional significance of this. We are also working to understand the importance of cell plasticity and differentiation (regulated by transcription factors and epigenetic modifying enzymes) to wound repair and normal versus pathological scar formation. Our main approach will be to manipulate expression of transcription factors, epigenetic proteins, and extracellular matrix components and receptors (a number of which are implicated in cancers) in our in vitro culture models (primary human cell isolations, some immortalised).

#### Recipient or parental organism

**Bacteria** - Attenuated and mobilization defective E. coli, unable to survive in the absence of specialised growth factors, and unable to colonize humans.

**Cells** - Including HEK-293T, primary human or mouse cells (dermal fibroblasts, keratinocytes or immune cells), or cell lines (BJ or HCA2 fibroblasts, HaCaT keratinocytes, 3T3, Kera-308)

#### Host/vector system

1) Production of lentiviral vectors by co-transfection of HEK293T cells with lentiviral transfer vectors, with 3rd generation self-inactivating lentiviral vectors that have been extensively modified to prevent viral replication outside the packaging cells (pLentilox system, pLX302, pLX304, pLenti-CMVtight-puro-DEST, pLenti-CMV-BLAST-DEST, pmD2.G, pRSV-REV, pMDLg/pRRE).

2) Transduction of cells (listed above) with viral particles produced from 1)

#### Origin & function

A number of the planned inserted genetic sequences can contribute to oncogenic transformation of cells (e.g. epigenetic modifying enzymes (e.g. HDACs), transcription factors (e.g. Sox9, Foxl2), cell-cell adhesion molecules (e.g. Ephrins), signalling receptors and intracellular signalling molecules (e.g. TLRs, integrins, ERK), and a cell line immortalisation strategy (hTERT). Although a 3rd generation lentivirus approach will be used to transduce cells with these genetic sequences, the nature of the genes deems this work is CL2.
This project will not use larger GMOs, only cultured cell systems.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1) Solid: All solid waste will be rinsed in P3 Steril or equivalent (i.e. recommended and validated germicide for enveloped viruses and mammalian cell lines – (Refer to 1) Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008, (CDC), 2) Conform to Regulation (EC) No 1907/2006 (REACH), and 3) Webber et al, 20, 12, Infection control and Hospital Epidemiology 2015), left overnight and then autoclaved (moist heat for 25 min at 121oC). The autoclave is serviced twice a year. All autoclaved solids will be placed in yellow bags for subsequent incineration by KCL contractor.

2) Liquid: All liquid waste will be treated with P3 Steril or equivalent, put down the sink and rinsed with copious quantities of water.

Spillage procedure: Wear laboratory coat and nitrile gloves. Use prepared solution P3 Steril or equivalent. If a spill occurs in a centrifuge, leave for 30 min for aerosols to settle. Cover spill with 10% P3 Steril solution starting from the outer edges of the spill. If any glass, needle or other sharp material is present, use a dustpan and brush or forceps to collect them; place in Sharps bin (yellow lid). Wipe up inactivated liquids with paper towels. Rinse surface with 10% P3 Steril and then water, wipe up with more paper towels. Place towels in yellow waste bag. All re-usable contaminated equipment (e.g. centrifuge following containment failure, the forceps or dustpan and brush used in the clean-up) should be carefully cleaned with sterilising solution and then with water. Report all significant spills via KCL reporting system, AIRS.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The project was reviewed by the Faculty of Life Sciences and Medicine Biological Safety Committee on 4th February 2021 and approved it as a Class 2 GM activity.

Project Containment

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<td>L3 L4</td>
<td>L2 L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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Project Ref 386/21.5
Plasmid and Viral Vectors for Gene Delivery to Human and Mammalian Cells in vitro

Cyclin D1 and Cdk4 are tumour promoters and can drive tumour progression, putting them into viruses would require CL2.

Historical Significant Changes

Historical Date of Additional Info

Historical Significant Changes

Historical Date of Additional Info

Recipient or parental organism

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

To make and use recombinant retroviral and lentiviral vectors, for the study of proteins involved in regulation of the cell cycle in mammalian cells.

Human diploid fibroblast cell line MRC5, human embryonic kidney cells (293T), H9C2 rat myoblast cells, U2OS and HeLa cancer cell lines, primary neonatal rat cardiac myocytes, human smooth muscle cells and human breast cancer cell line MCF7.

HPASMCs- Human pulmonary arterial smooth muscle cells (HPASMCs) are isolated from human pulmonary arteries. HPASMC are cryopreserved at passage one and delivered frozen. HPASMC are characterized by immunofluorescence with antibodies specific to α-smooth muscle actin. Cultured PASMC play an important role in vascular disease research and can be used to identify new therapeutic targets to treat pulmonary vascular disease.

1) Production of lentiviral vectors by co-transfection of HEK293T cells with lentiviral transfer vectors, with either 2nd generation packaging vectors (HIV-1 and VSV-G) or 3rd generation packaging vectors (pLP1, pLP2 and pLP/VSVG).
2) Transduction of cells (listed above) with viral particles produced from 1)
3) Transduction of human MRC5 fibroblasts, 293T, U2OS, HeLa, neonatal rat cardiomyocytes, H9C2 myoblasts, HAP1 cells, human smooth muscle cells or MCF7 breast cancer cells with viral particles produced from 2).

Cyclin D1 and Cdk4 are tumour promoters and can drive tumour progression, putting them into viruses would require CL2.
For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Disinfection**: 1% Virkon overnight for both liquid and solid waste.
- **Autoclave**: Solid waste is autoclaved at 121°C for 30min prior to disposal in clinical waste stream.
- **Liquid waste**: Disinfected liquid waste is dispelled down the drain with copious amounts of water

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<td><strong>Laboratory Activities</strong></td>
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</table>

- **Animal Units** | **Large Scale Activities** | **Human Clinical Applications** |
| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

**Project Ref**: 386/98.1

- **Date Ackn’d**: 22/02/2001
- **CU2 Project Title**: EVALUATION OF ADENOVIRAL VECTORS FOR GENE THERAPY OF HEAD AND NECK CANCER. 1.MINIMAL RESIDUAL DISEASE IN THE MUSCLE BED
- **Date Project Ceased**: 02/03/2022

- **Class**: Class 2
- **CultureVolClass2**: non-GMM
- **Consent Granted**: not applicable
Project notified under transitional arrangements

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes
Project transferred to GM543 on 08/09/2003 PROJECT TRANSFERRED

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

02/03/2022
Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Project Ref 386/99.1

Date Ackn'd | CU2 Project Title
---|---
22/02/2001 | ISOLATION & CHARACTERISATION OF GENES INVOLVED IN THE REGULATION OF APOPTOSIS

Date Project Ceased
18/01/2006

Consent Granted
Non-GMM | not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

Large Scale Activities

Human Clinical Applications
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

---

**Project Ref:** 386/trans1  
**Date Ackn’d:** 19/02/1993  
**CU2 Project Title:** IN VITRO STUDIES OF HEPATITIS B VIRUS  
**Date Project Ceased:** 01/04/2003  
**Class:** Class 3  
**Consent Granted:** yes  
**Project notified under transitional arrangements:** Y  
**Withdrawn:** N
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ] N

If yes, tick to confirm that it is attached to this form [ ] N

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ] N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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- **Laboratory Activities**
  - L2 L3 L4 L2 L3 L4

- **Glass Houses**
  - L3 L4 L2 L3 L4

- **Growth Rooms**
  - L2 L3 L4

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**Project Ref** 386/trans2

- **Date Ackn'd** 14/03/2001
- **CU2 Project Title**
  - UTILISATION OF MOLECULAR TECHNIQUES TO STUDY THE PATHOGENESIS OF HEAD AND NECK CANCER (PREVIOUSLY, STUDY OF THE MOLECULAR PATHOGENESIS OF ORAL CANCER)
  - Class CultureVolClass2 CultureVolumeClass3-4
  - Class 2

- **Date Project Ceased** 08/09/2003

- **Consent Granted**
  - Non-GMM: not applicable

- **Project notified under transitional arrangements** [ ] Y

- **Withdrawn** [ ] N

- Tick if notifying a connected programme of work [ ] N

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 543/01.1

Date Ackn'd 08/09/2003

CU2 Project Title
EXPRESION OF VIRAL, BACTERIAL AND MAMMALIAN DNA SEQUENCES IN MAMMALIAN CELLS USING NON-VIRAL DNA TRANSFER SYSTEMS

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Title: Pathogenesis and treatment of infections at mucosal surfaces

Mucosal surfaces of the oral cavity, gastrointestinal and genital tracts are the main routes of entry of infectious microorganisms as well as being the sites for mucosal infections that may also have systemic effects. This activity will investigate the mechanisms by which both pathogenic microorganisms and those that form part of the normal mucosal flora may cause disease. In addition novel approaches to treatment by using genetically engineered commensal bacteria to deliver microbicidal agents will be investigated.

Recipient or parental organism:
1. E.coli
2. Streptococcus mutants
3. Streptococcus pneumoniae
4. Streptococcus gordonii
5. Streptococcus mitis
6. Fusobacterium nucleatum
7. Tannerella forsythensis
8. Porphyromonas gingivalis
9. Enterococcus faecalis
10. Lactobacillus spp
11. Candida albicans

Host/vector system:
1. Fragments of genomic DNA derived either directly or by polymerase chain reaction amplification derived from Streptococcus spp., T. Forsythensis, F. nucleatum will be used for mutagenesis to generate microorganisms that are defective in gene function either as a result of random mutagenesis or targeted mutagenesis.

2. Gene fragments identified as having functions of interest as above, may be cloned and used for expression of the gene products in E.coli. To understand function of such gene products, mutagenesis may also be performed with expression in E.coli. Similarly, DNA encoding proteins with antimicrobial potential e.g. single domain antibodies, cyanovirin and derived from human, animal, plant or algae sources will be used for expression of the gene product in heterologous hosts. The aims are to identify genes that contribute to virulence of mucosal pathogens and to express proteins that may be used for treatment or prevention of mucosal infections.

The microorganisms that will be modified are part of the normal flora to be found at mucosal surfaces in healthy individuals. Mutagenised microorganisms, Streptococcus spp., T. Forsythensis, F. nucleatum, Candida albicans show or are expected to show loss of function compared with wild type organisms. Streptococcus pneumoniae is a class 2 pathogen and work with virulent strains (i.e. TIGR4 derivatives) will be carried out in a microbiological safety cabinet.

Engineered Streptococcus gordonii and Lactobacillus strains may have comparable colonizing activity to wild type strains but cannot transfer antibiotic resistance since the genes are stably integrated into the chromosome of the modified host and cannot be mobilised. To reduce exposure and risk for the researcher to negligible levels, liquid cultures will be handled in a microbiological safety cabinet.

In all studies, genetically modified material will be destroyed on site by autoclaving.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from GMM cultures will be aspirated and treated with Haztabs (1 table/litre = 2,500 ppm chlorine) for 12 hours. Concentrated solutions will be made freshly on the day of use according to manufacturer’s instructions. Inactivated culture supernatents will be discharged to the drainage system. Solid waste will be double bagged, sealed and placed in a closed metal container. Waste will then be autoclaved in the closed metal container at 136°C for 30 minutes. This will result in complete inactivation of GMMs. Autoclave tape will be used to indicate that the material has been autoclaved. Autoclave function is monitored by services performed at 3 month intervals by Health Services Associates. Autoclaved waste will be placed in yellow bags for off-site incineration by White Rose.
The local GMSC approved the Risk Assessment for this project on and signed off the component activities on 11th March 2006 and 11th July 2006.

The class of activity is 2.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Is an emergency plan required according to regulation 20?  [N]

If yes, tick to confirm that it is attached to this form  [N]

Tick to confirm that you have attached a risk assessment to this form  [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment  [N]

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### Project Containment

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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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### Project Ref  543/07.1

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Tick if notifying a connected programme of work  [Y]

Withdrawn  [N]

Historical Significant Changes  PROJECT TRANSFERRED TO GM386 ON MERGER OF GM543 WITH G

Historical Date of Additional Info

Significant Change ID

02/03/2022
### Project Additional Information

**Purposes of the contained use**
Identification and study of the host contribution to bacterial infection and pathogenesis. This is done by infection of the fruitfly Drosophila melanogaster with the class 2 pathogen Mycobacterium marinum. Both host (Drosophila) and pathogen (M. Marinum) can be genetically modified.

**Recipient or parental organism**
- Mycobacterium marinum
- Drosophila melanogaster

**Host/vector system**
- M. marinum vectors: pVK173T, pFPV2, and equivalents/derivatives thereof.
- D melanogaster: crippled P-element-derived transposable elements (carried in vector pUAST and equivalents/derivatives thereof).

**Origin & function**
- M. marinum: transgenes encoding fluorescent proteins such as dsRed and GFP, from corals or jellyfish, used for tracking bacteria cells.
- D melanogaster: transgenes encode a variety of molecules (signal-transduction molecules, fluorescent proteins, etc.) of heterologous (human, mouse, jellyfish, coral, bacteria) or homologous origin; used to alter the course of infection and hence to be informative as to the genetic basis of susceptibility to infection or of pathogenesis.

**Evaluation of foreseeable effects**
- M. marinum: in our hands, fluorescent derivatives typically grow more slowly and are slightly less pathogenic than wild-type strains; they will also be resistant to some aminoglycoside antibiotics (apramycin or kanamycin), the net effect should be a mild decrease in pathogenicity for these strains, since primary treatment for M. marinum does not typically depend on aminoglycosides.
- D melanogaster: transgenic files should exhibit reduced fitness relative to other laboratory strains; since lab strains of drosophila are already poorly-adapted for life in the environment, these flies should present no environmental risk.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
We are very careful to prevent release of any pathogen-infected Drosophila.

1. Individual infected flies are tracked.
2. In most cases flies are infected, placed in culture vials and never again removed. Once the experiment is completed, the entire vial (including contained flies) is frozen to kill flies, then autoclaved to kill bacteria, and finally incinerated.
3. In cases where an experiment requires removal of live infected flies as samples, all flies within the vial are anesthetized before the vial is opened, preventing inadvertent escapes.
4. Fly-traps will be placed at frequent intervals in the fly room to attract and contain escaped flies.

Using these techniques, in the course of 6 years doing similar experiments at Stanford University, I observed fewer than 10 escapes of a total of more than 100,000.
Infected flies. All of these escapes were due to defective vial closures; I have now changed the type of vial-closure to one that is more secure, so fewer escapes are expected.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids: Waste will be double-bagged and autoclaved at 124°C for 15 minutes. Appropriate autoclave indicator tape will be used to monitor autoclave function. After autoclaving, waste will be incinerated by White Rose. Waste containing live flies as well as pathogens will be frozen for at least 6 hours at -20°C before autoclaving.

Liquid: Addition of disinfectant (chlorine bleach to 5% or 1 haztab/litre) followed by pouring down designated sink.

Both procedures are expected to achieve 100% killing.

Monitoring will be done by attempting to culture M. marinum using standard techniques either after autoclaving or after addition of bleach, as appropriate.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

GMSC approved at meeting of 1 May 2007.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Human Clinical Applications

Large Scale Activities

Project Ref 543/09.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022

Page 6926 of 15326
Pathogenesis and treatment of infections at mucosal surfaces

Mucosal surfaces of the oral cavity, gastrointestinal and genital tracts are the main routes of entry of infectious microorganisms as well as being the sites for mucosal infections that may also have systemic effects. This activity will investigate the mechanisms by which both pathogenic microorganisms and those that form part of the normal mucosal flora may cause disease. In addition novel approaches to treatment by using genetically engineered commensal bacteria to deliver microbicidal agents will be investigated.

Recipient or parental organism

1. E. coli
2. Streptococcus mutans
3. Streptococcus pneumoniae
4. Streptococcus gordonii
5. Streptococcus mitis
6. Fusobacterium nucleatum
7. Tannerella forsythensis
8. Porphyromonas gingivalis
9. Enterococcus faecalis
10. Lactobacillus spp
11. Candida albicans
12. Epithelial cells
13. T cells
14. Knockout mouse

Host/vector system

2. Host: Streptococcus spp.: Vector: pALH109, pALH122, pNE1gfp, pEVp3, pSMB55, pMHL120, pCMG8 or related plasmids
3. Host: T. forsythensis; Vector: pMUF. R II or related plasmids
4. Host: F. nucleatum; Vector: pKH90, pORI9, pHS19, pSH17 or related plasmids
5. Host: C. albicans SC5314 and CAI4 strains; Vector: Cip10
8. Host: Knockout Mouse (C57/BL6)
1. Fragments of genomic DNA derived either directly or by polymerase chain reaction amplification derived from Streptococcus spp., T. Forsythensis, F. nucleatum will be used for mutagenesis to generate microorganisms that are defective in gene function either as a result of random mutagenesis or targeted mutagenesis.

2. Gene fragments identified as having functions of interest as above, may be cloned and used for expression of the gene products in E. coli. To understand function of such gene products, mutagenesis may also be performed with expression in E.coli. Similarly, DNA encoding proteins with antimicrobial potential e.g. single domain antibodies, cyanovirin and derived from human, animal, plant or algae sources will be used for expression of the gene product in heterologous hosts.

3. DNA encoding partial or complete HIV-1 genome to be used for the production of pseudovirus (non-replicating) or intact HIV-1.

The aims are to identify genes that contribute to virulence of mucosal pathogens and to express proteins that may be used for treatment or prevention of mucosal infections.

**Evaluation of foreseeable effects**

The microorganisms that will be modified are part of the normal flora to be found at mucosal surfaces in healthy individuals. Mutagenised microorganisms, Streptococcus spp., T. Forsythensis, F. nucleatum, C. albicans show or are expected to show loss of function compared with wild type organisms. Streptococcus pneumoniae is a class 2 pathogen and work with virulent strains (i.e. TIGR4 derivatives) will be carried out in a microbiological safety cabinet.

Engineered Streptococcus gordonii and Lactobacillus strains may have comparable colonizing activity to wild type strains but cannot transfer antibiotic resistance since the genes are stably integrated into the chromosome of the modified host and cannot be mobilised. To reduce exposure and risk for the researcher to negligible levels, liquid cultures will be handled in a microbiological safety cabinet.

HIV-1 pseudovirus is non-replicating. Intact HIV-1 is infectious and therefore work will be carried out in microbiological safety cabinets in the class 3 laboratory.

In all these studies, genetically modified material will be destroyed on site by autoclaving.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

SOP for use of autoclave external to CL3 laboratory:

All material for disposal will be autoclaved outside of the Cat 3 facility on a cycle that reaches 126°C. Autoclave bins with properly fitted, locking lids containing the waste will be taken from the CL3 laboratory to the autoclave immediately prior to autoclaving. The following procedures will be followed:

i) two trained staff must be available to carry out the procedure

ii) the autoclave room must be available for use.

iii) one member of staff will act to open doors; one will move the autoclave bins from the CL3 facility onto the transport trolley.

iv) the autoclave bins will be sprayed with 70% alcohol before being removed from the CL3 facility and placed on a tray on a trolley outside the CL3 laboratory. Gloves used for transferring the bins must be placed on top of the closed bins

v) autoclave bins from the CL3 laboratory will be taken directly to the autoclave and placed in the autoclave, the lids are then unlocked & turned over to allow steam penetration
vi) when all autoclave bins have been loaded into the autoclave, the tray will also be placed in the autoclave and the autoclave cycle will be started.

vii) at the end of the cycle, the print-out should be kept as a record of adequate sterilisation.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste from GMM cultures will be aspirated and treated with Haztabs (1 tablet/litre = 2,500 ppm chlorine) for 12 hours. Concentrated solutions will be made freshly on the day of use according to manufacturer’s instructions. Inactivated culture supernatants will be discharged to the drainage system. Solid waste will be double bagged, sealed and placed in a closed metal container. Waste will then be autoclaved in the closed metal container at 136°C for 30 minutes. This will result in complete inactivation of GMMs. Autoclave tape will be used to indicate that the material has been autoclaved. Autoclave function is monitored by services performed at 3 month intervals by Health Services Associates. Autoclaved waste will be placed in yellow bags for off-site incineration by White Rose.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Local GMSC approved the Risk Assessment for this project on and signed off the component activities on 11th March 2006 and 11th July 2006

The class of activity was 2.

The Risk Assessments relevant to this revision were approved, subject to minor changes, by the Dental Institute GMSC on 7th July 2009.

HIV pseudotype virus production was assessed as a class 2 activity and Production of Replication competent HIV stocks was assessed as a class 3 activity.

**Project Containment**

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**Project Ref** 543/94.1

**Date Ackn’d** 02/03/2022 **CU2 Project Title** **Class** **CultureVolClass2** **CultureVolumeClass3-4**

Page 6929 of 15326
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## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref 543/97.1

Date Ackn'd 08/09/2003

CU2 Project Title

OVER EXPRESSION OF GLUTOMINE FRUCTOSE-6-PHOSPHATE AMINO TRANSFERASE IN HUMAN MESANGIAL CELLS: A POTENTIAL MECHANISM OF MESANGIAL CELL INJURY

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

GM543/00.1

Historical Date of Additional Info 02/03/2000

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
A COMPARISON OF ADENO AND HERPES BASED VIRAL VECTORS FOR GENE DELIVERY TO THE MYCARDIUM

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

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Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 697/01.1

Date Ackn'd 21/09/2007
CU2 Project Title ANALYSIS OF THE IMMUNE RESPONSE TO PLU1 ANTIGEN USING
Class 2
CultureVolClass2
CultureVolumeClass3-4

20/03/2022
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref 697/01.2**

**Date Ackn’ed**

**CU2 Project Title**

EXPRESSION IN MAMMALIAN CELLS OF GENES ASSOCIATED WITH BREAST CANCER - USING RETROVIRAL VECTORS

**Date Project Ceased**

**Class**

Class 2

**Culture Vol**

**Class 2**

**Volume Class 3-4**

**Non-GMM**

Consent Granted

Not Applicable

**Project notified under transitional arrangements**

Y

**Withdrawn**

N

**Tick if notifying a connected programme of work**

N

**Historical Significant Changes**

TRANSFERRED FROM GM 697 - 21/9/07
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Large Scale Activities**

**Human Clinical Applications**
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**Name**

MED IMMUNE LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

MILSTEIN BUILDING

**Road Name**

GRANTA PARK

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB1 6GH

**Country**

ENGLAND

**Tel Number**

01223 471471

**Fax Number**

01223 471472

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

19/04/2001 06/12/2002
Date Accident Notified
08/01/2021

Accident ID
36.00

Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Cultures up to 50 L volumes. Chloros (Sodium hypochlorite) at 2% v/v final concentration. Virkon (Potassium monopersulphate/anionic surfactant) at 1% w/v and 20 minutes contact time. Autoclaving for 35 minutes at 121 degrees C (1 bar pressure) is also available for solid waste and cell paste.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**Project Additional Information**

**Purposes of the contained use**

To obtain a stably transfected cell lines with a reporter gene constructs containing a transcription factor responsive element fused to a reporter gene. The binding of specific transcription factors to the reporter gene enhancer will provide a direct measurement of activation of the appropriate pathway. The reporter cell lines would be used for invitro cell-based assays that incorporate signal transduction through the required signalling pathway.

**Recipient or parental organism**

Cell lines to be used for transfection with reporter genes are human and of haemopoietic origin. Such cell lines include cells of erythroid origin (eg.TF-1), basophilic (eg.KU812), lymphocytic (eg.Jurkat, Ramos), monocytic (eg. THP-1, U937) and myeloid origin (eg. KG-1, HL-60). There is no evidence that these cells contain active viruses or blood-borne pathogens that represent an identifiable risk for human infection. These cells are unlikely to be oncogenic. The examples of cell lines given have been in continuous culture for > 20 years. In this time, no hazards associated with their use have been reported. However, these cells may contain as yet unidentified viruses or other pathogens and may acquire pathogens during culture as such these cells have been assessed as requiring handling under level 2 containment to minimise any potential risks to the operator. There is no identifiable potential for harm to the environment. Accidental release would not introduce anything into the environment that could easily be transferred to other hosts. The cells require specific nutrients not available outside of culture media and are sensitive to physical conditions present in the environment, thus would be unlikely to survive in the environment.

**Host/vector system**

AAV Helper-Free Systems - Details & Specifications

The first step is cloning the gene of interest into an appropriate plasmid vector. For most applications, the DNA of interest is cloned into one of the ITR vectors (pAAV-MCS). The inverted terminal repeat (ITR) sequences present in these vectors provide all of the cis-acting elements necessary for AAV-2 replication and packaging.
The recombinant expression plasmid is co-transfected into the AAV-293 cells with helper plasmids encoding adenovirus-derived genes and AAV-2 replication and capsid genes, which together supply all of the trans-acting factors required for AAV replication and packaging in the AAV-293 cells. Recombinant AAV-2 viral particles are prepared from infected AAV-293 cells and may then be used to infect a variety of mammalian cells.

**Origin & function**

The reporter vectors are commercially available.

Introduction of the NFκB pathway enables endogenous NFκB to bind to the kappa enhancer element located upstream of the reporter gene on the vector. Binding of NFκB enhances the association of the cell's general transcription machinery with the HSV-TK promoter fused downstream of the Kappa enhancer element. This results in high induction levels of reporter gene transcription.

The SRE responsive element is used to monitor the induction of the serum response element and the mitogen-activated protein (MAP) kinase signal transduction pathway. Addition of serum or growth factors to the cell culture medium induces the binding of transcription factors to the SRE, which activates the transcription of the appropriate reporter gene.

The CRE responsive element is used to monitor the activation of cAMP binding protein (CREB) and cAMP-mediated signal transduction pathways. Several signal transduction pathways are associated with the cAMP response element (CRE), including jun-N-terminal kinase (JNK), p38 and protein kinase A (PKA). Induction of these pathways enables endogenous transcription factors, such as CREB or ATF, to bind CRE, and in turn activate transcription of the appropriate reporter gene.

SEAP and luciferase are highly sensitive enzymic reporters that will be used to provide quantitative data on the absolute induction level of the appropriate signalling pathways. The SEAP gene encodes a truncated form of human placental alkaline phosphatase that lacks the membran-anchoring domain, thereby allowing the protein to be efficiently secreted from transfected cells. After transfection of the SEAP reporter vector into cells, the appropriate reporter pathway can be activated using an appropriate stimulus. The addition of a suitable substrate into the cell culture medium will lead to its dephosphorylation by SEAP and the generation of an appropriate signal.

The firefly luciferase (luc) gene was derived from Photinus pyralis. After transfection of the luciferase reporter vector into cells the appropriate reporter pathway can be activated using an appropriate stimulus, and this results in intracellular accumulation of the product. The addition of a suitable substrate and lysis agent or substrate solution into the cell culture will result in lysis of the cells and the generation of an appropriate signal.

The pcDNA3.1 vector from which the NEO gene was derived is also commercially available. The NEO gene is a bacterial aminoglycoside phosphotransferase (APH) that was derived from the transposon Tn5. Neomycin is an aminoglycoside that blocks protein synthesis in mammalian cells by interfering with ribosomal function. Expression of the NEO gene in mammalian cells results in detoxification of Neomycin. The reporter vectors have been modified with the NEO gene so that a stably transfected cell line could be generated.

**Evaluation of foreseeable effects**

Genetic modification of human cells of haemopoietic origin with the modified reporter gene vectors as described would not increase the potential hazards of these cells to either human health or the environment. Any unidentified risks to human health associated with these cells will be minimised by using good microbiological practice and handling the cells under level 2 containment conditions. The cells require specific nutrients not available outside of culture media and are sensitive to physical conditions present in the environment, thus would be unlikely to survive in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste would be placed in a suitable container and autoclaved prior to disposal.
All liquid waste will be treated for at least 30 min with a chlorine-based disinfectant (2% Chloros v/v or 1% Virkon w/v) before being disposed of down the sink. Spills will be contained using absorbent material (such as paper towels), then Virkon powder added to the spill and left for 5 minutes. The used absorbent material would be disposed of by autoclaving. The area will then be wiped with a chlorine-based disinfectant (2% Chloros v/v or 1% Virkon w/v). Autoclaving or treatment with a chlorine-based disinfectant will be expected to kill 100% of the treated cells. Tests have shown that autoclaving or treatment with 1% Virkon w/v as described above reduced cell viability to 0%. 

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Safety Committee of the 12th December 2003 found that the risk assessment covering this work identifies the potential risks. The committee agrees with the assessment of activity class 2 and that the use of level 2 containment measures are sufficient to protect human health and the environment. The committee approved this work. The company safety committee was informed of the intention to perform this work on 20/2/04 and made aware of the risks, containment and procedures associated with its completion. There were no additional concerns raised.

Project Containment

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Project Ref 387/06.1

Date Ackn’d  27/04/2006  CU2 Project Title  Procedures utilising plasmid and phagemid DNA encoding a single chain (scFv) antibody - Pseudomonas exotoxin A (scFv-PE38) fusion for manipulation, culturing, expression and purification from E. coli.

Date Project Ceased

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Non-GMM  Consent Granted  Not Applicable

Project notified under transitional arrangements  N
Project Additional Information

**Purposes of the contained use**

To use laboratory strains of E. coli, as a host for the expression of antibody single chain Fv (scFv) fragments fused to the truncated mutant form of Pseudomonas exotoxin A, known as PE38, to obtain sufficient material for purification and use of scFv-PE38 in assays for cell killing of malignant B-cells.

**Recipient or parental organism**

Containment level 1, laboratory strains of E. coli such as:
- E. coli TG1 F' traD36 lacI (lacZ)M15 proA+B+/supE (hsdM-mcrB)5 (rk-mk+McrB-) thi (lacproAB)
- E. coli HB2151 nai thi-1 ara (lac-proAB) [F' proAB+ lacIq lacZM15]
- E. coli ORIGAMI (ara-leu) 7697 lacX74 phoA pvull phoR araD139 ahpC galE galK rpsL
- F [lac+lacI pro] gor522::Tn10trxB (Kanr, StrR, TetR)
- E. coli BL21(DE3) F- ompT hsdSB(- mB-) gal dcm (DE3)

Laboratory strains of E. coli are disabled and unable to survive for long periods outside of laboratory culture conditions. Such strains lack any known virulence factors and are non-colonising.

**Host/vector system**

Name and description of vector(s) carrying the gene:
- pCantab6 phagemid, based on pUC119, containing the pMB1 and M13 replicons, LacZalpha and M13 intergenic regions, ampr, M13 leader sequence, scFv gene, 6 His and c-myc tags and M13 p3g.
- pUC119, containing the pMB1 and M13 replicons, LacZalpha and M13 intergenic regions, amp, M13 leader sequence, 6 His and c-myc tags.

**Origin & function**

Pseudomonas exotoxin A is a bacterial toxin composed of three domains: domain Ia (amino acids 1-252) is the cell binding domain; domain II (amino acids 253-364) is responsible for translocation into the cytosol; domain III (amino acids 400-613) ADP-ribosylates elongation factor 2, arresting protein synthesis and causing cell death in eukaryotic cells. Domain Ib (amino acids 365-399) is a minor domain and its function is unknown. PE38 is a modified form in which all of domain Ia and amino acids 365-380 of domain Ib have been deleted.

Antibody scFv domains that target B-cell cell surface proteins, such as CD22, or control scFv antibodies targeting non-human proteins, or haptens.

**Evaluation of foreseeable effects**

There is a potential for the transfer of the genetic material to other hosts. The host strains are F' positive and vectors contain the f1 origin of replication. As such in the presence of a helper phage such as M13K07, vector could be packaged can thus transfer the plasmid to other F' strains. These will be handled using good microbiological practice at containment level 2 and disposed of by a specified route and method potential for transfer is minimised.
Potential risk of transformed host transiently colonising staff through aerosols and low-level splashes. This could potentially result in delivery of B-cell targeted toxin to skin and mucous membranes. The fusion gene products have the potential to induce cytotoxic effects if delivered to the site of action in sufficient quantity. PE38 is only toxic once it has been transferred into the cell cytoplasm, this can only occur when binding is targeted to a cell surface epitope by the attached scFv. The risk of harm is reduced if the target antigen is elevated on malignant B-cells but present at low levels on non-malignant B-cells. The toxin is immunogenic and exposure is likely to generate neutralising antibodies to the drug. However, providing good microbiological practice at containment level 2 is followed and steps where the production of aerosols is likely are performed in a microbiological safety cabinet, the likelihood of the gene product reaching the site of action in sufficient quantity is minimal.

The anti-GITC PE38 fusion poses no identifiable risk as the target is not present in humans. However in practice it will be handled as above. The host E. coli strains used for this are disabled laboratory strains, provided the host carrying these genes is handled using good microbiological practice and disposed of or spills decontaminated by the specific route and method there is no identifiable risk of transfer or harm to the environment. Provided the hosts carrying these genes are handled using good microbiological practice the risk to human health should be minimal at containment level 2 as a precaution until more is known about the toxic effects of the fusion protein.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste will be placed in a suitable container and autoclaved (at >121 degrees C for longer than 15 min) in a validated autoclave prior to disposal.

All liquid waste will be treated for at least 30 min with a suitable, validated disinfectant (2% Chloros v/v or 1% Virkon w/v) before being disposed of to drains.

Spills will be contained using absorbent material (such as paper towels), then Virkon powder added to the spill and left for 5 minutes. The used absorbent material would be disposed of by autoclaving. The area will then be wiped with a suitable disinfectant (2% Chloros v/v or 1% Virkon w/v).

Autoclaving at >121 degrees C for longer than 15 min or treatment with a chlorine-based disinfectant will be expected to kill 100% of the bacterial cells. In house tests have shown that autoclaving or treatment with 1% Virkon w/v as described above reduced cell viability to 0%.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

The GMSC held on 8th March 2006 found that the risk assessment identified potential hazards and that the control measures described are sufficient to protect human health and the environment. The committee agreed with the activity class 2 rating and approved the work, subject to HSE notification and consent, to be undertaken only in a designated containment level 2 laboratory. Work to be undertaken only by staff authorised by the manager responsible for this work.

**Project Containment**
Project Additional Information

**Purposes of the contained use**

Primary cells reach senescence after a limited number of population doublings, to use the same consistent cells throughout a research project, primary cells with extended replicative capacity, or immortalised cells are required. This risk assessment describes the use of modified lentiviral particles expressing sequence encoding either human telomerase (hTERT) or simian virus 40 (SV40) T antigen for the generation of stable immortalised primary cell lines to overcome senescence of these cells and to extend their use for subsequent in vitro studies.

**Recipient or parental organism**

Hazard Group 2 mammalian and primary human cells isolated from human tumours or normal tissue and covered by appropriate COSHH risk assessments.
The use of commercially available lentiviral particles expressing hTert or SV40 T antigen:

SV40 large T antigen (Simian Vacuolating Virus 40 Tag) is an oncogene derived from the polyomavirus SV40 which is capable of transforming a variety of cell types. The transforming activity of TAg is due in large part to its perturbation of the retinoblastoma (pRB) and p53 tumor suppressor proteins. In addition, TAg binds to several other cellular factors, including the transcriptional co-activators p300 and CBP, which may contribute to its transformation function.

3rd generation lentiviral vectors will be generated by a commercial source. All vectors used in this type of technology are self inactivating which minimises any risk. Lentiviral vectors fused to generate lentiviral particles from commercial sources have been designed to maximise their biosafety features. The envelope of HIV-1 has been completely removed from the vector and replaced with the VSV-G gene from Vesicular Stomatis Virus to pseudotype (envelope) the particles. Only five genes from HIV-1 are used in the system (gag, pol, rev, tat and vpr). These genes are required for packaging the virus particles. To minimise the threat of producing recombinant and new replication-competent virus, the genes are separated on to five different plasmids and contain no significant areas of homology. The expression vector contains a deletion in the 3’ LTR that results in self-inactivation of the virus after transduction of the target cell. Additionally, the four packaging plasmids may allow expression in trans of the proteins required to produce virus but none of the plasmids contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are present in the packaged virus and therefore, are not expressed in the transduced target cell. It also minimises the chance of new replication-competent virus being produced. Virus will be produced by commercial sources and not on site.

Human Telomerase Reverse Transcriptase (hTERT for short) maintains telomere ends by addition of the telomere repeat TTAGGG. Without telomerase and TERT in humans DNA is unable to replicate ultimately causing cellular senescence, otherwise known as cell death. This is the basis for the aging process in humans. As cells die they are no longer able to replicate newer, fresher cells, hence causing aging. Hence when telomeres shorten cells die. Using methods to enhance hTERT in humans we can increase the health of telomeres and hence maintain replicative capacity of cells.

**Origin & function**

Human Telomerase Reverse Transcriptase (hTERT for short) maintains telomere ends by addition of the telomere repeat TTAGGG. Without telomerase and TERT in humans DNA is unable to replicate ultimately causing cellular senescence, otherwise known as cell death. This is the basis for the aging process in humans. As cells die they are no longer able to replicate newer, fresher cells, hence causing aging. Hence when telomeres shorten cells die. Using methods to enhance hTERT in humans we can increase the health of telomeres and hence maintain replicative capacity of cells.

**Evaluation of foreseeable effects**

What are the potential hazards associated with the recipient host?
Primary cells are derived from unfixed human tissue, although some there is a potential risk of human infection from agents they may harbour and will be treated as Hazard Group 2.

What are the potential hazards arising from the gene product?
The expression of SV40Tag or hTERT are both oncogenic and will transform and/or immortalise cells allowing them to overcome replicative senescence. This is a property that we want to take advantage of to increase the lifespan of these primary cells.

What are the additional potential hazards arising from the combination of gene and host?
No additional hazards these are self inactivating viruses and pose no risk following integration into the host genome.

What is the potential for the transfer of the gene to other hosts? i.e. with no controls in place
Lentiviral particles can infect almost all human cells (including non-dividing cells), however, the recombinant retroviral vectors used to generate viral particles are self inactivating, replication deficient in that they are unable to replicate and do not express any of their endogenous genes. Once within the mammalian cell no viral particles would be produced. The virus could not therefore be transferred to another host.

What is the potential for harm to human health (in the event of exposure)? i.e. with no controls in place
The only realistic route of exposure likely to lead to engraftment is via a sharps injury and even in such a scenario the likelihood of that outcome is still low given that the cells are non-syngeneic with those handling them, although this may be more likely with tumour cells (whether recombinant or not).

What is the potential to disseminate and harm the environment (in the event of accidental release)? i.e. with no controls in place
These do not pose any identifiable risk to the environment because the reagent containing lentivirus is unable to replicate unless supplied with helper plasmids hence they cannot replicate outside of laboratory conditions.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste must be inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04) or where inactivation is not possible as Hazardous Biological waste (EWC 18 01 03). Inactivation should be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

Note: Occasions where inactivation is impossible due to the high volume of material, e.g. from robotic systems or due to the inability to prove inactivation, as is the case with large sealed filtration units. In such cases we use a registered waste contractor to remove as hazardous biological waste for incineration.

Both non-hazardous and hazardous biological waste are sent for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This work should be classified at Activity Class 2, because of the potential for adventitious agents to be present in the primary cells. There is a slight chance that recombinant cells could be able to divide more quickly or disseminate more widely in the event of accidental engraftment; however, this would not affect the risk control measures requirement. The only realistic route of exposure likely to lead to engraftment is via a sharps injury and even in such a scenario the likelihood of that outcome is still low given that the cells are non-syngeneic with those handling them, although this may be more likely with tumour cells (whether recombinant or not).

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The generation and use of lentiviral particles for mediating gene modulation in Hazard Group 1 & 2 and Activity Class 1 & 2 cells and their subsequent use in vitro and in vivo.

To enable the generation of lentiviral particles encoding Open Reading Frames (ORFs), cDNAs or specific gene sequences and their subsequent use in vitro and / or in vivo. The goals of this body of work are to enable the generation and use of third generation, recombinant lentiviral vectors expressing ORFs, cDNAs, specific gene sequences and / or shRNAs and their subsequent use to: 1) Efficiently generate cell and disease relevant models lines for assessment of target biology 2) To validate literature data 3) To create new models for use in the oncology and other therapeutic areas and 4) To identify and / or validate ORFs / cDNAs or specific gene sequences as potential drug targets.

Mammalian cell lines including Hazard Group 1 and 2 and Activity Class 1 and 2 genetically modified cells all of which are covered by appropriate risk assessments.

Third generation (or greater) lentiviral vectors will be used (ref: SACGM compendium of guidance part 2-11 P119 sections 8- 30).
“Third Generation” lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase biosafety. These include the vif, vpr, vpu and nef accessory genes which are not required for in vitro replication. The tat gene is also deleted and the Tat-responsive promoter present in the 5’ LTR is replaced with heterologous promoters, for example with the Rous sarcoma virus U3 region. An additional biosafety feature is achieved by deletion of the rev gene from the viral transfer vector. Viral packaging is achieved by providing three
helper constructs in trans containing gag, pol and rev sequences. An additional biosafety feature is that these vectors are self-inactivating (SIN), whereby the U3 region of the 3’ LTR (which contains the major viral promoters and enhancers) is copied to the 5’ end of the provirus during reverse transcription. Deletion of enhancer and promoter elements from the 3’ U3 region in the vector construct will result in a provirus that is entirely devoid of U3 enhancer sequences, therefore reducing the potential for transactivation of cellular genes as a result of insertion. Furthermore, such vectors are not easily mobilisable as a result of a superinfection with wild-type virus.

**Origin & function**

Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and/or shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins) will be expressed in self inactivating lentiviral expression vectors. Guidance on inserts that may have greater risk of adverse effects on human health can be found in SACGM compendium of guidance part 2-2 p37-46.

Of particular note:

**Oncogenes** (ref: SACGM compendium of guidance part 2-2 p38 sections 6-9)

Genes encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation. Full length cDNA encoding wild type and disease relevant mutants of these types of genes will be expressed in third generation SIN lentiviral vectors. Any use of lentiviral particles encoding oncogenic inserts will require appropriate controls and operator training.

Examples of genes that may be categorised as oncogenes include:

- Growth factors, or mitogens,
- receptor tyrosine kinases,
- cytoplasmic tyrosine kinases,
- cytoplasmic serine/threonine kinases and their regulatory subunits,
- regulatory GTPases
- transcription factors.

**Knockdown of tumour suppressors** (ref: SACGM compendium of guidance part 2-2, p42, paragraph 24).

Knockdown of genes encoding known tumour suppressors can contribute to cellular transformation. However, shRNA expression systems rarely completely silence the targeted gene. shRNAs targeting these types of genes or gene sequences with tumour suppressive functions will only be expressed in third generation SIN lentiviral vectors. Any use of lentiviral particles capable of reducing tumour suppressor expression will require appropriate controls and operator training.

Examples of genes that may be categorised as tumour suppressors include:

- p53 Nuclear, transcription factor
- RB1 Nuclear, transcription modifier
- APC Cytoplasmic, function unknown
- ATM Unknown location, kinase
- WT1 Nuclear, transcription factor
- NF1 Cytoplasmic, GTPase activating protein
- NF2 Cytoplasmic/cytoskeletal, membrane linkage
- P16 INK4 Nuclear, cyclin dependant kinase inhibitor
- VHL Nuclear, adaptor

Selectable markers – examples (but not restricted to):

- Ampicillin resistance: E.coli derived
Evaluation of foreseeable effects

Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and/or shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins) will be expressed in self inactivating lentiviral expression vectors. Guidance on inserts that may have greater risk of adverse effects on human health can be found in SACGM compendium of guidance part 2-2 p37-46.

Of particular note:
- Oncogenes (ref: SACGM compendium of guidance part 2-2 p38 sections 6-9).
  Genes encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation. Full length cDNA encoding wild type and disease relevant mutants of these types of genes will be expressed in third generation SIN lentiviral vectors. Any use of lentiviral particles encoding oncogenic inserts will require appropriate controls and operator training.
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  • NF2 Cytoplasmic/cytoskeletal, membrane linkage
  • P16 INK4 Nuclear, cyclin dependant kinase inhibitor
  • VHL Nuclear, adaptor
- Selectable markers – examples (but not restricted to);
  • Ampicillin resistance: E.coli derived
  • Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
  • Puromycin resistance (PAC): Puromycin acetyl transferase is derived from Streptomyces alboniger

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste must be inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04) or where inactivation is not possible as Hazardous Biological waste (EWC 18 01 03). Inactivation should be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

Note: Occasions where inactivation is impossible due to the high volume of material, e.g. from robotic systems or due to the inability to prove inactivation, as is the case with large sealed filtration units. In such cases we use a registered waste contractor to remove as hazardous biological waste for incineration.

Both non-hazardous and hazardous biological waste are sent for incineration.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The BioSafety Committee classified this work at Activity Class 2 because of the use of HG/AC2 hosts and also the potential and/or unknown hazard of the inserted gene sequences rather than the use of the proposed lentiviral vectors. They were prepared to approve the work at AC2, since they saw no likelihood for the introduction of any Containment Level 3 requirements due to changes in infectivity.

They recommended that a note was included to allow reclassification to AC1 for some resultant cell lines, e.g. "Note: Where there is an opportunity to reclassify the culture and use of resulting cell lines as Activity Class 1, i.e. HG/AC1 hosts with non-hazardous sequences, a further GM risk assessment should be completed."

Project Containment

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Project Ref 387/11.1
To use plasmids encoding:

1) Open Reading Frames (ORFs), cDNAs or specific gene sequences
2) silencing RNAs (e.g. siRNAs for the knockdown of Open Reading Frames (ORFs), cDNAs or specific gene sequences by RNA Interference (RNAi)

To create stable or transient recombinant hazard group or Activity Class 1 or 2 cell lines, and their subsequent use in vitro and/or in vivo.

This Risk assessment covers work where either the parental cell already requires Containment Level 2, or where the genetic insert is considered to have the potential to be hazardous, or increases the hazard of the parental cell.

Recipient or parental organism

The experimental work proposed here involves the generation of engineered plasmids and the transfection into mammalian or insect cell lines in vitro. Transfections may be transient or undergo selection to establish stable pools and/or clonal lines.

Following the transfection of the cell lines with gene specific plasmids;

- RNA and protein may be extracted from the cells to determine expression levels of target gene/product
- supernatants may be harvested for purification
- other function/cell based assays may be performed.
- Cell lines may be used for antibody selections in phage display
- Cell lines may be used in vivo for antibody generation, or for animal models

Host/vector system

The plasmid vectors to be used are derived form commercially available pUC or pBR322/pBR325 derived vectors, hence are high copy number, non-mobilisation deficient, contain ColE1 origin, with strong or moderate cellular or viral promoters (beta-actin, MV, SV40, EF1a) and one or more selectable marker (G418, zeocin, hygromycin resistance).
Examples of these vectors are given below.

pcDNA family:
Contains SV40 origin and early promoter expressing the neomycin resistance gene. pUC origin of replication, an F1 intergenic region and ampicillin resistance marker. Heterologous expression under control of CMV promoter.

pEE family:
Mammalian expression vectors containing the glutamine synthetase (GS) gene downstream of the SV40-early promoter. The gene of interest sequence is cloned downstream of the cytomegalovirus (CMV) immediate early promoter and upstream of the SV40 poly A terminator. The ampicillin resistance gene (beta-lactamase) is also present for propagation and selection in E.coli.

pDEST family (may contain oriP):
Mammalian expression vectors containing the ampicillin resistance (beta-lactamase) gene for propagation and selection in E.coli. An antibiotic resistance gene is also present downstream of the SV40-early promoter and upstream of the SV40 poly A terminator. The pUC origin of replication is present for propagation in E. coli and the EBV origin of replication (oriP) may be present for episomal replication in EBNA expressing cells. The gene of interest sequence is cloned downstream of the cytomegalovirus immediate early (CMVIE) promoter and upstream of the SV40 poly A terminator. The vector also contains the SV40 origin and an F1 intergenic region.

plEx insect cell vectors (Novagen):
The plEx vectors are designed for cloning and high-level expression of proteins in transiently transfected Spodoptera-derived insect cells. To drive target gene expression, the vectors employ an optimal combination of two transcription elements derived from AcNPV baculovirus, the hr5 enhancer and the IE1 immediate early promoter. This promoter/enhancer combination recruits endogenous insect cell transcription machinery, thereby avoiding the need for using baculovirus.

In this risk assessment both "non-hazardous" and "hazardous" inserts are covered.

Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and/or siRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins) will be expressed.

Guidance on inserts that may have greater risk of adverse effects on human health can be found in SACGM Compendium of Guidance part 2-2 p37-46.

Of particular note:
Over-expression of Oncogenes (ref: SACGM compendium of guidance part 2-2 p38 sections 6-9).

Genes encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation. Examples of genes that may be categorised as oncogens include:

- p53 Nuclear, transcription factor
- RB1 Nuclear, transcription modifier
- APC Cytoplasmic, function unknown
- ATM Unknown location, kinase
- WT1 Nuclear, transcription factor
- NF1 Cytoplasmic, GTPase activating protein
- NF2 Cytoplasmic/cytoskeletal, membrane linkage
- P16 INK4 Nuclear, cyclin dependant kinase inhibitor
- VHL Nuclear, adaptor
- Selectable markers - examples (but not restricted to);
  - Ampicillin resistance; E coli derived
### Evaluation of foreseeable effects

**What are the potential hazards associated with the recipient host?**

Hazard Group and Activity Class 1 mammalian cell lines in continuous culture have been found unable to establish colonies in immuno-competent human adults following intradermal inoculation, ingestion or inhalation. Not known to be allergenic or toxic. Sf9 and Sf21 insect cells are non-pathogenic and there is no evidence that these cells contain active viruses or pathogens that represent an identifiable risk for human infection.

Hazard group 2 mammalian cells, primary human cells isolated from human tumours or normal tissue and covered by appropriate risk assessments. These may harbour viruses or other pathogens.

**What are the potential hazards arising from the gene product?**

The expression of the majority of genes is predicted to generally have little or no adverse effect. However, the precise outcome is difficult to predict. Expression of proto-oncogenes and oncogenic derivatives of these genes may transform normal epithelial and fibroblast cells and potentially enhance proliferation and tumourgenesis of cancer cell lines. Many Hazard Group 1 cancer cell lines in use already harbour mutations within these genes.

Knockdown of tumour suppressor genes may contribute to cellular transformation of normal epithelial and fibroblast cells and potentially enhance proliferation and tumourgenesis of cancer cell lines. Multiple cancer cell lines many of which already harbour mutations within one or more of these genes.

**What are the additional potential hazards arising from the combination of gene and host?**

The expression of the majority of genes is predicted to generally have little or no adverse effect. However, the potential outcomes of down regulating particular targeted genes should be carefully assessed. For example, knocking down the expression of a tumour suppressor gene in a mammalian cell could contribute to cellular transformation.

However, one of the features of RNAi knockdown systems is that the targeted gene is rarely completely silenced. Indeed, it is not unusual for there to be varying degrees of target gene down-regulation within an experimental population and therefore it is important to consider the ramifications of heterogeneous expression of the targeted genes.

**What is the potential for the transfer of the gene to other hosts? i.e. with no controls in place.**

Once within the host cell the plasmids are either stably integrated or transiently expressed and are not mobilisable.

**What is the potential for harm to human health (in the event of exposure)? i.e. with no controls in place.**

Genetically modified cells could have the potential to impair normal biological pathways and systems. However, the immune system would quickly recognise these cells as foreign and mount an immune defensive response. Recipient strains that are able to infect or colonise human hosts should be avoided i.e work should not be performed on ones own cells or those of other laboratory staff.

**What is the potential to disseminate and harm the environment (in the event of accidental release)? i.e. with no controls in place.**

There is no evidence to suggest that the host or genetically modified cell line will be able to propagate outside of a controlled laboratory environment without defined growth media and temperature requirements. There are no gene products that are known to be harmful to humans or to the environment.
In Vivo Waste Management

We would be doing the cell-based genetic modifications in our main laboratories and some of the resulting cells may be transferred over to our animal facility to administer into animals.

We would only send untreated GM waste off site from our main laboratories and only in two situations, which relate solely to medium to high scale cell culture, not in vivo work. These are:

• where the high volume of waste demands it, i.e. where we have robotic systems generating high volumes of plastic ware that we are unable to autoclave and;
• where we have large sealed filter housing units that we are unable to validate that inactivation has been successful without breaking open the unit and creating an increased risk of contamination.

Our animal facility is quite separate from the main laboratories and has its own set of waste handling procedures. In fact, no untreated GM waste, either solid or liquid, is transported off-site from our animal facilities; it will always be treated on-site prior to off-site incineration.

Treatment would be by autoclaving to 121°C for 45 minutes as a minimum (or 131°C for liquids).

Containment

We have separate isolation cages for our GM in vivo work, which is completely sealed with separate negative pressure air supply and a HEPA filter inlet and outlet. Our building is a designated building, regulated under the Animal (Scientific Procedures) Act 1986 and is therefore designed to prevent escape and to ensure complete containment of animals. We are also an AAALAC accredited establishment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

There are occasional requirements to transfer untreated waste off site for incineration with a waste contractor approved to carry hazardous biological waste. This situation would only occur if a project was scaled up and would be when we did not have the onsite facilities to deal with the volume or type of consumables.

I request a derogation for to transfer untreated waste off site for incineration.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste is inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04) or where inactivation is not possible as Hazardous Biological waste (EWC 18 01 03). Inactivation should be by autoclaving or chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

Note: Occasions where inactivation is impossible due to the high volume of material, e.g. from robotic systems or due to the inability to prove inactivation, as is the case with large sealed filtration units. In such cases we use a registered waste contractor to remove a s hazardous biological waste for incineration.

Both non-hazardous and hazardous biological waste are sent for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The Biosafety Committee classified this work at Activity Class 2 because of the use of HG/AC2 hosts and also the potential and/or unknown hazard of the inserted gene sequences rather than the use of plasmids, which are unknown to cause harm to human health or the environment. They were prepared to approve the work at AC2, since they saw no likelihood for the introduction of any Containment Level 3 requirements due to changes in infectivity.

They recommended that a note was included to allow reclassification to AC1 for some resultant cell lines, e.g. "Note: Where there is an opportunity to reclassify the culture and use of resulting cell lines as Activity Class 1, i.e. HG/AC1 hosts with non-hazardous sequences, further GM risk assessment should be completed."

### Project Containment

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### Project Ref 387/11.2

**CU2 Project Title**

The generation and use of adenoviral particles for mediating transient gene modulation in Hazard Group 1 & 2 and Activity Class 1 & 2 cells and their subsequent use in vitro and in vivo

**Date Ackn’d** 03/05/2011

**Date Project Ceased**

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Class CultureVolClass2 CultureVolumeClass3-4

Consent Granted
**Project Additional Information**

**Purposes of the contained use**

To enable the generation and subsequent use of adenoviral particles encoding Open Reading Frames (ORFs), cDNAs, or shRNAs directed to specific gene sequences both in vitro and/or in vivo.

The goals of this body of work are to enable the generation and use of third generation ‘gutless’ adenoviral vectors expressing ORFs, cDNAs, shRNAs or specific gene sequences and their subsequent use to: 1) Efficiently generate cell and disease relevant models lines for assessment of target biology 2) To validate literature data 3) To create new models for use in the oncology and other therapeutic areas and 4) To identify and/or validate ORFs/cDNAs or specific gene sequences as potential drug targets.

**Recipient or parental organism**

Adenoviral vectors are viral-based gene delivery systems that can efficiently transduce a wide range of target cells resulting in high-level gene expression. They are non-integrating vectors so expression in dividing cells is progressively lost.

The experimental work proposed here involves the generation of adenoviral particles and the addition of adenovirus particles encoding gene sequences to mammalian cell lines (Hazard group 1 and 2 and Activity class 1 and 2 cells) in vitro and the direct administration in vivo. Following the transduction of the mammalian cell lines with adenovirus, RNA and protein will be extracted from the cells to determine expression levels of target gene or gene product and functional assays will be performed.

Following the direct administration of adenoviral vectors in vivo, animals will undergo behavioural analysis (as detailed in project licence) and/or tissues/cells will be harvested and used in downstream functional assays.

**Host/vector system**

Mammalian cell lines including Hazard Group 1 and 2 and Activity Class 2 genetically modified cells all of which are covered by appropriate risk assessments.

**Vector**

Third Generation "Gutless" Adenoviral Vectors will be used (ref: SACGM compendium of guidance part 2-7 p 80 sections 5).

Third Generation vectors have been constructed by deleting the majority of the viral genes (E1, 2 and 3 genes) and retain only packaging sequences (Amalfitano et al., 1998) and the latest vectors have all or nearly all of the virus genes removed. These so-called 'gutless' vectors (Hardy et al., 1997; Kumar-Singh & Chaberlain, 1996; Leiber et al., 1999, Morsy et al., 1998; Steinwaerder et al., 1999) originally retained only the ITR and packaging sequences and required helper virus and appropriate complementing cells for propagation, followed by careful purification to remove helper virions. This design was based on finding that all adenoviral packaging proteins could be supplied in trans (Morsey et al., 1998). The vectors are transfected into a cell line like HEK293 that is able to supply the proteins required for replication in trans along with a helper virus that has deletions in its genome.

The psi (packaging) sequence is often flanked by LoxP sequences which results in the deletion of the psi sequence from the adenovirus helper genome following the expression of cre recombinase in the packaging cell line which substantially reduce the packaging of the helper genome into the virion.

**Origin & function**

In this risk assessment both "non-hazardous" and "hazardous" inserts are covered.

Open reading frames, cDNAs, and gene sequences encoding potential drug targets or therapeutic proteins or shRNAs designed to knockdown the expression of specific gene sequences will be expressed in adenoviral expression vectors.
Guidance on expression of inserts that may have greater risk of adverse effects on human health can be found in SACGM compendium of guidance part 2-2 p 37-46.

Of particular note:

Oncogenes (ref: SACGM compendium of guidance part 2-2 p 38 sections 6-9).

Genes encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation. Full length cDNA encoding wild types and disease relevant mutants of these types of genes will be expressed in adenoviral vectors.

Knockdown of tumour suppressors (ref: SACGM compendium of guidance part 2-2, p42 paragraph 24).

Knockdown of genes encoding known tumour suppressors can contribute to cellular transformation. However, shRNA expression systems rarely completely silence the targeted gene.

Any use of adenoviral particles capable of increasing expression of potential oncogens or reducing tumour suppressor expression will require appropriate controls and operator training.

Evaluation of foreseeable effects

What are the potential hazards associated with the recipient host?

These will vary depending on the cell type and can be known or unknown. Primary human samples are screened for the presence of the high risk human pathogens hepatitis virus and HIV by the supplier. Samples containing such pathogens should not be used.

In general, Hazard Group 2 cells and Activity class 2 cells present a greater risk than Hazard Group 1 cells, usually due to their potential to harbour human pathogens.

What are the potential hazards arising from the gene product?

The knockdown of or increased expression of the majority of genes is predicted to generally have little or no adverse effect. However, the precise outcome is difficult to predict. Knockdown of tumour suppressor genes may contribute to cellular transformation normal epithelial and fibroblast cells and potentially enhance proliferation and tumourigenesis of cancer cell lines and expression of proto-oncogenes and oncogenic derivatives of these genes will transfrom normal epithelial and fibroblast cells and potentially enhance proliferation and tumourigenesis of cancer cell lines. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines many ofwhich harbour mutations within these genes.

What are the additional potential hazards arising from the combination of gene and host?

Adenoviral gene transfer is one of the most reliable methods for introducing genes into mammalian cells. Because infection by adenovirus is no cell-cycle dependent, you can deliver your gene to primary as well as transformed cell lines. Following infection, your target gene is transiently expressed at high levels since many cells receive multiple copies of the recombinant genome. Expression is transient because adenoviral DNA normally does not integrate into the cellular genome so expression in dividing cells is progressively lost. However persistent expression in non-dividing cells has been observed in vivo (Chen, H. H. et al., (1999) Human Gene Ther. 10:365-373).

Adenoviruses are capable of infecting a wide variety of proliferating and quiescent cell types from many different animal species including humans, non-human primates, pigs, rodents, mice, and rabbits. Published reports suggest that nearly all human cell types - including skin, muscle, bone, nerve and liver cells - are susceptible to infection by adenovirus. Replication-deficient adenovirus may theoretically recombine with the wild-type virus to result in a replication - competent virus. However with third generation or gutless vector systems this risk is minimal. Several recombination events would be required and this is unlikley to occur.

The following 3 boxes look at the theoretical potential for harm to human health. Do not refer to ways risks are minimised.

What is the potential for the transfer of the gene to other hosts? i.e. with no controls in place.

Adenovirus can infect almost all human cells (including non-dividing cells). Adenoviruses are unusually stable to chemical or physical agents and adverse pH or...
temperature conditions, allowing for prolonged survival outside of the body of water. However, the recombinant vectors used to generate viral particles are replication
deficient in that they are unable to replicate and do not express any of their endogenous genes and require packaging components to be provided in trans.

What is the potential for harm to human health (in the event of exposure)? i.e. with no controls in place.

Wild-type adenoviruses are respiratory pathogens and in vivo infections are generally limited to the epithelial cells lining the respiratory tract, they most commonly cause respiratory illness, but may also cause other illnesses such as gastroenteritis and conjunctivitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection. Wild type Adenovirus is generally associated with mild respiratory infections in children, and it is thought that the majority of the population is likely to have antibodies to the wild type virus.

Adenoviruses are highly stable in even adverse conditions, allowing prolonged survival outside the body. Adenoviruses are most commonly transmitted by direct contact, such as a handshake. Persons who are infected may shed the virus for months or years, without experiencing any symptoms. Many people have been exposed to adenoviral infections throughout their lives and have anti adenoviral immunity.

However, Adenoviral vectors (Third generation) are rendered replication-deficient by removal of the majority of viral genes including the E1a gene, which is essential for the virus to replicate. If the virus cannot reproduce, it cannot sustain the illnesses listed above. The only potential for harm to human health could be through direct exposure to or injection of the recombinant adenoviral particles. However, even with injection the immune system would most likely recognise this as foreign and would mount an immune defensive response. Recipient strains that are able to infect or colonise human hosts should be avoided i.e. work should not be performed on one's own cells or those of other laboratory staff. The particles are unable to penetrate through gloves and would be unlikely to penetrate the dead layers of skin cells.

What is the potential to disseminate and harm the environment (in the event of accidental release)? i.e. with no controls in place.

Adenoviral vectors do not pose any identifiable risk to the environment because the reagent is unable to replicate unless supplied with helper plasmids and hence they cannot replicate outside of laboratory conditions.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**In vivo Waste Management**

We would be doing the cell-based genetic modifications in our main laboratories and some of the resulting cells may be transferred over to our animal facility to administer into animals.

We would only send untreated GM waste off site from our main laboratories and only in two situations, which relate solely to medium to high scale cell culture, not in vivo work. These are:
- Where the high volume of waste demands it i.e. where we have robotic systems generating high volumes of plasticware that we are unable to autoclave and;
- Where we have large sealed filter housing units that we are unable to validate that inactivation has been successful without breaking open the unit and creating an increased risk of contamination.

Our animal facility is quite separate from the main laboratories and has its own set of waste handling procedures. In fact, no untreated GM waste, either solid or liquid, is transported off-site from our animal facilities; it will always be treated on-site prior to off-site incineration.

Treatment would be by autoclaving for 121°C for 45 minutes as a minimum (or 131°C for liquids).

**Containment**

We have separate isolation cages for our GM in vivo work, which is completely sealed with separate negative pressure air supply and a HEPA filter inlet and outlet. Our building is a designated building, regulated under the Animal (Scientific Procedures) Act 1986 and is therefore designed to prevent escape and to ensure complete containment of animals. We are also an AAALAC accredited establishment.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

There are occasional requirements to transfer untreated waste off site for incineration with a waste contractor approved to carry hazardous biological waste. The situation would only occur if a project was scaled up and would be when we did not have the onsite facilities to deal with the volume or type of consumables.

I request a derogation for to transfer untreated waste off site for incineration.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste is inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04) or where inactivation is not possible as Haardous Biological Waste (EWC 18 01 03). Inactivation should be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

Note: Occasions where inactivation is impossible due to the high volume of material, e.g. from robotic systems or due to the inability to prove inactivation, as is the case with large sealed filtration units. In such cases we use a registered waste contractor to remove as hazardous biological waste for incineration.

Both non-hazardous and hazardous biological waste are sent for incineration

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Please enter comments on the GM safety committee on the risk assessment

The BioSafety Committee classified this work as Activity Class 2 by reference to the following:

(ref: SACGM compendium of guidance part 2 p84 sections 23). "Many adenovirus vectors will be considered low risk GM activity class 1 and can be handled at Containment Level 1. This means that virus preparations could be handled on the open bench. However, adenoviruses are robust and transmitted effectively in aerosols and droplets, even if disabled or attenuated. Therefore, measures might be required to control aerosol generation and airborne dissemination."

However since Activity Class 2 and Hazard Group 2 will be used and the inserts pose additional risk this work should be classified as 2.

There was some discussion about the ability of the virus for prolonged survival outside the body and the committee felt that there should be some mention of the means of surface decontamination and if a dedicated hood was advisable.

They were also prepared to approve the work at AC2, since they saw no likelihood for the introduction of any Containment Level 3 requirements due to changes in infectivity.

They recommended that a note was included to allow reclassification to AC1 for some resultant cell lines, e.g. "Note: Where there is an opportunity to reclassify the culture and use of resulting cell lines at Activity Class 1 i.e. HG/AC1 hosts with non-hazardous sequences, a further GM risk assessment should be completed."

---

Project Containment
Project Additional Information

**Purposes of the contained use**

The keratinocyte cell line (ATCC, CCD KERTr) will provide a continual cell source for in vitro experiments to explore the biology around inflammatory skin disease.

**Recipient or parental organism**

Cells are primary human skin epithelial cells, keratinocytes (immortalized). Human keratinocyte cell line (CCD 1106 KERTr) will be cultured using standard tissue culture techniques. Cells may be used in antibody selections and in several in vitro cell based assays. Human keratinocyte cell line is available from ATCC (CRL-2309 ). These calls should be assigned a Containment Level 2 category.

**Host/vector system**

**Vector:**
The pLXSN16E6E7 vector contains the human papilloma virus (HPV) type 16 E6 and E7 genes under control of the Moloney murine leukemia virus (MoMuLV) promoter-enhancer sequences. The vector also contains a gene controlling resistance to neomycin transcribed from the SV40 promoter. This line produces the amphotropic retrovirus LXSN16E6E7 which encodes the HPV16 E6 and E7 open reading frames, and which can be used to stably infect and immortalize many cell types. Therefore, this RA could potentially cover any human cell line that has been immortalized with this retroviral system.

**Origin & function**
The pLXSN16E6E7 vector contains the human papilloma virus (HPV) type 16 E6 and E7 oncogenes under control of the Moloney murine leukemia virus (MoMuLV) promoter-enhancer sequences. The E6 & E7 oncogene products will immortalize the recipient cell.

**Evaluation of foreseeable effects**

Potential hazards associated with the recipient host:
These will vary depending on the cell type and can be known or unknown. Potential common pathogens might be human pathogens such as Epstein Barr virus or rhinovirus. Primary human samples are screened for the presence of the high risk human pathogens hepatitis virus and HIV by the supplier. Samples containing such pathogens should not be used.

Potential hazards arising from the gene product:
The expression of the majority of genes is predicted to generally have little or no adverse effect. However, the precise outcome is difficult to predict. The work outlined in this assessment covers therapeutic transgenes and gene sequences expressed by the recombinant OV by their very nature these will have a therapeutic effect. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines and expression of a therapeutic transgene will often result in cancer cell death or arrest and render the cells more immunogenic in vivo which would result in greater clearance.

Potential hazards arising from the combination of gene and host:
The knockdown of or increased expression of the majority of genes is predicted to generally have little or no adverse effect. Gene products will only ever be expressed in cells that are able to support viral replication (tumour cells) and the greater the expression levels the more likely the cell is permissive to viral replication and will ultimately die due to this property. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines many of which harbour mutations within these genes or expressing these genes through other delivery vectors (adeno- or lentiviral transduction). Enhanced tumour cell killing is a beneficial additional effect of combination of host and gene.

Potential for transfer of gene to other hosts:
The recombinant OV agent can infect most human cells (including non-dividing cells). However, normal cells will not support viral replication and the virus will be inactivated. In cancer cells where replication is permissible the virus will be propagated and will result in cell lysis and death and release of virus which will go on to infect other surrounding cells. By their very nature, the replication component virus cells that support viral replication will ultimately die due to viral replication, and the level of viral replication will drive the level of the therapeutic transgene which will also enhance immune clearance in vivo or cell killing.

Potential for harm to human health in the event of exposure:
The recombinant OV agent has very low pathogenicity and is not considered a human pathogen. Human infection following acute exposure to large quantities of virus causes at most mild symptoms. No human-to-human
Transmission has ever been observed.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste is inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04) or where inactivation is not possible as Hazardous Biological waste (EWC 18 01 03). Inactivation will be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee were happy in principle with this GMO RA at AC2 but further information is needed regarding any virus testing done on the line, initial isolation reference etc etc. If the host cell has been in use for a long period, and there is subsequent testing for virus production available there may be the possibility to down grade to AC1.

Project Containment

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Project Ref 387/13.1

Date Ackn'd 02/03/2022
The use of a recombinant replicative Oncolytic Virus (OV), as a novel cancer therapeutic agent in vitro (in Hazard Group 1 & 2 cells), in vivo use and ex vivo analysis

Recombinant Oncolytic Virus strains (the production of which are described in Medimmune's GMO risk assessment G398) will be used to infect tumour cells. This work is described in MedImmune's GMO risk assessment G400.

The recombinant OV is a replication competent virus that can efficiently infect a wide range of target cells. In normal cells however, viral replication is inhibited and effectively shut down due to an intact intrinsic anti-viral response and intact interferon response pathway. In tumour cells with defects in the anti-viral response and interferon pathways, the recombinant OV is able to replicate, resulting in expression of viral genes and transgenes and production of viral progeny. They are non-integrative viruses so expression in dividing cells is progressively lost in cells that do not support viral replication. The GMO in this instance would be the virally infected tumour cells.

The experimental work proposed here involves the addition of recombinant OV particles encoding therapeutic gene sequences to mammalian cell lines (Hazard Group 1 and 2 and Activity Class 1 and 2 cells) in vitro and the direct administration (intra-tumoral or intravenous) in vivo using rodents. Following the transduction of the mammalian cell lines with recombinant OV, RNA and protein will be extracted from the cells to determine expression levels of viral RNA, target gene RNA or gene product and functional assays will be performed.

Following the direct administration of recombinant OV in vivo, animals will undergo monitoring and tumour measurements (as detailed in project licence) and / or tissues / cells will be harvested and used in downstream functional assays.

Recipient or parental organism

Recombinant Oncolytic Virus strains (the production of which are described in Medimmune's GMO risk assessment G398) will be used to infect tumour cells. This work is described in MedImmune's GMO risk assessment G400.

The recombinant OV is a replication competent virus that can efficiently infect a wide range of target cells. In normal cells however, viral replication is inhibited and effectively shut down due to an intact intrinsic anti-viral response and intact interferon response pathway. In tumour cells with defects in the anti-viral response and interferon pathways, the recombinant OV is able to replicate, resulting in expression of viral genes and transgenes and production of viral progeny. They are non-integrative viruses so expression in dividing cells is progressively lost in cells that do not support viral replication. The GMO in this instance would be the virally infected tumour cells.

The experimental work proposed here involves the addition of recombinant OV particles encoding therapeutic gene sequences to mammalian cell lines (Hazard Group 1 and 2 and Activity Class 1 and 2 cells) in vitro and the direct administration (intra-tumoral or intravenous) in vivo using rodents. Following the transduction of the mammalian cell lines with recombinant OV, RNA and protein will be extracted from the cells to determine expression levels of viral RNA, target gene RNA or gene product and functional assays will be performed.

Following the direct administration of recombinant OV in vivo, animals will undergo monitoring and tumour measurements (as detailed in project licence) and / or tissues / cells will be harvested and used in downstream functional assays.

Host/vector system

Vector: An attenuated recombinant Oncolytic Virus is the vector system (please see details in section 17).

Origin & function

Specific Open Reading Frames, cDNAs and gene sequences encoding therapeutic gene sequences or therapeutic proteins (e.g. cytokines) and / or si/shRNAs or miRNAs
(designed to knockdown the expression of cancer causing genes) and reporter genes can be inserted into recombinant Oncolytic Virus. Examples of transgene inserts include immune-modulating cytokines which would augment the anti-tumour and anti-viral immune response. Inserts will generally have a neutral or therapeutic effect.

**Evaluation of foreseeable effects**

Potential hazards associated with the recipient host:
These will vary depending on the cell type and can be known or unknown. Potential common pathogens might be human pathogens such as Epstein Barr virus or rhinovirus. Primary human samples are screened for the presence of the high risk human pathogens hepatitis virus and HIV by the supplier. Samples containing such pathogens will not be used.

Potential hazards arising from the gene product:
The expression of the majority of genes is predicted to generally have little or no adverse effect. However, the precise outcome is difficult to predict. The work outlined in this assessment covers therapeutic transgenes; gene sequences expressed by the recombinant OV, by their very nature, will have a therapeutic effect. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines and expression of a therapeutic transgene will often result in cancer cell death or arrest and render the cells more immunogenic in vivo which would also result in greater clearance.

Potential hazards arising from the combination of gene and host:
The knockdown of or increased expression of the majority of genes is predicted to generally have little or no adverse effect. Cells expressing these gene products will be able to support viral replication (tumour cells) and the greater the expression levels the more likely the cell is permissive to viral replication and will ultimately die due to this property. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines many of which harbour mutations within these genes or expressing these genes through other delivery vectors (adeno- or lentiviral transduction). Enhanced tumour cell killing is a beneficial additional effect of combination of host and gene.

Potential for transfer of gene to other hosts:
The recombinant OV agent can infect most human cells (including non-dividing cells). However, normal cells will not support viral replication and the virus will be inactivated. In cancer cells where replication is permissible the virus will be propagated and will result in cell lysis and death and release of virus which will go on to infect other surrounding cells. By their very nature, the replication competent virus cells that support viral replication will ultimately die due to viral replication, and the level of viral replication will drive the level of the therapeutic transgene, which will also enhance immune clearance in vivo or cell killing.

Potential for harm to human health in the event of exposure:
The recombinant OV agent has very low pathogenicity and is not considered a human pathogen. Human infection following acute exposure to large quantities of virus causes at most mild symptoms. No human-to-human transmission has ever been observed.

Potential to disseminate and harm the environment in the event of accidental release:
Only attenuated recOV will be used and this poses minimal risk to the environment. (Please see Section 17 for further details)

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Note: Rodents will be used for the in vivo work.
All in vivo work will be carried out under Containment Level 2 conditions using rodents. All handling of recOV agents will be in Class II safety cabinets and all treated animals will be kept in separate red-line isolation cages, which are completely sealed with separate negative pressure air supply and HEPA filters on the inlet and outlet. Our building is a designated building, regulated under the Animal (Scientific Procedures) Act 1986 and is therefore designed to prevent escape and to ensure complete containment of animals. We are also an AAALAC accredited establishment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We require a derogation to send untreated GM waste off site from our main laboratories in two situations, which relate solely to medium to high scale cell culture, not in vivo work. These are:
- Where the high volume of waste demands it i.e. where we have robotic systems generating high volumes of plasticware that we are unable to autoclave and;
- Where we have large sealed filter housing units that we are unable to validate that inactivation has been successful without breaking open the unit and creating an increased risk of contamination.
We will treat all other waste as described in Section 12.
All GMO waste is inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04) or where inactivation is not possible as Hazardous Biological waste (EWC 18 01 03) under an HSE derogation, see Section 11. Inactivation will be by autoclaving (121°C for 45 minutes as a minimum, or 131°C for liquids) or by chemical treatment (1% final Virkon for at least 30 minutes or other validated method).

In vivo Waste Management

We would be doing the cell-based genetic modifications in our main laboratories and some of the resulting cells may be transferred over to our animal facility to administer into animals (rodents).

Our animal facility is quite separate from the main laboratories and has its own set of waste handling procedures. In fact, no untreated GM waste, either solid or liquid, is transported off-site from our animal facilities; it will always be treated on-site prior to off-site incineration.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

Please enter comments on the GM safety committee on the risk assessment

The BioSafety Committee agreed with the risk assessments in principle, i.e. as a classification of Activity Class 2, subject to some minor modifications. They acknowledged the need for further discussions with the author on potential intellectual property and commercial confidentiality concerns for the preparation of the CU2 form.

Note: The BioSafety Committee were advised by the BSO of subsequent changes prior to the risk assessments being sent for signatures.

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Project Ref 387/13.2

Date Ackn'd 24/07/2013  Date Project 02/03/2022

CU2 Project Title The generation and subsequent use of AC1 or AC2 cell lines by retroviral transduction

Class 2  Consent Granted

CultureVolClass2 < 1 Litre  CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

1) To generate genetically modified cell lines via retroviral delivery systems (e.g., MMLV-based, MSCV-based) or to purchase cell lines from external sources that have been generated using such retroviral delivery systems, and to culture these cells for use in vitro and in vivo experiments.
2) To perform subsequent genetic modification of these cell lines via further retroviral transduction.

Overview of different types of GMOs that will be produced:
Retroviral vectors (RV) are viral-based gene delivery systems that can stably deliver genes or shRNA into cells with up to 100% efficiency. RV particles bind to target cells using an envelope protein that allows for release of the RV RNA containing the gene or gene silencing sequence into the cell. The RVs RNA is then converted into DNA using an enzyme called reverse transcriptase by a process called reverse transcription. The DNA pre-integration complex enters the nucleus and integrates into the target cell's chromosomal DNA. Gene delivery is stable because the target gene is integrated in the chromosome and is copied along with the DNA of the cell every time the cell divides.

The experimental work proposed here involves the generation of retroviral particles and the addition of retrovirus particles encoding gene sequences to mammalian cell lines in vitro and the direct administration in vivo. Following the transduction of the mammalian cells with recombinant retrovirus, RNA and protein will be extracted from the cells to determine expression levels of target gene or gene product and functional assays will be performed. Extracellular vesicles may also be removed from the conditioned media and used to assess delivery of the vesicle content to other mammalian cells and in vivo models. These vesicles may also be analysed biophysically, proteomically and genetically. Following the direct administration of retroviral vectors in vivo, animals will undergo behavioural analysis (as detailed in project licence) and/or tissues/cells will be harvested and used in downstream functional assays.

Recipient or parental organism

Mammalian cells including Hazard Group 1 and 2 and Activity Class 1 and 2 genetically modified cells all of which are covered by appropriate risk assessments.

Host/vector system

Taken from the SACGM compendium of guidance part 2.11 p116-126:

To date most genetic modification work involving retroviruses has involved the development of transduction vectors derived from competent oncogenic retroviruses and lentiviruses. Many such retroviral transduction systems are manipulated in cDNA form and give rise to defective vectors. It is important to consider the hazards posed by the virus from which these vector systems are derived in order to make an accurate assessment of the risks posed to human health.

Wild-type retroviruses fall into a range of ACDP hazard groups (see Table 2.11.2). The appropriate containment level should be adopted as a minimum requirement when handling wild-type viruses. Please note that this risk assessment only covers vectors derived from the HG1 and HG2 wild-type retroviruses. The Hazard Groups of some retroviruses is shown in Table 3 (see in accompanying documentation).
Oncogenic retroviral vector systems generally consist of two main components – a transfer vector and a packaging system. The transfer vector is usually a proviral cDNA in which viral coding sequences have been deleted and foreign DNA inserted. The packaging system commonly consists of one or more helper constructs that express viral genes needed to generate infectious viral particles.

The generation of replication competent virus (RCV) and insertional mutagenesis as a result of proviral integration poses major safety issues when handling retroviral vectors. RCV can be generated by recombination events between the vector and the components of the packaging system (including both the packaging constructs themselves and endogenous proviruses present in the cell line used). Proviral integration can result in the activation of cellular genes adjacent to the integration site or insertional disruption of tumour-suppressor functions (features central to oncogenesis by retroviruses not carrying a cellular oncogene). Retroviral vector systems have therefore been developed and refined in order to reduce the likelihood of RCV generation and proviral transactivation. Consequently, there is a range of systems that vary in their safety profile.

Oncogenic Retroviral Vectors.

The majority of these vectors have been derived from competent oncogenic retroviruses, such as ALV, MoMLV and FeLV, that efficiently infect actively dividing cells. “First Generation” retroviral vectors contain a packaging system that is essentially a retroviral cDNA itself, encoding viral gag, pol and env genes but with its packaging sequence deleted. This construct is either cotransfected with the transfer vector, or is stably incorporated into the host-cell chromosomes generating a helper cell line. Such systems are inherently the most hazardous since a single recombination event would be sufficient to generate RCV. The 3' LTR is deleted in “Second Generation” packaging systems, improving biosafety by reducing the possibility that the packaging construct will be mobilised as well as reducing the likelihood of RCV generation, as two recombination events are required. With “Third Generation” systems, the 5’ LTR is also deleted and the packaging sequences are divided between two constructs, with gag/pol encoded by one construct and env by the second. This significantly reduces the likelihood of RCV generation, by increasing the number of recombination events that are required to reconstitute a competent viral genome. Two component packaging systems of this type should be used wherever possible. Additional biosafety can theoretically also be achieved by using self-inactivating (SIN) transfer vectors, although there are some publications suggesting that the use of SIN vectors confers no advantages with respect to cellular transformation (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2835037/).

Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and/or shRNAs or microRNAs (designed to knockdown the expression of open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins) will be expressed in retroviral expression vectors.

Guidance on inserts that may have greater risk of adverse effects on human health can be found in SACGM Compendium of Guidance part 2-2 p37-46.

Examples of such inserts include: "exogenous sequences that may have harmful biological activity (for example toxins, cytokines and growth factors, genes that may alter the growth status of cells (such as oncogenes, cytokines and growth factors), sequences which may be involved in the control of expression of such inserts (for example promoters and control regions) and other products that may have no inherent harmful activity but may have other adverse effects (such as allergens or antigenic proteins)"

Of particular note:

Overexpression of Oncogenes (ref: SACGM Compendium of Guidance part 2-2 p38 sections 6-9).

Genes encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation. Full length cDNA encoding wild type and disease relevant mutants of these types of genes will be expressed in retroviral vectors. Any use of retroviral particles encoding oncogenic inserts will require appropriate controls and operator training.

Examples of genes that may be categorised as oncogenes include:

See Table 1 in accompanying documentation


Knockdown of genes encoding known tumour suppressors can contribute to cellular transformation. However, shRNA expression systems rarely completely silence the targeted gene. shRNAs targeting these types of genes or gene sequences with tumour suppressive functions will be expressed in retroviral vectors. Any use of retroviral particles capable of reducing tumour suppressor expression will require appropriate controls and operator training.

Examples of genes that may be categorised as tumour suppressors include:

See Table 2 in accompanying documentation

Any use of retroviral particles encoding such increased risk inserts will be classed as an AC2 activity, and the appropriate controls should be adhered to. Use of retroviral particles encoding "non-harmful inserts" will be classed as an AC1 activity.
Evaluation of foreseeable effects

Potential hazards associated with the recipient host:

These will vary depending on the cell type and can be known or unknown. Potential common pathogens might be human pathogens such as Epstein Barr virus or rhinovirus. Primary human samples are screened for the presence of the high risk human pathogens hepatitis virus and HIV by the supplier. Samples containing such pathogens should not be used.

Note: Hazard Group 2 cells and Activity class 2 cells present a greater risk than Hazard Group 1 cells, usually due to their potential to harbour human pathogens.

Potential hazards arising from the gene product:

The expression of the majority of genes is predicted to generally have little or no adverse effect. However, the precise outcome is difficult to predict. Expression of proto-oncogenic derivatives of these genes will transform normal epithelial and fibroblast cells and potentially enhance proliferation and tumourigenesis of cancer cell lines. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines many of which harbour mutations are present in human colon carcinoma cell lines DLD-1, LoVo and HCT116; human prostate cancer cell lines LNCaP and PC-3; human lung cancer cell lines Calu-6 and SKLU-1; and human pancreatic cancer cell line MIAPaCa2, A549 lung cancer cells, SW480. Knockdown of tumour suppressor genes may contribute to cellular transformation of normal epithelial and fibroblast cells and potentially enhance proliferation and tumourigenesis of cancer cell lines. Multiple cancer cell lines already in culture have mutations or are null for tumour suppressor genes (esp. TP53, PTEN and TSC1 and 2). Cells expressing shRNA against these gene products pose no greater risk than Hazard group 1 or 2 cancer cell lines many of which already harbour mutations within one or more of these genes. For example, LnCAP, PC3, 87Mg all have PTEN mutations or are pten null.

Potential hazards arising from the combination of gene and host:

Guidance from SACGM compendium of guidance: Viruses and viral vectors with a human host-range carrying potentially oncogenic sequeces or shRNAs targeting tumour suppressor genes may pose risks to human health and safety over and above those hazards associated with the recipient strain itself. The potential outcomes of downregulating particular targeted genes should be carefully assessed. For example, knocking down the expression of a tumour suppressor gene in a mamalian cell could contribute to cellular transformation. However, one of the features of RNAi knockdown systems is that the targeted gene is rarely completely silenced. Indeed, it is not unusual for there to be varying degrees of target gene downregulation within an experimental population and therefore it is important to consider the ramifications of heterogeneous expression of the targeted genes. In particular, the use of virus vector strains that are capable of modifying host chromatin (eg retroviruses and lentiviruses) represent a hazard to the operator and appropriate controls will be required.

Potential for transfer of gene to other hosts:

Retroviral particles can infect almost all human cells (except non-dividing cells). However, the recombinant retroviral vectors used to generate viral particles are replication-deficient in that they are unable to replicate and do not express any of their endogenous genes. Once within the mammalian cell no viral particles would be produced. The virus could not therefore be transferred to another host. Once exception to this could be where an individual's genome contains endogenous viral genes through a previous retroviral infection that then recombine with the genes delivered by the retrovirus to produce replicative-competent virus. This scenario is very unlikely, especially with the separation of viral genes as exploited in 2nd generation and above systems.

One risk that should be considered is the subsequent addition of a retroviral packaging mix to cell lines that have already been manipulated though retroviral transduction, which has the potential to mobilise the integrated genes. This includes the use of the MembranePro reagent for the generation of virus-like particles. The risk is that the cells will produce infectious (but replication-deficient) retrovirus particles, so these cell lines (and the conditioned media) would need to be handled with the considerations afforded to the conditions/cell lines used to generate retroviral vectors, and retroviral particles.

Potential for harm to human health in the event of exposure:
The only potential for harm to human health could be through direct exposure to or injection of the recombinant retroviral particles. However, even with injection the immune system would most likely recognise this as foreign and would mount an immune defensive response. Recipient strains that are able to infect or colonise human hosts should be avoided i.e. work should not be performed on one's own cells or those of other laboratory staff. The particles are unable to penetrate through gloves and would be unlikely to penetrate the dead layers of skin cells.

Potential to disseminate and harm the environment in the event of accidental release:

Retroviral vectors do not pose any identifiable risk to the environment because the reagent is unable to replicate unless supplied with helper plasmids and hence they cannot replicate outside of laboratory conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None at this stage, although waste derogations may be sought should we intend to scale up in the future.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste is inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04) or where inactivation is not possible as Hazardous Biological waste (EWC 18 01 03). Inactivation will be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee agreed that retroviral transduction of cell lines was unlikely to result in a classification above AC2, providing the host cell lines did not contain HG3/4 adventitious agents. The AC2 classification was accepted. Note: Host cells are always checked by individual risk assessments to establish whether HG/AC 1/2.

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Purposes of the contained use

Generation and use of isogenic cell lines generated with replication-incompetent adeno-associated viruses (AAV) containing human genomic DNA as a method of altering endogenous gene expression in human cell lines.

Cell lines, containing endogenous exons of the gene of interest will be replaced by exons encoded in the designed pAAV vector resulting in changes at the endogenous locus causing either:

1. Exon loss to knock-out gene function
2. Point mutations (coding/noncoding)
3. Introduction of a SNP/small deletion/small insertion into the endogenous gene
4. Complex modifications e.g translocations and amplifications
5. Tagged version of the endogenous gene using a reporter gene e.g. luciferase/SNAP Tag

The goals of this work will allow assessment of target biology and creation of new models for use in oncology and other therapeutic areas.

Adeno-associated viruses (AAVs) are replication-deficient paroviruses, which have traditionally required co-infection with a helper adenovirus or herpes virus for productive infection. The AAV Helper-Free System allows the production of infectious recombinant human adeno-associated virus (AAV) virions without the use of a helper virus. The AAV Helper-Free System takes advantage of the identification of the specific adenovirus gene products that mediate AAV replication and the demonstration that these gene products can be introduced into the host cell by transfection.

In the AAV Helper-Free System, most of the adenovirus gene products required for the production of infective AAV particles are supplied on the helper plasmid, pHelper (i.e. E2A, E4, and VA RNA genes), that is co-transfected into cells with human AAV vector DNA. The remaining adenoviral gene product is supplied by the AAV-293 host cells, which stably express the adenovirus E1 gene.2

By eliminating the requirement for live helper virus the AAV Helper-Free System provides a safer, purer and more convenient alternative to retroviral and adenoviral gene delivery systems. In the targeting vector the sequence between the ITR's can be customised to create specific genetic modifications.

There will be 3 main types of GMO created:
• GMO's containing knock-out of gene function by exon loss/modification - genomic DNA surrounding the region to be deleted and insertion of a selection cassette to knock-out gene function
• GMO's containing insertion of a SNP/small deletion/small insertion into the endogenous gene - genomic DNA encompassing the region containing the SNP/small deletion/small insertion of interest and a selection cassette
• GMO's containing a tagged version of the endogenous gene using a reporter gene e.g. luciferase-genomic DNA encompassing the region to be targeted, a reporter gene and a selection cassette.

In every case, the complete gene from the endogenous locus is not overexpressed or contained in the targeting vector. The technology specifically targets the endogenous gene locus and therefore we only the region of interest is targeted. For example, if we want to create a point mutation in exon 1 of a gene we do not need to insert a complete cDNA with the exon 1 mutation; we just need to insert the mutated version of exon 1 with surrounding genomic DNA.

Recipient or parental organism

Mammalian cell lines including Hazard Group 1 and 2 and Activity Class 1 and 2 genetically modified cells all of which are covered by appropriate risk assessments.

Host/vector system

AAV Helper-Free Systems - Details & Specifications

The first step is cloning the gene of interest into an appropriate plasmid vector. For most applications, the DNA of interest is cloned into one of the ITR vectors (pAAV-MCS). The inverted terminal repeat (ITR) sequences present in these vectors provide all of the cis-acting elements necessary for AAV-2 replication and packaging. The recombinant expression plasmid is co-transfected into the AAV-293 cells with helper plasmids encoding adenovirus-derived genes and AAV-2 replication and capsid genes, which together supply all of the trans-acting factors required for AAV replication and packaging in the AAV-293 cells. Recombinant AAV-2 viral particles are prepared from infected AAV-293 cells and may then be used to infect a variety of mammalian cells.

Origin & function

Guidance on expression of inserts that may have greater risk of adverse effects on human health can be found in SACGM compendium of guidance part 2-2 p37-46

Of particular note:

Oncogenes (ref: SACGM compendium of guidance part 2-2 p38 sections 6-9).
Genes encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation. Exons encoding wild type and disease relevant mutants of these types of genes will be expressed in AAV vectors, but not over-expressed.
Knockout of tumour suppressors (ref: SACGM compendium of guidance part 2-2, p42, paragraph 24).
Knockout of genes encoding known tumour suppressors can contribute to cellular transformation. The level of knockout of tumour suppressor will be specific to each cell line.

Any use of AAV capable of increasing expression of potential oncogenes or reducing tumour suppressor expression will require appropriate controls, operator training and a separate risk assessment.

Evaluation of foreseeable effects

Potential hazards associated with the recipient host:
These will vary depending on the cell type and can be known or unknown. Primary human samples are usually screened for the presence of high risk human pathogens such as hepatitis virus and HIV by the supplier although this may not always be the case. Unscreeded samples can be safely used at CL2 as long as they are not high risk samples or have been tested and shown to contain human pathogens.

Potential hazards arising from the gene product:
The knockdown of or increased expression of the majority of genes is predicted to generally have little or no adverse effect. However, the precise outcome is difficult to predict. Knockdown of tumour suppressor genes may contribute to cellular transformation of normal epithelial and fibroblast cells and potentially enhance proliferation and tumourigenesis of cancer cell lines. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines many of which harbour mutations within these genes.

Potential hazards arising from the combination of gene and host:
AAV gene transfer is one of the most reliable methods for introducing genes into mammalian cells. Following infection, there may be transient expression of the partial gene
product at high levels since many cells receive multiple copies of the recombinant genome, but this will not be full length cDNA. This transient expression is rapidly lost and in most cases there is not a promoter to drive expression of the gene fragment. AAV are capable of infecting a wide variety of proliferating and quiescent cell types from many different animal species including humans, non-human primates, pigs, rodents, mice, and rabbits. Published reports suggest that nearly all human cell types—including skin, muscle, bone, nerve, and liver cells—are susceptible to infection by AAV but it is limited to dividing cells. Recombinant AAV cannot recombine to form replication competent AAV. There are few risks that arise from the combination of gene and host that are not already present in the host cell line. Potential for transfer of gene to other hosts: There is no evidence that when the inserted genetic material recombines with the host cell lines genomic DNA that any sequences of the AAV viral genome are incorporated. In the recombinant AAV viral particles, the only AAV genomic sequences present are the ITR’s and there is no evidence that these are incorporated into the host genome during homologous recombination. There are very few hazards associated with removing gene function, e.g. tumour suppressors, since only one allele of the gene would be targeted rendering the infected cell heterozygous. The tumorigenic effect is only seen when both alleles have been removed. There is low risk associated with producing a tagged version of the endogenous gene using a reporter gene. The highest level of risk comes from introducing SNP/small deletion/small insertion of interest. The introduction of a single mutation in the inserted genetic material provides a low risk of transforming any cell line, potentially becoming oncogenic when recombined into a genes endogenous locus. However, the expression levels of this altered version of the gene will generally be lower as it is expressed under the control of its endogenous promoter. Even if an end-user were to be infected, there is a very low risk of the infected cells being correctly targeted due to the low efficiency of the targeting process without the use of a selection marker. If the cells are correctly targeted, only one allele of the gene would be targeted making the infected cell heterozygous. These cells are very unlikely to be cancerous – at worst they will be one step along the path to transformation of the cells. Potential for harm to human health in the event of exposure: Wild type AAVs are not categorised by ACDP and so are Hazard Group 1. AAVs are defective by nature and are not associated with human illnesses so the hazards to human health can be expected to be low. The AAV system being used (Stratagene Helper-Free AAV system) is a ‘gutless’ system which does NOT require wild-type adenovirus to supply helper functions. The main hazard associated with AAV vectors arises from the inserted genetic material. Potential to disseminate and harm the environment in the event of accidental release: AAV vectors do not pose any identifiable risk to the environment because the reagent is unable to replicate unless supplied with helper plasmids and hence they cannot replicate outside of laboratory conditions. If the project is successful, it would result in in vivo work, in mouse models. All in vivo work will be carried out under Containment Level 2 conditions using rodents. All handling of AAV agents will be in Class II safety cabinets and all treated animals will be kept in separate red-line isolation cages, which are completely sealed with separate negative pressure air supply and HEPA filters on the inlet and outlet. Our building is a designated building, regulated under the Animal (Scientific Procedures) Act 1986 and is therefore designed to prevent escape and to ensure complete containment of animals. Only Home Office-approved operatives, trained in safe handling, will be used to undertake in vivo work, to minimise the risk of infection from potentially infectious adventitious agents from the host cell lines. Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

If the project is successful, it would result in in vivo work, in mouse models. All in vivo work will be carried out under Containment Level 2 conditions using rodents. All handling of AAV agents will be in Class II safety cabinets and all treated animals will be kept in separate red-line isolation cages, which are completely sealed with separate negative pressure air supply and HEPA filters on the inlet and outlet. Our building is a designated building, regulated under the Animal (Scientific Procedures) Act 1986 and is therefore designed to prevent escape and to ensure complete containment of animals. Only Home Office-approved operatives, trained in safe handling, will be used to undertake in vivo work, to minimise the risk of infection from potentially infectious adventitious agents from the host cell lines. For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste is inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04). Inactivation will be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

No non-inactivated waste will be sent off site.

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Since some of the inserts are potentially hazardous and may be inserted into infectious hosts, the committee decided that this should be classified as AC2.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Project Ref** 387/14.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>26/08/2014</td>
<td>Culture of EndoC-BetaH1 cell line and use in in vitro experimental procedures</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

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Project notified under transitional arrangements N
The cells will be used in a range of in vitro assays to characterise the target and investigate the activity of potential drugs.

No new GMOs will be created. Human foetal pancreatic buds were transduced with a lentiviral vector that expressed SV40LT under the control of the insulin promoter. SV40LT-expressing beta cells proliferated, formed insulinomas, and were then transduced with human telomerase reverse transcriptase (hTERT). "A genetically engineered human pancreatic beta cell line exhibiting glucose-inducible insulin secretion. Ravassard et al., J Clin Invest. 2011; 121(9):3589–3597 doi:10.1172/JCI58447".

Active protein is required to give the transformed phenotype of the cells. The virus stocks used to generate cell lines have all tested negative for the presence of replication competent particles hence the cell lines cannot release lentiviral particles. The lines have been passaged numerous times since their generation and would therefore be expected not to expressing vector particles themselves. No work with the lentivirus itself will be carried out at MedImmune.

In 2013 it was discovered by the collaborator that the EndoC-betaH1 cell line was contaminated with Murine Xenotropic retrovirus BXV1. Bxv1 endogenous MLV is produced as an active virus and is prevalent in the most common mouse lines e.g. C57/B16, BALB/C & DBA but also found in some cell lines e.g. RAW 264, hybridomas & VCAP. Bxv1 virus is on the HSE approved list of biological agents as a human pathogen hazard group 2 agent and therefore procedures must be performed at containment level 2.

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<tr>
<td>Mammalian virus pTrip RIP TcDNA, pTrip RIP hTERT LoxP and pTrip RIP TcDNA LoxP. All vectors used are not mobilisable and do not contain the gag, pol or env. genes. All have tested negative for the presence of replicative competent particles.</td>
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<td>SV40 large T antigen under control of the rat insulin II promotor. The cells will be used in a range of in vitro assays to characterise the target and investigate the activity of potential drugs</td>
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Potential hazards arising from the gene product:
SV40 Large T antigen originates from the SV40 virus. SV40 large T antigen is a multifunctional protein, essential for viral DNA replication and for regulation of viral gene expression. Reported functions of this protein include ATPase and helicase activities. p53s binding by the SV40 large T antigen may be involved in mediating the oncogenic action of this virus. In order to interact with p53, it would need to be delivered to the nucleus. If expressed however, the SV40 fragment may be a biologically active substance, and is potentially oncogenic which might have a deleterious effect if delivered to a target tissue. The SV40 large T antigen is under the control of the rat insulin II promoter to restrict expression to the beta cell. The hTERT is the human Telomerase Reverse Transcriptase. This gene has been extensively used in association with SV40LT to immortalise human cells. It will prevent shortening of human telomeres and therefore will be useful to prevent senescence of the cell line and thus can be crucial for establishment of stable cell lines.

Potential hazards arising from the combination of gene and host:
Host cells are human derived cells and should be cleared by normal immune response however the contaminating mouse Bxv1 virus is on the HSE approved list of biological agents and is therefore classified as human pathogen Hazard Group 2 agent.

Potential for transfer of gene to other hosts:
The lentivirus stocks used to generate cell lines have all tested negative for the presence of replication competent particles hence the cell lines cannot release lentiviral particles. The lines have been passaged numerous times since their generation and would therefore be expected not to be expressing lentivirus vector particles themselves. No work with the lentivirus itself will be carried out at MedImmune

Potential for harm to human health in the event of exposure:
The lentivirus stocks used to generate cell lines have all tested negative for the presence of replication competent particles hence the cell lines cannot release lentiviral particles. The lines have been passaged numerous times since their generation and would therefore be expected not to be expressing lentivirus vector particles themselves. No work with the lentivirus itself will be carried out at MedImmune

The contaminating component Bxv1 endogenous MLV is classified as Hazard Group 2 therefore can cause human disease and may be a hazard to employees; it is unlikely to spread to the community and there is usually effective prophylaxis or treatment available. It also has the potential to infect other cell lines.

Potential to disseminate and harm the environment in the event of accidental release:
Accidental spill of the contaminating component Bxv1 endogenous MLV would not harm the environment as viral particles could not survive outside of the lab environment. Correct disposal of treated cell waste will kill host cells and therefore destroy associated viral particles.

To prevent contamination of other cell lines by Bxv1 endogenous MLV we suggest a 24 hour exclusion from labs working on drug product expressing cell lines for those who have handled EndoC-BetaH1 cell line as a precaution.

8.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste is inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04) or where inactivation is not possible as Hazardous Biological waste (EWC 18 01 03). Inactivation will be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

In addition: Correct disposal of treated cell waste will kill host cells and thus destroy associated viral particles.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

EndoC-betaH1 cell line is contaminated with Bxv1 endogenous MLV which is produced as an active virus. The contaminating component Bxv1 endogenous MLV is classified as a human Hazard Group 2 agent and therefore can cause human disease and may be a hazard to employees; it is unlikely to spread to the community and there is usually effective prophylaxis or treatment available. It also has the potential to infect other cell lines if not correctly handled at Containment Level 2.

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Project Ref 387/14.2

Date Ackn’d 01/09/2014

CU2 Project Title

The culture and use of recombinant strains of Vaccinia Virus (VACV), an orthopoxvirus, generated by reverse engineering for in vitro cell-based assays, in vivo use and ex vivo analysis

Date Project Ceased 02/03/2022

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

02/03/2022 Page 6979 of 15326
Project Additional Information

Purposes of the contained use

To use recombinant vaccinia, an oncolytic virus, to selectively infect mammalian cells in vitro and/or in vivo to assess oncolytic potency in tumour and normal cells and to analyse levels of viral replication, selectivity of infection, oncolytic potency, viral cell viability and mechanisms of tumour cell lysis.

Recipient or parental organism

Recombinant oncolytic VACV will be used to infect tumour and normal cells.
VACV is a replication competent oncolytic virus that can efficiently infect a wide range of target cells, however viral engineering has rendered some of the rVACV variants tumour selective. The experimental work proposed here involves the addition of VACV particles encoding therapeutic gene sequences to mammalian cell lines (Hazard Group 1 and 2 and Activity Class 1 and 2 cells) in vitro and the direct administration in vivo. Following the transduction of the mammalian cell lines with rVACV, RNA and protein will be extracted from the cells to determine expression levels of viral RNA, target gene RNA or gene product and functional assays will be performed.
Following the direct administration of rVACV in vivo, animals will undergo monitoring and tumour measurements (as detailed in the project licence) and/or tissues/cells will be harvested and used in downstream functional assays.

Host/vector system

Hosts: The host organism would be normal and cancer cell lines of human and mouse origin. These would include Hazard Group 1 and 2 cell lines and Activity Class 1 and 2 genetically modified cells all of which are covered by appropriate risk assessments.
Vectors: Recombinant attenuated Western Reserve (WR) or Lister strains of Vaccinia are the backbone vectors for carrying the genes and are covered by appropriate risk assessments. Attenuation mechanisms are outlined briefly below and fall under the categories of Disabled and attenuated vectors and Conditionally replicative vectors (outlined in SACGM Compendium of Guidance, Section 2.10, p106-107). Various permutations of VACV have been created and attenuated for virulence by the deletion of the following genes, alone or in combination:
o Thymidine Kinase (TK): TK deletion from VACV engenders enhanced specificity for tumour cells. TK-deleted VACV is highly attenuated in non-dividing cells but is able to replicate robustly in transformed cells.
o Vaccinia Growth Factor (VGF): VGF is a viral ortholog of EGF that encourages increased metabolic activity in cells and promotes viral replication and spread. Deletion of VGF from vaccinia virus severely attenuated viral replication in vivo, and increases the LD50 of the virus by over 2 logs. VACV that are deleted for both VGF and TK appear to replicate specifically in tumour cells.
B18R: B18R is a Type I IFN decoy receptor encoded by VACV. Deletion of the B18R gene enables infected cells to generate a stronger type I IFN response in response to VACV infection and protects surrounding nontransformed cells from viral-mediated cell killing by eliciting the antiviral state. Tumour cells often have defects in the type I IFN response, rendering them still susceptible to oncolysis by B18R deleted VACV.

Origin & function

Specific Open reading frames, cDNAs and gene sequences encoding therapeutic gene sequences or therapeutic proteins (e.g. cytokines and antibodies) and/or si/shRNAs or miRNAs (designed to knockdown the expression of cancer causing genes) and reporter genes can be inserted into rVACV. An example of a transgene insert includes (but is not limited to) immune modulating cytokines such as human or mouse granulocyte-macrophage colony-stimulation factor (GM-CSF), a cytokine for stimulating proliferation of haematopoietic stem cells which would augment the anti-tumour and anti-viral immune response.

Guidance on inserts that may have a greater risk of adverse effects on human health can be found in SACGM compendium of guidance, Part 2-2, p37-46. Inserts will generally have a therapeutic effect.

Evaluation of foreseeable effects

Potential hazards associated with the recipient host:
These will vary depending on the cell type used and can be known or unknown. Potential common pathogens might be human pathogens such as Epstein Barr virus or rhinovirus. Primary human samples are screened for the presence of the high risk human pathogens: hepatitis virus and HIV by the supplier. Samples containing such pathogens should not be used.

Note: Hazard Group 2 cells and Activity Class 2 cells present a greater risk than Hazard Group 1 cells, usually due to their potential to harbour human pathogens.

Potential hazards arising from the gene product:
The expression of the majority of genes is predicted to generally have little or no adverse effect. However, the precise outcome is difficult to predict. The work outlined in this assessment covers therapeutic transgenes and gene sequences expressed by rVACV. By their very nature these will have a therapeutic effect. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines and expression of a therapeutic transgene will often result in cancer cell death or arrest and render the cells more immunogenic in vivo which would result in greater clearance.

Potential hazards arising from the combination of gene and host:
The knockdown of, or increased expression of, the majority of genes is predicted to generally have little or no adverse effect. Gene products will only ever be expressed in cells that are able to support viral replication (tumour cells) and the greater the expression levels, the more likely the cell is permissive to viral replication and will ultimately die due to this property. Cells expressing these gene products pose no greater risk than Hazard Group 1 or 2 cancer cell lines, many of which harbour mutations within these genes or expressing these genes through other delivery vectors (adeno- or lentiviral transduction). Enhanced tumour cell killing is a beneficial additional effect of combination of host and gene.

Potential for transfer of gene to other hosts:
Vaccinia can infect many cells, although the majority of variants used here are disabled/attenuated and conditionally replicative vectors, i.e. replicate selectively in tumour cells. Normal cells will not support viral replication and the virus will be inactivated. In cancer cells where replication is permissible the virus will be propagated and will result in cell lysis and death and release of virus which will go on to infect other surrounding cells. By their very nature, the replication component virus cells that support viral replication will ultimately die due to viral replication and the level of viral replication will drive the level of the therapeutic transgene which will also enhance immune clearance in vivo or cell killing. Vaccinia replication takes place in the
cytosol so there is no chance of stable integration or recombination with the host genome. Additionally many of the variants that will be used are disabled and attenuated vectors. Poxviruses have a large number of genes, many of which are dispensable for growth in vitro and cause attenuation when disrupted.

Potential for harm to human health in the event of exposure:

Typical characteristics of the poxvirus family include a large dsDNA viral genome (varying from 130 to 300kb in size) which is enclosed in a multi-membrane virion, making them some of the largest known viruses. Replication takes place within the cytoplasm of permissive cells and all the enzymes required to initiate viral gene transcription are carried within the virion. Other general features include the induction of virus containing pustular, epidermal lesions, although the severity of the disease is dependent on the host organism and poxvirus species. (Ref: SACGM, Table 2.10. Host range of poxviruses and the typical symptomatic consequences of infection. *Less common adverse reactions to Vaccinia virus inoculation in humans, p104).

VACV normally has no serious health effects in humans, although it can cause disease of the skin when used as a vaccine vector. Vaccinia virus is usually injected in the dermis where a localised lesion appears (a “take”) and then scabs over and heals in about 10-14 days. The vaccination is accompanied by fever, rash, lymphadenopathy, fatigue, myalgia and headaches in some patients. Accidental infection with the virus can occur through contact between the vaccination lesion and broken skin (inadvertent inoculation). Serious complications such as ocular vaccinia, myopericarditis, eczema vaccinatum (a papular, vesicular and pustular rash that is very infectious, 38 cases per million doses), progressive vaccinia (progressive necrosis at the vaccination site, 3 cases per million doses), postvaccinal CNS disease (headache, lethargy, seizures and coma, 12 cases per million doses), foetus malformations and abortion (very rare) sometimes occur after vaccination. Complications are more serious in immunosuppressed individuals and the smallpox vaccine usually causes one death for every million doses.

Contraindications to vaccine are their use in immunocompromised individuals, individuals with certain skin (e.g. eczema) and cardiac diseases and pregnant women. However it must be noted that VACV is a poxvirus that has an extensive safety record and has been used widely as a vaccine vector in vaccination campaign for the irradication of smallpox and is therefore considered to pose minimal risk to human health.

Vaccinia virus may cause disease in situations whereby immunity may be reduced, i.e. during pregnancy, in people with active skin disorders such as eczema or psoriasis, or in immuno-compromised individuals such as those infected with HIV, those individuals who have undergone splenectomy or are on high dose steroid treatment or immune suppressive chemotherapy. It is well documented that vaccinia can be passed to close contacts of vaccine recipients generally with little adverse consequence. Therefore, although an individual with a laboratory-acquired infection is unlikely to receive the virus dose given for vaccination purposes, close contacts, particularly those with contraindications for vaccination, may also be at risk.

The viruses that will be used in this assessment do not cause full blown Vaccinia infection but tend to cause more localised infections such as a pustule or blister (with or without necrosis and scarring) particularly on the hands, mouth or around the eyes. It is possible for one infected area to contaminate and infect another (e.g. finger to eye). A wide range of photographs of Vaccinia virus infections are available at: www.bt.cdc.gov/training/smallpoxvaccine/reactions/default.htm and by searching for “smallpox vaccine” or Vaccinia virus on the internet. SHE should maintain a list of people who work with Vaccinia and all people that work with Vaccinia will be given a letter for their family doctor to be kept with their general practice notes, to prompt their general practitioner to consider Vaccinia if they develop such a blister or sore.

Potential to disseminate and harm the environment in the event of accidental release:

Poxviruses are highly stable and resistant to dehydration; infectious virus can be stored in dried powder form. Transmission is usually via aerosol or direct contact and infectious virus can survive for protracted periods in dried scab material shed from epidermal lesions. In the event of any release into the environment, genetically modified poxviruses might persist and could be transmitted to other humans or animal species.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All in vivo work will be carried out under Containment Level 2 conditions using rodents. All handling of rVACV agents will be in Class II safety cabinets and all treated animals will be kept in separate red-line isolation cages, which are completely sealed with separate negative pressure air supply and HEPA filters on the inlet and outlet. Our building is a designated building, regulated under the Animal (Scientific Procedures) Act 1986 and is therefore designed to prevent escape and to ensure complete containment of animals. Only Home Office-approved operatives, trained in safe handling, will be used to undertake in vivo work, to minimise the risk of infection from potentially infectious adventitious agents from the host cell lines.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste is inactivated prior to disposal as Non-hazardous Biological waste (EWC 18 01 04) or where inactivation is not possible as Hazardous Biological waste (EWC 18 01 03). Inactivation will be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

It is well known that Vaccinia and other poxviruses have the capacity to survive for considerable periods in dried material such as detached vaccination scabs, but it is less well appreciated that survival in aqueous solutions can be for several weeks. Live virus can also be isolated from solid surfaces and fabric for as long as two weeks after contamination. For laboratory workers, ingestion, inoculation via needles or sharps, and droplet or aerosol exposure of mucous membranes or broken skin are possible routes of infection. Laboratories working with Vaccinia and other poxviruses should have suitable local rules to control these potential sources of infection, including suitable procedures for decontamination of equipment and surfaces.

It is imperative that strict hygiene is adhered to, including hand-washing after work and the use of an alcohol gel to limit transfer. All door handles and equipment must be wiped down with 20% Trigene Advance as a precautionary measure.

Laboratory coats should be disposable or will require to be washed at a high temperature (at least 60°C) or autoclaved frequently as VV is relatively heat resistant, can survive for long periods on fabrics, and has been shown to transfer from fabrics to other objects by direct contact.

Other information

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 0.02% sodium hypochlorite, 30% isopropanol, 40% ethanol, 0.02% glutaraldehyde, 0.01% benzalkonium chloride, 0.0075% iodine, 30% Sanytex and 0.0075% ortho phenylphenol.

The virus is resistant to solvent/detergent combinations (TNBP/Triton X-100 and TNBP/ Tween 80) and longer incubation periods (between 10 minutes and 24h depending on the solvent/detergent used) are necessary to inactivate the virus.

PHYSICAL INACTIVATION: The virus is inactivated by dry heat at 95 ºC for 2 hours. The heat-sensitive fraction of the virus is inactivated by moist heat at 60 ºC while the heat-resistant fraction may take higher temperatures to fully inactivate it. The virus in its aerosol form is also sensitive to UV light (254 nm).

SURVIVAL OUTSIDE HOST: The dried virus can survive up to 39 weeks at 6.7% moisture and 4°C

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The Biosafety Committee asked the author to expand on the goals, give an explanation as to why they were doing this work, is it oncolytic, and is it being used for tumour cell lines. The Committee agreed this would be AC2 and therefore require an HSE notification.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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Animal Units

<table>
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<th>Human Clinical Applications</th>
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Project Ref 387/16.1

Date Ackn’d 14/01/2016

CU2 Project Title Culture and use of Klebsiella pneumoniae genetic knockout and reporter strains for in vitro assays

Class 2

CultureVolume Class 3-4 < 1 Litre

Non-GMM Consent Granted

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

Klebsiella pneumoniae strains, including genetic knockout strains, will be cultured for use in phage display cell selections to isolate antibodies or antibody mimetics to cell surface antigens. Candidate antibodies and/or mimetics will subsequently be tested in in vitro screens, including phage ELISA, in vitro cell binding assays and opsonophagocytic killing assays.

K. pneumoniae, including capsular polysaccharide knockout (e.g. deltapCpSB) and lipopolysaccharide (LPS) O-antigen knockout (e.g. deltaWaaL) strains (allelic replacement with an antibiotic resistance gene), will be cultured with and without antibiotics for use in phage display cell selections to isolate antibodies or antibody mimetics to cell surface antigens. It is proposed that capsular polysaccharide and LPS O-antigen knockout strains will improve access to cell surface antigens by depleting cell surface carbohydrates. Candidate antibodies and/or mimetics will be tested in in vitro cell binding assays for cross-reactivity to wild-type K. pneumoniae strains.

Subsequently, candidate antibodies and/or mimetics will be further characterised in in vitro assays and assayed in an opsonophagocytic killing assay, utilizing K. pneumoniae reporter strains, e.g. luciferase.

Recipient or parental organism

K. pneumoniae is listed on the HSE’s Approved List of Biological Agents as a Hazard Group 2 bacteria, hence it has also been assessed as Hazard Group 2 in biologic risk assessment B605. It primarily poses a risk to immunocompromised individuals. Many strains have the ability to produce extended-spectrum beta lactamases (ESBL) and are resistant to many classes of antibiotics such as aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and trimethoprim/sulfamethoxazole. This work proposes to use GMOs derived from cultured strains available from American Type Culture Collection (ATCC) and National Collection of Type Cultures (NCTC). Examples include but are not restricted to: K. pneumoniae ATCC 43816, K. pneumoniae NCTC 9135, K. pneumoniae NCTC 9181, K. pneumoniae NCTC 9187 and K. pneumonia NCTC 11357.

Host/vector system

Vector:

pDMS 197 - a non-mobilisable suicide vector containing an R6K origin of replication, an antibiotic resistance gene (e.g. Gentamicin) and a sacB gene (Edwards et al., 1998, Gene: 207:2, 149-157). It integrates into the host chromosome and subsequent removal of the vector DNA, including the R6K origin of replication and the sacB gene, results in replacement of the wide-type allele with a mutant allele, such as the Gentamicin resistance gene.

Origin & function

Selectable antibiotic resistance markers - examples (but not restricted to):

- Gentamicin resistance: bacterial derived aminoglycoside phosphotransferase gene.
- Blasticidin resistance: bsd or bsr (blasticidin S deaminase) gene isolated from Aspergillus.
- Hygromycin B resistance: acts as an aminocyclitol to inhibit protein synthesis by disrupting translocation and promoting mistranslation.
- Puromycin resistance (PAC): Puromycin acetyl transferase is derived from Streptomyces alboniger.
- Luciferase reporter gene: bacterial derived luxCDABE operon. The bacterial luciferin-luciferase system is encoded by a set of genes labelled the Lux operon. Five genes (LuxCDABE) are active in the emission of visible light.
Evaluation of foreseeable effects

Potential hazards associated with the recipient host:
K. pneumoniae is categorized as a Hazard Group 2 bacteria, by the HSE, and predominantly poses a risk to immunocompromised individuals and those that are hospitalised. Drug resistant isolates are increasing with current evidence implicating plasmids as the primary source of the resistance genes (Hudson et al., 2014, PLoS One, 9:6, e99209). There is the potential but very low risk of drug-resistance genes from K. pneumoniae being transferred to laboratory strains of E. coli. K. pneumoniae is commonly present within environmental flora and fauna and poses limited risk to healthy individuals (Podschun & Ullmann, 1998, Clin Microbiol Rev: 11:4, 589-603).

Potential hazards arising from the gene product:
The luciferase reporter gene does not pose a risk to human health or the environment. It has been successfully used for over 10 years without report of adverse events.
The antibiotic resistance gene product does not pose a risk to human health or the environment. However, if the gene was transferred from K. pneumoniae to another human pathogen it could compromise the clinical use of that antibiotic. This transfer is extremely unlikely as the resistance gene is stably integrated into the host chromosome.

Potential hazards arising from the combination of gene and host:
There are no additional hazards identifiable from the combination of these genes (antibiotic resistance and luciferase reporter) and K. pneumoniae. With regard to the knockout antibiotic resistant strains, these have been created to knockout capsular polysaccharide and LPS O-antigen. It has been reported that capsular polysaccharide knockout mutants are severely attenuated and unable to cause pneumonia or urinary tract infections (Tomas et al., 2015, JBC, 290:27, 16678-16697). LPS O-antigen knockout mutants also have reduced virulence (Hsieh et al., 2012, PLoS One, 7:3, e33155).

Potential for transfer of gene to other hosts:
There is the potential but very low risk of genes from K. pneumoniae being transferred to other hosts. However, this risk is extremely low for the antibiotic and luciferase reporter genes due to their non-mobilisable nature and integration into the host chromosome.

Potential for harm to human health in the event of exposure:
K. pneumoniae is primarily an opportunistic pathogen and infections are predominantly seen in immunocompromised individuals, particularly those with impaired respiratory defenses, i.e. diabetics, alcoholics and those suffering from malignant and obstructive pulmonary diseases. Infections are primarily hospital acquired (nosocomial) but outside hospital the most common conditions caused by K. pneumoniae are pneumonia, typically in the form of bronchopneumonia and bronchitis, and urinary tract infections. For the luciferase reporter strains this risk remains. For the knockout antibiotic resistant strains, these have been created to knockout capsular polysaccharide and LPS O-antigen. It has been reported that capsular polysaccharide knockout mutants are severely attenuated and unable to cause pneumonia or urinary tract infections (Tomas et al., 2015, JBC, 290:27, 16678-16697). LPS O-antigen knockout mutants also have reduced virulence (Hsieh et al., 2012, PLoS One, 7:3, e33155).

Potential to disseminate and harm the environment in the event of accidental release:
K. pneumoniae is commonly present within environmental flora and fauna and poses limited risk to healthy individuals (Podschun & Ullmann, 1998). It can persist in the environment and also has the potential to infect the majority of mammalian species.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All Class 2 GMO waste must be inactivated prior to disposal as Non-Infectious Healthcare Waste (EWC 18 01 04) or where inactivation is not possible as Infectious Healthcare Waste (EWC 18 01 03). Inactivation should be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

Note: Where inactivation is not possible, the HSE will be contacted for derogation. It is not anticipated that this would be likely.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

K. pneumoniae is listed on the HSE’s Approved List of Biological Agents as a Hazard Group 2 bacteria, hence it has also been assessed as Hazard Group 2 in biologic risk assessment B605. The genetic modification of K. pneumoniae to express either antibiotic resistance or a luciferase reporter gene does not alter the risk associated with the recipient host.

Project Containment

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Animal Units

| L2 L3 L4 L2 | L3 L4 L2 | L3 L4 L2 |

Large Scale Activities

| L2 L3 L4 L2 | L3 L4 L2 | L3 L4 L2 |

Human Clinical Applications

Project Ref 387/16.2

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<th>CultureVolumeClass3-4</th>
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<td>17/02/2016</td>
<td>Culture and use of Salmonella enterica subsp. Enterica serovar typhimurium genetic knockout and reporter strains for in vitro assays</td>
<td>Class 2</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

02/03/2022
Salmonella enterica subsp. enterica serovar typhimurium (S. typhimurium), including genetic knockout strains, will be cultured for use in phage display cell selections to isolate antibodies or antibody mimetics to cell surface antigens. Candidate antibodies and/or mimetics will subsequently be tested in in vitro screens, including phage ELISA, in vitro cell binding assays and opsonophagocytic killing assays. 

S. typhimurium, including capsular polysaccharide knockout (e.g. deltaYih) and lipopolysaccharide (LPS) O-antigen knockout (e.g. deltaWaaL) strains (allelic replacement with an antibiotic resistance gene), will be cultured with and without antibiotics for use in phage display cell selections to isolate antibodies or antibody mimetics to cell surface antigens. It is proposed that capsular polysaccharide and LPS O-antigen knockout strains will improve access to cell surface antigens by depleting cell surface carbohydrates. Candidate antibodies and/or mimetics will be tested in in vitro cell binding assays for cross-reactivity to wild-type S. typhimurium. Subsequently, candidate antibodies and/or mimetics will be further characterised in in vitro assays and assayed in an opsonophagocytic killing assay, utilizing S. typhimurium reporter strains, e.g. luciferase.

Recipient or parental organism

S. typhimurium is listed on the HSE's Approved List of Biological Agents as a Hazard Group 2 bacteria and has been assessed as a Hazard Group 2 bacteria in B607v1.

S. typhimurium causes diarrhea and paediatric blood stream infections (bacteremia). Infections are especially common in a hospital setting; many of which can be severe. Patients with severe onset HIV are at the highest risk of infection while malnourished children are the next most susceptible group. Along with these susceptible groups, other links have been found between those suffering from malaria, sickle cell anemia, and people recently treated with gastric acid suppression. It poses limited risk to healthy individuals. This work proposes to use GMOs derived from cultured strains available from (but not limited to) American Type Culture Collection (ATCC) and National Collection of Type Cultures (NCTC).

Host/vector system

pACYC177 - a non-mobilisable plasmid vector containing the p15A origin of replication, an ampicillin antibiotic resistance gene and a kanamycin resistance gene. It integrates into the host chromosome and results in allelic replacement.
Selectable antibiotic resistance markers - examples (but not restricted to):
- Gentamicin resistance: bacterial derived aminoglycoside phosphotransferase gene.
- Blasticidin resistance: bsd or bsr (blasticidin S deaminase) gene isolated from Aspergillus.
- Hygromycin B resistance: acts as an aminocyclitol to inhibit protein synthesis by disrupting translocation and promoting mistranslation.
- Puromycin resistance (PAC): Puromycin acetyl transferase is derived from Streptomyces alboniger.

Luciferase reporter gene: bacterial derived luxCDABE operon. The bacterial luciferin-luciferase system is encoded by a set of genes labelled the Lux operon. Five genes (LuxCDABE) are active in the emission of visible light.

Potential hazards associated with the recipient host:
S. typhimurium is categorized as a Hazard Group 2 bacteria, by the HSE. It is a rod-shaped, Gram-negative, flagellated facultative anaerobe that is mostly present in the mammalian GI tract. It is a Non-Typhoidal Salmonella serotype that causes diarrhea and paediatric blood stream infections (bacteremia). Acute gastroenteritis is the most common symptom in infected patients. Patients with severe onset HIV are at the highest risk of infection while malnourished children are the next most susceptible group. Along with these susceptible groups, other links have been found between those suffering from malaria, sickle cell anemia, and people recently treated with gastric acid suppression.

Drug resistant isolates are increasing with current evidence implicating plasmids as the primary source of the resistance genes (Garcia et al., 2014, DOI: 10.1371/journal.pone.0089635). There is the potential but very low risk of drug-resistance genes from S. typhimurium being transferred to other laboratory bacterial strains. typhimurium is commonly present within environmental flora and fauna and poses limited risk to healthy individuals.

Potential hazards arising from the gene product:
The luciferase reporter gene does not pose a risk to human health or the environment. It has been successfully used for over 10 years without report of adverse events. The antibiotic resistance gene products do not pose a risk to human health or the environment. However, if the genes were transferred from S. typhimurium to another human pathogen it could compromise the clinical use of those antibiotics. This transfer is extremely unlikely as the resistance gene is stably integrated into the host chromosome.

Potential hazards arising from the combination of gene and host:
There are no additional hazards identifiable from the combination of these genes (antibiotic resistance and luciferase reporter) and S. typhimurium. With regard to the knockout antibiotic resistant strains, these have been created to knockout capsular polysaccharide and LPS O-antigen. It has been reported that capsular polysaccharide knockout mutants have reduced resistance to killing by human serum (Marshall et al., 2015, DOI: 10.1128/IAI.00634). LPS O-antigen knockout mutants also have reduced virulence (Bender et al., 2013, DOI: 10.1371/journal.pone.0073287).

Potential for transfer of gene to other hosts:
There is the potential but very low risk of genes from S. typhimurium being transferred to other hosts. However, this risk is extremely low for the antibiotic and luciferase reporter genes due to their non-mobilisable nature and integration into the host chromosome.

Potential for harm to human health in the event of exposure:
S. typhimurium is a Non-Typhoidal Salmonella serotype that causes diarrhea and paediatric blood stream infections (bacteremia). Acute gastroenteritis is the most common symptom in infected patients. Patients with severe onset HIV are at the highest risk of infection while malnourished children are the next most susceptible group. Along with these susceptible groups, other links have been found between those suffering from malaria, sickle cell anemia, and people recently treated with gastric acid suppression. There is limited risk to healthy individuals.

For the luciferase reporter strains this risk remains. For the knockout antibiotic resistant strains, these have been created to knockout capsular polysaccharide and LPS O-antigen. It has been reported that capsular polysaccharide knockout mutants have reduced resistance to killing by human serum (Marshall et al., 2015, DOI: 10.1128/IAI.00634). LPS O-antigen knockout mutants also have reduced virulence (Bender et al., 2013, DOI: 10.1371/journal.pone.0073287).

Potential to disseminate and harm the environment in the event of accidental release:
S. typhimurium is commonly present within environmental flora and fauna and poses limited risk to healthy individuals. It can persist in the environment and also has the potential to infect the many common mammalian species (cattle, pigs, sheep, horses, rodents and galliformes).

Evaluation of foreseeable effects

Potential hazards associated with the recipient host:
S. typhimurium is categorized as a Hazard Group 2 bacteria, by the HSE. It is a rod-shaped, Gram-negative, flagellated facultative anaerobe that is mostly present in the mammalian GI tract. It is a Non-Typhoidal Salmonella serotype that causes diarrhea and paediatric blood stream infections (bacteremia). Acute gastroenteritis is the most common symptom in infected patients. Patients with severe onset HIV are at the highest risk of infection while malnourished children are the next most susceptible group. Along with these susceptible groups, other links have been found between those suffering from malaria, sickle cell anemia, and people recently treated with gastric acid suppression.

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Potential hazards arising from the gene product:
The luciferase reporter gene does not pose a risk to human health or the environment. It has been successfully used for over 10 years without report of adverse events. The antibiotic resistance gene products do not pose a risk to human health or the environment. However, if the genes were transferred from S. typhimurium to another human pathogen it could compromise the clinical use of those antibiotics. This transfer is extremely unlikely as the resistance gene is stably integrated into the host chromosome.

Potential hazards arising from the combination of gene and host:
There are no additional hazards identifiable from the combination of these genes (antibiotic resistance and luciferase reporter) and S. typhimurium. With regard to the knockout antibiotic resistant strains, these have been created to knockout capsular polysaccharide and LPS O-antigen. It has been reported that capsular polysaccharide knockout mutants have reduced resistance to killing by human serum (Marshall et al., 2015, DOI: 10.1128/IAI.00634). LPS O-antigen knockout mutants also have reduced virulence (Bender et al., 2013, DOI: 10.1371/journal.pone.0073287).

Potential for transfer of gene to other hosts:
There is the potential but very low risk of genes from S. typhimurium being transferred to other hosts. However, this risk is extremely low for the antibiotic and luciferase reporter genes due to their non-mobilisable nature and integration into the host chromosome.

Potential for harm to human health in the event of exposure:
S. typhimurium is a Non-Typhoidal Salmonella serotype that causes diarrhea and paediatric blood stream infections (bacteremia). Acute gastroenteritis is the most common symptom in infected patients. Patients with severe onset HIV are at the highest risk of infection while malnourished children are the next most susceptible group. Along with these susceptible groups, other links have been found between those suffering from malaria, sickle cell anemia, and people recently treated with gastric acid suppression. There is limited risk to healthy individuals.

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Potential to disseminate and harm the environment in the event of accidental release:
S. typhimurium is commonly present within environmental flora and fauna and poses limited risk to healthy individuals. It can persist in the environment and also has the potential to infect the many common mammalian species (cattle, pigs, sheep, horses, rodents and galliformes).
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All Class 2 GMO waste must be inactivated prior to disposal as Non-Infectious Healthcare Waste (EWC 18 01 04) or where inactivation is not possible as Infectious Healthcare Waste (EWC 18 01 03). Inactivation should be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

Note: Where inactivation is not possible, the HSE will be contacted for derogation. It is not anticipated that this would be likely.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

S. typhimurium is listed on the HSE's Approved List of Biological Agents as a Hazard Group 2 bacteria. The genetic modification of S. typhimurium to express either antibiotic resistance or a luciferase reporter gene does not alter the risk associated with the recipient host.

Note: All work with HG2/AC2 infectious agents at our MedImmune Cambridge facilities is undertaken at Containment Level 2, following standard procedures including our Laboratory Code of Practice.

Working with infectious agents takes place in full compliance with Containment Level 2 requirements, including use of Class II safety cabinets to minimise the risk from aerosols and mandatory wearing of PPE (lab coat, safety glasses, gloves) for working with infectious agents.

Project Containment

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Project Ref 387/17.2

Date Ackn'd CU2 Project Title  Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
The generation and use of recombinant adeno associated virus (rAAV) particles for mediating gene modulation in Hazard Group 1 & 2 and Activity Group 1 & 2 cells and their subsequent use in vitro and in vivo.

Project Additional Information

**Purposes of the contained use**

To enable the generation and use of recombinant adeno associated virus (rAAV) particles encoding 1) open reading frames (ORFs), cDNAs or specific gene sequences and 2) short hairpin RNAs (shRNAs) for the knockdown of ORFs, cDNAs or specific gene sequences by RNA Interference (RNAi) and their subsequent use in vitro and in vivo. RNAi is a phenomenon whereby small fragments of double stranded RNA trigger the sequence specific degradation of homologous mRNAs (see http://www.nature.com/focus/rnai for further reading). These vectors will be used to validate literature data and to identify and/or validate ORFs/cDNAs or specific gene sequences as potential therapeutics.

Gene products, cDNAs, ORFs, specific sequences and/or shRNAs will be cloned into available rAAV plasmids, or obtained commercially or from within the broader MedImmune/AZ organisation. These will be packaged into rAAV particles by commercial CROs or packaged in-house using commercially available helper-free systems (e.g., http://www.clontech.com/ES/ProductsNiral_TransductionAAV_Vector_Systems/ibcGetAttachment.jsp?dtemId=93546&fileId=6846850&siteex=10023:22372:US)

rAAV particles encoding genes of interest or shRNAs will then be used to transduce mammalian cells (Hazard Group 1 & 2 and Activity Class 1 & 2) for further studies or administered directly in vivo.

**Recipient or parental organism**

Mammalian and insect cells limited to Hazard Group 1 & 2 and/or Activity Class 1 & 2 genetically modified cells, all of which are covered by appropriate risk assessments. Wild-type AAV are not categorised by the ACDP therefore Containment Level 1 is sufficient.

**Host/vector system**

Wild-type AAV are not categorised by the ACDP therefore Containment Level 1 is sufficient. Wild type AAV have no known link to any human illness. rAAV have a long history of safe use and at the time of writing are being used in 173 clinical trials as gene delivery vectors in man http://www.abedia.com/wiley/vectors.php.
AAV are dependoviruses i.e. are not capable of replication without the presence of a helper infection, such as coinfection with adenovirus or herpes simplex virus. In this work rAAV particles will be prepared using a "helper-free" system in which the genes required for rAAV production are provided in plasmid form rather than from co-infection with another virus.

Wild-type AAV is able to integrate into human chromosome 19 at a specific site. However rAAV encoding genes of interest will be prepared by engineering of a "gutless" AAV vector in which the AAV replication and capsid genes are replaced by the coding sequence of interest, with the replication and capsid genes needed for AAV production being provided by plasmids. This means that the resulting rAAV particles do not possess AAV replication genes themselves and no longer have the ability to integrate into chromosomal DNA. Note that integration into DNA may occur in a passive way at chromosome break points but overall the risk of insertional mutagenesis is low.

To date, 13 natural AAV serotypes have been identified from human and non-human primate sources [Srivastava, A. (2016). In vivo tissue-tropism of adeno-associated viral vectors. Current Opinion in Virology, 21: 75-80.]

http://www.sciencedirect.com/science/article/pii/S1879625716300992]. This work will include the use of all natural AAV serotypes. Given the low pathogenicity of AAV, the major potential hazards posed by rAAV depend on the inserted genetic material.

Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and/or shRNAs (designed to knockdown the expression of open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins) will be expressed using rAAV vectors.

Guidance on inserts that may have greater risk of adverse effects on human health can be found in SACGM compendium of guidance part 2-2 p37-46.

Of particular note:

Overexpression of Oncogenes (ref: SACGM compendium of guidance part 2-2 p38 sections 6-9). Genes encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation.

Full length cDNA encoding wild-type and mutants of these types of genes will be expressed in rAAV. Any use of rAAV particles encoding oncogenic inserts will require appropriate controls and operator training. Examples of genes that may be categorised as oncogenes includes growth factors or mitogens (e.g. c-Sis), receptor tyrosine kinases (e.g. EGFR, PDGFR), cytoplasmic tyrosine kinases (e.g. Src-family), cytoplasmic serine/threonine kinases (e.g. Raf kinase) and their regulatory subunits, regulatory GTPases (e.g. Ras protein), transcription factors (e.g. myc gene) and telomerase complex components (e.g. TR, TERT).

Knockdown of tumour suppressors (ref: SACGM compendium of guidance part 2-2, p42, paragraph 24). Knockdown of genes encoding known tumour suppressors (e.g. TERT, TR).

Potential hazards associated with the recipient host:

Health and Safety
Executive

These will vary depending on the cell type and can be known or unknown. Potential common pathogens might be human pathogens such as Epstein Barr virus or rhinovirus. Primary human samples are screened for the presence of the high risk human pathogens hepatitis virus and HIV by the supplier. Samples containing such pathogens should
not be used. Note: Hazard Group 2 cells and Activity class 2 cells present a greater risk than Hazard Group 1 cells, usually due to their potential to harbour human pathogens.

Potential hazards arising from the gene product:
The expression of the majority of genes is predicted to generally have little or no adverse effect. However, the precise outcome is difficult to predict. Expression of proto-oncogenes and oncogenic derivatives of these genes will transform normal epithelial and fibroblast cells and potentially enhance proliferation and tumourigenesis of cancer cell lines. Cells expressing these gene products pose no greater risk than Hazard group 1 or 2 cancer cell lines many of which harbour mutations within these genes. For example, the T24 bladder cancer cell line has a H-Ras mutation, or K-ras mutations are present in human colon carcinoma cell lines DLD-1, LoVo and HCT116; human prostate cancer cell lines LNCaP and PC-3; human lung cancer cell lines Calu-6 and SKLU-1; and human pancreatic cancer cell line MIAPaCa2, A549 lung cancer cells, SW480. Similarly, expression of telomerase components may immortalise cells, however many cell lines in culture are already immortalised and by over expression of telomerase. Knockdown of tumour suppressor genes may contribute to cellular transformation of normal epithelial and fibroblast cells and potentially enhance proliferation and tumourigenesis of cancer cell lines. Multiple cancer cell lines already in culture have mutations or are null for tumour suppressor genes (esp. TP53, PTEN and TSC1 and 2). Cells expressing shRNA against these gene products pose no greater risk than Hazard group 1 or 2 cancer cell lines many of which already harbour mutations within one or more of these genes. For example, LnCAP, PC3, U87Mg all have PTEN mutations or are pten null.

Potential hazards arising from the combination of gene and host:
Guidance from SACGM compendium of guidance: Viruses and viral vectors with a human host-range carrying potentially oncogenic sequences or shRNAs targeting tumour suppressor genes may pose risks to human health and safety over and above those hazards associated with the recipient strain itself. The potential outcomes of downregulating particular targeted genes should be carefully assessed. For example, knocking down the expression of a tumour suppressor gene in a mammalian cell could contribute to cellular transformation. However, one of the features of RNAi knockdown systems is that the targeted gene is rarely completely silenced. Indeed, it is not unusual for there to be varying degrees of target gene downregulation within an experimental population and therefore it is important to consider the ramifications of heterogeneous expression of the targeted genes.

Potential for transfer of gene to other hosts:
rAAV are relatively stable and resistant to dehydration. They can infect many cell types, including most human cells, however the rAAV vectors are replication deficient so after transduction there would be no further production of virus particles.

Potential for harm to human health in the event of exposure:
The only potential for harm to human health could be through direct exposure to or injection of the rAAV. However, even with injection the immune system would most likely recognise the rAAV as foreign and would mount an immune defensive response. Recipient strains that are able to infect or colonise human hosts should be avoided i.e. work should not be performed on ones own cells or those of other laboratory staff. The rAAV particles are unable to penetrate through gloves and would be unlikely to penetrate the dead layers of skin cells. Most humans have had prior AAV infection and possess neutralising antibodies to AAV (e.g. 80% of individuals are seropositive for AAV-2).

Potential to disseminate and harm the environment in the event of accidental release:
AAV vectors are non-enveloped DNA viruses that are relatively stable and resistant to dehydration. They could potentially survive in the environment for long periods, however they are defective by nature and unable to establish an infection or replicate in the absence of a helper virus or helper functions.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All Class 2 GMO waste must be inactivated prior to disposal as Non-Infectious Healthcare Waste (EWC 1801 04) or where inactivation is not possible as Infectious Healthcare Waste (EWC 1801 03). Inactivation should be by autoclaving or by chemical treatment with 1 % final Virkon for at least 30 minutes (or other validated method). Note: Where inactivation is not possible, the HSE will be contacted for derogation. It is not anticipated that this would be likely. Both non-hazardous and inactivated hazardous waste is sent for incineration.

Is an emergency plan required according to regulation 20?  

 Tick if you are claiming exemption from disclosure for section of the risk assessment

Trademarked

Draft

Please enter comments on the GM safety committee on the risk assessment

The BioSafety Committee agreed that this work is classified as Activity Class 2. Work can only be performed at Containment Level 2 due to the nature of gene sequences and host cell lines (Hazard Group 2 or Activity Class 2). Note: All work with HG2/AC2 infectious agents at our MedImmune Cambridge facilities is undertaken at Containment Level 2, following standard procedures including our Laboratory Code of Practice. Working with infectious agents takes place in full compliance with Containment Level 2 requirements, including use of Class II safety cabinets to minimise the risk from aerosols and mandatory wearing of PPE (lab coat, safety glasses, gloves) for working with infectious agents.

Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<td>Human Clinical Applications</td>
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Project Ref 387/18.2
Use of lentiviral systems to overexpress prion-like proteins: amyloid-beta, Tau, alpha-synuclein, TDP-43 and Huntingtin, in mammalian primary cells and cell lines.

The goals of this body of work are to enable the generation and use of third (and later) generation, recombinant lentiviral vectors to modulate gene expression by either over-expressing ORFs, cDNAs or specific gene sequences. These vectors will be used to: 1) efficiently generate cell and disease relevant models lines for assessment of target biology; 2) validate literature data; 3) create new models for use in the oncology and other therapeutic areas and 4) to identify and/or validate ORFs/cDNAs or specific gene sequences as potential drug targets. Specifically, this assessment covers the generation and use of recombinant lentiviral particles expressing ORFs/cDNAs/gene specific sequences for in vitro use and for the generation of stable cell lines (and subsequent use of such stable lines both in vitro).

Gene products, cDNAs, ORFs/specific sequences will be cloned into available lentiviral transfer plasmids or obtained commercially or from within the broader MedImmune/AZ organisation. These will be packaged into self-inactivating third generation lentiviral particles by commercial CROs or in house using commercially available 3rd generation packaging systems (e.g. http://tools.invitrogen.com/contentlisfs/manualsvirapoweUentiviral_system_man.pdf). Lentiviral particles encoding genes of interest will then be used to transduce mammalian cells (Hazard group 1 and Activity class 1 cells only) for further studies.

Prion-like proteins are proteins which exhibit some of the characteristics of prion proteins (e.g. are able to cause the transmission of aggregate forms of prion-like protein between cells) but are not prion proteins. The critical factor for a protein to be considered a prion is infectivity, which necessarily means inter-individual transmissibility. Infectivity has only been demonstrated for prion diseases and not to other diseases showing prion-like propagation mechanisms in experimental models.
The cells that we want to use may be generated in-house, bought in from commercial sources or brought in from collaborators. The prion-like proteins that we want to include in this assessment are based in the current ongoing projects and are: amyloid-beta, Tau, alpha-synuclein, TDP-43 and Huntingtin.

### Recipient or parental organism

Cell lines and primary rodent cell cultures including Hazard Group 1 and Activity Class 1 genetically modified cells all of which are covered by appropriate risk assessments, e.g. HEK, CHO and SHSY5Y cells.

### Host/vector system

Third generation (or greater) lentiviral vectors will be used (ref: SACGM compendium of guidance part 2-11 P119 sections 8-30).

"Third Generation" lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase biosafety. These include the vif, vpr, vpu and nef accessory genes which are not required for in vitro replication. The tat gene is also deleted and the Tat-responsive promoter present in the 5' L TR is replaced with heterologous promoters, for example with the Rous sarcoma virus U3 region. An additional biosafety feature is achieved by deletion of the rev gene from the viral transfer vector. Viral packaging is achieved by providing three helper constructs in trans containing gag, pol and rev sequences (figure 1).

An additional biosafety feature is that these vectors are self-inactivating (SIN), whereby the U3 region of the 3' L TR (which contains the major viral promoters and enhancers) is copied to the 5' end of the provirus during reverse transcription. Deletion of enhancer and promoter elements from the 3' U3 region in the vector construct will result in a provirus that is entirely devoid of U3 enhancer sequences, therefore reducing the potential for transactivation of cellular genes as a result of insertion. Furthermore, such vectors are not easily mobilisable as a result of a superinfection with wild-type virus.

The 3rd generation Lentiviral Expression Systems includes the following key safety features:

- The Lenti expression vectors contain a deletion in the 3' L TR (~U3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.

- The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).

- The VSV-G gene from Vesicular Stomatitiis Virus is used in place of the HIV-1 envelope (Burns et al., 1993; Emi et al., 1991; Vee et al., 1994). Alternative envelope proteins (eg. Rabies g protein) that provide different transduction profiles may also be used. Envelope proteins other than VSVG generally decrease the overall permissive transduction profile (compared to VSVG) and are therefore generally considered of no increased risk.

- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).

- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain L TRs or the 4J packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replicationcompetent virus can be produced.
The lentiviral particles produced using these systems are replication-incompetent and only carry the gene of interest. No other viral species are produced.

Expression of the gag and pol genes from helper plasmids are Rev dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al., 1998).

A constitutive promoter (RSV promoter) has been placed upstream of the 5' LTR in the Lenti expression vectors to offset the requirement for Tat in the efficient production of viral RNA (Dull et al., 1998). The TransLenti Viral Packaging System is based on lentiviral vectors developed by Kappes and Wu (Kappes et al. 2001; Kappes et al. 2003; Wu et al. 2000; Wu et al. 2001). This newest (4th) generation lentiviral system includes a significant number of safety features designed to enhance its biosafety and to minimize its relation to the wild-type, human HIV-1 virus. More significantly, the reverse transcriptase (RT) and integrase (IN) proteins are split from the native Gag-Pol polypeptide structure and are provided in trans from a separate plasmid producing a novel class of HIV-based vectors. Instead of expressing Gag-Pol, the TransLenti Viral system contains a plasmid that expresses Gag/Gag-Pro and one that expresses Pol (RT & IN) fused to Vpr. Removing the RT & IN from the packaging construct prevents the lentiviral replication machinery from functioning. This system, in contrast to the standard third generation vectors, prevents the generation of recombinant viral particles that possess the required functional Gag-Pol structure for DNA mobilization and the emergence of replication competent lentivirus.

The TransLenti Viral Packaging System includes the following key safety features:

- The expression vectors contain a deletion in the 3' LTR (~U3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell (Shimada et al. 1995 and Zufferey et al. 1998).
- The number of genes from HIV-1 that are used in the system has been reduced (Le. gag, pol, rev, tat and vpr).
- The VSV-G gene from Vesicular Stomatitis Virus is used to pseudotype the vector particles (Yee et al. 1994). The HIV-1 envelope has been completely removed from the vector.
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids.
- Although the packaging plasmids allow expression in trans of genes required to produce viral progeny (e.g. gag, pol, rev, tat, env) in the TLA-HEK293T producer cell line, none of them contain LTRs or the I.jJ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replicationcompetent virus has been shown to be produced.

3rd generation packaging systems for lentiviral production are based on those originally published by Dull T et al (J Virol. 1998 Nov; 72(11):8463-71.). There are several sources of these vectors. The lentiviral vectors vary depending on source but all have 3rd Generation or greater safety features built in.

**Origin & function**

Open reading frames, cDNAs and gene sequences encoding prion-like proteins amyloid-beta, Tau, alpha-synuclein, TDP-43 and Huntingtin will be expressed in self inactivating lentiviral expression vectors. Only inserts that have minimal risk those that will not increase the risk of the resulting cell lines above Cat1 Activity class 1 will be covered by this risk assessment. Inserts will be cloned into pCDH lenti packaging vectors. Guidance on inserts that may have greater risk of adverse effects on human health can be found in SACGM compendium of guidance part 2-2 p37-46. use of these types inserts will be covered by G345.
Potential hazards associated with the recipient host: These will vary depending on the cell type and can be known or unknown. All cells are considered minimal risk and are Hazard Group 1/2 or Activity Class 1/2.

Potential hazards arising from the gene product: The five proteins that are studied under this risk assessment are classified as prion-like proteins. Prion-like proteins are self-propagating proteins which have the transmission feature of prion proteins, this results in pathologic species being taken up by cells and normal protein within that cell being converted into the pathologic form. Prion-like proteins are not prion proteins. A detailed review of the five proteins has been made for a proper evaluation of the risks.

Amyloid-beta (AD): Is a fragment of 39-42 amino acids resulting from the cleavage of Amyloid precursor protein by several secretases [1]. The role of AD is not well understood but animal models lacking its expression don't show any loss of physiological function. There are several forms of AD, AD-40 which is soluble and AD-42 which has high in vitro propensity to misfold and aggregates, both soluble and insoluble forms have been associated with Alzheimer's disease (AD) and cognitive decline [2, 3]. AD-42 is present in AD patient's brains in the form of plaques but its presence has been also shown outside of the brain, in the cerebrospinal fluid (CSF). It is considered a prion-like protein as misfolded aggregates of AD can seed a chain reaction of misfolding and those aggregates shown the biochemical characteristics of prions (e.g., insoluble and proteinase partial resistance)[4, 5]. Furthermore, transmission from cell to cell has been achieved [6] and so has been the spreading of pathology and the replication of the cognitive decline in animal models after injection of AD [7-9]. Despite that, there is no evidence of AD being naturally transmitted between individuals or iatrogenic transmission of the disease and we estimate that the risk of "infection" is extremely small.

Tau is primarily a cytoskeletal stabilising protein abundantly expressed in the central nervous system (CNS) and much less abundantly expressed in the peripheral nervous system [10]. Inclusions of detergent insoluble tau protein are the pathological hallmark of several neurodegenerative diseases (Tauopathies). It has been shown by several groups that misfolded, insoluble tau can enter a cell and "corrupt" the soluble intracellular tau in a prion-like manner causing aggregation of the intracellular tau in a so called "seeding" reaction [11-13]. Species of tau shown to be capable of seeding have been derived from recombinant protein [11, 13], transgenic mice over expressing human tau [11], and from human tauopathy brain samples [13]. In all published work this seeding effect has relied on over expression of human tau in the recipient cells. Seeding is induced by using concentrations of exogenous tau much higher than is physiologically relevant. Indeed, exogenous aggregates must be injected directly into the brain of transgenic mice before any seeding effect is seen. Cell death has never been observed in cells which have undergone tau seeding either in vivo or in vitro. There is no direct evidence of a person ever having been "infected" with tau pathology, so we estimate that the risk of "infection" is extremely small.

Alpha-synuclein is a small protein of 140 amino acids highly expressed in the brain, with a pre-synaptic localization [14, 15]. The alpha-synuclein protein is considered to be a natively unfolded protein that can become misfolded and aggregates forming D-sheet structures [16, 17]. The presence of insoluble alphasyneuclein aggregates in Lewy bodies are indeed a hallmark of Parkinson's disease (PD) [18]. Aggregated material from a recombinant source can be taken up by cells, however, sustained persistence and seeding in the culture required over expression of the alpha-synuclein in the host cells [19]. Similarly, material from diseased brains and recombinant aggregates have been transmitted into transgenic mouse models accelerating the transgene generated pathology [20, 21]. Although the same pathology spread was reported in wild type animals, those results have been now disproved [22]. There is no direct evidence of a person ever having developed PD iatrogenically or due to exposure to contaminated material, so we estimate that the risk of "infection" is extremely small.

TDP-43 is an RNA binding protein of 414 amino acids localized in the cell nucleus in normal conditions.
Misfolded and aggregated TDP-43, accompanied by a mislocalization to the cytoplasm, has been described in a wide range of neurodegenerative diseases including tauopathies and synucleinopathies but mostly in Amyotrophic lateral sclerosis and Frontotemporal lobar degeneration [23]. Both wild type and dominantly inherited mutations of TDP-43 are associated with disease and in vitro experiments with recombinant protein have demonstrated a higher propensity to aggregate when mutations are present [24, 25]. Furthermore, either synthetically produced or brain purified TDP-43 aggregates have been transduced into cells have shown cellular uptake of TDP-43 [26]. Importantly, no evidence has been reported so far that prove transmission of any type of TDP-43 aggregates into animal models. Therefore, we estimate that the risk of “infection” by exposure to TDP-43 is negligible.

Huntingtin is a protein of unknown function which is highly expressed in the CNS and also expressed in several tissues throughout the body [27]. Wild-type Huntingtin contains a polyglutamine tract in the N-terminal of 6-35 glutamine residues. However, in individuals affected by Huntington's disease (an autosomal dominant genetic disorder), it contains more than 36 glutamine residues (highest reported repeat length is about 250) [28, 29]. This expansion leads to spontaneous aggregation of the Huntingtin protein, massive cell death in the CNS and the clinicopathological features of Huntington's disease. Although a genetically inherited condition, recent literature has pointed towards a "prion-like" spread of pathology within the brains of HD patients, whereby aggregated, pathogenic Huntingtin can leave a cell, enter a neighbouring cell and induce further aggregation. It has been shown that aggregated mutant Huntingtin derived from cell culture and from patient samples can induce seeding in a cell model over-expressing wild-type Huntingtin. Seeding models have all relied on high levels of over-expression of the wild-type protein in recipient cells [30, 31]. Seeding is induced by using concentrations of exogenous Huntingtin much higher than is physiologically relevant. There is no direct evidence of a person ever having been "infected" with Huntington's pathology and Huntington's disease has never been reported in an individual with less than 36 polyglutamine residues. Cell lines expressing wild-type Huntingtin pose no risk to the user as they are expressing a non-pathogenic protein, so we estimate the risk as negligible.

For all of the named prion-like proteins above, it is important noticing that the published cell to cell and animal propagation models for prion-like proteins require high concentration of misfolded protein combined with high levels of host protein over-expression to occur, so accidental transmission to normal expressing individuals are extremely unlikely [32,33]. Additionally, transmission to any in vivo model requires injection of the material, which makes the risk almost negligible under a no-sharp restriction.

Antibiotic Resistance Genes and Tags

There are thought to be no associated risks with these gene products. However, the clinical use of similar antibiotics could be compromised if the genes were transferred to a human pathogen. This is thought to be very unlikely because the plasmid vectors used are non-mobilisable. As for the tags, these are not known to be allergenic or toxic.

Potential hazards arising from the combination of gene and host

None, it is not thought to alter the resulting cell line classification.

Potential for transfer of gene to other hosts:

Lentiviral particles can infect almost all human cells (including non-dividing cells). However, the recombinant retroviral vectors used to generate viral particles are self-inactivating, replication deficient in that they are unable to replicate and do not express any of their endogenous genes. Once within the mammalian cell no viral particles would be produced. The virus could not therefore be transferred to another host.

Potential for harm to human health in the event of exposure:

The only potential for harm to human health could be through direct exposure to or injection of the recombinant lentiviral particles. However, even with injection the immune system would most likely recognise this as foreign and would mount an immune defensive response. Recipient strains
that are able to infect or colonise human hosts should be avoided i.e. work should not be performed on ones own cells or those of other laboratory staff. The particles are unable to penetrate through gloves and would be unlikely to penetrate the dead layers of skin cells.
Potential to disseminate and harm the environment in the event of accidental release: Lentiviral vectors do not pose any identifiable risk to the environment because the reagent is unable to replicate unless supplied with helper plasmids and hence they cannot replicate outside of laboratory conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All Class 2 GMO waste must be inactivated prior to disposal as Non-Infectious Healthcare Waste (EWC 1801 04) or where inactivation is not possible as Infectious Healthcare Waste (EWC 18 01 03). Inactivation should be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).
Note: Where inactivation is not possible, the HSE will be contacted for derogation. It is not anticipated that this would be likely.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Upon discussion with the BioSafety Committee it was decided to assign AC2 due to the potential health risks from prion-like agents.
Note: All work with HG2/AC2 infectious agents at our Medimmune Cambridge facilities is undertaken at Containment Level 2, following standard procedures including our Laboratory Code of Practice.
Working with infectious agents takes place in full compliance with Containment Level 2 requirements, including use of Class II safety cabinets to minimise the risk from aerosols and mandatory wearing of PPE (lab coat, safety glasses, gloves) for working with infectious agents.

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</thead>
<tbody>
<tr>
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<td>L3</td>
<td>L4</td>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

This risk assessment is to cover genetically modified cell lines that have been received or bought from third parties - where the method used for introducing the genetic modification cannot be established, when every effort has been made to elucidate the technology used in the cell line generation.

As the facts around generation of the GMO cell line cannot be established, it should be assumed that the most risky methods have been used (i.e. early Oncogenic gamma Retroviral Vectors systems) and worked on at Activity Class 2.

- This RA covers Hazard group 1 and 2, or activity class 1 and 2 parental cell lines that have been genetically modified by a third party using a process that cannot be traced or verified.
- Cells can be stored, resuscitated and undergo routine subculture.
- Use is limited to in vitro assays (no in vivo).
- The user should think through the potential risks and repercussions carefully when planning experimental work - specifically around the possibility of re-generating replication competent virus in processes such as Invitrogen Membrane Pro.
- When the intended use carries increased risk due to the unknown nature, then the scientist should evaluate if remaking the cell line inhouse is the preferred option.
Recipient or parental organism

Host: Hazard group 1 and 2, or activity class 1 and 2 parental cell lines covered by existing Biological or GMO RA.

Host/vector system

Vector: As the methods of genetic modification are unknown for the cell lines being assessed, the most hazardous scenario should be assumed, e.g. early "first generation" y-retroviral systems derived from Moloney Monkey Leukaemia Virus (MMLV); Murine Stem Cell Virus (MSCV); Feline Leukaemia Virus (FeLV) and Avian Leukosis Virus (ALV) that are packaged with <3 plasmids and are not self-inactivating (SIN).

Taken from the SACGM compendium of guidance part 2.11 p116-126:

To date, most genetic modification work involving retroviruses has involved the development of transduction vectors derived from competent oncogenic retroviruses and lentiviruses. Many such retroviral transduction systems are manipulated in cDNA form and give rise to defective vectors. It is important to consider the hazards posed by the virus from which these vector systems are derived, in order to make an accurate assessment of the risks posed to human health.

Vector Systems and their design:

These vector systems generally consist of two main components - a transfer vector and a packaging system.
- The transfer vector is usually a proviral cDNA in which viral coding sequences have been deleted and foreign DNA inserted.
- The packaging system commonly consists of one or more helper constructs that express viral genes needed to generate infectious viral particles.

The generation of replication competent virus (RCV) and insertional mutagenesis as a result of proviral integration poses major safety issues when handling retroviral vectors. RCV can be generated by recombination events between the vector and the components of the packaging system (including both the packaging constructs themselves and endogenous proviruses present in the cell line used). Proviral integration can result in the activation of cellular genes adjacent to the integration site or insertional disruption of tumour-suppressor functions (features central to oncogenesis by retroviruses not carrying a cellular oncogene). Retroviral vector systems have therefore been developed and refined in order to reduce the likelihood of RCV generation and proviral transactivation. Consequently, there is a range of systems that vary in their safety profile.

Oncogenic Retroviral Vectors

The majority of these vectors have been derived from competent oncogenic retroviruses, such as AIV, MoMlV and Fe IV, that efficiently infect actively dividing cells.

"First Generation" retroviral vectors contain a packaging system that is essentially a retroviral cDNA itself, encoding viral gag, pol and env genes but with its packaging sequence deleted. This construct is either cotransfected with the transfer vector, or is stably incorporated into the host-cell chromosomes generating a helper cell line.

Such systems are inherently the most hazardous since a single recombination event would be sufficient to generate RCV.

Origin & function

Inserts may be:
- Open reading frames
- cDNAs and gene sequences encoding potential drug targets or therapeutic proteins
- shRNAs or microRNAs designed to knockdown the expression of proteins
- those caused by use of CRISP/Cas9, TAIENs etc. where insertions or deletions in genes may arise due to genetic engineering
non-coding DNA elements such as response elements, promoters, enhancers etc. Guidance on inserts that may have greater risk of adverse effects on human health can be found in SACGM compendium of guidance part 2-2 p37-46.

Examples of such inserts include: "exogenous sequences that may have harmful biological activity (for example toxins, cytokines and growth factors, genes that may alter the growth status of cells (such as oncogenes, cytokines and growth factors), sequences which may be involved in the control of expression of such inserts (for example promoters and control regions) and other products that may have no inherent harmful activity but may have other adverse effects (such as allergens or antigenic proteins))."

Of particular note:
Overexpression of Oncogenes (ref: SACGM compendium of guidance part 2-2 p38 sections 6-9).

Genes encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation. Full length cDNA encoding wild type and disease relevant mutants of these types of genes will be expressed in retroviral vectors. Any use of retroviral particles encoding oncogenic inserts will require appropriate controls and operator training.

Examples of genes that may be categorised as oncogenes include:
- growth factors/mitogens eg C-Sis
- receptor tyrosine kinases eg EGFR, PGFR, VEGFR, HER2/neu
- cytoplasmic tyrosine kinases eg Src family, Syk-ZPA-70 family, BTK family and the Abl gene in CML
- cytoplasmic serine threonine kinases and their regulatory subunits eg Raf kinase and cyclin dependant kinase
- Regulatory GTPases eg Ras protein
- transcription factors eg myc gene

Knockdown of tumour suppressors (ref: SACGM compendium of guidance part 2-2, p42, paragraph 24).

Knockdown of genes encoding known tumour suppressors can contribute to cellular transformation. However, shRNA expression systems rarely completely silence the targeted gene. shRNAs targeting these types of genes or gene sequences with tumour suppressive functions will be expressed in retroviral vectors. Any use of retroviral particles capable of reducing tumour suppressor expression will require appropriate controls and operator training.

Examples of genes that may be categorised as tumour suppressors include:
- Cellular tumor antigen p53 P53
- Retinoblastoma-associated protein RB1
- Adenomatous polyposis coli protein APC
- Serine-protein kinase ATM ATM
- Wilms tumor protein WT1
- Neurofibromin NF1
- Merlin NF2/SCH

Health and Safety Executive
- Cyclin-dependent kinase inhibitor 2a CDK4/1/Multipple tumor suppressor 1/MTS-1/p16-INK4a
- von Hippel-Lindau disease tumor suppressor VHL

Please note that:
Even if the insert is considered "non-hazardous" due to unknown nature of the generation method used - the cell lines will be worked on at containment level 2.

Work using infectious protein type inserts (tau, alpha synucleon etc) are specifically excluded from this risk assessment and should be considered under a new GMO RA.

Evaluation of foreseeable effects

Retroviral vectors do not pose any identifiable risk to the environment because the reagent is unable to replicate.
unless supplied with helper plasmids and hence they cannot replicate outside of laboratory conditions. The genetically modified cells are no more risk than hazard group 2 cell lines and would rapidly die off outside of a sterile, warm, humid and enriched environment. These will vary depending on the cell type and can be unknown. Potential common pathogens might be human pathogens such as Epstein Barr virus or rhinovirus. Note: Hazard Group 2 cells and Activity class 2 cells present a greater risk than Hazard Group 1 cells, usually due to their potential to harbour human pathogens.

The expression or abrogation of the majority of genes is predicted to generally have little or no adverse effect. However, the precise outcome is difficult to predict. Expression of proto-oncogenes and oncogenic derivatives of these genes will transform normal epithelial and fibroblast cells and potentially enhance proliferation and tumourigenesis of cancer cell lines. Cells expressing these gene products pose no greater risk than Hazard group 2 cancer cell lines many of which harbour mutations within these genes. For example, the T24 bladder cancer cell line has a H-Ras mutation, or K-ras mutations are present in human colon carcinoma cell lines DLD-1, LoVo and HCT116; human prostate cancer cell lines LNCaP and PC-3; human lung cancer cell lines Calu-6 and SKLU-1; and human pancreatic cancer cell line MIAPaCa2, A549 lung cancer cells, SW480.

Knockdown of tumour suppressor genes may contribute to cellular transformation of normal epithelial and fibroblast cells and potentially enhance proliferation and tumourigenesis of cancer cell lines. Multiple cancer cell lines already in culture have mutations or are null for tumour suppressor genes (esp. TP53, PTEN and TSC1 and 2). Cells expressing shRNA against these gene products pose no greater risk than Hazard group 2 cancer cell lines many of which already harbour mutations within one or more of these genes. For example, LnCAP, PC3, 87Mg all have PTEN mutations or are pten null.

Guidance from SACGM compendium of guidance:
The potential outcomes of downregulating particular targeted genes in host cells should be carefully assessed. For example, knocking down the expression of a tumour suppressor gene in a mammalian cell could contribute to cellular transformation. However, one of the features of RNAi knockdown systems is that the targeted gene is rarely completely silenced. Indeed, it is not unusual for there to be varying degrees of target gene downregulation within an experimental population and therefore it is important to consider the ramifications of heterogeneous expression of the targeted genes.

Even the oldest recombinant retroviral vector systems used to generate viral particles are replication-deficient in that they are thought unable to replicate and do not express all of their endogenous genes. Once within the mammalian cell no viral particles would be produced therefore the insert could not therefore be transferred to another host. One exception to this could be where the host cells genome contains endogenous viral genes through a previous retroviral infection that then recombine with the genes delivered by the retrovirus to produce replicative-competent virus. This scenario is very unlikely, but theoretically possible.

Another risk that should be considered is the subsequent addition of a retroviral packaging mix to cell lines that have already been manipulated through retroviral transduction, which has the potential to mobilise the integrated genes. This includes the use of the MembranePro reagent for the generation of virus-like particles. The risk is that the cells will produce infectious (but replication-deficient) retrovirus particles, so these cell lines (and the conditioned media) would need to be handled with the considerations afforded to the conditions/cell lines used to generate retroviral vectors, and retroviral particles.

Under no circumstances should cells genetically manipulated by unknown methods be used with membrane-pro reagent or have retroviral packaging plasmids added to them. The user should think through the potential risks and repercussions carefully when planning experimental work. When the intended use carries increased risk due to the unknown nature then the scientist should evaluate if remaking the cell line in-house is the preferred option.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All Class 2 GMO waste must be inactivated prior to disposal as Non-Infectious Healthcare Waste (EWC 1801 04) or where inactivation is not possible as Infectious Healthcare Waste (EWC 1801 03). Inactivation should be by autoclaving or by chemical treatment with 1 % final Virkon for at least 30 minutes (or other validated method).

Note: Where inactivation is not possible, the HSE will be contacted for derogation. It is not anticipated that this would be likely.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Upon discussion with the BioSafety Committee it was decided that work could be proceed at Activity class 2, only after every effort has been made to investigate the technologies used to generate the cell line. Wherever possible and practical cell lines should be re-made in-house so the technology and risks posed are clear and understood.

Note: All work with HG2/AC2 infectious agents at our Medimmune Cambridge facilities is undertaken at Containment Level 2, following standard procedures including our Laboratory Code of Practice.

Working with infectious agents takes place in full compliance with Containment Level 2 requirements, including use of Class II safety cabinets to minimise the risk from aerosols and mandatory wearing of PPE (lab coat, safety glasses, gloves) for working with infectious agents.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<td>L2 L3 L4 L2</td>
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Project Additional Information

Purposes of the contained use
To use genetically-modified human Rhinovirus (HRV) in the infection of mammalian cells in vitro, to enable in vitro assays to be performed, such as (but not limited to):

- HTRF, Mirrorball assays, flow cytometry, western blotting, staining, proliferation assays.
- Cells may hazard group 1 or 2; or genetically modified activity class 1 or 2
- Cells may also be cultured with or without stimulating factors e.g. cytokines or growth factors
- Cell populations may be sorted using, for example, magnetic beads or flow-cytometry
- Protein or RNA may be extracted and used in assays such as RT-PCR, ELISAs
- Once isolated, cells, lysates or supernatants may be frozen and stored for culture or used in assays at a later date.

Recipient or parental organism
Human rhinovirus with non-hazardous genetic modifications, such as the insert of a fluorescent protein (e.g. GFP) will be covered. For hazardous inserts a further risk assessment will need to be undertaken.

Human rhinovirus is on the HSE approved list of biological agents and is classified as a human pathogen Hazard Group 2 agent (http://www.hse.gov.uk/pubns/misc208.pdf). Hazard Group 2 reflects the infectious nature of human rhinovirus, and the need for appropriate containment to minimise risk to operator health when performing the procedures outlined herein. There are >100 serotypes of HRV.

Human rhinoviruses (HRV) are a major cause of colds in humans. In healthy individuals, the symptoms are upper respiratory tract symptoms such as runny or blocked
nose, runny eyes and a general feeling of lethargy. Most individuals will recover within a few days. Viral shedding will occur a few days before symptoms appear and may continue for 3-4 weeks after infection.

HRV are able to survive outside mammalian cells on non-porous surfaces for several days and hands for several hours and still remain viable and infectious, especially when humidity is high (Gwaltney 1982). There is evidence that hands are a very important method of transmission of rhinoviruses (Ansari 1991) and that as few as 10 viral particles is sufficient to cause an infection (D’Alessio 1984).

Host/vector system

Human rhinovirus (HRV) is both the vector and the organism. An example of a GM insert to be covered by this risk assessment is the HRV-C15-GFP virus. This was made at MedImmune, Gaithersburg, Maryland, US. In brief, DNA encoding eGFP flanked by the viral protease recognition sites (to allow for the cleavage of free GFP from the viral polyprotein) was inserted into the wild-type virus DNA and subsequently generated as per wild-type rhinovirus.

Origin & function

In this risk assessment only inserts expected to be non-hazardous are covered, including: open reading frames (ORFs), cDNAs or specific gene sequences, e.g. Emerald green fluorescent protein (EmGFP) is an example of a non-hazardous protein. EmGFP has been isolated from the jellyfish Aequorea victoria.

Guidance on inserts that may have greater risk of adverse effects on human health can be found in SACGM compendium of guidance part 2-2 p37-46.

Evaluation of foreseeable effects

Potential hazards associated with the recipient host:

Potential hazards arising from the gene product:

The gene products covered are non-hazardous, and not anticipated to increase the hazard or risk posed by the recipient host virus.

Potential hazards arising from the combination of gene and host:

There are no additional hazards anticipated from the genetically modified rhinovirus over wild-type virus, and inclusion of a foreign gene may negatively impact growth and reproduction kinetics.

Potential for transfer of gene to other hosts:

The reporter viruses remain replication competent, and can produce progeny virus. However they are likely to have reduced growth kinetics given that they are encoding a foreign gene. HRV reporter virus (such as HRV-C15-GFP) do not tolerate the foreign genes well and lose the encoded gene insert within two passages, reverting to wild-type virus.

Therefore while recombination with rhinovirus serotypes is theoretically possible in the unlikely event of co-infection, it is unlikely that the gene modification would successfully spread or be maintained.

Potential for harm to human health in the event of exposure:
Human rhinoviruses (HRV) are a major cause of colds in humans. Transmission is airborne, via nasal passages, although hands and possibly eyes have been implicated too. However, to a small subset of the population, the common cold can present a serious health risk; especially in the very young or old, or in people with respiratory problems such as asthma and COPD. Although HRV itself is usually self-limiting, those suffering from asthma and COPD are 70% and 50% more likely to be hospitalised due to exacerbations as a result of HRV infection. HRV infection is also associated with pro-inflammatory cytokine production and enhanced mucous production from airway epithelial cells.

The addition of non-hazardous genetic modifications is not expected to increase the risk of infection or alter the natural history of the virus.

As vulnerable persons are more likely to have problems associated with viral infection, it is important that medical/occupational health advice is taken to ensure that careful consideration of the situation and potential implications is taken when any persons responsible for caring for neonates, the elderly or immuno-compromised persons, is thinking of working with rhinovirus. There is a risk of generating aerosols through processes such as, but not limited to centrifugation, plate washing and pipetting.

Potential to disseminate and harm the environment in the event of accidental release:
GM rhinovirus are likely to have reduced growth kinetics with respect to wild-type virus, given that they are encoding a foreign gene. HRV reporter virus (such as HRV-C15-GFP) do not tolerate the foreign genes well and lose the encoded gene insert within two passages, reverting to wild-type virus. Therefore while recombination with rhinovirus serotypes is theoretically possible in the unlikely event of co-infection, it is unlikely that the gene modification would successfully spread or be maintained.

Rhinovirus is rapidly degraded at room temperature and cannot survive on surfaces for longer than 1 week, therefore contact with receptor/host would have to occur within this timeframe for infection to be spread. All rhinovirus covered by this risk assessment are lab strains, although no disabling mutations have been introduced. Rhinovirus growth and replication are optimal at 33°C so are unlikely to survive outside of this temperature. Based on this, there is potential to disseminate and harm the environment (in the absence of control measures), however this is thought extremely unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All Class 2 GMO waste must be inactivated prior to disposal as Non-Infectious Healthcare Waste (EWC 18 01 04) or where inactivation is not possible as Infectious Healthcare Waste (EWC 18 01 03). Inactivation should be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method).

Note:Where inactivation is not possible, the HSE will be contacted for derogation. It is not anticipated that this would be likely.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
The Biosafety committee agreed that this work should be undertaken at Containment Level 2.

Note: All work with HG2/AC2 infectious agents at our MedImmune Cambridge facilities is undertaken at Containment Level 2, following standard procedures including our Laboratory Code of Practice.

Working with infectious agents takes place in full compliance with Containment Level 2 requirements, including use of Class II safety cabinets to minimise the risk from aerosols and mandatory wearing of PPE (lab coat, safety glasses, gloves) for working with infectious agents.

Activity Class 2 as the host organism is assigned AC2 by the ACDP approved list of biological agents, and the genetic modification is not known to be disabling or attenuating.

**Project Containment**

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**Project Ref** 387/20.1

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<td>Use of recombinant adeno associated virus (rAAV) particles to overexpress prion-like proteins: amyloid-beta, Tau, alpha-synuclein, TDP-43 and Huntingtin, in mammalian primary cells and cell lines</td>
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Non-GMM| Consent Granted
Not Applicable

Project notified under transitional arrangements

**Project Additional Information**

02/03/2022
Purposes of the contained use

The goals of this body of work are to enable the generation and use of recombinant AAV vectors to modulate gene expression by either over-expressing ORFs, cDNAs or specific gene sequences related to the already stipulated prion-like proteins. Specifically, this assessment covers the generation and use of recombinant AAV particles expressing ORFs / cDNAs / gene specific sequences for in vitro use and for the generation of stable cell lines (and subsequent use of such stable lines both in vitro). Gene products, cDNAs, ORFs, specific sequences will be cloned into available rAAV plasmids, or obtained commercially or from within the broader MedImmune/AZ organisation. These will be packaged into rAAV particles by commercial CROs or packaged in-house using commercially available helper-free systems.

Recipient or parental organism

Cell lines and primary rodent cell cultures including Hazard Group 1/2 and Activity Class 1/2 genetically modified cells all of which are covered by appropriate risk assessments, e.g. HEK, CHO and SHSY5Y cells.

Several different natural AAV serotypes have been described and these differ in their capsid structure, leading to different cell transduction profiles and specificities.


In this body of work, the particular AAV serotype chosen for the preparation of a particular rAAV will depend on the tropism of the natural AAV serotypes and the specific aims of the experiment, therefore this risk assessment will cover rAAV using all natural AAV serotypes. There is no difference in risk associated with any particular AAV serotype.

Host/vector system

Wild type AAV have no known link to any human illness therefore Containment Level 1 is sufficient. AAV are dependoviruses i.e. are not capable of replication without the presence of a helper infection, such as co-infection with adenovirus or herpes simplex virus. Wild-type AAV is able to integrate into human chromosome 19 at a specific site but with very low efficiency.

In this work rAAV particles will be prepared using a "helper-free" system in which the genes required for rAAV production are provided in plasmid form rather than from co-infection with another virus. The rAAV encoding genes of interest will be prepared by engineering of a "gutless" AAV vector in which the AAV replication and capsid genes are replaced by the coding sequence of interest, with the replication and capsid genes needed for AAV production being provided by plasmids. This means that the resulting rAAV particles do not possess AAV replication genes themselves and no longer have the ability to integrate into chromosomal DNA. Note that integration into DNA may occur in a passive way at chromosome break points but overall the risk of insertional mutagenesis is low. To date, 13 natural AAV serotypes have been identified from human and non-human primate sources [Srivastava, A. (2016). In vivo tissue-tropism of adeno-associated viral vectors. Current Opinion in Virology, 21: 75-80. http://www.sciencedirect.com/science/article/pii/S1879625716300992]. This work will include the use of all natural AAV serotypes. Given the low pathogenicity of AAV, the major potential hazards posed by rAAV depend on the inserted genetic material.

rAAV have a long history of safe use and at the time of writing are being used in 173 clinical trials as gene delivery vectors in man http://www.abedia.com/wiley/vectors.php.

Origin & function

The five proteins encoded by the genetic material being studied under this risk assessment are classified as prion-like proteins. Prion-like proteins are self-propagating proteins which have the transmission feature of prion proteins, this results in pathologic species being taken up by cells and normal protein within that cell being converted into the pathologic form. Prion-like proteins are not prion proteins. A detailed review of the five proteins has been made for a proper evaluation of the risks.

Amyloid-beta (AB): Is a fragment of 39-42 amino acids resulting from the cleavage of Amyloid precursor protein by several secretases [1]. The role of AB is not well understood but animal models lacking its expression don’t show any loss of physiological function. There are several forms of AB, AB-40 which is soluble and AB-42 which has high in vitro propensity to misfold and aggregates, both soluble and insoluble forms have been associated with Alzheimer’s disease (AD) and cognitive decline [2, 3].
AB-42 is present in AD patient's brains in the form of plaques but its presence has been also shown outside of the brain, in the cerebrospinal fluid (CSF). It is considered a prion-like protein as misfolded aggregates of AB can seed a chain reaction of misfolding and those aggregates shown the biochemical characteristics of prions (e.g., insoluble and protease resistant [4, 5]). Furthermore, transmission from cell to cell has been achieved [6] and so has been the spreading of pathology and the replication of the cognitive decline in animal models after injection of AB [7-9]. Despite that, there is no evidence of AB being naturally transmitted between individuals or iatrogenic transmission of the disease and we estimate that the risk of "infection" is extremely small.

Tau is primarily a cytoskeletal stabilising protein abundantly expressed in the central nervous system (CNS) and much less abundantly expressed in the peripheral nervous system [10]. Inclusions of detergent insoluble tau protein are the pathological hallmark of several neurodegenerative diseases (Tauopathies). It has been shown by several groups that mis-folded, insoluble tau can enter a cell and corrupt the soluble intracellular tau in a prion-like manner causing aggregation of the intracellular tau in a so-called seeding reaction [11-13].

Species of tau shown to be capable of seeding have been derived from recombinant protein [11, 13], transgenic mice over expressing human tau [11], and from human tauopathy brain samples [13]. In all published work this seeding effect has relied on over expression of human tau in the recipient cells. Seeding is induced by using concentrations of exogenous tau much higher than is physiologically relevant. Indeed, exogenous aggregates must be injected directly into the brain of transgenic mice before any seeding effect is seen. Cell death has never been observed in cells which have undergone tau seeding either in vivo or in vitro. There is no direct evidence of a person ever having been infected with tau pathology, so we estimate that the risk of infection is extremely small.

Alpha-synuclein is a small protein of 140 amino acids highly expressed in the brain, with a pre-synaptic localization [14, 15]. The alpha-synuclein protein is considered to be a natively unfolded protein that can become misfolded and aggregates forming B-sheet structures [16, 17]. The presence of insoluble alpha-synuclein aggregates in Lewy bodies are indeed a hallmark of Parkinson's disease (PD) [18]. Aggregated material from a recombinant source can be taken up by cells, however, sustained persistence and seeding in the culture required over expression of the alpha-synuclein in the host cells [19]. Similarly, material from diseased brains and recombinant aggregates have been transmitted into transgenic mouse models accelerating the transgene generated pathology [20, 21]. Although the same pathology spread was reported in wild type animals, those results have been now disproved [22]. There is no direct evidence of a person ever having developed PD iatrogenically or due to exposure to contaminated material, so we estimate that the risk of infection is extremely small.

TDP-43 is an RNA binding protein of 414 amino acids localized in the cell nucleus in normal conditions. Misfolded and aggregated TDP-43, accompanied by a mislocalization to the cytoplasm, has been described in a wide range of neurodegenerative diseases including tauopathies and synucleinopathies but mostly in Amyotrophic lateral sclerosis and Frontotemporal lobar degeneration [23]. Both wild type and dominantly inherited mutations of TDP-43 are associated with disease and in vitro experiments with recombinant protein have demonstrated a higher propensity to aggregate when mutations are present [24, 25]. Furthermore, either synthetically produced or brain purified TDP-43 aggregates have been transduced into cells have shown cellular uptake of TDP-43 [26]. Importantly, no evidence has been reported so far that prove transmission of any type of TDP-43 aggregates into animal models. Therefore, we estimate that the risk of infection by exposure to TDP-43 is negligible.

Huntingtin is a protein of unknown function which is highly expressed in the CNS and also expressed in several tissues throughout the body [27]. Wild-type Huntingtin contains a polyglutamine tract in the N-terminal of 6-35 glutamine residues. However, in individuals affected by Huntington's disease (an autosomal dominant genetic disorder), it contains more than 36 glutamine residues (highest reported repeat length is about 250) [28, 29]. This expansion leads to spontaneous aggregation of the Huntingtin protein, massive cell death in the CNS and the clinicopathological features of Huntington's disease. Although a genetically inherited condition, recent literature has pointed towards a prion-like spread of pathology within the brains of HD patients, whereby aggregated, pathogenic Huntingtin can leave a cell, enter a neighbouring cell and induce further aggregation. It has been shown that aggregated mutant Huntingtin derived from cell culture and from patient samples can induce seeding in a cell model over-expressing wild-type Huntingtin. Seeding models have all relied on high levels of over-expression of the wild-type protein in recipient cells [30, 31]. Seeding is induced by using concentrations of exogenous Huntingtin much higher than is physiologically relevant. There is no direct evidence of a person ever having been infected with Huntington's pathology and Huntington's disease has never been reported in an individual with less than 36 polyglutamine residues. Cell lines expressing wild-type Huntingtin pose no risk to the user as they are expressing a non-pathogenic protein, so we estimate the risk as negligible.
For all of the named prion-like proteins above, it is important noticing that the published cell to cell and animal propagation models for prion-like proteins require high concentration of misfolded protein combined with high levels of host protein over-expression to occur, so accidental transmission to normal expressing individuals are extremely unlikely [32, 33]. Additionally, transmission to any in vivo model requires injection of the material, which makes the risk almost negligible under a no-sharp restriction.

Antibiotic Resistance Genes and Tags
There are thought to be no associated risks with these gene products. However, the clinical use of similar antibiotics could be compromised if the genes were transferred to a human pathogen. This is thought to be very unlikely because the plasmid vectors used are non-mobilisable. As for the tags, these are not known to be allergenic or toxic.


16. Fauvet, B., et al., Characterization of semisynthetic and naturally Nalpha-acetylated alpha-synuclein in vitro and in intact cells: implications for aggregation and cellular...


**Evaluation of foreseeable effects**

All cell lines transduced by rAAV and so expressing prion-like protein will potentially become more hazardous.
rAAV are relatively stable and resistant to dehydration, therefore there is potential for persistence on contaminated surfaces. The rAAV can infect many cell types, including most human cells, however the rAAV vectors are replication deficient so after transduction there would be no further production of virus particles. Integration into the host genome is extremely unlikely. The only potential for harm to human health would be through direct exposure to or injection of the rAAV. However, even with injection the immune system would most likely recognise the rAAV as foreign and would mount an immune defensive response. Most humans have had prior AAV infection and possess neutralising antibodies to AAV (e.g. 80% of individuals are seropositive for AAV-2). The rAAV particles are unable to penetrate through gloves and would be unlikely to penetrate the dead layers of skin cells. Mammalian cells are unable to infect or colonise human hosts due to normal immune clearance.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

rAAVs generated will not be used to transduce larger GMOs

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be solidified with VernaGel 100 (a super absorbent powder) and disposed directly into a sharps bin (contained in the class 2 microbiological safety cabinet), autoclaved on site, and sent for specialist incineration as mentioned above. Solid waste e.g. plasticware, shall also be disposed via a sharps bin, autoclaved on site, and sent for specialist incineration. This is to avoid prion-like protein entering the water system and ensuring full deactivation. All waste material will be double contained during transfer to the on site autoclave facility.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

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**Comments**

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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
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<th>Glass House</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 390

Data Premises Notified: 07/11/1990 (Originally)

Transferred from 1992 Regs?: Y

Transitional Premises Class: 1

Data Premises Closed: N

Emergency Plan Required?: N

Non-GMMs: N

Withdrawn: N

Name: TATE & LYLE CITRIC ACID

Name 2: 

Department: BIOPROCESS DEVELOPMENT

Campus Estate or Research Centre: 

Building: 

Road Name: DENISON ROAD

District: 

Town: SELBY

County: NORTH YORKSHIRE

Postcode: YO8 8EF

Country: ENGLAND

Tel Number: 01757 703691

Fax Number: 01757 701468

E-mail: 

HSE Division: YORKSHIRE AND NORTH EAST

Comments: 

Date at Which Additional Info Submitted: 02/03/2022
## Premises Addresses

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<tr>
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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify)  
Tick if confidential

<table>
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<tr>
<th>Laboratory</th>
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- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
The maximum culture volume that could be released at any one time is 15 litres. Disposal of small quantities of solid and liquid waste is by autoclaving at 121 degrees C for at least 20 minutes. Autoclaves have temperature probes and automated air bleeds. Alternatively, small quantities of solid waste (e.g., biomass samples) are dried in a static oven (no internal fan) at 100 degrees C.

Disposal of 15 litre vessel wastes at the end of fermentations is by neat phenolic type disinfectant. The vessel is then emptied through the sample point into stainless steel buckets. The buckets are then autoclaved at 121 degrees C for 45 minutes as the second stage of waste inactivation.

No organisms that are especially tolerant of heat or disinfectants are used.

<table>
<thead>
<tr>
<th>Other(s)</th>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 391**

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**Name**

INTERVET UK LTD/MSD ANIMAL HEALTH

**Name 2**

ANIMAL HEALTH

**Campus Estate or Research Centre**

WALTON MANOR

**Road Name**

WALTON

**Town**

MILTON KEYNES

**County**

BUCKINGHAMSHIRE

**Postcode**

MK7 7AJ

**Country**

ENGLAND

**Tel Number**

01908 665050

**Fax Number**

01908 664778

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

GM280 Closed and merged with GM391 on 31/03/2005.

**Date at Which Additional Info Submitted**

02/03/2022
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee
<table>
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- **Bacteriology**
  - Parasitology
  - Transgenic Birds

- **Virology**
  - Transgenic Animals
  - Transgenic Fish

- **Mycology**
  - Transgenic Invertebrates
  - Transgenic Plants

- **Other(s)**

- **Tick if confidential**
For activities involving GMMs, describe the waste management measures which will apply to the activity

For the GM work, currently performed on this site, the maximum culture volume that could be released at any one time would be 2000 litres once every two years. This waste, as with all other potentially active waste, is transferred to a kill tank where it is deactivated by steam sterilisation at 126 degrees C for 60 minutes. This deactivation method was validated by: control system validation (checking for correct functioning of all inputs and outputs of the system; pressure hold testing of the vessel; Twelve point thermocouple temperature mapping on a full vessel. The deactivation is monitored both physically and microbiologically. Correct execution of the temperature/time cycle acts as a failsafe for allowing the discharge valve to be opened. In addition, every time the effluent tank has gone through a sterilisation cycle (approximately once a week) a sample is taken and checked for microbiological sterility.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref  391/01.1

Class  CultureVolClass2  CultureVolumeClass3-4
Class 2  > 500 litres

Non-GMM  Consent Granted  not applicable

Project notified under transitional arrangements  N

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information
### Purposes of the contained use

Cultivation of GMMs to produce inactivated vaccines for livestock and/or poultry. Laboratory scale culture for process development and vaccine development. Larger scale growth for production of commercial product.

### Recipient or parental organism

**E. coli K12 and its derivatives:** strains DH10B, DH5alpha, XL1Blue, XL10, SURE, TG1, JM109, MC4100, or equivalent.

### Host/vector system

**pBR322 and its derivates:** pGEMT, pGEX series, pET series, pQE series, pMAL series, pACYC177, p15A or equivalent

**pUC and its derivates:** pBluescript, pBC, pACYC184 or equivalent.

### Origin & function

Gene(s) for antigenic proteins of Escherichia coli, Salmonella spp, Pasteurella spp, Clostridium spp. Mannheimia spp, Haemophilus spp, Brachyspira spp and Actinobacillus spp, EXCLUDING known toxins (except where genetically wholly and irreversibly detoxified on another HSE notified centre), cytokines, superantigens and hyperallergens.

### Evaluation of foreseeable effects

The GMOs compared to the parent E. coli K12 strains should have:

- (a) no increase in infectivity or pathogenicity;
- (b) no reversal of disabling mutation; and
- (c) in the event of inadvertent release, no increase in fitness for environmental survival or spread.

Consequently, overall there is a negligible likelihood that the insert in the GMOs could arise in harm with negligible consequences for the ecosystem, rare species, ubiquitous species or spread and persistence.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid culture waste from small scale and solid waste from laboratory experiments and production areas will be decontaminated by autoclaving using a validated decontamination cycle giving 100% kill.

Liquid waste from production areas is transferred to a kill tank where it is deactivated by steam sterilisation at 126 degrees C for 60 minutes. This deactivation method is validated by control system validation with correct execution of the temperature/time cycle as a failsafe for allowing opening of the discharge valve.

Solid waste is decontaminated by autoclave (commissioned with TST test and 12 point thermocouple tests followed by monthly TST test and 6 monthly review with 12 point thermocouple tests) ultimately disposed of by incineration (contract disposal) and decontaminated liquid waste is discharged to the sewer system.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N
The risk assessments were discussed by the genetic modification committee on 9 November 2001. Some minor comments were made and the risk assessments modified slightly to the versions attached. The committee considered the risk assessments and considered that Class 2 categorisation under "the contained use (2000) regulations" was appropriate given the nature of the GMMs involved and the level of containment appropriate to the work.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
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Animal Units

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Project Ref 391/01.2

Date Ackn'd 29/11/2001

CU2 Project Title

Project noted under transitional arrangements N

Inactivation by Homologous Recombination of Salmonella spp genes

Class 2

Consent Granted not applicable

> 500 litres

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Cultivation of GMMs to produce inactivated vaccines for livestock and/or poultry. Laboratory scale culture for process development and vaccine development. Larger scale growth for production of commercial product.

Recipient or parental organism

Salmonella enterica serovars Enteritidis, Typhimurium, Hadar, Anatum, and derivatives thereof in which genes have been inactivated by internal deletion, multiple point mutation or antibiotic cassette insertion.

The genetic modifications are not expected to enhance either the existing pathogenic traits of the parent organism, or the fitness of the GMM. It is expected that pathogenicity and fitness will be reduced or at most unaffected by the proposed alterations. It is not expected that the potential for environmental harm will be increased by the genetic alterations proposed.

Host/vector system

Salmonella enterica spp described above with the following non-replicating or conditionally replicating (eg. thermoawbaurucw) suicide vectors: pSC101-ts, R6K, pLD55, pKD20, pKD46, pMB1, ColE1, RP4, p15A, oriV or derivatives thereof.

Origin & function

Gene(s) for antigenic proteins of Salmonella spp. EXCLUDING known toxins, cytokines, superantigens and hyperallergens.

Evaluation of foreseeable effects

The GMMs compared to the parent Salmonella strains should have:-
(a) no increase in infectivity or pathogenicity;
(b) no reversal of disabling mutations; and
(c) in the event of inadvertent release, no increase in fitness for environmental survival or spread.

Consequently, overall there is a negligible likelihood that the genetic modification in the GMMs could result in harm with negligible consequences for the ecosystem, rare species, ubiquitous species or spread and persistence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid culture waste from small scale and solid waste from laboratory experiments and production areas will be decontaminated by autoclaving using a validated econtamination cycle giving 100% kill.

Liquid waste from production areas is transferred to a kill tank where it is deactivated by steam sterilisation at 126 degrees C for 60 minutes. This deactivation method is validated by control system validation with correct execution of the temperature/time cycle as a failsafe for allowing opening of the discharge valve Solid waste is decontaminated by autoclave (commissioned with TST test and 12 point thermocouple tests followed by monthly TST test and 6 monthly review with 12 point thermocouple tests) and ultimately disposed of by incineration (contract disposal). Decontaminated liquid waste is discharged to the sewer system.
The risk assessments were discussed by the genetic modification committee on 9 November 2001. Some minor comments were made and the risk assessments modified slightly to the versions attached.

The committee considered the risk assessments and considered that Class 2 categorisation under "the contained use (2000) regulations" was appropriate given the nature of the GMMs involved and the level of containment appropriate to the work.

Project Containment

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Animal Units

- L2
- L3 L4
- L2 Yes

Large Scale Activities

- L2
- L3 L4
- L2 Yes

Human Clinical Applications

- L2
- L3 L4
- L2

Project Ref 391/03.1

EVALUATE THE UTILITY OF A BACULOVIRUS EXPRESSION SYSTEM FOR THE PRODUCTION OF IRREVERSIBLY GENETICALLY INACTIVATED CLOSTRIDIAL TOXINS.

Class 2 > 500 litres

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: N
### Project Additional Information

**Purposes of the contained use**

| Cultivation of GMMs to produce vaccines for livestock and/or poultry. Laboratory scale culture for process development and vaccine development. Larger scale growth for production of commercial product. |

**Recipient or parental organism**

| Recombinant baculovirus engineered to express genetically inactivated clostridial toxins in cultured Sf9 or Sf21 insect cells. |

**Host/vector system**

| Recombinant baculovirus engineered to express genetically inactivated clostridial toxins in cultured Sf9 or Sf21 insect cells. |

**Origin & function**

| Genes for toxins of clostridial species supplied, by others, as detoxified by deletion or multiple point mutation. |

**Evaluation of foreseeable effects**

| The baculovirus used contains mutations that render it sensitive to conditions within its normal lepidoptera host and which greatly reduce the infectivity of the baculovirus for insect cells. Neither of these attenuating lesions is likely to be affected by the expression of inactivated clostridial toxins. The GMO therefore presents no foreseeable adverse effect. |

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

**For only GMMS - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| N/A |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Liquid culture waste from small scale and solid waste from laboratory experiments and production areas will be decontaminated by autoclaving using a validated process. |
decontamination cycle giving 100% kill.

Liquid waste from production areas is transferred to a kill tank where it is deactivated by steam sterilisation at 126 degrees C for 60 minutes. This deactivation method is validated by control system validation with correct execution of the temperature/time cycle as a failsafe for allowing opening of the discharge valve.

Solid waste is decontamination by autoclave (commissioned with TST test and 12 point thermocouple tests followed by monthly TST test and 6 monthly review with 12 point thermocouple tests) ultimately disposed of by incineration (contract disposal) and decontaminated liquid waste is discharged to the sewer system.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessments were discussed by the genetic modification committee on 18 June 2003. Some minor comments were made and the risk assessments modified accordingly. Final version attached.
The committee considered the risk assessments and considered that Class 2 categorisation under "the contained use (2000) regulations" was appropriate given the nature of the GMMs involved and the level of containment appropriate to the work.

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Project Ref 391/05.1

Date Ackn'd 16/09/2005

CU2 Project Title Inactivation, by homologous recombination, of Campylobacter jejuni genes.

Class 2

Culture Vol Class 2 > 500 Litres

Class Volume Class 3-4

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The *C.jejuni* strains developed during this programme of work may be used as a live vaccine for use in chickens under high containment to reduce colonisation by wild type *C.jejuni*.

**Recipient or parental organism**

*C.jejuni*  
*E.coli* K12 and derivatives

**Host/vector system**

*C.jejuni* - non-replicating or conditionally replicating (e.g. thermosensitive) suicide vectors e.g. pGEMT  
*E.coli* - non-mobilisable e.g. pGEMT.

**Origin & function**

Gene(s) for: proteins of *C.jejuni* excluding known toxins, cytokines, superantigens, hyperallergens. These genes being inactivated by internal deletion, multiple point mutation or antibiotic cassette insertion.

**Evaluation of foreseeable effects**

- Is there an increase in infectivity or pathogenicity? - No  
- Could any disabling mutation within the recipient be overcome due to the insertion of a foreign gene? - No  
- Does the foreign gene encode a virulence determinant from a related organism? - No  
- If the foreign gene is a virulence determinant could it contribute to the pathogenicity of the GMM? - No  
- Is treatment available? - Yes  
- Will the genetic modification affect susceptibility of the GMM to treatment? - No [Any change in susceptibility will not be clinically significant. The antibiotic resistance in this instance is kanamycin from *C.jejuni* and so is a naturally occurring combination. Furthermore the treatments of choice are either erythromycin or ciprofloxacin and there is no implication from kanamycin resistance for treatment.]

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste up to 5L or small scale solid laboratory waste are decontaminated by autoclave using a validated decontamination cycle (commission with TST test and 12 point thermocouple tests; ongoing monitoring by monthly TST test and 6 monthly review with 12 point thermocouple tests) giving 100% kill.

Liquid waste from production areas is transferred to a kill tank where it is decontaminated by steam sterilisation at 1260C for 60 minutes. This method is validated by control system validation with correct execution of the temperature/time cycle as a failsafe for allowing opening of the discharge valve.

Autoclaved solid waste is collected by a licensed clinical waste disposal agent for final destruction by incineration. Decontaminated liquid waste is discharged to the sewer system.

Animal carcass:- Contained in leak proof packaging as clinical waste and collected by a licensed clinical waste disposal agent for final destruction by incineration.

Bedding and semi-liquid animal waste from the bottom of the isolator:- Fumigated in isolator and then contained in leak proof packaging as clinical waste and collected by a licensed clinical waste disposal agent for final destruction by incineration.

The isolator:- Soaked in disinfectant (currently Virkon) and then washed. The washings are discharged to sewer.

Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The risk assessment was discussed by the genetic modification safety committee. Some minor comments were made and the risk assessments modified accordingly. The final version is attached. The committee considered that a Class 2 categorisation under the contained use regulations was appropriate given the nature of the GMMs involved and the level of containment appropriate to the work.

**Project Containment**

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<td>L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

02/03/2022
Establishment of attenuation of salmonella genetically modified mutants and attenuated salmonellas as vaccine vectors.

**Project Additional Information**

**Purposes of the contained use**

The salmonella strains developed during this programme of work may be used as a live vaccine for use in chickens under high containment to reduce colonisation by wild type C. jejuni or other pathogens of the chicken.

**Recipient or parental organism**

Salmonella spp
E. coli K12 and B strains and their derivatives.

**Host/vector system**

- Salmonella(1) expressing a C. jejuni gene on expression vector pYA3341Asd+(3)
- E. coli K12 and derivatives + pBR322 or pUC or pYA292 derived plasmids
- E. coli B and derivatives + pBR322 or pUC or pYA292 derived plasmids

1. Such as but not limited to strain X3987 (infect immun, 62, 5519), a triple mutated attenuation asd cya crp
1. Such as but not limited to cjA, a surface expressed solute binding protein
3. Such as but not limited to pYA3341Asd+(3), a derivative of pYA292 (Gene, 94, 29)

**Origin & function**

Gene(s) for immunogenic proteins of C. jejuni and other bacteria excluding known cytokines, superantigens, hyperallergens, and toxins except where these have been demonstrably and irreversibly inactivated.
Evaluation of foreseeable effects

The GMMs compared to the parent Salmonella strains should have:-

a) no increase in infectivity or pathogenicity;
b) no reversal of disabling mutation(s); and
c) in the event of inadvertent release, no increase in fitness for environmental survival or spread.

Consequently, overall there is a negligible likelihood that the genetic modification in the GMMs could result in harm with negligible consequences for the ecosystem, rare species, ubiquitous species or spread and persistence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory waste:
Liquid waste up to 5L or small scale solid laboratory waste are decontaminated by autoclave using a validated decontamination cycle (commission with TST test and 12 point thermocouple tests; ongoing monitoring by monthly TST test and 6 monthly review with 12 point thermocouple tests) giving 100% kill.

Liquid waste from production areas is transferred to a kill tank where it is decontaminated by steam sterilisation at 1260°C for 60 minutes. This method is validated by control system validation with correct execution of the temperature/time cycle as a failsafe for allowing opening of the discharge valve.

Solid waste is autoclaved and collected by a licensed clinical waste disposal agent for final destruction by incineration. Decontaminated liquid waste is discharged to the sewer system.

Clinical waste:
Animal carcasses:- Contained in leak proof packaging as clinical waste and collected by a licensed clinical waste disposal agent for final destruction by incineration.

Bedding and semi-liquid animal waste from the isolators:- Fumigated in isolator and transferred to leak proof packaging as clinical waste and collected by a licensed clinical waste disposal agent for final destruction by incineration.

The isolator:- soaked in disinfectant and then washed. The washings are discharged to sewer.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
The risk assessment was discussed by the genetic modification safety committee on 1st September 2005, some minor comments were made and the risk assessments modified accordingly. The final version is attached. The committee considered that a Class 2 categorisation under the contained use regulations was appropriate given the nature of the GMMs involved and the level of containment appropriate to the work.

**Project Containment**

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**Project Ref** 391/11.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
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<th>Class</th>
<th>CultureVol</th>
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<tbody>
<tr>
<td>06/09/2011</td>
<td>Animal studies with Bacillus subtilis spores expressing the C-terminal domain of the alpha toxin gene from Clostridium perfringens type A</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Class 2</td>
</tr>
</tbody>
</table>

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

A B. subtilis recombinant has been created that expresses the C-terminal domain of the alpha toxin (cpa 247-370) from Clostridium perfringens type A on the surface of the spore. This recombinant was constructed by the group of Prof S C using genetic technology developed at Royal Holloway College, University if London. This recombinant needs to be evaluated in chicken to demonstrate any potential for raising immune responses against alpha toxin in this species.
Recipient or parental organism

B. subtilis strain PY79, a derivative of the type strain 168. It is non-pathogenic and has GRAS (US FDA) status.

Host/vector system

Not relevant as the scope of experiments is limited to animal trials with the GMM that has been establishments previously by Professor C at the University of London. For the animal trials no genetic manipulations will be performed and vectors are therefore not involved.

Origin & function

The inserted gene codes for the C-terminal domain of the alpha toxin (cpa 247-370) from C. perfringens type A. This portion of the alpha toxin has no haemolytic or toxic activities (Titball et al. 1993, FEMS Microbiology Letters, 110, 45-50). However, antibodies raised to this non-toxic portion of the alpha toxin have been shown to protect mice against challenge with active whole alpha toxin (Stevens et al, 2004, Journal of Infectious Diseases, 190, 767-773).

Evaluation of foreseeable effects

B. subtilis is non-pathogenic and has GRAS (US FDA) status. It has no pathogenic traits and offers no risk to humans or animals. The inserted gene codes for the C-terminal domain of the alpha toxin (cpa 247-370) from C. perfringens type A. This portion of the alpha toxin has no haemolytic or toxic activities (Titball et al. 1993, FEMS Microbiology Letters, 110, 45-50), however it is responsible for the binding of the toxin to cell membranes (Naylor et al. 1999, Journal of Molecular Biology, 294, 757-770). There is therefore a very minor possibility that the expression of this protein could alter the tissue tropism of the recipient microorganism. However, the identical GMM described here has already been administered to mice with no detectable adverse effects on their health (Hoang et al. 2008, Infection and Immunity, 76, 5257-5265).

DNA transfer from B. subtilis to related micro-organisms has been described under a selection pressure (antibiotics). This would occur by transformation to similar species. Genetic transfer of this kind is recognised in most bacteria and is not unique to B. subtilis. Normally, transfer of genes is enhanced if the clone is carried on a plasmid vector since plasmid-borne transfer is exponentially more frequent. In this construct the recombinant DNA sequence is stably maintained within the chromosome and not on a plasmid thus the probability of genetic transfer is minimal.

Should recombinant spores be released accidentally, then as a soil organism it might be expected to survive indefinitely, in contrast to other species such as E. coli which would have limited long-term survival. While spores can survive 100s-1000s of years (Nicholson. 2002, Cellular and Molecular Life Sciences, 59, 410-416) it must be emphasized that the soil is rich in other bacteria and this GM B. subtilis would have to compete to survive. The type of strain used is a derivative of strain 168, a laboratory strain that has been routinely pasaged for over 60 years (Piggot and Coote. 1976, Bacteriology Reviews, 40, 908-962). It will have lost many of its original properties found in the soil-form. Thus, it is highly unlikely that the lab strain, even if released, would be able to compete with resident soil organisms. The C-terminal domain of the alpha toxin has no known environmentally hazardous properties

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable and reusable labware and laboratory waste are autoclaved at 134°C for 20 minutes. For non-autoclavable labware and surfaces a 70% alcohol, 1% Nuevo, 1% Virkon or a 1% Bioceleanse solution with a contact time of at least 10 minutes will be used.

Animal containment isolators are cleaned by removal of the solid waste, which is disposed of as clinical waste gassing with formaldehyde to a minimum concentration of 3000 ppm for a minimum of 8 hours, washing with hot water and a final gassing with formaldehyde as above. The clinical waste is disposed of by a licensed contractor. Animal carcasses are disposed of by incineration by an approved specialist company.
The GM safety committee have reviewed the risk assessment and agree with the assignment of this project to class 2.

### Project Containment

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### Project Ref 391/11.2

- **Date Ackn'd**: 07/09/2011
- **CU2 Project Title**: Animal studies with avian metapneumovirus (AMPV) expressing foreign genes
- **Class**: Class 2
- **Culture Vol Class**: < 1 Litre
- **Class Culture Vol Class**: Class 2
- **Class Culture Vol Class 3-4**: Consent Granted

- **Historical Significant Changes**: N
- **Historical Date of Additional Info**: Withdrawn N
- **Significant Change ID**: N
- **Date of Significant Change**: N

**Tick if notifying a connected programme of work**: N

**Project notified under transitional arrangements**: N
### Project Additional Information

#### Purposes of the contained use

To evaluate safety and immunogenicity in chickens under controlled laboratory conditions

#### Recipient or parental organism

**Parental Strains:**
Avian meta pneumovirus from the UK and Germany

#### Host/vector system

Not relevant since the scope of experiments is limited to animal trials with GMM that have been established previously by Dr N at the University of Liverpool. For the animal trials no genetic manipulations will be performed and vectors are therefore not involved

#### Origin & function

The genes inserted code for the major protective antigen of the model virus

#### Evaluation of foreseeable effects

**Hazards associated with the recipient microorganism**

AMPV does not infect humans. After 20 years of animal experiments, humans have been exposed to high aerosol doses, yet there has been no evidence of human infection. Also personnel exposed to high levels of virus during AMPV outbreaks on commercial poultry farms have not displayed any evidence of infection or disease. The AMPV #8544 strain causes mild to moderate clinical disease in turkeys for up to 10 days after which there is full recovery. In chickens, #8544 causes little disease. LAH strain is completely attenuated in chickens and turkeys. Commercial poultry are the most susceptible species hence any escape into the general environment would be highly unlikely to lead to infection in the much less susceptible wild bird population. As confirmed by field surveys, one very extensive in Italy, AMPVs of subtype A and B has never been detected in wild bird populations.

**Hazards arising directly from the inserted gene product (e.g. cloning of a toxin or oncogene)**

The protein expressed from the inserted gene is involved in tropism and host range, effects on these traits with AMPV as a vector can not be excluded with 100% certainty but the protein as such has never been associated with causing harmful effects to humans. Further, any effect on tropism or host range of the recipient microorganism due to this protein is considered very unlikely since mononegavirales such as AMPV and NDV are not able to incorporate the protein into the virion. Related genes have been expressed in numerous viral vector systems including adeno, herpes, pox and paramyxovirus species such as Newcastle Disease Virus (NDV). In none of these cases altered pathogenic properties were observed.

The potential hazards of sequences within the GMM being transferred to related microorganisms.

Very unlikely, indications for genetic transfer between AMPV strains or between AMPV and other members within the family of paramyxoviridae have never been observed.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not Applicable**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory waste will be autoclaved using a validated discard cycle (134°C for 20 mins) prior to incineration off site by an approved licensed contractor.

Animal containment isolators will be cleaned by removal of the solid waste, which is disposed of as clinical waste (see below), gassing with formaldehyde to a minimum concentration of 3000ppm for a minimum of 8 hours, washing with hot water and a final gassing with formaldehyde as above. Animal carcases and waste are disposed of by incineration by an approved licensed specialist company. Animal waste and carcases will be placed in sealed bins prior to incineration off site by an approved contractor. Bins will be either surface disinfected with 1% Virkon or fumigated with formaldehyde (see above) prior to release to the contractor for incineration.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

The GM safety committee, having reviewed the risk assessment agrees with the assignment of this project to class 2

Project Containment

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Animal Units

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Project Ref 391/12.1

<table>
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<tr>
<th>Date Ackn'd</th>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>27/02/2012</td>
<td>Rhodococcus Equi Vaccine (REV) Working Seed Preparation</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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</tbody>
</table>

Date Project Ceased  
02/03/2022
**Project Additional Information**

**Purposes of the contained use**
The purpose of this activity is to produce vials of freeze dried working seed in GMP environment for subsequent use in process transfer validation batches.

**Recipient or parental organism**
The GMM is Rhodococcus equi which has been modified to delete four genes, ipdA, ipdB, ipdA2 and ipdB2, involved in the cholesterol catabolic pathway. The resulting ΔipdABA2B2 double deletion mutant (=strain RG2837) was impaired in growth on the steroid catabolic pathway intermediate 3αo-H-4A (3'-propionic acid)-5α-o-hydroxy-7αß-methylhexahydro-1-indanone (5OH-HIP). In addition, strain RG2837 appeared hampered for macrophage survival and could be safely administered intratracheally to foals at a dose at which the parent strain induced pneumonia (van der Geize et al, 2011, PLoS Pathogens 7 (8) e1102181).

**Host/vector system**
Not Applicable

**Origin & function**
The bacteria is an unmarked gene deletion mutant

**Evaluation of foreseeable effects**
The vaccine strain is an unmarked deletion mutant which is attenuated for macrophage survival and therefore unable to colonise the lungs of foals and to cause pneumonia.

Genes, encoding for enzymes that are involved in the cholesterol catabolic pathway were deleted. As a consequence these enzymes are absent and the intermediate 3αo-H-4A (3'-propionic acid)-5α-o-hydroxy-7αß-methylhexahydro-1-indanone (5OH-HIP) cannot be catabolised. This results in attenuation of its most important virulence trait i.e. survival in macrophages and as a consequence in its ability to cause pneumonia.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not Applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
None applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
All waste materials, consumable items and contaminated equipment will be autoclaved on transfer from the contained facility using a validated cycle of 126°C for 30 minutes, providing complete inactivation. The autoclave is validated in accordance with BS EN 285 and is subject to annual requalification. Temperature within the
autoclave load and chamber drain, and chamber pressure are continuously monitored and recorded using calibrated equipment for each autoclave run.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC, having reviewed the risk assessment agrees with the assignment of this project to class 2

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 391/12.2

Date Ackn’d 19/03/2012

CU2 Project Title Animal studies with defined microbiological vectors expressing a range of Campylobacter jejuni genes

Class Class 2

Culture Vol Class 2 < 1 Litre

Culture Volume Class 3-4 Non-GMM Consent Granted

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

To evaluate various previously created microbiological vectors, expressing one or more of a range of Campylobacter jejuni genes, as potential vaccines in chickens. The evaluation will involve the administration of the constructs, by the oral route, to the chickens and may include the assessment of induction of appropriate immune responses and effects on subsequent colonisation of the birds by C. jejuni in a challenge model.

**Recipient or parental organism**

A range of previously created microbiological vectors including bacteria and protozoa.

**Host/vector system**

Not relevant as the scope of the experiments is limited to animal trials with GMMs that have been previously created by other research teams in other facilities. For the animal trials no genetic manipulations will be performed and vectors are therefore not involved.

**Origin & function**

- **pal**: Peptidoglycan associated lipoprotein
- **jlpA**: Surface-exposed lipoprotein that actuates pro-inflammatory response via NF-kB and p38 MAP kinase
- **ciaA**: Putative amino acid transporter periplasmic solute-binding protein
- **fiaA**: Flagellin protein
- **pglE**: UDP-4keto-6-deoxy-GlcNAc c4 amino transferase - knockout mutant abolishes flagella function and inhibits invasion of epithelial cells therefore affecting C. jejuni ability to colonise the intestine
- **cadF**: Outer membrane fibronectin-binding protein which promotes bacteria-host interaction and facilitates colonisation in the intestine
- **flpA**: Adhesin
- **laj1**: colonisation associated proteins
- **laj2**: colonisation associated proteins
- **laj3**: colonisation associated proteins
- **laj4**: colonisation associated proteins

**Evaluation of foreseeable effects**

All of the microbiological vectors are non-pathogenic to humans and animals and have GRAS (US FDA) status or are not recognised to be human pathogens and infection occurs exclusively in chickens or are suicide vectors that are believed to be non-pathogenic in humans. The inserted genes code for adhesins and/or colonisation factors of C. jejuni. The products of these genes appear to contribute to the ability of C. jejuni to persist in the chicken gut for long periods. None of them appear to have any direct pathogenic effects, however, these factors may be responsible for the attachment of C. jejuni to cell membranes. There is therefore a very minor possibility that the expression of these proteins could alter the tissue tropism of the recipient microorganisms.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable
Disposable and reusable labware and laboratory waste are autoclaved at 134°C for 20 minutes. For non-autoclavable labware and surfaces a 70% alcohol, 1% Nuevo, 1% Virkon or a 1% Biocleanse solution with a contact time of at least 10 minutes will be used. 3% Kilcox, with a contact time of 3 hours, will also be used as appropriate.

Animal containment isolators are cleaned by removal of the solid waste, which is disposed of as clinical waste, gassing with formaldehyde to a minimum concentration of 3000 ppm for a minimum of 8 hours, washing with hot water and a final gassing with formaldehyde as above. The clinical waste is disposed of by a licensed contractor.

Animal carcasses are disposed of by incineration by an approved specialist company.

The GM safety Committee, having reviewed the risk assessment agrees with the assignment of this project to class 2.

Project Containment

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Project Ref 391/12.3

Date Ackn'd 18/06/2012

CU2 Project Title Eimeria vector project

Class 2 CultureVolClass2 < 1 Litre

Consent Granted

Non-GMM

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

- Evaluation of the safety and efficacy of Eimeria as a vector for the expression of heterologous coding sequences

#### Recipient or parental organism

- Chicken Eimeria species including
  - Eimeria tenella
  - Eimeria acervulina

#### Host/vector system

- Not applicable, work will only be carried out with transformed parasites

#### Origin & function

- VP2 from infectious bursal disease virus (IBDV). This constitutes the major outer component of the virus capsid. gB, gD and gI from infectious laryngotracheitis virus (ILT).
- These are all vital envelope glycoproteins.

#### Evaluation of foreseeable effects

- The chicken Eimeria species are globally endemic parasites currently not rated by the Advisory Committee on Dangerous Pathogens (ACDP), but regarded as hazard group 1 by DEFRA. Chicken Eimeria are completely host and tissue specific and consequently pose no risk to human health or risk of spread in the environment other than through contact with chickens.
- Further, Eimeria spp are antigenically complex micro-organisms and their interaction with the host is mediated by a diverse series of proteins expressed in a sequential manner. Whilst it is considered possible that the expression of VP2 might increase the potential for modification of the host pathogen relationship, most likely related to an immunosuppressive effect, any risk to human health is considered minimal because of the exquisite host specificity of the recipient. Expression of any inserted genetic material is controlled by stage specific promoters, restricting expression to defined stages of the life cycle of the parasite. Thus completion of the full life cycle is unlikely in the event of any effect on host range.
- Genetic transfer of traits between different strains of the same species but not between different species has been recorded for Eimeria. It is therefore possible that heterologous sequences could be transferred to wild type strains of the same species, in the worst case this might involve transfer of the sequence from an attenuated (precocious) strain to a non attenuated strain.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- Not applicable

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For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Solid laboratory waste will be autoclaved using a validated discard cycle (134°C for a minimum of 10 minutes) prior to incineration off site by a licensed contractor.

Processed faecal suspensions will be sealed in plastic containers which will be surface disinfected using 3% Kilcox Extra prior to being sent off site for incineration by a licensed contractor.

Animal carcasses and isolator waste will be removed from the isolator in sealed plastic bags, sealed into plastic containers which will be surface disinfected using 3% Kilcox Extra prior to being sent off site for incineration by a licensed contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GM safety committee, having reviewed the risk assessment agrees with the assignment of this project to class 2

Project Containment

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Project Ref 391/13.1

Date Ackn'd 15/05/2013

CU2 Project Title Animal studies with double recombinants of Herpesviruses of turkeys expressing combinations of foreign viral genes

Class 2

CultureVolClass2 < 1 Litre

Non-GMM Consent Granted
Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To evaluate safety and efficacy in chickens under controlled laboratory conditions

Recipient or parental organism

Parental Strains:
The HVT parent strain is FC-126

Host/vector system

Not relevant since the scope of experiments is limited to animal trials with GMM that have been established previously. For the animal trials no genetic manipulations will be performed and vectors are therfore not involved.

Origin & function

Three of the foreign genes are viral envelope glycoproteins thought to play roles in the attachment of the virus to host cell receptors prior to fusion with the cell membrane and in the envelopment of the virus during egress form the cell. The final protein is a major structural protein and host protective immunogen.

Evaluation of foreseeable effects

HVT is currently classified in the subfamily of alphaherpesvirinae, and is also known as Meleagrid herpesvirus 1, turkey herpesvirus, or Marek's disease virus serotype 3. The virus was first described around 1970 as a herpesvirus infecting turkeys and sharing antigenic features in common with Marek's disease virus (MDV). Whilst MDV is highly pathogenic for chickens, HVT is non-pathogenic and could therefore be used for effective vaccination against infection and disease caused by MDV (Okazaki et al., 1970, Avian Diseases, vol. 14, p. 413-429). Since then, vaccination of chickens against MDV by using HVT has become part of the standard vaccination program of billions of chickens produced worldwide every year.

The HVT virion has all the features of a typical herpesvirus, and is about 160nm in size in its enveloped form. It possesses a large genome (approximately 159 kb) comprising linear double stranded DNA. The HVT genome has a long history of manipulation; in particular its non-pathogenic properties have lead to research into the use of HVT as a viral vector for expression and delivery of various proteins to the chicken. Examples are the expression of genes coding not only for antigens from other poultry pathogens such as: Infectious bursal disease virus (IBDV) (Darteil et al., 1995, Virology, vol. 211, p. 481-490), and Newcastle disease virus (NDV) (Sondermeijer et al., 2993, Vaccine, vol. 11, p. 349-358). But also the expression has been described of a parasite antigen (Cronenberg et al., 1999, Acta et al., 2007, Vaccine, vol. 25, p. 8529-8535) Such work has led to the development of commercial vaccines using such technology, including the Vaxxitech product range (Merial), the Vectormune HVT product range (Ceva) and the Innovax product range (Merck Animal Health), thus providing convincing data in a large number of birds of the safety of HVT vectored IBD, ND and ILT vaccines.
There are no perceived hazards associated with the recipient microorganism.

Hazards arising directly from the inserted gene product (eg cloning of a toxin or oncogene)
The gene products are involved in attachment and entry into host cells, replication and release. There are no reports in the literature indicating that these gene products have a direct influence on virulence. Overall the risk of hazards arising directly from the inserted gene products is considered low.

Hazards arising from the alteration of existing traits (eg alteration of pathogenicity, host range, tissue tropism, mode of transmission or host immune response)
HVT is a non-pathogenic alpha herpesvirus with a host range restricted to chickens and turkeys. Whilst it is considered unlikely that the introduction of coding sequences for two of the genes into HVT would significantly modify the existing host restriction because they originate from a virus causing an acute, highly contagious, infection limited to chickens and pheasants. For the remaining gene it is possible that the presence of the protein in the virus envelope could result in transmission to man. HVT however is typically cell associated and does not generate enveloped viral particles in the supernatant of infected cultures such as is seen with most other herpesviruses, although infectious cell-free virus has been detected in the feather dust of chickens vaccinated with HVT. Therefore the risk of hazards arising from alteration of the existing traits of the HVT vector is considered low.

The potential hazards of sequences within the GMM being transferred to related microorganisms. The HVT backbone of recombinant construct has been modified by the insertion of a fragment containing the heterologous gene and promoter sequence. There have been no gene deletions. The phenotype of the recombinant strains is therefore the same as that of the HVT backbone. If the inserted fragment with the heterologous gene would be lost, this would result in the wild type HVT, which is also avirulent.

There have never been reports of the recombination of HVT with other related herpesviruses of poultry, e.g. virulent (serotype 1) MDV or serotype 2, and the possibility of recombination with a virus capable of infecting humans is therefore considered extremely small. Genetic transfer to other organisms in the environment has never been described for herpesviruses, and is therefore considered to be unlikely.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Laboratory waste will be autoclaved using a validated discard cycle (134°C for a minimum of 10 mins) prior to incineration off site by an approved licensed contractor.

Animal containment isolators will be cleaned by removal of the solid waste, which is disposed of as clinical waste (see below), gassing with formaldehyde to a minimum concentration of 3000ppm for a minimum of 8 hours, washing with hot water and a final gassing with formaldehyde as above. Animal carcasses and waste are disposed of by incineration by an approved licensed specialist company. Animal waste and carcasses will be placed in sealed bins prior to incineration off site by an approved contractor. Bins will be either surface disinfected with 1% Virkon or fumigated with formaldehyde (see above) prior to release to the contractor for incineration.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

Y
The GM safety committee, having reviewed the risk assessment agrees with the assignment of this project to class 2.

### Project Containment

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### Project Ref 391/15.1

- **Date Ackn'd**: 11/11/2015
- **CU2 Project Title**: Development of a N-glycan based vaccine for use in chickens to aid in preventing infection and colonisation by Campylobacter jejuni
- **Class**: Class 2
- **Culture Volume**: 1-50 Litres

### Project Additional Information

- **Purposes of the contained use**: Proof of concept for the safety and efficacy of a N-glycan based vaccine and if successful the generation of safety, quality and efficacy data to be used for product registration
- **Recipient or parental organism**: Non-GMM

Project notified under transitional arrangements **N**
1. E. coli K12 W3110 laboratory strain (or other recognised non-pathogenic strains), which contains disabling mutations and is known to have a restricted ability to colonise the intestine and limited persistence in the environment. It has a history of safe commercial use and is not known to have adverse effects on micro-organisms or plants. The K12 strain used has a kanamycin cassette replacing the O-antigen polymerase (wzy) gene on the chromosome.

2. Avian pathogenic E. coli strains.

3. Avian 'commensal' E. coli strains

**Host/vector system**

E. coli vector pACYC 184: a commercially available cloning vector, origin of replication p15A, containing chloramphenicol (cat) and tetracycline resistance (tet) genes, which is mobilisation deficient.

**Origin & function**

The functional genes (pgl gene cluster of C. jejuni 81116): gne, pglK, pglH, pgll, pglJ, pgIB (with mutation in the WWDYG motif), pglA, pglC, pglDEFG and the waaC from the LOS locus upstream of gne transcribed in opposite direction) code for enzymes involved in polysaccharide biosynthesis and assembly. The mutation in pgIB (the oligosaccharyl transferase responsible for the transfer of glycan to the final protein) ensures that N-linked protein glycosylation does not occur. Instead the glycans are transferred to the E. coli lipid A to enable surface display on the outer membrane of E. coli.

The pgl gene cluster has been inserted into the tetracycline resistance gene on the parent plasmid and is constitutively expressed from the tetracycline resistance gene promoter. The tet gene was inactivated during the cloning process.

Functional operons with a reduced number of genes (including deletion of pgIB) within the cluster will also be evaluated.

**Evaluation of foreseeable effects**

i) Hazards associated with the recipient microorganism (eg bacterial host or viral vector)

Health and Safety

Executive

Contained Use Notification

E. coli K12 W3110 laboratory strain (or other recognised non-pathogenic strains), contain disabling mutations and is known to have a restricted ability to colonise the intestine and limited persistence in the environment. It has a history of safe commercial use and is not known to have adverse effects on micro-organisms or plants. The K12 strain used has a kanamycin cassette replacing the a-antigen polymerase (wzy) gene on the chromosome.

Avian pathogenic Escherichia coli. APEC is considered as both a primary and a secondary pathogen in the chicken and is a member of the extra-intestinal pathogenic E. coli (exPEC) pathotype along with human uropathogenic (UPEC) and neonatal meningitis-associated E. coli (NMEC). Data suggests that APEC strains can be readily transmitted to humans and there is evidence that some APEC strains are closely related to human exPEC strains.

Further, whole genome sequence analysis has revealed a high degree of similarity between APEC and exPEC (Zhu Ge et al (2014), Comparative genomic analysis shows that avian pathogenic Escherichia coli isolate IMTS5155 (02:K1:H5; ST complex 95; ST140) shares close relationship with ST95 APEC 01:K1 and human exPEC 018:K1 strains. PLoS ONE 9:11: e112048. DOI:10.1371/journal.pone0112048). PCR based phylotyping and multi-locus sequence typing have also revealed a link between APEC and human exPEC. Overall it is therefore considered that APEC possess zoonotic potential. (reviewed by Dziva and Stevens (2008), Coli bacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic Escherichia coli in their natural hosts. Avian Pathology, 37:4, 355-366.)
(ii) Hazards arising directly from the inserted gene product (eg cloning of a toxin or oncogene)

The inserted genes code for enzymes involved in the N-linked general protein glycosylation pathway of Campylobacter jejuni, resulting in the synthesis and transfer of a heptasaccharide glycan to lipid A which is surface expressed on the outer core lipooligosaccharide of the recipient E. coli. There is no indication of toxicity associated with the novel glycan.

(iii) Hazards arising from the alteration of existing traits (eg alteration of pathogenicity, host range, tissue tropism, mode of transmission or host immune response)

The biological significance of protein glycosylation in C. jejuni is unclear, however mutants with disrupted glycosylation have been constructed and shown to have both reduced capacity for attachment and invasion of human epithelial cell lines and impaired colonization of mouse and chicken intestinal tracts (Szymanski, Burr and Guerry (2002) Campylobacter protein glycosylation affects host cell interactions. J. Bacteriol. 70, 2242-2244; Hendrixson and DiRita (2004) Identification of Campylobacter jejuni genes involved in commensal colonization of the chick gastrointestinal tract. Mol. Microbiol. 52,471-484; Jones et al (2004) Adaptation of Campylobacter jejuni NCTC1168 to high-level colonization of the avian gastrointestinal tract. Infect. Immun. 72, 3769-3776; Karlyshev et al (2004) The Campylobacter jejuni general glycosylation system is important for attachment to human epithelial cells and in the colonization of chicks. Microbiology, 150, 1957-1964). Overall however these results are difficult to interpret due to alterations in the glycosylation of multiple C. jejuni proteins which could result in many variable effects including protection against proteolytic cleavage, solubility, protein assembly and antigenic variation as well as adhesion. Since the oligosaccharyl transferase gene pglB is either mutated or deleted from the inserted biosynthetic pathway Nglycosylation of E. coli proteins cannot occur, so the likelihood of any significant effect on colonisation potential through the modification of recognised E. coli adhesins is extremely low. Surface expressed recombinant glycan however could interact with lectin-like receptors on the host cell surface which has the potential to enhance bacterial adhesion. Even in the presence of an adhesin, specific virulence in E. coli is further associated with a combination of toxins and functional genetic elements which enable the strain to cause disease (Kaper, Nataro and Mobley (2004) Pathogenic Escherichia coli Nature Reviews: Microbiology 2, 123-140). It is thus unlikely that the presence of the recombinant glycan alone would be sufficient to enhance its virulence over that of the parental strain.

(iv) The potential hazards of sequences within the GMM being transferred to related microorganisms

Transfer of the operon to related microorganisms provides the potential for the introduction of a functional Nglycosylation pathway which whilst unable to glycosylate proteins could result in surface expression of recombinant glycan. The use of a non-mobilisable plasmid or the chromosomal integration of the operon minimise the likelihood of the successful transfer of the entire pathway. This pathway whilst highly conserved within Campylobacter sp. does not exist within other bacteria. It is therefore unlikely that this will provide any fitness advantage to the bacterium.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Laboratory waste materials will be subjected to autoclaving using an approved discard cycle (e.g. 134°C for 10 minutes) prior to disposal as clinical waste by an approved contractor. Re-usable glassware will be decontaminated by autoclaving using a validated cycle (e.g. 121°C for 20 minutes) before cleaning.
Small scale fermenters will be decontaminated using 1% Virkon. The vessel and associated connectors and tubing will be filled with freshly prepared 1% Virkon and left for a minimum of 12 hours. The condenser cannot easily be filled with Virkon and will therefore be removed and decontaminated by autoclaving or using Virkon separately. Animal carcasses and bedding will be placed into sealed bins, which will be surface disinfected or fumigated prior to removal from the animal room and sent for incineration by an approved contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project and its associated risk assessment has been reviewed by the GMSC and has been categorised as class 2.

Project Containment

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Project Ref 456/01.1

Date Ackn'd 11/01/2001

CU2 Project Title LIVE POXVIRUS VECTORED ANTI-VIRAL FELINE VACCINE 1.

Class Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Not Applicable

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

02/03/2022
### Project Additional Information

#### Purposes of the contained use

Evaluation of poxvirus vectored feline viral antigens for vaccination of cats.

#### Recipient or parental organism

- Non-modified poxvirus with no markers or attenuations prior to use. Biological Hazard and MAFF hazard group 1.
- Only produces disease in its natural host. Is not known to produce disease in humans.

#### Host/vector system

Host is poxvirus as described above.

#### Origin & function

- Inserted gene sequences derived from a feline virus, feline genes from feline peripheral blood mononuclear cells and marker genes derived from E. coli. The genes and their regulatory elements are not known to affect virulence, survival in the host, survival in the environment or increase resistance to therapeutic agents. The donor genes are not known to be involved in host range, virulence or expansion of tissue tropism.

- Insertion of the genes into the parental viruses confers an attenuated plaque phenotype.

- The inserted genes are intended to induce protection against the parental organism following vaccination of cats with the GMM.

#### Evaluation of foreseeable effects

The GMM is only known to cause disease in its natural host. The inserted sequences are not expected in increase the survivability of the GMM in the environment or increase its host range of tissue tropism. The use of category 2 containment reduces any risk to the environment and therefore exposing the natural host to effectively zero. The GMM is not known to cause disease in humans and category 2 containment reduces any risk of exposure to effectively zero.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be decontaminated by autoclaving using a validated temperature cycle designed to achieve 100% killing.

Liquid waste will be heat treated using a validated temperature cycle designed to achieve 100% killing.

No waste will be disposed off containing any live organisms.

The risk assessment and notification form was reviewed by the GMM Safety Committee on 15/12/00. All elements were debated and the committee found that the information provided was in accordance with the 2000 regulations. The containment level applied was deemed to be appropriate for the activities and scale in order to reduce all risk to effectively zero.

Please enter comments on the GM safety committee on the risk assessment

The risk assessment and notification form was reviewed by the GMM Safety Committee on 15/12/00. All elements were debated and the committee found that the information provided was in accordance with the 2000 regulations. The containment level applied was deemed to be appropriate for the activities and scale in order to reduce all risk to effectively zero.

Project Containment

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Project Ref 456/05.1

Date Ackn’d 29/04/2005

CU2 Project Title Storage of seed materials and associated laboratory work targeted at formulating an experimental vaccine.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Non-GMM Consent Granted

Not Applicable
Project Additional Information

Purposes of the contained use

To provide vaccine for potential evaluation in controlled laboratory studies or in the field.

Recipient or parental organism

a) The parental Mannheimia haemolytica is a field isolate originally cultured from bovine pneumonic lung tissues.

B) The parental Pasteurella multocida is a field isolate originally cultured from pneumonic lung tissue. This strain is highly virulent and is capable of replication in the bovine lung.

These are both classified as ACDP hazard group 2 and had no genetic modifications carried out prior to the production of the deletant strains.

Host/vector system

Not applicable.

Origin & function

Attenuation of the virulence of Mannheimia haemolytica and Pasteurella multocida by virtue of gene deletions, whilst maintaining a suitable immune response in the host animal.

Evaluation of foreseeable effects

The deletant strains are likely to retain the features of the parental organism with respect to host range, tissue tropism and mode of transmission. They will however be significantly attenuated with respect to virulence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The level of attenuation of these organisms is such that the measures applicable to containment level 1 are appropriate. However, since the mode of transmission is unlikely to have been affected, work with open vessels where there is a risk of aerosol generation will be carried out within a microbiological safety cabinet.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The risk assessment and notification form were reviewed by the GMSC on 31 January 2005. All elements were debated and the committee found that the information provided was in accordance with the 2000 regulations. The containment level applied was deemed to be appropriate for the activities and scale in order to reduce all risk effectively to zero.

**Project Containment**

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**Project Ref** 456/05.2

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<th>Project notified under transitional arrangements</th>
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<td>26/08/2005</td>
<td>The Development of a vaccine as an aid in preventing losses due to Marek's disease.</td>
<td>Class 2</td>
<td>1-50 Litres</td>
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Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N
**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Evaluation of immunogenicity, potency and safety in chickens.

**Recipient or parental organism**

The construct is a novel avian herpesvirus, consisting of regions of herpesvirus of turkeys (HVT) and of Marek's disease virus (MDV).

A. The parental HVT is ubiquitous in domestic turkeys and widespread in chicken populations where vaccination with HVT has been practiced since the early 1970s. It is not considered to be pathogenic for turkeys or chickens (Cainek BW HW Jr. Iowa State University Press, Ames, Iowa pp342-385).

B. The parental MDV causes a lymphoproliferative disease that affects the peripheral nervous system and other organs.

**Host/vector system**

Not applicable.

**Origin & function**

The construct contains MDV genes which are not known to affect virulence in and of themselves. Deletion of these genes has been shown to have no affect on the transforming potential of the virus. Further, these genes do not include the viral genes and regions known to be associated with virulence and oncogenicity in MDV. In the construct these have been replaced by the equivalent regions of HVT genome.

**Evaluation of foreseeable effects**

Neither of the parental strains are known to be human pathogens. The construct is expected to have similar traits to that of an approved avirulent vaccine strain of HVT.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Although the GMM has been shown to be avirulent, full evaluation of the potential for shed and spread is incomplete. In consequence, any animal work will be carried out in a facility with HEPA filtered extract air in order to provide enhanced protection of the environment. For studies involving subcutaneous or intramuscular vaccination of animals, syringes will be filled in a safety cabinet, but to reduce the risk of needlestick injury, injection of the animals will take place on the open bench.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid waste will be decontaminated by autoclaving using a validated temperature cycle designed to achieve 100% killing.

Liquid waste will be heat treated using a validated temperature cycle designed to achieve 100% killing.

No waste will be disposed of containing any live organisms.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

02/03/2022
The risk assessment were reviewed by the GMSC on 15 July 2005. All elements were debated and the committee found that the information provided was in accordance with the 2000 Regulations. The containment level applied was deemed to be appropriate for the activities and scale in order to reduce all risk effectively to zero.

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Project Ref 456/07.1

Date Ackn’d 13/04/2007

CU2 Project Title The development of a vaccine to provide passive protection to the new born calf against clinical disease caused by Cryptosporidium parvum.

Class Class 2

CultureVolClass2 1-50 Litres

Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Evaluation of potential constructs for the expression of the gene of interest, process development and scale up to 40L.

Recipient or parental organism

The recipient E. coli strain is a weakened laboratory adapted mutant and is regarded as non pathogenic. It is expected that the survivability of the bacterial host in the environment will be poor.

Host/vector system

The plasmid comprises a heat-inducible expression plasmid with a pUC origin of replication and tetracycline resistance gene plus the coding sequence for the gene of interest. The plasmid is non conjugative and there is therefore minimal risk of transfet to other micro-organisms.

Origin & function

The gene sequence has been obtained from Cryptosporidium parvum and codes for a structural protein. It is not a known virulence determinant.

Evaluation of foreseeable effects

Not applicable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be decontaminated by autoclaving using a validated temperature cycle designed to achieve 100% killing.

Liquid waste will be heat treated using a validated temperature cycle designed to achieve 100% killing.

No waste will be disposed of containing any live organisms.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The risk assessments were reviewed by the GMSC on 5th March 2007. All elements were debated and the committee found that the information provided was in accordance with the 2000 regulations. The containment level applied was deemed to be appropriate for the activities and scale in order to reduce all risk to effectively zero.
## Project Containment

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- L4

**Large Scale Activities**
- L2
- L3
- L4

**Human Clinical Applications**
- L2
- L3
- L4

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### Project Ref 456/trans1

**Date Ackn'd**
21/02/2001

**Date Project Ceased**

**CU2 Project Title**
DEVELOPMENT OF BACTERIAL DELEANT VACCINES

**Class**
Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM Consent Granted**
Not Applicable

**Project notified under transitional arrangements**
N

---

### Project Additional Information

#### Purposes of the contained use

#### Recipient or parental organism

#### Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref  456/trans2

Date Ackn'd  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4

02/03/2022  

Page 7061 of 15326
## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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**Name**

THE INSTITUTE OF SCIENCE AND TECHNOLOGY IN MEDICINE

**Name 2**

ISTM UNIVERSITY HOSPITAL OF NORTH STAFFORDSHIRE

**Department**

KEELE UNIVERSITY MEDICAL SCHOOL

**Campus Estate or Research Centre**

HARTSHILL CAMPUS

**Building**

FACULTY OF HEALTH

**Road Name**

THORNBURROW DRIVE

**District**

HARTSHILL

**Town**

STOKE ON TRENT

**County**

STAFFORDSHIRE

**Postcode**

ST4 7QB

**Country**

ENGLAND

**Tel Number**

01782 554226

**Fax Number**

01782 747319

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

**Date at Which Additional Info Submitted**

01/10/2004
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
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<th>Laboratory</th>
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<th>Large Scale</th>
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- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify) Tick if confidential

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<th>Bacteriology</th>
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<th>Microbiology Research</th>
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Maximum culture volumes are 1 litre (routinely only 10-15 ml) and all organisms are destroyed by autoclaving at 121 degrees C for fifteen minutes. The effectiveness of this procedure will be monitored periodically using a thermocouple to ensure correct operation of the autoclave. Autoclaved organisms that are incapable of growth in either solid or liquid media are deemed non-viable.

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Maximum culture volumes are 1 litre (routinely only 10-15 ml) and all organisms are destroyed by autoclaving at 121 degrees C for fifteen minutes. The effectiveness of this procedure will be monitored periodically using a thermocouple to ensure correct operation of the autoclave. Autoclaved organisms that are incapable of growth in either solid or liquid media are deemed non-viable.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 392/19.1**

**CU2 Project Title**

1. Use of lentivirus to alter the expression in cell lines of genes involved in the action of anti-cancer drugs.
2. Reprogramming of human somatic cells to stem cells using lentivirus or sendai virus in cell culture for in vitro modelling of patient cells.

**Date Ackn'd**

01/03/2019

**Date Project Ceased**

**Class**

Class 2

**CultureVol**

< 1 Litre

**Consent Granted**

Non-GMM

Project notified under transitional arrangements

**Withdrawn**

N

**Tick if notifying a connected programme of work**

N

---

**Historical Significant Changes**

**Historical Date of Additional Info**

02/03/2022
## Purposes of the contained use

1. **ANTI-CANCER DRUGS**
   Lentivirus will be used to help identify targets for the development of novel anticancer drugs and to understand the mechanism of action of the drugs.

2. **SOMATIC CELL REPROGRAMMING**
   'Replication-incompetent' 'genome-integrating' viral vectors (using Lentivirus or LeV) or 'replication-incompetent' 'non-integrating' viral particles (using Sendai virus or SeV) will be used to reprogramme human somatic cells to induced pluripotent stem cells.

## Recipient or parental organism

1. **ANTI-CANCER DRUGS**
   Third generation lentiviruses will be modified to encode constructs that either increase (cDNA) or decrease (shRNA) the expression of genes involved in the activity of cancer drugs. This may include both oncogenes and tumour suppressors. The lentiviruses will then be used to transduce cultured normal or cancer cell lines.

2. **SOMATIC CELL REPROGRAMMING**
   Primary human fibroblasts and primary blood cells (Peripheral blood mononuclear cells). Primary cells from patients will be screened through a rigorous ethical screening process that will exclude patients with risk of infection. Uncharacterised cell lines may still be associated with endogenous biological agents (e.g., blood borne viruses). HEK293FT cell line will be used to package the Lentivirus.

## Host/vector system

1. **ANTI-CANCER DRUGS**
   3rd generation Lentivirus particles from commercial vendors (e.g. Thermofisher or Origene) will be generated in a packaging cell line (eg HEK293T) following transfection with plasmids encoding the viral proteins. The viral particles will be used to transduce cultured established cancer cell lines including Ovcar4, COV-362, COV318, MDAMB-231, MDAMB468 cells.

2. **SOMATIC CELL REPROGRAMMING**
   ViraPower™ Lentiviral Expression System, (Thermofisher), is a third-generation system consisting of the following components: (1) A pLenti-based expression vector into which the reprogramming genes (Oct4, Sox2, Klf4, c-Myc/l-Myc) will be cloned, (2) the ViraPower™ Packaging Mix that contains an optimized mixture of the three packaging plasmids, pLP1, pLP2, and pLP/VSVG, and (3) an optimized HEK 293FT producer cell line (called BL-15) that stably expresses the SV40 large T antigen under the control of the human CMV promoter and facilitates optimal production of virus.
   Sendai Virus kit is supplied by Thermofisher (Cat. No. A16517, A16518). SeV vectors used in this kit consist of viral proteins NP, P, M, F (activated), HN, and L, and the SeV genome RNA, from which the F gene is deleted.

## Origin & function

1. **ANTI-CANCER DRUGS**
   The genetic material will be purchased from commercial sources, for example Thermofisher ("ViraPower") or Origene ("LentiORF, lenti shRNA"). Standard molecular biology techniques will be used to incorporate the desired genetic material into a plasmid encoding the relevant part of the lentivirus where this is not already commercially available.
The intended function is to either identify and validate potential novel drug targets or to analyse the mechanism of action of candidate drug molecules.

2. SOMATIC CELL REPROGRAMMING
LeV kit (ViraPower™ Lentiviral Expression System) is supplied by Thermofisher (Cat. No. K4950-00). The procedure is designed to produce ‘replication-incompetent’ ‘genome-integrating’ viral vectors.
SeV kit (Sendai Virus kit) is supplied by Invitrogen (Cat. No. A16517, A16518). The procedure is designed to produce ‘replication-incompetent’ ‘non-integrating’ viral particles.
Both procedures to achieve reprogramming of human somatic cells to induced pluripotent stem cells. Somatic cells such as patient blood cells or patient fibroblasts (post surgical tissues or from pre-screened commercial cell banks) will be used for this reprogramming.

Evaluation of foreseeable effects

1. ANTI-CANCER DRUGS
There is not anticipated to be any effect on the virus of introducing the genetic constructs into the viral genome. The virus is replication-defective and this is not expected to change. The infectivity of the virus is also not expected to change.
The effect of the genetic modification on the cultured cells transduced with the virus will be assessed with a range of cancer cell biology techniques. These will include cell growth assays, immunoblotting, microscopy, QPCR, assays measuring cell death, apoptosis, autophagy and metabolic changes (eg changes in branched chain amino acids). The anticipated changes in the transduced cells include changes in the growth characteristics, for example altering the ability of the cells to grow in an anchorage independent manner or independently of growth factors. The sensitivity of the cells to other anti-cancer drugs may be altered. This may increase the ability of these cells to form tumours in immunocompromised individuals.
If personnel are inadvertently exposed to lentivirus, it is possible that the cells infected by the virus become predisposed to cancer because the cells may acquire the deregulated growth characteristics described above. The virus is not able to replicate, so the infection is not expected to spread beyond the directly infectly cells. The risk assessment has identified that the proposed work should be considered class 2.

2. SOMATIC CELL REPROGRAMMING
The nature of the vector (both LeV and SeV being replication-incompetent) is such that they do not endow the genetically modified micro-organism with a phenotype likely to cause toxicity to humans, animals or plants, or likely to cause deleterious effects on the environment. There is a possibility that DNA could become integrated into genomic DNA of personnel if accidentally introduced although this is likely to occur with a very low frequency and not more than the standard mutation rate in cells. Bearing in mind that some of the targeted sequences are implicated in carcinogenesis, there is, therefore, a very small risk of the personnel incorporating cancer promoting factors into a small number of cells. Despite the inclusion of the safety features associated with the two types of systems, the virus produced with these systems can still pose some biohazardous risk since it can transduce primary human cells. Therefore, an SOP for containment level 2, class 2 GMMs will be in operation as outlined in Risk Assessment.

Characterisation of GMO from reprogramming will include
1. Identification of reprogrammed colonies - by live/imunostaining
2. Assessing growth rate and morphology for abnormalities
3. Absence of viral genome using PCR after >10 passages
4. Pluritest to confirm pluripotency of GMO

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Liquid waste containing high titre virus (viral stocks and from cells up to 1 passage after infection) will be collected into sealable polypropylene container containing 1/10 maximum volume of 10% Sodium dodecyl sulfate (final concentration at least 1% SDS) and left for at least 1 hour. Used tips and other small plasticware items will be placed in the same container. The liquid container will be closed and placed in a clinical waste bag for transport to the autoclave. At the autoclave, the bagged bottle will be placed inside the autoclave and the lid of the container will be loosened without opening the waste bag. After autoclaving, the liquid will be poured down a dedicated sink with copious amounts of water and solid items collected through a sieve transferred to normal clinical waste using gloves.

2. Liquid waste expected to contain a low titre of virus (eg from cells 2 passages after infection) will be collected into container containing 1/10 maximum volume of 10% Distel (final concentration at least 1%). After 24 hours, the liquid will be poured down the dedicated sink in the containment lab with copious amounts of water.

3. For large plastic solid wastes in contact with virus (eg. pipettes, culture flasks, pots, etc), the waste will be first soaked in 1% Distel in a sharps bin for 24hrs to inactivate the virus. After 24hrs, the lid will be attached but the seal left open to allow the liquid to be poured down the dedicated sink in the containment lab with copious amounts of water. The sharps bins will be sealed completely, placed in a clinical waste bag and sent for incineration unless it has come into contact with high titre virus (as defined above) in which case it will be placed inside an autoclave bag, with tissues to collect drips, then a further bag and inactivated by autoclaving and then sent for incineration.

4. Inactivation of waste by autoclave as indicted above will be for 60 min at 135°C. A Crossmark stick is included in every autoclave cycle to ensure correct operation. The autoclave is routinely calibrated to ensure it reaches the desired operating temperature and is in the same building as the containment laboratory.

5. The degree of virus inactivation is expected to be >4 log unit reduction by Distel alone and > 9 log unit reduction by disinfectant followed by autoclaving. Other methods may be necessary to inactivate virus before removing samples from the containment laboratory for analytical procedures. These will include treatment with 4% paraformaldehyde (eg prior to microscopy) or boiling in SDS (eg prior to electrophoresis). These methods will be validated by measuring the virus titre in the treated samples.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
1. Is autoclaving necessary?
Response - this is planned for high titre waste only. Because the virus may predispose anyone infected with it to cancer, a greater degree of cell kill than achieved by disinfection alone (4 log units) is desirable.

2. How will inactivation of the virus be confirmed before removing samples from the laboratory?
Response - for every experiment a point in the experimental protocol has been identified where it is anticipated the virus will be inactivated. These will be formally demonstrated to inactivate the virus before the samples are removed from the laboratory. Examples include treatment with 4% paraformaldehyde or 10% trichloroacetic acid or heating to 95 degrees C in 0.1% SDS.

3. How will viral stocks be stored?
Response - a dedicated space in a -80 freezer has been identified. Access to this will be restricted by a lock and samples will be clearly labelled to indicate the presence of viable virus. The laboratory door will also be labelled. Access to the laboratory area is restricted by card control.

**Project Containment**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

**Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
The maximum culture volume to be released at any one time is estimated to be two litres.

The deactivation method of preference is to autoclave. The autoclave is subject to a recorded system of routine maintenance and testing. The deactivation method by autoclave for particular waste streams will be validated by trial and analysis.

The ongoing monitoring will cover verification of autoclave service and performance records, verification of method compliance by staff, and spot analysis of deactivated waste. Heat sensitive tape will be used to designate autoclavable waste and indicate heat treatment.

Should experience or risk assessment indicate that deactivation by autoclave will not be suitable, other methods such as chemical destruction or sterilisation will be considered.

### For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Mycology**  **Transgenic**  **Transgenic**  **Other (please specify below)**

**Invertebrates**  **Plants**
### GM Centre Number: 396

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**Name**

| Name                  | HERIOT-WATT UNIVERSITY |

**Department**

| Department            | BIOLOGICAL SCIENCES  |

**Campus Estate or Research Centre**

| Building              |                        |

**Road Name**

| Town                  | EDINBURGH               |

**District**

| District              | RICCARQON              |

**Town**

| County                | EAST LOTHIAN           |

**County**

| Postcode              | EH14 4AS               |

**Country**

| Country               | SCOTLAND               |

**Tel Number**

| Tel Number            | 0131 451 3187          |

**Fax Number**

| Fax Number            | 0131 451 3009          |

**E-mail**

| HSE Division          | SCOTLAND               |

**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022 | 00:00:00 | Page 7074 of 15326 |

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07/11/1990

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07/11/1990
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 1 (GMMs)</td>
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Other (please specify)  
Tick if confidential

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<th>Parasitology</th>
<th>Transgenic Birds</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>
The maximum culture volume that could be released at any one time is 500 ml. GM waste is autoclaved within 24 hours. Autoclave performance is monitored at least weekly using Bacillus stearothermophilus spore strips (Oxoid).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

Project Ref 396/09.1

Date Ackn'd 25/08/2009

CU2 Project Title Construction and analysis of transgenic E. coli and Yersinia ruckeri bearing Y. ruckeri genes encoding potential enhancers of virulence with respect to salmonid fish

Date Project Ceased

Class 2 CultureVolClass2 < 1 Litre CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Farming of trout and salmon is an important commercial activity in the UK and is a sustainable means of ensuring an adequate supply of essential nutrients in the human diet. However, farmed fish are particularly susceptible to disease as they are stocked at high density and may be kept in less than optimal conditions. One particular disease of trout and salmon is called enteric redmouth (ERM) and is caused by the gram-negative bacterium Yersinia ruckeri. This bacterium occurs as a number of different strains, the most common of which is known as serovar O1, or the Hagermann strain. The disease has been largely controlled by application of an oral vaccine but in recent years, a novel strain called EX5 which is not controlled by the vaccine has made an appearance and is causing problems in fish farms. We have been analysing the difference between the O1 and EX5 strains by a proteomic-based approach and have noted a number of proteins that differ between the strains. For example, one of these is identified as the outer membrane protein OMPA, which appears to be greatly over-expressed in the EX5 strain relative to O1. We are interested in analysing the properties of the OMPA gene from EX5. We will also investigate those EX5 genes encoding other proteins (as yet unidentified) found to differ substantially in expression between O1 and EX5.

The sequence encoding the promoter and coding region of OMPA (and other proteins) from Yersinis ruckeri strain EX5 will be amplified by PCR methods and cloned into the vector pBluescript KS and replicated in the K12 E.coli strain XL1-Blue (Stratagene). This plasmid will then be recovered and introduced into Yersinia ruckeri strain O1. We will then monitor the effect of phagocytes and serum from vaccinated and unvaccinated fish (trout, Orynchus mykiss) on the genetically modified O1 strain, wildtype O1 and also EX5 Yersinia ruckeri. In addition we will introduce the modified O1 strain (and wildtype control strains) into vaccinated and unvaccinated trout by intraperitoneal injection, and monitor for signs of enteric redmouth disease.

Recipient or parental organism

Recipient organisms: Escherichia coli K12 XL1-Blue (Stratagene), Yersinia ruckeri O1 (Hagermann strain)
Parental organism: Yersinia ruckeri strain EX5

Host/vector system

Host system Escherichia coli K12 XL1-Blue (Stratagene), Yersinia ruckeri O1 (Hagermann strain)
Plasmid vector pBlueprint KS (Stratagene)

Origin & function

Initially we will concentrate on the gene encoding OMPA from Yersinia Ruckeri. OMPA is an outer membrane protein found in all gram-negative bacteria, required for outer membrane stability and may be involved in attachment of bacteria to host cells. Genes encoding other proteins that differ between the O1 and EX5 strains (as yet unidentified) will also be cloned and analysed in the same way. It is anticipated that these genes might encode, for example, elicitors of defense responses (so-called pathogen-associated molecular patterns) or secreted elicitors of pathogenicity. We may also isolate and characterise genes encoding homologous proteins from related bacteria (for example, Yersinia enterolitica).

Evaluation of foreseeable effects

The most potentially hazardous GMM to be produced will be Yersinia ruckeri strain O1 carrying plasmids bearing the OMPA gene (or other pathogenicity determining genes) from Yersinia ruckeri strain EX5. If OMPA (or other proteins) play a major role in the virulence of strain EX5, this GMM should resemble strain EX5 in its properties when fish are challenged with it; that is fish vaccinated against Yersinia ruckeri O1 will be susceptible to the recombinant line. The GMM will thus be as pathogenic (but no more than) the EX5 strain. Should an EX5-derived pathogenicity factor be transferred to related pathogenic microorganism, there is the possibility that virulence of these organisms could be enhanced. The genes in question will be plasmid-borne, so although the potential for gene transfer is relatively high under particular circumstances, there would have to be selection pressure for such plasmids to be retained in the environment. It is not considered probable that one single factor will convert a non-pathogenic organisms into a pathogenic one. Yersinia ruckeri is not a pathogen of humans or mammals, there are no risks to humans from any engineered strains of Yersinia ruckeri planned in this work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
No non-microbial GM work is planned, however the GMO's may be tested against (non-GM) fish. This will be done in our contained fish facilities, all materials (including water) will be autoclaved or disinfected prior to disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Manipulations that might cause aerosols will be undertaken in a safety cabinet. Solid waste, fish and bacterial cultures on plates will be autoclaved at 121 degrees C for 20 minutes. Autoclaved waste will be disposed of together with other waste from the School of Life Sciences. Liquid waste and water from infected fish tanks will be treated with Chloros before disposal in an appropriate drain. This disinfectant is routinely used for treatment of potentially infected liquids in our laboratories; microbiological safety is routinely monitored in the fish laboratories.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

The Heriot-Watt Genetic Modification Safety Committee (6th May 2009) has reviewed the risk assessment for this project and there were no comments of note made.

**Project Containment**

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**Project Ref**  396/13.1

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<td>19/02/2013</td>
<td>Expression and RNA based knockdown of components of intracellular signal transduction pathways in mammalian cells using retroviral vectors</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<td>02/03/2022</td>
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We plan to use retroviruses to express, or knock down the expression of, intracellular signalling molecules in cultured mammalian cells and investigate the effects of these interventions on signal transduction pathways and assayable cellular processes, such as gene expression, migration, proliferation etc. Our core area of research is the class I PI3K signal transduction network and its significance in cancer.

The retroviruses will be previously developed laboratory vectors that have been modified, according to the originators designs, to express, or knock down the expression of, intracellular signalling molecules, including protein and lipid kinases and derived mutants (e.g. PI3K, AKT, InsR), lipid and protein phosphatases (e.g. PTEN, SHIP2) and associated proteins (e.g. adaptor, scaffolding proteins, substrates).

We aim to generate the retroviruses by packaging in cultured mammalian cell lines (often HEK293T cells). We will then use these prepared retroviruses to infect commonly used tissue culture cell lines (eg HEK293, HeLa, L6, 3T3-L1, U87MG, 1321N1, MCF10A and MDCK cells) or embryonic stem cells, hepatocytes, fibroblast and myoblast cells derived from rodents, with the purpose of modifying gene expression in these target cells.

Viral vectors and viruses

Viral vectors will either be engineered expression vectors, containing cDNAs encoding signalling molecules to be transcribed and translated, or be 'knock-down' vectors, encoding specific short hairpin RNA molecules for RNA interference. Examples of the vectors that will be used include:

Lentiviral/Retroviral expression vectors

pHR-SIN-CSGW, derived from human immunodeficiency virus 1 (HIV) genome but with viral genes deleted (Ikeda et al, Nature Biotechnology, 2003). This vector contains the viral long terminal repeat (LTR) but with the U3 region deleted in the 3’ LTR to prevent viral enhancer and promoter transfer into target cells, thus rendering it a so-called ‘self inactivated vector’. Derivatives of this plasmid with altered restriction sites for cDNA insertion will be used. The vector also contains the Rev Response Element (RRE) to enhance expression, packaging signals, the HIV central polypurine tract (cPPT) to increase viral titre and a WPRE (Woodchuck hepatitis virus post-transcriptional regulatory element) to enhance mRNA stability. Expression of the inserted gene comes from a SFFV (spleen focus forming virus) promoter. This vector is routinely packaged as a 3 component system to avoid the production through recombination of replication competent viruses.
The retroviral vector pWZL hygro (Serrano et al, 1996, Cell, 88, p593), based upon Murine Moloney sarcoma virus, contains long terminal repeats, the viral gag gene and an inserted hygromycin resistance cassette, within a bacterial plasmid vector. To produce infective virus particles, the virus requires pol and env sequences provided by co-transfection or a packaging cell line.

 Lentiviral/Retroviral RNAi vectors

pLKO.1 (Addgene) is a replication incompetent self inactivating lentiviral vector for siRNA expression, encoding a puromycin resistance gene.

pMKO.1puro (Addgene) is a modification of the pQCXIN vector (Clontech) in which the puromycin resistance gene was introduced and the human U6 promoter to drive the expression of inserted cDNAs.

Origin & function

The expressed cargo in these lentiviruses will be either cDNAs encoding proteins of scientific interest, or short complementary hairpin sequences, designed to knockdown endogenous expression of such proteins by RNA interference. The expressed proteins of interest will be those involved in intracellular signalling and regulatory mechanisms, including protein and lipid kinases and derived mutants (e.g. PI3K, AKT, InsR), lipid and protein phosphatases (e.g. PTEN, SHIP2) and associated proteins (e.g. adaptor, scaffolding proteins and substrates).

Evaluation of foreseeable effects

The most hazardous GMM to be produced appear to be pantropic expression lentiviruses encoding active oncogenes and pantropic lentiviruses expressing interfering RNA to knock down the expression of tumour suppressor proteins. Accidental exposure to these viruses in a form that could be internalised by the user or others would carry a significant risk of viral integration into cellular DNA and disruption of the normal mechanisms regulating cell proliferation, with an increased risk of cancer development. Other unforeseen effects of modified cellular gene expression, unrelated to tumour formation, could also stem from accidental personal contamination with lentiviruses encoding poorly characterised proteins.

Thorough measures must be taken to avoid personal contamination. However, it should be considered that retroviral particles and infected cultured cells would not survive outside culture medium, viruses would be harmless unless internalised and even if viral gene expression were to occur in cells, tumour development would be unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No non-microbial GM work is planned.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste will be sterilised by same day autoclaving (121°C, twenty minutes), with some additional precautions.

Liquid waste, including culture medium that contains high titres of lentivirus, will be immediately mixed with disinfectant (Terminex) prior to autoclaving. Only disposable plastic culture ware and pipettes should be used. Solid waste will include disposable pipette tips contaminated with virus. These will be fully immersed in disinfectant for several hours before autoclaving. A treated waste sample will be included in each RT-PCR based contamination screen.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The GM risk assessment for this project was circulated to the Heriot-Watt Genetic Modification Safety Committee for review, and the Committee is content that the work as described falls under class 2, and that correct levels of containment and disposal are described. Dr Leslie has the appropriate experience to safely carry out this work and the laboratory where the work will be carried out is appropriate for category 2 work.

### Project Containment

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#### Project Ref 396/17.1

- **Date Ackn'd**: 12/04/2017
- **CU2 Project Title**: Adaptation, colonisation and pathogenicity determinants in Escherichia coli
- **Class CultureVolClass2 CultureVolumeClass3-4**
  - Class 2
  - < 1 Litre
- **Non-GMM Consent Granted**
- **Project notified under transitional arrangements**: N

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: N
- **Historical Date of Additional Info**: 
- **Significant Change ID**: 
- **Date of Significant Change**: 

02/03/2022
Project Additional Information

Purposes of the contained use

The overarching goal is to advance knowledge of bacterial factors which promote survival, persistence, resistance and infectivity of these bacteria and hence identify potential targets to take forward towards disease control.

To achieve this overall goal, specific targets include bacterial genes and genomic loci which are incorporates investigation of Hazard Group 1 (non-pathogenic) and Hazard Group 2 E. coli strains mainly of animal origin but also includes isolates from human and environmental sources. This panel of isolates includes pathogenic, opportunistic and antibiotic-resistant E. coli plus – for comparative purposes – related Enterobacteriaceae in which phenotypic or genomic characteristics (e.g. genome islands, plasmids, resistance determinants) exhibit similarities.

In order to adequately characterise E. coli strains a broad range of approaches will be employed:

i) Cloning of specific PCR- or restriction enzyme-derived fragments or synthetic oligonucleotides into cloning vectors for the purposes of producing reference standards for gene expression analyses by quantitative RT-PCR and for recombinant protein production.

ii) Cloning of specific PCR- or restriction enzyme-derived fragments or synthetic nucleotides into plasmid vectors to produce reporter constructs to monitor gene expression in response to environmental conditions.

iii) Mutation and (where required) complementation of selected genes, genome islands and plasmids to fulfil “molecular Koch’s postulates” for which the purpose is to demonstrate the contribution of gene products to fitness and/or pathogenicity.

iv) Introduction of selected genes, operons, genome islands, or plasmids into recipient E. coli strains to fulfil “molecular Koch’s postulates”.

v) Random transposon mutagenesis (e.g. Tn-Seq) as a “global” screen for fitness, colonisation and pathogenesis-associated factors and phenotypes.

Recipient or parental organism

For routine cloning and vector construction purposes, E. coli K12 strains (e.g. DH5alpha, TOP10 and TOP10F, XL1-Blue) will be used. These strains are multiple auxotrophs and lack abilities which permit their survival except under defined culture conditions in the laboratory.

Selected wild-type E. coli strains – mainly originating from agricultural species – will be targeted for loss- or gain-of-function investigations via mutagenesis & complementation and for expression analyses through introduction of reporter constructs. Genome sequence is available for >6000 E. coli and generally, cognate sequence is available for all strains to be subjected to genetic manipulation thus potential virulence and antibiotic resistance can be predicted.

We stock a panel of >200 E. coli isolates from which wild-type strains selected for genetic manipulation will include, for example:

- E. coli P4 (phylogroup A), 1303 (phylogroup A), P4-NR (phylogroup B1) which were originally isolated from bovine mastitis and are widely-employed in experimental infection studies;
- E. coli K71 (phylogroup B1) which was originally isolated from a bovine farm environment and is a non-pathogenic strain used for comparative purposes; widely-employed as in experimental infection studies;
- E. coli 26561 (phylogroup A) which is a multidrug resistant (MDR) strain.

Host/vector system

Vectors used will vary according to purpose and all vectors employed have been routinely used for genetic modification of many different E. coli strains as well as related bacterial species.

Examples of vectors for specific purposes include:

1. Cloning for recombinant protein production and for generating constructs: pBluescript II; pGEM; pGEX; pUC; pRSET.
2. Cloning large constructs such as genome islands: cosmids/BACs such as pBeloBAC
3. Mutagenesis: conditionally-replicating vectors such as pAJR
4. Complementation and monitoring Expression: low to moderate copy number vectors such as pACYC184

Origin & function

Genes inserted will be inserted for one or more of the following purposes:
a) express and purify relevant proteins for further study with an N- or C-terminal tag;
b) inactivation of genes targeted for mutagenesis using antibiotic resistance as a selective marker;
c) complementation a mutant strain.
d) monitoring expression/localisation of a promoter or protein sequence using enzymatic, fluorescent or luminescent protein reporter;
e) introduction of contiguous genomic regions for gain-of-function study in non-pathogenic strains.

Target genes will cover a range of functions such as sensors, regulators, transporters, adhesins, genome islands (e.g. secretion system or capsular polysaccharide). None of the genes we target are known to be involved in promoting human pathogenicity or persistence in the environment. Mutants are expected to create attenuated strains and reporter constructs are not expected to confer any increased risk. Complementation is intended to restore native, wild-type phenotype and cloning of genome islands will be carried out in highly auxotrophic laboratory strains.

Evaluation of foreseeable effects

Much of the work involves loss of function (mutagenesis), restoring native function (complementation) or production of individual recombinants none of which are expected to pose hazard.

Selected investigations may introduce a gene/locus into disabled strain. The targeted E. coli pathotypes are ACDP Hazard Group 1 (HG1) and HG2 and genes selected for cloning may represent any of a range of functional classes including metabolism, regulation, adhesion and toxicity thus cloned genes may confer function on recipient strains. However, pathogenicity of E. coli strains under investigation is typically conferred by multiple factors all of which are required co-ordinately to result in ability to cause disease – since recipient strains lack the combination of determinants required to confer pathogenicity, insertion of a single gene or of an operon encoding multiple components required for a single function has a very low possibility of increasing hazard in the recipient strain.

The greatest potential hazard is likely to be through introduction of antibiotic resistance gene(s) into wild-type strains. This is a necessary element of this work to be able to select for the appropriately modified organisms. All resistance determinants in use are naturally evolved genes which have become widely disseminated in a broad range of pathogenic, opportunistic and non-pathogenic (including commensal) strains and are widely distributed in global ecosystems.

The wild-type strains to be used represent pathotypes for which multiple determinants are typically required to confer pathogenicity. Inserted gene(s) and other genetic elements to be targeted are present in the natural “pan-genome” of E. coli and related bacteria hence novel factors are unlikely to be introduced with result of additional risk.

Vectors are not self-replicating and are all widely used in microbiology and molecular biology laboratories without harmful consequence.

The risks of the inserted materials are minimal with the application of routine safe working procedures.

E. coli strains are recognised as host-associated although some environmentally-adapted strains can survive outside warm-blooded hosts – this phenotype cannot yet be predicted for individual strains hence it is assumed that all E. coli will survive to some extent under ambient conditions. The strains targeted are animal pathogenic, commensal or environmental strains of no known human pathogenic potential; in some instances, human pathogenic E. coli strains will be used for comparative purposes.

All handling is carried out in a Containment Level 2 microbiology laboratory. Containment procedures will limit exposure to negligible levels and minimise any potential risk for human or environmental exposure. If exposure were to occur, strains pose no risk over and above natural strains of E. coli.

Evaluation of foreseeable effects

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Manipulations that might cause aerosols will be undertaken in a safety cabinet. Solid and liquid waste contaminated with microorganisms will be sterilised by autoclaving. Some liquid waste will be treated with hypochlorite disinfectant prior to disposal and surface decontamination will be done using Distel.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The Heriot-Watt Genetic Modification Safety Committee has reviewed the risk assessment for this project and there were no comments of note made.

Project Containment

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Project Ref 396/17.2

Date Ackn'd 30/11/2017

CU2 Project Title: Genetic modification of the causal organism of rapid blight in turf grasses, the straminopile protist Labyrinthula terrestris and related environmental accessions.

Class 2

Culture Vol Class 2: < 1 Litre

Class Culture Vol Class 3-4: Non-GMM Consent Granted

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Rapid blight is a newly emerging disease of amenity grasses in many parts of the world including the UK. A better understanding of the plant-pathogen interactions between monocotyledonous host plants such as Poa annua and this protist pathogen is required in order to design strategies to control this disease. The aims of this project are to generate transgenic lines of Labyrinthula terrestris, the causal organism of rapid blight in turf grasses, with constitutively expressed marker genes such as Green Fluorescent Protein, in order to understand the mechanisms by which L. terrestris and related species infect monocotyledonous plants, and how plants might defend themselves against L. terrestris. We will study plant-pathogen interactions during the infection process and in further physiological and biochemical studies, identify L. terrestris virulence factors and effectors. Subsequently, plant-pathogen interactions will be further explored by modulating gene expression of L. terrestris virulence factors, PAMPS and effectors in order to discover how grasses (for example Poa annua) defend themselves against this pathogen. DNA constructs will be generated either in generic E.coli cloning vectors (for example pBluescript KS) for transformation into Labyrinthula by electroporation or biolistics, or alternatively in binary vectors such as the pCambia series for transformation into Labyrinthula using disarmed non-pathogenic Agrobacterium tumefaciens (EHA 105 for example). For examples of methods to transform straminopiles, see Sakaguchi et al., Applied and Environmental Biology (2012), 78, 3193-3202; Cheng et al., Microbial Research (2012), 167, 179-186.

Recipient or parental organism

Initial recipient organisms are Escherichia coli K12 XL 1 blue (Stratagene), and Agrobacterium tumefaciens EHA 105. The final recipient organism will be the protist Labyrinthula terrestris and very closely related UK environmental strains and isolates. These environmental strains may possibly be new species but are not so far sufficiently characterised to establish this; examples of this are the groups Laby 10 and Laby 31, described by Chitrampalan et al., (European Journal of Plant Pathology, 001 10.1 007/s1 0658-015-0701-0).

Host/vector system

The host system will be Escherichia coli bearing pBluescript derived plasmids, and Agrobacterium tumefaciens bearing derivatives of the binary vector series pCambia.

Origin & function

Initially we will focus on commonly used marker genes; uidA encoding Beta glucuronidase, derived from E. coli, or the GFP gene encoding green florescent protein from Aequoria victoria, in order to monitor the growth and development of L. terrestris when invading plant tissue. The UidA and GFP genes will be expressed using the CaMV 35S promoter from the Cauliflower Mosaic Virus, or alternatively from the L. terrestris native ubiquitin promoter. Subsequently we will clone and analyse L. terrestris genes encoding PAMPS, virulence factors and effectors (as identified by biochemical and physiological studies on L. terrestris UK environmental accessions with different levels of virulence) by expressing these in L. terrestris and monitoring the plant-pathogen interaction in order to elucidate the role of specific L. terrestris genes in plant defense against L. terrestris.

Evaluation of foreseeable effects

The most potentially hazardous GMM to be produced may be an L. terrestris strain that is normally only weakly pathogenic towards grasses such as Poa annua bearing expressed genes for a pathogenicity factor from an aggressive donor strain of L. terrestris, which may convert the weak pathogen into a stronger pathogen, but no more virulent than the original donor strain of L. terrestris. Since these straminopile protists are not thought to have a high potential for gene transfer (they do not have plasmids, nor as far as known do they reproduce sexually, only clonally),
the risk for transfer to other organisms is not high. In addition, L. terrestris is not a pathogen of humans or animals, and will only infect certain monocotyledonous plants under specific conditions such as enhanced environmental salt concentrations.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No non-microbial GM work is planned, however GMOs will be tested against (non-GM) plants such as the grass Poa annua. This will be done in contained facilities and all materials (including plants and soil) will be autoclaved prior to disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Manipulations that might cause aerosols will undertaken in a safety cabinet. Solid waste, plants and soil and bacterial cultures on plates will be autoclaved at 121 degrees C for 20 minutes. Autoclaved waste will be disposed of together with other waste from the Institute of Life and Earth Sciences. Liquid waste and water from infected plants in bags will be treated with Chloros before disposal in an appropriate drain. This disinfectant is routinely used for treatment of potentially infected liquids in our laboratories.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Heriot-Watt Genetic manipulation safety committee (21st July 2017) has reviewed the risk assessment for this project and there were no comments of note made.

**Project Containment**

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02/03/2022
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### Transferred from 1992 Regs?

| Transferred from 1992 Regs? | Y |

### Transitional Premises

| Transitional Premises Class | 2 |

### Emergency Plan Required?

| Emergency Plan Required? | N |

### Non-GMMs Withdrawn

| Non-GMMs Withdrawn | N |

### Withdrawn

| Withdrawn | N |

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### HSE Division

| HSE Division | SCOTLAND |

### Comments

| Comments | |

| Date at Which Additional Info Submitted | 02/03/2022 |
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  
Tick if confidential

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<td>Animals</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.

---

**Project Ref 397/01.1**

**Date Ackn'd** 20/02/2001

**CU2 Project Title**

TO DEFINE THE EFFECT OF ADENOVIRAL OR ADENO-ASSOCIATED VIRAL (AAV) GENE TRANSFER OF CANDIDATE GENES ON BLOOD PRESSURE AND ORGAN DAMAGE IN THE STROKE PRONE SPONTANEOUSLY HYPERTENSIVE RAT

**Date Project Ceased** 26/10/2007

**Class** Class 2

**Culture Volume Class 2** < 1 litre

**Consent Granted** Non-GMM

**Project notified under transitional arrangements** N

**Historical Significant Changes**

PROJECT TRANSFERRED TO GM 318 (26/10/07)

**Project Additional Information**
The aim of the project is to assess the ability of a number of biological active genes to reduce blood pressure, cardiac hypertrophy and other phenotypic changes associated with hypertension in the SHRSP animal model.

Recipient or parental organism

All adenovectors to be used in this study are E1-deleted first generation adenoviral vectors based on the pJM17 system (McGrory, WJ Bautista, DS and Graham, FL: A simple technique for the rescue of early region 1 mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. This renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biologically agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. However, all the inserted genes are biologically active. None of the transgenes are proto-oncogenes. All transgenes will be under the control of the CMV promoter. There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore the natural topism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use. AAV vectors have received increasing attention for molecular interventions in vivo and for gene therapy applications due to their low level of immunogenicity in vivo and their ability to integrate into the genome, thus producing sustained expression of transgenes for prolonged periods of time. They also have the ability to infect both dividing and non-dividing cells. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This sytem is published (Zhang, X, de Alwesh, M, et al. High-tier rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow. AAV vectors are replication defective.

Host/vector system

The adenoviral vectors are generated by homologous recombination between pJM17 and shuttle vectors containing transgene expression cassette and flanking E1 sequences. Following homologous recombination in helper 293 cells (which express the helper E1 function in trans, replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by go-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M, et al. High-tier rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow. AAV vectors are replication defective.
The adenoviral vector DNAs are standard and originated from the laboratory of Dr Frank Graham (McGrory, W J Bautista, DS and Graham FL: A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 163: 614-617, 1988). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vivo to generate high-level gene expression in all cells transduced by the adenovirus. The AAV system is published (Zhang, X, de Alwesh, M., et al. High titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All transgenes will be constructed from full length cDNAs obtained from other research institutes and verified in our own laboratory prior to subcloning into the relevant vectors.

**Origin & function**

There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced. Consequence of environmental exposure - 'effectively zero'.

**Evaluation of foreseeable effects**

All measures as per HSE containment level 2 for both production of recombinant adenoviruses and AAV in the laboratory and animal experimentation.

Laboratory-based experiments: during the production of replication-defective adenoviruses, all solid waste (plastics etc.) are autoclaved prior to disposal. All liquid waste from tissue culture is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. The use of sharps is avoided.

Animal experiments: All instruments used in the preparation of animals for GM work will be sterilised by autoclaving. Solutions exposed to the viable GMOs will be disinfected with chlorine-based disinfectant. All plastic ware will be autoclaved prior to disposal. Animals receiving the GMO will be housed in separate cages during the procedure-kill time period. All animal carcasses will be disposed of by incineration. Animal bedding will be autoclaved prior to disposal and cages disinfected prior to being re-used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Passed with amendments by local GMSC 1.2.2001.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
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Animal Units: L2 L3 L4 L2 L3 L4 L2 L3 L4

Large Scale Activities: L2 L3 L4 L2 L3 L4 L2 L3 L4

Human Clinical Applications: L2 L3 L4

Laboratory Activities Glass Houses Growth Rooms

Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 397/01.2

Date Ackn'd 20/02/2001

CU2 Project Title TO DEVELOP AND TEST TROPISM MODIFIED ADENOVIRAL AND ADENO-ASSOCIATED VIRAL (AAV) VECTORS FOR SELECTIVE AND ENHANCED GENE TRANSFER TO VASCULAR ENDOTHELIAL CELLS IN VITRO AND VIVO

Date Project Ceased 26/10/2007

Class 2

Consent Granted: not applicable

Non-GMM

Tick if notifying a connected programme of work: N

Historical Significant Changes: PROJECT TRANSFERRED TO GM 318 (26/10/07)

Date of Significant Change

Project Additional Information

Purposes of the contained use

The aim of this project is to restrict the tropism of adenoviral and AAV vectors. Currently, both these vector types poorly transduced vascular cells in vitro and in vivo, but are highly permissive for transduction of non-vascular cells. We will use small targeting ligands (peptides) to develop tropism modified adenoviral and AAV vectors and test the ability of these modified viruses to provide cell selective gene transfer in vitro and in vivo. All adenoviral vectors will express reporter genes.

Recipient or parental organism
All adenovectors to be used in this study are E1-deleted first generation adenoviral vectors based on the AdEASY system (He, T-C., Zhou, S., de Costa, LT., Kinzler, KW, Vogelstein, B. A simplified system for generating recombinant adenoviruses. PNAS. 95:2509-2514, 1998). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and recombination with the expression cassette. Targeting ligands will be incorporated into fiber-deleted vectors as described by vonSeegern (Von Seegern DJ, Huang S, Fleck SK, Stevenson SC, Nemerow GR. Adenoviruses vector pseudotyping in fiber-expressing cell lines: Improved transduction of Epstein-Barr virus-transformed B cells. JOURNAL OF VIROLOGY 74; (1) 354-362 JAN 2000). E1 deletion renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also be purified on cesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. There will be a minimal risk of harmful effect of these viruses in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use.

AAV vectors have received increasing attention for molecular interventions in vivo and for gene therapy applications due to their low level of immunogenicity in vivo and their ability to integrate into the genome, thus producing sustained expression of transgenes for prolonged periods of time. They also have the ability to infect both dividing and non-dividing cells. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). Targeted AAV vectors will be prepared using published methods are produce replication-defective AAV vector particles (Girod A, Ried M, Wobus C, Lahm H, Leike K, Kleinschmidt J, Deleage G, Hallek M. Genetic capsid modifications allow efficient re-targeting of aden-associated virus type 2. Nature Medicine. 1999;5:1052-1056). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced.

In addition, we are restricting the tropism of AAV and Ad vectors not expanding it. There will be no foreseeable deleterious effect of enhanced gene transfer to endothelial cells.

**Host/vector system**

The adenoviral vectors are generated by recombination in vitro and shuttle vectors containing transgene expression cassette and flanking E1 sequences (He, T-C., Zhou, S., de Costa, LT., Kinzler, KW, Vogelstein, B. A simplified system for generating recombinant adenoviruses. PNAS. 95:2509-2514, 1998). Following transfection into helper 293 cells (which express the helper E1 function in trans, replication-defective first generation adenoviruses are produced. This methodology has been used for many years for the development of recombinant adenoviruses.

AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X,
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Origin & function

The adenoviral vector DNAs are standard and originated from the laboratory of Dr Bert Vogelstein (He, T-C., Zhou, S., de Costa, L.T., Kinzler, KW, Vovelstein, B. A simplified system for generating recombinant adenoviruses. PNAS. 95:2509-2514, 1998). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vitro and in vivo to generate high-level gene expression in all cells transduced by the adenovirus.


The targeting ligands (small peptides) have been isolated by phage display technology for their ability to bind vascular endothelial cells in vitro and in vivo. These peptides are selected for incorporation into the Ad and AAV vectors for tropism modification of viral vectors.

Evaluation of foreseeable effects

There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome.

AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01 of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced. In addition, we are restricting the tropism of AAV and Ad vectors not expanding it. There will be no foreseeable deleterious effect of enhanced gene transfer to endothelial cells.

Consequence of environmental exposure - 'effectively zero'.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures as per HSE containment level 2 for both production of recombinant adenoviruses and AAV in the laboratory and animal experimentation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory-based experiments: during the production of replication-defective adenoviruses, all solid waste (plastics etc.) are autoclaved prior to disposal. All liquid waste from tissue culture is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. The use of sharps is avoided.

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Passed with amendments by local GMSC 1.2.2001

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Project Containment

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Animal Units

| L2 Yes | L3 L4 L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 L2 L3 L4 |

**Project Ref** 397/94.2

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Withdrawn  

N

Tick if notifying a connected programme of work  

N

Historical Significant Changes

PROJECT TRANSFERRED TO GM 318 (26/10/07)
Project Additional Information

Purposes of the contained use

The aim of this project is to restrict the tropism of adenoviral and AAV vectors. Currently, both these vector types poorly transduced vascular cells in vitro and vivo, but are highly permissive for transduction of non-vascular cells. We will use small targeting ligands (peptides) to develop tropism modified adenoviral and AAV vectors and test the ability of these modified viruses to provide cell selective gene transfer in vitro and in vivo. All adenoviral vectors will express reporter genes.

Recipient or parental organism

All adenovectors to be used in this study are E1-deleted first generation adenoviral vectors based on the AdEASY system (He, T-C., Zhou, S., de Costa, T., Kinzler, KW, Vogelstein, B.A. simplified system for generating recombinant adenoviruses. PNAS. 95:2509-2514, 1998). In brief, adenoviruses are generated by recombination resulting in the deletion of the E1 region of the viral genome and replacement with the expression cassette. Targeting ligands will be incorporated into fiber-deleted vectors as described by von Seggern (von Seggern DJ, Huang S, Fleck SK, Stevenson SC, Nemereow GR. Adenovirus vector pseudotyping in fiber-expressing cell lines: Improved transduction of Epstein-Barr virus-transformed B cells. JOURNAL OF VIROLOGY 74: (1) 354-362 JAN 2000). E1 deletion renders the adenoviruses replication defective. However, with these first generation viruses there is still a small risk of recombination with the E1 region in 293 helper cells used to propagate the viruses. All stocks are therefore routinely tested for the presence of replication-competent adenovirus (RCA) and will be negative. All stocks will also purified on caesium chloride gradients. E1-deleted adenoviruses cannot replicate unless the E1 function is complemented in trans. Therefore the disabled vector is considered a biological agent unlikely to cause human disease and is assigned to hazard group 1 with containment level 1 as the minimum requirement. There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. Furthermore, the natural tropism of the adenoviral vectors are not altered from wild type adenoviruses in the viruses proposed for use. AAV vectors have received increasing attention for molecular interventions in vivo and for gene therapy applications due to their low level of immunogenicity in vivo and their ability to integrate into the genome, thus producing sustained expression of transgenes for prolonged periods of time. They also have the ability to infect both dividing and non-dividing cells. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper functions necessary for AAV packaging in trans. The cells are then infected with a replication-defective simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). Targeted AAV vectors will be prepared using published methods are produce replication-defective AAV vector particles (Girod A, Ried M, Wobus C, Lahm H, Leike K, Kleinschmidt J, Deleage G. Hallek M. Genetic capsid modifications allow efficient re-targeting of adeno-associated virus type 2. Nature Medicine, 1999:5 1052-1056). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient vector (below 10⁶⁹ particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced. In addition, we are restricting the tropism of AAV and Ad vectors not expanding it. There will be no foreseeable deleterious effect of enhanced gene transfer to endothelial cells.

Host/vector system

The adenoviral vectors are generated by recombination in vitro and shuttle vectors containing transgene expression cassette and flanking E1 sequences (He, T-C., Zious, S., de Costa, LT., Kinzler KW Vogelstein, B. A simplified system for generating recombinant adenoviruses PNAS. 95:2509-2514, 1998). Following transfection into helper 293 cells (which express the helper E1 function in trans, replication-defective first generation adenoviruses are produced. This methodology has been used for many years
for the development of recombinant adenoviruses. AAV vectors used in this study are serotype 2 and replication-defective through deletion of the rep and cap genes and insertion of the recombinant gene expression cassette within the AAV inverted terminal repeats (ITRs). The AAV vectors are generated by co-transfection of the AAV vector (carrying the expression cassette) and a helper plasmid carrying the helper function necessary for AAV packaging in trans. The cells are then infected with a replication-defective herpes simplex virus (HSV) to provide helper function for AAV replication (in place of helper adenovirus). Upon a cytopathic effect in the cells, AAV and HSV particles are harvested. The HSV is removed completely by filter sterilisation to leave recombinant replication-defective AAV particles capable of expressing the transgene. This system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating amplicons and gH-herpes vectors) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). All stocks of AAV vectors will be generated at the Western Infirmary, University of Glasgow, AAV vectors are replication defective.

Origin & function

The adenoviral vector DNAs are standard and originated from the laboratory of Dr Bert Vogelstein (He, T-C, Zhou, S., de Costa LT., Kinzler, KW, Vogelstein, BA simplified system for generating recombinant adenoviruses. PNAS. 95:2509-2514, 1998). The cytomegalovirus immediate early promoter is a standard viral promoter used for gene expression studies in vitro and in vivo to generate high level gene expression in all cells transduced by the adenovirus. The AAV system is published (Zhang, X, de Alwesh, M., et al. High-titer rAAV production from replicating amplicons and gH-herpes vectors; Girod A, Ried M, Wobus C, Lahm H, Leike K, Kleinenschmidt, Deleage G, Hallek M. Genetic capsid modifications allow efficient re-targeting and of adeno-associated virus type 2. Nature Medicine. 1999;5: 1052-1056) and has been used for AAV preparation in Glasgow and elsewhere (Institute of Child Health, London). The targeting ligands (small peptides) have been isolated by phage display technology for their ability to bind vascular endothelial cells in vitro and in vivo. These peptides are selected for incorporation into the Ad and AAV vectors for tropism modification of viral vectors.

Evaluation of foreseeable effects

There will be a minimal risk of harmful effect of these genes in organs/tissues. If the replication-defective adenovirus is inadvertently released into the general environment, immune cells will target any infected cells. It is generally accepted that the Ad will be inactivated almost immediately by the immune surveillance cells located in the peripheral system. The recombinant adenovirus will pose less of a threat once in the cell than most expression vectors as they will definitely not replicate or integrate into the host genome. AAV vectors are replication defective. There is some recombination between plasmids that may result in wild type like virus (not fully functional as they contain deletions). However, this is usually minimal (less than 0.01% of stocks). Furthermore, wild type AAV has no known pathogenicity and therefore no harmful consequences of generating even fully wildtype virus is anticipated. None of the sequences are likely to be harmful if expressed. If, by accident, they were introduced in humans via the recombinant vector, they are unlikely to be harmful since they are either reporter genes or a functional gene which would have no effect unless expressed at very high levels. Since we are only using small quantities of replication deficient factor (below 10e9 particles) accidental exposure to the vector is extremely unlikely to result in high levels of protein being produced. In addition, we are restricting the tropism of AAV and Ad vectors not expanding it. There will be no foreseeable deleterious effect of enhanced gene transfer to endothelial cells. Consequence of environmental exposure - 'effectively zero'.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All measures as per HSE containment level 2 for both production of recombinant adenoviruses and AAV in the laboratory and animal experimentation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory-based experiments: during the production of replication-defective adenoviruses, all solid waste (plastics etc) are autoclaved prior to disposal. All liquid waste from tissue culture is treated with chlorine-based disinfectants for at least 24 hours prior to disposal. The use of sharps is avoided.

Animal experiments: All instruments used in the preparation of animals for GM work will be sterilised by autoclaving. Solutions exposed to the viable GMOs will be disinfected with chlorine-based disinfectant. All plastic ware will be autoclaved prior to disposal. Animals receiving the GMO will be housed in separate cages during the procedure-kill time period. All animal carcasses will be disposed of by incineration. Animal bedding will be autoclaved prior to disposal and cages disinfected prior to being re-used.
Passed with amendments by local GMSC 1.2.2001.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment
GM Centre Number: 399

Data Premises Notified (Originally) 13/12/1990
Transferred from 1992 Regs? Y
Transitional Premises Class 1
Data Premises Closed 26/04/2005
Transitional Premises Emergency Plan Required? N
Non-GMMs N
Withdrawn N

Name
UNIVERSITY OF WALES COLLEGE OF MEDICINE

Name 2
PSYCHOLOGICAL MEDICINE

Campus Estate or Research Centre
Building

Road Name
HEATH PARK

Town
CARDIFF

County
CARDIFF

Postcode
CF4 4XN

Country
WALES

Tel Number
029 2074 24840

Fax Number
029 2074 7839

HSE Division
WALES AND SOUTH WEST

Comments
GM399 closed and merged with GM130 on 26/4/2005

Date at Which Additional Info Submitted
02/03/2022
## Premises Addresses

<table>
<thead>
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<th>Country</th>
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<td>PSYCHOLOGICAL MEDICINE</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 4 (GMMs)</td>
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<tr>
<td>Non-microbial</td>
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Other (please specify)

Tick if confidential

Bacteriology  Parasitology  Transgenic Birds  Microbiology Research  Gene Therapy

Virology  Transgenic Animals  Transgenic Fish
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Other(s)</td>
</tr>
</tbody>
</table>

Surplus, used and waste culture medium treated with (1% final conc.) sodium hypochlorite for 1 hour, prior to disposal. Cell remnants treated in the same manner.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 400

<table>
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#### Name

**MERCK SHARP & DOHME RESEARCH LABORATORIES**

<table>
<thead>
<tr>
<th>Name 2</th>
<th>NEUROSCIENCE RESEARCH CENTRE</th>
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**Campus Estate or Research Centre**

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**Road Name**

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<table>
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<tr>
<th>Tel Number</th>
<th>01279 440000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fax Number</td>
<td>01279 440700</td>
</tr>
</tbody>
</table>

**E-mail**

**HSE Division**

| EAST AND SOUTH EAST  |

**Comments**

All GM Activities ceased as of 22/02/2006.

**Date at Which Additional Info Submitted**

23/11/1990
## Premises Addresses

<table>
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<th>Date</th>
<th>Premises Closed</th>
<th>Name</th>
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<th>Building</th>
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<td>MERCK SHARP &amp; DOHME RESEARCH LABORATORIES</td>
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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)  

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
The maximum volume of material that could be released at any one time is less than 10 litres. Solid waste is inactivated by on-site incineration using temperatures in excess of 1000 degrees centigrade. The progress of the material through the incinerator is automatically monitored until the 'slag' is collected for removal. Liquid waste is inactivated by autoclaving through a cycle that kills all microorganisms. Again, this is electrically monitored to ensure the procedure has taken place correctly.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 400/04.1

**CU2 Project Title**

Generation and use of commercially available Lentiviral vectors to study proteins associated with diseases such as Alzheimer's, Schizophrenia, Anxiety, Depression and Epilepsy.

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tr>
<td>26/11/2004</td>
<td>Generation and use of commercially available Lentiviral vectors to study proteins associated with diseases such as Alzheimer's, Schizophrenia, Anxiety, Depression and Epilepsy.</td>
<td>Class 2</td>
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<table>
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<th>Date Project Ceased</th>
<th>Non-GMM</th>
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<table>
<thead>
<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>
## Purposes of the contained use

Terlings Park is a basic research site for Merck Sharp & Dohme specialising in neuroscience. Lentivirus will be used to transduce cells in vitro to identify those cells, to potentially knock-down specific gene expression, to express reporter genes or to express proteins thought to play a role in neurological diseases.

## Recipient or parental organism

293FT cells (Invitrogen) will be used to propagate virus (hazard group 2 cell line)

<table>
<thead>
<tr>
<th>Cells to be transduced:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazard group 1 cell lines eg CHO, PC-12, SHSY5Y, NG108, A549 or MCF7</td>
</tr>
<tr>
<td>Rodent, primary, tissue slices or neural progenitor cells</td>
</tr>
<tr>
<td>Human neural progenitor cells</td>
</tr>
</tbody>
</table>

## Host/vector system

<table>
<thead>
<tr>
<th>Vectors for packaging genes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLP1; pLP2 and pLP/VSV</td>
</tr>
<tr>
<td>Vectors for genes of interest:</td>
</tr>
<tr>
<td>pLenti6/V5-D-TOPO or</td>
</tr>
<tr>
<td>pLenti4/V5-DEST or</td>
</tr>
<tr>
<td>pLenti6/V5-DEST</td>
</tr>
</tbody>
</table>

## Origin & function

Fluorescent reporter genes such as GFPO or DSred (Invitrogen)

Tetracyclin-regulated suppressor protein (tet- off or Tet-on) (Clontech)

Ecdysone induction system (Clontech)

Any gene whose expression is to be decreased by the use of DNAs encoding small hairpin RNA (shRNA) structures from the lentiviruses (obtained from Human or mouse whole genome clone collections as part of a global Merck & Co. effort on RNAi methodology).

Reporter genes such as beta lactamase or aequorin with the promiscuous G protein, Galpha 15 (Aurora Biosciences and NIH).

## Evaluation of foreseeable effects

Cells would only be hazardous to human health if they gain entry to the body and are histocompatible with the individual or the individual is immunosuppressed.

Virus is infectious at high titre and should be treated with caution. Routes of transmission are broken skin, exposure to mucus membranes and self inoculation. Viral particles are replication incompetent and therefore infection should not spread from infected area. If an individual is immunologically compromised (for example, taking immunosuppressant drugs) then their risk of infection from lower titre virus is increased. If individuals who are already infected with a wild type lentivirus such as HIV are infected there is an increased risk of a recombination event resulting in the HIV expressing the recombinant gene.

Genes and their products

Reporter genes such as beta lactamase or GFP would not have a detrimental affect on human health and, as stated above, would be confined to the local area of infection. The effect of other genes studies is unknown and, for the majority of cases, is likely to be minimal. However the risk of an adverse effect occurring, for example cell proliferation, is still possible.
No foreseeable environmental harm (see attached Risk Assessment).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Control measures:
Only scientists with at least 6 months experience of working with Hazard group 1 biological agents and who have had specific training are allowed to work in the virus culture facility.
Laboratory is designed and operates to Containment Level 2 standards.
PPE: Double gloves (outer gloved changed regularly); disposable, one use, lab coats and safety glasses.
Any cuts or grazes are covered by a waterproof dressing prior to entry into the laboratory.
All solid waste is disinfected prior to placing in waterproof, waste containers (‘sharps bins’) which are wiped with disinfectant before removal from the lab for transport to the incinerator.
Any item that cannot be incinerated (equipment that has a high metal content) will be disinfected by either fumigation (Hydrogen peroxide) or liquid disinfectant.
All liquid waste is disinfected prior to disposal down the laboratory sink.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Liquid disinfectant, Trigene (Medichem International Ltd), used for surfaces and liquid waste
- On-site clinical incinerator (as governed by The Environment Agency) used for solid waste. Hydrogen Peroxide fumigation (Validated and performed by Bioquell) for Biological safety cabinets prior to annual K1 test or repair.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Following discussion the committee unanimously approved.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
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Animal Units  Large Scale Activities  Human Clinical Applications
LANCASTER UNIVERSITY

I.E.N.S.

BAILRIGG

LANCASTER LANCASHIRE LA1 4YQ ENGLAND

01524 592716 01524 843854

BIOLOGICAL SCIENCES

NORTH WEST

02/03/2022
### Premises Addresses

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<td>LA1 4YQ</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
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- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial
- Other (please specify)

Tick if confidential
Disinfection of surfaces and bacterial cultures. Autoclaving of solid wastes including disposable plasticware, followed by disposal via a commercial clinical waste management using incineration. The maximum culture volume of GMMs in an experiment is 1 litre.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment
Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref 401/03.1**

**CU2 Project Title**
MOLECULAR CELL BIOLOGY OF PARASITIC PROTOZOA

**Class**
Class 2

**CultureVolClass2**
< 1 litre

**Non-GMM Consent Granted**
not applicable

**Consent Granted**

Tick if notifying a connected programme of work

Project notified under transitional arrangements

Historical Significant Changes
**Project Additional Information**

**Purposes of the contained use**

This project focuses on understanding the relationship between shape, form and the biochemistry of parasite cells and their pathogenicity ie how they cause disease. We work with the parasites (Trypanosoma brucei) that cause African Sleeping Sickness in humans and the disease Nagana in domestic cattle; and Leishmania species, the causative agents of Leishmaniasis. There is no effective vaccines against these diseases and only a meagre armoury of toxic drugs.

Genes encoding proteins that are important in controlling cell shape and motility will be modified, expressed or manipulated in cultured forms of these parasites. We anticipate that a greater understanding of the basic molecular and cellular biology of these parasites may afford new opportunities for vaccine and/or drug development. We are also interested in using these flagellated protozoan cells as model organisms to study the assembly of the eukaryotic flagellum.

In order to carry out this work we need to:

1. Grow Leishmania species and T. brucei in liquid culture media for extraction of nucleic acids and proteins.
2. Generate genetically modified Leishmania and T. brucei to study gene function.

**Recipient or parental organism**

The species of trypanosome we use in the laboratory, Trypanosoma brucei brucei, is not pathogenic to man but causes the disease Nagana in domestic cattle.

Leishmania major causes Old World cutaneous leishmaniasis whilst Leishmania mexicana causes New World cutaneous leishmaniasis in humans.

These parasites do not produce spores. They are rapidly killed outside the culture vessel by dessication, osmotic shock, washing with water, detergents and cannot penetrate unbroken skin. Tsetse flies are the vectors for trypanosomes and sand-flies the vector for Leishmania spp. We do not maintain insectories with either vector and they are not endemic in the UK. Thus these parasites are effectively deficient in mobilisation capacity.

The parasites can potentially cause disease in UK animals. However this would have to be by direct inoculation into the host animal with no likelihood of infection via feed, contaminated surfaces or by inhalation. Given that these protozoan parasites are spread by vectors not endemic in the UK and that they are rapidly killed outside of culture vessels the overall risk is effectively zero.
The main hazard to human health is by direct inoculation of the laboratory worker. We control this by establishing a set of Good Working Practices and Procedures.

### Host/vector system

**TRYPANOSOMA BRUCEI BRUCEI STRAINS**

Use is mainly made of strains that have been engineered to express T7 RNA polymerase and tet repressor to facilitate inducible expression of transfected genes. Engineered genes are effectively silent in the absence of inducer.

**TRYPANOSOMA VECTORS**

Trypanosome vectors are based on a series of vectors constructed to provide prokaryotic tetracycline mediated inducible expression of either epitope tagged proteins or double stranded RNA molecules capable of down regulating expression of genes of interest via an RNAi mediated mechanism.

**LEISHMANIA STRAINS**

- Leishmania major
- Leishmania mexicana

**LEISHMANIA VECTORS**

Leishmania vectors are based on a series of plasmid based or integrative vectors that enable the deletion of genes by homologous recombination of expression of epitope tagged proteins.

### Origin & function

Recombinant DNA inserts will be derived by PCR amplification of T. brucei and Leishmania spp. genomic DNA.

The main types of inserts to be used are:

i) Cytoskeletal and other housekeeping genes involved in the construction of the cytoskeleton, mitotic spindle and flagellum of the protozoa. Usually these genes will be tagged to allow visualisation of the expressed proteins.

ii) Portions of genes arranged to produce double stranded RNA copies that will down regulate endogenous gene expression by means of RNA interference.

iii) Gene flanking sequences to engineer gene knockouts.

### Evaluation of foreseeable effects

The final GMOs are likely to be generally reduced in fitness compared to wild type organisms.

Therefore the risks of the genetically modified organism is likely to be equivalent to, or usually lower than that of the ‘wild-type’ strain.

The rationale for these conclusions are as follows:

- Sequences transferred to the GMO are parasite gene sequences.

- The mutants are genetically stable and the potential for transfer of genetic material between these and other organisms is negligible.

- Antibiotic markers will be used that are not related to the primary therapeutic agents for these parasites. The reporter genes and counter-selectable markers will be either neutral or lead to a reduction in fitness.
We consider that none of these manipulations will effect the vector or host range, enhance or broaden tissue tropism, or decrease susceptibility to host defence mechanisms.

The genes that are of interest to us are housekeeping genes and are not known virulence genes. Moreover, these parasites do not produce toxic products that are linked to human or animal pathogenicity.

It is highly unlikely that transfer of even cryptic genes could result in enhanced virulence or endow extended host range on any recipient species. The complex and necessary life cycle changes also mitigate against such a likelihood for any of these organisms.

There is no intention to release genetically modified organisms into the environment as part of this programme of work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste is treated with disinfectant (2% Trigene) or autoclaved for complete sterilisation prior to disposal according to procedures established by the University for the safe disposal of clinical waste. The autoclaves are routinely checked to verify the effectiveness of autoclaving.

Lancaster University has established procedures for the disposal of clinical waste. Briefly these are as follows:

Microbiological waste and viable genetically modified organisms are categorized as Group C waste. Such material must be autoclaved prior to disposal. After autoclaving waste material is placed into a yellow clinical waste sack (conforming to BS 6642:1985) labelled with the Departmental name, lab number and date of disposal and placed in the locked storage container situated outside the Biological Sciences building. Removal and disposal of waste from this storage facility is currently contracted to the Lancashire Ambulance Service who dispose of waste via the incinerator facility at the Royal Preston Hospital, Lancashire.

Given the class 2 containment and control measures it is unlikely that the GMMs will reach the environment and cause harm to humans or animals outside the laboratory.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The committee met to consider the risk assessment submitted by Dr McKean entitled "Molecular cell biology of parasitic protozoa II", on April 25, 2003.

A number of minor criticisms were made pertaining to the level of detail and justification made for some statements within the assessment, and these were conveyed to Dr McKean who subsequently acted to improve the assessment to our satisfaction.

The committee noted that the assignment of the work to Class 2 was based solely on the classification of the host organisms at ACDP category 2, rather than on any specific risk posed by the proposed genetic modification activity. We were satisfied that the proposed genetic manipulation would not create any additional risk associated with these organisms and that the containment procedures in place to satisfy their ACDP category 2 status were sufficient for assignment to GM class 2.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
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#### Project Ref 401/19.1

- **Date Ackn'd**: 11/10/2019
- **CU2 Project Title**: Generation and use of genetically modified reporter viruses to understand virus-host interactions
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Consent Granted**: Non-GMM
- **Historical Significant Changes**: Withdrawn
- **Tick if notifying a connected programme of work**: No

### Project Additional Information

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

02/03/2022
Purposes of the contained use

1. Transgene expressing lentogenic Newcastle disease virus:

Newcastle disease virus (NDV) is an avian paramyxovirus serotype 1 (APMV-1) belonging to the Avulavirus genus within the Paramyxoviridae family that causes an acute, highly contagious disease responsible for severe economic losses in domestic poultry, especially chickens. In this project, we will use the NDV vaccine strain LaSota to investigate virus-host interactions in avian cell lines.

The molecular biology of NDV has extensively been studied and reverse genetics tools to examine the gene functions have been developed. In this proposal, we aim to make use of the reverse genetics tools to express following genes individually in the backbone of LaSota strains of Newcastle disease virus (NDV) pre-expressing green fluorescence protein (GFP) gene:

- S gene of infectious bronchitis virus
- VP2 protein of infectious bursal disease virus
- HA gene of low pathogenic avian influenza virus
- gD and gB genes of infectious laryngeotracheitis virus

Purpose and Predicted Outcome: We aim to understand the potential of NDV to effectively express these genes and to investigate if recombinant NDV expressing these genes can be used as vaccine candidates in chicken. Based on previous studies (Shirvani, et al., 2018; Huang et al., 2004, Hu et al., 2017, Kanabagatt et al., 2014), it is predicted that such expression of genes will not enhance the pathogenicity of pre-attenuated LaSota-NDV strain and these genes may attenuate it further.

2. The generation of reporter gene expressing low pathogenic influenza viruses.

Influenza viruses are member of orthomyxoviruses and carry broad host spectrum, which span from multiple animal species (chicken, pigs) to humans. We will use a laboratory adapted vaccine strain of Influenza A virus PR8/33/H1N1, which is a low pathogenicity virus with known genetic characteristics and an intravenous pathogenicity index = 0.0. In this project, we will construct reporter viruses expressing green fluorescent protein (maxGFP), red fluorescent protein (turboRFP) and Gaussia Luciferase. No modification in the virus sequence will be made or no alternation in the cleavage site of the HA gene is sought that change virus tropism and virulence.

Purpose and Predicted Outcome: The purpose of these reporter viruses is to evaluate the effects of innate immune genes on virus replication kinetics. Experimentally, cell lines including chicken embryo fibroblast (DF-1), Madin-Darby Canine Kidney (MDCK) and chicken kidney cells (CKC) will be transfected with expression plasmids to express innate immune genes. These cells will then be infected with the reporter influenza viruses. The replication of these viruses will be monitored either by conventional plaque assay, flow cytometry or by high throughout image screening of fixed cells.

Recipient or parental organism

GMOs of following viruses are intended in this application. These viruses are either vaccine strains or low pathogenic.

Newcastle disease virus

Over 250 species of birds have been reported to be susceptible to natural or experimental infection with NDV (Capua and Alexander, 2004). ND is normally absent in poultry in the UK, but 20 or more cases of "pigeon paramyxovirus" have been reported every year since 1983. NDV does not appear to naturally infect cattle, and vaccine strains of NDV are highly attenuated in experimentally infected calves (Subbiah, M., et al., 2008). Although, NDV has been isolated from pigs with an influenza-like illness (Ding, Z., et al., 2010), experimental inoculation of piglets with a strain of NDV did not produce any clinical signs of disease (Malik et al., 1969). The NDV has a tendency to replicate in the cancerous tissue of several animals including humans. The disease is not lethal for human and only cause mild and self-limiting flu-like illness and conjunctivitis.

Low Pathogenicity Influenza Viruses
Influenza A viruses (IAV) are zoonotic pathogens with a natural reservoir almost entirely in birds but can be transmitted to human and other animals. ACDP proposes that: (a) highly pathogenic e.g. H5N1 and H7N7 and uncharacterised avian influenza viruses and (b) animal viruses closely related to novel viruses capable of infecting humans should be handled at Containment Level 3 (CL3). Work with these viruses is not intended at Lancaster in this application. Only GFP/RFP/Luciferase expressing low pathogenicity viruses such as strain PR8/33/H1N1 will be used which are further attenuated due to insertion of the reporter genes.

Foreseeable effects:
The reporter genes (GFP/RFP/Luciferase) are non-viral components and are not reported to increase the pathogenicity of viruses. Expression of viral genes in NDV have attenuated LaSota strains which is already a selected attenuated strain of NDV. Since wild type NDV can infect all birds, expression of additional genes from any other avian viruses will not enhance host-spectrum. The generated reassortment viruses will remain low pathogenicity viruses and the proposed gene modifications would not be expected to alter the virulence and therefore the risk is still at CL2. The reporter genes are not reported to be carcinogenic or toxic when exogenously expressed and no harmful consequences are envisaged from their expression in cell cultures.

Transfected cells producing the recombinant influenza and NDV must be maintained in tissue culture and cannot replicate outside the laboratory. NDV and low pathogenicity AIV can cause mild infections in human if accidentally exposed to the virus. GM viruses are cultured only inside CL3 facilities within routine handling within Class II Microbiological Safety Cabinets. The main hazard to human health is by direct inoculation of the laboratory worker. We control this by establishing a set of Good Working Practices and Procedures, and avoiding the use of sharps. As all culture media and cells will be decontaminated in 1% Virkon or Distel for overnight before disposal, no harm to human health or the environment is envisaged.

The GM and parental viruses can potentially cause disease in UK animals; however, this would have to be by direct inoculation into the host animal with no likelihood of infection via feed, contaminated surfaces or aerosol. This programme of work does not include deliberate infection of animal species with these viruses. Although experimentally NDV would be able to infect other animal species such as ruminants, rodents or birds, the likelihood of this occurring accidentally is very small due to laboratory confinements. In the event that this did occur, the recombinant virus would be unable to propagate (Subbiah, M., et al., 2008). Also, since infected ruminants and pigs are unlikely to excrete virus, the risk that the virus will disseminate or cause disease is low. Accidental release of virus could result in infection of birds, including poultry. However, the GMO is produced from a licensed vaccine strain of NDV and low pathogenicity influenza virus, which are non-pathogenic in poultry and expression of a marker gene is likely to attenuate the virus further. The bird population, particularly pigeons, are exposed to the avian paramyxovirus APMV-1 (a virus similar to NDV) and are likely to be immune to NDV.

1.) Transgene expression in Newcastle Disease Virus (NDV) LaSota Strain
The NDV vaccine strain LaSota will be used to investigate the expression of these genes (Fig. 1) and their subsequent roles in virus biology. The recombinant viruses will then be used to infect the avian or duck cell lines. Two proteins (HN and F) are well characterized and are major immunogenic proteins of NDV. Most of the neutralizing antibodies are derived against these proteins and no modification will be made in these proteins that determine the viral tropism. The pathogenicity of NDV is controlled by the sequence in cleavage site of the F protein, which remains the same (GRQGR - nonpathogenic) for all recombinant viruses proposed in this application. Since an individual protein of other viruses will be expressed as transgene, no recombination is expected. These genes have previously been cloned in the NDV backbone and no adverse effects have been reported and the NDV has either retained the infectivity or was further attenuated (Shirvani, et al., 2018; Huang et al., 2004, Hu et al., 2017, Kanabagatt et al., 2014). Using reverse genetics initially constructed by Ben Peeters at Central Veterinary Institute, The Netherlands (Ben et al., 1999), rescue of the virus will be performed as described before (Ayllon et al., 2013). Modification includes expression of viral genes (Fig. 1) in the junction of M and P genes. Infectious viruses will be produced only in complementing cell lines that contain three supportive plasmids, pNP, pP, and pL, as a source of replication complex. Therefore, the virus can only be rescued in specific cell lines where all the required components are present.

Fig. 1: Schematic presentation of insertion of transgenes in the genome of LaSota NDV through restriction digestion of intergenic regions between P and M genes.
References:


2.) Reporter genes expression by low pathogenic influenza virus

Reporter genes (GFP, RFP and Gaussia Luciferase) will be cloned into the segment 8 (NS gene) of the low pathogenicity AIV strain PR8/33/H1N1. The reporter NS segment will be generated by mutating the splice acceptor site in the NS1 protein gene to prevent splicing (Fig. 2). The reporter protein will be fused to the C-terminus of the NS1 protein coding region. A self-cleaving protease (2A) from porcine teschovirus-1 will be inserted in the C-terminus of the reporter genes. This 2A self-cleavage will allow the release of NS2 protein (also known as NEP, nuclear export protein) during influenza virus infections. The reporter genes named as maxGFP, turboRFP and Gaussia Luciferase have been used to generate three reporter plasmids. These reporter genes will only be incorporated in the NS gene segment as illustrated in the figure 2. The generated reassortment viruses will remain low pathogenicity viruses and the proposed gene modifications will not alter the virulence and therefore the risk is still at CL2 (Breen M, et al., 2016, and Perez et al., 2013; Eckert et al, 2014).

Using reverse genetics a recombinant influenza virus will be rescued from DNA copies of the negative sense RNA influenza genome. Each of the 12 plasmids (8 expressing viral genomes and 4 expressing viral polymerase) will be transfected in 293T cells (human embryonic kidney cells) and then the co-culture with permissive MDCK (Martin Darby Canine Kidney) cells (as shown by Neumann et al., 1999). The AIV level 2 influenza viruses will be rescued in CL3 laboratory. All tube labels of plasmids for the rescue of new viruses must checked by a second member of the lab before use in the virus rescue.

Fig. 2: Schematic representation of reporter gene insertion in the NS gene segment of low pathogenicity IAV

References

- Gabriele Neumann, Tokiko Watanabe, Hiroshi Ito, Shinji Watanabe, Hideo Goto, Peng Gao, Mark Hughes, Daniel R. Perez, Ruben Donis, Erich Hoffmann, Gerd Hobom, and Yoshiihoro Kawoaka Generation of influenza A viruses entirely from cloned cDNAs. PNAS August 3, 1999 96 (16) 9345-9350; https://doi.org/10.1073/pnas.96.16.9345
DNA inserts will be derived by PCR amplification of reporter genes (GFP/RFP/Luciferase) or viral genes (VP2, S, gD, gB, HA) of from cDNA (as described in Purpose of the contained use). The reporter genes will be amplified from plasmid DNA whereas the cDNA from viral RNA/DNA will be prepared from non-infectious extracted genetic material of the virus or by a synthetic approach. The reporter genes will allow visualization and detection of viruses. The viral genes, to be studied in this application, are surface proteins in their respective viruses. The primary function of these proteins is immunogenicity in susceptible hosts and most of the virus neutralization antibodies are raised against these proteins.

**Origin & function**

The final GMOs are likely to be of comparable or reduced fitness compared to wild type organisms. Therefore, the risk posed by the GMO is likely to be equivalent to, or usually lower than that of the ‘wild-type’ strain.

The rationale for these conclusions are as follows:

- The sequences transferred to the GMO are reporter gene sequences or genes from other viruses that will not increase virulence.
- The mutants are genetically stable and the potential for transfer of genetic material between these and other organisms is negligible.
- The reporter genes markers will be either neutral or lead to a reduction in fitness.
- We consider that none of these manipulations will affect the host range, enhance or broaden tissue tropism, or decrease susceptibility to host defence mechanisms.
- The genes that are of interest to us are not known virulence genes. Moreover, these GMOs will not produce toxic products that are linked to human or animal pathogenicity.
- It is highly unlikely that transfer of viral genes could result in enhanced virulence or endow extended host range on any recipient species.
- There is no intention to release genetically modified organisms into the environment as part of this programme of work or to use them in live animals.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**All waste will be chemically treated (1% Distel or 1% Virkon for 16h), autoclaved for complete sterilisation prior to sterilization (triple lock system) according to procedures established by the University for the safe disposal of clinical waste. The autoclaves are routinely checked to verify the effectiveness of autoclaving.**

Lancaster University has established procedures for the disposal of clinical waste. Briefly these are as follows:

Microbiological waste and viable genetically modified organisms must be autoclaved prior to disposal. After autoclaving waste material is placed into a yellow clinical waste sack (conforming to BS 6642:1985) labeled with the Departmental name, lab number and date of disposal and placed in a locked storage container. Removal and disposal of waste from this storage facility for incineration off-site is via a licensed contractor.

Given the Class 3 containment level and control measures in place it is very unlikely that the GMMs will reach the environment and cause harm to humans or animals outside the laboratory.
The proposed work is limited to low pathogenicity viral strains and will introduce genetic material that is unlikely to increase the virus pathogenicity or host range. The activities are to be conducted in a dedicated room within an ACDP Containment level 3 compliant lab, which exceeds the required level 2 containment and will minimise the risk of accidental release.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick if you have attached a risk assessment to this form Y

Tick if you have attached a risk assessment to this form N

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

If yes, tick to confirm that it is attached to this form N
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**Name**

UNIVERSITY OF CAMBRIDGE

**Name 2**

ANATOMY

**Campus Estate or Research Centre**

DOWNING STREET

**Town**

CAMBRIDGE

**District**

CAMBRIDGESHIRE

**Postcode**

CB2 3DY

**Country**

ENGLAND

**Tel Number**

01223 333893

**Fax Number**

01223 333 786

**E-mail**


**HSE Division**

EAST AND SOUTH EAST

**Comments**


**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity

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Maximum culture volume that can be released is less than one litre.

Autoclaving: Autoclaves are calibrated annually. Brownes tubes/indicator tape used for routine monitoring. Waste may also be incinerated if the risk assessment requires it. Disinfectants: Commercial disinfectants such as Virkon or Chloros are used at concentrations recommended by manufacturer. Working stocks replenished on a routine basis. Animal waste ultimately being destroyed by incineration. Incineration. Sealed containers are taken for incineration. Waste may have been autoclaved or treated with disinfectant beforehand. Records are kept of material sent for incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 403**

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**HSE Division**

| HSE Division    | EAST AND SOUTH EAST |

**Comments**

ACCENTUS IS THE PARENT COMPANY BUT THEY ARE STILL AEA TECHNOLOGY

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

-Give brief details of the genetic modification safety committee

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- Other (please specify)  
  
  - Bacteriology  
  - Parasitology  
  - Transgenic Birds  
  - Transgenic Animals  
  - Transgenic Fish  
  - Microbiology Research  
  - Gene Therapy  

- Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum amount of fermentation culture fluid produced at any time is 100 litres. Waste is deactivated by two methods:

* adding amounts of a commercial disinfectant powder (Virkon) to give the concentration recommended by the manufacturer;
* steam sterilising (121°C, 1 bar) the fermenter contents in situ for 20 or 45 minutes as required.

Monitoring the deactivation method is carried out either by weighing and recording details of the amount of disinfectant powder added or by recording the time and temperature of the steam sterilisation process with a calibrated thermocouple.

When necessary, the deactivation process is validated using standard microbiological techniques and spreading a known volume of deactivated culture fluid on rich nutrient agar and checking for no growth.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Name

YAMANOUCHI RESEARCH INSTITUTE

Name 2

Department

Campus Estate or Research Centre

Road Name

ARMSTRONG ROAD

District

LITTLEMORE PARK

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX4 4SX

Country

ENGLAND

Tel Number

01865 747100

Fax Number

01865 748974

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<td>Animals</td>
<td>Fish</td>
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Tick if confidential
The maximum culture volume for any single activity involving genetically modified bacteria or yeast will be 2 litres. For insect or mammalian cell lines a maximum culture volume of 3 litres will be used for any one activity. Waste in flasks or beakers is inactivated by addition of Presept (sodium dichloroisocyanurate) tablets. 5g tablet per 550 ml, for a minimum of one hour. Alternatively waste is aspirated into flasks containing Trigene II disinfectant concentrate at 1:100 dilution. Use of Presept tablets and Trigene II disinfectant have been validated by the manufacturers and relevant literature has been obtained. Monitoring of deactivation methods. No routine monitoring of the deactivating methods is considered necessary by our GMCS as the methods used are in excess of the minimum recommended by the manufacturers and the micro-organisms are all disabled and cannot survive outside of the laboratory environment.

For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
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Please enter comments of the GM safety committee on the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
GM Centre Number: 406

Data Premises Notified (Originally) 02/01/1991
Transferred from 1992 Regs? Y
Transitional Premises Class 1
Data Premises Closed N
Transitional Premises Emergency Plan Required? N
Non-GMMs N
Withdrawn N

Name
ELI LILLY & CO LTD

Name 2

Department
SITE SERVICES

Campus Estate or Research Centre

Building

Road Name
FLEMING ROAD

District
SPEKE

Town
LIVERPOOL

County
MERSEYSIDE

Postcode
L24 9LN

Country
ENGLAND

Tel Number 0151 448 6180
Fax Number 0151 448 6216

E-mail

HSE Division NORTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

  Give brief details of the genetic modification safety committee

  - Laboratory
  - Animal Unit
  - Growth Room
  - Glass House
  - Large Scale

  **Level 1 (GMMs)**

  **Level 2 (GMMs)**

  **Level 3 (GMMs)**

  **Level 4 (GMMs)**

  **Non-microbial**

  **Other (please specify)**

  - Bacteriology
  - Parasitology
  - Transgenic Birds
  - Microbiology Research
  - Transgenic Animals
  - Transgenic Fish
  - Gene Therapy

- Tick if confidential

02/03/2022
The maximum culture volume handled on the premises is 6000 litres. Under normal working practices, no live organisms are released to the environment and the fermentation system is designed for containment, including exit gas streams and in process sampling. Any waste is heat inactivated via a contaminated waste heat treatment vessel, prior to discharge into the drain; this includes steam condensate which has been in contact with the process equipment. This vessel has been validated as suitable for assuring the thermal destruction of E. coli, and the temperatures of the vessel contents and the exit stream are monitored. If the release of a large quantity of cold material should occur into this vessel, the exit valve will close until the temperature of the contents rise to the required temperature for release.

The maximum volume of live culture, which could theoretically be released at any one time, is 6000 litres. However the likelihood of this event is extremely low, given that for this to occur, the fermenter vessel would have to fail, and the safety devices on the waste heat treatment vessel would also have to fail, simultaneously.

---

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 407

Data Premises Notified (Originally) 02/01/1991

Transferred from 1992 Regs? Y

Transitional Premises Class 3

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

UNIVERSITY OF CAMBRIDGE

Name 2

Department

CLINICAL VETERINARY MEDICINE

Campus Estate or Research Centre

Building

Road Name

MADINGLEY ROAD

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB3 0ES

Country

ENGLAND

Tel Number

01223 337600

Fax Number

01223 337610

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Animal Unit</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 407/00.1

Date Ackn'd 24/01/2000

CU2 Project Title INHIBITION OF VIRULENCE FACTOR EXPRESSION BY TARGETTED REDIRECTION OF RIBONUCLEASE P

Class Class 3

CultureVolClass2 < 2 litres

CultureVolumeClass3-4

Non-GMM Consent Granted yes
**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Is an emergency plan required according to regulation 20?**

- No

If yes, tick to confirm that it is attached to this form

- No

Tick to confirm that you have attached a risk assessment to this form

- No
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**Project Ref**  407/01.1

Date Ackn’d: 16/05/2001

**CU2 Project Title**

IMMORTALISATION OF PRIMARY CELL CULTURES BY EXPRESSION OF TRANSFORMING GENES USING RETROVIRAL VECTOR MEDIATED GENE DELIVERY

Date Project Ceased

Class: Class 3

CultureVolClass2: 100 ml

Consent Granted: yes

Non-GMM

Project notified under transitional arrangements: N

Withdrawn: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To generate immortal cell lines by causing them to express one or more transforming/immortalising genes. Gene delivery will be achieved using pan-tropic (amphotropic) retroviral vectors containing one or more transforming/immortalising genes under a strong constitutive promoter. A non-origin binding, ts mutant (U19-tsa58) of SV40 T-Ag
and the human telomerase (hTERT) gene will be used in the first instance, but other transforming genes may be used in future.

Primary equine or ovine bone marrow cells will be used in the first instance, but other cell types may be used in future (eg rat neuronal cell progenitors).

Initially, the retroviral vectors will be provided by a collaborator and thus will not be prepared on this site. However, future use may require the production of the vectors in our own cat 3 laboratory.

Future work may involve the transformation and immortalisation of non-dividing cell types, necessitating the use of lentiviral (eg HIV or EIAV) based vectors.

**Recipient or parental organism**

Primary cell cultures from a variety of animal sources (eg Human, mouse, rat, cow, sheep). Finite lifespans. Not knowingly infected with any organism likely to pose a human or environmental hazard. Human cells will only be used from screened sources.

**Host/vector system**

| Moloney murine leukaemia virus derived vectors packaged in Ecotropic, amphotrophic or pan-tropic (VSV-G) envelope glycoprotein. |
| HIV or EIAV based vectors (non-replication competent, with all viral structural genes deleted) packaged in VSV-G protein. |

**Origin & function**

| Vector backbone: Retroviral LTR, packaging signal, selective marker (Hgr or Neo genes), polyadenylation site. |
| Purpose: Propagation, delivery and expression of genes contained within the vector. |
| Origin: Commonly used MLV vectors pBABE and pZIP derivatives. HIV and EIAV derived vectors. |

| Inserted genes: SV40 T-Ag (U19-tsa58 mutant), human telomerase gene (hTERT). |
| Purpose: Transformation and immortalisation of recipient cell lines. |

**Evaluation of foreseeable effects**

Possible deleterious effects include:

1. the accidental infection of the operator with the transforming viral vector;
2. the propagation of replication competent mutant viruses generated by recombination in the producer cell lines or by recombination with endogenous/adventitious agents present in the recipient lines. These viruses could conceivably infect and transfer the transforming genes to the operator in the event of a containment breach. This could conceivably lead to oncogenesis.

Evaluation 1: Handling the viruses at containment level 3 makes the likelihood of such an event effectively zero. The consequences of the event are unlikely to be severe as multiple events are required to cause oncogenesis in an immunocompetent individual. The vectors are not replication competent and cannot therefore establish a productive infection capable of perpetuating itself.

Evaluation 2: The vectors are generated using a multicomponent packaging line and are therefore unlikely to recombine to produce replication competent mutant (RCM) viruses. Multiple recombination events would be required to generate a replication competent virus containing the transforming genes. Since only one round of infection is possible and the titres of vector are fairly low (10^{4}/ml) there is very limited opportunity for such an event to occur in the recipient cell lines. It is highly unlikely that RCMs would be capable of replicating in the non-murine cell lines. After the vectors have been used to infect the recipient cell lines, the culture supernatants will be tested (by serial passage and gene specific PCR) to confirm that no RCMs have been generated. Resultant cell lines will be handled at containment level 2 for the duration of the studies.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard operating procedures for disposal of category 2 and 3 waste have been provided previously.

Solid waste: (Category 1, 2 and 3)
Sterilised by autoclaving in unsealed biohazard bags contained within stainless steel containers with loose fitting lids. Final disposal: landfill.

Liquid waste: (Category 2 and 3)
Collected in 1 litre polypropylene bottles containing 1 ml of QC Clear/D(superscript TM) broad-spectrum disinfectant. Volume not to exceed 800 ml per bottle. Autoclaved under standard conditions. Final disposal: Down the drain.

Glassware:
Contaminated bottles, etc are sterilised by autoclaving. Glass pipettes are completely submerged for at least 1 hour with 1% Virkon before being machine washed. The Virkon solution is changed once a week, or twice a week during hot weather or periods of heavy usage. Activity indicator colour is checked daily. Workers are expressly forbidden to discard pipettes containing growth medium because it will inactivate the Virkon. The conditions are compliant with manufacturers recommendations. Only disposable plastic pipettes are used for cat 3 work.

Autoclaving:
All autoclaves are serviced annually by an authorised agent using an 'in load' thermocouple monitor. Browne TST Control Integrators for steam autoclaves (complete colour change indicates that the strip was exposed to steam at 121C for at least 15 mins or 134C for 5.3 mins. Order code 2342) are included with all runs of category 3 waste. Steam exposure indicators are included in the centre of all category 1 and 2 waste runs. All three autoclaves are operated using the standard conditions of 30 min @ 128C. 100% kill is anticipated under these conditions.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
- The committee agree with Containment already assigned for these vectors at University College London and consider that Laboratory Level 3 Containment is appropriate for the generation of cell lines proposed in the present proposal.
- The use of glassware and sharps must be avoided at all stages of these Level 3 procedures.
- When it is confirmed that the transfected cell lines do not produce replication-competent virus, these lines may then be handled at Laboratory Level 2 Containment.

**Project Containment**

<table>
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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Project Ref** 407/03.1

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<td>11/07/2003</td>
<td>MOLECULAR CLONING AND EXPRESSION OF FULL-LENGTH, NATIVE AND MODIFIED PRP PROTEIN SEQUENCES.</td>
<td>Class 2</td>
<td>1-50 litres</td>
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<tr>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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**Historical Significant Changes**

<table>
<thead>
<tr>
<th>Historical Date of Additional Info</th>
<th>Significant Change ID</th>
<th>Date of Significant Change</th>
</tr>
</thead>
</table>

**Project Additional Information**

**Purposes of the contained use**

The purpose of the contained use is to express normal and modified sequences of PrP protein from assorted species, from plasmid vectors in which the sequences are inserted, via both prokaryotic and eukaryotic expression systems.
### Recipient or parental organism

Eukaryotic transfection and expression will be carried out using standard laboratory cell lines or dissociated primary cell cultures.

Prokaryotic transformation and expression will be conducted using disabled laboratory strains of E. coli E.g. XL1-Blue, DH5-Alpha, BL21 (DE3).

### Host/vector system

The vectors used in these procedures will be E. coli compatible plasmids of non-mobilisable or mobilisation defective phenotype.

### Origin & function

The PrP sequences to be used will be derived from genomic material or existing plasmids, and subsequent modifications will be carried out using standard molecular biology techniques. The PrP DNA sequences will be expressed as protein that will be detected on cell surfaces, in cell preparations or harvested as a source of recombinant protein for laboratory use. The attached RAs indicate that the primary species of interest will be murine, ovine, cervid and bovine. However, this work may expand to include PrP genes from any other species.

The function of PrP is not fully understood. It is a copper binding protein with a super-oxide dismutase activity. It is expressed in cells of the CNS and PNSD and lymphoid system. PrP is associated with transmissible spongiform encephalopathies, and it is currently believed that a modified form of the protein may itself constitute the infectious agent involved.

### Evaluation of foreseeable effects

Recombinant PrP is not a toxic substance and the vectors to be used do not encode toxic material. This has been established by use of similar reagents in a number of other laboratories generating similar protein. Therefore, it is reasonable to assume that there will be no toxicity or hazardous effect associated with the use of these reagents. In addition, recombinant PrP has not been shown to be infectious (Hill, Antoniou and Collinge, 1999).

The eukaryotic cell lines to be used cannot survive in the normal environment. As such, they would not be able to act as a vector as they cannot survive and colonise an external host effectively.

There is an extremely remote possibility that a host bacterium strain, even if disabled, could colonise the gut through ingestion and produce protein in situ, or pass on the plasmid of interest. The work is classified as Class 2 and the containment procedures required should eliminate any such risk. In addition, in the unlikely event that colonisation does occur, there is negligible risk as recombinant PrP protein is considered non-hazardous.

There is a theoretical risk that altering the PrP DNA sequence, or the context in which it is expressed, could alter the conformation of the protein to a disease specific form that may be regarded as hazardous. An example of this would be the generation of recombinant sequences that are analogous to mutations associated with human prion disease. The escape of a bacterial strain containing such a hazardous protein and the colonisation of an operator would be the worst-case scenario of this type of work.

The risk associated with this theoretical possibility is considered to be negligible because of the following:

- the infectivity of prion disease is subject to a species barrier that for some cases cannot be overcome.
- requires specific routes of infection that are unlikely to occur within the given working practices of the laboratory.
- the materials in use are subject to levels of containment and methods of disposal suitable for reducing to a minimum any chance of contact by the operator.

All these factors indicate that the risks to operator health and to the environment are negligible from this course of work.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
We would like to derogate the requirement of restricted access to laboratories to allow this work to proceed within a designated area of our Category 1 laboratory for particular parts of the proposed work, specifically the use of naked DNA and prokaryotic organisms. Furthermore, this derogation is sought only for GMMs based on sequences considered particularly low risk such as murine and ovine PrP DNA sequences. The Category 1 laboratories in our Department meet Category 2 criteria for all aspects of equipment, waste handling/disposal and other safety aspects except for the issue of limited physical access to the laboratory. Limited access to the GMMs (and hence the associated risks) can be provided by continuous supervision of the materials by the operator, by use of additional rooms that are lockable for certain phases of the work where supervision is impractical (eg overnight culture in a lockable hot-room) and storage of materials in areas with restricted access. This form of supervisory restriction of access at the point of work, combined with restricted access to storage areas, is sufficient to provide the necessary protection intended for Category 2, as outlined in the HSE Guidance documents. The low risk of the GMMs being handled, the limited opportunity for operator risks to occur, the non-pathogenic nature of recombinant proteins and species source for the DNA of these specific forms of PrP, means that this aspect of containment can be justifiably derogated for the prokaryotic usage described in this project.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste that contains PrP related material listed under the genetic manipulation regulations will be decontaminated and/or disposed of in an appropriate manner.

Specifically:
Solid waste will be placed into a clinical waste bag for incineration.

Spent bacterial or eukaryotic cell culture waste will be inactivated by autoclaving before disposal by normal drains.

Solutions of recombinant PrP, together with buffers and solutions from techniques such as SDS-PAGE and dialysis, that have been exposed to recombinant PrP, will be disposed of in accordance with COSHH regulations.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Decision:
Class 2, with derogation of physical access restrictions to allow work to be carried out in the CVS N lab subject to tightened access control measures.

Category:
Class 2, Notification required.

Comments:
Summary - this work assessed as Class 2 and should be notified to the HSE. The purification of recombinant ovine and mouse PrP may be performed in the CVS N lab, subject to the production of a written procedure based COSHH assessment detailing how the material is to be handled, processed, stored and disposed of. Spill response, personal protection and access control should also be specified. A specific area of the lab should be designated for this work. The bovine and cervid PrP work should be performed in the dedicated TSE lab where possible. Use of facilities outside this lab should comply with the relevant parts of the COSHH assessment described above.
Project Containment

Laboratory Activities

- L2: Yes
- L3
- L4

Glass Houses

- L2
- L3
- L4

Growth Rooms

- L2: Yes
- L3
- L4

Animal Units

- L2
- L3
- L4

Large Scale Activities

- L2
- L3
- L4

Human Clinical Applications

- L2
- L3
- L4

Project Ref 407/07.1

Date Ackn'd 30/01/2007

CU2 Project Title

Investigations into the mechanism of pathogenicity and the rational design of novel vaccine delivery vectors

Class 2

Culture Volume

Class 2

1-50 Litres

Non-GMM

Consent Granted

Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work Y

Historical Significant Changes

- Significant Change ID

- Date of Significant Change

Project Additional Information

Purposes of the contained use

This program of work has the objective of identifying genes from a range of hazard group 2 pathogens that are associated with virulence using mutagenic techniques and animal infection studies. Mutants will be investigated to determine the mechanisms behind any associated phenotypic change and assessed for their suitability as vaccine vectors.

Recipient or parental organism

- Salmonella enterica (HG 1 + 2 strains only), Bordetella pertussis, B.parapertussis, B. bronchiseptica, Streptococcus equi, S.suis, S.pneumoniae, Staphylococcus aureus, Listeria monocytogenes, Campylobacter jejuni and Rhodococcus equi. Many of the mutants made will be attenuated for virulence, as the mutations will be in metabolic...
genes, genes involved in the protection of bacteria from host defence mechanisms or genes that encode mechanisms for signalling to the host. However, some mutants will be as virulent as the parent.

Host/vector system

Animal hosts: Mice and possibly chickens in future.

Bacterial hosts: Salmonella enterica (HG 1 + 2 strains only), Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica, Streptococcus equi, Streptococcus suis, Streptococcus pneumoniae, Staphylococcus aureus, Listeria monocytogenes, Campylobacter jejuni and Rhodococcus equi. Vectors: Non-mobilisable or mob-defective plasmids appropriate for each species of bacteria.

Origin & function

This program of work has the objective of identifying genes from a range of hazard group 2 pathogens that are associated with virulence. The genes will be identified using library generating gene knockout technologists (such as transposon mutagensis) followed by screening individual mutants or pools of mutants for pathogenicity in a suitable animal model (e.g. mouse infection models). The genes identified in these screens will then be specifically knocked out in the original wild-type organism using defined allelic replacement techniques and complemented using chromosomal or plasmid-expressed copies of the gene in question to confirm that no other spurious mutations are responsible for any new phenotype. The precise identities of these genes cannot be specified in advance of the screening procedure. However, each gene that is identified will be risk assessed to ensure that the subsequent manipulations will not pose a substantially higher risk or require reclassification of the activity to a higher category (i.e. Class 3) or alter the host organism in a way that may increase it pathogenic potential (e.g. environmental stability as vaccine vectors. They will be transformed with expression plasmids containing prokaryotic or eukaryotic promoters (e.g. CMV promoters) to express recombinant proteins (as potential antigens and/or immunomodulatory proteins) and selectable markers (e.g. antibiotic resistance genes). To study the contribution of the different arms of the immune system to the response to these mutants, they will be used individually or in pools, to infect conventional, gene-targeted and transgenic mice, by various routes of infection (e.g. intravenous, intraperitoneal, oral or intranasal routes). This work is covered by a Home Office Project Licence. At various time points post-infection, mice will be killed and organs will be examined for bacterial counts and parameters such as gene expression in the bacteria and host immunological response to the bacteria. These experiments will use the parent strains of the mutants as controls.

Evaluation of foreseeable effects

It is expected that many of the mutants made will be attenuated for virulence, as the mutations will be in metabolic genes required for growth of the bacteria in vivo; genes involved in the protection of bacteria from host defence mechanisms; or genes that encode mechanisms for signalling to the host. However, some mutations may not affect the ability of the bacteria to grow and cause disease in vivo and hence those mutants will be as virulent as the parent strain. All of the proposed work will be performed at laboratory and animal containment level 2, unless there is deemed sufficient cause to raise or lower the containment level. Where this is the case, approval from the HSE will be sought in advance.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Detailed standard operating procedures for disposal of category 2 laboratory waste have been provided previously.

To summarise:

Solid Waste: (Category 2)
Laboratory waste is sterilised by autoclaving in unsealed biohazard bags contained within stainless steel containers with loose fitting lids. Final disposal: Landfill, double bagged in plain black polythene outer bag.
Liquid waste: (Category 2)
Collected in 1 litre polypropylene bottles containing 1ml of QC Clear/D™ broad-spectrum (Quat-NH4) disinfectant. Volume not to exceed 800 ml per bottle. Autoclaved under standard conditions. Final disposal: Down the drain.

Autoclaving:
All autoclaves are serviced annually by an authorised agent using an "in load" thermocouple monitor. Browne TST Control Integrators for steam autoclaves (complete colour change indicates that the strip was exposed to steam at 121°C for at least 15 min or 134°C for 5.3 min: Order code 2342). Steam exposure indicators are included in the centre of all category 1 and 2 waste runs. All autoclaves are operated using the standard conditions of 30 min @ 128°C. 100% kill is anticipated under these conditions.

Animal containment Lab waste:
Animal waste (faeces, bedding, carcasses) is double bagged in yellow clinical waste bags. These bags are stored in a large locked clinical waste bin or cold storage room and collected regularly by a commercial company (Vetspeed) licenced for GM waste disposal by incineration. Cages are sprayed with 3% Virkon and then processed at 70°C with acidified wash solution in a commercial cage washer. More details and justification for these conditions being sufficient to ensure effectively 100% kill are provided as a separate attachment.

Please enter comments on the GM safety committee on the risk assessment

Two risk assessments are attached, with comments at the ends. Here is a representative set of comments for one of the Ras (GM2006/08)

Decision: Class 2. Notification required for subsequent animal work.

Comments:
BAB: insert description - I thought pTECH used the tetC fragment to act as an adjuvant so the proteins are expressed as fusions with this. So not sure if 'under the control of the pTECH promoter element' is correct. TetC fragment is bigger than amp.
Happy with the rest as she has acknowledged there may be some risk of increased pathogenicity and given reasons for why this is unlikely to happen.

DT: I have no major issues with this assessment. However, re Q23 (impact of immunosuppression) there is good evidence that Campylobacter-associated disease, at least, is much more prevalent in the immunosuppressed population (I suspect this will also be true of Salmonella).

LT: The work as described here is OK at CL2 and would be covered under GM407/97.2, however, I assume it is intended to introduce these GMOs into an animal at some point. On checking the detail of GM407/97.2 and its subsequent notification updates, I find that such work will require notification.

Project Containment

<table>
<thead>
<tr>
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<td>L3</td>
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<td>L2</td>
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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

This notification is intended to cover the use of lentiviral vectors as gene delivery vectors for making transgenic cell lines and animals. Lentiviral vectors will be used to introduce a variety of transgenes into a variety of cell lines (including human cell lines); tissues and germ line cells from a range of animal model systems, e.g: mice, rats, chickens. These transgenes will include: 1. micro RNAs and short hairpin RNAs designed to knock down gene expression within the recipient host cell line; 2. Functional gene products (e.g. Fluorescent markers, reporter genes, growth factors, interferon related genes, pro-apoptotic genes, ion channels and receptors) under a range of constitutive or inducible expression control. 3. innate immune receptors (such Toll-like or Nod-like receptors) and proteins from the Nuclear Factor Kappa B family. Although most targets/proteins would be innocuous, some of these targets/products may result in a phenotypic change in the recipient cell that might have deleterious effects should the vector be accidentally administered to the operators' own tissues. Thus, the lentivectors would be considered class 2 GMMOs, but once used for transgene delivery, the resultant GM cell line/animal etc would be considered a class 1 GMO unless risk assessment suggested otherwise. Each transgene will be individually assessed by the local GM safety committee to determine whether its use constitutes a Class 1 or Class 2 activity that falls under this current “umbrella” notification. Those deemed to be Class 3 activities will be notified individually to the HSE.

**Recipient or parental organism**

Lentivector particles will be produced by transfection of a eukaryotic cell line such as HEK 293T (a derivative of the 293 cell line containing the temperature sensitive gene for SV40 T-antigen and adenovirus type 5 E1A (a colinear segment from nt 1 to 4344, integrated into chromosome 19). The lentivectors will then be used to transduce mouse or rat neuronal cells (in cell culture and in individual animals), mouse cells (in cell culture), horse cells (in cell culture) and chicken cells (in cell culture and in ovo).
The anticipated effect is that the transgenes will be integrated into the cell genome, will be expressed and will produce a phenotypic change in the transduced cells/tissues. Typical phenotypic effects would be activation of the cells, resulting in enhanced repair capability in neuronal tissues; and expression of transgene products that suppress the replication of avian viruses.

Host/vector system

The lentivectors will be based upon (or similar to) the commercial vector systems pLentilox and Lenti 6 vectors in widespread use as gene delivery vectors. These consist of a vector plasmid into which the transgene cassette is inserted. Transfection of this plasmid into 293T cells results in the production of the lentivector RNA that contains the signals necessary for packaging into virion-like particles and integration into subsequently transduced cells. They contain no other lentiviral gene products (and some of our derivatives have had the potentially oncogenic WRE element deleted or modified as described in Schambach Gene Therapy (2006) 13, 641–645). These vectors are classified as HG1 while those that contain the entire WRE are classified as HG2. Packaging into virion-like particles is achieved by cotransfection of separate plasmids that express the gag/pol genes of HIV-1, the HIV rev gene and the VSV G envelope protein (or other the envelope protein from another virus such as influenza. The vectors lack extensive regions of homology and are unlikely to undergo homologous recombination. The likelihood of replication competent virus being regenerated by recombination is negligible. The recipient hosts would not be expected to propagate any such virus even if it did occur.

Origin & function

The genetic material to be included in the transgene cassette includes:

1. Mammalian genes encoding selected transcription factors or growth factors under the control of tissue specific or ubiquitous promotors (e.g. Sox2, NOGGIN)
2. Diphtheria toxin receptor gene under tissue specific promotor (e.g. P0 for Schwann Cell and olfactory ensheathing cells.
3. Micro RNA expression cassettes driven by the chicken, mouse or horse U6 promoter and targeting a variety of viral and host gene products.
4. Constructs designed to inducibly express the transgene contingent on the presence of the influenza virus RNA polymerase. In the absence of polymerase/virus infection, the transgene should not be expressed. The transgenes will consist of fluorescent markers, interferon response genes (e.g. Mx) and genes intended to cause the cell to rapidly initiate apoptosis in response to virus infection (eg. See section 17 for details).
5. Mammalian genes encoding selected proteins from the NIKB signalling pathway (p65, IKBa) or innate immune receptors under the control of tissue specific or ubiquitous promotors.
6. Channel rhodopsin 2, NMDA receptors and sodium channels.

The examples listed above correspond to those for which RAs have been submitted to our GMSC for approval. Since this is a standard gene delivery methodology, we anticipate that others will be submitted in future. These will be assessed individually by our GMSC and overall monitoring of the various projects will be the responsibility of the BSO.

Evaluation of foreseeable effects

The lentivectors will integrate their transgene payload into the target cells and the transgene RNA will be transcribed. This will result in a phenotypic change in the recipient cell/tissue/animal. The likelihood of this posing any risk to human, animal or environmental health is extremely low/negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Some of the vectors are intended to be introduced into the germ-line of mice and chickens to produce transgenic animals. These would pose no environmental, human or animal health risk and would be maintained under standard animal laboratory containment level 1 conditions after administration of the lentivector.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste: Biologically contaminated laboratory waste is collected in biohazard bags contained within a stainless steel container. Used plastic pipettes and pipette tips
are flushed with 5% Virkon and then discarded into disposable plastic containers that are autoclaved when full or weekly, whichever is sooner. All solid waste is autoclaved under standard validated conditions before exiting the premises.

Liquid waste: Collected in 1 litre polypropylene bottles containing 1 ml of QC Clear/D™ broad-spectrum (Quat-NH4) disinfectant. Volume not to exceed 800 ml per bottle. All liquid waste autoclaved under standard conditions.

Autoclaving: The premises have extensive autoclave facilities that are serviced annually by an authorised agent using an “in load” thermocouple monitor. Two Browne TST Control Integrators for steam autoclaves (complete colour change indicates that the strip was exposed to steam at 121 °C for at least 15 min or 134 °C for 5.3 min. Order code 2342) are included in loads periodically (one in the centre and one visible from the top). The autoclave is operated using the standard conditions of 60 min @121°C or 30 min @ 134°C. 100% kill is anticipated under these conditions.

Final disposal:
Solid waste: Landfill, double bagged in plain black polythene outer bag.
Clinical waste: Such waste is not routinely inactivated by a validated means prior to removal for incineration. Incineration is performed by external contractor licensed for GM waste transport and disposal of Class 2 GM waste.
Liquid waste: Post-inactivation, down the wash-up room sluice.
5% Virkon used for pipette tip rinsing is refreshed regularly and disposed of down the drain after minimum of 15 min from last use.
Animal carcasses and bedding: Incineration as described above.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment

Proposal 2008/03. Transgenic strategies to generate influenza virus resistant chickens.
BAB: I am happy with this.
RB: As discussed, I suggest including text that states the potential functional elements (see Q13) will be / could be from different species.
AG: I am happy with GM2008/3 (there is a spelling mistake in the answer to Q13 - it should read caspases not casases).
LT: No comment – its my proposal. Will be notified when candidate gene products have been tested in vitro before lentivector construction is done. GM chicken transgenesis is to be done by collaborators at the Roslin institute not here at Cambridge.

LT: Class 2 because of use of 293T cells and wpre in lentivector.
Inserts not hazardous. Include in umbrella notification to HSE.
AG: I have no major issues.
BAB: I am not up with the room designations in the Innes - is the surgery at category 2? I am happy once the injection is done that the animals are containment level 1 but probably with the possibility of aerosols and injecting the operator and animal handlers this procedure should still be containment level 2.
D.T: where she appears to refer to the host (mouse primary cells, or HEK cells) as ACDP class 2. May be I am out of touch but I didn’t think ACDP applied to mammalian cells? Other than that I didn’t have any issues.

Proposal 2011/11. Recognition of infectious agents by Pattern Recognition Receptors
LT: This is very similar to a number of proposals that have been considered by the GMSC in recent months and only falls into class 2 because of the use of the lentivector delivery system. I will include it with the umbrella notification for lentiviral vectors that is to be submitted shortly.

02/03/2022
Project Additional Information

Purposes of the contained use

This work will make use of Lactobacillus lactis subsp. cremoris for the cloning and expression of genes of interest (including potentially / known virulence and antibiotic resistance proteins) from Staphylococcus spp. (including the human pathogen: Staphylococcus aureus). The L. lactis subsp. cremoris cells will be electroporated with pOri23: a plasmid with the gene of interest under control of a lactococcal promoter (P23) following standard laboratory procedures. The resulting recombinant L. lactis subsp. cremoris strain expressing the protein of interest will then be tested in a range of in vitro phenotypic assays in order to evaluate the function of the expressed protein.

Recipient or parental organism
This work will make use of Lactobacillus lactis subsp. cremoris as a recipient.

**Host/vector system**

The vector is pOri23: ermAM - macrolide-lincosamide streptogramin resistance gene (erythromycin resistant in E. coli and Lactobacillus), P23 L. lactis promoter, oriColE1 – E. coli replicon, repD/E lactobacillus replicon.

**Origin & function**

The origin of the genetic material will be from Staphylococcus spp. (including S. aureus). A range of different coding sequences (CDS) will be cloned in order to investigate their biological function. CDS to be cloned include potential adhesins, coagulases proteins, other virulence factors and CDS encoding antibiotic resistance.

**Evaluation of foreseeable effects**

Lactobacillus lactis subsp. cremoris is a common bacterium used in the production of cheese and other dairy products. As a genetically tractable organism of very low pathogenic potential it has been used for the cloning and expression of genes from a range of more pathogenic bacteria. Its low pathogenic potential also has enabled its use as the first genetically modified organism in human clinical studies. In particular this Gram-positive bacterium is not known to carry adhesins to mammalian matrix proteins and has a well-characterized genetic background. It is highly unlikely that the transfer and expression of a single staphylococcal protein would generate a Lactobacillus lactis subsp. cremoris strain with pathogenic potential towards humans. Furthermore any such GMO would be unlikely to be any more pathogenic that the original Staphylococcus spp. strain from which the gene originated.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All bacterial liquid cultures will be autoclaved in an suitable autoclavable container. All other bacterial cultures and contaminated materials will be autoclaved following standard Departmental practice for handling Category 2 pathogens (see below). Routine cleaning of surfaces and areas of work are carried out using 70% Ethanol solution. Thus all waste leaving the department will be completely inactivated.

Solid waste: General laboratory waste is collected in biohazard bags contained within a stainless steel container. All solid waste is autoclaved under standard conditions before exiting the premises.

Liquid waste: Collected in 1 litre polypropylene or glass bottles containing 1 ml of QC Clear/D™ broad-spectrum (Quat-NH4) disinfectant. Volume not to exceed 800 ml per bottle. All liquid waste autoclaved under standard conditions.

Autoclaving: The premises have extensive autoclave facilities that are serviced annually by an authorised agent using an “in load” thermocouple monitor. Two Browne TST Control Integrators for steam autoclaves (complete colour change indicates that the strip was exposed to steam at 121 °C for at least 15 min or 134 °C for 5.3 min) are included in loads periodically (one in the centre and one visible from the top). The autoclave is operated using the standard conditions of 60 min @121°C or 30 min @ 134°C. 100% kill is anticipated under these conditions.

Final disposal:

Solid waste: Landfill, double bagged in plain black polythene outer bag.

Liquid waste: Post-inactivation, down the wash-up room sluice.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
Decision: Approved conditionally. Initial work in E.coli can be done under existing notifications. However, transfer to Lactobacilli will require HSE notification.

Category: Class 2, Notification required.

Comments:
A.G. I have a couple of minor points.
Summary: gram should start with 'G'; mammal should be mammalian; lactocococcal should be lactococcal
Q17: Is this not yes? especially with relation to the antibiotic resistance determinants
Q31: What about the antibiotic resistance
I agree with a class 2 designation

BAB: I think Ewan is confused about what should go in the Insert description box - he has described what is in the plasmid vector, which for me should be in the box above. From his summary text what should be here is genes of interest (potentially known virulence and antibiotic resistance proteins) from Staph species which he has put in Q14. Apart from that I am happy with the classification to take it to containment level 2.

C.C: It should be made clear whether intermediate cloning steps will be performed in E. coli.
The E.coli<>L.lactis shuttle vector pOri23 is given as being non-mobilizable. It is in fact mobilization-defective (it is a derivative of pIL253, a L.lactis plasmid that can be mobilized by pIL205). This isn't likely to happen in the lab of course.
Class 2 activity fine (depending on the genes to be cloned, Class 1 activity could be justified)
P.M. looks fine to me

L.T: The only concern I have is whether the plasmid carrying the genes has the potential to enable them to be transferred to another less innocuous host species. I assume this is the reason that it has been moved up to Class 2. We have not notified Lactobacillus for Class 2 GM work, so this will need to be done.

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<td>Animal Units</td>
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<td>L4</td>
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**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
**Project Additional Information**

**Purposes of the contained use**

To use plasmid transfection methods to generate recombinant influenza viruses to study the effect of mutations in the influenza A virus genome on antigenicity, replicative fitness, host species tropism and transmission dynamics in cell culture and animal model systems.

**Recipient or parental organism**

Influenza A virus generated from plasmid clones corresponding to each of the 8 segments of naturally occurring wild-type strains, specifically: A/PR/8/34 (Cambridge) and A/WSN/33 vaccine strains; A/Duck/Netherlands/10/99 (H1N1); A/swine/Eng/1353 (H1N1)). Other low pathogenicity avian influenza strains and strains of equine influenza may also have virus rescue systems developed for them. This notification only applies to HG2 strains and class 2 genetically modified versions of these.

**Host/vector system**

pDual reverse genetics plasmids or derivatives thereof capable of producing:
A/PR/8/34 (Cambridge); A/WSN/33
A/Duck/Netherlands/10/99 (H1N1);
A/swine/Eng/1353/09 (H1N1);
A/England/195/09(H1N1)
**Origin & function**

The pDual reverse genetics plasmids (e.g. pRF483) are comprised of the pCDNA3 backbone carrying human or avian PolI promoter upstream of the negative sense full genome sequence from 1 of each of the 8 segments of the viruses named above. Downstream of these is the mouse RNA PolI terminator or HDV self-cleaving ribozyme sequence positioned to facilitate precise termination at the 3' end of the influenza genome sequence. This sequence is then followed by the CMV IE promoter in the reverse orientation to drive expression of a mRNA encoding the influenza gene product. Transfection of 8 plasmids (1 for each gene segment) allows the rescue of infectious virus with the genetic content dictated by the plasmids used. Substitution of one or more plasmids with those obtained from different strains, or carrying defined mutations allows the production of defined genetic variants that can then be studied to determine the effects of the sequence variation on antigenicity, replicative fitness, host range and transmission dynamics.

**Origin of viral sequences:** Wild-type sequences of the strains listed above. Sequence variants occurring in response to antigenic selective pressure, or selection by passage of virus strains in cells or tissues from non-cognate host-species (e.g. pig, horse, chicken, but excluding human) are amplified by PCR and sequenced (or generated commercially by gene synthesis). These are then cloned into the reverse genetics plasmid (e.g. pRF483) and used to generate viruses with defined mutations (either individually or in combination). These studies are restricted to HG2/Class2 influenza viruses e.g. low pathogenicity avian influenza viruses (excluding H5 and H7), swine influenza viruses, equine influenza viruses and human influenza viruses.

**Evaluation of foreseeable effects**

The virus rescue systems allow the production of as near as technically possible homogenous population of viruses from a defined set of plasmid encoded genes. The expectation is that such rescued viruses will behave like the viruses they were derived from. However, it is not uncommon for the rescued viruses to show decreased replicative fitness as their homogeneity limits their adaptive and interactive potential when compared to the quasispecies behavior of the original virus population. The application of virus rescue for reverse genetics allows the effects of mutations (such as those that occur naturally during virus growth) to be tested individually in a defined genetic background to determine their effect on antigenicity, replicative fitness and host-range. The foreseeable effects are: 1. No effect (synonymous mutations); 2. reduced susceptibility to neutralization by sera specific for the original strain; 3. increased replicative fitness in the cell lines from the species in which they have been passaged; 4. increased or decreased replicative fitness when propagated at different temperatures. The possibility of generating viruses that gain the ability to infect humans...
more efficiently than the parental virus is unlikely but possible (note: A/Swine/Eng/1353 is a close descendant of the pdm2009 strain A/England/195 and is already human tropic). The proposed work does not involve the deliberate introduction of mutations associated with human tropism into non-human tropic strains. All virus genes used will be sequenced prior to performing virus rescue experiments. The virus rescue system uses the HEK293 cell line which is of human origin. It is now generally considered to be necessary to verify the sequences of rescued viruses to confirm that no unintended mutations have occurred during the rescue process. This has the added benefit of confirming that mutations confirming human tropism are absent. Any unexpectedly showing signature sequences for human tropism (i.e ability to use alpha 2-6 sialic acid receptors) will not be cultured. Should it be decided in future to analyse such variants we deem this would require renotification to the HSE.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste is pretreated with QC-Clear D prior to autoclaving. All waste is autoclaved (25 min@134°C for mixed solid waste; 25min@121°C for liquid waste, using appropriate "in load" probe location. 100% kill expected) using the main department autoclave. Disposal is to landfill or down the sluice to the drain. The autoclave waste cycle is validated annually by 12 point in-load thermocouple. Each waste run is verified by visual inspection of the data print-out showing temperature and duration for each stage of the sterilization cycle. Each load contains a TST control strip* to verify the load has undergone the process. Success or failure is recorded in the autoclave logbook, along with action where relevant.

*Browne TST Control Integrators for steam autoclaves. Re-order code 2342. Complete color change indicates that the strip was exposed to steam at 121degC for at least 15 min (or 134degC for 5.3 min).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
GM4014_01 Rescue of recombinant influenza viruses. (Part 1 of 2). Barcoded influenza viruses.
BAB: This looks good to me. I did wonder if the 7 day restriction for contact with pigs was too long - is it
not usually a 48h restriction (eg people from Pirbright etc).
Response from LT: As there is the potential for infection of the worker rather than simple passive
carriage, 7 days seems more appropriate. This would be extended if the worker showed flu-like
symptoms to 7 days post recovery. Workers will also be vaccinated using the current trivalent vaccine
C.C: This seems fine, given that the only modifications are in the wobble position and are unlikely to
cause any phenotypic changes.
C.B: These look OK to me.
A.G: Assuming that all operators have received the appropriate training and are familiar with the
techniques and SOPs then I am happy with the risk assessments. I am not sure that anybody else
using the hoods that you refer to will come into contact with swine, however if they do then it might be
worth notifying them in advance of them using the hoods.
Response from LT: There will be a hood dedicated to working with influenza virus for the duration of
this project.
GM4014_02 Rescue of recombinant influenza viruses. (Part 2 of 2) Characterisation of cell culture
adapted influenza viruses.
BAB: This looks good to me. I did wonder if the 7 day restriction for contact with pigs was too long - is it
not usually a 48h restriction (e.g. people from Pirbright etc).
Response from LT: As there is the potential for infection of the worker rather than simple passive
carriage, 7 days seems more appropriate. This would be extended if the worker showed flu-like
symptoms to 7 days post recovery.
C.C: This also seems fine. I was concerned at the possibility that the adaptations may increase
virulence, but this is expected to be unlikely given the nature of the lab passaging. Has there been any
published work reporting this type of approach to generate attenuated strains? If so it'd be useful to
include a reference.
C.B: These look OK to me.
A.G: Assuming that all operators have received the appropriate training and are familiar with the
techniques and SOPs then I am happy with the risk assessments. I am not sure that anybody else
using the hoods that you refer to will come into contact with swine, however if they do then it might be
worth notifying them in advance of them using the hoods.
Response from LT: There will be a hood dedicated to working with influenza virus for the duration of
this project.
GM4014_04 Rescue of recombinant influenza viruses: Production of A/PR/8/34 virus carrying segment
4 (HA) from A/mallard/10-Nmkt/1999 or A/mallard/10-Cam/1999
BAB: On Q28b can you definitely say it would not disseminate or would this be a limited transmission
chain. This sort of goes with Q30 answer - unknown.
Response from LT: Changed to "unlikely" and now addressed in Q35.
You need to complete the Q36 justification.
Response from LT: Now completed.
Apart from that I am happy with CL2
CB: Looks fine to me.
DT: I am fine with the revised version.
A.G: I have looked over the application and it all looks fine to me.
### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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### Project Ref 407/15.1

**Date Ackn'd**: 13/04/2015  
**CU2 Project Title**: Animal models of neurodegenerative disease

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<th>CultureVolumeClass3-4</th>
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<tr>
<td>Class 3</td>
<td>&lt; 1 Litre</td>
<td>&lt; 1 Litre</td>
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</table>

**Non-GMM**: Yes  
**Consent Granted**: Yes  
**Project notified under transitional arrangements**: No

### Historical Significant Changes
- N

### Project Additional Information

**Purposes of the contained use**:  
The purpose of the contained use is to express normal and modified sequences of DNA encoding the prion protein (PrP) within invertebrate and mammalian hosts to study prion pathogenesis in laboratory animal models.

**Recipient or parental organism**
- **Invertebrate hosts**:  
  Drosophila melanogaster are small flies (about 3 mm in length and 2 mm in width) and belong to the order Diptera. Drosophila mature through complete metamorphosis.
with the mature female being slightly larger than the male. Similar to all insects Drosophila are covered in a chitinous exoskeleton, they have three main body segments and they have three pairs of segmented legs. They have one pair of wings and can walk and fly (excluding wingless mutants). Caenorhabditis elegans (C. elegans) are free-living (non-parasitic), transparent nematodes (roundworms), about 1 mm in length and were the first animal whose relatively small genome was completely sequenced. C. elegans is an unsegmented, vermiform, pseudocoelomate, that lacks a respiratory and a circulatory system. The majority of these nematodes are female hermaphrodites. Males have specialised tails for mating that include spicules. C. elegans is bilaterally symmetrical, has a cuticle (a tough outer covering), four main epidermal cords, and a fluid-filled pseudocoelom, (body cavity). The basic anatomy of C. elegans includes a mouth, pharynx, intestine, gonad, and collagenous cuticle. The four bands of muscles that run the length of the body are connected to a neural system that allows the muscles to move the animal's body only in the forward direction, (from the back to the front), so that any living, moving individual is always on either its left side or its right side when observed crossing a horizontal surface.

Vertebrate hosts
Laboratory mice are small mammals of the order rodentia, usually of the species Mus musculus. They are the most commonly used mammalian research model in areas such as bio-medicine, immunology, genetics, and infectious disease. Mice belong to the Euarchontoglires clade, which includes humans. The laboratory mouse genome has been sequenced and many mouse genes have human homologues. This relatively high homology with humans at the genetic level, their ease of maintenance and handling, and their high reproduction rate, make mice particularly suitable models for human-oriented research. Mice have sharp teeth and can bite if provoked.

Laboratory hamsters are typically stout-bodied, with tails shorter than body length, and have small, furry ears, short, stocky legs, and wide feet. They have thick, silky fur, which can be long or short. Hamsters have sharp incisors; they have an upper pair and lower pair that grow continuously throughout life. Hamsters are very flexible, but their bones are somewhat fragile. Hamsters have poor eyesight.

Host/vector system
Selected hosts will be made transgenic for PrP by appropriate transgenesis vector systems that give stable integration of DNA encoding the prion protein in the recipient’s genome. We will use currently available vectors that allow efficient transgenesis of the species in question, as well as any new vectors or transgenesis systems that may emerge in the future.

Origin & function
The normal form of the prion protein is expressed in hosts ubiquitously but principally by cells of the central and peripheral nervous system, and the lymphoreticular system. The physiological function of the normal form of the prion protein PrPC is not fully understood although it can function as a copper binding protein. PrP is associated with prion diseases, or transmissible spongiform encephalopathies. It is currently believed that PrPSc, a conformationally modified form of PrPC, may itself constitute the infectious prion agent.

The species forms of PrP to be studied here will include: ovine, murine, hamster, bank vole, porcine, cervid, bovine and human. However, the work may expand to include PrP genes from any other species. The PrP DNA sequences will be obtained by PCR cloning from the genomic DNA or mRNA from the species in question or from plasmids carrying the previously cloned genes or by direct DNA synthesis by a commercial gene synthesis company. The genes will be expressed as protein in the new invertebrate and mammalian host and will be detected on cell surfaces, in the cytosol, or secreted from cells, or in cultured cell preparations or harvested as a source of protein for laboratory use.

Evaluation of foreseeable effects
Drosophila melanogaster
In the unlikely event that PrP transgenic flies that express the prion protein manage to escape from our fly containment laboratory, there is a possibility that they will be able to survive for a limited amount of time and potentially breed with non-transgenic flies. However, only 25% of the resultant progeny could express the prion protein since expression requires the presence of the PrP transgene and the driver gene, which are encoded on different chromosomes in our transgenic flies. Escapees that express wild-type PrP are considered to present an extremely low level of risk. There is no evidence that wild-type PrP expression in the fly spontaneously mis-folds into an
infectious form. Furthermore, it is considered to be extremely unlikely that such flies would come into contact with a prion-infected animal and acquire exogenous prions un-aided. Escapees that express a mutant allele of PrP associated with spontaneous formation of prion disease (e.g. alleles associated with CJD, GSS and site directed mutants that knowingly disrupt the stability of the native conformation of the protein) and their progeny could potentially spontaneously generate infectious prions. However, the ability of such flies to generate infectivity that can infect a mammalian host has not yet been demonstrated experimentally. Escapees could be those that express wild-type or mutant forms of PrP that have knowingly been exposed to exogenous prions in the laboratory and such flies could potentially infect other hosts that express PrP. For this to occur the infection would be expected to occur by the oral route. However, it would be very unlikely that a prion-infected fly would escape from our fly containment laboratory and subsequently be eaten by another animal or human. Even if it did, the amount of prion infectivity in one fly would be considered to be relatively low and therefore present an extremely low risk by peripheral exposure.

**Caenorhabditis elegans**

In the wild, C. elegans are found in the soil and feed upon decomposing plant matter. In the laboratory, C. elegans are maintained in liquid culture or on agar plates supplemented with bacteria, using very specific methods. It is not likely that experimental C. elegans could survive for any appreciable time if they were to escape from such laboratory cultures or if they are removed unwittingly. As such, in the highly unlikely event that PrP transgenic C. elegans, with or without exposure to infectious prions, could escape from a worm containment laboratory, it is considered that this would pose little, if any, risk to the operator or the surroundings.

**Rodents**

Any prion infectivity work to be carried out in laboratory mice or hamsters will be in collaboration with groups already working with these animals and these infectious agents so that discussions on current ‘best practice’ can take place prior to commencement of the work. All prion-infected vertebrate hosts will be housed in CL-2 / CL-3 purpose-built animal containment facilities (for example, the APHA at Weybridge) that have been specifically designated as suitable for prion infectivity experiments. These types of experiments have been on-going for many years at the APHA CL-2 / CL-3 purpose-built animal containment facilities where prion-infected rodents are suitably maintained, controlled and monitored by experienced animal staff. The chances of a prion-infected vertebrate host escaping from such a facility is considered to be negligible.

---

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Two GMO classification levels and corresponding containment levels apply as follows: Low level (CL-2) applies to hosts carrying PrP genes with no history or expectation of causing spontaneous prion disease in the natural host. These will include, but will not be limited to, wild-type (normal alleles) of ovine, murine, hamster, bank vole, porcine, cervid, bovine and human PrP. Higher risk level (CL-3) applies where mutant alleles of PrP associated with spontaneous formation of prion disease (e.g alleles associated with inherited prion diseases of humans such as familial CJD, GSS, FFI, and site-directed mutants) that knowingly disrupt the stability of the native conformation of the protein that may induce a prion disease-specific form.

The intention is to expose these two classes of GMOs to infectious prion material from a variety of sources. Use of prion inocula from hosts not known to produce prions capable of causing disease in humans (e.g. ovine and murine PrPSc) will not result in alteration of the containment class used for the experiment. However, where the prion inocula is derived from human, bovine or cervid prion disease, or any species infected with bovine prion material, the activity will be deemed as Class 3, irrespective of the origin of the PrP gene in the host GMO.

This work will be carried out in CL-2 / CL-3 prion-dedicated containment laboratories with suitable control measures in place for use of invertebrate (e.g. Drosophila or C. elegans) or vertebrate hosts (e.g. rodents) of experimental prion disease.

With particular reference to the use of Drosophila, the fly laboratory (room 313) will contain 2 types of fly-trap (hanging sticky paper and bright yellow liquid traps) to trap any flies that escape from their containment vials. In addition, the fly containment facilities will have a double door arrangement with an internal fly screen to prevent the escape from the room of any flies that can fly. The PrP transgenic Drosophila used for Class 3 experiments will be first crossed with a wingless diver fly line to produce flightless flies that express PrP. The use of flightless Drosophila will significantly reduce the possibility of escape from the containment laboratory of flies potentially carrying prion agents that have a remote possibility of causing human disease. The fly containment laboratory will have sticky paper mats placed on the ground in the doorway area (on the inside) so that any flightless flies will be trapped as they attempt to cross this area in the unlikely event of escape from their containment vials. Only suitably experienced
research personnel will perform experiments with Drosophila inoculated with Category 3 prion material.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

1. Plasmid preparation: There is an extremely remote possibility that a PrP DNA transformed host bacterium strain, even if disabled, could colonise the gut through ingestion and produce protein in situ, or pass on the plasmid of interest. The molecular biology component involving murine and ovine PrP genes has been risk assessed to require Containment Level (CL) CL-2 and notified as such previously (with derogation see GM407/03.1). The PrP genes from human and bovine sources were specifically excluded from that derogation. They will therefore be manipulated in our CL2/3 prion laboratories for all procedures. However, we request permission to perform plasmid preparative procedures requiring centrifugation in the CL1 laboratory on the condition that to ensure restricted access, the centrifuge will not be left unattended. Sealed buckets will be used and opened in the class 2 MSC in room 313.

There is a theoretical risk that altering the PrP DNA sequence, or the context in which it is expressed, could alter the conformation of the protein to a disease specific form that may be regarded as hazardous. An example of this would be the generation of recombinant sequences that are, or are analogous to, mutations associated with human prion disease. The escape of a bacterial strain containing such a hazardous protein and the colonisation of an operator would be the worst-case scenario for this type of work.

The risk associated with this theoretical possibility is considered to be negligible because of the following:

a) the infectivity of prion disease is subject to a transmission, or species barrier, that for some cases cannot be overcome.

b) requires specific routes of infection that are unlikely to occur within the given working practices of the laboratory.

c) the materials in use are subject to levels of containment and methods of disposal suitable for reducing to a minimum any chance of contact by the operator.

2. Fly manipulation. All this work will be carried out in CL-2 / CL-3 prion-dedicated containment laboratories with suitable control measures in place. We would like to be permitted to house our Class 3 fly cultures in the incubators in our prion-dedicated CL-2 laboratory (rooms 309 and 310. See facility plan). The flies will not me manipulated in these labs and so there is an extremely low likelihood of accidental escape. Access will be restricted by locking the incubators (in addition to the CL2 PIN code access controls already in place). Although possible, we would prefer not to have to designate these rooms as CL3(TSE) because of the limitations this would place on their use by inexperienced students and future re-designation for other non-TSE purposes.

See Appendix I: Standard Practices for Arthropod Research at Containment Level 3

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste that contains prion-infected material (either CL-2 or CL-3 material) will be decontaminated and/or disposed of in an appropriate manner.

All solid waste will ultimately be placed into clinical waste bags for incineration. The items for disposal will be double bagged and fully sealed and clearly labelled before being taken to an appropriate clinical waste bin for removal from site and incineration.

Solutions of prion-infected material or samples of brain homogenate, together with buffers and solutions from techniques such as SDS-PAGE and ELISA that have been exposed to prion samples, will be disposed of in accordance with COSHH regulations. Specifically, all liquid waste will be de-contaminated in a solution of 2M NaOH for one hour or 1M NaOH overnight followed by suitable neutralisation [ie. addition of an equal volume of 2M or 1M HCl, respectively (NaOH + HCl = NaCl + H2O)] prior to disposal in a designated sink using copious amounts of water.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
ATTACHMENTS: GM2015_02_decision.doc, GMA2015_01_decision.dot, Prion facility.ppt, Appendix 1 Standard Practices, Appendix 2
Local ACGM use Proposal no: GM2015/02
Decision: Approved
Category: Class 2 GM. Significant change from previous notification. Notification required.
Comments:
BAB: GM2015_02 Q40 do they want to derogate from CL2 for all this work or only non-human that is not bovine??
They are proposing to work in room 309 which does not have a CL2 cabinet for the Ecoli work I think. Where they produce final high concentrations of bacteria in suspension and/or plasmids/proteins how are they proposing to limit aerosol formation and inhalation?
Where they work with human genotypes or mutants that are more likely to spontaneously cause PrPsc I think this needs to be done in a CL2 hood. Presumably all waste from this would need to either be chemically inactivated if liquid or incinerated if solid ie not the usual CL2 autoclave route that they have said they will use in Q28.
Response from LT. No derogation is requested (see comment below). A class 2 MSC is required for the components likely to generate an aerosol. The class 2 hood in room 313 is to be used for this purpose. This RA refers to the plasmid preparation. Solid waste disposal is by incineration (this has now been indicated as the final route of disposal). Chemical inactivation (2M NaOH or 20K ppm hypochlorite) for liquid as specified in TSE SOP.
LT. This is basically an update of a previously approved RA dealing with recombinant PrP purification. It revises the containment up to full Class 2 (or possibly CL3) from a previous derogated CL2 standard because they now intend to purify human PrP sequences. The preparation of the plasmids and proteins for the expression component requires full CL2 (including use of a class 2 MSC for any steps likely to generate an aerosol). The production of the drosophila transfer vectors arguably does not require CL2, as the plasmids will not be able to express protein except when crossed into the appropriate drosophila driver-line background. However, it makes sense to do all this work in the dedicated CL2 and CL3 TSE labs. Room 313 is to be designated as CL3(TSE) and its access controls upgraded. It is an ex-CL3 lab and already up to standard.
Rm 313 is where the Class 3 TSE infection experiments in drosophila will be performed (see GMA2015/01). I am uncertain whether the E.coli expression of human and bovine PrP is class 2 or class 3 (Class 3 seems excessive for WT proteins.) The modified versions that might have the propensity to spontaneously adopt the prion form might be viewed as Class 3. If so, the general derogation for TSEs pertaining to negative pressure and fumigation sealability would enable rooms 309 and/or 310 to also be designated CL3(TSE) simply by upgrading the access control. Ideally room 313 will be sufficient and this wont be necessary as it may pose problems in the future should we ever wish to re-task these rooms if there is reason to believe the rooms themselves have been contaminated. I will request clarification from the HSE regarding the classification of the E.coli expression of human PrP mutants in the CU2 notification.
DT: I have been through the documents and I agree that the this risk assessments appear to be appropriate. I don’t have any questions or points for consideration.
A.G. I have reviewed all of the documents that you have provided and I am happy that sufficient consideration has been made to the work that will be conducted and that sufficient security measures are in place. One minor point, the documents state that prions from other species may be used. For records and in case of an accidental release the group should inform you of the species if other species are used.
C.Can. I have gone through the documents and it all looks in place to me.
S.S. I am happy with the risk assessment, although I agree with Andrew that if prions from other species not currently mentioned are brought in then it would be a good idea to inform you/update the document accordingly.

Local ACGM use Proposal no: GMA2015/01
Decision: Approved
Category: Class 3 GMA. HSE notification required.
Comments:
BAB: GMA2015_01
Do the cabling ingresses into room 313 need to go through Roxtec gland plates or is the trunking sufficient?
Can they add in Section B that wingless flies are unlikely to breed with wild-types?
I am not sure which bits of the project they want derogated - all of it? Surely not the ones with forms of PrP that can spontaneously form human infectious PrP. Just thinking this through I am not sure it will make a difference to what they are going to do in practice anyway.
Response from LT: The trunking has been thoroughly sealed and will not allow flies to escape via any cable penetrations to other rooms in the suite. The comment regarding wingless flies mating habits could be included, but this is a post escape event and escape is already adequately prevented by other measures. The reference to derogation was a hangover from an earlier version and has been deleted.
C.Can. I have gone through the documents and it all looks in place to me.
L.T. I think Drs Bujdoso and Thackray have done a thorough job of their risk assessment and supporting documentation. The measures taken to reduce the hazard posed by the PrP transgenic flies (whether infected or uninfected) are proportionate to the risks. The facility (room 313) requires some upgrading before the Level 3 work can commence (double access door and covering vents with mesh).
DT: I have been through the documents and I agree that the this risk assessments appear to be appropriate. I don’t have any questions or points for consideration.
A.G. I have reviewed all of the documents that you have provided and I am happy that sufficient consideration has been made to the work that will be conducted and that sufficient security measures are in place. One minor point, the documents state that prions from other species may be used. For records and incase of an accidental release the group should inform you of the species if other species are used.
S.S. I am happy with the risk assessment, although I agree with Andrew that if prions from other species not currently mentioned are brought in then it would be a good idea to inform you/update the document accordingly.

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Project Ref 407/20.2

Date Ackn’d 04/09/2020

CU2 Project Title
Host interactions and motility of Klebsiella pneumoniae and Pseudomonas aeruginosa

Class 2
CultureVolClass2 < 1 Litre
CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

| The motility and interaction of the opportunistic pathogens Klebsiella pneumoniae and Pseudomonas aeruginosa with the eukaryotic host will be studied by infection assays, FACS and microscopy. Therefore, plasmids carrying antibiotic resistance markers for selection and expressing fluorescent proteins will be electroporated into strains of Klebsiella pneumoniae. Genes of interest will be deleted by allelic exchange mutagenesis. Genes will be inactivated by insertion of antibiotic resistance markers (e.g. tetracycline, chloramphenicol) by using a suicide plasmid. Mutants will be confirmed by PCR and DNA sequencing. Commercially available transposon mutants of Klebsiella pneumoniae (Ramage et al. 2017) and Pseudomonas aeruginosa (Liberati et al 2006) will be used. Functional genes and their natural promoters from Klebsiella pneumoniae or Pseudomonas aeruginosa will be cloned into plasmid vectors to allow complementation of the mutant strain. |

**Recipient or parental organism**

| Klebsiella pneumoniae and Pseudomonas aeruginosa are ACDP hazard group 2 pathogens. Both species are opportunistic pathogens and primarily only pose a risk to immunocompromised individuals and those that are hospitalised. Both are commonly present within the environmental flora and fauna and pose limited risk to individuals if correct sanitation procedures are followed. Klebsiella species rarely cause disease in healthy humans and most Klebsiella pneumoniae infections are self-limiting with symptoms relating to pneumonia (bronchitis) and urinary tract infections. Pseudomonas aeruginosa is able to infect the airway, urinary tract, burns, and wounds, and other blood infections. |

**Host/vector system**

| Escherichia coli |

**Origin & function**

| Plasmids carrying fluorescent proteins and antibiotic resistance markers for selection. Plasmids do not increase pathogenicity of Klebsiella pneumoniae or Pseudomonas aeruginosa and antibiotic resistance markers are chosen so that at least two different classes of antibiotics remain usable. The vast majority of the transposon mutants are expected to have reduced virulence when compared to the respective wildtype because most of the mutations result in loss of function, and there are no examples of mutants that substantially increase its virulence in such a way that the mutant strains would have to be considered as HG3 or above. |
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste will be deactivated by QC clear treatment followed by autoclaving using a validated waste cycle. The expected degree of kill is effectively 100% according to preliminary tests. This procedure is in accordance with the University policy, according to which all GMM must be inactivated before disposal.

In case the autoclave might be down or is unavailable, samples will be treated with 1 % final Virkon for at least 30 min.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick if you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The project and the risk assessments have been viewed and approved by the Department of Veterinary Medicine Biological and Genetic Modification Committee.

Minor comments from members of the Biological and Genetic Committee were included in the final version. e.g.

RB - In case of power cut/failure, samples will be moved to a different freezer in the same containment level two laboratory. In the unlikely event that the freezer defrosts, as all the contents are sealed in cryo-vials inside freezer boxes, the biological material will be kept contained.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L3 L4</td>
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</table>

02/03/2022
Project Additional Information

**Purposes of the contained use**
Identification of vaccine targets to prevent respiratory disease in racehorses. Respiratory disease affects 10% of all Thoroughbreds each month during their first two-years of life. The disease reduces performance and is an important cause of lost days in training, leading to significant cost to the racing industry. *Streptococcus zooepidemicus* is the pathogen most strongly linked to this disease, but there are currently no vaccines against it. We have studied the genomes of *S. zooepidemicus* and its close relative, *S. equi*, which causes strangles in horses. Recently, we developed a novel method, transposon directed insertion site sequencing (TraDIS), to identify genes important to *S. equi*, which has informed the design of better strangles vaccines. In this project, we will use this new method to identify key genes required for *S. zooepidemicus* to resist the equine immune response and attach to the equine trachea, prerequisites for this organism to cause respiratory disease. Our results will direct vaccine design to improve the health of young Thoroughbred racehorses.

**Recipient or parental organism**
*Streptococcus zooepidemicus* (wild type)

**Host/vector system**

**Origin & function**
Conferring erythromycin antibiotic resistance for selection carried on ISS1. Generating mutants by transposon mutagenesis. Allelic replacement mutagenesis using sections of *S. zooepidemicus* DNA for homologous recombination.
The transposon libraries generated will contain strains with an insertion in every gene of S. zooepidemicus (apart from essential genes, which will be lost in the process of generating the libraries), generating a knockout in the gene where the transposon has inserted, these strains express erythromycin resistance as part of the integration process. Allelic replacement mutants will have the gene of interest deleted from the genome and will lose the function of that particular gene these strains will be antibiotic sensitive.

### Evaluation of foreseeable effects

Not applicable

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste will be deactivated by autoclaving using a validated waste cycle. Liquid waste will be treated with QC clear D followed by autoclaving. The autoclave cycle for waste disposal will be run at 121°C for 15 minutes. This procedure is in accordance with the University policy, according to which all GMM must be inactivated before disposal. All waste will be ultimately incinerated as clinical waste. Spillages/leaks will be decontaminated with 70% ethanol or 1% virkon for 30 min, Swabs will be taken from spillage area, plated onto Strep select agar plates and incubated overnight at 37°C to confirm complete killing.

### Is an emergency plan required according to regulation 20?  

N

### Tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

N

The project and risk assessments have been viewed and approved by the Department of Veterinary Medicine Biological and Genetic Modification Committee. Comments from the committee include:

Although not on the ACDP list of biological agents, other Streptococci are Hazard Group 2 pathogens. HSE website for occupational risk of infection supports information given in RA. Disease similar to eg Streptococcus pyogenes, a group 2 pathogen. Experiments planned interrupt gene expression and do not introduce new expressed genetic material apart from erythromycin, thus I agree with the hazard group and containment level (2) specified in the RA. As the bacteria are penicillin resistant the erythromycin resistance does not interfere with treatment options if infection occurs.

### Project Containment

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02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 407/95.2

Date Ackn’d 28/08/1995

Date Project Ceased

Consent Granted

Project notified under transitional arrangements [Y]
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Project Containment

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Project Ref 407/95.4

**Date Ackn'd**: 28/08/1995

**CU2 Project Title**: GENE TRANSFER INTO RETINAL TISSUE AND CELL LINES

**Class**: Class 2

**CultureVolClass 2**: not applicable

**Consent Granted**: not applicable

**Project notified under transitional arrangements**: Y

**Effective Date of Additional Info**: 28/08/1995

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<tr>
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**Project Ref** 407/95.5

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**Date Project Ceased**

- Non-GMM
- Consent Granted: not applicable

- Project notified under transitional arrangements: Y

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N


**Historical Date of Additional Info**: 20/09/1999, 20/09/1999, 03/02/1997

**Project Additional Information**

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 407/97.1

Date Ackn'd 03/02/1997

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

EXPRESSION OF VIRAL GLYCOPROTEIN AND POLYMERASE, USING THE SEMLIKI FOREST VIRAL EXPRESSION SYSTEM

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements Y
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 407/97.2

Date Ackn'd 20/08/1997

CU2 Project Title
THE PATHOGENESIS AND IMMUNITY OF BACTERIAL INFECTION

Class 2
Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
GM407/99.4, GM407/00.2, GM407/97.2

Historical Date of Additional Info
04/11/1999, 29/08/2000, 15/01/2001

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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</table>
# Project Additional Information

## Purposes of the contained use

## Recipient or parental organism

## Host/vector system

## Origin & function

## Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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02/03/2022
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Name

GURDON INSTITUTE

Name 2

UNIVERSITY OF CAMBRIDGE

Campus Estate or Research Centre

THE WELCOME TRUST/CANCER RESEARCH

Road Name

TENNIS COURT ROAD

Building

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB2 1QR

Country

ENGLAND

Tel Number

01223 334088

Fax Number

01223 334089

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
**Premises Addresses**

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<td>CAMBRIDGES HIRE</td>
<td>CB2 1QR</td>
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Tick if confidential

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Mycology
Transgenic
Invertebrates
Transgenic
Plants
Other (please specify below)
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 408/01.1

Date Ackn'd
15/02/2001

CU2 Project Title
MODIFICATION OF GENES ASSOCIATED WITH STOMATIC NUCLEAR REPROGRAMMING AND/OR OCT4 EXPRESSION.

Non-GMM
Consent Granted
not applicable

Withdrawn
N

Tick if notifying a connected programme of work
N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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02/03/2022
**Project Ref** 408/04.1

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<td>Packing of retroviruses to perform siRNA in human tissue culture cells</td>
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To study the function of human proteins in cell culture by reducing their levels using siRNA.

**Recipient or parental organism**

Recipient/vector

- Amphotropic retroviruses
  - murine embryonic stem cell virus
  - extensively modified human lentivirus (commercial vector system)

Inserted material

- 21 nucleotide fragments of human cDNA.

**Host/vector system**

- Eukaryotic viruses/mammalian
Replication incompetent retroviruses

Cell Lines
HeLa
Ltert
Phoenix Packaging cell line
293T Packaging cell line

Origin & function

Origins:
Hairpin repeat of 21 nt human exon. Sequences that should stimulate siRNA in human cell lines. Hairpins derived from human cDNA library generated in Amsterdam and distributed by Cancer Research UK.

Intended function: To reduce the level of endogenous protein in cell culture in siRNA screens and generating stable cell lines.

Evaluation of foreseeable effects

The insertion of siRNA sequences into the recipient virus is not expected to alter its tropism, host range or virulence. They should not allow generation of replication-competent viruses. The siRNA sequences are expected to reduce but not eliminate the amount of endogenous protein.

The viruses to be packaged are extensively modified to prevent the generation of replication competent virus. The human lentivirus has only the gag, pol and rev proteins of the parental virus (see risk assessment).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

-

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard methods will be used.

Liquids: Treat with virkon according to manufacturers instructions. 100% kill expected. Dispose via drains.
Solid and mixed waste: Autoclave 121 degrees C, 100% kill expected. Disposed as standard waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Project Containment

Laboratory Activities Glass Houses Growth Rooms

L2 Yes L3 L4 L2 L3 L4 L2 L3 L4 L3 L4 L2 L3 L4

Animal Units Large Scale Activities Human Clinical Applications

L2 L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4

Project Ref 408/08.1

Date Ackn'd 28/01/2008

CU2 Project Title Packaging of mouse cDNA library into retroviruses for cell-based functional screening for reprogramming factors.

Date Project Ceased

Class 2 Culture Vol Class 2 Culture Volume Class 3-4

Consent Granted Not Applicable

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Historical Significant Changes

Project notified under transitional arrangements N

Project Additional Information

Purposes of the contained use

By using retrovirus expression vectors containing cDNA5 from mammalian cells, we would like to screen for reprogramming factors that can affect cell fate and proliferation.

Recipient or parental organism

Differentiated mouse cell lines
### Host/vector system
- pMX retroviral expression vector
- Phoenix Eco Cells (Murine-ecotropic)

pMX vector lacks gal-pol and envelope sequence. The pMX vector amplifies and produces retrovirus protein only in Phoenix Eco Cells. These cells produce gal-pol and envelope protein that can only infect mouse or rat cells.

### Origin & function
**Origins of the genetic materials involved:**
Expression library contains cDNA5 from mouse oocytes, primordial germ cells and embryonic germ cells. Embryonic germ cells are derived from primordial germ cells of mid-gestation embryos. The library contains all gene sequence expressed in these tissues and the cell line.

**Intended functions of the genetic material involved:**
Since all gene sequences expressed in the original tissues can be expressed in recipient cell lines, there would be various types of functions. However, only recipient cells that express a certain gene product will survive in vitro culture by virtue of de-differentiation and re-programming. These gene products presumably have a role in cell differentiation and proliferation.

### Evaluation of foreseeable effects
Some recipient cells transfected with the gene products are expected to acquire pluripotency by being reprogrammed. The reprogrammed cells are expected to be able to differentiate all kinds of tissues including germ U cells, neuronal cells and muscle cells, etc. The foreseeable effects can be evaluated by expression of pluripotent U cell-specific genes, such as Stella/Dppa3, Oct4 and Nanog.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**n/a**

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

To prevent the retrovirus into human, we have chosen the Phoenix Eco cells that produce envelope that can only infect mouse or rat cells. Retrovirus-encoding vector lacks sequences for its gag-pol and envelope, therefore the virus cannot replicate in any cells except the Phoenix cells.

All culture medium containing active retrovirus particle will be autoclaved after use.

All other liquids will be treated with 2% Virkon solution for 30 mm.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The type (form): Phoenix Eco cells and transfected recipient cells (cells)

Treatment: Autoclave (126 degrees, 25 mm)

Degree of Kill: 100%

Autoclave is tested and certified annually

All other liquids: 2% Virkon solution for 30mm according to manufacture’s guidelines.

Ultimate form and fate: Incineration

---

**Is an emergency plan required according to regulation 20?**  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y
Please enter comments on the GM safety committee on the risk assessment

Application approved.

Project Containment

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Animal Units

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Project Ref 408/11.1

Date Ackn'd
17/06/2011

CU2 Project Title
Cell fate determination in lung/stem progenitor cells and lentiviral RNAi-mediated gene silencing to study cell competition in mammalian cells

Date Project Ceased

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

1. Manipulation of gene expression in lung stem and progenitor cells growing in culture. The aim of the project is to identify signalling pathways and transcription factors which mediate the switch between proliferation and differentiation of the stem/progenitor cells.
2. To study the process of cell competition in mammalian cells growing in culture using Lentiviruses as a tool for manipulating gene expression.

Recipient or parental organism

The recipient cells will be:
1. Human, mouse or canine cell lines including HEK 293T (human embryonic kidney); MDCL cells (Madine-Durby Canine Kidney) or other human or mouse polarized epithelial cells.
2. Mouse wildtype lung progenitor cells in primary cultures.
3. Embryonic or adult wildtype mouse lungs and trachea primary cultures.

Host/vector system

Third generation lentivirus used for ectopic expression or knock-down (siRNA) of gene expression. Replication-incompetent. Self-inactivating (SIN). Gag, pol, env and rev are encoded on three additional plasmids to minimize the risk of replication-competent virus formation by homologous recombination. Replication-incompetent. Self-inactivating (SIN). Gag, pol, env and rev are encoded on three additional plasmids to minimize the risk of replication-competent virus formation by homologous recombination. VSV G coat protein. E1-deleted adenovirus used for ectopic expression or knock-down (siRNA) of gene expression.

Origin & function

Origins:
Mouse and human DNA. Commercially available Lentiviral shRNA Pooled Libraries (pre-assembled lentiviral particles) such as:
- MISSION LentiPlex Pooled Libraries (SIGMA or Open Biosystem, based on TRC shRNA collection);
- TRIPZ Lentiviral Inducible shRNAmir Library (Open Biosystems collection).
Well characterized reporter genes, such as the jellyfish enhanced Green Fluorescent Protein (eGFP) and bacterial β-galactosidase.

Intended Functions:
1) Silencing of specific genes e.g. the Wnt inhibitor Notum expression in target cells to characterize its role in cell competition.
2) Systematic genome-wide knockdown of target genes in target cells in order to screen for novel cell competition genes.
3) Control of cell fate specification, differentiation and self-renewal of progenitor cells.

Evaluation of foreseeable effects

Potential human health hazard of the GMO (virus-modified cells growing in culture):
There is no evidence that mammalian cells in culture per se can affect the operator. In addition to the cell lines used for most of these experiments, we will be obtaining patient samples which, by their nature, will not be completely characterized. There is a very small chance that the primary human cells will be infected with a transmissible virus which was not screened for in the hospital. The consequences of a researcher contracting such an infection could be very severe. We will only grow these cells in microbiological safety cabinet in a containment level 2 laboratory to ensure that the chance of this occurring is very low. The viruses that the cells will be infected with will be self-inactivating and contain no sequences for packaging proteins. Consequently it is highly unlikely that they become infectious even in human cells.

Potential human health hazard of the inserted genetic material.
It is possible that the manipulations could increase the oncogenic potential of target cells. There is a low risk that the researcher will accidently infect themselves with the virus particles (see risk assessment).

Potential human health hazard of the vectors
The lentivirus and the adenovirus can infect human cells. They are both replication-incompetent and will not result in a transmissible infection. There is a low risk that the researcher will accidentally infect themselves with the viral vector. This could result in the researcher being exposed to the inserted genetic material (potentially oncogenic), or the lentivirus incorporating into their DNA (random insertion).
Potential human health hazard of the resulting genetically modified micro-organism. The likelihood of these genetic modifications making mammalian cells growing in culture hazardous to humans is negligible. Even if this somehow occurred, the cells are killed after completion of the experiment and do not survive out of the culture conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Partially-characterized human primary cells will be used in some experiments. These will always be treated as containment level 2.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be inactivated by autoclave (degree of kill effectively 100% and liquid waste treated with virkon according to the manufacturer's guidelines. Autoclave is tested and certified annually, and during each run is shown to hold temperature correctly. All carcasses of mice injected with genetically-manipulated cells will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Application approved

Project Containment

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<tr>
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Project Ref 408/12.1

Date Ackn'd 02/03/2022
Silencing of specific non coding RNA or chromatin reader/modifier e.g. down regulation of the expression of the PCR2 components in target cells to characterize their role in gene regulation

Purposes of the contained use
Chromatin readers or effectors, and more recently non coding RNA, have been shown to modify the activation of certain genes, but not the sequence of DNA. Additionally, the chromatin proteins associated with DNA may be activated or silenced. This is why the differentiated cells in a multi-cellular organism express only the genes that are necessary for their own activity. Replication incompetent viruses will be used to ectopically express, or knock-down, candidate proteins or long non coding RNAs to test their ability to regulate gene expression.

Recipient or parental organism
The recipient cells will be:
Human or mouse cell lines e.g. mouse embryonic stem cells (E14, J1); colorectal carcinoma cell line (HCT116); HEK 293T (human embryonic kidney); mouse embryonic fibroblasts (MEF, NIH3T3, MRCS, WI38); mouse myoblast cell line (C2C12); breast cancer cell lines (MCF7, MDA-MB415, MDA-MB231, BT474, MCF10A, T47D); osteosarcoma lines (U2OS); lung cancer cell lines (HuH7, H1299, A549), human and mouse erythroid leukaemia cells (HEL, MEL, K562); human promyelocytic leukemia cells (HL60); Human Burkitt’s lymphoma cell line (P493-6)

Host/vector system
Lentiviral inducible shRNA silencing will use third generation lentivirus. Replication-incompetent. Self-inactivating (SIN). Gag, pol, env and rev are encoded on three additional plasmids to minimize the risk of replication-competent virus formation by homologous recombination.
Retroviral vector, based on Moloney murine leukemia virus (MMLV), will provide the viral package signal, transcription and processing elements. The viral env gene is produced by the package cell line. Transfection into a package cell line produces high-titer, replication-incompetent viruses.
Commercially available Lentiviral shRNA Pooled Libraries (pre-assembled lentiviral particles) such as:
- MISSION LentiPlex Pooled Libraries (SIGMA or Open Biosystem, based on TRC shRNA collection);
- TRIPZ Lentiviral Inducible shRNA mim Library (Open Biosystems collection).
- pMX Retroviral vector (Cell Biolabs)
- pLKO Tet On inducible vector (Addgene)

Origin & function
Origins:
Mouse and human DNA. Commercially available Lentiviral shRNA Pooled Libraries (pre-assembled lentiviral particles) such as:
- MISSION LentiPlex Pooled Libraries (SIGMA or Open Biosystem, based on TRC shRNA collection);
- TRIPZ Lentiviral Inducible shRNAmir Library (Open Biosystems collection).
- pMX Retroviral vector (Cell biolabs)
- pLKO Tet On inducible vector (Addgene)

Intended Functions:
Silencing of specific non-coding RNA or chromatin reader/modifier e.g. down regulation of the expression of the PCR2 components in target cells to characterize their role in gene regulation

Evaluation of foreseeable effects

Human health hazard identification - Identify any potentially harmful properties of:

i) The recipient micro-organism (for micro-organisms also give ACDP hazard group)
There is no evidence that mammalian cells in culture per se can affect the operator.
Also, the Replication-defective Lentiviruses that the cells will be infected with will be self-inactivating and contain no sequences for packaging proteins. Consequently it is highly unlikely that they become infectious even in human cells.
Overall Risk: Low

ii) The inserted (donated) genetic material
The inserted genetic material will knock-down the whole genome, one gene at a time. In addition in separate experiments we will knock-down specific inhibitors and modulators of Wnt signalling e.g. Notum, a Wnt signalling inhibitor expressed at high levels in some cancer cell lines. It is possible that such manipulations could be increasing the oncogenic potential of target cells.
Overall Risk: Medium

iii) The donor micro-organisms (where used/appropriate)
Not used.

iv) The vector* (see additional section below for viruses or viral vectors)
The Lentiviral transfer vector is replication incompetent. We use self-inactivating transfer vectors (with SIN 3’LTR, reducing the likelihood of RCV generation) and additionally we use 3rd generation packaging system (where the packaging sequences are divided in two separated constructs, also reducing the likelihood of RCV generation).
The Replication-defective Lentiviruses can infect human cells. However the event of operator infection is not likely. In particular no sharps will be used when handling lentiviruses so there is no risk that the researcher will accidently inject themselves with the viral vector.
Overall Risk: Low

v) The resulting genetically modified micro-organism
The likelihood of genetic modifications generating cell lines in culture that are hazardous to humans is negligible. In addition cells do not survive out of the liquid culture media.
Overall Risk: Effectively Zero
i) The recipient micro-organism
The cells are not hazardous and they will not survive outside the culture conditions.
Overall Risk: Effectively Zero

ii) The inserted (donated) genetic material
Since the viruses are replication defective they cannot spread between cells and animals and the consequence is therefore low.
Overall Risk: Effectively Zero

iii) The donor micro-organisms (where used/appropriate)
Not used.

iv) The vector* (see additional section below for viruses or viral vectors)
There is the risk that one of the genes silenced can result in an oncogenic effect for the target cell. If they escaped into the environment, the viruses would not survive for more than a matter of minutes. In principle, the viruses could infect mammalian cells and cause a cancer. However, no live animals are within the immediate environment of the experiments. Therefore, the probability of this occurring is incredibly low. The viruses cannot replicate and spread to other cells and they are therefore unlikely to cause any noticeable adverse effect for the environment.
Overall Risk: Effectively Zero

v) The resulting genetically modified micro-organism
The likelihood of genetic modification that make tumour cell lines in culture hazardous for environment is negligible, and even if this happened the cells are killed after completion of the experiment and they do not survive out of the culture media.
Overall Risk: Effectively Zero

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Every cell line will always be treated as containment level 2

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste that has been in contact with the Lentiviruses/Retroviruses or modified cells will be inactivated using standard laboratory measures (autoclaving material, using virkon inactivation for liquid). No sharps will be used when handling lentiviruses/retroviruses. Each researcher will receive individual training and this will be documented.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
## Project Containment

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### Project Ref 408/13.1

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<td>25/07/2013</td>
<td>Reprogramming and cell fate determination from pluripotent stem cells undergoing specification into primordial germ cells (PGC) or somatic cells, and their reversion to a pluripotent state</td>
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<table>
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<th>Date Project Ceased</th>
<th>Class</th>
<th>CultureVol</th>
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<th>CultureVolume</th>
<th>Class3-4</th>
<th>Non-GMM</th>
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</table>
The recipient cells will be:
1. Mouse, human and monkey, including HEK 293T (human embryonic kidney); MDCK cells (Madine-Durby Canine Kidney) or other human or mouse germ cells
2. Mouse or human embryonic stem cells or progenitor cells, including neuronal progenitor cells and primary embryonic fibroblasts.

**Host/vector system**

expression. Replication-incompetent. Self-inactivating (SIN). Gag, pol, env and rev are encoded on three additional plasmids to minimize the risk of replication-competent virus formation by homologous recombination. Self-inactivating (SIN).

Retroviral vector used for ectopic expression of transcription factors. The vector, based on Moloney murine leukemia virus (MMLV), will provide the viral package signal, transcription and processing elements. The viral Gag, pol, env gene is produced by the package cell line. Transfection into a package cell line produces high-titer, replication-incompetent viruses.

**Origin & function**

Origins:
Mouse and human DNA. Commercially available Lentiviral shRNA Pooled Libraries (pre-assembled lentiviral particles) such as:
- MISSION LentiPlex Pooled Libraries (SIGMA or Open Biosystem, based on TRC shRNA collection);
- TRIPZ Lentiviral Inducible shRNAmir Library (Open Biosystems collection).

Well-characterized reporter genes, such as the jellyfish enhanced Green Fluorescent Protein (eGFP) and bacterial β-galactosidase.

Intended Functions:
1) Expression or silencing of specific genes, for example of the key determinants of PGC specification, Prdm1, Tcfap2c, Prdm14 and Oct4 to induce PGC fate.
2) Systematic genome-wide knockdown of target genes in target cells in order to screen for novel genes regulating germ cell specification and their properties.
3) Control of cell fate specification, differentiation and self-renewal of progenitor cells.

**Evaluation of foreseeable effects**

Potential human health hazard of the GMO (virus-modified cells growing in culture):
There is no evidence that mammalian cells in culture per se can affect the operator. In addition to the cell lines used for most of these experiments, we may use established human progenitor cells and human induced pluripotent cells. There is a very small chance that the human cells will be infected with a transmissible virus which was not screened for in the human cells. The viruses that the cells will be infected with will be self-inactivating and contain no sequences for packaging proteins. Consequently it is highly unlikely that they become infectious even in human cells.

Potential human health hazard of the inserted genetic material.
It is possible that the manipulations could increase the oncogenic potential of target cells. There is a very low risk that the researcher will accidentally infect themselves with the virus particles (see risk assessment).

Potential human health hazard of the vectors
The lentivirus, retrovirus and adenovirus can infect human cells. They are replication-incompetent and will not result in a transmissible infection. There is a low risk that the researcher will accidentally infect themselves with the viral vector. This could result in the researcher being exposed to the inserted genetic material (potentially oncogenic), or the lentivirus incorporating into their DNA (random insertion).

Potential human health hazard of the resulting genetically modified micro-organism.
The likelihood of these genetic modifications making mammalian cells growing in culture hazardous to humans is negligible. Even if this somehow occurred, the cells are killed after completion of the experiment and do not survive out of the culture conditions.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Partially-characterized human induced pluripotent cells and established progenitor cells will be used in some experiments. Primary cells if included in the experiments will be treated as containment level 2.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be inactivated by autoclave (degree of kill effectively 100%) and liquid waste treated with virkon according to the manufacturer’s guidelines. Autoclave is tested and certified annually, and during each run is shown to hold temperature correctly.

All carcasses of mice injected with genetically-manipulated cells will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

application approved

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Animal Units

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 | L2 | L3 | L4 |

Project Ref 408/15.1

Date Ackn’d 01/04/2015

CU2 Project Title Expansion, genetic modification and generation of stable knockout human-derived cell lines

Date Project Ceased 02/03/2022

Class 2

Consent Granted
**Project Additional Information**

**Purposes of the contained use**

1- Generation of human cell lines and mouse embryonic stem cells stably expressing the Cas9 nuclease to allow the generation of single-knockout cells clones. The aim of the project is to identify novel genes involved in DNA damage response and repair, and the prevention of chromosomal translocation, a hallmark of many cancers.

2- Generation of 3D-cultures for epithelial-derived cancers and epithelial-derived diseases, and generation of mutation, knockout and knock-in alleles. The aim of the project is to investigate further the function of genes involved in cancer formation and development.

**Recipient or parental organism**

The recipient cells will be:

1- Human and mouse cell lines including HEK 293T (human embryonic kidney); HepG2 (human hepatocellular carcinoma), HeLa cells, U2OS (Human osteosarcoma cells), RPE-1 (human immortalised epithelial retina cells), HAP1 (human near-haploid cells), E14TG2a (mouse embryonic stem cells)

2- Human cells in primary cultures expanded in culture

**Host/vector system**

1- CRISPR plasmids (such as pSpCas9n-2A-GFP and pSpCas9-2A-GFP)

2- Second and third generation adenovirus, E1- and E3-deleted; E2 gene inactivated, further reducing the possibility of RCA generation


5- Second generation retrovirus. Replication incompetent. Self-inactivating
(SIN). 5'-LTR deleted. Gag, pol, env and rev are encoded on two additional plasmids to minimise the risk of replication-competent virus formation by homologous recombination. VSV-G coat protein
6- Adeno-associated virus
7- Sendai virus

Origin & function

Origins:
- bacterial Cas9 nuclease
- human and murine single-guide RNA (sgRNA)
- human DNA
- I-SceI (yeast endonuclease)
- Well-characterised reporter genes such as eGFP (jellyfish enhanced Green Fluorescent Protein), HA-tag (Human influenza hemagglutinin tag) or LacZ (bacterial ß-galactosidase)

Intended functions:
- Knocking down specific genes involved in cancer formation or tissue regeneration
- Systematic genome-wide knockdown of target genes in target cells in order to screen for novel interactors
- Systematic genome-wide knockdown and knock-out of target genes involved in preventing chromosomal translocation

Evaluation of foreseeable effects

Potential human health hazard of the GMO (genetically modified cells growing in cultures)
There is no evidence that mammalian cells in culture per se can affect the operator. However, in addition to cell lines of known safe history, human cells from patients will be used. Transplant donors will be screened for (at least) hepatitis B and C, HIV, Cytomegalovirus, Epstein-Barr virus, Toxoplasma and Syphilis. However patient samples will not be fully characterised and there is a very small chance that the primary human cells will be infected with a transmissible virus. The consequence of a researcher contracting such infection could be very severe. Such cells will only be grown at containment level 2 to ensure that the chance of this occurring is very low.

Potential human health hazard of the inserted genetic material
It is possible that the genetic manipulation could increase the oncogenic potential of target cells. There is a low risk that the researcher will accidently infect themselves with the virus particles (see risk assessment).

Potential human health hazard of vectors
Lentivirus and adenovirus can infect human cells. They are both replication-incompetent and will not result in transmissible infection. There is a very low risk that the researcher will accidently infect themselves with the viral vector. This could result in the researcher being exposed to the inserted genetic material (potentially oncogenic) or the lentivirus incorporating into their DNA
Also, one of the lentiviral vector used (pRRL sEF1a HA.NLS.Sce(opt).T2A.IFP) contains Woodchuck hepatitis Post-transcriptional Regulatory Element (WPRE) to enhance transgene expression. This element may be capable of expressing part of the X protein of WHV, which has potential oncogenic properties.

Potential human health hazard of the resulting GMO
The viruses that the cells will be infected with will be self-inactivating and contain no sequences for packaging proteins. Consequently it is highly unlikely that they become infectious even in human cells.

The likelihood of the genetic modifications making mammalian cells growing in culture more hazardous is negligible. Even if this somehow occurred, the cells are killed after completion of the experiment and do not survive outside the culture conditions.

Partially characterised human tissues and primary cells will be used in some experiments. These will always been handled at containment level 2.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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<thead>
<tr>
<th>Category</th>
<th>Description</th>
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<tr>
<td>Partially characterised human tissues and primary cells</td>
<td>Will be used in some experiments. These will always be handled at containment level 2.</td>
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<tr>
<td>Solid waste</td>
<td>Solid waste will be inactivated by autoclave (degree of kill effectively 100%). Autoclave is tested and certified annually, and during each run is shown to hold temperature correctly.</td>
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<tr>
<td>Liquid waste</td>
<td>Liquid waste will be treated with Virkon or other approved disinfectant, according to the manufacturer's guidelines.</td>
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Please enter comments on the GM safety committee on the risk assessment

Approved

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2</td>
<td>L3 L4</td>
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**Project Ref** 408/16.1

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<td>1) Reprogramming of human fibroblast cell lines to induce pluripotent stem cells 2) Human stem cells models of cerebral cortex development and disease</td>
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Non-GMM  Consent Granted

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Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
# Project Additional Information

## Purposes of the contained use

1) Reprogram somatic patient-derived cell lines for study using our cortical differentiation model
2) Human stem cell models of cerebral cortex development and disease. The aims of this project are to:
   1. Carry out lineage analysis and live imaging of single human and non-human primate neural progenitor cells, using GFP-expressing lentiviruses
   2. Express, using lentiviruses, different gene products to study their effects on neurogenesis and neurodegeneration.
   3. Express, using a single lentivirus construct, the Cas9 nuclease together with libraries of genespecific guide RNAs

## Recipient or parental organism

The recipient cells will be:
1) human fibroblast cell lines generated from patient skin biopsies
2) human and mouse cell lines: HEK 293T, human fibroblasts, human pluripotent stem cells, human neural stem cells, neurons and glial cells derived from pluripotent stem cells

## Host/vector system

1) modified, non-transmissible forms of mouse Sendai Virus (SeV) with Fusion protein deleted rendering the virus incapable of producing infectious particles (such as CytoTune-IPS 2.0 kit from ThermoFisher)
2) 3rd generation, replication-incompetent, self-inactivating lentiviruses, containing different promoter sequences and wild-type WPRE: pBOB and pLenti backbones, with CMV, synapsin1, EF1-alpha or PGK promoters; lentiCRISPRv2, with the human U6 promoter, the EFS-NS promoter and wild-type WPRE

## Origin & function

- Origins:
  - human genes (transcription factors Oct4, Sox2, Klf4 and c-Myc and genes involved in neurogenesis and neurodegeneration)
  - bacterial Cas9 nuclease
  - human and murine single-guide RNA (sgRNA)
  - Well-characterised reporter genes such as GFP (jellyfish Green Fluorescent Protein)
- Intended functions:
  - Expressing the transcription factors Oct4, Sox2, Klf4 and c-Myc in differentiated cell types, resulting in the effected cell reverting to an embryonic stem cell-like pluripotent state.
  - Expressing and/or knocking down specific genes involved in neurogenesis
Evaluation of foreseeable effects

Hazards to human health:
1) Sendai virus is ACDP class 1 organism and is a murine parainfluenza virus that is non-pathogenic to humans, yet genetically modified sendai viral vectors can transduce human and animal cells.

The Sendai virus in the CytoTune 2.0 kit is non transmittable, replication incompetent, and will not integrate into the genome: since Sendai virus is an RNA virus, it does not need to enter the nucleus for transcription. Sendai virus requires no integration for viral proteins to be made in the host cell. This eliminates the possibility of integration of the transgenes into the host genome and alteration of the host genome. As it is replication-incompetent, the virus is cleared from the reprogrammed cells by proliferation/passaging. The host cell can be cleared of the vectors and encoded genes thanks to the cytoplasmic nature of SeV: also functional temperature sensitive mutations have been introduced into the key viral proteins to aid clearance of the virus from the cytoplasm in culture.

Although the virus is replication incompetent, the encoded transgenes (Yamanaka reprogramming factors, c-Myc, Klf4, Oct3/4 and Sox2) could result in a short term expression of a potentially oncogenic gene within the infected cell. However only cells that come in direct contact with the packaged virus could become infected.

2) The lentiviral transfer vectors pBOB and lentiCRISPR v2 are replication incompetent and self-inactivating. The use of a 3rd generation packaging system (where the packaging sequences are divided in two separated constructs, reducing the likelihood of RCV generation), which obviates the need to include the Tat gene/protein, increases the safety of this system yet further. pBOB contains wild-type WPRE which is suspected to express part of the X protein which may have oncogenic properties; it is used due to necessity for robust expression in neural cells.

The replication-defective lentiviruses can infect human cells. However the event of operator infection is not likely. In particular no sharps will be used when handling lentiviruses so there is no risk that the researcher will accidently inject themselves with the viral vector.

Hazard to the environment:
1) Wild-type SeV is highly contagious in host species (i.e. rodents), but the infection does not persist in immunocompetent animals. However, as the virus does not survive for more than 3 hours outside of the host cells and culture conditions, the risk of infecting and causing disease in animals is negligible. In addition, in the extremely improbable event that an animal was infected with modified, non-transmissible SeV, it would not be able to spread beyond the infected cell as the virus lacks the Fusion protein.

The likelihood of genetic modifications generating cell lines in culture that are
hazardous to the environment is negligible. In addition cells do not survive out of the liquid culture media. Since SeV does not integrate into the host genome and remains in the cytoplasm, there is no risk of insertional mutagenesis. Also, the host cell can be cleared of the vectors and encoded genes thanks to the cytoplasmic nature of SeV; plus functional temperature sensitive mutations introduced into the key viral proteins.

2) The viruses cannot replicate and spread to other cells and are therefore very unlikely to cause any adverse effects. The likelihood of genetic modifications generating cell lines in culture that are hazardous to humans is negligible. In addition cells do not survive out of the liquid culture media.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimize any possible aerosol effects of the viral stocks, appropriately capped centrifuge bottles and screw-capped tubes will be used. Tubes will be centrifuged in capped buckets and the buckets will be opened in a MSC Class II.

Solid waste will be inactivated in Anistel (or other approved disinfectant) before autoclaving (degree of kill effectively 100%). Autoclave is tested and certified annually, and during each run is shown to hold temperature correctly.

Liquid waste will be treated with Anistel, Virkon or other approved disinfectant, according to the manufacturer's guidelines.

No sharps will be used when handling viruses. Virus will be used at containment Level 2 in a Class II biological safety cabinet, and with appropriate personal safety equipment to prevent mucosal exposure/splash (gloves, lab coat and safety specs).

All work surfaces will be wiped with Anistel 1% (or other approved disinfectant, according to the manufacturer guidelines).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Project Containment

Laboratory Activities

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<tr>
<th>Class</th>
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<th>L4</th>
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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 408/17.1

Date Ackn’d: 16/03/2017

CU2 Project Title

Investigating the molecular function of RNA-modifying enzymes in normal regulatory processes and in cancer

Class: Class 2

CultureVolClass2: < 1 Litre

CultureVolumeClass3-4

Non-GMM: Consent Granted

Project notified under transitional arrangements: N

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To use the CRISPR-Cas9 system in human cell lines in order to investigate the molecular function of RNA-modifying enzymes, especially terminal uridyl transferases, such as TUT4 and TUT7.

Recipient or parental organism

Mammalian cell lines (such as OV-90, PA-1, DU-145, H1299, A549, MCF-7, ...
### Host/vector system

3rd generation lentivirus, replication-incompetent, self-inactivating such as lentiCRISPRv2
helper vectors such as pMD2.G and psPAX2

### Origin & function

**Origins:**
- bacterial Cas9 nuclease
- human single-guide RNA (sgRNA)
- Well-characterised reporter genes such as GFP (jellyfish Green Fluorescent Protein)
- involved Fluorescent Protein)

**Intended functions:**
- Knocking down specific genes encoding for RNA-modifying enzymes

### Evaluation of foreseeable effects

**Hazard to human health:**
The lentiviral vector lentiCRISPR v2 is a 3rd generation system, where the packaging system is split into two plasmids (one encodes Rev and the other encodes Gag and Pol). Tat is eliminated in the 3rd generation system that makes it safer. lentiCRISPR v2 is replication incompetent and self-inactivating as it incorporates a SIN system. Replication-incompetent lentiviruses can infect human cells, however the risk is very low as no sharps will be used when handling lentiviruses.
lentiCRISPR v2 contains a mutated version of WPRE (mut6, see Zanta-Boussif et al, 2009, Gene Therapy 16, 605-619). mut6-WPRE cannot express part of the protein X, therefore doesn't present a risk to human health.
The inserted genetic material is in itself harmless. However, the Cas9 nuclease and the sgRNAs are both carried by the lentiviral vector and the targeted genes may have oncogenic properties. In the unlikely event of infection, there is a small risk of harm.
The likelihood of genetic modifications generating cell lines in culture that are hazardous to human health is negligible. In addition cells do not survive out of the liquid culture media.

**Hazard to the environment:**
The lentivirus cannot replicate and spread to other cells and is therefore very unlikely to cause any adverse effects.
The likelihood of genetic modifications generating cell lines in culture that are hazardous to the environment is negligible. In addition cells do not survive out of the liquid culture media.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimize any possible aerosol effects of the viral stocks, screw-capped tubes will be used; they will be centrifuged in capped buckets and the buckets will be opened in a MSC Class II.

Solid waste will be inactivated in Distel (or other approved disinfectant) before autoclaving (degree of kill effectively 100%). Autoclave is tested and certified annually, and during each run is shown to hold temperature correctly.

Liquid waste will be treated with Distel or other approved disinfectant, according to the manufacturer's guidelines.

No sharps will be used when handling viruses. Virus will be used at containment Level 2 in a Class II biological safety cabinet, and with appropriate personal safety equipment (gloves, lab coat and safety specs).

All work surfaces will be wiped with Distel 1% (or other approved disinfectant, according to the manufacturer guidelines).

---

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

---

Please enter comments on the GM safety committee on the risk assessment

Risk assessment approved at CL2

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Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Large Scale Activities</th>
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02/03/2022
We can generate human and non-human primate cerebral organoids containing cortical neurons from pluripotent stem cells (PSCs). This process consists of a number of distinct steps, including the directed differentiation of PSCs to form a complex population of cortical stem and progenitor cells and an extended period of cortical neurogenesis. Importantly, this system reproduces the diversity of cortical projection neurons found in vivo. The two aims of this project are to:

1. Carry out lineage analysis in organoids of single human and non-human primate neural progenitor cells, using GFP-expressing lentiviruses
2. Express or knock down/out, using lentiviruses, different gene products to study their effects on neurogenesis and neurodegeneration.

Wild-type cerebral organoids generated from well-characterised ESCs and iPSCs lines (Human, Chimp, Bonobo, Gorilla, Macaque) – e.g. already used in published work (Shi et al., 2012; Otani et al., 2016)

Third generation, replication-incompetent, self-inactivating lentiviruses, containing different promoter sequences and wild-type WPRE. Backbones: pBOB, pLB, pLKO.1, lentiCRISPRv2 and pLenti backbones

Promoters: CMV, synapsin1, EF1-alpha, U6, PGK, or EFS-NS
Expression of fluorescent protein (FPs) such as GFP or combination of several FPs, such as BFP, GFP, mRuby2. FPs may be fused to genes encoding factors affecting neural progenitor temporal identity or proliferation. Alternatively FPs might be expressed in combination with siRNAs or gRNAs and Cas9 protein aiming to knock down or knock out genes involved in neural progenitor and neuronal biology.

Origin & function

Hazard to human health:
Lentiviral transfer vectors such as pBOB and lentiCRISPR v2 are replication incompetent and self-inactivating. They are 3rd generation systems where the packaging system is split so to limit the risk of RCV generation. They contain wild-type WPRE which is suspected to express part of the X protein which may have oncogenic properties; it is used due to necessity for robust expression in neural cells. The replication-defective lentiviruses can infect human cells. However the event of operator infection is not likely. A small volume (1-2ul) of diluted suspension of lentivirus (estimated 7x10^4 TU/ml) is loaded in a glass micropipette for the injection of organoids. These glass micropipettes are extremely fragile and cannot easily puncture gloves. The risk of infection is therefore negligible.

Hazard to the environment:
The viruses cannot replicate and spread to other cells and are therefore very unlikely to cause any adverse effects. The likelihood of genetic modifications generating organoids in culture that are hazardous to the environment is negligible. In addition they do not survive out of the liquid culture media.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be inactivated in Anistel (or other approved disinfectant according to manufacturer's instructions) before autoclaving (degree of kill effectively 100%). Autoclave is tested and certified annually, and during each run is shown to hold temperature correctly.
Liquid waste will be treated with Anistel, Virkon or other approved disinfectant, according to the manufacturer's guidelines.
No sharps else than the glass micropipettes will be used when handling viruses. Virus will be used at containment Level 2 in a Class II biological safety cabinet, and with appropriate personal safety equipment to prevent mucosal exposure/splash (gloves, lab coat and safety specs).
All work surfaces will be wiped with Anistel 1%.

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]  

Please enter comments on the GM safety committee on the risk assessment
Application approved

**Project Containment**

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**Animal Units**  
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408/17.3

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<td>Modifying the cyclisation sequence of Zika virus and knocking out candidate host genes involved in the cyclisation of Zika virus</td>
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### Project Additional Information

#### Purposes of the contained use

During replication, the RNA-genome of Zika virus undergoes cyclisation by base pairing regions in the 5'UTR with corresponding regions in the 3'UTR. One of these regions is called Cyclisation sequence (5'CS and 3'CS). We have recently identified a few candidate host genes involved in the life cycle of Zika virus possibly by affecting the virus genomic cyclisation process. We wish to further characterise the role of these candidate genes by:

1. Deleting these genes in the host cells by CRISPR, and testing the ability of Zika virus to replicate in the knockout cells.
2. Introducing mutations in the genomic-RNA of Zika virus and testing the virus interaction with the candidate host genes.

#### Recipient or parental organism

- Commercial human cell lines such as JEG3, RPE1, Vero
- Commercial Aedes mosquito cell lines such as C6/36 or Aag-2
- Commercial human and mosquito cell lines such as JEG3, RPE1 or Aag2 modified using the CRISPR-Cas9 technology

#### Host/vector system

- Plasmids and 3rd generation lentiviral vectors for CRISPR-Cas9 editing of cells
- pACNR-based plasmids for modified positive strand Zika RNA

#### Origin & function

1. CRISPR-Cas9 is expected to knock-out selected genes in the target cells. In most cases, gene editing will have minimal effect on the biology of the cells or it may negatively impact their growth in culture.
2. Modified positive strand RNA Zika genome capable of infecting tissue culture cells; they will not be more pathogenic than the wild-type virus.
   - 5'-CS-M: A strain with 3 point mutations in the 5'CS that attenuate the basepairing ability of the 5'UTR and 3'UTR of the virus and hence severely reduces the ability of the Zika virus to replicate inside cells (relative replication efficiency 0.02 +/- 0.002)
   - 5'-CS-M + 3'-CS-M: A strain that contain the 3 point mutations mentioned above, plus 3 point mutations in the 3'CS that rescue the base-pairing ability of the 5'UTR and 3'UTR of the virus and hence partially-rescues the ability of the virus to replicate inside cells (relative replication efficiency 40.15 +/- 1.67) (see Liu et al, 2017, J. Virol. doi: 10.1128/JVI.00484-17)
   - Additional Zika strains with point mutations that are expected to reduce the pathogenicity of the virus
The Zika virus strain that contains a luciferase reporter gene. This strain is not more pathogenic than the wildtype virus.

- Zika virus with a mutated non-functional RNA polymerase. This strain cannot replicate inside its host and its pathogenicity is severely reduced.

**Evaluation of foreseeable effects**

**Hazard to human health:**
recipient and resulting GM micro-organisms and cells:
E.coli ACDP 1 and well-characterised commercial cell lines with a long history of safe use are not hazardous. Resulting cells are not fitter nor pathogenic than the recipient cells.

inserted (donated) genetic material:
1) CRISPR-Cas9: the inserted genetic material is in itself harmless. However the Cas9 nuclease and the sgRNAs are both carried by the lentiviral vector and the targeted genes may have oncogenic properties. In the unlikely event of infection, there is a small risk of harm.

2) Mutated Zika viruses have a similar or decreased pathogenicity.

vectors:
The lentiviral vector lentiCRISPR v2 is a 3rd generation system where the packaging is split into two plasmids (one encode Rev and the other encodes Gag and Pol). Tat is eliminated in the 3rd generation system that makes it safer. lentiCRISPR v2 is replication incompetent and self-inactivating as it incorporates a SIN system. Replication-incompetent lentiviruses can infect human cells however the risk is very low as no sharps will be used.

lentiCRISPR v2 contains a mutated version of WPRE (mut6, see Zanta-Boussif et al, 2009, Gene Therapy 16, 605-619); it has been suggested that WT WPRE (Woodchuck Hepatitis Post-transcriptional Regulatory Element) expresses a truncated X-protein that may have oncogenic properties. Mut6-WPRE cannot express part of the protein X, therefore doesn't present a risk to human health.

pACNR-based plasmids are not mobilisable. They carry modified positive strand Zika RNA genomes; Zika virus is ACDP 2. The plasmids are not infectious as they require in vitro transcription. Also they cannot cross cell membranes and will not produce any viruses. The replication of the modified Zika viruses is reduced and the pathogenicity is not greater than the pathogenicity of WT Zika virus.

No sharps will be used.

**Hazard to the environment:**
The cells and E. coli strains (recipients and resulting GMs) cannot survive outside lab culture conditions. Also, disinfection procedures prevent them to reach the environment.

The products of the inserted genetic material will not be detrimental to fauna or flora.

The lentiviral vectors cannot replicate and spread to other cells and are therefore very unlikely to cause any adverse effects.

There is a potential for Zika virus to infect mosquitoes Aedes aegypti and...
Aedes albopictus, but these are not native species in the UK.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste is inactivated using either chemical treatment (Anistel or approved disinfectant) according to the manufacturer’s validated conditions. Solid waste is inactivated by autoclaving using validated and recorded temperature cycles.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment 

Please enter comments on the GM safety committee on the risk assessment

Application approved

Project Containment

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Animal Units

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Human Clinical Applications

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Project Ref  408/19.1

Date Ackn’d  16/01/2019  
CU2 Project Title  Applying CRISPR/Cas9 genome editing and lentiviral gene delivery to study cancer  
Class  2  
CultureVol  ≤ 1 litre
Bacteria-derived CRISPR/Cas systems have recently been applied as novel tools to introduce site-specific double strand breaks (DSBs) in mammalian cells. When the DSB sites are repaired by nonhomologous end joining, small insertions/deletions are often introduced into the sites: hence, null mutations can be introduced into target genes. The CRISPR/Cas system consists of two components: Cas9 endonuclease and guide RNA (gRNA) molecules. Both elements can be expressed from separate transgenes transiently or constitutively. In this project, we use retro/lentivirus as a vector for delivering Cas9 and gRNA expression cassettes to target cells. The goal is to develop and utilize retro/lentiviral vectors for CRISPR/Cas9-based genome editing including the performance of genomewide genetic screens and the targeted disruption of genes of interest for mechanistic and translational studies.

In addition, we will use retro/lentiviral vectors for delivery of putative oncogenes or tumour suppressors (TS) into mammalian cells under the control of constitutive or inducible promoters. This will allow us to assess their oncogenic/TS potential in desirable cellular and genetic context.

**Recipient or parental organism**

- Mouse ES cells: AB1, AB2.2, JM8, R1, v6.5, KY1.1 and their derivative lines.
- Human ES cells: H1, H9, Shef lines and their derivative lines.
- Human iPS cells: Pathogen-negative hiPSC lines derived from various donor/somatic cells.
- Primary mouse cells from transgenic or wild type mice.
- Mouse and human cell lines and their derivative lines.
- Cell lines for virus production (PlatE for ecotropic retrovirus production, 293T for amphotropic retrovirus and lentivirus production)

**Host/vector system**

Specify plasmids, viral and cellular vectors etc to be used for delivery into
host (remember, these can also be genetically modified and be considered as resultant GMMs)

<Retroviral vector>
Mouse Moloney leukaemia virus-derived retroviral vector (e.g., pRetrox, pBabe, pMSCV)
Packaging plasmid mix for amphotropic retrovirus production (Clontech Retro-X system or equivalent)

<Lentiviral vector>
3rd generation lentiviral vectors (Invitrogen pLenti 7) and its derivatives (pLenti-CMV/TO Hygro DEST, Addgene #17291) and pHIV-ZsGreen (derivative of pSico, Addgene #11586).
4th generation lentiviral vectors (Clontech pLVX).
Packaging Mix for 3rd generation vectors such as Invitrogen ViraPower Lentiviral Packaging Mix)
Packaging Mix for 4th generation vectors such as Clontech Lenti-X packaging mix or Invitrogen ViraPower.

Origin & function

drug resistant markers (neo, puro, puro-lk, hyg, zeo, bsd)
fluorescent proteins (GFP, zsGreen, mCherry, dsRed, mRFP, mTagBFP, CFP, Venus, YFP, tagRFP657)
Cas9 endonuclease
CRISPR guide RNAs: 20-nt sequences fused with guide RNA scaffold sequences
Integration and expression (constitutive or inducible) of ORFs (wild type or tagged) of putative oncogenes and tumour suppressors.

Evaluation of foreseeable effects

Hazard to health:
Fluorescent proteins and Drug selection markers are active in humans. Transgenic animals (including monkeys) expressing fluorescent proteins and/or drug selection markers have been generated and appear to be healthy. There is no evidence to suggest a harmful effect.
CRISPR guide-RNA and Cas9 endonuclease: When both elements are coexpressed in the same cells, they form complexes and induce double strand breaks at the genomic sites that match 20-nt guiding sequences – active in humans. CRISPR guide RNAs and the Cas9 protein themselves are not harmful. Moreover, mouse guide RNAs are designed not to match the human genome to avoid targeting in the very unlikely case of researcher infection with the viral vectors.
Any of these genes are not directly oncogenic. However, when guide RNAs target, for example, tumour suppressor genes, the genes can be inactivated and this may eventually cause tumour formation.
Similarly, when human ORFs are overexpressed they might potentially have oncogenic or tumour suppressive function based on the genetic and cellular
context. These might eventually cause tumour formation however, since
development of fully blown tumour requires acquisition of multiple genetic
lesions this is highly unlikely.

Hazard to environment:
Any inserts are not directly hazardous. However, when guide RNA and Cas9
proteins are co-expressed in the same cells, they form complexes and can
induce double strand breaks at target sites. This may be hazardous to the
recipient cells/organism but not to the environment (as onward infection
cannot occur). Same applies for overexpression of genes with potentially
oncogenic or tumour suppressive function that could be potentially deleterious
for the recipient cells/organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid wastewill be inactivated by autoclaving (degree of kill effectively 100%) and liquid waste will be
treated with Distel or other approved disinfectant according to the manufacturer's instructions.
Autoclave is tested and certified annually and during each run is shown to hold temperature correctly.
Contingency plan: Backup autoclave in the building and in the adjacent department.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Approved after discussion regarding centrifugation in CL1 room and organisation of temporary CL2
arrangements

Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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### Purposes of the contained use

**Specific Objectives**

1. Modify recipient mammalian cell lines (including previously generated OCRL KO Lowe syndrome disease models) to carry Cas9 using a lentiviral expression system
2. Screen target genes of interest using commercially available gRNA vectors that will be transduced into the Cas9 modified cells using the lentiviral expression system

**Recipient or parental organism**

To generate the Lentivirus - HEK293T-LentiX
To be transfected with Cas9 and gRNA vectors - Mammalian cell lines, including:
- Unmodified immortalised RPE-1 (Human retinal pigmented epithelial) cells
- These RPE-1 cells modified for OCRL KO
- Unmodified immortalised HK2 (Human Kidney) cells
- These HK2 cells modified for OCRL KO

**Host/vector system**

For viral packaging in HEK293-T-LentiX:
- Third generation lentivirus (self-inactivating, replication incompetent)
- Packaging Vectors: pRSV-Rev (rev element), pRRE (gag/pol elements)
- Envelope vector: pMD2.G

Expression Vectors (packaged in HEK293-T-LentiX, delivered to model cell lines by lentiviral infection)
- Cas9
- Commercially available gRNA target plasmids for each gene of interest (e.g., Dharmacon Edit-R sgRNA or Transomic transEDIT-dual CRISPR)

**Origin & function**

Transfection of HEK293T-LentiX cells with viral packaging and Cas9 or gRNA expression vectors will allow the HEK293T-LentiX cells to generate Lentivirus for later use in target model cell lines. Viral infection of model cell lines with Cas9 will allow gRNA plasmids later transfected in to be processed and KO target genes of interest, while infection of model cell lines with gRNA target vectors will allow KO of specified target genes of interest in each Cas9 modified model system via the CRISPR/Cas9 pathway. They will also allow resistance to puromycin, geneticin or blasticidin, for the purpose of positively screening integrations. The inserted sequences themselves will integrate randomly and thus not target specific sequences in the genome. None of these inserted sequences have any potential oncogenic activity.

**Evaluation of foreseeable effects**

**Hazard to health:**
Recipient cells: The mammalian cell lines to be used are non-hazardous and will not survive outside of cell culture conditions.

Inserted genetic material: There is a small chance a researcher may be exposed and accidently infect themselves with the viral vector, however the genetic material is harmless. Neither the Cas9 nor sgRNAs would cause harm separately as editing only occurs in lines which have both at the same time. Additionally, given genome integration is not targeted, but random, there is a low risk of any insertional mutagenesis in tumour suppressor genes. However, as some of the genes targeted for knock-out are oncogenes, in the unlikely event of infection, there is a small risk of harm.

**Vector:**
The lentivirus that will be used to infect cells are replication incompetent, and contain no sequences for packaging or envelope proteins, therefore making them incapable of infected a recipient cell more than once. Thus, they will not be able to cause a transmissible infection. As they are using a 3rd generation system, four plasmids (rather than three) are required to be co-transfected into the same cell to generate viral particles, and the tat protein, which is essential for wild type retroviruses to replicate is completely absent from the system, making the protocol even safer. This makes the risk of infection to the user or colleagues extremely low, even during the production of the lentivirus when working according to biosafety conditions. Though there is a low risk that a researcher could accidently infect themselves, it is unlikely that they will be exposed to the inserted genetic material or the lentiviruses; no sharps will be used, so the risk of the researcher accidently injecting themselves with viral particles is negligible.

The commercial gRNAs, including those from Dharmacon and Transomic contain the Woodchuck hepatitis posttranslational regulatory element (WPRE), and so may be able to express part of protein X from the WHV. Several publications have suggested that truncated forms of these X proteins may have oncogenic properties, which while the virus could not replicate, could be inserted and result in a potentially oncogenic mutation. The Cas9 construct contains the mutated mut6 version of WPRE that cannot express part of protein X and thus presents no risk to public health.

**Resulting GM cells:**
Any risk that the resulting modified human cell lines would be hazardous to humans is negligible, and in the
extremely unlikely event that it happened, they would be unable to survive outside of culture conditions.

Hazard to the environment:
The mammalian cell lines to be used are non-hazardous and will not survive outside of cell culture conditions.
The inserted genetic material is not hazardous to the environment.
The vector viruses are replication incompetent and thus extremely unlikely to cause any adverse effects, they cannot spread to animals.
The resulting genetically modified cells: there is negligible risk that the genetic modifications would make the human model cell lines hazardous to the environment, and even if so they could not survive outside of culture conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be inactivated by autoclave (degree of kill effectively 100%). Autoclave is tested and certified annually, and during each run is shown to reach and hold temperature correctly.
Liquid waste is treated with approved disinfectant according to manufacturers instructions.
Backup autoclave in the building and in the adjacent building.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The Biological and Genetic Modifications Safety Committee approved the risk assessment after a minor change.

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<table>
<thead>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
RNA-modifying enzymes might play important roles in maintaining structure and stability of mitochondria RNAs, enabling efficient, accurate and dynamic protein synthesis in mitochondria. Defects in mitochondrial RNA modifications, arising from either mt-DNA mutations or mutations in nuclear-encoded mitochondrial modification enzymes, are associated with multiple mitochondrial diseases, the molecular mechanisms of action still remain poorly understood.

In this study, we propose to characterise NSUN2-mitochondria interdependence and investigate the biology of NSUN2 mediated m5C RNA modifications in mitochondrial translation and crucial mitochondrial functions for cell survival by utilizing CRISPR-Cas9 system and generating stable knockouts. We will also generate rescue mutants by using lentiviral based integration of the gene in KO mutants to further characterize the role of NSUN2. We will be studying the loss-of-function mutants (knockouts) and rescue of these mutants to understand its biological functions in mitochondrial physiology and human cancers.

Human tissue culture cells (such as U-2OS, lung cancer cell line such as PC_14, NCI-H358 etc.)

3rd generation lentivirus system
VB190206-1038ate (Vector builder)
**lentiCRISPRv2** (Addgene #52961)  
**pMD2.G** (Addgene #12259)  
**psPAX2** (Addgene #12260)

### Origin & function

Loss of function of the targeted gene – The gene is involved in regulating stress response which could be mediated via mitochondria. Mutation in this gene is not lethal but renders the cell sensitive to stress.

### Evaluation of foreseeable effects

**Hazard to Health:**
The cell lines are commercial cell lines with a long history of safe use. They cannot survive outside lab culture conditions. The GM cells are not fitter and/or more pathogenic than the recipient cell lines. In addition they cannot survive outside lab culture conditions.  
There is a low risk of accidental infection with the viral vector however the inserted gene (CRISPR-Cas9) doesn’t encode for a product with potentially harmful biological activity. Cas9 and the sgRNA are carried on the same plasmid however the targeted genes do not have harmful effects.  
The system is a 3rd generation system, the packaging system is split so to limit the risk of replication-competent virus; there is no requirement for Tat, which is essential for WT viruses. The lentivirus is replication-incompetent and self-inactivating, they cannot infect more than one cell. Any accidental infection would not be a transmissible infection.  
The vector is pseudotyped and the envelope plasmid encodes for VSV-G, giving the lentiviral vector a broad host-cell range rather than limiting the infection to cells expressing CD4 on their surface (as would be the case with wild-type HIV).  
The vector VB190206-1038ate used for the rescue experiment contains the WT WPRE that is capable of expressing part of the X protein of the Woodchuck Hepatitis Virus, which may have oncogenic properties, work using this vector must be done at CL2.  
The lentiCRISPRv2 vector contains the mut-6 mutated version of WPRE, that cannot express part of the protein X of the Woodchuck Hepatitis Virus, therefore doesn’t present a risk to human health. Work using this vector can be assigned to Class 1 and can be done at Containment Level 1.  
The fact that lab coat, gloves and safety specs are worn, and that no sharps are used when handling lentiviral vectors reduces further the risk of accidental injection. Also, only small amounts of lentivirus are handled at a time.  
**Hazard to Environment:**
WT and GM mammalian cells cannot infect or colonise animals and plants and they cannot survive outside lab culture conditions.  
The product of the inserted gene is not detrimental for natural flora and fauna.
The viruses are replication-incompetent and therefore unlikely to cause any harm to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be inactivated by autoclave (degree of kill effectively 100%) and liquid waste treated with 1% Distel for 30 minutes (or other approved disinfectant according to manufacturer’s guidelines). Autoclave is tested and certified annually, and each run is shown to reach and hold temperature correctly (134 degrees C for 3 minutes). Contingency plan: waste autoclave available in the adjacent building.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The Biological and Genetic Modifications Safety Committee approved the risk assessment after minor changes were made (clarification regarding the two lentiviral vectors used)

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<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Project Ref 408/20.1
Work in the lab will be split across two areas: (1) characterisation of the mechanism of Human Silencing Hub (HUSH)-mediated repression of endogenous target genes and repetitive elements; and (2) exploiting fluorescent reporter lines for different types of chromatin to characterise epigenetic pathways.

(1) HUSH: We will (i) clone individual genes encoding HUSH complex subunits into expression vectors, and deliver them to relevant cultured host cells; (ii) knockout HUSH complex components using CRISPR/Cas9 in host cells, and (iii) re-introduce either wild-type or mutant versions of HUSH subunits in these knockout cell lines using lentiviral delivery vectors. We will then characterise the effects of these modifications on the host cell. In addition, we will assess the requirement for additional cellular components in HUSH complex function by performing genome-wide CRISPR/Cas9 screens in fluorescent reporter lines previously used to characterise HUSH.

(2) Chromatin reporters: We will generate fluorescent reporter lines for epigenetic pathways other than the HUSH complex. This will be achieved either through CRISPR-mediated knock-in into a target genomic locus, or through lentiviral expression of a fluorescent protein (e.g. GFP). Resulting reporter lines will be interrogated through genome-wide CRISPR/Cas9 screens, and any resulting hits will be characterised in the same way as HUSH components (described above).

Recipient or parental organism

Mammalian tissue culture cell lines (e.g. HeLa, 293T, KBM7, HAP1, Jurkat, HuT78, SUPT1, CEMT4, Molt3, Molt4, HL-60, LCL, Daudi, U87, THP1, Ramos, U373, KG1, DG75, AKBM, Kasumi-3, NK-92, SH-SY5Y, SK-N-SH, hTERT RPE-1).

Human and murine embryonic stem cells
Established human and mouse organoids (e.g. intestinal organoids)
Bacteria: E. coli K-12 and derivatives (e.g. Top10, Sure 2, NEB Stable, DH5-alpha, DH10-beta, ElectroMAX DH10B, XL1-Blue).

Host/vector system

Non-mobilizable standard cloning vectors (e.g. TOPO, pMAX-GFP and pcDNA3), along with replication incompetent retroviral (e.g. pBABE, pGT0-GFP-pA and pMKO) and lentiviral vectors (e.g. pHIR-SIN, pSIREN and pTRIPZ), will be used to clone genes (including endogenous retroelements) encoding fluorescent proteins and epigenetic regulators.

2nd rather than 3rd generation lentiviral vectors will be used for most experiments involving exogenous gene expression. While 3rd generation systems are generally considered safer than 2nd generation systems because the packaging vector has been divided into two separate plasmids, the majority of lentiviral transfer plasmids available in the laboratory are 2nd generation, and therefore extensive time and work would be required to subclone all of these into 3rd generation transfer plasmids.

2nd and 3rd generation lentiviral vectors harbouring shRNAs or CRISPR sgRNAs or a CRISPR-associated protein (e.g. Cas9) will be used for gene knockout or modulation of expression of individual cellular genes, as well as for genetic screens. While both 2nd and 3rd generation lentiviral systems are used, 3rd generation systems will be used exclusively where delivery of both Cas9 and sgRNA in a single vector is required.

Retroviral vectors will also be used for gene disruption in gene-trap genetic screens (pGT0-GFP-pA), modulation of expression of individual genes (pMKO), or exogenous expression of fluorescent proteins or epigenetic factors (pBABE).

Origin & function

Inserts will be used to (i) express fluorescent proteins (e.g. GFP, mCherry, mScarlet, iRFP or HaloTag), (ii) introduce shRNAs or CRISPR sgRNAs or a CRISPR-associated protein (e.g. Cas9) for gene knockout or modulation of expression of individual cellular genes, as well as for genetic screens, (iii) encode mammalian proteins involved in epigenetic modulation, and (iv) express labelled mammalian genes to enable their visualisation, using fluorescent tags as well as antibiotic resistance genes for positive/negative selection (e.g. Puromycin, Blasticidin, Hygromycin or thymidine kinase), or to simplify biochemical analyses using tags such as HA, Strep, Myc, Flag, luciferase or a biotin ligase (e.g. BirA, BioID2 or BASU).

Genes encoding fluorescent proteins, Cas9 or sgRNAs have no known oncogenic activity. Genes encoding mammalian proteins or mutant versions of these proteins may have oncogenic activity. The inserted DNA will integrate randomly and therefore not target specific sequences in the genome. Inserts will be used to (i) express fluorescent proteins (e.g. GFP, mCherry, mScarlet, iRFP or HaloTag), (ii) introduce shRNAs or CRISPR sgRNAs or a
CRISPR-associated protein (e.g. Cas9) for gene knockout or modulation of expression of individual cellular genes, as well as for genetic screens, (iii) encode mammalian proteins involved in epigenetic modulation, and (iv) express labelled mammalian genes to enable their visualisation, using fluorescent tags as well as antibiotic resistance genes for positive/negative selection (e.g. Puromycin, Blasticidin, Hygromycin or thymidine kinase), or to simplify biochemical analyses using tags such as HA, Strep, Myc, Flag, luciferase or a biotin ligase (e.g. BirA, BioID2 or BASU). Genes encoding fluorescent proteins, Cas9 or sgRNAs have no known oncogenic activity. Genes encoding mammalian proteins or mutant versions of these proteins may have oncogenic activity. The inserted DNA will integrate randomly and therefore not target specific sequences in the genome.

Evaluation of foreseeable effects

Hazard to health:
Recipients will be various mammalian cells in culture. They are well characterised, have a long history of safe use, and are not hazardous to human health. They are unlikely to survive outside lab culture conditions. Bacteria: the bacterial strains we use are highly disabled and unable to replicate to any extent outside the lab, and are therefore considered ACDP hazard group 1.
There is a low risk that the researchers could accidently infect themselves with the viral vector, but the inserted genetic material is in itself harmless. Fluorescent proteins are not toxic, nor reported to have deleterious effects on exposure. The Cas9 nuclease or the sgRNAs can cause no harm separately as genome editing only occurs in cells expressing both simultaneously. Moreover, given that the integration in the genome will be random, there is a very low risk of insertional mutagenesis in tumour suppressor genes. Even though the following is not required, we will follow, as common sense suggests, all the standard safety procedures in our lab, which are: i) wearing gloves, labcoats and glasses; ii) handle viruses inside safety cabinets; iii) treat all the working surfaces within the cabinets with disinfectant and 70% ethanol after finishing work with viruses.
Viral gene delivery vectors (retroviruses, lentiviruses) to be used are those typically considered class 1 GMMs, and will all be self-inactivating, and therefore once packaged, are unable to propagate. A three-plasmid transient transfection system will be used to generate the recombinant retroviruses and lentiviruses, thereby decreasing the likelihood of recombination within the packaging cell lines to generate replication competent virus. The retroviral vectors are based on the Moloney murine leukaemia virus in which the gag, pol and env genes have been deleted. Gag and pol are supplied in trans on a packaging plasmid. We will generate VSV-G pseudotyped virus in order to deliver the virus particles to human cells. The lentiviral vectors are based on an HIV-1 self-inactivating vector (SIN) containing a deletion in the U3 region of the 3' LTR of the virus. In addition, the gag, pol, env, nef, vif,
vpu and vpr genes have been deleted. Again, a second packaging plasmid encoding gag, pol, rev and tat will be used along with the plasmid encoding VSV-G to make pseudotyped viruses that will enter human cells.

Lentiviral vectors contain the wild-type version of WPRE (Woodchuck hepatitis post-transcriptional regulatory element); it has been suggested that wild-type WPRE expresses a truncated X-protein that may have oncogenic properties.

There is a very low risk that the researchers will accidentally infect themselves with the viral particles. It is very unlikely that the researchers will be exposed to the inserted genetic material or the viruses and, therefore, incorporating foreign DNA into their genome. No sharps will be used when handling lentiviruses or retroviruses so there is no risk that the researcher will accidently inject themselves with the viral vectors.

The disabled nature of the bacterial hosts will not be altered by the plasmids used – no expression is anticipated and would not in any case be compatible with bacterial systems.

Expression of genes encoding epigenetic modifiers in cultured mammalian cells may alter the expression of cellular genes but these changes will not override the especially disabled nature of the cells.

As lentiviral and retroviral vectors containing are disabled, any accidental infection of the epithelial cells of the researcher would be localised. Transduced cells would not be able to produce viral particles. Due to the high turnover rate of epithelial cells, any localised infection would not be expected to be long lasting.

Expression of the genes described above is not expected to be detrimental to human health.

Hazard to environment:
Mammalian stem cells or cell lines are especially disabled and unable to survive or propagate outside of laboratory culture. They are not hazardous to fauna or flora.

Bacteria: the bacterial strains we use are highly disabled and unable to replicate to any extent outside the lab. Nevertheless, appropriate control measures, including disinfection of liquid cultures and autoclaving of bacterial plates, will be taken.

Fluorescent proteins are not toxic, nor reported to have deleterious effects on exposure.

Non-viral mammalian expression vectors are easily destroyed in the environment and therefore pose no risk on their own.

The viral vectors are all replication-disabled so will be unable to disseminate in the wider animal or human populations.

None of the inserts or deletions in cells, vectors or viruses will be able to alter the non-hazardous status of the recipients with respect to environmental risk.

None of the gene products used have hazardous properties and will therefore not pose an environmental risk.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be inactivated by autoclave (degree of kill effectively 100%) and liquid waste treated with 1% Distel for 30 minutes (or other approved disinfectant according to manufacturer’s guidelines). Autoclave is tested and certified annually, and each run is shown to reach and hold temperature correctly (134 degrees C for 3 minutes). Contingency plan: waste autoclave available in the adjacent building.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Biological and Genetic Modifications Safety Committee approved the risk assessment after a comment was added (justifying the use of 2nd generation lentivirus vector)

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Project Ref  408/21.1

Date Ackn'd  02/12/2021  CU2 Project Title  Gene editing of intestinal cells using viral vector loaded with shRNA or dCas9 and

Class  CultureVolClass2  CultureVolumeClass3-4

Class 2  < 1 Litre
The function of epithelia depends on the apical-basal polarisation of each cell. It is generally assumed that polarity complex proteins govern epithelial polarisation. These proteins, which were discovered in Drosophila, are evolutionarily conserved in organisms as diverse as worms and mammals. The recent discovery that the Drosophila adult midgut polarises in the absence of these conserved polarity proteins provides evidence for an alternative pathway in epithelial polarisation. Together with increasing evidence of the lack of defects in mammalian epithelia when polarity proteins are knocked out, this raises the question of whether the mammalian intestinal epithelium polarises independently of these conserved polarity factor proteins. To test this hypothesis, I plan to employ CRISPR Interference (CRISPRi) to silence polarity factors and their paralogues in mouse intestinal organoids to investigate whether the canonical polarity proteins are required for cell polarisation in the mouse intestinal epithelium. The efficiency of gene silencing will be measured by qRT-PCR and western blotting. Cell morphology, the localisation of polarity proteins and cell junction proteins will be characterised. This project will elucidate whether polarity in the mammalian intestinal epithelium depends on these conserved polarity proteins or whether it resembles that in the Drosophila intestine.

We plan to manipulate gene expression in intestinal organoids in vitro to understand the mechanisms that control intestinal cell polarity. We are targeting genes that modulate cell polarity in the intestinal cells. We will knock down Myo5b and polarity factor proteins (aPKC, Par3 and Par6) in wild type mouse intestinal organoids and study their role in mammalian intestinal epithelium polarity.

We plan to induce CRISPRi and gRNA to cells through lentiviral infection, which allows for stable integration. The workflow of CRISPRi starts with 1) sgRNA design& clone sgRNA into an expression vector, 2) double-transfect cells with dCas9 and sgRNA containing vectors and 3) induce dCas9 expression in transfected cells and assay gene of interest's transcript levels. Once validated in mouse intestinal organoids we will knockdown mammalian organoids or epithelial tissues that derived from wild type mouse embryonic cells, lung, bladder, and mammary tissue to determine whether these mammalian epithelia also require these polarity proteins to polarise.

If CRISPRi is ineffective, I plan to employ Lenti-X Tet-On 3G CRISPR/Cas9 System (632633, Takara Bio) to generate knockout cells by co-expressing an endonuclease Cas9 and a gRNA specific to the targeted gene. The Tet-On 3G system enables doxycycline (dox)-inducible Cas9 expression. Fully functional CRISPR/Cas enzymes will introduce a double-strand break (DSB) at a specific location based on a gRNA-defined target sequence. DSBs are preferentially repaired in the cell by non-homologous end joining (NHEJ), a mechanism which frequently causes insertions or deletions (indels) in the DNA. Indels often lead to frameshifts, creating loss of function alleles. CRISPR plasmids are listed in the below vectors section.

Recipient or parental organism

- HEK 293T cells
- Mouse primary cell lines:
  - Mouse intestinal organoids derived from wild type mouse intestinal stem cells
Wild type mouse embryonic cell lines and its derives
Wild type mouse mammary cell lines (EPH4 cell)
Wild type mouse primary mouse bladder epithelial Cells
Wild type mouse primary alveolar epithelial

**Host/vector system**

**CRISPRi plasmid vectors:**
1. pLenti-gRNA opti_EF1a-EGFP-CAAX (Addgene: 167936)
2. pLenti-Krab-dCas9 (1.1)-ecDHFR-EF1a-tagRFP-2A-tet3G (Addgene: 167935)

**CRISPR plasmid vectors:**
1. Lenti-X™ Tet-On® 3G CRISPR/Cas9 System, with Cas express under CMV Promotor (632633, Takara)
2. pLVX-hyg-sgRNA1 Vector System and with gRNA express under U6 Promotor (632630, Takara)
3. Third generation, replication- incompetent, self- activating lentiviruses, containing different promoter sequence and wild-type WPRE
4. Promotors: minimal CMV promoter, PGK promoter, EF1-alpha and tetON
5. 3rd generation transfer vector : pInducer20 (Addgene# 44012), Tet-pLKO-neo(Addgene# 21916) and pHIV-EGFP (Addgene# 21373)

**Helper vectors:** pMDLg/pRRE (Addgene 12251) contains Gag and Pol; pRSV-Rev (Addgene#12253) and pMD2.G. (Addgene#12259)

**Origin & function**

**Hazard to Health:**
The recipient microorganism(s) (standard bacterial strains, E/coli k-12 derivatives, which are commonly used for plasmid expansion) are not pathogenic to humans and unlikely to survive outside laboratory culture. The hosts can be assigned to ACDP hazard group 1. HEK293T cells and EPH4 cells, and wildtype intestinal organoid, embryonic cell lines and primary cells, are safe to use and cannot survive outside the lab culture conditions.

We do not anticipate any genes we tested will be oncogenic or have a distinguish effect on the progression of cancer when knockdown. There is a low risk that the researcher will accidentally infect herself/himself with the viral vector. The consequences of this are low. The plasmid vectors are all derivatives of standard lab vectors. They are not mobilizable.

The method of genetic modification involves the transduction of mammalian intestinal organoids in culture with lentivirus containing dCas9 and sgRNA. They are pseudotyped with VSVG and Rev and are capable of transducing many, possibly all, human and mouse cells types, including those of the researchers. No human gRNAs and Cas9 will be used, and introduction of dCas9 will not result in the production of double strand DNA break putting them at risk of later cancer development. So the risk is low. However, to expose the researcher to dCas9 and mouse sgRNA would have penetrate the skin, or eyes, or be swallowed.

The gene transfer vector contains a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) which contains an open-reading frame (ORF) encoding a truncated peptide of the woodchuck hepatitis virus X protein. It has been suggested that the X protein is pivotal to the generation of liver cancers, which are a very common consequence of infection with HBV in man. Many studies have suggested that the X protein is nonfunctional in the absence of a second enhancer, We2/En2 (WHV/HBV), which is not present in the PRe, and this has been confirmed recently in the context of a lentiviral vector. The expression of the X protein does not appear to be directly oncogenic, but there are studies that show that under some circumstances it can act as a weak cofactor for oncogenesis; for example, in combination with cellular oncogenes. Researchers must know the potential safety issues surrounding the use of an unmodified WPRE in their vectors.

**Evaluation of foreseeable effects**

The recipient microorganism(s) (standard bacterial strains, E/coli k-12 derivatives, which are commonly used for plasmid expansion) are not pathogenic to humans and unlikely to survive outside laboratory culture. The hosts can be assigned to ACDP hazard group 1. HEK293T cells and EPH4 cells, and wildtype intestinal organoid, embryonic cell lines and primary cells, are safe to use and cannot survive outside the lab culture conditions.
The genetically modified microorganisms are not more hazardous to health than the recipient microorganisms.

Hazard to the Environment:
The recipient cell lines and organoids would not survive and become established in the environment, they are unlikely to survive outside laboratory culture. The plasmid vectors are all derivatives of standard lab vectors. Neither the sgRNA, encoded dCas9, bacterial or mammalian transfectants are likely to have any deleterious effect on the environment. Also, lentivirus particles are relatively fragile, being easily inactivated by 0.1% detergent, chloroform, phenol, 1% bleach, 70% ethanol, 65°C for 30 min, pH <6.5 or >9.0, UV light, or autoclaving.

In a case of disposal of plasmid directly to the environment, the plasmid will get degraded. Plasmid on their own has no effect to a host as it can only be delivered to a host via transfection or viral transduction. Lentiviral particles are pseudotyped with VSVG and are capable of transducing many, possibly all, mammalian cells types.

In the very unlikely event of the release of virus from the Level 2 Containment facility, it could survive in the environment for a similar period of time to the wild-type virus. If the virus infected a susceptible host, then expression of the plasmid would occur in the infected cells.

Since the virus cannot replicate under normal circumstances, expression would be limited to the infected cell and once this cell divides to one of its daughter cells. The potential hazard to environment e.g. mouse live in wild, is also low. In order to cause hazard lentiviral vector with CRISPRi and gRNA have to be delivered to the same mouse. To induce the gene knockdown, the mouse expresses CRISPRi and gRNA needs to be treated with both TMP and DOX for a period of time. The probability of such occurrence is extremely low.

Therefore, no foreseeable adverse environmental effects are expected and risk to the environment is negligible.

The genetically modified microorganisms are not more hazardous to the environment than the recipient microorganisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be killed effectively 100% by autoclave (134°C for 3 minutes). Autoclave tested and certified annually and each run shown to reach and hold temperature correctly. Liquid waste material will be treated with Distel 1% for 30 minutes prior to sink disposal.

Two waste autoclaves on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee recommended the use of PEG-based solution for smaller lentiviruses as this has proven to be an effective alternative to centrifugation, with a lower risk associated to it.

The committee recommended making the risk assessment more concise as key information was sometimes diluted in the text.

The committee unanimously approved the RA pending the changes expressed above.
Project Additional Information

Purposes of the contained use

Bacteria-derived CRISPR/Cas systems have recently been applied as novel tools to introduce site specific double strand break (DSBs) in mammalian cells. When the DSB sites are repaired by non-homologous end joining, small insertions/deletions are often introduced into the sites, hence, null mutations can be introduced into target genes. The CRISPR/Cas9 system consists of two components: Cas9 endonuclease and guide RNA (gRNA) molecules. Both elements can be expressed from separate transgenes transiently or constitutively. In this project, we use retro/lentivirus as a vectors for delivering Cas9 and gRNA expression cassettes to target cells. The goal is to develop and utilize retro/lentivirus vectors for CRISPR/Cas9-based genome editing including the performance of genome-wide
genetic screens and the targeted disruption of genes of interest for mechanistic and translational studies. In addition, we will use retro/lentiviral vectors for delivery of putative oncogenes or tumour suppressors (TS) into mammalian cells under the control of constitutive or inducible promoters. This will allow us to assess their oncogenic/TS potential in desirable cellular and genetic context.

**Recipient or parental organism**

1. Mouse ES cells: AB1, AB2.2, JM8, R1, v6.5, KY1.1 and their derivative lines.
2. Human ES cells: H1, H9, Shef lines and their derivative lines.
3. Human iPS cells: Pathogen-negative hiPSC lines derived from various donor/somatic cells.
4. Cell lines for virus production (PlatE for ecotropic retrovirus production, 293T for amphotropic retrovirus and lentivirus production)
5. Human cell lines (cancer and non-cancer cells) with long history of safe use, such as A549, H1975, OE21, Kasumi, MV411, Panc-1, MCF10A

**Host/vector system**

- **Retroviral vector**
  - Mouse Moloney leukemia virus-derived retroviral vector (eg. pRetrox, pBabe, pMSCV)
  - Packaging plasmid mix for amphotropic retrovirus production (Clontech Retro-X system or equivalent)

- **Lentiviral vector**
  - 3rd generation lentiviral vectors (Invitrogen pLenti 7) and its derivatives (pLenti-CMV/TO Hygro DEST, Addgene 17291), pHIV-ZsGreen (derivative of pSico, Addgene 11586) and lentiCRISPR v2 (Addgene #52961), CRISPRi and CRISPRa system.
  - 4th generation lentiviral vectors (Clontech pLVX).
  - Packaging mix for 3rd generation vectors such as Invitrogen ViraPower Lentiviral Packaging Mix)
  - Packaging Mix for 4th generation vectors such as Clontech Lenti-X packaging mix or Invitrogen ViraPower

**Origin & function**

1. Drug resistant markers (neo, puro, puro-tk, hyg, zeo, bsd)
2. Fluorescent proteins (GFP, zsGreen, mCherry, dsRed, mRFP, mTagBFP, CFP, Venus YFP, tagRFP657)
3. Cas9 endonuclease
4. CRISPR guide RNAs: 20-nt sequences fused with guide RNA scaffold sequences
5. Integration and expression (constitutive or inducible) of ORFs (wild type or tagged) of putative oncogenes and tumour suppressors.

**Evaluation of foreseeable effects**

- Hazard to health:
  1. The recipient cell lines used here are not pathogenic and cannot survive outside culture conditions.
  2. Altered genetic material: Fluorescent proteins and drug selection markers are active in humans. Transgenic animals (including monkeys) expressing fluorescent proteins and/or drug selection markers have been generated and appear to be healthy. There is no evidence to suggest a harmful effect.
  3. CRISPR guide-RNA and Cas9 endonuclease: When both elements are co-expressed in the same cells, they form complexes and induce double strand breaks at the genomic sites that match 20-nt guiding sequences-active in humans. CRISPR guide RNAs and the Cas9 protein themselves are not harmful. Moreover, mouse guide RNAs are designed not to match the human genome to avoid targeting in the very unlikely case of researcher infection with the viral vectors.
Any of these genes are not directly oncogenic. However, when guide RNAs target, for example, tumour suppressor genes, the genes can be inactivated and this may eventually cause tumour formation. Similarly, when human ORFs are overexpressed they might potentially have oncogenic or tumour suppressive function based on the genetic and cellular context. These might eventually cause tumour formation however, since development of fully blown tumour requires acquisition of multiple genetic lesions this is unlikely.

3. Vectors: Both the retrovirus and lentivirus systems that will be used here are replication-incompetent; therefore, there is no risk of diseases associated with these viruses. However, they integrate into the genomes of infected cells and can cause insertional mutagenesis. There are generally two concerns associated with insertion of vectors: oncogene activation and tumour suppressor inactivation. Both viruses have been engineered to lack enhancer elements in LTR, which significantly reduce the risk of oncogenic activation. Tumour suppressor inactivation, which can theoretically lead to tumour formation. To our knowledge, this has never been observed in human operators. Note that retro-and lenti-viruses that lack the U3 enhancer element have been used in clinical trials of gene therapy of hematopoietic disorders and no leukaemia has been thus far observed. The lentiviral vectors contain WT WPRE. This element is capable of expressing part of the X protein from WHV and it is suggested that truncated hepadna virus Xproteins may have oncogenic properties. A vector containing wild type WPRE would be handled at CL2.

4. The GM cell lines are not fitter than the recipient strain and they are not pathogenic.

Hazard to the environment:
1. The recipient cell lines used here do not present a hazard to fauna or flora and cannot survive outside culture conditions.
2. Altered genetic material: Any inserts are not directly hazardous. However, when guide RNA and Cas9 proteins are co-expressed in the same cells, they form complexes and can induce double strand breaks at target sites. This may be hazardous to the recipient cells/organism but not to the environment (as onwards infection cannot occur). Same applies for overexpression of genes with potentially oncogenic or tumour suppressive function that could be potentially deleterious for the recipient cells/organism.
3. Vectors: The viral vectors are replication-disabled and couldn’t spread in animals.
4. The modified cell lines do not present a hazard to the environment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be inactivated by autoclaving (degree of kill effectively 100%) and liquid waste will be treated with Distel or other approved disinfectant according to the manufacturer’s instructions. Autoclave is tested and certified annually and during each run is shown to hold temperature correctly (134°C for 3 minutes).

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form  

02/03/2022
Meeting held 10 Dec 2021
- correct mistake on page 6 (the virus is disabled, not attenuated, wrong tick box)
- check with the researchers which centrifuge they are planning to use
- check that the researchers are not planning to use 2nd generation lentivirus packaging
RA approved subjct to the minor comments above addressed

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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**Name**

LABCORP EARLY DEVELOPMENT LABORATORIES LTD

**Department**

MUTAGENESIS & CELL BIOLOGY

**Campus Estate or Research Centre**

**Road Name**

WOOLLEY RD

**District**

ALCONBURY

**Town**

HUNTINGDON

**County**

CAMBRIDGESHIRE

**Postcode**

PE28 4HS

**Country**

ENGLAND

**Tel Number**

01480 892300

**Fax Number**

01480 892350

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Name change from Huntingdon Life Science Ltd notified 24/01/2017, name change from Envigo CRS Ltd 13/06/2019

**Date at Which Additional Info Submitted**

02/03/2022

02/03/2022  Page 7232 of 15326
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 410/13.1

**Date Ackn'd** 24/04/2013

**CU2 Project Title** Single Bio-distribution and Toxicity Study

**Date Project Ceased**

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<tr>
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**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**
Appropriate control measures will be employed to restrict the GMM to designated containment locations in order to minimise potential for exposure to staff and prevent escape into the environment.

Information included in this form takes into account the following considerations:
- □ Donor organism: genetic sequences obtained from human genome (Homo sapiens) and synthetic origin that have been inserted into human adenovirus type 5 (HAd5) genome to act as genetic expression modulators and induce selective replication of VCN-01 in tumor cells.
- □ Recipient (parental organism): Human Adenovirus type 5
- □ Final GMO: VCN-01

Wild-type human adenovirus (HAd) serotype 5 belongs to the C-subgroup type (Mastadenovirus, Adenoviridae). HAd consists of an icosahedral protein capsid of about 80nm in diameter with no lipid envelope. The faces of the icosahedron are formed by a structural protein called hexon. In each of the twelve vertices of the capsid there is a protein known as fiber, which binds to the virus’ primary cellular receptor, the Coxsackle-Adenovirus Receptor (CAR). For the virus to enter the cell, in addition to the fiber-CAR binding, it is also necessary that a protein attached to the virus fibers called penton base binds to the cell surface integrins. The viral genome is packaged within the capsid, and it is a linear double stranded DNA of 36 Kb which is released into the core after the capsid reaches the nuclear membrane.

Once in the nucleus, adenoviruses express regulatory genes responsible for the activation of cell cycle and subsequent transcription of other viral genes. The former are known as early gene 1 (Early 1). The E1 region is divided into E1A and E1B which encode E1A proteins (12S and 13S) and E1B, E1A protein binds to and inactivates proteins of the retinoblastoma (Rb) family, and thus the E2F transcription factor is released from the E2F-Rb complex, inducing cell cycle progression. E1A-13S protein in conjunction with E2F activates the transcription of E2 (DNA replication proteins), E3 (proteins that evade immune response) and E4 (proteins which functions are similar to the ones of E1, and that also control the transport and RNAs viral transcription). Most adenoviral vectors used in gene therapy are based on HAd5.

HAd Host-range

The host range of HAd5 is restricted to humans. It is also described that chimpanzees, swine, cotton rats (Sigmodon hispidus) and some kinds of hamsters (Mesocricetus aurata), are semi-permissive species for the replication of human adenovirus.
**HAd pathogenicity**

In terms of biosafety, HAdV5 are human adenoviruses classified as class 2 biological agents. HAd infections are mostly asymptomatic but may cause diseases of the respiratory, ocular and gastro-intestinal system, especially in children. Clinical studies using healthy human volunteers have also shown that infection with natural non-attenuated isolates of group C and other adenovirus serotypes does no cause severe disease, with self-limiting, profile and rarely requiring medical intervention. The incubation period for getting disease is 1-10 days, being lymphoid organs considered as natural repositories for the virus. Most of the human population is sero-positive for adenovirus, and HAd-C is a widespread species, which implies that any adenoviral infection is easily neutralized. Biosafety level 2 practices and containment facilities are recommended for all activities involving the virus and potentially infectious body fluids or tissues.

**Host/vector system**

Not applicable.

**Origin & function**

VCN-01 is an oncolytic adenovirus which is able to replicate and lyse selectively tumor cells.

VCN-01 is derived from wild-type human adenovirus (Had) serotype 5 (HAd5), whereby HAd5 backbone has been genetically altered to incorporate distinct modifications that confer tumour selectivity and anti-tumour activity: 1) Insertion of a tumour-specific promoter in E1A; 2) Partial deletion of the E1A gene; 3) Amino acid change in a adenovirus fiber protein; and 4) Insertion of the coding region of the human sperm hyaluronidase gene.

The genetic modifications in VCN-01 are the following:

1. Insertion of a tumour-specific synthetic promoter composed have been inserted at the E1A endogenous promoter region of the human adenovirus type 5 genome.
2. Partial deletion of the E1A gene (delta-24 mutation). Such deletion corresponds to a 24 base pairs sequence in the HAd5 ARM genomein the E1A protein sequence.
3. Mutation of the sequence that codes for amino acids in the adenovirus fiber protein that interact with heparan sulfate in eucariothic cells.
4. Insertion of an expression cassette for the human sperm hyaluronidase (PH20) cDNA.

Role of deleted or mutated genes/sequences:

- Role of the partial deletion of the E1A gene: The delta-24 mutation generates a truncated E1A viral protein unable to bind to cellular pRb. In normal cells, this binding is required for effective virus replication. Deregulation of the retinoblastoma pathway is a hallmark of tumour cells. Under these conditions E1A function is unnecessary. Therefore, VCN-01 is unable to activate viral replication in normal cells.

- Role of the adenovirus fiber protein modification: Different HAd5 capsid proteins binds to cellular membrane to promote internalization. VCN-01 capsid includes genetic modifications that benefit simultaneously from liver de-targeting properties and the tumour-targeting properties of the integrin-binding RGD peptide.

Role of inserted transgenes/sequences:

- Role of the tumour-specific synthetic promoter: E1A modified promoter has been inserted to confer selectively of viral replication only in tumour cells, where E2F pathway is constitutively over-activated. It restricts the expression of key master proteins in adenovirus to cancer cells and inhibits its expression in normal cells.

- Role of the human sperm hyaluronidase (PH20) cDNA: Hyaluronan (HA) is the major structural component of extracellular matrix (ECM). High levels of this compound are found in several types of cancer, including pancreatic cancer and are often correlated with invasive and metastatic behaviour. PH20 expression from VCN-01 facilitates spread of the agent during treatment and improves antitumour activity.

**Evaluation of foreseeable effects**

Wild-type adenoviruses are able to replicate in epithelial tissues whereas VCN-01 replication is restricted to tumour cells. Several in vitro studies have been conducted with...
VCN-01 to assess the selectivity of replication and of cytotoxicity in non-tumour cells. Its selectivity has been evaluated in terms of replication using human primary biopsies. Contrary to the non-selective wild-type, VCN-01 was not able to replicate and behaved as the non-replicating control virus. Additionally, some in vitro studies were conducted to determine VCN-01 selectivity using cytotoxicity assays in human normal cells. In these studies VCN-01 cytotoxicity has demonstrated to be significantly lower than wild-type adenovirus toxicity.

IDENTIFICATION OF CHARACTERISTICS AND MECHANISMS THAT MAY DIRECTLY OR INDIRECTLY LEAD TO ADVERSE ENVIRONMENTAL EFFECTS.

a) Possibility of recombination and mutation of VCN-01 in the subject (human host):

The likelihood that VCN-01 is genetically unstable is negligible. Reasoning:

- Possibility of GMO recombination: HAd5 and other adenoviruses have humans as their natural host. However, most of the human population is sero-positive for adenovirus, which implies that any adenoviral infection is easily neutralized. Moreover, such infections are mostly asymptomatic, auto-limiting and restricted to several permissive tissues. Studies have shown that for recombination between different adenoviruses genomes occur they need to be present in the same cell at very high number of copies. It is a highly unlikely event that a single cell of an infected animal contains high amounts of VCN-01 together with pre-existing forms of HAd5 or other adenoviruses, due to the differential target cell populations of the intended use by the proposed route of administration for VCN-01 compared to the one of native HAd5 infections (oral/respiratory tract).

- Possibility of GMO complementation: Transcomplementation between the genomes of VCN-01 and wild-type adenoviruses from subgroup C could take place in case of co-infection at a cellular level. In that situation, wild-type genomes could provide missing functions in a temporal and self-limiting way. However, co-infection of VCN-01 and an HAd5 at a cellular level is not expected, since the target cells of Adwt serotype subgroup C would be the broncoepithelial cells (the typical route for HAd subgroup C is the oral/respiratory), and VCN-01 would not be able to reach this cell type in preclinical models. Confirming this assumption, this phenomenon has never been described during the extensive preclinical experience with different oncolytic adenovirus.

- Possibility of GMO mutation: The genomic stability of VCN-01 has been thoroughly assessed. Data obtained in a bioamplification assay demonstrates that VCN-01 genome is stable even at high selective pressure.

b) Modifications in the GMOs that affect the viral life cycle by changes in virus structure or by non-structural changes in the context of the intended use for human host.

- Changes in binding and entry compared to the parental virus: VCN-01 contains a genomic modification in the adenovirus fiber protein. Nevertheless, such a modification only induces increased bioavailability at short time-points after infection. Overall, capsid modifications in VCN-01 have demonstrated not to increase its tropism for normal organs or modify its host range.

- Changes in transcription and translation compared to the parental virus: Because of its genetic modifications viral transcription and translation will be not possible in normal cells. Other viral proteins involved in transcription and/or translation are normally present in VCN-01. The likelihood that the modifications in VCN-01 will result in an increased transcription pace or decreased transcription specificity compared to wild-type adenovirus is negligible.

- Changes in assembly compared to the parental virus: The role of the modified genes contained in VCN-01 has been studied. None of these genes encode structural proteins or play a role in adenovirus genome packaging. The genes encoding structural proteins are not expressed by VCN-01 in normal (non-tumor) cells but packaging sequences are normally present and structural proteins expressed at normal levels in VCN-01 infected tumor (permissive) cells. The likelihood that the genetic modifications will result in a more effective assembly is negligible. An influence on viral assembly due to more efficient transcription and translation is also unlikely.

- Changes in replication compared to the parental virus: Because of the insertion of its genetic modifications, VCN-01 is not able to replicate in normal cells and its cytotoxicity is significantly reduced. In tumour cells, however, the proteins involved in viral replication are present and expressed by VCN-01 at levels equivalent to wild-type
(permissive) cells are equivalent to non-modified adenovirus.

- Changes in release and cell to cell spreading compared to the parental virus: Several adenoviral proteins are known to be involved in release spreading. These proteins are normally present in permissive (tumor) cells infected with VCN-01, whereas not expressed in normal (non-tumour) cells. The function of the genetic modifications does not include a known direct role in release and spreading in normal cells. The likelihood that deletion or insertions of these genes will enhance virus release and spreading of VCN-01 compared to wild-type HAd is negligible.

- Changes in cell, tissue and host tropism compared to the parental virus: VCN-01 includes a modification in the fiber sequence that provokes that the mechanisms of adenovirus entry in organs after in vivo administration change. However, tissue biodistribution of VCN-01 has been evaluated in mice and hamster, and after extensive analysis in different organs it has been concluded that biodistribution profile of VCN-01 at short time-points after systemic administration is very similar to that reported for wild-type adenovirus and other oncolytic adenovirus previously tested in clinical trials. In addition, the analysis of VCN-01 genome content at different time-points clearly indicates a fast kinetics of degradation of the virus in normal tissues where VCN-01 is not able to replicate. By contrast, the analysis of VCN-01 genome content in tumors demonstrates that virus reaches efficiently tumours and its levels increase significantly with time after both intratumour or intravenous administration. Therefore, although VCN-01 displays capsid modifications affecting its infectivity in RGD-expressing cells and tumour accessibility in vivo, these changes do not broaden the tropism for normal cells compared to wild-type adenovirus.

- Changes in route of transmission compared to the parental virus: All structural proteins that determine transmission capabilities of adenoviruses are normally present in VCN-01 genome and the proteins deleted in VCN-01 do not play a direct roles in transmission. Therefore the likelihood that the route of transmission of VCN-01 is altered compared to wild-type HAdV-C viruses is negligible.

c) Effects of the GMO compared to wild-type virus on the subject (human host)

- Changes in immuno-modulation compared to the parental virus: Any adenoviral gene implicated in immune-modulation has not been ablated or affected by the genetic modifications introduced in VCN-01. In addition, genetic modifications in VCN-01 are not expected to introduce new immunogenic domains in the virus that could rest in a modified immune response against the virus, and these assumptions have been further validated in preclinical studies using the relevant animal models.

- Changes in induction of cell death (apoptosis and necrosis) compared to the parental virus: Adenoviral genes implicated in apoptosis induction have not been manipulated in VCN-01.

- Changes in growth factor, cyto-/chemokine signalling compared to the parental virus: Host recognition of adenovirus occurs via different elements including the capsid components and the nucleic acids. VCN-01 does not contain any modification that could result in a modulation of such pathways.

- Changes in cell intrinsic signalling and intracellular homeostasis compared to the parental virus: Although VCN-01 uses a different entry pathway compared to wild-type HAd5, after the initial attachment step, VCN-01 induces identical pathways for internalization, endosomal maturation, endosomal escape and trafficking to the nuclear pore with respect to HAd5.

Pathogenesis compared to the parental virus in the subjects: Interactions of VCN-01 with host have been previously analyzed and, overall, experimental data demonstrate that the immune response induced by VCN-01 only undergoes productive replication in tumour cells, which results in significantly reduced toxicity after in vivo administration. As a consequence, the modifications contained in VCN-01 significantly reduce its pathogenesis compared to HAd5.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Justification</th>
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**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

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<td>None</td>
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</table>
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste associated with dosing of the GMM, residual GMM dose and disposable blood sampling equipment will be autoclaved at 121°C/20 minutes prior to disposal and secured in clinical waste bins and disposed of through the Company’s clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Animal wastes (both treated animal carcasses and used bedding) will be autoclaved as required. All animal wastes will be secured in clinical waste medibins and disposed of through the Company's clinical waste incineration route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The HLS GMM local risk assessment was approved by members of the HLS GMSC and signed off on 22 April 2013. The GMSC considered that an emergency plan was not required for work with the GMM associated with this notification.

Project Containment

<table>
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Project Ref 410/15.1

Date Ackn’d 20/05/2015

CU2 Project Title

Pre-clinical testing programme for Surv.m-CRA-1. Single IV dose biodistribution study, 4 week toxicity study with 8 week recovery period via the IV route. This includes sample preparation and supporting bioanalytical studies

Date Project Ceased

Class 2

Consent Granted

Non-GMM

Project notified under transitional arrangements N

< 1 Litre

Class Volume

Consent Granted
**Project Additional Information**

**Purposes of the contained use**

The GMO is a potential cancer treatment. These studies are required prior to clinical evaluation.

**Recipient or parental organism**

Human adenovirus type 5

**Host/vector system**

Not applicable

**Origin & function**

The adenovirus has 4 genetic inserts:
- Survivin promoter: regulates adenoviral Ela gene expression in a cancer-specific manner
- El a: transactivating the viral and cellular genes; critical for producing infective adenoviruses.
- CMV promoter: capable of significantly increasing the expression of a wide variety of genes, which cover a broad eukaryotic host range
- El El 9k: prevents early onset of cell death, providing extended periods for efficient adenoviral replication

**Evaluation of foreseeable effects**

Wild type Adenovirus serotype 5 (Ad5) is commonly contracted in childhood and causes mild respiratory symptoms conferring lifelong immunity (estimated 90%+ adults are seropositive for Ad5). Adenovirus does not have to be replication-competent to cause corneal and conjunctival damage. Surv.m-CRA-1 is conditionally replication competent virus with survivin promoter in its construct, which enables the vector to replicate only in tumour cells and to reduce the risk of infection to normal cells. Survivin is expressed highly in many tumours and in foetal tissue, it is also expressed during the G2-M phase of the cell cycle. Therefore replication may be possible in healthy individuals as well as in tumours.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not Applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Waste materials will be autoclaved on site (121 °C, 20mm) where necessary. Disposal will be via our clinical waste disposal route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The wording of the risk assessment was discussed by the GMSC at draft stage, and their recommendations incorporated into the final document.

Project Containment

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

<table>
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<tr>
<th>Class 2</th>
<th>Culture Volume Class 2</th>
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<tr>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Project Ref 410/17.1

Date Ackn'd 09/03/2017

CU2 Project Title Pre-clinical testing programme for PT3.9 and the contained use of 3 strains of modified pseudomonas aeruginosa bacteria (identified as Strain 3524, Strain 5538 and Strain 3633), susceptible to the phage.

Date Project Ceased

Class 2

< 1 Litre

Consent Granted

Non-GMM

Tick if notifying a connected programme of work N

Withdrawn N

Historical Significant Changes
### Project Additional Information

**Purposes of the contained use**

PT3.9 is in development for treatment of antibiotic resistant pseudomonas aeruginosa infection. The notification consists of the pre-clinical testing programme for PT3.9.

**Recipient or parental organism**

1) Phi33, which is a bacteriophage belonging to the PB1-like family of phage
2) *Pseudomonas aeruginosa*

**Host/vector system**

*Pseudomonas aeruginosa*

**Origin & function**

1) PT3.9 has been modified to improve its efficacy  
2) the 3 strains of *Pseudomonas aeruginosa* in use have been modified to increase their susceptibility to PT3.9

**Evaluation of foreseeable effects**

The 3 bacteriophages making up PT3.9 and the 3 strains of pseudomonas aeruginosa are considered to be equivalent, in terms of potential effects and overall risks. They are therefore considered together in the risk assessment

1) The PT3.9 bacteriophage is not anticipated to pose a risk to human health. It has the potential to replicate and infect pseudomonas aeruginosa, which is widespread in the environment. The sponsor indicates that the modified bacteriophage will not have any significant advantage over the wildtype bacteriophage, based on in-vitro data. Since there is a potential for the phage to persist, and since the work involve significant aerosolisation to perform the inhalation toxicology study, biosafety level 2 measures are
required to prevent the potential for adverse effects on the environment.

2) P. aeruginosa is a hazard group 2 pathogen. The modifications made to the strains in use are not indicated to affect the potential or scope of adverse effects on humans or in the environment.

Biosafety level 2 control measures will be employed throughout the programme of work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

e.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste materials will be autoclaved on site (121°C, 20min) where necessary. Disposal will be via our clinical waste disposal route.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The wording of the risk assessment was discussed by the GMSC at draft stage, and their recommendations incorporated into the final document.

Project Containment

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Project Ref 410/17.2
Development of influenza vaccines. The candidate influenza vaccine must be assessed for retroviral purity prior to release by the client. This work does not require use of the vaccine strain as such, but assessment of bulk virus seed containing the virus for presence of retroviral contamination.

Wild-type animal origin low pathogenic influenza viruses do not have the multi-basic amino acid peptide insertion at the HA cleavage site (PENPKTR*GLF), are not pathogenic to chickens and are not therefore designated a SAPO agent by DEFRA. As such the wildtype viruses are handled at CL2.

Virus to be generated by client and sent to Envigo within study samples.

HOST Wild-type animal origin low pathogenic influenza viruses do not have the multi-basic amino acid peptide insertion at the HA cleavage site (PENPKTR*GLF), are not pathogenic to chickens and are not therefore designated a SAPO agent by DEFRA. As such the wildtype viruses are handled at CL2.

VECTOR E. Coli (strains NEB 5-alpha F’Iq competent E.coli, Veggie NovaBlue Singles Competent Cells or Invitrogen Top 10 Cells) - Disabled non-pathogenic, ACDP category 1

pAD 3000 plasmid - non-mobilisable
The virus contains the hemagglutinin (HA) and neuraminidase (NA) genes reasserted influenza virus strain B/Indiana/25/2015 and the internal genes from the cold adapted, temperature sensitive, attenuated master donor virus (MDV) backbone A/Ann Arbor/6/60

Origin & function

Wild-type animal origin low pathogenic influenza viruses do not have the multi-basic amino acid peptide insertion at the HA cleavage site (PENPKTR*GLF), are not pathogenic to chickens. Hemagglutinin (HA) and neuraminidase (NA) genes reasserted influenza virus strain B/Indiana/25/2015. The HA protein on the surface of the virus is responsible for virus/cell attachment and fusion. The HA protein does NOT contain the 7 amino acid multi-basic peptide insertion at the HA cleavage site (PENPKTR*GLF), characteristic of highly pathogenic influenza viruses. The NA of influenza viruses has enzymatic activity and is responsible for cleaving the HA from the cell receptor thus releasing new virus particles budding from the cell surface. There are no known regions or sequences that have been shown to be solely responsible for a highly pathogenic phenotype. Internal genes PB2, PB1, PA, NP, M or NS from Influenza A/Ann Arbor/6/60 - these are all well characterised genes that are used in the commercial vaccine and have been shown to be safe. E. Coli (strains NEB 5-alpha F'Iq competent E.coli, Veggie NovaBlue Singles Competent Cells or Invitrogen Top 10 Cells) - Disabled non-pathogenic, ACDP category 1

pAD 3000 plasmid - non-mobilisable

Influenza A/Ann Arbor/6/60 - attenuated, cold adapted and temperature sensitive ACDP category 1

Evaluation of foreseeable effects

The wildtype virus is designated CL2 by HSE and DEFRA based on the following:
• presence of a HA cleavage site consistent with low pathogenic influenza viruses
• shown in assays not to cause pathogenicity in the chickens
Combination of the wildtype HA and NA genes with the internal genes of A/Ann Arbor/6/60 is known to result in highly attenuated cold adapted temperature sensitive viruses.
Potential for harm to human in the event of exposure - Potential for limited and mild upper respiratory tract infection
Potential to harm and disseminate to the environment - The HA and NA genes are from low pathogenic animal origin influenza strain that is NOT currently circulating in the UK. In this case there is a low risk to the environment if the 6:2 virus was accidently released because the HA and NA genes could potentially reassort with genes from currently circulating wild type influenza to generate an influenza virus with a novel HA that is not currently in the UK
Environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Surfaces will be deactivated using Virkon spray. All samples and waste materials will be inactivated by autoclaving or chemical treatment (e.g. Virkon) prior to disposal as clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC have reviewed the risk assessment and their comments have been incorporated into the final version as attached.

Project Containment

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Animal Units

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Project Ref 410/17.3

Date Ackn'd 15/12/2017

CU2 Project Title Pre-clinical testing programme for HB-201, consisting of toxicology studies and

Class 2

Culture Volume Class 2 Not Applicable

20/02/2022
**Project Additional Information**

**Purposes of the contained use**
The GMO is a potential cancer treatment. These studies are required as part of its approval process.

**Recipient or parental organism**
Lymphocytic choriomeningitis virus (LCMV), Armstrong strain (i.e. hazard group 2).

**Host/vector system**
Not applicable

**Origin & function**
Human papillomavirus 16 (HPV16) E6 and E7, expressed as a nonfunctional, non-oncogenic fusion protein E7E6. This acts as an antigen to provide cancer immunotherapy.

Additionally, HB-201 has been modified to contain three genome segments, i.e. one L and two S segments of which each is altered to carry either the viral GP protein plus one vaccine antigen or the viral NP protein plus one vaccine antigen. Consequently, HB-201 encodes for the full genomic information of LCMV (NP, GP, L and Z proteins) of three genome segments (instead of the naturally occurring two Segments) and are therefore infectious. However, due to the fact that only a small percentage of viral progeny packages (10%) all three genome segments, vector spread is severely compromised (i.e. attenuated) compared to bi-segmented wildtype LCMV and can be easily controlled by the host’s immune system.

**Evaluation of foreseeable effects**
The house mouse (Mus musculus) is the primary reservoir host of LCMV. Peripheral (IV, SC, IM) infection of most laboratory mouse strains is
asymptomatic. Conversely, direct intracerebral (IC) inoculation causes immuno-pathological T cell-mediated choriomeningitis and death within 6 - 9 days (Cole et al., 1972). In mice, besides vertical transmission, virus ingestion via the gastric route is the probable natural route of infection (virus is shed in urine and feces) and there are to our best knowledge no documented cases of human to human transmission with the only exception of human vertical transmission from infected mother to fetus (causing congenital hydrocephalus, chorioretinitis, mental retardation, and even more rarely, through organ transplantation. Human seroprevalence ranges from 3 - 5% in most industrialized countries (Welsh et al 2008) LCMV infections of humans can occur after exposure to fresh urine, droppings, saliva, or nesting materials from infected rodents. Transmission may also occur when these materials are directly introduced into broken skin, the nose, the eyes, or the mouth, or presumably, via the bite of an infected rodent. Post-natal acquired LCMV infection of immunocompetent human adults may be even asymptomatic or limited to a non-specific, self-limited viral syndrome with symptoms such as fever, cough, malaise, myalgia, headache, photophobia, nausea, vomiting, adenopathy, and sore throat.

The client's vaccine vectors code for Human papilloma virus-16 (HPV-16) proteins E7 and E6 (Genbank K02718). HPVs are restricted in their host range to human stratified epithelia at either cutaneous or mucosal sites. The HPV-16 proteins E7 and E6 are artificially expressed as a fusion protein that is rendered non-functional due to several mutations as described in Cassetti M et al, 2004. Thereby, the original activity of the individual proteins (Wieking et al., 2012) is abrogated which renders the expressed fusion protein E7E6 non-oncogenic. Thus, in contrast to functional individual HPV E6 and E7 proteins, E7E6 fusion proteins expressed from HB-201 is unable to interfere with cellular functions of p53, Rb (retinoblastoma protein) and PTPN13 (protein tyrosine phosphatase, non-receptor type 13) while still maintaining its immunogenicity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will either be autoclaved or chemically deactivated (e.g. immersion in Virkon solution) within the work room. All waste materials will then be disposed of via our clinical waste route.
Members of the GMSC were involved in the development of the risk assessment, and the comments and recommendations of all GMSC members were incorporated into the final version of the risk assessment.

**Project Containment**

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

**Project Ref 410/18.1**

- **Date Ackn'd**: 12/04/2018
- **CU2 Project Title**: Modified ORF virus (ORFV-MELAN-A): Preliminary Immunogenicity Study
- **Class**: Class 2
- **CultureVolClass2**: Not Applicable
- **Non-GMM Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**: N
- **Significant Change ID**: 02/03/2022
### Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**
The GMO is a potential skin cancer treatment. These studies are required as part of its approval process.

**Recipient or parental organism**
Parapox (Orf) virus strain D1701-V. This is apathogenic and avirulent in-vivo, it has been classified as S1 by the ZKBS (Az: 6790-01-1627).

**Host/vector system**
Not applicable

**Origin & function**
The inserted genetic material codes for human Melan-A, which is involved in melanosome biogenesis by ensuring the stability of GPR143. It plays a vital role in the expression, stability, trafficking, and processing of melanocyte protein PMEL, which is critical to the formation of stage II melanosomes.

**Evaluation of foreseeable effects**
ORFV-MELAN-A is not anticipated to reproduce in vivo, however the client has indicated that there is a possibility that the inserted genetic material could induce adverse autoimmune effects in the event of exposure.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
High titre waste will either be autoclaved or chemically deactivated (e.g. immersion in Virkon solution) within the work room. All waste materials will be disposed of via our clinical waste route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The comments of the GMSC have been incorporated into the attached risk assessment.

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications
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Project Ref 410/18.3

Date Ackn'd: 27/09/2018
CU2 Project Title: Pre-clinical testing programme for HB-202, consisting of toxicology studies and associated and qPCR work.

Class: Class 2
CultureVolClass2: Not Applicable
CultureVolumeClass3-4: Non-GMM Consent Granted

Project notified under transitional arrangements: N

Withdrawn: N
Tick if notifying a connected programme of work: N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
The GMO is a potential cancer treatment. These studies are required as part of its approval process.

Recipient or parental organism
HB-202 consists of 2 GMOs; TheraT(Pic)-E7E6 and TheraT(LCMV)-E7E6. TheraT(Pic) is based on the Pichinde virus, TheraT(LCMV) is based on Lymphocytic choriomeningitis virus (LCMV), Armstrong strain (i.e. both hazard group 2).

**Host/vector system**
Not applicable

**Origin & function**
Human papillomavirus 16 (HPV16) E6 and E7, expressed as a nonfunctional, non-oncogenic fusion protein E7E6. This acts as an antigen to provide cancer immunotherapy.

**Evaluation of foreseeable effects**
The risk of causing disease in man and/or potential toxicity cannot be formally excluded. However, based on all available information we believe that TheraT vectors carry a minimal risk of causing disease in man, consistent with their intended use as cancer immunotherapies in humans. In the unexpected event that an accidental infection of staff occurred, the course of infection would most likely be clinically unapparent and without risk of human to human transmission. There are no anticipated adverse effects from the donated genetic material (HPV E7E6).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will either be autoclaved or chemically deactivated (e.g. immersion in Virkon solution) within the work room as necessary. All waste materials will then be disposed of via our clinical waste route.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment
Members of the GMSC were involved in the development of the risk assessment, and the comments and recommendations of all GMSC members were incorporated into the final version of the risk assessment.

### Project Containment

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### Project Ref 410/18.4

**CU2 Project Title**

Pre-clinical testing programme for VCN-11, consisting of toxicology studies and associated immunoassay method development work. The immunoassay work additionally uses a second GMM, Ad5WT-PH20, as a reference.

**Date Ackn’d**

31/10/2018

**Date Project Ceased**

Class CultureVolumeClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

**Withdrawn**

N

Tick if notifying a connected programme of work Y

**Project notified under transitional arrangements**

N

### Project Additional Information

**Purposes of the contained use**

The GMM is a potential cancer treatment. These studies are required as part of its approval process.

**Recipient or parental organism**
### Host/vector system

Not applicable

### Origin & function

VCN-11 is an oncolytic adenovirus that is unable to replicate in normal cells but has been genetically altered to selectively replicate in and lyse tumour cells. VCN-11 is derived from wild-type human adenovirus serotype 5 (HAd5) where the HAd5 backbone has been genetically altered to incorporate five distinct modifications that confer tumour selectivity and anti-tumour activity as follows:

1. Insertion of a tumour-specific promoter (415 promoter) to confer selectivity of viral replication only in tumour cells and inhibit expression of viral proteins in normal cells.
2. Partial deletion of the E1A gene such that VCN-11 is unable to activate viral replication in normal cells.
3. Mutation of the sequence that codes for amino acids in the adenovirus fibre protein resulting in improved tumour infectivity and reduced infection of liver cells.
4. Insertion of the coding region of the human sperm hyaluronidase gene enhances dissociation of the Hyaluronan extracellular matrix in tumour cells, thus enhancing the intra-tumoral distribution of the oncolytic adenovirus and improving its therapeutic activity.
5. An albumin binding domain (ABD) inserted in the hypervariable region 1 of the hexon that enables the virus to bind to blood albumin of human, mouse and hamster origin, resulting in the evasion of pre-existing neutralizing antibodies against human adenovirus serotype 5.

Ad5WT-PH20 is a modified version of wild-type human adenovirus serotype 5 (HAd5) that has been genetically altered to incorporate a single change as follows:

1. Insertion of the coding region of the human sperm hyaluronidase gene (PH20) enhances dissociation of the Hyaluronan extracellular matrix in tumour cells, thus enhancing the intratumoural distribution of the oncolytic adenovirus and improving its therapeutic activity. PH20 is expressed only when the Ad5 is able to replicate, as PH20 expression is linked to the major late promoter via de splice acceptor IIIa after the fiber viral gene.

### Evaluation of foreseeable effects

Wild type Adenovirus serotype 5 (Ad5) is commonly contracted in childhood and causes mild respiratory symptoms conferring lifelong immunity (estimated 90%+ adults are seropositive for Ad5). Wild-type adenoviruses are able to infect and replicate in normal epithelial tissues, whereas following VCN-11 infection replication is restricted to tumour cells indicating that the overall risk from infection is low in healthy individuals. The expression
products of the inserted genes result in selective enhancement of antitumour activity. In vitro cytotoxicity studies with the related virus VCN-01 have demonstrated significantly lower cytotoxicity than wild-type adenovirus toxicity, therefore the same is expected to be true of VCN-11. The likelihood that deletion or insertions of these genes will enhance virus release and spreading of VCN-11 compared to wild-type HAd is considered to be negligible.

Although VCN-11 is not able to replicate in normal cells, the replication competency of VCN-11 in tumour cells was considered sufficient justification to assign this work to activity Class 2 and therefore we conclude that it can be safely used at laboratory and animal containment level 2.

Ad5WT-PH20 is modified version of wild-type human adenovirus serotype 5 (HAd5) that is fully replication competent and is therefore assigned to activity Class 2.

Wild-type human adenovirus serotype 5 (HAd5), while not a GMM, is fully replication competent and is therefore assigned to activity Class 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste associated with dose preparation and dosing of the GMM, residual GMM dose and disposable blood sampling equipment will be autoclaved at 121°C/20 minutes prior to disposal and secured in clinical waste bins and disposed of through the Company’s clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Disinfection of dissecting instruments following completion of post-mortem procedures can be made by chemical disinfection (eg. immersion in 2% Virkon for 30 minutes) followed by autoclaving. Animal wastes (both treated animal carcasses and used bedding) will be disposed directly into medibins and sealed. The outsides of clinical waste bins will be disinfected using 2% Virkon solution prior to disposal through the Company’s clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Immunoassay wastes will be autoclaved at 121°C/20 minutes prior to disposal and secured in clinical waste bins and disposed of through the Company’s clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Members of the GMSC were involved in the development of the risk assessment, and the comments and recommendations of all GMSC members were incorporated into the final version of the risk assessment.

**Project Containment**

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**Project Ref** 410/18.5

**Date Ackn'd** 27/12/2018

**CU2 Project Title** Pre-clinical testing program for AdsVEGF-R2 and AdsVEGF-R3 consisting of toxicology studies and associated immuogenicity and immunoassay development and analysis work.

**Class** Class 2

**Culture Vol** 

**Volume** < 1 Litre

**Non-GMM Consent Granted**

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

02/03/2022
Purposes of the contained use

The GMM is a potential cancer treatment. These studies are required as part of its approval process.

Recipient or parental organism

Adenovirus type 5 (Ad5)

Host/vector system

Not applicable

Origin & function

AdsVEGF-R2 and AdsVEGF-R3 are replication defective recombinant Adenovirus type 5 (Ad5 serotype) based gene transfer vectors with partial deletions of the E3 region and deletions of the E1A and E1B genes. They have expression cassettes for human soluble vascular endothelial growth factor receptors 2 and 3 respectively (sVEGFR2 and sVEGFR3 respectively). In addition to the sVEGFR coding DNA (cDNA) the expression cassette contains human cytomegalovirus (CMV) enhancer and promoter elements. In addition to the common characteristics AdsVEGF-R2 additionally contains simian virus 40 polyadenylation signal. In addition to the common characteristics AdsVEGF-R3 additionally contains bovine growth hormone polyadenylation signal (BGH pA). Both organisms were constructed using standard DNA manipulation techniques. The sVEGFR fusion protein constructs for both organisms were created by fusing cDNA’s of Ig domains 1 – 3 of hVEGFR2 and VEGFR3 respectively into the Fc-region of human IgG1. Initial virus stocks for both organisms were generated in HEK293 cells by cotransfection and plated plaques were further purified and amplified to generate the initial stocks.

Evaluation of foreseeable effects

The recombinant viruses are replication deficient due to the loss of the E1 and part of the E3 gene. However, the viral particles are themselves capable of transduction and will elicit an immune response should it enter a human. If a human is infected with either recombinant virus the transgene will be constitutively expressed in any infected cells however the infected cells should be rapidly cleared such that expression is only transient. Neither virus will integrate into the cellular genome and so the transgene will be gradually diluted from the cell population. VEGF-R2 is a human gene considered to be the main mediator of angiogenesis, vasculogenesis and vascular permeability. VEGF-R3 is a human gene involved in lymphangiogenesis.

The sponsor has indicated that the AdsVEGF-R2 material retains some degree of replication competence despite modification. Therefore containment level 2 is required.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste associated with dosing of the GMM, residual GMM dose and disposable blood sampling equipment will be autoclaved at 121°C/20 minutes prior to disposal and secured in clinical waste bins and disposed of through the Company’s clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Disinfection of dissecting instruments following completion of post-mortem procedures can be made by chemical disinfection (eg. immersion in 2% Virkon for 30 minutes) followed by autoclaving.

Animal wastes (both treated animal carcasses and used bedding) will be disposed directly into medibins and sealed. The outsides of clinical waste bins will be disinfected using 2% Virkon solution prior to disposal through the Company’s clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Immunoassay wastes will be autoclaved at 121°C/20 minutes prior to disposal and secured in clinical waste bins and disposed of through the Company’s clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Members of the GMSC were involved in the development of the risk assessment, and the comments and recommendations of GMSC members were incorporated into the final version of the risk assessment.

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
## Project Ref 410/19.1

**Date Ackn'd** 21/03/2019  
**CU2 Project Title** Pre-clinical testing program for TG6010 and TG6030 toxicology studies in rabbit (TG6010) and Cynomolgus Monkeys (TG6030)  
**Class** Class 2  
**CultureVol** < 1 Litre  
**Class CultureVol** Class 2 < 1 Litre  
**Class CultureVol Class 3-4**  
**Non-GMM Consent Granted**  
**Project notified under transitional arrangements** N

**Withdrawn** N  
**Tick if notifying a connected programme of work** Y

### Historical Significant Changes

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**  
Both GMM's are potential cancer treatments. These studies are required as part of the approval process.

**Recipient or parental organism**  
Copenhagen Recombinant Vaccinia Virus (VV)

**Host/vector system**  
Not applicable

**Origin & function**  
TG6010:  
TG6010 is a recombinant vaccinia virus (Copenhagen strain) deleted in the thymidine kinase (J2R) and ribonucleotide reductase (I4L) genes.  
The following genetic material was inserted:  
- human cytidine deaminase (hCD) DNA in the thymidine kinase locus of the VV genome.
Replicative and propagative characteristics of VV have been attenuated in TG6010 with the disruption of the thymidine kinase (J2R) and ribonucleotide reductase (I4L) genes which renders the modified organism dependent of highly dividing cells such as cancer cells (Parato K.A. et al., 2011). Deletion of thymidine kinase (TK) J2R gene to attenuate the virus and restrict its replication to actively dividing cells (i.e. tumour cells). Deletion of the I4L gene, the large regulating subunit of the ribonucleotide reductase (RR) gene to decrease the virus replication by acting on the viral deoxyribonucleic acid (DNA) synthesis in order to reduce toxicity and dissemination. Generation of TG6010 was performed by homologous recombination. The hCD gene was inserted into the thymidine kinase locus. Modification was conducted using plasmid vector pTG18657. The VV transfer plasmid pTG18657 contains an expression cassette encoding for the human hCD gene under the control of the p11K7.5 promoter flanking by sequences (BRG and BRD) surrounding the TK (J2R) gene. It was generated by cloning of the sequence encoding hCD in the plasmid pTG18495.

TG6030 is a recombinant vaccinia virus (Copenhagen strain) deleted in the thymidine kinase (J2R) and ribonucleotide reductase (I4L) genes. The following genetic material was inserted:

• the coding sequences for the light chain of a monoclonal antibody (4E03) blocking human CTLA4 immune checkpoint protein and for the human GMCSF glycoprotein in the ribonucleotide reductase (RR) locus of the VV genome.
• the coding sequence for the heavy chain of a monoclonal antibody (4E03) blocking human CTLA4 immune checkpoint protein in the thymidine kinase (TK) locus of the VV genome.

Replicative and propagative characteristics of VV have been attenuated in TG6030 with the disruption of the thymidine kinase (J2R) and ribonucleotide reductase (I4L) genes which renders the modified organism dependent of highly dividing cells such as cancer cells (Parato K.A. et al., 2011). Deletion of thymidine kinase (TK) J2R gene to attenuate the virus and restrict its replication to actively dividing cells (i.e. tumour cells). Deletion of the I4L gene, the large regulating subunit of the ribonucleotide reductase (RR) gene to decrease the virus replication by acting on the viral deoxyribonucleic acid (DNA) synthesis in order to reduce toxicity and dissemination. Generation of TG6030 was performed by homologous recombination. The coding sequence for the heavy chain of a monoclonal antibody (4E03) blocking hCTLA4 immune checkpoint protein was inserted in the thymidine kinase (TK) locus of the VV genome. Then, the coding sequences for the light chain of a monoclonal antibody (4E03) blocking hCTLA4 immune checkpoint protein and for the hGM-CSF glycoprotein were inserted in the
Modification was conducted using plasmid vectors pTG19348 and pTG19367. The VV transfer plasmid pTG19367 contains an expression cassette encoding for the heavy chain of the 4-E03 monoclonal antibody gene under the control of the p7.5 promoter flanking by sequences (BRG and BRD) surrounding the TK (J2R) gene. The VV transfer plasmid pTG19384 contains two expression cassettes encoding for the light chain of the 4-E03 monoclonal antibody and the human GM-CSF genes under the control of the p7.5 short promoter and pSE/L respectively. The cassettes are flanking by sequences (BRG and BRD) surrounding the TK (J2R) gene.

Evaluation of foreseeable effects

The deletion of the TK and RR activities in the vaccinia virus (VV), which restricts its replication to highly dividing cells such as tumour cells, should considerably reduce the pathogenicity and the dissemination of the virus compared to the wild type VV.

TG6010:
The hCD gene inserted in the VV has no harmful properties by itself. The hCD enzyme is ubiquitously and constitutively expressed in normal tissues. Variable levels of expression are reported in organs and fluids. As the enzyme is derived from human polymorphonuclear neutrophils, high concentration of hCD is found in mature neutrophils, with levels many times greater than in other blood elements such as lymphocytes. hCD is not suspected to induce toxicity. The in vivo effect of hCD was tested by injecting 20 mg (1 mg/kg) of hCD subcutaneously in female C57BL mice daily for 1 day or 4 days. Blood samples were then collected, and platelet numbers and cell counts, including differential counts, were obtained. No immediate harmful effect was observed.

The transfer plasmids used to generate the GMM have no harmful properties.

TG6030:
The coding sequences for the hGM-CSF cytokine inserted in the VV has no harmful properties by itself. The hGM-CSF cytokine is routinely used clinically by subcutaneous injection, primarily for bone marrow stimulation to reduce the complications of neutropenia and/or bone marrow transplantation. It was chosen as a potent stimulator of systemic anti-tumour immunity. It is well-tolerated in patients. The result is a transient stimulation of neutrophil and eosinophil cells. Clinical trials of hGM-CSF demonstrated this protein was well-tolerated at very high doses that increased white blood cell counts to over 50,000 cells/mL. The most common toxicity is mild bone pain.

The coding sequences for the light and the heavy chains of monoclonal antibody (4E03) blocking human CTLA4 immune checkpoint protein inserted in the VV have no harmful properties by themselves. Human monoclonal antibody blocking CTLA4 immune checkpoint inhibitor is a medicinal product already commercialised as ipilimumab through a non-viral vector based vectorized form. Then, pre-clinical and clinical experiments experience cumulated inform on potential harmful potential of human monoclonal
antibodies blocking CTLA4 immune checkpoint inhibitor. In its pre-clinical development, 66 cynomolgus monkeys were treated for ≥ 3months with ipilimumab for toxicology studies. Most pre-clinical studies were conducted at 3 or 10 mg/Kg and toxicities associated with ipilimumab in cynomolgus monkey were minimal, and included slight increases in circulating T cells, slight to moderate lymphocyte administration of multiple organs, lymph node hyperplasia, and some evidence of decreased spleen weight. In clinical trials, ipilimumab is most commonly associated with adverse reactions resulting from increased or excessive immune activity. The transfer plasmids used to generate the GMM have no harmful properties.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste associated with dosing of the GMM, residual GMM dose and disposable blood sampling equipment will be autoclaved at 121°C/20 minutes prior to disposal and secured in clinical waste bins and disposed of through the Company’s clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Disinfection of dissecting instruments following completion of post-mortem procedures can be made by chemical disinfection (e.g. immersion in 2% Virkon or 0.45% active chlorine bleach solution for 30 minutes) followed by autoclaving.

Animal wastes (both treated animal carcasses and used bedding) will be disposed directly into medibins and sealed. The outsides of clinical waste bins will be disinfected using 2% Virkon or 0.45% active chlorine bleach solution prior to disposal through the Company’s clinical waste incineration route.

Monitoring for residual viable GMM will not be performed.

Immunoassay wastes will be autoclaved at 121°C/20 minutes prior to disposal and secured in clinical waste bins and disposed of through the Company’s clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Members of the GMSC were involved in the development of the risk assessment, and the comments and recommendations of all GMSC members were incorporated into the final version of the risk assessment.

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Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

Both GMM's are potential cancer treatments. These studies are required as part of the approval process.

**Recipient or parental organism**
Copenhagen Recombinant Vaccinia Virus (VV)

Host/vector system
Not applicable

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste associated with dosing of the GMM, residual GMM dose and disposable blood sampling equipment will be chemically inactivated by soaking in 2% Virkon solution for a minimum of 30 minutes. The outsides of any dosing waste containers (e.g. designated sharps bins) will be sealed and chemically disinfected with 2% Virkon solution prior to removal from the treatment area then secured in clinical waste bins and disposed through the Company's clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Disinfection of dissecting instruments following completion of post-mortem procedures can be made by chemical disinfection (e.g. immersion in 2% Virkon or 0.45% active chlorine bleach solution for 30 minutes) followed by autoclaving.

Animal wastes (both treated animal carcasses and used bedding) will be disposed directly into medibins and sealed. The outsides of clinical waste bins will be disinfected using 2% Virkon or 0.45% active chlorine bleach solution prior to disposal through the Company's clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Animals rooms will be cleaned using 2% Virkon solution at the end of the animal housing phase of the studies.

Immunoassay wastes will be autoclaved at 121°C/20 minutes prior to disposal and secured in clinical waste bins and disposed through the Company's clinical waste incineration route. Monitoring for residual viable GMM will not be performed.

Is an emergency plan required according to regulation 20? N

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**Name**

ANIMAL HEALTH TRUST

**Name 2**

**Department**

CENTRE FOR PREVENTIVE MEDICINE

**Campus Estate or Research Centre**

**Road Name**

LANWADES PARK

**District**

KENTFORD

**Town**

NEWMARKET

**County**

SUFFOLK

**Postcode**

CB8 7PN

**Country**

ENGLAND

**Tel Number**

01638 750659

**Fax Number**

01638 750794

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 415/01.2

Date Ackn'd 18/10/2001

CU2 Project Title GENERATION OF EQUINE INFLUENZA VIRUS BY A REVERSE GENETICS SYSTEM

Date Project Ceased

Class Class 2

CultureVol Class 2 < 1 litre

Consent Granted not applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work Y

Historical Significant Changes

02/03/2022 Page 7268 of 15326
The project aim is to establish a plasmid-based reverse genetics system to generate equine influenza viruses entirely from cloned cDNA. This technique enables the relevance of amino acid changes in influenza virus proteins to be directly assessed. The system will be established for a well-characterised strain of the H3N8 subtype of equine influenza virus (A/eq/Newmarket/2/93). The plasmid-based reverse genetics system has been established for human influenza virus, and the plasmids for the rescue of A/Victoria/3/75 (H3N2) are available from Dr Thomas Zrcher (GiaoxSmithKline, Stevenage, UK) for use in this project. In this system, mammalian cells are transfected with 4 protein expression plasmids in which the influenza virus replication genes are expressed under the control of human cytomegalovirus (CMV) promoter and viral RNA transcription plasmids expressing all 8 influenza genes under the control of the human polymerase I (Poll) promoter. Assuming functional compatibility between the human influenza replication proteins and equine influenza virus genes (equine-human influenza reassortants have been generated by traditional methods), the human influenza system will be used as the basis for establishing the equine influenza system. Individual genes of the human influenza system will be generated de novo. Chloramphenicol acetyltransferase (CAT) and/or green fluorescent protein (GFP) reporter genes flanked by the non-coding sequences of an influenza gene will be used as a model RNA to demonstrate that protein expression plasmids expressing the viral replication genes NP, PB1, PB2 and PA are functional and to determine transfection efficiency. Transfection of mammalian cells with protein-expression plasmids encoding cDNA for each of the equine influenza proteins will be used to check expression of full-length proteins prior to subcloning into the vRNA-coding transcription plasmid. The T7 expression system may also be used to confirm expression of full-length proteins if this cannot be achieved by expression under the control of the CMV promoter.

If successful in generating recombinant virus, the relevance of specific amino acid mutations in the influenza virus proteins for the phenotype of equine influenza viruses will be assessed. Mutations will be introduced into the haemagglutinin (HA) gene and any effect on antigenicity examined in vitro. The mutations that will be introduced will be those observed in wild-type viruses, the aim being to compare the effects of different mutations in a common background to remove the influence of concomitant changes in other genes or other regions of the same gene.

Recipient or parental organism

Recombinant human and equine influenza A viruses. Human influenza A virus is an ACDP Hazard Group 2 agent; therefore the recombinant viruses will be handled at ACDP Containment Level 2

Host/vector system

Host/Vector systems are standard, well-defined systems including standard cloning strains of Escherichia coli, attenuated vaccinia virus, modified vaccinia Ankara T7 virus and non-human mammalian cells.

Origin & function

02/03/2022

Page 7269 of 1532
Genetic material obtained from influenza virus isolates. To be used in in vitro research only.

Evaluation of foreseeable effects

Mutations in the HA may potentially alter virus binding to, or entry into cells by changing the structure of the receptor binding site, which is well-defined for influenza HA. Only mutations observed in naturally arising variants of equine influenza will be introduced into the HA, and it is considered unlikely that natural variants occurring as a result of antigen drift will have alterations to tissue tropism or host range. Introduction of equine influenza HA and/or NA into a genetic background of human influenza virus is expected to alter the host range as equine influenza has a different receptor specificity to human influenza. It is expected that an equine-human reassortant will have reduced infectivity for humans. Such reassortants have been generated in vitro without the use of genetic manipulation techniques. Traditional equine influenza vaccines should provide susceptible species with adequate protection against an equine-human reassortant, as the major component of protective immunity is antibody to the (equine) HA.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Standard procedures for waste disposal in a Containment Level 2 laboratory. Solid waste (eg plasticware) will be autoclaved. Virus containing liquid waste will be decontaminated overnight with a 2% v/v solution of Virkon prior to disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been approved by the local GMSC.

Project Containment

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Animal Units

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02/03/2022
Purposes of the contained use

Viral vectors will be used to express genes which are associated with pluripotency in equine and canine cells to induce the formation of pluripotent stem cells. These cells will be used for in vitro modelling of inherited diseases in these species and for investigations into the therapeutic potential of these cells or their derivatives in the treatment of injuries and diseases.

Recipient or parental organism

Equine and canine cell lines

Host/vector system

Replication defective retroviral and/or lentiviral vectors

Origin & function

The viral vectors will express multiple genes which are associated with pluripotency. These will include oncogenes such as c-myc

Evaluation of foreseeable effects

The viral vectors to be used will be capable of infecting human cells. The vectors to be used will all be replication defective to minimise their risk and would be unable to spread if released to the environment. However, some of the vectors may contain oncogenes (e.g., c-ymyc) and would therefore have the potential to promote tumour formation if they came into direct contact with human cells during handling. To minimise this risk, the viral vectors containing oncogenes will be handled in class 2 microbiological safety cabinets. The use of sharps will not be required during the handling of the viral vectors.
The viral vectors will be used to genetically modify animal cells to induce pluripotency. The resulting cells will pose no risk to human health or the environment should they be released. Therefore they will be handled in containment level 1 facilities.

The induced pluripotent stem cells (or cells derived there from) may be transplanted into experimental animal models of injury or disease to assess the therapeutic potential of the cells. No additional genetic modifications will be made to the cells which would make them more likely to undergo uncontrolled growth. Therefore any animal models receiving these cells will pose no risk to human health or the environment and will not require special containment facilities.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| Not applicable |

Describe the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| The viral vectors containing oncogenes may pose a risk to human health and so all material which comes into contact with these lenti/retroviruses will be treated to ensure that all virus is killed |
| Surfaces will be cleaned with a 1% solution of virkon followed by 70% alcohol. |
| Liquid waste containing viral vectors will be inactivated by treatment with disinfectant for 1h. |
| Contaminated laboratory glassware will be immersed in disinfectant for 1h prior to washing and sterilisation by autoclaving |
| Contaminated plasticware including pipettes will be immersed in disinfectant for 1h prior to washing and sterilisation by autoclaving |
| Contaminated plasticware including pipettes will be immersed in disinfectant prior to being double bagged and autoclaved to ensure complete inactivation of any hazardous material |
| Either a 3% solution of virkon (peroxygen compound or a 10% solution of chloros will be used as a disinfectant. |
| Inactivation will be monitored regularly by the direct plating of treated liquids onto permissive cell lines and PCR analysis for viral sequences being performed or monitoring viral reporter gene (e.g green fluorescent protein) expression directly where applicable. |

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The GMO committee reviewed the application on the 7th February 2011 and agreed the work involved expression of an oncogene, and therefore has an increased risk to human health, then the work should be carried out at level 2. This is in agreement with the discussions at the 16th meeting of the scientific advisory committee on genetic modification on the 18th March 2009. An area at the AHT currently used for level 2 work was identified (room 70 within the centre for preventive medicine) in which the work would be performed.

**Project Containment**

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**Project Ref 415/15.1**

**Date Ackn’d** 29/01/2015

**CU2 Project Title**

Generation of plasmids containing the phospholipase A2 genes from Streptococcus equi and sub species equi (S.equi), and S.equi sub species zooepidemicus (S.zooepidemicus) and expression of the recombinant fusion proteins

**Class**

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**Project notified under transitional arrangements** N

**Withdrawn** N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The plasmids containing phospholipase A2 genes will be used to express recombinant proteins in E. coli. These proteins will be used in vitro to stimulate different subsets of equine immune cells, and the effects will be studied.

02/03/2022
will allow for the furthering of our knowledge of the pathogenic factors produced by S. equi and the immune response in the horse.

**Recipient or parental organism**

Competent E. coli K-12 derivatives such as DHSa-like and Bl21-DE3

**Host/vector system**

The plasmids will be produced and protein expressed in competent E coli (DHSa-like and Bl21-DE3). The vectors used will be pGEX, pET, or pMAL

**Origin & function**

The plasmids transformed into E coli will express phospholipase A2. proteins from S. equi and S. zooepidemicus.

**Evaluation of foreseeable effects**

The plasmid produced will be transformed into E. coli. The risk of transfer to other bacteria is low. The recipient E. coli strains are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. E. coli K-12 derivatives are recognised as non-colonising and disabled, and may be considered to be equivalent to hazard group 1. The vectors are considered to be non-mobilisable. Gene transfer is thus a remote possibility. Phospholipases are a group of enzymes that bring about changes in host cell membrane composition through the hydrolysis of fatty acids. This causes release of arachidonic acid, a precursor of eicosanoids (e.g prostaglandin, leukotrienes) leading to activation of inflammatory cascades. Therefore, the protein is likely to be toxic and cause an inflammatory response, and so if the recombinant protein breached the skin it is possible that this would be detrimental to humans, and so poses a risk. To minimise this risk, the bacteria expressing the plasmid will be cultured in plastic rather than glass flasks, and no sharps will be used during the handling of either the bacteria ~ expressing the protein, or the protein itself. The bacteria will be handled in class 2 microbiological cabinets at containment level 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation required

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The E. coli expressing the phospholipase A2 proteins may pose a risk to health, therefore all material that comes into contact with these bacteria will be treated to ensure all bacteria are killed. Surfaces will be cleaned with a 1% solution of virkon, followed by 70% alcohol. Liquid waste will be inactivated by treatment with disinfectant for 24 hours. Contaminated laboratory culture flasks will be immersed in disinfectant for 24 hours prior to washing and sterilisation by autoclaving. Should pipettes or other laboratory equipment become contaminated, where possible, they will be immersed in disinfectant prior to being double bagged and autoclaved to ensure all bacteria are killed. Where this is not possible (e.g. the non-removable interior of an incubator), the contaminated area will be sectioned off and thoroughly cleansed with a 1% solution of virkon, followed by 70% alcohol.
Either a 10% solution of chloros, or a 1:50 ANIGene HLV4D solution (previous name TriGene) will be used as disinfectant. Inactivation will be monitored by direct plating of treated liquids on to agar plates and incubated at 37°C for 24 hours. Where no growth is observed after 24 hours in culture, decontamination will be deemed complete.

The risk assessment covers the expression of phospholipase A2 toxins derived from Streptococcus equi and Streptococcus zooepidemicus in E. coli. The committee reviewed the application on the 13th November 2014 and agreed that because of the nature of the toxins the application was correctly considered to be a class 2 activity. The committee also agreed that it was prudent that no sharps would be used when handling the bacteria and the expressed proteins.

Project Containment

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Project Ref 701/99.1

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Class: 2
Consent Granted: Non-GMM: Yes
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form
Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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Project Ref 701/99.2

Date Ackn'd 25/05/1999

CU2 Project Title MUTATIONAL ANALYSIS OF THE EQUINE ARTERITIS VIRUS PROTEINS GL + M; EFFECTS UPON VIRUS REPLICATION AND PATHOGENESIS.

Class 3

Non-GMM Consent Granted yes

Project notified under transitional arrangements Y

Historical Significant Changes GM415/01.1

Historical Date of Additional Info 20/07/2001

Project Additional Information

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Name**

RADIATION PROTECTION DIVISION OF THE CENTRE FOR RADIATION, CHEMICAL AND ENVIRONMENTAL HAZARDS

**Name 2**

PART OF THE HEALTH PROTECTION AGENCY

**Department**

RADIATION EFFECTS

**Campus Estate or Research Centre**

**Building**

**District**

CHILTON

**Town**

DIDCOT

**County**

OXFORDSHIRE

**Postcode**

OX11 ORQ

**Country**

ENGLAND

**Tel Number**

01235 822648

**Fax Number**

01235 833891

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

04/11/2003
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Laboratory</th>
<th>Animal Unit</th>
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The maximum individual culture volume is 25 ml. Liquid waste is deactivated by disinfectant (Virkon) according to manufacturers instructions. Waste is deactivated by placing in sealed hazard bags and autoclaved. Verification of deactivation is by autoclave tape and monitoring of autoclave by thermocouple.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 419**

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**Name**

GLASGOW CALEDONIAN UNIVERSITY

**Name 2**

**Department**

DEPARTMENT OF LIFE SCIENCES

**Building**

CHARLES OAKLEY BUILDING

**Road Name**

COWCADDENS ROAD

**District**

**Town**

GLASGOW

**County**

RENFREWSHIRE

**Postcode**

G4 0BA

**Country**

SCOTLAND

**Tel Number**

0141 331 3000

**Fax Number**

0141 331 3208

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee

  - Laboratory
  - Animal Unit
  - Growth Room
  - Glass House
  - Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

- Other (please specify)
  - Tick if confidential

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
Maximum culture volume which would be released is 12 L per day. Waste is deactivated by autoclaving at 126 degrees C for 30 minutes. The autoclave receives regular servicing at which time all instrumentation is recalibrated. In addition TTS and Spore Strips are used periodically to ensure continued effectiveness of the system.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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**Project Ref  419/16.1**

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Tick if notifying a connected programme of work

Project notified under transitional arrangements

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Aim:** This project aims to utilise genetically altered bacterial strains, typically associated with nosocomial infections (Staphylococcus and Enterococcus species), to investigate the cellular responses to situations that pose a stress condition (e.g., antibiotic-induced stress and stresses encountered within the host during colonisation or infection, for example pH).

**Methods:** Global regulatory genes and virulence genes of candidate Staphylococcus and Enterococcus species will be inactivated by targeted gene deletion or disruption, using for example TargeTron Gene Knockout System (Sigma). Initially the Enterococcus faecalis virulence gene efA will be deleted using, for example, the TargeTron Gene Knockout system (Sigma). Other deletion mutants targeting specific global regulatory genes will be acquired from other laboratories for use in this project and not constructed within the laboratory.

Resistance to the antibiotic linezolid will be generated in the reference strain of Staphylococcus aureus ATCC 29213 using a naturally occurring linezolid resistance plasmid. This GMM will be acquired from another laboratory and not constructed within the laboratory.

**Recipient or parental organism**

**Parental strains:**

i) Enterococcus faecalis ATCC 47077 / OG1RF (complete genome GenBank: CP002621.1). This strain is a rifampicin and fusidic acid resistant derivative of OG1, which is widely used by the research community. Hazard Group 2 organism.

ii) Staphylococcus aureus ATCC 29213. Staphylococcus aureus subsp. aureus ATCC 29213 is one of the most commonly used strains in drug discovery research and for quality control (Soni et al., 2015). It is sensitive to a large variety of antimicrobials, including methicillin. Hazard Group 2 organism.


**Host/vector system**

Enterococcus faecalis ATCC 47077 / OG1RF – no vector, deletion mutant. Targetted gene deletion to be generated using a commercially available system, eg TargeTron Gene Knockout System (Sigma) or equivalent.

Staphylococcus aureus ATCC 29213 will be given the plasmid cfr (a naturally occurring resistance plasmid of S. aureus), which codes for linezolid resistance.

**Origin & function**

GMM which will be generated using the host strains listed above:

1) Enterococcus faecalis ATCC 47077 / OG1RF; deletion of relA or relA/relQ (GTP diphosphokinase genes) (Abranches et al., 2009). RelA/RelQ are involved in the synthesis/hydrolysis of ppGpp (guanosine 3’-diphosphate 5’-diphosphate), which is a mediator of the stringent response (bacterial stress response) that coordinates a variety of cellular activities in response to changes in nutritional abundance. This GMM will be acquired from another laboratory and not constructed within Dr Lang’s laboratory.

Enterococcus faecalis. Journal of Bacteriology 191(7);2248-2256.

2) Enterococcus faecalis ATCC 47077 / OG1RF; deletion of the virulence factor gene efaA (Enterococcus faecalis endocarditis specific antigen). Enterococcus faecalis endocarditis specific antigen (EfaA), a well-known virulence factor of E. faecalis originally detected in connection with studies of endocarditis (Lowe et al., 1995). Using a mouse model system, it has been shown that an EfaA mutant of E. faecalis OG1RF is less virulent than the wild-type strain (Singh et al., 1998). This GMM will be constructed in Dr Lang's laboratory at Glasgow Caledonian University.


3) Staphylococcus aureus ATCC 29213; insertion of the naturally occuring plasmid cfr (Locke et al., 2014).
The plasmid cfr is a naturally occurring plasmid of S. aureus that conveys resistance to the antibiotic linezolid. Plasmid DNA isolated from strain 1128105 was previously transformed into the strain S. aureus ATCC 29213. This GMM will be acquired from another laboratory and not constructed within Dr Lang's laboratory.

Evaluation of foreseeable effects

1) Enterococcus faecalis ATCC 47077 / OG1RF; deletion of relA and relQ; these mutants are defective in the regulatory pathways associated with a stringent (stress) response. There is no known increase to the innate hazard/risk of these mutants relative to the parental strain (Enterococcus faecalis ATCC 47077 / OG1RF; Hazard Group 2 organism).

2) Enterococcus faecalis ATCC 47077 / OG1RF; deletion of efaA; using a mouse model system, it has been shown that an EfaA mutant of E. faecalis OG1RF is less virulent than the wild-type strain (Enterococcus faecalis ATCC 47077 / OG1RF; Hazard Group 2 organism) (Singh et al., 1998). References: Singh KV, Coque TM, Weinstock GM, Murray BE (1998). In vivo testing of an Enterococcus faecalis efaA mutant and use of efaA homologs for species identification. FEMS Immunol Med Microbiol. 21(4):323–31.

3) Staphylococcus aureus ATCC 29213 cfr+; isolate will be resistant to the antibiotic linezolid, but virulence will not be altered. There is no known increase to the innate hazard/risk of this mutant relative to the parental strain. The parental strain, S. aureus ATCC 29213 (Hazard Group 2), is one of the most commonly used strains in drug discovery research and for quality control. To limit the potential for horizontal gene transmission of the resistance plasmid, the GMM will be cultivated in pure culture only and all discard will be isolated from other biological waste. The organism is sensitive to a large range of clinically used antibacterials, including methicillin, therefore, despite the acquisition of resistance to the antibacterial linezolid other treatment options are available.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Working environment: To reduce the hazard posed to the workers and environment, all manipulations will be undertaken in the Micro-Containment Suite (MCS) C122 using Class II Microbial Safety Cabinet (MSC II) and cultures incubated in a bacterial cell incubator, also located in the MCS.
Access to the MCS is restricted via keycard entry to users who are carrying out the work described in the specific Risk Assessments and a training log is retained within C122 alongside the Risk Assessments.
Waste disposal: Solid media cultures (agar plates) will remain within the MCS in sealed containers and separate from other biological waste prior to autoclave destruction. The solid waste will be transported in sealed and labelled containers to the autoclave (located on the floor above the Micro-Containment Suite), using a hoist system, and destruction recorded using a biological waste destruction log system.
Liquid cultures (broth cultures) will only leave the MCS once inactivated using an appropriate disinfectant, eg 10% Anistel for 24 hours. Once inactivated, the waste will be sent for autoclave disposal, as outlined above. Contaminated materials decontaminated by immersion in appropriate disinfectant, eg 10% Anistel for 24 hours then bagged and autoclaved.
Discard is undertaken according to GMM Risk Assessment and MCS guidelines. All discards will be labelled GMM for disposal purposes. Waste is removed daily from the MCS for autoclaving using a hoist system (autoclave located the floor above the Micro-Containment Suite), autoclaved and discarded following a strict and traceable protocol. Only trained and registered personnel are permitted access to the MCS, domestic staff are not permitted access and are not allowed to dispose of any waste from this area.

Accidental spillages: Small spillages: contaminated surfaces will be treated with an appropriate disinfectant; eg 10% Anistel. Contaminated materials decontaminated by immersion in appropriate disinfectant, eg 10% Anistel for minimum 60 minutes or bagged and autoclaved.

Large spills: Anyone working in the MCS will be alerted to the spill. A Microbial Spill Kit (available in MCS) will be used to cover the area twice the size of the spill with disinfectant soaked tissue or surrounded with dry disinfectant. The contaminated tissue will be bagged for autoclave destruction and the area re-cleaned with disinfectant solution (2% Anistel or equivalent). If broken glass is involved, forceps or a brush will be used to transfer the glass to a sealable autoclavable discard pot. The pot will be labelled and discard technicians notified. (PI and Biological Safety Advisor) and (GMO officer) will be notified and the incident recorded through the accident recording system.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Your application to GCU GMSC has been approved for submission to HSE as a Class 2 project with some minor amendments indicated in the document

Project Containment

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Project Ref: 419/16.2

Date Ackn’d: 22/12/2016

CU2 Project Title: The generation of human induced pluripotent stem cells (and targeted cell lineages derived by differentiation from these cells) through the delivery of episomal vectors into human primary cells by non-viral transfection methods

Class: Class 2

Culture Volume: Class 2 < 1 Litre

Consent Granted: Non-GMM

Date Project: 02/03/2022

Page 7288 of 15326
Project Additional Information

Purposes of the contained use

To reprogram human skin fibroblasts, derived from skin biopsies from patients undergoing elective surgery, or from diabetic and non-diabetic individuals, and from involved (diseased) and uninvolved regions of skin. The patients are not tested for HBV, HCV or HIV, requiring Class 2 categorisation. The IPS cells will then be differentiated to target cell lineages, as required, using modified serum, specific substrata and combinations of growth factors and cytokines, and used in numerous studies requiring difficult or impossible to source human cell types.

Recipient or parental organism

Human skin fibroblasts, derived from skin biopsies from patients undergoing elective surgery for breast cancer, or from diabetic and non-diabetic individuals, and from involved (diseased) and uninvolved regions of skin from diabetic patients. The anonymised patients are not tested for HBV, HCV or HIV, although their clinical background is available on request.

Host/vector system

The system uses episomal DNA plasmid vectors, as described in Risk Assessment 24/15/AG/LS. There is no integration of the genetic material into the host cell genome. The non-viral DNA plasmid vectors contain the EBNA 1 (Epstein-Barr virus nuclear antigen 1) expression cassette and the OriP origin of replication. Though EBNA 1 is a viral protein, the vectors themselves are DNA plasmids which enter the cell via lipid-based transfection, or Amaxa electroporation, and do not act as viruses or retroviruses.

Epi5 Reprogramming Vectors
These are oriP/EBNA1 vectors containing 5 reprogramming factors (Oct4, Sox2, Lin28, Klf4, and L-Myc) which replicate extra-chromosomally only once per cell cycle.

- pCE- hOCT3/4: Oct 4
- pCE-hSK: Sox2, KLF4
- pCE-hUL: L-Myc, Lin28

Epi5 p53 & EBNA Vectors
Vectors expressing mp53DD (a dominant negative mutation of p53) and EBNA1, which together improve the reprogramming efficiency of the system.

- pCE-mP53DD: mp53DD
- pCXB-EBNA 1: EBNA 1
The vectors will be delivered to the recipient cells, using either Lipofectamine 3000 (ThermoFisher Cat No L3000 - please see MSDS appendices 5 and 6 to risk assessment, 24/15/LS/AG), or using Amaxa II electroporation and kit VAPD-1001 as described in the amendment (dated 18/05/16; received and approved 23/05/16) to the same.

Origin & function

The episomal vectors are of commercial origin (ThermoFisher C-004-SC, MSDS Appendix 7, 24/1S/AG/LS) and contain the reprogramming factors Oct4, Lk4, Sox2, L-Myc and Lin28, as well as mpS3DD and EBNA1.

Oct3/4 is encoded by POUSF-1, and is a transcription factor associated with maintenance and self-renewal of pluripotent stem cells.

Sox2 is encoded by SOX2, and is a transcription factor associated with maintenance and self-renewal of pluripotent stem cells.

Klf4, encoded by KLF4, is a transcription factor regulating proliferation, apoptosis, differentiation and pluripotent reprogramming.

L-Myc, encoded by MYCL 1, is a transcription factor implicated in pluripotent reprogramming and lung cancer. Full function is unknown.

Lin28, encoded by LIN28, enhances translation of IGF-2 (insulin-like growth factor 2) mRNA

MpS3DD, encoded by MpS3DD, is a dominant negative mutation of pS3 protein, providing temporary high efficiency pS3 knockdown for improved reprogramming.

EBNA1, encoded by EBNA1, is involved in gene regulation, extrachromosomal replication and episome genome maintenance.

Evaluation of foreseeable effects

The oncogenic potential of the genes to be introduced to achieve reprogramming:

POUSF1: Aberrant expression of this gene in adult tissues is associated with tumorigenesis.


SOX2: Implicated in proliferation and tumorigenicity in melanoma and lung cancer.


KLF4: Oncogene involved in cell migration and invasion, also implicated as a tumour suppressor.


MYCL 1: Implicated in lung cancer.


LIN28: Associated with chemoresistance and oncogenesis.

Hsu KF, Shen MR, Huang YF, Cheng YM, Lin SH, Chow NH, Cheng SW, Chou CY, Ho CL. Overexpression of the RNA-binding proteins Lin28B and IGF2BP3 (IMP3) is associated with chemoresistance and poor disease outcome in ovarian cancer. Br J Cancer. 2015 Jul 28;113(3)


EBNA1: Increases cell survival and proliferation. Implicated in cancers included B-ceillymphoma and epithelial tumours.

Frappier L. Contributions of Epstein-Barr nuclear antigen 1 (EBNA 1) to cell immortalization and survival. Viruses. 2012 Sep;4(9):1537-47.

The introduction of the genes is intended to induce a state of pluripotency in the recipient cells, which will increase their proliferative and tumorigenic capabilities. Pluripotent cells are also able to generate teratomas when injected into immunedeficient mice, and may pose a similar risk with humans. The modified cells therefore pose a greater risk to human health than the original host cells.

The cells only pose a teratoma risk when injected or ingested, so needles or sharps will not be used for any aspect of pluripotent cell culture. It is highly unlikely that the the procedures followed post-transfection will create significant aerosols. If good laboratory practice and the recommendations listed in the risk assessment are followed, ingestion or inhalation of the material is highly unlikely. Further, the plasm ids used to re-programme the cells are essentially eliminated on subsequent culture due to their episomal nature, as indicated in Appendices 1 and 2 of the current risk assessment (24/15/AG/LS). The cells are highly unlikely to survive outside of a specified tissue culture environment and should not pose an increased risk compared with the original (unmodified) primary cells if good laboratory practice and the recommendations for decontamination listed in the risk assessment are followed.

The use of primary human cells which have not been tested for viral contamination leads to containment requirements (Class 2). It is not possible to predict how the reprogramming factors used may interact or impact on such contaminants. Culture work is to be performed at low scale and in low volumes, reducing the overall risk from handling the cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Colonies should be picked using pipette tips and not needles or sharps. Adherence to good laboratory practice and correct disposal as detailed in the risk assessment (Appendix 8) and relevant COSHH forms. Spent tissue culture media, reagents and plastics are treated overnight with Presept inside fume hood, as is done with all cultures from any source. Presept is a chlorine releasing agents which operates with a complete biocidal spectrum. Liquids are then disposed of in a standard sink with plenty of water, and culture plastics are disposed of as standard laboratory plastic. Class II safety cabinet should be utilised for transfection and maintenance of cells, and PPE (laboratory coat and latex/nitrile gloves) should be worn. Correct decontamination procedures are essential, together with adherence to the protocols set out in the risk assessment and COSHH forms to inactivate the cells. Culture work is to be performed at
low scale and in small volumes which further reduces the risk associated with the material.

The cells will undergo a morphological change and acquire new characteristics upon being successfully modified. Visual confirmation of pluripotent cell morphology is the first indicator of this, which will be followed by characterisation techniques such as alkaline phosphatase staining, flow cytometry (SSEA3, SSEA4, TRA-1-60, TRA-1-81), immunocytochemistry (Oct4, Sox2, Nanog), RT-qPCR (tools such as pluripotency array cards), and differentiation to cells representing the three germ layers. Given the culture protocol and usage, and adherence to good laboratory practice for Class II organisms, the modified cells are not seen as presenting a greater risk to the user than the starting material. The ultimate form of the iPSCells will, of course, depend upon their directed differentiation to targeted cell lineages, which is achieved using modified media, specific substratum and defined combinations of cytokines and/or growth factors delivered at key stages of differentiation (see amendment 02 dated 24/10/16; receipted and approved 26/10/16).

These cells will also be subject to Class II containment (above), none of the iPSCells, or their targeted cell lineages, will be employed as human therapeutics.

Working environment: To reduce the hazard posed to the workers and environment, all manipulations will be undertaken in the Micro-Containment Suite (MCS) C122 using Class II Microbial Safety Cabinet (MSC II) and cultures incubated in a cell incubator, also located in the MCS.

Access to the MCS is restricted via keycard entry to users who are carrying out the work described in the specific Risk Assessments and a training log is retained within C122 alongside the Risk Assessments.

Discard is undertaken according to GMM Risk Assessment and MCS guidelines. All discards will be labelled GMM for disposal purposes. Both solid and liquid waste will be fully decontaminated using 10% Anistel for 24hours then bagged and autoclaved by the individual user and removed from the MCS for autoclaving using a hoist system (autoclave located the floor above the Micro-Containment Suite), autoclaved and discarded following a strict and traceable protocol. Any solid human tissue will be disposed of in accordance with our in-house guidelines in adherence with the Human Tissue Act Scotland 2006. Only trained and registered personnel are permitted access to the MCS, domestic staff are not permitted access and are not allowed to dispose of any waste from this area.

Accidental spillages: Small spillages: contaminated surfaces will be treated with an appropriate disinfectant; eg 10% Anistel. Contaminated materials decontaminated by immersion in appropriate disinfectant, eg 10% Anistel for minimum 60 minutes or bagged and autoclaved.

Large spills: Anyone working in the MCS will be alerted to the spill. A Microbial Spill Kit (available in MCS) will be used to cover the area twice the size of the spill with disinfectant soaked tissue or surrounded with dry disinfectant. The contaminated tissue will be bagged for autoclave destruction and the area re-cleaned with disinfectant solution (2% Anistel or equivalent). If broken glass is involved, forceps or a brush will be used to transfer the glass to a sealable autoclavable discard pot. The pot will be labelled and discard technicians notified. Dr L (PI and Biological Safety Advisor) and Prof S (GMO officer) will be notified and the incident recorded through the accident recording system.
Reasons for recommendation of amendment: Two amendments were submitted for the original GMRA reference 24/15/AG/LS. These were circulated to the GMSC and the following feedback comments provided.

1. Location of work to change to Micro-containment suite (MCS) utilising viral disposal procedure. Training will be required for access.
2. Human tissue disposal procedures may require to be added for any tissue obtained, but cells may be decontaminated and disposed of via the local guidelines in MCS.
3. Disposal via original RA is not permitted and details provided for CU2 form should be used.
4. Plan is required for disposal, shipping and receipt of GMM cells from Biobank.

Risk assessment to human health: Cells may contain adventitious agents which under the COSHH may fall in to hazard group 3. No attempt will be made to seek and identify any pathogenic agents in the cells provided by the Biobank. Both primary and GM iPS cells of human origin will be utilised. This cell type is considered medium/high hazard level. Both cell types will be or have inserted vectors expressing biologically active molecules. As per original risk assessment, these molecules are growth factors or proteins that have been shown to increase proliferation and have demonstrated evidence of tumourigenesis. In addition, as the iPS cells are already modified we do not know the affect this has had on any adventitious agents present. For this purpose containment level 2 safety cabinets are required for manipulation to protect the user. Risk is further minimised by no use of sharps and the use of small volumes.

Risk assessment to the environment is deemed low/negligible. Escape and survival highly unlikely. Containment measures applied above sufficient.

Review of control measures: Shipment protocols for GMM iPS cells containing unknown biologic agents will be adhered to as per Biobank procedure and GMM legislation.

Final assignment of containment measures and risk class:
Containment levels required correspond to Level 2 with no additional measures. Risk posed to human health is considered low. This gives an overall risk classification of Class 2. Containment level 2 and Risk class level 2 therefore CU2 submission required. This was dated 24/10/16

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**Name**

LABCORP EARLY DEVELOPMENT LABORATORIES LIMITED

**Department**

BIOTECHNOLOGY SERVICES

**Campus Estate or Research Centre**

CORNING HAZELTON EUROPE

**Building**

BIOTECHNOLOGY SERVICES

**Road Name**

OTLEY RD

**District**


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<td>YORKSHIRE</td>
<td>HG3 1PY</td>
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**Tel Number**

01423 500011

**Fax Number**

01423 569595

**E-mail**

**HSE Division**

YORKSHIRE AND NORTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref** 421/01.1

<table>
<thead>
<tr>
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<th>CU2 Project Title</th>
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<th>CultureVolumeClass3-4</th>
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<td>30/04/2001</td>
<td>GENE THERAPY VECTOR DISTRIBUTION STUDY IN MICE USING QUANTATIVE PCR TO DETERMINE CONCENTRATION OF VECTOR IN SPECIFIC ORGANS</td>
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Tick if notifying a connected programme of work N

Historical Significant Changes
**Project Additional Information**

**Purposes of the contained use**
To determine the tissue concentration of a gene therapy vector in the organs of mice treated with a modified human herpes simplex viral vector.

**Recipient or parental organism**
The recipients in the study are BALB-C mice.

**Host/vector system**
The vector is a replication competent herpes simplex type-1 virus. The strain is JS-1 ECACC Accession Number 01010209. It is deleted for the neurovirulence factor ICP34.5 and for the immune avoidance factor ICP47. The transgene codes for human GM-CSF the expression of which is under the control of the human cytomegalovirus immediate early promoter.

**Origin & function**
The human GM-CSF gene product will be delivered by intratumoural injection in human clinical trials not covered by this application. Covance are acting as subcontractors for the safety determination studies on this agent. The agent was produced by Biovex under a previous successful notification to HSE.

The function of the gene product is as an anti-tumour therapy.

**Evaluation of foreseeable effects**
HSV is not usually transmitted through the aerosol route. Infection is by contact. Humans are the only natural host for HSV.

All operations will be performed under class 2 containment levels. It is unlikely that the vector will reach the environment, if it did it could not cause harm to humans or animals which it came into contact with. Access to the facility will be restricted and appropriate disinfection and waste management procedures will be instituted. These measures will reduce the likelihood of hazards to negligible.

It is not thought that the modified virus would pose a serious risk to the environment. HSV is a human virus that can be propagated by lytic infection. No integration of the viral genome with the cellular genome occurs during replication.
The virus is fragile, and is rapidly inactivated by disiccation, lipid solvents and mild detergents. The fact that contact is required for transmission attests to the instability of the virus outside the host.

It is unlikely that the Oncovex HGMSCF vector will be harmful to humans, animals, or plants. The vector is modified so that replication only occurs in rapidly dividing cells and not in non-dividing cells. Considerable literature shows that HSV 1 deleted for ICP34.5 is non-pathogenic in animals and humans. Approximately 80% of the human population are seropositive for HSV-1. Infection is by contact, and transmission by the aerosol route is not thought to occur. The therapeutic gene delivered, human GM-CSF is present in normal, healthy humans.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The study will involve the administration of the vector to 18 mice by I.V. injection and 18 mice by subcutaneous injection. Animals will be killed, organs removed and nucleic acid extracted. Nucleic acids will then be subjected to quantitative PCR analysis.

Doses will be prepared in a microbiological class II cabinet. Laboratory gowns, gloves, face masks will be worn, care will be taken not to generate aerosols.

In the animal room trained technicians will wear gowns latex/nitrile gloves and respirators. Care will be taken to prevent needlestick injuries and all waste will be transferred immediately to containers for autoclaving. Animals will be housed in an exclusive room with filtered air extract. After use cages will be disinfected and the room washed with hypochlorite solution.

Necropsy will be performed in ventilated workstations and staff will wear safety glasses, gowns and gloves and will be protected by glass screens. All workstations will be disinfected after use. Snapfrozen tissues will be received in the biotechnology department where small samples will undergo nucleic acid extraction in class II cabinets.

The vector will be inactivated by this step.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All animals cages will be disinfected using hypochlorite solution. All waste including food, bedding and carcasses will be double bagged and autoclaved within the facility prior to removal as clinical waste by White Rose Environmental. All surfaces will be disinfected with hypochlorite solution or "Virkon" used instruments will be autoclaved and disposed of in 'Sharps' boxes as clinical waste. Any re-usable equipment will be separately autoclaved. All laboratory coats will be autoclaved after use. This organism is an enveloped virus and hence is rapidly inactivated in air and highly susceptible to disinfectants and/or autoclaving. No viable organisms would survive de-contamination procedures.

Is an emergency plan required according to regulation 20?  

- [ ] If yes, tick to confirm that it is attached to this form

- [ ] Tick to confirm that you have attached a risk assessment to this form

- [ ] Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
The Genetic Modification Safety Committee has been consulted throughout the development of the protocol and representatives of the various groups involved (animal technicians, necropsy, dispensary and biotechnology departments) attended the meeting at which the completed risk assessment was presented. All members were happy that the risk assessment was adequate and accurate and that the intended control procedures would result in a negligible risk to operatives. A study startup meeting will be arranged prior to commencement to reinforce control procedures and adherence to them. This meeting will involve all study operatives.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<tr>
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**Project Ref 421/01.2**

- **Date Ackn’d**: 18/10/2001
- **CU2 Project Title**: RECOMBINANT OVINE ADENOVIRUS
- **Class**: Class 2
- **Culture Volume Class**: ≤ 1 litre
- **Non-GMM Consent Granted**: Not applicable
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

- **Purposes of the contained use**: To demonstrate freedom from adventitious agents, toxicology and biodistribution
- **Recipient or parental organism**

02/03/2022
The parent organism is Ovine Adenovirus

Host/vector system

OAdV 623 is a recombinant adenovirus which contains the full viral genome and a transgene containing a prostate specific promoter (derived from zgenes - the human prostate specific membrane antigen and the rat probasin gene) and the therapeutic gene, purine nucleoside phosphorylase (PNP).

Origin & function

The product of the therapeutic gene (PNP) will be expressed in human prostate cells and convert a non-toxic pro-drug fludarabine phosphate into the toxic agent 2-fluoradenine.

Evaluation of foreseeable effects

OAV has been shown to infect human cells, but is unable to complete its full replication cycle, therefore representing minimal risk to the worker. The therapeutic gene will be expressed at very low levels, if at all, in not target tissue. The gene product is non-pathogenic for humans. OAV 623 was derived from a virus that was recovered from a sheep in Western Australia. Due to lack of evidence OAV seroconversion in the UK, we have to assume that this virus is not endemic to the UK. Although the virus is not fatal in itself, secondary infections can be. Therefore, although the organism is considered Class 1, after reviewing potential environmental effects it is classified as Class 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All open manipulations will be performed in a Class II Safety Cabinet. All potentially contaminated material will be autoclaved before disposal. Any spillages will be disinfected with Virkon, and all materials to mop up spillages will be autoclaved. In vivo studies will be conducted in rodent animal houses. All bedding and carcasses will be autoclaved or collected in specifically identified bins for off-site incineration. Staff who have contact with sheep will be excluded from working with this organism.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All animal cages will be disinfected using hypochlorite solution. All waste, including bedding and carcasses, will be double bagged and either autoclaved or placed in identified bins which will be sealed and removed by White Rose Environmental for incineration. All surfaces will be disinfected with hypochlorite solution or Virkon. All other materials which come into contact with the organism will be autoclaved, including lab coats.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The risk assessment prepared for this study was reviewed and approved by the Genetic Modification Safety Committee. A study start-up meeting will be held prior to starting work with this organism.

Project Containment
### Project Ref 421/03.1

<table>
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<tbody>
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<td>Class 2</td>
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#### Project notified under transitional arrangements

#### CU2 Project Title

IN VITRO & AND IN VIVO TESTING OF GENETICALLY MODIFIED OVINE ADENOVIRUS (OADV) TO DEMONSTRATE FREEDOM FROM ADVENTITIOUS AGENTS, TOXICOLOGY & BIODISTRIBUTION

#### Purposes of the contained use

To demonstrate freedom from adventitious agents, toxicology and biodistribution.

#### Recipient or parental organism

The parental organism is an Ovine Adenovirus

#### Host/vector system

OAdV 220 is a recombinant adenovirus which contains the full viral genome and a transgene containing the RSV (Rous Sarcoma Virus) promoter, such that the PNP enzyme will be constitutively expressed.

#### Origin & function
The product of the therapeutic gene (PNP) will be expressed in human prostate cells and convert a non-toxic agent 2-fluoroadenine.

### Evaluation of foreseeable effects

OAV has been shown to infect human cells, but is unable to complete its full replication cycle, therefore representing minimal risk to the worker. The therapeutic gene will be expressed at very low levels, if at all, in not target tissue. The gene product is non-pathogenic for humans.

OAV220 was derived from a virus that was recovered from a sheep in Western Australia. Due to lack of evidence of OAV seroconversion in the UK, we have to assume that this virus is not endemic to the UK. Although this virus is not fatal in itself, secondary infections can be. Therefore, although the organism is considered Class 1, after reviewing potential environmental effects, it is classified as Class 2.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All open manipulations will be performed in a Class II safety cabinet. All potentially contaminated materials will be autoclaved before disposal. Any spillages will be disinfected with Virkon, and all materials used to mop up spillages will be autoclaved. In vivo studies will be conducted in Rodent Animal Houses. All bedding and carcasses will be autoclaved or collected in specifically identified bins for off-site incineration. Staff who have any contact with sheep will be excluded from working with this organism.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All animal cages will be disinfected using hypochlorite solution. All waste, including bedding and carcasses will be double bagged and either autoclaved or placed in identified bins which will be sealed and removed by White Rose Environmental for incineration. All surfaces will be disinfected with hypochlorite solutions or Virkon. All other materials which come into contact with the organism will be autoclaved, including lab coats.

---

**Is an emergency plan required according to regulation 20?**
- Yes: 
- No: 

**If yes, tick to confirm that it is attached to this form**

**Tick to confirm that you have attached a risk assessment to this form**
- Yes: 
- No: 

**Tick if you are claiming exemption from disclosure for section of the risk assessment**
- Yes: 
- No: 

### Please enter comments on the GM safety committee on the risk assessment

The risk assessment prepared for this study was reviewed and approved by the Genetic Modifications Safety Committee. A study start-up meeting will be held prior to starting work with this organism.

### Project Containment

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Project Ref 421/03.2

Date Ackn’d 18/07/2003

CU2 Project Title TESTING CELL BANKS OF MARINE VIBRIO SPP FOR IDENTITY, PURITY AND STABILITY.

Class Class 2

CultureVOL Class 2 < 1 litre

Consent Granted not applicable

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To determinate freedom from adventitious agents and identity and stability testing of the host organism and transgene product.

Recipient or parental organism
The parental organism is a marine Vibrio, which has not been fully characterised but has been classified as most similar to Vibrio aguillarum.

Host/vector system
The parental organism, similar to Vibrio aguillarum, contains a plasmid which expresses the non-toxic B subunit of E. coli endotoxin. The plasmid is mobilisation defective preventing its transfer to other organisms.

Origin & function
The product of the transgene is the non-toxic B subunit of E. coli which is immunogenic and hence will be used as the basis for a vaccine against E. coli endotoxin.

Evaluation of foreseeable effects
Although not fully characterised, the host organism is very closely related to a marine Vibrio which is poorly tolerant of the saline conditions and is non-pathogenic to humans. There is a risk of marine fish infection though the laboratory is over 60 miles from the sea. The gene product is non-toxic to humans or animals.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All open manipulations will be performed in a class II safety cabinet. All potentially contaminated material will be autoclaved before disposal. Any spillages will be disinfected with Virkon. Any in vivo studies will be conducted in Rodent Animal Houses conforming to class II handling requirements. All bedding and carcasses will be autoclaved or collected in specifically identified bins for off-site incineration.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All open manipulations will be performed in a Class II Microbiological Safety Cabinet. All potentially contaminated material from this work will be autoclaved before disposal, reducing the likelihood of an escape of the GMO. The use of mobilisation-defective or non-mobilisable vectors would mean that the active transfer of 'foreign' DNA is not expected. Should in vivo studies be required then:

All animal cages will be disinfected using hypochlorite solution. All waste, including bedding and carcases, will be double bagged and either autoclaved or placed in identified bins which will be sealed and removed by White Rose Environmental for incineration. All surfaces will be disinfected with hypochlorite solution or Virkon. All other materials which come into contact with the organism will be autoclaved, including lab coats. No viable organism could survive the sterilisation and disinfection procedures employed.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment prepared for this study was reviewed and approved by the Genetic Modification Safety Committee.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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Project Ref 421/03.3

02/03/2022
**GENETICALLY MODIFIED (ATTENUATED) DENGUE VIRUS VACCINES WILL BE ADMINISTERED TO MONKEYS SUBCUTANEOUSLY TO DEMONSTRATE SAFETY**

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**Project notified under transitional arrangements**

**Withdrawn**

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</table>

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

To demonstrate safety of the vaccines C2D 1 and C2D 2.

**Recipient or parental organism**

The parental organism is dengue (a flavivirus). Dengue is transmitted by Mosquito (ie requires injection) and not ingestion or inhalation.

**Host/vector system**

The candidate vaccines are live attenuated vaccines which retain antigenic activity but replicate poorly, are temperature sensitive and have reduced virulence in vitro and in vivo.

Dengue C2D 1 is a live attenuated GMO engineered using the backbone dengue 2 PDK53 (non structural protein and capsid) with structural proteins (premembrane and envelope) of dengue wild serotype 1 (live attenuated virus parental strain). This vaccine is developed on VERO cells.

Three mutations in nonstructural gene regions of the PDK-53 viral genome are responsible for the in vitro attenuated phenotypic markers and attenuated mouse neurovirulence phenotype of the DEN-2 PDK-53 virus.

The genetic loci encoding the attenuated phenotypic markers of the Mahidol candidate DEN-2 PDK-53 vaccine virus have been defined experimentally by the CDC. This strain showed good safety during clinical study with previous live attenuated vaccine studies.

Dengue C2D 4 is the same as above but has the structural proteins (premembrane and envelope) of dengue wild serotype 4 (live attenuated virus parental strain) in place of the same proteins serotype 1.

**Origin & function**

The intended function is to vaccinate against dengue fever.
Both vaccine GMO candidates are live-attenuated and some reactogenicity could be expected after a first injection related to viral replication. The parent DEN-2 PDK-53 vaccine virus replicates efficiently (MID50=5PFU) in humans and is safe and immunogenic in adult volunteers (Bharmarapravati et al., 1996). The chimeric GMO viruses CD2 1 and CD2 4 viruses which express the structural genes of heterologous flaviviruses (nonstructural replication machinery of the DEN-2 PDK-53 genotype) and premembrane and envelope of attenuated serotypes 1 or 4 should retain the attenuated phenotypic markers of DEN-2 PDK-53 virus (Huang et al 2000). These attenuated phenotypic markers of DEN-2 PDK-53 virus includes small plaque size and temperature sensitivity in LLC-MK2 calls and relative to the homologous wild-type DEN virus, poor replication efficiency in Aedes albopictus C6/36 cells, and avirulence for newborn mice following intracranial inoculation 104 PFU of virus (Huang et al., 2000).

It has been shown in previous clinical studies that Dengue attenuated virus vaccines (monovalent, bivalent, trivalent or tetravalent vaccine (Sabchereon et al., 2002) can induce within 2 weeks after vaccination dengue like symptoms with transient and mild fever, headache, myalgia, eye pain, rash associated with decrease of leukocytes and platelet counts and increase of transaminases levels.

If a harmful event were to occur, minor dengue-like symptoms are likely to be seen.

It is noted from the literature that exposure to one strain of Dengue virus and subsequent seroconversion can lead to the development of more serious (haemorrhagic) Dengue fever on subsequent exposure to a different serotype against which there would be no cross-protection. The major risk therefore is to laboratory personnel being injected accidentally and then being exposed to a mosquito bite infected with a different Dengue virus serotype. It is felt that this risk is remote and can be managed through proper handling procedures.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All open manipulations will be performed in a class II safety cabinet.
All potentially contaminated materials/clothing will autoclaved or soaked in hypochlorite/Virkon before disposal by incineration. Any spillages will be disinfected with Virkon and all materials used to mop up spillages will be autoclaved and incinerated. The study will be conducted in designated primate animal houses. All bedding and carcasses will be autoclaved or collected in specifically identified bins for off-site incineration.

Staff will wear protective clothing, including respirators, gloves, overalls, aprons, face shields which will be treated as above if contaminated and disposed of by incineration.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All animals cages will be disinfected using hypochlorite solution.
All waste, including bedding and carcasses will be double bagged and either autoclaved or placed in identified bins which will be sealed and removed by White Rose Environmental for incineration. All surfaces will be disinfected with hypochlorite solution or Virkon. All other materials which come into contact with the organism will be autoclaved including lab coats.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N
# Project Containment

<table>
<thead>
<tr>
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<th>Human Clinical Applications</th>
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## Project Ref 421/06.1

**Date Ackn’d** 25/04/2006

**CU2 Project Title** MVA-Measles.

**Class** Class 2

**CultureVolClass2** < 1 Litre

**Non-GMM** Not Applicable

**Consent Granted**

**Project notified under transitional arrangements** N

---

**Withdrawn** N

**Tick if notifying a connected programme of work** N

---

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

## Project Additional Information

### Purposes of the contained use

Repeat dose and local tolerance study in the rat to assess toxicity and immunogenicity of MVA-measles vaccine. Intended therapeutic use is the prevention of measles infection in man.

### Recipient or parental organism

MVA-measles is the test system and consists of MVA (Modified Vaccinia Ankara) as the vector. MVA is an attenuated vaccinia virus and is widely considered as the...
vaccinia of choice for clinical investigation because of its high safety profile.

### Host/vector system

**MVA (Modified Vaccinia Ankara)**

### Origin & function

The MVA-measles constructs contain the following measles antigens: F-antigen (responsible for fusion with the host cell), N-antigen (the nucleocapsid protein) and the H-antigen) hemagglutinin responsible for virus binding to suitable receptors on the host cell and essential cofactor for F-protein-mediated fusion.

For re-categorisation of the MVA-measles construct the client has performed "human cell growth" assays in which preliminary data suggests there are no growth anomalies.

### Evaluation of foreseeable effects

Accidental human exposure may lead to an inflammatory response or irritation to the eye. Potential for allergic reaction.

Affected eyes should be irrigated thoroughly, skin should be washed thoroughly with soap and water and rinsed with 80% ethanol. Medical attention should be sought.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

When handling test compound in bulk respiratory protection or working under a laminar air flow cabinet is required. The wearing of safety glasses is required as are disposable gloves when handling the test material. Environmental characteristics of the test material have not been fully evaluated. Release to the environment should be avoided. The test compound can be denatured or inactivated by a variety of organic solvents. None of the components of the test compound are listed as carcinogenic.

Release into the environment is unlikely and if so will be in small quantities. Spillages should be contained using absorbent material. Spill area should be cleaned thoroughly with water and afterwards 80% Ethanol.

The inability of MVA to replicate, as demonstrated in the in-vitro and in-vivo studies detailed above, indicates that the impact on the environment should there be an accidental release will be minimal, localised and of a minor nature. Existing control measures for the disposal of waste materials, carcasses and test compound should be adequate to ensure that there is minimal risk of exposure to the environment.

Test material should be stored in a refrigerated environment and should be maintained within sealed containers. These should be placed within and additional impervious containers. Such measures will prevent other test materials being cross-contaminated.

Test material will be supplied within sterile glass vials incorporating a rubber septum. There will not be any requirement to conduct any dilutions or formulations procedures. The test material will be supplied ready for use and will only be required to be withdrawn from the vial immediately prior to administration.

Storage measures should be appropriate to ensure the glass vials cannot be damaged. They should be placed within a second impervious, sealed container (polypropylene) to prevent and where necessary contain any accidental damage/spillage.

Administration of the test material will be conducted within a dedicated work area separate to other studies and test materials. Existing personal protection worn by operational staff within rodent rooms will be sufficient to provide adequate protection against accidental spillages.

Lab staff will wear appropriate safety equipment including, lab coats and gloves.
All potentially contaminated material will be inactivated by chemical disinfection or by autoclaving at 121 degrees C for a minimum of 15 minutes. The likelihood of release is therefore low.

In the event of spillage, the contaminated work area will be disinfected with a viracidal agent.

Where applicable, activities involving the virus and potentially infectious fluids will be performed using Biosafety Level 2 contamination facilities. Procedures where the risk of aerosolisation is high should be performed inside a class 2 biological safety cabinet.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The test compound can be denatured or inactivated by a variety of organic solvents. All materials exposed to the GMO will be autoclaved using validated means, leading to inactivation of the GMO and incineration. Animals treated with GMO will be disposed of by incineration, according to the conditions of the GMO agreement with the external contractor.

All stored GMO material will be held in waterproof secondary containers so that in the event of leakage of the primary storage vessels the GMO is contained and can be dealt with as described in Section 8.

Existing control measures for the disposal of waste materials, carcasses and test compound should be adequate to ensure that there is minimal risk of exposure to the environment.

Test material should be stored in a refrigerated environment and should be maintained within sealed containers. These should be placed within and additional impervious containers. Such measures will prevent other test materials being cross-contaminated.

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Test material will be supplied within sterile glass vials incorporating a rubber septum. There will not be any requirement to conduct any dilutions or formulations procedures. The test material will be supplied ready for use and will only be required to be withdrawn from the vial immediately prior to administration.

Storage measures should be appropriate to ensure the glass vials cannot be damaged. They should be placed within a second impervious, sealed container (polypropylene) to prevent and where necessary contain any accidental damage/spillage.

Administration of the test material will be conducted within a dedicated work area separate to other studies and test materials. Existing personal protection worn by operational staff within rodent rooms will be sufficient to provide adequate protection against accidental spillages.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
First risk assessment for this study was thought by members of the GMO committee not to be good enough. A re-assessment was carried out and the committee agreed this was an accurate assessment. It was agreed that facilities at the Harrogate site were suitable in respect of containment for this study to proceed after notification.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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**Project Ref** 421/06.2

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<td>Immuno VEX HSV-2 used against Herpes simplex virus in the rat.</td>
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**Non-GMM Consent Granted**

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<tr>
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<th>Not Applicable</th>
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</table>

**Project notified under transitional arrangements**

Tick if notifying a connected programme of work

Tick if you are claiming exemption from disclosure for section of the risk assessment

**Project Additional Information**

**Purposes of the contained use**

The project is a GLP preclinical toxicity and repro-toxicity study of an HZV-2 vaccine candidate, Immuno VEXHSV-2, by subcutaneous administration to Crl:CD(SD) rats.
Intended therapeutic use is the prevention of herpes simplex viral infections in man.

Recipient or parental organism

Immuno VEXHSV-2 is a replication impaired vaccine based on HSV-2 strain HG52. It has deletions in the genes that encode vhs, ICP47, UL43 and ICP34.5. Deletion in these genes render the virus avirulent in mouse models as demonstrated by intracranial, intranasal and subcutaneous administration of doses up to 10 (6) pfu in mice and guinea pigs. It would therefore be anticipated that Immuno VEXHSV-2 would have greatly reduced pathogenicity in humans compared to wild type HSV-2.

Host/vector system

The strain of HSV-2 used to produce Immuno VEXHSV-2 is HG52.

Origin & function

Immuno VEX HSV-2 contains deletions with the vhs, ICP47, UL43 and ICP34.5 genes. Deletions in either of the ICP34.5 or vhs genes have been shown to render the virus avirulent and greatly reduce levels of reactivation in animal models compared to wild type virus (Perng et al, 1995; 1996; Strelow and Leib, 1995, Strelow et al. 1996). Deletion of ICP34.5 from HSV has been shown to provide the greatest level of attenuation of any single gene deletion that still allows the virus to replicate efficiently in culture. Deletions in the ICP47 gene have also been shown to greatly reduce the virulence of HSV (Goldsmith et al., 1998). Deletions in the ICP47 gene have also been shown to greatly reduce the virulence of HSV (Goldsmith et al., 1998). Deletions in the ICP47, UL43 and ICP34.5 genes will not alter the host range of Immuno VEX HSV-2 compared to the wild type, parental virus. Dose of Immuno VEX HSV-2 up to 1x10 (6) pfu have been shown to be non-toxic when administered via the intranasal, intracranial and subcutaneous routes in both mice and guinea pigs. This is in contrast to the wild type HSV-2 strain HG52 which has an LD50 of 1x10 (2) pfu when administered intracranially (MacLean et al., 1991).

There are no exogenous genes inserted into ImmunoVEX HSV-2.

Evaluation of foreseeable effects

Accidental infection in the laboratory is by splash to a mucosal surface, or the eye, or by entry through broken skin. HSV can infect various parts of the eye including the cornea and retina.

Except in neonates and the immuno-compromised, wild type HSV infection is not systemic but is limited to epithelial cells at the infection site and to the sensory ganglia that innervate that site. The standard treatment for HSV-2 infection are the anti-viral agents acyclovir and valacyclovir. The thymidine kinase (TK) gene required for the activity of these drugs is intact and expressed from ImmunoVEX HSV-2. Thus ImmunoVEX HSV-2 is sensitive to the standard drugs used to treat HSV-2 infections, so can be used in the unlikely event of an accidental infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

When handling test compound in bulk respiratory protection or working under a laminar air flow cabinet is required.

The wearing of safety glasses is required as are disposable gloves and masks when handling the test material.

Any cuts should be covered with a suitable dressing.

Persons handling the virus must wash their hands with a suitable anti-viral soap before touching their skin or eyes.

Any spills must be immediately treated with a suitable disinfectant (such as 1% Virkon solution).

Syringes should be placed in a sharps bin and destroyed by incineration.

The work area should be cleaned with a suitable disinfectant on completion of the work.
None of the components of the test compound are listed as carcinogenic.

The vector is fragile and cannot survive for long periods outside the host. Infectivity is eliminated by a temperature of greater than 56°C maintained for ~20 hours, or exposure to pH of less than 4. It is also rapidly inactivated by desiccation, lipid solvents, mild detergents and bleaches and common disinfectants. The fact that direct contact is required for transmission attests to the instability of the virus outside the host.

Existing control measures for the disposal of waste materials, carcasses and test compound should be adequate to ensure that there is minimal risk of exposure to the environment.

Test material should be stored in a frozen (-80°C) environment and should be maintained within sealed containers. These should be placed within and additional impervious containers. Such measures will prevent other test materials being cross-contaminated.

Administration of the test material will be conducted within a dedicated work area separate to other studies and test materials.

Existing personal protection worn by operational staff within rodent rooms will be sufficient to provide adequate protection against accidental spillages.

Lab staff will wear appropriate safety equipment including, lab coats and gloves.

All potentially contaminated material will be inactivated by chemical disinfection. The likelihood of release is therefore low.

In the event of spillage, the contaminated work area will be disinfected with a viricidal agent.

Where applicable, activities involving the virus and potentially infectious fluids will be performed using Biosafety Level 2 contamination facilities. Procedures where the risk of aerosolisation is high should be performed inside a class 2 biological safety cabinet.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The test compound can be denatured or inactivated by chemical disinfection.

All materials exposed to the GMO will be disinfected using a suitable chemical disinfectant, leading to inactivation of the GMO and incinerated.

Animals treated with GMO will be disposed of by incineration, according to the conditions of the GMO agreement with the external contractor.

All stored GMO material will be held in waterproof secondary containers so that in the event of leakage of the primary storage vessels the GMO is contained and can be dealt with as described in section 8.

Existing control measures for the disposal of waste materials, carcasses and test compound should be adequate to ensure that there is minimal risk of exposure to the environment.

Test material should be stored in a frozen environment and should be maintained within sealed containers. These should be placed within and additional impervious containers. Such measures will prevent other test materials being cross-contaminated.
Administration of the test material will be conducted within a dedicated work area separate to other studies and test materials.

Existing personal protection worn by operational staff within rodent rooms will be sufficient to provide adequate protection against accidental spillages.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The attached assessment was considered by members of the Covance Harrogate GMO committee and it was agreed that the containment level for this study should be level 2 and that facilities at the Harrogate site were suitable in respect of containment for this study to proceed after notification.

Project Containment

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Animal Units

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Project Ref 421/06.3

Date Ackn'd 01/11/2006

CU2 Project Title

Characterisation of the RNA sequence of the haemagglutinin gene in the region coding for the cleavage site in the influenza vaccine strain NIBRG-14

Date Project Ceased

Class 2 CultureVolumeClass

< 1 Litre

Consent Granted

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

Historical Significant Changes

02/03/2022 Page 7313 of 15326
The influenza vaccine strain NIBRG-14 is an attenuated strain of H5N1 avian pandemic flu. The attenuation results in part from modification of the haemagglutinin which eliminates the pathogenicity in avian species. The present work is to monitor batches of vaccine to ensure the genetic modification is intact.

NIBRG-14 pandemic vaccine reassortant has been produced using 6 reassortant RNAs (Excluding the haemagglutinin (HA) and neuraminidase (NA) from the human strain A/PR/8/34 (PR8). PR8 has had over 100 passages in each of the mice, ferrets and embryonated chicken eggs. The result of such a passage history is complete attenuation of the virus and its inability to replicate in man.

PR8 grows to high titre in embryonated chicken eggs and since the late 1960’s it has been used to produced ‘high growth reassortants’ in combination with the prevailing influenza A vaccine strain. Such parental strains possess phenotypic markers of vaccine safety, such as temperature sensitivity, cold-adaptation and attenuation in ferrets or rodents and moreover have a demonstrated attenuated phenotype in man.

Due to the nature of influenza viruses, vectors are made by introducing different combinations of the 8 RNAs making up the influenza genome (reassortants). In NIBRG-14, 6 reassortant RNAs derive from PR8 and 2 are derived from H5N1 though the haemagglutinin is modified to remove avian pathogenicity. The products of the inserted genes is, at minimum, the modified HA (H5) and NA (N1) of the pandemic strain virus. The H5 HA has been derived from a highly pathogenic H5 strain by reverse genetics, the HA has been modified so that the multiple basic amino acids at the HA cleavage site, which are associated with high pathogenicity, have been reduced to a single basic amino acid. Any protein derived from the wild type strain, on its own is rather inherently infectious nor harmful.

The origins of the genetic materials are as described above. The intended functions are that the HA and NA genes code for proteins which are immunogenic but are contained in a virus which is non-pathogenic and non-transmissible hence act as a vaccine strain.

Published information indicates that a reassortant between PR8 and a wild-type human influenza virus is likely to be avirulent in man. Although such information is difficult to interpret because the genetic composition of the reassortants was not clear, it is known that the degree of attenuation increases as reassortants include more PR8 genes. The NIBRG-14 reassortant, contains six out of eight viral genes from PR8 and the NA and modified HA genes of the H5N1 virus. Furthermore, the H5 HA retains a preference for α2,3 linked residues (see below), so the ability of the NIBRG-14 to bind to and replicate in human cells should be minimal. It is therefore envisaged that NIBRG-14 reassortant would be attenuated for humans compared to the H5 wild type. Furthermore, it is clear that such reassortants are expected to be of low chicken and animal pathogenicity compared to the highly pathogenic parental wild strains, and this has been borne out by experience to date. Nevertheless, as the factors affecting pathogenicity (see below) are not fully understood, genetic manipulation to remove the polybasic sites theoretically could have unpredicted effects on both transmissibility and pathogenicity.

Although it is considered that, an H5N1/PR8 reassortant such as NIBRG-14 will be either attenuated or possibly non-infectious for man, an indirect hazard may exist through secondary reassortment with a human or animal influenza virus as influenza viruses are known to exchange genes by the process of reassortment. In order for secondary reassortants to be generated several events need to occur; firstly infection of the laboratory staff with the reassortant strain; secondly, for an infected worker to
have a mixed infection with a wild type influenza virus, and thirdly for a reassortment event to take place. In practice, manufacturers, and associated laboratories, have 30 years of experience with large scale production of vaccines based on PR8 reassortants and no reported cases of human illness although it should be noted that data are not available on whether human infections may have occurred. Staff working with this strain will be vaccinated with current seasonal influenza vaccines to reduce the chances of infection with wild-type virus.

It should also be considered that poultry and pig farmers are continually exposed to animal influenza viruses and there have been few documented cases of human infection in this population with a reassortant between an avian or porcine and a human influenza virus (es) in human cells is considered to be minimal.

The influenza HA is responsible for attachment of virus to the target cell and has specificity for sialic acid receptors on cell surface molecules. The HA present on human influenza A viruses preferentially bind to receptors containing α2,6 linked sialic acid residues, whereas avian influenza viruses preferentially bind to α2,3 linked sialic acid (Rogers and D'Souza, 1989). Human tracheal cells have mainly α2,6 linked residues (Nelson et al, 1993), so the acquisition of an avian HA by PR8 virus is expected to minimise potential binding to human respiratory epithelial cells. Although the α2,3 receptor specifically of avian viruses will reduce the efficacy of such binding, it may not completely prevent infection in man. Moreover, the presence of avian-like receptors has been demonstrated in human respiratory tract epithelium (Matrosovich et al, 2004). Beare and Webster (1991) found that extremely large quantities of avian viruses (between 10^6.8 and 10^9.2 egg infectious doses) were needed for replication in man and, since replication was poor, that it was not possible to induce person-to-person transmission.

The HA of influenza virus must be cleaved into HA1 and HA2 by host cell proteases for a productive infection. Pathogenicity of H5 influenza A viruses for chickens is largely determined by the nature of the amino acids at the HA cleavage site. H5 viruses with multiple basic amino acid sequences are highly pathogenic and their HA can be effectively cleaved by ubiquitous furin-like proteases, which are expressed in most organs of birds and humans. In contrast, the HA of H5 viruses of low pathogenicity for birds and certain laboratory animals contain a single basic residue at the cleavage site, a feature common to all other subtypes of influenza HA, and which can only be cleaved by trypsin-like proteases, which are restricted to certain cell types, e.g. epithelial cells lining the respiratory tract of man and the gut of birds. Thus, HA cleavability influences tissue specifically and is a major determinant of pathogenicity for H5 viruses in chickens and certain laboratory animals. Multiple basic amino acids at the cleavage site have not been observed for any other HA subtype.

The available evidence suggests that virulence of the 1997 and later H5N1 viruses for man is related to the presence of the HA multiple basic amino acids. It is therefore considered imperative to remove them and has been done in the NIBRG-14 strain. This procedure will also increase the safety of the reassortants for avian species as cleavage site modifications have resulted in reduced killing of avian embryos.

The PR8/H5N1 6:2 reassortants created by reverse genetics for H5N1 vaccine production such as NIBRG-14 do not contain the gene constellation considered necessary for pathogenicity in chickens, mice and ferrets and in contrast have internal genes that confer sensitivity to the innate immune response.

By virtue of PR8 attenuation, avian receptor specificity, loss of multiple basic amino acids at the HA cleavage site and the absence of other H5N1 genes associated with pathogenicity in humans (ie NS1 or PB2 genes), it is envisaged that an H5N1xPR8 2:6 reassortant (NIBRG-14), while possibly infectious for man and ferrets, will have only a theoretical probability of causing harm to human health. On the basis of these arguments, reassortants derived from H5 strains in which the multiple basic amino acid HA cleavage site has been removed, using PR8 as the recipient virus, likely would be similarly attenuated.

If staff are exposed to aerosols containing high titre reassortant virus, there is a possibility that sub-clinical infections could result. If this happened, it is unlikely that a reassortant virus would transmit to human contacts as it is likely that replication will be attenuated and virus shedding, if it occurs, may be below the titres considered to be needed for human infection.

However, although there is no precedent, as described above there is a theoretical possibility of secondary reassortant with normal influenza human viruses and that such reassortant viruses may be replication-competent in humans, whilst having avian-like coat proteins. Although very unlikely, the secondary reassortant could become adapted to human infection and transmission in which case the public health consequences would be serious. All staff will be vaccinated against seasonal flu to reduce still further the likelihood of this occurring.
Environmental consideration

Influenza A viruses are endemic throughout the world in some agricultural animals (swine and horses) and some populations of wild birds. Of the influenza A viruses, a number can cause highly pathogenic disease in domestic poultry including H5 strains. Infections have occurred in the big cats following consumption of dead chickens infected with H5N1 viruses.

In the case of an H5N1 reassortant, the virus will have avian receptor specificity, and thus birds would be the species theoretically most susceptible. The contribution of the six PR8 internal genes to replication and virulence in birds is unknown.

However, it has been demonstrated by the use of reverse genetics that acquisition of only one PR8 gene by an avian influenza virus can abolish virus replication in ducks. Experimental evidence has demonstrated that PR8 virus is attenuated in not only humans (see above) but also chickens. Furthermore, a reassortant between PR8 (internal protein genes) and the 1997 Hong Kong H5N1 virus (NA and HA with a single basic aminio acid) was barely able to replicate in chickens ans was not lethal. Similar studies have been performed with the 2003 Hong Kong H5N1 virus at the WHO Collaborating Centre, Memphis, USA, where the 6:2 PR8 reassortant did not replicate or cause disease signs in chickens. The removal of the multiple basic amino acids from the H5xPR8 reassortant in both studies probably played a major role in reducing the risk for chickens.

Although replication occurs in chicken embryos, for reasons that are unknown, the risk of environmental transmission via such replication in nature is remote.

Pigs are uniquely susceptible to infection by all strains of influenza A virus because they have both alpha 2,3 and alpha 2,6 receptors in abundance. Although pigs are not susceptible to infection with PR8, it is conceivable that pigs are susceptible to infection by an H5N1 reassortant, as viruses with avian receptor specificity are known to replicate in this species. It is also possible that these species would be susceptible to secondary reassortments between the H5N1 reassortant and a pig virus. There is infact evidence that triple reassortants between avian, pig and human influenza viruses can circulate in pigs in the USA.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste must be considered infectious and must be decontaminated daily.

Liquids are inactivated by autoclaving within the facility. The liquid is then disposed of down the sink using large amounts of water followed by disinfectant.

Solid wastes are inactivated by autoclaving. The biohazard autoclave bags should not be taped closed prior to autoclaving. After autoclaving, all waste leaving the facility must be double bagged before placing in the designated bins.

Daily Waste
Prior to commencement of work within the class II safety cabinet (in the class III facility), a medium sized pipette tip waste box shall be filled with approximately 2L of an appropriate disinfectant and carefully placed, ensuring airflow is not restricted, within the class II safety cabinet. All consumables and reagents should be disposed of into this box after use. At the end of the work for that day, the pipette tip box should be sealed, cleaned with 70% ethanol, placed into an autoclave bag and autoclaved.

Decontamination Spills
Spills with in the class II safety cabinet

In the event of a spill, all surfaces and items shall be disinfected and cleaned before being removed from the class II safety cabinet.

If the spill results in puddles:

The area will be flooded with an appropriate disinfectant, and left for 30 minutes

The area is then wiped clean with water followed by 70% ethanol.
All items in contact with the spill are autoclaved.

After a spill is decontaminated, the area shall be thoroughly cleaned and dried. Residual materials can support the growth and multiplication of microorganisms, and can jeopardise the product protection normally provided by the class II safety cabinet.

Spills in Incubators

An appropriate disinfectant is added to the tray of water at the bottom of the incubator.
The materials in the incubator are removed and disinfected by wiping them with appropriate disinfectant and placed in another incubator.
Contaminated materials are placed in a biohazard waste bag prior to autoclaving.
Incubator internal surfaces are wiped with an appropriate disinfectant, followed by water and then surfaces wiped with 70% ethanol.

Spills in centrifuges or in the laboratory

Work is stopped immediately. All persons must evacuate the room immediately removing all laboratory clothing in the airlock prior to exit. No entry into the laboratory is permitted. The room would then be decontaminated using a validated procedure involving a portable hydrogen peroxide generator which can be operated from outside the laboratory via ports in the laboratory wall.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

First risk assessment for this study was thought by members of the GMO committee not to be good enough.
A re-assessment was carried out and the committee agreed this was an accurate assessment. It was agreed that facilities at the Harrogate site were suitable in respect of containment for this study to proceed after notification.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
Project Ref 421/08.1

Date Ackn'd 12/03/2008

Project Title
The project is a GLP preclinical toxicity and repro-toxicity study of an HSV-2 vaccine candidate, ImmunoVEXHSV-2 by sibcutaneous administration to Balb/c mice

Class 2
CultureVol
Not Applicable

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Immuno VEXHSV-2 is a replication competent herpes simplex virus type-2 (HSV-2) based vector, ImmunoVEXHSV-2 contains deletions with the vhs, ICP47, UL43, US5 and ICP34.5 genes. Deletions in either of the ICP34.5 or vhs genes have been shown to render the virus avirulent and greatly reduce levels of reactivation in animal models compared to wild type virus.

Recipient or parental organism

The GMO will be injected subcutaneously into laboratory mice in laboratory conditions.

Host/vector system

N/A

Origin & function


Approximately 15% of the human population are seropositive for HSV-2 infection is by direct contact, and transmission by aerosols is not thought to be a natural infection route. HSV-2 infection in the immune-competent host is restricted to the epithelium and sensory nerves. HSV-2 rarely causes severe problems unless the virus enters the brain or the eye which may lead to encephalitis or retinopathy respectively.

Primary infection occurs most commonly in genital mucosal tissue. The major characteristic of HSV is its ability to establish a latent infection in the dorsal root ganglia of the sensory neurones innervating the primary infection site, from where it can reactive and cause secondary disease symptoms. In these latently infected neurones the virus remains as an episome for the duration of the host's life—it does not integrate into the host's genome.

**Evaluation of foreseeable effects**

Accidental infection in the laboratory is by splash to mucosal surface, or the eye, or by entry through broken skin. HSV can infect various parts of the eye including the cornea and retina. Personal protective equipment will be in place to minimise the risk. Except in neonates and the immuno-compromised, wild type HSV infection is not systematic but is limited to epithelial cells at the infection site and to the sensory ganglia that innervate that site. Very rarely (< 1 in 106 people/year) the virus enters the CNS and causes meningoencephalitis; or may disseminate and cause multiorgan disease. This type of wide spread viremia is limited to immune-compromised patients. The standard treatment for HSV-2 infection is acyclovir, either administered topically or orally.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A - materials are micro-organisms.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Existing control measures for the disposal of waste materials, carcasses and test compound should be adequate to ensure that there is minimal risk of exposure to the environment.

Waste that has been or is potentially contaminated with GMOs must be collected in appropriate containers (eg autoclave bags for autoclave waste, hermetically sealed units for GMO waste that can not be inactivated) separately from other waste. This applies to all forms of disposable waste eg personal protective equipment (PPE), laboratory equipment, animal cage tray liners, animal tissues/carcasses etc.

All materials exposed to GMO will be disposed of by incineration, according to the conditions of the GMO agreement with the external contractor.

Non-disposable items must be treated (by autoclave or effective disinfectants) in order to render any GMO contamination inactive.

Any spills must be immediately treated with a suitable disinfectant (such as 1% Virkon solution). Syringes should be placed in a sharps bin and destroyed by incineration. The work area should be cleaned with a suitable disinfectant on completion of work.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

Y

**Tick to confirm that you have attached a risk assessment to this form**

N

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
Committee members had not further comments to add following review of the risk assessment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tr>
<td>12/03/2008</td>
<td>Repeat dose study in the rat to assess toxicity and local tolerance to MVA_BN® anthrax vaccine. Intended therapeutic use is the prevention of anthrax infections in man.</td>
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<th>Class</th>
<th>CultureVolClass2</th>
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<tr>
<td>Significant Change ID</td>
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<tr>
<td>Date of Significant Change</td>
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Project Additional Information

Purposes of the contained use

MVA is an attenuated Vaccinia virus and is widely considered as the Vaccinia virus strain of choice for clinical investigation because of its high safety profile. MVA-BN® is claimed not to replicate in humans due to its inability to fully replicate in a vaccinated individual. In addition, it neither does result in the reversion of the attenuated property of the backbone vector MVA-BN® nor does it result in a higher virulence of MVA-BN®. Recombination is reduced by decreased homology with the viral gene sequence homology.
**Recipient or parental organism**
The GMO will be injected subcutaneously into laboratory rats in laboratory conditions.

**Host/vector system**
N/A

**Origin & function**
The parental organism is MVA-BN which is highly attenuated Vaccinia virus. This provides the backbone vector and is not classified as a GMO.

The MVA virus backbone is generated by 516 serial passages on chicken embryo fibroblasts of the Ankara strain of vaccinia virus (CVA) which results in the deletion of 31 kilobases of genomic sequence.

The test article to be used in this study (MVA-mBN157B) is a recombinant MVA-BN product which contains genetic information which encodes the protective antigen (PA) of bacillus anthracis. This additional antigen does solely have anthrax-specific translocation properties (which are not biologically active since it is the only anthrax-specific antigen encoded in the GMO).

**Evaluation of foreseeable effects**
Accidental human exposure may lead to a mild inflammatory response or irritation to the eye. Potential for allergic reaction.

No occupational exposure limit or short term exposure limit has been evaluated.

The inability of MVA to replicate indicates that the impact on the environment, should there be an accidental release, will be minimal, localised and of a minor nature.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
N/A materials are micro-organisms.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Existing control measures for the disposal of waste materials, carcasses and test compound should be adequate to ensure that there is minimal risk of exposure to the environment.

Waste that has been or is potentially contaminated with GMOs must be collected in appropriate containers (eg autoclave bags for autoclave waste, hermetically sealed units for GMO waste that can not be inactivated) separately from other waste. This applies to all forms of disposable waste eg personal protective equipment (PPE), laboratory equipment, animal cage tray liners, animal tissues/carcasses etc.

All materials exposed to GMO will be autoclaved using validated means, leading to inactivation of the GMO and incinerated.

Animals treated with GMO will be disposed of by incineration, according to the conditions of the GMO agreement with the external contractor.

Non-disposable items must be treated (by autoclave or effective disinfectants) in order to render any GMO contamination inactive.
The test compound can be denatured or inactivated by a variety of organic solvents. Spillages should be contained using absorbent material. Spill area should be cleaned thoroughly with water and afterwards with 80% ethanol.

In the opinion of the committee final classification of the organism should be designated as Class 2 GMO. The reason is that, as stated, the environmental impact has not been fully investigated. Details of discussions were as follows:

Based on the fact that MVA-BN® is non-replicating, has been demonstrated not to replicate in primate, murine or human cell lines, and is so far found to be safe in humans. MVA-BN® can be classified as a Class 1 organism. MVA-mBN157B can also be considered as a Class 1 organism since the only additional antigen it is encoding for is the protective antigen (PA) of bacillus anthracis. This additional antigen does solely have anthrax-specific translocation properties (which are not biologically active since it is the only anthrax-specific antigen encoded in the GMO), does not result in the reversion of the attenuated property of the backbone vector MVA-BN® and does not result in a higher virulence of MVA-BN®. Thus, MVA-mBN157B can be classified as a Class 1 organism. However, as the environmental impact has not been fully investigated, the overall classification of the organism should be class 2.

**Project Containment**

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**Project Ref** 421/08.3

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<tr>
<td>09/09/2008</td>
<td>Repeat dose subcutaneous administration toxicity study in the mouse</td>
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**Date Project Ceased**

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Historical Significant Changes

Historical Date of Additional Info
name of GMO changed to T-VEC
24/07/2012

Project Additional Information

Purposes of the contained use
The objective of the study is to determine the toxicity of the test article, OncoVEXGM-CSF, following 5 repeated subcutaneous administrations (on days 1, 4, 7, 10 and 13) to the mouse followed by a 24 hour, or 28 day observation period. Analysis of various tissues and bodily fluids will also be conducted.

Recipient or parental organism
CDI mouse for use in laboratory work to evaluate toxicity

Host/vector system
OncoVEXGM-CSF is replication competent herpes simplex type-I virus (HSV-1) used for treatment of solid tumours

Origin & function
OncoVEXGM-CSF was generated from the wild type is JS-1 strain of HSV-1 (ECACC Accession Number 01010209). Strain JS-1 is a recently prepared clinical isolate from a reactivating individual. The genes encoding 1CP34.5 and ICP47 have been deleted from Onc0VEXGM-CSF. 1CP34.5 is known as the neurovirulence factor. The deletion of ICP34.5 allows the vector to replicate in dividing cells but prevents replication in non-dividing cells. Deletions in the ICP34.5 gene have been shown to render the virus avirulent and greatly reduce levels of reactivation in animal models compared to wild type virus (Perng et al, 1995; 1996). In fact, deletion of 1CP34.5 from HSV has been shown to provide the greatest level of attenuation of any single gene deletion that still allows the virus to replicate efficiently in culture. The vector is also deleted for ICP47. The role of ICP47 is to block antigen presentation to MHC class I and II molecules by blocking transporter associated with antigen processing (TAP1 and TAP2). Deletions in the ICP47 gene have also been shown to greatly reduce the virulence of HSV (Goldsmith et al., 1998).

Onco0VEXGM.CSF contains the coding sequence for human GM-CSF (hGM-CSF), a cytokine involved in the stimulation of T-cells. hGM-CSF expression is under the control of the human cytomegalovirus immediately early promoter (HCMV IE) and this expression cassette has been inserted into the 1CP34.5 locus of the JS-1 genome. Both copies of the 1CP34.5 gene have been removed as the majority of the virus sequence has been confirmed by Southern blot analysis.

Evaluation of foreseeable effects
It is not thought that the modified virus would pose a serious risk to the environment. HSV is a human virus that can be propagated by lytic infection. No integration of the viral genome with cellular genome occurs during replication. The virus is fragile and is rapidly inactivated by desiccation, lipid solvents and mild detergents. The fact that contact is required for transmission attests to the instability of the virus outside the host.

It is unlikely that OncoVEXGM-CSF will be harmful to humans, animals or plants. The vector is modified so that replication only occurs in rapidly dividing cells and not in non-dividing cells. Approximately 80% of the population are
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Existing control measures for the disposal of waste materials, carcasses and test compound should be adequate to ensure that there is minimal risk of exposure to the environment. Animal carcasses, animal bedding, laboratory blood samples and laboratory urine samples are bagged and placed in hermetically sealed units which are then sprayed with virkon. The units are then placed in labelled 'Taylor Bins' and removed offsite for incineration. Collections are available 4 days per week with a fleet of approximately 60 appropriately labelled specific bins, each containing up to 75kg material. The contents of bins are tipped into the incinerator using an automatic lifting system with no manual handling involved. Incineration is undertaken by SRCL (formally White Rose Environmental), Knostrop Leeds, registered with the HSE as a premises handling GMM’s. GMO consignment notes are available for all GMO waste movements. An application for derogation from the use of an autoclave on site is hereby made.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All comments from genetic modification risk assessment incorporated into current version of risk assessment

Project Containment

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**Project Ref**  421/09.1

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**Non-GMM Consent Granted**  Not Applicable

**Project notified under transitional arrangements**  N

**Withdrawn**  N

**Tick if notifying a connected programme of work**  N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**
The aim of this project is to isolate viral DNA from a human adenovirus serotype 5 (Ad-5)-based conditionally replication competent adenovirus (CRAd) named As(1/PPT-E1A) and perform a PCR on the DNA to evaluate whether unwanted recombinants have occurred during the production steps.

**Recipient or parental organism**

N/A - test material will not be dosed to organisms/cell lines

**Host/vector system**

Modified adenovirus serotype 5 used for treatment of prostate cancer.

**Origin & function**
The prostate-specificity of I/PPT was first evaluated in an adenoviral vector with the luciferase reporter gene, Ad (I/PPT-Luc). An extensive screening of 17 cell lines was performed and the results showed that Ad (I/PPT - E1A) confers absolute prostate specificity (Cheng et al., 2004).

The I/PPT comprises the following:

1) an H19 insulator (I) shielding the PPT sequence from the LITR/E1A enhancer sequence, preventing the LITR/E1A enhancer sequence overlapping with the virus encapsidation sequence needed for virus assembly.

2) the PPT sequence, a chimeric sequence comprising the prostate specific antigen enhancer (PSAe) followed by the prostate-specific membrane antigen enhancer (PSMAe) and the T cell receptor y-chain alternate reading frame protein promoter (TARPp).

This yields gene expression which is restricted to cells of prostate origin and is highly active in prostate cancer cells both in the presence and absence of testosterone.
Evaluation of foreseeable effects
As the GMO is specific to replication in human prostate cells, including prostate cancer cells the risk to human health is considered low. Human adenoviruses do not replicate in animal cells and therefore, this also means that the risk to animal/plant health and environment should also be considered to be low. The precautions to be followed when manipulating this GMO, which are detailed in the selections below, should also minimise the risks to the environment and to human/animal health.

Ad5 infects the upper respiratory tract. Causing a mild non-chronic disease (common cold symptoms). Individuals with a functional immune system clear an adenovirus infection within a few days. Furthermore Ad5 is considered endemic in the Western World, and infection is usually acquired during childhood, which means that most individuals have neutralizing antibodies against the virus and may therefore not even notice if they get infected (the immune system very quickly clears the infection).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All potentially contaminated material will be inactivated by chemical disinfection, 1% Virkon for 15 minutes or autoclaving at 121°C for a minimum of 15 minutes. The likelihood of release is therefore low.
In the event of spillage the area will be covered by an impregnated chemical disinfectant (as above) cloth for at least 15 minutes, the cloth will then be autoclaved 121°C for a minimum of 15 minutes.
Where it is not possible to autoclave or chemically deactivate contaminated materials, they will be placed into hermetically sealed containers, marked with biohazard tape and disposed of by incineration through SRCL. All stored vessels the GMO is contained and can be dealt with as described above.
An application for derogation from the use of an autoclave on site is hereby made.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Risk assessment reviewed by GMO Committee and confirmed as acceptable.

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02/03/2022
The genetically modified virus will be the test material in biosafety studies. It will be in the form of supernatant containing infectious virus particles. These studies will look for the presence of contaminating viruses or reverse transcriptase activity indicative of retroviral contamination.

Recipient or parental organism

Influenza virus A/Puerto Rico/8/34 (Pr8) (recipient virus)

Wild type Influenza virus A/Indonesia/5/2005 have been inserted onto plasmids and mixed with the genes from PR8

Host/vector system

N/A

Origin & function

Influenza virus A/Purto Rico/8/34 (PR8) recipient virus is completely attenuated following hundreds of passages in eggs, mice and ferrets. It is therefore unable to replicate in man

Wild type Influenza virus A/Indonesia /5/2005 (an avian H5N1 influenza strain and the donor virus) is the virus of interest but is considered highly pathogenic in man.
Due to the nature of influenza and the fact that they have different genes on different segments of their genome, reassortants of the virus can occur naturally if two strains infect one host.

Using reverse genetics, the HA (H5 and NA (N1) genes from A/Indonesia/5/2005 have been inserted onto plasmids and mixed with the genes from PR8. It is the HA and NA proteins that are recognised by the immune system.

The H5 gene in the modified virus has a deletion and mutation in the multiple basic amino acids at the cleavage site, which results in a loss of pathogenicity associated with H5. The resulting virus reassortant should elicit an immune response that will protect against wild type A/Indonesia/5/2005 but will not be pathogenic in man.

Evaluation of foreseeable effects

There is considered to be a low probability that the reassortant virus will reassort again with a third influenza virus and produce a pathogenic strain that could replicate in man. There is considered to be a low chance of a worker becoming infected with the reassortant strain as the virus does not readily infect man and all staff working with this virus will be vaccinated with the seasonal influenza vaccine, reducing the chance of a co-infection further still.

This virus grows well in vitro and staff will be handling high titre virus. However, this virus does not readily infect man. There may be a possibility of a sub-clinical infection with low virus shedding but replication is attenuated in humans and it is not believed that the virus could replicate to high levels. The severity of the infection would be greatly reduced compared to the wild-type virus.

Influenza viruses can also infect birds and pigs. This reassortant virus has a high replication rate in chicken embryos but with minimal pathogenicity. However, data from other PR8 and H5N1 reassortants show that those viruses were barely able to replicate in chickens and were not lethal. The acquisition of one gene from PR8 was enough to abrogate virus replication in ducks.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In the event of spillage the area will be covered by an impregnated chemical disinfectant (1% w/v Virkon) cloth for at least 20 minutes, the cloth will then be autoclaved 121º for a minimum of 15 minutes. If fumigation is required, the appropriate SOP will be followed.

All potentially contaminated material will be inactivated by chemical disinfection (1% w/v Virkon for 20 minutes) and/or autoclaving at 121º for a minimum of 15 minutes in the autoclave located in the BDL3 laboratory. The likelihood of release is therefore low. Disposal methods will follow documented laboratory procedures.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The original risk assessment assigned in the final Activity Class as 3. The committee recommended this was amended to a 2+. Following discussions with HSE Biological Agents Unit Covance was advised to submit as Activity Class 2. This was on the basis that the virus and system is well characterised, widely used and proven to be relatively safe.

The location of the unitacentrifuge was also discussed but after consultation with the HSE it was agreed the location was acceptable.

It was discussed if staff could be vaccinated against H1N1 but to date vaccinations are not available.

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**Project Ref** 421/16.1

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Non-GMM Consent Granted

Tick if notifying a connected programme of work N

**Project Additional Information**
### Purposes of the contained use

To determine the toxicity, biodistribution and shedding profile of the test RP1, following subcutaneous administrations in Crl:SD rats.

### Recipient or parental organism

Crl:SD rats

### Host/vector system

RP1

### Origin & function

RP1 is a disabled recombinant herpes simplex type virus (HSV-1) containing the therapeutic genes.

#### Evaluation of foreseeable effects

**Hazards associates with the host/recipient/vector:**

Humans are the only natural host for HSV-1. Other species can be infected experimentally, but these appear to be "dead end" hosts- there are no natural non-human reservoirs for the virus. The deletion of ICP34.5 and ICP47 will not alter the host range of RS1 compared to the wild type, parental virus.

RP1 is attenuated and has a limited if any replicative capacity in normal cells in vivo. The virus is enveloped and hence is fragile and readily inactivated outside the host. Infectivity is eliminated by a temperature of greater than 56°C maintained for ~20 hours, or exposure to pH of less than 4. It is also rapidly inactivated by desiccation, lipid solvents, mild detergents and bleaches and common disinfectants. The fact that direct contact is required for transmission attests to the instability of the virus outside the host.

The doses will be prepared in a class 2 microbiological safety Hazards arising directly from the inserted gene: There are no known hazards directly associated with these genes.

RP1 contains the coding sequence for encoding ***** (*****), a cytokine involved in the stimulation of T-cells. ***** expression is under the control of the human cytomegalovirus immediately early promoter (HCMV IE) and this expression cassette has been inserted into the ICP34.5

RP1 also expresses a truncated ***** (***** envelope glycoprotein, incorporated as a fusion protein.

Hazards arising from the alteration of existing pathogenic traits:

RP1 is a disabled, non-pathogenic version of HSV-1, modified so that replication occurs selectively in tumour cells. Considerable literature shows that HSV-1 deleted for the neurovirulence factor is non-pathogenic in...
humans and animals. Because the virus is attenuated in normal cell, no adverse effects would be expected with exposure to individuals who may come into contact with it. Additionally, wild type HSV-1 is a universally prevalent virus that rarely causes severe problems. The potential for recombination with wild type HSV-1 is low, as this could only occur if both strains were present and replicating in the same cell. If this were to happen, any resulting virus would itself be non-pathogenic due the deletion of the neurovirulence factor ICP34.5. Consideration relating to whether an inserted sequence, that does not give rise to a harmful phenotype in the recipient, could give rise to harm as a result of natural gene transfer to other, possibly related, organisms: RP1 is attenuated and has a limited if any replicative capacity in normal cells in vivo. The virus is enveloped and hence is fragile and readily inactivated by desiccation, lipid solvents and mid detergents. Direct contact is required for transmission. There is no indication that transfection between viruses could occur.

Estimation of the severity of the harmful event were it to occur: Accidental parenteral inoculation, droplet exposure of mucous membranes or contamination of broken skin.

Wild type HSV-1: 7-10 days.

RP1 is attenuated and has a limited if any replicative capacity in normal cells in vivo. The virus is enveloped and hence is fragile and readily inactivated. RP1 is expected to be sensitive to the anti-viral agents such as the tyrosine kinase inhibitors (TKI) Acyclovir, valacyclovir, famciclovir and cidofovir. RP1 is also known to be susceptible to common disinfectants – 1% sodium hypochlorite, iodine solutions containing ethanol, 70% ethanol, glutaraldehyde and formaldehyde. In the event of accidental exposure through a splash to the eyes or mucous membranes, flush with copious amounts of clean water for at least 15 minutes. In the event of exposure to broken skin or a needle stick, thoroughly clean the site with soap and water or use a skin disinfectant. See a physician for monitoring for signs of infection. Acyclovir or other TKI anti-viral drugs may be administered prophylactically.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Disposal of affected sharps:** Used needles and syringes should be disposed of in the yellow sharps bins and placed with other GMO waste in dedicated wheeled bins for incineration.
Inactivation of the GMO:
All potentially contaminated material will be inactivated by chemical disinfection (1% Virkon for 30 minutes) and/ or autoclaving at 121ºC for a minimum of 15 minutes. The likelihood of release is therefore low. Disposal of GMO waste in dedicated wheeled bins for incineration. Where it is not possible to autoclave or chemically deactivate contaminated materials, they will be placed into hermetically sealed containers, marked with biohazard tape and disposed of by incineration through a licensed contractor.

Please enter comments on the GM safety committee on the risk assessment

1. In section ‘Hazards arising directly from the inserted gene’ This paragraph states what the inserted Genes are, but does not detail what/if any hazards are known.
Amended
2. I've just added a couple of comments to the assessment: Named RPM1 in header and is it possible to get these contact times consistent for Virkon in the assessment; we typically use 20 minutes for viral inactivation in virology, I think it would save confusion.
Amended to 30 minutes

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Project Containment

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Project Ref 421/19.1

Date Ackn’d 17/07/2019
CU2 Project Title The test article is a modified Newcastle Disease virus, strain NDV 73T modified with

Class 2
CultureVolClass2 < 1 Litre
CultureVolumeClass3-4

02/03/2022 Page 7332 of 15326
human granulocyte-macrophage colony stimulating factor (GM-CSF). It is being used in clinical studies to infect tumour cells and subsequently cause reductions in the size of solid tumours.

**Project Additional Information**

**Purposes of the contained use**

A QPCR assay will be developed and validated using the recombinant NDV MEDI5395 as a positive control. Subsequently, human patient samples containing this GMO will be assessed for the quantity of the GMO using molecular biology techniques. No in-vivo activities to be undertaken.

**Recipient or parental organism**

Modified Newcastle Disease virus of strain NDV 73T.

**Host/vector system**

NDV 73T is derived from NDV MK-1 07, a commercial poultry vaccine that was first marketed in 1948. MK-1 07 virus was further studied by Cassel and Garrett in the 1960s as an oncolytic agent, and subsequently passaged 73 times in Ehrlich ascites tumour cells to generate an adapted oncolytic virus called NDV 73-T. NDV 73T was modified using reverse genetics to produce recombinant NDV 73T (r73T) virus and additional r73T derivatives were also generated that have sequence modifications that attenuate the virulence. Attenuation of virulence has been demonstrated by intra-cerebral pathogenicity index tests in chicken host. Only derivatives with an ICPI <0.4 were selected.

**Origin & function**

These rNDV viruses are produced by standard reverse genetics methodologies i.e. transfecting modified viral genomic cDNA into a tissue culture cell and resulting recombinant virus is then amplified in specific pathogen free embryonated chicken eggs or human cell lines. The major virulence factor is the F protein cleavage site which is modified by removing the native protein cleavage site. In addition, therapeutic transgene(s) have been inserted into the viral genome to further attenuate the virus whilst providing oncolytic activity. Recombinant NDV 73T viruses are recovered by reverse genetics with various modifications in:

1. F protein cleavage site, the native site has been modified to ensure the virus has ICPI < 0.4
2. Transgene(s) are inserted into the viral genome for enhancing immuno-modulation and virulence attenuation.
(3) F and HN chimera are constructed that contains the ectodomain, transmembrane domain of other viruses and cytoplasmic domain of 73T. The other viruses include PIV5 (dog is a natural host) and PPMV-1 (ICPI < 0.025, pigeon is a natural host).

(4) Other mutations may be introduced into F, HN and V genes to ensure genetic stability and virulence attenuation in chickens.

Evaluation of foreseeable effects

Hazard identification in respect of human health and environmental safety

Hazard associates with the host/recipient/vector:
Vaccine strains of NDV with pathogenicity index <0.4 are considered to be non-pathogenic to poultry and humans however are categorised as ACDP hazard group 2. The modifications made to this virus do not increase the likelihood of pathogenicity.

Hazard arising directly from the inserted gene:
None - the genetic changes to the viral RNA sequence or insertion of therapeutic transgenes is for the purpose of attenuating virulence levels. rNDV with demonstrated low virulence levels is less hazardous than wild type NDV. NDV that is characterised and has an ICPI value of less than 0.4 is categorised as ACDP hazard group 2.

Hazard arising from the alteration of existing pathogenic traits:
None - modifications make the virus less virulent however NDV that is characterised and has an ICPI value of less than 0.4 is categorised as ACDP hazard group 2.

Consideration relating to whether an inserted sequence, that does not give rise to a harmful phenotype in the recipient, could give rise to harm as a result of natural gene transfer to other, possibly related, organisms:
Genes would be unable to recombine with other hosts.

Estimation of the severity of the harmful event were it to occur:
Minimal. Newcastle disease virus (NDV) is a paramyxovirus that is pathogenic in birds but causes only mild symptoms in humans and is therefore considered to pose minimal risk to human health. Laboratory exposure to NDV purified virus or by extensive contact with infected birds (such as eviscerating infected poultry in processing plants) can cause mild transient conjunctivitis and influenza-like symptoms in humans, and outbreaks of this population have been limited primarily to laboratory workers and vaccination teams exposed to large quantities of virus. No human-to-human transmission has been observed. The disease has not been reported in people who rear poultry or consume poultry products.

Health and Safety Executive

Newcastle disease virus (NOV) otherwise poses no hazard to human health. Interest in the use of NOV as an anticancer agent has arisen from the ability of NOV to selectively kill human tumour cells with limited toxicity to normal cells. No treatment for NOV exists, but the use of prophylactic vaccines and sanitary measures reduces the likelihood of outbreaks.

NOV is only considered a select agent! specified animal pathogen, and is thought to pose an environmental risk, if it has a virulence level (ICPI- Intra-cerebral pathogenicity index) of >0.4. Below this level NOV is not considered a specified animal pathogen. The virus in this assessment is thought to have similar risks to the current vaccines used for NOV. NOV that is characterised and has an ICPI value of less than 0.4 is categorised as ACOP hazard group 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Inactivation of the GMO:

All potentially contaminated material will be inactivated by chemical disinfection (1% Virkon for 20 minutes) and/or validated autoclaving at 121°C for a minimum of 15 minutes. The likelihood of release is therefore low. Disposal methods will follow documented laboratory procedures.

Where it is not possible to autoclave or chemically deactivate contaminated materials, they will be placed into hermetically sealed containers, marked with biohazard tape and disposed of by incineration through licensed contractor, Stericycle (SRCL) Leeds.

Is an emergency plan required according to regulation 20?  

N  

If yes, tick to confirm that it is attached to this form  

N  

Tick to confirm that you have attached a risk assessment to this form  

Y  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Y  

Please enter comments on the GM safety committee on the risk assessment  

N/A
Committee Meeting 16 May 2019

If you think this GMO is associated to a risk, you need to update accordingly the section Hazard as the vector is non-pathogenic, and no hazard associated to the inserted gene or the modifications done therefore is this a class 2?

Add extraction to study procedure/aims to make it clear that only extracted DNNRNA will be used for QPCR, so the higher risk is before/during extraction

Confirmed that the material did not require a SAPO as it has an ICPI less than 0.4
(consultant)
Confirmed this as a Class 2
Confirmed that it did not require a SAPO license
Confirmed that that it was not subject to the requirements of the ATCSA; referance made to the foot note to schedule 5:
Excludes non-viable microorganisms - e.g., in fixed tissues
Excludes medicinal products - e.g., vaccines, immunotoxins, future gene therapy agents?
Excludes clinical specimen for diagnostic purposes
Must be disposed of as soon as reasonably practicable following diagnosis
Covers GM forms of listed micro-organisms if still capable of causing serious harm to humans or animals - require same or higher containment level
Covers micro-organisms that have been modified by any means - passage, mutagenesis, protoplast fusion
Excludes established vaccine strains even if not part of a medicinal product
Recommended additions: Validation of the Autoclave and that it should stat whether sharps are to be used or not in the activity
All containment arrangements discussed and agreed.

Project Containment

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**Project Ref** 421/21.1

<table>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>16/12/2021</td>
<td>The GMO is a live virus vaccine of Herpes Simplex Type 2 (HSV-2) virus that has</td>
<td>Class 2</td>
<td>Not Applicable</td>
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The parent virus is Herpes Simplex Type 2. It has been modified to remove the ICP0 gene, which encodes infected cell protein 0. This is a critical regulatory protein involved in controlling the switch between latency and replication. 205 amino acids have been deleted from the ICP0 gene, including the nuclear localisation signal (NLS) and the oligomerisation domain, which are essential for the ICP0 to serve as a potent co-activator of viral mRNA synthesis. The removal of the ICP0 gene makes the virus sensitive to interferon, which would be present in humans. In addition, this deleted region has been replaced with a Green Fluorescent Protein (GFP) gene to facilitate the visualisation and tracking of the virus.

### Purposes of the contained use

The following methods will be used for batch release and stability testing:

- Plaque Assay (Class 2 Activity)
- Interferon (IFN) sensitivity assay (Class 2 Activity)

### Class 1 Activities:

- Host cell protein ELISA
- Host cell DNA QPCR
- DNA Size distribution
- Picogreen assay
- DNA extraction
- ICP0 deletion assay
- GFP insertion assay
- BCA total Protein assay
- Residual BSA ELISA
- Residual Benzonase ELISA
- Residual Doxycycline ELISA

# Project Additional Information

## Recipient or parental organism

The parent virus is Herpes Simplex Type 2. It has been modified to remove the ICP0 gene and add a GFP gene. Pre-formulated vector supplied by the client. The methods will then be used for batch release and stability testing of the HSV-2 vaccine (RVx201).
### Host/vector system

N/A

### Origin & function

The GMO is a live virus vaccine of Herpes Simplex Type 2 (HSV-2). The parent virus is Herpes Simplex Type 2. It has been modified to remove the ICP0 gene, which encodes infected cell protein 0. This is a critical regulatory protein involved in controlling the switch between latency and replication. 205 amino acids have been deleted from the ICP0 gene, including the nuclear localisation signal (NLS) and the oligomerisation domain, which are essential for the ICP0 to serve as a potent co-activator of viral mRNA synthesis. The removal of the ICP0 gene makes the virus sensitive to interferon, which would be present in humans. In addition, this deleted region has been replaced with a Green Fluorescent Protein (GFP) gene to facilitate the visualisation and tracking of the virus.

### Evaluation of foreseeable effects

#### Hazards associates with the host/recipient/vector:

Wild type HSV-2 causes genital herpes. The deletion of the ICP0 gene in this GMO severely affects its ability to replicate. It is still capable of replication, but in order to achieve full replication competency it requires propagation in a complementary cell line that contains ICP0.

#### Hazards arising directly from the inserted gene:

The inserted gene encodes for Green Fluorescent Protein. This is a well characterised gene, which allows the virus to fluoresce, and is not considered to be hazardous.

#### Hazards arising from the alteration of existing pathogenic traits:

The deletion of the ICP0 gene reduces the virus’s ability to replicate and therefore reduces its pathogenicity. The addition of the GFP gene has no impact on pathogenicity.

#### Consideration relating to whether an inserted sequence, that does not give rise to a harmful phenotype in the recipient, could give rise to harm as a result of natural gene transfer to other, possibly related, organisms:

It is highly unlikely that this GMO will come into contact with wild type HSV-2 during the course of this work. The presence of wild type HSV-2 is required for gene transfer as the GMO contains a deletion and reinstatement of the gene is only possible with wild type virus. Recombination with the cell culture that grows the virus is unlikely to occur due to the absence of overlapping sequences. Transfer of the GFP gene is not considered likely due to the lack of overlapping sequences, and GFP is not considered to be a hazardous gene.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Wild type HSV-2 is classified as Hazard Group 2, requiring Containment Level 2 in the UK. The modifications to this virus, described here, reduce its ability to replicate and therefore its pathogenicity. This assessment covers different activities that require different levels of containment. Where the GMO is grown in the presence of the complimentary cell line, Containment level 2 is required (Plaque assay and IFN-sensitivity assay). Where no cells are present (and hence no ICP0 gene) Containment level 1 is sufficient with the disinfection procedures detailed below.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The GMO will be inactivated by soaking in 1% (w/v) of Virkon for 20 minutes. All waste materials that have come in contact with the GMO will be autoclaved at 121°C for 15 minutes.
Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Human Clinical Applications

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Project Ref 421/21.2

The replication attenuated TheraT vector is based on Pichinde virus strain passage 18 (PICV p18; GenBank Access)

Class CultureVolClass2 CultureVolumeClass3-4

- Class 2
- Not Applicable

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

RNA from the TheraT (PIC) E6E7 will be extracted and used as a positive control in the development and validation of an RT-qPCR assay.

**Recipient or parental organism**

The recipient Pichinde virus (PICV) virus belongs to the family of Arenaviridae which are enveloped ambisense RNA viruses containing two RNA genome segments ("bi-segmented"): segment L, which encodes an RNA dependent RNA polymerase and a zinc finger binding "Z" protein (GenBank EF529747.1) and segment S, which encodes a nucleoprotein (NP; GenBank EF529746.1) and a glycoprotein (GP).

**Host/vector system**

Hazards associated with the host/recipient/vector:

The natural host of parental virus PICV are rice rats (Oryzomys albicularis) from which PICV was isolated in Colombia. Since PICV is geographically restricted by its natural host, there is little to no preexisting immunity against PICV in the general human population. In general, PICV is not known to cause diseases in humans (Arenaviruses: Biology and Immunology; MBA Oldstone 2013 Springer Publishing; Center of Disease Control and Prevention publication New World Arenaviruses).

TheraT vectors developed by HOOKIPA Biotech GmbH are based on PICV passage 18 (PICV p18; GenBank Access. Nr. EF529747.1 and EF529746.1). PICV p18 was established by passaging PICV 18 times in spleen of inbred guinea pigs. Infection of inbred guinea pigs with PICV p18 is an established model which allows to recapitulate clinical and pathological features of infection with another arenavirus. Animals infected with the guinea pig-adapted PICV strain p18 develop a severe disease that mimics Lassa fever. In contrast to strain p18 of PICV, the low spleen passage strain p2 causes only limited febrile illness in infected animals.


Arenaviruses generally have rodent reservoirs and are not adapted to human hosts. Accordingly, human clinical isolates of arenaviruses bear the hypothetical risk of adaptation with a consequent increase in pathogenicity for humans (Center of Disease Control and Prevention publication "Biosafety in Microbiological and Biomedical Laboratories 5th edition" Section VIII-E: Viral Agents). In contrast, the genetic traits of the p18 variant of Pichinde virus, which differentiate it from p2, reflect the adaptation of the parental p2 variant to guinea pigs and not to humans or primates. As expected, adaptation of Pichinde virus to guinea pigs was reported not to augment its pathogenicity of non-human primates (Jahrling PB et al. Infect Immun. 1981 May;32(2):872-80).

Accordingly, the adaptation of the ancestral Pichinde virus p2 to p18, which was used for TheraT vector generation, does not interfere with attenuation which is based on inefficient packaging of all three TheraT genomic segments. Therefore TheraT vectors based on Pichinde Virus exhibit impaired replication kinetics when compared to parental PICV (PICV wt) in cultured human cells, in immunodeficient mice and in guinea pigs which are highly susceptible to infection with PICV strain p18.

**Origin & function**

For TheraT, a proprietary vaccine vector platform of Hookipa Biotech GmbH, the originally bi-segmented arenavirus genome was modified by segregating the essential viral nucleoprotein (NP) and viral glycoprotein (GP) from one genomic segment onto artificially duplicated segments. Each TheraT vector thus contains three genome segments, including one large (L) genomic segment and two S segments in which the S segments encode either PICV GP or NP but not both. The genetic design of these S segments prevents intersegmental recombination and reversion to a functional wild type-like single S segment encoding both PICV GP and NP. As all four original PICV
genes encoded on L and S-segments are required for virus replication, all three genetic segments are required for generation of replication-competent progeny TheraT vectors. Attenuation is based on inefficient packaging of the three genetic segments.

The encoded antigens are based on HPV strain 16 proteins E7 and E6. The proteins are artificially expressed as a fusion protein that is rendered non-functional due to several mutations as described in Cassetti M et al, 2004. Therefore, the original activity of the individual proteins is abrogated and the expressed fusion protein E7E6 non-oncogenic.

### Evaluation of foreseeable effects

Hazards arising directly from the inserted gene:

- The HPV antigens encoded by the vector were modified by various mutations to disable the wild-type function. There are no anticipated adverse effects from the inserted genes.

Hazards arising from the alteration of existing pathogenic traits:

- Insertion of the antigen sequence is not expected to alter pathogenicity of TheraT vector. Attenuation is based on inefficient packaging of all three genomic segments and is independent of the transgene.

Consideration relating to whether an inserted sequence, that does not give rise to a harmful phenotype in the recipient, could give rise to harm because of natural gene transfer to other, possibly related, organisms:

- During the replication cycle, arenaviruses exists exclusively in the form of RNA and do not normally integrate into the host genome. Likewise, any other form of gene transfer is not expected to happen.

Estimation of the severity of the harmful event were it to occur:

- TheraT vectors are attenuated, replication competent viral vectors based on PICV. These vaccine vectors were genetically engineered to allow for expression of genes of interest against which a cytotoxic T-cell response is induced and confer an attenuated replication competent phenotype. Any unintentional infection of humans would be expected to lead to a transient infection associated with activation of the immune system.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**N/A**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Disposable items will be placed in autoclave bags, or sharps bins held within the laboratory where the work is being performed. Sharps bins will be sealed prior to removal from the laboratory. Containers and bags will be identified as containing biohazard waste.
Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
No comments arose from the committee

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Project Ref 421/22.1

Date Ackn'd 13/01/2022

CU2 Project Title
The GMO is a pseudotyped lentivirus expressing the S-Protein of SARS-CoV-2 and will be used in this project as part of a neutralisation assay. Modified HEK293 cells will be infected with these viruses in order to establish an effective assay

Class 2
Non-GMM Consent Granted

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
**Project Additional Information**

### Purposes of the contained use
The lentivirus will be used to infect cells that express the ACE-2 receptor. The assay will be used to identify and quantify antibodies that neutralise via the S-protein of SARS-CoV-2 by preventing infection of the lentivirus and expression of the luciferase gene.

### Recipient or parental organism
The GMO is a lentivirus virus that has been modified to remove the Envelope gene. A gene encoding luciferase has been inserted in its place. In addition, the virus was produced in the presence of the plasmid encoding the S-Protein from SARS-CoV-2. This protein is therefore expressed on the surface of the lentivirus and allows the lentivirus to bind to and infect cells expressing the ACE-2 receptor. The gene for the SARS-CoV-2 Spike protein however is not incorporated into the pseudovirus.

### Host/vector system
The lentivirus is well characterised. It is developed from Human Immunodeficiency virus type 1 (HIV-1) but has been genetically modified to remove the envelope gene. It is therefore unable to target its usual receptor CD4. Progeny virions are therefore non-infectious. Lentiviral vectors as categorised as Hazard Group 2 requiring Containment Level 2. In addition, it has a self-inactivating deletion in the U3 region of the 3' LTR which minimises the chance of production of replication-competent particles via a recombination event. Due the retroviral replication process, the 3'LTR serves as the template for the generation of the 5'LTR in the viral progeny. Thus the proviruses (integrated copies of the viral genome) that are established in infected target cells lack functional 5'LTR sequences, including the viral transcriptional promoter and enhancer. Therefore, further replication of the virus cannot occur even if the HIV envelope gene was present.

### Origin & function
The inserted gene encodes the luciferase protein. There are no hazards associated with this gene/protein and it is well characterised.

### Evaluation of foreseeable effects
The inserted gene encodes the luciferase protein. There are no hazards associated with this gene/protein and it is well characterised.

The gene for the SARS-CoV-2 Spike Protein was present during production of the pseudovirus and therefore the pseudovirus expresses the Spike Protein. However, this gene is not present in the pseudovirus. There are no hazards associated with the Spike Protein as the rest of the SARS-CoV-2 virus is not present.

Hazards arising from the alteration of existing pathogenic traits:

The lentiviral particles have been modified in order to remove genes required for any infection into its target cell. This significantly reduces any risk associated with the parent virus. The addition of the S-protein from SARS-CoV-2 changes the tropism of the Lentivirus from CD4+ T-cells to cells expressing ACE2, but the gene for the S-protein is only expressed transiently during the original pseudovirus production and is not contained within this lentivirus pseudovirus therefore does not allow it to replicate or increase its pathogenicity.

Consideration relating to whether an inserted sequence, that does not give rise to a harmful phenotype in the recipient, could give rise to harm as a result of natural gene transfer to other, possibly related, organisms:

The genes are unlikely to transfer and require specific culture conditions and the other plasmids to be present in order to produce complete virus particles.
Due to the deletion of replication and pathogenic genes there is little risk of the GMO establishing more harmful forms of the vector. There is a self-inactivating deletion in the U3 region of the 3’ LTR which minimises the chance of production of replication-competent particles via a recombination event. Estimation of the severity of the harmful event were it to occur: The GMO is considered infectious however it is non-pathogenic and replication deficient so will not likely cause a hazardous event.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All potentially contaminated material will be inactivated by chemical disinfection (minimum 1% w/v Virkon for 20 mins) and/or autoclaving at 121°C for a minimum of 15 minutes. The likelihood of release is therefore low. Disposal methods will follow documented laboratory procedures. Where it is not possible to autoclave or chemically deactivate contaminated materials, they will be placed into hermetically sealed containers, marked with biohazard tape and disposed of by incineration through a licensed contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
GM Centre Number: 423

Data Premises Notified (Originally) 11/04/1991

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed 03/02/2009

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

UNIVERSITY OF CAMBRIDGE

Name 2

Department

INSTITUTE OF BIOTECHNOLOGY

Campus Estate or Research Centre

Building

Road Name

TENNIS COURT RD

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB2 1QT

Country

ENGLAND

Tel Number 01223 334160

Fax Number 01223 334162

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
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Tick if confidential

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<td>Fish</td>
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02/03/2022
The maximum culture volume that could be released at any one time is 50 litres resulting from a fermentor failure. The waste is deactivated by autoclaving in situ and the process is physically monitored by measuring temperature, pressure and time. Validation of our autoclaves is obtained using TST control integrators. Cells of Escherichia coli are destroyed at 120 degrees C.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Name

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Name 2

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Department

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Campus Estate or Research Centre

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HSE Division

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Page 7349 of 15326
### Premises Addresses

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### Premises Conditions

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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref 424/02.1**

<table>
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<td>TO CONSTRUCT RETROVIRAL VECTORS, CAPABLE OF INFECTING HUMAN CELLS THAT EXPRESS GENES ENCODED BY CULTANEOUS AND OTHER HUMAN PAPILLOMAVIRUSES (HPV)</td>
<td>Class 2</td>
<td>1-50 litres</td>
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Date Project Ceased: 12/07/2006

Class 2

Non-GMM

Consent Granted

Tick if notifying a connected programme of work: N

Historical Significant Changes: transferred to centre 774 on 12/7/06.
**Project Additional Information**

**Purposes of the contained use**

We aim to investigate the effects of expressing HPV genes in human epidermal keratindyes. We will investigate cellular responses to viral gene expression in the context of ultraviolet radiation. The main purpose of these experiments is to determine whether the viral encoded activities could account for effects in skin cancer.

**Recipient or parental organism**

HPV genes will be propagated in bacteria (E. coli K12 strains HB101 and sure). DNA will then be introduced into suitable mammalian retroviral packaging cell lines (murine) for virus production. Recombinant viruses will then be used to transduce human cells.

**Host/vector system**

- pBabe Puro/pBabe-bsd retroviral vectors, propagated in recombination deficient bacteria (E. coli HB101/Sure).
- Phoenix cells: murine retro viral packaging cell line.

**Origin & function**

All HPV DNA sequences used are previously cloned in plasmid vectors and freely available.

Likely that the HPV encoded sequences will modify the cell cycle, differentiation status and apoptotic responses of the infected human cell.

**Evaluation of foreseeable effects**

Recombinant virus is potentially hazardous. Contact will therefore be minimised. Work will be carried out in a safety cabinet thereby reducing the risk of aerosol. Sharps will be avoided. If contact does occur; recombinant virus is replication defective, effect thereby localised; immune rejection of virally infected cells of human/murine origin; handling only one virus at a time will minimise changes of recombination.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All work will be carried out in purpose built and equipped laboratory space designated and reserved for such experiments.

All liquid waste will be decontaminated with 1% Virkon or 5% sodium hypochlorite. Solid waste will be autoclaved. A 100% kill will result.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Liquid waste - decontaminated with 1% Virkon or 5% Sodium Hypochlorite. Then to drains.
- Solid waste - autoclaved followed by incineration. 100% kill is expected in both cases.
The committee considers this work to be class II and therefore all retroviral work will be carried out in our Cat II suite, Room 1.18.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<td>Animal Units</td>
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<td>Human Clinical Applications</td>
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**Project Ref** 424/02.2

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<td>IMMORTALISATION OF PRIMARY CULTURES OF HUMAN EPITHELIAL AND MESENCHYMAL CELLS USING STABLE VIRUS PRODUCING PT67/PBABELHYGROH TERT ......</td>
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Tick if notifying a connected programme of work N

Historical Significant Changes

TRANSFERRED TO GM CENTRE 774 - 12/7/06.

Tick if you are claiming exemption from disclosure for section of the risk assessment N
**Project Additional Information**

**Purposes of the contained use**

The purpose of the contained use is to generate immortalised human cell lines derived from the skin that can be used for research and drug screening.

**Recipient or parental organism**

Recipient cells will include human oral, anogenital and cutaneous keratinocytes. Hair follicle, germinative epithelium, matrix, outer root sheath keratinocytes, dermal papilla and connective tissue sheath fibroblasts as well as sebocytes from sebaceous glands.

**Host/vector system**

The pBABE retroviral vector consists of the long terminal repeat (LTR) packaging sequence from the Moloney murine leukemia virus (MoMuLV). Because the env and reverse transcriptase genes have been deleted and as there is no origin of replication this vector is considered by the ACGM to be non-mobilisable and incapable of further replication in the target cell.

**Origin & function**

It is intended that infecting cultured human cells with hTERT and SV40LT inserts will result in the production of immortalised cell lines. Therefore, the foreseeable effects of this activity are the generation of immortalised human cell lines.

**Evaluation of foreseeable effects**

Foreseeable effects will be the immortalisation of skin derived cell lines.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

PT67 packaging cells are considered to be disabled hosts and are not considered to be pathogenic to humans or animals and would have a very limited ability to survive in the environment.

pBabe will be used to express hTERT and SV40LT in human primary cell lines. The human cell lines in both their primary and transformed state are unable to survive outside of the tissue culture flask and are therefore, unlikely to spread or cause damage to the environment. If a human or animal were to be infected by one of these transformed human cell lines, these cells would be rejected by the normal immune system.

All work will be carried out in purpose built and equipped laboratory space designated and reserved for such experiments.

All liquid waste will be decontaminated with 1% Virkon or 5% Sodium Hypochlorite. Solid waste will be autoclaved. A 100% kill will result.

Retroviral supernatants for infection of cell cultures will be produced in volumes of <(less than) 100 ml.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Retroviral vectors to be used in this study are considered by the ACGM to be non-mobilisable and incapable of further replication in the target cell.

Human cell lines in both their primary and transformed state are unable to survive outside of the tissue culture flask and are therefore, unlikely to spread or cause damage to the environment. If a human or animal were to be infected by one of these transformed human cell lines, these cells would be rejected by the normal immune system.
As sharps will not be used in these studies the risk of inoculation into humans is negligible. All work with retroviral supernatants will be carried out by the worker wearing laboratory coat, facemask and double gloves. The risk of human infection is considered negligible.

All liquid waste will be decontaminated with either 1% Virkon or 5% Sodium Hypochlorite. Solid waste will be autoclaved followed by incineration. Members of the MuLV group of viruses require high titre to establish infection in immunologically competent animals and so the risk of harm to either the environment associated with accidental release of the vector is low.

The committee considers this work to be class II and therefore all retroviral work will be carried out in our Cat II suite, Rm 1.18.

### Project Containment

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee considers this work to be class II and therefore all retroviral work will be carried out in our Cat II suite, Rm 1.18.
GM Centre Number: 431

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Name

UNIVERSITY OF WALES COLLEGE OF MEDICINE

Name 2

ORAL SURGERY MEDICINE & PATHOLOGY

Campus Estate or Research Centre

Road Name

HEATH PARK

Town

CARDIFF

County

CARDIFF

Postcode

CF4 4XY

Country

WALES

Tel Number

029 2074 2903

Fax Number

029 2074 4869

E-mail

HSE Division

WALES AND SOUTH WEST

Comments

GM431 CLOSED DOWN AND MERGED WITH GM 130 ON 26/04/2005.

Date at Which Additional Info Submitted

02/03/2022
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify)

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

02/03/2022
Small volumes, max. 10 ml, produced in each "experiment". Surplus, used and waste culture medium autoclaved before disposal as clinical waste as per UWCM/Trust policy. Cell remnants treated in the same manner. Autoclaves operated in accordance with departmental SOP. Autoclaves regularly serviced and tested. Autoclaves subject to independent annual inspection and testing.

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 431/01.1**

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<tr>
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<td>16/07/2001</td>
<td>FACTORS INFLUENCING THE PATTERNING AND SURVIVAL OF MAMMALIAN EPITHELIAL STEM CELLS</td>
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Historical Significant Changes

Transferred to GM130 on 26/04/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Tick if notifying a connected programme of work

Withdrawn

N
### Project Additional Information

#### Purposes of the contained use

To identify and study the behaviour of mammalian epithelial stem cells. After the basic epithelial stem cell patterns have been established, work will examine how intrinsic stem cell patterns are altered by the forced expression of certain genes that are putatively associated with control of stem cell growth and differentiation. This project will use combined techniques of stem culture, retroviral transduction, tissue reassembly to determine: a) the size and distribution of stem cell territories in a range of human and murine epithelia of differing complexities, b) the influence of stromal/epithelial interactions in determining stem cell patterns, c) how stem cells are altered in malignancy, and d) how stem cell behaviour is influenced by the expression of genes associated with cell differentiation and cell death.

#### Recipient or parental organism

The recipient or parental organism for the standard cloning are disabled non-pathogenic E coli strains. The recipient cell lines for expression of the proteins are standard cell culture lines which cannot survive out of culture and which are kept free from any adventitious agents as far as can be determined by our routine assays for contaminating agents which are problematic in tissue culture, e.g. mycoplasma.

#### Host/vector system

<table>
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#### Source of Nucleic Acid:

- GFP cDNA
- Alkaline phosphatase cDNA
- Galactosidase cDNA
- RAS human cDNA
- Bcl-2 related human cDNAs

#### Description of DNA to be manipulated:

- Modified jellyfish gene, Mammalian placenta & E.Coli - Lineage markers
- Mammalian GTPase & Mammalian homologs of C.Elegans ced genes - Potential modifiers of Stem cell behaviour

#### Evaluation of foreseeable effects

To introduce modified oncogenes and anti-oncogenes into mammalian cells in culture, the cDNA of the gene in question is sub-cloned into a replication defective retroviral vector plasmid. This is transfected into a "packaging" cell line which therefore secretes retroviral virions capable of coding for the inserted sequence. These are able to infect human cells but are not capable of further cycles of replication. There is therefore no risk of subsequent spread of infection. The viruses are extremely fragile and are extremely sensitive to desiccation, the recipient cell lines are unable to survive outside culture conditions.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cells and media which may contain infectious virus are handled by designated specially trained workers in a class II biological safety cabinet equipped with external ducting. All liquid waste will be sterilised at point of use by sodium hypochlorite, as per College and Trust sterilisation and disinfection policy. All solid waste will be sterilised in an adjacent autoclaving facility which is regularly tested by Cardiff and Vale Trust engineers.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Risk assessment suitable and sufficient, agree with assigned containment level and classification.

**Project Containment**

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<th>Growth Rooms</th>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 431/04.1

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<td>09/12/2004</td>
<td>Modulation of the interplay between extracellular matrix and cells by adenoviral gene transfer</td>
<td>Class 2</td>
<td>1-50 Litres</td>
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The function and the structure of the extracellular matrix (ECM) is controlled by regulatory molecules as well as by modifying enzymes which can crosslink or cleave matrix constituents. Some of these molecules have functions in the ECM and also control cells residing in the matrix by regulating the availability of cytokines or by direct effects on cells: (1) decorin (see below) can regulate collagen fibril formation as well as TGF-B availability and has direct effects on cell differentiation and survival (Kresse & Schonherr, 2000, J. Cell Physiol. 189:266). (2) Tissue transglutaminase does not only influence cell-matrix interactions but also has GRPase activity and via this activity can contribute to the regulation of intracellular signalling cascades that control cell spreading and motility (Stevens et al., 2004, J. Cell Sci. 117:3389). To investigate the contribution of these distinct functions to biological effects, we plan to use adenoviruses for the expression of wild type or mutant components in different types of cells. Adenoviral gene transfer is necessary (a) to mimic the situation in vivo (because naturally these molecules are induced and not constitutively expressed), (b) the cells used in the investigation (e.g., endothelial cells, primary fibroblasts, macrophages) are difficult to transfect and easily damaged, and (c) adenoviral infection allows a reproducible induction of a specific molecule.

Small leucine-rich proteoglycans (SLRPs) are multifunctional regulatory molecules of the extracellular matrix. Members of this family are decorin, biglycan, fibromodulin, and lumican. In the past, SLRPs were primarily considered as organizers of collagenous networks. More recently their interactions with TGF-B and their direct effects on cells have come into focus. We showed with the help of adenoviral vectors that decorin induces capillary formation and survival of endothelial cells, but it induced programmed cell death in tumour cells (Schonherr et al. 1999, Eur. J. Cell Biol. 78:44; Tralhao et al. 2003 FASEB J., 17:464). Our studies on endothelial cells further indicated that protein kinase B (Akt) is involved in decorin-mediated signalling leading to cell differentiation and survival (Schonherr et al., 2001, J. Biol. Chem 276, 40687). In collaboration with Dr. P. at this institution we plan to use adenoviruses that have already been prepared to investigate signalling cascades in other cell systems. These adenoviruses have already been generated under approval of the Genetic Modification Sub-Committee (GM 312/DWT.pB/3) of the Institution of HSE notification. These adenoviruses will allow us to carry out in-depth analysis of decorin-induced intracellular signalling pathways(s). We will use these viral vectors to transfect endothelial cells and fibroblasts in culture. The already existing adenoviral vectors will allow us to carry out in-depth analysis of decorin-induced intracellular signalling pathways(s). We plan to use adenoviral vectors containing different mutated forms of SLRPs to analyse which parts of these molecules have specific functions. Therefore, we will use the cDNAs of the SLRPs which are available in our laboratory to construct new adenoviral vectors. The resulting replication deficient viruses will subsequently be used in different cell culture models to analyse the function of SLRP mutants.

Transglutaminases are enzymes that have extracellular as well as intracellular functions. One of the best characterised members of this family of multifunctional enzymes is tissue transglutaminase (TGase2). In the extracellular matrix, this enzyme can catalyse the formation of isopeptide bonds leading to protease resistant high molecular weight complexes, while intracellularly, the enzyme acts as a G-protein in signal transduction and mediates PKCa activation via regulation of PLC81. Recent evidence demonstrates that TGase2 controls cell morphology, differentiation and survival by modulating cell-matrix interactions. We have shown that fibroblasts deficient in TGase1 are defective in cell spreading, migration and matrix assembly (Stevens et al., 2004 J.Cell Sci. 117:3389). Recent work by us and others also showed that lack TGases2 have delayed healing of skin wounds and have a deficiency in TGF-B1 production (Szondy et al., 2003. Proc. Natl. Acad. Sci. USA 100:7812). While previous cell culture data also implicated the enzyme in the activation of latent TGF-B the mechanism has not been elucidated. At present it is unclear whether the effects in the wound healing...
relate to defects in fibroblasts or in cytokine release by macrophages and also which of the activities of the enzyme is are essential for normal wound healing. We plan therefore to generate different adenoviruses which contain transglutaminase and different transglutaminase to determine whether we can rescue the phenotype of cells isolated from mice lacking transglutaminase and to analyse which activity of the enzyme is relevant for which biological function.

Recipient or parental organism

Adenovirus is an ACDP category 2 pathogene. Ad5 is primarily associated with mild upper respiratory tract infections of childhood. Deletion of the E1 gene region renders the adenovirus replication deficient. Replication deficient adenoviral vectors by their nature have a limited potential for lateral spread, although this could be facilitated by co-infection with wild-type adenovirus or gene rescue from helper cells.

To generate new replication deficient adenoviruses containing transgenes preliminary prokaryotic cloning steps are necessary. Genetically disabled E. coli K12 strains specifically developed for the applications in recombinant DNA cloning experiments will be used. These strains have limited potential to colonise the gut or survive in the environment.

Host/vector system

E. coli strains JM109, TOP 10, INV a F’ and DH5a will be used.

A commercial Ad5 vector system will be used for the construction of the replication deficient adenovirus (rendered replication deficient through deletion of the complete E1a and E1b gene function and further disabled by partial deletion of E3 gene). The Ad5 based AdEasy vector system from Stratagene. This system comprises pAdEasy-1, pShuttle, pShuttle-CMV, pShuttle-CMV-LacZ (control).

Replication deficient adenoviruses will be propagated in HEK-293 cells (a helper cell line expressing the E1 helper function). The resulting adenoviral vectors will be used to infect target cells in vitro.

Origin & function

All DNA are human and mouse cDNA clones previously generated by us or gifts from collaborators. DNA sequences are known and will be confirmed prior to generation of recombinant virus.

Replication deficient adenoviruses containing wild type human decorin and variant forms of decorin and variant forms of decorin have been previously generated with the AdEasy System in Germany or the USA (Schonherr et al. 1999, Eur. J. Cell Biol. 78:44; Tralhao et al. 2003FASEB J. 17:464). New adenoviral vectors containing additional mutations in the transgene will be prepared using the same technology. In addition, adenoviral vectors for other members of this protein family, ie human biglycan, fibromodulin, and lumican will be generated. The effect of transgenes cloned into these vectors on endothelial cells and fibroblast will be investigated using a number of established biological assays to study extracellular matrix assembly and angiogenesis. New adenoviral vectors containing mutants of human decorin using the same technology will be prepared.

Replication deficient adenoviruses containing mouse dominant negative and positive Akt were gifts from Boston USA (Fujiiy W & Walsh K, 1999, J Biol. Chem. 274:16349). These adenoviruses will be used to investigate the decorin signalling in target cells in culture. In addition, replication deficient adenoviruses approved by Genetic Modification Sub-Committee (GM 312/DWT./PB/3) will be used in collaboration to investigate pathways in these cells in culture.

Replication deficient adenoviruses containing human and mouse transglutaminase or mutants thereof will be generated to determine whether we can rescue in vitro in cell culture assays the phenotype of cells isolated from mice lacking transglutaminase and to analyse which activity of the enzyme is relevant for which biological function.

Evaluation of foreseeable effects

From experiments using adenoviral gene transfer of decorin we already know that the expression of decorin leads to differentiation and survival of normal cells (endothelial cells, primary fibroblasts). In addition, we and others have shown that decorin expression in tumour cells leads to a more differentiated phenotype or programmed cell death. Therefore, the transient expression of decorin in cells in culture does not present a foreseeable hazard. Furthermore, as these experiments should mimic the
situation in vivo, no large scale over-expression of the transgene is planned and thus fairly low concentrations of the adenoviruses will be used.

The generation of other adenoviral vectors containing the cDNA of molecules related to decorin is of low risk, because none of the SLRPs has been shown to have a transforming capacity. In contrast eg the lack of lumican or decorin expression in mammary tumours has been shown to be associated with a bad prognosis. In addition, over expression of biglycan in the pancreas carcinoma cells cause G1-arrest.

Potentially the largest risk is the use of the existing adenovirus containing active Akt which can enhance cell survival and proliferation. However, Akt alone is not sufficient to cause a malignant transformation of human primary cells. In addition, active Akt will only be used infrequently and only at a dose that results in activity mimicking the normal levels of activation in response to decorin. The adenovirus transducing the dominant negative form of Akt (which prevents Akt signalling) will be used to much greater extent. The potential hazard presented by these experiments is perceived to be low.

The generation of other adenoviral vectors containing the cDNA of transglutaminase which is a protein cross linking enzyme is also perceived to be very low. Transglutaminases have no transforming effect on cells. These enzymes strongly promote cell adhesion and several studies have shown that the malignant potential of cancer cell lines is reduced upon transfection with vectors directing constitutive expression of transglutaminase.

The host vector systems which will be used in the experiments are commercially available and well characterised. As the used adenoviruses are replication deficient they can only replicate in host cells which contain E1 sequences that have been deleted from the viral vector (these sequences are present eg in HEK-293, which are used for virus propagation). It is highly unlikely that the replication deficient viruses would regain spontaneously the ability for replication. In addition, large numbers of virus particles would be necessary for a sustained infection. Expression using the adenoviral vectors is only transient and is lost in rapidly replicating cells. Therefore, adenoviral vectors are unlikely to cause any permanent change in infected cells.

A potential hazard is the exposure of humans to recombinant virus. All manipulations of the virus will be done in a class 2 biological safety cabinet in the designated laboratory facility. The access will be limited to staff trained ACGM level 2 procedures. Any disposable materials that had contact with the virus will be disinfected chemically as well as by autoclaving prior to disposal. The use of sharps will be avoided to prevent accidental injury related risk. Therefore, any risk of the exposure of humans or the environment is very low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus in solution (tissue culture media, buffers etc.) will be inactivated by adding no less than equal volume sodium dichloroisocyanurate (Actichlor) at a concentration of 2500 ppm. After 4 h, solutions can be discarded. Nevertheless, the solutions will be autoclaved ed prior to being discarded through the main drain. Pipettes immersed in 2500 ppm sodium dichloroisocyanurate for 4h will be transferred to plastic bags for autoclaving. All plastic ware is autoclaved after virus exposure. Actichlor is effective against all viruses and the high concentration overcomes the high organic contents of media.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Risk assessment suitable and sufficient once additional information had been provided with the tables of vector, host and DNA.

Project Containment

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Data Premises Notified (Originally) 12/06/1991
Transferred from 1992 Regs? Y
Transitional Premises Class 1
Data Premises Closed 15/02/2002
Transitional Premises N
Emergency Plan Required? N
Non-GMMs N
Withdrawn N

Name
IMPERIAL COLLEGE OF SCIENCE TECHNOLOGY MEDICINE

Name 2

Department
PHYSICS

Campus Estate or Research Centre
BLACKETT LABORATORY

Building

District

Road Name
PRINCE CONSORT RD

Town
LONDON

County
GREATER LONDON

Postcode
SW7 2BZ

Country
ENGLAND

Tel Number 0207 594 7704
Fax Number 0207 589 0191

E-mail

HSE Division
LONDON

Comments
GM433 MERGED WITH GM8 FROM 15/2/2002

Date at Which Additional Info Submitted
15/02/2002
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify)  
Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**

- **Microbiology Research**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

All disposable items are bagged and then autoclaved in an Astell Scientific Sterimate autoclave located within the laboratory suite. The effectiveness of the heat treatment is tested regularly using control indicator strips (Prestige Medical). The autoclave unit is also tested annually. The autoclaved bags are disposed of through a central college facility. Cell cultures, 1 litre or less, are deactivated using a standard sodium hypochlorite procedure.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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| Comments                         | FORMERLY KNOWN AS LIFE TECHNOLOGIES LTD |
| Date at Which Additional Info Submitted | 14/12/2001 |
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)

Non-microbial

Other (please specify)  
Tick if confidential

- Bacteriology  
- Parasitology  
- Transgenic Birds  
- Microbiology Research  
- Virology  
- Transgenic Animals  
- Transgenic Fish  
- Gene Therapy
In routine laboratory experiments, at most 2 litres of liquid culture medium is disposed of at any one time. Cultures are autoclaved for 20 minutes at 121 degrees C to inactivate them. A 100 ul sample from inactive culture which contained genetically modified E.coli is transferred to an LB plate containing the relevant genetic selection, spread out and then left at 37 degrees C overnight to verify efficiency of inactivation. Inactive cultures are disposed of in the laboratory sink.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 440

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#### Name

UNIVERSITY OF LIVERPOOL

#### Name 2

PHYSIOLOGY

#### Campus Estate or Research Centre

CROWN STREET

#### Road Name

CROWN STREET

#### District

#### Town

LIVERPOOL

#### County

MERSEYSIDE

#### Postcode

L69 3BX

#### Country

ENGLAND

#### Tel Number

0151 794 5305

#### Fax Number

0151 794 5337

#### E-mail

#### HSE Division

NORTH WEST

#### Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify)  

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
All solid waste resulting from GM work is sealed in bags and autoclaved prior to incineration. Autoclaving is carried out in a regularly serviced and tested machine. Liquid culture waste, up to a maximal volume of 4 litres at a time is inactivated by autoclaving or by treatment with bleach prior to disposal. Inactivating efficiency is tested by re-plating of inactivated waste and examination of bacterial growth by colony counting. These are well established and standard procedures.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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### Name

UNIVERSITY OF WALES COLLEGE OF MEDICINE

### Name 2

UNIVERSITY HOSPITAL WALES

### Department

MEDICAL MICROBIOLOGY AND PHL

### Campus Estate or Research Centre

### Building

### Road Name

HEATH PARK

### Town

CARDIFF

### County

CEREDIGION

### Postcode

CF14 4XN

### Country

WALES

### Tel Number

029 2074 3521

### Fax Number

029 2074 2161

### E-mail

### HSE Division

WALES AND SOUTH WEST

### Comments

GM446 CLOSED DOWN AND MERGED WITH GM130 ON 26/04/2005

### Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: [ ]

- Give brief details of the genetic modification safety committee

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

- **Other (please specify)**: [ ]

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

- **Tick if confidential**: [ ]
For activities involving GMMs, describe the waste management measures which will apply to the activity

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Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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### Name

**NATIONAL INSTITUTE OF AGRICULTURE BOTANY**

**Department**

**PATHOLOGY**

### Campus Estate or Research Centre

**Building**

### Road Name

**HUNTINGDON ROAD**

### Town

**CAMBRIDGE**

### County

**CAMBRIDGESHIRE**

### Postcode

**CB3 0LE**

### Country

**ENGLAND**

### Tel Number

**01223 342200**

### Fax Number

**01223 277602**

### E-mail

**HSE Division**

**EAST AND SOUTH EAST**

### Comments

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

- Chair of GMSC & Biological Safety Officer (BSO)
- Management Representative
- Deputy BSO
- NIAB Safety Committee Representative (H&S Manager)
- NIAB Facilities Manager
- Co-opted members as necessary:
  - Member of other NIAB Teams
  - Supervisory Medical Officer (SMO)
  - Representative of Study Managers

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<th>Animal Unit</th>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste is disposed of by verified methods.
E.g. small quantities of GM waste (laboratory and growth rooms) are autoclaved, to sterilize all GM material, before disposal to landfill.

Larger quantities of GM waste (glasshouse) are disposed of by an approved external incineration contractor for certified disposal.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Principal Investigators for all NIAB GM projects must complete a risk assessment and submit it to the Genetic Manipulation Safety Committee (GMSC) for approval. Only once the GMSC are satisfied that a risk assessment has been conducted in accordance with the genetic manipulations regulations will work on a project proceed.
Study of the infection process of Fusarium spp. (culmorum/graminearum) in Maize (Zea mays) using reporter genes

Maize varieties will be infected with Fusarium spp. harbouring the reporter genes uid A (GUS), GFP and DsRed. Maize tissues will be examined/ assayed for the presence of the reporter genes/reporter gene products in order to determine the extent of systemic infection and whether sytemic infection significantly contributes to Fusarium contamination of grain.

Recipient or parental organism

GMO1: Parental organism is Fusarium graminearum.
GMO2: Parental organism is Fusarium culmorum.

Both GMOs are natural pathogens of cereals such as maize, wheat, barley and oat and are endemic within Europe.

Origin & function

For GMO1 and GMO2 the inserted DNA will be a selectable gene conferring resistance to hygromycin (hph) and uidA (GUS) and Green Fluorescent Protein (GFP) and DsRed reporter genes (includes promoters etc.) Both reporter constructs were created in at Rothamsted Research (GFP, and JIC (DsRed), and have been routinely used to investigate the interaction between Fusarium spp. and small grain cereals. Hygromycin resistance will be used to select transformed Fusarium colonies, the reporter genes will be used to determine sytemic spread of the Fusarium species in Maize tissues.

Evaluation of foreseeable effects

The organisms studied are endogenous plant-pathogens on cereals across Europe and as such would be expected to survive and spread if accidentally released. The expression of the selection and marker genes will not alter the pathogenicity or fitness of the fungus, and will not confer any selective advantage in nature if accidentally
released. We are not aware of any reports where expression of these genes has been found harmful to human health or to the environment. Furthermore F. graminearum and F. culmorum are not considered pathogenic to humans (Hazard Group 1 organisms) or animals, however the toxins produced by the fungi are toxic if ingested. The modifications to the Fusarium strains should not alter their pathogenicity to humans or their ability to disseminate. It is expected that the GMMs will be able to produce toxins; however, infected plants will not be consumed. Health hazards from inhalation of Fusarium spores have not been reported and the toxins are not known to be carcinogenic. Fusarium spores may be irritant to skin and eyes and appropriate operating procedures are in place to prevent this with any Fusarium spp handled.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GM Fusarium species will be used to inoculate maize seed/seedlings and as such they are plant-associated GM micro-organisms.

All manipulations of fungal cultures will be performed in a Class II Microbiological Cabinet.

Inoculated plants will be contained in dedicated spore proof growth facilities.

All operations will adhere to NIABs level 2 containment guide lines (attached SOP_GMM_001 – 009)

Both facilities are already approved for work with licensed (non indigenous) plant pathogens. (PHL 200/6871 (08/2012)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All work will be conducted in Level 2 Containment Facilities (MSC Class 2, and sporeproof growth room) no derogation requested. Material for microscopic examination will be transported in triple wrapped containers (see SOP)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work will follow NIAB standard operating procedures for working in level 2 containment facilities SOPs SOP_GMM_01 – 009 attached.

Waste will be in the form of fungal spores and mycelia on agar plates and associated contaminated waste such as tubes, filters, pipette tips, microscope slides, plant material, plant growth medium and containers.

Fungal growth medium and associated waste will be put in autoclaveable biohazard bags and sterilized by passage through a waste autoclave cycle (121 C for 30 minutes - degree of kill 100%).

All surfaces will be wiped with either a fresh 70% EtOH solution or a 10% Trigene solution dependent on surface type and detailed in attached SOPs (degree of kill greater than 99.999%).

All other consumables and plant material will be placed in autoclaveable biohazard bags and sterilized by passage through a waste autoclave cycle (132 C for 90 minutes) before disposal with general waste (landfill) (degree of kill 100%). Waste is autoclaved in a certified autoclave, load temperatures are recorded throughout the autoclave cycle and checked before waste disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

Comments were received on the need to specify the exact facilities being used for level 2 operation, this has been done.

**Project Containment**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

1. Maximum culture volume that could be released at any one time: 1 litre.
2. a) Solid waste: autoclaved and incinerated (121 degrees C; 15 mins)
   b) Liquid waste: Vircon treated (3%) followed by autoclaving and disposal down sink.
   c) Glassware: Vircon treated (3%) for 24 hours; autoclaved as before.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 448/04.1**

**CU2 Project Title**

RESEARCH INTO THE BIOSAFETY ISSUES SURROUNDING THE USE OF PLANT VIRUSES AS VECTORS TO EXPRESS FOREIGN GENE IN PLANT.

**Class**

Class 2

**CultureVolClass2**

< 1 litre

**CultureVolumeClass3-4**


**Non-GMM Consent Granted**

not applicable

**Consent Granted**

Project notified under transitional arrangements

**Historical Significant Changes**

Withdrawn

**Tick if notifying a connected programme of work**

---

02/03/2022
### Project Additional Information

#### Purposes of the contained use

To use potato virus X (PVX) modified with GFP (and truncated versions of this gene) to investigate the stability of genetic inserts in the PVX expression vector.

#### Recipient or parental organism

The plant virus PVX will be used. This virus is indigenous to the UK and is mechanically transmissible, infecting solanaceous plants. Vioral vectors based on PVX are available from the Sainsbury Laboratory, John Innes Centre, Norwhich. The constructs contain PVX under the CaMV 35S promoter and are cloned into a binary vector for the purposes of agroinoculation.

#### Host/vector system

The host plant will be Nicotiana benthamiana. Infection will be via agroinoculation, either from liquid cultures or via colonies from plates. PVX can only be spread by mechanical means.

#### Origin & function

A PVX virus vector will be used, containing the gene (and truncated versions of the gene) encoding green fluorescent protein (GFP) from the jellyfish Aquoria victoria. GFP will be used as a reporter gene to assess the stability of the construct during passaging experiments.

#### Evaluation of foreseeable effects

It is anticipated that expression of the inserted gene will not exceed 2% of total cell protein. None of the components of the vector system (Agrobacteria, PVX, GFP) pose a risk to human health. It is highly unlikely that either the PVX or GFP sequence will help either laboratory strains of E. coli or disarmed Agrobacteria strains overcome any disabling mutations. There is potential for PVX to survive outside containment and interact with wild type virus. It is generally believed that virus carrying extra genetic elements will be less fit than their wild type equivalents and that the gene will be lost. The aim of this work is to provide proof that this happens.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A (the PVX system serves as a transient expression system, the plant will not be transformed)

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Waste material will be in the form of liquid cultures and agar plates (and the associated lab ware that will have come into contact with the modified organisms), and, in addition, there will be infected plant material, soil and pots. Prior to autoclaving, all plastic ware and glassware for reuse will be soaked for 24h in 3% Virkon. All contaminated material will be autoclaved at 121 degrees C for 20 min using a regularly serviced and validated machine situated within the facility. Work surfaces will be wiped down with 3% Virkon after a procedure has been completed and any spills will also be treated with 3% Virkon. The treatment described have been shown to achieve 100% kill (see attached documents). The material used to wipe down surfaces and clear up spills will then be autoclaved, as above, before disposal. Treated waste will then be disposed of by incineration.

Is an emergency plan required according to regulation 20? ✗

If yes, tick to confirm that it is attached to this form ✗
The attached risk assessment and operating procedures have been examined and discussed and the Genetic Modification and Biological Safety Committee agree that the work should proceed under the conditions stated.

**Project Containment**

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**Project Ref** 448/05.1

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<td>Investigating genes involved in symptom induction in the tuber necrotic strain of Potato virus Y (PVY)</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**
Purposes of the contained use

In order to study the genes involved in symptom induction in the tuber necrotic strain of Potato virus Y (PVY), a clone containing a full-length genome copy of a UK isolate of PVYNTN will be constructed from which infectious RNA transcripts can be synthesised in vitro. The transcripts will be established by mechanical inoculation to tobacco and be transferred by mechanical inoculation to potato. Gene swap experiments will then be conducted between the clone isolate and isolates, which are unable to cause necrotic tuber symptoms.

Recipient or parental organism

PVY. This is a single stranded, monopartite RNA virus, belonging to the Potyvirus genus. PVY is naturally transmitted in a non-persistant fashion by aphids and can be mechanically transmitted, the host range is generally limited to the Solanaceae and it is distributed. The initial clone should produce wild-type necrotic symptoms on potato tubers, the subsequent modifications are expected to make an isolate that causes less damaging symptoms on potato, with no tuber necrosis. The changes are not expected to alter the host range nor the survivability of the modified virus compared to the wild type.

Host/vector system

The host plants will be Nicotiana spp and Solanum tuberosum. The plants will be mechanically inoculated with the in vitro RNA by rubbing the leaves with a mild abrasive (eg celite).

Origin & function

The isolate of PVY that will be used to make the infectious clones is called v942490 and originated from symptomatic potatoes grown in the field in the UK in 1998. The clone will contain the whole of the genome of the isolate, which is translated in the plant into a single polyprotein; the polyprotein is cleaved into 9 gene products, which allow full systemic infection of the plant. The plasmid will contain the PVY genome cloned adjacent to an RNA polymerase (T7 and SP6) promoter sequence. Prior to use the plasmid will be purified, cut using a restriction enzyme to linearise the plasmid at the 3' end of the PVY genome insert. This plasmid will then be used to make in-vitro RNA transcripts (using T7 or SP6 RNA polymerase) of the PVY genome which should be infectious when mechanically inoculated to host leaf.

Evaluation of foreseeable effects

It seems unlikely that the cloned PVY genome will have any effect on the E. coli strain used; it is unlikely that it will complement any of the disabling mutations. Full-length clones of potyviruses are difficult to establish due to the length of the genome (10kb) and the established plasmids are often found to rearrange within the bacterial clones, thus it is unlikely that the plasmid will persist in E. coli. The PVY transcripts from the initial clone will have the same sequence as PVY isolate V942490; an isolate found within the UK. This isolate belongs to the tuber necrotic strain (NTN), which is the causal agent of potato tuber necrotic ringspot disease (PTNRD) and is able to cause superficial necrotic rings on the surface of the tubers of susceptible cultivars. The genome of these strains is recombinant containing regions of other PVY strains (PVY-N and PVY-O). Future genome rearrangements will aim to deconstruct the recombinant genome and aim to 'knockout' the ability of this isolate to cause tuber symptoms. Thus the aim of the project is to modify the isolate to generate less damaging symptoms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The GMOs in use are the transformed E. coli and the modified PVY isolates, the plants will not be transformed.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste material will be in the form of liquid cultures and agar plates (and the associated lab ware that will have come into contact with the modified organisms), and in addition, there will be infected plant material and the soil/pots in which the material was grown. Prior to autoclaving, all plastic ware and glassware for reuse will be soaked for 24 hours in 3% Virkon. All contaminated material will be autoclaved at 121 degrees C for 20 minutes using a machine (which is serviced annually on contract and for which there is validation data) situated within the facility. Work surfaces will be wiped down with 3% Virkon after procedure has been completed (eg inoculating plants) and
any spills will also be treated with 3% Virkon. The treatments described have been shown to achieve 100% kill (see attached documents). The material used to wipe down surfaces and clear up spills will also be autoclaved as above before disposal. Treated waste will then be disposed of by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The attached risk assessment and operating procedures have been examined and discussed by members of CSL Genetic Modification Biological Safety Committee. They reached agreement that the work could proceed under the conditions stated.

Project Containment

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<th>Growth Rooms</th>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Project Ref 448/06.1

Date Ackn’d 04/01/2006

CU2 Project Title

The aim of the study is to investigate the genes involved in symptom induction in the pathogen Pepino mosaic virus (PepMV) in the tomato host and indicator hosts.

Date Project Ceased 02/08/2019

Class 2

Culture Volume

< 1 Litre

Non-GMM Not Applicable

Consent Granted

Project notified under transitional arrangements N
The study aims to produce clones from which full-length infectious transcripts can be generated for PepMV. These transcripts will be inoculated to tomato, Nicotiana benthamiana, N. glutinosa, N. tabaccum and Datura stramonium to investigate symptom induction. Subsequently the clone will be altered by removing the tomato strain (causes symptoms in tomato) sequences and systematically replacing it with corresponding pepino strain (no symptoms in tomato) sequence. These new clones will be inoculated as before and symptoms on tomato and other indicator hosts will be investigated. The aim is to identify which regions of sequence are involved in symptom induction.

Pepino mosaic virus (PepMV) belongs to the genus potexvirus, it was first described infecting pepino (Solanum muricatum) plants in Peru. In 1999 the virus was found in tomato (Lycopersicon esculentum) crops in Europe. It has filamentous particles of around 510 nm in length that contain one genomic ssRNA molecule (6410 nucleotides). It is transmitted readily by inoculation of sap to many solanaceous species. No vector is known but the virus is easily transmitted from plant to plant through contact.

The host plants will be Lycopersicon esculentum, Nicotiana benthamiana, N. glutinosa, N. tobacco and Datura stramonium. The plants will be mechanically inoculated with the in-vitro synthesised RNA (from the plasmid) by rubbing the leaves with a mild abrasive (eg celite). One of the JM series of E. coli strains will be used. These are laboratory-adapted strains, and have biological limitations which mean they are unlikely to survive in the gut, lung or elsewhere. These strains have a history of safe use. A non-mobilisable plasmid vector (pUC 19) will be used which is defective in one or more functions required to transfer to other hosts.

The isolates of PepMV that will be used to make the infectious clones are from tomato in UK, tomato in North America and pepino in Peru. The clone will contain the whole of the genome of the isolate, which is translated in the plant into 5 gene products, which allow full systemic infection of the plant. The plasmid will contain the PepMV genome cloned adjacent to an RNA polymerase (T7 and SP6) promoter sequence. Prior to use the plasmid will be purified, cut using a restriction enzyme to linearise the plasmid at the 3' end of the PepMV genome insert. This plasmid will then be used to make in-vitro RNA transcripts (using T7 or SP6 RNA polymerase) of the PepMV genome, which should be infectious when mechanically inoculated to host leaf.

It seems unlikely that the cloned PepMV genome will have any effect on the E coli strain used; it is unlikely that it will complement any of the disabling mutations.
Full-length virus clones are often difficult to establish due to the length of the genome (6kb). The established plasmids are often found to rearrange within the bacterial clones, thus it is unlikely that the plasmid will persist in E. coli. The transcripts from the initial clone will have the same sequence as the PepMV isolates found within the UK. This isolate belongs to the tomato strain. Future genome rearrangements will aim to deconstruct the genome and aim to 'knockout' the ability of this isolate to cause foliage symptoms on tomato. Thus the initial aim of the project is to modify the isolate to generate less damaging symptoms. To carry out reciprocal modifications to prove which genes modulate symptoms it will be necessary to modify the Pepino strain to enable it to generate symptoms, but these symptoms are likely to be identical to those caused by the tomato strain.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The GMOs in use are the transformed E. coli and the modified PepMV isolates, the plants will not be transformed.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste material will be in the form of liquid cultures and agar plates (and the associated lab ware that will have come into contact with the modified organisms), and, in addition, there will be infected plant material and the soil/pots in which the material was grown. Prior to autoclaving, all plastic ware and glassware for reuse will be soaked for 24 hours in 3% Virkon. All contaminated material will be autoclaved at 121 degrees C for 20 minutes using a machine (which is serviced annually on contract and for which there is validation data) situated within the facility. Work surfaces will be wiped down with 3% Virkon after a procedure has been completed (eg inoculating plants) and any spills will also be treated with 3% Virkon. The treatments described have been shown to achieve 100% kill (see attached documents). The material used to wipe down surfaces and clear up spills will also be autoclaved as above before disposal. Treated waste will then be disposed of by incineration.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

**Please enter comments on the GM safety committee on the risk assessment**

The attached risk assessment and operating procedures have been examined and discussed by members of CSL Genetic Modification Biological Safety Committee. They reached agreement that the work could proceed under the conditions stated.

**Project Containment**

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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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02/03/2022
## Project Ref 448/13.1

### CU2 Project Title
- **Use of Mengovirus vMC0 (ATCC-VR-2310) as a sample process control for detection of enteric viruses in food and environmental samples**

### Purposes of the contained use
- For use as a sample process control when detecting enteric viruses in food and environmental samples

### Recipient or parental organism
- We do not possess the parental organism

### Host/vector system
- N/A

### Origin & function
- vMC0 mengovirus (ATCC-VR-2310). This virus has been modified by the removal of the poly(c) tract

### Evaluation of foreseeable effects
- Exposure of experimenters to mengovirus vMC0 could occur during its use as a process control. There is no available information on the infectivity of this strain for humans.

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*Page 7392 of 15326*
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid material for disposal (pipette tips, microcentrifuge tubes, pipettes etc) will be incinerated according to Fera's waste management procedure. All liquid waste will be disinfected in a 1% solution of Virkon® Rely+On for at least 10 minutes.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

There were no comments from the committee

Project Containment

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Animal Units

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| Data Premises Notified (Originally) | 23/10/1991 |

### Transferred from 1992 Regs?

| Transferred from 1992 Regs? | Y |

### Transitional Premises

| Transitional Premises Class | 1 |

### Data Premises Closed

| Emergency Plan Required? | N |

### Non-GMMs

| Non-GMMs | N |

### Withdrawn

| Withdrawn | N |

### Name

| NORTH EAST SURREY COLLEGE OF TECHNOLOGY |

### Department

| APPLIED SCIENCE AND CONSTRUCTION |

### Campus Estate or Research Centre

| NESCOT |

### Building

### Road Name

| REIGATE ROAD |

### District

| |

### Town

| EWELL |

### County

| SURREY |

### Postcode

| KT17 3DS |

### Country

| ENGLAND |

### Tel Number

| 0208 394 1731 |

### Fax Number

| 0208 394 3030 |

### HSE Division

| EAST AND SOUTH EAST |

### Comments

### Date at Which Additional Info Submitted

| 02/03/2022 |

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Page 7394 of 15326
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify) Tick if confidential

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<th>Microbiology Research</th>
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<tr>
<td>Virology</td>
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<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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### Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

| Max culture vol 500ml, deactivation: autoclaving @ 20 lb/sq in. for at least 30 minutes | Validation: test plating of autoclaved cultures | Monitoring: autoclave test strips and periodic re-evaluation |

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<td>Other (please specify below)</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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**Project Ref** 453/13.1

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<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Date Project Ceased</th>
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</thead>
<tbody>
<tr>
<td>01/05/2013</td>
<td>Interaction between small molecules produced by the fish pathogen Yersinia ruckeri and the fish host</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

The fish pathogen Yersinia ruckeri is the causative agent of Enteric Redmouth (ERM) disease in salmonids, known as Yersiniosis, and can cause losses in aquaculture. However, only few pathogenic mechanisms of Y. ruckeri have been described. Previous work by Dr Deng's research group has shown that Y. ruckeri produces several small organic molecules that may have a role in virulence, and will be the subject of these studies.

Goals of the project:
1. to determine the structures of these small organic molecules;
2. to understand how these molecules are biosynthesised;
3. to understand the impact of these molecules on Y. ruckeri pathogenicity.

Recipient or parental organism

The wild-type Y. ruckeri strain is not a human pathogen but is a fish pathogen and therefore it is handled at Containment Level 2. This project will generate several mutant strains in which various genes involved in the biosynthesis of the potential virulence factor(s) will be knocked out. In some cases, the genes knocked out will be reintroduced into the mutant strains, in knockin experiments, to help verify the functions of the encoded proteins in vivo.

Host/vector system

The vectors used to knockout the target genes in Y. ruckeri will be pK18mobSac and pK18mobGII. The vector used to knockin the target genes into Y. ruckeri will be pRK404. These three vectors are well described and (i) are either non-mobilisable (e.g. pUC series and their derivatives) or mobilisation-defective (e.g. pBR322 and its derivatives) in bacteria; (ii) contain only selective markers that are already in routine use in standard cloning vectors; or (iii) contain no recognisable harmful sequences.

Origin & function

We will generate knockout mutant strains of Y. ruckeri. The deleted or modified genes will be those associated with the biosynthetic pathways which direct the biosynthesis of small molecules that may be involved in virulence. In some cases the genes will be re-introduced into the mutants to verify function.

Evaluation of foreseeable effects

Yersinia ruckeri is a Containment level 2 fish bacterial pathogen, not known to cause disease to humans.

In this study the focus is on the biosynthetic pathway of siderophores as virulence factors in Y. ruckeri, which will be studied initially by knockout mutagenesis. Genetic manipulation of secondary metabolism in Y. ruckeri is expected to decrease virulence compared with the wild-type strain. Precedents to support this conclusion can be found in the literature, where knockout mutants of different genes in Y. ruckeri relating to biosynthesis result in significant reductions of virulence (Appl Environ Microbiol. 70, 5199-5207. Microbiol. 2007, 153.483.489. J Bacteriol. 2011, 193, 944-951).

The genes inserted as selection markers will confer resistance to the antibiotics kanamycin and tetracycline, which are not commonly used to treat Y. ruckeri infections in fish, for which there is an effective vaccine used in aquaculture.
The genetic insertions for the knock-ins are stable in the genome and plasmid maintenance is not required. The knock-in mutant strains of Y. ruckeri may revert or partially revert to a virulent phenotype. Whilst the risks of the knock-ins generating a more virulent phenotype seem very low, this cannot be completely excluded and therefore whilst likely negligible it cannot be stated there is no risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: 2% Trigen/Distel (active ingredient = TM307).
Glassware: soaked with 2% Trigene/Distel or autoclaved.
Plasticware: autoclaved.
Sharps: contaminated sharps are placed in CinBins, bagged and incinerated.

Liquid waste, after inactivation, will be disposed of down the drain. Solid waste, after inactivation will be sent off site for incineration.

In the event of spillage, the contaminated area will be flooded with concentrated Trigene/Distel (to 2% final concentration), mopped up with paper towels, and reswabbed with 2% Trigene/Distel. Contaminated material will be autoclaved.

The use of Trigene/Distel has been validated experimentally to kill > 99.99% of cells.

The autoclave in the Chemistry section is validated at least annually by means of biological indicators. On a daily basis, when in use, monitoring is by thermocouple temperature recordings and autoclave strips included in the loads.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This application was approved at Containment Level 2 by the local Genetic Modification Safety Committee. They were satisfied that generally the knock-out mutants were likely to be less virulent than wild type strains and potentially this work could be at Containment Level 1, but that it was not possible to be sure there was no risk associated with the knockin mutants, in terms of reversion to virulence.

Project Containment
<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3</td>
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<tr>
<td></td>
<td>L2 L3</td>
<td>L2 L3</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4</td>
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<tr>
<td></td>
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</tbody>
</table>
### Data Premises Notified (Originally)
- **Date:** 05/12/1991
- **Transferred from 1992 Regs:** Y
- **Transitional Premises Class:** 1
- **Data Premises Closed:** N
- **Transitional Premises Emergency Plan Required:** N
- **Non-GMMs:** N
- **Withdrawn:** N

### Name
- **NORTH EAST WALES INSTITUTE**

### Name 2
- **Department:** MULTIDISCIPLINARY RESEARCH & INNOVATION CENTRE

### Campus Estate or Research Centre
- **Name:** PLAS COCH, MOLD ROAD
- **Town:** WREXHAM
- **County:** CLWYD
- **Postcode:** LL11 2AW
- **Country:** WALES
- **Tel Number:** 01978 290666
- **Fax Number:** 01978 290008
- **E-mail:**
- **HSE Division:** WALES AND SOUTH WEST

### Comments

### Date at Which Additional Info Submitted
- **02/03/2022**
The premises is located at THE NORTH EAST WALES INSTITUTE, MRC SCIENCE HEALTH AND MEDICAL STUDIES, PLAS COCH, MOLD ROAD, WREXHAM, CLYWD, LL11 2AW, WALES.

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<tr>
<td>Level 2 (GMMs)</td>
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<td>Level 4 (GMMs)</td>
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<tr>
<td>Non-microbial</td>
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</tbody>
</table>

Other (please specify)

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity:

- Maximum culture volume is one litre.
- Deactivation: for > 5ml, by autoclaving 115 degrees C for 20 minutes.
- for < 5 ml, by exposure to bleach for >24h (5 litres of bleach).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 456

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Data Premises Closed</td>
<td>17/04/2009</td>
<td>Transitional Premises</td>
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<td>Non-GMMs</td>
<td>N</td>
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<tr>
<td>Emergency Plan Required?</td>
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#### Name

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#### Name 2

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<th>RESEARCH &amp; DEVELOPMENT</th>
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#### Campus Estate or Research Centre

<table>
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<th>Fax Number</th>
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#### Comments

All work transferred to GM391 on 17/04/2009

#### Date at Which Additional Info Submitted

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<th>Date</th>
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Premises Addresses

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<th>Department</th>
<th>Name 2</th>
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<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
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<td>UXBRIDGE</td>
<td>UB9 6LS</td>
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<td>N</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

Project Ref 456/01.1

Date Ackn'd 11/01/2001

CU2 Project Title LIVE POXVIRUS VECTORED ANTI-VIRAL FELINE VACCINE 1.

Class 2

Volume < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

This project has transferred to GM391

Significant Change ID

Date of Significant Change
### Purposes of the contained use

Evaluation of poxvirus vectored feline viral antigens for vaccination of cats.

### Recipient or parental organism

Non-modified poxvirus with no markers or attenuations prior to use. Biological Hazard and MAFF hazard group 1.

Only produces disease in it's natural host. Is not known to produce disease in humans.

### Host/vector system

Host is poxvirus as described above.

### Origin & function

Inserted gene sequences derived from a feline virus, feline genes from feline peripheral blood mononuclear cells and marker genes derived from E. coli. The genes and their regulatory elements are not known to affect virulence, survival in the host, survival in the environment or increase resistance to therapeutic agents. The donor genes are not known to be involved in host range, virulence or expansion of tissue tropism.

Insertion of the genes into the parental viruses confers an attenuated plaque phenotype.

The inserted genes are intended to induce protection against the parental organism following vaccination of cats with the GMM.

### Evaluation of foreseeable effects

The GMM is only known to cause disease in it's natural host. The inserted sequences are not expected in increase the survivability of the GMM in the environment or increase its host range of tissue tropism. The use of category 2 containment reduces any risk to the environment and therefore exposing the natural host to effectively zero. The GMM is not known to cause disease in humans and category 2 containment reduces any risk of exposure to effectively zero.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not claimed.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not claimed.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be decontaminated by autoclaving using a validated temperature cycle designed to achieve 100% killing.

Liquid waste will be heat treated using a validated temperature cycle designed to achieve 100% killing.

No waste will be disposed off containing any live organisms.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**
The risk assessment and notification form was reviewed by the GMM Safety Committee on 15/12/00. All elements were debated and the committee found that the information provided was in accordance with the 2000 regulations. The containment level applied was deemed to be appropriate for the activities and scale in order to reduce all risk to effectively zero.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
</tr>
</tbody>
</table>

Project Ref 456/05.1

Date Ackn'd 29/04/2005

CU2 Project Title

Storage of seed materials and associated laboratory work targeted at formulating an experimental vaccine.

Date Project Ceased 17/04/2009

Class 2

Culture Vol Class 2 1-50 Litres

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work N

Historical Significant Changes

This project has transferred to GM391

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use
To provide vaccine for potential evaluation in controlled laboratory studies or in the field.

Recipient or parental organism
a) The parental Mannheimia haemolytica is a field isolate originally cultured from bovine pneumonic lung tissues.
B) The parental Pasteurella multocida is a field isolate originally cultured from pneumonic lung tissue. This strain is highly virulent and is capable of replication in the bovine lung.

These are both classified as ACDP hazard group 2 and had no genetic modifications carried out prior to the production of the deletant strains.

Host/vector system
Not applicable.

Origin & function
Attenuation of the virulence of Mannheimia haemolytica and Pasteurella multocida by virtue of gene deletions, whilst maintaining a suitable immune response in the host animal.

Evaluation of foreseeable effects
The deletant strains are likely to retain the features of the parental organism with respect to host range, tissue tropism and mode of transmission. They will however be significantly attenuated with respect to virulence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
The level of attenuation of these organisms is such that the measures applicable to containment level 1 are appropriate. However, since the mode of transmission is unlikely to have been affected, work with open vessels where there is a risk of aerosol generation will be carried out within a microbiological safety cabinet.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid waste will be decontaminated by autoclaving using a validated temperature cycle designed to achieve 100% killing.
Liquid waste will be heat treated using a validated temperature cycle designed to achieve 100% killing.
No waste will be disposed of containing any live organisms.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
The risk assessment and notification form were reviewed by the GMSC on 31 January 2005. All elements were debated and the committee found that the information provided was in accordance with the 2000 regulations. The containment level applied was deemed to be appropriate for the activities and scale in order to reduce all risk effectively to zero.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tr>
<th>Animal Units</th>
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<tr>
<td>L2</td>
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### Project Ref 456/05.2

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<tbody>
<tr>
<td>26/08/2005</td>
<td>The Development of a vaccine as an aid in preventing losses due to Marek's disease.</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th></th>
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<tbody>
<tr>
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<th>Class</th>
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<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>Class 2</td>
<td>1-50 Litres</td>
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<tr>
<th>Non-GMM</th>
<th>Consent Granted</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Not Applicable</td>
</tr>
</tbody>
</table>

Tick if notifying a connected programme of work | N

### Historical Significant Changes

This project has transferred to GM391

### Project Additional Information

Purposes of the contained use

Evaluation of immunogenicity, potency and safety in chickens.
The construct is a novel avian herpesvirus, consisting of regions of herpesvirus of turkeys (HVT) and of Marek’s disease virus (MDV).

A. The parental HVT is ubiquitous in domestic turkeys and widespread in chicken populations where vaccination with HVT has been practiced since the early 1970s. It is not considered to be pathogenic for turkeys or chickens (Cainek BW HW Jr. Iowa State University Press, Ames, Iowa pp342-385).
B. The parental MDV causes a lymphoproliferative disease that affects the peripheral nervous system and other organs.

<table>
<thead>
<tr>
<th>Recipient or parental organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>The construct is a novel avian herpesvirus, consisting of regions of herpesvirus of turkeys (HVT) and of Marek’s disease virus (MDV).</td>
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| A. The parental HVT is ubiquitous in domestic turkeys and widespread in chicken populations where vaccination with HVT has been practiced since the early 1970s. It is not considered to be pathogenic for turkeys or chickens (Cainek BW HW Jr. Iowa State University Press, Ames, Iowa pp342-385). |

<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>The construct contains MDV genes which are not known to affect virulence in and of themselves. Deletion of these genes has been shown to have no effect on the transforming potential of the virus. Further, these genes do not include the viral genes and regions known to be associated with virulence and oncogenicity in MDV. In the construct these have been replaced by the equivalent regions of HVT genome.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Evaluation of foreseeable effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither of the parental strains are known to be human pathogens. The construct is expected to have similar traits to that of an approved avirulent vaccine strain of HVT.</td>
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<table>
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<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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<tbody>
<tr>
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<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
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<tbody>
<tr>
<td>Although the GMM has been shown to be avirulent, full evaluation of the potential for shed and spread is incomplete. In consequence, any animal work will be carried out in a facility with HEPA filtered extract air in order to provide enhanced protection of the environment. For studies involving subcutaneous or intramuscular vaccination of animals, syringes will be filled in a safety cabinet, but to reduce the risk of needlestick injury, injection of the animals will take place on the open bench.</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All solid waste will be decontaminated by autoclaving using a validated temperature cycle designed to achieve 100% killing. Liquid waste will be heat treated using a validated temperature cycle designed to achieve 100% killing. No waste will be disposed of containing any live organisms.</td>
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<table>
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<tr>
<th>Is an emergency plan required according to regulation 20?</th>
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<tbody>
<tr>
<td>N</td>
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<table>
<thead>
<tr>
<th>If yes, tick to confirm that it is attached to this form</th>
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<th>Tick to confirm that you have attached a risk assessment to this form</th>
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<th>Tick if you are claiming exemption from disclosure for section of the risk assessment</th>
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<table>
<thead>
<tr>
<th>Please enter comments on the GM safety committee on the risk assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>The risk assessment were reviewed by the GMSC on 15 July 2005. All elements were debated and the committee found that the information provided was in accordance with the 2000 Regulations. The containment level applied was deemed to be appropriate for the activities and scale in order to reduce all risk effectively to zero.</td>
</tr>
</tbody>
</table>
Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
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L2 | L3 | L4
L2 | L3 | L4
L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4
L2 | L3 | L4
L2 | L3 | L4

Project Ref 456/07.1

CU2 Project Title
The development of a vaccine to provide passive protection to the new born calf against clinical disease caused by Cryptosporidium parvum.

Date Ackn'd 13/04/2007
Date Project Ceased 17/04/2009

Non-GMM Consent Granted Not Applicable
Project notified under transitional arrangements N

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
This project has transferred to GM391

Project Additional Information

Purposes of the contained use
Evaluation of potential constructs for the expression of the gene of interest, process development and scale up to 40L.

Recipient or parental organism
The recipient E. coli strain is a weakened laboratory adapted mutant and is regarded as non pathogenic. It is expected that the survivability of the bacterial host in the environment will be poor.

Host/vector system

02/03/2022 Page 7414 of 15326
The plasmid comprises a heat-inducible expression plasmid with a pUC origin of replication and tetracycline resistance gene plus the coding sequence for the gene of interest. The plasmid is non-conjugative and there is therefore minimal risk of transfer to other micro-organisms.

### Origin & Function

The gene sequence has been obtained from Cryptosporidium parvum and codes for a structural protein. It is not a known virulence determinant.

### Evaluation of foreseeable effects

As discussed.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be decontaminated by autoclaving using a validated temperature cycle designed to achieve 100% killing.

Liquid waste will be heat treated using a validated temperature cycle designed to achieve 100% killing.

No waste will be disposed of containing any live organisms.

---

**Project Containment**

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All solid waste will be decontaminated by autoclaving using a validated temperature cycle designed to achieve 100% killing.

Liquid waste will be heat treated using a validated temperature cycle designed to achieve 100% killing.

No waste will be disposed of containing any live organisms.

---

The risk assessments were reviewed by the GMSC on 5th March 2007. All elements were debated and the committee found that the information provided was in accordance with the 2000 regulations. The containment level applied was deemed to be appropriate for the activities and scale in order to reduce all risk to effectively zero.
**Project Ref**: 456/trans1

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<tr>
<th>Date Ackn'd</th>
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<td>DEVELOPMENT OF BACTERIAL DELETANT VACCINES</td>
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<th>Non-GMM Consent Granted</th>
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**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**: This project has transferred to GM391

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref 456/trans2**

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<td>VACCINIA VIRUS EXPRESSION OF ANTIGEN</td>
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Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements Y

Date Project Ceased

17/04/2009

Withdrawn N

Tick if notifying a connected programme of work N
**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

---

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**

- [N] No

**If yes, tick to confirm that it is attached to this form**

- [N] No
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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### Name

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<tr>
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| Name 2 | SCOTTISH GOVERNMENT | Department | MARINE LABORATORY |

<table>
<thead>
<tr>
<th>Campus Estate or Research Centre</th>
</tr>
</thead>
</table>

| Road Name | 375 VICTORIA RD | Building | PO BOX 101 |

| Town | ABERDEEN | District | TORRY |

| Tel Number | 01224 876544 | Fax Number | 01224 295511 |

| HSE Division | SCOTLAND |

### Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

<table>
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<th>Town</th>
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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

- **Tick if confidential**

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 457/01.1

**Date Ackn'd** 12/02/2001  
**CU2 Project Title** EXPRESSION OF INDIVIDUAL PROTEINS FOR CHARACTERISATION AND ANTIBODY PREPARATION

**Date Project Ceased** 16/07/2008  
**Class** Class 2  
**Culture Vol Class**  
**Culture Volume Class**

**Non-GMM**  
**Consent Granted** not applicable

**Project notified under transitional arrangements** Y

**Withdrawn** N  
**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Class**

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**Non-GMM**

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

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Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 457/09.1

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<tr>
<td>18/11/2009</td>
<td>Construction of a full length salmonid alphavirus clone, recovery of recombinant</td>
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<td>&lt;1 Litre</td>
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<tr>
<td></td>
<td>infectious virus in cell culture and subsequent infection of fish and cell cultures</td>
<td>Class 2</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Withdrawn [N] 

Tick if notifying a connected programme of work [N]
The overall objective of this project is to determine genetic loci in Salmonid Alphavirus RNA virus genomes which determine the phenotypic properties of these viruses in cell cultures and in vivo models. Such properties include, for example, ability to infect cells, ability to stimulate interferon and to activate other cellular genes, ability to trigger apoptosis, and ultimately ability to cause disease. These properties can be assessed both in vitro by infection of cells in culture and in established salmonid infection models. One key approach to be used will be to compare viruses with specific genetic modifications to parental virus. This will involve swapping sequences between strains which are known to exhibit different virulence properties and by deleting/mutating specific areas of sequence. Another key approach will be to insert reporter genes into the virus genome. In all cases, this work will involve engineering complete or partial cDNA copies of the viral genome. The virus to be used is salmonid pancreas disease virus (SPDV). This virus is the most significant viral disease of Atlantic salmon aquaculture in Scotland where it is responsible for considerable economic losses. The ubiquity of the pathogen, impact of the disease and lack of official control measures render it a threat to the long term sustainability of salmon aquaculture, in addition to raising welfare issues. This work will contribute to understanding the fundamental biological processes influencing the outcome of SPDV infection which is key to developing control and mitigation strategies that underpin a sustainable industry that can coexist with ubiquitous pathogens.

The alphavirus genome consists of a single strand of positive sense RNA of around 12 kb. This RNA codes for two open reading frames. The first codes for the non-structural viral replicase proteins (nsP1, 2, 3 & 4), the second for the viral structural proteins (C, E1, E2 & E3). Expression of the replicase proteins is under the control of a promoter (P) in the 5’ non-coding region of the virus. Expression of the structural proteins is under the control of a subgenomic promoter (SP) which is activated later in infection and leads to a high level of expression from this second open reading frame. The full genome sequence of SAV isolates is available and a strategy for generation of recombinant Sleeping Disease Virus (SDV) has previously been reported (Moriette et al 2006). The recovery of recombinant virus from cDNA is usually based on the transfection of cells with positive stranded RNA generated in vitro from SP6 or T7 driven full length cDNA transcripts. An alternative approach is to transfect cells with plasmids and provide T7 polymerase in situ by eg infecting cells with a recombinant vaccinia viral strain which expresses the T7 viral polymerase.

A similar strategy to those previously reported is planned for generation of recombinant SPDV. A plasmid will be generated that encodes the entire SAV genome under the control of a T7 promoter. Plasmids will be used to generate positive sense genome copies using T7 polymerase that will be used to transfect permissible cell lines. This is expected to result in the generation of infectious virus.

Recipient or parental organism

The GMM recipient organisms generated in this project include:
I) E.coli host cells transformed with plasmid DNA.
II) Eukaryotic cell lines transfected with plasmids for the purpose of virus expression.
III) Recombinant viruses in some cases bearing reporter genes as described above.
GMM type I risks

Only disabled E. coli K12 strains as defined in the ACGM Compendium of Guidance will be used as hosts. The project and risk assessment explicitly excludes use of other host species and other non-K12 strains of E. coli, including E. coli B strains such as BL21 and its derivatives. E.coli K-12 derivative strains may be considered as equivalent to ACDP hazard group 1 and are not considered pathogenic to humans or animals. They are expected to have limited survival in the environment and often have auxotrophic requirements unlikely to be satisfied outside of laboratory culture. None of the genes intended for expression encode any protein of detriment to human health nor derive from any organism known to cause a human health risk. Proteins will not be expressed in E.coli which in this instance is being used purely for the generation of plasmid DNA.

GMM type II risks

Generation of replicating virus is not considered to pose any greater risk to the recipient cell line than routine characterisation and isolation of fish alphavirus. Both fish and mammalian derived cell lines (eg BHK) may be used as host cell lines for initial generation of recombinant viruses, but this will occur at temperatures permissive for SPDV infection (<25ºC). These cell lines are well characterised and in routine laboratory use for isolation of viruses. No known human health or environmental hazards associated with the proposed cell lines has been identified.

GMM type III risks

Recombinant viruses will be used to re-infect cell lines for the purposes of studying viral pathogenesis as described above. Risks to the recipient cells will be no greater than those incurred in the primary generation of infectious virus described above. Recombinant viruses may also be used to infect live fish in established SPDV challenge models. Atlantic salmon are susceptible to SPDV. The focus on use of only naturally occurring and identified gene changes in generated viruses coupled to the fact that other changes are unlikely to dramatically increase the fitness of the virus will likely result in no dramatic increase in the pathogenicity of viruses above that of the wild type isolates.

GMM Risks type II

Inserted genes will be derived from SPDV. The intention is to generate replicatively active virus in cell lines. Generation of replicating virus is not considered to pose any greater risk to the recipient cell line than routine characterisation and isolation of fish alphavirus. Furthermore, virus will be replicated at temperatures below 25ºC will be used as host cell lines for generation of recombinant viruses: no fish viruses adapted to these lower temperatures are known to cause any hazard to humans.

GMM Risks type III

No risks beyond those described above are anticipated in infection of tissue cultured cells or live fish with recombinant SPDV.

Host/vector system

GMM type I risks

The only vectors that will be used will be well-characterised vectors that (i) are either non-mobilisable (eg pUC series and their derivatives) or mobilisation-defective (eg pBR322 and its derivatives) in bacteria; and (ii) contain only selective markers that are already in routine use in standard cloning vectors; and (iii) contain no recognisable harmful sequences. The primary purpose of this part of the work is to generate plasmids from which proteins will not be expressed in host E.coli strains used for propagation of plasmid. Fish pathogens are generally adapted to low temperatures and their proteins are often rendered inactive at the elevated temperatures associated with E.coli culture (37ºC). In the unlikely event that biologically active inserted proteins are produced, consequences for human health are not anticipated since gene products or indeed wild type SPDV are not known to cause any hazard to humans.

Origin & function

02/03/2022
The intention is to generate recombinant SAV viruses using a reverse genetics approach. Using this, SPDV will be genetically modified to identify and characterise genetic loci that determine the phenotypic properties of this important virus of Atlantic salmon. Specific gene loci will be targeted. Sequences may be swapped between different natural isolates that have varying phenotypes or they may be mutated or deleted. The only foreign genes that will be inserted are non-harmful marker or reporter genes. Changes in phenotypes will be assessed both in vitro and in vivo.

Recombinant viruses will be used to re-infect cell lines for the purposes of studying viral pathogenesis as described above. Risks to the recipient cells will be no greater that those incurred in the primary generation of infectious virus described above. Recombinant viruses may also be used to infect live fish in established SPDV challenge models. Atlantic salmon are susceptible to SPDV. The focus on use of only naturally occurring and identified gene changes in generated viruses coupled to the fact that other changes are unlikely to dramatically increase the fitness of the virus will likely result in no dramatic increase in the pathogenicity of viruses above that of the wild type isolates.

Evaluation of foreseeable effects

Virulent viruses will be selected for study. It is unlikely that any significant increase in virulence will be observed in this study since most changes would be expected to reduce virulence of the wild type virus strain.

RNA viruses exist as populations of genotypes and are selected for fitness by the systems in which they replicate. The predominant genotype in an RNA virus population is that most fit to survive the selective pressures encountered during virus replication and dissemination. Genotypes with all possible base changes at all possible positions are likely to be generated naturally in the course of replication of RNA viruses. The cDNA clones of the laboratory strains of SPDV to be used in this project represent single genotypes selected from natural isolates by molecular cloning. It is likely that these cDNAs represent genotypes abundant in the original natural virus isolate and therefore genotypes most fit for replication. Most point mutations or deletions introduced into such viruses can be expected to attenuate fitness. However, base changes in the structural, replicase genes or non-coding sequences of avirulent virus cDNAs which change the sequence to that of a more virulent virus cDNA would be expected, at least in some cases, to increase virulence but only to a level seen in wild type isolates. With the exception of reporter genes, known to be harmless to humans, the engineered recombinant viruses to be generated will either contain only sequences found in natural virus isolates or specific point mutations or deletions; they are therefore likely to remain non-infective for humans. Any transfer of genetic material will only be between different strains of naturally occurring viruses. The studies in this project are therefore unlikely to change the host range or tropism of these viruses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment and Control Measures

Aquarium Control Measures

The waste water treatment system provides treatment of waste water from the disease aquarium facility. It caters for a flow of up to 10m3/hour of freshwater and sea water. The treatment is Ozone and there is a sodium hypochlorite solution standby dosing system and generator in the event of a plant failure or power failure which automatically activates on plant failure. Once the water has passed through the system it leaves the degassing tank and flows to the domestic sewage works at Nigg Bay. Once at Nigg Bay sewage works the sludge is removed and incinerated to produce electricity and the waste water is then treated by UV irradiation. However this supplementary treatment process is not considered as part of the treatment system.

Entry to the aquaria facility is highly controlled and monitored. Specific documented procedures exist for entry/exit and disinfection of all material originating from the
aquarium. This includes onsite autoclaving and subsequent incineration of all potentially infectious material generated within the facility. Specific risk assessments are generated for all planned experiments. These are scrutinised and signed off by a local bio-security officer. Additional precautions to be taken in the aquaria during experimentation with GMMs would include collection of all waste water and chemical treatment (eg hypochlorite) in the aquaria prior to discharge of waste via the effluent treatment plant described above.

Laboratory Control Measures

Standard tissue culture protocols will be used to grow and maintain stocks of viruses and to assay for virus infectivity. These involve typical volumes of infectious material of <500 ml. All tissue culture work will be carried out in a microbiological safety cabinet. In other procedures where aerosols may be generated, for example homogenisation of tissues, a microbiological safety cabinet will also be used. Laboratories are designed to comply with ACDP containment level 2. All surfaces (benches, walls, floors) are impervious to water, easy to clean and resistant to acids, alkalis, solvents and disinfectants. All tissue culture procedures are conducted within contained laminar flow hoods to minimise risk to fragile cultures. All GMM solid waste is collected and autoclaved prior to incineration. All liquid waste is chemically disinfected with Microsol prior to autoclaving.

Building Physical Measures

Laboratories are designed to comply with ACDP containment level 2. All surfaces (benches, walls, floors) are impervious to water, easy to clean and resistant to acids, alkalis, solvents and disinfectants. All tissue culture procedures are conducted within contained laminar flow hoods to minimise risk to fragile cultures.

Inactivation and disposal

All contaminated material solid and small quantities of sealed liquid waste, will be placed in identifiable biohazard (blue) autoclave bags, transferred securely to the autoclave and inactivated on site by autoclaving prior to incineration by a third party. Reusable laboratory equipment, such as glassware will be soaked in Microsol (10%) immediately after use, prior to washing and drying. Liquid waste will be treated with Microsol (10%) or Virkon and autoclaved prior to disposal down the sink. Sharps (in sharps bin, eg needles, syringes, scalpels) – will be autoclaved and disposed of via the clinical waste stream for incineration. All solid waste from the aquaria is autoclaved and disposed of via the clinical waste stream for incineration.

Validation and Monitoring of control methods

The waste autoclave is monitored under the UKAS quality system and is serviced and calibrated annually. Individual run data is recorded to ensure the appropriate temperature is reached (121°C) for each run.

Aquarium

Previous experimental work to determine the efficacy of aquaria ozone disinfection system demonstrated that a single pass through the ozone system produced a 3.75 log reduction of IPNV isolate 975/99. This virus represents one of the hardiest viruses known in terms of its ability to persist in the environment.

The plant is checked twice daily and measurements taken for the following:-

-Ozone alarm system (daily check recorded)
-Ozone Redox Potential (ORP), (daily check recorded). FRS Standard Operating Procedures (SOP’s) state that ORP readings must be above 700mV. Fraser D.I., Munro P.D. and Smail D.A. (2006) Fisheries Research Services Internal Report No 13/06 DISINFECTION GUIDE VERSION IV PRACTICAL STEPS TO PREVENT THE
INTRODUCTION AND MINIMISE TRANSMISSION OF DISEASES OF FISH, recommend that a ORP of between 600-750mV is used to disinfect effluent water.


-Ozone generator flows daily (the generator that is running should have the float at the red line).
-Generator pumps daily
-Ambient and exhaust generator readings daily (below 0.1ppm)

These records are kept in the Ozone plant room.

There are 2 ozone generators one duty and one standby in case of failure etc., both generators are serviced on a 6 monthly basis.

The sodium hypochlorite dosing pumps are tested every month when the ozone system is shut down to change the activated carbon. Fraser D.I., Munro P.D. and Smail D.A. (2006) Fisheries Research Services Internal Report No 13/06 DISINFECTION GUIDE VERSION IV PRACTICAL STEPS TO PREVENT THE INTRODUCTION AND MINIMISE TRANSMISSION OF DISEASES OF FISH, recommend that processing plant effluent is treated with a dose of 1000ppm sodium hypochloride solution for 10min to disinfect the effluent. At FRS the sodium hypochlorite standby dosing pumps 1000 ppm sodium hypochloride solution, minimum contact time 12 hours.


There are 2 pumps with auto change over one duty and one standby, that pump the waste water through the contact chamber.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Comments from the GMSC were included in the risk assessment. The GMSC comprises Senior management, Union and Biosecurity experts. External advice was also sought from Prof ********* (university of Edinburgh), a collaborator and acknowledged alphavirus expert. Most comments were minor edits and did not fundamentally change the scope or outcome of the assessment. The GMSC and Prof ********* agreed the risk assessment was suitable and categorisation of the work as class 2.

Project Containment

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### Name

- **Name**: OXFORD UNIVERSITY HOSPITALS NHS TRUST
- **Name 2**: JOHN RADCLIFFE HOSPITAL

### Address

- **Campus Estate or Research Centre**: Headley Way
- **Road Name**: Headley Way
- **District**: Headington
- **Town**: Oxford
- **County**: Oxfordshire
- **Postcode**: OX3 9DU
- **Country**: England

### Contact Information

- **Tel Number**: 01865 741166
- **Fax Number**: NONE
- **E-mail**: NONE
- **HSE Division**: EAST AND SOUTH EAST

### Comments

- **Date at Which Additional Info Submitted**: 02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

[ ]

Give brief details of the genetic modification safety committee

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[ ] Tick if confidential

- **Bacteriology**
  - Parasitology
  - Transgenic Birds
  - Microbiology Research

- **Virology**
  - Transgenic Animals
  - Transgenic Fish
  - Gene Therapy
Mycology

Transgenic

Invertebrates

Transgenic

Plants

Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Site 1: Maximum culture released at any one time is one litre. Supernatant is treated with bleach, left for 2 hours then disposed to drain with copious amounts of water. Approximately one bacterial transformation and one large scale probe preparation is performed each year.

Site 2: All waste autoclaved @ 121 deg c for 15 minutes. Autoclave is serviced every six months. Thermocouple testing is done. Autoclave log kept and autoclave tape used.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 462/05.1

CU2 Project Title

A clinical trial to assess the benefit of two different types of virus-based vaccines (vaccinia and fowlpox) in the setting of early stage colorectal cancer.

Class CultureVol

Class 2 < 1 Litre

Consent Granted

Not Applicable

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022  Page 7435 of 15326
Project Additional Information

Purposes of the contained use

During the trial we will be delivering genetically modified virus vaccines to human volunteers to try to stimulate their immune system to fight off any residual cancer cells. We will be specifically delivering the virus vaccines, PANVAC-V and PANVAC-F to patients who have had an operation to remove a colorectal cancer.

Recipient or parental organism

Human volunteers (cancer patients).

The viruses have been grown in chick embryo dermal cells at the provider company in the USA.

Host/vector system

The genetically modified viruses that we will use will be PANVAC-V and PANVAC-F. The virus backbones used are a) a vaccinia virus derived from the Syeth vaccination strain (class 2 containment) and b) a fowlpox virus (class 1 containment). Vaccinia has been found to be an excellent primer of the immune system but cannot be used repeatedly because of the antibody response against the vaccinia's own proteins. However experiments have determined that using repeated fowlpox vaccinations after the vaccinia prime leads to repeated boosting of the primary response with continuing increases in specific immune activity to at least five boost injections.

The virus vaccines will be administered by injection under the skin of cancer patients that have had their primary cancer removed.

Origin & function

Each of these viruses has been modified so that they also contain the genes of five human proteins: carcino-embryonic antigen (CEA), mucin-1 (Muc-1), lymphocyte function antigen 3 (LFA3), ICAM-1 and B7.1.

CEAcand MUC-1 are over-expressed in solid cancers but are not carcinogenic in their own right. The remaining three proteins are natural human co-stimulatory molecules, which allow maximum activation of the immune system.

The intended functions are to stimulate the immune system to fight residual cancer cells that carry the proteins CEA and MUC-1.

Evaluation of foreseeable effects

The main foreseeable event is stimulation of the immune system. Volunteers may experience reddening at the site of inoculation and mild flu-like symptoms for 24-48 hours.

The fowlpox virus is unable to replicate in human hosts and for this particular strain is no longer capable of replicating effectively even in fowl hosts. Therefore we do not anticipate any problems with PANVAC-F.

The vaccinia backbone has a slight risk of vaccinia-related illness as recognised during the worldwide smallpox eradication program. However the incidence of these effects were in the order of 14 per million vaccinations.

The vast majority of people accidentally or, in the case of the trial, intentionally infected with vaccinia will suffer very few ill effects because of the effectiveness of the antibody response in humans against this organism. However the patients that are immunosuppressed and those with a history of severe eczema there is an increases risk of vaccinia related illnesses including generalised vaccinia and vaccinia eczematum (a skin condition associated with vaccinia which can be serious). For this reason we will exclude patients that are HIV positive and we will screen patients for immunoglobulin status. An occupational health assessment will also be performed so that workers can be screened to ensure that they are not put at risk of problems in the unlikely event of accidental infection.
The inserts are human proteins whose functions are well characterised. Modification by insertion into the viral vectors is not expected to change their biological activities.

Stringent procedures under clinical trial conditions have been designed to prevent any release into the environment. The virus does not appear to spread effectively by aerosol spray and there is no evidence of shedding of virus from vaccinated individuals. Stand operating procedures (SOPs) have been set up within the unit for all activities surrounding the trial from receipt of the material, storage, administration, clean down in the lab, disposal of contaminated waste, and what to do in the event of any incident. The unit has experienced in gene therapy trials using class I organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Class II containment requires disposal by validated means and an autoclave sited in the building where the waste is generated. The unit where this work is to take place (Cancer Research UK, Churchill Hospital) do not have an autoclave. Currently class I waste is placed in sealed bins and collected as soon as possible for offsite disposal by an independent contractor approved for disposal of GM waste. Separating class I waste (fowlpox derived) from class II (vaccinia) for this trial would not be practical.

Derogation is requested so as to allow this route of disposal to be extended to class II waste from clinical trials. Autoclave facilities on the Churchill site are located some distance from the Unit. Transport of waste across site would be difficult with inherent risk of delays and errors. It would require transport across areas used by staff, patients and hospital visitors. We believe in current system of off-site disposal in sealed robust bins, which works well for class I waste to date, is the safest route of disposal at this time.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste contaminated with vaccine will be disposed of according to the local SOP ie transferred immediately into robust bins, sealed and collected by an approved external contract for off site incineration (subject to approval of the derogation requested above). Waste material will include the vials in which the vaccines arrive, syringes and needles used for injection and the dressings applied to the injection site. Expected degree of kill: effectively 100%.

Preparation areas will be cleaned with 2% Virkon according to local SOPs. Should any spills occur volumes will be very small, inactivation will be with Virkon. Absorbant materials used in cleaning will then be disposed of as above. Expected degree of kill: Effectively 100%.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

GMSC approval at Class II activity under the Genetically Modified (CU) Regulations 2000.

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Comments

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Maximum culture volumes are 1000 ml (routinely only 10-15 ml) and all organisms are destroyed by autoclaving at 121 degrees C for 15 mins. Genetically modified micro-organisms are typically more fragile than wild type organisms and are rendered non-viable at temperatures well below this value. The effectiveness of the procedure will be monitored at monthly intervals using steriliser control tubes ("Browne Tubes") suitable for autoclaves operating at up to 126 degrees C.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 463/14.1

Date Ackn'd CU2 Project Title
11/02/2014 Genetic modification of Plasmodium falciparum

Date Project Ceased

Class CultureVol Class 2 CultureVolume Class 3-4
Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Purposes of the contained use

The aims of this work are to transfect exogenous DNA into Plasmodium falciparum for in vitro studies to:

1. Provide for reporter gene studies on the (i) structure and function of nucleic acid regulatory sequences and (ii) action of antimalarial agents
2. Label genes with tags to provide for isolation of interacting proteins, cell localisation and trafficking studies
3. Explore the function of genes through knock-out and knock-in studies

Recipient or parental organism

The recipient organism is Plasmodium falciparum (ACDP hazard group 3*), the aetiological agent of malaria in humans. The * refers to this organism being listed in the Certificate of Exemption accompanying the ACDP Approved List for the classification of microorganisms – this organism is not transmissible though aerosol routes.

Host/vector system

Plasmid vector- these are members of the pUC series plasmids (eg pBluescript SK+). Novel sequences introduced are listed below. Otherwise no other modifications to the ampicillin or kanamycin selection cassette, multiple cloning site and bacterial origin of replication are made to this plasmid.

Origin & function

a) Plasmodium coding or non-coding sequences:

(i) P. falciparum coding sequences

Full (or partial) open reading frames would be derived from P. falciparum, and therefore would not increase level of risk of the GMO. Of the P. falciparum sequences to be used, the functions of only a few are linked to virulence (e.g. several naturally occurring variants of the PfEMP1 protein are known to bind to human endothelial receptors CD36 and ICAM-1) but since these forms exist in nature, they would not increase the level of risk of the resultant GMO. Some constructs designed for stable integration are, by design, incomplete open reading frames. Such constructs would not alter the pathogenicity of the final GMO because they aim to delete or disrupt the target gene, causing loss of function. Knock-in of endogenous sequences to complement loss-of-function produced by these knock out experiments will be required to replace an existing function and therefore are unlikely to affect pathogenicity.

(ii) Non-coding regulatory sequences:

These are necessary for expression of the selectable marker or inserted coding sequence, and are not likely to alter the pathogenicity of the final GMO, since they are not translated. These sequences may be transcribed (e.g. 5’ and 3’ untranslated regions of genes), but their transcription will lead only to the expression of genes that naturally occur in the parasite, or of non-pathogenic reporter genes, so the pathogenicity of the resultant GMO is not considered likely to be increased.

b) Reporter genes/tags:

Coding or flanking sequence of genes will be placed up or downstream from reporter genes/tags. None of these reporter genes are likely to affect the virulence or therapeutic drug sensitivity of the transfected organisms, since the altered proteins thus expressed will either contain portions of proteins normally expressed by the parasite and/or reporter gene proteins which are not hazardous (eg. luciferase and GFP). The reporters are standard, used in many different experimental systems and are highly unlikely to affect the pathogenicity of an organism. Specifically, for each reporter gene:

(i) HA epitope tag: a short (10-12 amino acid) epitope derived from influenza haemagglutinin. It is highly immunogenic and therefore easily detected with commercial
antibodies. In itself it has no biological function.

(ii) Myc epitope tag: a short (10 amino acid) epitope from human c-myc, detected with commercial antibodies as for the myc tag. In itself it has no biological function.

(iii) LUC: Phontinus pyralis (firefly) luciferase (or derivatives) is an enzyme which catalyzes production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function.

(iv) GFP and derivatives: Aequorea victoria (jellyfish) green fluorescent protein emits green light when excited by blue/UV light, and has no other known biological function. Its derivatives emit other colours of light, and again, have no other known functions.

c) Drug resistance genes and other selectable markers: P. falciparum sequences will be inserted alongside one or more drug selectable markers. Parasites expressing the transferred genes can then be selected in vitro by addition of the appropriate drug to the culture medium.

(i) The human dihydrofolate reductase (DHFR) gene provides resistance to pyrimethamine or antifolate analogues (e.g. WR99210). The resultant parasites will by definition be resistant to pyrimethamine, but since (a) the P. falciparum pyrimethamine-resistant DHFR is a naturally occurring mutation and (b) for this reason pyrimethamine is not used as a treatment for infection in the UK (and most regions globally), this does not affect the therapeutic drug sensitivity of the organism. Note also that transfected drug resistant genes confer a less-resistant folate-resistant phenotype than naturally occurring resistant alleles. Reduced levels or altered timing of gene expression are the probable cause (Lakshmanan et al (2005) EMBO 24(13):2294-305 and Crabb and Cowman (1996) PNAS 93(14):7289-94)

(ii) Other selectable markers: Genes from various bacteria confer resistance to: bleomycin, phleomycin, neomycin (geneticin, G418), tetracycline, blasticidin. In all cases, the drug used for selection would never be used for treatment of an unlikely accidental infection with the GMO, so these would not alter the ability to treat disease.

Evaluation of foreseeable effects

P. falciparum is one of five Plasmodium spp. aetiological agents of human malaria - left untreated, human malaria can cause a life-threatening illness.

The genetic modifications proposed (see previous section) specifically outline our risk evaluation with respect to any potential alteration to the hazards to human health of this organism. This, and the attached risk assessment, lead us to conclude that the transgenic Plasmodium falciparum pose no more of a hazard to human health and the environment than an unmodified Plasmodium falciparum. Given known issues with plasmid loss following genetic modification and often reduced replication rates following transfection, realistically these GMOs are actually considered less likely to be a hazard.

Presented below is information that considers effects on the risk of genetic modification to alterations to host range/tropism, altered interactions with a human host immune system and susceptibility to antimalarial therapy. These will outline our basis for an evaluation that insertion of these exogenous sequences will not affect the interaction with the human host. We do not outline here the issue of transmission. Our studies will not explore the transmission of the parasite by the mosquito vector - a risk analysis is thus primarily centred around the extant risk of the unmodified parasite - and as advised in note 6 is considered fully in the attached risk assessment.

a) Alterations to host range and tissue tropism
The inserted genes will not alter the host range of the transfected P. falciparum populations. Any genes inserted which could possibly affect tissue tropism (specifically, var genes encoding members of the clonally variant Plasmodium falciparum erythrocyte membrane protein 1, PfEMP1) are naturally occurring P. falciparum genes, and therefore do not change the tissue tropism.

b) Altered interactions with host defences
No alterations in the interaction of genetically manipulated P. falciparum with host defences are anticipated. Protein will be expressed in both transient and stable transfectants. To interact with the human immune system, these proteins must be exported to the host cell surface. Protein expressed here (such as PfEMP1) undergo clonal antigenic variation as a means of immune escape, and as such the expression of a constant epitope at the same site would only reduce virulence of the GMO.

c) Susceptibility to antimalarial drugs
Laboratory clones of P. falciparum will be rendered resistant to selection drugs: pyrimethamine (and antifolate analogues), bleomycin, phleomycin, neomycin (geneticin, G418), tetracycline and blasticidin. None of these drugs would be used for treatment in the event of accidental infection (HPA guidelines direct the use of quinine, and doxycycline/clindamycin or Malarone or an artemesinin combination therapy) The drug-resistance markers used to select transfected parasites will not therefore affect...
treatment of any accidental infections. Parasites in use within the laboratory are thus sensitive to standard chemotherapeutic treatment of malaria in the UK.

Pyrimethamine resistance will not compromise therapy as this would not be used as a first line treatment in this country in any case. Inserted sequences from other Plasmodium genes and reporter genes would not affect drug sensitivity, since these gene products are not involved in drug resistance.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable here.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The recipient organism is P. falciparum (ACDP Hazard Group 3). P. falciparum has a Certificate of Exemption accompanying the ACDP Approved List as this organism is not transmissible though aerosol routes. The secure cell culture facilities therefore do not include the following measures:

1. Laboratory sealable for fumigation.
2. Negative pressure relative to the immediate surroundings.
3. The naturally occurring organism does not represent any hazard of infection through aerosol transfer, therefore face masks are not required for this work.

The Code of Practice for use of the cell culture suite does, however, require the following control measures to be in place - based on the extant risk of the parasite to human health;

1. Use of regularly maintained and tested Class II microbiology safety cabinets (annual serice contract includes testing and validation of HEPA filters and KI dicus tests).
2. Use of microbiology jacket and non-latex gloves.
3. No sharps in the cell culture facility.
4. Safe and effective autoclave of materials within the cell culture facility (dedicated autoclave within the CL3 facility is subject to annual service contract with validation of both pressure and temperature).
5. Induction and probationary training supervised by experienced user.
6. All work is carried out in a secure cell culture facility, with access restricted to named individuals and swipe card access to the main laboratory and keypad access to cell culture suite.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Both solid and liquid waste are generated from work with transgenic P. falciparum.

Solid waste includes soiled plasticware/paperware and microscope slides. Soiled plasticware/paperware waste is treated with a 1-5% Virkon multi-purpose disinfectant solution for at least 10 minutes – this provides for complete lysis of the infected erythrocyte and parasite. This waste is secured in an autoclave bag loosely sealed at the neck, which is then contained in a second autoclave bag (left unsealed) to reduce risk of plasticware penetrating out of the bag. These are stored in an upright plastic bin (covered with a lid when not in use) until ¾ full. A trained user then takes the material into the cell culture lobby (no more than 5M) to the autoclave. The cycle employed is 121-125 degrees C for at least 15 minutes. The autoclave is regularly maintained and validated under an annual contract. Regular monitoring of activity using autoclave tape that changes colour on reaching the correct temperature for the appropriate time. The autoclaved material is then disposed of with other laboratory waste – this is removed for incineration via a commercial contract held by the University. Glass slides are air-dried and fixed with methanol – this kills the parasites. The slide is then disposed of via yellow hard sharps bins – again taken for incineration via a commercial contract.

Liquid waste is collected via vacuum into traps containing c 500mL of 10% Virkon. These are regularly changed (at least twice a week), with monitoring of effectiveness provided by changes from the pink/red colouring agent. Liquid waste is disposed of via drains with copious volumes of cold water.

An in vivo determination of the degree of kill after Virkon treatment is not possible as addition of Virkon to cultures of parasites congeals the lysed cells to a slurry. The only means of absolute detection of living parasites would require be injection of this slurry into a primate, and the Virkon itself would be toxic. By means of an in vitro assessment, no intact parasite or red blood cell is visible via light microscopy of a 5% haematocrit 2% parasitaemia culture (typically maintained in the laboratory) with an equal volume of 1% Virkon solution after 10 minutes treatment at room temperature. The stages of parasite used in this laboratory absolutely require a viable erythrocyte -
and under the conditions provided in our disinfectant procedures, these are not available.

The risk assessment (Project ID 54) was considered at a full meeting of the GMSSC on 3 February 2014. In advance of this meeting, drafts of the risk assessment were discussed informally with the Chair. For reasons of simplicity and clarity, it was agreed that the PI would remove those sections dealing with standard cloning in disabled E. coli strains through which transformation vectors for use in P. falciparum were generated. These activities, which are clearly Class 1 and do not require notification to HSE, are covered in a separately approved risk assessment (Project ID 41). Based on a discussion of the P. falciparum risk assessment (Project ID 54) it was agreed that this work should be notified to HSE as a Class 2 activity, supported by the risk assessment and full Codes of Practice. The committee approved the risk assessment and felt that the codes of practice for working with P. falciparum within the secure culture facility were both well-considered and appropriate. Within the codes of practice, some modifications to the training records were requested so that it could be clearly identified what competences had been achieved by registered workers following training. I can confirm that the necessary changes have been made to the codes of practice. The membership of the GMSSC includes a representative of the trades union recognized by Keele University and I can confirm that the outcomes of the meeting on 3 February 2014 have been disseminated to all applicable unions (UCU, UNITE and UNISON). The GMSSC also includes representatives of the Joint Workplace Safety Committee and I can confirm that the outcomes of the GMSSC meeting will be reported appropriately at the next meeting of the JWSC. (Paul Eggleston, Chair - GMSSC).

Please enter comments on the GM safety committee on the risk assessment

The risk assessment (Project ID 54) was considered at a full meeting of the GMSSC on 3 February 2014. In advance of this meeting, drafts of the risk assessment were discussed informally with the Chair. For reasons of simplicity and clarity, it was agreed that the PI would remove those sections dealing with standard cloning in disabled E. coli strains through which transformation vectors for use in P. falciparum were generated. These activities, which are clearly Class 1 and do not require notification to HSE, are covered in a separately approved risk assessment (Project ID 41). Based on a discussion of the P. falciparum risk assessment (Project ID 54) it was agreed that this work should be notified to HSE as a Class 2 activity, supported by the risk assessment and full Codes of Practice. The committee approved the risk assessment and felt that the codes of practice for working with P. falciparum within the secure culture facility were both well-considered and appropriate. Within the codes of practice, some modifications to the training records were requested so that it could be clearly identified what competences had been achieved by registered workers following training. I can confirm that the necessary changes have been made to the codes of practice. The membership of the GMSSC includes a representative of the trades union recognized by Keele University and I can confirm that the outcomes of the meeting on 3 February 2014 have been disseminated to all applicable unions (UCU, UNITE and UNISON). The GMSSC also includes representatives of the Joint Workplace Safety Committee and I can confirm that the outcomes of the GMSSC meeting will be reported appropriately at the next meeting of the JWSC. (Paul Eggleston, Chair - GMSSC).

Project Containment

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<th>Human Clinical Applications</th>
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Project Ref 463/14.2

Date Ackn'd 18/03/2014

CU2 Project Title Molecular cell biology of Leishmania and Trypanosoma brucei

Class 2

CultureVol Class 2 1-50 Litres

CultureVolume Class 3-4 < 1 Litre

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Purposes of the contained use

- To target specific genes for disruption by RNA interference or by homologous recombination to generate null, loss-of-function or gain-of-function mutants for phenotypic analyses, by the introduction of antibiotic resistance genes into chromosomal sites in the genomes of Leishmania or T. brucei.
- To clone Leishmania and Trypanosoma brucei genes into plasmid vectors engineered for prokaryotic expression: to express and purify the target proteins for further analyses.
- To evaluate the ability of compounds to kill parasites in vitro, towards development of new drugs

Recipient or parental organism

- Leishmania major MHOM/IL/81 Friedlin
- Leishmania mexicana
- Leishmania donovani MHOM/SD/62/IS-CL2D
- Leishmania infantum MCAN/es/98/LLM-724
- Leishmania (Viannia) braziliensis MHOM/BR/75/M2904
- Leishmania amazonensis
- Trypanosoma brucei brucei Lister 427 and GVR35
- Trypanosoma brucei rhodesiense

Host/vector system

Non-mobilisable plasmid shuttle vectors: pUC based such as the pBS or pSK series including eukaryotic sequences to allow chromosomal integration or expression of introduced genes. Plasmid vector p2T7Ti will be used for RNA interference in T. brucei.

Origin & function

The sources of the genetic material are as follows:

i) Genomic DNA from Leishmania spp. or T. brucei e.g. the BBS1 gene which encodes a component of the flagellum-associated BBSome complex. Leishmania and T. brucei genomic DNA and parasite lines will be obtained from Prof. D. F. Smith and Prof. Paul Kaye at the University of York, UK.
ii) Reporter genes or tagging epitopes: Parasite sequences may be placed adjacent to these to study expression or to create tagged proteins. Standard reporter genes and epitope tags (functions as indicated by names) include:
- c-myc epitope [Myc tag], human
- luciferase [LUC], firefly (Phontinus pyralis)
- green fluorescent protein [GFP and derivatives], jellyfish (Aequorea victoria)

iii) Drug resistance and selection markers: these permit the maintenance of transfected plasmids
- E. coli tetracycline repressor gene (TET) -- confers regulatable gene expression
- Hygromycin phosphotransferase (HYG) – hygromycin resistance
- Bleomycin resistance gene (BLE) – phleomycin resistance
- Puromycin acetyltransferase (PAC) – puromycin resistance
- Bacterial neomycin phosphotransferase (NEO) -- neomycin resistance

**Evaluation of foreseeable effects**

Species of Leishmania are causative agents of human leishmaniasis; the parasite species L. donovani, L. infantum, L. braziliensis are designated hazard group 3 (ACDP categorisation). The remaining species covered by this notification, L. major and L. mexicana, are hazard group 2 (ACDP categorisation). The trypanosome subspecies Trypanosoma brucei rhodesiense is a causative agent of human African trypanosomiasis, while T. brucei brucei is non-infective to humans but is listed by DEFRA as a Specified Animal Pathogen (cattle), therefore a SAPO licence is required. For susceptible individuals, both leishmaniasis and African trypanosomiasis are treatable (as detailed in the Keele University Code of Practice, Dec 2013). The pathogenicity of Leishmania and T. brucei species and the hazards these impose to experimental work in the School of Life Sciences are covered by the Keele University Code of Practice.

Genetically-modified and parental lines of Leishmania and T. brucei will be subject to the same safety protocols; the environmental risk is therefore no greater than with unmodified parasites. The life cycle of Leishmania involves transmission of extra-cellular parasite stages between hosts by a sandfly vector, followed by intracellular maintenance within macrophages in the mammalian host. No other organism is known to be at risk from infection. A sandfly colony is maintained in a different laboratory in the Huxley building and safety procedures are detailed in the Code of Practice to prevent accidental infection of the vector with Leishmania. Permission is not requested at the current time to undertake experimental infections of sandflies with Leishmania. The life cycle of T. brucei involves transmission of extra-cellular parasite stages between hosts by a tsetse fly vector. There are no tsetse fly colonies at Keele and the insect is geographically restricted to regions of sub Saharan Africa, therefore insect transmission of parasites from the laboratory is not possible.

Transgenic Leishmania and T. brucei will be resistant to one or more antibiotics used for selection (tetracycline, hygromycin, phleomycin, puromycin, neomycin). This is irrelevant to therapy against human disease where other drugs of choice (e.g. pentavalent antimonials, amphotericin B, miltefosine) are used for treatment.

The target genes for manipulation will be wild type Leishmania or T. brucei sequences that have been fully characterised by DNA sequencing. The products of these genes are not known to have a role in the progression of human disease but this possibility cannot be discounted for those genes coding for proteins of unknown function. The GMOs may express proteins at a level significantly higher than wild type parasites that have not been genetically-modified.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

The ACDP Approved List of biological Agents (2013) classifies L. donovani, L. braziliensis and T. brucei rhodesiense as CL3 species to which derogation can be applied, as these pathogens are not transmissible through the aerosol droplet route. This is also detailed in Appendix 3.2 of the HSE publication "Biological agents: managing the risks in laboratories and healthcare premises". The main physical control measures that are not required are:

i) Laboratory sealable for fumigation
ii) Negative pressure relative to the pressure of the immediate surroundings
iii) Extract and input air from the laboratory should be HEPA filtered
iv) Microbiological safety cabinet/enclosure

In practice, measures (iii) and (iv) will be in place in the CL3 laboratory. A Class II microbiological safety cabinet will be used in the dedicated laboratory for sterile culture of the organism but is not a requirement for operator safety. Activities will involve small scale, standard laboratory techniques with no aerosol generating procedures.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM waste is of 2 types: liquid and solid.
Liquid GMM waste: is treated by chemical disinfection, using Distel detergent diluted 1:10 with contact time >12 hours. This gives 100% kill, as validated by testing described in the attached risk assessments.
Solid GMM waste: is autoclaved at 134 degrees C for 15 minutes at 27psi pressure. This gives 100% kill of Leishmania and Trypanosoma brucei, as validated by testing described in the attached documentation. All solid waste is sealed in bags and transported directly from the culture laboratory to the autoclave contained within the CL3 suite. This waste is handled by authorised personnel only. After autoclaving, the material is stored in a designated bin until collected for incineration.

Is an emergency plan required according to regulation 20?  N

Tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment (Project ID 57) was considered at a full meeting of the GMSSC on 3 February 2014. In advance of this meeting, drafts of the risk assessment were discussed informally with the Chair. Further clarification on various issues was also sought from experienced former colleagues of Dr Price at The University of York. Based on a discussion of the proposed work as a whole, it was agreed that it should be notified to HSE as a combination of Class 2 and Class 3 activities, dependent on the ACDP hazard group classifications of the relevant species, and supported by a copy of the approved risk assessment and full Codes of Practice. The committee approved the risk assessment and felt that the codes of practice for working with both Leishmania and Trypanosoma within the secure culture facility were both well-considered and appropriate. Within the codes of practice, some modifications to the training records were requested so that it could be clearly identified what competences had been achieved by registered workers following training. The GMSSC also noted that Dr Price will be submitting a COSHH CBA1 notification for first use of non-genetically modified parasites and a separate SAPO application to DEFRA covering the work with the cattle pathogen T. brucei. The membership of the GMSSC includes a representative of the trades union recognized by Keele University and I can confirm that the outcomes of the meeting on 3 February 2014 have been disseminated to all applicable unions (UCU, UNITE and UNISON). The GMSSC also includes representatives of the Joint Workplace Safety Committee and I can confirm that the outcomes of the GMSSC meeting will be reported appropriately at the next meeting of the JWSC. (Paul Eggleston, Chair - GMSSC).

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Project Ref 463/19.1

Arbovirus production from infectious clones and genetic modification to examine the replication and infectivity of these viruses and their interactions with mammalian and invertebrate host cells.

Date Ackn’d 05/04/2019

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The PI has 15 years experience working with CL2 viruses and 11 years working with CL3 viruses in containment.

The purpose of the contained use is several fold and described below. The research carried out focuses primarily on the interaction of virus minority variants with the host cell. We use RNA viruses as a model system, in particular we use positive stranded RNA viruses of the Togaviridae (Alphaviruses) and Flaviviridae family. These are of primary interest as they infect both arthropod and mammalian hosts, which allows us to study evolution and host adaptation during a transmission cycle. We will primarily use Semliki forest virus and Sindbis virus from the Alphaviruses and Zika virus from the Flaviviruses as our model organisms, but other ACDP2 alphaviruses may also be used as listed below.

1. To generate stocks of viruses from infectious clones.
   We use infectious clones electrophoresed into Vero cells to generate wild-type viruses as repeated cell culture passages of viruses can alter the specific infectivity of the viruses in certain cells. Therefore initially, stocks will be created from viruses that are generated using from plasmids using in vitro transcription and electroporation of the viral RNA. The resulting viruses will be stored at -80 degrees until used for viral infections.

2. To determine infection and replication competence of viruses in particular cell types.
   The viruses generated above are used to infect different cell lines including but not limited to the mammalian cell lines.
Infections will be used to measure replication and infection by using standard growth curves and measure virus titer using standard plaque assays. The RNA of viruses will also be used for next generation sequencing to determine the effect of different cell types on RNA secondary structure and the production of minority variants.

To determine the effect of RNA secondary structure on the generation of virus minority variants

Infectious clones will be modified to generate viruses that have the secondary structure altered while retaining the protein coding region of the viruses. These viruses will be used to generate infections in the cell lines described above to determine how the secondary structure affects the production of minority variants.

To determine the effect of changes in mutation rate/fidelity in the virus

Mutations identified in Venezuelan equine encephalitis virus and West Nile virus (Kautz et al. 2018, Van Slyke et al. 2015) that result in changes in replication fidelity or alterations in the mutation rate of specific mutations, will be introduced into the infectious clones. Our experience with such mutations indicates that they will result in attenuation of the viruses. We will investigate the method of attenuation and the effect of these mutations on virus replication and virulence as well as the interaction of these viruses with the host immune system.

To determine the effect of minority variants on host cell interactions

Specific mutations and deletions that are found during natural replication will be incorporated into the infectious clones. These altered viruses will be rescued and assessed for their ability to replicate and infect other cells. Our experience of such mutants is that many are not viable and those that are able to produce virus show evidence of attenuation.

To infect mosquitoes to determine competence for particular viral mutations

The specific mutations associated with minority variants and with replication fidelity/mutation change will be used to establish infections in Aedine and Culicine mosquitoes. The only GM organism will be the virus, the mosquitoes will be wild-type and not GMO modified. Experimental infections will be carried out in small containers within a biosafety cabinet and mosquitoes will only be manipulated when anaesthetized and on ice. Once the mosquitoes have been incubated for 12-21 days, mosquitoes will be dissected and the resulting tissues tested for the presence of virus using the appropriate cell culture assays.

Recipients or parental organisms

Viruses:
The alphaviruses are a diverse group of small, spherical, enveloped viruses with single-stranded, positive-sense, RNA genomes and have been isolated from all continents except Antarctica. They belong to the family Togaviridae, and include 29 recognized species.
The ACDP2 alphaviruses are all classed as arthralgic alphaviruses that cause mild to severe arthritis upon infection, sometimes accompanied by mild febrile illness with a maculopapular rash. The ACDP2 viruses that we propose to use are Sindbis virus, Semliki Forest virus, Ross River virus, O’nyong nyong virus and Bebaru viruses. These viruses are not responsible for significant illness or fatalities. In addition, these viruses are not easily aerosolized and the major route of transmission is mosquito bite. Infection routes in the laboratory are generally as a result of sharp exposure or mucosal infection. The two alphaviruses that will be worked with primarily will be Sindbis virus and Semliki forest virus. Semliki Forest (SFV) and Sindbis viruses (SINV) are arboviruses that were isolated from mosquitoes in Uganda and Egypt respectively. In humans these viruses generally cause a self-limiting mild febrile illness. SFV has only been identified as causing more than a mild self-limiting illness on two occasions. One was a lab infection, where the worker was found to be immune deficient. The second was when the serum samples of individuals having persistent headaches, fever, myalgia and arthralgia were found to have SFV. It is unclear why these strains of SFV had a different manifestation to most SFV strains. SINV causes a mild febrile illness, but has not been associated with any human disease. Of the other alphaviruses that are proposed to be used, Ross River virus
causes some mild arthralgic illness and O'nyong nyong virus has been associated with some outbreaks of mild arthralgic disease. Bebaru is a sub-type of SINV and is not generally associated with any human infections.

The only Flavivirus we propose to use is Zika virus. Flaviviruses, like Alphaviruses are enveloped viruses that are transmitted by a variety of species. Zika virus is found within the mosquito-borne group of flaviviruses and is typically transmitted by Aedine mosquitoes. Until 2014 Zika was considered an obscure virus in the Flaviviridae family. However, a large outbreak in Brazil resulting in serious birth complications associated with infection of Zika in pregnant women resulted in a concerted effort to understand the virus. Zika was first described in Uganda in 1947 and while there were numerous people with antibodies against the Zika virus, there was no evidence of significant outbreaks of the virus. The virus causes a mild febrile illness in adults and the major risk factor appears to be to the fetus if the mother is infected during pregnancy. Zika is transmitted by the Aedes aegypti mosquito and is primarily found in the Americas and Asia. Sporadic cases have been observed in Europe but these are a result of exposure during travel.

For all the above viruses, wild-type sequences of the viruses will be used to generate infectious clones, i.e. virus in the form of cDNA inserted into a bacterial plasmid with either a T7 or Sp6 bacterial promoter, or a CMV promoter. These full-length plasmids will be sequenced to confirm the genome of the virus and to ensure that no inadvertent mutations are included. Following confirmation of the correct sequence, the plasmid will be linearised, transcribed into RNA and electroporated into cells. To validate particular RNA secondary structures or mutations described above, deliberate mutagenesis will be performed using a variety of different protocols. All plasmids that are mutagenized will be sequenced to confirm that no other mutations are generated. These viruses containing specific mutants will be linearised, transcribed and electroporated into cells. Stocks of both wild-type and viruses containing mutations will be harvested and used for future work.

These viruses will be used to initiate cell infections with various cell types including but not limited to Vera cells, MRC-5 cells, U4.4 cells and C6/36 cells. In addition, some mosquito infections will be carried out. Mosquitoes will be infected with an artificial bloodmeal containing virus. Mosquitoes will be held for up to 21 days. Mosquitoes will be sampled by taking legs/wings, salivary deposit, midguts and salivary glands.

Host/vector system

Prokaryotic Hosts: All strains of E. coli used in this work are apathogenic, commercially available strains (e.g. Sure, JM109, DH-strains, Rosetta, TopTen etc.) classified as 'especially disabled hosts' by ACDP. These strains are only able to survive in the controlled environment of the lab and are used for propagation of the plasmids described above.

Eukaryotic hosts: Eukaryotic cells will be used to propagate the viruses, at no point will the introduction of the virus alter the host cell genome. These eukaryotic cells include cell lines of vertebrate or arthropod origin (for example, Vero cells (African Green monkey cells) or U4.4 cells (Aedine embryonic cells). Mosquito hosts such as Aedes aegypti and Aedes albopictus will also be used, once infected mosquitoes will be held for up to 21 days before being sampled. Once the mosquito tissue has been processed, the viral sample is handled as any other viral sample.

Origin & function

Viruses in plasmid forms will be obtained from collaborators at Leeds University and Glasgow University as well as from the WRCEVA reference collection at UTMB in Galveston, who already have the plasmids available. Prior to use the plasmids will be sequenced to confirm the sequence expected. The function of the genetic material is to produce viable virus for further study. These GMO organisms contain either the wild-type sequence, or mutations and deletions that are produced during normal viral replication, or mutations that our preliminary data shows cause attenuation of the virus. As these are transported in a DNA form there are no additional risks from transporting normal DNA samples.

The genetic material produced from this is designed to generate viral particles and these will function as viable virus once rescued.
The expected nature of this work is as described below. For all our experiments, any unexpected observations with viruses rescued from the plasmids will result in all work with that virus ceasing and a risk assessment being performed. As a routine measure all work will be carried out at containment level 2.

1. To generate stocks of viruses from infectious clones.

We do not expect any change from wild-type viruses during this portion of the work. Use of plasmids to generate viruses to create large scale stocks of particular strains of viruses is a common tool in virology. There have been no indications that this will lead to any change from that expected for wild-type viruses. Therefore this carries no more risk than working with wild-type viruses at CL2.

2. To determine infection and replication competence of viruses in particular cell types.

Although it is always possible that infection in different cell type could result in a change in the virus, our experience with both Alphaviruses and Flaviviruses is that in both mammalian and insect cell lines, infection is usually 2-3 days in length. Mammalian cells will produce observable cytopathic effect within 2-5 days, whereas infection with insect cells is asymptomatic and does not produce any overt sign of virus. It is not expected that there will be any changes from wild-type, therefore there this is a low risk procedure.

3. To determine the effect of RNA secondary structure on the generation of virus minority variants

RNA viruses have optimised their genome to maximise replication efficiency. We therefore expect that any mutation that alters the secondary structure of these viruses to attenuate the viruses. While we have no preliminary data to determine this, our hypothesis is that the secondary structure influences the production of specific minority variants. Therefore, it is likely that altering the secondary structure will impair the ability of the virus to generate minority variants, which has the potential to attenuate the virus by altering the distribution of important minority variants that are used to facilitate replication and transmission. Given that we expect this to attenuate the virus this aim is also low risk.

4. To determine the effect of changes in mutation rate/fidelity in the virus

Mutations identified in Venezuelan equine encephalitis virus and West Nile virus (Kautz et al. 2018, Van Slyke et al. 2015) will be introduced into the infectious clones. Our experience with such mutations indicates that they will result in attenuation of the viruses. This attenuation has been observed in many different systems including poliovirus, and influenza virus. Ultimately, it is likely that any change in the RNA-dependent RNA-polymerase from the wild-type will result in attenuation of the virus, as RNA viruses have optimised their RNA-dependent RNA-polymerase to maximise efficiency and replication. Given that we expect this to attenuate the virus this aim is lower risk than that in aim 1.

5. To determine the effect of minority variants on host cell interactions

Specific mutations and deletions that are found during natural replication will be incorporated into the infectious clones. These altered viruses will be rescued and assessed for their ability to replicate and infect other cells. Our experience of such mutants in Venezuelan equine encephalitis virus is that many are not viable and those that are able to produce virus show evidence of attenuation. Given these previous results we expect that any mutations incorporated will result in changes in the ability of the virus to infect cells. Similar results were observed in West Nile virus (personal communication) suggesting that these minority variants only function well when the wild-type virus is also present in the cell. Again given the expected attenuation of the viruses this is lower risk than that in aim 1.

6. To infect mosquitoes to determine competence for particular viral mutations

For wild-type viruses, it is expected that between 50-90% of mosquitoes will be infected with the virus, and while these viruses are transmissible following infection of mosquitoes, safe handling of mosquitoes and the use of primary containment and a glove box reduces the risk. For the viruses that have been modified from wild-type, we again expect that any modification will reduce the ability of the virus to infect mosquitoes. In our experience, mosquitoes will be infected in the midgut, but will be unable to disseminate to the rest of the body and therefore unable to generate virus to be transmitted to another host. Our data on Venezuelan encephalitis virus shows that any change to the RNA-dependent RNA-polymerase results in reduction of disseminated mosquitoes to 5-15% compared to 80-90%.
for wild-type (Warmbrod et al. submitted). Given this, we recognize that this may represent a slightly higher risk than wild-type if these results <;Ire not observed. However, our expectation is that any modification from wild-type generally results in attenuation in both in vitro and in vivo hosts. Therefore this aim is low risk, but any change from the expected risk will result in cessation of all work until a further risk assessment is carried out.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Mosquitoes will be maintained for up to 21 days following bloodmeals. Mosquitoes will be sampled by removing the legs/wings of mosquitoes. Salivation of the immobile mosquito into FBS and then removal of the salivary glands and the midgut. All manipulations prior to the removal of legs/wings will be done on ice to prevent mosquito movement. All manipulations will be performed in the glovebox. All samples will be put in individual tubes with safe-lock tubes including a stainless-steel ball for trituration.

The major risk is preventing mosquito escape once infected with the virus. All mosquitoes will be manipulated in a separate room with a double door and a net on the inner door. All manipulations of mosquitoes will be performed in a sealed glove box. Mosquitoes will be contained within primary containment with no more than 50 mosquitoes, which allows accurate counts to be maintained, in a sealed carton with a mesh lid. Mosquitoes will only be manipulated outside the primary containment when they are cold anaesthetized and cannot fly. They will also be manipulated on ice at all times to prevent recovery from the cold anaesthetization. Once the mosquitoes have been manipulated they will be placed back into the primary container where they are sealed. They are then placed in a secondary Tupperware container and placed in an incubator. Accurate counts of mosquitoes are maintained at all times. If any mosquito is missing, the person cannot leave the room until all mosquitoes are accounted for. An insectocutor can also be placed within the room or within the man trap if deemed necessary.

If a mosquito escapes outside the glove box, the user should call Dr. Forrester-Soto from the phone within the room. They must stay within the laboratory and try and catch the mosquito. No-one can enter or exit during this time. If after 3 hours the mosquito cannot be caught, a CO2 trap will be set up and left in the room for 24 hours. All captured mosquitoes will be killed following capture by the trap.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All alphaviruses and flaviviruses are killed when exposed to a 5% bleach solution or to the antimicrobials, cavicide or virkon. Specific protocols have been validated for killing of viruses and inactivation of viruses for further work. These methods have been validated for alphaviruses and published (Patterson et al. 2018). We have not validated these methods for flaviviruses, but prior to use, we will follow the same protocol to validate these methods.

Waste methods for the laboratory are described below.

All waste materials must be decontaminated prior to disposal from the cell culture virology room.

a. Paper waste generated in the MSC

   Paper waste generated in the BSC should be placed in a biohazard bag within the BSC. When the bag is 2/3 full, loosely tape, surface decontaminate, remove from BSC and place in biohazard bag located in the laboratory for further decontamination via the autoclave. .

b. Biological waste generated in the MSC

   Biological waste (pipette tips, serological pipettes, tubes, flasks and cell culture plates) must be placed in an autoclave-safe pan containing an appropriate disinfectant (CavicideNirkon, 5% Microchem or 5% freshly prepared bleach). All TLtubes, flasks and culture plates must be opened; pipette tips and serological pipettes must be rinsed (aspirate disinfectant and eject) so that the any virus within the pipette tip is also disinfected, prior to disposal in
autoclave pan. Do not overfill autoclave pans, the lid must close appropriately. 

i. Once work is complete all pans should be sealed with autoclave tape. Surface decontaminate the entire pan and remove from the BSC and store in a secured area for 24 hours.

ii. Following a 24 hour contact time; without opening the lid on the pan, drain the pan of all liquids down the sink in the laboratory. Discard solid waste in the biohazard bag located in the laboratory for autoclaving. Generally, 30 minutes is sufficient contact time for this concentration of bleach to kill alphaviruses and flaviviruses. However, 24 hours is used to ensure complete kill of any infectious material.

c. Vacuum traps
Vacuum traps must contain 10% fresh bleach (the bleach must be prepared daily). It must have a HEPA filter installed between the second flask (overflow flask) and the vacuum pump or in-house connection. At the end of the procedure the tubing must be rinsed with bleach followed by water. The waste must stand for at least 30 min in the flask before pouring it down the drain and cleaning the flask.

d. Waste generated in the Lab
i. All waste from the Lab must be considered infectious and therefore, must be decontaminated. Waste will be placed in autoclave bags in the laboratories prior to autoclaving. Autoclave bags must be loosely closed or taped when they are no more than 2/3 full. They must then be double bagged prior to autoclaving and placed in a lock-proof container such as an autoclave pan. Waste must not be left in the corridors.

ii. Autoclave waste must not be left in the autoclave. Users must ensure that waste is removed within 24 hours after the completion of the cycle.

e. Infected mosquitoes
i. All mosquitoes must be counted to ensure all infected mosquitoes are accounted for. As all mosquito manipulations should be performed in the glove box and the secondary container should only be opened in the glove box the most likely escape is in the glove box. Escaped mosquitoes in the glove box must be killed using paper towels present in the glove box for that eventuality. In the event of an escape outside of the glove box containment, personnel will not be allowed to leave the room until the mosquito is killed. As described above, in the event that the mosquito cannot be found after 3 hours, a CO2 trap will be placed in the room to collect the mosquito, after 24 hours, any mosquitoes will be killed. Mosquito tissues for processing are treated in the same way as the infected cell culture, inactivated using the correct buffer for downstream processing.

ii. The remaining mosquitoes are placed in 70% ethanol before being autoclaved to inactivate all viruses present in the samples.

Please enter comments on the GM safety committee on the risk assessment

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
Risk assessments have been written in consultation with (Chair of the Keele University Genetic Modification Safety Sub-Committee, GMSSC), (University Biological Safety Advisor) and (Head of Department of Occupational Health and Safety). As there is currently no virology work being carried out at Keele, an external academic with expertise in this area (University of Glasgow) was also consulted to ensure processes were suitable and robust. Full consideration has been given to any risks to human and animal health and release into the environment and the necessary containment measures needed to reduce risk to a negligible level. The notification documents have been approved by all members of the GMSSC which includes trade union representation.

### Project Containment

**Laboratory Activities**

- L2: Yes
- L3
- L4
- L2

**Glass Houses**

- L3
- L4
- L2

**Growth Rooms**

- L3
- L4

**Animal Units**

- L2
- L3
- L4
- L2

**Large Scale Activities**

- L3
- L4
- L2

**Human Clinical Applications**

- L3
- L4

### Project Ref 463/20.1

**Date Ackn’d**: 21/05/2020

**CU2 Project Title**: Production of SARS-Cov-2 virus like particles (VLP’s) containing a reporter system for initial screen of treatments and antivirals

**Class**: Class 2

**CultureVolClass2**: < 1 Litre

**CultureVolumeClass3-4**: Non-GMM

**Consent Granted**: Yes

**Project notified under transitional arrangements**: Yes

### Project Additional Information

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
### Purposes of the contained use

The notifier has 15 years experience working with CL2 viruses and 11 years working with CL3 viruses in containment.

The purpose of the contained use is to identify compounds that prevent infection of cells and therefore infection of SARS-CoV-2. The VLP’s will be used to infect cells in the presence of different compounds and the degree of reporter gene recorded.

### Recipient or parental organism

**Viruses:**

The coronaviruses are a group of viruses that typically circulate within wild animals and occasionally emerge to jump into humans. There are 6 known human infecting coronaviruses, of which SARS-CoV-2 is the latest to emerge and has caused significant human infection. Coronaviruses are a group of RNA viruses, that unusually contain a proof-reading domain. This allows them to replicate their large genome with greater ease. Although SARS-CoV-2 is a relatively new addition to the list of human coronaviruses, we know that the virus has a propensity for ACE2 receptors on human cells. The virus causes a wide range of symptoms from severe to mild, with the worst being Severe Acute Respiratory (SAR) distress which requires hospitalization and a prolonged stay on a ventilator and may be fatal. Initial reports suggest that co-morbidities such as advanced age, obesity, cardiovascular and cerebrovascular disease as well as diabetes contribute to severe disease. However, it has become apparent that age is not a good predictor of severe disease. There is emerging evidence that the amount of virus the person is exposed to and potentially host genetics could play roles in determining the severity of disease.

All coronaviruses are transmitted through droplet transmission or aerosol transmission. The major route of transmission in the environment is contact with aerosols generated by an infected person. In the lab with wild-type virus the major source of infection would be any aerosol-producing technique. This includes but is not limited to, centrifugation, pipetting and vortexing. All of these will be done under conditions designed to reduce aerosol transmission and in a microbiological safety cabinet, or in the case of centrifugation in sealed buckets.

Recent work with the viruses has also identified that fomites can play a role in Coronavirus transmission. It appears that the virus is stable on metal and plastic surfaces for up to 3 days. All areas in contact with virus will therefore be wiped down with Virkon or Cavicide following any manipulations in the MSC.

### Host/vector system

**Prokaryotic Hosts:** All strains of E. coli used in this work are apathogenic, commercially available strains (e.g. Sure, JM109, DH-strains, Rosetta, TopTen etc.) classified as ‘especially disabled hosts’ by ACDP. These strains are only able to survive in the controlled environment of the lab and are used for propagation of the plasmids described above.

**Eukaryotic hosts:** Eukaryotic cells will be used to propagate the viruses and at no point will the introduction of the virus alter the host cell genome. These eukaryotic cells include but are not limited to cell lines of vertebrate origin (for example, Vero cells (African Green monkey cells) and 293T cells (kidney epithelial cells)).

### Origin & function

**Pseudoviruses** will be generated by the incorporation of the envelope and spike proteins into the plasmid pcDNA3.1 (commercially available from Invitrogen). A second plasmid pNL4-3Luc(env-) has been developed to have a reporter gene in conjunction with genes encoding the HIV proteins gag and pol. The plasmid is available on request from the NIH AIDS Institute and has been previously used with the H5 protein from avian influenza (Skidmore et al. Med. Chem. Commun. 2015, 6,640).

The plasmids will be transfected into cells together to produce a pseudotype virus with the luciferase reporter gene in complex with the HIV gag and pol proteins (the envelope protein has been replaced with the luciferase reporter gene). This pseudotype virus will produce envelope proteins consistent with the SARS-CoV-2 virus.

Once the pseudotype virus has been produced, it will be harvested, and titrated for luciferase expression in lieu of identification of virus titer.
The pseudovirus system will then be used to test agents that may prevent virus entry into cells and therefore the amount of luciferase expressed in cells. This will be used to validate the fractions of the heparin that have been identified as having potential to interact with the virus. Other promising compounds will also be tested using this system.

This system has been successfully used at CL2 level with Henipa and Nipah viruses as well as with SAPO agents such as Newcastle disease virus (NDV). (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2994542/, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4061091/, ). Some concerns have been raised with the use of this system with HIV envelope proteins as to the possibility of recombinant (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5372769/). However, as this system will be used in the absence of any HIV envelope proteins, this possibility is remote. To confirm that there is no possibility of revision, we will test the pseudotyped produced virus using Gag, Pol and Luciferase primers. We will also use SARS-Cov-2 non-structural and envelope primers, with the expectation that if the Gag, Pol, Luc and SARS-CoV-2 envelope proteins are PCR positive and the non-structural protein PCR is negative that there will be no issues with the safety of the virus. Due to the small potential of human infection in immunocompromised personnel, all procedures will be carried out at CL2 levels in a MSC.

**Evaluation of foreseeable effects**

The expected nature of this work is as described below. For all our experiments, any unexpected observations with viruses rescued from the plasmids will result in all work with that virus ceasing and a new risk assessment being performed. As a routine measure all work will be carried out at containment level 2.

1. To generate stocks of pseudotype virus.

   This has been used successfully in our collaborators’ laboratory in San Raffaele in Italy, and the plasmid contains no non-structural proteins that would facilitate viral replication. Initially we will verify that the pseudovirus has been generated as expected, and a set of PCR's that amplify the Coronavirus envelope protein, the HIV gag protein and the luciferase gene will be used to verify that this pseudotype virus is stable. At each infection a control infection will be carried out and RNA will be harvested and verified using these PCR's. We will also use PCR to amplify a gene encoding a non-structural Coronavirus protein to ensure that the circulating transmission in the human population is not impacting these studies.

2. Validation of compounds inhibiting pseudotype entry into cells.

   As most of these compounds already exist and have been well characterised there are few issues associated with this work. However, in the event that a compound causes an increase in reporter gene expression, all experiments with that compound would be stopped and an additional risk assessment performed.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Coronaviruses are killed when exposed to a 5% bleach solution or to the antimicrobials, caviocide or virkon. Specific protocols have been validated for killing of viruses and inactivation of viruses for further work. These methods have been validated for alphaviruses and published (Patterson et al. 2018), both the validation methods and confirmation of inactivation. We have not validated these methods for Coronaviruses, but prior to use, we will follow the same protocol to validate these methods for Coronaviruses.

Waste methods for the laboratory are described below.
All waste materials must be decontaminated prior to disposal from the cell culture virology room.

a. Paper waste generated in the MSC

Paper waste generated in the BSC should be placed in a biohazard bag within the BSC. When the bag is 2/3 full, loosely tape, surface decontaminate, remove from BSC and place in biohazard bag located in the laboratory for further decontamination via the autoclave.

b. Biological waste generated in the MSC

Biological waste (pipette tips, serological pipettes, tubes, flasks and cell culture plates) must be placed in a hard-sided autoclave-safe pan containing an appropriate disinfectant (Cavicide/Virkon, 5% Microchem or 5% freshly prepared bleach). All tubes, flasks and culture plates must be opened; pipette tips and serological pipettes must be rinsed (aspirate disinfectant and eject) so that the any virus within the pipette tip is also disinfected, prior to disposal in autoclave pan. Do not overfill autoclave pans, the lid must close appropriately.

i. Once work is complete all pans should be sealed with autoclave tape. Surface decontaminate the entire pan and remove from the BSC and store in a secured area for 24 hours.

ii. Following a 24 hour contact time; without opening the lid on the pan, drain the pan of all liquids down the sink in the laboratory. Discard solid waste in the biohazard bag located in the laboratory for autoclaving. Generally, 30 minutes is sufficient contact time for this concentration of bleach to kill alphaviruses and flaviviruses. However, 24 hours is used to ensure complete kill of any infectious material.

c. Vacuum traps

Vacuum traps must contain 10% fresh bleach (the bleach must be prepared daily). It must have a HEPA filter installed between the second flask (overflow flask) and the vacuum pump or in-house connection. At the end of the procedure the tubing must be rinsed with bleach followed by water. The waste must stand for at least 30 min in the flask before pouring it down the drain and cleaning the flask.

d. Waste generated in the Lab

i. All waste from the Lab must be considered infectious and therefore, must be decontaminated. Waste will be placed in autoclave bags in the laboratories prior to autoclaving. Autoclave bags must be loosely closed or taped when they are no more than 2/3 full. They must then be double bagged prior to autoclaving and placed in a leak-proof container such as an autoclave pan. Waste must not be left in the corridors.

ii. Autoclave waste must not be left in the autoclave. Users must ensure that waste is removed within 24 hours after the completion of the cycle.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
Risk assessments have been written in consultation with Chair of the Keele University Genetic Modification and Biological Agents Safety Sub-Committee, GMBASSC, University Biological Safety Advisor and Head of Department of Occupational Health and Safety.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2 Yes</td>
<td>L3</td>
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<tr>
<th>Animal Units</th>
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<td>L2</td>
<td>L3</td>
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Project Ref 541/14.1

Date Ackn'd 01/03/2019

CU2 Project Title

Study of Plasmodium biology: genetic and biochemical screenings of essential and critical genes for asexual and initial sexual development

Class

Class 3

Culture Vol Class

< 1 Litre

Non-GMM

Consent Granted

Yes

Project notified under transitional arrangements

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

Transferred from GM541 01/03/2019

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

The containment is required for the cultivation of erythrocytic stages of Plasmodium parasites. Parasites are maintained in in-vitro cell cultures by providing human red blood cells to support their growth. Erythrocytic stages are maintained in in-vitro cell cultures by providing human red blood cells to support their growth. Erythrocytic stages are used in the presented research project to study the biology of Plasmodium strains competent to infect humans. This project aims to discover and validate targets useful...
for malaria therapeutic interventions by applying genetic biochemical and cellular techniques presented.

Recipient or parental organism

Plasmodium is an apicomplexa parasite that completes its life cycle infecting alternatively mosquitoes (genus Anopheles) and vertebrates. In vertebrates it causes malaria. Human malaria is a deadly disease that affects about a half of the world population. The present project aims to study essential and critical genes for parasite asexual development and sexual differentiation. The strains under study are P. falciparum, vivax and knowlesi. In mosquitoes and humans Plasmodium respectively accomplishes sexual and asexual development. Plasmocium developmental stages used in this project are the erythrocytic stages and the initial phases of sexual development in absence of the vector. These stages are cultivated in vitro by providing human red blood cells (RBC), essential to support parasite growth. In fact Plasmodium is an obligate intracelular parasite.

Host/vector system

Since there is no way to maintain Plasmodium except inside red blood cells (RBCs), the parasites will be cultivated, transfected and stored inside RBCs. No forms/stages of erythrocytic parasite development can persist in the environment following RBC lysis. This project will use laboratory strains with known drugability and drug resistance. Genetic modification will be achieved by electroporation of purified plasmids constructed and analyzed in E. coli into RBCs. All the plasmids used in this project derive from pBlueScriipt and therefore are non-mobilisable.

Origin & function

Fragments of DNA that encode for fluorescent proteins such as GFP, yFP and RFP derivates will be used as probes for transfected parasites, for promoter functions, for protein tagging and for trafficking detection. These fragments originate from jellyfish and corals. They enable detection and visualization of parasites using fluorescence microscopy or flow cytometry. Fragments of DNA that encode for SB100x transposase, TALEN, endonucleases, recombinases, recombinin, will be used in this project. SB100X derives from Sleeping Beauty transposases and was produced and characterized by the Izsak's laboratory (Nat Genet. 2009 June; 41(6): 753-76L1). SB100X enables the insertion of a DNA fragment flanked by specific inverted repeats into the genome. TALEN is a chimera of TALE (Transcrition activator -like effectors from gene product recognizes specific DNA sequences and digests in the proximity of them. Homing endonucleases, such as Scel, specifically recognize and digest long stretches of DNA sequence (Gimble & Thorner, Nature, 1992, vol 35:35; hybridized short RNA molecule (Mali P. et al., Science, 2013, vol339, pp823-826). Cre recombinase is a tyrosine recombinase enzyme derived from the P1 Bacteriophage (Abreseth & Hoess Journal of Biological Chemistry 1984, 259: 1509-1514) and Flp is a recombinase enzyme derived from Saccharomyces cerevisiae (Broach & Hicks, Cell 1980, vol 21: pp. 501-508). These recombinases excise and insert DNA fragments flanked by specific DNA sequences called Lox and Frt, respectively. All these gene products enable genome editing and recombinaton. These molecules will be used to generate parasites that are defective in metabolic functions. Other fragments of DNA included in our plasmids encodes for specific drug resistance, such as human dihydrofolate reductase (hDHFR), yeast dihydroorotate dehydrogenase (yDHOXD) and blasticidin-S deaminase (BSD); Tetracycline transactivator proteins, and specific Plasmodium proteins or protein fragments under biochemical characterization. Accessory DNA fragments are used for optimal plasmid segregation and Plasmodium expression, as minimal centromer, Rep20, Promoters and 3’ untranslated regions.

Evaluation of foreseeable effects

There is no risk in the genetic modificatons included in this project for multiple reasons (1) The modifications will not cause a gain of function, or increase the wildtype danger, infectivity, virulence and environment or disinfectant resistance. In fact, used approaches aim to disable paraites in any of their vital functions and virulence, in order to gain access to unknown gene functions. (2) drug resistance genes (above listed), used to select transfected parasite, will be the only gain of functions. Nevertheless, despite the introduction of drug resistance genes, strains used in the present project will remain pharmacologically treatable, if therapeutic intervention is needed for accidental events. (3) There is no possibility to spread the modified parasite, wild type or mutant can survive in the environment. In fact, minimum changes is salts concentration of the media or air exposure will result in hemolyzed or dried RBC with consequent death of parasites (i.e. tap water kills parasites by RBC lysis). In order to reduce the risk of accidental injuries (like cuts or punctured wounds) that may expose to infection, glassware will be avoided as much as possible (details in the code of practice), and sharps (blades or needles) must not be used during Plasmodium culturing.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We request some derogations to CL3 containment level in accordance to the fact that cultivated stages cannot cause airborne transmission and the vector is absent from the natural environment in the United Kingdom. In particular, the requested derogations regard the following CL3 requirements: sealable laboratory for fumigation (M2), entry to laboratory via airlock (M4), negative pressure relative to the pressure in the immediate surroundings (M5) and HEPA filtered air from the laboratory (M6)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Treatment of contaminated items.
- Pippette tips and Graduated Pipettes: Place into polypropylene jars containing 2% Virkon. Incubate for 24 hours and then Virkon solution will be poured down the sink and solid waste disposed into autoclavable 'clini' bags.
- Microfuge tubes, Disposable cuvettes, Electroporation cuvettes, flasks and plates: they should be placed in a separate autoclavable 'clini' bag within the microbiological safety cabinet.
- 'Clini' autoclavable bags: When 3/4 full the neck of the autoclavable bag is loosely tied and moved into the autoclave tins and autoclaved within the suite. After autoclaving it is sent off site for incineration.
- Glass slides used in preparing blood smears of Plasmodium-infected red blood cells: they should be placed in a sharps bin. Plasmodium in blood smears on glass slides is inactivated by means of the fixative used to fix the blood film to the slide prior to staining. Because of this inactivation the glass slides can be disposed of by placing in a sharps bin and entering the clinical waste route.
- Liquid Waste: Cuvettes, glasks and universal/falcon tubes containing liquid waste are to be emptied or aspirated into jars containing 2% Virkon and after 24 hours incubation disposed down into the sink.

Disinfection procedures.
- Surface sterilisation; Hard surfaces such as the work surface of the safety cabinet or benches must be disinfected with 70% Ethanol.
- Equipments and their parts must be disinfected with 1% Distel.
- Spillages: Suspected contamination on hands, gloes or work surfaces must be disinfected immediately with 70% Ethanol. Minor spillages (up to ~ 10 ml) should be treated with 70% Ethanol and wiped with towels. Large spillages (>10ml) must be treated with Virkon powder and then wiped with 70% Ethanol soaked towels.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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02/03/2022
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<th>Animal Units</th>
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<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
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**Name**

UNIVERSITY OF NOTTINGHAM

**Name 2**

UNIVERSITY PARK GM CENTRE

**Department**

PHARMACEUTICAL SCIENCES

**Campus Estate or Research Centre**

**Road Name**

UNIVERSITY PARK

**District**

**Town**

NOTTINGHAM

**County**

NOTTINGHAMSHIRE

**Postcode**

NG7 2RD

**Country**

ENGLAND

**Tel Number**

0115 951 5100

**Fax Number**

0115 951 5102

**HSE Division**

MIDLANDS

**Comments**

GM66, 171, 232, 594, 605 & 738 have merged with GM470 to make one main centre on 18/02/2005.

**Date at Which Additional Info Submitted**

12/07/2002
### Significant Change

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>

Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref**  11/01.1

**CU2 Project Title**: The Production of recombinant ovine and bovine PrP for the generation of monoclonal antibodies specific for conformational isomers of PrP

**Class Culture Vol**: Class 2 1-50 Litres

**Consent Granted**: Not Applicable

**Project notified under transitional arrangements**: N

**Historical Significant Changes**: Project transferred from GM11

---

02/03/2022
### Purposes of the contained use

The biological function of the prion protein PrPC is not known. It is thought that the disease isoform of this molecule, PrPSc, may be the infectious agent for BSE in cattle and scrapie in sheep. We wish to produce recombinant truncated forms of ovine and bovine PrP. Once purified monomeric truncated PrP will be converted to the -form for use as a target for antibody isolation in order to produce -form PrP conformational specific antibodies. Such antibodies may also be specific for the disease isoform PrPSc.

### Recipient or parental organism

The proposed E. coli hosts Novablue DE3 and TG1 are routinely used lab strains of E. coli that are inherently safe K12 derivatives. The PrP gene inserts, from bovine or ovine source, are truncated and do not possess the GPI anchor domain or the export signal domain. The truncated versions would therefore be significantly different from the disease and are likely to pose a reduced risk to human or animal health.

### Host/vector system

The PTrcHis, pRSET, pET, pMal expression vector systems will be used in the K12 derived E. coli host strains Novablue DE3 and TG1.

### Origin & function

The truncated PrP genes are from bovine or ovine origin. These would be produced in the E. coli host cells using the expression vectors PTrcHis, pRSET, pET, and pMal which produce target proteins fused to either a poly-His tag or maltose binding protein, these tags would then be used to affinity purify the PrP-fusion proteins.

### Evaluation of foreseeable effects

Due to the truncated nature of the inserts in the constructs proposed here and that the expressed proteins are structurally distinct from the disease isoforms (they need prolonged incubation at low pH in vitro to produce the conformational change thought to be central to the propagation of PrPSc, i.e. from a predominantly a-helical structure to a predominantly -structure) the hazards to human health are low and containment level 2 is appropriate.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Solid waste:**

Disposable plastic ware will be used wherever possible, disposable solid waste (including E. coli plates) to be put in biohazard burn bins and sent for incineration (100% kill). Reusable contaminated solids (including E. coli growth flasks) to be soaked in 20,000 ppm available chlorine of sodium hypochlorite for at least 2 hours, except for contaminated metal equipment which will be soaked in 2m sodium hydroxide for at least 2 hours.

**Lique waste:**

All liquid waste (including E. coli cultures) will be treated with 20,000 ppm available chlorine of sodium hypochlorite for at least 2 hours. Spills would be cleaned up with paper towels which will be treated as solid waste, contaminated spill areas will be soaked in 20,000 ppm available chlorine of sodium hypochlorite for at least 2 hours.
except for contaminated metal which will be soaked in 2M sodium hydroxide for at least 2 hours. Where soaking is not possible, contaminated areas will be wiped down with one of the above disinfectants (as appropriate) at least 3 times allowing drying between applications for at least 1 hour. All work using the listed constructs in E. coli will be carried out in the BSE suite (biology Dept., University fo Leicester) and the SOP for this lab followed.

Disinfectant to be used, exposure time and working concentration:
2M sodium hydroxide or 40% (v/v) Haychlor industrial bleach (Chloros hypochlorite bleach). A 40% (v/v) solution of Haychlor industrial bleach contains 20,000 to 64,000 ppm available chlorine (manufacturers data). Decontamination procedures obtained from “transmissible spongiform encephalopathy agents: safe working and the prevention of infection. Advisory Committee on Dangerous Pathogens, Spongiform Encephalopathy Advisory Committee. ISBN 0-11-322166-5.

N.B. The above waste treatment/decontamination procedures are to ensure that contaminating recombinant PrP is destroyed, these procedures will also ensure that viable E. coli are unlikely to remain after treatment. For example University's guidelines from "Genetically Modified Organisms" and "Hazardous Biological Agents" state that solutions containing 1,000 ppm available chlorine (e.g 1% Chloros) are suitable for routine disingection of bench tops and other surfaces. 2.5% Chloros or equivalent is suitable for pipette jars and spillages of blood etc. 10% Chloros or equivalent should be used for blood and other materials with a high organic content if the presence of infectious agents is suspected.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Agreed:
1 That a designation of Class 2 is appropriate
2 That the risk assessment is sound and that work should be carried out at Containment Level 2
3 That the proposer should forward the proposal to HSE for prior approval of the project.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<td>L3 L4 L2 L3 L4</td>
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</tbody>
</table>
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

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**Historical Significant Changes**

GM171/01.1 Transferred to GM470 on 18/02/2005

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**Class**

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**CultureVolClass2**

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**Non-GMM**

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**Project notified under transitional arrangements**

| Y |

---

**Tick if notifying a connected programme of work**

| N |

---

**Date of Significant Change**

18/02/2005

---

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 171/01.10

Date Ackn'd 18/02/2005

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Consent Granted

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes GM171/01.10 has transferred to GM470 on 18/02/2005

Historical Date of Additional Info

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
Project Ref 171/01.11

Date Ackn'd 18/02/2005

CU2 Project Title HIGH LEVEL EXPRESSION OF HETEROTRIMERIC G PROTEIN SUBUNITS, B-ARK MINIGENE AND G-PROTEIN-COUPLED RECEPTORS IN MAMMALIAN CELLS USING THE SINDBIS VIRUS EXPRESSION SYSTEMS

Class 2

CultureClass2 VolumeClass3-4

Non-GMM

Consent Granted Not Applicable

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes GM171/01.11 has transferred to GM470 on 18/02/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form 

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 171/01.12

Date Ackn'd 18/02/2005

CU2 Project Title EXPRESSION IN MAMMALIAN CELLS OF CONSTITUTIVELY ACTIVE FORMS OF

Class 2

CultureVolClass2 

CultureVolumeClass3-4
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 171/01.13

Date Ackn’d 18/02/2005

CU2 Project Title

EXPRESSION OF CONSTITUTIVELY-ACTIVE FORMS OF NOS 1-3, AKT, ERBB2-4, ERA/B, PARP, HIF1A IN MAMMALIAN CELLS

Date Project Ceased

Date of Significant Change

Withdrawn N

Historical Significant Changes

GM171/01.13 Transferred to GM470 on 18/02/2005

Tick if notifying a connected programme of work N

Consent Granted Not Applicable

Project notified under transitional arrangements Y

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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### Project Ref 171/01.14

**CU2 Project Title**: HIGH LEVEL OF TRANSCRIPTION FACTORS IN EUKARYOTIC CELLS USING ADENO-X OR ADEO-X TET-OFF (CLONTECH) ADENOVIRAL-MEDIATED GENE TRANSFER

**Class**: Class 2

**Consent Granted**: Not Applicable

**Project notified under transitional arrangements**: Y

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**: GM171/01.14 Transferred to GM470 on 18/02/2005

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Project Ref  171/01.15

Date Ackn'd  18/02/2005  CU2 Project Title  HIGH LEVEL EXPRESSION OF PROTEINS INVOLVED IN ENERGY BALANCE/LIPID  Class 2
Date Project Ceased

METABOLISM IN EUKARYOTIC CELLS USING ADENO-X OR ADEO-X TET-OFF (CLONTECH) ADENOVIRAL-MEDIATED GENE TRANSFER

Non-GMM
Consent Granted
Not Applicable

Project notified under transitional arrangements

Withdrawn
N

Tick if notifying a connected programme of work
N

Historical Significant Changes
GM171/01.15 Transferred to GM470 on 18/02/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 171/01.16

Date Ackn’d 18/02/2005

CU2 Project Title

EXPRESSION OF THE SIGNALLING PROTEINS P110 (P13-KINASE) AND MEKK IN MAMMALIAN CELLS

Class 2

Consent Granted Not Applicable

Project notified under transitional arrangements Y

Withdrawn N

Historical Significant Changes GM171/01.16 Transferred to GM470 on 18/02/2005
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Project Ref 171/01.17

Date Ack'n'd 18/02/2005

CU2 Project Title MEDIATORS AND MODULATORS OF APOPTOTIC CELL DEATH

Class 2

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements Y

Historical Significant Changes
GM171/01.17 Transferred to GM470 on 18/02/2005

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Project Ref 171/01.18

Date Ackn'd 18/02/2005

CU2 Project Title EXPRESSION IN MAMMALIAN CELLS OF CONSTUITIVELY-ACTIVE FORMS OF:

Class 2

CultureVolClass2

CultureVolumeClass3-4

Class 2

CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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<td>L3 L4 L2 L3</td>
<td>L4 L3 L4 L3</td>
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**Laboratory Activities**
- L2: General Laboratory
- L3: High Biosafety Level
- L4: Maximum Biosafety Level

**Glass Houses**
- L3: Intermediate Biosafety Level
- L4: High Biosafety Level

**Growth Rooms**
- L2: General Growth
- L3: High Growth
- L4: Maximum Growth

**Animal Units**
- L2: General Animal
- L3: Intermediate Animal
- L4: High Animal

**Large Scale Activities**
- L2: General Large Scale
- L3: Intermediate Large Scale
- L4: High Large Scale

**Human Clinical Applications**
- L2: General Human
- L3: Intermediate Human
- L4: High Human

**Project Ref** 171/01.19

**Date Ackn’d** 18/02/2005

**CU2 Project Title** CO-EXPRESSION IN MAMMALIAN CELLS OF APP695 AND UBIQUITIN + 1

**Date Project Ceased**

**Class** Class 2

**Culture Vol Class**
- Class 2
- Class 3-4

**Culture Volume Class**
- Non-GMM Consent Granted: Not Applicable

**Project notified under transitional arrangements** Y

**Historical Significant Changes**

- GM171/01.19 Transferred to GM470 on 18/02/2005

**Significant Change ID**

**Historical Date of Additional Info**

**Date of Significant Change**

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Project Ref 171/01.2

Date Ackn'd 18/02/2005

CU2 Project Title EXPRESSION OF MAMMALIAN CELLS OF NATIVE-NEGATIVE AND CONSTITUTIVELY ACTIVE FORMS OF NOTCH AND DELTA

Date Project Ceased

Class 2

CultureVolClass2

Consent Granted Not Applicable

Class CultureVolumeClass3-4

Non-GMM

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes GM171/01.2 Transferred to GM470 on 18/02/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 171/01.20

Date Ackn'd 18/02/2005

CU2 Project Title THE EXPRESSION OF GFP REPORTER CONSTRUCTS AND EUKARYOTIC

Class 2
VECTORS CONTAINING WILD TYPE TY, P, E AND DOMINANT NEGATIVE FORMS OF P85 (P13-K SUBUNIT), IRS PROTEINS AND FORKHEAD TRAN-FACT IN MAMMALIAN CELL LINES

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**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
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Historical Significant Changes
GM171/01.21 Transferred to GM470 on 18/02/2005
**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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02/03/2022
### Project Ref 171/01.3

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- **Non-GMM Consent Granted:** Not Applicable
- **Project notified under transitional arrangements:**

#### Historical Significant Changes

- **GM171/01.3 has transferred to GM470 on 18/02/2005**

#### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Animal Units

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Project Ref 171/01.4

Date Ackn'd 18/02/2005

CU2 Project Title CLONAL ANALYSIS OF RODENT CELLS IN ORGAN CULTURE EXPRESSING THE

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2
Date Project Ceased
DELTA RECEPTOR TRANSFECTED WITH ECOPTROPIC REPLICATION DEFICIENT RETROVIRUSES

Non-GMM Consent Granted
Not Applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes
GM171/01.4 has transferred to GM470 on 18/02/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Project Ref** 171/01.5

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**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

| Laboratory Activities | Glass Houses | Growth Rooms |
## Project Ref 171/01.6

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<td>HIGH LEVEL TRANSIENT EXPRESSION OF K-ATP CHANNEL SUBUNITS IN RAT AND HUMAN CELLS, USING ADENO-X OR ADENO-X-TET-OFF (CLONTECH) ADENOVIRAL-MEDIATED GENE TRANSFER</td>
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**Historical Significant Changes**

- GM171/01.6 has transferred to GM470 on 18/02/2005

**Project Additional Information**

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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<th>Laboratory Activities</th>
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<td>L3 L4</td>
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### Project Ref 171/01.7

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02/03/2022  Page 7498 of 15326
Date Project Ceased
GM171/01.7 has transferred to GM470 on 18/02/2005

Non-GMM Consent Granted
Not Applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes
GM171/01.7 has transferred to GM470 on 18/02/2005

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Historical Significant Changes

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref 171/01.8

Date Ackn’d 18/02/2005

CU2 Project Title RAF-1 KINASE FUNCTION AND CROSS-CASCADE ACTIVATION

Class Class 2

CultureVolClass2 Class 2

CultureVolumeClass3-4

Consent Granted Not Applicable

Project notified under transitional arrangements Y

Historical Significant Changes GM171/01.8 has transferred to GM470 on 18/02/2005

Withdrawn N

Tick if notifying a connected programme of work N

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Ref: 171/01.9

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#### Historical Significant Changes
GM171/01.9 has transferred to GM470 on 18/02/2005

### Project Additional Information

#### Purposes of the contained use

#### Recipient or parental organism

#### Host/vector system

#### Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Project Ref 171/03.1

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<tr>
<td>18/02/2005</td>
<td>INDUCIBLE EXPRESSION OF CELL SIGNALLING MOLECULES IN</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>
This aim is to express cell signalling molecules in hormone-secreting cell lines, to examine their functional roles in hormone secretion in these cells. The tet-on inducible expression allows for control levels of expression, with activated transcription in the presence of tetracycline (Tc) or analogues.

The cell lines used for packaging of the replication deficient retroviral vectors and for the expression of signalling-molecule cDNAs have a history of safe use. Alterations in the properties of the cells used in this work as a result of expression of these cDNAs will be negligible if any. The genetically modified cells are very unlikely to pose more of a hazard than the parental cell lines.

Genes of interest will be inserted into the expression vector (pRev-TRE, Clontech). Both this and the pRev-TET-on (Clontech) vector are considered as non-mobilisable. Both these vectors will be propagated in a suitable E. coli strain (eg DH5alpha, XL-1 Blue), which are recognised as non-colonising and disabled. The pRevTRE vector and the pRevTET-on plasmid will be independently transfected into the packaging cell line (RetropackTM PT67, Clontech), which produces viral proteins from stably integrated genes. Viral lysates will be used to infect cultured mammalian cell (PC-12, alphaTC, RINm5F, INS-1E), which do not have the ability to infect or transfer the inserted DNA to other cells.

cDNAs for a number of cell signalling protein molecules, including GTP binding proteins, have been cloned. These will be inserted into the pRev-TRE expression vector (Clontech), into a site not specifically situated to facilitate expression in E. coli. It is highly improbable that the proteins will be expressed within the bacterial cells. The E. coli strains used for propagation are not considered pathogenic to humans or animals and they are expected to have a low probability of survival outside of the laboratory culture environment - they always have auxotrophic requirements which are unlikely to be satisfied outside the laboratory.

The pRevTRE vector and the pRevTET-on plasmid will be independently transfected into the packaging cell line PT67, which allows for the safe, efficient production of high titer, infectious replication-incompetent retrovirus.

Phage produced by the pRevTet-on vector will be used to establish stable Tet-On cell lines expressing the "reverse" tet repressor (rTetR). Once established, the cell line (hormone-secreting) will be infected with the pRevTRE phage containing the inserted gene sequence. Transcription of the gene will only begin upon integration into a
stable (r)TA expressing line and in the presence of Tc or the Tc derivative doxycycline (Dox).

Evaluation of foreseeable effects

The RetroPack PT67 packaging cell line provides the genes necessary for recombinant virus particle formation: gag (core structural proteins), pol (reverse transcriptase, integrase), and env (coat glycoproteins). The recombinant virus produced by this cell line contain the products of these genes but lack the genes themselves, thus these retroviral particles can infect target cells and transmit the gene of interest but cannot replicate (replication-ioncompetence) within these cells. Retroviral production from subsequently infected cell lines is prevented. All retroviral packaging cells from Clontech have been safety-tested for replication incompetence, and a product analysis certificate accompanies the RetroPack PT67 cells.

Retrovirus such as Moloney murine leukaemia virus (MoMuLV) do not naturally infect human cells, however recombinant virus packaged from the MoMuLV-based vector described here maybe capable of infecting human cells and therefore could have deleterious effect if they were to infect a laboratory worker. The viral supernatants produced could, depending on the gene insert, contain potentially hazardous recombinant virus. Users are advised not to create retroviruses capable of expressing known or potential oncogenes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All transfections and manipulations of cells will be performed in a Class II biological safety cabinet (identifiable to other users), preventing release of aerosols. Protective clothing, double gloves to be worn at all times.

Because of viral nature of the work, no glassware or sharps will be used, only disposable plasticware. All potential infectious waste (solutions and microbiological plates) will be de-contaminated and autoclaved prior to disposal. Due to the labile lipid-derived nature of membranes of cells etc, the use of alcohol, detergents or bleach will be sufficient for decontamination. In the event of spillage, the area will be effectively disinfected.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee considered that the molecules being expressed using the retroviral vectors pose no significant risk to the people carrying out the procedures or to the environment. However because the ACDP guidelines indicate that the minimum level of containment for the use of retroviral vectors is at level 2 this work has to be carried out at this level.

Project Containment

02/03/2022
Project Additional Information

Purposes of the contained use

The aim of this work is to knock down expression of genes by introducing synthetic "genes" (random DNA sequences less than 100bp), encoding shRNA, these are processed by the cell to generate silencing RNAs (siRNAs). The siRNA leads to the degradation of the targeted mRNA and hence knockdown expression of that gene. shRNA libraries will be used to carry out a genetic screens (when coupled with an intracellular fluorescent reporter system) in tissue cultured cell lines. The shRNA is delivered to the cells by transfection with eukaryotic expression vectors or infection with replication defective adenoviral or lentiviral vectors. This work is directed at identifying components of signal transduction pathways.

Recipient or parental organism

The shRNA library will be constructed in E. coli. The E. coli strain used for propagation are not considered pathogenic to humans or animals and they are expected to have a low probability of survival outside of the laboratory culture environment - they always have auxotrophic requirements which are unlikely to be satisfied outside the laboratory. They are considered to be ACDP group 1. shRNA libraries will also be constructed in commercially-available, replication-deficient adenoviral vectors and
relication-defective lentiviral vectors.

The cell lines used for packaging of the replication-deficient retroviral vectors and for the expression of signalling-molecule cDNAs have a history of safe use. Alterations in the properties of the cells used in this work as a result of expression of these cDNAs will be negligible, if any. The genetically modified cells are very unlikely to pose more of a hazard than the parental cell lines.

Host/vector system

The shRNA library will be constructed in E. coli (ACDP group 1 strains). Commercially available eukaryotic expression plasmids with optimal antibiotic selection will be used eg pSilencer (Ambion), pSiren (BD Biosciences), pSiEx(Novagen), psiStrike (Promega). These vectors are mobilisation defective.


Plasmid or virus will be used to infect transfect the cell line of interest (eg HeLa, HEK293, nB2A, IN157, MCF-7, PC12, ES cells, and primary cells such as human macrophage and fibroblasts) (ACDP group 2).

Origin & function

The shRNA genes are small oligonucleotides synthesised invitro. The shRNA genes (small, random DNA sequences) are cloned downstream of a eukaryotic RNA polymerase III promoter consequently they cannot be expressed in E. coli and not affect their pathogenicity. DNA from the shRNA libraries generated will be used to transfect/infect eukaryotic cells to carry out genetic screens. Using a promoter-specific intracellular fluorescent reporter system in eukaryotic cells, changes in the level of expression from that promoter (detected by a change in fluorescence) will indicate that a component of the signal transduction pathway has been affected by the particular siRNA expressed in that cell.

Evaluation of foreseeable effects

The recombinant virus particles are capable of infecting human cells and could have a deleterious effect if delivered to a target tissue. However, as the virus is unable to replicate, only the initially infected cells would be affected, making serious effects very unlikely unless the infection was massive. The packaging cell lines to be used are supplied along with the commercially available vectors and contain the genes necessary for recombinant virus particle formation. Recombinant virus produced in the packaging cell lines contain the gene products provided in trans by the packaging cells, but they lack the genes themselves. The viral particles produced by the packaging cells can infect target cells and transmit the gene of interest but cannot replicate within these cells. Virus production from cells infected subsequently is therefore prevented. (The packaging cell lines provided by commercial suppliers are safety tested for replication incompetence, and a product analysis certificate is provided).

Primary human macrophage and fibroblast cells will be used in some experiments. These will be obtained from donors outside the school and grown to only low passage numbers to avoid any risk of transformation and accidental introduction into the original donor.

The Modified E. coli and viral vectors are not hazardous to the environment and in the event of accidental release from containment are unlikely to survive and propagate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All transfections and manipulations of cells will be performed in a Class II biological safety cabinet (identifiable to other users), preventing release of aerosols. Protective
clothing, double gloves to be worn at all times. Because of viral nature of the work, no glassware or sharps will be used, only disposable plastic ware. All potential infectious waste (solutions and microbiological plates) will be either disinfected [using 2% Virkon] or autoclaved, prior to disposal via drains or incineration as appropriate. Due to the labile lipid-derived nature of membranes of cells etc, the use of alcohol, detergents or bleach will be sufficient for decontamination. In event of spillage, the spill will be absorbed onto paper towel, the area will be effectively disinfected with Trigene (1:100 dilution) or Virkon (1:100 dilution). Towels to autoclaved prior to disposal. Disinfectants are effective against the organisms in question. Data on the appropriateness and effective degree of kill for these detergents are provided by Brindle Microbiological Consultants (Trigene) and Antek International; a subsidiary of Du Pont International (Virkon). The autoclave is subject to calibration and validation by a service company twice yearly.

Project Containment

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Animal Units

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Project Ref 232/00.7

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<td>30/11/2000</td>
<td>EXPRESSION OF NON-TOXIC PROTEINS IN MAMMALIAN CELLS USING</td>
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Project Additional Information

Purposes of the contained use

The intention is to express proteins in mammalian cells and tissues, to achieve high levels of transfection (-100%). The adenovirus system achieves this high transfection efficiency.

For transfection of cultured cells, the retroviral system allows efficient selection, and low level constitutive expression of genes using the Tet-off system, thereby enabling much more representative data. The proteins which I will be using are non-toxic and not oncogenic.

Recipient or parental organism

Adenoviral systems: Overview of system.

Inserts are cloned into a shuttle vector in E.coli. The insert DNA is then recloned into a predigested plasmid containing the Adeno-X viral DNA, and DNA of successful recombinants cloned and purified. The DNA is then transfected into an amphototrophic packaging cell line, which provides E1 sequences in trans, enabling production of an 'infectious' virus. The cell supernatant is then used directly for infection of cells and organs.

This is the Adeno-X system from Clontech, based on disabled Adenovirus 5. The Adenovirus lacks E1 sequences and is hence replication incompetent, and these are provided in trans by the cell line. The virus is produced in the supernatant of the packing cell line, but is replication defective, hence an access factor of -3.

Retroviral system: Overview of system

Inserts are cloned into a shuttle plasmid vector in SE.coli. The resulting plasmid is then transfected into an amphotrophic packaging cell line (with or without the Tet-on or Tet-off plasmids), and virus is produced by the cell line. The supernatant, containing 'infectious' virus, is collected and can be used for transformation of cell lines. It is then possible to select for stable integration of the retroviral DNA into the genome of the cell line (which does not support viral replication) using antibiotic selection. The retroviral system is the Retro-X system, and Tetracycline-regulated derivatives available from Clontech, based on the MuLV retrovirus. These systems use defective virus DNA, with the gag, pol and env genes provided in trans from a packaging cell line; the gag, pol and env genes are the product of separate integrations into the packaging cell line; and so a functional virus cannot be produced as a result of a single recombination event. The virus is produced in the supernatant of the packaging cell line, and can be used at this stage to infect cells. However, the virus is replication defective, hence an access factor of -3.

Host/vector system

Cytochromes P450 are membrane bound chemical metabolising enzymes. These are present endogenously, and have been expressed as transgenes, with no ill effects. AhR, AIP, PPAR and coactivator proteins are signalling molecules which are expressed endogenously, and are extremely unlikely to have any adverse biological effect. Latrophilin receptor is a transmembrane receptor which is expressed endogenously, and it is extremely unlikely to have any adverse effects.

Reporter proteins such as LacZ, GUS, luciferase, GFP and RFP have been expressed as transgenes and are know to be harmless. Fusion of these proteins with...
cytochromes P450, AhR, AIP or PPar are highly likely to be harmless, and there is no basis for proposing a damaging protein arising

**Origin & function**

The intention is to express proteins in mammalian cells and tissues, to achieve high levels of transfection (-100%). The adenovirus system achieves this high transfection efficiency. For transfection of cultured cells, the retroviral system allows efficient selection, and low level constitutive expression of genes using the Tet-off system, thereby enabling much more representative data. The proteins which I will be using are non-toxic and not oncogenic.

**Evaluation of foreseeable effects**

The work to be carried out will use only small scale cultures (11 maximum) and therefore no more than 10^12 organisms will be used at any one time. Normal laboratory containment procedures are sufficient to contain even total spillage of such a culture and since no especial risk of air-borne transmission exists, no special containment within the ACGM2 suite will be required. There are no sensitive populations of either plants or animals or humans in the area surrounding the laboratory and so there is no specific risk associated with accidental release. The research labs are not near a main road or any other building that may be liable to catastrophic explosion, and so a breach in the physical fabric of the laboratory is not seen as a likely event. All organisms are heat sensitive and so in the event of fire they would be destroyed. In the event of a release, gene spread is limited due to the use of non-self mobilisable vectors and the cloned genes are not known to confer any harmful properties and are therefore highly unlikely to cause a problem if released. The viruses are easily destroyed, and there should be minimal chance of environmental contamination. The virus would in any case be unable to replicate. There are no other environmental considerations.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMS - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The viruses are only infectious immediately after the packaging in specialised mammalian cells, which contain several genes necessary for the infectivity of the virus. The virus particles can only go through one round of infection, and are then unable to produce viable virus. Thus the stage where there is an ability to infect other cells is tightly defined, this stage will normally occur inside a class 2 cabinet (to ensure sterility of the cells and supernatant), and there is little opportunity for aerosol formation. The infectious virus will be clearly labelled. Infection of tissue samples may take place on the open bench; the experiments will be carefully designed to minimise the production of aerosols. HSE guidance confirms that these expression systems are very well characterised, and suitable for class 2 containment. The viruses are also relatively fragile, and are destroyed by ethanol, detergent, or UV. The work will be carried out at ACDP/ACGM Class 2 containment level. Waste will be disinfected using a validated protocol, e.g.

1) Soaking in 1% Stericol for >30 minutes
2) Soaking in 1% Chloros for >30 minutes
3) Autoclaving

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
### Project Containment

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**Large Scale Activities**

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**Human Clinical Applications**

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### Project Ref 232/00.8

**Date Ackn’d:** 15/02/2001

**CU2 Project Title:** EXPRESSION OF PPAR, GABA, AHR, GLUTAMATE AND GROWTH HORMONE RECEPTORS, CYTOCHROME P450 GENES, DELTA-LATROINSECTOTOXIN AND ASSOCIATED GENES.

**Class:** Class 2

**Culture Vol Class 2:**

**Consent Granted:** not applicable

**Project notified under transitional arrangements:** N

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### Project Additional Information
### Purposes of the contained use

These proteins are expressed for a variety of structural and functional studies of P450 and other genes involved in chemical carcinogenesis and of molecules involved in the process of neurotransmission.

### Recipient or parental organism

The cloning hosts will be E.coli hosts JM109, HMS 174 and derivatives all RecA-K12 derivatives or BL21 and derivatives. These are disabled and therefore considered non-pathogenic to humans, animals or plants. The strains are all laboratory adapted and so are unlikely to survive outside the laboratory. Induction experiments utilise a host lysogenic for bacteriophage DE3 or else superinfection with the phage CE6. In both instances these phage are disabled cloning strains carrying tetracycline resistance.

### Host/vector system

The plasmid vectors used are pBR derivatives encoding ampicillin or chloramphenicol and are considered non-mobilisable. The typical host used are pT7-7 and pRSET series of plasmids. Expression is by T7 polymerase induced using IPTG.

### Origin & function

These proteins are expressed for a variety of structural and functional studies of P450 and other genes involved in chemical carcinogenesis and of molecules involved in the process of neurotransmission.

The eukaryotic receptor genes are membrane bound, multicellular proteins that are unlikely to be able to functionally express in E.coli which lacks the ability to perform membrane insertion, transport, assembly and other post-translational processing required for functional expression. These proteins are expressed as polypeptide fragments that do not form functional receptors in E.coli. These clones are therefore unlikely to show a biological effect. These proteins all also require the ligand activation.

Functional P450 protein has no known biological effect.

The reporter genes are encoding for products such as beta-galactosidase, luciferase and Green Fluorescent protein that are well characterised and known to be non-toxic. Delta latroinsectotoxin is an insect specific toxin which has no mammalian toxicity. The toxin is degraded when eaten and is only possibly active when injected. It is therefore of no risk to insects in the wild.

### Evaluation of foreseeable effects

The work to be carried out will use only small scale cultures (11 maximum) and therefore no more than 10 12 organisms will be used at any one time. Normal laboratory containment procedures are sufficient to contain even total spillage of such a culture and since no especial risk or air-borne transmission exists, no special containment within the ACGM 2 suite will be required. There are no sensitive populations of either plants or animals or humans in the area surrounding the laboratory and so there is no specific risk associated with accidental release.

In the event of a release, gene spread is limited due to the use of non-self mobilisable vectors and the cloned genes are not known to confer any harmful properties and are therefore highly unlikely to cause a problem if released.

The chance of release of bacterial plasmid hosts into the environment is reduced to negligible levels by employing the above working practices. The recombinant strain is unlikely to survive in the natural environment as growth media supplements are required for growth.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- Small scale cultures (11 maximum)
The work will be carried out at ACDP/ACGM Class 2 containment level. Waste will be disinfection using a validated protocol, e.g.
1) Soaking in 1% Stericol for >30 minutes
2) Soaking in 1% Chloros for >30 minutes
3) Autoclaving

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Project approved by GM Safety Committee which includes representatives of all groups of University employees. Decision reported to University Safety Committee for consideration. Project details assessed by appropriate expert members of committee.

Project Containment

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<th>Growth Rooms</th>
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Animal Units

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Large Scale Activities

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Project Ref 232/00.9

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<td>15/02/2001</td>
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Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

Transfection techniques are used to express receptor and reporter genes in mammalian cells. These transfected cells are then used for functional studies.

**Recipient or parental organism**

Growth and construction of the transfection plasmids in *E. coli* is carried out as for Project I except that pcDNA1 constructs need to be grown in MC1061/P3 which is a RecA+E. coli strain carrying amber stop codon suppressed tetracycline and ampicillin antibiotic resistance genes and a functional chloroamphenicol antibiotic resistance gene.

**Host/vector system**

The cell systems used are both derived from primary tissue (e.g. rat hepatocytes) and also well characterised cell lines (e.g. HEK392). None of the mammalian cell lines could survive outside laboratory culture conditions.

**Origin & function**

The plasmids used for transfection carry either a CMV (cytomegalovirus) promoter (e.g. pcDNA derivatives) or a Rous Sarcoma virus promoter RSV (e.g. pRBK derivatives), together with a combination of selectable markers including tetracycline and ampicillin for bacterial selection and kanamycin resistance for selection in mammalian cells.

The receptors and reporter genes expressed in these experiments are non-toxic.

**Evaluation of foreseeable effects**

The work to be carried out will use only small scale cultures (11 maximum) and therefore no more than 10-12 organisms will be used at any one time. Normal laboratory containment procedures are sufficient to contain even total spillage of such a culture and since no especial risk of air-bourne transmission exists, no special containment within the ACGM 2 suite will be required. There are no sensitive populations of either plants or animals or humans in the area surrounding the laboratory and so there is no specific risk associated with accidental release.

The research labs are not near a main road or any other building that may be liable to catastrophic explosion, and so a breach in the physical fabric of the laboratory is not seen as a likely event. All organisms are heat sensitive and so in the event of fire they would be destroyed.

In the event of a release, gene spread is limited due to the use of non-self mobilisable vectors and the cloned genes are not known to confer any harmful properties and are therefore highly unlikely to cause a problem if released.

The chance of release of mammalian or bacterial plasmid hosts into the environment is reduced to negligible levels by employing the above working practices.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Small scale cultures (11 maximum).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The work will be carried out at ACDP/ACGM Class 2 containment level. Waste will be disinfected using a validated protocol, e.g.
1) Soaking in 1% Stericol for >30 minutes
2) Soaking in 1% Chloros for >30 minutes
3) Autoclaving

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project approved by GM Safety Committee which includes representatives of all groups of University employees. Decision reported to University Safety Committee for consideration. Project details assessed by appropriate expert members of committee.

Project Containment

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Project Ref 232/03.1

Date Ackn'd 18/02/2005

CU2 Project Title PHYSIOLOGY AND PATHOGENICITY OF CAMPYLOBACTER GENE PRODUCTS

Date Project Ceased

Class 2 CultureVolClass2 < 1 Litre Non-GMM Consent Granted Not Applicable
The aim of the project is to evaluate the role of specific Campylobacter genes associated with the physiology and pathogenicity of the bacterium in vivo. To do this gene sequences and their promoters will be reintegrated into the bacterial genome in order to knock-out or alter their function. Campylobacter genes for which we can ascribe a role will be manipulated in an attempt to express them in laboratory strains of E. coli to study the structure and function of the proteins they encode.

**Recipient or parental organism**

- Host: E. coli Top10 cells (Invitrogen) (ACDP 1)
- Host: E. coli BL21(DE3) (Novagen) (ACDP 1)

All the recipient Campylobacter species to be used in this study are wild type ACDP group 2 organisms. No multiply-antibiotic resistant isolates will be used which would preclude therapy with standard antibiotic regimes should infection occur.

- Host: Campylobacter jejuni (ACDP 2)
- Host: Camplylobacter coli (ACDP 2)
- Host: Camplylobacter lari (ACDP 2)
- Host: Campylobacter upsaliensis (ACDP 2)

**Host/vector system**

- Vector: pCR2.1 Topo plasmid (Invitrogen)
- Vector: Approved vectors based on colE1 replicating plasmids (eg pUC18, pBS and pBC)
- Vector: pET series of expression plasmid vectors (Novagen)

All the Campylobacter transforming DNAs will constitute integrative constructions carrying antibiotic selection either to mark a targeted gene knock-out or as second cistron to the functional Campylobacter gene to be reintroduced. These DNA constructions will be assembled in E. coli using the plasmid vectors cited above.

**Origin & function**

Genomic DNAs of the following:

- Campylobacter jejuni (ACDP 2)
- Camplylobacter coli (ACDP 2)
- Campylobacter lari (ACDP 2)
Campylobacter upsaliensis (ACDP 2)  
Campylobacter bacteriophage DNA

The target gene sequences are expected to encode bacterial cell-surface associated proteins or be responsible for the biosynthesis of surface associated components (e.g., lipopolysaccharide, capsular polysaccharide, lipid and glycosylation modifications of proteins). Gene functions may also include products responsible for intracellular DNA modifications.

**Evaluation of foreseeable effects**

Campylobacter enteritis is the most common form of bacterial food poisoning in the world. Campylobacter infection occurs through ingestion (fecal oral route) and results in a self-limiting diarrhoeal disease. A few rare post-infection cases have been associated with the later development of a paralysing autoimmune disease (Gillian Barre syndrome). There is no specific risk of transmission by aerosol known for these organisms. Campylobacter jejuni is known to encode a cytolethal distending toxin. However, the toxin contains multiple sub-units all of which would have to be co-expressed. The host Campylobacter species are initially wild type and have the potential to cause human disease. It is therefore proposed that all experiments will be performed under containment conditions ACDP 2. The resulting GMMs are unlikely to pose any further risk to human health as they will contain DNAs of similar origin and that the ACGM containment level be 2, consistent with the host and donor DNAs.

Birds carry thermophilic Campylobacters in their gut without any known detrimental effect, such that most wild birds and domestic poultry are colonised. It is possible that the modified Campylobacters could survive in avian species if they were to be released into the environment. Therefore it is proposed that all experiments will be performed under containment conditions ACGM 2.

When expressing the Campylobacter genes in E. coli it is possible that some of these genes could increase their fitness and/or human or animal colonisation potential. However, the auxotrophically crippled organisms (E. coli K-12 ACDP 1) will still retain limited survivability in the environment. The majority of genes are not known to be toxic, and when expressed in isolation form the remaining Campylobacter host genes are unlikely to produce any deleterious effects over the parental organism. Owing to the potential for increased expression levels in E. coli the containment level for this work is proposed to be ACGM level 2 consistent with the ACDP level of the donor organisms.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Local Incineration: solid wastes are incinerated at 1000 degrees C for 360 minutes until ash (100% kill). These are discarded as solid waste.

Autoclave: Liquid wastes are autoclaved at 121 degrees C for 30 minutes until to achieve 100% kill (internal temp and pressure monitors) and discarded to the drain once cool. These measures are coupled with condensate thermocouples and thermologue test strips.

Chemical Treatment: 2% Virkon is used to remove contamination from solid surfaces. This concentration will kill the bacterial rapidly as recommended by the supplier. After 15 minutes contact the disinfectant is diluted and the liquid waste discarded to drain.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N
Project approved at Containment level 2 by GM Safety Committee, which includes representatives of all University employees. The project details were assessed by expert members of the committee and the decision was reported to the general safety committee.

**Project Containment**

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**Project Ref** 232/trans1

- **CU2 Project Title:** STRUCTURAL STUDIES ON RECOMBINANT FRAGMENTS OF INVASIN AND INTIMIN
- **Class:** Class 2
- **Culture Vol Class 2:** Not Applicable
- **Culture Volume Class 3-4:** Not Applicable
- **Non-GMM Consent Granted:** Not Applicable
- **Date Ackn'd:** 18/02/2005
- **Date Project Ceased:**
- **Withdrawn:** N

**Historical Significant Changes**

GM232/00.1. GM232/trans1 transferred to GM470 on 18/02/2005

**Historical Date of Additional Info**

15/03/2000, 18/02/2005

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units
| L2 | L3 | L4 |
Large Scale Activities
| L2 | L3 | L4 |
Human Clinical Applications
| L2 | L3 | L4 |

Project Ref 232/trans3

Date Ackn'd 18/02/2005  
CU2 Project Title USE OF BOILUMINESCIENCE FOR PHYSIOLOGICAL STUDIES OF BACTERIA

Class 2
Non-GMM Consent Granted Not Applicable
Project notified under transitional arrangements

Tick if notifying a connected programme of work
Historical Significant Changes
GM232/00.3, GM232/trans3 transferred to GM470 on 18/02/2005

Historical Date of Additional Info
22/09/2005, 18/02/2005

Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Project Containment

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Project Ref 232/trans4

Date Ackn'd: 18/02/2005

CU2 Project Title:
CHARACTERISATION OF THE BIOCHEMISTRY AND GENETICS OF ENVIRONMENTAL GENE REGULATION IN STAPHYLOCOCCUS

Class:
Class 2

CultureVolClass2:

CultureVolumeClass3-4:

Non-GMM Consent Granted:
Not Applicable

Project notified under transitional arrangements:
Y

Withdrawn:
N

Tick if notifying a connected programme of work:
N

Historical Significant Changes:
GM232/00.4, GM232/trans4 transferred to GM470 on 18/02/2005

Historical Date of Additional Info:
22/09/2005, 18/02/2005

Date Ackn'd:
18/02/2005

Date Project Ceased:

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project notified under transitional arrangements **Y**

Withdrawn **N**

Tick if notifying a connected programme of work **N**

**Historical Significant Changes**
GM232/00.5, GM232/trans5 transferred to GM470 on 18/02/2005

**Historical Date of Additional Info**
22/09/2000, 18/02/2005

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 232/trans6

Date Ackn'd  18/02/2005

Date Project Ceased

Withdrawn  N

Tick if notifying a connected programme of work  N

DEVELOPMENT OF BIOLUMINESCENT AND FLUORESCENT DERIVATIVES OF PSEUDOMONAS SPP FOR STUDIES OF GENE REGULATION

Class 2

Non-GMM

Consent Granted  Not Applicable

Project notified under transitional arrangements  Y

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### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**
  - Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- **Is an emergency plan required according to regulation 20?** N
- **If yes, tick to confirm that it is attached to this form** N
Project Containment

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Animal Units

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Project Ref 470/00.2

Date Ackn’d 12/09/2000

CU2 Project Title STUDIES IN GENE REGULATION IN PORPHYOMONAAS GINGIVALIS

Class Class 2

CultureVolClass2

CultureVolumeClass3-4 non-GMM

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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**Project Ref** 470/01.1

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<tr>
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<tr>
<td>22/02/2001</td>
<td>CONSTRUCTION OF LABORATORY SAFETY AND VACCINE STRAINS OF NEISERIA MENINGITIDIS, NEISERIA GONNORHOEAEA, NEISERIA LACTAMICA, NEISERIA SUBFLAVA AND OTHER NEISERIA SPP.</td>
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The objectives of the project are to construct stable, disabled strains of N. meningitidis, N. gonorrhoeae and other Neisseria groups, by genetically modifying genes which are essential for growth of the organism in the host. Further manipulations will make the strains unable to repair the mutations. These strains can then be further developed as candidate live attenuated and vesicle vaccine strains for meningococcal infections by upregulating certain protective antigens and removing endotoxin, LOS, and capsule genes.

**Recipient or parental organism**

The modified genetic material will ultimately be reintroduced into wild type Neisseria meningitidis, Neisseria gonorrhoeae or non-pathogenic commensal Neisseria spp.

**Host/vector system**

Genetically manipulated material will be maintained in disabled E.coli strains: XL1-Blue, JM109, DH5aF°, SCS110

All strains are unable to maintain colonisation of the human gut and are disabled.

Examples of cloning vectors to be used include:
(1) pUC19:- colE1 origin, non-mobilisation, non-conjugative
   pCRII  ampicillin resistant, kanamycin resistant
   pGIT-5
   pGEM
   pET
   etc.

(2) pCVD422:- Suicide vector in bacteria not carrying -pir (contains pir dependent origin, of replication). Self mobilisable vector but can only replicate in E.coli -pir strains like SM10. Carries ampicillin resistance and the SacB gene from Bacillus strains which metabolises sucrose to generate a compound lethal to Gram negative organisms. All vectors (except pCVD422) are non-mobilisable. PCVC422 cannot replicate in non lambda pir strains.

**Origin & function**

The genetic material to be modified will be isolated from clinical isolates of Neisseria meningitidis or Neisseria gonorrhoeae - ACDP hazard group2 pathogens and a range of non-pathogenic commensa Neisseria spp. The genetic material will be used to inactivate genes e.g. aromatic amino acid biosynthetic genes essential for prolonged growth or survival of Neisseria meningitidis in the human host.

**Evaluation of foreseeable effects**

**Manipulations in E.coli** - All manipulations are in disabled hosts using non-mobilisable or mobilisation deficient vectors. None of the E.coli strains used will be able to colonise humans. Expression of genes derived from Neisseria meningitidis will be carried out using disabled, non-colonising E.coli hosts and non-mobilisable vectors. All genes expressed with the intention of purifying proteins will be housekeeping genes or genes involved in DNA repair/uptake, global regulation nutritional requirements, or surface LOS/capsule expression, all found in most prokaryotes. Therefore in the unlikely event of ingestion of an over expressing E. coli strain it is unlikely to cause harm.

**Manipulation of Neisseria sp.**

The neisseria strains to be used are wild type and therefore should be handled under containment level 2. However as an additional safety precaution to protect laboratory staff and eliminate the risk of environmental contamination, we intend to handle all Neisseria strains in the Institute category 3 facility (both wild types and mutants). The inactivated gene will be inserted in the exact position of the wild type gene and will have no potential for expression of the target gene above the normal wild type levels at any time during the construction. At no stage will antibiotic markers which could compromise therapy for meningococcal or gonococcal infection be introduced into the wild type organisms.

All cloned DNA will be fragments of well characterised non secreted housekeeping genes involved in bacterial nutrition, regulation of gene expression or structural components of the cell. None of the target genes on their own are known virulence determinants although some may regulate certain virulence genes. These genes will in addition be further deleted to completely abolish expression and normal cellular activity of the gene product in the Neisseria host. All the gene products are highly unlikely to have a deleterious effect on humans and since their expression will be completely abolished in the Neisseria host by the manipulations there is no possibility of any of the products being produced. It should also be noted that homologs of these genes and their protein products are present in most if not all bacteria including those of the human normal flora and therefore could be classed as being present in the environment in high levels.

Taken together, these measures are considered to reduce the likelihood of any potential hazard arising from the proposed genetic manipulation effectively to zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste generated will be autoclaved prior to disposal. No viable counts are expected after the treatment. Laboratory lab coats will be autoclaved prior to laundering.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The submission was approved by the committee without modification.

**Project Containment**

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<td>Large Scale Activities</td>
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**Project Ref** 470/01.10

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<td>INTRODUCTION OF VECTORS CONTAINING DIFFERENT ANTIBIOTIC RESISTANCE AND GENES INVOLVED IN THE ADAPTATION TO ENVIRONMENTAL CHANGES FROM OTHER GROUP 1 AND GROUP 2 ORGANISMS INTO STREPTOCOCCUS SPP</td>
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Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 470/01.11

Date Ackn’d: 07/03/2001

CU2 Project Title:
CONSTRUCTION OF ISOGENIC MUTANTS AND CHROMOSOMAL FUSIONS TO REPORTER GENES IN STREPTOCOCCUS SPP USING TRANSPOSONS AND SUICIDE PLASMIDS

Class: Class 2

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 470/01.12

Date Ackn'd 07/03/2001

Date Project Ceased

CU2 Project Title

CLONING OF GENES FROM STREPTOCOCCUS SPP INTO GENETICALLY DISABLED ESCHERICHIA COLI USING A RANGE OF VECTORS

Class 2

Consent Granted not applicable

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 470/01.13

Date Ackn'd: 07/03/2001

CU2 Project Title: CLONING AND EXPRESSION OF LUXR/I HOMOLOGUES AND OF THE QUORUM

Class: Class 2
SENSING RELATED GENES ISOLATED FROM GROUP 1 AND GROUP 2 BACTERIA INTO DISABLED ESCHERICHIA COLI
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 470/01.14

CONSTRUCTION OF ISOGENIC MUTANTS AND CHROMOSOMAL FUSIONS TO REPORTER GENES IN CHROMOBACTERIUM VIOLACEUM USING TRANSPOSONS AND SUICIDE PLASMIDS

Date Ackn’d 07/03/2001

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref:** 470/01.15

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<td>CONSTRUCTION OF ISOGENIC MUTANTS AND CHROMOSOMAL FUSIONS TO REPORTER GENES IN KLEBSIELLA SPP. USING TRANSPOSONS AND SUICIDE PLASMIDS.</td>
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- **Non-GMM Consent Granted:** not applicable
- **Project notified under transitional arrangements:** Y

**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? ☒

If yes, tick to confirm that it is attached to this form ☒

Tick to confirm that you have attached a risk assessment to this form ☒

Tick if you are claiming exemption from disclosure for section of the risk assessment ☒

Please enter comments on the GM safety committee on the risk assessment

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### Project Ref 470/01.16

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 470/01.17

Date Ackn'd 07/03/2001

CU2 Project Title CONSTRUCTION OF ISOGENIC MUTANTS AND CHROMOSOMAL FUSIONS TO REPORTER GENES IN PSEUDOMONAS SPP USING TRANSPOSONS AND SUICIDE PLASMIDS

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID GM470/01.17a

Date of Significant Change 03/06/2010
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
CLONING OF GENES FROM PSEUDOMONAS SPP. INTO GENETICALLY DISABLED ESCHERICHIA COLI USING A RANGE OF VECTORS

Date Ackn'd 07/03/2001

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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ANTIBIOTIC RESISTANCE AND QUORUM SENSING RELATED GENES FROM OTHER ACDP HAZARD GROUP 1 AND 2 ORGANISMS INTO VIBRIO CHOLERAE

Date Project Ceased

Tick if notifying a connected programme of work

 Projekt notified under transitional arrangements

Historical Significant Changes

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Project Ref 470/01.2

INTRODUCTION OF THE QUORUM SENSING LUX REPORTER PLASMID PSB401 AND OTHER PLASMIDS ALSO CONTAINING QUORUM SENSING RELATED GENES INTO HAEMOPHILUS SPP.

Class 2

Consent Granted

not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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02/03/2022
**Project Ref**: 470/01.20

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**Project notified under transitional arrangements**: Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 470/01.21

**Date Ackn'd** 07/03/2001

**CU2 Project Title** CHARACTERISATION OF HELICOBACTER PYLORI PATHOGENESIS

**Class** Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**
Date Project Ceased

Tick if notifying a connected programme of work

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Project Ref** 470/01.22

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<td>07/03/2001</td>
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Withdrawn N  
Tick if notifying a connected programme of work N  
Historical Significant Changes  
Historical Date of Additional Info  
Significant Change ID  
Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

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02/03/2022
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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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- Animal Units
  - L2
  - L3
  - L4

- Large Scale Activities
  - L2
  - L3
  - L4

- Human Clinical Applications
  - L2
  - L3
  - L4

**Project Ref 470/01.25**

- **Date Ackn’d:** 07/03/2001
- **CU2 Project Title:** ANALYSIS OF THE EFFECT OF MUTATION OR OVER-EXPRESSION OF REGULATORY GENES ON CELL PHYSIOLOGY AND VIRULENCE GENE EXPRESSION IN DISABLED AND WILD-TYPE DIARRHOEAGENIC E.COLI, AND RELATED BACTERIA
- **Class:** Class 2
- **Consent Granted:** not applicable
- **Project notified under transitional arrangements:** Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Project Ref  470/01.26

Date Ackn'd 07/03/2001

Date Project Ceased

Non-GMM not applicable

Consent Granted

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Animal Units

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Project Ref 470/01.27

Date Ackn'd 07/03/2001  
CU2 Project Title CLONING OF QUORUM SENSING RELATED GENES FROM VIBRIO SPP.

Class 2  
CultureVolClass2  
CultureVolumeClass3-4
**Project Additional Information**

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<thead>
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<tr>
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<td>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</td>
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Animal Units

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Project Ref 470/01.28

Date Ackn’d 07/03/2001

CU2 Project Title

ISOLATION AND CHARACTERISATION OF STAPHYLOCOCCAL GENES INVOLVED IN IRON UPTAKE AND THE REGULATION OF VIRULENCE DETERMINANT EXPRESSION

Class

Class 2

Consent Granted

not applicable

Tick if notifying a connected programme of work N
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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02/03/2022
CLONING OF QUORUM SENSING GENES HOMOLOGOUS TO LUXR AND LUXL OR OTHER QUORUM SENSING RELATED GENES FROM KLEBSIELLA SPP INTO GENETICALLY DISABLED ESCHERICHIACOLI USING A RANGE OF VECTORS
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Project Ref  470/01.3

Date Ackn'd CU2 Project Title

15/02/2001 INTRODUCTION OF A RANGE OF VECTORS CONTAINING EXCLUSIVELY

Class CultureVolClass2 CultureVolumeClass3-4

Class 2
HOMOLOGUES OF THE LUX/R QUORUM SENSING GENES OR OTHER QUORUM SENSING RELATED GENES FROM GROUP 1 AND GROUP 2 ORGANISMS INTO VIBRIO SPP.

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Project Ref** 470/01.30

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

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02/03/2022
Project Ref 470/01.31

Date Ackn'd 14/03/2001

Date Project Ceased

CU2 Project Title ISOLATION AND CHARACTERISATION OF STAPHYLOCOCCAL GENES INVOLVED IN IRON UPTAKE AND THE REGULATION OF VIRULENCE DETERMINANT EXPRESSION

Class 2

CultureVolClass2 Class CultureVolumeClass3-4

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID GM470/01.31a

Date of Significant Change 03/06/2010

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Animal Units

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Withdrawn N

Tick if notifying a connected programme of work N

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref** 470/01.33

- **Date Ackn’d**: 14/03/2001
- **CU2 Project Title**: CLONING AND SELECTION OF KNOWN REGULATORY AND VIRULENCE-ASSOCIATED GENES FROM BIOLOGICAL HAZARD GROUP 2 PROTEOBACTERIA IN DISABLED E.COLI
- **Class**: Class 2
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 470/01.34

Date Ackn'd 14/03/2001

Date Project Ceased

Class 2

CultureVolClass2

CultureVolumeClass3-4

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Is an emergency plan required according to regulation 20?  N

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Project Ref  470/01.35

Date Ackn'd  14/03/2001  CU2 Project Title  ANALYSIS OF THE EFFECT OF MUTATION OR OVER-EXPRESSION IN

Class  Class 2  CultureVolClass2

CultureVolumeClass3-4
DISABLED AND WILD-TYPE DIARRHOEAGENIC E.COLI, AND RELATED BACTERIA UPTO ACDP HAZARD GROUP 2.

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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**Project Ref** 470/01.36

Date Ackn’d: 14/03/2001

Date Project Ceased

CU2 Project Title: INVESTIGATION OF THE EXPRESSION AND REGULATION OF VIRULENCE DETERMINANTS IN DIARRHOEAGENIC E.COLI UPTO ACDP HAZARD GROUP 2

Class: Class 2

CultureVolClass2: not applicable

Non-GMM Consent Granted

Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
**Project Ref** 470/01.4

**Date Ackn’ed** 15/02/2001

**CU2 Project Title** INTRODUCTION OF A RANGE OF VECTORS CONTAINING DIFFERENT ANTIBIOTIC RESISTANCE AND QUORUM SENSING RELATED GENES FROM OTHER GROUP 1 AND GROUP 2 ORGANISMS INTO CHROMOBACTERIUM VIOLACEUM.

**Class** Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM** not applicable

**Consent Granted**

**Project notified under transitional arrangements** Y

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Historic Significant Changes**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Please enter comments on the GM safety committee on the risk assessment

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Project Ref 470/01.5
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref**  470/01.6

- **Date Ackn’d**: 15/02/2001
- **CU2 Project Title**: CONSTRUCTION OF ISOGENIC MUTANTS AND CHROMOSOMAL FUSIONS TO REPORTER GENES IN BURKHOLDERIA SPP USING TRANSPOSONS AND SUICIDE PLASMIDS
- **Class**: Class 2
- **Culture Vol Class 2**: not applicable
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

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Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

Large Scale Activities

Human Clinical Applications

**Project Ref** 470/01.8

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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Project Ref 470/01.9

Date Ackn’d 15/02/2001

CU2 Project Title CONSTRUCTION OF ISOGENIC MUTANTS AND CHROMOSOMAL FUSIONS TO REPORTER GENES IN VIBRIO SPP. (EXCEPT V. CHOLERAE) USING TRANSPOSONS AND SUICIDE PLASMIDS

Class 2

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

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Tick to confirm that you have attached a risk assessment to this form

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02/03/2022
The aim of this project is to develop genetic systems to facilitate molecular studies in the clostridia. These will allow basic studies of cell signalling, metabolic processes and virulence gene regulation in the human anaerobic pathogen. Clostridium difficile and expression studies of metabolic genes in Clostridium sporogenes and in the non-pathogenic Clostridium acetobutylicum and Clostridium beijerinkii. These studies will involve 1) mutation of clostridial genes with plasmids or transposons, 2) complementation of mutants with homologous genes from clostridia or those from other bacteria or eukaryotic sources; 3) overexpression in clostridia of specific proteins (excluding known virulence determinants) from clostridia, other bacteria or eukaryotes to investigate effects on clostridial physiology/metabolism and to generate proteins for biochemical studies and antibody generation; 4) introduction of reporter constructs into clostridia for gene expression studies and, 5) expression of clostridial genes (excluding known virulence determinants) or homologues from other bacteria or eukaryotes in E. coli to generate proteins for biochemical studies and antibody production.

Disabled E. coli strains may be used as initial hosts for preparation of constructs but the modified genetic material will ultimately be reintroduced into wild type Clostridium difficile, Clostridium sporogenes, or the non-pathogenic Clostridium acetobutylicum or Clostridium beijerinkii.
The E. coli host strains to be used as cloning hosts are not known to be pathogenic. They have multiple auxotrophic requirements which are unlikely to be met in the environment where they are considered unlikely to survive. These strains can be considered equivalent to ACDP Hazard Group 1 organisms.

Clostridium difficile strains to be used, eg. CD630, CD3, and CD6 are ACDP Hazard Group 2 pathogens capable of causing gastrointestinal infection in humans; infection risk is associated with use of broad spectrum antibiotic therapy and pathogenesis is associated with production of exotoxins. Other strains eg CD37 (non-pathogenic in animal models) are non-toxigenic human clinical isolates likely to have reduced virulence for humans. These organisms may survive under appropriate conditions in the environment.

Clostridium sporogenes is a ACDP Hazard Group 2 clostridial species. Certain strains have been implicated in cases of gas gangrene. Other strains (formerly known as Clostridium oncoclyticum) have been used in the 1960s in human clinical trials for the treatment of brain tumours. The strain to be used here, Clostridium sporogenes NCIMB 10696, is believed to belong to the former category. It was isolated from soil and has previously been used in the testing of sporicidal agents in the quality control of media. It is known to produce toxins.

Clostridium acetobutylicum ATCC, 824, N1-4082 and Clostridium beijerinkii NCIMB 8052 are saccharolytic, non-pathogenic, non-colonising, non-toxigenic bacteria of ACDP Hazard Group 1. Although non-pathogenic these organisms may survive under appropriate conditions in the environment.

**Host/vector system**

**Vectors:** for cloning in E. coli and for use as suicide vectors in clostridia:

- Non mobilisable pUC- and pMTL-derived plasmid vectors, other vectors including Super Cos, Lambda or others which are Bom-/(Nic-), Mob- and Tra-.
- Mobilisation defective: Pinpoint, pBR322, pET30, ptrc99A, pAlterEx2, pJDC9, pMal, pLAFR or other vectors Bom+//(Nic+) but Tra- and Mob- which can efficiently be mobilised if they are co-resident with certain other plasmids.

For cloning/expression in clostridia:

- pMTL500E (based on the pAM 1 replicon) ans pMRL540E (based on the pCB102 replicon), and their derivatives, are non-mobilisable vectors which replicate in both E. coli and clostridia. pMTL9301 and derivatives are mobilisable shuttle vectors which replicate in E. coli and clostridia. Replicons from other plasmids eg pGK12, pCB101, and pBP1, other shuttle vectors eg pMK4 derivatives and plasmids previously used to deliver transposons in other Gram positive bacteria eg Tn917 derivatives may also be used.

Antibiotic resistance markers will include genes encoding resistance to ampicillin, erythromycin, tetracycline, chloramphenicol, kanamycin and spectinomycin.

**Origin & function**

C. difficile.

The genetic material for construction of defined mutants will be isolated from clinical isolates of C. difficile as detailed above. Genes to be mutated or overexpressed will include those involved in cell-cell signalling eg luxS, two component or other gene regulators or metabolic genes. Complementation studies may use gene homologues from clostridia, other bacteria upto ACDP Hazard Group 2 or eukaryotic sources. Reporter constructs may employ reporter genes from bacterial or eukaryotic sources.

C. sporogenes, C. acetobutylicum and C. beijerinkii.

Work with these clostridia will focus on expression studies with known metabolic genes, eg carboxypeptidase, nitroreductase and cytosine deaminase.

E.coli.

Genes for cloning in E. coli will include known metabolic genes or cell signalling genes eg luxS from clostridia, eukaryotic homologues and homologues from other bacteria upto ACDP Hazard Group 2. For construction of reporter fusions, promoters of known virulence determinants eg clostridial toxins may be cloned but no attempt will be made to clone full length genes of these determinants or to express known clostridial virulence determinants in E. coli.
C. difficile and C. sporogenes.

Introduction and/or expression of homologues of known metabolic or cell signalling genes in these clostridia is considered highly unlikely to enhance the virulence of the pathogen or its ability to survive in the environment. Random mutation using transposons or deliberate inactivation or overexpression of gene regulators could potentially reduce or enhance virulence determinant expression. All mutants generated by transposon mutagenesis and those where regulators are deliberately mutated or overexpressed will be fully assessed for production of known virulence determinants eg toxin production and will be handled in a Class 1 microbiological cabinet until fully characterised.

None of the antibiotic markers to be used are routinely used for therapy of clostridial infections so therapy would not be compromised in the unlikely event of accidental infection with a GMO.

C. acetobutylicum and C. beijerincki.

Modification of these organisms is considered extremely unlikely to alter their non-pathogenic status or their ability to survive in the environment.

E. coli

The E. coli strains to be used are disabled and the clostridial genes or homologues to be cloned are not likely to have a detrimental effect on human health. In the unlikely event of infection the consequence of an infection with recombinant E. coli containing these genes is likely to be very low. Modification of the E. coli strain is considered highly unlikely to enhance their survival in the environment.

Evaluation of foreseeable effects

Not applicable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste generated will be autoclaved to achieve 100% kill. Autoclaved waste will ultimately be disposed to landfill after further heat treatment and processing by approved waste disposal contractors.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The assessment was passed by the committee with minor modification.

Project Containment
The key aims of this research are:

1. Identification & modulation of genes involved in key physiological processes.

The organism is widely distributed in the environment and as a consequence is commonly found in raw foods. As it is very difficult to prevent, or control, the frequency of C. botulinum, and to apply a defined level of control. In order to more effectively prevent botulism it is imperative to understand those environmental factors that affect the ability of the organism to grow and/or elaborate toxin when present in food. Moreover, as the ability to spores represents one of the, if not the most important, virulence factors. Thus analysis/modulation of the processes that regulate growth, sporulation, toxin production and germination will be a major aspect of this project. The major form of modulation will be seek inactivation of the target gene through either gene disruption, or via antisense RNA. Overexpression of selected gene products may also be pursued, however, in this case the genes studied would not encode a product that could potentially cause harm to human health or the environment, nor result in hyper production of toxin. The effects of modulation will be measured phenotypically, by enzyme assay where appropriate, and using transcriptomics and proteomics.

2. Characterise putative quorum sensing mechanisms.

Preliminary data has shown that the organism contains 35 two-component regulators, in addition to numerous orphan responses regulators and sensor kinases. Such
gene products play a pivotal role in the co-ordination of bacterial gene expression in response to both environmental signals and bacterial derived cell signals, termed autoinducers (Ais). With respect to the latter, homologues of genes involved in bacterial cell signal generation are also evident, including the agr system of staphylococcus aureus that mediates AI-1 synthesis and the ubiquitous LuxS system responsible for bacterial AI-2 production.

3. Develop genetic tools.
The existing genetic system available in C.botulinum are rudimentary in nature. To achieve the above goals more effective tools will be required; in particular vectors that may be employed to bring about gene replacement. These may be used to replace the neurotoxin gene with a useful reporter gene, such as gusA. The use of such a strain in subsequent experiments would further minimise the risks associated with the envisaged studies.

Recipient or parental organism

Category 1 disabled hosts
Escherichia coli (disabled K12 derivatives) and Bacillus subtilis 168. These hosts will be used in experiments aimed at transferring plasmids into C botulinum, (ii) for heterologous expression of proteins of C.botulinum (other than intact BoNT), or (iii) as intermediate hosts to in vivo methylate plasmids before transformation of C. botulinum. Work will also be undertaken using various strains of non-toxinogenic Clostridium sporogenes, and in particular strain 10696 which is classified as Class 1 by the ATCC.

Category 2 The sequenced strain proteolytic C.botulinum type A Hall A (ATCC 3502) will be employed as the primary recipient strain. In addition, we intend to study a number of different strains of proteolytic culture collection, with whom we are collaborating.

Host/vector system

pUC or pMTL-based vectors (based on the ColE1 replicon) will be used for all cloning and expression work in E.coli K12. With the exception of the pET vector series (which is nic+) all vectors to be employed are inc mob minus, and approved in Part 2A Annex II of ACGM Compendium.

The shuttle vectors to be employed in clostridia will carry both Gram-negative and Gram-positive derived components. Thus they will carry the ColE1 replicon (derived from either pUC of pMTL vectors), and in some instances the pBR322-derived bla gene. Neither element functions in clostridial species. Antibiotic resistance genes will include the C.perfringens catP gene, the pAMB1-derived erm gene, the enterococcal Spec gene, and the Tn916-derived tet gene. For those plasmids that need to be introduced by conjugative mobilisation, the plasmids will also possess the oriT region of plasmid RK2. Such plasmids are not self transmissible, but may be mobilised from bacterial donors if the donor carries Tra &Mob functions on a second element.

The use of transposons for generating mutants will also be explored. These will include elements isolated from Gram-positive organisms (eg.Tn552 and Tn916), Gram-negative elements adapted for use in Gram-positives (eg. Tn 10), transposons generated from clostridial IS (Insertion Sequence) elements to which an antibiotic resistance gene (catP) has been added. These would include ISCb1 recently discovered in C. beijerinckii NCIMB 8052, ISCce1 from C. cellulolyticum and group II intron L1. LtrB of Lactococcus lactis.

Inserts
The inserts to be employed will be sequence specific segments of DNA, generated from the C.botulinum genome by PCR. In no instance will a gene be cloned which is perceived to encode a product that has the potential to be harmful to human health or the environment. Under no circumstances will the intact botulinum neurotoxin genes be cloned. In the majority of cases, only non-functional genes will be introduced into Clostridium ie; internal fragments of genes, or genes which have been inactivated by internal deletions/insertions.

In some instances heterologous genes may also be introduced. These could include homologues of regulatory genes or sporulation/germination genes from B.subtilis, but in the main will be genes encoding innocuous reporter enzymes.

Origin & function
Our major focus is to bring about the inactivation of genes as a means of ascribing function and identifying regulatory networks. Therefore, in the main we will be introducing inactive portions of genes consisting of internal fragments (single cross-over knock-out), genes that have been inactivated by insertion or deletion (double cross-over), or fragments encoding the 5'-non-coding region and a portion of complementation studies. These will be expressed either from their native promoter, placed under the inducible (ITPG) control of fac or a similar promoter.

Regulatory Genes:
The genes to be targeted will be transcriptional regulators and sensor kinases. These will form part of two-component regulators (there are estimated to be 35 such systems in the genome sequence), or may be orphan. The only transcriptional regulator that has been characterised in C.botulinum is cntR (clostridial neurotoxin regulator). It is absolutely required for production of toxin. Its inactivation will therefore lead to severe depression of toxin production. It may of course additionally lead to the repression of other genes linked to toxin production.

Genes associated with Spore Formation:
Homologues of B.subtilis involved in both sporulation and germination will be targeted, dependent on the genes seen to be up and down regulated by inactivation of specific response regulators/sensor kinases. These will include homologues of the key spo and ger genes, and other factors implicated in cell differentiation.

Genes involved in cell-cell communication:
All clostridial genomes, including C.botulinum, contain luxS homologues, the gene responsible for production of AI-2, a furanone signalling molecule. AI-2 production has been shown to occur in C.difficile and C. perfringens, and to participate in the regulation of virulence factors. The effect of inactivating luxS, by both knock-out and antisense diffusible signalling molecules that regulate the production of toxins and other virulence factors, but efforts to purify these presumed peptide molecules have been unsuccessful.

Intriguingly, we have identified with C.botulinum two separate unannotated ORFs capable of encoding small proteins (44-54 aa), which share homology with the precursor (AgrD) of the Staphylococcus aureus autoinducer peptide (AIP). AIP regulates the temporal production of virulence factors. In both cases, the identified clostridial agrD genes are preceded by a gene encoding an agrB homologue, the S.aureus transmembrane protein required for synthesis and processing of agrD to AIP. The effect of inactivating these genes, by both knock-out and antisense technology, will be evaluated.

Reporters Genes:
To aid in our analysis of gene regulation, we will also introduce reporter genes encoding innocuous enzymes which have been placed under transcriptional control of C.botulinum gene promoters. These will include lacZ (B-galactosidase), gusA (B-glucuronsidase), xylE (catechol 2,3 monooxygenase) luxAB (luciferase), and other well characterised reporter genes. These will be introduced either into the chromosone, or on autonomously replicating plasmids.

Evaluation of foreseeable effects:
B.subtilis and the proposed E.coli disabled E.coli K12 derivatives strains are all in the biological agents hazard group1. The introduction of C.botulinum genes with known harmless functions will not increase the hazard. C botulinum genes encoding proteins with homologies to known proteins with toxic or harmful properties and genes with unknown functions are to be introduced into the above hosts. These GM stains will be handled at containment level 2 to ensure the overall risk is negligible. A functional BoNT will not be cloned or introduced into these strains.

C.botulinum is not infectious, and therefore classical concerns of virulence, and any enhancement of such properties, are not appropriate. As the principal modifications to be undertaken are the inactivation of genes (regulators and homologues of genes involved in the sporulations process, spore formation and germination) the majority of GMMs will be less hazardous than the recipient itself. It is theoretically possible that certain manipulations could effect the levels of neurotoxin produced, although this in itself may not make the organism anymore virulent/hazardous.

All work with C.botulinum cells (vegetative/spores), supernatants and any media that may contain BoNT will take place in a containment level 2 work suit designated for work with C.botulinum and the above mentioned hosts. This room will have key code access and will only be available for use by members of staff trained in handling C.botulinum. Risk assessments and standard operating procedures will be produced for all aspects of research undertaken. Work is performed in an anaerobic airtight cabinet or in a Class II safety cabinet, thus the risk of exposure to the toxin and to contract botulism is minimized in an appropriate manner. In case of exposure to the toxin, there is a toxin antidote available in Queens Medical Centre which is adjacent to University of Nottingham. In case of exposure of wounds or cuts to C.botulinum,
antibiotic treatment will be available with a penicillin-based antibiotic. The use of the E.coli based ampicillin cassette on shuttle vectors does not confer ampicillin resistance to C.botulinum, and therefore, does not interfere with antibiotic treatment. For the above-mentioned reasons, the risks to staff are considered to be minimized to a very low level.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste C.botulinum liquid cultures (in volumes not exceeding 100ml) will be disinfected overnight in 1% virkon in the anaerobic cabinet before double bagging, autoclaving on sitr then sent for incineration. Contaminated plasticware and solid media will be double bagged, autoclaved on site then incinerated.

Autoclaving at 125°C for 15 mins followed by incineration will give 100% kill of bacteria and inactivation of B0NT (which is inactivated at 100°C for 3 minutes). Both incineration and autoclave procedures are expected to give a 100% kill of all GMMs.

The autoclave has a chart readout which is checked after each discard cycle and is also validated on a six monthly cycle in accordance with HTM 2110.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Written comments on this Class 2 draft assessment has been received from several members of the committee and these were discussed at the GMSC "C" meeting on 19th November 2004. The general consensus was that there were no major safety issues associated with the genetic modifications proposed. The main issues were operational to ensure safe handling and disposal of the organism and in particular culture supernates containing botulinum toxin. The assessment required modification to address these issues. The committee were informed that the work with this organism would be performed in a dedicated laboratory on B floor CBS and that appropriate containment measures to minimise the risk of exposure to toxin or Clostridial spores would be applied. Issues of vaccination of workers involved in this project against C. botulinum neurotoxins was raised and it was confirmed that the following discussion with the University's Occupational Health Advisor, vaccination could not be made available via Occupational Health though it was subsequently identified that anti-yoxin was readily available in QMC. It was agreed that it was important to limit the number of workers involved in the project to minimise risk and the PI confirmed that the maximum number of workers likely to be involved would be four. In light of the above discussion, an amended assessment was circulated and approved by the committee.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022
The scientific aims of this project are to investigate the mechanisms of dimorphic and phenotypic switching in Candida, a fungus that can be pathogenic to humans. Although the project will not address pathogenicity per se, it is possible that switching mechanisms may help to understand the basis of pathogenicity. Candida albicans is a diploid species which makes genetic manipulation more complicated than with a haploid organism. In some cases therefore, Candida glabrata will be used. C. glabrata is a haploid organism that behaves with some similarity to C albicans but is less virulent and undergoes the dimorphic switch to the filamentous form less efficiently.

Strains of Candida will be transformed with vectors containing genes or gene regulons (promoters) derived from Candida with the aim of either deleting genes or producing strains having that gene under a regulatable promoter. Down-regulation of a gene may also be achieved using RNAi approaches. No null mutations in a gene are known to have produced a strain of Candida that has enhanced virulence. In any case, the virulence of Candida is known to by polygenic, ie involves several factors, so alteration in one gene is highly unlikely to lead to a major shift in virulence. The plasmid vectors that are constructed will be amplified in disabled strains of Escherichia coli although the genes are highly unlikely to be expressed in E. coli because only yeast promoters will be used and they are unlikely to function in E. coli. Genes to be regulated by these methods include genes involved in protein secretion and secretion stress (eg HACA) or genes that are putatively involved in control of gene expression due to effects on chromatin (eg DNA methylation or histone modification) or RNA function (eg MTR4).
Candida strains. *C. albicans* is an ACDP category 2 organism. The *C. albicans* strains CA14 (ura3), CA18 (ura3, ade2), RM1000 (his1, ura3) and BWP17 (ura3, his, arg) are the standard hosts for DNA transformations world-wide. BWP17 is the favoured host for the work proposed as it is widely used and is triply auxotrophic. These auxotrophies make *C. albicans* avirulent. Transformation with URA3 plasmids partially restores the virulence of CA14, but this restoration is not complete because the genes neighbouring URA3 remain inactivated. This is enough to partially (but not completely) attenuate virulence. The ura3 marker is recycled for subsequent rounds of transformation and, therefore by necessity, some of the transformants we create are not genetically disabled, although their virulence is slightly lower than the wild type strains of *C. albicans* carried by most individuals. At no time will we need to create *C. albicans* mutants that are free of disabling mutations. The BWP17 strain could be used to generate strains that are up-regulated in gene function. But, the resulting strains would be disabled and, anyway, it is unlikely that production of such strains in non-disabled hosts would have enhanced virulence because virulence is polygenic. Genetically disabled *C. glabrata* strains might be used in some experiments, and it will be entirely appropriate to use similar precautions to those used for our *C. albicans* experiments.

Bacterial strains. The *E. coli* strains to be used are all Rec A- derivatives such as DH5a, XL1-Blue, SURE and TOP10. These strains are disabled and non-colonising and are equivalent of ACDP category 1 organisms (ie non pathogenic to humans or animals). They have limited survivability in the environment as they require specific nutrient supplements not required by wild-type organisms.

Recipient or parental organism

**Host/vector system**

Candida vectors. These are pUC and pBR322-derived plasmids that will be maintained in *E. coli* host before transfer to a fungal host organism. The plasmids contain the Candida URA3 gene to compliment the ura3 auxotrophy in the recipient Candida strain, leaving the other auxotrophies (his and arg in strain BWP17) unaffected. Similarly, other vectors enable the regeneration of the ura marker (to regenerate a triply auxotrophic strain). Due to the nature of the promoters there is unlikely to be any expression of eukaryotic genes in the bacterial strains mentioned. Vectors are integrated into the Candida genome and considered non-mobilisable.

Bacterial vectors. These are pUC derivatives encoding ampicillin, zeomycin or other antibiotic resistance markers. Considered to be non-mobilisable. Hazards arising from mobilisation are unlikely due to poor survivability of *E. coli* strains outside the laboratory.

Origin & function

The Candida strains will only be used in the contained laboratory environment. The aim of the study is to understand the molecular basis of dimorphism in Candida, ie how Candida changes between yeast and filamentous forms.

Candida genes will be isolated and self-cloned back into Candida (having passed through the stage of cloning in disabled strains of *E. coli* using conventional gene cloning approaches. The inserted genes will be used to delete genes from the Candida genome by recombination. So, the strains of Candida that are generated will be deficient (either deleted or down-regulated) in gene function. Up-regulated expression of some genes will only be studied in a multiply disabled strain (above). Genes to be regulated by these methods include genes involved in protein secretion and secretion stress (eg HACA) or genes that are putatively involved in control of gene expression due to nutrient availability (eg GCN4), effects on chromatin (eg DNA methylation or histone modification) or RNA function (eg MTRA).

Evaluation of foreseeable effects

As a scientific level, the studies will reveal the molecular basis of dimorphism and provide possible tools which might be exploited in the future in control of dimorphism (ie design of anti-Candida drugs).

In relation to safety, the most hazardous GMMs to be produced will be derivatives of the ACDM category 2 organism *C. albicans*. There is no indication, however, that any strain produced will be more virulent than the natural commensal strains of *C. albicans*. All strains used are disabled and there is no indication from the literature of any strain, in which a gene has been deleted or down-regulated, having enhanced virulence. The GMM strains will remain disabled due to auxotrophies. None of the target genes are likely, on their won, to have an impact on virulence because of the recognised multigenic nature of virulence in *C. albicans*. Thus, the GMM organisms are considered to be of the same ACDP category 2 as the host strain.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All solid waste and plastics exposed to GM material will be autoclaved prior to disposal. All liquid waste and glassware exposed to GM material will be autoclaved prior to disposal/wash up. Accidental spills will be contained using paper towels and any Candida will be destroyed by Trigene (2%) disinfectant which is effective for killing fungi including C. albicans. The paper towels will be autoclaved.

Class 2 autoclave disposal facility is available in the same laboratory area. The autoclave is subject to a regular programme of annual service and testing for effectiveness and checks are made on completion of cycle (on daily basis).

Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form Y

Please enter comments on the GM safety committee on the risk assessment

The local GM committee (University of Nottingham) GMSCB reviewed the risk assessment on 6th October 2005. They agreed with the assessment of the activity as being Class 2. Questions were raised regarding the related safety issues of a possible need for health screening for workers, potential contraindications to work and the need to provide information to staff who work on the project. Advice was sought from Occupational health on these matters. Minor amendments to the risk assessment were made on the basis of the advice provided.

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
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<td>L2 L3 L4 L2</td>
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Project Ref 470/05.3

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022 Page 7600 of 15326
Characterisation and analysis of lung epithelial, airway smooth muscle/myofibroblast, hepatocyte and human umbilical vein endothelial cell (HUVEC) immortalisation using Retro- and Lentiviral expression systems. (A05/08)

Date Project Ceased

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The overall goal is to characterise the function of the tumour suppressor gene LIMD1. LIMD1 is involved in suppression of cell growth and thus tumour growth and may constitute a novel targets for cancer therapy. In addition we will also screen primary epithelial cells for novel gene (including LIMD1) which induced immortalisation. Furthermore, in collaboration with 'other named staff' above we will use reagents indicated below to immortalize the primary cell lines, bronchial epithelial cells (HBEC), primary hepatocytes, primary Human Umbilical Vein Endothelial Cells (HUVEC) and airway smooth muscle/myofibroblasts.

Recipient or parental organism

The bacterial host will be DH5 alpha.

Infecitve virus is produced by lipid-mediated transfection of retroviral and lentiviral expression vector into the Bosc23 packaging cell line (to produce ecotropic retrovirus) and the PT67 packaging cell line (to produce amphotropic retrovirus) and the 293gp cell line (to produce amphotropic lentivirus) and collection of culture supernatant. These packaging cell lines express envelope and gag and pol proteins derived from the 10A1 Moloney murine leukemia viral genome and HIV1 viral genome respectively.

The recipient transformed cell lines are HEK293, MCF7, U2OS, SAOS2, A549, MDA-MB435, HeLa, HepG2, ECV304, 293T. The recipient primary human cells to be transduced by retrovirus and lentivirus are bronchial epithelial cells (HBEC), primary hepatocytes, primary Human Umbilical Vein Endothelial Cells (HUVEC) and smooth muscle/myofibroblasts. The work with the immortalised cell lines and primary human cells, which is routinely done in our department, is done at containment level 2.

Host/vector system

Retroviral vectors: gives to <30% transduction efficiency in most cell types, and requires active cell division. In addition, there is integration into the host genome. Unlike adenovirus, recombinant retrovirus is very easy to develop. Retroviral vectors to be used: pBABEpuro, pSuper.retro pWZL and pLXSN (these vectors are retroviral and derived from the Moloney murine leukemia virus (MMLV)).

Lenvitivirus: gives to >80% transduction efficiency, thus lentiviral vectors can mediate the efficient delivery, integration and stable expression of transgenes in dividing as well as nondividing cells in vitro. As such, they open exciting possibilities for both basic research and the genetic treatment of human diseases. As with retrovirus, recombinant lentivirus is very easy to develop. We will also use the Lentiviral based vector pHR.
Both vectors are non-mobilisable and replication defective.

Origin & function

The genetic material ('inserts', see below) have been produced in my lab via molecular biology techniques such as RT-PCR from cellular RNA.

Inserts to be used: LIMD1 (LIM Domain Containing Protein 1), BAF (Barrier-to-autointergration Factor), Grbn7 (Growth Receptor Binding Protein 7), FHOD1 (diaphanous-related formin homology 2 domain containing protein 1).

HIF1alpha (Hypoxia-inducible factor 1alpha), PHD1, 2 and 3; (HIF-pprolyl-hydroxylases, 1, 2 and 3) these genes are not considered proto-oncogenes according to the ACGM classification and will not affect the virulence of the recipient line.

We will also use the Bmi-1 oncogene to induce immortalisation but not transformation of primary human bronchial epithelial cells, primary hepatocytes, primary Human Umbilical Vein Endothelial Cells (HUVEC) and airway smooth muscle/myofibroblasts.

hTERT (catalytic subunits of telomerase), Hras (Harvey rat sarcoma virusl (v-Ha-ras) oncogene homolog, simian virus 40 large T antigen (SV40LT), simian virus 40 small t antigen and Human Papilloma virus E6/E7 will also be used to induce immortalisation.

We will also introduce different combination of the above lenti/retro constructs into the indicated primary cells to induce various levels of transformed phenotype. It is important to state that such transformed lines produced will be no more transformed or pathogenic than HeLa cells which are widely used throughout biomedical research in category 2 facilities.

Evaluation of foreseeable effects

The various inserts will not alter the pathogenicity of the cells. The virus is replication defective and unstable Standard cell culture practice will prevent introduction into humans.

It is important to note that immortalisation of the primary cultures indicated does not cause transformation. As stated above such cells can only survive in very specific in vitro tissue culture conditions. Cell lines created this way will remain none-pathogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We will apply all the measures specified as requirements for containment level 2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All transfections and manipulations of cells will be performed in a Class II biological safety cabinet (identifiable to other users), preventing release of aerosols. Protective clothing, double gloves to be worn at all times. Because of virus nature of the work, no glassware or sharps will be used, only disposable plastic-ware. All potential infectious waste (solutions and microbiological plates) will be either disinfected [using Trigene] or autoclaved,, prior to disposal via drains or incineration as appropriate. In the event of spillage, the spill will be absorbed onto paper towel, the area will be effectively disinfected with Trigene (1:100 dilution). Towels will be autoclaved prior to disposal.

Disinfectants are used in accordance with manufacturers recommendations and manufacturers data shows that the disinfectants are effective against the organisms in question. Data on the appropriateness and effective degree of kill for these detergents are provided by Brindle Microbiological Consultants (Trigene).
The autoclave is subject to calibration and validation by a service company twice yearly.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee approved the assessment as a class 2 activity for lenti and retroviral infection of tissue culture and was satisfied that the location of the work conformed to the required containment level.

**Project Containment**

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<tr>
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<th>Growth Rooms</th>
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</thead>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref** 470/06.1

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<th>Date Project Ceased</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
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<tr>
<td>08/02/2006</td>
<td>A novel human in vitro model for the study of Hepatitis C virus [C05.10]</td>
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Tick if notifying a connected programme of work N

Withdrawn N

Historical Significant Changes

Historical Date of Additional Info
Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Post-graduate research into the pathogenesis of hepatitis C virus (HCV), outlined below:
Hepatitis C virus (HCV) is a major global health problem. The virus was discovered in the early 1990s by molecular techniques and has never been able to be cultured in the laboratory. This seriously hinders progress towards understanding pathogenesis and developing therapeutics.

A new full length HCV replicon (JFH1) has been constructed from wild-type virus and shown to replicate and produce small amounts of infectious HCV when transfected into cell lines. However, studies in cell lines have only limited relevance when compared to primary cells in vivo. We will transfect primary liver cells with the subgenomic replicon of this clone (pSGR-Luc-JFH1) using established methods, to assess:

(a) establish whether replication occurs in the cells and, if so, to what level and;
(b) to establish the effect of the subgenomic replicon on the phenotype and function of infected cells;

We will also transfet cell lines with full-length replicons and aim to infect in vitro cultures of primary liver cells with the virus produced:

c) to establish whether infectious virus is produced from the infected cells and to what extent, and also;
d) to establish the effect of the full-length replicon on the phenotype and function of infected cells;

Depending on the results of this work, further studies on the viral life cycle and effects may be carried out in subsequent studies. In particular, it is anticipated that if viral replication and/or virus particle production is supported in these cultures, we plan also to infect primary human hepatocytes (only) with different genotypes of wild-type (non-GM) HCV. Further studies are likely to involve study of virus binding and entry, through the use of HCV plasmids with modified envelope protein (E1/E2) genes.

If the human hepatocyte cell culture system described above successfully supports replication of and production of HCV, we also plan to introduce hepatitis B virus to the system in similar ways (ie both wild-type and plasmid-encoded virus and viral genes) to use the model to assess differences in HBV genotypes and their natural pathogenesis in liver cells).

Recipient or parental organism

The replicons will be transfected into primary hepatocytes and standard hepatocyte cell lines including Huh7 cells (containment level 1/2 rising to containment level 3 when transfected). Infectious virus produced by Huh7 cells transfected with JFH1 will be introduced to cultures of primary human hepatocytes and primary human stellate cells (all at containment level 3).

Host/vector system

No modification to the HCV replicons will be carried out during our experiments. Transfection of eukaryotic cell lines will be by electroporation (no vector required).

Transfer of genetic material into host mammalian cells will be by transfection of RNA. Multiple RNA copies will be produced from linearised plasmid using an isothermal in vitro RNA polymerase reaction, therefore cloning or use of bacterial hosts will not be carried out.

Origin & function

No modification to any of the genetic material will be carried out during our experiments.
pJFH1
Other than both structural and non-structural wild-type HCV genes, the only addition is that of T7 (an RNA polymerase promoter) and Xbal (a restriction site) in the 5’ and 3’ untranslated regions [see below]

pLuc-JFH1
This full-length bicistronic construct has been engineered with the firefly luciferase gene (see below) at the 5’ end followed by the encephalomyocarditis virus (EMCV) internal ribosome entry site which ensures follow-on translation of the normal HCV 2a wild-type genes. The firefly luciferase gene (luc) has no interaction with the HCV genes or host cell and simply will produce light which can be quantitated in order to permit assessment of the amount of replicon translated in the cells.

pSGR-Luc-JFH1
Other than non-structural wild-type HCV genes, the only additions are that of the luciferase cassette (as above), followed by the EMCV IRES which directs expression of the HCV non-structural genes in the second cistron.

The control plasmids contain various sequences or mutations which prevent replication (or virus production). For example, GND represents a disabling mutation in the active site motif of NS5B polymerase. These are commonly used to ensure that any RNA (or virus) detected is the product of novel replication (or virus particle) production within the transfected (or infected) cells.

Details of the construction of the pSGR-Luc-JFH1 plasmid, JFH1 and Luc-JFH1 replicons are as follows:

Sequence map of JFH1 full-length replicon (Wakita et al. 2005 Nature Medicine). There is a T7 promoter at the 5’ end and an X-Bal restriction site in the 3’ UTR (not shown):

- the Luc-JFH1 replicon (Wakita et al. 2005 Nature Medicine) is as the pSGR-Luc-JFH1 plasmid, but with the addition of the core, E1, E2 and p7 genes between the EMCV IRES and the non-structural genes.

Sequence map of pSGR-Luc-JFH1 (Targett-Adams and McLaughlan, 2005 in press). There is a T7 promoter at the 5’ end and an X-Bal restriction site in the 3’ UTR (not shown):

Luc EMCV NS3 4B 5A 5B

Control plasmids are as follows:

Sequence maps of full-length chimeric control plasmids (Lindenbach et al. Science 2005):

- FL-J6/JFH contains structural genes from the J6 strain (genotype 2a) and FL-H77/JFH contains structural genes from the H77 strain (genotype 1a)

Replication incompetent full-length plasmids with the GND-mutation will also be used as negative controls (see section 5.3).

Sequence map of pSGR-Luc-JFH1/GND [replication-incompetent control plasmid]
(Targett-Adams and McLaughlan, 2005 in press). There is a T7 promoter at the 5’ end and an X-Bal restriction site in the 3’ UTR (not shown):

GND
Luc EMCV NS3 4A 4B 5A 5B

Other JFH1 plasmids, with reporter genes inserted for the purpose of tracking the protein product, may also be used in the future, as they become available. None will be constructed in-house. HCV 1b subgenomic replicons. (replication incompetent) may be used as control plasmids for pSGR-JFH1.

Different genotypes of wild-type (unmodified) HCV, if used, would be recovered from human serum with Ethical consent and will be characterised by the Trent HCV Study Group.

The Genbank accession number for the consensus sequence of JFH1 is ABO47639

**Evaluation of foreseeable effects**

HCV is assigned to category 3 in the ACDP listings, with modifications to BSL 3 requirements possible. HCV is a blood-borne virus spread almost solely by percutaneous exposure (infection may theoretically occur via mucous membrane exposure although only one case of possible transmission via an eyesplash of infected material has every been reported). In approximately 80% of those exposed it causes a chronic infection characterised by liver inflammation and steatosis. This can progress to fibrosis, cirrhosis, liver failure or hepatocellular carcinoma. Non-genotype 1 viruses (from one of which this agent was derived) respond better to antiviral treatment and there is now evidence that antiviral treatment early in the course of infection is more more likely to result in the virus becoming undetectable.

The replicons are constructed from an HCV genotype 2a wild-type virus consensus sequence. They are called pJFH-1 and pLUC-JFH1 (full length) and pSGR-Luc-JFH1 (subgenomic). As the full-length replicon expresses only wild-type HCV genes (with or without the non-toxic luciferase protein) it is anticipated that the hazards associated with the virus it may produce are similar to those of wild-type HCV. Neither the plasmid nor wild-type HCV is known to integrate with the genome of the recipient cell.

The luc gene is not known to have any toxic or harmful effects, even when expressed to high levels in animal models. There is no evidence to show that the EMCV IRES would lead to relative over-expression of the HCV genes in a human host.

All workers with human-derived tissue and cells should be immunised and have a documented response to hepatitis B vaccine. There is no available vaccine for hepatitis C. Pre-employment or routine screening is not required since, due to the long incubation period, baseline status can be determined if an incident occurs. In addition, the virus would be identifiable (by PCR) as having derived from the working environment.

If any percutaneous or mucocutaneous exposure occurs or is suspected to have occurred (including eyesplash), standard first aid should be administered. Eyes should be washed using the available sterile eye-way solution. Cuts, abrasions, penetrating injuries or contaminated mucous membranes should be washed under running water and any bleeding encouraged (without scrubbing).

Occupational Health will be notified of the exposure and baseline blood samples for storage should be taken as soon as possible. Follow-up blood samples for molecular diagnosis and serology will be required at 6, 12 and 24 weeks after the incident, or sooner if illness develops.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We are applying for a derogation as there is currently no autoclave sited within the CL3 suite or lobby. The room arrangements are detailed in the Code of Practice. We consider it is not reasonably practicable to provide an additional autoclave in D31 [CL3 lobby] for the following reasons.

* D31 is too small to accommodate an autoclave.
* Costs of purchase and installation will be considerable.
* It will not be necessary to transport waste beyond D38 and access to the adjacent corridors is controlled so that only authorised personnel have access.
The following procedural controls will be implemented which we consider will adequately control risk:

* All solid CL3 will be autoclaved at the end of each working session, regardless of the amount of waste produced.
* All waste will be retained in the CL3 laboratory until the autoclave is available.
* Waste will only be handled by the CL3 trained user prior to autoclaving.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Although only the full-length replicon (pJFH1) is capable of producing infectious virus, all waste generated within the CL3 laboratory will be handled in the same way and only by trained personnel, as follows:

According to advice taken from "Guidance for Clinical Health Care Workers: protection against infection with blood-borne viruses" (1998, DoH publication), "HIV and hepatitis viruses are susceptible to heat and wherever possible equipment must be sterilised by conventional procedures employing moist or dry heat. For heat-labile articles and surfaces which cannot be sterilised it will be necessary to employ methods of chemical disinfection. Recommendation of disinfectants for the purpose of inactivation of HIV and hepatitis viruses is restricted by lack of adequate data for many chemical agents. The methodology for evaluating the activity of compounds against HIV and hepatitis viruses is complex, not least because of the need to simulate "real life" conditions where organic contamination is likely to be present".

Manufacturer's validation data for our current disinfectant (Trigene) is available. Testing against Hepatitis B surface antigen (BHsAg) has been carried out by the PHLS (now HPA) to show that addition of a 1% dilution of Trigene (for 5 minutes) neutralised a 5% dilution of serum which was highly reactive for HBsAg.

While there is no available test for the validation of disinfectants or sterilisation against HCV, evidence of action against HBsAg is accepted as a standard substitute and statements to this effect are available. The mode of action of Trigene is not only to disrupt the cell wall and virus envelope, but it will also inactivate and break down nucleic acids on contact, via direct alteration of the secondary structure.

**Solid waste**

Plastic consumables (except pipettes), gloves and contaminated paper towels will be double-bagged into autoclave bags and autoclaved for at least 15 minutes at 121 degrees C.

Currently, there is a well-established procedure for electronic monitoring of the cycle temperatures and for validating that the cycle has run correctly by examining the resultant print-out. A record is kept of each cycle and there is an SOP in the case of suspected autoclave breakdown or failure. All personnel involved will be trained in the handling of CL3 waste. The current systems for autoclave validation will continue to be used (ie printout on every run and when services), however, if a cycle containing CL3 waste is known or suspected to have failed, the procedures for handling the contents will be as for all CL3 waste until successfully autoclaved. Successfully autoclaved material will be sent for incineration. Servicing and external validation is carried out by Lab3. Total Laboratory Service (Lab3 Limited) every 6 months.

**Liquid waste**

Liquid waste containing toxic chemical/materials will be disposed of by existing validated methods for the disposal of the chemicals used. Non-toxic liquid waste will be aspirated into sealed containers containing disinfectant (Trigene). This will be left for at least 12 hours prior to discard into drains. Daily renewal of fresh disinfectant solution to a known level will ensure the final concentration is not less than 5%.

**Spillages**

Spillages that contain, or potentially contain, virus particles, will be subjected to a 5% solution of Trigene (ie final concentration when diluted with the spillage) for 30 minutes as recommended by the manufacturer's guidelines. The decontaminated spill will be mopped up with paper towels and disposed of in a yellow bag for incineration. The worker who deals with the spillage must wear protective clothing ie gloves and a disposable apron and, in the case of extensive floor spillages, protective footwear.

**Other material**
Cells potentially harbouring the full-length (infectious) replicon and/or associated virus particles will not be removed from the CL3 facility unless killed. This will be by fixation, using glacial acetic acid, methanol or paraformaldehyde, or by fatal disruption, using a chaotropic agent such as Trizol, Triton X-100 or guanidine-isothiocyanate extraction. Denatured cell extracts may then be removed for PCR, luciferase activity analysis or for fluorescence microscopy. Once analysed, cell extracts will be disposed of by the most suitable route depending on what has been mixed with the extract during processing but as live virus will not be present in these extracts, CL3 disposal will not be required.

This project was discussed at the meeting of University of Nottingham Genetic Modification Safety Committee “C” on 14th November 2005. The assessment had been circulated to committee members prior to the meeting. This project will use existing full length or subgenomic HCV constructs provided by collaborators and no genetic modification of these constructs will be undertaken. The constructs would be obtained as purified plasmid DNA. No other specific concerns were raised regarding the safety of these constructs and the remainder of the discussion focussed on the procedures to be used in handling transfected cells in which HCV virus was to be replicated.

All work with cell lines containing live virus was to be performed in a Cat 2 laboratory (D38, Boots Building).

It was recognised that it would be necessary to apply for a derogation to allow use of the autoclave in D30 for inactivation of Catr 3 waste. This risk assessment and Code of Practice for the Cat 3 suite was modified to include the necessary procedures to ensure safety.

Once viral RNA had been isolated from transfected cells, further analysis would be done in a designated Cat 2 laboratory in QMC where routine diagnostic HCV work is performed. Given that the RNA extraction kits used for isolation of viral RNA contain chaotropic agents, it was considered unlikely that RNA extracts would contain viable HCV virus, but such extracts would be transported, stored, handled and inactivated according to protocols which took this possibility into account.

The assessment was approved by the committee as a Class 3 activity.

It was agreed that an update on progress of work under this assessment would be presented at each subsequent meeting of GM committee C.

**Project Containment**

<table>
<thead>
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Project Additional Information

Purposes of the contained use

The lab is interested in a number of areas:
Using human embryonic stem cells to model genetic and epigenetic based disease.
Improving differentiation of human embryonic stem cells to therapeutically useful lineages.
Understanding and minimising epigenetic consequences of in vitro manipulation of embryos and embryonic stem cells.

Recipient or parental organism

Non-colonising, non-pathogenic laboratory strains of K-12 derived E.coli: DH5a, HB101, Sbtl3. ACDP Hazard Group 1 will be used for generation of reporter and regulatory gene constructs.

CULTERED MAMMALIAN CELLS ASSIGNED TO ACDP HAZARD GROUP 1

Non-human cell lines and primary cells; or well characterized human cell lines:
Somatic cells (ovine, bovine, murine fibroblasts)
Stem cells (mouse embryonic stem cells)
Immortal cell lines (NTERA and 2103Ep Embryonal Carcinoma cells)

CULTERED MAMMALIAN CELLS ASSIGNED TO ACDP HAZARD GROUP 2

Primary somatic cells (human fibroblasts)
Stem cells (human embryonic stem cells)
Human Embryonic Kidney 293 cells

Some of these cell lines have a theoretical (but not specific) risk of carriage of human pathogens (eg. Primary fibroblasts, embryonic stem cells) and require containment level 2. HepB vaccination is available through the University's Occupation Health department and all staff involved in this work are vaccinated.

Host/vector system

pUC based, non mobilise cloning vectors (eg pBluescript, pGEM and pCR-TOPO vector families) will be used for generation of constructs in disabled E. coli strains.

For manipulation of mammalian cells, a second generation, inducible lentiviral expression system will be used, which requires three separate plasmids. All auxiliary viral genes are deleted i.e. vpr, vif, vpu and nef. Transfection into human embryonic kidney (HEK) 293 cells (from ATCC and produced in this system are replication incompetent. The plasmids are:

1. Transfer plasmid. Includes inserts, psi element, LTR (long terminal repeat) and WPRE (woodchuck hepatitas B regulatory element – this eliminates the need for rev in packaging system and improves the biosafety)
2. Packaging plasmid including gag and pol genes
3. Envelope plasmid including env and vsv-g genes

The complete sequence of all plasmids and inserts to be used is known and can be provided.

The transgenic inserts and shRNA sequences in the transfer plasmid are the only sequences that will be transferred to target cell types described in section 5.1. These sequences are under continual repression from KRAB domain and TET Repressor sequences and repression is only relieved when doxycycline (or tetracycline) is added, thereby increasing the safety of the vector system.

The originating laboratory (see http://tronolab.epfl.ch/) has extensive experience in using this system and supplies researchers worldwide. Their recommendation is that this viral system should be used at biosafety level 2 and therefore assigned to ACDP hazard group 2.

Origin & function

1) Reporters:
GFP (green fluorescent protein)
Luciferase (an enzyme that oxidizes exogenously applied luciferin, releasing photons)
2) Selection markers:
Neomycin and puromycin antibiotic resistance genes.
Thymidine kinase (involved in dTTP synthesis).
3) Gene Modulators and Modifiers:
GATA-4 is a zinc-finger transcription factor which binds a GATA sequence and exerts an effect in embryogenesis and cardiogenesis.
NKX2.5 is a homebox gene involved in heart development.
MEF (myocyte enhancer factor) 2c is a transcription factor involved in muscle development.
TBX-5 is a transcription factor that binds T box sequences and is implicated in heart and upper limb development. Hairpin loops that form double stranded RNA can downregulate the expression of the corresponding gene by inducing the degradation of the gene’s mRNA.
Tet Repressor (TR) bind to tet operator sequences and inhibit transcription KRAB domain from Drosophila is fused to TR sequence. On binding of TR-KRAB to tet operator sequences, repression of the approx 3 kilobases of transgenic/ viral sequences occurs.

Proteins produced (once the above expression system is induced) are believed to be non-toxic and non-virulent.

Evaluation of foreseeable effects

There are no significant perceived hazards to human health or the environment associated with generation of the proposed constructs in disabled E. coli strains.
Following manipulation of mammalian cells, the most dangerous combination of inserted material would be expression of a transcription factor such as DNA methyltransferase 1 may elicit change in phenotype by altering methylation pattern. However, the viral system we will employ is second generation and replication defective. Moreover, the inducible nature means that only when operator intervention occurs (addition of doxycycline) will the transgenes be expressed. The viral system utilises expression of the WPRE (woodchuck hepatitis B regulatory element), which may have oncogenic properties. Accordingly, extra care is needed when handling naked DNA containing such sequences, as prescribed in the Nottingham University's COP for Biological Agents and GMM, part 3.4. Given the properties of the vector system to be used and the inserted material, we believe that mammalian cells containing these sequences will not be any more pathogenic or virulent than prior to transfection. In addition, the lentiviral particles to be generated are replication defective and are unlikely to survive in the environment. The specified gene inserts will have negligible effect on the environment, especially as the constructs are inducible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid and solid waste containing or exposed to GMO's will be transferred to CBSII prep room A81 for autoclaving to achieve 100% kill, prior to incineration. 121 degrees C for 15 minutes at 2 atmospheres is sufficient to inactivate mammalian cells and E.coli and the human pathogens that present a theoretical risk in our containment level 2 human cell cultures. The autoclave has a chart readout which is checked after each discard cycle and is also validated on a six monthly cycle in accordance with HTM 2110. In the event of spillages, surfaces will be disinfected with 1/100 dilution of Trigene Advance which has been validated by the manufacturer to produce a log5 kill of E.coli and to neutralise human retroviruses.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project was circulated for comments to members of University of Nottingham Genetic Modification Safety Committee “C”. The consensus view from comments received was that the risk assessment was comprehensive and adequately described and addressed the risk of the proposed manipulations in E.coli and mammalian cells. The potential hazards associated with use of primary human cell lines were acknowledged. In addition, the potential oncogenic hazard associated with the WPRE (woodchuck hepatitis B regulatory element) was identified and appropriate precautions for handling this element were identified. The investigators indicated that they considered that the proposed project should be undertaken as a class 2 activity. Recent guidelines from HSE which indicated that there is still some uncertainty over stability of the lentiviral vector systems in general, supported this view. The assessment was formally approved by the committee as a class 2 activity at a meeting held on 18/06/07.

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms
Project Ref 470/08.1

Comparative functional genomics of Escherichia coli strains and pathotypes (DO8-01).

Project Additional Information

Purposes of the contained use

The scientific goals and major aims of the project are to compare the whole cell transcriptomics, interactomics, and phenotypic responses of different E. coli strains that have gene knockouts in a range of genes encoding transcription factors and other metabolically important genes, and/or have affinity tagged chromosomally encoded transcription factors which will be used for ChiP-chip (chromatin immunoprecipitation on DNA chip) work. Reporter genes will be engineered in to the chromosome to monitor specific gene expression.

Recipient or parental organism

The major genetic manipulations will be to make gene knockouts in the strains, or to affinity tag, or add in reporter genes that are transcriptionally fused to these genes. These manipulations will be achieved using chromosomai engineering.

Host/vector system

Disabled laboratory K-1 strains (examples: TGI, TG2, XL-Blue), or K-1/B strains (examples: BL21, Origami) will be used as hosts for cloning of genes and/or overexpression of
proteins and in plasmid construction. These are all ACDP HG I
Strains used for chromosome engineering will be E. coli K-12 laboratory strains or strains which have had minimal manipulation made to them: EMG2, MG1655 (genome sequenced), W3110 (genome sequenced), (all ACDPI), and E. coli pathotypes- including the genome sequenced strains: CFT073 (Uropathogenic E. coli), 042 (Enterococcal aggregate E. coli), H10407 (Enterotoxigenic E. coli), E2348/69 (Enteropathogenic E. coli) (ACDP2), APEC01 (Avian pathogenic E. coli) andVT negative 0157: H7 Sakai and EDL933. Both of the 0157:H7 strains have both genes encoding the shigella-like toxin (Stxin and Sbc2) deleted making them ACDP2. Other ACDP2 E. coli pathotypes may be used.

VECTORS
Standard non-mobilisable p15A, pSc101, IncW and ColE1 replicon derived plasmids will be used for cloning and overexpression work which will be carried out in E. coli K-12 laboratory strains only. Plasmids include the pUC plasmids (ampicillin resistance), pACYC184 (chloramphenicol and tetracycline resistance), and GATEWAY vectors (kanamycin resistance- Invitrogen)
Specific gene knockout vectors will include pEXI OOT (Ampicillin resistance and sucrose sensitivity, from sacB, Schweitzer and Huang, 1995, Gene 158: 15-22) or derivatives (Lee et al., submitted) pACBSR (chloramphenicol resistance and lambda red recombinase, Herring et al., 2003, Gene 331: 153-i 63) pKD3 (Kanamycin resistance), pKD4 (Chloramphenicol resistance), pKD2O and pKD4G (both ampicillin resistance and lambda red recombinase) (Datsenko and Wanner 2001, Proc. Natl. Acad. Sci. USA 98: 6640-6645)
The pET series of vectors (ampicillin resistance) will be used to overexpress proteins for raising antibodies if required.

Origin & function
None of the genes that are to be manipulated or overexpressed in order to raise antibodies are known to be toxic, or allergenic. These genes are common to K-12 and pathogenic E. coli strains and can be considered to be core function genes.
The genes that will be inserted will be antibiotic resistance cassettes, which will mark chromosomal alterations; Tag sequences such as FLAG, Protein A, 6x histidine or 12x histidine will be fused to pre-existing genes, and reporter genes, such as green fluorescent protein (gfp) or bioluminescence (lux) genes. Examples of genes which will be tagged or deleted include rpoS (stress/stationary phase regulator), fur (iron uptake regulator), soxR/S (oxidative stress response regulators), fThDC (motility master regulators) and genes involved in metal ion homeostasis.

Evaluation of foreseeable effects
With the exception of the E. coli K-12 laboratory strains, which are recognized as disabled and are ACDP1, and would be used as the host strain in cloning or overexpression work, the parental/recipient organisms are pathogenic E. coli strains classified as ACDP2 and contain no known disabling mutations that could revert. The organisms as such pose the same risk to humans as the original wild-type, but complete deletions of regulator genes or affinity tagging of these genes on the chromosome may attenuate the organisms. Although highly unlikely because the regulators regulate core metabolic function, there is also the possibility that deletion of a gene encoding a regulator may derepress expression of genes in the host that are normally repressed. The organisms are not listed as an animal pathogen controlled by DEFRA.
The E. coli K-12 laboratory strains are defective in environmental survival and capability to establish in the mammalian gut. The pathogenic E. coli strains are human pathogens, and are already present in the environment. The 0157 strains used in this study are defective in verotoxin production because the genes for the toxins have been deleted and therefore the strains are less of a potential hazard than 01 57:H7 already present in the environment.
Identification of further regulators by ChiP-chip and transcriptomics is envisaged in the project. These regulators are likely to be core metabolism regulators, but may include pathogenicity regulators. Deletion of the genes encoding these regulators may lead to expression of pathogenicity factors that are normally switched off until the correct environmental signals are perceived by the bacterium. Any potential expression of a normally repressed pathogenicity gene will not make the strain more hazardous because it already has the capacity to express these genes. It may be disadvantaged compared to the wild-type because regulation of pathogenicity factors has evolved to control their expression, which may have a fitness cost to the bacterium.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
not applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste is routinely inactivated by autoclaving (134°C for 30 minutes). Contaminated plasticware is triple bagged, tagged and taken away by White Rose environmental in locked, leakproof containers for incineration. Contaminated sharps are discarded in Sharpsafe containers before being triple bagged and disposed of as detailed above. These procedures are detailed in the local code of practice. All liquid waste is routinely autoclaved at 134°C for 30 minutes, prior to disposal to drain. Small spills of liquid waste or contamination of equipment is disinfected chemically. These procedures are detailed in the local code of practice. The standard disinfectant used is Virkon (1% WN solution). The disinfectant will only be used to disinfect lightly contaminated areas and not for treatment of substantially contaminated areas or for making safe contaminated organic matter such as paper towels used to absorb spilled cultures- these materials will be autoclaved prior to disposal. Multiple independent tests on pathogenic bacteria including E. coli have shown that 1% solutions of Virkon are effective in achieving at least a b5 reduction in bacterial count under the appropriate conditions. See http://www.antecint.co.uk/main/virkons.htm

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All members of GMSCD were asked to comment on the assessment. Several comments concerned with clarifying aspects of the proposed work were received and incorporated into the revised assessment Therefore the committee do not have any outstanding concerns and approved the project as a Class 2 activity.

Project Containment

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Project Ref 470/08.2

Date Ackn'd 02/03/2022
SVMS_CPW1: Veterinary bacterial pathogens and Zoonoses: virulence genes, pathogenicity, vaccines and immunity.

**Project Additional Information**

**Purposes of the contained use**

This CU2 accompanies the connect programme for working with a range of hazard group organisms groups ACDP1 & 2.

The aim of this connected programme is to gain further insight into mechanisms & pathogenicity and immunity of common bacterial pathogens & livestock species.

**Scope:**
- The study of the role of specific genes by their disruption.
- The function of specific genes by their cloning and transfer to new donors.
- The development of vaccines by employing a combination of both disruption of genes and expression of pathogen antigens in vaccine strains constructs.
- The effect of changes in bacterial gene expression on host immune responses by either disruption, over expressions, or fusion to carrier proteins.
- The over production of specific proteins for the purpose of purification.

Genes will be cloned either into the genome or as episomal clones in plasmid vectors. The specific methods used in any process will be assessed in the individual risk assessments to ensure they fall under or within class 2 activities for genetic modification. The connected programme outlines the restrictions and scope of the work to be carried out. It lists the genera of organisms (section 7 below).

Infection studies will also be carried out under ASPA regulations. Where this is done all work will under class-2 containment. Organisms used in infection are expected to be & equivalent virulence to that of the wild-type parental strains. Full risk assessments and operating procedures for working with animals with these infections will be in force during this work. Waste material from infected animals will be treated as hazard group-2 infectious material and treated accordingly (see below).

The contained use is to reduce the risk of accidental release or spread of GMOs into the environment. Work is to be restricted class 2 activities and below. The work will not generate organisms higher than ACDP2.

**Recipient or parental organism**

The major genetic manipulations will include
- Disruption of genes by insertion of antibiotic markers, tags and fusion proteins.
- The cloning and transfer to new donors within Genera.
- The development of vaccines by a combination of gene disruption of genes and expression of pathogen antigens in vaccine strains constructs. This expression will be across genera and each construct will
Over expressions, of non toxins proteins in Laboratory strains of e coli for the purpose of their purification. These manipulations will be run under the CPW and achieved using a range of standard molecular methodologies. The individual processes will be reviewed independently by GMSCd.

### Host/vector system

Hosts as listed in CPWI: Laboratory E. coli (classi); Salmonella onterica, Campylobacter sp., E. coli (avian serovars), Streptococcus (class I and 2), Lactococcus lactis, Corynebacterium (ulcerans, bovis and pyogenes), Clostridium (perfringens, pyo genes and difflidie), &achyspira sp. & Lawsonia sp. Restrictions on transfer of genes between genera and species are outlined within the CPW.

Vectors: Host vector systems will be detailed in the individual risk assessments held under this CPW. These will be reviewed by the University GM committee prior to work commencing. Vectors will be standard sequencing, expression and gene fusion vectors used in molecular bacteriology studies.

### Origin & function

The origins and restrictions on the transfer of the genetic material are outlined in the CPW in general they cover.

- Transfer of genes from the listed bacterial genera into laboratory strains of E. coli for their manipulation using cloning vectors, expression vectors, over expression and fusion vectors.
- Transfer of antibiotic genes, appropriate for the genera to be studied, to form insertion knockouts in genes to allow study of function.
- Use of low level expression vectors for trans-complementation of genes. None & the genes that are to be manipulated or over-expressed are known to be toxic, or allergenic. These genes are common core function genes. Where genes are involved in virulence they are part of multi-gene systems and individual doning is unlikely to increase virulence of recipient strains.

The details of these will be in the individual risk assessments held under this CPW. These will be reviewed by the University GM committee (GMSCd) prior to work commencing. Vectors will be standard sequencing, expression and gene fusion vectors used in molecular bacteriology studies. Example risk assessments are attached.

### Evaluation of foreseeable effects

With the exception of the E. coli laboratory strains, some Streptococci and Lactococci listed in the CPW and which are recognized as ACDPI. The other parental/recipient organisms are pathogenic and are classified as ACDP2. The GM microorganisms will pose the same risk to humans and animals as the original wild-type. Complete deletions of regulator genes or affinity tagging of these genes on the chromosome may attenuate the organisms. Although highly unlikely because the regulators regulate core metabolic function, there is the possibility that deletion of a gene encoding a regulator may derepress expression of genes in the host that are normally repressed.

The organisms are not listed as an animal pathogen controlled by DEFRA.

The E. coli laboratory strains are defective in environmental survival and capability to establish in the mammalian gut.

The pathogenic strains are veterinary and human pathogens, and are already present in the environment.

The Salmonella Typhi strain listed is defective in the am operon with multiple lesions and has previously been derogated through the HSE to be used in laboratory studies at ACDP2 (see attached letter and section 11).

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation has already been sought for Salmonella Typhi arc vaccines strains used as controls and in laborai&i1 based vaccine studies. See attached paper work letter from HSE November, 1993. Salmonella Typhi arc strains have been used successfully in human clinical trials without major health risks. This CPW includes there use as vectors for antigens by cloning but places limits work to avoid complementation of the arc lesions.
12. Describe the waste management

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Infectious GM waste will be either autoclaved [121°C 15 minutes] to render non infectious or treated with disinfectant [see below] then sent for incineration at SRCL. The autoclave has a chart readout which is checked after each discard cycle and is also serviced, calibrated & validated [early].

General decontamination will be with either Virkon (1%) or Tn-gene (1%). Bactericidal efficiency of Trigene has been tested for 1 minute contact time for 1% at 1 minute or up to 10 minutes in the presence of high organic load. Validated by the manufacturer to produce a > log5 kill of the organisms to be used. An exception to this is Clostridium difficile where the current literature suggests 2% Trigene solution. Increased concentrations will be used in work with organisms in the presence of blood, serum 10% Tn-gene or Virkon.

In the event of a need arising to send untreated GM waste for off site incineration it will be triple bagged and sent to SRCL [formerly White Rose] who are a GM facility notified under GM CU Regs [GM Centre 779].

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

Please enter comments on the GM safety committee on the risk assessment

All members of GMSCD were asked to comment on the CPW comments received have been acted on. The committee do not have any outstanding concerns for this CPW or the attached risk assessments as Class 2 activities.

Project Containment

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Project Ref 470/08.4

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
Generation of gene knock-out cell lines using the recombinant Adeno-associated virus (rAAV) vector system.

The aim of this project is to develop a variety of permissive human hepatoma cell lines with defined putative HCV cellular receptor(s) knock-outs. The unavailability of an efficient culture system for HCV has restricted the study of the mechanisms of HCV infection and development of effective antiviral agents. Several research groups have applied gene silencing technology by using the short interfering RNA (siRNA) approach in their HCV entry studies (Bartosch, et al. 2003, Zhang, et al., 2004, Lavillette, et al., 2005, Bartosch, et al., 2005, Lavillette, et al., 2005, Evans, et al. 2007). However, the expression of all genes of interest was down-regulated in the range of 60-90%. Although this approach provided some useful information, the interpretation of this method can be difficult because of incomplete inactivation of the studied genes. Therefore, generating HCV receptor gene knockout cell lines will generate a valuable resource which will allow detailed and novel investigation of the exact roles and mechanisms that HCV receptors play in HCV entry.

Recipient or parental organism
Non-colonising and disabled E. coli strains e.g ToplOF' or other K12 derivatives equivalent to ACDP hazard Group 1 will be used for generation of mutant constructs. Mammalian cell lines that will be used in this project are Huh7, Human hepatoma cell line and HEK-293 (Human Embryonic Kidney epithelial-derived cell line) which are only able to survive under specialised laboratory culture conditions. And are considered equivalent to ACDP hazard group 1.

Host/vector system
Non-viral vectors; The vectors pCDNA3.1 Directional TOPO (Invitrogen), pGEMT-Easy (Promega) will be used. These vectors are non-mobilisable. Viral vectors Name: Adeno-associated Virus (MV) serotype 2 recombinant vectors*, pHelper (carrying adenovirus-derived genes) and pAAV-RC (carrying AAV-2 replication and capsid genes) Adeno-associated virus (AAV) is a human parvovirus. It is replication-deficient parvovirus, which have traditionally required co-infection with a helper adenovirus or herpes virus for productive infection. AAV possesses a single stranded DNA genome of 4.7 kb. The wild-type virion possesses two open reading frames (OREs), termed rep and cap, flanked by two inverted terminal repeats (ITRs). The rep ORE encodes proteins involved in viral replication, and the cap ORE encodes proteins necessary for viral
packaging. In most rAAV vectors these OREs are deleted and replaced with a gene expression cassette of interest. The MV helper free system (Strategene) allows the production of infectious recombinant human adeno-associated virus-2 (AAV-2) virions without the use of a helper virus. The system takes advantage of the identification of the specific adenovirus gene products that mediate AAV replication and the demonstration that these gene products can be introduced into the host cell by transfection. In the AAV Helper-Free System, most of the adenovirus gene products required for the production of infective AAV particles are supplied on the plasmid pHelper (i.e. E2A, E4, and VA RNA genes) that is co-transfected into cells with human AAV-2 vector DNA. The manufacturers recommend use of this vector system at Biosafety level 2. The AAV-2 ITR-containing plasmids (pAAV-MCS, pAAV-LacZ and pAAV-hrGFP and pAAV-IRES-hrGFP) do not share any regions of homology with the rep/cap-gene containing plasmid (pAAV-RC), preventing the production of wild-type AAV-2 through recombination.

Origin & function

Antibiotic resistance markers, and reporter genes present in the plasmids to be used for construct generation are from commercial sources and their functions and properties well characterised.

Human DNA will be used as the source of genetic material for generation of gene knockouts in the mammalian cell lines. Genes that will initially be targeted for knockout include:

Scavenger receptor BI (SR-B1), a 509—amino acid polypeptide belonging to the CD36 superfamily, which includes cell surface membrane proteins that bind chemically modified lipoproteins and often many other types of ligand. SRB1 is highly expressed in the liver hepatocytes and steroidogenic tissues (Acton, at at., 1996). It is reported that SRB1 interacts (cellular protein binding) with Hepatitis C virus (HCV) envelope glycoprotein E2 via hypervariable region 1 (HVR1) of E2 (Scarselli, at at, 2002).

CD81, a tetraspanin (membrane protein) which is expressed on hepatocytes and B lymphocytes, was shown to bind to the HCV glycoprotein protein E2 (Pileri, eta!., 1998)

CLDN-1 isa 21 1-amino-acid protein with 4 transmembrane spanning domains that is highly expressed in the liver and is a key component of tight junction strands.

Evaluation of foreseeable effects

None of the proposed manipulations is considered likely to alter the properties of the recipient organisms in a way that will increase their hazard to human health or ability to survive in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste containing or exposed to GMOs will be autoclaved to achieve 100% kill prior to incineration. The autoclave is validated using thermocouples on a yearly cycle and is tested every three weeks using internal temperature sensors.

All liquid waste will be treated with Trigene (2% vol/vol) as per manufacturer’s recommendation. In the event of spillages, surfaces will be disinfected with 1/100 dilution of Trigene which has been validated by the manufacturersto produce a > log5 kill of E. coli and to neutralise a range of viruses including human Adenovirus type 5 and retroviruses.
This assessment was circulated for comments to members of University Genetic Modification Safety "C". The consensus view was that this was a well focussed assessment which adequately addressed the risks of the proposed modifications in both E. coli and mammalian cells. The AAV vector system has advantages over other systems in terms of safety of use but the recommendation by the manufacturers that this should still be used at Biosafety 2 was recognised by the workers involved who indicated the work involving this vector should be a Class 2 activity. This was endorsed by the committee. The investigators were notified of this decision by the chair of GM committee "C" and this decision will be recorded in the minutes of the next meeting of this committee.

Please enter comments on the GM safety committee on the risk assessment

This assessment was circulated for comments to members of University Genetic Modification Safety "C". The consensus view was that this was a well focussed assessment which adequately addressed the risks of the proposed modifications in both E. coli and mammalian cells. The AAV vector system has advantages over other systems in terms of safety of use but the recommendation by the manufacturers that this should still be used at Biosafety 2 was recognised by the workers involved who indicated the work involving this vector should be a Class 2 activity. This was endorsed by the committee. The investigators were notified of this decision by the chair of GM committee "C" and this decision will be recorded in the minutes of the next meeting of this committee.

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Project Ref 470/08.5

Date Ackn'd 28/08/2008
Date Project Ceased

Development of novel models of glutamatergic central nervous system disorders using in vivo shRNA and transgenic approaches.

Consent Granted Not Applicable

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

Altered glutamatergic neurotransmission is implicated in numerous psychiatric disorders, including schizophrenia and anxiety. The first aim of this project is to use lentiviral delivery of short hairpin RNA (shRNA) to cause selective, partial knockdown of specific components of the brain glutamatergic system (e.g. Excitatory Amino Acid Transporter 2; EAAT2, and Vesicular Glutamate Transporter 1; VGLUT1) in order to create novel models of hypo- and hyper-glutamatergic states. These models could be used to investigate the neurobiological and neurochemical bases of psychiatric illnesses, and assess the efficacy of novel treatment strategies.

The first aim is to perform in vitro studies in immortalised and primary cell lines in order to determine the extent of shRNA-mediated transporter knockdown (at mRNA, protein, and functional levels) and select candidate shRNA sequences for future in vivo use.

The shRNA lentiviral vectors are designed to only target mouse specific gene and will not cross target any other species including rat and human.

The second main aim of the project is to use this same technology to create cre inducible conditional shRNA driven mouse knock-downs for VGLUT1.

Recipient or parental organism

Recipient cell lines are HEK293T, HEK293, HeLa, Neuro-2A (mouse neuroblastoma) and C8-D1A (mouse astrocyte), obtained from ATCC or ECACC. Human cell lines are used for viral production, transduction control and GFP producing unit control determination/viral titre. All work with cell lines that have been transduced with lentivirus will be performed at containment level II. Lentivirus will also be introduced into mouse ES cells. Once transduced these cells are non-productive with respect to viral particles.

Host/vector system

The parental lentiviral plasmid vector to be used for this project is pLKO.1-puro (Sigma MISSION). Recombinant lentiviral particles containing the pLKO.1-puro vector will be generated (by HEK293 cells) using Lentiviral packaging mix. This includes (1) a packaging vector that contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions, and (2) the vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector which provides the heterologous envelope for pseudotyping.

Safety features of this system are that (1) no single plasmid contains all the genes necessary to produce packaged lentivirus and resultant particles are replication incompetent, (2) a deletion in the U3 portion of the 3’ LTR eliminates the promoter-enhancer region and further negates the possibility of viral replication, and (3) lentiviral genes not necessary for shRNA packaging have been removed.
This proposal is designed for delivery of mouse-specific short hairpin RNA (shRNA) to cause selective, partial knockdown of specific components of the brain glutamatergic system (Excitatory Amino Acid Transporter 2; EA.AT2, and Vesicular Glutamate Transporter 1; VGLUT1) in order to create novel animal models of hypo- and hyper-glutamatergic states. The expressed shRNA's that will decrease glutamate transporter expression.

**Origin & function**

The shRNA proposed for use are only specific for mouse sequences and will not act against other species. There are no foreseeable effects.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Standard containment level 2 procedures to be applied.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Tissue culture plastics & solid waste will be decontaminated by the addition of 2% Trigene solution for a minimum of 1 hour, then placed into yellow clinical waste sacks for incineration.

Liquid waste — 2%Trigene overnight then disposal down sink. For spill wipe down with Trigene and then with 100% ethanol. All tissue can then be disposed in yellow waste bags and sent for incineration.

Use of Trigene as a disinfectant for inactivation of lentivirus is in accordance with manufacture’s protocols and is supported by manufacturer’s independent efficacy testing data.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

**Project Containment**

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The overall goal is to reprogram differentiated somatic mammalian cells to a pluripotent state by using molecular components present in amphibian and fish oocytes and eggs. The aim is to transduce primary and immortalised mammalian with specific transcription factors known to regulate pluripotency as well as cDNA libraries derived from axolotl, xenopus, sturgeon and lungfish.

Recipient or parental organism


Somatic cells (ovine, bovine and murine fibroblast)

Insect cell lines: SF9

Amphibian: fish oocytes and eggs.

Host/vector system

Bacteria - Noncolonising, non-pathogenic laboratory strains of E.coli: DH5a, XL1, Blue, Stbl2, Stbl3, DH10bac. ACDP Hazard Group 1.

Vectors: pUC based cloning vectors (pBluecript, pGEM, pCR-TOPO vector families ) (Non mobiliasable) Retrovirus vectors: pMXs, pBASE (all derived from Moloney murine leukeamia virus) ACDP HG 1
Lentivirus vectors: pLenti6/TR, pFUW, pWPXld, pLVTHM, pLVCT (3rd generation HIV-1-based). In addition these vectors require pMD2.G (envelope vector) and psPAX2 (packaging vector) ACDP HG 2

Baculovirus vectors: pFastBac ACDP HG1

Phages: R408 and EX-Assist

1) Reporters: GFP and other colour/stability/localisation variants, dsRED, luciferase, LacZ
3) Gene Modulators and Modifiers: Pluripotency transcription factors and gene modulators (eg Oct4, Nanog, Klf4, Sox2, Rex1), modulators of cell immortalisation (hTERT, SV40LT), epigenetic modifiers (eg DNA methyl transferases, DNA demethyltransferases, histone methyl transferases, histone acetyltransferases, histone deacetylases, histone demethylases, polycomb proteins, histone variants), hairpin loops for RNAi (against transcription factors, modulators and epigenetic modifiers), inducible expression systems (eg tTA, rtTA and TRE sequence for Tet-off/Tet-on systems).
4) Promoters/regulatory elements for expression for reporters, markers or modulators:
   Consitutive promoters (eg PGK, EF1a, CAG, CMV). Tissue specific promoters/ regulatory elements active in haematopoietic development (eg Brachyury, SCL), germ cell development (eg, Vasa, Dazl) and pluripotency (eg, Nanog, Oct4, Rex1)
5) Site specific recombinase genes (eg Cre, Fip) and recognition sequences (eg Lox, FRT)

Origin & function

Many proteins (for the described genes) are reasonably believed to be non-toxic and non virulent. Some cDNA libraries will contain full-length coding sequences. Some encoded proteins may be oncogenic or modulators of growth and differentiation, although overexpression of only one gene is unlikely to result in oncogenic transformation. Some products (eg c-myc) can cause increased cell proliferation and may thus be oncogenic on overexpression, whereas other inserts will cause cell immortalisation (TERT, SV40LT) but not transformation. Others (eg DNA methyltransferases) can modulate gene expression and thus can cause altered cellulose phenotypes upon overexpression.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste. Plasticware, pipettes and small tubes are disinfected by submerging in 5% trigene 12 hours and is then drained and disposed of as clinical waste for incineration. Agar plates and solid bacterial pellets will be sent for autoclaving to the Pathology dept at the QMC Hospital site (this is the general hospital autoclaving facility which routinely tested and calibrated for sterilisation of biohazards) before going for incineration. Validation systems for autoclaves. 121 c for 15 minutes at 2 atmospheres is sufficient to inactivate mammalian cells and E.coli. We will check that the autoclave tape has changed colour at the end of the procedure

LIQUID

Disinfection for 12 hours with 5% trigene prior to disposal to drains. Trigene is Bactericidal, Fungicidal, Virucidal, Mycobacterycidal and sporidical. It has been independantly tested for the manufacturer against a wide range of microorganisms and was found to be effective against them all at a dilution of 1/20 (5%) and most at 1/50 (2%, except <cycobacterium tuberculosis var bovis).

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
The main area of concern for the GMSC was the use of the lentivirus in mammalian expression systems in association with the use of oncogenes (Specifically c myc) The GMSC felt therefore that Class 2 was appropriate for this project.

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**Project Ref**  470/09.1

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- Non-GMM Consent Granted
- Project notified under transitional arrangements

**Project Additional Information**
Purposes of the contained use

Contained use is used to reduce the risk of accident spread of GMOs into the environment. Work is to be restricted to class 2 activities and below. The work will not generate organisms of higher than class-2 risk (organisms listed in section 7 below). The aim of the programme is to gain insight into the basic virology, epidemiology, diagnosis, pathogenesis, immune evasion and host immune responses of veterinary viruses affecting livestock, wildlife and companion animals.

Several generic approaches will be used. These are described below. Individual combinations of end host, vector and donor virus allowed are outlined in the CPW document. Section numbers refer to the CPW document.

<table>
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<th>3.1 Cloning, Maintenance and manipulation of whole viral genomes</th>
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<td>3.1.2 Mutation/ deletion/ substitution/ tagging of whole virus genomes for functional studies</td>
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| 3.3 Stable transfection of host cells with constructs encoding viral genes |  |
| 3.4 Passage of recombinant viruses in mammalian host cells. |  |
| 3.5 Inoculation of animals with infectious or non-infectious constructs |  |

Recipient or parental organism

Donor Viruses
Herpes Viruses Including:
* Ovine herpesvirus2 (OvHV-2), ACDP 2;
* Alcelaphine herpesvirus-1 (AIHV-1), ACDP 2;
* Elephant Herpesviruses (EEHV-1,-2,-3,-4,-5), ACDP 2;
* Equine Herpesviruses (EHV-1,-2,-4), ACDP 2;

Retroviruses including:
* Koala retrovirus (KoRV), ACDP 2;
* Gibbon ape leukaemia virus (GALV), ACDP 2;
* Murine leukaemia virus (MuLV), ACDP 2;
* Feline Leukaemia virus (FeLV), ACDP 2;
* Feline immuno deficiency virus (FIV), ACDP 2;
* Porcine endogenous retroviruses (PERV), ACDP 2;
* Bovine endogenous retroviruses (BERV), ACDP 2;
* Ovine endogenous retroviruses (OERV), *Jaagsiekte (JSRV), ACDP 2;

Arenavirus:
Lymphocytic choriomenigitis virus, ACDP 3.

Influenza viruses:
Mammalian influenza viruses (low pathogenicity), ACDP 2;
Avian influenza viruses (low pathogenicity), ACDP 2;
Avian Influenza viruses (high pathogenicity), ACDP 3 DEFRA SAPO group 4.

Orbiviruses
Bluetongue virus, DEFRA SAPO group 3
End Host Organisms
Standard mammalian cell lines (e.g. CHO, P815, rabbit kidney cell 13, Cos 7, HEK 293), ACDP 1; Prknery Avian
(chicken and duck) respiratory epithelial cells, ACDP 1; Primary mammalian (pig and human) respiratory epithelial
cells, ACDP 1; Primary ovine and bovine tissue epithelial and fibroblast cells, ACOP 1; Primary Equine Leukocytes,
ACDP 1; Standard insect cell lines (e.g. Sf9), ACDP 1.
BacTera: F. coli K12 Group I, ACDP 1.
Animals to be Infected: Equus caballus, Mus musculus, Oryctolagus cuniculus, Rattus norvegicus, Ovis aries, Mesocricetus auratus, ACDP I
Justification of Hazard group:
ACDP 1, unlikely to cause disease in humans.
ACDP 2, can cause disease in humans but unlikely to spread to the community, effective prophylaxis is available
ACDP3, Can cause severe disease in humans. Either ow risk of community spread or prophylaxis available
DEFRAJSAPSO group 3, exotic to the UK, moderate risk of spread
1DEFRAJSAPSO group 4, exotic to the UK, high risk of spread
Note that viruses masked with an * are not classified under either The SAPO or ACDP systems and have been
assigned a provisional risk category based on their risk to humans and animals -

Host/vector system
Host vector systems Will be detailed in the individual risk assessments held under this CPW. These will be reviews
the University GM committee prior to work commencing. Vectors will be standard sequencing, expression and gene fusion vectors used in molecular virology studies.
Vectors listed below with ACUP hazard group and justification of Hazard Group.
1. Standard bacterial cloning vectors and expression vectors for individual gene products
(ag. E. coli derived plasmids with antibiotic cassettes, transposons and markers), ACDP 1, unlikely to cause disease
in humans.
2. Bacterial Artificial Chromosomes (SAC), ACDP 1, Unlikely to cause disease in humans.
3. Vaccinia virus (NYVAC). ACDP 2, can cause disease in humans but unlikely to spread to the community,
affective prophylaxis is available. Local rules for the use of vaccinia virus will be in force.
4. Saculovirus (AcNPV), ACDP 1, Unlikely to cause disease to humans.
5. Adenovirus (AdS), ACOP 1, can cause disease in humans but unlikely to spread to the community.

Origin & function
The origins and restrictions on the transfer of the genetic material are outlined in the CPW in general they cover.
Transfer of genes and whole viral genomes from the listed viruses into laboratory strains of E. coli for their
manipulation using cloning vectors, expression vectors, over expression, fusion vectors and bacterial artificial Chromosomes
Transfer of genes or whole viral genomos from the listed viruses into mammalian cell lines using3g cloning vectors, expression vectors, overexpression vectors and fusion
vectors
Transfer of genes from the listed viruses into insect cell lines using baculovirus vectors
Infection of animals with constructs derived from genes or whole viruses from the listed viruses.
1. Develop reagents using genetic modification techniques to study the epidemiology of viruses, with the aims of improving or establishing new diagnostic tests and
characterising virus-encoded nucleotide and amino acid
sequences that determine host range and pathogenicity.
2. Use genetic manipulation and subsequent expression of viral genomes, individual genes, gene fragments and control elements to study the V function in vitro and in vivo.
3. Determine the role of manipulated genes in pathogenicity and protective immune responses. Thus the antigenicity of infectious or non-infectious constructs encoding individual viral genes or expressed recombinant protein may be used in vitro or in vivo, including inoculation of animals. The details of these will be detailed in the individual risk assessments held under this CPW. These will be reviewed by the University GM committee prior to work commencing. Vectors will be standard sequencing, expression and gene fusion vectors used in molecular bacteriology studies. Example risk assessments are attached.

**Evaluation of foreseeable effects**

The viruses, vectors and recipient hosts have been assigned to hazard group categories as outlined in section 6. Full details of the justifications are contained in the CPW. Where live virus is to be utilised the corresponding containment level laboratory or animal housing facility will be used. Local rules for viruses that cause disease in humans will be in place and where applicable will outline additional measures that may include vaccination (where available or work within microbiological safety cabinets. These precautions are outlined in the CPW and supporting documents.

**Containment and control measures for GMOs that are not micro-organisms (e.g. GM animals & plants)**

Most of the CMOs that will be created under this CPW are micro-organisms. My cell lines expressing genes or viruses will be held and manipulated under category 2 laboratory containment. It is not anticipated that any hazards greater than appropriate for this level of containment will be created.

GMO animals will not be created. Animals infected with viral GMO’s will be held under category 2 containment facilities or category I faculties (horses) with containment and waste disposal precautions as outlined in the CPW.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation within this application, not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste management measures include,

(i) Autoclaving and then innerMion at SRCL.
(ii) Contaminated plastic waste triple bagging and then incineration by SRCL (formerly White Rose Environmental)
GM Centre 779 licensed to accept GM waste up to Class 2.
(iii) Sharps disposal in sharp safe containers and disposal through HSE licensed contractors (White Rose HS approved contractors).
(iv) Use of Virkon & Trigeno Advance disinfectants in accordance with manufacturers protocols approved and tested disinfectant sprays to clean surfaces.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
In addition to review by the local GMSC the CPW was sent for external review by individuals with specific experience of using other organisms in the CPW. Amendments were made to the CPW to reflect comments of experts and those of the committee and local rules will be produced where appropriate in accordance with SAGCM guidance before work with the particular organism commences. The GMSC has given initial approval for the CPW at Class 2 but will review all subsequent risk assessments and local rules as they are produced.

**Project Containment**

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**Project Ref 470/10.1a**

- **Date Ackn'd**: 22/12/2010
- **CU2 Project Title**: Derogation to several GM projects see paper file
- **Class**: Class 2
- **CultureVolClass2**: Non-GMM
- **CultureVolumeClass3-4**: Consent Granted
- **Date Project Ceased**:
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Project notified under transitional arrangements**: N

**Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**

02/03/2022
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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The next generation of tissue engineering devices will use growth factors to generate replacement tissues which are of greater similarity to natural tissue. These include growth factors which generate bone and blood supply such as Bone Morphogenic Proteins (BMPs) and Vascular Endothelial Growth Factor (VEGF) as well as a range of other growth factors involved in wound healing or human development.

Growth factors work by stimulating transcription factors inside cells.

Many growth factors are not commercially available. Those which are can be prohibitively expensive costing up to £100,000 for experimentally useful quantities. A reliable in house way to make growth factors will enable the University of Nottingham's Tissue Engineering and Human Development research groups to conduct cutting edge experiments which are not currently financially viable.

Routine molecular cloning will be conducted in non-pathogenic recipient microorganisms such as attenuated E. coli and K. lactis. The genes will be cloned in deemed safe episomal constructs (plasmids from manufacturers with published MSDS).

- DH5α a K-12 E. coli derivative. It is non-colonising and disabled. It is hazard group 1 (SACGM2.3 pp.4) because it is not pathogenic to humans or animal and is rec. Cloning work in these cells will not give expression of any growth factor or oncogenic protein because expression requires DE3 modified strains or helper phage.

- BL21 is a strain derived from B E. coli that has been used in laboratory protein expression for over 30 years. It is rec+ and so has greater risk of transfer of genes into its genome. The HSE commissioned study stated that where BL21 is used to produce proteins which do not give rise to bacterial pathogenicity it may be classed as class-1 (ref: ACGM Newsletter 30, Nov. 2001 and SACGM 2.3 pp4). Commercially available derivatives of this strain include DE3 modified BL21-origami, BL21-rossetta.
BL21-shuffle, Small volume work such as plating and protein expression screening will be classified as level 1. When expressing potential growth factors and oncogenes in large volumes >100mL the potential for significant aspirates and spillage rises and so level 2 procedures will be applied as a precaution.

- Yeasts: Kluyveromyces lactis cells purchased from NEB is the GG799 strain. The FDA classifies K. lactis as "generally recognised as safe" (ref: Panuwatsuk 2003 Biotechnology and Bioengineering 81 (6) 712 -9). Recombiantion events are extremely rare except where intentionally transformed. Selection of modified strains is by ability to use acetamide as a nitrogen source and so does no confer selective advantage outside laboratory settings. K. lactis expressing growth factors will be important for safety. Small volumes and plates for screening can be treated as level 1 (SACGM 2.3 pp3) wheras volumes > 100mL potential for significant aspirates and spillage arises and so level 2 procedures will be applied as precaution.

Mammalian cell expression hosts

- C2C12 cells, human ES, mouse ES cells have minimal ability to colonise humans or survive outside the laboratory so are classified as hazard group 1 (SACGM 2.5). However when treating these cells with growth factors there is a possibility of transformation. When conducting these experiments the cells will be treated as Hazard group 2.

- HEK293T (human embryonic kidney) cells with integrated genes for growth factors or transcription factors pose little risk of colonisation nor are likely to survive outside the laboratory. Their excreted products could be harmful and will be treated with the extra caution advised (SACGM 2.5 pp.10) Therefore these cell lines will be treated as hazard group 2 with additional precautions for workers and disposal protocol.

- MEF (Mouse Embryonic Fibroblast) expressing membrane bound growth factors or receptors. MEFs are derived from disease free and classified mice strains. Modification in a connected program of work to incorporate NOTCH and other membrane bound growth factors and receptors will pose little risk once modified. The cells will act similarly to feeder layers and will be mitomycin C treated to inhibit further replication, they will be classified as Hazard Group 1.

Plasmid vectors to be used in this work are non-mobilisable. No vector includes any mammalian promoters and therefore in the extraordinarily unlikely event of transfer to a human cell the gene would not be expressed. The vectors to be included are:

- E. coli expression vectors such as PDuet are based upon pUC vectors and have a variety of different points of origin including: ColE1, P15A, CloDF13, RSF1030, COLA, mini-F/RK2. Proteins expression is under control of T7lac expression genes and prophylaxis could include a glucose drink. T7 ara an alternative expression system has no background expression.

- E. coli pGEM vectors (of pUC origin) or equivalent will be used to make RNA for in situ hybridisation probes. They use T7 and S6 promoters.

- pKLAC is a plasmid with an E. coli origin for replication and gene manipulation. The plasmid is a pBR322 derivative using the pMB1 origin of replication. When linearised it can recombine with the K. lactis (yeast) genome. Protein expression is under the control of lac promoters and prophylaxis could include a glucose drink.

Growth Factors

- BMP - Bone morphogenic protein family: Growth factors known to enhance differentiation of adult stem cells into bone. They have a pleiotropic role in development reflecting a diverse range of developmental pathways including spinal cord formation and limb bud formation (arms and legs).

- VEGF - Vascular endothelial growth factor family: Cytokines that stimulate new blood vessel formation.

- FGF - Fibroblast growth factor family: Growth factors which stimulate growth and proliferation of cells involved in development and wound repair. Fgf8 is known to play a key role in spinal cord formation and limb bud formation (arms and legs).

- Wnt - Wingless (like Hedgehog) family: Growth factors involved in embryonic development and maintenance of adult stem cell niches.
• Shh - Hedgehog family growth factors involved in embryonic development particularly spinal cord formation and limb bud formation (arms and legs).
• TGFβ - Transforming growth factor beta (TGF-β) family: Growth factors such as NODAL and activin which controls proliferation and cellular differentiation in a number of developmental pathways. TGFβ3 may be significant in limb development.
• IGF - Insulin like growth factor: Growth factors which trigger similar cellular response as insulin and which are involved in a number of developmental pathways.
• BDNF, NGF, NT3 - Brain derived neurotrophic factor, Nerve growth factor and neutrophin-3 respectively are all neurotrophin family of growth factors involved in brain development and neuron survival.
• NOGGIN, CHORDIN - Secreted decoy receptors which compete for BMP proteins so giving an inhibitory effect

**Evaluation of foreseeable effects**

The genes themselves will not provide any selectable advantage for GMOs such as E. coli and K. Lactis, and the strains used are uncompetitive compared to native E. coli and K. lactis. The vectors are not horizontably transmissible.

A remote risk exists of the genes enhancing the growth profile of HEK293T cells but these will be handled using Containment level 2 procedures and need special cell culture medium and so cannot proliferate in the environments.

The genes listed include growth factors that all have a role in differentiation and cell proliferation. The vectors to be used for expression of these genes are not mobilisable and so are generally considered safe but the proteins themselves pose a significant risk for temporary harmful transformation. Because there are no genetic changes the alteration in cell activity should not persist after removal of the growth factor. The proteins will be handled like hazardous chemicals and personal protective equipment will protect the worker.

When the genes are part of naked DNA such as used in gene cloning extra care will be required when handling these sequences and procedures prescribed in University of Nottingham COP for Biological Agents and GMMs part 3.4 will be followed. For example PCR hoods which blow towards the operator to keep the workbench gene free will not be used, a bunsen burner or safety cabinet will be used instead.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste exposed to GMOs such as petri dishes and plasticware will be placed in autoclave bags, sealed and then transferred in sealed containers to A81 for autoclaving prior to transfer off site (SRCL Ltd) for incineration.

Small volumes of liquid waste (<5mL) will be autoclaved and incinerated as for solid waste.

125°C for 15 minutes at 2 atmospheres is sufficient to give 100% kill of E. coli, K. lactis, mammalian cells and human pathogens. The autoclave has a chart readout which is checked after each cycle and is also validated on a six monthly cycle in accordance with HTM 2110.

Larger volumes of liquid waste potentially carrying growth factors or transcription factors will be treated with Trigene for >2hr at recommended concentration before disposal down the drain.
It was felt that the assessment covering use of plasmid expression vectors was very thorough and comprehensive. It was agreed that the GMOs generated were unlikely to have any additional harmful traits which could be disseminated in the environment in the event of accidental release and that the control measures to limit exposure and the proposed waste disposal measures were appropriate. Due consideration was also given to limiting the scale of the work during screening of expression and to the potential hazardous properties of the purified, expressed proteins. Further information was sought concerning proposed use of the second generation lentiviral vectors. Additional details were included in a revised version of the assessment which was recirculated for additional comment. Further discussion on local protocols for containment of the work using designated/dedicated MSCs and standardised disinfection and waste disposal then took place and these were agreed and also included in the final assessment. Due to the nature of the work, and particularly expression of potential oncogenes and specific fusion proteins it was agreed that overall the project is a Class 2 activity.

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Project Ref 470/12.1

- Date Ackn’d: 11/01/2012
- CU2 Project Title: Molecular Cell Biology and Genetics of Trypanosome Species B11-06
- Class: Class 3
- Culture Volume: 1-50 Litres
- Consent Granted: Yes
- Project notified under transitional arrangements: N
African trypanosomes are parasite of the blood which cause human 'sleeping sickness' and a major disease of cattle in sub-Sahara Africa. To evade the host immune system, trypanosomes use a system of antigenic variation in which surface coat identity is regularly switched through the expression of 1 of ~2000 alternative coat genes. This requires a specialised genome structure that includes a large number of chromosomes. This project focuses on the mechanisms that ensure the stable inheritance of the unusual genome and control of the system of antigenic variation. In addition to the major disease-causing species, a number of related non-pathogenic trypanosomatid organisms are used for evolutionary comparison.

In addition, because of their position on the tree of life, African trypanosomes are important model systems for understanding the evolution of the eukaryotic cell. The recent sequencing of the trypanosome genome has highlighted features of the trypanosome cytoskeleton that are predicted to be key nodes in their cell biology.

The major aims of the project are:
1) characterisation of the machinery of genome segregation in African trypanosomes (both the proteins and genetic elements involved in chromosome stability)
2) understanding the control of antigenic variation of African trypanosomes and the expression of the major surface proteins
3) analysis of the evolution of the major surface protein families
4) studies of motor proteins involved in cell division, motility and endocytosis

In order to fulfil the aims of the project, the following types of GMOs will be generated:

- knockdown (RNAi) and knockout (gene deletion) of individual endogenous genes for phenotypic analysis
- expression of epitope-tagged endogenous proteins (either by integration of tag to native loci or via ectopic expression of transgenes from inducible promoters)
- creation of modified chromosomes by directed integration of selectable markers
- generation of libraries of RNAi mutants to screen for specific phenotypic defects

Trypanosoma brucei rhodesiense and T.b. gambiense cause potentially serious disease in man, and other Trypanosoma species are potential animal pathogens. However, trypanosomes are not robust outside of culture. They do not form spores, resistant cysts or other dormant stages and are rapidly killed by desiccation, osmotic shock or detergents. The insect vectors required for transmission of Trypanosoma brucei (tsetse flies) are not present in the UK and we do not maintain an insectory. The organisms cannot penetrate unbroken skin. There is effectively zero probability of release by aerosol formation, since the parasites are killed by dehydration and cannot cross mucous membranes. Direct inoculation of a laboratory worker is the only effective hazard of handling, and this will be managed by the measures detailed in the SOPs attached.

Trypanosomes do not perform genetic exchange in culture. As such, it is not foreseeable that any GM made in a trypanosome species could be accessed by other organisms and the potential for release of the GMO itself is negligible.

Other GMOs:
- cloning and manipulation (without expression) of trypanosomal DNA fragments in E. coli in order to generate the genetic modifications above
- production of yeast artificial chromosomes containing large fragments of trypanosome chromosomes and subsequent modification and purification of these YACs (without expression of trypanosomal genes)
- exogenous expression of specific trypanosome proteins in bacterial, yeast or Leishmania tarentolae expression systems for production of purified protein (for biochemical analysis or antibody production)

Escherichia coli (K12/B strain), Saccharomyces cerevisiae, and Leishmania tarentolae are HG1 organisms and all of the GMs to be made are non-mobilisable. There is no foreseeable alteration to the hazard from these organisms as a result of the proposed GM.

**Host/vector system**

The following host systems will be used for GMO:
- Trypanosoma brucei rhodesiense [Hazard Group 3]
- Trypanosoma brucei brucei, T. brucei gambiens [HG2]
- Trypanosoma evansi, T. congolense, T. equiperdum, T. vivax (other African trypanosomes) [not classified by ACDP; treated as HG2]
- Crithidia deanei, C. fasciculata, C. oncopelti, C. bombi (arthropod parasites of the order Trypanosomatida) [HG1]
- Leishmania tarentolae (lizard parasite) [HG1]
- Escherichia coli, laboratory K12 or B strain derivatives [HG1]
- Saccharomyces cerevisiae S288C strain derivatives [HG1]

These hosts will be modified with a variety of non-mobilisable cloning, tagging and expression vectors. Below are examples of vectors currently in use. Similar vectors, antibiotic cassettes or epitope tags may be substituted if required, providing these do not increase the hazards associated with the work (e.g. no antibiotic resistance cassettes against current treatments will be used).

**T. brucei:**
- p2T7-based – plasmid for tetracyclin-inducible RNAi in trypanosomes; integrates stably into genome [hygromycin or phleomycin resistance markers + ampicillin for bacterial growth] [LaCount (2000) Mol Biochem Parasitol 111:67-76]
- pENT-based – integrating plasmid for epitope tagging at endogenous locus. Common epitopes are fluorescent protein (GFP, dsRed or similar) and/or short antigen, such as Myc, HA, FLAG or TV-tags. [hygromycin, puromycin, blasticidin, or neomycin] [Kelly (2007) Mol Biochem Parasitol 154:103-9]

**E. coli:**
- pQE30-based – inducible expression plasmid for [amp] [Qiagen]

**S. cerevisiae:**

**Other:**
- pLEXSY-based – episomal vector for expression and purification of proteins in Leishmania tarentolae [hyg, phleo, neo] [Jena Bioscience]

**Origin & function**

For knockdown of genes by RNA interference (without library production), tagging of endogenous loci, or expression of genes of interest, DNA fragments (or whole CDSs, in the case of expression), will be generated by PCR from genomic DNA isolated by standard methods from the species under study. In the case of RNAi library production, DNA fragments will be again isolated from genomic DNA, but by direct fragmentation and cloning of gDNA.

The vast majority of genes will be inserted into the species from which they are derived (with the exception of expression constructs for protein purification). No genes are anticipated to increase pathogenicity, virulence, host range or transmission.

Most of the genes to be targeted for modification are involved in core cellular activities. Examples include genes encoding for proteins in the mitotic spindle and kinetochore, motors necessary for the beating/assembly of the flagellum, components of the transcriptional machinery and structural proteins of the nucleus. Those involved in the control of antigenic variation are also likely to be homologues of core polymerase or chromatin binding proteins. GM for this project will involve production of
mutant (knockdown/knockout) or epitope-tagged versions of genes, which will be either phenotypically neutral or deficient.

Evaluation of foreseeable effects

In the vast majority of cases, GM for this project will involve only production of mutant (knockdown/knockout) or epitope-tagged versions of genes in their endogenous species. These will at most be phenotypically neutral, and more likely detrimental to cell growth. No modifications proposed are anticipated to increase pathogenicity, virulence, host range or transmission. All modifications to trypanosomatids are likely to result in reduced or unchanged fitness and/or virulence. Moreover, trypanosomes have a complex life cycle with several essential differentiated forms and it is highly unlikely that the transfer of even cryptic genes would result in increased virulence. The reporter genes/epitopes to be used are already widely used and have no intrinsic harmful activity. The parasites themselves do not produce toxic products or immune effectors that are linked to human pathogenicity. Virulence and host-specificity in eukaryotic parasites are complex traits with multiple genetic determinants. It is highly unlikely, in all but one case (detailed here), that any transfer of material from one Trypanosoma strain/species would result in increased pathogenicity or transmissibility. The notable exception is in the development of resistance to normal human serum, which is toxic to T. brucei brucei, T. evansi and T. equiperdum. In T. brucei rhodesiense, a single gene, SRA, confers human serum resistance. In the worst case scenario, transfer of SRA to a non-human infective trypanosome species would confer human serum resistance to this GMO. The resultant GMO would still have no greater host range than T. brucei rhodesiense, for which the containment is already adequate. However, to mitigate this risk, no genes homologous to SRA (or non-specific genetic material such as libraries) will be transferred from human infective parasites to non-infective trypanosomes.

The reporter genes/epitopes to be used are not related to primary therapeutic agents for these parasites. The organisms are not believed to express effectors of the human immune response.

Release of GMOs into the environment forms no part of this programme of work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

JUSTIFICATION FOR SPECIFIC DEROGATIONS

In accordance with ACDP guidance regarding the use of Trypanosome brucei rhodesiense, the following physical measures, which would normally be associated with full Containment Level 3, are considered unnecessary for safe and contained usage of parasites in C4d.

1) Laboratory not sealable for fumigation
   There is no foreseeable need to fumigate the laboratory. In the event of a major spillage, the bulk of material would be soaked up on absorbent paper towels then autoclaved, killing any parasites. The area would be wiped down with disinfectant. Any aerosols created that were not cleaned in this initial effort would dry out, killing both host cell and parasite. The area of the laboratory would be left isolated for a period to ensure effective drying. Effective parasite transmission is only possible via direct inoculation of viable parasites.

2) No negative pressure, HEPA filtered extract, microbiological safety cabinet or specified measures to control aerosol dissemination
   The organism presents no hazard of infection by the airborne route. Class II microbiological safety cabinets are in use in the dedicated laboratory, they are necessary for sterile culture of the organism; they are not required for operator safety. Activities involve small scale, standard laboratory techniques with no aerosol generating procedures.

3) Autoclave not within the laboratory
   Although room C4d contains the necessary equipment for the proposed work on the parasites, an autoclave cannot be reasonably accommodated within the room. However, a dedicated autoclave facility is in place directly opposite C4 containing regularly serviced and validated autoclaves. Using the procedures outlined in SOP-BW-#03 (attached) all biological waste from C4d can be safely transported to this facility and sterilised. All material for autoclaving is either double-sealed in leak-proof containers or pre-treated with microbiocide and then single-sealed, to manage risk of spills or other contact with material.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material that has been in contact with live trypanosomes is sterilised by autoclaving prior to disposal. An autoclave for sterilisation of waste is located in C3 directly across the corridor from the C4d. To avoid potential release or exposure of workers prior to autoclaving, material is either sealed in a leak-proof container, or disinfected with a microbiocidal agent proven to kill trypanosomes prior to transport.
All contaminated waste is autoclaved on a standard waste cycle (134°C, 15min) for solid waste or standard sterilisation cycle (121°C, 15min) for disinfected liquid waste. Liquid waste will be treated with 5% Trigene for 2 hours prior to autoclaving. Trigene has been validated as achieving effective 5 log kill at 0.25% in growth medium. Autoclave runs are recorded and checked to ensure effective completion of the cycle. The autoclave is serviced, calibrated and validated every six months by a competent person. More details if required are contained in SOP-BW-#03.

13.

Please enter comments on the GM safety committee on the risk assessment

<table>
<thead>
<tr>
<th>B11-06- Molecular cell Biology and genetics of Trypanosomes PI B W.</th>
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<tbody>
<tr>
<td>It was noted that the general safety and housekeeping arrangements for handling of these SAPO organisms have been approved by DEFRA and are all in place. First use of these organisms has been notified to HSE under COSHH Regs and a derogation has been obtained to not apply all of the measures in CL3 [eg sealability of room, HEPA ON extract]. Subject to approval by the GMSC the project will be notified to HSE as a Class 3 GM activity and similar derogations will be applied for. The PI confirmed that all organisms will be handled to the same standards irrespective of whether they are human or animal pathogen. Members considered that potentially the most risky scenario would be if the SRA gene which allows resistance to human serum and therefore human infectivity were to be transferred to a strain which did not carry this gene. The PI explained that this was highly unlikely to occur by accidental action and that this was not part of the planned experimental work. Genetic exchange is not possible between trypanosome strains except in the fly host (which will not be present) so even if strains were accidentally combined in the lab, no gene transfer would occur. Although sharps will not be used in the procedures, the committee requested that details of prophylactic treatment to be used in case of accidental inoculation should be obtained from Oxford and the UBSA would ensure these were sent to Nottingham Occupational Health so they are aware of the treatment measures required. This information will also be included in SOPs and training given to workers. The PI confirmed that sealed flasks will be used to grow the organisms. The project was approved by the GMSC as a Class 3 activity [with aforementioned derogations].</td>
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Project Containment

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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
The aim of this work is to discover and characterize novel effector proteins from bacterial pathogens by high-content screening in mammalian cell lines, followed by detailed biochemical and genetic analysis of putative effectors. We will express individual bacterial proteins in mammalian cells, examining the cells for phenotypes by automated microscopy. Detailed characterisation of proteins of interest will then be performed, including the making of bacterial mutants and marked bacterial strains.

Mammalian cell lines including: HeLa (human, cervical carcinoma), U2OS (human, osteosarcoma), HEK293 (human, embryonic kidney), Caco2 (human, colorectal adenocarcinoma). Mouse embryonic fibroblasts, murine primary cultures and immortalised lines (various knockouts from collaborators). Bacterial pathogens including: Salmonella Typhimurium; non-verotoxigenic E. coli; Shigella flexneri; Listeria monocytogenes; Staphylococcus aureus; Chlamydia trachomatis.

Lentiviral particles: pHR-SIN-CSGW or similar – 2nd generation, non-replicative transduction vectors. Laboratory strains of Saccharomyces cerevisiae. Non-pathogenic. Laboratory strains of E. coli used for routine cloning and construction. Non-pathogenic.

Mammalian expression vectors including pCMV-3xFLAG, pEGFP-C3, pCDNA-derived vectors and similar. Non-mobilisable.

Yeast expression vectors for 2-hybrid screening including pGBT9 and pGAD or similar. Non-mobilisable.

Bacterial expression vectors including pDsRed2, pSA10, pWSK29, pKD46 and pCP20 or similar. Non-mobilisable.

Lentiviral vectors: pHR-SIN-CSGW, pLKO.1 or similar – 2nd generation vector constructs. Non-mobilisable.
The expression of genes from bacterial pathogens in mammalian cells is intended to reveal novel effector functions, via perturbation of normal cell morphology or phenotype. Transient or stable transfection of bacterial effectors is anticipated to yield cells with inhibited growth and altered cellular phenotype. Genes will originate from Salmonella Typhimurium; non-vero toxigenic E. coli; Shigella flexneri; Listeria monocytogenes; Staphylococcus aureus or Chlamydia trachomatis. Gene sets will be enriched for suspected effector proteins and known toxins or oncogenes will not be cloned and expressed. Genetic material to be introduced into bacterial pathogens will be from self, or known markers, fluorescent proteins or epitopes. No virulence factors will be transferred from any pathogen to another pathogenic organism without further notification.

Other proteins and epitope tags that may be introduced into mammalian cells or bacteria include:
- DsRed2 – A bright red fluorescent protein, derived from Discosoma sp. red fluorescent protein.
- EGFP – a brighter, codon-optimised version of green-fluorescent protein
- mKate2 – a monomeric, far-red fluorescent protein, derived from the sea anemone Entacmaea quadricolor
- c-myc, FLAG, HA and poly-histidine epitope tags.

**Evaluation of foreseeable effects**

The majority of genes we plan to study are associated with aspects of bacterial virulence. As such they co-operate with other gene products to co-ordinate virulence processes such as invasion, intracellular survival or extracellular attachment. Individual genes, particularly secreted effectors are unlikely to convey entirely new virulence properties upon organisms lacking the companion proteins (chaperones, secretion apparatus, other effectors).

Expression of tagged effectors in the native pathogen, or effector mutants might alter pathogenicity, but we consider it unlikely that this would result in a more virulent organism. Overexpression of native virulence factors/effectors following complementation of expression in modified bacterial pathogens will be minimized by the use of inducible, low-copy number plasmids. In most circumstances over-expression of effectors results in growth retardation/sickness of the host strain, and to maintain near wild-type protein levels low-copy number vectors are preferred. Therefore it is unlikely that complemented or modified organisms would have increased virulence compared to wild-type bacteria.

Addition of a novel virulence gene from a known bacterial pathogen into a lentiviral vector may represent an additional risk to human health. It is possible that expression of a single gene might yield a toxic effect to cells infected with the virus. However, in general pathogenic effector function is highly dependent upon the action of other effectors. To date only a single oncogenic effector has been observed. It is the nature of effector proteins that they are directly transferred into the host cytoplasm, therefore this would happen only from direct expression in the eukaryotic cells undergoing primary lentiviral infection. It is unlikely that such effectors would be transmitted/transferred to neighbouring cells, so even toxic, lytic effectors would be restricted in their spread throughout tissues.

Expression of a single bacterial effector protein would likely be insufficient to cause overt disease in an infected person and would not rescue viral replication.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

TriGene Advance Disinfectant has been validated by the manufacturer as bactericidal for E. coli, L. monocytogenes, Staphylococcus spp, Shigella spp. and Salmonella spp. Recommended dilutions for high-level contamination are 1:100 (for surfaces) or 1:10 (for highly contaminated liquids and suspensions). Contact times are given by the manufacturer as 30 minutes. These data have been validated under conditions equivalent to or exceeding those of use e.g. in bodily fluids such as blood and vomit, where protein levels are high.

To ensure a robust safety margin we will use TriGene at 1:50 for surface disinfection and 1:10 for decontamination of liquid wastes and glassware. Contact times will be increased to a minimum of 60 minutes. Treated liquids will be disposed of via a laboratory sink.

Contaminated solid waste will be placed into autoclavable bags within a solid-sided plastic container. When full, bags will be loosely sealed (to allow steam entry), a lid placed upon the container and transported on a trolley to the autoclave. Material is autoclaved in the plastic container to avoid further handling. Autoclaves are validated every 6 months. Autoclaved material will then enter the clinical waste stream for incineration.
Following discussion with the GMSC the PI modified his experimental approach from library-based screening to a more targeted genomics and PCR approach. The GMSC requested that vectors with inducible expression should be used wherever possible. The GMSC considered that the project could be covered by two risk assessments, one to cover work with the pathogenic bacteria and the other to cover mammalian cell expression work and use of lentiviral vectors. Other issues discussed for inclusion in local laboratory rules and training were:
- Symptoms to watch out for in case of accidental infection.
- Procedures capable of generating aerosols of pathogens that can infect by this route should be carried out in an MSC as appropriate.
- Dedicated autoclave bins will be used for disposal which will avoid any double handling by autoclave operators.

The GMSC approved the activity as Class 2 with some aspects being class 1.

Project Containment

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Animal Units

- L2
- L3
- L4

Large Scale Activities

- L2
- L3
- L4

Human Clinical Applications

- L2
- L3
- L4

Project Ref 470/12.3

Date Ackn'd 28/12/2012

CU2 Project Title

- Plant model system for the study of pollen food allergy

Class 2

Consent Granted

Non-GMM

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use
The aim of the project is to define the parameters that characterise the extrinsic allergenicity of plant pollens and assess the mechanisms of immune sensitisation [in-vivo & in-vitro] of Bet v 1 & LTP family of proteins that are both implicated in food allergy.

Recipient or parental organism
Initially these studies will be done in Arabidopsis but may progress to include Tobacco plants in the future.

Host/vector system
The vector system for transfer into the plant system will use plant binary transformation vectors in agrobacterium tumifaciens. Arabidopsis plants will be transformed by floral dip and selected by their ability to grow on kanamycin or hygromycin.

Origin & function
Bet v 1, the major allergen from birch (Fagales), belongs to one of the most well studied families of pollen allergens and is responsible for spring pollinosis in the Northern hemisphere. Therefore large European populations are exposed to this allergen daily. Plant lipid transger proteins (LTPs) comprise a ubiquitous class of plant proteins involved in food allergy mainly triggered by fruits of the Rosacea family. In contrast with the sensitisation via birch pollen, the sensitisier for the LTPs, described mainly in Mediterranean populations has not been identified.

Evaluation of foreseeable effects
The GM plants will be more allergenic than the wild type Arabidopsis plants to individuals that are specifically allergic to Bet v 1 or Art v 3.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
GM plants will be grown in controlled environment rooms where access is restricted. Atopic individuals in general and those allergic to pollen or food allergy of the Rosacea family will be excluded from this room.

- Mesh filters on drains checked and cleaned regularly.
- Sticky mats will be placed the entrance to the room and to the facility.
- Dedicated lab coats.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Plant material will be bagged within growth room and sent for incineration

Is an emergency plan required according to regulation 20?  No

If yes, tick to confirm that it is attached to this form  No

Tick to confirm that you have attached a risk assessment to this form  Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment  No

Please enter comments on the GM safety committee on the risk assessment

The committee acknowledged that whilst this was extremely low risk project the genetically modified plant was potentially more harmful to susceptible individuals that the unmodified Arabidopsis and therefore notification was required. The control measures proposed should reduce risk to low/effectively zero.

Project Containment

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Project Ref 470/13.1

Date Ackn'd 19/04/2013

Date Project Ceased

CU2 Project Title B 13-01 To test the ability of Proteus mirabilis isolates to swarm across catheter surfaces

Class 2

CultureVol Class 2 1 Litre

Consent Granted

Project notified under transitional arrangements No

Withdrawn No

Tick if notifying a connected programme of work No

Historical Significant Changes
### Project Additional Information

#### Purposes of the contained use

Proteus mirabilis forms part of the normal flora of the gut, but it is also the cause for 40% of catheter-associated urinary tract infections in long term catheter users. Currently the relative contributions of swimming through fluid environment or swarming through the catheter—urethra interface to catheter-associated urinary tract infection (CAUTI) is unknown. In this study, modified Proteus strains deficient in swarming or swimming will be compared for their ability to migrate across catheter material in vitro.

#### Recipient or parental organism

GM strains of Proteus mirabilis are a gift from collaborators at the University of Brighton. Their genetic characteristics are detailed below. No further genetic modification will be carried out.

#### Host/vector system

Recipient or parental organism

- **Swarming-deficient mutants** were generated from *P. mirabilis* strain B4 with a mini-Tn5Km2 transposon and the pUT suicide delivery vector. The delivery vector pUT, carrying mini-Tn5Km2, was transferred to *P. mirabilis* strain B4 from the *Escherichia coli* S17.1Apir donor strain by conjugation.

#### Origin & function

- **f/hA** is the genetic basis for non-swimming, non-swarming mutant NS63.
- **G37** — Non-swarming mutant: WbdN from *E. coli* - putative glycosyl transferase
- **G93** - Non-swarming mutant: SurA from *S. enterica serovar typhimurium* - survival protein precursor; encodes a peptidyl-prolyl-cis-trans isomerase

#### Evaluation of foreseeable effects

- **Origin & function**

  - The identification of f/hA is the genetic basis for non-swimming, non-swarming mutant NS63.
  - G37 — Non-swarming mutant: WbdN from *E. coli* - putative glycosyl transferase
  - G93 - Non-swarming mutant: SurA from *S. enterica serovar typhimurium* - survival protein precursor; encodes a peptidyl-prolyl-cis-trans isomerase

- **Due to the nature of the transposon mutagenesis used to create the mutants, detailed above, *P. mirabilis* mutant strains will have a reduced fitness in respect to the wild type so will not carry any further risk outwith the Hazard category assigned to the wild type [ACDP HG 2]**

- **Mutants generated using the transposon mutagenesis procedure are stable and unlikely to revert to wild type. *P. mirabilis* does not cause infections in normal healthy individuals and is already ubiquitous in environment. Insertion of transposons results in an attenuated phenotype and the vector cannot replicate in the recipient.**

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- **N/a**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid cultures will be treated with 1% Distel for 15mins prior to discharge to drains — manufacturer's validation data shows 5log kill. Agar plates/solid media autoclaved — in accordance with NUHT protocols — autoclaves validated in accordance with HTM 2010

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The committee reviewed the assessment and asked for some clarification on the antibiotics to be used for selection. These were satisfactorily addressed by the PI and the GMSC approved as a class 2 activity

**Project Containment**

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**Project Ref** 470/13.3

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<tr>
<td>19/12/2013</td>
<td>Adenovirus-mediated gene transfer of vascular growth factors and their receptors into mesenteric and muscle tissues. [GMSC Number B13-04]</td>
<td>Class 2 &lt; 1 Litre Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>

Date Project Ceased:  

Project notified under transitional arrangements
### Project Additional Information

**Purposes of the contained use**

The purpose of the research is to investigate the signalling pathways through which vascular growth factors and their receptors, delivered via non-replicative adenovirus particles, induce vascular remodelling in mesenteric and muscle micro-vessels. Specifically it will investigate the role of the different vascular growth factors on increased permeability and endothelial growth and migration in rodent (rat and mouse) microvessels in vivo. It is expected that mesenteric and muscle cells will express the recombinant protein and the vessels will respond accordingly. This work is part of a program to identify novel therapeutic targets for permeability associated diseases, including cancer, diabetes, psoriasis, etc.

**Recipient or parental organism**

The adenovirus is serotype 5, and has been rendered replication deficient due to deletion of the E1 gene essential for assembly of the infectious virus particles, and deletion of the dispensable E3 gene required for evasion of the host immune system. The appropriate level of containment for replication deficient adenovirus containing harmless genes is Level 1 under SACGM guidelines, but as the inserted genes encode for growth factors that may have a proto-oncogenic effect, the containment should be Level 2. Non-replicative adenoviral particles possessing genes encoding for human and mouse vascular growth factors and their receptors will be produced in a packaging cell line and purified. The recombinant adenovirus will be used to directly infect rodent mesenteric and muscle tissues with the vascular growth factors/receptors by injection of the particles to determine the role of these on vascularisation, angiogenesis, permeability of microvessels, and growth and migration of endothelial cells. There is sufficient homology between the encoded human proteins and the rodent ligands/receptors for functional interactions to occur.

**Host/vector system**

The genes of interest have been cloned into shuttle vectors (constructed at the University of Bristol) and have been incorporated into the AdEasy vector by homologous recombination in E. coli, followed by purification of the DNA. The DNA will be transfected into HEK-293 cells to package the DNA and assemble the virus particles. The E1 gene is provided in trans by the HEK-293 cells.

**Origin & function**

Vascular Endothelial Growth Factors (VEGF) A & C. VEGF-A is a growth factor that induces angiogenesis, vasculogenesis, endothelial cell growth and migration. It also plays a significant role in vascular-related diseases. VEGF-C also promotes angiogenesis and endothelial cell growth, but also has a role in lymphangiogenesis and can affect the permeability of blood vessels.

Soluble VEGF receptor 1 (sVEGFR1) acts as a decoy receptor for soluble VEGF-A.

Endothelial nitric oxide synthase (eNOS) generates nitric oxide in blood vessels that regulates vasodilation by relaxing smooth muscle.

Soluble Delta-like Ligand 4 (sDLL4) and Netrin act as axonal guidance molecules. Netrin has also been shown to play a role in angiogenesis, capillary maturation, and cancer regulation. DLL4 also promotes VEGFR2 expression.

Enhanced Green Fluorescent Protein (eGFP) will be used as a control gene.

Angiopoietin 1 (Ang1) is a growth factor that promotes angiogenesis.

Soluble Tie2 (sTie2) is a soluble receptor for Ang1, and is involved in vascular stabilisation and remodelling. Soluble (sEphrinB2) is a soluble ligand of the EphB4 receptor.

Soluble Ephrin B-type Receptor-4 (sEphB4) is a soluble receptor that binds EphrinB2, and plays a role cell migration, cell-cell interactions, angiogenesis, erythropoiesis, and
in numerous developmental processes, particularly in the nervous system. It also plays a role in axon guidance.

**Evaluation of foreseeable effects**

As all the genes encode for growth factors, or their receptors, they are all equally hazardous as all theoretically have the potential to be proto-oncogenic. A range of animal cells, including human, can be infected by the adenovirus particles. The greatest risk would be associated with accidental infection of someone during handling of the viral particles. Thus, the viral particles will be handled at containment level 2. No sharps will be used during the production and purification of the viral particles, but they will need to be injected into rodent tissues via a syringe. However, in this worst case scenario, no further infectious virus would be produced by the individual's infected cells, the virus would be rapidly inactivated by the host defence systems, and we will be modulating single genes (modulation of multiple genes is generally thought to be involved in transformation of cells), thus reducing the risks involved further. The adenovirus is replication defective due to deletion of the E1 gene. The virus also lacks the E3 gene. There is no possibility of the virus reverting or acquiring either gene via complementation. However, the viral particles produced, although non-pathogenic, are infectious and capable of infecting a variety of animal species, including humans. Adenovirus is capable of surviving within the environment. Infection is unlikely to cause any obvious symptoms, but the encoded genes are potentially harmful. Potential routes of transmission are by aerosols, waste disposal, and by needle-stick injury. No vaccines against adenovirus serotype 5 are available.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste will be soaked in 10% Distel before placing into yellow burn bins for incineration. Plasticware will be sluiced thoroughly with 10% Distel and left for 24 hours before disposal in yellow burn bins. Syringes (needles) will be sluiced with 10% Distel before disposal in sharps bins prior to incineration. All solid animal waste, including bedding, will be autoclaved prior to incineration in yellow burn bins. Liquid waste will be treated with 10% Distel for 24 hours prior to disposal via the drainage. Spillages will be contained with paper towelling and soaked in 10% Distel for at least 5 minutes before placing into yellow burn bins where they will be left for at least 24 hours before incineration. The spill site will also be wiped down with 70% ethanol.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

GMSC noted that Adenovirus with E1/E3 deletions could be assigned to Class 1 however because the inserts include growth factors Class 2 is appropriate for production, purification and packaging of the modified virus.

**Project Containment**
**Project Additional Information**

**Purposes of the contained use**

To produce recombinant animal/insect/plant/fungal/bacterial proteins for use in allergenicity functional-relationship studies.

**Recipient or parental organism**

E.coli will be transformed with conventional shuttle vectors and after DNA isolation and sequencing the amplified vector will be then transferred to yeast (Pichia pastoris or other), bacteria (mainly E.coli systems), insect cells (drosophila, Spodoptera frugiperda or other using baculovirus system), plants (Arab)

**Host/vector system**

- E.coli to be used JM109, XL1-Blue, BL21, DH10B, MC106 all K12 derivatives and recognised as non-colonising and disabled, ACDP hazard group 1.
- Pichia (pastoris, methanolica), Sacharomyces, Schizosaccharomyces pombe and others are not considered pathogenic to human and animals, ACDP hazard group 1.
- Drosophila melanogaster, Spodoptera frugiperda are common insect cells used in expression and are not considered as pathogenic to human and animals, ACDP hazard group 1.
- Agrobacterium tumefaciens is the most common host to be used in plant transfection is not considered as pathogenic to human and animals, ACDP hazard group 1.
Arabidopsis is innocuous to small mammals and insects when eaten, and is unattractive to large mammals. Arabidopsis is self-compatible, nearly exclusively inbreeding and exhibits cleistogamy (within-flower pollination prior to the flower opening). Outcrossing does not normally occur. The system assessed here is generic plant expression system other than pollen. A pollen specific HSE notified risk assessment "Plant model system for the study of pollen allergy" is already in place (D12-11).

Chlamydomonas reinhardtii and Synechococcus elongates are the most common algae hosts used in photosynthesis studies and are not considered as pathogenic to human and animals, ACDP hazard group 1.

Chinese Hamster Ovary (CHO) cell line derived from DG44 cells and Jurkat cell line (human T lymphocyte) are commonly used in mammalian expression systems and are not considered as pathogenic to human and animals, ACDP hazard group 1. They are well characterised with low risk of endogenous infection with a biological agent presenting no apparent harm to laboratory workers.

Vectors

Plant, bacterial, algal, yeast vector systems — all non-mobilisable. Baculo- virus ACDP HG 1

Origin & function

The product of expression will be in most cases a well characterised allergenic protein. Potentially any protein expressed in a recombinant system will render the system more allergenic. The project will focus on allergenicity of plant proteins in general and in particular in proteins contained in pollen and N/S storage proteins from seeds that are ubiquitous to many organisms. Some major allergens from fungi are also of interest.

Most allergens described at WHO-IUIS are of interest (www.allergen.org). Examples of allergens current being used are: Bet v 1, Ber e 1, Mal d 1, Asp f 1, Asp f 2, Art v 1, Ara h 1, Der p 1, Der p 2, Phi p 1, Phi p 5, Phi p 12.

Evaluation of foreseeable effects

Although the recipient organisms are ACDP HG 1, the insertion of genes that express allergenic proteins is of concern. From point of view of human health it will depend on each allergen and on the susceptibility of the lab worker.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Agar plates /bacterial pellets will be sterilised by autoclaving prior to sending for incineration. Autoclave subject to annual validation.

Liquid waste and cultures will be treated with Distel [10%] overnight prior to disposal to drain, plastic ware will be treated with 2% Distel overnight prior to off site incineration [5log kill as per manufacturers data]

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The GMSC view was that this project was class 2 on the basis that the modified GMMs would produce proteins that were known allergens and could produce allergenic response in susceptible individuals as such an MSC would be required for procedures that involved aerosol generation such as sonication/disruption of bacteria to harvest protein.

### Project Containment

<table>
<thead>
<tr>
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<th>Growth Rooms</th>
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### Project Ref 470/15.1

<table>
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<tr>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
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<td>24/12/2015</td>
<td>Construction of new reporter parasite lines to enhance high throughput anti-parasitic drug discovery (D15-06)</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
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<th>Non-GMM</th>
<th>Consent Granted</th>
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Tick if notifying a connected programme of work  N

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<th>Project notified under transitional arrangements</th>
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<tr>
<td>N</td>
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</table>

#### Project Additional Information

**Purposes of the contained use**

Using parasites lines expressing reporter proteins to allow growth inhibition studies upon administration of antiinfective drugs. This will significantly increase the throughput of the screening experiments and minimize the reagents needed in this sort of experiments and significantly reduce the number of vertebrate animals used in
Recipient or parental organism

The goal of this project is to stably insert and express reporter genes in T. gondii. Commercially available luciferase genes include the secreted Gaussia and nanoluc secreted luciferases and a click-beetle Luc-GFP fusion, all of which we have successfully used in the past. These reporter proteins under the control of their own promoter will be targeted to the UPRT locus using CRISPR/CAS9 (Shen et al., 2014. mBio 5(3):e01114-14). UPRT is a nonessential gene in T. gondii and as such provides a neutral location for insertion of reporters. This project utilizes parasite line T. gondii RH strain as a recipient strain. The inserted DNA codes for widely used reporter proteins which are non-toxic, non-oncogenic and generally regarded as safe. The constructs are thus unlikely to cause any deleterious effect on human health or the wider environment.

- Three strains of Toxoplasma gondii (RH, Me49, NED) will be constructed. The three strains represent different genotypes, but all are of the same species, and belong to the same biological hazard category Level 2.
- Recipient micro-organism (Hazard Group 2); The biological agent is a protozoan parasite called Toxoplasma gondii strain RH, category level 2, will be constructed with reporter bioluminescent gene.
- Recipient micro-organisms (Hazard Group 1);

We will be using 3 E. coli strains listed below to amplify our plasmids in DB3.1 for the ccdB gateway vectors

F- gyrA462 endA1 glnV44 4(ear-recA) mcrB mrr hsdS20(rB-, m8-) ara14 galK2 lacY1 proA2 rpsL20(Smr) xyl5 Bleu mll

TOPO10 (Invitroden)
F- mcrA 4(mrr-hsdRMS-mcr8C) cp8OlacZAM15 4lacX74 nupG recA1 araD139 4(ara-leu)7697 galE15 galK16 rpsL(StrR) endA1 Mach/
- 4recA1398 endA1 tonA 0804lacM15 4lacX74 hsdR(rK- mK+)

Host/vector system

The following vectors will be used:

1-Vector (CRISPR; Toxoplasma expression) with plasmid (pSAG1::CAS9-U6::sgUPRT) and gene/insert (SAG1 5' UTR).
2-Vector (Gateway cloning) with plasmid (pUPRT::DHFR-D) and gene/insert (DHFR cassette).
3-Vector (CRISPR; Toxoplasma gondii) with plasmid (pSAG1::CAS9-U6::sg290860-6) and gene/insert (CRISPR sg290860-6).
4-Vector (Toxoplasma Expression) with plasmid (pCAT-YFP-TetR) and gene/insert (YFP-TetR).

Origin & function

All vectors and plasmids will be bought from addgene (https://www.addgene.org/).
- Inserted genes code for reporter proteins which give a luminescent, fluorescent or chromogenic readout indicating the number of the parasites and/or an indication of the amount of reporter protein.

Evaluation of foreseeable effects

None of the combinations are any more hazardous to others.
- There are no harmful properties associated with the plasmids which we are using as it does not replicate. Moreover the insertion of reporter genes is not toxic and targeted gene (UPRT) is a nonessential gene (no related to virulence) in T. gondii and as such provides a neutral location for insertion of reporters and hence will not be any alteration in the virulence of the agent or in its ability to cause disease. The GMMs that will be generated pose no additional...
risk over and above that of the wild type strains. This genetic modification will not increase the agent's pathogenicity or affect its susceptibility to antiparasitics or other effective treatments. The additional genetic material added to the wild type T. gondii does not in itself constitute an additional risk nor does its enable mobilisation of genetic material to another recipient.

- None of the genes that will be inserted into T. gondii by integration into the chromosome have any intrinsic pathogenic potential. These genes are only tracking genes to enable the fluorescent identification and quantification of the organism in response to the tested drugs in drug screening experiments. Based on scientific evidence no change in the pathogenicity of the organism occurs (Shen et al MBio. 2014 May 13;5(3). pii: e01114-14. doi: 10.1128/mBio.01114-14.)

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

We will inactivate the parasite in the lab by exposure to 5% Distel solution. This is in accordance with the local risk assessment form we follow in the school, university safety guidelines and is also based on scientific literature.

For solid waste:
- Autoclaving and then incineration following the School code and using HSE approved contractors [SRCL] for incineration of Class 2 material. [In accordance with previously obtained Derogation] Contaminated plastic waste disposed of by triple bagging and disposal via HSE licensed contractors.
- Sharps disposal if they are required to be disposed of in sharp safe containers and disposal through HSE licensed contractors

For liquid waste:
- Procedures are detailed in the local code of practice. Autoclaving or disinfectant appropriate to volume and type of material to be disposed of following SVMS local rules.
  Autoclaves will be run at 134°C for 10 minutes

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

**Please enter comments on the GM safety committee on the risk assessment**

The following points were raised some in independent comments.
- Consensus from several reviewer comments that this is clearly a class 2 activity parental organism is ACDP HG 2, but the final GMO would be no more hazardous than the non-modified.
- This would not be covered under current Class 2 connect programs of work held by the School and so it will require separate notification to the HSE.
Toxoplasma gondii is a single-celled parasite that infects 1/3 of the world population and causes disease in livestock. T. gondii is an obligatory intracellular protozoan parasite belonging to the phylum Apicomplexa similar to malaria parasites Plasmodium. The aim of the research project is to understand the fundamental biology of the parasite development and multiplication in host cells and to use this as a model to understand apicomplexan biology. The main focus will be on the proteins involved in parasite motility, division and invasion into the host cells.

Future work will involve in vivo work with Toxoplasma to test the virulence and growth of transgenic parasites in a rodent model. Relevant risk assessment for this work will be undertaken prior to work commencing.

Recipient or parental organism
Toxoplasma gondii wild type strains e.g T. gondii RH [ACDP Hazard Group 2] and mutant derivatives:
Plasmid vectors used will be non-mobilisable and most are either tagging/conditional vectors or expression vectors. No viral vectors will be used.

T. gondii:
- pCTG vector and derivatives: pCTG contains a chloramphenicol-resistance marker and a GFP expression cassette. Derivatives include vectors containing resistance cassettes such as those for phleomycin, mycophenolic acid and pyrimethamine resistance, as well as fusion proteins such as RFP (DsRed, mCherry and other variants), YFP and other GFP-derived fluorescent protein variants, HA, c-myc, mDHFR, destabilization domain and Ty1 epitope tags. Genes-of-interest may be driven by inducible promotors that include TetR-binding operator sequences (tetO), or by a range of parasite-specific promoter sequences. (See van Dooren et al, PNAS, 105(36): 13574, 2008) for a description of the base vector.
- pTCY vector and derivatives: pTCY contains a chloramphenicol-resistance marker and a YFP expression cassette that is used as a negative selection marker. Derivatives of this vector are used primarily in generating knockouts of genes-of-interest in Toxoplasma or in generating homologous integration events. (See van Dooren et al, PNAS, 105(36): 13574, 2008) for a description of the base vector.
- pPR2-HA3 vector and derivatives: pPR2-HA3 contains a pyrimethamine-resistance cassette and is used for gene replacements. (See Sheiner et al, PLoS Pathog, 7(12): e1002392, 2011) for a description of the pPR base vector that is further modified to include a 3xHA (haemagglutinin) epitope tag and additional restriction endonuclease sites. (See Huynh et al, Eukaryot Cell, 8(4): 530, 2009) for a description of the base vector.
- pLIC-YFP/DHFR vector and derivatives. pLIC-YFP/DHFR contains a pyrimethamine-resistance cassette and is used for 3' gene replacements. Derivatives include vectors containing mycophenolic acid, phleomycin and chloramphenicol resistance cassettes, and HA, destabilization domain, c-myc and Ty1 epitope tags. (See Huynh et al, Eukaryot Cell, 8(4): 530, 2009) for a description of the base vector.
- PSB and TOX cosmids modified by recombineering to introduce resistance cassettes such as those for pyrimethamine, chloramphenicol, phleomycin and mycophenolic acid resistance cassettes and epitope tags. The recombineering procedure and base vectors are described in Brooks et al, Cell Host Microbe, 7(1): 62, 2010.
- loxP flanking sequences is used to induce Cre recombinase excision, and CRISPR/Cas9 sgRNAs will be used to direct Cas9 excision (See Sidik et al, PLoS One, 27:9(6):e100450, 2014).

E. coli:
- pBluescript / pUC based high copy plasmid is used for molecular cloning in bacteria. These plasmids contain a selectable antibiotic resistance marker: ampicillin or kanamycin.
- pGEX4-T-1 / pET series are used for recombinant protein production. These plasmids contain a selectable antibiotic resistance marker: ampicillin or kanamycin.

Origin & function

Examples of the families of T. gondii genes that will be studied include motor proteins like myosin and kinesin gene families involved in motility and cell proliferation and cell division related genes like Centrin, Cyclin and associated kinases. The source and functions of some of the commonly used genes used as selection markers in Toxoplasma gondii are as follows:
- mutant Toxoplasma DHFR gene (pyrimethamine resistance): a drug-resistant form of dihydrofolate reductase/thymidylate synthase, which reduces dihydrofolic acid to tetrahydrofolic acid.
- the Streptomyces BLE gene (phleomycin resistance): binds with high affinity to phleomycin drug to prevent it from interfering with an organism's DNA.
- E. coli-derived chloramphenicol acetyltransferase (chloramphenicol resistance): acetylates chloramphenicol drug which prevents the drug from binding to the prokaryotic ribosome (in the case of Toxoplasma, likely the apicoplast ribosome), its site of action.
- Toxoplasma HXGPRT (mycophenolic acid resistance and 6-thioxanthine sensitivity): adds a phosphoribosyl group to purine bases such as hypoxanthine, xanthine and guanine, providing the parasite with a means of salvaging purines. In the context of Toxoplasma genetic modification, its presence allows the bypass of a second mode of purine salvage that occurs via inosine 5'-monophosphate dehydrogenase, an enzyme sensitive to mycophenolic acid. In the case of negative selection, HXGPRT converts the drug 6-thioxanthine to toxic nucleotides that become incorporated into the parasite's DNA.
- Toxoplasma UPRT (FUDR sensitivity): adds a phosphoribosyl group to the pyrimidine base uracil, thereby functioning in pyrimidine salvage. UPRT catalyses the conversion of FUDR to its toxic nucleotide, which interferes with RNA and DNA synthesis.

Most of the drugs used to select transgenic parasites are not used as a treatment to cure T. gondii, and their use therefore poses little risk for drug sensitive toxoplasma in clinical settings. However pyrimethamine can be used to select transgenic parasites and treat toxoplasmosis.

Tetracycline-controlled expression utilizes modified T. gondii promoters that contain tet operator sequences derived from E. coli, in cell lines expressing variants of the E. coli-derived tet repressor (TetR) protein (see Meissner et al, Science 298(5594): 837, 2002; van Poppel et al, Int. J. Parasitol 36(4): 443, 2006). Other promoters used in transgene expression are derived for T. gondii. Cre Recombinase, and Cas9 (of the CRISPR/Cas9 system) will be expressed in both the parasites, and the host cells with cell penetrating peptides attached (e.g. Penetratin) to allow optimisation of approaches for generating recombination and gene editing in Toxoplasma.

Standard reporter proteins will be used including: fluorescent proteins (GFP and derivatives); epitope tags (e.g. HA, cMyc, Ty); enzyme reporters (e.g. BirA* (mutant biotin ligase), APEX (a modified ascorbate peroxidase)).

Commonly used antibiotic resistance markers e.g ampicillin, kanamycin will be introduced into disabled E.coli hosts.

**Evaluation of foreseeable effects**

T. gondii is a unicellular protozoan parasite that infects all warm-blooded animals and invades all nucleated cells. The infection is either asymptomatic or causes mild flu-like symptoms which are quickly resolved in healthy persons.

In the UK about 30% of the adult population is seropositive for T. gondii and thus immune to further infection. Immunosuppressed persons or pregnant women are at greater risk but these persons are not allowed to work in the facility.

Tagging of T.gondii proteins with fluorescent or epitope tags is considered highly unlikely to affect the virulence or transmissibility of the recipient organism.

Targeted deletion or knockdown of genes involved in motility, proliferation or cell division is considered most likely to decrease the fitness of Toxoplasma strains and would therefore be unlikely to enhance virulence or transmissibility. Where deletion strains are complemented, episomally, there is the possibility that there may be over-expression of a complementing gene but given the complex, multistage life cycle of this organism, it is considered unlikely that this alone would be sufficient to increase virulence or transmissibility.

Given the disabled nature of the host E.coli strains to be used, none of the planned modifications is anticipated to increase their pathogenicity or ability to survive in the environment.

In the unlikely event that any of the proposed modifications resulted in a more hazardous GMM, the proposed containment, avoidance of sharps and disinfection protocols will ensure that any additional risk is effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the solid waste[agar plates/pellets] will be autoclaved using a programmable autoclave with a program of 15 minutes pulsed free steaming and 30 minutes at 132°C. An autoclave [validated in accordance with BS2646] is available directly opposite to laboratory suite C4

Liquid tissue culture waste — 5% Distel for 2hrs — 5 log kill

Surfaces - 5% Distel 10 minutes
This assessment was circulated to all GMSC Central members for comments and also reviewed in detail by one member who currently runs a project involving GM in another human parasite. A biological risk assessment for work with T.gondii was also prepared by the applicants and the laboratory area and equipment where the work would be performed was also inspected by the University Biosafety Adviser and confirmed as meeting the required standard. Additional detail was sought on the classes of genes which were to be investigated in Toxoplasma gondii to define the scope of the work and this information was added to the assessment. Clarification of the use of some anti-parasitic agents e.g pyrimethamine for selection of T.gondii during manipulation was also sought but this drug had previously been used in GM work with T.gondii and overall the risk that its use would compromise therapy was considered to be very small. There was also some discussion over the likely effect of the proposed modifications on virulence and transmissibility of T.gondii and the E.coli hosts to be used for construct generation and recombinant protein expression. Given the complex life cycle of T.gondii, and the targeted approach for gene deletion/regulation it was considered unlikely that any of the planned modifications would enhance virulence or transmissibility of the resulting GMMs above that of the wild type. The modified assessment was approved as a Class 2 activity.

Please enter comments on the GM safety committee on the risk assessment

This assessment was circulated to all GMSC Central members for comments and also reviewed in detail by one member who currently runs a project involving GM in another human parasite. A biological risk assessment for work with T.gondii was also prepared by the applicants and the laboratory area and equipment where the work would be performed was also inspected by the University Biosafety Adviser and confirmed as meeting the required standard. Additional detail was sought on the classes of genes which were to be investigated in Toxoplasma gondii to define the scope of the work and this information was added to the assessment. Clarification of the use of some anti-parasitic agents e.g pyrimethamine for selection of T.gondii during manipulation was also sought but this drug had previously been used in GM work with T.gondii and overall the risk that its use would compromise therapy was considered to be very small. There was also some discussion over the likely effect of the proposed modifications on virulence and transmissibility of T.gondii and the E.coli hosts to be used for construct generation and recombinant protein expression. Given the complex life cycle of T.gondii, and the targeted approach for gene deletion/regulation it was considered unlikely that any of the planned modifications would enhance virulence or transmissibility of the resulting GMMs above that of the wild type. The modified assessment was approved as a Class 2 activity.

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Project Ref 470/17.1

Date Ackn'd 30/03/2017

CU2 Project Title Reverse genetics of low pathogenicity influenza A viruses

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted Not Applicable

02/03/2022
The scientific goal of the project is to better understand the role of non-structural and 'accessory proteins' in the replication and pathogenesis of influenza A virus. The role of the non-structural protein, NS1, in replication and pathogenesis of influenza A viruses has been established for several years. This highly multifunctional protein continues to be extensively studied. For more than 25 years, it was believed that the eight RNA genome segments of influenza A viruses (PA, PB1, PB2, NP, HA, NA, M and NS) between them encoded only 10 proteins. However, in 2001, the first accessory protein (PB1-F2) was discovered. This small protein (usually up to 90 amino acids long) is translated from an alternative open reading frame of the PB1 gene. Further accessory proteins encoded by the PB1 gene segment (PB1-N40), the M gene segment (M42), the N gene segment (NS3) and the PA gene segment (PA-N155, PA-N183, PA-X) have since been identified. Many of these accessory proteins are not found in all influenza A virus strains suggesting that they are not essential for virus replication but may have an influence on pathogenicity of different strains.

Reverse genetics of influenza A viruses was first established as a technique in 1999. Different methods have been developed, but the principle is that each of the gene segments of influenza A is cloned into a plasmid and these 8 plasmids are co-transfected into a mammalian cell together with 4 plasmids encoding the proteins (PA, PB1, PB2 and NP) required for replication of the virus gene segments. Use of ambisense plasmids means that the expression of the 4 proteins required for replication can be combined with the plasmids encoding the relevant gene segments (an 8-rather than 12-plasmid system). The recombinant viruses generated are then cultured in mammalian cells or embryonated hens' eggs (frequently the more permissive host for influenza viruses).

Swapping of gene segments and/or introducing point mutations or deletions into the gene segment of interest allows the effect on the virus phenotype to be studied. A reverse genetics system is well established for the laboratory-adapted human influenza A virus A/Puerto Rico/8/34 (H1 N1) ('PR8') and this is frequently used to provide a conserved background into which genes from a virus strain of interest can be swapped and the effects on the virus' properties observed (if indeed the virus generated is replication competent). Reverse genetics systems have also been developed to generate a purely equine influenza recombinant virus (e.g. Quinlivan M et al. 2005. Attenuation of equine influenza viruses through truncations of the NS1 protein. J Virol 79:8431-8439).

We propose to use reverse genetics to study the different forms of accessory proteins, with a particular focus on equine and avian influenza A viruses. Comparisons will be made of the ability of viruses with different forms of accessory protein to replicate and induce or inhibit innate immune responses in in vitro cell cultures.

Recipient or parental organism

Plasmids expressing influenza A gene segments will be requested from collaborators including the PR8 system and an A/equine/Kentucky/5/02 (H3N8) system. Additional plasmids expressing equine or avian influenza A gene segments will be generated. Plasmids will be amplified in E. coli and transfected into 293T cells to generate recombinant influenza virus. At no point will viruses be generated that possess a multi-basic cleavage site in the haemagglutinin (HA) molecule, which is the key feature of highly pathogenic avian influenza (HPAI). The sequence of the genes encoded by each plasmid preparation generated will be confirmed before recombinant viruses are generated.

Host/vector system

1) E.coli ACDP 1
All bacterial host strains are disabled or non-colonising and are routinely used for research purposes in many laboratories (e.g. TG1, XL1-blue, Novablue DE3, DH10B,
HB2151).

2) Cell lines

- Well established and characterised continuous cell lines will be used to generate (293T cells) and replicate (Madin-Darby canine kidney cells) recombinant influenza viruses. These will be routinely handled at containment level 2 in accordance with the ACDP guidelines.

3) Embryonated hens’ eggs may be required to replicate recombinant influenza viruses that fail to replicate in MDCK cells. Again, these will be routinely handled at containment level 2.

The vectors are non-mobilisable plasmids.

Origin & function

Each plasmid used in generation of recombinant virus contains an influenza virus gene segment under the control of an RNA or DNA promoter. The eight gene segments encode viral structural proteins, viral proteins involved in replication of the RNA genome and non-structural proteins.

The encoded proteins are not known to be toxic when individually expressed.

Evaluation of foreseeable effects

The recipient E.coli strains that will be produced are attenuated laboratory strains that each only contain plasmids for one gene — there is negligible chance of viral particles being formed from these and the gene products are not themselves toxic. The bacteria are unlikely to survive outside a laboratory.

The cells to be used in this work are established well characterised laboratory cell lines. These cell lines cannot survive outside the laboratory.

Vector —

Inserted material — the individual proteins expressed by the plasmids are not in themselves harmful.

The final GMMs to be generated (recombinant influenza A viruses) could theoretically undergo reassortment with a wild-type influenza A virus. However, the chances of this occurring are extremely unlikely as it requires both the recombinant virus and the wild-type virus to simultaneously infect a host cell.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Please note there is an existing derogation in place for this site as follows;

Part II Table 1 a

8. Autoclave required in the building (for waste that you have identified by risk assessment that is suitable to send off site for inactivation)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste to be sent off site as per derogation; Solid Medium

Cell pellets and tissues infected with GM viruses

Waste that will be inactivated on site;

Concentrated class 2 bacterial cultures by validated autoclave or disinfection Tissue cultures and associated plastics

Note - Our orginal derogation document stated we would use trigene. As this is no longer availabale we have moved to Distel and we have validation documentation for this product.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]
8 Comments
1. This looks OK to me, the only comment is of the routine use of Distel at 10%, this should not be required for surface disinfection, 1% would be better.
2. A few minor points - I checked the Viral Connected programme of work and influenza virus reverse genetics is specifically mentioned as not being covered under that CPW. The ACDP flu guidelines referred to also specifically mention that some HA subtypes should be handled at higher containment than class II and it needs to be made clearer under this assessment that the parent viruses and viruses being produced don't fall into those categories (ie exactly which HA subtypes are present on what virus as quite a few are mentioned).
3. From AB email:
   Sent: 30 August 2016 08:49
   Subject: RE: D16-04 Reverse genetics influenza.
   Yes — happy with this. And I just checked with J that I understood it.
4. This looks like it would be HG2 work. Check this with author. If not covered by CPW would require notification.

5. Comments sent back to PI
   HI J
   Can you respond to the following comments regarding the assessment submission D16-04 Reverse Genetics Influenza. Sorry this got a bit delayed due to changes in
   • A few minor points - I checked the Viral Connected programme of work and influenza virus reverse genetics is specifically mentioned as not being covered under that CPW.
   • The ACDP flu guidelines referred to also specifically mention that some HA subtypes should be handled at higher containment than class II and it needs to be made clearer under this assessment that the parent viruses and viruses being produced don't fall into those categories (i.e. exactly which HA subtypes are present on what virus as quite a few are mentioned).
   • comment is of the routine use of Distel at 10%, this should not be required for surface disinfection, 1% would be better.
   • If the points above can be answered as clarified as required in the assessment this can be signed off. However would be a HG-2 recombinant pathogen and as the work
is not directly covered by the CPW it would need to be notified to the HSE and that will incur a fee to the HSE to notify this or to alter the CPW which would require review (ca. £900 last time one was put in).

6.

Delayed posting fault on workspace meant no posted when sent in. response from JD

From:
Sent: 23 October 2016 10:30
To:
Subject: RE: D16-04 Reverse Genetics Influenza.
Hi M

Apologies for the long time to respond to this — it got a bit buried as a result of various other things taking priority unfortunately, but I do really need to progress this for S PhD.

Please see responses below. Do I have to pay the fee to HSE, in which case I would use Sanjeev's cost centre = A138Q8.

I attach the revised version here.

From:
Sent: 14 September 2016 11:27
To:
Cc:
Subject: D16-04 Reverse Genetics Influenza.

Hi J

Can you respond to the following comments regarding the assessment submission D16-04 Reverse Genetics Influenza.

• A few minor points - I checked the Viral Connected programme of work and influenza virus reverse genetics is specifically mentioned as not being covered under that CPW. Yes, hence the dash

• The ACDP flu guidelines referred to also specifically mention that some HA subtypes should be handled at higher containment than class II and it needs to be made clearer under this assessment that the parent viruses and viruses being produced don't fall into those categories (i.e. exactly which HA subtypes are present on what virus as quite a few are mentioned). The ACDP guidance refers to wild-type viruses. I have added the following to section 5. 'Only established reverse genetics systems will be used and where these are based on virus subtypes for which the wild-type virus falls into the category of 'Viruses that should NOT be handled at containment level 2' in the ACDP guidance, these will have been manipulated so that they may be handled at CL2 (see 'mitigating circumstances' in the ACDP guidance). To ensure that the latest advice is followed, before any novel construct is generated, the ACDP advice and WHO guidelines will be referred to.'

• comment is of the routine use of Distel at 10%, this should not be required for surface disinfection, 1% would be better. I know I got this from somewhere, but cannot remember where — have changed to 1%.

• If the points above can be answered as clarified as required in the assessment this can be signed off. However would be a HG-2 recombinant pathogen and as the work is
not directly covered by the CPW it would need to be notified to the HSE and that will incur a fee to the HSE to notify this or to alter the CPW which would require review (ca. £900 last time one was put in).

7.

requested further clarification
And actually reading it, I would want more info! I am not really happy with this statement; 'Viruses that should NOT be handled at containment level 2' in the ACDP guidance, these will have been manipulated so that they may be handled at CL2 (see 'mitigating circumstances' in the ACDP guidance).
It still doesn't tell me what they are doing to ensure that virus that require a higher containment level are not used. Thanks

8.

Discussed with PI response and addition here new version uploaded above under any edits.
Had a quick chat and we have added new text see added in yellow. The construct is made externally such has relevant changes. And there are also ways, although expensive, where you don't need to go anywhere near a wild-type virus, you can just get all the genes synthesised and manipulate them at the plasmid level.

Addition to section 5:
at The Pirbright Institute has established a reverse genetics system in which recombinant virus is generated with the internal genes of avian influenza but the HA and NA from PR8. These constructs have already been generated under relevant HSE approved containment and ACGM management systems and Dr S will supply the plasmids.
In addition, it is possible to synthesise gene segments so that manipulations such as removing the multi-basic cleavage site can be undertaken at the plasmid level.
Therefore, only ACGM class 2 virus will be handled at SVMS in this project.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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</tbody>
</table>

**Project Ref** 470/18.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
</tr>
</thead>
<tbody>
<tr>
<td>22/02/2018</td>
<td>Propagation of Togaviruses and Flaviviruses for investigations of virus replication and host response to infection</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Date Project Ceased: 02/03/2022
Tick if notifying a connected programme of work

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

**Purposes of the contained use**

The aim of this project is to develop viral culture models to study the consequences of infection of permissive cells with Zika virus, Sindbis Virus and Semliki Forest Virus. These viruses, belong to the Flaviviridae and Togaviridae families are categorised as HG2 organisms. Semliki Forest Virus and Sindbis Virus are selected as model organisms or similar pathogens such as Chikungunya virus (CHIKV), which are categorised as HG3. Infection will be performed in transformed cell lines and primary neuronal cells, including ex vivo rat primary neuronal cells, to investigate the epiphenomenon and pathogenesis of these neurotropic virus infections. Nociception will be assessed using ion flux assays. The site of virus replication will be assessed using fluorescent reporter assays.

Virus particles will be generated from molecular clones provided by external collaborators (Andres Merits, University of Tartu; Andrew Tuplin, University of Leeds). These molecular clones will be transcribed into viral RNA, then transfected into permissive cells to generate virus particles. These viruses will be used to infect appropriate target cells (infected cells will be assessed for gene expression, localisation of virus replication complexes. Genetic modification will be required to produce attenuated variants of SFV, SIN and ZIKV as control preparations. Additionally, viral genes will be sub-cloned into mammalian expression vectors for investigation of protein function.

**Recipient or parental organism**

Escherichia coli cells for propagation of plasmid containing cloned alphavirus/flavivirus genomes (Class 1): TOP10 (Invitrogen) E.coli Genotype F- mcrA A(mrr-hsdRMS-mcrBC) cp8OlacZAM15 AlacX74 recA1 araD139 A(araleu)7697 galU galK rpsL (StrR) endA1 nupG NEB10 (NEB) E.coli DH10OBtm derivative; A(araleu)7697 araD139 fhuA AlacX74 galK16 galE15 e14- cp80dIacZAM15 recA1 relA1 endA1 nupG rpsL (StrR) rph spoT1 A(mrr-hsdRMS-mcrBC)

Stellar Competent Cells (Takara Clontech) E.coli HSTO8 derivative; F—, endAl, supE44, thi-1, recA1, relA1, gyrA96, phoA, 080c1 lacZA M15, A(lacZYA-argF) U169, A(mrr-hsdRMS-mcrBC), AmcrA, A—

Mammalian cells used to propagate human viruses (Class 2):

Zika virus, Sindbis Virus and Semliki Forest Virus will be propagated from molecular clones in mammalian cell culture. The following cultured cell lines may be used for propagation:

COS-7 (BSL2 - contains SV-40 viral DNA sequences) - an African Green Monkey fibroblast immortal cell line, derived from CV-1 simian cells transformed by an origin-defective mutant of SV40 (Simian Vacuolating virus 40).

HEK 293T (BSL-2 contains Adeno and SV-40 viral DNA sequences) - a Human Embryo Kidney epithelial-derived cell line.

Both cell lines produce the SV40 T antigen that allows replication of SV40 origin-containing plasmid during transient expression experiments. The cell-lines are only able to survive under specialised laboratory culture conditions.

HuH7 and (BSL1) hepatocyte derived cellular carcinoma.

HeLa (BSL2 - contains human papilloma virus) — derived from a cervical epithelial adenocarcinoma of a 31 year old black female.

SK-HEP-1 (BSL1) - of human liver endothelial adenocarcinoma origin from a 52 year old Caucasian male.

BHK-21 [C-13] (ATCCO CCL-10Tm) (BSL1) - derived from Syrian golden hamster kidney.

CHO (BSL1) — derived from a biopsy of an ovary of an adult Chinese hamster.

I HepG2 (BSL1) - derived from a liver hepatocellular carcinoma of a 15 year old Caucasian male.
A549 (BSL1) - derived from explant culture of lung carcinomatous tissue from a 58 year old Caucasian male.

Host/vector system

Vectors containing virus genomes: The BSL2 viral genomes, or fragments thereof, are directly cloned into non-mobilisable vectors, including pGEMT, pcDNA3, pCAGG and phCMV and their derivatives. pcDNA3.1 contains the CMV immediate early promoter to facilitate protein expression and the T7 promoter to enable viral RNA transcription.

Origin & function

The inserted genomes facilitate completion of the entire replication cycle of the Zika virus, Sindbis Virus and Semliki Forest Viruses. The genomes include the structural proteins required for virus assembly (viral capsid, envelope glycoproteins), and the non-structural gene products including virus-encoded polymerases, proteases and helicases. No directly toxic gene products, e.g. toxins, cytokines, growth factors, allergens, hormones or oncogenes, are encoded by these viral genomes.

Evaluation of foreseeable effects

The mutations introduced into these genomes will be used to attenuate virus replication. As such none of the GMM created during this project are anticipated to be more hazardous than the wild-type cloned virus genome of Zika virus, Sindbis Virus or Semliki Forest Virus (all BSL2).

Genetic modification of the target viruses using reverse genetics approaches are unlikely to introduce new hazards associated with the pathogenicity, host range, tropism or transmission of these viruses. All attenuation mutations are anticipated to maintain these traits while reducing replication and pathogenicity. However, no individual gene is over-expressed in these experiments over and above that required for virus replication. None of the gene products are identified as direct oncogenes or toxins.

Viral particles generated in mammalian cells will carry recombinant RNA genome and are capable of infecting human (and animal) cells. The Zika virus, Sindbis Virus and Semliki forest Virus are all categorised on the ACDP Approved List as HG2. The viruses can infect cells expressing appropriate receptor molecules and could therefore infect human cells (including neuronal cells) if accidentally injected into humans. Although they can cause human disease and may present a hazard to laboratory staff working with these pathogens, there is little risk of spread to the community, and the consequences of infection are low. Upon cell entry and uncoating the genome will be transcribed and proteins synthesized. Infection can result in arthralgia, which is treatable with analgesics.

In the event of a release of these blood borne viruses on-going transmission is highly unlikely. These viruses are vector-borne, transmitted by mosquitoes of the Culex/Culiesta genera (Sindbis virus) and Aedes genus (Zika virus and Semliki Forest Virus), which would be required to transmit the viruses between human hosts.

Propagation of the Vectors in E- coli: The vectors are non-mobilisable. The E coli K-12 derived laboratory strains are non-colonising and disabled. Transfer of plasmids containing viral genomes to other plasmids or bacterial strains would not create a pathogen of concern.

Transfer of viral sequences to environmental viruses. Species belonging to the viral families Flaviviridae and Togaviridae are documented to undergo recombination. Recombination of these viral cloned virus genomes with other viruses of these families could result in novel virus variants with altered pathogenic properties. However, this would require contact with wild-type Zika virus, Sindbis virus or Semliki Forest virus. None of these pathogens are present in the UK and as such the likelihood of these recombination events is low. The consequences to pathogenicity would also be low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Plastic consumables (except pipettes), gloves and contaminated paper towels will be placed into autoclave bags and autoclaved for at least 10 mins at 134oC. Autoclaves are validated annually.

Plastic pipettes or other hollow instruments will be discarded into dilute, working concentration disinfectant solution for manufacturer's recommended contact time to discarding for incineration/liquid waste containing toxic chemical / materials will be disposed of by existing validated methods for the disposal of the chemicals used.

Non-toxic liquid waste will be aspirated into sealed containers containing disinfectant (Distel). This will be left for at least 12 hours the manufacturer's recommended contact...
time prior to discard into drains. Daily renewal of fresh disinfectant solution to a known level will ensure the final concentration is not less than 5% Waste will then be disposed of as clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
While the proposed work will produce viable recombinant viral particles it is unlikely that the recombinant viruses will be any more virulent than their wild-type counterparts. The containment procedures and waste disposal procedures in place make exposure of personnel or the environment with recombinant viruses highly unlikely. I see no reason why this work should not be approved.

This is not my area of expertise but I agree with Karl that exposure to the virus would be mitigated by the systems in place. The work has been previously carried out with cloned viruses and the point mutations are likely to disable/attenuate the particles over enhance virulence.

I agree, this looks OK to approve.

K earlier comments cover the main point - i.e. modified virus almost certainly less hazardous than WT virus. Containment plans for handling wild-type virus seem appropriate.
Looks ok by me (it is my area of expertise).

I agree that the planned modification of the wild type viruses is unlikely to generate viruses that are any more pathogenic than the original strains, and can be handled safely using the proposed containment and disposal procedures. It seems unlikely that recombination with wild type viruses in the environment is an issue given the fact that these viruses are not naturally present in the UK. Transmission by insect vectors is also highly unlikely given the specific vectors involved do not exist in the UK. I am happy that this assessment can be approved.

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<table>
<thead>
<tr>
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<th>Glass Houses</th>
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</tr>
</thead>
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<tr>
<td>L2</td>
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### Project Ref 470/18.2

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<th>Non-GMM</th>
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</thead>
<tbody>
<tr>
<td>20/06/2018</td>
<td>Heterologous expression of fungal secondary metabolite biosynthesis genes in Aspergillus species</td>
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<td>&lt; 1 Litre</td>
<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Project notified under transitional arrangements: No
Genome sequencing of fungal species has revealed the presence of thousands of yet uncharacterised gene clusters required for the biosynthesis of secondary metabolites. In order to identify metabolites produced from these gene clusters, heterologous gene expression with subsequent metabolite extraction is required. Respective biosynthesis genes will be amplified by PCR from genomic DNA of the originating organism or produced by gene synthesis. After a cloning of genes into Aspergillus expression plasmids, the plasmids will be amplified in Escherichia coli, purified and used for transformation of Aspergillus expression platform strains. Transformants will be analysed for the genomic integration of expression vectors and selected strains will be cultivated for induction of gene expression. Metabolites will be purified by extraction with organic solvents from culture supernatants and mycelium and subjected to HPLC analytics. In addition, the mycelium will also be used for purification of the proteins produced from recombinant gene expression.

**Bacterial strains.** The E. coli strains to be used are all Rec A- derivatives such as DH5α, 10-p, XL1-Blue, SURE and TOP10. These strains are disabled and non-colonising and are equivalent of ACDP category 1 organisms (i.e. non-pathogenic to humans or animals). They have limited survivability in the environment as they require specific nutrient supplements not required by wild-type organisms. For protein expression using a pET vector system E. coli BL21(DE3) Rosetta2 cells will be used as these strain contain an endogenous phage T7 polymerase (DE3) and a plasmid that codes for rare E. coli tRNA sequences (Rosetta2).

**Fungals strain.** All Aspergillus strains have now been classified as category 2. Therefore, all work will be carried out under safety category 2. Parental wild type strains such as A. oryzae RIB-40, A. terreus SBUG844, A. niger A1144 and A. fumigatus CBS144.89 will be used as recipient strains.

**Host/vector system**

Bacterial vectors. These are generally pUC and pBR322 derivatives encoding ampicillin, zeomycin, kanamycin or other antibiotic resistance markers. Vectors include pUC19, pJET1.2, pET43 or pBluescript. They are considered to be non-mobilisable. Hazards arising from mobilisation are...
unlikely due to poor survivability of the E. coli derivatives outside the laboratory.

Fungal vectors: Plasmids re-isolated from E. coli will be used for fungal transformation. These vectors cannot replicate in Aspergillus species and require integration into the genome. As this happens either by homologous recombination or by non-homologous end-joining repair mechanisms, plasmids become part of a chromosome and have no self-mobilisation ability.

Origin & function

Genes of fungal origin will be used for cloning and expression that produce enzymes with metabolic activity and substrate conversion. The genes will derive from fungal gene clusters assumed from in silico prediction to be responsible for the production of secondary metabolites. A priority will be given to genes that code for enzymes belonging to the classes of polyketide synthases, non-ribosomal peptide synthetases (NRPS), NRPS-like enzymes and terpene synthases. Depending on gene cluster composition, additional genes such as monoxygenases, methyltransferases or reductases will be cloned for recombinant expression in Aspergillus species.

Evaluation of foreseeable effects

It is unlikely that the risk classification of E. coli strains used in this study will increase as no specific virulence determinants will be cloned and/or expressed. The promoter sequence used in generation of expression constructs for fungal transformation are of eukaryotic origin and not recognised by E. coli. In addition, most genes will derive from fungal genomic DNA and open reading frames are interrupted by intron sequences that cannot be spliced by E. coli. In pET gene expression and protein production only genes will be cloned of which the function is known and which are not increasing the risk potential of E. coli.

Resulting strains from fungal transformations with secondary metabolite biosynthesis gene will most likely not increase the pathogenic potential of the transformants. However, as this project aims in the identification and characterisation on novel secondary metabolites, biological activity of the resulting substances is unknown and needs to be tested. However, gene expression will be regulated by the composition of the growth medium and initial culture volumes will not exceed 100 ml to keep the maximum amount of metabolites produced at low level. In conclusion, as Aspergillus species are already classified as category 2, this classification will not be exceeded in recombinant strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Surface disinfection with 2% Distel before and after work. Validated by manufacturers data.

Autoclaved Waste

Solid Waste — waste pipette tips/swaps/spreaders within CLII cabinets which may be contaminated with spores will first be collected in jars containing 2% Distel and soaked overnight before autoclaving.

All Liquid waste will be autoclaved

Autoclave waste loads validated annually.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
This would appear to be a pretty standard Class II assessment, based solely dependent on the HG2 classification of the Aspergillus species in use. E. coli GM work is not designed to express any heterologous proteins in the main, although if required, any proteins expressed in E. coli are unlikely to affect its pathogenicity. Aspergillus work is not designed to express any known factors that would be expected to increase host pathogenicity and will not provide the host strains with increased resistance to any antibiotic agents that might be used to treat any infection. Sufficient precautions appear to be suggested in the advent of any significantly toxic metabolite being produced as a result of the proposed GM work. All work will be carried out in designated CL2 laboratory spaces.

Reply Edit Delete Like 09 Feb, 2018
User icon: plzah
A H
I agree, this is a CL2 activity with the appropriate safeguards in place. The main risk still remains infection / allergens from Aspergillus, and this is unlikely to be increased by the work proposed.

Reply Edit Delete Like 09 Feb, 2018
User icon: plzww
B W
Agree with comments above. E. coli work is straight-forward and v. low risk. Aspergillus work contains an (identified) risk due to necessary uncertainty regarding toxicity, but in my opinion this is well managed in proposed work.

Reply Edit Delete Like 12 Feb, 2018
User icon: Add a picture of yourself
L C
Comment from A C
There does not seem to be any expected increased risk from the modifications and I think this is well described. Biggest issue for me is preventing exposure to spores (possible allergen risk is the main concern), but also containment of the GM spores so they don’t escape into the environment – They consider breech of containment would not cause problems because of the nature of the modifications and because the genes they are looking at are widely distributed in the environment anyway -which again I happy with.
So I think hazard/containment has been considered appropriately but there is just a bit of inconsistency in different sections of the form.

In Part 1 section 9 – they say they will use CL2 containment to limit contact of GMMs (primarily spores) with humans and the environment.

Are you confident that the final GMMs will not be hazardous to humans or the environment, even in the event of a total breach of containment?

If YES you must justify your answer

If the answer is ‘No’ or you are in any way unsure, Part 2 of this form must be completed.

Yes – Specific cultivation conditions are required to induce the expression of recombinant genes. These conditions (high starch content, doxycycline) are unlikely to be present in the environment. In addition, gene clusters derive from fungal species ubiquitous in the environment. Therefore, the resulting metabolites from heterologous gene expression, though yet not characterised, are already produced in the environment by the originating fungal species. However, an allergenic potential of spores produced by fungal GMMs will remain, but not increase in comparison to the parental strains.

A containment level 2 facility and use of good microbiological practice will be sufficient to limit contact of arising GMMs with humans and the environment. All exposed glassware, solutions and plasticware will be autoclaved following use, prior to disposal or wash up. Lab areas in which manipulations occur will be disinfected with 2% Distel on a regular basis.

In Part 2 section 2/3 they also mention use of an MSC to limit human exposure to GM spores (and presumably dispersal).

Should the MSC also be mentioned in Section 1 Part 9? Also given the risk of allergy to spores is considered the same if they are GM or non-GM, do they routinely work with non GM spores in the MSC?

Assignment of a provisional containment level that is adequate to protect against hazards to human health.

This step will involve considering the containment level necessary to control the risk of the recipient microorganism (i.e. the ACDP Hazard Group of the recipient micro-organism) and making a judgement about whether the modification will result in a GMM, which is more hazardous, less hazardous, or about the same. Sometimes it may help to compare the GMM with the relative hazard presented by other organisms that would fall within the same ACDP Hazard Group as the GMM.

The highest risk for distribution of the GMMs from this project occurs from airborne spores. To keep exposure risk at a minimum, all work with spores will be performed under a class II safety cabinet.

Fungi growing in liquid cultures are forming mycelium with a very low potential for infection. Due to the general classification of Aspergillus species in risk category II, all work will be performed in category II lab space.

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02/03/2022
The major aims of this project are: (A) modifying the genome of producing Actinomadura strains in order to prevent or enhance the production of metabolites of interest, (B) cloning the relevant genes within suitable vectors for their characterisation, modification, and expression, (C) transiently or stably transforming selected E. coli, Streptomyces, or Actinomadura hosts with the appropriate recombinant constructs containing Actinomadura genes, and (D) obtaining the expression of the recombinant biosynthetic enzymes and the production of the respective natural or synthetic products.

The work to be carried out on Actinomadura will include the mutation, deletion or insertion of appropriate DNA sequences within the host's genome in order to obtain genetically-modified strains with depleted or enhanced production of the target metabolites, or the production of novel ones. Due to the well-known difficulty in the growth and manipulation of some Actinomadura species and to their poor productivity, standard microorganisms such as E. coli and Streptomyces lividans will be initially employed in order to easily obtain and engineer the constructs to be later introduced in Actinomadura, or as final hosts for the efficient expression of the desired biosynthetic enzymes and/or their products.

The molecular biology techniques to be used for the purposes above include, but are not limited to: molecular cloning, DNA mutagenesis (both random and site-directed), gene knock-out and knock-in, DNA deletion and insertion, chromosomal integration, overexpression, insertion of reporter and selection genes.

The Actinomadura strain in our possession is an unidentified specimen previously isolated by an external group. Its genome has been fully-sequenced and a database search followed by phylogenetic analysis placed the organism evolutionarily close to Actinomadura macra.
Three strains belonging to the genus Actinomadura are classified ACDP Hazard Group 2 pathogens for humans, as they are known to be responsible for some cases of actinomycetoma, a chronic subcutaneous infection of the skin and connective tissue, generally acquired by inoculation of fungal or bacterial spores through a breach in the skin. The initial symptoms of an actinomycetoma lesion appear as a small, painless, subcutaneous nodule which softens to form a sinus releasing seropurulent or purulent discharge, often containing characteristic large, white to yellow granules. Multiple nodules are formed gradually and later ulcerate and drain through sinus tracts, while the surrounding tissue becomes swollen and deformed. A few antibiotics are successfully used in the treatment of the infection. The strain used for the purposes of this project will be treated as Class 2 pathogens.

The Streptomyces strains to be used will be derivatives of S. lividans 1326 (e.g. TK24), one of the two model organisms for streptomycetes and the best genetically-characterised Streptomyces strain. Its lack of methylated-specific restriction systems and its low endogenous protease activity make this organism ideal for cloning and expressing heterologous proteins, including complete natural product biosynthetic pathways. Streptomycetes are infrequently pathogen and do not have an ACDP hazard group classification although, in rare cases, some species have been associated with mycetoma in humans. The strain used for the purposes of this project will be treated as Class 2 pathogens.

The E. coli strains to be used will be laboratory derivatives of B or K12 (e.g. DH5α, BL21, BAP1, G1724) which are considered to be disabled, non-colonising and non-pathogenic for humans, animals, and plants. All of these strains have multiple auxotrophic requirements which are unlikely to be met in the environment, where their survival is considered unlikely. All of these strains are considered ACDP Hazard Group 1, and introduction of replicative plasmids and vectors containing DNA from other organisms into these laboratory strains is not considered to raise this classification.

### Host/vector system

Plasmid vectors suitable for cloning, expression, and/or chromosomal integration in Actinomadura, Streptomyces, or E. coli will be employed, as well as shuttle vectors for two or more of the strain used.

The starting vectors will be routinely-used, engineered versions of natural plasmids (e.g. ColE1 derivatives such as pUC family, pBluescript family, pJ702) or further derivatives thereof (e.g. pJ773, pSE101, pSET152, pUWLKS, pUZ8002) which, with the exception of pSET152 and pUZ8002, are non-mobilisable. pUZ8002 is a conjugative vector providing transfer functions for pSET152 in Streptomyces, but possesses only very low levels of self-transfer.

Derivatives of the plasmids above featuring Actinomadura and/or Streptomyces DNA will also be used, which will be obtained as a result of this project.

### Origin & function

The genes involved in this project will be antibiotic resistance genes to allow for the selection of the desired recombinant strains, reporter genes to monitor gene expression or visually tag the bacteria, and Actinomadura genes (or parts thereof) coding for putative or known biosynthetic enzymes involved in the production of secondary metabolites.

None of the genes above or the products they encode for has been shown or predicted to be harmful for humans, also when over-expressed.

Selectable markers will include resistance genes against (3-lactams (e.g. ampicillin, carbenicillin), aminoglycosides (e.g. kanamycin, gentamicin), tetracyclines and natural cyclic oligopeptides (e.g. thioestrepton, an antibiotic for Streptomyces).

Reporters genes will be those routinely used in research, encoding for example 3-3-galactosidase, fluorescent proteins, luciferase, and tyrosinase (converting the amino acid tyrosine into the dark pigment melanin). The genes to be inserted into the aforementioned hosts will originate from Actinomadura or Streptomyces and will encode for one or more modules of the mega-enzymatic complexes involved in the recognition, activation and polymerisation of the amino acid units forming the desired secondary metabolites.

None of the inserted genes has been shown or suspected to alter any pre-existing traits of the host organism.

None of the antibiotics to be used in this project (e.g. ampicillin, kanamycin, tetracycline and thioestrepton) is routinely used for the treatment of actinomycetoma.

Genes that will be expressed as part of this project are the resistance markers mentioned above (e.g. ampicillin, kanamycin, tetracycline and thioestrepton), reporter genes such as those coding for 3-3-galactosidase, fluorescent proteins, luciferase, as well as genes for the mega-enzymatic complexes involved in the production of secondary metabolites.

One or more genes involved in the production of secondary metabolites in Actinomadura and/or Streptomyces may be overexpressed to increase the production yields of desired metabolites.

The genes to be (over)expressed will be controlled by inducible systems (e.g. lac, PBAD) and their expression activated upon addition of suitable inducers (e.g. IPTG, arabinose).
None of the inserted genes or the products they encode for has been shown or predicted to be harmful for humans when (over)expressed or alter any pre-existing traits of the host organism in a harmful way.

The Actinomadura and Streptomyces strain used in the course of this project are wild type organisms, and therefore able to survive in their natural environment. The genetically-modified versions of these organisms will generally make them less fit in the environment, either by loss of function (such as losing the capacity to produce antimicrobial metabolites, etc.) or by metabolic overburden (overproduction of desired metabolites at the expense of other biological functions).

The E. coli strains to be used have multiple auxotrophic requirements which are unlikely to be met in the environment, where their survival is considered unlikely. The genetically-modified versions of Actinomadura and Streptomyces strain used in the course of this project will not differ significantly from their wild type parents, as they will be likely producing a natural product or engineered version of it. However, these modifications are likely to be at the cost of their ecological fitness, which is not important in the laboratory. Such GGMOs are therefore very unlikely to have direct consequences on the environment.

The E. coli strains to be used will be laboratory derivatives of B or K12 (e.g. DH5a, BL21, BAP1, GI724) which are considered to be disabled, non-colonising and non-pathogenic for humans, animals, and plants, and introduction of replicative plasmids and vectors containing DNA from other Actinomadura or Streptomyces into these laboratory strains is not considered to increase the hazard they pose.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste (liquid culture, agar plates, other in vitro cultures and consumables contaminated with bacterial culture) will be autoclaved at 126 °C for 15 min.
The disinfection procedure for Actinomadura was validated with 5% Chemgene (Medi-Mark Scientific) for less than 10 minutes

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

A number of minor issues were requested to be addressed by the committee, this included the proposed use of RPE, information on spills and as below:
Section 7. The genetically-modified versions of these organisms will feature the appropriate selection markers to ensure their dependency on laboratory conditions' Needs further clarification as per WS comments Approved in principle as a Class 2 activity, when changes made
Note — The committee agreed that the use of RPE was not required.

Project Containment

Laboratory Activities

Glass Houses

Growth Rooms

02/03/2022
The aims of the project are to delete surface protein genes from S. marcescens to test the theory that their induction reduces the effect of Bdellovibrio predation. Initially we used wild type S. marcescens and Bdellovibrio in serum to detect gene expression changes in S. marcescens during a period of "growback" after predation. This study identified pilus, outer membrane and capsule genes to be critical for avoiding predation. We also have an interest in deleting Type VI secretion machinery. We now want to make direct deletions of these genes in S. marcescens and then test the resulting deletion mutants in a) (commercial) human serum and b) in our zebrafish larval infection model for sensitivity or resistance to predation by Bdellovibrio.

We will also have to construct and test complemented strains of the deletion mutants with the genes returned.

Recipient bacterium into which the gene deletions will be finally delivered is wild type Serratia marcescens which is a clinical isolate with carbapenem resistance. We are removing surface virulence genes in our deletion experiments to...
make the strain less virulent.

Host/vector system

The plasmids being used are Steptomycin and Apramycin (separate) versions of the broad host range conjugable but non-self mobilizable plasmid pKNG1 01. The plasmid is mobilisable by the S17-1 E. coli host. The reference for this plasmid is as follows:


Origin & function

We are deleting individual gene systems for the production of fimbriae which are surface pilus like fibres.
We are deleting individual genes for the production of capsule on the surface of the Serratia.
We are deleting individual genes for outer membrane modification.
We are receiving strains with the Type VI genes (an injection system of Serratia) deleted - produced by Dr Coulthurst.
We will be adding mCherry, mNeon, mCerulean genes to produce coloured bacteria by expression at the deletion site.

Evaluation of foreseeable effects

There will be a reduction in virulence as we are deleting surface gene products that are known to be pathogenicity determinants. They are also surface factors which may ameliorate the interaction with Bdellovibrio bacteria. None of the genes we are inserting have toxic properties. The mNeon, mCherry and mCerulean are non-toxic fluorescent proteins engineered from an original fluorescent protein of jellyfish and routinely used to colour bacteria. The antibiotics on the plasmids Apramycin (is a Kanamycin like antibiotic) and Streptomycin are conventional antibiotics. They are on plasmids that replicate in the S17-1 il-pir donor strains of E. coli but the design of the experiment is that they are on a suicide plasmid in Serratia so when the experiment to construct the strains in complete, the plasmids will be lost and the Serratia will be non-resistant. During the screen for plasmid loss, there will be some antibiotic expression but the hazard is not increased as these are not clinical treatments for Serratia infections.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste run, 3 mins at 134 °C (1.52 Bar) in C floor Med School Sapphire autoclave
Distel is used at 10%

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
* I get the point that the zebra fish are not "animals" for home office licencing purposes but really that section of the RA does need filling in (this is about risk not where they fit in the legislation) and actually a quick google search for this pathogen in fish does indicate it is a pathogen (hence what happens to the water etc they are incubated in does matter and it shouldn't just go down the sink) *

"If autoclaving waste water - a fluid cycle on the autoclave and appropriate additional safety precautions will of course be required. I am sure it is in hand but autoclaving larger volumes of fluids has the potential to go horribly wrong, hence my comment."

### Project Containment

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### Project Ref 470/19.2

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<td>&lt; 1 Litre</td>
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### Historical Significant Changes

- **Withdrawn**: N
- **Date of Significant Change**: 02/03/2022

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02/03/2022

Page 7677 of 15326
### Project Additional Information

#### Purposes of the contained use

Influenza D virus (IDV) was first isolated from pigs with respiratory disease in 2011. However, it was subsequently determined that cattle are likely to be the reservoir host as there is higher prevalence of IDV among cattle in all countries where studies have been conducted. Little is known about the pathobiology of IDV. The virus is potentially involved in respiratory disease complex in cattle; experimental infection of cows causes limited disease. Like influenza A virus (IAV), IDV appears to be capable of infecting a broad range of mammalian species but unlike IAV, there is no evidence that birds are infected. Although antibodies against IDV have been detected in horses and people, virus has only been isolated in nature from pigs and ruminant species. The virus is genetically similar to influenza C virus (ICV). In ICV and IDV; there are 7 rather than 8 gene segments with segment 4 encoding a single surface glycoprotein, the hemagglutinin-esterase-fusion (HEF) glycoprotein, rather than the two surface glycoproteins separately encoded by segments 4 and 6 in IAV. Influenza C virus (ICV), which occurs in people and pigs, is relatively under-studied as it is rarely isolated and usually only associated with mild disease.

The nine known viral proteins expressed by the seven IDV genome segments include the polymerase proteins (P3, PB1 and PB2), which form the RNA-dependent RNA polymerase complex and are expressed by the three largest gene segments (1-3) and the nucleoprotein (NP) expressed by segment 5 (Figure 1). These four proteins are associated with the RNA gene segments in the mature virus particle and are required for virus replication. As previously mentioned, a single surface glycoprotein (HEF) is encoded by segment 4. The HEF glycoprotein possesses receptor binding, receptor destroying and membrane fusion activities. Segment 6 encodes two matrix proteins (M1 and CM2). The M1 protein forms a stabilising layer inside the lipid envelope, which is acquired from the host cell membrane, while the CM2 protein forms ion channels in the envelope. Finally, segment 7 encodes two proteins, the non-structural protein NS1, and NS2. By analogy with the other influenza viruses, it is likely that both proteins play various roles in virus replication. The NS1 protein has been most extensively studied in IAV; it interacts with the host's innate immune system, either up- or down-regulating the production of pro-inflammatory cytokines depending on the virus strain and the host cell type.

Reverse genetics systems to generate infectious virus particles in which a specific gene is modified in order to determine its function were first published for IAV in 1999. The earliest approaches involved transfecting mammalian cells with 12 plasmids — 8 expressing template RNA for each gene segment and 4 expressing the 4 viral proteins required for virus replication (Figure 2). If isolation of effects on the 4 polymerase complex proteins is not required, use of 8 ambisense plasmids to produce viral RNA and protein from the same plasmid is more efficient. Typically, a co-culture of 293T and MDCK cells is transfected as 293T cells have higher transfection efficacy and MDCK cells are more permissive to virus growth. The virus produced is then amplified by culture in either MDCK cells or embryonated hens' eggs. Although ICV is little studied, a reverse genetics system using a similar approach to the IAV system was reported around 10 years ago.

The aim of this project is to take a comparative approach to study the role of the IDV NS1 protein in modulating the innate immune response in cell cultures from different host species.

#### Recipient or parental organism

Plasmids expressing each of the seven gene segments of IDV will be generated by amplifying cDNA from virus isolates held at SVMS or provided by collaborators using primers including a Sapl restriction endonuclease site. The cDNA will be cloned into a plasmid designed for generating recombinant IAV, e.g. pDZ. Plasmids will be amplified in E. coli and transfected into 293T cells (or co-cultured 293T and MDCK cells) to generate recombinant influenza virus (as described above). To amplify recombinant viruses generated, the supernatant from transfected cells will be inoculated onto MDCK cell monolayers or into embryonated hens' eggs. After generating the wild-type virus, segment 7, encoding the NS1 protein will be modified and the effects on virus replication and induction of innate immune responses in cells determined. The importance of the NS1 protein will be tested by introducing a stop codon so that NS1 is not produced (1'NS1). If variation in NS1 proteins is observed in nature, these changes will be introduced to determine whether there is an effect on virus replication of innate immune responses.

#### Host/vector system

E.coli ACDP 1

All bacterial host strains are disabled or non-colonising and are routinely used for research purposes in many laboratories (e.g. TG1, XL1-blue, Novablue DE3, DH10B, HB2151).

E. coli strain genotype

Novablue DE3 endA1 hsdR17(rK12— mK12+) supE44 thi-1 recA1 gyrA96 relA1 lac (DE3)F’[proA+B+ lac! qZ7M15::Tn10] (TetR)

02/03/2022 Page 7678 of 15326
XL1-blue recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F proAB lacIqZAM15 Tn10 (Tetr)].
TG1 supE thi-1 delta (lac-proAB) delta (mcr-hsdSM)5 (F- Km) F' traD36 proAB lacIqZAM15
DH1OB F- mcrA A (mr-hsdRMS-mcrBC) [9801acZAM15 AlaC74 recA1 endA1 araD139 A (ara, leu)7697 galU galK A- rpsL napG /pMON14272 /pMON7124
HB2151 K12, ara, del(lac-pro), thi/F'proA+B+, lacIq, lacZdelM15

Cell lines
- Well established and characterised continuous cell lines will be transiently transfected to generate (293T cells) and replicate (Madin-Darby canine kidney cells) recombinant influenza viruses. These will be routinely handled at containment level 2 in accordance with the ACDP guidelines.

Embryonated hens’ eggs
- Inoculation into embryonated hens’ eggs may be required to generate larger or higher titre stocks of recombinant influenza viruses. Again, these will be routinely handled at containment level 2.

The vectors are non-mobilisable resistance plasmids e.g. pDZ. The pDZ plasmid was derived from the protein expression plasmid pCAGGs. It has a human RNA polymerase I promoter and a mouse terminator sequence that encodes the negative sense genomic RNA. In ambisense, it has a polymerase II transcription cassette consisting of a chicken 3-actin promoter and polyA signal to encode the viral proteins from the same viral gene. The sequence of the genes encoded by each plasmid preparation will be confirmed before recombinant viruses are generated.

Origin & function
- Each plasmid used in generation of recombinant virus contains an influenza virus gene segment under the control of either an RNA or DNA promoter or, in the case of the ambisense system both. The seven gene segments encode viral structural proteins, viral proteins involved in replication of the RNA genome and non-structural proteins as described in section 4. The encoded proteins are not known to be toxic.

Genes;
- Influenza D virus segments 1 to 7
- Ampicillin or kanamycin resistance markers

Evaluation of foreseeable effects
- The inserted genes do not alter any existing traits of the bacterial host. The plasmids used encode resistance to standard antibiotics (ampicillin and kanamycin).
- Influenza D virus segments 1 to 7 will be expressed in transiently transfected mammalian cells. Genes will be expressed from mammalian promoters after transient transfection. None of the genes have any known known properties.
- The recipient micro-organism is not capable of infecting any plant, animal or insect species (see part 1).
- The final GMM (recombinant influenza D virus) is an enveloped RNA virus and therefore unlikely to survive long in the environment, similarly to other influenza viruses, for example influenza A viruses only survive for around 5 minutes on the hands and 24-48 hours on stainless steel or plastic (Weber & Stilianakis, 2008). Therefore, the risk of exposure of susceptible animals to infectious virus in the event of unexpected environmental contamination would be very low.


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation from Schedule 8, Table 1a, containment measure 8, Autoclave required in building.
In line with previously issued derogation against the above containment measure we would like to derogate this work against the requirement to have an autoclave in the building.
All solid medium containing GMM's will be placed in a burn bin and sent for validated incineration as infectious GM waste. These waste procedures are reflected in our local codes of practice and induction training.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All CL2 liquid waste will be autoclaved before final disposal.
Solid medium containing GMMS [1 &2], [e.g. agar plates, bacterial slopes/stabs], cell pellets [in eppendorf tubes] and tissues infected with GM Viruses and other solid waste which cannot be effectively disinfected will be placed in a burn bin and sent for validated incineration as infectious GM waste. This is in accordance with our existing derogation and SVMS local rules.
Contaminated recycleable materials (e.g. glassware) will be autoclaved prior to cleaning and recycling

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<td>Tick to confirm that you have attached a risk assessment to this form</td>
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<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
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Please enter comments on the GM safety committee on the risk assessment
Looks fine to me. 12 plasmid system?! Rather you than me...

No major comments.
One comment though on submission. I wondered if this was effectively an amendment for current reverse genetic work with Al.
I discussed with the school BSO RT and it was felt that it a full document assessment aided clarity for review. Can we discuss in meeting?

The existing connected programme of work specifically excludes influenza reverse genetics as there are and have been significant concerns about "gain of function" experiments in Influenza A viruses. There is an existing Influenza A GM risk assessment but these D viruses are very different in terms of genetics, risk and epidemiology and very much lower concern. We felt it would be a lot simpler keeping these separate than tangling it up with the endless discussion of human and avian pandemic influenza's.
Rachel clarified my immediate concern. I agree with the 12 constructs... insane

5.
Nothing more to add.

6.
No further comments.

7.
Again, no major concerns, but like others I can't understand the 12 plasmid approach (and 4 genes seems to be repeated...). How do you troubleshoot this?! If 'only' 11 plasmids get transfected, how do you work out which is missing?! Is there any reason why the genes all have to be encoded on separate plasmids?

8.
I'm not going to comment on whether this will work or not, especially as I won't be doing it. Though apparently at least one group have got it to work. Safety wise it seems ok.
I've transfected 15 plasmids previously in a screen, would have to be a pretty effective transfection! RA wise is fine.

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**Project Ref** 470/19.3

- **Date Ackn'd**: 30/05/2019
- **CU2 Project Title**: The characterisation of regulatory networks, and identification of genes involved in virulence and biofilm formation, in Acinetobacter spp., Proteus spp. and Klebsiella spp.
- **Class**: 2
- **Culture Vol**: Class 2, ≤ 1 litre
- **Class Culture Vol**: 2
- **Culture Volume**: Class 2, ≤ 1 litre
- **Non-GMM**: Consent Granted
- **Project notified under transitional arrangements**: No
- **Withdrawn**: No
- **Tick if notifying a connected programme of work**: Yes
Bacteria adhere to almost every surface and in the context of healthcare, medical-device biofilm-centred infections pose an enormous threat, particularly from multi-antibiotic resistant super-bugs. Following an initial reversible surface-attachment step, bacteria become irreversibly surface-attached leading to the formation of antibiotic tolerant/resistant biofilms that are almost impossible to eradicate. Consequently, a better understanding of bacterial-surface interactions will aid the discovery and development of novel anti-attachment materials that can be used as coatings on implanted medical devices.

The aim of the project is to understand how Proteus spp., Acinetobacter spp., and Klebsiella spp. sense polymer surfaces, and more generally how biofilm formation, and pathogenesis, is regulated by answering the following questions:

1. How do bacteria regulate their behaviour in response to the polymer surface?

We will study the expression of key master regulators using lux/gfp reporter fusions in combination to DIC and fluorescence video microscopy techniques.

2. What is the role of flagellar rotation, type IV pilus, quorum sensing, two-component systems and cell wall stress in surface sensing?

Strains with mutations in different components of the flagellar apparatus will be created by homologous recombination and biofilm formation and assessed using video microscopy. In addition, we will construct gene fusions and fluorescent fusions.

3. Which other genes/pathways are involved in surface recognition, biofilm formation, and pathogenesis? Screen Tn5 transposon mutant libraries on the biofilm RPs for increased/decrease swarming motility and/or increased/decreased biofilm formation to identify genes involved in surface detection using swarming assays and biofilm formation assays.

Acinetobacter spp. GMM:

Acinetobacter baumanii deletion mutations in targeted genes will be generated by allelic exchange using plasmid vectors which will result in unmarked deletion strains. This will involve conjugation of parental Acinetobacter strains with a conjugation-efficient E. coli strain (32163, that is also a diaminopimelate auxotroph) containing a suicide plasmid that is unable to replicate in Acinetobacter. The plasmid integrates into the chromosome, resulting in Trimethoprim resistant Acinetobacter (occasionally we will use a variant of the plasmid that contains Apramycin resistance). A second plasmid is then conjugated into these Acinetobacter, this confers Tetracycline (or Hygromycin) resistance, and expresses a restriction enzyme that causes a double strand break only in the presence of the suicide plasmid. The resulting deletion mutants will have the second plasmid cured by plating on sucrose, resulting in strains that have no additional resistance cassettes, no replicating plasmids, and that vary from the parental Acinetobacter only by the removal of one gene.

Initial construction will be in E. coli and antibiotic resistance cassettes will be introduced into Acinetobacter mutant strains whilst being constructed. Where possible antibiotic selection used will be for antibiotics not used clinically and will not result in an increase in the hazard classification group, and, where possible, resistance markers
will be removed by use of recombinases or sensitivity enrichment protocols. Mutants will be complemented by introduction of intact copies of genes on plasmids or by insertion into the chromosome. It may be necessary or advantageous to regulate expression of the complementing gene using an inducible or constitutive promoter, and these may be incorporated into the complementation construct. The isogenic mutants and their complemented counterparts will be used in a wide range of phenotypic testing. The impact of gene mutations will also be tested in bioassays such as those to monitor biofilm formation in vitro under Containment Level 2 within the designated laboratory areas in the Centre for Biomolecular Sciences and the Food Sciences building.

Transposon libraries using the mini-Tn5 construct will be used to screen for the genes that are important in virulence factor production (mainly genes involved in biofilm formation, quorum sensing molecules production and phase-variation). This will involve the use of the plasmid pRL27 which contains a Tn5. The plasmid requires the pir gene, and as Acinetobacter do not have this gene the Tn5 is transposed into the chromosome where it is stable due to loss of the tnp gene. The Tn5 is known to randomly insert throughout the genome and confers kanamycin resistance to the Acinetobacter. The resulting strains will be screened for phenotypes of interest (e.g. biofilm, phase variation or quorum sensing inhibition) and the sites of Tn5 insertion sequenced. The resulting mutants of interest will likely have the Tn5 inserted within a gene of interest, thereby disabling the gene.

An A. baumannii strain AB5075-UW transposon mutant library which belong to the three-allele transposon mutant library generated by Gallagher et al. (2015) will also be utilised. The transposon used to disrupt the genes is T26, which contains a TcR gene. Details about the transposon used can be found at: http://www.gs.washington.edu/labs/manoil/transposons/transposons.pdf.

In addition, to study the effect of mutations in target genes in Acinetobacter spp. including A. baumannii we will make gene reporter fusions in both plasmids and in the chromosome, using reporter genes such as luxABCDE, gfp, yfp, mNeonGreen, mRuby3, DsRed, lacZ, phoA etc. The expression of these genes will be monitored in laboratory cultures (A. baumannii only). These experiments will all be performed under controlled conditions in CL2 laboratories. In the worst case scenario, the mutations, fusions, or antibiotic resistance genes are unlikely to increase the level of risk of the resulting strains beyond what is acceptable for hazard group 2.

Considering that A. baumannii is an opportunistic human pathogen and that the isogenic mutants are unlikely to be more virulent than the parent strains, the consequence of human infection by any of these mutants is low, and in the unlikely event that human infection were to occur, the antibiotic therapies currently available would be sufficient treatment. Antibiotics used to treat A. baumannii infections that will not be used for this work include colistin, imipenem, meropenem and tigecycline.

Proteus spp. GMM:
Proteus mirabilis deletion mutants in targeted genes will be generated by allelic exchange using plasmid vectors. Initial construction will be in E. coli and antibiotic resistance cassettes will be introduced into mutant strains while being constructed. Where possible antibiotic selection used will be for antibiotics not used clinically and will not result in an increase in the hazard classification group, and, where possible, resistance markers will be removed by use of recombinases or sensitivity enrichment protocols. Mutants will be complemented by introduction of intact copies of genes on plasmids or by insertion into the chromosome. It may be necessary or advantageous to regulate expression of the complementing gene using an inducible or constitutive promoter, and these may be incorporated into the complementation construct. Transposon libraries using the mini-Tn5 construct will be constructed to screen for the genes that are important in virulence factor production (mainly urease activity and swarming motility). The nature of these transposon mutations are obviously unknown but will include incorporation of a kanamycin resistance cassette.

The isogenic mutants and their complemented counterparts will be used in a wide range of phenotypic testing and also in C. elegans, G. mellonella and eukaryotic cell lines.

The impact of the mutations will also be tested in bioassays such as those to monitor biofilm formation in vitro under Containment Level 2 within the designated laboratory areas in the Centre for Biomolecular Sciences.

In addition, to monitor the expression of target genes in Proteus spp. including Proteus mirabilis we will make gene reporter fusions in both plasmids and in the chromosome, using reporter genes such as luxABCDE, gfp, yfp, mNeonGreen, mRuby3, DsRed, lacZ, phoA etc. The expression of these genes will be monitored in laboratory cultures (P. mirabilis only). These experiments will all be performed under controlled conditions in CL2 laboratories.

Klebsiella spp. GMM: This work will require the production of a range of plasmids that will then be transferred into Klebsiella. The plasmids will be produced and maintained in attenuated (HG1) strains of E. coli.

Klebsiella deletion mutants will be generated by allelic-exchange resulting in unmarked deletion strains. This will involve conjugation of parental Klebsiella strains with a conjugation-efficient E. coli strain ((32163) containing a suicide plasmid that is unable to replicate in Klebsiella. The plasmid integrates onto the chromosome, resulting in Trimethoprim-resistant Klebsiella (occasionally a variant of the plasmid that contains Apramycin resistance will be used). A second plasmid will be conjugated into these Klebsiella, conferring Tetracycline (or Hygromycin) resistance, which expresses a restriction enzyme that causes a double strand break only in the presence of the suicide plasmid. The resulting deletion mutants will have the second plasmid cured by plating on sucrose, resulting in strains that have no additional resistance cassettes.
replicating plasmids, and that vary from the parental Klebsiella only by the removal of one gene. These gene deletion mutants will then be genetically complemented by having a copy of the deleted gene introduced into the chromosome at a non-native site, using a variant of the same plasmid system used for the deletion mutagenesis. The resulting strains will not contain any additional resistance cassettes or replicating plasmids. The inserted genes will be present at single-copy level on the chromosome, and will be under the control of a copy of their native promoter so should be expressed at wild-type levels.

A range of Klebsiella strains that will include fluorescent and luminescent markers (luxABCDE, gfp, yfp, mNeonGreen, mRuby3, DSRed, lacZ, phoA etc.) will be constructed. Some of them will have the fluorescent/luminescent marker genes under the control of a constitutive promoter, while others will have the reporter gene under the control of a promoter from Klebsiella. These will be introduced via the same suicide vector system described above, resulting in strains that contain no additional resistance cassettes or replicating plasmids.

In addition, a transposon system to generate libraries of randomly mutated Klebsiella will be utilised. This will involve the use of the plasmid pRL27 which contains a Tn5. The plasmid requires the piri gene, and as Klebsiella do not have this gene then the Tn5 is transposed into the chromosome where it is stable due to loss of the tnp gene. This Tn5 is known to randomly insert throughout the Klebsiella genome. The Tn5 confers kanamycin resistance to the Klebsiella. The resulting strains will be screened for phenotypes of interest include reduction of biofilm formation and the sites of Tn5 insertion sequenced. The resulting mutants will likely have the Tn5 inserted within a gene of interest, thereby disabling the gene.

For all three genera (Proteus spp., Acinetobacter spp. and Klebsiella spp.) GMM:

It may also be necessary to express some proteins in E. coli (e.g. BL21/DH5a) for purification and biochemical analysis and/or for complementation of in-frame deletion mutants. Wherever possible these will be done with the incorporation of a regulatory system so that the protein is only expressed in the presence of either IPTG or arabinose, two compounds unlikely to be found outside the laboratory. This work will also largely be done in E.coli, but it may be necessary to shuttle these constructs into A. baumannii or K. pneumoniae to examine protein expression. Due to the need for a precise set of environmental requirements (such as the need for IPTG or arabinose induction) and the low chance that these vectors could accidentally shuttle between strains in the laboratory, it is unlikely that protein-expressing strains will increase the virulence of the organism.

It is also proposed that some gene products will be altered, usually by truncation or amino acid substitution, in order to further elucidate the function of the unknown gene product, such as protein binding domains. These alterations are unlikely to result in a gene product that is more hazardous than the original wild-type strain and will most likely result in attenuated function.

Host/vector system

All of the E. coli strains we propose to use (e.g. DH5a, BL21, JM109, (32163 and S17-1) are K12 derivatives which are considered to be non-colonising and non-pathogenic for humans and animals. All of these strains have multiple auxotrophic requirements which are unlikely to be met in the environment where their survival is considered unlikely. All of these strains are considered equivalent to ACDP Hazard Group 1.

A. baumannii, P. mirabilis and K. pneumoniae are ACDP Hazard Group 2 organisms and are recognised as human and animal pathogens and are capable of causing human infections. While infectious, these organisms are opportunistic pathogens and requires a compromised immunity in order to establish an infection.

A. baumannii, P. mirabilis and K. pneumoniae host strains to be used include (but are not limited to) the common laboratory strains A. baumannii AB5075-UW, P. mirabilis Hauser 1885 and K. pneumoniae NCIMB10104.

A range of A. baumannii, P. mirabilis and K. pneumoniae clinical strains that have been isolated from patients will also be used in experiments. As such, they have not been disabled and can cause a variety of diseases including UTIs, lung infections and sepsis. They typically infect the elderly, and patients in intensive care units, rarely causing infection in healthy individuals. Acinetobacter spp. clinical isolates include (but are not limited to) A. baumannii 17978 and 19606, clinical/environmental isolates of Acinetobacter iwoffii, and clinical/environmental isolates of Acinetobacter baylyi. Klebsiella spp. clinical isolates include K. pneumoniae 52.145 (Seq type ST66, Capsule type K2), 43816 (Seq type ST493, Capsule type K2), MGH78578 (Seq type ST38, Capsule type K52), NJST258-1 (Seq type ST258, Capsule type cps-2), clinical/environmental isolates of A. baumannii, K. pneumoniae, K. oxytoca and K. variicola.

The antibiotic sensitivity profiles of these strains will already be known or will be determined before they are used. Specific note for Klebsiella spp.:

Klebsiella isolates are exhibiting rising levels of antibiotic resistance in the clinic, which is why they are of immense importance. However, the primary strain we will focus on, K. pneumoniae 52.145, is sensitive to beta-lactams (including ampicillin and penicillin), carbapenems, tetracyclines, and aminoglycosides. It has been relatively well-studied in the literature for its interactions with host immune cells, and a complete genome sequence is available. It is considered to be highly virulent, as it contains gene for all the known major virulence factors found in Klebsiella species, but it is unlikely to cause disease in healthy individuals.

A variety of vectors may be used in E. coli, Acinetobacter spp., P. mirabilis and/or Klebsiella spp. for the manipulation and creation of novel strains are detailed in section
5.2.2.

The genome insertion plasmids insert into the chromosome of a range of bacteria but the conditions for this to occur are very stringent and unlikely to be met outside of the laboratory or by accident, for example the Tn7 derived plasmids require an accessory plasmid to be co-conjugated in order for insertion to occur. The self-mobilizable vectors require a stringent set of conditions that are unlikely to occur outside of the laboratory. Vectors which are required for the expression of a protein will have a regulatory component (wherever possible) so that protein expression is linked to the presence of either IPTG or arabinose, which are unlikely to be found outside the laboratory.

Transposon libraries have been or will be created using the Gram-negative specific transposon mini-Tn5, which requires unique conditions for transposition and subsequent mutation to occur. These conditions are likely only to be met in laboratory environments and are unlikely to occur in the environment, and spread to environmental bacteria is considered highly unlikely.

All the plasmids carry at least one resistance marker against the following antibiotics: aminoglycosides (kanamycin, gentamicin, streptomycin, spectinomycin, apramycin, hygromycin B), 13-lactams (ampicillin, carbenicillin), chloramphenicol, trimethoprim, tetracycline, and mercuric ions.

Example of plasmid vectors to be used for:

a) Complementation of mutations and biosensors in A. baumannii:

- pMO557M: This is an Acinetobacter shuttle vector, which contains colE1 origin of replication (E.coli), pWH1266 origin of replication (A. baumannii), araC, pBAD promoter and has hygromycin B resistance gene. This vector is low-copy and is non-mobilisable (contains oriT and no tra genes). This vector will be used for fluorescence tagging, for genetic complementation and for promoter fusions. Genes which will be cloned under their native promoter in this vector include the quorum sensing gene abal and csuR (tip of the csu pili, involved in surface attachment), fluorescent proteins for bacterial in vivo tagging (mNeonGreen, mRuby3 or E2-Crimson), and csu promoter in frame with the bioluminescent reporter genes (luxCDABE).

- pUC18-miniTn7-hygromycin B: miniTn7 is contained in a delivery plasmid, pUC18T with CoLE1 origin of replication, OriT, ampicillin resistance cassette and hygromycin-B 4-0-kinase (hph) gene for antibiotic resistance selection. Used for mutant complementation in the chromosome. This plasmid in non-mobilisable and can't transfer without a helper plasmid either as it does not contain the transposable gene. This vector will be used for fluorescence tagging and for the construction of two promoter fusions: briefly the abar gene (quorum sensing transcriptional regulator) will be cloned together with the promoter of the neighbour gene abam (a quorum sensing transcriptional regulator) and the bioluminescent reporter operon (luxCDABE). Alternatively, the abar and abam genes (both quorum sensing transcriptional regulators) will be cloned together with the promoter of the neighbour gene abal (a quorum sensing synthetase) and the bioluminescent reporter operon (luxCDABE) to measure the transcriptional activity of abal. In addition, dslRed express and gfp genes will be cloned in this vector for in vivo tagging.

b) Mutations (Tn5 or in frame deletion), complementation of mutations and biosensors in P. mirabilis:

- pGEMTeasy (Promega): multipurpose cloning vector (Ampr, on, f1 on, lacZ). This vector will be used to express biosynthetic genes involved urease production (ureC) and in flagellation synthesis (fhaA) for complementation of in frame deletion mutants.

- pCVD442 and pDM4: these are a suicide vectors (containing oriR6K, sacB and Ampr for pCVD442 and Cmr for pDM4). These vectors do not replicate in Proteus mirabilis and will be used to generate in frame deletion mutants of the ureC and fhaA biosynthetic genes, resulting in a strain incapable of producing urease or a non-flagellated strain (for pCVD442: see Zunino et al., 2007 https://doi.org/10.1111/j.1574-695X.2007.00285.x).

- pUT-miniTn5: oriT tnp Tn5 [oriR6K, KanR], which results in the insertion of the Tn5 into the genome of Proteus. The vector does not replicate in Proteus and the resulting strain will contain a tnp5 insertion stably maintained in a fixed chromosomal location as the tnp gene will be lost after Tn insertion.

- Plasmids vectors used to generate mutants and complementation of mutants in Klebsiella and Acinetobacter spp.:

- pGP1-Scel: oriR6K TpR mob+ carries I-Scel cut site, which cannot replicate in Klebsiella or Acinetobacter (due to oriR6K).

- pDA1-Scel-SacB: oripBBR1, TetR mob+ carries I-Scel gene, which replicate in Klebsiella and Acinetobacter, but the final mutant strains will not contain the plasmid as they will have been cured via growth on sucrose-containing plates.

- pRL27: oriT TetR tnp Tn5[oriR6K, KanR], which is temperature sensitive and results in the insertion of the Tn5 into the genome of Klebsiella and Acinetobacter. The resulting strains will not contain a replicating plasmid, and the Tn5 insertion is stable in a fixed chromosomal location as the tnp gene (required for movement of the Tn5) is lost after Tn insertion.

d) E.coli molecular cloning vectors for the creation of genetic constructs (e.g. pBlueScript II, pGEM series, pUC series) which are or non-mobilisable plasmid vectors.

All the plasmids carry at least one resistance marker against the following antibiotics: aminoglycosides (kanamycin, gentamicin, streptomycin, spectinomycin), (3-lactams (ampicillin, carbenicillin), chloramphenicol, trimethoprim for Acinetobacter spp. and Proteus spp., or hygromycin, apramycin and trimethoprim for Klebsiella spp.

Origin & function
The nature of this project is to identify novel genes which affect the surface adherence, biofilm formation, pathogenicity and motility of Proteus spp., Acinetobacter spp. and Klebsiella spp. There are likely to be genes which are unknown and in-frame deletion mutants of these genes will need to be complemented with intact copies of the gene. This gene will in most cases be under the control of the native promoter but it may be necessary to put under the control of a constitutive or inducible promoter. The expression vectors used in Proteus spp. will contain chloramphenicol, ampicillin, kanamycin or gentamicin resistance markers, hygromycin-B 4-0-kinase (hph) or ampicillin resistance markers in Acinetobacter spp., and trimethoprim, tetracycline, apramycin or hygromycin in Klebsiella spp.

Non-native genes to be inserted into A. baumannii, P. mirabilis or Klebsiella spp. will include reporter genes that may be used singly or in combination:

- Luciferases (e.g. lux, luc, gluc) which encode bioluminescent proteins.
- Fluorescent proteins (e.g. gfp, mNeonGreen, mRuby3, DSRed, cfp, mTurquoise2, tdTomato, mSapphire or mCerulean).
- lacZ which encodes beta-galactosidase.

These genes do not encode for any toxins, and will not result in increased bacterial growth. These are typically energy-intensive proteins to produce, and if they result in an effect on cell replication/survival rates, they are usually detrimental. All of these marker genes have been well-studied in a variety of organisms including the above-mentioned species.

These genes have a role in virulence and the complementation of mutants of these genes (even if under the control of an inducible or constitutive promoter) is not expected to increase the virulence beyond that of the wild-type. This list is not exhaustive and it is expected that other potential candidates will be included, but again, none of them when complemented should result in a strain with greater virulence than the wild-type.

For Acinetobacter spp., Proteus spp. and Klebsiella spp., genes which will be investigated will code for regulatory proteins involved in cell-to-cell communication (Quorum Sensing) or will code for sensory proteins controlling bacterial cell surface behaviour (swarming and swimming motility, and biofilm formation).

Examples of genes which would be targeted for deletion in Acinetobacter spp. are:

- Abal/Abar/AbAM (Quorum sensing system)
- Two component regulatory systems (OmpR/EnvZ, GacA/GacS, BfmR/BfmS, PilR/PilS, PhoR/PhoQ, AdeR/AdeS)
- Other sensory proteins (KdpD, PilH, PUG, QseB BaR, CopR)
- CsuA-E (chaperone husher protein)
- Diguanylate cyclases and phosphodiesterases
- Structural proteins involved in the formation of fimbriae, exopolysaccharides or polyamines (e.g. 1,3 diaminopropane).

Genes which will be investigated in Proteus spp. will code for cell division proteins, involved in flagella synthesis, virulence factors synthesis (e.g. urease) or will code for sensory proteins controlling bacterial cell surface behaviour (swarming and swimming motility, and biofilm formation).

Examples of genes which we would like to target for deletion in Proteus spp. are:

1. Flagellar genes:
   - fliDC (master regulator switch of flagella motility).
   - motAB (flagellar stator).
2. Che (Chemotaxis) system:
   - MCPs (Methyl Accepting sensory Proteins) (PMI2808; PMI2809).
3. Virulence genes:
   - zapAB (IgA degrading protease).
   - hpmAB (hemolysin). uraABC (urease).
4. Self-identity genes:
   - idsA, idrAB (cell-to-cell communication).
5. Cell division genes: minCDE, ftsZA.
6. Fimbrial genes:
MRP: mrpA PMF: pmfA UCA: ucaA
In Klebsiella spp. a range of genes that we predict to have a role in either biofilm formation or other virulence traits will be investigated. Examples include: polyamine synthesis enzymes; cyclic-di-GMP related proteins: diguanylate cyclases and phosphodiesterases; structural proteins involved in the formation of structures such as fimbriae; and genes for proteins involved in the synthesis and/or transport of exopolysaccharides such as cellulose and poly-glutamic acid. We will also look at the roles of the synthesis of polyamines (such as putrescine and spermidine) by Klebsiella by deletion of the genes encoding enzymes needed for polyamine synthesis.

Evaluation of foreseeable effects

It is anticipated that mutation of the majority of the genes to be studied will reduce rather than enhance the virulence of the host strains. However, mutation of the regulatory genes which may co-ordinately control expression of a number of virulence factors, as it may happen by Tn mutagenesis, has the potential to generate strains which may express elevated levels of some gene products. However, Acinetobacter spp., Proteus spp. and Klebsiella spp. all have several gene regulators which interact to control gene expression and in a number of cases more than one regulator appears to control gene expression of a single gene. Consequently, mutation of a single or even multiple regulators is considered highly unlikely to result in a strain which will have significantly enhanced virulence given the multifactorial nature of the infection process and importance of predisposing factors in most cases of an infection. Introduction of additional antibiotic resistance markers is considered unlikely to affect the therapeutic potential in the unlikely event of laboratory acquired infection or breach in CL2 physical containment.

The reporter genes to be introduced are not considered likely to enhance the virulence of the recipient strains or to affect the therapeutic potential.

The E. coli strains to be used for construct generation and protein expression are considered disabled and are unlikely to survive for significant periods of time outside the laboratory. None of the complementation constructs to be generated is considered likely to enhance the virulence of the E. coli hosts.

Any gene in section 5.3.1 which has been mutated could be deliberately cloned in a broad-range cloning vector (e.g pUC18, section 5.2.2.) and over-expressed in E. coli (DH5a, TOP10 or BL21) for protein function analysis. The resulting strain is expected to be non-virulent and non-pathogenic. It may also be necessary to overexpress some of these genes in Acinetobacter spp, Proteus mirabilis or Klebsiella spp.

The genes to be inserted into A. baumannii, P. mirabilis or K. pneumoniae are largely to complement the deleted gene of interest. They will be, where possible, under the regulation of the native promoter and it is therefore unlikely that such complementation would increase the virulence and pathogenicity of the organism beyond that of the wild-type. Sometimes it may be necessary to have the Gene Of Interest (GOD under the control of an inducible or constitutive promoter of appropriate strength. This may increase the production of a single GOI but in these cases this is considered unlikely to increase the virulence or pathogenicity of the organism beyond the wild-type.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

SOLID WASTE:
Centre for Biomolecular Studies
Solid media used for the culture of microorganisms will be autoclaved before incineration. All contaminated plastic ware will be autoclaved and incinerated in the same manner. All contaminated glassware will be autoclaved and then washed before reuse. Contaminated insect and/or nematode work will be sealed in autoclave bags and then autoclaved. All reusable sealed containment equipment will be decontaminated by autoclaving before being reused. The autoclaves have chart recorders for monitoring load and drain temperatures.
Food Sciences
All solid infectious waste (culture plates, contaminated tissues, gloves etc) will be sterilised by autoclaving (126°C for 15min on a validated cycle) before off-site incineration (via SRCL).
LIQUID WASTE:
Liquid waste will be autoclaved before disposal via the drains.
ANIMAL STUDY WASTE:
Cages will be autoclaved and washed before re-use. Bedding and animal carcasses will be inactivated by autoclaving prior to disposal. Water bottles will be soaked overnight in 1% Virkon solution.

Is an emergency plan required according to regulation 20? \(N\)

If yes, tick to confirm that it is attached to this form \(N\)

Tick to confirm that you have attached a risk assessment to this form \(Y\)

Tick if you are claiming exemption from disclosure for section of the risk assessment \(N\)

Please enter comments on the GM safety committee on the risk assessment
Person A
Can the PI confirm how control of susceptible groups will be managed?
I think the autoclave cycle is 162°C for 10 mins, can this be checked?

Person A
The GM assessment notes that A. baumannii has been designated a red alert human pathogen due to its extensive antibiotics resistance spectrum. Can this information be entered into the BARA.
GMRA Section 5. Can the antibiotics used clinically that will not be used for this work be listed.
GMRA 5.1 The host has been entered as HG3 rather than 2
GMRA assessment refers to the infection of animals but the associated paperwork has not been provided
GMRA autoclave run time needs checking

Person B
The discard autoclave cycle is 126°C for 10 min.
The disabled E. coli hosts should be ACDP HG 1 and the wild type A. baumannii is ACDP HG 2. These need changing on the form.
4. **Person B**

Correction:
The discard autoclave cycle is 126oc for 15 min.

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5. **Person C**

Based on comments and clarifications above I am happy with this.

I see no reason why this is any different in risk form wild type so controls suitable for wit should suitable for the GM.

With additional waste management caveats.

---

6. **Person D**

The Antibiotic resistance profile on the GM RA is indeed a bit confused - which antibiotic resistance genes/plasmids are you putting into these? This information would be needed if someone did manage to infect themselves with it. Its also not clear if you are working with well characterised strains (ie ones in which you know their antibiotic resistance profiles) or field isolates (where you won't know).

Minor point c elegans is not an insect - it is a nematode. I also personally prefer people to give common and latin names for things like your Galleria melona (wax wing moth larvae) as it took me a while to remember what these are.
Person E
It says in part 5 It may also be necessary to express some proteins to further characterise the function of the molecule*. What proteins are they, and what is the consequence of them being expressed if there was an exposure.

Person D
new version generally looks ok to me (it is making more sense combined)

Person F
The RA specifies the containment that will be used to control spread of infection from and between the experimental animals. However I would like clarification as to if the procedures involve imaging. If this is the case the control measures involving the individually Ventilated Cage and Class 1 MSC may not be able to provide the required containment. This is because the optical imaging in the IVIS requires animals to be moved from the MSC to the imaging kit and imaged in the open room outside any filtration controls.
No major issues with this. The GM proposed is either routine TN based random mutagenesis or directed mutagenesis of genes for the species listed. However the actual use of specific vectors is unclear. If there are references for the methods then these could be cited. I am finding hard to define what's being used for what out of the vector list. i.e. pBBR1 is self-mobilisable therefore poses greater risk for transfer but what is it used for?

I am less worried regrading which specific genes are being knocked out as in effect all genes (except essential) are potential KO targets. In section 5.3.2 if anything it overplays the uniqueness of risk from GM inserts in regulators. While this potential exits via simple point mutation which is happening as a natural process in all bacteria. If these KOs were beneficial they would be expected to develop naturally. They are correct in their view that the risk form this is low.

Overall, this looks reasonable and I see no reason why it shouldn't be a class 2 assessment.

Main GMRA
Agree w/ Michael re. clarity over what some of the listed vectors are to be used for.
I'm unclear on the animal usage in the main GMRA. Unless I'm missing it, it's not mentioned until we get to section 8 (control measures) - perhaps some descriptive overview of what's to happen with this earlier in the RA?
Section 8.6 - C. elegans is not an insect and what developmental stage is G. mellonella to be used at? Presumably not as adult moths? Are these actualit required for this work?
Section 11 - mentions mammalian cell lines. Maybe it's me, but haven't seen this mentioned before this. Needs more explanation surrounding this - actually required for this work?
Supplementary GM animal RA
No mention of C. elegans or G. mellonella here. Inconsistent with main GMRA?

Sorry, just noticed that the version uploaded isn't the most up to date version of the GMRA - section 8.6 should state that the G. mellonella is the larvae only. The waxmoth...
and nematode work won't be done in the BSU, but in the lab. In terms of plasmids, Laura is using:
pGP1-Scel (suicide vector) and pDA1-Scel-SacB (used for curing the strains of the integrated pGP1-Scel plasmid) and the transposon carrying plasmid pRL27.

Person C
I have just seen the changes. They help make combinations clearer but now pose the following query.
While the listed insert genes appear low risk...
However I am surprised by the use of self-mobilisable vectors for complementing of genes under native promoters. pBBR1 seem particularly broad host range. Is these and the other self mobile vector routine for the complementation process.
Could this not be in a less mobile vector system for complementation?
The general approach for GM is to reduce risk options in any constructs and one of the min risks here is mobilisation of previously non mobile genes. When I first read the assessment it was unclear what the role of these vectors was?
Is there a non mobile vector that can be substituted.
If not we need to define the genes cloned for complementation more clearly.
As said the TCRs listed probably pose limited risk but the paper work suggest other inserts.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
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Animal Units
Large Scale Activities
Human Clinical Applications

Project Ref  470/21.1

Date Ackn'd  | CU2 Project Title  | Class  | CultureVolClass2  | CultureVolumeClass3-4

02/03/2022  |  |  |  |  |
The overriding scientific goal of this project is to determine the role(s) of individual MERS-CoV proteins in the context of a viral infection in vitro.

Therefore, the major aims of this project are to:
1) Establish the reverse genetic system to create recombinant MERS-CoV
2) Create a library of MERS-CoV mutants for use in experiments to determine the role(s) of individual MERS-CoV proteins in various aspects of MERS-CoV biology
3) Create reporter viruses expressing markers to assay MERS-CoV replication rapidly
4) Use recombinant MERS-CoV in assays (based on preliminary data).

Recipient or parental organism
Recipient organisms are:
1. Disabled commercially available laboratory strains of Escherichia coli (E. coli), such as DH5a, will be chemically transformed to express the plasmids.
2. Common continuous human cell lines, such as Vero E6, HEK293T, HeLa or Huh7 cells.
3. In the final step, Vero E6 cells will be transfected with the full-length MERS-CoV genome and N mRNAs to create virus. There is no evidence that any of these cell lines poses a danger to human health. However, full Class 3 precautions (including use of a Class I MSC) will be used.
4. Cell lines will be infected with MERS-CoV mutants and full Class 3 precautions (including use of a Class I MSC) will be used.

Host/vector system
The segments of the MERS-CoV genome will be cloned into plasmids in order to propagate them in E.coli. Commercially available plasmids, such as pUC19, that contain Ampicillin or Kanamycin resistance along with appropriate restriction sites will be used for this purpose.

Origin & function
The inserted genes will all be wild-type or mutated versions of coronavirus genes.

Coronaviruses have three groups of gene products that will be expressed:
1) Non-structural proteins, named nsp1 – nsp16, synthesised initially as 2 large polyproteins (1a and 1b). These proteins carry out biochemical functions critical for virus replication – such as components of the RNA-dependent-RNA-polymerase that transcribes and copies the viral genome. They also include proteins that induce subcellular rearrangements necessary for virus replication. Some have unknown/unclear functions.

2) Structural proteins. These proteins make up the physical structure of the virion.

3) Accessory proteins. These proteins usually have functions that allow viral survival, such as inhibition of immune signalling pathways. Some have unknown/unclear functions.

These genes have important functions in coronavirus replication, but only when expressed all together in the context of a virus infection.

**Evaluation of foreseeable effects**

Because the MERS-CoV genes all have functions that are required for some (in some cases unknown) aspect of MERS-CoV biology, deleting or modifying a gene is most likely to make the virus less ‘fit’ biologically. Whether that be replication, pathogenicity, host range, tropism, transmission and/or changing the host immune response. Indeed, in some cases, it may be that no infectious virus is recoverable as the gene in wild-type configuration is absolutely essential for virus replication. Under the ‘ideal’ replication conditions of tissue culture, it may be that deletion or modification of some genes has no significant effect on MERS-CoV biology. But, this means the virus is only as dangerous as the wild-type. Also, to reemphasise, I do not intend to mutate MERS-CoV in any way that may be considered ‘gain of function’ – such as swapping MERS-CoV genes for those of any other virus.

The only genes that may be inserted for reporter assays will be GFP, RFP, luciferase or similar. None of these have any known biological effects.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be autoclaved prior to removal from the CL3 suite and disposed of using normal approved disposal routes. E.g. liquid waste will be disposed of in the sink.

Waste container(s) of at least 1:100 Distel (final dilution) will be used for liquid waste, tips and other small items prior to autoclaving.

All containers will be sprayed with 2% Distel for 30 mins prior to removal from the MSC, therefore, the contents will also have at least 30 minutes contact time.

Distel is validated by the manufacturer at a 1:100 dilution for 30 mins against feline coronaviruses and a validation experiment will be conducted to confirm Distell activity against MERS before work begins.

**Is an emergency plan required according to regulation 20?** N

**Tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
See Biological Risk Assessment

Project Containment

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Project Ref 470/22.1

Date Ackn’ed: 05/01/2022

CU2 Project Title: Quantification of coronavirus entry and fusion using a lentivirus reporter system

Class Culture Vol Class 2 Volume Class 3-4

Class 2 < 1 Litre

Consent Granted

Project notified under transitional arrangements

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The aim of this activity is to quantitatively assess the early stages of the coronavirus replication cycle. This technique was previously used in my postdoctoral laboratory (Coleman CM & Sisk JM et al, 2016. Journal of Virology).

Specifically, the project will utilise a lentivirus reporter pseudotyped with viral entry proteins to quantify entry and virion fusion. The core of these pseudoviruses is a lentivirus lacking the entry protein (Env) and most of the accessory proteins. The pseudoviruses are then pseudotyped with a virus entry protein to create single-cycle...
virions. In this case, the pseudoviruses also carry a reporter protein (HIV-1 Vpr fused to β-lactamase, which is packaged into the virion) to allow for quantification of virus fusion.

Ultimately, the aim is to use this system to quantify virus entry and fusion under various experimental conditions, including:

a) In normal cells – both immortalised cell lines and primary human cells
b) In cells with a knockdown of cellular proteins
c) In the presence of drug treatments predicted to block virus entry/ fusion

The main advantage to this approach is that it does not require high containment virus handling, as would be required for working with wild-type viruses, while also allowing for specific quantification of the steps of the virus replication cycle.

Recipient or parental organism

Recipient organisms are:

1. Disabled strains of Escherichia coli (E. coli), such as DH5α, will be chemically transformed to propagate the plasmids. These E. coli are commonly used for this purpose and have all pathogenicity genes removed and so pose no risk to human health.
2. Immortalised eukaryotic cell lines, such as HEK293T, HeLa or Huh7 cells. No cell lines with confirmed productive chronic infections will be used. There is no evidence that any of these cell lines poses a danger to human health. However, as they are derived from human cells, Class II precautions will be taken out of an abundance of caution.
3. Primary human cells. These will be obtained from commercial sources, certified free of any human pathogen. There is no evidence that any of these cell lines poses a danger to human health. However, as they are derived from human cells, Class II precautions will be taken out of an abundance of caution.
4. Immortalised or primary human cells (from 2 and 3 above) that have been knocked-down specific cellular proteins. This will be achieved by transfection of siRNA. If a stable line is required, separate approval will be obtained.

Host/vector system

The viral vector contains:

1. The lentiviral structural proteins and replication incompetent genome
2. A reporter protein that is incorporated into the lentivirus virion
3. An entry protein from another virus that is expressed on the surface of the lentivirus

I will deal with each in turn to describe how the lentivirus is attenuated:

1. The second generation lentiviral packaging vector is based on HIV-1. This plasmid will produce a lentiviral genome and some structural proteins. However, the HIV-1 Env (HIV-1 entry protein) and most of the accessory proteins (that confer pathogenic qualities upon the virus) have been removed and, therefore, is replication incompetent. The plasmid, and resultant genome, contain only the bare minimum to form a virion: HIV-1 Gag/Pol, Tat and Rev only.
2. The reporter protein has no effect, positive or negative, on the safety of the viral vector.
3. The entry proteins from other viruses confer the ability to infect specific cell types upon the viral vector. However, this ability is not genetically inheritable by the vector for 2 reasons: a) this is not provided as part of the lentivirus packaging vector and b) the entry protein plasmids are NOT incorporated into the viral vector in themselves (they lack a lentivirus packaging sequence).

Origin & function

The inserted genes have the following functions:

1. The pCMV-R8.2 has multiple genes that code for proteins involved in HIV-1 virion assembly, integration and expression. These will be expressed in the transfected HEK293T cells and transduced cells (thought the time limitations of the experiment should exclude this).
2. HIV-Vpr-Lactamase is a HIV-1 accessory protein that serves as the reporter for the virion fusion assay. This gene includes a Myc tag. This gene will be expressed only in transfected HEK293T cells.
3. Virus entry protein genes. These genes encode proteins that allow entry of various viruses. This gene will be expressed only in the transfected HEK293T cells.

All of these plasmids also contain an ampicillin resistance gene that confers resistance upon transformed bacteria.

Finally, the pMM310-Myc contains a gene for eukaryotic cell selection – resistance to the antibiotic zeocin.

All genes will be overexpressed in the transfected 293T cells and, indeed, are designed to be overexpressed to ensure efficient vector production.

The plasmids contain the following genes.

pCMV-R8.2, a second generation lentiviral vector (these are also the genes that, theoretically, will be expressed upon transduction by the lentiviral vector):

- HIV-1 Gag/Pol
- HIV-1 Tat
- HIV-1 Rev
- Ampicillin resistance gene

pMM310-Myc:

- HIV-1-Vpr-Blam-Myc
- Ampicillin resistance gene
- Zeocin resistance gene

Viral entry protein plasmids:

- A coronavirus entry protein – either SARS-CoV or MERS-CoV spike. Or another virus entry protein – currently have VSV-G and Ebolavirus GP as controls for different entry pathways
- Ampicillin resistance gene

**Evaluation of foreseeable effects**

The sources of hazard related to alteration of existing traits in this protocol are as follows:

1) Expression of Amp/Kan resistance in E. coli strains during plasmid propagation, making the E. coli resistant to one of these antibiotics.

2) (In some cases) Expression of antibiotic resistance genes in eukaryotic cell lines, making them resistant to said antibiotic.

3) Expression of lentivirus, coronavirus and/or host surface proteins by eukaryotic cells that may alter the structure/function/appearance (in the immunological sense) of the cytoplasmic membrane of the cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

9 Comments
1. No major issues with this - seems thorough, but not my area of expertise. (Should there be separate BARAs in place for the LV system, primary cells etc, or at least the ref number should be cited)?
   o Reply
   o Edit
   o Delete
   o Like
   o 13 Sep, 2021
2. Need to clarify section 8.2 on disinfectant. If Distel is validated disinfectant for the laboratory work and work use then unclear why they need to indicate bleach. As indicated in comments need to confirm validations if used. Otherwise appears OK
   o Reply
   o Edit
   o Delete
   o Like
   o 17 Sep, 2021
3. Generally pretty thorough (and a fairly routine method for this type of work). Only section that really needs tidying up is the disinfectant - Distel is fine (bleach is unnecessary). Probably simpler to just autoclave all GM waste (rather than disinfect chemically and then autoclave - not necessary at CL2)
   o Reply
   o Edit
   o Delete
   o Like
   o 17 Sep, 2021
4. Read, no additional comments
   o Reply
   o Edit
   o Delete
   o Like
   o 20 Sep, 2021
5. Proposed work has been designed to be inherently safe (compared to working with wild type viruses). Measures to attenuate the lentiviruses appear to be thorough. The remaining risks appear to have been thoroughly considered and appropriate measures put in place to minimise them. As with most RAs the waste management procedures will need to be revisited regarding a replacement Distel.

6. The building is not completed, where will this work take place?
   Disposal route needs to be clear that waste will be autoclaved rather than disinfected
   I'd prefer Bleach removed unless there is a good reason for it
   This assessment will require notification to the HSE.

7. no further comments

8. I think this is logically presented and argued, and beyond some of the questions above, I'm happy with it.

9. Have asked PI to address minor comments and return via me for signoff.

---

**Project Containment**

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Project Ref 470/trans1

INTRODUCTION OF A RANGE OF ANTI BIOTIC RESISTANT VECTORS CONTAINING EITHER MODIFIED GENES OR GENES FROM OTHER GROUP 1 AND 2 ORGANISMS INTO YERSINIA PSEUDOTUBERCULOSIS AND YERSINIA ENTEROCOLITICA

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Project Ref 470/trans10

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Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Withdrawn Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

**Project Ref** 470/trans2

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- Non-GMM
- Consent Granted: not applicable

- Project notified under transitional arrangements: Y

**Project Additional Information**

- Date Project Ceased
- Withdrawn: N
- Tick if notifying a connected programme of work: N

- Historical Significant Changes
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 470/trans3

Date Ackn’d 13/02/1994

CU2 Project Title INVESTIGATION OF THE CONTROL OF SECRETED VIRULEMCE DETERMINANTS AND MOBILITY BY QUORUM SENSING IN SERRATIA SPP

Class 2

CultureVolClass2

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Project Ref 470/trans4

Date Ackn’d 13/02/1994

CU2 Project Title

CLONING OF GENES FROM YERSINIA PSEUDOTUBERCULOSIS AND Y ENTEROCOLITICA INTO DISABLED E COLI USING A RANGE OF VECTORS

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 470/trans5

Date Ackn'd: 07/02/1994
CU2 Project Title: INVESTIGATION OF EXPRESSION AND REGULATION OF VIRULENCE DETERMINANT EXPRESSION IN AEROMONAS SPP

Class: Class 2
Non-GMM Consent Granted: not applicable
Project notified under transitional arrangements: Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
# Project Ref

**470/trans6**

## Date Ackn’ed

07/02/1994

## CU2 Project Title

INVESTIGATION OF THE CONTROL OF SECRETED VIRULENCE AND DETERMINANTS AND MOTILITY BY QUORUM SENSING IN AERomonas spp

## Date Project Ceased

## Class CultureVolClass 2 CultureVolumeClass 3-4

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## Non-GMM Consent Granted

not applicable

## Project notified under transitional arrangements

Y

## Withdrawn

N

## Tick if notifying a connected programme of work

N

## Historical Significant Changes

## Historical Date of Additional Info

## Significant Change ID

## Date of Significant Change

## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 470/trans7

Date Ackn'd 07/02/1994

CU2 Project Title INTRODUCTION OF THE QUORUM SENSING LUX REPORTER PLASMID PSB401 AND OTHER PLASMIDS ALSO CONTAINING QUORUM SENSING RELATED GENES INTO KLEBSIELLA PNEUMONIAE

Class 2

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements Y

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 470/trans8

Date Ackn'd 07/02/1994
CU2 Project Title
TRANSFORMATION OF ISOGENIC MUTANTS ANS CHROMOSOMAL FUSIONS TO REPORTER GENES IN HAEMOPHILUS SPP USING TRANSPOSONS AND SUICIDE PLASMIDS

Class 2
Non-GMM not applicable

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
### Project Ref: 470/trans9

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#### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<tr>
<td>L2</td>
<td>L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
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<td>L2</td>
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Project Ref 594/01.1

<table>
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<tr>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>18/02/2005</td>
<td>PATHOGENESIS OF, AND HUMAN IMMUNE RESPONSE TO, ACUTE BACTERIA INFECTIONS: IDENTIFICATION, CLONING, EXPRESSION AND MUTAGENESIS OF GENES FROM CLASS 2 BACTERIA, INCLUDING NEISSERIA</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
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Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

We are interested in studying the role of individual bacterial genes in (a) the pathogenesis of bacterial infection (b) the host immune response to products of these genes, and (c) killing by colicins. The studies are designed to increase our understanding of host-pathogen interaction and develop new preventative and therapeutic methods against invasive bacterial disease. We attempt to detect proteins of interest (usually vaccine candidates or antimicrobials) from sequence data or genomic expression libraries (eg in the phage based x-ZapII) and identify their encoding genes. We then amplify the genes by PCR, clone them in plasmid vectors and express them under the influence of IPTG. These will be used to carry out studies on the characterisation of the molecular, immunochemical and functional properties of the antigens of interest.

Recipient or parental organism

Neisseria: including Neisseria meningitidis, N. gonorrhoea and commensal Neisseria (eg N. lactamica)
Campylobacter: Campylobacter jejuni and C. coli.
Klebsiella: K. pneumoniae ssp and K. oxytoca.

Host/vector system

Hosts:

All are E. coli, derivatives of strain K12: Examples:
JM109 (recA1, endA1, gyrA96, thi, hsdR17 (rK-,mK+), relA1, supE44, (lac-proAB), [F’, traD36, proAB, lacIqZM15].
XL10-Gold (Tetr, (mcrA) 183, (mcrCB-hsdSMR-mrr) 173, endA1, SupE44, thi-1, recA1, gyrA96, relA1, lac hte, [F’proAB lacIqZ M15 Tn10 (Tetr) Amy Camr].
XL-1 Blue (endA1, hsdR17 (rK-, mK+) relA1, supE44, thi-1, gyrA96, (lac-) [F’ proAB lacIqZ M15 Tn1].

Vectors (examples)

1. Plasmid vectors that will be used for studies of protein over-expression from native and inducible promoters (native constructs - pGEM-T, pBr4, pBR322, pUC18/19; inducible vectors - pBAD, pET etc) are considered non-Mobil sable or mobilisation defective. These plasmids will remain in the transformed host and will confer antibiotic resistance genes to the host. Examples of antibiotics to be used are tetracycline, kanamycin, chloramphenicol or ampicillin.

pBluescript: pUC19 derivative, which is non-mobilisable. It contains a colE1 origin of replication, T3 and T7 promoters, and an ampicillin resistance gene. It has an F1
origin that contains an initiator and terminator for M13 DNA replication and the lacZx peptide for blue/white selection.

pGEM: pUC19 derivative, which is non-mobilisable. It contains the coIE1 origin of replicatio, SP6 and T7 promoters, ampicillin resistance marker and the lacZx peptide for blue/white selection.

pREP4: pACYC-184 derivative, which is non-mobilisable. It contains a kanamycin resistance gene and lacIq for constitutive expression of the lac repressor.

pQE30: pDS-56 derivative, which is non-mobilisable. It contains an ampicillin resistance gene and a 6x Histidine tag for Ni/NTA purification of expressed proteins.

PET and pBAD: non-mobilisable expression vectors that contain; ampicillin or kanamycin resistance genes and a 6x Histidine tag for Ni/NTA purification of expressed proteins (pET); or an arabinose inducible promoter for regulated, inducible expression (pBAD).

PCRT7/NT-TOPO and its related plasmids: pUC derivative, which is non-mobilisable. It contains an ampicillin resistance gene and a 6x Histidine tag for Ni/NTA purification of expressed proteins.

2. Phages:

zapII: a based expressed phagemid useful for cloning genomic DNA in the range of 2-10 kb. zapII contains the pBluescript replicon. At the junction between n and the pBluescript DNA sequences there is an initiator and terminator for M13 replication.

Transposons: Tn5 derivatives (eg EZ::TN transposomes from Cambio) which have been constructed to transpose once. Plasmid vectors that will be used for studies of protein over-expression from native and inducible promoters (native constructs - pGEM-T, pBR322, pUC18/19; inducible vectors- pBAD, pET etc) are considered non-Mobilisable or mobilisation defective. These plasmids will remain in the transformed host and will confer antibiotic resistance genes to the host. Examples of antibiotics to be used are tetracycline, kanamycin, chloramphenicol or ampicillin.

Origin & function

Origins of the geneic material are the above mentioned bacterial pathogens. They include:

Neisseria: including Neisseria meningitidis, N. gonorrhoea and commensal Neisseria (eg N. lactamica)
Campylobacter: Campylobacter jejuni and C. coli. Helicobacter pylori
Klebsiella: K. oxytoca and K. pneumoniae ssp.

Evaluation of foreseeable effects

All of the E.coli host strains that we propose to use are K12 derivatives, which are recognised to be non-colonising and are considered to be non-pathogenic to humans or animals. In addition, they have multiple auxotrophic requirements which are unlikely to be met in the environment and so they are assumed to have very limited survivability in the environment.

The pathogens are mostly human commensal and/or opportunistic bacteria. They are common colonisers of skin and mucosal surfaces. Normal healthy individuals, when exposed, usually become transiently colonised with no consequences. However, in susceptible individuals, the pathogenic bacteria can cause invasive disease and in some cases (particularly N. meningitidis) they are capable of causing life threatening diseases.

The plasmid vectors used are all non-mobilisable and are, therefore, unlikely to be transferred to other environmental bacteria. The genes cloned from these bacteria will initially be of unknown function. It is unlikely, however, that any DNA sequence will confer on the host a significant increase in fitness or pathogenicity to the host due to the multiple disablements present in the host and the multifactorial nature of pathogenicity.

The antibiotic cassettes that we propose to use as selectable markers to facilitate inactivation of genes in the bacteria will confer resistance to antibiotics that are not in use.
in either the treatment or prophylaxis of disease. Furthermore, the mutants can be constructed in such a way that the selectable marker can be subsequently removed where appropriate.

The proposed E. coli host strains are all disabled E. coli K12 derivatives are are in the biological agents hazard group 1. Although the introduction of cloned genes might conceivably increase the fitness or survivability of these organisms it is assumed to be very unlikely. However, because of the small potential increase in perceived hazard the recombinant organisms will be treated as biological agents hazard group 2 and, therefore, containment level 2 precautions will be applied. The organisms will, therefore, be provisionally classified as hazard group 2.

All the bacteria included in the study are in the biological agents hazard group 2. After inactivation of the genes of interest, it is highly unlikely (although theoretically possible) that there will be an increase in the virulence or fitness of the organism.

All manipulations of live bacteria (including host E. coli in which genes of interest are overexpressed) will be contained using containment level 2. Genetically modified bacteria and, where appropriate, host E. coli in which genes of interest are overexpressed will be handled in a class 1 cabinet.

The containment precautions to be used are considered sufficient to reduce the risk of accidental infection, or release to the environment, to negligible or zero.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**N/A**

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**N/A**

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All bacteria will be handled and disposed of using rigorous procedures, including autoclave of all cultures and contaminated materials. All genetically modified E. coli will be cultured in volumes of less than 100 ml (except for large scale production in an E. coli expression host) in the Microbiology laboratory. Genetically modified bacteria will always be contained and handled in a class 1 microbiological safety cabinet within a category 2 laboratory. Genetically modified pathogenic bacteria will be cultured in volumes of less than 100 ml in sealed containers that can be autoclaved. All liquid cultures will be transferred in sealed containers to be autoclaved before disposal via the sink. Solid media on which modified bacteria have been cultured will also be transferred in sealed containers to be autoclaved before disposal. All liquid non-recombinant E. coli cultures will be sterilised in a 2% (v/v) phenolic disinfectant (Hycolin) for 24 hours before disposal down the sink waste. Solid media on which genetically modified E. coli have been grown will be autoclaved before disposal. If, in the unlikely event the genetically modified E. coli should escape the risk will still be negligible, as the host E. coli are disabled and unable to colonise a human host or persist in the environment. The risk of escape of genetically modified test pathogens will be negligible because of the level of containment that we propose to use. In the unlikely event of an escape of these bacteria the genetically modified organism is unlikely to survive for more than a very short time in the environment. The risk of transfer of vectors from genetically modified E. coli to environmental organisms is also low due to the non-mobilisable nature of the vectors to be used.

To reduce the likelihood of transmission of GMMs to people in the laboratory, in addition to the precautions detailed above (recombinant test bacteria and, where appropriate, recombinant E. coli to be contained and handled in class 1 cabinet) laboratory personnel will wear gloves to reduce the likelihood of contamination of hands. All waste produced while handling genetically modified test bacteria will be transferred immediately by the person handling the organism to be autoclaved. Contaminated laboratory coats will also be autoclaved.

These measures are considered to reduce the likelihood of any potential hazard effectively to zero.
The risk assessment were discussed thoroughly, and passed, at the appropriate committee.

Project Containment

<table>
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<tr>
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</tr>
</thead>
<tbody>
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Animal Units

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Project Ref 594/02.1

Date Ackn'd: 18/02/2005

CU2 Project Title: THE REGULATION OF CD95 (FAS) EXPRESSION BY MEMBRANE TRAFFICKING

Class: Class 2

Consent Granted: Not Applicable

Class Culture Vol Class 2 Culture Volume Class 3-4

< 1 Litre

Non-GMM

Project notified under transitional arrangements: N

Historical Significant Changes: GM94/02.1 TRANSFERRED TO GM470 ON 18/02/2005
### Project Additional Information

#### Purposes of the contained use

CD95 is a cell surface protein that transduces apoptotic signals critical to B cell development. Loss of CD95 function may also be important in the genesis of B cell lymphoma. We have made the novel observation that expression of CD95 in certain B cell lymphomas appears to be regulated by membrane trafficking (1). This project will investigate the mechanisms controlling trafficking of the CD95 molecule. The project is based on the creation of cell lines expressing tagged recombinant wild type or mutated CD95. Retroviral vectors are the most efficient tools for creating such cell lines.

#### Recipient or parental organism

The commercially available retroviral packaging cell line PT67 (expressing viral proteins gag, pol and env in trans) will initially be used to facilitate the production of replication incompetent retrovirus that can be used to introduce CD95 into other mammalian cells lines which will be used for trafficking studies.

Recombinant virus will be used to transduce the Burkitt's lymphoma - like cell lines Mutu I and Mutu III will also be transferred into immortalised (virus-negative) human breast cancer cell lines MCF-7.

Future studies may require introduction of constructs into additional, known virus-negative human cell lines.

All cell lines to be used are considered to be equivalent to ACDP hazard Group I and are routinely handled using Class I containment precautions.

#### Host/vector system

pRevTet off and pRevTreat are commercially available retroviral vectors derived from moloney murine leukaemia virus and moloney murine sarcoma viruses respectively. The expression of cloned inserts within pRevTet off is tightly controlled using tetracycline. Both retroviruses are defective, that is they lack the genes necessary (gag, pol and env) to undergo a full found of replication outside their packaging cell line PT67N.

The cell line PT67N provides the genes in trans that are missing in the retroviral vector. This is a commercially available standard packaging cell line.

Virus containing the cloned insert is produced transiently in the packaging cell line and used to transduce the target cell lines Mutu I, Mutu III or MCF-7.

#### Origin & function

Human CD95 is readily available as a cloned reagent from investigators. A CD95/CFP (cyan fluorescent protein) fusion protein will be generated as well as fusion proteins containing mutations within CD95 itself. These constructs will be cloned into the pRevTet off vector under the tight control of the tetracycline operator and used to transduce the target cell lines. Trafficking of the wild type fusion protein and the effect of mutations on trafficking will then be observed.

#### Evaluation of foreseeable effects

Modification of the retroviral packaging cell line PT67 or the Burkitt's lymphoma - like cell lines Mutu I and Mutu III by introduction of recombinant CD95 is considered highly unlikely to affect the ability of these cell lines to establish themselves in a mammalian host. These GMOs are therefore considered to pose no significant risk to human or animal health or the environment.

The effects of expressing mutant forms of CD95 in cells already expressing wild type protein are unknown, however, the possibility of generating a trans-dominant mutant within an amphotropic retrovirus must not be discounted. CD95 is not an oncogene but deletion of CD95 has been linked to development of lymphoma. This situation could potentially arise if workers were accidentally exposed to packaged recombinant retrovirus and the virus became integrated into mammalian cells. This likelihood will be minimised by use of a Class 1 Microbiological safety cabinet for all manipulations involving packaged recombinant retrovirus and derivation of stable cell lines. The risk from accidental exposure of workers to recombinant retrovirus is also likely to be further reduced by the nature of the defective retrovirus which dictates that replication is only possible within the packaging cell. Once integrated into mammalian cells the retrovirus is unable to replicate and produce further virus.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Once the cell lines have been established they are considered to pose no threat to human or animal health or the environment as the defective retrovirus can not undergo replication and viral particles are not produced. It is therefore requested that to make effective use of these reagents, once the cell lines have been established they are classified as equivalent to ACDP hazard Group 1 organisms allowing their use under containment procedures, appropriate for Class 1 activities.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste will be autoclaved to achieve a 100% kill. Autoclave waste will then be removed from the site by licensed disposal contractors prior to disposal to landfill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The initial assessment was modified to recognise the potential risk to human health of packaged retrovirus to be used for transfection into cell lines for trafficking studies and this part of the work was considered to require the additional containment precautions of a Class 2 activity plus the use of a Class 1 microbiological safety cabinet.

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Project Ref 594/03.1

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<td>18/02/2005</td>
<td>GENERATION OF PSEUDOTYPE VIRUSES CARRYING HEPATITIS C VIRUS OR</td>
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<td>1-50 Litres</td>
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02/03/2022
The aim of this project is to develop a single cycle infection system to study the role of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) glycoproteins in cell attachment and entry. The unavailability of an efficient culture system for HCV has restricted the study of the mechanisms of HCV infection and development of effective antiviral agents. Several research groups have developed systems to generate infectious pseudoparticles displaying heterologous viral glycoproteins in their functional form onto retroviral core particles. Recently, Bartosch et al. (2003) described such a system for producing pseudotype particles carrying functional HCV glycoproteins on their surface.

The pseudotype retrovirus systems utilise replication-defective packaging and transfer vectors. The transfer vectors containing the transgene are rendered replication-defective by inactivating critical genes involved in viral replication (e.g., the env, nef and vpr genes), which still retain the viral cis-acting sequences such as LTRs, packaging sequences and regions involved in reverse transcription. The transfer vectors are propagated by trans-complementing constructs (packaging vectors) which supply in trans the packaging proteins whose genes have been deleted in the transfer vector. The packaging vectors are also replication defective as they lack cis-acting sequences such as LTRs, packaging sequences, some structural genes (e.g., gag and pol) and regions involved in reverse transcription. The trans-complementing factors on the packaging vectors can be segregated and supplied as two separate complementing genomes, the first one for the Gag-pol proteins and the second one for the viral glycoproteins. For the generation of pseudotype particles, the glycoproteins derived from the virus of interest are used.

The project will utilise existing retroviral transfer/packaging vectors and vectors encoding HCV or HIV glycoproteins supplied in trans to produce infectious replication-competent pseudotype viruses. Three packaging/backbone vectors will be used: two derived from HIV and the other from murine leukaemia virus (MLV). Three systems will be used because they have been shown to have different efficiencies in different cell types.

Recipient or parental organism

HCV pseudotype particles generated in mammalian cells will carry recombinant RNA genome and are capable of infecting human (and possible animal) cells. The particles are likely to be hepatotropic due to the presence of HCV glycoproteins on their surface and could infect the target cells if accidentally injected into humans. Alternative routes of transmission are unlikely to be successful since the HCV envelope proteins will determine the tropism, and route of entry. Upon cell entry and uncoating the genome will be transcribed and proteins synthesized. However, the packaged RNA genome is incapable of replication and therefore no virus progeny will be produced.

HIV pseudotype particles generated in mammalian cells will carry recombinant RNA genome and are capable of infecting human (and possible animal) cells. The HIV envelope proteins will therefore confer the ability to infect cells expressing CD4 plus one of the known co-receptor molecules (e.g., CCR5 and CXCR4), which are
predominantly, but not exclusively, expressed on cells of the immune system. The particles could therefore infect the target cells if accidentally injected into humans. Upon cell entry and uncoating the genome will be transcribed and proteins synthesized. However, the packaged RNA genome is incapable of replication and therefore no virus progeny will be produced.

Host/vector system

1st generation HIV vector (pNL4-3.Luc.R-.E-): HIV is a category 3 human pathogen that is predominantly transmitted via sexual contact, vertical transmission (from infected mother to infant) and also via contaminated blood and blood products (eg needlestick injury). The transfer vector, pNL4-3.Luc.R-.E, is a disabled (ie replication-incompetent) retroviral vector carrying the provirus sequences derived from HIV isolate NL4-3. The construct has been rendered replication defective by introducing two frameshift mutations into the vpr and env genes. In addition, a reporter gene has been introduced into the nef gene, rendering the vector nef. Two versions of the vector will be used. One has the reporter gene firefly luciferase (lux), whilst the other contains green fluorescent protein (GFP). This vector retains all the cis-acting elements (such as LTRs, packaging sequences and regions involved in reverse transcription) and encodes functional Gag-pol proteins necessary for particle assembly and secretion. In addition, it also encodes all the remaining viral proteins.

The pseudotype virus particles will be recovered from the vector backbone by co-transfecting mammalian cell lines with vector expressing CMV promoter-driven HCV envelope proteins E1 and E2 or HIV gp120/41 (see below). These pseudotype particles will package the original defective backbone pNL4-3-derived RNA.

2nd Generation HIV packaging/reporter vectors (Packaging vector pCMV D R8.91, lentiviral reporter vector pWPT-GFP: pCMV D R8.91 is a disabled (ie replication-incompetent) HIV-1 retroviral vector that has been rendered replication defective by removing the accessory genes vpr, vif, vpu and nef as well as the env gene. This acts as a source of reverse transcriptase and gag proteins, which assemble and package transcripts produced from the pWPT-GFP vector. This vector is the only genetic material transferred to the target cells. It comprises the transgene cassette (GFP) flanked by cis-acting elements necessary for its encapsidation, reverse transcription and integration. The vector is a self-inactivating (SIN) HIV-1-derived vector, which loses the transcriptional capacity of the viral long terminal repeat (LTR) once transferred to target cells. This minimises the risk of emergence of replication competent recombinants (RCR) and avoids problems linked to promoter interference. The post-transcriptional regulatory element of woodchuck hepatitis virus has been inserted to enhance transgene expression.

MLV (MLV-CMC pr-gag-pol and MLV-CMVpr-GFP): MLV is an amphotropic virus but is non-pathogenic to humans. Vectors derived from MLV are frequently used in gene transfer/therapy experiments. Here we propose to use a three vectors systems for generating HCV pseudotype particles. The three vectors are: the MLV-CMV/pr-gag-pol packaging vector (which serves as a source of retroviral packaging proteins and is transcribed via a CMV promoter), a transfer vector, pMLV-CMVpr-GFP (carrying CMV promoter-driven GFP transcriptional unit flanked by MLV LTRs and a packaging signal), and a plasmid expressing HCV glycoproteins (see below). Complete pseudotype particles will be produced from mammalian cells co-transfected with the three plasmids.

Vectors containing the HCV glycoprotein (envelope) genes: The HCV envelope genes E1 and E2 derived from various genotypes have been amplified via PCR and directly cloned into pcDNA3 based vectors that contain the CMV early promoter to facilitate mammalian protein expression.

E.coli host. The vectors will be maintain in the TOP10F strain of E. coli (F mcrA [mrr-hsdRMS-mcrBC], 80lacZ M15, lacX74, deoR, recA1, araD139 [ara-leu]7697, galU, galK, rpsL, endA1, nupG) or other K-12 derived laboratory strains that are non-colonising and disabled. The survival of these bacteria in the environment is expected to be low as they have multiple auxotrophic requirements that are unlikely to be met outside of laboratory culture: the recA1 disablement of TOP10F means that it is unable to repair DNA by homologous recombination and is UV sensitive, and the [ara-leu] 7697 mutation shows that it is auxotrophic for leucine, and unable to grow in the presence of arabinose.

Origin & function

pNL4-3.Luc.R-.E-: The transfer vector, pNL4-3.Luc.R-.E, is a disabled (ie replication-incompetent) retroviral vector carrying the provirus sequences derived from HIV isolate NL4-3. The construct has been rendered replication defective by introducing two frameshift mutations into the vpr and env genes. In addition, a reporter gene has been introduced into the nef gene, rendering the vector nef. The original pNL4-3 clone was derived from NY5 (5’) and LAV (3’) cloned directly from genomic DNA into pUC18.
This vector retains all the cis-acting elements (such as LTRs, packaging sequences and regions involved in reverse transcription) and encodes functional Gag-pol proteins necessary for particle assembly and secretion. In addition, it also encodes all the remaining viral proteins, apart from vpr, as well as a reporter gene. Production of pseudotype viruses occurs when this plasmid is co-transfected with the viral glycoprotein-carrying pcDNA3 vectors.

MLV-CMVpr-gag-pol: The MLV-CMVpr-gag-pol packaging vector serves as a source of retroviral packaging proteins and is transcribed via a CMV promoter. Production of pseudotype viruses occurs when this plasmid is co-transfected with the viral glycoprotein-carrying pcDNA3 and the MLV-CMVpr-GFP vectors.

MLV-CMVpr-GFP: The MLV-CMVpr-GFP vector (carrying CMV promoter-driven GFP transcriptional unit flanked by MLV LTRs and a packaging signal), acts as a transfer vector. Production of pseudotype viruses occurs when this plasmid is co-transfected with the viral glycoprotein-carrying pcDNA3 and the MLV-CMVpr-gag-pol packaging vector.

Vectors containing the HCV glycoprotein (envelope) genes: The HCV envelope genes E1 and E2 derived from various genotypes have been amplified via PCR and directly cloned into pcDNA3 based vectors that contain the CMV early promoter to facilitate mammalian protein expression. This vector acts as a source of viral glycoproteins for pseudotype formation.

Evaluation of foreseeable effects

The vectors may be able to undergo recombination with endogenous retroviruses present in the culture cells (or accidentally infected host), or package transcripts from endogenous retroviral sequences. Also, the vectors could undergo recombination with wild-type virus. However, the resulting recombinant would not be more pathogenic than the wild-type virus.

Pseudotype viruses could in principle cause infection, but would not replicate. The potential target cell would be dictated by the envelope proteins incorporated into the pseudotype virus (ie HCV envelope proteins). Infection by pseudotype virus would result in reverse transcription then possible integration of the packaged genome. This in turn might lead to the production of viral/reporter proteins, but as the genome is defective, new infectious virus would not be produced. The effect of GFP or luciferase expression on the host cell is unknown, whilst some of the HIV gene products have been shown to have some detrimental effects (eg apoptosis, toxicity etc), at least in vitro. Whilst, cells producing viral proteins would be targeted by the host immune response (eg cytotoxic T-cells), it cannot be assumed that exposure to the GMM will not cause harm.

Containment and control measures for GMOs that are not microorganisms (eg GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

HCV E1E2 pseudotypes produced using the pNL4-3 system: The parental virus for the pNL4-3 vector is a category III organism. Infection by the pseudotype virus would lead to the production of viral proteins and reporter proteins. The pathogenic potential of these proteins is largely unknown, although some effects have been reported in in vitro studies. Whilst the resulting pseudotype virus produced is infectious, it is not able to replicate. Infection could lead to insertion of the packaged genome into the host chromosome. However, it lacks known transforming sequences. The risk of recombination between the HCV E1E2 containing vector and the pNL4-3 is low. As the inactivating mutations within the pNL4 backbone are small deletions there is a small risk of reversion and subsequent production of wild type virus. However, the cell lines used to produce the pseudoviruses are incapable of supporting HIV replication. Even so, the viruses are infectious and are capable of integration and subsequent protein production. There is a small but real risk of the mobilisation of endogenous retroviral sequences and the formation of replicative competent retroviruses. Therefore we propose that the containment level for this aspect of work is Level 2, with the following additional precautions.

All work involving the pseudotype viruses to be carried out within a Class 1 or Class II safety cabinet located in a room where access is restricted at times when manipulations involving these viruses are taking place. The work will involve standard laboratory procedures, however, considering the routes of infection of the viruses (parenteral), no sharps or glassware will be used when handling the pseudotype viruses. All solid waste materials will be autoclaved and liquid waste disinfected with gluteraldehyde. Training records for all new staff using these methods will be kept.

HCV and HIV pseudotypes produced using the 2nd Generation HIV packaging/reporter vectors (Packaging vector pCMV DR8.91, lentiviral reporter vector pWPT-GFP):
pCMV D R8.91 is a disabled (i.e. replication-incompetent) HIV-1 retroviral vector that has been rendered replication defective by removing the accessory genes vpr, vif, vpu and nef as well as the env gene. This acts as a source of reverse transcriptase and gag proteins, which assemble and package transcripts produced from the pWPT-GFP vector. This vector is the only genetic material transferred to the target cells. It comprises the transgene cassette (GFP) flanked by cis-acting elements necessary for its encapsidation, reverse transcription and integration. The vector is a self-inactivating (SIN) HIV-1-derived vector, which lose the transcriptional capacity of the viral long terminal repeat (LTR) once transferred to target cells. This minimises the risk of emergence of replication competent recombinants (RCR) and avoids problems linked to promoter interference. The post-transcriptional regulatory element of woodchuck hepatitis virus has been inserted to enhance transgene expression. Complete retroviral particles are produced in the presence of a third vector containing the viral glycoproteins under study. Infection of actively replicating cells allows reverse transcription of the RNA genome and subsequent integration of the GFP marker protein. Upon accidental transmission, the transcribed GFP cDNA may become integrated into recipient cells. The transfected cells will contain viral genes on split plasmids. Details of the constructs and the demonstration that they do not give rise to replication competent viruses are available in Zufferey et al Nat Biotechnol. 15: 871-875. There is evidence that such replication defective retroviruses are not pathogenic when tested in mice. Therefore we propose that the containment level for this aspect of work is Level 2, with the following additional precautions:

All work involving the pseudotype viruses to be carried out within a Class 1 or Class II safety cabinet located in a room where access is restricted at times when manipulations involving these viruses are taking place. The work will involve standard laboratory procedures, however, considering the routes of infection of the viruses (parenteral), no sharps or glassware will be used when handling the pseudotype viruses. All solid waste materials will be autoclaved and liquid waste disinfected with gluteraldehyde. Training records for all new staff using these methods will be kept.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2: All work involving the pseudotype viruses to be carried out within a Class 1 or safety cabinet located in a room where access is restricted at times when manipulations involving these viruses are taking place. All solid waste materials will be autoclaved at 134-137 degrees C, 21.-2.2 bar pressure with a holding time of 5 minutes. A chart record is maintained for each run. Autoclaves are also subject to quarterly thermocouple testing. All liquid waste known to be, or potentially infected with category 2 viruses will be discarded into a 500 ml polyethylene discard pot containing 50 ml of 100% Gigasept, so that when full with liquid waste the final concentration of Gigasept will be 10%. An expiry date for the gigasept, which should be two weeks from the date of first use, will be written on the surface of the discard pot. If the discard pot is not full before this expiry date then the discard pot will be filled to 500 ml with water and left for 24 hours to inactivate virus. Preparation of working dilutions of Gigasept will be carried out within the class 1 cabinet.

Containment level 3: All liquid waste known to be, or potentially infected with category 3 viruses will be discarded into a 500 ml polyethylene discard pot containing 50 ml of Gigasept, so that when full with liquid waste the final concentration of Gigasept will be 10%. An expiry date for the gigasept, which should be two weeks from the date of first use, will be written on the surface of the discard pot. If the discard pot is not full before this expiry date then the discard pot will be filled to 500 ml with water and left for 24 hours to inactivate virus. Preparation work working dilutions of Gigasept will be carried out within the class 1 cabinet. All solid waste will be autoclaved prior to disposal. Autoclaving of waste will take place using a destructive cycle of 126 degrees C for 45 minutes. The maximum load will be one autoclave bag (containing a maximum of 2 laboratory gowns) together with 4 discard pots. The autoclave is checked on a 6 monthly basis by independent thermocouple testing using typical loads. Cabinet generated solid waste will be accumulated in paper bags placed within the cabinet. At the end of each working period the waste will be placed into an autoclave bag and transferred to the autoclave. Solid waste bags will be left open and screw-capped lids loosened prior to autoclaving to allow steam penetration. Discard of all Gigasept solutions will take place using the designated sink within the main virology laboratory.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
RA approved by the local GMSC on 7 July 2003.

Project Containment

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<th>Growth Rooms</th>
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<td>Human Clinical Applications</td>
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Project Ref 66/00.1

Date Ackn'd 18/02/2005

CU2 Project Title

Studying gene expression, growth, quorum sensing and motility in Pseudomonas aeruginosa

Class 2

Culture Vol Class 2

< 1 Litre

Consent Granted

Not Applicable

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Project Transferred from GM66 to GM470 on 18/02/2005

Project notified under transitional arrangements Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

#### Project Containment

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Project Ref 66/01.1

CONVERSION OF FIBROBLASTS TO MYOBLASTS USING RETROVIRAL DELIVERED MYO D

Date Ackn’d 18/02/2005

Date Project Ceased

CU2 Project Title

Class Consented to

Class 2

Consent Granted

Not Applicable

Tick if notifying a connected programme of work N

Withdrawn N

Historical Significant Changes

GM66/01.1 Transferred to GM470 on 18/02/2005

Project notified under transitional arrangements Y

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Large Scale Activities

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Human Clinical Applications

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Project Ref 66/04.1

Date Ackn'd 18/02/2005

CU2 Project Title INVESTIGATION OF MOLECULAR BASIS OF ASEXUALITY/SEXUALITY IN ASPERGILLUS FUMIGATUS

Date Project Ceased

Class 2 CultureVol 2 Class 3-4 < 1 Litre

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work N

Historical Significant Changes GM66/04.1 transferred to GM470 on 18/02/2005

Historical Date of Additional Info

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The project aims to investigate whether the filamentous fungus *Aspergillus fumigatus* has the potential to undergo sexual reproduction. Recent genome analysis has revealed the presence of a series of genes involved with sexual reproduction, some of which are known to be expressed. However, the fungus is only known to reproduce by asexual means. Resolving this question is of major significance to understanding the population biology of this species in the wild and related management of the fungus. Insights into the sexual potential of the species might also allow exploitation of the sexual cycle in classical genetic studies to study inheritance of traits such as fungicide sensitivity/resistance or pathogenicity determinants.

There are two broad areas in which recombinant gene technologies will be used.

A) Cloning of putative genes involved in sexual development from *A. fumigatus* into E. coli hosts and vectors. This comprises genes regulating sexual compatibility, genes involved in sexual pathway signalling and genes involved with fruit body development.

B) Transformation of fungal genes involved in sexual development into fungal hosts. This encompasses:

- Transformation of putative sexual genes from *A. fumigatus* into class 1 ADCP host strains which lack the ability to undergo sexual reproduction as a result of previous gene disruption. This will allow complementation studies to assay the functionality of the *A. fumigatus* genes.
- Transformation of known functional sexual genes from class 1 ADCP host strains into *A. fumigatus*. These genes will be either under the control of native, constitutive or inducible promoters. Such studies may result in enhancement/restoration of sexual development in *A. fumigatus*, allowing possible exploitation of the sexual cycle.

Such studies have already been performed with sexual and asexual relatives of the plant pathogens *Cochliobolus* and *Bipolaris* species in the USA.

**Recipient or parental organism**

**Fungal strains.** *Aspergillus fumigatus* is a common environmental species, widespread in soil and in decomposing vegetation. It is not normally a risk to healthy persons. However, it occasionally causes aspergillosis, an invasive disease of the lungs and other organs, upon opportunistic infection of immunocompromised patients. It may also cause opportunistic infections of patients with existing medical disorders. The spores may in addition cause allergic reactions in certain patients. Thus the fungus is classified as ADCP class 2 (a potentially pathogenic microbe). More detail available in risk assessment.

**Bacterial strains.** The E.coli strains to be used are all Rec A-derivatives such as DH5a, XL1-Blue, SURE and TOP10. These strains are disabled and non-colonising and are equivalent of ADCP category 1 organisms (ie non pathogenic to humans or animals). They have limited survivability in the environment as they require specific nutrients supplements not required by wild-type organisms.

**Host/vector system**

**Fungal vectors.** These are pUC and pBR322-derived plasmids that will be maintained in E. coli host before transfer to a fungal host organism. Some plasmids will have fungal promoters that are either constitutive or induced under certain environmental conditions. Vectors are integrated into the fungal genome and considered non-mobilisable or mobilisation defective.

**Bacterial vectors.** These are pUC derivatives encoding ampicillin, zeomycin or other antibiotic resistance markers. Considered to be non-mobilisable.

**Origin & function**

Inserts are genes involved with sexual development in filamentous fungi. These may be categorised into three main areas:

a) Genes involved with determination of sexual compatibility. Examples include mating-type (MAT) genes determining sexual identity, pheromone precursor genes involved...
with production of short chain amino-acid pheromones; pheromone processing genes involved with enzymatic modification of pheromone precursor molecules.

B) Genes involved with sexual signalling pathways. Examples include pheromone receptor genes encoding transmembrane surface receptors, a series of MAP kinase elements involved with signal transduction, and final transcription factors activating sexual development.

C) Genes involved with fruit body development. These include genes encoding hormonal factors regulating hyphal morphogenesis, and transcriptional factors altering developmental pathways.

These genes are not virulence determinants so unlikely to alter the pathogenicity of the host organism. The antigenicity of these proteins is not known. Previous work with similar genes involved with sexual development in fungi has not revealed any risk, so the protein products are considered unlikely to pose a toxic hazard.

**Evaluation of foreseeable effects**

A fumigatus is classified as an ACDP class 2 organism (a microbe potentially capable of causing human disease). It is a common environmental fungus associated with decomposition of organic matter in soil and vegetation. It is not normally a risk to healthy persons. However, it occasionally causes aspergillosis, an invasive disease of the lungs and other organs, upon opportunistic infection of immunocompromised patients. It may also cause opportunistic infections of patients with existing medical disorders. The spores may in addition cause allergic reactions in certain patients.

The potentially most hazardous GMMs to be constructed in this work are the transformed strains of the ACDP class 2 fungus Aspergillus fumigatus. However, it should be stressed that these will be transformed with genes involved in sexual reproduction, which themselves have no known link to virulence or pathogenicity. They are not involved with toxin production, so unlikely to alter the pathogenicity of the host organism. Also the strains to be transformed will be pre-screened to ensure that they are sensitive to the main antifungals (Amphotericin B anditraconazole) used in clinical treatments.

The genes under study, those involved with sexual reproduction in ascomycete fungi, are widespread in numerous ascomycete fungi in the wild with the same functional gene families present in many harmless fungi. Thus potential transfer of such genes is not considered a risk, as it is stressed again that the genes under study do not related directly to the pathogenic potential of A. fumigatus.

There is the potential hazard that resultant GMM strains of A. fumigatus may have enhanced sexual fertility, which may be spread into wild-type strains of A. fumigatus through sexual recombination, promoting gene flow within the species. This might conceivably include flow of genes linked to virulence. However, there is no known occurrence of a sexual cycle of A. fumigatus in the wild and it is thought that A. fumigatus reproceses only by asexual means. Thus, if sex does occur in the wild it is at likely to be of very low occurrence if at all. Therefore there is low risk of spread of genes promoting sexual fertility. The hazard of spread of antibiotic resistance genes, used as selective markers, is thus also considered low risk.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste and plasticware exposed to GM material will be autoclaved prior to disposal.
Liquid waste and glassware exposed to GM material will be autoclaved prior to disposal/wash up.
Class 2 autoclave disposal facility available in same lab suite.

2% trigene will be used to swab benches and disinfect as required. It has been shown by the manufacturer to be effective for control of filamentous fungus and is used in accordance with their instructions.
Standard procedure for spillage/breach of containment involves absorbing onto paper towels followed by cleaning of area with disinfectant (2% Trigene). Paper towels then sent for autoclave.
The local GM committee approved the assessment of Class 2, subject to inclusion of some minor additions to the assessment which have been made. There were concerns raised about health of workers in respect of anyone who may be immuno-compromised or have other allergy/lung disorders. The UoN Occupational health physician was consulted and has advised that staff who are working on the projects will complete a health screening questionnaire and be approved as fit for the work by OH Department.

In addition the assessment was submitted to 4 other scientists for review:
Dr Alastair Chambers, School of Biology, University of Nottingham
Dr Marcos Alcocer, School of Biosciences, University of Nottingham, Loughborough, Leicestershire
Dr M Dickinson, School of Biosciences, University of Nottingham, Loughborough, Leicestershire
Dr S Avery, School of Biology, University of Nottingham, University Park, Nottingham

All supported assignment to Class 2 and any comments made were incorporated into the assessment.

**Project Containment**

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**Project Ref** 738/00.1

**Date Ackn'd**
18/02/2005

**CU2 Project Title**
Transfection of immortalised tumour cells to enhance/reduce tumourgenicity & evaluation of GM cell lines in vitro/in vivo incl' breeding transgenic mice

**Date Project Ceased**
02/03/2022

**Class**
Class 2

**CultureVolClass2**
Non-GMM

**CultureVolumeClass3-4**
Consent Granted
Not Applicable
 Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
GM738/00.1 transferred to GM470 on 18/02/2005

Project notified under transitional arrangements Y

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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**Name**

WEST MIDLANDS REGIONAL GENETICS SERVICE

**Name 2**

BIRMINGHAM WOMANS HOSPITAL NHS TRUST

**Department**

DNA LABORATORIES

**Campus Estate or Research Centre**

QUEEN ELIZABETH MEDICAL CENTRE

**Road Name**

QUEEN ELIZABETH MEDICAL CENTRE

**District**

**Town**

BIRMINGHAM

**County**

MIDLANDS

**Postcode**

B15 2TG

**Country**

ENGLAND

**Tel Number**

0121 627 2710

**Fax Number**

0121 627 2711

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

Premises closed

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

**Give brief details of the genetic modification safety committee**

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**

**Tick if confidential**

02/03/2022
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**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum volumes used are 150 ml per time. The waste is autoclaved. All autoclaves are on a full service contract. Each run is monitored and there is a cut out if the run falls below the set times and temperatures. Each week the monitoring is checked by an engineer.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 472

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### Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

No bacterial culture will be greater than 20 ml in volume. After processing, all bacterial pellets and plasticware used for processing will be sealed and added to a "Sinbin" (normally used for "sharps" disposal). This will be sealed on the same day and then transported to the hospital incinerator. All surfaces will be decontaminated with Virkon immediately after use.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment
| Data Premises Notified (Originally) | 11/03/1992 | Transferred from 1992 Regs? | Y | Transitional Premises Class | nones | Data Premises Closed | N | Non-GMMs | Y | Withdrawn | N |

Name
B & K UNIVERSAL GROUP LIMITED

Name 2

Department

Campus Estate or Research Centre

Road Name

Building

Town
HULL

District
GRIMSTON

County
HUMBERSIDE

Postcode
HU11 4QE

Country
ENGLAND

Tel Number
01964 527 555

Fax Number
01964 527 006

E-mail

HSE Division
YORKSHIRE AND NORTH EAST

Comments

Date at Which Additional Info Submitted
02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
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For activities involving GMMs, describe the waste management measures which will apply to the activity

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Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 477**

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**Name**

AGRICO UK LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

CASTLETON OF EASSIE

**Town**

FORFAR

**District**

**County**

ANGUS

**Postcode**

DD8 1SJ

**Country**

SCOTLAND

**Tel Number**

01307 840551

**Fax Number**

01307 840245

**HSE Division**

SCOTLAND

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory
Animal Unit
Growth Room
Glass House
Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology
Parasitology
Transgenic Birds
Microbiology Research

Virology
Transgenic Animals
Transgenic Fish
Gene Therapy
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

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**Name**

UNIVERSITY OF STIRLING

**Department**

BIOLOGICAL SCIENCES

**Building**

COTTRELL BUILDING

**District**

**Town**

STIRLING

**County**

STIRLING

**Postcode**

FK9 4LA

**Country**

SCOTLAND

**Tel Number**

01786 473171

**Fax Number**

01786 464994

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

**Level 1 (GMMs)**

**Level 2 (GMMs)**

**Level 3 (GMMs)**

**Level 4 (GMMs)**

Non-microbial

Other (please specify)

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
Contaminated materials are sterilised either by autoclaving (solids, small liquid volumes) or by treatment with hypochlorite solution (liquid cultures).

Operations involving animals (fish) are conducted in contained laboratories and all effluent is treated with a hypochlorite drip prior to release. The sterility of the effluent is monitored at regular intervals. Transgenic animals are destroyed by incineration at the end of each experiment.

Genetically modified plants are autoclaved prior to disposal.

Both buildings have autoclaves on site that are tested annually. Chemical disinfection of materials is carried out with a standardised hypochlorite solution according to local rules.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 479/13.1

Date Ackn'd 29/07/2013

Date Project Ceased

CU2 Project Title The use of lux-marked non-toxigenic strain of Escherichia coli O157:H7 and a lux marked strain of Salmonella enterica serovar typhimurium to study the effect of environmental parameters on the survival and activity of possible pathogens

Class 2

CultureVol

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Tick if notifying a connected programme of work
E. coli O157:H7 is a toxigenic strain of E. coli and ingestion of the organism may occasionally lead to severe illness including life threatening hemolytic-uremic syndrome. E. coli O157:H7 is a class 3 organism according to the ACDP classification and as such requires level 3 containment for safe use, a requirement that can limit studies on this organism due to the expense of constructing and maintaining such facilities. A naturally occurring strain of E. coli O157:H7, retaining the O157 and H7 phenotypes but which is non-toxigenic, since it lacked the toxin genes stx1 and stx2, was previously chromosomally marked with the lux CDABE cassette using transposon mutagenesis to provide a low-risk substitute to verocytotoxin producing E. coli O157:H7 in environmental survival studies (Ritchie et al 2003). A strain of S. enterica serovar typhimurium containing a plasmid borne lux gene fusion is also available enabling safer and more accurate detection and quantification of this organism (Lewis et al. 2006). The purpose of this project is to use a luminescence assay to determine how a range of environmental parameters effect the survival and activity of the bacteria. This project will not involve the creation of any new GMOs.


Recipient or parental organism

The recipient E. coli strain was a naturally occurring isolate of E. coli O157:H7 which was shown via PCR to lack the genes stx1 and stx2 which encode cytotoxins. The recipient Salmonella strain was S. enterica serovar typhimurium DT104 which is a well characterised and common pathogen in humans.

Host/vector system

The recipient E. coli strain was mated with E. coli s17 lamda pir which contained a suicide plasmid harbouring a transposon Tn5 derivative with a luxCDABE cassette. The absence of the suicide plasmid in the transposon mutated E. coli O157:H7 strain was confirmed by an inability to grow on ampicillin (the plasmid conferred ampicillin resistance) and via small scale plasmid DNA preparations. The recipient S. enterica strain was transformed, via electroporation, with the broad host range plasmid pBBR1MCS-5 in which the lux CDABE genes from Photorhabdus luminescens had been cloned. Gentamycin selection was used to confirm transformation since the plasmid encodes gentamycin resistance.

Origin & function

The genetic material transferred to the E. coli O157:H7 strain included a gene for kanamycin resistance enabling antibiotic selection for successful transposon mutagenesis together with the lux CDABE cassette from Photorhabdus luminescens encoding the ability to produce bioluminescence. The genetic material transferred to the S. enterica strain strain included a gene for gentamycin resistance enabling antibiotic selection for successful transformation.
together with the lux CDABE cassette from Photorhabdus luminescens encoding the ability to produce bioluminescence.

Gentamycin is not generally used by General Practitioners to treat cases of Salmonella infection. Gentamycin is not systemically active when given orally, as it is not taken up to any great extent from the small intestine. The therapeutic use of this antibiotic for Salmonella is thus largely limited to intravenous or intramuscular injection in a hospital setting. In addition, gentamycin has ototoxic and nephrotoxic properties (Gronroos et al., 2008; Kusunoki et al., 2004) and is not likely to be a drug of first choice for Salmonella infections. A number of other antibiotics are available for the treatment of Salmonella infections, for example cefotaxime and ciprofloxacin (The British National Formulary). The potential impact of the introduction of gentamycin resistance into S. Typhimurium is very limited. Many Salmonella infections do not require antibiotic treatment and due to the toxic side effects of gentamycin and a lack of efficacy when administered orally it is not widely used for treating Salmonella infections. Alternative antibiotics are available and the genetically modified strain of Salmonella remains a hazard group 2 microorganism.


Evaluation of foreseeable effects

There are no hazards arising directly from the inserted DNA since the genes do not encode the production of any toxins or other virulence factors or extend the host range of the bacteria. In the case of the genetically modified strain of E.coli, the likelihood of the inserted DNA being transferred to related organisms is small and in any case they would not increase the pathogenicity of the recipient organisms. The naturally occurring non-toxigenic strain of E.coli O157:H7 does not pose a risk to the environment and the genetic modification performed has not resulted in any increased potential for environmental damage. The likelihood of the inserted material in the modified strain of S.enterica being transferred to other organisms is greater since the plasmid containing the cloned DNA has a broad host range and is mobilisable if the RK2 transfer function is provided in trans (Kovach et al 1995). However, even if transfer did occur it would not increase the pathogenicity of the recipient organisms or their ability to cause environmental damage.

It is well-established that the toxin genes stx1 and stx2 play a key role in virulence of entero-hemorrhagic strains of E.coli O157:H7. The lack of stx genes in the naturally occurring non-toxigenic strain of E.coli O157:H7 means that it is a low risk to human health. However, if this bacteria were to acquire the ability to produce toxins via plasmid transfer from another organism it has the potential to cause disease since it retains the intimin phenotype and can colonise human guts (Ritchie et al. 2003). In such circumstances the genetic modification of the strain increases the risk to humans marginally since kanamycin would no longer be an effective antibiotic with which to treat any disease. It should be noted, however, that kanamycin is actually very rarely used as a treatment for E. coli O157:H7 infections in any case.

S.enterica serovar Typhimurium DT104 is recognised as a major cause of food poisoning worldwide and is assigned to Hazard Group 2. The wild type strain is characterised by multiple drug resistance and hence the genetic modification does increase marginally the risk to human health since gentamycin would no longer be an effective treatment in case of infection.

References:

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This work will produce a variety of wastes including contaminated pipette tips, agar plates and liquid cultures. Contaminated pipette tips are immediately placed in a solution
of sodium hypochlorite (12-14% v/v aqueous solution) and subsequently autoclaved using a cycle including an initial steam purge and kill step of 121 °C, 15 psi for 20 minutes. All petri dishes and liquid wastes are killed via autoclaving as described above. Autoclave tape is routinely used and the efficacy of the kill cycle regularly checked by autoclaving a thick culture (approx 1 E9 colony forming units per ml) and subsequently plating on non selective agar and checking for growth at 37 °C over a 4 day period. All autoclave use is documented and autoclaves are subject to regular inspections and maintenance.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved as Class 2 (13/06/2013)

**Project Containment**

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**Animal Units**

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

**Large Scale Activities**

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

**Human Clinical Applications**

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |
Data Premises Notified (Originally) 08/04/1992

Transferred from 1992 Regs? Y
Transitional Premises Class 1

Data Premises Closed Transitional Premises Emergency Plan Required? N

Non-GMMs Y
Withdrawn N

Name

MRC/UNIVERSITY OF NEWCASTLE CENTRE DEVELOPMENT IN CLINICAL BRAIN AGEING

Name 2

NEWCASTLE GENERAL HOSPITAL

Department

Campus Estate or Research Centre

Building

MRC BUILDING

Road Name

WESTGATE ROAD

District

Town

NEWCASTLE UPON TYNE

County

TYNE AND WEAR

Postcode

NE4 6BE

Country

ENGLAND

Tel Number 0191 273 5251

Fax Number 0191 272 5291

E-mail

HSE Division YORKSHIRE AND NORTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) [Tick if confidential]

Laboratory Animal Unit Growth Room Glass House Large Scale

Bacteriology Parasitology Transgenic Birds Microbiology Research
Virology Transgenic Animals Transgenic Fish Gene Therapy

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity:

All solid waste is inactivated by an on-site autoclave. Liquid waste (not more than 0.5L) is inactivated by exposure to Virkon (according to the manufacturer's instructions).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 482**

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**UNIVERSITY OF WALES COLLEGE OF MEDICINE**

**Name**

UNIVERSITY OF WALES COLLEGE OF MEDICINE

**Department**

INSTITUTE OF NEPHROLOGY

**Campus Estate or Research Centre**

**Building**

HEATH PARK

**Road Name**

HEATH PARK

**Town**

CARDIFF

**County**

CEREDIGION

**Postcode**

CF14 4XN

**Country**

WALES

**Tel Number**

02920 748446

**Fax Number**

02920 748470

**E-mail**

**HSE Division**

WALES AND SOUTH WEST

**Comments**

GM482 CLOSED DOWN AND MERGED WITH GM130 ON 26/04/2005

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<td>Large Scale</td>
<td>Tick if confidential</td>
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</table>

Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research |
|-------------|--------------|------------------|-----------------------|
Virology     | Transgenic Animals | Transgenic Fish | Gene Therapy |
For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
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<tr>
<th>Mycology</th>
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<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td>Other(s)</td>
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</table>

All small volumes produced will be autoclaved and then disposed of in yellow bags for incineration, as per UWCM/Trust policy. Cell remnants and solid waste will be treated in the same manner. Autoclaves operated in accordance with the departmental SOP and serviced and tested by the Trust Works Dept.

Larger volumes of liquid waste - no greater than 1 litre - will be disinfected by the addition of bleach (hypochlorus acid) to a final concentration of 10% before disposal.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
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Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

Project Ref 483/01.1

Date Ackn'd 19/02/2001  
Date Project Ceased 29/09/2003

CU2 Project Title  
CONSTRUCTION OF LABORATORY SAFE STRAINS OF NEISSERIA MENINGITIS. CONSTRUCTION OF A LIVE ATTENUATED VACCINE FOR NEISSERIA MENINGITIS GROUP B

Class 2 CultureVolClass2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022
### Purposes of the contained use

- Preparation of microorganisms
- Evaluation of virulence and protection in mice evaluation of immune responses in mice

### Recipient or parental organism

- Strain B16B6, Neisseria meningitidis. It is possible some mutants will be based on strains of other stereotypes, for instance H44/76, but all will be Neisseria meningitidis group B.

### Host/vector system

- E coli

### Origin & function

The objectives of the project are to construct stable, disabled strains of N. meningitidis by genetically modifying genes that are essential for growth of the organism in the host. These strains can then be used safely for experiments that at present involve virulent, clinical isolates. Furthermore by modifying the expression of global regulators of virulence (such as fur and lux homologues), surface expressed determinants such as LOS and Opc, and recombination or DNA uptake systems (such as recA and comA respectively) construct an enhanced live attenuated vaccine.

The initial E.coli and Neisseria recombination work will be performed in the Institute of Infections and Immunity at the University of Nottingham. Certain reagents, including transformation stocks of Neisseria containing mixtures of parental, single recombination event-generated recombinants, and cells carrying the desired deletions, will be stored at Cantab as a back-up source of materials. Neisseria cultures grown at Cantab will consist of auxotrophic mutant strains, strains that are rendered more biosafe or have disrupted cell division and strains that are altered for the expression of the fur. The cultures grown at Cantab will then be transported to Churchill to be tested in a mouse model for safety, efficacy and immunogenicity.

The strains to be used in the in vivo evaluation experiments will either be deletion mutants in one of the genes specified in the list below, or combinations of deletions of two or more of these genes. The design of the final vaccine strain will be based on the experimental findings using the single and multiple deleted constructs. The strains may still have resistance genes to kanamycin, erythromycin or tetracyclin in the deleted portion of the organism. This is a transient stage, as the final vaccine will not contain antibiotic resistance genes. None of the antibiotic resistance genes present in the constructs is used to treat Neisseria meningitidis clinically.

### Evaluation of foreseeable effects

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<tbody>
<tr>
<td>asd</td>
<td>Genes involved in the synthesis of the essential nutrient diaminopelic acid (DAP). A lesion in the gene asdA, encoding the enzyme aspartate B-semialdehyde dehydrogenase would render the organism auxotrophic for DAP. Since this compound is an essential constituent of the cell wall for Gram-(ve) and some Gram +(ve) organisms and is absent from mammalian tissues, mutants would undergo lysis after about 3 rounds of division in mammalian tissues.</td>
</tr>
<tr>
<td>aro</td>
<td>Mutations in any of the genes in the aro pathway would render the organism auxotrophic for aromatic amino acids. In addition any such mutation would refer a requirement for two other compounds, (1)p-aminobenzoic (pAB) and (2) 2,3-dihydroxybenzoate (DHB) neither of which are found in mammalian tissue. Any such mutant would be unable to proliferate in mammalian tissue.</td>
</tr>
<tr>
<td>pur</td>
<td>Mutations in any of the genes in the pur pathway would render the organism auxotrophic for purines essential constituents of nucleic acids. Many of the intermediates of the pathway are unavailable in mammalian tissues.</td>
</tr>
<tr>
<td>pyr</td>
<td>Mutations in any of the genes in the pyr pathway would render the organism auxotrophic for pyrimidines essential constituents of nucleic acids. Many of the intermediates of the pathway are unavailable in mammalian tissues.</td>
</tr>
<tr>
<td>fur</td>
<td>The product of the fur (ferric uptake regulation) gene controls the expression of numerous genes. Among these genes are those involved in iron uptake via high</td>
</tr>
</tbody>
</table>
affinity siderophore systems and many genes involved in virulence, such as toxins. Mutations in the fur locus may cause loss of viability of the resulting strain. Although fur is a negative regulator of gene expression it is thought that this loss of viability may be explained by the fact that in some organisms certain essential genes are positively regulated by fur. To overcome this potential problem we intend to place fur under different control. This would give low basal level of expression (required for some essential genes) and full control of fur expression by turning on or off the promoter fused to fur. Likely promoters to use are lactose, chloramphenical and tryptophan.

Cap: Gene needed to give the organism its final capsule, containing polysaccharides. Non-capsuled strains are more serum-sensitive and may lead to better immune responses. Non-capsuled stains which occur naturally are not known to cause disease.

rec: There are many genes involved in recombination but the central gene is recA. RecA is essential for all homologous recombination pathways. Mutations in recA would render the strain UV sensitive as well as unable to carry out homologous recombination to repair constructed deletions.

galE - The galE gene is essential for normal LOS/PLS in N.meningitidis and N.gonorrhoea. Without the essential galactose residue in the LOS the side chains cannot be synthesised. The result of this is a LOS with a greatly reduced size. Such mutants would no longer be resistant to the effects of human serum.

com - Mutations in this locus would render the organism unable to take up exogenously added DNA. This mutation would (along with the recA mutation) make the biosafe strain genetically isolated and therefore more stable.

min - Mutations in either of the minC, minD or minE will cause aberrant cell division and the production of DNA-less minicells or membrane vesicles.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid cultures will be chemically inactivated using Lifeguard at a ration of 1:1. This was shown to kill all measurable organisms after 5 minutes (sensitivity 99.99%). All solid waste, including mouse bodies, will be double bagged and autoclaved. Mouse bodies will then be incinerated. All sharps will be put in sharp bins and autoclaved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee considered that although the risk to the health of the operator from infection with the recombinant Neisseria was negligible, the wearing of gloves and safety glasses, the use of a Class 2 safety cabinet and the avoidance of sharps where possible, would reduce the risk to essentially zero. The use of sharps cannot be avoided when working with animals, but extra care should be taken.
<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L3 L4 L2 L3 L4</td>
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**GM Centre Number: 484**

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Name

LABCORP EARLY DEVELOPMENT LABORATORIES LIMITED

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Campus Estate or Research Centre

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Comments

Name change from Huntingdon Life Sciences Ltd notified 24/01/201, name change from Envigo CRS ltd 13/06/2019

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Tick box]

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify)  
Tick if confidential

- **Bacteriology**
- **Parasitology**
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- **Microbiology Research**
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment □

Tick if you are claiming exemption from disclosure for sections of the risk assessment □

Please enter comments of the GM safety committee on the risk assessment
Data Premises Notified: 30/04/1992 (Originally)

Data Premises Closed: 15/01/2007

Transferred from 1992 Regs?: Y

Transitional Premises Class: 1

Emergency Plan Required?: N

Non-GMMs: Y

Withdrawn: N

Name:

UNIVERSITY OF LIVERPOOL

Department:

SCHOOL OF CLINICAL SCIENCES

Building:

UCD/DUNCAN BUILDING

District:


Town:

LIVERPOOL

County:

MERSEYSIDE

Postcode:

L69 3GA

Country:

ENGLAND

Tel Number: 0151 706 4528

Fax Number: 0151 706 4080

E-mail: none

HSE Division: NORTHWEST

Comments:

Date at Which Additional Info Submitted: 02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

02/03/2022
Liquid waste is treated via one of the following routes:

**Domestos**
Contains 1000,000 ppm available chlorine on manufacture. For discharging spent medium final dilution will be 10% (if the indicator, present in the medium fails to decolorise then there is insufficient active chlorine present and additional bleach should be added).
For spillage - 10000 ppm ie 10% v/v
For contaminated glassware - 25000 ppm ie 2.5% v/v
Dilute in tap water.

**Virkon**
Use at a 1% solution and is effective in discard jars with 10 mins soaking time (solution is active when pink, inactive if colourless).
For fluid spillages Virkon powder can be applied direct.
Presept
(Bacterial, Yeast)
Dichloroisocyanurate Na salt  1,000 ppm  General lab use
2,500 ppm  Pipette jars, spent media
10,000 ppm  Blood spillages

Other Waste
All other waste including agar is autoclaved, then disposed of by incineration. The autoclave is validated annually and a certificate of calibration is issued after calibrated thermocouple test. Manual recording of the autoclave process is documented where a chart recorder is not available.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 485/02.1

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Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

TRANSFERRED TO GM 554 - 15/1/07

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

---

**Project Additional Information**
**Purposes of the contained use**

The aim of the project is to evaluate in malignant human glioma cell lines cytotoxicity, dose response effect, and IL12 secretion of a recombinant Semliki Forest Virus vector (SFV) expressing transgene human IL12.

**Recipient or parental organism**

The recipient micro-organism are immortalised human malignant brain tumour cells (cell lines U87MG, LNZ308, T98).

**Host/vector system**

The vector is based on SFV, an insect alphavirus naturally infecting and replicating in subtropical mosquito species only. The human IL-12 gene has been inserted downstream from non-structural genes of SFV, whose structural genes encoding essential capsid and membrane proteins have been deleted and are provided by a helper vector. Recombinant SFV vectors are produced by recombination in the packaging cell line HEK293.

**Origin & function**

The inserted cDNA encodes human IL-12, an immunostimulatory cytokine identified as an activator of the cytolytic function of NK cells, antigenspecified cytotoxic T cells, and a potent inducer of IFNγ production.

**Evaluation of foreseeable effects**

Natural SFV infection only occurs when infected mosquitoes take a blood meal from viraemic vertebrate hosts. There is virtually no risk of natural spread of SFV from one human to another or from an animal to a human in the absence of the competent mosquito vector. There are no known mosquito species susceptible to SFV in the UK and therefore the risk of virus transmission to wildlife is considered negligible. Humans infected with SFV by mosquitoes may develop mild symptoms with uncomplicated and complete recovery. The present SFV virus is not mobilisable because it is replication incompetent. Replication disabled SFV mutants have been produced by deleting structural virus genes, which are provided in trans by a helper vector for recombination in a suitable cell line. This ensures that the r recombinant SFV undergoes only one cycle of infection without further replication. In theory, replication competent virus (RCV) may be produced by recombination in the packaging cell line, but the real chance of such recombination is virtually nil because of the multiple deletions in the virus genome. In addition, the SFV vector does not encode resistance to any drug or antibiotic. The human IL-12 gene cannot be incorporated into the virus envelope, and thus the possibility of alteration of tissue tropism or host range of SFV is considered negligible. Furthermore, liposomal encapsulation further reduces natural SFV tropism. All virus preparations will be produced at the facilities of Regulon, Inc., in Palo Alto, CA, USA. Sterile recombinant SFV in suspension is delivered ready to use. The inserted human IL12 gene does not encode a pathogenic protein as proven by prior in vitro and in vivo data. No harmful biological activity of hIL12 is known.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste which may contain viable SFV vectors, such as used medium, SFV infected cells, and plastic disposable objects (flasks, pipettes, etc.) will be collected and autoclaved. The autoclaved waste will be then subjected to the normal waste management cycle in the laboratory. The autoclave is managed by a contractor company (Priorclave Tactrol). Autoclave testing and quality assurance are performed and recorded yearly. The autoclave is located in the laboratory tract immediately across the GM rooms and run by lab staff.
Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment  

Reviewed by the University of Liverpool Department of Medicine Biosafety Committee.  

Project Containment  

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Project Ref 485/03.1  

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Withdrawn N  

Tick if notifying a connected programme of work N  

Historical Significant Changes  

TRANSFERRED TO GM 554 - 15/1/07  

Historical Date of Additional Info  

Significant Change ID  

Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

PKC has been shown to be a key signalling molecule in pathophysiology of the pancreas. The aim of this study is to examine Ca (2+) dependent/independent translocation of GFP tagged PKC isoforms in living mouse pancreatic acinar cells. A variety of PKC isoforms will be expressed in pancreatic acinar cells using replication deficient adenoviruses.

**Recipient or parental organism**

During production of replication deficient adenoviruses following Host organisms will be used: E.coli derivatives: XL1 BLUE, DH10B and a cells line QB1-HEK-293 (supplied by Obliogen, Nottingham, UK). All hosts are well characterised, non-pathogenic and are included by Obliogen (Nottingham, UK) in a standard kit for production of replication deficient adenoviruses. Finally replication deficient adenoviruses will be used to transfect freshly isolated pancreatic acinar cells maintained in primary culture. These cells are short lived, and therefore will not be able to survive outside tissue culture conditions and do not present any danger to environment.

**Host/vector system**

Vestors to be used in this project pEGFP (Clontech, Oxford, UK), pCDNA 3 (Invitrogen, Paisley, UK), pCR259 and pCR276 (Qbiogene, Nottingham, UK) pGEM, pSHUTTLE (Promega, Southampton, UK).

Recombinant PKC - GFP constructs will by produced and incorporated into virus transfer vectors. The production of replication deficient adenoviruses will require standard E. coli derivatives (XL1 Blue, DH10B) and HEK-293 cell lines.

All viral vectors contain necessary components for Tn7 transposition events. Viral vectors: pCR259, a 4488bp adenoviral transfer vector containing coding sequences for Ampicillin resistance, Tn7 transposition CMV promoter, origin of replication and a multiple cloning site. Transpose-Ad 294 vector is a 38275 bp vector containing the adenoviral genome (minus regions E1-3 needed for replication) and coding sequences for Tn7 transposition.

**Origin & function**

E. coli derivatives (XL1 Blue (Stratagene California, USA), DH10B (Qbiogene, Nottingham, UK) and QB1-HEK-293 cell lines will be purchased from Obliogen (Nottingham, UK) other vectors (see above) will be purchased from Clontech (Oxford UK), Invitrogen (Paisley, UK) and Promega (Southampton, UK). PKC encoding vectors, PKC-GFP encoding vectors and/or virus transfer vectors of these constructs and/or E1-E3 deficient recombinant viral plasmids will be received from collaborating laboratories. There are a few laboratories that developed PKC-GFP constructs (eg laboratory of Professor Saito in Tohoku University in Japan, Laboratory of Professor R. Rizzulto in University of Ferrara in Italy, Laboratory of Professor T Mayer in Stanford University, USA). We will receive constructs from one or more of these highly reputable laboratories. The overall aim of the project is to elucidate the role of PKC activation in pathophysiology of acute pancreatitis. A variety of PKC isoforms from different sources will be expressed in pancreatic acinar cells using replication deficient adenoviruses.

The cells will be stimulated by substances that could potentially be responsible for triggering acute pancreatitis (bite acids, ethanol and high doses of calcium releasing secretagogues). The activation/translocation of PKC isoforms will be monitored using fluorescence microscopy.

**Evaluation of foreseeable effects**

The PKC constructs encode functional regulatory proteins. This necessitates containment level 2. The constructs are linked to non-hazardous reporter proteins (eg GFP). Viruses containing constructs of interest could potentially be able to infect human cells. However these viruses would not be able to replicate in human cells. The recombinant adenoviruses made with Transpose-Ad expression system kits are replication deficient viruses that have deletions in the E1 and E3 regions; they will not replicate in cells other than complementing cells (QB1-HEK-293).

The E.coli derivatives to be used are disabled, non-pathogenic in man and considered ACDP group 1. The DNA inserted plasmid is eukaryotic in origin, contains a promoter which does not encode for a protein and hence is not hazardous, the marker proteins (eg green fluorescent protein) have no known hazardous effects. Eukaryotic genes delivered are unlikely to give a selective advantage to the E. coli host and it is unlikely that they would be processed in bacteria correctly as they contain eukaryotic signals eg for mRNA processing.
Established mammalian cell lines require defined media and conditions for growth, and therefore are unable to survive outside these conditions.

Isolated pancreatic acinar cells are short living cells, and unable to survive more than 24 hours after isolation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Both liquid and solid GM waste produced during the stages of virus production including and following transfection of QB1-HEK293 cells will be autoclaved to achieve 100% kill prior to disposal. Autoclaving will be done at the end of each working day when contained work has been carried out. The autoclave is managed by laboratory staff and maintained by Priorclave engineers. The autoclave is services and calibrated every half year by Priorclave engineers. The autoclave is located in the laboratory suite (room 1.05a in Henry Wellcome Gastro Laboratories). Both liquid and solid waste will then be sent for incineration.

Solid waste produced at earlier stages of the virus production (including bacterial culture plates, disposable plastic pipettes, pipette tips and microfuge tubes) will be disposed of into autoclave bags in dedicated bins for regular autoclaving. Autoclaved waste will be later incinerated. Disposable culture vessels and other contaminated glass and plastic ware will be soaked in disinfectant overnight, then sent for incineration. Waste media produced before stages of virus production (before transfection of QB1-HEK 293 cells) will be chemically disinfected (1% Vircon overnight) prior to disposal in dedicated sinks. Re-useable glass and plasticware will be disinfected overnight or autoclaved. Benches will be disinfected routinely before and after procedures and at the end of each day.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

On its meeting held on 27 January 2003 the Genetic Manipulation Committee of Departments of Physiology and Human Anatomy and Cell Biology of The University of Liverpool considered application for contained use of PKC constructs and replication deficient adenoviruses containing PKC constructs. The Committee considered that containment level 2 is appropriate for stages of laboratory work involving transfection of HEK 293 cells, maintenance of these cells in culture, isolation of viruses and transfection of pancreatic acinar cells. These procedures will be conducted in Henry Wellcome Gastro Laboratories facilities shared by Department of Medicine and Department of Physiology, located on the premises of Department of Medicine. Other stages of experimentation will require containment level 1. The Genetic Manipulation Committee considered and approved Risk Assessment and waste disposal procedures for this project.

Project Containment

Laboratory Activities Glass Houses Growth Rooms

02/03/2022
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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GM Centre Number: 486

Data Premises Notified (Originally) 05/05/1992

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Name

UNIVERSITY OF CAMBRIDGE

Name 2

UNIVERSITY BIOMEDICAL SERVICES

Department

Campus Estate or Research Centre

CAMBRIDGE BIOMEDICAL CAMPUS

Building

THE ANN MCCLAREN BUILDING

Road Name

90 FRANCIS CRICK AVENUE

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB2 0BA

Country

ENGLAND

Tel Number 0

Fax Number 0

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

UBS Health and Safety Committee. This is chaired by the Director of Facilities, meets three times per year.
Biological/GM safety is a standing agenda item.
UBS has a Bio/GM Safety Committee. It meets virtually or face-to-face, as required. It reports into the UBS H&S Committee.
UBS has a Biological Safety Officer (BSO).
The UBS Bio/GMSC is comprised of representatives from management, academics, technicians and other experts as required. Additional expertise is sought from the BSOs and GMSCs from those departments that make use of and extend their GM activities to these facilities. These departments assess their GM activities through their own GMSC/BSO and then in collaboration with UBS as the provider of these biomedical facilities.

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<th>Glass House</th>
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Other (please specify) | Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research | Gene Therapy
Virology | Transgenic Animals | Yes | Transgenic Fish | |
Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below) | |
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 490

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Name
UNIVERSITY OF ABERDEEN

Name 2

Department
INSTITUTE OF MEDICAL SCIENCES

Campus Estate or Research Centre

Buildings

Road Name
FORESTER HILL

Town
ABERDEEN

County
ABERDEENSHIRE
Postcode
AB25 2ZD

Country
SCOTLAND

Tel Number
01224 272000
Fax Number
01224273144

E-mail

HSE Division
SCOTLAND

Comments
Premises merged with GM227 29/09/2016 & class 2 projects transferred to this centre

Date at Which Additional Info Submitted
02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale
<table>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 17/trans1
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
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- **Animal Units**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Large Scale Activities**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Human Clinical Applications**: L2 L3 L4 L2 L3 L4 L2 L3 L4

## Project Ref 227/01.1

- **Date Ackn'd**: 29/09/2016
- **CU2 Project Title**: ANALYSIS OF NOVEL MOBILE DNA ELEMENTS FROM GUT BACTERIA
- **Class**: Class 2
- **Non-GMM**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: Yes

- **Withdrawn**: No
- **Historical Significant Changes**: Project transferred on merger with GM227

## Additional Information

- **Historical Date of Additional Info**: N
- **Significant Change ID**: N
- **Date of Significant Change**: N

02/03/2022  Page 7789 of 15326
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- **Is an emergency plan required according to regulation 20?**
  - [ ] Yes
  - [x] No

- **If yes, tick to confirm that it is attached to this form**
  - [ ] Yes
  - [ ] No

- **Tick to confirm that you have attached a risk assessment to this form**
  - [ ] Yes
  - [ ] No

- **Tick if you are claiming exemption from disclosure for section of the risk assessment**
  - [ ] Yes
  - [ ] No

**Please enter comments on the GM safety committee on the risk assessment**

### Project Containment

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<th>Growth Rooms</th>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 227/95.1

Date Ackn'd 29/09/2016

CU2 Project Title PREVENTION OF SALMONELLOSIS IN HUMANS AND FARM ANIMALS BY

Class CultureVolClass2 CultureVolumeClass3-4

Class 2
Date Project Ceased
NATURAL DIETARY COMPONENTS

Non-GMM Consent Granted
Not Applicable

Project notified under transitional arrangements
Y

Withdrawn
N

Tick if notifying a connected programme of work
N

Historical Significant Changes
Project transferred on merger with GM227

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 227/96.1

Date Ackn’d: 29/09/2016

CU2 Project Title: TRACKING OF GENETICALLY MARKED STRAINS OF GUT BACTERIA IN STIMULATED RUMEN CONDITIONS

Class: Class 2

CultureVolClass2: Not Applicable

Consent Granted:

Project notified under transitional arrangements: Y

Historical Significant Changes: Project transferred on merger with GM227

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
## Project Ref: 227/99.1

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<td>29/09/2016</td>
<td>FATE OF GREEN FLUORESCENT PROTEIN LABELLED SALMONELLA ENTERIDITIS IN THE RAT INTESTINE</td>
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- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: Yes
- **Historical Significant Changes**: Project transferred on merger with GM227

### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 490/00.2

Date Ackn'd 17/07/2000  ANALYSIS OF ACID REGULATION OF GENE EXPRESSION IN COMMENSAL  Class 2

Class CultureVolClass2 CultureVolumeClass3-4
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**Withdrawn**  N  
**Tick if notifying a connected programme of work**  N

**Historical Significant Changes**
**Historical Date of Additional Info**
**Significant Change ID**
**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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## Project Ref 490/00.3

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<td>24/08/2000</td>
<td>MOLECULAR AND CELL BIOLOGY OF OOMYCETE SPECIES</td>
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Non-GMM Consent Granted: not applicable

Historical Significant Changes

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Withdrawn

Tick if notifying a connected programme of work

Project notified under transitional arrangements
**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Project Containment**

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Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

N

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment
Project Ref 490/00.4

Date Ackn'd 03/10/2000

CU2 Project Title INVESTIGATION OF THE MOLECULAR BASIS FOR OSMOREGULATION IN LISTERIA MONOCYTOGENES

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]
If yes, tick to confirm that it is attached to this form [ ]
Tick to confirm that you have attached a risk assessment to this form [ ]
Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2</td>
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**Project Ref** 490/01.1

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<tr>
<td>18/10/2001</td>
<td>DIFFERENTIATION OF STEM CELLS TO EXPRESS A PANCREATIC PHENOTYPE</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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</table>
Date Project Ceased

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements N

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The purpose of the contained use is to determine the potential of stem cells that are present, in low abundance, in a variety of tissues to transdifferentiate to pancreatic endocrine cells. This will be done by using adenoviruses to introduce into mammalian cells in culture transcription factors that are known to regulate the development of insulin secreting cells of the endocrine pancreas.

Recipient or parental organism

The recipient or parental organism is primary rat or human liver cells in culture.

Host/vector system

The vector system is recombinant replication deficient adenovirus. These are deleted in the E1A and E3 region so preventing any possibility of infection. The gene of interest is inserted in the E1A region so recombination in this region will remove the inserted gene.

Origin & function

The genetic material involved includes the gene sequences encoding the transcription factors PDX1, Ngn3, NeuroD, Pax4, Pax6, and Nkx6.1 and the marker protein EGFP and its derivatives.

Evaluation of foreseeable effects

The work will involve the use of recombinant replication defective adenoviruses. Wild type adenoviruses are common causes of infections resulting in diarrhoea in infants, and upper respiratory tract infections and conjunctivitis in children and adults. Such infections are self-limiting. Around 70% of the population have evidence of previous infections with adenovirus and immunity is lifelong. Its natural tropism is for the respiratory tract. There are no reports of formation of wild type virus from recombinant adenovirus in vivo. There is no evidence that adenoviruses of the serotypes used in the proposed work cause infections in animals apart from minor infection in cotton rats, which are not indigenous to the UK or Europe.

The genes will be inserted under control of the CMV promoter and will give moderate levels of expression. The gene can not be mobilised.

The transcription factors used in this work would be active if expressed in human cells. If the lung (the most likely route of entry into humans) took up the adenoviruses and the transcription factors were expressed, it is possible that isolated stem cells within the lung could be converted to a pancreatic endocrine like phenotype.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The liquid waste will be disposed by adding presept (to a resulting concentration of 5%) to waste for 1 hour (effectively 100% kill). Presept tablets are used routinely to inactivate biological material in liquid cultures. To confirm the efficacy of this procedure, Min6 cells (a cell line derived from the endocrine pancreas of the mouse) were resuspended in 10 ml of culture medium and presept was added to give a final concentration of >2500 ppm as recommended by the suppliers. The cell suspension was then added to a tissue culture flask and incubated overnight at 37 degrees C in a humidified tissue culture incubator. No cell growth was observed.

Plasticware will be collected in a marked bag and autoclaved (effectively 100% kill).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Approved as activity class 2 by GM safety committee.

Project Containment

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Project Ref 490/01.2

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<tr>
<td>18/10/2001</td>
<td>USE OF RECOMBINANT ADENOVIRUSES TO EXPRESS RAT H09290 IN</td>
<td>Class 2</td>
<td>≤ 1 litre</td>
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**Project Additional Information**

**Purposes of the contained use**

We have identified a novel MAL-related protein which we hypothesise plays a role in targeting proteins into secretory granules. In order to determine the function of this protein we need to express it in mammalian cells. The most efficient method of expressing proteins in mammalian cell lines is using adenoviral vectors. This will allow expression of our novel protein in a high percentage of cells allowing functional analysis to be carried out.

**Recipient or parental organism**

The recipient cells are mammalian cell lines, primarily B-cells but may also include other endocrine cells as well as fibroblast and epithelial cell types.

**Host/vector system**

The vector system is recombinant, replication deficient adenovirus. The vector DNA is deleted in E1A and E3 regions preventing production of infectious viral particles in any cell except for those that can supply the missing E1A genes.

**Origin & function**

The genetic material encodes a novel protein, H09290, which is related to MAL, a protein that may function in regulating secretion from the apical surface of epithelial cells. In addition we have identified an isoform of H09290 which is identical except for the N-terminal region. Both these MAL-related proteins will be inserted into the adenoviral vector to allow expression in a variety of mammalian cell lines in order to determine their functional relevance.

**Evaluation of foreseeable effects**

The recipient microorganism is replication deficient and can only produce infective virus if propagated in early passage HEK293 cells that supply the missing E1 gene. Once these viruses are used to infect other mammalian cells which do not contain E1-encoded trans-complementing factors, the virus is unable to replicate and produces only a transient non-cytopathic infection. There are no reports of formation of wild-type virus from recombinant virus in vivo. Wild-type adenovirus causes upper respiratory tract infections and approximately 70% of the population shows evidence of previous infections with immunity being lifelong following primary infection. Replication deficient Ad vectors have no mechanism for long term maintenance in cells and expression in the lining of the epithelium declines with time and is limited to ~2 months. In the absence of episomal replication of E1-deleted Ad5 in normal human cells long term maintenance would require integration into the genome which only occurs at a frequency of 1 per 10 (to the power of 5) pfu in exponentially growing cultures of primary human cells. In addition, deletion of the E3 gene makes the virus less able to establish and maintain an infection within cells. E3 normally inhibits translocation of MHC molecules to the cell surface so deletion of E3 may be expected to enhance presentation of viral antigens to the immune system. While there is evidence that infection of cells with E3-deleted virus results in a greater, localised inflammatory
response, this could have the beneficial effect of accelerating the clearance of infected cells. Hazards associated with the recipient organism are therefore minimal.

The inserted gene is not anticipated to be harmful. It is a membrane protein that shuttles between the Golgi and the plasma membrane. It is not thought to enter the nucleus or act as a transcription factor and is therefore not likely to affect gene expression or act as an oncogene. In vitro experiments with the MAL homologue have shown that prolonged over-expression is toxic to cells and results in cell death. Short-term over-expression of MAL (which would be the likely result following accidental exposure) increases the level of secretion to the apical cell membrane, causing membrane ruffling. It is not known whether H09290 will perform a similar role in lung epithelia but if it did the outcomes would be to either increase the normal secretory process or cause cell death which would result in clearance of infected cells. In addition the gene of interest is inserted in the E1A region so if recombination were to occur with wild-type adenovirus this gene would be lost. The inserted gene should not alter the tropism of the adenovirus or give it harmful properties.

There is no evidence that human Ad serotypes can naturally infect animals and replication is very limited in, for example, mouse cells. Replication has been shown to occur in the lungs of experimentally infected cotton tail rats when administered a high dose of virus. Such doses are not likely to occur outside the laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from viral preparations and cell infections will be put into bottles for subsequent autoclaving at 121 degrees C for 15 min. Small tips, centrifuge tubes will be immersed in Klorsept 87 for a period of 24 h. Larger plastics will be double bagged and autoclaved at 121 degrees C for 15 minutes. Autoclaving will produce effectively 100% kill. After autoclaving the material will be placed into an orange bag, sealed and designated for continuous feed auger (CFA) prior to landfill.

Autoclaves are tested daily by insertion of indicator strips that only change colour when exposed to the correct temperature for the appropriate period of time. These are inserted into a full load into the autoclave at the beginning of each day to ensure the autoclave is performing adequately.

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee recommended this project be assigned to Category 2.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022
### Project Additional Information

**Purposes of the contained use**

To define the mechanisms by which mutations in the sequestosome 1 gene cause Paget's disease of bone.

**Recipient or parental organism**

The GMOs used will include the E. coli strains NM522 or JM109 (both Promega), DH5a (Gibco), XL1-blue, SOLR or XL10-Gold (all Stratagene), INVaF or TOP10 (both inVitrogen). The natural environment of E. coli is the gut but these experimental strains are laboratory adapted E. coli K-12, being classified as disabled hosts in the ACGM guidelines, and are unlikely to survive in the human gut or elsewhere.

**Host/vector system**

The organisms listed above will be transfected with plasmid vectors which have had the cDNA's for human and murine sequestosome 1 introduced into the multiple cloning site. The plasmids used for sequestosome 1 expression will include pBluescript II SK- (Stratagene), pcDNA3.1/V5-His TOPO or pcDNA3.1-CT-GFP-TOPO (inVitrogen), pTRE2 (Clontech), pTet-Off (Clontech), pUBI (Nucleic Acids Res 1996; 24:1787-1788) and pUC-H2-LTR (J Cell Biol 1993; 122:685-701), pGEM, pGEX, pCMV-script, pCVM-tag, pUC derived cloning and expression vectors.
Origin & function

The wild type sequestosome 1 gene is normally expressed in many cell types. Neither the wild type nor the mutant sequestosome 1 is thought to cause cell transformation. Inoculation or ingestion of plasmids or cell lines expressing sequestosome 1 is not thought to represent a significant hazard to human health for several reasons. First, it is extremely unlikely that inoculated plasmids or cell lines would survive attack by the immune system. Even if the cell lines and plasmids were able to evade immune destruction, it is very unlikely that sustained expression of the recombinant protein would occur in relevant target cells such as osteoclast precursors. Even if expression did occur in target cells, we know that humans who normally express the mutant sequestosome 1 in all cells do not develop appreciable pathology (i.e. Paget's disease of bone) until at least the age of 55 years. In order to minimise the risk of accidental inoculation, through skin abrasions or needlestick injuries, gloves will be used by laboratory workers and the use of sharps (e.g. needles and scalpels) will be prohibited when handling cultures of cells.

Evaluation of foreseeable effects

The expression constructs will be used to generate transgenic mice which express wild type sequestosome 1 and mutant sequestosome 1. These studies are the subject of a separate application which has been classed as non-notifiable by the University of Aberdeen Foresterhill GM safety committee.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The expression constructs will be used to generate transgenic mice which express wild type sequestosome 1 and mutant sequestosome 1. These studies are the subject of a separate application which has been classed as non-notifiable by the University of Aberdeen Foresterhill GM safety committee.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (from bacterial and mammalian cell cultures will be autoclaved at 121 degrees C for at least 15 minutes before disposal. Plastics and other solid disposable waste will be autoclaved at 121 degrees C for at least 15 minutes before disposal. Glass bottles and other non-disposable vessels will be soaked in the appropriate concentration of Haztabs (10,000 ppm free chlorine) and extensively rinsed before being autoclaved at 121 degrees C for at least 15 minutes before re-use. Haztabs at a concentration of 10,000 ppm free chlorine is the recommended concentration of chlorine to disinfect liquid waste according to manufacturer's guidelines (Guest Medical). Prestige Medical TST Control Integrators will be placed inside the autoclave with each load of GMM waste. The Prestige Medical TST Control changes colour from yellow to purplish blue when the correct sterilisation parameters have been met (>121 degrees C for 15 minutes). The expected killing of micro-organisms and mammalian cells exposed to these conditions is 100%.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The goal is to examine the chitin structure of the cell walls of Aspergillus fumigatus chitin synthase mutants created elsewhere. We are studying specific genes with negligible roles in virulence of A. fumigatus. The chitin synthase mutants are either unaffected in virulence or have decreased virulence relative to the parent wild type strain.
The wild type strain (parental strain) of Aspergillus fumigatus that will be used is 237. No genes will be modified within this study, but the genes already modified are chsA, chsB, chsC, chsD, chsE, chsF, chsG (chs = chitin synthase). Various chitin synthase mutants will be analysed that have originated from this wild type strain, the strain numbers of which are H-452, H-458, H-460, H-466, H-473, H-480, H-484, and P-1. No vectors will be used in this study. The mutants have been created by gene inactivation by the insertion of a positive selectable antibiotic marker (encoding the hygromycin resistance gene HPT) or auxotrophic markers that are not used clinically and which has no effect on cell virulence. The mutants have been created by integrative transformation with selectable markers and are known to be either attenuated or unaffected in virulence.

### Host/vector system

No genes will be modified within this study, but the genes already modified are chsA, chsB, chsC, chsD, chsE, chsF, chsG (chs = chitin synthase). Various chitin synthase mutants will be analysed that have originated from this wild type strain, the strain numbers of which are H-452, H-458, H-460, H-466, H-473, H-480, H-484, and P-1. No vectors will be used in this study. The mutants have been created by gene inactivation by the insertion of a positive selectable antibiotic marker (encoding the hygromycin resistance gene HPT) or auxotrophic markers that are not used clinically and which has no effect on cell virulence. The mutants have been created by integrative transformation with selectable markers and are known to be either attenuated or unaffected in virulence.

### Origin & function

The strains will be provided from other academic groups in the UK and in France. No genetic manipulations will be carried out at Aberdeen. The goal is to examine the chitin structure of the cell walls of Aspergillus fumigatus chitin synthase mutants. These mutants have been created elsewhere and would be grown in enclosed, sealed bottles in relatively small volumes and then immediately killed with alkali and acids to extract the acid and alkali-resistant chitin. The wild type and mutant strains will be grown and analysed (mainly by transmission electron microscopy).

### Evaluation of foreseeable effects

The strains to be used are attenuated by either (1) auxotrophy or (ii) due to disruption in chitin synthase genes. Wild type strains that have not been subjected to genetic manipulation may be used as controls. This is essentially no risk to the environment. Aspergillus fumigatus spores are very common in all environments since it is a part of the normal microflora of all plants and decaying vegetation. It is not a primary pathogen and is asexual and cannot cross with other A. fumigantus or Aspergillus strains. Because the fungus is sporogenic additional precautions will be taken to prevent the release of spores. All cultures will be grown in bottles with screw-top seals and will be killed immediately for harvesting for chitin preparations. There is no requirement to use heavily sporulating cultures and this will be minimised. Procedures will be used to minimise possible exposure to air-borne spores. Heavily sporing cultures will not be opened, but rendered safe by autoclaving. Therefore there is minimal opportunity for the spores to be released. Our experiments are of minimal risk because we do not have to make DNA or enztme preparations or carry out any treatments that require the fungus to be viable. Once biomass has been generated boiling in strong alkali or acid treatments is used in the first steps of the chitin extraction procedures. This is an extremely harsh, lethal treatment that completely sterilises the cultures. Again it is stressed that the practical concern for release of spores is the possible contamination of other work in the lab rather than a health hazard. Many labs manipulate the fungus in the open. Accidental breakages will follow the same guidelines as those used to deal with other category 2 microorganisms in the laboratory. The fungus is sensitive to all decontaminating agents and disinfectants currently used. Cultures will be killed by autoclaving or chemical extraction.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Because even acute exposure to high numbers of spores does not lead to disease it will not be necessary to confine work to safety containment cabinets. All cultures are in sealed dishes or bottles and are killed by autoclaving prior to cleaning. No agar plate (Petri-dish) cultures will be used. Cultures that are heavily sporing will not be opened and used for sub-cultures. Because even acute exposure to high numbers of spores does not lead to disease it will not be necessary to confine work to safety containment cabinets. Accidental breakages will follow the same guidelines as those used to deal with other category 2 microorganisms in the laboratory. The fungus is sensitive to all decontaminating agents and disinfectants currently used. Cultures will be killed by autoclaving or chemical extraction.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Our standard procedures for use of Presept have been validated experimentally to kill > 99.99% of cells and spores. Viability tests were performed on killed cultures. Autoclaves on level 1 of the Institute of Medical Sciences are validated at least annually by means of biological indicators. In addition we have validated killing at 99.99% by plating samples harvested from the centre of autoclaved samples. In daily use, monitoring is by thermocouple temperature recordings and autoclave strips included in the loads. Bench-top autoclaves in our laboratory are also tested regularly. Our laboratory manager tests these autoclaves annually. We have validated killing at >99.99% by plating.
samples harvested from the centre of autoclaved samples. Presept (1 tablet per 500 ml). During experiments cultures will be killed in 0.1 M Na OH or 0.1 M HC1. The samples are also autoclaved in hydrogen peroxide and glacial acetic acid during the chitin extraction - the chitin residue being the only biochemical known that can stand these harsh treatments. The experimental procedure itself can therefore be considered an extreme form of disinfectant.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Foresterhill GM Committee has now considered the application for Genetic Modification work. The project was considered to be appropriate as an Class 2 level of work.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 490/04.2

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<th>CU2 Project Title</th>
<th>Date Project Ceased</th>
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<th>CultureVolumeClass3-4</th>
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<th>Project notified under transitional arrangements</th>
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<tbody>
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<td>The interactions between bacteriophages and their bacterial hosts.</td>
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</table>

Historical Significant Changes

Tick if notifying a connected programme of work Y

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

To date most of our studies in this area have focussed on Streptomyces phages. Thanks to bacterial genomic sequencing we have hypothesised a more general nature to some of our discoveries and we now wish to test some of these theories. We now propose to use several known human pathogens in our research.

**Recipient or parental organism**

Several organisms will be manipulated; Salmonella typhimurium SL 1344 and LT2, Thermobifida fusca, Streptomyces sp. Mycobacterium smegmatis (mc2155), M. avium, M. marinum, M bovis BCG, E. coli K12.

**Host/vector system**

Chromosomal genes in S. typhimurium and in E. coli will be mutated using the lambda red pcr targeting gene replacement system; the red genes will be introduced via pKD20 or pKD46 or similar. Gene replacement in T. fusca and S. coelicolor will reply on homologous recombination via DNA introduced by mating on suicide vectors derived from E. coli CoIE1 derived plasmids (eg pSET151). We also intend to use site-specific integrating vectors (eg pSET152). Mutations in the mycobacterial chromosomes will also be generated by homologous recombination with DNA cloned on suicide plasmid vectors or on phasmids. E. coli vectors used will be derived from CoIE1, p15A, or defective lambda phage.

**Origin & function**

All the genes we will be studying were discovered through research on host-phage interactions. All the genes when mutated in S. coelicolor give an altered response to phage infection. Some of the genes (the pgl genes) are involved in a phage resistance phenotype in S. coelicolor whilst others (such as pmt1 and ppm1) are required to synthesise the phage receptor. In S coelicolor the pgl genes involve proteins with the following predicted functions; a protein kinase (pglW), DNA methyltransferase (pglX), an ATPase (pglY) and a protein of unknown function (pglZ). Homologues of these genes will be knocked out in S. typhimurium, T. fusca and in the genetic elements SXT and CTnR391 (derived from V. cholera and P. rettgeri respectively and transferred by conjugation into E. coli). In each case an antibiotic resistance marker (usually kanamycin, chloramphenicol, apramycin, hygromycin or spectinomycin resistance genes) will replace the pgl gene. The pgl homologues from these organisms will also be cloned into E. coli and Streptomyces. The pmt1 and ppm1 genes appear to be involved in a protein glycosylation pathway, which is common to all the actinomycetes. Pmt1 and ppm1 homologues (and other genes involved in the glycosylation pathway) in mycobacteria will be replaced by antibiotic markers, kanamycin or hygromycin. The pmt1 and ppm1 genes from mycobacteria will also be inserted in E. coli and streptomyces.

**Evaluation of foreseeable effects**

It is unlikely that any of these manipulations described here will result in any increase in the hazardous nature of these organisms. If anything their fitness will be reduced either due to sensitivity to phage infection or due to defects in synthesising key cell wall proteins. Indeed other people's attempts to knockout the pmt1 gene in M. tuberculosis so far have failed. The knockout mutants will contain antibiotic resistance genes but these will no confer resistance to any known therapy for any disease caused by the organisms in question. Cloning of the heterologous glycosylation genes in E. coli K12 strains or in Streptomyces is not likely to confer any degree of pathogenicity on these bacteria. We intend to use E. coli K12 containing the mobilisable SXT and CTnR391 elements which originate from V. cholera and P. rettgeri. These elements are known to encode several antibiotic resistance genes and several genes of unknown function. Given these known properties E. coli containing these elements will be used under class 2 containment to eliminate any possibility of spread.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

To date most of our studies in this area have focussed on Streptomyces phages. Thanks to bacterial genomic sequencing we have hypothesised a more general nature to some of our discoveries and we now wish to test some of these theories. We now propose to use several known human pathogens in our research.
The class II safety cabinet will be used for manipulating Mycobacterium avium, M. bovis BCG and Thermobifida fusca at all times. This is because they are known to cause pulmonary disease and there is a clear danger of infection via an airborne route. For S. typhimurium, E. coli, M. smegmatis and M. marinum there is little danger from the airborne route and these organisms will normally be manipulated on the bench with protective clothing and gloves.

The class II safety cabinet will always be used whenever aerosols are expected, such as vigorous mixing or resuspending cells after centrifugation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Trigene kills E. coli, S. typhimurium, Streptomyces sp. T. fusca, M. smegmatis and 5% kills M avium and M Bovis BCG. We expect to get 100% inactivation and validation will be routinely tested. Information on testing of most of these organisms is described at http://www.medichem.co.uk/medical_products.html. Streptomyces sp and Thermobifida fusca are not mentioned in this information but we have performed our own tests on Streptomyces and found 1% Trigene to be sufficient for 100% killing. The concentration of Trigene for killing of T. fusca will be thoroughly tested before GM work begins.

In practice the lab will routinely use 5% Trigene for killing of liquid cultures and for disinfection of contaminated glassware and spills. Disinfection will take place in containers with lids for no less than 24 hours before disposal. Disinfected cultures and glassware will be disposed of down the sink with plenty of water.

Plates will be taped together, placed in a double autoclave bags, sealed and sent for autoclaving on the premises for sterilising.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee accepted the scientific procedures and hazard assessments as reasonable. They requested that only one type of disinfection should be used throughout the laboratories for simplicity sake, which we have implemented by converting to the use of Trigene. The committee also requested a clear statement of when the safety cabinet should be used and when it is safe to work at the bench.

The risk assessment was passed pending these changes. It was decided that the labs where the work will take place would be inspected for compliance with level 2 containment prior to receipt of microbes classified as ACDP hazard group 2.

Project Containment

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<td>L3</td>
<td>L4</td>
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<td>L2</td>
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<td>L2</td>
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<td></td>
<td>L3</td>
<td>L4</td>
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</tbody>
</table>
Project Additional Information

Purposes of the contained use

Beta secretase (BACE) is the enzyme involved in the generation of the toxic beta amyloid peptide, leading to neurodegeneration in Alzheimer's disease. The aim of the project is to generate an approach that allows BACE1 inhibitors to be tested in vitro and in vivo. Proteins relevant to beta amyloid formation will be expressed both in vitro and in vivo. Recombinant adenoviruses will be used as a gene expression system to express the proteins, APPswe/lon (with V717I/K670N/M671L mutations), Tau (with mutations P301L and R406W) and BACE1.

Recipient or parental organism

The recipient micro-organism is recombinant replication-deficient adenovirus. These are deleted in the Early Regions 1 (E1) and 3 (E3) of wild-type Ad5 adenovirus and form a kit Stratagene (AdEasy XL Adenoviral Vector System). The target organisms will be SH-SY5Y neuroblastoma cells, primary mouse neuronal cultures and live recipients (mice).

Host/vector system

pShuttleCMV vectors containing the transgenes of interest will be used for recombinant into pAdEasy constructs which will allow for the production of virus in HEK293 cells (contain the necessary machinery for the replication of virus).
The recombinant adenoviruses to be used will express the transgenes APPswe/lon, Tau and BACE1. These will be fused to different fluorescent proteins, EGFP, ECFP and dsRed (Clontech). The APPswe/lon transcript contains two mutations (London mutation V717I and Swedish mutations K670N and M671L) which make it more susceptible to cleavage by BACE1 (Beta secretase 1). APPswe/lon when expressed in eukaryotic cells gives rise to Beta amyloid plaques in brain tissue which is a pathological indication in Alzheimer's disease. BACE1 is to be used in the human wild-type form. BACE1 is known to cleave APPlon/swe to form Beta amyloid plaques. The Tau protein is known to be associated with the formation neurofibrillary tangles in the brain. The form used in this study contains two mutations (P301L and R406W).

BACE1 inhibitors will be tested both in vitro and in vivo on neuronally derived cells, expressing the three above transgenes, to assess whether the production of APP-derived toxic peptides and subsequent cell death is reduced.

Evaluation of foreseeable effects

Recombinant adenoviruses developed in this way cannot in human tissue (all Ad5 vectors E1a and E1b deficient). No stable disabling mutation is likely to result due to the lack of propagation in human cells.

Expression of the inserted genes is transient and the risk genomic integration is negligible (1 per 105 pfu in exponentially growing cultures). Wild-type adenoviruses are common causes of infections resulting in diarrhoea in infants, and upper respiratory tract infections and conjunctivitis in children and adults are self limiting. Around 70% of the population have evidence of previous infections with adenovirus and immunity is lifelong. It's natural tropism is for the respiratory tract. There is no evidence to suggest that inhaled adenoviral particles can spread to brain tissue. There are no known reports of wild type virus from recombinant adenovirus in vivo. Adenoviruses are sensitive to heat and can be inactivated in 1 hour at 56°C. There is no evidence that human Ad serotypes can naturally infect animals and replication is minimal in mouse cells for example.

In the unlikely event that viral particles are accidentally inhaled, it has been shown that endogenous BACE1 in human lung tissue is almost absent (Rossner et al, 2001) and therefore cleavage of mutant APP into the known neurotoxin Beta Amyloid is negligible. Even should cleavage occur, there are no symptoms in AD patients relating to cardiovascular or respiratory problems despite considerable Beta Amyloid expression in blood. High titer viruses expressing BACE1 and APP in this study will be handled separately to insure exposure to both viruses simultaneously is kept to a minimum.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

SH-SY5Y neuroblastoma cells and primary mouse neuronal cultures will be kept in sealed flasks in designated incubators. These flasks will only be opened within Class II tissue culture hood at containment level 2.

Recombinant adenoviruses will be used to infect mice for short term studies. Since expression of the transgenes is transient, we do not consider the animals to be genetically modified. However, animals following adenoviral injection will be kept in separate designated rooms. No adenoviral particles will be present in mice when they are euthanised due to the short life expectancy of the adenovirus. Because the virus cannot replicate in cells that do not contain the E1- encoded trans-complementing factors, the virus will not propagate in vivo mice. Please see attached GMM form for additional measures.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (from bacterial and mammalian cell cultures) will be autoclaved (Institute of Medical Sciences prep room) before disposal down the sink. Plastics and other solid waste will be autoclaved before disposal. Glass bottles and other non-disposable vessels will be soaked in the appropriate concentration of Trigene (according to manufacturers recommendations).

Validation of the autoclaving process involves the use of independent thermocouples placed at the centre of the load, to determine whether the correct time and pressure have been reached for the required time. To verify inactivation of the adenovirus, neat autoclaved cell culture waste will be added to HEK293 cells to determine a cytopathic effect.
Trigene will be used to clean bench tops. Glass bottles and culture vessels will be soaked in trigene and extensively rinsed before being autoclaved in the IMS autoclave facility for re-use.

The proposal has been assessed and approved as Class II activity by the genetic modification safety committee (see attached risk assessment).

Project Containment

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Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tbody>
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Project Ref 490/06.2

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<tr>
<td>23/08/2006</td>
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</table>

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
Flow cytometry can be used to analyse and sort fluorescently labelled genetically modified cells or micro-organisms on the basis of differential fluorescence or other parameters such as cell size and granularity. Additional fluorescent can be used to identify and quantify expressed specific molecules and to define the phenotype and function of the modified cells. This notification is for generic approval for work undertaken by FACS facility involving unspecified GMO's requiring level 2 containment measures where confidentiality may be an issue (eg IP on gene modified).

1. Well characterised fish, rodent or mammalian cell lines such as HL60, COS, CHO J774 etc,
2. Primary cells such as leukocyte subsets, epithelial or endothelial cells.
3. Micro-organisms such as E.coli, candida, or saccharomyetes (attenuated or wild type) in hazard groups C1 or C2 according to ACDP.

1. Commercially available or well characterised transient or stable transfection systems or regulated expression systems.
2. Commercially available or experimental fluorescent reporter genes such as GFP, YFP or other fluorescent proteins.
3. C1 or C2 hazard group adenoviral or lentivrus vectors

The genetic materials involved will be for research applications in biology or medicine. Intended function of the research will be studies on the function of intracellular or secreted proteins and the effects of mutations, overexpression, deletion or functional inactivation of genes on biological processes.

The client will be required to provide a risk assessment containing specific information on GMM and potential risks both to human health and the environment, consistent with assignment of work to containment level 2, plus evidence of CU2 notification before work is started. If client is from out-with UK, equivalent risk assessments and written evidence that UK containment level 2 measures are appropriate for GMO will be required from the client.

The following issues will be confirmed with client prior to work being started.

i) Hazards specific to recipient organisms.
ii) Hazards specific to inserted/disabled/deleted gene.
iii) Likelihood of gene transfer to other organisms.
iv) Likelihood of inserted/disabled/deleted gene increasing pathogenicity of the recipient organism.

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None
Waste management measures sufficiently stringent to contain and inactivate (100%) any organism covered by level 2/3 containment will be standard. Protocols will be confirmed as appropriate with client before work commences.

Fluid waste will be collected into 1:200 Chloros which is standard procedure for fluid waste from FACS equipment. Chlorine levels in waste will be increased to provide 100,000 ppm available chlorine (10% chloros 1 hour minimum). Where 10% chloros cannot be used for fluid waste (corrosion risks), 1% Virkon (1 hour minimum) will be used before flushing waste to drain.

Solid waste will be biohazard double bagged for autoclaving or yellow bag incineration. Autoclave situated within laboratory suite (4.07) is fitted with internal probes that allow external monitoring of temperature, humidity, time and pressure. The efficacy of the sterilisation cycle is tested with Prestige indicator strips. Solid waste that cannot be autoclaved or decontaminated will be incinerated.

Spills
70% ethanol, 10% chloros or 1% Virkon as appropriate for size of spill and surface/material affected. Absorbent swabs will be double bagged and incinerated.

Cleaning protocols
All cytometers are routinely cleaned internally using 1:200 Chloros and are sterilised using 70% ethanol. External surfaces, pipettes and work surfaces around instruments are cleaned with 70% alcohol.

Risk assessment has been discussed and approved by Foresterhill Genetic Modification Committee.

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</tbody>
</table>
Studies of growth, cell wall synthesis and tropic responses of the fungus, Cryptococcus neoformans. The genes have been deleted in C. neoformans Serotype A strains of opposite mating types, H99 (alpha) and KN99 (a). H99 (alpha) is a clinical isolate that has become the reference strain for this Serotype. KN99 was created through a series of back-crosses between an a-type clinical isolate and H99 to create a congenic a-strain that is estimated to be 96.9% similar to H99. In murine and rabbit models of Cryptococcal meningitis, KN 99 was no more virulent than the clinical isolate, H99.

Recipient or parental organism
The genes have been deleted in H99 and KN99 by the stable insertion of linear, PCR-generated constructs, carrying genes for hygromycin, neomycin of nourseothricin (antibiotic) resistance, into the target genes. No plasmid vectors were used and the use of antibiotics is not required for the maintenance of mutant strains.

Origin & function
The origins of antibiotic resistance genes used as genetic markers are bacterial and are stably integrated into the C. neoformans genome. They were used to disrupt fungal genes that function within the calcium-signalling and response pathway and include calcium channel genes (CCH1, MID1, PMR1/ECA1), calcineurin and its regulators (CNA1, CNB1, CBP1) and genes involved in the biosynthesis of chitin and chitosan (CHS1-8, CSR1-3), which may function downstream of calcium-signalling.
Previous work using the filamentous fungus, Candida albicans, with mutations that impaired calcium-signalling suggested that perturbation of this pathway results in the inability of the micro-organism to sense and respond to its environment (Brand et al, 2007). We now seek to test whether this process is conserved in another filamentous fungus, C. neoformans, by growing hyphal filaments for short periods (<24H) and observing any changes in cell composition, enzyme phosphorylation status and growth behaviour using standard biochemical tests and microscopy.

**Evaluation of foreseeable effects**

In studies carried out in animal models, mutants with impaired calcium-signalling in C. neoformans and other fungi were severely reduced in virulence (Odom et al, 1997, Bates et al, 2005, Steinbach et al, 2006). Perturbation of calcium-signalling also affects survival of C. neoformans at human body temperature (Odom et al, 1997). The inactivation of calcineurin has been identified as a target in the development of anti-fungal drugs (Sanglard et al, 2003). In previous work using the human opportunistic pathogen, C. albicans, it has been shown that disruption of calcineurin (CNA1) results in the failure of the organism to survive in serum (Blankenship et al, 2005). We have shown that disruption of CCH1, MID1, FIG1 (calcium channels), CNA1, CNB1, CRZ 1 (signalling) in C. albicans reduce the ability of the micro-organism to sense and respond to the environment (Brand et al, 2007). It is therefore likely that C. neoformans strains with mutations in this pathway are similarly disabled and that the observed reduction in virulence in C. neoformans is a human pathogen in ACDP Hazard Group 2. It is ubiquitous in the environment and is commonly isolated from soil and decaying wood. The infectious particles are desiccated spores and yeast cells that become airborne when decaying material is disturbed. Particles enter the respiratory tract through inhalation and are carried and cleared asymptomatically by immunocompetent hosts. If the host immune system is severely impaired, for example, due to AIDS, colonisation by C. neoformans Serotype A can lead to the development of Cryptococcal meningitis. No transmission of Cryptococcosis between animals or humans has been recorded. C. neoformans is a micro-organism of low pathogenic potential in healthy individuals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid spills will be inactivated using 1% Trigene. The contaminated area is flooded with Trigene (to a final concentration of 1%, mopped with paper towels and reswabbed with 1% Trigene. Contaminated material is autoclaved. Liquid and solid waste, glassware and plasticware will be autoclaved. Our standard procedures for the use of Trigene have been validated experimentally to kill >99.99% of C. neoformans vegetative cells. Viability tests have been performed on Trigene-exposed cultures. Central autoclaves are validated at least annually by means of biological indicators. In addition we have validated killing at >99.99% by plating samples harvested from the centre of autoclaved samples. In daily use, monitoring is by thermocouple temperature recordings and autoclave-indicator tape included in the loads. Bench-top autoclaves are monitored on a daily basis using autoclave-indicator tape and by checking external temperature and pressure gauges. Our Laboratory Manager tests bench-top autoclaves on a monthly basis using Brownes tubes, which are included in the load and change colour on exposure to correct autoclave conditions. Killing by these autoclaves has been validated at 99.99% by plating from solid and liquid samples taken from the centre of the autoclave. After inactivation normal routes for disposal are used for liquid and solid waste. Killed liquid waste is discarded down the sink. Disposal of inactivated solid waste is by continuous feed auger processing (heat treatment and maceration followed by landfill). Contaminated sharps are placed in Cin-bins, bagged and incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

Approved as class 2 activity.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
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<tbody>
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<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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</table>

**Animal Units**

| L2 L3 L4 L2 L3 L4 |

**Large Scale Activities**

| L2 L3 L4 L2 |

**Human Clinical Applications**

| L2 L3 L4 |

### Project Ref 490/07.2

- **Date Ackn'd**: 11/07/2007
- **Expression of RANK genes in osteoclasts using viral vectors.**
- **Date Project Ceased**: 09/10/2017
- **Consent Granted**: Not Applicable
- **Tick if notifying a connected programme of work**: N
- **Project notified under transitional arrangements**: N

**Non-GMM Consent Granted**

- **Class CultureVolClass2 CultureVolumeClass3-4**
  - Class 2 < 1 Litre

### Project Additional Information

**Purposes of the contained use**

To safely and efficiently transduce RANK (wildtype and mutant - see attached risk assessment for details) genes into osteoclasts and osteoclast precursors, using replication-deficient lentiviral vectors. In related experiments, we will also use commercially available adenoviral Cre Recombinase to knockout the RANK gene in osteoclasts generated from mice in which the RANK gene is flanked by two loxP sites.
Recombinant adenoviruses are considered unlikely to cause disease. They will only propagate in cells that contain and express the E1-encoded trans-complementing
lentiviral vector stocks using derivatives specific for protoviral components such as VSV-G.

Stock supernatant into mitotically active indicator cells (eg 293FT cells) and monitoring for syncitia formation or by DOR analysis of cells that have been transduced with
after infection of a cell they are incapable of further replication. It will be possible to demonstrate that the lentiviral vectors are replication deficient by plating 5% of the vector
Lentiviruses require close contact for their transmission and their survival in the general environment is poor. Replication deficient lentivirus cannot propagate so therefore,
transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project, these risks will be made clear to all personnel in
wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal route of HIV 1
human health due to the reasons described in section 6 above. In carriers of wildtype HIV there may be a theoretical risk of recombination of the lentiviral vector with

We would not expect any specific symptoms as the result of an accidental infection with the genetically modified micro-organisms described in this application since the
lentiviral vectors and recombinant adenoviruses cannot replicate. There is a very small theoretical risk that, if the lentiviral expression plasmid was accidentally injected into an
individual carrying wildtype HIV, the vector could recombine, via the truncated LTR’s, with the wildtype virus to produce replication competent virus, carrying the RANK
genes. The likelihood of this occurring is extremely low since a) the use of sharps will be prohibited, b) plasmid DNA does not enter cells readily and c) the plasmid would
have to be internalised by a CD4+, HIV infected T cell. The lentiviral vectors themselves are capable of transducing a broad range of cell types, (as a result of the VSV-G
gene) and therefore the RANK genes will be stably integrated into any cell type that the vectors are exposed to. However, this is not thought to pose a significant risk to
human health due to the reasons described in section 6 above. In carriers of wildtype HIV there may be a theoretical risk of recombination of the lentiviral vector with
wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal route of HIV 1
transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project, these risks will be made clear to all personnel in
the laboratory.

Lentiviruses require close contact for their transmission and their survival in the general environment is poor. Replication deficient lentivirus cannot propagate so therefore,
after infection of a cell they are incapable of further replication. It will be possible to demonstrate that the lentiviral vectors are replication deficient by plating 5% of the vector
stock supernatant into mitotically active indicator cells (eg 293FT cells) and monitoring for syncitia formation or by DOR analysis of cells that have been transduced with
lentiviral vector stocks using derivatives specific for protoviral components such as a VSV-G.

Recombinant adenoviruses are considered unlikely to cause disease. They will only propagate in cells that contain and express the E1-encoded trans-complementing
factors. For all other somatic cell types that are susceptible to adenoviruses leads to a transient non-cytopathic infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (from mammalian cell cultures) will be autoclaved before disposal down the sink. Plastics and other solid disposable waste will be collected in autoclavable bag within the biological safety cabinet, sealed and then sprayed with virkon (1% as per manufacturer’s instructions). The bag will then be removed from the safety cabinet into a clean autoclave bag, sealed and taken to the autoclave in a sealed container. The degree of kill following autoclaving will be 100% since there is a high expectation of kill of GM microorganisms exposed to 121 degrees for 15 minutes. The autoclaved waste will then be disposed of by continuous feed auger.

Glass bottles and other non-disposable vessels will be soaked in the appropriate concentration of Virkon (1%, according to the manufacturers recommendation) in a bucket with a sealed lid, rinsed and then autoclaved for future use.

Validation will involve regular testing of the autoclave to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time. At the start of the project we will validate the inactivation of lentiviral vectors following autoclaving HT1080 cells will be grown in the presence of increasing (10 fold) amounts of inactivated waste. The cells will then be selected for using the antibiotic blasticidin and incubated for 10 days before being stained with Crystal violet. The identification of any blasticidin-resistant colonies demonstrates the presence of active lentiviral vector within the ‘inactivated’ waste.

The virucidal disinfectant, Virkon, has been validated for inactivation of lentivirus at a concentration of 1%. Details of Virkon testing from independent studies can be found at http://www.antecint.co.uk/main/virkonvi.htm

Project Containment

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</table>

Animal Units | Large Scale Activities | Human Clinical Applications

This application was approved at Containment Level 2 by the local Genetic Modification Safety Committee.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
Survival and pathogenicity of Escherichia coli O157:H7

This strain of E. coli O157:H7 is a naturally occurring, non-toxigenic isolate. The isolate was originally obtained as an environmental isolate and is likely to exist naturally in the human gut of a proportion of the population. The genetic modification subsequently carried out was to introduce marker/reporter genes (stably into the chromosome) which allow us to detect and monitor them in environmental matrices safely in a contained, laboratory environment.

Recipient or parental organism

Escherichia coli O157:H7 strain 3704.

This strain of E. coli O157:H7 is a naturally occurring, non-toxigenic isolate. The isolate was originally obtained as an environmental isolate and is likely to exist naturally in the human gut of a proportion of the population.

Host/vector system

E. coli O157:H7 strain 3704 Tn5 luxCDABE was constructed by biparental mating of a spontaneous rifampin-resistant mutant of E. coli O157:H7 strain 3704 with a donor strain, followed by suicide plasmid delivery and transposon mutagenesis. The donor strain, E. coli S17 aprt luxCDABE Km2 contains the luxCDABE cassette from Photobacterium luminescens and the antibiotic resistance genes for ampicillin and kanamycin. The rifampin-resistant mutant of E. coli O157:H7 strain 3704 was made by plating serial dilutions of an overnight culture onto Luria-Bertani (LB) plates containing rifampin (100 μg ml-1). Transconjugants in which Tn5 luxCDABE had inserted into the chromosome were initially selected on the basis of growth on LB containing rifampin (100 μg ml-1) and kanamycin (50 μg ml-1) and then by visible bioluminescence in the
The absence of the plasmid (which conferred ampicillin resistance) was confirmed by small-scale plasmid DNA preparations and by the lack of growth of the transconjugant LB plates containing ampicillin (50 μg ml-1). The stability of the lux phenotype was examined by successive subculturing of the selected E.coli O157:H7 strain 3704 Tn5 luxCDABE in LB broth without addition of kanamycin and subsequent confirmation of colony growth on LB agar with versus without kanamycin (50 μg ml-1). To confirm that mutagenesis had not disrupted the O157, 57 or intimin phenotype of the strain, multiplex PCR was performed.

E.coli O157:H7 strain 3704 was kindly provided by Fiona Thompson-cartier (E.coli reference laboratory, University of Aberdeen, United Kingdom). The strain was originally isolated from a farm drain and has been proven to be nontoxigenic due to the absence of toxin activity (by Verocell assay) and toxin genes (by PCR) (F. Thomson-Carter, unpublished observation).

Introduced genes are luxCDABE genes (from Photorhabdus luminescens) and natural antibiotic resistance genes, which do not confer harmful properties to the micro-organism or human health.


<table>
<thead>
<tr>
<th>Origin &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>The luxCDABE cassette was originally obtained from Photorhabdus luminescens and luminescence encoded by these genes will be used to detect and monitor the marked E. coli O157:H7 strain in environmental samples</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Evaluation of foreseeable effects</th>
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<tr>
<td>E.coli O157:H7 strain 3704 was kindly provided by Fiona Thompson-Carter (E.coli reference laboratory, University of Aberdeen, United Kingdom). The strain was originally isolated from a farm drain and has been proven to be nontoxigenic due to the absence of toxin activity (by Verocell assay) and toxin genes (by PCR) (F. Thomson-Carter, unpublished observation). It is therefore categorised as a Hazard Group 2 micro-organism. Introduced genes are luxCDABE genes (from Photorhabdus luminescens) and natural antibiotic resistance genes, which do not confer harmful properties to the micro-organism or human health.</td>
</tr>
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<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
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In the event of a culture being split, the liquid must be absorbed into paper towels which must then be autoclaved. The contaminated area must then be thoroughly cleaned using Hibicet.

All contaminated equipment must be cleaned thoroughly using 10% Hibicel solution.

Contaminated clothing must be autoclaved before being washed.

All waste must be autoclaved at 121º for 60 mins. Liquid waste can be autoclaved in sealed containers. Solid waste should be placed into a double autoclave bag. Once autoclaved, the waste can be incinerated.

As the autoclaves are out with the lab, all waste must be transferred by the person who generated it in sealed boxes. The same operative must also empty the autoclave once finished as well as noting the disposal number for the waste.

Astell autoclaves have a temperature probe and digital readout to show autoclave has reached the required temperature and pressure.
TST control indicator strips may also be used. Cultivation techniques will confirm inactivation of GMM before waste is collected for incineration by disposal company contracted to University of Aberdeen.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The Old Aberdeen Genetic Modification Safety Committee approved the risk assessment on March 11th 2009

**Project Containment**

<table>
<thead>
<tr>
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<td>L2 L3 L4</td>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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**Project Ref** 490/09.2

<table>
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<th>CultureVolClass2</th>
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<tr>
<td>07/10/2009</td>
<td>Lentivirus delivered silencing of genes involved in the electrotactic response of mammalian cells</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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Date Project Ceased: 23/02/2021

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N
Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To safely and efficiently knockdown proteins involved in both cell division and migration induced by electric field application in mammalian cell lines. Genes will be silenced using replication-deficient lentiviral vectors which are created from the commercially available MISSION TRC shRNA constructs and the lentiviral packaging mix, both by Sigma Aldrich. Please see: http://sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/learning-center/getting-started.html

Recipient or parental organism

HEK293T cells will be used as the packaging cell line for the lentiviral vectors.
Cell lines where gene knockdowns will be performed are HEK293, HELA and possibly chronic wound human fibroblasts, SHSY5Y and PC12 cells.

Host/vector system

Pseudo-typed lentiviral particles will be produced in HEK293T cells using a third-generation packaging system, which has many features that lead to enhanced biosafety. Recombinant lentiviruses produced with the MISSION lentiviral Packaging Mix (Sigma Aldrich catalog number SHP001) have not been shown to produce replication competent viral particles because of designed safety features. It is a three plasmid system consisting of:
1. The packaging vector, which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions.
2. The vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector, which provides the heterologous envelope for pseudotyping.
3. The shRNA transfer vector (pLKO.1-Puro), which contains the sequence of interest as well as the cis acting sequences necessary for RNA production and packaging.
This vector contains a sequence encoding a puromycin resistance gene.
The multi-plasmid approach results in no single plasmid containing all the genes necessary to produce packaged lentivirus.
The Lentiviral Packaging Mix contains the first two components, it is designed to be co-transfected along with a compatible lentiviral transfer vector in order to create high titer pseudo-typed lentiviral particles used. Resulting particles are replication-incompetent and deletion in the U3 portion of the 3' LTR eliminates the promoter-enhancer region, further negating the possibility of viral replication. The system has also removed virulence genes which are not necessary for shRNA packaging. In addition, the lentiviral vector contains a self-inactivating 3' LTR that renders it unable to produce the infectious virus once it integrates into the host chromosome. These features combined have improved biosafety and handling. This information appears on-line in: http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/learning-center/mission-faqs/biosafety.html
HEK293, HELA, human fibroblasts, SHSY5Y and PC12 cells will be transduced with the pseudo-viral particles. In most cases and in order to rule out compensatory effects of the protein knockdowns, the resulting cell ines will be phenotyped and then disposed at the end of the experiment. In the remaining cases, stable cell lines will be generated. In these cases, no additional hazards compared with any other transformed cell line are present as the lentiviral vector contains a self-inactivating 3' LTR that in all cases renders it unable to produce infectious virus once integrated into the host chromosome.

Origin & function

Mammalian targeted genes will be the serine threonine phosphatases PPP4, PP2A and PP1 catalytic (c) and regulatory subunits; the Na+/H+ antiporter (SLC9A1); METTL10; kinases YLK4 and STK28A; ZNF365 and PDE4DIP.
- PP4c and its regulatory subunits are involved in microtubule organization at centrosomes, maturation of spliceosomal snRNPs, apoptosis, DNA damage and cell migration.
- PP2Ac and its regulatory subunits are implicated in the negative control of cell growth and division.
- PP1 is essential for cell division, and participates in the regulation of glycogen metabolism, muscle contractility and protein synthesis.
- The Na+/H+ antiporter (SLC9A1) is involved in pH regulation to eliminate acids generated by active metabolism or to counter adverse environmental conditions. Plays an important role in signal transduction.
- Q5JPI9 (encoded by METTL10) is a predicted protein of unknown function. It may be involved in vesicle transport processes.
- ULK4, STK28L ZFP465 and PDE4DIP genes are involved in neurite outgrowth and cell migration.

Cells resulting from the phosphatase protein knockdown experiments may have disrupted progression of mitosis and may present altered migration competence. Cells resulting from SCL9A1 gene silencing may present compromised pH regulation and electrotactic competence. And METTL10 knockdown may affect vesicle transport.

Cells resulting from ULK4, STK28L, ZFP465 and PDE4DIP gene silencing may show neurite outgrowth and cell migration.

**Evaluation of foreseeable effects**

We would not expect any specific symptoms as the result of an infection with the genetically modified microorganisms described in this application since the lentiviral vectors cannot replicate.
No sharps will be used in any of these experiments.
The vectors have been designed so that they are incapable of recombining with the wild-type virus in any way that would produce viable virus. Because viral structural genes have been placed on different genetic units, multiple recombination events must occur before a replication competent helper virus is generated. Furthermore, areas of homology among the units expressing the helper virus proteins have been minimized. Also, heterologous promoters for the helper virus proteins are used. Resulting particles are replication-incompetent and deletion in the U3 portion of the 3’ LTR eliminates the promoter-enhancer region, further negating the possibility of viral replication (http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/learning-center/mission-faqs/biosafety.html)

However, highly improbable viral replication-competence will be checked by subjecting cell cultures to puromycin resistance after treating these cells with media collected from cells that have been treated with viral supernatant which would lead to puromycin resistance. Additionally, cell media will be immunoblotted in order to rule out the presence of the viral protein p24.
The transfected cultured cell lines will not survive outside the culture medium and all liquid and solid waste will be autoclaved on site. This rules out the possibility of escape into external environment and survival, establishment or dissemination therein.


Factors such as congenital immunodeficiency caused by serious illness or chemotherapy would increase the susceptibility to infection by micro-organisms, including the genetically modified micro-organisms described in this application. In carriers of wild type HIV there may be a theoretical risk of recombination of the lentiviral vector with wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project, these risks will be made clear to all personnel in the laboratory.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
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<tbody>
<tr>
<td>1% Virkon</td>
<td>1% Virkon will be added to biological wet waste and plastics and this waste will be left over night prior to rinsing and disposal in double hazardous waste bags. Virkon is a unique, synergised oxidising system based on potassium monopersulphate which is proven effective against all major pathogens known to man such as viruses HIV/AIDS, hepatitis B, polio, lassa and rift valley fevers. Proven against a wide range of antibiotic-resistant strains. For more info, please see <a href="http://www/rmsupply.co.uk/Virkon_lab_products.htm">http://www/rmsupply.co.uk/Virkon_lab_products.htm</a>.</td>
</tr>
<tr>
<td>All bags with the material previously inactivated as described above will then be taken to the central autoclaving facility. Autoclaving is by validated (internal temperature probe linked to printout) autoclave, reserved only for inactivation of waste (effective 100% kill). The autoclave undergoes annual testing to ensure correct operation. Waste will be transferred to the autoclave in covered containers.</td>
<td></td>
</tr>
</tbody>
</table>

02/03/2022
This application was approved at Activity Class 2, Containment Level 2 by the local Genetic Modification Safety Committee.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

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**Project Ref 490/11.1**

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<td>25/11/2011</td>
<td>Lentiviral mediated gene knock-down of YAP in myogenic cells</td>
<td>Class 2</td>
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<td>Date Project Ceased</td>
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<td>Non-GMM</td>
<td>Consent Granted</td>
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Withdrawn

Tick if notifying a connected programme of work

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

**Purposes of the contained use**

To safely and efficiently deliver shRNA targeted against YAP gene into myogenic cells using replication-deficient lentiviral vectors.

**Recipient or parental organism**

For transformation of the lentiviral cloning vector, disabled k12-derived E.coli (XL-1 Blue, Stratagene) will be used. The 293T cell line (a fast-growing cell line derived from human embryonic kidney cells, containing the large T-cell antigen) will be used as the packaging cell line for the lentiviral vectors.

The target cells that will be transduced with the lentiviruses encoding shRNA against Yap are:
1) Primary mouse skeletal muscle myofibres (this preparation will include the associated muscle stem cells).
2) The well characterised myogenic mouse cell lines C2C12 and the mouse embryonic fibroblast C3H/10T1/2.
3) Mouse and human mesenchymal stem cells, derived from bone marrow, synovium, periosteum and adipose sources.

**Host/vector system**

VSV-G pseudotyped lentiviral vectors will be produced virus by co-transferring the 293 T cell line with the expression construct and 2 lentiviral packaging plasmids each individually carrying genes encoding the necessary viral structural and replicatory proteins (2nd generation packaging system).

**Origin & function**

YAP is ubiquitously expressed transcriptional coactivator that regulates the expression of genes involved in cell proliferation (cyclins, growth (Akt) and apoptosis (caspase 3, BIRC5, Survivin). Members of the TEAD family of DNA binding proteins are known to drive the expression of at least some of the genes that YAP regulates. Overexpression of constitutively active YAP (YAP S127A and YAP SS) promotes cell proliferation and tumour formation in Drosophila and a variety of mammalian cells/tissues, leading to the consideration of YAP as an oncogene. Knock-down of Yap can protect against cancer formation in certain cells types (Zhou et al., 2011 Moi Med Report). In stem cells, YAP influences pluripotency and cell fate decisions, with over expression of wt YAP enhancing pluripotent induction of mouse fibroblasts and forced expression of YAP S127A leading to an expansion and delayed differentiation of several progenitor cell populations in vivo. Knock-down of Yap in embryonic stem cells leads to enhanced differentiation and loss of pluripotency. Whole body knock-out of Yap is embryonic lethal in mice. YAP activity is negatively regulated by the 'Hippo pathway', consisting of the core kinases; mammalian ste20-like kinase (MST1/2) and large tumour suppressor (LATS1/2). LATS1/2 inhibits the transcriptional activity of YAP via phosphorylation at serine residue 127, which promotes the binding of YAP to 14-3-3 proteins and translocation out of the nucleus.

The insertion of these genes is not expected to alter the tropism of the lentiviral vector or give it harmful properties. Accidental uptake of the lentiviral vectors into human cells could result in expression of the YAP shRNA. However, this is not likely to cause any pathogenic effect since:
1) DNA integration is unlikely to cause tumorigenesis as silencing of Yap is a well established mechanism for tumour suppression in a variety of cancer cell lines and in vivo models.
2) Knock-down of Yap has been shown to induce apoptosis in vivo in a variety of cell types. Apoptosis of infected cells is likely to be beneficial as it will limit the number of progeny containing the integrated DNA.

**Evaluation of foreseeable effects**

We would not expect any specific symptoms as the result of an accidental infection with the genetically modified micro-organisms described in this application since the lentiviral vectors cannot replicate. There is a very small theoretical risk that, if the lentiviral expression plasmid was accidentally injected into an individual carrying wildtype HIV, the vector could recombine, via the truncated LTR’s, with the wildtype virus to produce replication competent virus, carrying the YAP shRNA. The likelihood of this occurring is extremely low since a) the use of sharps will be prohibited, b) plasmid DNA does not enter cells readily and c) the plasmid would have to be internalised by a CD4+, HIV infected T cell. The lentiviral vectors themselves are capable of transducing a broad range of cell types (as a result of the VSV-G gene) and therefore the YAP shRNA will be stably integrated into any cell type that the vectors are exposed to. However, this is not thought to pose a significant risk to human health due to the reasons...
described in section 6 above. In carriers of wildtype HIV there may be a theoretical risk of recombination of the lentiviral vector with wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project; these risks will be made clear to all personnel in the laboratory. Lentiviruses require close contact for their transmission and their survival in the general environment is poor. Replication deficient lentivirus cannot propagate so therefore, after infection of a cell they are incapable of further replication. It will be possible to demonstrate that the lentiviral vectors are replication deficient by plating 5% of the vector stock supernatant into mitotically active indicator cells (eg293T cells) and monitoring for syncitia formation or by PCR analysis of cells that have been transduced with lentiviral vector stocks using primers specific for pro-viral components, such as VSV-G.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (from mammalian cell cultures) will be autoclaved before disposal down the sink. Plastics and other solid disposable waste will be collected in autoclavable bag within the biological safety cabinet, sealed and then sprayed with Virkon (15, as per manufacturer's instructions). The bag will then be removed from the safety cabinet into a clean autoclave bag, sealed, and taken to the autoclave in a sealed container. The degree of kill following autoclaving will be 100% since there is a high expectation of kill of GM microorganisms exposed to 121°C for 15 minutes. The autoclaved waste will then be disposed of by continuous feed auger. Glass bottles and other non-disposable vessels will be soaked in the appropriate concentration of Virkon (1%, according to the manufacturer's recommendation) in a bucket with a sealed lid, rinsed and then autoclaved for future use. Validation will involve regular testing of the autoclave to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time. At the start of the project we will validate the inactivation of lentiviral vectors following autoclaving. HT1080 cells will be grown in the presence of increasing (10 fold) amounts of inactivated waste. The cells will then be selected for using the antibiotic blasticidin and incubated for 10 days before being stained with Crystal Violet. The identification of any blasticidin-resistant colonies demonstrates the presence of active lentiviral vector within the 'inactivated waste'. The virucidal disinfectant, Virkon, has been validated for inactivation of lentivirus at a concentration of 1%. Details of Virkon testing from independent studies can be found at http://www.antecint.co.uk/MAIN/virkonvi.htm

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This application was approved at Containment Level 2 by the local Genetic Modification Safety Committee
Project Ref 490/11.3

Lentivirus-mediated genetic modification of mammalian cells for the study of mesenchymal stromal cells

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Recipient or parental organism

For cloning of the different genes into the lentiviral cloning vectors, disabled K12-derived E. coli (One Shot Stbl3, Invitrogen or XL-1 Blue, Stratagene) will be used. The HEK293FT cell line (Invitrogen) and the HEK 293 LTV cell line (Cell Biolabs) (fast-growing cell lines derived from human embryonic kidney cells, containing the large T-cell antigen) will be used as the packaging cell lines for the lentiviral vectors.
VSV-G pseudotyped lentiviral vectors will be produced using the Cell Biolabs Virasafe Lentiviral Expression System and/or the ViraPower lentiviral expression kit (Invitrogen) by co-transfecting the 293FT and the HEK293 LTV cell lines with the expression construct and a lentiviral packaging mix containing 3 plasmids, each carrying genes encoding the necessary viral structural and replicatory proteins (to improve safety).

The live lentivirus and the lentivirus-infected cells will be cultured in a CL-2 biocabinet, and housed in a CL-2 CO2 incubator used solely for virus-infected cells.

**Origin & function**

<table>
<thead>
<tr>
<th>GFP</th>
<th>mCherry</th>
<th>LacZ</th>
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<tbody>
<tr>
<td>is a 27kDa protein that exhibits bright green fluorescence when exposed to blue light.</td>
<td>is a protein emitting bright red fluorescence when excited by orange light.</td>
<td>encodes the bacterial protein beta-galactosidase, which catalyses the metabolism of lactose to glucose and galactose.</td>
</tr>
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</table>

GFP, mCherry and LacZ are commonly used reporter genes whose expression can be deleted by fluorescence (GFP, mCherry), by using a substrate such as X-gal that is cleaved by beta-galactosidase to yield an insoluble blue product (LacZ), or by immunohistochemistry using antibodies against GFP, mCherry and beta-galactosidase. The insertion of these genes should not alter the tropism of the lentiviral vectors or give them harmful properties. Uptake of the lentiviral vectors into human cells could result in expression of the inserted genes.

Inoculation or ingestion of lentiviral vectors expressing reporter genes such as GFP, mCherry or LacZ is not thought to represent a significant hazard to human health nor to cause any pathogenic effect for the following reasons:

1. GFP, mCherry or LacZ activation does not lead to cell transformation.
2. It is highly unlikely there will be system-wide effects since these are genes encoding for proteins not present normally in the mammalian organisms, and therefore no biochemical or metabolic pathway should be affected by them.

**Evaluation of foreseeable effects**

We would not expect any specific symptoms as the result of an accidental infection with the genetically modified micro-organisms described in this application since the lentiviral vectors cannot replicate. The lentiviral vectors themselves are capable of transducing a broad range of cell types (as a result of the VSV-G gene) and therefore the reporter and expression genes will be stably integrated into any cell type that the vectors are exposed to. However, this is not thought to pose a significant risk to human health due to the reasons described in section 6 above; also, since lentiviral vectors will only be handled in a class II safety cabinet and sharps will be used in extremely controlled conditions, the risk of accidental injection, inhalation or ingestion of the lentiviral vectors is extremely low. The likelihood of this occurring is therefore extremely low also since plasmid DNA does not enter cells readily.

There is a very small theoretical risk that, if the lentiviral expression plasmid was accidentally injected into an individual carrying wildtype HIV, the vector could recombine, via the truncatedLTRs, with the wildtype virus to produce replication competent virus, carrying the expression or reporter genes. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project; these risks will be made clear to all personnel in the laboratory.

Lentiviruses require close contact for their transmission and their survival in the general environment is poor. Replication deficient lentiviruses cannot propagate so therefore, after infection of a cell they are incapable of further replication. It will be possible to demonstrate that the lentiviral vectors are replication deficient by plating 5% of the vector stock supernatant into mitotically active indicator cells (eg 293FT cells) and monitoring for syncitia formation or by PCR analysis of cells that have been transduced with lentiviral vector stocks using primers specific for pro-viral components, such as VSV-G. The insertion of the genes discussed in section 6 above is not expected to alter the tropism of the lentiviral vector or give it harmful properties.

The bacterial organisms are E. coli K12 derivatives; they have a proven safe history of use in routine cloning work and are unlikely to survive in the human gut or elsewhere. None of the genes expressed here would be expressed in these bacterial cells since the genes lack a bacterial promoter. Even if expression did occur, it would not be anticipated to confer any pathological advantage to the bacterial host. There is unlikely to be any hazard to the environment from these bacterial organisms.

The use of sharps will be generally prohibited, and allowed only in particularly planned and controlled circumstances.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be autoclaved before disposal down the sink. Plastics and other solid disposable waste will be collected in autoclavable bag within the biological safety cabinet, sealed and then sprayed with Virkon, (1:100, following manufacturer's recommendations). The bag will then be removed from the safety cabinet into a clean autoclave bag, sealed and taken to the autoclave in a sealed container, where they will be autoclaved at 121°C for 15 minutes. The degree of kill following autoclaving will be 100%. The autoclaved waste will then be disposed of by continuous feed auger.

Glass bottles and other non-disposable vessels will be soaked in the appropriate concentration of Virkon (1:100 dilution, according to the manufacturer's recommendations) in a bucket with a sealed lid, rinsed well and then autoclaved at 121°C for 15 minutes.

Validation will involve regular testing of the autoclave to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time.

To validate the inactivation of lentiviral vectors following autoclaving. HT1080 cells will be grown in the presence of increasing (10-fold) amounts of inactivated waste. The cells will then be selected for, using the antibiotic blasticidin and incubated for 10 days before being stained with Crystal Violet. The identification of any blasticidin-resistant colonies demonstrates the presence of active lentiviral vector within the 'inactivated waste'.

Regarding waste coming from in vivo experiments, harvested biological materials (tissues and body fluids) will be either fixed in 4% paraformaldehyde or preserved in PBS for further analysis or culture expansion of the freshly isolated samples. In the latter case, tissues will be transported back to our lab in appropriate CL-2 safety containers.

At the end of all experiments, any remaining lentiviruses, unfixed tissues or cells will be disinfected in 1% Virkon and then autoclaved at 121°C for 15 minutes prior to disposal.

The virucidal disinfectant, Virkon, has been validated for inactivation of lentivirus at a concentration of 1%. Details of Virkon testing from independent studies can found at http://www.antecint.co.uk/MAIN/virkonvi.htm

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was approved at Containment Level 2 by the local Genetic Modification Safety Committee

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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</table>
Salmonella enteritidis and S. typhimurium continue to be a major worldwide cause of disease. Their pathogenicity has been linked to virulence factors, of which flagella and fimbriae are considered to be important in early association of the bacteria with the gut epithelium and immune system. However, the exact roles of these virulence factors remain unclear.

To evaluate the roles of these bacterial factors in pathogenic interactions with the host gut and immune system, genetically altered (GA) salmonella strains unable to express virulence factors, including flagella [FliC], fimbriae [sef21 (type 1), sef17, sef14, lpf or pef] or combination thereof were prepared at the Veterinary Laboratories Agency (Weybridge, Addlestone, Surrey KT15 3NB). Strains with disabled flagella (che- or mot-) or containing in addition Green Fluorescence Protein (GFP) as a marker have also been developed. These GA strains will be applied to epithelial or immune cells in culture to monitor their effects on key molecular signalling systems and gene activation, in particular production of pro-inflammatory or survival factors. Alternatively, they will be used to orally infect mice (normal or genetically altered [deletions in key bacteria response pathways, such as Toll-like Receptors] mice). The ability of the strains to colonise the gut, interact with and modulate cross-talk between epithelial and immune cells, interfere with the local immune system, invade and spread systemically and cause acute and long-term illness will be evaluated.

The overall aims of the studies are to establish the roles of flagellin and fimbriae in short- and long-term host responses to infection and identify possible new therapeutic strategies to prevent or ameliorate infection by salmonella.
### Host/vector system

Deletion mutants: DNA fragments, which encoded relevant regions for flagella or fimbriae were amplified by PCR. These fragments were sub-cloned separately into pCRSCRIPT. Antibiotic resistance gene cassettes were cloned separately into these fragments, either by direct sub-cloning or by splice overlap extension PCR. The insertionally inactivated genes were sub-cloned on to appropriate PERFORM vectors and electroporated into Escherichia coli. The PERFORM vectors were transferred by conjugation from host E. coli (Allen-Vercoe et al, 1999). The deletion mutants carry no recombinant vector sequence (confirmed using Southern Blot Analysis). The nature of the recombination event led to complete loss of vector sequence from the strains.

Insertion mutants: Green Fluorescence Protein (GFP): GFP plasmid DNA, purified from E. coli XL1-blue, was electroporated into Salmonella (Wild-type or GA) strains.

### Origin & function

The GA salmonella strains are unable to express flagella [FliC] or individual fimbriae [sef21 (type 1), sef17, sef14, lpf or pef] or combinations thereof. Alternatively, they have disabled flagella (che- or mot-) or strains (wild-type and GA) in addition contain Green Fluorescence Protein (GFP) as a marker.

GA strains will be applied to cells in culture or given orally to mice (wild-type or GA [for example mice with deletions of key bacterial-response pathways]) to monitor and compare their effects on host cell metabolism and pathogenicity.

The studies will establish the requirements and roles of flagellin and fimbriae for effective and appropriate in short- and long-term host gut and immune responses to infection. This should facilitate development of new therapeutic strategies to combat or ameliorate infection by salmonella.

### Evaluation of foreseeable effects

Deletion of fimbriae will either have little or no effect on the pathogenicity of salmonella or will reduce its ability to infect. Experimental studies in vitro and in vivo with GA strains unable to express selected fimbriae support this (Allen-Vercoe et al. 1999; Robertson et al. 2000).

Results from studies with aflagellate salmonella are equivocal. The infection caused by aflagellate salmonella in salmonella-resistant mice is slightly more severe than that triggered by the parent strain (Vijay-Kumar et al, 2006). This appears to be due to failure of the host immune system to properly recognise or respond to aflagellate salmonella [lack of signalling via flagellin / TLR5 axis]. In contrast, the differences in pathogenicity of flagellate or aflagellate salmonella are much less marked in salmonella-susceptible mice, which are often used to model of severe systemic salmonellosis in humans. Furthermore, aflagellate salmonella is much less infective than wild-type salmonella for rats and chicks (Allen-Vercoe et al. 1999; Robertson et al. 2003).

The GA salmonella have specific antibiotic resistance cassettes within the targeted genes and thus have resistance to ampicillin, chloramphenicol, zeocin, kanamycin, tetracycline, trimethoprim or combinations thereof. They remain susceptible to the action of other antibiotics. In humans, treatment of salmonellosis with antibiotics is done only if the infection spreads from the intestines or otherwise persists. The antibiotics used would be ampicillin, gentamicin, trimethoprim/sulfamethoxazole, ceftriaxone, amoxicillin, or ciprofloxacin.

Human health could possibly be at slightly greater risk if exposed to aflagellate S. enteritidis or S. typhimurium as compared to its wild-type counterpart. However, the effects of the aflagellate salmonella strains we hold still remain far less severe than those caused by the most pathogenic of wild-type salmonella strains.

Recent studies have revealed that a proportion of the human population have polymorphisms in TLR5. If this prevents activation of TLR5 by flagellin, the susceptibility and reactivity of these individuals to wild-type salmonella is likely to be increased in a manner similar to that which we have observed with aflagellate salmonella.


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Culture waste, tissue homogenates, containers and vessels, dilutions, agar plates and extracts derived during the study and associated laboratory waste will be autoclaved as will be caging, tissue, carcass, faeces and urine, bedding material, culture and food waste collected during animal experiments. Isolators are sterilised by being sprayed down with 1% Virkon or are sprayed down with Alcide, prepared in accordance with the manufacturer’s instructions. A kill efficacy of 100% is expected and all facilities are regularly monitored to ensure effective operation / killing

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Original notification
GM227/95.1 Prevention of salmonellosis in humans and farm animals by natural dietary compounds [15 Feb 95].

Notification under the transitional provisions of the GMO Contained Use Regulations 2000
GM227 Role of fimbriae and flagella in pathogenicity of Salmonella enteritidis in the rat or mouse [14 Feb 01].

Present Application (CPU2): Movement of project from the GM Centre at the Rowett (Centre GM227) to GM Centre at Foresterhill (GM490).

The Committee discussed with Drt how he felt the aflagellate mutants of S. enteritidis and S. typhimurium may increase the severity of infection compared to wildtype strains. The Committee were satisfied, following Dr ’s explanation, that the overall risk to human health from these aflagellate strains was much less severe than that posed by most wildtype strains of salmonella and thus remained within the limit of acceptable risk for activity class 2 organisms.

This project was approved at Activity Class 2, Containment Level 2 by the Local Genetic Modification Safety Committee.
Project Additional Information

Purposes of the contained use

The goal of the work is to examine the virulence of Fusarium oxysporum urease mutants. These mutants have been created elsewhere and would be grown in enclosed, sealed bottles in small volumes (<200ml). Conidia of the different strains will be used for virulence assays on mice, as well as for microscopy analysis.

Recipient or parental organism

Fusarium oxysporum is a soil-borne fungus that is a common part of the plant decomposing microflora and can act as a plant pathogen. It can occasionally cause opportunistic infections in severely immunocompromised humans. Every one of us is likely to be exposed to spores of this fungus every day from environmental sources.
Host/vector system

Fusarium oxysporum targeted mutants lacking the genes ureG (urease accessory protein), ure1 (structural urease) or dur3 (urea transporter), as well as complemented strains of these mutants have been created elsewhere. The mutants have been generated by gene inactivation via the insertion of a positive selectable antibiotic marker (encoding the hygromycin or phleomycin resistance gene, hph or phleo, respectively). GFP- and cherryFP-labelled transformants were also created and will be used for microscopy analysis.

Origin & function

DNA fragments for construction of the GMMs, containing the sequences flanking the genes ureG (urease accessory protein), ure1 (structural urease) or dur3 (urea transporter), the complete sequences of these genes, the positive selectable antibiotic markers encoding the hygromycin or phleomycin resistance genes, and the GFP- and cherryFP genes were all generated elsewhere by PCR amplification. No transfer of these DNA sequences will be performed in this study.

Evaluation of foreseeable effects

The urease mutants under investigation attenuate the fungus, preventing infection of plants. It is anticipated that the mutants will also be attenuated in humans compared to the wildtype strain but these data are yet to be confirmed and published. Sporulating cultures will not be used in this project, thus preventing the risk of aerosol generation in these experiments. In any event, individuals who are severely immunocompromised will not be permitted to work on the project.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures are in bottles (liquid cultures) and are killed by autoclaving prior to cleaning. Chemical activation of spills and cleaning bench surfaces and equipment will be performed using 2% Trigene, as recommended by the manufacturer. Validation will involve regular testing of the autoclave to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time. At the start of the project we will validate the inactivation of the GM F. oxysporum by plating samples on solid YPD laboratory medium. This process will be repeated monthly and records kept of test results. Trigene inactivation will again be validated by plating samples on solid YDP agar. This process will be repeated monthly and records kept of the test results.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This application was approved at Containment Level 2 by the local Genetic Modification Safety Committee. They were satisfied that since there is no intention to generate spores in cultures of GM F. oxysporum there was no requirement for the use of a safety cabinet to handle the cultures.
**Project Containment**

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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**Project Ref** 490/12.3

Date Ackn'd 12/11/2012

CU2 Project Title

Generation of human induced pluripotent stem cells from peripheral blood mononuclear cells for directed differentiation to functional osteoclasts

Class 2

Culture Volume

< 1 Litre

Non-GMM

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

09/10/2017

Withdrawn N

Tick if notifying a connected programme of work N

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

To safely generate induced pluripotent stem cells (iPSC) from human peripheral blood mononuclear cells (PBMCs) using Sendai viral vectors.

**Recipient or parental organism**

Sendai virus (SeV): No modification/production of the viral vectors will be performed on site. We will purchase the viral vectors as part of a ready to use kit. Sendai virus is ACPD class 1 organism and is a murine parainfluenza virus that is non-pathogenic to humans, yet these genetically modified sendai viral vectors can transduce human and animal cells. The Sendai virus in the CytoTune kit is a non-transmissible virus, with the Fusion protein deleted. Therefore they are no longer capable of producing infectious particles from infected cells. (ref: Li, H.O. et al. A cytoplasmic RNA vector derived from nontransmissible Sendai virus with efficient gene transfer and expression. J. Virol.)
Peripheral blood mononuclear cells: human peripheral blood mononuclear cells purchased from commercially available sources (Frozen PBMC, STEMCELL, cat# PB003F). This would help us to optimise and standardise protocols for iPSC generation.

Host/vector system

CytoTune™-iPS Reprogramming System uses vectors based on replication incompetent Sendai virus (SeV) to safely and effectively deliver and express key genetic factors necessary for reprogramming somatic cells into iPSCs. The CytoTune™-iPS Reprogramming Kit contains four SeV-based reprogramming vectors, each capable of expressing one of the four Yamanaka factors (i.e., Oct4, Sox2, Klf4, and c-Myc) and are optimized for generating iPSCs from human somatic cells.

Origin & function

Unlike other viral transduction methods, SeV (belonging to the paramyxoviridae family) does not integrate in the host genome and remains in the cytoplasm (ref: Fusaki, N. et al., Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 85, 348-362, 2009). The SeV vectors replicate in the form of negative-sense single-stranded non-segmented RNA in cytoplasm of infected cells and does not go through a DNA phase. Reprogramming would be achieved by transfecting PBMCs with the four Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc, ref: Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676, 2006).

Oct4, Sox2, Klf4, and c-Myc will each be delivered in separate SeV vectors. Oct4 regulates the pluripotency of embryonic stem cells and under and overexpression has been associated with cell differentiation. Sox2 acts cooperatively with Oct4 to regulate pluripotency. Klf-4 is a transcription factor that regulates transcription factor expression during development. C-Myc, with roles in regulating cell proliferation and apoptosis, is a proto-oncogene and is overexpressed in many cancers. Together, they are a recognised cocktail of genes required for reprogramming somatic cells to a stem cell phenotype. The expression of these genes results in cells adopting a phenotype whereby they have self-renewal capacity and the ability, under managed conditions to proliferate indefinitely.

Evaluation of foreseeable effects

Although human is not the natural host for the SeV, and the virus is non-pathogenic to humans, appropriate care must be taken to prevent the potential mucosal exposure to the virus, since the vectors are capable of transducing human cells.

Given that the deletion of the fusion protein within the CytoTune sendai virus results in a non-transmissible virus, the hazards associated with these vectors are as a result of the nature of the inserted genes. Overexpression of any of these genes can result in dysregulation of cell proliferation, differentiation and survival and therefore potentially transform the cells. Therefore, following centrifugation and for all handling, the tubes containing CytoTune sendai viral particles will be opened in the Class 2 safety cabinet to avoid exposure to aerosolised particles.

Serologically positive rodents (the natural host for sendai virus), animals that have already been infected with wild type SeV, may be able to make infectious CytoTune™-Sendai virus. Sendai virus affects the respiratory tract and causes severe respiratory disease in rodents. It can be spread by contact and airborne transmission including through air circulation systems. We will not be performing any experimental work with animals, however, as a rule it is recommended that after handling sendai viral vectors or infected animals, a period of at least 3 hours should elapse before contact with other animals.

Once reprogrammed to iPSCs, there will be no additional hazard of handling the human cells (above that of handling the untransduced cells) given that the sendai virus is non-transmissible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be autoclaved before disposal down the sink. Plastics and other solid disposable waste will be collected in autoclavable bag and autoclaved, collected in "orange waste bag stream" for maceration to make it unrecognisable and further heat treated before being placed in landfill. All waste will be transferred to the autoclave facilities in a sealed container. The degree of kill following autoclaving will be 100% since there is a high expectation of kill of sendai viral particles exposed to 121ºC for 15 minutes. Trigene disinfectant (2%, to be validated prior to start of the project) will be used to deal with spills.

Validation will involve regular testing of the autoclave to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time. Sendai viruses are inactivated at temperatures above 37ºC. At the start of the project and at monthly intervals, we will validate the inactivation of sendai viral vectors following autoclaving by incubating cultures of Hela cells with autoclaved liquid waste. We will then perform reverse transcription on RNA isolated from the Hela cells, followed by PCR using primers to specifically amplify the viral genome to determine whether any virus had been incorporated into the cells.

Records will be kept of the results from each test.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project was approved at Activity Class 2 by the local Genetic Modification Safety Committee

Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 490/12.4

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<td>&lt; 1 Litre</td>
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Date Project 02/03/2022  Page 7842 of 15326
The main aim of the work is to examine interaction of host neuronal cells and fluorescently labelled HSV-1 in in vivo and in vitro experiments and their response to stress (surgery). These GM HSV-1 mutants were created elsewhere, would be used predominantly for confocal microscopy analysis.

Herpes simple virus-1 - linear double DNA stranded virus - is well spread pathogen in normal population. In some populations almost 90% of healthy adults are serologically positive and as such they have been previously exposed to HSV-1 without any local or systemic symptoms or sequela. It can occasionally cause opportunistic infections in severely immunocompromised individuals. As GM modification of WT virus is adding fluorescent tags to the normal WT genes, it is unlikely that GM virus would have different virulence characteristics than WT virus.

The GM HSV-1 were generated elsewhere by insertion of final constructed plasmid [fluorescent tags - gene for green fluorescent protein (GFP), red fluorescent protein (RFP) and Cherry red] together with HSV-1 RE infectious DNA into Vero cells, using Lipofectamine 2000 (Invitrogen, Carlsbad,CA) as per the manufacturer's instructions. These GFP-, RFP- and Cherry red-labelled transformants will be used for microscopy analysis.

Origin & function

Published in vitro replication kinetics and in vivo infectivity of fluorescent HSV-1 mutants seems to mimic and certainly it is not more severe the infection with wild type virus (in Srividya Ramachandran, Jared E. Knickelbein, Christina Ferko, Robert L. Hendricks, Paul R. Kinchington: Development and pathogenic evaluation of recombinant herpes simplex virus type 1 expressing two fluorescent reporter genes from different lytic promoters. Virology 378 (2008) 254–264.).

In any event, individuals who are severely immunocompromised and pregnant woman will not be permitted to work on the project. Any exposure of laboratory personell with WT or GM HSV-1 will be treated effective prophylaxis, which is available (Acyclovir tablets).
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

LIQUID WASTE - All liquid will be drained with a pastette to waste beaker and will be treated with 1% Virkon solution, left 12 hours or overnight before flushing to drains with copious amounts of water. Beaker will be placed on white biohazard tray so that any spillage can be easily identified and dealt with. All waste will be clearly labelled.

SOLID WASTE - HSV-1 contaminated plastics will be placed in autoclave bag along with any other associated solid waste. Contaminated surfaces will be sprayed with 1% Virkon solution and wiped clean with paper towel. Autoclavable waste will be removed from lab as soon as work is completed double bagged and blue tagged and autoclaved at 121 degree C for 15 minutes.

Autoclave is fitted with internal probes that allow external monitoring of temperature, humidity, time and pressure. The efficiency of the sterilisation cycle is tested with Prestige indicator strips.

Degree of kill is 100% when using autoclave for decontamination of solid waste, 1% Virkon for a minimum of 1 hour will be used for liquid waste. This is standard method for inactivation of a wide spectrum of micro-organisms including viruses.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

This application was approved at Containment Level 2 by local Genetic Modification Safety Committee

Project Containment

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Project Ref 490/12.5
Characterisation of a C-type lectin receptor

This application covers a range of specific projects with the overall aim to define the ligand and range of mycobacterial species that are recognised by a C-type lectin receptor, and to determine the physiological role of this receptor in vitro and in vivo. In particular, this project will utilise a range of non-pathogenic mycobacterial species including both wild-type and GM Mycobacterium smegmatis and M. bovis BCG.

Recipient organisms include M. bovis BCG strains Tokyo and Pasteur and M. smegmatis mc2155. All mycobacterial strains used in this project are either listed as hazard group 2 (M. bovis BCG) or are not listed (M. smegmatis). M. smegmatis is a non-pathogenic soil organism, but due to its taxonomic grouping it is being treated as a group 2 organism. M. bovis BCG is routinely used as a vaccine against M. tuberculosis in humans.

The risk of life-threatening infections with M. smegmatis and M. bovis BCG is negligible in healthy individuals. However, M. smegmatis has been reported to cause localized skin and soft tissue infections following traumatic injury or cardiac surgery. Immunocompromised and elderly individuals are more susceptible to M. bovis BCG, which can cause pulmonary infections.

In some of the experiments, tissues which may contain small amounts of live GM bacteria are removed from infected animals and transported back to the IMS in sealed containers for various experiments including the determination of bacterial burden and histology (IMS 4.08 and 4.27). Frozen, unfixed sections may be analysed by fluorescence microscopy (IMS 4.28). These sections would contain very small amounts of bacteria therefore present minimal risk. Local GM regulations will be posted in all locations.
In other experiments small amounts of fixed GM mycobacterial cells will be analysed by FACS (<106 cells per sample, IMS 4.08A). Local GM safety procedures have been established for the decontamination of samples and equipment. Fixed samples are expelled directly into 5% Tri-gene. Local GM regulations will be posted in all locations.

Origin & function

The nature of the mycobacterial ligand is currently unknown but possible candidates include genes encoding pathways responsible for the production of cell wall components such as lipids, PIM1,2, PIM6, MAME, LAM, man-LAM, TDM, which could be involved in host cell recognition. More commonly, we will be utilising mycobacterial strains expressing fluorescent markers, such as GFP or commonly used markers for studying immunological processes, such as ovalbumin.

Evaluation of foreseeable effects

It is extremely unlikely that gene deletion and complementation with heterologous genes will alter the virulence of the GMM compared to that of the parent organism. In fact, deletion of cell wall components is most likely to reduce virulence, as has been demonstrated in the literature (Mahon RN et al, 2012, Smith I, 2003).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Liquid waste: 5% Trigene (as per the manufacturer’s instructions)
- Glassware: Soaked with 5% Trigene or autoclaved.
- Plasticware: autoclaved
- Sharps: Contaminated sharps are placed in CinBins, bagged and incinerated.
- Slides from microscope facilities: Slides are transported to/from microscope rooms in plastic petri dishes with their tops sealed with tape or parafilm, and then disposed of as per Sharps.
- Animals and Tissues: All carcases are autoclaved within the MRF, and remaining tissues are autoclaved prior to disposal.
- FACS facility: Fixed samples are expelled directly into 5% Trigene and equipment will be decontaminated with 5% Trigene.

The degree of kill achieved will be >99.99% This is based on previous disinfection validation procedures and information from the equipment and chemical suppliers. Validation will involve sampling inactivated cultures and culturing the samples in rich media plates. Survival of mycobacteria will be assayed by growth at 37°C (M. smegmatis, and M. bovis BCG).

Once GMMs have been inactivated, killed liquid waste is discarded down the sink. Inactivated solid waste is discarded by CFA processing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
his application was approved at Containment Level 2 by the local Genetic Modification Safety Committee.

**Project Containment**

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**Project Ref** 490/13.1

- **Date Ackn'd**: 04/02/2013
- **CU2 Project Title**: Interspecies interactions and mechanisms of killing of the fungus, Candida albicans, by the bacterium, Pseudomonas aeruginosa
- **Date Project Ceased**: 23/02/2021
- **Date of Significant Change**: N
- **Historical Significant Changes**: Yes
- **Historical Date of Additional Info**: Yes
- **Significant Change ID**: N
- **Project notified under transitional arrangements**: N
- **Consent Granted**: Non-GMM

**Project Additional Information**

Candida albicans and Pseudomonas aeruginosa are opportunistic pathogens that can be co-isolated from the cystic fibrosis lung. They have also been used as a model system by several labs for the study of interspecies communication. Previous work by Dr. B has shown that killing of C. albicans hyphae, but not yeast, by the bacterium can occur through the activity of bacterial secreted factors but is more efficient in the presence of the bacterium. The rate of killing is also affected by alterations in the glycosylation state of the fungal cell-wall (Brand et al, 2008).
a. GOALS OF THE PROJECT:
1. To determine the role of P. aeruginosa quorum-sensing (QS) signalling in the killing of C. albicans;
2. To elucidate the mode-of-action in the killing of C. albicans by P. aeruginosa;
3. To characterise inter-species interactions in the context of mixed-species biofilms.

Recipient or parental organism
The P. aeruginosa GM strains used in these experiments are generated in the well-described wild-type backgrounds PAO1 (Stover et al. 2000) and PA14 (Rahme et al. 1995), two fully sequenced strains (www.pseudomonas.com). The strains will be sent to the University of Aberdeen by Dr. S D, using triple-layer packaging and UN3373 labelling, according to Packaging Instruction P1650 for the transport of a Biological Substance Category B.

Host/vector system
Although vectors will be used to deliver DNA elements into the P. aeruginosa genome in order to generate mutant strains, this work will be carried out at the University of Nottingham. The strains used in Aberdeen will not contain vectors and no vectors or plasmids will be used or held in Aberdeen.

Origin & function
We will be using strains which have been genetically modified by gene deletion, gene mutation or gene reporter fusions. The deleted or modified genes will be those associated with quorum sensing in P. aeruginosa. Specifically these are genes regulating, and regulated by, the las operon, the rhl operon and the pqs operon. These include lasI, lasR, rhlI, rhlR, pqsA, pqsB, pqsC, pqsD, pqsH, pqsE, pqsR, pqsL, chic (chitinase), cbpD (chitin binding protein), pvdA, pvdD, pvdH, mexA, mexB, mexH, mexI, opmD, rhiA, phzB1, phzB2, phzA1, phzA2, phnA, phnB, phoB, phoR, lecA, lecB, rsaL, pchA, pchE, catA, catB, hcnA, hcnB, hcnC, rpoS, qscR.

Evaluation of foreseeable effects
a. P. aeruginosa is an environmental bacterium that lives naturally in water, moist soil and swamp conditions. It can become an opportunistic human pathogen in patients with compromised epithelial and immune defences, such as sufferers of cystic fibrosis or those with wounds or burns. P. aeruginosa falls within the ACDP Hazard Group 2. Transmission to people within the laboratory could occur via ingestion, wounds and the eye mucosa, particularly in wearers of extended-use soft contact-lenses. Gloves should be worn to minimise the likelihood of mouth/eye/wound transmission from the hand. Soft contact-lens wearers will use safety goggles when handling the organism. The pathogenic P. aeruginosa can be transmitted via the respiratory tract to people who are severely immunocompromised or are suffering from cystic fibrosis. Such people will not be permitted to work with the organism. P. aeruginosa work is routinely carried out on open-top benches in Class 2 laboratories but risk of exposure to aerosols will be avoided. P. aeruginosa cultures will be centrifuged in sealed-bucket centrifuges and the tubes allowed to sit for 10 minutes prior to opening to allow the aerosols to settle. In the event of equipment failure, such as a burst tube, the centrifuge bucket and the tubes it contains will be removed to a microbiology safety cabinet for decontamination. Safety goggles will be worn during the vortexing of cultures. Lab workers are made aware of these risk factors and are asked specifically in the Local Regulations to make their supervisors (or Occupational Health) aware if they are exposed or susceptible to any such risk factors.

b. The genes inserted as selectable markers will confer resistance to the antibiotics gentamicin or tetracycline, which are not commonly used to treat infection by P. aeruginosa. The compounds, gentamicin and tetracycline, will not be used routinely in Aberdeen, since the insertions are stably in the genome and plasmid maintenance is not required. The genes inserted as fluorescence markers will be standard fluorescent proteins (eg, GFP) used for localisation studies by microscopy. Genes inserted as promoter reporters will be commonly used constructs such as the lacZ gene, which produces a colour change in the cells. The genes inserted for investigation of protein function will be P. aeruginosa genes that have been modified by point mutation with the aim of disabling QS signalling (see c below). The risks arising from the insertion of these genes are low.

c. The virulence factors produced by P. aeruginosa are regulated through the QS pathways, which will be disabled by mutation or deletion of the regulators and effectors of these pathways. The mutation or deletion of QS genes results in an overall reduction of potentially harmful production of toxins (Williams and Camara, 2009). The QS mutants to be used have been shown, in many animal experiments, to be significantly less virulent than wild-type organisms (Rumbaugh et al. 1999; Rumbaugh et al. 2009). The isogenic mutants are extremely unlikely to be more virulent than the parent strains, even in susceptible patients. In addition, a human infection by any of these mutants can be eradicated by antibiotic therapy.
d. The mutants used will have QS genes chromosomally disrupted, so there is little chance of the natural transfer of the mutated gene to other organisms.

In summary, the “Likelihood of Hazard” is medium, since we are handling a potential pathogen and it is not possible to exclude accidents entirely, although there have been no accidents involving P. aeruginosa since the start of work on wild-type cells in the AFG in 2003. The “Consequence of Hazard” is low, since serious infections only occur in individuals with pre-disposing conditions (cystic fibrosis, open wounds/burns or severely compromised immune systems. Therefore the “Estimation of Risk to Humans” is low.

Quorum-sensing mutants have been shown to be significantly less virulent than their wild type counterparts. If these mutants were to enter the environment they would provide no extra risk than those posed by natural populations of P. aeruginosa found in the environment. In addition, natural QS mutants can be isolated from both the environment and human cystic fibrosis infections so quorum sensing mutation is natural within P. aeruginosa populations.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Liquid waste: 2 % Trigene/Distel (active ingredient = TM307).
- Glassware: Soaked with 2 % Trigene/Distel (TM307) or autoclaved.
- Plasticware: autoclaved.
- Sharps: Contaminated sharps are placed in CinBins, bagged and incinerated.
- Slides from microscope facilities: Slides are transported to/from microscope rooms in plastic petri dishes with their tops sealed with tape or parafilm, and then disposed of as per Sharps as they carry minimal volumes of cultured cells (<20μl) and present minimal risk.
- Samples used for analysis by scanning electron microscope will be fixed in 2.5 % glutaraldehyde and 3 washes with ethanol, which has been established experimentally to kill 99.99 % P. aeruginosa cells.
- Liquid waste, after inactivation, will be disposed of to drain. Solid waste, after inactivation will be sent off site as part of the “orange bag” waste stream. The waste will be macerated to make it unrecognisable and further heat treated before being placed in landfill.
- In the event of spillage, the contaminated area is flooded with concentrated Trigene/Distel (to 2 % final conc.), mopped up with paper towels, and reswabbed with 2 %. Killing at >99.99% has been validated experimentally. Contaminated material is autoclaved.
- The use of Trigene/Distel has been validated experimentally to kill >99.99% of cells. Viability tests were performed on Trigene/Distel-exposed cultures. Central autoclaves are validated at least annually by means of biological indicators. In addition we have validated killing at >99.99% by plating samples harvested from the centre of autoclaved samples. In daily use, monitoring is by thermocouple temperature recordings and autoclave strips included in the loads. Bench-top autoclaves in our laboratory are also tested regularly. We test these autoclaves monthly, and in addition they are examined by external experts twice a year. We have validated killing at >99.99% by plating samples harvested from the centre of autoclaved samples.
- No viable cells have been recovered after fixation in 2.5 % glutaraldehyde and 3 ethanol washes, as used for scanning electron microscopy.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
This application was approved at Containment Level 2 by the local Genetic Modification Safety Committee. They were satisfied that generally the work could be carried out on the open bench. Following experiments to determine the risk posed by aerosols after centrifugation, it was agreed that tubes containing more than 10 ml culture should be allowed to sit for 10 minutes following centrifugation prior to opening the tube. In the event of a split tube, the centrifuge bucket would be opened in a Class 2 microbiology safety cabinet for decontamination.

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Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: N
Project Additional Information

Purposes of the contained use

The objective is to understand how zygomycete fungal pathogen interacts with innate immune systems and how calcineurin is involved in this interaction. The two calcineurin mutants have been created at Duke University, Durham, NC, USA. Wild-type and mutants will be grown on agar plates for spore or yeast production. The spores or yeast then will be co-cultured with macrophages in vitro to examine the interactions.

This experiment is to elucidate 1) how spore size affect the host-pathogen interactions and 2) how calcineurin is involved in the host-pathogen interactions.

Recipient or parental organism

Mucor circinelloides is a soil-borne fungus that is common part of microflora. Mucor is also known as bread mould and a common lab contaminant. Therefore exposure to these organisms with high spore counts in immunocompetent individuals does not normally cause problems. Zygomycete fungi are potential human pathogens in immunocompromised individuals and are therefore ACDP Hazard Group 2. Under certain circumstances such as neutropenia due to solid organ or bone marrow transplant, and in poorly controlled diabetic patients this fungus is a potential life-threatening pathogen of high morbidity. Rare cases have been reported in individuals with no apparent underlying predisposition but systemic infection does not occur in healthy individuals so is readily treated with anti-fungal agents. The strains (CBS277.49 and NRRL3631) used in this study are not virulent in the heterologous murine host.

Two wild-type strains will be used: CBS277.49, a larger spore producer and NRRL3631, a small spore producer

Host/vector system

Mucor circinelloides has one calcineurin B regulatory subunit (CnbR) and three calcineurin A catalytic subunit (CnaA, CnaB, and CnaC). MSL9 and MSL10 are cnaA mutants and MSL7 and MSL8 are cnbR mutants

Origin & function

We will be using strains of Mucor circinelloides which have been genetically modified by gene replacement. The cnbR and cnaA genes were replaced with the auxotrophic marker gene, pyrG, that is originated from its own genome and has no effect on virulence.

Evaluation of foreseeable effects

The strains to be used are attenuated by either (i) auxotrophy or (ii) due to disruption in calcineurin genes. Wild type strains that have not been subjected to genetic manipulation may be used as controls.

Mucor circinelloides spores are very common in all environments since it is a part of the normal microflora of all plants and decaying vegetation. The main hazard is from the inhalation of large numbers of spores leading to sensitisation over many years (e.g. "farmers lung") or invasive mucormycosis in a few severely immunocompromised patients who would normally already be in intensive care. These patients include organ and bone-marrow transplant patients and cancer patients. In terms of diabetics, it is usually associated with poorly controlled diabetes involving ketocidosis. Individuals will be made aware of the risks of the project and will be asked to inform their supervisor or Occupational Health if they have any concerns. Although extremely rare, mucormycosis generally affects the skin, sinuses or lungs. It is anticipated that the calcineurin mutants used in this study will be less pathogenic than their wildtype counterparts.

We will therefore prevent the release of spores. All cultures are in sealed dishes or bottles and are killed by autoclaving prior to cleaning. Cultures that are heavily sporylating will only be opened in a microbiology safety cabinet and used for sub-cultures although even acute exposure to high numbers of spores does not lead to disease. It is noted that mucoraceous fungi do not produce large numbers of aerially dispersed spores, such as with Aspergillus species.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste: 1% Trigene
Glassware: soaked with 1% Trigene or autoclaved.
Plasticware: autoclaved
Sharps: Contaminated sharps are placed in CinBins, bagged and incinerated.

Our standard procedures for use of Trigene have been validated experimentally to kill >99.99% of cells. Viability tests were performed on Trigene-exposed cultures. Autoclaves on level 1 are validated at least annually by means of biological indicators. In addition we have validated killing at >99.99% by plating samples harvested from the centre of autoclaved samples. In daily use, monitoring is by thermocouple temperature recordings and autoclave strips included in the loads. Bench-top autoclaves in our laboratory are also tested regularly. Our laboratory Manager tests these autoclaves annually. We have validated killing at >99.99% by plating samples harvested from he centre of autoclaved samples.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This application was approved at Containment Level 2 by the local Genetic Modification Safety Committee

Project Containment

<table>
<thead>
<tr>
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<th>Growth Rooms</th>
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<td>Human Clinical Applications</td>
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Project Ref 490/13.3

Date Ackn'd 19/08/2013  Project Title Protein expression in mammalian cells using lentiviral constructs

Class 2  CultureVolClass2 < 1 Litre  CultureVolumeClass3-4

02/03/2022  Page 7852 of 15326
Project Additional Information

Purposes of the contained use

To examine how overexpression of factors regulating translation affect neuron function.

Recipient or parental organism

- E. coli K12 derivatives DH5-alpha, XL-1 blue, JM-109 will be used for amplification of the pLKO.1 and perceiver plasmids containing expression cassettes for shRNA and protein expression, respectively.
- HEK293TN cells, primary rodent neurons, neuronal cell lines including SH-SY5Y, HCN-A94 and PC12 cells, transformed mammalian cell lines including HeLa and U2OS cells.

Host/vector system

- System Biosciences’ (SBI) lentivectors are a set of third generation lentivectors developed for gene therapy. The multi-plasmid system consists of three plasmids that make up the packaging mix, and one plasmid, the lentivector, containing the expression cassette and elements for the synthesis of the production of viral RNA. The packaging mix is composed of pPACKH1-GAG, pPACKH1-REV and pVSV-G, and these are transfected together with a lentivector into HEK293TN cells to produce infective particles. The viral RNA expressed from the lentivector is then packaged by the proteins produced by the packaging mix into viral particles.

Origin & function

We have discovered mutations in the promoter of the translation initiation factor eIF4E that increase eIF4E expression in patients with autism and schizophrenia (Neves-Pereira et al., J. Med. Genet. 46;759-765) and there is evidence from others that overexpression of eIF4E predisposes to autism. eIF4E is central to the control of translation, and localized post-synaptic translation is involved in long-term potentiation and instrumental in memory and learning. We will examine how overexpression of eIF4E affects neuron function. We have already tested the effects of eIF4E overexpression using plasmid-based overexpression. Although findings are promising we were hampered by low transfection efficiency in achieving our aim, the analysis of the effects of candidate protein expression on neuronal differentiation, RNA and protein expression. This problem can be solved by using lentivirus-based constructs for protein overexpression. We will express eIF4E, an essential translation initiation factor conserved in humans and rodents in neuronal and other cells.

Evaluation of foreseeable effects

- Lentivectors have several biosafety features that prevent replication or recombination with wild-type virus in any way that would produce viable virus. They have a deletion in the enhancer of the U3 region of 3'LTR that ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target.
cells. The RSV promoter (in HIV-based vectors) and the CMV promoter (in FIV-based vectors) upstream of 5’LTR in the lentivector allow efficient Tat-independent production of viral RNA, reducing the number of genes from HIV-1 that are used in this system.

- The number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev). The corresponding proteins are expressed from different plasmids that lack packaging signals. The packaging plasmids share no significant homology to any of the expression lentivectors, the pVSV-G expression vector, or any other vector, to prevent generation of recombinant replication-competent virus. None of the HIV-1 genes (gag, pol, rev) are present in the packaged viral genome, as they are expressed from separate plasmids lacking packaging signal. Therefore, the lentiviral particles generated are replication-incompetent.
- Produced pseudoviral particles will carry only a copy of your expression construct.
- This multi-plasmid approach results in no single plasmid containing all the genes necessary to produce packaged lentivirus.

The eIF4E protein is not toxic. Elevated expression of eIF4E has been found in cancer tissues and eIF4E overexpression promotes tumor formation (De Benedetti, A. and Graff, J.R.. eIF-4E expression and its role in malignancies and metastases. Oncogene 23: 3189-3199). Recently it was also described that mice overexpressing eIF4E develop autistic traits (Santini et al., Nature 493, 411-415).

As cells used are not able to colonise humans, and no sharps will be used to avoid accidental injection the eIF4E sequence does not present any additional risk.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Adding 1% Virkon to biological wet waste and plastics and leaving overnight prior to rinsing and disposal in double hazardous waste bags. Then all bags will be tagged with blue tags and labelled with the lab number prior to autoclaving and disposal. Inactivated liquid waste will then be disposed with normal liquid waste. Solid waste will be macerated and further heat treated before being placed in landfill.

Autoclaving is by validated (internal temperature probe linked to printout) autoclave, in the IMS building, reserved only for inactivation of waste (effective 100% kill). The autoclave undergoes annual testing to ensure correct operation.

The virucidal disinfectant, Virkon, has been validated for inactivation of lentivirus at a concentration of 1%. Details of Virkon testing from independent studies can be found at [http://www2.dupont.com/RelyOn/en_US/products/virkon.html](http://www2.dupont.com/RelyOn/en_US/products/virkon.html) or requested through [http://www2.dupont.com/RelyOn/en_US/sales_support/contact.html](http://www2.dupont.com/RelyOn/en_US/sales_support/contact.html).

Monitoring measures: HT1080 cells will be grown in the presence of increasing (10 fold) amounts of inactivated waste. The cells will then be selected for using the antibiotic blasticidin and incubated for 10 days before being stained with Crystal Violet. The identification of any blasticidin-resistant colonies demonstrates the presence of active lentiviral vector within the ‘inactivated’ waste.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y
This project was approved at Activity Class 2 by the local Genetic Modification Safety Committee

### Project Containment

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### Project Ref 490/13.4

**Date Ackn'd**
30/10/2013

**CU2 Project Title**
Antimicrobial mechanism of the Rab32-dependent pathway in macrophages

**Date Project Ceased**

**Class**
Class 2

**CultureVolClass2**
< 1 Litre

**CultureVolumeClass3-4**

**Non-GMM Consent Granted**

**Project notified under transitional arrangements**
N

### Additional Information

#### Purpose of the contained use
To investigate the effect of the Salmonella Typhimurium effector GtgE on the Rab32/BLOC-3 pathway

#### Recipient or parental organism
1) S. Typhimurium wild type strains SL1344, LT2 and 14028S.
2) Standard laboratory strains of disabled E. coli, such as DH5 and XL-1 blue, will be used for standard molecular biology procedures. The E. coli strain CC118 lambda-pir will be used for cloning pSB890-derived plasmids and the E. coli strain beta-2163 Δnic35 (described in Babic et al. doi:10.1016/j.resmic.2008.06.004) for conjugative transfer of pSB890-derived plasmids.

3) Human cell lines such as Hela or THP-1 and mouse cell lines such as RAW 264.7.

4) VSV-G pseudotyped replication-defective retroviral vectors based on Moloney murine leukemia virus.

**Host/vector system**

Non-mobilisable low copy plasmids (such as pWSK129 and pACYC184) and high copy plasmid, such as pUC, pcDNA3 or pEGFP, will be used in standard molecular biology procedures to transform disabled E. coli strains. The conjugative plasmid pSB890, which carries an R6K origin of replication only functional in strains expressing the pir gene, will be used for homologous recombination and allelic exchange. pLZRS retroviral vector, which contains contains long terminal repeat and packaging sequences from the Moloney murine leukemia virus, will be used to clone host transport factors (such as Rab32), will be used to generate VSV-G pseudotyped virus, and transduce mouse RAW 246.7 macrophages.

**Origin & function**

Fluorescent proteins (e.g., GFP and mCherry) will be cloned in low copy plasmids such as pWSK129 and pACYC184. The resulting plasmid will be transformed into and S. Typhimurium strains to be able to visualize the bacteria by live imaging microscopy.

We have previously shown that the S. Typhimurium effector GtgE is a cysteine protease that targets Rab32 and antagonize the Rab32/BLOC-3 pathway. Introduction of gtgE into S. Typhi allows S. Typhi to survive in mouse macrophages as a consequence of antagonizing the Rab32/BLOC-3 pathway (Spanò and Galán, Science, 338, 960). The anti-microbial mechanism associated to this transport pathway remains completely unknown. It is intended to to delete Salmonella type III secretion effector genes from S. Typhimurium. To this purpose the flanking regions (~800 bp) of specific Salmonella type III secretion effector genes will be cloned into the pSB890 plasmid and the plasmid transformed into the E. coli strain CC118 lambda-pir. The plasmid will be then transformed in the E. coli strain beta-2163 Δnic35 and conjugation with S. Typhimurium strains will be performed. Homologous recombination and allelic exchange will be performed as described in “Genetic Analysis of Pathogenic Bacteria”, Cold Spring Harbor Laboratory Press.

**Evaluation of foreseeable effects**

E. coli: The bacteria to be used in the protocol are disabled through the use of multiple stable disabling mutations that make them highly unlikely to survive in the environment and unable to colonize the intestine. Salmonella type III secretion effector genes tagged with specific epitopes, such as 3xFlag, or flanking regions of Salmonella type III secretion effector genes are unlikely to alter existing pathogenic traits in E.coli disabled strains.

S. Typhimurium: The manipulations of the bacterial genes to be carried out will introduce loss of function mutations and will not increase the pathogenesis of the bacteria. Fluorescent proteins are not been reported to alter any existing pathogenic traits. Salmonella type III secretion effector genes tagged with specific epitopes, such as 3xFlag, or flanking regions of Salmonella type III secretion effector genes are unlikely to alter existing pathogenic traits in S. Typhimurium as they include only homologous DNA and commonly used epitope tags. They are unlikely to alter stability or pathogenicity of the recipient strains.

Mammalian cells: Host transport factor genes, such as the small GTPase Rab32 gene, do not alter any existing pathogenic traits in cell cultures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated waste (solid and liquid) will be inactivated by autoclaving at +121°C for 15 min which is expected to result in 100% kill. The autoclaves are serviced and calibrated twice per year to confirm that the necessary levels of temperature and pressure are maintained for the required time. Further validation will be on a monthly basis using an autoclave indicator stick will be included in the waste.

Inactivated solid wastes will be sent off site as part of the "orange bag" waste stream. The waste will be macerated to make it unrecognizable and further heat treated before being placed in landfill.

Spills will be treated with Haz-Tab solutions containing 10,000ppm chlorine

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

This project was approved at Activity Class 2 by the local Genetic Modification Safety Committee

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Project Ref 490/14.1

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<td>19/03/2014</td>
<td>Characterisation of testicular somatic cells and germ cells derived from pluripotent stem cells (ES and iPS cells)</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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Date Project Ceased: 02/03/2022
To derive testicular somatic cells and germ cells from pluripotent stem cells. We will transduce genes specifying these cells into ES/iPS cells and tissues dissected from mice, using replication-deficient lentiviral vectors. Moreover, for generation of iPS cells from mouse skin fibroblasts, pMX replication-deficient retroviral vector will be used.

For cloning of genes specifying somatic or germ cells into viral vectors, E. coli K-12 based strains, such as DH5α, XL-1 Blue, α-Select, Stable2, will be used. Platinum-E (Plat-E) and HEK293 cell lines will be used for packaging retroviruses and lentiviruses, respectively. Using these viral vectors, we will transduce primary cells isolated from mouse gonads. We will also transduce mouse ES and iPS cells to differentiate into a specific gonadal cell-type, such as Sertoli cells, Leydig cells and germ cells.

Recipient or parental organism

For cloning of genes specifying somatic or germ cells into viral vectors, E. coli K-12 based strains, such as DH5α, XL-1 Blue, α-Select, Stable2, will be used. Platinum-E (Plat-E) and HEK293 cell lines will be used for packaging retroviruses and lentiviruses, respectively. Using these viral vectors, we will transduce primary cells isolated from mouse gonads. We will also transduce mouse ES and iPS cells to differentiate into a specific gonadal cell-type, such as Sertoli cells, Leydig cells and germ cells.

Host/vector system

pMX retroviral vector (Kitamura et al., Exp Hematol.11, 1007-1014. 2003) that carries 5’ and 3’ LTR, but lacks gag, pol and env will be used for generating mouse iPS cells. Platinum-E (Plat-E) cells will be used as an ecotropic retrovirus packaging cell line. We also use commercially available 3rd generation lentiviral vectors that contain SIN (self-inactivating) mutation in the LTR and lack gag, pol, env and rev. For packaging, the lentiviral vectors will be co-transfected with additional plasmids, pRRE, pVSV-G, and pRev into HEK293 cells (human embryonic kidney cells transformed with sheared adenovirus 5 DNA).

Origin & function

To derive testicular somatic cells and germ cells from pluripotent stem cells, ES/iPS cells are transduced with lentiviral vectors carrying genes required for differentiation of Sertoli cells, Leydig cells and germ cells. The precise identity of these genes cannot be specified at the moment because they will be identified by future high-throughput gene expression profiling experiments.

A number of genes are expressed in the testis as shown by other groups. Some of them are originally thought to be oncogenes and therefore unregulated expression in mammalian cells may cause transformation when transduced. The eukaryotic systems have been designed that functional protein would be expressed only when lentiviral vectors were introduced to mammalian cells.

Genes required to generate iPS cells are Klf4, Sox2, Oct4 and Myc. These genes encode transcription factors involved in many aspects of cell differentiation, e.g. neurogenesis, epithelial cell differentiation, and most notably formation of pluripotent stem cells in the inner cell mass (ICM) of early embryos. Myc is a well-known oncogene, but the retrovirus carrying the myc cDNA protein will not infect humans because the virus is ecotropic.

Evaluation of foreseeable effects

Although the viruses primarily produced are infectious; the retrovirus are ecotropic (infects only rodents) and the lentivirus is amphotropic (infects mammals including...
humans), secondary viruses will not be produced from infected cells because gag, pol, env and rev genes are absent. We would not therefore expect any specific symptoms as the result of an accidental infection with the genetically modified micro-organisms described in this application.

However, if the lentiviral vector were accidentally injected into carriers of wildtype HIV, there may be a theoretical risk of recombination of the vector with wildtype virus to produce replication competent virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project, these risks will be made clear to all personnel in the laboratory.

Genetically modified micro-organisms could not survive outside laboratory conditions and thus there is no hazard to the environment. Therefore, the chances of any of the retroviral vectors (ectropic retroviruses or VSV-G pseudotyped lentiviruses) coming into contact with rodents is extremely low. All waste will be inactivated prior to leaving the premises and cultures containing retroviruses will not be removed from the laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from mammalian cell cultures will be autoclaved before disposal down the sink. Plastics and other solid disposable waste will be collected in autoclavable bag within the biological safety cabinet, sealed and then sprayed with 1% Virkon (according to manufacturer’s instructions). The bag will then be removed from the safety cabinet into a clean autoclave bag, sealed, and taken to the autoclave in a sealed container. The degree of kill following autoclaving will be 100% since there is a high expectation of kill of GM microorganisms exposed to 121°C for 15 minutes. The autoclaved waste will then be disposed of by continuous feed auger.

Glass bottles and other non-disposable vessels will be soaked in the appropriate concentration of 1% Virkon in a bucket with a sealed lid, rinsed and then autoclaved for future use.

Validation will involve regular testing of the autoclave to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time. At the start of the project, we will produce retrovial and lentiviral vectors carrying the EGFP gene, and then validate the inactivation of these viral vectors following autoclaving. Proliferative mouse embryonic fibroblasts will be grown in the presence of increasing (10 fold) amounts of inactivated waste. The cells will be incubated for 10 days. The identification of any green fluorescent colonies demonstrates the presence of active viral vectors within the ‘inactivated’ waste.

The virucidal disinfectant, Virkon, has been validated for inactivation of lentivirus at a concentration of 1%. Details of Virkon testing from independent studies can be found at http://www.antecint.co.uk/MAIN/virkonvi.htm.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This application was approved at Activity Class 2 by the local Genetic Modification Safety Committee.

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#### Project Ref 490/14.2

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<td>19/03/2014</td>
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<td>Class 2</td>
<td>≤ 1 Litre</td>
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#### Project Additional Information

**Purposes of the contained use**

At present, very little is known about how dermatophytes cause disease, due to poor infection models (having to use zoophilic species rather than anthropophilic species) and poor genetic tools. Dermatophyte infections are the most common fungal infections in the World, affecting up to 20% of the population at any one given moment. Some individuals have recurrent infections, but it is not yet known whether these infections represent new infections or reactivation of a latent infection. At present there are few research groups working on these pathogens; however, the recent public release of the genome sequences of seven dermatophytes by the Broad Institute and the Hans Knoll Institute, Jena, will change this, stimulating research in this field.
The scientific goals of this study are to investigate the pathogenesis of dermatophyte infections, including regulation and mechanisms of cell wall biosynthesis, and response to nutrients and stresses, by using targeted gene disruption to create null mutants or to over express specific genes. Mutants expressing an iRFP (infra-red fluorescent protein) reporter will also be created to allow in vivo imaging in experimental models. The study also intends to study the host immune response to dermatophytes using mutants which lack or over express specific genes, to determine which fungal cell wall components are important in the host-pathogen interactions.

Dermatophyte fungi cause superficial skin, hair, and nail infections (e.g. tinea, ringworm, jock itch and athlete's foot). Dermatophyte infections can affect the skin on almost any area of the body, such as the scalp, legs, arms, feet, groin, and nails. These infections are usually itchy. Redness, scaling, cracking of the skin, or a ring-shaped rash may occur. Dermatophyte infections are usually spread through direct contact with an infected person or animal. Clothing, bedding and towels can also become contaminated and spread the infection. Symptoms typically appear between 4 and 14 days following exposure (www.cdc.gov). The most common causes of these infections are Trichophyton species, Microsporum species and Epidermophyton floccosum. All dermatophyte fungi are classified as ACDP Hazard Group 2.

**Recipient or parental organism**

| Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum canis and Epidermophyton floccosum (dermatophytes) isolates. |

**Host/vector system**

Vectors to carry out transformation will have either the Hygromycin B phosphotransferase (hbp) gene, the neomycin phosphotransferase (nptII) gene (Yamada et al., 2008 J. Dermatol Sci), or the nourseothricin acetyltransferase (NAT) dominant selectable marker (Alshahani et al. 2010 Med Mycol).

**Origin & function**

The genetic material involved will represent plasmid DNA obtained from collaborators and/or created by the Aberdeen Fungal Group, University of Aberdeen or will be polymerase chain reaction (PCR) amplified DNA fragments, neither of which can be autonomously replicated in fungal cells.

The plasmid DNA or PCR fragments will contain DNA regions identical to sections Genes in the fungal genome allowing integration of the DNA into the fungal genome through homologous recombination. In addition the plasmid DNA or PCR product will also contain a selectable marker allowing cells which have taken up the new genetic DNA to be selected in culture.

**Evaluation of foreseeable effects**

To date, any stable gene disruptions made in dermatophyte fungi have either led to reduced growth rates or have had no effect on growth. Similarly, virulence of the strains has either been reduced or unaffected.

The dominant selectable markers to be used in this study have not been shown to affect the virulence of dermatophytes.

There do not appear to be any hazards associated with altering potential pathogenic traits of these fungi. With gene disruption, we are aiming to create strains which have reduced virulence, and with overexpressing strains we are aiming to produce strains which stimulate the host immune response to clear the fungus (again reducing virulence). To date, all genetically modified dermatophytes have either shown reduced or unaffected virulence.

There is no evidence for any ability of dermatophytes to be capable of transferring genetic material with other organisms. Where strain typing has been attempted, there is little evidence for huge strain differences, suggesting relatively stable genomes and a lack of evidence for exchange of genetic material.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management:
Liquid waste: add 1% Distel (previous name: Trigene) as per the manufacturer’s instructions. In the event of spillage, the contaminated area is flooded with concentrated Distel (to 1% final conc.), mopped up with paper towels, and reswabbed with 1% Distel. Contaminated materials are autoclaved.

Glassware: Soaked with 1% Distel or autoclaved.

Plasticware: autoclaved

Animals and Tissues: All carcases will be autoclaved within the MRF, and remaining tissues are autoclaved prior to disposal.

Contaminated waste, with the exception of carcases in the MRF, will be autoclaved either in the research laboratories or in the IMS level 1 wash-up facilities.

Once GMMs have been inactivated, normal routes of disposal are used for liquid and solid waste. Killed liquid waste is discarded down the sink. Inactivated solid waste is discarded by CFA processing.

Distel:
Our standard procedures for use of Distel have been validated experimentally to kill >99.99% (degree of kill) of all dermatophyte species relating to this application (Trichophyton species, Microsporum species and Epidermophyton floccosum) (www.tristel.com).

Autoclaves:
Autoclaves on in the central autoclave facility are validated at least annually by means of biological indicators. In daily use, monitoring is by thermocouple temperature recordings and autoclave strips included in the loads.

Bench-top autoclaves in our laboratory used for inactivation of waste are tested regularly (using Browne tubes for assessing whether time and temperature within autoclave has been reached) and serviced.

Samples from autoclaved or Distel-treated, inactivated waste will be regularly (once a month) plated and assessed for fungal viability. A written record will be kept of all test results.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
Genetic modification safety committee did not make additional recommendations on the risk assessment, but emphasised the need to enforce wearing of protective garments (gloves and lab coats) when handling dermatophyte fungi.

**Project Containment**

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**Project Ref** 490/14.3

**Date Ackn’ed** 27/05/2014

**CU2 Project Title** Insight into filamins from filamin pathological mutations

**Class** Class 2

**CultureVol** < 1 Litre

**Class CultureVol** Class 2 < 1 Litre

**Consent Granted**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

a) The scientific goal of the project is to understand how cells sense the mechanical cues in the extracellular space and how they are transmitted to the cytoskeleton and, in turn control the re-arrangement of extracellular matrix. The role of the cytoskeletal proteins filamins will be analysed in detail. Initially overexpression or knocking down of filamins family members will be performed and the resulting modifications induced in the cells will be studied using biochemical and microscopical techniques. Then this kind of analysis will be expanded and applied to the filamins interactors with the final aim to characterise the complete signalling pathway involved in the mechanosensitive.
mechanism.

**Recipient or parental organism**

Human cells lines such as Hek293T, Hela and HT1080 will be used as final recipient of the plasmids. In case there is a need for a non human cell type, NIH 3T3, mouse MEF or CHO will be used.

**Host/vector system**

Pseudo-typed lentiviral particles will be produced in HEK293T cells using a third-generation packaging system, which has many features that lead to enhanced biosafety. Recombinant lentiviruses produced with the MISSION Lentiviral Packaging Mix (Sigma Aldrich catalog number SHP001) have not been shown to produce replication competent viral particles because of designed safety features. It is a plasmid system consisting of:

1. The packaging vector, which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions.
2. The vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector, which provides the heterologous envelope for pseudotyping.
3. The shRNA transfer vector (pLKO.1-Puro), which contains the sequence of interest as well as the cis acting sequences necessary for RNA production and packaging. This vector contains a sequence encoding a puromycin resistance gene.

The multi-plasmid approach results in no single plasmid containing all the genes necessary to produce packaged lentivirus. The lentiviral vector contains the first two components; it is designed to be co-transfected along with a compatible lentiviral transfer vector in order to create high-titer pseudo-typed lentiviral particles used. Resulting particles are replication-incompetent and deletion in the U3 portion of the 3’ LTR eliminates the promoter-enhancer region, further negating the possibility of viral replication. The system has also removed virulence genes which are not necessary for shRNA packaging.

In addition, the lentiviral vector contains a self-inactivating 3’ LTR that renders it unable to produce infectious virus once it integrates into the host chromosome. These features combined have improved biosafety and handling. This information appears on-line in: http://www.sigmaaldrich.com/life-science/functional-genomics-and-mi/shrnawww/learning-center/mission-faqs/biosafety.html

HEK293, HELA, HT1080, NIH-3T3, mouse MEF or CHO cells will be transduced with the pseudo-viral particles. In most cases and in order to rule out compensatory effects of the protein knockdowns, the resulting cell lines will be phenotyped and then disposed at the end of the experiment. In the remaining cases, stable cell lines will be generated. In these cases, no additional hazards compared with any other transformed cell line are present as the lentiviral vector contains a self-inactivating 3’ LTR that in all cases renders it unable to produce infectious virus once integrated into the host chromosome.

**Origin & function**

In the overexpression experiments all filamin family members will be investigated. A large range of proteins will be tested in the suppression experiments (i.e. using the shRNA to suppress their expression in cultured cells). In this case proteins that are known to interact with filamins will be examined. So far there are around 90 proteins described as Filamin interactors. Among them there are cytoskeletal proteins (FILIP, ILK, TRIO) transmembrane receptors (ITGB1, ITGB3, ITGB7, CFTR, CASR) protein kinases (SRC, MAPK14), E3 ligases (ASB2) and deubiquitinases (OTUD1).

The majority of the genes used in this project do not have a known oncogenic potential. The effect on cell differentiation/proliferation of the overexpression or suppression of filamins is not well characterized, however the available data indicate that both the overexpression and the removal of filamins proteins have adverse effect on cell growth. Some of the filamin interacting proteins to be used in the suppression experiments have been involved in cell proliferation in some specific conditions (SRC, Integrins) but the only one shown to be a tumour suppressor is BRCA2. This is the only protein among the filamin interactors that could promote cell proliferation if silenced and will not be studied in this project.

**Evaluation of foreseeable effects**

The GMM and transfected cells will be of very low hazard to the environment as they cannot survive outside of laboratory. The use of the heterologous envelop protein such VSVg in place of the native HIV-1 decreases the risk for human health, however it may broaden the host and tissue tropism and it could be a concern for the environment. The frequency of recombinant competent lentivirus generation using lentivirus vectors is very low, however to minimize the risk only relative small volume will be used.

We would not expect any specific symptoms as the result of an infection with the genetically modified micro-organisms described in this application since the lentiviral vectors cannot replicate.

No sharps will be used in any of these experiments.
The vectors have been designed so that they are incapable of recombining with wild-type virus in any way that would produce viable virus. Because viral structural genes have been placed on different genetic units, multiple recombination events must occur before a replication competent helper virus is generated. Furthermore, areas of homology among the units expressing the helper virus proteins have been minimized. Also, heterologous promoters for the helper virus proteins are used. Resulting particles are replication-incompetent and deletion in the U3 portion of the 3' LTR eliminates the promoter-enhancer region, further negating the possibility of viral replication (http://www.sigmaaldrich.com/life-science/functional-genomics-and-rna/shrna/learning-center/mission-faqs/biosafety.html)

However, highly improbable viral replication-competence will be checked by subjecting cell cultures to puromycin resistance after treating these cells with media collected from cells that have been treated with viral supernatant which would lead to puromycin resistance. Additionally, cell media will be immunoblotted in order to rule out the presence of the viral protein p24.

The transfected cultured cell lines will not survive outwith the culture medium and all liquid and solid waste will be autoclaved on site. This rules out the possibility of escape into external environment and survival, establishment or dissemination therein.


Factors such as congenital immunodeficiency and secondary immunodeficiency caused by serious illness or chemotherapy would increase the susceptibility to infection by micro-organisms, including the genetically modified micro-organisms described in this application. In carriers of wildtype HIV there may be a theoretical risk of recombination of the lentiviral vector with wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project, these risks will be made clear to all personnel in the laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Virkon will be added to biological wet waste and plastics and this waste will be left over night prior to rinsing and disposal in double hazardous waste bags. Virkon is a unique, synergised oxidising system based on potassium monopersulphate which is proven effective against all major pathogens known to man such as viruses HIV/AIDS, hepatitis B, polio, lassa and rift valley fevers. Proven against a wide range of antibiotic-resistant strains. For more info, please see: http://www.rmsupply.co.uk/Virkon_lab_products.htm.

All bags with the material previously inactivated as described above will be then be taken to the central autocalving facility. Autoclaving is by validated (internal temperature probe linked to printout) autoclave, reserved only for inactivation of waste (effective 100% kill). The autoclave undergoes annual testing to ensure correct operation. Waste will be transferred to the autoclave in covered containers.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
This application was approved at Activity Class 2, by the local Genetic Modification Safety Committee.

**Project Containment**

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**Project Ref** 490/14.4

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<td>Inflammatory signals regulate neuroendocrine control of growth and energy balance through re-modelling of mammalian hypothalamus</td>
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<td>Non-GMM</td>
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Tick if notifying a connected programme of work

Project notified under transitional arrangements

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to determine if nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) is involved in the development of inflammation in the brain that occurs as seasonal animals adapt to the lighting of their environment. This inflammation is thought to cause hypothalamic remodelling, involved in the regulation of seasonal body weight changes and the timing of reproduction. Similar inflammation has been reported to occur in conditions of chronic over nutrition. The aim of the project is to generate replication incompetent lentiviral vectors that will cause modified NFkB signalling; specifically, vectors will be produced that will (1) express the dominantly negative nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IkBa) that is a potent inhibitor of NFkB expression or (2) facilitate...
constitutive expression of IKKβ that will stimulate NFκB overexpression.

Recipient or parental organism

Lentiviral vectors will be delivered to rats intracerebrally using a stereotaxic frame to allow reproducible site directed delivery to the hypothalamus using coordinates according to the Bregma line and brain maps. The virus will be delivered through a Hamilton syringe with blunt needle which will be placed into the frame whilst empty. The syringe will be loaded from stock tubes held in clamps keeping fingers clear of the needle and lowered slowly to a precise depth using a screw mechanism which is part of the frame set up, and done whilst the rats are under general anaesthetic. At the end of a surgery day, the syringe and needle will be removed from the frame and discarded as a single unit into a sharps bin. These measures should reduce needle stick injury risk.

Barriers to prevent escape of rats will be present at exits, drains and vents or other ducts or possible escape routes. Unauthorised or untrained personnel will not have access to the lentiviruses. These lentiviruses will be clearly labelled and stored in screw-capped tubes within a sealed container in an appropriate fridge or freezer with biohazard warning labels.

The rats will be monitored for food intake and body weight for a period of at least 4 weeks following inoculation with the lentiviruses, with the objective being to see differential responses compared to control rats. The rats may be kept for up to 16 weeks before killing. Brains will be removed and immediately frozen on dry ice. The carcasses will be disposed of to waste as described below. The brains will be transported to the RI NH and stored at -80°C. Brains will be cryosectioned at the RI NH and processed for gene expression analysis.

Although extremely unlikely, the potential exists for the transfer of genetic material from the infected animal to other animals, with increased tropism conferring the capability to infect a wide range of mammalian cells due to the incorporated VSV-G envelope protein, in the first 48-72 hours post-infection through transfer of virus from body fluids. After this time risk of active virus transfer reduces to negligible levels as does the potential for transfer of genetic material between the GM rat and other organisms due to the viral incompetence to replicate, short half-life and inefficient transduction rates, thus the risk of transmission outside the laboratory is effectively zero. The viruses will not infect microbial organisms.

Host/vector system

Lentivirus will have been generated off site (e.g. Vector Biolabs) using Invitrogen’s ViraPower™ Lentiviral Expression System that allows creation of a replication incompetent, HIV-1-based lentivirus. The ViraPower™ Lentiviral Expression System is a third-generation system based on lentiviral vectors developed by Dull et al., 1998*. This third-generation lentiviral system includes a significant number of safety features designed to enhance its biosafety and to minimize its relation to the wild-type, human HIV-1 virus. These safety features are as described by Invitrogen are copied below:

The pLenti expression vector contains a deletion in the 3′ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.

• The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).
• The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope.
• Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).
• Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
• The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.
• Expression of the gag and pol genes from pLP1 has been rendered Rev dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al., 1998).
• A constitutive promoter (RSV promoter) has been placed upstream of the 5′ LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA (Dull et al., 1998).

Origin & function

The inserted genes IKKβ (CA) and IκBα (DN) impact directly on the expression of NF-κB which is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and cell survival. As such, many different types of human tumours have misregulated NF-κB. Active NF-κB turns on the expression of genes that keep the cell proliferating and protect the cell from conditions that would otherwise cause it to die via apoptosis. Defects in NF-κB results in increased susceptibility to apoptosis leading to increased cell death. In tumor cells, NF-κB is active either due to mutations in genes encoding the NF-κB transcription factors themselves or in genes that control NF-κB activity (such as IκB genes). NF-κB also affects cancer progression by controlling epithelial to mesenchymal transition and metastasis and controls vascularisation of tumours via VEGF and its receptors. NF-κB pathway mutations are rare in solid tumours but they do occur: increased IKKβ and NF-κB expression has been seen in prostate cancer; mutations in IκBα, and IKKβ have been seen in breast cancer. IKKβ induced NF-κB was found in intestinal epithelial cells to have an essential role in tumour formation in mice. NF-κB can act to stimulate or inhibit liver cancers. H-Ras mediated initiation of tumourigenesis requires IKKβ -mediated NF-κB activation in a mouse model of melanoma. IKKβ and NF-κB are crucial cofactors in lung cancer. NF-κB has also been shown to be activated in cancer stem cells, where it promotes a pro-inflammatory environment, inhibits apoptosis and stimulates cell proliferation but CSCs are a minor subpopulation of cancer cells. NF-κB induces Lin28 which inhibits processing of Let7 miRNAs which are down-regulated in cancer and usually act as tumour suppressors. In general NF-κB acts as a survival factor for transformed cells that otherwise would become apoptotic or senescent. Elevated NF-κB expression in cancer cells provides a survival mechanism for cells by up-regulating anti-apoptotic genes.

Tumourigenesis is a multistage process requiring the interaction of many mutated genes which also lessens the risk from delivery of a single oncogene or tumour suppressor gene to a target tissue. The principle route of transmission that could lead to transduction of laboratory workers by packaged viral particles is inoculation. Therefore, use of sharps will be prohibited except where essential for viral delivery to rats. Other possible routes of transmission such as inhalation, ingestion and eye splashes are less likely to lead to tumourigenesis because of the required very high titres, which will not be achieved under the given experimental conditions. Consequently, the risk of tumourigenesis is very low.

Evaluation of foreseeable effects

Advisory Committee on Dangerous Pathogens (ACDP) – no appropriate level from list but level 2 should be sufficient for this type of lentivirus because it is replication-incompetent and following injection, no new replication-competent virus can be produced, but instead results in "self-inactivation". The modifications made to this virus include VSV-G envelope protein incorporation which increases tropism conferring the capability to infect a wide range of mammalian cells. The virus does not express stable disabling mutations but see (b) below. Effects of the lentivirus itself are unknown but not considered to be serious as the gene delivery method has been developed with human gene therapy in mind.

These lentiviruses are adequately attenuated to be considered class 1 but the inserts contain oncogenic potential thus, pre-injection work and until the end of the quarantine period they should be considered as class 2 and appropriate control measures used for class 2 GMMs adhered to. For animal work following the quarantine period, containment level 1 would suffice (Karlen & Zuffery, 2007; Reuter et al, 2012). The waste brain tissues will be autoclaved and disposed of in the orange bag clinical waste stream.

i) Risks associated with virus vectors and virus handling, injection and during the quarantine period

The live virus would be able to transduce many tissues should it come in contact with them but is unable to replicate within them. The major hazard is therefore represented by the packaged virus prior to infection of the rats. The two potential transmission routes are by external exposure (either skin lesions or mucous membranes) and by accidental injection/inoculation using sharps. Given that the viruses are replication incompetent and will only infect at low efficiency in sub optimal conditions any potential harm would be extremely limited. The risks will be further minimised by control measures described below. The risks of harm from this virus type have been reduced through several modifications from the original HIV-1 virus. These include use of only three HIV-1 genes gag, rev and pol. Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with

(a) The two potential transmission routes are by external exposure (either skin lesions or mucous membranes) and by accidental injection/inoculation using sharps. Where
a worker has active eczema, chapping or sepsis, they should consult a competent person (e.g., a medical professional or occupational health provider) before embarking upon, or continuing activities.

(b) Given that virus is replication incompetent and will only infect at low efficiency in suboptimal conditions any potential harm would be extremely limited. As described above, the risk of tumourigenesis is negligible and also difficult to ascribe to infection, so for these reasons health surveillance of people working on the project is not necessary.

Recombinant lentiviral vectors can only be produced in complementing cells such as HEK 293T that have been transfected with the three packaging plasmids. The vectors described here pose a low risk to animal/plant health and the environment. The virus is replication-incompetent and following infection, no new replication-competent virus can be produced and so adverse effects on other animals are negligible. The active virus would be expected to be cleared from the infected rats within 48-72 hours after which time the rats should have the gene stably integrated into the genomic DNA and would not cause harm to other animals. Lentivirus is an enveloped virus that is highly susceptible to dehydration and sensitive to UV light, unbuffered pH and is rapidly inactivated outside a host. The modifications made to this virus including VSV-G envelope incorporation are not thought to confer a significant increased risk of its survival or clinical stability as the virus remains highly susceptible to dehydration, pH and UV light, and so the virus is thought not to pose a risk to the environment.

Risk level: Low

ii) Risks associated with handling animals, bedding, carcasses, tissues or fluids and waste after the quarantine period for the GMMs.

Following the 72 hour quarantine period the animals can be treated as a normal lab animal where containment level 1 would be adequate. The bedding can be treated as uncontaminated but this and all waste, tissues, fluids and carcasses will be disposed of as described in the additional information section below.

Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Soiled bedding, cages and any other contaminated materials from the quarantine period will be handled as infectious material in a biological safety cabinet and decontaminated by autoclaving and washing. Carcasses from this period will be considered as infectious and should be disposed of by incineration. Waste for removal by contractors for incineration should first be autoclaved then stored and transported in sealed bags or containers.

Surfaces and equipment will be cleaned by treatment with disinfectant e.g. Sodium hypochlorite (use 1-10% dilution of fresh bleach), or 1% VirKon S, and/or autoclaving for 30 minutes at 121°C and incineration or appropriate disposal for radioactive materials. Waste tissues will be autoclaved and incinerated. Animal carcasses will be incinerated, unless the animal has died within the quarantine period and then the carcass will be autoclaved before incineration. Rat bedding will be autoclaved. Cages will be autoclaved, washed and re-autoclaved before reuse.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The Foresterhill GM Committee approved this application at activity class two following lengthy discussions about how the lentiviral vectors would be safely injected into the rats without risk to the individual performing the surgery. The Committee are satisfied that the PI has evaluated the risks thoroughly and that all appropriate precautions and training will be employed throughout the course of the project.

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

**Project Ref 490/15.1**

- Date Ackn’d: 02/04/2015
- Date Project Ceased: 12/12/2018
- CU2 Project Title: The use of standard DNA recombination techniques in studies of CNS development and cell behaviour
- Class: Class 2
- CultureVol: ≤ 1 Litre
- CultureVolume: Class 3-4
- Non-GMM
- Consent Granted

**Historical Significant Changes**

- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**

- Purposes of the contained use:
  - To understand the implication of potential causative genes in the processes of embryonic development, eNS
formation and function, and normal cell behaviours.

Recipient or parental organism

**Sendai virus (SeV):** We will purchase the ready-to-use Sendai viral vectors from Life Technology. Sendai virus is an ACDP class 1 organism and is a murine parainfluenza virus that is non-pathogenic to humans, yet these genetically modified Sendai viral vectors can transduce human and animal cells. In the modified kit (CytoTune®-iPS 2.0), the virus can infect a wide range of cell types, but with the F gene deleted, they can’t produce infectious particles from infected cells. Importantly, human is not the natural host for sendai virus thereby the virus is non-pathogenic to human.

**Lenti-virus:** Pseudo-typed lentivirus will be used to achieve stable gene knockdown. We will purchase a third-generation packaging system, MISSION Lentiviral Packaging Mix from Sigma (SHP001) to produce virus particles. This packing system is based on multiple-plasmid approach and significantly lower the risk to produce replication competent viral particles by a single step. In addition, the vector contains a self-inactivating 3’ LTR that prevents the produce of infectious virus.

**Human lymphoblastoid Cell lines:** These cell line are immortalized human B cell lines which are currently used by the Wellcome funded UK10K and MRC UK epigenetic schizophrenia programmes.

Host/vector system

We will use the CytoTune®-iPS 2.0 Sendai Reprogramming Kit, which is newly released by Life Technology but has been rapidly used and well-referenced worldwide. The virus is non-integrating and only requires incubation as short as one overnight compared to multiple days of transductions required by other protocols. This kit is based on a modified, non-transmissible form of Sendai Virus, with the Fusion protein deleted. The viral vectors maintain full infectivity to a wide range of cells, however they are no longer capable of producing infectious particles from infected cells because the viral genome lacks the F gene. Additionally, the vectors and transgenes can be eliminated from the host cells which will lead to an integration-free reprogramming. Importantly, human is not the natural host for sendai virus thereby the virus is non-pathogenic to human.

We will also use a third-generation packaging system, MISSION Lentiviral Packaging Mix (Sigma, catalog number SHP001) to produce lenti-virus particles. This system has not been shown to produce replication competent viral particles because it only contains the minimal components (1+2, see following) and won’t work without co-transfection along with compatible lentiviral transfer vector. This unique design together with a lacking of 3’LTR assures very low risk of producing infective viral particles.

1. The packaging vector, which contains the minimal set of lentiviral genes required to generate the virus structural proteins and packaging functions.
2. The vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector, which provides the heterologous envelope for pseudolyping.

Lentivirus transduction will served as a backup method of Sendai virus to derive human iPSCs. The ViraPower Lentiviral expression system (Invitrogen) will be used to produce recombinant lentivirus containing Yamanaka factors essential for iPSe derivation. Again, this multi-plasmid approach efficiently prevent the produce of packaged lentivirus which greatly increases biosafety.

Origin & function

The state-of-art CytoTune®-iPS 2.0 Sendai Reprogramming Kit developed by Life Technology ensures both the virus vector and transgene eliminated after integration and produce an "integration-free" re-programming. Sendai virus does not integrate in the host genome. Lenti-virus mediated transduction serves as a backup strategy. Due to the
vector structure, the produced lenti-viruses are replication incompetent, and could not produce viral particles in infected cells, thus preventing system wide effects.

The Yamanaka factors (Oct4, Sox2, Klf4, c-Myc) are a recognised cocktail genes to force the cultured human lymphoblastoid cells to acquire the properties of pluripotent stem cells. Oct4, Sox2, Klf4, and c-Myc will each be delivered in separate SeV vectors. Oct4 regulates the pluripotency of embryonic stem cells and under and overexpression has been associated with cell differentiation. Sox2 acts cooperatively with Oct4 to regulate pluripotency. Klf-4 is a transcription factor that regulates transcription factor expression during development. C-Myc, with roles in regulating cell proliferation and apoptosis, is a proto-oncogene and is overexpressed in many cancers.

Together, they are a recognised cocktail of genes required for reprogramming somatic cells to a stem cell phenotype. The expression of these genes results in cells adopting a phenotype whereby they have self-renewal capacity and the ability, under managed conditions to proliferate indefinitely.

Evaluation of foreseeable effects

Although human is not the natural host for the SeV, and the virus is non-pathogenic to humans, appropriate care must be taken to prevent the potential mucosal exposure to the virus. Similarly, the adopted strategy will ensure that the produced lentiviruses are replication incompetent, preventing subsequent production of lentiviral particles in infected cells. There would be a potential risk of mobilisation and transmission of the recombinant lentivirus to individuals with wildtype HIV virus by accidental injection. Thus, individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project and these risks will be made clear to all personnel in the laboratory. In addition, sharps are strictly prohibited to use in this project. Another potential hazard associated with these vectors are as a result of the nature of the inserted genes. Overexpression of any of these genes can result in dysregulation of cell proliferation, differentiation and survival and therefore potentially transform the cells. Therefore, following centrifugation and for all handling, the tubes containing CytoTune sendai viral or lentiviral particles will be opened in the Class 2 safety cabinet to avoid exposure to aerosolised particles.

Serologically positive rodents (the natural host for sendai virus), animals that have already been infected with wild type SeV, may be able to make infectious CytoTune™-Sendai virus. Sendai virus affects the respiratory tract and causes severe respiratory disease in rodents. It can be spread by contact and airborne transmission including through air circulation systems. As a rule it is recommended that after handling sendai viral vectors or infected animals, a period of at least 3 hours should elapse before contact with other animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Solid waste including culture plates and disposable plastic ware will be inactivated with disinfectant (Precept, Johnson and Johnson, 29fl) for at least one hour prior to being rinsed with water and detergent and sent for wash and autoclave.
2. Glassware and non-disposable plastic ware will be treated with disinfectant (Precept, Johnson and Johnson, 29fl) for at least one hour prior to being rinsed with water and detergent and sent for wash and autoclave.
3. Liquid waste (i.e. cultured medium) will be treated with disinfectant (Precept, Johnson and Johnson, 59FL) for at least one hour prior to disposal down the sink.

Validation will involve regular testing of the autoclave to demonstrate, using independent thermocouples placed aile
centre of the load, that the correct time and pressure have been reached for the required time. Sendai viruses are inactivated at temperatures above 37°C. The "degree of kill" of chemical disinfectant is expected at 99.99%. At the start of the project and at monthly intervals, we will validate the inactivation of sendai viral and lentiviral vectors following autoclaving by incubating cultures of Hela cells with autoclaved liquid waste. We will then perform reverse transcription on RNA isolated from the Hela cells, followed by PCR using primers to specifically amplify the viral genome to determine whether any virus had been incorporated into the cells. Records will be kept of the results from each test.

Project Containment

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Project Ref 490/16.1

Date Ackn'd 21/01/2016

CU2 Project Title Pattern recognition receptors in immunity and homeostasis: transduction of cell lines and primary murine cells using lentiviruses

Class Class 2

CultureVol Class 2 < 1 Litre

Consent Granted

Project notified under transitional arrangements
Project Additional Information

Purposes of the contained use

To safely and efficiently transduce genes, either unmodified, mutated and/or fused to tag sequences (see attached risk assessment for details) of C-type lectin receptors, pathogen recognition receptors, and phagosome maturation markers, using lentiviruses into murine and human cell lines and murine primary cells.

Recipient or parental organism

Cloning of the various genes into the lentiviral cloning vectors will require the use of the disabled K12-derivied E.coli. A fast growing version of the human embryonic kidney (HEK) 293T cell line, containing the large T-cell antigen, will be used as the packaging cell line for the production of the replication incompetent lentivirus vectors. The packaged ecotropic lentivirus vectors will then be used to transduce murine primary and cell lines, and packaged amphotropic lentiviruses will be used for human cell lines and both murine primary cells and cell lines.

Host/vector system

VSV-G (amphotropic) and MLV (ecotropic) pseudotyped lentiviral vectors will be produced using the ViraSafe lentiviral expression kit (Cell Biolabs) by co-transfecting the 293T cell line with the expression construct and a lentiviral packaging mix containing 3 plasmids, carrying the minimal number of genes encoding the necessary viral structural and replicatory proteins (to improve safety).

Origin & function

The lentivirus vectors expressing wild type or mutated genes from murine and human C-type lectin-like receptors of the Dectin-1 and Dectin-2 clusters, members of the scavenger receptor family, Collectins, Natural Killer lectin-like receptor family, the complement pathway and the Toll-like receptor family will be used to transduce primary murine and human cells and cell lines. These cells will then be assessed for functions involved in immunity and homeostasis. The interaction of the transduced cells with various pathogens, other cell types, and cellular or serum components will be examined. To evaluate the temporal dynamics of uptake of pathogens, inert particles, and cellular components, we will perform experiments using murine macrophage cell lines transfected with murine phagosome maturation regulators (e.g. members of the Rab GTPase family) fused to fluorescent tags (e.g. green fluorescent protein (GFP). All of these experiments are performed in vitro and will only be performed in primary murine cells and cell lines. To enable functional determination of the various receptor domains and identified polymorphisms we will transduce cells with wild-type and/or receptors carrying specific mutations, which may also be tagged or co-expressed with. Functional mutants of specific members of the Rab GTPase family will also be used. Various combinations of receptors and murine phagosome maturation markers may be simultaneously introduced into cells derived from wild type or genetically altered mice allowing the combinatorial effects on cellular function to be assessed.

Lentiviral transduction will result in stable integration of the receptors or phagosome maturation regulation encoding genes in murine primary cells and cell lines. Stable integration of only the receptor encoding genes, using the amphotropic lentivirus system, will be in human cell lines. None of the receptor genes under study are known to be oncogenic and their expression will not affect the tropism of the lentivirus. However, several studies have linked members of the Rab GTPase family and functional mutations of these proteins (mainly activating mutations) with cancer. In particular, there is evidence showing that the phagosome maturation regulators under study are overexpressed in certain types of cancer and associated with tumour development and/or metastatic ability. Consequently, only the murine ecotropic MLV based lentiviral system for the expression of the Rab GTPase and phagosome maturation markers will be used. These Rab GTPases and phagosome maturation markers are not
Evaluation of foreseeable effects

Accidental infection with the genetically modified micro-organisms described in this application are not expected to give rise to any specific symptoms since the lentiviral vectors are replication incompetent. There is a very small theoretical risk that, if the lentiviral expression plasmid was accidentally injected into an individual carrying wildtype HIV, the vector could recombine, via the truncated LTRs, with the wildtype virus to produce replication competent virus, carrying the immune recognition receptor genes. The likelihood of this occurring is extremely low since a) the use of sharps will be prohibited, b) plasmid DNA does not enter cells readily and c) the plasmid would have to be internalised by a CD4+, HIV infected T cell. Only the VSV-G containing lentiviral vectors (amphotropic viruses) are capable of transducing a broad range of cell types and therefore the expressed genes will be stably integrated into any cell type that the vectors are exposed to. However, this is not thought to pose a significant risk to human health due to the reasons described in section 6 above. In carriers of wildtype HIV there may be a theoretical risk of recombination of the lentiviral vector with wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project; these risks will be made clear to all personnel in the laboratory.

Lentiviruses require close contact for their transmission and their survival in the general environment is poor. Replication deficient lentivirus cannot propagate so therefore, after infection of a cell they are incapable of further replication. It will be possible to demonstrate that the lentiviral vectors are replication deficient by plating 5% of the vector stock supernatant into mitotically active indicator cells (eg 293FT cells) and monitoring for syncitia formation or by PCR analysis of cells that have been transduced with lentiviral vector stocks using primers specific for pro-viral components, such as the VSV-G.

All phagosome maturation genes, such as those from the Rab GTPase family, lysosomal-associated membrane proteins and proton transporter families will only be expressed in the MLV lentivirus vector system (ecotropic viruses specific for rat and mouse). This is due to the potential of these genes to cause phenotypic changes causing tumourogenesis.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (from mammalian cell cultures) will be treated with 5% Chemgene (for high level disinfectant) before disposal down the sink. Plastics and other solid disposable waste will be collected in an autoclavable bag within the biological safety cabinet, sealed and then sprayed with Chemgene (2%). The bag will then be removed from the safety cabinet placed into a clean autoclave bag, sealed, and taken to the autoclave in a sealed container. The degree of kill following autoclaving will be 100% since there is a high expectation of killing of GM microorganisms exposed to 121ºC for 15 minutes. The autoclaved waste will then be disposed of by CFA processing. Glass bottles and other non-disposable vessels will be soaked in the appropriate concentration of Chemgene (2%, according to the manufacturer’s recommendation) in a bucket with a sealed lid, rinsed and then autoclaved for future use.

Validation will involve regular testing of the autoclave to demonstrate, using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time. At the start of the project we will validate the inactivation of lentiviral vectors following autoclaving. NIH3T3 cells (for the murine ecotropic lentiviruses) or HEK293T cells (for the amphotropic lentivirus) will be grown in the presence of increasing (10 fold) amounts of inactivated waste. The cells will then be selected for using the antibiotic puromycin and incubated for 10 days before being . The identification of any puromycin-resistant colonies demonstrates the presence of active lentiviral vector within the ‘inactivated’ waste.

The virucidal disinfectant, Chemgene, has been validated for inactivation of lentivirus at a concentration of 2%. Details of Chemgene testing can be accessed at http://www.medi-mark.co.uk/images/uploads/CHEMGENE_HLD4H_TEST_SUMMARY.pdf

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
This application was approved at Containment Level 2 (GM-15-010) by the local Genetic Modification Safety Committee.

**Project Containment**

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**Project Ref**: 490/20.1

- **Date Ackn'd**: 07/08/2020
- **CU2 Project Title**: Genetic modification of mammalian cells for the study of cancer
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Date Project Ceased**: 07/08/2020
- **Non-GMM Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N

**Project Additional Information**
Cancers are heterogeneous tissues comprised of multiple components, including tumour cells and microenvironment cells. They arise from extensive mutation and deregulation of genes, leading to loss of control of key cellular processes. In addition, for tumours to grow, become invasive and metastasize they must develop a blood supply through the process of angiogenesis and evade the host immune system. The tumour microenvironment (TME) has a critical role in tumour initiation, promotion and progression. Fibroblasts are the predominant cell type in the TME. There is evolving evidence showing that in breast cancer, cancer associated fibroblasts (CAFs) contribute to tumorigenesis via bidirectional crosstalk with tumour cells, and actively involved with metabolic reprogramming, angiogenesis and therapy resistance of the tumour. Through transcriptomic screening of tumour and CAFs we have identified genes required for processes associated with carcinogenesis; cancer cell migration, invasion proliferation and angiogenesis. These include mediators of intracellular signalling events and cell-cell adhesion. The aim of our research is to understand the complex connections between breast CAFs and cancer cells/other TME component such as endothelial cells, and to explore their therapeutic potential in cancer.

Objectives:
- To identify and track CAFs and other relevant cell types present in multicellular culture models using reporter genes.
- To use hTERT overexpression to establish immortalized cell lines from primary fibroblasts and/or epithelial cells derived from patients which allows long term or more complex in vitro studies
- To use shRNAs/CRISPR and micro-RNA derivatives determined from on-going transcriptomics screens and bioinformatics mining of, for example, TCGA, to knock down expression of genes/microRNAs involved in carcinogenesis e.g. those associated with proliferation, invasion and migration

Recipient or parental organism

**E.coli**

We will use the E.coli strain One Shot Stbl3 (Invitrogen), XL-1 blue (Stratagene), one shot or subcloning Dh5α (Invitrogen), or related disabled E. Coli strains to amplify the plasmid vectors. The natural environment of E. coli is in the gut but these experimental strains are laboratory adapted E coli K-12, being classified as disabled hosts in the ACGM guidelines, and are unlikely to survive in the human gut or elsewhere.

- **Lentivirus**

Lentiviruses are a type of retrovirus. They are unique from other types of retroviruses because they can infect both dividing and non-dividing cells and are stably integrated into the host genome. This is because their pre-integration complex (or virus shell) encodes a localisation sequence that is recognised by the import machinery of the nucleus, enabling the virus to get through the intact membrane of the nucleus of a target cell. Lentiviral vectors that will be used in this project are HIV-derived viral vectors. HIV derives its virulence from 9 genes. Of these nine genes, five or six have been removed (depending on viral expression system used), leaving only the viral genes that are essential for its gene transfer ability. Furthermore, the provirus is broken into pieces, leaving the vectors replication defective.

Tropism of the lentiviral vector depends on the envelope (env) protein that is expressed. Lentivirus coated with e.g. the envelope protein from murine leukemia virus (MLV) is ecotropic, i.e. able to infect murine cells, but not human cells. Lentivirus coated with e.g. the envelope protein from vesicular stomatitis virus (VSV-G) is pantropic, i.e. able to infect both murine and human cells.

We will use pantropic virus. This will be used because i) pantropic virus is needed to infect human cells in culture, and ii) the pantropic VSV-G envelope protein brings stability to the lentiviral vector yielding higher functional titres following Lenti-X™ Concentrator and a standard speed centrifugation. We will avoid concentration of immortalisation viruses where possible by using viral supernatants directly.

- **In vitro target cells**

Host cells will include primary fibroblasts or cancer epithelial cells, generated in-house using well established protocols, commercially available HUVECs (https://www.cellapplications.com/human-umbilical-vein-endothelial-cells-huvec), plus established human cancer cell lines e.g. MCF-7, T47D, MDA-MB-231 and others
available from the European Collection of Authenticated Cell Cultures (https://www.phe-culturecollections.org.uk/collections/ecacc.aspx). The cell lines used are not capable of survival outside the laboratory and are non-transplantable due to immuno-incompatibility. They are therefore, considered to be highly disabled hosts.

Host/vector system

Replication-defective retroviral vector system:
The Phoenix ecotropic and amphotropic retroviral transduction systems (packaging producer line is derived from 293T human embryonic kidney cell-line), or equivalent, 3 plasmid packaging system will be used. The transducing particles produced are simple enveloped RNA virus vectors that consist of gag (coding for the core proteins), pol (coding for the viral RNA reverse transcriptase) and env (coding for the viral envelope). Long terminal repeats (LTRs) harbour sequences that aid viral genome integration and transcription of the inserted genes. The ecotropic particles cannot transduce human cells and are therefore categorized as ACDP Hazard group 1. The amphotropic particles can transduce human cells and therefore are categorized and Hazard group 2.

In the 3 plasmid packaging system one construct harbours gag and pol and a separate construct harbours env. A third construct is then transfected into the producer cells that contains: (a) the gene of interest plus the retroviral packaging signal to allow production of particles capable of transducing human target cells; and (b) sequences within the Long Terminal Repeats (LTRs) that aid viral genome integration and transcription of the inserted genes. Only sequences within the LTRs are integrated in the genome of target cells. In this packaging system, the functions required for production of transducing units are separated in three different plasmids making the potential for recombination and virus production in the target cells virtually impossible.

The retroviral vectors to be used with this system are pBABE and pBabe derivatives or equivalent MLV-derived retroviral vectors. The plasmids are non-mobilisable but are designed to be integrated into the host genome. Vectors carry drug resistance markers or reporter genes that are non hazardous to humans.

Replication-defective lentiviral vector system:
Where mitosis-independent transduction is required lentiviral systems will be used. These systems are based on HIV-1. However, viral genes such as vpr, vif, vpu and nef are deleted and the HIV envelope gene is replaced with Vesicular stomatitis virus G protein (VSV-G) to increase the host range of the virus. Replication defective transducing units are produced by co-transfection of a transgene vector harbouring the gene of interest together with at least two separate non-overlapping packaging constructs containing gag, pol and env making the potential for recombination virtually impossible. Biosafety is further increased by the use of self-inactivating vectors. The particles can transduce human cells therefore they are categorized and Hazard group 2 and will be handled under Containment level 2 conditions.

The plasmids to be used with the lentiviral system pLKO.1 (addgene), pGIPZ, pLEX (Open Biosystems) or equivalent self-inactivating (SIN) lentiviral vectors due to deletion of part of the HIV-1 3’ LTR – upon integration the provirus lacks a promoter so full-length vector sequence can no longer be transcribed. Vectors carry drug resistance markers or reporter genes that are non-hazardous to humans.

- Ready-to-use lentivirus
  - pCSII-CMV-hTERT-IRES-Hygro second generation lentivirus ready to infect human cells, will be used (provided by collaborators in Leeds or Glasgow). The lentivirus contains the hTERT gene under the control of a CMV promoter and an internal ribosome entry site (IRES) to allow simultaneous translation of a hygromycin resistance gene, flanked by 5’ and 3’LTR sequences, as well as an origin of replication and ampicillin resistance gene.
  - pGIPZ-tGFP (Dharmacon), a replication-incompetent lentiviral vector designed specifically for the expression of shRNAs in mammalian cells. The Human U6 promoter drives RNA Polymerase III transcription for generation of shRNA transcripts. Selection of shRNA expressing cells is possible using turbo GFP under the control of a CMV promoter.

- Production of lentivirus

Viral vectors will be transfected into a packaging cell line such as HEK293T-based Lenti-X™ HTX (Clontech; www.clontech.com), which has been modified to stably express the SV40 large T antigen under the control of the CMV promoter. This enables replication of the plasmids containing an SV40 origin of replication to high copy number in the transfected cell, thus achieving a high viral titre.
- 2nd generation lentivirus

This will NOT be used for production of lentivirus containing hTERT or other inserts that are potentially harmful.

Plasmids that are co-transfected into packaging cells to produce virus:
1. expression plasmid
2. packaging plasmid encoding gag, pol and rev viral genes
3. envelope plasmid

1. Lentiviral expression plasmids

Lentiviral expression constructs are created by cloning a gene of interest into a vector sequence that is flanked by LTRs and the Psi-sequence. The LTRs are needed for the integration of the gene of interest into the host genome and the Psi-sequence is required for packaging RNA transcribed from the gene of interest in virions.

- pLKO.1 vector, a replication-incompetent lentiviral vector designed specifically for the expression of shRNAs in mammalian cells. The Human U6 promoter drives RNA Polymerase III transcription for generation of shRNA transcripts. For stable selection in mammalian cells pLKO.1 contains the Puromycin resistance gene (PuroR) which is driven by the Human phosphoglycerate kinase promoter (hPGK).

2. Lentiviral packaging plasmids

- psPAX2 (Addgene plasmid #12260), encoding pol, gag and rev genes. Transcription of viral genes gag, pol and rev within psPAX2 is mediated via the human cytomegalovirus (hCMV). For bacterial selection psPAX2 contains the Ampicillin resistant gene.

3. Lentiviral envelope plasmids

- pMD2.G (Addgene plasmid #12259). This plasmid encodes the coding sequence for the envelope G glycoprotein from Vesicular Stomatitis Virus (VSV-G) to allow production of pseudotyped lentivirus with a broad host range. The human b-globin intron and polyadenylation (beta-globin pA) signal enhance expression of the VSV-G gene in mammalian cells and allows efficient transcription termination and polyadenylation of mRNA, respectively.

- 3rd generation lentivirus

This will always be used for production of lentivirus containing hTERT or other inserts that are potentially harmful, and may be used for any other gene to be modified.

Plasmids that are co-transfected into packaging cells to produce virus:
1. expression plasmid
2. packaging plasmid encoding gag and pol viral genes
3. packaging plasmid encoding rev viral gene
4. envelope plasmid

1. Lentiviral expression plasmids

Lentiviral expression constructs are created by cloning a gene of interest into a vector sequence that is flanked by LTRs and the Psi-sequence. The LTRs are needed for the integration of the gene of interest into the host genome and the Psi-sequence is required for packaging RNA transcribed from the gene of interest in virions.
A suitable expression plasmid will be used, such as:

- pLKO.1 vector, a replication-competent lentiviral vector designed specifically for the expression of shRNAs in mammalian cells. The Human U6 promoter drives RNA Polymerase III transcription for generation of shRNA transcripts. For stable selection in mammalian cells pLKO.1 contains the Puromycin resistance gene (PuroR) which is driven by the Human phosphoglycerate kinase promoter (hPGK).
- Other plasmids will be added as required and The Foresterhill Biological Safety Committee informed of the expression plasmids to be used.

2. Lentiviral packaging plasmid encoding gag and pol

- pMDLg/pRRE (Addgene plasmid #12251, Didier Trono) or equivalent. A 3rd generation LV packaging plasmid that includes gag, coding for the virion main structural proteins; pol, responsible for the retrovirus-specific enzymes; and RRE, a binding site for the Rev protein which facilitates export of the RNA from the nucleus. This plasmid does not include Rev.

3. Lentiviral packaging plasmid encoding Rev

- pRSV-Rev (Addgene plasmid #12253, Didier Trono) or equivalent. A Rev cDNA expressing plasmid in which the joined second and third exons of HIV-1 rev are under the transcriptional control of RSV U3 promoter.

4. Lentiviral envelope plasmid

- pMD2.G (Addgene plasmid #12259). This plasmid encodes the coding sequence for the envelope G glycoprotein from Vesicular Stomatitis Virus (VSV-G) to allow production of pseudotyped lentivirus with a broad host range. The human b-globin intron and polyadenylation (beta-globin pA) signal enhance expression of the VSV-G gene in mammalian cells and allows efficient transcription termination and polyadenylation of mRNA, respectively.

Origin & function

- hTERT (Human Telomerase Reverse Transcriptase), the catalytic subunit of the enzyme telomerase. Telomerase prevents shortening of telomeres at the tips of chromosomes during replication by adding TTAGGG sequence repeats to maintain telomeres. Overexpression of TERT is widely used to immortalise cells.
- Reporter genes such as EGFP and derivatives, dsRed and derivatives, Firefly luciferase (Fluc), β-galactosidase under the control of mammalian or viral promoters (e.g. CMV or MLV 5' LTR) to enable tracking of cells.
- Inserts encoding wild-type or mutant genes involved in normal development, cell signalling, angiogenesis or cancer. cDNAs may be expressed as fusions with a fluorescent molecules EGFP or dsRed; in non-tagged or tagged form (N- or C-terminal MYC, HA, FLAG, or HIS-tag); cDNA libraries may be used. Expression will be constitutive via mammalian or viral promoters (e.g. CMV or MLV LTR) or inducible (e.g. TET ON or TET OFF systems).
- Small hairpin RNA molecules (shRNAs) or derivatives of microRNAs to knockdown genes involved in normal development, cell signalling, angiogenesis or cancer under the control of U6 or H1 promoters. shRNA and miRNA libraries may be used.
- Inserts encoding function-blocking peptides or antibody fragments to inhibit the function of proteins involved in normal development, cell signalling, angiogenesis or cancer. Mammalian expression vectors harbouring cDNAs and reporter genes will be obtained from commercial sources and collaborators. Lentiviral vectors will be obtained from commercial sources and collaborators (lentiviral vectors will be SIN vectors. Plasmids will be propagated in E. coli K-12 derivatives.
- Inserts encoding reporter genes such as EGFP and derivatives, dsRed and derivatives, Firefly luciferase (Fluc), β-galactosidase under the control of mammalian or viral promoters (e.g. CMV or MLV 5' LTR) to enable tracking of cells.
- TERT (Telomerase Reverse Transcriptase), is the catalytic subunit of the enzyme telomerase. Telomerase prevents shortening of chromosomes during replication by adding TTAGGG sequence repeats to maintain telomeres. Cell immortalization is typically achieved through the overexpression of TERT, particularly for cells that are most affected by telomere length, such as human cells (Lundberg AS. 2000; Fridman AL, 2008).

Evaluation of foreseeable effects

(a) hazards associated with the recipient organism including ACDP hazard group and the effects of any stable disabling mutations

- Bacterial expression
The bacterial organisms are E. coli K12 derivatives and are unlikely to survive in the human gut or elsewhere. None of the genes expressed here would be expressed in...
these bacterial cells since the genes lack a bacterial promoter. Even if expression did occur, it would not be anticipated to confer any pathological advantage to the bacterial host.

- Lentiviral vectors
  (i) Second (pre-made in the PI’s former lab) or (ii) ready-to-use third generation lentiviral systems will be used for generation of pantropic virus.

Second generation lentiviral system carry the following safety features:
1. The number of lentiviral genes necessary for packaging, replication and transduction is limited to four (Gag/Pol/Rev/Tat) all lacking packaging signals. The plasmids share no significant homology to the expression vector, preventing the generation of replication-competent virus.
2. None of the Gag, Pol, or Rev genes will be present in the packaged viral genome, thus making the mature virus replication-incompetent.
3. The packaging plasmids share no significant homology to any of the expression lentivectors, the pVSV-G expression vector (pMD2.G), or any other vector, to prevent generation of recombinant replication competent virus.
4. An enhancer deletion in the U3 region of 3′ΔLTR ensures self-inactivation of the lentiviral vector following transduction & integration into the target cell’s genomic DNA.

Third generation lentiviral expression systems include the following additional safety features:
1. The number of lentiviral genes necessary for packaging, replication and transduction is further reduced to three (Gag/Pol/Rev). It utilizes a chimeric 5′ LTR to ensure transcription in the absence of Tat.
2. Gag/Pol and Rev are encoded on separate plasmids.

All of these safety features mean that there is no likelihood of this work resulting in the production of replication competent lentiviruses.

Third generation lentiviral systems are considered safer than second generation systems but may be more difficult to use because they require transfection with four separate vectors in order to create functional lentiviral particles.

Our current protocol employs a 2nd generation lentiviral system (Addgene) to modify TERT and insert GFP/RFP, generated by the PI at her former lab. A supply of pre-made virus exists and when exhausted, we will use a 3rd generation commercial ready to use system (e.g. from OriGene, Dharmacon, ThermoFisher) for TERT, shRNA and CRISPR modification in future.

- Target cells
  None of the target cells have inherent pathogenic properties and they would be unable to survive if introduced accidentally into non-immunocompromised lab workers. The risk associated with accidental injection of target cells is therefore negligible in immune-competent individuals, since the cells would be eliminated by the host’s immune system.

(b) hazards arising directly from the inserted gene
- TERT
  This may cause pathogenic effects, since TERT is considered an oncogene. It is expressed in around 90% of malignant cancer. While telomerase does not drive the oncogenic process, it is permissive and required for the sustained growth of most advanced cancers.
- Non-mammalian reporter genes, such as GFP/RFP and other fluorescent variants are non-pathogenic proteins. The gene products would not be expected to cause any hazard to human health.

(c) hazards arising from the alteration of existing pathogenic traits
It is not thought that the inserted genes will alter the tropism of the lentiviral vectors. Thus, they will not be able to infect additional cell types and will stay non-replicative.
Pathogenic traits are not present in commercially available disabled strains of E coli K12: they do not have the type of glycocalyx required for the attachment to the mucosal surface of the human colon and they do not express capsular (K) antigens which are important for colonisation and virulence. The genes in the plasmids will not introduce pathogenic traits into the bacteria.

(d) likelihood and effects of natural gene transfer to other organisms

Bacterial strains are multiply disabled and therefore there is no foreseeable risk of gene transfer to workers or the environment. Should plasmid DNA accidentally enter the body it is highly unlikely that it would lead to expression since the DNA is not readily taken up by cells and will be degraded rapidly.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The following precautionary measures will be used for work with lentivirus:

- Lab users are made aware of the risks when receiving lab induction/training and through Local Rules and Risk Assessments.
- Individuals carrying the HIV virus, or any other immuno-compromised individuals, will not be allowed to work on the project. There is a very small risk that, if the lentiviral expression plasmid was injected into an individual carrying wildtype HIV, the vector could recombine, via the truncated LTRs, with the wildtype virus to produce replication competent virus. Lab users are asked to contact the Occupational Health Service if (s)he has any medical condition or takes any medication that causes his/her immune system to be compromised.
- The risk of accidental injection, inhalation or ingestion will be minimised by wearing gloves, handling stocks and cultures containing viral particles in a Class II safety cabinet. No needles and sharps will be used.
- For transduction of human cells with genes such as TERT known to have potential pathogenic effects, ready-to-use lentivirus will be collected from collaborators and used.
- The operator will use appropriate CL-2 safety equipment, such as coat, gloves, sleeves, protective goggles (during the lentivirus handling).

Factors such as congenital immunodeficiency and secondary immunodeficiency caused by serious illness or chemotherapy would increase the susceptibility to infection by micro-organisms, including the genetically modified micro-organisms described in this application. In carriers of wild-type HIV there may be a theoretical risk of recombination of the lentiviral vector with wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised will not be allowed to work on this project, these risks will be made clear to all personnel in the laboratory.

We would not expect any specific symptoms as the result of an infection with the genetically modified micro-organisms described in this application since the lentiviral vectors cannot replicate.

All liquid waste (from mammalian and bacterial cell cultures will be treated with Virkon (1:50, following manufacturer’s recommendations) and left for at least 30 minutes before disposal down the drain with copious amounts of water. This is from the first media change after transfection and for 10 days (or two passages) after target cell infection.

Plastics and other solid disposable waste will be collected in an autoclavable bag within the biological safety cabinet, sealed and then sprayed with Chemgene (1:50, following manufacturer’s recommendations). The bag will then be removed from the safety cabinet into a clean autoclave bag, sealed, and taken to the autoclave in a sealed container.
Glass bottles and other non-disposable vessels will be soaked in the appropriate concentration of Chemgene (1:50 dilution, according to the manufacturer’s recommendation) in a bucket with a sealed lid, rinsed well and then autoclaved.

Validation will involve regular testing of the autoclave to demonstrate, using independent thermocouples placed at the centre of the load, that the correct temperature and pressure have been reached for the required time.

To validate the inactivation of lentiviral vectors, a standard viral culture test will be used after each transduction procedure. Cells able to be transduced by the virus will be grown in the presence of increasing (10-fold) amounts of inactivated waste. Any transduced cells will then be selected for using appropriate antibiotic treatment for 10 days prior to detection of colonies e.g. by staining with Crystal Violet. The identification of any antibiotic-resistant colonies demonstrates the presence of active lentiviral vector within the ‘inactivated’ waste.

100% expectation of kill of GM microorganisms exposed to 121ºC for 15 minutes.

Liquid and solid waste will be disposed of according to the normal Foresterhill waste procedures as described.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee were happy that the hazards of the project have been correctly identified, and that appropriate safe systems of work will be used to control those risks identified. This application was approved at Containment Level 2 by the local Genetic Modification Safety Committee.

Project Containment

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Project Ref 490/20.2

Date Ackn’d 11/11/2020  CU2 Project Title Investigation of molecular and cellular mechanisms regulating neurogenesis and Development

Class 2  CultureVolClass2 Class 2  CultureVolumeClass3-4 < 1 Litre

02/03/2022  Page 7883 of 1532
The main goal of this project is to examine the functions of risk genes for brain developmental disorders or RNA metabolism related genes in regulating neural stem cell and brain development. GM micro-organisms will be used to manipulate the expression of risk genes genetically and to label neural stem cells and their progenies. We will infect cells with virus (Lentivirus, retrovirus, AAV, Adenovirus) carrying shRNA or cDNA, and fluorescence protein by micro injection into the embryonic or adult mouse brain, or human brain organoids.

Bacteria: K12 derivative E. coli strains such as DH5α, Stella or XL10-gold
Replication incompetent recombinant Retrovirus
Third-generation, replication incompetent, recombinant Lentivirus
Replication incompetent recombinant Adenovirus (serotype 5)
Replication incompetent recombinant Adeno-associated virus (AAV)
Mammalian cell lines and organoids: HEK293T, HEK293GP, Adult mouse neural stem cells, human iPSCs induced forebrain organoids.
Animal: Mouse

For knockdown experiments, retroviral vector pUEG-shRNA (Human U6 promoter-shRNA, Ef1α promoter and EGFP), lentiviral vector cFUGW-shRNA (Human U6 promoter-shRNA, human Ubiquitin C promoter-EGFP), or adeno associated viral vector AAV-CMV-GFP (CMV promoter- EGFP) will be used. For overexpression experiments, retroviral vector cUXIE (Ubiquitin C promoter-cDNA-IRES-EGFP), lentiviral vector cFUGW (Ubiquitin promoter -EGFP – IRES- cDNA) will be used. EGFP in all vector can be replaced by different colour of fluorescence protein such as mCherry, RFP, Tdtomato. All vectors contain ampicillin resistance for selection in E.coli.
Each virus will be packaged as follows:

*Retrovirus: retroviral transfer plasmid, and plasmid expressing envelope gene VSV-G will be co-transfected into HEK293GP packaging cell line (stably expressing Gag-Pol). Virus will be concentrated using ultra-centrifugation and stored at -80 °C.

*Lentivirus: 3RD generation lentiviral transfer plasmid (FUGW), packaging plasmid (pMDLg/pRRE and pRSV-Rev) and envelope plasmid (for expressing VSV-G) will be co-transfected into HEK293T packaging cell line. Virus will be concentrated using ultra-centrifugation and stored at -80 °C.

*AAV: AAV transfer plasmid, helper plasmid (pAD) and Rep/Cap plasmid (Serotype 5 or 2/9) will be co-transfected into HEK293T packaging cell line. Virus will be purified using chloroform treatment, PEG/NaCl precipitation and chloroform extraction method, and then be stored in -80°C

* Replication incompetent recombinant Adenovirus (serotype 5) will be purchased.

**Origin & function**

The following genes will be modified to knockdown or overexpress their expression.

**Genes implicated in brain developmental disorders, including:**

*Cyfip1: This gene encodes a protein that regulates cytoskeletal dynamics and protein translation. This protein also interacts with the Fragile X mental retardation protein (FMRP) and translation initiation factor 4E to inhibit protein translation. A large chromosomal deletion including this gene is associated with increased risk of schizophrenia and epilepsy in human patients. Reduced expression of this gene has been observed in various human cancers and the encoded protein may inhibit tumour invasion.*

*Fnbp1l: The protein encoded by this gene binds to both CDC42 and N-WASP. This protein promotes CDC42-induced actin polymerization by activating the N-WASP-WIP complex and, therefore, is involved in a pathway that links cell surface signals to the actin cytoskeleton. Diseases associated with FNBP1L include Malignant Breast Melanoma and Familial Hypocalciuric Hypercalcaemia. Intelligence genes. High expression during early development.*

**Genes involved in RNA metabolism, including:**

*Mettl5 (Methyltransferase Like 5) linked to Intellectual Developmental Disorder, Autosomal Recessive 72 and Epilepsy, Familial Temporal Lobe, 4. Implicated in intellectual disability disorder;*  

*PTBP1: associated with pre-mRNAs in the nucleus and appear to influence pre-mRNA processing and other aspects of mRNA metabolism and transport. Diseases associated with PTBP1 include Human T-Cell Leukaemia Virus Type 2 and Spastic Ataxia 4;*  

*hnrNPG: RNA-binding protein that plays several roles in the regulation of pre- and post-transcriptional processes. Implicated in tissue-specific regulation of gene transcription and alternative splicing of several pre-mRNAs. Diseases associated with RBMX include X-Linked Hereditary Ataxia and Fragile X-Associated Tremor/Ataxia Syndrome;*  

*Pus7: PUS7 (Pseudouridine Synthase 7) Diseases associated with PUS7 include Intellectual Developmental Disorder With Abnormal Behaviour, Microcephaly, And Short Stature and Non-Specific Syndromic Intellectual Disability. Among its related pathways are Gene Expression and tRNA processing. Gene Ontology (GO) annotations related to this gene include nucleic acid binding and RNA binding*  

*Adat3: This gene encodes a subunit of a tRNA-specific adenosine deaminase. This heterodimeric enzyme converts adenosine to inosine in the tRNA anticodon. A mutation in this gene causes a syndrome characterized by intellectual disability and strabismus. This gene shares its 5’ exon with the overlapping gene, secretory carrier membrane protein.*  

*NSUN2: This gene encodes a methyltransferase that catalyzes the methylation of cytosine to 5-methylcytosine (m5C) at position 34 of intron-containing tRNA(Leu)(CAA) precursors. This modification is necessary to stabilize the anticodon-codon pairing and correctly translate the mRNA. Diseases associated with NSUN2 include Mental Retardation, Autosomal Recessive 5 and Dubowitz Syndrome.*  

**Other genes, including:**

*Hopx: homeodomain protein that lacks certain conserved residues required for DNA binding. It was reported that choriocarcinoma cell lines and tissues failed to express this gene, which suggested the possible involvement of this gene in malignant conversion of placental trophoblasts. Studies in mice suggest that this protein may interact...*
with serum response factor (SRF) and modulate SRF-dependent cardiac-specific gene expression and cardiac development. Marker for quiet stem cell in many tissues; ChR2: Channelrhodopsin is a subfamily of retinylidene proteins (rhodopsins) that function as light-gated ion channels. They serve as sensory photoreceptors in unicellular green algae, controlling phototaxis: movement in response to light. Expressed in cells of other organisms, they enable light to control electrical excitability, intracellular acidity, calcium influx, and other cellular processes. We will overexpress this gene on organoids before transplantation to validate the functional connectivity.

Evaluation of foreseeable effects

Bacteria:
The K12 derivative bacteria are widely used in routine culture work for cloning and have a proven safe history. There will be no expression of functional proteins in bacteria and therefore the inserts should not confer any advantage to the bacterial host. The bacteria are disabled, and the plasmid vectors classified as non-mobilizable, and therefore are not transferred between organisms.

Viral vectors:
All retroviral, lentiviral (3rd generation), adenoviral and adeno-associated viral vectors that will be used in this project are replication incompetent and have a history of safe use. All viral vectors are designed with low homology to prevent recombination with the host. The multi-plasmid transfection system ensures minimal likelihood of recombination, and lentiviral vectors are self-inactivating. For the concentration of virus, a ultracentrifuge with sealable buckets will be used in case of spillage. These detachable buckets will be opened and closed inside category II tissue culture hoods. After concentration, virus will be aliquoted into sealed tubes.

Inserted genes:
Reduced expression of Cyfip1 has been observed in various human cancers. Therefore, CYFIP1 might have a function to inhibit tumour invasion. Other than knockdown of CYFIP1, there is no evidence that knockdown or overexpression of listed genes to increased risk of specific cancers.

Target cells:
The eukaryotic cell lines are standard cell lines with a long history of safe use. They cannot survive outside culture conditions. Genetically modified brain organoids will pose no hazard to human health or the environment since they are unable to colonise humans or survive outside of laboratory culture. For the mouse work, virus will be stereotaxically injected into the mouse brain using neurosyringe. Neuro syringes is ultrafine needle (32gauge) but has dull end and a protective sleeve of needle. Also, only 0.5-1 µl of virus will be injected, therefore there is limited risk of accidental sharp injury or spillage due to direct human handling of the virus. However, there is a possibility that virus aerosol is produced during loading of virus into syringe. Therefore, loading should be performed in the biosafety cabinet.

Effects of accidental inoculation of laboratory workers:
No specific symptoms are expected as the result of an infection with the genetically modified micro-organisms described in this application since the viral vectors cannot replicate. Any genetically modified cells would be eliminated by the immune system in immunocompetent individuals. In carriers of wild-type HIV there may be a theoretical risk of recombination of the lentiviral vector with wildtype virus. Thus, there would be the potential risk of mobilisation and transmission of the recombinant lentivirus to other individuals via the normal routes of HIV-1 transmission. Individuals known to be HIV-positive or to be immunocompromised are asked to contact Occupational Health before working on this project. This will be made clear to all personnel in the laboratory.

Infected mice will be housed in secure cages in a containment level 2 room in a full barrier facility.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be decontaminated with 10% chemgene (final concentration) within the microbiological safety cabinet for a minimum of 20 minutes prior to disposal through the laboratory waste sink. The treatment with 10% chemgene completely disrupts the virus thereby eliminating virus activity. Solid waste will be decontaminate by
immersion in 10% chemgene solution for at least 20 minutes. The solid waste will then be autoclaved under vacuum air extraction with rapid steam heating to 121°C for 25 minutes. 100% kill is expected to be achieved.

The autoclaves are tested regularly, using independent thermocouplers placed at the centre of the load, to ensure a sufficient temperature and pressure are maintained for the required time. Validation is performed by spreading samples from autoclaved solutions on agar plates and monitoring for bacterial growth. To check the lab autoclave is working correctly, indicator tape is included in the runs.

All infected liquid and solid waste will be sterilized by 10% chemgene. Validation by the manufacturer indicates that this will result in inactivation of all organisms detailed in this proposal.

Liquid and solid waste will be disposed of according to the normal Foresterhill waste procedures as described.

The committee were happy that the hazards of the project have been correctly identified, and that appropriate safe systems of work will be used to control those risks identified. This application was approved at Containment Level 2 by the local Genetic Modification Safety Committee.

Please enter comments on the GM safety committee on the risk assessment

The committee were happy that the hazards of the project have been correctly identified, and that appropriate safe systems of work will be used to control those risks identified. This application was approved at Containment Level 2 by the local Genetic Modification Safety Committee.

Project Containment

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Project Ref 490/21.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

03/06/2021 Deletion of duplicated metabolic genes of Staphylococcus epidermidis to explore their Class 2 < 1 Litre
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Staphylococcus epidermidis (multidrug resistant clinical isolates).

Host/vector system

pMAD vector

Origin & function

To enable the targeted deletion of the 6 genes listed in section 6 we will utilise the pMAD shuttle vector for generating gene inactivation mutants in naturally non-transformable gram-positive bacteria. This vector was derived from the cloning of shuttle vector pE194ts:pBR322 and the NaeI-BglII DNA fragment containing the extended multiple cloning site from the pMTL22 plasmid (Arnaud et al., 2004). This vector features the bla and emrC genes for antibiotic resistance.

Evaluation of foreseeable effects

S. epidermidis strains used for this work are clinical isolates derived from blood cultures of patients who suffered from bloodstream infections during their admission in the intensive care unit of Aberdeen Royal Infirmary. These infections are largely the result of inevitable contamination during invasive procedures or insertion of medical devices (cannulation, intubation or catheterisation) in intensive care where patients are already susceptible to a range of opportunistic infections, all compounded by the ‘immune-paralysis’ associated with sepsis (McGahee and Lowy., 2000). S. epidermidis is otherwise one of the most predominant commensal microorganisms which colonises the skin and mucous membranes. Unlike S. aureus, S. epidermidis is not classified as Hazard Group 2 pathogen by the ACDP. However, S. epidermidis strains isolated in intensive care units are known to carry multiple resistance genes giving rise to Multidrug Resistant Staphylococcus epidermidis (MRSE). For this reason, precautionary measures for handling and disposal of S. epidermidis will be the same as those expected for HG 2 pathogens, notwithstanding that multidrug resistance in staphylococci does not necessarily result in increased bacterial fitness or virulence (Geisinger and Iseberg, 2017). For this project we will create targeted deletion mutants of wild-type clinical isolates of S. epidermidis (free of disabling mutations).

Selection of deletion mutants requires the insertion of use of a dominant selectable marker such as Spc (spectinomycin resistance). Spectinomycin is not used in clinical practice and therefore the use of this marker does not compromise antimicrobial therapies.

For this project we will create targeted deletion mutants of wild-type clinical isolates of S. epidermidis. To date there is no evidence to indicate that antimicrobial resistance...
or virulence of staphylococci can be increased through targeted deletion of any gene(s). If anything, these strains are expected to display antimicrobial resistance and virulence levels that are less than (or at worse equal) to the wild-type clinical isolates. It is important to note that multidrug resistance has been often associated with a reduction in pathogenicity in Staphylococcus and beyond. (Geisinger and Isberg, 2017).

The risk of DNA transfecting human cells is extremely low. In any event DNA replication and expression of genes borne on the shuttle vector cannot be expressed in human cells in the absence of eukaryotic origin of replications and promoters.

References

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| n/a |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| n/a |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Small spills will be inactivated using laboratory disinfectant, Chemgene, according to the instruction of the manufacturer (https://www.starlabgroup.com/GB-en/gloves-safety/laboratory-disinfectant_WebPSUB-159946/chemgene-hld4l-conc-clear-unfragranced-5-litre_SLXTM309-C.html#tab=downloads).

Large cultures will be inactivated by adding neat Chemgene to a concentration of 10%.

Cultures on petri dishes will be inactivated by autoclaving (121°C, 15psi, 15-20min).

Our standard procedures for use of Chemgene have been validated experimentally to kill >99.99% of cells. Viability tests were performed on Chemgene-exposed cultures. Autoclaves are validated at least annually by means of biological indicators. In addition, we have validated killing at >99.99% by plating samples harvested from the centre of autoclaved samples. In daily use, monitoring is by thermocouple temperature recordings and autoclave strips included in the loads.

Once the genetically modified microorganisms have been inactivated, normal routes of disposal are used for liquid and solid waste: 1) solid waste is incinerated; 2) liquid waste is discarded down the sink.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

### Please enter comments on the GM safety committee on the risk assessment

The safety committee noted that activities associated with this work should be assigned to Class Activity 2 given the requirement for CL2 containment measures to control the risk (refer to section 7 for explanation of risk).

The safety committee noted that a higher concentration of laboratory disinfectant (10% Chemgene) is required for inactivation of GM S. epidermidis grown in larger cultures and also for routine bench top and equipment decontamination.
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**Project Ref**: 490/97.2

**Date Ackn'd**: 04/07/1997

**CU2 Project Title**: PRODUCTION OF RECOMBINANT ADENOVIRUS

**Class**: Class 2

**CultureClass2**: Consent Granted

**CultureVolumeClass3-4**: not applicable

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  490/99.2

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**Non-GMM Consent Granted**

*not applicable*

**Project notified under transitional arrangements**

*Y*

**Withdrawn**

*N*

**Tick if notifying a connected programme of work**

*N*

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

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Animal Units

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**Name**

UNIVERSITY OF EAST LONDON

**Name 2**

**Department**

FACULTY OF SCIENCE & HEALTH

**Campus Estate or Research Centre**

STRATFORD CAMPUS

**Road Name**

ROMFORD RD

**Building**

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

E154LZ

**Country**

ENGLAND

**Tel Number**

0180 590 7000

**Fax Number**

0208 223 4959

**E-mail**

**HSE Division**

LONDON

**Comments**

Date at Which Additional Info Submitted:

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<tbody>
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<td>Other(s)</td>
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Maximum culture volume to be released at one time: 1 litre.
Waste deactivation: by autoclaving at 15 lbs/sq inch pressure and 121 degrees C for at least 20 minutes.
Validation of the deactivation method: colour change of autoclave tape.
Monitoring of deactivation method: all waste is labelled, autoclave tape attached, and placed in appropriate containers prior to deactivation in an autoclave room.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### MRC INSTITUTE OF HEARING RESEARCH

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### Comments

Centre closed

### Date at Which Additional Info Submitted

14/10/2004
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Transgenic Fish</td>
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</table>
Maximum culture volume for disposal is between 500 ml to 1000 ml liquid LB media. Autoclaving is used for waste deactivation. Contaminated fluids are autoclaved before disposal at 126 degrees C, for 20 minutes. Contaminated LB plates and solid waste are placed in either yellow biohazard bags and incinerated, or autoclaved, wrapped and placed in the normal bins for disposal.

At regular intervals, to monitor the adequacy of a disinfectant or the disinfectant method, the Kelsey and Maurer in-use test is used. Whenever a new disinfectant, dilution, or deactivation procedure is brought into use, the Kelsey and Maurer in-use test is also performed.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Other(s)

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 497

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**Name**

AVENTIS CROP SCIENCE LTD

**Department**

PLANT SCIENCE RESEARCH

**Campus Estate or Research Centre**

FYFIELD ROAD

**Town**

ONGAR

**County**

ESSEX

**Postcode**

CM5 OHW

**Country**

ENGLAND

**Tel Number**

01277 301301

**Fax Number**

01277 301180

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022

Page 7900 of 15326
### Premises Addresses

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<td>RHONE POULENC AGRICULTURE LTD</td>
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<td>CM5 OHW</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
All waste materials and laboratory ware are sterilised by autoclaving or in some instances by treatment with hypochlorite.

The maximum culture volume for GMMs used at any one time in any single vessel is 2 litres; this is the maximum volume that could foreseeably be released accidentally.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

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**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

Date Premises Closed  Name  Department  Name 2  Campus Estate or Research Centre  Building  Road Name  District  Town  County  Post-code  Country  Withdrawn
30/06/2016  MUREX BIOTECH LTD  CENTRAL ROAD  TEMPLE HILL  KENT  DARTFORD  DA1 5LR  ENGLAND  N

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)  Tick if confidential

Bacteriology  Parasitology  Transgenic Birds  Microbiology Research
Virology  Transgenic Animals  Transgenic Fish  Gene Therapy
The maximum culture volume for the class 1 activities in 50 litres. This is done occasionally (around 3-4 times per annum) but volumes are more typically 2 litres or less. The worst case scenario would occur if a 50 l fermenter were to break or leak its entire contents. In this unlikely event, the spill would be contained within the area and decontaminated in situ. Waste or spills would be deactivated by a variety of methods dependent on the situation. These range from heat inactivation by autoclaving in validated autoclaves to use of approved disinfectants such as sodium hypochlorite (presept tablets) or fumigation with formaldehyde gas for room sterilisation.

Deactivation is in accordance with published procedures, but the Murex GMSC agrees that it would be good practice to have internally validated these methods and has instigated a programme of experimental work to provide scientific backing to the practices adopted.

<table>
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<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Date at Which Additional Info Submitted**: 23/01/2002
Premises Addresses

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<td>HARPENDEN</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
All genetically modified plant material must be disposed of by one of the following procedures:

1. Autoclaving on a waste cycle (30 minutes at 15 lbs/in², 121 degrees C). The inbuilt autoclave monitoring systems must be checked to ensure the cycle was completed and records of each run kept to the extent that it is noted such output was checked. All autoclaves must be correctly maintained and users appropriately trained. Service records for autoclave equipment must be retained.

2. Milling, grinding, or processing of material to such an extent that the organism is rendered non-viable. Where the non-viability cannot be judged by eye, e.g., in the case of small seed where the extent of destruction cannot be ascertained an appropriate germination test must be carried out using a recorded and approved protocol. All such disposals and assessments should be recorded.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Name
SCOTTISH AGRICULTURAL SCIENCE AGENCY

Name 2

Department

Campus Estate or Research Centre
EAST CRAIGS

Road Name
82 CRAIGS ROAD

Town
EDINBURGH

Country
SCOTLAND

County
EAST LOTHIAN

Postcode
EH12 8NJ

Tel Number
0131 244 8863

Fax Number
0131 244 8940

E-mail

HSE Division
SCOTLAND

Comments

Date at Which Additional Info Submitted
20/09/2002
### Premises Addresses

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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities
- Give brief details of the genetic modification safety committee
  
  - Laboratory
  - Animal Unit
  - Growth Room
  - Glass House
  - Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial
- Other (please specify) Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

<table>
<thead>
<tr>
<th>Project Ref</th>
<th>504/02.1</th>
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<td>USE OF PLASMIDS CONTAINING FULL LENGTH TRANSCRIPTS OF POTATO SPINDLE TUBER VIROID (PSTVD) IN E. COLI TO PREPARE DNA AND RNA PROBES FOR DIAGNOSTIC WORK.(CA REJECTED NOTIFICATION )</td>
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<td>Project notified under transitional arrangements</td>
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</table>
# Project Additional Information

**Purposes of the contained use**

Probes are used to diagnose PSTVd in samples of imported and UK-derived potato material submitted for testing. These probes are produced from full length transcripts of PSTVd in E. coli.

**Recipient or parental organism**

The host organism is a disabled laboratory strain of E. coli (JM 109 or JM 101)

PSTVd is a non-indigenous viroid pathogen of potato, comprising approximately 359 nucleotides. It causes disease in potatoes and tomatoes and has been found in a number of other plants. It is not present in the UK and is a quarantine organism listed in EC Plant Health legislation. PSTVd is held under plant health containment for use as a positive control in diagnostic assays.

**Host/vector system**

For the RNA probe the plasmid pSP65 containing a full length dimmer of PSTVd is used. For the DNA probe an M13 bacteriophage cloning vector containing full length PSTVd is used.

**Origin & function**

The full length PSTVd sequences were produced in the USA and tyrailed in Peru.


**Evaluation of foreseeable effects**

The resulting PSTVd RNA and DNA sequences may be infectious, so present the same risk to potatoes and tomatoes as wild-type PSTVd.

There are no foreseeable effects on human health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All material will be autoclaved or sterilised in 20% sodium hypochlorite (equipment that has been in contact with the GMM) prior to disposal. Both disposal methods are used in the laboratory to kill wild type PSTVd.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The work requires a GM Plant Health Licence.

**Project Containment**

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<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

<table>
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<th>Human Clinical Applications</th>
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02/03/2022
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<td><strong>Emergency Plan Required?</strong></td>
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</table>

**Name**

SWANSEA UNIVERSITY

**Department**

SCHOOL OF BIOLOGICAL SCIENCES

**Road Name**

SINGLETON PARK

**Town**

SWANSEA

**County**

SWANSEA

**Postcode**

SA2 8PP

**Country**

WALES

**Tel Number**

01792 295361

**Fax Number**

01792 295447

**HSE Division**

WALES AND SOUTH WEST

**Comments**

Date at Which Additional Info Submitted:

02/03/2022
## Premises Addresses

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<th>Road Name</th>
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<th>Town</th>
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<td>SINGLETON PARK</td>
<td>GROVE, WALLACE &amp; MARGAM</td>
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<td>SWANSEA UNIVERSITY</td>
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<td>INSTITUTE OF LIFE SCIENCE 1 &amp; 2</td>
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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Chair (Senior Lecturer in Biochemistry)
- University Biological & GM Safety Advisor
- Occupational Hygienist/Scientific Safety Officer
- Representative College H&S Lead
- College Biological and GM Safety Officers (from College of Science, Engineering and School of Medicine)
- College Academic and Technical Representatives
- Occupational Health Advisor
- Recycling and Waste Officer
- Environment Officer

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 1 (GMMs)</td>
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<td>Level 4 (GMMs)</td>
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</table>
The maximum culture volume for disposal of liquid waste is 2 litres. Solid waste is deactivated by autoclaving. Liquid waste is deactivated either by autoclaving or by addition of disinfectant Virkon. Validation and monitoring involves use of autoclave tape, and for disinfectant, reference to the manufacturer's instructions supplemented with testing for live micro-organisms post deactivation.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref** 507/07.1

**Date Ackn'd** 29/11/2007

**CU2 Project Title** Biofilm formation, adhesion and antimicrobial resistance of Staphylococci.

**Class** Class 2

**CultureVolClass2** 1-50 Litres

**CultureVolumeClass3-4** Not Applicable

**Date Project Ceased** 02/03/2022

**Non-GMM Consent Granted** Not Applicable
### Project Additional Information

#### Purposes of the contained use

The aim is to investigate S. aureus and S. epidermidis adhesion, biofilm formation and development of antibiotic resistance. The project will examine the differential expression of S. aureus and S. epidermidis adhesins and intercellular adhesive mechanisms, the global regulatory circuits like agr, sigB and sar, and antibiotic resistance in relation to biofilm formation and infections associated with implanted biomaterials. The long-term objective is to improve our understanding of staphylococci infections which will lead potentially to the development of strategies for the prevention and improved treatment of such infections.

#### Recipient or parental organism

- **Staphylococcus strains; ADCP Group 2 micro-organisms:** S. epidermidis 1457, S. epidermidis 5179, S. epidermidis 1585 and clinical S. aureus and S. epidermidis isolates as the parental hosts.
- S. aureus RN4220 restriction deficient cloning host.
- S. carnosus TM300 cloning host for protoplast transformation.
- Escherichia coli K12 cloning strains; ADCP Group 1 micro-organisms: E. coli TOP 10 Cloning host; E. coli BL21AI Expression host; E. coli MC1061 Expression of erythromycin resistance of Tn917 in E. coli

#### Host/vector system

- pTVlts for transposon Tn917; pRN3208 for transposon Tn551; pBT1, pBT2 replicon in staphylococci for allelic exchange mutagenesis; pRB474 shuttle vector for cloning in E. coli/staphylococci; pASI shuttle vector for cloning in E. coli/staphylococci; pCX15 inducible gene expression in staphylococci under control of xylA promoter; pWBG636 conjugative mobilisation of plasmids; ClosTron system for allelic exchange mutagenesis; pBluescript II SK E. coli cloning vector; pCRII TOPO & pCR2.1 TOPO cloning vectors for PCR fragments in E. coli; pENTR/D-TOPO entry vector for Gateway technology in E. coli; pDEST1 7 expression vector: N-terminal His6-Tag.

#### Origin & function

DNA will derive from S. aureus and S. epidermidis. Genetic modification will be carried out to determine the function of genes involved in biofilm formation and antimicrobial resistance of these bacteria. Marked transposons will be mobilised from E. coli into S. aureus and S. epidermidis. Mutants which demonstrate either susceptibility to various agents or a diminished capacity for virulence will be selected using laboratory models of disease. The genes which have been disrupted by these transposon insertions will then be identified by genetic analysis. To determine if these disrupted genes play a role in the actual observed phenotype, the wild-type gene will be cloned back into the mutants; restoration of the parental phenotype will then be examined. In addition, the genes may also be mutated by site-directed mutagenesis to create unmarked, non-polar mutations, in order to clarify their role in virulence and resistance. This gene knockout and complementation approach will be used to elucidate the genetic basis for virulence, pathogenesis and overall environmental fitness in S. aureus and S. epidermidis.
S aureus and S. epidermidis are ACDP Hazard Group 2 bacterial species capable of causing opportunistic human infection. Likelihood of causing infection in healthy laboratory worker is low and therapy is available to treat any potential infections. The likelihood of escape of bacteria from the laboratory or transfer to susceptible individuals is low given the level of containment and disinfection procedures employed during handling of these bacteria. S. carnosus is used as starter for fermentation of sausages and has no hazards for human health, Escherichia coli K12 cloning strains to be used in the project have no associated hazard.

The plasmid vectors to be used are either: (i) narrow-host range E. coli vectors for DNA sequence analysis (ii) shuttle vectors for self-cloning of DNA in staphylococci which require electroporation or protoplast transformation or conjugation for their introduction, or (iii). These vector systems given the level of containment and disinfection procedures employed during handling of these vectors and recipient hosts carry a very low associated hazard. Genes involved in virulence, biofilm formation and antibiotic resistance are identified by a mutagenesis procedure which will stop expression of the recombinant gene. Self-cloning of DNA will be used to restore expression within the mutants and check the function of the encoded DNA. The genes explored are widely distributed in naturally occurring populations of staphylococci. All other sub-cloning of DNA will be performed in E. coli K12 cloning strains which are highly attenuated and will not represent an increased hazard risk after insertion of DNA from S. aureus and S. epidermidis.

Since mutagenesis and self-cloning of DNA will be the genetic modification performed, the recombinant strains will not present a pathogenicity hazard greater than the original host organisms, S. aureus or S. epidermidis. The only interspecies transfer of DNA to be performed will be from S. aureus or S. epidermidis to E. coli K12 cloning strains. The E. coli K12 cloning strains are harmless and transfer of DNA into them will not represent an increased hazard.

As outlined above the genetic modification performed, mutagenesis and self-cloning of DNA will not increase the hazard risk above that of the wild type parental strains. S. aureus and S. epidermidis are ACDP Hazard Group 2 micro-organisms capable of opportunistic human infection, but for which treatment exists in healthy individuals. Hence they pose a low level of risk.

### Evaluation of foreseeable effects

S aureus and S. epidermidis are ACDP Hazard Group 2 bacterial species capable of causing opportunistic human infection. Likelihood of causing infection in healthy laboratory worker is low and therapy is available to treat any potential infections. The likelihood of escape of bacteria from the laboratory or transfer to susceptible individuals is low given the level of containment and disinfection procedures employed during handling of these bacteria. S. carnosus is used as starter for fermentation of sausages and has no hazards for human health, Escherichia coli K12 cloning strains to be used in the project have no associated hazard.

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### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMM cultures (liquid and on plates), and contaminated plasticware, glassware, paper towels/spill material will decontaminated by autoclaving for 20 minutes at 134 degrees celsius in the designated autoclave in room 508. 100% of the GMMs will be inactivated using this method of heat/steam treatment.

The GMO authorised autoclave on the ground floor, room 026 will be used if the autoclave in room 508 breaks/out of order. The autoclaves will be tested/checked according to the regulations.

Hands will be disinfected after handling any cultures, and also before leaving the laboratory using disinfectant hand scrub, HDSIO, available at each sink.

The disinfectant for routine use is sodium dichloroisocyanurate (NaDCC) at 0.25% made up according to manufacturers' specification. All work areas will be disinfected using 0.25% NaDCC immediately after use. This is currently being reviewed as new products are being introduced to the market.

### Is an emergency plan required according to regulation 20? N

### If yes, tick to confirm that it is attached to this form N

### Tick to confirm that you have attached a risk assessment to this form Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment N
A special meeting of the Working Group was convened:

TO CONSIDER

The GM Risk Assessment for the Institute of Life Sciences Building.

explained the requirements concerning the risk assessment of the ILS and tabled an assessment that detailed the control measures to be carried out for handling Containment Level II work in laboratories (ILS 5D, Floor).

The findings of the assessment were discussed, in particular the requirements of wearing appropriate and suitable personal protective clothing for laboratory staff undertaking Containment Level I and Containment Level II working within the same laboratory explained the minimum safety requirements.

It was agreed that there would be a need to slightly amend the ‘local safety rules’ to include new rules governing the need to wear designated laboratory coats whilst working on the th Floor.

Following further discussion the following ACTION was AGREED

1. Action: is to complete HSE Form cu2 2000 (rev 2005) Notification of intention to conduct individual contained use activities” and forward a completed copy to all Committee members.
2. Action: is to speak with MB to confirm who should sign the HSE form
3. Action: is to ensure that once the form has been signed it is sent to the HSE and that signed a copy be sent to the Group Secretary.
4. Action: to update local safety rules and disseminate to all staff.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

Large Scale Activities

Human Clinical Applications

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<td>Investigation of cytochrome P450, sterol biosynthesis and beta oxidation pathways in fungi</td>
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<th>Tick if notifying a connected programme of work</th>
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<tbody>
<tr>
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</table>
**Project Additional Information**

### Purposes of the contained use

The aim is to investigate GM fungi of Candida spp., Fusarium spp. and Aspergillus spp. which have alterations (through methods such as gene insertion and gene knockout) in the pathways involved in, and associated with cytochromes P450, sterol biosynthesis and beta oxidation to study the pathways affected and to produce novel products. Azole drugs interact with cytochromes P450 and genetic modifications will address mode of action, susceptibility and resistance.

### Recipient or parental organism

<table>
<thead>
<tr>
<th>Organism</th>
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<tbody>
<tr>
<td>Candida spp., Fusarium spp. and Aspergillus spp.</td>
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</tr>
<tr>
<td>E. coli – lab adapted strains with long history of safe use</td>
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</table>

### Host/vector system

Vectors used for transformation in Candida spp. will be episomal shuttle vectors. In Aspergillus spp., general use vectors, based on vectors such as pGEM-T easy or pCR2.1 will be used. In all cases, the vector can contain an origin of replication that allows for manipulation in E. coli, but there is no origin of replication for the host organism. Genetic manipulation will be via homologous recombination. Most recipient strains have pyr auxotrophy and pyrG from related fungi, e.g. A. nidulans, used as marker. A similar homologous integration system to Aspergillus is used in Fusarium.

### Origin & function

Genes associated with cytochromes P450, sterol biosynthesis and beta oxidation and related pathways from Candida spp., Fusarium spp. and Aspergillus spp.

Genes have the potential to be involved with anti-fungal drug resistance. Antibiotic/Antifungal selection genes such as kanMX and hgh/hgt will also be
No genes known to be involved with the pathogenicity of this organism will be modified as part of this project.

**Evaluation of foreseeable effects**

As similar genes to those in the host will be self-replicated within the fungi, the hazard risk will be similar to that of the wild-type or less. Genes will also be inserted to produce other products, such as hydroxy fatty acids, are currently not known to play a role in pathogenicity and are present no greater risk of hazard than the wild-type.

As only the sterol and beta-oxidation pathways (and pathways associated with them) are to be altered, the recombinant strains will not present a pathogenicity hazard greater than the original hosts.

The genetic modifications to Candida spp., Fusarium spp. and Aspergillus spp. performed will not increase the hazard of each species above that wildtype.

As outlined previously, each fungal species is an ACDP Hazard Group 2 pathogen and represent a low risk to healthy individuals. There are also antifungal treatments available to treat infection in said individuals. Therefore the risk posed by exposure to these GMM is low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste will be contained in sealed containers and inactivated by autoclave prior to disposal to foul waste. Solid waste will be autoclaved prior to disposal in appropriate skip (for subsequent incineration)

**Is an emergency plan required according to regulation 20?**

- N

**If yes, tick to confirm that it is attached to this form**

- N

**Tick to confirm that you have attached a risk assessment to this form**

- Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- N
The committee thought this was a well thought out and detailed risk assessment. The panel agreed that modification of the pathways listed would not be expected to alter virulence of the pathogens or the hazards posed.

With respect to the environmental risk – the review panel noted that there is a site of special scientific interest near to the University but that Fusarium are not expected to be a pathogen of these wild plants (and the modified form no more than wild type), there are no significant crops of maize or wheat in the immediate area and that the GMMs are not expected to be more harmful than wild-type.

The panel requested the following clarifications:
- In the event of a spillage during centrifugation – rotors should be opened within a microbiological safety cabinet.
- Once autoclaved, waste will be placed in designated skip for final disposal via incineration

The review panel were satisfied that the control measures in the proposal were appropriate to minimise the risk and approved the assessment subject to minor modification.

**Project Containment**

<table>
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<tr>
<td>Animal Units</td>
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<td>Human Clinical Applications</td>
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**Project Ref** 507/19.1

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<td>Host-pathogen interactions important in the movement of Campylobacter jejuni from the broiler chicken gut to edible tissues (CampAttack)</td>
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**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Previous research done in our research group identified key genes involved in extra-intestinal spread, which is where Campylobacter leaves the gut and infects organs such as the liver. Genes responsible for increased invasion have also been studied. The key genes will be knocked out of C. jejuni strain M1, this should make the strains less invasive in vitro. The strains will be compared to the wild type and the significance of the genes determined. If invasion is reduced then in the future a vaccine could be developed using the information obtained from these experiments.

**Recipient or parental organism**

Campylobacter jejuni M1.

**Host/vector system**

Vectors being used are:

- pUC19-pGEMT easy
- pRDH315==� contains CAT cassette and H pylori rpsl gene (sensitive to streptomycin)
- pRDH316==� contains kanamycin cassette and H pylori rpsl gene (sensitive to streptomycin)
- pKM46==� contains kanamycin cassette with flanking region of CJ 0046 pseudogene
- pCM46==� contains CAT cassette with flanking region of CJ 0046 pseudogene

**Origin & function**

Genes thought to be involved in invasion and/or inflammation are being knocked out individually. The genes identified are involved in movement/chemotaxis, metabolism and membrane association.

**Evaluation of foreseeable effects**

Campylobacter is a class 2 bacterium, and the knock-out mutants will be less invasive than the wild-type. There is no greater risk than any other class 2 bacteria and the mutants are not more harmful than the wild type. The genetic modifications performed to Campylobacterspp. will not increase the hazard of each species above that wild-type. As outlined previously the risk of a healthy individual becoming infected with Campylobacter is low and any if infected the majority of infections are self-limiting. Therefore, the risk posed by exposure to these GMM is low. When the appropriate selections are used, cassettes and plasmids introduced to the recipient are highly stable. Throughout experimentation and the handling of GM bacteria, the selection will be maintained, therefore, any hazard as a result of phenotypic or genetic instability is low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be contained in sealed containers and inactivated by autoclave. Solid waste will be autoclaved. All waste will be autoclaved promptly. The degree of kill is 100% by autoclaving. Following autoclaving, waste is placed into tiger bags (yellow/black striped) and disposed into a locked autoclave only skip. The skip is collected by Veolia and is taken to Viridor, Trident Park Cardiff where it is incinerated.

The autoclaves are serviced, as well as calibrated and validated, yearly. Test strips and tubes are used to test the efficiency of the autoclaves and autoclaved samples.
have been sent to the waste contract company to test and have been shown to be negative. Any spills should be dealt with in the same manner as bacterial spills in containment level 2, flood the area with disinfectant (2% Distel solution) and mop up with paper towels, dispose of in autoclave bags.

**Please enter comments on the GM safety committee on the risk assessment**

GMO PANEL – 3rd July 2019
Review and consideration of GMO1903
1. The Sub-Committee asked for clarification in the risk assessment who the project partner was and which elements of work are taking place on site or off site. They also requested clarification of how knock out strains would be transported between the partner lab and Singleton campus.
2. Clarification on whether cells would be centrifuged following infection was requested.
3. The panel commented the likelihood of illness is in fact high if a person becomes infected and requested details of control measures to prevent exposure to reduce the potential of exposing people to a ‘low’ risk. They also requested details of the disinfectant procedures.
4. There is a need to detail how the control measures prevent the exposure, resulting in the potential exposure of people to a low risk. Information on disinfectant procedures and what is being used was also requested.
5. More detailed information on containment measures was requested.
6. Further information on the average culture volume and frequency e.g. no of experiments per week was requested.
7. The panel felt that more detail was required in relation to the disposal of waste and how reporting of any spills or accidents would be completed.

GMO PANEL - 2nd October 2019
Review and consideration of GMO1903
The panel noted the updated risk assessment, following comments provided from the previous panel and approved the project to proceed.

**Project Containment**

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<th>Growth Rooms</th>
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**Name**

UNIVERSITY OF ABERTAY DUNDEE

**Name 2**

**Department**

MOLECULAR & LIFE SCIENCES

**Campus Estate or Research Centre**

**Road Name**

40 BELL STREET

**Town**

DUNDEE

**District**

PERTH AND KINROSS

**County**

**Postcode**

DD1 1HG

**Country**

SCOTLAND

**Tel Number**

01382 308000

**Fax Number**

01382 308663

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Tick if confidential

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 508/96.1

Date Ackn'd 22/02/2001

CU Project Title

GENETIC MODIFICATION OF ELM TREES AND OTHER PLANT GENOMES

Class 2

Culture Vol Class 2

Culture Volume Class 3-4

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
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<th>Laboratory Activities</th>
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02/03/2022
GM Centre Number: 509

Data Premises Notified (Originally) 19/10/1992

Transferred from 1992 Regs? Y

Transitional Premises Class none

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Name

ADVANTA SEEDS UK LTD

Name 2

Department

PLANT BREEDING STATION

Building

Campus Estate or Research Centre

Road Name

District

BOOTHBY GRAFFOE

Town LINCOLN

County LINCOLNSHIRE

Postcode LN5 OLF

Country ENGLAND

Tel Number 01522 811040

Fax Number 01522 810694

E-mail

HSE Division MIDLANDS

Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|-------------|-------------|-------------|-------------|
Level 1 (GMMs) | Level 2 (GMMs) | Level 3 (GMMs) | Level 4 (GMMs) | Non-microbial
Maximum culture volume used - 1 litre.

Waste deactivation - cultures are autoclaved at 121 degrees C, 1 atmosphere steam pressure to obtain a suitable and calculated Del.

Monitoring - digitally controlled cycle with two independent thermocouples to control, including a printout of each temperature cycle for each sterilisation.

Validation - periodic use of spore strips.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 510/20.1

Date Ackn'd: 12/03/2020

CU2 Project Title: Identification and functional analysis of virulence determinants from enteric bacterial and Microsporidia pathogens

Class: Class 2

CultureVolClass2: < 1 Litre

CultureVolumeClass3-4: Non-GMM

Consent Granted
**Project Additional Information**

**Purposes of the contained use**

This project will involve identifying new virulence genes in the following enteric pathogens and also characterise the functions of known virulence genes. The overall purpose is to determine the role of newly-discovered and established pathogen genes in virulence and pathogenicity of important human pathogens.

Pathogens that will be used in this study:

- All pathogens used in this project are hazard group (HG) II pathogens and their use will be strictly regulated under containment II guidelines. The pathogens being investigated are:
  - Pathogenic E.coli strains including enteropathogenic E.coli (EPEC) E2348/69 0127:H6 which is the causative agent of infantile diarrhoea, predominantly in developing countries; and Class I shiga-toxin-minus enterohemorrhagic E. coli 0157:H7
  - Salmonella enteric serovar typhimurium SL344 - which causes non-typhoid gastroenteritis in humans
  - Trachipleistophora hominis - a microsporidian parasite that may infect immunocompromised patients
  - Encephalitozoon cuniculi - a microsporidian parasite that may infect immunocompromised patients

Genetic modification of the last two Microsporidian parasites will be attempted but currently no GM is possible as these parasites only exist within larger host cells and are dependent on host cells for their survival.

Pathogen genes will routinely be expressed in non-pathogenic disabled E.coli K12 and BL21 strains or mammalian cell lines that are classified as Hazard Group I, but will be manipulated and treated as Class II.

**Recipient or parental organism**

The recipient and parental organisms are as following

(i) Pathogenic species

The GMM in this study are all ACDP Hazard Group (HG) II pathogens and their use will be strictly regulated under containment II guidelines. Typically, these pathogens produce a self-limiting non-lethal diarrhoea in adults, lasting just a few days. The pathogens being investigated are:

- Pathogenic E.coli strains including enteropathogenic E.coli (EPEC) E2348/69 0127:H6 which is the causative agent of infantile diarrhoea, predominantly in developing countries; and Class I shiga-toxin-minus enterohemorrhagic E. coli 0157:H7
- Salmonella enteric serovar typhimurium SL344 - which causes non-typhoid gastroenteritis in humans
- Trachipleistophora hominis - a microsporidian parasite that may infect immunocompromised patients
- Encephalitozoon cuniculi - a microsporidian parasite that may infect immunocompromised patients

Genetic modification of the last two Microsporidian parasites will be attempted but currently no GM is possible as these parasites only exist within larger host cells and are dependent on host cells for their survival.
(ii) Laboratory-strain E. coli K12 which as defined by the Compendium of Guidance (Part 2) are non-colon ising and cannot survive for long outside the laboratory. They can be considered as equivalent to ACDP Hazard Group 1.

(iii) E. coli BL21 and derivatives: The same arguments for K12 broadly exist for BL21 expression strains. Stemming from a HSE-commissioned study, ACGM Newsletter 30 (Nov. 2001) states (in para 3) that "BL21 can be considered broadly equivalent to K12 strains and that in most cases work which uses this host can be considered as a class 1 activity ....... but the cloning of a bacterial pathogenicity determinant into BL21 will need careful consideration and may in some cases warrant classification as class 2". Since this project involves cloning genes from ACDP Hazard Group 2 pathogens, their expression in BL21 will require CL2.

(iv) Mammalian hosts: Cultured mammalian cells used in this study include: HeLa, Caco-2, RAW 264.7, T84, J774, Cos-7 and HEK293T. These cell lines are highly unstable and require stringent conditions for growth. They cannot reproduce outside of controlled laboratory conditions and the transfected pathogen genes will not alter this susceptibility. In addition, as defined in the SACGM compendium of Guidance; section 2.5, para 5) in the unlikely event of injection of mammalian cells into a lab user, the immune system would rapidly reject the cells. Therefore, no additional hazards have been identified beyond those associated with the cloned gene products themselves. All cell lines will be treated as ACDP Hazard Group II.

Host/vector system

For pathogen expression the following vectors will be used - pACYC184, pSK(bluescript) and ptrc99a
Gene knockouts will be made with the suicide vector such as - pCVD442
For K12 and BL21 expression - common expression vectors such as pET expresion vectors will be used

Origin & function

Most of the genes cloned and expressed in the pathogens used in this project and in K12 strains will be of pathogen origin. When expressed in the pathogen (usually a deletion mutant) the complemented gene will be from the same species. Therefore, for almost all experiments, no unforeseen genetic interactions are predicted. In addition, the complemented genes are almost exclusively under the control of inducible promoters and hence would only be expressed weakly outside of the lab.

The intended function of the genetic material will be to either complement a deficient gene in the same strain or carry where mutagenesis of the gene may be performed to understand the genes functions. In addition, in disabled E.coli cloning or expression strains, and mammalian cells, the virulence genes will be expressed to purify the protein or understand the cell biological effects of the proteins in these class I cells.

Origin of genetic material:
Genomic DNA or RNA extracted from pathogenic strains as indicated in the above descriptions
Manipulations
(i) Cloning, expression, and manipulation of known or putative virulence genes in E. coli non-pathogenic strains such as K12
(ii) Expression of defined virulence genes in E. coli BL21 or BL21 -derived strains, from standard vectors where the cloned gene is expressed from a phage T7 promoter only upon IPTG induction of a chromosomally inserted T7 RNA polymerase gene.
(iii) Use of suicide plasmid vectors to construct genetically-defined mutants of pathogenic strains by either gene inactivation or allelic replacement.
(iv) Complementation of defined mutants of mutants by reintroducing the corresponding wild-type gene on
mobilisation-defective vectors. Some experiments may express defined genes in the wild-type strains, to examine multicopy effects.

(v) Examining the effects of mutant strains and purified gene products on standard cultured mammalian cells, such as HeLa, Caco-2, T84, HEK293, and J774 cells. Knockout mammalian cells with defined defects in particular pathways will be used in some experiments.

(vii) Expression of pathogenic genes in mammalian cells by transfection of non-mobilizable plasmids to investigate the virulence gene function.

**Evaluation of foreseeable effects**

While most of the work in this project will involve expression of virulence genes in disabled lab-strains of E. coli, the pathogens used in this project may be genetically altered by gene knockout, expression of virulence genes from a plasmid or in-cis or in-trans mutagenesis of virulence genes to elucidate their functions. These pathogens all belong to ACDP Hazard Group 2 and since only genes from the same or very closely-related strains will be introduced into these hosts, it is difficult to envisage how the proposed work could increase the virulence of these species. The plasmids used in this study are all non-mobilisable and therefore the risk of transfer of the plasmids to other bacteria is very small.

Where a gene is overexpressed from a plasmid, the gene will be typically under the control of an inducible promoter, and would only be weakly expressed outside of the lab. Therefore, no additional risks above and beyond those involved in handling ACDP Hazard group 2 pathogens have been identified.

Pathogenic Microsporidia, Microsporidia are weakly pathogenic towards healthy humans but can pose a threat to immuno-compromised patients. At present no methods are available to genetically modify microsporidia species, including those species used in this study. Attempts will be made to design protocols to make gene deletion possible with Microsporidia. The risks of increasing virulence of a Microsporidia species following genetic modification are extremely low as these pathogens are highly dependent on host cells for survival and have a finely tuned evolutionary relationship with the host. Gene deletion in Microsporidia will target important genes and will likely decrease virulence while gene expression studies will involve tagging pre-existing genes. Thus, the any attempted genetic manipulation will likely have little effect on Microsporidia pathogenicity compared with the parent strain.

In all cases, the pathogens and GM-modified class I organisms will be treated under Class II containment procedures and therefore given the low inherent risk of these GMMs, combined with the containment procedures in place, the overall risk is extremely low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The procedures in place are suitable for ACDP Hazard group II material

GM waste is of 2 types: liquid and solid.

All contaminated materials, including waste destined for incineration, will be inactivated as follows:

Liquid GMM waste:

[1] Treatment with a disinfectant, e.g. Distel™ or Virkon™ at 5x the recommended concentration, diluted 1:10 (final
concentration 10,000ppm available chlorine, 100% kill, contact time> 2 hours).
[2] Followed by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware.

Solid GMM waste:
[1] is autoclaved at 134 degrees C for 15 minutes at 27 psi pressure. This gives 100% kill.
All clinical waste is sealed in bags and transported on robust trays from the containment facility to the autoclave. This waste is handled by authorised personnel only. After autoclaving, the material is transferred to yellow bags for incineration

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref  510/20.2

Date Ackn'd  12/03/2020

Date Project Ceased  

CU2 Project Title  The Study of The Kinetoplastid Lipid Biosynthesis: Regulation of Virulence and Host-Pathogen Interactions

Class  Class 2

CultureVolClass2  < 1 Litre

CultureVolumeClass3-4  

Class  Non-GMM

Consent Granted  

Project notified under transitional arrangements  N
Sphingolipids are essential signalling molecules and integral structural components of eukaryotes plasma membranes. Differences in the sphingolipid metabolism between protozoan parasites and host cells have received major attention as a promising drug target towards the development of selective and safe new anti-protozoa Is. While many protozoan parasites can synthesise their own sphingolipid, there is strong evidence that they rely on salvaging host cell sphingolipids as well. The proposed work will look systematically into the enzymes involved in sphingolipid metabolism and attempt to decipher the mechanism of host-pathogen interactions and exploit differences for drug discovery projects.

The proposed projects will utilise genetic manipulation to knock out essential and semi-essential genes involved in lipid metabolism with direct impact on the virulence of the parasite; these genetic manipulations result in genotypic and phenotypic parasites of less capacity to induce infections while remaining a subject of interest of the experimental systematic studies.

Purpose of GM: Reverse genetic methods will be used to identify and characterise parasite proteins involved in lipid biosynthesis and trafficking, which will then be functionally analysed in transgenic Trypanosoma, using both molecular and biochemical methods.

The work will focus on the use of the following protozoan parasite:

[1] Kinetoplastid, Trypanosoma brucei brucei (a separate SAPO licence application has been submitted)

Recipient or parental organism

Escherichia coli, Saccharomyces cerevisiae and Schizosaccharomyces pombe are all are standard laboratory strains and approved hosts in widespread use for cloning and expression studies.

[1] E. coli K12 strains: BL21, JM101

Host/vector system

1) E. coli K12 strains: pUC or pAT153 based, e.g. pGEX and pET
2) S. prombe strains: pUC based, e.g. REP series
3) S. cerevisiae strains: pRS or pESC based
4) T. brucei pXS, p2T7

Origin & function
Genetic material:
Genomic DNA or RNA extracted from T. brucei brucei using conventional techniques.
The strains to be used will be:
T. brucei brucei (Lister 427, transgenic lines 29-13 and BF-4)
Genetic manipulation:
The open reading frames of target T. brucei genes (i.e. those encoding enzymes in the sphingolipid and other lipid biosynthetic pathways) will be amplified by PCR and cloned into unique restriction sites downstream of vector promoter signals, prior to transfection into E. coli.
Plasmid vectors will be pUC or pA T153 based such as the pGEX or pET series for the production of fused and nonfused recombinant proteins. The replicon is from colE1. The plasmids are non-mobilisable and include a selectable marker for resistance to ampicillin. Cloning in the pGEX vectors introduces target genes downstream of a glutathioneS-transferase gene for expression of a C-terminal fusion peptide. Transcription is inducible from the upstream tac promoter. Cloning in the pET vectors places target genes under the control of strong bacteriophage T7 transcription and translation Signals. Expression is induced by providing a source of T7 RNA polymerase in the host cell, under the control of the inducible lacUV5 promoter.
In practice, plasmids are cloned into JM101 (F'traD36 for no transfer of F, lacZDM15 for blue/white selection of recombinants, supE44 amber suppressor) and selected recombinants transferred into a lambdaDE3 lysogen of BL21 (F' ompT rB-mB-) containing the T7 polymerase gene cassette for expression.
The amplified fragments will be used to create null/non-functional DNA constructs that will be used to create the mutant strains of the T. brucei for further investigations to ascertain function in growth and infectivity. T. brucei brucei will only be grown in suspension.

Evaluation of foreseeable effects
T. brucei brucei is a cattle pathogen.
T. brucei brucei is not a plant pathogen.
The risk of infection and dissemination is effectively zero due to the absence of the intermediary insect vectors and containment.
accidental self-injection is minimal as the use of sharps prohibited. Safety protocols described within Code of Practice and Standard Operating Procedures.
Transgenic T. brucei will also be resistant to one or more antibiotics but this is irrelevant as T. brucei is non-infective to man. The objective of the project is directed towards the down regulation of specific endogenous gene expression which is more likely to make the transgenic organism less virulent and less able to survive in a host.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM waste is of 2 types: liquid and solid.
All contaminated materials, including waste destined for incineration, will be inactivated as follows:
Liquid GMM waste:
[1] Treatment with a disinfectant, e.g. Distel™ or Virkon™ at 5x the recommended concentration, diluted 1:10 (final concentration 10,000ppm available chlorine, 100% kill, contact time>2 hours).
Followed by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware.

Solid GMM waste:

1 is autoclaved at 134 degrees C for 15 minutes at 27 psi pressure. This gives 100% kill. All clinical waste is sealed in bags and transported on robust trays from the containment facility to the autoclave. This waste is handled by authorised personnel only. After autoclaving, the material is transferred to yellow bags for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 510/20.3

Date Ackn'd 12/03/2020

CU2 Project Title The Study of The Apicomplexan Biosynthesis: Regulation of Virulence and Host-Pathogen Interactions

Class 2

CultureVolClass2 < 1 Litre

Class 3-4

Consent Granted Not Applicable

Project notified under transitional arrangements N
Purposes of the contained use

Sphingolipids are essential signalling molecules and integral structural componenets of eukaryotes plasma membranes. Differences in the sphingolipid metabolism between protozoan paraSites and host cells has received major attention as a promising drug target towards the development of selective and safe new anti-protozoa Is. While many protozoan paraSites can synthesise their own sphingolipid, there is strong evidence that they rely on salvaging host cell sphingolipids as well. The proposed work will look systematically into the enzymes involved in sphingolipid metabolism and attempt to decipher the mechanism of host-pathogen interactions and exploit differences for drug discovery projects.

The proposed projects will utilise genetiC manipulation to knock out essential and semi-essential genes involved in lipid metabolism with direct impact on the virulence of the parasite; these genetiC manipulations result in genotypic and phenotypic parasites of less capacity to induce infections while remaining a subject of interest of the experimental systematic studies.

Purpose of GM: Reverse genetiC methods will be used to identify and characterise parasite proteins involved in lipid biosynthesis, which will then be functionally analysed in transgenic Toxoplasma, Neospora and Sarcocystis, using both molecular and biochemical methods.

The work will focus on the use of the following protozoan parasites:

1. Toxoplasma gondii: Nicolle and Manceaux (ATCC® 50174) RH and RHdeitaku80
2. Neospora caninum: Nc-LiV [NC-Liverpool]

Recipient or parental organism

Escherichia coli, Saccharhormyces cerevisiae and Schizosaccharomyces pombe are all are standard laboratory strains and approved hosts in widespread use for cloning and expression studies.

1. E. coli K12 strains: BL21 , JM101
2. S. prombe strains: LH 121 , THTS 18 and THTS22
3. S. cerevisiae strains: YPH499, YPH499-HIS-GAL-AUR1, AGO, RCD113, ISC1
4. Toxoplasma gondii: Nicolle and Manceaux (ATCC® 50174) RH and RHdeitaku80
5. Neospora caninum: Nc-LiV [NC-Liverpool]
8. Bovine Turbinate (ATCC): BT (ATCC® CRL-1390™)
E. coli K12 strains: pUC or pAT153 based, e.g. pGEX and pET
S. prombe strains: pUC based, e.g. REP series
S. cerevisiae strains: pRS or pESC based
Toxoplasma, Neospora and Sarcocystis: pEXP, pminiHXGPR, p5RT70tetR

Genetic material:
- Genomic DNA or RNA extracted from Toxoplasma, Neospora and Sarcocystis using conventional techniques.

Genetic manipulation:
- The open reading frames of target Toxoplasma, Neospora and Sarcocystis genes (i.e. those encoding enzymes in the sphingolipid and other lipid biosynthetic pathways) will be amplified by PCR and cloned into unique restriction sites downstream of vector promoter signals, prior to transfection into E. coli.
- Plasmid vectors will be pUC or pAT153 based such as the pGEX or pET series for the production of fused and nonfused recombinant proteins. The replicon is from colE1. The plasmids are non-mobilisable and include a selectable marker for resistance to ampicillin. Cloning in the pGEX vectors introduces target genes downstream of a glutathioneS-transferase gene for expression of a C-terminal fusion peptide. Transcription is inducible from the upstream tac promoter. Cloning in the pET vectors places target genes under the control of strong bacteriophage T7 transcription and translation signals. Expression is induced by providing a source of T7 RNA polymerase in the host cell, under the control of the inducible lacUV5 promoter.
- In practice, plasmids are cloned into JM101 (F'traD36 for no transfer of F, lacZDM15 for blue/white selection of recombinants, supE44 amber suppressor) and selected recombinants transferred into a lambdaDE3 lysogen of BL21 (F' ompT rB-mB-) containing the T7 polymerase gene cassette for expression.
- The amplified fragments will be used to create null/non-functional DNA constructs that will be used to create the mutant strains of the Toxoplasma, Neospora and Sarcocystis for further investigations to ascertain function in growth and infectivity. The parasites will be grown in human foreskin fibroblasts (toxoplasma) or bovine turbinate (Neospora and Sarcocystis).

Evaluation of foreseeable effects
- Theoretically, laboratory infections with the animal and human pathogens Toxoplasma, Neospora and Sarcocystis could be caused accidentally if large numbers of proliferative parasites were inoculated through the skin or via an open wound. To avoid this the use of sharps is to be avoided. Ingestion of cysts could also result in infection, to avoid this, encysted forms will not be studied.
- Safety protocols are described within the Code of Practice. Adherence to these render the risks of infection and dissemination effectively zero. Notably, Toxoplasma is endemic to the U.K. The down regulation of specific endogenous gene expression by the reverse genetics proposed is most likely to make the organism less able to survive in the environment.
- The risk of infection and dissemination is effectively zero due to the absence of the definitive hosts and containment. Toxoplasma, Neospora and Sarcocystis are not plant pathogen.
- No known potential and in the absence of infection of host cells, risk of transfer of genetic material must be regarded as very low or negligible.
- The objective of the project is directed towards the down regulation of specific endogenous gene expression which is more likely to make the transgenic organism less virulent and less able to survive in a host.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM waste is of 2 types: liquid and solid.
All contaminated materials, including waste destined for incineration, will be inactivated as follows:

Liquid GMM waste:
1. Treatment with a disinfectant, e.g. Distek™ or Virkon™ at 5x the recommended concentration, diluted 1:10 (final concentration 10,000ppm available chlorine, 100% kill, contact time > 2 hours).
2. Followed by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware.

Solid GMM waste:
1. Is autoclaved at 134 degrees C for 15 minutes at 27 psi pressure. This gives 100% kill.
All clinical waste is sealed in bags and transported on robust trays from the containment facility to the autoclave. This waste is handled by authorised personnel only. After autoclaving, the material is transferred to yellow bags for incineration.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 510/20.4
To determine the underlying mechanisms responsible for influenza A (H1 N1) virus induced asthma exacerbation by assessing epithelial cell damages.

Madin-Oarby Canine Kidney (MOCK) cells will be used for H1 N1 propagation. Mammalian cells which will be infected with dilution of H1 N1 include, immortalised epithelial cells (A459 and BEAS-2B), and primary epithelial cells obtained from bronchial brushing of healthy and asthmatic donors.

Origin & function
Influenza A virus (H1 N1), which is going to purchased from ATCC, was obtained from Nasopharyngeal specimen from a patient positive for Flu A in Virginia, 2009. Epithelial cells will be infected with H1 N1 at different dilutions (max Mal 5).

Evaluation of foreseeable effects
Cell Pathologic Effect (CPE) of include rounded refractile cells with cell degeneration. H1 N1 will be manipulated in a Class II Microbiological safety cabinet. Written records of staff training will be obtained and checked before commencement of work by individuals. Disposable plasticware (pipettes especially) will be used for mammalian cell culture. Precausions are full personal protective equipment including laboratory gowns, gloves and safety google.
Virus stock and virus-infected cells will be securely stored in -80°C freezer and locked after hours. All virus-contaminated wastes are disposed of in virkon solution and autoclaved for destruction. It is highly unlikely that any higher pathogenic strains will arise as a result of the use of these GMO strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All virus-contaminated wastes are disposed of in virkon solution and autoclaved for destruction.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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### Project Ref 510/20.5

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<td>Transduction of mammalian cells using lentiviral vectors</td>
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This project assesses the biological function of putative cancer driver genes by lentiviral transduction, and also involves identifying potential oncogenes and tumour suppressors by genome-scale screen in human cancer cell lines. Typically the methodology will involve a gene highly over or under-expressed in cell lines, and reversing the expression by either over-expressing the gene or expressing a small hairpin RNA (shRNA) or CRISPR single guide RNA (sgRNA) to silence expression of the gene in question. Lentiviral particles will be generated on site containing either a cDNA, appropriate shRNA or sgRNA. cDNAs may occasionally contain a mutation where a dominant negative or constitutively active version of the gene is required. Cells will be infected by titrated lentiviral particles followed by antibiotic selection or sorting by flow cytometry. Downstream analysis may involve proliferation, apoptosis and/or cell cycle assays at the NHC, and development of in vivo xenograft models in collaboration with external collaborators with appropriate animal licence and facilities. RNA, DNA and protein would typically be extracted from infected cells in each case to verify appropriate infection and to validate cDNA/shRNA expression or CRISPR/Cas9 gene editing. For genome-scale screen, a CRISPR library with various sgRNAs or pooled shRNA library is used to create a pool of cells which will then undergo in vitro selection to screen for constructs which either promote or inhibit cell proliferation. The purpose is to perturb the natural state of target genes identified from our OMICS screens and to assess the functional effects on cancer cells.

Packaging cell line 293T is derived from the well-characterised 293 cell line (Pear et al., PNAS 1993; 80; 8392; ATCC CRL-11268). This cell line stably and constitutively expresses a temperature-sensitive version of the SV40 large T antigen. Lentivirus is produced by co-transfection of the 293T cell line with transfer, packaging and envelope plasmid vectors. Human cancer cell lines will be transduced with the supernatant generated by the packaging cells. These cell lines originally derived from cancer patients, and are purchased from cell-line bank such as DSMZ. The cell-line cells are only able to survive if maintained in tissue culture in the NHC laboratory or if injected into immunodeficient animals (in collaboration with external collaborators).
Lentiviral packaging system (2nd generation) derived from HIV is going to be used to produce lentiviral particles. This recombinant lentivirus has pseudotyped with the VSV-G envelope protein and its tropism has a broadened host range. This system was specifically engineered for biosafety by separating lentiviral genes into three plasmid vectors, with packaging and envelope genes away from viral LTR on the transfer plasmid. The plasmids remain in the packaging cell line, effectively precluding the production of replication competent virus in target cells or should the viral vector escape containment. The plasmids have been engineered not to contain any regions of homology to each other, to prevent undesirable recombination events which might result in replication competent virus being produced. The viral vector cannot produce any new viral particles after the initial infection in host cells, without genes encoded in the envelope and packaging vectors. Also self-inactivation (SIN) is achieved by deleting a large portion of the viral 3' LTR. The deleted 3' LTR of the viral genome is transferred into the 5' LTR after one round of reverse transcription, and this deletion abolishes transcription of the full-length virus after it has incorporated into host cells and minimizes, the risk of generating replication-competent lentivirus.

Origin & function

cDNA sequences of human origin, appropriate shRNA or sgRNA sequences will be cloned into lentiviral vectors to assess potential contribution to tumourigenesis of candidate genes identified from our OMICS screens. cDNA sequences may include partial or entire gene sequences, or mutant forms of the gene sequences. shRNAs and sgRNAs are smaller than 100 bases in length, which are designed to target specific DNA. Target genes in cancer cell lines include proto-oncogenes and tumour suppressors such as FOX01, TP53 and other candidate genes. In genome-scale screen, many of the target genes will have unknown function but may include known oncogenes and tumour suppressors regulating cell proliferation and apoptosis. In CRISPR/Cas9 gene editing, Cas9 nuclease enzyme and a sgRNA are integrated into host genome, causing single or double-stranded breaks on target genetic locus. Genetic breaks are then repaired with a template for precise gene editing, or by non-homologous end joining mechanism leading to silencing of the target genes. The aim is to use either overexpression or knock-down/knock-out or gene editing to assess the potential contribution to tumourigenesis of each candidate gene identified from our genomics screens. Once a stably infected cell line is produced, experiments performed will depend on the particular gene selected but will typically involve a panel of proliferation, apoptosis and/or cell cycle, as well as collection of RNA/DNA/Protein for further profiling analysis at the NHC and potentially in vivo engraftment in collaboration with external collaborators.

Evaluation of foreseeable effects

The lentivirus is capable of transducing a wide range of mammalian cells, but it would not transfer genetic material to microbial organisms. Release of the viral vector into the environment is very unlikely due to the standard procedures employed in the tissue culture facility. Even should such an event occur the virus is replication incompetent and self-inactivating. It is highly unlikely that the genetically modified cell lines could transfer the viral vector into the environment since they have very limited ability to propagate outside of the cell culture facility, and no means to mobilise the transfected DNA sequences to other organisms. The transfer vector contains sequences that will incorporate into the host cell genome, but cannot produce functional viral particles without the genes encoded in the envelope and packaging vectors. With replication-competency and SIN it is not possible for viruses to replicate and produce more virus after the initial infection. Potential hazard of harmful sequences within the virus being transferred to related viruses is negligible, since there are only very small regions of homology between the viral vector and wildtype viruses, making recombination events unlikely. The lentivirus would have the ability to infect human tissues should it come in contact with them. The major hazard is therefore represented by the packaged lentivirus prior to infection of the target cells, and residual virus in the medium of transfected cells. The main potential transmission routes are by external exposure to high tit res (either skin lesions or mucous membranes). In addition, oncogenic effects could arise if tumour suppressor genes are silenced by certain
shRNAs or sgRNAs, particularly in genome-scale screens, as well as the over-expression of potentially oncogenic cDNAs. Therefore there is a theoretical possibility that they could render the cells neoplastic. If shRNAs, sgRNAs or cDNAs were to be transfected directly into living human tissue, the detrimental effects that they could cause are uncertain but unlikely on their own to transform affected cells. Tumourigenesis is a multistage process requiring the interaction of many mutated genes which also lessens the risk from delivery of a single genetic change to a target tissue.

The virus is generally weak and does not survive long outside of buffered solutions. Infection requires optimal conditions and the use of supplements in cell culture. Likelihood of harm is low as the virus is non-replicable and self-inactivating. With lab procedures for avoiding contamination, in particular no sharps, risk is generally no greater than handling human tissue. Reversion to replication-competent lentivirus is extremely unlikely given that several recombination events would be needed to reconstitute an active viral genome, the viral genes are present on three different plasmids which have minimal sequence homology and the viruses are self-inactivating following insertion. Given that the lentivirus is replication incompetent and SIN, and will only infect at low efficiency in sub-optimal conditions, potential harm would be extremely limited.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (culture and media) will be decontaminated by autoclaving or by Virkon solution (>1% w/v final working concentration of Virkon for at least 2 hours prior to disposal to sewers). All contaminated plasticwares and materials will be properly disinfected by autoclaving or by 2% w/v Virkon solution for at least 2 hours prior to incineration. Spillages will be soaked with tissue paper, which will afterwards be decontaminated with 2% w/v Virkon solution for at least 2 hours. Contaminated surfaces will be decontaminated with 1% w/v Virkon solution followed by 70% Ethanol.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

Laboratory Activities | Glass Houses | Growth Rooms

02/03/2022
Trypanosomiasis and leishmaniasis are caused by parasites which belong to the genera Trypanosoma and Leishmania, respectively. These diseases affect a large number of people across the world, and could be fatal if not properly treated. These diseases are also animal infective, where they also cause weight loss and significant number of deaths in domestic and wild animals, posing a restraint on agricultural outputs and economic prosperity, especially in resource poor communities. Trypanosomiasis and leishmaniasis require an insect vector, tsetse fly and sand fly respectively for disease transmission.

There is yet no known vaccine for these diseases. Both diseases can be treated with available chemotherapy, however, this approach is faced with many challenges, including drug resistance, toxicity, high treatment cost and the lack of guaranteed supply. Hence, there is a need for new strategies to combat these diseases.

Membrane-bound phosphatases (also called ectophosphatases) such as acid phosphatase are type I membrane protein that is, single span transmembrane proteins that have their N-terminus exposed to the extracellular space and C-terminus cytosolic. They catalyse the hydrolysis of orthophosphate monoesters and generate Pi as final product.

The roles of the ectophosphatases in parasite-host interactions during the trypanosomatids infections have been predicted but largely understudied.

A multidisciplinary approach will be used to investigate the unique roles of Trypanosoma and Leishmania ectophosphatases, and attempt to decipher the mechanism of...
host-pathogen interactions and exploit differences for drug discovery.

The proposed projects will utilise genetic manipulation to knock out or overexpress essential Trypanosomatid ectophosphatase genes with direct impact on parasite viability and virulence.

This research will focus on the use of the following protozoan parasite:

[1] Leishmania
[2] Trypanosoma (a separate SAPO licence application has been submitted)

**Recipient or parental organism**

Standard laboratory strains of host organisms approved and widely used for cloning and expression studies will be deployed throughout this research.

**E coli:**
- XL1 blue strain of Escherichia coli (E. coli)
- Top 10 E. coli
- Chemically competent Rosetta (DE3) cells

Leishmania Mexicana:
- (MNYC/BZ/62/M379 strain and various transgenic lines)

Trypanosoma brucei brucei and various widely used transgenic lines:
- Wild type T. b. brucei, strain Lister 427 (s427; MiTat 1.2/BS221)
- s427-WT derived TbAT1 knockout (tbat1−/−)
- B48 which was derived from a TbAT1-KO strain
- Aquaporin (aqp2/aqp3, aqp1-3) null strains

**Host/vector system**

Standard and approved laboratory vectors will be used. For example:

- pET151D/TOPO expression vector system
- pHD1336
- Leishmania pX, pTEX

**Origin & function**

All materials that will be used for genetic manipulation in this research will come from the open reading frames of Trypanosoma and Leishmania ectophosphatases, which will be amplified using the polymerase chain reaction-based techniques, the product will be cleaned before cloning into the restriction sites downstream of the promoter signals of the appropriate vector. This assembly (vector plus inserted gene) will be used for the transformation of E. coli.

The nature of modification is as follows. The pET system will be used for the cloning and expression of recombinant proteins in E. coli. Briefly, plasmid vectors (pET System) with selectable antibiotic cassette will be used for the production of fused and non-fused recombinant proteins. The insert (target) ectophosphatase gene will be cloned in the pET vector under the control of a strong bacteriophage T7 transcription and translation signals, expression of this gene will be induced by providing a source of T7 RNA polymerase in the host (E. coli) cell.

The T7 RNA polymerase should convert all of the cell's resources towards expression of the target ectophosphatase gene after a few hours of induction. In order to clone the target ectophosphatase gene, non-expression hosts that do not contain the T7 RNA polymerase gene will be initially used, thus eliminating plasmid instability caused by the production of proteins that are potentially toxic to the host cell. Once established in the non-expression host, the plasmid will then be transferred into the expression hosts, which contain a chromosomal copy of the T7 RNA polymerase gene under lacUV5 control, and IPTG will be added for the expression of the target ectophosphatase gene.
This will enable the over-production of the corresponding protein (ectophosphatase) of interest. The protein will be purified for onward chemical and molecular characterization.

In addition, for proof of concept, the amplified fragments will also be used for the transfection of the appropriate Trypanosomatid to create either an overexpressed or null/non-functional construct that will be used to create the mutant strains of the appropriate Trypanosomatid for further investigations into the essentiality in terms of its function and chemical inhibition.

**Evaluation of foreseeable effects**

There is a possibility of laboratory infections with Leishmania due to accidental skin or open wound inoculation with a large population of infective parasites, but considering that the use of sharps is prohibited in this work, the chances of this kind of accidental infection happening is effectively zero.

- T. brucei on the other hand is a cattle pathogen. Also, both Leishmania and T. brucei, require a vector (sand fly and tsetse fly respectively) for disease transmission; the absence of these parasite vectors in the UK makes the risk of infection by these parasites effectively zero.

- There will be a strict adherence to safety protocols described within the Code of Practice and Standard Operating Procedures operational at the NHC.

- Moreover, the absence of these tropical insect vectors makes it impossible for these parasites to disseminate within the environment.

- In addition, there is no known report of similar genetic modifications resulting in the capacity of similar organisms to survive, establish, and disseminate with and or displace other organisms.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

GM waste is of 2 types: liquid and solid.

All contaminated materials, including waste destined for incineration, will be inactivated as follows:

**Liquid GMM waste:**

1. Treatment with a disinfectant, e.g. Distel™ or Virkon™ at 5x the recommended concentration, diluted 1:10 (final concentration 10,000ppm available chlorine, 100% kill, contact time > 2 hours).
2. Followed by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware.

**Solid GMM waste:**

Solid wastes will be autoclaved at 134 degrees C for 15 minutes at 27 psi pressure. This gives 100% kill.

All clinical waste is sealed in bags and transported on robust trays from the containment facility to the autoclave. This waste is handled by authorised personnel only. After autoclaving, the material is transferred to yellow bags for incineration.

**Bacteria inoculation.**
GM E. coli will be disinfected with the appropriate solutions e.g. 100% ethanol followed by sterilization of inoculating loops with naked flame, autoclaving of GM E. coli and contaminated lab equipment like glassware, culture flask, etc. These will ensure 100% kill. UV light on the microbiological safety cabinet will be turn on upon completion of each task.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment  

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
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GM Centre Number: 511

Data Premises Notified (Originally) 03/12/1992

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed 08/05/2013

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

MRC PROTEIN PHOSPHORYLATION UNIT

Name 2 UNIVERSITY OF DUNDEE

Department BIOCHEMISTRY

Campus Estate or Research Centre MSI/WTB COMPLEX

Building

Road Name DOW STREET

District

Town DUNDEE

County PERTH AND KINROSS

Postcode DD1 5EH

Country SCOTLAND

Tel Number 01382 344 241

Fax Number 01382 223778

E-mail

HSE Division SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 511/00.1**

<table>
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<td>21/02/2000</td>
<td>EXPRESSION OF PROTEIN KINASES &amp; PHOSPHATASES &amp; ASSOCIATED PROTEINS INVOLVED IN INTRACELLULAR SIGNALLING PATHWAYS IN MAMMALIAN CELLS USING ADENOVIRAL VECTORS</td>
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**Date Project Ceased**

01/05/2013

**Consent Granted**

Non-GMM: not applicable

**Project notified under transitional arrangements**

Y

**Withdrawn**

N

**Historical Significant Changes**

TRANSFERRED TO GM6 ON 01/05/2013

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
RNAi knockdown and expression of protein kinases and phosphatases and associated proteins involved in intracellular signalling pathways in mammalian cells using retrovirus vectors

Commonly used tissue culture cell lines (HEK293, HeLa, COS cells) or embryonic stem cells, hepatocytes, fibroblast and myoblast cells derived from rodents.

The retroviral vectors to be used for the studies are called pHR-SIN-CSGW, pLK0.1 (Stewart et al., 2003), pMKO.1puro (Masutomi et al 2003) or pRS (commercially available origene vector). Please use full risk assessment for more information on these systems.
The inserts will be either RNAi constructs designed to remove intracellular mRNAs for their target genes or the cDNAs coding for the normal intracellular versions of kinases, phosphatases, etc. All these proteins are components of signalling pathways and so could affect cell growth. However, none will be known oncogenic versions of the proteins and so should not lead to cellular transformation. It is not known whether any of the proteins are capable of altering the properties of any naturally occurring viruses in any way that would increase their pathogenicity. However, from the known properties of the proteins this would not be predicted. The transfected cultured cell lines will not survive outwith the culture medium and all liquid and solid waste will be autoclaved on site. This rules out the possibility of escape into external environment and survival, establishment or dissemination therein.

**Evaluation of foreseeable effects**

The RNAi constructs are designed to remove intracellular mRNAs for their target genes and so the predicted direct effect of this will be the removal of the corresponding protein from the cells infected with the virus. This is predicted to interfere with the signalling pathway and this will what will be tested. In the majority of cases it is predicted that the removal of a component from the signalling pathway will lead to the loss of ability for that signalling pathway to function. In some cases it might be that the loss of an inhibitory component could lead to an upregulation of that pathway. Both effects are likely to be deleterious to the cell in the long run and it is not predicted that cell infected with the viruses will beat a selective advantage relative to their parent cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Autoclaving is by validated (internal temperature probe linked to printout) autoclave, in the same building, reserved only for inactivation of waste (effective 100% kill). The autoclave undergoes annual testing tyo ensure correct operation. Waste will be transferred to the autoclave in covered containers.

**Is an emergency plan required according to regulation 20?**

N

**Tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Project Containment**

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02/03/2022

Page 7960 of 15326
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02/03/2022
GM Centre Number: 514

Data Premises Notified (Originally) 05/01/1993

Transferred from 1992 Regs? Y

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

PLYMOUTH MARINE LABORATORY

Name 2 MARINE BIOLOGICAL ASSOCIATION

Department

Campus Estate or Research Centre

Building

Road Name PROSPECT PLACE

District THE HOE

Town PLYMOUTH

County DEVON

Postcode PL1 3DH

Country ENGLAND

Tel Number 01752 633412

Fax Number 01752 633101

E-mail

HSE Division WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted 02/03/2022
### Premises Addresses

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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

**Give brief details of the genetic modification safety committee**

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

**Other (please specify)**

**Tick if confidential**

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
Maximum culture volume = 3 litres. All solid waste deactivated by autoclaving. All liquid waste deactivated by either autoclaving, or deactivation by 'Virkon' or similar. Samples of deactivated waste periodically plated onto suitable medium to assess viability.

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<tr>
<th>Virology</th>
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<th>Transgenic Fish</th>
<th>Gene Therapy</th>
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<td>Transgenic Invertebrates</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity:

Maximum culture volume = 3 litres. All solid waste deactivated by autoclaving. All liquid waste deactivated by either autoclaving, or deactivation by 'Virkon' or similar. Samples of deactivated waste periodically plated onto suitable medium to assess viability.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref** 514/05.1

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<td>14/07/2005</td>
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<td>studies of the involvement of AHLs in the development of marine microbial</td>
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<td>communities.</td>
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Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to investigate the role of N-acyl homoserine lactone (AHL) bacterial signalling molecules in the development of microbial communities. This work does not involve the creation of new GMMs, but will utilise GM bacteria constructed at Nottingham University (UK) and Umea University (Sweden). The GMMs will be used in studies of the involvement of AHLs in biofilm formation of mixed species microbial communities.

**Recipient or parental organism**

- E. coli JM109 (recA1 supE44 and A1 hsdR17 gypA96 relA1 thi D (lac-proAB)).
- C violaceum wild type strain ATCC31532 was obtained from the American Type Culture Collection (Rockville, MD USA).
- V anguillarum NB 10, serotype 01, is a clinical isolate from the Gulf of Bothnia, Sweden.

**Host/vector system**

- pSB401 - AHL reporter plasmid; luxR "::luxCDABE (Apr) in pACYC184 (Winson et al., 1998)
- pSB536 - AHL reporter plasmid; ahyrR "::luxCDABE (Apr) in pUCP18 (Swift et al 1997))
- pSB1075 - AHL reporter plasmid; lasR "::luxCDABE (Apr) in pIC18 (Winson et al., 1998)
- pNQflaC4-gfp27 - gfp labelled plasmid; lac::gfp mut-s (CmR) from pJBA88 (pUC18-based plasmid)
- pDM42 - AHL reporter plasmid; pNQflaC4 derivative carrying luxR-Plux+RBSII::gfp+mut3*-TO; CmR- from pJBA88
- pDM44 - AHL degrading plasmid; pNQflaC4 derivative carrying a PA1/04/03::aaiA gene fusion; CmR - from pJBA27

**Origin & function**

- C. violaceum CV026 is defective in AHL production due to a mini-T5 insertion in the AHL synthase gene, creating an AHL reporting strain (McClean et al., 1997).
- Mutations to V. anguillarum are inframe deletions of quorum sensing genes. (DM21 (vanl); DM35 (vanR) CmR; DM27 (vanM); DM28 (VanlM); DM34 (vanN) CmR; DM59 (vanU); AC11 (vanO) CmR; AC10(vanT)

**Plasmid carrying strains are:**

- JM109/pSB401; JM109/pSB536; JM109/pSB1075 - AHL reporter strains
- NB10/pNQflaC4-gfp27; DM21/pNQflaC4-gfp; DM27/pNQflaC4-gfp - wild type and mutant gfp-labelled strains
- NB10/pDM442; DM21/pDM42; DM27/pDM42; DM28/pDM42 - wild type and mutant AHL reporter strains
- NB10/pDM44 - wild type strain with AHL degrading enzyme

**Evaluation of foreseeable effects**

The E. coli strain used in classified as a ‘disabled or non-colonising host’. Work to date with these GMMs has suggested that the insertion of AHL reporter plasmids into this strain has not affected the pathogenicity of this organism.

Chromobacterium are ACDP hazard group 1, rarely causing disease in humans. Again, work to date with CV026 has suggested that the mutation to the AHL synthase gene has not affected the pathogenicity of this organism.

Vibrio sp. (except V. cholerae) only rarely causes disease in humans.
Deletion of genes vanI, vanM, vanN, vanR and vanIR were found to have no effect on the virulence of the bacteria in a fish infection model; there was no increased nor decreased virulence (Milton et al., 1997; 2001).

VanT is not essential for virulence (Croxatto et al. 2002).

In a separate study of brine shrimp, there were no significant differences between the virulence of the wildtype NB10 and GMMOs containing mutations in vanl, vanR, vanM, vanN and vanO (Defoirdt et al., 2005).

From these studies, it seems that the alterations made to V. Anguillarum are unlikely to increase the level of risk over an equivalent to hazard group 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

After growth, cultures (liquid, agar, biofilm material) will be killed by autoclaving prior to disposal. Autoclaves are situated within the GM laboratory, and are tested on a yearly basis.

Any spillages will be contained within the laboratory and dealt with using an appropriate disinfection procedure.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee agree with the applicant that the risk assessment should stand as class II, for the benefit of the individuals involved with the project, and to the institution.

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Name**

UNIVERSITY OF WOLVERHAMPTON

**Name 2**

**Department**

SCHOOL OF BIOMEDICAL SCIENCE & PHYSIOLOGY

**Campus Estate or Research Centre**

CITY CAMPUS SOUTH

**Building**

MA BUILDING

**Road Name**

WULFRUNA STREET

**District**

**Town**

WOLVERHAMPTON

**County**

MIDLANDS

**Postcode**

WV1 1SB

**Country**

ENGLAND

**Tel Number**

01902 321000

**Fax Number**

01902 322680

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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Tick if confidential

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 515/00.1

Date Ackn’d 20/12/2000

CU2 Project Title

ANALYSIS OF BIocide SUSCEPTABILITY OF ESCHERICHIA COLI 0157:H7 - DETERMINATION OF ENVIRONMENTAL STRESSES ON THE BIocide

SUSCEPTABILITY OF THIS STRAIN - USING BIOLUMINESCENCE MONITORING OF HIGHLY

Class 2

Culture Vol

< 1 litre

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Historical Significant Changes

Withdrawn

 Tick if notifying a connected programme of work

N

Historical Date of Additional Info

Significant Change ID
<table>
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## Project Additional Information

### Purposes of the contained use

Organism used is E.coli serotype 0157:H7 atcl 4388 does not possess shiga-like toxin I or II, does not possess the genes for these toxins - therefore is not pathogenic - considered equivalent to ACDP Hazard Group 2

### Recipient or parental organism

E.coli serotype 0157:H7 ATCC Thy - auxotroph, non-reverting. (See sheet attached to notification)

### Host/vector system

Non-mobilicable PUC-BASED plasmids containing luciferase - encoding genes
- PSB311
- PSB100
  (See sheet attached to notification)

### Origin & function

To allow bioluminescent monitoring of the organism by virtue of the luciferase genes borne by the plasmid

### Evaluation of foreseeable effects

The plasmids under use have an extensive history in being used by workers and are not considered to have human health risks associated with them. They are non-mobilisable.

The E.coli strains have been specifically disabled, are toxin-less, are not pathogenic to humans or animals and have limited survival in the environment. The cloned DNA is unlikely to alter the pathogenicity of the host.

Overall in terms of severity or consequence of harmful effect were it to occur: It is considered that any unforeseen events will have no environmental impact.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Materials are bagged and autoclaved. Autoclave's activities are charted autoclave is regularly checked for efficiency using bacillus spore check. Waste is removed as clinical waste for subsequent incineration.

### Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y
## Project Containment

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### Project Ref 515/17.1

**Date Ackn'd**: 14/06/2017

**CU2 Project Title**: High throughput drug screening assay using Green Flourescent Protein (GFP) expressing Leishmania spp.

**Class**: Class 2

**CultureVolClass2**: < 1 Litre

**Non-GMM Consent Granted**: Consent Granted

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

### Project Additional Information

**Purposes of the contained use**

The project aim is to utilise the GFP expressing Leishmania spp to screen novel drug treatments for this parasite. Drug assays will be performed against the extracellular promastigote stage of the parasite and the intracellular amastigote stage that have been allowed to infect a human THP-1 cell line in in vitro culture.
Recipient or parental organism

Leishmania mexicana (MNYC/BZ/62/M379) – ATCC PRA-416
Leishmania aethiopica (MHOM/ET/72/L100) – ATCC PRA-417
Leishmania tropica (MHOM/SU/58/OD) – ATCC PRA-418
Leishmania major (MHOM/SU/73/5ASKH) – ATCC PRA-419

Host/vector system

Host cells: Human acute monocytic leukaemia cells (THP-1)

Cell lines are to be used in tissue culture and as such are only capable of replicating in culture. If accidentally injected into the operator they would be antigenically incompatible and rapidly destroyed.

Origin & function

The insert included in pRib1.2αNeoGFP encodes for protein that confers resistance to the aminoglycoside G418 (Geneticin) allowing for the selection of the GFP expressing organisms

pRib1.2αNeoGFP encodes for GFP protein.

The GFP protein fluoresces under blue light.

The proteins expressed may be considered to have little chance of causing harmful reactions in laboratory workers.

The inserted gene product; GFP do not have any oncogenic, cytotoxic or allergenic effects as far as the author is aware. So the risk for human health of transfer to related microorganisms is not significant.

Evaluation of foreseeable effects

The hosts; THP-1 monoytic leukaemia cell lines are to be used in tissue culture and as such are only capable of replicating in culture. If accidentally injected into the operator they would be antigenically incompatible and rapidly destroyed.

The cell lines have been provided by ATCC and are well characterised.

The cloned DNA inserts are unlikely to alter the pathogenicity of the cloning host and therefore containment level 2 is appropriate throughout the procedures.

Leishmania is a BSL-2 organism and care must be taken when handling this parasite. Infection is usually transmitted by the bite of an infected sandfly although laboratory transmission is possible through needle stick injury. The work has been risk assessed and no sharps will be used during the culture of the organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste will be disposed of in an appropriate container in the tissue culture lab and destroy by autoclaving. A complete degree of kill is expected when carrying out these procedures. This will be validated by re-culturing the autoclaved organisms to confirm complete kill. Waste will then be disposed of via special clinical waste collection.

Liquid waste will be disinfected with Trigene disinfection solution. Disposed disinfected liquid waste in container located in MB418 for disposal by technical staff. A complete degree of kill is expected when carrying out these procedures. This will be validated by re-culturing Trigene treated organisms to confirm complete kill. Waste will then be disposed of via special clinical waste collection.

The Genetically Modified Organisms Safety Committee has approved your risk assessment containment level 2 for the project "High throughput drug screening assay using GFP expressing Leishmania spp." subject to the following conditions:

- This project requires containment level 2 and therefore, it requires notification to the HSE. Before starting the project, the Principal Investigator, Dr Wayne Heaselgrave, must notify to the HSE the intention to carry out this project in the University of Wolverhampton. Dr Heaselgrave must present to the GMO safety committee (by e-mail to the secretary Dr Armesilla) evidences of the notification and the acknowledge of notification from the HSE before starting any work.
- Work can begin 45 days after acknowledgment from the HSE of receiving the notification, unless HSE require that work does not begin.
- The Principal Investigator (Dr Wayne Heaselgrave) must report to the GMO safety committee (by e-mail to secretary Dr Armesilla) any correspondence with the HSE.
- No work on this project will be carried out by undergraduate or master students.
- PhD students or other members of staff should not undertake any work on this project until receiving appropriate training from the PI. The PI should get approval by the GMO safety committee for PhD students or other members of staff to work in this project by providing to the committee in writing the names of new researchers required to work in the project and the training provided. Moreover, Dr Wayne Heaselgrave should submit to the committee (by e-mail to secretary Dr Armesilla) a risk assessment including the additional worker/s, indicating the nature of the training. This risk assessment must be approved by the G MOSC before any new staff or PhD students start working on the project.
- The project can only be carried out in the containment level 2 laboratory MB418.

Project Containment

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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
### GM Centre Number: 516

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#### Name

CABI BIOSCIENCE UK CENTRE

#### Department

BIODIVERSITY AND MOLECULAR BIOLOGY

#### Road Name

BAKEHAM LANE

#### Town

EGHAM

#### District

SURREY

#### County

TW20 9TY

#### Postcode

ENGLAND

#### Tel Number

01784 470111

#### Fax Number

01491 829100

#### E-mail

bioscience@cabi.org

#### HSE Division

EAST AND SOUTH EAST

#### Comments

Date at Which Additional Info Submitted

16/10/2003

Page 7976 of 15326
**Premises Addresses**

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**Premises Conditions**

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities
  
  Give brief details of the genetic modification safety committee

  - Level 1 (GMMs)
  - Level 2 (GMMs)
  - Level 3 (GMMs)
  - Level 4 (GMMs)
  - Non-microbial
  - Other (please specify)

Tick if confidential
Maximum culture volume 100 L of soil and 1 L of liquid culture.

The waste will be deactivated by autoclaving, which will be monitored by the placing of thermologs in the centre to ensure the required temperature has been reached. The deactivation method will be monitored by taking samples from autoclaved material and attempting to culture from these samples.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 516/11.1**

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Tick if notifying a connected programme of work

Withdrawn

Date Project Ceased

02/03/2022
Historical Significant Changes

**Historical Date of Additional Info**

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Project Additional Information

**Purposes of the contained use**

CABI's responsibilities as an international Depository Authority (IDA) within the Budapest Treaty (1977) require the acceptance of organisms, which fall within the remit of the GRC, for safe deposit and/or patent storage. This means that CABI may accept Hazard Group 1 or 2 organisms (provided that they are considered no more hazardous than the wild type) from third parties for long-term secure storage. Microbes will be handled according to relevant Standard Operating Procedures (SOPs) and Technical Operational Procedures (TOPs) and the culture plates/vials of the relevant organisms only opened within a suitable Class 2 biological safety cabinet. We will not be undertaking genetic manipulation, per se, we are only going to be "handling" organisms that have been genetically manipulated by third parties who wish CABI to process the samples for optimal long-term storage.

**Recipient or parental organism**

All GMMs to be accepted will be limited to ACDP (UK) Hazard Group 1 & Group 2 organisms. Appropriate GMM risk assessments will be required from the depositor to demonstrate that the organism belongs to ACDP (UK) Hazard Group 1 or group 2 and that the GM is no more hazardous than the wild type of that organism.

**Host/vector system**

This will be dependent on the specific case.

**Origin & function**

Not known currently: i.e. the nature of CABI's position as a signatory of the Budapest Treaty means that anyone can request to send a strain for safe deposit and/or patient storage. At the point of receiving the request, CABI will require the submission of an appropriate GMO RA in order to determine whether, or not, the strain will be accepted for preservation. Clear records will be maintained and the Biological Safety Officer will be informed (where appropriate the BSO will convene a meeting of the Biological Safety Committee in order to assess the request). CABI's current class 1 licence is for a GMM that was created through a specific project. This upgrading of the licence is not with an aim to undertake actual GMM work. As described above, it is purely in recognition that advances in biotechnology mean that we are increasingly likely to be asked, by third parties, to accept (and process) their GMMs for optimal long-term storage.

**Evaluation of foreseeable effects**

As we only accept GMMs of hazard group 1 & 2 carrying supporting documentation that specifically states that they are no more hazardous than the relevant wild type, there should be no adverse effects. Further, the plates/vials of the isolates will only be opened within an appropriate Class II biological safety cabinet - and by trained staff (and in accordance with relevant SOPs and TOPs) wearing appropriate PPE - so those undertaking the work (and anyone else should not be exposed to the organisms).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material to be autoclaved after use and clearly marked for disposal by incineration, where appropriate, and in accordance with existing TOPs and SOPs

Is an emergency plan required according to regulation 20? 

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The risk assessment review noted the potential handling of hazard category 2 GMM and although agreeing they shall not be manipulated per se, concurred an application for hazard class 2 activities is to be made. TO's and SOP's to be updated to reflect additional training and procedures required where appropriate. Staff nominated for class 2 works shall be trained and their records updated.

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02/03/2022
### GM Centre Number: 517

| Data Premises Notified (Originally) | 22/01/1993 | Transferred from 1992 Regs? | Y | Transitional Premises Class | 1 |
| Data Premises Closed               | 21/06/2004 | Transitional Premises       | N | Non-GMMs                  | N |
|                                     |           | Emergency Plan Required?   |   | Withdrawn                 | N |

#### Name

ST BARTHOLOMEWS AND THE ROYAL LONDON SCHOOL OF MEDICINE AND DENTISTRY

#### Campus Estate or Research Centre

#### Road Name

51-53 BARTHOLOMEWS CLOSE

#### Town

LONDON

#### District

WEST SMITHFIELD

#### County

GREATER LONDON

#### Postcode

EC1A 7BE

#### Country

ENGLAND

#### Tel Number

0207 601 7352

#### Fax Number

0207 726 4248

#### HSE Division

LONDON

#### Comments

Date at Which Additional Info Submitted

02/03/2022
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Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Laboratory</th>
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<th>Large Scale</th>
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Other (please specify)  
Tick if confidential

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<th>Microbiology</th>
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<tr>
<td>Mycology</td>
<td>Transgenic</td>
<td>Fish</td>
<td>Other (please specify below)</td>
</tr>
</tbody>
</table>

| Other(s) |

For activities involving GMMs, describe the waste management measures which will apply to the activity

Details of waste management are as follows:
All bacterial cultures and tissue culture flasks are autoclaved at 121°C with a 20 minute holding time before disposal by incineration. The autoclaving process is monitored by Browne's tubes or autoclave tape. Autoclaves are serviced annually. Some tissue culture fluid is inactivated by Chloros (10000 ppm free chlorine) overnight, monitored by colour change of the medium, before disposal via the sink. All animal waste is incinerated at source.

Tick to confirm that you are attaching a summary of the risk assessment
Please enter comments of the GM safety committee on the risk assessment
| Data Premises Notified (Originally) | 22/01/1993 | Transferred from 1992 Regs? | Y |
| Data Premises Closed | N |
| Transitional Premises Class | 1 |
| Emergency Plan Required? | N |
| Non-GMMs | N |
| Withdrawn | N |

**Name**

SALISBURY DISTRICT HOSPITAL

**Department**

WESSEX REGIONAL GENETICS LABORATORY

**Building**

**District**

OLDSTOCK

**Town**

SALISBURY

**County**

WILTSHIRE

**Postcode**

SP2 8BJ

**Country**

ENGLAND

**Tel Number**

01722 336262

**Fax Number**

01722 338095

**E-mail**

**HSE Division**

WALES AND SOUTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

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For activities involving GMMs, describe the waste management measures which will apply to the activity

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The maximum culture volume released at any one time is 1 litre. The supernate is poured into 2% Virkon and stored 2 hours - o/n before disposal down the sink with copious water. All solids and contaminated disposables are collected in designated 'yellow' bags and incinerated.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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<td>0207 584 9467</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Level 4 (GMMs)</th>
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<th>Transgenic Birds</th>
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<tbody>
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 521

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#### Name

| INSTITUTE OF OPHTHALMOLOGY |

#### Name 2

| UNIVERSITY COLLEGE LONDON |

#### Department

| MOLECULAR GENETICS |

#### Campus Estate or Research Centre

| Building |

#### Road Name

| 11-43 BATH STREET |

#### Town

| LONDON |

#### County

| GREATER LONDON |

#### Postcode

| EC1V 9EL |

#### Country

| ENGLAND |

#### Tel Number

| 0207 608 6820 |

#### Fax Number

| 0207 608 6863 |

#### E-mail

| LONDON |

#### Comments

| GM CENTRE CLOSED & ALL WORK TRANSFERRED TO GM14 |

#### Date at Which Additional Info Submitted

| 02/03/2022 |
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Premises Conditions

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Give brief details of the genetic modification safety committee

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 521/00.2

Date Ackn’d 19/02/2001

CU2 Project Title PRODUCTION AND USE OF RECOMBINANT ADENO-ASSOCIATED VIRAL VECTORS

Date Project Ceased 24/04/2012

Class CultureVolClass2 CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes PROJECT TRANSFERRED TO GM 14 & GM CENTRE CLOSED

Historical Date of Additional Info

Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment
L2 L3 L4 L2 L4 L2 L3 L4
Animal Units
L2 L3 L4 L2 L3 L4 L2 L3 L4
Large Scale Activities
Laboratory Activities Glass Houses Growth Rooms
Human Clinical Applications

Project Ref 521/01.1

Date Ackn'd 19/02/2001

Date Project Ceased 24/04/2012

CU2 Project Title TRANSFECTION AND EXPRESSION OF HUMAN ONCOCGENES IN MAMMALIAN CELLS

Class 2

Consent Granted not applicable

Non-GMM

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

PROJECT TRANSFERRED TO GM 14 & GM CENTRE CLOSED

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 521/01.2

Date Ackn'd: 20/02/2001
CU2 Project Title: PRODUCTION AND USE OF RECOMBINANT HUMAN IMMUNO - DEFICIENCY
Class: Class 2
CultureVolClass2: 
CultureVolumeClass3-4: 
VIRUS (RHIV) VECTORS

Date Project Ceased
24/04/2012

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements Y

Withdrew N

Tick if notifying a connected programme of work N

Historical Significant Changes
PROJECT TRANSFERRED TO GM 14 & GM CENTRE CLOSED

Historical Date of Additional Info

 Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref 521/01.3**

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<td>28/09/2001</td>
<td>TRANSFECTION AND EXPRESSION OF POTENTIAL HUMAN ONCOGENES IN EUKARYOTIC AND PROKARYOTIC CELLS</td>
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**Historical Significant Changes**

PROJECT TRANSFERRED TO GM 14 & GM CENTRE CLOSED
Project Additional Information

Purposes of the contained use
To prevent the exposure of humans to risks associated with the Eukaryotic expression of human oncogenes. It is not likely that DNA or cells will infect humans and therefore there is no foreseeable effect. All activity will be Class 2 with special provisions of "no shards".

Recipient or parental organism
Disabled strain of E.Coli and tissue culture cell lines as detailed below.

Host/vector system
pBI-EEFP, pIIRES type bicostronic vectors, pTREX toruse in Eukaryotic cells.
In cell lines, Hela, MCF7, HRIE7, ARPE19, CAL02, A431, MRC5, HOP2.cos-1, cos 7. MDCK, CHU, CHUKI, NIH3T3, SWISS 3T3, EPNT.
For prokaryotes pBluescript, pCRII, pBLUNJ, pRSET, TrCHISA, pGEX, pUBEX in TOP10F, E.Coli XL-Blue, E.Coli JM83, BL21, DHSx

Origin & function
Human oncogenes have originally been cloned from human tissues, the precise function of which are largely unknown. They will be studied during this project.

Evaluation of foreseeable effects
So long as DNA or transfected cells are not introduced directly to humans there are no foreseeable effects. Class 2 activity is determined due to the expression of human (potential) oncogenes. We will not use any vectors which will directly infect human or animals such as viruses. We will in all instances adhere to precautions and procedures described in 'Guidance and Construction of Recombinants containing potentially oncogenic nucleic acid services.' Para 13-15 GMAC Category 2 containment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All end waste from this project will be autoclaved. Autoclave indicator tape will be used to determine that effective autoclaving has taken place. We have previously determined that autoclave is 100% effective in killing all tissue culture cell lines and all strains of E.Coli used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Similar work to that described in this notification and in the accompanying risk assessment is currently being undertaken by the Institute. See GM 521/01.1 and is happy that the work can be carried out safely.

**Project Containment**

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 521/02.1

- **CU2 Project Title**: TRANSFECTION AND EXPRESSION OF POTENTIAL HUMAN ONCOGENES IN EUKARYOTIC AND PROKARYOTIC CELLS
- **Class**: Class 2
- **CultureVol**
  - Class 2: 1-50 litres

- **Non-GMM Consent Granted**: not applicable

- **Date Ackn'd**: 28/01/2002
- **Date Project Ceased**: 24/04/2012
- **Withdrawn**: N
- **Historical Significant Changes**
  - PROJECT TRANSFERRED TO GM 14 & GM CENTRE CLOSED

**Project Additional Information**

**Purposes of the contained use**: To prevent the exposure of humans to risks associated with the eukaryotic expression of human oncogenes. It is not likely that DNA or Cells will infect humans and therefore there is no foreseeable effect. All activity will be Class II with specific provisions of "no sharps".

**Recipient or parental organism**
Disabled strains of E.coli and tissue culture cell lines as detailed below

### Host/vector system

**HOST:**  pCDNA3  Tet on/off E1A-deleted (replication deficient) adenovirus expressing GFP pGST, pMBP, pBS, pUC, pSP, pGL2, pCAT, pCH110  
**VECTORS:**  E.coli DH5alpha, BL21.  Drosophila SL2 cells HeLa, OSH50T, jurkat, A20, WEHI, raji, RJ225, jijoye, C113, 293, HEK, Y79, WERI, mel202, 270, 285, 290, OCM-1, OCM-3, OCM-8, OMM-1-3, 92.1SUM520E, SUM 159, MCF - 7T47DD, MDA231, 435, 468, HBL, FRTL-5, PRL

### Origin & function

Human oncogenes have originally been cloned from human tissues. The precise function of which are largely unknown

### Evaluation of foreseeable effects

So long as DNA or transfected cells are not introduced directly to humans there is no foreseeable effects. Class II activity is determined due to the expression of human (Potential) oncogenes. We will not use any vectors which will directly affect human or animals such as viruses. We will in all instances adhere to precautions and procedures. Described in "Guidance and construction of recombinants contains potentially oncogenic nucleic acid sequences“ Para 13 - 15 GMAC Category II Containment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste from this project will be autoclaved. Autoclave indicator tape will be used to determine that effective autoclaving has taken place. We have previously determined that autoclaving is 100% effective in killing all tissue culture cell lines and all strains of E.coli used.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

### Project Containment

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**Laboratory Activities**

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**Glass Houses**

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**Growth Rooms**

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02/03/2022
**Project Ref** 521/02.2

**Date Ackn'd** 19/11/2002

**Date Project Ceased** 24/04/2012

**Project Title** USE OF RECOMBINANT LENTIVIRAL VECTORS TO EXPRESS A RANGE OF NON-ONCOGENIC SIGNALLING PROTEINS AND ADHESION MOLECULES IN EUKARYOTIC CELLS

**Class** Class 2

**Consent Granted** not applicable

**Project notified under transitional arrangements** N

**Recipient or parental organism**

Strains of E. coli bacteria and a range of human and tissue culture cell lines as detailed below.

**Host/vector system**

Hosts.
- E. coli DH5 alpha
- E.coli DB3.1

**Purposes of the contained use**

Purpose of contained use is to minimise/prevent the exposure of humans to risks associated with the eukaryotic expression of a variety of signalling proteins expressed through a human lentiviral gene transfer system. It is not likely that transduced cell lines will produce viable progeny and therefore only handling of the producer cell line with expression vectors and isolated viral particles can be considered a risk. All this activity will be carried out in class II biological safety cabinets and in category containment laboratory facilities.
293FT kidney epithelial cells (producer cell line) supplied from invitrogen
Target cell lines
Primary cultures of rat brain and retinal endothelial. Primary aortic and lung rat endothelial cells. Cells. GP8/3.9, RBE4 and GPNT cells (rat endothelial cell lines). LD7.4 rat RPE cell lines. ARPE19, hRPE7 human RPE cell lines.
CHO cells. COS cells.

Vectors
pENTR4 (invitrogen)
pLENT16/V5 (invitrogen)

Origin & function
A range of cell adhesion molecules and signalling proteins have originally been cloned from human/rat tissues. The functions of all the proteins are generally known. No human oncogenes will be used.

Evaluation of foreseeable effects
As long as the DNA or transfected cell are not directly introduced to humans there is no foreseeable effects. Class II activity is determined to minimise exposure to virus-producing cell lines.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All GMO waste from this project will be autoclaved immediately. Autoclave indicator tape will be used to determine that autoclaving has reached correct temperature and pressure. We have previously determined that autoclaving results in 100% kill of the GMO used in this project.

Project Containment

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02/03/2022
**Project Ref**: 521/03.1

**Date Ackn’d**: 14/11/2003

**CU2 Project Title**: TRANSFECTION AND EXPRESSION OF POTENTIAL HUMAN ONCOGENES IN EUKARYOTIC CELLS

**Class**: Class 2

**CultureVol**: 1-50 litres

**Non-GMM**: not applicable

**Consent Granted**: Project notified under transitional arrangements

**Withdrawn**: N

**Historical Significant Changes**: PROJECT TRANSFERRED TO GM 14 & GM CENTRE CLOSED

**Recipient or parental organism**: Tissue culture cell lines as detailed below.

**Host/vector system**: The vector is the pLNC-cmyc ER virus. The virus will be used to transduce primary epithelial cells.

**Origin & function**: Human c-myc was originally cloned from human cells, but its precise functions are largely unknown. The virus will be destroyed during the project.

**Purposes of the contained use**: The purpose of the contained use is to prevent the exposure of humans to risk associated with the eukaryotic expression of c-myc. It is not likely the DNA or cells will infect humans and therefore there is no foreseeable effects, all activity will be class II with specific provisions of "no sharps".
Evaluation of foreseeable effects

So long as the DNA or transfected cells are not introduced directly to humans there are no foreseeable effects. Class II activity is determined due to the expression of human (potential) oncogenes. We will not use any vectors which will directly infect humans or animals. We will in all instances adhere to precautions and procedures described in "evidence and construction of recombinants containing potentially oncogenic nucleic acid sequences" PARA 13-15 GMAC category II containment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid cultures of viruses, or cells infected with viruses including all tissue culture plasticware will be autoclaved for 30 mins at 121 degrees C under 115lb/sq in pressure. This is effective in producing 100% kill of viruses as assessed by replating 10(6) CFU of autoclaved viruses on indicator cell lines and observing the appearance of no transduced cells. Each autoclaved sample will be marked with autoclave indicator tape. Each load will not be regarded as safe unless indicator tape shows that correct autoclaved parameters have been achieved. In addition we will also include thermosticks with each load to ensure the centre of the load has reached the correct working temperature and pressure. Autoclaves are routinely serviced to ensure correct working temperatures and pressures. Autoclaved material will be marked "inactivated GM waste" discarded to clinical waste and removed from the site by an authorised contractor.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

None

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<th>Animal Units</th>
<th>Large Scale Activities</th>
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The purpose of the contained use is to prevent the exposure of humans to risks associated with the expression of human signalling molecules - some with a potential transforming activity. It is not likely that DNA or cells will infect humans and therefore there are no foreseeable effects. All work will be carried out according to class II rules as specified further in the accompanying risk assessment.

Recipient or parental organism
Replicon-deficient Ad5 vectors and tissue culture cell lines as detailed below.

Host/vector system
Vectors code for Adenovirus 5 which are replication deficient since they lack the E1 gene. Target gene sequences will be inserted at its place. Production of recombinant virions will be done in HEK293 cells which complement the genes required for replication. Final experiments will be undertaken in endothelial, epithelial and fibroblast cell lines of human or rodent origin, and primary rodent endothelial cells.

Origin & function
All target sequences to be used encode signalling molecules involved in the transmission of extracellular signals to the cytoskeleton, nucleus or the junctions of the cell. The molecules to be used are dominant-negative mutants of the small GTPases. Furthermore, we are going to use dominant-negative versions of MAP kinase pathway components which again are generally characterised by a growth-attenuating effect. We also envisage the occasional use of activated versions of these molecules, some of which have been shown to exhibit mid transforming activity in a 3T3 foci assay.

The precise function in the target cell lines is unknown and should be determined during the course of this project.
Evaluation of foreseeable effects
As long as target DNA is not stably introduced into the human genome there are no foreseeable effects. Class II has been applied due to the expression of a potential human oncogene (e.g. activated rac1).
The Ad5 vectors to be used stay episomal in host cells; therefore - even in the unlikely event of infection - target sequences cannot be integrated into the human genome. The Ad5 vectors to be used are also replication deficient; therefore no amplification of virions can take place in the unlikely event of the recombinant virus being taken up by humans.
We will in all instances adhere to precautions and procedures described in 'Guidance and Construction of Recombinants Containing Potential Oncogenic Nucleic Acid Sequences' Para 13-15 GMAC category II containment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste will be collected separately in bins and then autoclaved. Autoclave indicator tape will be used to determine that effective autoclaving has taken place. Liquid waste will be kept to a minimum, treated with 1% Virkon and hypochloride for a 12h period before being discarded. This mixture is highly effective against all 18 virus families affecting man and animals. Phenol red in the media will change to colourless and indicate that the inactivation procedure is effective.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The local GMSC met and discussed this proposal, and is satisfied as to the proposed level of containment and competence of the investigators.

Project Containment

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Animal Units
L2 L3 L4 L2 L3 L4 L2 L3 L4

Large Scale Activities
L3 L4 L3 L4 L2 L3 L4

Human Clinical Applications
L3 L4 L3 L4
The identification of novel targets for treating neovascular eye disorders

In most cases, mammalian gene sequences will be used. Polyomavirus represents the only pathogenic donor organism, the middle T antigen being its transforming oncogene.

Cultured mammalian cells will be transfected with bacterial plasmids containing mammalian gene sequences or transduced with viral vectors containing mammalian gene sequences.

ViraPower Lentiviral expression systems (Invitrogen) will be used for cloning/expression of VEGF120/121, VEGF164/165 and mutant variants, VEGF188/189, PIGF and mutant variants, VEGFR-1/2, neuropilin-1/2, notch ligands and notch receptors and their mutant variants, PV-1 and mutant variants, moesin and mutant variants, polyoma middle T antigen and mutant variants.
Vectors will be introduced into bacteria by heat transformation or by electroporation using a BioRad GenePulser Xcell electroporator. Vectors will be introduced into yeast cells by electroporation. Vectors will be introduced into mammalian cells in a class 2 safety cabinet either by electroporation using an Amaxa Nucleofector II, or by chemical transfection using lipid based transfection reagents.

Origin & function

Of the inserts to be used, VEGF, PIGF and Notch ligands are growth factors, and VEGFR1/2, neuropilin-1/2 and notch are growth factor receptors; Hey-1 and Hey-2 are nuclear proteins and downstream genes of the notch pathway; moesin, PV-1, radixin and caveolin are structural proteins, linked to the actin cytoskeleton, and it is thought that certain mutant variants of moesin and PV-1 may disrupt cytoskeletal arrangements and affect cell structure; integrins are involved in cell-cell and cell-matrix interactions; C5α, LFA1, CD46, CD55 and Mac-1 are involved in the immune system, C5α Mac-1, CD46 and CD55 in the complement cascade, LFA1 in immune cell recruitment RBP4 is a specific retinol-binding protein; polyoma middle T antigen is a transforming oncogene, and Src, PI3K and Ras are components of the polyoma middle T signalling pathway, and it is thought that mutant variants of polyoma middle T, Src, PI3K and Ras may disable aspects of their activity and therefore affect cell transformation. MicroRNA sequences are designed with the aim of causing reduced expression of a target gene.

Evaluation of foreseeable effects

VEGF is a pro-angiogenic and pro-inflammatory growth factor which may exert harmful effects if over-expressed at high levels in the tissue, but with low severity because of the short half-life of the protein. VEGFR1/2 and neuropilin-1/2 are receptors and co-receptors, respectively, which mediate VEGF signalling. Notch ligands and Notch receptors are growth factors and receptors, respectively, and unregulated expression of their mutants variants has been linked to tumorigenesis, but with low severity and a self-limited localisation. C5α, LFA1, CD46, CD55 and Mac-1 may cause transient local inflammation if expressed at high levels locally, but with low severity. Polyoma middle T-antigen is a transforming oncogene with the potential to induce tumour growth, although the severity would be likely to be low and limited to immune-deficient individuals. Src, PI3K and Ras are involved in the Polyoma middle T-antigen signal transduction pathway and may be involved in its transforming ability, indicating a potential for harm, but effects would be limited, localised and transient with low severity. The use of low volumes of culture medium and replication-deficient lentivirus reduce the risk to humans. Sharps will not be used when handling potentially harmful sequences; this will significantly reduce the risk of DNA transfer through needlestick or other puncture injury. Other proteins including the structural proteins such as PV-1 and nuclear proteins such as Hey-2 have no documented risk of causing diseases in humans.

pPICZ yeast expression vectors contain the TEF1 and EM7 promoters, which drive the expression of the gene coding for resistance to the antibiotic Zeocin in yeast and prokaryotes, respectively. Insert expression in pPICZ vectors is controlled by the AOX1 promoter, which is induced by methanol. Gene expression is therefore suppressed in the absence of methanol. Bacterial plasmid vectors contain the prokaryotic lac, EM7, T7 and SP6 promoters, and the cytomegalovirus (CMV) immediate-early promoter and the simian virus (SV) 40 promoter for expression in mammalian cells. All bacterial plasmid vectors used are non-mobile, and are therefore unable to transfer into wild-type bacteria in the environment.

The ViraPower Lentiviral expression systems which will be used are derived from HIV-1 but have a number of inbuilt safety features. There are no longer terminal repeats (LTR) in the packaging plasmids, so that HIV-1 structural genes are only expressed in the producer cell and never packaged into virions. Secondly, viral particles are replication incompetent, and they only carry the gene of interest, so that no other viral proteins are produced. Additionally, these viruses have a deletion in the 3' LTR which makes the virus “self-inactivating”; transduced and integrated lentiviral vectors are no longer capable of producing a packagable viral genome. The insertion of genes would be unlikely to affect the behaviour of the viral vectors.

Cultured mammalian cells transfected with bacterial plasmids containing mammalian gene sequences or transduced with viral vectors containing mammalian gene sequences may display altered signalling, growth or viability but due to the fact that these cell lines rely on specific culture conditions and would be unlikely to survive outside the laboratory culture conditions, this would not pose a risk to humans. The insertion of genes would be unlikely to alter the recipient organisms' ability to survive in the environment, compete with other organisms or transfer to them the inserted sequences.

The majority of materials used in this project are not considered hazardous to handlers. The transforming oncogene polyoma middle T antigen and the VEGF isoforms and receptors have a potential to cause harmful effects. In the case of the vectors used for VEGF, the risk of natural gene transfer is very low. The most hazardous GMM is disabled lentivirus containing the polyoma middle T sequence, and any procedure in which this construct is handled, particularly with a view to transducing cells, is likely to
be the most hazardous

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The inactivation measures which will be used, as appropriate, are chemical disinfection with a 1% solution of Virkon disinfectant for 10 minutes or a 105 bleach (sodium hypochlorite) solution, treatment with UV light, and autoclaving. Virkon disinfectant has been proven to kill 99.999% of microorganisms in less than 10 minutes, and is the disinfectant of choice for inactivating viruses. Sodium hypochlorite is a commonly-used wide-spectrum disinfectant. Autoclaving involves treatment with high pressure steam at 121°C for 15-20 minutes, which kills all bacteria and viruses. The inactivation of bacteria and yeast should be confirmed by spreading treated liquid waste onto the appropriate solid growth medium, incubating overnight at the optimum temperature and checking for micro-organism growth. The inactivation of virus should be confirmed by attempting to infect host cells and testing for infectivity or expression of proteins coded by the viral vector. Inactivated contaminated solid waste should be disposed of in Biohazard bags, and inactivated contaminated sharps in Biohazard sharps bins. Inactivated contaminated liquid waste may be disposed of in normal waste water drains.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All points were addressed before the risk assessment was finalised

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Production and use of recombinant lentiviral (rLV) vectors.

Our group aims to contribute to the development of novel methodologies of human disease treatment through identification of novel targets of diseases and their characterisation using in vitro and in vivo systems. We are particularly interested in cancer and ocular disorders. Recently our studies have identified that the small leucine rich proteoglycan family members may inhibit tumourigenesis. To examine this possibility, it is essential to analyse the effect of their activation in in vitro and in vivo cancer models. To this end, the third generation lentivirus system is ideal because it is very safe and can easily produce stable expression. So far, there is no effective treatment of many types of cancer. If the activation is effective, this may provide a novel strategy of cancer treatment.

Virus:
The third generation lentiviruses such as Lenti-X system (Clontech), SIN third generation (Gentarget, OpenBiosystem),

Cells:
Non-transformed cells 293, NHU-hTERT

ATCC supplied established human cancer cell lines such as Hela, EJ28, J82, RT112, 253 JBV, and MCF7.

Corneal cells: epithelium, endothelium, and fibroblast

Animals: mouse

To construct viral vectors, the following intermediate constructs will be made using general lab strain of E. coli. There is no potential for harm to health and/or harm to the
Commercially available packaging vectors are used such as pMDL, prev, and pVSVG.

Lentiviral transfer vectors containing inserted genes of interest will be constructed using commonly used safe vectors such as pLVX-Tight-Puro (Clontech), LT-Exp1 (Gentarget), plenti vectors (Invitrogen), and Express-In™ (Openbiosystem).

**Origin & function**

Origin: human, mouse, Xenopus (OMD, PRELP), P1 bacteriophage (Cre recombinase), E.coli (Tet-on)

Functions:
OMD and PRELP are secreted proteins in the small elucine rich proteoglycan family. These proteins regulate cellular signalling from extracellular space. We expect that overexpression of these genes show negative effect on tumour cell growth.

Cre recombinase has the activity to induce recombination through the Cre-lox system.

Tet-on (for inducible system) is used for suppression of spontaneous expression of introduced genes.

**Evaluation of foreseeable effects**

The third generation lentivirus system has been recognized as a very safe system. The inserted DNA and virus are unlikely to be pathogenic. However, the most hazardous GMO is recombinant lentivirus. There is the possibility of mutagenesis caused by random insertion of viral sequences, but the risk of foreseeable adverse effects is very low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Infection of mice will be performed in the animal facility. The protocols are set out in the Home Office Animal Project Licence (PPL70/6750). The mice will be always kept in the animal facility.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The viruses, cells and animals cannot survive in natural conditions. They will be killed by standard methods.

E. coli and eukaryotic cells are inactivated by treatment with Virkon. Also, all waste and used glassware are autoclaved. The autoclaved waste will be discarded using plastic bags. We expect to kill 100% of cells and mice. The conditions are approved to kill E. coli, mammalian cell lines, and mice. Mammalian cells are confirmed by lysis of structure. Death of mice is confirmed by eye. The dead mice will be packed in plastic bags. The bags are temporarily kept in designated -20 freezer in the animal facility. Then, the dead animals are incinerated by the designated special facility.

**Is an emergency plan required according to regulation 20?**

Y

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y
Prof O has submitted a new Class 2 risk assessment using third generation lentiviruses. The risk with this proposal lies in the possibility of random positional integration into the genome and the unpredictable effects this could have. The committee reviewed the risk assessment forms and the following points were raised.

GMM1 & 2
- Section 1.11 mentions xenopus but not section 1.13
- Section 2.06 states there is no risk. Clearly there is a risk but this is controlled.
- Section 2.09 - The risk should be low not zero

HSE Risk assessment
- As the form will be submitted to the HSE it must be accurate.
- Section 2.13 needs to be more specific. It is not acceptable to state "such as Virkon" or "suitable bags".
- Section 2.07 again states there is no risk. This needs to be amended
- We need to make it clear in the risk assessment why we believe the proposal is Class 2, i.e. because of the level of virus titre being used and the injection into animals.

CU2 form
- The sentences relating to the Home Office need to be removed. The HSE are interested in the disposal of the carcasses not how the animals will be killed

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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<td>L3</td>
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Animal Units
- L2 Yes

Large Scale Activities
- L3

Human Clinical Applications
- L3
Data Premises Notified: 02/02/1994 (Originally)  
Transferred from 1992 Regs?: Y  
Transitional Premises Class: 1  
Data Premises Closed: 10/07/2007  
Transitional Premises Emergency Plan Required?: N  
Non-GMMs: N  
Withdrawn: N

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</tr>
<tr>
<td>County</td>
<td>SUFFOLK</td>
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<td>Postcode</td>
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<td>01284 812200</td>
</tr>
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<td>Fax Number</td>
<td>01284 811191</td>
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<td>EAST AND SOUTH EAST</td>
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Comments: CHANGE OF NAME FROM IACR-BROOMS BARN TO THE ABOVE AS OF 1/1/2003

Date at Which Additional Info Submitted: 17/12/2002
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
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Tick if confidential

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<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tbody>
<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>
2L liquid culture.

Deactivation of waste.
Bacterial culture
- Deactivation: autoclave 121 degrees C for a minimum of 25 minutes
- Validation: exceeding standard method for deactivation of bacterial cultures
- Monitoring: 3M autoclave tape. Autoclave serviced every 6 months.

Plant in-vitro culture
- Deactivation: Autoclave 121 degrees C for a minimum of 25 minutes
- Monitoring: 3M autoclave tape. Autoclave serviced every 6 months.

Plant material and soil from glasshouse
- Deactivation: autoclave 121 degrees C for a minimum of 60 minutes
- Validation: deactivated waste buried in registered land-fill site also used for field trial GM waste.
- Monitoring: Browns Tubes. Autoclave serviced every 6 months.

Please enter comments of the GM safety committee on the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

For activities involving GMMs, describe the waste management measures which will apply to the activity
GM Centre Number: 525

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Name

UNIVERSITY OF WALES COLLEGE OF MEDICINE

Name 2

DERMATOLOGY

Campus Estate or Research Centre

Road Name

HEATH PARK

Town

CARDIFF

District

CARDIFF

County

Wales

Postcode

CF14 4X

Country

Wales

Tel Number

029 2074 2883

Fax Number

029 2076 2314

HSE Division

WALES AND SOUTH WEST

Comments

GM525 CLOSED DOWN AND MERGED WITH GM130 ON 26/04/2005

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify)  
Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
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<th>Transgenic Invertebrates</th>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Autoclave: The autoclaves used are in Public Health and are regularly tested and serviced.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
### GM Centre Number: 530

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**Name**

UNIVERSITY OF LONDON

**Name 2**

UNIVERSITY OF LONDON

**Department**

SCHOOL OF PHARMACY

**Campus Estate or Research Centre**

**Road Name**

29/39 BRUNSWICK SQUARE

**District**

GREATER LONDON

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

WC1N 1AX

**Country**

ENGLAND

**Tel Number**

0207 753 5800

**Fax Number**

0207 753 5941

**E-mail**

**HSE Division**

LONDON

**Comments**

GM CENTRE CLOSED & ALL WORK TRANSFERRED TO GM14

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below)
---|---|---|---
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 530/01.1**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
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<td>INDUCTION AND EXPRESSION OF PROTEINS IN CULTURES OF MAMMALIAN CELLS USING SEMLIKI FOREST VIRUS DERIVED DNA AS A VECTOR</td>
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<th>Class</th>
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<td>24/04/2012</td>
<td>Class 2</td>
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Non-GMM | Consent Granted |
---|---|
| | not applicable |

Tick if notifying a connected programme of work

Withdrawn

Historical Significant Changes

PROJECT TRANSFERRED TO GM14 & GM CENTRE CLOSED

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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- **Project notified under transitional arrangements**: Y

#### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
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<td>Animal Units</td>
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Withdrawn  N  Tick if notifying a connected programme of work  N
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Project Containment

Laboratory Activities
- L2
- L3
- L4

Glass Houses
- L2
- L3
- L4

Growth Rooms
- L2
- L3
- L4

Animal Units
- L2
- L3
- L4

Large Scale Activities
- L2
- L3
- L4

Human Clinical Applications
- L2
- L3
- L4

Project Ref 530/08.1

Date Ackn’d 15/04/2008

CU2 Project Title Propagation and use of tumour replicating viral vectors for use in cancer gene therapy.

Date Project Ceased 24/04/2012

Class
- Class 2

CultureVol
- < 1 Litre

Class Culture
- Class 2

Volume
- < 1 Litre

Consent Granted Not Applicable

Non-GMM

Tick if notifying a connected programme of work N

Historical Significant Changes PROJECT TRANSFERRED TO GM14 & GM CENTRE CLOSED

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
### Purposes of the contained use

The purpose of this project is to develop strategies to improve the in vitro and in vivo function of viral vectors for potential use in cancer gene therapy.

### Recipient or parental organism

Well characterised, ACDP Class 1 mammalian cell lines (e.g. A549, CaLu 6, CT26, B16-F10, HeLa) well characterised, ACDP Class 2 mammalian cell lines (e.g. HEK293, COS-7, SVEC 4-10, 2F2B) Murine embryonic stem cell line (El4Tg2a) and murine primary cell C57B16, Oalt/b/c and CD1 immunocompetent mice

Athymic (nude) immunodeficient mice

### Host/vector system

1. Wild-type Reovirus is replication competent. However, its replication is selective to cells that have an activated Ras signalling pathway (Curr Opin Mol Ther. 2006 Jun; 8(3):249-60)

2. Tumour-replicating Adenovirus. ICOVIR-5 (Ad-DM-E2F-K-Delta24RGD) is an optimised oncolytic adenovirus that combines Ela transcriptional control by an insulated form of the E2F promoter with Delta24 mutation of Ela to improve the therapeutic index of AdDelta24RGD (Mol Ther. 2007 Sep; 15(9): 1607-15)

### Origin & function

Reovirus will be provided by Oncolytics Siotech Inc. Calgary, Canada (Reolysin). This product is currently being used in clinical trials and we wish to investigate its oncolytic potential in cancer gene therapy. The virus is not genetically modified.

ICOVIR has an Ela gene deletion in the retinoblastoma (Rb) protein-binding region, substitution of the Ela promoter for E2F-responsive elements and an RGD-4C peptide motif inserted into the adenoviral fibre to enhance adenoviral tropism.

### Evaluation of foreseeable effects

Reovirus will infect human cells however, it is only replication competent in cells with an activated ras signalling pathway therefore, the risk to human cells is minimised. Ras mutations and overexpression of the ras signalling pathway are most frequently associated with a transformed phenotype and the development of malignancy. However, there is a theoretical risk that ras signalling may be activated in growth factor-stimulated cells, cells that proliferate (wound healing) or in people with cellular proliferative disorders. Therefore we cannot exclude the possibility that Reovirus (Reolysin) might infect and replicate in apparently normal cells, causing lysis. Furthermore, the release of tumour associated antigens following lysis may modify the host immune system to infected cells. However, this is unlikely to result in adverse disease. Reovirus will also be readily transmitted via aerosols. Therefore, to protect people from the potential risk of infection all work associated with the virus will be carried out at Class 2.

ICOVIR is replication defective in normal, healthy cells, However, if ICOVIR were to recombine resulting in replication competence it would be comparable to wild type adenovirus since it contains no additional genetic material. All strains of adenovirus 5 are non-oncogenic. Wild-type Ad5 may cause mild respiratory diseases in children. Primary infection of adenovirus is thought to generate life-long immunity and it is thought that the majority of the adult population are likely to have antibodies to the wild-type virus. Although replication has been shown to occur in the lungs of experimentally infected cotton rats administered with high doses of virus, there is no evidence that adenoviruses can naturally colonise non-human hosts and therefore pose no significant harm to animals, plants or ecosystems.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Wild type Reovirus or Adenovirus-infected animals may excrete (shed) adenovirus (especially in the first 72 hours after infection). Precautions must be taken not to create aerosols when emptying animal waste material, washing cages or cleaning the room.

Handling is carried out in Class 2 microbiological cabinet.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No derogation required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated with a 1% Yukon solution for at least 3 hrs. It can then safely be disposed of in the laboratory sink. All surfaces coming in contact with liquid waste will be washed with 1% Virkon and/or 70% isopropanol and/or a mild detergent (e.g. SDS).

Plastic tips, simplettes, syringes, etc or glassware that was used to hold or dispense virus will similarly be soaked in a: 1% Virkon solution for at least 3 hrs, but overnight in most cases. The liquid will be disposed of in the laboratory sink, and the remaining solid waste will be collected in the laboratory solids bins.

1% Virkon has activity equivalent to 1000 p.p.m. chlorine without hazards, corrosion potential and loss of activity due to organic challenge.

(Altvirai Res. Inactivation of adenovirus types 5 and 6 by Virkon S. 2004 Oct;64(1):27-33.)

All other solid waste will be inactivated by autoclaving. The autoclave will be validated by external contractors at least once per year and the day to day operation monitored using the instrument readouts of temperature, pressure and time. The autoclaves used for waste also indicate a failed cycle if the required parameters are not met.

Please enter comments on the GM safety committee on the risk assessment

The committee feel that the proposed research using replication deficient viruses and tumour replicative competent viruses that are dependent upon ras signaling for adenoviral infection are safe. This research is a continuation of work currently being conducted within other UK institutions, using similar protocols. In these proposed experiments reporter genes are used as markers for transfection efficiency and translation. All cell lines used in these experiments are well characterized and if handled correctly, according to well developed protocols, will not pose any difficulties concerns, even for those cell line assigned at class 2 containment. The proposed experiments are very timely and need to be conducted without undue delay. There are no additional unknown risks associated with using nude mice in these proposed studies as similar studies have been carried out using the very similar experimental protocols as outlined in this proposal.

What happens if humans with cancer (in early stages people might not know that they have cancer) would work with tumouronly replicative viruses? The worst case scenario would be that of the wild-type viruses.

Is it really likely that all of these cell lines will be used? Yes, cell lines held by this laboratory have been acquired ‘specifically for their different properties/species specificity.

The School requires double HEPA filters for Class II Microbiological Safety Cabinets used for GM work.

Other points of clarification were dealt with at the draft stage for the Risk Assessment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<td></td>
<td>L4</td>
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</tbody>
</table>
We aim to identify the molecular targets for our small antibacterial molecules using a multicopy suppression strategy. This requires the creation of a shot-gun library of Clostridium difficile DNA in specific vectors. These will then be transferred back into Clostridium difficile with the aim of identifying clones that are resistant to our molecules. Further to this our potential targets will be cloned to confirm their role in resistance. An alternative approach to this will be by creating a transposon library of random mutants and testing these for sensitivity against our compounds.

Clostridium difficile strains are ACDP hazard category 2. Clostridium difficile R20291 is regarded as the PCR ribotype 027 reference strains. Strains produced in this study are not likely to be more virulent than this. Clostridium difficile 630 and 630 delta erm will also be used in this study but are known to be less virulent than strain R20291. Initial cloning will take place in E.coli and plasmids will be screened to ensure those containing toxin genes will not be placed in C.difficile as the additive effect is unknown. C. difficile only presents a hazard when the normal bacterial flora is compromised and is carried by 25% of the population with no effect to health.

Recipient or parental organism

Clostridium difficile strains are ACDP hazard category 2. Clostridium difficile R20291 is regarded as the PCR ribotype 027 reference strains. Strains produced in this study are not likely to be more virulent than this. Clostridium difficile 630 and 630 delta erm will also be used in this study but are known to be less virulent than strain R20291. Initial cloning will take place in E.coli and plasmids will be screened to ensure those containing toxin genes will not be placed in C.difficile as the additive effect is unknown. C. difficile only presents a hazard when the normal bacterial flora is compromised and is carried by 25% of the population with no effect to health.

Host/vector system

The disabled E.coli strains TOP10, JM109 and CA434 will be used as the plasmid host and are not pathogenic. Due to the differences in codon usage between E.coli and C.difficile, it is very unlikely that expression of C.difficile genes will occur in E.coli. Also due to the random fragmentation of the genomic DNA the required elements for
expression may not be cloned with the genomic DNA. The pMTL vectors based on the CoE1 replicon will be used as shuttle vectors in E.coli and C.difficile. These plasmids are nic mob minus and are derived from Gram positive and negative components, many of which (except the Gram positive replicon) do not function in clostridia. These plasmids will also contain antibiotic resistance markers which are regularly used in the laboratory environment and E.coli. The plasmids are not self-transmissable.

**Origin & function**

Genomic DNA will be extracted from the Clostridium difficile sequence strains CD630 and R20291. Fragmented segments of this DNA will then be cloned into pMTL80000 series vectors for propagation in E.coli and conjugation into Clostridium difficile. The approach of creating mutants for testing, either by deletion of a gene or insertion of a transposon will make the GMM less virulent. The majority of the genes to be expressed are innocuous bacterial enzymes used for basic metabolic functions and they are unlikely to function in isolation or in E.coli.

**Evaluation of foreseeable effects**

**C.difficile:**
The duplication of metabolic genes in the multicopy suppression studies are highly unlikely to increase virulence or survival in the environment. The effect of gene dosage on the only known virulence determinants of C. difficile, toxins A and B is unknown. Expression levels of the genes encoding toxin A and B vary widely in clinical strains and the experiments here are unlikely to exceed clinical levels even in the worst case scenario. Random mutagenesis or directed gene deletion may increase or decrease virulence factor expression. None of the antibiotic markers used correspond to antibiotics that are routinely used to treat C. difficile infection.

**E.coli:**
The strains used are disabled, non-pathogenic laboratory strains which are unlikely to cause disease even with the addition of fragmented clostridial DNA or genes. As such infection with recombinant E.coli or survival in the environment is deemed highly unlikely.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste will be autoclaved to achieve 100% kill and will then be sent to landfill after processing by approved waste disposal contractors.

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<th>Is an emergency plan required according to regulation 20?</th>
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<tbody>
<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
<td>N</td>
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<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
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<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>N</td>
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Please enter comments on the GM safety committee on the risk assessment
The GMSC has considered the proposal for work with C. difficile, and after initial discussion to clarify the text of the risk assessment we have no concerns about the genetic modification as planned, and note that it is highly unlikely that a GMO more hazardous than the host will be generated. The main concerns expressed were regarding the safe transport, handling and disposal of the pathogenic host organism. Acceptable safe working practices have been cited in the risk assessment, and reference has been included to indicate the expertise and experience of the key post-doc who has safely and successfully worked with C. difficile, and with other Level 2 and 3 pathogens for 11 years. One committee member has extensive experience of pathogen work including MRSA and has no problem with this straightforward application using category 2 bacteria. The GMSC has approved this proposal.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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- **Animal Units**
  - L2 L3 L4 L2 L3 L4 L2 L3 L4

- **Large Scale Activities**
  - L2 L3 L4 L2 L3 L4 L2 L3 L4

- **Human Clinical Applications**
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### GM Centre Number: 531

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**Name**

ROYAL BOTANIC GARDEN

**Name 2**

**Department**

SCIENCE

**Campus Estate or Research Centre**

**Building**

20A INVERLEITH ROW

**Town**

EDINBURGH

**District**

EAST LOTHIAN

**County**

EH3 5LR

**Country**

SCOTLAND

**Tel Number**

0131 552 7171

**Fax Number**

0131 552 0382

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<tr>
<th>Mycology</th>
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<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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<td>Other(s)</td>
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</table>

| a) Up to 10 litres of media containing GMOs will be handled at any one time; |
| b) all waste will be deactivated by autoclaving as monitored by autoclave indicator tape; |
| c) the autoclave will be maintained and checked at intervals as recommended by the manufacturer. |

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 532

<table>
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#### Name

CHARLES RIVER UK LTD

#### Name 2

#### Department

#### Campus Estate or Research Centre

#### Road Name

MANSTON ROAD

#### District

#### Town

MARGATE

#### County

KENT

#### Postcode

CT9 4LT

#### Country

ENGLAND

#### Tel Number

01843 823388

#### Fax Number

01843 823297

#### E-mail

enquiries@criver.co.uk

#### HSE Division

EAST AND SOUTH EAST

#### Comments


#### Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory
Animal Unit
Growth Room
Glass House
Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

Bacteriology
Parasitology
Transgenic Birds
Microbiology Research

Virology
Transgenic Animals
Transgenic Fish
Gene Therapy
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment
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**Name**

CAMPDEN AND CHORLEYWOOD FOOD RESEARCH ASSOCIATION

**Campus Estate or Research Centre**

**Road Name**

STATION ROAD

**Town**

CHIPPING CAMPDEN

**District**

**County**

GLOUCESTERSHIRE

**Postcode**

GL55 6LD

**Country**

ENGLAND

**Tel Number**

01386 842000

**Fax Number**

01386 842100

**E-mail**

information@campden.co.uk

**HSE Division**

WALES AND SOUTH WEST

**Comments**

**Date at Which Additional Info Submitted**

31/05/2005
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Full heat sterilisation facilities eg autoclaves available and always used for live organisms. Typical waste cycles are 90 mins at 121 degrees C.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GENESYS LTD**

**Emergency Plan Required?**

- **Data Premises Notified** (Originally): 04/10/1993
- **Transferred from 1992 Regs?**: Y
- **Transitional Premises Class**: 1
- **Data Premises Closed**: N
- **Transitional Premises**: N
- **Non-GMMs**: N
- **Withdrawn**: N

**Name**

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**Town**

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<td>CAMBERLEY</td>
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**HSE Division**

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<td>EAST AND SOUTH EAST</td>
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**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

| Date Premises Closed | Name               | Campus Estate or Research Centre | Building            | Road Name        | District | Town              | County       | Post-code       | Country    | Withdrawn |
|----------------------|--------------------|---------------------------------|---------------------|------------------|----------|-------------------|--------------|----------------|------------|-----------|-----------|
| 19/02/2007           | GENESYS LTD        |                                 | SUITE 5 ST          | 40 LYNCHFORD     |          | FARNBOROUGH       | HANTS        | GU14 6EF       | ENGLAND    | N         |
|                      |                    | House                           | ROAD                |                  |          |                   |              |                |            |           |
| 19/02/2007           | GENESYS LTD        |                                 | SUITE 5 ST          | 40 LYNCHFORD     |          | FARNBOROUGH       | HANTS        | GU14 6EF       | ENGLAND    | N         |
|                      |                    | House                           | ROAD                |                  |          |                   |              |                |            |           |
| GENESYS LTD          |                    |                                 | INNOVATION          | ALBANY PARK      |          | CAMBERLEY         | SURREY       | GU16 7PL       | ENGLAND    | N         |
|                      |                    | House                           |                     |                  |          |                   |              |                |            |           |

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)  

Tick if confidential
Virology | Transgenic Animals
---|---
Mycology | Transgenic Invertebrates
Other(s) | Transgenic Fish

Transgenic Plants
Gene Therapy
Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste is inactivated either by overnight incubation with hypochlorite (primarily 20 litres spent fermentor media) or by autoclaving (0.25 litres in shake flasks). The efficacy of the waste treatment is confirmed by plating representative samples.

| ![Tick icon] | Tick to confirm that you are attaching a summary of the risk assessment

| ![Tick icon] | Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
## GM Centre Number: 539

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### Name

| LEICA BIOSYSTEMS NEWCASTLE LTD |

### Name 2

#### Department

#### Campus Estate or Research Centre

- BALLIOL BUSINESS PARK WEST

#### Road Name

- BENTON LANE

#### Town

- NEWCASTLE UPON TYNE

#### County

- TYNE AND WEAR

#### Postcode

- NE12 8EW

#### Country

- ENGLAND

#### Tel Number

- 0191 215 0567

#### Fax Number

- 0191 215 1152

#### E-mail

#### HSE Division

- YORKSHIRE AND NORTH EAST

### Comments

#### Date at Which Additional Info Submitted

- 02/03/2022

Page 8045 of 15326
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- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**
  - [ ]

- **Give brief details of the genetic modification safety committee**

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For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
**GM Centre Number: 540**

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**Name**

NEWCASTLE UNIVERSITY

**Name 2**

UNIVERSITY SAFETY OFFICE

**Campus Estate or Research Centre**

**Road Name**

KING'S GATE

**Town**

NEWCASTLE UPON TYNE

**District**

TYNE AND WEAR

**Country**

ENGLAND

**Tel Number**

0191 222 6320

**Fax Number**

0191 222 6276

**E-mail**

none

**HSE Division**

YORKSHIRE AND NORTH EAST

**Comments**

**Date at Which Additional Info Submitted**

10/08/2001
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02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
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Tick if confidential
Virology
Transgenic Animals
Mycology
Transgenic Invertebrates
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 40/16.1

Date Ackn'd 19/07/2017

CU2 Project Title Phenotypic characterisation of mutant derivatives of Mycobacterium tuberculosis using an attenuated host strain

Class 2
CultureVolClass2 1-50 Litres
ClassVolumeClass3-4
Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Transferred from GM 40

Historical Date of Additional Info
Significant Change ID
Date of Significant Change

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to characterise the function of genes encoded by Mycobacterium tuberculosis. In order to achieve this in as closely related organism as possible to the virulent M. tuberculosis we will utilise a highly attenuated and genetically validated M. tuberculosis generated by Prof W Jacobs (Albert Einstein College of Medicine, New York). The M. tuberculosis mc(2)7000 strain is an unmarked derivative of the mc(2)6306 M. tuberculosis strain harbouring disabling deletion mutations in RD1 region (associated with the attenuation of bacilli Calmette-Gurin (BCG) vaccine) and panCD (associated with pantothenate synthesis). RD1 mutations limit the ability of M. tuberculosis to grow within animal tissue and have been validated in humans through the use of the BCG vaccine, panCD mutations limit growth in vivo by restricting the availability of essential nutrients to the bacteria. Evidence supporting these statements is to be found in the supporting documents. Additional parallel studies will be performed in Mycobacterium bovis BCG, an avirulent strain of M. bovis, and similar HG2/1 mycobacteria (e.g. Mycobacterium smegmatis, Mycobacterium marinum, Mycobacterium thermoresistibile).

We will exploit these background strains in phenotypic screens. Nulling deletion mutations will introduced in protein coding genes within M. tuberculosis and additionally complement with a mycobacterium specific inducible expression vector. The mutants will enable us to identify the function of these genes using basic phenotypic characterisation studies. Potential foreign genes, which are exploited already within the field as validation markers and reporters, will be cloned into mycobacterium specific vectors to create viable test strains (e.g. luxAB genes from Vibrio harveyi). Antimicrobial testing and mutant generation using these strains will assist our efforts in mode of action studies of novel anti-tuberculosis compounds. Additionally genes of interest will be expressed at high levels in disabled host bacterium (E. coli) to enable further biochemical and biophysical analysis of the target protein.

**Recipient or parental organism**

The parental Mycobacterium tuberculosis H37Rv strain is a fully sequenced laboratory strain of TB classified as a HG3 organism. The route of transmission is via aerosol but can also infect via oral routes. This strain will not be used in the notified activities. The highly attenuated M. tuberculosis mc(2)7000 strain and its derivatives, classified as hazard group 2, will be used. The GM activities in this notification are growth in vivo, complementation, and storage of GM mc(2)7000 strains.

Other Mycobacterium related strains with potential opportunistic activities will also be used;
- Mycobacterium marinum (HG2)
- Rhodococcus equi (HG2)
- The vaccine strain;
- Mycobacterium bovis BCG
- Non-pathogenic;
- Mycobacterium smegmatis
- Mycobacterium thermoresistibile (HG2)
- (HG1)
- Disabled laboratory strains of E. coli including K12/MC1061 or similar/derivatives thereof will be utilised in construction of genetic elements using standard protocols.TOP10, XL-10 Gold (host for routine cloning); BL21, C41, BL21 star (for protein expression).
The pJV53 and pAL70 series of vectors for generating marked and unmarked mutants in Mycobacterium spp. including M. tuberculosis mc(2)7000 will be used (van Kessel JC, Hatfull GF Nat Methods. 2007. 4(2):147-52, Cascioferro et al., Appl Environ Microbiol. 2010; 76(15): 5312-5316). These vectors carry a hygromycin resistance gene for selection which can be removed by successive passaging of the organism in the absence of the selective marker.

phaE181 phage is a recombinant phage that contains a derivative of the mariner-based Tn5371. The recombinant phage phAE181 is derived from a temperature sensitive mutant of the lytic Mycobacteriophage TM4, which can infect many slow and fast growing Mycobacterium species, including M. tuberculosis, M. bovis, M. smegmatis and M. marinum. The phage can replicate inside a mycobacterial host (with a productive lytic cycle) at 30°C, but not at the non-permissive temperature of 37°C. Tn5371, which contains a hygromycin resistance gene (hyg), can thus be delivered by infecting the target Mycobacterium host at 30°C, followed by selection of Tn-mutants on hygromycin-containing plates at 37°C (Kriakov et al., 2003 J Bacteriol 185(16):4983-91).

DNA sequences will be introduced into Mycobacterium spp. including M. tuberculosis mc(2)7000 using well characterised and harmless non-mobilisable (Parsons et al., 1998 Mol. Microbiol. 28: 571-582) shuttle vectors that can replicate in E. coli and mycobacteria (e.g. pJEM15, pMV261, pMV306, pW16, pPR27 and their derivatives). These vectors contain an E. coli origin of replication (e.g. pJEM15, pMV261, pMV306, pW16, pPR27 and their derivatives). Additionally pMIND and pMEND derivatives will be used in complementation and overexpression studies (Blokpoel et al. Nucleic Acids Res. 2005.33(2):e22. 10.1093/nar/gni023). pNV18 and pNV19 are constructed by inserting a 1.8 kb DNA fragment carrying the pAL5000 origin of replication (Snapper et al. 1990 Mol. Microbiol. 4:1911-1919; Stolt 1997 Nucl. Acids Res.25:3840-3846) into the unique Nhel site of pK18 or pK19 (Pridmore 1987 Gene 56:309-312) for expression in Rhodococcus equi.

E. coli recombinant protein expression for crystallographic and biochemical studies will utilise the non-mobilisable pET expression systems.

Origin & function
Source: M. tuberculosis H37Rv
Genetic material: DNA fragments encoding various well defined genes involved in cell wall metabolism and function, e.g. transferases, fatty acid biosynthesis, polyketide biosynthesis, siderophore biosynthesis, p450 enzymes, core biosynthetic genes, regulatory enzymes and shock response genes. The intention is not to work with any sequences where insertion or deletion could be reasonably foreseen to increase the hazardous characteristics of the organism.
Source: Depending on marker - E. coli for B-galactosidase, luminescent vibrios for luciferase, jellyfish for GFP, Streptomycyes for agarases.
The genetic material that are to be expressed are marker protein which are highly unlikely to have any biological effect, eg GFP, luciferase, B-galactosidase, agarase.
Antibiotic resistance genes expressed from their native promoters.

Evaluation of foreseeable effects
1. Hazards to human health:
The parental strain M. tuberculosis H37Rv is capable of causing TB, the deletions of RD1 and panCD are well documented in peer-reviewed papers as attenuating the ability of these strains to colonise and cause infection in mammals (see accompanying summary of relevant literature). Furthermore, there is no reduction in the susceptibility of the GMMs to frontline treatments for TB. The likelihood of the 10 disabling gene deletions being overcome is
extremely unlikely. The possibility of generating a fully infectious TB strain is therefore effectively zero. Subsequent genetic modifications by over-expression of potential target gene candidates by plasmid expression or through further gene deletions are unlikely to modify the bacteria's virulence or infective route as long as the mutations are not rescuing or reverting the original RD1 and panCD deletion genotype. The majority of GMMs will render the strains less efficient at causing disease. It is not anticipated that any GMMs will show increased virulence.

2. Possible impact on the environment:
The disabling mutations were introduced by multiple gene replacement events in the H37Rv chromosome. The likelihood of mobilisation of the genetic modification from the mc(2)7000 derivatives, or transfer into other species in the environment in the event of a release is diminutive. This is reduced further still when the waste control measures are taken into consideration. All potentially infected material is autoclaved prior to disposal. 100% killing of GMMs is required. Disinfectants such as 2% Trigene and 10% Chloros are available for spills/disinfection of contaminated surfaces. Potential modes of transmission to the environment: Drains, aerosols, faulty autoclaves, and carried on individuals. These risks are reduced to moderated/unlikely, firstly by the disabling mutations within the bacteria, which reduce the likelihood of survival outside the laboratory (panCD) and the control measures used in the contained environment (see BioCOSHH and risk assessment).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The parental Mycobacterium tuberculosis H37Rv strain is a fully sequences laboratory strain of TB classified as a HG3 organism. This strain will not be used in any part of this notification. The HG2 classified M. tuberculosis mc(2)7000 strain and its derivatives will be used at our premises. The M. tuberculosis mc(2)7000 strain is an unmarked derivative of the mc(2)6306 harbouring disabling deletion mutations in RD1 region (associated with the attenuation of bacilli Calmette-Gurin (BCG) vaccine) and panCD (associated with pantothenate synthesis). RD1 mutations limit the ability of M. tuberculosis to grow within animal tissue and have been validated in humans through the use of the BCG vaccine, panCD mutations limit growth in vivo by restricting the availability of essential nutrients to the bacteria. Therefore we seek a derogation to allow the reclassification of the M. tuberculosis mc(2)7000 strain to HG2. Similar derogations for work with mc(2)7000 and its parental strain have been granted to existing projects in the GM public register, including project Ref 8/15.2 & Ref 552/13.2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials used in the CL2 facility are to be regarded as potentially contaminated and disposed of accordingly to ensure 100% killing. All solid waste will be autoclaved in a validated autoclave before leaving the laboratory and disposed of in clinical waste bags for clinical waste disposal. Liquid waste is pre-treated with Surfanios at 5% final concentration and left overnight before autoclaving and disposal of as clinical waste. Minimum 126°C degrees for 45 minutes, 10 minutes free-steam. Activities will be separated from those which use Virkon for chemical disinfection, as Virkon is documented as being ineffective against Mycobacteria.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
All materials used in the CL2 facility are to be regarded as potentially contaminated and disposed of accordingly to ensure 100% killing. All solid waste will be autoclaved in a validated autoclave before leaving the laboratory and disposed of in clinical waste bags for clinical waste disposal. Liquid waste is pre-treated with Surfanios at 5% final concentration and left overnight before autoclaving and disposal of as clinical waste. Minimum 126°C degrees for 45 minutes, 10 minutes free-steam. Activities will be separated from those which use Virkon for chemical disinfecction, as Virkon is documented as being ineffective against Mycobacteria.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Project Containment

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Project Ref  540/00.1

Date Ackn'd  12/05/2000

Date Project Ceased

CU2 Project Title  GENETIC ANALYSIS OF STREPTOCOCCUS EQUI (SUBSP.EQUI) (99/56)

Class  Class 2

CultureVolClass2  

CultureVolumeClass3-4  

Consent Granted  not applicable

Non-GMM

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements  Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Project Ref 540/00.2

Date Ackn'd 13/11/2000

Date Project Ceased

CU2 Project Title DEVELOPMENT OF MOLECULAR TOOL FOR THE ANALYSIS OF GENE FUNCTION AND EXPRESSION IN AVIRULENT BACILLUS ANTHRACIS STRAIN UM23CL-2

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? 
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 540/01.1

Date Ackn'd 26/01/2001
CU2 Project Title INTERACTION OF MODIFIED STRAINS OF SALMONELLA TYPHIMURIUM WITH
Class 2

Class CultureVolClass2 CultureVolumeClass3-4
Class 2

26/01/2001 INTERACTION OF MODIFIED STRAINS OF SALMONELLA TYPHIMURIUM WITH
Class 2

02/03/2022 Page 8060 of 15326
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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- Large Scale Activities
  - L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

- Human Clinical Applications
  - L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

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**Project Ref** 540/01.2

- **Date Ackn’d**: 04/09/2001
- **CU2 Project Title**: MOLECULAR GENETIC INTERVENTIONS TO INTERFERE WITH REPPLICATION OF PRIONS
- **Class**: Class 2
- **CultureVolClass2**: < 1 litre
- **Non-GMM**: not applicable
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: N

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- **Date Project Ceased**: 02/07/2002
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
**Project Additional Information**

**Purposes of the contained use**

The aim of our work is to develop two novel molecular genetic strategies to treat or prevent prion diseases, by interfering with the prion protein (PrP) conversion process, during which the cellular prion protein (PrPc) undergoes conformational changes transforming it into an abnormal, protease-resistant isoform (PrPsc). PrPc is encoded by the cellular PRNP gene and is co-translationally translocated into the lumen of the endoplasmic reticulum (ER), trafficking through Golgi and trans-Golgi network to reach the outer face of the cell membrane, from where it is internalised into endosomes and targeted to lysosomes for degradation. The conversion of PrPc into PrPsc is thought to occur on the cell membrane or in early endosomes. Replication of the agent causing transmissible spongiform encephalopathies is tightly associated with this post-translational conversion step and according to the now widely accepted “prion hypothesis” PrPsc itself represents the infectious agent. One of our strategies of interfering with conversion is creation of a "phenotypic knockout" of PrPc by expression of mini-genes encoding PrPc-specific single-chain antibodies fused with an ER-retention signal, thereby depleting the cell surface of PrPc molecules, and the other is overexpression of mini-genes encoding dominant negative mutants of PrPc that follow the identical pattern of subcellular trafficking and are capable of blocking the conversion of wild-type PrPc into PrPsc.

**Recipient or parental organism**

Initial cloning hosts will be disabled E. coli K12 derivatives such as XL1-blue and HP101. These are non-colonising and have auxotrophic and/or other mutations that limit their survivability outside laboratory media in the very unlikely event of escape (Compendium of Guidance 2000, Part 2A, Annex II). As such, they are equivalent to ACDP hazard group 1.

The host for expression of genetically modified material (anti-PrP single chain antibodies; dominant negative PrP mutants) is the mouse neuroblastoma cell line Neuro2a, which is not known to have any associated pathogens and has a long history of safe use. These cells are not known to contain any adventitious agents and they have pracitically no survivability outside tissue culture medium and do not colonise humans. For some experiments, Neuro2a cells will be infected with the Chandler strain of mouse scrapie agent (Bosque & Prusiner [2000] J Virol 74:4377). Hazards associated with infection of cells by mouse scrapie have been assessed in a COSHH risk assessment. Transfection of scrapie-infected cells with the GM sequences that are expected to inhibit production of pathogenic PrP isoforms are expected to result in a lower hazard than that involved in scrapie infection of non-transfected cells. Even if the GM sequences lack the predicted inhibitory activities, it is difficult to envisage how they could result in a greater hazard than those associated with infection of cells with scrapie, and therefore no additional GM hazards have been identified in relation to infection of cells with scrapie.

The host for introduction of the foreign genes in the germline of mammals is the mouse (Mus musculus).

**Host/vector system**

The phagemid vector pSEX81 (http://www.ncbi.nlm.nih.gov/entrez/viewer.cgi?val=2462915) is a pUC119 derivative comprising a ColE1 origin but is Bom-/(Nic-), Mob- and Tra- and therefore non-mobilisable (ACGM compendium of Guidance, Part 2A, Annex II). The mammalian expression vector plasmid pCMV/myc/ER comprising the HCMV promoter (http://www.invitrogen.com/content/vectors/pcmvmycer.pdf) is a pUC18 derivative and therefore non-mobilisable (ACGM compendium of Guidance, Part 2A, Annex II). The mammalian expression vector plasmids pL15TK (Holscher et al, J Virol 1998; 72:1153-9) and MoPrP.Xho (Borchelt DR et al, Genet Anal 1996; 13:159-63) comprising the HCMV promoter or the Prnp promoter, respectively are pUC19 and pBluescript derivatives, respectively, and therefore non-mobilisable (AC GM compendium of Guidance, Part 2A, Annex II). All the vectors are widely used in many laboratories and no specific hazards have been identified in relation to any of the vector sequences.

The GM mice will be constructed in a collaborating institution abroad (German Cancer Research Centre, Heidelberg, Germany) and then imported. The gector plasmid MoPrP.Xho (Borchelt DR et al, Genet Anal 1996; 13:59-63) allows for targeted expression in brain and myocardial cells of transgenic mice and is designed to give an expression level and pattern matching that of the endogenous Prnp gene.

**Origin & function**

The mini-genes encoding single-chain antibodies will be based on the variable regions of heavy chain and light chain immunoglobulin cDNA segments of murine hybridoma cells producing monoclonal antibodies directed against PrPc. Heavy chain and light chain segments will be joined via a flexible linker peptide. Expression of such single-chain antibodies fused with M13 pIII coat protein should enable recombinant phage to bind to the epitope of the parent monoclonal antibody, thus...
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Tissues will be collected (on death of the animal) and any unused material will be destroyed by incineration, using the services of a hospital-approved clinical waste contractor.

Records will be kept of numbers in each cage. All control measures required for Animal containment level 1 (Compendium of Guidance, Part 3E).

The mice will all be identified by earmarks and will be checked daily. The containment described below as well as the operator protection to be used when working with the live animals or derived tissues will render the risk of any putative harmful effects from the transgene product to humans effectively zero.

The containment level is required according to Compendium Part 2A, Annex III, Table 5A. The assessment is that containment level 1 is suitable for this work. Mice will be housed at all times in secure cages in a lockable room, minimising the risk of release. Appropriate facilities and procedures are in place to prevent any escape of animals, such as door guards, use of a closed-cage "mini-barrier" system (Fittlinger Co., Hockenheim, Germany), providing SPF-like conditions via closed-circuit ventilation of individual plastic cages operating at sub-atmospheric pressure, and the absence of floor drains and windows in the room. The mice will all be identified by earmarks and will be checked daily. Records will be kept of numbers in each cage. All control measures required for Animal containment level 1 (Compendium of Guidance, Part 3E).

Tissues will be collected (on death of the animal) and any unused material will be destroyed by incineration, using the services of a hospital-approved clinical waste contractor.

Codes of Practice under the Animals (Scientific Procedures) Act 1986 which equate at least to containment level 1 as described in the Compendium of Guidance, Part 3E.

Evaluation of foreseeable effects

Published data on Prnp null mice and derived cells as well as other cell culture studies demonstrate that deletion of PrPc, as intended in the present project, does by itself lead to the de novo creation of PrPsc but actually prevents the formation of PrPsc, infectivity and pathology that is typically seen in wild-type cells and animals upon scrapie agent inoculation (Sailer A et al, Cell 1994;77:967-968). Furthermore, PrPc depletion by Prnp gene knockout is not directly cytotoxic but may render cells slightly more vulnerable to oxidative stress (Brown DR et al, Exp Neurol 1997;146:104-112). Based on this, it is anticipated that depletion of PrPc by expression of mini-genes encoding ER-anchored anti-PrP single chain antibodies in transfected mammalian cell cultures, as intended in the present project, will not lead to cellular toxicity or de novo creation of PrPsc but will rather protect cells from scrapie agent replication and pathology.

Evaluation of foreseeable effects

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It is also anticipated that expression of novel dominant negative PrP mutants in mammalian cells or mouse brain will inhibit conversion of wild-type PrPc into PrPsc, as has been the case with the previously characterised prototype mutant PrPc - 114-121 (Holscher et al, J Virol 1998; 72:153-9), thus protecting from scrapie agent replication and pathology. The likelihood of the expressed products leading to (rather than inhibiting) production of pathogenic isoforms of PrP is extremely low and even if this could occur the likelihood of the expressed product reaching a target tissue (CNS) and causing a pathogenic effect is so remote as to be almost negligible. Nevertheless, because it is not possible to be 100% certain about predicted functions prior to performing experiments, combined with the consequences that could be involved in the extremely unlikely event of a hazard arising, to err on the side of caution the risks are assessed as medium/low rather than effectively zero. This is consistent with the recommended containment level 2 in the Compendium of Guidance, Part 2A, Annex III, Table 5A, for work involving expression of modified human or animal PrP genes where there is no expectation that the modified protein will have the ability to convert human PrP to pathogenic isoforms.

For introduction into the mouse germline only such PrP deletion mutants that have been well characterised and shown to lack harmful effects in cultured cell lines will be considered, greatly reducing the likelihood that any harmful effects will arise in vivo. The transgene products are unlikely to be secreted with saliva, urine or faeces. Furthermore, it is anticipated that the transgene products will not display any toxic or allergenic potential, nor induce any behavioural changes or act as a disease reservoir. There is some risk of allergenicity associated with mouse work in general and workers showing an allergic response to the animals are not required to come into contact with them although the expressed sequence is unlikely to be allergenic in itself since it is only expressed in muscle or CNS (Borchelt DR et al, Genet Anal 1996; 13:159-163). The containment described below as well as the operator protection to be used when working with the live animals or derived tissues will render the risk of any putative harmful effects from the transgene product to humans effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The transgenic mice expressing dominant negative versions of PrPc under the control of the Prnp gene promoter will be maintained to the standards prescribed in the Codes of Practice under the Animals (Scientific Procedures) Act 1986 which equate at least to containment level 1 as described in the Compendium of Guidance, Part 3E. This containment level is required according to Compendium Part 2A, Annex III, Table 5A. The assessment is that containment level 1 is suitable for this work. Mice will be housed at all times in secure cages in a lockable room, minimising the risk of release. Appropriate facilities and procedures are in place to prevent any escape of animals, such as door guards, use of a closed-cage "mini-barrier" system (Fittlinger Co., Hockenheim, Germany), providing SPF-like conditions via closed-circuit ventilation of individual plastic cages operating at sub-atmospheric pressure, and the absence of floor drains and windows in the room. The mice will all be identified by earmarks and will be checked daily. Records will be kept of numbers in each cage. All control measures required for Animal containment level 1 (Compendium of Guidance, Part 3E).

Tissues will be collected (on death of the animal) and any unused material will be destroyed by incineration, using the services of a hospital-approved clinical waste contractor.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

02/03/2022
Inactivation of any liquid waste or any contaminated plasticware or glassware will be by autoclaving at 121 degrees C for 20 minutes in a laboratory autoclave. The autoclave will be serviced on a 6 monthly basis and on an annual basis will be tested by thermocouple mapping employing a representative typical load. All autoclave runs will be monitored using TST (Time, Steam, Temperature) indicator test strips (Albert Browne Ltd, TST class 6 emulating indicator 121 degrees C for 20 min) meeting international standard ISO11140 part 2 to verify temperature and time.

In the event of spillage of possibly contaminated material, the area will be disinfected using 1% Virkon. The used paper towels will be autoclaved as above. As all work is performed in a separate laboratory, the wider environment is unlikely to become contaminated.

Control measures relating to TSE infectivity: As explained above, it is extremely unlikely that any TSE infectivity will be created as a result of the GM work to be performed in this project. Nevertheless, in view of the theoretical risk of spontaneous formation of PrPsc and scrapie infectivity by expression of the PrP deletion mutants (expected to behave as dominant negatives) and in view of using scrapie-infected mouse cell cultures in some of the experiments, disinfection of any remaining cellular material cell culture medium and plastic dishes will be carried out using methods approved for inactivation of TSE agents (incubation in sodium hypochlorite with release of 20,000 ppm chlorine for 1 hour, known to lead to 100% kill; joint ACDP/SEAC 1998 publication "Safe working and the prevention of infection") followed by incineration using an approved hospital contractor. In addition to the controls already mentioned, use of sharps will be avoided whenever alternatives exist.

In the event of spillage from the flasks, the area can be effectively disinfected and the wider environment is unlikely to become contaminated. The disinfectant used will be sodium hypochlorite with release of 20,000 ppm chlorine to be applied for all spillages for a period of 1 hour, after which this will be collected into a suitable container for disposal via the yellow bag route for incineration.

Tissues from transgenic mice will be collected (on death of the animal) and any unused material will be destroyed by incineration, using the services of a hospital-approved clinical waste contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approval granted.

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02/03/2022
Project Additional Information

**Purposes of the contained use**
The aim of the project is to identify antimicrobial activity in vertebrate gastro-intestinal (GI) tract material, using a sensitive assay involving a mutant of S. typhimurium.

**Recipient or parental organism**
Salmonella typhimurium strain CS015 (phoP102::Tn10d-Cam)

**Host/vector system**
No vectors will be used in this study. The GM strain CS 015 (phoP102::Tn10d-Cam) will be supplied to us from our collaborator (SI Miller). Ref: Gunn SS & Miller SI (1996) J of Bacteriology 178 6857-6864

**Origin & function**
Origin: Transposon Tn10d-Cam is a 1.5kb DNA element specifying chloramphenicol resistance (cam) and is defective for transposase (d). Ref: Elliott T and Roth JR (1988) Mol Gen Genet 213:332:338

Nature of modification: The GMM has been generated by our collaborator by insertion of the transposon Tn10d-Cam into phoP of wild type Salmonella typhimurium. This modification increases the sensitivity of the strain to killing by anti-microbial peptides. Ref: Miller SI, Kukrai AM, Mekalamos JJ (1989) PNAS 86 5054-5058.

Intended function: The detection of anti-microbial activity.
Evaluation of foreseeable effects

The recipient organism wild-type Salmonella typhimurium is classified as ACDP hazard group 2 that is very widely distributed in nature, infecting a wide range of zoonotic hosts. It is a very commonly encountered pathogen that causes infection via the oral route, involving in the vast majority of cases a self limiting gastroenteritis that resolves within 5-7 days without treatment, particularly in otherwise healthy individuals. Except in rare cases where bacteremia or other complications may occur, treatment with antibiotics is usually not advised but if necessary ampicillin or amoxicillin is usually recommended. As outlined below, the mutant S.typhimurium strain to be used in this project is very likely to be strongly attenuated in virulence and is therefore likely to be less hazardous than the parent wild-type strain. However since this has not been directly tested in humans, the mutant strain will be treated as equivalent to ACDP hazard group 2.

Salmonella strain CS015 has acquired chloramphenicol resistance but carried a mutation in phoP. This alteration has resulted in a strain with reduced pathogenicity ie the strain is attenuated for virulence in mice (and by extrapolation in humans), is unable to survive within macrophages and is sensitive to killing by antimicrobial cationic peptides. Ref: Miller SI, Kukrai AM, Mekalamos JJ (1989) PNAS 86 5054-5058. It is difficult to envisage how the phoP::Tn10d-Cam mutation could increase the ability of S.typhimurium to survive, disseminate or displace other organisms in the environment. Although the inserted transposon carries a Cmr marker, Cmr is already widely encountered among enteric bacteria in nature (Gallardo et al (1999) J Med Microbiol 48 367-374; Liberti and Loiacono (2000) Int J Antimicrob Agents 16 347-8). Moreover, any possible advantage that might be conferred by the Cmr marker, would probably be vastly out-weighed by the increased sensitivity of the mutant strain to killing by cationic peptides, which would likely to place it at a selective disadvantage in competition with wild-type strains in the guts of animals or poultry. S.typhimurium does not infect plants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Control measures to protect human health and the environment.

Since the GMM is classed as activity Class 2, they will be handled at containment level 2 (small scale, laboratory activities), as defined in ACGM Compendium of Guidance.

Contaminated Material (plastics, gloves, paper waste, bacterial cultures, agar plates, sharps, glassware).
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, Temperature) test strips (Albert Browne Ltd, TST class 6 emulating indicator 121 degrees C for 20 min) meeting international standard ISO11140 part 2.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved 1 October 2001.
The objectives of this programme are to construct and characterise mutants of Mycobacterium bovis BCG. These mutants will be used to analyse well-defined, non-toxic, mycobacterial cell wall components (proteins, lipids, etc) and genes that contribute to the basic physiology of mycobacteria. This project is of special interest because Mycobacterium tuberculosis, the causative agent of tuberculosis, is a major cause of death worldwide. The use of M. bovis BCG as a model organism to study M. tuberculosis coelocal genetics and physiology is well documented and will aid in the development of new classes of drugs against tuberculosis.

M. bovis BCG (Pasteur) will be the recipient organism. This is a widely used live attenuated vaccine strain used to protect against tuberculosis. The ACDP categorisation
of pathogens classifies M. bovis BCG as hazard group 2; although under clinical conditions for immunisation laboratory Containment Level 2 is not necessary. Staff working on the programme have previously been immunised with BCG. Studies to identify the genetic determinants of attenuation of virulence in M. Bovis BCG (Pasteur) have revealed two large tandem duplications and 14 large unlinked genome deletions in M. Bovis BCG (Pasteur), totalling c. 200 kb and encompassing 119 open reading frames. These deletions reflect a progressive adaptation of M. bovis BCG to laboratory conditions resulting in their compromised ability to survive within the host.

Host/vector system

DNA sequences will be introduced into M. bovis BCG using well characterised and harmless non-mobilisable shuttle vectors that can replicate in E. coli and mycobacteria (eg pJEM15, pMV261, pVV16, pPR27). These vectors contain both an E. coli and mycobacterial origin of replication (from the pUC cloning vector series and plasmid pAL5000, respectively) and antibiotic resistance markers (eg kanamycin and gentamycin). Only antibiotic resistances that are already present in very widely used bacterial cloning vectors will be exploited. Some of the vectors also contain the mycobacterial hsp60 promoter, the reporter genes lacZ (encoding B-galactosidase), phoA (encoding alkaline phosphatase) and gfp (encoding green fluorescent protein) or the counter-selective amrnker sacB (causing sucrose sensitivity to mycobacteria).

Origin & function

Only DNA sequences encoding non-toxic products of known function will be inserted into the vectors for introduction into M. bovis BCG. These products will be involved in the synthesis of cell wall components (eg. B-keto-acyl synthase and mycolyl transferase) and/or contribute to basic cellular physiology (eg essential metal ion homeostasis). The latter will include metal transporting P-type ATPases and SmtB-related metal-sensor proteins that occur in a wide range of bacteria, including non-pathogenic strains.

All sequences will be well characterised prior to introduction into M. bovis BCG and will be generated by PCR using specific primers designed to amplify known DNA sequences (no random sequences will be introduced). The DNA sequences will include defined sequences, amplified by PCR, from M. tuberculosis (ACDP hazard group 3) DNA which has been provided by Colorado State University (USA) through the Tuberculosis Research Materials Contract of The National Institutes of Health and M. smegmatis (ACDP hazard group 1) DNA. The sequence of the M. tuberculosis genome is complete and contains no toxin-life genes.

Introduced DNA sequences will either (i) remain on self replicating plasmids to drive reporter gene expression or for expression from the mycobacterial hsp60 promoter, or (ii) be used to direct allelic replacement mutagenesis and hence generate gene deletion mutants of M. bovis BCG.

Evaluation of foreseeable effects

M. bovis BCG is a widely used live attenuated vaccine strain used to protect against tuberculosis. Staff working on this programme have previously been immunised with BCG. We will only introduce very short (<4kb) DNA sequences into M. bovis BCG using well characterised harmless vectors. Introduced DNA will be well defined genes (or parts of genes) that encode non-toxic cell wall components and that contribute to basic physiology (such as the sensing and transport of essential metal ions). It is therefore very unlikely that these sequences would restore virulence to M. bovis BCG which has numerous unlinked genetic alterations. Only non-mobilisable vectors will be used to introduce DNA sequences and hence it is highly unlikely that these sequences will be transferred to another organism. For reasons outlined above, the likelihood of harm in the event of exposure would be no greater than that with non-GM M. bovis BCG.

Environment

It is difficult to envisage how either the vectors or the inserts could confer a harmful phenotype or competitive advantage to M. bovis BCG. The environmental hazards associated with the project are therefore considered no greater than those associated with handling non-GM M. bovis BCG. The project involves small-scale work for research purposes and all contaminated material will be completely inactivated by autoclaving prior to disposal. In the highly unlikely event of release into the environment no risks are envisaged.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste materials (bacterial liquid cultures/Agar plates) will be completely inactivated (100% kill) by autoclaving. All autoclave runs are validated by monitoring with a chart recorder and the autoclaves are validated annually by Thermocouple testing. All solid waste will subsequently be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approval granted.

Project Containment

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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 540/02.1

Date Ackn'd 01/03/2002

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Consent Granted

Non-GMM not applicable

Project notified under transitional arrangements N

Class 2

USE OF RECOMBINANT REPLICATION-COMPETENT SENDAI VIRUS (SEV) AS A NEW GENE TRANSFER AGENT FOR GENE THERAPY OF INHERITED DISEASES

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 litre

Number of pages: 1

02/03/2022
### Project Additional Information

**Purposes of the contained use**

Recombinant SeV will be used to transfer reporter and therapeutic genes to different cell types in vitro and compared to currently available gene transfer vectors.

**Recipient or parental organism**

Sendai virus (SeV) is an enveloped virus with a nonsegmented negative-strand RNA genome of 15384 nucleotides and is a member of the family Paramyxoviridae. The SeV genome contains six major genes: nucleoprotein (NP), phosphoprotein (P) and large protein (L) from a ribonucleoprotein (RNP) with the SeV genomic RNA. The matrix protein (M) engages in the assembly of viral particles. Two envelope glycoproteins, hemagglutinin-neuraminidase (HN) and fusion protein (F) mediate the attachment of virions and penetration of RNPs into infected cells. SeV replication is independent of nuclear functions and does not have a DNA phase. Therefore, it does not transform cells by integrating its genetic information into the cellular genome. SeV has been reported to naturally infect rodents (causing upper respiratory tract infections) and has never been reported to infect humans.

**Host/vector system**

Transgenes are normally inserted immediately before the ORF of the viral 3'-proximal nucleocapsid (NP) protein gene in a full-length SeV cDNA copy. The inserted gene is flanked by "gene start signal" and polyadenilation/stop sequences and recognised by the viral RNA polymerase.

**Origin & function**

The Sendai genome derives from the Z-strain. The recombinant virus has been developed by a Japanese biotech company, DNAVEC.

The recombinant SeV will carry different transgenes:
1. Bacterial transgenes, such as beta-galactosidase or CAT;
2. Eukaryotic transgenes such as luciferase (from Photinus pyralis) and GFP (from jellyfish);
3. Therapeutic genes

**Evaluation of foreseeable effects**

1. Recombinant SeV does not have an altered tropism compared to wild type virus. However, insertion of a transgene reduces the replication speed as well as the final virus titre in a way that is proportional to the size of the transgene, carried. In vivo a remarkably attenuated replication and pathogenicity were generally seen (Sakai et al., FEBS Lett 1999; 456: 221-226).
2. Virus replication and transmission to neighbouring cells has been demonstrated in the host species (rodents). SeV has never been reported to infect humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Recombinant Sendai virus is stored at -80 degrees C in 1-2ml vials. In vitro transfection experiments of cultured or primary cells will be carried out in a class II safety cabinet. After use the cabinets are routinely UV irradiated. Single-use plasticware is used. All GM contaminated material (including materials destined ultimately for incineration) will be completely inactivated by autoclaving before final disposal. This will allow a 100% degree of virus "kill", according to manufacturer's guidelines. The work involves standard laboratory protocols and there are no unusual procedures that require additional containment measures. Personnel will wear labcoats and gloves.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Safety committee is satisfied that the recombinant virus does not pose a risk to human health, for reasons that are outlined clearly in the attached risk assessment. The committee is also satisfied that the risk assessment recognises all likely environmental risks and that it specifies adequate control measures to contain all identified risks.

Project Containment

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Project Ref 540/03.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
USE OF ATTENUATED SALMONELLA TO DELIVER RECOMBINANT YERSINIA F1 PROTEIN IN THE LABORATORY AND FOR IMMUNISATION OF MICE.

Purposes of the contained use

To study the immune response to Yersinia F1 protein by infection of mice and cells in tissue culture to evaluate the potential of F1 protein as a candidate vaccine antigen.

Recipient or parental organism

Attenuated AroA Salmonella typhimurium SL3261 (vaccine strain).

Host/vector system

The vector system consists of the low copy number plasmid pLG339(Kan) into which the 4 genes of the caf operon of Yersinia pestis (caf1 structural gene, the caf1A and caf1M export and assembly genes and the regulatory caf1R gene) has been cloned to create the plasmid pAH34L.

Origin & function

The caf1 operon was derived from Yersinia pestis strain MP6 and codes for the capsule structural F1 protein which is a surface capsular structural protein whose function in virulence is thought to be conferring resistance to phagocytosis. Recombinant Salmonella typhimurium SL3261/pAH34L express the F1 protein on the surface and it has been reported that infection of mice has been shown to induce immunity that protects the mice against challenge with virulent Yersinia pestis. The recombinant Salmonella typhimurium SL3261/pAH34L has been used previously as a vaccine strain to protect mice against a lethal challenge with Yersinia pestis with no untoward side effects in the mice so vaccinated (Titball, RW, et al., (1997) Infection and Immunity 65: 1926-1930).

Evaluation of foreseeable effects

Salmonellae, including SL3261, that are attenuated by mutations in genes such AroA of the aromatic amino acid biosynthetic pathway are safe vaccines in animals and have been used in trials in humans. No wild-type strains will be used for this work. The likelihood that the expression of recombinant proteins such as F1 protein by the host strain will alter the strain and lead to pathogenic traits is negligible. No untoward side effects were observed in mice vaccinated with the recombinant Salmonella typhimurium SL3261/pAH34L strain, cf. Titball, RW, et al., (1997) Infection and Immunity 65: 1926-1930.

The only host strain that will be used will be attenuated Salmonella typhimurium strain SL3261, which is defined as disabled in the Compendium of Guidance (Part 2A, Annex II, para 11). This strain has been used as a live oral vaccine strain in mice. It has a non-revertible AroA deletion mutation (as well as other auxotrophic mutations) that confers a requirement for nutrients that cannot be supplied by mammalian hosts. Consequently, inoculation of mice with even a large infective dose results in a mild
self-limiting infection that can persist for only a limited period (usually < 2 weeks) before the organism is cleared completely. During this time no signs of distress are apparent and the host mounts an effective immune response to SL3261. The strain has been widely used as a safe live oral candidate vaccine delivery system for various recombinant antigens. Moreover, very similar AroA attenuated S. typhimurium strains have been used in human vaccine trials with no adverse reactions. Therefore, even infection with a very high dose would be unlikely to produce a symptomatic infection in normal healthy adults. However, the possibility that a more prolonged or even symptomatic infection might occur in immunocompromised individualxs cannot be excluded, since such data are not available.

In nature, S. typhimurium infections occur via the oral route (eg in contaminated food) and it has never been documented to be transmitted by an aerosol route. During infection experiments in mice, the recombinant SL3261 will be shed in faeces. Infection of laboratory or animal house personnel by contaminated faeces would require direct transmission of faeces to the mouth and it is highly unlikely that a sufficiently high infective dose would be transmitted unintentionally by this route (particularly in the case of the attenuated SL3261 strain) to establish an infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The recombinant attenuated S. typhimurium SL3261 strain will be grown in carefully controlled conditions in a containment level 2 laboratory. All contaminated material will be completely inactivated by autoclaving (100% kill). All autoclave cycles are validated by monitoring with a chart recorder. The autoclaves are serviced twice annually and their efficiency validated annually by thermocouple testing. Waste will consist of contaminated laboratory glassware as well as discarded residues from small scale culture media or solid media cultures and contaminated disposable plastics such as centrifuge tubes and pipettes.

Mice infected with the recombinant attenuated S. typhimurium strains are maintained in the airborn infection suite in the Comparative Biology Centre which equates to containment 2 to the standards prescribed in the Codes of Practice under the Animals (Scientific Procedures) Act 1986. The animals are maintained in closed cages in rooms with self closing doors. Access is by two-keypad controlled doors and through a further two doors. The doors are all flush to the floor. The floor drains are screwed down to the floor and have very small drainage holes, much too small for a mouse. Records are kept of the numbers of mice in each cage. Dead mice and other animal waste (eg bedding) are treated as clinical waste and are disposed of by autoclaving within the airborne infection suite followed by incineration, in accordance with Comparative Biology Centre standard practice for dealing with infected carcasses. Cages used for infected mice are also autoclaved prior to washing.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Approved 19 December 2002.

Project Containment
### Project Additional Information

**Purposes of the contained use**

The production of conditionally immortal human cell lines for use in drug discovery and development. Primary cells derived from muscle, fat, liver and microvascular endothelial cells will be infected with a replication incompetent retrovirus containing a combination of immortalising genes.

**Recipient or parental organism**

Non-self human muscle, fat, liver and microvascular endothelial cells obtained from volunteer subjects and covered by ethical permission and appropriate consent, or purchased from a supplier company.

**Host/vector system**

Viral transduction of primary human cells in Newcastle will involve their infection with up to two amphotropic retroviruses (produced in London) based on murine leukaemia virus (MLV) A strain. One vector was constructed using the pZipNeoSV(X)1 backbone, which additionally confers resistance to neomycin (G418), while another vector was.
constructed using the pBabe-Hygro backbone, which additionally confers resistance to hygromycin B. Both viruses are disabled so incapable of producing infectious virus.

All virus stocks will have been tested to confirm absence of replication competent virus before transfer from London.

**Origin & function**

The shuttle vectors employed in the immortalisation process will encode either temperature-sensitive simian virus (SV40) large T antigen (designated U19ts58) or the catalytic component of human telomerase (hTERT), and will be transduced into human cells as either LT alone or as LT + hTERT. The product of each of these genes is capable of extending the lifespan of and/or immortalising human cells. The SV40 large T antigen gene (LT) contains two mutations: tsA58 point mutation renders the T antigen thermolabile and severely attenuated at the physiological temperature 37 degrees C but wild-type at 33.5 degrees C, while the U19 double mutation ablates binding to SV40-origin DNA sequences present in the vector, and thus prevents autonomous viral replication. The hTERT and LT viruses encode resistance to hygromycin B and G418 respectively, and thus infected cells can be selected on the basis of resistance to these antibiotics.

**Evaluation of foreseeable effects**

The overall level of risk is low - the viruses are infectious to humans and human cells in vitro, but no competent virus would be produced in the event of infection since the recombinant virus is replication defective. Work at containment level 2 will be sufficient to prevent infection. All procedures involving use of virus will be performed in a designated class 2 containment hood within a secure exclusively designated laboratory containing its own equipment. In normal individuals, the LT virus would be recognised as foreign on inoculation, would be readily complement-inactivated, and therefore would not be allowed to integrate into the genome and produce the LT product. However, the hTERT virus is not inactivated by complement. Therefore, neither metal sharps nor glassware will be used in working with these viruses, to minimise the risk of inoculation into the bloodstream. The highest (although still low) risk associated with this type of virus is probably insertional mutagenesis and the subsequent activation or inactivation of host genes proximal to the insert that regulate cell growth in a positive or negative manner. Provided that human cells transduced with such virus are free of helper activity and are tested for the absence of replication-competent genetically modified virus, they pose no/low risk.

Transduced cells will be maintained under containment level 2 conditions and segregated from untransduced cells. Absence of active virus in transduced cells will be confirmed prior to use and at intervals of 3 months during continuous culture.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All Class 2 activities will be performed using full containment level 2.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All plasticware contaminated with GMMs will be decontaminated by soaking in 1% Virkon for at least 24 hours, drained and transferred to a segregated autoclave bag. Virkon has been demonstrated to be an effective virucidal agent against related retroviruses showing the same protein, sugar and membrane lipid content as MLV, as dilutions ranging from 1/100 to 1/1400. However, the efficiency of Virkon action can be decreased by the presence of organic matter (ACGM Compendium of Guidance 2000, Part 3A, Annex III). Therefore, all detergents will be made fresh daily, their correct preparation being ensured by the strict following of standard operating procedures adhering to the manufacturer's guidelines. Additionally, a record of the preparation of all disinfectant solutions will be kept. All paper, gloves etc. will be collected in a segregated autoclave bag. Once each bag is full, or 2-weekly if this time is shorter, the bag will be removed by autoclaving at 121 degrees C for 20 min (100% kill) in the discard validated autoclave within the Xcellsyz Ltd facility. Autoclaving at 121 degrees C for 20 min has been found to inactivate all organisms encountered.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Formal approval by the Microbiological Hazard and Genetic Modification Safety Advisory Sub-Committee. Can proceed once CU2 notification acknowledged by HSE.

Please enter comments on the GM safety committee on the risk assessment

Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

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Animal Units

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Project Ref 540/03.3

Date Ackn'd: 14/04/2003

CU2 Project Title

IMMORTALISATION OF AVIAN CELLS BY RETROVIRAL TRANSFER OF THE TELOMERE MODIFYING GENES TELOMERE REVERSE TRANSCRIPTASE (TERT) AND A TELOMERASE RNA (TR)

Class 2

CultureVolClass2 < 1 litre

Non-GMM: not applicable

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Project Additional Information
Purposes of the contained use

Telomeres are necessary for chromosome stabilisation and their maintenance determines the mortality of a cell. The aim of this work is to manipulate telomere size and maintenance in avian cells in vitro by constitutive expression of the TERT and TR genes. We will examine the effects of the transfected genes on telomere length, telomerase activity, cell growth and cell immortality. We will use the Pantrophic Retroviral Expression System (BD Biosciences, Clontech), which provides a highly efficient gene delivery system.

Recipient or parental organism

1. Avian primary cell lines. These cell lines are primary cell lines established from embryonic tissues. They are non-self lines, which cannot colonise humans and therefore pose no hazards to human health.

2. Packaging cell lines GP2-293 and GP2-293Luc. Both lines are human embryonic kidney (HEK 293) based cell lines. These human cell lines are established non-self lines, which cannot colonise humans and therefore pose no hazards to human health.

The GP-293Luc packaging cell line is a cell line that has been modified to encode luciferase activyr. This cell line functions as an experimental control to verify that the viral transfection system is functioning properly and that the avian cells can be infected.

Host/vector system

1. The avian cell lines are primary cell lines established from avian embryonic tissues.

2. The packaging cell lines, GP2-293 and GP2-293Luc, employed during these procedures are commercial cell lines and purchased from BD Biosciences, Clontech.

3. The viral vectors pLXRN, pLNHX, pLPCX and pVSV-G are commercial vectors purchased from BD Biosciences, Clontech. The viral vectors are propagated in the disabled Escherichia coli host DH5a, using ampicillin selection. The copy number is low.

Origin & function

Origin: The pLXRN, pLNHX and pLPCX viral vectors contain elements from Moloney murine leukaemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV). These viral vectors are disabled in that they lack the genes env, gag and pol necessary for viral particle formation and replication. It is unlikely that introduction of the TERT or TR genes into these vectors will decrease their stability. Plasmid VSV-G encodes the G envelope glycoprotein of the vesicular stomatitis virus and lacks the genes gag and pol.

The hTERT cDNA was a gift of the Geron Corporation, USA; the avian TR cDNA sequence was a gift of JL Chen, John Hopkins University, Baltimore (Chen JL et al 2000 Cell 100 503-514); the avian TERT cDNA is being cloned in the laboratory from an avian cDNA library.

Intended Function: Immortalisation of avian cells by retroviral transfer of the telomere modifying genes TR and TERT.

Evaluation of foreseeable effects

The Pantrophic packaging cell line (GP2-293 produces virus that can infect both mammalian and non-mammalian cells (Burns et al 1993, PNAS 90 8033-8037). This is due to the fact that virions are pseudotyped with the envelope glycoprotein from the VSV-G virus. VSV-G mediates viral entry through lipid binding and plasma membrane fusion (Emi et al 1991 J Virol 65 1202-1207). It is therefore possible that non-replicating virus could infect the target cells of organisms other than humans, transmit the TERT and TR genes and cause a biological effect.

The health risks associated with the inserted genes are low. This is because:
(i) the virus cannot replicate in cells that lack the viral structural genes gag and pol, necessary for viral particle formation and replication. Thus virus would be confined to the original infected cell;
(ii) the TERT or TR genes do not induce a transformed phenotype and do not render cells tumorigenic (Morales et al 1999 Nat Gen 21 115-118; Franco et al 2001 Exp Cell

Nonetheless it is possible that if the transfected cells are already pre-disposed to tumour formation eg by a separate oncogenic sequence, then a tumour may result. However, pathogenicity is extremely unlikely as the virus cannot replicate in target cells that lack the viral structural genes.

The virions produced by co-transfection of GP-293 with recombinant vector and pVSV-G can infect and transmit the TERT and TR genes to the avian (target) cells. However, the virions cannot replicate because the avian cells lack the viral genes.

The probability of the virus reverting to wild type is low because the viral gag and pol genes are supplied only from the genome of the packaging cell line GP-293, and the viral envelope protein env, is expressed by a separate vector pVSV-G. Thus at least two independent recombination events are necessary for reversion to wild type. The chance of producing replication-competent virus is also reduced by the use of minimal viral sequences.

We will however test for the presence of replication-competent virus by a) using indicator cell lines for plaque estimation and b) monitoring the supernatant of the cultures infected with the recombinant retroviral particles for reverse transcriptase activity. In the unlikely event of replication competent virus being detected, the stock will be inactivated by autoclaving as described in Section 12.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Control measures to protect human health and the environment:
All work will be done under full containment level 2 facilities.
Production of virus particles and viral infection of host cells is performed at small scale (<10 (to the power of 7) cells) under sterile conditions in closed plastic tissue culture vessels. All cell culture work will be done in a Class ii safety cabinet with HEPA microfilter. Needles will not be used during the procedures and protective clothing (laboratory coats and gloves) will be worn at all times.

Contaminated material:
All material that is possibly contaminated with virus will be collected in closed vessels for transfer to the autoclave and will be inactivated by autoclaving at 121 degrees C for 20 min. This will result in 100% kill. All autoclave runs will be monitored using TST (Time, Steam and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121 degrees C for 20 min) meeting international standard ISO11140 part 2. The autoclave is tested on an annual basis using an independent thermocouple mapping employing a representative typical load.
In the event of spillage of possibly virus-contaminated material the area will be disinfected using 1% Virkon. All paper material used in the clean-up will be autoclaved as above.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
# Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<td>L3</td>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L3</td>
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**Project Ref** 540/03.4

**Date Ackn'd**

27/05/2003

**CU2 Project Title**

GENE INACTIVATION AND EXPRESSION IN SPECIES OF ORAL STREPTOCOCCI AND LACTOCOCCI

<table>
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<th>Class</th>
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<th>CultureVolumeClass3-4</th>
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**Non-GMM**

Consent Granted

not applicable

**Project notified under transitional arrangements**

N

**Withdrawn**

N

**Tick if notifying a connected programme of work**

N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Streptococci of the "viridans group" are normal commensal organisms found in the mouths of humans and other mammals. S. mutans and S. sobrinus are known to be associated with dental caries in humans and this project is concerned with investigations of features of these organisms that may contribute to disease, with the aim of developing novel preventive or therapeutic approaches. The pathogenesis of dental caries relates to the ability of the organisms to colonise dental plaque and, when present in sufficient numbers and exposed to a carbohydrate source, to damage tooth enamel by reducing the local pH. The project is thus principally concerned with
structural and regulatory genes for enzymes and proteins associated with the adhesion of streptococci, metabolism of carbohydrates and formation of sucrose-derived polymers.

Recipient or parental organism

“Viridans streptococci” are generally considered of low pathogenic potential and normally exist in the mouth and naso-pharynx as commensals. They do not normally multiply or survive outside the body. They are occasional opportunistic pathogens and have been associated with cases of infective endocarditis but are regarded as being of low pathogenic potential. Available information indicates that infections in otherwise healthy individuals are readily treatable with antibiotics, with penicillins being the antibiotics of first choice. The project will be focused on the mutans group of streptococci that are associated with dental caries, specifically S. mutans, S. sobrinus and S. downei. It is believed that the pathogenesis of this disease is due to the ability of these organisms to form organic acids at low pH. A number of surface proteins and extracellular enzymes are known to polymerise glucose and fructose using sucrose as the substrate (GTFs and FTFs) have been linked with the potential of S. mutans and S. sobrinus to cause caries in experimental animals. The mutans streptococci are not known to produce any toxins. Some experiments will be performed with other oral streptococci for purposes of comparison. In particular, S. gordonii strain Challis, which has been used extensively for genetic manipulation in other laboratories. It is also anticipated that certain experiments will involve expression in Lactococcus lactis, an organism that is not considered a human pathogen and is approved as Generally Regarded as Safe (GRAS) for use in the food industry. All streptococci are placed in ACDP Hazard Group 2.

Host/vector system

Insertional inactivation experiments in oral streptococci will utilise either linear PCR amplicons incapable of replication or plasmids incapable of replication in streptococci. Insertional inactivation and cloning experiments will utilise markers for resistance to antibiotics that are not of clinical importance for treatment of infections caused by the bacteria. Specifically, resistance to penicillin will not be introduced to streptococci. Vectors used for complementation experiments will be those based on naturally-occurring streptococcal replicons.

Origin & function

The project is principally concerned with structural and regulatory genes for enzymes and proteins associated with the adhesion of streptococci, metabolism of carbohydrates and formation of sucrose-derived polymers.

Experiments will be undertaken to inactivate genes of species of oral streptococci by insertional inactivation or gene replacement and to transfer DNA between bacteria for expression of complementation of inactivated genes.

Evaluation of foreseeable effects

Natural isolates of oral streptococci shown considerable variation in properties such as acidogenicity and production of extracellular enzymes. It is difficult to envisage how the inactivation of specific genes or alterations in their regulation could enhance the ability of the host strains to colonise humans or otherwise cause disease. Furthermore, the risk of infection with the host bacteria is low. Therefore the level of risk is identified as low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste, disposable plastics, laboratory glassware, or other contaminated materials, including waste destined for incineration, will be autoclaved to inactivate GMMs (100% kill) prior to disposal or cleaning and recycling. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, Temperature) test strips. (Albert Browne Ltd., TST class 6 emulating indicator 121 degrees C for 20 min) meeting international standard ISO11140 part 2.
Please enter comments on the GM safety committee on the risk assessment

Approved 14 April 2003.

Project Containment

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Animal Units

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<td>L2 L3 L4</td>
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Project Ref 540/04.1

Date Ackn’d: 29/04/2004

Date Project Ceased:

CU2 Project Title: Investigating stress responses in Candida albicans and Candida glabrata.

Class: Class 2

Culture Volume Class 2: 1-50 Litres

Non-GMM: Not Applicable

Consent Granted: Not Applicable

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID: 540/04.1a

Date of Significant Change: 09/02/2021
**Purposes of the contained use**

Candida albicans is a human pathogen in ACDP Hazard Group 2. C. albicans causes superficial infections of mucosal epithelia (thrush) (Odds [1988] Candida and Candidosis, Balliere Tindall). C. albicans can also cause systemic infections which can be fatal, but these only occur in severely immunocompromised patients. To keep things in perspective, it is important to realise that C. albicans is carried commensally in 50% of the healthy population and most Candida infections arise as an overgrowth of the strains resident in the normal microflora of the gut and vaginal tract. It is a micro-organism of low pathogenic potential that rarely affects healthy individuals. It only becomes a potential medical problem when the immune system of an individual is significantly impaired. Furthermore, as with most pathogenic micro-organisms, the virulence of C. albicans strains is likely to become attenuated rather than enhanced after prolonged laboratory culture. The organism does not form spores, therefore there is no transmission by air-currents. C. glabrata is closely related to C. albicans, and generates similar types of infections in humans, but undergoes the yeast-hypha morphogenesis less efficiently, and is considered to be less virulent than C. albicans. Furthermore, genetically disabled C. glabrata strains will be used in these experiments, and it will be entirely appropriate to use similar precautions to those used for our C. albicans experiments.

The C. albicans strains CA14 (ura3) and RM1000 (his1, ura3) are the standard host for DNA transformation world-wide. These auxotrophies makes C. albicans avirulent (Leberer et al. [1996] PNAS, 93, 13217). Transformation with URA3 plasmids partially restores the virulence of CA14, but this restoration is not complete because the genes neighbouring URA3 remain inactivated. This is enough to partially (but not completely) attenuate virulence. The ura3 marker is recycled for subsequent rounds of transformation (Fonzi, & Irwin (1993) Genetics, 134, 717). Therefore by necessity, some of the transformants we will create are not genetically disabled, although their virulence is slightly lower than the wild type strains of C.albicans carried by most individuals. C.albicans strain CA18 (ura3, ade2) will also be used in some experiments. CA18 is transformed with ADE2 or URA3 containing plasmids, leaving one remaining disabling mutation in the transformants. This is sufficient to render all of the transformants completely avirulent.

Pathogenicity in C. albicans and C.albicans and C.glabrata is a polygenic trait involving numerous physiological characteristics (e.g. rapid growth, cell wall, morphogenesis, secreted hydrolases, phenotypic switching). Therefore, (i) no single Candida gene (or combination of genes) will turn E.coli, S.cerevisiae or S. pombe into a pathogen, (ii) a reduction in any one of these parameters can attenuate C.albicans or C.glabrata virulence, and (iii) an increase in any one of these parameters is very unlikely to increase C.albicans/C.glabrata virulence. Furthermore, C.albicans strains that have been cultured in the laboratory tend to display reduced pathogenicity compared to strains carried commensally by most individuals.

The likelihood of natural gene transfer to other species is essentially zero. The plasmids that will be used are not mobilisable and the GMMs are highly unlikely to come into contact with other micro-organisms under the experimental conditions to be used. Furthermore, despite the recent discovery of sex in C.albicans strains mate very inefficiently in vivo (Hull et al., (2000) Science 289, 307-310), probably because the specific growth forms that mate most efficiently aren't maintained at 37 degrees (Miller & Johnson (2002) Cell 110, 293-302). In addition, C.albicans must be homozygous at the mating type locus for them to be competent for mating, and the vast majority of clinical isolates are heterozygous at the mating type locus.

E.coli, S.cerevisiae and S.pombe. Only disabled E.coli strains (E.coli K12-Compendium of Guidance 2000, Part A, Annex ii; BL21-ACGM Newsletter 30, Nov 201) and especially disabled S.cerevisiae and S.pombe strains which are unable to colonise humans and are non-pathogenic will be used. In addition, pathogenicity in C.albicans and C.glabrata is a polygenic trait involving numerous physiological characteristics (e.g. rapid growth, cell wall, morphogenesis, secreted hydrolases, phenotypic switching). Therefore, no single Candida gene (or combination of genes) will turn E.coli, s.cerevisiae or S.pombe into a pathogen.

**Host/vector system**

<table>
<thead>
<tr>
<th>Hosts</th>
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<tbody>
<tr>
<td>2. S.cerevisiae and S.pombe. These yeast are considered especially disabled, non-pathogenic and non-colonising (Compendium of Guidance 2000, Part A, Annex II).</td>
<td></td>
</tr>
<tr>
<td>3. C.albicans (recipient strains carrying disabling markers CAI4; ura3: CAI8; ura3, ade2: RM1000; his1, ura3: derivatives of these strains carrying further disabling markers, e.g. in transcription factors, signalling pathways.)</td>
<td></td>
</tr>
<tr>
<td>4. C.glabrata (recipient strains carry single or multiple disabling markers such as ade2, his3, ura3, trp1 or lys2).</td>
<td></td>
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</tbody>
</table>
Vector system
1. Non-mobilisable or mobilisable defective E.coli vectors (including pBR322, pUC18/19, pBluescript, pGEMT, pGEX; lac-based expression plasmids such as pET vectors (Compendium of Guidance 200, Part A, Annex II).
2. Non-mobilisable S.cerevisiae vectors (including multicopy 2um vectors; single copy Ycp replicating vectors; single copy integrating Yip vectors; single copy GAL-based expression vectors).
3. Non-mobilisable S.pombe vectors (low copy replicating and integrating vectors (Rep/Rip).
4. Non-mobilisable C.albicans vectors (low copy replicating and integrating vectors (YPB1, Cip and Clp20); low copy replicating and integrating vectors for ectopic expression (YPB-ADHpt, pACT1); integrating expression vectors regulated by methionine (pEXPa is a MET3 vector), or maltose (pMRP is a MAL2 vector). These vectors are routinely used in by methionine (pEXPa is a MET3 vector), or maltose (pMRP is a MAL2 vector). These vectors are routinely used in many Candida laboratories.

Origin & function
The genetic material involved in this project is of yeast (C.albicans and C.glabrata) origin. In most cases we will simply express specific native yeast genes or mutant versions, cloned from genomic sequences, or express native or mutant yeast genes where the genes have been fused with standards tags (eg. MYC, 6 histidine residues, HA, FLAG, GFP, YFP). Candida genes involved in stress responses will also be deleted or disrupted to assess their role in stress responses. In addition, well characterised reporter genes will be fused to yeast promoters to allow studies of expression in yeast. In some cases we will use #Candida genomic and cDNA libraries. Candida genes will also be expressed in the yeasts S.pombe and S.cerevisiae to adress whether any of the proteins involved in stess responses are conserved. Candida genes involved in the project include
1. Stress-signalling genes (e.g. HOG1)
2. Transcription factor genes (e.g. SK01)
3. Auxotrophic markers (e.g. URA3, ADE2, LYS2)
4. Regulated promoters (e.g. MET3, MAL1, MAL2) and "constitutive" promoters (e.g. ACT1, ADH1),
5. Reporter genes (e.g. yEGFP, Renilla luciferase, StLacZ, SaLexA)
6. Candida genomic and cDNA libraries.

Evaluation of foreseeable effects
As described above pathogenicity in C.albicans and C.glabrata is a polygenic trait involving numerous physiological characteristics (e.g. rapid growth, cell wall, morphogenesis, secreted hydrolasis, phenotypic switching). Therefore, (i) no single Candida gene (or combination of genes) will turn E.coli, S.cerevisiae or S.pombe into a pathogen, (ii) a reduction in any one of these parameters can attenuate C.albicans or C.glabrata virulence, and (iii) and increase in any one of these parameters is very unlikely to increase C.albicans/C.glabrata virulence. Furthermore, C.albicans strains that have been cultured in the laboratory tend to display reduced pathogenicity compared to strains carried commensally by most individuals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMM contaminated waste will be inactivated and disposed of as follows:
Liquid waste: Presept tablets (1 tablet per 500ml)
Solid waste: autoclaved
Glassware: Soaked with Presept tablets (1 tablet per 500 ml) or autoclaved
Standard procedures for use of Presept have been established in the laboratory.
Plasticware; autoclaved
Sharps: Contaminated sharps are placed in CinBins, bagged and incinerated.
Validation and Monitoring of Inactivation

The use of Presept has been validated experimentally in many major Candida laboratories to kill >99.99% of cells. We will validate this procedure in our laboratory by performing viability tests on killed cultures. We will monitor this procedure monthly by repeating viability tests on killed cultures. We will also re-validate the procedure should the conditions of use change, for example if the volumes to be inactivated increase, larger amounts of protein material are involved, or if we need to switch to a different chemical.

Upon autoclaving GMM waste samples are subjected to autoclaving at 134 degrees C for 30 min (100% kill). This procedure has been validated to completely inactivate all organisms encountered. We will again validate this procedure for Candida as above. The autoclaves are validated by annual thermocouple mapping and each run is to be monitored by continuous chart recording of the temperature/time profile.

Once GMMs have been inactivated, normal routes of disposal are used for liquid and solid waste. Killed liquid waste is discarded down the sink. Inactivated solid waste is discarded by CFA processing.

If spillage were to occur the contaminated area will be flooded with Virkon (peroxyenic acid) (freshly diluted 1 in 50), mopped up with paper towels, and reswabbed with Virkon (freshly diluted 1 in 50). Killing at >99.99% has been validated experimentally. Contaminated material is autoclaved. In addition Virkon will be used for routine cleaning of bench tops and laboratory equipment. Virkon is preferred to Presept for swabbing benches, because it is less nasally invasive.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The projects are passed by the University GM Committee prior to being sent to the HSE.

**Project Containment**

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Animal Units

| L2 | L3 | L4 |
| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |
| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |
| L2 | L3 | L4 |

Project Ref 540/04.2
The purpose of this project is to provide a system for the high-level expression (mg quantities from a litre) of proteins from Listeria species for protein structure and function studies. As part of this project recombinant plasmids containing the Listeria genes of interest will be supplied by collaborating laboratories. These genes will then be sub-cloned, where necessary, into more vectors that support high-level protein expression, and introduced into suitable E. coli hosts. Proteins will be expressed in these hosts for the purpose of in vitro protein structure and function studies only. No live wild-type Listeria strains will be used or stored. In order to characterise the relevant protein's sturcture and function some PCR-induced site-directed mutagenesis may also be undertaken as part of this project.

Listeria have been classified as ACDP hazard group 2 organisms. They are, however, very commonly encountered pathogens in nature that very rarely, if ever, cause disease in normal health adults, implying low levels of virulence compared to many other group 2 organisms. However, they are capable of causing serious infections in individuals with certain predisposing conditions (very young or very elderly, immunocompromised individuals, and pregnant women), and therefore caution is warranted in dealing with any potentially pathogenic factor from these organisms.

The recipient organisms for this study are E. coli strains K12 and its derivatives and BL21 and its derivatives. Use of these organisms is discussed in the Host/Vector system section that follows this.

The parental organisms for this study are the pathogenic species of the Listeria genus, Listeria monocytogenes and Listeria ivanovii. As mentioned previously, at no time in this project will wild-type strains of Listeria be handled or stored. All genetic material from Listeria will be cloned into non-mobilisable or mobilisation-deficient plasmids by collaborating laboratories prior to entry into the laboratory.

E. coli strains K12 and its derivatives and BL21 and its derivatives only will be used as hosts during this project. All cloning and plasmid amplification will be done using E. coli K12-derivative hosts. These hosts are classed as disabled, non-pathogenic, and have minimal colonisation potential. For protein expression both K12-derivative hosts and BL21-derivative hosts may be used. BL21-derivatives can be broadly considered equivalent to disabled K12-strain. However, special consideration is required in the case of cloning bacterial pathogenicity determinants into BL21, as is described in this notification. More detail is given below (foreseeable effects).
Only vectors that are non-mobilisable or mobilisation-defective in bacteria will be used. For cloning this includes pGEM-T (Promega), and for expression this includes vectors of the pET (Novagen) and pGEX (Amersham) series. Given that gene products to be studied in this project are potentially biologically active, only plasmids that specifically support expression of protein to an intracellular location will be used. Vectors that specifically support secretion of gene products from the host organism will be excluded from this study.

**Origin & function**

Cloned sequences will initially be derived from Listeria genomic DNA, by PCR. This work will be conducted in collaborating laboratories. All DNA sequences to be handled in this project will already be cloned into non-mobilisable or mobilisation defective plasmids and maybe transformed into a K12-derivative or BL21-derivative bacterial strains. Specific modifications and intended functions will vary depending on the particular experiment, but at all times they will be restricted to facilitating standard, routine, well established molecular biology procedures with the intent of expressing recombinant proteins for biochemical and biophysical structure and function studies.

**Evaluation of foreseeable effects**

1. **Risk to Human Health**
   The use of only K12-derivative and BL21-derivative hosts, which have limited ability to survive outside the laboratory environment, combined with non-mobilisable or mobilisation-deficient plasmids that are in common use limits the risks to human health from the host/vector system. However, some of the gene products to be studied have specific roles in the pathogenic/virulence mechanisms of Listeria species. Whilst it is difficult to envisage how any of the gene products could enhance mammalian cell invasion by the disabled E. coli strains to be used as hosts in this study, the possibility that some of the gene products may pose a hazard when expressed at high levels cannot be excluded. Of particular concern are proteins that may be secreted during expression in E. coli. To minimise the likelihood of any such hazard, sequences encoding recognisable secretion signals will be removed from Listeria genes sub-cloned into expression vectors. This will avoid active secretion and minimise release of product from disabled E. coli hosts.

2. **Environmental Harm**
   The details of the host/vector system in reference to human health implications also applies here. In the very unlikely event of GMM escape, the use of only K12-derivative and BL21-derivative hosts, which have limited ability to survive outside the laboratory, combined with non-mobilisable or mobilisation-deficient plasmids that are in common use, limits any possible environmental impact. As also noted above, some of the gene products to be expressed might have detrimental effects if they are delivered in sufficient quantities to mammal cells/tissues or other eukaryotic cells, but the biological containment measures noted will minimise the likelihood of any hazard arising and therefore the previsionally assigned containment level 2 will be sufficient to contain the risk.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work will be conducted in accordance with the requirements for containment level 2, as described in the Compendium of Guidance 2000, Part 3A Section 1.

All contaminated waste, disposable plastics, laboratory glassware, or other contaminated materials, including waste destined for incineration, will be autoclaved at 121 degrees C for at least 30 min to inactivate GMMs (100% kill) prior to disposal or cleaning and recycling. Autoclaves, available within the building the work is taking place, will be validated by annual thermocouple mapping and each run will be monitored by continuous recording of the temperature/time profile.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
This application has been passed by the University GM Committee.

Please enter comments on the GM safety committee on the risk assessment

This application has been passed by the University GM Committee.

Project Containment

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Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if notifying a connected programme of work

N

Project Ref 540/04.3

Identification and analysis of virulence determinants from the attaching and effacing (A/E) family of enterobacterial pathogens, by cloning genes and constructing mutants in various E. coli hosts, Citrobacter rodentium, non-pathogenic yeast hosts, and various mammalian cell hosts

Class 2

< 1 Litre

Class Culture Vol

Class 2

< 1 Litre

Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

The objectives of the project are to understand the pathogenic mechanisms of members of the family of attaching and effacing pathogens involving closely related species that infected humans - enteropathogenic E. coli (EPEC) and enterohaemorrhagic E. coli (EHEC; but see below), rabbits (REPEC), pigs (PEPEC), dogs (DEPEC) and mice (Citrobacter rodentium) which for convenience are hence forth described collectively 'A/E bacteria'. Please note that the description 'A/E bacteria' as used here specifically excludes verotoxigenic E. coli strains. Genetically attenuated derivatives of EHEC strains where the Shiga-like (Vero) toxin genes have been deleted will be used in some experiments, but wild-type EHEC or other verotoxigenic strains will not be used.

Recipient or parental organism

(i) Disabled E. coli K12 strains as defined by the Compendium of Guidance (Part 2, Annex II Para 11): these are non-colonising and cannot survive for long outside the laboratory. They can be considered as equivalent to ACDP Hazard group 1. It is difficult to envisage how any of the cloned genes could increase the virulence of these strains. Therefore no additional hazards have been identified beyond those associated with the cloned gene products themselves.

(ii) E. coli BL21 and derivatives: Stemming from a HSE-commissioned study, ACGM Newsletter 30 (Nov. 2001) states (in para 3) that "BL21 can be considered broadly equivalent to K12 strains and that in most cases work which uses this host can be considered as a class 1 activity……, but the cloning of a bacterial pathogenicity determinant into BL21 will need careful consideration and my in some cases warrant classification as class 2". Since this project involves cloning genes from ACDP Hazard group 2 pathogens, their expression in BL21 will require CL2. Use of expression vectors dependant on the T7 RNA polymerase will greatly minimise the likelihood of expression in the very unlikely event of transfer to other hosts.

(iii) The 'A/E bacterial' hosts that will be used are either non pathogenic for humans or ACDP Hazard group 2 pathogens. Since only genes from very closely strains of the same species, or the very closely related ACDP Hazard Group 1 species C. rodentium, will be introduced into these hosts. Therefore, no additional risks above and beyond those involved in handling ACDP Hazard group 2 pathogens have been identified.

(iv) Yeast hosts: Only non-pathogenic yeast species as defined by the Compendium of Guidance (Part 2, Annex II, para 7) will be used. It is diffic ult to envisage how any of the cloned genes could, in isolation, confer these species with pathogenic traits. Therefore no additional hazards have been identified beyond those associated with the cloned gene products themselves.

(v) Mammalian hosts: Cultured mammalian cells are highly unstable and are rapidly inactivated in the absence of controlled laboratory conditions and the transfected genes will not alter this susceptibility. Therefore no additional hazards have been identified beyond those associated with the cloned gene products themselves.

(vi) Other GMOs: Genetically attenuated strains of Shigella flexneri, Yersinia pseudotuberculosis, Y. enterocolitica and Salmonella typhimurium will be used in some experiments, but not as hosts for cloned EPEC genes. The hazards associated with handling these strains are no greater (indeed significantly less) than those associated with handling the wild-type parent strains, which are ACDP hazard group 2 pathogens.

Host/vector system

Various hosts will be used, as described in section 7 above. Most of the vectors to be used are standard vectors with a long history of safe use in the laboratory. These include non-mobilisable or mobilisation-defective E. coli vectors such as pSK (bluescript), pACYC184, pLG339, pBR332 and derivatives including, for example, the pET expression vector series. The non-mobilisable yeast vector pREP-1 and standard nonmobilisable or mobilisation defective mammalian expression vectors such as pcDNA-3. Drug resistance markers used in these vectors are already widely distributed in nature. Moreover, the use of nonmobilisable or mobilisation defective vectors minimised likelihood of transfer to other species.

Some experiments will employ the non-self transmissible but mobilisable 'suicide' vectors pCVD442, pKNG101, and pTnMod-RCm. These vectors will be maintained only
in disabled E. coli K12 hosts containing a chromosomally-inserted Lambda gene, which is required for replication of these plasmids. These suicide vectors are used to construct mutants in 'target' strains to which they are mobilised by transfer functions encoded by chromosomally-inserted genes in the donor, disabled, E. coli K12 host. Thus, in the very unlikely event of unintended mobilisation to a different strain or species these vectors would be incapable of replicating in most cases. In the even more unlikely event of unintended mobilisation to another E. coli strain already carrying a Lambda phage actively expressing the gene that permits plasmid replication, it is difficult to envisage any detrimental effects arising from expression of the cloned genes in isolation from other 'A/E bacterial' genes. Moreover, horizontal transfer of genes between different E. coli strains already occurs in nature.

### Origin & function

| (i) | Cloning, expressing, and manipulating 'A/E bacterial' genes in disabled E. coli K12 strains. |
| (ii) | Expressing defined 'A/E bacterial' genes in E. coli BL21 or BL21-derived strains, from standard vectors where the cloned gene is expressed from a phage T7 promoter only upon IPTG induction of a chromosomally inserted T7 RNA polymerase gene. |
| (iii) | Expressing defined 'A/E bacterial' genes in the non-pathogenic yeast hosts Saccharomyces cerevisiae or Schizosaccharomyces pombe. |
| (iv) | Use of standard transposon (Tn) mutagenesis procedures to construct Tn mutant libraries of 'A/E bacterial' strains and screening these to identify potentially novel factors involved in interactions with mammalian cells (see below). |
| (v) | Use of suicide plasmid vectors to construct genetically defined mutants of 'A/E bacterial' strains by either gene inactivation or allelic replacement. |
| (vi) | Complementation of defined mutants of the 'A/E bacterial' strains by reintroducing the corresponding wild-type gene on mobilisation-defective vectors. Some experiments may express defined genes in the wild-type 'A/E bacterial' strains, to examined multicopy effects. |
| (vii) | To explore potential strain-specificities of particular interactions, complementation of mutant strains may include corresponding genes from the various 'A/E bacterial' strains, as well as hybrid genes encoding proteins with domains from different 'A/E bacterial' strains. |
| (viii) | Examining the effects of mutant strains and purified gene products on standard cultured mammalian cells, such as HeLa, CaCo-2, T84, HEK293, and J774 cells. Mutant mammalian cells with defined defects in particular pathways will be used in some experiments. |
| (ix) | Transfecting mammalian cells with defined plasmids transiently expressing defined EPEC or mammalian proteins, or defined mutants of these. |
| (x) | For comparative purposes only, some experiments may examine the effects of the ACDP Hazard group 2 pathogens Shigella flexneri, Yersinia pseudotuberculosis, Y. enterocolitica and Salmonella typhimurium SL1344 in mammalian cells. These experiments will employ mutant strains that were attenuated by Drs P Sansonetti (Pasteur Inst, France), H. Wolf-Watz (Umea, Sweden), G. Cornelis (Brussels, Belgium) and B. Finlay (Vancouver, Canada), using genetic manipulation to inactivate key virulence genes. Consequently, these GMOs are also included in this risk assessment. |

### Evaluation of foreseeable effects

1. **Risk of human health**
   Most of the GMMs will be incapable of causing harm to animals, either because they will be unable to survive outside the laboratory and/or are non-pathogenic for animals. However some of the GMMs constructed in 'A/E bacterial' hosts may be capable of inducing A.E lesions and diarrhoea in humans. The 'A/E bacterial' hosts that will be used are either non pathogenic for humans or ACDP Hazard group 2 pathogens. Moreover, giventh requirement for the concerted action of many different 'A/E bacterial' genes products in disease (Deng et al., 2004 PNAS 101:3579) it is difficult to envisage how any of the cloned genes could significantly increase the virulence of the GMMs above that associated with wild-type human-specific strains, which are highly prevalent in nature. Therefore, the hazards associated with these GMOs will be the same as those associated with corresponding wild-type 'A/E bacteria' strains.

2. **Environmental Harm**
   GMMs constructed in disabled E. coli K12 strains, E. coli BL21-derived strains (which can be considered broadly equivalent to K12 strains) and mammalian cell hosts are very unlikely to survive for long outside the laboratory and will be unable to establish, disseminate and/or replace other organisms to any significant extent. In cases where large quantities of a particular gene product will be expressed to facilitate protein purification, the likelihood of unusually large quantities being delivered to a target tissue will be minimised by the pET expression vector system that will be used (ie expression from a phage T7 promoter in non-colonising BL-21 derived hosts that require the addition of IPTG to induce the chromosomally-inserted T7 RNA pol gene required for high level expression of the cloned gene product). GMMs constructed in non-pathogenic yeast hosts or in 'A/E bacterial' hosts could survive in the unlikely event of escape from the laboratory. However, it is difficult to envisage how any of the cloned gene products could increase the virulence of these hosts, or confer novel virulence traits, and it is equally difficult to envisage how any of the cloned gene products could increase the fitness of these hosts and their abilities to compete with or displace other organisms. While some of the gene products to be expressed might have...
detrimental effects if they are delivered in sufficient quantities to mammalian cells/tissues or other eukaryotic cells, but the biological containment measures noted will minimise the likelihood of any hazard arising and therefore the provisionally assigned containment level 2 will be sufficient to contain the risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
only the bacterial, yeast or mammalian cell lines described in section 7 will be used as hosts.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All work will be conducted in accordance with the requirements for containment level 1, as described in the Compendium of Guidance 2000, Part 3A Section 1.

All contaminated waste, disposable plastics, laboratory glassware, or other contaminated materials, including waste destined for incineration, will be autoclaved at 121 degrees C for at least 30 min to inactivate GMMs (100% kill) prior to disposal or cleaning and recycling. Autoclaves, available within the building the work is taking place, will be validated by annual thermocouple mapping and each run will be monitored by continuous recording of the temperature/time profile.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The University Genetic Modification Committee has approved this GM risk assessment.

Project Containment

<table>
<thead>
<tr>
<th></th>
<th>Laboratory Activities</th>
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</tr>
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Project Ref  540/04.4

Date Ackn'd  02/03/2022  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4
The Regulation of Flagellar Assembly in Bacteria

Date Project Ceased

26/10/2004

Class 2 1-50 Litres

Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

This project will involve the study of the regulation of protein secretion by regulatory factors during flagellar assembly in S. typhimurium. The project will involve the use DNA recombinant technologies to create targeted deletions, insertions and point mutations directly on the bacterial chromosome.

Recipient or parental organism

The organisms to be used in this study are Salmonella enterica a ACDP Hazard group 2 organism, Escherichia coli K12 strains which are equivalent to ACDP hazard group 1 and are in the ACGM ‘disabled’ hosts category. The majority of work to be performed will use wild-type strains of S. enterica serovar Typhimurium including LT2, SL1344 and 14028s. Although these strains were originally natural isolates, they have been maintained and passaged in the laboratory for many decades and are likely to have adapted to laboratory conditions. Moreover, LT2 is known to be an attenuated strain of S. typhimurium attributed to a mutation in the stationary phase sigma factor o (-5) (Wilmes-Riesenberg, Foster and Curtis (1997) Inf. And Imm. V 65 p 203-210). Nevertheless, LT2 is formally classified as an ACDP Hazard Group 2 organism along with SL1344 and 14028s. However, taking into consideration the high dose required for S. typhimurium to cause symptomatic infections, the hazard risks associated with the strains LT2, SL1344 and 14028s are considered to be low.

Host/vector system

Mobilisable suicide vectors derived from the R6K vector are dependent upon the RP4 mob functions encoded on the chromosome of E. coli strain SM10 pir, these vectors are unable to replicate in recipient cells which do not provide this essential protein, or be mobilised if the recipient cell is unable to provide the RP4 transfer functions. Examples of mobilisable vectors to be used in this study are derivatives of the vectors pACYC184, pSC101, pBR322, R6K. Other vectors to be used such as pUC19 and pET28 are non-mobilisable requiring manipulation to achieve transfer to other strains. Chromosomally located genetic markers will be mobilised using bacteriophage P22-mediated transduction. The bacteriophage P22 derivative HT105 int201 packs at a higher frequency than wild-type P22, chromosomal DNA allowing for the transfer of selectable genetic markers by transduction into other strain backgrounds. No hazard to human health has been identified in using any of these vector systems or P22 mediated transduction.

Origin & function

The work during this project will involve the genetic manipulation of genes of interest on the bacterial chromosome. The majority of genes to be worked with are to be found in S. typhimurium LT2. Introduction of the reporter genes such as lacZYA from E. coli and the lux operon from P. luminescens will be used to monitor gene expression.
The lacZYA genes will be introduced into S. typhimurium strains using the Mu derived transposons MudJ and MudK while the lux operon will be introduced using a transposon system derived from the transposon Tn5. Antibiotic markers to be used during this study are all widely used in microbiology laboratories world-wide and are all derived from naturally occurring sources. The recombinant in vitro generated sequences to be used are also found naturally in a number of prokaryotes and are routinely used in DNA recombinant technology to overexpress proteins to high levels. During this study these sequences will be used to "tag" proteins of interest on the bacterial chromosome and purify these proteins from S. typhimurium LT2 under normal expression conditions avoiding overexpression. These reporter genes, antibiotic markers and recombinant sequences are widely used and no role in pathogenicity of micro-organisms has been established, leading to the conclusion that there associated hazards are low.

Evaluation of foreseeable effects

The worst case scenario, in the event of a laboratory infection of Salmonella enterica serovar Typhimurium, strains SL1344 and 14028s, is a self-limiting gastroenteritis. The individual will have diarrhoea, vomiting and a slight fever for 3-5 days. However infection will only result with the ingestion of the bacteria in excess of 10 (to the power of 6) bacteria. Full recovery would be expected. Strain LT2 is known to be an attenuated strain of S. enterica serovar Typhimurium. Therefore infection is highly unlikely and if it was to occur an extremely high dose, greater than that required for strains SL1344 and 14028s, would be required for any symptoms to develop.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work will be conducted in accordance with the requirements for containment level 2, as described in the Compendium of Guidance 2000, Part 3A section 1. All contaminated glassware and waste including any waste destined for incineration, will be completely inactivated by autoclaving (100% Kill) for at least 20 minutes at 121°C at full steam penetration. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The university Genetic Modification Committee has approved this GM Risk Assessment:
Local GM reference: GM 04/08

Project Containment

<table>
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02/03/2022
The aim of the project is to investigate trace metal ions homeostasis in the pathogenic fungus Cryptococcus neoformans. Trace metals are linked to capsule and melanin production, which are essential for the survival of this organism within humans. The sensing and acquisition of trace metals must therefore be a fundamental aspect of the ability of C. neoformans to cause infection. These studies will define metal homeostasis pathways in C. neoformans so that their role in the pathogens interaction with the host can be determined.

Cryptococcus neoformans (serotypes A and D) will be the recipient organisms that are classified as pathogens in ACDP hazard group 2. C. neoformans is a free living saprophytic yeast that has been isolated from a variety of environmental sources such as the soil, avian excreta and decaying wood. The life cycle of this fungus does not require human infection and is not a commensal organism. It can, however, cause serious disease in immuno-compromised individuals such as those with AIDS and patients receiving steroids or organ transplant therapies.
DNA sequences will be introduced into C. neoformans using the standard techniques of plasmid transformation or conjunct using the bacterium A. tumefaciens. C. neoformans cDNA will also be expressed in E.coli for protein purification purposes. All vectors are well characterised, widely used and contain standard antibiotic resistance markers. These include mobilisable defective C. neoformans vectors (pCnTel1, pM8), non-mobilisable S. cerevisiae vectors (pRS series) and non-mobilisable or mobilisable defective E.coli vectors (pBluescript, pUC and pET series).

Origin & function

Candidate genes within the C. neoformans genome will be mutated, tagged and/or deleted to determine the role of these genes in metal ion homeostasis. Genes will be tagged with various well characterised epitopes (eg. FLAG, GFP) to facilitate cellular localisation, detection via western blotting and isolation using immunoprecipitation techniques. The deletion of C. neoformans genes and the introduction of epitope coding sequences will involve the introduction of widely used dominant antibiotic resistance markers such as the nourseothricin and neomycin resistance cassettes. Mutated C. neoformans genes will be generated by amplifying and ligating the relevant sequences into E.coli vectors so that they can be modified before being reintroduced into the C. neoformans genome. Tagged and mutated genes will usually be expressed from their native promoters and occasionally from a constitutive promoter (eg. ADH promoter). Tagged and mutated C. neoformans genes will usually be integrated into the C. neoformans genome but will sometimes be expressed from a self-replicating plasmid. Reporter genes (such as lacZ) will also be introduced into the C. neoformans genome to analyse metal dependent gene regulation. In some cases, the ability of the C. neoformans genes to complement homologous genes in the yeast S. cerevisiae will be tested by expressing those genes in S. cerevisiae using standard yeast expression vectors.

Evaluation of foreseeable effects

Human infection occurs by inhalation of aerosolised particles from environmental sources. In healthy individuals, the resulting pulmonary infection initiates an inflammatory response that eliminates the yeast. If the pulmonary infection cannot be contained, significant disease can occur with dissemination of the yeast throughout the body. Infection of the central nervous system results in meningoencephalitis that is fatal if not treated. The anti-fungal drugs, amphotericin B, flucytosine and fluconazole are recognised effective treatments for cryptococcosis. The only documented cases of human to human transmission have involved organ transplantation with infected tissues.

The genetic manipulation of C. neoformans will involve the disruption of individual genes and the integration of coding sequences that allow for the identification and visualisation of proteins. It is highly unlikely that these integration events will generate a variant with increased virulence. Gene disruptions are likely to have a negative influence on the trafficking of metal ions and therefore on those virulence factors that depend on metal enzymes. In addition, any imbalance of trace metal metabolism will likely result in a reduction in the fitness of C. neoformans. The majority of experiments will be carried out using genetically disabled strains that will be unable to survive for long periods outside the laboratory. In addition, as with many laboratory-cultured organisms, C. neoformans becomes attenuated for virulence by laboratory growth. In any event, the accidental release of a non-disabled strain from a laboratory would not be predicted to add to the level of C. neoformans that humans are exposed to from the environment.

This study will also use genetically disabled strains of the yeast S. cerevisiae and the bacteria E.coli and A. tumefaciens to express and amplify C. neoformans DNA. These are non-pathogenic organisms and are unable to colonise humans. The pathogenicity of C. neoformans is a ploygenic trait so that the expression of a single C. neoformans gene is not going to convert to S. cerevisiae or E.coli into pathogenic organisms. In addition, C. neoformans genes will only be expressed using mobilisation defective plasmids.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work will be carried out at containment level 2. C. neoformans strains will be maintained as non-desiccated haploid yeast. Any procedures that may result in aerosols will be carried out using sealed containers or carried out in designated Class II safety cabinets.
All materials contaminated with GMM's, including liquid waste, solid waste, glassware, plastic ware and sharps in CinBins will be autoclaved. Autoclaved sharps in CinBins will then be incinerated. Autoclaving involves the GMM's contaminated waste being subjected to 121°C for 30 min, which kills 100% of the contaminating organisms. The autoclaves are validated by annual thermocouple mapping and each run is monitored by continuous chart recording of the temperature/time profile. Following sterilisation, normal routes of disposal are used for liquid and solid wastes. Liquid waste is discarded in the sink and solid waste by CFA processing.

Accidental spills of GMM's will be treated with Virkon (peroxycetic acid) (freshly diluted 1 in 50), wiped up and retreated with Virkon. Contaminated material will be autoclaved. Virkon will be used for routine cleaning of bench tops and laboratory equipment.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

The University Genetic Modification Safety Committee has approved the GM Risk Assessment.

Local GM reference: GM 06/03

Project Title: Analysis of the role of trace metal ion homeostasis genes in the virulence of Cryptococcus neoformans using gene disruption and modification.

Project Containment

<table>
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<th>Glass Houses</th>
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Project Ref 540/07.1

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<td>28/03/2007</td>
<td>Rescue of live virus from an infectious clone of Hepatitis C virus J6/JFH1 and</td>
<td>Class 3</td>
<td>&lt; 1 Litre</td>
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02/03/2022
characterisation of the virus, virus infected cells and products.

We intend to study the assembly of the J6/JFH hepatitis C virus in cell culture, to investigate the interaction of virus and host cell lipoprotein synthesis and to assess the influence of lipid modulating agents on the infectious process.

Huh7.5 cells. This cell line was derived from the human hepatoma cell line Huh7 following transfection with an HCV replicon. A clone supporting high level replicon replication, Huh7.5, was selected. The replicon was cleared from the cells by interferon treatment (Blight, K.J., McKeating, J.A. and Rice, C.M. 2002, J Virol 76, 13001-14).

Recipient or parental organism

Recipient or parental organism

pFLHCV J6/JFH1 is a pUC19 vector bearing a DNA copy of the J6/JFH1 recombinant hepatitis C virus as an insert (Kato et al.,2003 Gastroenterology 125. 1808; Lindenbach et al., 2005 Science, 309, 623)

Host/vector system

The recombinant pFL J6/JFH1 was produced by Lindenbach et al (Science. 2005;309(5734):623-6). It is a pUC19 plasmid which contains as an insert a DNA copy of a full length copy of HCV hepatitis C virus (HCV) genome which is a hybrid containing the non-structural protein genes of the HCV JFH1 strain and the structural protein genes of HCV J6 inserted downstream of a T7 promoter. JFH1 is the only known HCV strain capable of producing infectious progeny virus in human cells in culture. J6 is capable of replication in Huh7.5 cells but does not produce infectious progeny virus.

Origin & function

The recombinant pFL J6/JFH1 was produced by Lindenbach et al (Science. 2005;309(5734):623-6). It is a pUC19 plasmid which contains as an insert a DNA copy of a full length copy of HCV hepatitis C virus (HCV) genome which is a hybrid containing the non-structural protein genes of the HCV JFH1 strain and the structural protein genes of HCV J6 inserted downstream of a T7 promoter. JFH1 is the only known HCV strain capable of producing infectious progeny virus in human cells in culture. J6 is capable of replication in Huh7.5 cells but does not produce infectious progeny virus.

The three plasmids are identical except that in pFL-J6/JFH(GND) a point mutation has been introduced changing amino acid 2476 from H to L. This is an “adaptive mutation” which increases colony formation when the plasmid is transfected into cells.

HCV has a single stranded positive sense RNA genome and the pFL J6/JFH1 DNA plasmid is not capable of generating infectious virus in the absence of a T7 polymerase, to transcribe an RNA copy, and a mechanism of cutting the transcribed RNA precisely at the 3’ end of the HCV genome. However an in vitro generated RNA copy of the cDNA insert, cut out of the plasmid with the endonuclease Xba-1 followed by mung bean nuclease to generate the correct 3’ end and transfected into Huh7.5 cells, has
been shown to replicate and the cells release infectious, recombinant HCV (Kato et al., 2003, Gastroenterology125, 1808). We intend, therefore, to generate RNA copies of the virus genome in this manner and transfect them into Huh7.5 cells in order to generate a replicating system in order to study virus assembly and interaction with the host lipoprotein export system.

Evaluation of foreseeable effects

An RNA copy of the hybrid virus genome has been shown to replicate well when transfected into Huh7.5 cells. Huh7.5 cells are the only cell type known to support the replication of this virus in culture. The recombinant virus has been inoculated into chimpanzees and SCID mice and produces infections which are similar to those produced by wild type virus strains (Lindenbach et al. PNAS. 2006 103, 3805-9). In these experiments the virus has exhibited neither attenuation nor enhanced virulence and consequently the risks are similar to those associated with the wild-type virus, which is an ACDP hazard group 3 organism. The pFL-J6/JFH (GND) and pFL-J6/JFH (H2476) products are each attenuated by point single mutations, which could revert to produce a wild-type phenotype. The risks associated with such reversions would be similar to those associated with the wild-type virus.

Hepatitis C virus is a blood borne pathogen and the risks of infection have been intensively studied and are well characterised. We have established and approved protocols for the handling of hepatitis C virus in containment level 3. The replication of this recombinant clone in cell cultures presents no additional risk. The maximum titres expected from published studies with J6/JFH1 ((Lindenbach et al. PNAS. 2006 103, 3805-9). are one thousand-fold lower than the wild type material we routinely handle (Nielsen et al., 2004, Journal of General Virology 85, 1497-1507).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The project (risk assessment section 2.1) requires the determination of the density of cell culture derived J6/JFH HCV by density gradient ultracentrifugation. The containment level 3 laboratory does not contain an ultracentrifuge and is not equipped with the necessary power supply to run one. An ultracentrifuge is available in the adjacent containment level 2 virology laboratory. We request a derogation to allow ultracentrifugation to be carried out in this machine. The machine will be restricted for HCV use for the duration of this project. A label indicating that the use of the machine is restricted for HCV work will be posted on the machine and staff using the laboratory, all of whom work under the jurisdiction of will be acquainted with the restriction.

Material to be centrifuged will be loaded into sealed plastic centrifuge tubes and loaded into a dedicated sealed rotor within the microbiological safety cabinet within the containment level 3 laboratory. The sealed rotor will be disinfected with 1% Virkon prior to removal from the cabinet. The disinfected ultracentrifuge rotor will be placed into a lidded metal box for transport to and from the ultracentrifuge and under no circumstances will the rotor be opened outside of the microbiological safety cabinet in the containment level 3 laboratory. After centrifugation the rotors will be washed in 1% Virkon and will be housed within the category three laboratory at all times. The centrifuge chamber will be swabbed with 1% Virkon after each use.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The waste generated will be contaminated plasticware, paper, gloves and other soft discard which will be accumulated in Cin Bins. When full the bins will be closed but not sealed and transferred into an en suite autoclave and killed off. On occasion, use will be made of small quantities of disinfectant (1% Virkon) for swabbing surfaces. In all such cases the paper towels used will then be autoclaved. Waste disinfectant and container will also be autoclaved.

All waste will be inactivated by en suite autoclaving (100% kill) prior to disposal by incineration. The autoclave will be validated by annual thermocouple mapping and each run will be monitored by continuous chart recording of the temperature/time profile and the records retained for inspection.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
The University GM Safety Committee has approved the GM Risk Assessment:

Local GM reference: GM 06/42.

Principal Investigator:

Project Title: Rescue of live virus from an infectious clone of Hepatitis C virus J6/JFH1 and characterisation of the virus, virus infected cells and products.

### Project Containment

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### Project Ref 540/07.2

**Date Ackn'd** 15/05/2007

**CU2 Project Title**

The Use of Herpes Virus Saimiri vectors to investigate the role of HF-kB transcription factor family in the pathogenesis of liver diseases in vitro and in vivo.

**Date Project Ceased** 26/02/2016

**Class**

- Class 2

**CultureVolClass2**

- < 1 Litre

**Consent Granted**

- Not Applicable

**Project notified under transitional arrangements**

- N
**Project Additional Information**

**Purposes of the contained use**

We intend to use the Herpes Virus Saimiri as a vector system to deliver protein components of a family of proteins called NF-κB to investigate the functions of these proteins in the development of inflammation driven chronic liver disease.

**Recipient or parental organism**

Disabled E. coli K12 will be used in cloning work to generate the necessary viruses. Viruses will be used on human, rat and mouse primary liver myofibroblasts as well as on the human cell lines LX2 and Cos. In addition, the viruses generated will be used on fibroblastic cell lines of mouse and human origin. In addition, the virus will be used to infect mice to study the effects on liver disease using in vivo models. Control C57/Black 6 strain male mice as well as various mice that have knockouts of the various NF-κB family members (supplied by Birmingham University and JAX lab, Maine, USA) will be used in these experiments.

**Host/vector system**

Herpes Saimiri virus strain A11-S4 – this infects squirrel monkeys persistently without causing disease. (Griffiths et al Current Gene Therapy, 2006, 6, 1-15)

**Origin & function**

NF-κB proteins come in 5 different forms; namely p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2). These proteins bind in different combinations to gene promoters and regulate gene transcription. They are often bound in the cytoplasm by inhibitory proteins known as inhibitors of NF-κB or IκBs. There are seven IκB proteins namely IκBa, IκBβ, IκBγ, IκBε, Bcl-3 and the precursor proteins p100 and p105. NF-κB dimers are released from IκB proteins when the latter are phosphorylated by the actions of the Inhibitor of NF-κB Kinases (or IKKs). There are three IKKs namely IKKa (IKK1), IKKβ (IKK2) and NEMO (IKKy). For a general review of the NF-κB pathway please see Hayden and Ghosh 2004, Genes and Development, 18, 2195-2224.

The human cDNAs for the various NF-κB family members along with functionally inactive mutants (with inert tags such as FLAG, green fluorescent protein (GFP) or hemagglutinin (HA) epitope tag added for identification) will be inserted into Herpes Virus Saimiri using a Bacterial Artificial Chromosome (BAC) system. Examples of these will include but not be restricted to FLAG tagged p50 in addition to a mutated form of p50 (where residues 267, 269, 308, 310 are all mutated to alanine) that does not bind DNA in addition to GFP-tagged p65 and various mutated forms where serine residues have been mutated to alanine thereby restricting the ability of these proteins to activate gene transcription. These different forms of the proteins will enable us to dissect out subtle regulatory pathways that would otherwise be impossible to study.

The HA tag used consists of 9 amino acids of the much larger hemagglutinin protein (containing more than 550 amino acids) from the influenza virus. This epitope is highly unlikely to have the biological activity of the full length protein and is routinely used by researchers around the world for the purpose of identifying and tracking proteins. We, therefore, do not envisage that the addition of this epitope will alter the biological properties of the virus in any way and the risk assessment below will not, therefore, make any special reference to this.

**Evaluation of foreseeable effects**

The inserted genes will encode for the human forms of the various NF-κB family proteins and their inhibitors listed above. In addition, functionally inactive forms of the proteins will be used as controls. We do not expect these genes to fundamentally alter the biology of the attenuated virus strain A11-S4. It is difficult to be precise about the properties of the final genetically modified viruses that will be produced. However, what is known is that p50, for example, has both anti-inflammatory and pro-inflammatory properties (Bonizzi and Karin 2004, Trends in Immunol, 25, 280-288) and there is no evidence that it can act as a proto-oncogene (Karin 2006, Nature, 441, 431-436). We, therefore, cannot accurately predict the overall effects of an attenuated virus strain A11-S4 carrying the p50 protein. The attenuated virus strain A11-S4 carrying the mutated form of p50 should be inactive. On the other hand the attenuated virus strain A11-S4 carrying the p65 form of NF-κB may have the opposite effect and act to
promote the production of various inflammatory genes. However, the various mutants of p65 that we plan to use should have attenuated effects. It is impossible to predict in
detail what the biological properties of viruses carrying all the different members of the NF-κB family will be as the exact functions of many of the subunits and inhibitors in
different cell types has not been studied in detail. Indeed this is the aim of the current project in the context of liver fibrosis. In terms of the in vivo arm of the project, the
exact forseeable effects are again difficult to define in detail. Although NF-κB subunits are frequently increased in many cancers none of the NF-κB subunits are known to
be proto-oncogenes per se (Karin 2006, Nature, 441, 431-436). Over expression of the p50 subunit may lead to defects in the production of pro-inflammatory cytokines
such as TNF-α and this may lead to immunosuppression of the animal. On the other hand, over expression of the p65 subunit may have the opposite effect and lead to
increased inflammation. Ikβα over expression may lead to general inhibition of NF κB in the animal, which again may be anti-inflammatory. However, the effects in terms of
morbidity and mortality of the animals with each of the subunits and inhibitors and IKKs are impossible to predict. Of note mice infected with the virus expressing luciferase
in the Smith et al 2005 study were Nude mice that were naturally immunosuppressed. These mice were alive and well at 10 weeks when the study was terminated. We,
therefore, do not anticipate any major problems from the over expression of NF-κB family members or for that matter the mutated forms (which should be inert). However,
all animals infected with the virus will be carefully monitored for signs of toxicity and animals will be euthanized if there are significant

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Animals will be maintained in accordance with the Codes of Practice under the Animals (Scientific Procedures) Act 1986 which equate at least to containment A as

Mice will be maintained in closed cages in rooms with self-closing doors. Access to the facility is via two swipe card and key pad controlled doors. The doors are all flush to
the floor. The floor drains are screwed down to the floor and have very small drainage holes, much too small for a mouse. The mice are all checked daily and records are
kept of the numbers in each cage.

Animals infected with GM virus will be stored and handled only inside Containment Level 2 animal room and laboratory suite in the animal facility. Isolators will be used for
additional safety. The animal facility is isolated from the rest of the university and access is by card and keypad. Rooms will be maintained at negative pressure relative to
the surroundings.

All personnel dealing with the animals will be required to wear protective clothing including but not limited to lab coats, gloves and removable footwear. They will also be
expected to have read and understood any risk assessments pertaining to this project.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All contaminated materials including animal waste and carcasses and any waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of
waste or cleaning and recycling of reusable laboratory equipment, such as glassware. All waste material will be transported in robust leak proof containers. Autoclaves will
be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6
emulating indicator 121ºC for 20 min). Records will be kept.

Is an emergency plan required according to regulation 20?  

<table>
<thead>
<tr>
<th>No</th>
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Tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

<table>
<thead>
<tr>
<th>No</th>
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</table>
The University GM Safety Committee have approved these two GM Risk Assessments:

Local GM reference: GM 06/44
Principal Investigator:
Project Title: The Use of a Herpes Virus Saimiri Vector to Investigate the Role of NF-κB Subunits in Liver Disease.
Principal Investigator:
Project Title: The Use of a Herpes Virus Saimiri Vectors to Investigate the Role of the NF-κB Subunits in Liver Disease In Vivo Using Mouse Models.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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- **Animal Units**
  - L2 Yes
  - L3 L4
  - L2 L3 L4

- **Large Scale Activities**
  - L2
  - L3 L4
  - L2

- **Human Clinical Applications**
  - L2
  - L3 L4
  - L2

### Project Ref 540/08.1

- **Date Ackn'd**: 15/02/2008
- **CU2 Project Title**: Deletion of stress response genes in Salmonella enterica serovar Typhimurium strains LT2, SL1344 and SL 14028s.
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: No

### Project Additional Information

- **Withdrawn**: No
- **Tick if notifying a connected programme of work**: No

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

02/03/2022
Purposes of the contained use

Deletion of stress response genes in ACDP Hazard group 2 organisms Salmonella enterica serovar Typhimurium strains LT2, SL1 344 and SL14028s. After passage in the laboratory for many years these strains have mutations that adapt them to these in vitro conditions and the risk for any hazardous effects is considered low.

Recipient or parental organism

The organisms used and generated here are the enteric bacterial strains Salmonella enterica serovar Typhimurium strains LT2 s [Wilmes-Riesenberg, Foster and Curtis (1997) Inf. and 1mm. v. 65 p. 203-210], SL1344 and SL14028s. Both the parental strains used and the derivatives thereof can be considered non-hazardous to humans and to the environment.

Host/vector system

The hosts organisms used in this study are the Salmonella enterica serovar Typhimurium strains LT2, SL1344 and SL14028s. Vectors to be used are derivatives of the plasmid vectors pACYC184, pSCI 01, pBR322, R1 and R6K. Other vectors to be used such as pUC19 and pET28 are non-mobilisable plasmids requiring manipulation to achieve transfer to other strains. Chromosomally located genetic markers will be mobilised using bacteriophage P22-mediated transduction. No hazard to human health has been identified in using any of these vector systems or P22 mediated transduction.

Origin & function

The organisms to be used in this study are Salmonella enterica a ACDP Hazard group 2 organism, Escherichia coli K-12 strains which are equivalent to ACDP hazard group 1 and are in the ACGM “disabled” hosts category. The majority of work to be performed will use wild-type strain S. enterica serovar Typhimurium including LT2, SL1344 and 14028s. Although these strains were originally natural isolates, they have been maintained and passaged in the laboratory for many decades and are likely to have adapted to laboratory conditions. Moreover, LT2 is known to be an attenuated strain of S. typhimurium used widely in bacterial genetic studies. The attenuation of LT2 has been attributed to a mutation in the stationary phase sigma factor (Sigma 5) (Wilmes-Riesenberg, Foster and Curtis (1997) Inf, and 1mm. v. 65 p. 203-210). Nevertheless, LT2 is formally classified as an ACDP Hazard group 2 organism along with 1344 and 14028s.

The intention is to synthesize with PCR chromosomal DNA fragments and combine the DNA fragment with a DNA fragment that encodes for a resistance gene such as aphA or cat, such that the assembled DNA fragments can be integrated into the chromosomes of the wild-type strain S. enterica serovar Typhimurium including LT2, 5L1344 and 14028s by direct selection. Furthermore, the DNA fragments contain a recognition sequence of the FLP site-specific DNA recombinase that allows for, by site-specific recombination, the deletion of the resistance cassette, as devised by Datsenko and Wanner (Proc. Nati. Acad. Sci. U S A. 97, 6640-5, 2000). After deletion of the relevant genes, such as ppk, ppx, relA, spoT, relBE, dinJ yafQ, yefM yoeB, priF yhvA, hicAB, mazEF or chpB, the resulting strains will be analysed for changes in physiological traits, such as levels of translational errors (Kramer EB, Farabaugh PJ. RNA 13: 87-96, 2007) and levels of carbonylated proteins (Oncor Oxyblotting method).

Evaluation of foreseeable effects

Salmonella enterica serovar Typhimurium host strains LT2, SL1344 and SL14028s have been approved as ACDP Hazard group 2 organisms and will be handled in accordance with the requirements for containment level 2 as described in the SACGM Compendium of Guidance 2007. The derivatives of the host strains constructed in this project will carry deletions of genes coding for stress response elements. These stress response elements are thought to help the cells cope with environmental stress. Therefore, the deletion of the corresponding genes are likely to debilitate further the survival of the bacterial cells in the environment. Therefore, we foresee that the mutations constructed in this project will reduce the potential health or environmental hazards connected with the use of the above-mentioned host strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work will be conducted in accordance with the requirements for containment level 2 as described in the SACGM Compendium of Guidance 2007.
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

EThe University GM Safety Committee has approved the GM Risk Assessment:
Local GM reference: GM 07/27.
Principal Investigator
Project Title: Deletion of stress response genes in Salmonella enterica serovar Typhimurium strains LT2, SL1 344 and SL14028s.

Project Containment

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Project Ref 540/08.2

Date Ackn'd 15/02/2008

CU2 Project Title Differentiation of primary human stem cell lines.

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4
The purpose of this project is to obtain a series of cell lines from various organs, in particular the developing nervous system. These lines will be used to identify which genes and gene products are required to promote differentiation into an adult cell phenotype. Ultimately these cell lines will be used in developing strategies for the development of stem cell therapies for CNS disorders and additionally so that we can study the effects of various environmental and endogenous chemicals in the development of neuropsychiatric disease. We are therefore aiming to isolate ventral midbrain (mesencephalon), cortical plate (early forebrain), early hippocampus (memory in the adult), hindbrain, spinal cord, early muscle, liver, heart, spleen, kidney, early lung, skin, and early gonadal tissue.

Whilst these cell lines show a capacity to divide short term, they are only likely to divide a finite number of times and in order to prevent us having to re-derive cell lines we wish to generate cell lines capable of long term division. Transfection of a single gene of human telomerase (bTERT) provides a cell with the capacity to undergo infinite cell divisions without causing the cell to become transformed into a cancerous phenotype. In addition, expression of hTERT provides a level of genomic stability and a capacity to resist cell stress. We will therefore transfect the primary cell lines at low passage number with hTERT under the control of a Tet operon using a disabled lentiviral vector along with the Tet repressor to conditionally immortalise the cell lines. This will allow us to use tetracycline to switch on hTERT expression in stock cells allowing the immortalised phenotype. Removal of tetracycline will switch off hTERT expression reverting the cells to essentially wild type for biochemical study.

In order to determine those factors which control cellular differentiation of stem cells a functional genomic approach will be used. Using siRNA libraries and an ORF library in lentiviral vectors, cells will be transfected and functional and morphological assays used to identify those genes that promote or inhibit differentiation. To achieve this, cells will have been first transfected with viral vectors containing Green Fluorescent Protein (or its derivatives) under the control of cell specific promoters (e.g. CNPase for oligodendrocytes) to allow the cells to be imaged using fluorescence microscopy, or sorted using FACS. These cells will also be used for biochemical and morphological analysis in order to determine how similar they are to adult tissues and the original primary lines. Following the establishment of the cell lines we will then determine the effects of various chemicals on how these cells respond under optimal growth conditions, and under conditions of stress. Cells will be exposed acutely to high levels of specific chemicals and also to low levels over a prolonged period. A variety of the above methods used will then be used to determine how the cells respond to chemical exposure from simple cell survival to determining the levels of specific genes.

Recipient or parental organism

Different primary human stem cell lines derived from early human embryos. Virus will be produced in human 293 cells and its derivatives. The 293FT Cell Line in which the viral particles will be produced is derived from the 293F Cell Line, and stably expresses the SV40 large T antigen. Expression of the SV40 large T antigen is controlled by the human cytomegalovirus (CMV) promoter and is high-level and
constitutive. Studies have demonstrated maximal virus production in human 293 cells expressing SV40 large T antigen (Naldini, L. (1998). Lentiviruses as Gene Transfer Agents for Delivery to Non-dividing Cells. Curr. Opin. Biotechnol. 9, 457-463.). The 293 Cell Line is a permanent line established from primary embryonal human kidney transformed with sheared human adenovirus type 5 DNA (Graham, F. L., et al (1977) Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5. J. Gen. Virol. 36, 59-74). The E1A adenovirus gene is expressed in these cells and participates in transactivation of some viral promoters, allowing these cells to produce very high levels of protein. The 293FT Cell Line is derived from the 293F Cell Line which is a fast growing variant of the 293 cell line, adapted to grow in serum free media.

Host/vector system

The vector system will be restricted to “third generation” HTV-1 based vectors where the structural genes and genes for replication of the virus are placed on separate plasmids for co-transfection or are stably integrated into the packaging cell lines. For most of the work we will use lentiviral HIV-1 derived Virapower vectors based on those developed originally by Naldini and colleagues (Dull, T., Zufferey, R., Kelly, M., Mandel, R. J., Nguyen, M., Trono, D., and Naldini, L. (1998). A Third-Generation Lentivirus Vector with a Conditional Packaging System. J. Virol. 72, 8463-8471.). Other viral vectors with a similar composition will be used as necessary.

The basic pVirapower vectors contain:
- Rous Sarcoma Virus (RSV) enhancer/promoter for Tat-independent production of viral mRNA in the producer cell line (Dull et at, 1998)
- Modified (zU3) HIV-1 5’ and 3’ Long Terminal Repeats (LTR) for viral packaging and reverse transcription of the viral mRNA (Dull et at, 1998)
- HIV-1 psi (NI) packaging sequence for viral packaging
- CMV promoter for high-level expression or the human Ubiquitin promoter, UbC, for constitutive but physiological expression of the gene of interest.
- Two recombination sites, attRI and attR2 downstream of the RSV promoter for recombinational cloning of the gene of interest from a clone
- Chloramphenicol resistance gene (CmR) for counterselection
- The ccdB gene located between the attN. sites for negative selection
- Ampicillin resistance gene for selection in E. coli
- uc origin for high-copy replication of the plasmid in E. coli

Origin & function

hTERT will be obtained from Invitrogen as isolated plasmid DNA of human origin encoding a full length h-TERT cDNA (isoform 1). This will be cloned by Invitrogen to add a—V5 epitope tag for detection purposes by cloning into the viral vector pLenti6.2/V5-DEST or pLenti4/TO/V5-DEST. The hTERT sequence will provide unlimited cell replication on transfer and expression in cells.

The vector pLenti6/TR contains a tandem tetracycline repressor gene (Tet) that binds to tet operator sequences to repress transcription of the gene of interest in the absence of tetracycline (Postle, K., Nguyen, T. T., and Bertrand, K. P. (1984). Nucleotide Sequence of the Repressor Gene of the Tn10 Tetracycline Resistance Determinant. Nuc. Acids Res. 12, 4849-4863.). This will allow control of target gene (i.e. hTERT) expression.

The siRNA library will consist of short 30-50bp small interfering RNA sequences designed to inhibit gene expression, each siRNA species having been designed by computer programs to inhibit a specific gene. These sequences do not encode any proteins but provide a library of sequences designed to inhibit all potential genes in the genome. The siRNA library will be carried in a third generation lentiviral vector.

An ORF library in a lentiviral vector system will be used. Each clone represents a unique full length human coding sequence. As such, given the size of the library of over 5,000 ORFs, it is very likely that human oncogenes will be present in the library. These are however the normal cellular coding sequences that are expressed in a wide variety of cell types in the body, particularly during cell division. The purpose of this library will be to induce differentiation of cells to an adult phenotype by the presence of
the gene. The reporter genes will include green fluorescent protein (GFP) from Aequorea Victoria, and its various derivatives including proteins such as enhanced (EGFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP), normally under the control of a CMV promoter. In certain instances these proteins will be under the control of a specific human gene promoter (for example the tyrosine hydroxylase gene). These fluorescent protein expressing viral stocks will be directly obtained from commercial suppliers such as Invitrogen as a custom service. The purpose of these will be to provide simple non-invasive detection of transfected cells in culture.

**Evaluation of foreseeable effects**

The major routes of transmission are through accidental inoculation via needle stick injury, exposure to open wounds and aerosolisation of high titre virus stocks (SACGM Compendium of Guidance). If delivered by these routes the virus will produce limited infection of cells if sufficiently high titres of virus are delivered.

Transfection with hTERT, the gene for the catalytic subunit of human telomerase, immortalises normal human cells and permits continued cell division. It does not induce a transformed phenotype and does not render cells tumorigenic (Morales et al., 1999 Nature Genetics 21: 115-118). The hTERT gene is expressed naturally in human germ line cells, stem cells, lymphocytes and other tissues. It does not induce genomic instability, and it is not an oncogene. Therefore, hTERT overexpression is not considered to pose a hazard to human health. The V5 epitope is not considered to be functional. The final viral vector will therefore be capable of infecting a wide range of dividing and non-dividing cells and providing continued expression of hTERT. Reporter gene constructs based on green fluorescent protein, (GFP from Aequorea Victoria) and similar safe, non-toxic fluorescent proteins from other organisms (e.g. Discosoma spp. mCherry (Shaner et al Nature Biotechnology 22, 1567 - 1572 (2004)) will be used. Transfection with GFP and other similar proteins is considered safe as there is widespread use of these proteins both in Vitro and in VIVO with no deleterious effects. These will be coupled to genomic fragments coding for the promoter regions of various genes which will provide specific markers of cellular differentiation (e.g tyrosine hydroxylase gene promoter). This will provide a series of viral vectors able to transfect a wide range of cell types and produce fluorescent protein expression only in specific cell types to non-invasively monitor cell differentiation.

The siRNA genomic library is composed of short 30-30mer oligonucleotide sequences, each designed to inhibit a specific human gene. The insertion of these sequences into a lentiviral vector will allow knockdown of a specific gene in a very broad range of dividing and non-dividing cells.

The ORE lentiviral library contains the full length coding sequence of a broad range of genes each single gene under the control of the viral or mammalian promoter contained within a lentiviral vector. As such, given the size of the library of over 5,000 ORFs, it is possible that human oncogenes will be present in the library. These are however the normal cellular coding sequences that are expressed in a wide variety of cell types in the body, particularly during cell division. These clones will deliver a specific gene to the target cell and provide constitutive expression of the ORF.

In summary, all inserted genes code for proteins with known or suspected physiological effects with the exception of the reporter constructs (e.g. GFP). However, pathological or pharmacological effects are neither known nor suspected. The siRNA sequences will reduce the expression of the specific gene that they target. In the event of exposure, the GM virus could gain access to human cells. However, direct consequences to human health of exposure to the virus are low, and the risk of transfer is very low (see SACGM Compendium of Guidance, Part 2 “Risk assessment of genetically modified microorganisms (other than those associated with plants)”, 2.11, and above). The health risks of the inserted genes are expected to be minor and self limiting due to the use of replication incompetent virus (see SACGM compendium of guidance, Section 2.11.7 and 2.11.9). The overall likelihood of hazard is therefore low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**not applicable**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**not applicable**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All work will be carried out at Containment Level 2: All work will be done in accordance with the rules of good microbiological practice and only fully trained and competent individuals will be permitted to work. A “signing off” procedure will be used to monitor staff and their ability to work safely and within the directions of the risk assessment.
and this will be monitored on a regular basis. Production of virus particles will be provided by an external supplier, viral stocks being shipped to Newcastle from the supplier (e.g. Invitrogen). Viral infection of primary cells will be performed at small scale (< 1 o pfh) under sterile conditions in closed plastic tissue culture vessels and all work will be done in a Class 2 microbiological safety cabinet within a Containment Level 2 room, in order to control hazards associated with possible adventitious agents and to avoid contamination of cells. Protection levels provided by the flow cabinet are monitored on a 6 monthly basis in accordance with the appropriate legislation (BSEN 12469:2000). No hypodermic needles will be used. Used “sharps” (pipette tips, serological pipettes) will be collected in a yellow “CinBin” type container placed within the plenum of the MSC immediately after use. The container and all sharps content will be autoclaved prior to disposal by the clinical waste route. The cells will be harvested by protease digestion and centrifugation, and DNA, RNA and protein will be isolated for analysis. Certain transfected cells will be fixed in paraformaldehyde to visualise GFP etc. All waste that is possibly contaminated with virus will be inactivated by incubation with a final concentration of at least 1% Virkon for 30 minutes and all of this waste will be collected in dedicated bags and these transferred to an autoclave using closed containers and will be inactivated by autoclaving at 121°C for 20 mm. This procedure will ensure 100% killing efficiency. Autoclaving will be done at the end of each working day or on completion of work, whichever is the earliest. The temperature and time during each autoclave run will be monitored using indicator strips (TST control integrator, class 6 claiming indicator 121°C for 20 mm, Albert Browne Ltd, UK). The autoclave will be serviced and tested on an annual basis using an independent thermocouple mapping test. The viral killing activity of the autoclave can be tested using GFP-expressing indicator viruses. Cells will be transduced with the indicator viruses autoclaved as above, and green fluorescence will be monitored in an inverted fluorescence microscope or using green fluorescence in a FACS analyser. In the event of spillage of possibly virus-contaminated material, the area will be disinfected using 1% Virkon. The used paper towels and any other disposable material will be collected in dedicated bags and autoclaved as above. As all work is performed in a separate small tissue culture room the wider environment is unlikely to become contaminated. A spill kit will be available in the Containment Level 2 Laboratory for this purpose. All autoclaved waste will be incinerated using the yellow bag route as-cluval waste using an approved contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The University GM Safety Committee has approved the GM Risk Assessment: Local GM reference: GM 07/30.
Principal Investigator: Project Title: Differentiation of primary human stem cell lines.
**Project Additional Information**

**Purposes of the contained use**

Research into the molecular mechanisms of ageing via the production of reactive oxygen species. We wish to establish which pathways are involved in mitochondrial dysfunction in cellular senescence. To this end, we intend to monitor mitochondrial function and intracellular signalling pathways in living cells during the onset of senescence.

**Recipient or parental organism**

1. Bacterial strain Stbl3 and Top10 (Invitrogen) for amplification of constructed plasmids. Both bacterial strains are E.coli K12 strain derivatives.
2. Producer cell line 293FT for the generation of lentiviral virus (Invitrogen).
3. Human fibroblast cell lines MRC-5 (from ATCC), MRChTERT (Saretzki G. et. al., Oncogene 1999, 18:5148-5158) and fibroblasts and neurons derived from aging mice. All human cell lines are established non-self lines, which cannot colonise humans.

**Host/vector system**

ViraPower™ Lentiviral Expression System from Invitrogen

**Origin & function**

This project will construct lentiviral expression plasmids to express:

1. Mitochondrially targeted photoactivateable GFP (mt-PAGFP, Karbowski et al., Nature 2006, 443: 658-662). This is a mutated (T203H) form of codon enhanced GFP (EGFP; Patterson and Lippincott-Schwartz, 2002, Science 297 1873-1877) fused to a human COX VIII mitochondrial targeting sequence.
2. Mitochondrially targetted calcium sensor, D3cpv (4mt-D3cpv, Palmer et al., 2006, Chem & Biol. 13; 521-530). This is a 'cameleon' probe consisting of 4 tandem repeats of the human COX VIII mitochondrial targeting sequence fused to ECFP (a cyan coloured variant of EGFP) which is fused to a circularly permuted 173 Venus (an
engineered yellow fluorescent protein) via an engineered calmodulin binding peptide (initially derived from smooth muscle light chain myosin) and calmodulin peptide binding domain (initially based on Calmodulin sequence). The expressed protein enables live cell monitoring of changes in calcium levels in the mitochondria.

3. Cytoplasmically expressed calcium sensor, D3cpv (Palmer et al., 2006, Chem & Biol. 13; 521-530). This engineered gene is the same as that described in 2. above, except it has no mitochondrial targeting sequences.

4. AmCyan and mCherry- PGC1α fusion proteins. AmCyan is an engineered fluorescent protein derived from Anemonia majano cyan fluorescent protein (Matz et al, Nature Biotechnology, 17: 969-973). mCherry is an engineered version of dsRed, a red fluorescent protein from Discosoma species (Shaner et al., 2004, Nature Biotechnology 22, 1567-1572). PGC1α is a transcriptional coactivator which is involved in activating mitochondrial biogenesis and expression of ROS-detoxifying genes. It is expressed in heart, skeletal muscle and brown fat, where its expression is greatly enhanced in cold exposure, leading to expression of uncoupling proteins which increase proton leak and thermogenesis (Puigserver et al., 1998, Cell 92, 829-839).

5. mCherry- UCP2 fusion protein. UCP2 encodes an uncoupling protein which when expressed lowers mitochondrial membrane potential and subsequently mitochondrial ROS production. It is widely expressed in adult human tissues (Fleury et al., 1997, Nature Genetics, 15; 269-272)

Evaluation of foreseeable effects

The viral vector would be able to transduce many tissues should it come in contact with them. The major hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of transfected cells. The two potential transmission routes are by external exposure (either naked skin or mucous membranes) and needle-stick injury. Both are made extremely unlikely by the standard procedures employed in the tissue culture facility. The DNA in the packaged virus vectors have been modified to be “self-inactivating” (Yu et al., 1986, PNAS 83(10):3194-3198). A deletion has been made in the 3’LTR (called “delta U3”) which generates integrated viral genomes with defective 5’ and 3’ LTR’s. Thus, there is no transcription of the viral sequences in the target cell and no packagable viral genome is produced (Invitrogen).

Therefore, the foreseeable effects upon release of the packaged virus into the environment would be infection of the cells it came into contact with and expression of the transgene in those cells only.

Regarding the transgenes themselves, no health risks have been described or suspected to arise from UCP2 or PGC1α expression, and fluorescent proteins have been used in overexpression studies for more than a decade and are generally regarded as safe.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

All autoclaved material will be disposed of via an approved contractor by the clinical waste route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The University GM Safety Committee has approved the GM risk assessment:

Local GM reference: GM 07/16.
Principal Investigator: Prof Thomas von Zglinicki.
Project Title: To establish the relationship between nutrients, mitochondrial dysfunction and telomere loss in cellular senescence.

**Project Containment**

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**Project Ref** 540/08.4

Date Ackn'd 08/04/2008

CU2 Project Title

Transfection of human fibroblasts with wildtype and mutated genes of human telomerase (hTERT) and telomere modifying genes (TRF1, TRF2) by retroviral transfer.

Class

Class 2

Consent Granted

Not Applicable

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**
We discovered recently (Ahmed et al., JCS 2008, in press) that hTERT, the catalytic subunit of human telomerase protects mitochondria by decreasing oxidative stress and lowering apoptosis levels. That could have potentially important implication for the sensitivity of cancer cells to different drugs and treatments. As telomerase has both telomere-dependent and —independent functions we don’t know whether the effect we see is, at least partially, dependent on its catalytic and telomere-maintaining function. That’s why we want to employ different mutants with either a defect telomere binding, lacking catalytic activity or various import signals for the shuttling between organelles. Most of these mutants are cloned into retroviral vectors and include the use of retroviral particles for high transfection efficiency.

The GMM’s will be human diploid fibroblasts transfected with wildtype and different mutants of the catalytic subunit of human telomerase (hTERT). The wildtype TERT greatly extends the lifespan and the cells continue to proliferate beyond their Hayflick limit (replicative lifespan). As telomerase stabilises and continuously maintains telomeres protects the cells from any genomic instability. These cells are therefore highly stable but do not pose any major risk for human health or the environment. They are not viable outside their special growth medium and culturing conditions. The mutants that are not able to bind telomeres will have telomerase activity but the telomeres will not be maintained and these cells will have the same replicative lifespan as the parental fibroblasts. We will analyse whether these mutants are still able to enter the mitochondria and protect these from oxidative stress and apoptosis. The same will be done with the 2 mutants that have either a defective nuclear or mitochondrial localisation sequence. The former, again, as the previously described mutants, are not able to enter the nucleus and maintain telomeres and will be analysed regarding their mitochondrial localisation and functions. The latter mutant is not able to enter the mitochondria. It can maintain telomeres but will be excluded from the nucleus upon oxidative stress. cannot, however, enter mitochondria. These transfected fibroblasts will be phenotypically (regarding replicative lifespan) similar to those transfected with the wild type hTERT. Transfection with telomere binding proteins increases the level of these proteins with no major phenotypes expected, whereas the dominant-negative telomere binding proteins (TRF1 and TRF2) change the structure of telomeres and lead to a DNA damage response and growth arrest resembling senescence. No adverse effects of any of the described GMMs on human health or for the environment are expected.

The expression of wt-hTERT in normal diploid fibroblasts maintains telomeres and increases the replicative lifespan of cells. The various mutants have mutations in different localisation domains or the catalytic function. The dominant-negative mutant suppresses any endogenous telomerase and cells go into senescence in long term. Any fl5fàtective effects of the mutant for mitochondrial function will be assessed. With the help of the mutants with missing localisation domains the subcellular shuttling of the protein and its protective function in mitochondria (Ahmed et al., J of Cell Science 2008, in press) will be analysed. No adverse effects on human health or for the environment are expected from these GMOs.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The GMMs are human somatic fibroblasts that are not able to survive or spread outside their defined laboratory conditions as they need special growth medium, adhere to plastic and need special tissue culture incubators. Protective clothing and gloves will be worn at all times, class 2 safety cabinets will be used, the access for the tissue culture facility is restricted to specially trained and supervised personnel only and no sharp instruments will be used. All GMMs will be disposed of using special waste containers and autoclaving. All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The University GM Safety Committee has approved the GM Risk Assessment: Local GM reference: GM 07/28.

Principal Investigator:

Project Title: Transfection of human fibroblasts with wildtype and mutated genes of human telomerase (hTERT) and telomere modifying genes (TRF1, TRF2) by retroviral transfer.

Project Containment

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Project Ref  540/08.5

Date Ackn'd  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4

02/03/2022  Page 8113 of 15326
Cloning and expression of genes encoding proteins localized and functioning in the mitochondrial homologues of Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis.

Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis are parasites of the digestive tract (Entamoeba and Giardia) or urogenital tract (Trichomonas). Entamoeba and Giardia are transmitted through the fecal-oral route by cysts. Only the mature cysts are infectious as they need to survive passage through the highly acidic gastric juice. T. vaginalis has no cyst stage and must be transmitted by direct intimate physical contact, as in sexual intercourse, to allow transfer. All of these are wild type infectious parasites of hazard group 2.

Recipient or parental organism

Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis are parasites of the digestive tract (Entamoeba and Giardia) or urogenital tract (Trichomonas). Entamoeba and Giardia are transmitted through the fecal-oral route by cysts. Only the mature cysts are infectious as they need to survive passage through the highly acidic gastric juice. T. vaginalis has no cyst stage and must be transmitted by direct intimate physical contact, as in sexual intercourse, to allow transfer. All of these are wild type infectious parasites of hazard group 2.

Host/vector system

Hosts will be infectious parasites of hazard group 2. All vectors are non-mobilisable and shuttle vectors between E. coli and named parasites. These include, E.histolytica: pAS'A3'NEO and pEhHYG-tetR; G. lamblia: pNLop*-GltetR and CWP1 containing vector; and T. vaginalis: TagVag-47. None of these represent a risk to human or other organisms.

Origin & function

All of the cloned DNA will be derived from Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis. Inserted DNA will compromise PCR amplified genes which encode proteins that are candidates to function in the mitosomes of Entamoeba or Giardia, or the hydrogenosomes of Trichomonas. These proteins include mitochondrial Hsp70, Cpn 60, proteins involved in iron sulphur cluster assembly, proteins involved in energy generating pathways, and candidate organelle transport proteins. These genes will be cloned to provide material for sequencing to verify gene identity, and sub-cloned into the respective vectors for investigating the cellular localisation of recombinant tagged proteins (analysed by microscopic studies) and other functions. The goal of the project is to understand how the Entamoeba, Giardia or Trichomonas mitochondrial homologous function. None of our work is linked to the pathogenicity of the three species and none of the genes encode known virulence determinants. We will be using this genetic material to make mutants to facilitate localization and analyses of expression including cellular localization, inducible expression, loss of function and gain of function.
Evaluation of foreseeable effects

These will be genetically modified organisms of wild type infectious parasites of hazard group 2. It is not envisaged that the mutants will modify the pathogenicity of the host strains. All of the cloned genes are associated with very specific structural or metabolic functions, none of which are known to be linked with pathogenicity. They are involved in central metabolic pathways and have no known deleterious effects on humans, animals or the environment. The genes to be cloned are already universally found in eukaryotic cells where they mediate housekeeping and metabolic functions in mitochondria. None of these mutations are expected to modify the host range nor tissue specificity. None of the GMOs are expected to increase the virulence of these strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All of this work will be carried out in a dedicated laboratory under full containment level 2 conditions as specified in the Compendium of Guidance 2007.

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermo couple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

OR

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GM Safety Committee has approved the GM risk assessment:

Local GM reference: GM08/08.

Principal Investigator: Dr Robert Hirt.

Project Title: Cloning and expression of genes encoding proteins localized and functioning in the mitochondrial homologues of Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis

The University GM Safety Committee has approved the GM risk assessment:

Local GM reference: GM08/08.

Principal Investigator: Dr Robert Hirt.

Project Title: Cloning and expression of genes encoding proteins localized and functioning in the mitochondrial homologues of Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis.
**Project Additional Information**

**Purposes of the contained use**

We aim to understand the molecular mechanism(s) of the enlargement of the bacterial cell envelope during cell's growth and division in the human pathogen Streptococcus pneumoniae (the pneumococcus). Bacteria have a cell envelope of a highly complex structure. In Streptococcus pneumoniae the cell envelope contains a multi-layered peptidoglycan (murein) sacculus to which several surface polymers (teichoic acid, capsular polysaccharide, surface proteins) are covalently attached. So far 91 different capsular polysaccharides have been identified in clinical isolates (giving rise to the same number of serotypes) and their structures are known. Capsule genes are not essential for pneumococcal growth, as non-encapsulated strains are viable without any growth defect in the laboratory. However, capsule genes are required for pneumococcal virulence and survival in the host.
Within this project we are particularly interested in the biosynthesis of capsular polysaccharides and their attachment to the cell wall peptidoglycan. Although all steps in capsular biosynthesis will be addressed, the focus will be on the membrane-associated steps including attachment the capsule to the cell wall. We aim to determine the chemical structure of the linkage unit residing between the repeating units of the capsular polysaccharide and the peptidoglycan. We also want to identify and characterize the so far unknown enzyme(s) catalyzing the attachment of capsule polysaccharides to peptidoglycan (named "capsule ligases" in this application), enzyme(s) for the polymerization of the capsular polysaccharides and transporters for the capsule precursors.

To reach our goals we will perform the following experiments:
- Inactivation of potential capsule genes in S. pneumoniae. The presence of a capsule is not essential for pneumococcal growth; therefore capsule genes are not essential for pneumococcal growth in the laboratory. Inactivation of capsule genes will be done using standard procedures. For this, either part of a pneumococcal capsule gene or the upstream and downstream regions of will be amplified by PCR and will be cloned into a shuttle plasmid transformed into E. coli. Plasmids will isolated and transformed into S. pneumoniae to interrupt the target gene by single or double cross-over (the plasmids cannot replicate in S. pneumoniae).
- Testing for capsule attachment and capsule structure in the mutants. Cell wall of encapsulated pneumococcal strains will be isolated by a standard procedure at the beginning of which cells are boiled in SDS solution. This will kill the bacteria. The purified cell wall and cell wall fragments will be analyzed by high-pressure liquid chromatography (HPLC), mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR). The attachment of capsular polysaccharides to cell wall will also be analyzed by detection of capsular polysaccharides by specific antiserum in culture supernatants and on bacteria after SDS-PAGE and Western-Blot. Also the lipid-linked capsule polysaccharide precursors will be extracted from cells and will be analyzed by HPLC, MS, and NMR spectroscopy. These will also be used as substrates in enzyme assays.
- We will also express candidate genes for capsule genes (for example, capsule ligases) in Escherichia coli. E. coli cells expressing the capsule enzymes will be lysed and the enzymes will be purified for in vitro enzyme assays and biochemical characterization.

Recipient or parental organism

1) Streptococcus pneumoniae is an ACDP hazard group 2 pathogen. It is currently a leading cause of pneumonia, meningitis and bloodstream infections in elderly persons, and among the main pathogen causing middle ear infection in young children under the age of 3 years. The bacterium is carried asymptotically in the nasopharynx of healthy children (20-40%) and, less frequently (5-10%), of healthy adults, with colonization beginning shortly after birth (Austrian, 1986, J. Antimicrob. Chemother. 18:35-45). It has been very well established form many decades that the capsule is a crucial virulence factor of S. pneumoniae as the capsule is essential for survival in vivo. To date at least 91 different capsular types have been described and their structures as well as the genes responsible for their biosynthesis are known. In all serotypes, the production of capsule requires the presence of multiple genes which are most often clustered at a single locus on the pneumococcal chromosome. A serotype 2 S. pneumoniae strain called strain D39, which was originally isolated in 1916, has been widely used for studies on S. pneumoniae virulence. The complete genome sequence of strain D39 has been reported (Lanie et al., 2007, J. Bacteriol. 189:38-51). The use of the S. pneumoniae strains with other capsular types would be limited to comparative analysis. Although non-encapsulated strains have been associated with superficial infections, such as conjunctivitis, clinical isolates from other infection sites are encapsulated, and spontaneous non-encapsulated derivatives of these strains are largely avirulent (Kadioglu et al., 2008, Nature Reviews Microbiology 6: 288-301; and references therein). The prevailing animal model for pneumococcal virulence is the intraperitoneal mouse model of infection which has been used in numerous studies for more than 60 years. The virulence of S. pneumoniae is related to the thickness of capsule in a particular strain and serotype (MacLeod and Krauss, 1950, J. Exp. Med. 92:1-9). Moreover, defined mutants in which less capsular polysaccharide is attached to the bacterial cell wall show markedly reduced virulence (Morona et al., 2006, Proc. Nat. Acad. Sci. USA 103:8505-8510). Thus, our proposed inactivation of capsule genes will most likely reduce the virulence of the bacterium.

2) E. coli strains: E. coli K12 strains and derivatives (e.g. DH5(alpha), XL1-Blue or MC1061) are defined as disabled or non-colonising in the SAGCM Compendium of Guidance (Part 2.2, point 33) and is therefore equivalent to an ACDP hazard group 1 organisms. E. coli BL21 and derivatives are described in ACGM Newsletter 30 (Nov. 2001, para 3) as being “broadly equivalent to K12 strains and that in most cases work which uses this host can be considered as a class 1 activity………, but the cloning of a bacterial pathogenicity determinant into BL21 will need careful consideration and may in some cases warrant classification as class 2”. In this project potential single capsule genes will be introduced into E. coli strains. The products of capsule genes are not toxic. The introduction of a single pneumococcal capsule gene into E. coli cannot result in capsule formation in E. coli.
The following host strains will be used, or derivatives thereof:
S. pneumoniae: we will use mainly strain D39 (capsule type 2), but also strains with other capsule type for comparison.
Escherichia coli: K12, BL21.
The following vector systems will be used:
E. coli plasmid vectors based on pUC, pBAD, pBR322, pET, pQE.

Origin & function
Standard resistance markers (cat, kan, spc, tet, erm), epitope tags (such as HA, c-myc, 6xHis, etc.), artificial promoter constructions (e.g. Pxyl or Pspac) for insertion duplication mutagenesis or for expression plasmids. Capsule genes will be derived from host organism, introduced either on a plasmid or inserted directly into the host genome. The organisms that will provide the DNA to be PCR amplified will be from any of the host organisms mentioned above.

Evaluation of foreseeable effects
1. Effects on Human Health
Characteristics of the vector system and any hazards associated with it
Most of the vectors to be used are standard vectors with a long history of safe use in the laboratory. All plasmids used are mobilisation minus. These include E. coli-S. pneumoniae shuttle vectors (pJDC9, pBT2, pPP2, pLSE4) and E. coli expression vectors such as pUC, pBAD, pBR322, pET, pQE, pJDC9 and derivatives. Genetic markers (cat, bla, kan, spc, tet, erm) will involve the disruption or manipulation of specific genes involved in capsule biosynthesis, as such is unlikely to pose any increased hazard above the handling of the wild type strain.

Source and characteristics of the inserted gene product and any hazards arising directly from its use.
Drug resistance markers used in these vectors are already widely distributed in nature.
Strain construction will involve plasmid derivatives passing through E. coli for amplification prior to reintroduction into the host bacterium. It is therefore possible that these constructs could have biological effects on E. coli, but it is difficult to envisage the hazard being significantly greater than the handling of the parent strains from which the DNA was isolated. In general the GMs proposed fall into two categories; firstly, gene disruptions where no functional gene product should be produced. These plasmids will then be used to generate chromosomal disruptions in the chromosomes of the host bacterium, in so doing the plasmid replicon will either be destroyed, or will be non-replicating in the target host, where they will integrate into the genome and lie dormant. Secondly, genes will be cloned in plasmids in such a way as to be controlled by artificial promoters, e.g. T7 or Pspac. These will then be used as expression systems to allow the purification of the encoded gene product.

The genes of interest encode proteins involved in capsule biosynthesis in S. pneumoniae. Since capsular genes are generally not present in the E. coli hosts used, it is highly unlikely that introduction of single pneumococcal capsular genes into E. coli will have any effect on pathogenesis.

Hazards arising from the alteration of any existing pathogenic traits
Since capsular polysaccharide represent a virulence determinant in S. pneumoniae it is highly unlikely that the inactivation of capsular genes will lead to significantly increased virulence, the reverse is more likely.

Potential hazards of sequences within the GMM being transferred to related micro-organisms
It is difficult to envisage how any of the cloned gene products could confer or increase the virulence of a recipient strain. Transfer of the GMMs to related organisms where possible is unlikely to represent any significantly increased hazard. If any GMM were to be transferred the most probable outcome would be the selective loss of this marker as it would either be redundant or more probably deleterious to the new host.

The overall likelihood that, in the event of exposure, the GMM could cause harm to human health
The likelihood of the GMMs causing harm to humans should be no greater (and in most cases significantly less) than that associated with the parent organisms. As outlined above S. pneumoniae is wide-spread among healthy persons. The bacterium could potentially cause infections in young children and immuno-compromised persons (for example, in elderly persons). The greatest risk would be to introduce S. pneumoniae into the blood-stream which could potentially cause systemic infection. To reduce
this risk the use of sharp items will be minimized.
The genetic manipulations that are to be performed in the project will predominantly involve the deletion or disruption of specific genes functioning in capsular biosynthesis. These are expected to result in the reduction in the amount of cell wall capsular material, and as such are unlikely to enhance virulence.

2. Environmental Effects
Capacity of the GMM to survive, establish, disseminate with and or displace other organisms
In the case of E. coli the strains used are genetically marked by multiple mutations leading to at least auxotrophy such that they are unlikely to be able to survive outside the laboratory environment. S. pneumoniae strains colonize the nasopharynx of humans. There are very few reports on animals, e.g. horses, with pneumococcal infections, and the pneumococci are not known to be living free in the environment. The GMMs derived from this project are likely to be even less able to persist outside the laboratory environment than the parent strain, as they will have lost genes which function to optimise viability, this also would mean that the strains are unlikely to be more hazardous than the parent.

Ability of GMM to cause harm to animals, plants or other organisms
As detailed above [Characteristics of the GMO(s)], the GMMs are likely to be less able to cause harm than the parent strains. None of the hosts to be used are known to be significant colonisers or cause harm to other organisms. It is therefore difficult to envisage how the cloned gene products would alter this.

Potential for transfer of genetic material between the GMM and other organisms
The genetic manipulations proposed are designed to be stable in the host genome, thus transfer of the manipulated genes is very unlikely to occur due to the absence of a direct route of transmission to other species, incompatibility of DNA restriction modification systems and finally the low likelihood of expression of the genetic material outside the intended organism. In some instances it may be necessary to use plasmids to introduce the genetically manipulated material, the potential for transfer of these markers will be minimised by the used of narrow host range plasmid replicons. It is however unlikely that the gain of the markers by another organism would provide a sufficient advantage for it to be maintained in the population for a significant period of time.

Potential hazard as a result of phenotypic or genetic instability
It is difficult to identify any hazard that would be increased by genetic instability; the most likely result being that the strains would revert to the parental state or would be further damaged in function. The E. coli strains and S. pneumoniae strains D39 that are contained in this proposal are well-documented strains with a long history of safe use, thus instability is unlikely to result in pathogenic strain creation. The use of the remaining S. pneumoniae strains with other capsular types would be limited to comparative analysis.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste containing GMM includes bacterial cultures and supernatants of bacterial cultures present in or at glass or plastic lab equipment. 1% virkon, 1% chloros, 1% chlorosan or 70% ethanol disinfectant will be used to clean up spillages and all of the waste materials will be autoclaved.

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.
The University GM Safety Committee has approved the GM Risk Assessment:


Principal Investigator: Dr Waldemar Vollmer.

Genetic manipulation of Streptococcus pneumoniae capsule biosynthesis: construction of S. pneumoniae mutants and heterologous capsule gene expression in Escherichia coli.

Project Containment

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Project Ref 540/09.2

Date Ackn’d 28/05/2009

CU2 Project Title Generating induced pluripotent stem cells (iPSCs) using lentiviral vectors

Class 2

Culture Vol Class 2 < 1 L

Non-GMM Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

02/03/2022
Project Additional Information

Purposes of the contained use

Induced pluripotent stem cells (iPSC) with properties similar to embryonic stem cells can be generated from skin fibroblasts by co-expression of various combinations of transgenes (for instance OCT4, SOX2, NANOG and LIN28; or OCT4, SOX2, KLF4 and c-MYC). We will obtain or produce replication deficient lentiviral vectors encoding the cDNAs (OCT4, SOX2, NANOG, KLF4, c-MYC, KLF4, TERT). These vectors will be used to transduce human primary cells with the aim of deriving iPSC from them. The factors we are going to introduce into the primary cells and cell lines are novel and their biological effects are not fully investigated. Some of the factors (c-MYC) are known to induce tumorigenesis upon activation in somatic cells. There is therefore at this stage a possibility that they could render the cells neoplastic and hence the reason for containment under level 2.

Recipient or parental organism

Primary human cells for transduction will include those obtained from the cornea, hair follicle, oral mucosa, blood, cord blood, muscle or skin biopsies, full-term human placenta or other tissues. Human cell lines will include well established fibroblast cell lines (BJ foreskin fibroblasts from ATCC, and others), BJ fibroblasts immortalised by the expression of telomerase, as well as standard laboratory cell lines like HT1080, HeLa, HEK293T, NIH3T3 fibroblasts, lymphoid, myeloid lines and others.

Host/vector system

For cloning, sequencing and prokaryotic expression of recombinant DNA sequences, disabled E. coli K12 derived bacterial host strains (e.g. TOP10, SURE, JM109, DH5, etc) will be used in conjunction with non-mobilisable vector systems e.g. Lambda gt and charon-based bacteriophage vectors and pUC/M13mp-based plasmid, filamentous phage or phagemid vectors. These host/vector systems have been widely used over many years and have a long history of safe application.

Replication-defective lentiviral vectors (rLVs) will be constructed based on the human immunodeficiency virus type-1 (HIV) genome, but they have been specifically engineered for biosafety by separating the packaging signals and viral LTR’s on the expression plasmid from the viral structural and expression genes (gag, pol and rev from HIV-1 and the VSV-G gene from Vesicular Stomatitis Virus in place of HIV-1 env) encoded on three separate plasmids which remain in the packaging cell line, effectively precluding the production of replication competent virus in the target cell or should the viral vector escape containment. The plasmids expressing these gene products carry no packaging signals or LTRs and so cannot themselves be mobilised with the vector and have been engineered not to contain any regions of homology to each other or to the viral vector, to prevent undesirable recombination events which might result in replication competent virus being produced. Additionally, we will use non-integrating variants of these lentiviral vectors, produced by using mutations affecting the catalytic active site of integrase. Such non-integrating lentivectors have a much reduced risk of causing insertional mutagenesis.

Origin & function

We will obtain or produce replication deficient lentiviral vectors encoding the cDNAs (OCT4, SOX2, NANOG, KLF4, c-MYC, KLF4, TERT). These vectors will be used to transduce human primary cells with the aim of deriving iPSC from them. The factors we are going to introduce into the primary cells and cell lines are novel and their
biological effects are not fully investigated. Some of the factors (c-MYC) are known to induce tumorigenesis upon activation in somatic cells. The function of the genetic material will be transduction of primary cell types highlighted above with the aim of producing iPSC.

**Evaluation of foreseeable effects**

Two potential risks to humans may arise from the hazards of working with the naked plasmid vector + insert DNA containing the potentially oncogenic DNA and the supernatant containing the viral particles from the packaging cell line. The amount of DNA is relatively low and it is envisaged that the risk of naked DNA entering the human body is relatively low. Linearized cloned DNA is usually introduced into the cells using different transfection techniques, however the transfection efficiency even under optimal culture conditions is not high, therefore transfection of researcher’s cells with naked DNA under accidental/non-optimal conditions would be rather low.

The viral vector would be able to transduce many tissues should it come in contact with them. The major hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of transfected cells. The two potential transmission routes are by external exposure (either naked skin or mucous membranes) and needle-stick injury. Some of the genes that will be studied are novel and potentially could have oncogenic effects or interfere with the ability of the cells to survive in culture. If those genes were to be transfected directly into living human tissue any detrimental effects that they might cause are uncertain. However, the genes will only be transfected into cell lines or primary cells which have no ability to survive outside tissue culture environment. Even in the event of needle stick injuries or aerosol exposure, these cells (which will exist in very low numbers) will be destroyed by the human immune system.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not Applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All GMO waste materials including all tissue culture fluid etc generated in this project MUST be autoclaved. No other inactivation or waste disposal routes will be used. Spillages must be cleaned up with disinfectant but the tissues and other waste materials must then all be autoclaved.

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

Is an emergency plan required according to regulation 20?  N  

If yes, tick to confirm that it is attached to this form  N  

Tick to confirm that you have attached a risk assessment to this form  Y  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N  

Please enter comments on the GM safety committee on the risk assessment
The University GM Safety Committee has approved the GM Risk Assessment:

Local GM reference: GM 09/02.

Principal Investigator: Dr Majlinda Lako.

Generating induced pluripotent stem cells (iPSCs) using lentiviral vectors.

**Project Containment**

<table>
<thead>
<tr>
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<th>Growth Rooms</th>
</tr>
</thead>
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**Animal Units**

| L2 | L3 | L4 |

**Large Scale Activities**

| L2 | L3 | L4 |

**Human Clinical Applications**

| L2 | L3 | L4 |

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**Project Ref** 540/09.3

**Date Ackn'd** 03/09/2009

**CU2 Project Title**

Adenovirus-mediated over expression of eukaryotic genes in cultured human skeletal muscle cells

**Class** Class 2

**Culture Vol** Class 2

Consent Granted

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**

02/03/2022
Purposes of the contained use

We are interested in examining the role of various genes in the development of type 2 diabetes. In order to understand how the functions of these genes may contribute to the pathogenesis of type 2 diabetes it is necessary to examine the downstream effects of these proteins, by overexpression of certain proteins using the adenovirus system in cultured human skeletal muscle cells. One of the problems associated with genetic manipulation of these cells is the low level of transfection efficiency, a problem which can be overcome by using adenoviral-mediated overexpression of the proteins of interest. This will allow us to assess the contribution of these proteins to insulin signalling and action in this cell type.

Recipient or parental organism

Recombinant adenovirus will be transduced in non-self cultured human skeletal muscle cells. The recombinant adenovirus will be propagated in HEK293 cells, a human embryonic kidney cell line.

Host/vector system

Adenovirus vector (E1-deleted) using the freely available AdEasy system. The gene of interest will be cloned into the shuttle vector, pAdTrack-CMV. This vector does not contain any of the components of the adenovirus. The recombinant plasmid will be co-transformed into E.coli with the adenoviral backbone plasmid, AdEasy-1, allowing homologous recombination to occur. The AdEasy-1 adenoviral vector is derived from the human adenovirus serotype 5 (Ad5) and is an E1 and E3 double-deletion vector. E3 is not necessary for viral production therefore, AdEasy-1-derived recombinant adenoviruses can be propagated in packaging cells which express E1, ie. HEK293.

Origin & function

cDNA sequences originating in humans will be cloned into the adenoviral vectors. Some examples include:
- TCF7L2, full-length and tagged with GFP. This is a transcription factor involved in the Wnt signalling pathway. It has been implicated in blood glucose homeostasis through regulation of proglucagon. While the Wnt signalling pathway has been implicated in cancer, it has been demonstrated that TCF7L2 itself, appears to act as a transcriptional repressor that restricts colorectal cancer cell growth (Tang W et al, Proc Natl Acad Sci 2008. 105; 9697-9702.). Therefore it is not believed to be oncogenic. This will be obtained as an adenovirus from our collaborator, Dr Guy Rutter.
- GLUT4, full-length and tagged with GFP. This is an insulin-regulated glucose transporter required for insulin-stimulated glucose disposal and is naturally expressed at relatively low levels in cultured human skeletal muscle cells.
- CDKAL1, full-length and tagged with GFP. This is a novel gene, identified through genome-wide association studies as being associated with the development of type 2 diabetes.
- FTO, full-length and tagged with GFP. This is a novel gene, associated with obesity and the development of type 2 diabetes. Bioinformatic analyses have shown that FTO shares sequence motifs with Fe(II)- and 2-oxoglutarate-dependent oxygenases implying that it may play a role in nucleic acid demethylation (Gerken T et al, Science 2007. 318: 1469-1472). Studies are ongoing to determine how nucleic acid methylation status may be linked to increased fat mass. There is no evidence that FTO may be oncogenic.

The properties of each gene which will be expressed in the adenoviral vector will vary significantly. However, from the published literature and our own previous laboratory experience, they are not anticipated to be hazardous when used within this system. The final genetically modified viral vector will be infection competent but replication incompetent. All new stocks of recombinant adenovirus will be screened by PCR to ensure that replication competent virus particles have not been generated by recombination in the packaging cell line.

Evaluation of foreseeable effects

The properties of each gene which will be expressed in the adenoviral vector will vary significantly. However, from the published literature and our own previous laboratory experience, they are not anticipated to be hazardous when used within this system. The final genetically modified viral vector will be infection competent but replication incompetent. All new stocks of recombinant adenovirus will be screened by PCR to ensure that replication competent virus particles have not been generated by recombination in the packaging cell line.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The GMM will be inactivated by the following:

i. Plasticware:
   Disposable plastics to be put in CinBins to be autoclaved prior to incineration. Autoclaving will be done after each packaging.

ii. Glassware
   Glassware will be swabbed with Virkon before autoclaving in Denley boxes. Swabs will be disposed of in a CinBin and autoclaved prior to incineration.

iii. Liquid Waste
   Liquid waste to be treated with Virkon and autoclaved prior to disposal down sink.

iv. Paper, gloves and other soft discard
   This will be put in a CinBin and autoclaved after each packaging.

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves are validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form
N

Tick to confirm that you have attached a risk assessment to this form
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment
N

Please enter comments on the GM safety committee on the risk assessment

The University GM Safety Committee has approved the GM Risk Assessment:

Local GM reference: GM 09/07.

Principal Investigator: Prof Mark Walker.

Adenovirus-mediated over expression of eukaryotic genes in cultured human skeletal muscle cells.

Project Containment

<table>
<thead>
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02/03/2022
Familial cylindromatosis (FC-OMIM 132700) is an autosomal dominant inherited, cutaneous tumour syndrome that occurs due to mutations in a tumour suppressor gene named CYLD. Recent advances in the understanding of the function of CYLD have raised possibilities of medical treatments such as aspirin, but these have not been validated in keratinocyte models.

We plan to use lentiviral transfection techniques that have been shown to be effective in transfecting keratinocytes for the development of in vitro models for drug screening:

a. CYLD deficient keratinocyte lines:
   • We plan to compare cell survival, proliferation, and apoptosis in a human keratinocyte cell line (HaCat) with and without functional CYLD in the presence of different aspirin-like compounds. Lentiviral delivery of short hairpin RNA targeting CYLD will be used to knockdown CYLD in the keratinocyte line.

b. Primary tumour cell lines:
   • We intend to reintroduce CYLD into primary human cylindroma tumour lines that are CYLD deficient. Cell survival, proliferation, and apoptosis will then be compared in the presence of different aspirin-like compounds with and without CYLD. Lentiviral delivery of CYLD cDNA will be used on the primary tumour cell lines.
Cell lines in culture: Human embryonic kidney cell line 293T carrying the SV40 large T (packaging cell line, hereinafter referred to as "HEK-293T"), Human keratinocyte cell lines (HaCat), primary keratinocyte tumour lines and human epidermal neural crest stem cells will be used. We will only use samples, or cells obtained from biopsies of volunteers or patients not known to carry infectious diseases, or with a history of safe use in laboratories.

Host/vector system

There are two types of lentiviral vectors that we intend to use: Trans-Lentiviral™ GIPZ for knockdown work and Trans-Lentiviral™ pLEX for overexpression work. (The term "trans" is used to emphasise the separation of the genes used for the packaging virus across multiple plasmids as a safety measure to reduce the likelihood of a recombination event). These are similar in structure, with pGIPz illustrated below, and are obtained from Openbiosystems. The Trans-Lentiviral packaging mix - contains an optimized mixture of five packaging plasmids (pTLA1-Pak, pTLA1-Enz, pTLA1-Env, pTLA1-Rev and pTLA1-TOFF) to facilitate viral packaging of the transfer vector following co-transfection into HEK-293T producer cells. These plasmids supply the helper functions as well as structural and enzymatic proteins in trans required to produce the lentivirus. The transfer vector will contain short hairpin RNA sequences, specific to the gene being targeted, CYLD, in the context of knockdown work, and cDNA (again CYLD) in the context of overexpression work. Empty vectors will be used for matched controls. These sequences are linked via an internal ribosomal reentry site (IRES) to green fluorescent protein (GFP), allowing monitoring of transfection efficiency.

Origin & function

Knockdown of CYLD in keratinocyte cell lines

CYLD, a ubiquitin hydrolase enzyme, is a tumour suppressor gene and therefore at this stage a remote possibility that they could render the cells neoplastic. Short hairpin RNA targeting human CYLD will be obtained in a pGIPz vector from Openbiosystems. This will be used to knockdown CYLD expression in keratinocyte lines such that it serves as CYLD deficient keratinocyte model in which potential therapeutic agents can be tested. pGIPz with a non silencing vector will be used in matched controls. The best candidates will be tested in the cylindroma cell lines below, which are a relatively precious resource.

Overexpression of CYLD in cylindroma cell lines

CYLD DNA sequences originating in humans or other eukaryotes will be purchased already cloned into replication deficient lentiviral transfer vectors described above and expressed in the primary cylindroma cell lines also described above. This will enable us to have an isogenic control of the cylindroma cell line with CYLD that is important for finding drugs that target CYLD deficient cells. CYLD is a tumour suppressor gene, and reintroduction of CYLD into the tumour cells should diminish their existing neoplastic status.

Evaluation of foreseeable effects

CYLD is a tumour suppressor gene and knockdown of this gene in HEK-293T cells using liposomal methods previously has not significantly altered the proliferation of such cells in tissue culture. We do not anticipate oncogenic transformations occur when these genes are singly knocked down in the neural crest cells or keratinocyte lines that we describe in this proposal. Only cells in tissue culture will be infected with the viral construct. These cells have no ability to survive outside tissue culture environment. Even in the event of a sharps injury, these cells (which will exist in very low numbers) will be destroyed by the human immune system.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Keratinocytes and neural crest stem cells, like any mammalian cells are extremely sensitive to rapid and complete inactivation by the disinfectants specified below which will be used in accordance with the manufacturer's instructions. Only disposable plastic pipettes will be used in the tissue culture facilities. Extra precautions will be taken by
disposing all liquid and solid contaminated waste in Virkon, prior to autoclaving.

All GMO waste materials including all tissue culture fluid etc generated in this project will be autoclaved. No other inactivation or waste disposal routes will be used. Spillages must be cleaned up with disinfectant and the tissues and other waste materials must then all be autoclaved prior to incineration. All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GM Safety Committee has approved the GM Risk Assessment:

Local GM reference: GM 09/11.

Principal Investigator: Prof Maya Sieber-Blum.

Generating human cell lines with and without CYLD using lentiviral vectors.

**Project Containment**

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<th>Growth Rooms</th>
</tr>
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<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2 L3 L4 L2</td>
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**Project Ref** 540/09.5

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<td>30/11/2009</td>
<td>Use of Viral Vectors for Optogenetic Control of Cortical Network Oscillations in Rodents</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Recently new optogenetic methods have been developed that make it possible to target and modulate the activity of endophenotypically distinct groups of neurons with pulses of light. Viruses that encode for light-activated molecules can be injected into the CNS of rats and/or mice to target specific key cell types such as the parvalbumin-expressing interneurons that are critical for the generation of certain types of cortical network activity (Cardin et al 2009; Han et al 2009; Sohal et al 2009). Slices can then be prepared from treated animals after a few days and electrophysiological experiments performed. The key advantage of this technique is that specific cell types are genetically labelled so it is possible to directly control the activity of individual cell types in the network.

Rattus norvegicus/Mus musculus
It is essentially impossible for the modified genetic material to be transferred to other rats/mice if mating occurred.
The only phenotypic differences between the GMO and the wild type animal are that, upon exposure to focused, intense light (~10mW/mm²) the infected neurons will fire action potentials, and that the infected neurons will express fluorescent proteins (e.g. eGFP, mCherry).
Given the nature of the modification, it is unlikely that the GM animal poses any greater risk than wild type mice/rats.

Host/vector system
HEK293FT human embryonic kidney cell lines will be used as hosts for viral production.
E.coli strains such as DH5α will be used for plasmid production.
NB Production of plasmids and virus will be provided by a secondary supplier outside of Newcastle in all instances.
MIT (Boston) create self-inactivating lentiviruses by performing triple-transfection of HEK293FT cells with:
- a plasmid encoding for the gene of interest embedded in the lentiviral main backbone (e.g., FCK-hCmC for lentivirus).
- a plasmid encoding for packaging proteins for viral synthesis (e.g., pCMV-dR8.74 or pCMV-dR8.91 for lentivirus).
- a plasmid encoding for the viral coat protein (e.g., VSV).
No virus-producing cell lines are enduringly kept; we have found it most convenient, safe, and efficient to acutely triply-transfect fresh HEK293FT cells with plasmids each time we need to produce virus. No helper virus is needed for any of these protocols. The host range of the virus includes mice, rats, primates, and humans. We expect there to be no replication-competent virus.
MIT will, if required, periodically test for the presence of replication-competent lentivirus in producer cells by the following method: After 3 washes to remove original virus, supernatant that has been in the dishes for at least 12 hours with 293FT cells that have been infected with replication-defective virus expressing a measurable reporter
gene (GFP, dsRed2, neomycin, puromycin, or others) will be used to infect a second set of 293FT cells. These cells will be maintained for several weeks and screened for drug resistance or for expression of marker gene by flow cytometry. These highly sensitive assays are capable of identifying a single infected cell. These tests will be performed once for each construct used, since it is expected that the properties of each plasmid will be stable. The lentivirus constructs we use originated from the labs of Didier Trono and David Baltimore, and represent some of the safest and easiest-to-use vectors available for the delivery of genes into mammalian tissue. The resultant lentiviruses are replication-deficient, and are used in many labs at MIT and around the world, at this time. Furthermore, they are designed to be extraordinarily unlikely to become replication competent, with >2/3 of the viral genome deleted from the final packaged vector (including the abolition of all virus-production genes). These viruses have self-inactivating properties to prevent integrated genes from being repackaged, in the event a subsequent infection ever occurs. The lentivirus will be directly injected into target areas of the CNS of rats/mice under general anaesthesia using stereotactic surgery. 0.5-2 μl will be used, and the wound sutured. Virus and vectors will be injected (according the atlas of Paxinos and Watson) of adult rats/mice using a stereotaxic frame. Following local administration, animals will be allowed to recover. Animals injected with virus will be maintained in the CBC until transfer under deep anesthesia to labs within the Institute of Neuroscience (rooms MG144, MG183).

REFERENCES
Lentivirus

Origin & function
The lentiviral vector employed is based on HIV and has been attenuated by removal of over 2/3 of the viral genome along with parts of the 3'UTR (SIN type virus) and by having any viral packaging proteins and other proteins coded by up to three separate plasmids e.g.: a plasmid encoding for the gene of interest embedded in the lentiviral main backbone (e.g., FCK-hCmC), a plasmid encoding for packaging proteins for viral synthesis (e.g., pCMV-dR8.74 or pCMV-dR8.9), and a plasmid encoding for the viral coat protein (e.g., VSV). The nature of the modifications to the virus are such that it stable as both the parental vector and also the recombinant vector. The vector therefore does not present a major risk of homologous recombination (SACGM 2.11.14).

Ion channels/pumps to be employed are based on either rhodopsins from the unicellular green alga Chlamydomonas reinhardtii (Chr-1, Chr-2, ChiEF)(Boyden et al. Nat Neurosci. 2005 Sep;8(9):1263-8) or on the light-driven chloride pump halorhodopsin from the archaeabacterium Natronomas pharaonis (Han and Boyden, PLoS One. 2007 Mar 21;2(3):e299).

The modifications made to the naturally-occurring opsin are meant to optimize their expression and trafficking to the cell membrane in mammalian cells. These include: codon optimization for expression in mammalian cells, addition of an ER trafficking sequence. Such opsins, which allow modulation of neuronal voltage and activity have been safely used in a variety of model organisms, from C. elegans to mouse to rat to non-human primates (NHPs) without evidence of toxicity (Han et al. 2009, in particular, tested for safety in NHPs).

Fluorescent reporter proteins based on green fluorescent protein (GFP from Aequorea victoria), and other similar safe, non-toxic fluorescent proteins from other organisms (e.g. Discosoma spp. mCherry (Shaner et al Nature Biotechnology 22, 1567 - 1572 (2004)) will be used.

Evaluation of foreseeable effects
The self-inactivating lentivirus vector systems to be used are such that they have been extensively modified and are regarded as being stable, as are the inserted sequences. The lentiviral vector system to be used is considered to be “Third Generation” as defined by the SACGM Compendium of Guidance 2.11.9 as the accessory genes (vif, vpr, vpu, and nef) have been deleted along with the tat gene. The risk of dissemination of the described vector is extremely low due to it being replication incompetent and self-inactivating (SIN) (SACGM Compendium of Guidance, Part 2.11). The extensive modification of the viral vector also prevents homologous recombination with other related viruses (see 3A). All cultures are maintained under sterile conditions and it is unlikely that any hazardous sequences if they were present could be transferred to related organisms.

In the event of exposure, the GM virus could gain access to a limited number of human cells. However, direct consequences to human health of exposure to the virus are
expected to be negligible, because of the benign nature of the inserted genes. As detailed in the SACGM “transformation has not been seen when using lentivirus systems in a broad range of in vitro studies and animal studies using both in vivo and ex vivo protocols. Liver tumours have been observed following administration of lentiviral vectors to foetal and neo-natal animals”. Furthermore, the use of replication incompetent virus (see SACGM compendium of guidance, 2.11.9) ensures that only limited cells will be infected. The overall likelihood of hazard is therefore low. The animals are maintained to the standards prescribed in the Codes of Practice under the Animals (Scientific Procedures) Act 1986 which equate at least to containment level 1 as described in General Provisions of Part 3D in the Guide to the Genetically Modified Organisms (Contained Use) Regulations, 2000. The assessment is that containment level 2 is suitable for this work. The overall likelihood of hazard is therefore low. The only risk of the animals is allergenicity which is no greater than normal non-GM animals. Workers showing an allergic response to the animals are not required to come into contact with them. Consequently, there is a low risk to human health. All staff involved are regularly assessed for signs of allergy.

Mit the supplier will periodically test for the presence of replication-competent lentivirus in producer cells by the following method: After 3 washes to remove original virus, supernatant that has been in the dishes for at least 12 hours with 293 cells that have been infected with replication-defective virus expressing a measurable reporter gene (GFP, dsRed2, neomycin, puromycin, or others) will be used to infect a second set of 293 cells. These cells will be maintained for several weeks and screened for drug resistance or expression of marker gene by flow cytometry. These highly sensitive assays are capable of identifying a single infected cell. These tests will be performed once for each construct used, since it is expected that the properties of each plasmid will be stable.

Hypodermic needles will be used and therefore suitable training and precautions will be taken to minimise the potential for self inoculation. Needles to be used are of very narrow gauge (<30G) and flexible to allow insertion into soft tissue and as such are of lower risk than standard needles. Viral vectors will be injected using blunt needles (They are the WPI 36 guage blunt needle (NF36 BL-2) and the Hamilton 33 or 30 blunt needle (7803-05/00)) therefore minimising the risk of accidental injury. However, work will be conducted under the same safety controls as if using sharps. Virus injection is conducted by a controlled microinjection unit and there is no direct contact with the syringe or needle during injection.

Used “sharps” (pipette tips, scalpel blades, staples) will be collected in a yellow “CinBin” type container placed within the plenum of the MSC immediately after use. The container and all sharps will be autoclaved prior to disposal by the clinical waste route. Blunt needles will be used for injection but will be handled as if sharps. Denley boxes will be used to transport all materials to the autoclave.

All waste that is possibly contaminated with virus will be inactivated by incubation with a final concentration of at least 1% Virkon for 30 minutes and all of this waste will be collected in dedicated bags and these transferred to an autoclave using closed containers and will be inactivated by autoclaving at 121°C for 20 min. This procedure will ensure 100% killing efficiency. Autoclaving will be done at the end of each working day or on completion of work, whichever is the earliest. Temperature and time during each autoclave run will be monitored using indicator strips (TST control integrator, class 6 emulating indicator 121°C for 20 min, Albert Browne Ltd, UK). The autoclave will be serviced and tested on an annual basis using an independent thermocouple mapping test.

In the event of spillage of possibly virus-contaminated material, the area will be disinfected using 1% Virkon. The used paper towels and any other disposable material will be collected in dedicated bags and autoclaved as above. As all work is performed in a separate room, the wider environment is unlikely to become contaminated.
will be available in the surgery unit and in all other locations where the work will take place.

Is an emergency plan required according to regulation 20?  No

If yes, tick to confirm that it is attached to this form  No

Tick to confirm that you have attached a risk assessment to this form  Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment  No

Please enter comments on the GM safety committee on the risk assessment

The University GM Safety Committee has approved the GM Risk Assessments:
Local GM references: GM 09/15 and GM 09/16 (Connected risk assessments).
Principal Investigator: Dr Fiona Le Beau.
GM 09/15 Use of Viral Vectors for Optogenic Control of Cortical Network Oscillations.
GM 09/16 Optogenic control of cortical network oscillations in small animals.

Project Containment

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Animal Units

| L2 Yes L3 L4 L2 | L3 L4 L2 L3 L4 |

Large Scale Activities

| L2 Yes L3 L4 L2 | L3 L4 L2 L3 L4 |

Human Clinical Applications

|                | L2 L3 L4 |

Project Ref 540/10.1

Date Ackn'd 06/01/2010

CU2 Project Title Use of Viral Vectors for Optogenic control of CNS function in monkey

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements No

Withdrawn No 02/03/2022
Recently new optogenetic methods have been developed that make it possible to target and modulate the activity of endophenotypically distinct groups of neurons with pulses of light. Viruses that encode for light-activated molecules can be injected into the CNS of rats and/or mice to target specific key cell types such as the parvalbumin-expressing interneurons that are critical for the generation of certain types of cortical network activity (Cardin et al 2009; Han et al 2009; Sohal et al 2009). Slices can then be prepared from treated animals after a few days and electrophysiological experiments performed. The key advantage of this technique is that specific cell types are genetically labelled so it is possible to directly control the activity of individual cell types in the network.

Rattus norvegicus/Mus musculus

It is essentially impossible for the modified genetic material to be transferred to other rats/mice if mating occurred. The only phenotypic differences between the GMO and the wild type animal are that, upon exposure to focused, intense light (~10mW/mm²) the infected neurons will fire action potentials, and that the infected neurons will express fluorescent proteins (e.g. eGFP, mCherry). Given the nature of the modification, it is unlikely that the GM animal poses any greater risk than wild type mice/rats.

HEK293FT human embryonic kidney cell lines will be used as hosts for viral production. E.coli strains such as DH5a will be used for plasmid production.

MIT (Boston) create self-inactivating lentiviruses by performing triple-transfection of HEK293FT cells with:
- a plasmid encoding for the gene of interest embedded in the lentiviral main backbone (e.g., FCK-hCmC for lentivirus).
- a plasmid encoding for packaging proteins for viral synthesis (e.g., pCMV-dR8.74 or pCMV-dR8.91 for lentivirus).
- a plasmid encoding for the viral coat protein (e.g., VSV).

No virus-producing cell lines are enduringly kept; we have found it most convenient, safe, and efficient to acutely triply-transfect fresh HEK293FT cells with plasmids each time we need to produce virus. No helper virus is needed for any of these protocols. The host range of the virus includes mice, rats, primates, and humans. We expect there to be no replication-competent virus.

MIT will, if required, periodically test for the presence of replication-competent lentivirus in producer cells by the following method: After 3 washes to remove original virus, supernatant that has been in the dishes for at least 12 hours with 293FT cells that have been infected with replication-defective virus expressing a measurable reporter gene (GFP, dsRed2, neomycin, puromycin, or others) will be used to infect a second set of 293FT cells. These cells will be maintained for several weeks and screened for drug resistance or for expression of marker gene by flow cytometry. These highly sensitive assays are capable of identifying a single infected cell. These tests will be
The lentivirus constructs we use originated from the labs of Didier Trono and David Baltimore, and represent some of the safest and easiest-to-use vectors available for the delivery of genes into mammalian tissue. The resultant lentiviruses are replication-deficient, and are used in many labs at MIT and around the world, at this time. Furthermore, they are designed to be extraordinarily unlikely to become replication competent, with >2/3 of the viral genome deleted from the final packaged vector (including the abolition of all virus-production genes). These viruses have self-inactivating properties to prevent integrated genes from being repackaged, in the event a subsequent infection ever occurs.

The lentivirus will be directly injected into target areas of the CNS of rats/mice under general anaesthesia using stereotactic surgery, 0.5-2 μl will be used, and the wound sutured. Virus and vectors will be injected (according the atlas of Paxinios and Watson) of adult rats/mice using a stereotoxic frame. Following local administration, animals will be allowed to recover. Animals injected with virus will be maintained in the CRC until transfer under deep anaesthesia to labs within the Institute of Neuroscience (rooms MG144, MG183).

**REFERENCES**

**Lentivirus**


**Origin & function**

The lentiviral vector employed is based on HIV and has been attenuated by removal of over 2/3 of the viral genome along with parts of the 3'UTR (SIN type virus) and by having any viral packaging proteins and other proteins coded by up to three separate plasmids e.g.:

- a plasmid encoding for the gene of interest embedded in the lentiviral main backbone (e.g., FCK-hCmC),
- a plasmid encoding for packaging proteins for viral synthesis (e.g., pCMV-ΔR8.74 or pCMV-ΔR8.9), and
- a plasmid encoding for the viral coat protein (e.g., VSV).

The modifications to the virus are such that it stable as both the parental vector and also the recombinant vector. The vector therefore does not present a major risk of homologous recombination (SACGM 2.11.14).

**Evaluation of foreseeable effects**

The self-inactivating lentivirus vector systems to be used are such that they have been extensively modified and are regarded as being stable, as are the inserted sequences. The lentiviral vector system to be used is considered to be "Third Generation" as defined by the SACGM Compendium of Guidance 2.11.9 as the accessory genes (vif, vpr, vpu, and nef) have been deleted along with the tat gene. The risk of dissemination of the described vector is extremely low due to it being replication incompetent and self-inactivating (SIN) (SACGM Compendium of Guidance, Part 2.11). The extensive modification of the viral vector also prevents homologous recombination with other related viruses (see 3A). All cultures are maintained under sterile conditions and it is unlikely that any hazardous sequences if they were present could be transferred to related organisms.

In the event of exposure, the GM virus could gain access to a limited number of human cells. However, direct consequences to human health of exposure to the virus are expected to be negligible, because of the benign nature of the inserted genes.

As detailed in the SACGM "transformation has not been seen when using lentivirus systems in a broad range of in vitro studies and animal studies using both in vivo and
The overall likelihood of hazard is therefore low.

The animals are maintained to the standards prescribed in the Codes of Practice under the Animals (Scientific Procedures) Act 1986 which equate at least to containment level 1 as described in General Provisions of Part 3D in the Guide to the Genetically Modified Organisms (Contained Use) Regulations, 2000. The assessment is that containment level 2 is suitable for this work.

The only risk of the animals is allergenicity which is no greater than normal non-GM animals.

Workers showing an allergic response to the animals are not required to come into contact with them. Consequently, there is a low risk to human health. All staff involved are regularly assessed for signs of allergy.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The self-inactivating lentivirus vector systems to be used are such that they have been extensively modified and are regarded as being stable, as are the inserted sequences. The lentiviral vector system to be used is considered to be “Third Generation” as defined by the SACGM Compendium of Guidance 2.11.9 as the accessory genes (vif, vpr, vpu, and nef) have been deleted along with the tat gene. The risk of dissemination of the described vector is extremely low due to it being replication incompetent and self-inactivating (SIN) (SACGM Compendium of Guidance, Part 2.11). The extensive modification of the viral vector also prevents homologous recombination with other related viruses (see 3A). All cultures are maintained under sterile conditions and it is unlikely that any hazardous sequences if they were present could be transferred to related organisms.

In the event of exposure, the GM virus could gain access to a limited number of human cells. However, direct consequences to human health of exposure to the virus are expected to be negligible, because of the benign nature of the inserted genes.

As detailed in the SACGM “transformation has not been seen when using lentivirus systems in a broad range of in vitro studies and animal studies using both in vivo and ex vivo protocols.....liver tumours have been observed following administration of lentiviral vectors to foetal and neo-natal animals”. Furthermore, the use of replication incompetent virus (see SACGM compendium of guidance, 2.11.9) ensures that only limited cells will be infected.

The overall likelihood of hazard is therefore low.

Workers showing an allergic response to the animals are not required to come into contact with them. Consequently, there is a low risk to human health. All staff involved are regularly assessed for signs of allergy.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not Applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste that is possibly contaminated with virus will be inactivated by incubation with a final concentration of at least 1% Virkon for 30 minutes and all of this waste will be collected in dedicated bags and transferred to an autoclave using closed containers and will be inactivated by autoclaving at 121°C for 20 min. This procedure will ensure 100% killing efficiency. Autoclaving will be done at the end of each working day or on completion of work, whichever is the earliest.

Temperature and time during each autoclave run will be monitored using indicator strips (TST control integrator, class 6 emulating indicator 121ºC for 20 min, Albert Browne Ltd, UK). The autoclave will be serviced and tested on an annual basis using an independent thermocouple mapping test.

In the event of spillage of possibly virus-contaminated material, the area will be disinfected using 1% Virkon. The used paper towels and any other disposable material will be collected in dedicated bags and autoclaved as above. As all work is performed in a separate room, the wider environment is unlikely to become contaminated. A spill kit will be available in the surgery unit and in all other locations where the work will take place.
The University GM Safety Committee has approved the GM Risk Assessments:
Local GM references: GM 09/15 and GM 09/16 (Connected risk assessments).
Principal Investigator: Dr Fiona Le Beau.
GM 09/15 Use of Viral Vectors for Optogenic Control of Cortical Network Oscillations.
GM 09/16 Optogenic control of cortical network oscillations in small animals.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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| Project Ref 540/10.2 |

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<td>17/02/2010</td>
<td>Genes involved in metal homeostasis in Staphylococcus aureus</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
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Withdrawn N

Historical Significant Changes
Historical Date of Additional Info
Project Additional Information

Purposes of the contained use

We will prepare cultures of non-clinical strains of Staphylococcus aureus for biochemical analysis to identify proteins involved in metal homeostasis of this organism. S. aureus will be cultured in small quantities for the isolation and analysis of nucleic acids and proteins. This will involve liquid culture of S. aureus, centrifugation, gel electrophoresis, liquid chromatography, MALDI-TOF-MS mass fingerprinting. Genes encoding proteins involved in metal homeostasis will be genetically inactivated, followed by biochemical and phenotypic analysis.

Recipient or parental organism

The organism for study is the hazard group 2 biological agent Staphylococcus aureus, using the common lab strain 8325-4 and derivatives thereof (RN4220, SH1000). Strain 8325-4 is a derivative of the NCTC8325 parent strain that was UV-cured of prophages phi12 and phi13 (which encode virulence genes chip, scin & sak) and phi11 (Novick & Richmond, 1965, J. Bacteriol. 90, pp 467-480).

Host/vector system

Several non-mobilisable plasmid vectors will be used for allelic replacement and subsequent complementation:

Plasmid pOB (Horsburgh, MJ et al., 2002, Mol. Microbiol. 44, 1269-1286), a suicide vector derived from the E. coli plasmid pGEM3zf incorporating an erythromycin resistance cassette, can be used for integration of mutant alleles into the restriction-deficient strain RN4220.

Plasmid pAUL-A is an E. coli – S. aureus shuttle vector constructed (Chen, JD & Morrison, DA, 1987, Gene 55, 179-187) by fusion of a fragment from the E. coli vector pJDC9 containing a multiple cloning site, transcription terminators and erythromycin resistance cassette, with a fragment from the native S. aureus plasmid pE194 containing a temperature-sensitive Gram-positive replication origin. This plasmid can also be used for integration of mutant alleles into the host genome, as it can be maintained in S. aureus at permissive temperatures and integrants acquired by selection at nonpermissive temperatures.

Plasmid pMK4, an E. coli – S. aureus shuttle vector constructed (Sullivan, MA et al., 1984, Gene 29, 21-26) by fusion of fragments from the E. coli vector pUC9, containing E. coli replication origin and ampicillin resistance cassette, and the native S. aureus plasmid pC194, containing a Gram-positive replication origin and a chloramphenicol resistance cassette, is used for complementation of mutants.

Phi11 is a serogroup B bacteriophage native to S. aureus NCTC8325 that is used for transduction of the mutant allele from the restriction deficient RN4220 strain to the
Origin & function

The intended mutations will involve interruption of a gene through insertion of a selectable marker. DNA sequences flanking the target gene will be amplified by polymerase chain reaction from S. aureus genomic DNA and cloned into plasmid vectors. Resistance determinant gene will be sub-cloned from commercially available vector (e.g. kanamycin resistance gene from pUC4K) and integrated into the target gene on this vector. S. aureus RN4220 is transformed with this construct using standard protocols (see 'Staphylococcus: Molecular genetics,' 2008, Ed. Lindsay, JA) and the mutant allele incorporated into the genome by homologous recombination. The locus is transduced into the host 8325-4 strain by using the bacteriophage phi11. Integration site is confirmed by PCR methods. Selectable markers will consist of antibiotic resistance determinants.

The initial target genes for insertional inactivation are the two chromosomally-encoded isozymes of superoxide dismutase (MnSOD). Future gene targets for insertional inactivation will be determined from preliminary results with wild type and will encode metalloenzymes and/or metal homeostasis factors, allowing phenotypic analysis of mutant strains in order to elucidate gene function.

Evaluation of foreseeable effects

The initial target genes for insertional inactivation are the two isozymes of MnSOD, named sodA and sodM. Strains in which these genes have been inactivated have previously been generated and analysed elsewhere, leading to reduced virulence in a mouse abscess model (Karavolos, MH et al., 2003, Microbiology 149, 2749-2758). These genes will either be inactivated as described or the mutant strain acquired from a collaborator. Future gene targets for insertional inactivation will be determined from preliminary results with wild type and will encode metalloenzymes and/or metal homeostasis factors. Some of these metalloproteins have the potential to be virulence factors, either through the involvement of the encoded proteins in host recognition/attachment or immune defence (e.g. SodA/M), or as regulators of important bacterial processes (e.g. peroxide sensor PerR, iron regulator Fur). In most cases, due to the importance of effective metal homeostasis to cell survival, and the role of metal starvation as a mechanism of immune defence against pathogenic infections (Corbin et al., 2009, Science 319, 962), strains in which such genes are inactivated would be expected to exhibit equivalent or reduced virulence relative to the host strain. However, in a small number of cases, such as the inactivation of a negative regulator of virulence gene expression, the mutant strain has the potential for increased virulence.
The University GM Safety Committee has approved the GM Risk Assessment:

Local GM references: GM 10/02.

Principal Investigator: Dr K W

Genes involved in metal homeostasis in Staphylococcus aureus.

Project Containment

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Animal Units

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Project Ref 540/10.3

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<td>12/08/2010</td>
<td>Application of lentiviral vectors in the characterisation of the pluripotent and differentiated state of induced pluripotent stem cells and human embryonic stem cells</td>
<td>Class 2</td>
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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) are able to differentiate into any cell type of the body and hence are important tools for i) providing in vitro models to study the cellular effects of development and disease and ii) developing cell therapies in tissue regeneration strategies. We have derived hESC lines from surplus human embryos and wish to research the molecular mechanisms that regulate pluripotency and differentiation into particular cell lineages (with emphasis on generating chondrocytes (cartilage) and osteoblasts (bone) and researching the developmentally regulated mechanisms that enable germ cells to enter meiosis). We will also carry out comparative studies with induced pluripotent cells generated from somatic cell types. We will begin by assaying a diverse selection of somatic cell types in order to investigate i) those that are most conducive to genetic reprogramming and ii) identify if different genetic factors / experimental conditions are required for different cell types. For this programme of work we will need to genetically modify hESCs and somatic cells by transfection with relevant genes, lineage-specific promoter elements and reporter gene sequences. In parallel we carry out comparative experiments with embryonic and somatic cells obtained from mouse and equine sources as part of our programme to identify species differences of stem cell behaviour.

Because of the low efficiency of transfection associated with hESCs and the primary cell types that we are using, we will employ lentiviral transfection technology to achieve a satisfactory level of transfection efficiency. Whilst this work will be carried out in parallel and will be complimentary to our existing programmes investigating the clinical application of stem cells this is purely a research project and lentivirally-transfected cells will not be used in the clinic.

Aims:

1) To generate iPSCs by lentiviral transfection of somatic cells.

2) Lentiviral transfection of ESCs with genetic reporter constructs in order to monitor the live cell pluripotent / differentiation status of hESCs and their derivatives.

3) Lentiviral transfection of molecular components involved in the driving differentiation of hESC and iPSC to cell lineages.

4) To investigate the regulation of meiosis-specific genes.

Recipient or parental organism

Cell lines in culture:

Human embryonic kidney cell line 293FT are a human embryonic kidney cell line optimised for viral transfection.
http://tools.invitrogen.com/content/sfs/manuals/293ft_cells_man.pdf

hESC lines including NCL1-5 (available from the UK Stem Cell Bank), hESC lines that have been derived at Newcastle University and are yet to be banked in the UK Stem Cell Bank, hESC lines that will be derived at Newcastle University and banked in the UK Stem Cell Bank during the course of this project. Primary human chondrocytes, primary mesenchymal stem cells derived from bone marrow and haemarthrosis aspirates, primary human skin fibroblasts, human amnion fibroblasts (HAF006), primary...
keratinocytes. iPSCs which are generated from the above mentioned somatic cells.
Mouse embryonic fibroblasts, derived in house or purchased from a commercial source.
In-house derived equine umbilical cord, keratinocytes and skin fibroblasts
All of these cells are unable to survive outside tissue culture conditions and are antigenically incompatible if accidently introduced to humans.

**Host/vector system**

**Lentiviral Packaging Vectors:**

- **pMDLg/pRRE**
  - Genes: gag and pol. Encodes structural proteins (gag) and enzymes (reverse transcriptase, integrase & protease) for viral replication. There is also a rev response element (RRE) in the encoded RNA.
  - Promoter: CMV

- **pMD2.VSVG**
  - Gene: VSV-G. Encodes envelope glycoprotein from the vesicular stomatitis virus.
  - Promoter: CMV

- **pRSV-Rev**
  - Gene = Rev. Rev interacts with transcribed viral RNAs (via the RRE) to facilitate nuclear export such that translation may take place. Rev is therefore beneficial for the expression of structural proteins to make daughter virions.
  - Promoter RSV

**Lentiviral Transfer Vectors:**

- **pCCLsin.PPT.Prom.[insert].Wpre**
  - The insert and viral DNAs are engineered into sites downstream of strong constitutive promoters (designated as Prom) (RSV, CMV, CAG and SV40) for some experiments cell lineage-specific promoters will be engineered into the vector. In both cases there will be no attempt to maximise expression. Insert refers to the gene or reporter of interest.
  - All of the vectors are based on the pUC19 backbone, defective in transfer to other hosts and are Bom-, Tra-Mob- (Follenzi and Naldini 2002)


**Origin & function**
Either genomic or cDNA sequences originating in humans and reporter sequences will be cloned into the lentiviral vectors using standard laboratory techniques. These will include both entire gene sequences, partial gene sequences and promoter sequences. Some of the factors we are going to introduce into the cell lines are novel and their biological effects are not fully investigated and as such there is the possibility that they could render the cells neoplastic.

Generation of iPSCs: the following genes have been identified as being involved in the reprogramming of somatic cells to a pluripotent phenotype. As such as part of our optimisation experiments we will transfect somatic cells with combinations of these genes in order to assay the most appropriate protocol for each cell type:

- SOX2- is involved in the maintenance and self-renewal of hESC and is one of many factors that are involved in the reprogramming of somatic cells to a pluripotent phenotype (Yu et al 2007, Takahashi et al 2007). cDNA will be generated from pluripotent hESCs.
- OCT4- is involved in the maintenance and self-renewal of hESC and is one of many factors that are involved in the reprogramming of somatic cells to a pluripotent phenotype (Yu et al 2007, Takahashi et al 2007). cDNA will be generated from pluripotent hESCs.
- NANOG- is involved in the maintenance and self-renewal of hESC and is one of many factors that are involved in the reprogramming of somatic cells to a pluripotent phenotype (Yu et al 2007, Takahashi et al 2007). cDNA will be generated from pluripotent hESCs.
- LIN28- is involved in regulating the expression of genes that control cell cycle and is widely expressed during the early stages of embryogenesis and is one of many factors that are involved in the reprogramming of somatic cells to a pluripotent phenotype (Yu et al 2007). cDNA will be generated from pluripotent hESCs or mature oocytes.
- KLF4- is expressed in many cell types and is involved in regulating proliferation, terminal differentiation and apoptosis. Depending on the cellular context KLF-4 can act as an oncogene or a tumour suppressor. It is one of many factors involved in the reprogramming of somatic cells to a pluripotent phenotype (Takahashi et al 2007). cDNA will be generated from pluripotent hESCs.
- c-MYC- is involved in regulating cell cycle progression in stem cells and during development and tissue homeostasis. It is one of many factors involved in the reprogramming of somatic cells to a pluripotent phenotype (Takahashi et al 2007). cDNA will be generated from pluripotent hESCs.
- Tbx3- is involved in regulating stem cell pluripotency and has been shown to increase the efficiency of iPSC generation (Han et al 2010). cDNA will be generated from pluripotent hESCs.

Cell lineage-specific promoter sequences will be cloned from human genomic DNA (purchased from a commercial supplier e.g. Promega). Experiment-dependent promoter sequences will be used to drive reporter elements and hence will be indicative of the pluripotent / cell lineage status of the cell.

Meiosis-specific genes will be sourced from human oocytes
- E2F6 is a transcription factor involved in suppressing expression of the meiosis-specific cohesin proteins SMC1B and Stag3 (Storre et al, 2005).
- Rec8-myc: Rec8 is a meiosis-specific cohesin protein, which will be tagged with myc to facilitate detection
- Histone H2 is a core nucleosomal histone, which maintains its function when tagged with a fluorescent protein and thereby provides a means to visualise chromosome dynamics by live cell imaging.
- Histone H1foo is an oocyte-specific linker histone
- Stra8 is a transcription factor expressed by spermatogonia
- Sycp1 is expressed during early meiosis of spermatocytes
- Pgx2 is expressed during late meiosis
- Prm1 is a post-meiotic transcription factor
- SFRS10 (cDNA) is a splicing factor expressed in meiosis
- TRA2A (cDNA) is a splicing factor expressed in meiosis
- SAM68 (cDNA) is a splicing factor expressed in meiosis
- T-STAR (cDNA) is a splicing factor expressed in meiosis
- ASPP1 (cDNA) is an apoptosis factor expressed in meiosis
• RBMX (cDNA) is a splicing factor expressed in meiosis
• hnRNP G-T (cDNA) is a splicing factor expressed in meiosis

siRNA experiments specific to meiosis-specific genes will be carried out by designing short-hairpin (sh) sequences in conjunction using commercially available software (eg Ambion or Sigma-Aldrich) and cloning in to lentiviral vectors as described by Manjunath et al 2009.

Reporter elements will be:

• EGFP – Aequorea Victoria jellyfish
• Venus/YFP - Vibrio fischeri
• RFP – Discosoma species of coral

Reporter cassettes will be obtained from plasmids gifted by Dr Owen Jones (Manchester University). These and related fluores are non-toxic and have no biological activity.

• Luciferase cDNA cassette will be generated from cDNA of stably-transfected CHO/N2-luc cell line (Wong et al 2003, Oldershaw et al 2008) and engineered into the appropriate vector

Overexpression of cell lineage-specific transcription factors will be used to drive differentiation toward particular cell types:

• L-SOX5, SOX6 and SOX9 cDNA will be generated from primary chondrocytes.
• CBFA1 and OSTERIX cDNA will be generated from osteoblasts obtained by differentiation of human bone marrow mononuclear cells.
• Notch signalling transmembrane receptors, Notch1, Notch2, Notch 3 and Notch4 and transmembrane ligands, Jagged1, Jagged2 and Delta are involved in mediating cell fate decisions during embryogenesis (Brennan & Gardner). Overexpression of receptor and ligand sequences obtained from cDNA of cell lineages known to express specific components will be used to delineate the mechanisms by which ES cell differentiation is regulated by Notch signalling.


Evaluation of foreseeable effects

Bacterial hosts for cloning and sequencing:

Disabled E. coli K12 derived bacterial host strains (e.g. TOP10, SURE, JM109, DH5, etc) (non-pathogenic to human) will be used in conjunction with non-mobilisable vector systems. Vectors are based on a pUC19 backbone which is Tra- and Bom- and thus mobilisation defective. These host/vector systems have been widely used over many years and have a long history of safe application. ACDP classification of the host is containment level 1. Cloning, amplification and isolation of plasmids is necessary to later package the virus. The gene product will not be expressed because the vectors lack the necessary promoters for the genes to be expressed in E.Coli. Also, the absence of eukaryotic translation/post-translational modification system would prevent the formation of a functional protein.

Packaging cell line:

Human embryonic kidney cell line 293FT are a human embryonic kidney cell line optimised for viral transfection.
http://tools.invitrogen.com/content/sfs/manuals/293ft_cells_man.pdf

These cells are unable to survive outside tissue culture conditions and are antigenically incompatible if accidently introduced to humans.

Host cells to be transfected with lentiviral particles are:

1) hESC lines including NCL1-5 (available from the UK Stem Cell Bank)
2) hESC lines that have been derived at Newcastle University and are yet to be banked in the UK Stem Cell Bank
3) hESC lines that will be derived at Newcastle University and banked in the UK Stem Cell Bank during the course of this project
4) Primary human chondrocytes isolated from cartilage tissue obtained through ethical consent of patients undergoing surgical procedures
5) Primary mesenchymal stem cells derived from bone marrow and haemarthrosis aspirates. Bone marrow mononuclear cells will be purchased from a commercial supplier (eg Lonza) and used to derived mesenchymal stem cells
6) Primary human foreskin fibroblasts. FED1 was generated by Dr Mary Herbert's group at Newcastle University in conjunction with the Bio-manufacturing facility at Newcastle University, this line is HTA approved. NUFF1 was purchased from a commercial source.
7) Human amnion fibroblasts (HAF006)
8) Primary keratinocytes obtained by Dr Mary Herbert's group with ethical approval
9) iPSCs which are generated from the above mentioned somatic cells.
10) Mouse embryo fibroblasts
11) NIH 3T3 cells

All host cells are unable to survive outside tissue culture conditions and are antigenically incompatible if accidently introduced to humans.

It is possible that infection may occur should the virus come into contact with broken skin or mucous membranes and as such the greatest risk to the operator comes from needlestick injury or from inhalation of aerosols. It is possible that some of the genes that are transfected might have oncogenic effects and there is no guarantee on the detrimental effects that could be caused.

Overexpression of transcription factors expressed by differentiated cell types (e.g. L-SOX5, SOX6, SOX9, CBFA1 and OSTERIX) are involved in regulating the differentiation status of the cell and hence would be unlikely to render the cells neoplastic within the context of the experiments described in this proposal. Notch signalling components described can promote or suppress tumorigenesis depending upon the cellular context. The gene products will be biologically active when transfected into mammalian cells. However, the recipient transfected cells will either be embryonic or somatic stem cells that have a history of safe use and may be regarded as posing minimal risk. Meiotic-specific genes would unlikely have a physiological effect since they will not be presented in a cellular environment that will perpetuate the effects of their expression. These hosts can be considered especially disabled hosts because the cell lines are unable to colonise the worker (i.e. not their own cells)
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All genetically modified organisms including bacteriological and mammalian cells will be inactivated by Virkon® disinfectant. 2% (w/v) and then autoclaved before disposal.

The designated Microbiological Safety cabinet and centrifuge will be disinfected by thoroughly wiping down with 2% (w/v) Virkon® solution followed by 70% ethanol before and after every use. The Microbiological Safety cabinet will also be irradiated for 5 minutes with UV after every use.

Solid waste (e.g. cultureware, pipette tips, used gloves etc) will be autoclaved. Prior to autoclaving, waste will be stored in designated yellow waste bins. Autoclaving all of the waste will take place as soon as possible, in the nearest available autoclave (same building/floor) by an authorised person. Once autoclaved, all bags will be disposed of by incineration.

Liquid waste (e.g. cultures in flasks or spent media) will be sterilised in the culture flasks on in designated waste bins by addition of Virkon® to a concentration of 2% (w/v) and left overnight. Virkon®-disinfected solutions will be autoclaved and then disposed of through the incineration route.

Virally-treated cells, tissues and packaging cells (293 cells) will be disposed of (following the removal and appropriate disposal of culture medium) by addition of 2% (w/v) Virkon® solution. Cultureware will subsequently be disposed as per solid waste.

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

**Is an emergency plan required according to regulation 20?**

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

**Please enter comments on the GM safety committee on the risk assessment**

The University GM Safety Committee has approved the GM Risk Assessments:

Local GM references: GM 10/08.

Principal Investigator: Dr Rachel Oldershaw.

GM 10/08 Application of lentiviral vectors in the characterisation of the pluripotent and differentiated state of induced pluripotent stem cells and human embryonic stem cells.
The aim of this project is to study the role of specific components from the polysaccharide utilisation systems of Bacteroides thetaiotaomicron (Bt), a prominent member of our indigenous gut microbiota. Bt is able to use a wide range of plant and animal derived polysaccharides as its major source of carbon and energy. The genes that encode the machinery to access and degrade these polysaccharides are organised into co-regulated polysaccharide utilisation loci (PULs), each of which is specific for a particular polysaccharide. The key features that define a PUL are a pair of outer membrane associated carbohydrate binding and transport proteins, a number of carbohydrate-active catabolic enzymes and a closely linked inner membrane spanning sensor-regulator system.

In this project we will study the role of specific genes within different PULs by creating ‘clean’ deletions of these genes and studying the phenotype of the these knockout strains. Complemented mutant strains containing a single copy of the deleted gene reinserted at a specific site in the genome will be also be made.
The protocol to be used to create the knockout strains is an adaptation of the standard counter-selectable allelic exchange procedure employed in bacterial genetics based on a series of two recombination events. The method of counter-selection is based on sensitivity of Bacteroides spp. that contain a functional copy of the tdk gene (BT2275 in B. theta VPI-5482), which encodes a thymidine kinase (Tdk) involved in the pyrimidine salvage pathway, to the nucleotide analogue 5-fluoro-2-deoxy-uridine (FUDR).

Briefly, FUDR is imported into the bacterial cell and phosphorylated by Tdk, forming 5-fluoro-2-deoxy-uridine monophosphate (FdUMP), an event that activates its ability to bind irreversibly to an enzyme in the de novo thymidine biosynthetic pathway, ThyA. Thus, in the presence of Tdk FUDR ‘poisons’ the de novo biosynthetic pathway for thymidine. In this gene deletion protocol, genetic recombination must be conducted in a B. theta parent strain that has a deletion of tdk. This strain although not truly ‘wild-type’ due to its deletion of tdk, does not exhibit any auxotrophies (i.e., it still has a functional de novo pyrimidine pathway, which it can use during in vitro or in vivo growth in the absence of pyrimidines).


**Recipient or parental organism**

Bacteroides thetaiotaomicron VPI-5482 (Type strain), lacking the tdk gene (see section 6 above for description of tdk gene product). Bacteroides thetaiotaomicron is a prominent member of our resident gut microbiota and is known to play a significant role in maintaining health and nutrition, but is also an opportunistic pathogen when outside its normal niche (colon) and as such is classified as ACDP hazard group 2. It can cause bacteremia, peritonitis, endocarditis and wound infections. Can invade tissues after surgical or accidental trauma or tissue destruction (from infection with other organisms or malignancies). Risk of infection from other routes is negligible.

**Host/vector system**

Cloning host: Escherichia coli S17-1pir (conjugal K12 lysogenized for pir)

Vectors: The vectors to be used are both mobilisable, but replication limited ‘suicide’ vectors (pExchange-tdk and pNBU2-bla-ermGa/b). Both vectors can be maintained only in disabled E. coli K12 hosts containing a chromosomally inserted Lambda pir gene, which is required for replication of these plasmids. Thus, in the very unlikely event of unintended mobilisation to a different strain or species these vectors would be incapable of replicating in most cases. In the even more unlikely event of unintended mobilisation to another E. coli strain already carrying a Lambda phage actively expressing the pir gene that permits plasmid replication, it is difficult to envisage any detrimental effects arising from expression of the cloned genes as; i) they are not involved in any harmful traits (they are polysaccharide utilisation genes); ii) would have limited activity when expressed in isolation i.e. without the other co-regulated genes that make up an individual polysaccharide utilisation locus.

In addition, the drug resistance markers used in these plasmids are already widely distributed in nature.

Details of vectors:

- **pExchange-tdk** comprises: an E. coli R6K replication origin, an E. coli RP4 mobilization region, an ampicillin resistance gene (bla) for selection in E. coli, an erythromycin resistance gene (ermB) for selection in Bacteroides spp., and a copy of the BT2275 tdk gene (encodes Bt thymidine kinase) and promoter region, which upon chromosomal integration restores FUDR sensitivity (see section 2.1 above) and allows eventual counter-selection against pExchange-tdk.
- **pNBU2-bla-ermGa/b**. Bacteroides species integrative vector. Derivative of pKNOCK-bla-ermGb (see below) into which a PCR-amplified intN2-att fragment was inserted using engineered SalI and KpnI ends (intN2 encodes a Bacteroides integrase gene). MCS sites can be used to insert fragments into pNbu2-erm-a/b and the entire plasmid construct can then be conjugated into B. thetaiotaomicron and insertions made into either of the two NBU2-targeted tRNAser attachment sites (Wang et al. 2000. J. Bacteriol. 182: 3559–3571). pKNOCK-bla-ermGb suicide vectors - Base suicide vectors contain ermG for selection on erythromycin in Bacteroides spp. and bla for selection on ampicillin in E. coli. Vectors are derivatives of pKNOCK-cm (Alexeyev, 1999. Biotechniques 26: 824-828). The pBR325 cat gene was removed from pKNOCK-cm by MluI digestion and replaced with a PCR-amplified, bla-ermG-containing fragment from pGERM (Salyers et al. 2000. Methods 20: 35-46). The added bla-ermG fragment, with MluI ends, was inserted into the pKNOCK-cm MluI sites in both possible orientations ("a" and "b" designation).

**Origin & function**

The genes to be deleted from B. thetaiotaomicron are involved in polysaccharide utilisation in this bacterium and other gut Bacteroides spp. and enable these organisms to use a wide range of plant and animal derived glycans as a source of carbon and energy in the gut. They are therefore pivotal to the bacterium’s ability to survive in its specific niche and thus it is difficult to envisage how deletion of these genes would create a GM bacterium that was more hazardous than the wild-type strain.
In terms of cloning of these genes in E. coli it could be argued that they could endow a recipient organism with the ability to survive in a non-natural niche such as the human gut. However, B. thetaiotaomicron requires at least 6 different genes to utilise each polysaccharide, and as only one gene will be cloned in E. coli at a time for the complementation studies it is highly unlikely that a single gene product would confer any enhanced survival traits on the recipient organism.

During the allelic exchange protocol to delete specific genes from B. thetaiotaomicron one of the steps involves selection for a single crossover event where the whole suicide vector is inserted into the chromosome, thus creating a Bt strain that carries both the ampicillin (bla) and erythromycin resistance genes (ermG). However this is selected against in the next step so that the final GM Bt strain contains no extraneous vector sequences.

**Evaluation of foreseeable effects**

The polysaccharide utilisation genes that are to be disrupted/deleted are not involved in the ability of B. thetaiotaomicron to cause disease and even if they were, the removal of these genes as proposed would be more likely to create a less pathogenic strain than the wild-type. The risk of these genes altering existing pathogenic traits of this organism is therefore negligible.

The use of suicide vectors that can only replicate in specific E. coli hosts for these experiments minimises the likelihood of transfer of the genes to other species. The mobilisable suicide vectors require the pir gene product for their replication and this would not be available in most other species, minimising the risks of transfer when using these vectors. Furthermore, in the unlikely event of transfer to another species it is difficult to envisage how a single polysaccharide utilisation gene could confer/increase virulence of the recipient organism.

The likelihood of the GMMs causing harm to humans should be no greater (and in most cases significantly less) than associated with the wild type B. thetaiotaomicron or E. coli strains. This is risk extremely low as the E. coli strain to be used (S17-1λpir) is non-pathogenic and while B. thetaiotaomicron is an opportunistic pathogen its ability to cause disease is limited to very specific circumstances such as gut injury where the number of bacteria getting into the bloodstream is very large.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not Applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All GMM contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

**Is an emergency plan required according to regulation 20?**  

N

**If yes, tick to confirm that it is attached to this form**  

N

**Tick to confirm that you have attached a risk assessment to this form**  

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  

N
The University GM Safety Committee has approved the GM Risk Assessments:

Local GM references: GM 10/20.

Principal Investigator: Dr David Bolam.

Deletion of genes encoding components of the polysaccharide utilisation apparatus from the human gut bacterium Bacteroides thetaiotaomicron.

Project Containment

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<th>Laboratory Activities</th>
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Project Ref 540/10.5

Date Ackn’d 03/11/2010

CU2 Project Title Use of Viral Vectors for Optogenic Control of Human Cortical network Oscillations

Class 2

CultureVolClass2 ≤ 1 Litre

Consent Granted

Non-GMM

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022
## Purposes of the contained use

Recently new optogenetic methods have been developed that make it possible to target and modulate the activity of endophenotypically distinct groups of neurons with pulses of light. Viruses that encode for light-activated molecules can be injected into slices of acutely resected human brain tissue to target specific key cell types such as the parvalbumin-expressing interneurons that are critical for the generation of certain types of cortical network activity (Cardin et al 2009; Han et al 2009; Sohal et al 2009). Slices can then be incubated with the virus for 4-8h and electrophysiological experiments performed. The key advantage of this technique is that specific cell types are genetically labelled so it is possible to directly control the activity of individual cell types in the network.

## Resected brain tissue from patients with epilepsy or tumours.

The only phenotypic differences between the virus-infected tissue and normal brain tissue is that, upon exposure to focused, intense light (~10mW/mm^2) the infected neurons will fire action potentials, and that the infected neurons will express fluorescent proteins (e.g. eGFP, mCherry).

Given the in vitro nature of the modification, and the short life of the virus (c.2-4h in cerebrospinal fluid at room temperature) to be used it is unlikely that the GM tissue poses any risk.

## Host/vector system

**Host:**
Recombinant Herpes simplex virus. The bacterial host strains used to prepare the plasmids for recombinant HSV-1 generation are E. coli K12 derivatives such as DH5a, HB101, MC1061.

The cell lines used to generate recombinant virus are human 293 and 293T cells and derivatives, HeLa, VERO, and the VERO cell derivative 2-2, which contains the HSV IE 2 gene (Smith et al., Virology 1992; 186:74-86). None of this work will be performed at Newcastle.

**Vector system:**

The plasmid vectors are HSVPrpUC-based HSV amplicon vectors detailed in Neve et al., Biotechniques 2005; 39:381-391, which includes figures showing the map of their amplicon vector HSVPrpUC and an overview of the packaging procedure. They use 2-2 (VERO cells containing the HSV IE 2 gene) as a packaging line, and 5dl1.2 (partial IE 2 deletion mutant of HSV-1; McCarthy et al., J Virol 1989; 63:18-27)) as a disabled helper virus. Thorough characterization of each viral vector stock involves measuring helper plaque-forming units per ml (pfu/ml) on VERO 2-2 cells and wild type HSV-1 pfu/ml on VERO cells to ensure the absence of any wild type HSV-1. These methods are detailed in the following reference: Lim F, Neve RL. Generation of high-titer defective HSV-1 vectors. In: Current Protocols in Neuroscience. New York: Greene Publishing Associates and Wiley-Interscience, 1999, pp. 4.13.1-4.13.17.

Since using the 2-2/dl1.2 packaging system (from 1997), Neve and co-workers have never detected replication-competent viruses in their stocks. They have generated over 2000 ml of virus @ 108 lu/ml and have never observed any production of replication-competent viruses.

## Origin & function

The HSV.p1005 virus employed is a modified HSVPrpUC with an added cistron expressing eGFP. This dual-cistronic virus produces a separate transcript (separate promoter and an SV40 poly-A signal) for GFP. The cassettes are in a nose-to-tail orientation. The target gene is still driven by the IE 4/5 while the eGFP is driven by a CMV promoter. Co-expression is generally >90%. Therefore, this construct is good for electrophysiology.

Ion channels/pumps to be employed are based on either rhodopsins from the unicellular green alga Chlamydomonas reinhardtii (Chr-1, Chr-2, ChIEF)(Boyden et al. Nat Neurosci. 2005 Sep;8(9):1263-8) or on the light-driven chloride pump halorhodopsin from the archaebacterium Natronomas pharaonis (Han and Boyden, PLoS One. 2007 Mar 21;2(3):e299).

The modifications made to the naturally-occurring opsin are meant to optimize their expression and trafficking to the cell membrane in mammalian cells. These include: codon optimization for expression in mammalian cells, addition of an ER trafficking sequence. Such opsins, which allow modulation of neuronal voltage and activity have
been safely used in a variety of model organisms, from C. elegans to mouse to rat to non-human primates (NHPs) without evidence of toxicity (Han et al. 2009, in particular, tested for safety in NHPs).

Fluorescent reporter proteins based on green fluorescent protein (GFP from Aequorea victoria), and other similar safe, non-toxic fluorescent proteins from other organisms (e.g. Discosoma spp. mCherry (Shaner et al Nature Biotechnology 22, 1567 - 1572 (2004)) will also be used. While this technique cannot provide the degree of cell specific transfection possible with the use of cre transgenic animals, we will use partially cell subtype specific promoters targeting interneurons (DLX) and principal cells (CamKII and TH).

**Evaluation of foreseeable effects**

The bacterial host strains are E. coli K12: DH5α, HB101, MC1061 for production of plasmids (N.B. These will be held by our collaborators at MIT and no plasmids or bacteria will be held in Newcastle).

The cell lines are 293 cells and derivatives (e.g. 293T), HeLa, VERO (African green monkey), and the VERO cell derivative 2-2, which contains the HSV-1 IE 2 gene for the production of virus (none of these cells will be held in Newcastle).

Viruses to be used are replication incompetent (SACGM 2.9.10).

NB. Production of plasmids and virus will be provided by a secondary supplier outside of Newcastle in all instances (MIT). MIT will, periodically, test for the presence of replication-competent HSV-1 derivatives: Culture supernatants of VERO 2-2 cells which have been infected with a replication-defective virus (HSV-1 dl1.2) will be used to infect a second set of normal VERO cells. The second set of VERO cells will be grown for several weeks to identify infected cells by means of a plaque assay. This is a sensitive technique since a single colony, arising from a single replication-competent virus, can be detected readily.

The major routes of transmission are through accidental inoculation via needle stick injury, exposure to open wounds and aerosolisation of high titre virus stocks (SACGM Compendium of Guidance, part 2.6.1). If delivered by these routes the virus will produce a very limited infection of cells due to the replication incompetent nature of the virus. There is the potential for exposure of the eyes to viral particles due to aerosolisation in the event of a spillage, which would lead to infection of epithelial tissue. The external parts of the eye are however ectodermal in nature and do not directly connect to the neuroectodermal parts of the eye which connect to the central nervous system. Given that the replication incompetent nature of the recombinant virus and the lack of direct connection and isolated blood supply, CNS infection and therefore expression of viral gene expression products is highly unlikely. The insert sequence is also considered to be safe and therefore the likelihood of any hazard is low.

Given the high levels of infection of the population with wild type HSV-1 & 2, the potential for interaction is however unknown (SACGM 2.9.29). However, the extensive modification of the viral plasmid vector, the use of a replication incompetent helper virus and the presence of the packaging gene IE2 in the VERO 2.2 packaging cell line also prevents homologous recombination with other related viruses. All cultures are maintained under sterile conditions and it is unlikely that any hazardous sequences if they were present could be transferred to related organisms. Whilst there is the remote possibility that the recombinant virus could be recombined with wild type HSV-1 or HSV-2 from infected individuals, the nature of the insert and the limited expression would not produce any additional hazard over and above the wild type virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not Applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Any waste that is possibly contaminated with virus will be initially inactivated by incubation with a final concentration of at least 1% Virkon for 1h and all of this waste will be collected in dedicated bags and autoclaved (100% kill) prior to being disposed of by the clinical waste route.
All other contaminated materials will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GM Safety Committee has approved the GM Risk Assessments:

Local GM references: GM 10/18.

Principal Investigator: Dr Miles Whittington.

Use of Viral Vectors for Optogenic Control of Human Cortical Network Oscillations.

**Project Containment**

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**Project Ref** 540/11.1

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Date Project Ceased

02/03/2022 Page 8152 of 15326
The purpose of this research is to examine the role of various Candida cell wall structures on signalling and activation of human immune cells in healthy individuals and patients with Chronic Mucocutaneous candidiasis – a primary immune deficiency with selective susceptibility to infections with Candida species.

The project will be conducted in the Musculoskeletal Research group (MRG) of the Institute of Cellular Medicine (ICM), with optimal available containment level 2 laboratory space, equipment and intellectual expertise. The project will be conducted on human blood mononuclear cells obtained from peripheral blood and buffy-coats of human blood donors. After separation, peripheral blood mononuclear cells and/or monocytes will be stimulated with a range of Candida albicans mutants (a kind gift from Professor Neil Gow, University of Aberdeen) defective in various cell wall structures such as mannan, glucan and chitin to assess their effects on cytokine production and cell signalling pathways mediated by various cells surface receptors. Toll-like receptors (TLR 2, 4, 6) and C-type lectin receptors (mannose receptor, beta-glucan, dectin-1, dectin-2) will be investigated by measuring production of relevant cytokines and signalling pathway activation. Results will be confirmed by competitive blocking using TLR ligands and anti-receptor antibodies where available. Results obtained on cells from normal human blood donors will subsequently be investigated on cells from patients with a primary deficiency coined Chronic Mucocutaneous Candidiasis, to assess underlying defects which predispose them to Candida infections.

The Candida strains listed below are a kind gift from Professor N G, Aberdeen University in whose Laboratory these strains were generated. Work on these strains has been extensively published (see references below). The host organisms is NGY152 Candida albicans CAI-4 serotype (Wild-Type Strain); the following Candida albicans mutant strains will be used:

- CDH15 (mnn4 delta + Clp10) = mnn4 mutant (absence of phosphomannan)
- CDH13 (mnn4 + Clp10-MNN4) = matched control strain
- NGY355 (pmr1 delta) = pmr1 mutant (absence of phosphomannan & reduced O- and N-linked glycans)
- NGY356 (pmr1 delta + Clp10-PMR1) = matched control strain
- NGY357 (och1 delta + Clp10) = och1 null (defects in outer chain N-linked mannosyl chains)
- NGY358 (och1 delta + Clp10-OCH1) = matched control strain
- NGY337 (mnt1-mnt2delta + Clp10) = mnt1 and mnt2 mutant (defects in O-mannosylation)
- NGY335 (mnt1-mnt2 delta + Clp10-MNT1) = matched control strain
- NGY336 (mnt1-mnt2 delta + Clp10-MNT2) = matched control strain
C. albicans is a human commensal and opportunistic pathogen in ACDP Hazard Group 2. C. albicans causes superficial infections of mucosal epithelia (thrush) (Odds [1988] Candida and Candidosis, Balliere Tindall). C. albicans can also cause systemic infections which can be fatal, but these only occur in severely immunocompromised patients. To keep things in perspective, it is important to realise that C. albicans is widely distributed. C. albicans is carried commensally by at least 60-80% of the healthy population and most Candida infections arise as an overgrowth of the strains resident in the normal microflora. It is a micro-organism of low pathogenic potential that rarely affects healthy individuals. It only becomes a potential medical problem when an individual’s immune responses are significantly impaired. Furthermore, as with most pathogenic micro-organisms, the virulence of C. albicans strains is likely to become attenuated rather than enhanced after prolonged laboratory culture.

The C. albicans strain CAI4 (ura3) is a standard host for DNA transformation world-wide. The special growth requirements (auxotrophy) makes this C. albicans avirulent (Leberer et al. [1996] PNAS, 93, 13217). Transformation with URA3 plasmids partially restores the virulence of CAI4, but this restoration is not complete because the genes neighbouring URA3 remain inactivated and their virulence is slightly lower than the wild type strains of C. albicans carried by most individuals. It is important to note that although a large number of C. albicans mutants have been analysed, including virulence attributes, metabolic and cell wall genes, there is NO report of a mutation that increases the virulence of this species. This is unsurprising, since all evidence to date indicates that virulence in C. albicans is a multi-factorial process at the molecular level, with no single virulence factor of over-riding importance. Therefore, mutations in wild isolates are expected to have a neutral or negative effect upon virulence. A limited number of experiments will examine antifungal drug responses in a commercially acquired Candida strain (ATCC 18804) which we have used in this Laboratory since 2004.

Our work with antifungal agents includes manipulation of antifungal-resistant isolates, but it is important to note that Candida species have no effective mechanism for horizontal gene transfer that might allow dissemination of resistance traits. This difference from antibacterial resistance is well reflected in the clinic, where increased prophylactic and therapeutic usage of antifungal agents has led to an increase in prevalence, but not incidence of infections due to species with low intrinsic antifungal susceptibility. No increase in resistant strains of C. albicans has been found.

To summarise:
The likelihood of hazard is low, since we are handling a commensal and opportunistic fungal pathogen and the C. albicans strains used in this project are all disabled host strains of lower virulence. Most individuals already harbour wild type C. albicans strains. The consequences of any hazard arising from accidental contamination is also low since C. albicans infections are of low severity with mild, treatable symptoms and serious infections only occur in individuals with severely compromised immune systems. Staff and students employed on this project are informed of this situation to eliminate any with a compromised immune system. The estimation of overall risk to human health is low.

Host/vector system

Vector: Clp10 (Candida Integrative plasmid 10) is a convenient and efficient integrating vector for C. albicans (Murad et al, Yeast 2000,16:325). The plasmid is based on pBluescript KS+ (Stratagene, Cambridge, UK). It carries the marker CaURA3, has numerous convenient cloning sites and lacks a C. albicans replication origin. The ribosomal protein 10 coding region (RP10) provides homology to target chromosomal integration. An artificial StuI site was introduced beside the natural NcoI site in the RP10 coding region to provide two alternative unique restriction sites for chromosomal integration. The sequence of Clp10 is available with the GenBank Accession No. AF181970.
Integration and expression: The RP10 locus was chosen to target genomic integration because there appear to be two closely related RP10 loci and as a result, the disruption of one RP10 allele in this diploid fungus does not significantly affect the growth of C. albicans Clp10 transformants on rich or defined media. As RP10 is expressed at high levels, Clp10 integrates relatively efficiently by homologous recombination. Consistent with this, Clp10 transformed C. albicans Cal4 and Cal8 about 20 times more efficiently than a control plasmid. Finally, chromosomal integration at a highly expressed locus promotes efficient expression of genes integrated using Clp10 by avoiding unfavourable position effects. The increased stability of plasmids such as Clp10 reduces population heterogeneity due to plasmid loss or copy number variance.

Hosts: Hazards associated with C. albicans are not anticipated to have harmful effects or only effects of low severity. This is because C. albicans is widely distributed, with most individuals already harbouring wild type C. albicans strain while serious infections only occur in individuals with severely compromised immune systems. Pathogenicity in C. albicans is a polygenic trait involving numerous physiological characteristics (e.g. rapid growth, cell wall, morphogenesis, secreted hydrolases, phenotypic switching) so that no single C. albicans gene (or combination of genes) will increase its pathogenicity. On the contrary, vectors such as Clp10 are known to attenuate C. albicans and reduce pathogenicity, thus reducing the risk of hazards which are not anticipated to have harmful effects or only effects of low severity.

Origin & function

The C. albicans transformations in the strains we will acquire have an inactivated specific target gene to create a null mutant. The target genes which have been attenuated affect cell wall components such as mannann, glucan and chitin content which influence the cell wall structure. In none of the cases in question is this likely to increase virulence. As described above, it has been shown in numerous cases that C. albicans virulence is either decreased or remains unaffected by gene knockouts. No null mutations have been described that increase the virulence of C. albicans.

The special growth requirements (auxotrophy) makes this C. albicans strain CA14 (ura3) avirulent (Leberer et al. [1996] PNAS, 93, 13217). Transformation with URA3 plasmids partially restores the virulence of CA14, but this restoration is not complete because the genes neighbouring URA3 remain inactivated, although their virulence is slightly lower than the wild type strains of C. albicans carried by most individuals. It is important to note that although a large number of C. albicans mutants have been analysed, including virulence attributes, metabolic and cell wall genes, there is NO report of a mutation that increases the virulence of this species.

There are few papers reporting the isolation of C. albicans from the wild, suggesting that C. albicans might be obligately associated with mammals. The risk of infection of wild mammals (such as rodents) through accidental release of C. albicans is very low because to succumb to such infections, immunocompetent animals must be injected intravenously with relatively high infective doses (>10^4 CFU/g body weight). Therefore, none of the strains used in this project are likely to present a hazard to the environment.

Evaluation of foreseeable effects

As described previously the C. albicans mutant strains that we will acquire have individual inactivated target genes to create a series of null mutants with defective cell wall structures. In none of the cases in question does this likely to increase virulence. As described above, it has been shown in numerous cases that C. albicans virulence is either decreased or remains unaffected by gene knockouts. No null mutations have been described that increase the virulence of C. albicans.

As indicated above, pathogenicity in C. albicans is a polygenic trait involving numerous physiological characteristics (e.g. rapid growth, cell wall, morphogenesis, secreted hydrolases, phenotypic switching). Therefore, (i) no single C. albicans gene (or combination of genes we will be working with) will turn E. coli or S. cerevisiae into a pathogen, (ii) a reduction in any one of these parameters is likely to attenuate C. albicans virulence, and (iii) an increase in any one of these parameters is highly unlikely to increase C. albicans virulence. Furthermore, C. albicans strains that have been cultured in the laboratory tend to display reduced pathogenicity compared to strains carried commensally by most individuals.

Our work with antifungal agents includes manipulation of antifungal-resistant isolates, but it is important to note that Candida species have no effective mechanism for horizontal gene transfer that might allow dissemination of resistance traits. This difference from antibacterial resistance is well reflected in the clinic, where increased prophylactic and therapeutic usage of antifungal agents has led to an increase in prevalence, but not incidence of infections due to species with low intrinsic antifungal
susceptibility. No increase in resistant strains of C. albicans has been found.

The likelihood of natural gene transfer to other species is essentially zero. Furthermore, despite the recent discovery of sex in C. albicans, the risk of transfer between C. albicans strains is very low. This is because C. albicans strains mate very inefficiently in vivo [Hull et al., (2000) Science 289, 307-310], probably because the specific growth forms that mate most efficiently aren’t maintained at 37°C [Miller & Johnson (2002) Cell 110, 293-302]. In addition, C. albicans must be homozygous at the mating type locus for them to be competent for mating, and the vast majority of clinical isolates are heterozygous at the mating type locus.

In summary, the modified Candida organisms are extremely unlikely to cause harm to human health for the reasons described above.

As described previously the C. albicans mutant strains that we will acquire have individual inactivated target genes to create a series of null mutants with defective cell wall structures. In none of the cases in question does this likely to increase virulence. As described above, it has been shown in numerous cases that C. albicans virulence is either decreased or remains unaffected by gene knockouts. No null mutations have been described that increase the virulence of C. albicans.

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In summary, the modified Candida organisms are extremely unlikely to cause harm to human health for the reasons described above.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Emergency procedures for spillage of infectious materials.
- Instructions, spills kits, PPE must be used where required.
- Instructions on laminated sheet are available in the CL2 lab.
- Notify other workers and isolate the area (if required).
- Evacuate lab if risk of airborne infection.
- Allow aerosols to settle.
- Contain spills with tissues or granules.
- Cover with disinfectant (liquid or granules).
- Allow sufficient contact time before clean up.
- Sweep up debris gently (do not use brush).
- Pick up broken glass carefully (e.g. forceps or swabs).
- Put debris in a suitable container for safe disposal.
- Disinfect contaminated surfaces with 1% Virkon.

Spillage procedures for accidental exposure of infectious materials (eg injection, ingestion or inhalation).
- Remove contaminated clothing as quickly as possible and leave in lab.
- Remove contamination from skin, eyes and mouth by thorough washing with water.
- Minor cuts and small puncture wounds should be encouraged to bleed.
- Wash wounds with soap and water wiping away from the wound.
- Dress wounds.
- Use PPE if required when helping injured persons.
- Seek help where required - First aid, GP or Hospital.
- Emergencies should be taken straight to hospital and call ambulance if necessary (Security x 6666).
- Explain incident and biological agents or hazards to medical staff.
- Report all accidents immediately or as soon as practicable to the USO.

In the event of an accident or spillage the procedure will be immediately implemented. In the event of any injection injury or other significant accidental exposure seek medical attention immediately.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware.

Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

"I declare that this risk assessment has been scrutinised and approved by the School GM Safety Committee"

Project Containment

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Project Ref 540/11.2

Use of lentiviral vectors in mammalian tissues, cells and cell lines to study disease pathogenesis in vitro and in vivo

Date Ackn'd 05/12/2011

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Consent Granted

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

This application encompasses several areas of research involving several groups within the Institute of Cellular Medicine (ICM) at Newcastle University that will utilise the same general principle of lentiviral gene delivery as a tool to understand disease pathogenesis.

We plan to use various commercial viral vectors (eg from Sigma-Aldrich, Thermo-Scientific), or similar vectors from collaborating laboratories to generate lentiviral vectors encoding the corresponding cDNAs for expression or shRNAs targeted to selected cell components including, but not limited to the examples mentioned below in the different programmes of research. These vectors will be used to obtain infectious, but replication-incompetent lentiviruses for the transduction of mammalian tissues, cells and cell lines. The resulting GMOs will be used for various applications as described below. The programmes of research covered by this notification (PIs; see section 16 for details) are:

- Matrix biology in arthritis (ADR & DAY)
- In arthritis, dysregulated cell signalling and/or gene expression can lead to cartilage damage, typically due to the action of proteolytic enzymes (proteinases) such as matrix metalloproteinases (MMPs) as well as serine proteinases. There is evidence that several cell signalling pathways that are activated by pro-inflammatory stimuli drive the transcriptional activation of genes that are involved in the degradative mechanisms of arthritis.

We wish to validate laboratory observations that specific molecules are important in the overall mechanisms of cartilage and bone destruction. This could be a protein within a signalling pathway or a specific receptor, proteinase, inhibitor or a microRNA. Some genes, and indeed proteins, are rapidly turned over making conventional siRNA problematic. To this end we need to create stable, inducible RNAi knockdown of selected target genes in connective tissues and cells to a) provide prolonged and sustained gene silencing; b) confirm the specificity of small molecule inhibitors for their target (i.e., an inhibitor of protein X should not have any additional effect in a cell lacking protein X); c) to define the cellular pathology following loss/inhibition of specific cell signalling, receptor proteinase or microRNA constituents. We will aim to validate identified genes/targets using lentiviral approaches to drive over-expression of genes and/or mutant versions (eg. Kinase-dead ver, both constitutive and conditional, in human primary cultures and cell lines, to identify downstream targets using molecular biology methods such as microarray and/or chromatin immunoprecipitation approaches. Where indicated, limited in vivo experiments in rats and mice will be performed to further validate target candidates.

- Function of antigen presenting cells in health and disease (AK/JP)
- In order to mount immune responses, specialised mammalian cells termed antigen presenting cells (APC) capture
components (antigens) of invading pathogens. These components are digested by limited proteolysis within the endocytic compartment of the APC and then re-expressed as small peptide fragments on the APC surface in association with MHC molecules. These peptide/MHC complexes are recognised by CD4+ T lymphocytes (T cells) which then differentiate and expand into a number of specialised effector cells capable of limiting or destroying the pathogen. In some diseases which are termed autoimmune, APC capture and present self-components initiating immune responses targeted to various tissues within the body.

One specialised APC type is the B lymphocyte (B cell). Unlike other APC, each individual B cell clone expresses a unique receptor (BCR) for pathogen recognition. Recent evidence suggests that B cells have a unique APC function which is essential for the initiation of several autoimmune diseases including Rheumatoid Arthritis and Primary Biliary Cirrosis. We have recently compared immune activation following antigen presentation by B cells and other APC. This has involved cloning and of several BCRs recognising different antigens and their expression in various immortalised B cell lines. We now wish to extend our findings by expressing various immune related gene products, including antigen-specific BCR (or constructs to silence immune related gene products) in various ex-vivo mammalian immune cells including APC, T and B cells.

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Public Register

Health and Safety

Executive
CU 2 2000 (rev 11/08) Page 3 of 14
Epithelial to mesenchymal transition (EMT)
The process of epithelial to mesenchymal transition (EMT) involving biliary epithelium occurs in some chronic liver diseases and contributes significantly to the organ fibrosis. Fibrosis within organs is triggered by the microenvironment consisting of various growth factors and cytokines which are upregulated during the ongoing disease state. One of the major profibrotic agents is transforming growth factor-beta (TGFβ) and thus we stimulate cell cultures with this cytokine to initiate and monitor EMT in vitro. We have used several markers of EMT to characterise the process however the most reliable one is S100A4 expression which is an early marker of the transition. S100A4 is not expressed in healthy epithelium but appears shortly after addition of TGFβ or in diseased tissue sections which we have shown on protein and mRNA level. The role of this protein is not entirely discovered but it regulates apoptosis (by interaction with p53), migration (it binds heavy chain of myosin and associates with cytoskeleton) and cell differentiation. S100A4 has been shown to play an important role during the process of EMT in renal fibrosis whereas there is very few data regarding the liver. We wish to knockdown S100A4 in our in vitro primary cell culture and study the behaviour of the cells after stimulation with TGFβ. It would be very interesting to know whether inhibition of this marker can prevent epithelial cells from becoming fibroblasts upon stimulation with a profibrotic agent. We would like validate targets like S100A4 via gene silencing or over-expression. It is anticipated that additional groups may want to use these materials for related or similar projects in the future, and appropriate amendments to this risk assessment will be made.

Recipient or parental organism

see below

Host/vector system

Cell lines in culture: 293T Human embryonic kidney cell line (packaging cell line; Pear et al., PNAS 1993; 90; 8392)
SW1353 human chondrocyte cell line. Human embryonic stem cells lines (Newcastle)Murine C3H10T1/2 cells
Primary human cells generated from various tissues including joint tissues (cartilage, bone, synovium), kidney, liver, and immune cells, Human mesenchymal stem cells, Immortalized mouse cell fusions, Primary rodent cells (Mice and Rats) including transgenic animals (Home Office Licence to ADR: PPL/604173 "Joint tissue remodelling in health and
We will be using second and subsequent generation lentivirus vectors derived from FIV and HIV. They are replication incompetent and self inactivating (SIN). The systems separate the packaging signals and viral LTRs on the expression plasmid from the viral structural and expression genes (gag, pol and rev from FIV or HIV and viral envelope glycoproteins such as the VSV-G gene from Vesicular Stomatitis Virus, in place of HIV or FIV env). The viral structural and expression genes and the envelope glycoprotein genes are separated on at least two additional plasmids. Examples of vectors to be used are listed below:

Lentiviral Vector 1 (LV1): the Open Biosystems TransLentiTM Viral GIPZ&TRIPZ Packaging System, allows creation of a replication-incompetent HIV-1-based lentivirus which can be used to deliver and express shRNA in either dividing or non-dividing mammalian cells (Ref.: Shimada et al., Development of Vectors Utilized for Gene Therapy for AIDS. AIDS. 1995; 4). It uses a replication-incompetent lentivirus based on the trans-lentiviral system developed by Kappes et al. (Ref.: Kappes J.C., Wu X. Safety considerations in vector development. Somat Cell Mol Genet. 2001; 26(1-6):147-58.).

Lentiviral Vector 2 (LV2): pHR-SINcPPT-SIEW replication incompetent lentiviral system, obtained from Dr Heidenreich (NICR) (Ref.: Demaison et al., High-level transduction and gene expression in hematopoietic repopulating cells using a human immunodeficiency virus type 1-based lentiviral vector containing an internal spleen focus forming virus promoter. Hum Gene Ther. 2002, 13:803-13). This vector will be cotransfected together with pCMV8.9.1 (carrying HIV-1 gag, pol and rev/tat genes) and pVSV-G (carrying the VSV-G gene for pseudotyping).

Lentiviral Vector 3 (LV3): MISSION shRNA Lentiviral particles, Sigma-Aldrich. The Mission vector system represents a 2nd generation lentiviral system and will be used as an alternative especially when only limited work is envisaged. MISSION TRC transfer vectors contain a modified, self-inactivating 3’ long terminal repeat (SIN/LTR) which renders the resulting lentiviral particles replication incompetent. Specific features include: 1) multiple plasmids are used such that no single plasmid contains all the genes necessary to produce packaged lentivirus. Resultant particles are replication-incompetent. 2) Deletion in U3 portion of 3’ LTR which eliminates the promoter-enhancer region. This avoids promoter interference issues and further negates the possibility of viral replication. 3) Key virulence genes vif, vpr, vpu and nef are deleted. To monitor transfection efficiency we wish to also use a green fluorescent protein, from copepoda Pontellina plumata, expressing lentivirus pLKO.1-puro-CMV-TurboGFP.

Lentiviral Vector 4 (LV4): miRIDIAN shMIMIC Lentiviral microRNAs, Thermo Scientific. The miRIDIAN shMIMIC microRNA designs are created for each human microRNA described in the miRBase database. A CMV promoter drives strong transgene expression, a TurboGFP(nuc) Marker helps to visualize transduction. The SIN-LTR is a Selfinactivating long terminal repeat that results in replication-incompetent viral particles. Puromycin resistance permits antibiotic selective pressure and propagation of stable integrants whilst an IRES (internal ribosomal entry site) allows expression of TurboGFP and puromycin resistance genes in a single transcript.

Lentiviral Vector 5 (LV5): pDBR. (Calderon-Gomez et al 2011, Eur. J. Immunol. 41, 1696-1708.) This is is modified version of pL3.7 (Rubinson et. al 2003. Nature Genetics. 33, 401-6) in which the U6-shRNA/CMV-GFP cassette has been removed and replaced with a multiple cloning site. This replication incompetent vector will be cotransfected with the commercially available, 2nd generation packaging vector (containing TAT; psPax2; http://www.addgene.org/mammalianmrii/Packaging/) and VSV-G (carrying the VSV-G gene for pseudotyping) outlined in LV2.

New Lentiviral Vectors: We will also employ new generation, commercially available vectors systems with improved properties and safety measures as these become available. These will provide improved safety and thus reduced risk to users.

The transfer vector systems are derived from FIV or HIV and have been specifically engineered for biosafety by
separating the packaging signals and viral LTR’s on the expression plasmid from the viral structural and expression
genes (gag, pol and rev from FIV and the VSV-G gene from Vesicular Stomatitis Virus, or similar alternatives, in place
of FIV or HIV env) encoded on three or four separate plasmids, which remain in the packaging cell line, effectively
precluding the production of replication competent virus in the target cell or should the viral vector escape
containment. FIV vectors are included as they may be more efficient for some cell types. It does not imply any
different containment or risk considerations than HIV based vectors. The plasmids expressing these gene products
carry no packaging signals or LTRs and so cannot themselves be mobilised with the vector and have been
engineered not to contain any regions of homology to each other or to the viral vector, to prevent undesirable
recombination events which might result in replication competent virus being produced.
The viral vector and recombinant virus have a broad host range when pseudotyped with e.g. the VSV-G envelope
protein. The normal tropisms of HIV and FIV have been broadened to include multiple tissues from a broad range of
species. Pseudotyping with alternatives to VSV-G, for example measles virus derived glycoproteins, will produce
particles with more restricted tropisms.

Origin & function

 Either genomic or cDNA sequences originating in humans or other eukaryotes or shRNA sequences will be cloned
into the lentiviral vectors. These will include both entire gene sequences as well as partial gene sequences. As well
as “wild type” sequences, we will include sequences that are known to be mutated (either by deliberate genetic
manipulation or by use of cDNA derived from cell lines harbouring known mutations). In some instances clones will be
purchased from Open Biosystems. A few examples of the gene sequences to be used are outlined below:
shRNAs:
• TRB1 (pseudo-kinase)
• HDAC6 (histone deacetylase)
• S100A4 (calcium-binding protein)
• AKT3 (protein kinase)
• NF-κB (transcription factor)
cDNAs:
• MMP-14 (protease)
• ST14 (protease)
• PRSS23 (protease)
• NF-κB (transcription factor)
• PAR-2 (G protein coupled receptor)
• antigen receptors
• other immune genes (cytokines, costimulatory molecules)

These sequences will be inserted into replication deficient lentiviral vectors described above and expressed in the
cells above. The fluorescent protein of the lentivirus vector will enable us to visualise the sub-cellular localisation,
trafficking and cellular consequences of expression of these genes, facilitating our analysis of their function.

Evaluation of foreseeable effects

 The only viral sequences in the transfer vectors are the 3’ and 5’ long terminal repeats (LTR’s), # packaging signal
and the Rev response element (RRE) required for the production of viral particles. Deletion of accessory genes vpr,
vpu, vif and nef mean that the vector is unable to replicate once it has transduced the target cell. In addition third
generation lentivirus are deleted for the tat gene and carry a SIN deletion of the 3’ LTR ($U3) which results in “selfinactivation”
of the lentivirus following transduction of the target cell, precluding adventitious activation of the vector by
endogenous retroviruses and minimising the risk of recombination with ERVs. These vectors also contain a bacterial 
origin of replication and ampicillin resistance gene, an internal promoter such as CMV or SFFV promoter driving the 
gene of interest linked via an IRES element with a gene encoding a fluorescent protein for FACS-mediated sorting. 
Reversion to wild type virus is extremely unlikely given that several recombination events would be needed 
reconstitute an active viral genome, the viral genes are present on three different plasmids which have minimal 
sequence homology and the viruses are self-inactivating following insertion (i.e. the LTR’s are destroyed upon 
genome insertion). There is a long history of the safe use of these viral vectors.

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and the viruses are self-inactivating following insertion (i.e. the LTR’s are destroyed upon genome insertion). There is 
a long history of the safe use of these viral vectors.

**Containment**

While containment level 1 is sufficient to control risks associated with the GM work, in accordance with the ACGM 
Compendium of Guidance 2000, Part 2A, Annex VI, a parallel BioCOSHH assessment has considered hazards 
associate with possible adventitious agents when using mammalian cell culture. The BioCOSHH assessment 
concludes that containment level 2 should be used to control these hazards and consequently all work involving 
mammalian cell culture will be carried out under full containment level 2. This does not alter the assignment of the GM 
activity class. Established standard operating procedures (SOP1, attached) for work at containment level 2 will be 
employed.

**Controls**

In accordance with the SACGM Compendium of Guidance, GMM mammalian cells will be handled at containment 
level 2 and using microbiological safety cabinets (MSC) for operations likely to generate aerosols. All GM virus waste 
will be properly inactivated by autoclaving using established procedures (SOP2, attached). Spillages will be soaked 
with tissue paper, which will afterwards be autoclaved. Contaminated surfaces will be decontaminated with a 1-2% 
virkon solution (SOP2, attached). 
The use of sharps will be prohibited. Only plastic pipettes will be used in the tissue culture facilities. Furthermore, all 
contaminated materials and media including waste destined for subsequent incineration will be autoclaved (see 12). 
The generation of aerosols will be avoided or contained by use of MSC’s.

Given the uncertainty about the hazards of most potentially oncogenic sequences and the small quantities used 
(microlitre/microgram quantities), prevention of exposure or total enclosure is not “reasonably practicable”. Therefore 
the following measures are applied in the light of this GM risk assessment, will be used:

1) Good microbiological practice will be used at all times and strongly emphasised by senior staff. Designated 
workers will be trained in good microbiological practice before commencing any work with potentially oncogenic DNA 
sequences. They will be made fully aware of the potential hazards of such work and what to do in the event of 
accidental exposure.

2) Access to the laboratory where novel DNA fragments which could potentially be oncogenic is handled, will be 
limited to authorised personnel and designated workers on this project?

3) All experimental procedures involving naked DNA will be performed so as to minimise aerosol production. 
Procedures which are likely to generate aerosols such as the use of sonicators, vigorous shaking and mixing etc. will 
be avoided, or where necessary, will be carried out in closed containers or a class II MSC.

4) Procedures using lentiviral particles, packaging cell lines and exposed cells will only be carried out in the tissue 
culture suite. Normal procedure for handling biological material in the TC suite will be largely sufficient for handling the 
cells and particles. Specific items of relevance here include:
Safety cabinets are routinely disinfected prior to and following use.
Gloves and lab coats to be worn throughout.
Designated microbiological safety cabinets to be used.
No wild type virus will be used in same MSC.
Each packaging lentivirus will be monitored for their ability to produce infectious particles. Cell culture supernatants will be tested 3-5 days after infection for the presence of infectious particles by addition to 293T cells followed by monitoring the expression of encoded genes or their function (e.g. positivity for fluorescent protein by FACS).
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment such as glassware (SOP3, attached).
Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

"I declare that this risk assessment has been scrutinised and approved by the School GM Safety Committee"

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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
Project Additional Information

**Purposes of the contained use**

The work performed in the fibrosis laboratory in ICM is concerned with different aspects of transcriptional regulation, cell and organ function as well as therapeutic modalities of the Nuclear factor kappaB (NF-kappaB) family of proteins and their regulators. This includes processes such as transcription and DNA replication, regulation of signalling cascades and appropriate functioning of various organs. NF-κB is a family of transcription factors that come in 5 different forms; namely p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2). These proteins bind in different dimer combinations to gene promoters and regulate gene transcription. They are often bound in the cytoplasm by inhibitory proteins known as inhibitors of NF-κB or IκBs. There are seven IκB proteins namely IκBα, IκBβ, IκBγ, IκBε, Bcl-3 and the precursor proteins p100 and p105. NF-κB dimers are released from IκB proteins when the latter are phosphorylated by the actions of the Inhibitor of NF-κB Kinases (or IKKs). There are three IKKs namely IKKα (IKK1), IKKβ (IKK2) and NEMO (IKKγ). For a general review of the NF-κB pathway please see Hayden and Ghosh 2004, Genes and Development, 18, 2195-2224.

Growing evidence presented by multiple groups has culminated in the hypothesis that pathogenesis of disease in organs which are susceptible to fibrosis i.e. liver, skin, kidney and lung follows a common course; rounds of chronic inflammation/damage → organ fibrosis → increased susceptibility to develop cancer. This is known as the "inflammation-fibrosis-cancer axis". NF-kB family members can influence every step of this disease continuum, therefore we aim to understand the molecular events underpinning this axis in multiple organs which are susceptible to inflammatory and fibrotic disease, as common pathways are likely to exist, thus allowing us to identify generic drug targets.

We have previously reported that the absence of one of the NF-κB subunits, p50, in a mouse model results in increased inflammation and fibrosis (scarring) in the liver compared to controls in response to chemical injury. In addition these mice develop more liver tumours than controls. We have also shown that the absence of the c-Rel, a different NF-κB subunit, results in protection from the effects of liver injury on hepatic inflammation and fibrosis. However, in liver regeneration models these mice exhibit a reduced capacity to regenerate. The individual NF-κB subunits have discrete biological functions and these data highlight the importance of studying the exact role of each subunit and their post translational modifications in the pathogenesis of liver fibrosis, cancer and regeneration. In addition, the role of the
The role of this project is to investigate the exact gene targets and regulatory mechanisms of the different components of the NF-κB pathway mentioned above in the pathogenesis of liver disease. An important tool in allowing us to achieve this will be the use of adenoviral vectors to deliver wild type and functionally inactivated forms of the NF-κB subunits or their associated signaling proteins to the primary cells in vitro as well as liver of animals that will be used in models of liver fibrosis, regeneration and cancer (see associated GM 10/31.form for use of adenoviruses in animals).

### Recipient or parental organism

Adenovirus containing constructs expressing NF-κB proteins or mutants which have inhibitory or stimulatory functions will be given intravenously to both normal and genetically modified mice e.g. gene knockout or knock-in mice for members of the NF-kappaB family or their associated adapter molecules, or stimulating receptors including:

- Nfkb1 knockout mice
- TLR3 knockout mice.
- Mkk4 knockout mice.
- RelB knockout mice
- TLR4 knockout mice.
- Mekk1 knockout mice.
- c-rel knockout mice
- TLR9 knockout mice.
- TNF Receptor knockout mice.
- 3xNF-kappaB-luciferase reporter mice
- Myd88 knockout mice.
- IL-6 knockout mice.
- TpL2 knockout mice.
- ASK1 knockout mice
- IL-10 knockout mice.
- TLR2 knockout mice.
- Lys (TRIF-deficient mice) knockout mice.
- RelA+/- (Hets).
- Knockin of mNfkbp1 gene (S340A).

Or mouse cells: primary inflammatory and liver cell types which will be used for expression of the adenovirally encoded mutant/wt NF-kB subunits and their associated signalling or adapter molecules are:

1. Mouse/rat hepatocytes
2. Mouse/rat hepatic stellate cells
3. Mouse/rat endothelial cells
4. Mouse/rat myofibroblasts
5. Mouse/rat Bone marrow
6. Mouse/ rat fibroblasts
7. Mouse/rat Inflammatory cells
The Adenovirus being used; ad5-attenuated is replication deficient and will not lyse human cells. Adenovirus will either be obtained from a commercial supplier or collaborator. Additionally the adenovirus may be propagated in Vero/293 cells. Adenovirus will either be obtained from a commercial supplier or collaborator as described in GM 10/30 (virus vector and cell culture work).

The constructs used in this study will be expressing NF-kB Family proteins or mutants which have inhibitory or stimulatory functions. Although not all of these proteins are currently been expressed in adenovirus, many have been used in adenovirus or other systems such as baculovirus or Herpes Virus Saimiri without causing adverse effects or leading to immortalisation of cells, examples of which are described in GM 10/30 (virus vector and cell culture work).

The specific genetic material to be studied includes:

Human cDNAs for the various NF-κB family members (listed below) or mutant forms of these proteins or interacting/regulatory proteins (some may be conjugated with inert tags such as FLAS, green fluorescent protein (GFP) or haemagglutinin (HA) 9 amino acid epitope added for identification) will be inserted into adenovirus. The intention is to express these genes in mammalian cells and use various techniques to analyse their effect on gene expression, transcription factor function, the cell cycle and apoptosis. We also intent to express mutated versions of NF-kB transcription factors which may interfere with dimerisation and/or DNA binding.

The cDNAs encode for wild-type or mutant forms of:

1) RelA
6) IKKa
2) p50
7) IKKb
3) p52
8) NEMO
4) RelB
9) IkBa
5) C-Rel
10) Other kinases or phosphatases that may be shown in future studies to affect NF-kB signalling pathways.

We intend to express mutated forms of above proteins where mutations affect the ability of particular serine or threonine residues to be phosphorylated (i.e. mutation into alanine). We may also generate cDNAs where same serines or threonine have been mutated into aspartic or glutamic acid thus generating a constitutively active phosphomimic. It is impossible to predict what function these novel mutations may have on the cells transduced. The reason for this is the fact that phosphorylations of different subunits have different functions in different cells types. E.g. phosphorylation of S536 on RelA signals for termination of NF-kB activity in macrophages, whereas the same modification in hepatic stellate cells and hepatocytes has the opposite function.

Adenovirus will be given to mice by intravenous injection via the appropriate vessel e.g. tail vein or intra-hepatic or transduced cells may be administered via the appropriate route e.g. via the tail vein or intra-hepatic and then liver injury models or controls will be performed. We will then determine whether expression of these genes affects the injury process by assessing liver pathology using histochemical and biochemical techniques. We will measure downstream targets of our expressed proteins e.g. markers of inflammation, cell cycle and apoptosis by qRT-PCR.

These studies will allow us to discern the role of the individual NF-kB subunits and their post-translational modifications in the pathogenesis of liver disease and enable us to dissect out subtle regulatory pathways that would otherwise be impossible to study.

Evaluation of foreseeable effects

The Adenoviral vectors to be used are "attenuated" through deletion of the E1 region of the genome (containing E1A and E1B) and through insertions and deletions within the E3 gene that inactivate it. This has several affects on the virus. Firstly deletion of the E1A gene eliminates the potential for viral transformation of cells, since the E1A gene product is absolutely required for this process. It is also required for activation of all other early genes (E1B, E2, E3 and E4). The E1B gene product (also deleted) co-operates with E1A in transformation. The E2 region (E2A and E2B) contains DNA binding (E2A) and DNA polymerase (E2B) activities that are absolutely essential for viral replication. The E3 gene product helps in viral avoidance of the immune system by binding to the major histocompatability complex MHC polypeptides in the endoplasmic reticulum. However, this gene is also inactivated in this strain of adenovirus, resulting in the virus being highly susceptible to immune surveillance. The E4 gene product is required for formation of an active complex between the E1B gene product and the E4 gene product.

The above characteristics mean that the recombinant virus is totally unable to replicate in E1A- deficient cells of any organism. Under normal circumstances no human or
animal cells contain the E1A gene product, resulting in the inability of this virus to replicate in any naturally occurring organism in the external environment (human or non-human mammalian). The recombinant virus is also unable to recombine with a wild-type virus in a way that could generate a functional virus (recombination with a wild-type virus would, by the nature of the recombination event, remove the E1 region from the wild-type virus thereby inactivating its ability to replicate). Taken together, the above means that the recombinant virus is essentially unable to propagate in the external environment.

The inserts to be used in this study, based on the NF-kB pathway and associated proteins have not been reported to cause immortalisation or transformation in human cells. The effects of over-expressing some of the various NF-kB pathway gene products have not been previously characterised in terms of immortalisation or transformation, however. It is possible, therefore, that changing the expression of genes from this pathway could be harmful. The inhibition or activation of the NF-kB pathway could result in activation or inhibition of pathways that regulate the cell cycle and apoptosis, i.e. they could potentially be oncogenic. NF-kB also regulates inflammation and immune function. It is also possible that expression of these proteins could therefore produce adverse inflammatory effects and immune suppression. However, while the inserts would be expected to have biological activity the most likely effect if a human became infected could be a localised increase in inflammation. Although, as described above, these genes are known to play a role in cancer they have not been shown to be transforming in their own right in human cancer cells. In vivo: Viral particles capable of transducing a wide range of mammalian cells will be used in our experiments and these represent the hazardous material. However, because of its incompetence to replicate, short half-life and inefficient transduction rates, the risk of transmission outside the laboratory is effectively zero. Although there is only very low risk associated with an infected animal, this work still involves the use of an infectious agent that is able to infect human cells. Risks associated with working with infected mice could include might scratching/bite during handling or their waste products might be infectious. All researchers will wear gloves and laboratory coats, handling will be kept to a minimum and bedding will be incinerated to minimise risks. Additionally transfer of a replication deficient virus via a scratch or bite represents a very low risk. The biggest risk is a needle stick injury as a researcher might stab themselves or someone else with a syringe and inject harmful material. To control this risk animals will be restrained in appropriate restraining tubes, needles will not be re-sheathed but disposed of directly into sharps bins (for incineration) and only one person will give the injection to minimise the risk of a needle stick injury. All staff and students will undergo training in loading syringes and injecting virus and for the handling and disposal of needles. Personnel will also be instructed in the emergency procedures in the event of accidental contamination through needle stick injury or wounding.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All handling and packaging of the virus will be carried out in CL2 labs in class 2 microbiology safety cabinets so as to minimise aerosol spread. Any transport of materials to the animal facilities will use clearly labelled leak proof, drop-tested containers. All GM virus or animals infected with GM virus will be stored and handled only inside Containment Level 2 animal room and laboratory suite (Room MB108) in the CBC animal facility. Isolators will be used for additional safety. The animal facility is isolated from the rest of the University and access is by card and keypad. Holding and procedures rooms are maintained at negative pressure relative to the surroundings. The PI and the research team will control the specific risks of the project.

1. All handling and packaging of the virus will be carried out in CL2 facilities in class 2 microbiology safety cabinets so as to minimise aerosol spread. Transport of infectious materials to the animal facilities will use clearly labelled leak - proof and drop – tested containers. All GM viruses or animals infected with GM virus will be stored and handled only inside Containment Level 2 animal room and laboratory suite (Room MB108) in the CBC animal facility. Isolators will be used for additional safety. The animal facility is isolated from the rest of the University and access is by card and keypad. Holding and procedures rooms are maintained at negative pressure relative to the surroundings. The PI and the research team will control the specific risks of the project.

2. Replication defective viral vectors are used to ensure proliferation occurs only in special packaging cell lines or in the presence of complimentary plasmids;

3. All in vitro work is conducted in CL2 facilities with CL2 operating procedures applied in full to prevent infection of the worker or release of virus into the external environment.

4. Good microbiological practice will be used at all times and strongly emphasised by senior staff. Designated workers will be trained in good microbiological practice and working at CL2 before commencing any work with potentially infective materials. They will be made fully aware of the potential hazards of such work and what to do in the event of accidental exposure.

Staff carrying out the techniques will wear PPE (lab coats, gloves, lab specs) at all times during work. Staff will also be encouraged and expected to attend the USO biological and GM safety training courses in order to keep up with the most appropriate procedures. All personnel dealing with the animals will be required to wear protective clothing including but not limited to lab coats, gloves and removable footwear. They will also be expected to have read and understood any risk assessments pertaining to this project and to have documented this.

5. The users will also use microbiological safety cabinets to prevent risk of exposure by inhalation route. All aspects of work will be contained in sealed tubes during
centrifuging, storage and transport. Any sharps will be discarded into the autoclave bins within the class II cabinets and sealed prior to removing. All cabinets will be disinfected using 1% Virkon to ensure no exposure by subsequent users.

6) Sharps use will be kept to a minimum, as needle stab injury is a possible risk. The use of sharps in CL2 facilities is strongly discouraged and all precautions will be taken to identify procedures or devices that might be used in these projects which could cause an injury and infection of the researchers. Where so identified safer alternative methods or equipment will be sought. Where the use of such items is essential staff / students will be appropriately trained in their handling and disposal; sharps will be discarded into bins within the class II cabinets and sealed prior to removing for autoclaving and incineration.

7) The CBC is a controlled access area and measures are in place to ensure animals are not removed from the department. The animals are maintained in closed cages in rooms with self-closing doors. Access is via at least 3 additional self-closing doors and an external access door controlled by a keypad and swipe card. The doors are all flush to the floor. The floor drains are screwed down to the floor and have very small drainage holes, much too small for a mouse. The mice are all checked daily and records are kept of the numbers in each cage. All virally infected animals will be killed, at the end of the experiment, with the carcass being incinerated.

8) Bedding may be contaminated with cells, virus or waste products, however, it is unlikely that these cells or virus would be viable and, as these have limited ability to survive outside the laboratory, the risk can be considered negligible however as a precaution all bedding materials will be incinerated after autoclaving.

9) Workers showing an allergic response to the animals are not required to come into contact with them. Consequently, there is a low risk to human health. All staff involved are regularly assessed for signs of allergy.

10) Additional control measure concerning waste materials derived from routine culture and preparation of cells for implant are details on the accompanying form GM risk assessment GM10-30.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In vitro use: All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

In vivo use: All contaminated materials, including bedding, waste destined for incineration and animal carcasses, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Waste will be transported in leak proof containers. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min). Autoclaved waste will then be incinerated following standard university procedures. Records will be kept for all inactivation and preventative procedures

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessments associated with this project have been scrutinised and approved by the local genetic modification safety committee. The members of the committee were satisfied that all foreseeable risks associated with the GM hosts and vectors had been identified and that the risks to human health and the environment were minimised and controlled by the declared use of facilities, equipment and operating procedures for containment of the GM organisms.

02/03/2022
This risk assessment covers research projects at the Northern Institute for Cancer Research utilising the same or functionally very similar lentivirus vectors and mammalian host cells. In addition these projects share similar aims and use the same approaches. We are submitting a generic risk assessment for these projects after discussions with HSE inspector. The projects involve the assessing the biological function of candidate genes implicated in malignant progression which emerge from ongoing genomics projects characterising collections of primary tumour material. Some projects are screens to assess the functional relevance of various genes/proteins systematically in a more global fashion.

Typically the methodology will involve a gene highly over or under-expressed in a particular cell line and reversing the expression by either overexpressing the gene or expressing a shRNA to knock-down expression of the gene in question. Lentiviral particles will be generated on site containing either a cDNA or appropriate shRNA.
cDNA sequences of humans or mice origin and/or the appropriate shRNA sequences will be cloned into the lentiviral vectors. These may include partial or entire gene cDNAs or shRNAs may be used to create a pool of cells with several integrated constructs which will then undergo in vitro or in vivo selection to screen for constructs which either promote or inhibit leukemic cell proliferation or engraftment.

Recipient or parental organism

Packaging cell line 293T (and thereof derived cell lines) is derived from the well-characterised 293 cell line (Pear et al., PNAS 1993; 80; 8392; ATCC CRL-11268). This cell line stably and constitutively expresses a temperature-sensitive version of the SV40 large T antigen. This cell line will be used to produce lentiviral particles. These vectors alone are replication incompetent owing to their lack of envelope and gag-polymerase encoding sequences. Virus is therefore produced by co-infection of the 293T cell line with lentiviral vector along with one or more viral envelope encoding plasmids (e.g. VSV-G or constructs derived from the measles virus [MV] hemagglutinin and fusion protein genes [HΔ24 and FΔ30]) and a plasmid encoding the HIV or FIV gag-pol and the regulatory genes including rev, nef and vif. Plasmid DNA will be produced in standard K12 E-coli hosts but mammalian promoters would preclude expression of ORFs.

Mammalian cell lines and primary mammalian cells will be used for infection with the supernatant generated by the packaging cells. Cell lines such as MT2 or MT4 producing infectious particles will not be used for transduction. Most cell lines are derived from solid tumours or leukaemias. All cell lines have a long history of safe use and are commonly handled in UK labs at CL2 or CL1. All facilities at Newcastle University where these materials are handled are at CL2 standard. Use of new cell lines will be registered with the UBSO as a minor addition to the risk assessment summarising their properties and status. The mammalian cells and somatic stem cells are only able to survive if maintained in tissue culture in the laboratory, or if injected into inbred syngeneic or immunodeficient animals.

Host/vector system

The transfer vector systems are derived from FIV or HIV and have been specifically engineered for biosafety by separating the packaging signals and viral LTR's on the expression plasmid from the viral structural and expression genes (gag, pol and rev from FIV and the VSV-G gene from Vesicular Stomatitis Virus, or similar alternatives, in place of FIV or HIV env) encoded on three or four separate plasmids, which remain in the packaging cell line, effectively precluding the production of replication competent virus in the target cell or should the viral vector escape containment. FIV vectors are included as they may be more efficient for some cell types. It does not imply any different containment or risk considerations than HIV based vectors. The plasmids expressing these gene products carry no packaging signals or LTRs and so cannot themselves be mobilised with the vector and have been engineered not to contain any regions of homology to each other or to the viral vector, to prevent undesirable recombination events which might result in replication competent virus being produced.

The viral vector and recombinant virus have a broad host range when pseudotyped with e.g. the VSV-G envelope protein. The normal tropisms of HIV and FIV have been broadened to include multiple tissues from a broad range of species. Pseudotyping with alternatives to VSV-G, for example measles virus derived glycoproteins, will produce particles with more restricted tropisms.

Origin & function

cDNA sequences of humans or mice origin and/or the appropriate shRNA sequences will be cloned into the lentiviral vectors. These may include partial or entire gene sequences, or mutant forms of the gene sequences, under the control of constitutive promoters. In screening applications, many of the genes or targets of the shRNAs will have unknown function but may include known oncogenes or tumour suppressors. In the case of mutated genes, these will comprise of characterised sequences which have deliberately mutated by site directed mutagenesis. An example of a mutation that is constitutively activating might be a KRASv12 mutation which has been demonstrated to result in overactive ras signalling and therefore again should be considered to pose a greater hazard than the wildtype sequence. Other targets may include known proto-oncogenes/tumour suppressors such as RUNX1, ERG, GRIK2, PARK2, FOXO3, CRLF2, TP16, TP53 or TERT.

The aim is to use either overexpression or knock-down of gene-expression to assess the potential contribution to tumourigenesis of each candidate identified from our genomics screens. Once a stably infected cell line is produced experiments performed will depend on the particular gene selected but will typically involve a panel of typical proliferation, apoptosis and/or invasion assays as well as collection of RNA/DNA/Protein for further profiling analysis and potentially in vivo engraftment.

Evaluation of foreseeable effects

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Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

I declare this risk assessment has been scrutinised and approved by the School GM Safety Committee

Project Containment

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<th>Human Clinical Applications</th>
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02/03/2022
This risk assessment covers research examining Urinary tract infections (UTI) caused by uropathogenic Escherichia coli (UPEC) are one of the most prevalent microbial diseases in the UK. These infections are often self-limiting so a key public health challenge is to reduce antibiotic usage. The project will utilise an in vitro system to monitor phenotypic adaptation of UPEC during prolonged growth conditions. The objective is to increase our understanding of how recurrent infection occurs and identify feasible biomarkers for application in management strategies.

To achieve this, our experimental plan aims to establish continuous-cultures of laboratory and clinically derived UPECs in growth media supplemented with and without antibiotics. We will monitor phenotypic adaptation to this environment through bacterial growth rate, flagella and fimbriae expression, bacterial persistence and activation of host innate defence systems. This controlled modelling approach will enable us to plot the time course of phenotypic adaptation and so link phases of phenotypic adaptation UPEC to susceptibility to both innate and external bactericidal agents.

The genetic manipulations we will carry out in doing experiments to investigate these problems are all aimed at improving basic understanding of UPEC pathogenesis in a model growth system. We will apply a wide range of genetic modifications, including gene knock outs, insertion of reporter genes, such as LacZ and gfp (and its variants), insertion of regulated promoters and mutagenesis with transposons. Resistance genes will be used as selective markers for these constructions but these will comprise well tried and tested genes that do not constitute a threat to the evolution of resistant pathogens.

Strains to be used:
- Escherichia coli K-12
- Escherichia coli BL21
- Escherichia coli CFT073 - ACDP Class 2 approved
E. coli K-12 and BL21 strains: E. coli K-12 strains and derivatives (e.g. MG1655, W3110, C600 MC1000, JM109, DH5) are defined as disabled or non-colonising in the SACGM Compendium of Guidance (Part 2A, Annex 11, para 11) and is therefore equivalent to an ACDP hazard group 1 organism. E. coli BL21 and derivatives are described in SACGM Newsletter 30 (Nov. 2001, para 3) as being "broadly equivalent to K12 strains and that in most cases work which uses this host can be considered as a class 1 activity, but the cloning of a bacterial pathogenicity determinant into BL21 will need careful consideration and may in some cases warrant classification as class 2".

E. coli CFT073 and clinical isolates: Although CFT073 was originally a natural pathogenic UPEC isolate, it has been maintained and passaged in the laboratory for several decades. CFT073 will still be handled as a potential pathogen following the same protocols we will use to handle the known clinical isolates. Taking into consideration the mode of infection required for healthy individuals for UPEC to cause symptomatic infections, the hazard risks associated with CFT073 and the clinical isolates are still considered to be low.

Host/vector system

Vectors to be used:
- E. coli plasmid vectors such as pUC, pBR322, p15A, R1, F, RK2/RP4, RSF1010 and pSC101;
- E. coli bacteriophages P1, λ and M13

All vectors to be used are standard vectors with a long history of safe use in the laboratory, or derivatives thereof. These include non-mobilisable or mobilisation-defective E. coli K-12 vectors such as pUC, pACY184, pLG339, pBR332 and derivatives including, for example, the pET and similar expression vectors. We also use non-mobilisable derivatives of plasmids R1 and F. Moreover, the use of non-mobilisable or mobilisation defective vectors minimises the likelihood of transfer to other species. Some experiments will employ the non-self transmissible but mobilisable 'suicide' vectors e.g. pK18 mob. These vectors will be constructed in standard E. coli K-12 hosts that do not support the mobilisation of the plasmid. These suicide vectors are constructed to allow redundancy of target plasmid, i.e. in the very unlikely event of unintended mobilisation to a different strain or species these vectors would be unlikely to be able to replicate outside E. coli and so should not constitute any greater risk. Moreover, horizontal transfer genes between different E. coli strains already occurs in nature. However all these plasmid derivatives are essentially listed as Group 1 in the SACGM Compendium of Guidance 2000 (Part 2A Annex 11, 25, 26 and 35). Bacteriophage manipulations using P1, λ and M13 are confined to E. coli. Genetic manipulations involving generalised or specialised phage transduction would be used to move genetic markers from various backgrounds within a species (e.g. P1). The marker will usually involve the disruption or manipulation of specific genes involved in essential cellular processes, as such is unlikely to pose any increased hazard above the handling of the wild type strain, which would then be transduced to other genetic backgrounds.

Origin & function

The vectors will contain drug resistance markers already widely distributed in nature. Resistance markers (cat, bla, aphA, spc, tet, and hyg), reporter genes (such as lacZ, lux, gfp (and derivatives), etc.), epitope tags (such as FLAG, HA, c-myc, 6xHis, etc), artificial promoter constructions (e.g. PBAD). Genes will, in general, be derived from the host organism (e.g. genes directly involved in pathogenicity, introduced by either a plasmid replicon or inserted directly into the host genome.

Evaluation of foreseeable effects

Drug resistance markers used in these vectors are already widely distributed in nature. Strain construction will involve plasmid derivatives passing through E. coli K-12 for amplification prior to reintroduction into the host bacterium. It is therefore possible that these constructs could have biological effects on E. coli K-12 and UPEC, but it is difficult to envisage the hazard being significantly greater than the handling of the parent strains from which the DNA was isolated. In general the GMs proposed fall into two categories; firstly, gene disruptions where no functional gene product should be produced. These plasmids will then be used to generate chromosomal disruptions in the chromosomes of the host bacterium, so doing the plasmid replicon will either be destroyed, or will be non-cloned in plasmids in such a way as to be controlled by artificial promoters, e.g. pBAD, ppt and synthetic lac is not functional, or used as expression systems to allow the purification of the encoded gene product. Since the genes to be targeted are though to be associated directly with pathogenesis, it is highly unlikely that their manipulation and removal will benefit pathogenesis.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The work is category 2 it will be carried out under full containment level 2 (see Compendium of Guidance, Part 3) conditions this will include the use of microbiological safety cabinets where appropriate and full PPE compliance.

Furthermore a strict policy of hand washing on leaving the laboratory when handling E. coli/UPEC is in place. All contaminated materials, including waste destined for incineration and spent culture media, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Annual thermocouple mapping will validate autoclaves and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Where this option of decontamination is not possible (e.g. small spills on benching or floor and minor contamination of microscopes or spectrophotometers) an alternate chemical decontamination method will be employed; i.e Peroxygen compounds - VIRKON at a concentrate of 1%.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Reviewer 1 - SB Institute for Genetic Medicine.
Following my review this looks like a very well written proposal and seems suitable to me to be approved as a Class 2 project under CL2 conditions.

Reviewer 2 - CM Institute for Ageing and Health
Following review attached are my thoughts*, very few comments as this seems a fairly comprehensive assessment. Only thing to do is update references to current SACGM. I agree with CL2 if this is looking at wild type E. coli.

* The attached document contained grammatical improvements not containment control improvements.

Reviewer 3 - AK Institute for Cellular & Molecular Biosciences. Please find attached below a Class 2 GM project from PA & JH on UPEC. It has been through the ICAMB GM committee and suggested revisions have been incorporated and we approve this project for submission.

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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
**Project Ref** 540/14.1

**Date Ackn'd** 04/03/2014

**CU2 Project Title** Investigating regulatory networks in prokaryotic cells: routine cloning, manipulation and transcriptional expression of DNA sequences from other hazard group 1 and 2 microorganisms in disabled E. coli strains using a range of vectors

**Class** Class 2

**CultureVol** < 1 Litre

**Class Culture** Class 2

**Class Volume** Class 3-4

**Non-GMM** Not Applicable

**Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

### Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

### Project Additional Information

#### Purposes of the contained use

The principles and goals of Synthetic Biology are to design and employ useful DNA parts to construct, standardise, modify and re-structure nucleic acid sequences, genes and natural biological systems in order to understand and provide new solutions for public health, energy and environmental challenges. E. coli and Pseudomonas spp may serve as prokaryotic platforms for construction of biological logic and memory circuits for further facilitating synthetic biology studies. This notification is for routine cloning, manipulation and transcriptional expression of DNA sequences from other microorganisms (ACDP hazard group 1 and 2) into disabled E. coli or Pseudomonas spp, parental strains or isogenic mutants. To establish bacterial platforms the procedures involve (i) sub-cloning, DNA sequencing, site-directed mutagenesis or transposon random mutagenesis; (ii) construction or genomic insertion of fusions with harmless reporter genes such as lux, phoA, gfp, lacZ; (iii) construction of recombinant plasmids for subsequent transformation or mutagenesis of prokaryotic cells (e.g. Pseudomonas spp); (iv) construction of recombinant plasmids for the purpose of transcriptional expression of bacterial DNA sequences; (v) construction of biological logic and memory circuits in prokaryotic platforms.

### Recipient or parental organism

Two bacterial species are involved in this notification which are Escherichia coli and Pseudomonas spp. Disabled E. coli K12, B strains and their derivatives such as DH5a, S17-1, BL21 (DE3) as defined in the Compendium of Guidance will be used. They are considered as ACDP group 1 and not pathogenic. Recent research commissioned by the HSE (Chart et al. 2000), An investigation into the pathogenic properties of E. coli strains BLR, BL21, DH5a and EQ1 (J. Applied Microbiology, 89, p1048 – 1058) showed that BL21 is unlikely to be pathogenic, lacking any of the pathogenic mechanisms associated with E. coli strains involved in enteric infections. BL21 may be considered unlikely to colonise and establish a persistent infection in the gut of a healthy individual. The Pseudomonas spp. strains used in this notification have been isolated from a wide variety of sources including historical clinical environments that over the years became well-established laboratory strains. They include ACDP hazard groups 1 (P. fluorescens, P. putida, P. syringae) and ACDP hazard groups 2 (P. aeruginosa).
Pseudomonas aeruginosa is a Gram-negative bacterium commonly found in soil and ground water. It rarely affects healthy people and most community-acquired infections are associated with prolonged contact with contaminated water. Although P. aeruginosa is an opportunistic pathogen (i.e. more likely to infect patients who are already very sick as opposed to healthy individuals), it can cause a wide range of infections, particularly among immunocompromised people (HIV or cancer patients) and persons with severe burns, diabetes mellitus or cystic fibrosis. Serious Pseudomonas infections usually occur in people in the hospital and/or with weakened immune systems. Infections of the blood, pneumonia, and infections following surgery can lead to severe illness and death in these people. The most significant potential routes of exposure to Pseudomonas are from the direct contact with the bacteria, but other routes of exposure may take place depending on the strain and circumstances. The risks of infection are very low for healthy individuals and the infections could be efficiently eradicated by antibiotic therapy.

Host/vector system

Bacterial hosts used in this notification are mentioned as above.
A range of vectors as defined in the Compendium of Guidance will be used to introduce recombinant DNA sequences into E. coli. Non mobilisable: pACYC184, pUC series and their derivatives (pUC19, pUC57), or other vectors Bom-/(Nic-), Mob- and Tra-.
Mobilisable defective: Pinpoint, pBR322, pET30, pTrc99A or other vectors Bom+//(Nic+) but Mob- and Tra- which can efficiently be mobilised if they are co-resident with certain other plasmids.
Shuttle vectors: carrying simultaneously dual E. coli (R6K, ColE1 or p15A) and broad host range replicons (e.g. Pseudomonas) such as pVS1 derivatives (pME60xx series), pRO1600 derivatives (pUCpXx series, pFLP2, etc.) and ColE1 derivatives (e.g. pME3087, pME3088, pHKBS1, etc.).
Mobilisable Suicide vectors: these plasmids with R6K replication origin (e.g. pGP704, pRL27, pLM1, pKNG101, pDM4, etc.) can only replicate in E. coli supplying with pir gene (e.g. strain S17-1 λpir) and have narrow host range. These plasmids will only be used for construction of recombinant plasmids in E. coli for subsequent conjugative to defined bacterial strains such as Pseudomonas spp.
A range of suicide vectors that are unable to replicate in Pseudomonas spp. will be used for the construction of the mutations of Pseudomonas.
Non mobilisable: pACYC184, pUC19, pUC57, or other vectors Bom-/-(Nic-), Mob- and Tra-
Mobilisable defective: Pinpoint, pBR322, pET30, pTrc99A or other vectors Bom+/-(Nic+) but Mob- and Tra- which can efficiently be mobilised if they are co-resident with certain other plasmids.
Mobilisable Suicide vectors: these plasmids can only replicate in E. coli (e.g. strain S17-1 λpir) but not in Pseudomonas and are used to mobilize plasmids from E. coli into Pseudomonas including R6K derivatives (e.g. pGP704, pRL27, pLM1, pKNG101, pDM4, etc.). The suicide vectors may also contain transposons such as Tn5 (pUT-miniTn5 and derivatives) and Tn7 (pUC18R6KT-mini-Tn7T and derivatives), Tn917 (pTV series) or other of similar nature in order to create insertion mutants.
A range of vectors will be used to introduce recombinant genes and DNA parts into Pseudomonas spp once a functional replicon in these organisms has been incorporated into the vector when required.
Non mobilisable: pUCPxx derivatives (e.g. pUCP18), pBRR1 derivatives (e.g. pBBR1MCS-x series, pME6000, etc.) or other vectors Bom-/-(Nic-), Mob- and Tra-
Mobilisable defective: Pinpoint, pBR322, pET30, pTrc99A or other vectors Bom+/-(Nic+) but Mob- and Tra- which can efficiently be mobilised if they are co-resident with certain other plasmids.
Shuttle vectors: carrying simultaneously dual E. coli (R6K, ColE1 or p15A) and Pseudomonas or broad host range replicons such as pVS1 derivatives (pME60xx series), pRO1600 derivatives (pUCpXx series, pFLP2, etc.) and ColE1 derivatives (e.g. pME3087, pME3088, pHKBS1, etc.).
Mobilisable Suicide vectors: these plasmids can only replicate in E. coli (e.g. strain S17-1 λpir) but not in Pseudomonas and are used to mobilize plasmids from E. coli into Pseudomonas including R6K derivatives (e.g. pGP704, pRL27, pLM1, pKNG101, pDM4, etc.).

Origin & function

In order to fit the principles and goals of Synthetic Biology, genes and DNA parts from ACDP hazard group 1 and 2 organisms will be cloned into these vectors, and housed either in E. coli or in Pseudomonas spp. For example, the bacterial quorum sensing (QS) systems from other gram-negative bacteria (e.g. elastase, LasA protease rhamnolipids, type III flagellar-mediated swimming motility and pyocyanin in Pseudomonas aeruginosa) will be cloned into E. coli; the restriction-modification (RM) systems from E. coli will be cloned into P. aeruginosa or P. putida for methylation of the introduced plasmid for further studies; DNA partition (parAB) and DNA transportation systems from other bacterial organisms (e.g. Neisseria) will be introduced into bacterial hosts for DNA manipulation; transcriptional regulators (e.g. LazI, AraC and TetR) or promoters (e.g. cIa and cI434 from E. coli bacteriophages A and 434, respectively) from various ACDP hazard group 1 and 2 microorganisms will be cloned for constructing logic circuits or gene regulatory networks; computer-aided designed (CAD) DNA sequences without harmful biological functions will be cloned for constructing nucleic acid structures. The majority of genes and DNA parts for this project have been described in the standard biological parts website (http://parts.igem.org/DNA). Occasionally,
novel promoters, aptamers and regulators (such as novel quorum sensing (QS) genes (luxI/R) from other gram-negative bacteria) will also be cloned into E. coli. The vectors may contain fusions reporter genes such as lux, phoA, gfp, lacZ and antibiotic resistance markers (cat, bla, kan, spe, tet, erm) that are already in routine use in standard cloning vectors.

The vectors may contain transposons such as Tn5, Tn7, Tn917 or other of similar nature with strong transcriptional terminators in order to create random insertion mutants or targeted chromosomal fusions (e.g. mini-Tn7 with reporter genes insertion in the downstream of PA5548 of P. aeruginosa (Nature Method 2005, 2:443). The suicide vectors could also contain reporter genes such as lux, phoA, gfp, lacZ. For the chromosomal deletion/insertion mutants, only antibiotic markers (cat, kan, tet, erm) that are already in routine use in standard cloning vectors will be used.

**Evaluation of foreseeable effects**

Although the E. coli hosts are not known to be pathogenic and could be considered to be analogous to biological agents in hazard group 1, the introduction of genes such as QS systems, DNA partition systems, or transcriptional regulators from group 1 and 2 microorganisms into these strains could potentially make them pathogenic and so be classified as hazard group 2.

Pseudomonas species used in these experiments are hazard group 1 apart from P. aeruginosa which is hazard group 2. The well-characterised vectors that will be used in Pseudomonas spp. (i) are non-mobilisable (e.g. pUCPxx and pBBR1 series and their derivatives), mobilisation-defective (e.g. pBR322 and its derivatives), shuttle vectors between E. coli and Pseudomonas (e.g. pME60xx series) or mobilisable suicide vectors (R6K derivatives from E. coli to Pseudomonas); and (ii) contain only selective markers that are already in routine use in standard cloning vectors; and (iii) contain no recognisable harmful sequences. There will be no possibility of viral particles being produced in Pseudomonas and the likelihood of unintended transfection of eukaryotic cells and unintended transformation of other prokaryotic cells is extremely low. These vectors are unlikely to be maintained outside the laboratory in the absence of selective pressure. Therefore the level of risk of the recipient strains should not increase over an equivalent to hazard group 2. The introduced genes and standard DNA parts from ACDP hazard group 1 and 2 microorganisms such as RM systems, QS systems or DNA partition systems genes are unlikely to increase the level of risk of the resultant strains over an equivalent to hazard group 2. Genes such as reporters or routine used antibiotic resistance genes are under the control of their own promoter or native Pseudomonas promoters and their expression levels are not expected to be above native levels of expression therefore the level of expression of downstream targets are not expected to increase. It is difficult to envisage how the mutagenesis or chromosomal fusions could enhance the virulence of the Pseudomonas spp. which should not be above the equivalent to hazard group 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only GM micro-organisms will be involved.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Decontamination and autoclaving will be carried out where required. All solid waste materials will be disposed of into the autoclave bags, yellow clinical waste bags or sharps bins as required. All used sharps will be placed immediately after use into a sharps bin. Sharps bins will be located on the bench where the sharps are used so that they can be disposed of directly after use. All bacterial contaminated waste including both solid and liquid forms will be autoclaved before disposal.

a) Decontamination.
1% Trigene will be used for disinfection. The surfaces of the working bench and the microbiological safety cabinet will be decontaminated with 1% Trigene after use. Laboratory benches will be swabbed with 1% Trigene after any activity.

b) Autoclaving.
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Spillages of any GMOs or any of their contaminated products will be localised by spillage kits and disinfected with 1% Trigene. In the event of any injection injury or other serious accidental exposure seek first aid or medical attention if required.
**Project Containment**

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**Historical Significant Changes**

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**Historical Date of Additional Info**

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**

In this project interactions of cell wall-deficient bacteria with their murine and human hosts will be investigated and compared with those of their walled counterparts. Several mutations have previously been shown by our group to predispose Bacillus subtilis to grow in the absence of a cell wall, as so-called L-form bacteria. The mutations were identified in genes involved in synthesis of the cell wall, such as murA-F, polyisoprenoid synthetic pathway, such as ispA, as well as genes involved in the production of bacterial membrane, such as accDA. Interestingly, B. subtilis L-forms were able to divide independently of FtsZ, a protein conserved amongst bacteria and essential for division in rods. In this project mutations in genes involved in synthesis of bacterial cell wall and membrane, as well as those responsible for division will be introduced into bacterial species specified in section 7, to encourage their growth without the cell genes, such as lacZ, gus and gfp (as well as its many variants), insertion of regulated promoters, using non-clinically relevant antibiotic-resistance genes as selective markers. L-form bacteria obtained during the course of this project will be used to extend our knowledge about cell wall-deficient bacteria in general and to establish how the cell wall affects interactions with host cells in comparison to their rod-shaped or coccal counterparts. Effects on pathogen invasiveness and uptake, as well as host responses, such as cytokine production and intracellular signalling pathways, will be investigated using gentamycin protection assay, microscopy, FACs analysis, q-PCR, Western blotting, ELISA assays, protein arrays and DNA microarrays. Most of the work will involve murine macrophage-like cell lines RAW264.7 and J774 as well as human macrophage-like cells lines U937 and THP1. Epithelial cell lines such as HEK293 and HeLa might also be tested. In the later stages of the project isolated murine and human macrophages might be used as well as murine animal models. It is predicted that pathogenicity of L-forms will be severely compromised in comparison to their wall-sufficient counterparts, because the introduced mutations will affect essential cell processes such as generation of protective cell wall and division.

**Recipient or parental organism**

All of the organisms listed below are ACDP hazard group 2:

- Listeria monocytogenes strains EGD, EGDe, 10403S and derivatives
- Enterococcus faecalis V583 and derivatives
- Enterococcus faecium Aus0085 and derivatives
- Salmonella enterica serovar Typhimurium C5 and SL1344 and derivatives
- Staphylococcus aureus strains Wichita, MW2, Newman, COL and derivatives

**Host/vector system**

- E.coli/Listeria shuttle vectors: pMAD (allelic replacement vector), pPL2 (site specific Listeria integrative plasmid pMK-pMK4 (site-specific Listeria integrative plasmid) and their derivatives
- Staphylococcus aureus bacteriophage 80
- E. coli/Staphylococcs aureus and Enterococcus shuttle vectors pBR322 series, pDL277 (expression, cloning vectors), pMUTIN4 (lacZ fusion integrative vector), pSG series (N-terminal GFP fusion integrative vector), E. coli/Salmonella pBR322, pBAD series, pAT153, pGEX series (expression, cloning vectors)
- Salmonella pGP704 and pLac (gene disruption vectors)
- Salmonella P22HTint bacteriophage

**Origin & function**

- Resistance markers (such as cat, bla, kan, neo, spc, tet, ble, apr, and hyg), Reporter genes (such as lacZ, gus, gfp (and derivatives), etc.), Epitope tags (such as HA, c-myc, 6xHis, etc.), artificial promoter constructions (e.g. PxyL or PsPac). Genes will in general be derived from the host organism (e.g. genes directly involved in cell division, chromosome segregation, cell wall biosynthesis, metabolism), introduced either on a plasmid replicon or inserted directly into the host genome. Homologous genes from other bacteria might be introduced in some cases for convenience of manipulation.
The genes of interest predominantly encode proteins involved in essential cellular processes such as cell wall biosynthesis and division that we would like to understand better. Cell wall-defective mutants are likely to be resistant to antibiotics, which target cell wall components or their synthesis, such as most β-lactam antibiotics. However, their susceptibility to antibiotics that target other sites than the cell wall in order to reach their target (e.g., Gentamycin, daptomycin, metronidazole). Moreover, cell wall-defective mutants are also expected to be severely compromised in their ability to infect host and cause disease. Cell wall plays a critical role in protection of bacteria from the environment and the host immune system. Bacteria deprived of the cell wall have to be cultivated in a carefully designed osmoprotective internalin A (InlA) and ActA, T3SS1 and T3SS2 type III secretion systems of Salmonella spp. Secretion and correct folding of others, such as listeriolisin O (LLO) and phosphatidylinositol-specific phospholipase C (PI-PLC) or malfunction in cell wall-defective mutants, which are therefore unlikely to pose a significant threat. Mutants defective in other highly conserved metabolic functions such as division are very unlikely to become more pathogenic than the parent strain and in fact the opposite is more probable.

The threat that cell-wall defective bacteria may pose to environment is negligible, because these organisms die outside carefully designed osmoprotective medium.

All of the vectors to be used are standard vectors with a long history of safe use in the laboratory. These include non-mobilisable or mobilisation-defective Salmonella pBR322, pBAD series, pAT153, pGEX series. Streptococcal?Enterococcal pDL277, pSG series and pMUTIN4 and Listerial pMAD. The use of non-mobilisable or mobilisation defective vectors minimises the likelihood of transfer to other species.

The gene disruption vectors pGP704 and pLac are non-self transmissible but mobilisable in a host engineered to carry the bacteriophage lambda pir gene product. Listerial pPL2 and pLMK series also are non-self transmissible but mobilisable in a host that carry PSA phage.

Genetic manipulations involving generalised or specialised phage transduction would be used to move genetic markers from various genetic backgrounds within a species. (e.g. Staphylococcus aureus bacteriophage 80:: or salmonella-specific bacteriophage P22HTint). The marker will usually involve the disruption or manipulation of specific genes involved in essential cellular processes, as such is unlikely to pose any increased hazard above the handling of the wild type strain, which would then be transduced to other genetic backgrounds.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration and spent culture media, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Annual thermocouple mapping will validate autoclaves and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile. Where this option of decontamination is not possible (e.g. small spills on benching or floor and minor contamination of microscopes or spectrophotometers) an alternate chemical decontamination method will be employed (VIRCON and/or Distel) where necessary. When the work is completed hands will be sanitised with NovaClenz sanitiser (VWR)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The aim of our work is to functionally and structurally characterise Clostridial proteins involved in the virulence and pathogenicity mechanisms of this important human and animal pathogen. Our goal is to gain further insight into the mechanisms used to cause disease that can be exploited in the future to control or prevent infection.
Recipient or parental organism

Wild type and antibiotic sensitive variants of ACDP hazard group 2 Clostridial strains, mainly C. difficile but C. perfringens and C. sordellii may be included.

Disabled Escherichia coli K12 strains.

Host/vector system

1. Transfer into Clostridia of integrative transposons by conjugation from E. coli. In this case no vector will remain in the bacteria, with the exception of remnants of the transposon, which will be inactive.

2. Clostridia / Plasmid pMTL960. This plasmid replicates in both E. coli and Clostridia. It carries a chloramphenicol resistance gene which is active in both E. coli and Clostridia.

Origin & function

1. We intend to knock-out any gene, with particular focus on genes relation to sporulation, cell wall components and pilins. This procedure will not introduce foreign DNA into the recipient, other than an antibiotic resistance cassette and a transposon. The antibiotic resistances used will not be those used for therapy in infection, i.e. they will not encode vancomycin or metrinidazole resistance.

2. As we have to complement any mutation in a Clostridial strain, we will introduce on a plasmid, the wild type version of the gene into the mutant strain. As we intend to knock-out essentially any gene in the genome, we can summarise the intended functions of the genes as "normal cell metabolism" and "pathogenesis". For the purposes of plasmid introduction, the genes of interest are initially introduced in E. coli for conjugation with Clostridia.

3. We will also introduce a series of marker genes into the Clostridia, e.g. fluorescent protein, luciferase.

Note: no active toxin genes will be transferred into any Clostridia recipient.

Evaluation of foreseeable effects

1. Creation of mutants. As we intend to create mutants with decreased virulence, which we will determine using in vitro assays, e.g. cell culture techniques, we predict that the mutants will be less virulent than the wild-type strains. That also expands to the ability to survive in the environment. As an example, our collaborators have made knock-outs in a complex of 2 proteins we are also studying; in both cases, the resulting strains do not sporulate or have attenuated sporulation (personal communication). Similarly, knocking out the main S-layer protein results in cells that die and can't grow. We expect similar results as we look at other proteins involved in these processes. It is unlikely that any introduced genetic material could be transferred to related microorganisms. This is because the transposons which are inserted into the chromosome do not retain their ability to retarget another gene once inserted into the genome, i.e. they are rendered non-transposable.

2. Complementation of mutants. There is a very small chance that, by introduction of a gene into a strain defective in that gene, that the increased gene dosage will change the virulence of the strain. It is possible that some complemented mutants might have increased virulence, but we consider this unlikely. Clinical symptoms are directly associated with the production of toxins and although other components seem to vary between strains, no direct association with increased virulence has been established (see Hunt & Ballard, 2013, Micro Mol Biol Rev 77:567-581 for a review of virulence factors and variations between strains). There have been no reports to date that increasing the expression of essential cell wall proteins, pilis or genes involved in sporulation results in an increase virulence. The strains created will only survive in the laboratory under antibiotic selection. If such a strain was to infect a person or animal, the plasmid would be rapidly lost and the strain would not be any more virulent than the wild-type and, as explained above, is expected to be less pathogenic. Similarly, it is unlikely that the genes present on the plasmids could be transferred to another micro-organism, as the plasmids are easily lost once the antibiotic pressure is removed.

3. Introduction of marker genes. All marker genes used will be non-toxic and would not be expected to increase the virulence of the strain.
4. E. coli. The genes of interest will be included in E. coli for conjugation with Clostridia. This assessment excludes deliberate in-frame insertion of expressible DNA downstream of a prokaryotic promoter with the intention of expressing proteins (either alone or as fusion proteins) within E.coli. However, it is recognised that inserted DNA may contain sequences that have promoter activity in E. coli, but: (i) inserts are likely to be out-of-frame, or inverted with respect to the vector reading frame; (ii) codon usage will bias against high-level expression in E. coli. Moreover, the vectors to be introduced both in E.coli and Clostridia, will be low copy number so high-level expression is unlikely. The combination of the above factors greatly reduces the likelihood of biologically active proteins being produced in E.coli. Further, even if biologically active proteins are produced, as toxins are excluded from these experiments, consequences for human health are minimised.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cultures will be inactivated by either chemically or by heat serialisation (autoclaving)
Autoclaving (used for all waste: solid and liquid culture waste, agar plates etc)
134oC 30 minutes
Each run is monitored by Departmental staff
Autoclave is tested annually by use of a 12 point thermocouple test
Chemical sterilisation (used for liquid cultures when autoclaving is not possible such as to clear accidental spills and disinfect working areas)
Addition of Virkon to a final concentration of 1%; leave overnight; dispose of waste down sink.
Validation – manufacturer’s data, best standard practice in clinical and laboratory settings.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment has been scrutinised and approved by the University GM safety committee

Project Containment

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### Project Additional Information

**Purposes of the contained use**

This project will involve the creation of transgenic lines of the wheat pathogen *Zymoseptoria tritici* as well as other GMMs. The aim of the project is to investigate the molecular mechanisms that enable *Z. tritici* to infect its host. *Z. tritici* causes septoria tritici blotch disease that is characterised by necrotic blotches on wheat leaves. We are interested in how environmental signals are sensed and regulate the morphological changes of *Z. tritici* that are required for its virulence. *Z. tritici* is a dimorphic fungus that can exist in a yeast or filamentous form, with the latter being essential for host infection. Initially we will focus on the role of nutrients (particularly nitrogen and carbon) in the development of *Z. tritici*. The growth of *Z. tritici* on agar plates and in liquid media containing different nutrients will be analysed to determine those conditions that induce morphological change. Genes that are involved in nutrient sensing and morphological change will be identified through a combination of targeted and random mutagenesis by the introduction of a selectable marker into the *Z. tritici* genome using *Agrobacterium tumefaciens* mediated transformation. Epistatic analysis will identify any genetic interactions between the identified genes. Genes will be tagged with various epitopes (e.g. FLAG, GFP) to facilitate protein cellular localisation, detection via western blotting and isolation using immunoprecipitation techniques. Tagged and mutated genes will usually be expressed from their native promoters, occasionally from a constitutive promoter and will be integrated into the *Z. tritici* genome. The levels of mRNA originating from genes of interest will be analysed by standard methods (e.g. northern analysis). In some cases, the ability of *Z. tritici* genes to complement the homologous genes in the yeast *Saccharomyces cerevisiae* will be tested by expressing those genes in *S. cerevisiae* using standard yeast expression vectors. Several *Z. tritici* genes will be expressed in *E. coli* (BL21) for protein isolation purposes. *Z. tritici* mutants of interest will be analysed for their ability to infect wheat by collaborators at other institutions such as Dr. J R (Rothamsted Research), Dr. A C (Syngenta AG) and Dr. A S (Durham University). The growth of wheat and testing of transgenic lines of *Z. tritici* will be undertaken by collaborators in appropriate containment plant pathogen glasshouse or growthroom facilities and will not be tested in Newcastle (See section 10).
Zymoseptoria tritici strain IPO323, a modified Ku70 delta strain of IPO323 (Bowler et al., Mol. Plant Pathol. 2010 11: 691–704) and UK environmental isolates received from academic and commercial research labs (e.g. Rothamsted Research; Syngenta AG). These strains will be used to generate mutants that contain a gene disruption for functional studies. Note: Zymoseptoria tritici is the asexual state (anamorph) that was previously named Septoria tritici. Mycosphaerella graminicola is the sexual state (teleomorph).

Saccharomyces cerevisiae. This is the commonly used model yeast considered to be especially disabled and non-pathogenic (Compendium of Guidance 2000, Part A, Annex II).


Agrobacterium tumefaciens strains containing a disarmed Ti plasmid and therefore considered disabled [e.g. AGL-1, EHA105 (Hellens and Mullineaux, Trends Plant Sci. 2000 5: 446-451)] will be used to transform Z. tritici.

Recipient or parental organism


Non-mobilisable S. cerevisiae vectors: Examples that are routinely used include multicopy 2u vectors, low copy centromeric vectors and single copy integrating vectors (Compendium of Guidance 2000, Part A, Annex II).

Non-mobilisable or mobilisable defective E. coli vectors: Examples that are routinely used will include pBR322, pBluescript, the pUC vector series and the pET vector series (Compendium of Guidance 2000, Part A, Annex II).

Agrobacterium vectors: Mobilisable plasmids (e.g. pNOV2114, pCHYG) containing antibiotic resistance cassettes will be transformed into A. tumefaciens for transfer to Z. tritici strains according to the following reference (Motteram et al., Mol. Plant Microbe. Interact. 2009 22: 790–799).

Host/vector system

The genetic material that will be used in this project will originate from reference Z. tritici strain IPO323. Generally, Z. tritici genetic sequences will be amplified using PCR and then cloned into E. coli vectors so that they may be modified by mutation or gene tagging before being reintroduced into the Z. tritici genome. Reporter genes (such as lacZ) will be introduced into the Z. tritici genome to analyse gene regulation. Genomic and cDNA libraries will also be generated and used.

Origin & function

The pathogenicity of Z. tritici is a polygenic trait so that the expression of a single Z. tritici gene is not going to convert S. cerevisiae or E. coli into pathogenic organisms. In addition, Z. tritici genes will only be expressed using mobilisation defective plasmids (Compendium of Guidance 2000, Part A). The aim of this project is to dissect at the molecular level the mechanisms that link nutrient availability to the morphology and virulence of Z. tritici. Most, if not all, of the Z. tritici transformations that will be

Expression in Z. tritici.

Agrobacterium mediated transfer will be used to integrate targeted or random single copies of DNA cassettes into the Z. tritici genome. The transformation of Z. tritici using A. tumefaciens is an efficient way to generate genomic integrations and thereby disrupt gene function (Motteram et al., Mol. Plant Microbe. Interact. 2009 22: 790–79). Dominant markers containing genes that confer resistance to antibiotics such as hygromycin, neomycin and nourseothricin will be introduced to disrupt Z. tritici genes. The targeted inactivation of genes involved in nutrient acquisition or the control of morphology, are expected to reduce the fitness of Z. tritici. Mutants generated by random integration of antibiotic resistance markers will be screened for defects in nutrient acquisition or the control of morphology. The majority of these mutants will also be expected to reduce the fitness of Z. tritici with a consequent loss of virulence. In addition, mutated or tagged Z. tritici genes that are expressed from their native promoters will be reintroduced into the Z. tritici genome. It is predicted that mutated or tagged Z. tritici genes will have wild type or reduced activity. Furthermore, wild type Z. tritici genes expressed from their native promoters will be reintroduced into the Z. tritici genome to test the ability of these sequences to complement mutant strains. These genes will be expressed at wild type levels (to restore the wild type phenotype) which is highly unlikely to increase the virulence of Z. tritici over the wild type strains.

Expression of Z. tritici genes in S. cerevisiae and E. coli.

The pathogenicity of Z. tritici is a polygenic trait so that the expression of a single Z. tritici gene is not going to convert S. cerevisiae or E. coli into pathogenic organisms. In addition, Z. tritici genes will only be expressed using mobilisation defective plasmids (Compendium of Guidance 2000, Part A). The aim of this project is to dissect at the molecular level the mechanisms that link nutrient availability to the morphology and virulence of Z. tritici. Most, if not all, of the Z. tritici transformations that will be
undertaken will inactivate a specific gene target that have a negative influence on nutrient sensing and those virulence factors that respond to nutrient levels. Examples are genes that code for homologues of nitrogen sensing pathways in other fungi that regulate growth and morphology such as those in the protein kinase A (PKA) and mitogen-activated protein kinase (MAPK) pathways. Consequently, any mutations that alter nutrient sensing are likely to result in a reduction in the fitness of Z. tritici, which will likely reduce the ability of a mutant strain to survival within the environment. Furthermore, fungi that are maintained under laboratory conditions tend to exhibit reduced virulence (Franzot et al., 1998 Infect. Immun. 66: 89-97). The transfer of Z. tritici genes that will be expressed in E. coli is unlikely as these genes will be expressed from non-mobilisable or mobilisable defective plasmids. The transfer of chromosomally integrated tagged, mutated or disrupted genes within Z. tritici will only occur via sexual reproduction. It is conceivable that if laboratory strains were to escape into the environment that they could mate with environmental strains of Z. tritici. As described in section 3A.4, it is likely that all integrated variant alleles will either have no influence or reduce the fitness of Z. tritici. Therefore it is unlikely that the progeny of an environmental Z. tritici strain and a strain generated in the course of this work would be more virulent than a wild-type strain.

Human Risk
Z. tritici is a wheat pathogen that humans commonly come into contact with without causing disease. It is therefore highly unlikely that a variant strain of Z. tritici that is generated in the course of this work will pose a threat to healthy individuals. In addition, only disabled S. cerevisiae, and E. coli strains will be used (S. cerevisiae, E. coli K12 – Compendium of Guidance 2000, Part A, Annex II; E. coli BL21-ACGM Newsletter 30, Nov. 2001).

Environmental Risk
Z. tritici has been isolated from UK environmental sources and therefore laboratory strains would be predicted to survive within the same ecological niches as wild-type isolates. As described in section 3A.4, the generated strains will be most likely less fit than wild-type isolates. All E. coli and S. cerevisiae strains are highly disabled (S. cerevisiae, E. coli K12 – Compendium of Guidance 2000, Part A, Annex II; E. coli BL21-ACGM Newsletter 30, Nov. 2001).

All fungal work will be carried out at containment level 2. Infection by Z. tritici is initiated by the germination of spores on wheat. Spores are produced both asexually (pycnidiospores) and sexually (ascospores) by structures known as pycnidia and pseudothecia, respectively. These structures only form on wheat leaves and do not form when Z. tritici (anamorph) or M. graminicola (teleomorph) are cultured in liquid medium or on agar plates, growth conditions that will be used during the work at Newcastle. The virulence studies using wheat leaves will not be undertaken at Newcastle but by collaborators (see section 2.1) that have experience of these experiments and will be covered by their control measures. Z. tritici will be maintained as non-desiccated haploid fungi. Any procedures that may result in aerosols will be carried out using sealed containers or carried out in designated Class 2 safety cabinets. All materials contaminated with GMMs, including liquid waste, solid waste, glassware, plastic ware and sharps in CinBins will be autoclaved. Autoclaved sharps in CinBins and solid waste including agar plates will then be incinerated. Following sterilisation, liquid waste will discarded in the sink. Accidental spills of GMMs will be treated with Virkon (peroxycenic acid) (2%) overnight, wiped up and retreated with Virkon. Contaminated material will be autoclaved. Virkon (2%) will also be used for routine cleaning (1-2 hours contact time) of bench tops and laboratory equipment. All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment such as glassware. Autoclaves are validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile. In the event of spillage of a large culture (2 litres), all liquid will be absorbed using towels, which will then be autoclaved and then incinerated. The contaminated area will then be treated with Virkon (peroxycenic acid) (2%) overnight, wiped up and retreated with Virkon (2 hours). The Virkon contaminated towels will also be autoclaved and then incinerated.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
Decontamination and autoclaving will be carried out where required. All solid waste materials will be disposed of into the autoclave bags, yellow clinical waste bags or sharps bins as required. All used sharps will be placed immediately after use into a sharps bin. Sharps bins will be located on the bench where the sharps are used so that they can be disposed of directly after use. All bacterial contaminated waste including both solid and liquid forms will be autoclaved before disposal. a) Decontamination. 1% Trigene will be used for disinfection. The surfaces of the working bench and the microbiological safety cabinet will be decontaminated with 1% Trigene after use. Laboratory benches will be swabbed with 1% Trigene after any activity. b) Autoclaving. All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile. Spillages of any GMOs or any of their contaminated products will be localised by spillage kits and disinfected with 1% Trigene. In the event of any injection injury or other serious accidental exposure seek first aid or medical attention if required.

The University Biological Safety Committee unanimously agreed that the risk assessment for the project "Identification of genes involved in the virulence of the wheat pathogen Zymoseptoria tritici using gene disruption and modification" submitted by Dr R was appropriate and adequate and represented a Class 2 Contained Use Activity with transgenic plant pathogens.

Project Containment

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Project Ref 540/16.2

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<tr>
<td>04/03/2016</td>
<td>Reprogramming of human cells using Sendai virus vectors</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
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The purpose of this project is to generate induced pluripotent stem cells (iPSCs), which can then act as a model for prostate development and carcinogenesis. These cells will then be used both in vitro and in vivo by transplantation into immunodeficient mice to form prostate like glands.

Sendai viruses are included on ACDP Approved List of Biological Agents (see www.hse.gov.uk/pubns/misc208.pdf) and in the Health and Safety Executive (http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part2.pdf) the use of these viral agents is categorised as Hazard Group 1 (microorganisms that are not hazardous to human health).

The CytoTune®-iPS 2.0 Reprogramming System uses vectors based on a modified, non-transmissible form of Sendai virus (SeV) to safely and effectively deliver and express key genetic factors necessary for reprogramming somatic cells into iPSCs (Oct4, Sox2, Klf4 and c-Myc). There are 3 vectors containing the transgenes: Klf4, Oct4, Sox2, and c-Myc. These genes are known to contribute to the maintenance of pluripotency and the regulation of cell growth and apoptosis. Abnormal expression of c-Myc is also to function as a known oncogene. These reprogram target cells into iPSCs.

SeV vectors used in this kit consist of viral proteins NP, P, M, F (activated), HN, and L, and the SeV genome RNA, from which the F gene is deleted. The F gene is responsible for binding of Sendai virus to cell membrane and budding of new viral particles. Because SeV infects cells by attaching itself to cell surface receptor sialic acid, present on the surface of many cell types of different species, the vectors are able to transduce a wide range of cells. However, they are no longer capable of producing infectious particles from infected cells, because the viral genome lacks the F-gene.

In addition, the presence of functional mutations such as temperature sensitivity in the amino acid sequence of several SeV proteins (SeV/TSΔF, SeV/TS12ΔF, and SeV/TS15ΔF) renders the vectors easily removable from transduced cells. The mutations are in the M, HN, P and L proteins whilst the F gene is completely deleted (see below):
SeV/TSΔF mutations:
M protein - G69E, T116A, A183S
HN - A262T, G264, K461G
P - L511F
L - N1197S, K1795E
F - Deleted
SeV/TS12ΔF:
P - D433A, R434A, K437A
F - Deleted
SeV/TS15ΔF:
P - D433A, R434A, K437A
L - L1361C, L1558I
F - Deleted

The presence of functional mutations causing temperature sensitivity in the amino acid sequence of several SeV proteins (SeV/TSΔF, SeV/TS12ΔF, and SeV/TS15ΔF) renders the vectors easily removable by incubating cells at 38–39°C for 5 days. Sendai virus is cleared from cells by a combination of dilution and RNA degradation by the host cell. It has been shown that Sendai virus vectors are naturally removed from iPS cells induced using the conventional method, by passaging and culturing using common methods (Fusaki N. et al. (2009) Proc Jpn Acad Ser B Phys Biol Sci 85(8): 348-362). Natural removal was also observed for iPS cells induced using Sendai virus vectors simultaneously carrying three reprogramming factors and temperature-sensitive Sendai virus vectors simultaneously carrying three reprogramming factors.

Multiple sites of essential genetic code (as described above) to generate a wild type SeV are missing, making the chance of spontaneous conversion vanishingly small. Exposure to wild type virus is possible in mouse studies, where the Sedai virus is a natural pathogen, appears to be still a perceivable, if not minuscule risk. Moreover, the use of temperature sensitive mutations, dilution and degradation of the virus product in expansion of cells thereafter, checking of measurable of virus by PCR before committing to murine work will be effectively remove this risk of reversion to wild-type.

SeV is a non-human pathogen and does not induce disease in humans. SeV is responsible for respiratory tract infection in mice, hamsters, guinea pigs and rats, with infection passing through both air and direct contact routes (Baker, DG. 1998. Clinical Microbiology Reviews. 11:231-266). The virus is easily killed by drying or disinfectants/detergents. One of the tests for the validation of legitimate iPSCs include xenograft formation but given the above limitations in SeV transmission, any residual virus particles are rapidly diluted out by cell proliferations and routine checking for SeV by PCR is used to confirm loss from cells.

The host cells will be human primary culture or cell lines and, once reprogrammed will undergo monitoring of the dilution and heat sensitive erradiation according to the above methods, formally documented with PCR confirmation that the cells are clear of transgenes. Only "cleared" cells will be maintained and taken forward for additional experiments.

Origin & function

The SeV vector system is intended to express the transgenes Klf4, Oct4, Sox2 and c-Myc in primary mammalian cells in order to generate iPSCs.

Evaluation of foreseeable effects

1. Deletion of the F gene means the vector cannot bud out from the infected cell to infect other cells. These cells are replication incompetent following the first round of infection, as described in the above section.

2. There are temperature sensitive mutations to disable replication of the virus and ultimate loss of virus delivered transgenes. As described above, the virus contains temperature inducible mutations and there is dilution of virus particles that removes the virus from the host cells, including removal of known oncogene c-Myc.

3. The viral vector would be able to transduce many tissues should it come in contact with them. The major hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of infected cells. The two potential transmission routes are by external exposure (either skin lesions or mucous membranes; only in the case of very high titres and aerosol production) and by accidental injection/inoculation using sharps.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
NOTE: In accordance with the SACGM Compendium of Guidance 2000, Part 2A, Annex VI, a parallel BioCOSHH assessment has considered hazards associate with possible adventitious agents when using mammalian cell culture. The BioCOSHH assessment concludes that containment level 2 should be used to control these hazards and consequently all work involving mammalian cell culture will be carried out under full containment level 2. This does not alter the assignment of the GM activity class. Established code of practice (SOP) for work at containment level 2 will be employed.

GMM mammalian cells will be handled at containment level 2 and using microbiological safety cabinets. All GM virus waste will be properly inactivated by autoclaving. Spillages will be soaked with tissue paper, which will afterwards be autoclaved. Contaminated surfaces may be decontaminated with either a 1% virkon solution or 70% ethanol.

Only plastic pipettes will be used in the tissue culture facilities. Furthermore, all contaminated materials and media including waste destined for subsequent incineration will be autoclaved.

The use of sharps will be prohibited and all precautions will be taken to identify procedures or devices that might be used in these projects which could cause an injury to the researchers. Where so identified safe alternative methods or equipment will be sought.

The generation of aerosols will be avoided or contained.

Given the uncertainty about the hazards of most potentially oncogenic sequences and the small quantities used (microlitre/microgram quantities), prevention of exposure or total enclosure is not “reasonably practicable”. Therefore the following measures are applied in the light of this GM risk assessment, will be used:

(1) Good laboratory techniques will be strongly emphasised. Designated workers will be trained in good laboratory techniques before commencing work with potentially oncogenic DNA sequences. They will be made fully aware of the potential hazards of such work.

(2) Procedures using Sendai virus particles and exposed cells will only be carried out in the tissue culture suite. Normal procedure for handling biological material in the TC suite will be largely sufficient for handling the cells and particles. Specific items of relevance here include:

- Safety cabinets are routinely disinfected prior to and following use
- Gloves and lab coats to be worn throughout
- Designated microbiological safety cabinets to be used (class II)
- No wild type virus to be used

Staff competence will be checked during regular inspections of tissue culture facilities and during their project presentations involving sendai virus work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
1. This looks like a well-written form to me. It would be good to include a little more description of the primary cells and cell lines that will be used (Section 3A.1.1).
2. I am slightly unclear as to the final destination of the transduced cells. In Section 1 on the project, it states the purpose is "to generate induced pluripotent stem cells (iPSCs), which can then act as a model for prostate development and carcinogenesis." However, later on in Section 3B1.3 it says "Theoretically viable SeV may infect rodent cells. The chances of carrying viable virus from the hood, changing of lab clothes, lethal affects of drying, maintenance of a pathological titre to reach a rodent is vanishing." I'm not sure why this is mentioned unless the iPSCs mentioned earlier are to be introduced into rodents. If the iPSCs are to be introduced into rodents, then it needs to be described as this will no doubtably require sharps, as well as the consideration of infecting rodent cells in vivo.

The final risk assessment is approved by the committee for notification.

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### Project Ref 540/16.3

- **Critical mechanisms in inflammatory diseases**
- **Class**: 2
- **Consent Granted**
- **Project notified under transitional arrangements**: N
- **Date Project Ceased**: 05/05/2016

### Project Additional Information

02/03/2022
## Purposes of the contained use

Inflammation is a hallmark of many diseases including autoimmune/hyperimmune disorders, infection, cancer and a determining factor for the success of organ transplantation. Inflammation is a process orchestrated by multiple cell types and soluble factors including cytokines/growth factors and alters many fundamental cellular processes. Our research program focuses on understanding the role of amino acid metabolism in inflammation and its influence to adaptive immune responses. The GM program proposed in this application aim to: 1. understand critical molecular and cellular mechanisms during an inflammation process through modifying expression of genes of interest in relevant cell types (e.g. macrophages, fibroblast etc); 2. To establish experimental inflammatory disease models by establishing GM cells expressing nominal antigens in order to monitor adaptive immune responses or by manipulating gene expression in an adult mice to induce diseases such as cancer or prime immune response against a defined antigen;

The approaches include 1. ectopic expression of genes of interest or their mutant; 2. knocking down gene expression using siRNA; 3. establishing cell lines by immortalize primary cells with ectopic expression of oncogenes. 4. gene editing by introducing DNA modifiers such as Cre recombinase into cells or mice. 5. injecting viral vector carrying antigen genes into mice to prime immune response.

We propose to use replication deficient viral vectors including murine retroviral vectors, lentiviral vectors and adenoviral vectors in this program application.

## Recipient or parental organism

### Host:
1. Cells including established cell lines and primary cells from human, mouse and other mammalian origin
2. Mouse

### Host/vector system

| Vectors  | 1. Moloney murine leukemia virus (MMLV) based mouse retroviral vectors, GAG Pol Env deleted making the vector replication deficient. Using certain pseudotyping such as amphotropic ENV will increase the host range in comparison to original MMLV.  
2. HIV based lentiviral vector (pLenti-Trip) (obtained from Dr and original from pLenti Invitrogen), GAG Pol Env, vpr, vpu, vif, nef and tat gene are deleted and carry a deletion of the 3’ L TR (ΔU3) which results in “selfinactivation. Original pLenti with Plasmodin resistant gene were from Invitrogen and modified by Dr. To enhance nuclear import, a DNA fragment containing a central polypurine tract sequence and the central termination sequence, which together form a triple-stranded DNA flap (TRIP) was inserted in to pLenti vector before CMV promoter. Vector is replication deficient and insertion of TRIP fragment will facilitate integration. Using certain pseudotyping such as VSV glycoprotein will increase host range of lentiviral vector in comparison to original HIV.  
3. FLV based lentiviral vector (initially developed by Norian Lab in Stanford). GAG, Pol, Env deleted making the vector replication deficient. Using certain pseudotyping such as VSV glycoprotein will increase host range in comparison to original FLV  
4. Adenoviral vector left-hand ITR, E1a, and partial E1b sequence deleted making the vector replication deficient. |

### Origin & function

Protein coding sequences: DNA sequence coding proteins from human, mouse and other mammalian origins as well as bacteria and virus origin will be introduced to hosts to express the protein of interest or their mutants. Inserts can be classified as
1. Oncogenic, which will directly cause cells to immortalize or transformation;
2. Cell signal modifiers, which include cell surface receptors, intracellular kinases, kinase inhibiting proteins, secreted proteins such as cytokines and growth hormones such as interferons and inteferon receptors;
3. metabolic enzymes such as amino acid and lipid catabolism enzymes,
4. transcription factors and orphan receptors such as IRF3 and AHR;
5. cytoskeleton protein and nucleus scaffold protein such as modified GFP tagged actin;
6. cell basic machinaries such as molecular cheparones and mitochondria structure proteins;
7. genetic modifiers and protein modifiers such as Cre recombinase and biotin ligase (BirA);
8. viral antigens such as influenza A virus nucleoprotein, VSV glycoprotein etc;

Non-protein coding sequence: Inserts can be classified as
1. siRNA will be designed against genes of interest from human, mouse and other mammalian origin as well as viral origin to suppress the expression of genes of interest;
2. genetic modifiers such as a sequence designed for gene editing using crispr technology.

MMLV based murine retroviral vectors will be used to transduce human and mouse cells including established cell lines and primary cell lines. Examples of inserts include oncogenes in order to facilitate cell immortalization, viral antigen in order to monitor immune response in mice, metabolic enzymes such as indoleamine 2,3-dioxygenase (100), siRNA for gene supression.

Lentiviral vectors will be used to transduce tumor cell lines (mouse or human) and to deliver genes into mice. Examples include viral antigen in order to monitor immune responses or metabolic enzymes such as 100

Adenoviral vectors will be used to deliver cre recombinase to cells or animal tissues. Cre recombinase will be used to delete genes flanked with two 10xP sites. Only non-oncogenic/non toxic inserts will be carried by adenoviral vectors.

**Evaluation of foreseeable effects**

Viral vectors

Modification of viral vector cause virus to be replication deficient thus suitable to be used as gene transfer vehicle. while introducing a pseudotyping other than its original ENV protein will increase the host range for retro and lentiviral vectors.

- MMLV based vectors - GAG, POL and ENV are deleted
- Lentiviral vectors - GAG, POL and ENV are deleted. Accessory genes vpr, bpu, vif and nef are also deleted so that the vector is unable to replicate. Deletion of the tat gene and deltaU3 deletion of the 3' L TR results in 'selfinactivation' of the lentivirus following transduction of the target cell.
- Adenoviral vector - left-hand ITR, E1a and partial E1 b sequences are deleted which limit the virus replication capacity.

The highest risk inserts are the oncogenic sequences - these are only inseted into the MMLV retroviral vector

Two potential risks to humans may arise from the hazards of working with the naked plasmid vector + insert DNA containing the potentially oncogenic or other hamful sequence and the supernatant containing the viral particles from the packaging cell line. However, the transfection efficiency even under optimal culture conditions is negligible, and therefore transfection of researcher's cells with naked DNA under accidental/non-optimal conditions would be effectively zero.

Cell lines/animal work

Introducing oncogenes will directly cause cell lines to immortalize or transform. If during an unlikely event these cells were inoculated into the human organism, then these cells are recognised as non-self and will be destroyed by the immune system. Other sequences will alter cellular proliferation or differentiation as part of basic cellular machinernary or responses to extracellular signals (eg, cell signaling modifiers and metabolic enzymes). Some may
play roles in disease progress or pathogenesis. E.g., overexpressing cytokine and transcription factor gene may lead to deregulation of inflammatory response and overexpressing of a tryptophan metabolite enzyme may promote tumorogenesis by creating a favorable immune suppressive microenvironment. The gene products will be biologically active when transfected into mammalian cells and probably expressed at a level higher than seen in the wild type cells. However, the recipient transfected cells will be either primary human or mouse cells or well-characterized stable eukaryotic cell lines in tissue culture that have a history of safe use and these hosts can be considered disabled hosts because the cells are unable to colonize the worker (i.e., not their own cells). Genetic modifiers such as Cre recombinase and sequences designed to edit genes using CRIP technology will change the host cell genetic composition permanently and the physiological effects is gene dependent. Viral vectors carrying Cre recombinase gene is expected to delete the DNA sequence between two 10xP sites in a cis form in host DNA.

Introduction of viral antigen into the cells will change the antigenicity of the cell when injected into an animal.

Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Animals that have been exposed to GM viral vectors are not autoclaved but are treated as ‘inactivated’ GM waste. They will be disposed of by placing in a yellow "CinBin", separately from other types of clinical waste and will be held in a cold store/freezer until the allocated waste company collects them. The "Cin Bin" provides a secure means of storage and transport of the waste, the plastic containers are robust and leakproof and the lids cannot be removed once they have been fixed on. The waste company Healthcare Environmental Group is a GM registered carrier and the waste will be destined for incineration which will provide the validated means of GM virus inactivation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
The GMSC advised that lentiviral/retroviral and adenoviral vectors should be separated into two separate risk assessments due to the different types or risks. This has been implemented and the two risk assessments are submitted with this notification.

Further details of training were required - this has been added and the information is in the submitted risk assessments.

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**Project Ref** 540/16.4

**Date Ackn'd**: 23/06/2016

**CU2 Project Title**: Transduction of mammalian cells using viral vectors

**Class** 2

**Culture Vol/Class 2**: < 1 Litre

**Culture Volume Class 3-4**: Consent Granted

**Non-GMM**: Consent Granted

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

02/03/2022
### Purposes of the contained use

The projects involve assessing the biological function of candidate genes implicated in malignant progression and/or neurodegeneration which emerge from ongoing genomics projects characterising collections of primary tumour/brain material.

Lentiviral particles will be generated on site using either a cDNA or appropriate shRNA. cDNAs may occasionally contain a mutation where a dominant negative or constitutively active version of the gene is required. The purpose is to perturb the natural state of the gene in question and to assess the effects on cells. Downstream analysis may involve cell cycle analysis, proliferation, cytotoxicity, apoptosis or differentiation assays, and development of in vivo xenograft models. RNA, DNA and protein would typically be extracted from infected cells in each case to verify appropriate infection and to validate cDNA/shRNA expression. A lentiviral pool expressing various cDNAs or shRNAs may be used to create a pool of cells with several integrated constructs which will then undergo in vitro or in vivo selection to screen for constructs which either promote or inhibit cell proliferation or engraftment.

### Recipient or parental organism

Cell lines in culture: 293T Human embryonic kidney cell line (packaging cell line), adherent and suspension human and mammalian cell lines (including cancer cells) (such as HeLa, MEF, MRC7, SK-N-SH, DAOY, MON, KD, Molt-4, RPMI-8402, MN-60, Pre-B-697) immortalised normal cells and primary mammalian cells.

### Host/vector system

We will be mainly using third and subsequent generation lentivirus. They are replication incompetent and self inactivating (SIN). The systems separate the packaging signals and viral LTRs on the expression plasmid from the viral structural and expression genes (gag, pol and rev from FIV or HIV and viral envelope glycoproteins such as the VSV-G gene from Vesicular Stomatitis Virus, in place of HIV or FIV env). The viral structural and expression genes and the envelope glycoprotein genes are separated on at least two additional plasmids.

### Origin & function

cDNA sequences of human or mouse origin and/or the appropriate shRNA sequences will be cloned into the lentiviral vectors. These may include partial or entire gene sequences, or mutant forms of the gene sequences, under the control of constitutive promoters. Many of the genes or targets of the shRNAs will have unknown function but may include known oncogenes or tumour suppressors. As an example, we will use mutant B-RAF and N-Ras constructs to generate primary human fibroblasts with oncogene-induced senescence phenotype.

The aim is to use either overexpression or knock-down of gene-expression to assess the potential contribution to tumourigenesis of each candidate identified from our genomics screens. Once a stably infected cell line is produced, experiments performed will depend on the particular gene selected but will typically involve a panel of typical proliferation, apoptosis and/or invasion assays as well as collection of RNA/DNA/Protein for further profiling analysis and potentially in vivo engraftment.

### Evaluation of foreseeable effects

Known oncogenes such as mutant B-RAF or N-Ras or shRNAs that will silence known tumour suppressor genes may be inserted into primary cells and cell lines. There is therefore a theoretical possibility that they could render the cells neoplastic. If during an unlikely event these cells were inoculated into the human organism, then these cells are recognised as non-self and will be identified by the immune system. Inserted gene products will be both genomic, cDNA sequences or small interfering double stranded RNA sequences originating in humans or rodents, including those which could be implicated in tumour progression, i.e. oncogenic and tumour suppressor DNA sequences. Specific functions will vary depending on the particular experiment, but will include functions that are known to contribute to the maintenance of pluripotency and/or differentiation and the regulation of cell growth and apoptosis. The gene products will be biologically active when transfected into mammalian cells and probably expressed at a level higher than seen in the wild type cells. However, the recipient transfected cells will be either non-self primary human or mouse cells or well characterised stable eukaryotic cell lines in tissue culture that have a history of safe use and these hosts can be considered disabled hosts because the cells are unable to colonise the worker (i.e. not their own cells).
Two potential risks to humans may arise from the hazards of working with the naked plasmid vector and insert DNA containing the potentially oncogenic DNA and the supernatant containing the viral particles from the packaging cell line. However, the transfection efficiency even under optimal culture conditions is negligible, and therefore transfection of researcher’s cells with naked DNA under accidental/non-optimal conditions would be effectively zero. The potential oncogenes will only be sufficiently oncogenic to induce tumourigenicity in immortalised cell lines or in pre-malignant cells or give a growth advantage to cells in culture without inducing tumourigenicity. Tumourigenesis is a multistage process requiring the interaction of many mutated genes which also lessens the risk from delivery of a single oncogene or tumour suppressor gene to a target tissue. The principle route of transmission that could lead to transduction of laboratory workers by packaged viral particles is inoculation. Therefore, use of sharps will be prohibited. Other possible routes of transmission such as inhalation, ingestion and eye splashes are less likely to lead to tumourigenesis because of the required very high titres, which will not be achieved under the given experimental conditions. Consequently, the risk of tumourigenesis is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Spillages will be soaked with tissue paper, which will afterwards be autoclaved. Contaminated surfaces may be decontaminated with trigene solution. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC suggested that for all experiments using the transduced material, lack of infectious particle production is confirmed by the addition of cell culture supernatant to 293T cells. Additional information on the monitoring and validation of autoclaves was added. The GMSC committee agreed on the assignement of this work to activity class 2.

Project Containment

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We aim to utilise a commercially available ‘immortalisation’ kit that utilises Lentiviral vectors to transfect Simian Virus 40 (SV40) T antigen to induce immortalization. We have previously successfully transfected these cells with Lentiviral agents. SV40 T antigen has been shown to be the simplest and most reliable agent for the immortalization of many different cell types, and its mechanism of action is relatively well understood.

We will culture these cells according to well-defined tissue culture protocols. The cells will be transferred to an appropriate well plate and transfected with Lentiviral vectors (incubated overnight), following manufacturers protocols. The next day we will remove the viral supernatant and add the appropriate volume of complete growth medium to the cells and incubate. After 72 hours incubation we will subculture the cells. After 10-15 days we will screen for positive transgene expression (i.e. Western blotting, RT-PCR).

Recipient or parental organism

Primary human airway (subglottic sub site) epithelial cells cultured in submerged culture at passage 0 - 2. Taken from theatre patients via brushings.

Host/vector system
Ready to use Lentiviral particles will be purchased from abm inc. USA. Particles are produced by abm inc using a standard protocol meeting BioSafety Level 2 requirements https://www.abmgood.com/SV40-Cell-Immortalization.html#documents

Immortalisation is induced using simian virus 40 (SV40) T antigen (pLenti SV40-T)
The virus is replication incompetent. The following features ensure replication incompetence –
An enhancer deletion in the U3 region of 3ΔLTR ensures self-inactivation of the lentiviral vector following transduction and integration into the target cell’s genomic DNA. The number of lentiviral genes necessary for packaging, replication and transduction is limited to three (Gag/Pol/Rev), and their expression is derived from different plasmids, all lacking packaging signals. These plasmids share no significant homology to the expression vector, thus preventing the generation of replication-competent virus by recombination events. None of the Gag, Pol, or Rev genes will be incorporated into in the packaged viral genome, thus making the mature virus replication-incompetent.

Origin & function

This Lentiviral vector is derived from abm inc. as a pre made virus for immortalisation utilising simian virus 40 (SV40) T antigen. This gene is expressed under the control of the CMV promoter.

Evaluation of foreseeable effects

We envisage that the Lentiviral vector will infect the primary epithelial cells and transfect Simian Virus 40 (SV40) T antigen to induce immortalization of the cells. It is expected that this will be achieved by inactivating the tumor suppressor genes (p53, Rb, and others) that can induce a replicative senescent state in cells. Recent studies have also shown that SV40 T antigen can induce Telomerase activity in the infected cells.

The major hazard is presented by the packaged virus prior to infection of the target cells and residual virus in the medium of transfected cells. The lentiviral system is pseudoviral in nature; its coat proteins are derived from the vesicular stomatitis virus (VSV). The VSV G coat-protein confers significant stability and increased pleiotropy. Thus it is possible that infection may occur should the lentivirus come into contact with broken skin (such as needle-stick injury) or mucous membranes. Inhalation is also a potential source of exposure. However, the risk of harm to the worker is virtually zero. Contact with mucosal membranes, skin and inhalation will be prevented utilising standard class two containment protocols. Needle stick injury is a potential source of harm, however no needles are to be used at any point in the use of this agent. In addition the virus is replication incompetent so infection would be localised

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Containment level 2 behaviour and activities will be used to control for any associated hazards related to this work. We have dedicated human cell culture facilities within our laboratories. Standard operating procedures for work at containment level 2 will be employed.

Infection of mammalian cells will be undertaken in dedicated cell isolation and culture facility within Prof Pearson’s laboratory (room M1.086). Standard operating procedures for work at containment level 2 will be employed including class 2 microbiological safety cabinets. Notifications will be placed in the work area to inform staff that viral work may occur in this area. When virus is being used signs will be placed on hoods, doors and incubators indicating this and will be used only for these samples until full decontamination is undertaken as described below.

We already have protocols in place and risk assessments for epithelial cell culture.

Work will be carried out in a dedicated microbiological safety cabinet within a dedicated room, and only trained authorized personnel will be allowed to work with lentiviral particles.

The following measures will be applied:
1. Good microbiological practice will be used at all times and strongly emphasised by senior staff.
2. Personal protection equipment will be used all the time while performing lentivirus-involving experiments. These include Howie-style lab coat and gloves.
3. All experimental procedures will be performed so as to minimise aerosol production. Procedures which are likely to generate aerosols such as the use of sonicators, vigorous shaking and mixing etc. will be avoided, or where necessary, will be carried out in closed containers or the microbiological safety cabinet.
4. Procedures using lentiviral particles, packaging cell lines and exposed cells will only be carried out in room M1.086. Normal procedure for handling biological material will be largely sufficient for handling the cells and particles.
5. The safety cabinet is routinely disinfected prior to and following use.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Two main virucidal disinfecting agent will be used: 1% (v/v) Trigene Advance for routine surfaces and tools disinfection (e.g. safety cabinet surface, centrifuge rotor, pipette), and for disinfection of liquid waste (e.g. spent medium) and for submersion of other disposable waste (pipette tips, conical tubes, and other culture ware). Trigene is a best available virucidal disinfectant. This includes killing numerous types of viruses including HIV virus, and thus lentivirus.

Solid waste (e.g. cultureware, pipette tips, used gloves) will be stored in appropriate yellow bins before autoclaving. Once autoclaved, bins bags will be disposed of by incineration via the yellow clinical waste route.

Liquid waste (e.g. cultures in flasks or spent media) will be sterilised in the culture flasks in designated waste buckets by addition of Trigene to a concentration of at least 1% (w/v) for overnight disinfection. Trigene-disinfected solutions will be disposed of as inactivated waste via the designated drain in the tissue culture room.

Culture flasks will be disposed of (following removal, appropriate disinfection and disposal of culture medium) by addition of Trigene solution inside the flasks and firmly closing the flask lids. These flasks will be left overnight by the sink on the disinfection bench. After leaving overnight, solutions will be discarded as inactivated waste while flasks will be placed in the designated yellow bins for autoclaving before disposal via clinical waste route.

Non-diposable contaminated items (e.g. centrifuge buckets etc.) will be disinfected by complete submersion in Trigene solution overnight. Items will then be rinsed in water and dried.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Requested that the evaluation of risk in section 3A.2.3 should be upgraded from 'effectively zero' as this does not reflect insert being oncogenic.

For 3A5 the Phrase "In addition the virus is replication deficient so the contamination would be localised." Should be changed to "In addition the virus is replication incompetent so the contamination would be localised."

Recommended that centrifuge buckets are decontaminated with Trigene rather than Virkon as Virkon can corrode and changing disinfectants for different jobs may be confusing.

Changes implemented and the GMSC agreed the classification as activity class 2.

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02/03/2022
Project Ref 540/16.6

Screening for compounds that suppress the loss of mitochondrial dynamics in response to human cell infection with Listeria listeriolysin

Project Additional Information

Purposes of the contained use
To identify the molecular pathway that causes the loss of mitochondrial dynamics as cells become infected with listeriolysin. The intention is to use this information to determine novel targets to protect cells against listeria infection

Recipient or parental organism
The host organism is Listeria monocytogenes EGDe (BUG1600). For the production of the constitutive cGFP expression in strain BUG2539, the plasmid pAD1 was chromosomally integrated and selected by resistance to chloramphenicol. This resulted in constitutive expression of cGFP from the pHYPER promoter. Production of the strain is characterised in Balestrino et al. App Env Micro 2010, 76, 3225-6.

Host/vector system
No vectors will be used and no genetic manipulation will be performed. This application is solely to use strains of Listeria that have been previously modified.

Origin & function
The two strains of Listeria monocytogenes (background EGO) both constitutively express soluble Green Fluorescent
Protein which is necessary to visualize and measure the bacterial number in close proximity to human Hela or U20S cells grown on either 96 or 384 well plates. One is a standard strain constitutively expressing gfp. The second is a negative control for expression of the ILO toxin, which has been engineered with a deletion in the Hly gene encoding ILO. These two cell lines will be used in a large screen to identify components that promote the loss of mitochondrial dynamics on infection as follows: human cells will be transfected with a druggable targets siRNA library approx. 3 days prior to infection with the Listeria bacteria with an approx. multiplicity of infection of 50. After 1 hr of infection, cells will be treated with 5 nM tetramethylrhodamine methyl ester, a compound that concentrates in the mitochondrion as a function of the membrane potential. Images will be captured post-incubation and the effect on fission calculated by determining mitochondrial aspect ratio (a measure of mitochondrial fragmentation).

**Evaluation of foreseeable effects**

The gram-positive bacterium Listeria monocytogenes is a ubiquitous, intracellular pathogen which has been implicated within the past decade as the causative organism in several outbreaks of foodborne disease. Listeriosis, with a mortality rate of about 24%, is found mainly among pregnant women, their fetuses, and immunocompromised persons, with symptoms of abortion, neonatal death, septicemia, and meningitis. The organism has a multifactorial virulence system, with the thiol-activated hemolysin, listeriolysin 0, being identified as playing a crucial role in the organism's ability to multiply within host phagocytic cells and to spread from cell to cell. The organism occurs widely in food, with the highest incidences being found in meat, poultry, and seafood products. Only standard strains of Listeria monocytogenes will be used.

Infectious Dose: Given the widespread distribution of Listeria in foods, ingestion of the bacteria is likely to be a common event. However in general the incidence of listeriosis is low from 2 to 8 sporadic cases per million in Europe and US. The minimum dose required to cause clinical infection in humans seems to be significantly higher than in other food borne pathogens. The approximate infective dose of Listeria monocytogenes has been estimated to be 10 to 100 million colony forming units in healthy hosts, and only 0.1 to 10 million colony forming units in individuals at high risk of infection [Farber, J. M., Ross, W. H., & Harwig, J. (1996). Health risk assessment of Listeria monocytogenes in Canada. International Journal of Food Microbiology, 30(1-2), 145-156].

Listeria monocytogenes is classified as a Hazard Group 2 (HG2) pathogen. It is difficult to envisage how constitutive expression of the chromosomally encoded GFP could increase the virulence of the Listeria monocytogenes host strain beyond that of the parent HG2 strain, in the event of accidental exposure to humans. Indeed one of the strains to be used has been attenuated by the deletion of the hly gene encoding listeriolysin 0 which as stated already is a major virulence factor. Hence the hazards of accidental human exposure to this GM strain will be significantly reduced compared to those of the parent.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Disinfection and autoclaving will be carried out where required. All waste materials will be disposed of into yellow clinical waste bins or sharps bins as required . All used sharps will be placed immediately after use into a sharps bin. Sharps bins will be located on the bench where the sharps are used so that they can be disposed of directly after use. All infected waste will be autoclaved before collection by external waste disposal contractor

a) Disinfection.
1% Virkon/Trigene will be used for disinfection. The surfaces of the microbiological safety cabinet will be disinfected with 1% Virkon/Trigene after use. Laboratory benches will be swabbed with 1% Virkon/Trigene after any activity.
b) Autoclaving.
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) and test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min). In addition the TST strips are kept for record inspection.
L. monocytogenes can be inactivated by ozone, high pressure (500MPa), and high temperatures (at least 70°C for 2 minutes).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No Comments

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Animal Units

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 | L2 | L3 | L4 |

Project Ref 540/16.7

Date Ackn'd 28/09/2016

CU2 Project Title Transduction of mammalian cells using lentiviral vectors

Class 2

CultureVolClass2 < 1 Litre

Class VolumeClass3-4

Consent Granted
This risk assessment aims to cover all research projects at the Institute of Genetic Medicine utilising the same or functionally very similar lentivirus vectors and mammalian host cells. The projects involve assessing the biological function of candidate genes implicated in multiple cellular functions/Scientific areas. Some projects are screens to assess the functional relevance of various genes/proteins systematically in a more global fashion. Typically, the methodology will involve a gene highly over or under-expressed in a particular cell line and reversing the expression by either overexpressing the gene or expressing a shRNA/CRISPR to knock-down/knock-out expression of the gene in question, respectively. Lentiviral particles will be generated on site containing either a cDNA or appropriate shRNA/CRISPR. cDNAs may occasionally contain a mutation where a dominant negative or constitutively active version of the gene is required. Cells will be infected by titrated lentivirus particles followed by antibiotic selection or sorting by FACS. The purpose is to perturb the natural state of the gene in question and to assess the effects on cells. Downstream analysis may involve cell cycle analysis, proliferation, cytotoxicity, apoptosis or differentiation assays, and development of in vivo xenograft models. RNA, DNA and protein would typically be extracted from infected cells in each case to verify appropriate infection and to validate cDNA/shRNA/CRISPR expression. A lentiviral pool expressing various cDNAs or shRNAs/CRISPRs may be used to create a pool of cells with several integrated constructs which will then undergo in vitro or in vivo selection to screen for constructs with particular relevant functions (i.e., genetic screens) (e.g. of cell functions tested: differentiation, proliferation, tumorigenic potential, etc).

Recipient or parental organism

293 Human embryonic kidney cells (packaging cell line; Pear et al, PNAS 1993; 90:8392), adherent and suspension human and mammalian cell lines (including cancer cells such as Ishikawa, HEC-1A, AN3CA, HCC-38, MFE-296, MFE-319, EFO27, MCF7, DAOY, MON, KD), immortalised normal cells (e.g., MCF-10A, 184B5) and primary mammalian epithelial cells (e.g., mouse embryonic fibroblasts -MEFs-).

Lentiviral constructs will be expanded using E. coli K12 derived bacterial host strains (e.g. DH5). 293T packaging cells will be used to create lentiviral particles by transfection. Packaging cell line 293T (and thereof derived cell lines) is derived from the well-characterised 293 cell line (Pear et al., PNAS 1993; 80; 8392; ATCC CRL-11268). This cell line stably and constitutively expresses a temperature-sensitive version of the SV40 large T antigen. This cell line will be used to produce lentiviral vectors. These vectors alone are replication incompetent owing to their lack of cell and gag-polymerase encoding sequences. Virus is therefore produced by co-infection of the 293T cell line with lentiviral vector along with one or more viral envelope encoding plasmids (e.g. VSV-G or constructs derived from the measles virus [MV] hemagglutinin and fusion protein genes [HΔ24 and FΔ30]) and a plasmid encoding the HIV or FIV gag-pol and the regulatory genes including rev, nef and vif. Mammalian cell lines and primary mammalian cells will be used for infection with the supernatant generated by the packaging cells. Most commercial cell lines that will be used are derived from solid tumours or leukaemias, have a long history of safe use and are commonly handled in UK labs at CL2 or CL1. All facilities at Newcastle University where these materials are handled are at CL2 standard. The mammalian cells and somatic stem cells are only able to survive if maintained in tissue culture in the laboratory, or if injected into inbred syngeneic or immunodeficient animals.

Host/vector system

The transfer vector systems are derived from FIV or HIV and have been specifically engineered for biosafety by separating the packaging signals and viral LTR's on the
expression plasmids from the viral structural and expression genes (gag, pol and rev from FIV and the VSV-G gene from Vesicular Stomatitis Virus, or similar alternatives, in place of FIV or HIV env) encoded on three or four separate plasmids, which remain in the packaging cell line, effectively precluding the production of replication competent virus in the target cell or should the viral vector escape containment. FIV vectors are included as they may be more efficient for some cell types. It does not imply any different containment or risk considerations than HIV based vectors. The plasmids expressing these gene products carry no packaging signals or LTRs and so cannot themselves be mobilised with the vector and have been engineered not to contain any regions of homology to each other or to the viral vector, to prevent undesirable recombination events which might result in replication competent virus being produced.

The viral vector and recombinant virus have a broad host range when pseudotyped with e.g. the VSV-G envelope protein. The normal tropisms of HIV and FIV have been broadened to include multiple tissues from a broad range of species. Pseudotyping with alternatives to VSV-G, for example measles virus derived glycoproteins, will produce particles with more restricted tropisms.

### Origin & function

The factors we will introduce into the cell lines are non-coding shRNAs, non-coding RNAs (e.g., microRNAs), CRISPRs and coding cDNAs originating in humans or rodents. The use of shRNAs/CRISPRs will generate single cells with stable gene expression knockdown/knockout. On the contrary, cDNA constructs will be used to generate single cells with stable expression of any particular gene. Gene knockdown/knockout performance will depend upon shRNA/CRISPR target specific and efficient recognition.

cDNA sequences of humans or mice origin and/or the appropriate shRNA/CRISPR sequences will be cloned into the lentiviral vectors. These may include partial or entire gene sequences, or mutant forms of the gene sequences, under the control of constitutive promoters. In screening applications, many of the genes or targets of the shRNAs will have unknown function but may include known oncogenes or tumour suppressors. In the case of mutated genes, these will comprise of characterised sequences which have deliberately mutated by site directed mutagenesis. An example of a mutation that is constitutively activating might be a KRASv12 mutation which has been demonstrated to result in overactive ras signalling and therefore again should be considered to pose a greater hazard than the wildtype sequence. Other targets may include known proto-oncogenes/tumour suppressors such as RUNX1, ERG, GRIK2, PARK2, FOXO3, CRLF2, TP16, TP53 or TERT.

The aim is to use either overexpression or knock-down/knock-out of gene-expression to assess the potential contribution to cellular processes (e.g., tumourigenesis, differentiation, etc) of each candidate identified from our genomics screens. Once a stably infected cell line is produced experiments performed will depend on the particular gene selected but will typically involve a panel of typical proliferation, differentiation, apoptosis and/or invasion assays as well as collection of RNA/DNA/Protein for further profiling analysis and potentially in vivo engraftment.

### Evaluation of foreseeable effects

The only viral sequences in the transfer vectors are the 3' and 5' long terminal repeats (LTR's), the packaging signal and the Rev response element (RRE) required for the production of viral particles. Deletion of accessory genes vpr, vpu, vif and nef mean that the vector is unable to replicate once it has transduced the target cell. In addition third generation lentivirus are deleted for the tat gene and carry a SIN deletion of the 3’ LTR (U3) which results in “self-inactivation” of the lentivirus following transduction of the target cell, precluding adventitious activation of the vector by endogenous retroviruses and minimising the risk of recombination with ERVs. These vectors also contain a bacterial origin of replication and ampicillin resistance gene, an internal promoter such as CMV or SFFV promoter driving the gene of interest linked via an IRES element with a gene encoding a fluorescent protein for FACS-mediated sorting.

In particular global screens may affect any gene expressed in the test system. Consequently, known oncogenes or shRNAs that will silence known tumour suppressor genes may be inserted into primary cells and cell lines. There is therefore at this stage a theoretical possibility that they could render the cells neoplastic. If during an unlikely event these cells were inoculated into the human organism, then these cells are recognised as non-self and will be destroyed by the immune system. Inserted gene products will be both genomic, cDNA sequences or small interfering double stranded RNA sequences originating in humans or rodents, including those which could be implicated in tumour progression, i.e. oncogenic and tumour suppressor DNA sequences. Specific functions will vary depending on the particular experiment, but will include functions that are known to contribute to the maintenance of pluripotency and / or differentiation and the regulation of cell growth and apoptosis. The gene products will be biologically active when transfected into mammalian cells and probably expressed at a level higher than seen in the wild type cells. However, the recipient transfected cells will be either primary human or mouse cells or well characterised stable eukaryotic cell lines in tissue culture that have a history of safe use and these hosts can be considered disabled hosts because the cells are unable to colonise the worker (i.e. not their own cells).
Two potential risks to humans may arise: 1) the hazards of working with the naked plasmid vector + insert DNA containing potentially oncogenic DNA or shRNA/CRISPR against any tumour suppressor and 2) the supernatant containing the viral particles from the packaging cell line.

1) The amount of DNA is relatively low and it is envisaged that the risk of naked DNA entering the human body is relatively low. Linearized cloned DNA is usually introduced into the cells using different transfection techniques, however the transfection efficiency even under optimal culture conditions is not high, therefore transfection of researcher’s cells with naked DNA under accidental/non-optimal conditions would be rather low.

2) The viral vector would be able to transduce many tissues should it come in contact with them. The major hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of transfected cells. The two potential transmission routes are by external exposure (either naked skin or mucous membranes) and needle-stick injury. Some of the genes that will be studied are novel and potentially could have oncogenic effects or interfere with the ability of the cells to survive in culture. If those genes were to be transfected directly into living human tissue any detrimental effects that they might cause are uncertain. However, the genes will only be transfected into cell lines or primary cells which have no ability to survive outside tissue culture environment. Even in the event of needle stick injuries or aerosol exposure, these cells (which will exist in very low numbers) will be destroyed by the human immune system.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste materials including all tissue culture fluid etc generated in this project MUST be autoclaved. No other inactivation or waste disposal routes will be used. Spillages must be cleaned up with disinfectant but the tissues and other waste materials must then all be autoclaved.

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Upon the advice of the committee Dr Llobet-Navas has extended the scope of his original risk assessment to allow this notification to cover other appropriate lentiviral activities that may take place in the future in the Institute for Genetic Medicine.

The committee advised that, although the risk assessment covers 2nd generation lentivirus vector systems or better, 3rd generation (or safer) vectors should be used in preference to 2nd generation where possible.

Project Containment
Project Ref 540/16.8

Date Ackn’ed 20/10/2016

CU2 Project Title Transduction of mammalian cells using lentiviral vectors

Date Project Ceased

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrew N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

This risk assessment covers research projects in our lab utilising lentivirus vectors and mammalian host cells. The projects involve assessing the biological function of candidate genes implicated in neuronal function or brain tumour progression. Genetic modification will occur in vitro and in vivo.

Lentiviral particles will be generated on site containing either a cDNA or appropriate shRNA. cDNAs may occasionally contain a mutation where a dominant negative or constitutively active version of the gene is required. The purpose is to perturb the natural state of the gene in question and to assess the effects on cells. Downstream analysis may involve cell cycle analysis, proliferation, cytotoxicity, apoptosis or differentiation or neuronal function assays, and development of in vivo xenograft models. RNA, DNA and protein would typically be extracted from infected cells in each case to verify appropriate infection and to validate cDNA/shRNA expression. A lentiviral pool expressing various
cDNAs or shRNAs may be used to create a pool of cells with several integrated constructs which will then undergo in vitro or in vivo selection to screen for constructs which effectively modify cellular function.

Controlling Abnormal Network Dynamics using Optogenetics (CANDO) is a major multi-site cross-disciplinary project to develop a cortical implant for optogenetic neural control. The goal of this project is a first-in-human trial in patients with focal epilepsy. This seven-year, £10M award from the Wellcome Trust and EPSRC involves collaboration between 13 research groups based in Newcastle, Imperial College and University College, London. The role of my lab in this collaboration is to design and produce molecular biological tools to support neurophysiology research, particularly lentiviral vectors for gene delivery. Optogenetics is a powerful tool to understand and experimentally manipulate neural activity. Ectopic expression of channelrhodopsin (ChR2) allows light-evoked stimulation of neurons in the adult mammalian brain. The channelrhodopsin gene will be subcloned under a cell-specific or ubiquitous promoter and tagged with a reporter such as green fluorescent protein (GFP).

Research by our group and others has demonstrated that tumour cells derive their energy from fatty acid sources as well as carbohydrates. Our research focuses on either upregulating or downregulating cellular signalling factors and factors involved in fatty acid bioenergetics with relevance to brain tumours. This work aims to increase understanding of tumour cell biology and identify potential new drug candidates. In view of this goal, our research involves creating and inserting shRNA for genetic knockdown experiments and also overexpression constructs for evaluating the contribution of particular gene products in fatty acid metabolism and signalling. This project aims to modify brain-derived cells to evaluate or modify their oncogenic capacity. The aim is to use either overexpression or knock-down of gene-expression to assess the potential contribution to tumourigenesis of multiple candidate genes. Once a stably infected cell line is produced, experiments performed will depend on the particular gene selected but will typically involve a panel of typical proliferation, apoptosis and/or invasion assays as well as collection of RNA/DNA/Protein for further profiling analysis and potentially in vivo engraftment.

The third-generation self-inactivating lentivirus we intend to produce in the course of this project was developed in the Trono and Baltimore labs to eliminate replication competence while maintaining the ability of the vector to infect both dividing and non-dividing cells. The lentiviral vectors employed do not present risk of homologous recombination (SACGM 2.11.14).

The transfer vector systems are derived from FIV or HIV and have been specifically engineered for biosafety by separating the packaging signals and viral LTR’s on the expression plasmid from the viral structural and expression genes (gag, pol and rev from FIV and the VSV-G gene from Vesicular Stomatitis Virus, or similar alternatives, in place of FIV or HIV env) encoded on four separate plasmids, which remain in the packaging cell line, effectively precluding the production of replication competent virus in the target cell or should the viral vector escape containment.

The viral vector and recombinant virus have a broad host range when pseudotyped with e.g. the VSV-G envelope protein. The normal tropism of HIV has been broadened to include multiple tissues from a broad range of species. Pseudotyping with alternatives to VSV-G, for example measles virus derived glycoproteins, will produce particles with more restricted tropisms.

The viral vector is disabled. >2/3 of all genes are deleted, including all genes involved in viral replication and genes encoding the original viral coat proteins. The vector is a third-generation self-inactivating (SIN) design. This design produces a disabled, replication-incompetent lentiviral vector that lacks the genes encoding for the original packaging proteins, structural proteins, reverse transcriptase and integrase, and additional virulence factors present in HIV-1. Deletion of accessory genes vpr, vpu, vif and nef mean that the vector is unable to replicate once it has transduced the target cell. In addition third generation lentiviruses are deleted for the tat gene and carry a SIN deletion of the 3’ LTR (the U3 region) which results in “self-inactivation” of the lentivirus following transduction of the target cell, precluding adventitious activation of the vector by endogenous retroviruses and minimising the risk of recombination with ERVs. Finally, a pseudotyped envelope (e.g. vesicular stomatitis glycoprotein) has been substituted for the original HIV-1 envelope protein.

Packaging cell line 293T (and thereof derived cell lines) is derived from the well-characterised 293 cell line (Pear et al., PNAS 1993; 80; 8392; ATCC CRL-11268). This cell line stably and constitutively expresses a temperature-sensitive version of the SV40 large T antigen. This cell line will be used to produce lentiviral particles. These vectors alone are replication incompetent owing to their lack of envelope and gag-polymerase encoding sequences, as well as their self-inactivating nature. Plasmid DNA will be produced in standard competent E.coli hosts (e.g. Stbl3, Stbl2, DH5 alpha, etc).
Virus will be added to cells in vitro to create stably-transduced cultures. The viruses are third-generation, self-inactivating constructs and are not replication competent.
Virus will also be injected directly into the brains of adult mice. Mice are themselves containment systems, since they are non-permissive hosts for this virus.
Mammalian cell lines and primary mammalian cells will be used for infection with the concentrated or unconcentrated supernatant generated by the packaging cells. Cell lines such as MT2 or MT4 producing infectious particles will not be used for transduction. Most cell lines are derived from normal brain tissue or solid tumours.

Host/vector system

Replication-incompetent self-inactivating lentivirus will be produced by transfection of HEK293T cells with plasmids such as pMDLg/pRRE (Addgene #12251) and pRSV-REV (Addgene #12253), which encode viral packaging proteins; pMD2.G (Addgene #12259), which encodes the pseudotyped VSVg coat protein, or pMD.RVG.CVS24.N2c (Addgene #19712), which encodes the pseudotyped rabies glycoprotein coating; and pEMCV-IRES-GFP (Addgene #12254) or similar third-generation lentivirus plasmid, which allows for the expression of the gene of interest and a reporter tag for identification of infected cells. Each plasmid will be transfected along with three viral production genes (for example, pMDG, pRSV-REV and pMDLg/pRRE) into HEK293T cells by calcium chloride coprecipitation. HEK293T will assemble the third-generation lentiviral vector and release it into the media.
The plasmids used for transfection of HEK293T cells are commercially available and have been modified to increase the safety and efficiency of viral production. The lentiviral constructs we use originated from the labs of Didier Trono and David Baltimore, and represent some of the safest and easiest-to-use vectors available for the delivery of genes into mammalian tissue [1-3]. The resultant lentiviruses are replication-deficient, and are used in many labs around the world at this time for in vivo applications [3-4]. Furthermore, they are designed to be extracellularly unlikely to become replication competent, with >2/3 of the viral genome deleted from the final packaged vector (including the abolition of gag/pol and wild-type envelope genes). These viruses also have self-inactivating properties to prevent integrated genes from being repackaged, in the event a subsequent infection ever occurs.
References

Origin & function
cDNA sequences of human or mouse origin and/or the appropriate shRNA sequences will be cloned into the lentiviral vectors. These may include partial or entire gene sequences, or mutant forms of the gene sequences, under the control of constitutive or cell-specific promoters. Many of the genes or targets of shRNA will have unknown function but may include known oncogenes or tumour suppressors.
For the first project, we aim to use optogenetics to evoke neural activity. Optogenetics is a powerful tool to understand and experimentally manipulate neural activity. The genes of interest to be inserted into the vector will therefore include opsins for the purpose of optogenetic stimulation of mammalian neurons. Ectopic expression of channelrhodopsin (ChR2) and its variants allow light-evoked stimulation of neurons in vitro and in the adult mammalian brain.
Although this is not an exhaustive list, inserts in the vector plasmid construct may include genes such as:
ChR2 (H134R) channelrhodopsin with standard peak activation and decay kinetics
ChR2 (L132C) CATCH construct for eliciting calcium currents
ChR2 (E123A) ChETA construct for faster closure kinetics
ChR2 (I170V) ChIEF construct for faster closure kinetics
ChR2 (T159C) TC mutant for a bigger photocurrent
ChR2 (C128T) Step-Function Opsin for bistable on-off control
Chronos – a sensitive blue-light-activated excitatory opsin
Chrimson – a sensitive red-light-activated excitatory opsin
eNpHR3.0 – chloride pump which inhibits neuronal activity with peak excitation at 590nm and fast decay time
ACR2 – chloride channel which inhibits neuronal activity with peak excitation at 470nm
ArchT – proton pump which inhibits neuronal activity with peak excitation at 590nm

For the second project, we aim to increase or decrease mitochondrial activity and metabolic gene expression by manipulating expression of pro-metabolic transcription factors such as PGC1α, transporters such as CPT1α, and enzymes such as acyl CoA dehydrogenases. The goal of this manoeuvre is to explore the intrinsic metabolic limitations on energy-intensive cellular activities such as firing action potentials (in the case of neurons) or cell cycle progression (in the case of tumour cells). In addition, we will explore the role of metabolism on cellular activities by using shRNA delivered to cells in vitro. The shRNA constructs will be cloned into a third-generation lentiviral vector plasmid and the resulting viral particles will be delivered to cells.

Although this is not an exhaustive list, inserts in the vector plasmid construct may include genes such as:

- PGC1α – enhances the metabolic capacity of cells dependent upon oxidative respiration
- PPARG – acts as a transcription factor to regulate pro-metabolic gene expression
- CPT1α – acts as a transporter of acyl-carnitines across the mitochondrial membrane for beta-oxidation
- Medium-Chain acyl CoA dehydrogenase – acts as an enzyme to break down fatty acids for energy
- shRNA targeting any of the above genes, for example

For the third project, we aim is to use either overexpression or knock-down of gene expression to oncogenically transform mammalian cells, particularly to assess the contribution of metabolic changes to malignant behaviour and to assess the effects of oncogenic transformation on cellular metabolic strategy. Once a stably infected cell line is produced, experiments performed will depend on the particular gene selected but will typically involve a panel of typical proliferation, apoptosis and/or invasion assays as well as collection of RNA/DNA/Protein for further profiling analysis and potentially in vivo engraftment.

Although this is not an exhaustive list, inserts in the vector plasmid construct may include genes such as:

- H-Ras – an oncogene
- EGFR – a growth factor receptor
- p53 – a tumour suppressor
- shRNA against any of the above genes, for example
- HPV exons 6 and 7 – encodes gene products that disrupt tumour suppressor signalling

Promoters used in the vector plasmid construct for both projects may include ubiquitous promoters (such as CMV, EF1, etc) or cell-specific promoter regions (such as hThy1, hSyn1, CamKII, PV, GFAP, etc).

Fluorescent reporter proteins based on green fluorescent protein (GFP from Aequorea victoria), and similar safe, non-toxic fluorescent proteins from other organisms (e.g. Discoma spp. mCherry (Shaner et al. Nature Biotechnology 2004) will be used. Transfection with GFP and other fluorescent reporter proteins are considered safe as there has been widespread use of these proteins both in vitro and in vivo with no deleterious effects.

Evaluation of foreseeable effects

There are potential routes of transmission by which the viral vector or its products could be delivered, and these risks can be controlled with appropriate measures. The viral vector would be able to transduce many types of tissues should it come in contact with them. The major hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of infected cells. The two potential transmission routes are by external exposure (either skin lesions or mucous membranes; only in the case of very high titres and aerosol production) and by accidental injection/inoculation using sharps. If genes were to be transduced directly into living human tissue the detrimental effects that they could cause are uncertain but unlikely on their own to transform affected cells. Given that virus is replication incompetent and will only infect at low efficiency in sub optimal conditions any potential harm would be extremely limited. These risks will be further minimised by measures described below.

All those involved with this project will be trained in biological safety, GM safety and will have been advised by the PI and the project license holder on the detailed risk assessment for the use of sharps that has been prepared for this work. To minimize risk, blunt needles are used for injection and virus delivery is conducted by a controlled microinjection unit so there is no direct contact with the syringe or needle during injection.

The inserted genes include reporters such as enhanced green fluorescent protein (eGFP), which allows for identification of infected cells, and opsins such as channelrhodopsin, which allows for optical stimulation of neuronal activity. In addition, known oncogenes or shRNAs that will silence known tumour suppressor genes may be inserted into primary cells and cell lines. There is therefore a theoretical possibility that they could render the cells neoplastic. If during an unlikely event these cells were inoculated into the human organism, then these cells are recognised as non-self and will be identified by the immune system. Inserted gene products will be both genomic, cDNA sequences or small interfering double stranded RNA sequences originating in humans or rodents, including those which could be implicated in tumour progression, i.e. oncogenic and tumour suppressor DNA sequences. Specific functions will vary depending on the particular experiment, but will include functions that are known to
Contribute to the maintenance of pluripotency and/or differentiation and the regulation of cell growth and apoptosis. The gene products will be biologically active when transfected into mammalian cells and probably expressed at a level higher than seen in the wild type cells. However, the recipient transfected cells will be either primary human or mouse cells or well characterised stable eukaryotic cell lines in tissue culture that have a history of safe use and these hosts can be considered disabled hosts because the cells are unable to colonise the worker (i.e. not their own cells).

Two potential risks to humans may arise from the hazards of working with the naked plasmid vector + insert containing the potentially oncogenic DNA and the supernatant containing the viral particles from the packaging cells. However, the transduction efficiency even under optimal culture conditions is negligible, and therefore transfection of researcher's cells with naked DNA under accidental/non-optimal conditions would be effectively zero.

The individual potential oncogenes will only be sufficiently oncogenic to give a growth advantage to cells in culture without inducing tumourigenesis. Tumourigenesis is a multistage process requiring the interaction of many mutated genes which also lessens the risk from delivery of a single oncogene or tumour suppressor gene to a target tissue. The principle route of transmission that could lead to transduction of laboratory workers by packaged viral particles is inoculation. Therefore, blunt needles and pipet tips will be used. Other possible routes of transmission such as inhalation, ingestion and eye splashes are less likely to lead to tumourigenesis because of the required very high titres, which will not be achieved under the given experimental conditions. Consequently, the risk of tumourigenesis is negligible.

The self-inactivating lentivirus vector systems to be used are such that they have been extensively modified and are regarded as being stable, as are the inserted sequences. The lentiviral vector system to be used is considered to be “Third Generation” as defined by the SACGM Compendium of Guidance 2.11.9 as the accessory genes (vif, vpr, vpu, and nef) have been deleted along with the tat gene. The risk of dissemination of the described vector is extremely low due to it being replication incompetent and self-inactivating (SIN) (SACGM Compendium of Guidance, Part 2.11). The virus will be injected into mice or rats, which are non-permissive hosts for this vector. The extensive modification of the viral vector also prevents homologous recombination with other related viruses. All cultures are maintained under sterile conditions and it is unlikely that any hazardous sequences if they were present could be transferred to related organisms. The restricted location of the virus within the CNS of injected animals and the non-permissibility of rodents as hosts makes it unlikely that experimental animals would be able to transfer genetic material to other animals. Whilst it is possible for the virus to infect other organisms, this is considered unlikely (SACGM Compendium of Guidance, Part 2 “Risk assessment of genetically modified microorganisms (other than those associated with plants)”. Viral particles capable of transducing a wide range of mammalian cells will be produced by the packaging cell line and these represent the hazardous material. However, because of its incompetence to replicate and inefficient transduction rates, the risk of transmission outside the laboratory is effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

IN VITRO WORK:
In accordance with the SACGM Compendium of Guidance, GMM mammalian cells will be handled at Containment Level 2 and using microbiological safety cabinets. All GM virus waste will be properly inactivated by autoclaving. Spillages will be soaked with tissue paper, which will afterwards be autoclaved. Contaminated surfaces may be decontaminated with either a 1% virkon solution or 70% Ethanol.

IN VIVO WORK:
Viral infection of animals will be performed under sterile conditions within a surgical suite equating to Containment Level 2, in order to control hazards associated with possibly adventitious agents and to avoid contamination. Used bedding and other materials from animal cages will be autoclaved for sterilization before leaving the CBC to be incinerated. Please see related documents (GM Risk Assessment Forms 2 and 5, relevant Home Office licenses) for more detail on how these procedures will be carried out.

Hypodermic needles will be used and therefore suitable training and precautions will be taken to minimise the potential for self inoculation. Viral vectors will be injected using blunt needles, therefore minimising the risk of accidental injury. A detailed risk assessment has been prepared and all staff will be required to demonstrate proficiency in all procedures before surgical work with viral vectors is allowed to commence.
GENERAL:
Used blunt needles, pipette tips, serological pipettes will be collected in a “CinBin” type container inside the biosafety cabinet and placed within the plenum of the MSC immediately after use. The container and its contents will be autoclaved prior to disposal by the clinical waste route. Blunt needles will in fact be used for injection but will be handled as if sharps. Biohazard boxes will be used as a secondary containment system to transport all materials to the autoclave.
All waste that is possibly contaminated with virus will be inactivated by incubation with a final concentration of at least 1% Trigene for 30 minutes and all of this waste will be collected in dedicated bags and these transferred to an autoclave using closed containers and will be inactivated by autoclaving at 121°C for 20 min. This procedure will ensure 100% killing efficiency. All autoclaved waste will be incinerated using the yellow bag route as clinical waste using an approved contractor.

INACTIVATION OF GMOs:
Dead animals are collected and treated as clinical waste and are disposed of by incineration following autoclaving.
Animals will be perfused with para formaldehyde (PFA) for experimental purposes and it is therefore inappropriate to autoclave this material. Although the PFA is expected to kill any residual GM virus present in the animal, it is not possible to accurately validate whether inactivation of GM virus has been effective. For this reason any PFA-perfused carcasses will be disposed of as GM waste. This waste will be collected by SCRL who are a GM registered carrier and who are aware of the arrangements and will be disposed of by incineration. All associated documentation for waste disposal will clearly state that this is GM waste. Waste will be securely stored and transported in yellow ‘Cin bin’ containers which have securely fastened lids.
Other waste that is possibly contaminated with virus will be inactivated by incubation with a final concentration of at least 1% Trigene for 30 minutes and all of this waste will be collected in dedicated bags and these transferred to an autoclave using closed containers and will be inactivated by autoclaving at 121°C for 20 min. This procedure will ensure 100% killing efficiency.
Temperature and time during each autoclave run will be monitored using indicator strips (TST control integrator, class 6 emulating indicator 121ºC for 20 min, Albert Browne Ltd, UK). The autoclave will be serviced and tested on an annual basis using an independent thermocouple mapping test.

Please enter comments on the GM safety committee on the risk assessment
The committee requested that:
Details of attendance on Biosafety and GM safety training courses were stated.
A definition of 'third generation' vector was included e.g. at least 3'SIN deletion and no gag, pol, env and regulatory genes
There is less rigidity in the list of inserts to allow flexibility of the risk assessment allowing other similar inserts to be included.
It was clear that blunt needles are to be used for all vectors, not just those containing the oncogenic sequences.
These were all addressed and included in the attached final documentation.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</thead>
<tbody>
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<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
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### Project Ref 540/17.1

**Date Ackn’d** 05/05/2017  
**CU2 Project Title** Use of fluorescent tagged plasmid with known sequence in an E.coli strain isolated from wastewater to assess transmission of mobile genetic elements in a closed wastewater treatment bioreactor.

**Date Project Ceased**

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<td>Class 2</td>
<td>≤ 1 Litre</td>
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**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Withdrawn** N

**Tick if notifying a connected programme of work** N

### Project Additional Information

**Purposes of the contained use**

Six wastewater treatment bioreactors have been established to monitor the dissemination of an antibiotic resistance plasmid throughout the reactors from a strain of E. coli to be seeded into the incoming wastewater stream. To distinguish antibiotic resistance genes that occur naturally in the wastewater, the seeded strain will carry a copy of plasmid RP4 into which the gene encoding the green fluorescent protein (GFP) has been cloned (Musovic et al., AEM, 76, 4813-4818 (2010) & EM Rpt 6, 125-130 (2014)). It will be transferred by conjugation to a wild type E. coli strain isolated from wastewater as the seed organism to investigate the fate of broad host range IncP-1 plasmid during wastewater treatment. The work will be performed in tandem with an on-going bioreactor experiment to study the dynamic of antibiotic resistance genes (ARGs) associated with mobile genetic elements (MGEs) during treatment processes. pRP4 encodes resistance genes for ampicillin, tetracycline and kanamycin. Mass balance of gfp-labelled pRP4- Plac::gfp plasmid will be quantified using flow cytometry (Medical School Flow Cytometry Core Facility) to monitor the conjugal transfer kinetics of the plasmid and the harboured ARGs between commensal bacterial. The experiment will involve the seeding of E. coli donor strain into six wastewater treatment bioreactors to track the transmission of the RP4 plasmid over a time series within the operating treatment cycle. Additionally, the prevalence of the antibiotic resistances encoded by pRP4 will be monitored by plate counts. The frequencies of the target resistances and the GFP signal will provide the required data from horizontal transfer in the bacterial cell population of the individual reactors.

**Recipient or parental organism**

An E.coli strain isolated from domestic wastewater will be used as the seed organism to host the GFP-tagged pRP4 plasmid. The identity of the E. coli was confirmed by the Microbiology Department at the Freeman Hospital and is susceptible to 17 clinical antibiotics including major classes of beta-lactams antibiotics (e.g. Aztreonam,
Meropenam, Ertapenam), trimethoprim, ciprofloxacin, etc. A serotype test has also been carried out and has confirmed that the strain is not of the O157 serotype and therefore not shiga toxigenic.

Host/vector system

Plasmid RP4::Plac::gfp (Musovic et al., 2010) has a molecular size 56.4 kb, and encodes resistance against the antibiotics ampicillin, tetracycline and kanamycin. The used of the native pRP4 (ie without the gfp gene) is not subject to GM regulations or other restrictions.

Origin & function

The gfp gene has been introduced onto pRP4 to facilitate the monitoring of the transfer of the antibiotic resistance genes associated with pRP4 within the population of cells within the bioreactor. The gfp gene, obtained originally from Aequorea victoria, is widely used as a visual and quantifiable reporter gene and is not associated with any toxicity. Its role here is to monitor transfer to other cells in the bioreactor population. Plasmid RP4, subgroup alpha (Thomas, 1981) and tagged with a mini Tn5 insertion of a gfpmut3b gene, is derived from Pseudomonas putida KT2440 (Barth et al., 2015). The complete nucleotide sequence pRP4 has been published (Pansegrau et al., 1994). The plasmid will be obtained from Dr Musovic, Technical University of Denmark. The gfp gene will be expressed from the Plac promoter at a medium level of expression. Plate mating assays will be used to transfer the gfp-labelled plasmid to the indigenous E. coli. The resulting transconjugant will be used as the donor strain for the subsequent seeding experiments to track the fate and migration of the plasmid and the associated resistance genes to other indigenous bacteria found in wastewater treatment system. This is a natural process that occurs in wastewater treatment processes. The gfp-labelled pRP4 plasmid will not be subjected to any further modifications.

Evaluation of foreseeable effects

pRP4 is a well-characterised resistance plasmid belonging to the IncP-1 group, which is a promiscuous conjugal plasmid with the ability transfer and replicate in broad range of hosts especially to Gram-negative bacteria. The ability of the pRP4 to replicate and to be maintained in the recipient organisms poses a potential environmental and health risk if released. However, the work will be restricted to Containment Level 2 laboratories in both the Baddiley-Clark and Cassie Buildings. RP4 vector will be introduced in a closed bioreactor systems under contained use and all remnant materials, including the reactor outflow, will be inactivated by autoclaving. The only exception will be small (millilitre volumes) samples taken for FACS and DNA extraction, which will be inactivated during processing. The primary hazard is the potential of the commensal E. coli isolated from domestic wastewater being a pathogenic strain (ACDP Hazard Group 2), which may be carried through the biotreatment processes (activated sludge, secondary treatment biofilms and final effluent) but at much lower concentrations, equivalent to ACDP Hazard Group 2 (Compendium of Guidance). Prof David Graham has an internationally recognised reputation for running bioreactors using wastewater input streams, and the only variation in the proposed experiments is the presence of the gfp gene. As a result there will be no increase in the risk to human health. Ms Jong also has more than two year of experience running wastewater bioreactors. Therefore, under the proposed contained use, the recombinant organism is unlikely to represent an increased risk to human health. Level of risk is predicted to be low/medium.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min). Contaminated materials used in laboratory during the experiment and waste will be disposed of via and appropriate route e.g. sharps in sharps bins, bulk materials in autoclaves. All outflow from bioreactors will be inactivated by means of autoclaving. A limited number of samples will be used for analytical studies (PCR, FACS etc), and these will be inactivated during processing and autoclaved after use.

02/03/2022
The only comment was "My only issue with the risk assessment is that it does not mention the possibility of the strain being HG3. Since it is an unknown strain, it would be useful to have some kind of assurance that it is not verocytotoxigenic (ie HG3). They may already know this from the identification that was done at the Freeman, but it would be worth stating it explicitly in the risk assessment." The confirmation that the strain was tested at the Freeman Hospital Microbiology Department to confirm it was not verocytotoxigenic was added to the GMM risk assessment form as requested.

**Project Containment**

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**Project Ref** 540/18.1

- **Date Ackn'd**: 17/05/2018
- **CU2 Project Title**: Role of histone modifications and kinases in mitosis
- **Class**: Class 2
- **Culture Vol Class 2**: < 1 Litre
- **Culture Volume Class 3-4**: Not Applicable
- **Non-GMM Consent Granted**: Not Applicable
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**: 02/03/2022
Lentiviral particles will be generated on site containing either a cDNA or an appropriate shRNA. cDNAs may occasionally contain a mutation where a dominant negative or constitutively active version of the gene is required. The purpose is to perturb the natural state of the gene in question and to assess the effects on cells. Downstream analysis may involve cell cycle analysis, proliferation, cytotoxicity, apoptosis or differentiation assays. Stable cell lines will be obtained by antibiotic selection based on resistance genes encoded by the plasmids. Localization and protein-protein interactions will be determined by immunofluorescence microscopy and immunoprecipitation and/or western blotting respectively. RNA, DNA and protein would typically be extracted from infected cells in each case to verify appropriate infection and to validate cDNA/shRNA expression. A lentiviral pool expressing various cDNAs or shRNAs may be used to create a pool of cells with several integrated constructs which will then undergo in vitro or in vivo selection to screen for constructs which either promote or inhibit cell proliferation.

Established human cell lines: including HeLa, U2OS, HEK293, hTERT-RPE-1, MDA-MB231, Daudi, K562, Jurkat, MCF-7, U937, THP-1, Ramos, PC3,
Established mouse cell lines: NIH-3T3
Established hamster cell line: CHO

We will be using second and subsequent generation lentivirus and retrovirus. They are replication incompetent and self-inactivating (SIN). The systems separate the packaging signals and viral LTRs on the expression plasmid from the viral structural and expression genes (gag, pol and rev from FIV or HIV and viral envelope glycoproteins such as the VSV-G gene from Vesicular Stomatitis Virus, in place of HIV or FIV env). The viral structural and expression genes and the envelope glycoprotein genes are separated on at least two additional plasmids.

cDNA sequences of humans or mice origin and/or the appropriate shRNA sequences will be cloned into the lentiviral vectors. These may include partial or entire gene sequences, or mutant forms of the gene sequences, under the control of constitutive promoters. Many of the genes or targets of the shRNAs will have unknown function but may include known oncogenes or tumour suppressors, such as cDNAs encoding human or mouse genes, and mutants thereof: Haspin, Aurora B, MCAK, Pik1, Aurora A, Aurora C, Sgo1, Sgo2, TESO/RCC2, Rad5, WDR5, Hec1, Dsn1, Kn1, PP1, PP2A, RepoMan, INCENP, Survivin, Borealin, Histone H3, H4, H2A, H2B, H2AZ, H2AX, CENP-A, CENP-B, CENP-E, Rad21, Scc2, SMC1, SMC2, SMC3, SMC4, SA1, SA2, FLJ35779, ZNF198, astrin, Chk1, DikZIP, PRC1, Mklp2, Mklp1, KIf4, Bub1, BubR1, Mad1, Mad2, CTCF, TAF3, TAF5, DIDO3, BHC80, ING2/4, BPTF, EGFP; CRISPR Cas9 targeting factors and shRNAs for these genes.

The aim is to use either overexpression or knock-down of gene-expression to assess the potential contribution to cell cycle progression of each candidate identified from our genomics and proteomics screens. Once a stably infected cell line is produced experiments performed will depend on the particular gene selected but will typically involve a panel of typical proliferation, apoptosis cell biology assays as well as collection of RNA/DNA/Protein for further profiling analysis.
unable to produce infectious virus once it integrates into the host chromosome. Deletion of accessory genes vpr, vpu, vif and nef mean that the vector is unable to replicate once it has transduced the target cell. In addition third generation lentivirus are deleted for the tat gene and carry a SIN deletion of the 3’ LTR (deltaU3) which results in “self-inactivation” of the lentivirus following transduction of the target cell, precluding adventitious activation of the vector by endogenous retroviruses and minimising the risk of recombination with ERVs.

The origin of the virus the mechanism of attenuation, and its stability in both the parent viral vector and the recombinant vector:

The transfer vector systems are derived from FIV or HIV and have been specifically engineered for biosafety by separating the packaging signals and viral LTR’s on the expression plasmid from the viral structural and expression genes (gag, pol and rev from FIV and the VSV-G gene from Vesicular Stomatitis Virus, or similar alternatives, in place of FIV or HIV env) encoded on three or four separate plasmids, which remain in the packaging cell line, effectively precluding the production of replication competent virus in the target cell or should the viral vector escape containment. FIV vectors are included as they may be more efficient for some cell types. It does not imply any different containment or risk considerations than HIV based vectors. The plasmids expressing these gene products carry no packaging signals or LTRs and so cannot themselves be mobilised with the vector and have been engineered not to contain any regions of homology to each other or to the viral vector, to prevent undesirable recombination events which might result in replication competent virus being produced.

The viral vector and recombinant virus have a broad host range when pseudotyped with e.g. the VSV-G envelope protein. The normal tropisms of HIV and FIV have been broadened to include multiple tissues from a broad range of species. Pseudotyping with alternatives to VSV-G, for example measles virus derived glycoproteins, will produce particles with more restricted tropisms.

The probability of reversion to wild type is negligible. No single plasmid contains all the components necessary to produce viral particles. 2nd and 3rd generation viral plasmids will be used which means that 3 or 4 separate plasmids are required to produce virus. Reversion to wild type is extremely unlikely given that several recombination events would be needed reconstitute an active viral genome, the viral genes are present on three different plasmids which have minimal sequence homology and the viruses are self-inactivating following insertion (i.e. the LTR’s are destroyed upon genome insertion).

All potential routes of transmission of the virus known, eg those that may occur during a laboratory accident. The viral vector would be able to transduce many tissues should it come in contact with them. The major hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of infected cells. The two potential transmission routes are by external exposure (either skin lesions or mucous membranes; only in the case of very high titres and aerosol production) and by accidental injection/inoculation using sharps.

The viral vector can infect humans or human cells in vitro. However, the severity of harm would be minor given that infection of human tissue if achieved would be with non-replicable virus and low-efficiency. Likelihood of harm Low given lab procedures for avoiding contamination, in particular no sharps, etc. The virus is generally weak and does not survive long outside of buffered solutions. Infection requires optimal conditions and the use of additives in cell culture. Immune-competent mice show sterilising immunity with wild-type virus infection; humans would also show such sterilising immunity. Risk is generally speaking no greater than that of handling human tissue or bodily fluids i.e. relatively safe given that proper precautions are taken to avoid infection during specialised training.

The GM virus would have the ability to infect human tissues should it come in contact with them. The major hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of transfected cells. The main potential transmission routes are by external exposure to high titres (either skin lesions or mucous membranes). Some of the genes that will be studied could have oncogenic effects. If those genes were to be transfected directly into living human tissue the detrimental effects that they could cause are uncertain but unlikely on their own to transform affected cells. Given that virus is replication incompetent and will only infect at low efficiency in sub optimal conditions any potential harm would be extremely limited.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Spillages will be soaked with tissue paper, which will afterwards be autoclaved. Contaminated surfaces may be decontaminated with either a 1% virkon solution or 70%
The GM safety committee agreed the classification of the risk assessment as activity class 2. The ability to use 3rd generation vectors was explored.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
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</table>

Animal Units
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

Project Ref 540/18.2

Immortalisation of mammalian cell cultures

Date Ackn'd 19/09/2018

Date Project Ceased

Consent Granted Not Applicable

Project notified under transitional arrangements N
Purposes of the contained use

To expand the life span of rare patient cell lines by releasing them from dependence on the growth factors normally necessary for their proliferation.

Recipient or parental organism

Host organism - Primary fibroblasts, PA317 and HEK293 cells

E.coli will be used to produce and propagate the viral vectors and associated production of plasmids which encode replication factors for viral production in HEK293 cells and PA317 cells. However, the viral vectors are not active in prokaryote, are not replicative as a double stranded DNA molecule and do not contain prokaryotic promoters adjacent to the inserted genes. Plasmid expression in prokaryotes is therefore limited to plasmid replication factors and antibiotic resistance, as found in standard molecular biology techniques and so this part of the process poses no risk above standard molecular cloning.

The target cells which will be infected with the virus are primary fibroblast cultures. These cells will be infected at moderate titre and it is anticipated that the viral vectors will have a biological effect, but will not replicate under these conditions due to the absence of essential replication factors. The effect of the virus is not anticipated to induce changes in these cells which will increase their pathogenicity, toxicity or infectivity. Once transduced excess virus will be removed and destroyed. The major risk is posed by the high titre virus, both before transduction and immediately following transduction where a significant quantity of infective virus particles is present. Following measurements of biological activity, cells will be disposed of via a standard route (see disposal SoP in appendix 2).

Human embryonic kidney cell line 293FT (HEK293) is a cell line optimised for viral transfection and serves as the standard cell line for viral production and packaging. Plasmids supplying the replication and packaging functions which have been deleted from the wild-type virus, or are normally provided by co-infecting adenovirus, will be transfected into the cells with the replication defective viral genome. The HEK293 cells are not anticipated to become more pathogenic or toxic through the process of viral production.

The major risk is posed by the high titre virus produced by the cells. These cells are widely used in this application and have a long history of safe use. Following viral production HEK293 cells will be disposed via a standard route consistent with CL2 containment PA317 cell line was derived from TK- NIH/3T3 cells by cotransfection with packaging construct DNA (pPAM3) carried in pBR322 and the herpes simplex virus thymidine kinase (TK) gene carried in pBR322. Introduction of retroviral vectors into these cells, by infection or by transfection, results in production of retrovirus virions with an amphotropic host range that are capable of infecting cells of many mammalian species. Virions produced by this line have been used successfully to transfer genes into humans. The PA317 cells are not anticipated to become more pathogenic or toxic through the process of viral production. The major risk is posed by the high titre virus produced by the cells. Following viral production PA317 cells will be disposed via a standard route consistent with class II containment.

Host/vector system

Vector system

The retroviral vector pLXSN has been widely used and has a history of safe use. The vector is detailed below:

pLXSN contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus.
MoMuSV), and is designed for retroviral gene delivery and expression (Miller & Rosman (1989) Biotechniques; 7(9): 980-990). Upon transfection into a packaging cell line, pLXSN can transiently express, or integrate and stably express, a transcript containing 4′+ (the extended viral packaging signal), the gene of interest and a selectable marker. The 5′ viral L TR in this vector contains promoter/enhancer sequences that control expression of the gene of interest in the multiple cloning site. The SV40 early promoter (PSV40e) controls expression of the neomycin resistance gene (Neor), which allows antibiotic selection in eukaryotic cells. pLXSN also includes the Col E1 origin of replication and E. coli AmpR gene for propagation and antibiotic selection in bacteria, although we do not plan to propagate the viral vector in E.coli.

pLXSN is deleted (relative to the wildtype retrovirus) for the gag, pol and env genes. This means that whilst the packaged viral particles are infectious, they remain unable to replicate outside the highly specific and engineered conditions of the packaging cell line.

**Origin & function**

The E6E7 region of human papilloma virus 16 (HPV16) has previously been isolated and cloned into pLXSN (Halbert et al. (1991) JVirol65: 473-478). This region of the HPV16 genome has been shown to be sufficient to transform cells in culture when expressed from this vector. The intention is to infect cells with a high titre virus and select those which take up large amounts of the virus, and so become resistant to G418 (the vector also expresses the Neor gene). Those cells which survive extended selection in G418 are generally transformed by their concomitant extended exposure to the E6E7 genes.

**Evaluation of foreseeable effects**

The viral vector would be able to transduce many tissues should it come in contact with them. The major hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of transfected cells. The two potential transmission routes are by external exposure (either naked skin or mucous membranes) and needle-stick injury. Both are made extremely unlikely by the standard procedures employed in the CL2 tissue culture facility. Hypodermic needles or other sharp objects are not permitted in the viral handling laboratory or the MSC (see appendix 2), making needle stick injury very unlikely. Whilst pipette tips are used in the tissue culture facility, these are routinely filter tips, making contamination of the pipette barrels very unlikely. Standard use of surgical gloves and laboratory coats make external exposure to virus unlikely. However, there is a risk of contamination of the arms of the laboratory coat by an aerosol generated within the MSC. This necessitates the use of disposable covers for the laboratory coats whilst handling the packaged viral vector. External exposure by virus is unlikely to cause transfer of the viral vector since it is not expected to penetrate the epidermal layers of the skin. Direct exposure of virus to mucous membranes is difficult to envisage since all material must be sterilised using 1% Virkon prior to removal from the MSC, precluding the generation of aerosols containing viral particles in the vicinity of laboratory workers. Virally transduced genes will only be transfected into cell lines or primary cells which have no ability to survive outside tissue culture environment. Even in extreme events of needle stick injuries, these cells (which will exist in very low numbers) will be destroyed by the human immune system. Small numbers of cells that might exist in aerosols during the cell destruction also pose minimal risk of direct entry into the human body. These cells will be handled at CL2 and with all the measures described in Section 4 and appendix 1 and 2, the risk to human health will be minimised.

The following potential adverse consequences have been identified:

1. Random integration of virus into the genome. Although there is a risk of activating neighbouring oncogenes, this is unlikely since the viral vectors are both transcriptionally inert (other than the inserted sequences) and modified to reduce the frequency of integration.

2. Expression of E6/E7 genes. Retrovirus is unlikely to infect non-dividing cells, even at low efficiency. The risk that
E6/E7 will be expressed in these cells is therefore very low, even following exposure to high titre virus, itself an unlikely event. Furthermore, this would be a transient expression which in a non-replicative virus is unlikely to result in transformation of the cell. In vitro, cells must be transduced at high titre and placed under selective pressure with G418 in order to achieve transformation. It is unlikely that exposure to small quantities of virus will have harmful consequences.

The viral vectors are non-replicative, and any exposure would be to very small quantities of virus. The severity of these adverse events is likely to be both transient and treatable. The likelihood of harm is therefore negligible and the severity of harm is minor, a level of risk of effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

In accordance with ACGM and ACDP requirements virus lysates and infected mammalian cells will be handled at containment level 2 and using microbiological safety cabinets. These, like any micro-organism, are extremely sensitive to rapid and complete inactivation by the disinfectants specified below which will be used in accordance with the manufacturer's instructions. Only plastic pipettes will be used in the tissue culture facilities, sharps must be excluded, the generation of aerosols prevented and all waste must be inactivated as below before disposal. Furthermore, the following measures, contained in the Compendium, Part 3A, Annex I paragraph 10, applied in the light of this GM risk assessment, will be used.

1. Good laboratory techniques will be strongly emphasised. Designated workers will be trained in good laboratory techniques before commencing work with viruses. They will be made fully aware of the potential hazards of such work.
2. Access to the laboratory where viruses are handled, will be limited to authorised personnel and designated workers.
3. Gloves will be worn at all times. Where users are handling high titre viral stocks, packaging cell lines or newly infected cell cultures (which will have a high viral titre in the medium) disposable plastic oversleeves are to be worn in the hood and disposed of as for gloves.
4. Sharps will be avoided, as stab injury is a possible means of inoculation of virions. Hypodermic needles, glassware, scalpels, scissors, sharp-edged metal/plastic boxes and microscope slides/coverslips are not permitted within the designated virus room or MSC. See appendices 1-3 for details and SoP.
5. The generation of aerosols will be avoided. All experimental procedures involving virions will be performed so as to minimise aerosol production. Procedures which are likely to generate aerosols such as the use of sonicators, vigorous shaking and mixing etc. will be avoided, or performed within the MSC.
6. Procedures using the viral particles, packaging cell lines and exposed cells will only be carried out in the designated tissue culture suite. Normal procedure for handling biological material in the TC suite will be largely sufficient for handling the cells and particles (see appendices 1 and 2 for details and SoP).
7. All liquid waste to be treated with 2-3% Virkon (see appendix 2).
8. Safety cabinets are routinely disinfected prior to and following use using 70% ethanol. These should also be routinely swabbed down with 1% Virkon as well as exposed to UV radiation.
9. Solid waste to be treated with 1% Virkon, collected into an autoclave bag within the MSC and autoclaved prior to disposal.
10. Gloves and designated lab coats to be worn throughout. Gloves to be disposed as solid waste and lab coats sterilised (autoclaved) before going to the laundry.
11. Designated microbiological safety cabinets to be used for all viral work.
12. Individual culture medium bottles for each cell line.
13. No wild type virus to be used in these facilities.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste, including sharps bins, disposable plastics, laboratory glassware, or other contaminated materials will be autoclaved at 121°C for at least 30min to inactivate GMMs (100% kill) prior to disposal or cleaning and recycling. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous recording of the temperature/time profile. Culture media from cells will be treated with 2-3% Virkon for a minimum of 3 hours before disposal via drain.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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### Project Ref 540/19.1

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<tr>
<td>05/04/2019</td>
<td>Cloning and manipulation of Staphylococcus aureus and Staphylococcus epidermidis strains to study protein secretion and general physiology</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Non-GMM Consent Granted</td>
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</table>

Date Project Ceased

Project notified under transitional arrangements N
This risk assessment covers research projects in our lab utilising bacteria specifically Staphylococcus aureus, Staphylococcus epidermidis for the purpose of facilitating molecular biology procedures such as (i) sub-cloning, DNA sequencing, or site-directed mutagenesis; (ii) construction of chromosomal mutants; (iii) construction of fusions with harmless reporter genes such as GFP or tags such as HA and His; (iv) construction of chromosomal insertions with antibiotic resistance cassettes (v) construction of recombinant plasmids for subsequent purpose of over-producing proteins in Staphylococcus strains.

The genetically modified strains will be cultured in small to medium quantities for the isolation and analysis. This will involve solid and liquid culture of the strains, centrifugation, extraction of RNA, DNA and protein, microscopy, gel electrophoresis, western blotting, mass spectrometry and immunological studies.

Recipient or parental organism

We will use a number of strains of Staphylococcus aureus and Staphylococcus epidermidis including those that are MRSA.

Staphylococci are non-spore forming, facultative anaerobic, gram-positive bacteria from the group of cocci which occur naturally in humans and animals. Out of the 30 species which make up the genus Staphylococcus, the so-called "coagulase-positive" staphylococci have the highest pathogenic potency. The most important representative of the coagulase-positive staphylococci is Staphylococcus (Staph.) aureus. As staphylococci occur naturally on the skin and in the mucosa of animals and humans (particularly in the nasal area), food is mainly contaminated by secondary contamination through people. Meat and meat products offer favourable growth conditions for staphylococci. Other typical foods involved in staphylococcal intoxications are milk and dairy products, egg-containing preparations, salads, creams, cake fillings, ice-cream and pastry goods. Because of the heat stability of the enterotoxins, which are also resistant to the decomposition enzymes to be found in the gastrointestinal tract, the staphylococci can be destroyed through sufficient heat treatment but not the toxins possibly contained in the food.

PATHOGENICITY: Opportunistic pathogen, normal flora; produces a variety of syndromes with a range of clinical manifestations; clinically different in general community, newborns, menstruating women, and hospitalized patients; food intoxication is characterized by abrupt/violent onset, severe nausea, cramps, vomiting, and diarrhea using lasting 1-2days; animal bites can result in localized infections; may cause surface or deep/system infections in both community and hospital settings; surface infections include impetigo, folliculitis, abscesses, boils, infected lacerations; deep infections include endocarditis, meningitis, septic arthritis, pneumonia, osteomyelitis; systemic infection may cause fever, headache malaise, myalgia; newborns are susceptible to scalded skin syndrome (SSS) caused by exfoliative toxins; may be colonized during delivery resulting in sepsis meningitis; toxic shock syndrome is an acute multi-system illness caused by TSST-1 a super antigen; characterized by sudden onset, high fever, vomiting, profuse watery diarrhea, myalgia, hypotension erythematous rash

EPIDEMIOLOGY: Occurs worldwide; particularly in areas where personal hygiene is suboptimal; in hospitals by development of antibiotic-resistant strains

HOST RANGE: Humans; to a lesser extent, warm-blooded animals

INFECTION DOSE: Virulence of strains varies greatly

MODE OF TRANSMISSION: Contact with nasal carriers (30-40% of population); from draining lesions or purulent discharges; spread person-to-person; ingestion of food containing staphylococcal enterotoxin (food may be contaminated by food handlers hands); from mother to neonate during delivery

INCUBATION PERIOD: Variable and indefinite, commonly 4-10 days; disease may not occur until several months after colonization; interval between eating food and onset of symptoms is usually 2-4 hours (30 min to 8 hours)

COMMUNICABILITY: As long as purulent lesions continue to drain or carrier state persists; auto-infection may continue for the period of nasal colonization or duration of active lesions

RESERVOIR: Human; patients with indwelling catheters or IVs act as reservoirs for nosocomial infections; food borne - occasionally cows with infected udders
ZOONOSIS: Yes - direct or indirect contact with infected animals
VECTORs: None
DRUG SUSCEPTIBILITY: Many strains are multi-resistant to antibiotics and are of increasing importance; methicillin resistant (MRSA) strains have caused major outbreaks world-wide; Vancomycin resistant (VRSA) are being increasingly isolated; sensitivity must be determined for each strain.
Staphylococcus aureus only rarely causes a severe or life threatening disease in a laboratory situation. In particular people who are immunocompromised need to take care and a separate risk assessment may need to be conducted.
Staphylococcus epidermidis belongs to the group of coagulase negative Staphylococci that occurs naturally on the skin of animals and humans. S. epidermidis can however become an opportunistic pathogen if the skin barrier is breached and the bacterium enters the bloodstream. Occurs worldwide; particularly in areas where personal hygiene is suboptimal; associated with noscomial infections; in hospitals by development of antibiotic-resistant strains. Staphylococcus epidermidis only rarely causes a severe or life threatening disease in a laboratory situation.
S. aureus and S. epidermidis are both hazard group 2 biological agents.

Host/vector system

pRAB11, pIMAY, pORI. None of these vectors are mobilisable and only vectors that are either non-mobilisable or mobilisation-defective in bacteria as defined in the Compendium of Guidance will be used.

Origin & function

Genomic sequences will originate from Staphylococcus aureus or Staphylococcus epidermidis. Specific modifications and intended functions will vary depending on the particular experiment, but in all cases they will be restricted to facilitating routine molecular biology procedures such as those provided as examples in section 6.

Evaluation of foreseeable effects

The vectors that will be used, will be well-characterised vectors and that contain only selective markers that are already in routine use in standard cloning vectors. Level of risk effectively zero

The genes of interest inserted into S. aureus or S. epidermidis will be native to the genome of strains and will not present any additional hazard to human health. The antibiotic resistance cassettes used in our experiments will confer resistance to erythromycin and/or chloramphenicol, gentamicin, kanamycin and apramycin. These antibiotics are used to combat a range of bacterial infections in humans. Neither are recommended as a first choice in the treatment of Staphylococcal infections in humans. However, the Erm genes responsible for erythromycin resistance also mediate inducible resistance to clindamycin, which is used in the management of Staphylococcal infections, particularly those of the skin/skin structure. Clindamycin resistant strains are already circulating in the environment and other effective antibiotics are available. Level of risk Medium

It is unlikely that the genetic modification will alter significantly the fundamental properties of the parental micro-organism such as pathogenicity, infectivity, virulence, survivability or host range. Deletion of genes coding for secretion systems and toxins may be expected to reduce virulence as described in:


Anti-bacterial toxins will be overproduced in Staphylococcal strains. These toxins do not target humans or animals but specifically inhibit the growth of other Staphylococci so the modified strains are not expected to be more pathogenic. They may, however potentially outcompete other Staphylococcal strains present in the microbiota. Level of risk Medium

Transfer of genetic material may occur between related microorganisms. However, as stated above, the genes of interest inserted into S. aureus/S. epidermidis will be native to the genome of S. aureus or S. epidermidis (we will not cross-introduce genes). Antibiotic resistant strains are already circulating in the environment. Level of risk Medium

The genetically modified micro-organisms are likely to cause similar effects on human health (and the environment) as the wild type parent. Therefore, the containment measures for the parental micro-organisms (ie Containment Level 2) will be required to reduce the risks to research and
support staff and to the broader environment to an acceptable level. It is extremely unlikely that the genetic modification will alter significantly the fundamental properties of the parental micro-organism such as pathogenicity, infectivity, virulence, survivability, host range and/or response to prophylaxis/treatment. Therefore, additional Containment Level 3 control measures such as sealability for fumigation, HEPA filters on extract air and autoclave within laboratory suite are not merited for the level of risk i.e. not reasonably practicable. Level of risk Medium

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The work is small scale, for research purposes only. The work will be carried out in a laboratory which has been designated as containment 2 compliant and access to the laboratory (CBCB 3.34 and 3.30) and storage area (CBCB 1.09) is limited to personnel who require an authorised swipe-card for entry to the building at all times and the laboratories outside of normal working hours. All personnel will wear suitable PPE (e.g. Howie lab coats and gloves). Effective disinfectants will be made available at all times, namely 70% ethanol and 1% Virkon solutions used following manufacturer’s instructions. Virkon disinfectant contains oxone, potassium peroxomonosulphate, sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. Virkon will be used to decontaminate equipment and work surfaces at completion of work, at the end of the day, and following spills of viable materials. If a spill occurs, cover the spill with paper towels and soak the towels with a 1% Virkon disinfectant. Allow the material to soak for approximately 1 hour before discarding materials in biohazard bag for autoclaving. Bench tops are impervious to water and resistant to solvents, acids, alkalis, and chemicals used for surface decontamination. Skin should be decontaminated immediately when contamination suspected. Procedures will be carried out in such a way as to keep aerosol production to a minimum, for example centrifugation will take place in sealed centrifuge buckets, but there is no requirement for specific measures to control aerosol dissemination.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee suggested that the specific strains were removed from the risk assessment to allow for some flexibility but to ensure that MRSA strains are included within the scope.

Project Containment

<table>
<thead>
<tr>
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</tr>
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02/03/2022
# Project Additional Information

## Purposes of the contained use

The primary aim of this project is to investigate gene regulation and cell-cell sensing mechanisms in Pseudomonas spp. using molecular genetic tools. In addition, Pseudomonas spp. will be used as prokaryotic models for studying population behaviours such as biofilm formation using targeted mutagenesis by suicide plasmids, random mutagenesis by transposons (using λpir dependent vectors in a disabled Escherichia coli strain as DNA donor) or by labelling cells with fluorescent reporter genes carried on plasmids. The project will involve the construction and analysis of isogenic mutants and chromosomal fusions with reporter genes, such as lux, phoA, gfp, lacZ etc., and cloning of genes and DNA sequences for genetic complementation of mutants or expression of proteins in Pseudomonas spp belonging to other ACDP hazard group 1 and 2 species.

## Recipient or parental organism

The Pseudomonas spp strains used in these experiments have been isolated from a wide variety of sources including historical clinical environments that over the years became well-established laboratory strains. They include ACDP hazard groups 1 (P. fluorescens, P. putida, P. syringae) and ACDP hazard groups 2 (P. aeruginosa). Pseudomonas spp are Gram-negative bacteria commonly found in soil and ground water. It rarely affects healthy people and most community-acquired infections are associated with prolonged contact with contaminated water. Although P. aeruginosa is an opportunistic pathogen (i.e. more likely to infect those patients who are already very sick as opposed to healthy patients), it can cause a wide range of infections, particularly among immunocompromised people (HIV or cancer patients), neonates and...
persons with severe burns, diabetes mellitus or cystic fibrosis. Serious Pseudomonas infections usually occur in people in hospitals and/or with weakened immune systems. Infections of the blood, pneumonia, and infections following surgery can lead to severe illness and death in these people. The most significant potential routes of exposure to Pseudomonas are from direct contact with the bacteria, but other routes of exposure may take place depending on the strain and circumstances. The risks of infection are very low for healthy individuals and infections can be efficiently eradicated by antibiotic therapy.

Host/vector system

A range of vectors will be used to introduce recombinant genes and DNA sequences into Pseudomonas spp once a functional replicon in these organisms has been incorporated into the vector when required.

Non mobilisable: pUCPxx derivatives (e.g. pUCP18) or other vectors Bom-/(Nic-) Mob- and Tra-.

Shuttle vectors: carrying simultaneously dual E. coli (R6K, CoIE1 or p15A) and Pseudomonas or broad host range replicons such as pVS1 derivatives (pME60xx series), pRO1600 derivatives (pUCPxx series, pFLP2, etc.).

Mobilisable Suicide vectors: these plasmids can only replicate in E. coli (e.g. strain S17-1 λpir) but not in Pseudomonas and are used to mobilize plasmids from E. coli into Pseudomonas including R6K derivatives (e.g. pGP704, pRL27, pLM1, pKN501, pDM4, etc.) and CoIE1 derivatives (e.g. pME3087, pME3088, etc.) in order to create chromosomal insertions. The suicide vectors may also contain transposons such as Tn5 (pUT-miniTn5 and derivatives) and Tn7 (pUC18R6KT-mini-Tn7T and derivatives), Tn917 (pTV series) or other of similar nature in order to create insertion mutants. The likelihood of horizontal gene transfers from non-mobilisable is negligible. The likelihood of horizontal gene transfer from the shuttle or mobilisable suicide vectors to non-intended microorganisms outside the laboratory is likely a hazard. However, these vectors are unlikely to be maintained outside the laboratory due to the absence of antibiotic selective pressure or replication-required elements. At worst, the level of risk of the recipient strains should not increase over an equivalent to hazard group 2.

Origin & function

Only genes and DNA sequences from ACDP hazard group 1 and 2 organisms will be cloned into these vectors and housed in Pseudomonas spp ACDP hazard groups 1 and 2 organisms. For example, the quorum sensing systems from other ACDP hazard group 1 and 2 organisms (including Pseudomonas spp) will be cloned into P. aeruginosa or P. putida for mutant complementation or gene function study; DNA partition (parAB) and DNA transportation systems from other bacterial organisms (E. coli) will be introduced into Pseudomonas for DNA manipulation; regulatory genes and their responding promoters such as lacI, ampR, tetR or their homologs from other ACDP hazard group 1 and 2 organisms will be cloned for studying gene regulatory networks. The vectors may contain antibiotic resistance markers (cat, kan, tet, erm) that are already in routine use in standard cloning vectors. The vectors may contain transposons such as Tn5, Tn7, Tn917 or other of similar nature with strong transcriptional terminators in order to create random insertion mutants or targeted chromosomal fusions (e.g. mini-Tn7 with reporter genes insertion in the downstream of PA5548 of P. aeruginosa (Nature Methods 2005, 2:443)). The suicide vectors could also contain reporter genes such as lux, phoA, gfp, lacZ. For the chromosomal deletion/insertion mutants, only antibiotic markers (cat, kan, tet, erm) that are already in routine use in standard cloning vectors will be used. Once a stably genome modified bacterial strain is produced, experiments will depend on the particularly selected genes but will typically involve a panel of phenotype proliferation, biological assays as well as collection of DNA/RNA/Protein for further profiling analysis. The majority of genes and DNA sequences have been described in literatures or in the standard biological parts website (http://parts.igem.org/DNA). Occasionally, novel promoters, aptamers and regulators (such as quorum sensing (QS) genes from other gram-negative bacteria) will also be cloned into Pseudomonas spp.

Evaluation of foreseeable effects

The mutations (such as in-frame deletions, transposon/antibiotic markers insertions) will disable the functions of study genes such as quorum sensing (QS) communication pathways and the reporter fusions are used to study expression activity from native promoters. These are unlikely to increase the level of risk of the resultant strains over an equivalent to hazard group 2. The introduced genes and standard DNA sequences from ACDP hazard group 1 and 2 microorganisms such as isogenic or homologous QS systems or DNA partition systems genes are unlikely to increase the level of risk of the resultant strains over an equivalent to hazard group 2. These genes have no harmful biological activity to produce extra toxins, cytokines or allergens other than native biological elements. Genes such as luxI/R homologs or reporters are under the control of their own promoter or native Pseudomonas promoters and their expression levels are not expected to be above native levels of expression therefore the level of expression of downstream targets are not expected to change. Use of well-characterised vectors for mutagenesis and introduction of genes are unlikely to increase the level of risk of the resultant strains over an equivalent to harzard group 2. It is difficult to envisage how the use of vectors, mutagenesis, chromosomal fusions, or introduced genes described above could enhance the virulence of the Pseudomonas spp. which would not be above hazard group 2.
Only GM micro-organisms will be involved.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Decontamination and autoclaving will be carried out. All waste materials will be disposed of into the autoclave bags for autoclaving or yellow clinical waste bags for professional removal. All bacterial contaminated waste including solid agar plate and liquid broth will be autoclaved before disposal.

a) Decontamination.
Chemgene disinfectant will be routinely used for disinfection. The surfaces of the laboratory bench and the microbiological safety cabinet will be decontaminated with Chemgene after use. Spillages of any GMMs or any of their contaminated products will be disinfected with Chemgene.

b) Autoclaving.
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. The cycle parameters of 121°C 15psi for 15min will be used. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Risk assessment has been peer reviewed by Newcastle University GMSC and activity class 2 classification agreed. Minor comments received asking for clarification of assessment of inherent environmental risks prior to selection of containment and control measures. This has been addressed by the Principal Investigator

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Risk assessment has been peer reviewed by Newcastle University GMSC and activity class 2 classification agreed. Minor comments received asking for clarification of assessment of inherent environmental risks prior to selection of containment and control measures. This has been addressed by the Principal Investigator

Project Containment

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<td>L3</td>
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Animal Units

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<td>L2 L3 L4 L2</td>
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Project Ref  540/20.2
### Project Additional Information

**Purposes of the contained use**

The project specifically aims at identifying novel immune mechanisms to fight bacterial infections and specific bacterial survival strategies.

**Recipient or parental organism**

Salmonella typhimurium and Listeria monocytogenes

**Host/vector system**

Only non-mobilisable or mobilisation-defective low copy vectors will be utilised to produce small amount of proteins. These vectors encode determinants of resistance to ampicillin, chloramphenicol or kanamycin, selective markers that are in routine use. They contain no recognisable harmful sequences. This vector system was previously shown to have no effect on the level of risk to the host.

**Origin & function**

The inserted sequences will be derived from PCR amplification of either genomic DNA or plasmid DNA. Fluorescent proteins will be used as a marker for infected cells and it will in no way contribute to the infection process. Gene manipulation would not confer any survival or reproductive advantage over the wild-type

**Evaluation of foreseeable effects**

The expression of heterologous proteins is not anticipated to alter any pathogenic traits of the host. In general, chromosomal gene manipulation of Salmonella and Listeria will be aimed at inactivating virulence pathways, hence decreasing the ability to infect and not conferring any survival advantage compared to the wild-type. There is a potential risk that genetic alteration of certain genes, such as transcriptional regulators, may result in increased expression of pathogenic traits. The virulence phenotype of bacterial pathogens is usually dependent on the complex regulation and interactions of multiple genes and virulence factors. The risk associated with this scenario will be minimized by evaluating the target regulatory gene roles and its impact on pathogenesis (for instance bioinformatically or by comparison with published data) prior to deletion of the gene. Additionally, Hazard Group 2 control measures already in place would be sufficient to contain the organism. When GM strains will be used for infections, mice will be regularly checked for symptoms (e.g. body temperature, fur, behaviour) to avoid any unnecessary pain.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste materials will be disposed of into autoclave bags, yellow clinical waste bags or sharps bins as required. All pathogen infected waste will be autoclaved before disposal. Food, water and bedding that have been in contact with any animal during the infection trial will be placed in autoclavable bags and sterilized via the standard autoclaving route. Cages used for housing animals will also be sterilized by autoclaving. Deceased, infected animals will be placed in autoclavable bags, frozen and will subsequently be incinerated off site using standard procedures implemented at Newcastle University. All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

Risk assessment has been peer reviewed by Newcastle University GMSC and activity class 2 classification agreed. GMSC queried use of 70% ethanol and suggested a propriatry one that has been adequately tested on the microorganisms used. Updated to Trigene/Chemgene where appropriate.

Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Yes

Tick if you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

Risk assessment has been peer reviewed by Newcastle University GMSC and activity class 2 classification agreed. GMSC queried use of 70% ethanol and suggested a propriatry one that has been adequately tested on the microorganisms used. Updated to Trigene/Chemgene where appropriate.

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Project Ref 540/21.1

Date Ackn'd 02/03/2022  
CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4
### Role of cell wall integrity and secreted protein toxins in Mycobacterium abscessus colonisation and virulence

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**Project notified under transitional arrangements**

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#### Purposes of the contained use

This risk assessment covers research projects in our lab utilising bacteria specifically Mycobacterium abscessus for the purpose of facilitating molecular biology procedures such as (i) sub-cloning, DNA sequencing, or site-directed mutagenesis; (ii) construction of chromosomal mutants; (iii) construction of fusions with harmless reporter genes such as GFP or tags such as HA and His; (iv) construction of chromosomal insertions with antibiotic resistance cassettes and their subsequent removal to leave unmarked deletions (v) construction of recombinant plasmids for subsequent purpose of complementing deletion mutants with a wild-type or mutated copy of the gene. The genetically modified strains will be cultured in small to medium quantities for the isolation and analysis. This will involve solid and liquid culture of the strains, centrifugation, extraction of RNA, DNA and protein, microscopy, gel electrophoresis, western blotting, mass spectrometry and immunological studies.

#### Recipient or parental organism

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<th>Recipient or parental organism</th>
<th>Purpose of the contained use</th>
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<tr>
<td>Mycobacteria are non-sporeforming, non-motile, aerobic, Gram-positive, rod-shaped bacilli belonging to the family Mycobacteriaceae. There are over 170 recognised species within the Mycobacterium genus, the majority being environmental organisms isolated from water and soil sources and all sharing the characteristic complex cell wall organisation. The most well-known species is Mycobacterium tuberculosis associated with the human disease TB and has the highest pathogenic potential of the genus. M. abscessus is rapidly-growing non-tuberculosis mycobacteria (NTM), that are particularly associated with the opportunistic infection of vulnerable individuals with respiratory pathologies, e.g. cystic fibrosis and bronchiectasis. M. abscessus is an airborne pathogen but can also cause skin and soft tissue infections. People with open wounds may be at risk for infection by M. abscessus. Rarely, individuals with underlying respiratory conditions or impaired immune systems are at risk of lung infection. PATHOGENICITY: Opportunistic pathogen, environmental organism; able to survive initial innate immune response through resistance towards phagocytosis by host macrophages. Infection of respiratory tissue leads to localized inflammation and granuloma formation by recruited immune cells. Persistence by M. abscessus leads to localized tissue destruction by inflammatory pathways. Tissue necrosis leads to reductions in respiratory capacities of the individual – breathing difficulties, persistent coughing, etc. M. abscessus can also cause skin and soft tissue infections, presenting as cutaneous nodules, pustules and abscesses. M. abscessus can also rarely cause meningitis and cerebral abscesses. EPIDEMIOLOGY: Occurs worldwide, especially prevalent in East Asia however incidence is increasing globally; resistant to wide range of antibiotic classes. HOST RANGE: Primarily humans, can potentially extend to some animals. INFECTIOUS DOSE: Virulence of strains varies greatly. MODE OF TRANSMISSION: Exact mechanism yet to be established; contaminated water sources known to be significant risk factor (i.e. contaminated shower heads).</td>
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**Project Additional Information**
either through biofilm formation or aerosolization. Fomites (dust particles) have been implicated in person-to-person transmission between high risk individuals.

**INCUBATION PERIOD:** Ranges from 1-15 days (mean = 5.5 days).

**COMMUNICABILITY:** As long as persistence continues in the infected individual’s respiratory tissues granuloma will remain, and person may be considered infectious and transmissible between vulnerable persons.

**RESERVOIR:** Environmental sources, colonisation of medical devices or surfaces through biofilm formation.

**ZOONOSIS:** Unknown

**VECTORS:** Unknown

**DRUG SUSCEPTIBILITY:** Cell wall physiology and antibiotic resistance genes makes therapeutic treatment via antimicrobials difficult. M. abscessus is thought to be resistant to the following antimicrobials – amoxicillin-clavulanic acid, cefepime, ceftriaxone, ciprofloxacin, doxycycline, imipenem, minocycline, moxifloxacin, tobramycin and trimethoprim-sulfamethoxazole.

Most effective antimicrobials M. abscessus are thought to be the following, clarithromycin, amikacin, cefoxitin and linezolid.

M. abscessus only rarely causes a severe or life threatening disease in a laboratory situation. In particular people who are immunocompromised need to take care and a separate risk assessment may need to be conducted.

M. abscessus is not listed in the HSE approved list of biological agents, however we are self classifying it as HG2 based on the description above. It was previously classified as the same species as M. chelonae. See https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3322061/; M. chelonae is included in the list as HG2.

M. abscessus is also classified as HG2 in the PHE culture collection: see https://www.phe-culturecollections.org.uk/products/bacteria/detail.jsp?refid=NCTC+10882&collection=nctc

**Host/vector system**

pORBIT, pMIND, pMV261. The vectors that will be used, will be well-characterised vectors and that contain only selective markers such as hygromycin and kanamycin that are already in routine use in standard cloning vectors. Only vectors that are non-mobilisable or mobilisation-defective in bacteria as defined in the Compendium of Guidance will be used.

**Origin & function**

The genes of interest inserted into M. abscessus will be native to the genome of strains and will not present any additional hazard to human health. The antibiotic resistance cassettes used in our experiments will confer resistance to hygromycin, kanamycin and/or chloramphenicol. These antibiotics are not recommended as a first choice in the treatment of M. abscessus infections in humans. Multi-drug resistant M. abscessus strains are circulating in the environment and other effective antibiotics are available.

Genes will be expressed from vectors at close to native (chromosomal) level, native promoter sequences will be used and is therefore unlikely that the cloned genes will confer any additional advantage for host colonisation. The toxins we will work on that use the ESX-4 T7SS are proteins that are strongly predicted to target other bacteria and not the host (e.g. cell wall hydrolases) and are therefore unlikely to present any additional risk to human health. GFP is non-toxic and has a long history of safe use – no hazards identified.

**Evaluation of foreseeable effects**

The vectors that will be used, will be well-characterised vectors and that contain only selective markers that are already in routine use in standard cloning vectors. Level of risk effectively zero

The genes of interest inserted into M. abscessus will be native to the genome of strains and will not present any additional hazard to human health. The antibiotic resistance cassettes used in our experiments will confer resistance to chloramphenicol, gentamicin and kanamycin. These antibiotics are used to combat a range of bacterial infections in humans. None are recommended as a first choice in the treatment of M. abscessus infections in humans. Level of risk Low

It is unlikely that the genetic modification will alter significantly the fundamental properties of the parental micro-organism such as pathogenicity, infectivity, virulence, survivability or host range. Deletion of genes coding for secretion systems and toxins may be expected to reduce virulence as described in:


Use of non-mobilisable or mobilisation-defective vectors in the mycobacterial hosts that very limited ability to survive outside the laboratory greatly reduces the likelihood of transfer to related microorganisms. There would be no advantage if a gene encoding an individual component of the T7SS were transferred to a related microorganism because at least six gene products are required to form a functional T7SS (we will not make any constructs that include all of these genes together). If a T7SS toxin were transferred to a related organism it would require that organism to have a T7SS that is sufficiently closely related to M. abscessus that it could recognise and secrete that toxin. This is unlikely because the T7SSs where examined are very specific and where examined are not able to report ‘foreign’ T7 substrates (e.g. Species-specific secretion of ESX-5 type VII substrates is determined by the linker 2 of EccC5. Bunduc CM, Ummels R, Bitter W, Houben ENG. Mol Microbiol. 2020 Jul;114(1):66-76; Identification of a substrate domain that determines system specificity in mycobacterial type VII secretion systems. Phan TH, Ummels R, Bitter W, Houben EN. Sci Rep. 2017 Feb 16;7:42704; EssC is a specificity determinant for Staphylococcus aureus type VII secretion. Jäger F, Kneuper H, Palmer T. Microbiology. 2018 May;164(5):816-820). Level of risk Low

The genetically modified micro-organisms are likely to cause similar effects on human health (and the environment) as the wild type parent. Therefore, the containment measures for the parental micro-organisms (ie Containment Level 2) will be required to reduce the risks to research and support staff and to the broader environment to an acceptable level. It is extremely unlikely that the genetic modification will alter significantly the fundamental properties of the parental micro-organism such as pathogenicity, infectivity, virulence, survivability, host range and/or response to prophylaxis/treatment. Therefore, additional Containment Level 3 control measures such as sealability for fumigation, HEPA filters on extract air and autoclave within laboratory suite are not merited for the level of risk i.e. not reasonably practicable. Level of risk Medium

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The work is small scale, for research purposes only. The work will be carried out in a laboratory which has been designated as containment 2 compliant and access to the laboratory and storage area (Catherine Cookson Building, Floor 2 laboratory 2023 and room 2016B) is limited to personnel who require an authorised swipe-card for entry to the building at all times and the laboratories outside of normal working hours.

All work will be conducted in a class 2 microbiology safety cabinet. All personnel will wear suitable PPE (e.g. Howie lab coats and gloves). A 1:10 dilution of the Distel disinfectant will be made available at all times and used following manufacturer's instructions. Distel will be used to decontaminate equipment and work surfaces at completion of work, at the end of the day, and following spills of viable materials. If a spill occurs, cover the spill with paper towels and soak the towels with a 1:10 dilution of Distel disinfectant. Allow the material to soak for approximately 1 hour before discarding materials in biohazard bag for autoclaving. Skin should be decontaminated immediately when contamination suspected.

Cold Storage (-80ºC, -20ºC and 4ºC) will be in restricted areas and will be suitably labelled (with strain, date and owner) containers.

All flasks and plates will be appropriately labelled (with strain, date and owner) and stored in a dedicated fridge in the CL2 laboratory (room 2023). All flasks and plates will be placed in a secondary container during storage time in the fridge to prevent accidental spill.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials used are to be regarded as potentially contaminated and disposed of accordingly to ensure 100% killing. Liquid waste is autoclaving and disposal of via sink disposal routes. Minimum 126ºC degrees for 45 minutes, 10 minutes free-steam. All waste materials will be disposed of into the autoclave bags, or sharps bins as required. Solid waste in autoclave bags and sharps bins are autoclaved before disposal.

a) Disinfection.

Distel laboratory disinfectant will be used for disinfection (https://www.lab-shop.co.uk/downloads/datasheets/382752_TM308%20-%20Specification.pdf). The surfaces of the microbiological safety cabinet will be disinfected with 70 Distel laboratory disinfectant at a 1:10 dilution after use. Laboratory benches will be swabbed with a 1:10 dilution of Distel laboratory disinfectant after any activity.

b) Autoclaving

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips.
The GMSC asked for clarification about the restriction of storage areas and recommended secondary containment for storage of samples. Regarding disinfection the GMSC queried the use of two different disinfectants and recommended Distel used at 1:10 dilution as this is the manufacturer's recommendation. These points have been addressed in the risk assessment and procedures.

**Project Containment**

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**Project Ref** 540/97.1a-f

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Tick if notifying a connected programme of work Y
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
Project Ref 540/97.2

Date Ackn'd 21/04/1997

CU2 Project Title PRODUCTION OF RECOMBINENT VACCINIA CARRYING GENES OF HEPATITAS C VIRUS (HCV)

Class 2

CultureVol Class 2

CultureVolume Class 3-4

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Project Ref 540/97.3A

Date Ackn'd 21/04/1997  
CU2 Project Title INTERACTION OF MICROORGANISMS WITH INTESTINAL CELL WALLS-RISK  
Class 2  
CultureVolClass2  
CultureVolumeClass3-4
### Project Additional Information

#### Purposes of the contained use

- Recipient or parental organism

#### Host/vector system

#### Origin & function

#### Evaluation of foreseeable effects

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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- **Animal Units**
  - L2 L3
  - L4 L2

- **Large Scale Activities**
  - L2 L3
  - L4 L2

- **Human Clinical Applications**
  - L2 L3
  - L4 L2

**Project Ref** 540/97.3B

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- **Date Project Ceased** 26/02/2016
- **Withdrawn** N
- **Tick if notifying a connected programme of work** N

**Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 540/97.5

Date Ackn'd 21/04/1997

CU2 Project Title USE OF ATTENUATED GONOCOCCI AS LIVE VACCINES

Date Project Ceased 03/10/2002

Class 2

Culture Vol Class 2

Consent Granted not applicable

Non-GMM

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

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INVOLVED IN INSULIN SIGNALLING IN CULTURED HUMAN MUSCLE CELLS

Date Project Ceased
13/11/2006

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment.

**Project Containment**

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**Project Ref 540/trans1**

- **Date Ackn’ed**: 11/06/1985
- **CU2 Project Title**: GENETIC ANALYSIS OF EXTRACELLULAR VIRULENCE DETERMINANTS OF STREPTOCOCCUS PYOGENES
- **Class**: Class 2
- **CultureVolClass2**: not applicable
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Animal Unit</th>
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<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
<th>Level 4 (GMMs)</th>
<th>Non-microbial</th>
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<tbody>
<tr>
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</table>

### Other (please specify)

- **Bacteriology**
  - Parasitology
- **Virology**
  - Transgenic Animals
- **Mycology**
  - Transgenic Invertebrates

<table>
<thead>
<tr>
<th>Transgenic Birds</th>
<th>Transgenic Fish</th>
<th>Transgenic Plants</th>
<th>Microbiology Research</th>
<th>Gene Therapy</th>
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<tr>
<td>Other (please specify below)</td>
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</tr>
</tbody>
</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity.

- Tick to confirm that you are attaching a summary of the risk assessment
- Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.

**Project Ref 141/01.1**
TO EVALUATE TRANSDUCTION EFFICIENCY OF LEN

Date Ackn'd: 25/05/2017

Date Project Ceased:

CU2 Project Title: TO EVALUATE TRANSDUCTION EFFICIENCY OF LEN

Class: Class 2

CultureVolClass2: Class 2

CultureVolumeClass3-4:

Non-GMM: Not Applicable

Consent Granted:

Project notified under transitional arrangements:

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes:
Transferred from GM141

Historical Date of Additional Info:

Significant Change ID:

Date of Significant Change:

Project Additional Information

Purposes of the contained use:

Recipient or parental organism:

Host/vector system:

Origin & function:

Evaluation of foreseeable effects:

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants):

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification):

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate):
Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L3</td>
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Animal Units

<table>
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<th>Growth Rooms</th>
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</thead>
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</table>

Project Ref 141/01.2

Date Ackn'd 25/05/2017

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Transferred from GM141
## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

### Is an emergency plan required according to regulation 20?  
- [ ] Yes
- [x] No

### If yes, tick to confirm that it is attached to this form  
- [x] Yes
- [ ] No

### Tick to confirm that you have attached a risk assessment to this form  
- [x] Yes
- [ ] No

### Tick if you are claiming exemption from disclosure for section of the risk assessment  
- [x] Yes
- [ ] No

### Project Containment

<table>
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</table>

02/03/2022

Page 8255 of 15326
This project intends to utilise the well characterised ability of Pseudomonas aeruginosa to form a biofilm mode of growth to establish whether bacterial biofilms could be used as a method to transfer proteins for gene therapy usage.

Pseudomonas aeruginosa has been shown to preferentially assume a biofilm mode of growth, embedding itself in a gelatinous organic polymer matrix composed primarily of alginate. There is a lack of understanding of the in situ regulation of biofilm matrix polymer formation nor whether the genetic promoters of the biofilm could be utilised to express non-biofilm genes in a formed biofilm. This project intends to address both aspects.

The contained use activity is required as part of this study involving vectors constructed at the Paterson Institute encoding the algC or the algD gene that encodes the enzyme phosphomannomutase or GDP-mannose dehydrogenase respectively that are both critical enzymes in the formation of the biofilm under the genetic control of the tetracycline response element or their natural promoters driving a non biofilm gene.

In the first experiments we must determine the activity our algC or algD promoter that has been inserted into our non-mobilisable vector. This can only be done in the wild-type strain (PAO1) as we require all the genetic components required for biofilm formation to be intact. Furthermore PAO1 is genetically identical to the delta strains of
P. aeruginosa (PAO1 algC and PAO1 algD) that is also being used in this study. It is hypothesised that when the transformed wild-type P. aeruginosa is grown under conditions that will allow the formation of the biofilm that expression of a non biofilm reporter gene such as B-galactosidase or green fluorescent protein will increase as this will be placed downstream of our algC or algD promoter.

We will then compare this data to that acquired by the transformation of the PAO1 alg C and the PAO1 algD (avirulent defined mutants) with the algC or algD gene under the control of the tetracycline promoter where it is hypothesised that addition of tetracycline to the medium will allow the induction of a biofilm. The final stage of this project will then look at whether the algC or algD promoter can be utilised to drive expression of non-biofilm genes within this tetracycline induced biofilm.

Recipient or parental organism

Pseudomonas aeruginosa strain PA01. The most widely used laboratory stain with complete physical and genetic maps being available and the genome sequenced. The original strain was isolated from a wound in 1955 by B Holloway described in detail in 1969 in Bacteriology Review. The environmental bacterium is a gram negative prototrophic bacteria that like many is an opportunistic human pathogen that causes bacteraemia in burn victims, urinary tract infections in catheterised patients and is the predominant cause of morbidity and mortality in cystic fibrosis patients.

Host/vector system

All vectors are based on non mobilisable vector systems. They will either be pUC, pGEM, pBluescript, pSP (Promega), pPROTet (Clonetech) based vectors.

Origin & function

E. coli - galactosidase gene or Aquea Victoria green fluorescent protein (GFP) under the control of the O, aerygubisa algC or algD promoter that normally promotes the expression of phosphomannomulase or GDP mannose dehydrogenase respectively in the wild type organism.

Evaluation of foreseeable effects

The algC and algD promoters regulate the formation of the P. aerugosa biofilm and so therefore it is foreseen that when the bacteria is grown under conditions to form a biofilm the algC and algD promoters will drive expression of the downstream B-galactosidase for GFP gene.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Animals will not be used in these experiments.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, agar plates, cell pellets) are rendered inactive by either autoclaving or disinfection using 1% Virkon and are subsequently incinerated along with clinical waste.

Liquid waste [culture & medium - volumes <51] is disinfected using Virkon at either 2% or 3% for a minimum contact time of 1 hour prior to disposal to sewers.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

The committee have reviewed the amendments to the risk assessment 02/614 to include the use of Pseudomonas aeruginosa strain PA01 as a host and agree with its conclusions.

Project Containment

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Animal Units

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Large Scale Activities

<table>
<thead>
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<th>L4</th>
</tr>
</thead>
</table>

Human Clinical Applications

<table>
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</tr>
</thead>
</table>

Project Ref 141/06.1

Date Ackn'd 19/10/2006  
CU2 Project Title Modulating signalling through the Notch and Wnt pathways in cell culture by adenoviral, lentiviral or retroviral transduction.

Class 2

Consent Granted Not Applicable

Non-GMM

Project notified under transitional arrangements N

Historical Significant Changes TRANSFERRED FROM GM CENTRE 141

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To evaluate the effects of activation or inhibition of expression of the Notch and Wnt signalling pathways in mammalian cell lines and mammalian cell lines. Notch and Wnt signalling pathways are highly conserved cell-fate determination pathways, and play a central role, at the cellular level, regulating morphology, proliferation, motility and cell fate. Abnormalities in the components of these pathways are associated with a number of developmental disorders and cancer. Modulating the expression
of the pathways will help to understand the intricacies of the signalling system and understand the effects on cell development and cancer induction. If any of these translated sequences were over expressed, after self inoculation, in a target cell it could possibly result in transformation of that cell. Although this has not been proven, there is a risk factor associated with the production of vectors expressing such sequences.

**Recipient or parental organism**

Adenovirus serotype Ad5-Adenoviridae are classified as ACDP hazard group 2

Lentivirus - Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3

Retrovirus - MuLV based

**Host/vector system**

<table>
<thead>
<tr>
<th>Non-viral systems</th>
<th>Recombinant plasmid vectors are replication incompetent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-viral systems</td>
<td></td>
</tr>
<tr>
<td>Bacterial Host-Escherichia coli k-12 derivaties (TG1, TOPO 10 series and DH5-alpha)</td>
<td></td>
</tr>
<tr>
<td>Plasmid vectors-pcDNA3.1, pBluescript (Strategene) and TOPO (Invitrogen) based plasmid vectors.</td>
<td></td>
</tr>
<tr>
<td>Viral systems</td>
<td>Adenoviral vectors deleted for E1 (cell transformation and tumourgenicity) and E3 genes (non-essential genes for in vitro growth). E1 is provided in trans by 293T packaging cell line.</td>
</tr>
<tr>
<td>Viral systems</td>
<td>Adenoviral vectors deleted of all viral lytic cycle genes. Coding sequence (except for E1) provided by helper virus and E1 provided by 293T cells.</td>
</tr>
<tr>
<td>Viral systems</td>
<td>Replication defective E1 vectors in themselves can be considered unlikely to cause disease according to the HSC’s advisory committee’s compendium of guidance on genetic modification.</td>
</tr>
<tr>
<td>Viral systems</td>
<td>Lentiviral and retroviral vectors are deleted of any viral expressed sequences. Genes for packaging provided by Transient plasmid transfection in to producer cells.</td>
</tr>
</tbody>
</table>

**Origin & function**

| Reporter genes, including GFP, dsRed, Luciferase, will be used to assess modulation of the notch and wnt pathways in vivo. |
| Genetic material of human, murine, rat and Xenopus origin will elucidate the relative contributions of wnt and notch signalling on the differentiation of mesenchymal stem cells to chondrogenic, dipogenic or osteogenic lineages. |
| Due to the nature of the inserted genetic material (i.e oncogenic) containment level 2 controls and procedures will be employed in this project. |

**Evaluation of foreseeable effects**

The Adenoviral, Retroviral and Lentiviral based vectors are replication incompetent. Thus whilst they pose an infection risk to humans and other species, they are unable to initiate further rounds of replication/infection cycles.

If self-inoculation occurs with adenoviral constructs the chances of seroconversion are minimal due to double deletion of genetic material from replication deficient plasmid. The most likely course of events is that a localised immune response will clear the inoculated pathogen without any chances of further contamination. The retroviral and lentiviral vectors contain no viral genes so are essentially "gutless" shells. The chances of seroconversion are again minimal especially as the retrovirus is a murine pathogen.

The heterologous genes do not encode viral specific proteins, nor do they interfere with the known activities of the virus and as such are unlikely to have an effect upon the basic nature of the recombinant adenovirus and lenti or retrovirus. However since they are oncogenic the appropriate containment level to be used is CL2.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable
No animals will be used in this project

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste materials (e.g. gloves, plastic ware, agar plates, cell pellets) are rendered inactive by autoclaving and are subsequently incinerated along with clinical waste. Surfaces are disinfected with 1% Virkon solution.

Plastic ware that can be effectively disinfected is treated with 1% Virkon solution for a minimum of 1 hour prior to incineration.

Glassware is treated with 1% Virkon for a minimum of 1 hour prior to cleaning.

Liquid waste (culture & medium - volumes < 5l) is disinfected by the addition of Virkon to give a 1% using Virkon at either 2% or 3% for buffered solutions (i.e. culture media). A minimum contact time of 1 hour is allowed prior to disposal to sewers.

Validation

Autoclaving

The discard autoclave is subject to regular three monthly service and annual validation. Records of service and validation are kept on site.

Disinfectant

For the modified bacteria, Lentivirus/retrovirus/adenovirus, Virkon is routinely used according to the manufactureres (Antek) guidelines. Antek report a 4 log kill for retrovirus at a dilution of 1:2000 i.e. 0.05% after 30 minutes. Our protocols require 2% & 1% Virkon which is excess of the validation concentration.

For bacterial systems we have carried out in-house tests and have shown that Virkon will provide effective kill at a concentration of 1% after 10 minutes contact time. We routinely allow a minimum contact time of 1 hour to ensure complete inactivation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC assessed the project as GM Class 2.
The following points were highlighted.
The production of virus to be carried out in a dedicated virus containment room.
The recovery of viral particles/DNA by ultracentrifugation would involve the use of sharps. Only suitably trained and experienced persons allowed to carry out this specific procedure.

Project Containment

02/03/2022
Project Ref 141/06.2

Date Ackn'd 25/05/2017

CU2 Project Title USE OF LENTIVIRAL VECTORS CONTAINING shRNA

Class 2

Cultures Vol

Class Culture Vol

Class 2 < 1 Litre

Consent Granted Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Transferred from GM141

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

shRNA technology will be used to reduce the expression of genes of interest in primary murine haemopoietic cells and murine and human haemopoietic cell lines. Lentiviral vectors will be used to deliver the sequences of interest. Targets will be identified through proteomic assessment of proteins involved in stem cell biology and oncogenic transformation of haemopoietic cell lines. An example of such a target is the protein FMIP which is involved in M-CSF signalling.

Recipient or parental organism

Lentivirus parental organism - Human Immunodeficiency virus 1 (HIV-1) is classified as ACDP hazard group 3.

Host/vector system

Bacterial host - Escherichia coli k-12 derivatives (e.g. DH5-alpha).
Plasmid vector - pSuper and Bluescript (Strategene) or similar based vectors which are Bom-, Mob- and Tra-
Viral vectors - Recombinant Lentiviral vectors pseudotyped with VSV-G envelopes will be used in 293T cells.

Origin & function

Origins - genes of interest will arise from studies in primary murine haemopoietic cells and murine and human haemopoietic cell lines.

Genes of interest will be identified using proteomic analysis of stem cell differentiation and the process of transformation by leukaemic oncogenes. Protein profiles will be obtained by Mass Spec analysis of differentiating cells and the consequences of leukaemic oncogene expression on the proteome will be assessed. This will enable the identification of proteins that change during these processes. shRNAi will then be designed to target these genes.

An example of such a target is the protein FMIP which is involved in M-CSF signalling.

It is not intended to target known tumour suppressor genes or genes that could be inferred as such.

The project will be reviewed on a six or twelve month basis, in this regard: the exact frequency being dependent on the progress of this work.

Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors based on the HIV lentivirus. These self inactivating vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk to humans and other species, they are unable to initiate further rounds of replication/infection cycles. The chances of seroconversion are minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability it is thought there is NO significant increase of the likelihood of transfection via airborne routes of exposure. Besides the control measures identified are appropriate to guard against the associated risks.

The target genes do not encode viral specific proteins, nor do they interfere with the known activities of the virus and as such are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

However since they are oncogenic by ACGM definition the appropriate containment level to be used is CL2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

No animals will be used in this project.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, agar plates, cell pellets are rendered inactive by autoclaving and are subsequently incinerated along with clinical waste. Surfaces are disinfected with 1% Virkon solution.

Plastic ware that can be effectively disinfected is treated with 1% Virkon solution for a minimum of 1 hour prior to incineration.

Glassware is treated with 1% Virkon for a minimum 1 hour before cleaning.

Liquid waste (culture & medium - volumes <5l) is disinfected by the addition of Virkon to give a 1% using Virkon at either 2% or 3% for buffered solutions (i.e. culture media). A minimum contact time of 1 hour is allowed prior to disposal to sewers.

Validation

Autoclaving

The discard autoclave is subject to regular three monthly service and annual validation. Records of service and validation are kept on site.
Disinfectant
For the modified bacteria, and Lentivirus, Virkon is routinely used according to the manufacturers (Antek)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC assessed the project as GM Class2.
The following points were highlighted

The recovery of viral particles/DNA by ultracentrifugation should not involve the use of sharps.

The project needs to be reviewed on a 6 or 12 monthly basis, depending on the progress of the work, in the context of reviewing any genes of interest which may be of uncertain function, in case this affects the risk assessment in any way (e.g. unknown tumour suppressor genes)

It was confirmed that the expression of shRNA may block normal self renewal and differentiation. It may also lead to the induction of cell death.

Project Containment

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<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2</td>
<td>L2 L3 L4 L2</td>
<td>L3 L4</td>
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</table>

Project Ref 141/06.3

Date Ackn'd 25/05/2017
cu2 project title Expression of Rho GTPases using Retroviral Vectors

Date Project Ceased

Class 2

CultureVolClass2 < 1 litre

Non-GMM

Consent Granted Not Applicable

02/03/2022
Retroviral vector systems will be used to transfect well-studied cell lines either transiently or to establish stable cell lines. The project hopes to elucidate further, the role of Rho GTPases in tumourgenesis.

pLZRS- is derived from the Moloney murine leukaemia virus (MMLV); the env pol sequences being replaced by the LacZ gene; which in turn was replaced with a multiple cloning site, followed by an internal ribosomal entry sequence (IRES) and either neomycin or zeocin resistance gene.

Host/vector system

Bacterial host- Escherichia coli K-12 derivatives (e.g. Dalpha; TOPO 10)
Cell line host - Phoenix Retrovirus producer cells: eco and amphotrophic versions

Origin & function

Genes of interest will be generated by PCR from available plasmids or cDNA libraries. They will be of mammalian origin, including human. Examples include RAS, Raf, Asef.

From a functional perspective RAS is a "worst case scenario" being a known oncogene. Other oncogenes will also be used.

Evaluation of foreseeable effects

The vectors used are disabled vectors based on the Moloney murine leukaemia virus. They are used in conjunction with eco or amphotrophic versions of the Phoenix cell line. The systems used are designed such that viral particles produced in the packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication. The chances of seroconversion is minimal.

The amphotrophic cell line will produce virus capable of infecting human cells: but not capable of replication. The ecotropic cell line produces virus which are capable of infecting murine cells only: and not capable of initiating further rounds of replication.

It is thought there is no significant increase in the likelihood of transfection via airborne routes of exposure. Besides the control measures identified are appropriate to guard against the associated risks.

The target genes do not encode viral specific proteins, nor do they interfere with the known activities of the virus and as such are unlikely to have any effect upon the basic nature of the recombinant virus.

However since some of the target genes are oncogenic the appropriate containment level to be used is CL2.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| Not applicable. |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick if you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC assessed the project as GM Class 2.
The following points were asked for:

References for the target gene-supplied by author as Appendix 1

The project to be reviewed on a 6 or 12 monthly basis, depending on progress of the work.

Project Containment

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Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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</thead>
<tbody>
<tr>
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</tbody>
</table>
We have identified a number of genes that are overexpressed in childhood leukaemias. One of these, a cathepsin, appears to be involved in B-cell differentiation and may be involved in cell adhesion and/or migration. We propose to study this by (i) transducing human leukaemic cell lines with lentiviral vectors (either expression or RNAi) (ii) transducing human haematopoietic cells obtained from cord blood. We hope to show that overexpression of the protein leads to an invasive phenotype in leukaemic cell lines and altered development in HSC's.

Recipient or parental organism

Lentivirus parental organism - Human immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3.

Host/vector system

Bacterial host - BL21 (DE3)pLysS
Plasmid vector - Bluescript plasmids (Stratagene)
Viral vectors - A minimum of a 3-plasmid system will be used. A SIN-lentivector with a pseudotyped VSV-G capsule will be derived using 293T packaging cells.

Origin & function

Origins - gene of interest - The cathepsin, asparaginyl endopeptidase (AEP). This is expressed at various levels during the normal maturation of a lymphoblast and is not oncogenic. The cDNA has been cloned from a human cDNA pool and will be expressed in both its active and inactive forms and shRNA will be synthesised prior to insertion.
The recombinant viral vectors are highly disabled vectors based on the HIV-1 lentivirus. These self-inactivating vectors have had regulatory and accessory genes deleted ensuring viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication / infection cycles. The probability of seroconversion is minimal. The VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability. It is thought that there is NO significant increase in the likelihood of transfection via the airborne route of exposure. The control measures utilised are appropriate to guard against this eventuality and associated risks. The target gene does not encode viral specific protein, nor does it interfere with known activities of the virus and so is unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Evaluation of foreseeable effects

No animals will be used in this project at this site.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration.

Virkon is routinely used as per manufacturer's recommendations:

Solid surfaces are disinfected with 1% Virkon solution.

Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration.

Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

The autoclave undergoes annual validation. Records are kept on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The GMSC assessed the project as GM Class 2.

It was noted that there is considerable experience of using such lentiviral vector systems are already in the Institute. The project needs to be reviewed within 12 months, depending on the progress of the work, to ensure that the risk assessment remains valid.

Responsibility as defined in legislation lies with the Head of of research group. They have managerial control and responsibility for ensuring suitable and sufficient risk assessments are undertaken and that work is conducted in accordance with Institute Codes of Practice, which have been drawn up to ensure compliance with HSE guidance.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
</tr>
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**Project Ref** 141/07.2

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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>25/05/2017</td>
<td>Understanding tumour promoting roles of myofibroblasts in the stroma of human invasive carcinomas.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td></td>
<td>Not Applicable</td>
<td></td>
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Withdrawn N

Historical Significant Changes Transferred from GM141

Project Additional Information
We wish to investigate the roles of non-cancer cells within tumour. It is already known that these non-carcinoma cells promote tumour growth. We intend to culture the non-cancer cells and modify expression levels of particular candidate genes using lentiviral siRNA and retroviral cDNA expression vectors. This technology will help us elucidate molecular mechanisms by which the non-carcinoma cells promote tumour growth.

Recipient or parental organism

Lentivirus parental organism- Human Immunodeficiency virus-i (HIV-1) is classified as ACDP hazard group 3 Moloney Murine Sarcoma virus (MMSV) parental organism - hazard group 1. Causal agent of sarcoma in mice and rats.

Host/vector system

Bacterial host- E. Coli- XL1O Gold, Stbl3, and DH5 alpha.

VECTORS: PLKO1 siRNA lentiviral vector; pWPI lentiviral cDNA expression vector; pHAGE-CMV-eGFP lentiviral expression vector; PBabe retroviral cDNA expression vectors (puromycin, Zeomycin); pWZL Blast retroviral cDNA expression vector. Retroviral expression vectors MSCV IRES GFP; pLV-tetR1KRAB-red; iDuetl 01 (tetEF. GFP. PGK. hygro) - MI NON-mobilisable. Viral vectors/Host systems- SEE ADDITIONAL SHEET.

Origin & function

Genes of interest are: Human SDF-1/CXCL12 cDNA; human CXCR4 cDNA; human CXCR7 cDNA; humanTGF-beta cDNA; human EphB2 cDNA; human p16INK4A cDNA. The first 4 genes and their gene products play a role in tumour progression and proliferation. EphB2 and p16INK4A play a role in the regulation of cell positioning and proliferation. More detailed functional information for the above are given in appendix 2 of the risk assessment.

Evaluation of foreseeable effects

The recombinant lentiviral vector utilised is a highly disabled vector based on HIV. It is a self-inactivating vector which has had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells (HEK 293T) are replication incompetent. Thus whilst they pose an infection risk; in that they can integrate into the chromosome, they are unable to pop-out and initiate further rounds of replication/infection cycles. The probability of seroconversion is thought to be minimal. Whilst the VSG envelope extends the cellular tropism and confers greater environmental survivability, it is thought that there is no increase in the likelihood of transfection via the airborne route of exposure. Besides, the control measures utilised for this work are appropriate to guard against the associated risks. It is worthy of note that the HIV specific envelope protein that targets CD4 T cells is exchanged for the ubiquitous VSV-g (Vesicular stomatis virus C protein), which makes it more visible to the immune system.

The target genes do not encode viral specific proteins, nor are they likely to interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus. Likewise the recombinant Moloney reirovirus used (MMSV) is similarly disabled and is replication incompetent. MMSV is thought to be fragile and has poor environmental survivability capabilities. Again workplace precautions and controls will minimise the likelihood of risk to human health and the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration.
Virkon is routinely used as per the manufacturer’s recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer’s recommendations). The autoclave undergoes annual validation. Records are kept on site.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
Y

Please enter comments on the GM safety committee on the risk assessment

The GMSC agreed with the classification of this project as GM Class 2. They asked the principal investigator (P1) to provide details of the genes of interest; in order to better consider the risks to human health and the environment. These are given in Appendix 2 and 3 of the risk assessment.

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Project Ref 141/08.1

Date Ackn’d 25/05/2017  
Date Project Ceased

CU2 Project Title Knockdown of genes in normal haematopoietic and leukemic stem cells.

Class 2

CultureVolClass2 < 1 Litre  
CultureVolumeClass3-4

Non-GMM  
Consent Granted Not Applicable

Project notified under transitional arrangements  
N
Purposes of the contained use

Microarray experiments previously performed have identified a number of candidate genes that may be critical regulators of normal haematopoietic and/or leukemia stem cells (LSC5). This project seeks to evaluate the role of these genes in these cell types in murine and human model systems using genetic knockdown experiments. Briefly, murine or human bone marrow cells will be transduced using lentiviral vectors containing shRNAs and cells will then be functionally evaluated using a combination of in vitro and in vivo techniques, the latter involving transplantation of cells in to mice. Three lentiviral vector systems will be used: (i) pLB, which provides constitutive expression of shRNAs from a Pol III promoter, (ii) pSicoR, which provides constitutive expression of shRNAs from a Pol lII promoter with the option of irreversible Cre-mediated conditional inactivation of knockdown, and (iii) pSL1K, which provides the option of inducible knockdown from a tetracycline-responsive promoter.

Recipient or parental organism

Lentiviral parental organism- Human Immunodeficiency virus-i (HIV-i) is classified as ACDP hazard group 3

Host/vector system

Bacterla host:
Ecoli Stbl 3 (for generating lentiviral system plasmid)
Ecoli DH5 alpha (for all other plasmids)
Lentiviral vector systems:
pLB- see appendix 1 risk assessment
pSicoR- see append ix 1 risk assessment pSLIK-see appendix 3 risk assessment
used with 293 FT cells

Origin & function

Origins- genes of interest- Human
Microarray experiments have identified the genes of interest as candidates for regulation of normal haematopoietic and/or leukaemia stem cells (LSC5).
These include:
Bbx- an HMG-box transcription factor of unknown function
Bmprl- a TGFb family receptor
Transcription factors- Myb; E2f6; Mycn
Cell cycle regulatory proteins Cdkl, 2 and 6
RNA binding protein- Igf2bp3
Chromatin regulatory proteins- Arid2; Cbx 5; Hmgb3; Smarc2 and 5
Evaluation of foreseeable effects

The recombinant viral vector vectors are highly disabled vectors based on the HIV lentivirus. They are self inactivating vectors, which have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal (Details of the origins and deletions within the lentiviral constructs are given in appendix 1 and 3 of the risk assessment).

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated risks.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Knock-down of some of the target genes which are cell cycle inhibitors or tumour suppressors may accentuate oncogenesis. Whilst knock-down of others, which are potential oncogenes, may inhibit oncogenesis.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of Virkon); work carried out in containment 2 laboratories or Home office inspected animal facilities; work within Class2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Whilst no transgenic mice are generated in this project; transduced cells will be transplanted in to C571 BL6 mice by intravenous injection. This procedure will be carried out by specifically trained staff only.

The Biological Resource unit (BRU) houses all mice in sealed individually ventilated cages. All handling is within cabinets. The rooms are secured with sealed drains and close sealed doors. There are no open ducts within the room. Corridors are sealed with electronically locking flush fitting doors. All corridor risers are sealed. The unit is inspected regularly by Home Office inspectors to ensure that it meets the required standards.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site.

Virkon is routinely used as per the manufacturer’s recommendations:Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation).

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

02/03/2022
A query as to the risk to workers from any free virus whilst administering transduced cells to mice was raised. This is answered in appendix 8 of the risk assessment. The need to refer workers to the Institute GM Code of Practice and the local Virus room Code of Practice was raised. This was subsequently inserted in to section 7 vi and section 9.2 of the risk assessment. The GMSC agreed that it was a GM Class 2 project.

Project Containment

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<tr>
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**Project Ref 141/08.2**

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<td>25/05/2017</td>
<td>Initiating leukaemia with primary human bone marrow cells.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<table>
<thead>
<tr>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Applicable</td>
<td>N</td>
<td>N</td>
</tr>
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</table>

Historical Significant Changes

Transferred from GM141

Project Additional Information
### Purposes of the contained use

The project aims to investigate the biology of human leukaemia stem cells (LSCs) using a xenogeneic murine model system. Briefly, leukaemia is experimentally initiated in mice by infecting primary human bone marrow cells with retroviruses which carry oncogenes that are associated with human leukaemia. The transduced bone marrow cells are then transplanted into irradiated recipient immune deficient mice.

### Recipient or parental organism

Murine Stem Cell Virus (MSCV) viral vector system was derived from the Murine Embryonic Stem Cell Virus (MESV) and the LN retroviral vectors (first described in Hawley et al., (1992) Journal of Experimental Medicine 176:1149). The vectors achieve stable, high-level gene expression in hematopoietic and embryonic stem cells through a specifically designed 5’ long terminal repeat (LTR). This LW is from the murine stem cell PCMV virus, and it differs from the M0MuLV LTR used in other retroviral vectors by several point mutations and a deletion. These changes enhance transcriptional activation and prevent transcriptional suppression in embryonic stem and embryonal carcinoma cells. As a result, the LTR drives high-level constitutive expression of a target gene in stem cells.

### Host/vector system

- **Bacterial host:**
  - E.coli DH5 alpha (for amplifying plasmid stocks)

- **Viral vector system:**
  - MSCV used with Phoenix A and Phoenix GP cells

### Origin & function

- **Origins- genes of interest- Human**
- **Function of genes of interest- oncogenes associated with human leukaemia**
  - A full list of these genes is given in appendix 2 of the risk assessment

### Evaluation of foreseeable effects

The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication! infection cycles. The probability of seroconversion is minimal (Details of the origins and deletions within the MSCV constructs are given in appendix 1 of the risk assessment).

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated risks.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of Virkon); work carried out in containment 2 laboratories or Home office inspected animal facilities; work within Class2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Transduced cells will be transplanted into immune deficient mice by intravenous injection. This procedure will be carried out by specifically trained staff only.

The Biological Resource unit (BRU) houses all mice in sealed individually ventilated cages. All handling is within cabinets. The rooms are secured with sealed drains and close sealed doors. There are no open ducts within the room. Corridors are sealed with electronically locking flush filling doors. All corridor risers are sealed. The unit is inspected regularly by Home Office inspectors to ensure that it meets the required standards.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site.

Virkon is routinely used as per the manufacturer’s recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

Liquid waste (culture and medium) is disinfected by the addition of Viricon to give a 1% final working concentration. 2% or 3% solutions of Viricon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

A query as to the risk to workers from any free virus whilst administering transduced cells to mice was raised. This is answered in appendix 5 of the risk assessment.

The need to refer workers to the Institute GM Code of Practice and the local Virus room Code of Practice was raised. This was subsequently inserted in to appendix 6 of the risk assessment.

The GMSC agreed that it was a GM Class 2 project.

Project Containment

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<td>L2 Yes</td>
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Project Ref 141/08.3
Recombinant Lentiviruses will be used to express genes involved in stress signalling pathways in primary mouse cells and mammalian cell lines. The gene products may be involved in signal transduction eg p38MAPK and JNK, or downstream target genes eg the dusp family of dual-specificity phosphatases. We will not use Lentiviruses to express genes that we could reasonably predict to have potentially harmful effects in humans eg oncogenes and pro-inflammatory cytokines.

The transduced primary mouse cells will be transplanted into mice and investigated in in vitro systems. Mouse, mouse primary cells and mammalian cell line responses to environmental stresses (e.g. chemotherapeutic agents) will be evaluated.

Recipient or parental organism

Lentiviral parental organism- Human Immunodeficiency virus-I (HIV-1) is classified as ACDP hazard groups

Host/vector system

Lentiviral vector System:
- pCMV-VSVG
- pMDL9/RRE
- pRSV-REV
- pRRL

Host : HEK 293 cells

Origin & function

cDNA5 encoding:
- p38 MAPKalpha, JNK1, JNK2, ERK1, ERK2
duspi, dusp4, dusp8, duspl0
- pten
These genes are signal transduction components, either kinases or phosphatises.
The origins of these genes are murine and human

Evaluation of foreseeable effects

The recombinant viral vector vectors are highly disabled vectors based on the -(IV lentivirus. They are self inactivating vectors, which have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication? infection cycles. The probability of ser000nversion is minimal

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated risks.
The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building ( either by autoclaving or use of Virkon); work carried out in containment 2 laboratories or Home office inspected animal facilities; work within Class2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The Biological Resource unit (BRU) houses all mice in sealed individually ventilated cages. All handling is within cabinets. The rooms are secured with sealed drains and close sealed doors. There are no open ducts within the room. Corridors are sealed with electronically locking flush fitting doors. All corridor risers are sealed. The unit is inspected regularly by Home Office inspectors to ensure that it meets the required standards.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Virkon is routinely used as per the manufacturer's recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. My contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers( as per manufacturer's recommendation).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GMSC agreed that this was a GM Class2 project. They asked that the COSHI-I assessment be completed for the use of cell lines in this body of work. This has been done and is enclosed.

### Project Containment

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<th>Project notified under transitional arrangements</th>
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### Project Additional Information

**Purposes of the contained use**

Previous studies, such as microarrays, have identified a number of candidate genes that may be critical regulators of normal haematopoietic cells development. This project seeks to evaluate the role of these genes in murine model systems using genetic knockdown of forced expression experiments. Briefly, murine cell populations will be transduced using lentiviral vector, retroviral vectors or transfected with normal plasmids containing shRNAs or the cDNA of the gene of interest. The cells will then be...
functionally evaluated using a combination of in vitro and in vivo techniques, the letter involving transplantation of cells in to mice.

**Recipient or parental organism**

Lentivirus parental organism- Human Immunodeficiency virus-1 (HIV-1 is classified as ACDP hazard group 3

Murine stem Cell Virus- derived from the Murine Embryonic Stem Cell Virus (MESV) and the LN retroviral vectors (first described in Hawley et al, (1992) Journal of experimental medicine 176:1149

**Host/vector system**

Recombinant lentiviral vectors pseudotyped with VSV-G envelopes, will be used with 293T cells

MSCV used with Phoenix A and Phoenix GP cells

**Origin & function**

Candidate regulator genes for normal haematopoietic cell development will be obtained in the form of cDNA originating from murine genomic or RNA libraries

**Evaluation of foreseeable effects**

The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal (details of the origins and deletions within the MSCV and lentiviral constructs are given in appendix 1 of the risk assessment)

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated residual risks.

The target genes do not encode viral specific protein, Nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of Virkon) work carried out in containment 2 laboratories or home office inspected animal facilities; work within class2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site.
Virkon is routinely used as per the manufacturer’s recommendations –

Solid surfaces are disinfected with 1% Virkon solution.
Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration.
Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation)

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<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
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Please enter comments on the GM safety committee on the risk assessment

A query as to the risk to workers from any free virus whilst administering transduced cells to mice was raised. This is answered in appendix 4 of the risk assessment.

The committee agreed it was a GM Class 2 project

### Project Containment

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### Project Ref

141/09.1

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**Project Additional Information**

**Purposes of the contained use**

Viruses have been explored as agents to target cancer either in a gene therapy setting (through the delivery of cytoxic gene payloads or alike) or as attenuated strains which replicate within tumour cells leading to the subsequent destruction of that cell. The replication competent virus approach is being explored in the clinical setting with a number of different viruses including reovirus and adenoviruses. Whilst attractive, there are a number of potential barriers which the viruses need to efficiently overcome in order to drive tumour destruction including efficient tumour cell targeting, infection and replication. This project seeks to use gene-modified replication competent viruses to investigate whether the approach can be improved in model systems.

**Recipient or parental organism**

Adenovirus type 5 (Replication Competent): ACDP Hazard Group 2
The actual genetic modification work will be carried out by colleagues in The Netherlands. In this project the already modified virus will be used for in vitro and in vivo (mouse) experiments.

The modification removes the ability for the modified virus to infect via the coxsackie B adenoviral receptor (CAR).
The modification enables the virus to infect cells via the Carcinoembryonic antigen (CEA), which is highly expressed in a number of solid tumours, but only in the luminal side of the gastro intestinal tract of a normal human adult. Thus the tropism of the virus has been affected. This characteristic of the virus targeting CEA is realised by the incorporation of a 58 aa affibody specific for the tumour antigen.
The immunogenicity of the affibody is currently unknown - as such, if immunogenic, it may further drive the immune response against the virus.

**Host/vector system**

Not applicable - the already modified virus will be supplied by collaborators based in the Netherlands

**Origin & function**

Gene-modified adenovirus targeted to CEA. The gene modification involves the deletion of amino-acids within the fibre domain which disrupts the normal infective route of the virus (through CAR) and incorporates an affibody for targeting. An affibody is effectively a very small antibody type domain that is small enough to permit efficient expression on the adenoviral fibre without significantly reducing viral titre. Viruses directed to the Her2/neu antigen have been described (Myhre S et al. (20090 Gene Therapy 16:252-261). In effect, these modifications of the virus re-direct the virus to target known tumour antigens thereby improving the specificity of targeting of the virus.

Origin of the affibody.
Affibodies have been generated using combinatorial protein approaches - effectively the antigen binding domain was generated from synthesised library cDNA's and tested using a screening approach to bind antigen. The cDNA of the affibody was then cloned into the H1 loop of the adenoviral fibre domain which contains mutations destroying
natural CAR binding and then introduced into the Ad5 genome by homologous recombination replacing the endogenous Ad5 wild-type fiber. The Ad5 genome is then used to generate functional viruses using standard methods.

**Evaluation of foreseeable effects**

The virus is essentially wild-type but with mutations of the fibre domain which prevents binding to the normal virus receptor (coxsackie adenovirus receptor - CAR) and incorporation of a 58 aa affybody specific for the tumor antigen. Initially, the target antigen used will be Carcinoembryonic antigen (CEA) which is highly expressed on a number of solid tumors but only within the luminal side of the gastro-intestinal tract of the normal human adult. The fibre attenuation prevents the normal route of viral infection and the CEA specificity will result in targeting to CEA expressing cells. These viruses have only recently been generated and part of this proposal is to test the level of specificity of infection driven by the affybody.

The affybody/fibre mutations will modify the range of cell the virus will be able to infect. Normal targeting should be severely reduced and targeting of target antigen cells increased; consequently, this will alter the tropism of the virus. The mutations will otherwise have no major effect upon the individuals except that the immunogenicity of the affybody is currently unknown - as such, if immunogenic, it may further drive the immune response against the virus.

Currently we do not have any information on the shedding of the modified virus during animal experiments. As the virus lacks CAR binding, it is possible that more virus could be shed since there isn't the extensive pool of cells to soak up the virus. However, all work using this virus will be performed in the class 2 containment room within BRU. Waste disposal procedures are stringently adhered to (see section 12).

Further viral modifications may be generated at a later date. But these will be submitted to the local GMSC for consideration.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Solid waste material (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration.

- Virkon is routinely used as per the manufacturer's recommendations:-
  - Solid surfaces are disinfected with 1% Virkon solution.
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  - Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

- Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

- The autoclave undergoes annual validation. Records are kept on site.

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**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N
With regard to the animal work: clarification on viral shedding and treatment of waste bedding was asked for, the information provided was supplied and incorporated in to the risk assessment.

Please enter comments on the GM safety committee on the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

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Project Ref 141/09.2

Date Ackn'd: 25/05/2017

CU2 Project Title: RNAi knockdown of genes involved in stress responses using the BLOCK-iT Lentiviral miR RNAi Expression System from Invitrogen

Class: Class 2
Culture Volume: < 1 Litre
Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

We wish to study the role of the transcription factor ATF2 in tumour cells. By knocking down the expression of ATF2 in a variety of cell lines, we will assess the contribution of ATF2 to the transformed phenotype. Knockdown will be achieved using Lentiviral based gene transfer. Genetic modifications include the sub-cloning steps involved in the production of a Lentiviral expression plasmid, co-transfection of this expression vector together with packaging vectors into a host cell line to produce virus, and subsequently, use of Lentiviral stocks to infect target cell lines that will stably express the miRNAi of interest.

Recipient or parental organism

Lentiviral parental organism - Human Immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3

Host/vector system

Bacterial host - E.coli XL1-Blue
Plasmids - all have pUC origin of replication and all are mobilisation defective.

Lentiviral vector system- commercially available BLOCK-IT Lentiviral miR RNAi Expression System fro Invitrogen.
HEK 293 cells is packaging cell line.
The ‘Block-It Lentiviral miR RNAi expression system’ (Invitrogen cat no K4937-00) is a four plasmid virus production system and consists of:
pLenti6/V5 DEST - packaging vector into which the target sequence of interest is sub-cloned.
pLP1 - encodes Gag (viral structural protein) and Pol (viral replication protein).
pLP2 - encodes Rev (viral transcription factor)
pLP/VSVG - encodes the VSV-G glycoprotein for production of pseudotyped virus.
Target cells for viral vector are:- Ramos, Raji, Nmalwa, SudHL4, CRL 2261

Origin & function

ATF2 - Human - both tumour suppressing and tumour promoting phenotypes have been described for ATF2 gene. It functions as a transcription factor.

Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors based on the HIV lentivirus. They are self inactivating vectors, which have had regulatory and accessory genes deleted, ensuring that viral particles produce in packaging cell lines are replication incompetent. This whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated risks.

The target gene does not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of Virkon); work carried out in containment 2 laboratories or Home office inspected animal facilities; work within Class 2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Animal work will be carried out by trained staff only. Intravenous injection of cell lines stably expressing miRNA of interest only. NO live virus will be injected in to mice, therefore shedding of virus is not an issue.

The Biological Resource unit (BRU) houses all mice in sealed individually ventilated cages. All handling is within cabinets. The rooms are secured with sealed drains and close sealed doors. There are no open ducts within the room. Corridors are sealed with electronically locking flush fitting doors. All corridor risers are sealed. The unit is inspected regularly by Home Office inspectors to ensure that it meets the required standards.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-pont thermocouple tests on an annual basis. Records are kept on site.

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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Since the project employs a lentiviral system with a gene that shows both tumour promoting and suppressor activities it was thought GM Class 2 was appropriate and this agreed with the applicants classification.

Project Containment

Project Ref 141/10.1
Human tumours are frequently found to contain activating mutations in the ras family of oncogenes. Expression of oncogenic ras in cells leads to aberrant signalling and acute activation of the AP-1 family of transcription factors. We wish to study the signalling events occurring downstream of oncogenic ras and subsequent changes in gene expression with a particular focus on ATF2-dependent gene expression. To do this we need to differentiate between events occurring early (within a few hours), after ras expression, and those occurring later (several days). This will be achieved by generating high-titre recombinant adenoviruses that express oncogenic ras. This allows the synchronous infection of all the cells in a sample followed by a rapid onset of ras gene expression (within 8 hours). Given the hazardous nature of the ras oncogene we are proposing to undertake and employ a range of control measures, as detailed in the risk assessment.

Recipient or parental organism

Adenovirus: ACDP Hazard Group 2

Origin & function

Human cDNA encoding HRasG12V, KRasG12V. These are oncogenes frequently found to be mutated in human cancers. Their expression causes constitutive activation of MAPK and PI3K signalling pathways and acute activation of the AP-1 family of transcription factors. We will carry out experiments intended to gain a better understanding of their role in tumourigenesis of interest and these cause aberrant expression and acute activation of the AP-1 family of transcription factors.
Flp recombinase. Required to initiate a recombination event to remove the STOP cassette and allow expression of ras in cells co-infected with recombinant ras-Adenovirus.

Evaluation of foreseeable effects

The recombinant Adenovirus lacks the E1 region and so is replication-incompetent. However, it is possible that an adverse recombination event occurring within the 293 packaging line, (or theoretically in human tissues, since adenovirus is ubiquitos in humans), could lead to generation of a recombination-competent virus (RCV). We plan to use a well characterised PCR-based assay to screen our virus stocks for RCV (indicated by the presence of the E1a gene). We will carry out this test each time we expand the virus. Furthermore we will check our stocks by infection of either HeLa or A549 cells which do not contain the E1 region. Only if RCV is present will this lead to cytopathic effects in the infected cells.

The recombinant Adenovirus possesses a STOP cassette located upstream of ras which essentially prevents its expression. Gene expression requires removal of the STOP cassette by a recombination event mediated by Flp recombinase. Flp will be expressed by means of a second recombinant adenovirus. Therefore, ras gene expression only occurs if both the ras-expressing virus and the Flp-expressing virus co-infect the same cell. This will significantly reduce the risk of workers accidentally expressing oncogenic ras in their own tissues. We will undertake not to handle open tubes of the ras-expressing and the Flp-expressing virus stocks at the same time.

Stringent disinfection regimes are applied in the laboratory along with autoclaving of all solid contaminated waste. The use of biosafe rotors and microbiological safety cabinets are also employed to protect human health and the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail " alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user.

Virkon is routinely used as per the manufacturers recommendations:-
Solid surfaces are disinfected with 1% Virkon solution
Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Usually the contact time is longer, typically overnight.
Liquid waste (culture medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

The autoclave undergoes annual validation by a third party. Records are kept on site.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form
The committee asked for additional information on the STOP cassette and the possibility of read-through: the techniques employed to detect replication competent adenovirus; and whether both viruses are handled at the same time. This additional information is included in the Appendices 2 and 3 of the risk assessment.

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<td>Use of lentiviral vectors to investigate the biological function of 5T4 and related molecules</td>
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<td>Non-GMM</td>
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**Historical Significant Changes**

- Transferred from GM141

**Project Additional Information**

02/03/2022
Purposes of the contained use

5T4 and related molecules will be introduced or knocked down in cell lines to investigate their biological functions. These goals will be achieved with the use of lentiviral vectors which are able to infect dividing and non-dividing cells and stably integrate into their genome. The vectors will encode the desired genes under constitutive or inducible promoters. Gene knock down will be achieved by the use of vectors encoding for shRNA against target genes. The system for lentiviral vector production is available from Invitrogen. The system utilises SIN (self inactivating) vectors which have had regulatory and accessory genes deleted to ensure that viral particles produced in packaging cell lines are replication incompetent.

Recipient or parental organism

Lentiviral parental organism- Human Immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3.

Host/vector system

Bacterial: E.coli One Shot Stbl3’
Plasmids: pLP, pcDNA, pLenti, pENTR, pDONR, pMDLg/pRRE, pRSV-Rev and pMDg.2 plasmid vectors for packaging of lentiviral particles.

Origin & function

Human and mouse 5T4 coding sequence cloned from cDNA of respective species and is deposited in NCBI database. The sequences of other 5T4 related genes will be cloned from cDNA obtained from human or mouse cell lines. siRNA sequences targeting genes of interest will be designed and/or purchased from Invitrogen. Fluorescent protein genes are commercially available. High expression of 5T4 is very strongly expressed in normal placental trophoblast. We therefore believe 5T4 is "Oncogenic by ACGM definition" and this is highlighted in the risk. Assessment (section 7 (iv))

Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors based on the HIV lentivirus. These self inactivating vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular topism and confers greater stability and environmental survivability it is though that there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the control measures utilised are appropriate to guard against the associated risks.

The target gene does not encode viral specific protein, nor does it interfere with known activities of the virus and so is unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Contaime and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowe prior to disposal to sewers. (as per manufacturer's recommendations).

The autoclave undergoes annual validation. Records are kept on site.

Please enter comments on the GM safety committee on the risk assessment

Committee agreed with the GM Class 2 classification of the work.

It asked if 5T4 was expressed in foetal cells. 5T4 is expressed in normal placental trophoblasts although the risk to any pregnant females is considered to be very low, due to the nature of the viral vector system used which generates replication competent viral particles. No pregnant females work on project. This will be kept under review.

**Project Containment**

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<td>L3</td>
<td>L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
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**Project Ref** 141/11.2

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<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<td>25/05/2017</td>
<td>Lentiviral and retroviral transfection of fibroblast cells and epithelial cells</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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Consent Granted
**Project Additional Information**

**Purposes of the contained use**

We seek to investigate the role of normal and activated fibroblasts in tumour-stromal interactions upon knockdown or overexpression of genes of interest. Fibroblasts are known to exert tumour-promotive as well as tumour-suppressive functions depending on cell and expression level context. We intend to co-culture fibroblast cells with breast cancer cell lines and analyse proliferation, migration and molecular markers with Western blot, real-time PCR and Elisa.

**Recipient or parental organism**

- Lentivirus parental organism - Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.
- Retroviral system parental organism - Moloney Murine Leukaemia virus (MMLV)

**Host/vector system**

- Bacterial host: E. coli DH5 alpha and Stbl3
- Viral vector/Host system - 3 plasmid based systems will be used
- Lentiviral system is a self-inactivating lentivector system with a pseudotyped VSV-G capsule. Viral particles will be produced using HEK 293T packaging cell line.
- Retroviral system: pBABE system with packaging plasmids pCMV-VSVG and pUMVC3-gag-pol. Viral particles produced are replication incompetent. System is available from Adgene. (More detailed descriptions of vector system characteristics are given in Appendix 1 of risk assessment)
- Target cell lines for recombinant virus are Fibroblast cell lines 522 and 544 (see Appendix 4 of risk assessment) and breast cancer cell lines (MCF7, T47D, ZR75.1, MDA-MB -231!, MDA-MB-468). Other tumour, non-tumour epithelial and stromal cell may be investigated. Amendment and GMSC approval would be sought beforehand.

**Origin & function**

- Human genes TGF beta receptor2 has suspected tumour promoter and suppressor functions. See appendix 2 of risk assessment for more detail. (Obtained from colleagues in PLKO1 plasmid as a knockdown). Lentiviral vector system will be utilised to generate knockdown versions of this gene; whilst the MSCV retroviral system will be used to over express these genes in the target cells.
- Cyclin D1 is a tumour promoter gene (cDNA from Adgene) will be knocked down and over expressed with the same systems as above; within the breast cancer cell lines.
given in section 4 of the risk assessment.

**Evaluation of foreseeable effects**

The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, control measures utilised are appropriate to guard against the associated risks.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user.

Virkon is routinely used as per manufacturer's recommendations:-

Solid surfaces are disinfected with 1% Virkon solution.

Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Usually the contact time is longer, typically overnight.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

The autoclave undergoes annual validation by a third party company. Records are kept on site.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

Please enter comments on the GM safety committee on the risk assessment

The committee agreed with the GM Class 2 classification.
### Project Additional Information

**Purposes of the contained use**

The work involves the study of Rho proteins (a family of GTPases involved in many cell signalling functions), their regulators and pathway-related genes along with their mutant forms. Frequently we use either overexpression or RNAi knockdown as a method of study. We have identified some important cell lines where lentiviral transduction may be required to get acceptable expression levels either transiently or to produce stable cell lines.

**Recipient or parental organism**

Lentivirus parental organism - Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.
Bacterial host: E. coli NEB 10-beta (DH10B derivative) from New England Biolabs

Viral vector/Host system - 3 plasmid based systems will be used from sigma and/or Invitrogen (PLkO.1 and pLenti respectively).

Lentiviral system is a self-inactivating lentivector system with a pseudotyped VSV-G capsule. Viral particles will be produced using HEK 293T packaging cell line.

Target Cells: Well established cell lines such as MDCK II (dog) and human cancer cell lines (e.g. breast cancer cell line MDA 231 and osteo-sarcoma cell line HOS and MG63).

Cloning will typically be subcloning of existing vectors (pcDNA) or if not available from a cDNA library. This will include versions of mammalian species including human, mouse and dog. Target siRNA sequences are designed from existing databases.

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Origin & function

Cloning will typically be subcloning of existing vectors (pcDNA) or if not available from a cDNA library. This will include versions of mammalian species including human, mouse and dog. Target siRNA sequences are designed from existing databases.

Evaluation of foreseeable effects

The recombinant Lentiviral viral vector systems consist of disabled vectors. They have had HIV regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. The systems employed will be Self Inactivating (SIN) vector systems. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and enironmental survivability, it is though there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the risk control measures utilised are appropriate to guard against the associated risks. These include the use of safety cabinets, biosafe rotors and prescribed disposal routes for waste, wearing of appropriate PPE and no use of any sharps.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation by a third party company. The performance test results generated during validation are kept on site.

Virkon is routinely used as per the manufactur's recommendations:-

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Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer's recomendations).
The committee asked for additional information in the form of maps of the lentivector systems which are to be employed. These were supplied - see maps attached to risk assessment.
The committee agreed with the GM Class 2 classification

Please enter comments on the GM safety committee on the risk assessment

The committee asked for additional information in the form of maps of the lentivector systems which are to be employed. These were supplied - see maps attached to risk assessment.
The committee agreed with the GM Class 2 classification

Project Containment

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Animal Units

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Project Ref 141/11.4

Date Ackn'd: 25/05/2017

Date Project Ceased

CU2 Project Title
Constitutive and Inducible Protein Knockdown and Overexpression of Drug Targets Using Lentiviral Vectors in Lung and Colorectal Cancer Cell Lines

Class 2

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes
Transferred from GM141

Historical Date of Additional Info

Significant Change ID

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
**Project Additional Information**

**Purposes of the contained use**

Novel molecular based therapeutics work by targeting enzymes, such as kinases or substrate transporters. However, these drugs frequently have off-target effects and it is important to know exactly what the mechanism of action of the drug is. To demonstrate that a drug is acting through its main target one can either knock-down the target by RNAi or overexpress a dominant negative version of the protein and show that this produces the same effect as the drug (both approaches are required for convincing proof of on target effects). This also allows for dissection of downstream pathways to ascertain the effectors responsible for drug related changes. The work needs to be carried out in a variety of cell lines and for a variety of targets due to the varied nature of Clinical and Experimental Pharmacology, which not only depends on the results obtained with different drugs but also the availability of drugs which depends on collaborations with pharmaceutical companies. Some of the cell lines we work with, mainly small cell lung cancer cell lines, are impossible to transfect with conventional transfection approaches (e.g. Lipofection or electroporation and therefore a lentiviral based approach has been chosen. As knocking-down/overexpressing some of the targets of interest may prove lethal to the cells ability to carry out inducible knock-down/overexpression is key to this proposal.

**Recipient or parental organism**

Lentivirus parental organism - Human Immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3

**Host/vector system**

Bacterial Hosts: E. coli DH5 alpha and ccdB survival 2 (genotypes are given in appendix 2 of the risk assessment).

Plasmid Vectors:
- pENTR/pSUPER+; pENTR/pTER+ (used for RNAi delivery into cell lines expressing target mRNA/protein via lipid based transient transfection).
- pENTR4; pENTR4-FLAG (used for protein expression studies).
- pLenti X1 GFP-Zeo; pLenti X2 Puro; pLenti CMV TetR Blast; pLenti X2 Puro; Lenti CMV TetR Blast; pLenti CMV Puro; pLenti CMV/TO Puro; pLenti CMV/TO GFP-Zeo; psPAX2; oMD2.G (used for lentiviral particle generation)

Viral vector system: HEK293 cells will be used to generate lentivirus particles

The lentivirus vector system is a Self-inactivating (SIN) vector system. Disablement characteristics of the system is given in Appendix 4 of the risk assessment.

**Origin & function**

Wild-type and mutated (activating or inactivating mutations) genes of the following proteins TDP2, MCT-1, MCT-4, c-Myc, p110alpha, p110beta, AKT1, AKT2, AKT3, PDPK1, K-Ras and other PIP3 interacting proteins. Also Bcl-2 family members including Mcl-1, Bcl-2, Bcl-xL, Bax, Bak, Bim, Bid, Bad, Puma and Noxa.

The genetic material is mainly human in origin, but some genes may be animal in origin (mainly murine). The genetic material will be in the form of cDNA.

The function or suspected functions of these genes are listed in Appendix 3 of the risk assessment. They are also listed below.

Biological function of proteins of interest.

TDP2 - Resolves DNA-topoisomerase adducts induced by topoisomerase poisons such as etoposide.

Overexpression may result in resistance to topoisomerase. Unlikely to be oncogenic.
MCT-1 and MCT-4 - Monocarboxylate transporters. Involved in the influx and efflux of molecules such as lactate from cells. Unlikely to be oncogenic.

C-Myc - Transcription factor involved in expression of numerous prosurvival and proliferation genes. Frequently activated in cancer and known to be oncogenic when overexpressed in presence of other mutations which promote cell survival.

K-Ras - GTPase known to activate the prosurvival and proliferation signalling pathways PI3K and MAPK. Frequently activated in cancer and oncogenic when activated.

P110 alpha and beta - PI3K catalytic subunits which generate secondary messenger PIP3. Frequently activated in cancer and oncogenic when activated.

AKT1, 2 and 3 - Main PI3K downstream effector which promotes survival, proliferation and migration. Frequently activated in cancer but not oncogenic by itself.

PDPK1 - Required for activation of AKT downstream of PI3K signalling. Frequently activated in cancer but not oncogenic by itself.

Other PI3K interacting proteins - Biological function not clear but likely to be activated in cancer and could potentially be oncogenic.

Mcl-1, Bcl-2 - Anti-apoptotic (pro-survival) Bcl-2 family members which inhibit Bax and Bak. Frequently upregulated in cancer but not oncogenic by themselves.

Bax and Bak - Pro-apoptotic proteins which cause release of cytochrome C from mitochondria. Frequently downregulated in cancer but require other mutations for their tumour suppressor function to be apparent.

Bim, Bid, Bad, Puma and Noxa - Pro-apoptotic BH3 only proteins which inhibit anti-apoptotic Bcl-2 family members and therefore activate Bax and Bak. Frequently downregulated in cancer but require other mutations for their tumour suppressor function to be apparent.

**Evaluation of foreseeable effects**

The recombinant Lentiviral viral vector system consists of disabled vectors. They have had HIV regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. The system employed is a Self Inactivating (SIN) vector systems. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the risk control measures utilised are appropriate to guard against the associated risks. These include the use of safety cabinets, biosafe rotors and prescribed disposal routes for waste, wearing of appropriate PPE and no use of any sharps.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation by a third party.
company. The performance test results generated during validation are kept on site.

Trigene is routinely used as per the manufacturer's recommendations:
Solid surfaces are disinfected with 2% Trigene solution.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The committee asked for further details of the animal work which was supplied - see Appendix 7 of risk assessment. The committee asked for justification on the use of Trigene disinfectant. This was supplied - see Appendix 8 of risk assessment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</thead>
<tbody>
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Animal Units: L2 L3 L4 L2 L3 L4 L2 L3 L4

Large Scale Activities: L2 L3 L4 L2 L3 L4 L2 L3 L4

Human Clinical Applications: L2 L3 L4 L2 L3 L4

Project Ref 141/12.1

Date Ackn'd 25/05/2017

CU2 Project Title: Over-expression of kinases using ViraPower HiPerform T-Rex Gateway expression system (Invitrogen)

Class 2

Culture Vol Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements  N

Tick if notifying a connected programme of work  N
Over-expression or knockdown of kinases to study the role of various kinases in cell lines and patient samples. Genetic modifications include the sub-cloning steps involved in the production of a Lentiviral expression plasmid, co-transfection of this expression vector together with packaging vectors into a host cell line to produce virus, and subsequently, use of Lentiviral stocks to infect target cell lines that will stably express the protein of interest. Downstream analysis of stable cell lines will involve Western blotting, FCAS analysis and microscopy.

Lentivirus parental organism-Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3

Bacterial: E.coli Oneshot stbl3; E. coli ccdB survival 2; E.coli TOP10; E.coli Oneshot Omnimax. Bacterial hosts are disabled. Genotypes of bacterial host are given in Appendix 2 of the risk assessment.

Viral vector system: Lentivirus (VSV-G) with 293FT as host.
This system is a self-inactivating (SIN) vector system. The system is a 4-plasmid system. Details of the biosafety features of this system are given in appendix 4 of the risk assessment.

Target cells: ATCC catalogue lung cancer cell lines and patient B-cell lines or fibroblasts, HTCC116 DLD-1 colon cancer cell lines and additional catalogue colon and breast cancer cell lines.

The likelihood of the presence of adventitious agents in patient derived cells is considered in Appendix 7 of the risk assessment.

Biological Functions of Proteins of Interest are given below. They are all of human origin.

PKC - Protein kinase C (PKC) is a family of serine- and threonine-specific protein kinases. PKC family members phosphorylate a wide variety of protein targets and are known to be involved in diverse cellular signalling pathways PKC family members also serve as major receptors for phorbol esters, a class of tumor promoters.

MLK4 - MLK4 is a member of the mixed lineage family of kinases activated by environmental stress, cytokines and growth factors. This family of kinases lies upstream of
the kinase MKK4 and these kinases are critical regulators of the JNK pathway. In addition MLKs can activate the MKK3/6-p38 pathway and the Raf/Mek/Erk pathway.

FRK - The protein encoded by this gene belongs to the TYR family of protein kinases. This tyrosine kinase is a nuclear protein and may function during G1 and S phase of the cell cycle and suppress growth.

PAK3/7 - Both are Ser/Thr p21 activated kinases that have been described to regulate the Raf-Mek-Erk pathway and play a role in regulating the cell cycle.

Abl 1/2 - Is well studied tyrosine kinase that plays a role in CML and other cancers where the kinase is constitutively activated by a translocation to create a fusion protein BCR-ABL. Activated form can be oncogenic.

Cs1g2 - This is an undescribed kinase that is in the casein kinase family.

**Evaluation of foreseeable effects**

The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whils the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, control measures utilised are appropriate to guard against the associated risks. These include the use of Class II safety cabinets, disinfection of liquid biological waste and autoclaving solid waste. The use of sharps is minimised in this project. Their use is not thought to be necessary. Any use will conform to the measures outlined in the Institute's Code of Practice.

The target genes do not encode viral specific protein nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation (12-point thermocouple testing) by a third party company. Records are kept on site.

Trigene is routinely used as per the manufacturer's recommendations:
- Solid surfaces are disinfected with a 1 in 50 dilution of Trigene.
- Plastic ware that can be effectively disinfected is treated with a dilution of 1 in 50 dilution of Trigene overnight.
- Any contaminated glassware is treated with a 1 in 50 dilution of Trigene.
- The contact time is overnight.

Liquid waste (culture and medium) is disinfected by the addition of Trigene to give a working dilution of 1 in 50; and left overnight before disposal to sewers.
The committee thought that this risk assessment was comprehensive and supplied all the relevant information.

### Project Containment

<table>
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</thead>
<tbody>
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<td>L3 L4 L2</td>
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</table>

- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

### Project Ref 141/12.2

- **Date Ackn'd**: 25/05/2017
- **CU2 Project Title**: Mycobacterial vaccines for the immunotherapy of cancer

<table>
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<tbody>
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<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
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- **Withdrawn**: N
- **Historical Significant Changes**: Transferred from GM141
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to develop a vaccine for childhood Acute Lymphoblastic Leukemia (ALL). As BCG is safely used as a childhood vaccine against tuberculosis, a childhood ALL specific recombinant BCG vaccine will be developed and tested in a mouse model. BCG has been shown to be an effective vaccine vector due to its adjuvant properties and ability to cross-present antigens to the MHC class I processing pathway. The TEL/AML fusion protein occurs in 25% of cases of childhood ALL. T-cell peptide epitopes have been identified in this protein and will be cloned into wildtype BCG to make a recombinant vaccine.

**Recipient or parental organism**

*Mycobacterium Bovis BCG Pasteur strain (BCG) (attenuated with a long history of safe use)*. ATCC 35734.

Nucleotide sequence, AF095590

**Host/vector system**

Plasmid vectors:
- pCR3.1 (non-mobilisabe).

Bacterial hosts:
- *Echerichia coli DH5 alpha and TOP 10 series.*
- *Mycobacterium Bovis BCG Pasteur strain (BCG) (attenuated with a long history of safe use).* ATCC 35734

**Origin & function**

Genes of interest will be of human origin. Genes of interest will be junctional peptides from the TEL-AML1 and BCR-ABL. Either the whole gene, gene fragments or peptides will be used.

The TEL-AML1 fusion peptide is a small 30aa sequence taken from the TEL-AML1 fusion protein which is an oncogene fusion protein that is an initiating lesion in childhood ALL. However, this protein will not be used. The fusion peptide which will be cloned into the BCG and is not known to have any biological activity.

**Evaluation of foreseeable effects**

The BCG strain used in this project is a close relative of the commercially available BCG vaccine strain which has been used to vaccinate millions of patients. This strongly suggests that there would be minimal risk from the BCG strain used in this work.

The expressed tumour proteins should not be functional in the bacterial strain and given the nature of the vaccine approach, the bacterial vector with human proteins should be rapidly degraded by the innate immune system and used to prime an immune response. The risk to human health from the GMM is likely to be minimal.

The genetic insert would not alter the pathogenicity of the BCG but may impact upon deleteriously upon the growth of the bacteria since the expression of heterologous genes is commonly associated with reduced growth rates of the individual vector.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation (12-point thermocouple testing) by a third party company. Records are kept on site.

All liquid laboratory waste will be inactivated prior to removal from the Class II microbiological safety cabinets employed for this work.

Virkon is routinely used as per the manufacturer's recommendations:- 
Solid surfaces are disinfected with 1% Virkon solution. 
Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. 
Any contaminated glassware is treated with 1% Virkon for a minimum of 1 hour prior to cleaning. Usually the contact time is longer, typically overnight. 
Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendations).

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</table>

Project Ref 141/12.3

Date Ackn'd | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4
02/03/2022 | | | | |
Lentiviral transfection of fibroblast and epithelial cell lines using custom made lentiviral particles to over express or down regulate genes involved in the autophagic tumour/stromal model of cancer.

The aim of this project is to overexpress or knock-down genes of interest using lentiviral transduction in both fibroblast and epithelial cell lines to investigate the importance of these genes in both cancer cells and the associated stromal compartment in order to investigate effects on cancer cell growth, tumour-stoma interactions in co-cultures between stromal fibroblast cells and cancer cells, and in cancer cell metabolism.

For stable knock down experiments, lentiviral particles containing shRNA sequences targetting genes of interest will be custom made or purchased from Santa Cruz or Genocopia. The lentiviral particles are a pool of concentrated transduction ready viral particles containing target specific constructs encoding the specific shRNA under the control of a CMV promoter. Details of the viral vectors are provided in appendix 1 of the risk assessment.

For overexpression, pre-made or custom made lentiviral particles will be purchased from GeneCopoeia. The full coding sequences of the candidate genes will be inserted by the company into the viral vectors under the control of a CMV promoter and a puromycin resistance gene for selection purposes after transduction.

The viral particles are prepared by the company in the following manner. An OmicsLink™ ORF lentiviral expression plasmid (GeneCopoeia Cat. No EX-EGFP-Lv105) was constructed using GeneCopoeia proprietary RecJoin™ technology. This plasmid was co-transfected into 293Ta cells (GeneCopoeia Cat Np CLv-PK-01) with the Lenti-Pac HIV Packaging Mix (GeneCopoeia Cat. No HPK-LvTR-20). Lentivirus-containing supernatants were harvested 48 hours after transfection. Pre-made lentivirus titer ranges from 10^7 copies/ml for crude version to 10^9 copies/ml for purified lentivirus. Lentifact lentivirus production process involves stringent quality control processes including sequence verification of the insert, qRT-PCR based titer estimation, etc.

We intend to use these particles on a variety of cell lines in both monoculture and co-culture of fibroblast cells with breast cancer cell lines and look at cell metabolism, autophagy, mitophagy, proliferation, migration and molecular markers with Western blot, immunocytochemistry and flow cytometry, as well as biochemical assays of products of metabolism such as lactate and ketones.

Recipient or parental organism

Lentivirus parental organism- Human Immunodeficiency virus (HIV-2) is classified as ACDP hazard Group 3.

Host/vector system

Target Cells for Viral vector
Fibroblast cell lines BJ5TA, BJ1, 218, 522 and 544
Epithelial cancer cell lines MCF7, MCF10A, MDA-MB-231, SKBR3, T47D, BT20, BT474, MBA-MB-468 (all breast cancer cell lines) see appendix 2 in risk assessment for more details of the cell lines to be used. Other epithelial cell lines may be investigated, and amendment to the risk assessment would be obtained beforehand.

### Origin & function

**Genes of interest:**
- cDNA: Human Caveolin -1 and related genes
- cDNA: Human Autophagy/mitophagy related genes - including ATG16L, LAMP, TOM-20, LC3
- cDNA: Human Lactate transporter genes including MCT1 and MCT4
- cDNA: Human Transcriptional regulator genes - Including CAPER
- cDNA: Human genes associated with glycolysis and glucose transport - including GLUT-1
- cDNA: Human genes associated with senescence such as WNT5a, FBX032 and the CDK family of genes
- siRNA: to target Caveolin and related genes
- siRNA: to target MCT1, MCT4 and related genes
- siRNA: to target CAPER and associated transcriptional regulators.

As we will be studying a variety of similar and related genes within the above families, we will review the risk assessment when we look at other genes to ensure the assessment is still valid.

For knock-down experiments - Lentiviral particles containing shRNA sequences targetting genes of interest will be purchased from Santa Cruz or Genocopia.

For over-expression custom made particles will be purchased from Genocopia.

### Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors based on the HIV lentivirus. They are self inactivating vectors, which have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated risks.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the building (either by autoclaving or use of disinfectant); work carried out in containment 2 laboratories, work within Class 2 microbiological safety cabinets, routine disinfection of work surfaces; all staff are suitably trained in GM work and employing the necessary risk control measures.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on...
an annual basis. Records are kept on site. Trigene is routinely used as per manufacturer’s recommendations:-
Solid surfaces are disinfected with Trigene trigger spray solution.
Plasticware that can be effectively disinfected is treated with final concentration of v/v 1% Trigene for a minimum of 1 hour prior to incineration.
Any contaminated glassware is treated with 1% Trigene solution for a minimum of 1 hour prior to cleaning.
Liquid waste (culture and medium) is disinfected by the addition of Trigene to give a 1% final working concentration.
A minimal contact time of 1 hour is allowed prior to disposal to sewers.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The committee agreed that this was a Class 2 project.
It was the committee’s view that the assessment was adequate for the risk profile of the work.

Project Containment

![Project Containment Table]

Project Ref  141/12.4

![Project Ref Table]

Date Ackn’d  25/05/2017

Date Project Ceased

CU2 Project Title

Lentiviral transfection of prostate epithelial cells

Class  Class 2

CultureVolClass  < 1 Litre

CultureVolumeClass  Consent Granted

Project notified under transitional arrangements  N
Previous studies have identified the attraction to, and the ability to cross the bone marrow endothelium as being pivotal in the metastatic process of prostate cancer. Previous studies have identified a number of candidate genes involved in targeting and crossing the endothelial membrane towards bone marrow stroma. This project seeks to evaluate these candidate genes in established cell line models of prostate epithelial metastasis. Cell lines used will be human bone metastatic cell line PC-3 and non-bone metastatic cell line LNCaP to assess action of these genes. Cell lines will be transduced with genes of interest or their corresponding siRNA pools using lentiviral vectors and analysed using a combination of human bone marrow stroma co-culture assays which model binding, transendothelial migration, colony formation and expansion.

Recipient or parental organism

Lentivirus parental organism - Human Immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3

Host/vector system

Bacterial host: E.coli JM109

Viral vector system: 3 generation system (ref: Dull & Zuerly et al (1998) J. Virology voi72# 11 p8463-8471) consisting of 3 packaging plasmids and a transfer vector containing the cassette of interest.

Host cell; HEK293

Target cells for viral vector:-
Human bone metastatic cell line PC-3
Non-bone metastatic cell line LNCaP

Origin & function

Ephrin receptors:-EphA1, EphA2, EphA4, EphB, 2EphB3, EphB4, EphB6
Eph receptors are a group of receptors activated by the ephrin and form the largest known subfamily of receptor tyrosine kinases (RTKs). Both Ephs and their corresponding ephrin ligands are membrane-bound proteins that require direct cell-cell interactions for Eph receptor activation. Subsequent downstream pathways have been shown to regulate motility, cell adhesion and cell polarity through the Rho family of GTPases.
Eph/ephrin signalling regulates a variety of biological processes including the guidance of axon growth cones, formation of tissues boundaries, cell migration, and contact inhibition of locomotion (CIL). Additionally, Eph/ephrin signalling has recently been identified to play a critical role in several processes including long-term potentiation, angiogenesis, and stem cell differentiation.
Genes listed are expressed in prostate epithelial cells.
Ephrin A1, A3, A4, A5; Ephrin B1, B2, B3:- The Ephrin family are a family of proteins that serve as the ligands of the Eph receptors, which compose the largest known subfamily of receptor protein-tyrosine kinases (RTKs). Both ephrins and Eph receptors are membrane-bound proteins binding and activation of Eph/ephrin intracellular signalling pathways only occur via direct cell-cell interaction. Subsequent downstream pathways have been shown to regulate motility, cell adhesion and cell polarity through the Rho family of GTPases.

Eph/ephrin signalling regulates a variety of biological processes including the guidance of axon growth cones, formation of tissue boundaries, cell migration, and contact inhibition of locomotion (CIL). Additionally, Eph/ephrin signalling has recently been identified to play a critical role in several processes including long-term potentiation, angiogenesis, and stem cell differentiation.

2 classes of ephrin:
- Ephrin A - GPI (glycosylphosphatidylinositol) linked ephrins
- Ephrin B - Transmembrane linked ephrins

Gene listed are expressed in prostate epithelial cells.

Caveolin: Cav-1 Cav-2:- Family of integral membrane proteins which are the principal components of caveolae essential for receptor-independent endocytosis. Caveolins act as scaffolding proteins within caveolins have also been shown to have lipid binding properties and mediate aspects of cholesterol and fatty acid metabolism.

The caveolin gene family has three members in vertebrates: CAV1, CAV2, and CAV3, coding for the proteins caveolin-1, caveolin-2, caveolin-3, respectively.

Evaluation of foreseeable effects

The recombinant Lentiviral vector system consists of disabled vectors. They have had HIV regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is though there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the risk control measures utilised are appropriate to guard against the associated risks. These include the use of safety cabinets, biosafe rotors and prescribed disposal routes for waste, wearing of appropriate PPE and no use of any sharps.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation by a third party company. The performance test results generated during validation are kept on site.

Virkon is routinely used as per the manufacturer's recommendations:
- Solid surfaces are disinfected with 1% Virkon solution.
- Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.
Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as manufacturer's recommendations).

The committee agreed with the classification. It asked the author for more information about the genes of interest and these were supplied and are listed in section 7 of this form and as a table appended to the risk assessment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
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**Project Ref** 141/13.1

**Date Ackn'd** 25/05/2017

**CU2 Project Title**

Using fluorescent and luminescent reporters to monitor and isolate breast cancer (stem) cells in vitro and in vivo

**Date Project Ceased**

**Class**

Class 2

**Consent Granted**

Non-GMM

**CultureVolumeClass2**

< 1 Litre

**Project notified under transitional arrangements**

N
**Project Additional Information**

**Purposes of the contained use**

Primary aim: to monitor and isolate breast cancer (stem) cells in vitro and in vivo. Micrometastases are a major problem in breast cancer as they are resistant to standard therapies and have the potential to kill the host. It is known that breast cancer cells preferentially metastasise to the lungs, liver and bone, however the timing of this progression and the processes underpinning it are not clear. Understanding how and when breast cancer cells metastasise and how micrometastases are regulated in their environment will aid the development of new strategies to inhibit their formation or progression.

We plan to monitor cellular dissemination of breast cancer (stem) cells using in vivo imaging and isolation from mouse tissues. We have obtained bicistronic luciferase and green (GFP) and red (RFP) fluorescent cDNA vectors for lentiviral transduction from Standford University, USA (FULG and FULT vectors from (Liu et al., 2010)) for this purpose. We will transfect both breast cancer and cell lines and primary breast xenografts with the fluorescent vectors and inject them into mice. We will then monitor the spread of breast cancer (stem) cells to metastatic sites. The advantage of the bicistronic vector is that injection of luciferein can be used to detect as few as 10 cells in a metastatic site and the GFP/RFP+ cells can be localised in the excised tissues and sorted from host cells by FACS.

**Recipient or parental organism**

Lentivirus parental organism- Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.

**Host/vector system**

Bacterial: E. coli Stbl3 (from Invitrogen)

Viral vectors; pMDLg/pRRE, pRSV-Rev, pMD2.G, pCMVdelta8.91 lentiviral packaging plasmids

Lentiviral system: This is a self-inactivating lentivector system with a pseudotyped VSV-G capsule. Viral particles will be produced using the HEK293T packaging cell line.

FLUG and FULT plasmids contain multifunctional reporter genes firefly luciferase and fluorescent proteins

Target cells: Continuous human breast cancer cell lines: MCF-7; T47D; MDA-MB-231; BT474 - All from ATCC Primary human tissue samples from Christie Hospital Biobank.

**Origin & function**
The FULG and FULT plasmids are described in Liu et al, 2010 (PNAS vol. 107 p18115-18120). They are multifunctional reporter genes used to analyse disease models by linking in vivo and ex vivo assays. They contain both firefly luciferase (Luc+) for whole body tracking of cells via bioluminescence imaging, and fluorescent proteins to allow intravitral imaging and ex vivo analyses. In FUKG and FULT Luc2 is fused to either enhanced GFP (eGFP) (FULG) or the red fluorescent protein tomato (FULG).

Evaluation of foreseeable effects

The recombinant Lentiviral vector systems consist of disabled vectors. They have had HIV regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. The systems employed will be Self-inactivating (SIN) vector systems. Thus whilst they may pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the risk control measures utilised are appropriate to guard against associated risks. These include the use of safety cabinets, biosafe rotors and prescribed disposal routes for waste, wearing of appropriate PPE and no use of any sharps.

The reporter genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The mice used in this study will not be GM animals. GM human tumour cells will be injected into mice. These will not have the ability to infect the animals cells.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user.

The autoclave undergoes annual validation (12-point thermocouple testing) by a third party company. Records are kept on site. It also undergoes a quarterly maintenance regime as recommended in BS2646.

Any plasticware that can be effectively disinfected is treated with 1% virkon and left overnight before entering the clinical waste route.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturers recommendations) but it is usual to leave overnight until disposal to drains.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
The Committee agreed with the classification of the work as GM Class 2. It is asked for confirmation that no sharps would be used during lentiviral particle generation. This was confirmed by the research group concerned.

### Project Containment

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### Project Ref 141/13.2

- **Date Ackn’d**: 25/05/2017
- **CU2 Project Title**: Use of Retroviral Transduction
- **Class**: Class 2
- **CultureVolClass2**: ≤ 1 Litre
- **CultureVolumeClass3-4**: Not Applicable
- **Non-GMM**: Consent Granted
- **Tick if notifying a connected programme of work**: N
- **Project notified under transitional arrangements**: N

#### Withdrawn

- **Historical Significant Changes**: Transferred from GM141
- **Historical Date of Additional Info**: Not Applicable
- **Significant Change ID**: Not Applicable
- **Date of Significant Change**: Not Applicable

### Project Additional Information

**Purposes of the contained use**

We seek to investigate the role of normal and cancer-activated fibroblasts in tumour-stromal interaction using knockdown or overexpression of genes of interest. Fibroblasts are known to exert tumour-promoting as well as tumour-suppressing functions dependent upon micro-environmental conditions and the metabolic state of the contributing cell types. We intend to co-culture fibroblast cells with breast cancer cell lines and analyse cell functionality (proliferation, migration, invasion, stem cell expansion) as well as biochemical states (molecular markers by western blot, real time PCR and Elisa).
Recipients or parental organism

Moloney Murine Leukemia Virus (MMLV)-based retroviral vectors pBabe, pWZL and pLNCX used to generate recombinant viral particles using a HEK293T based packaging cell line.

Host/vector system

Phoenix-amphotropic HEK293T packaging cell line. Provides the env gene and protein which determines the virus particle infectivity range. In this case- amphotropic.

Retroviral vectors pBabe; pWZL and pLNCX MMLV-based retroviral vectors

Details of the host vector system are given in Appendix 1 of the risk assessment

Origin & function

The host-vector system will produce recombinant virus particles which will then be used to infect a range of cell lines. These cell lines are human breast cancer cell lines and human and murine fibroblast cell lines.

The genes of interest comprise a wide range including those involved in proliferation, migration, invasion and stem cell expansion; as well as biochemical states. The known or suspected functions of these genes are listed in Appendix 2 of the risk assessment.

Evaluation of foreseeable effects

The recombinant virus has its structural genes deleted, which disables the virus and so prevent its growth in the absence of complementing cell lines or co-transfection of appropriate plasmids.

The amphoteric envelope, conferred by the packaging cell line, changes cellular tropism to most mammalian cells including human. It may be able to confer greater stability and environmental survivability of the virus. However, appropriate work practices will prevent the replication incompetent viruses from being released in to the environment.

The absence of the use of sharps in this work should help minimise risks to workers

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user.

Virkon is routinely used as per the manufacturer's recommendations:-

Solid surfaces are disinfected with 1% Virkon solution

Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to being placed in to the clinical waste route and then goes for incineration

Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Usually the contact time is longer, typically overnight.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).
The autoclave undergoes annual validation by a third party. Records are kept on site.

The committee asked to be informed of the genes that the project studies, as work progressed. This was agreed by the risk assessment author.

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**Project Ref 141/15.1**

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<th>Project notified under transitional arrangements</th>
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<tr>
<td></td>
<td>Non-GMM</td>
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Tick if notifying a connected programme of work N

Historical Significant Changes

- transferred from GM141

Historical Date of Additional Info

02/03/2022
Purposes of the contained use

Microarray and single cells expression profiling experiments previously performed have identified a number of candidate genes that may be critical regulators of normal and tumorigenic prostate progenitor cells. Genes expressed in the stromal compartment of the prostate may also have a critically important function in the regulation of normal prostate and tumour-initiating cells. This project seeks to evaluate the role of these genes in these cell types in murine and human model systems using genetic knockdown experiments. Briefly, murine or human prostate cells will be transduced using lentiviral vectors containing shRNAs and cells will then be functionally evaluated using a combination of in vitro and in vivo techniques, the latter involving transplantation of cells in to mice. One retroviral vector system will be used: pSHAG-MAGIC2, which provides constitutive expression of the shRNA from MSCV promoter. Two lentiviral vector systems will be used: (i) pLKO.1, which provides constitutive expression of shRNAs from a PolIII promoter, and (ii) pSicoR, which provides constitutive expression of shRNAs from a Pol III promoter with the option of irreversible Cre-mediated conditional inactivation of knockdown. In some cases, we would like to ensure that the genetic alteration is restricted to specific prostate lobe/area in vivo. For this, adenovirus or lentivirus will be injected intraprostatically to genetically modified mice and their control counterparts.

Recipient or parental organism

Bacterial hosts: Escherichia coli Stbl3 (for use with lentiviral vector system plamids)
   Escherichia coli Dh5 alpha (for general cloning purposes)
Bacterial recipients are none-infective strains and have multiple auxotrophic requirements. Growth and survivability is unlikely out of laboratory growth medium.
Transformation with plasmids used in this work will not alter this phenotype.

Target cell lines—Primary murine and human prostate cells (please see appendix 7 for cell use criteria)
Growth and survivability of modified and un-modified cells is unlikely outside of laboratory growth medium.

Host/vector system

Bacterial hosts: Escherichia coli Stbl3 (for use with lentiviral vector system plamids)
   Escherichia coli Dh5 alpha (for general cloning purposes)

Lentivirus pLKO.1 and pSicoR systems: parental organism is Human Immunodeficiency virus (HIV-1) and is classified as ACDP hazard group 3. Used with HEK 293 FT cells.
More details can be found in appendix 1 and 3 of the risk assessment.

The pSHAG-MAGIC2 retrovirus system: parental organism is Murine Stem Cell Virus. Used with Phoenix-E and Plat-E cells.
More details can be found in appendix 3 and 4 of the risk assessment.

Target cells: primary murine and human prostate cells

Origin & function

shRNAs expressed will target the genes listed in appendix 2 of the risk assessment, for knockdown. The function of these genes in leukaemia stem cells is not known formally, however, genes such as Pten, Brca1, and ATM are cell cycle/dna damage regulators/tumour suppressors so their knockdown may accentuate oncogenesis.
**Evaluation of foreseeable effects**

The recombinant viral vectors are highly disabled vectors based on the HIV or MSC virus. These vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

For the lentivirus, whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability it is thought that there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the control measures utilised are appropriate to guard against the associated risks.

The target genes do not encode viral specific protein, nor does they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus or MSC retrovirus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration.

Virkon is routinely used as per the manufacturer's recommendations:-

Solid surfaces are disinfected with 1% Virkon solution.

Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration.

Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

The autoclave undergoes annual validation. Records are kept on site.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment
The committee agreed with the classification. It asked for clarification on the regime for training workers in this work. This was supplied to the committees satisfaction. Likewise the committee asked for a standard operating procedure for dealing with breakages in centrifuges used in this work. Again this was supplied to the committees satisfaction.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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**Project Ref 141/15.2**

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<th>CU2 Project Title</th>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>25/05/2017</td>
<td>Investigating Transcriptional Networks in Lung Cancer</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Not Applicable</td>
<td>N</td>
<td>N</td>
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Withdrawn N

Historical Significant Changes

Transferred from GM141

**Project Additional Information**

**Purposes of the contained use**

The proposal concerns the immortalisation of primary cells that can be readily expanded in a number for experimental investigations. Specifically, the primary cells of interest will be those that comprise the tumour microenvironment. This
is a broad set of cell types but the aim of the work is to attempt to generate mixed cultures of cells that re-capitulate key aspects of the tumour microenvironment including immune suppression. Consequently, cells that would be of interest include tumour and non-tumour stromal cells including fibroblasts and the diversity of immune cells. We have expertise in disaggregating tumours and expanding T cells for in vestion and also immortalising B cells employing EBV. We also have performed short-term tumour cell growth for co-culture assay. However, in the absence of immortalisation, cell number is usually a limiting factor for the breadth of potential experiments that can be performed. For this project, we propose to exploit the directed expression of single genes that have been shown to immortalise a range of primary cells.

Recipient or parental organism

Primary human tumour or peripheral blood cells

Host/vector system

Retroviral vectors.
Retroviral viral gene transfer vectors have been extensively engineered to eliminate all virus protein coding sequences within the vector itself. Vector particle are generated by co-expression of gag/pol and env genes with the retroviral vector within a packaging cell. The gag/pol and env genes are encoded on separate plasmids and engineered to reduce homologous sequences with the retroviral vector. This degree of engineering reduces the possibility of generating a replication-competent retrovirus to essentially zero based upon the extensive history of use of retroviral vector technology in the pre-clinical and clinical situation.

Lentiviral vectors.
Lentiviral vectors have been engineered as described below:
An enhancer deletion in the U3 region of 3'L1LTR ensures self-inactivation of the lentiviral vector following transduction and integration into the target cell’s genomic DNA.
• The number of lentiviral genes necessary for packaging, replication and transduction is limited to three (Gag/Pol/Rev), and their expression is derived from different plasmids, all lacking packaging signals. These plasmids share no significant homology to the expression vector, thus preventing the generation of replication-competent virus by recombination events.
• None of the Gag, Pol, or Rev genes will be incorporated into the packaged viral genome, thus making the mature virus replication-incompetent.

Origin & function

The genes to be used are sourced commercially with sequences as listed below.

Immortalising genes.

SV40 large and small T antigen sequences. Full sequence available here: http://www.abmgood.com/SV40-CellImmortalization.html
hTERT. Full sequence available here: http://www.abmgood.com/hTERT-Cell-Immortalization.html
siRNA targets to aid immortalisation:
pRB siRNA. Full sequence available here: http://www.abmgood.com/Myc-p53-Rb-Ras-Cell-Immortalization.html

Evaluation of foreseeable effects

Both retroviruses and lentiviruses are lack any potentially immune avoidance gene products that are encoding within the wild type viruses hence they are unlikely to have the same degree of immune protection as that afforded to the
wild type virus. The envelope glycoproteins lend to be derived from other viruses which possess different and, most
often, increased immunogenicity profiles compared to the retrovirus lentivirus envelope glycoproteins. Indeed, the
VSVg envelope commonly used to pseudotype lentiviral vectors is sensitive to complement mediated destruction in
human serum. Overall, the replication deficient virus vectors are most likely to possess an increased immunogenicity
profile as compared to the wild type vector suggesting that in the immune-competent individual, these vectors are
more likely to be subjected to immune-mediated clearance.

For the target cells ..

The genes to be expressed facilitate the modified cell 10 overcome cellular senescence and to allow the transduced
cell to become immortalised.

The modified cells require specific conditions to survive including maintenance at 37 degrees C and cultured in
incubators flushed with C02. Outside of this environment, the cells could survive in media for a period of time but
would be unlikely to expand in number. However, with evaporation, solute concentrations would build within the
remaining media and the cells would either die of that level of toxicity or die through drying out. Outside of the media
environment, cells would last only a short period of time (hours) before dying due to dehydration.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a
validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes
for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is
displayed on the autoclave at the end of each cycle and this is monitored by the end-user.
The autoclave undergoes annual validation (12-point thermocouple testing) by a third party company.
All liquid laboratory waste will be inactivated prior to removal from the Class II microbiological safety cabinets
employed for this work.

Virkon is routinely used as per the manufacturer's recommendations: Solid
surfaces are disinfected with 1% Virkon solution.
Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration.
Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Usually the
contact time is longer, typically overnight.

liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration.
2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to
disposal to sewers. (as per manufacturer's recommendations).

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form
Then committee agreed with the classification and that the assessment was comprehensive and well written.

**Project Containment**

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<td></td>
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</table>

**Project Ref**: 141/15.3

**Date Ackn'd**: 25/05/2017

**CU2 Project Title**: Primary Cell Immortalisation

**Class**: Class 2

**CultureVol**: < 1 Litre

**ClassCultureVol**: Class 2 < 1 Litre

**ClassVolume**: Class 2

**Consent Granted**: Not Applicable

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**: Transferred from GM141

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

This project involves over expression of the PDGFRαIB in human lung cancer cells from the ATCe. The PDGFR gene is implicated in lung cancer and we wish to introduce and express these genes in a small range of cell lines. The
gene will be introduced into the cell by chemical means e.g. lipofectamine. Downstream analysis, involving cell sorting and various assays will be carried out. Other genes of interest involved in lung cancer may be used subsequently (e.g. AMPK, 81M, NKIRAS2) but the Biosafety Committee will be informed beforehand. We also intend to transfect a small range of cell lines with microRNAs. miRNAs will be introduced into the cell lines using premade Lentiviral particles resulting in integration into the cell genome. The modified cells will then be analysed using standard laboratory assays and techniques such as western blotting. Whilst we have identified the use of two miRNAs which we intend to use, other miRNAs may be used later in the project. The Biosafety Committee will be informed beforehand if this occurs. We also plan to stably silence important oncogene targets of the identified microRNAs using the shRNA lentiviral particles. We will purchase ready for use Lentiviral particles from Santa Cruz Biotechnology. Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

### Recipient or parental organism
Lentiviral systems: Lentivirus parental organism- Human Immunodeficiency virus-1 (HIV-1) is classified as ACDP hazard group 3

### Host/vector system
Bacterial Host Vector system: E.coli TOPO 101 pCMV expression vectors
Viral/Host systems: Custom made lentiviral particles from Santa Cruz Biotechnology for transduction of shRNA constructs into target cells
microRNA lentivector expression systems from System Bioscience (SBI), involving co-transfection of expression and packaging vectors in HEK.293 cells, resulting in pseudo-viral particles harbouring miRNA expression constructs for transduction in to target cells
Target Cells: A549 lung cells from ATCC,
H292 lung cells from ATCC

### Origin & function
Human PDGFR Gene eDNA Clone (full-length ORF Clone), expression ready, C-HA-tagged
Vector: pCMV3-C-HA
HA Tag Sequence: TATCCTTACGACGTGCCTGACGCC
Species: Human
Gene Synonym: PDGFR Bela and PDGFR-Alpha
Gene Bank ref 10: NM_002609.3 and NM_002609.4

### Health and Safety
PDGFR is implicated in lung cancer in humans. Other genes implicated in human lung cancer, such as AMPK, 81M and NKIRAS2 and genes of similar functional profile, may also be investigated.

### Evaluation of foreseeable effects
The recombinant viral vectors are highly disabled vectors based on the HIV lentivirus. They are self-inactivating vectors, which have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal. Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. The autoclave has an integral "pass" or "fail" alert for each cycle processed in the machine. This is displayed on the autoclave at the end of each cycle and this is monitored by the end-user. The autoclave undergoes annual validation (12-point thermocouple testing) by a third party company. All liquid laboratory waste will be inactivated prior to removal from the Class II microbiological safety cabinets employed for this work. Virkon is routinely used as per the manufacturer's recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Usually the contact time is longer, typically overnight. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
The Committee asked for more information on the lentivirus systems intended for use. This was supplied to their satisfaction. The Committee agreed with the classification of the work as GM Class 2.

**Project Containment**

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</table>

**Project Ref** 141/16.1

**Date Ackn'd** 25/05/2017

**CU2 Project Title** Lentiviral infection of pancreatic cells and the surrounding stroma

**Class** Class 2

**CultureVol** ≤ 1 Litre

**CultureVolume** Class 3-4

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes** Transferred from GM141

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Lentiviral vector packaging systems will be used to transduce cells which are otherwise difficult to modify using retroviral or other methods of transfection. This will mostly be employed within a pancreatic cancer setting, focusing on the tumour cells and surrounding stromal cells.
Recipient or parental organism

Lentivirus parental organism- Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.

Host/vector system

Lentiviral vector systems:-
Lentiviral vector packaging systems have divided the essential functions amongst multiple plasmids to reduce the risk of generating replication-competent lentivirus (RCL). The split-genome packaging system is designed so that multiple recombination events between the components are required for autonomous replication. Clinical trials using a split-genome packaging system have shown that this strategy effectively eliminates the creation of RCLs (see Levine et. al. PNAS, 103: 17372-17377, 2006). Commercially available 3rd generation lentiviral vector systems separate the viral envelope, env (e.g. VSV-G) from the gag-pro-pol, which encodes structural and enzymatic functions. We will be using pLB, pSicoR and pSLiK which lack gag, pol, env,tat, rev and other accessory viral genes.
We will also be using GIPZ shRNA Lentiviral Particles which are produced using the Trans-Lentiviral Packaging System. The Trans-Lentiviral Packaging system provides an even higher level of safety over 3rd generation packaging systems by further splitting the viral pol (reverse transcriptase (RT) and integrase (IN) functions) from gagpro. Because the RT and IN enzymes are provided in trans to gag-pro, additional recombination events are necessary to produce RCLs

Target cells:-
Cells will be cultured from primary animal or human sources or through immortalised cell lines. Cell lines of human origin will be verified using the Institute's human cell line authentication service. The source tissue of the cells includes pancreas, kidney, spleen, liver, breast, pleural effusion, lymphocyte, brain, pericardial effusion, ascites, lymph node and endothelium. Human patient material will be acquired via the MCRC Biobank. The Institute prohibits the collection of human tissue known, or highly likely, to be infected with a human pathogen. Any deviation from this rule must be approved by the Institute Director and Biosafety Committee.

Origin & function

PKN2 (Protein Kinase N2) is a PKC-related serine/threonine-protein kinase and Rho/Rae effector protein that participates in specific signal transduction responses in the cell. Plays a role in the regulation of cell cycle progression, actin cytoskeleton assembly, cell migration, cell adhesion, tumour cell invasion and transcription activation signalling processes.
Other genes of similar function or implicated in the same cellular processes to that named may be investigated. But information as to their name and nature will be provided to the Biosafety Committee beforehand.
GFP (Green Fluorescent Protein) is a Energy-transfer acceptor. Its role is to transduce the blue chemiluminescence of the protein aequorin into green fluorescent light by energy transfer. Fluoresces in vivo upon receiving energy from the Ca2+-activated photoprotein aequorin.

Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors based on the HIV virus. These vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/ infection cycles. The probability of seroconversion is minimal.
For the lentivirus, whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environment metal survivability it is thought that there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the control measures utilised are appropriate to guard against the associated risks.
The target genes do not encode viral specific protein, nor does they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration.

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The autoclave undergoes annual validation. Records are kept on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee agreed with the classification.
It asked for details of the target cells which were subsequently provided and incorporated into the risk assessment.

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications
Project Ref 141/16.2

**Dissecting factors driving tumour-promoting or tumour-inhibitory inflammation in mouse models of cancer**

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Non-GMM Consent Granted

Tick if notifying a connected programme of work N

**Project notified under transitional arrangements**

Withdrawn N

**Historical Significant Changes**

Transferred from GM141

Project notified under transitional arrangements N

**Project Additional Information**

**Purposes of the contained use**

The project aims to investigate the immunomodulatory properties of factors derived from cancer cells on innate and adaptive immune cells typically found in the tumour microenvironment. Briefly, mouse cancer cell lines will be transduced using standard retro and lentiviral vector packaging systems such as pFB, pMSCV, pRevTRE or pLKO.1 to variably induce the upregulation or downregulation of candidate factors that might impact on the ability of the cancer cells to modulate inflammation and subvert immune surveillance. The factors will typically involve inflammatory and immune mediators but might also include known oncogenes or tumour-suppressors in order to examine how specific mutations impact the ability of the cancer cells to modulate the inflammatory and immunogenic properties of cancer cells.

**Recipient or parental organism**

Lentivirus parental organism- Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.

Moloney Murine Leukaemia Virus parental organism is classified as hazard group 1.

**Host/vector system**

Lentiviral vector systems:-

Lentiviral vector packaging systems have divided the essential functions amongst multiple plasmids to reduce the risk of generating replication-competent lentivirus (RCL). The split-genome packaging system is designed so that multiple
recombination events between the components are required for autonomous replication. Clinical trials using a split-genome packaging system have shown that this strategy effectively eliminates the creation of RCLs (see Levine et al. PNAS, 103:17372-17377, 2006). Commercially available 3rd generation lentiviral vector systems separate the viral envelope, env (e.g. VSV-G) from the gag-pro-pol, which encodes structural and enzymatic functions. We will employ systems such as pFB, pMSCV, pRevTRE and PLKO.1. These systems are designed as described above with the gag and pol elements provided in trans using GP-2 or Phoenix cells.

Retraviral vector systems:- GP2-293 (Clonetech@631458) is an HEK-derived cell line engineered to express the Moloney Murine leukemia Virus (MoMuLV) essential viral packaging components by stably integrating, gag and pol genes. The viral envelope must be supplied in trans. High titre recombinant retrovirus particles are produced by transient co-transfection of pFB or pMSCV-based retraviral expression vector and a plasmid that expresses a viral envelope, such as pVSV-G. These commercially available vectors with proven safety profile are in use over many years for producing infectious but defective retroviral particles. These replication deficient VI Ps will be used to transduce the target cancer cells to study the impact of the encoded transgene expression on its immunogenic properties.

The final genetically modified retroviral and lentiviral particles will be replication incompetent and therefore will not be able to propagate and establish a clinical infection. However, because they are infective they will be treated under containment level-2. Their properties are not expected to be altered by any of the inserts we will introduce into the vectors.

The potential route of transmission of the viral particles in the laboratory is mainly through skin contact that could arise from splashes, touching the objects or surfaces that are contaminated with culture media. Precautionary measures such as wearing laboratory coats and gloves and working in a laminar flow safety cabinet will prevent transmission through direct contact or inhalation of aerosol. Airborne transmissions are minimised since all cell culture work will be conducted in a class 2 microbiological safety cabinet and all centrifugation steps will be carried out in specialised sealed centrifuge buckets to prevent the generation of aerosols. Filter tips will be employed to prevent cross contamination of pipettes. No sharps or needles will be used so there is not risk of percutaneous transmissions. Accidental spillages will be dealt with by cleaning with appropriate disinfectant solutions (i.e. 1% Virkon). Any viral particle suspensions will be disposed of only after completely neutralising the virus using 1% Virkon for at least 24 hours. Contaminated consumables will be autoclaved prior to incineration.

Target Cells:- Well-characterised mouse cancer cell lines such as melanoma (8 16, 5555), colon-carcinoma (CT26) and breast-carcinoma (4T1), will be used in this project and are (or in the process to be) commercially available. B16 (ATCC® CRl6475™) and 5555 melanoma cells are a mixture of spindle-shaped and epithelial-like cells derived from C57BU6J mice. CT26 (ATCC® CRL-2638™) is an induced, undifferentiated colon carcinoma cell line derived from Balb/c mice. Product data sheets and certificate of analysis are available on the ATCC website.

Origin & function

The specific gene that we are using in this project is mouse Cycloxygenase-2 gene that was supplied commercially by SourceBioscience. COX-2 is an enzyme that is responsible for formation of prostanoids.

Other genes may be employed in this project encoding for molecules that may play a role in inflammation and immunity. In this event the Biosafety Committee will be notified beforehand.

Evaluation of foreseeable effects

The recombinant viral vectors are highly disabled vectors. These vectors have had regulatory and accessory genes deleted ensuring that viral particles produced are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication infection cycles. The probability of seroconversion is minimal.
For the lentivirus, whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability it is thought that there is no significant increase in the likelihood of transfection via the airborne route of exposure. Besides, the control measures utilised are appropriate to guard against the associated risks. The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the recombinant lentivirus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Virkon is routinely used as per the manufacturer's recommendations: Solid surfaces are disinfect with 1% Virkon solution.

Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration.

Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer's recommendations).

The autoclave undergoes annual validation. Records are kept on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee agreed with the classification of the work.
The research group was asked to notify the Committee of the intended use of additional genes of interest Likewise if the work progressed in to animals (mice) then appropriate assessment would be required beforehand.

Project Containment

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02/03/2022
**Project Ref**: 141/93.1A

**Date Ackn’d**: 25/05/2017

**CU2 Project Title**: TRANSFER OF GENES INTO HAEMOPOIETIC CELLS:

**Class**: Class 2

**CultureClass Vol**: Class 2

**CultureVol Class-4**: Class 3-4

**Non-GMM**: Not Applicable

**Consent Granted**: Project notified under transitional arrangements

**Withdrawn**: No

**Tick if notifying a connected programme of work**: No

**Historical Significant Changes**: GM141/96.1, GM141/98.1, Transferred from GM141

**Historical Date of Additional Info**: 11/11/1996, 16/06/1998

**Significant Change ID**: 0

**Date of Significant Change**: 0

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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</tr>
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</table>

Project Ref 168/18.2

Date Ackn'd: 01/10/2020

CU2 Project Title: Experimental evolution of bacterial pathogens and their mobile genetic elements

Class: Class 2

CultureVolClass2: < 1 Litre

CultureVolumeClass3-4: 

02/03/2022
In vitro experimental evolution is an experimental tool for studying the evolutionary dynamics of microbes, such as bacteria. Replicate experimental population of microbes are propagated under controlled laboratory conditions for several 100s or 1000s of generations and the evolution of their phenotypes observed over time. We are interested in how bacterial populations adapt to their abiotic and biotic environment. Genome sequencing is used to determine the mutations targeted by natural selection that underpin the observed evolutionary adaptation. These mutations are then introduced into the ancestral genotype to test their phenotypic effects. Labelled strains of the ancestral genotype allow these to be distinguished in mixed cultures, allowing for changes in Darwinian fitness to be measured by direct competition. We will use these approaches to understand how opportunistic bacterial pathogens, Escherichia coli and Pseudomonas aeruginosa, adapt to their biotic and abiotic environment. Specifically, we will test how bacterial pathogens adapt following acquisition of mobile genetic elements (e.g. plasmids), and how bacterial pathogens adapt to different microenvironments known to be present within hosts or natural environments.

Recipient or parental organism

- Pseudomonas aeruginosa
- Escherichia coli

Host/vector system


Origin & function

During this project we will generate the following GMMs:
1) Variants of bacterial strains carrying selectable markers. This allows us to:
   a) select for transconjugants (i.e. bacteria which are carrying the plasmid).
   b) identify differently marked strains when in co-culture, for example, during competition experiments.
2) Variants of bacterial strains in which we have deleted genes that were the target of natural selection during the experimental evolution.
3) Variants of plasmids labelled with derivatives of green fluorescent protein (GFP), enabling their identification using fluorescence.
4) Variants of bacterial strains carrying vectors allowing complementation, e.g. by reintroducing different alleles of a gene into a strain in which that gene has been deleted.
5) Generating these strains will also require us to use standard laboratory E. coli cloning strains such as DH5-alpha and constructs such as pUC18.

Inserted genes:
- aacC1 (encodes gentamicin acetyltransferase-3-1), confers resistance to the antibiotic gentamicin
- strpAB (encodes aminoglycoside 3-phosphotransferase), confers resistance to the antibiotic streptomycin
- aphA1 (encodes aminoglycoside 3-phosphotransferase II), confers resistance to the antibiotic kanamycin
- aadA (encodes aminoglycoside 3-adenyllyltransferase), confers resistance to the antibiotic spectinomycin
- gfp and derivatives (encodes green fluorescent protein)
- telAB (and similar), confers tellurite resistance
- lacZ (encodes beta-galactosidase), results in production of blue pigment when grown on X-gal

These inserts encode enzymes that modify antibiotics rendering them non-functional (aacC1, strpAB, aphA1, aadA), that detoxify tellurite (telAB), or that produce fluorescent or chromogenic markers (gfp, lacZ). None of them are likely to pose any direct risk to humans, animals, or plants. The insertion cassettes are designed to encode the gfp variants under the control of the lactose-inducible PA1/04/03 promoter followed by the resistance gene under its own native level promoter. Total expression of inserted proteins is unknown, however as the inserts are generally of low copy number, total amount of protein produced should be similarly low and unlikely to pose any additional risk.

Evaluation of foreseeable effects

P. aeruginosa and E. coli are designated as Class II organisms by ATCC and as Hazard Group 2 by the Advisory Committee on Dangerous Pathogens (ACDP).

E. coli is a commensal bacterium and part of a healthy mammalian gut flora but may also cause opportunistic infections. The project uses environmental and clinical isolates of E. coli. Healthy individuals are not typically at a high risk of infection. Infection may occur where the user is immuno-compromised (e.g. undergoing treatment for cancer or HIV) or via infection of existing wounds or burns. Certain strains of E. coli can cause diarrhoea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses (CDC). Infections of healthy people are readily treatable with antibiotics and most patients recover within 10 days.

P. aeruginosa is an environmental bacterium inhabiting freshwater and soil environments, but also causes opportunistic infections. Healthy individuals are not typically at risk of infection. Infection may occur where the user is immuno-compromised (e.g. undergoing treatment for cancer or HIV, cystic fibrosis patients) or has exposed burns. Infections of healthy people are readily treatable with antibiotics and most patients recover within 10 days.

The addition of resistance markers may increase the risk that infections are harder to treat with antibiotics. However, such risks will be mitigated by selecting resistance gene markers against antibacterials that are not used clinically (e.g. streptomycin, gentamycin).

The inserted genes will be introduced using a mini-Tn7 transposon system or homologous recombination system, both of which introduce the marker into a known location in the genome (confirmed using PCR). Labelling the bacterial chromosome poses little risk of horizontal gene transfer as the delivery vectors are unable to replicate and therefore are rapidly lost. The E. coli cloning strains are highly compromised laboratory strains and are uncompetitive outside of the lab environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Chemical disinfection using Virkon or Distel: company testing shows effectively 100% killing of related strains of P. aeruginosa and E. coli.
Autoclaving using standard cycles effects 100% killing. Material contaminated with GMOs is autoclaved at 134 degrees C for 20 minutes. This cycle kills all plant materials and (soil) microorganisms, and has been validated by BMM Western (the autoclave manufacturer). In all autoclave runs, the temperature is monitored and recorded by a probe placed in the centre of one of the bags being autoclaved. The autoclave is checked and tested by BMM Western every 3 months. Autoclave conditions are automatically recorded and checked and archived for 3 years. The autoclave is regularly serviced and maintained.

Project Containment

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<td>L2 L3 L4 L2 L3 L4 L2</td>
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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 207/12.2

Date Ackn'd 11/05/2014

CU2 Project Title Imaging and manipulation and analysis of fungi expressing recombinant fluorescent and luminescent proteins in vitro

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Project transferred from GM207 on 11/05/2014

This Project proposal was considered and approved by the University of Sheffield Biosafety Committee on 27th June 2018.

Please enter comments on the GM safety committee on the risk assessment

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project transferred from GM207 on 11/05/2014
Project Additional Information

Purposes of the contained use
To image analyse and manipulate fungi expressing recombinant fluorescent and luminescent proteins in vitro

Recipient or parental organism
A range of containment level I and II fungi and disabled E. coli K12. Some of the fungi are pathogens of plants and humans.

Host/vector system
Standard host/vector systems used for yeast and filamentous fungi

Origin & function
Green fluorescent protein, other fluorescent proteins and the luminescent protein aequorin derived from Aequoria victoria and other marine organisms. These proteins will be used to label fungal proteins and organelles, and measure intracellular calcium, in fungal cells.

Evaluation of foreseeable effects
There is no possibility for increasing the pathogenicity of the pathogenic organisms with the experiments performed on them in the proposed work. The other non-pathogenic fungi to be used are model organisms that are considered generally safe to work with. The genetic material to be inserted into the recipient fungi and E. coli are not involved in pathogenesis and are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of the cloning host or normal human defence mechanisms. Gene transfer is possible but unlikely to be hazardous

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solids (e.g. plastic-ware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (bloack bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for
treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

SPILLAGES

Particular care should be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that involves broken glass) unless they are aware of the potential risks and trained safe working practices.

If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste stream.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The genetic modification safety committee agreed that the appropriate containment and control measures have been assigned.

**Project Containment**

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

**Project Ref** 540/01.4

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Date Project Ceased: 02/03/2022
### Project Additional Information

#### Purposes of the contained use

The objectives of this programme are to construct and characterise mutants of *Mycobacterium bovis* BCG. These mutants will be used to analyse well-defined, non-toxic, mycobacterial cell wall components (proteins, lipids, etc) and genes that contribute to the basic physiology of mycobacteria. This project is of special interest because *Mycobacterium tuberculosis*, the causative agent of tuberculosis, is a major cause of death worldwide. The use of *M. bovis* BCG as a model organism to study *M. tuberculosis* coelomocyte genetics and physiology is well documented and will aid in the development of new classes of drugs against tuberculosis.

#### Host/vector system

DNA sequences will be introduced into *M. bovis* BCG using well characterised and harmless non-mobilisable shuttle vectors that can replicate in *E. coli* and mycobacteria (eg pJEM15, pMV261, pVV16, pPR27). These vectors contain both an *E. coli* and mycobacterial origin of replication (from the pUC cloning vector series and plasmid pAL5000, respectively) and antibiotic resistance markers (eg kanamycin and gentamycin). Only antibiotic resistances that are already present in very widely used bacterial cloning vectors will be exploited. Some of the vectors also contain the mycobacterial hsp60 promoter, the reporter genes lacZ (encoding B-galactosidase), phoA (encoding alkaline phosphatase) and gfp (encoding green fluorescent protein) or the counter-selective marker sacB (causing sucrose sensitivity to mycobacteria).

#### Origin & function

Only DNA sequences encoding non-toxic products of known function will be inserted into the vectors for introduction into *M. bovis* BCG. These products will be involved in the synthesis of cell wall components (eg. B-keto-acyl synthase and mycolyl transferase) and/or contribute to basic cellular physiology (eg essential metal ion homeostasis). The latter will include metal transporting P-type ATPases and SmtB-related metal-sensor proteins that occur in a wide range of bacteria, including non-pathogenic strains.

All sequences will be well characterised prior to introduction into *M. bovis* BCG and will be generated by PCR using specific primers designed to amplify known DNA sequences (no random sequences will be introduced). The DNA sequences will include defined sequences, amplified by PCR, from *M. tuberculosis* (ACDP hazard group 3) DNA which has been provided by Colorado State University (USA) through the Tuberculosis Research Materials Contract of The National Institutes of Health and *M. smegmatis* (ACDP hazard group 1) DNA. The sequence of the *M. tuberculosis* genome is complete and contains no toxin-life genes.
Introduced DNA sequences will either (i) remain on self replicating plasmids to drive reporter gene expression or for expression from the mycobacterial hsp60 promoter, or (ii) be used to direct allelic replacement mutagenesis and hence generate gene deletion mutants of M. bovis BCG.

Evaluation of foreseeable effects

M. bovis BCG is a widely used live attenuated vaccine strain used to protect against tuberculosis. Staff working on this programme have previously been immunised with BCG. We will only introduce very short (<4kb) DNA sequences into M. bovis BCG using well characterised harmless vectors. Introduced DNA will be well defined genes (or parts of genes) that encode non-toxic cell wall components and that contribute to basic physiology (such as the sensing and transport of essential metal ions). It is therefore very unlikely that these sequences would restore virulence to M. bovis BCG which has numerous unlinked genetic alterations. Only non-mobilisable vectors will be used to introduce DNA sequences and hence it is highly unlikely that these sequences will be transferred to another organism. For reasons outlined above, the likelihood of harm in the event of exposure would be no greater than that with non-GM M. bovis BCG.

Environment

It is difficult to envisage how either the vectors or the inserts could confer a harmful phenotype or competitive advantage to M. bovis BCG. The environmental hazards associated with the project are therefore considered no greater than those associated with handling non-GM M. bovis BCG. The project involves small-scale work for research purposes and all contaminated material will be completely inactivated by autoclaving prior to disposal. In the highly unlikely event of release into the environment no risks are envisaged.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste materials (bacterial liquid cultures/Agar plates) will be completely inactivated (100% kill) by autoclaving: All autoclave runs are validated by monitoring with a chart recorder and the autoclaves are validated annually by Thermocouple testing. All solid waste will subsequently be incinerated.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Approval Granted

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms
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<thead>
<tr>
<th>Project Ref</th>
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<td>Date Ackn'd</td>
<td>20/02/2001</td>
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<td>EXPRESSION CLONING OF CDNAs ENCODING RECEPTORS FOR VITAMIN D OR PARATHYROID HORMONE, AND FOR THE VITAMIN D-24-HYDOXYLASE</td>
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**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 541/01.10

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Date Project Ceased

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 541/01.11

Date Ackn’d: 20/02/2001
CU2 Project Title:
RETROVIRAL PROPAGATION AND INFECTION OF MAMMALIAN CELLS

Class: Class 2
CultureVolClass2: 
CultureVolumeClass3-4: 
Non-GMM: not applicable
Consent Granted:
Project notified under transitional arrangements: Y

Withdrawn: N
Tick if notifying a connected programme of work: N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref** 541/01.12

**Date Ackn'd** 20/02/2001  
**CU2 Project Title** THE BIOSYNTHESIS OF EUKARYOTIC MEMBRANE PROTEINS

**Class** Class 2  
**CultureVolClass2**  
**CultureVolumeClass3-4**  
**Non-GMM** not applicable  
**Consent Granted**  
**Project notified under transitional arrangements** Y

**Withdrawn** N  
**Tick if notifying a connected programme of work** N

**Historical Significant Changes**  
**Historical Date of Additional Info**  
**Significant Change ID**  
**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  541/01.13

Date Ackn’d  20/02/2001
CU2 Project Title  STUDIES ON THE TRANSCRIPTION CONTROL OF GENE EXPRESSION IN
Class  Class 2
CultureVolClass2
CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

### Project Ref 541/01.14

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- Non-GMM Consent Granted: **not applicable**
- Project notified under transitional arrangements: **Y**

Withdrawn | N |
Tick if notifying a connected programme of work | N |
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
<th>Laboratory Activities</th>
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02/03/2022
Project Ref: 541/01.15

Date Ackn'd: 20/02/2001

CU2 Project Title:
IN VIVO EXPRESSION OF HUMAN CARTILAGE OLIGOMERIC MATRIX PROTEIN IN MAMMALIAN CELLS

Class: Class 2

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 541/01.16

Date Ackn'd 20/02/2001  CU2 Project Title CHARACTERISATION OF NOVEL GENES ENCODING EXTRACELLULAR MATRIX  Class 2  CultureVolClass2  CultureVolumeClass3-4

20/02/2001  CHARACTERISATION OF NOVEL GENES ENCODING EXTRACELLULAR MATRIX

Class 2

Page 8349 of 15326
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Project Ref  541/01.17

Date Ackn'd  20/02/2001

CU2 Project Title  MOLECULAR AND BIOCHEMICAL BASIS OF CHONDRODYSPLASIAS

Class  Class 2

Culture Vol Class 2

Culture Vol Class 3-4

Non-GMM  not applicable

Consent Granted

Project notified under transitional arrangements  Y

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

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02/03/2022
Project Ref: 541/01.18

Date Ackn'd: 20/02/2001

CU2 Project Title: AGGRECAN G3 DOMAIN ECPRESSION STUDIES

Class: Class 2

CultureVolClass2: Class Culture Volume 2

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

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Animal Units

Large Scale Activities

Human Clinical Applications

**Project Ref**  541/01.19

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AND EXTRACELLULAR MATRIX MOLECULES USING PLASMID-DERIVED VECTORS IN E.COLI HOSTS AND MAMMALIAN TISSUE CULTURE CELLS

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

N

Withdrawn

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Project Ref 541/01.2

Date Ackn’d 20/02/2001

CU2 Project Title

EXPRESSION AND FUNCTION OF P-GLYCOPROTEIN AND MULTIDRUG RESISTANCE PROTEIN IN THE HUMAN PLACENTA

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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If yes, tick to confirm that it is attached to this form N

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Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref**  541/01.20

**CU2 Project Title**
OVER EXPRESSION OF ANTIBODY FRAGMENTS IN ESCHERICHIA COLI

**Class**  Class 2

**Culture**

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**Non-GMM**
not applicable

**Consent Granted**

**Project notified under transitional arrangements**  Y

**Withdrawn**  N

**Tick if notifying a connected programme of work**  N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

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Animal Units

Large Scale Activities

Human Clinical Applications

**Project Ref 541/01.21**

Date Ackn'd: 20/02/2001
CU2 Project Title: CD105 PROMOTER ACTIVITY IN VASCULAR ENDOTHELIAL CELLS
Class: Class 2
CultureVolClass2: 
CultureVolumeClass3-4: 
**Date Project Ceased**

**Non-GMM Consent Granted**

- Project notified under transitional arrangements

**Withdrawn**

- Tick if notifying a connected programme of work

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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**Project Ref** 541/01.22

**Date Ackn’d**

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**Project notified under transitional arrangements**

| Y |

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

20/02/2001

**Date Project Ceased**

**Withdrawn**

| N |

**Tick if notifying a connected programme of work**

| N |

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

| N |

**Tick to confirm that it is attached to this form**

| N |

**Tick to confirm that you have attached a risk assessment to this form**

**Is an emergency plan required according to regulation 20?**

| N |

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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02/03/2022
### Project Ref 541/01.23

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- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

### Project Additional Information

#### Purposes of the contained use

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**
Evaluation of foreseeable effects

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**Project Ref**  541/01.24

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<th>IMMUNOMODULATORY MOLECULES FOR LONGTERM EXPRESSION OF TRANSGENES IN THE CENTRAL NERVOUS SYSTEM</th>
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**Historical Significant Changes**
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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**Project Ref 541/01.25**

Date Ackn’d: 20/02/2001

CU2 Project Title: GENERATION OF RECOMBINANT ADENOVIRAL VECTORS WITH REGULATED CELL-TYPE SPECIFIC EXPRESSION

Class: Class 2

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 541/01.27

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Project Ref** 541/01.28

- **Date Ackn'd**: 20/02/2001
- **CU2 Project Title**: GENETICALLY MODIFIED CANCER CELL LINES
- **Class**: Class 2
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

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If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022 Page 8372 of 15326
Project Ref 541/01.29

Date Ackn'd 20/02/2001

Date Project Ceased

CU2 Project Title PREVENTION OF ARTERIAL REMODELLING AFTER CORONARY ANGIOPLASTY IN A PORCINE MODEL

Class 2

Culture Vol Class 2

Culture Volume Class 3-4

Non-GMM not applicable

Consent Granted

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

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Project Ref 541/01.3

Date Ackn'd 20/02/2001  
CU2 Project Title REGULATION OF CYTOKINE GENE EXPRESSION  
Class 2  
CultureVolClass2  
CultureVolumeClass3-4
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Project notified under transitional arrangements

**Project Additional Information**

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

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- Animal Units: L2 L3 L4 L2 L3 L4 L2 L3 L4
- Large Scale Activities: L2 L3 L4 L2 L3 L4 L2 L3 L4
- Human Clinical Applications: L2 L3 L4

**Project Ref** 541/01.30

- Date Ackn’d: 21/02/2001
- Date Project Ceased: 05/02/2004
- Date of Significant Change: 02/03/2022

- Project notified under transitional arrangements: Y

**Project Title:** ANALYSIS OF PROLACTIN GENE REGULATORY ELEMENTS

- Class: Class 2
- CultureVolClass2: not applicable
- Consent Granted: not applicable

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

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02/03/2022
Project Ref: 541/01.31

Date Ackn'd: 21/02/2001

CU2 Project Title: GLUCOCORTICOID RECEPTOR FUNCTION IN PITUITARY AND OTHER TISSUES

Class: 2

CultureVolClass: 2

CultureVolumeClass: 3-4

Non-GMM: not applicable

Consent Granted: Y

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

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Project Ref 541/01.32

Date Ackn’dCU2 Project TitleClassCultureVolClass2CultureVolumeClass3-4
21/02/2001CLONING AND EXPRESSION OF MEMBRANE PROTEINS FROM THR HUMAN GI Class 2
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Project Ref** 541/01.33

**Date Ackn’d:** 21/02/2001

**CU2 Project Title:** GENERATION OF RECOMBINANT ADENOVIRUS EXPRESSING IMMUNE MODULATORY MOLECULES FOR LONG TERM EXPRESSION OF TRANSGENE IN THE CENTRAL NERVOUS SYSTEM

**Class:** Class 2

**Culture Vol Class:**

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**Non-GMM:** not applicable

**Project notified under transitional arrangements:** Y

**Withdrawn:** N

**Tick if notifying a connected programme of work:** N

**Historical Significant Changes**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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02/03/2022

Page 8382 of 15326
Project Ref: 541/01.34

Date Ackn'd: 21/02/2001

CU2 Project Title: GENERATION OF RECOMBINANT ADENOVIRUSES EXPRESSING IMMUNE STIMULATORY MOLECULES (FU3, MDC) FOR USE IN AN IMMUNOTHERAPEUTIC APPROACH FOR THE TREATMENT OF SOLID TUMOURS

Class: Class 2

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Project Additional Information:

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Project Ref** 541/01.35

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Date Project Ceased: INSENSITIVITY SYNDROMES

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

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Project Ref 541/01.36

Date Ackn’d 21/02/2001

CU2 Project Title HUMAN PROLG1-1 PEPTIDE PRODUCTION IN VITRO AND IN VITRO: RELEVANCE TO IGF-1 ACTION

Class 2

Non-GMM Not applicable

Consent Granted

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

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Host/vector system

Origin & function

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02/03/2022
Project Ref 541/01.37

Date Ackn'd 21/02/2001
Date Project Ceased 05/02/2004

CU2 Project Title GENETICALLY-ENGINEERED EUKARYOTIC CELL-LINES: THEIR USE TO GENERATE PROPROTEINS AND RELATED ENDOPROTEOLYTIC PRODUCTS

Class 2
Consent Granted not applicable

Non-GMM

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

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Project Ref 541/01.38

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

21/02/2001 USE OF GROWTH FACTORS TO PREVENT NEURONAL DEGENERATION Class 2

02/03/2022 Page 8389 of 15326
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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### Project Ref  541/01.39

- **Date Ackn’d**: 21/02/2001
- **CU2 Project Title**: GENE THERAPY FOR THE LESCH NYHAM SYNDROME
- **Class**: Class 2
- **CultureVolClass2**: not applicable
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**
- **Historical Date of Additional Info**: 05/02/2004
- **Significant Change ID**: Withdrawn

**Date of Significant Change**: 02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

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<th>Growth Rooms</th>
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### Project Additional Information

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 541/01.40

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### Project Additional Information

**Purposes of the contained use**

- *Recipient or parental organism*

- *Host/vector system*

- *Origin & function*

- *Evaluation of foreseeable effects*

- *Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)*

- *For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)*

- *Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)*
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 541/01.41

**Date Ackn’d** 21/02/2001

**CU2 Project Title**

GENERATION OF DEFICIENT ADENOVIRUSES EXPRESSING THERAPEUTIC PROTEINS AND/OR IMMUNOMODULATORY MOLECULES UNDER CELL TYPE SPECIFICALLY FOR LONG TERM GENE EXPRESSION IN THE BRAIN AND PITUITARY

**Class** Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

Consent Granted not applicable

Project notified under transitional arrangements Y

**Withdrawn** N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
**Project Ref** 541/01.42

**Date Ackn'd** 20/02/2001

**CU2 Project Title** GENERATION OF RECOMBINANT ADENOVIRUS EXPRESSING THE BACTERIAL ENZYME CARBOXYPEPTIDASE G2 (CPG2) IN EITHER A SECRETED OR MEMBRANE ANCHORED FORM FOR EXPRESSION IN THE CNS

**Class** Class 2

**Culture** Culture Volume Class 2

**Class 3-4**

**Non-GMM Consent Granted** not applicable

**Project notified under transitional arrangements** Y

**Withdrawn** N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 541/01.43

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Project Ref 541/01.44

Date Ackn’d 21/02/2001

Date Project Ceased 05/02/2004

CU2 Project Title
GENERATION OF RECOMBINANT ADENOVIRUS EXPRESSING GLI FOR THE TREATMENT OF PARKINSON'S DISEASE

Class CultureVolClass2 CultureVolumeClass3-4
Class 2

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
**Project Additional Information**

1. **Purposes of the contained use**

2. **Recipient or parental organism**

3. **Host/vector system**

4. **Origin & function**

5. **Evaluation of foreseeable effects**

   - Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

   - For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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**Is an emergency plan required according to regulation 20?** [N]

**If yes, tick to confirm that it is attached to this form** [N]

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**Tick if you are claiming exemption from disclosure for section of the risk assessment** [N]

**Please enter comments on the GM safety committee on the risk assessment**

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**Project Containment**

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02/03/2022
THE ROLE OF NMDA RECEPTOR SUBUNITS IN DEVELOPMENT AND PLASTICITY OF THE BARREL CORTEX

Date Ackn’d: 21/02/2001

Date Project Ceased: 05/02/2004

Class: Class 2

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Please enter comments on the GM safety committee on the risk assessment

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Project Ref 541/01.46

Date Ackn'd 21/02/2001

CU2 Project Title CONSTRUCTION OF A RECOMBINANT HERPES VIRUS VECTOR CONTAINING A

Class 2

CultureVolClass2

CultureVolumeClass3-4

02/03/2022
CELL TYPE SPECIFIC INDUCIBLE PROMOTER FOR THE TREATMENT OF GLIOBLASTOMA

Date Project Ceased: 05/02/2004

Project notified under transitional arrangements: Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Please enter comments on the GM safety committee on the risk assessment

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Animal Units
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Project Ref 541/01.47

Date Ackn’d 21/02/2001
Date Project Ceased 05/02/2004

USE OF IL-1 RECEPTOR TYPE II AND A P65 MUTANT TO INHIBIT THE INNATE IMMUNE RESPONSE TO ADENOVIRAL VECTORS

Class CultureVolClass2 CultureVolumeClass3-4
Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022 Page 8407 of 15326
PHARMACOLOGICAL MODULATION OF HETEROLOGOUSLY EXPRESSED ION CHANNELS, PUMPS AND GAP JUNCTIONS
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Date Project Ceased

Tick if notifying a connected programme of work

Withdrawn

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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- **Large Scale Activities**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Human Clinical Applications**: L2 L3 L4 L2 L3 L4 L2 L3 L4

**Project Ref**: 541/01.5

- **Date Ackn’d**: 20/02/2001
- **CU2 Project Title**: MOLECULAR MECHANISMS OF MAMMARY GLAND FUNCTION
- **Class**: Class 2
- **Culture Class**: Class CultureVolClass2 CultureVolumeClass3-4
- **Non-GMM**: Consent Granted: not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**

- **Historical Date of Additional Info**: Withdrawn: N
- **Date of Significant Change**:
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

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Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
**Project Ref** 541/01.50

Date Ackn'd 21/02/2001

**MU2 Project Title**

MOLECULAR MECHANISMS GOVERNING NORMAL AND ABERRANT HAEMATOPOIESIS

Date Project Ceased

**Class** Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

Non-GMM not applicable

Consent Granted

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Withdrawn N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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Animal Units

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<th>Project Ref</th>
<th>541/01.52</th>
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**Project Title**

GENERATION OF RECOMBINANT ADENOVIRUS VECTORS EXPRESSING REDUCTASE ENZYMES SUCH AS P450 REDUCTASE FOR THE ACTIVATION OF PRODRUGS TO CYTOTOXIC PRODUCTS FOR THE TREATMENT OF SOLID TUMOURS

**Date Ackn’d**

21/02/2001

**Class**

Class 2

**Non-GMM**

Consent Granted: not applicable

**Date Project Ceased**

Withdrawn: N

**Tick if notifying a connected programme of work**

N

**Project notified under transitional arrangements**

Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 541/01.53

THE USE OF HUMAN CYTOCHROME P450 2B6 AND NADPH : CYTOCHROME C(450) REDUCTASE GENES IN A RETROVIRAL VECTOR MEDIATED GENE THERAPY STRATEGY FOR THE TREATMENT OF CANCER

Class 2

Consent Granted

not applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

N

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

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Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref  541/01.54

Date Ackn’d  21/02/2001  CU2 Project Title  EXPLOITING EUKARYOTIC INITIATION FACTOR 4E OVEREXPRESSION TO  Class  2  CultureVolClass2  Class 2  CultureVolumeClass3-4
ENHANCE THE AMPLITUDE OF THERAPEUTIC GENE TRANSLATION BEARING 5'-UTR SEQUENCES FROM HUMAN VEGF GENE

Date Project Ceased: 05/02/2004

Tick if notifying a connected programme of work: N

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 541/01.55

Date Ackn’d: 21/02/2001

Date Project Ceased:

CU2 Project Title:
EFFECT OF HYPOXIA ON GENE EXPRESSION AND ANGIOGENESIS IN HUMAN AND MURINE CELLS IN VITRO AND AS SOLID TUMOURS GROWN IN VIVO

Class

Class 2

Consent Granted

not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 541/01.56

Date Ackn'd 21/02/2001

Date Project Ceased

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
**Evaluation of foreseeable effects**

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [N]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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**Project Ref**  541/01.57

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<td>USE OF CTLA4LG AND CD28 PROTEIN TO SUPPRESS IMMUNE RESPONSES</td>
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TO RECOMBINANT ADENOVIRUSES

Date Project Ceased: 05/02/2004

Non-GMM Consent Granted: not applicable

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 541/01.58

Date Ackn’d: 10/08/2001

CU2 Project Title: GENETIC MANIPULATION OF TRYPANOSOMA BRUCEI SPP. INCLUDING T.B. RHODESIENSE, T.BRUCEI, T.GAMBIENSE

Date Project Ceased: 01/04/2004

Class CultureVol: Class 3

CultureVolumeClass: up to 2 Litres

Non-GMM Consent Granted: yes

Tick if notifying a connected programme of work N

Historical Date of Additional Info: Project transferred to GM207 from 01/04/2004

Historical Significant Changes: Project transferred to GM207 from 01/04/2004

Significant Change ID: 541/01.58a

Date of Significant Change: 17/09/2007
**Project Additional Information**

**Purposes of the contained use**

Identified proteins enriched in particular stages of the trypanosome life cycle will be expressed or their genes manipulated in cultured insect or bloodstream forms of the parasite. This will allow investigation of the development of Trypanosoma brucei spp. through its life cycle at the cellular and molecular level. Our aim is to improve understanding of the function and regulation of these molecules, which it is expected will be clarified by these experiments. This may identify strategies to control the transmission of these parasites in Africa.

**Recipient or parental organism**

GMO: Cultured insect or bloodstream forms of the parasite. Insect forms are non-pathogenic, non-transmissible. Bloodstream forms require tsetse fly transmission. These are only indigenous in Africa. No tsetse fly colonies are maintained at the University of Manchester. Trypanosome do not produce toxins and we expect most genetic manipulation of the parasite to reduce fitness and therefore virulence.

**Host/vector system**

Parental organism: Trypanosoma brucei spp. blood and insect forms

Vector system: pGEM or pBluescript based bacterial shuttle vectors engineered to enable parasite gene expression or modulation in the parent organism using a tetracycline regulated inducible expression system. These are non mobilisable shuttle vectors. Specific vectors are pET series, pGem, pGemT-easy, pLew100, pZJM, pBluescript ll, p2T7, pH5D41 and pH430

**Origin & function**

1. T.brucei spp. genes or proteins displaying specificity, enrichment or implicated in the involvement in particular life cycle stages of the parasite. Their function is expected to be in progression of the parasite through its developmental cycle and, potentially, parasite metabolism.
2. Reporter gene expression to allow selection of transgenic parasites or detection of the expression pattern or location of those proteins described in (1). This will include genes for resistance to antibiotics used for parasite selection and green fluorescent protein gene fusions for protein localisation.

**Evaluation of foreseeable effects**

Where gene expression of particular molecules implicated in life cycle regulation are expressed we anticipate differentiation phenotypes to be observed. Specifically we will use markers for each life cycle stage to detect reduced ability to initiate differentiation, enhanced differentiation rates of failure in the programme of differentiation events at particular points. Transgenic parasites harbouring regulatory molecules with specific mutations to enable the isolation of interacting partner molecules (for example substrate-trapping mutants) or which generate altered differentiation phenotype will be used for protein isolation or examined for altered pattern of gene/protein expression. In each case transgenic bloodstream and insect form parasites will be used and differentiation between these stages examined. However, it is not possible to differentiate parasites from the insect form back to the bloodstream form in the absence of the tsetse fly vector. Therefore the life cycle cannot be completed in the laboratory. These genes will be expressed using a tetracycline inducible expression system. Therefore, in the absence of tetracycline the genes are effectively silenced.

Antibiotic resistance genes used for selection of transgenic parasites are not those used for clinical treatment of the parasite and will otherwise have no foreseeable effect. Reporter genes (e.g. green fluorescent protein) are for localisation and assay of expression pattern for particular parasite genes or sequences. There is no foreseeable consequence of this.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

T. brucei spp. does not produce spores. They are rapidly killed outside of the culture vessel or blood by desiccation osmotic shock (washing with water, detergents) and cannot penetrate unbroken skin. Parasite genes are expressed or perturbed using a tetracycline inducible system and will be effectively silenced outwith the culture vessel.
Tsetse flies are required for transmission and completion of the life cycle; these are indigenous only in sub Saharan Africa. Thus the parasites, whilst mammal infective as the bloodstream forms are effectively deficient in mobilisation capacity.

The wild type bloodstream form parasite is mammal infective and virulent. It is highly unlikely that genetic modification will enhance this; rather gene manipulation is likely to reduce virulence and transmissibility.

We request derogation from Containment class III to containment class II for specific containment measures.

CONTAINMENT MEASURE:
1. Isolation. The laboratory is separate from other laboratories in the building being fully contained within a locked laboratory, with access restricted to authorised lab personnel.
2. Laboratory sealable for fumigation. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSHH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for sealability for fumigation.
3. Entry via airlock. Trypanosomes can only be transmitted by blood-blood contact or by skin puncture. They cannot survive in air and do not form spores. We request derogation as risk assessment does not indicate a requirement for this measure.
4. Negative pressure. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSHH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for airflow.
5. Filtration of extract and input air. Trypanosomes do not form spores and cannot undergo airborne transmission. Handling of bloodstream form parasites is most commonly as in vitro cultured forms. These are opened only in a microbiological safety cabinet with HEPA filtration. We request derogation to level 2.
6. Microbiological safety cabinet. Trypanosomes do not form spores and cannot undergo airborne transmission. Handling of bloodstream form parasites is most commonly as in vitro cultured forms although purification of parasites from blood is not practical in a safety cabinet. However there is no generation of aerosols. The laboratory is self contained providing containment. We request derogation to level 2.
7. Autoclave in the laboratory suite. There is a central sterilisation facility in the Stopform building, but no autoclave facility within the laboratory. However, all parasites, and material that has come into contact with parasites is rendered non-infective by soaking in 2% Virkon for 12h prior to transport to the autoclave facility. Transport is in sealed containers. Therefore we request derogation to level 2.
11. Shower. Trypanosomes are only transmitted by blood-blood contact or by inoculation. Skin penetration of the naked parasite is not possible. Parasites cannot survive in the external environment. Therefore we request derogation to level 2.

REQUEST FOR DEROGATION OF ANIMAL FACILITIES
The School of Biological Sciences BSU operates as a first class animal containment facility. Laboratory rodents infected with Trypanosoma brucei spp. will be contained in accordance with the regulations defined for Containment level III. However we request derogation to containment level II for specific regulations:

1. Isolation. The BSU is separate from other laboratories in the building being fully contained within a locked laboratory, with access restricted to authorised BSU personnel.
2. Laboratory sealable for fumigation. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSHH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for sealability for fumigation because trypanosomes do not produce spores, cannot be transmitted in an airborne form, or by aerosol. Blood-blood contact is required.
4. Entry via airlock. Trypanosomes can only be transmitted by blood-blood contact or by skin puncture. They cannot survive in air and do not form spores. We request derogation as risk assessment does not indicate a requirement for this measure.
5. Negative pressure. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSHH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for airflow.
6. Filtration of air. Trypanosomes do not form spores and cannot undergo airborne transmission. Transmission can occur only by blood-blood contact or inoculation. Standard operating procedures minimise risk of aerosol. We request derogation to containment level II with respect to HEPA filtration of the animal enclosure.
7. Microbiological safety cabinet. Trypanosomes do not form spores and cannot undergo airborne transmission. Operating procedures limit the possibility of aerosol. The
BSU is self contained providing containment. We request derogation to level 2.

11. Shower. Trypanosomes are only transmitted by blood-blood contact or by inoculation. Skin penetration of the naked parasite is not possible. Parasites cannot survive in the external environment. Therefore we request derogation to level 2.

14. Control of disease vectors. Trypanosomes can only complete their life cycle in the tsetse fly. These are not indigenous in the UK, or outside sub Saharan Africa. There are no tsetse flies maintained at the University of Manchester.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

A laboratory scale project employing good microbiological practice and good occupational safety and hygiene.

Disenfection with 2% aqueous Virkon solution according to University Policy and Guidance document. Material that has come into contact with parasites is inactivated by soaking in hypochlorite (1:500) for 24h.

Liquid waste and solid waste (other than sharps and animal waste) is sterilised in an autoclave maintained and validated by the manufacturer's service organisation for the destruction of clinical waste prior to transfer by a licensed operator to a commercial incinerator site authorised to treat GM waste. Sharps and animal waste are transferred directly to the incinerator for destruction.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GM committee, at a meeting held on 29.4.97, endorsed the decision of the local GM committee (dated 16.4.97) and the two outside experts from other local committees that this project requires Containment Level 2 facilities that are available in laboratories 2.681 and 2.682 of the Stopford Building. No special problems were identified that could not be contained by Good Microbiological Practice. It was noted that Group II organisms were involved and that HSE approval was required before work could start.

**Project Containment**

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Genes encoding proteins likely to be important in controlling cell shape, motility, metabolism and nuclear organisation of the African trypanosome will be modified, expressed or manipulated in cultured insect or blood stream forms of the parasite. Our aim is to study fundamental aspects of parasite molecular cell biology - in particular, cell differentiation, cell motility, mitosis and control of antigenic variation. Overall, we believe that these experiments will assist in providing insight to basic parasite biology and to pathogenicity mechanisms. Given the emerging T.brucei spp. sequence information, these experiments may identify strategies and targets important for future control measures of these parasites.

GMO: Cultured insect or bloodstream forms of the parasite. Insect forms are non-pathogenic and non-transmissible. Bloodstream forms require tsetse fly transmission. These are only indigenous to Africa. There are no tsetse fly colonies maintained at the University of Manchester.

Trypanosomes do not produce toxins and we expect most genetic manipulation of the parasite to reduce fitness and therefore virulence.

Parental organism: T.brucei spp blood and insect forms.
Vector system: Non-mobilisable shuttle vectors engineered to enable parasite gene expression or modulation in the parent organisms using a tetracycline regulated inducible expression system.
Vectors used: pET series, pGem, pGem T-easy, pLew100, pZJM, pBluescript II.
1. T. brucei spp. genes or proteins displaying specificity, enrichment or implicated in the involvement in particular life-cycle stages of the parasite. Their function is expected to be in differentiation, motility, cytoskeleton, metabolism or nuclear organisation.

2. Reporter gene expression to allow selection of transgenic parasites or detection of the expression pattern or location of these proteins described in (1). This will include genes for resistance to antibiotics used for parasite selection and green fluorescent protein gene fusions for protein localisation.

**Evaluation of foreseeable effects**

We expect to see phenotypes dependent upon the specific situation. These will range from paralysis (flagellum motility); abnormal shape/division defects (mitotic and cytoskeletal proteins); antigenic coat abnormalities (nuclear regulatory proteins); general growth phenotypes (metabolic proteins). Often, the outcome will be a lethal phenotype particularly in initial gene experiments using RNA interference. In many cases, transgenic bloodstream and insect form parasites will be used and differentiation between these stages examined. However, it is not possible to differentiate parasites from the insect form back to the bloodstream form in the absence of the tsetse fly vector. Therefore, the lifecycle cannot be completed in the laboratory. These genes will be expressed using a tetracycline inducible expression system. Therefore, in the absence of tetracycline, the genes are effectively silenced.

Antibiotic resistance genes will be used for selection of transgenic parasites are not those used for clinical treatment of the parasite and will otherwise have no foreseeable effect.

Reporter genes (e.g., green fluorescent protein and epitope-tagged proteins) are for localisation and assay of expression pattern for particular parasite genes or sequences. There is no foreseeable consequence of this.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

T. brucei spp. does not produce spores. They are rapidly killed outside of the culture vessel or blood by desiccation osmotic shock (washing with water, detergents) and cannot penetrate unbroken skin. Parasite genes are expressed or perturbed using a tetracycline inducible system and will be effectively silenced outwith the culture vessel. Tsetse flies are required for transmission and completion of the life cycle; these are indigenous only in sub Saharan Africa. Thus the parasites, whilst mammal infective as the bloodstream forms are effectively deficient in mobilisation capacity.

The wild type bloodstream form parasite is mammal infective and virulent. It is highly unlikely that genetic modification will enhance this; rather gene manipulation is likely to reduce virulence and transmissibility.

We request derogation from Containment class III to containment class II for specific containment measures.

**CONTAINMENT MEASURE:**

1. Isolation. The laboratory is separate from other laboratories in the building being fully contained within a locked laboratory, with access restricted to authorised lab personnel.
2. Laboratory sealable for fumigation. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSSH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for sealability for fumigation.
3. Entry via airlock. Trypanosomes can only be transmitted by blood-blood contact or by skin puncture. They cannot survive in air and do not form spores. We request derogation as risk assessment does not indicate a requirement for this measure.
4. Negative pressure. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSSH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for airflow.
5. Filtration of extract and input air. Trypanosomes do not form spores and cannot undergo airborne transmission. Handling of bloodstream form parasites is most commonly as in vitro cultured forms. These are opened only in a microbiological safety cabinet with HEPA filtration. We request derogation to level 2.
6. Microbiological safety cabinet. Trypanosomes do not form spores and cannot undergo airborne transmission. Handling of bloodstream form parasites is most
commonly as in vitro cultured forms although purification of parasites from blood is not practical in a safety cabinet. However there is no generation of aerosols. The laboratory is self contained providing containment. We request derogation to level 2.

8. Autoclave in the laboratory suite. There is an autoclave in the laboratory for sterilisation of small volume waste. Most sterilisation is carried out within the central sterilisation facility in the Stopford building. However, all parasites, and material that has come into contact with parasites is rendered non-infective by soaking in 2% Virkon for 12h prior to transport to the autoclave facility. Transport is in sealed containers. Therefore we request derogation to level 2.

11. Shower. Trypanosomes are only transmitted by blood:blood contact or by inoculation. Skin penetration of the naked parasite is not possible. Parasites cannot survive in the external environment. Therefore we request derogation to level 2.

REQUEST FOR DEROGATION OF ANIMAL FACILITIES

The School of Biological Sciences BSU operates as a first class animal containment facility. Laboratory rodents infected with Trypanosoma brucei spp. will be contained in accordance with the regulations defined for Containment level III. However we request derogation to containment level II for specific regulations:

1. Isolation. The BSU is separate from other laboratories in the building being fully contained within a locked laboratory, with access restricted to authorised BSU personnel.

2. Laboratory sealable for fumigation. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSSH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for sealability for fumigation because trypanosomes do not produce spores, cannot be transmitted in an airborne form, or by aerosol. Blood: blood contact is required.

4. Entry via airlock. Trypanosomes can only be transmitted by blood: blood contact or by skin puncture. They cannot survive in air and do not form spores. We request derogation as risk assessment does not indicate a requirement for this measure.

5. Negative pressure. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSSH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for airflow.

6. Filtration of air. Trypanosomes do not form spores and cannot undergo airborne transmission. Transmission can occur only by blood-blood contact or inoculation. Standard operating procedures minimise risk of aerosol. We request derogation to containment level II with respect to HEPA filtration of the animal enclosure.

7. Microbiological safety cabinet. Trypanosomes do not form spores and cannot undergo airborne transmission. Operating procedures limit the possibility of aerosol. The BSU is self contained providing containment. We request derogation to level 2.

11. Shower. Trypanosomes are only transmitted by blood-blood contact or by inoculation. Skin penetration of the naked parasite is not possible. Parasites cannot survive in the external environment. Therefore we request derogation to level 2.

14. Control of disease vectors. Trypanosomes can only complete their life cycle in the tsetse fly. These are not indigenous in the UK, or outside sub Saharan Africa. There are no tsetse flies maintained at the University of Manchester.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

A laboratory scale project employing good microbiological practice and good occupational safety and hygiene.

Disinfection with 2% aqueous Virkon solution according to University Policy and Guidance document.

Liquid waste and solid waste (other than sharps and animal waste) is sterilised in an autoclave maintained and validated by the manufacturer's service organisation for the destruction of clinical waste prior to transfer by a licensed operator to a commercial incinerator site authorised to treat GM waste. Sharps and animal waste are transferred directly to the incinerator for destruction.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y
On 6.9.2000, the local GM committee considered a risk assessment submitted as a 5-yearly review of this project. The two outside experts from other local committees had questioned whether the two laboratory-attenuated strains (427 and 927) of Trypanosoma brucei brucei could be considered as specially disabled. However it was confirmed that the strains could not survive outside the culture medium and that their ‘procyclic’ forms, exclusively used in this work, were not infective to either mammals or rodents. Nevertheless, the fact that these organisms are categorised by ACDP as Hazard Group 2 requires that the experiments be performed under Containment Level 2 conditions. This decision was endorsed by the University GM committee at a meeting held on 11.10.2000.

Subsequently, in view of the fact that the range of properties of strains of organisms classified as Trypanosoma brucei brucei is not clearly differentiated from the range of properties of strains classified as Trypanosoma brucei rhodesiense, (a member of Hazard Group 3) it was decided to apply for approval of this work as a class 2 activity, with derogation as described in section 11.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref** 541/01.6

**CU2 Project Title**

APOPTOSIS GENES IN MAMMARY GLAND

**Date Ackn’d**

20/02/2001

**Date Project Ceased**

**Class**

Class 2

**CultureVolClass2**

Consent Granted

**Consent Granted**

not applicable

**Project notified under transitional arrangements**

Y

**Tick if notifying a connected programme of work**

N

**Historical Significant Changes**

Withdrawn

N

**Date of Significant Change**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022  
Page 8434 of 15326
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**Project Ref 541/01.7**

**Date Ackn'd**
20/02/2001

**CU2 Project Title**
ANALYSIS OF STRUCTURE, FUNCTION AND ASSEMBLY OF DESMOSOMAL GLYCOPROTEINS

**Class**
Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM**
not applicable

**Consent Granted**

**Tick if notifying a connected programme of work**
N

**Project notified under transitional arrangements**
Y

**Withdrawn**
N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref  541/01.8

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Date Project Ceased

Tick if notifying a connected programme of work

Withdrawn

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 541/01.9

Date Ackn’d: 20/02/2001

CU2 Project Title: DEVELOPMENT OF A RETROVIRAL MEDIATED TRANSFECTION ASSAY TO DISSECT THE MITOTIC CHECKPOINT

Class: Class 2

Culture Vol Class 2: not applicable

Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Adenoviral vectors expressing interferon alpha, endostatin, angiostatin or antisense CD105 mRNA will be developed by co-transfection of the shuttle vector and plasmid pJM17 into the embryonic kidney 293 cells. Transduction efficiency of the vectors will be first tested on endothelial, breast cancer and smooth muscle cells in vitro followed by their application in mouse models of angiogenesis and restenosis. Inhibition of CD105 in the endothelial cells by the antisense CD105 vector may lead to inhibition of angiogenesis and hence the regression of the tumour. Selective vascular expression of the interferon alpha, endostatin or angiostatin driven by the CD105 promoter will suppress angiogenesis and tumour growth. Application of these vectors in a mouse restenosis model may also inhibit the formation of neo-intima.

Recombinant adenoviruses with deletions in the E1 and E3 regions generated by homologous recombination between the shuttle vector pMV60 and plasmid pJM17 are replication deficient. The viruses will be used to infect cultured endothelial and smooth muscle cells and finally applied in mouse models of angiogenesis and restenosis. The viral vectors will be able to inhibit angiogenesis and tumour growth and reduce the formation of neo-intima in the mouse models.
Hosts: mouse vascular endothelial cells and smooth muscle cells; human vascular endothelial cells and smooth muscle cells; MCF-7 breast cancer cells; 293 embryonic kidney cells.

Vector: recombinant adenovirus type 5.

Origin & function

The vectors (Ad 5) will be generated by cotransfection of plasmid pJM17 and the shuttle vector pMV60 into 293 embryonic kidney cells. Plasmid pJM17 contains the genome of Ad 5 with deletions in the E1 and E3 regions. The antisense Ad 5 for CD105 may act as an inhibitor of CD105 gene expression in endothelial cells and smooth muscle cells. The Ad5/IFNalpha, Ad5/endostatin and Ad5/angiostatin may function as inhibitors of angiogenesis, tumour growth and enointima formation.

Evaluation of foreseeable effects

CD105 is a gene expressed in vascular endothelial cells and smooth muscle cells and regulates TGFB signalling. The antisense Ad 5 will be constructed to express a small fragment (< 1 kb) of CD105 antisense mRNA. The antisense Ad 5 may be able to down-regulate CD105 gene expression in the cultured endothelial and smooth muscle cells and the mouse models. Since CD105 is pro-angiogenic and correlated with tumour progression, down-regulation of CD105 expression is likely to suppress angiogenesis and tumour growth. Endostatin and angiostatin are natural peptides which are present in the body. The two peptides are known to inhibit tumour angiogenesis. The Ad5/endostatin and Ad5/angiostatin will exhibit antitumour effects when applied in the mouse tumour models. IFNalpha is used for tumour therapy, but it possesses various side-effects. The Ad5/IFNalpha driven by the CD105 promoter will restrict the expression of IFNalpha to the vasculature only, thus reduce its non-specific effects on other tissues. IFNa side-effects include fever, malaise and myalgia.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the culture media, washing solutions such as PBS, used plasticware, eg tissue culture flasks, pipettes and tips will be inactivated in a validated autoclave within the building. The mouse carcasses will be bagged in 800 gauge yellow bags, labelled and transported to a licensed incinerator. These processes of waste treatment will give 100% kill. The individuals who perform this project will receive regular medical check up.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GM Committee met on 13 September 2001 and agreed that this application would be satisfactory provided that certain changes (now incorporated into the application) were made. Reasons for arriving at conclusions were to be given, and particular alternative was to be given to assessing the risk of experiments employing the human interferon gene. Such changes have now been made to the satisfaction of the Chair of the committee.
The genes for a number of proteins involved in sulfonate and sulfate ester metabolism in Pseudomonas putida, Pseudomonas aeruginosa, and E. coli have been cloned and studied in the laboratory. The project involves molecular genetic, biochemical and physiological approaches to examine the role of these genes and their products in Pseudomonas species, with special emphasis on their relevance in the soil sulfur cycle.

The organisms to be transformed are Pseudomonas putida S-313 (Zurrer et al (87) Appl. Env. Microbiol. 53:1459-1463) and Pseudomonas aeruginosa PA01 (Holloway (55) J. Gen. Micro. 13:572-581). P. putida strains are non-pathogenic (ACGM, Annex II), whereas P. aeruginosa is an opportunistic pathogen that attacks...
Host systems used for the cloning experiments are E. coli K-12 derivatives (strains DH5α, JM109, XL1-Blue), which are disabled strains (ACGM, annex II). Expression experiments will be carried out in E. coli BL21(DE3) (Annex II), and gene transfer to pseudomonads will be done with strain S17-1 (hsdR thi recA mob+ pro) (Simon et al (83) Bio/Technology 1:784-791). Vector systems to be used include non mobilisable vectors of the pUC series and pBluescript (Annex II), expression vectors of the pET series (Novagen) and mobilisable vectors of the pUCP series (West et al (94) Gene 148:81-86) and the pBBR series (Kovach et al. (95) Gene 166:175-176). The mobilisable vectors used are not self-mobilisable, but rely on the presence of mob genes which have been integrated into the host chromosome to enable conjugative transfer. The host used in this case, strain S17-1, is auxotrophic for proline, and therefore unable to survive outside the laboratory. Other mobilisable vectors used include those the pKNOCK series (Alexayev, 1999, Biotechniques 26:824-828) and pUT series (De Lorenzo et al., 1994. Meth. Enzymol. 235:388-405), both of which contain the R6Kα replicon, and can only replicate in hosts containing the pir gene.

Origin & function

The genetic material involved in the study comprises genes involved in sulfur metabolism in soil pseudomonads (Pseudomonas putida, Pseudomonas aeruginosa). These genes encode enzymes for the desulfurisation of sulfonates and sulfate esters, and the transport systems required for uptake of the substrates into the cell. The presence of additional copies of the sulfonatase and sulfatase genes, or their deletion, is not expected to give a survival advantage to the strains used.

Evaluation of foreseeable effects

The presence of additional copies of the sulfonatase and sulfatase genes, or their deletion, is not expected to give a survival advantage either to the Pseudomonas strains used or to the disabled hosts used as intermediates in the cloning experiments.

Pseudomonas aeruginosa is a class 2 pathogen, and appropriate measures are required for its containment. The strain used (strain PA01) has been in laboratory cultivation for many years and is thought to display reduced virulence, but this is not experimentally proven or documented, and cannot be relied on.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be disposed of using a validated autoclave facility within the building. Solid waste will be disposed of using a licensed incinerator for genetically modified organisms. Small laboratory waste (pipette tips, Eppendorf tubes etc) will be immediately treated with disinfectant (Virkon) as required for Class 2 organisms, and subsequently incinerated in the GM-licensed incinerator.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

(a) E. coli BL21 is a problem strain. Its genotype does not indicate it is either auxotrophic or recombination-deficient. ACGM Compendium annex II para 12 indicates it is not a K12 or B strain derivative. Apparently this is in error, and it is known to be a B strain. It recommends an access factor of 10⁻³.

(b) E. coli S17-1 is clearly disabled, but what is the significance of mob+? This is normally part of a plasmid, does its presence in the host chromosome have significance for plasmid mobilisation?

(c) The overall risk assessment of 10-15 is correct and would normally warrant containment level 1 as proposed here. However, P. aeruginosa remains a Category 2 pathogen unless the proposer can provide convincing evidence that the incorporation of sulfonatases etc. render it otherwise. Therefore, this part of the proposal requires containment level 2 facilities to be consistent with its hazard group.

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Animal Units

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Project Ref 541/06.1

- Date Ackn'd: 04/01/2006
- CU2 Project Title: The use of recombinant Salmonella to examine gene function and expression.
- Class: Class 2
- CultureVolClass2: < 1 Litre
- Non-GMM: Not Applicable

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

The objectives of this project are to understand the biology of Salmonella, gain insights into the mechanism by which they cause disease, and develop more effective vaccines. This will involve cloning, expression and disrupting Salmonella genes. This research will be conducted using plasmids in disabled E. coli K12 hosts, and also re-introducing cloned DNA sequences back into strains of Salmonella.

Recipient or parental organism

E. Coli K12 strains (incl. DH5alpha, JM101, JM109, SURE & S17-1 lambda-pir), are disabled hosts, ACGM 2000 part 2A, annex II. Well characterised attenuated Salmonella hosts: Derivatives of WT S. typhimurium (SL3261), S. enteritidis (Se795aroA) & S. typhi (541Ty) attenuated by lesions in genes of the aromatic amino acid biosynthetic pathway, in the stress-response gene htrA, or both. They carry the same attenuations as disabled strains listed in ACGM 2000, part 2A, annex II. The S. typhi strains have been shown to be innocuous in humans and are considered disabled. The only WT hosts to be used are the well-studied S. typhimurium strains SL1344 and C5 (ACDP HG 2).

Host/vector system

Cloning & expression vectors: All derived from those listed in the ACGM 2000, part 2A, annex II as 'non mobilisable' (eg pUC series, pGEM series & derivatives) or 'mobilisation defective' (pBR322 & derivatives). Gene disruption vectors: Suicide vector pFUSE (Baumier et al., 1996) carries the mob region of RP4 allowing it to be mobilised by the transfer functions provided by an RP4 derivative integrated into the donor cell chromosome (E. coli S17-1 lambda-pir). It cannot to replicate in recipient cells lacking the essential pir gene, or be mobilised if the recipient cell provides RP4 transfer functions; hence is unable to replicate in/mobilised from the Salmonella strains.

Origin & function

Only DNA sequences constituting genes or parts of genes from Salmonella or E. coli that encode non-toxic products of known function will be inserted into the vectors for introduction into Salmonella. These products will contribute to basic cellular physiology such as essential metal ion homeostasis (including metal-transporting P-type ATPases and SmtB/MerR-related metal-sensing proteins). Introduced DNA will be expressed either individually as full-length proteins or sub-regions, and/or as fusions to other genes (within the vector) encoding non-toxic products, including the reporter genes lacZ (encoding beta-galactosidase) and gfp (encoding green fluorescent protein from jelly fish).

All sequences will be well characterised prior to introduction into Salmonella strains and will be generated by PCR using specific primers designed to amplify known DNA sequences (no random sequences will be introduced).

Introduced DNA sequences will either i) remain on self-replicating plasmids to drive reporter gene expression or for expression of genes from their natural promoter or from vector promoters, or ii) be integrated into the genome to generate gene disruption mutants of Salmonella.

Evaluation of foreseeable effects

Human health:

The attenuated Salmonella strains employed are specifically designed to be given as oral vaccines. They can survive in and colonise the host gut sufficiently to induce an immune response however the ability to colonise the host is very dose dependent, and requires large numbers. There are no recorded instances of aro or htr mutants spreading between hosts. The wild-type S. typhimurium strains to be employed are of the type which could cause food poisoning if ingested in a large dose. These strains have been safely used for 20 years and the likelihood of hazard can therefore be considered low. We will only introduce very short (<4 kb) DNA sequences into Salmonella using well characterised harmless vectors. Introduced DNA will be well defined genes (or parts of genes) that encode non-toxic products that contribute to basic physiology (such as the sensing and transport of essential metal ions).
For reasons outlined above, the likelihood of harm in the event of exposure would be no greater than that with non-GM Salmonella and can be considered negligible.

Environment:
The disabled host strains will not replicate in the environment. Infections doses for man and animals are high and the probability of accidental infection low. The host strains are not known to be infectious for plants. The wild-type S. typhimurium strains may be able to replicate in the environment. The chance of transfer of the vectors to other organisms in the environment can be considered low. It is difficult to envisage how any of the genetic manipulations proposed in this project could confer a harmful phenotype or competitive advantage to the host strains. The environmental hazards associated with the project are therefore considered no greater than those associated with handling non-GM Salmonella. The project involves small-scale work for research purposes and all contaminated material will be completely inactivated by autoclaving prior to disposal. In the highly unlikely event of release into the environment no risks are envisaged.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All contaminated waste materials (bacterial liquid cultures/agar plates) will be completely inactivated (100% kill) by autoclaving: All autoclave runs are validated by monitoring with a chart recorder and the autoclaves are validated annually by thermocouple testing. All solid waste will subsequently be incinerated.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The committee agreed that they represent realistic assessments of the risks involved, and that the facilities available and the experience of the proposers are adequate to control these risks. It was emphasised that the personnel involved must undergo Health Surveillance screening before work commences.

Project Containment

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Project Title: Investigating gene function in the bacterium Campylobacter jejuni.

Purposes of the contained use:
The aims of the work are to determine the role of individual gene products in Campylobacter jejuni in order to understand how this organism colonises animals and causes disease in humans. Findings will facilitate design of strategies to reduce illness caused by the organism. The work will involve cloning, expression and disruption of C. jejuni genes.

Recipient or parental organism:
Escherichia coli BL21 and K12 strains and derivatives.
Campylobacter jejuni commonly used laboratory strains: NCTC 11168, NCTC 81128, 81176

Host/vector system:
Cloning and expression vectors: All derived from those listed in the ACGM 2000, part 2A, annex II as 'non mobilisable' (eg pUC series, pGEM series & derivatives) or 'mobilisation defective' (pET)
Gene disruption vectors: Suicide vectors unable to replicate in recipient campylobacter cells.

Origin & function:
Only specific Campylobacter genes or gene fragments will be inserted into vectors - no random sequences will be introduced.
Campylobacter genes or gene fragments will be cloned in Escherichia coli, disrupted through insertion of antibiotic resistance cassettes and reintroduced into Campylobacter strains whereupon through homologous recombination the disrupted non-functional gene will replace the chromosomal functional gene.
Full length functional Campylobacter genes will be cloned into Escherichia coli and reintroduced into Campylobacter strains constructed as immediately above to restore a functional gene. Again the full length functional Campylobacter gene will be recombined onto the Campylobacter chromosome.

Full length genes will be cloned into Escherichia coli and expressed as full-length proteins and as fusions to other genes (within the vector) encoding non-toxic products on plasmid vectors.

**Evaluation of foreseeable effects**

**Human health:**
Expression of Campylobacter genes in Escherichia coli is unlikely to render such disabled strains more harmful.

The wild type Campylobacter strains could potentially cause gastrointestinal illness if ingested. The strains employed are well characterised laboratory strains and have been used world wide for 20 years and therefore with good microbiological practice the likelihood of hazard is low. The gene knockouts constructed will only disable strains and complementation of gene knockouts can only restore potential hazards to wild type levels. Therefore the likelihood of harm in the event of exposure is no greater than with non-GM Campylobacter and can be considered negligible.

**Environment**
The disabled host strains will not replicate in the environment. Infections doses for man and animals are high and the probability of accidental infection low. The host strains are not known to be infectious for plants. The wild-type Campylobacter strains may be able to replicate in the environment. The chance of transfer of the vectors to other organisms in the environment can be considered low. It is difficult to envisage how any of the genetic manipulations proposed in this project could confer a harmful phenotype or competitive advantage to the host strains. The environmental hazards associated with the project are therefore considered no greater than those associated with handling non-GM Campylobacter. The project involves small-scale work for research purposes and all contaminated material will be completely inactivated by autoclaving prior to disposal. In the highly unlikely event of release into the environment no risks are envisaged.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
All contaminated waste materials (bacterial liquid cultures/agar plates) will be completely inactivated (100% kill) by autoclaving: All autoclave runs are validated by monitoring with a chart recorder and the autoclaves are validated annually by thermocouple testing. All solid waste will subsequently be incinerated.

**Is an emergency plan required according to regulation 20?** No

**If yes, tick to confirm that it is attached to this form** No

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment** No
The committee agreed that they represent realistic assessments of the risks involved, and that the facilities available and the experience of the proposers are adequate to control these risks. It was emphasised that the personnel involved must undergo Health Surveillance screening before work commences.

### Project Containment

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**Project Ref** 541/06.3

- **Date Ackn’d**: 20/04/2006
- **CU2 Project Title**: The use of recombinant fungi to study allergen function.

**Project Additional Information**

**Purposes of the contained use**

The objectives of this project are to understand the biology of Aspergillus and to gain insights into how allergen proteins produced by this fungus work. In this instance allergenicity genes may also have a function in virulence. This will involve cloning and knockout of allergen and/or virulence genes from Aspergillus and expression of allergen proteins in small amounts in auxotrophic strains of Pichia pastoris. Gene knockout experiments involve cloning fungal genes in disabled E. coli K12 derivatives and reintroduction of DNA back into Aspergillus.
Recipient or parental organism

Escherichia coli K12 strains (incl DH5alpha, JM101, JM109, SURE & S17-1 lambda-pir), in the ACGM 2000 disabled hosts category, part 2A, annex II.
Well characterised Pichia pastoris His Arg auxotrophs
Laboratory strains of Aspergillus fumigatus (Af293, CEA10) (ACDP Hazard Group 2), A nidulans (Glasgow strains) and A. clavatus (NRRL1) (both ACDP Hazard group 1).

Host/vector system

Cloning and expression vectors: All derived from those listed in the ACGM 2000, part 2A, annex II as 'non-mobilisable' (eg pUC series, pGEM series & derivatives) or 'mobilisation defective' (pBR322 & derivatives). plCz alpha series plasmids for P. pastoris (Invitrogen) - non-mobilisable shuttle vector for E. coli and P. pastoris.
All vectors used for Aspergillus are integrative and based on E. coli vectors with no fungal origin of replication.

Origin & function

Only DNA sequences constituting genes or parts of genes from Aspergillus will be inserted into vectors. Allergen coding sequences (taken from the IUIS approved list of Aspergillus fumigatus allergens) will be cloned into Pichia expression vectors in E. coli before being transferred to Pichia. Flanking regions of allergen or selected known virulence genes with no coding sequence will be placed either side of a selectable marker (either Aspergillus pyrG or dominant selectable marker Hygromycin resistance from pCB1004) by PCR and transformed into Aspergillus. Avirulent and non-allergic strains of Aspergillus will be generated by gene knockout.

All sequences will be well characterised prior to introduction into Aspergillus or Pichia strains and will be generated by PCR from genomic DNA or total RNA using specific primers designed to amplify known DNA sequences (no random sequences will be introduced).

Introduced DNA sequences will either (I) remain on self-replicating plasmids to drive reporter gene expression or for expression of genes from their natural promoter or from vector promoters, or (ii) be integrated into the genome to generate gene disruption mutants of Aspergillus.

Evaluation of foreseeable effects

Human Health:
Aspergillus fumigatus is a known pathogen of immunocompromised individuals. It is also capable of being an allergen. However A. fumigatus spores are extremely common in the environment and respiratory exposure to this organisms is almost constant. These strains of A. fumigatus have been used safely in the laboratory for many years and there are no recorded instances of fungal infection resulting from this work during this time. The modifications to the organism proposed in this project would reduce allergenicity and/or virulence and thus the risk of harm due to exposure to organisms would be no greater than with non-GM Aspergillus and can be considered negligible. In the unlikely event that gene knockout causes increased virulence (as assessed by growth rate and sporulation in the first instance) live cultures will be destroyed, a small spore stock retained at -80 and HSE will be informed of the existence of the strain. GM strains produced in this project should pose no extra threat to immunocompromised individuals or women of childbearing age who are expecting a child or planning a pregnancy - exposure to wild-type Aspergillus is almost constant in the natural environment and strains produced in this project should be disabled with respect to virulence and pathogenicity.

Environment:
The disabled host strains will not replicate in the environment. Infection of immunocompetent individuals is extremely unlikely and the probability of accidental infection almost zero. The chance of transfer of the vectors to other organisms in the environment can be considered low. It is difficult to envisage how any of the genetic manipulations proposed in this project could confer a harmful phenotype or competitive advantage to the host strains. The environmental hazards associated with the project are therefore considered no greater than those associated with handling non-GM Aspergillus. The project involves small-scale work for research purposes and all contaminated material will be completely inactivated by overnight treatment with Virkonp, autoclaving and incineration prior to disposal. In the highly unlikely event of release into the environment no risks are envisaged.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste materials (bacterial liquid cultures/agar plates) will be completely inactivated (100% kill) by autoclaving: All autoclave runs are validated by monitoring with a chart recorder and the autoclaves are validated annually by thermocouple testing. All liquid cultures are treated overnight with 2% Virkon prior to autoclaving. All solid waste will subsequently be incinerated.

All staff will be trained in handling sporulating fungi. GM Aspergillus will be treated with especial caution to reduce any airborne spore production - The GM strains will be handled exclusively as liquid cultures unless spore production is required for storage. Sporulation will be limited on solid media by limiting culture time to 24h at 37 degrees C and 48 h at 30 degrees C. Where sporulation is needed to produce stocks culture will be undertaken in narrow necked tissue culture flasks to limit spore escape. Flasks and other potentially sporulating cultures will be handled in a class 2 hood equipped with a UV light (in tissue culture room, ATR5) and the UV will be switched on for 1h after sporulating cultures have been opened. All work undertaken in type 2 safety cabinet according to the SOPs in Appendix 6. No culture larger than 200 ml used. All liquid waste sterilised using PreSept tablets prior to autoclaving at 121 degrees C for 30 mins. All solid waste autoclaved as above. Conveyance of culture sin double containment unbreakable sealed containers. All cultures specifically marked as “GM”. Spills to be mopped up with 10% bleach then 2% Virkon and waste autoclaved as GM waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee approved the application subject to provision of the following additional information.
1. Procedures to cover increased pathogenicity and methods for the control of spore formation.
2. Negative information, particularly where the project would have no known effect on pregnancy, childbirth and on immunocompromised individuals.

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Project Ref 541/07.1
The project is to investigate the mechanisms by which regulatory proteins control the activities of stress-activated signalling pathways. cDNAs encoding mammalian protein kinases, regulatory proteins and transcription factors will be expressed in mammalian cells. Plasmids expressing siRNAi will also be used to knock down the expression of endogenous proteins in mammalian cells and to determine their function in order to obtain high transfection efficiencies we will use lentiviral and adenoviral vectors.

The recipient of the GMO’s will be established human, mouse, and rat cell lines (including HEK293, HeLa, N1E-115, NIH3T3, PC12 H1299, HepG2, INS1, U20S, Rin5F, C2C12) and primary cultures of rodent cells including neurones, myocytes, embryonic fibroblasts and embryonic stem cells.

Adenoviral expression vector pAdEasy will be used for protein expression in mammalian cells. Lentiviral Vectors (Pwpi, pLVTHM, pLKO.1) will be for protein and siRNA expression in mammalian cells. All vectors are replication incompetent. The HEK293 (human) cell line is used to generate the adenoviruses and lentiviruses.

The adenovirus features deletion of the complete E1 region and the majority of the E3 region that constitutes the early transcription portion of the genome. The E1 region allows the virus to replicate while the E3 region prevents proper function of the MHC class 1 proteins so that the hosts immune response to the virus is compromised. The virus is therefore replication incompetent and only grows in complementing cells such as HEK293 that contain the appropriate E1 sequences. The lentiviral vectors used contain deletions in their 3’ UTR which results in self-inactivation of the lentivirus after transduction of the target cells. The number of genes from HIV-1 that are used in the system has been reduced to four (gag, pol, rev, tat) and all viral auxiliary genes associated with virulence (e.g. vpr, vif, vpu and nef) have been deleted. The VSV-G gene from Vesicular Stomatis Virus is used in place of the HIV-1 envelope. The lentiviral particles produced in this system are replication-incompetent and only carry the gene
of interest. The genes encoding structural and other components required for packaging the viral genome are separated into three plasmids which have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus. None of the HIV-1 structural genes are present in the packaged viral genome, and thus, are not expressed in the target cells.

The adenoviral and lentiviral vectors will be used to express protein under the control of a constitutive CMV and E1Flpha promoters (pAdEasy, pWPI, pLVTMMH). Human, mouse or rat cDNAs encoding protein kinases e.g. JNK, ERK, p38, PKA, CAMK, S6K, c-Abl, PKB, TOR, ULK1; regulatory proteins e.g. JIPs, Arrestin, Ser1, TRB, eIFs; and transcription factors e.g Jun, ATF2, p73, Elk-1, will be cloned into the vectors. Exceptions are bacterial Cre and beta-galactosidase and GFP from Aequoria Victoria. Beta-galactosidase and green-fluorescent protein (GFP) will be used as reporter genes. Cre recombinase will be used to excise segments of genes that have been flanked by loxP recombinase sites. Protein kinases transfer phosphate groups to substrates. Regulatory proteins bind to protein kinases and regulate their activities. Transcription factors bind gene promoters and regulate transcription. The c-Abl, JNK and p38 pathways are pro-apoptotic in response to stress stimuli but in some cell types can promote proliferation of differentiation. The ERK and mTOR pathways promote cell growth, while ERK, PKB and PKA can signal cell survival. Wild-type and mutant signalling proteins will be expressed to examine loss and gain of functions.

Lentiviral vectors (pLVTHM, pLKO.1) featuring RNA polymerase III promoters H1 and U6 will be used to express siRNAs in cells. siRNA is generated by the cellular machinery from a short hairpin RNA that is encoded by a small DNA insert in the vector. siRNA will be targeted to knock-down the expression of the signaling proteins described above.

Evaluation of foreseeable effects

Potential laboratory accidents would include ingestion and droplet exposure to mucous membranes (e.g. splashes in eye or mouth, direct injection). The viruses can transduce human cells but they are replication defective so will not colonise the host. The expressed proteins and siRNAs are biologically active and will affect signalling pathways involved in cell growth, differentiation and apoptosis. While not themselves oncogenic they may promote tumorigenesis. In addition some of the lentiviral vectors include the Woodchuck WPRE to enhance transgene expression. This is reported to promote tumorigenesis. All experimental procedures are carried out in a Class II laminar flow biological safety cabinet, therefore the chance of transmission in a Laboratory accident is low. In addition, tumorigenesis is a complex multifactor process that involves more than one oncogenic event.

The lentivirus includes the VSV-G protein that may potentially allow infection of the natural hosts of VSV, which include livestock. VSV-G may also stabilise the virus. However, the laboratory is in the city centre and there are no livestock nearby.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Good laboratory practice will be used including use of gloves and labcoats. All work will be performed in a Class II laminar flow biological safety cabinet in a tissue culture lab that is separated from the main lab. It will be communicated to all users of the tissue culture lab that Class 2 activity is taking place. Clearly labelled incubators will be used to culture the lentivirus infected cells. Liquid waste is soaked in 2% Virkon for at least 30 minutes prior to disposal down the sink. The viruses are rapidly inactivated after exposure to chemical germicides such as Virkon. Solid waste is removed daily from the laboratory and is incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
This application has been fully discussed at two local GM safety committee meetings. The classification of the project as a Class II rather than Class I was fully discussed at the first meeting and due to the use of lentiviruses and the nature of the genes of interest it was decided that Class II classification was appropriate. The nature of the inserts, host vector systems, likelihood of harmful effects and potential stabilization of the virus were all fully explored. Amendments to the first draft recommended at the initial meeting were subsequently discussed at the second meeting when the committee approved the project and HSE notification.

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Project Ref 541/07.2

Date Ackn’d | CU2 Project Title
---|---
19/03/2007 | Modulation of gene expression towards analysis of effects of extracellular matrix organisation and cell fate, using gene delivery systems.

Date Project Ceased

Class | CultureVolClass2 | CultureVolumeClass3-4
-----|------------------|-------------------
Class 2 | < 1 Litre        | Not Applicable

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use
To retrovirally transduce human, guinea pig, and rat cells with genes or shRNA oligonucleotides.

Recipient or parental organism
Human primary and established cell lines, such as mesenchymal stem cells (MSCs), smooth muscle cells (SMCs), human dermal fibroblasts (HDFs), and retinal pigmented epithelial cells (ARPE-19).
Rat established cell lines, such as rat foetal lung cells (RFL-6). Guinea pig primary cells, such as MSCs.
Retrovirus derived from Moloney murine leukaemia virus (MMLV) and murine stem cell virus (MSCV).

Host/vector system
Vectors: Commercially available retroviral and lentiviral self-inactivating vectore package into transducible viral particles by specific eukaryotic packaging cells. The lentiviral and retroviral expression vectors do not contain sequences required for virus packaging. Genes for packaging are provided by transient transfection of helper vectors or the packaging cell line.

Origin & function
Genetic material - subcloned from cDNA library, for example:
ERG (ets-related gene) and isoforms. From mouse kidney.
HDAC (histone deacetylase) family members. From human brain.
FGFR (fibroblast growth factor receptor) family members. From mouse brain.

Intended functions: to regulate differentiation of MSCs.

Genetic material - Human total RNA by RT-PCR, for example:
Elastin
Fibulin family members
Fibrillin family members

Intended functions: Recombinant expression for use in structural and functional assays.

Genetic material - Commercially synthesised oligonucleotides to form shRNA complexes to, for example:
Fibulin family members
Fibrillin family members

Intended functions: Downregulation of protein expression to monitor role of protein in ECM assembly and cell differentiation.

Evaluation of foreseeable effects
The majority of the gene products expressed have no known deleterious effects although some do have oncogenic properties. The split function of the viral vectors however greatly reduces the risk of recombination events occurring and the self-activating properties of the vectors themselves inhibit production of virus once the transgene becomes stably incorporated into the genome of the target cells, eliminating the risk of undesired infection. Therefore it is highly unlikely that replication competent virus will be produced. Good laboratory practice in accordance with the risk assessment of the experimental procedure will ensure that the worker or others within the laboratory will not be exposed to the virus.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Spillages to be cleaned with 1% Virkon. |
| All liquid waste to be soaked in 1% Virkon for 24 hours. |
| All solid waste to be autoclaved. |
| The area to be cleaned with 70% ethanol afterwards. |
| It is anticipated that this will give 100% kill. |

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All three risk assessments (GAW2, CMK2, CMK3) were initially considered at local GM safety committee meeting on 12/01/07. In addition to some minor amendments that were completed during the meeting, the committee requested clarification on the known or suspected physiological, pathological and/or pharmacological effects of the shRNA complexes from the fibulin and fibrillin families of proteins in the risk assessment CMK2.

This has now been answered fully in section 1.7, p47 of CMK2. All three risk assessments were approved in a supplementary meeting of the local GM committee meeting on 07/02/07.

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02/03/2022
Project Ref 541/07.3

Date Ackn'd
04/09/2007

CU2 Project Title
Improving the scope of bone marrow transplantation for metabolic and related disease.

Date Project Ceased

Class
2

CultureVolClass2
1-50 Litres

CultureVolumeClass3-4

Non-GMM
Not Applicable

Consent Granted

Tick if notifying a connected programme of work
Y

Withdrawn
N

Historical Significant Changes
Project notified under transitional arrangements
N

Historical Date of Additional Info

Significant Change ID
541/07.3a

Date of Significant Change
30/06/2010

Project Additional Information

Purposes of the contained use
The project will investigate methods to develop gene delivery strategies using self-inactivating HIV-1 based lentiviral vectors in mouse models of lysosomal storage disorders to a) improve stem cell engraftment, homing and enzyme delivery and b) reduce cellular substrate storage to improve phenotype.

Recipient or parental organism
Mice used in this study are wild type, heterozygous or mutant for mucopolysaccharide disease (MPS) type I, II, IIla and IIlb and metachromatic leukodystrophy MLD. Other mice for use in xenogenic transplantation studies of human stem cells will be immunodeficient NOD/SCID/gammaC chain (IL2R) -/- or SCID/Rag2 -/- /C3R -/- mouse strains, designed to be able to develop a partly functional human immune system. See 8. and A in additional comments.

Host/vector system
Hosts: Mammalian cells: human and mouse primary cells isolated from bone marrow (endothelial, mesenchymal and haematopoietic stem cells) and other cell types from eg. mouse skin, heart, and liver HeLa and HEK293 T, HUVECs, Jurkant, U937, HL60, microglial cells. Mice (see above)
Vector System: Disabled replication incompetent lentiviral vectors.

Origin & function
The inserted genes in the lentiviral vectors are either full length alpha-L-iduronidase, iduronate sulfatase, sulphamidase, alpha-N-acetylglucosaminidase, arylsulfatase (lysosomal enzymes) or factor VIII and factor IX. The lysosomal enzymes are deficient in different lysosomal storage diseases and are involved in the breakdown of the glycosaminoglycans dermatan sulphate and heparan sulphate. Factor VIII and factor IX are clotting factors which will
be used as markers. The transgenes used in this project occur naturally in mammalian cells and are not known to have any harmful physiological or pharmacological properties nor affect host or human defence mechanisms.

Lentiviral vectors containing short (23mer) antisense sequences of the genes encoding dermatan sulphate and heparan sulphate proteoglycans, CD26 and CXCR4 will also be used. Down-regulation of these genes would not have any harmful physiological or pharmacological properties nor affect host or human defence mechanisms.

eGFP (enhanced green fluorescent protein) or LacZ are used as markers to assess transduction efficiency of the lentiviral vectors. They are not known to have any harmful physiological or pharmacological properties nor affect host or human defence mechanisms.

2) Lentiviral vectors

A number of methods are employed to make lentiviral vectors non-mobilisable. The viral components are partitioned onto 3 (2nd generation) or 4 (3rd generation) plasmids with little or no shared homology to limit the risk of recombination. This ensures that there is no risk of replication competence beyond a single transfection. The HIV virulence genes are removed making the vector capable of a single infection but not of altering gene expression significantly as the wild-type virus does. The HIV specific envelope protein that targets CD4 T cells is exchanged for a ubiquitous envelope protein (VSV-G Vesicular stomatis virus G protein) which makes it more visible to the immune system and also expands the host range to mice, sheep and other mammals, although human cells remain the most permissive to transduction. Removal of the CD4 tropism removes the ability of lentiviral vectors to evade the immune system (normally achieved in wild-type virus by infecting and killing CD4 reactive T cells). Self inactivating long terminal repeats (LTR) are designed to minimise the risk of viral mobilisation from the chromosomal location once integrated and inactivates the viral LTR promoter at the same time.

**Evaluation of foreseeable effects**

Replication competent virus is a very small but potential risk. This is tested for by serial infections of HeLa cells with viral stocks, and two rounds of removal of supernatant and transfer to uninfected stocks after 48 hours. In addition other techniques can be employed such as Q-PCR.

The major risks of working with lentiviral vectors are associated with exposure to open wounds or by needle stick injury- A plaster should be placed over any open wound on the hands or lower arms even though all experiments are conducted with the appropriate PPE ie, gloves and a lab coat. Needle stick injury would only occur during animal inoculation. Any sharps or needles will not be used at any other stage in experiments involving lentiviral vectors — except when delivering vector to animals. Respiratory infection is effectively zero even when using high titre stocks. In addition, all experiments using lentiviral vectors will be conducted in a class II cabinet.

In the event of needle stick injury and the lentiviral vector was delivered into the hand then some cells in the skin would express the transgene. Some limited systemic spread is possible if delivered into a vein but large animal experiments have shown that even at the highest titres we intend to use, this will be relatively small. Also, the transgenes used in this project would not have harmful physiological or pharmacological properties nor affect host or human defence mechanisms. If skin cells express CFP for example (which is quite immunogenic) then it is likely that the host immune system will mount a T cell and humoral response which will slowly eradicate these cells to be replaced by others— as is seen in rodent and large animal experiments where vector is delivered to a few cells in a localised area. Insertional mutagenesis has been shown to be a small risk in retroviral vector systems, although lentiviral vectors appear to pose less of a risk to gene upregulation than retroviral vectors as they preferentially integrate into the 5' region of active genes and are thus most likely to abrogate gene expression.

Lentiviral vector half-life (the time taken for half of the lentiviral vector to become incapable of infection) at 37°C is less than 30 mins and less than 24h at 4°C

See A in additional comments.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Viral vector transfected cells or animals receiving direct injections of viral vectors are not likely to pose a risk to users as the vectors are only capable of a single integration event and have been shown by RCR testing to be incapable of mobilisation from their site of integration. The main risk is from needle stick injury during injection. All staff will use appropriate PPE, animals will be anaesthetised during this procedure and staff appropriately trained in this procedure. Waste disposal will be carried out according to the SOP for waste disposal attached. In brief, needles will be disposed of in sharps bins, any directly contaminated material disposed of by autoclaving (on the central site) followed by the local clinical waste route (incineration) or sink in the case of liquid waste. Carcasses will be disposed of by incineration.

There is a small risk of mice receiving either transfected or untransfected human cells being able to harbour and transmit infections to humans. These models have been shown to be poor at propagating human pathogens in this manner. The major route if infection would be blood/blood contact as it is the blood system that is reconstituted with human cells. All these mice will be kept in SPF environments within IVC cage& They will only be handled by users with appropriate PPE, and workers will be notified of their increased pathogenic risk. Animals will be anaesthetised when withdrawing blood and staff appropriately trained in the procedure. In the event of transmission of
Infection to a human from a humanised mouse, this would be likely to be nothing more serious than the risk of working in a hospital. Hence workers may be at risk of contracting blood borne infections as mouse lung epithelia will not be humanised thus minimising risk of airborne infections such as cold or influenza viru&

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

[All class II waste on the production site will be treated with 2% virkon overnight prior to disposal via clinical waste route (incineration) or sink for liquid waste. This method is appropriate for 100% inactivation of viral vector and cell killing.

On the central site, GM contaminated solid waste will be autoclaved prior to incineration. Liquid waste will be treated with 2% virkon overnight prior to disposal via the sink. All GM animals will be bagged and incinerated according to local procedures for waste disposal. See SOP for waste disposal attached.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GM waste is to be autoclaved within the Stopford building following relocation of the project onto the main campus.

On the production site - Lentiviral vector waste (eg culture media, flasks, pipettes will be decontaminated in at least 2% virkon solution (final concentration) overnight (16hours) and then incinerated to give 100% kill at the site where viral vector production is undertaken.

The small amount of lentiviral vector waste generated by injections at the central site will be disposed of by autoclaving where appropriate prior to disposal via incineration to give 100% kill. GM animal carcasses and products will be disposed of by incineration. See SOP for waste disposal.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The committee agreed with the GM classification of the project and Level I work was approved.

There were several points raised concerning various aspects of the Level 2 work detailed in the proposal. Namely;

• waste management
• needle-stick injury risk
• disablement characteristics
• health surveillance requirements

The principal applicant was requested to amend the proposal according to the suggestions made by the committee.

Project Containment
Project Ref 541/07.4

CU2 Project Title
Modulation of cell signalling pathways to analyse their effect on cellular properties such as proliferation, apoptosis, polarity, migration, adhesion and fate, using gene delivery systems.

Class CultureVolume
Class 2 1-50 Litres

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID 541/07.4a
Date of Significant Change 11/08/2014

Project Additional Information

Purposes of the contained use

virally transduce human, mouse, and rat cells with genes, microRNAs or shRNA oligonucleotides.

Recipient or parental organism

Human primary and established cell lines, such as primary ductal carcinoma in situ derived breast epithelial cells, breast cancer cell lines (T 47D, SK BR 3, BT 474, Hs 578T, CAL51, MDA MB 468, BT 549, MDA MB 231, PMC 42). We will not be using any lymphocytic cell lines to minimise the risk of replication competent retrovirus being generated.

Mouse primary and established cell lines, such as mouse mammary epithelial cell lines (FSK7, HC1 1, C57MG) and mouse preaipocyte cell line (3T3;L1). Rat established cell lines, such as rat mammary epithelial cell line (RAC3I 1). Retrovirus derived from Moloney murine leukaemia virus (MMLV). Adenovirus (Ads). Lentivirus derived from HIV1.

Host/vector system

Vectors: Commercially available adenoviral, retroviral and lentiviral self-inactivating vectors, and well established retroviral vectors (pLNCX and pBABE) packaged into
transducible viral particles by specific eukaryotic packaging cells. The lentiviral, adenoviral and retroviral expression vectors do not encode proteins required for virus packaging. Proteins for packaging are provided by transient transfection of helper vectors or the packaging cell line.

**Origin & function**

Genetic material - subcloned from cDNA library, for example: Components of the Notch signalling pathway (Delta, Jagged, Notch, RBP-kappa, Numb, Hes, Hey, Deltex, Su(dx)) — human, mouse and rat. Components of the Wnt signalling pathway (Wnt, Frizzled, LRPS&6, Dishevelled, Axin, GSK3beta, beta-catenin, TCF, Lef1) — human, mouse, rat and Xenopus. Intended functions: to regulate proliferation, apoptosis and differentiation of human breast cancer cells.

Genetic material - Commercially synthesised oligonucleotides to express microRNAs to, for example: Components of the Notch pathway (Delta, Jagged, Notch, RBP-kappa, Numb, Hes, Hey, Deltex, Su(dx)). Components of the Wnt pathway (Wnt, Frizzled, LRPS&6, Dishevelled, Axin, GSK3beta, beta-catenin, TCF, Lef1). Intended functions: down regulation of protein expression to alter proliferation, apoptosis and differentiation in human breast cancer cells.

Genetic material - Commercially synthesised oligonucleotides to form shRNA complexes to, for example: Bcl-2 family members. Intended functions: downregulation of protein expression to monitor role of proteins in apoptosis. Genetic material - subcloned from complexes to, for example: IAP family members - human. Intended functions: to regulate apoptosis in human breast cancer cells. Genetic material - subcloned from cDNA library for example components of the spindle assembly checkpoint (BunM, BubR1, Mpsl, CenpF) and Histone H2B - human, mouse. Intended functions: to regulate cell division of human cell lines.

Genetic material - subcloned from cDNA library, for example: Genes within the adhesion signalling pathway regulating apoptosis, focal adhesion kinase and downstream components of the associated signalling cascade (PI3_Kinase, protein kinase b, the MAPKinases Jnk and p38) and apoptotic Bcl-2 proteins - human, mouse and bovine origin. Intended functions: to regulate apoptosis.

**Evaluation of foreseeable effects**

The majority of the gene products expressed are expected to affect cellular properties such as proliferation, apoptosis, migration, adhesion, polarity and fate. Also most have known or suspected oncogenic or tumour suppressor properties. The split function of the viral vectors however greatly reduces the risk of recombination events occurring and the self-inactivating properties of the vectors themselves inhibit production of virus once the transgene becomes stably incorporated into the genome of the target cells, eliminating the risk of undesired infection. Therefore it is highly unlikely that replication competent virus will be produced. Good laboratory practice in accordance with the risk assessment of the experimental procedure will ensure that the worker or others within the laboratory will not be exposed to the virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Spillages to be cleaned with 1% virkon.
All liquid waste to be soaked in 1% virkon overnight.
All solid waste to be autoclaved.
The area to be cleaned with 70% ethanol afterwards
It is anticipated that this will give 100% kill.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N
All five risk assessments (CHS4, CHS5, APG2, KRB I and SST1) were initially considered briefly at the local GM safety committee meeting on 22/06/2007 and in full at the meeting on 30/08/2007. At the second meeting the committee requested clarifications and amendments to all the assessments, in particular control measures, waste management and the biological activity of the inserted sequences were discussed at length. All five risk assessments were finally approved at a supplementary meeting of the local GM committee on 21/09/2007.

Project Containment

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Project Ref 541/07.5

Date Ackn'd 21/11/2007

CU2 Project Title
Genetic manipulation of embryonic and adult stem cell lines established and primary cell lines using viral and non-viral vectors.

Date Project Ceased

Class 2 CultureVol
1-50 Litres

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Gene transfer vectors will be used to deliver transgenic material (genes, siRNA and miRNA) to embryonic or adult stem cells in order to manipulate, analyse or promote phenotype, differentiation or immortalisation of such cells.

Recipient or parental organism

Human and mouse embryonic and adult stem cells (for example: mesenchymal stem cells, chondroprogenitors) and primary (for example: chondrocytes, fetal and adult fibroblasts) and established cell lines (for example; 293T, 3T3).

Human embryonic cells and tissues are obtained by donation after comprehensive pathogen screening at the clinic (HIV, Hepblc). Cell lines are also subjected to the same screening process once in the laboratory context. Human primary cells are donated from unscreened patients and are quaranteened pending the pathogen screening as described prior to genetic modification.

Retrovirus derived from Moloney murine leukaemia virus (MMLV) ACDP hazard group 2.

Adenovirus (Ad215) ACDP hazard group 2.

Adeno-associated Virus (AAV) ACDP hazard group 2.

Lentivirus derived from HIVISIV ACDP Hazard group I and EIAV IFIV ACDP hazard group I.

Host/vector system

Bacterial hosts derived from E.Coli strains DH5a, JM109, INVIO. Plasmid vectors derived from pcDNA3, pGL3, pENTRIA, etc.

Viral vectors- Recombinant lentiviral vectors based on HIV-I, EIAV, SIV, FIV pseudotyped with VSV-G, gp64, RRV, ENTV envelopes.

Retroviral vectors based on MLV, pseudotyped with MLVIVSV-G will be packaged by transient transfection in 293T cells.

Established adenoviral vectors (Ad215) will be produced by transient transfection of 293T (or derivative) cells.

AAV will be produced in an established transient 293T helper system.

Origin & function

Transgenic material - Human and mouse cDNAs of transcribed genes involved in aspects of transformation, self-renewal or differentiation will be expressed from pol II promoters. These genes would include transcription factors, signaling molecules, enzymes, growth factors and cytokines. Such genes would include: Transformation; hTERT, Bmi-I, Self-renewal; Oct4, Nanog, Sox2, Differentiation; FoxA2, Hnf4, GATA4, Sox9, FGF-2, FGF-1O, TGF-B. Only one potential oncogene will ever be expressed from a single cassette although reporter genes may also be expressed from bicistronic cassettes. These gene targets will also be subject to gene knockdown with siRNA and miRNA expressed from pol 111111 promoters. Reporter genes include; Flourescent- e.g. GFP, YFP, CFP, dsRed, Luminescent- e.g. firefly and renilla Luciferase, biochemical e.g. CAT, AFP. Mammalian promoter/enhancer elements will be used to drive reporter genes as previously described.

Evaluation of foreseeable effects

The recombinant viral vectors are all highly disabled vectors based on Lentivirus/reovirus: These self inactivating vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Whilst they pose an infection risk they are unable to initiate further rounds of replication/ infection cycles. The probability of seroconversion is minimal. Whilst the envelope pseudotyping extends the cellular tropism and confers greater stability viral vector will always be contained within a class II safety cabinet thus user exposure to liquid aerosol is impossible.

The exposure of non-human hosts to pseudotyped vector could result in initial infection but, again seroconversion is highly unlikely.

Adenoviral vectors are deleted of essential replication/packaging genes that are supplemented by an established packaging cell line (293T).

Adeno-associated Virus is defective by nature and has a replication disabled genome and is incapable of replication without helper adenovirus. In this instance the vectors used would require the provision of cap and rep genes in trans in order to replicate and disseminate. There is no significant chance of reversion to the wild-type.

Furthermore, the AAV8 & 5 subtypes derived from humans are not able to replicate or cause disease in any animal species therefore it highly unlikely that such a virus, if released into the environment, would represent a significant risk.
The majority of the gene products expressed are expected to affect cellular properties such as proliferation, apoptosis, migration, adhesion and fate. Also, most have known or suspected oncogenic or tumour suppressor properties. Gene products in plasmid form exist in non-mobilisable constructs and are therefore of minimal environmental risk. Viral vector expressing potential oncogenes are a potential environmental risk as host infection could elicit a transformation event. All work is carried out in a class II cabinet and all liquid waste inactivated by virkon treatment and solid waste autoclaved. Primary cells infected with viral vector containing potential oncogene would pose minimal threat as expression would most likely be restricted to the infected cell, which in itself would be rejected by the host immune system.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals will be used in this project at this site.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) are rendered inactive by autoclaving (100% kill) in a validated machine located in the same building. Subsequently the solid waste enters the clinical waste route and goes for incineration. Virkon is routinely used as per the manufacturer's recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations). The autoclave undergoes annual validation. Records are kept on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC assessed the project as GM Class 2. Some aspects of this work will be carried out in a clean room environment where there is a continual cascade of HEPA-filtered air under positive pressure. No aspect of viral production will be undertaken in the clean room only application of viral vectors to cells and as such there will be no possibility of generating a virus containing aerosol. All transfer of virus or virus transduced cells will be carried out using a secondary container from flow cabinet to incubator minimising the chances of spillage. Masks are worn at all times in the clean room which would avoid inhalation after spillage and any spill will be treated immediately with sterile biocide. This risk assessment was initially considered briefly at the local GM safety committee meeting on 22/01/2007 and in full at the meeting on 30/08/2007. At the second meeting the committee requested clarifications and amendments to all the assessments, in particular control measures, waste management and the biological activity of the inserted sequences were discussed at length. The risk assessment was finally approved at a supplementary meeting of the local GM committee on 21/09/2007.

Project Containment

02/03/2022
Project Ref 541/08.1

Date Ackn'd 08/04/2008

CU2 Project Title
Modulation of neural cell gene expression using viral gene delivery systems in vitro and in vivo to explore the neurophysiological processes involved in time-keeping (daily and seasonal,.....

Class
Class 2

CultureVol
≤ 1 Litre

Class Culture
Class2 Culture
Volume
Class3-4

Non-GMM

Consent Granted
Not Applicable

Project notified under transitional arrangements

Withdrew
N

Tick if notifying a connected programme of work
N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To virally transduce human and rodent cells and tissues with genes, microRNAs or shRNA oligonucleotides, both in vitro and in vivo.

Recipient or parental organism

In vitro work (primary and established cell lines, ex vivo tissues)
Established cell lines (HEK293, COS, AT120, SH-SYSY)
Primary cells will be isolated from rats, mice, and hamsters (for example, mouse fibroblasts and primary cortical neuronals).
Tissue slices will be isolated from rats, mice, and hamsters (for example, coronal brain slices through the hypothalamus or hippocampus).
In vivo work:
Extremely small volumes (nL-qL) of viral gene delivery particles introduced into specific tissues of rats, mice or hamsters with recovery and behaviour monitoring. Mice used for these experiments will either be commonly used laboratory strains (eg C57B6) or established transgenic lines housed at the University of Manchester (eg GPR5O knockout mice, 3xTg transgenic mice).
We will not be using any lymphocytic cell lines or tissues to minimise the risk of replication competent retrovirus being generated.

Viral delivery systems:
Retrovirus derived from Moloney murine leukaemia virus (MMLV).
Adenovirus (Ads).
Lentivirus derived from HIVI (3 generation, self-inactivating).

Host/vector system

Vectors: Commercially available adenoviral, retroviral and lentiviral self-inactivating vectors, and well established retroviral vectors (pLNCX and pBABE) packaged into transducible viral particles by specific eukaryotic packaging cells. The lentiviral, adenoviral and retroviral expression vectors do not encode proteins required for virus packaging. Proteins for packaging are provided by transient transfection of helper vectors or the packaging cell line.

Origin & function

Genetic material - subcloned from cDNA library:
Components of the molecular clock (e.g. Clock, Period, Cryptochrome, Rev-erb, BMAL, VIP, CKIE, CK1O) — mouse, rat, hamster. Intended functions: to regulate circadian patterns of gene expression in cells and tissues.
G-coupled protein receptors (e.g. GPR5O, MTI, MT2, ChR2) — mouse, rat and hamster. Intended functions: to modulate specific signalling pathways within the nervous system.
Components of the thyroid hormone signalling pathway (e.g. TRH, D102, D103) — mouse, rat, hamster. Intended functions: To modify local (hypothalamic) thyroid hormone availability.
Genes involved in cellular trafficking (e.g. PSD95, GFP-based reporters: pIN-G and pIN-ER) — mouse, rat, hamster. Intended functions: To visualise intracellular trafficking using GFP-based reporters.
Ion channels (e.g. Kv4, Cav2.2 and auxiliary Ky and CaV) — mouse, rat, hamster. Intended functions: To discretely modify neuronal ion homeostasis.
Reporter genes (eGFP, mRFP, luciferase, organelle-targeted GFP-based reporters) — from jellyfish, coral and firefly respectively. Intended purpose to act as reporters of gene expression when driven under specific gene promoters, to allow intracellular localisation of fusion proteins, and visualisation of organelle dynamics.

Genetic material - Commercially synthesised oligonucleotides to form shRNA complexes to:
Components of the molecular clock (e.g Clock, Period, Cryptochrome, Rev-erb, BMAL, VIP, CK1&), CK1O) — mouse, rat, hamster. Intended functions: to regulate circadian patterns of gene expression in cells and tissues.
G-coupled protein receptors (e.g. GPR5O, MTI, MT2, ChR2) — mouse, rat, hamster. Intended functions: To modulate specific signalling pathways within the nervous system.
Components of the thyroid hormone signalling pathway (e.g. TRH, D102, D103) — mouse, rat, hamster. Intended functions: To locally (e.g. within the hypothalamus) modify thyroid hormone availability.
Genes involved in cellular trafficking (e.g. P6D95) — mouse, rat, hamster. Intended functions: To alter intracellular trafficking of PS D95-associated proteins.
Ion channels (e.g. Kv4, Cav22 and auxiliary Ky and CaV) — mouse, rat, hamster. Intended functions: To discretely modify neuronal ion homeostasis.

Genetic material - cloned from genomic DNA:
Promoter region of molecular clock genes (e.g. Clock, Period, Cryptochrome, Rev-erb, SMAL, VIP) — human, mouse and rat. Intended functions: to drive the expression of eGFP or Luc reporter genes.
Gene inserts: The majority of the gene products expressed are expected to affect cellular properties such as circadian gene expression, intracellular trafficking, neuronal communication, and ion homeostasis. None of the genes are known to have direct oncogenic properties. As the purpose of this research is to modify physiological processes within the cells/tissues/animals, the gene inserts are expected to have effects on the host. However, these effects will be highly localised within the animals, due to the targeting on very small quantities of virus to specific sites of the nervous system. For example, we aim to modify thyroid hormone (T3) availability within the hypothalamus using a lentiviral delivery system. Within this line of research we would expect to observe a number of potential effects in the transfected animals, such as altered metabolic rate and feeding behaviour. Because we are using a lentiviral system, we would expect these effects to be relatively long-lasting. However, the risk posed to the environment, general public, animals care staff, and researcher working with the animals can be considered very low. Exposure of an individual to live virus could allow the gene insert to be expressed, but since viral particle cannot replicate gene expression would be highly localised to the site of exposure. None of the genes of interest have foreseeable hazardous consequences if expressed at localised areas of the skin and lungs, the most likely sites of exposure. Risks of exposure will be minimised at all stages of the work by employment of good laboratory practice and adherence to Class 2 containment measures.

Viral vectors: All viral systems to be employed involve split function of the viral vectors, which greatly reduces the risk of recombination events occurring. Lentiviral systems are also self-inactivating, so that the vectors themselves inhibit production of virus once the transgene becomes stably incorporated into the genome of the target cells, eliminating the risk of undesired infection. Therefore it is highly unlikely that replication competent virus will be produced. Good laboratory practice in accordance with the risk assessment of the experimental procedure will ensure that the worker or others within the laboratory will not be exposed to the virus.

Transfected animals: Although unlikely tranfected animals have the potential to pass skin or blood into their immediate environment, the risk from such contaminants is negligible. It is unlikely, but possible that viral particles could transfect skin cells or blood during injection of the virus. However, because the particles are non-replicable, shed skin/blood cells would not be able to pass on the viral vector. Animals will be housed in filter top cages to minimise the spread of bedding and shed skin, and all animal remains/waste will be autoclaved prior to incineration. The likelihood of escape of genetically manipulated animals into the environment is negligible due to the breeding and maintenance of these animals in dedicated facilities. However, even should an animal escape the risk to public health or the environment would be negligible. While it would be possible for these animals to breed with animals in the wild (although unlikely due to behavioural differences in lab-reared animals), the potential for GM animals to transfer transiently expressed genes is extremely low because these will not be targeted to reproductive organs (ie. do not enter the germline). Even should this occur, penetrance of the knockout or mutant loci into the wild population would be negligible because no (foreseeable) selective advantage would be passed on the offspring.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GM animals will be held in a purpose-built facility (Biological Services Unit; BSU) at the University of Manchester. The BSU has restricted swipe-card access at all times. All areas have sealed floors, temperature and humidity control, and filtered ventilation. All animals which have been injected with viral vectors will be housed within individual filter cages, and all animal carcasses, waste and bedding will be autoclaved and incinerated. Cages will be autoclaved and washed.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In vitro:
Spillages to be cleaned with 1% virkon
All liquid waste to be soaked in 1% virkon overnight.
All solid waste to be autoclaved.
The area to be cleaned with 70% ethanol afterwards.
It is anticipated that this will give 100% kill.

In vivo:
Spillages to be cleaned with 1% yukon.
All liquid waste to be soaked in 1% virkon overnight.
All solid waste to be autoclaved. The area to be cleaned with 70% ethanol afterwards. All animal remains and bedding will be isolated, autoclaved and incineration. Used cages will be soaked in 1% virkon overnight, then washed and autoclaved. It is anticipated that this will give 100% kill.

The risk assessments submitted herein (ASL/01) were considered initially at the local GM safety committee meeting on 17/01/2008, where the committee commented on the containment level of the programme of work. Specific comments were made about by the committee regarding the nature of the lentiviral system being used & the activity of the proposed inserts to be examined. Further meetings were subsequently held between the researchers, the local BSO & the University Biological Safety Adviser to discuss the details of containment measures and waste management. The risk assessments underwent a final review & approval at a specially convened meeting of the local GM safety committee on 26/02/2008.

**Project Containment**

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**Animal Units**

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**Project Ref** 541/08.3

- **Date Ackn’d**: 21/11/2008
- **CU2 Project Title**: The use of intestinal pathogens to study mucosal immunity, regulation and initiation.
- **Class**: Class 2
- **Culture Volume Class 2**: 1-50 Litres
- **Non-GMM Consent Granted**: Not Applicable

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**
The objective of this project are to understand the biology of immune responses to intestinal pathogens, gain insights into the mechanism by which they cause disease and identify molecules and pathways involved in elimination of the pathogens. This will involve infection of genetically-modified mice with different intestinal pathogens (either wild type strains, or genetically modified strains). Cells isolated from genetically modified mice will also be infected with different gut pathogens and their characteristics studied in vitro.

**Recipient or parental organism**

Genetically modified mice (listed below) or primary cells isolated from these mice (T-cells, dendritic cells, epithelial cells).

- Genetically modified mice, for example:
  - knockout for the protein Nod2
  - Conditional knockout for intrgrin β specifically in leukocytes. T-cells or dendritic cells.
  - Conditional knockout for arginase
  - Tagged with yellow fluorescent protein in cells expressing CD11c

**Host/vector system**

Genetically modified intestinal pathogens, for example:

- Listeria monocytogenes bacterial strain (described by Wollert et al, 2007, cell v. 129, p 891-902): modified so that the E-cadherin receptor of the bacterium recognizes mouse E-cadherin, therefore facilitating oral infection of mice in vivo.

Non-genetically modified intestinal pathogens, for example:
- Trichuris muris parasite
- Toxoplasma gondii parasite
- Trichinella spiralis parasite
- Heligmosomoides polygyrus parasite
Salmonella typhimurium bacteria
Yersinia enterocolitica bacteria

**Origin & function**

For genetically modified mice:

All mice are genetically altered by disruption of gene function by targeted homologous recombination. For complete knockout mice, homologous recombination occurs to disrupt the gene of interest. For conditional knockout mice, loxp sites are inserted to flank important regions of the gene of interest. In the presence of Cre recombinase (which is under the control of a cell type-specific promoter), the gene region between the loxp sites is removed, therefore disrupting the gene of interest. None of the genetically modified mice to be used are any more dangerous to humans or the environment than non-genetically modified strains.

For genetically modified micro-organisms:

Genetic modifications in the gut pathogens involve tagging of the pathogen with a non-toxic fluorescent protein to aid visualisation during experimentation, or facilitating oral infection of mice for example the Listeria monocytogenes strain listed above. None of the genetically-modified strains to be used are any more dangerous to humans or the environment than non-genetically modified strains.

**Evaluation of foreseeable effects**

**Micro-organisms**

For all intestinal pathogens detailed in Part 7, there is potential risk of hand-mouth transmission. This risk is extremely low, as all work involving these pathogens will be carried out in a Class II biological safety cabinet, all workers will wear protective lab coats that are only kept in Class II areas, and all workers will wear gloves. Additionally, all workers will be specifically trained in working with Class II organisms. Hands will be washed with anti-bacterial cleaner before and immediately after any work has been carried out.

There is no risk of contamination with needles, as no work will involve the use of needles.

Upon infection of mice with the intestinal pathogens detailed in part 7 (except for Toxoplasma gondii) there is the potential for the organism to be shed through the animal's faeces. However, the risk to animal workers of contamination is extremely low. All the handling and mice and changing of bedding will be carried out in a Class II biological safety cabinet, protective equipment will be worn at all times (gloves, mask), and hands will be washed immediately after removal of gloves. All waste bedding from the mice is bagged, tagged and incinerated off site.

In the extremely unlikely event of infection of a worker, mild sickness may occur. However, this will be very short-lived and the organisms expelled by a normal immune response. For work with Listeria monocytogenes, an additional infection risk is posed to pregnant women. Therefore female staff will be informed of this risk and will not be asked to work with Listeria or Listeria-infected mice if they may be at risk.

For genetic modification of the Listeria, Salmonella and Toxoplasma strains detailed in the attached risk assessments, the genetic modification does not in any way increase the potential risk to humans or the environment versus the risk of using the unmodified strain.

**Mice**

For the use of genetically modified mice detailed in part 7 (and use of cells from these mice), no increased risk over the use of non-modified mice is apparent (i.e. negligible). The genetic modifications do not cause any changes in the mouse that will increase risk to humans, or increase survival in the environment. In the unlikely event of escape of the genetically-modified mice into the environment, the mice are extremely unlikely to survive in the wild and replace indigenous mouse populations.
Similarly, infection of genetically modified mice with the genetically modified/non-modified micro-organisms will have no greater detrimental effects than the infection of non-genetically modified mice with non-genetically modified micro-organisms. Potential outcomes of infection of the genetically modified mice are:

- the GM mice will expel infection more quickly than wild type mice.
- the GM mice will show identical infection clearance compared to wild type mice.
- the GM mice will be more prone to infection than wild type mice.

If GM mice are protected from infection, this will not provide any significant survival advantage over wild type mice in the environment, as such infections are extremely rare. If mice are more prone to infection, the mice will certainly not have any selective advantage over wild type mice in the environment. In terms of animal welfare, all infected animals will be monitored regularly during infection, and humanely sacrificed if significant weight loss occurs (as detailed in our Home Office Animal Licence).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All genetically modified mice will be housed in wire or isolator cages within secure, designated animal units within the University of Manchester. Infected mice will be kept in isolator cages. Bedding changes and mouse retrieval are carried out in closed rooms, and mouse transport between animal facilities is carried out in sealed filtered transport cages within linked buildings (i.e. not outside), making escape of mice very unlikely.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For genetically modified micro-organisms:
- Spillages to be cleaned with 1% virkon
- All liquid waste to be soaked in 1% virkon
- All solid waste to be autoclaved
- The area to be cleaned with 70% ethanol afterwards

- It is anticipated that this will give 100% kill.

For genetically modified mice:
- Animal carcasses are placed in thick walled body bags that cannot be pierced by body parts of the mouse.
- These bags are tagged and incinerated off site.
- Waste bedding is double bagged in clinical waste bags, tagged and incinerated off site.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
Risk assessments for projects MAT2 (encompassing MT1-3) and SMC1 (encompassing SC1-6) were discussed at the local GM sub committee working party meetings on 01/09/08. At both meetings the working party asked for amendments to be made to the risk assessments for both projects, these mainly concerned control measures, waste management, assessing the potential risk of infected animals to animal workers, assessing potential risk alteration in pathogenicity of GM pathogens infecting GM mice. After all amendments had been made, risk assessments were approved by the local GM committee on 10/11/08.

**Project Containment**

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<th>Human Clinical Applications</th>
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**Project Ref** 541/09.1

- **Date Ackn’d**: 15/09/2009
- **CU2 Project Title**: Human placental studies to support an adenovirus-mediated vascular endothelial growth factor (VEGF) gene medicine for the treatment of fetal growth restriction (FGR)
- **Class**: Class 2
- **CultureVolClass2**: Not Applicable
- **CultureVolumeClass3-4**: Consent Granted
- **Non-GMM**: Yes
- **Consent Granted**: Yes
- **Withdrawn**: No
- **Tick if notifying a connected programme of work**: Yes

**Historical Significant Changes**

**Historical Date of Additional Info**

- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

- **Therapeutic Aim (Clinical Trial - Beyond This Project)**
- **Vascular endothelial growth factor (VEGF)** is a paracrine and endocrine agent, naturally expressed in several tissues and commonly known for its part-role in blood and
Lymph vessel growth and development, but less known for its vasodilatory properties. Fetal growth restriction (FGR) is sometimes characterised by reduced blood flow from the uterus to the placenta, dangerously compromising the delivery of oxygen and nutrients to the fetus during its development.

Our long-term aim (Phase 2 Clinical Trial) is to exploit the vasodilatory property of VEGF, by transfecting the uterine artery of pregnant humans that exhibit poor uterine artery Doppler (a clinical measure of maternal blood flow efficacy to the placenta/fetus), to improve maternal blood flow to the placenta, in clinical cases where neonatal morbidity and mortality outcome is dire.

Research Objective (This project application)
The aim of this current Phase Zero clinical trial proposal is to test for potential toxicological effects of VEGF transfection on human placental tissue in vitro; a side-effect which is theoretically possible in future in vivo administration studies, since the placenta occurs downstream of uterine artery blood flow. The currently proposed Phase Zero Clinical Trial will provide valuable data from which to pitch a safe dose of the VEGF viral vector in a subsequent Phase 1 Clinical Trial (rabbit, in vivo, London based project, UCL collaborators to notify) and Phase 2 Clinical Trial (early onset human FGR cases with a poor fetal and neonatal prognosis).

Recipient or parental organism
Not applicable

Host/vector system

Viral Vector. The vector to be used will be a replication deficient adenovirus, carrying either the human recombinant VEGF gene (a growth hormone with vasodilatory properties), or lacZ beta-galactosidase (for viral localisation in ex vivo tissue).

Host (in vitro) human Tissue: There are two proposed stages of work, with in vitro human placental tissue as the host in both cases:
Stage A. Dose ranging experiments of the viral vector in the in vitro human placental explant (tissue fragment) model (host) to determine appropriate starting dose of viral vector - applicable to stage B (below). 3mm³ pieces of human placental villous tissue will be cultured in 12 well plates for 7 days. On day 4, they will be exposed to one of 4 doses of adenoviral vector with recombinant human VEGF, or a PBS control, or the adenoviral vector control. Culture medium will be harvested and assayed for measures of host cellular death, differentiation and function. Host tissue fragments will be harvested at day 7 and their protein content measured. In parallel treated 12-well plates of cultured tissue, fragments will be fixed in zinc fix solution and their morphology analysed. Stage B. 5-hour in vitro dual perfusion of the human placental lobule (host). A high dose of adenoviral vector, determined from stage A (above), containing recombinant human VEGF will be administered to the maternal-side circulation of the in vitro dual placental lobule. Samples will be taken from the host in vitro fetal venous circulation to enable measurements of transplacental viral transfer (courier transfer of samples to Finland for assay; Biological Sample Category B UN3373, IATA 650 compliant packaging used with thermal control unit for dry ice shipment - compliant with UN1845; we are a licensed dry ice shipper with DHL). Other host in vitro venous sampling will occur to assay for tissue cellular integrity, differentiation, function and changes in paracellular permeability.

Origin & function

Stock viral vectors will be supplied in a ready to use form Ark Therapeutics Inc, Huopio, Finland.

Evaluation of foreseeable effects

The recombinant virus is replication deficient due to the loss of the E1 gene. The loss of the E1 gene suppresses assembly of infectious viral particles in host cells. 20% of normal healthy adults have the E1 insert in their respiratory epithelium (SACGM Compendium of Guidance, part 2.7, paragraph 14), raising the possibility of the adenovirus becoming replication competent, whilst also carrying the VEGF gene. However, the insertion of the VEGF gene in place of the E1 cassette ensures that any homologous recombination that restores the E1 sequence to the vector also deletes the VEGF insert, so the genetically modified component would be lost if RCAs were inadvertently be generated.

Furthermore, part of the E3 gene has also been deleted, making the virus less likely to establish an infection and spread the community, being more susceptible to immune surveillance. This dual gene deletion makes self-replication capability extremely unlikely. The vector will not replicate in other in vivo, or in vitro cells. The replication deficient characteristic means that there is also a reduced capacity for colonisation and if there was any accidental exposure to the environment, there would be
compromised productive infection.

Increased immune surveillance through E3 gene deletion may cause a more severe immune response than would be the case for the wild type virus (SACGM Compendium of Guidance, Part 2, paragraph 50). The vector is produced in a cell line, which minimises potential for recombination to create replication competent adenovirus.

Recombinant adenoviral vectors infect a variety of human cell types. The virus may enter the human body via cuts and sharps injury. The virus could transmit via the lungs if the virus becomes airborne, should aerosol generation be permitted. If a worker becomes infected by the AdVEGF virus, the transgene may be expressed in any infected cells. In the unlikely event of inhalation of aerosol, a small degree of transfection will occur in the airways, predominantly in the upper respiratory tract. However, infection will be the host’s immune response and the infected cells should be rapidly cleared, such that expression is only transient and localised. The recombinant adenovirus is designed to be replication deficient; therefore, should the virus infect the worker, it should not replicate. This is verified in paragraph 25 of the SACGM Compendium of guidance, Part 2, where it is stated that these strains are disabled and incapable of establishing a productive, transmissible infection in humans. Adenovirus can provoke a strong immune response and non-productive infection symptoms could include fever, rhinitis, pharyngitis, cough and conjunctivitis. However, manufacture and testing of the viral seed stocks (see below) ensure that potential for contamination with infectious productive adenovirus is low, so any symptoms will be very transient. In practice, aerosol generation will not occur, and even if this did occur, any inhaled dose would be very low, so symptoms are unlikely to occur. Furthermore, whilst the naturally occurring adenovirus is associated with mild respiratory infections in children, it is thought that the majority of the population is likely to have antibodies to the wild type virus, inferring life-long immunity. The wild type virus is ubiquitous, causing only a mild respiratory disease in humans which is self-liming and does not require any specific treatment.

The viral vector will be supplied to us in a ready to use state, by Ark Therapeutics, Kuopio, Finland (EU). Its production is described as follows: “Recombinant adenovirus type 5 was rescued in human embryonic cells by co-transfection of a plasmid containing the VEGF gene and an adenovirus backbone sub360. The resulting E1-E3 deleted viral progeny was plaque purified and an appropriate clone was selected and used to produce a crude viral lysate.” (Source: GMO Information Form: Recombinant adenovirus containing the short, mature form of human vascular endothelial growth factor D, 24th February 2009, Ark Therapeutics Oy, Kuopio, Finland).

Although not every batch of virus produced for non-clinical use will be tested by Ark Therapeutics, their testing of the viral seeds stocks and the derived lots that are tested indicate that replication competent adenoviruses are not expected to be detectable in test samples. It may be judged that considering the amounts that will be handled in this work the chance of a laboratory worker being accidentally exposed to a single RCA virus, let alone sufficient to produce a clinically relevant infection, is effectively zero.

The gene product, VEGF, is under the control of the CMV promoter and so is constitutively produced. VEGF is a human gene, which promotes vasodilation, angiogenesis and lymphangiogenesis and is naturally expressed in several tissues. However, the upregulation of VEGF production alone is unlikely to promote successful vascularisation, which needs to be balanced out with other angiogenic factors (Carmeliet, 2000, Nat Med 6, 1102-3). Hence, it is unlikely that infection with adenovirus expressing VEGF will be more hazardous than infection with wild-type adenovirus. The inappropriate expression of VEGF, might theoretically worsen pre-existing conditions such as diabetic retinopathy, or cancer but it is unlikely to cause any de novo disease. Indeed any transgene expression from this limited infection will be limited both in amount and duration, so the exacerbation of any underlying conditions in the worker is unlikely. Furthermore, any potential impact of transgene expression away from the site of infection will be further limited by sequestration in local tissues, the dilution and degradation of the produced VEGF in body fluids. For these reasons, the likelihood of VEGF gene recombination with any host endogenous adenoviruses is low and any scope for VEGF hypotensive effects in the host’s systemic circulatory system is negligible.

We do not anticipate that the worker’s immune response would in any way be compromised by any transient over-expression of the transfected protein, should infection and transfection happen. In fact the deletion of the E3 region, which encodes proteins involved in the evasion of the host’s immunity, will offer extra immune surveillance against the virus. The complete vector sequence has been determined and no novel or truncated reading frames, which may have an adverse effect on the safety profile of the product, have been introduced during manufacture. Adenovirus does not integrate into the host cell genome and the vector has no additional features that promote integration.

Health Surveillance is required: initial pre-project appointments with University Occupational Health have been carried through for all scientists working on this project. Routine health surveillance will occur once every 3 months for this project.
The use of a Class 2 MSC for viral stock handling and at other points where possible is a prudent measure, although not considered necessary from the perspective of virulence for this E1 deleted adenoviral strain.

Potential routes of transmission are eyes, mouth (ingestion), skin (puncture wound and lungs. Any previous skin break injuries will be covered prior to experimentation. In the laboratory, care must be taken to avoid the spread of viral material by aerosol, direct contact, or accidental injection.

Self-inspection and risk assessment: Laboratories will be routinely self-inspected (at least annually) and results recorded. Defects found at any time will be amended before work recommences. Risk assessments for each laboratory are undertaken to account for all activities performed. Risk assessment will include details of secondary containment of materials during transport between laboratories.

Signage: The laboratory will be clearly signed (biohazard sign plus a sign explaining the pathogen containment level and the level of GM working on the laboratory doors). Local rules relating to this project will be drawn-up and displayed within the GM laboratories. A list of current GM inductees will also be displayed.

Controls and segregation of activities: The dispensing and diluting of viral vector stocks will be done in Class 2 MSC. On other occasions, the worker will be protected from eye and mouth contact by the glass screen of the MSC or perfusion cabinet. Sharps will be avoided at the stage of the viral experimental process, during tissue exposure periods to the virus and at any subsequent stage where residual virus might be present in culture medium, perfusates and assay solutions. Sharps (pointed forceps and sutures) will have been used during the tissue cannulation process in “Stage B”, prior to working with the viral vectors, but these will have been used at a separate workstation and will have been decontaminated and put out of use at the point of viral working. The GM laboratory doors have digi-locks fitted and their codes are known only to GM inducted personnel. The MSC and tissue incubator used for this project will be separate to those on other GM projects in this laboratory (room 330). For perfusion work, one whole bay of this laboratory will be reserved for this project and the perfusion cabinet will be located against a wall, away from the central bench, shared by bays.

GLP, hygiene and PPE: Good laboratory practice will be used throughout. PPE, including Howie type lab coats and gloves will be used. Before leaving the laboratory, used gloves will be discarded and hands will be washed thoroughly with soap and water.

Administration of the virus to tissue fragments in “Stage A” will be done in a Class 2 MSC, whereas viral handling with the perfused tissue model in “Stage B” will be otherwise controlled, when reasonably practicable to do so. Perfusion in “Stage B” will occur within containment trays within containment trays within the perfusion cabinet (not a Class 2 Containment apparatus). Aerosol generation risk will be zero and drip splashes minimised in “Stage B”, since we will: control viral stock handling (Class 2 MSC for aspirating into syringe, syringe with luer-locking cap for transit to perfusion cabinet), control pressurised viral administration (manual syringe plunging) to sealed perfusate tubing line (luer locking of syringe to a three way tap, so no needle involved) and control post-experimental transfected tissue dissection (Class 2 MSC). All viral work in “Stage A” will be performed in a Class 2 MSC. Aspirated wash solution will be collected into a secondary contained 2.5 litre sealed waste bottle, containing an appropriate quantity of Virkon to give a 2% solution following maximum experimental aspiration; bunged and held under negative pressure by an aspirator pump fitted with a 0.22µM syringe filter at the air inlet port, avoiding potential viral dissemination into the laboratory atmosphere. Spent culture media (sample) collection and aliquoting will occur into gasket sealed sample vials. Vials will be put into secondary containers for storage at -80ºC. At the end of tissue culture, tissue fragments will either be rendered non-infective by dissolving in 0.3M sodium hydroxide (for subsequent protein assay), or they will be zinc fixed (for imaging).

In “Stage B”, perfusion of exposed human placental tissue will be conducted in trays and all venous perfusate samples will be treated as potentially infectious. Samples will be collected into polypropylene tubes and capped in the perfusion cabinet before being centrifuged. Our SOP on centrifugation instructs the user to leave the centrifuge unopened for 30 minutes in the event of a tube break, so eliminating the risk of aerosol leak into the laboratory. This SOP also advises on spillage disinfection. All aliquoting of supernatants (virus containing) will occur in a Class 2 MSC. This control is a prudent measure, although not actually necessary, as aerosol generation is not anticipated. Storage of supernatants will occur at -80ºC in gasket sealed sample vials, within secondary sealed containers. All assays will be performed in a Class 2 MSC. 96-well plate reading of assayed samples will occur in another laboratory commissioned for GM working.
Generally, during placental perfusion, placental venous perfusate from the maternal-side and fetal-side will be aspirated into a secondarily contained and sealed waste bottle in a controlled manner. This will contain significant levels of virus, especially immediately after the transfection period. This non-pressurised venous perfusate will be channelled into a drip-point and collected into a secondary contained beaker within the perfusion cabinet (not a class II containment piece of apparatus). In reference to “System of Work – M10” for Containment Laboratory Level 2, Schedule 8, Part II of the GMO (CU) Regulations 2000, there is a negligible risk of aerosol generation at this point in the procedure, since the elution from the placenta is not pressurised and is without mechanical force (unlike examples given of mechanical process, including shaking, centrifugation and sonication). Nonetheless, splash risk from droplets has been evaluated (see end of this section). During viral tissue perfusion, the collection vessels will be continually aspirated into a secondary contained 2.5 litre sealed waste bottle, containing an appropriate quantity of Virkon to give a 2% solution following maximum experimental aspiration; bunged and held under negative pressure by an aspirator pump fitted with a syringe filter at the air inlet port, safely containing the waste and preventing viral release into the laboratory atmosphere. At the end of perfusion experimentation, using blunt ended scissors and blunt ended forceps, the perfused placental tissue (approximately 150g) will be transferred to the biological safety cabinet, cut up into small cubes, and either zinc fixed for imaging, or immersed in 2% Virkon overnight, drained and sent to clinical waste (supervised burn).

Viral-containing droplet production and decontamination evaluation during perfusion:

To ensure that droplet splashes were minimal and fully contained during perfusion, a simulation experiment was performed with a low hazard dye, bromophenol blue. The simulation perfusion was carried out, substituting the virus with the dye. An A3 sheet of white paper was taped to the inside of the perfusion cabinet sash, adjacent to the dripping perfusate (perfusate drips 15 cm into an aspirated beaker, placed in a secondary containment tray). We also took this opportunity to evaluate the syringe/three way-tap “luer locking system” that we thought would protect from viral spray risk during virus administration to the perfusion inflow line. We found that this was very effective at preventing any dye from leaking under the pressure generated from syringe plunging. Perfusion was performed for 30 minutes at normal flow rates. The A3 paper was inspected at the end of this period for contamination and was found to be clear of dye.

There was a minor risk of splashes from drips, beyond the inside wall of the glass collection beaker into the secondary containment tray. However, repositioning of the aspiration tube inside the beaker, to a vertical position, prevented splashing onto the white secondary containment tray. We evaluated that flooding the secondary containment tray with a 2% Virkon solution prior to controlled aspiration would be a reasonably practicable measure if splashes were found to occur during the course of an experiment and we would have plenty of Virkon solution at hand before each experiment. Also, we found that channeling the dripping perfusate, using parafilm, which had been trapped into the placental perfusion clamp assembly prior to experimentation, was very effective at ensuring all drips were controlled into the beaker, before dye administration occurred. We will use this method routinely.

2% Virkon treatment of the disassembled apparatus, beaker, aspirator, secondary containment trays and the inside of the perfusion cabinet (non-disposable parts of perfusate delivery system, clamp apparatus and non absorbent cabinet surfaces, i.e. all surfaces) would be an easily achievable SOP after experimentation. The perfusate dye (dye/virus) distributor, a disposable part associated with the luer-locking syringe mechanism, occurring just upstream of perfusate delivery to the placental tissue, would undergo 2% Virkon soaking, but would then need to be routinely subjected to autoclave treatment, to ensure penetrating decontamination.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon tablets (working concentration = 2% minimum of 2 hours) will be used to render waste culture media and perfusate disinfected. 2% Virkon will be used to wipe all trays, MSC, perfusion cabinet and equipment surfaces and will be used to soak smaller disposable consumables items. Following at least 2 hours of disinfection, the 2% Virkon solutions will be poured into the normal drainage system. Used culture plates and their ‘net-well’ inserts, used viral administration syringes, three way taps, perfusate line and cannulae will be autoclaved at 123°C held for 40 minutes, within the building, before being disposed of into normal clinical waste for incineration. All metal re-usable equipment (placental clamp apparatus, blunt forceps and scissors) will be soaked in 2% Virkon for 10 minutes and then autoclaved, as above.

Culture medium and perfusate spillage within trays, during experimentation, will be dealt with by aspirating the liquid into the controlled waste bottle, as normal liquid waste (see above). Trays will then be flooded with a 2% Virkon solution then aspirated to the controlled waste bottle, in the same way. Non-contained spillage will be dealt with according to our displayed SOP podter “Permitted Disinfectants”. Waste paper towing and Virkon powder scoping utensils, used to clear surfaces after spillage
disinfection, will be bagged, sealed, autoclaved (as above), before being disposed of into normal clinical waste for incineration.

It is expected that all solutions, materials and transfected tissue will be completely killed after the above measures have been undertaken. 2% Virkon disinfectant solutions will be made up weekly and will be discarded thereafter, or if the pink colouration fades. All autoclaving of waste will be logged following the checking of time, temperature and vent settings, prior to switch on. Arrangements will be made periodically to have the autoclave tested for functionality by a trained engineer. Records of function testing will be retained alongside the autoclave.

After reviewing the assessments the committee raised several points which required clarification:
1. Address the effects of possible homologous recombination with wild type E1 gene. Would this make the GM Adenovirus replication competent, whilst continuing to carrying genetic insert gene (VEGF)?
2. Should transfection occur in human, explain scope for hypotensive effects in the systemic vasculature.
3. Evaluate the potential for droplet splashes in phase B (perfusion of placental lobule) of the proposed wok.
4. Describe the likely replication competency of the supplied virus.
5. Describe segregation of projects sharing the GM lab (330).
6. For aspirated viral containing waste, explain how you might disinfect the solution that enters the waste bottle, to disable the infection hazard in the most immediate and controlled manner.

All these points have now been addressed to the satisfaction of Genetic Modification Safety Committee and are encompassed in the current version of the Risk Assessment.

Project Containment

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<thead>
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Project Ref 541/09.2
Purposes of the contained use

A broadly cross-reactive meningococcal vaccine developed by Novartis Vaccines is currently undergoing phase 3 outer membrane proteins – factor H binding protein (fHBP) fused with a further protein GNA2091), GNA2132 (fused with a further protein GNA1030 and Neisserial adhesion A (NadA). The vaccine also has an outer membrane vesicle component. fHBP is a virulence factor that binds the human complement factor H thereby enhancing bacterial survival in the host. The function of GNA2132 is unknown though it has been implicated in heparin binding, which may also lead to enhanced survival within the host. NadA is avirulence factor involved in host cell adhesion and invasion.

In order to predict the efficacy of the vaccine in England and Wales it is necessary to characterise native case isolates for fHBP, GNA2132 and NadA in terms of a) genetic variation, b) surface expression levels and c) serum bactericidal antibody (SBA) susceptibility to pre and post-vaccination sera – the gold standard assay for predicting Men B vaccine efficacy (there is no reliable animal model for meningococcal disease). To enable the evaluation of surface expression, e.g. by Whole cell ELISA and Immunoblotting, it is necessary to compare wild type native isolates against negative control (gene-knockout) isolates such that e.g. non-specific antibody binding may be accounted for. Similarly, in order to evaluate SBA susceptibility, knockout isolates of a) each gene, b) different combinations of 2 genes and c) all of the genes, will be required to help elucidate the SBA activity against any single antigen.

Single and multiple knock out strains have been developed for these antigens, for the purpose outlined above, by Novartis vaccines (Sienna, Italy). It is our intention to obtain these isolates from Novartis for use in the present study.

Recipient or parental organism

The knockout strains will be required for comparison with the wild type strains used in the immunoassays and are therefore required to be wild-type in all respects other than that of the gene/s being disabled. The GMM constructs will therefore be treated as wild -type. A deficit of one or more of the antigens - fHBP, GNA2132 and NadA is likely to significantly reduce invasiveness and the ability in the host.

Host/vector system

fHbp, NadA and gna2132 knockout isolates and combination thereof were constructed at Novartis Vaccines, Siena, Italy. Briefly, In order to generate Neisseria meningitides mutant strains lacking the main antigens contained in the vaccine, three knockout plasmids were constructed for the deletion of all or part of the respective antigen-coding gene and replacement by allelic exchange with an antibiotic resistance cassette. Using standard cloning procedures, upstream and downstream flanking
regions of the gna2132 and fhbp genes were amplified by PCR from the MC58 genome and cloned on either side of an erythromycin resistance cassette into the pBluescript (Pharmacia) cloning vector, generating the knockout plasmids pBSUDgna2132:Erm and pBSUDfhbp:Erm, respectively. For the generation of the NadA knockout construct, the nmb1994 gene and surrounding upstream and downstream regions were amplified by PCR from the MC58 genome and cloned into the pBluescript cloning vector, and subsequently an internal HincII fragment at the 5’ end of the coding region was substituted with the erythromycin resistance cassette, generating pBS961:Erm. For generation of the single knockout mutants, the knockout plasmids were linearised and transformed into the wildtype strains. Erythromycin resistant colonies were selected and checked by PCR for correct insertion due to a double homologous recombination event and colonies with correct PCR profile were further analysed by Western Blot for the lack of expression of the respective antigen.

For generation of a single Nm derivative strain lacking the expression of two or three antigens, a stepwise deletion by allelic replacement strategy was used in which double-knockouts were constructed using Erythromycin and Kanamycin cassettes and triple-knockouts were constructed using Erythromycin, Kanamycin and Chloramphenicol cassettes.

**Origin & function**

The function of the genetic material, i.e. antibiotic resistance genes, is to enable the selection of isolates in which the genes of interest have been successfully removed.

The erythromycin (Erm) cassette contains a Streptococcus pneumoniae gene for rRna adenine N-6-methyltransferase. This protein produces a dimethylation of the adenine residue at position 2058 in 23S rRNA, resulting in reduced affinity between ribosomes and erythromycin resulting in erythromycin resistance.

The Kanamycin (Kan) cassette contains the Campylobacter coli aphA-3 gene. The gene product is an aminophosphotransferase (APH) that inactivates kanamycin by transferring the γ-phosphate of ATP to the hydroxyl group in the 3’ position of the pseudosaccharide.

The chloramphenicol (Cin) resistance cassette contains the Campylobacter coli chloramphenicol acetyltransferase (cat) gene. The enzyme chloramphenicol acetyltransferase covalently links acetyl groups to the chloramphenicol molecule. This in-turn prevents chloramphenicol from binding to the ribosome – its site of action.

The knockout isolates and their respective antibiotic cassettes are listed in the table below:

<table>
<thead>
<tr>
<th>Genes Knocked Out</th>
<th>Strain</th>
<th>Clonal complex</th>
<th>Sequence type</th>
<th>Country</th>
<th>Type</th>
<th>resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fhbp MC58KOfhbp</td>
<td>32</td>
<td>74</td>
<td>UK</td>
<td>B:15:Pl.7,16b</td>
<td>Erythromycin</td>
<td></td>
</tr>
<tr>
<td>Fhbp 44/76KOfhbp</td>
<td>32</td>
<td>32</td>
<td>Norway</td>
<td>B:15:Pl.7,16</td>
<td>Erythromycin</td>
<td></td>
</tr>
<tr>
<td>Fhbp NZ98/254KOfhbp</td>
<td>41/44</td>
<td>42</td>
<td>New Zealand</td>
<td>B:4:Pl.4</td>
<td>Erythromycin</td>
<td></td>
</tr>
<tr>
<td>nadA MC58KOnadA</td>
<td>32</td>
<td>74</td>
<td>UK</td>
<td>B:15:Pl.7,16b</td>
<td>Erythromycin</td>
<td></td>
</tr>
<tr>
<td>nadA 5/99KOnadA</td>
<td>8</td>
<td>1349</td>
<td>Norway</td>
<td>B:2b:Pl.5,2</td>
<td>Erythromycin</td>
<td></td>
</tr>
<tr>
<td>nadA NMBKOnadA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gna2132 MC58KOfna2132</td>
<td>32</td>
<td>74</td>
<td>UK</td>
<td>B:15:Pl.7,16b</td>
<td>Erythromycin</td>
<td></td>
</tr>
<tr>
<td>Gna2132 8047KOfna2132</td>
<td>41/44</td>
<td>42</td>
<td>New Zealand</td>
<td>B:4:Pl.4</td>
<td>Erythromycin</td>
<td></td>
</tr>
<tr>
<td>Gna2132 NZ98/254 KOgna2132</td>
<td>269</td>
<td>275</td>
<td>Norway</td>
<td>B:NT:Pl.22,9</td>
<td>Erythromycin</td>
<td></td>
</tr>
<tr>
<td>Gna2132 UK013KOgna2132</td>
<td>269</td>
<td>36</td>
<td>UK</td>
<td>B:NT:Pl.3</td>
<td>Erythromycin</td>
<td></td>
</tr>
</tbody>
</table>
The pathogenic traits of these strains are likely to be lessened since isolates lacking nadA (that previously harboured the gene) are likely to be less invasive whilst fHbp deficient isolates are less likely to survive within the host due to the loss of a virulence factor shown to be important in this respect. Strains deficient in gna2132 may also be less likely to survive within the host since the GNA2132 antigen is has been shown to bind hepan - also proposed to enhance survival within the host.

The single gene knockout strains were all constructed using Erythromycin resistance cassettes. Double-knockouts were constructed using Erythromycin and Kanamycin cassettes and the triple-knockouts were constructed using erythromycin, kanamycin and chloramphenicol cassettes. These antibiotics do not constitute first line therapeutic/prophylactic antibiotics against meningococci (curent guidelines include penicillin, cefotaxime or ceftriaxone for therapy and rifampicin, ciprofloxacin or ceftriaxone to eradicate carriage in close contacts/exposed individuals). The presence of one or more exogenous antibiotic resistance genes may also place a disadvantageous burden on these strains.

Meningococci are obligate human pathogens and survive only within the human nasopharynx.
The local GMO committee reviewed the risk assessment and local application form at length and were generally happy with the contents. The committee asked for more detail of working with meningococci to be added; in particular the means of controlling aerosol production and ensuring worker safety training. This detail has been added to the risk assessment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3</td>
<td>L2 L3 L4 L2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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</table>

**Project Ref** 541/09.3

- **Date Ackn’d**: 17/11/2009
- **CU2 Project Title**: Modulation of cell signalling pathways to analyse their effect on cellular properties such as proliferation, apoptosis, polarity, migration, adhesion and fate, using gene delivery systems
- **Class**: Class 2
- **Culture Vol/Class 2**: < 1 Litre
- **Class Culture Volume Class 3-4**: Non-GMM
- **Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

- **Purposes of the contained use**: To virally transduce human, mouse and rat cells with genes, microRNAs or shRNA oligonucleotides
**Recipient or parental organism**

- Human primary, established cell lines, and tissue explants, such as epithelial cells, fibroblasts, vascular smooth cells and organotypic cultures of lung, and synovium, and primary human peripheral blood derived cells.
- Mouse primary, and established cell lines, and tissue explants, such as mouse monocytes, macrophages, lymphocytes, vascular smooth muscle cells and organotypic cultures of lung.
- Rat primary, and established cell lines, and tissue explants, such as Rat1 cells.

**Host/vector system**

Vectors: Commercially available adenoviral, retroviral and lentiviral self-inactivating vectors, and well established retroviral vectors (pLNCX and pBABE) packaged into transducible viral particles by specific eukaryotic packaging cells. The lentiviral, adenoviral and retroviral expression vectors do not encode proteins required for virus packaging. Proteins for packaging are provided by transient transfection of helper vectors or the packaging cell line.

**Origin & function**

- Genetic material - subcloned from cDNA library, for example: expression vectors for glucocorticoid receptor, HIF1a, macrophage migration inhibitory factor, Notch receptors and their signalling network.
- Genetic material - Commercially synthesized oligonucleotides to express microRNAs to, for example: to modulate expression of glucocorticoid receptor, Notch receptors and their signalling network, HIF1a.
- Genetic material - Commercially synthesized oligonucleotides to form shRNA complexes to, for example: glucocorticoid receptor, and its signalling partners, Notch receptors and their signalling network, HIF1a.

**Evaluation of foreseeable effects**

The majority of the gene products expressed are expected to affect cellular properties such as proliferation, apoptosis, migration, adhesion, polarity and fate. The split function of the viral vectors however greatly reduces the risk of recombination events occurring and the self-inactivating properties of the vectors themselves inhibit production of virus once the transgene becomes stably incorporated into the genome of the target cells, eliminating the risk of undesired infection. Therefore it is highly unlikely that replication competent virus will be produced. Good laboratory practice in accordance with the risk assessment of the experimental procedure will ensure that the worker or others within the laboratory will not be exposed to the virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Spillages to be cleaned with 1% virkon.

All liquid waste to be soaked in 1% virkon overnight.
All solid waste to be autoclaved. The area to be cleaned with 70% ethanol afterwards. It is anticipated that this will give 100% kill.

<table>
<thead>
<tr>
<th>Is an emergency plan required according to regulation 20?</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
<td>N</td>
</tr>
<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
</tr>
<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>N</td>
</tr>
</tbody>
</table>

Please enter comments on the GM safety committee on the risk assessment

All risk assessments were initially considered at local GM committee meetings for the Stopford and AV Hill Buildings in June and August 2009. The committee requested clarifications and amendments to all the assessments, in particular control measures, waste management and the biological activity of the inserted sequences were discussed at length. All the risk assessments were finally approved at meeting of the local GM committee on the following dates:

- DWR003 approved 1/9/09
- TWO001 approved 1/9/09
- AWO002 approved 4/8/09

**Project Containment**

<table>
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<th>Laboratory Activities</th>
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<tr>
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<td>L3</td>
<td>L4</td>
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<tr>
<td></td>
<td>L2</td>
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<td>L3</td>
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<td></td>
<td>L2</td>
<td>L4</td>
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<th>Animal Units</th>
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<tr>
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<td>L3</td>
<td>L4</td>
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<tr>
<td>L3</td>
<td>L2</td>
<td>L2</td>
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<td>L4</td>
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**Project Ref** 541/11.1

<table>
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<tr>
<th>Date Ackn’d</th>
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<th>CultureVol</th>
<th>Class Culture Vol</th>
<th>CultureVolume</th>
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</thead>
<tbody>
<tr>
<td>18/05/2011</td>
<td>Creation of recombinant fungi to study growth, nutrition and pathogenicity</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Date Project 02/03/2022
The purpose of this research is to gain an insight into the growth and nutrition of filamentous fungi. The work will primarily involve Aspergillus and will involve knocking out, placing under conditional promoters, or tagging with N or C terminal fusion with green fluorescent protein or derivatives genes thought to play a role in the growth and nutrition of filamentous fungi. The work will also involve the expression of some of these proteins in auxotrophic strains of Pichia pastoris to enable these proteins to be purified and characterised. Gene knockout/conditional promoter constructs will involve cloning into disabled E. coli K12 derivatives and reintroduction back into host fungus. Auxotrophic and antibiotic resistance markers will be used for selecting recombinant strains. Strains may also be tested for changes in the ability to infect insect larvae such as the Wax Moth, an alternative system to using animal models.

**Recipient or parental organism**

Escherichia coli K12 strains (including DH5 alpha, JM101, JM109, SURE) are disabled.

Pichia pastoris His and Arg auxotrophs.

Aspergillus strains including Aspergillus fumigatus (ACDP Hazard group 2) and Aspergillus nidulans (ACDP) Hazard group 1) and ACDP Hazard group 1 soil/compost residing filamentous fungi.

**Host/vector system**

Cloning and expression vectors are all "non-mobilisable (e.g. pUC-based such as pGEM and pBluescript series and derivatives and the yeast pPIC series)."

**Origin & function**

For knockout vectors, promoter replacement vectors and for C or N terminal green fluorescent protein tagging, a selectable marker such as PyrG or hygromycin resistance will contain upstream and downstream sequence of the target gene of the host and will be cloned in E. coli before transforming into the host fungus.

For expression in Pichia pastoris, gene sequence compromised of the ORF will be cloned into E. coli before transforming into P. pastoris for methanol-induced gene expression.

All host sequences will be generated by PCR using specific primers from host genomic DNA or cDNA derived by reverse transcription of mRNA or synthesised synthetically by a commercial company following codon optimisation.

Cloned DNA will be verified by sequencing prior to introduction into the gene expression organism and introduced sequences will be integrated into the host genome. Proteins encoded by the cloned DNA will be overexpressed using an inducible promoter system.
Human health:

With the exception of Aspergillus fumigatus, all the fungi in this study are ACDP Hazard group 1 and do not pose a significant threat to human health. Aspergillus fumigatus (ACDP Hazard group 2) is an opportunistic pathogen that can cause a threat to immunocompromised individuals. In addition, it is also capable of being an allergen. However, A. fumigatus spores are extremely common in both indoor and outdoor environments and respiratory exposure is almost constant. The modifications proposed are in genes related to growth and nutrional capability of this organism and would either have negligible effect or would impair growth compared to the parental strain and thus recombinant strains would pose no increased risk to individuals. Recombinant strains may be tested for virulence in an invertebrate pathogenicity model (e.g. wax moth larvae) or animal model (e.g. mouse) and in the unlikely event that virulence is shown to be increased, the strain will be destroyed. Recombinant A. fumigatus strains generated should pose no greater threat to immunocompromised individuals or women of child-bearing age or who are expecting or planning a pregnancy. Respiratory exposures to wild-type A. fumigatus is constant in both indoor and outdoor environments.

Environment:

A. fumigatus as well as being extremely common in the air (spores) is also extremely common and ubiquitous in soil and decaying plant material. The recombinant strains will have no positive phenotypic effect that would make them advantageous in the environment compared to the wild-type strain and are likely to be less competitive. Pichia pastoris auxotrophic strains are unlikely to survive in the environment without supplementation and recombinant gene expression is methanol induced and unlikely to occur outside the laboratory. In addition, all manipulations are on a small laboratory scale and only small amounts of recombinant material will be generated. All work is carried out in a class II laboratory and all open manipulations where spores may escape are conducted in a Microbiological Class II safety cabinet that is routinely maintained under a service contract. In addition, biomass and materials are destroyed by contact with Trigene and autoclaving prior to disposal. In the unlikely event of release into the environment, no risks are envisaged.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Some of the work will involve studying the ability of genetically modified strains to infect insect larvae such as Wax moth larvae. The larvae themselves are not genetically modified. Infected larvae will be contained in sealed plastic boxes and destroyed by autoclaving prior to disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste will be completely inactivated by autoclaving (121 degrees for 15 min). Autoclave runs are validated by monitoring on a chart recorder and autoclaves are maintained annually and checked by thermocouple testing. In addition, liquid wastes are treated overnight with 10% Trigene prior to autoclaving. All research members will be trained in the safe handling of sporulating fungi to minimise the release of spores into the air. For long term storage, small quantities of spores will be stored frozen in glycerol at -80 degrees C. In addition all cultures of genetically modified organism will be clearly labelled and disposed as soon as possible. All manipulation of organisms will be carried out in a Microbiological Class II safety cabinet which is routinely maintained and sterilised under a service contract. All containers (petri dishes, flasks etc) containing cultures are sealed with parafilm. Any spills will be immediately treated with 10% trigene and contaminated material autoclaved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The project was discussed at the local GM/Biohazard safety committee held on 10.02.11. Committee chair (BSO). It was commented commercially sensitive box has been ticked. However, notifications are on public register, GDR stated that work described on forms was not commercially sensitive. Noted that spores will not naturally infect wax moth larvae, hence need for injections this information needs to be added under environmental risk section. Suggested replacing animal from with description of the procedure used to inject, including the hazards, estimated risk and control measures. Under section 7 of the CU2 form, virulence genes need to be mentioned with a discussion of possible outcomes of oer expression. Other minor amendments were also noted.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tbody>
<tr>
<td>L2  Yes L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
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</table>

**Project Ref**  541/12.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>03/01/2012</td>
<td>The investigation of the biochemical and biological effects of proteins on haemopoietic cells utilizing lentiviral vectors</td>
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</table>

<table>
<thead>
<tr>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
</tr>
</tbody>
</table>

Non-GMM  Consent Granted

Project notified under transitional arrangements  N

Withdrawn  N  Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**
**Purposes of the contained use**

We are involved in the systematic analysis of stem cell differentiation and oncogenic transformation of haemopoietic cell lines via proteomic and phosphoproteomic analysis. Protein profiles obtained by Mass Spec analysis of differentiating cells and the consequences of leukaemic oncogene expression on the proteome will allow potential targets to be identified. Lentiviral vectors will be employed to either express genes of interest or reduce their expression via delivery of shRNA whilst simultaneously expressing a mutated form of the protein of interest. The work will be undertaken in primary haemopoietic cells and murine and human cell lines. The genes will be assessed for their effects on the growth, survival, motility and differentiation capabilities of the cells.

**Recipient or parental organism**

Target cells will be either primary or well established immortalised murine or human haemopoietic cells. Cell lines include; Baf3, FDCP-Mix, NFS60- and K562.

**Host/vector system**

Replication disabled self inactivating lentiviral vectors will be used to transfect either primary or well established immortalised murine or human haemopoietic cells. The lentiviral expression system use a third-generation HIV derived lentiviral system. The system consists of three packaging plasmids and a transfer vector containing the gene of interest. The structural complexity of the lentiviral genome has been designed to minimize its relation to the wild-type, human HIV-1 virus to enhance biosafety. The key safety features are:

1. The expression vector contains a deletion in the 3’ LTR that is copied to the 5’ LTR upon integration leading to self-inactivation of the lentivirus. Once targeted into the transduced target cell, the lentiviral genome is no longer capable of producing packagable viral genome hence cannot be rescued by replication competent virus.

2. The number of genes from HIV-1 that are used in the system has been reduced to three. Rev is on a separate vector to gag, and pol. This means that 5 genes are absent including tat which is essential for viral replication.

3. Viral particles are packaged in Envelope proteins from vesicular stomatitis virus rather than HIV-1.

4. Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gag, pol, rev, env) none of them contain LTRs or the \( \Psi \) packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus are never expressed in the transduced target cell. No new replication-competent virus can be produced.

**Origin & function**

The genes we will be experimenting with will be identified through our Mass spectrometry screening programme of stem cell differentiation and leukaemogenic transformation. This means we will be investigating Mutine and human cDNA of various genes which will include:

CD45, a haemopoietic specific phosphatase involved in the regulation of cytokine signalling; FMIP, a protein involved in RNA transport and M-CSF signalling.

**Evaluation of foreseeable effects**

The GMOs in this proposal will consist of replication defective lentiviral vectors and human and murine haemopoietic cells.

Although the inserts within the lentiviral vectors will be expressed at levels high enough to achieve biological activity and could feasibly have a deleterious effect if delivered to a target tissue the fact that the DNA has been introduced without the ability to infect other cells and that the cells would rapidly induce an immune response make it very unlikely that any harmful effects would be seen (VSV are inactivated by Complement and \( \alpha \)-galactosyl natural antibody mediated mechanisms in human serum). The modifications to the lentivirus should not have any affect on host defence mechanisms or the tropism or host range. On packaging into the VSV coat proteins the lentivirus will be capable of infecting a variety of mammals however the virus will be replication defective and will therefore not cause an infectious disease. The safety features mentioned above insure that integrated vector DNA cannot be rescued by replication competent virus hence is unlikely to become mobilized. Although the genes will be expressed, or reduced in the presence of the shRNA, we do not expect them to have a deleterious effect. For example, although CD45 is involved in signal transduction it is
expressed on all hematopoietic cells being a defining marker of the hematopoietic system. Thus increased expression should not modulate the mature hematopoietic cell function to any extent. Further, there is no evidence of involvement of CD45 in complement activation processes.

Retroviruses are fragile structures and do not survive for long periods in the environment. Eukaryotic haemopoietic cells and the established cell lines to be used are fragile requiring distinct conditions for survival not available in the environment (temperature, growth factors etc). The cell lines would therefore not survive in the environment. The most likely routes of transmission however will be by inoculation by sharps, contact with open wounds and exposure to aerosols. Local rules requiring the dressing of open wounds, the use of gloves and the avoidance of the use of sharps will minimise transmission by the first two routes. The use of a class II biological safety cabinet will provide adequate protection against aerosols.

The conceivable routes of transmission to the environment would occur during waste disposal, accidental spillage or via aerosol production. The use of a class II biological safety cabinet will prevent the danger posed by aerosols. Standard laboratory practices of disinfecting liquid waste with Virkon (Antec International) and autoclaving solid waste will adequately control the risk during waste disposal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| Not Applicable |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| No application for derogation from full containment measures |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The waste material within this project includes the following GMMs E. coli K12, replication incompetent lentiviruses, murine cell lines. Further waste includes plasticware and other disposables used during experimental protocols. All waste material is treated with Virkon (Antec International) as per manufacturers instructions. A 1% solution gives effectively 100% kill of all the GMMs in this project within 30 minutes. (Manufacturers and independent testing). Liquid waste will be treated with a solution of 1% Virkon for a minimum of 2 hours before disposal down the sink. Solid waste treated with a solution of 1% Virkon for a minimum of 2 hours before disposal via autoclaving. Autoclaves are routinely run on a 30 minute cycle at 121°C. This ensures effectively 100% kill. The autoclaves have a data logging system. The autoclaves are serviced annually by LTE Scientific, Oldham which includes a UKAS calibraton and load validation.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

**Please enter comments on the GM safety committee on the risk assessment**

The committee sought clarification of the waste disposal methods. Satisfactory answers were received by the PIs representative.

**Project Containment**

| Laboratory Activities | Glass Houses | Growth Rooms |

02/03/2022
<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<th>CultureVolumeClass3-4</th>
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<td>23/03/2012</td>
<td>Modulation of transforming growth factor-beta (TGF-B1) mediated cell signalling</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<tr>
<td></td>
<td>pathways using adenoviral gene delivery systems to analyse their effect on cellular</td>
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<td></td>
<td>properties in the peritoneum such as proliferation, apoptosis, migration, fate and</td>
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<td></td>
<td>protein synthesis</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
- Project notified under transitional arrangements N

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

**Purposes of the contained use**

To virally transduce mouse peritoneum in vivo with replication deficient adenovirus expressing TGF-B1 transgene. Insertion of the transgene is in the E1 site which prevents a rescue of replication potential after possible subsequent recombination. The transgene is under the control of a mouse cytomegalovirus (CMV) immediate early promoter and terminated by the SV40 polyadenylation signal. The mouse CMV promoter is functional in a number of mammalian cells including human.

**Recipient or parental organism**

Mouse peritoneum

**Host/vector system**

The adenovirus was constructed by intracellular homologous recombination of a recombinant plasmid (comprising the cDNA of TGFβ inserted into the multiple cloning site of shuttle plasmid pACCMV using BgIII) and an adenovirus derivative (PJM17) in 293 cells. The shuttle plasmid contains a partial adenovirus type 5 genome with a human CMV promoter, a multiple cloning region and the SV40 polyadenylation signal inserted into the E1 region. Adenovirus derivative PMJ17 carries the entire Ad5 genome with a deletion/substitution in E3 and insertion of pBR322 in E1. Each of these will limit the risk of production of wild-type virus after recombination.
The adenovirus leads to expression of porcine transforming growth factor - beta 1 (TGF-β1) which is involved in fibrosis (scarring), and angiogenesis (blood vessel growth). AdTGF-β1 was created with TGF-β1 cDNA mutated at residues 223 and 225 so that the transgene product does not bind to latency-associated protein (LAP) and is therefore synthesized as in a biologically active form. The gene is under the control of the mouse cytomegalovirus (CMV) immediate early promoter and terminated by the SV40 polyadenylation signal.

TGF-β1 is a multifunctional cytokine that is central to increased collagen production and has been used topically to aid wound healing. When delivered to an organ or tissue, it may induce scarring/fibrosis. TGF-β1 has pro- and anti-wound inflammatory actions and can induce epithelial to mesenchymal transdifferentiation in certain epithelial-like cells. In a mouse model, intraperitoneal administration of both AdTGF-β1 or control adenovirus results in a transient, mild peritonitis that had resolved by day 4. Transient peritoneal expression of the ADTGF-β1 will induce fibrotic changes in the peritoneum. Expression of the transgene will peak around day 7 and then will return to baseline around day 14.

**Evaluation of foreseeable effects**

The gene product expressed is expected to affect cellular properties such as proliferation, apoptosis, migration, fate and protein synthesis. There would be no transfer of genetic material with other organisms and there would be no harmful products of gene expression. Peritoneal cells will be transiently transfected with replication-deficient adenovirus and will show no phenotypic or genetic instability or any other harmful effect. It is highly unlikely that replication competent virus will be produced. Good laboratory practice in accordance with the risk assessment of the replication competent virus will be produced. Good laboratory practice in accordance with the risk assessment of the experimental procedure will ensure that the worker or others within the laboratory will not be exposed to the virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

This adenovirus will be used at level 2 biohazard containment. All bench and animal work will be carried out in a level 2 biological safety cabinet. Spillages to be cleaned with Trigene. All liquid waste will be soaked in Trigene overnight. All solid waste to be contained separately and autoclaved. Animal waste is autoclaved prior to incineration via the clinical waste route. The area to be cleaned with Trigene afterwards. It is anticipated that this will give 100% kill.

Mice will be kept in appropriate cages in a separate category 2 area of the animal unit. Appropriate measures are in place to prevent animals escaping with barriers in place. Any escaped animal can be detected and recaptured. All experimental procedures will be carried out to minimise the chances of escape.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Spillages to be cleaned with Trigene. All liquid waste to be soaked in Trigene overnight. All solid waste to be contained separately and autoclaved. The area to be cleaned with Trigene afterwards. It is anticipated that this will give 100% kill.

Animal waste is autoclaved prior to incineration via the clinical waste route. Sharps and any other consumables used in the adenovirus dilution and administration will be classed as clinical waste and autoclaved. Used cages will be sealed whole, autoclaved and cleaned by the animal technicians. Autoclave load is monitored to ensure steam penetration and load temperature is validated for killing efficacy and regular tests will confirm total inactivation of contaminated waste has occurred.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

02/03/2022
Q: Does the virus affect inflammation
A: The people who developed the model have not seen any signs of TGF-beta1 down regulating inflammation.

Q: As adenovirus is shed in the urine how would bedding be treated.
A: Bedding and cage are autoclaved in a containment class 2 autoclave and then the bedding is disposed of as clinical waste and the cage is washed as normal.

Q: How is stock of concentrated virus diluted.
A: The concentrated virus is transported to the BSF in a double boxed container and diluted in a class 2 cabinet in the BSF.

Q: Where is the gene inserted in the adenovirus
A: SaH will check this and amend the form

Q: The gene is under control of a mouse promoter. Can this work in humans
A: SaH will check this and amend the form

The appropriate changes were made and the committee are happy with the form and a

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
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**Project Ref** 541/12.3

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<td>Generation of adult cardiomyocytes from skin fibroblasts using cell reprogramming approach</td>
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<td>02/03/2022</td>
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The main objective of this project is to generate human adult cardiomyocytes from skin fibroblasts to translate our findings in animal models to a human relevant model using human induced pluripotent stem cell (iPS) and iPS-derived cardiomyocytes (iPS-CM) technologies.

We will first reprogram skin fibroblasts to form inducible pluripotent stem cells (iPS) by expressing a cocktail of transcription factors: Oct4, ASox2, Mef2c, Klf4 (Takahashi et al., Cell. 2007;131(5):861-72). Then cardiomyocytes will be generated from the iPS using spontaneous differentiation protocol (Guan et al., Circ Res. 2007;100(11):1615-25). iPS formation will only be achieved if a highly efficient gene transfer system is used (Guan er al., Nat Protoc. 2009;4(2):143-54.) Lentiviral vector constructs have proven to be very productive in terms of transduction due to their ability to infect both replicating and non-replicating cells, including stem cells. It has been reported that lentiviral system is a method of choice for gene delivery system for generating iPS from fibroblasts because it integrates into the genome which make the gene transfer highly efficient and stable compared to the adenovirus system (Narsinh et al., Circ. Res. 2011, 108:1146-1156). Therefore we will use this method in this project.

Recipient or parental organism
Human and mouse

Host/vector system
Host cells will be: HEK293 cells, primary human/mouse skin fibroblasts, E.coli DH5a, JM109, TOP10, BJ5183, XL10-Gold, XL1 Blue.
Vectors will be: pHAGE-mSTEMCCA, pHAGE-hSTEMCCA, oHDM-Hgpm2, pHDM-tat1b, pRC/CCMV-rev1B, pHDM- VSV-G

Origin & function
Oct 4 (human and mouse): This gene encodes a transcription factor containing a POU homeodomain. This transcription factor plays a role in embryonic development, especially during early embryogenesis, and it is necessary for embryonic stem cell pluripotency. Stem cells maintain expression of Oct4, consistent with the stem cell hypothesis of carcinogenesis.

Sox 2 (human and mouse): This gene encodes a member of the SRY-related HMG-box (SOX) family of transcription facotrs involved in the regulation of embryonic development and in the determination of cell fate. The product of this gene is required for stem-cell maintenance in the central nervous system, and also regulates gene expression in the stomach. Stem cells maintain expression of Sox2, consistent with the stem cell hypothesis of carcinogenesis.

Mef2c (human and mouse): This locus encodes a member of the MADS box transcription enhancer factor 2 (MEF2) family of proteins, which play a role in myogenesis. The
encoded protein, MEF2 polypeptide C, has both trans-activating and DNA binding activities. This protein may play a role in maintaining the differentiated state of muscle cells. Mef2c is not expressed in cancer cells.

Klf4 (human and mouse): Krueppel-like factor 4 is a protein that in humans is encoded by the KLF4 gene. In embryonic stem cells (ESCs), KLF4 has been demonstrated to be a good indicator of stem-like capacity. It is suggested that the same is true in mesenchymal stem cells (MSCs). KLF4 was not detected in proliferating or cancer cells, indicating a non-cell autonomous effect of KLF4 on proliferation and carcinogenesis (Tetreault et al., Gastroenterology. 2010;139(6):2124-2134).

Lentivirus packaging genes (HIV-gag, HIV-env, HIV-tat, HIV-rev and VSV-G): The HIV lentivirus RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and nine genes (gag, pol, and env, tat, rev, nef, vif, vpr, vpu, and sometimes a tenth vpr, which is a fusion of tat env and rev), encoding 19 proteins. Three of these genes, gag, pol, and env, contain information needed to make the structural proteins for new virus particles. During viral replication, the integrated DNA provirus is transcribed into mRNA, which is then spliced into smaller pieces. These small pieces are exported from the nucleus into the cytoplasm, where the are translated into the regulatory proteins Tat (which encourages new virus production) and Rev. As the newly produced Rev protein accumulates in the nucleus, it binds to viral mRNAs and allows unspliced RNAs to leave the nucleus, where they are otherwise retained until spliced. At his stage, the structural proteins Gag and Env are produced from the full-length mRNA. The full-length RNA is actually the virus genome; it binds to the Gag protein and is packaged into new virus particles (Pollard et al., Annu. Rev. Microbiol. 52: 491-532).

**Evaluation of foreseeable effects**

The viral vector is replication defective and the insertion of the genes listed above will not alter the properties of the final GM virus. Possible hazards associated with this vector may be caused by stable expression of the transgenes, insertional mutagenesis and potential for generation of replication competent virus. Lentiviral stocks may be contaminated with replication-competent viruses (RCV), generated by rare spontaneous recombination events in the propagating cell lines. However, the use of third generation vector using 5 plasmids approach as described above will significantly minimize the likelihood of RCV generation. Nonetheless, each preparation is screened for the presence of wild type contamination. Batches in which replication competent viruses are found at this level will be discarded by autoclaving and decontamination using 1% Virkon solution.

The viral vector has the potential to deliver its genetic material into humans. However, the expression vector is replication-incompetent in any mammalian cells since the part of the viral genome critical for viral replication has been deleted. Therefore, the potential hazard will be mainly due to the activity of the inserted genes.

The most likely route of accidental infection with a retrovirus will be via inadvertent percutaneous inoculation due to spilled reagent during pipetting, skin contact and ingestion. To minimise the risk workers must use suitable lab coats. Gloves must be worn at all times. Upon leaving the lab both gloves and lab coats must be removed and hands washed in the wash hand basin provided. Lab coats should not be worn or taken outside the laboratory except for autoclaving and cleaning and the safety procedures in place for this must be followed. All work will be performed in a Class 2 lab using Class 2 microbiological safety cabinet. Sharp material, i.e. glass pipette will be used to isolate stem cell colonies. To minimise inadvertent exposure due to the use of sharp materials the following procedures must be followed:

* Sharp materials should be used with extreme care and only when necessary.
* Ensure that sharp materials are not left exposed, but always removed promptly in the sharp bin
* Avoid injuries during preparation of Pasteur pipette
* Consider double gloving with a larger pair of gloves innermost for optimum comfort. Double gloving does not prevent sharps injury but has been shown to effect up to a six-fold decrease in inner glove puncture.
* Sharp materials must be discarded into a yellow sharps container which is clearly marked with the British safety number 7320 and UN3291. When the sharps container is 2/3 full the lid must be closed securely. The identification label must be completed to identify user, date and department. The closed bin must immediately be moved to the decontamination facility in room 1.402 stopford building which is located very close to our laboratory (1.302 stopford building).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste material will be packaged into biohazard clear autoclave bags and then decontaminated by autoclave before being discarded as clinical waste. Liquid waste will be soaked in 1% virkon for at least 1 hour before being discarded into general laboratory sink. Sharp materials will be discarded into a yellow sharps container which is clearly marked with the British safety number 7320 and UN3291. When the sharps container is 2/3 full the lid must be closed securely. The identification label must be completed to identify user, date and department. The closed bin must immediately be moved to the decontamination facility in room 1.402 stopford building which is located very close to our laboratory (1.302 stopford building).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The nature of the inserted genes and the VSV-G surface protein used in the virus packaging system have been discussed. It’s agreed that due to the activity of inserted genes in regulating cell growth and development all the work using these lentiviral vectors is classified as class 2 activities. The committee has suggested to precisely defined the safety measure in particular in the use of sharp materials Standard operating procedure (SOP) will be generated by the investigator.

Project Containment

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Project Ref 541/14.1

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<td>Study of Plasmodium biology: genetic and biochemical screenings of essential and</td>
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The containment is required for the cultivation of erythrocytic stages of Plasmodium parasites. Parasites are maintained in in-vitro cell cultures by providing human red blood cells to support their growth. Erythrocytic stages are maintained in in-vitro cell cultures by providing human red blood cells to support their growth. Erythrocytic stages are used in the presented research project to study the biology of Plasmodium strains competent to infect humans. This project aims to discover and validate targets useful for malaria therapeutic interventions by applying genetic biochemical and cellular techniques presented.

Plasmodium is an apicomplexa parasite that completes its life cycle infecting alternatively mosquitoes (genus Anopheles) and vertebrates. In vertebrates it causes malaria. Human malaria is a deadly disease that affects about a half of the world population. The present project aims to study essential and critical genes for parasite asexual development and sexual differentiation. The strains under study are P. falciparum, vivax and knowlesi.

In mosquitoes and humans Plasmodium respectively accomplishes sexual and asexual development. Plasmocium developmental stages used in this project are the erythrocytic stages and the initial phases of sexual development in absence of the vector. These stages are cultivated in vitro by providing human red blood cells (RBC), essential to support parasite growth. In fact Plasmodium is an obligate intracelular parasite.

Fragments of DNA that encode for fluorescent proteins such as GFP, yFP and RFP derivates will be used as probes for transfected parasites, for promoter functions, for protein tagging and for trafficking detection. These fragments originate from jellyfish and corals. They enable detection and visualization of parasites using fluorescence microscopy or flow cytometry.

Fragments of DNA that encode for SB100x transposase, TALEN, endonucleases, recombinases, recombinase will be used in this project. SB100X derives from Sleeping Beauty transposases and was produced and characterized by the Izsvak's laboratory (Nat Genet. 2009 June ; 41(6): 753-76L1). SB100X enables the insertion of a DNA fragment flanked by specific inverted repeats into the genome. TALEN is a chimera of TALE (Transcription activator -like effectors from gene product recognizes specific
DNA sequences and digests in the proximity of them. Homing endonucleases, such as Scel, specifically recognize and digest long stretches of DNA sequence (Gimble & Thorner, Nature, 1992, vol 35: hybridized short RNA molecule (Mali P. et al., Science, 2013, vol339, pp823-826). Cre recombinase is a tyrosine recombinase enzyme derived from the P1 Bacteriophage (Abremski & Hoess Journal of Biological Chemistry 1984, 259: 1509-1514) and Flp is a recombinase enzyme derived from Saccharomyces cerevisiae (Broach & Hicks, Cell 1980, vol 21: pp. 501-508). These recombinases excise and insert DNA fragments flanked by specific DNA sequences called Lox and Frt, respectively. All these gene products enable genome editing and recombination. These molecules will be used to generate parasites that are defective in metabolic functions.

Other fragments of DNA included in our plasmids encodes for specific drug resistance, such as human dyhydrofolate reductase (hDHFR), yeast dihydroorotate dehydrogenase (yDHODH) and blasticidin-S deaminase (BSD); Tetracycline transactivator proteins, and specific Plasmodium proteins or protein fragments under biochemical characterization. Accessory DNA fragments are used for optimal plasmid segregation and Plasmodium expression, as minimal centromer, Rep20, Promoters and 3’ untranslated regions.

Evaluation of foreseeable effects

There is no risk in the genetic modifications included in this project for multiple reasons (1) The modifications will not cause a gain of function, or increase the wildtype danger, infectivity, virulence and environment or disinfectant resistance. In fact, used approaches aim to disable parasites in any of their vital functions and virulence, in order to gain access to unknown gene functions. (2) drug resistance genes (above listed), used to select transfected parasite, will be the only gain of functions. Nevertheless, despite the introduction of drug resistance genes, strains used in the present project will remain pharmacologically treatable, if therapeutic intervention is needed for accidental events. (3) There is no possibility to spread the modified parasite, wild type or mutant can survive in the environment. In fact, minimum changes is salts concentration of the media or air exposure will result in hemolyzed or dried RBC with consequent death of parasites (i.e. tap water kills parasites by RBC lysis). In order to reduce the risk of accidental injuries (like cuts or punctured wounds) that may expose to infection, glassware will be avoided as much as possible (details in the code of practice), and sharps (blades or needles) must not be used during Plasmodium culturing.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We request some derogations to CL3 containment level in accordance to the fact that cultivated stages cannot cause airborne transmission and the vector is absent from the natural environment in the United Kingdom. In particular, the requested derogations regard the following CL3 requirements: sealable laboratory for fumigation (M2), entry to laboratory via airlock (M4), negative pressure relative to the pressure in the immediate surroundings (M5) and HEPA filtered air from the laboratory (M6)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Treatment of contaminated items.
- Pippette tips and Graduated Pipettes: Place into polypropylene jars containing 2% Virkon. Incubate for 24 hours and then Virkon solution will be poured down the sink and solid waste disposed into autoclavable 'clini' bags.
- Microfuge tubes, Disposable cuvettes, Electroporation cuvettes, flasks and plates: they should be placed in a separate autoclavable 'clini' bag within the microbiological safety cabinet.
- 'Clini' autoclavable bages: When 3/4 full the neck of the autoclavable bag is loosely tied and moved into the autoclave tins and autoclaved within the suite. After autoclaving it is sent off site for incineration.
- Glass slides used in preparing blood smears of Plasmodium-infected red blood cells: they should be placed in a sharps bin. Plasmodium in blood smears on glass slides is inactivated by means of the fixative used to fix the blood film to the slide prior to staining. Because of this inactivation the glass slides can be disposed of by placing in a sharps bin and entering the clinical waste route.
- Liquid Waste: Cuvettes, glasks and universal/falcon tubes containing liquid waste are to be emptied or aspirated into jars containing 2% Virkon and after 24 hours incubation disposed down into the sink.

Disinfection procedures.
- Surface sterilisation; Hard surfaces such as the work surface of the safety cabinet or benches must be disinfected with 70% Ethanol.
- Equipments and their parts must be disinfected with 1% Distel.
- Spillages: Suspected contamination on hands, gloves or work surfaces must be disinfected immediately with 70% Ethanol. Minor spillages (up to ~10 ml) should be treated with 70% Ethanol and wiped with towels. Large spillages (>10 ml) must be treated with Virkon powder and then wiped with 70% Ethanol soaked towels.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project was first presented to the FLS GM/Biohazard local committee on 28/06/2013 and discussed fully at a sub-committee meeting on 28/08/2013. It was decided that the work will take place in a separate Containment level 2 laboratory with restricted access (swipecard and PIN) and a limited number of registered users. It is in fact the suite used for the schedule 5 pathogens and toxins. The room cannot be fumigated and although it is under negative pressure, this is not currently monitored.

IR was asked to list the derogations from containment level 3 that are being requested - essentially fumigation monitored negative pressure.

As there is no vector present, the only route of infection is for the red blood cells containing the plasmodium (at a certain stage in their life cycle) to enter the blood stream via a cut or wound. IR was asked to consider the possibility of avoiding the use of glassware as much as possible. Although slides and cover slips would be used, the latter can be handled with forceps. No needles or blades will be used.

The need to use live Plasmodium - containing RBCs in experiments was discussed at length. They will need to be viewed under a fluorescent microscope and IR (and LG) to look into the access arrangements in the lab that will house the new CL2 flow cytometer that will be in the MCCIR labs.

The HSE notification form and code of practice will also need to be updated after the GM forms had been amended. IR was asked to revise the forms according to all the suggestions made and the group would look at them again before sending the project to the University advisory group.

The sub-committee met again on 11.10.2013 and 23.10.2013 to consider the amendments and the forms were approved locally prior to the University GM/Biohazard Advisory Group meeting in 08.11.2013.

The Advisory group noted that:
(a) the project was to creat mutant of Plasmodium spp, the causative agent of malaria, that are defective in growth and metabolic processes using a transposase based system to generate random mutants;
(b) the agent is a HG3, however, specific derogations for some of the control measures for working at ful CL3 are being sought from HSE;
(c) the culturing would be undertaken in a highly restricted dedicated laboratory also used for home office restricted biological agents;
(d) analysis will also be undertaken in restricted access, dedicated communal cell sorter facility, however, when cell sorting using live plasmodium work is being undertaken, no other user will be using the dedicated area other than the lab manager and the PI;
(e) there were a number of precautions in place to prevent needle stick injuries.

Agreed:
(i) blood from the blood transfusion center should be used for culturing malaria;
(ii) the final version of the occupational health guidance should be incorporated into the Appendix 2 of the Code of Practice

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<table>
<thead>
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02/03/2022
This project aims to characterize staphylococcal genes encoding enzymes that are involved in the biosynthesis of wall teichoic acids (WTA) by recombinant, knock-out as well as complementation strategy. This project will involve cloning, expressing and genetically disrupting these genes. This research will be conducted using plasmids in disabled E. coli K12 hosts, and also re-introducing cloned DNA sequences back into strains of Staphylococcus aureus.

Recipient or parental organism

Escherichia coli K12 strains (incl. DH5alpha, JM109, SURE & S17-1 lambda-pir, well characterised attenuated S. aureus hosts: strain RN4220. (Nair D, Memmi G, et al. Whole-genome sequencing of Staphylococcus aureus strain RN4220, a key laboratory strain used in virulence research, identifies mutations that affect not only virulence factors but also the fitness of the strain. J Bacteriol. 2011). The only wild-type hosts to be used are the well studied S. aureus strains USA300 and Newmann (ACDP haxzard group 2).

Host/vector system

Expression vectors: pET-28a carries the N-terminal His Tag sequence followed by thrombin site and three cloning sites (see The pET-28a-c(+) vectors carry an N-terminal His Tag®/thrombin/T7.Tag® (annex 1).
Gene disruption vectors: Suicide vector pKO-R1 (annex II) will be used to create knockout mutant by allelic exchange.

Plasmid pRB474, a vector is used for cloning and expression of staphylococcal genes in S aureus.

Origin & function

Only DNA sequences constituting genes or part of genes from Staphylococcus aureus that encode wall teichoic acids biogenesis genes will be inserted into the vectors for introduction into S. aureus. Introduced DNA will be expressed either individually as full-length proteins or sub-regions, and/or as fusions to other genes (within the vector) encoding non-toxic products.

All sequences will be well characterised prior to introduction into Staphylococcus strains and will be generated by PCR using specific primers designed to amplify known DNA sequences (no random sequences will be introduced).

Introduced DNA sequences will either (i) remain on self-replicating plasmids for expression of genes or (ii) be integrated into the genome to generate gene disruption mutants of Staphylococcus aureus.

Evaluation of foreseeable effects

Human health:

The S. aureus lab strain RN4220 were recently sequenced and found to carry many mutation leads to decreased virulence. The wild-type S. aureus strains to be employed are of the type which could cause infection if exposed in a large dose. These strains have been safely used for many years and the likelihood of hazard can therefore be considered low. We will only introduce very short (<4 kb) DNA sequences into Staphylococcus using well characterised harmless vectors. Introduced DNA will be well defined genes (or parts of genes) that encode genes involved in the biogenesis of wall teichoic acid biogenesis. Since it was found that the deletion mutant deficient in wall teichoic acids (WTA) or WTA glycoepitopes are less virulent and more susceptible to antibiotics (ref 1,2), we expect that new mutants deficient in other WTA biogenesis genes will have similar phenotype: decreased virulence and more susceptible to antibiotics.

For reasons outlined above, the likelihood of harm in the event of exposure would be no greater than that with non-GM Staphylococcus and can be considered negligible.

Environment:
The disabled host strains will not replicate in the environment. Infections doses for man and animals are high and the probability of accidental infection low. The host strains are not known to be infectious for plants. The wild-type S. aureus strains may be able to replicate in the environment. The chance of transfer of the vectors to other organisms in the environment can be considered low. It is difficult to envisage how any of the genetic manipulations proposed in this project could confer a harmful phenotype or competitive advantage to the host strains. The environmental hazards associated with the project are therefore considered no greater than those associated with handling non-GM Staphylococcus aureus. The project involves small-scale work for research purposes and all contaminated material will be completely inactivated by autoclaving prior to disposal. In the highly unlikely event of release into the environment no risks are envisaged.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste materials (bacterial liquid cultures/agar plates) will be completely inactivated (100% kill) by autoclaving. All autoclave runs are validated by monitoring with a chart recorder and the autoclaves are validated annually by thermocouple testing. All solid waste will subsequently be incinerated.
The risk assessment GM-GX001: Characterization of genes involved in biosynthesis of wall teichoic acids from Staphylococcus aureus, was reviewed by the Medicine (Stopford, AV Hill and CTF) Local GM/Biohazard Committee on the 10th April 2014. Minor corrections were recommended and the amended risk assessment was reviewed at the following meeting on the 8th May 2014. The committee were satisfied that the risk assessment covered all the relevant points and was signed by the chair.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 Yes L3 L4 L2</td>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### Project Ref 541/14.3

<table>
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<th>Date Ackn'd</th>
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<tr>
<td>01/09/2014</td>
<td>Investigation into the molecular recognition by lymphocytes using lentiviral vectors</td>
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<th>Class2</th>
<th>VolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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### Historical Significant Changes

Historical Date of Additional Info

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### Purposes of the contained use

To virally transduce primary human lymphocytes with genes, microRNAs or shRNA oligonucleotides

### Recipient or parental organism

Human primary lymphocytes including natural killer (NK) and T lymphocytes isolated from PBMCs

### Host/vector system

**Lentiviral structural vectors:** pMDLg/pRRE (gag-pol packaging plasmid), pMD2.g (VSV-G envelope plasmid), pRSV-Rev (post-transcriptional regulatory plasmid)

**Lentiviral transfer vector** pLenti-PGK-Neo-DEST Transfer plasmid (PGK promoter) or pLenti-CMV-Neo-DEST Transfer plasmid (CMV promoter)

Host: HEK293T/17 cells (Human embryonic Kidney 293, large T Antigen, clone 17)

Retroviral vectors: The pQCXIN vector contains a CMV/MSV hybrid promoter in the 5' LTR to drive high titers during the packaging step. They also contain a self-inactivating 3' LTR to reduce promoter interference and drive high expression of your transgene from the internal CMV promoter. Expression of an antibiotic resistance genes is achieved through ribosome binding to an internal ribosome entry site (IRES).

Host: Using AmphoPack293 as the host system, TNA from the vector is packaged into infectious, replication-incompetent retroviral particles since pQCXIN lacks structural genes (gag, pol and env) necessary for particle formation and replication; however, these genes are stably integrated as part of the packaging cell genome. Once a high titer clone is selected, these retroviral particles can infect target cells and transmit the gene of interest but cannot replicate within these cells due to the absence of viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

### Origin & function

Genetic material: subcloned from cDNA library, for example: Components of the NK cell inhibitory and activatory pathways, such as MICA, KIR2DL1, ICAM, and HLA-Cw4, and immuno-signalling and cytoskeletal proteins, such as LAT, TCRζ, tubulin and myosin.

Intended functions: to inhibit or activate NK cells and to regulate immuno-signalling and cytoskeletal pathways in NK and T cells.

### Evaluation of foreseeable effects

These gene products expressed can affect many cellular signalling pathways, including those involved in cell proliferation, differentiation, adhesion, migration and apoptosis. The exact effects of expression of these proteins will be dependent on the cell type. However, expression would be very limited due to the replication-incompetence and self-inactivation features engineered into the lentiviral transduction system. Furthermore, the extreme vulnerability of lentiviral vectors to environmental conditions and cellular immunity makes any risk to human health extremely small. Good laboratory practice in accordance with the risk assessment of the experimental procedure will ensure that the worker or others within the laboratory will not be exposed to the virus.

In the retroviral system, expression would be very limited due to the replication-incompetence and self-inactivation features engineered into the retroviral transduction system. Furthermore, the extreme vulnerability of retroviral vectors to environmental conditions and cellular immunity makes any risk to human health extremely small. Good laboratory practice in accordance with the risk assessment of the experimental procedure will ensure that the worker or others within the laboratory will not be exposed to the virus.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Spillages to be cleaned with 1% virkon  |
| All liquid waste to be soaked in 1% virkon overnight. |
| All solid waste to be autoclaved. |
| The area to be cleaned with 70% ethanol afterwards |

It is anticipated that this will give 100% kill.

Is an emergency plan required according to regulation 20?  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Tick if you have attached a risk assessment to this form  Y

Please enter comments on the GM safety committee on the risk assessment

The project was originally considered at the local GM/Biohazard safety committee meeting held on 09.07.2013 and the committee asked for clarification on the exact biological role of the various genes to be inserted and the viral vectors being used. The risk assessments were revised and separated into 2 applications.

DMD-3: Investigation into the molecular recognition by Lymphocytes using lentiviral vectors.

DMD-4 Investigation into the molecular recognition by Lymphocytes using retroviral vectors

These were assessed by the relevant academic expert before being approved and noted at the local committee meeting held on 08.07.2014

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Project Ref  541/14.4

Date Ackn'd  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4

02/03/2022  Page 8502 of 15326
The aim of this project is to use the commercially available Sendai Virus kit to reprogramme human primary cells into iPSCs that can then be used for any further studies. The Sendai Virus, SeV, (mouse parainfluenza virus type 1, hemagglutinating virus of Japan (HVJ)) is a nonsegmented negative-strand RNA virus belonging to the Paramyxovirus family. CytoTune™-iPS Reprogramming System, that we will be using, uses vectors based on replication incompetent Sendai virus (SeV) to safely and effectively deliver and express key genetic factors necessary for reprogramming somatic cells into iPSCs.

Human primary cell (embryonic and adult skin fibroblasts, peripheral blood cells) will be used. Tissues or BioBanks, and patient biopsies via collaborations are all sources that will be used to obtain the cell line.

The Sendai Virus, SeV, (mouse parainfluenza virus type 1, hemagglutinating virus of Japan (HVJ)) is a nonsegmented negative-strand RNA virus belonging to the Paramyxovirus family. SeV vectors used in this kit consist of viral proteins NP, P, M, F (activated), HN, and L, and the SeV genome RNA, from which the F gene is deleted. Because SeV infects cells by attaching itself to cell surface receptor sialic acid, present on the surface of many cell types of different species, the vectors are able to transduce a wide range of cells. However, they are no longer capable of producing infectious particles from infected cells, because the viral genome lacks the F-gene. In addition, the presence of functional mutations such as temperature sensitivity in the amino acid sequence of several SeV proteins (SeVfTS6F, SeVrrS12t:.F, and SeVFt515t:.F) renders the vectors easily removable from transduced cells.

SeV vector can express the reprogramming genes without chromosomal integration. The Sendai virus vectors commercially bought contain RNA sequence that will express factors hOct3/4, hSox2, hKlf4, and hc-Myc. After
transduction, the viral vectors will cause the cells to express these four gene proteins. ALL the four transcription factors, Oct4, Sox2, cMyc, and Klf4 are both necessary and sufficient to induce pluripotent gene expression and therefore reprogramme somatic cells into a stem cell like state (iPSCs). ALL 4 vectors are required for reprogramming and the frequency of all 4 vectors getting into the same cell, executing reprogramming and generating a sustained colony in OPTIMISED culture conditions in vitro is only 0.01-<1%. There is no evidence that Sox-2 alone could reprogramme skin cells to neural especially as any skin cells infected would be terminally differentiated transient or dieing. The likelihood of any of these individual factors having effects on skin cells is negligible even in the unlikely event of skin contact since the RNA virus does not insert so is not propagated. No evidence that this has occurred during reprogramming is available even for integrating viruses (e.g.lenti).

Outside the generation of iPSC cells these factors have other functions. c-myc is a well recognised oncogene, Krueppel-like factor 1 is a transcription factor that is necessary for the proper maturation of erythroid (red blood) cells. SOX2, is a transcription factor that is essential for maintaining self-renewal, or pluripotency, of undifferentiated embryonic stem cells. Sox2 has a critical role in maintenance of embryonic and neural stem cells. Sox2 binds to DNA cooperatively with Oct4 at non-palindromic sequences to activate transcription of key pluripotency factors. Oct 4 is critically involved in the self-renewal of undifferentiated embryonic stem cells. All 4 are needed for reprogramming making this a low frequency event ( see later).

The virus is replication incompetent and the genes are only expressed in a transient manner so expression will not be constant enough to cause any significant changes via infection. PPE will always be worn (gloves and lab coat) as per University guild lines and regulations, so no skin will actually be exposed. In the extreme situation that an infection occurs to the skin should not cause any significant damage. Infection will occur only in the upper dead or terminally differentiated layers of the skin. Therefore, if infection occurs, it will be transitory as the cells are sloughed naturally.

The SeV vectors used in this kit are non-integrative, non-transmissable and not pathogen to humans and so the risk is very low. The viral vectors maintain full infectivity to a wide range of cells, including human cells, however they are no longer capable of producing infectious particles from infected cells because the viral genome lacks the F gene. Fusion PROTEIN (F) fuses the viral envelope with cell membrane when the virus enters the cell but the virus can't make any more. The gene encoding the F protein is deleted from the CytoTune™reprogramming vectors, rendering them incapable of shedding infectious particles from infected cells, and therefore it is replication incompetent. Therefore although c-myc is an oncogene the likelihood of shedding c-myc containing particles is negligible.

PPE will always be worn (gloves and lab coat) as per University guild lines and regulations, so no skin will actually be exposed. In the extreme situation that an infection occurs to the skin should not cause any significant damage, as the virus is replication incompetent as well as a non-integrative vector with transient expression only. This means that this system is much safer than most lentiviral vector system even with the presence of c-myc. The risk of teratomas is negligible. It is actually quite hard to generate these using >1 million cells injected in vivo, (~50% success). ALL 4 vectors are required for reprogramming and the frequency of all 4 vectors getting into the same cell, executing reprogramming and generating a sustained colony in optimised culture conditions in vitro is only 0.01-<1%.

Evaluation of foreseeable effects

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All liquid waste to be soaked in 1% virkon overnight
All solid waste to be autoclaved.
The area to be cleaned with 70% ethanol afterwards.
It is anticipated that this will give 100% kill.

13.
The project SJK-1 (risk assessment submitted with this notification) was initially presented to the FLS GM/Biohazard local committee held on 08.07.2014. The committee made the following comments:
How is the virus disabled? The committee felt that "F-protein deletion" was not sufficient explanation, and asked for more details and further information on why this would prevent new virus being formed.
To address concerns about local infection of the operator, if it splashes/spills onto broken skin.
Origin of the human cells to be used in the study, and screening status of the samples.
Details as to why a class II microbiological safety cabinet is being used in the project.
Details required as to how the samples are manipulated - e.g. use of sharps, etc.
The committee was unable at this stage to determine whether the project was GM class 1 or 2.
The committee chair (Dr. G) subsequently met with the project PI (Professor K) in order to address these concerns. The project was amended and resubmitted as GM class 2. Dr. G finally signed the project off on 10.10.2014.

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 541/16.1
The main objective of the project is to generate gene knock-out cells appropriate for cardiovascular research using the Crispr-Cas9 lentiviral system. Crispr-Cas9 is a technique that can enable us to precisely define the functional aspect of genes. Lentiviral vector constructs have proven to be very productive in terms of transduction due to their ability to infect both replicating and non-replicating cells, including stem cells. We will study the functional mechanistic aspects of genetic variants associated with cardiovascular development and functions. In these stem cell models, the genotypes of embryonic stem or induced pluripotent stem cells (iPSC) will be edited with the Crispr-Cas9 lentiviral system. Alternatively, genotypes of fibroblast cells will be edited prior to iPSC generation. We will generate embryonic stem, iPSCs or fibroblast cell lines with stably integrated plasmids containing Cas9 nuclease gene and specific guide RNA (gRNA) sequence against target genes. Through transduction with lentiviral particles, the transduced cells will be selected by media containing puromycin/reporter gene products for obtaining a population of cells with the plasmid successfully integrated/high expression of Cas9 nuclease and gRNA. The stem or iPSC cells with edited genotypes will be differentiated into cell types that are related to cardiovascular development and functions. For efficiency testing, cell lines that can be easier maintained and transduced/transfected will be transduced/genome edited/assayed first (eg HEK293, HepG2 etc). For negative controls, Crispr-Cas9 plasmids without or with scrambled gRNA sequence may be used.

Recipient or parental organism

Parental organism is a 2nd generation lentivirus vector derived from HIV-1.

Host/vector system

Host cells will be human/mouse ESC, iPSC or fibroblasts, HEK293, HepG2, E.coli DH5a. TOP10 Vectors will be: pLentiCRISPRV2 (modified), pCMV-VSV-G, pMD2G, psPAX2,
CRISPR-Cas9 gene and guide RNA CRISPR consists of two components. a "guide" RNA (gRNA) and a non-specific CRISPR-associated endonuclease (Cas9) The gRNA is a short synthetic RNA composed of a "scaffold" sequence necessary for Cas9-binding and a user-defined -20 nucleotide spacer" or "targeting" sequence which defines the genomic target to be modified. Therefore, one can change the genomic target of Cas9 by simply changing the targeting sequence present in the gRNA CRISPR was originally used to "knock-out target genes in various cell types and organisms, but modifications to the Cas9 enzyme have extended the application of CRISPR to selectively activate or repress target genes, purify specific regions of DNA and even image DNA in live cells using fluorescence microscopy. Furthermore the ease of generating gRNAs makes CRISPR one of the most scalable genome editing technologies and has been recently utilized for genome-wide screens (https://www.addgene.org/CRISPR/guide/)

Lentivirus packaging genes (Gag, Pol, tat, rev and VSV-G/Env). The HIV lentivirus RNA genome consists of at least seven structural land marks (LTR, TAR RRE, PE, SLIP, CRS, and INS), and nine genes (gag, pol env, tat, rev, nef, vif, vpr, vpu, and sometimes a tenth ev, which is a fusion of tat env and rev) encoding 19 proteins. Three of these genes gag pol, and env. contain information needed to make the structural proteins for new virus particles. During viral replication the integrated DNA provirus is transcribed into mRNA, which is then spliced into smaller pieces

These small pieces are exported from the nucleus into the cytoplasm. where they are translated into the regulatory proteins Tat (which encourages new virus production) and Rev as the newly produced Rev protein accumulates in the nucleus. It binds to viral mRNAs and allows unspliced RNAs to leave the nucleus, where they are otherwise retained until spliced. At this stage the structural proteins Gag and Env are produced from the full-length mRNA. The full-length RNA is actually the virus genome. It binds to the Gag protein and is packaged into new virus particles (Pollard et al, Annu Rev. Microbial 52 491-532)

Evaluation of foreseeable effects

The viral vector is replication defective and the insertion of the genes listed above will not alter the properties of the final GM virus. Possible hazards associated with this vector may be caused by stable expression of the transgenes, insertional mutagenesis and potential for generation of replication competent virus. Lentiviral stocks may be contaminated with replication-competent viruses (RCV), generated by rare spontaneous recombination events In the propagating cell lines. However, the use of second generation vector using 3 plasmids approach will significantly minimize the likelihood of RCV generation. Nonetheless, each preparation is screened for the presence of wild type contamination. Batches in which replication competent viruses are found at this level will be discarded by autoclaving and decontamination using 1% Virkon solution. The viral vector has the potential to deliver its genetic material into humans. However, the expression vector is replication-incompetent in any mammalian cells since part of the viral genome critical for viral replication has been deleted. Therefore the potential hazard will be mainly due to the activity of the inserted genes or insertional mutagenesis.

The genes (including annotated/unannotated genes non-coding RNA genes and transcribed/untranscribed non-genic genomic regions) of interest (targeted by the gRNA) are those related to cardiovascular development and functions selected/discovered from genetic studies. Most of the candidate genes In this study are of unknown functions while some genes are known as regulators of cell growth differentiation/development or physiological functions in the cardiovascular system. We will target both human and mouse genes whenever appropriate if a candidate gene is later known to be involved in oncogenesis. we will review the risk assessment.

The most likely route of accidental infection with a retrovirus will be via inadvertent percutaneous inoculation due to spilled reagent during pipetting, skin contact and ingestion. To minimise the risk workers must use suitable lab coats.
Gloves must be worn at all times upon leaving the lab both gloves and lab coats must be removed and hands washed in the wash-hand basin provided. Lab coats should not be worn or taken outside the laboratory except for autoclaving and cleaning and the safety procedures in place for this must be followed. All work will be performed in a Containment level 2 lab using Class II microbiological safety cabinet.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid waste material will be packaged into biohazard clear autoclave bags and then decontaminated by autoclave before being discarded as clinical waste liquid waste will be soaked in 1% Virkon for at least 1 hour before being discarded into general laboratory sink sharp materials will be discarded into a yellow sharps container which is clearly marked with the British safety number 7320 and UN 3291. When the sharps container is 2/3 full the lid must be closed securely. The identification label must be completed to identify user, date and department. The closed bin must be immediately moved to the larger clinical waste collection bins in room 3 44 CTF building (for 3 23b CTF) or room 5 018 AV Hill BUilding (for 5 019m-n AV Hill)

Is an emergency plan required according to regulation 20? No

If yes, tick to confirm that it is attached to this form No

Tick to confirm that you have attached a risk assessment to this form Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment No

Please enter comments on the GM safety committee on the risk assessment
It was agreed that due to the nature of the vector being used (ie, the gRNA and Cas9 genes are located in the same plasmid) and the activity of the inserted genes in targeting genes encoding products involved in cell growth and development, all the work using these lentiviral vectors is classified as class 2 activities.

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Animal Units Large Scale Activities Human Clinical Applications
The aim of this project is to generate a recombinant vaccinia virus expressing a bacterial enzyme, for use in gene-directed enzyme prodrug therapy (GDEPT). GDEPT is a two-step gene therapy approach where the gene for a non-endogenous enzyme is directed to target tissues. The enzyme is expressed intracellularly where it is able to activate a subsequently administered prodrug. In this instance, a GDEPT strategy will be employed with the aim of improving the therapeutic ratio of cancer chemotherapy. This involves delivery a non-secreted form of the carboxypeptidase G2 (CPG2) gene of Pseudomonas spp. strain RS16 specifically to solid tumours where it can be used to activate nitrogen mustard prodrugs to release an active drug producing DNA-DNA interstrand cross-links and cell death.

Specific delivery of CPG2 to tumour cells will be achieved through the use of the Copenhagen strain of vaccinia virus. The CPG2 gene will be inserted into the vaccinia virus genome such that it disrupts the viral thymidine kinase (TK) gene. The virally encoded TK is utilised for the synthesis of the thymidine nucleotide, required for viral DNA synthesis and ultimately viral replication in quiescent cells. Dividing cells transiently express TK in order to produce the components required for division during the synthesis phase of the cell cycle. As such the majority of rapidly dividing cancer cells constitutively express TK and the pool of nucleotides is consistently high. Therefore deletion of viral TK forces the virus to rely on cellular TK, most highly expressed in cancer cells thereby contributing to a restriction of tissue tropism of the virus for productive replication.

To generate the TK deleted recombinant virus, the open reading frame for a non-secreted form of the CPG2 will be cloned into a recombination transfer vector so that it will be control of a synthetic vaccinia virus promoter. This transfer vector will be transfected into vaccinia virus strain Copenhagen infected cells to permit specific insertion of the vaccinia promoter-CPG2 cassette at the TK locus of the viral genome, mediated by homologous recombination. Successful recombinant virus will be recovered through plaque purification in conjunction with selection utilising marker genes included alongside the inserted CPG2 cassette. Marker genes will be those for a yellow fluorescent protein-guanine phosphoribosyltransferase fusion protein (YFP-gpt) and red fluorescent protein (RFP). Following recombinant purification, the virus will be passaged in cells transiently expressing the Cre recombinase gene of the P1 bacteriophage for Cre/LoxP recombination based excision of the marker genes from the viral genome, leaving only the VACV promoter-CPG2 cassette. The resultant virus will be amplified and used to produce virus stocks. The recombinant virus will then be tested in vitro for...
confirmation of transgene expression and activity.

Recipient or parental organism

Vaccinia virus strain Copenhagen. The Copenhagen strain is an attenuated vaccinia virus strain which was utilised as the smallpox vaccination strain during the 1950’s and 60’s in Northern European countries.

Host/vector system

VACV recombination transfer vector containing synthetic VACV promoter used to drive hologous recombination in infected cells

Origin & function

Carboxypeptidase G2 (CPG2) is a zinc-dependent metalloenzyme from Pseudomonas spp. strain RS16 that cleaves the glutamic acid moiety from folic acid and its analogues

Evaluation of foreseeable effects

The insertion of the CPG2 gene into the vaccinia virus genome will further attenuate the vaccinia virus resulting from a restriction of tropism. The recombinant virus will be used for cancer tissue specific expression of the CPG2 enzyme, where it can be used to activate nitrogen mustard prodrugs to release an active drug producing DNA-DNA interstrand cross-links and cell death.

The inserted transgene, CPG2, does not interfere with any known activities or modulate the immunogenicity of vaccinia virus. Additionally, CPG2 will be inserted into the vaccinia virus genome such that it specifically disrupts the viral thymidine kinase (TK) gene. TK deleted vaccinia virus is highly attenuated in normal cell lines, and permits only limited replication at the site of inoculation in immunocompetent individuals. Thus, the final TK deleted vaccinia virus vector will have a highly restricted tropism, limiting the potential effects of both viral pathology associated with systemic disease and the side effects of transgene expression.

The complete host range of wild-type vaccinia virus includes many vertebrate species. A similar host range is potentially possible for the recombinant virus. However, owing to the viral TK deletion during transgene insertion there would be a limited viral replication especially with regards to the dissemination of the virus in the host and replication in internal organs required for vaccinia virus pathology and transmission.

To minimise risks to the environment and human health control measures will be employed. These control measures include rendering all solid and liquid waste inactive within the building (either by autoclaving or use of disinfectant); routine disinfection of work surfaces; all work to be performed in a containment level 2 facility, including use of a class II microbiological safety cabinet for all virology work; all staff are suitably trained in GMO work and employing the necessary risk control measures; workers will inspect hands and exposed skin for abrasions or wounds, and cover them with appropriate dressings, prior to putting on PPE.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste (e.g. gloves, plastic ware, etc.) will be rendered inactive by autoclaving (100% kill) in a validated machine located within the same building. Subsequently the solid waste will enter the yellow bag clinical waste stream and go for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site.

Liquid waste will be decontaminated via chemical disinfection with 1% sodium hypochlorite or 5% Virkon (50% potassium peroxomonosulfate 5% sulfamic acid and 15% sodium alkylbenzene sulfonate) for a contact time of at least 2 hours prior to disposal to drain.

Disinfection of surfaces will also be performed with either of the chemical disinfectants listed above, followed by wiping with 70% ethanol.

Clothing or fabric material that may have come into direct contact with the virus should be autoclaved prior to being collected for washing.
The committee agreed that this was a class 2 project. It was the committee's view that the assessment was adequate for the risk profile of the work.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

### Project Ref  541/18.1

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>26/07/2018</td>
<td>Modulation of chromatin conformation, epigenetic marks and gene transcription to analyse their effect on cellular properties such as proliferation, apoptosis, polarity, migration, adhesion and fate, using viral gene delivery systems</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td></td>
<td>Not Applicable</td>
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<td>N</td>
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<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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<td>N</td>
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</table>
### Purposes of the contained use

To virally transduce mammalian cells with genes, microRNAs, shRNA oligonucleotides or CRISPR/Cas9 constructs.

### Recipient or parental organism

| Human primary and established cell lines, such as MCF7, T470, BT474, SKBR3, MCF10AHEK293 |
| Other mammalian primary and established cell lines, such as primary murine myeloid cells, murine RAW 264.7, c17.2, BV-2 and C3H/1 OT112 cell lines |
| Retrovirus derived from Moloney murine leukaemia virus (MML V). Adenovirus (Ad5). Lentivirus derived from HIV1 |
| Adeno Associated Virus |

We will not use primary lymphocytes to minimise the risk of generating replication competent retrovirus or lentivirus.

### Host/vector system

Vectors: Commercially available defective viral vectors, which may include adenoviral, adenovirus associated viral, retroviral and lentiviral self-inactivating vectors, and well established retroviral vectors with a history of safe use (such as pLNCX and pBABE) packaged into transducible viral particles by specific eukaryotic packaging cells. The lentiviral, adenoviral, adenovirus associated viral and retroviral expression vectors do not encode proteins required for virus packaging. Proteins for packaging are provided by transient transfection of helper vectors or the packaging cell line.

### Origin & function

| Genetic material - subcloned from cDNA library, for example: cDNAs from genes or gene transcripts involved in stem cell potency or differentiation e.g. Oct3/4 and Nanog |
| Genetic material - commercially synthesised oligonucleotides to express microRNAs to, for example: miRNA from genes or gene transcripts involved in ocular physiology e.g. bestrophin 1 |
| Genetic material - commercially synthesised oligonucleotides to form shRNA complexes to, for example: CBFb/RUNX transcription factors which will cause decreased migration of cancer cells; shRNA against H2A.Z is predicted to reduce chromatin accessibility, leading to decreased transcription factor and activator binding at key developmental genes; shRNA against murine Pu.1, Cebpa, Runx1, or Spi1 transcription factors to knock them down. These factors promote haematopoietic cell differentiation. |
| Genetic material - commercially synthesised oligonucleotides to form gRNA complexes to guide CasSo Intended functions: to generate null mutations at the targeted genetic locus or to introduce specific mutations by homologous recombination |

We will not include genetic material into the defective viral vectors that changes the tropism of the virus or encodes a toxic protein, such as diptheria toxin.

### Evaluation of foreseeable effects

The majority of the gene products expressed are expected to affect gene transcription and thus will have an effect on cellular properties such as proliferation, apoptosis, migration, adhesion, polarity and fate. Also many have known or suspected oncogenic or tumour suppressor properties.

The split function of the viral vectors however greatly reduces the risk of recombination events occurring and the self-inactivating properties of the vectors themselves inhibit production of virus once the transgene becomes stably

02/03/2022
incorporated into the genome of the target cells, eliminating the risk of undesired infection. Therefore it is highly unlikely that replication competent virus will be produced. Good laboratory practice in accordance with the risk assessment of the experimental procedure will ensure that the worker or others within the laboratory will not be exposed to the virus. This will include:
- viral production within a Class 2 Safety Cabinet;
- the wearing of gloves and a howie-style labcoat;
- the use of sealed containers when centrifuging the virus to concentrate it;
- the opening of sealed containers within a Class 2 Safety Cabinet;
- not using sharps during viral production;
- not vortexing solutions that contain viral particles.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Spillages to be cleaned with 1% virkon.
All liquid waste to be soaked in 1% virkon overnight.
All solid waste to be autoclaved.
The area to be cleaned with 70% ethanol afterwards.
It is anticipated that this will give 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Both risk assessments (VIR-PXS-02 and VIR-SPB-04) were considered at local GM safety committee meeting (05/07/2017) where approval was given subject to minor amendments, in particular expansion of information around potential health effects following accidental exposure. Neither of the projects fitted within existing connected programmes of work so were considered together as a new connected programme.

Project Containment

<table>
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<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
This project aims to functionally characterize the genomic alterations found in prostate cancer. The target cells for this project will be either well-characterized prostate cell lines, or primary material collected from patients via the MCRC Biobank. Lentiviral, retroviral and Adenoviral expression vectors will be used to introduce point mutations, deletions, and reporter gene fusions and to over-express or knockdown genes that are found to be altered in cancer. Non-viral and lentiviral CRISPR platforms will be used to edit target cell genomes.

Well characterised prostate cell lines and/or primary human prostate cells obtained from patients via the MCRC Biobank.

Lentiviral vector systems: 3rd generation (4-plasmid) systems will be employed for this work. More specifically, they are pMDLg/RRE, pRSV-Rev, pMD2.G used together with a transfer vector e.g. pLJM, pLKO, pLenti-puro, pLenti-CRISPRV2.

Virus replication and production requires co-transfection of all 3/4 vectors into a suitable producer cell line (HEK293). This means the risk of producing...
replication-competent virus is negligible because it is very unlikely that all the necessary components will be re-combined into a single viral genome. These systems have a good track record with regard to safety, and production of replication-competent virus is very unlikely. These systems have the usual deletions associated with their development (e.g. U3 deletion)

2nd generation (3-plasmid) lentiviral vector systems will only be used if it is unavoidable for technical reasons.

Retroviruses will be produced by transfecting the transfer vector along with an envelope vector into the packaging cell line, GP2 (Clontech). This leads to the production of a disabled virus since the components for replication are supplied in trans during packaging. The transfer vectors employed will be pBabe series, pQCX series (Clontech), pMSCV series, pRetroX series (Clontech).

Adenoviruses expressing either Cre recombinase or markers eg GFP with be purchased from the University of Iowa. For in-house production we will use the Adenovirus Dual Expression kit (TaKaRa #6170) which generates replication incompetent virus lacking the E1 region. These essential gene products are supplied in trans during packaging by HEK293 cells, but the E1 genes are not transferred to progeny virus

**Origin & function**

We are attempting to model the genetic changes that occur in cancer. Therefore our genes of interest are either over- or under-expressed in human cancers. This project aims to characterise known/novel oncogenes (e.g. c-myc, n-myc, med12, mutant TP53, kras, hras, GSK3B, MTOR) or tumour suppressors (e.g. PTEN, wt TP53, BRCA2, Rb1, ATF2).

Changes in expression of these classes of gene alter the proliferation and survival of cells to promote the development of tumours in humans.

Cas9 is a nuclease that can be directed to specific regions in the target genome by a guide RNA. The researcher designs the guide RNA sequence such that Cas9 molecule will target to a particular locus and delete the gene of interest. Modification of this system also allows a gene of interest to be over-expressed or epitope tagged. Risks to human health depend on the gene being targeted, but since most of our genes of interest are cancer-associated, the CRISPR systems we will use may have the potential to be oncogenic. The control measures employed in our laboratory make accidental transfer of the CRISPR machinery to lab workers highly unlikely.

Cre and Flp are recombinases which promote recombination of DNA in a sequence-specific manner. Accidental transfer of these genes to lab workers would not be anticipated to have any serious or lasting effects, since the recognition elements for these enzymes occur only infrequently in the human genome.

Marker genes GFP, RFP, Puro, Neo, Hygro express non-toxic gene products - either fluorescent proteins or antibiotic resistance genes used to identify and track genetic modification events

**Evaluation of foreseeable effects**

GM virus:-

The Lenti and Retrovirus produced will be replication-defective and this will limit the extent of any accidental infection of lab workers. Nevertheless, since the recombinant virus has the potential to deliver cancer-associated genes to humans, it should be considered hazardous and potentially oncogenic. However the working practices employed in department (principally the use of PPE, Class 2 MSCs and no sharps policy) effectively reduce this risk to zero.

Furthermore both retroviruses and lentiviruses are lacking any potentially immune avoidance gene products that are encoded within the wild type viruses hence they are unlikely to have the same degree of immune protection as that afforded to the wild type virus. The envelope glycoproteins tend to be derived from other viruses which possess different and, most often, increased immunogenicity profiles compared to the retrovirus / lentivirus envelope glycoproteins. Indeed, the VSVg envelope commonly used to pseudotype lentiviral vectors is sensitive to complement mediated destruction in human serum. Overall, the replication deficient virus vectors are most likely to possess an increased immunogenicity profile as compared to the wild type vector suggesting that in the immune-competent individual, these vectors are more likely to be subjected to immune-mediated clearance.

The Adenovirus system used lacks the E1 region and is replication incompetent. This will limit the extent of any accidental infection of lab workers. Nevertheless, since the recombinant virus has the potential to deliver cancer- associated genes to humans; it should be considered hazardous and potentially oncogenic. However the working practices employed in department (principally the use of PPE, Class 2 MSCs and no sharps policy) effectively reduce this risk to zero.

Target cells:-

The genetic modifications will not alter the ability of our mammalian cell cultures to survive in the general environment. The modified cells require specific conditions to survive. They are maintained at 37 degrees C and cultured in a humidified atmosphere containing 5%CO2. Outside of this environment, the cells could survive in media for a period of time but would not expand in number. In non-sterile conditions the culture would be rapidly killed by contaminating micro-organisms. The GM cells cannot tolerate evaporation of the medium. Solute concentrations would build within the remaining media and the cells would either die of that level of toxicity or die through drying out. Outside of the media environment, cells would last only a short period of time (hours) before dying due to dehydration.
The GM cells have no ability to colonize a human host. During accidental transfer of the cells to a lab worker (e.g. needle stick), the cells would be rapidly eliminated by an immune response. Lab workers are never permitted to culture their own cells.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

In relation to waste processing we request the following derogation Schedule 8 Part 2 Table 1a: Autoclave required in the building

Although an Autoclave is present, all plastic ware that has been used with live GMMs we wish to treat it with an appropriate volume and concentration of disinfectant (1% Virkon) for at least one hour (typically overnight), prior to it being sent directly for incineration. In this way any residual GMMs that may be present are killed at the point of use, rather than transporting them alive to an autoclave within the same building.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid laboratory waste will be inactivated prior to removal from the Class II microbiological safety cabinets employed for this work. Virkon is routinely used as per the manufacturer’s recommendations (99.99% kill):- Solid surfaces are disinfected with 1% Virkon solution. Plastic ware is disinfected with 1% Virkon for a minimum of 1 hour (typically overnight) after which it is removed from the tissue culture laboratory and sent for incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour (typically overnight) prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer’s recommendations).

**Is an emergency plan required according to regulation 20?**

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**Tick to confirm that you have attached a risk assessment to this form**

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**Tick if you are claiming exemption from disclosure for section of the risk assessment**

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</table>

**Project Containment**

<table>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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**Animal Units**

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

**Large Scale Activities**

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

**Human Clinical Applications**

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |
### Purposes of the contained use

This project aims to functionally characterize genes and genomic alterations in breast cancer and associated stromal cells. The target cells for this project will be either well characterized breast or stromal cell lines, or primary samples collected from patients via the MCRC or similar biobanks. Non-viral and lentiviral CRISPR platforms will be used to introduce point mutations, deletions, fusions or copy number changes. Lentiviral expression vectors will be used to over-express of knock-down genes that are found to be altered in breast cancer or associated stromal cells.

### Recipient or parental organism

The recipient cells will be either well characterized breast or stromal cell lines and/or primary human breast cancer or stromal cells obtained via the MCRC Biobank or other biobanks.
- Well characterised cell lines will present a low risk to workers; as they are free of human pathogens and are tested for authenticity and mycoplasma by on-site testing. They will have a history of safe use.
- Primary human cells: obtained from patients whose clinical history will be known. As such this will prohibit collection from patients who present an increased risk through infectious disease e.g. BBVs.

More precisely patient samples will be collected only from those where the information available asserts that the only disease they have is cancer.

We will employ pseudotyped envelope systems such as VSVg to produce lenti- or retroviral particles and to ensure transduction of established cell lines and primary human breast and stromal cells.

The lentiviral vectors employed will be e.g. pCCL, pBABE, pTRIZ, pLV-Puro, pLenti-CRISPRV2.

Other lentiviral vector systems will be added as the technologies evolve.
3rd generation (4-plasmid) lentiviral vector systems will be preferentially employed for this work. If it is unavoidable for technical reasons, 2nd generation (3-plasmid) lentiviral vector systems will be used. The risk of producing replication-competent recombinants is negligible because viral production requires cotransfection of 3/4 vectors into a suitable producer cell line (e.g. HEK293T). Moreover, these systems have deletions and modifications in specific regions associated with activating viral transcription (e.g. U3 deletion, truncated 5'LTR). Therefore it is very unlikely that all the necessary components will be re-combined into a single viral genome. These systems have a good track record with regard to biosafety. The lentiviral vectors employed will be e.g. pCCL, pBABE, pTRIZ, pLV-Puro, pLenti-CRISPRV2. Other lentiviral vector systems will be added as the technologies evolve.

We are attempting to understand the role of cancer-associated genes during tumour development and progression. Therefore our genes of interest are either over-, or under-expressed, down regulated or mutated in human cancers. Or its oncogenic potential is unknown. The genes of interest are:

1) NOTCH related genes (including NOTCH1-4, DLL1/3/4, JAG1/2, DTX1-4).
   NOTCH activation and deregulation of NOTCH signalling is observed in many cancers and their progression. Also mutations within and upstream of the PEST domains of NOTCH1-4 receptors have been observed. Mutations and amplifications of NOTCH signalling often increases the expression of target genes, resulting in the progression of cancer.

2) Other breast cancer related genes such as PIK3CA, STAT3, IL1B, IL1R, ILRA, ESR1, PGR, HER2, GATA3, ALDH1A1-3.

3) Recombinant and fusion proteins (e.g. NOTCH1-IgG Fc decoys).

The lentiviruses produced will be replication-defective and this will limit the extent of any accidental infection of lab workers. Nevertheless, since the recombinant virus has the potential to deliver cancer-associated genes to humans, it should be considered hazardous and potentially oncogenic. However, the working practices employed in department (principally the use of PPE, Class 2 MSCs and no sharps policy) effectively minimises the risks to workers. Furthermore lentiviruses are lacking any potential immune avoidance gene products that are encoded within the wild type viruses; hence they are unlikely to have the same degree of immune protection as that afforded to the wild type virus. The envelope glycoproteins tend to be derived from other viruses (e.g. VSV) which possess different and, most often, increased immunogenicity profiles compared to the lentivirus envelope glycoproteins. Although VSVg has a broader host range, VSVg envelope is indeed commonly used to pseudotype lenti- and retroviral vectors because it is sensitive to complement-mediated destruction in human serum. Overall, the replication-deficient viral vectors are most likely to possess an increased immunogenicity profile as compared to the wild type vector suggesting that in immune-competent individuals, these vectors are more likely to be subjected to immune-mediated clearance.

The genetic modifications will not alter the ability of our cell cultures to survive in the general environment. The modified cells require specific conditions to survive. They are maintained at 37 degrees C and cultured in a humidified atmosphere containing 5%CO2. Outside of this environment, the cells could survive in media for a period of time but would not expand in number. In non-sterile conditions the culture would be rapidly killed by contaminating microorganisms.
The GM cells cannot tolerate evaporation of the medium. Solute concentrations would build within the remaining media and the cells would either die of that level of toxicity or die through drying out. Outside of the media environment, cells would last only a short period of time (hours) before dying due to dehydration. The GM cells have no ability to colonize a human host. If accidental transfer of the cells to a lab worker occurred (e.g. through an open wound), the cells would be rapidly eliminated by an immune response. Lab workers are never allowed to culture their own cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**Not applicable**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Although an autoclave is present, any plasticware (e.g. plastic pipettes) that has been in contact with live GMMs and can be effectively disinfected will be immersed in disinfectant for a minimum of 1 hour. (typically overnight); prior to it being sent for incineration. In this way any residual GMMs that may be present are killed at the point of use rather than being transported to an autoclave.

Any plastic ware that can not be disinfected as described above will be sent for autoclaving.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid laboratory waste will be inactivated prior to removal from the Class II microbiological safety cabinets employed for this work.

Virkon is routinely used as per the manufacturer's recommendations (99.99% kill):- Solid surfaces are disinfected with 1% Virkon solution.

Plastic ware is disinfected with 1% Virkon for a minimum of 1 hour (typically overnight) after which it is removed from the tissue culture laboratory and sent for incineration.

Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour (typically overnight) prior to cleaning.

Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

**Is an emergency plan required according to regulation 20?**

- [ ] N

**If yes, tick to confirm that it is attached to this form**

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] N

**Please enter comments on the GM safety committee on the risk assessment**

The Committee agreed that this was a GM Class 2 project and that the risk assessment was suitable and sufficient for the work described.

**Project Containment**

02/03/2022
The main objective of this project is to explore/establish an experimental/computational framework to study epigenetic inheritance in cancer relevant settings. In particular, we will generate from a single non-transformed 'parental' cell line a battery of cellular models where the introduction of oncogenic factors (H-RAS(G12V), SRAF(V600E), MYC, etc) will drive cellular transformation. To do so, we will use a variety of well characterized cell lines (HA1 E, HA1 ER, HeLA, among others) that will be transduced with lentiviral vectors carrying the oncogenes under study. Moreover, we plan to use lentiviral delivery systems to over-express or knockdown genes altered in cancer whether we obtain results that support us doing so using CRISPR/CAS9 technologies. Finally, we will use non-viral and lentiviral CRISPR/CAS9 platforms to edit target genomes. All the cell lines generated throughout our research program will be used to analyse the generation and
propagation of innate/acquired resistant phenotype to a given drug at a single-cell level. In particular, we will explore dynamic changes in the expression, intracellular localisation and segregation of non-coding RNAs and its link with epigenetic events supporting drug resistance.

Recipient or parental organism

Well characterized cell lines and eventually primary human material sourced from the MCRC biobank.

Host/vector system

We will use a 3rd generation Lentiviral packaging systems in which the packaging components are split across 4 different plasmids. Viral replication required to generate infective particles is only achieved by the concomitant cotransfection of the 4 plasmids into a single cell from a suitable lentiviral producer cell line (293T). The immediate consequence of the use of such a system is that the risk of producing replication competent viral particles is negligible due to the low probability that the 4 vectors recombine into a replication competent viral genome. These systems are widely used and have a good record regarding safety as the production of replicative competent viruses is highly unlikely. In particular we will use the following system:

- pMDLgplRRE (Addgene) expresses HIV-1 Gag-Pol
- pRSV-REV (Addgene) expresses HIV-1 REV
- pMD2.G (Addgene) expresses VSV-G
- pLEX_307 (Addgene) modified to express H2B-GFP instead of puromycin and carrying the mutant/wild-type ORF

Origin & function

The Target Accelerator Pan·Cancer mutant collection is a library of cancer associated ORFs. Although the library will be obtained from Addgene, it has been created in the Broad Institute and it is showcased in several publications (For details regarding each library component please refer to https://www.addgene.org/kits/boehm-target-accelerator-cancer-collection/). Our project aims to characterize the effect of cancer related oncogenes (BRAF, k-RAS, etc) or tumor suppressors (PTEN, BRCA2, etc) in the acquisition of epigenetic mechanisms of drug tolerance/resistance. A large body of evidence suggests that the overexpression or silencing of the aforementioned genes may cause changes in proliferation and survival of cells promoting the potential development of tumors. Although most of the genes that will be used are oncogenic, the accidental transfer and propagation of these genes to operators is highly unlikely.

The Crispr/Cas9 system enables the modification of the genome in a site-directed manner. Based on the research needs the Cas9 nuclease can be programmed to operate on a sequence dependent basis as a guide RNA dictates their targeting to alter/mutate a gene of interest. Modifications of the system includes epitope-tagging and expression control (over-expression and knock-down). We believe that risk to human health are highly associated to the genes to be modified. The security measures employed in our lab make accidental transfer highly unlikely. In addition, selection markers will be used in most of our systems such as GFP, RFP. Puromycin resistance, G418 resistance, etc to track efficiency of transduction. Those markers express non-toxic gene products therefore they cannot be considered a hazard to human health.

Evaluation of foreseeable effects

The potential for generation of replication-competent lentivirus is addressed by the design of the vectors and by safe laboratory practice. In terms of vector design. 3rd generation lentiviral systems provided by Addgene separate transfer, envelope, and packaging components of the virus onto 4 different vectors. The transfer vector encodes the gene of interest and contains the sequences that will incorporate into the host cell genome, but cannot produce functional viral particles without the genes encoded in the envelope and packaging vectors. Unless recombination
occurs between the packaging, envelope, and transfer vectors, and the resulting construct is packaged into a viral particle, it is not possible for viruses normally produced from these systems to replicate and produce more virus after the initial infection.

As we are going to generate a pooled ORF library and use it as such to transduce cells, we do not believe that there is a risk associated to the ORF expressed from the lentivirus as its contribution to the global pool will be minimal. Furthermore, we will screen transduced cell lines using a life/death approach thus reducing the complexity of the final output. In the eventuality that transduced cells get in direct contact with the operator, we anticipate that those cells will be recognized as non-self by the operator immune system and will be rapidly cleared.

Nevertheless, as little recombinant viruses has the potential to deliver cancer-associated genes to humans, they should be considered potentially oncogenic. In the unlikely event of accidental infection with a single or pooled lentivirus, infected cells can be readily identified and monitored by tracking GFP expression. As stated above, the lentivirus will not be able to replicate unless a highly unlikely recombination event takes place involving all 4 plasmids. Therefore, from a health and safety perspective, only a multi-hit transformation event (highly unlikely) could be potentially unsafe to the operator. At this point, it is important to stress that the working practices in place in our lab reduces the risk to accidental contamination to a minimum. Furthermore, any exposure of a worker is expected to result in a normal immune response, thus the non-self material will be cleared by the immune system.

All manipulation will be performed in a Class II biological safety cabinet and a specific cell culture incubator will be designated for working with lentivirus. All members of staff involved in this project will be trained in the safe handling of viral stocks. Controls will be in place to minimize accidental infection via skin contact or injection. The use of aspirators, scalpels and needles will be entirely banned whilst working with viral stocks.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All members of staff involved in lentiviral production and experimentation will be trained thus minimizing the risks. Virucidal disinfectant will be permanently available for decontamination (spills, waste fluids, plasticware).

Any solid material which cannot be effectively disinfected will be autoclaved on site and then sent for incineration via an approved hazardous waste contractor.

All liquid laboratory waste will be inactivated prior to removal from the Class II MSC employed for this work. Viriton is routinely used as per the manufacturer's recommendations (99.99% kill); Solid surfaces are disinfected with 1% Viriton solution.

Plasticware is disinfected with 1% Viriton for a minimum of 1 hour (typically overnight) after which it is removed from the tissue culture laboratory and sent for incineration.

Any contaminated glassware is treated with 1% Viriton solution for a minimum of 1 hour (typically overnight) prior to cleaning.

Liquid waste (culture and medium) is disinfected by the addition of Viriton to give a 1% final working concentration. 2% or 3% solutions of Viriton are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).
The GM Safety committee discussed the risk assessment, agreed that this was GM class 2 work and suggested some minor amendments to aid clarity.

Please enter comments on the GM safety committee on the risk assessment

The GM Safety committee discussed the risk assessment, agreed that this was GM class 2 work and suggested some minor amendments to aid clarity.

### Project Containment

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### Project Ref 541/19.3

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
Date of Significant Change

Project Additional Information

Purposes of the contained use

The overall purpose of the work is to investigate the effect of changes of the glycoprotein genotype of human cytomegalovirus (HCMV) on the phenotypic characteristics of the laboratory strain Merlin of HCMV in order to better understand the pathogenesis of congenital HCMV infection.

In this work the genome of HCMV strain Merlin held within a bacterial artificial chromosome (BacMid) will be used to exchange the glycoprotein sequence of the parental strain with alternate genotypes of individual HCMV glycoproteins. GM HCMV strains will be propagated in cell culture and the resulting virus will be characterized by growth kinetics, cytopathogenic effect, enzyme linked lectin assay, and cytokine production in a discrete cell culture model, to characterize the effect of change of virus glycoprotein sub-type on glycosylation of surface glycoprotein, viral replication kinetics and induction of innate immune responses.

Recipient or parental organism

It is important to state that the GMM will not create new strains of virus as the glycoprotein alterations will only produce strains of virus that are already represented in the normal, circulating, populations of wild-type virus. The worst case scenario is, therefore, a strain of HCMV equivalent to a wild-type clinical strain in virulence. As such, the recombinant virus would not infect immunocompetent, HCMV seropositive individuals. If immunocompetent, HCMV seronegative individuals were exposed they may develop silent infection (approximately 95% of cases) or a self-limiting infectious mononucleosis syndrome. HCMV seronegative individuals who contract infection in pregnancy would be at risk of transmission of the virus to their fetus.

However, HCMV strain Merlin contains disabling mutations in genes RL13 and UL128, which were repaired first singly and then in combination to produce BACs containing a complete wild type (WT) gene complement. Growth of the wild type virus in cell culture necessarily induces changes in the RL13 and UL128 loci thereby reducing the virulence and capability of causing infection of humans. The potential for causation of human disease is thus significantly reduced and as infectious virus is only produced on transduction of cells by the BacMid DNA, attenuated virus will be generated during ongoing culture.

Host/vector system

The genome of human cytomegalovirus strain Merlin inserted into a bacterial artificial chromosome will be propagated in disabled E.coli strain SW102 and mutated in that background using homologous recombination with a maker cassette amplified from plasmid pEP-Kan using polymerase chain reaction (PCR). To reconstitute virus, BacMid DNA will be purified from E.coli and used to transfect human embryo lung fibroblast cells (HEL). The BacMid can only give rise to infectious virus when propagated in permissive human cells. HCMV strains will be propagated in HEL (MRC-5 cells).

Origin & function

Only glycoproteins present in HCMV strains that exist in nature will be used and will result in altered genotype that already exists in nature. Altered virus is anticipated to pose no more risk of infection and disease than any existing wild type HCMV isolate. No or only subtle change in pathogenicity of the laboratory adapted strain is anticipated. In immunocompetent (and non-pregnant) individuals the altered virus is anticipated to pose no more risk of infection or of disease than any of the currently circulating HCMV strains.

Evaluation of foreseeable effects

Change of genotype of a single glycoprotein is highly unlikely to significantly change the transmissibility or virulence of the recombinant virus as glycoproteins require interaction with a cluster of other glycoproteins to exert their effects. One such complex, (glycoprotein complex II) is known to be involved in virus entry into host cells and changes in the action of this complex are anticipated to be in the efficiency of infection of cells and more subtle change in the interaction with the innate immune system. It is possible that recombinant viruses may have altered tissue tropism in vivo. This would allow the recombinant virus to enter and replicate in alternate cell types to the HEL cells used for propagation in vitro. The altered cell tropism could potentially result in the cell tropism observed with existing wild-type strains of HCMV. Immunocompetent (and non-pregnant) individuals this pose no more risk than the risks of infection with existing circulating strains of HCMV and would likely be of lower risk than wild type strains because of the attenuation of the virus by growth in cell culture.

The DNA sequence corresponding to the modified glycoprotein gene is unlikely to present a hazard if they were to recombine into a wild type HCMV as the recombinant...
would only acquire genes that would already exist within currently circulating HCMV genes. Inadvertent recombination would generate a virus that would represent a similar hazard to the intended GMM. Therefore, it is unlikely that specific containment measures (over and above those inherent in biohazard category 2 containment) will be required to prevent cross-contamination.

The HCMV genome was derived from a wild type strain of HCMV and has the characteristics of wild type virus save for known mutations that allow growth of the virus in culture. The mutations serve to attenuate the virus relative to the parental wildtype strain of virus. Return of the recombinant virus to full wild type virulence would require reversion and this is not achievable within cell culture, and is extremely unlikely to be achieved by re-combination within a human host.

The HCMV strains produced will be transmissible by aerosol/droplet as per normal person-to-person contact. For this reason, viruses will be handled exclusively within a class II microbiological safety cabinet inside a containment level 2 laboratory. Workers will wear labcoats and disposable gloves.

The cell lines containing virus are unlikely to survive in the environment as they require specific laboratory growth media and conditions in order to survive. HCMV is unlikely to survive for more than a few minutes in the external environment. The virion lipid envelope is essential for virus infectivity and is readily disrupted on drying in the external environment. More prolonged environmental survival (several hours) has been reported when the virus is contained within bodily fluid.

If the recombinant virus did survive in the environment, it would join similar wild type virus which only circulate in the environment by direct person: person transmission. It is important to emphasize that the alteration in glycoprotein types in the recombinant virus will only generate glycoprotein types which are already circulating glycoprotein types.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To prevent environmental release, anticipated 100% kill of viral particles will be contained through appropriate decontamination and disposal procedures. The disinfection, decontamination and discard policy of the virology unit (Local Policy 02) will be followed. Briefly, small plastic disposables such as pipette tips, Eppendorf tubes and plastic pastettes will be discarded into dry, screwtop, disposal jars. The dry discard jars will be removed for autoclaving, after one week or when full, whichever is the soonest.

For contaminated material which is not suitable for disposal in the single use dry jar, such as larger volumes of liquid or known “high titre” material, then a liquid disinfectant will be employed (Distel 5%, Tristel Solutions Ltd., Snailwell, Cambridgeshire). Virkon powder (Day Impex Ltd., Colchester, Essex) will be used as a dry powder for containment and decontamination of fluid spills.

All other material for decontamination by autoclaving will be placed in the appropriate discard bin provided in each laboratory. When full, bins will be lidded and transported to the decontamination suite for autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The committee agreed that this was GM class 2 and directed that even though the work will generate recombinant forms of virus representative of the glycoprotein types already found within the circulating wild-type HCMV virus strains, the fact that the viruses were being produced using recombinant techniques meant that notification was required, even though the Department already hold internal authorisation for handling wild type strains of HCMV in teaching and research.
**Project Additional Information**

**Purposes of the contained use**

In broad terms the aim of the work is to enhance the understanding of cancer and its development in humans which may lead to better treatment of the disease and better outcomes for patients. In this context Lentiviral vectors will be produced for use in basic research and be applied to human primary and established cell lines and/or murine or other mammalian primary or established cell line basic research and pre-clinical studies. Transduced cells and lentiviral particles may also form the inoculum for in vivo work.

**Recipient or parental organism**
lentiviral vectors derived from HIV-1 which are commercially available will be used.
The strategy will be to employ a hierarchy of choice to encourage the use of the most bio-safe systems first. Thus
researchers are expected to utilise self-inactivating vectors (SIN) where possible; and 3rd generation systems rather
than 2nd generation systems.
The use of systems in the lower parts of the hierarchy (eg 2nd generation systems) must be justified on technical
grounds (e.g. higher ranked systems do not provide a high enough titre). The choice of systems will be scrutinised by
the Biosafety Committee.
Genetic material - Virus genome components sourced as cloned inserts on plasmid backbones or engineered
inpackaging cell lines in which the viral genes are integrated. These materials will be well characterised and sourced
from commercial suppliers or may be shared between researchers.

Host/vector system

Vectors Will be used to transduce:
Well characterised human and murine cell lines. These present a low risk to workers: as they are free of human
pathogens and are tested for authenticity and mycoplasma by on-site testing. They will have a history of safe use.
Primary human cells: obtained from patients (where their clinical history will be known): usually via the MCRC Biobank
(although other Biobanks may be used) . As such this will mostly prohibit collection from patients who present an
increased risk through infectious disease e.g. BBVs. In what we anticipate to be rare or exceptional circumstances
there may be a need to obtain material from patients whose cancer has some sort of clinical association with another
disease. For example hepatocellular carcinoma has a clinical association with Hepatitis: cervical cancer with Herpes
infection). In such cases the work, having been suitably risk assessed, will need to be authorised by the Director of
the Institute.
Primary human cells may also be obtained from "healthy volunteers." (lab workers are not allowed to culture their
own cells and use it in their own research).

Live mice - The majority of in vivo work carried out will involve the use of lenti-transfected cell lines where the
expectation is that there to be no Lentivirus carry over due to the number of passages and time that has elapsed to
prepare the cellular inoculum. This part of the work. may involve the use of sharps (needle and syringe, blades).
Accordingly appropriately trained staff (usually BRU trained animal technicians) carry out this part of the work.
Some aspects of the work the inoculum may be the viral vector particles themselves. Where possible the use of
sharps will be avoided. For example by delivering the inoculum by inhalation via a pipette. If direct injection of viral
particles is required, as above, this part of the work will be carried out by experienced workers ( usually BRU trained
animal technicians) employing anaesthetics, restraining devices or scruffing to minimise the mobility of the mice and
so reduce the likelihood of a sharps injury and needle safe devices if appropriate.

Origin & function

The employment of lentiviral based genetic modification systems in vitro and in vivo will enable us to study a number
and range of genes involved in cancer. In this respect almost all of the genes of interest will be oncogenes or
potential oncogenes, tumour suppressors or involved in cell cycle and/or cell signalling pathways. Use of lentiviral
vector and CRISPR sytems will be used to
- Introduce, knockdown, over express, silence or otherwise edit genes that we study to further our understanding of
the role they play in cancer development and/or treatment
- Study genes involved in the immune systems response to cancer
- Generate receptors targeting cancer cells e.g Chimeric antigen receptor; T-cell receptor
Lentiviral systems may be used in conjunction with other gene modification systems ( e.g siRNA knockdown or
CRISPR applications) either in parallel or to deliver components of such systems ( e.g stable expression of shRNA or
Genetic material from a variety of sources will be used:
- Subcloned from cDNA libraries (e.g., cDNAs from genes or gene transcripts involved in signaling pathways)
- Commercially synthesized oligonucleotides to express micro-RNAs and/or shRNA complexes
- Commercially synthesized oligonucleotides to form gRNA complexes to guide Cas9 in Lenti viral based CRISPR
- Commercially synthesized oligonucleotides that encode a construct (e.g., chimeric antigen receptor etc.)
- Human and/or murine or other mammalian DNA isolated from samples, or supplied by research colleagues or those that are commercially available
- Marker genes (e.g., GFP and luciferase which may originate from a range of organisms (e.g., bacteria, yeast, jellyfish etc.)

Similarly, Lenti-based CRISPR systems will be used which are commercially available.

Only genetic material known to be involved in the development and progression of cancer or its treatment using CAR-T cells will be studied. Using lentiviral vectors to express toxins or other biologically active molecules will not be included in this programme.

Evaluation of foreseeable effects

GM virus: The lentiviruses produced will be replication-defective and this will limit the extent of any accidental infection of lab workers. Nevertheless, since the recombinant virus has the potential to deliver cancer-associated genes to humans, it should be considered hazardous and potentially oncogenic. However, the working practices employed in the Institute (principally the use of PPE, Class 2 MSCs and minimisation of the use of sharps policy) effectively minimises the risks to workers.

Furthermore, lentiviruses are lacking any potential immune avoidance gene products that are encoded within the wild type virus; hence they are unlikely to have the same degree of immune protection as that afforded to the wild type virus. The envelope glycoproteins tend to be derived from other viruses (e.g., VSV) which possess different and, most often, increased immunogenicity profiles compared to the lentivirus envelope glycoproteins. Although VSVg has a broader host range, VSVg envelope is indeed commonly used to pseudotype lentivirus because it is sensitive to complement-mediated destruction in human serum.

Overall, the replication-defective viral vectors are most likely to possess an increased immunogenicity profile as compared to the wild type vector suggesting that in immune-competent individuals, these vectors are more likely to be subjected to immune-mediated clearance.

Target cells:

The genetic modifications will not alter the ability of our cell cultures to survive in the general environment. The modified cells require specific conditions to survive. They are maintained at 37 degrees C and cultured in a humidified atmosphere containing 5% CO2. Outside of this environment, the cells could survive in media for a period of time but would not expand in number. In non-sterile conditions, the culture would be rapidly killed by contaminating microorganisms. The GM cells cannot tolerate evaporation of the medium. Solute concentrations would build within the remaining media, and the cells would either die of that level of toxicity or die through drying out. Outside of the media environment, cells would last only a short period of time (hours) before dying due to dehydration.

The GM cells have no ability to colonize a human host. If accidental transfer of the cells to a lab worker occurred (e.g., through an open wound), the cells would be rapidly eliminated by an immune response. Lab workers are never allowed to culture their own cells.

Only work with lentiviral vectors with oncogenic/transformatory potential, or constructed for the use programming autologous T-cells to target cancer cells where a GM class 2 classification is indicated will be covered by this connected programme. Lentiviral vectors pre-presentation different risks to workers will not be considered in this programme and will be notified separately.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NIA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Any plasticware (e.g. plastic pipettes) that has been in contact with live GMMs and can be effectively disinfected will be immersed in disinfectant for a minimum of 1 hour. (typically overnight); prior to it being sent for incineration. In this way any residual GMMs that may be present are killed at the point of use rather than being transported to an autoclave.

Any plasticware that cannot be disinfected as described above will be sent for autoclaving.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid laboratory waste will be inactivated prior to removal from the Class II microbiological safety cabinets and/or laboratory employed for this work.

Virkon and Distel is routinely used as per the manufacturer's recommendations (99.99% kill):- Solid surfaces are disinfected with 1% Virkon solution. Plastic ware is disinfected with 1% Virkon for a minimum of 1 hour (typically overnight), after which it is removed from the tissue culture laboratory and sent for incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour (typically overnight) prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers. (as per manufacturer's recommendations).

Solid or other waste that cannot be effectively disinfected (as per section 11) and thus requires sterilisation, is collected in an autoclave bag, sealed with tape or cable-tie and sent for autoclaving. The autoclave is located on the same site and is transported to it via leak proof containers. The autoclave is validated annually and undergoes regular maintenance.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee agreed that the classification of these assessments were GM Class 2 (it was agreed that other elements, e.g. the use of adenovirus vectors, were class 1)

Project Containment

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02/03/2022
Project Ref  541/97.1

Date Ackn'd  17/10/1997

Date Project Ceased  05/02/2004

CU2 Project Title  Studies on the virulence of Erwinia amylovora

Class  Class 2

Consent Granted  not applicable

Project notified under transitional arrangements  Y

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

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Please enter comments on the GM safety committee on the risk assessment

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Project Ref 541/97.2

Date Ackn’d 17/10/1997  CU2 Project Title Use of viral vectors for research on gene therapy

Class CultureVolClass2 CultureVolumeClass3-4

Class 2
Date Project Ceased

05/02/2004

Tick if notifying a connected programme of work  Y

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements  Y

Withdrawn  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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02/03/2022
Project Ref: 541/97.5

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Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 541/98.1

Date Ackn'd CU2 Project Title

| 03/02/1998 | MOLECULAR DETERMINATION OF THE ADHESION OF LISTERIA | Class 2 |

Class CultureVolClass2 CultureVolumeClass3-4
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tbody>
<tr>
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<td>L4</td>
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### Project Ref 541/trans1

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<tr>
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<tr>
<td>21/02/2001</td>
<td>ISOLATION OF FUNGAL (CANDIDA AND ASPERGILLUS) GENES ENCODING ANTIGENIC PROTEINS. NOTE: TITLE CHANGED FROM ISOLATION OF CANDIDA ALBICANS GENES ENCODING ANTIGENIC PROTEINS</td>
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<table>
<thead>
<tr>
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<table>
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<tr>
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<tr>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  No

If yes, tick to confirm that it is attached to this form  No

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  No

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
**Project Ref**: 541/trans2  

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<td>21/01/1994</td>
<td>CONSTRUCTION OF PHAGE ANTIBODY LIBRARIES</td>
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**Non-GMM Consent Granted**: not applicable

**Tick if notifying a connected programme of work**: N

**Project notified under transitional arrangements**: Y

### Project Additional Information

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**
  - organisms
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>L2 L3 L4</td>
<td>L3 L4 L2 L3</td>
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<td>Large Scale Activities</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4 L3</td>
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<td>Human Clinical Applications</td>
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Project Ref 541/trans3

Date Ackn'd 21/01/1994
CU2 Project Title CLONING IMMUNODOMINANT ANTIGENS OF BORDETELLA PERTUSSIS IN
Class 2
Culture Vol Class 2
Culture Volume Class 3-4

02/03/2022
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<th>Human Clinical Applications</th>
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<tr>
<td>L2 L3 L4</td>
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<td>L2 L3 L4</td>
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</table>

**Project Ref** 541/trans4

Date Ackn’d: 21/01/1994

CU2 Project Title: CLONING STREPTOCOCCAL ANTIGENS IN LAMBDA VECTORS: CLONING ENDOCARDITIS - SPECIFIC STREPTOCOCCAL ANTIGENS IN LAMBDA GT 11

Class: Class 2

Consent Granted: not applicable

Project notified under transitional arrangements [Y]

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Ref: 541/trans5

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<tr>
<td>21/01/1994</td>
<td>IDENTIFICATION AND CHARACTERISATION OF NOVEL AND MUTATED GENES CONTROLLING EXTRACELLULAR MATRIX DEPOSITION IN EUKARYOTES (FORMERLY &quot;EXPRESSION OF NORMAL AND MUTATED EXTRACELLULAR MATRIX MOLECULES&quot;)</td>
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- **Non-GMM Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

#### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

L2

Large Scale Activities

L2

Human Clinical Applications

L2

Molecular Cell Biology of Trypanosomes (Formerly Cloning of

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<td>21/01/1994</td>
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GENES ENCODING CYTOSKELETAL PROTEINS OF TRYPANOSOME BRUCEI

Project notified under transitional arrangements

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref 641/97.1**

Date Ackn’d 15/05/1997

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

PHASE II TRIAL TO STUDY EFFECTS OF VACCINIA WITH LIVE RECOMBINANT VACCINIA VIRUS EXPRESSING HUMAN PAPILLOMA 16 & 18 E6 & E7 PROTEINS (TA-HPV)

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
<th>Laboratory Activities</th>
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02/03/2022
Investigation of the Biological effects of Hepatitis C Virus proteins on Hepatocytes

Class 2
Consent Granted

Project transferred from GM79
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  79/01.1

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<tr>
<td>10/09/2004</td>
<td>Investigation of the role of molecules involved with cell fate decisions</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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### Project Additional Information

**Purposes of the contained use**

To generate retroviral particles containing DNA sequences of molecules involved with cell fate decisions. To use these retroviruses to transfect mammalian primary or immortalised cells.

**Recipient or parental organism**

1. Primary cells of mammalian origin.
2. Immortalised cell lines.

Not genetically modified and no foreseeable adverse effects.

**Host/vector system**

1. Retroviral vectors such as PMX and pMSCV (see attached risk assessment)
2. Packaging cell lines such as phoenix

Expression of retroviral vectors in packaging cell lines will generate replication - defective viruses. Once these viral particles have been used to transfect recipient cells, supernatants from these cells will be screened for the presence of infectious virus.

**Origin & function**

cDNA of mammalian origin coding for molecules involved with cell fate decisions

Including: notch molecules 1-4

- notch ligands jagged 1/2, delta
- notch signalling molecules delta, csl
- notch modulatory molecules - the fringe family
- mutant forms of the above molecules

Notch molecules are thought to be involved with cell fate decisions in the presence of certain cytokines, notch delays differentiation and promotes the proliferation of primitive cell types. Notch 1 is a known oncogene and truncated, constitutively active forms of notch 1 have been found in T lymphoblastic neoplasms. Molecules which potentiate notch signalling may be expected to delay differentiation also.
Following retroviral transfection, cells will be monitored for the presence of infectious retroviral particles on a regular basis. Once it has been established that no infectious particles are generated by host (or recipient) cells, then transfected cells will be used for experimentation. Primary cells are routinely screened for the presence of infectious agents to reduce the risk of co-infection with more than one retrovirus. Primary cells will not be used from lab workers or their family members to the risk of engraftment of transfected cells likely routes of transmission of replication - deficient virus include aerosols and sharps injuries. See attached risk assessment. Accidental inoculation with replication - deficient virus is likely to result in the destruction of virus by the host immune system and therefore long-term injury is unlikely. Experimental waste containing virus particles and transfected cells will be autoclaved to minimise any foreseeable environmental effects.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

See section 7. Analysis of transfected cells will be performed under category 1 conditions only when the absence of infectious viral particles has been established.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Packaging cell lines, cell culture supernatants containing retroviral particles and transfected target cells will be treated with 1% virkon solution which will destroy any retrovirus or retroviral containing cells. (A 0.05% virkon solution is sufficient for retroviral destruction according to the manufacturer). Solid waste is also treated with 1% virkon solution for a minimum of 1 hour. Both liquid and solid waste is autoclaved in order to ensure a 100% kill of GM material. Departmental autoclaves are serviced annually using a thermocouple. Refer to attached virkon data sheet for further information.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The local GMSC considered that the work using retroviral vectors required containment level 2 because of the potential oncogenic properties of the inserts. Other aspects of the project e.g. construction of plasmids in disabled Ecoli K12 strains have been assessed separately by the GMSC.

Project Containment

<table>
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02/03/2022
The folate pathway is an essential but complex metabolic pathway in malaria parasites that is involved in DNA synthesis and amino-acid conversion. We have identified and characterised most of the genes involved in this pathway and now aim to investigate the individual roles of these genes by transformation experiments. This will encompass experiments where individual genes are disabled, to assess whether their activities are mandatory for survival, and experiments where certain of these genes are modified, to assess the role of naturally occurring polymorphisms in a controlled genetic background. We have evidence that different parasite lines differ in their ability to make use of preformed folate from the host, and transformation provides a powerful route to investigate such a difference. This has been demonstrated in other laboratories where transformation is now routinely used to investigate basic questions about the biology of the parasite.

Laboratory strains of Plasmodium falciparum that have been in vitro culture for many years, both in this and numerous other laboratories will be used. The genetic modification introduced by transformation will render them more resistant to the drugs pyrimethamine, neomycin or blasticidin (used as the plasmid markers), but will have no effect on their susceptibility to a range of antimalarial drugs currently in clinical use. The risk of laboratory infection is extremely low, but such drugs will be able to clear infection rapidly. The targeted gene modifications in the folate pathway are likely to reduce their general viability relative to the untransformed host, or leave it unaffected. The modifications will not alter host specificity.

Recipient or parental organism
Laboratory strains of Plasmodium falciparum that have been in vitro culture for many years, both in this and numerous other laboratories will be used. The genetic modification introduced by transformation will render them more resistant to the drugs pyrimethamine, neomycin or blasticidin (used as the plasmid markers), but will have no effect on their susceptibility to a range of antimalarial drugs currently in clinical use. The risk of laboratory infection is extremely low, but such drugs will be able to clear infection rapidly. The targeted gene modifications in the folate pathway are likely to reduce their general viability relative to the untransformed host, or leave it unaffected. The modifications will not alter host specificity.

Host/vector system
The plasmid vectors to be used are derived from the commercially available pBluescript and pGEM series of E. coli vectors. These vectors are modified to include the necessary elements for targeting the genes of interest and introduced via electroporation. They are maintained by selection using the drugs mentioned above. If such selection is removed, plasmids are lost, unless integration has occurred at the target gene locus.
1. Plasmid sequences plus partial sequences of target P. falciparum folate pathway genes designed to disrupt the corresponding gene of the recipient cell.
2. Plasmid sequences plus sequences of target P. falciparum genes designed to alter the target gene at known polymorphic loci.
3. Plasmid sequences plus putative promoter regions of P. falciparum designed to test the effect of possible varying levels of expression on folate gene products.
4. Plasmid sequences that carry reporter activities such as Green Fluorescent Protein (GFP) or Chloramphenicol Acetyltransferase (CAT) to rapidly test for successful transformation.

All plasmids are constructed via cloning in standard disabled E.coli hosts (principally XL1-Blue, Stratagene).

**Evaluation of foreseeable effects**

No new potential hazards are identified as a result of the genetic modification. As with untransformed P. falciparum, the only route for transmission to humans will remain as direct inoculation into the bloodstream of the operator. The natural route of infection, via the bite of the Anopheles mosquito, is excluded by the complete absence of the latter from the working environment (mosquitoes are not bred in this Institute). Even if such a mosquito were present, it could not feed from culture flasks. Moreover, it would be unable to ingest sexual forms of the parasite for onward reproduction and transmission of the parasite, as only asexual forms will be cultured. Airborne contamination, e.g. by aerosol spread from cultures, is also not a risk with this organism, and there is no possible person to person transmission. Viability of these organisms for experimental purposes is totally dependent upon careful maintenance of stringent laboratory culture conditions and there is no known or foreseeable risk to the environment, as the parasite cannot survive in any environment outside of the tissue culture medium and the narrowly confined parameters of temperature, pH, and gas atmosphere (5% CO/5% O/90% N).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We request derogation from full Class 3 containment because the Risk Assessment indicates that the following features of level 3 containment are not required, given the absence of an airborne contamination risk when working with this organism - see section 2 (Growth and propagation of the parasite); 3 (Assessment of risks to human health), and 4 (Assessment of risk to the environment), of the Risk Assessment.

1. Fumigation, and thus a sealable laboratory.
2. Airlock entry to the laboratory.
3. Negative pressure relative to the surrounding laboratories, and HEPA filtered air extract.
4. Autoclave in the culture facility. Biological material is completely killed by verified disinfection procedures before removal from the facility in sealed plastic boxes. These are transported directly to the nearby Departmental Autoclaving suite and the material sterilised in situ.
5. Specific measures to control aerosol dissemination.
6. Shower within the culture facility.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

1) Parasite microscope slides are treated with methanol + flaming before staining with dye (also in methanol) and viewing, rendering material on the slide harmless. After use, the slide are routinely treated with bleach (Chloros, in appropriate dilution) before disposal and areas such as the microscope stage are wiped with 70% ethanol after use.
2) Disposal of solid culture materials (plastic pipettes/tips/tubes/tissue culture flasks) is by placing the items in a separate labelled double biohazard bag kept in the tissue culture room which is subsequently directly removed from autoclaving. In the case of liquid waste (culture supernatants and residual cells), treatment is for 16-24hrs in a solution of bleach (Chloros) dilute to a final volume containing 10% free chlorine (1:3 v/v) before disposal. The container is then placed for autoclaving. Spills for infected material must be attended to immediately. The volume is reduced by use of dry paper towels (which must be placed for autoclaving) followed by treatment of the affected area with diluted (1:3 v/v) Chloros bleach followed by a wipe with paper towel soaked in 70% ethanol. The latter is important, as bleach is detrimental to many surfaces including stainless steel; for such surfaces, Virkon disinfectant can be used in place of the bleach, either as a solution or as powder.
3) Degree of kill: the parasite is an extremely fastidious organism entirely dependent for viability upon careful maintenance within host red blood cells in a stringent tissue culture medium under the atmosphere of a special gas mix. Lysis of the red cells, removal of the medium, exposure to normal air (as opposed to the 5% CO/5% O mix
needed for culture), increase in temperature above approx. 39°C, small changes in pH away from physiological, all lead to parasite death within a very short time. As established in microscopy, Chloros lyses all host cell within a short period, penetrating the parasites and killing them 100%. It is not possible to restore a viable culture from infected red cells treated in this way. Autoclaving also kills the parasite 100%. Autoclaving is carried out in our central sterilising facility in equipment employing data logging of all autoclaving cycles. The autoclaves are also checked regularly by internal indicator tablets included in the items for disposal that confirm the proper length and temperature of the cycle. In addition, thermocouple tests of appropriate loads are carried out as part of the annual autoclave service that is carried out by the manufacturer.

Please enter comments on the GM safety committee on the risk assessment

This project has been considered by the UMIST GMSC. The Committee believes that the containment measures and working practices described in the Risk Assessment are appropriate for this work on Plasmodium falciparum.

**Project Containment**

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<td>Date Ackn'd</td>
<td>10/09/2004</td>
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<tr>
<td>CU2 Project Title</td>
<td>MOLECULAR AND CELL BIOLOGY OF CANDIDA ALBICANS</td>
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<tr>
<td>Class</td>
<td>CultureVol</td>
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<tr>
<td>Non-GMM</td>
<td>Consent Granted</td>
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<tr>
<td>Project notified under transitional arrangements</td>
<td>N</td>
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</table>
The application covers a range of specific projects with the overall goal to study the regulation and mechanisms of cell wall biosynthesis and its role in yeast-hypha morphogenesis of Candida albicans. More specific goals:
1/ Role of glycosylation
2/ Role of the Ras/cAMP-dependent pathway
3/ Role of specific O- and N-glycosylation enzymes in cell wall structure and adhesion

Recipient organisms include:
1/ E. coli - many multiply disabled K12 derivatives
2/ S. cerevisiae - many multiply disabled, non-pathogenic S288C derivatives
3/ C. albicans (CA14, ura3; CA18, ura3 ade2 abd derivatives of these strains with further disabling markers
(eg. glycosylation enzymes, components of the Ras/cAMP-dependent pathway).

Host/vector system

Vectors include:
1/ Many non-mobilisable E. coli vectors (including pUC 18/19, p8LUESCRIPT, lac-based expression plasmids such as pET vectors)
2/ Many non-mobilisable S. cerevisiae vectors (including YEps, YCps, Y1ps and single-copy GAL-based expression vectors)
3/ Specific non-mobilisable C. albicans vectors (single-copy integrating vectors PMB7, pDDB57, integrating expression vectors regulated by methionine pEXPa)The genetic materials in the modifications are all derived from naturally occurring organisms of E. coli, S. cerevisiae and C. albicans. More specific Candida genes include:
1/ Glycosylation genes (eg. CaSRB1, PMT1, PMT2, MNN1, 2, 3, and 4)
2/ Regulated promoters (MET3, MAL2)
3/ Auxotrophic markers (URA3, ADE2, LYS2)
4/ Components of the Ras/cAMP-dependent pathway (PDE1, PDE2, TPK1, 2, 3, BCY1)
5/ Candida genomic and cDNA libraries

Origin & function

The genetic materials in the modifications are all derived from naturally occurring organisms of E. coli, S. cerevisiae and C. albicans. More specific Candida genes include:
1/ Glycosylation genes (eg. CaSRB1, PMT1, PMT2, MNN1, 2, 3, and 4)
Candida albicans is a human pathogen in hazard group 2. Can dida albicans causes superficial infections of mucosal epithelia (thrush). However, at no time in the last 20 years since work with Candida began, has a member of any lab had a thrush infection caused by a lab strain. C. albicans also causes infections in severely immunocompromised patients and these can be fatal. However, it is important to remember that C. albicans is widely distributed, being carried by over half of the population. It rarely affects healthy individuals. IOt only becomes a potential medical problem when the immune system of an individual is impaired. Furthermore, as with most pathogenic microorganisms, the virulence of Candida albicans strains is likely to become attenuated rather than enhanced after prolonged laboratory culturing.

The strain Candida albicans CA1-4 (ura3) is the standard host for DNA transformation worldwide. Its uridine auxotrophy makes it avirulent (Leberer et al. 1996, PNAS, 93, 13217). Transformation with URA3 plasmids partially restores the virulence of CA1-4, but the restoration is not complete because the genes neighbouring URA3 remain inactivated. The URA3 marker is recycled for subsequent rounds of transformation (Fonzi and Irwin, 1993, Genetics, 134, 717). Therefore by necessity, some of the transformants we create are not genetically disabled, although their virulence is lower than the wild-type strains of C. albicans carried by most individuals.

C. albicans CA1-8 (ura3, ade2) is transformed with ADE2 or URA3 containing plasmids, leaving one remaining disabling mutation in the transformants. This is sufficient to render all of the transformants completely avirulent.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

We follow all safety guidelines for operation at containment level 2. Briefly:

All waste materials will be inactivated before disposal:

* liquid waste - autoclaving at 121 degrees C for 15 minutes (proven to effectively 100% kill fungal cells). The Department of Biomolecular sciences has two large autoclaves, one of which is to be replaced in January 2001. The next thermocouple tests will be carried out on both autoclaves when the engineers are in the department to install the new autoclave. The autoclaves have always been serviced annually by BMM Weston/Drayton Castle, thermocouple testing of various loads of waste is to be included in the annual service contract. The new autoclave will have a data logging system, the old autoclave has a chart recorder to monitor the conditions. Autoclave indicating rubes are placed in bins of contaminated materials each week.

* glass ware - soaked with Virkon (see attached information provided by the manufacturer) proven to effectively 100% kill fungal cells)

* plasticware - autoclaved under the conditions shown above. Before inactivation, plastics will be double-bagged for transport to autoclaves

* sharps will be minimised, but any contaminated sharps will be placed in separate CinBins, bagged and incinerated.

Once GMMs have been inactivated, normal routes of disposal will be used. These measures will reduce the likelihood of all hazards to negligible. The overall risk is therefore effectively zero with the proposed containment and control measures.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N
The UMIST GM safety committee has considered this project. The work with E. coli K12 and Saccharomyces cerevisiae is Class 1 but the Committee thought that the Candida albicans experiments required containment level 2 and were thus Class 2 activities.

**Project Containment**

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<td>L2 Yes</td>
<td>L3 L4 L2</td>
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- Animal Units: L2 L3 L4 L2
- Large Scale Activities: L2 L3 L4 L2
- Human Clinical Applications: L2 L3 L4 L2

**Project Ref** 79/04.1

- **Date Ackn'd**: 10/09/2004
- **CU2 Project Title**: AN INVESTIGATION OF THE MORPHOLOGICAL AND TRANSLATIONAL CHARACTERISTICS OF YEAST
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**
- **Historical Date of Additional Info**: Project transferred from GM79

**Project Additional Information**
### Purposes of the contained use

The application covers various specific projects which all have the overall aim of understanding the relationship between cellular proliferation and protein synthesis. More specific goals include an investigation of the control of translation initiation in response to stress as well as an analysis of downstream effect such as flocculence and colony morphology.

### Recipient or parental organism

Recipient organisms include:

- C. albicans - strains such as CA1-4 (ura3) and CA1-8 (ura3 ade2). These strains are standard lab strains with auxotrophic phenotypes and highly attenuated virulence. These strains cannot survive without media containing high concentrations adenine and uracil, and given the stringent practises for autoclaving liquid and solid media prior to disposal in our institute, there is no possibility that these strains could escape into the environment.

### Host/vector system

Vectors include:

Specific non-mobilisable C. albicans (single integrating vectors and integrating expression vectors regulated by methionine).

### Origin & function

The genetic materials are all derived from naturally occurring organisms of E. coli, S. cerevisiae, and C. albicans. More specific Candida genes include:

1. Protein synthetic genes (e.g. GCD1, GCD6, CDC33)
2. mRNA decay genes (e.g. DCP1, CCR4)
3. Auxotrophic markers (e.g. URA3, ADE2, LYS2)
4. Candida genomic and cDNA libraries

These genes have homologues in all eukaryotic organisms. As a result there could be absolutely no effect of a gene transfer because the DNA sequences included in our constructs are part of the genomes of all known eukaryotic organisms.

### Evaluation of foreseeable effects

Candida albicans is a human fungal pathogen in Hazard Group 2. It is a micro-organism of a very low pathogenic potential that very rarely effects healthy individuals. It becomes infectious, however, in severely immunocompromised patients like HIV positive individuals or organ transplant patients. Candida albicans can cause superficial infections of mucosal epithelia (thrush). However, in the 20 or more years that C. albicans has been studied in labs, there has never been a thrush infection caused by a laboratory derived strain.

In fact the lab strain CA1-4 which is the standard lab strain worldwide is auxotrophic for uracil. This strain cannot therefore survive in the absence of high concentrations uracil and this makes the strain avirulent. It might be anticipated that transformation of this strain with plasmids bearing the URA3 gene restores the virulence of CA1-4, but the strain is still severely disabled (e.g. Chen et al., 2004. Mol Microbiol. 51:551-65). The lab strain CA1-8 is auxotrophic for both uracil and adenine. Transformation of this strain therefore would only ever complement one of these auxotrophies, thus this is sufficient to render all transformed strains avirulent. Even though these strains are avirulent, the stringent practises for autoclaving liquid and solid cultures prior to disposal eliminates the possibility of strains escaping into the environment alive.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- **N/A**

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- **N/A**
We follow all safety guidelines for operation at containment level 2. Briefly:

All waste materials will be inactivated prior to disposal. Liquid - autoclaving at 121 degrees centigrade for 15 minutes which has been proven to effectively kill 100% of fungal cells. The Department of Biomolecular Sciences has a large autoclave which replaced two autoclaves in January 2001. The autoclave is serviced annually by BMM Weston/Drayton Castle and thermocouple testing of various loads of waste is included in the annual service contract. The autoclave has a data logging system and autoclave indicator tubes are placed in bins of contaminated waste on a weekly basis.

Glassware - soaked with 1% Virkon (see information provided by the manufacturer - proven to effectively kill fungal cells)

Plasticware - autoclaved under the conditions described above. Before inactivation plastics will be double-bagged for transport to the autoclave. The use of sharps will be minimised, but any contaminated sharps will be placed in separate CiniBins, bagged and incinerated.

Once GMMs have been inactivated, normal routes of disposal will be used. The proposed containment and control measures will eliminate the hazards, making the overall risk effectively zero (see risk assessment).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

None.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 79/95.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022

Page 8561 of 15326
The investigation of the Biochemical and Biological Effects on Oncogenes on Haemopoietic Cells

<table>
<thead>
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<th>Class 2</th>
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Non-GMM Consent Granted

- Not Applicable

Project notified under transitional arrangements

- Y

Historical Significant Changes

Project transferred from GM79

Historical Date of Additional Info

Significant Change ID

Withdrawn

- N

Tick if notifying a connected programme of work

- N

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref**  79/99.1

- **CU2 Project Title**: Identification & physical & genetic characterisation of plasmid stability determinants from gram-positive bacteria
- **Class**: Class 2
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: Y
- **Withdrawn**: N
- **Historical Significant Changes**: Project transferred from GM79

Date Ackn’d: 10/09/2004

Date Project Ceased: 

Tick if notifying a connected programme of work: N

Tick if you are claiming exemption from disclosure for section of the risk assessment: N

Tick if you have attached a risk assessment to this form: 

Tick to confirm that it is attached to this form: N

Is an emergency plan required according to regulation 20?: N

If yes, tick to confirm that it is attached to this form: N

Tick to confirm that you have attached a risk assessment to this form: 

Tick if you are claiming exemption from disclosure for section of the risk assessment: N
Project Additional Information

Purposes of the contained use

To generate retroviral particles containing DNA sequences of molecules involved with cell fate decisions. To use these retroviruses to transfect mammalian primary or immortalised cells.

Recipient or parental organism

1) Primary cells of mammalian origin.
2) Immortalised cell lines.

Not genetically modified and no foreseeable adverse effects.

Host/vector system

1) Retroviral vectors such as PMX and pMSCV (see attached risk assessment)
2) Packaging cell lines such as phoenix

Expression of retroviral vectors in packaging cell lines will generate replication - defective viruses. Once these viral particles have been used to transfect recipient cells, supernatants from these cells will be screened for the presence of infectious virus.

Origin & function

cDNA of mammalian origin coding for molecules involved with cell fate decisions including: notch molecules 1-4
notch ligands jagged 1/2, delta
notch signalling molecules delta, csl
notch modulatory molecules - the fringe family
mutant forms of the above molecules

Notch molecules are thought to be involved with cell fate decisions in the presence of certain cytokines, notch delays differentiation and promotes the proliferation of primitive cell types. Notch 1 is a known oncogene and truncated, constitutively active forms of notch 1 have been found in T lymphoblastic neoplasms. Molecules which potentiate notch signalling may be expected to delay differentiation also.

Evaluation of foreseeable effects

Following retroviral transfection, cells will be monitored for the presence of infectious retroviral particles on a regular basis. Once it has been established that no infectious particles are generated by host (or recipient) cells, then transfected cells will be used for experimentation. Primary cells are routinely screened for the presence of infectious agents to reduce the risk of co-infection with more than one retrovirus. Primary cells will not be used from lab workers or their family members to the risk of engraftment of transfected cells likely routes of transmission of replication - deficient virus include aerosols and sharps injuries. See attached risk assessment. Accidental inoculation with replication - deficient virus is likely to result in the destruction of virus by the host immune system and therefore long-term injury is unlikely. Experimental waste containing virus particles and transfected cells will be autoclaved to minimise any foreseeable environmental effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

See section 7. Analysis of transfected cells will be performed under category 1 conditions only when the absence of infectious viral particles has been established.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Packaging cell lines, cell culture supernatants containing retroviral particles and transfected target cells will be treated with 1% virkon solution which will destroy any retrovirus or retroviral containing cells. (A 0.05% virkon solution is sufficient for retroviral destruction according to the manufacturer). Solid waste is also treated with 1% virkon solution for a minimum of 1 hour. Both liquid and solid waste is autoclaved in order to ensure a 100% kill of GM material. Departmental autoclaves are serviced annually using a thermocouple. Refer to attached virkon data sheet for further information.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The local GMSC considered that the work using retroviral vectors required containment level 2 because of the potential oncogenic properties of the inserts. Other aspects of the project e.g. construction of plasmids in disabled Ecoli K12 strains have been assessed separately by the GMSC.

Project Containment

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Project Ref 8/12.7

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<td>Molecular analysis of fungal virulence</td>
<td>Class 2 &lt; 1 Litre</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements [N]
### Purposes of the contained use

The specific aim of this work is to identify, sequence, disrupt and analyse the expression of fungal genes involved in virulence. To achieve this genes will be isolated from genomic DNA by PCR, heterologous hybridisation, mutant complementation and related techniques. This will require construction of Aspergillus and Candida libraries in Saccharomyces cerevisiae/Escherichia coli shuttle vectors e.g. YEp24. Genes of interest will be sequenced and characterised further. As part of this further analysis gene disruption and replacement experiments will be performed. In addition these lesions will be complemented using the homologous gene. This will be done by DNA mediated transformation of viable fungal cells using non-mobilisable vectors carrying appropriate nutritional (for complementation of auxotrophy) or antibiotic (e.g. hygromycin and phleomycin) markers. Immuno compromised mice will be infected with GM Aspergillus and Candida spp by nasal droplet, or intravenous injection, and after sacrifice tissues excised and analysed for fungal gene expression.

### Recipient or parental organism

**Escherichia coli:** Strains in common laboratory use eg DH5alpha, XL-10 will be used as cloning tools. These strains, which are generally derivatives of the K12 strain, have a widespread and long history of safe laboratory use. They also contain numerous mutations eg thi-1 which render the strains auxotrophic and therefore unlikely to survive outside the laboratory environment. ACDP HG1

Aspergillus nidulans: there are a huge number of classically produced mutant strains of A. nidulans. These have been constructed in many strain backgrounds and often have been crossed many, many times. These strains have a long history of safe laboratory use and usually contain auxotrophic mutations eg argB that would prevent survival outside the laboratory. ACDP HG1

Aspergillus fumigatus: AF237, D141 and ATCC46645, 293, CEA10 and auxotrophic derivatives thereof eg pyrG will be used to construct knock-out, regulatable and reconstituted strains. These backgrounds have a long and safe history of use worldwide. Additionally the pyrG auxotrophies would prevent survivial outside the host. ACDP HG2

Candida albicans: SC5314, CAI4 and CAI10 and auxotrophic derivatives thereof eg his3/his3, ura3/ura3 will be used to construct knock-out, regulatable and reconstituted strains. ACDP HG2

Candida glabrata: ATCC2001 and auxotrophic derivatives thereof eg his3, ura3, trp1 will be used to construct knock-out, regulatable and reconstituted strains. ACDP HG2

Pichia pastoris: A methylotrophic yeast used as a protein expression system, commercially sourced. GS115 and SMD1163 and auxotrophic derivatives thereof eg his4 will be used to express selected fungal proteins. These strains have a long and safe history of laboratory use worldwide. ACDP HG1

Saccharomyces cerevisiae: the majority of laboratory strains are derived from three major wild type lineages S288C,

We also seek permission to use other strains of the spp described above that have essentially the same characteristics as those described.

### Host/vector system

- E. coli and S. cerevisiae vectors including pUC, pBluescript, YE, Y1 and YC series and similar
- We will use a series of C. glabrata episomal vectors (Kitada et al, Gene 175:105, 1996)
- Mobilisable Aspergillus vectors will not be used.
We will construct Aspergillus and Candida libraries in Saccharomyces cerevisiae/Escherichia coli shuttle vectors e.g. YEp24. Genes of interest will be sequenced and characterised further. As part of this further analysis gene disruption and replacement experiments will be performed. In addition these lesions will be complemented using the homologous gene. It is not possible to list all of the genes that will be inactivated as we are attempting to characterise complex processes required to maintain fitness en vivo, e.g. nutrient sensing, adherence, metabolic plasticity etc. Disruption of these genes is by definition likely to result in a reduced ability to survive in vivo and therefore to cause disease. Inserted genes will either be from the same species, e.g. used to complement a null allele to create a reconstituted strain, tags added to selected genes e.g. GFP, TAP, HA, etc. Or heterologous antibiotic genes eg ampR, bleR, hygR as markers of transformation.

Evaluation of foreseeable effects

The most hazardous GMM will be a reconstituted Hazard group 2 Fungal pathogen from the list of hosts e.g. Aspergillus fumigatus. This will occur where gene disruption and replacement experiments have been performed and lesions complemented using the homologous gene and where DNA mediated transformation of non-mobilisable vectors is used carrying appropriate nutritional genes for complementation of auxotrophy. Any antibiotic or antifungal selection markers will not be the same as those used for frontline therapy.

The worst case GMO constructed is not anticipated to be any more hazardous than the wild type organisms. It is unlikely that an accidental release would harm the environment. Although classified as Hazard group 2, GM Class 2 the Aspergillus and Candida spp used are opportunistic pathogens that cause disease principally in immuno compromised individuals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste - Treated with virkon at a minimum of 1% concentration after dilution with the waste for a minimum of 30 minutes. Disposal after treatment will be to drains. This has been independently validated as an effective method of inactivation of the fungal pathogens in use and Aspergillus spores. http://www.therapeuticresource.ca/sporicidal.html

Solid waste - Plastic pipette tips will be disposed of into a 1% virkon solution after use for a minimum of 30 minutes before being drained and subject to autoclave treatment detailed below.

Solid Waste - Solid waste will be placed into autoclave bags and taken in lidded containers to the autoclave for treatment at 134 degrees for up to 30 minutes holding time. This waste will then be placed in orange clinical waste bags for disposal as clinical waste by the authorised Imperial College waste contractor. The Autoclave is serviced quarterly.

Annually a worst case scenario mock load is prepared and placed in the autoclave. An engineer using a 12pt thermocouple load temperature NAMAS test apparatus ensures that every test point within the load achieves a temperature known to be effective at inactivating pathogenic microorganisms. At the same time the autoclaves load and chamber sensors are calibrated against the test rig. In all cases a minimum of a 10^5 reduction in microbiological burden is expected.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
The Committee were satisfied with the risk assessment and had no comments.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2</td>
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**Project Ref** 80/07.3

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<td>Inducible shRNA--mediated knockdown of B-Raf gene expression in melanoma cell lines.</td>
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</table>

**Historical Significant Changes**

Transferred from GM141

**Project Additional Information**

*Purposes of the contained use*

The aim of the project is to assess the effect of blocking the expression of B-Raf and related signalling proteins on the growth of melanoma cell lines in a 3-dimensional model which more closely replicates the tumour.
microenvironment. We have previously shown that 5iRNA-mediated gene knockdown of B-Raf inhibits melanoma cell growth. However, this culture model is not compatible with siRNA oligonucleotides. Therefore, we wish to create melanoma cell lines which stably integrate 5hRNA sequences into their genome for long-term gene knockdown.

Recipient or parental organism

The recipient cell lines will be primary human melanocytes or immortalized human melanoma cell lines. Each of these cell lines are commercially available, have been maintained in cell culture for many years, are not known sources of human pathogens, and are unlikely to survive outside the laboratory environment.

Host/vector system

The vector to be used is a lentivirus derived from HIV-1. However the vector has been modified in several ways to ensure that the virus is replication-defective, and will not form active viral particles unless it is co-transfected into a packaging cell line with helper plasmids.

Origin & function

The genetic material that will be cloned into the lentiviral vector will be DNA that encodes a short hairpin RNA molecule directed against B-Raf or related genes which support melanoma progression. Expression of the shRNA molecule will result in gene-specific knockdown, most likely promoting growth inhibition or cell death in the transfected cells. We also propose to use a lentiviral vector encoding the bacterial Tet-repressor protein, so we can achieve inducible expression of 5hRNA in the cell lines under study. Once Tet-repressor and shRNA constructs have been stably introduced into cells, shRNA expression and subsequent gene knockdown will only occur if tetracycline is added to the culture medium.

Evaluation of foreseeable effects

The lentiviral vectors which we propose to use do have the potential for infecting any human cell. Through good lab ‘1’ practise (GLP) the risk of these vectors affecting the user or others in the laboratory is extremely low. In the unlikely event of infection, the viral particles will not replicate, as discussed above, The expression of these shRNA molecules is hypothesized to target the growth of melanoma cells, and would likely induce cell death. In the case of infection with a lentivirus encoding the Tet-repressor, expression of this protein would have little effect on human cells, as Tet-repressor promoter elements are not present in human genes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We are not requesting derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All transfections and infections of cells will be performed in a designated Class II biohazard safety cabinet, which will be disinfected with 1% Virkon after use. Virkon is a chemical disinfectant that kills 99.999% of organisms in less than 10 minutes. The cabinet will prevent unwanted infection of the cell cultures as well as release of the viruses into the environment. Infected cells will be grown in a designated incubator. Pipettes, containers and plasticware contaminated with viruses will be left completely submerged in 1% Virkon before disposal. Cells and growth medium will be treated with 1% Virkon before disposal according to the Institute’s Waste Index and Disinfection protocols. HIV-1 is transmitted through blood and a limited number of bodily fluids, none of which will be encountered in the course of this work. Further, sharps will not be used. To date, no one has been identified as infected with HIV due to contact with an environmental surface (see http://www.cdc.gov/hiv/resources/qa/qa35.htm). No living material infected with HIV-1 derived lentivirus will be transported other than between cabinet, microscope and incubator in the tissue culture room 5C10.1 (ACGM2), but cell extracts will be transported between room 5C10.1, laboratories 4N19-21 (ACGM1) and the equipment room on the 4th floor (ACGM1). Viral preparations will be double-contained and transported between room 5C10.1 and the —80oC freezer (room 4S4, ACGM1) for storage. In the event of a spillage, chlorine-releasing disinfectant granules (HAZ-TAB) or 1% Virkon will be used for immediate disinfection.
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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GM Centre Number: 542

Data Premises Notified (Originally) 29/07/1987

Transferred from 1992 Regs? Y

Transitional Premises Class 3

Data Premises Closed

Transitional Premises Emergency Plan Required? Y

Non-GMMs Y

Withdrawn N

Name

THE FRANCIS CRICK INSTITUTE

Name 2

Department

Campus Estate or Research Centre

THE RIDGEWAY

Building

Road Name

MILL HILL

District

Town

LONDON

County

GREATER LONDON

Postcode

NW7 1AA

Country

ENGLAND

Tel Number 0208 959 3666

Fax Number 0208 906 4477

E-mail

HSE Division LONDON

Comments

Change from National Institute for Medical Research 01/04/2015

Date at Which Additional Info Submitted

09/05/2003
Premises Addresses

<table>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

Membership
1. A Chairman to be appointed by the Executive.
2. Representatives appointed by management (such as the Group Leader of a laboratory, Floor or Quadrant manager or senior members of staff) with responsibility for or use biological agents activities and/or the use of radiation.
3. Representatives, chosen by the Staff Forum from all persons having access to the biological agent facilities, or who might otherwise be exposed to work with biological agents, such as research workers, administrative, technical and ancillary staff, students or visiting workers.
4. The Biological Safety Officer.
5. The Deputy Biological Safety Officer.
6. An Occupational Health representative will be a member of the Committee. The representative will automatically receive agendas and minutes, although attendance only needs to be as appropriate.
7. Where appropriate, at least one Committee member is to liaise between the BRSC and the Crick Health and Safety committee.
8. The Radiation Protection Officer and/or the Radiation Protection Advisor and the Laser Safety Officer.
9. Co-opted members to supplement internal expertise may be needed to assess particular proposals.
| Level 1 (GMMs) | Yes | Yes |
| Level 2 (GMMs) | Yes | Yes |
| Level 3 (GMMs) | Yes | Yes |
| Level 4 (GMMs) | Non-microbial |

**Other (please specify)**

| Bacteriology | Yes | Parasitology | Yes | Transgenic Birds | Microbiology Research |
| Virology | Yes | Transgenic Animals | Yes | Transgenic Fish | Gene Therapy |
| Mycology | Yes | Transgenic Invertebrates | Yes | Transgenic Plants | Other (please specify below) |

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

02/03/2022
Studies on biological clocks and sleep

Circadian (circa- approximately, -diem day) rhythms are a fundamental property of the life of a cell. When held in temporal isolation, organisms from unicells to humans exhibit behavioural and physiological rhythms that persist with a period of approximately 24 hours. These rhythms are driven by cell-autonomous molecular circadian clocks. They confer selective advantages to organisms by facilitating anticipation, and thereby adaptation to, the alternating day/night cycle. The competitive value of circadian clocks has been demonstrated in prokaryotes and higher plants, whilst disturbance of circadian timing in humans, as seen in rotational shift workers for example, carries significant long-term health costs.

We have developed novel tools, based on variants of fluorescent proteins and the luciferase enzyme, that allow us to probe the inner workings of the clockwork in more detail than previously possible. Since we now want to study the clockwork in neuronal systems and in primary cells, lentiviral systems are necessary to introduce relevant transgenes into the host cell genome for stable expression, especially in intact organotypic tissue slice cultures. Moreover, we also wish to perturb the clockwork by knocking down the expression of various ‘clock genes’ (and their modifiers such as peroxiredoxin proteins), and lentiviral systems allow this in neuronal and ‘difficult-to-transfect’ cell lines, including primary cells.

HIV1-based lentiviruses, modified for safety, have emerged as powerful tools for effecting changes in gene expression in terminally-differentiated cultured cells such as neurons and organotypic tissue slices. Therefore, we seek authorization to use such vectors to attain our goals in relation to clock research.
The lentivirus is produced by co-transfecting the packaging plasmid mix and the transfer vector into human HEK293FT cells. The recipient cells will be various murine and human cells in culture, including primary cells. Both the recipient cells and the producing cells require specific cell culture conditions to grow and are not harmful. They cannot colonise the environment nor cause disease by colonizing human or animal hosts and thus are classified as ACDP Group 1. Cells from human volunteers, which may be used in this project are uncharacterised and although themselves present no greater risk, may contain adventitious infectious agents and therefore will be handled at Containment Level 2 within the host laboratory.

Host/vector system

The ViraPower™ Lentiviral Expression System (Invitrogen), facilitates highly efficient, in vitro or in vivo delivery of a target gene to dividing and non-dividing mammalian cells using a replication-incompetent lentivirus. Similar systems have been developed by multiple companies and use very similar (if not identical) components, e.g. Sigma Mission shRNA pLKO based system. This new generation of lentiviral systems has been used extensively throughout the World and includes important features designed to enhance its biosafety:

• The pLenti expression vector contains a deletion in the 3′ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
• The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).
• The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns et al., 1993; Emi et al., 1991; Yee et al., 1994).
• Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).
• Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
• The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.
• Expression of the gag and pol genes from pLP1 has been rendered Rev dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al., 1998).
• A constitutive promoter (RSV promoter) has been placed upstream of the 5′ LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA (Dull et al., 1998).

References

activate components of the circadian clock we will use cloned cDNA that will be over-expressed. The genes in question are neither oncogenes nor tumour suppressor genes and thus accidental infection of a human host by these viruses is likely to have no greater consequence than infection by an empty virus. We will also express bioluminescent and fluorescent proteins as 'reporters' of the clock within cells, which are derived from firefly and jelly fish sequences respectively. These have a long history of safe use in various non-replicative lentiviral systems.

A theoretical exception to this principle is a retrovirus encoding a prion-forming protein, which could theoretically set up a prion infection in the host. Therefore, proteins with a potential to undergo prion formation (e.g. mutant PrPsc) will not be used.

The short hairpin RNA (shRNA) sequences used to inactivate genes in the host are derived from the host cell genome and tested in conventional (non-lentiviral) vectors before use to test their function in cell lines. They will be directed to genes that function in the circadian clockwork, usually as transcription factors that switch oscillating genes on/off over the 24 hour day. These genes are neither oncogenes nor tumor suppressor genes and thus accidental infection of a human host by these viruses is likely to have no greater consequence than infection by an empty virus.

List of genes / fusions encoding 'reporter' proteins that will be expressed from lentiviral vectors:

- Basic fluorescent proteins: GFP, RFP, dsRED, CFP, YFP
- Redox-sensitive fluorescent proteins: roGFP1/2, roGFP1/2-Grx1, roGFP1/2-Trx, roGFP1/2-Prx1, roGFP1/2-Prx2, roGFP1/2-GAPDH
- NAD/NADH-sensitive fluorescent proteins: SuperFrex
- H2O2-sensitive fluorescent proteins: Hyper, Hyper-2, Hyper2-Grx1, Hyper2-Trx, Hyper2-Prx1, Hyper2-Prx2, Hyper2-GAPDH
- Bioluminescent proteins: Luciferase (Luc2), OxyLUC
- Light-sensitive ion channels: Channelrhodopsin, Halorhodopsin (and variants)
- Light-sensitive expression systems: Lite-On
- Voltage/Calcium sensing fluorescent proteins: Cameleon, Case12, GCAMPS, Arch3, PROPS, VFSP3
- pH-sensitive fluorescent proteins: pHluorin, synapto-pHluorin, pHred

List of genes encoding proteins that function in the circadian clockwork. The expression of these genes will be inhibited by shRNA-containing lentiviral vectors, or the genes will be over-expressed by lentiviral vectors:

- Mouse or human Clock, Bmal1/2. Encode clock-relevant transcription factors.
- Mouse or human peroxiredoxins (Prx1, 2, 3, 4, 5, 6). Encode clock-relevant antioxidant response proteins.
- Mouse or human catalase. Encode clock-relevant antioxidant response proteins.
- Mouse or human superoxide dismutase. Encode clock-relevant antioxidant response proteins.
- Mouse or human thioredoxins (Trx1, 2). Encode clock-relevant antioxidant response proteins.
• Mouse of human glutaredoxin (Grx). Encode clock-relevant antioxidant response proteins.
• Mouse of human glutathione reductases. Encode clock-relevant antioxidant response proteins.
• Mouse or human GAPDH, Enolase. Encode clock-relevant metabolic enzymes.

Evaluation of foreseeable effects

Inactivation of most genes involved in circadian clock function by RNA interference (RNAi) will have no significant effect on the cell, and if anything, make them more susceptible to undergo apoptosis. The genes that will be disabled are not known oncogenes. Thus, the risk associated with viruses encoding such genetic elements is predicted to be less than that or empty lentiviruses.

Gain-of-function of clock components by overexpression is unlikely to translate to a survival benefit for accidentally-transduced cells, let alone serve as an oncogenic event. The same is true for bioluminescent and fluorescent reporter proteins; these have no other recognised function than to produce light. Thus, the risk posed by gain-of-function vectors is likewise less than that of empty viruses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture materials and labware will be autoclaved and incinerated which eliminates 100% of the infectious material. All solid waste will be collected in double autoclave bags, sealed with autoclave tape, labelled with the users name and the room number and will be autoclaved and incinerated. Culture fluids will be treated with 2% Virkon for 16 hours (which eliminates 100% of infectious material) and disposed to drains. Alternatively, disinfected liquid waste will be gelled with Vernagel in sealed containers labelled with autoclave tape, and taken to the autoclave room for transfer to the incinerator. Spills will be sprinkled with Virkon powder/Trigene and Vernagel reagent to solidify. Recyclable labware will be soaked in 1% Virkon for 16 hours (which eliminates 100% of infectious material). Bench/cabinet surfaces will be wiped down with 10% Trigene and 70% ethanol (which eliminates 100% of infectious material).

Is an emergency plan required according to regulation 20? 

N

If yes, tick to confirm that it is attached to this form 

N

Tick to confirm that you have attached a risk assessment to this form 

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment 

N

Please enter comments on the GM safety committee on the risk assessment
The project has been reviewed by the Institute of Metabolic Science's biological and genetic modification safety committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

**Project Containment**

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**Project Ref** 170/12.2

Date Ackn'd 24/04/2017

CU2 Project Title

Interactions between viral replication and metabolism

Class 2

≤ 1 Litre

Class 2

< 1 Litre

Consent Granted

Non-GMM

Tick if notifying a connected programme of work N

Historical Significant Changes

Project transferred from GM170 24/04/2017

Withdrawn N

Date Project Ceased

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

Herpesviruses are ubiquitous pathogens of man and all vertebrates. In humans, herpes simplex virus (HSV) causes oral and genital lesions and occasionally more serious diseases such as keratitis and encephalitis. Following primary infection latent virus resides in sensory neurones and can reactivate from these cells to facilitate recurrent disease/
transmission. Murine gammaherpesvirus 68 is a gamma-2 herpesvirus, originally isolated from small free-living rodents in Slovakia. It behaves as a natural pathogen in conventional laboratory mice and has been used extensively as a model for the study of gammaherpesvirus pathogenesis. MHV-68 causes an infectious mononucleosis-like illness but is otherwise avirulent in adult, immunocompromised mice. Spread between mice is rare, even when housed in the same cage; intimate contact is probably required for virus transmission. Infection of humans has not been documented. In mice the virus productively infects epithelial cells and establishes a latent infection in B lymphocytes. The object of this research is to identify metabolic processes that influence the kinetics of virus replication in vitro and viral dissemination in vivo using reporter gene expressing HSV and MHV-68. This will be achieved by infecting mammalian cell lines and both wild type and genetically altered mice. The contribution of individual virus encoded gene products to such interactions will be assessed using viral mutants with specific gene truncations or deletions. In addition, viral gene products cloned into replication defective retroviral and/or Adenovirus vectors to facilitate the transduction of mammalian cells will be used to probe such interactions further.

Recipient or parental organism

Parental viruses: HSV 1/2 (HG2 pathogens), MHV68 (HG1 pathogen)
The biological functions of HSV-1, HSV-2 and MHV-68 genes are largely undefined however HSV and MHV-68 do not encode known toxins or proven oncogenes. In common with other well studied and genetically related herpesviruses it is expected that both HSV and MHV-68 will encode gene products including miRNAs that affect multiple cellular pathways/processes.

HSV encodes some 80 different gene products and at least 15 miRNAs involved in various aspects of virus replication, cell modification and immune evasion. The detailed mode of action of these virus encoded products is in many cases poorly understood. A number of gene products are known to be toxic in the expressing cell, for example the HSV-1 UL41 gene shuts off host protein synthesis and overexpression of a number of immediate early gene products prevent cell division (e.g. ICP0).

MHV-68 encodes some 70 different gene products and at least 8 miRNAs involved in various aspects of virus replication, cell modification and immune evasion. The detailed mode of action of these virus encoded products is in many cases poorly understood. A number of gene products are know to be toxic in the expressing cell, for example MHV-68 shuts off host protein synthesis and the virus can induce cell division and proliferation of B cells. In addition, specific gene products are involved in immune evasion, such as the K3 gene product that specifically degrades mouse (but not human) MHC class I, and M3, which is a pan chemokine binding protein.

Standard mammalian cell lines such as NIH 3T3, HEK (human embryonic kidney) 293, MEF (murine embryonic fibroblasts), HeLa and BHK (baby hamster kidney) cells. All mammalian cell lines used have a history of safe use and it is unlikely that they will carry adventitious pathogens.

Host/vector system

Bacterial artificial chromosomes (BAC): The use of full-length infectious herpesvirus genomes cloned as BAC has significantly improved the process of genetic manipulation of the genomes and is an approach widely used throughout the herpesvirus research community. The risks posed by working with either naked viral DNA or genomes cloned as BACs are low since the specific infectivity of DNA is many orders of magnitude lower than that of fully formed virus particles. Herpesvirus genomic DNA is however capable of infecting human cell lines in culture if introduced in certain ways (e.g. by transfection procedures) and therefore there is a potential risk of infection when working with or handling virus genomes cloned as BACs. The risk is very low because a route of direct delivery of the BAC DNA into cells is necessary for infection and would be highly unlikely during normal laboratory handling. Non mobilizable bacterial genomic vectors to be used: pUC series, HSV cloned as bacterial artificial chromosome in a derivative of pBAC108L (disabled F plasmid), MHV-68 cloned as bacterial artificial chromosome in a derivative of pBAC108L.
Bacterial hosts will be multiply disabled E.coli K12 derivatives eg DH5 alpha, DH10B, JC8679 for all bacterial cloning. These derivatives (HG1 pathogens) are disabled and do not compete with normal gut flora.

Adenovirus vectors: The parent vector is an Ad-5 recombinant in which the E1 region is replaced by a lacZ coding cassette. This virus grows only in helper cells (eg 293 cells) that provide the E1 gene product in trans, and is an ACDP hazard group 1 organism. All insertions will be in the E1 region, replacing the lacZ cassette. This effectively eliminates the possibility of generating a replication competent virus carrying the foreign insert by recombination with a wild type virus. Adenoviruses are spread via the respiratory aerosol route and this route represents the major risk of infection.

Retrovirus vectors: Third generation lentivirus vectors lack pol, tat, env, rev and gag genes and can only be packaged following complementation with helper plasmids expressing RT, IN, tat, rev together with the VSV G protein. These are expressed from different plasmids so reconstitution of wild type virus is not possible. In addition, the vector genome has a self inactivation LTR to reduce the risk associated with insertional mutagenesis. Host range and virus stability is expanded by incorporation of the VSV G protein.

Herpesvirus vectors: Herpes simplex virus deleted for the gH gene is replication defective and cannot complete a full round of replication. Such vectors can be propagated on gH expressing complementing cell lines that do not result in the generation of detectable levels of replication competent virus since there is no homology between the virus and the gH expression cassette in the CR1 cell line. Following infection of noncomplementing cell lines the virus can only go through a single round of replication and produces non infectious virus progeny. This vector is classified as a class I GMO by the HSE.

Technique used to introduce insert or vector into host:
Infection of mammalian cell lines and mice with recombinant HSV or recombinant MHV-68 containing gene disruptions and/or expressing reporter genes.
Cloned genomic fragments of HSV or MHV-68 will be cloned directly into pUC-based vectors and transformed into E.coli K12 derivatives. HSV or MHV-68 cloned as a bacterial artificial chromosome will be transformed into E.coli K12 derivatives and purified plasmid DNA used to reconstitute virus following transfection of mammalian cell lines.

Retroviral and Adenovirus vector mediated transduction of mammalian cell lines with HSV or MHV-68 encoded gene products.

Origin & function

Investigation into the interaction between viral replication and cellular metabolic processes may involve:
1. Genomic cloning of HSV1/2 or MHV68 sequences into plasmid and replication defective viral vectors.
2. Inactivation/modification of virus encoded genes and non-coding RNAs, including alteration of their regulation of expression, using full-length infectious herpesvirus genomes cloned as bacterial artificial chromosomes (BAC).
3. Cloning of reporter genes (e.g. GFP, betagalactosidase, Cre recombinase, luciferase) under latent or lytic cycle promoter control. Each of these proteins has no known biological effects in eukaryotes.
4. The expression of virus and/or cellular gene products in a glycoprotein H (gH) deleted HSV-1 (Class I GMO as agreed with Dr Mark Bale at the HSE) vector. This gH deleted vector is severely disabled and can only be propagated in a gH complementing cell line. The severely disabled nature of such a gH deleted vector makes it very unlikely that it will pose a risk greater than wild type HSV-1 since any cell entering a productive replication cycle will be killed and no infectious progeny will be produced.

Evaluation of foreseeable effects

HSV-1/2 (HG2): Recombinant viruses containing mutations of individual gene products or encoding reporter gene cassettes are most likely to be less virulent than wild type parental virus.
MHV-68 (HG1): recombinant viruses containing mutations of individual gene products or encoding reporter gene...
Retrovirus vectors: There is a low risk of aerosol transmission and efficient transduction would require breach of mucosal membrane or the skin surface. This would result in transduction of a small number of cells as the virus is defective and cannot spread.

Adenovirus vectors: Deliberate attempts to deliver foreign genes in vivo using adenovirus vectors (in gene therapy experiments) establish that this is an inefficient process. In the event of accidental infection:

1. A small number of cells would express the foreign gene. This might result in death of those particular cells if the gene product was cytotoxic.

2. There is a very small risk that a pre-existing adenovirus infection might result in complementation of a recombinant vector by wild type virus. Transient growth of the recombinant might then occur. Infected cells would be killed by virus growth or by immune response to infection. There is no expectation that a herpesvirus gene product would exacerbate an adenovirus infection or modify tissue tropism (note the receptor binding proteins of herpesviruses are integral membrane proteins which cannot assemble into an adenovirus virion). Given these factors, the proposed recombinants are of no greater risk than the parental adenovirus vector but given the uncertainty about the functions of herpesvirus gene products, category 2 containment is appropriate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All disposable culture materials and labware will be autoclaved and incinerated which eliminates 100% of the infectious material. All solid waste will be collected in double autoclave bags, sealed with autoclave tape, labelled with the users name and the room number and will be autoclaved and incinerated. Culture fluids will be treated with 2% Virkon for 16 hours (which eliminates 100% of infectious material) and disposed to drains. Alternatively, disinfected liquid waste will be gelled with Vernagel in sealed containers labelled with autoclave tape, and taken to the autoclave room for transfer to the incinerator. Spills will be sprinkled with Virkon powder/Trigene and Vernagel reagent to solidify. Recyclable labware will be soaked in 1% Virkon for 16 hours (which eliminates 100% of infectious material). Bench/cabinet surfaces will be wiped down with 10% Trigene and 70% ethanol (which eliminates 100% of infectious material).

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
The project has been reviewed by the Institute of Metabolic Science's biological and genetic modification safety committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

**Project Containment**

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**Project Ref** 542/01.1

Date Ackn'd: 24/01/2001

CU2 Project Title: STRUCTURAL PROPERTIES OF RECOMBINANT PRION PROTEIN MOLECULES

Date Project Ceased: 

Class: Class 2

Consent Granted: Not Applicable

Non-GMM: 

Project notified under transitional arrangements: N

Withdrawn: N

Historical Significant Changes: GM542/03.2

Historical Date of Additional Info: 09/05/2003

Significant Change ID: 542/04.3

Date of Significant Change: 21/10/2004

**Project Additional Information**

Purposes of the contained use:

CL3: Expression of recombinant prion protein sequences (r-Prp) in bacterial and yeast hosts; protein isolation and purification using conventional protein chemistry techniques of cell lysis and centrifugation to produce samples of non-viable prion protein. Further handling of all protein samples will be at CL3, except for wt sheep r-Prp and its natural variants, which will be handled at CL2.
Recipient or parental organism

a) E.coli  b) P.pastoris

Host/vector system

Ecoli B and K12 strains, plus pTrcHis vector (IPTG induced): (for sheep and mouse Prp)
P.past. SMD1163 strain (Invitrogen) (methanol induced): (for human Prp).

Origin & function

The DNA material comes from a) (sheep and mouse r-Prp) Dr J hope, Institute of Animal Health, Compton, UK and b) (human r-Prp) Prof Stephan Weiss, Genecentre, Ludwig-Maximillian University, Munich, Germany.

Sequences of cDNA for sheep and mouse r-Prp will be expressed in E.coli to produce purified recombinant prion protein r-Prp lacking glycosylation and GPI anchor. This material has beh conformation of the non-transmissible cellular Prpc protein. The sequence will be modified to include FLAG, and/or hexaHis tags to aid purification. Single point mutations will be made of individual residues in sheep r-Prp, which are candidates for specific structural roles eg V115M to give the 3F4 monoclonal antibody epitope, as occurs naturally in hamster and mouse Prp. Truncation and deletions of large fragments, and concatenation of sequences will be performed for X-ray structural and spectroscopic studies of the conformational properties of the sheep r-Prp molecule as a model for the self-association of the non-transmissible prion protein in its wild-type cellular form.

The yeast expression system has been used (LMU, Munich) to express the monomeric and covalent dimeric form of the human prion protein r-Prp(Hu), with similar purification tags. This glycosylated material will be used for structural studies by X-ray diffraction.

Evaluation of foreseeable effects

Purpose of work:

1. To crystallise recombinant prion protein (recPrp) in its natural, cellular non-transmissible form, alone and as a complex with an existing monoclonal antibody.

2. To express and purify the recPrp in its wild type and various related forms for structural studies.

3. The aim is to use the structural information from the Xray analysis to interpret the known properties of the prion protein in solution. This will entail repeating some of the published biophysical and spectroscopic experiments using sheep r-Prp. In no case has any of these studies generated material with transmissible or infective properties.

Foreseeable effects:

A GMO expression or prion protein is classified as a potential hazard. Extensive published literature indicates that the proposed in vitro experimental conditions for handling the recombinant Prp are unable to bring about conversion of the natural form of Prp-c to an infective or transmissible form, Prp-sc. The genetic manipulation in E.coli will be performed using the sequence of sheep Prp, since this form is without known hazard; also there is no evidence for transmissibility of sheep scrapie in the human system. The recPrp(hu) dimer (expressed in yeast), and the rec-Prp(mouse) dimer (expressed in E.coli) have been shown to be non-toxic in neuronal cell culture. The recombinant prion protein materials will be used in vitro only. No scrapie protein material from any infected animal source will be involved, hence no hazards are foreseen. All expression and purification work will be performed at CL3. Wild-type sheep r-Prp and its natural variants (eg C151) will be handled at CL2. Other species, dimers and sheep r-Prp including mutations will be handled at CL3.

Also see Risk Assessment, attached.
Not-applicable.

For Class 2

Containment Measure 8 - Autoclave required in building.

An autoclave is an out-building serving the containment 4 laboratory has been adapted to take prion waste and we would wish to use this machine rather than adapt one in the main building. Waste will be decontaminated as far as possible and transported to the autoclave in robust sealed containers.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste from the CL3 activities in the expression of recombinant prion protein will be pooled and autoclaved at 137 deg C in the special NIMR facility attached to the CL3/CL4 laboratory where all the contained work will be performed. These are standard conditions for the complete killing of bacteria and yeast. No toxic or transmissible material from animal sources is involved.

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<td>Tick to confirm that you have attached a risk assessment to this form</td>
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Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment

The Biological Safety Committee have approved this project for work at Classes 2 and 3.

### Project Containment

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**Project Ref** 542/01.2

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## Project Additional Information

### Purposes of the contained use

Generation of virus vectors; administration of vectors to tissue cultures and to intact tissue in situ.

### Recipient or parental organism

GMO: Replication-defective Sindbis virus. No adverse effects foreseen.

Recipient: rodent cells in culture; laboratory rats and mice.

### Host/vector system

Sindbis virus, replication defective.

### Origin & function

Reporter genes and probes derived from Aequoria and coral fluorescent proteins and firefly luciferase, and fusion proteins including various neurotransmitter receptors and signalling proteins of rat and mouse. These will serve as probes to determine activity-dependant activation and/or redistribution of proteins of interest, to monitor levels of intracellular messengers, and to ascertain the function of genes and gene products implicated in synaptic transmission and plasticity.

### Evaluation of foreseeable effects

None likely. The virus system to be used generates only replication-defective virus, not functional virus. Even normal virus can only be spread to humans via mosquitoes, and there is no obvious way for mosquitoes to take up our reagents.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Waste material will be managed according to the attached code of practice.

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This project has been approved by the NIMR Biological Safety Committee for work at Containment Level 2.

Project Containment

**Project Ref**  542/03.1

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<td>03/01/2003</td>
<td>ANALYSIS OF FV1-LIKE RESTRICTION OF LENTIVIRAL REPLICATION IN NON-HUMAN PRIMATE CELLS.</td>
<td></td>
<td>Class 2</td>
<td>1 litre</td>
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Withdrawn | N | Tick if notifying a connected programme of work | N |

Historical Significant Changes
### Project Additional Information

**Purposes of the contained use**

We plan to study a series of genes from non human primates that are capable of blocking lentivirus replication at a post-penetration, preintegration step in the viral life cycle. For this purpose we intend to set up a series of assays allowing us to monitor HIV-1, SIV, FIV and EIAV replication in a one-step assay.

**Recipient or parental organism**

Viral vectors will be derived from HIV-1, SIV, FIV and EIAV.

**Host/vector system**

Viral vectors will be prepared by co-transfecting three plasmids into 293T cells. The first plasmid encodes the viral genome, and includes cis-acting sequences required for viral transcription, packaging, reverse transcription and integration and carries one or more reporter genes. It does not encode viral genes required for replication such as gag, pol or env. The second plasmid provides (in trans) the products of gag and pol but lacks an RNA packaging signal. The third plasmid provides (also in trans) env function via the VSV-G protein. Two days after transfection virus is harvested from the cell supernatant.

**Origin & function**

Recovered virus will be used to infect a variety of cell types. These will be cultured for 2-4 days, fixed in formaldehyde and scored for virus integration either by FACS analysis or by LacZ staining. This assay will form the basis of our attempts to probe the interaction between the restriction gene and its viral target.

**Evaluation of foreseeable effects**

Lentiviruses are associated with a number of pathological effects in humans and other animals. By comparison, the lentiviral vectors we are proposing to employ will be relatively harmless. Lentiviral pathogenicity required high level virus replication; the vectors we will be using are incapable of productive infection, giving rise to only one round of replication. They have been designed in such a fashion as to reduce the chances of recombination to yield infectious virus to effectively zero. However it is still theoretically possible that they might cause injury by a mechanism of insertional activation of cellular oncogenes. This remote risk applies equally to humans as well as other animals. This risk aside no other adverse effects are foreseen.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be autoclaved (121 degrees C for 15 min) and liquid waste will be treated with chloros (>2,500ppm for at least 60 min) according to the local code of practice followed. Cells will be treated with 3.5% formaldehyde prior to FACS analysis.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee have reviewed this project and have agreed with the classification of Class 2.

Project Containment

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Project Ref 542/03.4

Date Ackn'd 12/11/2003

CU2 Project Title PRODUCTION OF HIV-ENV/INFLUENZA HA RECOMBINANT GENES FOR THE EXPRESSION OF RECOMBINANT GLYCOPROTEINS (RGPS) FOR STRUCTURAL (X-RAY CRYSTALLOGRAPHY) STUDIES AND INCORPORATION INTO INFLUENZA

Class CultureVolClass2 CultureVolumeClass3-4

Class 3 up to 2 litres

Non-GMM Consent Granted yes

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

Vaccinia recombinants containing the envHA genes (VACeH) will be made and used to assess the feasibility of expressing proteins from such constructs. Experiments will be performed on protein expressed in VACeH-infected cells to assess the receptor-binding and fusion properties of the expressed proteins as indicators of their correct folding. If the expressed proteins function as predicted (based on what is known of the functions of the parent env and HA), cells will be cultured in bulk, infected with VACeH and used as a source of glycoprotein for purification for structural analyses. Additionally, mammalian cell clones will be established that constitutively express the envHA glycoprotein (based on the vector system pEE14tpa). The latter work can be conducted at containment level 1. Attempts to produce recombinant influenza viruses for therapeutic use in HIV-infected humans and studies of any viruses produced will initially be carried out at containment level 3 (see risk assessment). When (if) such studies establish the safety of the viruses permission will be sought to use them at lower containment levels.

**Recipient or parental organism**

Escherichia coli strain DH5a (for growth of plasmids).
Vaccinia strain vRB12 (to allow selection of VACeH recombinants).
A variety of transformed-mammalian cells (eg CV-1, BHK, CHO, NSO, 293TY, MDCK).
The plasmid-based reverse genetics system developed for the human H1N1 influenza virus, WSN.
The organisms indicated are those in use currently (in relation to other approved activities involving genetic modification) - we may wish to change specific reagents if better ones become available.

**Host/vector system**

pRB21 - vector used to allow production of vaccinia recombinants.
pEE14tpa - vector used to transform mammalian cells for constitutive expression of envHA.
pHH21-vector, a series of plasmids used to produce recombinant influenza viruses.

**Origin & function**

Influenza HA and HIV env-genes cloned into a variety of vectors (under approved activities involving genetic modification) already exist at NIMR. They are used for protein expression, functional and structural studies. In addition, the elements to construct the "conditionally lethal gene" (the HIV-2 LTR and the Thymidine kinase gene from Herpes Simplex 1) for inclusion in recombinant influenza viruses have already been cloned at NIMR.

The env-HA gene recombinants will be produced by PCR amplification of the relevant gene segments of pre-existing HA- (X31 in the first instance) and HIV env-gene (NL43 and JRFL in the first instance) clones. The fragments will then be stitched together using PCR-SOE (Splice Overlap Extension) and cloned into a vaccinia shuttle vector (pRB21 in the first instance) and mammalian expression vector (pEE14tpa in the first instance) for amplification in a disabled E. coli strain (DH5a in the first instance).

CV-1 cells will be infected with an attenuated strain of vaccinia (vRB12 in the first instance) and then the pRB21 constructs will be transfected in to allow production of vaccinia recombinants. The recombinants will then be used to infect CV-1 cells in bulk culture to produce rgps for purification.

The pEE14tpa constructs will be used to transfect mammalian cells (CHO in the first instance) which will then be treated with the drug Methyl-sulphoximine (MSX) to allow
selection of clones which express the rgps of interest constitutively. The rgps will be purified for functional and structural studies. envHA genes known to express in the recombinant vaccinia system will be modified by using specific primers in PCR to allow incorporation of the genes into the pHH21 vector system and propagation of these vector constructs in E. coli for subsequent generation of influenza virus recombinants. By replacing the influenza HA gene with that of envHA, recombinant influenza should infect cells expressing CD4 and certain chemokine receptors (rather than epithelial cells of the respiratory tract that express suitable sialic acid receptors). When the influenza NA gene is also replaced with the “conditionally lethal gene” and ganciclovir is administered (in culture or in whole body systems) only CD4 expressing cells that are infected with HIV should be killed. Such recombinant viruses may ultimately play a role in the treatment of HIV-infected humans.

See attached papers:
Blasco & Moss (pRB21/vRBV12) - 1995, Gene, 158, 157-162
Neumann et al (influenza reverse genetics) - 1999, Proc Natl Acad Sci USA 96, 9345-50

Evaluation of foreseeable effects

The disabled E. coli hosts will be transformed to ampicillin resistance by all vectors used and all genes to be used in the study have been shown to be stable when cloned. In particular, neither HIV-env nor influenza-HA genes are harmful in bacteria and both proteins (when produced in bacteria) are functionally inactive. It is therefore unlikely that the rgps (envHAs) will be active.

Vaccinia-envHA recombinants (VACeH) will express rgps in infected cells. Such cells will not express constitutively as vaccinia induces a lytic infection. Within the Division of Virology HSE approval has been given for the expression of HIV-env and influenza-HA using vaccinia-recombinant systems. This means that vaccinia has already been given the receptor binding (CD4 and chemokine receptors for HIV and sialic acid for influenza) and fusogenic properties of the other viruses without increasing vaccinia's virulence. The vaccinia recombinants to be produced here should have the receptor binding properties of HIV and, to some extent (to be assessed), the fusogenic properties of influenza.

Mammalian cells transfected with the pEE14tpa constructs will be resistant to MSX and will express rgps constitutively in a soluble condition.

The recombinant influenza viruses should show the receptor binding characteristics of HIV (human CD and chemokine coreceptors) and fusion characteristics of influenza. This will result in altered host cell tropism and may prevent influenza from being an aerosol-transmitted (respiratory) virus. The overall pathogenesis of the recombinant influenza viruses (ability to grow to high titre and spread to a wide range of cell types) are likely to be reduced compared to the parental virus WSN.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable at this stage, but would need to be able to use in a clinical setting when (if) the project goes to a human trial stage.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For work carried out at containment level 1 the relevant Codes of Practice will be used. Liquid waste (to include culture supernatant following removal of expressed rgps) to be treated with Chloros prior to disposal, solid waste to be bagged, autoclaved and incinerated.

For work carried out at containment level 2 the relevant Codes of Practice will be used. All work with vaccinia will be carried out in class II microbiological safety cabinets (laboratories 268b/202 - containment 2) or, exceptionally, in class 1 cabinets (RV2 - containment 3).

For work carried out at containment level 3 the relevant Code of Practice will be used. All work relating to the generation of the recombinant influenza viruses and their purification from bulk cultures will be carried out in class i microbiological safety cabinets (RV2).
The Secretary emphasised prior to the discussion that this project should be treated as Commercial - in - Confidence.

Dr Kioussis asked, on behalf of the committee, whether the virulence of the vaccinia and the env/HA influenza recombinant viruses would be changed by the presence of the fusion protein.

Dr Daniels answered that both parental proteins (influenza HA/gp160) have been expressed in the vaccinia system and used for vaccination purposes. The virulence of the vaccine strains has not been increased above that seen for the parental vaccinia vaccine strain.

Dr Kioussis also asked that since the ENV/HA influenza virus will have an altered tropism due to the presence of the gp120 component of gp160, will this increase or decrease infectivity?

Dr Daniels replied that what has been learned from expressing ENV/HA in the vaccinia system has confirmed what was anticipated, ie it retains the receptor binding characteristics of gp160 but the fusion characteristics of influenza HA. As a consequence of this, the tropism of the proposed recombinant influenza virus to be produced will be altered compared to the wild type influenza. The recombinant influenza will be tropic for CD4+ cells and therefore have a more limited host range than wild type influenza. As a consequence of this the recombinant influenza should be attenuated and no longer be transmissible by aerosol routes. Further to this CD4+ cells of the host immune system are poor hosts for wild type influenza. Therefore it is anticipated that the recombinant influenza will not grow to the same high titre as the wild type. Additionally the majority of the human population will have immunity to influenza that will still function against the products of the genes conserved (6 or 7 out of 8 depending on the particular influenza recombinant) between the wild type and the recombinant influenza. In view of this the recombinant form can be considered an attenuated influenza vaccines.

The committee were satisfied with Dr Daniels answers to these questions and recommended that the project be forwarded to the HSE for consideration as a class 3 activity.

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INVESTIGATION OF GENE FUNCTION AND REGULATION IN MYCOBACTERIUM BOVIS BCG.

The analysis of gene function and regulation in Mycobacterium bovis BCG will involve the construction and characterisation of deletion mutants, the characterisation of individual proteins and their interactions, and the characterisation of gene expression and regulation using reporter genes.

Constructs of selected genes which have been deleted or interrupted by an antibiotic selection marker will be transformed into M. bovis BCG. The resultant recombinants will be screened to identify those in which the gene has been deleted by allelic exchange. Phenotypic analysis of the resultant mutants, and complemented strains in which the mutated gene is reintroduced on an integrating plasmid containing a further antibiotic selection marker, will include growth characteristics in bacteriological media under various conditions and in macrophages and mice to assess any changes in virulence. They will also be used to test their ability to vaccinate against challenge with a virulent M. tuberculosis strain in mouse models of TB.

Tags allowing purification of individual proteins, possibly along with any associated proteins, will be added to the protein of interest via cloning on a plasmid which will then be reintroduced into M. bovis BCG. Following growth, sterile cell lysates will be prepared and the protein of interest isolated in vitro.

Gene expression and its regulation will be investigated by introducing plasmids containing fragments of M. bovis BCG or M. tuberculosis DNA linked to reporter genes which allow ready monitoring of expression levels.

Recipient or parental organism
Mycobacterium bovis BCG.

Host/vector system
For knockout construction, plasmids based on pBluescript which do not replicate in mycobacteria will be used for delivery of the mutation.
For complementation and introduction of tags or reporters, plasmids will be based on the mycobacterial integrating vectors or on replicating plasmids containing the replicon of the mycobacterial plasmid pAL5000 or pMF1.

**Origin & function**

Origin is M. bovis BCG or M. tuberculosis.

For knockout construction the intended function is to permit recombination to delete genes of interest from the chromosome thus creating a mutant strain defective in a defined gene or genes.

For complementation the intended function is to restore a specific gene or genes to confirm that the phenotype of a mutant strain is due to the targeted mutation rather than a secondary mutation elsewhere on the chromosome.

For tag insertions the intended function is to permit the isolation of an individual protein, and maybe associated proteins, from cell lysates of M. bovis BCG for further analysis in vitro.

For reporter insertion the intended function is to drive the expression of a readily assayed reporter gene by mycobacterial expression signals to study regulation of gene expression.

**Evaluation of foreseeable effects**

Hazards to human health.

The recipient microorganism is Mycobacterium bovis BCG, the strain that is used as a live vaccine to protect against TB. All work with this organism is carried out under the Containment Level 2 Code of Practice.

Deletion of genes involved in virulence is expected to decrease virulence in comparison with the parent strain. BCG has genomic deletions compared with the parental M. bovis strain, which result in attenuation. It is most unlikely that deleting further genes will result in increased virulence although this cannot be formally excluded.

For tag insertions, the DNA being added will result in the addition of a harmless tag to a single protein. However, in some cases the protein will be expressed from a non-native promoter and therefore we cannot rule out the possibility of generating a strain with increased virulence owing to the unnatural expression of the protein. However, since pathogenicity in mycobacteria is not due to the acquisition of a single gene, for example a toxin, it is most unlikely that such an increase in virulence will result.

For reporter insertions, the DNA being added will result in the expression of a harmless reporter gene and therefore the virulence of the pathogen is not expected to be altered.

The parent strain to be used is the vaccine strain M. bovis BCG, which is sensitive to all anti-TB drugs. None of the antibiotics used to make the genetic constructs are front-line TB antibiotics, hence the transfer of these resistance markers will not compromise any treatment.

All work will be carried out at Containment Level 2.

Hazards to the environment

The genetically modified organisms would be expected to survive in the environment but not to displace other organisms. They would not be pathogenic to other species.
The control measures used for the human health risk mean that any escape is highly unlikely, and hence that the estimated environmental risk is effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be autoclaved at 121 degrees C in accordance with the code of practice for the containment level 2 laboratory where the work is carried out, to give 100% kill.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

This project has been passed by the Biological Safety Committee for work at Class 2.

**Project Containment**

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**Project Ref** 542/04.1

<table>
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<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>22/03/2004</td>
<td>STUDIES ON THE LIFE CYCLE OF THE HUMAN PAPILLOMAVIRUS AND</td>
<td>Class 2</td>
<td>1-50 litres</td>
<td></td>
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</table>
### Purposes of the contained use

In order to study these viruses, individual genes of these viruses will be cloned into expression vectors and studied individually at first, and eventually in concert. This is to provide a clear picture of the workings of these viruses in the cell.

### Recipient or parental organism

**List of recipient strain**
- **Bacteria**
  - E.coli strains: DH5α, DH10B, Sure, TG1, TB1
- **Viruses**
  - E1-deleted adenovirus
  - Defective retroviruses-all the viral genes have been removed from the vector
  - Defective lentiviruses-all the viral genes have been removed from the vector and self-inactivating
- **Cell lines**
  - HeLa, W12, S12, SiHa, CaSki - All derived from human cervix
  - U2OS, Saos-2 - All derived from human bone
  - FSK, NEK - All derived from human skin
  - COS, CHO - All derived from hamster

### Host/vector system

**List of vectors**
- Vectors without promoters: pSP72, pSP64, pGEM,
- Vectors with promoters that function in mammalian cells: MV11, pCDNA, pBabeNeo, pBabePuro, pBabeHygro, pBabeBlast, LXSN, pJ5 p81, p277, p274
- Vectors with promoters that function upon induction in bacteria: pGEX2T, pMALc, pET

### Origin & function

The genetic materials that are involved are of either viral or human origins. They are genes that will be used in the context of understanding how they affect the life cycle of HPV or AAV. They are not expected to alter pathogenicity, host range, tropism of mode of transmission of the cell of the viruses.
### Evaluation of foreseeable effects

Three types of organisms will be genetically modified, namely bacteria, viruses and human cell lines. Bacteria will be modified to carry either viral or cellular genes of interest. The effect of these genes on the bacteria is not the aim of the exercise. Instead, the bacteria will be used as a convenient host that carries genes of interest that will be eventually extracted from the bacteria and then transferred into human cell lines for analyses.

Human cell lines that receive genes of interest either via infection by recombinant viruses described above or via transfection will be generated. They will either be short-lived cells, which means that they will not be propagated for more than a month or they will be made to be stable lines, which means that they will be cultured for longer than a month. Whether short-lived or stable lines are to be generated will depend on the nature of the experiment and also on the property of the gene of interest that is introduced into the cell.

There is no single or even several GMM that will be made that can be deemed as particularly hazardous because the vectors that will be used are themselves already very safe (e.g., self-inactivating vectors, defective/non-replicating viruses), and the genes of interest that will be studied are not known to be toxic or oncogenic. Instead, several of the genes of interest will be studied as "anti-oncogenic" or tumour suppressor genes.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable to this programme.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

This project has been passed for work at Class 2

### Project Containment

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</table>
Project Additional Information

**Purposes of the contained use**
Modification of listeria monovytogenes to include a protein epitope for the study of ovalbumin specific immune responses.

**Recipient or parental organism**
Listeria monovytogenes is a Gram positive bacterium that causes disease in mice and humans generally when administered in high doses (about 10 (to the power of 8) bacteria) orally. The organism can be pathogenic for pregnant or immunosuppressed individuals. In sublethal infections of mice the bacterium is cleared by innate and specific immune responses and the animal retains long-term immunity.

**Host/vector system**
The host sequence is derived from the ovalbumin gene that is fused to the signal sequence and promoter of the Listeriolysin hly gene. Ovalbumin is non hazardous for humans or animals and will not alter the pathogenicity of the host.

**Origin & function**
Coding sequence introduced into Listeria is derived from ovalbumin. The intended function is for ovalbumin to elicit specific immune responses in the context of a pathogen, which would allow the role of memory T cells in protection against pathogen-associated antigen.

**Evaluation of foreseeable effects**
No effects beyond the effects of Listeria parent organisms are expected.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Containment and control measures as detailed in the attached code of practice covering the use of Listeria will be applied.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management measures are described in detail in the attached Code of Practice covering the use of Listeria monocytogenes.

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Animal Units

| L2 Yes | L3 L4 L2 L3 L4 L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 L2 L3 L4 L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 |

Project Containment

The Biological Safety Committee has passed this project for work at Class 2.

Project Ref 542/04.4

Date Ackn'd 08/11/2004

CU2 Project Title Cloning of eph receptor/ephrins and reporter fluorescent proteins into the Semliki Forest Virus (SFV) vector system Generation of SFV-eph/ephrin infectious particles

Class 2 CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Date Project Ceased

02/03/2022

Page 8598 of 1532602/003/2022
### Project Additional Information

#### Purposes of the contained use

To investigate the role of eph receptor/ephrins in hippocampal plasticity.

#### Recipient or parental organism

**Donor organism:** mouse

#### Host/vector system

**Vector system:** modified Semliki Forest Virus vector system (replication incompetent, conditionally infectious). Plasmids used: pSFV2gen and pSFV-Helper2.

**Host cells:** BHK-21 cells

**Tissue to be infected:** mouse/rat hippocampal slices.

#### Origin & function

**Origin of vector:** wild-type SFV

**Origins of inserted genes:** mouse embryonic cDNA (eph receptor/ephrins) green fluorescent protein (aequoria victoria)

#### Evaluation of foreseeable effects

The eph receptor/ephrin proteins are not inherently toxic, nor do they have any known oncogenic or allergenic functions. They are normally widely expressed in diverse cell and tissue types in both vertebrates and invertebrates. They are unlikely to have significant harmful effects although this would be impossible to exclude, especially in view of their known roles in development and signalling properties. However, the disabled virus vector systems to be used should render this risk negligible. The viral genome is encoded on two separate plasmids, making virions produced replication incompetent. Additionally, specific mutations in the helper plasmid mean that these virions only become infectious following treatment with high, non-physiological concentrations of chymotrypsin. Under careful test conditions, non replication competent infectious particles have been seen to be generated when using this dual-safety system.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This work has been assigned to Containment 2.

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection and waste disposal: 1) Effective disinfectants must be available at all times. The disinfectant of choice will be a chlorine based one. However see notes on
the disinfection of delicate equipment. 2) Work surfaces must be cleaned and disinfected after use. 3) All waste materials must be rendered non-infectious before disposal. 4) Material for autoclaving must be transported by nominated persons direct to the discard autoclave in robust containers. Care must be taken to ensure that the external surface of the container is rendered safe prior to removal from the contaminated area. The container must then be placed immediately into the autoclave by the nominated person. 5) Pipettes and other disposable items must be treated with disinfectant. 6) Liquid wastes must be thoroughly disinfected before disposal via the drains.

Disinfection:
- For routine decontamination (Infected material)
  1) Chloros - 2,500 ppm available chlorine changed daily and made up fresh weekly. Note: Chloros is readily deactivated by organic material. Caution: Not be be used on equipment subject to corrosion.
  2) For delicate equipment - 2% glutaraldehyde. Caution: This material is toxic and must be used with care. The Safety Section must be consulted before use.
  3) For the decontamination of the microscope objective 70% ethanol with an overnight contact time is suitable.
  4) Follow the disinfection by washing with a suitable detergent.
  Adequate contact time must always be allowed for effective disinfection.
  If necessary, contact the Safety Section for further advice.

For spillages and accidents
- Minor spillages - 1) Precept granuals 2) 10,000 ppm available chlorine 3) 2% glutaraldehyde
  Followed by washing with a suitable soap.

AUTOCLAVE CODE OF PRACTICE
1) The temperature at the centre of any load must be maintained at the following minimum times/temperatures: 121ºC = 15 minutes, 126ºC = 10 minutes and 134ºC = 3 minutes.
2) Instructions for the use of the autoclave must be displayed locally.
3) Containers must be of a solid construction and allow for adequate steam penetration throughout the contents.
4) If autoclave bags are in use, then these must be supported in solid containers. The opening must be turned back over the supporting container to allow steam penetration. Discord container lids must be removed unless the steriliser cycle has been designed to cope with the tops in place.
5) Effluent discharge: a) The drainage system must avoid dispersal of splashes and steam into the working area. b) Exhaust from the chamber, together with any discharge from the vacuum pump and any chamber cooling the water, should lead to a cooling/dilution tank which in turn should be connected directly to the foul drain. This pipe should be vented.
6) CYCLE FAILURE
   Should the autoclave fail during the cycle, the safety section must be informed immediately and no attempt must be made to open the chamber until a proper risk assessment has been made.
7) The autoclave must be filled with a safety interlock to prevent access to the contents whilst the temperature of the material inside is above 80ºC.
8) Tests - An annual calibration and validation (under worst load conditions) shall be carried out by an approved contractor. A thermocouple test must be carried out monthly to confirm the satisfactory operation of the autoclave. This test should be carried out under worst load conditions.
   The performance of the autoclave will be recorded to ensure that it meets laid down requirements.
9) Any fault with the autoclave, such as steam leaks, perished seals, etc., must be reported immediately.
10) As the autoclave falls within the provision of the Pressure Systems and Transportable Gas Container Regulations 2000, work on it must only be carried out by a competent person in accordance with the manufacturer's requirements. All such work must be adequately documented and the records retained.
   A written maintenance system must be established and periodic routine examinations of the autoclave must be carried out with the manufacturer's instructions and a Certificate of Examination provided.
11) Unloading - Operators must wear a visor and heat-resistant gloves together with an apron and laboratory coat whilst removing materials from the autoclave. Loads must be easy to handle or be able to be transferred directly to a trolley for final disposal.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Project Containment

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Project Ref 542/07.1

Date Ackn’ed 16/10/2007

CU2 Project Title Genetic manipulation of the human malaria parasite Plasmodium falciparum.

Class 3

Culture Class

Volume Class

<1 litre

Consent Granted Yes

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
**Purposes of the contained use**

We plan to transfect *P. falciparum* parasites with plasmid DNA (vectors) containing all or part of selected *P. falciparum* genes. The products of these genes are important in the maturation of the parasite, the invasion process and/or development within the invaded erythrocyte. We plan to use vectors of the pH, pARL, pLN, pSSPF, pXL-BACII-hDHFR and pHTH series, or their derivatives, which contain different selectable markers for cloning and selection in bacteria and subsequently the malaria parasite. These vectors are designed to allow: single crossover homologous recombination events where the entire vector is inserted into the parasite genome; double crossover events where the input DNA directly replaces the target parasite gene(s); autonomous plasmid replication and expression (as episomes) or insertion of the target gene with a tag, into a non-essential site within the parasite genome to allow expression of the tagged target gene independent of the native gene. A fuller description is given in the Risk Assessment (attached). The target genes will be those that encode proteins located on the surface of mature parasites or the infective stage of the parasite (the merozoite), proteases important in parasite development and merozoite release, organelle specific proteins and factors involved in the parasite’s metabolism. All of these are involved in maturation of the parasite, the invasion process or developmental events in the invaded erythrocyte.

**Recipient or parental organism**

*P. falciparum* is a protozoan parasite of the phylum Apicomplexa, and is one of the agents responsible for causing human malaria. It is normally found in tropical and sub-tropical regions and is transmitted by mosquitoes of the *Anopheles* spp. It is possible to cultivate the asexual and sexual erythrocytic stages (human host) in tissue culture in vitro.

**Host/vector system**

**Host:**
The host sequence is derived from genes encoding proteins involved in the development and maturation of the parasite, parasite metabolism, or recognition and invasion of erythrocytes by the merozoite.

**Vector:**
The vectors are of the pH, pARL, pLN, pSSPF, pXL-BACII-hDHFR and pHTH series or their derivatives, which are derived from pUC series plasmids of *E. coli*. These have been modified to include drug resistance cassettes, 5' and 3' malarial untranslated regulatory sequences (UTR) and appropriate multiple cloning sites. The vectors may also contain sequences encoding reporter proteins; these include widely used enzyme reporters such as firefly luciferase and chloramphenicol acetyl transferase (CAT), fluorescent proteins (e.g. GFP, DsRed, RFP varieties) or epitope tags (e.g. cmyc, HA3, FLAG or Tyl).

**Origin & function**

**Origin of coding sequence:**
Culture adapted cloned *P. falciparum* parasite lines;
3D7, D10, A4, CIO, FCBI, T996 T994
Parasite lines imported: Dd2at and 3D7

**Intended function:**
1) To disrupt the gene of interest in order to ascertain its function (i.e. perform a gene knock out).
2) To ‘tag’ the gene with a marker in order to detect and study the function and activity of the gene product.
   1) This can be done using sequences for GFP or related proteins cloned into a part of the target sequence in order to produce a fusion protein that can be detected by optical techniques such as fluorescence.
   2) Similarly this can be done using short peptide epitope tags (e.g. HA, cmyc, FLAG, Tyl tags). Gene products modified with these tags can be readily detected using immunochemical techniques.
3) Further functional studies on the protein by gene modification or truncation, or replacement with orthologous genes of other species of malaria parasites. This will help to determine the parts of the molecule that are required for its biological function. These truncated genes can also be tagged as in b).
4) To overexpress genes of interest which are otherwise of low abundance (as episomes or integrated into a non
essential site of the parasite genome)
Origin of vector: pHH, pARL, pLN, pSSPF, pXL-BACII-hDHFR and pHTH plasmid series. These contain selectable
markers (drug-resistance) and regulatory or mRNA stabilising elements (5' and 3 malaria UTRs).

Evaluation of foreseeable effects
The main concern when considering this project is that P. falciparum is pathogenic to man. A code of practice (COP) is
already in place (attached) to minimise accidental infection of laboratory workers. The parasite can only be transmitted through the mosquito secondary host or by direct
contact with infected blood by injection or through cuts. No P. falciparum work on these premises involves exposure to sharps such as needles or broken glass. All parent
parasite lines have been screened for drug susceptibility and are known to be sensitive to quinine sulphate. This characteristic will not be altered by any of the work
described above. In the unlikely event that a person is believed to have been accidentally infected, treatment with the appropriate anti-malarial drug will commence
immediately. All used equipment and reagents are decontaminated before leaving the containment 3 tissue culture room (see COP) and thus no live modified parasite will
discarded and transported out of the room. If it is necessary to transport live parasites to another facility, e.g. for centrifugation or microscopy, the parasites are sealed
(in a centrifuge bottle or sealed coverslip on a microscope slide) and transported in a sealed container.
Work with P. falciparum is undertaken under containment 3 guidelines and modification of the parasite does not pose greater risks of infection to humans. These modified
parasites will not be put through the mosquito cycle and thus there is no risk that they will enter the wild population. Most of the genes under study are unique to P.
falciparum and thus it would be difficult to transfer these genes to another organism The parasite will only be cultivated in vitro; no insect transmission will be undertaken.
The work with live parasites is carried out in a sealed room so there will be no possibility of mosquitoes being infected and infecting the wider populace. The parasite is not
viable ex vivo except in the special in vitro culture conditions used. All parasite material is rendered non-infectious with reagents such as phenol or detergents before further
analysis is carried out in non-category 3 containment facilities. The vectors contain drug-resistance cassettes. The drugs most used in the parasite selection process
(WR99210, neomycin, blasticidin and puromycin) are not in general use for the clinical treatment of humans or animals and thus the problem of drug resistance appearing
in the humananimal population is not an issue. Less often, pyrimethamine is also used in selection of transgenics. Resistance to this drug in wild parasite populations is
widespread, and it is not recommended for use alone for either prophylaxis or treatment of acute malaria. Ampicillin is used in the cloning of the vector in the bacterial host.
This antibiotic has been in widespread use in standard laboratory molecular cloning protocols for a number of years and there have been no recorded cases of drug
resistance linked to using these vectors in bacteria.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
The work with live parasites is required to be performed under Containment 3 facilities. However, once the parasite has been treated with denaturing reagents, such as
phenol or detergents, the parasites are non-infectious and can be worked with under Containment 1 facilities.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Refer to Code of Practice, attached. All lab ware (pipettes, flasks, tips and tubes) and culture waste (spent growth medium) are treated by immersion in sodium
hypochlorite solution (2,500 pp million of chlorine) for a minimum of 30 minutes. The medium is rendered colourless, or dark brown precipitates (parasitised erythrocytes)
are observed as the killing process occurs. Lab ware is also autoclaved at 121° C for 15 minutes before being discarded. There is 100% destruction of infectious material
after these treatments.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
This project has been approved by the Biological Safety Committee for work at Class 3. Please see attached comments.

**Project Containment**

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**Project Ref** 542/09.1

- **Date Ackn’d:** 21/08/2009
- **CU2 Project Title:** Investigation into xenotropic murine leukaemia virus-related virus (XMRV) and its role in disease

**Class**

- **CultureVolClass2:** Class 3
- **CultureVolumeClass3-4:** < 1 Litre

**Consent Granted**

- **Non-GMM:** Yes

**Historical Significant Changes**

- **Historical Date of Additional Info:** 21/08/2009

**Project Additional Information**

- **Purposes of the contained use:**

  In 2006 a novel retrovirus was isolated from patients with familial prostate cancer (Urisman et al. PLoS Pathog 2006). This virus is very similar to several endogenous murine leukaemia viruses (MLV) found in mice and murine cell lines, and was named Xenotropic MLV-related virus (XMRV). The presence of XMRV seems to correlate...
with a deficiency in Rnase L, a molecule involved in the anti-viral response induced by interferon. At this time, it is not known whether the prostate cancer in these patients is linked to infection by XMRV and whether this is related to a loss of Rnase L function. The goals of this project are to investigate the link between XMRV and prostate cancer as well as with Rnase L.

A plasmid containing a DNA copy of the XMRV RNA genome under the control of the CMV promoter has been synthesized previously (XMRV VP62 viral molecular clone, Dong et al. PNAS 2007). When transfected into mammalian cells, this plasmid will express viral properties and nucleic acid which will assemble into viral particles. For the purposes of creating a safer, replication incompetent XMRV stock, deletions and mutations will be made in the RNA packaging signal and/or promoter regions of the XMRV sequence in the VP62 plasmid. This modified VP62 plasmid will then be used to transfect mammalian cells (along with an MLV based vector and in some cases a plasmid expressing a viral envelope gene) to produce pseudotyped virus-like particles.

**Recipient or parental organism**

VP62/pcDNA3.1 plasmid - encodes full length XMRV genomic DNA. This will be mutated to contain deletions in the RNA packaging signal and/or U3 promoter regions to create a modified VP62 plasmid.

**Host/vector system**

Vectors to be used:
- KB4 - contains the murine leukaemia virus (MLV) gag pol sequences. These are structural and enzymatic proteins of the virus.
- LacZ-LTR - contains the gene lacZ, which encodes the protein β-galactosidase, under the control of the MLV long terminal repeat (LTR)
- VSV-G - encodes the vesicular stomatitis virus G protein (viral envelope protein)
- MLV-X - encodes the xenotropic MLV viral envelope proteins
- FBdelMOSAF - encodes the Moloney MLV viral envelope proteins

Three plasmids i) either KB4 or modified VP62, ii) LacZ-LTR and iii) one of the envelope plasmids, will be co-transfected into 293T cells (human embryonic kidney cell line) to produce pseudotyped retroviral particles. These virus like particles will be used to infect various mammalian cell lines including Mus dunni (murine cell line), D17 (canine osteosarcoma cell line), 293T (human embryonic kidney cell line), He La (human cervical carcinoma cell line). However, they will be capable of a single integration event only and are not capable of causing a spreading infection. The RNA genome to be packaged, the viral envelope and other viral proteins are encoded on separate plasmids thus eliminating the possibility of reversion to replication competence with a single recombination event. These clones can therefore be used to synthesize virus-like particles at containment level 2.

**Origin & function**

Plasmids expressing various genes from MLV have been used for several years to synthesize retrovirus-like particles, both for basic scientific research and for gene therapy. We will now include a plasmid expressing the genes from XMRV, a recently identified retrovirus, in order to study this novel virus.

In some instances, it may be desirable to produce replication competent virus by transfecting the parental VP62/pcDNA3.1 plasmid into mammalian cells. As the consequences of an XMRV infection are unclear at the present time, this will be done in containment level 3 facilities. These viral preparations will be treated in a similar manner to HIV.

**Evaluation of foreseeable effects**

Our genetic modifications of the VP62 plasmid will significantly decrease the hazard associated with the XMRV clone: By removing the U3 region, the viral promoter will be deleted and by removing the packaging signal, the encapsidation of viral RNA will be severely diminished. Loss of either function will lead to replication incompetency. We therefore feel that it is safe to work with this modified XMRV clone in a Microbiological Safety Cabinet at containment level 2.

It is unlikely that the XMRV sequences will be transferred to any other microorganism.

The effects in humans of infection with replication competent XMRV are under investigation.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All tissue culture waste (cells that have been transfected or infected, culture supernatants that may contain virus or virus-like particles and plastic waste), will be treated with Chloros or Super Q disinfectant and autoclaved.

To prove that this treatment is sufficient to kill virus, supernatant containing virus-like particles will be treated with Chloros or super Q prior to infecting cells, and viability measured using our infectivity assay for LacZ activity.

When we use the three plasmid system, no infectious virus should be produced after the initial integration event. Also, as no experiments require long term passage of cells, it is extremely unlikely that any recombination events could occur. (Reference: The SACGM Compendium of guidance Part 2: Risk assessment of genetically modified microorganisms (other than those associated with plants)).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Institute Biological Safety Committee have reviewed this application at their meeting on 13th August 2009 and endorsed the risk assessment and the class 3 assigned

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Project Ref 542/10.2
We plan to transfect P. knowlesi parasites with plasmid DNA (vectors) containing all or part of selected P. knowlesi genes. The products of these genes are important in the maturation of the parasite, the invasion process and/or development within the invaded erythrocyte. We plan to use vectors of the pHH, pARL, pLN, pSSPF, pXL-BACII-hDHFR and pHTH series, or their derivatives, which contain different selectable markers for cloning and selection in bacteria and subsequently the malaria parasite. These vectors are designed to allow: single crossover homologous recombination events where the entire vector is inserted into the parasite genome; double crossover events where the input DNA directly replaces the target parasite gene(s); autonomous plasmid replication and expression (as episomes) or insertion of the target gene with a tag, into a non-essential site within the parasite genome to allow expression of the tagged target gene independent of the native gene. A fuller description is given in the Risk Assessment (attached).

The target genes will be those that encode proteins located on the surface of mature parasites or the infective stage of the parasite (the merozoite), proteases important in parasite development and merozite release, organelle specific proteins and factors involved in the parasite's metabolism. All of these are involved in maturation of the parasite, the invasion process or developmental events in the invaded erythrocyte.

P. knowlesi is a protozoan parasite of the phylum Apicomplexa, and is one of the agents responsible for causing human malaria. It is normally found south-east Asia and is transmitted by mosquitos of the Anopheles spp. It is possible to cultivate the asexual and sexual erythrocytic stages (human host) in tissue culture in vitro.

The host sequence is derived from genes encoding proteins involved in the development and maturation of the parasite, parasite metabolism, or recognition and invasion of erythrocytes by the merozoite.

Vector:
The vectors are of the pHH, pARL, pLN, pSSPF, pXL-BACII-hDHFR and pHTH series or their derivatives, which are derived from pUC series plasmids of E. coli. These have been modified to include drug resistance cassettes, 5' and 3' malarial untranslated regulatory sequences (UTR) and appropriate, multiple cloning sites.

The vectors may also contain sequences encoding reporter proteins: these include widely used enzyme reporters such as firefly luciferase and chloramphenicol acetyl transferase (CAT), fluorescent proteins (e.g. GFP, DsRed, RFP varieties) or epitope tags (e.g. cmyc, HA3, FLAG or Ty1).
### Origin & function

**Origin of coding sequence:**
Culture adapted cloned *P. knowlesi* parasite lines:
A1, Nuri, Washington and H strain
Parasite lines cloned and adapted from Malaysian clinical isolates

**Intended function:**

a) To disrupt the gene of interest in order to ascertain its function (i.e. perform a gene knock out).

b) To 'tag' the gene with a marker in order to detect and study the function and activity of the gene product.

1) This can be done using sequences for GFP or related proteins cloned into a part of the target sequence in order to produce a fusion protein that can be detected by optical techniques such as fluorescence.

2) Similarly this can be done using short peptide epitope tags (e.g. HA, cmyc, FLAG, Ty1 tags). Gene products modified with these tags can be readily detected using immunochemical techniques.

C) Further functional studies on the protein by gene modification or truncation, or replacement with orthologous genes of other species of malaria parasites. This will help to determine the parts of the molecule that are required for its biological function. These truncated genes can also be tagged as in b).

D) To overexpress genes of interest which are otherwise of low abundance (as episomes or integrated into a non-essential site of the parasite genome)

**Origin of vector:** pHH, pARL, pLN, pSSPF, pXL-BACII-hDGFR and pHTH plasmid series. These contain selectable markers (drug-resistance) and regulatory or mRNA stabilising elements (5' and 3' malaria UTRs)

### Evaluation of foreseeable effects

The main concern when considering this project is that *P. knowlesi* is pathogenic to man. A code of practice (COP) is already in place (attached) to minimise accidental infection of laboratory workers. The parasite can only be transmitted through the mosquito secondary host or by direct contact with infected blood by injection or through cuts. No *P. knowlesi* work on these premises involves exposure to sharps such as needles or broken glass. All parent parasite lines have been screened for drug susceptibility and are known to be sensitive to chloroquine. This characteristic will not be altered by any of the work described above. In the unlikely event that a person is believed to have been accidentally infected, treatment with the appropriate anti-malarial drug will commence immediately. All used equipment and reagents are decontaminated before leaving the containment 2 tissue culture room (see COP) and thus no live modified parasite will be discarded and transported out of the room. If it is necessary to transport live parasites to another facility, e.g. for centrifugation or microscopy, the parasites are sealed (in a centrifuge bottle or sealed coverslip on a microscope slide) and transported in a sealed container.

Work with *P. knowlesi* is undertaken under containment 2 guidelines and modification of the parasite does not pose greater risks of infection to humans. These modified parasites will not be put through the mosquito cycle and thus there is no risk that they will enter the wild population. Most of the genes under study are unique to *P. knowlesi* and thus it would be difficult to transfer these genes to another organism.

The parasite will only be cultivated in vitro; no insect transmission will be undertaken. The work with live parasites is carried out in a sealed room so there will be no possibility of mosquitoes being infected and infecting the wider populace. The parasite material is rendered non-infectious with reagents such as phenol or detergents before further analysis is carried out in non-category 2 containment facilities.

The vectors contain drug-resistance casettes. The drugs most used in the parasite selection process (WR99210, neomycin, blasticidin and puromycin) are not in general use for the clinical treatment of humans or animals and thus the problem of drug resistance appearing in the human/animal population is not an issue. Less often, pyrimethamine is recommended for use alone for either prophylaxis or treatment of acute malaria.

Ampicillin is used in the cloning of the vector in the bacterial host. This antibiotic has been in widespread use in standard laboratory molecular cloning protocols for a number of years and there have been no recorded cases of drug resistance linked to using these vectors in bacteria.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not applicable**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

The work with live parasites is required to be performed under Containment 2 facilities. However, once the parasite has been treated with denaturing, such as phenol or...
detergents, the parasites are non-infectious and can be worked with under Containment 1 facilities.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Refer to Code of Practice, attached. All lab ware (pipettes, flasks, tips and tubes) and culture waste (spent growth medium) are treated by immersion in sodium hypochlorite solution (2,500 pp million of chlorine) for a minimum of 30 minutes. The medium is rendered colourless, or dark brown precipitates (parasitised erythrocytes) are observed as the killing process occurs. Lab ware is also autoclaved at 121°C for 15 minutes before being discarded. There is 100% destruction of infectious material after these treatments.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Biological Safety Committee approved the project at Class 2 for work at Containment 2

Project Containment

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Animal Units

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

Project Ref 542/10.3

Date Ackn'd 07/09/2010

Date Project Ceased

CU2 Project Title An examination of the properties of the 1918 pandemic influenza virus glycoproteins

Class

Class 3

Consent Granted Yes

Project notified under transitional arrangements N
Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The parental strain used as the background for reverse genetics experiment is classified as Hazard Group 2 and has a very long history of safe use - it was derived from the first human influenza virus isolated as a virus comparable of replication in the mouse brain following intracerebral inoculation. This is an inconsistent phenotype that was observed in some, but not all, influenza viruses isolated in the 1930s.

The genes from the 1918 virus that will be inserted into this background are those of the HA and NA of the 1918 pandemic virus.

The Contained Use will involve the propagation in the allantoic cavity of embryonated hens’ eggs and subsequent purification of virus using standard centrifugation techniques. The biophysical assays are to measure using the Octet apparatus involves detection of virus binding by interferometry.

Recipient or parental organism

The recipient virus is a long-established laboratory strain of a 1933 influenza virus. To our knowledge, no adverse events have been reported using this strain of virus.

The 1918 was responsible for first pandemic 20th century and was highly virulent in humans.

Host/vector system

A/WSN/33 is the host/vector system

Origin & function

Influenza viruses have an RNA genome composed of eight RNA segments. The engineered virus will have either six or seven of its RNA segments derived from the widely used laboratory strain of influenza virus A/WSN/33 (H1N1). The other RNA segment or pair of segments will be derived from the reconstructed virus derived from the H1N1 virus that was responsible for the 1918 pandemic. The gene or gene pair will be the segment that encodes the haemagglutinin glycoprotein either on its own or in combination with the gene that encodes the neuraminidase glycoprotein. The haemagglutinin is responsible for binding of the virus to receptors on the host cell and the fusion of the virus lipid envelope with the lipid envelope of the cell. The neuraminidase is responsible for the release of progeny virus from an infected cell and the disaggregation of viruses as they leave the infected cell. The haemagglutinin is the major determinant of neutralising antibodies; the neuraminidase is the target of the antiviral drugs, oseltamivir and zanamivir. The project is to examine the interaction of the engineered virus with receptor analogues.

Evaluation of foreseeable effects

The viruses produced may be hazardous to humans. The parental virus is not considered likely to pose a hazard but the newly constructed viruses containing the glycoproteins from the 1918 virus may have increased virulence for humans. No increase has been observed in mouse experiments with the viruses that we will use. Single gene replacement of the HA and the NA in two other viruses with a different genetic backgrounds, in contrast, has been observed to result in an increased virulence in a mouse model.
Our risk assessment of handling the WSN virus with the 1918 HA and/or NA genes is based therefore on the studies in mice but we recognise that the mouse is not an ideal model of human infection.

The GMO as a replication competent influenza virus is able to re-assort in mixed infections and it is not possible to predict the behaviour of the resultant progeny. To ensure that any such risk is minimised, no other influenza viruses will be handled at the same time as the virus is being propagated, or during its use. In addition, anyone with influenza-like symptoms will be excluded from using the virus. As an additional precaution against mixed infection all workers will be offered seasonal influenza vaccination. In addition, in the event of accidental exposure anti-viral drugs will be available; the 1918 neuraminidase and the WSN neuraminidase are both sensitive to zanamivir and oseltamivir.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Work will be carried out under Con 4 conditions as per the attached manual.

Solid waste material is collected and placed in secure boxes and autoclaved. This will be followed by offsite of incineration.

Liquid waste is chemically treated with Super Q, collected in an effluent tank and treated at 134°C

See manual for further details

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Biological Safety Committee have improved this project for work at Class 3 (see attached comments)

Project Containment

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<th>Glass Houses</th>
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02/03/2022
### Project Ref 542/11.1

**Date Ackn’d** 07/03/2011

**CU2 Project Title** Vaccination of laboratory mice with Recombinant DNA Vaccinia virus (rVV) expressing heterologous viral proteins

**Class** Class 2

**CultureVolClass** < 1 Litre

**CultureVolumeClass** Class 2

**Consent Granted**

#### Project notified under transitional arrangements

**Non-GMM**

### Project Additional Information

**Purposes of the contained use**

Vaccinia virus has been used extensively as a vaccine against smallpox, in a global campaign that led to the eradication of smallpox. Vaccinia virus is since being used as a vector for heterologous microbial proteins in recombinant forms that are used for vaccination against the original donors of those proteins. The purpose of the contained use of rVV strains is to study the immunological basis underlying vaccination of laboratory mice with rVV carrying either the envelope gene of Friend murine leukaemia virus (F-MuLV env) or the hemagglutinin gene of influenza A virus (IAV HA) or a green fluorescent protein (GFP) gene.

**Recipient or parental organism**

The parental microorganism is VV, a member of the poxvirus family.

**Host/vector system**

The production and selection of infectious VV recombinants expressing foreign genes was carried out with the use of plasmid vectors. These vectors contain all or part of the VV thymidine kinase (TK) gene interrupted by multiple unique restriction endonuclease sites placed adjacent to the TK promoter. After transfection of vaccinia virus-infected cells with such plasmids, homologous recombination occurs between the VV sequences flanking the chimeric gene and the same sequences within the virus genome. Recombinants formed in this manner have the chimeric gene inserted within the body of the vaccinia virus TK gene under control of a vaccinia virus promoter. Since recombinants have an interrupted TK gene, they are selected on the basis of their TK-phenotype and then checked for the presence and expression of the foreign gene.
Two rVV strains will be used, each expressing either the envelope gene of Friend murine leukaemia virus (F-MuLV env) or the hemagglutinin gene of Influenza A virus (IAV HA) or a green fluorescent protein (GFP) gene. The expressed foreign proteins serve as a source of antigenic peptides for immunisation. Although both proteins have biological functions in their native context (virus binding to its receptor and membrane fusion), their functions do not manifest in the heterologous context of rVV. Therefore, these foreign proteins expressed by rVV are considered biologically inactive.

**Origin & function**

**Intrinsic effects of VV**

The known effects of VV infection are derived from vaccination studies. Typically, vaccinia multiplies in the basilar epithelium after vaccination, causing a local cellular reaction. A papule appears 4-5 days after vaccination secondary to local replication of the virus. The papule becomes pustular within 7-10 days and reaches a maximum size of 2-4 cm; this is known as a Jennerian pustule. At this time, associated axillary lymphadenopathy and mild fever may occur. The pustule contains fluid with live viral particles that can spread by direct contact. Two to 3 weeks after vaccination the pustule dries from the centre and forms a scab. A characteristic scar that is approximately 1 cm in diameter usually remains as evidence of prior vaccination. Revaccination yields a similar, yet accelerated, course of events. No evidence exists for systemic viremia during administration of vaccinia virus in immunocompetent individuals. Most adverse reactions to vaccinia administration involve the skin and central nervous system (CNS). Progressive vaccinia also known as vaccinia necrosum is a rare complication in which viremia can lead to metastatic infection of the organs, necrosis of the skin, and, in some cases, death in immunosuppressed patients, particularly those with T-cell deficiencies. In children younger than 15 years who have eczema, vaccinia virus can also replicate rapidly in the eczematous lesions, leading to eczema vaccinatum (also termed Kaposi varicelliform eruption). The sequelae of eczema vaccinatum include prolonged hospital stays and, occasionally, death.

VV does not pose any hazard to the environment other than to human health. Since the resumption of smallpox vaccination by the US military in 2002 several cases of VV infection have been reported and it is therefore accepted that VV exist in the environment.

**Effects of genetic modification of rVV**

Insertion of the foreign proteins resulted in TK gene inactivation of rVV to be used in this study. In comparison with wild-type VV TK-deficient strains of rVV have been well-documented to be attenuated in laboratory mice. However, despite the wealth of data on the degree of attenuation in laboratory mice achieved by the deletion of the TK gene, similar data from human studies are lacking, and it currently thought, but not proven that TK-deficient strains of rVV are also attenuated in humans.

As the foreign proteins expressed by the rVV lack biological function in this context, other than the provision of immunogenic peptides, their presence does not modify the properties of rVV.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Derogation from full Class 2 "Autoclave required I (Table 1a Part II, Section 8) the building"

(see attached)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be autoclaved at 121°C in accordance with the code of practice for the containment level 2 laboratory where the work is carried out, to give 100% kill

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

02/03/2022
The Biological Safety Committee have approved this project for work at Class 2 (see attached documents)

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Y

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Project Ref 542/11.2

Date Ackn'd 14/10/2011

CU2 Project Title A new perspective on Toxoplasma gondii immunity

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
**Purposes of the contained use**

We will investigate the generation and recognition of Toxoplasma antigens and epitopes derived from them, through use of transgenic and knock-out Toxoplasma parasites and Toxoplasma-specific CD8 T cells derived from cloned mice.

**Recipient or parental organism**

Toxoplasma gondii

None of the genetically modified Toxoplasma strains are expected to present an increase in hazards to humans compared to the initial parent strains used to derive the genetically modified form from. The inserted gene products are all derived from different Toxoplasma strains or encode non-infectious, non-hazardous proteins (e.g. green fluorescent protein, ovalbumin).

**Host/vector system**

Toxoplasma gondii/All Toxoplasma GMOs used in this study were either commercially available or were previously constructed. The 4 transgenic strains we plan on constructing will use the vector gra1-PCNA-YFP/HXGPRT.

**Origin & function**

Toxoplasma gondii poses a containable threat for human health (see next paragraph). None of the Toxoplasma forms to be kept in the laboratory (tachyzoites and bradyzoites) pose a threat for the environment as they are short-lived and will not survive outside of their host (tissue culture cells for tachyzoites, mouse brains for bradyzoites). None of the genetically modified Toxoplasma strains are expected to present an increase in hazard to humans compared to the initial parent strains used to derive the genetically modified form from.

Toxoplasma gondii is an obligate intracellular protozoan parasite, which infects a diverse range of tissues and organs within many animal species. Although the sexual cycle of this organism occurs only in cats, the asexual forms are capable of infecting human. Toxoplasma infection can be contracted by three routes:

- Ingestion of sporulated oocysts from material contaminated with feline faeces.
- Oral ingestion of bradyzoites (tissue cysts) from infected animal tissue.
- Direct introduction of tachyzoites (or infected cells) into the bloodstream, or direct contact with the eye.

In most cases human infection is readily controlled by the cellular immune response and remains asymptomatic (approximately one-third of all British become infected at some point in their life without ever realizing it, usually from cat litter boxes, gardening, or eating rare meat). Individuals unable to mount an effective immune response are potentially at risk, however. This includes:

- Pregnant women - while not themselves unusually prone to Toxoplasma infection or clinical disease, parasite invasion across the placenta can result in severe neurological abnormalities in the fetus; congenital toxoplasmosis as a cause of birth defects.
- Individuals infected with the Human Immunodeficiency Virus (HIV; the AIDS virus). Toxoplasmic encephalitis (in most cases a reemergence of latent bradyzoite cysts rather than a new infection) is a leading cause of death in AIDS patients.
- Other individuals who are immunosuppressed, either through infection of the immune system, or by treatment with immunosuppressive drugs for cancer chemotherapy, treatment of allergic disorders, organ transplant, etc

**Evaluation of foreseeable effects**

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All material in contact with Toxoplasma tachyzoites, as well as Toxoplasma bradyzoite cysts will be decontaminated (chlorine based disinfectant or by autoclaving) after use. Chlorine based disinfectant or autoclaving destroys the parasite’s infectious potential

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Institute Biological Safety Committee have approved this project for work at Class 2

**Project Containment**

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**Project Ref 542/13.1**

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Date Project Ceased: 02/03/2022
Embryonic stem cells (ESC) are established from preimplantation embryos and have the unique potential to self-renew indefinitely and to generate all of the differentiated cell types that compromise the embryo (mesoderm, endoderm and ectoderm cells). The ability to maintain ESC in vitro offers a tremendous opportunity to study the molecular mechanisms of mammalian lineage specification and provides exciting clinical potential including the establishment of novel in vitro models for drug discovery and sources of cells for regenerative medicine. Moreover, the recent description of a method to establish induced pluripotent stem (iPS) cells from foetal and adult somatic cells by ectopic expression of a small set of transcription factors represents a breakthrough in the field of ESC research. iPS cell approaches offer the possibility of generating disease specific cells to model and ultimately treat diseases such as Alzheimer's disease, Parkinson's disease and diabetes. Moreover, fundamental questions regarding the plasticity of cell identity can be addressed by reprogramming, transdifferentiating and directing the differentiation of cells via gene overexpression or downregulation to manipulate cell fate.

Our objective is to understand the molecular mechanisms that regulate early embryonic cell fate specification and to generate functional cells to model and facilitate the treatment of human diseases. Towards this goal, we plan to work with human and mouse iPS, ES and extraembryonic cells to identify shared and divergent mechanisms that drive lineage specification in these species. These fundamental insights would facilitate the generation of therapeutically relevant cell types such as cardiomyocytes, neurons, pancreatic cells, haematopoietic stem cells, etc. We plan to use highly efficient vectors (including recombinant viral vectors derived from gamma-retroviruses, lentiviruses and adenoviruses) to genetically modify numerous human and mouse cell types towards the following specific aims:

- **PROJECT 1. Factors controlling cell fate specification of pluripotent stem cells**
- **PROJECT 2. Transcription factor mediated reprogramming of somatic and embryonic stem cells**
- **PROJECT 3. Gene targeting in human embryonic stem cell**

### Recipient or parental organism

- **E. coli, K12 derived strains** - disabled
- **Mammalian cells (primary cells and cell lines)** - disabled
  - Human, murine embryonic stem cell lines (H9, H2, H7, HuES3, HuES9, HuES12, …)
  - Human, murine induced pluripotent stem cell lines (produced by the lab)
  - Human murine primary cells (embryonic and adult skin fibroblasts, keratinocytes, peripheral blood cells, …)
  - Human, murine somatic cell lines (HEK-293T, HCT-116, …)

### Host/vector system

- **Gamma oncoretroviruses** - MuLV derived, replication defective
- **Lentiviruses** - HIV-1 derived, replication defective
- **Adenoviruses** - Ad5 derived, replication defective
- **Sendai viruses (SeV)** - Paramyxoviridae-neuraminidase (HN) and M genes inhibiting the formation of non-transmissible virus-like particles (NTVLP)
- **pUC derived plasmids** (pWPT, pR8.2, pMD2.G, …) - non-mobilisable, carrying insert and recombinant viral genome

### Origin & function
Type of inserts:
1. Human, murine, xenopus cDNA (x - TAG sequences) coding for transcription factors, signal transducers, growth factors and surface receptors.
2. shRNA and miRNA sequences against cell endogenous coding sequences
3. Reporter genes (GFP, RFP, LacZ, Luciferase, possibly fused to cDNA (type 1 inserts),…)
4. Selection genes (Neomycin, Puromycin, Hygromycin,..)
5. Functional non-coding sequences (promoter, enhancer, insulators, IRES, WPRE, of mammalian or viral origin)
6. Neutral non-coding sequences (introns, isolated exons)

- cDNAs (Type 1 inserts) will code for transcription factors, signal transducers, growth factors and receptors that function in pluripotency and lineage specification. shRNAs (Type2 inserts) will produce non-coding RNA sequences targeting endogenous mRNAs. Expression of both insert types may enhance/block commitment and differentiation of pluripotent stem cells (PSCs towards one of the three primary germ layers and further differentiated cell types.
- Reporter genes (Type 3 inserts) will code for proteins with fluorescent or enzymatic properties allowing easy identification of genetically modified (GM) cells. They are thought to have no deleterious biological effect.
- Selection genes (Type 4 inserts) will produce enzymatic proteins able to inactivate specific antibiotic families allowing selection of GM cells. They are thought to have no deleterious biological effect.
- Functional non-coding sequences (Type 5 inserts) will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES). They will be used (independently or in combination) in viral expression cassettes to control transgene expression.
- Neutral non-coding sequences (Type 6 inserts) will be used to facilitate homologous recombination at defined genomic loci (intronic/exonic genome sequences). They are thought to have no deleterious biological effect

Evaluation of foreseeable effects:
TYPE1, cDNA
Type1 inserts will code for transcription factors, signal transducers, growth factors and receptors that are expected to play a role in pluripotency, lineage specification and differentiation from pluripotent stem cells (OSCs). It is difficult to predict in vivo consequences of ectopic expression of these genes on human health. However, since they are key regulator of cell identity, their overexpression could lead to pathological modifications of cell phenotype/function. Moreover, several of these genes are involved in oncogenic processes and uncontrolled expression could subsequently initiate tumor transformation. However, malignancy is a complex multistep process that involves multiple genomic alterations and a single hit is unlikely to alone trigger the oncogenic process. Experiments involving co-transduction or even co-expression by the same vector of several inserts should consequently be considered a higher risk for personnel. Notably, reprogramming experiments of somatic cells via co-transduction with OCT4, SOX2, MYC and KLF4 could imply a specific risk since accidental genetic modification could theoretically lead to teratoma formation.

TYPE2, shRNA/miRNA
Type2 inserts will produce non-coding RNA sequences able to inhibit expression from the group of genes mentioned as Type1 insert. As for Type 1 insert, it is difficult to predict in vivo consequences of ectopic expression of these sequences on human health. However, since they target key regulators of cell identity, their expression could lead to pathological modifications of cell phenotype/function. Notably, several miRNAs have been implicated in oncogenic processes.

TYPE3, reporter gene
Type3 inserts will code for proteins with fluorescent or enzymatic properties allowing easy identification of genetically modified (GM) cells. They are thought to have no deleterious biological effect on expressing cells except acute toxicity at a very high concentration.

TYPE4, selection gene
Type4 inserts will code for enzymatic proteins able to inactivate specific antibiotic families allowing selection of GM cells. They are thought to have no pathological effect on expressing cells except acute toxicity at a very high concentration.

TYPE5, functional non-coding sequence
Type5 inserts will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers
TYPE6, neutral non-coding sequence
Type6 inserts do not have any foreseeable deleterious biological effect. They will be used for gene targeting projects to promote homologous recombination at defined genomic loci.

Evaluation of foreseeable effects of the Viral Vectors:

GAMMA-RV: amphotropically packaged MuLV derived Gamma-Retroviruses
Gamma-retroviruses stably integrate into the host cell genome. This property is associated with a risk of insertional mutagenesis, i.e. ectopic activation/inhibition of host gene expression after localized provirus integration. For example, development of leukemia was reported in 4 patients where hematopoietic progenitor cells were transduced by MLV vectors encoding the γc-IL2R chain in the context of SCID-X1 gene therapy. Moreover, the genes were used to generate gamma-RV vectors are functional in pluripotency and represent an additional oncogenic risk. Recombinant particles will be VSV-G packaging alters the tropism of the retroviral particles, requiring interaction only with phospholipids present in the plasma membrane of all cells rather than interaction with a cognate receptor as occurs with ecotropic packaging, thus, VSV-G pseudo-typed gamma-retroviruses acquire the ability to efficiently infect human cells. Moreover, VSV-G pseudo-typing improves stability of the viral particle and may represent an aerosol means of transmission in addition to percutaneous risk.

The above risks will be tempered by the fact we will use replication defective gamm-RV unable to propagate further after transduction. In addition, in the event of an accidental contamination (percutaneous, aerial), the amount of viral vectors available to effectively contaminate personnel will be smaller and transduction much less efficient compared to in vitro transduction protocols used on cultured cells. Notably, VSV-G pseudotyping results in complement sensitivity increasing the likelihood of immunological neutralisation in human hosts. Moreover, gamma-RV vectors are only efficient in transducing proliferating cells that limits the number of potential accidental targets. Together these considerations suggest that only very few cells may be effectively transduced through accidental contamination. Moreover, malignancy is a complex multistep process that involves multiple genomic alterations. It is thus unlikely that any single hit corresponding to an accidental infection would initiate transformation. Notably, our protocol will involve co-transduction of several inserts that are coded by separate vectors, making accidental multiple infection of the same human cell unlikely. Finally, cells exposed to non-percutaneous accidental infection (stratum corneum, respiratory epithelium) are terminally differentiated cells with limited lifespan and high turnover, which greatly limits the risk of effective tumoral transformation.

Adenovirai: Ad5 derived Adenoviruses
Replication competent virus (RCV) could be generated by recombination with the E1A sequences in the complementing cell line (293A) or another virus. However, all inserts will be integrated into the site of disablement (E1A deletion) and any recombination would result in the loss of the inserts. Adenoviral vector infection is of short duration and integration events leading to long-term transgene expression extremely rare. However risk associated with transient expression should be considered. However, our protocol will involve co-transduction of the inserts encompassed by separate vectors, making accidental multiple infection of the same human cell unlikely as well as concomitant reprogramming. In addition, cells exposed to non-percutaneous accidental infection (stratum corneum, respiratory epithelium are terminally differentiated cells with limited lifespan and high turnover, which greatly limits the risk of effective tumoral transformation. Strict adherence to Class2 containment and safety measures (see appendixD) will be undertaken to minimize the risk of infection (percutaneous, aerosol) during handling of high titer adenoviral vectors. However, the adenoviral vector purification procedure will involve collection of CsCl gradient purified vectors by plastic tube punctured with a needle. This procedure will be assigned the higher risk and will be performed by experienced personnel only. Alternatively, filter based methods may be used to reduce risk.

SENDAI: ΔF temperature sensitive Sendai virus derived vectors
Sendai virus does not stably integrate into the host cell genome thereby avoiding the risk of insertion mutagenesis commonly associated with retroviral vectors. However, Sendai virus can infect cells including human cells very efficiently and the vector is self-replicative in the cytoplasm of transduced cells. Moreover, proteins encoded by
DeltaF/TS-SeV used for this project are functional in pluripotency and represent oncogenic risk. However these risks will be tempered by using all the necessary aerosol containment and also by using infection defective DeltaF/TS-SeV unable to propagate further after transduction and with limited capacity for cytoplasmic replication. In addition, in the likelihood of an accidental contamination (percutaneous, ariel), the amount of viral vectors able to effectively contaminate personnel will be smaller and transduction much less efficient compared to in vitro transduction protocols used on cultured cells. Notably, DeltaF/TS-SeV express viral proteins (including HN on the cell surface) increases the likelihood of immunological neutralisation in human hosts. Additionally as the virus life cycle is cytoplasmic and of short duration, transgene expression will be transient in nature. Moreover, malignancy is a complex multistep process that involves multiple genomic alterations. It is thus unlikely that any single hit corresponding to an accidental infection would initiate transformation. Finally, cells exposed to non-percutaneous accidental infection (stratum corneum, respiratory epithelium) are terminally differentiated cells with limited lifespan and high turnover, which greatly limits the risk of effective tumoral transformation.

Environment considerations
VSV-G pseudotyping of recombinant gamma-RV particles would enable vectors to infect a variety of animal cell types including those of different animal species. Moreover, environmental stability tends to be increased by VSV-G pseudotyping compared to gamma-RV encompassing the native envelope. However, recombinant gamma-RV will be replication defective and cannot produce progeny viruses able to spread.

Adenoviruses are generally species specific. Indeed, serotype 5 Adenoviruses infect humans and are not known to infect other animals. Therefore, the risk of accidental infection of other organisms is very low. Because recombinant adenoviruses will be replication incompetent, they could not spread. The native Sendai virus is a known pathogen for rodents and occasionally pigs and the common route of infection is aerial and lung airway epithelium. DeltaF/TS-SeV may be able to infect a variety of animal cell types including those of different animal species. However, DeltaF/TS-SeV will be F protein defective and temperature sensitive and thus could not produce progeny viruses able to spread. Nevertheless, a risk of mobilization of recombinant vectors by native sendai virus infection may remain (notably, latent enzootic infections has been detected in laboratory mouse strains), All precautions will be taken to avoid DeltaF/TS-SeV vectors to enter animal facilities. It is very unlikely that vector genomes would be propagated significantly in natural rodent populations hosting wild type Sendai virus.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

During the production and concentration of recombinant lentiviral particles and viral transduction of cells, CL2 conditions will be strictly adhered to. Experimental work involving direct virus handling (production, transduction) will be carried out by trained personnel only. If culture supernatants are demonstrated virus free (gamma-RV; serial eventually removed from CL2 containment to carry on further work in CL1 containment rooms following good laboratory practises, using class ll microbiological safety cabinet for opened handling of cells and autoclaving/disinfection of GM discarded cells at the end of the process.

**Human Health**

- All gamma-RV (i.e. Vectalys), lentiviral (produced by the lab) and adenoviral (i.e. Hybrid systems) batches will be tested for absence of replication competent particles.
- Standard measures (absence of sharps, needles) and good laboratory practises will be undertaken to decrease the likelihood of percutaneous infection (virtually to zero)
- All work involving opened viral containers (handling of virus batches, cell cultures, contaminated tips, waste bottles will be performed within a class ll microbiological safety cabinet to decrease the likelihood of aerolised transmission of recombinant viral particles.
- All work including waste disposal will be carried out under the relevant Code of Practice (COP).

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- None required

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- All work including waste disposal and inactivation measures will be carried out under the relevant Code of Practice (COP), attached

**Is an emergency plan required according to regulation 20?**

- N

**If yes, tick to confirm that it is attached to this form**

- N
The Biological Safety Committee have approved this project for work at Containment level 2. (see attached comments)

Please enter comments on the GM safety committee on the risk assessment

[The Biological Safety Committee have approved this project for work at Containment level 2. (see attached comments)]

Project Containment

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<tr>
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Animal Units
Large Scale Activities
Human Clinical Applications

Project Ref 542/14.1

Date Ackn'd 29/04/2014
CU2 Project Title
Host innate immune mechanisms shape the defence to intracellular pathogens

Class 2
Culture Volume
Class 2
< 1 Litre

Consent Granted
Project notified under transitional arrangements

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information
### Purposes of the contained use

1. We will investigate the functional consequences gamma interferon induced p65GTPases have on the host defence to intracellular pathogens (Salmonella typhimurium and Shigella flexneri).
2. We will identify novel players that tune the recognition of intracellular pathogens and thereby influence the resulting immune response (Salmonella typhimurium).

### Recipient or parental organism

**Salmonella typhimurium and Shigella flexneri**

Wild-type Salmonella Typhimurium causes gastroenteritis and can cause septicemia in humans. Species of the genus Shigella are the cause of shigellosis, a diarrhoeal disease that is endemic in developing countries. The major site of Shigella pathology is the colon, where bacteria invade the intestinal mucosa, spread to the adjacent epithelial cells and cause much tissue damage, fluid secretion and inflammation.

None of the bacterial strains we will use present an increase in hazard to humans compared to the initial parent strains.

### Host/vector system

All bacterial strains used in this study are published and common laboratory strains.

### Origin & function

We will obtain the bacterial strains from collaborators (DH and SM, Imperial College).

1. We want to probe if host p65 GTPases influence the propagation of these bacteria in mouse and human tissue culture cells. We will infect mouse or human cells with these bacterial pathogens and analyse the fate of the bacteria by measuring their growth and checking for host immune consequences by standard cell biological and biochemical assays (immunoblots, immunofluorescent microscopy) (Salmonella typhimurium and Shigella flexneri).
2. We want to study what consequences inflammasome recognition has on the resulting long-term immune response and if novel cytokine molecules fine-tune this response. We will infect mice or cells with bacterial pathogens and study the production of these cytokines. We will investigate which molecules on which cells sense these cytokines and how that fine-tunes the specific immune response to these pathogens. This work will be done in vitro in murine cells as well as in vivo mouse models.

### Evaluation of foreseeable effects

Both Salmonella typhimurium and Shigella flexneri cause disease in humans by ingestion only. Common laboratory practices prevent accidental ingestion of laboratory material including these bacterial pathogens.

In the extremely rare case that accidental ingestion should occur, for both Salmonella and Shigella common medical advice can be sought. For both Salmonella and Shigella in the case of infection treatment consists of fluid replacement therapy combined with the potential administration of antibiotics. None of the laboratory strains used in this work will cause more severe human disease than the wild-type strains.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All material in contact with Salmonella and Shigella will be decontaminated by bleach or autoclave. Both methods destroy the infectious potential of the pathogens.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All material in contact with Salmonella and Shigella will be decontaminated according to the relevant Code of Practice attached.
There was a meeting of the Biological Safety Committee to discuss this project. The Committee approved the project for work at Class 2.

**Project Containment**

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**Project Ref** 542/14.2

- **Date Ackn’d**: 29/04/2014
- **CU2 Project Title**: Reverse genetics of the novel zoonotic avian influenza A/H10N8 virus to examine the properties of the haemagglutinin and neuraminidase genes
- **Consent Granted**: Yes
- **Project notified under transitional arrangements**: No

**Historical Significant Changes**

- **Historical Date of Additional Info**: 29/04/2014
- **Significant Change ID**
- **Date of Significant Change**
**Project Additional Information**

**Purposes of the contained use**

The aim is to investigate the balance between the receptor binding properties of the HA and NA glycoproteins of the H1N9 virus and the receptor destroying (sialidase) activity of the NA. The ability of the virus to bind to both human and avian sialic acid receptor analogues has shown that two patterns of binding emerge. One that is typically avain and one that has increased binding to the human receptor. Moreover, based on sequence signatures of the H10N8 NA, it is likely that the NA plays a role in the binding of virus to receptor. We need to assess the contribution of both the HA and the NA to the virus binding activity. To do this it is proposed to produce a virus in which the ability of the NA to bind sialic acid has been modified or removed. Binding of receptor through the NA glycoprotein affects the balance between the virus haemagglutinin (HA), the primary receptor binding protein, and the NA, with sialidase (receptor destroying enzyme) with additional receptor binding activity. If the NA mediates significant binding of virus to the receptor then the analysis of the antigenic properties of the virus can be compromised. Antigenic analysis of the virus is key to the development of effective vaccines against influenza.

Our purpose is to examine the ability of genetically modified virus to interact with virus receptor analogues in biophysical studies, to examine how these affect the ability of virus to infect cells in culture, to examine the antigenic properties of the modified viruses in in vitro studies and to examine the antibody response in ferrets of the modified viruses.

**Recipient or parental organism**

The genetic background into which the novel H10 HA and N8 NA genes are used to be inserted is one of two well-characterised influenza viruses, A/PR/8/34 or A/WSN/33. These are the usual background into which influenza reverse genetics is carried out.

**Host/vector system**

The reverse genetic viruses will be propagated in tissue culture and/or in ovo.

**Origin & function**

The genes from the zoonotic H10N8 virus that will be inserted into one of the above genetic backgrounds are those of the haemagglutinin (HA) and the neuraminidase (NA).

The HA is responsible for binding of the virus to the cell and the fusion of the virus to the cell membrane to enable the virus nucleic acid to enter the cell and replicate.

The NA is responsible for the release of progeny virus from the infected cell and is the target for the new anti-neuraminidase antiviral drugs. The NA gene of the zoonotic H10N8 virus is expected not only to cleave sialic acids from glycoconjugates but also to bind sialylated glycoconjugates.

**Evaluation of foreseeable effects**

The novel H10N8 virus in China is a low pathogenicity avian influenza virus that has emerged as a zoonotic infection in two cases in China, one was fatal but the patient had several co-morbidities. Alteration of the virus through reverse genetics is likely to influence its ability to infect humans and spread between humans.

The genetic background can reduce the virulence of wild-type viruses. The PR8 background is known to attenuate many viruses and, for example, reduces the levels of virus replication of reverse genetics-derived novel H7N9 viruses in the respiratory tract of ferrets (personal communication) and is less virulent in ferrets causes far fewer disease signs; the ferret is the optimal animal model for human influenza. However, changing of the balance between the receptor binding activities of the HA and the NA might promote infection and transmission.

It is unlikely that changes will affect its pathogenicity for poultry or wild birds, there is no plan to introduce nucleotides that encode a poly-basic cleavage site of the HA into the HA gene which is associated with highly pathogenic influenza viruses of H7 or H5 viruses. No other sub-types have been associated with 'typical' highly pathogenic...
avian influenza viruses.

The GMO as a replication competent influenza virus is able to reassort in mixed infections and it is not possible to predict the behaviour of the resultant progeny. To ensure that any such risk is minimised, no other influenza viruses will be handled in the same microbiological safety cabinet at the same time as the virus is being propagated, or during its use. In addition, anyone with influenza-like symptoms will be excluded from using the virus. As an additional precaution against a mixed infection, all workers will be offered seasonal influenza vaccination. In addition, in the event of accidental exposure anti-viral drugs will be available.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Management  and Containment of virus.

All this work is to be carried out under Containment 3 conditions to contain virus and prevent exposure of the worker. Further all work is carried out in accordance with the manual the Management of the Containment 4 Facility at the National Institute for Medical Research.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

There was a meeting of the Biological Safety Committee to discuss this project. The Committee approved the project for work at Class 3.

Project Containment

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Infection of cells with Listeria monocytogenes to study both the cell biology of infection as well as the immune response.

**Project Additional Information**

**Purposes of the contained use**
Modification of Listeria monocytogenes to introduce the green fluorescent protein GFP.

**Recipient or parental organism**
Listeria monocytogenes is a Gram positive bacterium that causes disease in mice and humans generally when administered in high doses (about 10^6 bacteria) orally. The organism can be pathogenic for pregnant or immunosuppressed individuals. In sublethal infections of mice the bacterium is cleared by innate and specific immune responses and the animal retains long-term immunity.

**Host/vector system**
The green fluorescent protein is introduced in a chromosomally integrated pAD vector.

GFP is non hazardous for humans or animals and will not alter the pathogenicity of the host.

**Origin & function**
The sequence for the green fluorescent protein (GFP) is introduced into Listeria. This allows visualisation of Listeria by immunofluorescence.

**Evaluation of foreseeable effects**
No effects beyond the effects of Listeria parent organism are expected.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Containment and control measures as detailed in the Containment 2 code of practice will be applied see attached.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management measures are described in detail in the Containment 2 Code of Practice.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee approve this project at Class 2 for work at containment level 2.

Project Containment

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Animal Units

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Project Ref 542/17.1

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<td>&lt; 1 Litre</td>
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</table>
Retroviral replication requires stable insertion of the viral genetic material into cellular genome. The catalytic events associated with this process are carried out by the virus-derived enzyme integrase (IN). A multimer of IN assembles on viral DNA ends forming a stable nucleoprotein complex known as the intasome (reviewed in ref. 1). Over the past 10 years, we determined a range of crystal and cryo-EM structures that illustrated the basic mechanism of the retroviral integration process. Unfavorable biochemical properties of HIV-1 IN necessitate the use of hyperactive and/or solubilizing mutations, which, by their nature, dramatically change the properties of the protein. Therefore, most of our studies focused on IN from Prototype Foamy Virus (PFV, a member of the Spumavirus genus), which is soluble and active in vitro. Unfortunately, PFV IN is highly divergent from its counterpart from the human lentivirus HIV-1. Recently, we discovered that the IN from the Maedi-Visna Virus (MVV), an ovine lentivirus, readily assembles into functional intasomes in vitro (2). Because MVV belongs to the same genus as HIV, it serves an excellent model for the human pathogen. Based on our structures, we designed a series of MVV IN mutants, the functionalities of which will need to be characterized in the context of viral infection. We are planning to do these experiments using a single-cycle MVV vector system.

Given that MVV is not known to be infectious to humans, and that the vector system is highly disabled, with vector particles requiring transfection with 4 separate vectors (expressing VSV-G envelope, GFP reporter, GAG-POL, and Rev) the planned experiments will pose no significant risk to personnel or to environment. Two types of experiments are envisaged: 1) Experiments with modified MVV vector particles. We will introduce a range of point mutations into the IN coding region (present within the packaging construct) and compare abilities of the resultant viruses to transduce human HEK293T, HeLa or sheep CPT-Tert cells. The packaging constructs are used to produce structural and enzymatic components of the viral particles, but their genetic material, plasmid DNA itself or RNA transcripts are not incorporated into viral particles, which makes this type of experiment inherently safe. 2) Experiments with modified human cells. We will knock-down expression of a range of cellular genes using short interfering RNA (siRNA) or short-hairpin RNA (shRNA) constructs, to test their potential importance for retroviral integration. In the first instance we are planning to knock-down PSIP1 and CPSF6 genes. Upon knock-down, cells will be subjected to infection with GFP MVV vectors. Following FACS analysis or antibiotic selection cells will be either discarded or processed for isolation of genomic DNA. References: 1. Lesbats, P., Engelman, A.N. and Cherepanov, P. (2016) Retroviral DNA Integration. Chem Rev, 116, 12730-12757. 2. Ballandras-Colas, A., Maskell, D.P., Serrao, E., Locke, J., Swuec, P., Jonsson, S.R., Kotecha, A., Cook, N.J., Pye, V.E., Taylor, I.A., Andresdottir, V., Engelman, A.N., Costa, A. and Cherepanov, P. (2017) A supramolecular assembly mediates lentiviral DNA integration. Science, 355, 93-95.

Recipient or parental organism

Human HEK293T, HeLa or sheep CPT-Tert cells

Host/vector system

We are planning to produce MVV vector particles by transfection of HEK293T cells with 4 constructs (maps of the plasmids are attached to this document):

1) pMD2.G, a VSV-G envelope glycoprotein expressing plasmid
2) MVV Gag-Pol expression vector (and its mutant forms carrying point mutations within the IN coding region)
3) GFP transgene construct (self-inactivating [SIN] and full-length LTR versions) with CMV-GFP-WPRE expression cassette
4) Visna Rev expression plasmid.
Retrovirus derived enzyme integrase (IN).
Green Flourescent protein. This is non hazardous fro humans or animals and will not alter the pathegenicity of the host.

Evaluation of foreseeable effects

MVV vector particles should be considered most hazardous GMM produced in this project. The risk to human health from the use of any lentiviral vectors is gauged to be low and is primarily a risk only to the researchers involved in the work. The ability of the pseudotyped virus to transduce both dividing and non-dividing primary human cells does mean there is a potential risk of gene disruption by insertional mutagenesis if exposure was to occur. However, following any such accidental exposure, it is not likely that any incidental reduction in the expression of a single tumour suppressor will be deleterious to the afflicted cell. It has been experimentally established that multiple genetic alterations (between 4 and 6) are required to obtain neoplastic transformation, and there are cell intrinsic safeguard mechanisms that impede the accumulation of such alterations. Moreover, the risk of actual exposure is low as (a) lentiviruses require close contact or percutaneous inoculation for their transmission; (b) their survival in the general environment is poor as they cannot readily survive outside of closed controlled cell culture conditions and they are rapidly inactivated by dehydration or other environmental insult.

The cell lines used in these experiments are extremely sensitive to environmental conditions such as temperature, gas atmosphere, desiccation, pH, extensive light exposure, substratum, and medium composition. Therefore, the cell lines to be used are not expected to survive outside the culture dish and incubator. Therefore, it is considered that these micro-organisms pose no significant hazard to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management will be in line with the measure outlined in the institutes' containment level 2 code of practice (see attached)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

Comments from committee meeting 30 May 2017

- Good scientific review
- Clarification required whether SIN or not
- Add plasmid map – a description of the organism
- Final assignment of containment measures and risk class – explain that once infected cell has gone through 3 passages - CL1

Project Containment
Understanding the host-pathogen interaction of Cryptosporidium, a protozoan parasite that causes diarrheal disease

The aim of this project is to investigate the host-pathogen interactions of the protozoan parasite Cryptosporidium, which is a major cause of diarrheal disease in humans and other animals. These parasite strains will be used to answer fundamental questions regarding infection, including: How does the immune system recognize and respond to a crypto infection? How does the parasite evade immune detection and cause disease?

All strains were derived from wild-type Cryptosporidium parvum and Cryptosporidium tyszzeri strains and the following genetic modifications have been made using Cas9 driven homologous recombination:

- expression of the fluorescent proteins mCherry, mNeon, tdTomato, and mScarlet
- expression of the luminescent proteins nanoluciferase and luciferase
- expression of the CRE recombinase protein (to enable genetic recombination)
In order to select for transgenic parasites, the neomycin resistance gene has been also been inserted into the parasite genome of all strains.

At the Francis Crick Institute, my research group will work with the above strains, and new transgenic strains that we develop as the project proceeds, including:
- lines with HA epitope tags on the C- and N-termini of proteins
- lines where genes have been removed or knocked out
- lines where Cas9 has been inserted into the genome (to increase gene editing efficiency)
- lines with proteins fused to affinity-purification tags (e.g. SNAP-tag technology from NEB)

### Recipient or parental organism
Cryptosporidium parvum and Cryptosporidium tyzzeri strains.

### Host/vector system
Cryptosporidium parvum and Cryptosporidium tyzzeri strains.

### Origin & function
All strains were derived from wild-type Cryptosporidium parvum and Cryptosporidium tyzzeri strains.

### Evaluation of foreseeable effects
Cryptosporidium parvum is infectious to humans and causes moderate to severe diarrheal disease. There is a drug available for treatment, nitazoxanide, which has good efficacy with the exception of patients that are severely immunocompromised.

Cryptosporidium tyzzeri is infectious to rodents and is commonly found in mice across the world. There is one reported case of zoonotic human infection, but the methods and conclusions from this report are highly dubious Rašková JCM 55(1) 2013:360-362.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management will be in line with the measure outlined in the institutes' containment level 2 code of practice (see attached)

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |
As far as I can tell there is nothing of concern in the proposal. But what must be got right are development of codes of practice to protect the investigators.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref** 542/20.1

**Date Ackn’ed** 22/05/2020

**CU2 Project Title** Directed Evolution with Sindbis Virus (VEGAS)

**Class** Class 2

**CultureVolClass2** < 1 Litre

**CultureVolumeClass3-4** Non-GMM

**Consent Granted** Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To use Sindbis virus packaged in mammalian cells to carry out directed evolution of proteins of interest in mammalian cells (primarily the baby hamster kidney cell line BHK21) using a protocol called VEGAS. We want to use the Sindbis virus because of its poor replication fidelity—each time the virus replicates, it introduces mutations to its genome at a high rate. This allows us to generate a large number of different variations of our gene of interest. In our first experiments, we will be evolving a bacterial protein—Cas13d. However, future work may use other CRISPR proteins, or other RNA binding proteins.
Different variations of the Sindbis virus will naturally be created as the Sindbis virus mutates. These Sindbis viruses will also carry our transgene, along with any mutations that it has accrued.

**Recipient or parental organism**

Mammalian cell lines, primarily baby hamster kidney cells

**Host/vector system**

Replication incompetent Sindbis virus

3 plasmids from addgene will be used to produce replication incompetent virus.

- **pTSin EGFP plasmid** contains the entire replication competent Sindbis genome with the structural genome components replaced by Enhanced Green Fluorescent Protein (EGFP) in an MCS locus.
- **pSin Helper plasmid** contains the Sindbis glycoprotein genes and can be converted to mRNA for initiating Sindbis virus packaging.
- **pSin Capsid plasmid** contains the Sindbis capsid gene and can be converted to mRNA for initiating Sindbis virus packaging.

**Origin & function**

Infection with Sindbis virus causes a rash-arthritis syndrome with symptoms including fever, rash, headache and joint pain. The disease is a self-limiting febrile illness that resolves in 1-2 weeks. Relatively little is known about the pathogenesis of this virus. However, the pathogenesis of other related alphaviruses is well understood and disease manifestations are thought to result from a combination of direct cellular damage caused by virus replication and indirect immunemediated damage in the target tissues. After inoculation via mosquito bite, alphaviruses spread through the circulation to liver, spleen, muscle, lymph nodes and connective tissues around bones and joints. The viral replication is followed by an inflammatory response characterized by infiltration of lymphocytes, Natural Killer (NK) cells, neutrophils and, especially, macrophages to the target tissues.

Use of a fourth plasmid pSRE-SSG plasmid which expresses the Sindbis Structural Genome (SSG) via the serum response element (SRE) promoter, is required to create replication competent virions. This plasmid will not be used and thus only replication incompetent virus will be produced in these experiments.

Thus we will be producing viral stocks that are infectious, but they are incapable of productive infection outside of our specific cell lines due to the viruses not containing the structural part of their genome.

The Cas13d transgene they carry will be inert in a mammalian system as mammalian cells do not express the necessary guide RNAs to make the Cas13d active. The Sindbis virus will not come in to contact with bacterial cells, which may express the relevant guide RNA.

Furthermore, as this is an arthropod borne virus, the natural carrier of the virus (Culex spp. mosquitoes) will not come in contact with the virus. We will not perform procedures using sharps, and so there is an extremely low risk of transmission for the virus.

**Evaluation of foreseeable effects**

Infection with Sindbis virus causes a rash-arthritis syndrome with symptoms including fever, rash, headache and joint pain. The disease is a self-limiting febrile illness that resolves in 1-2 weeks. Relatively little is known about the pathogenesis of this virus. However, the pathogenesis of other related alphaviruses is well understood and disease manifestations are thought to result from a combination of direct cellular damage caused by virus replication and indirect immunemediated damage in the target tissues. After inoculation via mosquito bite, alphaviruses spread through the circulation to liver, spleen, muscle, lymph nodes and connective tissues around bones and joints. The viral replication is followed by an inflammatory response characterized by infiltration of lymphocytes, Natural Killer (NK) cells, neutrophils and, especially, macrophages to the target tissues.

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**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
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| N

Please enter comments on the GM safety committee on the risk assessment
Comment 1
I have read the proposal as the virus will not be replicative competent based on packaging on different plasmids, I think the use of cat 2 lab for the production is a good idea (but in reality, might not be necessary).

Comment 2
If they naturally create different variations of the virus could they potentially produce a variant that does replicate in wild type cells. If so do they have a way to test this to be sure this isn’t going to happen?

Response
Regarding the potential for viruses to gain replication competency: this an extremely low risk scenario. The structural genome is separated from the Sinbis particles that we produce and the Sinbis virus does not have the machinery necessary to incorporate the structural genome back in to its own genome. We don't think there is even such machinery in existence. So the virus would have to mutate in such a way that the whole structural genome is re-evolved de novo - something that would take an obscene amount of time even if it were possible to happen.

Comment 3
The project briefly mentions that one of the plasmids they intend to use expresses GFP. My guess is that fluorescence will be used as a marker to identify transfected cells, but there is no explicit description of how fluorescence will be used experimentally. It does not appear that they will do any microscopy. Will they use the GFP to sort cells?

Response
Regarding the GFP viruses: these virus will be the first virus we produce, but we'll quickly stop producing them once we're confident that we are able to produce the Sindbis virus efficiently. We have no intention of sorting these cells, so flow cytometry STP don't need to be involved. We will use basic microscopy to identify GFP+ cells in our CL2 room. Future viruses will not encode GFP, but dCas13d.

Comment 4
I believe the proposal is sound as is the work proposed. As far as I can tell, I think the authors have rightfully described potential issues and measures to avoid that. I think CL2 is the correct level of containment based on the proposed work. I do not have anything else to add at this stage.

Comment 5
The work described is outside my area of expertise however, as it’s a new methodology and involving a viral system which is introducing a high rate of mutation, I would err on the side of caution-and ensure all work is done in cat 2 with a high degree of monitoring.

Comment 6
I thought this was a well written, comprehensive proposal. I have no problem with this being assigned to CL2.

Comment 7
They specify there are no problems for individuals with eczema in working with this. If someone has broken skin and the virus becomes modified is there no risk? Have they specified users will wear gloves when working in CL2 (I know they should be but maybe they should note broken skin should be covered?).

Response
Regarding eczema/broken skin: we will certainly be using lab coats and gloves as required by CL2 procedures. We will not be using sharps in any of the procedures, so it is unlikely that there would be an incident in which skin is broken. However, all skin will be adequately covered by the appropriate PPE.
We want to understand the lifecycle of SARS-CoV-2, the causative agent of COVID-19. While a novel pathogen that must be handled under high levels of biological containment, VLP assays will allow biological understanding of SARS-CoV-2 entry and release without requiring handling of infectious replication competent virus.

SARS-CoV-2 is a large (29kB), positive sense, single stranded RNA virus. Its genome encodes 29 separate proteins from 14 separate open reading frames (ORFs). A major 5' ORF encodes polyproteins that are predicted to be proteolytically processed into 16 non-structural proteins that assemble to form an enzymatic replicase/transcripase complex (e.g., Proteases (Nsp3 and Nsp5), primases (Nsp7 and Nsp8), RNA-dependent RNA polymerase (Nsp12), helicase (Nsp13), exoribonuclease (Nsp14), endonuclease (Nsp15)). Subgenomic RNAs at the 3' end of the genome encode 4 structural proteins (Spike (S), Membrane (M), Envelope (E) and Nucleocapsid (NC)) and 9 accessory factors (Gordon, et al., 2020). The capsid formed by NC possesses the ability to package the RNA-genome. Like SARS-CoV, SARS-CoV-2 enters human cells through binding the plasma membrane receptor Angiotensin Converting Enzyme (ACE)-2.

Study of the cell biology of other pathogenic viruses have been significantly derisked by using VLP, rather than viral or pro-viral, assays. E.g., VLP systems have allowed...
work on HIV and Ebola under BSL-2 conditions (Hoenen et al., 2014; Burgt et al., 2013; Watanebe et al., 2003). Because of this safety enhancement, VLPs have also been utilised in human vaccine studies for diseases spanning influenza to HIV (e.g., Fuenmayor et al., 2017; Lopez-Macias et al., 2011; Doan et al., 2005; Tacket et al., 2003). In VLP assays, structural components of the virus are expressed in-trans from individual mammalian expression vectors, rather than from live virus or a provirus (a cDNA copy of the full length viral genome). Typically, these structural proteins assemble within cells and are released as particles. Inclusion of the viral envelope proteins (in this case, S, M and E) allows the released particles to enter target cells, but because no viral genome is packaged and no enzymatic proteins are included, these particles can neither mount a productive infection, nor replicate the target cells. VLP assays have been described for SARS-CoV, where by S, E, M and NC proteins are expressed in mammalian cells, and particles can be harvested from the supernatant (Tseng et al., 2014; Hsieh et al., 2005). Given the extensive similarity between SARS-CoV and SARS-CoV-2, (S, 76% identical (95% identical in transmembrane and cytosolic region); M, 91% identical; E, 95% identical; NC, 91% identical), VLP assays likely represent a viable experimental strategy to study SARS-CoV-2 biology. Despite this enhancement in safety, we must be minded that VLPs have the potential to trigger an immune response and as this is a novel virus we don’t know the immunological consequences of exposure to the viral structural proteins.

REFERENCES:


Gordon et al., (2020) bioRxiv, DOI: https://doi.org/10.1101/2020.03.22.002386


Recipient or parental organism

Plasmids for the VLP system will be transfected into mammalian cells (e.g., Hek293, CaCo-2 and Vero, and their derivatives). The combination of 4 different stable plasmids supplying viral structural proteins, with no regions of homology to prevent undesirable recombination, no genome or viral RNA sequences and no enzymatic
genesynthesis and cloned using standard molecular techniques into individual mammalian expression vectors (e.g., pCR3.1 and derivatives). To enable single-round infectivity assays to be performed, a 580 bp packing sequence from SARS-CoV (Hsieh et al., 2005) or the analogous sequence from SARS-CoV-2 will be inserted into the 3' UTR of a 5th mammalian expression vector encoding GFP (e.g., pEGFP-N1) or a similar reporter (e.g., luciferase, pOL3) to allow VLP-mediated transduction of target cells. Packaged mRNAs from these transcripts will be our surrogates of infection in the target cells and will allow us to discern whether modifications (e.g., co transfection with alternate non-enzymatic proteins, siRNA/CRI/SPR reagents, or host cell overexpression plasmids) result in altered VLP transfer to target cells. In total, we will express approx. 6kB of DNA out of 29kB of viral genome. Separating this into 4 separate mammalian expression plasmids and not including any viral RNA or proviral cDNA will limit the risk of recombination. It may be necessary to additionally transfect a vector encoding a generic viral envelope protein (e.g. VSVG), as is used to pseudotype retro- or lentiviral particles. While ACE-2 is the receptor for this virus very few commonly used cell lines express this protein (https://web.expasy.org/cellosaurus/sars-cov-2.html) and to maximise transfer of VLPs to target cells, we will use VSVG pseudotyping to expand the range of target cells in case necessary target cells are refractory to transduction with SARS-CoV-2 VLPs. To facilitate biological understanding of the other viral gene products, mammalian expression vectors encoding non-enzymatic genes may be co-transfected into producer cells. Importantly, no viral RNA or proviral DNA will be included in these studies.

Example Procedure
Typically, 600,000 293T cells grown in 6-well tissue culture plates will be transfected with 500 ng each of pCR3.1-S, pCR3.1-E, pCR3.1-M and pCR3.1-NC. 48 hrs later, supernatants (approx 2 ml) containing the recombinant VLPs will be harvested, clarified by centrifugation using aerosol-tight lids on centrifuge buckets (300 x g for 2 minutes). These will be opened only within a Class 2 MSC and decontaminated after use. Supernatants will be filtered through a 0.45 micron syringe filter. VLP-containing supernatants will be layered on top of a 20% sucrose cushion and centrifuged in a refrigerated benchtop centrifuge at 20,000 x g for 2 hours. The supernatant will be removed and the pellet will be resuspended in denaturing 2xLDS-sample buffer. Alternatively, VLP containing supernatant will be used to transduce 300,000 target cells grown in 6 well plates in the presence of 0.8 ug/ml polybrene. 48 hours after transduction, viral supernatants will be removed, the monolayer washed in PBS and the cells lysed for assessment of reporter activity. Routes of exposure include inhalation and ingestion. Access to these routes is minimised by proper working practices and by using appropriate PPE. All VLP generating work will be performed under containment level 2 (CL2) conditions in a dedicated viral TC-suite, using a class II microbiological safety cabinet.

Containment level 2 (CL2) is provisionally assigned to this project in order to protect human health. Production of VLPs and their use for infection of target cells will be carried out in a CL2 laboratory with safeguards in place, and a standard operating procedure (SOP) defining the safe handling of biohazardous material, decontamination and waste disposal. It is not thought that coronaviruses are airborne, however they may be transmitted by contact or by procedures generating droplets or aerosols. We assume that the VLP’s transmission routes are similar. CL2 work will be carried out in class II microbiological safety cabinets (MBSC) with personnel wearing protective equipment (gloves, labcoat, and eye protection). Cuts and abrasions will be covered. No material that came in contact with VLPs will leave the MBSC without decontamination. No sharps will be used. Transport, centrifugation and storage will be carried out in closed and sealed and decontaminated containers not to be opened outside of CL2. Procedures that may allow aerosolisation (eg vortexing) will not be permitted. Any spills will be decontaminated using 1% Virkon, a recognised all-purpose antibacterial and antiviral product. Surfaces and instruments will be disinfected using an approved disinfectant as defined by the CL2 SOP. All contaminated disposables will be autoclaved prior to disposal off site. Fixation of transduced cells with 4% PFA or methanol will allow downstream analysis at CL1. Cell lysates will be generated at CL2 using 2xLDS-sample buffer or washed with PBS to remove VLPs and lysed in 1% Tx100 containing luciferase assay buffer (Promega). Liquid waste will be decontaminated using 1% Virkon.

Origin & function
We will express ORFs encoding SARS-CoV-2 S, M, E and NC transcripts. Sequences will be purchased from commercial suppliers or will be obtained by custom genesynthesis and cloned using standard molecular techniques into individual mammalian expression vectors (e.g., pCR3.1 and derivatives). As outlined above, a 580bp
packaging sequence from SARS-CoV (or the analogous region from SARS-CoV-2) will be obtained by genesynthesis and cloned into the 3' UTR of reporter vectors to allow packaging of their transcripts. STR-profiled, mycoplasma free cell lines (described above) will be obtained from Crick Cell-Services STP. These lines will be used as transfection hosts for the above described cDNAs and will generate the VLPs. These cells will also be transduced with VLPs that have packaged the reporter constructs, as outlined above and luciferase activity or GFP transduction will be analysed in these cells after lysis.

Evaluation of foreseeable effects

The most dangerous of the GMM are the SARS-CoV-2 VLP particles since these are capable of infecting human cells. However, there is no risk of viral production or productive infection as genomic components are not transferred and the VLPs will lack 25 of the 29 viral proteins. There is no risk of insertional mutagenesis as the plasmids expressing these genes are standard mammalian expression vectors and unlike retro and lentiviruses, coronaviruses do not replicate by integrating into the genome. The risks identified here relate to the fact that this is a novel emerging pathogenic infection and we don’t yet understand the immunological consequences of exposure to the structural proteins that will present within the VLPs.

Hazards to human health associated with the recipient microorganism (e.g. bacterial host or viral):
Biosafety measures render VLPs single-round infectious and replication incompetent. Given the absence of enzymatic components, there is no risk insertional mutagenesis. The genes inserted in any packaged vector (e.g. luciferase, or GFP) do not encode for toxic or hazardous proteins and only standard reporters with a long history of safe laboratory use will be used. The packaging sequence itself (580nt) is a fragment of Nsp15, an endoribonuclease, and will be placed in a 3' UTR, preventing its translation. The recipient organism (cultured human cell lines) will be cultured under CL2 conditions and so present minimal risk to human health.

Hazards to human health arising directly from the inserted gene product (e.g. cloning of a toxin gene or oncogene):
The genes used in this study are not expected to cause malignant transformation as they are not capable of performing insertional mutagenesis. There is a risk that viral structural proteins transferred by the VLP would be recognised as foreign and could cause an immune response if transferred to humans. As VLPs are replication incompetent, productive infection will not be possible.

Hazards to human health arising directly from the alteration of existing traits (e.g. alteration of pathogenicity, host range, tissue tropism, mode of transmission or host immune response):
Use of the WT viral envelopes are required for generation of the VLPs. Pseudotyping with the heterologous amphotrophic or ecotrophic viral envelope proteins will allow transduction of a wide range of cell types from many species, including humans, and this will only be used if infection of target cells cannot be achieved with WT VLPs. We do not anticipate pseudotyping will alter the pathogenicity of the VLPs, however, it will increase the tropism. The mode of transmission will be unaltered and while a host-immune response is possible, it is likely to be limited by the lack of productive infection.

Hazards to the environment associated with the recipient microorganism (e.g. bacterial host or viral vector):
The genetically modified mammalian cells are highly unlikely to survive outside the controlled environment of tissue culture and thereby pose no threat to the environment. Due to the replication-incompetence of the VLPs, they do not pose any hazards to the environment.

Hazards to the environment arising directly from the inserted gene product:
The genes to be packaged do not encode for toxic or hazardous products that could affect the environment.

Hazards to the environment arising from the alteration of existing traits (e.g. alteration of pathogenicity, host range or tissue tropism):
None identified

The potential hazards to human health of sequences within the GMM being translated to related microorganisms:
We do not envisage a route by which sequences inserted into the GMO could be translated to related microorganisms and thus do not identify a hazard here.

The potential hazards to the environment of sequences within the GMM being transferred to related microorganisms:
The vectors are not able to transfer genetic material to other microorganisms.
Are any of the work procedures likely to generate aerosols?
No

How will waste materials be disposed of?
Producer and target cells will be inactivated using 1% Virkon. Tissue culture waste will be inactivated using Chemgene. Liquid waste and any spills will be decontaminated using 1% Virkon. Surfaces and instruments will be disinfected using Chemgene. Solid waste will be autoclaved prior to disposal off site

Will it be necessary to use sharps?
No

Have any disinfectants been validated under the actual conditions of use?
Yes

Does the nature of this work preclude it being undertaken by any workers who have a serious skin condition (e.g. eczema) or other health problems that might make them more susceptible to infection (e.g. some kind of immunological defect)?
Yes

Will workers receive any vaccinations or health surveillance?
Yes

Consideration of whether there is a need to assign additional measures over and above the provisional level of containment.
Standard CL2 health surveillance.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste management will be in line with the measure outlined in the institutes' containment level 2 code of practice (see attached)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Comment 1
In my view the proposal should indeed be characterised as CL 2 as the possibility is there to infect human cells and despite no RNA or enzymatic activities, the VLP structural proteins could induce an immune reaction of unknown amplitude.

I understand that they might want to use pseudo vector env to boost infectivity of VLP, I am not sure what they will learn here as I thought they wanted to use structural protein to study the normal natural infection route of the Cov.2 which is though the ACE.2 receptor. By using VSVG they will boost infectivity of any human cell type with or without ACE.2 receptor. Also as there is no info on what will be studied using the VLP particles infected cells, it is difficult to understand why pseudo-vector is a potential option. Thus, will asked clarification on the use of pseudo vector env such as VSVG.

Full PPE should clearly be used by investigators.

Comment 2
I have no safety issues with the proposal (privately I think CL1 would not be unsafe but working under CL2 is probably a good idea from the culturing perspective). Any coronavirus antigen would likely be in far too low a concentration to stimulate a dangerous immune response. However, I have at least one concern from the scientific perspective but with no information about the scientific aims I don’t know how important this is. I worry that the use of VSV-G, amphotropic or ecotropic might give cell entry unrepresentative of coronavirus entry. Further, in the absence of RNA transcription, will enough GFP/luc be expressed to yield a useful marker of entry?

Response to comments 1 and 2
Re the need for pseudotyping, we want to use VLPs to study the natural viral assembly route (budding). However, once the VLPs are released, we are less concerned about how they get into other cells. We want to couple a reporter to the budding virus and want to maximise infection of target cells as a surrogate for how many viruses were produced. While ACE-2 is the receptor for this virus very few commonly used cell lines express this protein (https://web.expasy.org/cellosaurus/sars-cov-2.html I’ve listed Caco-2 and Vero as models that do) and VSVG pseudotyping will help us transduce a broader range of target cells, some of which (eg 293 derivatives) will be useful for large scale screening for factors affecting viral production, but which aren’t naturally infected by SARS-CoV-2.

Re what will be studied in infected cells, the GMRA described using the packaging sequence of SARS-CoV and/or SARS-CoV-2 inserted into the 3'-UTR of luciferase or GFP-reporter vectors to link mRNAs from these transcripts to the budding particle. These will be our surrogates of infection in the target cells.

Comment 3
Reviewed and no comments from me.

Comment 4
Only found one potential issue with the proposal: I think Simon needs to OK the use of facemask (written under Comments). We do not use facemasks even in CL3 with an airborne pathogen, so I don’t know why someone should use it in CL2, with a replication incompetent VLP. I am convinced beyond doubt that the work is CL2, no higher or lower.
Response
It has been agreed that the use of face masks will not be permitted for this work.

Comment 5
I thought it looked fine. There were I just put a couple of points in the attached.
- Do the [cultured] cells express ACE-2 receptors naturally or are they engineered?
- I'm not sure. If someone is unaccustomed to wearing a face mask they may be more inclined to fiddle with it. May be counter productive (this comment addresses the possible use of face masks).

Response
These two questions have been answered previously in the responses to comments 1 and 4 respectively.

Comment 6
I have no comments to add, allocation of CL1 & CL2 work is fine.

Comment 7
“To facilitate biological understanding of the other viral gene products, mammalian expression vectors encoding these genes may be co-transfected into producer cells”.

Response
Sorry, I meant any of the many non-structural proteins that may alter the viral biology in the producer cell, not the infected cell. The proposal is to make VLPs, not a virus. How about saying that ‘non-enzymatic viral gene products may be transfected into producer cells to investigate their role on VLP biogenesis’

Comment 8
Given the absence of transferred enzymatic activity or the viral genome, replication will not be possible. If something went wrong and replication occurred is there something in their method that would indicate this has happened and therefore further processing could be terminated.

Response
It's not clear what could go wrong here, so the comment is a little non-specific. Without replicating enzymes, replication won't be possible. The only thing that could bring this about would be if someone co-transfected a provirus (which doesn't exist) or infected the culture with live virus (which I would hope no one would do!)
Comment 9
"To boost infectivity, it may be necessary to additionally transfect a vector encoding a generic viral envelope protein (e.g. VSVG), as is used to pseudotype retro- or
lenti-viral particles".
This virus model is new is there any way this could result in modifications and or induction of replication?

Response
No, without a genome present, there is nothing for the envelope supplied in-trans to incorporate into. In total, we will be expressing 6kB of DNA out of 30kB of genome and
on 4 separate plasmids, so addition of a 5th plasmid expressing VSVG isn’t likely to induce replication.

Comment 10
"Aerosol-tight containers".
Are they referring to aerosol tight lids on centrifuge buckets? If so are these only being opened inside the MSC and decontaminated before removal for others to then use?

Response
I will add the aerosol tight lids on centrifuge buckets and the MSC direction for opening/closing. This will be useful for spinning down supernatants. I was actually meaning
that a sealed container would be used to transfer from one room to another if needed, because I don’t think many CL2 rooms have a refrigerated microcentrifuge, which we
would need.

Comment 11
"We do not anticipate pseudotyping will alter the pathogenicity of the VLPs, however, it will increase the tropism. The mode of transmission will be unaltered and while a
host-immune response is possible, it is likely to be limited by the lack of productive infection".
Given that this virus could potentially act differently to the other coronaviruses and test models being used to provide guidance on the safety for this study. Are there
methods to be sure that a productive infection isn’t occurring?

Response
I appreciate the concern, although am not sure that I understand the syntax around the “test models”. However, I come back to the point that without supplying the
replicative machinery, it won’t be possible to mount a productive infection. We are also not generating virus, we are generating VLPs. It would be possible to utilise some of
the Covid19 testing protocols in place at Crick (or even develop our own), but as this relies on looking for viral RNA, if we don’t supply the viral RNA, then we won’t be able
to find it.

Comment 12
"facemask (seems prudent)". Is it not the case that recommendations from HSE would suggest this isn’t necessary and could result in complacency?

Response
This comment has been answered previously in the response to comment 4

Comment 13
It appears that all the required proteins should never be present together to produce replicating virus. However as this virus could behave unexpectedly would be it
possible for them to determine safely if they have altered the system in any way to increase pathogenicity, transmission or induce viral replication.

Response
Again, this proposal is not to generate virus, it is to generate VLPs, hence it is hard to see how these VLPs could behave unexpectedly. To test pathogenicity and
transmission you would need an animal model, which wouldn’t be sensible to set up, especially as you would be asking to infect it with replication-incompetent VLPs. The
inclusion of a packaged Luciferase or GFP reporter (as outlined in the molecular biology section) would allow us to determine if infectivity was enhanced, so I can bring this
up.
Purposes of the contained use

The present project aims to examine the impact of Kaposi's sarcoma-associated herpesvirus (KSHV) infection on host telomeres to elucidate mechanisms of viral oncogenesis. To this end, infection of cancer cell lines with KSHV de novo will be carried out to analyse the effect of infection on these model systems.
The work will include the use of well-established genetically modified KSHV strains such as rKSHV.219 (Myoung et al., 2011) as well as KSHV derived from bacterial artificial chromosomes (BACs) such as BAC16 (Kevin et al., 2012).


Origin & function

Kaposi's sarcoma-associated herpesvirus (KSHV) is a human herpesvirus formally known as HHV8 and is listed as a hazard group pathogen by the Advisory Committee on Dangerous Pathogens' Approved List of biological agents. KSHV HHV8 infection is asymptomatic in immunocompetent individuals. KSHV can be transmitted via sexual contact and non-sexual routes, such as transfusion of contaminated blood and tissues transplants, or via saliva contact. There is now a general consensus that salivary transmission is the main route of transmission.

Potential infection of uninfected lab worker through oral ingestion of high titres of free virus. Normal containment level 2 (CL2) procedures and low titre of virus are sufficient to avoid this. About 5-15% of individuals in the UK are infected with KSHV (Cesarman et al., 2019). Upon profound immune suppression, such as AIDS or immunosuppressive treatment post-transplantation, individuals infected with KSHV have a significantly higher risk of developing certain virus-associated cancers.


KSHV life cycle is divided into lytic and latent phase. During latent phase, the virus does not replicate and persists in the form of an episome (a plasmid-like genome). This is the predominant form of the virus in infected cells and it does not enter productive replication without specific drug treatments or ectopic expression of viral gene products. For the majority of the work only the latent phase form the virus will used. However, in order to generate new KSHV-infected cell lines, a small amount of virus in its lytic will be produced by transfecting a doxycyclin inducible plasmid containing the main lytic promoter RTA into cells. After that, the cells still need to be treated with doxycyclin as well as sodium butyrate to initiate production of virions. This is the only stage where the virus has even a theoretical capacity to infect humans. The low amount of virus required for such procedures and local standard operating procedures (SOPs) are sufficient to mitigate this risk.

None of the new gene products which we anticipate to insert into the KSHV genome have any documented health hazard associated with them. This includes the aforementioned fluorescent or antibiotic selection markers such as GFP.

Modification of viral gene products for the purpose of interrogation of biological mechanisms as described in sections above may include alterations which have not been performed previously. Therefore, by default, it is a theoretical possibility that these modifications induce a change in the hazard associated with the virus. No such incident of drastic changes in pathogenicity has been reported in the literature thus far and the work is carried out with low amounts of the virus using established SOPs to prevent infection in the first place.

We do not anticipate any hazard to the environment as the gene products which will be inserted into the viral genome do not have the capacity of altering the behaviour of the pathogen. No broadening of viral tropism has been reported upon experimental modification of KSHV. We therefore deem it unlikely that the experiments carried out in
this project will result in generation of virions which may pose any increased hazard to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management will be in line with the measure outlined in the institutes’ containment level 2 code of practice (see attached)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee raised no concerns with this proposal although it asked for further clarification on routes of transmission and whether specific disinfectants would be required for this work. Additional information has been added to this application regarding transmission routes and the existing disinfectants approved for use at the institute have been confirmed as suitable for the work.

Project Containment

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Animal Units

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Project Ref 542/21.3

Date Ackn’d 22/07/2021  CU2 Project Title Exploration of Zika target cell susceptibility and resistance factors  Class 2  CultureVolClass2 < 1 Litre  CultureVolumeClass3-4
### Project Additional Information

**Purposes of the contained use**

Researching questions around target cell identity, susceptibility and resistance

**Recipient or parental organism**

Human and mouse primary tissue, cells and cell lines derived from glioblastoma tumours and embryo brain tissue.

**Host/vector system**

Zika Virus (HG2)

**Origin & function**

1) Fluorescent reporter transgenic strains (Zika-mCherry). These incorporate an mCherry reporter cassette within the Zika virus structural sequences. The transgenic strain is well characterised (Mutso et al., 2017 doi: 10.1099/jgv.0.000938) and is mildly attenuated compared to wildtype strains, as evidenced by a tendency to eject the mCherry reporter cassette over serial passage in culture.

2) Adapted Zika strains. We have derived adapted Zika virus strains, incorporating single base substitutions predominantly in virus structural sequences, through serial passage of the wildtype and mCherry reporter virus in glioma stem cells. This process of directed evolution in vitro is predicted to confer attenuated replication and transmission in other human cell types compared to the host-adapted wildtype virus.

**Evaluation of foreseeable effects**

The GMO variants described are predicted to achieve reduced viral levels and pathogenicity in hosts compared to wildtype virus strains. Human infection would require direct inoculation, and ongoing transmission of this bloodborne virus depends on Aedes Aegypti mosquitoes not native to the UK. Infection of non-pregnant adults is typically asymptomatic (80%) or associated with mild flu-like symptoms (20%): more serious sequelae including Guillain Barre syndrome are reported but very rare. This is reflected in the HG 2 classification

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Lipid enveloped viruses are sensitive to 70% (v/v) ethanol, sodium hypochlorite, formaldehyde, glutaraldehyde, phenolics, iodophors, and quaternary ammonium compounds. Zika virus will be inactivated with an equal volume of 2% Virkon for a minimum contact time of 10 minutes. Virkon solution is then disposed down the sink. Solid waste (excluding pipettes) will be placed in the appropriate waste disposal bags prior to disposal by a licensed contractor. Pipettes and stripettes will be placed in a dedicated pipette waste bin prior to disposal. Pre-treatment (autoclaving) of solid waste prior to disposal as medical waste. See attached COP for CL2 work

Is an emergency plan required according to regulation 20? [N] 

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

See attached

**Project Containment**

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**Project Ref 542/22.1**

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### Project Additional Information

**Purposes of the contained use**

To target and inhibit gene expression in *Klebsiella pneumoniae* to define novel essential gene functions, map gene-gene interactions, and to discover novel molecular tools to probe infection mechanisms. Constructing genetic knockout and knockdowns are standard ways of demonstrating the link between genes and phenotypes in bacteria.

**Recipient or parental organism**

*Klebsiella pneumoniae*

**Host/vector system**

*Klebsiella pneumoniae*

**Origin & function**

1. CRISPR interference mutants - where expression of endogenous genes (one or more) will be selectively inhibited using an inactivated *Streptococcus pyogenes* dCas9 protein expressed from an integrated or episomal vector.

2. Deletion mutants - where specific endogenous genes (one or more) will be selectively deleted from the genome.

3. Spontaneous mutants - where point mutations, deletion or duplications will be selected and propagated by screening for mutants that arise when cells are exposed to particular stress conditions (e.g. low pH, low oxygen, antibiotics, etc).

4. Recombinant mutants - where specific genes (one or more) derived from the same species or other bacteria will be introduced using an episomal or integrative vector. This will be predominantly for complementation.

**Evaluation of foreseeable effects**

*K. pneumoniae* is both an opportunistic pathogen and a commensal organism. Inhibition, deletion, mutation or introduction of genes, is unlikely to alter the bacteria’s ability to infect humans and cause disease.

It is possible that by changing endogenous genes this species could become more host-adapted, but these are not likely to alter the susceptibility to available antibiotics. All gene products will be of bacterial origin and therefore pose very low human health risks.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste containing Klebsiella pneumoniae will be treated with either Distell or Virkon prior to disposal. Contaminated laboratory waste generated during this project will be autoclaved before being sent off site for disposal by our waste contractors.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No comments were received from the Genetic Modification Safety Committee relating to this project.

Project Containment

Laboratory Activities Glass Houses Growth Rooms

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Animal Units Large Scale Activities Human Clinical Applications

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Project Ref 542/94.1

Date Ackn’d 06/06/1994

CU2 Project Title SELECTION OF REGULATED MYCOBACTERIAL PROMOTERS

Class CultureVolClass2 CultureVolumeClass3-4

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Consent Granted

02/03/2022  Page 8650 of 15326
Project notified under transitional arrangements Y

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes GM542/94.1der
Historical Date of Additional Info 12/02/2002

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form N

02/03/2022
Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 542/94.2

Date Ackn'd: 14/06/1994

CU2 Project Title: VIRUS PHENOTYPE CONFERRED BY HIV GLYCOPROTEINS

Class: Class 3

Consent Granted: Yes

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Animal Units: Large Scale Activities: Human Clinical Applications
Project Ref: 542/94.3

Date Ackn’d: 17/08/1994

CU2 Project Title: RESCUE OF HIV PROTEINS

Date Project Ceased: 

Class: Class 3

Culture Vol Class: 

Culture Volume Class: 

Non-GMM: yes

Consent Granted: 

Project notified under transitional arrangements: yes

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes:

Historical Date of Additional Info:

Significant Change ID:

Date of Significant Change:

Project Additional Information:

Purposes of the contained use:

Recipient or parental organism:

Host/vector system:

Origin & function:

Evaluation of foreseeable effects:

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants):
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

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Large Scale Activities

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Human Clinical Applications

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**Project Ref 542/96.1**

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<tr>
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<td>TRANSFECTION OF THE HUMAN MALARIA PARASITE P.FALCIPARUM</td>
<td>Class 3</td>
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Withdrawn  N  

Tick if notifying a connected programme of work  N  

Project notified under transitional arrangements  Y  

02/03/2022
**Project Additional Information**

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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Project Ref 542/96.2

Date Ackn'd: 17/09/1996

CU2 Project Title: MUTAGENESIS OF INFLUENZA HAEMAGGLUTININ

Class: Class 2

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

 Tick to confirm that it is attached to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Project Ref: 542/99.1

Date Ackn'd: 30/03/1999

CU2 Project Title: ROLE OF PAPILLOMAVIRUS PROTEINS IN GENOME AMPLIFICATION

Class: Class 2

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Non-GMM: not applicable

Project Additional Information:

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
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Project notified under transitional arrangements  

Withdrawn  

Tick if notifying a connected programme of work
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
### Project Containment

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### Project Ref 542/99.3

- **CU2 Project Title**: CONSTRUCTION & CHARACTERISATION OF DELETION MUTANTS OF MYCOBACTERIUM TUBEROCOLOSIS

- **Class**: Class 3

- **Non-GMM Consent Granted**: yes

- **Project notified under transitional arrangements**: Y

### Project Additional Information

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref**

542/trans1

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**Project notified under transitional arrangements**

Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

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If yes, tick to confirm that it is attached to this form

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Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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</table>
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 542/trans3

Date Ackn’d 23/02/2001

CU2 Project Title
VACCINIA DRIVEN EXPRESSION OF HIV/SIV GLYCOPEPTIDES

Date Project Ceased

Class 2

Consent Granted

Non-GMM

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
<td>15/11/2001</td>
<td>USE OF MYCOBACTERIAL CLONING VECTORS TO INVESTIGATE THE EXPRESSION AND DELETION OF GENES IN MYCOBACTERIA</td>
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- **Date Project Ceased**: 07/02/2002
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Project notified under transitional arrangements**: Y

### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**
- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
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Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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#### Name
- KINGS COLLEGE

#### Name 2
- UNIVERSITY OF LONDON

#### Campus Estate or Research Centre
- TGH5 GUY'S HOSPITAL CAMPUS

#### Road Name
- TGH5 GUY'S HOSPITAL CAMPUS

#### Town
- LONDON

#### County
- GREATER LONDON

#### Postcode
- SE1 9RT

#### Country
- ENGLAND

<table>
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<th>Tel Number</th>
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#### Comments
GM548 MERGED WITH GM 543 ON 8/9/2003, GM 235, 295 & 543 CLOSED & MERGED WITH GM386

#### Date at Which Additional Info Submitted
02/03/2022
## Significant Change

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## Date of Additional Information (significant change only)

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## Premises Addresses

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<th>Town</th>
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities [ ]

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 1 (GMMs)</td>
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Tick if confidential [ ]
For activities involving GMMs, describe the waste management measures which will apply to the activity

 Tick to confirm that you are attaching a summary of the risk assessment

 Tick if you are claiming exemption from disclosure for sections of the risk assessment

 Please enter comments of the GM safety committee on the risk assessment

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**Project Ref 295/02.3**

<table>
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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The purpose of this activity is to study the intracellular signalling mechanisms that mediate the response of cells to growth factors and extracellular matrix molecules, including proliferation and migration, and thus play a role in the development of human cancers and in cellular differentiation.

**Recipient or parental organism**

1. E. coli
2. murine cells (NIH3T3, and the NIH3T3 variants 293T, GP+E86, GP+EnvAm12)
3. human primary keratinocytes and carcinoma cell lines
4. monkey cells (COS-7)

**Host/vector system**

1. Host: E. coli DH5a; Vector: pCEV27, pBABEneo/puro plasmid, pMSCVneo, pMSCVhygro, pLib, pRx-bsr, pRcCMV, pc1, pGET-T, pPCR-Script
2. Host: GP+E86 murine fibroblasts; Vector: pBABEneo/puro, pMSCVneo, pMSCVhygro, pLib, pRx-bsr plasmid
3. Host: GP+envAm12 murine fibroblasts; Vector: pBABEneo/puro, pMSCVneo, pMSCVhygro, pLib, pRx-bsr plasmid
4. Host: Murine and human mesenchymal cells (osteoblasts, chondrocytes, fibroblasts, and adipocytes) either as established cell lines or primary cultures, as well as osteoclasts, osteoclast precursors, haematopoietic stem cells, and embryonic stem cells. Vector: pBABEneo/puro plasmid, pMSCVneo, pMSCVhygro, pLib, pRx-bsr virus
5. Host: NIH3T3 murine fibroblasts; Vector: pBABEneo/puro virus
6. Host: HNEK, H357, A431, HN12, HN4 & MCF7 human keratinocytes; Vector: pBABEneo/puro virus
7. Host: 293T cells; Vector: PINCO (modified from plasmid LZRSpBMN-Z)

**Origin & function**

Mouse and human cDNAs encoding signalling intermediates, cell cycle control genes, and AP-1 transcription factors, as well as novel cloned target genes, will be obtained by PCR, or excised from existing plasmid vectors, subcloned into viral plasmids, and used to produce amphotropic/ecotropic viruses as a means of expressing them in the target cells (see above).

The intended function is to investigate growth factor and extracellular matrix-dependent signalling pathways that regulate cellular differentiation, proliferation and motility, all of which are key aspects of cancer development.

**Evaluation of foreseeable effects**

The vectors to be used in these studies are replication-defective recombinant retroviruses, and have been well characterised and extensively used (Morgenstern & Land, Nuc Acids Res 18: 3587, 1990; Gasperi et al., Journal of Leukocyte Biology 66: 263-267, 1999). They are non-mobilisable in mammalian cells, and are rapidly inactivated by serum. The cell lines (GP+E86 and GP+envAm12 are third generation packaging cell lines, and have been specifically constructed to prevent recombination events between introduced constructs and endogenous viral structural sequences. Investigators using this system have not observed production of any replication-competent virus (RCV) (Markowitz et al., Virology 167: 400, 1988). The recipient cell lines are free of helper virus, and transduction of these lines with replication-defective viruses will not generate RCV. In spite of these findings, assays to detect RCV will be carried out as a matter of course. Thus, although the vectors will be packaged with ecotropic and amphotropic envelopes, and could conceivably infect rodent (ecotropic) and human cells (amphotropic), such viruses would be incapable of replication. Furthermore, these viruses are unstable at ambient temperatures, with a half-life of around 2 hours. Some of the gene products encoded by cDNAs cloned into these vectors have oncogenic potential. Therefore, the assessment is that work with both ecotropic and amphotropic viruses will be carried out at level 2. The containment level and procedures proposed for the study, together with the inherent safety of the packaging systems, will make the environmental risk effectively zero.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Liquid waste from bacterial and vertebrate cell cultures will be aspirated and treated with bleach (Haztabs; 1 tablet per litre = 2,500 ppm chlorine) for 12 hours. The bleach will be made freshly on the day of use according to manufacturers instructions. This will result in at least 99.999% kill of bacteria and 100% kill of viral GMMs and cell lines which are extremely liable. In addition, the retroviruses and cell lines have a very short half life at room temperature. Inactivated culture supernatants will be discharged to the drainage system. Solid waste will be double bagged, sealed, and placed within and autoclaved in a closed metal container. This will prevent any accidental release of GMMs into the working environment. The waste will be autoclaved at 136 degrees C for 30 minutes. This will result in complete inactivation of GMMs. Autoclave function will be monitored by annual services, the use of autoclave indicator tape (which measures a temperature of 134 degrees C for 3 minutes) during every run. Autoclaved waste will then be incinerated by White Rose. |

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The GMSC accepted the risk assessments for these projects.

Class of Activity 2.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

| L2 | L3 | L4 |
| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |
| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |
| L2 | L3 | L4 |

Project Ref  295/03.1
**Project Additional Information**

**Purposes of the contained use**

Mycobacterium tuberculosis (MTb) is a class 3 human pathogen and requires level 3 containment facilities for use. The recombinant MTb expressing luciferase gene would be used to significantly decrease the experimental times for bacterial determination in infected cultures and BCG expressing either luciferase or green fluorescent protein (GFP) would allow additional flexibility in substituting pathogenic MTb in certain types of assays. Luciferase and GFP are biologically harmless genes but as the recipient strain is pathogenic the containment level 3 would be required for the use of recombinant strains as for the wild type MTb. The same applies to Mycobacterium bovis (MB) expressing luciferase gene which would be used to uncover differences in pathogenesis between these two closely related species.

**Recipient or parental organism**

The recipient organism is bacterium Mycobacterium tuberculosis (MTb) or the attenuated strain of Mycobacterium bovis (MB) known as BCG (Bacillary of Calmette and Guerin). MTb and MB are class 3 organisms and BCG is Class 2.

**Host/vector system**

pSMT1 and pSMT3, both non-mobilisable vectors.

**Origin & function**

Luciferase gene is of bacterial origin and its function in the intended recombinant strains of Mycobacteria is to serve as reporter gene for quantitative determination of bacteria. The green fluorescent protein (GFP) is derived from jellyfish and is a fluorescent marker for tracking down the recombinant bacteria during infection of cells.

**Evaluation of foreseeable effects**

The recombinant Mycobacterium tuberculosis and Mycobacterium bovis expressing luciferase and BCG expressing luciferase or GFP are generated several years ago in the laboratory of Douglas Young at Imperial College London (Lab GM77). They have been used since then worldwide and several publications have quoted the use of these strains. Both genes are reporter constructs. Luciferase is an enzyme that converts certain type substrates into chemiluminescent products and GFP is a fluorescent protein that can be easily identified by fluorimetric techniques such as flow cytometry (FACS). These two genes have been used in numerous experimental systems with no biologically harmful effects every being reported. The use of these two genes would normally require level 1 containment measures but as the recipient...
strains are class 3 (MTb and Mb) or class 2 (BCG) organisms, it is necessary to apply the class 3 or respectively, class 2 containment measures. There is no indication from the originator or from the others who have used these strains that they are in any way different in terms of virulence from the corresponding wild type strains. Therefore, the environmental or personal risk from using these strains is no different from that of wild type strain.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Re: APPLICATION FOR DEROGATION FOR AUTOCLAVE FACILITY FOR CONTAINMENT LEVEL 3 LABORATORY**

During a recent inspection by HSE, it was pointed out to us that there had been a change in the regulations defining what constitutes a laboratory suite, and as a result our containment level 3 facility (room 311, floor 28 Guy’s Tower) contravened the new interpretation under the Genetically Modified Organism (Contained Use) Regulations 2000 (CU2000). I therefore am applying for derogation from Schedule 8 of CU2000.

The relevant features, following the guidance attached to Newsletter 30 are:

a) Both the autoclave and the containment facility are located at the far end of the building remote from the main access to the floor. They are joined by a short corridor that is not used by students, non-scientific staff or members of the general public. There are no offices at this end of the building, only laboratories.

b) The infection with Mycobacterium tuberculosis and mycobacterium bovis occurs through respiratory route. The infectious dose has not been firmly established but it is thought that the size of aerosol-born droplets containing bacteria is critically important (i.e several droplets containing 3-5 bacteria each, if inhaled by an immuno-susceptible person may establish an infection). Only 5% of infected individuals develop the disease.

c) The highest concentration of bacteria ever present in the waste is $10^7$/ml which is the remaining stock solution from the experiment (the stock solution is 1 ml, thus the amount of this waste is always less than 1 ml). The unused stock solution is routinely treated with hycolin for several hours prior to disposal. Typical concentration of bacteria in the experimental liquid waste is $10^5$/ml or less.

d) The waste is in loosely tied autoclave bags contained in metal autoclave bins.

e) Following the inspection we have purchased autoclave tins with clip-on lids to replace the identical ones without clip-on lids.

f) as in (e) above.

g) The container will be placed on a trolley following an agreed procedure that has been in place for the last 2 years.

h) the removal of waste from the Containment Laboratory does not take place unless it is clear that the autoclave is available for use. After this waste has been loaded into the autoclave low level waste may also be added, but only by the staff loading the level 3 waste.

i) As in (h) above.

j) The container is placed directly in the autoclave and the tray that sits on the trolley is then also placed in the autoclave.

k) The only personnel that move the waste are trained for work at containment level 3.

l) The SOP (revised to include the use of clip-on lids) for use of the Containment facility is attached. This contains an emergency procedure to be followed in the event of a...
spillage during waste transport.

DECONTAMINATION

1. All glassware and plastics must be autoclaved on a cycle to reach 126 degrees C before removal from the laboratory suite. Autoclave bins with properly fitting clip on lids containing the waste in loosely tied autoclave bags must be taken from the CL3 laboratory to the autoclave immediately prior to autoclaving. The following procedures will be follows:

i) at least two trained staff must be available to carry out these procedures
ii) the autoclave is room 3245 must be available for use
iii) one member of staff will act to open doors; one will move the autoclave tins from the CL3 facility onto the transport trolley
iv) the autoclave tins will be removed from the CL3 facility and placed on a tray on a trolley outside the CL3 laboratory
v) autoclave bins from the CL3 laboratory will be taken directly to the autoclave and placed in the autoclave
vi) when all autoclave bins have been loaded into the autoclave, the tray will also be placed in the autoclave and the autoclave cycle will be started.

2. If a spillage of CL3 infectious material occurs during transport of waste, the area will be disinfected by treatment with Presept, and/or by swabbing with disinfectant.

i) Any contaminated clothing must be removed and any affected skin should be washed with soap and water. In the event of cuts, the First Aid Officer should be contacted.
ii) Notification of the Biological Safety Officer and the relevant Line Manager must then take place. They will decide if any further action is required.

The waste consisting of disposable plastics and liquid waste in volumes of 100 ml or less in plastic containers will be placed in plastic bags, sealed and transferred to metal tins with clip-on lids. The standard internal operating procedure for removal and autoclaving of waste will then be applied (for details please see above application for derogation of autoclave facility for CL3 room).

The autoclave will operate the cycle at 126 degrees C for 80 min at 2.5 bar. The waste will then be placed in bags and disposed to designated bins.

This procedure is effective for complete killing of Mycobacterium tuberculosis wild type as determined by re-plating of the autoclaved material on microbiological plates. Similar test will be conducted for GM strains of MTb and MB (ie following completion of the cycle the metal been containing the waste will be returned to CL3 room in a reverse of the removal of waste procedure and the waste placed in Microbiological Safety Cabinet. An aliquot of the liquid waste and a swab from the solid waste will be tested for the presence of live bacteria by microbiological methods. Only following the negative result of the test will the remaining waste be removed from the room in a repeat of standard procedure and autoclaved prior disposal. This test-procedure will be repeated each time when the nature or the quantity of the waste significantly differs from the routine waste.
The proposal was looked at by two members of Safety Committee who were satisfied with risk assessments and proposed risk categorization.

The class of activity is 2/3 as detailed in the KCL GM RA.

**Project Containment**

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**Laboratory Activities**

- Project Containment
- Glass Houses
- Growth Rooms

**Project Ref** 386/01.15

**Date Ackn'd** 08/09/2003

**Date Project Ceased** 04/03/2014

**Project Title** EXPRESSION OF MAMMALIAN GENES IN MAMMALIAN CELLS USING NON-VIRAL VECTORS

**Class** Class 2

**Non-GMM Consent Granted** not applicable

**Project notified under transitional arrangements** Y

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Date of Additional Info**

**Historical Significant Changes**

PROJECT TRANSFERRED BACK TO GM386 FROM GM543 ON MERG

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
ELUCIDATION OF THE CARCINOGENIC PROCESS IN THE UPPER AERODIGESTIVE TRACT

PROJECT TRANSFERRED BACK TO GM386 FROM GM543 ON MERG
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

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### Project Ref 386/02.5

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Withdrawn</th>
<th>Project notified under transitional arrangements</th>
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<tbody>
<tr>
<td>08/09/2003</td>
<td>1. TRANSIENT STIMULATION OF CELL GROWTH BY PROTEIN TRANSDUCTION OF GROWTH STIMULATORY AGENTS. 2. MODIFICATION OF MAMMALIAN CELLS WITH TUMOUR...</td>
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Historical Significant Changes: PROJECT TRANSFERRED BACK TO GM386 FROM GM543 ON MERG

02/03/2022
Purposes of the contained use

To determine the effect of SV40 Large T antigen and tumour-suppressor genes such as Apoptin (VP3), p53, p16, p19ARF and pRB proteins on a range of cells, when added exogenously as fusions with the TAT protein transduction peptide.

Recipient or parental organism

SV40 Large T:
Bacteria - Attenuated and mobilization defective E. coli (K-12) derivatives such as ER 1647, DH5-a and XL1 Blue, unable to survive in the absence of specialised growth conditions.
Human fibroblasts - Detroit 551 cells, non-immortalized, limited replicative life span, unable to colonize human hosts, unable to survive outside specialized tissue culture environs.

Apoptin (VP3), p53, p19ARF and pRB proteins:
Retroviral packaging cells: GP+E86, murine fibroblast derived with split helper function, ecotrophic for mouse, sensitive to human complement. PG13 murine fibroblasts derived, split helper function, restricted amphotropic, sensitive to human complement.
Established and primary mouse and human tissue culture cell lines: epithelial, fibroblasts and myeloid types. These cell lines are spontaneously immortalised, unable to colonise in human hosts, unable to survive outside special tissue culture environments.

Host/vector system

SV40 Large T:
* Cloning vector - pET22b (Novagen Ltd), in bacterial host, unable to replicate in nonbacterial cells. No expression of specific gene products in the absence of exogenously provided T7 polymerase AND isopropyl-thiogalacto-pyranoside (IPTG).

Apoptin (VP3), p53, p19ARF and PRB proteins:
* PBABE and MFG series of retroviral vectors with murine leukaemia virus derived LTR promoter. These vectors are produced by safe packaging cell lines such as GP+E68 and PG13. These vectors have an LTR promoter and infect only rodent cells (GP+E68) or a wide range of cells including human (PG13). No inherent pathogenicity other than low risk of insertional mutagenesis is associated with these viruses.
* Mobilisation defective pUC based vectors with large array of different promoters such as SV40, CMV. Bacterial expression vectors such as pTriEx-1, in disabled bacterial hosts such as E. coli K-12 auxotrophic mutants for propagation.
* Spontaneously immortalised human and mouse tissue culture cell lines, unable to colonise humans. There is no evidence of the ability of these cells to secrete transmissible agents. These cells are unable to supply helper functions to disabled retroviral vectors.

Origin & function

SV40 Large T
* The genetic material originates as mRNA from human and/or mouse cells. The indicated function of the genetic materials is growth stimulatory.

Apoptin (VP3), p53, p16, p19ARF and pRB proteins:
* The genetic material originates as mRNA from human and/or mouse cells. The indicated function of the genetic material is that the full length cDNA products are tumour suppressors, including FHIT, p16/INK4a, p19/ARF, p21/waf1 and p53. These are cloned into mobilisation defective plasmid vectors including bluescript pCDNA3.1, pTRE2, pSVNeo and/or pBABE replication incompetent retroviral vector and will be expressed in human and mouse tissue cancer cell lines to determine their ability to
apoptose.

Evaluation of foreseeable effects

Waste - All waste material is dealt with as described under Section 12 - This covers the deliberate removal of material from the laboratory environment.

Accidental escape into the environment;

a) from bacteria: All manipulations with bacteria will take place at containment level 2. Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. In the absence of combination stimulation of induction, expression of the protein in bacteria will be virtually zero. Protein preparations from induced bacteria will be via detergent lysis, to avoid aerosol production.

b) From tissue culture - All air is filtered (Hepa) before venting to the environment, the labs are provided with spill kits and SOPs for dealing with accidental spillage. All manipulations with human tissue culture cell lines take place at containment level 2. Cell lines are unable to survive outside the laboratory environment. White coats and gloves are mandatory. In the event of escape of disabled retroviral vectors, hazard is unlikely because they are unable to replicate in the absence of helper function and are sensitive to dessication and have a half life of only 5-8 hours under optimum laboratory conditions. In addition, PG13 derived vectors are unable to infect recipient permissive cells in the presence of normal complement. GP+E86 derived vectors are unable to infect cells other than mouse. In the event of infection and integration, the protein products of the cDNA being examined in this way (eg apoptin(VP3), p53, p16, p19ARF and pRB proteins) do not have deleterious effects in normal animal cells.

Accidental contamination of workers in the suite - Bacterial hosts are attenuated for growth in the absence of special growth media, and the ability to adhere to the gut lining. The vector DNA is unable to propagate or express in human cells. Established cell lines used are unable to colonize human hosts.

Security - The building has 24 hour security, and swipe card only access, visitors must report to the security and be escorted whilst in the building.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste - all contaminated waste is autoclaved in the departmental autoclave that is used only for waste. The moist heat autoclave is used at 121 degrees C for 40 minutes. Temperature validation provided by means of thermocouple probe. The autoclave is serviced annually by Meadowrose Scientific and documentary evidence of correct function stored on site. Under these conditions complete kill of organisms is guaranteed. The resultant sterile waste is double bagged in yellow bags and taken away and incinerated by Medical School contractors (White Rose).

Liquid Waste - All liquid waste is inactivated with 10,000 ppm of Chlorine for greater than 10 hours, after which it is disposed of down the designated sink. Under these circumstance complete kill of organisms is guaranteed.

Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GMSC approved the RA for this project Activity Class 2.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</table>

Project Ref 386/03.1

Date Ackn'd 12/01/2004

CU2 Project Title UTILISATION OF GENE MEDIATED APPROACHES TO DESTROY RESIDUAL CANCER.

Class 2

CultureVolClass2 1-50 litres

Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Historical Significant Changes Project transferred to GM543 on 12/01/2004  PROJECT TRANSFERRED

Project Additional Information

Purposes of the contained use

Studies designed to find effective ways of destroying tumour cells that remain in the body after surgery that are resistant to conventional radiotherapy.

Recipient or parental organism

Human, rat and mouse cells maintained in vitro. The overall containment level that will be applied to these studies is 2.
Host/vector system

Host: human, murine or rat squamous cells.

Vector systems:
A. Adenovirus and selectively replicating adenovirus.
B. Salmonella species modified to carry either the cytosine deaminase gene or anti-angiogenic peptides.

Origin & function

The vectors all induce lysis of rodent and human tumour cells. In the case of the salmonella derived vectors tumour lysis occurs after exposure of the host to the pro drug 5 Fluorocytosine. Treatment with vectors carrying anti-angiogenic peptides is designed to prevent outgrowth of blood vessel sprouts. It is anticipated that this treatment will prevent tumour outgrowth and spread.

Evaluation of foreseeable effects

Non-replicating adenoviruses or selectively-replicating adenoviruses and Salmonella species are all used with the intent of destroying tumour but not normal cells. There are no foreseeable hazards to human health or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste. Solid waste will be double-bagged and autoclaved at 121 degrees C for 90 minutes followed by incineration. Sharps are discarded into designated sharps containers for incineration off site by White Rose Environmental.

Surgical instruments. Surgical instruments will be treated with a chlorine-releasing agent (for example HazTabs) and then scrubbed and autoclaved. All our autoclaves are on a service contract and are tested annually to ensure they meet the required specification for temperature and time holding.

Liquid waste. Decontamination with a chlorine-releasing solution at a concentration of greater than 10,000 ppm available chlorine for greater than 10 minutes prior to disposal down a designated sink.

The disinfectant to be used will be a chlorine-releasing cyanurate (Haz Tabs or PreSept), an agent that has good activity against Salmonella species and adenovirus.

The activity of HazTabs against Salmonella was demonstrated by Bloomfield and Miles (Journal of Applied Bacteriology 1979, 46:65-73) using a capacity test which demonstrated a 9log reduction following treatment of a test innoculum of 2.5 x 10 (to the power of 10) cfu/ml of Salmonella typhi. When 20% plasma (equivalent to serum) was included in the test 3,000mg/l of available chlorine was needed to kill Staphylococci. As HazTabs generate 10,000 ppm available chlorine (equivalent to 7,000 mg/l) and the concentration of serum in media will typically be less than 2%, this is a good disinfectant for the work to be carried out and is unlikely to become exhausted (Bloomfield and Uso 1985, Journal of Hospital Infection, 6@20-30).

The efficacy of HazTabs against viruses is shown by studies incorporating dried suspension of Polio virus and demonstrating a 5 log reduction following treatment with 9,200 ppm available chlorine after 1 minute, and at 5 and 10 minutes no virus was recovered, (Tyler R., Alylisse G.A.J., Bradley C. Virucidal activity of disinfectants:
The work proposed will not generate "high-hazard" waste and we do not propose to test whether organisms can be grown from the waste. However, every care will be taken to ensure that the concentration of chlorine is appropriate and liquid waste is treated for a minimum of 10 minutes to compensate for the presence of serum.

The local GMSC approved this as a class 2 activity.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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</table>

**Project Ref** 386/98.1

- **Date Ackn'd**: 08/09/2003
- **CU2 Project Title**: EVALUATION OF ADENOVIRAL VECTORS FOR GENE THERAPY OF HEAD AND NECK CANCER. 1.MINIMAL DISEASE IN THE MUSCLE BED
- **Class**: Class 2
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**

PROJECT TRANSFERRED back to GM386 ON MERGER OF GM 543 W1

02/03/2022
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
## Project Containment

### Laboratory Activities
- L2
- L3
- L4

### Glass Houses
- L3
- L4

### Growth Rooms
- L2
- L3
- L4

### Animal Units
- L2
- L3
- L4

### Large Scale Activities
- L2
- L3
- L4

### Human Clinical Applications
- L2
- L3
- L4

### Project Ref: 386/trans2

**Date Ackn'd:** 08/09/2003  
**CU2 Project Title:** UTILISATION OF MOLECULAR TECHNIQUES TO STUDY THE PATHOGENESIS OF HEAD AND NECK CANCER (PREVIOUSLY - STUDY OF THE MOLECULAR PATHOGENESIS OF ORAL CANCER)  
**Date Project Ceased:** 04/03/2014  
**Class:** Class 2  
**Culture Vol Class 2:**  
**Culture Volume Class 3-4:**  
**Non-GMM:** not applicable  
**Consent Granted:**  

- **Withdrawn:** N  
- **Tick if notifying a connected programme of work:** N  
- **Project notified under transitional arrangements:** Y  
- **Historical Significant Changes:** PROJECT TRANSFERRED back to GM386 ON MERGER OF GM 543 W1

### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 543/01.1

Date Ackn'd 02/03/2022  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4
EXPRESSION OF VIRAL, BACTERIAL AND MAMMALIAN DNA SEQUENCES IN MAMMALIAN CELLS USING NON-VIRAL DNA TRANSFER SYSTEMS

22/02/2001

Date Project Ceased
08/09/2003

Withdrawn
N

Tick if notifying a connected programme of work
N

Transferred to GM386 on 08/09/2003

Project notified under transitional arrangements
Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 543/01.2

Date Ackn’d: 15/02/2001

Date Project Ceased: 07/02/2003

CU2 Project Title: ADENOVIRAL TRANSFER OF CYTOKINE GENES INTO RAT RETINA

Class: Class 2

Culture Vol Class 2: not applicable

Culture Volume Class 3-4: not applicable

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Tick if notifying a connected programme of work: N

Tick if you are claiming exemption from disclosure for section of the risk assessment: N

Tick to confirm that it is attached to this form: N

Tick to confirm that you have attached a risk assessment to this form: N

Is an emergency plan required according to regulation 20?: N

Page 8693 of 15326

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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02/03/2022
Project Title
Pathogenesis and treatment of infections at mucosal surfaces

Class
Class 2

Consent Granted
Not Applicable

Non-GMM
Not Applicable

Tick if notifying a connected programme of work
Y

Historical Significant Changes
PROJECT TRANSFERRED TO GM386 ON MERGER OF GM543 WITH GM386

Recipient or parental organism
1. E.coli
2. Streptococcus mutants
3. Streptococcus pneumoniae
4. Streptococcus gordonii
5. Streptococcus mitis
6. Fusobacterium nucleatum
7. Tannerella forsythensis
8. Porphyromonas gingivalis
9. Enterococcus faecalis
10. Lactobacillus spp
11. Candida albicans

Host/vector system

Purposes of the contained use
Mucosal surfaces of the oral cavity, gastrointestinal and genital tracts are the main routes of entry of infectious microorganisms as well as being the sites for mucosal infections that may also have systemic effects. This activity will investigate the mechanisms by which both pathogenic microorganisms and those that form part of the normal mucosal flora may cause disease. In addition novel approaches to treatment by using genetically engineered commensal bacteria to deliver microbicidal agents will be investigated.
2. Host: Strptococcus spp; Vector: pALH109, pALH122, pNE1gfp, pEVP3, pSMB55, pMHL120, pCMG8 or related plasmids.
3. Host: T.forsythensis; Vector: pMJF/ΔR II or related plasmids
4. Host: F. nucleatum; Vector: pKH90, pOR19, pH17 or related plasmids

Origin & function

1. Fragments of genomic DNA derived either directly or by polymerase chain reaction amplification derived from Streptococcus spp., T. Forsythensis, F. nucleatum will be used for mutagensis to generate microorganisms that are defective in gene function either as a result of random mutagensis or targeted mutagensis.
2. Gene fragments identified as having functions of interest as above, may be cloned and used for expression of the gene products in E.coli. To understand function of such gene products, mutagensis may also be performed with expression in E.coli. Similarly, DNA encoding proteins with antimicrobial potential e.g. single domain antibodies, cyanovirin and derived from human, animal, plant or algae sources will be used for expression of the gene product in heterologous hosts.

The aims are to identify genes that contribute to virulence of mucosal pathogens and to express proteins that may be used for treatment or prevention of mucosal infections.

Evaluation of foreseeable effects

The microorganisms that will be modified are part of the normal flora to be found at mucosal surfaces in healthy individuals. Mutagenised microorganisms, Streptococcus spp., T. Forsythensis, F. nucleatum, Candida albicans show or are expected to show loss of function compared with wild type organisms. Streptococcus pneumoniae is a class 2 pathogen and work with virulent strains (i.e. TIGR4 derivatives) will be carried out in a microbiological safety cabinet.

Engineered Streptococcus gordonii and Lactobacillus strains may have comparable colonizing activity to wild type strains but cannot transfer antibiotic resistance since the genes are stably integrated into the chromosome of the modified host and cannot be mobilised. To reduce exposure and risk for the researcher to negligible levels, liquid cultures will be handled in a microbiological safety cabinet.

In all studies, genetically modified material will be destroyed on site by autoclaving.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from GMM cultures will be aspirated and treated with Haztabs (1 tablet/litre = 2,500 ppm chlorine) for 12 hours. Concentrated solutions will be made freshly on the day of use according to manufacturers instructions. Inactivated culture supernatents will be discharged to the drainage system. Solid waste will be double bagged, sealed and placed in a closed metal container. Waste will then be autoclaved in the closed metal container at 136°C for 30 minutes. This will result in complete inactivation of GMMs. Autoclave tape will be used to indicate that the material has been autoclaved. Autoclave function is monitored by services performed at 3 month intervals by Health Services Associates. Autoclaved waste will be placed in yellow bags for off-site incineration by White Rose.
The local GMSC approved the Risk Assessment for this project on and signed off the component activities on 11th March 2006 and 11th July 2006. The class of activity is 2.

**Project Containment**

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Animal Units

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**Project Ref 543/07.1**

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<th>CultureVolumeClass3-4</th>
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<th>Consent Granted</th>
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<td>Class 2</td>
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| Date Project Ceased | | |
|---------------------|------------------|
| 04/03/2014          |                  |

Withdrawn N

Tick if notifying a connected programme of work Y

Historical Significant Changes

PROJECT TRANSFERRED TO GM386 ON MERGER OF GM543 WITH G

Historical Date of Additional Info

Significant Change ID

02/03/2022
### Project Additional Information

#### Purposes of the contained use

Identification and study of the host contribution to bacterial infection and pathogenesis. This is done by infection of the fruitfly Drosophila melanogaster with the class 2 pathogen Mycobacterium marinum. Both host (Drosophila) and pathogen (M. Marinum) can be genetically modified.

#### Recipient or parental organism

<table>
<thead>
<tr>
<th>Mycobacterium marinum</th>
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<tr>
<td>Drosophila melanogaster</td>
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</table>

#### Host/vector system

- **M. marinum vectors:** pVK173T, pFPV2, and equivalents/derivatives thereof.
- **D melanogaster:** crippled P-element-derived transposable elements (carried in vector pUAST and equivalents/derivatives thereof).

#### Origin & function

- **M. marinum:** transgenes encoding fluorescent proteins such as dsRed and GFP, from corals or jellyfish, used for tracking bacteria cells.
- **D melanogaster:** transgenes encode a variety of molecules (signal-transduction molecules, fluorescent proteins, etc.) of heterologous (human, mouse, jellyfish, coral, bacteria) or homologous origin; used to alter the course of infection and hence to be informative as to the genetic basis of susceptibility to infection or of pathogenesis.

#### Evaluation of foreseeable effects

- **M. marinum:** in our hands, fluorescent derivatives typically grow more slowly and are slightly less pathogenic than wild-type strains; they will also be resistant to some aminoglycoside antibiotics (apramycin or kanamycin), the net effect should be a mild decrease in pathogenicity for these strains, since primary treatment for M. marinum does not typically depend on aminoglycosides.
- **D melanogaster:** transgenic files should exhibit reduced fitness relative to other laboratory strains; since lab strains of drosophila are already poorly-adapted for life in the environment, these flies should present no environmental risk.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

We are very careful to prevent release of any pathogen-infected Drosophila.

1. Individual infected flies are tracked.
2. In most cases flies are infected, placed in culture vials and never again removed. Once the experiment is completed, the entire vial (including contained flies) is frozen to kill flies, then autoclaved to kill bacteria, and finally incinerated.
3. In cases where an experiment requires removal of live infected flies as samples, all flies within the vial are anethetized before the vial is opened, preventing inadvertent escapes.
4. Fly-traps will be placed at frequent intervals in the fly room to attract and contain escaped flies.

Using these techniques, in the course of 6 years doing similar experiments at Stanford University, I observed fewer than 10 escapes of a total of more than 100,000
Infected flies. All of these escapes were due to defective vial closures; I have now changed the type of vial-closure to one that is more secure, so fewer escapes are expected.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids: Waste will be double-bagged and autoclaved at 124C for 15 minutes. Appropriate autoclave indicator tape will be used to monitor autoclave function. After autoclaving, waste will be incinerated by White Rose. Waste containing live flies as well as pathogens will be frozen for at least 6 hours at -20C before autoclaving.

Liquid: Addition of disinfectant (chlorine bleach to 5% or 1 haztab/litre) followed by pouring down designated sink.

Both procedures are expected to achieve 100% killing.

Monitoring will be done by attempting to culture M. marinum using standard techniques either after autoclaving or after addition of bleach, as appropriate.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

GMSC approved at meeting of 1 May 2007.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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</tbody>
</table>

Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 543/09.1

Date Ackn'd 02/03/2022  
CU2 Project Title  
Class  
CultureVolClass2  
CultureVolumeClass3-4
Pathogenesis and treatment of infections at mucosal surfaces

Mucosal surfaces of the oral cavity, gastrointestinal and genital tracts are the main routes of entry of infectious microorganisms as well as being the sites for mucosal infections that may also have systemic effects. This activity will investigate the mechanisms by which both pathogenic microorganisms and those that form part of the normal mucosal flora may cause disease. In addition novel approaches to treatment by using genetically engineered commensal bacteria to deliver microbicidal agents will be investigated.

Recipient or parental organism

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1. E. coli</td>
<td>2. Streptococcus mutans</td>
<td>7. Tannerella forsythensis</td>
</tr>
<tr>
<td>4. Streptococcus gordonii</td>
<td>5. Streptococcus mitis</td>
<td>10. Lactobacillus spp</td>
</tr>
<tr>
<td>2. Host: Streptococcus spp.: Vector: pALH109, pALH122, pNE1gfp, pEVP3, pSMB55, pMHL120, pCMG8 or related plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Host: T. forsythensis; Vector: pMUF::R I or II or related plasmids</td>
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<td></td>
</tr>
<tr>
<td>4. Host: F. nucleatum; Vector: pKH90, pORI9, pHS19, pSH17 or related plasmids</td>
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<tr>
<td>5. Host: C. albicans SC5314 and CAI4 strains; Vector: Clp10</td>
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<tr>
<td>7. T-cell lines (MT-4, SupT1, C8166 and PM1); Vectors: p8.91, pCSGW, pMDG, pSVIII, pNL4.3, pLAI, pJRC52, pHYU2, pEGFP-vpr, Ad-Easy vector system pcDNA3, pGEX-3, pCAGGS, pCAGGS2, pGem-luc, pCX-EGFP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Host: Knockout Mouse (C57/BL6)</td>
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</tbody>
</table>
1. Fragments of genomic DNA derived either directly or by polymerase chain reaction amplification derived from Streptococcus spp., T. Forsythensis, F. nucleatum will be used for mutagenesis to generate microorganisms that are defective in gene function either as a result of random mutagenesis or targeted mutagenesis.

2. Gene fragments identified as having functions of interest as above, may be cloned and used for expression of the gene products in E. coli. To understand function of such gene products, mutagenesis may also be performed with expression in E.coli. Similarly, DNA encoding proteins with antimicrobial potential e.g. single domain antibodies, cyanovirin and derived from human, animal, plant or algae sources will be used for expression of the gene product in heterologous hosts.

3. DNA encoding partial or complete HIV-1 genome to be used for the production of pseudovirus (non-replicating) or intact HIV-1.

The aims are to identify genes that contribute to virulence of mucosal pathogens and to express proteins that may be used for treatment or prevention of mucosal infections.

Evaluation of foreseeable effects

The microorganisms that will be modified are part of the normal flora to be found at mucosal surfaces in healthy individuals. Mutagenised microorganisms, Streptococcus spp., T. Forsythensis, F. nucleatum, C. albicans show or are expected to show loss of function compared with wild type organisms. Streptococcus pneumoniae is a class 2 pathogen and work with virulent strains (i.e. TIGR4 derivatives) will be carried out in a microbiological safety cabinet.

Engineered Streptococcus gordonii and Lactobacillus strains may have comparable colonizing activity to wild type strains but cannot transfer antibiotic resistance since the genes are stably integrated into the chromosome of the modified host and cannot be mobilised. To reduce exposure and risk for the researcher to negligible levels, liquid cultures will be handled in a microbiological safety cabinet.

HIV-1 pseudovirus is non-replicating. Intact HIV-1 is infectious and therefore work will be carried out in microbiological safety cabinets in the class 3 laboratory.

In all these studies, genetically modified material will be destroyed on site by autoclaving.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

SOP for use of autoclave external to CL3 laboratory:

All material for disposal will be autoclaved outside of the Cat 3 facility on a cycle that reaches 126°C. Autoclave bins with properly fitted, locking lids containing the waste will be taken from the CL3 laboratory to the autoclave immediately prior to autoclaving. The following procedures will be followed:

i) two trained staff must be available to carry out the procedure

ii) the autoclave room must be available for use.

iii) one member of staff will act to open doors; one will move the autoclave bins from the CL3 facility onto the transport trolley.

iv) the autoclave bins will be sprayed with 70% alcohol before being removed from the CL3 facility and placed on a tray on a trolley outside the CL3 laboratory. Gloves used for transferring the bins must be placed on top of the closed bins

v) autoclave bins from the CL3 laboratory will be taken directly to the autoclave and placed in the autoclave, the lids are then unlocked & turned over to allow steam penetration
when all autoclave bins have been loaded into the autoclave, the tray will also be placed in the autoclave and the autoclave cycle will be started.

at the end of the cycle, the print-out should be kept as a record of adequate sterilisation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from GMM cultures will be aspirated and treated with Haztabs (1 tablet/litre = 2,500 ppm chlorine) for 12 hours. Concentrated solutions will be made freshly on the day of use according to manufacturer's instructions. Inactivated culture supernatants will be discharged to the drainage system. Solid waste will be double bagged, sealed and placed in a closed metal container. Waste will then be autoclaved in the closed metal container at 136°C for 30 minutes. This will result in complete inactivation of GMMs. Autoclave tape will be used to indicate that the material has been autoclaved. Autoclave function is monitored by services performed at 3 month intervals by Health Services Associates. Autoclaved waste will be placed in yellow bags for off-site incineration by White Rose.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Local GMSC approved the Risk Assessment for this project on and signed off the component activities on 11th March 2006 and 11th July 2006

The class of activity was 2.

The Risk Assessments relevant to this revision were approved, subject to minor changes, by the Dental Institute GMSC on 7th July 2009.

HIV pseudotype virus production was assessed as a class 2 activity and Production of Replication competent HIV stocks was assessed as a class 3 activity.

Project Containment

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<th>Laboratory Activities</th>
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<td>L2 Yes</td>
<td>L3 Yes</td>
<td>L4</td>
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<td>L2 L3 L4</td>
<td>L2 L4 L2 L3</td>
<td>L4 L2 L3 L4</td>
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</tbody>
</table>

Animal Units

| L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 |

Project Ref 543/94.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 543/94.2

Date Ackn’d 29/09/1994
CU2 Project Title
INVESTIGATION OF CELLULAR ADHESION, POSSIBILITY OF GENE THERAPY FOR TREATMENT OF CANCER AND CELL IMMORTALISATION

Date Project Ceased 10/02/2003
Class 2
CultureVolClass2
Class CultureVolumeClass3-4
Consent Granted not applicable
Project notified under transitional arrangements Y

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 543/97.1

OVER EXPRESSION OF GLUTOMINE FRUCTOSE-6-PHOSPHATE AMINOTRANFERASE IN HUMAN MESANGIAL CELLS: A POTENTIAL MECHANISM OF MESANGIAL CELL INJURY

Date Ackn'd 30/07/1997
Date Project Ceased 08/09/2003

Consent Granted not applicable
Project notified under transitional arrangements Y

Historical Significant Changes
Historical Date of Additional Info 02/03/2000

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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</table>

Project Ref 543/99.1

Date Ackn'd 05/05/1999

CU2 Project Title OVEREXPRESSION OF PC-1 (WILD & MUTATED FORM) IN HUMAN MESANGIAL

Class 2

CultureVolClass2 CultureVolClass3-4
**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**
- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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### Project Ref 543/99.2

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<tr>
<td>14/05/1999</td>
<td>TOLERANCE TO ALLOGRAFTS USING RECIPIENT BONE MARROW CELLS TRANSUCED WITH DONOR MHC GENES</td>
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<tr>
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<tr>
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Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project notified under transitional arrangements Y

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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</thead>
</table>

02/03/2022
## Project Ref 543/99.3

**Date Ackn'd**: 22/05/1995  
**CU2 Project Title**: A COMPARISON OF ADENO AND HERPES BASED VIRAL VECTORS FOR GENE DELIVERY TO THE MYOCARDIUM  
**Date Project Ceased**: 08/09/2003  
**Class**: Class 2  
**Culture Volume Class 2**:  
**Culture Volume Class 3-4**:  
**Non-GMM**: not applicable  
**Consent Granted**:  
**Project notified under transitional arrangements**: Y  
**Withdrawn**: N  
**Tick if notifying a connected programme of work**: N  
**Historical Significant Changes**:  
**Historical Date of Additional Info**: 30/09/99, 18/12/1995, 17/03/1998, 08/04/1999  
**Significant Change ID**:  
**Date of Significant Change**: 

### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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<tr>
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<td>Human Clinical Applications</td>
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Project Ref 543/trans1

Date Ackn'd 12/04/2001

CU2 Project Title EXPRESSION OF VIRAL OPEN READING FRAMES PLASMIDS FOR PROTEIN

Class 2

CultureVolClass2 ClassVolumeClass3-4
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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GM Centre Number: 546

Data Premises Notified (Originally) 21/01/1994

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed 12/02/2003

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

CHIROTECH TECHNOLOGY LTD

Department

HOLME PILOT PLANT

Campus Estate or Research Centre

Building

Road Name

HOLMEWOOD HALL

District

HOLME

Town

PETERBOROUGH

County

CAMBRIDGESHIRE

Postcode

PE7 3PG

Country

ENGLAND

Tel Number 01487 832405

Fax Number 01487 832464

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

<table>
<thead>
<tr>
<th>Date</th>
<th>Premises Closed</th>
<th>Name</th>
<th>Department</th>
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<th>Building</th>
<th>Road Name</th>
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<th>Town</th>
<th>County</th>
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<td>HOLMEWOOD HALL</td>
<td>HOLME PILOT PLANT</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify)  
Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
Maximum culture volume = 500 litres.
Waste deactivated using Virkon or NaOH. Waste deactivated monitored using flasks of culture media to which waste sample is added - checked for growth of culture organism.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 547

Data Premises Notified (Originally) 11/01/1994

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

FIRS LABORATORIES, RSR LTD

Name 2

Department

Campus Estate or Research Centre

PARC TY GLAS

Road Name

CARDIFF BUSINESS PARK

District

LLANISHEN

Town

CARDIFF

County

CITY OF CARDIFF

Postcode

CF4 5DU

Country

WALES

Tel Number 029 2076 5550

Fax Number 029 2076 4445

E-mail

HSE Division WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022

Page 8718 of 15326
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes GM547/99.3

Historical Date of Additional Info 08/04/1999

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
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<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 1 (GMMs)</td>
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<td>Other (please specify)</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid waste is autoclaved and liquid waste is treated with a chlorine releasing commercially available tablet, (Haztab).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
<tr>
<th><strong>Data Premises Notified</strong></th>
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**Name**

ROYAL BROMPTON & HAREFIELD NHS TRUST

**Name 2**

HAREFIELD HOSPITAL

**Campus Estate or Research Centre**

HEART SCIENCE CENTRE

**Road Name**

HILL END ROAD

**Town**

HAREFIELD

**County**

MIDDLESEX

**Postcode**

UB9 6JH

**Country**

ENGLAND

**Tel Number**

01895 828 726

**Fax Number**

01895 828 900

**E-mail**

**HSE Division**

LONDON

**Comments**

GM 549 MERGED WITH GM 309 ON 19/3/2004

**Date at Which Additional Info Submitted**

19/03/2004
## Premises Addresses

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Other (please specify)  

Tick if confidential

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<tr>
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<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Liquid waste (maximum culture volumes of 500 ml) will be chemically treated with an appropriate disinfectant (Hycolin, Chloros or Virkon), used at the manufacturers specified concentration for the appropriate length of time, before disposing of via the drainage system.

Solid waste will be placed into yellow, printed clinical waste bags meeting BS6642 and BS381C or sharps containers conforming to BS7320. Waste bags/bins will be secured using numbered ties and transported by designated staff to a registered on site facility for incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Name

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<tr>
<td>Name 2</td>
<td>WELLCOME TRUST SANGER INSTITUTE</td>
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Department

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Campus Estate or Research Centre

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District

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Town

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County

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Postcode

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Country

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Fax Number

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E-mail

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HSE Division

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Comments

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Date at Which Additional Info Submitted

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<th>02/03/2022</th>
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Page 8729 of 15326
Premises Addresses

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Premises Conditions

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02/03/2022
Contaminated dry waste is placed in suitable containers or bags and collected by an independent contractor for incineration. For the purposes of laboratory hygiene, some dry waste is disinfected using commercial disinfectants (Virkon, Tegodyne) according to manufacturers' recommendations prior to incineration.

Agar plates and semi-dry waste is collected in leak-proof containers and autoclaved prior to collection by an independent contractor for incineration.

Liquid waste is inactivated using commercial disinfectants (Virkon, Tegodyne) according to manufacturers' recommendations and disposed in laboratory sinks.

The maximum culture volume that could be released at any one time is 20 litres.

Validation of incineration is through the authorisation certification of the contractor.

Validation of autoclaving is through an independent annual validation and monitoring of fault condition indicators.

Validation of inactivation of liquid waste is through data supplied by the manufacturer.
TRANSCRIPTIONAL CHANGES ASSOCIATED WITH THE KNOCKDOWN OF GENE FUNCTION IN HAEMopoietic CELL LINES USING RETROVIRAL-BASED RNA INTERFERENCE (RNAI)

We will generate double-stranded oligonucleotides for genes involved in transcriptional regulation of the blood lineages including a number of known transcription factors which control cell division, growth and proliferation. We will insert these oligonucleotides into an RNAi compatible retroviral expression vector and transfect a human cell line K-562. We will monitor the suppression of target gene activity to identify downstream transcription changes using cDNA expression microarrays. In this way, we will be able to generate information which relate to the transcriptional networks which regulate the development of the blood lineages from stem cells.

The human K-562 cell line is a myelogenous leukaemia cell line established in 1970 (Univ. of Tennessee, U.S.A.) from a pleural effusion of a patient with chronic myeloid leukemia (CML) and has been characterised as a highly undifferentiated cell of the granulocytic type.

Retroviral vector pMSCVpuro (ClonTech cat. No. K1062-1) is based on the murine stem cell virus (MSCV), an amphotropic retrovirus deleted for all the gene products required for replication. The basic retroviral backbone used is derived from the Murine Embryonic Stem Cell Virus (MESV) and the LN retroviral vectors MoMuLV. Upon transfection into a packaging cell line, pMSCVpuro transiently expresses or intergrates and stably expresses, a transcript containing the extended viral packaging signal w the puromycin.
resistance gene, and a gene or oligonucleotide of interest inserted into multiple cloning sites. The vectors achieve stable, high-level gene expression in haemopoietic and embryonic stem cells through a specifically designed 5’ long terminal repeat (LTR). The 5’LTR is from the murine stem cell PCMV virus, and it differs from the MoMuLV LTR used in other retroviral vectors by several point mutations and a deletion. These changes enhance transcriptional activation and prevent transcriptional suppression in embryonic stem and embryonal carcinoma cells. The murine phosphoglycerate kinase (PGK) promoter controls expression of the puromycin resistance gene for antibiotic selection in eukaryotic cells. pMSCVpuro also contains the pUC origin of replication and E. coli Amp gene for propagation and antibiotic selection into bacterial hosts DH5a and HB101. These markers do not have deleterious effects on cells which carry them. pMSCVpuro will be transfected into a packaging RetoPack PT67 Cell line (Clontech cat. No K1060-D). Once in the cell, RNA from the vector is packaged into infectious, replication-incompetent retroviral particles. pMSCVpuro does not contain the gag, pol, and env structural genes necessary for particle formation and replication: these genes are stably intergrated into the PT67 genome. The intergration of these structural genes into the packaging cell lines minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

Origin & function

The human K-562 cell line ia a myelogenous leukemia cell line established in 1970 (Univ of Tennessee, U.S.A.) from apleural effusion of a patient with chronic myeloid leukemia(CML).
Retroviral vector pMSCVpuro and RetroPack PT67 packaging cell line are from a commercial company BD Biosciences Clontech (Palo Alto, California, USA; cat No, K1062-1 and cat. No, K1060-D respectively.) All the above mentioned genetic material are intended for scientific/medical research and have extremely limited use or survival outside of laboratory conditions.

Evaluation of foreseeable effects

The pMSCVpuro retrovirus is replication defective but will be packages with amphotropic protein. Packaging cell lines are helper virus free and the chance of the generation of replication competent retrovirus or wild type virus is low. These cell lines will have limited survival outside the laboratory culture conditions. However it is recognised that naked proviral DNA is potentially infective although this risk is negligible. It is likely that the supression of the genes of interest in the K-562 cell line using the proposed retroviral RNAi system may cause change in terms of the proliferative abilities of cells in culture (increased/decreased cell growth and division, possibly even cell death). Furthermore, there is a negligible risk of the modification of the human cell line K-562 by the retrovirus constructs rendering these cells pathogenic. There is no risk of introducing entire harmful functional genes into human target tissues. However the RNAi system employed could potentially result in suppression of endogenous gene expression of some functionally important oncogenes if introduced to the appropriate human target cells. However, this risk is very low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only GMMs are involved in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The genetically modified bacterial micro-organisms (GMMs) are being grown at small scale (typically 1ml) using good microbiological practice. Culture plates, tubes and other laboratory disposables in contact with the GMMs will be inactivated by incineration or autoclaving and cultures supernatants will be inactivated in Vikron or Tegodyne before disposal. pMSCVpuro constructs will only be transfected into packaging and K562 cell lines in tissue culture hood in a class 2 containment facility. Once pMSCVpuro constructs are converted into infective retroviral particles in packaging cell lines and are introduced into the K562 cell line, all culture flasks, tubes and other laboratory disposables and culture suprtnatants will be treated with Virkon or Tegodyne and autoclaved. Tissue culture hoods willl be cleaned with Virkon and/or Tegodynprior to and after use. Ultimately all laboratory disposables coming into contact with GMMs will be incinerated subsequently to the above forms of treatment. The above mentioned treatments will give 100% kill of all bacterial, retroviral and human cells used for experimentation. Routine monitoring procedures will include (i) assaying the ability of waste supernatants/swabs of laboratory equipment to produce viable bacterial colonies on agar plates and (ii) assaying the ability of waste supernatants/swabs of laboratory equipment to infect human cell lines with viable retroviral particles in cell culture under selective pressure. We will also monitor the presence of polymerase chain reaction (PCR). Proretroviral naked DNA will be handled using appropriate personal protective equipment including gloves and eye protection and sharps avoided where possible and care taken to avoid needlestick injuries.
The safety committee noted that suitable precautions for naked DNA had been considered. Approved as a class 2 activity 12th November 2002.

Please enter comments on the GM safety committee on the risk assessment

The safety committee noted that suitable precautions for naked DNA had been considered. Approved as a class 2 activity 12th November 2002.

Project Containment

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Project Additional Information

Purposes of the contained use

The aim is to establish the role of the putative human lung cancer tumour suppressor genes (TSGs) using conditional mouse models. The conditional mouse models will be generated by standard gene-targeting techniques in mouse embryonic stem (ES) cells (to insert loxP sites either side of the gene of interest). Recombination between the loxP sites will be achieved in vivo (using mice generated from these modified ES cells) by intranasal administration of an adenovirus expressing Cre recombinase. This will lead to ablation of the gene of interest only in lung tissues, and hence will more accurately recapitulate the sporadic nature of lung tumourigenesis in humans.

The reason for having contained usage is that the adenovirus, although replication-defective, is a pathogen of respiratory and gastrointestinal mucous and eye membranes, and may cause corneal and conjunctival damage.

Recipient or parental organism

The parental organism is an adenovirus expressing Cre recombinase, AdCreM1 (from Microbix; Cat: PD-01-43). AdCreM1 is deleted for all the gene products required for replication. AdCreM1 is a severely attenuated first-generation adenovirus (33 kb) containing a Cre expression cassette. It was constructed from the adenovirus type 5 (Ad5) deletion mutant (which contains complete deletions in the E1 and E3 portions of the adenoviral genome) by adding to the left end (via homologous recombination) the Cre expression cassette. The expression cassette contains the murine cytomegalovirus immediate-early gene promoter (MCMV), the cre open reading frame and the SV40 polyadenylation signal.

Host/vector system

The system that will be used is based on the method developed by Anton, Graham and colleagues (Anton, M. and Graham, F. L. Site-specific recombination mediated by an adenovirus vector expressing the Cre recombinase protein: a molecular switch for control of gene expression. J. Virol. 69:4600-4606, 1995). The adenovirus expressing Cre recombinase, AdCreM1 will be transfected into the packaging cell line, 293T*. Once in the cell, RNA from the vector is packaged into infectious, replication-incompetent adenoviral particles (within 7 to 12 days); the desired viral recombinants will be identified by screening individual plaques generated in a lawn of packaging cells. The recombinant viruses will be titered and then intranasally administered to mice.

* 293T cells are a highly transfectable derivative of the 293 cell line (human kidney epithelial cells transformed with adenovirus 5) into which the temperature sensitive gene for SV40 T-antigen has been inserted.

Origin & function

The function of the adenovirus is, once administered intranasally to the mouse, to infect the cells of the lung. Once inside the cell the adenovirus will produce the enzyme, Cre recombinase. This enzyme will then catalyse recombination between the loxP sites that have been inserted in the gene of interest, resulting in excision of the loxed segments of the gene and hence the ablation of its function. Thus we will see a loss of production of the protein of interest only in the lung (other body tissue will be unaffected), and determine whether such a situation leads to the development of lung cancer.

Evaluation of foreseeable effects

The effects of adenoviral infection of the mouse will be monitored in several ways:

1. The genetic background of the mice is such that at the ubiquitously-expressed Rosa-26 locus, they have a LacZ gene which has the promoter sequences separated from the coding sequences by a loxP-flanked STOP site. Thus exposure to Cre recombinase will excise the STOP signal and induce expression of the lacZ gene, which encodes the enzyme, B-galactosidase. Upon exposure to substrate, B-galactosidase produces a blue coloured strain. Thus in the initial stages, mice will be sacrificed at weekly intervals and tissue sections exposed to the substrate to confirm that the adenovirus has only infected the lung (ie blue staining will only appear in lung tissue, and no other body organ).

2. Mice that have been administered the adenovirus will be monitored daily for signs of ill health or discomfort by the staff at the animal house (and the visiting Vet). However, this adenoviral Cre recombinase system has been widely used for many years, and there are no reports in the scientific literature of any side-effects.
Mice that have been administered the adenovirus will also be routinely monitored for signs of distress/discomfort due to the formation of lung tumours and will be sacrificed if it is deemed they are suffering in any way.

Packaging cell lines are helper virus free and the chance of the generation of replication competent retrovirus or wild-type virus is low. The cell lines will have limited survival outside of laboratory culture conditions. There is negligible risk of the modification of these cell lines by the adenovirus constructs rendering these cells pathogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Signs and labels will be placed to indicate each area where adenovirus is used or stored (including Biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.).

When administering the adenovirus, the mice will be anaesthetised and instilled intranasally with 10-100 uL sterile phosphate-buffered saline (PBS) containing adenoviral particles at a viral dose of up to 1x10^8 pfu per mouse. The PBS will be administered drop-wise (20-25 uL over 15 sec) into the noses of mice using a regular pipetteman (0-200 uL) with the appropriate pipette tip. The pipette tips will be filtered so as to prevent aerosol formation. This entire procedure will be performed in a Class 2 Hood.

The mice will be housed in individually-ventilated cages (IVCs), in which the air entering and exiting the case is sterile-filtered. Since infected animals can excrete adenovirus (especially in the first 72 hours after infection), the changing of the food, water and bedding will be performed in Class 2 Hoods. All laboratory and animal staff coming into contact with the adenovirally-infected mice will wear protective clothing, including lab coats, masks, goggles, gloves, special training will be given to all animal husbandry personnel on adenovirus, the hazards associated with the work, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The most effective adenoviral germicides (with a minimum 15 min. contact time) are 1% sodium hypochlorite for bench surfaces and liquid wastes; and autoclaving for 1 hour at 121 degrees C or 250 degrees F (15 lbs per square inch of steam pressure) for plasticware, animal bedding and gloves, etc.

The pAdCreM1 vector will only be transfected into packaging cell lines in a tissue culture hood in a Class 2 containment facility. All culture flasks, tubes, other laboratory disposables and culture supernatants will be treated with 1% sodium hypochlorite. The plastic ware will subsequently be autoclaved before disposal.

Once the pAdCreM1 construct has been converted into infective adenoviral particles in packaging cell lines, it will be administered intranasally to mice (in a tissue culture hood in a Class 2 containment facility at the animal house) [this procedure is detailed in our Home Office-approved Project Licence]. The mice will then be returned to their individually ventilated cages, and the tissue culture hoods will be cleaned with 1% sodium hypochlorite after use. All case bedding from the adenovirally-treated mice will be handled in a Class 2 hood and disposed of by incineration. Any mice that die or are sacrificed, will also be incinerated.

The monitoring of adenoviral infection of mouse tissues will be examined by B-galactosidase staining (as detailed in Section 7: Evaluation of foreseeable effects).

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

02/03/2022
After review of the risk assessment, the investigator was asked whether a later, more completely disabled variant of AdCreM1 could be substituted for the intended purpose thus reducing the possibility of homologous recombination with wild type. The investigator replied that later variants that also expressed cre are not available.

The risk assessment was approved as a Class 2 activity requiring HSE notification on 9 January 2002.

### Project Containment

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### Project Ref 552/04.1

<table>
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<td>Inactivation of specific genes, complementation and expression of non-toxic reporter molecules in enteric bacteria.</td>
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<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<td>Class 2</td>
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<td>Non-GMM</td>
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### Project Additional Information
Purposes of the contained use

We will use classical gene replacement technology, based on PCR or suicide vector mediated gene conversion, to generate specific mutations in specific genes encoded within the genomes of selected enteric bacteria. Also we will introduce genes encoding non-toxic reporter molecules including Green Fluorescent Protein, Luciferase, Chloramphenicol acetyl transferase, beta-galactosidase and non-toxic vaccine antigens into different enteric bacteria. These reporter genes will be expressed from either constitutive or regulated bacterial promoters. The aim of the work is to use the reporter gene products to (1) track bacteria to different cells or sub-cellular locations within eucaryotic hosts; (2) to detect the activity of different promoters under different conditions.

Recipient or parental organism

The reporter genes will be supplied from existing plasmids as cloned DNA sequences or purchased from commercial suppliers. They will be introduced into any enteric bacteria other than those classified as Class III or above. Examples will be Escherichia coli (other than verotoxigenic forms), Shigella spp. Salmonella enterica (other than class III agents such as S. Typhi and S Paratyphi), Citrobacter spp etc.

Host/vector system

The host bacteria will be any enteric bacteria. The genes will either be introduced directly into the chromosome of the bacteria (via suicide vectors or PCR methodologies). Alternatively the genes will be cloned onto non-transferable plasmids such as those based on ColE1 replicons or pAYC184 (pUC plasmids for example).

Origin & function

The genetic material involved is from a variety of sources such as jelly fish (Green Fluorescent Protein), bacterial (beta-galactosidase, Luciferase, chloramphenicol acetyl transferase) but will not be cloned from these sources. We will use well-defined existing plasmids or genes from commercial sources. The DNA is simply to be used to report gene expression activity in enteric bacteria under different conditions.

Evaluation of foreseeable effects

The genes we have selected are all extremely well characterised and have been used without incident in thousands of research laboratories around the world. We will only use the named reporters associated with this application. If we decide to use any novel reporters we will amend the local risk assessment. To our knowledge there have been no foreseeable effects of these reporters other than high level expression could cause toxicity to the host bacteria (as with over expression of many proteins).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No applicable other than to disabled S. Typhi derivatives specifically derogated to CL2.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the experiments will be performed in a well maintained CL2 facility either in the laboratories or the in vivo facility at The Sanger Institute. A copy of the protocol for handling enteric pathogens in these facilities is enclosed for inspection and has been approved by the local safety committee. Briefly we have detailed policies for handling contaminated liquid and solid waste that involved the contained removal of materials for autoclaving. Hence, all contaminated material is autoclaved or inactivated using chemical disinfection procedures using a variety of accepted and validated disinfectants including phenolics (hycolin etc), Virkon S or chloros. All disinfectants are made up and utilised as described in the enclosed protocol. We routinely validate inactivation using a viable count method based on the killing of control cultures of bacteria within accepted ranges. All waste material is removed using clinical waste disposal methodologies in place at The Sanger Institute.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Project Containment

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Project Ref 552/04.2

Date Ackn'd: 08/07/2004

CU2 Project Title: Disabled derivatives of salmonella enterica serovar typhi

Class: Class 2

CultureVolumeClass2: 1-50 Litres

Non-GMM Consent Granted: Not Applicable

Project notified under transitional arrangements: No

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

The aim of the use is to study S. Typhi using a safer attenuated derivative suitable for use at CL2. This will facilitate further examination of (a) the vaccine potential of these derivatives (b) the mechanisms of pathogenesis of S. Typhi.

Recipient or parental organism

Both S. Typhi BRD948/CVD908-htrA (aroC aroD htrA) and ZH9 (aroA aroC ssaV) or similar derivatives have been extensively studied in human volunteers and have been shown so far to be safe and highly attenuated vaccine candidates. ZH9 is through phase II clinical study and is being prepared for an efficacy study in phase III in the field. Both BRD948 and ZH9 harbour at least three independently, non-reverting attenuating mutations. Both strains fail to colonise the human host and, as S. Typhi, are unable to colonise animals or plants. Both are base on the well characterised S. Typhi strain Ty2 which has been recently fully sequenced. Both strains harbour deletion mutations that are fully sequenced and inactivating. Both strains have been shown to be attenuated in mice as well as humans.


Host/vector system

Both BRD948 and ZH9 are fully antibiotic sensitive and harbour no foreign DNA.

Origin & function

All DNA is derived from S. Typhi Ty2 with no foreign DNA. The derivatives harbour specific DNA deletions intended to stably attenuate.

Evaluation of foreseeable effects

These derivatives have been shown to be highly attenuated in many scores of human volunteers and similar mutations attenuate S. Typhimurium in mice and other species. We see no reason why these organisms should loose there attenuated phenotype as the genetic lesions have been well characterised in multiple in vivo and in vitro systems. The derivatives have been used by different groups in the UK for many years safely at CL2. For example, BRD948 has originally recognised by HSE as disabled in 1993.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The microorganisms will be derogated to CL2 as they are unable to colonise the human host and are highly attenuated but are derived from S. Typhi. The derivatives now have an excellent safety record of being safely handled at CL2 with no evidence of human infection both in the UK and abroad. The microorganism will be handled at CL2 or a higher level of containment at all times.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

We operate a number of comprehensive and proven waste management measures which are conducted according to our local protocol for handling CL2 pathogens. This protocol has been in use for many years and involves autoclaving of clinical waste material, chemical inactivation of liquid waste followed by autoclaving and careful management of waste movement. The GMM will be used within a containment level 2 laboratory in which all bacterial cultures, glassware etc. is decontaminated prior to disposal. Thus drains, sinks etc. do not pose a mode of transmission to the environment. Air movement is also strictly regulated in the laboratory environment. Solid biological waste is autoclaved using temperatures, cycles and conditions appropriate for the inactivation of biological material. Autoclaving is performed by departmental
staff using approved conditions. Temperature is 134°C for 3 minutes per cycle. Glassware etc. is decontaminated, as described above, prior to being autoclaved.

The committee agreed with the classification and containment level assigned to this project.

Please enter comments on the GM safety committee on the risk assessment

The committee agreed with the classification and containment level assigned to this project.

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Project Ref 552/07.1

Date Ackn'd

23/10/2007

CU2 Project Title

The role of different capsules of Streptococcus pneumoniae in disease.

Date Project Ceased

Class

Class 2

Culture Vol Class 2

< 1 Litre

Consent Granted

Not Applicable

Project notified under transitional arrangements

N

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
Project Additional Information

**Purposes of the contained use**

This project will investigate the molecular and cellular basis of disease caused by the hazard group 2 respiratory pathogen *S. pneumoniae*. The basic approach is to identify and characterise capsular genetic loci with possible importance in the disease process. We will use a non-capsular *S. pneumoniae* TTGR4 isolate as recipient to generate a range of recombinant derivatives with the specific aim of expressing these distinct capsular genes/loci independently in one background strain only. Previous studies have involved methods where the different capsules are expressed in a variety of background strains; this has made direct comparisons of virulence or capsule expression difficult to analyse. This is because with a non-isogenic background the role of other pathogenic factors encoded on the chromosomes could not be ruled out as affecting the conclusions of any studies.

**Recipient or parental organism**

*Streptococcus pneumoniae* TIGR4 non-capsular mutant strain (and related non-encapsulated mutants) obtained from Tim Mitchell (Univ. Glasgow).

**Host/vector system**

*Streptococcus pneumoniae* chromosomal DNA will be supplied to us by Lotte Larnbertsen of the Statens Serum Institut, Copenhagen, Demark. Genes involved in capsule biosynthesis will be amplified from isolated chromosomal DNA from capsulated *S. pneumoniae* by PCR. Purified PCR products will be used to directly transform non-capsular naturally competent *S. pneumoniae* TIGR4. As the genes targeted for amplification by PCR will only be those involved with capsule biosynthesis no antibiotic resistance markers or non-*S. pneumoniae* DNA is transferred.

**Origin & function**

*Wild-type Streptococcus pneumoniae* is classified by ACDP as HG2. It can colonise the human respiratory tract and sometimes cause disease. The antibiotics of choice for treatment are Penicillins. The recipient strain, *Streptococcus pneumoniae* TIGR4, is penicillin sensitive. The donor DNA will not carry any antibiotic resistance markers. TIGR4 is also sensitive to erythromycin so this antibiotic would be a suitable treatment for workers sensitive to penicillin. Vaccines are available but they do not cover all capsular types and hence all *S. pneumoniae* strains.

Infectivity of *S. pneumoniae* TIGR4 compared to wild-type is greatly reduced due to this loss of capsule. Any recipient strains expressing capsule may regain a degree of virulence but virulence would not be enhanced compared to capsulated wild-type strain.

The aim of the experiment is to insert native genes in situ on the chromosome and no intermediate cloning steps or conjugative plasmids are required. Thus, it is unlikely that genes will be transferred to inadvertent recipients. Theoretically DNA could be exchanged by natural transformation.

The capsulated form of *S. pneumoniae* can elicit infections in some individuals. The capsules themselves are non-toxic. Researchers in the study will not be knowingly immune deficient, suffering from lung disorders etc. which could cause them to be at a greater risk from infection. An immune response mounted against *S. pneumoniae* TIGR4 recipient expressing capsule operons is not expected to be greater than that mounted against wild-type capsulated forms that exist as commensals in the general population. Immunity can be capsule-specific although non-capsular antigens can contribute to protection.

**Evaluation of foreseeable effects**

Wild-type *Streptococcus pneumoniae* is classified by ACDP as HG2. It can colonise the human respiratory tract and sometimes cause disease. The antibiotics of choice for treatment are Penicillins. The recipient strain, *Streptococcus pneumoniae* TIGR4, is penicillin sensitive. The donor DNA will not carry any antibiotic resistance markers.

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**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only 0MM will be utilised in this study.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A
Genetically modified S. pneumoniae TIGR4 will be grown on a small lab scale of up to 25 rids and will be handled and inoculated using aseptic technique and good microbiological practice. All lab disposables that come into contact with GMM will be chemically inactivated with Virkon (as per manufacturer’s protocol) before disposal by incineration. All contaminated glassware will be inactivated by autoclaving. Bench areas used will be cleaned using 1% Virkon (as per manufacturer’s protocol) before and after use. Autoclave facilities have undergone 12 point validation and give 100% kill. Chemical inactivation using 1% Virkon using manufacturer’s protocol will give 100% kill of bacteria.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Following review of the risk assessment the GMSC agreed that the investigator had satisfied all required criteria for a CL2 GMM activity. The few suggestions to changes in protocol that were made by the GMSC (for example, investigator initially proposed using crude chromosomal extract to introduce genes into non-capsulated S. pneumoniae. The GMSC suggested the use of purified PCR products, rather than use crude chromosomal extract, in order to avoid the risk of introduction of unknown genetic material and help maintain isogenicity of host strain. Following discussion with the investigator, where deemed relevant to reduce the risk of the investigation, these suggestions were incorporated into the attached risk assessment.

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**Project Ref** 552/07.2

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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
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<tr>
<td>26/10/2007</td>
<td>Assessment of the virulence of ACDP hazard group II enteric pathogens in vivo.</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td></td>
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</tbody>
</table>

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The aim of these experiments is to characterise the genetic factors expressed by *Clostridium difficile* that are associated with enteric infection or intestinal colonisation. Disease associated with *C. difficile* infections are normally acquired by oral infection. This work may lead to the development of new antibiotics or vaccines. Functional analysis of virulence-associated factors requires that their genes be mutated and the phenotype characterised. It is also essential to confirm that any reduction in virulence is indeed due to the mutation. This is done by reintroducing the wild type gene on a plasmid and confirming restoration of the wild type phenotype in the complemented strain. *E. coli* K12 routinely acts as an intermediate host for manipulating cloned DNA prior to reintroduction into the recipient pathogen. Antibiotic resistant genes and other non-toxic biological reporter genes (LacZ, CAT, GFP, Lux etc) can be introduced into the recipient during manipulation. The OMOs are, however, sensitive to the antibiotics recommended for therapy of infection.

**Recipient or parental organism**

*Clostridium difficile* strains 630 (as well as erythromycin sensitive variant), DI-1, CF5, CD196 and SM027 will be obtained from Prof. Brendan Wren (London School of Hygiene and Tropical Medicine).

**Host/vector system**

*Host bacteria*

*K-l2 derived E. coli* (for the purposes of this project will be used only under containment level 2 requirements).

*Clostridium difficile* strains 630, BI-l, CF5, CD196 and SM027

**Vectors**

- pMTL54O1F (AmpR, Mob, Tra-, Bom-, shuttle vector that can replicate in *B. coli* and *C. difficile*)
- pMTL54O2F1acZTTEEmIBtdRAM1 (AmpR, Enn’, Mob, Tra-, Bom- shuttle vector that can replicate in *B. coli* and *C. difficile*)
- pMTLOO7 (Cm’, Enn’, Mob’, Tra-, Bom- shuttle vector that can replicate in *B. coli* and *C. difficile*)
- pçMT, (Amp’, Mob, Tra-, Bom-, *E. coli* cloning vector)

**Origin & function**

The aim of the experiment is to inactivate target genes in *C. difficile*, the origin of the required genetic material will be *C. difficile*. Target genetic material will be cloned initially into *B. coli* K12 in order to produce the quantity of genetic material (vector plus insert) required for successful transfer and integration into *C. difficile*. Target genes introduced into *B. coli* K12 are unlikely to cause harm when removed from the normal environment of their natural host bacteria. However, it should be recognised that...
deliberate large scale production of some of the cloned proteins could be hazardous but this is not the aim of these experiments. Thus, the risk is negligible. It is not likely that an allergic response could be induced.

### Evaluation of foreseeable effects

**C. difficile** is classified by the ACDP as a hazard group 2 pathogen; it is able to cause disease in humans and domestic animals. In this work, inserted DNA will normally come from **C. difficile** with the exception of the non-toxic biological reporter genes. Inserted genes can encode potential virulence genes but during the construction of the GMMs virulence genes may be inactivated and attenuated variants generated. It is unlikely that the manipulation procedures will enhance the virulence of any of the GMMs to a higher level than that of the donor, as a consequence all work will carried out at containment level 2. Although the eventual aim of the experiment is to integrate genes (inactivation) onto the chromosome, inserted DNA will also be harboured on plasmids. However, such plasmids will be non-conjugative and consequently they will be unlikely to transfer on to unintended recipient bacteria.

Target genes will be cloned initially into E. coli 112. Genes and gene products are unlikely to cause harm out of the context of their natural host bacteria, **C. difficile**. However, it should be recognised that deliberate large scale production of some of the cloned proteins could be hazardous but this is not the aim of these experiments or within the remit of this assessment.

Although antibiotic resistance genes such as erythromycin will be used in the experiments as markers genetic transfer and gene inactivation, the GMMs will be sensitive to appropriate therapeutic antibiotics, such as vancomycin and metronidazole. Under certain conditions, **C. difficile** is able to form spores. These are resistant to certain traditional methods of inactivation, such as 70% ethanol. Spores, however, are sensitive to 1% Virkon, this will be used routinely for inactivation.

As **C. difficile** is classified as a hazard group 2 pathogen, it would be advisable for anyone knowingly immunosuppressed not to undertake this work. All individuals involved with these experiments will be expected to register with the Sanger Institute occupational Health Department and complete a pre employment health surveillance questionnaire.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only 0MM will be utilised in this study.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Not applicable**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Genetically modified **C. difficile** and E. coli K12 will be manipulated, cultured and handled by trained staff, experienced at working at CL2. Cultures will be handled and inoculated using aseptic technique and good microbiological practice.

All lab disposables that come into contact with 0MM will be chemically inactivated with Virkon (as per manufacturer’s protocol). Where the manufactures supplied data on inactivation is not applicable for intended use, viable counts of microorganism will be taken after exposure to working dilutions of Virkon to assess the percentage kill.

All contaminated glassware will be inactivated by autoclaving.

Bench areas used will be cleaned using 1% Virkon (as per manufacturer’s protocol) before and after use.

Autoclave facilities have undergone 12 point validation and give 100% kill. Chemical inactivation using 1% Virkon using manufacturer’s protocol will give 100% kill of bacteria.

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**Is an emergency plan required according to regulation 20?**

- [N] No

**If yes, tick to confirm that it is attached to this form**

- [N] No

**Tick to confirm that you have attached a risk assessment to this form**

- [Y] Yes

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [N] No

---

02/03/2022
Following review of the risk assessment, the GMSC agreed that the investigator had satisfied all required criteria for a CL2 0MM activity. Any suggested changes to risk assessment made by the GMSC were discussed with the investigator, where deemed relevant in reducing potential risk of the investigation, these suggestions were incorporated into the attached risk assessment.

**Project Containment**

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2 Yes</td>
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</table>

**Project Ref** 552/08.1

- **Date Ackn'd**: 06/10/2008
- **CU2 Project Title**: Plasmodium falciparum - erythrocyte interactions: GM Plasmodium falciparum parasites
- **Class**: Class 3
- **Culture**: 100ml
- **Consent Granted**: Being Processed
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**: 552/08.1a
- **Date of Significant Change**: 17/02/2010

**Project Additional Information**

- **Purposes of the contained use**: Genetically modified Plasmodium falciparum parasites will be generated in order to understand the molecular mechanisms of the parasite-erythrocyte interactions that play a major part in malaria pathogenesis and disease. A better understanding of these critical interactions may lead directly to the development of new prevention and control strategies.
measures such as new drugs and/or vaccines.

Receptor or parental organism

Plasmodium falciparum

Host/vector system

Plasmodium falciparum transfection vectors are not commercially available. Vectors are available from American type Culture connection (ww.mr4.org) and vectors to be used in this study will be obtained from this source, or from research colleagues.

Origin & function

Inserts:
Plasmodium falciparum merozoite proteins, including invasion ligands (EBL family, RBL family, AMA1), merozoite surface proteins (MSPs), invasion proteins (MyoA, MTIP, GAP45, GAP50) and components of the post-translational modification apparatus such as kinases and palmitoyl transferases that are involved in regulating erythrocyte invasion.

Constructs containing modified P.falciparum genes or fragments of such genes will be introduced into P.falciparum strains by transformation and selected for using anti-microbials. Constructs will either be maintained as episomes, or will integrate in a targeted manner into the P.falciparum genome. The effect of genetic modification will be assayed using standard cell biology (growth rate, microscopy, invasion assays) or biochemical (immunoprecipitation protein detection) techniques. By experimentally modifying specific P.falciparum genes, whether by tagging, gene ablation, or allele exchange, we will be able to generate and test hypotheses about the role of these genes in erythrocyte invasion and other parasite-erythrocyte interactions.

Evaluation of foreseeable effects

Potential source of additional risk in the generation of GM P. falciparum parasites is the introduction of drug resistance cassettes to P. falciparum strains, producing parasites that are resistant to one or more anti-microbial drugs, either anti-folates (pyrimethamine or derivatives such as WR99210), gentamicin or blasticidin. However, the impact of this is minimized by the fact that none of these drugs are in widespread use for P. falciparum control, and all strains generated will still be susceptible to more than one anti-malarial, including quinine, mefloquine and artesininin based compounds, and combinations thereof. In the extremely unlikely event of accidental worker infection (unlikely given the no sharps policy, the route of infection being only blood mediated, not aerosol mediated, and the use of CL3 techniques), GM P. falciparum parasite strains will therefore have exactly the same susceptibility profile to commonly prescribed anti-malarials as non-GM P. falciparum strains.

The other potential source of additional risk is the impact of experimental genetic modification on the virulence of the P. falciparum parasite itself. Based on past experience, it is our assessment that almost all genetic modifications planned will have if anything a negative impact on virulence. We will be modifying genes involved in the erythrocyte invasion process by tagging (GFP, epitope tags such as HA or c-myc), gene ablation or allele exchange (exchanging one natural allele variant for another or mutating specific codons in a gene to assess the effect on function). Erythrocyte recognition and invasion is essential for parasite growth and operates in a highly efficient manner in wildtype strains. Experimentally modified versions of proteins involved in erythrocyte invasion are therefore not likely to operate more efficiently than the unmodified proteins, and are much more likely to operate less efficiently. This has certainly been our past experience working with GM P. falciparum parasites in other institutions, and is the published experience of other labs — such modified lines tend to grow more slowly, and are rapidly outgrown by wildtype parasites if drug pressure is removed. We therefore think that in the majority of cases it unlikely that GM P. falciparum parasites will pose a risk or hazard to humans over and above the general risks of P. falciparum in vitro culture, and it anything the GM strains generated as part of this project are likely to be less fit than their wildtype counterparts.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We request derogation from the requirement to carry out this activity na laboratory sealable for furnigafon (as per "Biological agents: Managing the risks in laboratories and healthcare premises"; Appendix 3.2 Work with Hazard
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste in contact with live P. falciparum material is disinfected by treatment with Virkon, disposed of in biohazard bags, placed in an autoclave bin, sealed, and autoclaved prior to final disposal. Spillage containment kits will be maintained in the derogated CL3 room. MSCs will be disinfected after every use and given a final clean before shut-down each day.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

Mike Quail (08/09/08) - With the P. falciparum work they limit sharps to microscope slides. Has every alternative here (plastic/acrylic slides) been considered?
Response: The use of glass slides will only be permitted for the assessment of parasites that parasites have been inactivated by the staining process.

Christian Johnson (08/09/08) - Apart from some minor spelling corrections on part A I am quite happy for this assessment to stand as a class 3 GM activity.
Jo Butler (16/09/08) - I am completely happy with the risk assessments, though I did spot a few typos.
I think that the Class 3 assessment, GM Plasmodium falciparum parasites, is appropriately carefully argued and that all likely risks, including those from allele exchanges of erythrocyte invasion ligands, have been appropriately considered.
No other comments were received from the GMSC.

Project Containment

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Project Ref 552/09.1

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<tbody>
<tr>
<td>08/07/2009</td>
<td>The Pseudotyped lentiviral and retroviral infection of mouse embryonic stem (ES) and</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>
Project Additional Information

Purposes of the contained use

Analysing infection ability of different types of lentiviruses and retroviruses on the host cells.
GFP encoding lentiviral or retroviral vectors are introduced into 293 cells together with helper plasmids. The resulting supernatant, which contains virus, is used to infect target cells. Appropriate period after infection, cells are harvested for fluorescent detection and DNA, RNA or proteins are extracted.

Recipient or parental organism

Mouse embryonic stem cells: AB1, AB2.2, JM8 and their derivatives
Fibroblast cells: MEF, STO-derived cell lines.
   HEK293 and its derivatives.
   Primary mouse cells from various tissues.

Host/vector system

Vectors: 1, Lentiviral expression vector encoding enhanced green fluorescent protein (eGFP), SIN CSGW
   2, SIVmac (Simian Immunodeficiency Virus macaque) vectors (SIV-eGFP)
   3, MLV (Murine leukemia virus) vectors (MLV-eGFP)

Helper vectors: 1, pMDG (vesicular stomatitis virus envelope protein (VSV-G) expression vector),
   2, HIV-1 and HIV2 gag-pol expression vector (expressing packaging protein)
   3, SIV3+ (SIVmac gag-pol expression vector)
   4, pCIG3 N or B (MLV gag-pol expression vector)

Other viral vectors: FIV (Feline immunodeficiency virus and EIAV (Equine Infectious Anemia Virus)

Origin & function

Human or Mouse retrovirus which are self-inactive and infectable to mammalian cells

Evaluation of foreseeable effects

This viral system can infect most mammalian cells, but they are self-inactive, and have less risk to survive out of lab.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For liquid waste, decontamination will be carried out by addition of Virkon to waste solutions, which will then be stored overnight at room temperature in the Class II MSC. The resultant inactive waste will be disposed to drain.

All solid wastes will be autoclaved using a validated cycle prior to disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 552/09.2

Date Ackn’d 16/12/2009

CU2 Project Title Mouse models and screens to understand and visualize the consequences of chromosome mis-segregation

Date Project Ceased

Class CultureVolClass CultureVolumeClass

Class 2 < 1 Litre

Non-GMM Consent Granted
Chromosomal instability (CIN) occurs in the vast majority of cancers. The goal of this project is to find genes that protect the genome against numerical chromosome instability (whole chromosome losses and gains). For this purpose we will use established human cancer cell lines and mouse ES cells to perform screens in that aim to identify such genes. Additionally, we are developing tools to visualize chromosome segregation and ongoing CIN in vivo.

Recipient or parental organism

- MCF10A human breast cells
- MBL human mammary cells
- HT1080 cancer cell line
- HEK293T human embryonic kidney cells
- Mouse embryonic stem cells AB1, AB2.2, JM8 and their derivatives.

Host/vector system

1. Retroviral vectors
   a. Based on pRetrox or pBabe (Moloney-derived).
   b. Replication incompetent due to use of co-transfection of split genome vectors (Gag-Pol, Env) in 293T cells.
   c. Co-transfected with vector expressing Amphotropic envelope (pAmpho) or Ecotropic envelope (pEco) (Clontech), for expression in human or mouse cells, respectively.
   d. Self-inactivating LTRs to minimise risk of insertional activation of host genes and unwanted expression of the insert
   e. Chimeric Tet-ON/CMV promoter to restrict gene expression to the presence of doxycycline
   f. Puromycin (or a neomycin, blasticidin or zeocin) cassette under a constitutively active PGK promoter, allowing for selection of integration of the retroviral vector, while the introduced transgene is not expressed.

2. Lentiviral vectors
   a. 3rd generation HIV-based TRIPZ system (Open Biosystems).
   b. Replication incompetent due to use of co-transfection of split genome vectors (Tat, Gag, Pol, Rev, VSV-G) in 293T cells.
   c. Self-inactivating LTRs to minimise risk of insertional activation of host genes and unwanted expression of the insert
   d. Contain WPRE element expressing fragment of WHBV protein X (unmodified)
   e. Tightly-regulated Tet-ON promoter to express shRNA sequences only in presence of doxycycline

Origin & function
Inserts

a. Ras and c-Myc: In order to predispose cells for oncogenic transformation, cells will be transduced with either conditional RasV12 or conditional c-Myc. Both are well established oncogenes. Their expression will be tightly-regulated by use of the Tet-ON promoter, and only after selection of stable clones has been performed.

b. Mitotic checkpoint and machinery proteins p31CMT2, Cdc20, Mad1, Hec1, Mps1, BubR1, Bub3, ROD, Zwilch: I am studying the effect of several point mutants of the spindle checkpoint protein Mad2 which show decreased binding to other checkpoint proteins such as p31CMT2, Cdc20 and Mad1, which will be expressed in an inducible fashion. Other proteins that will be expressed are Hec1, Mps1, BubR1, Bub3, ROD complex. The role of these proteins in the development of cancer is still obscure, none of these proteins have been identified as potent oncogenes. Germline inactivation (as far as tested) has unequivocally resulted in early embryonic lethality in mouse models.

c. shRNAs to Mad2, Mps1 and Blm: Whereas loss of Mad2 or Mps1 expression results in cell lethality, rather than oncogenic transformation, Blm deficiency has been shown to result in a five-fold increase in tumour predisposition in a mouse model. As all three genes are important guardians of the genome, their loss will result in chromosome instability and are therefore likely to be oncogenic.

d. Fluorescent protein (GFP/CFP/YFP/RFP) fusions with Histone H2B, Tubulin and Centrin 3.

e. rTTA Tetracycline repressor protein.

Evaluation of foreseeable effects

Retroviral vectors retargeted to the amphotropic receptor, and lentiviral vectors are capable of transducing human cells, and becoming integrated into the genome of the host cell as a provirus. This is theoretically capable of causing insertional activation or disruption of host genes close to the site of insertion. Inappropriate activation of potentially harmful genes is minimised by the use of vectors containing deletions in the LTR regions (so-called self-inactivating or SIN vectors).

The presence in the lentiviral vectors of an unmodified post-transcriptional regulatory element (WPRE) derived from the woodchuck hepatitis B virus (WHBV) genome gives the potential for tumorigenesis due to expression of a fragment of WHBV gene X.

The retroviral vectors will be used to deliberately express the known oncogenes Ras and c-Myc, and several other genes involved in chromosome instability, which are potentially oncogenic. Lentiviral vectors will be used to express shRNA sequences capable of knocking down the expression of other mitotic checkpoint genes, an event which has been shown to be associated with tumour predisposition.

The following have also been taken into consideration:-

The viral vectors are produced by co-transfection with split genome helper plasmids, so are replication incompetent, with an extremely low risk of recombination events leading to infective capability.

The viral vectors will be produced at low titre, and will not be concentrated, e.g. by harvesting from a density gradient.

The potentially harmful gene inserts are under the control of a tightly-regulated inducible promoter, and will only be expressed in the presence of the antibiotic doxycycline.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid cultures will be chemically inactivated by the addition of a validated disinfectant (e.g. Virkon S) and leaving for the required time (>20 minutes), before being disposed via the drains.

Contaminated solid waste (culture dishes, serological pipettes, disposable gloves, etc) will be autoclaved using a validated cycle prior to disposal.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N
I note that the RA considers the work to be Class 2, with which I fully agree. (As pointed out in the RA, they are cloning potential oncogenes into amphi retro- and lentiviral vectors, and also their lentiviral vector contains an intact WPRE.).

This work doesn't seem to pose much of a safety risk. However it is quite a complex study so class 2 seems appropriate.

Please enter comments on the GM safety committee on the risk assessment

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Large Scale Activities

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Human Clinical Applications

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Project Ref 552/10.2

Date Ackn'd: 10/11/2010

CU2 Project Title:

Lentivirus vectors encoding sgRNAs to achieve knockdown of cancer gene expression

Class: Class 2

CultureVolClass2: < 1 Litre

Consent Granted: Non-GMM

Date Project Ceased: 18/08/2016

Tick if notifying a connected programme of work: N
**Project Additional Information**

**Purposes of the contained use**

Identification of genotypes in human cancer cell lines that are associated with sensitivity to anti-cancer therapeutics. The validity of sensitizing genotypes (to anti-cancer therapeutics) in a small subset of cell lines will be determined by using a shRNAi strategy to silence gene expression and the effect on cell growth measured. We will generate lentiviral particles from hairpin-pLKO.1 plasmids in the 293T packaging cells. These will be used to transduce human cancer cell lines and specificity of knockdown assessed by western blot or Q-PCR.

**Recipient or parental organism**

Human embryonic kidney 293 T cells (for virus propagation); Human cancer cell lines displaying sensitivity to targeted anti-cancer compounds - purchased from commercial repositories (ATCC, DSMZ, HPACC, ICLC, RIKEN and JHSF).

**Host/vector system**

Replication incompetent, disabled lentivirus, pseudotyped with VSV-G protein. The lentivirus will contain pLKO.1, an HIV-based plasmid that does not contain the WPRE (Woodchuck Post-transcriptional Response Element), with an insert that will be integrated into the host cells’ genomes. The lentiviral vector contains a self-inactivating 3' LTR that renders it further unable to produce infectious virus once it integrates into the host chromosome.

**Origin & function**

Two products will be generated: -
(1) Green Fluorescent Protein, which has a long history of use with no documented adverse events associated with it
(2) Short hairpin RNA (shRNA) molecules, which are designed to be capable of reducing the expression of targeted genes in a sequence specific manner. The use of shRNA molecules targeted to genes implicated in cancer, including known tumour suppressor genes

**Evaluation of foreseeable effects**

Lentiviral vectors pseudotyped with the VSV-G envelope protein are capable of transducing multiple human celltypes (i.e. not just the CCR, CD4+ cells of the immune system that are the normal target of wildtype HIV), and becoming integrated into the genome of the host cell as a provirus. This is theoretically capable of causing insertional activation or disruption of host genes close to the site of insertion. Inappropriate activation of potentially harmful genes is minimised by the use of vectors containing deletions in the LTR regions (so-called self-inactivating or SIN vectors).

The lentiviral vector system has ben developed with enhanced safety features. It is a three plasmid system consisting of : (1) pCMV-dR8.91 - the packaging vector, which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions. (2)pCMV-VSG - the vesicular stomatitis virus G-protein envelope vector, which provides the heterologous envelope for pseudotyping, and (3) pLKO.1 - shRNA transfer vector, which contains he sequence of interest as well as the cis acting sequences necessary for production and packaging of the RNA genome. The multi-plasmid approach results in no single plasmid containing all the genes necessary to produce packaged lentivirus. It also means that the resulting lentiviral vectors are, as far as reasonably foreseeable, entirely incompetent for replication and productive infection.

Lentiviral vectors will be used to express shRNA sequences capable of knocking down the expression of genes implicated in cancer, including known tumour suppressor genes. This has unpredictable, but potentially oncogenic outcomes, even as a single event.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

02/03/2022
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid cultures will be chemically inactivated by the addition of a validated disinfectant (e.g. Virkon S) and leaving for the required time (>20 minutes), before being diluted and disposed via the drains.

Contaminated solid waste (culture dishes, serological pipettes, disposable gloves, etc) will be autoclaved using a validated cycle prior to disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was discussed at a meeting of the Biological Agents and GM Safety Committee held on 22nd September 2010.

There was one specific comment from a member of the Committee not able to attend the meeting: "I have no problem with this a Class 2. I also note the absence of WPRE elements, but even if present these would still leave it at Class 2".

It was therefore approved by the Committee as a Class 2 GM activity.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L3</td>
<td>L4</td>
<td>L2</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
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Project Ref 552/11.1

Date Ackn'd 29/06/2011

CU2 Project Title Investigating host and viral genetic variation and the effect of such variation on virus pathogenesis in vitro and in vivo

Class 2

CultureVol Class 2 < 1 Litre

CultureVolume Class 3-4

Non-GMM Consent Granted

Date Project Ceased 02/03/2022
**Project Additional Information**

**Purposes of the contained use**

This connected programme of work aims to investigate how genetic variation in the host (gene mutation, gene expression differences, gene deletion and gene duplication) and in medically important human viruses affects the host response to infection, virus virulence and pathogenesis. The viruses we will investigate are all classified by the Advisory Committee on Dangerous Pathogens (ACDP) as hazard group two organisms and will include:

1. Human herpesviruses, including Kaposi's sarcoma associated herpesviruses (KSHV), Epstein Barr Virus (EBV), and Herpes Simplex Virus (HSV-1), both wild type viruses and viruses derived from recombinant bacterial artificial chromosomes.
2. Murine herpesviruses, including murine herpesvirus 68.
3. Human orthomyxovirus, influenza viruses
4. Human paramyxovirus, measles virus.
5. Lentiviral vectors

The result of sequencing host and virus genomes is the identification of mutations that may be linked to a particular phenotype of infection. However, such association must be mechanistically investigated to determine their true biological effect. This connected programme of work will facilitate such mechanistic investigation. Specifically we will study how herpesviruses are able to infect, persist in a latent state, reactivate into a lytic cycle of virus production and in some cases immortalise certain cell types. We will study how host genes prevent or limit infection of a variety of viruses.

This connected programme will use a number of standard molecular biology/virology methods to produce viruses in some cases derived from defined genotype recombinant sources such as herpesviruses BACs, reverse genetics influenza virus and virus engineered to express non-toxic marker genes. We will monitor virus infection in vitro by over-expression or ablation through RNAi different virus and host genes and in vivo by infection of wild type and gene knockout mice. These methods will be used to determine the phenotypic outcomes of virus and host interaction.

The foreseeable hazards of transduction of the eukaryotic cells with expression vectors to overexpress viral or host proteins, or to ablate their expression by RNAi, and/or the infection of these cells with wild type or recombinant viruses, would be the production of a cell line with altered properties able to establish an altered infection profile facilitating the selection of viruses with altered pathogenicity or host range, the infection of laboratory works and subsequent transmission to other people.

In vitro work:

**Lentiviral vectors:**

This project aims to clone human or viral genes of different functions and genotypes and produce plasmid expression and lentivirus vectors for gene delivery and expression in eukaryotic cells. Genes will be cloned from human and viral DNA or cDNA into bacterial vectors that contain bacterial and/or eukaryotic selectable markers and bacterial or eukaryotic expression signals. To generate lentiviral vectors the gene of interest will be transferred into a modified HIV vector, with viral genes deleted.
containing a CMV or SFFV promoter driving the expression of GFP and the single human gene from the bacterial plasmids. This will be transfected into eukaryotic cells together with an HIV packaging plasmid encoding gag/pol, tat and rev with virulence gene vif, vpu and nef deleted and an envelope expressing plasmid (VSV-G). We will use the lentivirus vector system to transduce cell lines and primary cells producing cells which transiently express the gene of interest and after selection or sorting produce stable cells.

Identifying host-virus interactions:

This work aims to identify the function and mechanism of action of host or viral genes which affect the phenotype of virus infection, using a number of different ADCP hazard group 2 viruses (see section 7.). These viruses will include those which are genetically modified to contain non-toxic marker genes, such as GFP or luciferase or that have been attenuated in comparison to parental strains.

In vivo work:

The aim of the in vivo experiments is to characterise host genetic factors that affect virus infection using a mouse model. We will study hazard group 2 viruses including influenza and herpes simplex virus, which may be genetically modified. The mice to be infected are housed in the Research Support Facility (RSF) building at the Wellcome Trust Sanger Institute (WTSI) in a purpose built CL2 facility. Mice are normally inbred but on occasions approved outbred mice may be used. The WTSI has a large scale programme for generating knockout mice. Some of the mice harbour defined mutations in different murine genes and some express reporter molecules such as drug resistance or B-galactosidase markers. All details of the infection procedures are approved by the Home Office Licence held by Professor G D/Dr S C and infections will be performed in accordance with this approved licence and appropriate project licence holder.

Recipient or parental organism

The recipient/parental viruses are all CL2 organisms, no CL3 work will be undertaken in this coordinated programme of work.

In vitro work:

Lentiviral vectors - The genes for expression will be from a variety of different cellular pathways and will include transcription factors, innate and adaptive immune system genes, oncogenes and tumour suppressor genes. The cloned genes will on occasion be modified by either single mutations, partial deletions and fusion to tags (e.g. antigen tags, GST functions) or controllable expression tags (e.g. tetracyclin inducible promoter plasmids or tamoxifen inducible fusion proteins). As this work will always focus on single human genes it is unlikely that such genes will have the capacity to alter bacterial cell function during cloning on their own. However, we are aware of the low risk posed by the expression of human genes that will alter cellular functions (i.e. oncogenes, hormones, tumour suppressor genes, cytokines, etc) in bacteria and their potential for plasmid mobilisation.

Cells transfected with lentiviral vectors, either for the production of virus vector stocks or following transduction, will contain viral genes on split plasmids therefore making the vector attenuated by lack of intact full length genomes that would require extensive recombination events to produce a replication competent virus. There is good evidence that such replication defective viruses are not pathogenic when tested in mice and a minimum of 3 recombination events are necessary to produce replication competent, recombinant virus. In addition such virus vectors are single cycle infection agents not able to form productive infections or to recombine with endogenous retroviruses and become productively infectious. Therefore, although exposure to such viral vectors is a possibility this would be a self limiting/dead end infection.

There is a very low likelihood that exposure to any components of this project will cause harm. The main risk is accidental infection and transfer of the human gene expressing lentivirus to the laboratory personnel directly handling the lentivirus. This risk is extreme low but in the event of such a transmission a subset of the human gene expressing clones may cause potential harm by insertional mutagenesis/overexpression of cellular genes by random integration acting to deregulate normal cell function, for example, expression of cytokines or oncogenes. The lentiviral vectors have a broad host range due to the receptor independent VSVG envelope. Again the likelihood that this will cause harm is low as the replicative defective nature of the HIV vectors ensures that even in the case of a breach of containment and accidental injection, no virus can spread within or between individuals. Also more than one genetic lesion is required for most cell transforming and altering events. As we will be working with only one human gene at a time the risks are low.
Level 2 containment for both the molecular biology and tissue culture work is sufficient for both the production of lentivirus plasmids and replication defective viral vectors. This level of containment is also sufficient for the transduced cell lines that result from exposure to the viral vector preparations.

Identifying Host-Virus Interactions.

For all viruses within this body of work genetic modifications will involve insertion of marker genes such as GFP, luciferase or deletion/modification of viral genes to cause attenuation. The foreseeable effects from the production of such recombinant viruses will be to produce a virus that is equivalent to or attenuated relative to wild type virus. It is very unlikely that a virus with increased pathogenicity will be created through the manipulation of viral genes. All viruses used are ACDP hazard group 2 and will be worked with in CL2 containment facilities.

Virus to be worked with include:

**HERPESVIRUSES (wild type and recombinant including from BAC):**
- Herpes Simplex Virus Type 1
- Kaposi's sarcoma associated herpesvirus
- Epstein Barr Virus
- Murine herpesvirus 68

These are transmitted primarily as cell free virus in saliva and through close physical contact. As such the potential for accidental exposure comes from virus containing medium that is ingested exposing the oral cavity. However, most people are already seropositive and latently infected with Human Cytomegalovirus and Epstein Barr Virus. Infection with any of the human viruses causes a self limited primary infecton with the potential for the virus to sporadically reactivate. Epstein Barr virus can cause infectious mononucleosis (glandular fever) in adults, which can have long term effects, including fatigue syndrome. Generally no disease symptoms arise, however herpes simplex virus causes cold sores and Kaposi's sarcoma associated herpesviruses and Epstein Barr Virus can cause lympho,as and other cancers. The primary risk of these viruses results from immunodeficiency or in the case of human cytomegalovirus infection of a seronegative mother during pregnancy. As such, the policy of immunosuppressed or pregnancy being exclusion criteria for work with these organisms will be enforced. Murine herpesvirus 68 and murine cytomegalovirus do not infect humans and do not cause disease in mice.

**ORTHOMYXOVIRUS (wild type, produced from reverse genetics systems and recombinant Influenza A)**

No influenza viruses classified above containment level 2 will be used. All people that work in the laboratories which use influenza will receive annual influenza vaccinations. Only low pathogenicity laboratory strains of influenza will be used and these may be genetically modified. Different strains of influenza virus will not be worked with at the same time to avoid the possibility of reassortment between viruses.

Influenza viruses are airbourne viruses transmitted primarily as foamites. Therefore the primary risk from GM influenza viruses is exposure to tissue culture medium either as an aerosol or as a large volume exposure to the nose, mouth and eyes (mucous membranes). These risks are minimised by the enforcement of no unsealed tubes and flasks outside of the Class II microbiological safety cabinets. A risk of onward transmission if infected exists, although due to the nature of the viruses used this will pose no greater risk to the general population than normal circulating influenza. Nevertheless, anyone with flu like symptoms will be requested to seek medical advice and minimise contact with others.

**PARAMYXOVIRUS**
- Measles
Measles is highly contagious and spread by coughing and sneezing. An infected person can transmit measles any time from about 4 days prior to the onset of the rash to 4 days after the onset. However, infection can be completely prevented by vaccination and therefore all workers in the laboratory who work with or in the environment where measles viruses are used will be required to have, or demonstrate existing immunity to measles.

The primary risk from GM measles virus is exposure to tissue culture medium either as an aerosol or as a large volume exposure to the nose, mouth and eyes (mucous membranes). These risks are minimised by the enforcement of no unsealed tubes and flasks outside of the class II microbiological safety cabinets.

In vivo work:
The GM viruses are to be used are those described above - these will include viruses harbouring specific mutations in selected viral genes and/or the expression of reporter genes. Some viruses will express non-toxic reporter molecules such as GFP or luciferase. Some viruses may be attenuated in comparison to their parental strains (see above).

The mice for infection may harbour mutations in specific murine genes and may also express non-toxic reporter molecules. The WTSI is currently undertaking mouse gene knock out of hundreds of genes using conventional gene replacement technology and some of these mice will be screened for altered susceptibility to infection using the virus in the coordinated programme of work and the associated risk assessments and Home Office licenses. None of the genetically modified mice pose a higher risk to human health or the environment than the parental strains.

We anticipate that mice will become infected by the viruses used for challenge and some will go on to develop clinical symptoms. Such mice will be humanely sacrificed according to the methods outlined in our Home Office Licence if their clinical symptoms reach a certain well documented level of severity. We foresee no unusual outcomes other than that some infections will be altered in severity or duration of infection in KO mice in comparison to wild type mice. If any of the mice show usually high susceptibility to infection they will be humanely sacrificed.

The primary risk will be infection of laboratory workers and other mice as a result of the infection of wild type or KO mice. We estimate this risk is very low and is managed by all work being carried out in purpose built CL2 facilities. Tissues and samples are fixed and virus inactivated before removal from the CL2 facilities, animal cadavers autoclaved before disposal and infectious material transported between the animal CL2 and virology CL2 laboratories in approved transport containers, according to risk assessments that are in place.

Host/vector system

Plasmids
Cloning plasmids will be pGEM derivatives, pUC series, pBLUESCRIPT series, pcDNA, pCMV/Zeo, and pEGFP-1. The lentiviral vectors use the tripartite packaging plasmid pCMVR89.1 (packaging plasmid), (pMDG (envelope VSV-G plasmid) and pCSGW (pHR-CMV-IRES-eGFP vector plasmid), pCSGW contains the promoter-mutated form of the woodchuck hepatitis virus post-transcriptional regulatory element (WRE), which is incapable of expressing the putative oncogene encoding protein X.

All plasmids are non-mobilisable.

Bacterial hosts
Bacterial hosts for all plasmid propagations within this programme of work are derived from E. coli K12, so are disabled in key bacterial functions (i.e. recombination defective) and are unable to survive in the environment. These will include E. coli - XL-Blue, TOPO10, JM109, SURE.

Host cell lines
Host cell lines and primary cells will include: 293T, HeLa, NIH3T3, B-cell lines from all stages of B-cell including BJAB, Ramos, Rajo Tom -1, DEL RPMI-8226, BCP-1, BCBL, HBL-6, BC3, T cell-lines including C8166, CEM, SupT1, monocytic cell lines including knockout cell lines, murine embryonic fibroblasts, PSNL 76/7-4.

NO cell lines or primary cells used will be from the workers.
The foreseeable effects of transduction of the eukaryotic cells with expression vectors, or the infection of these cells with recombinant retroviruses would be the production of a cell line with altered properties able to establish a tumour in the laboratory workers, but the likelihood of this hazardous event with the control measures in place is estimated to be LOW.

Mice
The mice may harbour deletions, which will have been characterised in the WTSI phenotyping pipeline and may also express non-toxic reporter gene products such as β-galactosidase. All infectious work will be carried out in purpose built CL2 facilities and animal cadavers will be autoclaved before disposal.

Origin & function

Origins of herpes viruses - Herpes simplex virus and murine herpesvirus 68 are supplied to us as a gift from Dr S E, University of Cambridge. KSHV and EBV are already available in the Kellam Lab.

Influenza viruses are supplied to us as a gift from Prof W B, Imperial College, London and P D, University of Cambridge.

Measles viruses are supplied to us as a gift from Prof P D, Department of Microbiology, Boston University School of Medicine.

The intended functions of the genetic modified material listed above is to attenuate or add a marker gene (e.g. GFP, luciferase) with the resulting GM virus having an altered phenotype in host cells or mice.

Lentivirus vector systems - These are standard laboratory reagents which are supplied to us as a gift from the Kellam laboratory at UCL. They originated from infectious retroviruses of humans and animals but have been extensively engineered to be self-inactivating and capable of only a single round of infection.

The function of retroviral expression vectors is to change the level of expression of host proteins within the cell.

Evaluation of foreseeable effects

Bacterial hosts
Bacterial host for all plasmid propagations within this programme of work are disabled in key bacterial functions (i.e. recombination defective) and are unable to survive in the environment or pass genetic material onto host bacterial flora following a breach of containment.

For the production of lentiviral vectors the lentiviral genome is split into three separate plasmids such that virus production is only initiated when all three plasmids are present in a eukaryotic cell. Therefore there is no risk of lentiviral particles being produced from bacterial culture.

OVERALL THE RISK OF ADVERSE EVENTS FROM THE HANDLING OF PLASMIDS OR BACTERIA (INCLUDING THOSE CONTAINING KNOWN OR POTENTIAL ONCOGENES) IS LOW.

Lentiviral Gene expression
For the lentiviral gene expressions and gene ablation vectors production of infectious viral particles only occurs following transfection of the three plasmid system into eukaryotic cells. Therefore the control of virus particle production can be contained both physically in a CL2 tissue culture laboratory and by prevention of virus production by the absence of all three plasmid vectors. Lentiviral vectors are therefore unlikely to be transmitted onto the laboratory workers during routine laboratory experiments involving the retroviral vector plasmids or following disposal of laboratory waste. The main risk is accidental exposure of laboratory workers to recombinant lentiviruses. As these lentiviral vectors are self-inactivating the risk of onwards transmission to another person is almost zero. The main risk is therefore alteration of the cellular function of the exposed person through insertional inactivation/activation of tumour suppressor genes/oncogene respectively, or the alteration of cellular function by gene overexpression or ablation from the transferred genetic material within the vector. THESE ARE EXTREMELY UNLIKELY EVENTS THAT HAVE ONLY OCCURRED IN A CLINICAL SETTING FOLLOWING PROLONGED INFECTION WITH HIGH TITRE RETROVIRAL VECTORS AND SELECTION IN VIVO FOR CELLS WITH ALTERED GROWTH POTENTIAL THEREFORE THE OVERALL RISKS ARE LOW

GM viruses
For all viruses within this body of work genetic modifications will involve insertion of marker genes such as GFP, luciferase or deletion/modification of viral genes to cause
attenuation. The foreseeable effects from the production of such recombinant viruses will be to produce a virus that is equivalent to or attenuated relative to wild type virus. It is very unlikely that a virus with increased pathogenicity will be created as we only intend to inactivate viral genes. All viruses used in this programme of work are ACDP hazard group 2 pathogens and both wild type and GM viruses will be handled in CL2 containment facilities. If available, laboratory workers will also receive vaccinations against parental virus strains. Immunocompromised or pregnant workers will not be allowed to work in the laboratory. OVERALL THE RISKS OF ADVERSE EVENTS FROM HANDLING GM VIRUSES WITHIN THIS PROJECT are low.

Mice
All infectious work will be carried out in purpose built CL2 facilities and animal cadavers will be autoclaved within this facility before disposal. If available, laboratory workers will also receive vaccinations against parental virus strains. Immunocompromised or pregnant workers will not be allowed to work in these facilities. OVERALL THE RISKS OF ADVERSE EVENTS FROM HANDLING GM VIRUSES FOR MURINE WORK ARE LOW.

Together the risk identified within this integrated programme of work can be minimised to acceptable LOW levels by working to existing laboratory standards and practices in designated Containment Level 2 laboratories. This is supported by the accompanying risk assessments.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
All mice are housed in a CL2 laboratory suite built for purpose. Animal cadavers will be autoclaved within this facility before disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
We operate a number of comprehensive and proven waste management measures which are conducted according to our local protocol for handling CL2 pathogens. This protocol has been in use for many years and involves autoclaving of solid waste material, chemical inactivation of liquid waste and careful management of waste movement. The GMM will be used within a containment level 2 laboratory in which all cultures/liquid waste is decontaminated prior to disposal using a final concentration of 1% Virkon solution for at least 16 hours (concentration validated by manufactureres) before disposal to the drains. Thus drains, sinks etc do not pose a mode of transmission to the environment. Air movement is also strictly regulated in the laboratory environment. Solid biological waste (including cadavers) is autoclaved using temperature cycles and conditions appropriate for the inactivation of biological material. Autoclaving is performed by departmental staff using approved conditions, (134 degrees C for 3 minutes per cycle). Plasticware etc is decontaminated with 1% Virkon solution prior to being autoclaved as described above.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Regarding the programme as a whole, the Biological Agents and GM safety Committee (BAGMSC) discussed the circumstances under which exclusion from the work activity should be considered, and agreed to adopt the wording "knowingly immunosuppressed".

SIBAGMRA11_06 and 07: The original risk assessments contained reference to lentiviruses being susceptible to desiccation. It was stated by BAGMSC that this has long since been established not to be the case, with dried blood smears being infectious for up to 14 days, and desiccation should not be relied upon as a control measure. This has now been deleted, and the team advised. Clarification was requested on the status of the WPRE, which is confirmed as protein x minus.

A change to the wording in Section 1.1 of SIBAGMRA11_06 has been introduced to satisfy a degree of confusion on the part of the Committee regarding transient vs stable expression from lentiviral vectors.

SIBAGMRA11_08: EBV can cause glandular fever which can have long lasting effects in adults as fatigue syndrome. Therefore, this is not a 'mild' risk and this needs to be considered in the risk assessment. Due to the possible persistence of measles virus within safety cabinets, it was agreed that there is a need to establish immunity to measles for those undertaking work and those in vicinity of work. Vaccination would then only be required for those without an appropriate titre level. If workers who are not immune to measles are to be excluded, the work with this virus must be restricted to room D2-34 only.

There were no further requests by BAGMSC for changes to be made to any of the other attached risk assessments.
Next generation sequencing has identified multiple genes harbouring activating heterosygous mutations in different types of human cancers. To validate these gene targets in a biological setting, we aim to introduce cDNAs encoding mutated proteins into human cancer cell lines using a retroviral vector. This will mimic heterozygous gene expression, thus replicating the consequences of the mutational event that occurs in cancer. Cell biology assays will then allow us to determine whether expression of the mutated gene is critical for malignant transformation.

The recipient used for retroviral vector production will be GP2-293 cells, a HEK293-based packaging cell line that stably expresses the viral gag and pol genes, and the VSV-G amphotropic envelope.

Retroviral vectors will be introduced in human haematopoietic cell lines (e.g. HL60, H562, HEL).

The parental organism is the retrovirus Mouse Embryonic Stem Cell Virus (MSCV), which in its native form is pathogenic only to mice.

The MSCV vector backbone is derived from the Murine Embryonic Stem Cell Virus (MSCV). The MSCV vector is a self-inactivating retroviral vector optimized for introducing and expressing target genes into pluripotent cell lines. It contains a specifically designed long terminal repeat from the murine stem cell PCMV virus. PVMV stands for PCC4-cell-passaged myeloproliferative sarcoma virus (Hilberg et al., 1987, Hawley et al., 1994) This LTR differs from the MMLCV LTR by several point mutations and a deletion. These changes enhance transcriptional activation and decrease transcriptional suppression in embryonic stem and embryonal carcinoma cells. As a result, the LTR drives high-level constitutive expression of a target gene in stem cells and other mammalian cell lines (Hawley et al., 1994).

Inserted cDNAs:-
SRSF2 (wild-type)
SRSF2-P95H
SF3B1-(wild-type)
SF3B1-K700E
To validate these gene targets in a biological setting, we aim to introduce cDNAs encoding mutated proteins into human cancer cell lines using a retroviral vector. This will mimic heterozygous gene expression, thus replicating the consequences of the mutational event that occurs in cancer. Cell biology assays will then allow us to determine whether expression of the mutated gene is critical for malignant transformation.

**Evaluation of foreseeable effects**

**Production of replication-competent retrovirus (RCR):** The viral genome is divided such that the backbone vector carries only the LTRs, the packaging signal and the transgene of interest, with all other elements required for viral vector production (gag, pol and amphotropic VSV-G envelope genes) stably integrated into the genome of the packaging cell line. This will significantly reduce the likelihood of recombination events leading to the production of replication competent viral particles, compared with multiple plasmid transfections. Batches of retroviral vector will be tested for production of cytopathic effect on monolayer cultures, and for gag-pol-env RT-PCR products, both of which would indicate the presence of RCR. If these are detected, the batch will be destroyed and the risk assessment will be reviewed.

**Insertional mutagenesis by vector:** Additional refinements of the vector system selected include the self-inactivation (SIN) system in which U3 enhancer sequences are completely removed from the 3' LTR thus reducing the chances of transactivation of cellular genes as a result of insertion. However, the risk of insertional activation or silencing cannot be excluded.

**Inserted genetic material:** The cDNAs that are to be studied have been identified in genomic screens as potential oncogenes. Both mutant and wild-type forms will be used. There is a risk that the oncogenic DNA could act as the first "hit" in an oncogenic pathway.

The viral backbone vector is a non-mobilisable plasmid which will be manipulated in E. coli K12. While the plasmid will contain a putative oncogene under the control of a eukaryotic promoter, the likelihood of harm by its introduction into workers or others is very low.

All of these effects are dependent upon the retroviral vectors (or the parental plasmids) being introduced into the lab workers by inoculation. This risk will be minimised by the work being carried out in a Containment Level 2 laboratory which operates a no sharps policy, confirmed by the assurance programme conducted by the Campus Health & Safety Service.

To prevent the development of aerosols, tubes and culture plates with viral supernatant will be sealed and centrifuged in certified aerosol-tight buckets, which will only be opened following transfer to a microbiological safety cabinet.

The risk to the environment would be by virtue of the VSV-G envelope retroviral particles being capable of infecting a wide variety of species and cell types. This would be unlikely given the labile nature of retroviruses, and the inactivation methods in place.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

*Not applicable*

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

*Not applicable*

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Liquid waste is treated by addition of Virkon to a final concentration of 1% (w/v), which is validated by the manufacturer to give 100% kill in all the species in this study. All solid waste is autoclaved with a hold time of 20 minutes at 134 Celsius in autoclaves which undergo 12-point 100% kill of thermostable strains of bacteria such as Bacillus stearothermophilus. All resulting material is destroyed by incineration. |
It was agreed by the BAGMSC, at its meeting held on 17th October 2012, that this risk assessment could be discussed via email, unless there were serious doubts raised about the validity of the classification. There were no such doubts raised in a number of emails corresponding to the quorum of the Committee, and was therefore approved as a Class 2 activity.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tr>
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**Project Ref** 552/12.2

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**Non-GMM Consent Granted**

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Purposes of the contained use

Genetic modification of organisms is critical to understand the impact of individual genes on the phenotype and lifestyle of the organism. This research is only just beginning in Chlamydia trachomatis. Generation of fluorescently labelled strains will help us to develop new techniques for understanding the lifecycle of this complex organism. It will also aid our understanding of the ease, degree and mechanisms of recombination in Chlamydia. Knockouts, either targetted or random as in TraDIS will help us to determine key genes for the lifestyle and infectious cycle of this organism.

### Recipient or parental organism

C. trachomatis is an HG2 pathogen, causing trachoma eye infections, sexually transmitted urogenital infections and invasive lymphogranuloma venereum (LGV) infections. This is a human-restricted pathogen.

### Host/vector system


For the TraDIS screen, transposon mutant libraries will be generated using derivatives of Tn5 and Mu that carry a gene conferring resistance to a single antibiotic, including kanamycin, gentamicin or chloramphenicol, but NOT tetracycline. Transposons will be introduced at one copy per cell by electroporation. The transposon derivatives possess outward-orientated promoters which allow the in vitro transcription of RNA at the insertion sites using genomic DNA extracted from pools of mutants. Whole genomic sequencing can then indicate the position of all the transposon insertion sites for many mutants simultaneously. Using this technology, pools of many mutants can be used to identify which genes are required for growth under different selective conditions. Transposon vectors will be pre-loaded with transposase enzyme to achieve the transposition event, obviating the need for the expression of a transposase gene, meaning that the inserted element cannot be re-mobilised.

### Origin & function

Transmission is thought to be by direct contact, although exact transmission methods for trachoma are unknown. Tissue tropism is epithelial for trachoma (eye)/STI strains and the invasive LGV strains can go on to infect lymphatic tissue. Conjunctivitis (through eye infection) can lead to scarring and trachoma; reproductive tract infections which may be asymptomatic, yet lead to pelvic inflammatory disease, scarring and infertility in females; proctitis in the case of LGV. The opportunity for infection in the laboratory is minimal, other than by the ocular route, which will be controlled by the use of microbiological safety cabinets and eye protection. There is a no-sharps policy in the laboratory, other than the use of capillaries for micro-manipulation, as detailed in the risk assessment.

Post-exposure treatment is via the use of a single dose of azithromycin, or doxycycline taken for up to 2 weeks. Tetracycline can also be used. There is no known resistance to these antibiotics in the clinical setting. The use of plasmids or transposon cassettes will induce resistance to a single antibiotic, but no constructs will be used which confer resistance to any of the frontline clinical treatments for Chlamydia infection.

It is not the aim of this project to specifically affect virulence. Little is known to date about the specifics of C.trachomatis virulence, but it is not anticipated that insertion of fluorescent cassettes as proposed will increase virulence. Almost all naturally occurring strains of C. trachomatis carry a plasmid, which is the basis of the recombinant
A plasmid proposed as a vector, therefore this is not anticipated to increase virulence. Potential creation of genome-wide knockouts (TraDIS) will involve the inactivation of genes, and will not create new functions. This is unlikely to increase pathogenicity.

Re-mobilisation of the inserted element cannot occur in the absence of the transposase exnzyme, so is unlikely to be capable of horizontal transmission to other bacterial species in the environment.

The vector itself is unlikely to be capable of causing direct harm to humans or other animals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste is treated by addition of Virkon to a final concentration of 1% (w/v), which is validated by the manufacturer to give 100% kill in the species in this study. All solid waste is autoclaved within the building, with a hold time of 20 minutes at 134 Celsius, in autoclaves which undergo 12-point thermocouple validation every three months. NB hold times fo 3 minutes at 134 Celsius are regarded as providing 100% kill of thermostable strains of bacteria such as Bacillus stearothermophilus. All resulting material is destroyed by incineration.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

Please enter comments on the GM safety committee on the risk assessment.
This risk assessment was discussed at a meeting of the BAGMSC held on 17th October 2012. The committee made the following requests for clarification:-
* Further detail on frequency of screening for antibiotic resistance should be added to section B1.3.

RESPONSE: There is no documented antibiotic resistance in clinical samples, antibiotic resistance screening of isolates is very difficult to perform and does not give consistent results.
* Clarify those techniques performed in the microbiological safety cabinet which result in aerosol generation (Section B3.3).
* Eyes are a target organ and eye protection needs to be stated in risk assessment and associated procedures as mandatory when working with Chlamydia, including protection from splashes as well as aerosols.
* Include symptoms in local handbook and re-iterate to those working with this risk assessment.

The risk assessment and local handbook have been amended in accordance with these requests.
NB The use of the genus name Chlamydia (chlamydia-like), which is referred to in the ACDP classification list, is no longer regarded as appropriate by the clinical and research community, and all species are reverting to Chlamydia

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### Project Containment

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### Project Ref 552/13.1

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Historical Significant Changes

Historical Date of Additional Info
### Project Additional Information

**Purposes of the contained use**

| Various activities in genomics research in the field of human health and disease, including, but not limited to expression of oncogenes, siRNA knockdown of genes implicated in disease pathways, and induction of cells into pluripotency, within the limits defined in the attached risk assessment. |

**Recipient or parental organism**

| Lentiviral and retroviral vectors based on a range of human and animal viruses, including HIV, EIAV, MMLV, MMTV and MSCV. The split vector systems and packaging cell lines to be used will conform to designs of second generation or later, and will be demonstrably incapable of replication in humans or the environment. Vectors may have wider tropisms compared to the parental organisms by virtue of the inclusion of amphotropic envelope proteins such as VSV-G. Wild-type WPRE elements in lentivector systems will be excluded wherever possible, but their inclusion would still be within the scope of the connected programme, as defined in the attached risk assessment. |

**Host/vector system**

| Viral vectors will be generated by introducing plasmids containing elements of the virus genome into established packaging cell lines, such as human embryonic kidney (HEK) 293 cells. In some cases, the essential packaging functions will be expressed stably by the packaging cell, with a single plasmid carrying the vector backbone being introduced by transfection, and in other cases the viral functions and the vector backbone will be encoded on separate plasmids which will be transfected together. Viral vectors produced in this way will then be used to transduce a range of human and animal (principally of mouse origin) cells. This will include established cell lines from various lineages, mouse embryonic stem (ES) and haematopoietic cells, and primary human samples. Transduced cells may be introduced subsequently into animal models of human disease. |

**Origin & function**

| As this is a connected programme of work, the genetic material to be introduced will be from a range of origins and have a variety of functions. This will include putative and known oncogenes and tumour-suppressor genes, short hairpin RNA (shRNA) molecules intended to inhibit the expression of a range of genes including those known or suspected to be involved in pathways of oncogenesis. |
Evaluation of foreseeable effects

The use of modern (i.e. second generation or later) vector systems in which reverse transcriptase, envelope protein and accessory genes are deleted from the sequences that are packaged into viral vector particles (and are provided in trans-, either on additional accessory plasmids or integrated into the genome of packaging cell lines) render them incapable of infection and of replication. However, it should be noted that a transduction event, even by a viral vector that is non-infectious and replication-defective, will result in integration of the inserted gene into the genome of the host cell. If the expression of the gene is driven by a constitutive promoter, that expression will be permanent. While the vector itself will not proliferate, cell division may result in a population of cells expressing the transgene.

Specific hazards associated with inserted genes include:-
Expression/over-expression of known or potential oncogenes: while oncogenesis is known to be a multi-factorial process, the risk of insertional activation of other oncogenes by the vector, or of insertional inactivation of tumour suppressor genes is unpredictable, and could act in concert to contribute to oncogenic pathways.

Expression of short hairpin RNAs designed to knock down known or potential tumour suppressor genes: similar risk to oncogene expression, above.

Expression of cytokines and growth factors: these proteins have the potential to cause inappropriate growth, differentiation or apoptosis of cells, which are associated with oncogenesis. Growth factors and cytokines may also be teratogenic, and have other effects on the immune response, so consideration will have to be given during the individual risk assessment as to whether expectant mothers and workers with other health issues should be excluded from such work.

The most severe outcome associated with these hazards is administration of the viral vector to a worker, the likelihood of which must be reduced as far as possible, for example by the adoption of a zero sharps policy (except during the administration of these vector systems to mouse models, or for activities such as excising gel slices where there is no direct contact with the GMM), and other control measures required at Containment Level 2. The risks to workers associated with the plasmids used to generate the viral vectors are considerably less than the virus itself, as the passive uptake of circular DNA into human cells is a very inefficient process, chromosomal integration will not occur, and the plasmids will not be maintained episomally in a dividing cell population.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Some aspects of this connected programme will include the administration of lentiviral and retroviral vectors, and cells transduced with such vectors, to GM mice (which represent no increased risk to human health or the environment compared to wild-type).

The animal facility (RSF) has been purpose built to house mice and was designed in consultation with the Home Office and HSE. Control measures include:
- Use of perimeter rodent control measures (bait boxes), to control escaped laboratory mice and prevent wild type mice entering the building.
- Rodent barriers to rooms containing mice to prevent escaped lab mice exiting the immediate area.
- Air scissors on the extract auger pipe for the automated bedding removal station to prevent large objects from passing through.
- Computerised auditing of the facility murine population, ensuring absence of a mouse (or mice) is quickly detected.
- SOPs to monitor for, react to, and record escapes.
- All rooms are subject to strict access control.
- Staff are trained to handle animals in accordance with Home Office requirements.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid waste contaminated with GM material will be bagged, and transported to autoclaves within the same building in closed rigid boxes within lidded trolleys. All of the autoclaves are subjected to annual inspection to written schemes, annual validation reports, and quarterly servicing. Waste runs are validated to achieve a minimum hold time of 3 minutes at 134°C, equivalent to a 30-log reduction in viability of indicator spores. Waste inactivated by autoclaving is removed from site for incineration or deep land-fill only after examination of the autoclave run report. Additional validation has been performed of the autoclaving of mouse carcasses to ensure the entire body has reached the validated temperature and hold time. Mouse carcasses are incinerated. All liquid waste is subject to treatment with Virkon-S, at a minimum concentration of 1% (w/v) and minimum contact time of 1 hour, before dilution and disposal via the drains. The degree of kill is effectively 100%.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The first draft of the risk assessment was discussed at the meeting of the Biological Agents & GM Safety Committee of 6th February 2013. The Committee were in agreement that creating a risk assessment for this connected programme of work was a sensible approach. JB suggested an additional sentence be inserted into the 'outline of process' section to indicate that all risk assessments will go the Committee for review and to decide if they fall within the scope of this connected programme of work. JB proposed that a list of those risk assessments that are applicable to this connected programme should be included as an Annex.

There was a discussion on whether the virus should be considered as the recipient organism or the vector system. IG stated that after discussion with HSE, the virus could be considered as the recipient.

JR asked if the vector system could be specified, JB agreed that ‘2nd generation or later vectors’ be stated in ‘Outline of Process’ and in answer to the question ‘have the pathogenic traits of the recipient organism been altered?’.

The risk assessment was amended, and approved by BAGMSC at the meeting held on 16th April 2013, subject to a further exception to the zero sharps policy in respect of excision of gel slices.

Project Containment

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The aim of the project is to identify the function of genes encoded by Mycobacterium tuberculosis. To this end we will exploit highly attenuated and genetically validated M. tuberculosis host bacteria generated by our collaborator Prof W J (Albert Einstein College of Medicine, New York). These host bacteria (MC(2)6030, MC(2)6030 and further mutant derivatives) harbour disabling deletion mutations in RD1 (associated with the attenuation of bacilli Calmette-Gurin BCG vaccine), panCD (associated with pantothenate synthesis) and other auxotrophic mutations within lysA. RD1 mutations limit the ability of M. tuberculosis to grow within animal tissue and have been validated in part in humans through use of the BCG vaccine, panCD mutations limit growth in vivo by restricting the availability of essential nutrients to the bacteria, essentially starving them.

We will exploit these background strains in phenotypic screens. Our collaborators have introduced null deletion mutations in ALL mapped protein coding genes within M. tuberculosis H37Rv (the most commonly-use lab strain with a fully -sequenced genome). We will exploit this library to identify the function of these genes using eukaryotic cell invasion assays performed at relatively high throughput (96-well and 384-well format) to observe the impact of such bacterial mutations on the eukaryotic host cell phenotype. All experiments will be conducted on simple cellular phenotyping platforms, available within the CL2 facilities at the WTSI.

Recipient or parental organism

Mycobacterium tuberculosis H37Rv is a fully sequenced laboratory strain of TB classified as a Hazard Group 3 pathogen. The route of transmission is by aerosol, but the host bacteria can also infect via oral or parenteral routes.

NB: This parental strain will not be used as part of this notified activity, and no further modifications to the strains MC(2)6020 and MC92)6030 and the derivatives thereof will be performed at our premises. The sole GM activity in this notification is growth, challenge of eukaryotic cell cultures, and storage of GM strains.
The gene deletions in these strains of M. tuberculosis were generated as follows. The gene of interest, disrupted by a selectable marker, is cloned into a conditionally replicating (temperature-sensitive) shuttle plasmid to generate a specialised transducing mycobacteriophage. The temperature-sensitive mutations in the mycobacteriophage genome permit replication at the permissive temperature of 30 degrees C but prevent replication at the non-permissive temperature of 37 degrees C. Transduction at a non-permissive temperature results in highly efficient delivery of the recombination substrate to virtually all cells in the recipient population. The deletion mutations in the targeted genes are marked with antibiotic-resistance genes that are flanked by gamma delta-res (resolvase recognition target) sites. The transductants which have undergone a homologous recombination event can be conveniently selected on antibiotic-containing media.

The specific strains of bacteria (MC(2)6030, MC(2)6020 and further mutant derivatives) harbour disabling deletion mutations in RD1 (associated with the attenuation of bacilli Calmette-Gurin BCG vaccine), panCD (associated with pantothenate synthesis) and other auxotrophic mutations within lysA. RD1 mutations limit the ability of M. tuberculosis to grow within animal tissue and have been validated in part in humans through use of the BCG vaccine. panCD mutations limit growth in vivo by restricting the availability of essential nutrients to the bacteria. Our collaborators have subsequently introduced null deletion mutations in all mapped protein coding genes within M. tuberculosis H37Rv (the most commonly-used lab strain with a fully-sequenced genome).

Origin & function
The only genetic material in the GMMs is a hygromycin resistance cassette flanked by resolvase recognition sites integrated into the host chromosome. No other exogenous sequences are present.

Evaluation of foreseeable effects
Since no additional changes will be made to the bacterial strains as part of this notified activity, the foreseeable effects arise from the strains which will be imported.

Forseeable effects on human health: Whereas the background strain H37Rv is capable of causing TB, the deletions of RD1, lysA and panCD are well-documented in peer-reviewed papers as attenuating the ability of these strains of M. tuberculosis to colonise and cause infection in mammals (see accompanying summary of relevant literature). Furthermore, no biologically active product will be generated in humans (with the exception of hygromycin resistance), and there is no reduction in the susceptibility of the GMMs to frontline treatments for TB.

Likelihood of disabling mutations being overcome: The specialised transduction mechanism used to generate the deletions is such that reversion or rescue of any deletion is extremely unlikely. Given that each strain has two such disabling deletions, the likelihood of generation of a fully infectious Tb strain is effectively zero.

Possible impact on the environment: Since the disabling mutations have been introduced by a gene replacement event in the Mtb chromosome, the likelihood of mobilisation of the genetic modification, or its transfer into other species in the environment in the event of a release is very small. This is reduced further still when the waste control measures are taken into consideration.

Theoretical modes of transmission to the environment: Drains, aerosols, faulty autoclaves, and carried on individuals, but these risks are moderated by (a) the disabling mutations within the bacteria, which reduce the likelihood of survival outside the laboratory, and (b) the control measures used in the contained environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid waste contaminated with GM material will be bagged, and transported to autoclaves within the same building in closed rigid boxes within lidded trolleys. All of the autoclaves are subjected to annual inspection to written schemes, annual validation reports, and quarterly servicing. Waste runs are validated to achieve a minimum hold time of 3 minutes at 134°C, equivalent to a 30-log reduction in viability of indicator spores. Waste inactivated by autoclaving is removed from site for incineration or deep land-fill only after examination of the autoclave run report.

All liquid waste will be subject to treatment with Surfanios, at a minimum concentration of 5% (v/v) and minimum contact time of 3 hours, before dilution and disposal via the drains. The degree of kill has been validated as a minimum of a 9-log reduction.

Activities will be separated from those which use Virkon for chemical disinfection, as Virkon is documented as being ineffective against Mycobacteria.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment
This risk assessment was compiled in conjunction with advice from Dr L M, Safety Advisor for TB Reference Laboratories, Public Health England, who provided specific advice on the use of appropriate disinfection methods, but agreed that the assessment was comprehensive and appropriate.

The risk assessment was discussed at length at the meeting of BAGMSC held on 6th September 2013, having been circulated to the Committee by email in advance of the meeting.

OH Physician stated the importance of the strains retaining antibiotic resistance, and further that the organisation should decide on the requirement for TB vaccination of affected staff, as is provided in the healthcare sector. The Committee agreed that the risk posed by these strains was sufficiently low to obviate the need for vaccination, but this would be discussed as part of the initial health surveillance interview.

The Committee agreed that the evidence for the effectiveness of the attenuation mutations was compelling, but the following requests for clarification were made:

1. There is no mention in the risk assessment of how the gene deletions in the MC(2)6020 or MC(2)6030 have been made. Please clarify whether any additional antibiotic resistance markers have been introduced as part of the process, and whether there is a risk of any reversion.

Response: The mutations within the Mycobacterium tuberculosis strains are null (totally inactivating) deletion mutations designed to be stable and minimise any risk of reversion. Furthermore, the deletion mutations were made with an antibiotic resistance cassette flanked by resolvase sites, which then deleted the antibiotic resistant gene, so the end mutants have no additional antibiotic resistance.

2. There was concern that if the activity is to be performed in an area shared with other work, there is a risk that staff may use Virkon inadvertently to disinfect TB, instead of the required Surfanios. Can you clarify how this will be controlled (e.g. by using a separate area)?

Response: We will be using room b in the CL3 suite E235 or E376 (Cgap CL3). If these TB strains are being used we will NOT be using other bacteria or viruses within the same suite at the same time. Indeed, we would decontaminate the rooms before or after switching to using these strains. We would also ensure that only Surfanios is available within the active room for disinfection.

It was agreed at the meeting that the activity should be assigned to Class 2, subject to satisfactory clarification of the above points.

Activity approved at Class 2, subject to this notification.

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**Project Ref** 552/14.1
Investigating SNP mutations in human lymphoblastoid cell lines using Cas9/CRISPR targeting

Purposes of the contained use

1) To validate computationally-predicted causal single-nucleotide polymorphisms (SNPs) by genome editing using a Cas9/CRISPR system.
2) To knockout specific loci to investigate regulatory elements and gene functions.

HapMap lymphoblastoid cell lines are an invaluable resource for studying genetic variation within populations and between individuals. It is possible to computationally predict SNPs that are responsible for a given phenotype. In this study, a given phenotype will be assessed by mRNA expression. To validate a causal SNP in vitro, a Cas9/CRISPR system and homologous recombination will be used to edit SNPs to assess the effect on phenotype (mRNA expression). To knockout a given locus, the Cas9/CRISPR system will be used to promote non-homologous end joining (NHEJ) to alter specific genomic targets. These processes will involve tranfection/nucleofection of lymphoblastoid cell lines with plasmids which will drive expression of pairs of locus-specific guide RNAs (gRNA) along with the mutant D10A Cas9 (nickase activity). gRNA and D10A Cas9 expression will be driven from the human U6 and cytomegalovirus (CMV) promoters, respectively. To promote homologous recombination, a single-stranded oligonucleotide harbouring the altered SNP sequence will also be co-transfected with the gRNA and D10A Cas9 plasmids. The use of pairs of gRNA co-expressed with D10A Cas9 and a single-stranded oligonucleotide homology donor in human cell lines has previously been demonstrated (1). Using Cas9/CRISPR to stimulate NHEJ in human cells has also been shown (1).

1. Ran et al., Cell 154, 1380-1389 (2013).

Recipient or parental organism

A selection of EBV-transformed human lymphoblastoid cell lines.

Host/vector system

Plasmid: Cas9(D10A)-NLS-IRES-puro (vector backbone is plRESpuro3 from Clontech).
Plasmid: U6 Bsal gRNA (vector backbone is pDONR201 from Life Technologies).
Homology donor: ~ 180 bp single-stranded oligo nucleotide

Origin & function

A selection of EBV-transformed human lymphoblastoid cell lines.
### Evaluation of foreseeable effects

**Forseeable effects on human health:**
Lymphoblastoid cell lines are EBV-immortalised B-lymphocytes transformed with Epstein-Barr virus (EBV). EBV causes infectious mononucleosis, which can lead to chronic fatigue in adult humans. Transmission is predominantly via the oral route and it is therefore unlikely that the virus would be transmissible under Containment Level 2 conditions. There is the potential for lymphoblastoid cells to shed EBV particles in culture, but by following the correct CL2 culture procedures the risk of transmission to humans is expected to be low.

The Cas9D10A would need to be co-expressed with a gRNA for it to be active in humans. This is extremely unlikely to happen, given that Cas9D10 and gRNA will be expressed for different plasmids. Also, given that the vectors are plamids and not viral backbones, the change of uptake in humans is extremely unlikely.

Consideration was also given to a theoretical, but extremely low risk off-target double strand breaks in the vicinity of a tumour suppressor gene, but this would not be expected to be sufficient to induce tumour formation.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not applicable**

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**None**

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid cultures will be chemically inactivated by the addition of a validated disinfectant (e.g. Virkon) and leaving for the required time (>20 minutes), before being diluted and disposed via the drains.

Contaminated solid waste (culture dishes, serological pipettes, disposable gloves, etc) will be autoclaved using a validated cycle prior to incineration off site.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

### Please enter comments on the GM safety committee on the risk assessment

The risk assessment was discussed at a meeting of the Biological Agents and GM Safety Committee held on the 5th December 2013. JR was aware of other projects in the pipeline and that checks should be made to ensure that this assessment fulfils all work envisaged. JB added that a connected programme of work should be considered.

The applicant did not want to broaden the scope as it covered all the work envisaged. The risk assessent was then circulated to the committee, a number of emails corresponding to the quorum of the committee were received in agreement of the classification as a Class 2 activity.

### Project Containment

| Laboratory Activities | Glass Houses | Growth Rooms |
This project will utilize the fish pathogen *Mycobacterium marinum* to investigate the pathogenesis of Mycobacterial and related infections, in the following ways:

1. To identify *M. marinum* genes that contribute to pathogenesis and infection
2. To identify host genes (zebrafish with equivalents in humans or mammals) involved in response to or controlling the infection.
3. To explore the immune and physiological response to infection and understand how this works in the context of intracellular Mycobacterial infections.

*Mycobacterium marinum* is a fish pathogen although serious systemic infections could occur in severely compromised humans, and is classified as Hazard Group 2. Superficial infections on the extremities/exposed surfaces can occur in immunocompetent humans causing a well-recognized disease called fish-tank, aquarium-tank, or swimmer’s granuloma.

Mode of transmission is through skin, particularly from sites of abraded skin. The host range is wide. Ectotherms get systemic disseminated disease. Warm-blooded animals get superficial, localized disease on the skin of their extremities. Human disease presents as a nodular or ulcerating lesion on the site of infection.

A number of very safe, well-tolerated antibiotics can be used effectively against *M. marinum*. We have tested the strain we use formally at a reference laboratory so that we...
can have its antibiotic sensitivity profile. It is susceptible to clarithromycin, rifampicin, ethambutol among other antibiotics.

M. marinum will be used to infect human derived cell lines such as THP1, Monomac6 an NHBE cells, human induced pluripotent stem cells (hiPSC), and Zebrafish embryos up to 5 days post-fertilisation.

Host/vector system

Vectors & inserts:
1. Extrachromosomal and integrating plasmids that express various fluorescent reporters and/or the antibiotic resistance markers for kanamycin and/or hygromycin. A variety of derivatives may be used in which 1) hygromycin resistance replaces Kanamycin and/or 2) other fluorescent proteins (eg, dsRed tdTomato, wasabi) replace gfp.
2. Transposon insertions throughout the genome; transposon MycoMar encodes a kanamycin resistance cassette, and transposon MycoMme encodes hygromycin resistance cassette flanked by gamma-delta resolvase sites. Strains carrying such insertions have been previously generated and lack the transposase required for further transposon mobilization.
3. Spontaneous mutants that have antibiotic resistance to isoniazid, rifampicin
4. Extrachromosomal and integrative plasmids that are used for genetic complementation of mutants. Eg. Complementation in which the mutation has been rescued by inserting the relevant native gene back into the genome at the L5 attB site. Sequences of several such commonly used vectors are attached.
5. Marinum mutants will be generated using various well-established mutagenesis techniques including targeted approaches driven by recombination (e.g. CRISPR).

Origin & function

Green fluorescent protein and beta-galactosidase will be used as reporter genes, and antibiotic resistance genes will be used as selectable markers. Cas9 will be used to generate sequence-specific mutations via the CRISPR.

Evaluation of foreseeable effects

Wild-type M. marinum is capable of causing infections in fish and other ectotherms in the environment. Some of the mutations will compromise the virulence and the infectivity of the pathogen. An increase in virulence or infectivity is unlikely and is not selected for. During complementation tests, we will be inactivating genes, some of which will be associated with virulence but there will be no attempt to alter host range or enahnce virulence beyond the level of that seen in the wild-type strain. Mutant strains could possibly revert back to wildtype virulence potential, however it is highly unlikely that any strain would mutate to an enhanced level of virulence. Furthermore, all mutant strains are treated with the same biosafety precautions as the fully virulent wild-type strain, so in the event of a reversion to virulence, no additional risk is posed to labworkers or the environment. Although some antibiotic resistance markers may be used they will not be those encoding resistance to currently commonly used antibiotics and have defined antibiotics that can be used to treat these M. marinum.

Transfer of DNA or antibiotic resistance to other organisms is very unlikely as the genes are not on a mobilisable element and mycobacterial DNA being GC-rich and having specialized promoters does not thrive in most other organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable. Zebrafish embryos may contain mutations, but these have been generated by techniques not covered by the definitions of the Contained Use Regulations.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste contaminated with GM material will be bagged, and transported to autoclaves within the same building in closed rigid boxes within lidded trolleys. All of the autoclaves are subjected to annual inspection to written schemes, annual validation reports, and quarterly servicing. Waste runs are validated to achieve a minimum hold time of 3 minutes at 134°C, (equivalent to a 30-log reduction in viability of indicator spores). Waste inactivated by autoclaving is removed from site for incineration or deep land-fill only after examination of the autoclave run report. All liquid waste from Mycobacterium species will be subject to treatment with BioCleanse, at a minimum concentration of 5% (v/v) and minimum contact time of 1 hour.
before dilution and disposal via the drains. The degree of kill has been independently validated as a minimum of 6 log reduction in viable organisms.

The risk assessment was circulated by email to the BAGMSC on 26th August 2014.

The following comments were received:-

CS: I'm happy with the work being classified as Class 2 as it will not be carried out in the RSF (answer to B4.5 - there would be a great potential for the generation of water droplets in the zebrafish room in the RSF which might carry the pathogen, and it is definitely a ‘damp environment - B2.4).
There is justification for using lass capillary needles with care.
It is important that gloves and masks are worn when the pathogen is being handled due to the potential to infect humans.

OHP: This is ok by me. Antimycobacterial drugs can be used in pregnancy.
Just to clarify at end of document (not just immunosuppressed individuals); may need to specify in text box that if eczema on hands or forearms, need to avoid work with this organism until eczema adequately treated. (This has no been revised).

JB: I note that M. marinum is correctly classified as HG2 and am happy that the suggested GM work will not increase the risk. I am therefore in agreement with it being classified as Class 2.

JO: Happy for this to be CL2.

JR No concerns - a very well written and clear application, and risks seem well controlled. The only thing that was somewhat puzzling was mention of the human derived lines (QB3.2) will be used for - not discussed at other points in the application, which focus on infection of 0-5 day zebrafish embryos. If the intent is to try to infect human stem cells (not sure whether this is possible with M. marinum or not), this either needs to be clarified in other sections (e.g. not listed as host in overview), or be filed in a separate assessment - happy with either option. (This has now been revised).

JL: Section on autoclaving needs amending. This has now been revised.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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Animal Units

Large Scale Activities

Human Clinical Applications
Luciferase-expressing trypanosomatid parasites as a toll in vaccine screen against nagana and visceral

Protozoan parasites of the Trypanosomatidae family are the causative agents for some of the most neglected diseases such as African trypanosomiasis and leishmaniasis. These parasites have a wide range of hosts and can infect both animals and humans. African trypanosomiasis is caused by Trypanosoma spp. parasites and is transmitted through the bite of infected Tsetse fly (Glossina spp.) vectors. Our research will focus on Trypanosoma congolense and T. vivax which are non-pathogenic to human but causes trypanosomiasis (also known as nagana) in animals. Nagana affects many economically important livestock species such as cattle and goat, causing a marked impact on agriculture and development of Africa. Related trypanosomatid parasites of the genus Leishmania cause leishmaniasis which is transmitted through the bite of infected sandfly (Phlebotomus spp and Lutzomyia spp) vectors. Over 20 Leishmania species are capable of infecting humans. Leishmaniasis can be subdivided into 3 main forms (cutaneous, mucocutaneous and visceral). Visceral leishmaniasis (VL) is the most severe form of the disease and causes an annual death toll of ~50,000. Our research will focus on Leishmania donovani and L. infantum which causes VL in human.

A common problem with these neglected diseases is that no vaccine is available and their control is largely dependent on chemotherapy/chemoprophylaxis treatments, as well as insect vector control. Treatments for these diseases have inherent limitations such as severe toxicity, drug resistance and prohibitively high costs. Thus, the development vaccines for VL and nagana is considered a safe and cost-effective method to prevent and reduce the impact of these diseases in endemic areas. To identify possible vaccination candidates, libraries of recombinant parasite cell surface proteins will be expressed and used to immunise rodent models of VL and nagana. Immunised animals will subsequently be infected with parasites to identify immunogens that confer protection to infections. Accurate quantification and monitoring of parasitaemia in animals following infections will be extremely challenging and labour-intensive, with respect to the number of proteins to be screened. The use of transgenic- parasites expressing luciferase will allow us to visualise and quantify parasitaemia through bioluminescence on an In Vivo Imaging System (IVIS, Perkin Elmer) without altering the infection pathology of the parasites. It will also have the advantage that the infection can be followed longitudinally, significantly reducing the number of
animals used for this research.

**Recipient or parental organism**

The recipient organisms will be Leishmania spp that are classified as ACDP Hazard Group 3* and animal African trypanosomes which include Trypanosoma congolense and Trypanosoma vivax. These parasites are both classified as SAPO Group 2 pathogens.

**Host/vector system**

Luciferase-expressing Trypanosoma vivax parasites: Firefly luciferase-expressing T. vivax parasites will be obtained from Pasteur Institute. This parasite strain has been published and is described in Genetic engineering of Trypanosoma (Dutonella) vivax and in vitro differentiation under axenic conditions. D'Archivio S, Medina M, Cosson A, Chamond N, Rotureau B, Minoprio P, Goyard S. PLoS Negl Trop Dis. 2011 Dec;5(12):e1461.

Briefly, these parasites were generated by transfecting the wild type T. vivax strain, TvY486, with a T. vivax-specific luciferase vector which was designed to express the luciferase reporter gene and the neomycin phosphotransferase gene that confers resistance to G418.

Luciferase-expressing Leishmania donovani parasites will be obtained from the same group from the Pasteur Institute which were produced in an analogous manner.

Luciferase-expressing Trypanosoma congolense parasites: Firefly or red shifted firefly luciferase-expressing T. congolense parasites will be generated from cultivated T. congolense parasites of the reference strain IL3000 and by using an appropriate T. congolense-specific vector.

**Origin & function**

Luciferase-expressing parasites of Leishmania spp and animal African trypanosomes will either be obtained from collaborators such as colleagues working in the Pasteur Institute (Paris) or selected on site.

These genetically modified strains of leishmania and trypanosomes will be used in experimental protocols aiming at identifying new vaccine candidates against trypanosomatid infections. Systematic screenings of recombinant proteins predicted to be associated with trypanosomatid cell surfaces will be performed by immunising mice and subsequently challenge them with trypanosomatid parasites. To determine if recombinant protein immunisations confer protection against trypanosomatid infections, parasite load of infected mice will be evaluated during the infection course of visceral leishmaniasis and animal trypanosomiasis.

Luciferase-expressing trypanosomatid parasites represent efficient tools for monitoring experimental infections in real time and at the whole organism level. Their use will highly improve the sensitivity and the spatial resolution of infectious processes studies and also provide reliable means to quantify parasites in vivo. They will also reduce the number of animals required for these experiments.

**Evaluation of foreseeable effects**

Hazard/risk to human associated with the recipient parasites depends on species. Over 20 different Leishmania species are pathogenic to humans with L. donovani and L. infantum classified as ACDP Hazard Group 3*. The animal African trypanosomes (T. congolense and T. vivax) are non-pathogenic to humans, but can cause disease in livestock and are classified as SAPO Group 2 pathogens. Genetic manipulation of L. donovani and L. infantum are not expected to increase their risk to humans, and may, in fact, attenuate virulence. Leishmania parasites are transmitted to the host through the bite of an infected sandfly. This insect vector is not indigenous to the United Kingdom and colonies are not maintained at the Sanger Institute. There are three different forms of leishmaniasis dependant on the species of Leishmania. All forms result in development of sores at the site of infection and can be accompanied by cold-like symptoms. The diseases they cause range from mild self-healing skin ulcers in cutaneous leishmaniasis through to the life-threatening visceral form. The cutaneous and mucocutaneous forms of leishmaniasis are self-healing but usually result in disfiguring scars. Visceral leishmaniasis is the most severe form of the disease due to disseminated infection of the bone marrow, liver and spleen and is often fatal. There is no effective vaccination available, and treatment heavily reliant on chemotherapy. Current drugs used for treatment include meglumine antimoniate, sodium stibogluconate, amphotericin B, ketoconazole, miltefosine, paramomycin and pentamidine. All stages (promastigotes and amastigotes) of the Leishmania life-cycle are hazardous and will be treated directly. The introduction of the luciferase gene is not expected to alter the virulence, infectivity and stability of the leishmania parasites.

Genetically modified Leishmania spp should maintain the same sensitivities to the standard drugs used for the treatment of leishmaniasis both in vitro and in vivo.

Hazard/risk to human arising directly from the inserted gene product is very limited as genetically modified trypanosomatids will express the reporter gene luciferase. The
The luciferase gene is not an oncogenic gene and its gene product is not known to be harmful to health. The luciferase inserted gene should not alter the pathogenicity of the kinetoplastid parasites to humans. In addition, mobilisation of the inserted genetic material within the same species of kinetoplastids would be highly unlikely. Transfer of genetic material in Leishmania spp. have only been known to happen when the parasites are in their sandfly hosts which are not used in our studies. It is also considered very unlikely that the luciferase gene could be horizontally transferred to other organisms; however, there is a theoretical but very limited risk that the antibiotic resistance associated with the luciferase gene could cause harm through transfer to another organism.

A conceivable hazard/risk to the environment that is associated with recipient trypanosomatids exists as these parasites can infect animals. Wild-type Leishmania spp. are known to parasitise animals such as dogs and rodents. Animal African trypanosomes can infect domestic animals such as cattle, sheep, goats, pigs, horses and dogs and wild animals. It is considered very unlikely that genetically modified kinetoplastid parasites will have a different host tropism to the parent. Hazard/risk to environment arising directly from the inserted gene product is not considered a risk, since the luciferase gene does not encode for hazardous products and does not cause gene silencing of a gene encoding a crucial metabolic enzyme in susceptible hosts.

Hazard/risk to environment arising from the alteration of existing traits is also extremely unlikely as the transgene encoding luciferase does not encode for a pathogenic determinant. Luciferase expression will not confer additional cell binding properties to trypanosomatid parasites and will not alter their host tropism nor mode of transmission. Insertion and the selection process of the luciferase gene in other trypanosome species such as Trypanosoma cruzi has, in fact, led to a decrease in virulence.

Potential hazard/risk of sequences within the GMM being transferred to related microorganisms is very unlikely as luciferase-expressing trypanosomes can only survive outside the laboratory in a suitable animal host which requires specific transmission vectors (in this case sandflies and tsetse flies) which are not endemic to UK. In addition, no theoretical modes of transmission of the genetically modified parasites to the environment are known and the environment cannot support their survival.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Application for derogation from infected mice to be held within the CL3 laboratory at all times:

1. Mice infected with Leishmania to be transferred from the CL3 to be housed at CL2 if capacity is required. Mice will not be handled at any time in CL2. Cages will not be opened and only handled to be removed from racks to fulfill Home Office observation requirements. Cages will be transferred back to CL3 for cleaning out and for mice to be handled in a Class I MSC only.

2. Mice to be transferred from the CL3 laboratory to the IVIS imaging CL2 laboratory and imaged in this area. Mice will be held in a sealed container with filtered CO2 provision and extraction at all times.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste which includes tissue culture flasks, media bottles and tubes, will be pre-treated with 1% (w/v) Virkon with a contact time of at least one hour to eliminate live trypanosomatid parasites. Treatment with 1% (w/v) Virkon kills the vast majority of organisms in 10 minutes and achieves 100% kill in 1 hour. Inactivated solid waste will be placed in a robust, secure and leak-proof container and then autoclaved. Solid waste such as used tips and pipettes will be collected in plastic bags loosely closed with tape, and transferred in an appropriate container for autoclaving. Sharps and syringes will be disposed of in suitable storage containers and placed, when nearly full, in an appropriate container for autoclaving.

Solid waste that does not require a disinfectant pre-treatment step such as other disposables, gloves, paper towels or tissues will be directly placed in an appropriate container before autoclaving. Infected animal cadavers will be stored frozen in bags until fully inactivated by autoclaving.
For liquid waste decontamination, Virkon will preferentially be used as it is the disinfectant commonly in use on site. Any liquid waste will be inactivated with a 1% (w/v) final concentration Virkon solution for at least one hour. Inactivated liquid waste will be diluted 1:5 with water before being disposed of down the drain.

Any spills involving trypanosomatid parasites will be inactivated using 70% (v/v) ethanol with a contact time of at least 1 hour. The degree of ‘kill’ achieved with 5 min exposure with 70% (v/v) ethanol is 100% of trypanosomes in liquid waste. 15-17.5% ethanol (v/v) has been shown to kill trypanosomes within 5 min exposure (Wang et. al. (2008) “Efficacy of common laboratory disinfectants and heat on killing trypanosomatid parasites.” Parasites and Vectors 1:35). As a precautionary measure, squirt bottles containing 70% (v/v) ethanol will be kept within easy reach in the containment room RI-125 of the Blue suite where the infection challenges and blood samplings will be performed and in the containment 2 laboratory B315 where in vitro culture work of the parasites will be performed. Bottles containing 2% Virkon giving a final concentration of 1% (w/v) can also be used for inactivation of spills.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Amendments/clarifications

Description: Please reference the associated RA for use of the wild type trypanosome.

B3.1 Centrifugation is given as a technique for concentration, but nowhere is any mention made of sealed leak proof buckets or rotors, or where these might be opened. This needs to be added in.

B3.4: Suggest that outer carriers should be opened in the MSC as primary vessel may have broken. If these will not fit in the MSC then double containment is required inside the transport box.

B4.3.2: What is ‘rinse flow’? Is this the Virkon from the previous sentence or something that will need decontaminating?

B4.3.2: Part (a) states treatment with 1% (w/v) Virkon kills most organisms in 10mins, but 100% in one hour. Why then is exposure to Virkon in the section above only for 5 minutes?

B4.3.2: a dwell time of 5min is given for chemical disinfection, while the data presented shows that 1hr is needed for fully effective killing. Why is a shorter time being proposed for use?

B4.3.2: Also we have “pre-treatment” with no description of the subsequent “treatment” – is this autoclaving or something else?

Approved by committee at SAPO2 subject to amendments

Leishmania GM Risk Assessment reviewed by BSGMSC:
15th-22nd January 2016

Amendments/clarifications

Description: Reference risk assessment for WT Leishmania work.

B1.2: Mention of human cancer cells could be confusing. Remove the first line from this paragraph?

B2.4 (third box): The Leishmania manual states that the eyes could be a route of transmission so would need to be protected from potential aerosols – add in sentence regarding all open work in MSC.

B3.3 (second box): Further to the above, state that that eye protection is not required as no open work is performed outside of the MSC at all. Although not required the handbook and the WT risk assessment already included safety specs as a PPE. I would prefer to keep it. Added a line about safety specs in 3.3 (first box).

Approved by committee at CL3 subject to amendments

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<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
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</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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Our goal is to develop and apply methods for genome-scale genetic manipulation of two human infective parasites, *P. falciparum* and *P. knowlesi*. By experimentally modifying the sequence or expression levels of specific Plasmodium genes, we will be able to generate and test hypotheses about the role of these genes in general parasite viability and fitness, as well as specific phenotypes including erythrocyte invasion, drug resistance, sexual development and transmission to mosquitoes, as well as other aspects of malaria biology. A better understanding of these critical interactions may lead directly to the development of new prevention and control measures such as drugs or vaccines.

This work covers the generation, propagation, and transmission of genetically modified *Plasmodium falciparum* and *Plasmodium knowlesi* parasites. Manipulations will include tagging with epitope tags or fluorescent proteins (e.g. GFP), gene knockout, and CRISPR/Cas genome editing. We will also explore approaches to increase or repress gene expression, including CRISPR/Cas approaches called CRISPR-i and CRISPR-a, as well as promoter modification and replacement, and overexpression from extrachromosomal elements.

Constructs containing modified Plasmodium genes or fragments of such genes will be generated and propagated in *E. coli* (vector generation is covered under a separate and linked GMM risk assessment). Constructs will then be introduced into Plasmodium strains by transfection and selected for using drugs. Constructs will either be maintained as episomes, or will integrate in a targeted manner into the Plasmodium genome. For CRISPR/Cas genome editing, parasites will be co-transfected with a plasmid to express the Cas9 endonuclease and specific guide RNA (gRNA), as well as a gene targeting vector. Note that Plasmodium parasites lack the non-homologous end-joining DNA repair (NHEJ) pathway, meaning that co-expression of Cas9 and gRNAs alone would not normally induce modifications in the genome. To facilitate NHEJ-mediated gene disruption, the two component system from bacteria will be introduced. To use CRISPR/Cas to regulate gene expression (CRISPR-a and CRISPR-i), we will first create parasite lines expressing a nuclease-dead version of Cas9 into which we will introduce either individual or pools of gRNA-expression plasmids to target Cas9 to specific regions of the genome.
The effect of genetic modification will be assayed using standard cell biology (growth rate, microscopy, invasion assays, flow cytometry), biochemical (immunoprecipitation, protein detection, mass spectrometry), or biological (mosquito susceptibility) techniques. Phenotyping of live GM \( P. \) falciparum parasites will only be carried out in approved \( P. \) falciparum CL3 facilities; all other phenotyping will use material that has been inactivated using documented validated methods, in accordance with the approaches used for non-GM \( P. \) falciparum parasites.

**Recipient or parental organism**

\( \text{Plasmodium falciparum} \)

\( \text{Plasmodium knowlesi} \)

**Host/vector system**

**Hosts:**

\( \text{Plasmodium falciparum} \) parasite strains. Standard lab-adapted strains will be used, primarily 3D7, as that is the strain from which the \( P. \) falciparum reference genome sequence was generated, or NF54, the parental strain of 3D7. In certain limited circumstances other lab-adapted strains will be used in place of 3D7, such as 7G8, HB3, DD2, or strains recently adapted from clinical isolates. In some cases these strains may be resistant to one of more currently used antimalarial drugs, but none are resistant to all frontline drugs so clinical treatment options are always available.

\( \text{Plasmodium knowlesi} \) parasite strains. Currently only a single \( P. \) knowlesi line is available for in vitro culture in human erythrocytes (PMID: 23267069); if others become available they will be tested and used if they have advantages, such as increased transfection efficiency. There is no currently known drug resistance in \( P. \) knowlesi parasites, and the in vitro cultured line is susceptible to all current frontline antimalarials.

**Vectors:**

\( \text{Plasmodium} \) transfection vectors are not commercially available, although some previously produced vectors are available from ATCC through the Malaria Research and Reference Reagent Resource Center (www.mr4.org). Vectors to be used in this study will be obtained from this source, or from colleagues, then adapted to contain the gene or gene fragments of interest. Essentially all vectors consist of three elements: antibiotic resistance gene with associated expression elements to allow propagation in \( E. \) coli (usually encoding Ampicillin or Kanamycin resistance); drug resistance cassette with associated \( \text{Plasmodium} \) expression elements to allow positive selection in \( P. \) falciparum and \( P. \) knowlesi (usually anti-folate drug resistance encoded by \( T. \) gondii or \( H. \) sapiens DHFR, or genes that enable resistance to Blasticidin or Gentamicin); an expression or gene targeting cassette to either express the gene of interest in \( \text{Plasmodium} \) falciparum or \( P. \) knowlesi or direct insertion of the construct to a specific genomic location in order to ablate or modify the endogenous gene. The vectors may be based on a number of scaffolds, including the \( \text{pJazz linear vector system} \) (Lucigen) that can accommodate large genomic inserts (on average ~8 kb). For \( \text{CRISPR/Cas} \) mediated genome editing, two additional components will be included – a cassette expressing Cas9 endonuclease and a cassette expressing target-specific gRNA. We currently plan to include both Cas9 and gRNA cassettes on a single vector that will be co-transfected into parasites together with a “donor homology” region supplied on a second vector (e.g. \( \text{pJazz linear vector} \) or conventional circular plasmid) or as a PCR product, which will direct the desired genome modification. However, alternative designs incorporating the gRNA into the donor homology construct are also possible.

**Origin & function**

**Inserts:**

Inserts will include the general vector components noted above – antibiotic resistance genes (Ampicillin or Kanamycin) for plasmid propagation in \( E. \) coli, selectable markers (DHFR, Blasticidin S Deaminase, or neo) for positive selection in \( P. \) falciparum and \( P. \) knowlesi, fluorescent or bioluminescent proteins (including GFP, mRFP, mCherry, BFP, iLOV, firefly luciferase, Nanoluc), and \( \text{Plasmodium} \) promoter regions (including PfCam, PbEF1alpha, PfSec12, PfHsp86, PfBip, among others).

CRISPR/Cas elements include the Cas9 nuclease, or the nuclease-dead version of dCas9 in isolation or as a fusion with a transactivation domain (for CRISPR-a applications), and the gRNA sequences (20 nucleotides of variable sequence to target Cas9).

In most organisms, CRISPR-mediated gene disruption uses the error-prone NHEJ pathway, which has been lost in \( P. \) falciparum. To enable this method, which would bypass the need for donor homology templates for each target gene, a well-characterised bacterial NHEJ system will be expressed.
A variety of Plasmodium falciparum or P. knowlesi inserts will be used, depending on the experimental goal. These will be either large genomic inserts of 4-8 kb in linear pJazz vectors, the complete coding region of an individual gene (e.g. as a fusion with a reporter protein for localization experiments), or partial fragments of genes, to serve as donor homology regions for gene knockout or gene editing. We aim to scale up these studies to the whole genome level, so genes and/or gene fragments incorporating every gene in the P. falciparum and P. knowlesi genome may be included.

**Evaluation of foreseeable effects**

Plasmodium falciparum is classified by the ACDP as a Hazard Group 3 human pathogen. Plasmodium knowlesi is classified as Hazard Group 2. Both organisms will be cultured in the same facility and under the same controls and procedures. The only way to infect humans in the lab is by injection into human blood circulation or by a bite from a late-stage (10 days+ post bloodmeal) infected mosquito, and there is no evidence for aerosol-based transmission. Therefore, sharps are banned in the lab, with the exception of glass microscope slides, used in a standardised process that inactivates the parasite. Work with P. falciparum infected mosquitoes is restricted to a high security CL3 insectary, where the mosquitoes are in double containment at all times, the only exception to this being during the feeding procedure, where they are singly contained. If infection did occur, there are several standard antimarial drugs that can be used for treatment. No vaccine is available.

GM Plasmodium strains will have no increased ability to infect humans relative to non-GM strains. In some GM parasites a product will be expressed, for example fluorescent or luciferase-based reporters, either individually or fused to parasite proteins. These products are unlikely to be biologically active in humans. There is no evidence that P. falciparum or P. knowlesi proteins can act as toxins or allergens in humans or can act as oncogenes.

Gene inserts could theoretically alter the pathogenicity of the modified line relative to the parental parasite line. However in the majority of cases these modifications will generate GM parasites that are essentially equivalent to non-GM lab or field strains or are less fit than their wild-type counterparts. Introduction of the transfection vectors will make the GM parasite resistant to one or more drugs used for positive selection (typically the antifolate WR99210, Blasticidin, or G418). However, none of these drugs used for plasmid selection in Plasmodium parasites are in wide use as frontline anti-malarials. In the case of introducing the bacterial NHEJ genes, there is no expectation that the NHEJ machinery will alter pathogenicity of the parasite, and the genes themselves are not associated with pathogenicity.

P. falciparum and P. knowlesi are only able to infect humans and certain species of non-human primates. Given the inability of free-living parasites to survive in the environment, and the absence of non-human primates from the Institute or its surroundings, there is no risk of release of genetically modified parasites to the environment. GM parasite stocks held in liquid nitrogen are not infectious in that state. The risk of releasing mosquitoes that are infected with GM parasites is negligible because we only work with infected mosquitoes in our CL3 insectary. This unit is highly secure requiring swipe card and PIN code to gain access, which is only granted to several highly skilled users who have gone through specific induction procedures. Infected mosquitoes within the unit are held within two containers, thus providing added security in the unlikely event of one containment method failing. In a brief moment, mosquitoes that have just been fed on parasites are briefly out of secondary containment, but these mosquitoes would take 10 more days to become infectious and would be highly unlikely to survive for 10 days in the insectary where there is no access to sugar or water. Additionally, infectious feeds take place beyond an additional door within the CL3 insectary that acts as a further barrier. Infected mosquitoes never leave the CL3 insectary while alive, so present negligible risk to the public. In the extremely unlikely case of an escape of a mosquito that carries GM parasites, the ability of the mosquito species we work with to transmit parasites requires temperature (26°C) and humidity (80%) conditions rarely seen in England.

Further detail is provided on the GM Risk Assessment 'Plasmodium experimental genetics: GM Plasmodium falciparum and Plasmodium knowlesi parasites'

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We request derogation from the following requirements:
- To carry out this activity in a laboratory sealable for fumigation
- To hold the laboratories at negative pressure relative to the pressure of the immediate surroundings
- An autoclave to be required in the laboratory suite
- Extract air to be HEPA filtered
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste in contact with P. falciparum and P. knowlesi culture whether GM or not, will be disinfected via approved methods and disposed of using WTGC waste policies.

Disinfection is primarily carried out with 1% Virkon for 20 minutes, but in certain circumstances (namely, when downstream work involves infecting mosquitoes as even slight Virkon exposure is detrimental to those stages) 5 minutes exposure to 5g/L Klorsept is used as an alternative. Both treatments have been validated to kill parasites and render them uninfectious, and specifics are listed in the CL3 biological safety procedures.

All biological waste that cannot be disinfected (e.g. blood bags) and all solid waste from the CL3 will be autoclaved prior to final disposal. Once a biohazard bag is full, the neck of the bag is folded over and the bag placed in an approved rigid sealed metal waste bin. All workers wear appropriate PPE when handling waste. Sealed full bins are kept inside the CL3 laboratory suites until ready to be collected in the designated waste area in the CL2 by the Waste Team.

On arrival of the waste team, the CL3 metal waste bins are passed to them, and taken in a sealed trolley for immediate autoclaving in the waste autoclaves in the same building. The trolley does not contain any other forms of waste during this operation.

All of the autoclaves are subjected to annual inspection to written schemes, annual validation reports, and quarterly servicing. Waste runs are validated to achieve a minimum hold time of 3 minutes at 134C, equivalent to a 30-log reduction in viability of indicator spores.

Waste inactivated by autoclaving is removed from site for destruction by incineration only after examination of the autoclave run report.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The Chair declared a conflict of interest as he may collaborate with the applicant in the future. The Deputy Chair therefore took over the chairing of this discussion. The Deputy Chair asked that the Chair be included in the discussions as the committee would benefit from his expertise.

Amendments:
Where appropriate, please cross reference relevant risk assessments covering CL3* Plasmodium and mosquito transmission work in general. You should include WT BA risk assessment for plasmodium and any other relevant plasmodium GM risk assessments, also the CRISPR connected program.

B2.4 Species of Anopheles mosquitoes have lived and bred in the wild in the East of England and in the past have been known to be infected by Malaria parasites. While the committee agrees that adequate measures are in place to prevent the release of mosquitos from the laboratory, the text needs to be amended to reflect this in this section (highlighted in pink).

B2.4 It is stated that mosquitos are double contained at all times – this can't be true for when mosquitos are actually handled. Please rephrase text appropriately.

B4.3.3: Where disinfectant is referred to as ‘bleach’ please name the disinfectant being used and indicate the concentration and contact time required for disinfection.

B4.3.3 Also indicate concentration and contact time for Virkon S

Typos
B1.2: ‘These products are unlikely to be biologically active…’
B3.4: ‘cryobank facilities in G140/141’ (not ‘in the G140/141’).

The committee commented that this was a well thought out and written risk assessment and that there didn’t seem to be any additional risks using GM parasites compared to the risks associated with using the wild-type parasites.

Approved at Containment Level 3* subject to amendments

Notification to HSE Required

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**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
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</thead>
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**Project Ref** 552/20.1

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Study of maternal-fetal interactions in placental (trophoblast) organoids and primary placenta/decidua cells pre and post wild-type (WT) and genetically-modified (GM) Plasmodium falciparum infection

GM Plasmodium falciparum parasites will be generated in order to monitor the infection of the placental organoids and primary cells from placenta and decidua. We propose to profile the transcriptome of the host (placental organoids, primary cells) and the fluorescence-labeled GM parasites during the course of infection by single-cell transcriptome profiling. This study of malaria parasite co-infection using human primary placenta organoid model and primary fetal and maternal cells will enable us to better understand the critical host-pathogen interaction mechanism that may directly lead to the development and/or control measures for new drugs and vaccine targets.

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### Project Additional Information

**Purposes of the contained use**

GM Plasmodium falciparum parasites will be generated in order to monitor the infection of the placental organoids and primary cells from placenta and decidua. We propose to profile the transcriptome of the host (placental organoids, primary cells) and the fluorescence-labeled GM parasites during the course of infection by single-cell transcriptome profiling. This study of malaria parasite co-infection using human primary placenta organoid model and primary fetal and maternal cells will enable us to better understand the critical host-pathogen interaction mechanism that may directly lead to the development and/or control measures for new drugs and vaccine targets.

**Recipient or parental organism**

Plasmodium falciparum

**Host/vector system**

Plasmodium transfection vector are not commercially available. Vectors are available from American Type Culture connection BEI resources (https://www.beiresources.org/Home.aspx) and vectors to be used in this study will be obtained from this source or from research colleagues.

**Origin & function**

Inserts or "genetic material" involved include the general vector components; antibiotic resistance genes (Ampicillin or Kanamycin) for plasmid propagation in E. coli, selectable markers (DHFR, Blasticidin S Deaminase, or neo) for positive selection in P. falciparum, fluorescent or bioluminescent proteins (including GFP, mRFP, mCherry, BFP, iLOV, firefly luciferase, Nanoluc), and Plasmodium promoter regions (including PfCam, PbEF1alpha, PfSec12, PfHsp86, PfBip, among others).

**Evaluation of foreseeable effects**

The inserted gene product will be expressed in GM P. falciparum infected red blood cells. These include the fluorescence reporter gene either individually or fused to parasite proteins. If using the CRISPR-cas9 approach, the Cas9 nuclease will be integrated and expressed within the parasite to generate double-strand breaks in the parasite genome. These products/proteins are not known to be biologically active in humans because Plasmodium-specific expression sequences and regulation is unique and very divergent from the human gene expression and regulation system. There is no evidence that P. falciparum proteins (either alone or fluorescence-tagged) can act as a toxin or allergen in humans.
Drug resistance cassette with associated Plasmodium expression elements to allow positive selection in P. falciparum (usually anti-folate drug resistance encoded by T. gondii or H. sapiens DHFR, or genes that enable resistance to Blasticidin or Gentamicin). An expression or gene targeting cassette to either express the gene of interest in P. falciparum or direct insertion of the construct to a specific genomic location in order to ablate or modify the endogenous gene. For CRISPR/Cas mediated genome editing, two additional components will be included: a cassette expressing Cas9 endonuclease and a cassette expressing target-specific gRNA. The planned GM parasites fused with a reporter construct will be resistant to the vector drug selection cassette, but this will typically not alter the overall virulence of the parasites. In the case of introducing the bacterial Cas9 NHEJ (non-homologous end joining) genes, there is no expectation that the NHEJ machinery or process is involved in pathogenicity as the overall impact on the parasites will be either to cause disruptive insertion or deletions. Therefore, GM changes will predominantly be neutral, or if any, result in a negative impact on pathogenicity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

B320, B319 and B213, are CL3 laboratories with approved structure and/or process derogations. These rooms are designed to comply with all of the requirements of CL3 with the following exceptions, which have been agreed by the Health & Safety Executive (HSE) on 18th July 2016:-

- The laboratories are not sealable for fumigation
- An autoclave is not present in either lab


Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste in contact with P. falciparum culture will be disinfected via approved methods and disposed of using WSI waste policies. Disinfection is primarily carried out with 1% (w/v) Virkon (final concentration) for 20 minutes. Treatments have been validated and specifics are listed in the CL3 laboratory handbook.

Sharps

Used slides and Femtotips® will be soaked in 100% methanol to inactivate any live parasite and then disposed in appropriate sharp bins which are then autoclaved as solid waste.

All biological waste that cannot be disinfected (e.g. blood bags) and all solid waste from the CL3* will be autoclaved prior to final disposal. Once a biohazard bag is full, the neck of the bag is folded over and the bag placed in an approved sealed metal waste bin. All workers wear appropriate PPE when handling waste. Sealed full bins should be kept inside the CL3* laboratory suites until ready to be collected in the designated waste area in the CL2 by the Waste Team.

Spillage containment kits will be maintained in the derogated CL3 room. MSCs will be disinfected after every use and given a final clean before shut down each day.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Risk assessment reviewed by Biological Safety Committee at meeting on 17th October 2019.

Comments / Amendments

Use of sharps in CL3 – the team is planning to use a microinjector for this piece of work and one member of staff will be trained to use it. The risk of a stick injury will be minimised by staff wearing cut proof gloves and the use of disposable microinjection glass capillaries.

Suggested that the microinjector should be used on a non-vibrating bench and the fact that the sensitivity of the microinjector may make the time allowed for work to take place quite restricted.

Brief description: ‘vector generation is covered under a separate and linked GMM risk assessment’ Please include reference.

1.2: Human blood, organoids & E.coli should be included in this section.
Included, please refer to section 1.2.

B1.1, box 1: No work is covered by SIBAGMRA09_BLOOD+TISSUEv3, this is a guidance document only.

B1.1, box1: Primary cells (decidual and placenta cells) & Human trophoblast organoid cultures from placenta are not required here as you have already stated in Brief description that these are covered by SIBAGMRA19_46.

B1.1, box1: Incorrect statement ‘Any risks associated with research involving the use of GM P. falciparum parasites are covered by risk assessment number SIBAGMRA15_37v2’. This risk assessment covers your team for these risks; you cannot work under another teams risk assessment.

B1.1: The risk from blood should be discussed throughout this section use SIBAGMRA09_BLOOD+TISSUEv3 for guidance.

B1.1, box 3: Reword the following as does not make sense ‘ensure processes Receive additional monitoring (if deemed necessary) and all risk is understood.’

B2.4, box 3: Include here that insectary is a separate area to the microscope room where work will be carried out.

B3.1, box 1: Large volume is this correct or a typo?

B3.3, box 1: It is not clear what the tweezers will be used for, ‘Using a tweezer’ this needs to be clarified.

B3.3, box 1: Latex gloves are not permitted on site please replace with nitrile.
B3.3, box 2: In the section that refers to using the centrifuge, it needs to be included that the sealed leak proof buckets are only opened inside an MSC.

B3.4, box 3: Include that the secondary transport container will contain enough absorbent material to absorb the full volume should a spill occur.

B4.5: Reference specific procedure by replacing ‘CL3 biological safety procedures’ with ‘CL3 laboratory handbook’.

B4.5: Add sharps disposal information.

B4.7: Reword first sentence so it excludes work rather than describing what is included.

B4.7: Exclude work above a specific volume of parasite culture.

B5: Please confirm that this risk assessment has been read and approved by team leader.

C1: Emergency procedures need to be more specific especially regarding glass breakage. Add further information regarding glass clean up, ensuring that any pieces of glass were wiped down with wet tissue and Klorsept. The person undertaking the cleaning should also wear the appropriate gloves.

Approved at Containment Level 3 subject to amendments and HSE approval

**Project Containment**

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02/03/2022
GM Centre Number: 553

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Name

| UNIVERSITY OF OXFORD |

Name 2

| UNIVERSITY OFFICES |

Campus Estate or Research Centre

| Building |

Road Name

| WELLINGTON SQUARE |

Town

| OXFORD |

County

| OXFORDSHIRE |

Postcode

| OX1 2JD |

Country

| ENGLAND |

Tel Number

| 01865 270811 |

Fax Number

| 01865 270816 |

E-mail

|                       |

HSE Division

| EAST AND SOUTH EAST |

Comments

Date at Which Additional Info Submitted

| 19/10/2000 | 30/01/2001 | 03/07/2001 | 30/07/2001 | 15/11/2001 |
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</table>
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 2 (GMMs)</td>
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Tick if confidential

Bacteriology  Parasitology  Transgenic  Microbiology
Birds  Research
Virology  Transgenic  Transgenic  Gene Therapy
Animals  Fish
Mycology  Transgenic  Transgenic  Other (please specify below)
Invertebrates  Plants
Other(s)  

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Please enter comments of the GM safety committee on the risk assessment

### Project Ref 116/05.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<tbody>
<tr>
<td>16/09/2017</td>
<td>Genesis of lentiviral Hepatitis C Virus (HCV) pseudotypes and Genesis of HCV DNA and RNA* (*which will eventually lead on to work at CL3 - this is to be submitted as a separate notification in due course when the facilities are ready locally)</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Not Applicable</td>
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</tbody>
</table>

#### Purposes of the contained use

Genesis of lentiviral HCV pseudotypes: To investigate how HCV infects liver cells and the role of the humoral immune response plays in controlling HCV replication. This assessment covers the generation of lentiviral pseudotypes using a disabled lentiviral vector by transient expression of HCV gps from an independent promoter.  

Genesis of HCV DNA and RNA: This seeks to generate synthetic HCV strain JFH RNA for subsequent recovery at CL3.

---

#### Host/vector system

**Genesis of lentiviral HCV pseudotypes:** Hosts include E. coli (Top 10 strains, Invitrogen); 293T embryonal kidney cell line; Human hepatoma cell lines (HepG2, Huh-7, HepH, HH29); Primary hepatocytes.

**Genesis of HCV DNA and RNA:** E. coli (JM 109 derivative strain, Top10, Invitrogen).
Genesis of lentiviral HCV pseudotypes: Vectors - pCDNA3 and pNL4.3R-E-lentiviral vector (based on a lab strain of HIV-1 from which the vpr and envelope genes have been deleted).

Genesis of HCV DNA and RNA: Vector - pUC19 (Stratagene).

Origin & function

Genesis of lentiviral HCV pseudotypes: Insert - HCV envelope gps - The E1E2 gps mediate viral attachment and fusion of the viral and host cell membranes necessary for infection of a target cell. The E1E2 region will be PCR amplified from clinical material and synthetic start and stop codons added to enable expression. Donor - Human (plasma samples and liver biopsies).

Genesis of HCV DNA and RNA: Insert - HCV DNA encoding the full length genomic sequence is not infectious for chimpanzees. However, synthetic RNA derived from plasmid DNA can lead to infection and seroconversion if delivered directly into the liver of a chimpanzee. The JFH strain of HCV will be used at this time. Donor - Human (plasma samples and liver biopsies).

Evaluation of foreseeable effects

Genesis of lentiviral HCV pseudotypes: 1) Transfected 'producer' 293T - Transfection of the pE1E2 expression constructs into 293T cells will result in the expression of HCV gps, which pose minimal risk to human health. Co-transfection of 293T cells with the lentiviral pNL4.3R-E- and vectors encoding the viral gps (HCV, VSV and MLV) will lead to expression of the reporter gene luciferase and the HIV proteins (gag, pol) which will drive assembly of pseudotyped particles. None of these viral proteins or luciferase have been associated with toxicity. 2) Lentiviral pseudotype infected target cells - The pseudotypes can only undergo a single cycle of infection and so the 'infected' target cells do not release new rounds of progeny virus. These cells do express lentiviral proteins and luciferase, which have no reported toxicity for humans. Accidental exposure by injection of these antigen expressing cells could lead to an immune response to the viral expressed genes. Whilst not harmful the detection of these immune responses is used in diagnosis of HIV infection, and so could lead to a false positive. Risks will be contained by handling materials at CL2. Environmental risks - The E coli hosts used are disabled and require a supplemented media for growth. The 293T and hepatoma cell lines are only viable within tissue culture media and in the presence of CO2. The pseudotyped viral particles are not desiccation resistant and are incapable of replication. No risk to the environment.

Genesis of HCV DNA and RNA: HCV JFH plasmids bearing E1E2 sequences will constitute new GMOs. The E1E2 ORF(s) will be derived from naturally occurring sequences present within infected patients and will be unlikely to possess biologic properties not already present within nature. DNA encoding HCV genomic length sequences are not infectious for chimpanzees and constitute minimal risk to human health. Synthetic HCV genomic RNA transcripts can be infectious for the chimpanzee if delivered intrahepatically. Other routes of delivery have not led to infection. Consequently the risk to human health at generating the RNA is minimal and can be contained at CL2. All experiments involving the delivery of the RNA into mammalian cells will take place at CL3 (to be submitted in due course). Environmental risk - The E. coli strains are disabled and are transfected using non-mobilisable vectors. The naked DNA generated by in vitro transcription will be unprotected from environmental RNAases and will degrade rapidly if released.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Surfaces to be wiped with 10% Trigene which has been shown to be an effective agent for inactivation of a wide range of enveloped viruses. Disposable plasticware will be rinsed in 10% Trigene, autoclaved and disposed of as clinical waste by incineration. Disposable gloves, paper towels etc will be deposited in autoclave bags and autoclaved before disposal.
Centrifuges and other equipment will be disinfected by wiping with 10% Trigene. If necessary the buckets can be totally immersed in this disinfectant.

Autoclaving of waste achieves 100% kill.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Originally the assessments were circulated in a different format with different titles, but the GMSC felt that those assessments were repetative and lacking in clarity. Comments were passed back to the Pis and meetings were held to discuss the comments in detail. The assessments were revised and recirculated for final approval. The GMSC has now approved the work.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>Human Clinical Applications</td>
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Project Ref 116/05.2

Date Ackn'd: 16/09/2017

CU2 Project Title: Cell culture propagation of Hepatitis C virus:

Class: Class 3

Culture Volume: <50mls

Non-GMM Consent Granted: Yes

Project notified under transitional arrangements: [N]

Tick if notifying a connected programme of work: [N]
**Project Additional Information**

**Purposes of the contained use**

This proposal seeks to deliver HCV strain JFH RNA into human liver derived cells and to characterise the resulting particles. This follows on from the recently notified Class 2 proposals. "Genesis of HCV virus DNA and RNA" & "Genesis of lentivirus pseudotype particles bearing HCV glycoproteins". This proposal includes expression of HCV strain JFH in human liver cells. Translation of viral RNA will lead to expression of HCV proteins, particle assembly and release.

**Recipient or parental organism**

As section below.

**Host/vector system**

Host: Primary and transformed human liver cells: screened and found to be negative for known human pathogens (HIV, HBV etc). After RNA transfection, the cells will be monitored for HCV infection for up to 14 days before being discarded.

Vector: The JFH strain of HCV is the only known HCV sequence capable of autonomous replication in hepatoma cell lines without adaptive mutation. Recent experiments suggest that the JFh strain can assemble and release viral particles at low titre in cell culture, which can be passaged in human cells. It is not known if these particles are infectious for humans.

**Origin & function**

Donor: HCV within clinical material from infected patients.

Genetic material: The investigators plan to transflect synthetic HCV RNA generated by run off transcription into liver cells to generate virus particles. Construction of these HCV genomes has been described in previously notified proposals. Plasmid DNA encoding the full length HCV genomic sequence is not infectious for chimpanzees. However, synthetic RNA derived from this DNA can lead to infection and seroconversion if delivered directly into the liver of a chimpanzee (Kolykhalov 1997).

**Evaluation of foreseeable effects**

Hazards to human health: Transfection and delivery of HCV RNA transcripts into human liver cells will lead to the expression of viral proteins which will result in the assembly of viral particles. Plasmid DNA encoding HCV JFH with heterologous E1E2 sequences will constitute new GMOs. The E1E2 ORF(s) will be derived from naturally occurring sequences present within infected patients and will be unlikely to possess biological properties not already present in nature. Synthetic HCV genomic RNA transcripts can be infectious for the chimpanzee if high doses are delivered intra-hepatically. Other routes of HCV RNA delivery have not led to infection. All experiments involving the delivery of genomic RNA into mammalian cells will take place under CL3 containment.
Environmental Hazards: The GMOs generated by this work pose no threat to the environment. The eukaryotic cells and cell lines used will be unable to survive in the external environment. The naked DNA used in electroporation will be unprotected from environmental RNAases and would be degraded rapidly if released into the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation - Fu

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 165/10.1

Date Ackn’d CU2 Project Title 21/07/2011 Lentiviral expression systems as a tool for studying intracellular signalling pathways in

Class CultureVol Class 2 CultureVol Class 3-4

Class 2 ≤ 1 Litre
This is a study undertaken in order to investigate the mechanisms of pro and anti-inflammatory signaling in immune cells such as macrophages, dendritic cells and lymphocytes.

We have previously generated lentivirus particles using 3rd generation transfer vectors and a 3rd generation packaging system. This system is designed to minimise the possibility of replication competent virus particles arising during lentivirus production, where the gag/pol and rev gene products are expressed from separate plasmids that are co-expressed with the envelope (VSVg) vector following co-transfection with the lentiviral backbone vector into packaging cells (KEK293T/17). Substitution of the HIV-1 envelope protein with the Vesicular Stomatitis Virus envelope glycoprotein (VSV G) not only results in the production of a higher virus titre with a significantly broadened host cell range but also contributes to an enhanced safety of the system. The resulting retrovirus is replication-defective. However, the improved safety features of 3rd generation packaging systems are off-set by considerably lower virus titre yields obtained with this configuration. Therefore to improve viral titres we obtained a 2nd generation packaging system from the Trono Lab (Switzerland), in this case, all the auxiliary genes are expressed from one plasmid, which also incorporates the transactivator gene tat (absent in the 3rd generation system), namely psPAX2 or PDCMV8.91. Together with the envelope protein encoding plasmid (pMD2.G) these 2nd generation packaging systems are compatible with packaging of 3rd generation lentiviral transfer vectors containing SIN LTRs and are widely used to produce small scale amounts of lentiviral particles of in vitro transduction of primary mammalian cells.

Viruses will be assembled and packaged in the HEK293T/17 cell line and the conditioned supernatants (containing mature lentivirus) will be concentrated by centrifugation and titred prior to use in experiments. Virus titres will be determined using a FACs-based assay using either GFP fluorescence assay or labelled tNFGR (truncated nerve growth factor receptor) marker genes to calculate the amount of lentivirus infectious units. This will be carried out in HT1080, Jurkat and SupT1 for 3-6 weeks and monitoring the culture supernatants for p24 expression by ELISA. A positive control for p24 protein will be incorporated in this assay. Once generated, lentiviruses will be used to infect a range of cell lines and primary cells over a range of MOI (multiplicity of infection). In the first instance we plan to study innate immune receptors, including TREM-1, DAP12, CD38, CD164, transmembrane protein123 (TMEM123), TLRs, tyrosine kinases., IRF family memebrs, NFKB signalling components (e.g. Myd88), p38 MAP kinase, and reporter genes such as TNF-luc, IL-6-luc, Stat3-luc. All these targets represent non-oncogenic, non-secreted cell components.

Recipient or parental organism

293T/17 cells (highly transfectable cell line derived from the 293T cell line established from human embryonic kidney cells)
RAW 264.7 (murine macrophage line)
HeLa (human epithelial carcinoma cell line)
HT1080 (human fibrosarcoma cell line)
MonoMac6 (monocytic cell line)
SUP T -1 (human T Lymphoblast cell line)
HUVECS (Human Umbilical Vein Endothelial Cells)

Primary human monocytes, acrophages, dendritic cells osteoclasts & lymphocytes derived from elutriated blood packs
Cells derived from dissociated human synovial membrane tissue taken from rheumatoid arthritis or osteoarthritis patients (primarily fibroblasts, monocytes, macrophages, dendritic cells & lymphocytes)
Alveolar macrophages from sarcoid patients

**Host/vector system**

pENTR/U6 vector
pENTR/4.1 vector
pRRLsin.PPTshCMV.GFPpre vector
pCMV-GIN-ZEO gateway vector
pCMV-GIN-ZEO gateway rc vector
pCMV-GIN-ZEO 234 gateway
pLentiPPT-DEST
p156RsinPPT.hCMV-GFP.WPRE (LV#5)
LV#5GR (gateway adapted LV#5)
pLent6/BLOCK-IT-DEST vector
pLP1 (packaging vector)
pLP2 (packaging vector)
pLP/VSVG (packaging vector-envelope protein)
pNTAP
pENTR 4.3
pCMVTag2
PGCsamln (retrovirus)
PcSGW
pMCsg
SMARTvector
pENTR4
pCCL-NGFR
pFELIX
pAGM
pscALPS
SIV3+
pSAX2
pMD.G
pDCMV8.91
pMD2.G

**Origin & function**

The gene and promoter sequences used are of human and rat origin. We have constructed lentiviral transfer vectors encoding (1) reporter genes (e.g. TNF-luciferase); (2) cDNAs (e.g., human Foxp3, p38) and; (3) shRNAs 9p38):
(1) Reporter genes will be used to measure promoter activities of the TNFα, IL-6 and Stat-3 genes in primary human immune cells (monocyte/macrophages, T cells). Constructs will contain the promoter(s) and deletion mutants thereof in the presence and absence of the 3′ UTR (untranslated region). These experiments will be short-term and result in destruction of target (infected) cells. We will also perform short-term (8-hour) co-culture experiments with adenovirus-infected primary human macrophages (expressing TNF-luciferase reporter genes) and primary human CD4 T cells transduced with Foxp3-expressing lentivirus. Macrophages will be infected with adenovirus for 1 hour and cultured for 24 hours in the absence of virus. Similarly, VD4 T cells will be transduced with lentivirus 14 days prior to co-culture and washed extensively prior to co-culture. The end-points for these experiments will necessitate cell lysis, resulting in destruction of the target (infected) cells. Lysates will be frozen prior to subsequent luciferase activity determination.

(2) Short-term (<4 weeks) transgene expression in human and rat immune cells using lentiviral vectors will be employed to study the role of human genes Foxp3, TREM-1, DAP12, CD38, CD164, transmembrane protein 123 (Tmem123), TLRs, tyrosine kinases, IRF family members, NFKB signaling components (e.g. Myd88), p38 MAP kinase and rat CD16A.

Immortalized human cell lines will be transduced and selected for stable expression of Foxp3. Such cell lines will be tested for replication competency using the p24 ELISA method, and all handling and disposal will be as outlined in the associated GM risk assessment.

(3) Lentiviral vectors expressing shRNA sequences (targeting the same genes as in (2) - above) and embedded in the miR30 backbone will be employed to perform specific gene knockdown in target cell populations.

The above-mentioned gene sequences will be inserted into lentiviral vectors in order to infect human and rat cells of the immune system.

**Evaluation of foreseeable effects**

None of these gene inserts transfer vector(s) should present a hazard. One of the transgenes, Jund is a member of the proto-oncogene Jun family however it is an atypical member as it has a negative effect on cell proliferation and thereby poses little risk (Hernandes et al., 2008; PGM081, section2).

The WPRE sequence located in transfer vectors encodes the potentially oncogenic X-protein sequence. There is a risk of recombination with other viruses and/or latent viral sequences in the target cells although the lentivirus produced is replication incompetent. Routine testing for replication competency of all virus produced will be performed to assess for this possibility and any such virus will be destroyed.

The use of Class II microbiological safety cabinets for II procedures involving the handling of lentivirus vectors will minimize the risk of accidental exposure to workers should a replication competent lentivirus arises during virus production and/or use.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste: inactivation by the addition of Microsol3 to a final concentration of 10%, or 1% (final concentration) Trigene/Virkon, as per manufacturers’ instructions. The effectiveness of each reagent is checked on any change in procedure. Inactivated waste will be disposed of via sink into the drains.

Solid waste: plasticware such as plates, culture flasks, are double bagged and either autoclaved at 136ºC for 6 minutes under vacuum. The run number is recorded and chart paper traces and printouts kept. The autoclaves are serviced bi-annually and validated yearly by contract using representatives dummy loads in order to check that all areas of the load are reaching the required temperatures. Autoclaved waste then proceeds via the Imperial College clinical waste route.
Part 1: PI CID number missing and the GM Centre is: GM165.
1.22: Change last strain to: Murine bone-marrow-derived cells.
1.25: Third before last paragraph, Typo: 'tehse'
1.3: Please answer the questions in this section
2.1.1.2: Associated hazards: The WPRE element has oncogenic potential and then there is the risk of recombination with other viruses. Although the vectors are replication incompetent.
2.1.1.3: Explain why the answer is 'no'
2.1.1.4: Answer is 'n/a'
2.1.1.5: Answer: None associated.
2.1.1.6: Answer: None associated.
2.1.1.7: Explain why the answer is 'none'
2.1.1.8: Answer: None, recombination incompetent
2.3.1.1: Change answer to "Working with/handling of viral vectors containing WPRE".
2.3.3.2.b: Question is unanswered
2.3.3.3.d: Leave aerosols to settle first, then disinfect and finally use soapy water.
2.3.3.4.a: Typo: 'tehn'
2.3.3.5, 2.3.3.6 and 2.3.3.7: Questions are unanswered.
2.3.3.8.b: Remove 'Latex gloves'
2.3.3.8.c: Change answer to "No other PPE is used".
2.3.6: Tick box: 2
Part 3: Fill in Tick box.
3.1.1.a: Waste container should be 'sealed' and remove reference to sharps.
3.1.1.b: Change Autoclave cycle details to 136°C for 6 minutes. Change 'temperature recorder' to 'print out'
Location of backup is: Basement and BSU, in event of a breakdown list the secondary autoclave.
3.2.a: Change answer to, "tubes carried in a rack".
3.31.a: Answer is 'None', delete the answers in rest of the section.
3.32: Answers are: n/a
3.4: Answer is 'no'
3.5.a: Spillage during transport: disinfect first.
3.5.b: Skin surface contamination: Use microsol wipe, then soapy water.
Medical intervention/prophylaxis: Change answer to section 2.3.5 of the form.
3.7.1: Remove staff that have left and add the CID numbers of those missing.
3.7.2: Question is unanswered: cleaners etc…
Agreed Class 2, Containment Level 2. This project is notifiable to the HSE.

All of the comments of the two committees have been addressed and the requested amendments incorporated into the risk assessments forwarded with this form.

**Project Containment**

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**Animal Units**

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<th>Human Clinical Applications</th>
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**Project Ref 165/97.2**

**Date Ackn’d** 21/07/2011

**CU2 Project Title** Investigation of intracellular signalling in human monocytes

**Class** Class 2

**Culture Vol Class 2**

**Culture Volume Class 3-4**

**Non-GMM** Consent Granted

Not Applicable

**Tick if notifying a connected programme of work** N

**Project notified under transitional arrangements** Y

**Historical Significant Changes** GM165/00.1, GM165/02.1 Transferred from GM165 on 21/07/2011

**Historical Date of Additional Info** 14/12/1999, 24/07/2002

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
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### Project Ref 165/98.1

**Date Ackn’d:** 21/07/2011

**CU2 Project Title:** ADENOVIRUS-MEDIATED GENE THERAPY OF COLLAGEN-INDUCED ARTHRITIS

**Class**  
Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM**

**Consent Granted**

Not Applicable

**Tick if notifying a connected programme of work**

N

**Project notified under transitional arrangements**

Y

**Historical Significant Changes**

Transferred from GM165 21/07/2011

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 207/06.2

Date Ackn’d 16/12/2015

CU2 Project Title The contribution of RNA secondary structure to the replication of mammalian positive-stranded RNA viruses

Class 2

CultureVolClass2 < 1 Litre

Consent Granted Yes

Project notified under transitional arrangements
### Project Additional Information

#### Purposes of the contained use

To carry out functional investigations of predicted RNA secondary structures in positive-stranded RNA viruses. For the investigations planned, infectious clones from hepatitis C virus (HCV), Theiler’s murine encephalitis virus (TMEV), human enterovirus (Coxsackie A21 CVA21) and other non-polio human enteroviruses will be manipulated to investigate a range of potential replication elements and other larger scale RNA structures associated with virus persistence. All manipulations are designed to modify RNA structure without altering the coding capacity of the region altered. All changes will therefore be made at synonymous sites in codons so that the proteins produced by the modified viruses are unchanged. The replication ability of viruses with modified RNA structure will be investigated in cell culture, and where appropriate, in a mouse model (TMEV).

#### Recipient or parental organism

**Bacterial**  
Disabled E. coli K12-derived strains for cloning mutated DNA copies of the viruses used for RNA structure investigations. All bacterial strains are unlikely to be harmful to man or the environment.

**Mammalian**  
A variety of standard continuous and primary cell lines will be used for virus expression. (3TC, Huh7 and a range of other human hepatoma-derived cell lines, Vero cells and other continuous or primary fibroblast cell lines).

#### Host/vector system

Infectious clones of TMEV, human enteroviruses and HCV.

#### Origin & function

Transfection of in vitro-generated RNA into mammalian cells allows the full replicative cycle of TMEV, other non-polio virus enteroviruses and HCV to be initiated. These cell lines will therefore produce infectious virus, whose replication abilities can be compared to those of wild-type viruses.

#### Evaluation of foreseeable effects

All bacterial strains are disabled and therefore unlikely to be harmful to man or the environment. All mammalian cell lines for virus expression are in standard use and are known to be non-harmful for human health or safety.

RNA transcribed from clones generates potentially infectious virus particles when transfected into the appropriate mammalian cell line, which are capable of being further propagated in cell culture and on passage into animals. However, none of the mutations introduced to investigate the role of RNA structure on virus replication alter protein coding of the viruses, and no steps lead to the production of chimaeric viruses, the insertion of foreign genes in the viral genomes. Furthermore, none of the mutants are
Likely to alter virus tropism or host range, nor increase infectivity, virulence or transmissibility. Since all mammalian expression uses transfected viral RNA sequences, no antibiotic resistance genes or gene products will be introduced into mammalian cells. We therefore anticipate that the mutant viruses will generally show impaired replication ability or at most, restored fitness to wild type levels.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Solids (eg plasticware such as pipettes, flasks, tubes etc).** All solids exposed to potentially infectious TMEV, CVA21 and other non-polio enteroviruses at containment level 2 will be immersed in 3% Trigene for >12 hours or autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) and then disposed of via the clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill. Used agar plates will be autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes). Any excess liquids will be discharged to drains, and solid then disposed of via clinical waste stream for incineration of microwave treatment or via the industrial (black bag) waste stream for landfill. All HCV-exposed solid materials in the containment level 3 laboratory will be autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes). Any excess liquids will be discharged to drains, and solid then disposed of via clinical waste stream for incineration or microwave treatment or via the industrial (black bag) waste stream for landfill.

- **Liquids (eg samples, culture supernatants, tissue culture media).** All culture fluids and other liquids containing potentially infectious TMEV, CVA21 and other non-polio enteroviruses at containment level 2 will be mixed with 3% Trigene for >12 hours or autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) and then disposed of through the drain. HCV culture fluids, and other liquid waste originating from work on HCV at containment level 3 will be mixed with 5% Trigene for >12 hours or autoclaved using a make safe cycle as specified in BS2646, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) and then discharged to drains.

- **Sharps (in sharps bin, eg needles, syringes, scalpels).** These will be autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), disposed via clinical waste stream for microwave treatment.

- **Animal bedding and carcasses.** These will be autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes). Carcases will be disposed of via clinical waste stream for incineration and bedding via clinical waste stream for microwave treatment or via the industrial (black bag) waste stream for landfill.

**Degree of kill:**

- **Autoclaving - effectively 100% kill of HCV.**
- **Disinfectant - Trigene is used for disinfection and has proven efficacy (> 5 log reduction in infectivity) against highly resistant non-enveloped viruses such as enteroviruses, and also flaviviruses (Sources: MediChem, Abbott Analytical)**
- **Microwaving or Incineration - not applicable, all waste is autoclaved or chemically disinfected prior to disposal by microwaving or incineration.**
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM to control the risks to human health and safety and to the environment.

**Project Containment**

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<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
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**Project Ref** 207/08.3

**Date Ackn’d** 20/10/2008

**CU2 Project Title** Use of bacteria to investigate the evolution of social behaviour and implications for bacterial disease

**Class**

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM**

**Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info** transferred from GM207 01/04/2009

**Significant Change ID**
### Project Additional Information

#### Purposes of the contained use

We wish to investigate several key questions about the evolution of social behaviour using the opportunistic pathogen Pseudomonas aeruginosa and go on to use our understanding of social evolution theory to understand observations of behaviour in bacterial infections. Specifically, we will investigate the extent to which social behaviour of bacteria effects their ability to infect hosts and cause disease. The social traits we focus on are the production of the iron-scavenging molecule pyoverdine and cell-to-cell communication systems. We will measure the extent to which clinical strains isolated from the sputa of cystic fibrosis patients exhibit these and other social behaviours.

### Recipient or parental organism

| Pseudomonas aeruginosa |

### Host/vector system

| Bacterial host/plasmid vector |

### Origin & function

#### Inserted genetic material:
- standard non-harmful reporter genes such as green fluorescent protein (GFP) gene derived from the jellyfish Aequorea Victoria and luciferase a gene derived from the firefly Photinus pyralis.
- Muc A gene which suppresses the production of alginate in wild type P. aeruginosa strains; insertion of this gene restores phenotype of mutant clinical strains to wild type phenotype.
- antibiotic resistance genes for selection purposes.

#### Gene deletions:
- Siderophore (pyoverdine and pyochelin) knockouts are no longer able to chelate bound iron. The ability to chelate iron is crucial to bacterial metabolism and the ability to colonise host tissue where iron is withheld by powerful chelators such as haemoglobin or transferring. The fitness and virulence of these strains is therefore, significantly reduced relative to the wild type in monoculture.
- Quorum sensing knockouts no longer have functioning QS systems. Bacteria with knockouts of these regulatory genes are less able to sense their bacterial neighbours and are disabled with respect to biofilm production, swarming motility and production of secreted virulence factors. As a result, strains that have QS systems disrupted have significantly reduced virulence relative to wild type in monoculture.

### Evaluation of foreseeable effects

The OMMOs with lux or gfp reporter genes inserted are likely to display comparable properties to wild type organisms. The GMMOs with social functions disabled by gene deletions or disruptions are either likely to have or have been demonstrated to have reduced fitness and virulence relative to wild type strains. Tissue tropism and host range are not likely to be affected. Consequently, harmful properties associated with the GMMOs are at the most comparable to those of the wild type organism. Antibiotic resistance genes will not be ones that are used therapeutically for P. aeruginosa.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (eg plasticware such as pipettes, flasks, tubes etc and agar plates and including lettuce leaves, warm oth pupae and C. elegans) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) — autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, eg needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for heat treatment.

Degree of kill:
Autoclaving - effectively 100% kill (annual validation using 12 point thennocouple of worst case loads)
Heat treatment or Incineration — not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM to control the risks to human health and safety and to the environment.

Project Containment

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Project Ref 31/01.7

02/03/2022
The aim of this project is to identify, and characterise the function of Streptococcus pneumoniae genes required for virulence. This necessitates the construction of plasmid vectors containing segments of S. pneumoniae DNA, which are used to transform wild type S. pneumoniae to make GMM strains with specific gene disruptions. The phenotype(s) of these strains will then be assessed using animal and cell culture models of disease.

Recipient or parental organism

The recipient microorganism in Streptococcus pneumoniae, which is a human pathogen. It is placed by the ACDP in Hazard Group 2.

Host/vector system

Host. Streptococcus pneumoniae.

Vectors: pJPC9111, pJPC9112, pLS1GFP, pLS70GFPcat, pucMUT, pDE1, pEVP3, pACH74, p10701

Origin & function

Source of Host Cells - Smithkline Beecham; Prof. James Paton, University of Adelaide
Source of Genetic material: inserts - PCR of Streptococcus pneumoniae DNA
Source of the material: vectors - Prof. James Paton, University of Adelaide; commercial sources.

Evaluation of foreseeable effects

The GMM is likely to pose no increased risk to human health in comparison with the wild type parental strain. On the contrary, since a putative virulence gene will have been mutated the GMM is likely to be attenuated in its ability to cause disease; and therefore be functionally disabled. The insertional mutagenesis protocol utilised in this project renders the GMM resistant to chloramphenicol, spectinomycin, erythromycin or kanamycin. However, there are a wide variety of alternative antimicrobial agents available which are effective against S. pneumoniae infections should infection by the GMM occur. S. pneumoniae is essentially restricted to the human nasopharynx and there is no recognised environmental source. The environmental survivability of the GMM is envisaged to be the same as the wild type, with the caviat that without...
appropriate selection pressure reversion to the wild type is likely, and there is no increased risk to the environment posed by the GMM compared to the wild type.

All work carried out under this project will take place in appropriate Containment level 2 premises, together with appropriate waste control and management systems.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Chemical disinfection of liquid GM waste: 2% hycolin solution, as per manufacturer's recommendations, overnight exposure. 100% kill validated by periodic plating out of cultures after exposure to hycolin disinfectant.

- Autoclaving of solid GM waste: Large autoclave; solid discard cycle 134 celsius for 30 minutes. 100% kill. Machine and cycle validated by thermograph and testing by manufacturer's engineer.

- Autoclaving of liquid GM waste: Large autoclave; liquid discard cycle 134 celsius for 30 minutes. 100% kill. Machine and cycle validated by thermograph and testing by manufacturer's engineer.

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## Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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Project Ref 332/trans2

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Withdrawn  

Tick if notifying a connected programme of work  

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

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**Tick if you are claiming exemption from disclosure for section of the risk assessment**

**Project Containment**

02/03/2022
Project Ref 553.02/97.1

Date Ackn'd 21/08/1997

Date Project Ceased

CU2 Project Title THE DEVELOPMENT OF DNA-MEDIATED TRANSFORMATION SYSTEMS FOR Erysiphe spp.

Class 2

Consent Granted not applicable

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Project notified under transitional arrangements Y
Evaluation of foreseeable effects

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Project Ref 553.06/97.1

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Date Project Ceased
21/03/2002

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
GM553/99.3, GM553/00.9

Historical Date of Additional Info
11/03/1999, 16/08/2000,

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Animal Units

- Large Scale Activities
- Human Clinical Applications

Project Ref 553.09/94.6

Date Ackn’d: 18/04/2001

CU2 Project Title: USE OF GENETICALLY MODIFIED VIRUSES TO STUDY ENDOCRINE AND IMMUNE ASPECTS OF BRAIN FUNCTION-NEUROPROTECTIVE GENE TRANSFER USING ADENOVIRUS

Class 2

Consent Granted: not applicable

Non-GMM

Project notified under transitional arrangements

Historical Significant Changes

- GM553/00.8, GM553/98.11, GM553/98.9, GM553.09/97.2, GM553.09/95.
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
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<tr>
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**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.
## Project Containment

### Laboratory Activities
- L2
- L3
- L4

### Glass Houses
- L2
- L3
- L4

### Growth Rooms
- L2
- L3
- L4

### Animal Units
- L2
- L3
- L4

### Large Scale Activities
- L2
- L3
- L4

### Human Clinical Applications
- L2
- L3
- L4

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**Project Ref 553/00.10**

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<td>05/06/2000</td>
<td>RECOMBINANT MOUSE CYTOMEGALOVIRUS (MCMV) TO STUDY VIRUS-HOST INTERACTIONS DURING A PERSISTENT VIRAL INFECTION</td>
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**Project notified under transitional arrangements**

**Y**

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**Historical Significant Changes**

- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

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Page 8834 of 15326
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

Project Ref 553/00.13

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Page 8835 of 15326
INFLUENZA VIRUS GENE EXPRESSION

Date Project Ceased: 26/10/2000

Class 2

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: Y

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purpose of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<tr>
<td>04/01/2001</td>
<td>ISOLATION AND EXPRESSION OF GENES FROM HUMAN PARASITIC NEMATODES. CLASS 2</td>
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<td>&lt; 1 litre</td>
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- **Date Project Ceased**: 31/07/2001
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change
Project Additional Information

Purposes of the contained use

The aim of this project is to isolate genes important in parasite survival in order to design vaccines against them. The strategy is concentrated on a family of protein that the parasitic nematode uses to maintain its environment within its human host. This appears to be a pore forming protein that in sufficient amounts lyses cells in a localised area.

This project involves the cloning and expression of the proteins of interest for subsequent purification and analysis.

Recipient or parental organism

The AD494 host is a K12 derivative of Escherichia coli and as such is regarded as a non-colonising, disabled host that is not pathogenic to humans and unlikely to survive outside laboratory culture.

Host/vector system

pET System available from Novagen
pET vectors are mobilisation defective plasmid vectors derived from pBR322

Origin & function

Excretory/secretory protein produced by Trichuris worms that induce pore formation in planar lipid bilayers in the host caecum.

The purpose of cloning and expressing the genetic material in the E.coli pET expression system is to produce active protein that can be purified for characterisation.

Evaluation of foreseeable effects

The insertion of the gene into the E.coli host is likely to make the E.coli more hazardous in so far as it is expressing a harmful protein. However it is a disabled host that is unlikely to colonise humans and survive in the environment. The modification is not expected to overcome disablement of the E.coli, nor effect host specificity, tissue tropism or susceptibility to host defence mechanisms. Exposure to the recombinant E.coli would therefore be expected to have only localised and transient effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill: Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licenced incinerator)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref** 553/01.1

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Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

- Significant Change ID: 553/05.4
- Date of Significant Change: 21/11/2005

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02/03/2022 Page 8839 of 15326
### Project Additional Information

#### Purposes of the contained use

The aim of this project are generally to study the control of expression, functions and cell biology of *Plasmodium falciparum* parasite encoded proteins. These questions will be studied through altering the expression of genes within the organism. This will be carried out by transfection with DNA, generating transient (episomal) expression of exogenous genes or stable integration (functional knockout of endogenous genes, or insertion of known coding sequences). Additionally, attempts will be made to inhibit gene expression through transfection with single or double stranded RNA. Using these methods, the project aims are:

- to study the functions of various *Plasmodium falciparum* genes and control sequences,
- to investigate the mechanism of antigenic switching, and endothelial adherence of variant antigen genes and their products, and
- to examine the mechanism of protein export of the variant antigens and other gene products which have a destination exterior to the parasite.

#### Recipient or parental organism

*Plasmodium falciparum* - the causative agent of human malaria

#### Host/vector system

Nucleic acid sequences will be transfected as either plasmid constructs or as RNA. Plasmids for transfection will be generated in disabled *Escherichia coli*. Vectors are pUC and pBS-based non-mobilisable bacterial plasmids.

#### Origin & function

**i) Target and control sequences from*Plasmodium falciparum* and *P. chabaudi* (rodent malaria):**

- *P. falciparum* -- var genes, exported proteins, non-coding regulatory regions, genes involves in transcriptional control or translational control
- *P. chabaudi* -- non-coding regulatory regions

Non-coding putative regulatory sequences should have no biological action. var genes code for erythrocyte surface proteins, which are involved in antigenic variation and in some cases, binding to various human host receptors. Other putative red cell surface proteins have unknown functions.

Sequences will be either placed adjacent to reporter genes or epitope to study gene expression, switching and trafficking, or alongside a selectable marker for stable transfectants:

**ii) Standard reporter genes and epitope tags: functions as indicated by names.**

- *E.coli* -- chloramphenicol acetyltransferase [CAT], Influenza virus -- hemagglutinin epitope [HA tag], firefly (Phontinus pyralis) -- luciferase [LUC]
- jellyfish (Aequorea victoria) -- green fluorescent protein [GFP; and derivatives]

**iii) Drug resistance and selection marker:**

- a) Dihydrofolate reductase (DHFR) from *Toxoplasma gondii* - pyrimethamine resistance, *P. falciparum* -- pyrimethamine resistance
- Human -- resistance to analog of pyrimethamine

#### Evaluation of foreseeable effects

The pathogenicity, virulence, infectivity and invasiveness of the modified parasites are not expected to be increased when compared to the parent microorganisms. The inserted genes will not affect host range and tissue tropism. No adverse effects are anticipated in terms of altered interaction with host defences. Parasites will remain fully susceptible to a range of first line treatment drugs.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

a) Laboratory not sealable for fumigation:
There is no foreseeable need to fumigate the laboratory.

b) No negative pressure, HEPA filtered extract, microbiological safety cabinet or specified measures to control aerosol dissemination:
The organism presents no hazard of infection by the airborne route.

c) Autoclave and some other equipment not within the laboratory:
Although the laboratory contains most of the necessary equipment for the work, some activities use specialist equipment that cannot reasonably be accommodated with the dedicated facility. In all cases a safe system of transport involving secondary containment is in use, any equipment used is cleaned and disinfected immediately on completion of the work and any contaminated items either returned to the dedicated laboratory or removed for disposal as waste.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

A) General procedures.
Consumables (mainly plasticware eg pipettes, flasks, tubes) *excluding radioactive items (see below) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Liquids (eg samples, culture supernatants, tissue culture media) *excluding radioactive items (see below) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

B) For waste items that are both radioactive and contain P. falciparum
Solids - disinfect with 2% Virkon for at least 30 minutes, discharge any excess liquid to drains as aqueous radioactive waste subject to limits contained in an authorisation granted under the Radioactive Substances Act 1993, dispose of solids as solid low level radioactive waste under an authorisation granted under the Radioactive Substances Act 1993.

Liquids - disinfect with 2% Virkon for at least 30 minutes, discharge to drains as aqueous radioactive waste subject to limits contained in an authorisation granted under the Radioactive Substances Act 1993.

C) Degree of kill
i) Autoclaving:
Effectively 100% kill (annual validation).

ii) Incineration:
Effectively 100% kill (licensed incinerator).

iii) Chemical disinfection with Virkon:
This is used only for the disposal of radioactive waste from metabolic and surface labeling experiments, the waste could possibly contain live parasites but cannot be autoclaved.

Current control measures of adding the chemical disinfectant Virkon, according to manufacturer's instructions, should be sufficient to kill any live organisms. Virkon is added to waste from parasite cultures because of the possible risk of contamination from blood products (known viruses, bacteria, etc.) and is not necessary to kill the...
parasites. Dilution in a sufficiently large volume of water kills the parasites. However, since both parasites and blood products are present, chemical disinfection is used.

Determination of the degree of kill (the percentage kill or log reduction in viability) after Virkon treatment would be impossible. The addition of Virkon to live (parental) cultures of parasites congeals the infected and uninfected red cells to a slurry. It would be impossible to remove the Virkon from this slurry and attempt to continue culture of any potentially living organisms (centrifugation reduces this slurry to a semi-solid, muddy lump and any traces of Virkon in a culture would render the host red blood cells inviable) making any living parasites undetectable. Any viability is thus reduced to below in vitro detectable limits. The only means of absolute detection of any living parasites would then be injection of this slurry into a primate, which would be highly unethical, and the Virkon itself would be highly toxic.

As stated above, the parasites have an absolute requirement for viable host red blood cells for their survival. Since Virkon contains a surfactant, no mammalian cells remain intact in a solution of Virkon (used as directed by manufacturer), and therefore no parasite can remain viable in this same solution.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
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<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 553/01.10

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Date Project Ceased: 02/03/2022
**Project Additional Information**

**Purposes of the contained use**
The project builds on recent advances made by our laboratory in the field of prion research. We have isolated novel, nucleic acid ligands for PrP that bind more strongly to the conformation associated with prion diseases such as BSE and CJD (PrPsc) than they do to the normal form found in healthy animals and humans (PrPc). These ligands have been patented and we are developing them for use in diagnostic and therapeutic applications relating to BSE and CJD. As part of this process, we need to be able to express and purify normal forms of PrP from humans, cattle and other species, in order to maximise the disease-selectivity of our ligands.

The genes encoding normal PrP will be amplified from primary cells using PCR and sequence-specific primers. The PrP genes isolated will then be sub-cloned into E. coli for expression with subsequent purification and refolding of recombinant PrP.

**Recipient or parental organism**
K12 and B derivatives of Escherichia coli - these are disabled strains that cannot colonise the human gut and have a history of safe use. These hosts may be considered equivalent to ACDP hazard group 1.

**Host/vector system**
E. coli bacterial host with plasmid vector.

**Origin & function**
The inserts will encode normal alleles of a widely-expressed gene of humans and other animals, PrP. Diseases associated with this gene are either:

a) genetic, in which specific mutations destabilize the structure of the encoded protein leading to increased possibility of spontaneous prion formation. Such alleles will not be used in this study.

b) infectious, in which abnormally folded PrP in the tissues of a human or animal infected with a transmissible spongiform encephalopathy (eg BSE) is associated with the ability to transmit the disease to healthy humans or other animals. No tissues from infected animals or humans will be used in this study.

**Evaluation of foreseeable effects**
The resulting genetically modified micro-organisms will be disabled strains of E. coli with normal human or animal PrP genes inserted. The exact pathogenesis of the prion diseases remains unclear and a strong hypothesis, as yet unproven, is that the infectious agent is simply a specific, mis-folded version of the normal PrP protein. If true, misfolding of normal PrP in vitro might yield infectious material. In spite of extensive attempts to generate infectious material from in vitro-expressed PrP in many laboratories world-wide, it has not been possible to do so to date. Moreover, the in vitro refolding methods to be used in this project are very widely used in the field and have been demonstrated not to yield infectivity. There is no reason to suspect that the genetically modified E. coli or the expressed PrP will be harmful but ACGM recommends a precautionary approach is taken and suggests that a level of containment equivalent to Containment Level 2 is appropriate for the time being for this type of work (see ACGM Compendium of Guidance, Part 2A, Annex III, Table 5A).
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Consumables (mainly plasticware eg pipettes, flasks, tubes ) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 156 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 165 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022

Page 8844 of 15326
Project Ref 553/01.2

Date Ackn'd 12/01/2001

CU2 Project Title INVESTIGATING HIV AND THE IMMUNE RESPONSE

Class CultureVolClass2 CultureVolumeClass3-4

Class 3 up to 20 ml

Non-GMM Consent Granted yes

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The main aim of this project is to understand the mechanisms by which HIV evades the immune response. Nef, an HIV accessory protein, is believed to play a major functional role in protecting infected cells against killing by CTLs and downregulation of MHC class 1 expression. Whilst various mechanisms have been proposed whereby nef regulates T cell activity and contributes to the pathogenesis of HIV, the function of nef at the molecular level remains poorly understood. This project is directed at a better understanding of the role of nef and how it interacts with host function. SIV will also be modified and investigated for comparative purposes.

Recipient or parental organism

Human immunodeficiency virus (HIV) and Simian immunodeficiency virus (SIV)

E coli K12 and B derivatives (disabled).

Human and mammalian cell lines.

Host/vector system

Proviral HIV and SIV DNA constructs will be made in disabled E.coli

Recombinant HIV and SIV will made by transfecting the provirus DNA into 293T cells, a human fibroblast cell line with high transfection efficiency for producing infectious virus. These viruses will be subsequently used to infect human and mammalian cell lines.

Origin & function
Constructs will comprise HIV and SIV under the control of the CMV promoter with
i) whole or partial deletions of the nef genes,
ii) point and/or multiple mutations in the nef gene,
iii) replacement of the nef gene with full length or partial sequences of nef from HIV isolated from patients infected with HIV (or SIV from macaques).

Standard reporter genes such as green fluorescent protein (GFP, and other coloured derivatives) and placental alkaline phosphatase (PLAP) will be inserted for detection of infected cells.

Nef downregulates the surface receptors CD4 and MHC 1, increases viral infectivity and stimulates T cell signaling pathways. The genetic modifications in nef are made for the purpose of studying changes in gene expression or in the modulation of immune response molecules.

There will be no switching of genes or transfer of genetic material between HIV and SIV.

**Evaluation of foreseeable effects**

The pathogenicity, virulence, infectivity and invasiveness of the modified viruses are not expected to be increased when compared to the parent microorganisms. The assessment of risk to human health and safety indicates no greater hazards than those with wild type unmodified viruses. In many cases the modified viruses are expected to be attenuated.

The genetic modifications will not affect host range and tissue tropism since there will be no alterations in genes coding for the envelope glycoproteins. No adverse effects are anticipated in terms of altered interaction with host defences, many of the modifications will result in improved immune response in infection because the mechanism by which the virus evades immune surveillance will be lost or reduced. Modified viruses will remain susceptible to anti-retroviral chemotherapy.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

There is no autoclave within the laboratory. An autoclave validated for disposal of waste is located within the same building and a safe system for transport of waste to the autoclave is used.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excelle liquids to drains, dispose of solids via clinical waste stream for incineration.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125oC for at least 15 minutes of 126-130oC for at least 10 minutes of 134-138oC for at least 3 minutes), discharge to drains.

Degree of kill
Autoclaving: effectively 100% kill (annual validation).
Incineration: effectively 100% kill (licensed incineration)

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

### Project Containment

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### Project Ref 553/01.3

- **Date Ackn'd**: 27/02/2001
- **CU2 Project Title**: CELLULAR IMMUNE RESPONSES TO FOREIGN ANTIGENS
- **Class**: Class 2
- **Culture Vol**: 1-50 litres
- **Consent Granted**: not applicable

### Project Additional Information

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
### Purposes of the contained use

Vaccinia virus and adenovirus recombinants will be used as plastic tools to facilitate studies into the function and interaction of genes involved in antigen presentation, processing and regulation. A wide range of gene sequences will be inserted into these vectors to gain a better understanding of their function and effect on the immune response. The role and responses of the various components in disease processes will also be studied. This will form the basis for the development of control strategies such as vaccines.

### Recipient or parental organism

- **K12 and B derivatives of Escherichia coli** - disabled strains that cannot colonise the human gut and have a history of safe use.
- **Mammalian cell lines** - low hazard, regarded as especially disabled hosts.

### Host/vector system

- **Western Reserve (WR) strain of vaccinia virus** - a replication competent strain that is able to infect humans and cause disease. Genes are inserted into the thymidine kinase (TK) locus.
- **E1 deleted Ad5 adenoviruses** - The adenovirus Ad5 vector has a deletion removing most of the E1 region and as such is unable to replicate except in complementing cell lines. Gene inserts are put into the E1 site. E1 deleted hosts are regarded as unlikely to cause disease.

### Origin & function

#### i) Cloning and expression of mammalian genes involved in immune responses will be undertaken to elucidate the biology and immunology of the systems:
- Fragments and full length proteins of MHC class I molecules, NK receptors, cell surface receptors expressed by T cells, molecules involved in antigen presentation and processing, tumour antigens.

#### ii) Many viral genes have the potential to modulate immune responses and their effects will be studied:
- Fragments and full length proteins of Human cytomegalovirus genes modulating immune responses, Herpes simplex virus genes modulating immune responses, Influenza A virus genes (also used to identify what the CTLs recognise and to expand CTLs in vitro).

#### iii) Interaction with host immune response plays an important role in the pathogenicity of some viruses. Pathogenesis and interaction with immune response will be studied:
- Individual whole HIV or SIV proteins and their truncations, also polypeptide and chimaeric fusions (joining several HIV or SIV proteins into one open reading frame), Dengue virus genes, Plasmodium falciparum genes.

#### iv) Understanding infectious disease processes leads on to the development of vaccines. Similar strategies are being used in the understanding of tumour processes and development of vaccines directed towards these:
- Genes from HIV, SIV, Dengue virus, Plasmodium falciparum, CTL epitopes (sequences coding for short peptide epitope derived from HIV, SIV, influenza virus, Plasmodium falciparum or melanoma-associated antigens).

#### v) Marker and selection genes. A variety of the commonly used marker and selection genes will be used. These include marker genes such as lack Z, luciferase, fluorescent tags (green, red, yellow fluorescent proteins), antibody epitope tags (Flag, histidine, myc). Fc portion of immunoglobulin, glutamine synthetase transferase (GST) etc and antibiotic resistance etc.

### Evaluation of foreseeable effects

For the mammalian cell line and bacterial hosts no significant hazards have been identified in the risk assessment. The resulting GMOs are not expected to carry any additional risks compared to that of the unmodified recipients.

Inserts code for normal mammalian and viral functional genes, or selective alterations of those genes, and standard markers. None of the products of gene expression are
expected to be harmful. Some of inserts are from pathogenic micro-organisms but these are not expected to be hazardous when expressed in isolation. None of the inserted sequences are expected to have toxic or oncogenic properties. Some of the inserts are referred to as tumour proteins and are called this because they are over-expressed or co-expressed in tumours. Whilst the function of these proteins is not known it is unlikely that they are oncogenic since they are also expressed in normal cells. In many cases the genes will be altered such that the expressed sequences will not be biologically active because, for example, they are synthesised as modified or disrupted coding sequences or the expression products will be rapidly degraded. There is no intention to modify the tissue topism or host specificity of the viral vectors and it is thought unlikely that any of the modifications would affect these.

In some cases the insertion of the foreign sequences may lead to altered interaction with host defence mechanisms. Some of the viral genes that will be expressed are known to modulate immune responses. In the majority of cases the effect will be to upregulate the immune response resulting in a less virulent virus. Some of the inserted sequences may downregulate the immune response. For recombinant adenovirus, since this is a replication defective adenovirus, downregulation of the immune response does not present significant additional risk. Particular attention will be paid to ensure no gene sequences are expressed in vaccinia virus whereby it is reasonably foreseeable this would increase the virulence of the virus by downregulating the immune response.

Insertion of the foreign sequences into the viral vectors is unlikely to result in additional hazards associated with replication or integration of the modified virus into a host cell, beyond those normally expected with the parent virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NOT APPLICABLE

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware e.g. pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

Sharps (e.g. needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - either autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Degree of kill:

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% (licensed incinerator)
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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<td>L3 L4 L2 L3</td>
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</tbody>
</table>

- **Animal Units**
  - L2

- **Large Scale Activities**
  - L2

- **Human Clinical Applications**
  - L2

**Project Ref 553/01.4**

- **CU2 Project Title**: STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE TYPE III SECRETION OF SHIGELLA FLEXNERI

- **Date Ackn'd**: 08/03/2001

- **Class CultureVol**: Class 2 1-50 litres

- **Non-GMM Consent Granted**: not applicable

- **Project notified under transitional arrangements**: N

- **Withdrawn**: N

- **Historical Significant Changes**: GM553/02.3

- **Historical Date of Additional Info**: 15/04/2002

**Project Additional Information**

02/03/2022
Purposes of the contained use

The aim is to study the detailed function of the type III secretion system of Shigella flexneri serotype 5a in relation to this organism's ability to invade eukaryotic cells in culture (as a simplified model system for how it causes disease). Type III secretion systems (secretons) are widely distributed, essential virulence determinants of Gram-negative bacteria. They are encoded by approximately 25 genes, which share homology with those encoding flagellar basal bodies. Type III secretion systems serve to translocate, upon contact with eukaryotic host cells, proteins from the bacterial cytoplasm into the host cell cytoplasm. The protein effectors of virulence are thought to be moved directly from the bacterial cytoplasm through the secreton (embedded in both bacterial membranes) to insert within the host membrane and form a pore through which other bacterial proteins are then translocated into the host cytoplasm. The following questions will be addressed:

1. What is the detailed structure of the secretion machinery and how is it assembled?
2. How tight is the connection between secreton and pore?
3. What signal(s) initially target Shigella substrate proteins to the secretion machinery and are they sufficient to mediate protein insertion/translocation in eukaryotic host cells?
4. How, in molecular detail, does type III secretion occur? and
5. What powers type III secretion and how is the energy utilised for substrate export?

Recipient or parental organism

Shigella flexneri serotype such as 5a and 2a, wild-type and mutants.

Host/vector system

Non-mobilisable plasmid vectors.

Origin & function

Inserts:
DNA from pWR1000 (S. flexneri 5a large virulence plasmid) carrying genes or fragments of genes from the ipa, mxi/or spa operons.

Normal/expected biological action of inserted DNA.RNA or transcribed/translated gene product: Formation of a part of the type III secretion machinery (each gene produce alone can not lead to biologically active structure int erms of secretion). Localisation in either the bacterial cytoplasm, inner or outer membrane of bacterial surface. Alternatively generation of a complete or a fragment of the known translocated effector proteins (IpaA-D and IpgD) in the bacterial cytoplasm.

Evaluation of foreseeable effects

The modifications to the Shigella flexneri are likely either not to affect the organism's fitness/virulence or to actually decrease it. Since the mutations made or planned expression of tenes resulting in the loss of a normal function, in no instance would the work to be undertaken be expected to increase fitness or virulence. Antibiotic markers will be used that are not the primary therapeutic agents. The reporter genes will be either neutral or lead to a reduction in bacterial fitness. Gene transfer in this species does not necessitate the use of vectors that will increase the subsequent movement of altered genes. In each instance the donor and recipient species are the same except during gene construction and manipulations in disabled E. coli. Therefore, no new or additional potential risks will be created through the movement of any virulence gene to a new potentially pathogenic species background. For these reasons none of these manipulations will affect the host range, enhance or broaden tissue tropism, or decrease susceptibility to host defence mechanisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - optionally pre-treat using 2% Virkon then always autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels)- dispose via clinical waste stream for incineration.

Degree of kill-
Autoclaving, effectively 100% kill (annual validation);
Incineration, effectively 100% kill (licensed incinerator).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications

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Project Ref 553/01.5
Variation in the genes that are expressed within bacterial populations has a significant impact on their ability to interact with their hosts and the environment. This is influenced by changes in the repertoire of genes that are expressed over time within clonal populations, and differences in the gene complement of different strains within a bacterial species. The aim of this project is to use the variability between bacterial strains and species as a marker for a functional genomics based approach to the investigation of bacterial pathogenesis. This has several inter-related aspects:

1) The presence of potentially unstable repetitive sequences is a marker for phase variable gene switching which is a marker for genes involved in niche adaptation, host interactions and immune evasion.
2) Comparative genome analysis (between strains and between species with different pathogenic potentials) reveals groups of genes that are potentially behaviour specific with regards to the capacity to cause disease and evade the immune response.
3) Analysis of genomes for sequences that have been recently acquired by horizontal gene transfer identifies genes that are likely to be involved in adaptation to, or altered interactions with, their hosts. Alternatively they are likely (although not certain) to increase the general fitness of the strain that has acquired them, which can itself be associated with alterations in virulence.
4) Intergenic elements that affect the expression of genes have been identified in association with recognised virulence genes, these may also be markers of altered virulence.
5) Similarities in the promoter regions of genes that regulate and co-ordinate expression can be used to identify genes that are co-expressed, and therefore part of a wider response of the bacteria to the host. Identifying these regulatory networks enables us to get a better understanding of the programmed response of the bacterium to the host during infection. Searching for common feature with those genes that are identified as virulence associated from the other analyses will extend the way in which we understand how these genes function.

We have advanced genome-based data for some of these groups of genes and are continuing to work on the others. However, sequence analysis alone can only generate hypotheses about the roles and functions of the genes that are identified. These now have to be investigated using traditional experimental laboratory methodology to determine their actual roles in virulence.
<table>
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<tr>
<th><strong>Recipient or parental organism</strong></th>
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<tbody>
<tr>
<td>Neisseria spp., Helicobacter spp., Haemophilus influenzae, and Escherichia coli.</td>
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</table>

The organisms that have been selected for study each possess recognized phase variable gene systems involved in virulence. Three (Haemophilus, Neisseria and Helicobacter) are naturally transformable, possess many variable genes, and switch genes largely through varying the length of similar simple sequence repeats. The fourth (E. coli) is included because although it does use phase variation, it contrasts with the other species in that it is clonal, has few phase variable genes, and mediates switching using a different molecular mechanism (local sequence inversion). In addition to being the most highly characterised Gram-negative bacterial species it is therefore being used as a comparative / control organism.

<table>
<thead>
<tr>
<th><strong>Host/vector system</strong></th>
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<tr>
<td>Bacterial plasmids.</td>
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<tr>
<th><strong>Origin &amp; function</strong></th>
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<tbody>
<tr>
<td>Two main approaches will be used in this work.</td>
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</table>

1) A common experimental strategy to address the functions of phase variable genes

The investigation of different phase variable genes will involve a common experimental strategy. The gene will be put into a permanent expression state using site directed mutagenesis. Genes will be altered to remove the sequences that mediate ON-OFF switching following cloning into standard 'disabled' laboratory strains of E. coli. These permanently switched-ON genes will either be used as they are, or linked to selectable antibiotic markers. The genes will also be knocked out either using insertion-deletion mutants containing selectable antibiotic markers, or by complete deletion of the open reading frame from the flanking sequences.

2) Strategy for the investigation of other candidate virulence genes identified by genome analysis

Candidate virulence genes identified by the other genomics approaches will be investigated using the classical knock-out and complementation strategy. Genes of interest as determined on the basis of genome sequence analysis (identified as being candidate virulence genes, or fitness changing genes acquired by recent horizontal transfer) will be deleted and the resultant phenotypes studied and their fitness compared with 'wild-type' bacteria.

Modifications will be made to host genes and these returned to the same host, there will be no transfer of genes between pathogenic species. The only foreign DNA to be inserted into a host will be that of standard reporter and selectable markers.

<table>
<thead>
<tr>
<th><strong>Evaluation of foreseeable effects</strong></th>
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<tr>
<td>Investigations into the functions of phase variable genes:</td>
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</table>

In each instance the mutants will differ from the wild-type bacteria in one of two ways. They will either lack the candidate virulence / niche adaptive genes, or they will invariably express these genes. No phenotype would be created by this process that would not be expected to occur as a result of the normal random switching of these genes in vivo. In addition, in each case the phase variability that was the reason for the selection of these genes for study, it itself a marker that the ON-OFF switching is itself adaptive. Each mutants' ability to make the environmental transitions for which each gene is adaptive, such as moving between different host niches, immune evasion, environment-host and host-to-host transfer, is likely to be seriously impaired. The overall fitness of these mutants for the aspects of its life style that involve these genes is likely to be seriously reduced. For this reason, the altered strains would be expected to be less fit and therefore ultimately less virulent that the 'wild-type' in these contexts.

Investigation of other candidate virulence genes:

In each case the mutants are expected to have 'neutral' or 'loss of function' phenotypes. Therefore these mutants are expected to have reductions in fitness and virulence when compared to the parent strains. However, although unlikely, there is a possibility that a gene that reduces virulence would be deleted but this is unlikely to necessitate
additional control measures for safe working. It should also be noted that it is likely that each of the single gene knock-out phenotypes that are viable have probably already been made by others who have been using the signature tagged mutagenesis methodology where these have been used in the same species. The main difference in mutants described in the currently proposed experiments is that we will know de-novo what the mutations that have been generated are.

General summary
The mutations that are to be introduced are each likely to result in a reduction rather than an increase in fitness and/or virulence. The majority of mutations will either fix a gene ON that it is normally present within the species and where the removed variability of the gene confers fitness, or will ablate expression of genes resulting in the loss of a normal function. Antibiotic markers will be used that are not normally the primary therapeutic agents. The reporter genes and counter-selectable markers will be either neutral or lead to a reduction in bacterial fitness. Gene transfer in these species does not necessitate the use of vectors that will increase the subsequent movement of altered genes. There will be no introduction of genes from one pathogenic species to another. For these reasons none of these manipulations will effect the host range, enhance or broaden tissue tropism, or decrease susceptibility to host defence mechanisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consuables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, dyringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving: effectively 100% kill (annual validation).
Incineration: effectively 100% kill (annual validation).

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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### Project Ref 553/01.6

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<th>CultureVol</th>
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<th>Project notified under transitional arrangements</th>
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<td>INVESTIGATING THE MOLECULAR GENETICS OF OVARIAN CANCER</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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**Purposes of the contained use**

The work is part of a wider project to understand the genetic changes in the pathogenesis of ovarian cancer. A strong candidate tumour suppressor gene has been identified and we wish to examine the effect of transfecting constructs containing this gene (RSK-3) into various cell lines to determine if it is a tumour suppressor gene. We have previously shown that RSK-3 can significantly inhibit colony formation in 4/6 ovarian cancer cell lines when a plasmid construct was transfected into cells. This result was obtained in spite of the low transfection efficiency (~20%) obtained when using plasmid vectors. Adenoviral vectors consistently show a higher degree of transfection efficiency, up to 100%, and greater gene expression. Therefore, the use of an adenoviral construct could provide more convincing evidence of the ability of RSK-3 to inhibit cell growth.

**Recipient or parental organism**

K12 and B derivatives of Escherichia coli - disabled strains that cannot colonise the human gut and have a history of safe use.

Mammalian cell lines - low hazard, regarded as especially disabled hosts.

**Host/vector system**

E1 deleted Ad5 adenovirus - the adenovirus Ad5 vector has a deletion removing most of the E1 region and as such is unable to replicate except in complementing cell lines. Gene inserts are put into the E1 site. E1 deleted hosts are regarded as unlikely to cause disease.

**Origin & function**

The inserted genes and gene sequences (RSK's) are of human origin and are serine Threonine kinases activated by the Ras-ERK pathway. Substrates phosphorylated by RSK include nuclear proteins such as c-FOS, histones and the CAMP-responsive-binding element protein (CREB), the oestrogen receptor and NFkB. RSK's are thought to regulate protein synthesis by phosphorylation of polyribosomal proteins and glycogen synthase kinase-3. It has been shown that the RSK's are involved in diverse functions as proliferation, growth arrest and differentiation.

**Evaluation of foreseeable effects**

For the mammalian cell line and bacterial hosts no significant hazards have been identified in the risk assessment. The resulting GMOs are not expected to carry any additional risks compared to that of the un-modified recipients.

The inserted sequences are being assessed as putative tumour suppressor genes and it is considered unlikely that the resulting genetically modified adenoviral vectors would cause harm. No alteration in tissue tropism, host specificity or interaction with host defences is anticipated. However, since RSKs have been shown to be involved in various functions including proliferation and are dominantly transforming, a cautious approach is being taken until experimental evidence supports the view that these are not likely to be harmful if delivered into human cells by the adenoviral vector.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plastic ware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125C for at least 15 minutes or 126-130C for at least 10 minutes or 134-138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.
Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incorporation, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Project Ref 553/01.8

Date Ackn’d 19/07/2001

CU2 Project Title VIRUS-INDUCED GENE DELIVERY AND SILENCING IN WOODY PLANTS

Class 2 Culture Volume Class 2 < 1 litre
Little is known about how trees resist viral pathogens. It is an open question as to how, over the duration of their lifetime, trees resist pathogens that evolve at a rate that is orders of magnitude faster than their hosts. This work aims to determine if a recently discovered mechanism that functions in virus resistance in herbaceous annual plants (such as tobacco and the weed, arabidopsis), also functions in woody perennial plants (ie trees).

The discovery that plants can use post-transcriptional gene silencing (PTGS) as a defence against viral pathogens has been one of the most interesting discoveries in plant pathology in recent years. To date, examination of virus-induced PTGS, also known as virus-induced gene silencing or VIGS, has been limited to herbaceous annuals of the Solanaceae and Brassicaceae. These studies have revealed that VIGS is systemic and persistent, but that VIGS is not dependent on either systemic infection by, or persistence of, the viral pathogen. It is thought that this defence strategy is widespread, and is likely to be used by many diverse plant genera. If this is true, the implication of VIGS as a defence mechanism in perennial plants are significant. The ability to mount and sustain a defence against pathogens, for the lifetime of the plant, could be an important component of survival for woody perennial plants, which may live for decades, or even hundreds, of years. A defence mechanism like VIGS could account for the durability of resistance to viral pathogens that is characteristic of many woody perennials. However, it remains to be determined if VIGS operates in woody perennial plants. This project will test the hypothesis that VIGS operates in a woody perennial. This will involve an examination of the relationship between woody perennial tree species, poplar and eucalyptus, and two viruses, Tobacco Rattle Virus (TRV) and Poplar Mosaic Virus (PopMV). TRV will be used as a vector to induce VIGS in woody plants as it represents the best-characterised vector for VIGS in plants. The interaction between TRV and Solanaceous and Brassicaceous plants have been extensively characterised, and there is now a substantial literature pertaining to TRV-induced VIGS in different plant species.

Tobacco Rattle Virus - TRV is a broad host range virus that is transmitted by nematodes. TRV can infect many species in the UK, but is not usually lethal to these plant species. In fact, while many plant species can be infected with TRV, few, if any, symptoms of viral infection are generally manifest. Hazards to the environment from natural isolates of virus are therefore very limited.

Poplar Mosaic Virus - PopMV is not a broad host range virus (like TV) and is transmitted manually, presumably by wounding brought about by insect feeding. As is the case with TRV, PopMV frequently does not even bring about viral symptoms on infected plants and is not lethal. The limited host range and low level of symptoms of PopMV means that there is likely to be a negligible risk to the environment from this virus.

K12 and B derivatives of Escherichia coli - disabled strains that cannot colonise the human gut and have a history of safe use.
In all instances viral genomic RNA are cloned as cDNAs in plasmid vectors. The plasmids are propagated in Escherichia coli. Viral RNA is unable to be expressed from the plasmid vectors in E.coli; therefore, viral RNA must be synthesised from purified plasmids in vitro. The plasmids function as templates for in vitro transcription reactions.

**Host/vector system**

The genes involved in transmission will be removed from TRV so that the virus can no longer be transmitted by nematodes. The PopMV coat protein gene is necessary for transmission of the virus from plant to plant and will be removed from the PopMV genome to prevent transmission. In both cases these genes will be replaced by a reporter gene (green fluorescent protein, GFP) which allows the virus to be detected in plants in which he virus is replicating.

To determine if TRV and PopMV can induce VIGS, the inserted gene encoding GFP as described above will be replaced by a portion of the gene encoding phytoene desaturase (PDS). In TRV three different RNA1 vectors will be made - one which contains the PDS sequence from tobacco, one which contains the PDS sequence from eucalyptus, and one which contains the PDS sequence for poplar. In PopMV two different vectors will be made - one that contains the PDS sequence from tobacco, and one that contains the PDS sequence for poplar. The PDS protein is an enzyme that catalyses a key step in carotenoid biosynthesis. Carotenoids are photoprotective pigments. Therefore, if the gene encoding PDS is silenced, no carotenoids are made and photobleaching occurs. Thus, photobleaching proves to be a simple means by which to test for whether TRV can cause VIGS of the PDS gene.

The PDS genes in TRV RNA2 and PopMV (described above) will be replaced by gene fragments corresponding to coding sequences for the following genes families:

i) Genes controlling flowering time, including LEAFY (which encodes a transcription factor) and TERMINL FLOWER-LIKE (which encodes a phosphatidyethanolamine-binding protein). Expression of these genes on viral vectors is hypothesised to alter flower time, either by overexpression or by VIGS.

ii) Genes involved in wood formation, including genes encoding MYB proteins, and lignin biosynthetic enzymes. Expression of these genes on viral vectors is hypothesised to alter wood quality or quantity, either by overexpression or by VIGS.

iii) Genes hypothesised to play a role in resistance to biotic or abiotic stress, including genes that encode leucine rich repeat (LLR) proteins. Expression of these genes on viral vectors is hypothesised to alter stress resistance, either by overexpression or by VIGS.

**Origin & function**

The insertion of foreign genes into the viruses is expected to decrease the fitness of the viruses as an infectious agent relative to the unmodified version of the virus. However in some cases the modified viruses may generate a wider range of symptoms on any plants that they do infect than does the unmodified virus. In particular:

i) VIGS induced loss of PDS activity results in photobleaching, this is likely to be deleterious to infected plants.

ii) Modifications resulting in alternations in flowering time and wood formation in infected plants result in changes in resource allocation that could be viewed as being deleterious to the plant; although, some of the changes in flowering time could be viewed as being beneficial to plant reproductive fitness. Overall the “health” of the plant (ie symptoms of disease) is likely to be unaffected by VIGS constructs designed to alter flowering time and wood formation.

iii) Constructs with modifications in genes hypothesised to play a role in resistance to biotic or abiotic stress, including genes that encode leucine rich repeat (LLR) proteins, are likely to increase the potential of the infected plants to succumb to abiotic or biotic stresses, the VIGS experiments would therefore have a deleterious effect on overall health or fitness of infected plants.

**Evaluation of foreseeable effects**

The genes involved in transmission will be removed from the TRV and PopMV such that in both cases the RNAs must physically applied to manually abraded plant leaves in order to be infectious.

The insertion of foreign genes into the viruses is expected to decrease the fitness of the viruses as an infectious agent relative to the unmodified version of the virus. However in some cases the modified viruses may generate a wider range of symptoms on any plants that they do infect than does the unmodified virus. In particular:

i) VIGS induced loss of PDS activity results in photobleaching, this is likely to be deleterious to infected plants.

ii) Modifications resulting in alternations in flowering time and wood formation in infected plants result in changes in resource allocation that could be viewed as being deleterious to the plant; although, some of the changes in flowering time could be viewed as being beneficial to plant reproductive fitness. Overall the “health” of the plant (ie symptoms of disease) is likely to be unaffected by VIGS constructs designed to alter flowering time and wood formation.

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**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 2, 1993 (either 121-125oC for at least 5 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Plants, plant material and soil - autoclave as specified in MAFF licence, discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Degree of kill
Autoclaving, effectively 100% kill (annual validation).
Incineration, effectively 100% kill (licensed incinerator).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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The European badger (Meles meles) acts as a wildlife reservoir for the Mycobacterium bovis the causative agent of bovine tuberculosis. Badgers infected with M bovis similarly develop tuberculosis. The Department for Environment, Food and Rural Affairs, DEFRA (formerly MAFF) are funding the Veterinary Laboratories Agency (VLA) Weybridge to develop a vaccine for tuberculosis in the badger. The attenuated vaccine, M. bovis Bacille Calmette Guerin (BCG) is a candidate vaccine for badgers, given its safety record and reported efficacy against tuberculosis (of both human and bovine type) in a number of studies. The challenge associated with the use of BCG as a vaccine for wildlife species is one of vaccine delivery.

The preferred route of delivery is oral and work is ongoing to develop strategies and formulations for the oral delivery of BCG. This project is directed at understanding the local immunological events in the gastrointestinal tract following ingestion of live BCG and is fundamental to developing a successful oral BCG vaccine.

Alternative well-characterised reporter genes may be inserted into BCG studied in a similar way.

Recipient or parental organism
Mycobacterium bovis bacille Calmette Guerin (BCG)

Host/vector system
Bacterial host M bovis BCG with plasmid vector
Green fluorescent protein (GFP) - from jellyfish, fluoresces under UV light.
LacZ - from Escherichia coli, produces beta galactosidase which reacts with the chromogenic substrate x-gal to produce a blue colour.
LuxAB genes - from Vibrio harveyi (or the American firefly luc gene), produce luciferases which are enzymes that catalyze production of light from luciferin and ATP.

Evaluation of foreseeable effects

The reporter genes are all well-characterised reporter genes with no known associated health hazards. No modification of genes involved in pathogenicity, virulence, host specificity or tissue tropism are being undertaken and there is no reason to expect the resulting genetically modified organism will differ in pathogenicity from the unmodified BCG strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes, culture plates etc) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (Either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration

Degree of kill:
Autoclaving, effectively 100% kill (annual validation).
Incineration, effectively 100% kill (licensed incinerator).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Project Ref** 553/02.2

**Date Ackn'd** 27/03/2002

**CU2 Project Title** MOLECULAR BASIS OF PARASITISM OF PLANT ASSOCIATED PSEUDOMONAS

**Class** Class 2

**CultureVol** < 1 litre

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The overall aims of this research programme are to understand the genetic basis of bacterial parasitism of plants and to understand at a mechanistic and evolutionary level how closely related bacteria have developed pathogenic or plant growth-promoting interactions with plants.

Plant pathogens and plant growth promoting Pseudomonas both enter into intimate interactions with plant hosts. The molecular basis of these interactions and of the different outcomes of these interactions, can only be described by comparing closely related model organisms. P. syringae and P. fluorescens are ideal organisms for this...
purpose: they share a high degree of similarity at a both genomic and physiological level; can be manipulated with the same molecular tools; the genomes of P. s. pv. 
tomato DC3000 and P. fluorescens SBW25 are being sequenced; and both P. s. pv. tomato and P. fluorescens enter into model interactions with the same host plant, the 
model plant Arabidopsis. Gene transfer between P. fluorescens SBW25, P. syringae pv. tomato and other relevant P. syringae strains, in conjunction with mutagenesis 
and complementation of genes involved in Pseudomonas-plant interactions, provided an extremely powerful explanatory tool for understanding the molecular basis of plant 
parasitism and plant-microbe interactions.

Recipient or parental organism

Pseudomonas savastanoi psv glycinea [pathogen of soybean (Glycine max)] and phaseolicola [pathogen of French bean (Phaseolus vulgaris)], P. syringae pathovars pisi 
[pathogen of pea (Pisum sativum)] syringae [pathogen of bean (P. vulgaris)], tabaci [pathogen of tobacco (Nicotiana tabacum) and N. clevelandii], and tomato [pathogens 
of tomato (Lycopersicon esculentum) and Arabidopsis thaliana] are all wild type strains.

P. s. pv. tomato mutant MXE is a kanamycin-resistant PT23 mutant with a transposon knockout of avrE.

Erwinia amylovora is a pathogen of most species of Maloidaea, such as apple and pear trees and the dspA/E-strain would be a kanamycin-resistant mutant with a 
transposon knockout of dspA/E.

Erwinia chrysanthemi is a pathogen of Chrysanthemum morifolium, but with a wide host range).

Escherichia coli strains (eg DH5, S17-1, Inv F’) and derivatives will be used for general cloning.

Host/vector system

Bacterial hosts identified above with plasmid vectors.

Origin & function

Various genes are known or are implicated to have a role in the complex interactions between micro-organisms and plants. These genes are instrumental in determining 
whether the outcome is a pathogenic or plant growth-promoting interaction with the plant. Such genes (including avirulence genes, regulatory genes from the type III 
secretion system and effector genes) will be transferred between different Pseudomonas and Erwinia. Mutagenesis and complementation of such genes will allow function 
to be elucidated.

Evaluation of foreseeable effects

The purpose of this work is to study the association between micro-organisms and plants and identify the genes and pathways that result in the association between either 
a pathogenic one or a plant growth promoting one. Transfer of such genes between pathogens and plant growth promoting bacteria is likely to result in a wide range of 
effects from reduced to enhanced virulence. In no case is the anticipated increased virulence expected to be significantly higher such as to warrant additional control 
measures compared to those required for wild type Pseudomonas pathogens. These are plant pathogens only and there is no reason to suspect that the modifications will 
result in any potential harm to humans or animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Plants, plant material and soil - autoclave as specified in MAFF licence, discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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Project Ref 553/02.4

02/03/2022
Investigation of the interaction between the nematode pathogen Microbacterium nematophilum and its host, Caenorhabditis elegans, will be carried out by conventional genetic methods and by attempting to introduce standard bacterial transposons, in order to disrupt and thereby clone bacterial genes that are involved in this interaction. Attempts will be made to introduce plasmids expressing Green Fluorescent Protein or other markers such as beta-galactosidase in order to facilitate examination of the bacteria during host infection.

Microbacterium nematophilum is a recently discovered bacterium that can act as a facultative pathogen of the soil nematode Caenorhabditis elegans. Infected worms become deformed and grow slowly, but are not killed by the infecting bacteria, so the pathogenic effects are minor.

A wild type strain of *M. nematophilum* will be modified using standard plasmid or phage vector systems.

Inserted DNA will carry selectable resistance markers and, in some cases, constructs encoding Green Fluorescent Protein or other standard histochemical staining markers. *E. coli* (disabled) and *Bacillus subtilis* will be used as sources of shuttle vectors for transformation. *E. coli* will also be the source of *lacZ*. Green Fluorescent Protein is a harmless protein isolated from jellyfish (*Aequoria victoria*).

Modification of *M. nematophilum* to create non-pathogenic, GFP-tagged or otherwise marked strains would, if anything, reduce the natural impact of this bacterium.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) discharge to drains.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal waste (nematodes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill (Note - nematodes are only 1mm long and not recognisable following autoclaving).

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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<td>L4</td>
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02/03/2022
## Project Additional Information

### Purposes of the contained use

Bacteria in the species Salmonella are orally acquired bacteria causing diseases ranging from gastroenteritis to Typhoid fever.  *S. enterica* serovar Typhimurium (*Salmonella typhimurium*) is widely used in rodent models to study Typhoid fever pathogenesis. Oral infection models in rodents have greatly increased our understanding of mucosal and systemic immunity to orally acquired bacteria. As the oral infection route is a common way humans acquire many pathogens, and the potential to exploit oral immunisation in developing novel vaccine strategies or tolerance to orally-delivered antigens, understanding the mechanisms underlying generating immunity to orally acquired infections is vital.

Orally acquired bacteria must penetrate the gut epithelium to cause infection. Exactly how this occurs during Salmonella infection is not known. Furthermore, the cell(s) involved in transporting Salmonella from the gut mucosa into the body during oral infection mice is unknown.

**Aims:**

1. To identify the cell(s) transporting orally acquired Salmonella into draining lymph nodes
2. To determine the capacity of these cells to stimulate and modulate Salmonella-specific T cells.

*Salmonella typhimurium* will be genetically modified to carry the marker genes that will make the Salmonellas fluoresce under UV excitation, enabling the bacteria to be identified in the cells harvested from the lymph. Immunological techniques such as flow cytometry and immunofluorescence microscopy will then be used to identify and characterise the cell types harbouring the orally administered *Salmonella*. The influence of the isolated *Salmonella*-laden cells on other cells of the immune system, such as T lymphocytes, will also be evaluated. To assess the interaction with antigen-specific T cells the *S. typhimurium* will in addition be genetically modified to express albumin from chicken egg white. This approach is a unique means to study the interaction of orally administered bacteria, including pathogens as well as vaccine candidates, with the cells of the gastrointestinal tract responsible for delivering them into host tissues and also the activation of antigen-specific cells of the immune system. *Escherichia coli* genetically modified in the same way will be used for control purposes.
<table>
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<th><strong>Recipient or parental organism</strong></th>
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<tr>
<td>Salmonella enterica serovar Typhimurium (S. typhimurium)</td>
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<tr>
<td>Escherichia coli</td>
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<th><strong>Host/vector system</strong></th>
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<tbody>
<tr>
<td>Bacterial hosts with plasmid vectors</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th><strong>Origin &amp; function</strong></th>
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<tbody>
<tr>
<td>i) GFP and DsRed - standard marker genes</td>
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<td>GFP - green fluorescent protein, from jellyfish, is a protein that fluoresces at a visible wavelength when excited by ultra violet light.</td>
</tr>
<tr>
<td>DsRed - red fluorescent protein, from reef coral, is a protein that fluoresces at a visible wavelength when excited by ultra violet light</td>
</tr>
<tr>
<td>ii) Ovalbumin (OVA) - albumin, from chicken egg white (ovalbumin; OVA), is a well-characterised protein widely used as a model antigen to monitor immune responses.</td>
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<tr>
<th><strong>Evaluation of foreseeable effects</strong></th>
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<tr>
<td>The inserted reporter genes are well-characterised with no known associations health hazards. Albumin is a well-characterised protein widely used as a model antigen to monitor immune responses; it has no other known biological activity and no adverse effects of using OVA as a model protein have been reported. No modification of genes involved in pathogenicity, virulence, host specificity or tissue tropism are being undertaken and there is no reason to expect the resulting genetically modified organisms will differ in pathogenicity from the unmodified strains.</td>
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Project Ref 553/02.6

Date Ackn'd: 04/09/2002

CU2 Project Title: CHEMICAL RETARGETING OF GENE DELIVERY VECTORS FOR THE TREATMENT OF CANCER AND OTHER HUMAN DISEASES

Class: Class 2

Consent Granted: not applicable

Project notified under transitional arrangements: N

Historical Significant Changes

Significant Date of Additional Info

Significant Change ID

Date of Significant Change

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Genetically modified viruses expressing therapeutic genes have great potential for the treatment of a variety of human diseases including cancer. Replication deficient adenoviruses have been widely studied as potential vectors for many years, however as a delivery vehicle they are less than perfect. This work aims to address the limitations of systemic virus delivery.

In the treatment of cancer, extended circulation of the virus within the blood stream is crucial for gene delivery to secondary tumours which may have spread to sites distant from the primary tumour. However many people will have been exposed to naturally occurring wild type adenovirus type 5 (Ad5), consequently the administration of therapeutic adenoviruses will result in their recognition and neutralisation by pre-existing antibodies before they have a chance to reach their target. The use of avian based adenoviral vectors will be developed to circumvent this problem, as will chemical coating of human adenovirus.

Due to the problems of efficiently penetrating large tumour masses, it is virtually impossible for a replication-defective virus to deliver a therapeutic gene to every tumour cell. Viruses which are able to replicate and move to neighbouring cells adjacent to the site of initial infection, thus spreading throughout the tumour, are much more likely to achieve a therapeutic cure. Conditionally replicating mammalian adenoviral vectors will be developed to address this aspect of improved therapy.

Adenovirus will initially bind to cells via a receptor (CAR) that is present on a wide range of tissue types and so much of the vector is lost through infection of healthy cells, which reduces the amount of virus able to reach the target tissue. Part of this project aims to further develop the viral vectors described above and chemically alter them to produce non-infectious polymer coated virus which can them be retargeted by attaching individual ligands that recognise specific human cell surface receptors, which are restricted to certain tissue or overexpressed on tumours.

Recipient or parental organism

i) Chicken Embryo Lethal Orphan (CELO) Virus - an avian adenovirus.
ii) Human adenovirus.

The adenoviruses will be targeted by chemical treatment. This involves coating of the virus with a polymer which will abrogate the binding to cell surface receptors. The coated virus will then be retargeted using human ligands. The receptors for several of the ligands used are expressed on normal cells at very low levels but are up-regulated or over-expressed on tumour cells.

Host/vector system

CELO propagated in chicken eggs, human adenovirus propagated in human cell lines.

Origin & function

Two strategies have been developed to regulate the replication of the human adenoviruses. The first involves mutant adenoviruses that have been designed to replicate only in cells carrying genetic mutations typical of cancer cells; the second strategy uses cancer-selective promoters to regulate expression of virus genes that are essential in replication, thereby restricting virus proliferation to tumour cells.

Gene inserts are:

a) standard reporter genes:
   - luciferase, from firefly and sea pansy
   - B-galactosidase, from E.coli
   - green fluorescent protein (or similar), from jellyfish
b) therapeutic genes (prodrug activating enzymes):
- nitroreductase, from E. coli, a flavoenzyme, the natural function of which is unclear, though one possibility is a role in xenobiotic metabolism. It can reduce a variety of quinone and nitroaromatic substances, including prodrugs such as CB 1954.
- cytosine deaminase, from E. coli, activates 5-fluorocytosine to 5-fluorouracil (5-FU), a well established anticancer drug.
- uracil phosphoribosyl transferase, from E. coli; enhances the efficacy of the cytosine deaminase system by improving the conversion of 5-FU to 5-dUMP in the cell.
- ganciclovir is an acyclic analog of the natural nucleoside 2'-deoxyguanosine and has substrate specificity for the viral Herpes Simplex Virus Thymidine Kinase (HSV-TK) enzyme, that is three orders of magnitude more efficient at monophosphorylating GCV than any human nucleoside kinase. GCV monophosphate is converted into a toxic GCV-triphosphate nucleotide by cellular kinases. Since GCV monophosphate is converted into a toxic GCV-triphosphate nucleotide by cellular kinases. Since GCV-triphosphate resembles 2'-deoxyguanosine triphosphate it is a substrate for DNA polymerase, however because of its structure it is a poor substrate for continued chain elongation and chain termination occurs.

Some of the conditionally replicating adenoviruses will incorporate the therapeutic gene under an additional level of control in the form of a tissue specific promoter (for example telomerase and hypoxia responsive promoters). These are more active in particular tumour types than in the tissue from which they originate.

As described earlier some of the adenoviruses will be retargeted by chemical treatment (rather than by genetic modification).

Evaluation of foreseeable effects

In summary,
- therapeutic genes are active only in the presence of pro-drugs,
- chemical coating of the viruses will lead to improved ability to evade immune responses,
- retargeting will result in restricted tissue tropism,
- there is no reason to suspect alteration in host range,
- the human adenoviruses will replicate only in tumour cells.

More specifically:

i) CELO virus

Adenoviruses are extremely species specific in their replication capacity, as a result avian adenoviruses including CELO are unable to replicate in mammalian cells. The retargeting of CELO virus will restrict the tropism to cells that express the appropriate human cell surface receptors. Consequently the tissue tropism of the retargeted virus is restricted compared to that of the natural virus, which potentially could enter the extensive range of mammalian cells that express CAR. However the retargeted virus having gained entry to the cells via the appropriate receptor will still remain unable to replicate in mammalian cells. In the absence of prodrug, expression of the prodrug-activating transgenes is not expected to have any harmful consequences. The reporter genes are well established as non-harmful.

ii) Conditionally replicating human adenoviruses (CRAds)

Human adenoviruses including Ad5 are extremely species specific in their replication capacity. There is no evidence that human serotypes can naturally infect animals. The polymer coat of the retargeted viruses will help the virus to evade antibody neutralisation, however the virus will only gain entry to cells expressing an appropriate receptor and as such will have a restricted tissue tropism in comparison with the wild type Ad5 or non-polymer coated CRAd. The new targeting moiety would both enhance and restrict the entry of the virus to cells which would otherwise be entered via initial CAR binding. Since only human targeting ligands are being investigated the host range of the retargeted virus will not be altered. Non-tumour cells that express receptors recognised by the targeting ligand will not support virus replication. In the absence of prodrug, expression of the prodrug-activating transgenes is not expected to have any harmful consequences. The reporter genes are well established as non-harmful. The risks from CRAds expressing tissue specific promoter driven therapeutic genes would be no greater than CRAds expressing a CMV driven therapeutic gene.

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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Genes encoding proteins likely to be important in controlling cell shape, motility, metabolism and nuclear organisation of the African trypanosome will be modified, expressed or manipulated in cultured insect or bloodstream forms of the parasite. Our aim is to study fundamental aspects of parasite molecular cell biology - in particular, cell differentiation, cell motility, mitosis and control of antigenic variation. Overall, we believe that these experiments will assist in providing insight to basic parasite biology and to pathogenicity mechanisms. Given the emerging T. brucei spp. sequence information, these experiments may identify strategies and targets important for future control measures for these parasites.

Recipient or parental organism

E. coli, Lambda phage, Crithidia fasciculate, Crithidia oncopelti, Crithidia deanei, Crithidia guillermei, Herpetomonas megaseliae, Herpetomonas roitmani, and Blastocrithidia culicis.

Leishmania major, Leishmania mexicana, Trypanosoma brucei brucei, Trypanosoma brucei gambiense and Trypanosoma congolense

Trypanosoma brucei rhodesiense.
Non-mobilisable shuttle vectors engineered to enable parasite gene expression or modulation in the parent organism using a tetracycline regulated inducible expression system.

**Origin & function**

The main focus of our work is genes that are likely to be important in controlling cell shape, motility, metabolism and nuclear organisation.

The main types of gene inserts to be used are:
- Cytoskeletal and other housekeeping genes involved in the construction of the cytoskeleton, mitotic spindle and flagellum of the protozoa. Usually these genes will be tagged to provide epitope or other marking of the expressed proteins.
- Portions of genes arranged to produce double stranded RNA copies that will down regulate endogenous gene expression by means of RNA interference.
- Flanking sequences will be used to engineer gene knockouts.

Most experiments will not involve cross species transfer. Some experiments will test putative homologous genes from other strains/species/genus for complementation of function or inactivation of function in the trypanosome. Given that the genes to be moved are involved in housekeeping functions are are not known (or likely to be) virulence genes the likely outcome of movement of genes is loss of virulence, and in the case of movement into a non-pathogenic strain will not result in a gain of virulence.

**Evaluation of foreseeable effects**

We expect to see phenotypes dependent upon the specific situation. These will range from paralysis (flagellum motility); abnormal shape/division defects (mitotic and cytoskeletal proteins); antigenic coat abnormalities (nuclear regulatory proteins); general growth phenotypes (metabolic proteins). Often, the outcome will be lethal phenotype particularly in initial gene experiments using RNA interference. In many cases, transgenic bloodstream and insect form parasites will be used and differentiation between these stages examined. However, it is not possible to differentiate parasites from the insect form back to the bloodstream form in the absence of the tsetse fly vector. Therefore, the lifecycle cannot be completed in the laboratory. These genes will be expressed using a tetracycline inducible expression system. Therefore, in the absence of tetracycline, the genes are effectively silenced.

Antibiotic resistance genes will be used for selection of transgenic parasites. These are not those used for clinical treatment of the parasite and will otherwise have no foreseeable effect.

Reporter genes (eg green fluorescent protein and epitope-tagged proteins) are for localisation and assay of expression pattern for particular parasite genes or sequences. There is no foreseeable adverse consequence of this.

In most instances the donor and recipient species are the same except during gene construction/manipulations in disabled E. coli. Usually genes transfer experiments will involve rescue of mutants to reveal shared functions or use of partial gene sequences (usually regulatory sequences) in order to control gene expression. This organism's pathogenicity is not based on toxins or other virulence factors whose genes could be inadvertently transferred between species. It is considered that no new or additional potential risks will be created through the movement of any cryptic gene to a new potentially pathogenic species background. The only known difference between T.b rhodesiense and the other trypanosomes is the possession of a human serum resistance gene. This is known and is not the focus of our studies.

The risks of the genetically modified organisms to human health and safety and to the environment are considered likely to be equivalent to, or usually lower than, that of the 'wild type' parental strain.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

These parasites do not produce spores and are fragile organisms. They are rapidly killed outside the culture vessel or blood by desiccation, osmotic shock, washing with water, detergents, and cannot penetrate unbroken skin. Tsetse flies are the vectors for trypanosomes and these are endemic only in the disease areas (Sub-Saharan regions).
Africa. We do not maintain insectories with these vectors. Thus these parasites are effectively deficient in mobilisation capacity.

The naturally occurring organism does not represent any hazard of infection through aerosol transfer. Aerosols are tiny droplets of liquid. These dry out within minutes under the laboratory conditions. Once dehydrated such material would not be viable.

ACDP guidance on work with T.b. rhodesiense:
The Schedule to the certificate of exemption accompanying the Approved List specifies a number of parasites, including T.b. rhodesiense, for which full Containment Level 3 need not be used. ACDP state that work with such parasites does not generally require an inward flow of air to the laboratory or the use of a microbiological safety cabinet as none of these agents is normally infectious by the airborne route. ACDP recommends that for working with these agents in research a separate room should be used or a designated area in a larger laboratory. In ACDP's guidance list is the notation that the laboratory need not be sealable for fumigation.

In relation to the individual control measures to be omitted from both laboratory and animal facilities:

a) isolated laboratory suite
A dedicated room for will be used, however this will be used for all the work with parasites detailed in this risk assessment (ie in Classes 1, 2 and 3). The room will at all times be operated to Containment Level 3 working practices and have a documented Code of Practice. This room is within the suite of rooms allocated to the research group undertaking this work.

b) Laboratory not sealable for fumigation.
There is no foreseeable need to fumigate the laboratory. In the event of a major spillage, the bulk of material would be soaked up on absorbent paper towels then autoclaved, killing any parasites. The area would be wiped down with disinfectant. Any aerosols created that were not cleaned in this initial effort would dry out, killing both host cell and parasite. The area of the laboratory would be left isolated for a period to ensure effective drying. Effective parasite transmission is only possible via direct inoculation of viable parasites.

c) No negative pressure, HEPA filtered extract, microbiological safety cabinet or specified measures to control aerosol dissemination.
The organism presents no hazard of infection by the airborne route. Class II microbiological safety cabinets are in use in the dedicated laboratory, they are necessary for sterile culture of the organism; they are not required for operator safety. Activities involve small scale, standard laboratory techniques with no aerosol generating procedures.

d) Autoclave and some other equipment not within the laboratory.
Although the laboratory contains most of the necessary equipment for the work, some activities use specialist equipment that cannot reasonably be accommodated within the dedicated facility. In all cases a safe system of transport involving secondary containment is in use, any equipment used is cleaned and disinfected immediately on completion of the work and any contaminated items wither returned to the dedicated laboratory:

i) Fluorescence microscopy: a fluorescent microscope is located within the same building.
ii) Storage: transfected parasite stocks are frozen and stored at -80 degrees C or in liquid nitrogen within the same building. The freezers are locked and accessed only by authorised personnel.
iii) Autoclaving: an autoclave validated for disposal of waste is located within the same building.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill

Autoclaving: Effectively 100% kill (annual validation)

Incineration: Effectively 100% kill (annual validation)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

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Project Ref  553/03.1
This project is directed at understanding antigenic variation in trypanosomes and how the organism evades the complement system in mammalian hosts.

i) Antigenic variation
The trypanosome wears a dense Variant Surface Glycoprotein (VSG) coat which shields invariant molecules on the trypanosome surface from the immune system. The trypanosome evades the immune system of the mammalian host, by continuously changing its VSG coat. As trypanosomes multiply in the blood of an infected mammal, eventually an antibody response is raised against a given VSG coat, killing all trypanosomes wearing this coat. However, at the population level, trypanosomes are continuously generated that have switched to a new (and temporarily unrecognisable) coat. These switch variants are not recognised by host antibodies, and can form the next wave of infection. Antigenic variation allows trypanosomes to form chronic infections in large mammals which can last for many years.

An individual trypanosome has about 1000 genes encoding different VSG coats. The active VSG gene is located in a VSG expression site, which is invariably located at a telomere or chromosome end. Each trypanosome has 20 VSG expression sites, of which only one is active. An individual trypanosome transcribes only a single VSG at a time. Switching the active VSG involves DNA rearrangement events slotting a previously inactive VSG gene into the active VSG expression site. Alternatively, trypanosomes can switch between the twenty VSG expression sites. We would like to understand how trypanosomes coordinate expression of these 20 VSG expression sites.

ii) Evasion of the complement system
In addition to evading the antibody response using antigenic variation, trypanosomes are also able to evade the complement system of the mammalian host. The mechanism of complement evasion is unclear, and something that we would like to investigate. The complement system has different functions. One of these is the opsonisation or coating of microbes, making them targets for macrophages with complement protein receptors. In addition, complement proteins can assemble on the surface of a microbe resulting in the formation of a membrane attack complex.

We have performed an initial analysis of complement evasion by T. brucei incubated in bovine serum and obtained promising results. However, unfortunately we are hampered by the lack of reagents against components of the bovine complement system. We would therefore like to continue our analyses using T. brucei incubated in
human serum. There are a much larger range of reagents against human complement proteins. In addition, these studies will be more relevant for understanding complement evasion in the trypanosomes causing African trypanosomiasis.

The T. brucei brucei 427 strain that we currently work with (and which is sensitive to human serum) grown very well in vitro. In contrast, T. brucei rhodesiense field isolates that are resistant to human serum do not grow in vitro. We would therefore like to create a T. brucei brucei strain that can grow well in vitro in human serum. We would like to genetically modify nonhuman infective T. brucei brucei strains, and insert virulence genes like the Serum Resistance Antigen (SRA) gene into these trypanosomes so that they become resistant to human serum. This will make T. brucei brucei comparable to the T. brucei rhodesiense that we already currently hold. However, these genetically modified trypanosomes will have the advantage of being manipulable in vitro instead of requiring expansion in laboratory rodents. In addition, the virulence genes would be regulatable, greatly increasing the safety of the experiment. We would therefore be able to study the pathogenic aspects of human infectivity in a manipulable in vitro system, and could avoid amplification in laboratory animals.

Recipient or parental organism

E. coli K12 or B derivatives
Trypanosoma brucei brucei 427

Host/vector system

Bacterial or parasite host with plasmid vectors

Origin & function

i) Antigenic variation
Single copy marker genes will be inserted into the repetitive VSG expression sites in order to follow the transcriptional behaviour of single expression sites. Constructs containing fluorescent marker genes like Green Fluorescent Protein (GFP), Yellow Fluorescent Protein (YFP) and DsRed, and drug resistance marker genes like the hygromycin, neomycin, blasticidin, and puromycin resistance genes into various regions of the T. brucei genome including the VSG expression sites. Marker genes introduced into VSG expression sites will allow monitoring of their transcriptional behaviour.

VSG expression sites include multiple genes of unknown function, in addition to the telomeric VSG gene. RNAi (double stranded RNA inhibition) will be used to inactivate the transcripts from various Expression Site Associated Genes (ESAGs), in addition to transcripts from other candidate genes possibly playing a role in antigenic variation. This technique allows the researcher to investigate the essentiality of different gene families.

ii) Evasion of the complement system
Virulence genes like the Serum Resistance Antigen (SRA) gene will be inserted into nonhuman infective T. brucei brucei strains so that they become resistant to human serum. These virulence genes would be inserted behind a tetracyclin inducible promoter, allowing the researcher to turn expression on and off. This will make T. brucei brucei comparable to T. brucei rhodesiense and facilitate study of the pathogenic aspects of human infectivity in a manipulable in vitro system avoiding amplification in laboratory animals.

Evaluation of foreseeable effects

i) Antigenic variation
The genes used here are completely neutral markers encoding fluorescent proteins, or are drug resistance genes for drugs that are not therapeutically relevant for African trypanosomiasis. There is therefore no biochemical mechanism that could explain how these genetic modifications could change the host range or virulence of the genetically modified trypanosome. The genetic modifications proposed would not change T. brucei susceptibility to the therapeutic drug suramin and there is no reason to believe that these genetic modifications would change the tissue tropism of the parasite. The double-stranded RNA inhibition (RNA) experiments resulting in the ablation of T. brucei RNA transcripts will incapacitate the parasites if the transcripts that are being targeted are essential. Nonessential transcripts being targeted will not result in a change to the trypanosome phenotype. The genetic modifications resulting in the introduction of marker genes into the VSG expression site could result in damage to the active VSG expression site. This would lead to reduced efficiency of antigenic variation. None of these procedures would lead to increased risk to human health and safety.
Evasion of the complement system

These T. brucei genetic modifications could make a trypanosome strain that lyses in human serum into one that doesn't lyse in human serum, making it potentially human infective. These genetically modified strains would become similar to the human infective T. brucei rhodesiense strains. All published evidence indicates that the SRA gene product is a receptor that interferes with internalisation of the Trypanosome Lytic Factor (TLF) present in human serum. There is no published evidence indicating that expression of the SRA protein could affect the tissue tropism of the trypanosomes. The SRA protein is a receptor for the Trypanosome Lytic Factor, and there is no known biochemical mechanism that could mediate changed drug susceptibilities in an SRA transgenic trypanosome. There is no known biochemical pathway that would confer susceptibility to the drug suramin, currently used in treating human trypanosomiasis, to T. brucei SRA gene transgenics.

These genetic modifications could increase risk to human health and safety and consequently additional containment and control measures have been assigned. However, even though these genetically modified organisms would be less safe than the original trypanosome, they would be much more safe to work with than human infective T. brucei rhodesiense as the tetracyclin inducible nature of virulence gene expression means that the researcher can restrict expression of these genes to the shortest period of time.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

These parasites do not produce spores and are fragile organisms. They are rapidly killed outside the culture vessel or blood by desiccation, osmotic shock, washing with water, detergents, and cannot penetrate unbroken skin. Tsetse flies are the vectors for T. brucei and these are endemic only in the disease areas (subSaharan Africa).

The naturally occurring organism does not represent any hazard of infection through aerosol transfer. Aerosols are tiny droplets of liquid. These dry out within minutes under the laboratory conditions. Once dehydrated such material would not be viable.

ACDP guidance on work with T. brucei sp.

The Schedule to the certificate of exemption accompanying the Approved List specifies a number of parasites, including T. brucei sp. for which full containment level 3 need not be used. ACDP state that work with such parasites does not generally require an inward flow of air to the laboratory or the use of a microbiological safety cabinet as none of these agents if normally infectious by the airborne route. ACDP recommends that for working with these agents in research a separate room should be used or a designated area in a larger laboratory. In ACDP's guidance list is the notation that the laboratory need not be sealable for fumigation.

In relation to the individual control measures to be omitted:

- Isolated laboratory suite
  A dedicated room will be used for working with T. brucei. The room will at all times be operated to derogated Containment Level 3 working practices and have a documented Code of Practice.

- Laboratory not sealable for fumigation
  There is no foreseeable need to fumigate the laboratory. In the event of a major spillage, the bulk of material would be soaked up on absorbent paper towels, then autoclaved, killing any parasites. The area would be wiped down with disinfectant. Any aerosols created that were not cleaned in this initial effort would dry out, killing both host cell and parasite. The area of the laboratory would be left isolated for a period to ensure effective drying. Effective parasite transmission is only possible via direct inoculation of viable parasites.

- No negative pressure, HEPA filtered extract, microbiological safety cabinet or specified measures to control aerosol dissemination.
  The organism presents no hazard of infection by the airborne route. Class II microbiological safety cabinets are in use in the dedicated laboratory, they are necessary for
sterile culture of the organism; they are not required for operator safety. Activities involve small scale, standard laboratory techniques with no aerosol generating procedures.

d) Equipment not within the laboratory
Although the laboratory contains most of the necessary equipment for the work, some activities use specialist equipment that cannot reasonably be accommodated within the dedicated facility. In all cases a safe system of transport involving secondary containment is in use, any equipment used is cleaned and disinfected immediately on completion of the work and any contaminated items either returned to the dedicated laboratory or removed for disposal as waste. The following activities are undertaken outside the dedicated laboratory:

i) Storage: transfected parasite stocks are frozen and stored temporarily at -80 degrees C in the adjacent Containment Level 2 laboratory before long term storage in liquid nitrogen within the Containment Level 3 laboratory. The samples will be in Nunc ampules in closed and sealed freezing boxes that are clearly marked as biohazard. The -80 degrees C freezer will be locked during use and accessed only by authorised personnel.

ii) Autoclaving: an autoclave validated for disposal of waste is located within the same building.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (121-125 degrees C for at least 15 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646 Part 3, 1993 (121-125 degrees C for at least 15 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (121-125 degrees C for at least 15 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - Dispose via clinical waste stream for incineration.

Degree of Kill

Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

### Project Containment

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<tr>
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<th>Human Clinical Applications</th>
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</table>

### Project Ref 553/03.10

- **Date Ackn'd**: 10/12/2003
- **CU2 Project Title**: USING RETROVIRAL VECTORS FOR THE CHARACTERISATION OF THE HYPOXIA-INDUCIBLE FACTORS (HIF) PATHWAY IN CANCER CELLS; AMPHOTROPIC VISUS.
- **Class**: Class 2
- **CultureVolClass2**: < 1 litre
- **Non-GMM Consent Granted**: N

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

### Project Additional Information

**Purposes of the contained use**

Replication defective amphotropic retroviruses will be used to assess the downstream biological effects of HIF-1, HIF-2 and degradation resistant HIF-1 in Renal cell carcinoma and other similar cell lines.

**Recipient or parental organism**

02/03/2022
For construction of retroviral vectors:
K12 or B derivatives of E. coli will be used as bacterial cloning hosts to generate plasmid expression clones in readiness for expression within specified packaging cell lines. These are disabled hosts that cannot colonise the human gut and have a history of safe use. These hosts may be considered equivalent to ACDP hazard group 1.

For packaging recombinant retroviral particles.
The packaging cell lines are of human origin, which are well characterised and authenticated and are obtained from commercial sources. Examples of packaging cell lines likely to be used are Phoenix 293 ampho cells, which generate amphotropic retroviruses. They can be regarded as low ghazard for GM activities and as hosts are suitable for Containment Level 1 precaution. However due to the presence of the E1a gene of Ad5 these cells will be handled at Containment Level 2 under COSHH.

For recombinant retroviral infection
Human renal cancer cell lines (and other similar cancer cell lines), eg 786-0 and RCC4 can be considered as especially disabled hosts and as such may be considered to be equivalent to ACDP hazard group 1.

Host/vector system
Genes of interest will initially be obtained from plasmids based on pcDNA1 Neo and pcDNA3. They will then be inserted into disabled retroviral expression vectors LZRS-IRES-GFP and/or pBMN-1-GFP. Vectors are based on pUC backbone and are non-mobilisable.

pBMN-1-GFP contains 5' and 3' LTR and IRES-GFP into which the gene of interest is inserted.

LZRS-IRES-GFP contains 5' and 3' LTR and IRES-GFP into which the gene of interest is inserted and also EBV nuclear antigen and origin of replication.

To generate amphotropic retroviruses the expression plasmids are transfected into Phoenix-Ampho cells, derived from HEK293 cells which have already undergone subsequent stable transfections with plasmids containing moloney packaging functions gag-pol and env genes. Non-moloney promoters are used to drive the gag-pol and env genes to minimise their inter-recombination potential. Stable integration of gag-pol and env genes into different sites of the 293 genome means that none of these genes are included in the viral particle thus generating a replication deficient virus.

Transfection with pBMN-1-GFP plasmid generates transient production of virus particles.

Transfection with LZmartin.wale@hpa.org.uk plasmid generates medium term production of virus particles over 20-3 months due to episomal nature of the plasmids.

Origin & function
Wild type HIF-1
Wild type HIF-2
HIF-1 double prole mutant resistant to degradation.

In normal cells HIF-1 and HIF-2 expression is regulated by degradation, the mutant would be constitutively expressed. In VHL defective renal cells HIF-1, HIF-2 and the mutant would be constitutively expressed.

HIF-1 and HIF-2 over-expression (constitutive or mutant forms) have been shown to have varying effects on tumourigenesis. Indeed, HIF-1 seems to retard tumour growth in certain models, while in normal mice mutant HIF-1 has promoted angiogenesis and thus hypervascularity without tumour formation. HIF-2 seems to have a possible role in the progression of Renal Cell Carcinoma; however, no specific genes to date have been assigned to this transcription factor that are not already also controlled by HIF-1, thus uncertainty remains in the field as to its role in tumour progression. It is not able to transform cells.

Standard markers such as enhanced green fluorescent protein (EGFP) from jellyfish will also be used. These are not known to have any harmful physiological or pharmacological properties or to affect pathogenicity of the micro-organisms.
**Evaluation of foreseeable effects**

No significant hazards have been identified from insertion of the foreign sequences into E. coli K12 or B derivatives. The vectors are based on the pUC backbone and are non-mobilisable. They are not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms. Gene transfer is possible, but unlikely to be hazardous. The resulting GMOs are not expected to carry any additional risks compared to that of the un-modified recipients and as such can be handled at Containment Level 1.

Transfection of the Phoenix-Ampho cell line is expected to result in the generation of a Retrovirus that is capable of infecting most mammalian cells and integrating into the host DNA but is incapable of replication. As the virus is replication defective there is no risk of transmission within the environment. ACDP states that replication defective amphotropic viruses contain functional oncogenes should be handled at Containment Level 2. Due to the uncertain role of HIF-1 in the progression of tumourigenesis they will be handled at Containment Level 2.

Calls infected with virus will be handled at Containment Levels 2. Once the original supernatant is removed, the viruses inside the cells integrate their DNA into the host genome within 24-36 hours, and thus the viruses themselves disassemble during this period. After this period, the cells can be handled at Containment Level 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| Not applicable. |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incinerational/dispose of solids via the industrial (black bag) waste stream for landfill.**

**Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.**

**Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.**

**Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.**

**Degree of kill:**
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)

**Is an emergency plan required according to regulation 20?**

- N

**If yes, tick to confirm that it is attached to this form**

- N

**Tick to confirm that you have attached a risk assessment to this form**

- Y
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

<table>
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- Animal Units
- Large Scale Activities
- Human Clinical Applications

**Project Ref**  553/03.2

- **Date Ack'n'd**: 13/02/2003
- **CU2 Project Title**: IDENTIFICATION OF RECEPTOR FOR HEPATITIS B VIRUS (HBV)

- **Class**: Class 2
- **Culture Vol**: < 1 litre
- **Consent Granted**: not applicable

**Withdrawn**: N

Tick if notifying a connected programme of work: N

**Project Additional Information**

**Purposes of the contained use**

We aim to isolate putative cellular receptor(s) for Hepatitis B Virus. We propose to develop a modified HIV1 minimal vector system for this purpose. Using the modified HIV1 minimal vector system, we would like to isolate cDNA corresponding to the putative receptor(s) from a human liver cDNA library expressed in 293T cells.
<table>
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<tr>
<th>Recipient or parental organism</th>
<th>mammalian cell line</th>
<th>HIV based viral vectors</th>
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<td><strong>Host/vector system</strong></td>
<td><strong>Mammalian cell line</strong></td>
<td><strong>HIV based viral vectors</strong></td>
</tr>
<tr>
<td><strong>Origin &amp; function</strong></td>
<td>A modified HIV minimal vector carrying a reporter gene and pseudotyped with HBV envelope will be produced. These vector particles will be used to transduce 293T cells (human kidney derived) expressing human liver cDNA library. Successfully transduced cells will be selected and cDNA(s) corresponding to HBV receptor(s) isolated from these cells.</td>
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<td><strong>Evaluation of foreseeable effects</strong></td>
<td>The vector particles are non-replication competent and self-limiting. The further modification of the minimal vector particles brings about only a change in the intracellular location of packaging and release of the vector particles, and such vector particles will be very much restricted in their host range (compared to standard HIV1 minimal vector particles). Hence they may be considered to be safer than the standard HIV1 minimal vector particles. The modified HIV1 minimal vector particles are therefore considered to present low risk to human health. However, since the envelope from a level 3 virus (HBV) is used, containment level 2 is considered appropriate.</td>
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<td><strong>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</strong></td>
<td><strong>NOT APPLICABLE</strong></td>
<td></td>
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<tr>
<td><strong>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</strong></td>
<td><strong>NONE</strong></td>
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Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Project Ref 553/03.3**

- **Date Ackn’d**: 13/02/2003
- **CU2 Project Title**: CO-EVOLUTION OF PSEUDOMONAS AERUGINOSA AND THE RNA BACTERIOPHAGE PP7
- **Class**: Class 2
- **Culture Volume Class 2**: < 1 litre
- **Consent Granted**: not applicable
- **Non-GMM**: N
- **Date Project Ceased**: Withdrawn
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: Not applicable
- **Historical Date of Additional Info**: Not applicable
- **Significant Change ID**: Not applicable
- **Date of Significant Change**: Not applicable

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**
Project Additional Information

Purposes of the contained use

The overall aim of this research programme is to understand the genetic basis of the co-evolution of a bacterial host and a virulent RNA bacteriophage. This research will investigate how host and phage mutants diversify and adapt to one another and, by specific molecular analysis of phage attachment processes, to identify host and phage mutations which give rise to resistance or increased virulence.

This proposal therefore seeks to examine how a bacterial host, P. aeruginosa PA01, and its bacteriophage, PP7, co-evolve when incubated for long periods together. During this time, mutations may occur in the host which make it more resistant to the bacteriophage, and similarly, mutations may occur in PP7 allowing it to overcome the host defences.

In order to interpret the significance of mutations in both host and bacteriophage at a population level, bacteria and bacteriophage isolated at different time points will need to be compared against standard references. These references are essentially the original PA01 and PP7 strains used to inoculate the experimental microcosms. However, in order to determine relative fitness between PA01 and evolved PA01 strains, the reference PA01 strains needs to be distinguishable from the evolved PA01 mutants to allow competition experiments (microcosm trials in which the two strains are grown together). In order to do this, we will utilise a PA01 derivative (PA01 panB) in which panB, a gene involved in the synthesis of pantothenate, has been deleted.

PP7 infects PA01 by binding to Type IV pili on the outer surface of the bacterium, and these pili are known to be involved in bacterial attachment to solid surfaces (biofilm formation). In order to determine how PA01 may acquire resistance to PP7 infection, it would be logical to look at possible mutations in Type IV pili expression, biofilm formation and regulation. We will therefore need to make use of a number of specific PA01 mutants for this purpose.

Recipient or parental organism

Pseudomonas aeruginosa PA01

Host/vector system

The GM mutants are all mini-transposon mutants, mini-transposon-derived mutants, or allelic replacement mutants.

Origin & function

Mutant strains

PA01 panB   Not-viable mutant in which panB, an essential gene in the biosynthesis of pantothenate has been deleted from the chromosome. Will not grow in minimal media without pantothenate; grows poorly in rich media without pantothenate; requires pantothenate in rich media for normal, wild type levels of growth;

pilA       Viable mutant which does not express Type IV pili (natural mutant);

wsp         Viable mutant in which the regulatory operon controlling bacterial attachment and biofilm formation has been lost (specific operon deletion with no marker inserted);

PA0164*     Viable mutant in which autolysis is up-regulated (no chromosomal marker as the Tet of the mini-transposon has been deleted);

PA0197       Viable mutant in which the mini-transposon (Tet ) has inserted into pilU; does not express Type IV pili;

PA01 162     Viable mutant in which the mini-transposon (Tet ) has inserted into pilT; does not express Type IV pili;

PA01*        Different isolate of PA01 which differs from PA01 and shows no autolysis;
Evaluation of foreseeable effects

The fitness of PA01 panB relative to the wild type PA01 strain is significantly reduced in media lacking exogenous pantothenate. The requirement for exogenous pantothenate means that PA01 panB would not be capable of colonising human surface wounds and subsequent infection. Therefore, PA01 panB is most unlikely to represent a significant risk to human health.

The fitness of the other GM PA01 strains is lower than that of PA01. The GM strains have mutations that destroy various virulence functions - such as PQS signalling, pili expression, attachment and biofilm formation. Although these functions are not important for the growth in laboratory situations, these are critical for survival in natural environments and in human infection.

The GM Ps. aeruginosa are not expected to be any more harmful than the parent wild type strain. In most cases the fitness and virulence will be reduce. No risks to the environment have been identified.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes). Any excess liquids discharged into drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Project Ref** 553/03.5

<table>
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<td>01/07/2003</td>
<td>IN VITRO EVOLUTION OF MYCOBACTERIAL PROMOTERS FOR EXPRESSION OF FOREIGN GENES IN MYCOBACTERIUM BOVIS BACILLUS CALMETTE-GUERIN (BSG)</td>
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<td>&lt; 1 litre</td>
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Mycobacteria have long been proposed as candidates for use as live vaccine vehicles, one of the main reasons being their outstanding adjuvant properties. Mycobacterium bovis BCG, an avirulent strain of M. bovis, has received tremendous attention in recent years in this regard. BCG has been used as a vaccine against tuberculosis since 1948. In 1991, it was estimated to have been given to 2.5 billion people, with few serious cases of complications having been reported, and thus it has a long-standing safety record. Other properties that have made BCG an outstanding candidate for live vaccine development include the fact that it can be given at birth or at...
anytime thereafter, a single inoculum can produce long-lasting immunity, it is also inexpensive which makes it an ideal candidate for developing countries.

Various foreign genes have indeed been expressed in BCG over the past decade, including bacterial and viral genes, and in some cases protective immunity to the antigens of interest has been reported in animal models. Extra-chromosomal and integrative vectors that are able to transform mycobacteria have been developed, and different mycobacterial promoters have been used to drive the expression of foreign genes in BCG. The most widely used promoter in these studies is the hsp60 promoter, however it has been reported that though it may be adequate for some antigens, its high activity is detrimental to the expression of others. We therefore undertook this study to try and develop mycobacterial promoters with a wide range of activities to drive the expression of foreign genes in BCG.

### Recipient or parental organism

**Mycobacterium bovis bacille Calmette Guerin (BCG)**

### Host/vector system

**Bacterial host M. bovis BCG with plasmid vector.**

### Origin & function

- **Reporter genes:**
  - Green fluorescent protein (GFP) - from jellyfish, fluoresces under UV light.
  - LacZ - from Escherichia coli, produces beta galactosidase which reacts with the chromogenic substrate X-gal to produce a blue colour.
  - LuxAB genes - from Vibrio harveyi (or the American firefly luc gene), produce luciferases which are enzymes that catalyze production of light from luciferin and ATP.

- **Promoter sequences from M. tuberculosis, M. smegmatis, and M. avium (shuffled by DNase digestion to yield a library of chimeric sequences).**

### Evaluation of foreseeable effects

The reporter genes are all well-characterised reporter genes with no known associated health hazards. The DNA fragments that will be inserted into the vectors are regulatory sequences required for initiating transcription of the reporter genes. No modification of genes involved in pathogenicity, virulence, host specificity or tissue tropism are being undertaken and there is no reason to expect the resulting genetically modified organism will differ in pathogenicity from the unmodified BCG strain.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Consumables (mainly plasticware eg pipettes, flasks, tubes)** - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

- **Liquids (eg samples, culture supernatants, tissue culture media)** - treat with Hycolin at a final concentration of 2% overnight then autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

- **Agar plates** - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least
10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incineration)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
appropriate containment and control measures have been assigned in accordance with the latest issues by ACGM.

**Project Containment**

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**Project Ref** 553/03.6

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<th>Date Ackn'd</th>
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<tr>
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<td>GENERATION OF SINGLE-CYCLE PSEUDOTYPED HIV-1 VIRIONS</td>
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Non-GMM Consent Granted:

not applicable

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

The aim of this project is to generate single cycle Env-pseudotyped luciferase reporter virus expressing the envelope protein from clinical and laboratory-adapted virus stocks in order to answer questions concerning the interplay between the Env protein and target cell components at a clonal level.

The resultant virus will be used for in vitro assays to assess inhibition of viral entry for polyanionic compounds used in topical microbicidal formulations, HIV-1 fusion inhibitors and also for assays assessing the neutralising activity of gp140 antibodies from mouse sera.

#### Host/vector system

- **Recipient or parental organism**: Initial transfection of 293T cells and resultant pseudovirus will then be used to transduce human glial cell lines (eg U87-CD4-CXCR4, U87-CD4-CCR5).

- **Origin & function**: The purpose of the modification is to produce HIV virus capable of mimicking the natural interactions under investigation but incapable of replication and therefore representing a significantly reduced hazard. The retrovirus (HIV-1) from which the pNL4-3 Env (-) Luc (+) construct was derived can cause disease in its natural host. HIV-1 is a human retrovirus, which causes severe immunodeficiency in humans (AIDS). Both plasmids have the potential to incorporate their genetic information into the genome of the target cells used for transfection. However, the design of the HIV -1 construct (containing a frameshift in the 5’ end of env) prevents production of infectious, replication-competent virions. Co-transfection with the env-carrying pcDNA3. 1/Zeo (+) plasmid allows rescue of infectious virus for env in the viral RNA (vRNA) and as such are only capable of a single round of replication, as no infectious viral progeny will be produced.

- **Evaluation of foreseeable effects**: The reporter gene (Luc) is all well-characterised with no known associated health hazards.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- **Not applicable**.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - treated with 2% Virkon final concentration, left overnight and then discharged to drains.

Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Disinfection, effectively 100% kill - Virkon has been tested by independent laboratories and been proven to be effective against a total of 20 virus families (including HIV/AIDS)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Project Ref** 553/03.7

**Date Ackn'd** 29/07/2003

**CU2 Project Title**

INFECTION OF HUMAN AND MURINE CELLS WITH RECOMBINANT, REPLICATION-DEFECTIVE RETROVIRUSES EXPRESSING A RANGE OF REGULATORY MOLECULES

**Date Project Ceased**

**Class**

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**Non-GMM** Consent Granted

yes

Project notified under transitional arrangements

N

**Withdrew**

N

Tick if notifying a connected programme of work

N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID** 553/05.6

**Date of Significant Change** 21/11/2005

**Project Additional Information**

**Purposes of the contained use**

To rescue recombinant, third-generation (self-inactivating), replication-defective lentivirus based on human immunodeficiency virus type 1 (HIV-1) virus, VSV-G/lentiviral replication-incompetent lentiviral and recombinant replication-defective amphotropic murine retroviruses all expressing various transcription factors, cytokine receptors, growth factors or other regulatory molecules. Use said recombinant viruses to transduce a range of human and murine haemopoietic cell lines and primary cells to identify and characterise the molecules and processes controlling the self-renewal, lineage commitment and differentiation of haematopoietic stem and progenitor cells. Murine and human cells infected with genes of interesting phenotype will be transplanted into NOD/SCID mice for further analysis.

**Recipient or parental organism**

For construction of retroviral vectors:

E. coli K12 and B derivatives (disabled)

For packaging recombinant retroviral particles:

293FT (Human embryonic kidney cell line 293F constitutively expressing SV40 T antigen from pUC-based plasmid pCMVSPORT6TAg.eno)

293T (similar to 293FT)

GP+envAM12 (murine cell line NIH3T3 + one plasmid containing the Moloney murine leukaemia virus gag and pol genes and a second plasmid containing the 4070A amphotropic env gene)

For recombinant retroviral infection:

human cell lines (such as established human leukaemic cell lines, eg MALM-6, KG1, HL-60, U937)

murine FDCPmix cell line (a multipotential haematopoietic cell line)

primary human cell cultures (from screened samples)

primary murine haematopoietic cell cultures.
The recombinant viruses are able to efficiently infect human and murine dividing and non-dividing cells but are replication defective. They contain transgenes driven by viral LTR or an internal eukaryotic promoter (such as CMV). There is also the facility for dual expression from RNA transcripts via an internal ribosome entry site to allow expression of a selectable marker. None of the selectable markers are considered to be hazardous to human health or the environment however the transgenes are likely to affect cell cycling and have the potential to be oncogenic. As such they could present a significant hazard to human health however the effect on the environment is likely to be less significant due to the inability of the virus to replicate and the instability of the virus outside of the laboratory environment. Human and murine cell lines and murine primary cell lines are not considered to be hazardous to human health or the environment due to the immune response and stringent nutritional requirements. Human primary cells although screened could still potentially harbour adventitious agents and as such could pose a threat to human health but would not be hazardous to the environment due to stringent nutritional requirements. Mice injected with mammalian cells previously infected pose no risk to the environment as the virus is unable to replicate and NOD/SCID mice are unable to survive outside of the labloratory environment.

Host/vector system

The ViraPower Lentiviral Expression System (Invitrogen)
Expression vector pLent6/V5 containing the gene of interest under the control of the human CMV immediate/early enhancer/promoter and elements that allow packaging of the construct into virions.

pLP1, pLP2 and pLP/VSV-G supply the helper functions as well as the structural and replication proteins in trans required to produce the replication-incompetent lentiviral particle.

VSV-G/lentiviral expression system
A lentiviral vector with viral genes deleted containing the Spleen Focus Forming Virus promoter driving expression of the gene of interest. (2) A lentiviral packaging plasmid encoding gag/pol, tat, rev and HIV-1 rev-response element (RRE), with virulence genes vif, vpr, vpu and nef deleted. (3) An envelope plasmid expressing only the VSV-G gene from Vesicular Stomatitis virus. The three plasmids are co-transfected into human 293T cells to produce the replication-incompetent lentiviral particles.

Amphotropic murine retroviral vectors.
Derived from the Murine Moloney Leukaemia Virus (eg p50-M-X-neo) and Murine Stem Cell Virus (eg MSCV-ires-GFP), recombinant murine retroviral vectors carrying the gene of interest.

All plasmids carry the ampicillin resistance gene driven by a bacterial promoter with the ColE1 origin of replication.

Viruses can only be generated in certain packaging cell lines which when transfected with a selection of vectors (2-4) provide all the genes necessary for efficient viral production. This allows the production of replication-defective viruses by expression of genes in trans and reduces the risk of replication competent viruses being generated. Infected cells will be checked for the absence of replication-competent helper virus.

Origin & function

Human genomic DNA and cDNA inserts, wild-type and mutated.
-- Wild-type transcription factors such as GATA family, Hhex, Pax5. Forced expression of these molecules is likely to influence the self-renewal and differentiation of haematopoietic cells, based on what is known about their normal function. The factors are not known to be transforming or oncogenic, although point mutations in GATA-1, have recently been reported in Down's syndrome-related acute megakaryoblastic leukaemia and Pax5 expression is deregulated in human medullablastomas.

-- Other regulatory molecules such as components of the notch pathway, cytokine receptors and growth factors. Modulation of the expression of these factors is expected to result in a transcriptional response within the nucleus and to alter the balance between self-renewal and lineage differentiation in stem and progenitor cells and perhaps also to alter the lineage output of apparently committed cells.

-- Other candidate regulatory molecules from differential expression analysis, expected to function in a similar manner to the genes described above. The function of these is currently unknown but is expected to have some impact on controlling haematopoietic cell self-renewal, growth and differentiation.
Known products of chromosomal translocations, their normal counterparts, or mutated versions with deletions, insertions and/or point mutations. These genes will include, but not necessarily be limited to, TEL-AML1, PML-RARa, PLZF-RARa, bcr-abl. These genes are suspected oncogenes by virtue of their expression as a result of specific translocations associated with different forms of human leukaemia.

Selective modifications of any/all of the above. Most of the modified versions of the proteins under study would be expected to display essentially the same biological activity in an experimentally inducible manner or to display a subset of the activities of their wild-type counterparts. However, the possibility of inadvertently generating novel biological activities cannot be excluded.

Standard reporter genes: such as neomycin resistance (neoR), beta-galactosidase (lacZ), jellyfish green fluorescent protein (GFP and derivatives) will not increase the level of risk to human health and the environment.

Viruses are either human or murine and the viruses have the capacity to infect, but not replicate in most mammalian cells. The transgenes are a variety of factors which are expected to influence self-renewal and differentiation of haematopoietic cells including but not limited to cytokine receptors and growth factors. Standard reporter genes may also be included into the virus such as neomycin resistance (neoR), beta-galactosidase (lacZ), jellyfish green fluorescent protein (GRP and derivatives).

Evaluation of foreseeable effects

E. coli K-12 and B derivates are disabled hosts, which cannot colonise the human gut, cannot survive outside of the alboratory and have a history of safe use. The vectors used for cloning are non-mobilisable, are based on pUC vectors and the genes are not expressed in bacteria. Therefore the risk from the cloning stage is negligible with respect to both human health and the environment. Cell lines and primary mouse cells could also be considered disabled hosts due to their inability to survive outside of the laboratory environment and the risks are negligible to both human health and the environment. Human primary cells pose a risk to human health due to possible adventitious agents. The risk from work with cells after infection is likely to be negligible to both human health, due to the immune system recognising the cells as non-self, and the environment, due to their inability to survive outside of the laboratory. The viruses are able to infect most mammalian cells but are unable to replicate. There are a number of safety features in the lentiviral vector systems that minimise the relation to wild type HIV-1 and the generation of replication competent virus. The major risk come from expression of the transgenes some of which have unknown effects but are likely to be oncogenic in nature.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose fo solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, ((either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1994 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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Project Ref 553/03.8

Date Ackn'd 13/11/2003

CU2 Project Title TO OBSERVE THE EFFECT ON MAMMALIAN HEART DEVELOPMENT OF BOTH, SHORT HAIRPIN RNA (SHRNA) TO KNOCK DOWN OR SILENCE GENE FUNCTION, AND CDNA TO OVER EXPRESS GENES, DELIVERED BY SELF-INACTIVATING...

Date Project Ceased 02/03/2022

Class 2

CultureVolClass2 < 1 litre

Non-GMM

Consent Granted not applicable
We propose to develop and optimise a lentivirus-based system as a tool to modulate candidate target genes in mouse tissues, cells (eg cardiac myocytes, embryonic stem cells) and embryos, in order to study their role in heart development. In the long term we will be able to combine this with high-throughput magnetic resonance imaging of embryos, enabling us to rapidly understand the functions of genes that contribute to heart development. Gene transfer using Lentiviruses will also enable us to study the function of gene promoters fused to appropriate reporters (eg lacZ - encoding b-galactosidase, or GFP encoding green fluorescent protein). Once gene transfer is achieved embryos and cells will be phenotyped using MRI, microscopy, or enzyme assays (eg lacZ) or epifluorescence (GFP).

For construction of retroviral vectors:
K12 or B derivatives of Escherichia coli, such as DB3.1, TOP10 and DH5α, will be used as bacterial cloning hosts to generate plasmid expression clones in readiness for expression within HEK293 derived packaging cell lines. These are disabled hosts that cannot colonise the human gut and have a history of safe use. These hosts may be considered equivalent to ACDP hazard group 1.

For packaging recombinant retroviral particles:
The HEK293 derived packaging cell line 293FT is of human origin, is obtained from commercial sources and is well characterised and authenticated. It contains the SV40 large T antigen driven by a CMV promoter, and is to be used for the packaging and expression of the viral vectors. They can be regarded as low hazard for GM activities and, as hosts are suitable for Containment level 1 precaution.

For recombinant retroviral infection:
Primary mouse cells (eg cardiomyocytes, skeletal myocytes, embryonic cells, stem cells) and embryos will be obtained from mice that are bred in a specific pathogen free unit, and have previously been obtained from approved commercial sources (eg Harlan) will be used for infections as target cells. None of these cells will be deemed high risk for blood borne pathogens. For the purposes of this assessment they can all be regarded as low hazard for GM activities and, as hosts are suitable for containment level 1 precautions. No human cells will be infected.

Host/vector system

pLenti-DEST Gateway Vector System (used for transgene expression)
pLENTILOX Vector (used for shRNA expression)
Both these plasmids are HIV-1 derived lentiviral vectors.

These lentivirus expression vectors are self-inactivating as they carry a deletion in the U3 region of the 3'LTR. Upon integration into the host genome, the 3'LTR is copied to the 5'LTR, rendering it transcriptionally inactive and therefore unable to generate a functional retrovirus. The HIV-1 Lentivirus based expression vectors lack any of the
packaging genes (gag, pro, pol, vif, vpr, tat, rev, nef, env) and require the use of a packaging cell line based on HEK293 (ie HEK293FT) and 3 other plasmids which provide gag, pol, rev and VSVG. The HIV-1 derived elements in these lentivirus expression vectors (ie 5’ and 3’ LTR’s and the psi packaging signal) allow packaging of the transgene itself. Importantly the tat gene, which is a crucial factor for HIV replication, is removed and replaced with a strong constitutive promoters, such as the RSV or CMV promoters in pLenti-DEST (invitrogen) and pLENTILOX respectively. Therefore the number of genes from HIV-1 has been reduced to 3, ie gag, pol, and rev. Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus.

These packaging genes are provided by co-transfection of packaging vectors pLP1 (provides gag/pol), pLP2 (provides rev), pLP/VSVG (provides VSV envelope) into the HEK293FT cell line along with the expression vector. By using this enhanced 4-plasmid system, virus particles can be produced that can infect human and mouse cells, but are replication deficient and cannot be transmitted from one cell to another. Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (eg gal, pol, rev, eng) in the 293FT producer cell line, none of them contain LTRs or the psi packaging sequence. This means that one of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced. The expression of gag/pol from pLP1 has been rendered rev dependent by the introduction of a rev-response element, and so only expresses with co-transfection of pLP2, which provides rev. Addition of the RRE prevents gag and pol expression in the absence of Rev. The HIV-1 env gene is replaced with a Vesicular Stomatitis Vierus envelope.

We are only infecting mouse cells/embryos from mice bred in a specific pathogen free unit, obtained originally from commercial suppliers. Therefore there is no chance that an endogenous mouse HIV-1 would recombine with the replication defective lentivirus as ordinary mice do not carry HIV-1 (it requires CD4 and a co-receptor (CXCR4/fusin or CCR-5) for infection - this is only present on human cells).

Overall then, only Lentiviral particles, which are unable to replicate, but which can deliver the gene of interest, are produced. Therefore the main risk is in their ability to deliver genetic material into a host genome and as described above, this is unlikely to pose any significant risk.

Origin & function

Standard reporter genes such as Lac Z (beta-galactosidase) from E. coli, GFP (Green fluorescent protein) from Jellyfish and Liciferase from Firefly have a history of safe use and are of no or negligible hazard to human health or the environment.

Murine transcription factors and other genes which have been indicted to play a role in heart development will be depleted or knock down using shRNA or over expressed using cDNA. These included:

1. Transcription factors that control heart development - for instance.

(a) Cited family members, (Cited2, Cited1, Cited4)
(b) TFAP2 isoforms, A, B, C, D, E
(c) FKBP family members (eg FKBP12, FKBP25)
(d) Aip1
(e) Hox genes
(f) polycomb group genes (eg Bmi1, Mel18, Mph1, Rae28; Nspc1,2)
(g) Nkx2.5
(h) Pax3
(i) retinoic acid receptors
(j) eHAND, dHAND
(k) Tbx isoforms
(l) Gata isoforms
2. Other genes that have been identified in human, zebrafish, or mouse genetic studies as affecting heart development:
For example, EVC1, EVC2, reptin, pontin, Jekyll, heart and soul, miles apart, gridlock, heartstrings, looptail, Gnas, Cyclin D isoforms, PTPN11, jagged, notch, lefty1, lefty2, nodal, inversin; and other genes identified in ENU mutagenesis screens.

Inserts are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. Importantly, in no case will we knock down genes that are known to function as tumour suppressors (eg RB, p53, BRCA1, BRCA2, INK4A etc) or overexpress genes that are known to promote tumour formation.

Evaluation of foreseeable effects
No significant hazards have been identified from insertion of the foreign sequences into E. coli K12 or B derivatives. The vectors are based on the pUC backbone and are non-mobilisable. They are not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms. Gene transfer is possible, but unlikely to be hazardous. The resulting GMOs are not expected to carry any additional risks compared to that of the un-modified recipients and as such can be handled at containment level 1.

Transfection of the HEK 293FT cell line with the 4 plasmids is expected to result in the generation of a Lentivirus that is capable of infecting most mammalian cells and integrating into the host DNA but is incapable of replication. As stated above none of the inserted DNA is expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. Therefore all work, which involves the handling of the virus, or cell lines and tissues infected with the virus, will be done at containment level 2, with the use of a microbiological safety cabinet for procedures which generate aerosols.

Infected embryos will be implanted into recipient pseudopregnant mice using standard methods. The embryos should only contain integrated virus, as the virus containing supernatant will have been washed away by several (at least 5) changes of medium. Although embryonic transfer is via mouth pipetting the risks of exposure are minimal, due to the length, complexity of tubing and cotton plug used in these procedures. However as a precaution two 0.2um filters will be placed in series along the length of the tubing.

The glass capillary tubes used to implant the embryos are potential sharps. As stated above the embryos posed a minimal risk as the virus should be integrated. Nevertheless, disposable gloves will be worn when handling all potential sharps that have come into contact with these embryos.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) discharge to drains.
Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Animal bedding - dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Sharps - (eg Glass capillary tubes) - collect in a suitable Sharps Containers for disposal. Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via clinical waste stream for incineration.

Routine Disinfection - (eg Hard surfaces, work benches, sinks etc) - Spray or wipe surface with 1% VIRKON, leave for 10 minutes and dry with paper towel to remove any remaining white deposit. Collect tissues and dispose of as above for consumables.

Embryos - are fixed in 4% paraformaldehyde for >2 weeks before embedding in agarose for MRI examination or in paraffin wax for histology, and subsequently disposed of in clinical waste stream for incineration.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incineration)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Animal Units

| L2 Yes | L3 L4 L2 | L3 L4 L2 | L3 L4 L2 |

Large Scale Activities

| L3 L4 L2 | L3 L4 L2 | L3 L4 L2 |

Human Clinical Applications

| L3 L4 L2 | L3 L4 L2 | L3 L4 L2 |
IDENTIFICATION OF PROTEINS REQUIRED FOR SECRETION IN LYMPHOCYTES

Retroviral and lentiviral delivery of a selection of human genes to cytotoxic T lymphocytes (CTLs) to assess their role in regulating secretion of stored proteins required for the immune effector functions.

FOR CONSTRUCTION OF RETROVIRAL VECTORS
K12 or B derivatives of E. coli will be used as bacterial cloning hosts to generate plasmid expression clones in readiness for expression within specified packaging cell lines. These are disabled hosts that cannot colonise the human gut and have a history of safe use. These hosts may be considered equivalent to ADCP hazard group 1.

FOR PACKAGING RECOMBINANT RETROVIRAL PARTICLES
The packaging cell lines are of human or murine origin, which are well characterised and authenticated and are obtained from commercial sources. Examples of packaging cell lines likely to be used are HEK293T cells (amphotropic) to generate lentivirus or PT67 to produce retrovirus. They can be regarded as low hazard for GM activities and as hosts are suitable for containment level 1 precaution.

FOR RECOMBINANT RETROVIRAL INFECTION:
Human primary lymphoid cells (eg CD8+ blasts or CTL) can be considered as especially disabled hosts and as such may be considered to be equivalent to ADCP hazard group 1 however the cells have the potential to contain adventitious agents and as such must be handled at containment level 2 under COSHH.

Lentiviral expression plasmid pHR-SINcPPT-SGVC containing 5' and 3' LTRs, packaging signal and Rev response element from HIV-1
p8.91 packaging plasmid containing Gag, pol, rev, tat from HIV-1
pMD-G envelope plasmid containing VSV-G envelope protein.

These three vectors are co-transfected into a packaging cell line to generate replication defective lentivirus.
This lentivirus expression vector is self-activating as it carries a deletion in the U3 region of the 3'-LTR. Upon integration into the host genome, the 3'-LTR is copied to the 5'LTR, rendering it transcriptionally inactive and therefore unable to generate a functional retrovirus. The HIV-1 Lentivirus based expression vector lacks any of the packaging genes (gag, pro, pol, vif, vpr, tat, rev, nef, eng), and requires the use of a packaging cell line based on HEK293 (ie HEK293T) and 2 other plasmids which provide gag, pol, rev, tat and VSVG. The HIV-1 elements in the lentivirus expression vectors (ie 5' and 3' LTRs and the psi packaging signal) allow packaging of the transgene itself. Therefore the number of genes from HIV-1 has been reduced to 4, ie gag, pol, rev and tat. Genes encoding the structural and other components required for packaging the viral genome are separated onto three plasmids. All three plasmids have been engineered not to contain any regions of homology and each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus.

The packaging genes are provided by co-transfection of packaging vectors p8.91 (provides gag/pol, rev and tat) and pMD-G (provides VSV-G envelope) into the HEK293T cell line along with the expression vector. By using this enhanced 3-plasmid system, virus particles are produced that can infect human and mouse cells, but are replication deficient and therefore cannot be transmitted from one cell to another. Although the two packaging plasmids allow expression in trans of proteins required to produce viral progeny (eg gal, pol, rev, env) in the 293T producer cell line, none of them contain LTRs or the psi packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced. The rev response element allows for rev-dependant nuclear export of unspliced viral mRNA. The HIV-1 env gene is replaced with a Vesicular Stomatitis Virus envelope.

Overall then, only Lentiviral particles, which are unable to replicate, but which can deliver the gene of interest, are produced. Therefore the main risk is in their ability to deliver genetic material into a host genome and as described above, this is unlikely to pose any significant risk.

Retroviral expression plasmid pMSCV containing 5' and 3' LTR and packaging signals. The LTR is from murine stem cell viral and prevents transcriptional suppression in embryonic stem and embryonal carcinoma cells. Therefore driving high levels of constitutive expression. Retroviral expression plasmid pLEGFP-C1 containing 5' and 3' LTR and packaging signals from Moloney murine leukemia virus.

Either of the above 2 plasmids will be transfected into PT67 packaging cells to generate replication defective retrovirus. PT67 packaging cell line is derived from NIH3T3 cells, which have subsequently been stably transfected with 2 plasmids that contain the gag/pol and env gene. This cell line produces viruses, which are dualtropic and have a broad mammalian host range. Split genome design lowers the possibility of generating replication-competent virus. Only the expression plasmids contain the LTRs and the packaging signals and therefore the generated virus contains one of the structural genes rendering it replication defective.

As with the lentivirus the main risk with retroviruses is their ability to deliver genetic material into the host genome. This is unlikely to pose any significant risk.

**Origin & function**

Mammalian proteins implicated in a specialised lymphoid secretory pathway such as:
- **Rab27a** (studies have shown that transgenic mice expressing Rab27aGFP are healthy)
- Rab geranylgeranyltransferase: a rab modifying enzyme that adds a geranyl geranyl group to Rab27a and 4 other Rab proteins.
- Rab7 and RILP: Both involved in moving lysosomes along microtubules.
- Tubulin-GFP: forms microtubules along which lysosome move
- Actin-GFP: forms the sub-cortical cytoskeleton.
- LYST: a protein required for the final step of secretion of secretory lysosome.
- AP-3: a clathrin binding adaptor protein involved in sorting proteins to the lysosome.
- Perforin: a pore forming protein used by lymphocytes to form pores in target cells.

Other good candidate genes such as the microtubule motors kinesins or clp proteins that attach lysosomes to microtubules.
All of the above and other candidate proteins will be involved in the secretion of lysosomes in immune cells. None of the available literature indicates any oncogenic or harmful effect of these proteins.

Standard reporter genes such as GFP (Green fluorescent protein) from jelly fish, luciferase from firefly, and B-galactosidase from E. coli. None of the above proteins are known to have harmful properties.

Standard antibiotic resistance genes such as Neomycin, hygromycin, puromycin and ampicilin.

**Evaluation of foreseeable effects**

No significant hazards have been identified from insertion of the foreign sequences into E. coli K12 or B derivatives. The vectors are based on the pUC backbone and are non-mobilisable. They are not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms. Gene transfer is possible, but unlikely to be hazardous. The resulting GMOs are not expected to carry any additional risks compared to that of the un-modified recipients and as such can be handled at containment level 1.

Transfection of the HEK 293T cell line with the 3 plasmids is expected to result in the generation of a Lentivirus that is capable of infecting most mammalian cells and integrating into the host DNA but is incapable of replication. As stated above none of the inserted DNA is expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. Therefore all work, which involves the handling of the virus, or cell lines and tissues infected with the virus, will be done at containment level 2, with the use of a microbiological safety cabinet for procedures which generate aerosols.

Transfection of the pT67 cell line with the retroviral expression plasmids is expected to result in the generation of a retrovirus that is capable of infecting most mammalian cells and integrating into the host DNA but is incapable of replication. As stated above none of the inserted DNA is expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. Therefore all work, which involves the handling of the virus, or cell lines and tissues infected with the virus, will be done at containment level 2, with the use of a microbiological safety cabinet for procedures which generate aerosols.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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**Degree of kill:**
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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**Project Ref**  553/04.1

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<th>Class</th>
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<th>Project notified under transitional arrangements</th>
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<td>18/02/2004</td>
<td>DEVELOPMENT OF PEDIATRIC HIV VACCINE FOR CENTRAL AFRICA USING RECOMBINANT BGC</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td></td>
<td>not applicable</td>
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Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info
We plan to develop recombinant Calmette-Guerin BCG (rBCG) as a candidate pediatric HIV vaccine. The avirulent strain of Mycobacterium bovis BCG offers as a vaccine vector some unique advantages and has a long record of safe use in more than two billion humans as a vaccine against tuberculosis. It is an excellent candidate vector for a pediatric HIV vaccine as it can be given safely at, or any time after birth, and indeed in many countries is given to newborns. Moreover, it can be delivered orally, the natural route of HIV infection of breast-fed babies born to HIV positive mothers, and is a potent adjuvant for Th1 responses able of inducing long-lasting immunological memory. Here, candidate rBCG constructed elsewhere will be used to vaccinate animals alone or in a prime-boost combination with other vectors expressing common HIV-derived immunogens.

The critical issues to be considered in developing rBCG technology include antigen localisation and long-term in vivo plasmid stability, both of which will be addressed in this work.

Recipient or parental organism

Mycobacterium bovis bacilli calmette-guerin (BCG)

Host/vector system

Vaccine strain BCG Pasteur,
lysine A (-) BCG strain (auxotrophic selection of plasmids pJH222 and pJH223)
Three E. coli-mycobacterial shuttle plasmids with kanamycin resistance gene as selectable marker will be constructed:
1) pMV261 - expression of foreign genes will be regulated by BCG hsp60 promoter, transfection will result in a multicopy and extrachromosomal plasmid.
2) pJH222 - expression of foreign genes will be regulated by alpha antigen promoter from Mycobacterium spp., transfection will result in a multicopy and extrachromosomal plasmid, plasmids harbor the complementing lysine gene regulated by BCG hsp60 promoter.
3) pJH223 - expression of foreign genes will be regulated by alpha antigen promoter from Mycobacterium spp., transfection will result in an integrated construct, plasmids harbor the complementing lysine gene regulated by BCG hsp60 promoter.

Origin & function

1. Multi-CTL epitopes. CTL recognize and kill cells displaying on their surface 8- to 10- amino acid-long peptides derived from microbial proteins. One way to minimise the amount of protein administered during vaccination is to engineer artificial proteins consisting of CTL epitopes. Compared to whole proteins, the use of CTL epitopes overcomes the potential risk of undesired biological activities of whole proteins and makes the possibility of reversion to virulence non-existent. The genes coding for short peptide epitope are derived from HIV, SIV, influenza or melanoma-associated antigens.

   gag group specific antigens - structural capsid proteins, RNA nuclear transport
   pol enzymes - protease, reverse transcriptase
   env envelope (gp41+ gp120=gp160) - external viral glycoprotein
   nef many functions - e.g. down regulation or up regulation of some specific cellular proteins
   tat viral transcription transactivator
   rev RNA transport and stability
   vif promotes virion maturation and infectivity
vpr promotes nuclear localisation, inhibits cell division
vpu promotes release of virions, degrades some specific cell proteins
vpx SIV homologue of vpr

3. Chimeric fusion proteins of the above Genes coding for SIV or HIV proteins or parts joint into one open-reading frame.

HIVA is derived from consensus HIV-1 clade A gag p24/p17 sequences and a string of clade A CTL epitopes. It does not contain the envelope gene and focuses solely on the induction of cell-mediated immune responses. Inserted in plasmid DNA and Modified Vaccinia Virus Ankara, HIVA was shown to be safe in normal BALB/c mice, severe combine immunodeficiency (SCID) mice, SIV-infected (immunocompromised) rhesus monkey and in over one hundred healthy human volunteers vaccinated with these constructs.

RENTA complements the HIVA immunogen and will join the present vaccine in a four-component DNA/HIVA-RENTA prime-MVA/HIVA-RENTA boost formulation in year 2004. RENTA is a fusion protein derived from consensus HIV-1 clade A sequences of tat, reverse transcriptase, nef and gp41. The natural biological activities of the HIV components have been inactivated.

4. reporter genes. Harmless commonly used marker genes such as GFP or beta-galatosidase may be inserted alone or in a combination with the above genes.

Evaluation of foreseeable effects

Mycobacterium bovis bacille calmette Guerin (BCG) is an attenuated strain of M.bovis. Wild type M. bovis is closely related to Mycobacterium tuberculosis (probably a subspecies) but is generally more pathogenic for animals than M.tb primarily causing tuberculosis in cattle. The BCG strain was attenuated by several hundred serial subcultures and named after the French scientists Calmette and Guerin. Wild type M. bovis is classified as an ACDP hazard group 3 agent with the BCG strain classified as hazard group 2 even though it has used since the 1920s to immunise humans against tuberculosis and currently is the most frequently administered vaccine in the world.

The inserted reporter genes are all well-characterised with no known associated health hazards.

Most of the foreign genes inserted into BCG will encode for epitopes derived from HIV, SIV, influenza and melanoma associated antigens. These sequences are unlikely to be hazardous to human health and pose no greater threat than BCG itself. Genes encoding for whole HIV or SIV proteins are likely to have some biological activity but as they only code for a small percentage of the overall HIV/SIV virus their risk to humans and the environment is likely to be insignificant.

No modification of genes involved in pathogenicity, virulence, host specificity or tissue tropism are being undertaken and so there is no reason to expect the resulting genetically modified organisms will differ in pathogenicity from the unmodified BCG strain.

Accidental inoculation is likely to result in priming of the immune system to HIV, SIV, influenza or melanoma which is unlikely to have any detrimental effects.

The greatest environmental hazard associated with exposure of animals to BCG is that cattle infected with BCG become positive for the tuberculin skin test. Although BCG does not persist in cattle and causes no disease, animals that become tuberculin positive through exposure to BCG would be deemed tuberculosis by the current TB screening measures in Great Britain and would be slaughtered as a result. This hazard is therefore an economical one.

Containment Level 2 will be sufficient to control the risk of exposure.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a makesafe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125C for at least 15 minutes or 126 - 130C for at least 10 minutes or 134 - 138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125C for at least 15 minutes or 126 - 130C for at least 10 minutes or 134 - 138C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, part 3, 1993 (either 121 - 125C for at least 15 minutes or 126 - 130C for at least 10 minutes or 134 - 138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125C for at least 15 minutes or 126 - 130C for at least 10 minutes or 134 - 138C for at least 3 minutes), discharge to drains OR dispose via clinical waste stream for incineration.

Animal carcasses - disposal via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form Y
Tick to confirm that you have attached a risk assessment to this form Y
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Please enter comments on the GM safety committee on the risk assessment

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**Project Additional Information**

**Purposes of the contained use**

The project proposes to unravel the contribution made by the fungus, Magnaporthe grisea, to the management of host imposed oxidative stress, that is, to understand how the pathogen survives in the hostile environment of the host, during successful infection. Also the role of fungal cutinase in penetration of the plant epidermis will be investigated.

The aim is to evaluate the contribution of catalase and cutinase activity in pathogenicity and virulence by "knocking out" the resident M. grisea catalase or cutinase gene(s) and replacing them with selectable markers. The knockouts will then be assessed for attenuated pathogenicity.

**Recipient or parental organism**

Escherichia coli strain DH5alpha is disabled and not harmful to animals or plants.

Agrobacterium tumefaciens strain LBA1126 is disarmed, having had the tumour-forming genes removed. It is considered to pose minimal risk to the environment. Magnaporthe grisea is the telemorph (sexually reproducing form) of the imperfect fungus Pyricularia grisea, the casual agent of rice blast. The telemorph is rarely encountered in nature. The strain to be used is Guy-11, which is widely used as a model plant pathogen. In the absence of a strain opposite mating type, guy-11 is not able to produce sexual (resting) spores. The asexual conidia that are produced are very limited viability and will not survive more than 24 hours or produce mature infection structures in the absence of host tissue. It is therefore considered unlikely to pose any risk to the environment in the UK.

**Host/vector system**

a) construct made from pBSC from stratagene

b) construct made from pGeenII (carries kanamycin resistance and R and LT-DNA border repeats and will express in fungal cells using donor vector pGPS3-Hyg (carries AmpR, HygR and an Aspergillus promoter and terminator)
Vectors have no harmful properties.

**Origin & function**

Two catalase genes (A and B) will be knocked out, and replaced by common selectable markers either as single or double knockouts. Neither of these genes has an implication for control of Magnaporthe as extra-cellular catalase activity is believed to allow the fungus to combat host-imposed oxidative stress, in fact this is expected to result in attenuated pathogenicity.

Two cutinase genes will be knocked out and replaced by common selectable markers either as single or double knockouts. Neither of these genes has an implication for control of Magnaporthe as cutinase is an enzyme which breaks ester linkages between cutin molecules. It is believed that Magnaporthe penetrates the host solely by mechanical means however as it carries 2 genes the group wish to investigate their function.

The standard marker genes used e.g. hygromycin B (an antibiotic) and Bialophos (a herbicide), will not be selected by those used to control this fungus commercially and are not expected to have harmful or physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. Gene transfer is possible but unlikely to be hazardous.

**Evaluation of foreseeable effects**

The E. coli and Agrobacterium strains used are not capable of causing disease in animals or plants and do not present any risk to the environment.

The transgenic fungus will be compromised in its ability to produce extra-cellular catalase or cutinase and is likely to have impaired host penetration function. These individual alterations are expected to result in attenuated pathogenicity, as extra-cellular catalase activity is believed to allow the fungus to combat host-imposed oxidative stress and loss of cutinase may impair host penetration, which will reduce the fungus ability to infect.

The transgenes introduced, as selectable markers will alter the sensitivity of the GMO to hygromycin B, and the herbicide Bialophus - neither of which is used to control this fungus commercially. The products of gene expression are not toxic. No fungicide resistance genes will be introduced.

It is not expected that loss of catalase or cutinase will have any significant effect on the fungus, other than attenuated pathogenicity. While it is possible that minor morphological changes that might result, there is no reason whatsoever to expect that the modified pathogen will be more virulent than the wild type.

The fungus is not a native species of the UK and in the absence of strains of fertile strains of the opposite mating type, the strain used for this work can produce only asexual spores of very limited viability. There is no known mechanism of genetic transfer between the GMO and other organisms.

The risk assessment indicated that modified fungus is no more hazardous than the wild type. However, since the work involves genetic modification of a pathogen with the potential to cause significant crop disease and serious economic losses, it is considered appropriate to work under containment level 2 conditions as a precautionary measure.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Conumables (mainly plasticware e.e. pipettes, flasks, tubes) - autoclaves using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125C for at least 15 mins or 126-130C for at least 10 mins or 134-138C for at least 3 mins). Discharge any excess liquids to drains, dispose of solids via industrial (black bag) waste stream of landfill.
Liquids (e.g. samples, culture, supernatants, tissue culture media) - autoclave using a makesafe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125°C for at least 15 mins or 126 - 130°C for at least 10 mins or 134 - 138°C for at least 3 mins), discharge to drains.

Agar plates - autoclave using a make safe cycle as spcified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 mins or 126-130°C for at least 10 mins or 134 - 138°C for at least 3 mins), discharge any excess liquids to drains, dispose of solids via industrial (black bag) waste stream for landfill.

Sharps (e.g. needles, syringes, scalples) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

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**Project Ref** 553/04.3

**Date Ackn’d** 06/05/2004  **CU2 Project Title** Using retroviral vectors for the characterisation of the hypoxia-inducible factors (HIF)  **Class** Class 2  **CultureVolClass2** < 1 Litre  **CultureVolumeClass3-4**
Replication defective amphotrophic retroviruses will be used to assess the biological effects of murine and human HIF, related hydroxylases, their respective substrates, selected fusions between these genes, activating and inactivating mutations thereof and inhibitory RNA to knock down expression of these genes in primary cells and mammalian cell lines.

For Construction of retroviral vectors:
K12 or B derivatives of E. coli will be used as bacterial cloning hosts to generate plasmid expression clones in readiness for expression within specified packaging cell lines. These are disabled hosts that cannot colonise the human gut and have a history of safe use. These hosts may be considered equivalent to ADCP hazard group 1.

For packaging recombinant retroviral particles:
The packaging cells are of human origin, which are well characterised and authenticated and are obtained from commercial sources. Examples of packaging cell lines likely to be used are Phoenix 293 ampo cells, which generate amphotropic retroviruses. They can be regarded as low hazard for GM activities and as hosts are suitable for handling at Containment Level 1. However due to the presence of the E1a gene of Ad5 these cells will be handled at Containment Level 2 under COSHH.

For recombinant retroviral infection:
Primary cells and cell lines of murine and human origin can be considered as especially disabled hosts and as such are suitable for handling at Containment Level 1. However due to the possible presence of adventitious agents primary cells and uncharacterised cell lines will be handled at Containment Level 2 under COSHH.

Host/vector system
enes of interest will initially be obtained from plasmids generally derived from IMAGE clones. They will then be inserted into disabled retroviral vectors, which are based on pUC and are non-mobilisable. Vectors include LZRS-IRES-GFP, Pbmn-1-GFP and pRetroSuper.

pBMN-1-GFP contains 5’ and 3’ LTR and IRES-GFP into which the gene of interest is inserted.

LZRS-IRES-GFP contains 5’ and 3’ LTR and IRES-GFP into which the gene of interest is inserted and also EBV nuclear antigen and origin of replication.
pRetroSuper contains the H1 RNA promoter driving transcription of siRNA and 5 Thymidine residues as a termination signal which has been inserted into the pRetro system. pRetro is a self inactivation pMSCV retroviral vector.

To generate amphotropic retroviruses the expression plasmids are transfected into Phoenix-Ampho cells, derived from HEK293 cells which have already undergone subsequent stable transfections with plasmids containing moloney packaging functions gag-pol and env genes. Non-moloney promoters are used to drive the gag-pol and env genes to minimise their inter-recombination potential. Stable integration of gag-pol and env genes into different sites of the 293 genome, means that none of these genes are included in the viral particle thus generating a replication deficient virus.

Transfection with pBMN-1-GFP and pRetrosuper plasmids generates transient production of virus particles.

Transfection with LZRS-IRES-GFP plasmid generates medium term production of virus particles over 2-3 months due to the episomal nature of the plasmid.

**Origin & function**

Inserts are designed to result in increased or decreased activity of normal mammalian genes and/or are standard marker genes to identify or select infected cells. Marker genes include EGFP and standard drug resistance markers such as those for puromycin. Inserts are not expected to affect pathogenicity of the cloning hosts or normal human defence mechanisms. The interventions planned are likely to produce variations in HIF activity that are, no bigger than those encountered in normal health.

Inserts code for either normal mammalian genes, or selective alterations of those genes, or for short inhibitory RNAs designed to knockdown expression of the endogenous genes, and/or standard marker genes to identify or select infected cells.

These genes include mouse and human HIF and related hydroxylases (eg PHD1-3 and FIH) and their respective substrates eg mouse and human HIF-1 alpha, human HIF-2alpha, human HIF-3 alpha, selected fusions between these genes and activating and inactivating mutations thereof (eg of the specific HIF prolyl and asparaginyl residues targeted by the hydroxylases, or of residues affecting the active site of the hydroxylases).

Based on previous published research, HIFs play a role in hypoxic gene regulation, especially pathways involving metabolism and angiogenesis (Harris 2002). Wild type HIF-1 and HIF-2 should be constitutively expressed in VHL defective renal cell lines and up-regulate or down-regulate a variety of consensus hypoxia responsive genes. HIF itself would be normally regulated and degraded in all other cell lines and tissue types using this system. The HIF-1 mutant would be constitutively expressed. HIF-1 and HIF-2 over-expression (constitutively or mutant forms) have been shown to have varying effects on tumourigenesis (Semenza 2002). Indeed, HIF-1 seems to retard tumour growth in certain models, while in normal mice mutant HIF-1 has promoted angiogenesis and thus hypervascularity without tumour formation (Elson et al., 2001). HIF-2 seems to have a possible role in the progression of Renal Cell Carcinoma; however, no specific genes to date have been assigned to this transcription factor that are not already also controlled by HIF-1, thus uncertainty remains in the field as to its role in tumour progression but it is not able to transform cells. The HIF hydroxylases module HIF activity. They are not equilibrium enzymes and so activity does depend on the amount of enzyme expressed. This is under dynamic control in normal physiology. HIF activity is also dynamically regulated over a very wide range in response to commonly encountered physiology stimuli.

Inserts code for either normal mammalian genes, or selective alterations of those genes, or for short inhibitory RNA designed to knockdown expression of the endogenous genes, and/or standard marker genes to identify or select infected cells.

These genes include mouse and human HIF and related hydroxylases (eg PHD1-3 and FIH) and their respective substrates eg mouse and human HIF-1 alpha, human HIF-2 alpha, human HIF-3 alpha, selected fusions between these genes and activating and inactivating mutations thereof (eg of the specific HIF prolyl and asparaginyl residues targeted by the hydroxylases, or of residues affecting the active site of the hydroxylases).

Based on previous published research, HIFs play a role in hypoxic gene regulation, especially pathways involving metabolism and angiogenesis (Harris 2002). Wild type HIF-1 and HIF-2 should be constitutively expressed in VHL defective renal cell lines and up-regulate or down-regulate a variety of consensus hypoxia responsive genes. HIF itself would be normally regulated and degraded in all other cell lines and tissue types using this system. The HIF-1 mutant would be constitutively expressed. HIF-1 and HIF-2 over-expression (constitutively or mutant forms) have been shown to have varying effects on tumourigenesis (Semenza 2002; Elson et al., 2001).
No significant hazards have been identified from insertion of the foreign sequences into E. coli K12 or B derivatives. The vectors are based on the pUC backbone and are non-mobilisable. They are not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms. Gene transfer is possible, but unlikely to be hazardous. The resulting GMOs are not expected to carry any additional risks compared to that of the un-modified recipients and as such can be handled at Containment Level 1.

Transfection of the Phoenix-Ampho cell line is expected to result in the generation of a retrovirus that is capable of infecting most mammalian cells and integrating into the host DNA but is incapable of replication. As the virus is replication defective there is no risk of transmission within the environment. ADCP states that replication defective amphotropic retroviruses expressing innocuous genes may be handled at Containment Level 1. However due to the uncertain nature of the genes involved in the HIF pathways as a precautionary measure these viruses will be handled at Containment Level 2.

Cell lines infected with the virus will initially be handled at Containment Level 2. Once the viral supernatant is removed, the viruses inside the cells integrate their DNA into the host genome within 24-36 hours, and thus the viruses themselves disassemble during this period. After this period there will be no infectious virus and cells can be handled at Containment Level 1.

Evaluation of foreseeable effects

Not applicable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Project Ref 553/04.4

Amnesia and autoimmunity: Animal models for antibody-mediated central nervous system disorders using replication defective adenovirus.

Date Ackn’d 07/10/2004

Date Project Ceased

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Replication defective E1/E3 deleted adenoviruses will be utilised to induce auto-antibodies to voltage gated potassium channels (VGKC) in mice to demonstrate a role for auto-antibodies in central nervous system (CNS) disorders.

Recipient or parental organism

For construction of adenoviral vectors:
E.coli K12 or B derivatives will be used as bacterial cloning hosts to generate plasmid expression clones in readiness for expression within specified packaging cell lines. These are disabled hosts that cannot colonise the human gut and have a history for safe use. These hosts may be considered equivalent to ACDP hazard group 1.

For packaging of recombinant adenoviral particles:
The packaging cell lines are of human origin (HEK293), which are well characterised and authenticated and are obtained from commercial sources. HeLa cells will be used to confirm that wild type virus is not present in the viral stocks. HEK293 and HeLa cells can be regarded as low hazard for GM activities and as hosts are suitable for Containment Level 1 precaution.

The recombinant replication defective adenoviral particles will then be used to inoculate mice in an attempt to provoke an immune response.

Host/vector system

Genes of interest, such as HBK5 (gene for voltage gated potassium channel), NIK (NF kappaB inducing kinase) and reporter genes, will be cloned into standard plasmid vectors. These vectors (pAdKS216 and pAdKS217) are versions of pAdTrack-CMV with alterations to the multiple cloning site.

pAdTrack-CMV is a shuttle vector containing a poly linker site, which is flanked by adenoviral sequences allowing homologous recombination with the pAdEasy vectors to occur in E. coli. The vector also contains a CMV promoter and a polyadenylation site flanking a polylinker site and also the sequence for enhanced GFP. This vector contains the origin of replication from pBR322.

pAdEasy contains all the adenoviral genes except E1 and E3. The version to be used also contains a chimeric CMV promoter, which drives 5-10 fold higher expression than normal CMV. This can lead to expression levels up to 30% of the cellular protein.

Homologous recombination takes place in E. coli BJ5183 cells and large scale amplification take place in E. coli DH10B cells.

Production of replication defective virus particles takes place in HEK293 cells.

Viruses expressing a variety of transgenes will be generated which include:
CMV driving EGFP
CMV driving HBK5
CMV driving HIK
CMV-EGFP and CMV-HBK5
CMV-EGFP and CMV-NIK
CMV-HBK5 and CMV-NIK

Origin & function

The voltage gated potassium channel protein (VGKC) is encoded by the HBK gene. This gene is expressed in several different endogenous cell types particularly in the
nervous system. It is a trans-membrane protein located in the plasma membrane and it is known to be expressed at high levels in neurons, in the hippocampus and in the molecular layer of the dentate gyrus in the mammalian brain. VGKCs control repolarisation and frequency of action potential in neurons, muscles and other excitable cells.

KF-kappaB inducing kinase (NIK) gene induces expression of NF kappa, which activates the immune system. NIK is also an endogenously expressed protein.

Standard marker reporter genes such as Lac Z, GFP etc will be used. These inserts have a history of safe use and are not expected to have harmful physiological or pharmacological properties or affect the pathogenicity of the host.

None of the genes are oncogenic nor will they be integrated into the host genome.

**Evaluation of foreseeable effects**

No significant hazards have been identified from insertion of the foreign sequences into E. coli K12 or B derivatives. The vectors are non-mobilisable. They are not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms. The resulting GMOs are not expected to carry any additional risks compared to that of the un-modified recipients and as such can be handled at Containment Level 1.

Transfection of HEK293 cells is expected to result in the generation of an adenovirus that is capable of infecting mammalian cells but cannot propagate any further. As the virus is replication defective there is no risk of transmission within the environment. BHK5 and NIK are both constitutively expressed in several mammalian cells, especially neurons. Over expression of HBK5 is not expected to have harmful properties. Over-expression of NIK could possibly activate the immune system. Therefore the risk of these viruses is increased, because the genetic inserts could give rise to an immune response, which could be deemed harmful. Therefore as a precautionary measure these viruses will be handled at Containment Level 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993. (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Project Ref 553/04.5

Date Ackn'd 09/11/2004

CU2 Project Title
Role of macrophage surface receptors in binding and phagocytic uptake of pathogenic bacteria: identification of novel bacterial ligands for class A scavenger receptors.

Date Project Ceased

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2 1-50 Litres

Non-GMM Consent Granted
Not Applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes
**Project Additional Information**

**Purposes of the contained use**

This project aims at analysing the role of macrophage pattern recognition receptors (PRR), in particular class A scavenger receptor (SR-A), in recognition and phagocytosis of pathogenic bacteria using two important Gram-positive pathogens Streptococcus pyogenes and Streptococcus agalactiae.

When streptococcal candidate surface protein ligands have been identified, the corresponding genes will be cloned in disabled E. coli and the proteins will be expressed and purified as glutathione-S-transferase (GST) fusions or as His-Tag proteins. Streptococcal mutants negative for expression of candidate surface protein ligands will be constructed by replacing the structural gene with a kanamycin resistance cassette (Km-2) or a chloramphenicol resistance cassette (cat). The streptococcal mutants negative for expression of candidate surface protein ligands and the wildtype (WT) strains will be used in experiments with purified WT and SR-A deficient murine macrophages to analyse the role of these streptococcal surface proteins in recognition and phagocytosis of streptococci by SR-A on macrophages. Moreover, the streptococcal mutants and WT strains will be inoculated into WT and SR-A deficient mice to analyse the role of streptococcal surface proteins in SR-A phagocytosis in vivo. In addition, streptococcal mutants defective in D-alanylation of LTA and surface capsular polysaccharide may also be used for analysis of interaction with SR-A both in vitro and in vivo. Alongside and subsequent to the analysis of SR-A interactions with streptococcal ligands, the roles of other macrophage PRR and cytokines may also be examined (for example, mannose receptor).

**Recipient or parental organism**

Attenuated E. coli strains such as DH5a, XL-1Blue, JM103, BL21 and KJ622.

Streptococcus pyogenes (group A streptococcus): Wildtype isolates of several serotypes, including M1, M3, M4, M5, M6, M22 and M28.

Streptococcus agalactiae (GBS - group B streptococcus): Wildtype isolates of several capsular serotypes, including Ia, III and V.

**Host/vector system**

Standard plasmid vectors will be used such as pJRS233, pLZ12Spec, pGEX, pET and pBR322.

pJRS233: Temperature-sensitive E. coli/streptococcal shuttle vector. This vector contains a temperature sensitive ori from plasmid pVE6004 that renders it unable to replicate at temperatures above 35 degrees C. It also contains the low-copy replicon pSC101 to allow work in E. coli at 37 degrees C. Further, pJRS233 also contains the erythromycin resistance gene (erm) and the polylinker of the plasmid pBluescript SK+ (Stratagene).
plZ12Spec: This plasmid is an E. coli/streptococcal shuttle vector that is not temperature sensitive in streptococci, and it contains the repA replicon and the spectinomycin resistance gene specR.

pGEX vectors (Amersham Biosciences): Non-mobilisable expression vectors for generation of GST fusion proteins. Expression is under the control of the tac promoter, which is induced by IPTG. Also contains an internal laq1 gene. The laq1 gene product is a repressor protein preventing expression until induction by IPTG.

pET vectors (Novagen): Mobilisable defective expression vectors based on the T7 promoter-driven system. Can be used to generate recombinant streptococcal surface proteins with N- or C-terminal His-Tags.

pBR322 vectors: Mobilisable defective expression vectors containing the origin of replication from pMB1. They contain a low-copy number regulatory region (rop) and also the amp® and tet® selectable marker regions.

Origin & function
Streptococcal surface proteins are likely to be virulence factors acting as adhesions, invasins and/or antiphagocytic factors, or may perform functions important for the survival of the bacteria. For example, members of the M protein family in S. pyogenes have been shown to possess antiphagocytic properties, allowing the bacterium to grow in whole human blood. Deletion of the gene(s) encoding M protein(s) renders S. pyogenes highly sensitive to phagocytosis in human blood. Members of the Alp protein family in S. pyogenes and GBS are also likely to affect virulence, possibly by acting as epithelial cell adhesins/invasins. It has been reported that the Alp family member protein enhances the virulence of GBS in a mouse model. Also, capsule negative mutants and mutants defective in D-alanyl LTA have been shown to be significantly less virulent than the parental strains in the mouse model.

In addition, streptococcal surface proteins may also perform functions important for survival of the bacteria. Thus, mutations in genes encoding surface proteins may be lethal or lead to bacteria that are less viable than the parental strain.

Evaluation of foreseeable effects

In E. coli:
Cloning and expression of genes encoding streptococcal surface proteins in attenuated E. coli is unlikely to give rise to harm. Surface proteins from streptococci are not expected to be exposed in the surface of E. coli, due to different mechanisms of surface protein attachment between Gram-positive and Gram-negative bacteria. The two major groups of surface proteins that have been identified in group A and group B streptococci are LPXTG proteins and lipoproteins. The LPXTG proteins are covalently attached to the streptococcal cell wall. When genes encoding LPXTG proteins are expressed in E. coli, the processed form of the protein is normally found in the periplasmic space, as shown for M proteins of S. pyogenes (Frithz et al., Mol Microbiol, 1989, 3:1111-9). Streptococcal lipoproteins are attached to the outer leaflet of the cellular membrane. Lipoproteins from Gram-positive bacteria are often toxic in E. coli (Sutcliffe and Russell, J Bacteriol, 1995, 177:1123-8), and it has been shown the N-terminal signal sequence of the lipoprotein is responsible for the toxicity. Therefore, genes encoding streptococcal lipoproteins will probably have to be cloned without the region encoding the N-terminal signal sequence. The resulting gene products are therefore expected to be found in the E. coli cytosol.

There are no reports in the literature indicating that isolated streptococcal surface proteins may act as toxins or that they in any other way may have harmful effects in humans. Therefore they are not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms. Gene transfer as described above is possible, but unlikely to be hazardous. The resulting GMOs are not expected to carry any additional risks compared to that of the un-modified recipients.

In Streptococci:
Isogenic mutants of S. pyogenes, and GBS negative for expression of candidate surface proteins will be generated by homologous recombination employing the temperature-sensitive E. coli/streptococcal shuttle vector pJR3233. The streptococcal gene will be replaced with either the kanamycin or the chloramphenicol resistance cassette. Deletion of genes encoding surface proteins in S. pyogenes and GBS will most probably decrease the virulence of these two organisms.
Transcomplementation if isogenic streptococcal mutants negative for surface protein expression will be performed with pLZ12Spec containing genes encoding surface proteins, including the promoter region and transcription termination signals. These derivatives are expected to restore the wildtype properties of the strain.

Revertants of isogenic streptococcal mutants may also be constructed. In this case, the streptococcal gene of interest and its flanking regions is cloned in pJRS233. The resulting pJRS233 construct is transformed into the isogenic mutant by electroporation and a revertant is recovered after homologous recombination between the plasmid and the bacterial chromosome, replacing the kanamycin or the chloramphenicol resistance cassette with the structural gene. Revertants of isogenic streptococcal mutants are also expected to obtain the wildtype properties.

The streptococcal WT strains, isogenic mutants, revertants and the transcomplemented strains may be used in experiments with purified WT and SR-A deficient mice to analyse the role of streptococcal surface proteins in recognition and phagocytosis of streptococci by SR-A on macrophages in vitro and in vivo.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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Project Ref 553/04.6

Date Ackn'd 19/11/2004

Date Project Ceased

Recombinant Influenza Virus Expressing HIV-1-Derived Proteins.

Class 2

Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The aim of the project is to develop candidate HIV-1 vaccines using engineered recombinant influenza viruses expressing HIV-1-derived immunogens. The focus is on induction of T cell responses and using prime-boost regimens heterologous vectors delivering a common immunogen. While the immunogen defines
vaccine specificity and provides a basic level of intrinsic immunogenicity, the choice of a vaccine vector and route of administration in great part determines the quality,
strength and longevity of elicited immune responses. Furthermore, functional properties of T cells primed by one vector can be further enhanced and/or developed by
heterologous boost administration.

Recipient or parental organism

E. coli K12 are disabled hosts that cannot colonise the human gut and will be used to manipulate plasmids containing sections of the influenza virus and transgenes.
Recombinant influenza virus based on influenza A/PR/8/34. Influenza A/PR/8/34 was isolated in 1934, is a laboratory-adapted strain which is non-virulent to people. When
given to human volunteers it caused no measurable disease symptoms.
HEK293T cells and MDCK cells will be used for rescue and amplification of the virus respectively. Cell lines have very strict nutritional requirements and are unlikely to
survive outside of these.
The recombinant influenza virus will be used to elicit an immune response to HIV.

Host/vector system

The 12 plasmid rescue method will be used to generate recombinant influenza viruses (Fodor et al., 1999). This involves the transfection into 293T cells of 8 plasmids,
encoding the 8 vRNA segments of the influenza genome and further 4 plasmids expressing the three influenza virus RNA polymerase subunits and the nucleoprotein.
Virus is expected to be generated by 24-48 hr post transfection. Recombinant viruses will be amplified on MDCK cells. The growth properties of the viruses will be
assessed on MDCK cells.
Advances in our understanding of the packaging of the influenza virus RNA genome together with technological innovations in reverse genetics allow the development of a
new generation of influenza virus vectors. The vector systems proposed by Horimoto et al. (2004) relies on a chimeric segment containing both the HA and NA genes,
joined via a promoter sequence. Since both HA and NA are encoded by a single segment with HA packaging signals, a foreign gene can be inserted into a NA-like
segment containing NA packaging signals.
The pPOLI-PR8 plasmid (Subbarao et al., 2003) will be modified by inserting the 3' end promoter sequence downstream of the HA stop codon followed by the insertion of
the NA ORF (Machado et al., 2003; Horimoto et al., 2004). After the NA stop codon 80 nucleotides of the |Haa coding region will be repeated followed by the HA
non-coding region (Watanabe et al., 2003). This construct, when transfected into 293T cells, will be transcribed by the cellular RNA polymerase 1 into an HA-like vRNA
with the capacity to encode both HA and NA proteins, after transcription by the influenza RNA-dependent RNA polymerase. Because the NA is expressed from the
chimeric construct, the NA segment is available for the expression of foreign genes. Transgenes will be inserted into pPOLINA-PR8, after removing most of the NA ORF,
maintaining 183 and 157 coding nucleotides at the 3' and 5' ends, respectively. These coding regions are required to ensure that the transgene vRA will be packaged into
viral particles.

Origin & function

Hiva Immunogen is derived from consensus HIV-1 clade A gag p24/p17 sequences and a string of CTL epitopes (Hanke and McMichael, 2000). The gene was made
synthetically to humanize the codons. In studies conforming to good laboratory practice requirements, the HIVA-expressing vaccines vectored by MVAand pTHr plasmid
DNA were shown to be non-toxic in BALB/c mice. Furthermore, acute toxicity and biodistribution of MVA HIV A were carried out in mice with severe combined
immunodeficiency (SCID) and SIV-infected rhesus macaques which again demonstrated that the MVA.HIVA vaccine was non-toxic and non-persistent. We have now
completed small phase I/II clinical trials approved by GTAC employing MVA.HIVA alone and in pTHRHIVA DNA prime-MVA.HIVA boost regimens in healthy HIV-1-uninfected
human and showed that the vaccine was safe. A number of other phase I/II trials are currently under way in Europe and Africa. In addition, a small safety/immunogenicity
trial in HIV-1-infected human subjects on highly active antiretroviral treatment in Oxford is underway, in which the vaccine has been so far safe. Thus, HIVA has been safe
in several hundred healthy HIV-1 uninfected and HIV-1-infected vaccinees.

Renta immunogen is a fusion protein derived from consensus HIV clade A sequences of Tat, reverse transcriptase, Nef and gp41. We inactivated the natural biological
activities of the HIV components and confirmed this formally in appropriate assays (Nkolola et al., 2004).

Reporter genes such as GFP, beta-galactosidase and luciferase are commonly used and have been shown to be harmless.
Multiple CTL epitopes - CTL recognize and kill cells displaying on their surface 8 - 10 - amino acid-long peptides derived from microbial proteins. One way to minimize the amount of protein administered during vaccination is to engineer artificial proteins consisting of CTL epitopes. Compared to whole proteins, the use of CTL epitopes overcomes the potential risk of undesired biological activities of whole proteins and makes the possibility of reversion to virulence non-existent. The genes encoding for short peptide epitope are derived from HIV, SIV, influenza, plasmodium or melanoma-associated antigens.

Individual whole SIV or HIV genes and their truncations - individual whole SIV or HIV proteins possess certain biological activities. However, the proteins on their own represent only an insignificant risk to people and or environment. In many instances, the protein biological activities are genetically inhibited.

gag group specific antigens - structural capsid proteins, RNA nuclear transport
pol enzymes - protease, reverse transcriptase
tat viral transcriptional transactivator
rev RNA transport and stability
vif promotes virion maturation and infectivity
vpr promotes nuclear localization, inhibits cell division
vpu promotes release of virions, degrades some specific cell proteins
vpx SIV homologue of vpr

Evaluation of foreseeable effects

Influenza virus is classified as a Hazard Group 2 organism by ACDP. The recombinant influenza virus is based on influenza A/PR/8/34, which was isolated in 1934 and has been passaged successively over 100 passages in ferrets, over 100 passages in mice and many times in embryonated chicken eggs. When given to human volunteers, it caused no measurable disease symptoms.

The inserted reporter genes are all well-characterised with no known associated health hazards.

Most of the foreign genes inserted into influenza A/PR.8/34 virus will encode for epitopes derived from HIV or SIV. These sequences are unlikely to be hazardous to human health and pose no greater threat than influenza itself. Genes encoding for whole HIV or SIV proteins are likely to have some biological activity but as they only code for a small percentage of the overall HIV/SIV virus their risk to people and the environment is likely to be insignificant. No modification of genes involved in pathogenicity, virulence, host specificity or tissue tropism are being undertaken and so there is no reason to expect the resulting genetically modified organisms will differ in pathogenicity from the unmodified influenza strain.

Accidental inoculation is likely to result in priming of the immune system to HIV, SIV and certain strains of influenza, which is unlikely to have any detrimental effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121 - 125ºC for at least 15 minutes or 126 - 130ºC for at least 10 minutes or 134 - 138ºC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121 - 125ºC for at least 15 minutes or 126 - 130ºC for at least 10 minutes or 134 - 138ºC for at least 3 minutes), discharge to drains.
Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121 - 125ºC for at least 15 minutes or 126 - 130ºC for at least 10 minutes or 134 - 138ºC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.
Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Animal bedding - EITHER autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121 - 125ºC for at least 15 minutes or 126 - 130ºC for at least 10 minutes or 134 - 138ºC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.
Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licenced incinerator)

Is an emergency plan required according to regulation 20? N
Tick if you are claiming exemption from disclosure for section of the risk assessment N
Tick if you confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Project Ref** 553/05.1

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<td>02/02/2005</td>
<td>Use of replication defective helper-free amphotropic retroviruses for the conditional immortalisation of primary human cells.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Non-GMM</td>
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02/03/2022
The aim of this project is to use replication defective amphotropic retrovirus to enable the conditional immortalisation of primary and cultured human cells from both normal subjects and cells isolated mainly from patients with metabolic or genetic bone diseases. Primary cells from patients with metabolic or genetic bone diseases and normal subjects. Primary cells may contain adventitious agents and under CoSHH will be handled at Containment Level 2. These cells will be screened prior to infection for endogenous helper virus using either the his-D reporter system or RT assay.

Retrovirus virions will be generated by employing the TE-Fly cell lines. TE-Fly cells are derived from TE 671 (human medulloblastoma) cells by transfection with CEB, a MLV gag-pol gene expression plasmid.

Further transfection has been performed to generate cell lines that also express env glycoproteins.

A - env 4070A amphotropic MLV
RD - env RD 114
GA - env from Gibbon Ape Leukemia virus

Finally cell lines will be transfected with expression vectors containing hTERT and telomerase. These cell lines will produce virions containing hTERT and Telomerase, which are capable of infection but are replication defective.

Temperature sensitive SV40 large T antigen (TsA58-U19) with mutation abrogating DNA binding; inactivates p53 and RB pathways, extends cell lifespan. hTERT catalytic subunit of human telomerase; extends cell life span by reconstituting telomerase activity.

Reporter genes confer neo- and hygro-mycin-resistance to transfected cells.
Cells infected with virions containing SV40 tag and hTERT are expected to be immortalised. Once the DNA has integrated, the cells may be considered as posing minimal risk of oncogenicity and they can normally be handled at Containment Level 1. Immortalised cell lines will be tested for the presence of retroviral virions both immediately after transduction and during long-term culture of the transduced cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), or inactivate using a final concentration of 2% Virkon for at least 1 hour following manufacturers guidelines, discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical inactivation, effectively 100% kill (manufacturers validation).

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
Project Ref 553/05.2

Date Ackn'd 22/02/2005

CU2 Project Title Expression/knock-down genes implicated in immune regulation in eukaryotic cells using lentiviral vectors

Class Class 2

CultureVolClass2 1-50 Litres

Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The aim of this project is to use self-inactivating lentiviral vectors to deliver genes, which have been implicated in immune regulation, to cells which are typically resistant to normal transfection methods. The cells to be transduced will include primary murine T cells or clones, embryonic stem cells and murine bone marrow dendritic cells.

Recipient or parental organism

Viral packaging cell line, such as 293T cell lines
These cells will be used for the production of virus

Murine cell lines, primary murine cells and established murine clones
These cells will be infected with the virus to enable efficient transfer of genes

Host/vector system

02/03/2022
The inserted genetic material will be cloned into one of the following self-inactivating lentiviral plasmid systems.
- pWPXL-GFP, pWPI-GFP (sourced from Trono Lab, Geneva)
- LentLox 3.7 (pLL3.7) (sourced from Van Parijs Lab, Cambridgeshire MA)
- pLVTH, pLVTH-siGFP, pLV-tTR-KRAB, pLV-tTR/KRAB-Red (sourced from Trono Lab, Geneva)

### Origin & function

Inserted genetic material will be mammalian (murine) origin of shRNA silencing constructs, derived from genes implicated by gene expression profiling and/or supportive literature in immune regulation. These genes include transcription factors such as FoxP3, T-bet, ROG and GATA-3, known to influence development of regulatory versus TH1 and TH2 effector cells; signalling molecules such as calcineurin and PLC-γ targetting pathways, implicated in T cell anergy and Smad 2/3/4 and 7 and Arkadia implicated in TGF-beta signalling; and ubiquitin ligases such as Cblb, GRAIL and Itch, and the molecule Carma-1 that appear to have opposing effects on the ability of T cells to signal for tolerance or immunity. Other examples include inhibitory receptor molecules implicated in mediating the effect of tolerogenic dendritic cells, such as PDL-1 and PDL-2, gp49B, PIRB and Pilr(alpha), and adhesion receptors CD147 and embikin. Standard reporter genes (eg GFP, EGFP, luciferase) and standard antibiotic resistance and mammalian marker genes will be used.

### Evaluation of foreseeable effects

Each of the lentiviral plasmid systems contain a feature where upon integration into the host genome, the 3'-LTR is copied to the 5'-LTR, rendering it transcriptionally inactive and therefore unable to generate a functional retrovirus, or mediate transcription of a host gene at the integration site.

The lentiviral particles are replication defective and therefore cannot disseminate in the environment. All non-essential genes have been removed from the system.

Genes encoding the structural and other components required for packaging have been separated into different plasmids and the chance of recombination has been minimised by the removal of any regions of homology between those plasmids.

The infectious virus particles will have a broad tropism due to the VSV-G envelope protein and therefore have the potential to infect laboratory workers. However as the vectors are replication defective and unable to disseminate beyond initial target cell, it is very unlikely that there would be any deleterious effect.

The inserts have been selected based on their implied relevance to immune regulation and as such could represent a risk to animal and human health. However, only murine sequences not known to interact with human receptors and ligands will be utilized minimising the hazard posed to human health. The constructs remain potentially hazardous for rodents but work will only be undertaken on cell cultures not on animals. As a precautionary approach for human health considerations ant to protect the environment, Containment level 2 will be applied while handling infectious viral particles.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate using a final concentration of 2% Virkon for at least 1 hour following manufacturers guidelines, discharge liquid to drains.
Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpals) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% (manufacturers validation)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 553/05.3

Date Ackn'd  15/04/2005
CU2 Project Title  Boosting Immunity with recombinant listeria monocytogens.

Date Project Ceased  02/03/2022
Class  Class 2
CultureVolClass2  < 1 Litre
Consent Granted  Not Applicable

02/03/2022  Page 8932 of 15326
### Project Additional Information

#### Purposes of the contained use

Attenuated (actA/inB double deficient) listeria monocytogenes expressing various T cell epitopes will be used to immunise mice with the aim of generating a T cell responses that will be evaluated in terms of vaccine-induced protective immunity against challenge infection.

#### Recipient or parental organism

Attenuated Listeria monocytogenes carrying in frame deletions of two bacterial virulence factor genes. ActA gene is required for cell-cell spread and inlB gene is required for liver infection.

#### Host/vector system

Standard bacterial vectors introduced by electroporation followed by selection for homologous integration into the L. monocytogenes chromosome between two divergent transcription unit.

#### Origin & function

Defined T cell epitopes derived from plasmodium berghei, plasmodium yoelii and mycobacterium tuberculosis will be less than 20 amino acids in length. Such as, CD8 epitopes from Plasmodium berghei CSP, Plasmodium yoelii MSP1, Plasmodium yoelii CS58-67 and CD4 and CD8 epitopes from mycobacterium tuberculosis 85A.

Hen ovalibumin lacking amino terminal 80 residues and also containing CD8+ T cell epitopes in the middle of the ovalbumin.

#### Evaluation of foreseeable effects

Virulent Listeria monocytogenes may cause gastroenteritis in healthy adults and more severe disease in immunocompromised individuals including abortion in pregnant women and is classified as a Hazard Group 2 organism by ACDP.

The attenuated strains which will be used in this project have been altered to carry deletions of the DNA encoding two important virulence factors. Due to the large deletions of two genes reversion to virulence is unlikely. Removal of these virulence factors attenuates the organism by more than 1000 fold in murine models of infection.

Although it is likely that the attenuated Listeria monocytogenes will be less virulent in humans than the parental form, due to the lack of clinical data in humans to support reduced virulence work will be performed at Containment Level 2.

Secretion of fusion proteins from the attenuated Listeria monocytogenes is unlikely to increase the virulence of the organism or have any other deleterious affects. The likelihood of developing an allergy to naturally occurring ovalbumin is small as the recombinant ovalbumin will not assume a native conformation due to the deletion and
addition mentioned above. In addition allergic reactions tend to be initiated upon exposure to protein antigens in the absence of infection.

The epitopes are less than 20 a mino acids in length and represent only a small portion of a single gene. Therefore it is highly unlikely that these epitopes will confer any of the pathogenic properties of Plasmodium or mycobacterium species to the recombinant Listeria.

Animals will be anaethetised prior to inoculation and all inoculations will be carried out in a Class II microbiological safety cabinet. Inoculations will be carried out with a 1ml syringe and the inoculation volume is 0.2ml, thus, up to five animals will be injected with the same needle. The needle will not be re-sheathed between inoculations and the attached syringe/needle will be disposed of into an appropriate sharps container after the syringe is empty. The needle will not be re-sheathed prior to disposal. Listeria will be shed at low levels for four to five days post inoculation therefore all bedding will be autoclaved prior to disposal. Animals are housed in individually ventilated cages and all handling will be done in a class II Microbiological Safety cabinet. Cages containing inoculated animals will be clearly labelled and husbandry staff made aware of the risks.

Contents and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquid (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge to drains, or inactivate using 1% Virkon following manufacturers instructions.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively, 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (manufacturers validation using Virkon)
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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### Project Ref 553/06.1

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<td>≤ 1 Litre</td>
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Non-GMM: Not Applicable

Withdrawn: N

Historical Significant Changes: coin case:4139519

Historical Date of Additional Info: 17/10/2008

Significant Change ID: 553/06.1.a

Date of Significant Change: 17/10/2008
### Purposes of the contained use

The aim of the project is to assess the immunogenicity of candidate HIV-1 vaccines vectored by recombinant viruses expressing HIV-1-derived immunogens.

### Recipient or parental organism

Development of vaccines against HIV-1

### Host/vector system

Not applicable

### Origin & function

1. HIV-1 and SIV Chimeric genes

   HIVA Immunogen HIVA is derived from consensus HIV-1 clade A gag p24/p17 sequences and a string of CTL epitopes (Hanke and McMichael, 2000). The gene was made synthetically to humanize the codons.

   RENTA Immunogen RENTA is a fusion protein derived from consensus HIV clade A sequences of Tat, reverse transcriptase, Nef and gp41. We inactivated the natural biological activities of the HIV components and confirmed this formally in appropriate assays (Nkolola et al., 2004).

2. Reporter genes. Harmless commonly used marker genes such as GFP or beta-galatosidase may be inserted alone or in a combination with other transgenes.

3. Multi-CTL epitopes. CTL recognise and kill cells displaying on their surface 8- to 10-amino acid-long peptides derived from microbial proteins. One way to minimise the amount of protein administered during vaccination is to engineer artificial proteins consisting of CTL epitopes.

4. Individual whole SIV or HIV proteins and their truncations: Individual whole SIV or HIV proteins possess certain biological activities. However, the proteins on their own represent only an insignificant risk to people and or environment. In many instances, the protein biological activities are genetically inhibited.

   - **gag**: group specific antigens - structural capsid proteins, RNA nuclear transport
   - **pol**: enzymes - protease, reverse transcriptase
   - **tat**: viral transcriptional transactivator
   - **rev**: RNA transport and stability
   - **vif**: promotes nuclear localisation, inhibits cell division
   - **vpu**: promotes release of virions, degrades some specific cell proteins
   - **vpx**: SIV homologue of vpr

### Evaluation of foreseeable effects

HIVA vectored by DNA and modified vaccinia virus Ankara (MVA) was safe and non-persistent in healthy and immunocompromized mice, healthy and SIV-infected rhesus macaques and in over 200 healthy and HIV-infected humans (Hanke et al., 2000, 2002, in press; Mwau et al., 2004). We have now completed small phase I/II clinical trials approved by GTAC employing MVA.HIVA alone and in pTHr.HIVA DNA prime-MVA.HIVA boost regimens in healthy HIV-1-uninfected humans and showed that the vaccine was safe. A number of other phase I/II trials are currently under way in Europe and Africa. In addition, a small safety/immunogenicity trial in HIV-1-infected human subjects on highly active antiretroviral treatment in Oxford is underway, in which the vaccine has been so far safe. Thus, HIVA has been safe in several hundred healthy HIV-1 uninfected and HIV-1-infected vaccinees.

Biological activities of RENTA components were inactivated and when vectored by DNA and MVA was safe in healthy mouse and monkeys (Nkolola et al., 2004). None of
The viral proteins are modified, therefore there is no possibility of change of virulence, invasiveness, host range, tropism or the ability to stimulate host defence mechanisms by the recombinant virus. Whole HIV-1/SIV envelope will not be used in these experiments.

Reporter genes such as GFP and luciferase have a history of safe use.

Compared to whole proteins, the use of CTL epitopes overcomes the potential risk of undesired biological activities of whole proteins and makes the possibility of reversion to virulence non-existent. The genes coding for short peptide epitope are derived from HIV, SIV, influenza, plasmodium or melanoma-associated antigens.

The HIV-1-derived transgene products are taken out of the HIV-1 virus context and/or are modified or biologically inactivated and are therefore harmless.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

<table>
<thead>
<tr>
<th>Not applicable</th>
</tr>
</thead>
</table>

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
<thead>
<tr>
<th>Consumables (mainly plasticware eg pepettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees for at least 10 minutes or 134-138 degrees C for at least 3 minutes), or use chemical disinfection according to manufacturers guidelines, discharge to drains.</td>
</tr>
<tr>
<td>Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.</td>
</tr>
<tr>
<td>Sharps (eg needles, syringes, scalpels) - dispose via infectious clinical waste stream for incineration.</td>
</tr>
<tr>
<td>Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees for at least 10 minutes or 134-138 degrees C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via infectious clinical waste stream for incineration.</td>
</tr>
<tr>
<td>Animal carcasses - dispose via infectious clinical waste stream for incineration.</td>
</tr>
</tbody>
</table>

**Degree of kill:**
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical disinfection with Virkon, used according to manufacturers instructions under standard conditions result in complete kill.
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Conf</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
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<tr>
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<td>L2</td>
<td>L3 L4</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Conf L3 L4 L2</td>
<td>L3 L4 L2</td>
<td>L3 L4</td>
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Project Ref 553/06.2

Date Ackn'd 13/02/2006

Date Project Ceased

CU2 Project Title BUILDING A FORMOSE REACTION IN A VESICLE

Class 2

Culture Vol Class 2 1-50 Litres

Consent Granted Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Significant Date of Additional Info

Significant Change ID

Date of Significant Change
### Project Additional Information

**Purposes of the contained use**

| Construction of several Vibrio Harveyi mutants |

**Recipient or parental organism**

| Vibrio Harveyi is non-pathogenic to humans but has severe pathogenic activity in a variety of shell fish, including prawns, shrimp and oysters. Infection causes vibriosis, resulting in tissue necrosis (due to production of exotoxin by the bacteria) and death. Shell fish are farmed in aquaculture and although treatment is possible using broad spectrum antibiotics, Vibrio Harveyi has the potential to cause environmental harm. By analogy with other Vibrio species, we have assigned Vibrio Harveyi to Containment Level 2. Vibrio Harveyi is ubiquitously found in the environment and infections are often linked to stressful environmental conditions of the hosts. |

**Host/vector system**

| Disabled E.Coli K12 and B derivatives - Class 1 |
| Wild type Vibrio Harveyi - Class 2 |
| Standard DNA Cosmids, P1 or lambda Phage |

**Origin & function**

| Mutations in the lux luminescence regulatory pathway will be obtained by gene replacement using Tn5 or Tn5lac transposons, conjugation of recombinant cosmids and other standard microbiological manipulation using disabled E.coli as an intermediate. |
| DNA transposons encodes a kanamycin resistance gene and transposase. |

**Evaluation of foreseeable effects**

| The inserts code for a transposase enzyme and resistance to the antibiotic kanamycin and/or standard marker genes such as Lac Z, GFP etc. None of the inserts nor any of the mutations in the lux luminescence regulatory pathway are expected to alter the pathogenicity of Vibrio Harveyi. Kanamycin is not the antibiotic of choice for treatment of this disease. |

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| Not applicable |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| Not applicable |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. |
| Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. |
Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

### Project Containment

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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<td>L2 Yes</td>
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### Project Ref 553/06.3

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<td>13/03/2006</td>
<td>Comparative genomics of plant-associated pseudomonas.</td>
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**Project Additional Information**

**Purposes of the contained use**

The overall aims of this research programme are to understand the genetic basis of bacterial parasitism of plants and to understand at a mechanistic and evolutionary level how closely related bacteria have developed pathogenic or plant growth-promoting interactions with plants and other soil eukaryotes.

**Recipient or parental organism**

- *Pseudomonas syringae* (various strains of)
- *Pseudomonas fluorescens* (various strains of)
- *Pseudomonas aeruginosa* (various strains of)

**Host/vector system**

- E. coli (such as)
- E. coli DH5α pir - cloning
- E. coli TOP10 (Invitrogen) - cloning
- E. coli BL21 DE3 - cloning, protein expression
- E. coli ROSETTA (DES3) (Novagen) - cloning, protein expression

- *Pseudomonas* (such as)
  - *P. syringae* pv. *Tomato* DC3000 - plant pathogen (tomato, Arabidopsis)
  - *P. syringae* pv. *Syringae* B728a - plant pathogen (bean)
  - *P. syringae* (savastanoi) pv. *Phaseolicola* 1448a - plant pathogen (bean)
  - *P. fluorescens* SBW25 - non pathogen
  - *P. fluorescens* Pf5 - non pathogen
  - *P. fluorescens* Pf01 - non pathogen
  - *Pseudomonas* sp. NZ17 - muchroom pathogen
  - *P. aeruginosa* PA01 - opportunistic pathogen - clinical isolate
  - *P. aeruginosa* PA14 - opportunistic pathogen - clinical isolate

- Mutant banks (such as)
  - *P. aeruginosa* PA01: ISphoAhah, ISlacZhah.
  - [Http://rod.brinkman.mbb.sfu.ca/glbrowse/uwgc_insertions/Mutant_Info.pdf](http://rod.brinkman.mbb.sfu.ca/glbrowse/uwgc_insertions/Mutant_Info.pdf)
  - *P. aeruginosa* PA01: miniTn5:lux
P. aeruginosa PA14: miniTn5::phoA, MAR2xT7. P. syringae pv. Tomato DC3000 regulatory mutants. Available from Arun Chatterjee Carol Bender and Alan Collmer (GacS, RpoN, HrpL, HrpS, RpoS, HrpV). Most mutants available from secondary sources contain a kanamycin resistant transposon insertion or are clean deletions created by homologous recombination.

Vectors: (such as)
pBBR1MCS and derivatives: (Labes et al. 1990)
pRU1156, pRU1144: (Karunakaran et al. 2005)
PET vectors (www.novagen.com)
pCR2.1 (www.invitrogen.com)

Fluorescent protein vectors: pMP4642/4655 (gfp), pMP4641 (cfp), pMP4658 (yfp) pME6010 (vector control), pMP4662 (rfp) (all Bloemberg et al. 2000)
miniTn5::uidA (miniTn::Km with promoterless uidA)
miniTn5::gfp::luxCDABE (miniTn::Km with promoterless gfp::luxCDABE)
miniTn5::ptac (miniTn::Km with outreading tac promoter)
pCre. The pCre plasmid encodes the enzyme Cre-recombinase and can be used to remove antibiotic resistance inserts from transposons such as Ispho Ahah and ISlacZhah when introduced into the same strain (Bailey and Manoil, 2002).

Complementation constructs will be constructed by amplifying and cloning the target genes into pCR2.1, followed by transfer to pUIC-I or pBBR1MCS. Protein expression constructs will be constructed by amplifying the coding region of the target gene and cloning into an appropriate pET vector. Epitope-tagged pET constructs may subsequently be cloned into pBBR1MCS for expression and characterisation in Pseudomonas.

We will obtain some complementing and epitope-tagged clones from existing clone collections. A FLEXgene collection of all P. aeruginosa PA01 ORFs, including a set of clones in bacterial protein expression vectors that add polypeptide tags including N-terminal 6xHis-(pDEST17), C-terminal 6xHis-(pDEST42), and N-terminal GST-(pDEST-GST), has been constructed using high-throughput methods, as described in LaBaer et al. (2005).

Origin & function

Candidate genes that are likely to have a key role in bacteria-plant interactions will be selected on the basis of comparative genomic and functional genomic analyses of model Pseudomonas strains. Seven criteria have been used to select genes.

1) Plant-induced - expression of genes is up-regulated during plant colonisation (Gas et al. 2003; Boch et al. 2002; Lindow et al. 2005)
2) Over-represented - certain protein domains are over-represented in Pseudomonas genomes. Genes containing these domains are likely to encode functions that have adaptively diversified. (Studholme et al 2005).
3) Under-represented - certain protein domains are under-represented in Pseudomonas genomes. Genes containing these domains may have been lost through negative selection. Remaining domains are likely to be under positive selection. (Studholme et al. 2005).
4) Novel architectures - one way bacteria evolve new functions is through the creation of novel domain architectures in which existing protein domains are recombined to give a new or altered function. (Studholme et al. 2005)
5) Secretion - proteins or protein products that have a direct role in plant-interactions are likely to be secreted. Secreted proteins can be identified through bioinformatic and functional analyses. (Preston et al. 2005).
6) Co-regulation - many genes involved in plant-bacteria interactions are coregulated through conserved global regulators such as GacS and RpoN, which in turn are subject to regulation by modification and modulation by proteins such as Lon protease and hydroxylases. These co-regulated genes can be identified on the basis of conserved promoter elements and regulation confirmed by examining expression in a regulatory mutant background.
7) Lateral gene transfer - some of the most interesting candidates for proteins involved in plant-interactions display features characteristic of laterally transferred genes and are most similar to genes present in phylogenetically distant organisms.

As genome sequence databases and the results of bioinformatic analyses are continually under review the list of inserts under consideration for further analysis is also...
under regular review. We have therefore assessed the risks associated with this project on an equivalent basis to the risks associated with a transposon mutagenesis approach to studying virulence in P. aeruginosa or P. syringae, followed by subsequent complementation and characterisation of disrupted genes.

Mutants will routinely be constructed by amplifying and cloning 2kb of flanking sequence from either side into pCR2.1, which will be assembled into a linear 4kb deletion construct before transfer to the suicide vector pUIC-M, and recombination into the target genome.

**Evaluation of foreseeable effects**

**For E. coli work (Class 1)**

The E. coli are K12 or B derivatives. These are disabled hosts that cannot colonise the human gut, have a history of safe use and cannot survive in the environment. Although BL21 has been shown to have slightly higher colonisation potential than K12 strains, it lacks pathogenicity determinants, and is not believed to present any risk to healthy adults.

Cloning vectors (pCR2.1, pET) are non-mobilisable pUIC and pME6010 vectors used are only mobilisable with the addition of a strain containing a helper plasmid for triparental mating.

Inserted genetic material will consist of 1-10kb of DNA from the pathogenic Pseudomonas strain. Although some cloned DNA will encode adaptive traits such as nutrient transport, secreted proteins or regulatory proteins, they will not increase the level of risk for these disabled GMMs.

**For Pseudomonas work (Class 2)**

P. fluorescens are not pathogenic to plants or animals.

P. syringae strains are plant pathogens that have never been isolated from human hosts, they are unable to grow at 37 degrees C. Gene inserts will be used to excise target genes and to restore function to mutants with deletions in target genes by complementation. Mutations are likely to reduce plant pathogenesis where they have any observable effect.

P. aeruginosa is an opportunistic pathogen of immuno-compromised patients. It is able to grow at 37 degrees C. It is occasionally associated with infections of epidermal surfaces, corneal surfaces, burns and wounds. It is highly unlikely to pose any risk to normally healthy laboratory workers using good laboratory technique. Gene inserts will be used to excise target genes and to restore function to mutants with deletions in target genes by complementation. Mutations are likely to reduce pathogenicity where they have any observable effect.

Fluorescent plasmids will be used to observe and track strains using microscopic techniques. The fluorescent proteins cloned in these vectors will not alter or enhance the pathogenesis of these organisms. Vectors used contain antibiotic resistance genes, giving marginal risk of transfer to other strains, this is not likely to increase risk above background levels. The antibiotic genes introduced in this study would be unlikely to increase resistance to antibiotics commonly used in clinical treatment. A worst case scenario would be that modifications or random mutations in cultured bacteria cause a minor increase in infectivity or antibiotic resistance and that a worker subsequently exposes themselves to the altered strain through an uncovered wound or burn, or through contact of contaminated fluids or surfaces with eyes. This is unlikely to pose a significantly increased risk to healthy laboratory workers for the reasons outlined above.

P. aeruginosa pathogenicity assays.

The model nematode Caenorhabditis elegans can be used to test organism to assess P. aeruginosa pathogenesis in simple laboratory test. C. elegans is a bacteriovorous soil microorganism that poses no risk to human health (the standard strain C. elegans N2 was isolated from mushroom compost).

P. aeruginosa strains have been shown to be infectious in various laboratory models, including mouse, nematodes (Caenorhabditis elegans, Panagrellus redivivus), insect (Drosophila melanogaster, Greater Wax Moth Galleria mellonella), amoebae and plant (Arabidopsis, alfalpfa). It should be noted that most of these are artificial models of infection that use large doses of bacteria, and unnatural inoculation methods, facilitating biofilm formation by this bacterium. P. aeruginosa and P. syringae are not typically regarded as vector-borne diseases, although they can be dispersed in a non-persistent, non-circulative manner by insects, soil micro-organisms and on seeds. The most frequent forms of transmission for P. syringae are through aerosols, rain-splash and seeds.

The consequence of the hazards being manifest are considered to be to be low and the likelihood of manifestation is negligible when containment assigned above is taken into account. In all cases, taking into account the control measures assigned above, the overall risks to the environment from the genetically modified micro-organisms produced in this work is low. Therefore additional containment or control measures are considered necessary to protect the environment other than those described to
protect human health and safety. These include containment of infected plants in growth chambers and measures to prevent insect transmission from infected plants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Plants, plant material and soil - autoclave as specified in Defra Plant Health Licence, discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

For waste items that are both radioactive and contain GMMs.

Consumables (mainly plasticware eg pipettes, flasks, tubes) - disinfect with 2% Virkon for at least 30 minutes, discharge any excess liquids to drains as aqueous liquid radioactive waste subject to limits contained in an authorisation granted under the Radioactive Substances Act 1993, dispose of solids as solid low level radioactive waste under an authorisation granted under the Radioactive Substances Act 1993.

Liquids (eg samples, culture supernatants, tissue culture media) - disinfect with 2% Virkon for at least 30 minutes, discharge to drains as aqueous liquid radioactive waste subject to limits contained in an authorisation granted under the Radioactive Substances Act 1993.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

### Project Containment

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#### Project Ref 553/06.4

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<td>Overexpression and knock-down of genes in human and murine haematopoietic cells using replication-incompetent retroviruses and lentiviruses.</td>
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</thead>
<tbody>
<tr>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
</tr>
</tbody>
</table>

Non-GMM Consent Granted: Not Applicable

Tick if notifying a connected programme of work: N

### Project Additional Information

**Purposes of the contained use**

The aim of this project is to modulate (overexpress or down-regulate) expression of critical transcription factors and other molecules such as cell cycle or gene expression.
regulators, which are thought to be involved in lineage commitment and differentiation of haematopoietic cells. Analysis of the effects on proliferation, differentiation and survival of the cells will be conducted at molecular and cellular levels.

**Recipient or parental organism**

Replication defective amphotropic Retrovirus - The recombinant pseudo-typed vectors expressing the gene of interest are amphotropic and can therefore infect human cells, however they are replication incompetent and do not express viral genes.

Replication defective Lentivirus - A number of features are designed to enhance biosafety. (i) The 3'LTR of the lentiviral vector has been deleted that results in self-inactivation of the lentivirus after transduction of the target cells without altering the vector titers. (ii) The current, third generation packaging unit of HIV-a based vectors conserves only three of the nine genes present in the genome of the parental virus: gag, pol, and rev, expressed from split genomes. This eliminates the possibility that a wild-type virus will be reconstituted through recombination. (iii) The VSV-G gene from Vesicular Stomatitis is used in place of the HIV-1 envelope protein. (iv) The lentiviral particles are replication incompetent and only carry the gene of interest.

The viruses are replication defective and pose no environmental danger. They are unstable, inactivated by detergent, UV light and ethanol and would not survive outside the laboratory environment.

Mouse and human primary cells and cell lines - The infected human and mouse cells will be recognised as non-self by the immune system and rapidly lysed by the complement and therefore are unlikely to be pathogenic. Moreover, once integrated into the transduced target cells, the retroviral and lentiviral genomes are no longer capable of producing packageable viral genomes. Cells are unable to survive outside tissue culture conditions and therefore pose no risk to the environment.

**Host/vector system**

Packaging cell lines, such as 293-GPG and 293T and Retroviral and Lentiviral vector systems. Packaging cell lines when transfected with vectors containing the genes of interest produce infectious viral particles, however these particles are only capable of one round of infection, as they do not contain the relevant viral genes necessary for replication. The viral particles are therefore replication defective.

As separate entities the packaging cell lines and the vectors pose no hazard to human health or the environment.

**Origin & function**

Human and mouse genomic wt DNA and cDNA inserts (coding for transcription factors such as SCL, GATA family members, Gfi1b, ETO-2, E2A, HEB, E2-2, Pax5, LEF1, Pu.1, CEBPa) will be overexpressed in the cells. We expect an alteration of the proliferation/differentiation balance or induction of apoptosis of the transduced cells and/or their progeny. In the course of the study, the above list could be extended to molecules identified as partners or targets of some of the transcription factors mentioned above. These could involve other transcription factors, co-factors, chromatin remodelling factors, cell cycle regulators, apoptotic factors, structural proteins, members of the general transcriptional machinery and other regulators of gene expression. They are all expected to modify the proliferation/differentiation balance or induce apoptosis.

Short-interfering RNA (siRNA) will be expressed to knock-down expression of some or all of the proteins mentioned above. We expect an alteration of the proliferation/differentiation balance or induction of apoptosis of the transduced cells and/or their progeny.

Modified forms (including truncations and point mutations) of some or all of the proteins mentioned above will be expressed. They are expected to display a subset of the activities of their wild-type counterparts. However, the possibility of inadvertently generating novel biological activities cannot be excluded. Inducible forms of chosen genes of siRNAs may also be used in the course of the study.

The bacterial Cre recombinase will be expressed in murine cells to specifically excise sequences flanked by loxP sites. Standard selection markers (GFP, antibiotic resistance, lacZ) will allow purification of the transduced cells and won't have any biological actions.

Of the transcription factors mentioned above, SCL is an oncogene. When ectopically expressed in T cells, it contributes to leukaemogenesis. Gfi-1b is a potential...
oncogene, as it was found to be the target of proviral integrations. Mutations in GATA1 have recently been reported in Down's syndrome-related acute megakaryoblastic leukaemia. The other molecules we propose to study have no known transforming or oncogenic properties.

The GFP (and derivatives) and LacZ DNA inserts and the Cre recombinase code for prokaryotic genes that have not been associated with harmful properties in humans. Outside a viral construct, none of this material is active as they lack promoter sequences.

**Evaluation of foreseeable effects**

Although retroviruses and lentiviruses can integrate into the genome of a cell they are generally considered to be of minimal risk to human health and the environment. However in this case a number of the genes being expressed have the potential to be involved in oncogenesis. For this reason it is felt that Containment Level 2 is necessary when working with the infectious viruses to protect human health. Use of sharps will be avoided while working with the viruses.

Infected cell lines pose minimal hazard once the virus has integrated into the host genome as no further viral particles can be produced. Therefore infected cell lines can be handled at Containment Level 1 48 hours post transfection.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Aga plates - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

- Animal Units
- Large Scale Activities
- Human Clinical Applications

Project Ref 553/06.5

Date Ackn'd 19/04/2006

CU2 Project Title

The use of replication impaired adenovirus, retroviral, lentiviral and CMV vectors to explore the genetic basis of biochemical pathways in physiological responses to environmental and innate stimuli.

Class CultureVolClass2 CultureVolumeClass3-4

- Class 2
- < 1 Litre
- Not Applicable

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Project Additional Information

Purposes of the contained use

The primary aim of the project is to explore the genetic basis of biochemical pathways that determine physiological responses to environmental and innate stimuli. This involves an integrated programme to target candidate genes by adenoviral, lentiviral and retroviral techniques and assess responses to a range of external stimuli including...
**Recipient or parental organism**

E. Coli - The E.coli are K12 or B derivatives e.g. DH5a will be used as cloning hosts to generate plasmid expression clones in readiness for transfection into packaging cell line. These are disabled hosts that cannot colonise the human gut and have a history of safe use.

Retrovirus – The virus carrying the inserts is replication defective and is therefore only capable of one round of infection. The viruses generated will be either ecotropic, amphotropic or pantropic depending on the envelope protein. The packaging cell lines used are second generation where the gag / pol and env genes are provided on separate vectors. The gene of interest is provided on a third vector. The possibility of producing replication competent virus using this system is minimal.

Lentivirus – The virus carrying the inserts is replication defective and is therefore only capable of one round of infection. The necessary genes are carried on four separate plasmids thus minimising the possibility of producing replication competent virus.

Adenovirus – The E1 deleted adenovirus carrying the genes of interest is replication defective and is only capable of one round of infection. CMV – Wild type and various mutants of Cytomegalovirus (CMV) will be used. The deletion of genes is not expected to increase the pathogenicity of the virus.

**Host/vector system**

Packaging cell lines and viral vectors systems - Cell lines are considered to be specially disabled hosts however the packaging cell lines will be used to generate viruses that are replication defective but capable of one round of infection.

Cell lines that have been exposed to infectious virus will still contain infectious viruses for a certain period of time (36 hours post transfection). However post 36 hours the viruses will have integrated their DNA into the host cell and the virus can then be deemed non infectious.

**Origin & function**

Inserts are designed to result in increased or decreased activity of normal mammalian genes. They either code for normal mammalian genes or selective alterations of those genes or for short inhibitory RNAs designed to knockdown expression of the endogenous genes. Standard marker genes to identify or select infected cells will also be used. These genes include mouse and human ligands for immune cells and receptors for immune cells and transcription factors involved in the regulation of immune ligands. Genes deleted from CMV - US2-US11, US6-US11, US2-US3 and pp65. The genes are believed to have a role in immune subversive functions of CMV by interfering with expression of MHC class I and/or MHC class II, rendering the target cell less immunogenic to circulating virus-specific T cells. Marker genes include EGFP and standard drug resistance markers such as those for puromycin. Inserts are not expected to affect pathogenicity of the cloning hosts or normal human defence mechanisms.

**Evaluation of foreseeable effects**

Retroviruses - Work, which involves handling of the infectious virus, such as the transfected packaging cell line or infecting mammalian cells, could pose a risk if there is free virus. Amphotropic retroviruses can infect human cells and integrate into the genome. Although these viruses would not be able to replicate, they might encode for oncogenes or cause oncogenic mutations during their integration. Both risks are small, particularly the latter, though this would increase with the number of infected cells.

However, known oncogenes should not be encoded in amphotropic or pantropic retroviruses capable of infecting human cells, and caution should be exercised with genes of unknown function and others like HIF genes where there are some indications that they could have a synergistic role in tumour progression. Lentivirus - The safety features make the chance of generating a replication competent retrovirus unlikely. However, there is a very small risk, which is difficult to quantify, that we might inadvertently produce an oncogenic inhibitory RNA into human cells by a single infection event. This risk exists because of the unknown function of the genes we would be investigating, and because some inhibitory RNAs have effects on multiple genes. Similar effects might apply to a transgene of unknown function and there is also a small risk that the integration site of the virus could trigger oncogenic potential.

Adenovirus - The viruses cannot replicate outside complementary cells and do not insert their DNA into the host genome. However, known oncogenes should not be encoded in these viruses, and caution should be exercised with genes of unknown function and others like HIF genes wherever there is some indication that they could have a synergistic role in tumour progression. The level of risk is small.

CMV - is a lymphotropic/epitheliotropic beta-herpesvirus that is carried as an asymptomatic life-long infection by the majority of individuals in all communities. Control of CMV infection is attributed to the potent cellular immune response that is detected in healthy immunocompetent virus carriers. CMV causes disease in severely immunocompromised individuals (e.g. end-stage HIV infection, Transplant recipients on immunosuppressant drugs). CMV is also associated with intrauterine infection and foetal developmental defects which may be severe and life threatening to the foetus/neonate. Those associated with the project who are pregnant or may intend to become pregnant should discuss this with the occupational health physician. The deleted genes are believed to have a role in immune subversive functions of CMV by interfering with expression of...
MHC class I and /or MHC class II, rendering the target cell less immunogenic to circulating virus-specific T cells. This property is diminished significantly for the RV798 strain and also in the RV35 and RV47 strains although to a lesser degree. The replacement of these genes with a prokaryotic reporter gene does not appear to alter the host range or pathogenicity of the recombinant virus. The loss of the pp65 gene appears to alter certain phenotypic traits of recombinant CMV (RVAd65) in human fibroblasts. This is manifest in delayed maturation although replication is at similar levels as wild type CMV (Schmolke et al. J Virol. 1995 Oct;69(10):5959-68), suggesting that this gene may be dispensable for growth in vitro. It is possible that these viruses will be more immunogenic and provoke a more vigorous immune response than parent CMV due to the less hindered presentation of a variety of antigens. One possibility to be considered is that an enhanced immune response may also result in greater inflammation at the site of infection. Embryos infected with Retrovirus or Lentivirus may generate transgenic animals, however once the virus has integrated into the host genome it is non-infectious and therefore the risks to humans and the environment are minimal and the work is assigned to Class 1. Ecotropic Retrovirus and Adenovirus work is assigned to Class 1. Cell lines, tissues and animal work is assigned to Class 1 36 hours post transfection. Amphotropic and pantropic Retrovirus, lentivirus and CMV work is assigned to Class 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. Liquids (e.g. samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. Agar plates – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. Sharps (e.g. needles, syringes, scalpels) - dispose via clinical waste stream for incineration. Animal bedding – EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill: Autoclaving, effectively 100% kill (annual validation) Incineration, effectively 100% kill (licensed incinerator) Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.
Project Additional Information

Purposes of the contained use

The aim of this project is to identify HIV CTL escape mutations and their impact in viral attenuation. For these purposes we need to express HIV recombinant virus containing specific CTL escape mutations. Escape mutations will be located in different conserved regions of the HIV proteome (Gag, Pol, Env and accessory proteins), depending on CTL responses of the patients. The construction of CTL escapes viral variants and the replication capacity assays will enable us to:

1. Identify epitopes and escape mutations within them that induce viral attenuation.
2. To associate viral attenuation for the CTL escape mutants with a better immune control for the remaining CTL responses.

Recipient or parental organism

E. coli K12 & B derivatives, such as DH5alfa, will be used as cloning host to generate plasmid expression clones. K-12, B strains are disabled hosts that cannot colonise the...
human gut. These E.coli strains are defective in cell wall components relevant to the ability to recognize and adhere to the mucosal surface of colonic cells (Curtiss, 1978), and have a history of safe use. These hosts may be considered equivalent to ADCP hazard group 1.

Packaging cells lines are of human origin, which are well characterised and authenticated and are obtained from commercial sources. They can be regarded as low hazard for GM activities.

Primary cells, such as Peripheral Blood Mononuclear cells (PBMCs), CD4 T cells and CD8 T cell clones from healthy donors will occasionally be used. These can be considered as especially disabled hosts and as such may be considered to be equivalent to ADCP hazard group 1. However Human Primary Cells and cell lines such as HEK293 have the potential to contain adventitious agents and as such must be handled at Containment Level 2 under COSHH.

Host/vector system

Vectors listed are derivatives from pUC non-mobilisable vectors. The inserted genetic material will be cloned into pNL4-3, p83-2 or pJM series vectors.

pNL4-3 - Full-length, replication- and infection-competent chimeric DNA derived from NY5 (5’) and LAV (3’) provirus strains.

p83-2 - Derived from pNL4-3. Contains a 5747 bp fragment with 155 bp of cellular flanking DNA and 5592 bp of DNA from HIV-1NY5 containing the 5’ LTR, complete gag, pol, and vif coding regions, and part of the vpr gene cloned into pDR8 (a derivative of pUC19).

p83-10 - Derived from pNL4-3. Contains a 4002 bp fragment with the LAV vpr (partial), tat, rev, vpu, env, and nef coding regions, 3’ LTR, and 34 bp of cellular DNA cloned into pDR8 (a derivative of pUC19).

p83-10GFP – Derived from p83-10 with Green fluorescent protein (GFP) cloned in frame.

pJM series - Derived from p83-2. Plasmids with specific HIV-1 deletions:
  - pJM11 with a deletion of gag PR,
  - pJM13 with a deletion of gag PR-RT,
  - pJM14 with a deletion in RT
  - pJM31 derived from pNL43 with a deletion in PRRT.

Transfection of plasmids and recombinant constructions derived from pNL43, p83-2 or pJM derivatives plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK293 or MT4 producer cell lines containing LTR packaging sequence. Replication-competent virus will be produced after transfection. Therefore, the risk associated is their ability to deliver genetic material into susceptible cell lines genomes only through specific techniques, such as electroporation or lipofectamine transfection and produce fully infectious particles, as described above.

MT4 - Human T cells isolated from a patient with adult T-cell leukemia. HTLV-I-transformed. RT production is negative or sufficiently low to assay for production of RT. Very useful for cytotoxicity inhibition assays for antiviral drugs. OKT4+, OKT4A+, and IL-2 receptor+.

MT2 - Human T-cell leukemia cells isolated from cord blood lymphocytes and co-cultured with cells from patients with adult T-cell leukemia. Transformed with and continuous producer of HTLV-I virions and should be handled accordingly. Line cloned for maximal cytopathic effects with LAV-1 and cured of mycoplasma.

HEK 293 - Primary human embryonic kidney cells transformed by sheared human adenovirus type 5 DNA. Cells grow as a monolayer. These cells express the transforming genes of Ad5, and are particularly well suited for titration of human adenoviruses, as they are susceptible to human adenovirus and highly permissive for adenovirus DNA.

JURKAT - Derived from Jurkat FHCRC. An IL-2 producing cell line, derived by incubating the cells at 41°C for 48 hours followed by a limiting dilution cloning over
macrophages.

JURKAT CypA -/- - cells show decreased HIV-1 replication with the onset of virus exponential growth delayed 4-5 days.

GHOSTX4 - Derived from HOS cells. Stably transduced with MV7neo-T4 retroviral vector, and stably co-transfected with the HIV-2 LTR driving hGFP construct and the CMV IE driving hygromycin-resistance construct. Co-receptor genes were introduced via retroviral infection. Indicator cells for HIV-1, HIV-2, or SIV infection with un-cloned, primary isolates, molecular clones, or pseudotyped virus. The puromycin-resistant cells are pools rather than clones for co-receptor expression.

Peripheral Blood Mononuclear cells (PBMCs), CD4 T cells and CD8 T cell clones from healthy donors will be used occasionally.

Origin & function

DNA or RNA extracted from blood samples from HIV seropositive patients.

Inserted material codes for escape mutations which are located in different conserved regions of the HIV proteome (such as Gag, Pol, Env and accessory proteins), structural viral proteins (eg. p24, p17) or viral enzymes (eg. protease, reverse transcriptase or integrase).

When expressed in E.coli, this is not expected to pose any significant risk, as there will be no protein expression due to the lack of inducer promoters.

Gene transfer is possible, significantly from the transfected producer cell lines, as the resulting production of infectious viral particles will be able to infect human cells. Accidental exposure could lead to the infection of human cells.

Evaluation of foreseeable effects

For E.coli work. No significant hazards have been identified above. The resulting GMO’s are not expected to carry any additional risks compared to the un-modified recipients. The insertion of the foreign sequences is not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms. Gene transfer as described above is possible, but unlikely to be hazardous. Assignment to provisional containment: Containment level 1 with Good Microbiological Practice and Good Occupational Health and Safety.

For viral work. The production of infectious viral particles from transfected cell lines (eg. MT4, MT2, HEK293) does pose a risk. Transfected plasmids will be integrated into host cell line genomes producing infectious HIV viral particles. The produced HIV CTL escape variants will contain mutations in highly conserved region of the HIV proteome, mainly structural and enzymatic proteins with an important role in the HIV life cycle. It has been described that amino acid changes in conserved regions of the HIV genome produce reduction in the replicative capacity of the mutant virus (Prado et al AIDS 2002). Our previous studies have shown that CTL escape mutants in highly conserved regions of the HIV proteome, such as p24 capsid (a protein that forms the viral core and is essential for the maintenance of the structure of the HIV particle) revert after transmission (Leslie et al Nat med 2004). In vivo reversion from mutant to wild type in the absence of immune pressure can be associated with a fitness cost to the mutant virus. Moreover, we have shown an in vitro fitness cost of the same p24 CTL escape mutants (Martinez-Picado JV 2006).

Thus, based in our previous results our hypothesis is that CTL escape mutations in conserved regions of the viral genome drive HIV variants into a state of viral attenuation. All CTL escape mutants to be tested are naturally occurring mutations in highly conserved proteins and the likelihood of increasing the risk of producing novel HIV particles by inadvertent recombination is low and has not been described in the literature so far. Additionally, HIV is already a highly variable RNA virus.

In conclusion, the resulting GMOs are not expected to pose any additional risks compared to the wild type and may therefore be handled at the same Containment Level. In the event of any adverse results the risk assessment will be reviewed. Assignment to provisional containment: Containment level 3 with Good Microbiological Practice and Good Occupational Health and Safety.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes).

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill: Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)

Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
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</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
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<td>L2</td>
<td>L3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L3</td>
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<td>L4</td>
<td>L2</td>
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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

1. **HBV:** To study the effects on the cell lines HepG2, Huh7, and HepaRG of (a) the transfection of plasmids expressing different regions of the HBV genome and (b) infection with HBV - the differential expression of various proteins in the cell line will be studied in the absence or presence of inhibitors, using a proteomics approach.

2. **HCV:** To study the morphogenesis and full infectious cycle of hepatitis C virus (HCV) in vitro, in the absence and presence of putative antiviral compounds.

**Recipient or parental organism**

1. **HBV:** The cell line HepG2.2.15, produces infectious HBV viral particles. HepG2.2.15 is a hepatoblastoma cell line (HepG2) transfected with a plasmid carrying the gene that confers resistance to G418, and four 5’ to 3’ tandem copies of the HBV genome positioned in such a way that two dimers of the genomic DNA are 3’ to 3’ with respect to one another. The isolated clone produces high levels of HBV e antigen and HBV surface antigen. HBV DNA is carried by these cells as chromosomally integrated sequences and episomally as relaxed circular, covalently closed DNA (cccDNA) and incomplete copies of the HBV genome.

2. **HCV:** Subgenomic replicons of the JFH1 genotype 2a strain cloned from an individual with fulminant HCV hepatitis replicate efficiently in cell culture. Huh 7.5.1 cells will be used to express wild type or modified infectious HCV viral particles derived from the pJFH1 plasmid.

**Host/vector system**

1. **HBV:** Host = HepG2.2.15 is a hepatoblastoma cell line (HepG2). Vector = The basic plasmid for use in the HBV studies is the 2XHBV plasmid. This plasmid contains two head to tail copies of HBV full-length DNA (sub-type adw) ligated into the pGEM3Z plasmid vector. Further we would use the 1.3X HBV DNA construct containing an over-length HBV genome of 4.2Kb. The individual genes encoding core, surface, polymerase, and the X protein will be subcloned into vectors for transfection studies.
2. HCV: Host = Huh 7.5.1 is a human hepatoma cell line. Vector = plasmids based upon pJFH1, a plasmid containing full length JFH1 cDNA

Origin & function

Gfp is a harmless fluorescing protein expressed in the jellyfish Aquoria victoria and is widely used as a reporter gene

Luciferase is a harmless enzyme that breaks down luciferin to produce visible light expressed by the firefly Photinus pyralis and is widely used as a reporter gene

HA tag is a widely used epitope tag derived from the influenza haemagglutinin gene for use in antibody studies

All other constructs will be modifications (mutations, deletions, over- or under-expression) to HBV or HCV genes not involved in pathogenesis.

Evaluation of foreseeable effects

HBV: Infectious HBV particles will be expressed in HepG2.2.15 (Human hepatoblastoma) cells. Modifications to the HBV construct will include expression of reporter genes such as luciferase, structural genes, such as core and surface proteins, and polymerase. None of these modifications are expected to increase pathogenicity or to alter tropism.

HCV: Infectious HCV particles will be expressed in Huh 7.5.1 (Human hepatoma) cells. Recombinants of the HCV include expression of marker genes such as gfp and luciferase, mutation of the GDD motif (abolishes RNA polymerase activity), overexpression and knockout of structural viral genes such as those coding for glycoproteins E1 and E2, NS5B, and expression of siRNA specific for inhibition of HBV and HCV viral genes. None of these modifications are expected to increase pathogenicity or to alter tropism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) – inactivate by chemical means (following manufacturers guidelines) then autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes).

Sharps (eg needles, syringes, scalpels) – reduce so as to minimize, dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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- **Animal Units**: L2, L3, L4
- **Large Scale Activities**: L2, L3, L4
- **Human Clinical Applications**: L2, L3, L4

**Project Ref**  553/06.8

<table>
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<tr>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<td>Modification of gene expression in tumour cell lines with adenoviral vectors to study their metastatic behaviour.</td>
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**Historical Significant Changes**
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**
Purposes of the contained use

The aim of this research is to determine the steps at which specific gene products influence metastasis. Our lab has established a series of steps for both pulmonary and liver metastasis including arrest in the organ, survival, proliferation and vascular remodelling. We now seek to understand how the tumour cells effect these functions in mouse models for metastasis by modifying the gene expression of tumour cells in tissue culture with adenoviral vectors and then injecting the tumour cells into mice to note how the altered expression impacts on metastasis. We will alter the expression of genes affecting survival, angiogenesis, and some proteases that we have previously shown to alter metastatic behaviour by tumour cells. We will also use adenoviral vectors to label tumour cells in order to track them in mice.

Recipient or parental organism

HEK 293 - Human embryonic Kidney cell line used for generating replication defective adenovirus

Human, rat or murine tumour or normal cells - infecting with Adenovirus

Cell lines can be considered as disabled hosts due to their strict environmental and nutritional requirements. Furthermore they are unable to colonise the lab worker due to host-cell line histocompatibility differences that would lead to rejection by the host's immune system.

Host/vector system

Replication defective adenovirus expressing reporter genes, mammalian genes and shRNA. Adenovirus 5 with deleted E1 and E3 genes is unable to replicate except within cells which provide the missing E1 and E3 genes, such as HEK 293 cells. The harvest of the Adenovirus from the replicative competent cells (HEK293) and its subsequent use in the infection of cells is the main point at which a risk might arise as this is the time when the largest titres are obtained and when an accident might have the greatest risk to a lab worker. This single point of infection posing a hazard puts some of the work into Containment Class 2 due to the possible tumour enhancing nature of some of the inserts. After this point no further infection can occur and no additional hazard is expected. Once the cell lines are infected they cannot support adenoviral replication and hence become minimal risk.

Origin & function

Reporter Genes

- Green fluorescent protein is a naturally fluorescent protein from the jellyfish, several variants of the natural gene have been produced that encode a protein with modified adsorption/emission characteristics and which have been optimised for higher level expression or better fluorescent properties for imaging, e.g. the "EGFP" or fluorescent properties such as mPlum or mCherry. FPs are widely used and no hazardous properties have been reported.

- Luciferase from firefly and sea pansy are widely used as reporter genes are not considered to have harmful properties.

Mammalian expression

Genes that are part of the inherent apoptosis pathways in cells such as DR4, DR5, caspas 3,8,9,10, FADD, GLIP,Bcls etc. The augmentation or elimination of expression of these genes in a tumour cell can enhance (or retard) their growth rate when transplanted into a murine host. They can lead to more metastatic tumours. These vectors will be handled under containment level 2 conditions.

Genes that affect angiogenesis such as ang 1 or 2, VEGF, Tiel or Tie2, VEGFR, TF, or HIF1a or b, for example. These genes in themselves are not oncogenic, but when expressed in tumour cells can lead to tumours with a more rapid growth rate.
Genes for protease expression especially of the matrix metalloproteinase family. Overexpression of some genes in this family in tumour cells can lead to more rapid tumour growth or more metastatic tumours.

Genes for the expression of tissue factor, an endogenous molecule involved in clotting initiation and angiogenesis. Overexpression of this gene product due to infection of the adenovirus in a host would be unlikely to be at sufficient high levels to result in any clotting disorders since this is widely expressed throughout the body. Expression in a tumour cell that lacked this gene might alter its ability to metastasize. Other consequences would not be apparent from the literature.

Induction of short hairpin RNAs to specifically downregulate gene expression of genes with homologous sequences. Unexpected effects often appear to be due to homology with microRNAs and hence in recent design of these vectors this lack of specificity is being eliminated by screening for this type of homology. There are some instances in which RNAi lacks specificity in that interferon may be induced, but there is no recognized hazard here.

All the above types of manipulations have been widely used and are not known to have resulted in any harmful effects. However expression of some of these genes while not oncogenic in themselves have been noted to alter tumour growth parameters. Antiapoptotic gene expression can enhance tumour growth and progression. HIF1 overexpression has been shown to lead to more rapid tumour growth. Overexpression of VEGF or ang2 can have a similar effect. Overexpression of some proteases can enhance metastasis and hence any vectors in this category will also be used under containment level 2 conditions.

Evaluation of foreseeable effects

The Adenovirus used has been disabled by deletion of the E1 and E3 genes and cannot replicate unless the E1 and E3 genes are provided in trans (as in the HEK293 cells). Therefore the possibility of the virus disseminating within the environment is minimal. However as with all viral delivery systems there is a single round of infectivity with viruses generated from HEK293 cells. Although none of the genes to be expressed are oncogenic by themselves, a number of them are involved in the apoptosis pathway and expression may affect growth and metastasis of the tumours. For this reason the genes listed in the appendix will be handled at Containment level 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**SELECT STANDARD PARAGRAPHS FROM THE FOLLOWING - ALTER FOR ON HOSPITAL OR SCIENCE AREA SITE**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.
Sharps (e.g., needles, syringes, scalpels) - dispose via chemical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Liquids (e.g., samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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**Project Additional Information**

**Purposes of the contained use**

Salmonella enterica serovar Typhimurium strains in which genes involved in the flagellar export apparatus have been mutated or deleted.

**Recipient or parental organism**

Salmonella enterica serovar Typhimurium strains are Hazard Group 2 bacteria, which can cause acute gastroenteritis and infect many mammalian species. Salmonella enterica serovar Typhimurium can only cause harm to animals in large doses (10^6 or higher). The control measures necessary to protect humans are adequate to protect the environment.

**Host/vector system**

**Origin & function**

flh, fli, and flg operon regions of Salmonella enterica serovar Typhimurium are involved in the flagellar export apparatus system.

**Evaluation of foreseeable effects**

The mutations will adversely affect the assembly of the flagellum, which should lead to a decreased or absent motility. Motility is not essential for virulence but it does enhance pathogenicity. Therefore it is expected that the mutants will have reduced pathogenicity compared to the wild type. As flagellin is an activator of the innate immune response it is possible that the mutant strains will be less immunogenic, however there are a number of other bacterial products that can activate the innate immune response. Therefore the mutant strains are not expected to be more hazardous than the wild type strains. Master flagellar regulator genes flhDC will be overexpressed from an inducible plasmid which should lead to the overexpression of the flagellar IM domain. In mutant strains lacking flgB this will lead to non-motile
strains with flagellar IM domain at enriched levels

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. Liquids (eg samples, culture supernatants, tissue culture media) – optionally pre-treat using 2% virkon then autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill: Autoclaving, effectively 100% kill (annual validation) Incineration, effectively 100% kill (licensed incinerator) Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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### Purposes of the contained use

The overall aim is to functionally characterise known and candidate tumour growth control genes using viral transfection systems.

### Recipient or parental organism

- **E.Coli K12 and B derivatives (disabled)**: will be used to generate the plasmids and the genes are unlikely to be expressed due to the presence of a mammalian promoter sequence.
- **Yeasts (s.cerevisiae, S.pombe, S.pastoris) and BL21**: in these non-pathogenic systems soluble proteins will be expressed.
- **Mammalian cell lines**: for protein expression and analysis
- **Insert cell lines (Sf21, SF9, D-Mel2)**
- **Packaging cell lines such as Phoenix 293T**: provide additional viral sequences necessary to generate replication deficient pseudoviruses.

### Host/vector system

- A variety of modern multi-vectored (9ie3 plasmid or 4 plasmid) systems will be used. These systems all feature split components to ensure there is no generation of replication competent virus.
- **Murine Stem cell based vectors**: high level expression in embryonic stem cells.
- **pRev TRE based vectors**: gene expression can be controlled by various concentrations of tetracycline or derivatives such as doxycycline.
- **pSUPER RNAi vectors**: expression of synthetic short RNAs can lead to specific suppression of gene expression.
Cancer promoting and cancer suppression (that antagonise tumour promoting genes) genes such as IGF2r, IGF2, IGFbps, Pten, PDK, Akt, MTOR, Raptor, Grb2,IRS1, Pat and amino acid transporters, IGFr, Hub genes, Foxo transcription factors, Akt phosphorylation substrates.

Selected modifications of any or all of the above.
Reporter genes such as lac Z, GFP etc

siRNA - validation of target and off target effects will be sought in transient systems using PCR and Western blots prior to using stable expression systems.

Origin & function

The pseudo-retroviruses are replication deficient by the nature of their design and as such cannot spread from one cell to another. However they are capable of one round of infection of mammalian cells, including human and insertion of the viral DNA into the host genome. There is a potential for adverse effects due to insertional mutagensis however this is expected to be minimal.

The genes to be analysed do not one their own initial cancer as a result of mutation or abnormal expression. However they can significantly promote or inhibit phenotypic consequences of cancer initiating genes (oncogenes or loss tumour suppressor genes).

Cell lines and primary cells (other than packaging cells) which have been exposed to the psudovirus pose a minimal risk once the virus has integrated into the genome due to its inability to replicate.

Evaluation of foreseeable effects

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Cell lines and primary cells (other than packaging cells) which have been exposed to the psudovirus pose a minimal risk once the virus has integrated into the genome due to its inability to replicate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Generation of germline genetic manipulations will be done by collaborators at their institutions.

There is minimal risk from the transgenics as the replication deficient virus will be integrated into the genome.

Therefore animal containment level 1

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe-cycle as specified in BS 2646-Part 3, 1993 (either 121-125°C for at least 15 minutes - or 126-130°C for at least 10 minutes for 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-25°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.
Degree of kill
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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**Project Ref  553/07.2**

**Date Ackn'd**  23/04/2007

**CU2 Project Title**
Infection of human and murine cells with recombinant, replication-defective retroviruses expressing a range of regulatory molecules.

**Date Project Ceased**

**Class** 2
**CultureVolClass2** < 1 Litre
**CultureVolumeClass3-4**

**Non-GMM** Consent Granted
Not Applicable

**Project notified under transitional arrangements** N

Withdrawn N
Tick if notifying a connected programme of work N

**Historical Significant Changes**
The overall aim of the project is to identify and characterise the molecules and processes controlling the safe-renewal, lineage commitment and differentiation of haematopoietic stem and progenitor cells. Regulatory genes, such as transcription factors. Growth factors, cytokine receptors and signalling molecules will be delivered to human and murine blood cells by using retroviruses and the effects on the survival, growth and differentiation of the recipient cells will be monitored. The genes may be either wild-type or in the form of the point mutants or chimeric factors generated by chromosomal translocations associated with human leukaemias, or selectively altered variants of any/all of these genes designed to modify and dissect their function.

Recipient or parental organism

E.coli K12 and B derivatives (disabled) - will be used to generate plasmids containing reporter genes, antibiotic resistance genes, regulatory genes of interest and certain retroviral genes.

293FT (human embryonic kidney cell line 293F constitutively expressing SV40 T antigen from pUC based plasmid pCMVSPORT6TAg.neo) and 293T (similar to 293F, T antigen increases viral particle yield). - used to generate pseudovirus.

GP+envAm12 (murine cell line NIH3T3 + one plasmid containing the Moloney murine leukaemia virus gag and pol genes and as second plasmid containing the 4070A amphotropic env gene)

Human cell lines (such as established human leukaemic cell lines, eg, MALM-6, KG1, HL-60, U937)

Murine cell lines (multipotential haematopoietic cell lines)

primary human cell cultures (from screened samples) and primary murine haematopoietic cell cultures

FACS sorted human and murine cells in culture. Cells may also be transplanted to recipient wild type and transgenic mice (transgenics covered by a separate risk assessment).

Host/vector system

The ViraPower Lentiviral Expression System (Invitrogen) generates recombinant, replication-defective lentivirus.

293FT cells transiently transfected with an expression vector (pLenti6/V5) containing the gene of interest under the control of the human CMV immediate/early enhancer/promoter and elements that allow packaging of the construct into virions. A mixture of three packaging plasmids (pL,P1, pLP2 and pLP/VSV-G) are co-transfected to supply the helper functions as well as the structural and replication proteins in trans required to produce the replication-incompetent lentiviral particle.

VSV - g/lentiviral expression system. Will be used for the expression of transcription factors PU.1, HoxB4 and GATA-1. Similar to that described above, it also incorporates a number of safety features. There is SIN factor in the vector and it utilises three plasmids rather than a four plasmid system: (1) A lentiviral vector with viral genes deleted containing the Spleen Focus Forming Virus promoter driving expression of the gene of interest. (2) A lentiviral packaging plasmid encoding gag/pol, tat, rev and HIV-1 rev-response element (RRE), with virulence genes vif,vpr, vpu and nef deleted. (3) An envelope plasmid expressing only the VSV-G gene from Vesicular Stomatitis virus. The three plasmids are co-transfected into human 293T cells to produce the replication-incompetent lentiviral particles.

Amphotropic murine retroviral vectors. Derived from the Murine Moloney Leukaemia Virus (eg p50-M-X-neo) and Murine Stem Cell Virus (eg MSCV-ires-GFP), recombinant murine retroviral vectors carrying the gene of interest will be transfected into murine GP+envAm12 packaging cell line to produce replication-defective
amphototropic retroviral particles.

For retroviral expression systems above, the resulting disabled particles are able to infect most mammalian cells including human, mouse and rat.

**Origin & function**

Mice and human genomic DNA and cDNA inserts, wild-type and mutated (see below, section 2 in appendix for details):

Wild Type transcription factors, such as GATA family, Pu.1, HoxB4

These factors exert their function within the nucleus and are expected to alter the balance between self-renewal and lineage differentiation in stem and progenitor cells.

Other regulatory molecules, such as components of the notch pathway, adaptor proteins (e.g. LNK), cyrokinw receptors and growth factors.

Acting primary outside the nucleus, modulation of the expression of these factors is expected to result in a transcriptional response within the nucleus and to alter the balance between self-renewal and lineage differentiation in stem and progenitor cells.

LNK has been shown to be involved in several intracellular signalling pathways that all regulate the survival and proliferation of hematopoietic cells.

Members of the Notch family may be involved in mediating cell-fate decisions during hematopoiesis.

Other candidate regulatory molecules from differential expression analysis, expected to function in a similar manner to the genes described above.

Known products of chromosomal translocations, their normal counterparts, or mutated versions with deletions, insertions and/or point mutations. These genes will include, but not necessarily be limited to, TEL~AML1, JAK2 and FLT3-1TD. These are suspected oncogenes by virtue of their expression as a result of specific translocations associated with different forms of human leukaemia or point mutations resulting in constitutively activated signalling pathways.

Selective modifications of any/all of the above.

Standard reporter genes: such as neomycin resistance (neoR), beta-galactosidase (lacZ), jellyfish green fluorescent protein (GFP and derivatives), and nerve growth factor (NGF)

**Evaluation of foreseeable effects**

The psuedo-retroviruses are replication deficient by the nature of their design and as such cannot spread from one cell to another. However they are capable of one round of infection of mammalian cells, including human, and insertion of the DNA into the cell genome. There is the potential for adverse effects due to insertional mutagenesis however this is expected to be minimal.

The majority of the genes to be analysed are likely to affect self renewal and lineage differentiation and as such are likely to affect survival and proliferation of cells. Other genes are suspected oncogenes. The potential for harm due to accidental exposure is therefore greater than a psuedo-retrovirus expressing a reporter gene.

Cell lines and primary cells (other than HEK 293 and GP+ env AM 12) which have been exposed to the psuedovirus pose a minimal risk once the virus has integrated into the genome due to its inability to replicate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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Liquids (eg sample, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

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Sharps (eg needles, syringes, scalps) - dispose via clinical waste stream for incineration.

Animal bedding - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

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Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
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Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Use of viral vectors and lentiviruses to study growth control of cells in tissue culture.

purposes of the contained use

The use of amphotropic retrovirus, lentivirus and adenovirus vectors to help study the functions of cellular and viral genes that affect the regulation of cell proliferation and cell survival.

Recipient or parental organism

E. coli - disabled hosts such as HB101, DH1, DH 5, JM101 and derivatives cannot colonise the human gut and have a history of safe use.

Mammalian cells - these are considered to be especially disabled hosts as they have specific nutritional requirements and the immune system should recognise the cells as foreign.

Packaging cell lines- Variants of HEK cells lines and other well characterised cell lines complement the missing genes to enable the viruses to be effectively packaged. Prior to transfection these cell can be considered to be especially disabled hosts, however once transfected they will be handled according to classification of the virus package.

host/vector system

Retrovirus (amphotropic) - replication defective but can infect and integrate into the genome.

Lentivirus - a variety of different systems may be used to generate the replication defective virus. Some contain the SIN function otheres contain the WPRE function. These viruses can infect and integrate into the human genome but are unable to replicate.

Adenovirus - E1 or E2 / E3 deletion. These viruses can infect cells but they do not integrate into the genome and they cannot replicate.
**Origin & function**

cellualle and viral genes encoding proteins that promote cell proliferation and/or decrease apoptosis.
cellualle and viral genes encoding proteins that inhibit cell proliferation and/or promote apoptosis.
siRNAs directed against cellualle and viral genes encoding proteins that promote cell proliferation and/or decrease apoptosis.
siRNAs directed against cellualle and viral genes encoding proteins that inhibit cell proliferation and/or promote apoptosis.

Only single growth-promoting (for example ras, myc, or E1A) and anti-apoptotic genes (such as mdm2, iASPP, mutant p53) will be transduced in this programme of work. The work will not include viral oncogenes which have multiple oncogenic activities such as the SV40 T antigen, nor will it include clusters of genes such as HPV16 E6-E7. Expression of siRNA limited to a single siRNA directed against this class of genes, or the expression of multiple siRNAs which affect the same regulatory pathway.

Ecotropic virus receptor enables entry of ecotropic viruses into cells expressing this receptor.

Also included are standard reporter genes such as GFP, luciferase and B-gal, and standard antibiotic selection genes.

The Cre and Cre recombinase gene may also be used for control of expression of the gene of interest (eg switching genes on and off).

**Evaluation of foreseeable effects**

The inserts to be used are involved in cell cycling and as such many act as oncogenes or tumour suppressor genes. Viruses provide an efficient means of transducing cells. In particular both amphotropic retroviruses and lentiviruses can integrate into the genome of infected cells. There is therefore the potential, should accidental exposure occur, to affect cell cycling (either to suppress or to proliferate) of infected cells. However as cancer is a multi step process and only individual oncogenes will be integrated the likelihood of adverse effects is low, especially when considering the exposure titre and the likelihood of stem cells being transduced.

Also included in this work is the transfection of human cell lines with a virus containing the ecotropic virus receptor. Should accidental exposure with this virus occur it would render those cells infected susceptible the infection by ecotropic viruses which cannot normally enter a cell.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |
| N/A |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

** SELECT STANDARD PARAGRAPHS FROM THE FOLLOWING - ALTER FOR ON HOSPITAL OR SCIENCE AREA SITE**

**Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125C for at least 15 minutes or 126-130C for a least 10 minutes or 134-138C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.**

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**Animal carcasses - dispose via clinical waste stream for incineration.**
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines.)

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Project Ref 553/07.4

Date Ackn'd  05/07/2007
CU2 Project Title Use of Chimeric HIV for assessment of candidate vaccine efficiency in mice.

Class 2
CultureVolClass2 < 1 Litre
Consent Granted Not Applicable

Project notified under transitional arrangements  N

Tick if notifying a connected programme of work  N

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

In chimeric HIV-1 the gp120 envelope is substituted with pg80 envelope of ecotropic murine leukaemia virus (MLV), therefore allowing chimeric HIVs to infect mouse cells through the cationic amino acid transporter, the MLV receptor. They do not enter/replicate in human cells. Their replication in mice overtime declines to zero. Thus, chimeric HIVs can serve as a challenge for mice immunized with candidate vaccines, which employ non-env HIV-1-derived genes as a source of immunogens.

**Recipient or parental organism**

HIV-1 with gp120 envelope gene deleted. A number of different HIV-1 clades will be used including B and D termed EcoHIV and EcoNDK respectively.

**Host/vector system**

HEK 293T cell line – used as a producer cell line for generating the chimeric viruses
E. coli – used for constructing the pseudoviruses and producing DNA

**Origin & function**

gp80 envelope gene from murine leukaemia virus - Envelope gp80 of ecotropic murine leukaemia virus facilitates entry of the chimeric HIV viruses into mouse cells expressing the cationic amino acid transporter, the MLV receptor.

**Evaluation of foreseeable effects**

HIV is a hazard group 3 virus for which the eventual prognosis following infection is generally fatal. The replacement of HIV-1 gp120 envelope with the envelope gene from murine leukaemia virus creates a chimeric viruses, such as EcoHIV-1 and EcoNDK, which can no longer enter human cells. Instead the chimeric virus can only gain entry into murine cells. The chimeric virus has been shown not to replicate in human lymphocytes in culture (ref A mouse model for study of systemic HIV-1 infection, antiviral immune responses and neuroinvasiveness. Potash et al. PNAS 2005; 102:3760-3766).

However as we are creating a novel animal virus with only one recombination step required to generate the parental organism which would be pathogenic to humans we believe Containment Level 2 to be appropriate for this work.

The likelihood of recombination is minimised by control measures both within the lab and within the cells being used.

Recombination in E.coli is unlikely as a) HIV-1 does not naturally infect E.coli, b) other constructs used within the laboratory only contain partial env genes and there are no
regions of homology between the sequences flanking partial env and the chimeric molecular clones.

Recombination in human 293 cells is unlikely because c) HIV-1 does not infect 293 cells; d) only partial HIV-1 env genes are used in the laboratory.

Recombination in the mouse is unlikely because e) HIV-1 does not infect mice efficiently if at all; f) there will be no HIV-1 present in Animal Facility, unless the personnel were infected with HIV-1, which would cause greater risk of HIV-1 infection itself than theoretical recombination; g) the EcoHIV and wt HIV would have to infect the same cells in the mouse body for a recombination to occur.

Recombination in humans accidentally exposed to EcoHIV h) EcoHIV does not replicate in human cells; and i) if the wt HIV-1 were present in humans, it would cause the disease without the need for recombination.

Possibility of cross-contamination of EcoHIV with wt HIV-1.
EcoHIV stocks will be grown exclusively in LEVEL-2 laboratory, in a dedicated incubator, in which the risk of accidental exposure to HIV-1 is zero. Purified plasmid DNA will be transfected into 293 cells. Contaminating wt HIV-1 does not infect 293 cells and thus would not be amplified. Theoretically, plasmid EcoHIV DNA might also be contaminated by plasmid carrying a molecular clone of wt HIV-1, but the likelihood is very small and even if this happened, the EcoHIV virus stock will only be used for injections into mice in which wt HIV-1 cannot replicate.

Therefore the greatest risk is due to the unknown nature of creating a novel animal virus, however research has been carried out in the USA with this virus with no unexpected side effects.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**N/A**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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- **Animal carcasses - dispose via clinical waste stream for incineration.**
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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**Project Ref** 553/07.5

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<tr>
<td>05/07/2007</td>
<td>Development of Aptamers against Herpes Simplex Virus type 2</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Non-GMM</td>
<td>Not Applicable</td>
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Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes

02/03/2022
The aim of the project is to develop aptamers (synthetic nucleic acid ligands) that are able to neutralize HSV-2 infection. The addition of reporter genes to the virus will enable accurate quantitative analyses of the virus neutralisation activity of the aptamers.

Herpes Simplex Virus type 2 (HSV-2)

Virus will be constructed by collaborators outside of the University.

Standard Reporter genes such as fluorescent proteins, β-galactosidase (Lac Z) and luciferase

HSV-2 is a human pathogen transmitted by sexual contact and is assigned to Hazard Group 2 by ADCP. Infections are asymptomatic in the majority of cases but can cause life threatening complications in immuno-compromised hosts.

The standard reporter genes are all well established and in common use. No known detrimental effects have been observed through their use.

Therefore the addition of reporter genes are expected to have no effect on HSV-2, which will remain in the same classification as the wild type organism.

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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**Project Ref** 553/07.6

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<th>Date Ackn'd</th>
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<th>CultureVolumeClass3-4</th>
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<tr>
<td>21/11/2007</td>
<td>Identification of proteins implicated in regulating T cell antigen receptor (TCR)</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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The use of self-inactivating, replication Incompetent lentivirus based expression vectors to generate stable cell lines expressing T-cell receptor signalling components or protein arginine methyltransferases (PRMT5) (wild type and null mutants that abolish methylation activity), and reporter gene as part of a bi-cistronic RNA. Future plans include the stable inactivation of these genes using lentiviruses expressing small interfering RNA (siRNA) and short hairpin RNA (ShRNA).

Replication defective Lentivirus — The lentiviral systems to be used are replication defective and self-inactivating. The number of genes used from HIV-1 has been reduced to either three or four (gag, pol, rev and tat). The env gene is VSV, which allows for amphotrophic infection. Genes have been separated on to three or four plasmids, which have minimal regions of homology, minimising the likelihood of recombination events. The main risk from these vectors is the possibility of gene knockout upon integration into the genome.

The viruses pose no environmental danger. They are unstable, inactivated by detergent, UV light and ethanol and would not survive outside the laboratory environment Mammalian cell lines — are especially disabled hosts and once the virus has integrated in the host genome pose minimal risk. The infected cells will be recognised as non-self by the immune system and rapidly lysed by the complement and therefore are unlikely to be pathogenic. Cells are unable to survive outside tissue culture conditions and therefore pose no risk to the environment Primary lymphoid cells (human) — are especially disabled hosts and once the virus has integrated in the host genome pose minimal risk. However, they may harbour adventitious agents and as such will be handled at Containment Level 2 under COSHH.

Host/vector system
Packaging cell lines, such as 293T and Lentiviral vector systems
Packaging cell lines when transfected with vectors containing the genes of interest produce infectious viral particles, however these particles are only capable of one round of infection, as they do not contain the relevant viral genes necessary for replication. The viral particles are therefore replication defective. As separate entities the packaging cell lines and the vectors pose no hazard to human health or the environment. The WPRE sequence is present in some of the vectors and in line with guidance Containment Level 2 will be used.

Origin & function
Inserts code for mammalian proteins implicated in specialised lymphocyte antigen receptor activation pathway. Examples include TCR signalling adapters (human SLP-76,
LAT, ADAP), protein tyrosine kinase (Lck), protein arginine methyltransferases (PRMT 1-8). Mice lacking expression of SLP-76 or LAT do not develop T cells. Mice lacking expression of ADAP develop T cells which are defective in adhesion pathways. Lck controls the activity of SLP-76, LAT and ADAP and mice lacking expression do not develop T cells. Mice lacking PRMT1 and PRMT 4 cause embryonic and perinatal lethality respectively.

Inserts code for siRNA and shRNA to knock out expression of the genes of interest mentioned above.

Standard reporter genes — such as, eGFP, Luciferase, B-galactosidase.

These are regularly used reporter genes that have a history of safe use.

Standard antibiotic resistance genes — such as, neomycin, hygromycin, puromycin and ampicillin.

**Evaluation of foreseeable effects**

The viral vectors are capable of one round of infection but are unable to replicate. Therefore, in the unlikely event of exposure there is the potential for the virus to integrate into the host genome at a point where they disrupt the function of a gene. As the vectors are self-inactivating they are unlikely to activate gene transcription at the site of integration.

The genes to be expressed are not expected to be toxic or oncogenic, however as they are involved in survival, proliferation, differentiation and death signalling pathways a cautious approach is being taken.

Inactivation of these genes using silshRNA is also not expected to be toxic or oncogenic, however as above a cautious approach is being taken.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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**Degree of kill:**
- **Autoclaving, effectively 100% kill (annual validation)**
- **Incineration, effectively 100% kill (licensed incinerator)**
- **Chemical, effectively 100% kill (following manufacturers guidelines)**

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y
Appropriate containment and control measures have been assed in accordance with the latest guidance issued by ACOM.

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### Project Ref  553/08.1

**Date Ackn’d**: 22/01/2008  
**CU2 Project Title**: Immortalisation of human and mouse cells using an Adenoviral-expressed temperature-sensitive SV40 mutant.  
**Class**: Class 2  
**Culture Volume Class 2**: < 1 Litre  
**Non-GMM Consent Granted**: Not Applicable  
**Withdrawn**: N  
**Project notified under transitional arrangements**: N  

---

### Purposes of the contained use

Replication defective (Ei or EiIE3 deleted) adenovirus will be used to deliver a temperature sensitive mutant of the A gene of SV40.
### Recipient or parental organism

Primary mammalian cells from various tissues. Mammalian cells are considered to be especially disabled hosts and unlikely to survive within the environment.

### Host/vector system

Replication defective adenovirus 5 cause transient transfections of human cells. They are unable to disseminate within the environment.

### Origin & function

Temperature sensitive mutant of the A gene from the 3V40 virus. The normal A gene from SV4O is involved in the viruses ability to transform cells. The temperature sensitive version conditionally transforms cells growing at 33°C. At 37°C the cells differentiate.

### Evaluation of foreseeable effects

The adenovirus is replication defective and therefore cannot disseminate, however the initial virus is capable of a single round of infection. Although at 37°C the insert has a much decreased ability to transform cells a precautionary approach is being taken due to the fluctuating temperatures within the airways (the natural infection route for adenovirus).

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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**Degree of kill:**
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

### Is an emergency plan required according to regulation 20?  

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

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02/03/2022

Page 8980 of 15326
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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**Project Ref 553/08.2**

- **Date Ackn’d**: 28/04/2008
- **CU2 Project Title**: Genetic modification of human embryonic stem cells and primary blood-derived leukocytes using retroviral and lentiviral vectors to study macrophage function and HIV infection.
- **Class CultureVolClass2 CultureVolumeClass3-4**: Class 2 < 1 Litre
- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Project Additional Information**

- **Purposes of the contained use**: The aim of this project is to use replication defective retrovirus and lentivirus to deliver genes, or selected mutants of those genes, or to knock down genes, which are implicated in the entry and exit pathways of HIV-1.
Viral packaging cell lines, such as HEK293, will be used for the production of virus capable of infecting human cells but incapable of replicating. Mammalian cells, such as primary human embryonic stem cells and blood derived macrophages.

**Host/vector system**

- Constitutive expression of the transgene-of-interest using Self-Inactivating lentivirus (pi-IR-SIN) vectors, including LentiLox
- Tetracycline-responsive, titratable control of expression of the transgene, using a commercially available retrovirus (Murine Moloney Leukaemia Virus)-based system (Clontech 632104);
- Inducible individual gene-knockdown, using a commercially-available retroviral (MLV), Tet-inducible RNAi system (Clontech 630926);
- Library gene-knockdown, using commercially available lentiviral shRNA libraries (Sigma MISSION RNAi)

**Origin & function**

The genes to be studied are primarily those involved in the entry and exit pathways of HIV-1 in monocytes and macrophages, such as:
- Receptors CD4, CCR5, CXCR4,
- Restriction factors, including APOBECs, especially APOBEC3G,
- Cytoskeletal transport, including Actin-GFP, Tubulin-GFP, motor proteins, which are implicated in HIV transport into the cell,
- Proteins involved in the budding/retention of the virus in intracellular vesicles, including tetraspanins CD9, CD81, CD53 and tetherin (CD317),
- Defined mutants of the above.
- shRNAs to downregulate expression of these genes individually or in groups.
- Possible future work may include a genome-wide shRNA screen.
- standard reporter genes (e.g. GFP, Luciferase, 6-galactosidase)
- standard antibiotic resistance genes (e.g. Neomycin, Hygromycin, Puromycin and ampicillin).

**Evaluation of foreseeable effects**

All the retroviruses and lentiviruses used in this project are replication defective due to the deletion of numerous genes and essential genes being separated on to plasmids with minimal homology. Therefore the viruses are capable of a single round of infection and integration into the genome but they are unable to replicate and disseminate within the environment.

Inserts code for mammalian proteins implicated in HIV entry/transport/exit. The proteins products of the genes to be studied are not expected to be toxic or cancer inducing in any way. However, the HIV receptors are also receptors for chemokines and as such the genes could modulate immune function, especially chemotaxis, in target cells. This could provide a growth/invasion advantage to cells.

ShRNA against mammalian proteins implicated in HIV entry/transport/exit. Initially, the shRNAs to be used are not targeting known tumour suppressors. However, there is potential for off-target effects with shRNAs, which could potentially have tumour-suppressive effects. This will be minimised by designing shRNAs with as little sequence homology as possible to off-target genes, including BLAST searches against genome databases.

In the immediate future our experiments will target individual genes. It is possible however that we may later want to perform a genome-wide screen using shRNA technology with our hES-derived macrophage system. In this case the risk of the shRNAs will be greater, as shRNAs against all possible genes would be employed, and may include unknown tumour suppressors.

Gene transfer is possible, significantly from the transfected producer cell lines, as the resulting Lentivirus/Retrovirus will be able to infect a wide range of cells, including human cells, as the VSV-G envelope enables amphotropic infection. However dissemination in the environment is limited due to the viruses being replication defective. Gene transfer from the individual packaging and expression plasmids is unlikely because they are based on the pUC series of plasmids and can be described as non-mobilisable.

Most of the genetic inserts are not likely to cause significant effect on an individual. However the WPRE represents a potential oncogenic risk, and some of the
genes-of-interest may modulate immune function. It is theoretically possible for these Lentiviruses to integrate into the genome of an individual on exposure. As they are self- inactivating they are not capable of activating gene transcription from the inactivated viral LTR. However they could in theory disrupt the function of a gene at the site of insertion. The worst-case scenarios could be 1) the WPRE exerting an oncogenic effect; or 2) the chance integration of the virus into a tumour suppressor, thus disrupting that gene’s function; or in the long-term 3) the potential interruption/knock down of a tumour suppressor gene. Even so this single event is unlikely to cause any tumour formation and so the overall risk is still considered low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding — Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by the ACGM.
The aim of this project is to generate single-cycle Env-pseudotyped virus expressing the envelope protein from clinical and laboratory-adapted HIV in order to answer questions concerning the interplay between the Env protein and target cell components at a clonal level. Resultant virus will be used in neutralisation (antibody) and viral inhibitory assays (e.g. gp41 inhibitory peptides).

Initial transfection of 293T cells and resultant pseudovirus will then be used to transduce human cell lines expressing CD4, CCR5 and/or CXCR4, such as PBMC5, U87.CD4.CCR5, U87.CD4.CXCR4, TZM-bl cells.
## Host/vector system

- **pNL4-3.Luc.R-E-** (developed by Dr Nathaniel Landau, Aaron Diamond AIDS research Center NY, USA) and pSG3deltaEnv backbone plasmid (available through the NIH AIDS Research & Reference Reagent Program) and mammalian expression plasmids encoding HIV Env (e.g. the Standard Reference Panel of Subtype B HIV-1 Env Clones available through the NIH AIDS Research & Reference Reagent Program).

## Origin & function

HIV-1 env insert is derived from primary and patient isolates. It encode the envelope protein of HIV-1.

- **pNL4-3.Luc.R-E-** Backbone plasmids encode full-length HIV virus with mutations in the env gene (e.g. insertion mutations, frameshift mutations, and/or stop codon mutations) that prevent viral progeny from producing and expressing Env protein. Additional HIV genes have been disabled in backbone plasmids (e.g. vpr and nef in pNL4-3.Luc.R-E-).

- **pSG3deltaEnv** - Backbone plasmids encode full-length HIV virus with mutations in the env gene (e.g. insertion mutations, frameshift mutations, and/or stop codon mutations) that prevent viral progeny from producing and expressing Env protein. Additional HIV genes have been disabled in backbone plasmids (e.g. vpu in pSG3deltaenv).

## Evaluation of foreseeable effects

The purpose of the modification is to produce HIV virus capable of mimicking the natural interactions under investigation but incapable of replication and therefore representing a significantly reduced hazard. The retrovirus (HIV-1) from which the backbone constructs were derived can cause disease in humans. However the design of these plasmids prevents production of infectious, replication-competent virions. Co-transfection with the env-carrying plasmid allows rescue of infectious virus for env in the viral RNA and as such are only capable of a single round of replication, as no infectious viral progeny will be produced.

Reporter genes (such as luciferase) are well characterised with no known associated health hazards.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- n/a

## For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- n/a

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Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)
Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Appropriate containment and control measures have been assied in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Project Ref**  553/08.4

- **Date Ackn'd**: 19/09/2008
- **CU2 Project Title**: Energy Metabolism in Heart Failure - Role of High-Energy-Phosphate Storage and Delivery via the Creatine Kinase/Phosphocreatine System
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

02/03/2022
The researchers propose to use replication defective lentivirus delivered transgenes (cDNA) to overexpress genes or short-hairpin RNA (shRNA) to knockdown expression of genes involved in cardiac energetics both in vitro and in vivo.

E. coli K12 or B derivatives will be used as bacterial cloning hosts (e.g. DH5a, XL-10, JM-109) to generate plasmids. These are disabled hosts, they cannot colonise the human gut and have a history of safe use. These hosts may be considered equivalent to ACDP hazard group 1.

Established cell lines (e.g. HL-1 mouse atrium, 3T3 fibroblasts, C2C12 myoblasts) have a history of safe use and can be considered as disabled hosts and therefore may be considered to be equivalent to ACDP hazard group 1.

Primary mouse and rat cells (e.g. cardiomyocytes, skeletal myocytes) will used as target cells for viral transduction and will be obtained from rats and mice that are bred in a specific pathogen free unit or from approved commercial sources (e.g. Harlan). None of these cells are deemed high risk for blood borne pathogens. These hosts may be considered equivalent to ACDP hazard group 1.

HEK 293 cells (or derivatives of) are of human origin and used for the production of replication deficient, infectious lentiviral particles. Untransfected cells may be considered equivalent to ADCP Hazard Group 1 as GM hosts, however they will be handled at Containment Level 2 due to the potential presence of adventitious agents, as required under the COSHH Regulations. Once transfected the cells will be considered equivalent to ADCP Hazard Group 2 due to the infectious viral particles.

Standard lentiviral systems will be used that generate amphotropic replication deficient viral particles, such as Open Biosystems packaging system. These systems have standard feature to maximise safety, such as:

- The expression vectors contain a deletion in the 3' LTR that results in “self-inactivation” of the lentivirus after transduction of the target cell.
- The number of genes from HIV-1 that are used in the system has been reduced (i.e. gag, pol, rev, tat and vpr).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto a number of plasmids minimizing the threat of recombinant replication competent virus production.
- None of the structural genes are actually present in the packaged viral genome, therefore no new replication-competent virus can be produced.
- The VSV-G gene from Vesicular Stomatitis Virus is used to pseudotype the vector particles. The HIV-1 envelope has been completely removed from the vector.
- Although the packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gag, pol, rev, env) in the TLA-HEK293T producer cell line, none of them contain LTRs or the \( \Psi \) packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
- The reverse transcriptase (RT) and integrase (IN) proteins are provided in trans producing a class of vectors that contain split gag-pol components on separate vectors.

Viral post-transcriptional regulatory elements – The lentiviral plasmid pGIPZ contains the woodchuck hepatitis B virus (WHV) regulatory element (WPRE) which increases transgene expression. The form of WPRE present in pGIPZ has been modified and is not capable of expressing part of the X protein from WHV.
The transducing vector will contain inserts including mammalian cDNA, shRNA oligonucleotide constructs, standard reporter genes (e.g. eGFP, Luciferase, β-galactosidase) and standard antibiotic resistance genes. The inserts have a role primarily in regulating energy metabolism within the cell and have no known role in tumour growth/suppression. Examples include Creatine Transporter, Creatine Kinase Isoenzymes (MM-CK, mito-CK, BB-CK), Adenylate Kinase, AMPK, PGC-1α and ANT The shRNA constructs will be used to deplete the target endogenous mRNA and therefore restrict gene function. However no shRNA will be used that depletes a known tumour suppressor. The products of the inserted genes are not considered to be inherently toxic. However, their expression by the GMM may result in an altered cardiac phenotype in murine hosts. Gene products are not expected to have harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms.

Evaluation of foreseeable effects

Insertion of the foreign sequences into E. coli is not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the host or normal human defence mechanisms. The resulting GMO's are not expected to carry any additional risks compared to that of the un-modified recipients. This work is assigned to Class 1.

Primary and continuous cell culture exposed to infectious virus poses minimal risk after the initial media change as once the virus has integrated into the genome it poses minimal risk due to the replication defective nature of the virus. There may be an alteration in the cellular energetics due to the inserted transgene however that is unlikely to overcome the strict biological requirements of the cell culture nor affect the response of the immune system. This work is assigned to Class 1.

HEK 293 cells producing infectious replication defective viral particles and work with the infectious viral particles poses the greatest risk within this project. The lentiviruses being generated have a wide tropism and will be able to infect human cells. It is theoretically possible for the lentivirus to integrate into the genome of the infected cells of an individual on exposure. In the unlikely event that normal human defence mechanisms were evaded upon accidental exposure to lentivirus (eg following needlestick injury), any potential effect of inserts on cellular metabolism is unlikely to be harmful and would remain localised. Infection cannot spread as the viruses are replication defective and self-inactivating making the integrated lentivirus incapable of activating nearby genes or producing a packageable viral genome. However in theory the function of a gene at the site of insertion could be disrupted. Even so this single event is unlikely to cause any tumour formation in humans and so the overall risk is still low. Further, the theoretical risk of oncogenesis resulting from the X protein of WHV has been eliminated in the pGIPZ vector with a modified WPRE element.

Assignment to provisional containment for virus work: Class 2.

Animals inoculated with lentivirus are not likely to exhibit different behavioural characteristics compared to uninfected animals nor do they pose any greater risk to human health from biting or scratching. There is effectively no risk of transfer from infected animal to open wounds as the virus is self-inactivating and cannot propagate once integrated into the genome of a host cell. The greatest risk to humans will be from any percutaneous injury sustained during the inoculation procedure. This risk will be reduced due to the animal being anaesthetized. Needles and syringes are restricted for use only when there is no alternative, ie for intramyocardial injection. Scalpels will only be used for the surgery preceding the inoculation via injection and will therefore not be exposed to virus. Appropriate control measures include wearing gloves and safe disposal of the intact needle-syringe assembly in a Sharps Waste Container placed adjacent to the animal. Post inoculation the animals will be held in Containment Level 1 facilities.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Animal bedding – EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

---

Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

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Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Animal Units

| L2 | L3 | L4 | L2 |

Large Scale Activities

| L2 | L3 | L4 | L2 |

Human Clinical Applications

| L2 | L3 | L4 | L2 |
Social traits, such as co-ordinated behaviour, anticompetitor toxins and iron-scavenging, can be important for the success of pathogenic bacteria. We are addressing under which conditions these traits are beneficial to the bacteria, and how the expression of these traits affects their pathogenicity in animal (caterpillar) hosts. Mutation rates can affect the evolution of these traits, and we are also investigating this question.

Pseudomonas aeruginosa is a hazard group 2 pathogen which can cause infection of immunocompromised individuals, people with cystic fibrosis, and can potentially establish an infection through burns and cuts. Infections can be fatal if untreated.

Recipient or parental organism
Pseudomoas aeruginosa is a hazard group 2 pathogen which can cause infection of immunocompromised individuals, people with cystic fibrosis, and can potentially establish an infection through burns and cuts. Infections can be fatal if untreated.

Host/vector system
All genetically modified strains of Pseudomonas aeruginosa to be used are knockouts. The majority of which are transposon-insert mutants (with associated tetracycline resistance cassettes from Escherichia coli) obtained from external sources.

mutS knockout was made by digesting the wildtype allele, liberating an internal fragment, and then ligating. This non-functional allele, and an associated kanamycin resistance cassette from E. coli, was then inserted back into the wild type background by conjugation from an E. coli donor.

Origin & function
bacteriocin (pyocin) mutant (pys2 knockout) - Bacteriocins cause cell lysis of susceptible P. aeruginosa strains.
pyoverdin mutant (pvdF knockout) - pyoverdin is the primary iron-scavenging siderophore of P. aeruginosa.
Quorum sensing (QS) mutants (lasR, rhlR knockouts) and knockouts of genes under the control of QS expression (lasB, rhlA; which encode elastase and rhamnolipid, respectively) - QS describes changes in gene expression in a wide range of genes (such as those coding for proteases (eg elastase) and surfactants (rhamnolipids)) in response to high concentrations of diffusible signal.

Type IV pili mutant (pilA knockout) - type IV pili are involved with social motility.

mutS knockout - results in less efficient DNA repair, and hence an approximately 100-fold higher mutation rate. strain.

Evaluation of foreseeable effects

Pyoverdin mutants have reduced ability to obtain iron, hence are likely to grow less well, and are expected to be less virulent, in humans. There is no reason to suspect the growth or virulence of the pyocin mutant would be altered in human hosts. Quorum sensing mutants do not express a range of traits, including many traits crucial for successful growth of bacteria in vivo, such as proteases (elastase), polysaccharides for biofilm formation and surfactants (rhamnolipid). They will therefore be less virulent. The type IV pili mutants have reduced motility and attachment, so would be less efficient at colonising human hosts. The mutS mutant typically shows reduced virulence in published in vivo studies using mouse models.

The antibiotic cassettes are not the antibiotics typically used for treatment of P. aeruginosa, hence they will not increase risks to human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

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Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Caterpillar carcasses - Autoclave using a make safe cycle as specified n BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for incineration.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 10% kill (licensed incinerator)
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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### Project Ref 553/08.6

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- **Non-GMM Consent Granted**: Not Applicable

- **Project notified under transitional arrangements**: N

- **Historical Significant Changes**: N

- **Historical Date of Additional Info**: 17/06/2010

- **Significant Change ID**: GM553/08.6a

- **Date of Significant Change**: 17/06/2010
### Project Additional Information

#### Purposes of the contained use

We propose to utilise the HSV-1 amplicon vector pHSV-ips to deliver a strong, but short lived, expression "pulse" of the key genes required for the induction of human iPSC cells from primary human fibroblasts.

#### Recipient or parental organism

- Disabled E. coli and yeasts, such as K12 derivatives and S. cerevisiae
- Mammalian cell lines, such as MRC5V2, HEK293, CHO, African Green Monkey cells (Vero 2-2, which also constitutively express ICP27) for generating amplicons glioma cells (G16-9) for amplicon titration
- Primary cells, such as human fibroblasts

#### Host/vector system

Fully defective, non-replicating viral system based on herpes simplex virus type 1 (HSV-1) amplicon vector.

The HSV genome lacking the packaging signal and ICP27 is inserted into a BAC. The packaging signal and the HSV origin of replication is inserted into a plasmid vector containing the transgenes of interest, selectable antibiotic markers and reporter genes to create an amplicon. Transducing amplicon particles are then generated by co-transfecting the amplicon with the helper plasmid containing the packaging gene ICP27 and the BAC containing the deleted HSV genome into Vero 2-2, which also constitutively express ICP27. As the BAC and the helper plasmid do not contain a packaging signal they cannot be incorporated into the transducing amplicon particles which are therefore non replicating.

This results in no detectable helper virus contamination (< 1 helper virus/10⁹ amplicon particles).

#### Origin & function

Genes known to generate induced pluripotent stem (iPS) cells by "reprogramming" primary cell types. A range of such genes have been described, including the transcription factors OCT4, SOX2, KLF4, C-MYC, NANOG and LIN28. These genes have been identified as potentially oncogenic.

Standard reporter genes, such as GFP, EFP and Lac Z etc

Standard antibiotic resistance genes, such as Neomycin and Hygromycin etc

TA-Advanced is a tetracycline controlled transactivator protein.

Cre, is a type I topoisomerase which catalyses site specific recombination between loxP sites.

HSV-TK (Herpes simplex virus thymidine kinase), phosphorylates gancyclovir enabling it to be incorporated into the cellular DNA.

### Evaluation of foreseeable effects

When expressed in combination the genes are expected to reprogram somatic cells to pluripotent cells. As the genes are suspected oncogenes accidental exposure could pose a problem, however a number of safety features have been incorporated into this work to minimise the risks.
The genes have been synthetically sequenced to have approximately 70% homology with the wild type sequence while maintaining protein homology. This will reduce the likelihood of homologous recombination with the wild type variants.

It is possible to excise the four transgenes from their promoter by cre recombinase as they are flanked by lox-P sites. This excision is verified by the expression of M-cherry. The cre recombinase used to excise the genes is under the control of a tet responsive element (pTight). TA-Advanced is a tetracycline controlled transactivator protein, which works in conjunction with a tet responsive element. In the presence of doxycycline the TA-Advanced binds to the TRE and activates expression of the Cre recombinase. In the absence of doxycycline the rtTA-Advanced is unable to bind the TRE and gene expression is blocked.

This vector also contains the HSV-TK (Herpes simplex virus thymidine kinase) suicide gene which allows any cells containing the plasmid to be selectively killed by the addition of gancyclovir.

Expression of Cre recombinase and TA-Advance is unlikely to have any deleterious effect.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Degree of kill:**
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

### Is an emergency plan required according to regulation 20?

<table>
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**If yes, tick to confirm that it is attached to this form**

| N |

**Tick to confirm that you have attached a risk assessment to this form**

| Y |

02/03/2022
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Project Ref 553/09.1

Date Ackn’d 25/02/2009

CU2 Project Title INVESTIGATION OF THE BINDING OF YEAST STRAINS TO C-TYPE LECTIN RECEPTORS AND RELATED MOLECULES

Class 2 CultureVolClass2 < 1 Litre CultureVolumeClass3-4

Non-GMM Consent Granted Not Applicable

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The objective of this work is to identify and define the multiple individual receptor-ligand interactions involved in the interaction of fungi with the immune system. *Candida albicans* will be modified such that they have deficiencies in selected genes, including those for cell wall synthesis. The strains are, therefore, deficient in components of...
the cell wall. This will allow us to determine which components interact with each specific immune receptor that we are studying. Some deleted strains will have the gene re-integrated to allow the experiments to be controlled with precision.

**Recipient or parental organism**

Saccharomyces cerevisiae is assigned to Hazard Group 1.

Wild type Candida albicans is assigned to Hazard Group 2.

**Host/vector system**

Standard integrative plasmids such as Clp10

**Origin & function**

URA3 selection to allow growth in medium deficient in uracil

Knock out and/or reintroductions of genes encoding cell wall components

GFP or derivatives a fluorescent standard marker protein

10 or16 amino acid epitopes derived from chicken ovalbumin

**Evaluation of foreseeable effects**

The C. albicans strains CAI4 (ura3), CAI8 (ura3, ade2), RM1000 (his1, ura3) and BWP17 (arg4, his1, ura3) are the standard hosts for DNA transformation world-wide. These auxotrophies makes C. albicans avirulent. Transformation with URA3 plasmids partially restores the virulence of CAI4, but this restoration is not complete because the genes neighbouring URA3 remain inactivated. This is enough to attenuate virulence partially but not completely. By necessity, some of the transformants are not genetically disabled, although their virulence is slightly lower than the wild type strains of C. albicans carried by most individuals. It is important to note that although a large number of C. albicans mutants have been analysed, including virulence attributes, metabolic and cell wall genes, there is NO report of a mutation that increases the virulence of this yeast. This is unsurprising, since all evidence to date indicates that virulence in C. albicans is a multi-factorial process at the molecular level, with no single virulence factor of over-riding importance. While one might imagine that inactivating a repressor might increase virulence this is NOT the case. Therefore, mutations in wild isolates are expected to have a neutral or negative effect upon virulence. Furthermore, none of the genetic modifications are expected to affect the effectiveness of current therapies (nystatin, fluconazole, amphotericin)

Inserts knock down the expression of genes encoding yeast cell wall components, or encode wild-type copies of these genes for re-introduction into mutant strains. Also selection marker genes such as URA3, allowing growth in medium deficient in uracil, have been introduced into these strains. Inserts are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. No harmful properties of these strains have been identified over the years that they have been used in various laboratories around the world.

Most of the C. albicans transformations have inactivated a specific target gene to create a null mutant. In none of the cases we are studying is this likely to increase virulence. As described above, it has been shown in numerous cases that C. albicans virulence is either decreased or remains unaffected by gene knockouts. No null mutations have been described that increase the virulence of C. albicans.

All the mutants have been generated by gene disruption with the URA3 gene marker and recycling of this in selective medium. Therefore, the original open reading frame has gone and is replaced with hisGdp1200 sequence scar. For the construction of the re-integrant control and to put back the URA3 gene, mutants are transformed with a StuI-digested plasmid, which is inserted in the RPS1 locus. This will remove the RPS1 open reading frame from one allele.

The ectopic expression of most specific genes is unlikely to affect virulence at all, and in some cases it will be expected to reduce C. albicans virulence by adversely affecting growth. The key point is that the probability of increasing virulence by ectopic expression of a single gene is very low, because pathogenicity is complex and polygenic trait requiring a high level of fitness of the C. albicans cell (Odds [1994] ASM News, 60, 313).

We propose to grow and use mostly strains that already exist and that have been used safely in a number of laboratories elsewhere. These strains have been in use in
other laboratories without problems developing. The strains that will be generated in our lab contain GFP with either 10 or with 16 amino acid extension sequences derived from a non pathogenic protein (chicken ovalbumin). These are not expected to present any changes in the properties of expressed GFP protein and thus the new strain is unlikely to behave any differently from the one that has been used safely in other laboratories.

Inoculation of mice – the use of needles may increase the risk of percutaneous injury occur. Systemic infection is rare in healthy individuals however any accident will be reported immediately to Occupational Health to assess whether antifungal treatment is required.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

### Project Containment

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### Project Ref 553/09.2

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<td>13/03/2009</td>
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<td>1-50 Litres</td>
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

Vaccinia Virus will be used to express selected genes from Foot and Mouth Disease Virus (FMDV) to enable crystallisation studies of the capsids.

**Recipient or parental organism**
**Host/vector system**

Standard plasmid vectors will be used to transfer the genes to the Vaccinia Virus by homologous recombination with the TK gene.

**Origin & function**

5' and 3' UTRs, coding sequence for the viral capsid and selected non-structural proteins of Foot and Mouth Disease Virus. Also specific mutations in the FMDV capsid with the intention of stabilizing its structure. Expression should generate virus-like particles of FMDV which lack genes essential for replication and are not infectious.

T7 RNA polymerase will be expressed in a separate Vaccinia virus to the FMDV genes. This is a bacterial promoter and will provide the polymerase necessary for the transcription of the FMDV genes.

Standard antibiotic resistance genes

**Evaluation of foreseeable effects**

Humans are not a natural host of Vaccinia Virus. Vaccinia infection is typically asymptomatic in healthy individuals but may cause a mild rash and fever. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous infection. Substantial safety data has been obtained in human subjects as the Wyeth strain was used as a vaccine for smallpox. Serious complications occurred mainly in immunosuppressed and extremely young individuals. Rare complications include eczema vaccinatum, disseminated vaccinia rash, progressive vaccinia and encephalitis.

Vaccinia virus can infect a broad range of cell types.

Inserts are placed into the TK region of the Vaccinia Virus thus knocking out the gene. This slightly attenuates the virus and the GM versions therefore pose a reduced risk when compared to the wild type.

Foot and Mouth disease virus does not infect humans and therefore poses minimal risk to workers, however FMDV is a DEFRA category 4 pathogen which infects livestock. At no point will full length FMDV RNA or cDNA be used. The sequences are all derived from synthetic genes. Only the genes required for capsid production will be expressed, no viable FMDV will be generated.

Expression of the FMDV capsid is unlikely to alter the envelope of the Vaccinia virus because although both viruses are enveloped vaccinia has a complex structure while FMDV has a simple icosahedral structure.

The FMDV genes are driven by bacterial T7 promoter and therefore are unlikely to be expressed in mammalian cells unless a second vaccinia virus expressing the T7 polymerase is present in the same cell.

In the unlikely event of recombination between the two GM Vaccinia viruses generating a single Vaccinia Virus expressing FMDV genes and T7 polymerase the resulting virus is likely to be less virulent as constitutive expression of FMDV proteins as toxic to the infective cell.

If the Vaccinia Virus expressing FMDV was accidentally released and subsequently infected cattle there is a remote possibility of the animals generating antibodies against FMDV but only if they were infected with the Vaccinia Virus expressing T7 polymerase as well. The animals would not be infectious for FMDV but would test positive in an antibody test.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps (eg needles, syringes, scalpels) – Use of sharps will be minimised. If they are used the sharps bin will be autoclaved prior to disposal via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

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02/03/2022
Use of Viruses to Deliver Transgenes for Cancer Therapy

Purposes of the contained use

Use of Hazard Group 2 viruses for delivery of transgenes to cells with the ultimate aim of using the viruses as cancer gene therapy.

Recipient or parental organism

Pox viruses, such as Vaccinia Virus Western Reserve and Wyeth – Hazard Group 2
Modified Vaccinia Ankara and Fowlpox may also be used to help boost immune responses – Hazard Group 1
Alpha viruses, such as Sindbis Virus – Hazard Group 2

Host/vector system

Homologous recombination between shuttle plasmids and the viral genome.

Origin & function

Antigen molecules and/or costimulatory molecules under transcriptional control of endogenous or synthetic promoters such as, but not limited to L6 tumour antigen, carcinoembryonic antigen (CEA), B7-1, intercellular adhesion molecule-1 (ICAM-1), and leukocyte function associated antigen-3 (LFA-3).
Cytokines such as, but not limited to granulocyte-macrophage colony-stimulating factor (GM-CSF), IL10 and IL2.
Prodrug-activating enzymes genes (PAE) (or variants of) will include genes such as, but not limited to nitroreductase, cytosine deaminase or uracil phosphoribosyl transferase (UPRT) under transcriptional control of endogenous or synthetic promoters.
Hyaluronidase or DNase expression will involve encoding human PH-20 (spam1), Hyal1, Hyal2, or DNase under the control of P7.5 vaccinia or PSE/L synthetic promoter.
within the TK site of attenuated vaccinia. MicroRNA (mir) binding sites will be used to target RNA for accelerated degradation within cells expressing the appropriate mirs. Anti angiogenic factors such as but not limited to Endostatin, soluble Ftl1 and delta4. Standard reporter genes such as β-galactosidase, luciferase, green fluorescent protein. Antibiotic resistance genes for selection purposes.

Evaluation of foreseeable effects

Humans are not a natural host of Vaccinia Virus. Vaccinia infection is typically asymptomatic in healthy individuals but may cause a mild rash and fever. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous infection. Substantial safety data has been obtained in human subjects as the Wyeth strain was used as a vaccine for smallpox. Serious complications occurred mainly in immunosuppressed and extremely young individuals. Rare complications include eczema vaccinatum, disseminated vaccinia rash, progressive vaccinia and encephalitis. Vaccinia virus shows an inherent selectivity for cancerous tissues relative to normal tissues and is therefore a good candidate for cancer therapy. The foreign genes are inserted into the thymidine kinase region of the virus. They are under the control of the P7.5, PSE/L or synthetic promoter which results in the disruption of the thymidine kinase gene reducing the virulence.

TK gene-deleted vaccinia viruses, including JX-594, are significantly attenuated in normal tissues in vivo and are, therefore, less toxic whilst maintaining anti-tumoural activity. This increased therapeutic index is thought to be due to over-expression of cellular TK in cancer cells, supplying TK in trans to the deletion mutant virus. JX-594 is a replication-competent, transgene-armed therapeutic vaccinia virus derived from the commonly used Wyeth vaccine strain (Dryvax®, Wyeth laboratories). It is designed to selectively replicate in and destroy cancer cells, while at the same time stimulating a systemic anti-tumoural immune response through the expression of its transgene, hGM-CSF, in the context of tumour lysis. Three genetic modifications are included in JX-594: (1) thymidine kinase (TK) gene deletion, (2) GM-CSF gene insertion under the control of the synthetic early-late promoter, and (3) lac-Z gene insertion.

Western Reserve strain has had the TK gene and the Vaccinia Growth Factor (VGF) gene deleted. While removal of just TK or VGF decrease viral pathogenicity to some extent, the double deletion has a dramatic effect on decreasing pathogenicity. vvDD has been shown to be non-toxic to nude mice. Accordingly this agent is a relatively safe starting point for introducing transgenes with additional anticancer function.

Sindbis virus is mildly pathogenic to humans causing poly-arthritis, mild fever and skin rash. Infection is self limiting. Other symptoms include headache and myalgia. There is no evidence for person to person transmission. The virus is normally transmitted via arthropod organisms.

Sindbis virus can localise to tumour tissues via interactions with the laminin-1 high affinity cell surface receptor. Increased expression of the high affinity Laminin receptor in tumours, coupled with a lack of free laminin in the tumour environment should allow differential localisation between the tumour and other sites of infection. microRNA binding sites should accelerate viral transcript degradation in cells expressing the appropriate microRNAs. Two significant toxicities observed in Sindbis infection are toxicity to macrophages in the synovium of the joints and toxicity to skeletal muscle. These two sites of toxicity show tissue-specific expression of microRNA molecules (Macrophages – Mir-142, Muscle – Mir-133 and Mir-206) Binding sites specific for these (and other) microRNA molecules will be incorporated into the virus genome to prevent replication at these sites and consequently to limit virus induced toxicity. The transgene will also be suppressed, by the same microRNA mechanism.

Antigen molecules and/or costimulatory molecules – certain self antigens (such as CEA and L6) are overexpressed in cancers and are consequently used as markers for certain cancers. Expression of these genes in vaccinia should boost immunity. It is not expected that immunity against these self antigens will be harmful. GM-CSF (granulocyte-macrophage colony-stimulating factor) is widely used in the clinics to rescue patients after chemotherapy-mediated suppression of their immune systems. It is reportedly the most effective cytokine for stimulating tumour-specific anti-tumoural immunity. It is not expected to be hazardous when expressed in vaccinia or Sindbis. Prodrug-activating enzymes genes convert non toxic prodrugs into active cytotoxic ones. In the absence of the prodrug moderate levels of the activating enzymes are not expected to be harmful. Some studies have indicated that high levels of nitro-reductase enzyme could have limited cytotoxicity in some cells.

DNase cleaves DNA and will be used to break down extracellular DNA within tumour nodules and improve virus spread. A recombinant DNase protein has been licensed for use to treat cystic fibrosis by reducing lung secretion viscosity. PH-20 (spam1) has endoglycosidase activity. It can cleave hyaluronic acid in the Extracellular Matrix resulting in a decrease in interstitial fluid pressure (Breken et al 1998). A recombinant protein has been developed by Halozyome http://www.halozyome.com/ and has been approved for use in the clinic to increase dispersion of sub-cutaneous injections.

Inclusion of hyaluronidase or DNase should allow the virus to spread more effectively through interstitial connective tissue, but replication will be mainly restricted to cycling cancer cells and pathology in normal tissues is expected to be lower than the wild type (class II) virus.

Anti angiogenic factors interfere with pathological angiogenesis and can be expressed without overt toxicity to existing tissues. Cytokines regulate the immune response towards the tumour. Some cytokines will stimulate an anti-tumour response (for example by promoting engraftment of T cells)
while others will suppress the immune response in order to allow the infected virus to spread more effectively through the tumour.

Standard reporter genes and antibiotic resistance genes are widely used and no hazardous properties have been reported.

In vivo experiments pose the greatest risk of accidental exposure due to percutaneous injury. Standard working practices such as immediate disposal of needles and not resheathing should minimise the risks.

Tissues from treated animals will be handled at the same level as the virus stocks.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding – EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

02/03/2022
Project Additional Information

Purposes of the contained use

Mutants of intercellular signalling genes in a number of Hazard Group 2 bacteria will be assessed for their effect on quorum signalling

Recipient or parental organism

Helicobacter pylori. This is a hazard group 2 microaerophilic bacterium that inhabits various areas of the stomach and duodenum. It causes a chronic low-level inflammation of the stomach lining and is strongly linked to the development of duodenal and gastric ulcers and stomach cancer. Over 80% of individuals infected with the bacterium are asymptomatic.
Pseudomonas aeruginosa. This is a hazard group 2 pathogen that can cause infection of immunocompromised individuals, people with cystic fibrosis, and can potentially establish an infection through burns and cuts. Infections can be fatal if untreated.

Actinomyces naeslundii T14V. This is a hazard level 2 bacteria that occupies the oral cavity. They have been implicated in periodontal disease and root cavities. These bacteria are also associated with good oral health.

Streptococcus oralis A hazard level 2 bacteria that is numerous in the mouth and throat. It is a common cause of endocarditis, which may be fatal if untreated, and is also implicated in dental plaque formation.

Salmonella typhimurium. A hazard level 2 bacteria that occupies the gut. It is a major cause of gastroenteritis, which may require hospitalisation, and may be fatal if untreated. Infection is established through ingestion.

Enterococcus faecalis A hazard level 2 opportunistic pathogen. It is a normal inhabitant of the intestinal tract and female genital tract but can develop virulence through a fluid gene transmission system whereby it can acquire virulence factors and antibiotic resistance.

Porphyromonas gingivalis A hazard level 2 anaerobic pathogen. It is commonly found in the human body and especially in the oral cavity, and is associated with periodontal lesions, infections, and adult periodontal disease. Approximately 70-90% of people pubescent and older have gingivitis, a possible precursor to adult periodontal disease, which is associated with Porphyromonas gingivalis and allows it to further infect the areas near the root of the teeth causing tooth decay and infection.

Staphylococcus aureus. A hazard level 2 bacterium that is a common coloniser of human skin and mucosa. Infection may develop through cuts and sores, and skin and wound infections, urinary tract infections, pneumonia and bacteraemia (blood stream infection) may then develop. In immunocompromised individuals infection can be severe, and antibiotic resistant S. aureus (MRSA) is a major cause of hospital secondary infections.

Host/vector system

Individual genes from the various Hazard Group 2 bacteria will be knocked out and replaced by the kanomycin resistance gene using standard DNA plasmids or cosmids.

Origin & function

Genes which have been identified as having a possible role in interspecies communication will be knocked-out by the insertion of the gene for kanamycin resistance using transposons. Examples of such genes are homologues of the luxS or luxP genes of Vibrio harveyi. The luxS gene produces the protein autoinducer 2, which is a quorum sensing signal protein and the luxP gene produces a protein which detects and transduces the quorum sensing signal.

Evaluation of foreseeable effects

Quorum signalling results in changes of gene expression in a wide variety of genes, including many which code for traits crucial for successful growth of bacteria in vivo, such as proteases (elastase), polysaccharides for biofilm formation and surfactants (rhamnolipid). The knock-out strains are expected to show some alteration to the changes in gene expression and are expected to be less virulent than the wild type strains as a result.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) –autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

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Project Ref 553/09.5
**Project Additional Information**

**Purposes of the contained use**

Lentiviruses and amphotrophic retroviruses will be used to deliver genes or short hair pin RNAs to investigate their roles in the insulin like growth factor signalling pathway.

**Recipient or parental organism**

- E. coli K12 or B derivatives - E.coli strains are disabled and cannot colonise the human gut.
- Mammalian cell lines - Minimal hazard for mammalian cell lines obtained from commercial sources that are well characterised and authenticated.
- Primary cell cultures - Primary human cells and human cell lines that are not fully authenticated and characterised may carry contaminating infectious agents. Primary cells from non primate sources are deemed to be of low hazard.
- Packaging cell lines - produce infectious replication defective viral particles after they have been transfected with multiple viral plasmids.

**Host/vector system**

Replication defective amphotrophic retroviruses and lentiviruses

Both these systems have the required viral genes (gag and pol, env) split onto separate plasmids. These have either been separately integrated into the genome of the packaging cell line (such as 293T) or will be transfected at the same time as the plasmid containing the transgene.

These systems produce infectious viral particles, which cannot replicate and are designed so to minimise the likelihood of generating replication competent virus through recombination.
Human and mouse genomic DNA and cDNA inserts encoding proteins in the IGF signalling pathway, and other proteins that regulate cellular processes including proliferation, cell survival and DNA repair. Also included are inserts encoding fusion proteins that regulate the subcellular localisation of the transgene product, such as the HA-ER-I-PpoI system in which the DNA binding domain of the estrogen receptor (ER) is fused to, and directs nuclear import of, the I-Ppo1 endonuclease (Berkovich et al Nat Cell Biol, 9: 683-690, 2007). In the course of the study, this list may be extended to molecules identified as partners or targets of components of the IGF axis. These could involve other cell surface receptors, co-factors, repair proteins, chromatin remodelling factors, cell cycle regulators, apoptotic factors, structural proteins, members of the general transcriptional machinery and other regulators of gene expression.

Short hairpin RNA (shRNA) will be expressed to silence expression of signalling proteins including components of the IGF axis, and or proteins involved in DNA repair. This is likely to influence cellular proliferation, apoptosis induction, and cell survival following DNA damage.

Standard selection markers (GFP, antibiotic resistance, lacZ) will allow identification of the transduced cells, but is unlikely to have any significant biological effect.

**Evaluation of foreseeable effects**

The Lentiviruses and Retroviruses are amphotrophic and can therefore infect human cells however the design of these systems is such that the viral particles are replication defective and are only capable of one round of infection.

The genes to be expressed or knocked down have the potential to affect proliferation, apoptosis and survival of cells and could therefore in some instances have detrimental effects should they be integrated into genomic DNA of a workers cells.

Once incorporated into the genomic DNA of the experimental cell lines the inserts pose minimal risk due to the replication defective nature of the viruses. The cells would be recognised as non-self by the immune system and rapidly lysed by complement.

Experimental procedures will minimise the likelihood of exposure to the infectious virus, such as the use of a micro-biological safety cabinet and sealed centrifuge tubes and rotors, however the use of needles will be required for inoculations and this poses a risk. Staff are trained in appropriate handling procedures and syringe-needle assemblies are disposed of immediately after use.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 15 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Animal bedding – EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Project Ref 553/09.6

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<td>&lt; 1 litre</td>
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Date Project Ceased

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The use of replication defective Lentiviral vectors to modulate expression of candidate genes to assess their role in tumourigenesis.

**Recipient or parental organism**

E.coli K12 or B derivatives

Mammalian cell cultures - especially disabled hosts which should be recognised as foreign by the human immune system. Due to the presence of adentitious agents certain cell lines will be handled at Containment level 2.

**Host/vector system**

Standard Lentiviral vector systems, which produce replication defective virions.

The system to be used is a third generation one. The expression vectors have a deletion in the 3’ LTR which results in self inactivation. The number of HIV genes have been reduced to gag, pol, rev, tat and vpr. The envelope protein is VSV-G which allows amphotrophic infection. The genes are split onto four different plasmids. No replication competent virus can be produced.

**Origin & function**

Mammalian genes thought to be involved in Hereditary Leiomyomatosis renal cell cancer (HLRCC) such as Fumerate Hydratase, succinate dehydrogenase and G-protein coupled receptors.

Fumerate Hydratase is a nuclear-encoded protein that catalyses a step of the Krebs cycle, converting fumarate to malate. It is also thought to be a tumor suppressor as inactivated protein is seen in tumours from patients suffering from Hereditary Leiomyomatosis renal cell cancer.

Succinate dehydrogenase is an enzyme also involved in the Krebs cycle catalysing the oxidation of succinate to fumarate.

G-protein coupled receptors are transmembrane signalling receptors, which pass signals across cellular membranes to activate internal pathways.

Other genes identified during the course of the project may also be expressed/knocked down.

shRNA constructs to knock down expression of the above genes.
Standard reporter genes and antibiotic resistance genes

**Evaluation of foreseeable effects**

E. coli K12 or B derivatives are disabled hosts which cannot colonise the human gut and have a history of safe use. Genes are not expected to be expressed in these systems due to the lack of bacterial promoter.

Mammalian cell cultures are especially disabled hosts which should be recognised as foreign by the human immune system. Due to the presence of adventitious agents certain cell lines will be handled at Containment Level 2. As lentivirus integrates into the host DNA and lacks essential genes for replication, mammalian cells, which have been infected pose minimal risk.

293 cells (and various derivatives) are used to produce viral particles which are capable of one round of infection. These cells will be handled at Containment Level 2 with the use of sharps minimised.

Lentiviral particles are infectious but replication defective however they are capable of integrating into the host genome where they may cause insertional mutagenesis. Control measures are in place to minimise the likelihood of exposure however needles are required for inoculations.

Inoculations using transduced cells pose minimal risk to the worker as the lentivirus will be integrated into the host genome and replication defective. The cells should be recognised as foreign by the human immune system.

Inoculations using lentivirus capable of one round of infection poses the greatest risk due to the use of needles. Workers are trained in these procedures including the policy of not re-sheathing needles and disposing of intact needle-syringe assembly into sharps bins immediately after use.

Accidental exposure to a single gene is considered to be low risk.

The genes themselves are involved in individual steps of the Krebs cycle and cellular signalling. Although the loss of fumarate hydratase is seen in HLRCC patients it is likely that other gene mutations are required for the progression of the disease.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycles as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS2646< Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Animal bedding - EITHER autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annul validation)
Incineration, effectively 100% (licensed incinerator)

Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

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Project Ref 553/09.7

Date Ackn'd 08/10/2009

CU2 Project Title
The use of retroviral and lentiviral vectors to facilitate transgene expression and/or gene silencing by RNA interference in mammalian cell and tissue culture systems

Date Project 02/03/2022

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 Litre

Non-GMM Consent Granted
**Project Additional Information**

**Purposes of the contained use**

Replication defective retroviral and lentiviral gene transfer vectors will be used to introduce transgene and/or RNA interference expression cassettes into mammalian cells to investigate the role. A variety of transgenes / RNAi targets associated with cell growth and survival, cell growth properties, apoptosis, key regulatory pathways, and fluid transport processes will be investigated.

**Recipient or parental organism**

E. coli K12 or B derivatives - E.coli strains are disabled and cannot colonise the human gut.

Mammalian cell lines - Minimal hazard for mammalian cell lines obtained from commercial sources that are well characterised and authenticated.

Adult mice - no hazard to human health

Fertilized mouse eggs prior to re-implantation to create transgenic mouse lines - no hazard to human health

**Host/vector system**

Replication defective ecotropic and amphotrophic retroviruses and lentiviruses

Third or later generation retro- and lentiviral systems (based on viruses from various species) incorporating self-inactivating 3' LTR (SIN) U3 deletion. Both these systems have the required viral genes (gag and pol, env) split onto separate plasmids. These have either been separately integrated into the genome of the packaging cell line (such as 293T) or will be transfected at the same time as the plasmid containing the transgene. These systems produce infectious viral particles, which cannot replicate and are designed so to minimise the likelihood of generating replication competent virus through recombination.

**Origin & function**

Mammalian and reporter gene sequences:

1) FOXP Forkhead Transcription Factors and associated interacting genes / proteins

FOXP Forkhead transcription factors have key roles in development and in human malignancy. Best studied in terms of cancer is FOXP1 which in its full length form is believed to act as a tumour suppressor and in an N-terminally truncated form has been shown to have oncogenic activity in a model of avian nephroblastoma.
2) HSP90 Heat Shock Proteins
HSP90 heat shock proteins are highly conserved molecular chaperones that have key roles in signal transduction, protein folding, protein degradation, and morphologic evolution. HSP90 proteins play important roles in folding newly synthesized proteins or stabilizing and refolding denatured proteins after stress.

3) Lymphoma Associated miRNA Factors
Several miRNA families are over or under expressed in lymphoma. Best studies examples include the miR-155 and miR-199 families.

4) Lymphoma Associated Antigens
Lymphoma-associated antigens have been identified through their humoral recognition by patients' the immune system and/or their presence in lymphomas despite restricted normal tissue expression.

5) Modulators Of Epithelial Ion Transport
Modulators of epithelial ion transport have a key role in regulating fluid balance across epithelia. Best studies examples of such modulators include ENaC the epithelial sodium channel, CFTR an epithelial chloride channel and members of the CLC family of calcium activated chloride channels and protein and RNA factors that activate or repress their activity.

6) Common causes of blindness
Mutations in a variety of genes result in retinal dysfunction and inherited blindness.

7) Commonly Used Reporter Transgenes
Commonly used, simple to measure reporter genes will be utilised to assist in vector development, and as experimental controls. Examples include firefly luciferase and similar transgenes, jellyfish green fluorescent protein and similar transgenes, E.coli LacZ, mammalian serum, blood clotting and red cell production factors such as alpha-fetoprotein, FIX and EPO.

**Evaluation of foreseeable effects**

The inserted mammalian and reporter gene sequences are normal or selective alterations of characterised mammalian genes or miRNAs and/or standard reporter genes. The inserted sequences are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of the packaging cell lines.

The majority of the sequences are expected to pose little or no risks in humans. However, some inserted sequences may have harmful properties in humans via the ability to act as tumour suppressor and/or oncogenes eg truncated FOXP1, or by their ability to affect normal human defence mechanisms by mediating immunotolerance eg FOXP3. Consequently, gene transfer of some of the sequences to humans may be hazardous.

The retroviral and lentiviral particles produced will have a broad tropism and be capable of infecting and transducing human and other mammalian cells, inserting viral and mammalian and/or reporter gene sequences (see below) into the host genome. This is an anticipated risk associated with the use of retroviral and lentiviral gene transfer vectors.

The viral particles have however been rendered non-replicable by a number of safety features:

1) The viral genomes are self-inactivating, carrying a deletion in the U3 region of 3’LTR. During integration into the host cell genome, the viral 3’ LTR is copied to the 5’ of viral genome rendering it transcriptionally inactive and unable to function as a replicative retroviral genome.

2) The viral genome does not contain any of the viral packaging or structural genes necessary for viral replication.

3) The viral packaging and structural genes factors necessary for viral particle production are supplied in the producer cell line in trans, either by the producer cell line itself or by co-transfection with packaging plasmids. Regions of homology (eg LTRs or packaging sequences) between these viral elements and the viral particle genome have been minimised to eliminate undesirable recombination events that could lead to the generation of replication competent virus (RCV).
4) In the case of lentiviral vectors, the viral particle genomes contain exogenous promoter sequences to permit transgene/RNAi factor expression. This allows viral tat gene, essential to wildtype lentiviral replication, to be completely eliminated from the packaging system. Thus no RCV can be produced.

Consequently, only retroviral and lentiviral particles which are unable to replicate, but which can deliver the transgene/RNAi insert of choice will be produced. These viral particles cannot contain additional viral genes as their sequences lack the LTR or packaging sequences necessary for their sequences to be incorporated into the viral particles.

Accidental human exposure to viral packaging cells carries minimal risk as the inserted sequences are not expected to affect the pathogenicity of the cells. It is anticipated that any cells would be rapidly cleared by the complement/immune system of any exposed individual.

Accidental human exposure to viral particles could lead to viral infection and the insertion of viral and mammalian and/or reporter gene sequences into the host genome. Importantly, in the context of human gene therapy clinical studies, high doses of retroviral and lentiviral vector particles have been administered in vivo with no observed complications. However, in the worst case scenario, integration within a tumour suppressor gene or the biological activity of the integrated sequences could lead to the generation of a tumour. Indeed, leukaemia like tumours have been observed in a small number (~10%) of individuals infused with bone marrow stem cells that had previously been treated ex vivo with retroviral vectors. Despite these observations it is considered highly unlikely that accidental human exposure to the viral particles described in this risk assessment would lead to the generation of a tumour. Major differences between the described worse case scenario and any accidental exposure exist including large differences in transgene/host interactions, likely exposed viral particle numbers, in vivo rather than ex vivo delivery route, consequent exposure of viral particles to complement/immune system, likely access to relevant precursor stem cells and immune status of individuals involved.

Consequently, it is not anticipated that accidental human exposure to viral particles is associated with significant risk. However, given the nature of some of the mammalian gene sequences (discussed above), gene transfer could be hazardous.

Retroviral and Lentiviral Transduction

Retroviral and lentiviral particles will be purified from packaging cell cultures and used to transduce mammalian cells to facilitate the production of new mammalian cell lines permanently expressing the desired transgene and/or RNAi inducing molecule. Gene transfer from viral particle to mammalian cell line is expected.

The inserted sequences are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of the transduced mammalian cell line. It is anticipated that any cells would be rapidly cleared by the complement/immune system of any exposed individual.

Accidental human exposure to viral particles during mammalian cell transduction could lead to viral infection and the insertion of viral and mammalian and/or reporter gene sequences into the host genome. These risk are discussed in detail above.

In some cases new transgenic lines will be created using viral particles. Due to the replication defective nature of the particles once integrated into the host genome the virus poses minimal risk to humans.

Where viral particles are used in vivo the greatest risk is from the use of needles however as detailed above it is not anticipated that accidental exposure would be linked to significant risk.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Transgenic mice will be housed and handled at Animal Containment Level 1.

Where no viral particles are present, standard practices will be employed. No further controls are required for safe handling.

In the presence of viral particles, animals will be housed in IVCs marked as biohazardous and handled at Containment Level 2. At first cage change, IVCs and contents will
be autoclaved. Thereafter standard practices only will be required due to the replication defective nature of the particles.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or via the industrial (black bag) waste stream for landfill.

Liquids (eg bacterial and mammalian culture media, culture supernatants, viral samples) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or disinfect with 2% Virkon for at least 30 minutes, discharge to drains.

Consumables (mainly plasticware eg pipettes, flasks, tubes) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or disinfect with 2% Virkon for at least 30 minutes, discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for sharps.

Animal bedding – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes). Dispose of solids via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection with Virkon, used according to manufacturers instructions under standard conditions, manufacturers validation [eg4.79] log reduction ([eg99.998]% kill).

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment
Project Ref 553/09.8

Date Ackn'd 12/10/2009
Date Project Ceased

Manipulation of the eukaryotic cell ubiquitin system by pathogens. 1) Influence of Paramyxovirus infection on deubiquitinating activity in eukaryotic cells. 2) Influence of Yersinia infection on deubiquitinating activity in eukaryotic cells.

Class 2 CultureVolClass2 1-50 Litres
Class CultureVolumeClass3-4
Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn
Tick if notifying a connected programme of work

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Ubiquitin processing enzymes play an important role in host-pathogen interactions and some pathogen genomes encode for unique deubiquitinating enzymes (DUBs).

1. The Paramyxoviruses human respiratory syncytial virus and human parainfluenza virus as representatives of the Pneumoviridae and Paramyxovirinae subfamilies, respectively, will be analysed for their potential to induce enhanced deubiquitinating activity in infected cells. Small active site modifiers specific for deubiquitinating enzymes will be used to monitor and analyse DUB activity in infected cells

2. The Yersinia Enterocolitica and Pseudotuberculosis as representatives of Enterobacteria will be analysed for their potential to induce or block deubiquitinating activity in infected cells. Small activity-based modifiers specific for deubiquitinating enzymes will be used to monitor and analyse DUB activity in infected cells. Active proteins will then be identified using tandem mass spectrometry. The discovery of novel mechanisms of how Yersinia enteropathogens exploit cellular functions will shed light on the biological role of Yersinia infection and provide a window for potential antiviral pharmaceutical intervention.
### Recipient or parental organism

**For Part 1:**
- Disabled E. coli, K12 and B derivatives and BL21
- Mammalian cell lines: Vero, LLCMK2, HEp2, 293T
- Parainfluenza Virus (PIV) Type I, II, III;
- Respiratory Syncytial Virus (RSV)

**For Part 2:**
- Disabled E. coli, K12 and B derivatives and BL21
- Mammalian cell lines: U937, THP-1 293T
- Yersinia Enterocolitica
- Yersinia Pseudotuberculosis

### Host/vector system

Standard bacterial cloning of E.coli strains using non-mobilisable plasmid vector systems

T7 or PolI expression vectors carrying whole cDNA clones of the viral genomes, respectively, will be transfected into mammalian cell lines in addition to eukaryotic expression plasmids coding for proteins required for transcription of viral mRNAs and replication of the viral genome. Additionally, expression vectors carrying whole open reading frames of the deubiquitylating enzymes or other mammalian proteins under study will be transfected into mammalian cell lines.

### Origin & function

**Part 1:**
- Bacterial and viral constructs may either include additional genes coding for nonhazardous reporter genes or tags (i.e. green fluorescent protein) or contain single or multiple sequence deletions.

**Part 2:**
- Bacterial constructs will encode human genes encoding for the deubiquitylating enzyme OTUB1 and C91S, a catalytically inactive form. These constructs may either include additional genes coding for nonhazardous reporter genes or tags (i.e. green fluorescent protein) or contain single or multiple sequence deletions.

- Mammalian constructs will include eukaryotic expression vectors carrying whole open reading frames of the deubiquitylating enzymes or other mammalian proteins under study

### Evaluation of foreseeable effects

**Part 1:**
- E. coli K12 or B derivatives are disabled hosts which cannot colonise the human gut and have a history of safe use. Genes are not expected to be expressed.

- Mammalian cell cultures are especially disabled hosts which should be recognised as foreign by the human immune system. These will be used for production and amplification of recombinant Paramyxoviruses as below. This will increase the level of containment to the degree of the resulting virus.

- Human Paramyxoviruses (PIV-I-III, RSV) infect the upper and lower respiratory tract epithelia causing common cold symptoms like sore throat, coughing and running nose and are often accompanied by malaise and fever. Recombinant viruses will either have single or multiple gene deletions within their genome, or additionally code for a nonhazardous, characterized reporter gene like green fluorescent protein (GFP). An analogous recombinant virus was produced in the USA, University of Utah, using reverse genetics technology (Roth et al. 2009, Antiviral Research) and was shown to be less infective than the wild type strain in cell culture systems. Therefore, the
inserted genetic material should not increase the level of risk for the GMMOs

Part2:  
E. coli K12 or B derivatives are disabled hosts which cannot colonise the human gut and have a history of safe use. Genes are not expected to be expressed

Mammalian cell cultures are especially disabled hosts which should be recognised as foreign by the human immune system. These will be used for infection studies increasing the required containment appropriate for the infecting organism as below.

Yersinia pseudotuberculosis mutant strains YPIII (pIB44) ΔypkA (Km-R), YPIII (pIB47) YpkA D270A (Tc-R) and the contact A mutant strain of Yersinia pseudotuberculosis (IP2777) containing Y591A, N595A and E599A mutations will be used for infection assays. The mutant strains have a lower level of virulence and are generally less viable as compared to wildtype.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.</td>
</tr>
<tr>
<td>Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.</td>
</tr>
<tr>
<td>Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.</td>
</tr>
</tbody>
</table>

For waste items that are both radioactive and contain GMMs

<table>
<thead>
<tr>
<th>Consumables (mainly plasticware eg pipettes, flasks, tubes) - disinfect with 2% Virkon for at least 30 minutes, discharge any excess liquids to drains as aqueous liquid radioactive waste subject to limits contained in an authorisation granted under the Radioactive Substances Act 1993, dispose of solids as solid low level radioactive waste under an authorisation granted under the Radioactive Substances Act 1993.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquids (eg samples, culture supernatants, tissue culture media) – disinfect with 2% Virkon for at least 30 minutes, discharge to drains as aqueous liquid radioactive waste subject to limits contained in an authorisation granted under the Radioactive Substances Act 1993.</td>
</tr>
</tbody>
</table>

**Degree of kill**  
Autoclaving, effectively 100% kill (annual validation)  
Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection with Virkon, used according to manufacturers instructions under standard conditions, manufacturers validation [eg4.79] log reduction ([eg99.998]% kill).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
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<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2</td>
<td>L3 L4</td>
</tr>
</tbody>
</table>

**Project Ref** 553/10.1

- **Date Ackn’d**: 12/01/2010
- **CU2 Project Title**: Generation of HIV Virus-Like Particles (VLP)
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **CultureVolumeClass3-4**: Non-GMM Consent Granted

**Date Project Ceased**: 12/01/2010

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**: N

**Historical Date of Additional Info**: 12/01/2010
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to generate single-cycle envelope-pseudotyped virus expressing the envelope protein from clinical and laboratory-adapted strains of HIV in order to answer questions relating to the development of broadly neutralizing antibodies. The level of production as well as the potency will be assessed by various different techniques.

**Recipient or parental organism**

Producer cell lines, such as HEK 293 cells
- Mammalian cells including primary human cells
- E. coli K-12 and B derivatives

**Host/vector system**

pNL 4.3.luc.R-E- is a plasmid which encodes HIV backbone however it has a number of mutations which render the resulting viral particles replication defective. There is a frameshift mutation in the envelope gene and also the Vpr gene which knocks out production of both of these proteins. In addition the luciferase gene has been inserted in the place of the Nef gene. Nef is a protein that co-ordinates host cellular activities to enable viral replication and it has been demonstrated that viruses without functional Nef cannot replicate. Therefore three of the genes are missing from the backbone plasmid.

Different HIV envelope proteins are supplied on separate plasmids which are co-transfected into the producer cell lines to generate replication defective virus like particles

**Origin & function**

Luciferase is a standard reporter gene with a history of safe use. Other standard reporter genes such as GFP may be used.

The envelope genes are all from strains of HIV.

The rest of the alterations to the virus knock out genes necessary for viral replication.

**Evaluation of foreseeable effects**

The purpose of the modification is to produce HIV virus capable of mimicking the natural interactions under investigation but incapable of replicaton and therefore representing a significantly reduced hazard. The retrovirus (HIV-1) from which the backbone constructs were derived can cause disease in humans. However the design of these plasmids prevents production of infectious, replication-competent virions. Co-transfection with the env-carrying plasmid allows rescue of infectious virus for env in the viral RNA and as such are only capable of a single round of replication, as no infectious viral progeny will be produced.

As there are two mutations and the Nef gene missing, the virus would have to undergo multiple successful recombination event in order to revert to a replication competent virus. The two plasmids have minimal regions of homology to minimise the risk from recombination. The viruses are not repeatedly passaged and therefore any mutations will not be maintained within the population. The Nef gene is not present in either plasmid and therefore the possibility of the virus like particles obtaining Nef is remote.

Reporter genes such as (luciferase) are well characterised with no known associated health hazards.
The main risk of exposure would be due to a percutaneous injury however as the virus is replication defective no disease can form but individuals may mount an immune response and as such may test positive for HIV. All individuals are trained in the handling and disposal of sharps to reduce to risk of exposure.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes) or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels ) - dispose via clinical waste stream for incineration.

Degree of kill: Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control safety measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

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</table>

02/03/2022
Project Additional Information

Purposes of the contained use

Replication defective Lentiviruses have become a standard research tool for genetically modifying mammalian cells. They are especially useful where the cells to be modified are difficult to transfect using conventional methods. These cells include non-dividing cells, cells that are not active or growing, and difficult to transfect cells such as neurons, primary cells and stem cells.

Recipient or parental organism

Mammalian cells both primary and standard cell lines

Host/vector system

Standard commercially available replication defective lentiviral systems based on viruses such as MoMLV, HIV and EIAV. These systems have had non-essential genes removed from the vectors and the remaining genes split onto separate plasmids with minimal homology to each other.
For example, those viral vectors based on HIV are lacking tat, vif, vpu, vpr, nef and the envelope genes has been replaced with VSVG-env. The remaining genes gag, pol, rev, VSVG-env, gene of interest (with cis-acting signal required for reverse transcription and integration) are generally divide onto a number of plasmids depending on the system used.

Due to the fact that none of the plasmids carrying gag, pol, rev and env have the cis-acting signal they are unlikely to be present in the packaged virus and the risk of generating replication competent viruses minimal.

Some of the vectors will also include a self-inactivating feature which results in the inactivation of the LTR upon integration and reduces the risk of transactivation of host genes.

Some of the viruses will contain WPRE as it is frequently included in lentiviral systems. Current HSE guidance will be followed in relation to the hazard posed by WPRE.

**Origin & function**

The genes to be expressed are potential oncogenes such as human telomerase reverse transcriptase (hTERT) and human papilloma virus 16 E7 (HPV16-E7) or other genes that potentially pose an increase in hazard.

hTERT is a ribonucleoprotein polymerase that maintains the length of telomeres. Telomerase expression plays a role in cellular senescence and is normally suppressed in postnatal somatic cells.

HPVs have been found in 90% of cervical cancers as well as other carcinomas. Viral early proteins E6 and E7 induce immortalization and transformation in a variety of rodent and human cell types.

**Evaluation of foreseeable effects**

The viral vectors are replication defective and therefore cannot disseminate should exposure occur. However the nature of these vectors is to integrate into the host cell genome where there is a potential for disruption of the host genes. In addition, the genes to be expressed effect the cell cycling and may lead to immortalization of the cell.

To minimise the risk sharps will be excluded from this work and all work with the virus will be performed in a Class II micro-biological safety cabinet.

Once the virus is integrated into the mammalian cells they poses no greater risk than other immortalised cell lines as the virus cannot replicate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via NHS clinical waste stream.

Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or
134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via NHS clinical waste stream.

Sharps (e.g., needles, syringes, scalpels) - dispose via clinical waste stream. NO SHARPS TO BE USED WITH VIRUS PREPERATION.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2</td>
<td>L3 L4</td>
</tr>
</tbody>
</table>

Project Ref  553/11.1

Date Ackn'd  25/01/2011

Date Project Ceased  

CU2 Project Title  To develop a protective vaccine against Staphylococcus aureus infections

Consent Granted

Class Consent Granted

CultureVol L2 < 1 litre

Project notified under transitional arrangements [N]
This project aims to measure
1) The immune responses elicited by irradiated Staphylococcus aureus strain Newman, and an isogenic spa mutant, which has been previously described. Briefly, isogenic spa::Tcr mutants were constructed by the insertion of a tetracycline resistance cassette into spa using allelic replacement. The mutant exhibits reduced virulence in mouse models. Immune responses elicited by spa mutant organisms are of interest in view of the possibility that Spa is an immune subversion protein.
2) The virulence of S. aureus strain Newman and bioluminescent strain Xen8.1 will in murine challenge models. S. aureus spa::Tcr will be (i) studied as an radiation inactivated vaccine (ii) used in immunological assays of vaccine efficacy.

Recipient or parental organism
S. aureus is a facultatively anaerobic, Gram-positive coccus that is able to cause a variety of skin and soft tissue infections (boils, wound infections) as well as invasive disease (septic arthritis, osteomyelitis, septicemia, endocarditis). Additionally, some strains carry heat stable enterotoxins mediating food poisoning. Rates of invasive infection in healthy adults without hospital exposure are low, invasive infection occurs most frequently following exposure of wounds and burns to S. aureus. It also commonly results following injection of the organism, as occurs commonly with intravenous drug users.

Origin & function
SPA is an exoprotein that is considered to be a virulence factor, as it:

- Binds the Fc region of immunoglobulins of most mammalian species.
- Binds Fab fragments of immunoglobulins independent of the heavy-chain isotype via an alternative site, conferring superantigen-like function.
- activates complement.
- spa deletion reduces the virulence of S. aureus in mice.

The Lux operon (derived from Vibrio fischeri) encodes the bacterial luciferase (light emitting) system enabling various in vitro and in vivo assays to be undertaken. It is a widely used and safe standard reporter gene system.
SPA mutations:

Since spa is involved in pathogenicity and virulence, and its deletion reduces virulence in mice, the genetic modification is expected to reduce virulence of the organism in humans relative to unmodified Staphylococcus aureus strain Newman.

Lux operon

Expression of the lux operon in the form of bioluminescent luciferase will have no harmful effects nor will it increase virulence of the host pathogen. It will help to:

A) Develop in vitro assays for inhibition of Staphylococcal growth by sera from vaccinated animals.
B) Track systemic spread of S. aureus infection in unimmunised and immunised mice.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

 Liquids (eg samples, culture supernatants, tissue culture media) – adjust to a final volume of 10% v/v with Microsol (Anachem). Leave material standing for 30 minutes, and discard to drains.

 Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

 Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration with their use minimised where possible.

 Animal bedding – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes). Dispose of solids via the industrial (black bag) waste stream for landfill.

 Animal carcasses - dispose via clinical waste stream for incineration.

 Degree of kill
 Autoclaving, effectively 100% kill (annual validation)
 Incineration, effectively 100% kill (licensed incinerator)
 Chemical disinfection with Virkon, used according to manufacturers instructions under standard conditions, manufacturers validation [eg4.79] log reduction ([eg99.998]%
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM

### Project Containment

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**Animal Units**

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**Large Scale Activities**

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**Human Clinical Applications**

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### Project Ref 553/11.10

<table>
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<tr>
<td>01/11/2011</td>
<td>Characterization of replication kinetics of transmitter/founder HIV-1 strains</td>
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<td>&lt; 1 Litre</td>
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<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
<th>Project notified under transitional arrangements</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
Project Additional Information

Purposes of the contained use

In this project we will assess macrophage and T cell infection by chronic and transmitter/founder (T/F) viruses as well as cell-to-cell spread by chronic and T/F viruses and the role of the accessory genes Vpu and Nef on cell-to-cell spread. These viruses will be capable of establishing a spreading infection.

The aims of the project will be able to produce the HIV-1 strains expressing reporter genes Luciferase, GFP, RFP or mCherry, or the strains deleted in vpu or nef by transduction of E. coli with a plasmid containing the HIV-1 genes as well as one of the reporter genes, or a plasmid deleted in vpu or nef. Selection will be with either ampicillin or kanamycin, depending on the plasmid. The resulting plasmid will then be transfected into HEK-293T cells and the resulting virus-containing supernatant harvested and used in macrophage and T cell infections. The level of infection will be established by assessing the expression of the relevant reporter genes or the level of HIV-1 Gag p24 protein or HIV-1 DNA where no reporter genes are present. The HIV-1 genes will either consist of wild-type patient-derived sequences or lab-adapted, chronic strains and in relation to the T/F infections viruses consisting of a patient-derived env gene cloned into the backbone of the lab-adapted NL4-3 strain will also be used.

Recipient or parental organism

E. coli strains are disabled and cannot colonise the human gut.

Packaging cell line HEK293T - Minimum hazard for human cell lines obtained from commercial sources that are well characterised and authenticated.

Primary human cells that are not fully authenticated and characterised from commercial sources that are well characterised and authenticated.

Primary human cells that are not fully authenticated and characterised may carry contaminating infectious agents.

Host/vector system

Standard plasmid vector systems are non hazardous.

Replication-competent HIV-1 vector capable of establishing a spreading infection in cells expressing CD4 and CCR5 or CXCR4 (ACDP Class 3). Infection, through exposure of blood and body fluids is terminal although controllable with lifetime antiretroviral therapy.

Origin & function

Mammalian and reporter genes.

Insertion of the reporter genes luciferase, GFP, RFP and mCherry into the HIV-1 plasmid and deletion of vpu and nef from the HIV-1 plasmid will have no effect on virus production.

Expression of Luciferase, GFP, RFP and mCherry in infected cells will have no effect on HIV-1 virulence.

Lack of expression of Nef from HIV-1 results in decreased virulence.

Lack of expression of Vpu from HIV-1 results in decreased virulence.
E.coli transformed with HIV-1 plasmids. No HIV-1 proteins will be expressed at this stage and the E.coli strain is disabled therefore the risk is minimal. The risks will be minimized by performing all work under good microbiology practices with PPE including lab coat, gloves and goggles. The use of sharps is prohibited.

HEK-293T cells transfected with HIV-1 plasmids. These cells produce the HIV-1 virus for use in future experiments. There is a recognised hazard at this stage of the work which is the possibility of human infection. The risk will be minimised by following good microbiology practices, performing all work in a Class 2 safety cabinet with PPE including lab coat, disposable apron, double gloves and goggles. The use of sharps is prohibited.

Primary cells and cell lines infected with replication-competent genetically-modified HIV-1. These cells produce HIV-1 virus and therefore there is a recognised hazard at this stage of the work which is the possibility of human infection. This risk will be minimised by following good microbiology practices, performing all work in a containment level 2 safety cabinet with PPE including lab coat, disposable apron, double gloves and goggles. The use of sharps is prohibited.

Contains and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes ) - soaked in 5% Trigene before autoclaving using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least three minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) wate stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - treated with 1% Trigene and autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least three minutes) before discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least three minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Any equipment, surfaces that may come into contact with the virus will be treated with 5% Trigene followed by 70% ethanol which inactivates the virus.

Degree of kill

Autoclaving, effectively 100% kill (annual validation)

Chemical disinfection with Trigene, used according to manufacturers’ instructions under standard conditions (effectively 99.998% kill)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

**Project Containment**

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**Project Ref 553/11.11**

<table>
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<tr>
<td>11/11/2011</td>
<td>Use of listeria DPL-1942 (ova) to examine CD8 T cells responses</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Non-GMM

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Murine cytomegalovirus induces a high frequency of antigen specific T cells, many of which can be triggered by proinflammatory cytokines in the absence of cognate antigen. We theorise that these "innate-like" T cells may impact on the development of the other infections. Listeria monocytogenes DPL-1942 carries a genetically modified epitope from chicken ovalbumin which can be recognised by CD8 T cells. This is the standard bacterial infection model used to study CD8 T cell responses because it is well characterized and many experimental reagents are available to track the ovalbumin-specific T cells. Tracking T cells specific for unmodified Listeria would be technically difficult. The ovalbumin modification is necessary since natural Listeria epitopes have not yet been identified for H-2b mice. Homogenisation and culture of
organs from infected mice can inform if MCMV enhances protection. This work will involve the study the immune response by infecting C57Bl/6, IL18 ko and IL 18ra ko mice with Listeria DPL-1942. Outcomes will be measured using culture of L.monocytogenes and cellular immune responses.

Recipient or parental organism

Listeria monocytogenes (attenuated)

Host/vector system

The L. monocytogenes DPL-1942 (ova) was constructed and described by Pope, C, et al in 2001. “A rLM (rLM-ova) was constructed that expresses a secreted form of OVA and an erythromycin-resistance marker. An Ag expression cassette was constructed that consists of the entire coding sequence of OVA fused to the signal sequence and promoter of the hly gene and an erythromycin resistance gene for selection. The Ag expression cassette was introduced into the Listeria, and double-crossed into the Listeria chromosome by homologous recombination. Integration of the Ag cassette into the LM genome and secretion of the OVA fusion protein by rLM-ova were confirmed by PCR and Western blot analyses, respectively.” Pope, C, et al. 2001. "Organ-specific regulation of the CD8 T cell response to Listeria monocytogenes infection". J. Immunol. 166, 3402-9.

Origin & function

The OVA ovalbumin sequence is derived from chicken and functions as a specific marker antigen. It is not expected to have any biological activity.

Evaluation of foreseeable effects

LM DPL 1942 is act A deficientThe attenuated actA mutant of Listeria monocytogenes can enter and multiplu in the cytosol of the infected host cell but is deficient in actin-dependent cell-to-cell spread. It has been found to be of attenuated virulence for inbred C3H mice: the LD50 after I.v injection is 1000-fold higher than that of the wild-type strain. Thus the GMO is significantly less pathogenic than wild type Listeria. There is no risk posed by transfer of OVA to this strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Innoculated mice will be held at the Animal Containment Level 2.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121- 1250c for at least 15 mins or 126-130oc for at least 10 mins or 134-138oc for at least 3mins) discharge any excess liquids to drains, dispose of solids via clinical waste stream for incinerator/ dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, part3, 1993 (either 121-1250c for at least 15 mins or 126-130oc for at least 10 mins or 134-138oc for at least 3mins), or inactivate by chemical means (following manufactures guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 646, part 3, 1993 (either 121-125oc for 15 mins or 126-130oc for at least 10 mins or 134-138oc for at least 3mins), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/ dispose of solids vis the industrial(black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose vis clinical waste stream for incineration.

Animal bedding - Either autoclave using a make safe cycle as specified in BS 2646, part 3, 1993 (either 121-125oc for at least 15 mins or 126-130oc for at least 10 mins, or 134-138oc for at least 3mins), dispose via the industrial (black bag) waste stream for landfill OR dispose vis clinical waste stream for incineration.

Degree of kill:

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<td>L3 L4 L2 L3 L4</td>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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**Project Ref** 553/11.2

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<th>Class CultureVolClass2 CultureVolumeClass3-4</th>
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<tr>
<td></td>
<td>Class 2 &lt; 1 Litre Non-GMM Consent Granted</td>
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</table>

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Purposes of the contained use

Replication defective retroviral and lentiviral gene transfer vectors will be used to introduce transgenes into human and murine primary cells and cell lines.

### Recipient or parental organism

- **E. coli K12 or B derivatives** - E. coli strains are disabled and cannot colonise the human gut.
- **Mammalian cell lines including primary cells** - Minimal hazard for mammalian cell lines obtained from commercial sources that are well characterised and authenticated.
- **Packaging cell lines** - produce infectious replication defective viral particles after they have been transfected with multiple viral plasmids.
- **Adult mice** - No hazard to human health.

### Host/vector system

Replication defective ecotropic and amphotropic retroviruses and lentiviruses

Third or later generation retro- and lentiviral systems (based on viruses from various species) incorporating self-inactivating 3' LTR (SIN) U3 deletion which is transferred to the 5' LTR after reverse transcription and integration in infected cells. This results in the transcriptional inactivation of the LTR in the provirus and reduces the risk of transactivating genes around the site of insertion.

The viral vectors are derived from numerous wild type viruses, such as MoMLV, HIV and EIAV. However non-essential genes have been removed from the vectors and the remaining genes split onto separate plasmids with minimal homology to reduce the risk of homologous recombination. For example lentiviral vectors derived from HIV are devoid of tat, vif, vpu, vpr nef and the envelope gene has been replaced by VSV-G envelope. Gag, pol and rev are generally on one plasmid, env on a second and the gene of interest is on a third plasmid along with cis-acting signals required for reverse transcription and integration. As the gag, pol, rev and env plasmids do not contain the packaging sequence none of the genes are present in the package viral genome. The likelihood of these viral particles obtaining the missing genes is very low and therefore the risk of replication competent viruses, being created is minimal.

**Baculvirus system** - baculviruses have a restricted host range and are not generally known to infect vertebrate cells and replication incompetent in vertebrate cells

### Origin & function

Mammalian and reporter genes.

- Human and mouse genomic DNA and cDNA inserts coding for transcription factors such as MAZ, GATA family members, TFII-I or MLL-fusion proteins, HOXAS, Meis1.
- MLL-fusion proteins and HOX9/Meis1 are known oncogenes.
- MAZ, GATA family members and TFII-I have no transforming or oncogenic properties.
- ETV6-AML1 and bcr-abl - suspected oncogenes.
- MAZ, GATA family members and TFII-I have no transforming or oncogenic properties.
Short interfering RNA (siRNA) will be expressed to knockdown expression of some or all of the proteins mentioned above. An alteration of the proliferation/differentiation balance or induction of apoptosis of the transduced cells and/or their progeny is expected.

Standard selection markers (GFP, antibiotic resistance, lacZ) will allow identification of the transduced cells, but is unlikely to have any significant biological effect.

**Evaluation of foreseeable effects**

The lentiviruses and retroviruses are amphotropic and can therefore infect human cells however the design of these systems is such that the viral particles are replication defective and are only capable of one round of infection.

The genes to be expressed or knocked down have the potential to affect proliferation, apoptosis and survival of cells and could therefore in some instances have detrimental effects should they be integrated into genomic DNA of a workers cells. Once incorporated into the genomic DNA of the experimental cell lines the inserts pose minimal risk due to the replication defective nature of the viruses. The cells would be recognised as non-self by the immune system and rapidly lysed by complement.

The lentiviral particles produced will have a broad tropism and be capable of infecting and transducing human and other mammalian cells, inserting viral and mammalian and/or reporter gene sequences (see above) into the host genome. This is an anticipated risk associated with the use of lentiviral gene transfer vectors. The viral particles have however been rendered non-replicative by a number of safety features.

Accidental human exposure to viral packaging cells carries minimal risk as the inserted sequences are not expected to affect the pathogenicity of the cells. It is anticipated that any cells would be rapidly cleared by the complement/immune system of any exposed individual.

Accidental human exposure to viral particles could lead to viral infection and the insertion of viral and mammalian and/or reporter gene sequences into the host genome. Importantly, in the context of human gene therapy clinical studies, high doses of retroviral and lentiviral vector particles have been administered in vivo with no observed complications. It is considered highly unlikely that accidental human exposure to the viral particles described in this risk assessment would lead to the generation of a tumour.

**Retroviral and Lentiviral Transduction**

Retroviral and lentiviral particles will be purified from packaging cell cultures and used to transduce mammalian cells to facilitate the production of new mammalian cell lines permanently expressing the desired transgene and/or shRNA inducing molecule. Gene transfer from viral particle to mammalian cell line is expected.

In some cases new transgenic lines will be created using viral particles. Due to the replication defective nature of the particles once integrated into the host genome the virus poses minimal risk to humans.

Where viral particles are used in vivo the greatest risk is from the use of needles however it is not anticipated that accidental exposure would be linked to significant risk.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified I BS2646, Part 3. 1993 (either 121-125°C for at least 14 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) wastestream for landfill.
Liquids (e.g., samples, culture supernatants, tissue culture media) - autoclave using a makesafe cycle as specified in BS2646, Part 3 1993 (either 121-125°C for at least 14 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (e.g., needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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</thead>
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Animal Units
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- L3
- L4

Large Scale Activities
- L2
- L3
- L4

Human Clinical Applications
- L2
- L3
- L4

Project Ref 553/11.3

Date Ackn’d CU2 Project Title Class Culture Vol
03/03/2011 Infection of Human And Murine Cells with Recombinant Replication Defective Class 2 < 1 Litre
Purposes of the contained use

Replication defective lentiviral gene transfer vectors will be used to introduce transgenes and short hair pin RNA into different mammalian cells.

Recipient or parental organism

E. coli K12 or B derivatives - E.coli strains are disabled and cannot colonise the human gut.
Mammalian cell lines - Minimal hazard for mammalian cell lines obtained from commercial sources that are well characterised and authenticated.
Primary cell cultures - Primary human and mice cells and human and mice cell lines that are not fully authenticated and characterised may carry contaminating infectious agents. Primary cells from non primate sources are deemed to be of low hazard.
Packaging cell lines - produce infectious replication defective viral particles after they have been transfected with multiple viral plasmids.
Adult mice - No hazard to human health

Host/vector system

Replication defective lentivirus
The viral vectors are derived from numerous wild type viruses, such as MoMLV, HIVand EIAV. However nonessential genes have been removed from the vectors and the remaining genes split onto separate plasmids with minimal homology to reduce the risk of homologous recombination. For example lentiviral vectors derived from HIV are devoid of tat, vif, vpu, vpr nef and the envelope gene has been replaced by VSV-G envelope. Gag, pol and rev are generally on one plasmid, env on a second and the gene of interest is on a third plasmid along with cis-acting signals required for reverse transcription and integration. As the gag, pol, rev and env plasmids do not contain the packaging sequence none of the genes are present in the package viral genome. The likelihood of these viral particles obtaining the missing genes is very low and therefore the risk of replication competent viruses being created is minimal. In addition, some of the later generation viral vectors also include a self inactivating feature, a deletion within the 3' L TR, which is transferred to the 5' LTR after reverse transcription and integration in infected cells. This results in the
Transcriptional inactivation of the LTR in the provirus and reduces the risk of transactivating genes around the site of insertion.

**Origin & function**

**Mammalian and reporter genes**

Human and mouse genomic DNA and eDNA inserts encoding JmjC-domain-containing protein.

JmjC is a histone demethylase that specifically demethylates Lys-27 of histone H3 and plays a central role in regulation of posterior development, by controlling HOX gene expression. The expression of JmjC is induced upon activation of the RAS-RAF signalling pathway. JmjC recruits to the INK4A-ARF locus and contributes to the transcriptional activation of p16INK4A in human diploid fibroblasts. Inhibition of JmjC expression in mouse embryonic fibroblasts results in suppression of p16INK4A and p19Arf expression and in cell immortalization.

JmjC2d is also a histone demethylase that specifically demethylates Lys-9 of histone H3. The majority of histone lysine methyltransferases contain a SET domain, which catalyzes the addition of methyl groups to the specific lysine residues. No oncogenic or tumourigenic properties have been associated with JmjC2d.

Jmjd2d is also a histone demethylase that specifically demethylates Lys-9 of histone H3. The majority of histone lysine methyltransferases contain a SET domain, which catalyzes the addition of methyl groups to the specific lysine residues. No oncogenic or tumourigenic properties have been associated with Jmjd2d.

MLL2 is a histone methyltransferase that methylates Lys-4 of histone H3. H3 lys-4 methylation represents a specific tag for epigenetic transcriptional activation. These genes are expressed on all cell types. Elevated levels of MLL2 in the breast and colon cells may be associated with malignancy.

**Tyrosine kinase 2 (TYK2) gene and variants of.** TYK2 is a member of the Janus-activated kinase family and plays a key role in the signal transduction pathway of many cytokines, including type I interferons, IL-6, IL-10 and IL-22.

TYK2-Pro1 104Ala that is protective for multiple sclerosis (MS). The germline allele encoding TYK2-Ala104 has been potentially linked to cancer. Given that the allele encoding TYK2-Ala1 104 is expressed in healthy individuals it seems unlikely that this variant has oncogenic properties per se but instead requires additional genetic variants or environmental factors to contribute to carcinogenesis.

**Regulator of G protein signalling 1 (RGS1) gene -** RGS1 may be involved in regulating B cell activation and proliferation, although it is expressed in various cell types and tissues. RGS1 over-expression has been correlated with melanoma progression and is a prognostic marker for primary cutaneous melanoma. RGS1 over-expression has not been directly associated with oncogenesis.

**Chemokine (C-X-C motif) receptor 4 (CXCR4) gene -** The CXCR4 protein has 7 transmembrane regions and binds stromal cell-derived factor-1 (SDF-1/CXCL12). The interaction with its ligand leads to an increase in intracellular calcium. CXCR4 plays a role in haematopoiesis, vascularisation and possibly cerebellar development and hippocampal-neuron survival. CXCR4 over-expression has been associated with the ability of the receptor to mediate metastasis of various cancers including prostate cancer, and it has been found 10 be a prognostic marker for leukaemia and breast cancer.

**Interleukin-12A (IL12A) gene -** IL-12A is a subunit of a cytokine that acts on T cells and NK cells and plays an important role in Th1 and Th2 cell differentiation. Polymorphisms in the non-Coding region of IL12A have been associated with the risk of cervical cancer and hepatocellular carcinoma. IL-12A overexpression or knockdown using the lentiviral system is unlikely to be oncogenic.

**Prostaglandin E receptor 4 (PTGER4) gene -** PTGER4 is a member of the G-protein coupled receptor family and binds prostaglandin E2 (PGE2). PTGER4 has been implicated in T cell factor signalling, skin immune responses and development of the circulatory system. The knockdown of PTGER4 accelerates B cell/lymphoma spreading in mice and a role for PTGER4 in inflammatory breast cancer cell invasiveness has been described.

**Interleukin-7 receptor alpha-chain (IL7RA) gene -** IL-7RA plays an important role during VDJ recombination during the development of lymphocytes and is important in T-cell differentiation and activation. The precise function of sIL-7RA remains to be elucidated. Genetic variation potentially leading to changes in IL-7RA expression has been linked with non-small cell lung carcinoma, however overexpression or knockdown of IL-7RA using the lentiviral system is unlikely.
to be oncogenic.
Cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1) gene - CYP27B1 is a member of the cytochrome
P450 superfamily that localizes to the inner mitochondrial membrane where it catalyzes the hydroxylation of 25-
hydroxyvitamin D3 to give rise to the active form of vitamin D3 that in turn regulates calcium homeostasis. Changes in
CYP27B1 expression has been linked with prognosis of prostate cancer.
M-phase phosphoprotein 9 (MPHOSPH9) gene - MPHOSPH9 over-expression has been observed in neuroblastoma
cell lines.
Tumour necrosis factor, alpha-induced protein 3 (TNFAIP3) gene - TNFAIP3 expression is upregulated by TNF and it
functions to inhibit NF-KB activation and TNF-mediated apoptosis. TNFAIP3 is a tumour suppressor gene and its
inactivation has been observed in several B-celllymphomas and in chronic lymphocytic leukaemia, leading to
constitutive NF-KB activation that is thought to be pathogenic.
Interferon regulatory factor 8 (IRF8) gene - IRF8 expression is a transcription factor involved in regulating responses
to type I interferons by binding to the interferon-stimulated response element. While the expression or knockdown of
IRF8 variants using the lentiviral system is unlikely to be oncogenic, changes in IRF8 expression have been observed
in human multiple myeloma.
Signal transducer and activator of transcription (STAT3) gene - STAT3 is a transcription factor that becomes activated
and translocates to the cell nucleus once it is phosphorylated by receptor associated kinases that themselves become
activated upon the binding of cytokines to their transmembrane receptors. STAT3 has been described to be involved
in the development and progression of head and neck cancers.
Ectopic viral integration site 5 (EVI5) gene - EVI5 is a regulator of cell cycle progression and plays a role during
interphase and cytokinesis. Expression of EVI5 variants using the lentiviral system is unlikely to be oncogenic. a
 genetic region including EVI5 has been associated with melanoma.
VAV1 gene - VAV1 is a guanine nucleotide exchange factor for the Rho family of GTP binding proteins and is involved
in haematopoiesis and T- and 8-cell development and activation. Expression of VAV1 variants using the lentiviral
system is unlikely to be oncogenic, studies in mice suggest that VAV1 may be implicated in lumphomagenesis and
metastasis.
Suppressor of cytokine signalling 1 (SOCS1) gene - SOCS1 is a negative regulator of cytokines that signal through
the JAKISTAT3 pathway. for example. The expression of SOCS1 variants using the lentiviral system is unlikely to be
oncogenic. SOCS1 expression has been suggested as a biomarker of aggressive laryngeal carcinoma.
Standard selection markers (GFP, antibiotic resistance, lacZ) will allow identification of the transduced celis, but is
unlikely to have any significant biological effect.

**Evaluation of foreseeable effects**

The inserted mammalian and reporter gene sequences are normal or selective alterations of characterised
mammalian genes or shRNAs and/or standard reporter genes. Most of the genetic inserts are not likely to cause
significant effect on an individual. However, since some of the genes to be expressed in these vectors are potential
oncogenes, they may cause host cells to proliferate.

The lentiviral particles produced will have a broad tropism and be capable of infecting and transducing human and
other mammalian cells, inserting viral and mammalian and/or reporter gene sequences (see above) into the host
genome. This is an anticipated risk associated with the use lentiviral gene transfer vectors. The viral particles have
however been rendered non-replicative by a number of safety features.

Accidental human exposure to viral packaging cells carries minimal risk as the inserted sequences are not expected
to affect the pathogenicity of the cells. It is anticipated that any cells would be rapidly cleared by the
complement/immune system of any exposed individual.

Accidental human exposure to viral particles could lead to viral infection and the insertion of viral and mammalian
and/or reporter gene sequences into the host genome. Importantly, in the context of human gene therapy clinical
studies, high doses of retroviral and lentiviral vector particles have been administered in vivo with no observed complications. It is considered highly unlikely that accidental human exposure to the viral particles described in this risk assessment would lead to the generation of a tumour.

Retroviral and Lentiviral Transduction

Retroviral and lentiviral particles will be purified from packaging cell cultures and used to transduce mammalian cells to facilitate the production of new mammalian cell lines permanently expressing the desired transgene and/or shRNA inducing molecule. Gene transfer from viral particle to mammalian cell line is expected in some cases new transgenic lines will be created using viral particles. Due to the replication defective nature of the particles once integrated into the host genome the virus poses minimal risk to humans. Where viral particles are used in vivo the greatest risk is from the use of needles however it is not anticipated that accidental exposure would be linked to significant risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Transgenic mice will be used and handled at Animal Containment Level 1.

Where no viral particles are present, standard practices will be employed. No further controls are required for safe handling.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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<thead>
<tr>
<th>Category</th>
<th>Treatment Measures</th>
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<td>Consumables (mainly plasticware eg pipettes, flasks, tubes)</td>
<td>Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes). Discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.</td>
</tr>
<tr>
<td>Liquids (eg samples, culture supernatants, tissue culture media)</td>
<td>Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes). Discharge to drains or chemically disinfect with Virkon.</td>
</tr>
<tr>
<td>Agar plates</td>
<td>Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (121-125°C for at least 15 minutes; 126-130°C for at least 10 minutes; or 134-130°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.</td>
</tr>
<tr>
<td>Sharps (eg needles, syringes, scalpels)</td>
<td>Dispose via clinical waste stream for incineration.</td>
</tr>
<tr>
<td>Animal bedding</td>
<td>Either Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-130°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.</td>
</tr>
</tbody>
</table>

Degree of kill

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical disinfection with Virkon. used according to manufacturers’ instructions under standard conditions (effectively 99.998% kill).
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

**Project Containment**

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**Project Ref** 553/11.4

**Date Ackn'd** 08/03/2011

**CU2 Project Title** Infection of human cancer cell lines with recombinant replication-defective lentiviruses expressing reporter genes and experimental genes of interest

**Class** Class 2

**Culture Volume** < 1 Litre

**Class Culture Volume** Class 2 < 1 Litre

Non-GMM Consent Granted

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Purposes of the contained use

Replication defective lentiviral gene transfer vectors will be used to introduce transgenes and RNA into cancer cell lines.

### Recipient or parental organism

- **E. coli K12 or B derivatives** - E. coli strains are disabled and cannot colonise the human gut.
- **Mammalian cell lines** - Minimal hazard for mammalian cell lines obtained from commercial sources that are well characterised and authenticated.
- **Primary cell cultures** - Primary human and human cell lines obtained from commercial sources that are well characterised and authenticated.
- **Packaging cell lines** - produce infectious replication defective viral particles after they have been transfected with multiple viral plasmids.
- **Adult mice** - No hazard to human health.

Replication defective virus from numerous wild type viruses, such as MoMLV, HIV and EIAV. However non-essential genes have been removed from the vectors and the remaining genes split onto separate plasmids with minimal homology to reduce the risk of homologous recombination. For example lentiviral vectors derived from HIV are devoid of tat, vif, vpu, vpr, nef and the envelope gene has been replaced by VSV-G envelope. Gag, pol and rev are generally on one plasmid, env on a second and the gene of interest is on a third plasmid along with cis-acting signals required for reverse transcription and integration. As the gag, pol, rev and env plasmids do not contain the packaging sequence none of the genes are present in the package viral genome. The likelihood of these viral particles obtaining the missing genes is very low and therefore the risk of replication competent viruses being created is minimal.

In addition, some of the later generation viral vectors also include a self inactivating feature, a deletion within the 3' LTR, which is transferred to the 5' LTR after reverse transcription and integration in infected cells. This results in the transcriptional inactivation of the LTR in the provirus and reduces the risk of transactivating genes around the site of insertion.

### Host/vector system

Replication defective lentivirus

The viral vectors are derived from numerous wild type viruses, such as MoMLV, HIV and EIAV. However non-essential genes have been removed from the vectors and the remaining genes split onto separate plasmids with minimal homology to reduce the risk of homologous recombination. For example lentiviral vectors derived from HIV are devoid of tat, vif, vpu, vpr, nef and the envelope gene has been replaced by VSV-G envelope. Gag, pol and rev are generally on one plasmid, env on a second and the gene of interest is on a third plasmid along with cis-acting signals required for reverse transcription and integration. As the gag, pol, rev and env plasmids do not contain the packaging sequence none of the genes are present in the package viral genome. The likelihood of these viral particles obtaining the missing genes is very low and therefore the risk of replication competent viruses being created is minimal.

In addition, some of the later generation viral vectors also include a self inactivating feature, a deletion within the 3' LTR, which is transferred to the 5' LTR after reverse transcription and integration in infected cells. This results in the transcriptional inactivation of the LTR in the provirus and reduces the risk of transactivating genes around the site of insertion.

### Origin & function

A range of genes including those known or thought to control growth and differentiation (e.g. CDX1, KRT20, BMI1, ALDH1 etc) will be transfected into human cancer cell
lines and cells isolated from fresh tumour material. Similarly, genes which are known or thought to be involved in the development and progression of cancer will also be studied.

**CDX1**
This gene is a member of the caudal-related homeobox transcription factor gene family. The encoded DNA-binding protein regulates intestine-specific gene expression and enterocyte differentiation. It has been shown to induce expression of the intestinal alkaline phosphatase gene, and inhibit beta-catenin/T-cell factor transcriptional activity.

**KRT20**
The protein encoded by this gene is a member of the keratin family. The keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells. This cytokeratin is a major cellular protein of mature enterocytes and goblet cells and is specifically expressed in the gastric and intestinal mucosa. It is perhaps the most commonly used diagnostic marker of colorectal epithelial cell differentiation.

**BMI1**
Component of the Polycomb group (PcG) multiprotein PRC1 complex, a complex required to maintain the transcriptionally repressive state of many genes throughout development. PcG PRC1 complex acts via chromatin remodelling and modification of histones; it mediates monoubiquitination of histone H2A 'Lys-119'. Rendering chromatin heritably changed in its expressibility.

**ALDH1**
Aldehyde dehydrogenase (ALDH) is an enzyme involved in intracellular retinoic acid production. ALDH1A is an important member of the ALDH family that includes 17 genes encoding different substrate specificities, of which ALDH1A1 catalyses the oxidation of retinal to retinoic acid. Retinoic acid signalling is linked to cellular differentiation during development and has important function in stem cell self-protection throughout an organism's lifespan. This gene has been used as a marker of cancer stem cells in breast, prostate and colorectal cancers.

Short-interfering RNA (siRNA) may be expressed to knock-down expression of some or all of the genes studied. We expect an alteration of the proliferation/differentiation balance or induction of apoptosis of the transduced cells and/or their progeny.

**Evaluation of foreseeable effects**

Modified forms (including truncations and point mutations) of some or all of the proteins mentioned above will be expressed. They are expected to display a subset of the activities of their wild-type counterparts. By definition, some of these genes will be considered as oncogenic and could be expected to have effects on proliferation, differentiation and survival of host cells.

The lentiviral particles produce will have a broad tropism and be capable of infecting and transducing human and other mammalian cells, inserting viral and mammalian and/or reporter gene sequences (see above) into the host genome. This is an anticipated risk associated with the use of lentiviral gene transfer vectors. The viral particles have however been rendered non-replicative by a number of safety features.

Accidental human exposure to viral packaging cells carries minimal risk as the inserted sequences are not expected to affect the pathogenicity of the cells. It is anticipated that any cells would be rapidly cleared by the complement/immune system of any exposed individual.

Accidental human exposure to viral particles could lead to viral infection and the insertion of viral and mammalian and/or reporter gene sequences (see above) into the host genome. This is an anticipated risk associated with the use of lentiviral gene transfer vectors. The viral particles have however been rendered non-replicative by a number of safety features.

Accidental human exposure to viral packaging cells carries minimal risk as the inserted sequences are not expected to affect the pathogenicity of the cells. It is anticipated that any cells would be rapidly cleared by the complement/immune system of any exposed individual.
Accidental human exposure to viral particles could lead to viral infection and the insertion of viral and mammalian and/or reporter gene sequences into the host genome. Importantly, in the context of human gene therapy clinical studies, high doses of retroviral and lentiviral vector particles have been administered in vivo with no observed complications. It is considered highly unlikely that accidental human exposure to the viral particles described in this risk assessment would lead to the generation of a tumour.

Retroviral and Lentiviral Transduction.
Retroviral and lentiviral particles will be purified from packaging cell cultures and used to transduce mammalian cells to facilitate the production of new mammalian cell lines permanently expressing the desired transgene and/or shRNA inducing molecule. Gene transfer from viral particle to mammalian cell line is expected.

In some cases new transgenic lines will be created using viral particles. Due to the replication defective nature of the particles once integrated into the host genome the virus poses minimal risk to humans.

Where viral particles are used in vivo the greatest risk is from the use of needles however it is not anticipated that accidental exposure would be linked to significant risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclaves using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains or chemically disinfect with Virkon.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose vial clinical waste stream for incineration.

Animal carcases - dispose via clinical waste stream for incineration.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% (licensed incinerator)
Chemical disinfection with Virkon, used according to manufacturers' instructions under standard conditions (effectively 99.998% kill)
Is an emergency plan required according to regulation 20?  
N
If yes, tick to confirm that it is attached to this form  
N
Tick to confirm that you have attached a risk assessment to this form  
Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  
N
Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
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- **Animal Units**
  - L2
  - L3
  - L4

- **Large Scale Activities**
  - L2
  - L3
  - L4

- **Human Clinical Applications**
  - L2
  - L3
  - L4

### Project Ref 553/11.5

**CU2 Project Title**

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<tr>
<td>16/06/2011</td>
<td>Generation and characterisation of induced pluripotent stem (iPS) cells and other stem cell types by transfection with reprogramming, chromatin, and epigenetic factors using viral vectors</td>
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**Class**

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<tr>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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**Non-GMM Consent Granted**

- Consent Granted

**Project notified under transitional arrangements**

- N

**Tick if notifying a connected programme of work**

- N
### Purposes of the contained use

Replication defective lentiviral gene transfer vectors will be used to introduce transgenes and RNA into human cells in order to generate iPS cells. Cellular signalling pathways, epigenetics, and senescence will be investigated in the produced iPS cells.

### Recipient or parental organism

- **E. coli K12 or B derivatives** - E. coli strains are disabled and cannot colonise the human gut.
- **Mammalian cell lines** - Minimal hazard for mammalian cell lines obtained from commercial sources that are well characterised and authenticated.
- **Primary cell cultures** - Primary human and mice cells and human and mice cell lines that are not fully authenticated and characterised may carry contaminating infectious agents. Primary cells from non primate sources are deemed to be of low hazard.
- **Packaging cell lines** - produce infectious replication defective viral particles after they have been transfected with multiple viral plasmids.

### Host/vector system

#### Replication defective lentivirus

Genes encoding the structural and other components required for packaging the viral genome are separated onto two plasmids. The packaging genes for lentiviral vectors are provided by co-transfection of packaging vectors and viral vector into a packaging cell line such as HEK293. By using this enhanced 3-plasmid system, virus particles can be produced that can infect human and mouse cells, but are replication deficient therefore cannot be transmitted from one cell to another. Although the two packaging plasmids allow expression of proteins required to produce viral progeny (e.g. gag, pol, env) in the HEK293 producer cell line, none of them contain LTRs or the psi packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. Therefore, no new replication-competent virus can be produced. The HIV-1 env gene is replaced with a Vesicular Stomatitis Virus G (VSV-G) envelope.

Because the number of HIV-1 genes are reduced to minimum (gag and pol) and that none of the 2 packaging plasmids contain LTRs or psi sequences, the number of recombination events required to generate a wild-type virus capable of infecting & replicating ensures that no replication competent virus can be produced.

Different transcription genes are supplied in separate plasmids which are co-transfected into the producer cell lines to express protein.

### Origin & function

**Mammalian and reporter gene sequences:**

- **hTER** - The ectopic expression of the telomerase catalytic subunit (human telomerase reverse transcriptase of hTERT) and subsequent activation of telomerase can allow cells to proliferate so that they can stay in a stem cell like state and divide repeatedly.

- **SV40LT** - encodes for a protein (Tag) that is a proto-oncogene derived from the polyomavirus SV40 which is capable of transforming a variety of cell types. The transforming activity of Tag is due in part to its perturbation of the retinoblastoma (pRB) and p53 tumour suppressor proteins, an activity that also helps to maintain a pluripotent state of cells and prevent cell apoptosis.

- **P-MIG** - The pMIG vector includes a sequence for an internal ribosome entry site, abbreviated IRES. This is a nucleotide sequence that allows for translation initiation in the middle of a messenger RNA (mRNA) sequence as part of the greater process of protein synthesis. IRES are often used by viruses as a means of shutting down translation.
hOCT4 - OCT4 is a homeodomain transcription factor of the POU family. It is expressed in mammalian early embryo and germ cells and can both stimulate and repress target gene transcription. OCT4 is critically involved in the self-renewal of undifferentiated embryonic stem cells and regulates the expression of multiple genes via interaction with other transcription factors present in pluripotent cells, e.g. SOX2.

hSOX2 - SOX2, also known as SRY (sex determining region Y)-box 2, maintains or preserves developmental potential.

hKLF4 - The Kruppel-like family of transcription factors (Klfs) has been extensively studied for their roles in cell proliferation, differentiation and survival. KLF4 promotes self-renewal in ES cells as well as inhibiting cell differentiation.

Standard selection markers (GFP, antibiotic resistance, lacZ) will allow identification of the transduced cells, but is unlikely to have any significant biological effect.

Evaluation of foreseeable effects

The inserted mammalian and reporter gene sequences are normal or selective alterations of characterised mammalian genes or miRNAs and/or standard reporter genes. Most of the genetic inserts are not likely to cause significant effect on an individual. However, some inserted sequences may have harmful properties in humans via the ability to act as a tumour suppressor and/or oncogenes eg SV 40 LT - encodes for a protein (Tag) that is proto-oncogene derived from the polyomavirus SV40 which is capable of transforming a variety of cell types. The transforming actiity of TAg is due in large part to its perturbation of the retinoblastoma (pRB) and p53 tumour suppressor proteins, an activity that also helps to maintain a pluripotent state of cells and prevent cell apoptosis.

The lentiviral particle produced will have a broad tropism and be capable of infecting and transducing human and other mammalian cells, inserting viral and mammalian and/or reporter gene sequences (see below) into the host genome. This is an anticipated risk associated with the use of lentiviral gene transfer vectors. The viral particles have however been rendered non-replicative by a number of safety features.

Accidental human exposure to viral packaging cells carries minimal risk as the inserted sequences into the host genome. It is considered highly unlikely that accidental human exposure to the viral particles described in the risk assessment would lead to the generation of a tumour.

Retroviral and Lentiviral Transduction

Retroviral and lentiviral particles will be purified from packaging cell cultures and used to transduce mammalian cells to facilitate the production of new mammalian cell lines permanently expressing the desired transgene and/or RNAi inducing molecule. Gene transfer from viral particle to mammalian cell lines is expected.

In some cases new transgenic lines will be created using viral particles. Due to the replication defective nature of the particles once integrated into the host genome the virus poses minimal risk to humans

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.
Liquids (eg samples, cultures supernatant, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines).

Project Containment

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<tr>
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<td>Animal Units</td>
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Project Ref 553/11.6

Date Ackn'd | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4 |
-------------|------------------|-------|-----------------|-----------------------|
20/06/2011   | Investigation of copine protein function and trafficking in neuronal cells using delivery | Class 2 | < 1 Litre |
Purposes of the contained use

Sindbis virus constructs will be used to express mutant versions of copine proteins in different cell lines and primary cultures of neurones, and study the consequences of the mutations on the cells.

Recipient or parental organism

E.coli strains that are disabled and cannot colonise human gut.

Mammalian cell lines (BHK, HEK 293, COS-7). Minimal hazard for mammalian cell lines obtained from commercial sources that are well characterised.

Primary cell culture - Primary rat cell lines can only survive in cell culture facility conditions.

Host/vector system

The Sindbis virus expression system was commercially available from Invitrogen and has been used worldwide to produce viruses for use in dissociated neuronal cultures and slices.

The system consists of two plasmids, pSinRep5 (used to express the gene of interest) and pDH(26S), a helper plasmid (produces structural proteins (capsid, p62, 6K and E1) required for viral encapsidation and release from the produced cell line). The E1 and E2 glycoprotein sequences from pDH(26S) that are required for viral infection of host cells, are derived from a neurovirulent strain of Sindbis virus cDNA (TE12), thus allowing for efficient infection of neurons in culture. RNA transcripts of these plasmids (capped and poly-adenylated) are generated in vitro and transfected into BHK cells, where they are translated as mRNA to produce encapsulated viral particles. Particles produced by these transfections have little or no plaque forming unit (pfu) capability associated with them.

In addition, we also plan to use the pSinRep5nsP2ser plasmid (with pDH(26S) as a helper), which has reduced cytotoxic effects of sindbis viral expression on cultured cells. In the Sindbis Expression System, the structural genes in the viral genome have been replaced by a cassette into which the gene of interest is cloned. The structural genes are provided on the helper plasmid and are not incorporated into the final Sindbis viral particles. Rather, the gene of interest is packaged, along with the replicase sequences. This is designed to render those particles non-infectious following a single round of infection. The risk of replication competent virus being created is minimal.

Origin & function
Mammalian and reporter genes.
The genetic material to be introduced into the virus will be derived from normal mammalian genes. None of the inserts, or mutations are expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms.

Copine protein family - Copines are Ca2+-dependent phospholipid-binding proteins that are thought to be involved in membrane-trafficking, and may also be involved in cell division and growth.

Standard selection markers and polypeptide tags (GFP, antibiotic resistance, lacZ) will allow identification of the transduced cells, but is unlikely to have any significant biological effect.

Evaluation of foreseeable effects

The inserted mammalian and reporter gene sequences are normal or selective alterations of characterised mammalian genes. The genetic inserts are not likely to cause significant effect on an individual.

Native Sindbis virus is endemic in Northern and Eastern Europe and is maintained in the avian population. Human infection, caused by mosquito bites, is rare even in the countries of origin, and causes mild fever with a rash, and may predispose to the development of arthritis. The virus is unstable and can survive for only a limited time outside the laboratory. However the Sindbis virus to be used here can only undergo one round of replication when co-expressed with the appropriate helper vector and in a suitable cell line. It is not anticipated that accidental human exposure would be linked to significant risk.

Consequently, considering the non-replicative nature of virus type to be used and complexity of the life cycle of sindbis virus, its accidental release into the environment would not cause any significant impact.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - treated with 1% virkon and discharge to drains or autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 - (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes).

Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Any equipment surfaces that may come into contact with the virus will be treated with 1% Virkon or 70% ethanol which inactivates the virus.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Chemical disinfection with Virkon, used according to manufacturers' instructions under standard conditions (effectively 99.998% kill)
Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]  

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

**Project Containment**

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<td>L3 L4 L2 L3</td>
<td>L4 L2 L3 L4</td>
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Animal Units | Large Scale Activities | Human Clinical Applications
L2 L3 L4 L2 L3 L4 L2 L3 L4

**Project Ref 553/11.7**

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<td>13/07/2011</td>
<td>Viral delivery of exogenous recombinant DNA to investigate receptor, channel and transporter proteins that underlie synaptic inhibition in neuronal cells</td>
<td>Class 2</td>
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Non-GMM | Consent Granted

Withdrawn [N]  
Tick if notifying a connected programme of work [N]

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Sinbis virus constructs will be used to express proteins in order to investigate the mechanisms that regulate synaptic inhibition in the brain.

**Recipient or parental organism**

E.coli strains that are disabled and cannot colonise human gut.

Mammalian cell lines (BHK, HEK 293, COS-7). Minimal hazard for mammalian cell lines obtained from commercial sources that are well characterised.

Primary cell culture - Primary rat neuronal cell lines and slice cultures can only survive in cell culture facility conditions.

**Host/vector system**

The Sindbis virus expression system was commercially available from Invitrogen and has been used worldwide to produce viruses for use in dissociated neuronal cultures and slices.

The system consists of two plasmids, pSinRep5 (used to express the gene of interest) and pDH(26S), a helper plasmid (produces structural proteins (capsid, p62, 6K and E1) required for viral encapsidation and release from the produced cell line). The E1 and E2 glycoprotein sequences from pDH(26S) that are required for viral infection of host cells, are derived from a neurovirulent strain of Sindbis virus cDNA (TE12), thus allowing for efficient infection of neurons in culture. RNA transcripts of these plasmids (capped and poly-adenylated) are generated in vitro and transfected into BHK cells, where they are translated as mRNA to produce encapsulated viral particles. Particles produced by these transfections have little or no plaque forming unit (pfu) capability associated with them.

In addition, we also plan to use the pSinRep5nsP2ser plasmid (with pDH(26S) as a helper), which has reduced cytotoxic effects of sindbis viral expression on cultured cells. In the Sindbis Expression System, the structural genes in the viral genome have been replaced by a cassette into which the gene of interest is cloned. The structural genes are provided on the helper plasmid and are not incorporated into the final Sindbis viral particles. Rather, the gene of interest is packaged, along with the replicase sequences. This is designed to render those particles non-infectious following a single round of infection. The risk of replication competent virus being created is minimal.

**Origin & function**

Mammalian and reporter genes.

The genetic material to be introduced into the virus will be derived from normal mammalian genes. None of the inserts are expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms.

GABA receptors (e.g. GABA-A, GABA-B): Activation of these receptors results in the opening of channels in the cell's membrane that allow specific ions to flow, such as potassium and chloride.

Ion transporter proteins (e.g. NKCC1, KCC2): establish the relevant ionic gradients.

Standard selection markers and polypeptide tags (GFP < antibiotic resistance, lacZ) will allow identification of the transduced cells, but is unlikely to have any significant biological effect.

**Evaluation of foreseeable effects**

The inserted mammalian and reporter gene sequence are normal or selective alterations of characterised mammalian genes. The genetic inserts are not likely to cause...
Native Sindbis virus is endemic in Northern and Eastern Europe and is maintained in the avian population. Human infection, caused by mosquito bites, is rare even in the countries of origin, and causes mild fever with a rash, and may predispose to the development of arthritis. The virus is unstable and can survive for only a limited time outside the laboratory. However the Sindbis virus to be used here can only undergo one round of replication when co-expressed with the appropriate helper vector and in a suitable cell line. It is not anticipated that accidental human exposure would be linked to significant risk.

Consequently, considering the non-replicative nature of virus type to be used and complexity of the life cycle of sindbis virus, its accidental release into the environment would not cause any significant impact.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - treated with 1% virkon and discharge to drains or autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes).

Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Any equipment surfaces that may come into contact with the virus will be treated with 1% Vinikon or 70% ethanol which inactivates the virus.

### Degree of kill

**Autoclaving**, effectively 100% kill (annual validation)

Chemical disinfection with Vinikon, used according to manufacturers' instructions under standard conditions (effectively 99.998% kill)

### Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4 L2 L4 L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

**Project Ref** 553/11.8

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>Culture Vol</th>
<th>Class</th>
<th>Culture Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>18/07/2011</td>
<td>Induction of pluripotent stem cells (IPS) formation by HSV-1 amplicon delivery of iPS and large T antigen genes</td>
<td>Class 2</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Class 3-4</td>
</tr>
</tbody>
</table>

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Herpes simplex type 1 amplicon technology will be used to deliver the iPS and pluripotency genes in order to improve iPS formation efficiency.

**Recipient or parental organism**

E. coli strains that are disabled and cannot colonise the human gut.
Mammalian cell lines including primary cells: Minimal hazard for mammalian cell lines obtained from commercial sources that are well characterised.

Host/vector system

The vector pEP4EO2SET2K, (carrying the iPS inducing genes and the SV40 large T antigen) will be ligated by standard enzyme digestion and ligation and/or LoxP-Cre recombined with pHGPuro (carrying the HSV-1 origin of replication and packaging signal), to generate an HSV-1-iPS inducing construct. The HSV-1-iPS construct DNA will be extracted and transfected into the packaging cell line Vero 2-2 along with the fHSVpac270 DNA and pEBHICP27 vectors. The fHSVpac270 DNA, which contains a modified HSV-1 genome, will furnish all the necessary genes to assemble infectious virions, apart from ICP27, which is encoded by pEBHICP27. However, fHSVpac270 cannot itself be packaged since it lacks the HSV-1 iPS vector can be replicated and packaged. The infectious particles thus generated will be released from the cells by sonication, using a dedicated instrument, and concentrated by ultracentrifugation on sucrose gradients. The HSV-1 iPS vector will be stably maintained as an episome, as it possesses the oriP and EBNA1 genes as a part of the pEP4EO2SET2K vector, and it will drive the expression of the iPS genes Oct4, KLF4, Sox2 and the SV40 large T antigen.

The HSV-1 iPS vector on its own cannot generate infectious particles and hence the system is safe.

Origin & function

Mammalian and reporter genes.

The SV40 large T antigen is an oncogene, capable of conferring transforming properties to primary cells (immortalisation).

The iPS inducing genes HLF4, Oct4 and Sox2 are transcription factors, able to alter the cellular gene expression pattern, to reprogram differentiated cells to a stem cell-like status.

Evaluation of foreseeable effects

The resulting GMOs (HSV-1 iPS transduced fibroblast cells are not expected to carry any additional risks to that of the un-modified recipients and could not survive outside the lab environment. However, secondary infection of the transduced cell with a wild type HSV-1 virus, could in theory generate infectious particles carrying the iPS and T antigen genes. Even in this event, the HSV-1 iPS particles would only be able to infect cells once, but would not be able to further spread, as they would lack the HSV-1 proteins necessary to replicate their genome, and assemble a functional viral capsid.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware e.g. pipettes, flasks, tubes) - treated with 1% Virkon followed by autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (121-125°C for at least 15 minutes; 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) - treated with 2% virkon for at least 30 minutes before discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (121-125°C for at least 15 minutes; 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Any equipment surfaces and non disposable items treated with 1% Virkon or 70% ethanol.
Degree of kill
Autoclaving, effectively 100% kill (annual validation)

Chemical disinfection with Virkon, used according to manufacturers' instructions under standard conditions (effectively 99.998% kill)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

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</table>

Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 553/11.9

Date Ackn'd 20/10/2011

CU2 Project Title Lentiviral expression systems as a tool for studying articular cartilage homeostasis

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N
## Project Additional Information

**Purposes of the contained use**

Lentivirus expressing a defined protein will be injected into mouse articular cartilage in order to understand what maintains homeostasis.

**Recipient or parental organism**

Mammalian cell lines including primary cells - Minimal hazard for mammalian cell lines obtained from commercial sources that are well characterised and authenticated.

Packaging cell lines - produce infectious replication defective viral particles after they have been transfected with multiple viral plasmids.

Adult mice - No hazard to human health.

**Host/vector system**

Replication defective lentiviruses

Third generation lentiviral systems (based on viruses from various species) incorporating self-inactivating 3' LTR (SIN) U3 deletion which is transferred to the 5' LTR after reverse transcription and integration in infected cells. This results in the transcriptional inactivation of the LTR in the provirus and reduces the risk of transactivating genes around the site of insertion.

The viral vectors are derived from numerous wild type viruses, such as MoMLV, HIV and EIAV. However non-essential genes have been removed from the vectors and the remaining genes split onto separate plasmids with minimal homology to reduce the risk of homologous recombination. For example lentiviral vectors derived from HIV are devoid of tat, vif, vpu, vpr, nef and the envelope gene has been replaced by VSV-G envelope. Gag, pol and rev are generally on one plasmid, env on a second and the gene of interest is on a third plasmid along with cis-acting signals required for reverse transcription and integration. As the gag, pol, rev and env plasmids do not contain the packaging sequence non of the genes are present in the package viral genome. The likelihood of these viral particles obtaining the missing genes is very low and therefore the risk of replication competent viruses being created is minimal.

Some of the vectors contain the woodchuck hepatitis virus PRE (WPRE) either in the mutate or wild type form. The wild type WPRE contains the gene X promoter and encodes a truncated X protein. Theses have the potential to have oncogenic effects.

**Origin & function**

Mammalian and reporter genes
Tissue inhibitors of metalloproteinases (TIMPs) are widely distributed in the animal kingdom. Four genes encoding TIMPs (1 to 4) were identified into human and mouse genomes. Was originally thought that TIMPs exert their regulatory activity on other classes of enzymes such as ADAMs (a disintegrin and metalloproteinases) and ADAM-TSs (a disintegrin and metalloproteinases with thrombospondin domains). These enzymes are important for ECM turnover during embryonic development, as well as during remodeling into adult tissues. Some enzymes are also necessary for the shedding of molecules anchored to the cell surface. Imbalance into the physiological crosstalk between enzymes and inhibitors may result in diseases associated with uncontrolled ECM turnover, inflammation, cell growth and migration, such as arthritis, cardiovascular disease or cancer.

Members of the Toll-like receptors (TLRs) family - TLRs were initially identified based on their sequence similarity with Drosophila Toll receptors. These receptors form part of the innate immune response. In general TLRs are thought to be involved in host defence against infection with different microbial products and the reason why we wish to use it is that it recycles TIMPs. Dominant negative versions would be expressed to stop internalisation of TIMPs.

Human TNF (Tumour Necrosis Factor) a multifunctional proinflammatory cytokine that belongs to the TNF superfamily.

CCN family proteins are involved in a number of biological processes in development, tissue repair, and tumor suppression; but their exact functions are still unspecified. The CCN family consists of 6 distinct proteins (i.e. cysteine-rich 61; Cyr61/CCN1, CTGF/CCN2, nephroblastoma overexpressed; Nov/CCN3, Wnt-inducible secreted proteins 1, 2, and 3; Wisp1-3/CCN4-6 with similar structures as described above, except for Wisp2/CCN5, which lacks the CT module. Connective tissue growth factor (CTGF) is the second member of the CCN family of proteins (CCN2) and its structure is characterized by 4 distinct modules, i.e. insulin-like growth factor binding protein-like (IGFBP), von Willebrand factor type C (VWC), thrombospondin type 1 repeat (TSP1), and a carboxyl terminal cysteine knot (CT).

Tenascin family members are also matricellular proteins and TNC has been shown to protect cartilage from degradation. We wish to understand these proteins and their role in the maintenance of articular cartilage.

Luciferase reporter constructs - Luciferase firefly gene is located downstream of promoter region. Used to monitor signalling in cells by assaying for luciferase activity.

Evaluation of foreseeable effects

The lentivirus amphotrophic and can therefore infect human cells however the design of these systems is such that the viral particles are replication defective and are only capable of one round of infection.

Some of the vectors contain the woodchuck hepatitis virus PRE (WPRE) either in the mutate or wild type form. The wild type WPRE contains the gene X promoter and encodes a truncated X protein. These have the potential to have oncogenic effects.

The lentiviral particles produced will have a broad tropism and be capable of infecting and transducing human and other mammalian cells, inserting viral and mammalian and/or reporter gene sequences (see above) into the host genome. This is an anticipated risk associated with the use of lentiviral gene transfer vectors. The viral particles have however been rendered non-replicative by a number of safety features.

Lentiviral Transduction

Lentiviral particles will be purified from packaging cell cultures and used to transduce mammalian cells to facilitate the production of new mammalian cell lines permanently expressing the desired transgene inducing molecule. Gene transfer from viral particle to mammalian cell line is expected.

Accidental human exposure to viral packaging cells carries minimal risk as the inserted sequences are not expected to affect the pathogenicity of the cells. It is anticipated that any cells would be rapidly cleared by the complement/immune system of any exposed individual.

There is a risk of accidental human exposure to viral particles due to the use of needles to inject the mice and could lead to viral infection and the insertion of viral and mammalian and/or reporter gene sequences into the host genome. To minimise the risk all staff are trained in the use of needles and the injections will only be performed...
Once. Importantly, in the context of human gene therapy clinical studies, high doses of lentiviral vector particles have been administered in vivo with no observed complications. It is considered highly unlikely that accidental human exposure to the viral particles described in this risk assessment would lead to the generation of a tumour.

Where viral particles are used in vivo the greatest risk is from the use of needles however it is not anticipated that accidental exposure would be linked to significant risk.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Mice will be injected and held at containment level 2 facilities.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

**Degree of kill**

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

**Please enter comments on the GM safety committee on the risk assessment**

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**
**Project Ref** 553/12.1

**Date Ackn’d** 06/01/2012

**CU2 Project Title** Targeting neural plasticity and auditory cortical connectivity using optogenetics

**Date Project Ceased**

**Class** Class 2

**Culture Vol** ≤ 1 Litre

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Use of Adeno-associated virus and replication defective lentivirus to selectively eliminate or activate specific neural circuits in a reversible fashion

**Recipient or parental organism**

E. coli K12 or B derivatives - E. coli strains are disabled and cannot colonise the human gut

Mammalian cells lines - Packaging cell lines such as HEK293 cells are especially disabled hosts and are of minimal hazard.

**Host/vector system**

Vectors containing essential genes (gag, pol and env) will be transfected into packaging cells (generally HEK293 derivatives) to generate lentiviral particles which are capable of infecting cells but are self-limiting due to their replication defective nature.
Adeno associated vectors appear to be defective and require co infection with a helper virus (such as Adeno virus or Herpes simplex virus) in order to replicate.

**Origin & function**

Microbial opsin genes encode light-activated channels which will be used to selectively eliminate or activate specific circuits in a reversible fashion. An example of such genes is ArchT from Halorubrum sp which encodes a light-driven proton pump. The vectors also contain the WPRE genes used to enhance expression of the transgenes.

Standard reporter genes and antibiotic resistance genes

**Evaluation of foreseeable effects**

The lentiviral particles produced will have a broad tropism and will be capable of infecting and transducing human and other mammalian cells, inserting viral and transgene sequences into the host genome. There is an anticipated risk associated with lentiviral gene transfer vectors however the viral particles have been rendered non-replicative by a number of safety features.

Adeno associated viruses are naturally replication defective requiring a helper virus for replication. Although wild type AAV can integrate into the host cell chromosome the gutless nature of the vectors means that integration is unlikely to occur. Long term expression is mainly due to the maintenance of episomes.

Both vectors contain the Woodchuck post regulatory element to enhance viral titre and expression. Some concern has been raised with regards to the oncogenic potential of this element.

These light activated channels are not expected to make the replication defective viruses more hazardous.

Standard reporter genes and antibiotic resistance genes are widely used and no hazardous properties have been reported.

Accidental exposure to the viral particles could lead to the insertion of viral and/or transgenes into the host DNA which in the worst case could lead to integration knocking out a tumour suppressor gene or the oncogenic expression of WPRE.

Exposure will be minimise by control measures however such as using MSC and minimising the use of sharps.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Animal bedding and carcases are removed from the site by a licenced contractor for incineration.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding – dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

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<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 553/12.11

Date Ackn’d 13/08/2012  

CU2 Project Title Interactions of myxoma viral proteins with host receptors

Class 2  

Culture Vol  

Class CultureVolClass2 CultureVolumeClass3-4

Non-GMM  

Consent Granted
Immunity against pathogens is maintained with a complex network of interactions between various cellular and humoral components of the immune system. Cell surface receptors play a major role in this process. They are not only involved in sensing the pathogen threat and turning on the immune system against it but also limiting the immune response from going out of control. Inhibitory cell surface receptors are expressed on many immune system cells to slow down the immune response when necessary and so to avoid self-destruction. However, these fine tuners of the immune system can occasionally be hi-jacked by microorganisms which down-regulate the immune response for their benefit. Common strategies used by pathogens involve inducing expression of host ligands for inhibitory receptors or using decoy ligands that are encoded in their genome to pair with the host receptor.

Myxoma virus - is a pox virus responsible for myxamotosis in the rabbit and hare population and has been used in Australia as a biological control for their alien rabbit population. It is endemic in the rabbit population of this country and sporadic outbreaks occur regularly. It is typically a fatal disease that is mainly spread by direct contact and it is recommended that pet rabbits are vaccinated against this disease. It is not pathogenic to humans and therefore doesn't have an ADCP grouping.

mammalian cell lines used for the production of the virus, such as BGMK and RK13, are especially disabled hosts and cannot survive outside the strict conditions of laboratory culture. They are unlikely to pose a hazard to the worker.

Host/vector system
Genetically modified viruses have been created elsewhere by homologus recombination.

Origin & function
Green Flourescent protein or other non hazardous marker genes.
M141R open reading frame deletion. M141 shares sequence homology to host CD200 which is involved in mediating the host immune response by binding to CD200 receptor.
M128R open reading frame deletion. M128 has a similar phenotype and is thought to bind to a similar inhibitory receptor.

Evaluation of foreseeable effects
No adverse effect is expected from GFP or other non hazardous marker genes as they have a history of safe use.
M141 and M128 are thought to be involved in the pathogenicity of the virus by binding to a receptor in the host that is involved in mediating the innate immune response. Interactions between other viral CD200 homologues and the CD200 receptor lead to a down regulation of the immune response in the host. Therefore knocking out these
genes may lead to a virus that will be less able to down regulate the immune response.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms
Purified virions will be used for biophysical characterisation using electron and fluorescence microscopy.

Recipient or parental organism

Bunyamwera Virus is an ACDP hazard group 2 agent whose natural host is the Aedes genus. The natural route of transmission to humans is via an insect bite.

Host/vector system

These viruses have been created elsewhere and are simply being produced from a variety of mammalian cell lines.

Origin & function

Fluorescent proteins, such as GFP or RFP have a history of safe use.

Truncation of the Gc protein, which is responsible for catalyzing the fusion of the viral envelope and the host membrane.
Bunyamwera virus has been found to be pathogenic to mice and occasionally humans, transmission is via an insect bite and may lead to a mild febrile illness with headache, joint and back pains but most infections are unrecognised.

Bunuamwera virus is not known to exist in the UK environment.

In the lab the main route of transmission would be via a percutaneous injury and as such the use of sharps will be minimised.

Truncation of the Gc gene will not impair the virus's ability to replicate and as such will behave similar to a wild type virus.

Addition of fluorescent proteins is not expected to alter the hazards associated with the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturer's guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM
The overall aim of the project is to investigate the therapeutic potential of inhibiting kinases and/or oncogenes in ovarian cancer cells. Our aim is to modify the expression of such genes in human and murine tumour cell lines and monitor the effects on the survival, apoptosis and microtubule dynamics of the recipient cells.

Recipient or parental organism

Human cell lines (such as SKOV3, Hey, A2780, OC316 cell lines)
Murine cell lines (MUKA) generated from a mouse model of low grade ovarian cancer following the deletion of PTEN and activation of KRAS.
Human and murine lymphocytes, including T cell, B cell and dendritic cell (DC)
### Host/vector system

For construction of retroviral vectors:

The pLZRS, pQCXIH and pLPC are self-inactivating retroviral vector containing an inactive 3' Long terminal repeat sequence (LTR) which becomes duplicated into the 5' LTR upon integration into the host genome. This ensures that the virus generated from this vector self inactivates and becomes replication-incompetent after integration into the host genome.

Lentiviruses, pHr-SIN-GFP, including 3rd generation VSV-G pseudotyped lentiviruses, similar to available commercial systems, such as sold by e.g. invitrogen. Commercially available adenoviruses expressing Cre will be used for recombination experiments in vivo. For packaging recombinant retroviral particles: 293FT (human embryonic kidney cell line 293F constitutively expressing SV40 T antigen from pUC-based plasmid pCMVSPORT6TAg.neo) and 293T (similar to 293FT). T antigen increases viral particle yield. GP+envAm12 (murine cell line NIH3T3 + one plasmid containing the Moloney murine leukaemia virus gag and pol genes and a second plasmid containing the 4070A amphotropic env gene)

### Origin & function

Wild-type and mutated murine and human genomic DNA and cDNA inserts:

- Wild-type kinase genes such as FER, FES, PTEN, SIK2 and CRMP2 or oncogenes such as TP53
- Mutated versions with deletions, insertions and/or point mutations. Selective modifications of any/all of the above.

- Standard reporter genes such as puromycin and neomycin resistance (puroR, neoR), beta-galactosidase (lacZ), jellyfish green fluorescent protein (GFP and derivatives), truncated hCD4, hCD25, tdTomato and nerve growth factor (NGF)

Short hairpin RNA interference sequences against the above mentioned genes (or other genes).

### Evaluation of foreseeable effects

Expression of the genes of interest, their mutants and inhibition of expression is likely to result in eukaryotic cell proliferation, quiescence, and apoptosis.

### Evaluation of foreseeable effects

Evaluate the effects of the activity on the environment, including potential impacts on human health and the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

In vivo growth of transplanted modified human and murine cells will be undertaken at Animal Containment Level 1

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138° for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138° for at least 3 minutes), discharge to drains.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Animal bedding - dispose via the clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Virkon 1% solution 30 minutes contact, effectively 100% kill

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM

### Project Containment

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<tr>
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### Project Ref  553/12.14

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<td>24/10/2012</td>
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<td>Class 2</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements [N]

Tick if notifying a connected programme of work [N]

Date Project Ceased

Withdrawn [N]

02/03/2022 Page 9069 of 15326
### Project Additional Information

#### Purposes of the contained use

The aim of the project is to identify molecules and associated mechanisms that regulate tissue stem cell function. Of particular interest in the transcriptional and extrinsic control of hematopoietic and epithelial (principally mammary and thymic) stem cells by transcription factors and signalling molecules, as well as the interaction of these mechanisms and the resulting changes in stem cell self-renewal, lineage priming, chromatin states and differentiation potential.

#### Recipient or parental organism

- **E. coli - K12 and B derivatives**
- Mammalian cells and primary murine stem cells
- Occasionally avian cell lines
- Packaging cells lines such as Phenix E and 293 T to generate retro and lentiviruses

#### Host/vector system

Standard second and third generation lentiviral plasmids using HIV-1–based packaging systems, including separate, non-homologous cistrons for envelope (typically VSV-G) and other viral proteins, and self-inactivating LTRs. WPRE elements will be included in the viral backbone to enhance protein expression.

#### Origin & function

- Genes involved in hematopoietic lineage specification (e.g. C/EBPs, PU.1, GATA-1/2, Klf1)
- Genes involved in lineage-specific progenitor proliferation (e.g. Epo/Epor, GM-CSF/GM-CSFR, M-CSF/M-CSFR)
- Genes involved in stem cell maintenance (e.g. Mpl/Thpo, Kit/Kitl, Imp1)
- RNAi-based constructs to effect knockdown of genes of interest above.
- Standard reporter genes such as GFP, Luciferase and beta-galactosidase
- Standard antibiotic resistance genes such as puromycin and neomycin

#### Evaluation of foreseeable effects

- No hazard expected from the expression of the genes in E. coli K12 and B derivatives
- No hazard expected from the over expression or knock down of genes in mammalian or avian cells
Potential hazard from the replication defective but infectious viral particles (for one round) due to the presence of WPRE and the potential for insertional mutagenesis. Viral particles are designed in such a way that they do not have the mechanisms for replication and as such no viral particles can be produced after the first round of infection.

Use of sharps is limited when working with virus particles.

Mammalian cell lines exposed to viral particles are not expected to pose any hazard after the initial infection. Infectious particles would have integrated into the host genome and any free viral particles removed in the washing stages.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Animal bedding – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Project Ref** 553/12.15

- Date Ackn'd: 26/11/2012
- CU2 Project Title: Transfection of eukaryotic (human and mouse) cells by replication defective lentivirus vectors
- Class: Class 2
- Culture: < 1 Litre
- Consent: Granted

**Project Additional Information**

- Purposes of the contained use: Use of lentiviral vectors to over-express and knock down genes involved in transplant rejection
**Recipients or parental organism**

| Human and murine primary T cells |
| 293 packaging cells |

**Host/vector system**

| second generation lentiviral vectors |

**Origin & function**

Inserted mammalian DNA will comprise non-oncogenic transgenes such as siRNA genes, suicide genes and marker genes.

**Evaluation of foreseeable effects**

The potential for generation of replication-competent lentivirus is addressed by the design of the vectors. The second and higher generations of lentiviral systems separate transfer, envelope and packaging components of the virus onto different vectors. In this system the transfer vector encodes the gene of interest and contains the sequences that will incorporate into the host cell genome, but cannot produce functional viral particles without the genes encoded in the envelope and packaging vectors. It is not possible for viruses normally produced from these systems to replicate and produce more viruses after the initial infection. Importantly, the inability to replicate reduces the likelihood of unintended spread and mutagenic or oncogene-activating or tumor suppressor gene-inactivating insertion, and thus greatly improves the safety of these vectors.

Negligible risk and effects of recombination or complementation.

The WPRE sequence is present in these vectors which has been shown to have potential oncogenic activity.

Primary cells exposed to the replication defective lentivirus will be used in vivo. There is low risk from this activity since any virus will have either integrated in to the host genome or have been washed free of the media.

Lentiviral particles may be used in vivo.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
Please enter comments on the GM safety committee on the risk assessment

## Project Containment

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## Project Ref 553/12.16

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### Project notified under transitional arrangements

N

### Historical Significant Changes

- [ ] Historical Date of Additional Info
- [ ] Significant Change ID
- [ ] Date of Significant Change

### Project Additional Information

**Purposes of the contained use**

Using mouse and human lentiviral shRNA in pooled or arrayed screens to conduct cell based loss of function screening as well as gain of function screening using cDNA.

**Recipient or parental organism**

Mouse and human cell types including primary cells, iPS cells, immortalized cells and secondary cells.
Host/vector system

HEK293 cells
GIPZ shRNA vector or other lentiviral expression vectors. These lentivirus expression vectors are self-inactivating as they carry a deletion in the U3 region of the 3'-LTR. Upon integration into the host genome, the 3'-LTR is copied to the 5'LTR, rendering it transcriptionally inactive and therefore unable to generate a functional retrovirus. Packaging vectors such as Gag-Pol and env. Other systems available may include additional vectors such as TetOn/Off and Tat-Rev

Origin & function

Genetic material from mouse and human. The shRNA and cDNA libraries we employ will naturally contain known human tumour suppressor and oncogenes and will be under the control of constitutively active mammalian promoters. As such it could pose the risk of cell transformation if it is accidentally administered.

As the TDI facility provides a service for all University Departments projects the facility will participate in should span very diverse fields of diseases, cell processes and tissue types.

As this is high throughput screening a large proportion of the work will be done by robotics.

Evaluation of foreseeable effects

The shRNA and cDNA libraries we employ will naturally contain known human tumour suppressor and oncogenes and will be under the control of constitutively active mammalian promoters. As such it poses the risk of cell transformation if it is accidentally administered. However, these risks are strongly mitigated by the biosafety features of the vectors as well as the risk reduction for lentiviral transductions.

Overall then, only lentiviral particles, which are unable to replicate, but which can deliver the gene of interest, are produced. They cannot contain any other genes (e.g. gag, pol, rev & env) because none of the vectors used to supply these elements contain LTR’s or the psi packaging signal required for these genes to be delivered. Therefore, the main risk is in their ability to deliver genetic material into a host genome and as described above, this is unlikely to pose any significant risk.

One further consideration, however, is that the Woodchuck hepatitis B virus regulatory element (WPRE), used to enhance stability of the transcript, is present in some lentivectors. This element may have oncogenic properties so virus produced using these vectors should be considered to have an accordingly higher level of risk. We might buy/use other libraries and packaging systems in future but they will incorporate at least the same biosafety features as the aforementioned libraries and packaging plasmids.

Once the viral particles are integrated into the genome of the cells they pose minimal risk due to the replication defective nature of the viral systems.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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02/03/2022
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Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers’ guidelines)

Is an emergency plan required according to regulation 20? No

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment No

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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**Project Ref** 553/12.17

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02/03/2022  Page 9076 of 15326
Project Additional Information

Purposes of the contained use

Inflammatory bowel diseases (IBD) are chronic diseases of the gastrointestinal tract that are thought to arise from a dysregulated immune response to the intestinal microflora. A growing number of genes are identified to be linked to human IBD including cytokines, genes involved in pathogen recognition or autophagy.

We will use self-inactivating, replication incompetent viruses (e.g. lentivirus or retrovirus) based expression vectors containing the genes of interest or sh/siRNA against them.

Recipient or parental organism

mammalian cell lines and primary cells

Host/vector system

Standard packaging cell lines such as HEK293
Standard commercially available replication defective retro and lentiviral systems

Origin & function

Genes known or suspected to be linked to inflammatory bowel disease such as cytokines, genes involved in pathogen recognition and autophagy, and sh/siRNA against those genes.

Evaluation of foreseeable effects

Viruses to be used are replication defective and have a number of safety features such as packaging genes divided onto different plasmids, self inactivation of the 3'UTR etc

The woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) sequence is present within many of the lentivirus vectors to be used. This sequence has been linked to liver tumours in mice.

Overexpression or knocking down genes suspected to be involved in IBD is unlikely to have any adverse effects.
Once the replication defective virus has integrated into the host genome it presents minimal risk and the cells can be used in subsequent experiments at class 1. Infectious virus will not be inoculated.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
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- Chemical, effectively 100% kill (following manufacturers guidelines)

- Is an emergency plan required according to regulation 20? [N]
- If yes, tick to confirm that it is attached to this form [N]
- Tick to confirm that you have attached a risk assessment to this form [Y]
- Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

**Please enter comments on the GM safety committee on the risk assessment**

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.
Project Additional Information

Purposes of the contained use

1) to investigate whether particular genetic sequences (either single genes, genetic mutations, or combinations of genes) observed by whole genome sequencing are responsible for drug resistance. This will be used to characterise novel mutations in known resistance genes in several bacterial species of major clinical relevance (S. aureus and members of the Enterobacteriaceae family).

2) to characterise gene transfer systems permitting exchange of resistance genes amongst clinically relevant Enterobacteriaceae.
Enterobacteriaceae - clinical isolates such as E. coli, Klebsiella spp, Enterobacter spp. Some isolates will be multidrug resistant but effective treatments remain available
Staphylococcus aureus - clinical isolates which may have multiple drug resistance but effective treatments remain available
Staphylococcus - Newman strain
E. coli K12 derivatives such as DH5alpha and DC10B - hazard group 1 lab strain

Host/vector system

The vectors to be used (such as pRMC2, pIMAYZ) are derivatives of those used in laboratories to study virulence and resistance genes. No attenuating effect on laboratory strains has been reported.

Origin & function

Gene inserts will be obtained from clinical isolates of Staphylococcus aureus. The genes to be inserted will be either housekeeping genes, chromosomal genes or genes on mobile genetic elements which have been incorporated into the host genome. Only the gene and ~150bp flanking regions will be inserted. These genes will be capable of causing resistance to a single class of antibiotics and are not known to cause any alteration in transmissibility or pathogenicity.

For the Enterobacteriaceae work
i) naturally occurring plasmids. These are frequently large plasmids which can include multiple resistance genes, as well as virulence factors and mechanisms for plasmid transfer.

ii) recombinant plasmid vectors including one or more genes conferring antimicrobial resistance to a single antibiotic, obtained as described in the overview above.

Evaluation of foreseeable effects

For the Staphylococcus aureus work:
The final GMM organism will be S. aureus Newman with resistance to a single class of antimicrobial. As this strain is fully susceptible, a broad range of antimicrobial treatment is still available. No increased transmissibility or virulence is anticipated.

For the Enterobacteriaceae work:
The recipient E.coli are K12 or B derivatives, such as DH5alpha and DH10B strains. These are disabled hosts that cannot colonise the human gut and have a history of safe use, and are assigned to ADCP hazard group 1. Insertion of the genetic content should (in the case of recombinant plasmid vectors) result in resistance to a single antibiotic class. In the case of naturally occurring plasmids from Enterobacteriaceae, several resistance genes may be transferred and possibly virulence factors. Although the isolates from which these plasmids will be obtained will be multiply drug-resistant, the susceptibility phenotype will be known prior to manipulation, and we can ensure that any transformant strains generated will be susceptible to some clinically useful anti-microbials. As an additional precaution, resultant modified strains will be handled as hazard group 2 organisms.

In vivo testing may be required when the results of the in vitro work are inconclusive. The challenges will be carried out intravenously in a class II micro biological safety cabinet. Dissemination of the organism is not expected in the short time scale of the experiments.

Evaluation of foreseeable effects

N/A

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

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Sharps (e.g., needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding – EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes) disposition via clinical waste stream for incineration.

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Degree of kill:
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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Gene therapy involves the treatment of disease through the delivery of exogenous nucleotides which interact with the host transcriptional or translational machinery to correct specific deficits in gene function. Genetic material can be delivered with high efficiency using viral vectors. We propose to utilize standard lentiviral and adeno-associated virus (AAV) vector technology for the production of lentiviral vectors and AAV vectors. These vectors will be used to deliver therapeutic genes and common reporter genes to mammalian cells in culture and to tissues in vivo.

The inserted genes will fall broadly into two categories:

a) Reporter genes, the product of which are non-toxic, typically fluorescent, and used to easily identify and evaluate the success of gene transfer. These genes are derived from a variety of organisms but have a long history of safe use.

b) Mammalian genes with important roles in phototransduction and/or a potential influence on retinal degeneration process.
derived from a variety of organisms but have a long history of safe use

b) Mammalian genes with important roles in phototransduction and/or a potential influence on retinal degeneration process.

**Host/vector system**

Lentiviral vectors – Vectors are attenuated, non-pathogenic, replication deficient, and bare little genetic homology with wild type virus. Additionally, vectors are self inactivating (SIN), so following integration any vector would be unable to be packaged by a latent wild type lentivirus if one were present. Viral genes required for vector packaging are coded on three separate plasmids during production, all but eliminating the possibility of recombination, and are provided in trans (i.e. not packaged). Virus genes which are not essential for packaging are not present. Vectors are integrating, and thus pose a theoretical risk of causing insertional mutagenesis following administration. No harmful chemicals or compounds are used during production or purification.

AAV vectors – AAV vectors are non-pathogenic, replication deficient and bare little genetic homology with wild type virus. AAV viruses do not contain genes required for independent replication. AAV genes required for packaging (rep and cap) and helper genes are provided in trans (i.e. not packaged) and on separate plasmids. No harmful chemicals or compounds are used during production or purification.

Both lentivirus vectors and AAV vectors may contain the WPRE sequence which has been linked to tumour growth in rodents. However the sequence used in these vectors has been modified from the wild type so that the X-protein is not produced and it is therefore not tumourogenic.

**Origin & function**

Inserts code for normal mammalian genes or selective alterations of those genes. Also standard marker genes such as lac Z, GFP, etc. Inserts are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms.

**Evaluation of foreseeable effects**

Resulting GMOs will express mammalian genes or marker proteins to investigate their use as gene therapy agents but will carry no additional hazards. Any transfer of genetic material to other organisms would be of minimal hazard. GMOs would not survive outside laboratory conditions. E.coli strains do not colonise the human gut; mammalian cells will not survive outside laboratory conditions. Vectors are non pathogenic and replication deficient.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

The inoculation of experimental models is assigned to Class 2 due to the combined use of syringe needles and lentiviral vector as indicated in the SACGM Compendium of Guidance. Workers will receive specific instructions and training in the safe use and disposal of contaminated syringes and syringe needles. All other work may be undertaken safely at Class 1.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

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134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding – dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

The assessment was originally assigned to Class 2 on the basis of the vector alone. This element was re-assigned to Class 1 as the system has intrinsic biological safety features but the inoculation stage was identified as requiring assignment to Class 2 due to the combination of a lentiviral system and the use of syringe needles.

Appropriate containment and control measures have therefore been assigned in accordance with the latest guidance issued by SACGM.

Project Containment

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<thead>
<tr>
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</table>
Use of viral vectors to deliver the appropriate factors to create iPS cells by reprogramming and induced neurons (iN) by direct differentiation of donor cells originating from genetically diverse mice. The effect of genetic variations on the characteristics and properties of the reprogrammed/differentiated cells will then be assessed.

Recipient or parental organism

Primary murine cells are low risk

Primary human cells are low risk but may contain adventitious agents and will be handled at containment level 2 due to CoSHH

Origin & function

Transcription factors which are known or suspected to reprogram somatic cells into embryonic stem-like pluripotent cells include:-

Myc - a known human oncogene that will be under the control of constitutively active mammalian promotors.

Klf4 - also associated with cellular transformation in some scenarios, so should be assigned a similar level of risk to Myc.

Klf2 is a newly reported reprogramming gene, which helps to reprogramme cells to a naive hES cell-like state, which is more closely related to mouse ES cells than normal.
hES cells. Sox2 - has been suggested to act by maintaining or preserving development potential. Oct4 - is a homeodomain transcription factor of the POU family. It is expressed in mammalian early embryo and germ cells and can both stimulate and repress target gene transcription. Oct4 regulates the expression of multiple genes via interaction with other transcription factors present in pluripotent cells. Together c-Myc, Klf4, Sox2 and Oct4 are capable of reprogramming cells to become pluripotent.

In addition other transcription factors may be used to achieve direct differentiation of somatic cells to neurons such as:-
- Neurons (iNs): Ascl1, Brn2 and Myt1l (10)
- Motor neurons (iMNs): Ascl1, Brn2, Myt1l, Lhx3, Hb9, Isl1 and Ngn2 (11)
- Dopaminergic neurons (iDAs): Ascl1, Nurr1 and Lmx1a (17)

Other reprogramming genes which could have oncogenic potential may be used in the future.

Evaluation of foreseeable effects

Together c-Myc, Klf4, Sox2 and Oct4 are capable of reprogramming cells to become pluripotent. As such they pose a greater risk in combination than individually. The risk of induction of pluripotency in the event of ingestion, inhalation or injection is very low, as even if the genetic material is successfully introduced into the genome of cells within the body, reprogramming is an inherently very inefficient process (~0.01% efficiency) even in defined tissue culture conditions, and the environment in which a reprogramming cell would find itself would not be conducive to the reprogramming process (other signalling events induced by the local environment would be likely to override the reprogramming process). In addition the genes are in four separate vectors decreasing the likelihood of all four entering the same cell in the event of accidental exposure. If, despite this low level of risk, reprogramming does occur within a laboratory worker, then it is possible that a teratoma could result, as is the case when iPS cells are injected into immunosuppressed mice. Other reprogramming genes which also have oncogenic potential may be used in the future.

EBV-episomal systems do not integrate into the host DNA and replicate once per cell cycle in the presence of drug selection. Once drug selection is removed episomes are lost at approx 5% per cell generation. As this is a plasmid based system the likelihood of the vector gaining entry into cells without very specific transfection methods is unlikely.

PiggyBac is also a plasmid based delivery system and the likelihood of the vector gaining entry into cells without very specific transfection methods is unlikely.

Replication defective adenovirus retains the ability to enter human cells and will express the foreign genes for a limited period of time, however it will be less pathogenic than the wild type due to the inability to replicate.

Replication defective retroviruses and lentiviruses have a number of modifications to decrease the risks such as separation of the gag, pol and env genes onto different plasmids (or stable expression in the packaging cell line), self-inactivating nature of the LTR’s and deletion of non-essential genes. However the vectors can integrate into the host genome and this potentially could inactivate a tumour suppressor gene. The WPRE element is also present in some of these vectors which may have an oncogenic property.

The inserts are not expected to increase the hazard from E. coli cells.

Murine and human somatic cells once tranduced with the vectors are not expected to present a risk as the vectors lack elements essential for replication and packaging.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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<tbody>
<tr>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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</table>

Project Ref  553/12.4
Experimental evolution of antibiotic resistance in pseudomonas aeruginosa

The evolution of antibiotic resistance poses an important threat to human health and welfare and the overall goal of this project is to study the underlying evolutionary processes that drive the spread of resistance in pathogen populations. To achieve this goal, we will carry out experiments in which we will challenge genetically modified populations of the pathogenic bacterium P.aeruginosa with evolving antibiotic resistance in controlled lab experiments. Specifically looking at costs and benefits of resistance mutations, compensatory adaptation, physiological interactions between antibiotics and mutation rate and resistance.

**Recipient or parental organism**

PSEUDOMONAS AERUGINOSA

**Host/vector system**

Standard E.coli (K12 and B derivatives) and vectors will be used to generate the plasmids for transfecting into the Pseudomonas. Also oligo-mediated recombineering to introduce mutations into the Pseudomonas.

**Origin & function**

- Reporter genes (such as eFPs and Lux) that have no known toxicity
- Antibiotic resistance marker (such as aac1 and amp)
- rpoB alleles containing rifampicin resistance mutations.
- A null mutation in a stress response gene (LexA).
- Knockout mutations of DNA repair genes.

**Evaluation of foreseeable effects**

Pseudomonas aeruginosa is a hazard group 2 micro-organisms that is an opportunistic pathogen of humans. This organism is primarily a danger to individual who suffer from cystic fibrosis or who have compromised immunity. This organism can be readily isolated from environmental samples, and healthy adults are considered to be at
negligible risk from infection from this organism.

Reporter genes (such as eFPs and Lux) have no known toxicity and are not expected to alter the pathogenicity of the Pseudomonas.

The antibiotic resistance marker (such as aac1 and amp) does not provide resistance to antibiotics that are currently used to treat Pseudomonas infections.

rpoB alleles containing rifampicin resistance mutations. Rifampicin is not used to treat Pseudomonas infections and rifampicin resistance mutations tend carry a fitness cost implying that Pseudomonas carrying engineered rifampicin resistance alleles will tend to be at a competitive disadvantage.

A null mutation in a stress response gene (LexA). This mutation disables a highly conserved stress response gene that helps to protect bacteria against antibiotics and oxidative stress.

Knockout mutations of DNA repair genes. There is no evidence that increasing mutation rates directly results in an increase in the virulence of bacterial pathogens.

We do not expect that any of these manipulations will increase the toxicity or pathogenicity of Pseudomonas. If anything, we expect that these manipulations will attenuate the virulence of this organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tbody>
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<td>L3 L4</td>
<td>L2 L3 L4</td>
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<td>Animal Units</td>
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<td>Human Clinical Applications</td>
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### Project Ref 553/12.5

<table>
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<tr>
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<tr>
<td>10/04/2012</td>
<td>Apoptotic and cell cycle responses following Helicobacter pylori infection</td>
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<table>
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<tr>
<th>Class CultureVolClass2 CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>Class 2</td>
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<tr>
<td>&lt; 1 Litre</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

<table>
<thead>
<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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<tbody>
<tr>
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<td>N</td>
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</table>

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
### Purposes of the contained use

Deregulation of cell controls affecting proliferation and/or apoptosis is a common event during host pathogen interactions. This program of work is to study the molecular mechanisms used by Helicobacter pylori to hijack and alter host defense mechanisms, cell cycle and apoptotic responses. Using strains of Helicobacter pylori deficient for genes that potentially target the host response (mainly contained within the cag-pathogenicity island of the bacterium) as well as strains that transgenically express the fluorescent marker enhanced green fluorescent protein (to follow the localization of the bacterium during infection), we will be able to pinpoint the molecular mechanisms used by this bacterium to modulate proliferation and apoptosis of the host cell.

### Recipient or parental organism

*Helicobacter pylori* - hazard group 2 micro-organism

### Host/vector system

Mammalian cell lines and primary cell cultures, multicellular gastric organoids, and animals will be inoculated with the genetically modified Hp to test the cellular interactions between host and pathogens.

### Origin & function

The strains generated will be knock-out or knock-in mutants of genes which are potentially involved in targeting the host response such as cagA, virb10, and VacA.

Inserted genetic material will encode non-infectious, non-hazardous proteins, such as standard marker proteins to allow for visualising localization during infection.

### Evaluation of foreseeable effects

Insertion of non-infectious, non-hazardous genes (e.g. standard reporter genes) is not expected to alter the pathogenicity of the bacteria.

Helicobacter strains mutated in the cag (deltaCags) and/or vacA gene products lack some of the components that enable the bacterium to produce the major virulence factors used to establish the infection. Therefore they have an attenuated virulence as compared to the wild type strain. None of the genetically modified Helicobacter strains are expected to present an increase in hazard to humans compared to the parental strains.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Carcasses will be sent for incineration via a licenced contractor

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Consumables (mainly plasticware eg pipettes, flasks, tubes)** - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding – Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Project Ref 553/12.6

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<td>31/05/2012</td>
<td>Extracellular Blocking Of Bacteria</td>
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<td>&lt; 1 Litre</td>
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</table>
Purposes of the contained use

The purpose of the contained use is for the handling of gram negative bacteria which have genetic modifications in their polysaccharide translocation machinery.

Recipient or parental organism

Hazard Group 2 gram negative bacteria, such as E. coli E69.

Host/vector system

Mutants created elsewhere.

Origin & function

Genes to be knocked out or mutated are involved in the polysaccharide translocation machinery and are expected to lead to a reduction in capsule formation.

Possible inclusion of standard antibiotic resistance genes or reporter genes.

Evaluation of foreseeable effects

Mutations of proteins involved in the polysaccharide translocation machinery are expected to prevent the formation of capsular polysaccharide on the cell surface. The hypothesis is that this will increase the antigenicity of the affected bacteria, allowing them to be more rapidly detected and cleared by the host immune system. The mutants act as a positive control for the chemical compounds to be tested.

In some cases the gene will be disrupted by the insertion of a standard molecular biology antibiotic resistance gene. These antibiotics are not routinely used to treat disease.

The inclusion of standard reporter genes would not be expected to increase the hazard of the bacteria.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
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Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

Use of replication defective Retrovirus and Lentivirus containing WPRE.

**Recipient or parental organism**

*E. coli* K12 or B derivatives - *E. coli* strains are disabled and cannot colonise the human gut

Mammalian cells lines - Packaging cell lines such as HEK293 cells are especially disabled hosts and are of minimal hazard.

**In vivo work**

**Host/vector system**

Vectors containing essential genes (gag, pol and env) will be transfected in to packaging cells (generally HEK293 derivatives) to generate lentiviral particles which are capable of infecting cells but are self limiting due to their replication defective nature.

**Origin & function**

Inserts will be standard reporter genes, such as GFP, standard antibiotic resistance genes, structural proteins and other non-functional harmless genes.

The vectors may also contain the WPRE genes used to enhance expression of the transgenes.
The lentiviral particles produced will have a broad tropism and will be capable of infecting and transducing human and other mammalian cells, inserting viral and transgene sequences into the host genome. There is an anticipated risk associated with lentiviral gene transfer vectors however the viral particles have been rendered non-replicative by a number of safety features.

The vectors may contain the Woodchuck post regulatory element to enhance viral titre and expression. Some concern has been raised with regards to the oncogenic potential of this element. Where possible the vectors will contain versions that have mutated start codons.

Standard reporter genes and antibiotic resistance genes are widely used and no hazardous properties have been reported.

Accidental exposure to the viral particles could lead to the insertion of viral and /or transgenes into the host DNA which in the worst case could lead to integration knocking out a tumour suppressor gene or the oncogenic expression of WPRE.

Exposure will be minimise by control measures however such as using MSC and minimising the use of sharps for in vitro work. Needles may be required for in vivo work.

<table>
<thead>
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For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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</table>

Project Ref  553/12.8

Assessment of the protective efficacy of candidate vaccines against human malaria (Plasmodium falciparum or P. vivax) using transgenic parasites

Date Ackn’d  20/06/2012

Class  Class 3
CultureVolClass2  < 1 Litre
CultureVolumeClass3-4  Non-GMM
Consent Granted  Yes

Project notified under transitional arrangements  N

Tick if notifying a connected programme of work  N

Historical Significant Changes
**Project Additional Information**

### Purposes of the contained use

The aim of this project is to assess the protective efficacy of candidate vaccines against *P. falciparum* or *P. vivax* antigens, using parasites that express fluorescent or bioluminescence reporter genes. These transgenic parasites enable easy visualisation within the mosquito vector. This research will assess the protective efficacy of these vaccines and look for immune correlates of protection against liver-stage, blood-stage and mosquito-stage malaria infection.

### Recipient or parental organism

*Plasmodium falciparum* is a hazard group 3 micro-organism and is the causative agent of malaria in human. It's life cycle is split between the human host and an insect vector. Transmission to human occurs via a bite by the infected mosquito vector and only female Anophele mosquitoes are involved as the males do not feed on blood.

### Host/vector system

The transgenic parasites have been made elsewhere using homologous recombination.

### Origin & function

Standard marker proteins, such as EGFP and Luciferase.

### Evaluation of foreseeable effects

*Plasmodium falciparum* is the causative agent for malaria and is assigned to hazard group 3 by ACDP.

Falciparum malaria symptoms include: complain of headached, fever and aches and pains all over the body, and diarrhoea and abdominal pain are sometimes present. Spleen and liver are often palpable on clinical examination. This may be misdiagnosed as influenza in non endemic areas, and, unless treated promptly, the clinical picture can deteriorate rapidly. Can progress to cerebral malaria: confusion, delirium, loss of consciousness. Incubation period: at least 7 days.

Standard marker proteins, such as luciferase and GFP have a history of safe use and are not expected to alter the pathogenicity of the parasites and therefore have no additional risks compared to the wild types.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

*P. falciparum* gametocytes will be cultured in human erythrocytes either in the insectary or in the blood-stage CL3 laboratory in the Jenner Institute. For dissections,
mosquitoes will be killed by drowning in 70% ethanol and then decapitation.

The GM parasites will be used to infect mosquitoes, but the mosquitoes are not in themselves genetically modified. Infected mosquitoes are maintained in a secure insectary at a containment level appropriate to the pathogenic nature of the parasites. Access to the insectary is through a sealed anteroom and all doors have rubber seals and brushes to prevent mosquitoes escaping from the insectary area.

Mature plasmodium gametocytes will be fed to mosquitoes in sealed pots using standard membrane-feeding techniques. Infected mosquitoes will be maintained for up to 16 days at 26°C before dissection. Mosquitoes must be kept in a pot within a sealed plastic container which is then placed in an enclosed mosquito cage (i.e triple contained). The cage itself will be kept in a locked incubator.

For dissections, mosquitoes will be killed by drowning in 70% ethanol and then decapitation.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Justification for containment level 3

Plasmodium falciparum is a hazard group 3 pathogen, normally requiring containment level 3. However, the HSE advisory committee on dangerous pathogens, annex 1 lists biological agents which may be used at less than the minimum containment conditions required by COSHH. Plasmodium falciparum is listed as an agent to which the derogation may apply.

For work with genetically modified Plasmodium falciparum parasite-infected mosquitoes the following derogations from full Class 3 containment are requested (the insect facility is classified as an animal facility)

1. The laboratory is not sealable for fumigation
2. Entry to lab via airlock is not required.
3. There is no negative pressure relative to the immediate surroundings
4. Extract and input air are not HEPA filtered
5. Autoclave is not required in the laboratory suite
6. Inactivation of GMMs in effluent from handwashing sinks and showers and similar effluents is not required
7. Incinerator for disposal of animal carcasses is not required (mosquitoes)

Justification: A 14-day developmental period is required within the mosquito post-infection before parasites are capable of infecting humans. At this point direct inoculation of the parasites into the human blood is required, through the bite of the insect. There is no air-borne route of infection. There is no aerosol formation. Parasites are inactivated by bleach, detergent, disinfectant, or desiccation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
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- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

1. Please can you confirm whether this notification is solely for P. falciparum or is it to include vivax as well.
2. If it is to include vivax we will need some more information on the micro-organism.
3. P. falciparum 3D7 has been sequenced but could you please provide any other information such as has it been in continuous blood culture for a number of years, has no resistance to major anti-malarial drugs, any other relevant information

response
1. Just for P. falciparum, not for P. vivax
2. N/A
3. 3D7 is not drug-resistant and infections are treatable with standard anti-malarials such as riamet and chloroquine (3D7 is the strain which is used for challenge and subsequent drug-cure of human volunteers in vaccine trials). It is a totally laboratory-adapted strain, having been in continuous culture under lab conditions for many years.

Project Containment

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Human Clinical Applications

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Project Ref 553/12.9
Use of viruses to determine the mechanisms that regulate interferon production

Purposes of the contained use
Use of recombinant replication-defective lentiviruses in overexpression and knockdown of genes thought to be involved in regulating interferon. As interferons play an important role in coordinating the mammalian host defence against viruses we will also be using wild type and genetically modified versions of viruses to challenge the mammalian immune response.

Recipient or parental organism
Replication defective retro and lentiviruses
encephalomyocarditis virus (EMCV),
herpes simplex virus 1 (HSV-1) - hazard group 2,
influenza A virus (flu, PR8 strain and derivatives thereof) - hazard group 1,
Sendai virus (SeV, cantell strain),
moloney murine leukaemia virus (MLV),
vaccinia virus (VV) - hazard group 2 and
varicella zoster virus (VZV) - hazard group 2
Mice (wild type and genetically modified)

Host/vector system
E. coli K12 or derivatives of
Mammalian cell lines
Standard plasmid vectors

Origin & function
encephalomycarditis virus (EMCV) can infect rodents and pigs that are the natural hosts of this picornavirus, which infects multiple body systems. It causes a variety of gastrointestinal, respiratory and systemic signs, as well as mortality. It is not known to infect humans.

Herpes simplex virus 1 (HSV-1) is a hazard group 2 micro-organism that can cause infection predominantly of mucosal surfaces (mouth, lips, etc.) in humans. In the majority of cases, infection (often acquired in early childhood) is not life threatening and passes asymptomatically. HSV-1 is susceptible to treatment with anti-viral drugs (nucleoside analoges). Primary infection can lead to persistent infection of the central nervous system without overt symptoms (latency). HSV is ubiquitous and it is estimated that one out of two people experience recurrent infection.

Influenza A virus (flu, PR8 strain and derivatives thereof) is a hazard group 1 micro-organism which infects birds and mammals and transmission occurs through aerosolised particles and close contact with infected animals. Symptoms are minor including chills, fever, sore throat, muscle pain, headaches, coughing but can develop into more severe and life threatening disease (pneumonia), especially in immune-compromised, old or very young individuals.

Sendai virus (SeV, cantell strain) induces lesions within the respiratory tract and naturally infects rodents and pigs.

Moloney murine leukaemia virus (MLV), naturally infects mice and causes leukemia.

Vaccinia virus (VV) is a hazard group 2 micro-organism and is relatively safe for humans as testified by its use as a vaccine against smallpox. Transmission could occur through inhalation of airborne virus in droplets or through skin injuries.

Varicella zoster virus (VZV) is a hazard group 2 micro-organism where primary infection occurs this results in chicken pox and very rarely lung or CNS involvement. Most UK adults are immune (from childhood infection) and all individuals working on the project have been confirmed to be immune by antibody titre. Transmission can occur via direct inoculation or direct contact with vesicle fluid from chicken pox patients.

Mammalian genes known or suspected to control the production of IFN, including genes linked to Aicardi-Goutieres-Syndrome (AGS) in humans, such as RIG-I, MDA5, MAVS, TREX1 and SAMHD1. These genes may be modified, for example by truncation or point mutation.

DNA and RNA viruses (listed above) genetically modified to lack one or multiple genes and to express reporter genes such green fluorescent protein, luciferase or beta-galactosidase, or strains expressing tags such as the FLAG-tag.

Reporter genes, such as GFP, luciferase and B-galactosidase

**Evaluation of foreseeable effects**

Genes known or suspected to control the production of IFN, including genes linked to AGS in humans are expressed in healthy individuals and none of them (including variants of them) is known as either an oncogene or a tumour suppressor gene. Therefore, overexpression and knockdown are highly unlikely to have oncogenic potential or pose any additional risks.

DNA and RNA viruses lacking one or more genes are typically non-infectious or attenuated when compared to the wild-type versions, therefore these recombinant viruses are not expected to pose any additional risks.

Standard reporter genes have a history of safe use and are not expected to pose any additional risks.

Animals challenged with cells transduced with replication defective viruses pose minimal risk. When challenged with replication defective retro/lentiviruses there is a potential hazard posed by the presence of WPRE and or insertional mutagenesis. When challenged with the genetically modified DNA / RNA viruses listed there are potential risks however these viruses are expected to be attenuated compared to the wild type and control measures and training should reduce the risks.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Animal bedding – EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.
Project Ref 553/13.1

Date Ackn'd 29/01/2013

CU2 Project Title Use of replication defective viruses to assess the role of genes thought to be involved in DNA replication

Class Class 2
CultureVolClass2 < 1 Litre

Consent Granted

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

The process of DNA replication is crucial for all dividing cells as the genetic material must be accurately copied before partition into two new daughter cells. Errors that occur during the process of DNA replication underlie the development of cancer, other diseases and genetic diversity. Identification of pleotropic mutations in replication genes that permit cellular viability while manifesting as altered cellular phenotypes are crucial for understanding this complex and vitally important process.

Recipient or parental organism

Packaging cell lines such as HEK293 cells (and derivatives of) are especially disabled hosts and require strict nutritional requirements for survival. They do however contain fragments of viral sequences.

Human primary cells, such as fibroblasts are especially disabled hosts and require strict nutritional requirements for survival.

Mammalian cell lines, such as retinal pigment epithelial cells are especially disabled hosts and require strict nutritional requirements for survival.
E.coli K12 and B-derivatives are disabled hosts and cannot colonise the human gut

**Host/vector system**

- 3rd generation replication defective lentiviral vectors expressing gag, pol, tat and VSV-G
- Adeno- associated virus

**Origin & function**

- Genes encoding DNA replication proteins such as the PCNA gene.
- Genes encoding proteins thought to interact with DNA replication proteins such as Fen-1, DNA2, PolD1, PolD2, MSH6, Lig1, Caf-1 p150, DNMT1
- Standard reporter genes, such as GFP and antibiotic resistance genes
- shRNA to inhibit genes transcription from DNA replication genes and genes which are thought to interact with DNA replication proteins

**Evaluation of foreseeable effects**

In the lentiviral vector systems essential genes are split onto multiple plasmids which have limited sequence homology. In addition none essential genes are removed from the vectors. As such it is highly unlikely that a recombination event could generate a replication competent virus. However the replication defective virus is infectious for one cycle and has the potential to integrate into the host genome.

Adeno associated virus also has its essential genes split onto a number of plasmids to reduce the likelihood of recombination generating replication competent virus. However the replication defective virus is infectious for one cycle.

Packaging cells lines once transfected with viral vectors produce infectious but replication defective viral particles.

PCNA is normally highly expressed in proliferating cells, but there are no reports that it has oncogenic potential

Vectors contain WPRE but it has the ATG and promoter sequence mutated so that it does not express any fragment of the potentially oncogenic protein X ORF. However it may be necessary to use vectors in the future where the WPRE is the standard gene commonly found in vector systems.

shRNA against some of the PCNA interacting proteins may potentially be more hazardous as there is some evidence that these proteins may have tumour surpressing funtions and thus their inactivation could increase the potential for the transduced cell to become transformed.

Once primary cells and cells lines have been transduced they do not pose any greater risk than the unmodified versions due to the replication defective nature of the viruses.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
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Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
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- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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**Project Ref** 553/13.10

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The purpose of this study is to look at how HCV stimulates innate immunity, how it inhibits the activation of innate antiviral mechanisms, and how innate antiviral mechanisms control HCV infection. As part of this project we will also study how HCV causes mixed cryoglobulinemia. Finally, we seek to understand at the molecular level how B cells and antibodies recognise HCV and HCV-infected cells.

#### Recipient or parental organism

Hepatitis C virus - Hazard group 3
mammalian cell lines, such as hepatome Huh-7 and primary human cells
293 cells for lentiviral production

#### Host/vector system

Plasmid encoding HCV RNA
Plasmids encoding the sequences necessary for replication defective lentiviral production.

#### Origin & function

Anti viral genes such as genes involved in pathogen detection, interferon induction and antiviral pathways
shRNA to genes such as those involved in pathogen detection, interferon induction and antiviral pathways
Immunoglobulin genes
reporter genes such as GFP, RFP, YPET, VENUS and Luciferase
WPRE in the lentiviral constructs

#### Evaluation of foreseeable effects

Growth of HCV in plasmids is used to conserve a consistent genetic make up of the virus as it rapidly mutates in mammalian cells. This also allows for selective alteration of the genetic structure using sequences found in patient samples. These samples can be considered to be of the same harm as the wild type virus. Addition of marker
proteins such as GFP or luciferase is not expected to increase the harm, in fact it causes a reduction in viral replication.

As part of the project the group will be looking at mammalian genes which are active or interact with the virus during infection. Lentivirus will be used as an agent to transfect mammalian cells to either overexress or knock down such genes. These genes may also be selectively mutated using site directed mutagenesis.

Lentiviral vectors are replication defective and as such are only infectious for a single round of infection. There is the potential presence of WPRE in the vectors which may have oncogenic potential and as such this work will require containment level 2. Knock down or overexpression of individual genes is not expected to increase the potential harm from the lentivirus. Expression of reporter genes has a history of safe use.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. |
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| HCV - will be inactivated by virkon. |
| Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill. |
| Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration - NO SHARPS WILL BE USED IN CL3 |

### Degree of kill:

- **Autoclaving**: effectively 100% kill (annual validation)
- **Incineration**: effectively 100% kill (licensed incinerator)
- **Chemical**: effectively 100% kill (following manufacturer's guidelines)

### Is an emergency plan required according to regulation 20? [N] |

### If yes, tick to confirm that it is attached to this form [N] |

### Tick to confirm that you have attached a risk assessment to this form [Y] |

### Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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### Project Ref 553/13.11

**Date Ackn'd**: 05/12/2013

**CU2 Project Title**: Study of cancer-induced bone disease and immune inflammation by over-expressing or silencing genes of interest

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**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

Use of lentiviral vectors to over express or knock down genes predicted to be involved with general tumour and immune biology.

**Recipient or parental organism**

E. coli K-12
**Mammalian cell lines and primary cells**

Lentiviral production cells based on HEK 293 cells

**Host/vector system**

Standard second and third generation replication defective lentiviral vector systems such as pLENTI

**Origin & function**

The transcribed gene product will either be a non-coding gene (e.g. microRNAs), protein-coding gene or the corresponding knockdown sequence.

Some of the genes could potentially be tumour suppressors and/or oncogenes and therefore an overexpression or knock-down may result in a decrease or increase in the malignant capability of the cell lines, particularly in their ability to proliferate, migrate and secretion of growth factors.

Other genes being looked at are involved in the innate immune system such as receptor genes including members of the killer cell immunoglobulin-like and leukocyte immunoglobulin-like receptor family (KIR and LILR) and HLA-molecules and genes which regulate their function into natural killer cell and T cell lines and antigen presenting cells (APC).

Commonly used reporter genes such as GFP, luciferase, etc.

Standard antibiotic resistance genes such as puromycin, hygromycin, etc

WPRE which is known to enhance shRNA function by stabilizing and promoting nuclear export of the shRNA transcripts and also increases lentiviral titres by facilitating packaging of the virus. However, there have been concerns about the use of WPRE, as it has been reported that it may lead to tumourigenesis, especially if it encodes the X protein.

**Evaluation of foreseeable effects**

Due to the nature of the project a number of the genes could either be tumour suppressors or oncogenes when they are over-expressed or if the gene sequence is used to knock out the corresponding gene. Therefore there is a potential increase in hazard when these genes are used in combination with lentivirus which is infectious for a single round after exposure. However working practices are such that exposure is unlikely to occur.

Once the virus has integrated into the cells it is incapable of generating more viral particles and as such these cells can be handled at containment level 1 (class 1) after 48 hours and a change of media.

Overexpression or knocking down of innate immune receptor genes is unlikely to have a harmful effect should accidental exposure occur, the greater risk is from the presence of the WPRE.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycles as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by

**Project Containment**

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Large Scale Activities</th>
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**Project Ref** 553/13.2
Use of replication defective viruses to assess the role of genes thought to be involved in neurodegenerative diseases

### Purposes of the contained use

Replication defective lentiviruses will be used to alter the expression of genes involved in the process of neurodegeneration. Directed differentiation of transfected stem cells and subsequent in vitro methods will be used to functionally characterise both molecularly and electrophysiologically the effect of these genes.

### Recipient or parental organism

Packaging cell lines such as HEK293 cells (and derivatives of) are especially disabled hosts and require strict nutritional requirements for survival. They do however contain fragments of viral sequences.

Mammalian primary cells, such as murine embryonic stem cells and progenitor cells are especially disabled hosts and require strict nutritional requirements for survival.

Mammalian cell lines are especially disabled hosts and require strict nutritional requirements for survival.

E. coli K12, B-derivatives and Saccharomyces cerevisiae are disabled hosts and cannot colonise humans - ADCP 1

### Host/vector system

3rd generation replication defective retro / lentiviral vectors which contain WPRE

### Origin & function

All the DNAs/gene products are well characterised and are either associated with neurodegeneration or neurotrophin receptor signalling. Plasmids generally encode fragments of known cDNAs, intact cDNAs for expression in mammalian cell lines, or mammalian genomic DNA. Where full cDNAs are used, none are predicted to alter pathogenicity of host organisms or to cause adverse effects in humans.
Standard reporter genes and antibiotic resistance genes such as GFP, luciferase, beta galactosidase, kanamycin, neomycin and ampicillin.

Wild type and mutated genes coding transcription factors potentially involved in the striatal lineage commitment/differentiation process. The expected action will be the modification of these processes.

ShRNAs that prohibits translation of specific mRNAs, reducing target protein levels in particular of the products of previously mentioned genes.

**Evaluation of foreseeable effects**

In the lentiviral vector systems essential genes are split onto multiple plasmids which have limited sequence homology. In addition non essential genes are removed from the vectors. As such it is highly unlikely that a recombination event could generate a replication competent virus. However the replication defective virus is infectious for one cycle and has the potential to integrate into the host genome.

Packaging cells lines once transfected with viral vectors produce infectious but replication defective viral particles.

Vectors contain WPRE which may have oncogenic potential.

ShRNA against the genes may affect the differentiation of neuronal cells and is not expected to pose any risk to the worker. Over expression or mutation of the genes is not expected to pose a risk to the worker.

Once primary cells and cells lines have been transduced they do not pose any greater risk than the unmodified versions due to the replication defective nature of the viruses

Risks posed by in vivo work are limited to the use of needles and the presence of the WPRE gene. All workers will have attended the home office course and suitably trained. Minimal risk posed to husbandary staff.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

02/03/2022
Animal bedding – EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref**  553/13.3

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<td>03/06/2013</td>
<td>A Drosophila model of Candida albicans gastrointestinal infection</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Date Project Ceased: 02/03/2022
Innate immunity is the first line host defence present in all metazoans. Given the extensive evolutionary conservation of innate immunity between fruit flies and mammals, we have established the fruit fly Drosophila melanogaster as a model to study gastrointestinal infection by the human fungal pathogen Candida albicans (C. albicans). The model can be used as a platform to explore virulence and pathogenicity of C. albicans at a level of detail unavailable in mammalian experimental models (see below). We want therefore, to use a library of C. albicans deletion mutants to discover the fungal attributes that contribute to pathogenicity in the gut and participate in the shift from a gastrointestinal (GI) infection to a systemic one during immunosuppression of the host.

Recipient or parental organism
C. albicans wild type reference strain SN152 and derived mutants with auxotrophies for histidine, leucine, and arginine.

Host/vector system
The two deletion mutant libraries have already been constructed elsewhere. Fusion PCR was used to synthesize gene disruption cassettes containing either C. dubliniensis HIS1 or C. maltosa LEU2 flanked by ~350 nucleotides matching sequences upstream and downstream of the target gene. The knock out therefore was performed as a homologous recombination event without using transposable elements. One of 48 20-nucleotide barcodes was included adjacent to the selectable markers. Heterozygous gene disruption strains were constructed by transformation of SN152 with a HIS1-marked gene disruption cassette; His+ transformants were screened by colony PCR for the presence of expected 5’ and 3’ junctions of the integrated DNA. Homozygous gene disruption strains were constructed by transforming the heterozygous knockout strain with a LEU2-marked gene disruption cassette; His+Leu+ transformants were screened for expected 5’ and 3’ junctions of the second disrupted allele and for absence of the original target ORF. At least two homozygous knockout isolates were obtained for each target gene.

Origin & function
Mutations are all deletions of normally expressed genes. Genes identified as contributing to pathogenicity via loss of immune response in the D. melanogaster model upon deletion will be re-integrated into genome to demonstrate restoration of response.

Evaluation of foreseeable effects
The mutations that were introduced do not affect the organism's fitness but they do not increase it, since they are either neutral or lead to loss of a normal function6,7. Recent paradigms using these libraries of C. albicans mutants did not indicate any incident of hyper-virulence20, 21. Hence, there is no indication to date suggesting any increase in pathogen fitness or virulence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes, vials with Drosophila larvae-pupae) - autolave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130° for at least 10 minutes or 134-138° for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - optionally pre-treat using 2% Virkon then always autolave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130° for at least 10 minutes or 134-138° for at least 3 minutes), discharge to drains.

Agar plates - autolave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130° for at least 10 minutes or 134-138° for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpeks) - dispose via clinical waste stream for incineration.

Degree of kill -
- autoclaving, effectively 100% kill annual validation;
- incineration, effectively 100% (licensed incinerator).

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

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**Project Containment**

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**Animal Units**

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Project Ref 553/13.4

Use of recombinant viral vectors for expression of exogenous genes in cells of the central nervous system in vivo

Class 2

Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Use of EnvA-pseudotyped, glycoprotein (G)-deleted mutant of the SADB19 strain of rhabdovirus expressing genes to help elucidate neural circuit function.

Recipient or parental organism

SADB19 is a naturally attenuated strain of Rhabdovirus used as an oral vaccine for wild animals throughout Europe. The attenuated strain has not been derogated be ADCP and therefore remains in the same hazard group at the wild type - Hazard group 3

Host/vector system

pcDNA-SAD-deltaG- transgene plasmids

Origin & function

EnvA envelope protein of avian sarcoma and leukemia virus.

Fluorescent reporter proteins, e.g. GFP, mCherry or tdTomato and other standard reporter genes.

Light-activated ion channels/pumps (and their reporter protein fusions), e.g. Channelrhodopsin-2 (ChR2) or the Archaerhodopsins (Arch and ArchT).

Enzymes (e.g. Cre or Flp recombinases, horse radish peroxidase).

‘Designer Receptors Exclusively Activated by Designer Drugs’ (DREADDs), e.g. hM4D(Gi), will be inserted into the plasma membrane of cells. DREADDs are only active in...
the presence of their synthetic ‘designer’ ligands.

Other receptors/ligands, e.g. TVA, the highly-specific cognate receptor for the avian sarcoma and leukosis virus envelope protein EnvA.

**Evaluation of foreseeable effects**

SADB19 is a naturally attenuated strain of Rhabdovirus and has been used as an oral vaccine strain throughout Europe since the 1970's with no known adverse effects. Research has shown that of the 16 species tested all remained free of clinical signs except a small percentage (5.7) of murine species and a high percentage of immunocompromised mice (nude and scid) when inoculated intramuscularly. Importantly in these animals the virus was not detected in the salivary glands and was not passed on to untreated controls in the same cage. In addition the vector to be used has the glycoprotein G deleted this renders the virus unable to spread from the initially-infected ‘primary’ cells. The Env gene is replaced with the EnvA gene from avian sarcoma and leukosis virus and mammals do not naturally express any known receptor for EnvA therefore pseudotyping the virus with this envelope will decreasing the risk to humans and other mammals.

Fluorescent reporter proteins, e.g. GFP, mCherry or tdTomato, or other standard reporter genes will accumulate intracellularly and/or be inserted into the plasma membrane, thereby labelling the transduced cells. These proteins have a history of safe use.

Light-activated ion channels/pumps (and their reporter protein fusions), e.g. Channelrhodopsin-2 (ChR2) or the Archaerhodopsins (Arch and ArchT), will accumulate intracellularly and be inserted into the plasma membrane.

Enzymes (e.g. Cre or Flp recombinases, horse radish peroxidase) will accumulate intracellularly.

‘Designer Receptors Exclusively Activated by Designer Drugs’ (DREADDs), e.g. hM4D(Gi), will be inserted into the plasma membrane of cells. DREADDs are only active in the presence of their synthetic ‘designer’ ligands.

Other receptors/ligands, e.g. TVA, the highly-specific cognate receptor for the avian sarcoma and leukosis virus envelope protein EnvA, will accumulate intracellularly and be inserted into the plasma membrane of cells.

None of the inserts are expected to increase the hazard of the virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.
Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Guidance sought from AHVLA with regards to SAPO status of SAD vaccine strain

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Animal Units

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Project Ref 553/13.5

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<td>Armed oncolytic viruses for the treatment of cancer</td>
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<td>&lt; 1 Litre</td>
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### Project Additional Information

**Purposes of the contained use**

Replicating and conditionally replicating (so called 'oncolytic') adenoviruses and Herpes simplex virus 1 expressing agents capable of further potentiating its anticancer effects.

**Recipient or parental organism**

Adenoviruses such as ColoAd1 which is an Ad11p/Ad3 chimeric group B adenovirus virus or conditionally replicating ones which are dependant upon tumour associated transcription factors or tumour-associated genotypic/phenotypic changes to allow replication.

Herpes simplex virus 1 (17+ strain) with both copies of the neurovirulence gene ICP34.5 gene removed from the virus in order to attenuate neurovirulence in mice and restrict tropism to transformed or malignant cells.

**Host/vector system**

Standard reporter genes such as luciferase, GFP (and its variants) and standard antibiotics

For Adeno virus work:
- Enzymes to increase spread of virus through solid tumour stroma
- Immune modulating agents
- Agents used to affect the immune system
- Prodrug activating enzymes such as E. coli nitroreductase (activated CB1954), cytosine deaminase/UPRT (activates 5-fluorocytosine) or Herpes virus thymidine kinase (activates gancyclovir)

Various promoters such as CMV, ubiquitin promoter or IRES or P2A sites to link expression of the transgene to that of specific virus proteins for strong ubiquitous expression.
### Tissue-specific and cancer-selective promoters.

For HSV work:
Antibodies for use as immune modulating agents.

Cytokines to modulate the tumour microenvironment such as interferon.

Vaccines consisting of a peptide antigen fused to co-stimulatory molecules.

### Evaluation of foreseeable effects

For Adenovirus work:
ColoAd1 has a wild-type Ad11p backbone with a chimeric E2 region and deletions in E3/E4 and was created by hyper-infection of HT20 colorectal carcinoma cells, with multiple adenovirus serotypes. The selection process involved the collection of progeny virus at short time points after infection. This favoured chimeras with the minimal genome necessary to permit replication in tumour cells (shorter genome faster replication). In comparison with Ad11, ColoAd1 is attenuated in normal cells through loss of genomic sequences. It is also further vulnerable to the immune system due to the absence of E3 genes.

Other conditionally replicating adenoviruses can be divided loosely into those that are dependent on tumour-associated transcription factors to regulate expression of key virus proteins, and those dependent on tumour-associated genotypic/phenotypic changes to complement functional deletions in the virus. Transgenes inserted into either E1 or E3 will render the virus either unable to replicate or more vulnerable to the immune system.

Wild type and conditionally replicating human adenovirus cannot be propagated in murine tissue. The performance of the viruses and combinations of viruses will be assessed by intravenous and intratumoural injection into nude and DCID mice that have been pre-implanted with human tumour xenografts. The Virus should replicate in the human tumour tissue but cease when the replication competent (or conditionally replicating) virus reaches a non-permissive cell (eg a mouse cell) replication of both viruses will cease.

For transgenes that are deemed to be harmless such as reporter genes, species specific antibodies and enzymes that have no damaging effect on tissues, strong ubiquitous promoters such as CMV may be used.

For transgenes with potential deleterious effects their expression will be carefully controlled using either tumour-specific promoters or by placing them under control of the adenovirus promoter. Such transgenes will only be employed in adenoviruses that are heavily attenuated and incapable of supporting a productive infection in humans or animals, for example in adenoviruses that have major deletions in their E3 regions and cannot resist rapid elimination by the immune system.

For HSV work:
Herpes simplex 1 with both copies of the neurovirulence gene ICP34.5 gene removed. ICP34.5 counteracts the interferon response in normal cells by causing inactivation of PKR. Many cancer cells have dysfunctional PKR signalling and thus an ICP34.5 disabled herpes virus is still capable of replicating selectively in tumours.

Antibodies for use as immune modulating agents. Antibodies used will be specific for murine antigens, therefore not pose a threat of interfering with the human immune system upon potential exposure.

Cytokines will be used to modulate the tumour microenvironment in an attempt to override immunosuppressive signals produced by tumour cells and associated Tregs and fibroblast cells. There is significant overlap between some cytokines/receptors between mice and humans, however, only cytokines with no cross reactivity between mice and humans will be used such that upon any potential exposure will not interfere with the human immune system.

Vaccines will consist of a peptide antigen fused to co-stimulatory molecules. In this case it is possible that mouse ligand could cross react with human receptor upon potential human exposure, however, any effects separate from ICP34.5 deleted HSV-1 should be against the cancer associated antigen only.
### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes of 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.  
  
  Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes of 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.  
  
  Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes of 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.  
  
  Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.  
  
  Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes of 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.  
  
  Animal carcasses - dispose via clinical waste stream for incineration. |
|---|
| Degree of kill:  
  Autoclaving, effectively 100% kill (annual validation)  
  Incineration, effectively 100% kill (licensed incinerator)  
  Chemical, effectively 100% kill (following manufacturers guidelines)  |

### Is an emergency plan required according to regulation 20?  
N

### If yes, tick to confirm that it is attached to this form  
N

### Tick to confirm that you have attached a risk assessment to this form  
Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

---

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidelines issued by ACGM

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**Project Containment**

02/03/2022
Elucidating the mechanism of chromosome segregation in Trypanosoma brucei

Faithful chromosome segregation is essential for the proliferation of all organisms. Although studies in conventional model eukaryotes have found that macromolecular kinetochore complexes assemble onto centromeric DNA to facilitate segregation, it is not known whether this mechanism applies to all eukaryotes. To uncover fundamental principles of eukaryotic segregation machinery, we study kinetochores in Trypanosoma brucei, an evolutionarily distant eukaryotic parasite. Using a localization-based screen and proteomics, we have identified 19 kinetochore proteins. We aim to characterize these proteins using various techniques both in vivo and in vitro to reveal the molecular mechanism of chromosome segregation in trypanosomes.

Trypanosoma brucei brucei. Procyclic forms of the parasites are non-infectious and T. brucei brucei strains are lysed by human serum. Use is mainly made of strains that have been engineered to express T7 polymerase and Tet repressor to facilitate inducible expression of transfected genes. Engineered genes are effectively silent in the absence of inducer.
Trypanosome vectors are based on a series of vectors constructed to provide prokaryotic tetracycline mediated inducible expression using either endogenous promoters or systems employing T7 polymerase and prokaryotic promoters/repressors.

**Origin & function**

Trypanosome genomic DNA is purified from Trypanosoma brucei brucei. Trypanosome genes/gene fragments will be expressed in E. coli/mammalian cells/insect cells to produce non-toxic recombinant products. Studies of gene function involve the targeted disruption of trypanosome genes through homologous recombination. In addition, RNAi gene knockdowns will be performed by expression of double-stranded RNA to endogenous genes via expression from opposing promoter vectors or as inverted repeat constructs. These are established technologies in trypanosomes. Trypanosome deletion mutants or RNAi cells, induced by tetracycline, are tested for phenotype during in vitro culture.

The main types of gene insert to be used are:

- Genes encoding kinetochore proteins that play a role in mediating the interaction between centromere DNA and spindle microtubules, as well as other mitotic proteins. Usually these genes will be tagged to provide epitope or other marking of the expressed proteins.
- Portions of genes arranged to produce double stranded RNA copies that will down regulate endogenous gene expression by means of RNA interference.
- Flanking sequences will be used to engineer gene targeting and knockouts.

**Evaluation of foreseeable effects**

The main focus of our work is genes that are likely to be important for chromosome segregation, cell cycle and nuclear organization. Final GMOs will contain one or more of the trypanosome genes of interest. In each expression system there is deliberate, in frame insertion of expressible DNA downstream of a strong promoter. However, the genes expressed are non toxic and will not increase the survival, replication or pathogenicity of the host strains.

Most experiments will not involve cross-species transfer. Some experiments will test putative homologous genes from other strains/species/genus for complementation of function or inactivation of function in trypanosomes. Given that the genes to be moved are involved in housekeeping functions and are not known (or likely to be) virulence genes, the likely outcome of movement of genes is loss of virulence, and in the case of movement into a non-pathogenic strain will not result in a gain of virulence.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes, vials with Drosophila larvae-pupae) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138° for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media - optionally pre-treat using 2% Virkon then always autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138° for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138° for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.
Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill -
autoclaving, effectively 100% kill (annual validation);
icineration, effectively 100% (licensed incinerator).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tbody>
<tr>
<td>L2</td>
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### Project Ref 553/13.8

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<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Date Project Ceased</th>
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<tr>
<td>10/09/2013</td>
<td>Examining the role of chromatin influencing DNA double-strand break repair pathway choice in mammalian cells</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

02/03/2022  Page 9125 of 1532
### Project Additional Information

**Purposes of the contained use**

Use of retro and lentivirus to elucidate the function of molecular events propagated in chromatin upon DNA damage detection, the influence these have on the execution of accurate DNA repair, and how defects in these events manifest in immune deficiency and cancer.

**Recipient or parental organism**

- E. coli K12 derivatives
- Mammalian cell lines such as 293 derivatives, U2OS, RPE, He La
- Primary cells

**Host/vector system**

- Adeno associated virus - replication defective
- Lentivirus - replication defective
- Retrovirus - replication defective

**Origin & function**

- Genes such as BRCA1, 53BP1, RIF1 and associated proteins thought to be involved in coordinating chromatin reorganisation during double strand break repair.
  
  RIF1 is thought to be the critical effector of 53BP1-dependent DNA repair by non-homologous end joining
  
  BRCA1 (Breast cancer 1 early onset gene) is responsible for repairing DNA and mutations of this gene are linked to an increase in risk of cancer.
  
  53BP1 (tumour suppressor p53 binding protein 1) mediates DNA repair and is underexpressed in some cancers
  
- Other genes expected to play a role in double stranded break repair
  
  WPRE (present in the lentiviral vectors) is a potential oncogene
  
- Reporter genes such as GFP, strep-Tag and FLAG
Standard antibiotic resistance genes

Evaluation of foreseeable effects

AAV virus - Wild type AAV are not thought to cause disease in human. Replication defective versions will be used. Genes required are separated onto 3 plasmids and a packaging cell line reducing the likelihood of recombination. The viral particles do not contain any of the viral genome but only the targeting construct comprising homology regions proximal to initiator/terminator sequences of the gene of interest coding sequence to an epitope tag, a selection marker, and recombination sites (loxP/frt) to facilitate the subsequent excision of selection markers if required. All transgene-expression related sequences of the gene to be tagged to facilitate accurate gene targeting. The transgene should integrate into a targetted region as opposed to remaining episomal. Expression of the tagging proteins is not expected to increase the hazard of the AAV.

Lentivirus - replication defective. The gene products required for viral particle generation are split onto 3 plasmids and a packaging cell line to reduce the possibility of recombination. The viral particles do not contain viral genes only cDNA or shRNA of interest and selection marker. Lentiviral particles randomly integrate.

Retrovirus - is replication defective and based on MoMLV (mouse leukemia virus). Essential viral genes are split between a shuttle vector and the packaging cell line to reduce the possibility of recombination. The viral particles do not contain viral genes only cDNA or shRNA of interest and selection marker.

Occasionally the genes to be over expressed/knocked out may be a tumour suppressor genes. Overexpression is not expected to be hazardous however knocking out the gene has the potential to be hazardous.

Expression of WPRE in the lentiviral vectors is potentially hazardous.

Generation and work with viral particles - Class 2

Transfected cell lines are not expected to be hazardous after initial round of integration

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
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- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

**Project Containment**

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**Project Ref** 553/13.9

<table>
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<tr>
<td>27/11/2013</td>
<td>Nano-scale localization studies of the cell wall synthesis and division machineries of Staphylococcus aureus by super-resolution microscopy</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
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<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

Withdrawn | N

Tick if notifying a connected programme of work | N

02/03/2022
This project aims to use two techniques of super-resolution microscopy (SIM and STORM, both available at the Department of Biochemistry) to characterize the localization of proteins required for cell and synthesis and cell division of S. aureus.

Recipient or parental organism
Staphylococcus aureus - Hazard group 2
strain RN4220: methicillin susceptible strain
strain NCTC8325-4: methicillin susceptible strains
strain COL: methicillin-resistant strain

Host/vector system
Genetically modified strains were created elsewhere using pMAD vector, pCNX (Derivative of the pCN51 vector) and pMGPII vector

Origin & function
Proteins involved in cell wall synthesis and division, such as PBP2 and PBP4 (involved in peptidoglycan biosynthesis), SpolIIIE and Slp (two DNA translocases required during cell division) and FtsZ and EzrA (two components of the divisome). If required other proteins will also be studied.

Fluorescent marker genes such as GFP, YFP and mCherry

Photswitchable and photoactivatable fluorescent fusions such as PAmCherry (red), PAGFP (green) and green-to-red photoswitchable mMaple.

Standard antibiotic resistance genes such as erythromycin, chloramphenicol and kanamycin.

Evaluation of foreseeable effects
Staphylococcus aureus are bacteria commonly carried on the skin or in the nose of healthy people without causing any disease. However in some cases can cause infections that are usually mild, superficial infections of the skin, however in some cases it does have the potential to cause serious life threatening illness.

Strains RN4220 and NCTC8325-4 are laboratory reference strains, susceptible to antibiotics normally used to control S. aureus infections. RN4220 has a mutation in the agr locus and NCTC8325-4 has a mutation the the sigmaB locus. Both mutations result in attenuated ability to cause infection. Strain COL is resistant to methicillin and
other beta-lactam antibiotics. However it is susceptible to a variety of antibiotics.

Addition of fluorescing genes is not expected to make the S. aureus more hazardous.

Deleting the genes of interest or mutating them is not expected to affect the pathogenicity of the bacterium.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration - Use of sharps is minimised in this project.

Degree of kill:
- Autoclaving, effectively 10% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

### Is an emergency plan required according to regulation 20?  

| N |

### If yes, tick to confirm that it is attached to this form

| N |

### Tick to confirm that you have attached a risk assessment to this form

| Y |

### Tick if you are claiming exemption from disclosure for section of the risk assessment

| N |

### Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

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Project Containment
### Project Ref 553/14.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tr>
<td>20/01/2014</td>
<td>Investigating novel molecular pathways to induce cardiac development and repair</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

- **Non-GMM Consent Granted**
- **Project notified under transitional arrangements**

#### Historical Significant Changes
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

#### Project Additional Information

**Purposes of the contained use**

Use of replication defective lentiviral vectors to aid the study of cardiovascular disease

**Recipient or parental organism**

- Mammalian cells such as primary epicardium cells.
- Standard cell lines.
- Lentiviral production cells based on 293 cells and their derivatives

**Host/vector system**

02/03/2022  

Page 9131 of 15326
Standard replication defective lentiviral vectors which contain the minimal genes necessary split onto 2 or 3 separate plasmids (second or third generation vector systems).

In certain cases the Tet ON system will be used in the viral vectors so that the gene of interest will only be expressed in the presence of doxycycline enhancing the safety features.

### Origin & function

c-myc appears to play a central role in cell growth regulation and cell metabolism and its overexpression is often observed in cancerous cells. It is also one of four genes which when combined are used to create induced pluripotent stem cells.

WPRE - Woodchuck post regulatory response element which is known to enhance shRNA function by stabilizing and promoting nuclear export and also increase lentiviral titres by facilitating the packaging of the virus. However, there have been concerns about the use of WPRE as it has been reported that it may lead to tumourigenesis especially if it encodes the X protein.

Standard antibiotic resistance genes such as ampicillin, puromycin and hygromycin etc.

Commonly used reporter genes such as GFP, luciferase etc.

Overexpression or knockdown of key factors or small molecules to stimulate proliferation, migration, differentiation and maturation, with the potential to promote cardiovascular repair and regeneration.

Expression or knockdown of a number of these genes could have potentially deleterious effects although use of control measures to minimise percutaneous and inhalation exposure will reduce the risks to minimal.

### Evaluation of foreseeable effects

Rhomboids are intramembrane serine proteases that cleave membrane proteins within the bilayer and are involved in a wide range of biological processes. Mammalian rhomboid targets include thrombomodulin, ephrinB4 and EGF but Rhomboids are generally highly specific with a limited number of substrates.

iRhom molecules lack intramembrane protease activity but are required for inactivation of Tumour Necrosis Factor α Converting Enzyme (TACE) and thus secretion of TNFα and various EGFR ligands.

EGFR ligands, such as EGF, amphiregulin and HB-EGF will lead to activation of downstream signalling cascades such as the MAPK and JNK pathways leading to processes such as migration or proliferation. As such a precautionary approach will be taken.

The diversity of biological functions already known to depend on rhomboids is reflected in evidence that rhomboids play a role in a variety of diseases including cancer, parasite infection, Parkinson's disease and diabetes.

Standard fluorescent protein reporter genes, such as enhanced green fluorescent protein (EGFP), will be transduced into cells using lentiviral vectors, to provide positive controls with which to assess the efficiency of lentiviral transfer. Fluorescent proteins are a common investigative tool and no hazards are associated with their use.

The silencing of rhomboid and rhomboid related genes can be achieved using a Cas9 CRISPR-associated nuclease expressed under an EFS promoter which is guided to specific sites within the host genomes of human and mouse cells by a customizable sgRNA (synthetic single-guide RNA), under a U6 promoter, DNA double strand breaks, catalysed by Cas9, create frame shift insertion/deletion (indel) mutations that result in a loss-of-function allele.

Some concern has been raised with regards to the potential oncogenic nature of the WPRE which is present in many lentiviral vectors and therefore precautionary approach is adopted with these lentiviral particles.
Other risks from lentiviral particles is from their potential to integrate within a tumour supressor region of the genome however that will be minimised due to the lack of sharps within this project.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment
The project aims to investigate possible interactions between Streptococcus pyogenes, the agent responsible for group A streptococcal infections, and vessels/sinuses of the lymphatic system that may provide routes of entry, dissemination or sequestration in mucosal tissue and draining lymph nodes.

Streptococcus pyogenes causes human disease, ranging from simple (asymptomatic) colonisation of the throat, to pharyngitis, cellulitis, to necrotising soft tissue infection, and bacteremia with septic shock and death. It can also cause immunological sequellae such as rheumatic fever, the pathogenesis of which is unknown.

Recipient or parental organism

Streptococcus pyogenes causes human disease, ranging from simple (asymptomatic) colonisation of the throat, to pharyngitis, cellulitis, to necrotising soft tissue infection, and bacteremia with septic shock and death. It can also cause immunological sequellae such as rheumatic fever, the pathogenesis of which is unknown.

Host/vector system

pDL278 and derivatives. Confers SpectinomycinR
pGHost5 and derivatives. Confers Erythromycin R
pUCMUT and derivatives (gene targeting/knockout vector) Confers kanamycinR
pICL18 and derivatives including pICL18.LUX.

**Origin & function**

Short non-functional segments of putative S. pyogenes virulence genes to facilitate homologous recombination and mutagenesis of target genes.

Plasmids used for complementation will carry coding sequences of putative virulence factors that are missing from the host organisms eg superantigen genes spea, smez, mf; enzymes such as spycop; regulatory genes such as covRS.

Plasmids carrying reporter genes will include genes for fluorescent proteins such as gfp and other spectral variants; genes encoding bioluminescent systems (the luciferases are enzymes that produce light as a by-product of oxidation of a specific substrate, eg luxABCDE, gluc, lucff)

**Evaluation of foreseeable effects**

The GMM's should be no more hazardous than the parent strains from which they were derived. Any excess risk is related to (a) the introduction of additional antimicrobial resistance genes that may impede the efficacy of antimicrobials if required, and (b) the inadvertent but rare genetic modification that results in unforeseen increase in virulence, for example in a regulatory gene. Hazards from antimicrobial resistance are minimised by use of antimicrobial markers that are not in clinical use. Mutations in regulatory genes can occur spontaneously in nature, and are selected for during invasive infection; as such the risk of such mutations arising in nature is possibly greater than the risk that they occur in the laboratory.

All strains are sensitive to penicillin (and its derivatives)

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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Animal bedding – EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.
Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM.

**Project Containment**

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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
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</tr>
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**Project Ref**  553/14.2

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<th>Class</th>
<th>Culture Vol</th>
<th>Class-2</th>
<th>Culture Vol</th>
<th>Class-3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<tbody>
<tr>
<td>26/03/2014</td>
<td>Generation of replication incompetent HIV-1 particles</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Date Project Ceased

Withdrawn N Tick if notifying a connected programme of work N

Historical Significant Changes

02/03/2022
## Project Additional Information

**Purposes of the contained use**

This project aims to produce replication incompetent fluorescent and non-fluorescent HIV-1 particles. These particles will be used for the superresolution microscopy studies of the distribution and dynamics of HIV proteins in the context of the free virus, initial virus-cell interactions during virus attachment and fusion as well as at the plasma membrane of HIV producing transfected cells.

**Recipient or parental organism**

- Mammalian cell line HEK293 is used for the production of the virus
- Mammalian cells to study the interaction of the virus

**Host/vector system**

- pCHIV (and derivatives there of) encode replication incompetent viral particle when placed in a permissive cell type such as HEK293 cells.

**Origin & function**

- pCHIV is based on the Human Immunodeficiency virus which is assiged to ACDP hazard group 3. The full DNA sequence of HIV-1 was placed into a plasmid vector pcDNA3.1 to create HIV-1NL4-3.

- pCHIV plasmid and all its derivatives encode for replication incompetent particles that express all HIV-1NL4-3 proteins but lacks Nef open reading frame as well as viral long terminal repeat regions (LTRs). These sequences are indispensable for initiation of reverse transcription as well as integration of resulting cDNA into the host genome. Nef is an important HIV accessory protein that is one of the first to be produced in HIV infected cells. It performs many numerous regulatory functions that are dispensable in vitro, but are essential for efficient virus spread and disease progression in vivo.

- A version pCHIV will also be used that lacks the env gene of HIV. This will subsequently be cotransfected with a second plasmid containing a different env gene, such as an env with a truncated C-terminal tail.

- Versions will also be created that have fluorescent marker genes, such as GFP or Cherry, added to Vpr

**Evaluation of foreseeable effects**

- Viral particles will be produced which are replication incompetent due to the lack of LTR genes and a functional Nef, they will however still be able to infect mammalian cells.

- To ensure that the plasmid preparations have not regained the LTR genes or a functional Nef (highly unlikely), the preparations will be tested by restriction digest and the results compared with the plasmid map to confirm there are no significant sequence changes.

- The likelihood of the virus gaining functional genes. This is unlikely as each viral preparation is created in a transient manner.

- As the LTR genes are required for integration it is unlikely that these viral particles will be able to integrate into the host genome and therefore insertional disruption of a
tumour suppressor gene is unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100 % kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

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<th>CultureVolumeClass3-4</th>
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<tr>
<td>26/03/2014</td>
<td>Expression of anti-disease agents and gene editing components in cell lines</td>
<td>Class 2</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

### Project Additional Information

#### Purposes of the contained use

Use of replication defective lentiviral vectors to target and disrupt the interactions of the oncogenic proteins in mammalian cells

#### Recipient or parental organism

E. coli - K12 derivatives
mammalian cell lines
Primary mammalian cells
Packaging cell lines, such as HEK293

#### Host/vector system

Standard replication defective retro and lentiviral systems, such as MLV-ecotropic pCAG-Eco, pCNV-d8.2, pBabe, pMiG, pGIF/pFUW

Origin & function
Inserts code for engineered Variable Heavy (VH antibody domains or peptide aptamers, which are designed to be specific for various disease-causing proteins. They have no known oncogenic or other harmful effects, with a safe history of use. Other genes which may be implicated in leukaemias and other blood based diseases. Viruses may also encoding cas9 & gRNA for gene editing with the objective to knock in or knock out genes to assess their roles in leukaemias and other blood based diseases, such as CRISPR technology. Woodchuck hepatitis regulatory element for enhanced expression is present in the lentiviral vectors. Also standard marker/reporter genes such as lac Z, GFP, and localisation sequences etc. may be included and that also have a safe history of use. Standard antibiotic resistance genes, such as Kanamycin, Ampicillin etc.

Evaluation of foreseeable effects

Retrovirus and lentivirus particles are generated to transduce the mammalian cells. They are replication deficient as packaging elements necessary for their propagation are supplied by a packaging cell line or as additional helper plasmids, which lack packaging signals. Therefore, the resulting virus particle does not contain the structural genes required to produce replication-competent virus post-transduction, minimising hazard. The lentiviral vectors contain the Woodchuck hepatitis regulatory element (WPRE) for enhanced expression. This element may have oncogenic potential and as such work with these viruses is assigned to class 2.

Standard reporter genes and antibiotic resistance genes have a history of safe use and are not expected to increase the risk.

Inserts code for engineered variable heavy chain antibody domains or peptide aptamers which are designed to be specific for various disease causing proteins. They have no known oncogenic or other harmful effects.

Gene editing technologies, such as CRISPR allow targeted gene editing. They require sequence specific homology to allow specific point mutations which are user defined effectively causing a frameshift insertion/mutation. It is not expected that this mutation would be any more hazardous than that posed by insertional mutagenesis of the virus itself or the presence of the WPRE gene.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
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- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

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Animal Units: L2 | L3 | L4

Large Scale Activities: L2 | L3 | L4

Human Clinical Applications: L2 | L3 | L4

Project Ref 553/14.4

Use of Lentivirus to evaluate transgenes in cell types with poor transfection efficiency (e.g. cardiomyocytes)

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N
### Project Additional Information

#### Purposes of the contained use
Lentivirus will be used to transfect mammalian cells, such as cardiomyocytes or other excitable cells with genes linked to cardiovascular disease and/or genes useful for visualisation of cellular signals.

#### Recipient or parental organism
- **E. coli K12 derivatives**
- Primary and cell lines of mammalian cells.
- Packaging cell line, such as HEK293 cells.

#### Host/vector system
Standard vectors for the generation of lenti and retroviral particles such that the necessary genes are split onto a number of plasmids which have limited homology to prevent minimise recombination.

#### Origin & function
Genes of interest that will be useful in understanding how cardiomyocytes change their behaviour in response to disease causing mutation.

- Standard reporter genes, such as GFP etc
- Altersations to standard reporter genes which allow them to respond to second messengers, such as Ca++, ATP, cAMP and membrane potential etc.
- Light sensitive channels, such as Channel rhodopsin, and Halorhodopsin and derivatives there of.
- Standard antibiotic resistance genes, such as hygromycin and neomycin
- Genetic sequences that allow controlled gene expression, such as Tet etc
- Sequences that allow site specific recombination, such as Cre etc

#### Evaluation of foreseeable effects
The viral particles are infectious but cannot replicate. Therefore they are not transmissible from one individual to another. However, viral particles have the potential to integrate into the host genome which may cause intentional mutagenesis. In addition, viral particles contain WPRE which may have the potential to be oncogenic.
Inserts are genes thought to be involved in cardiovascular disease and may be either over expressed or knocked down. In addition genes that interact with these proteins may be altered.

Expression of reporter genes have a history of safe use and there is no indication that the alterations, such as calcium signalling sequence would affect this.

Genes of interest are not expected to be oncogenic and are unlikely to lead to a clinical significant event because:-
1) many of the genes we work with are only expressed in the heart, and cause disease by affecting the way other components of the heart cell behave. The same machinery does not exist in other cell types so the potential to adversely affect cell behaviour is diminished.
2) In the case of mutations in genes that cause multisystem disease (i.e. where targets are present in other tissues) skin and other epithelial defects are rarely associated with inherited cardiomyopathy or arrhythmia, even though all cells in the body are affected. Since it is impossible for the virus to infect more than all the cells in the body, it is unlikely that accidental exposure could lead to a more significant outcome than the natural history of the condition.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - dispose of solids via clinical waste stream for incineration.

Liquids (eg samples, culture supernatants, tissue culture media) – inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - dispose of solids via clinical waste stream for incineration.

Sharps (eg needles, syringes, scalpels) - No sharps will be used.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.
The focus of our research is the origin of the haemopoietic stem cells (HSCs) during mouse embryonic development Runx1 is absolutely required for HSCs generation. How Runx1 exerts its role in the onset of haemopoiesis is not known and is the focus of our studies.

Recipient or parental organism
E. coli K-12 and B derivatives - class 1
mammalian cell lines - class 1
HEK293 derived cells for packaging replication defective virus
### Host/vector system

Standard Replication defective recombinant Lentivirus and retrovirus which have the essential genes separated onto different plasmids. Some versions will be ecotropic and some will be amphotropic.

### Origin & function

Modulation of genes (overexpression or down regulate) or mutation using gene editing systems such as CRISPR and TALENS

- Runx1- regulated target genes (such as SPI1, Gfi1, Gfi1b)
- Genes encoding transcription factors directly or indirectly mediating Runx1 expression (such as Notch1, Hoxb4)
- Genes encoding Runx1-interacting proteins (such as Gata1, Cbfb)
- Other molecules such as cell cycle or gene expression regulators could be included

Woodchuck hepatitis virus post-transcription regulatory element (WPRE)

Standard antibiotic resistance genes

### Evaluation of foreseeable effects

Based on previous reports, expression of wild type and mutated Runx1, or other Runx genes, may affect the differentiation, maturation, and proliferation of mouse hematopoietic cells in vitro and in vivo. Expression of Runx1 target genes or upstream regulators (to be identified) may have an effect on in vitro and in vivo hematopoietic differentiation. In addition, we expect an alteration of the proliferation/differentiation balance or induction of apoptosis of the transduced cells and/or progeny.

Molecules identified as partners or targets or Rnx2, 2 and 3. These could involve other transcription factors, co-factors, chromatin remodelling factors, cell cycle regulators, apoptotic factors, structural proteins, members of the general transcription machinery and other regulators of gene expression. They are all expected to modify the proliferation/differentiation balance or induce apoptosis.

WPRE is a potential oncogene and as such could be hazardous in a viral system which integrates into the host genome.

CRISPR/TALENS allows for user defined genome editing (usually insertion or deletion) which results in a loss of function. Although, CRISPR/Cas vectors used are derived from the bacteria strains (S. pyogenes, S. thermophiles, T. denticola or N. meningitidis) they only contain small portions of the genome (similar to lentiviral vectors based on HIV) which has subsequently been codon optimised for expression in mammals. The genome sequences of many E. coli strains are not available so it is not known whether these strains contain intact Cas genes and/or CRISPR loci. However, introducing CRISPR vectors into these hosts is unlikely to cause any recombination due to the fact that all CRISPR and Cas-expressing genes within these vectors contain sequences that are codon-optimized for mammalian expression and thus do not have any sequence resemblance to their prokaryotic counterparts. The probability of DNA exchange between a vector and the host chromosomes can be further reduced (although not eliminated) by using a recA minus strain of E. coli, such as NEB Stable, DH5a and DH10b.

Standard antibiotic resistance genes and reporter genes have a history of safe use.

As a number of the genes have the potential to affect proliferation and differentiation their expression in lentiviral vectors which can integrate into the genome is potentially hazardous.

Primary cells and cell lines which have been infected with replication deficient lentiviral/retroviral particles are not expected to be hazardous after the particles have integrated into the genome and any residual particles removed by washing.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Degree of kill:

- Autoclaving effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturer's guidelines).

Is an emergency plan required according to regulation 20?  N

Tick to confirm that you are claiming exemption from disclosure for section of the risk assessment  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

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### Project Additional Information

**Purposes of the contained use**

The creation of a replication defective lentivirus to study interferon induction both during viral infections and autoinflammatory diseases.

**Recipient or parental organism**

- E. coli - K12 or B derivatives Hazard Group 1
- Mammalian cells - standard cell lines and primary cells
- Producer cells - generally derived from HEK293 cells
- In vivo - direct inoculation of virus or of virus infected cells

**Host/vector system**

Use of full length HIV (pNK4-3 with a disrupted env gene (by the insertion of EGFP) on one plasmid (pNL4-3-deltaE-EGFP) and the env gene, such as VSV-G or THOV-G, on a second plasmid. Production of infectious but replication incompetent viruses occurs in producer cells, such as 293 cells.

Other similar systems may be used, such as pNL4-3.Luc. R-E- where the Luciferase gene has been inserted into the Nef gene and there are 2 frameshift mutations which render it Env- and Vpr-.

**Origin & function**
EGFP and Luciferase are standard reporter genes and have a history of safe use. Other standard reporter genes may also be used in similar vector systems.

The vector contains the genetic material for HIV-1 and as such produces virions which are infectious. The viral particles are replication defective due to either the deletion on the env gene or a frameshift mutation which inactivates the env gene. The addition of reporter genes is not expected to cause an increase in pathogenicity.

All these vectors are first generation there is the potential for recreation of a replication competent virus with either wild type env or the VSV-G or THOV-G envelope genes, however this is considered unlikely due to the following reasons.

Possibility for homologous recombination in E. coli:
- HIV-1 does not infect E. coli;
- all plasmid manipulations will be carried out in a laboratory where wt HIV-1 is not present;
- recombination deficient E. coli strains are used for all recombinant DNA procedures to minimize spontaneous losses of genetic information;
- plasmids with partial or full env genes are not used in the laboratory;
- there are no flanking homology regions between the VSV-G or THOV-G gene-containing plasmids and the lentivectors and homologous recombination is therefore impossible;
- and d) wt HIV-1 cannot infect 293T cells so all wt HIV-1 would be generated only from the cell(s) in which the recombination took place and therefore the amount of wt HIV-1 would be small.

Possibility for homologous recombination in target cells:
- a) wt HIV-1 would need to be present in the same target cells, but this is impossible as we are working in a Class 2 facility where HIV-1 is absent;
- b) we are not using unscreened human cells (such as cells from blood) that may contain HIV-1 if the donor of these cells is HIV-1 positive;
- c) VSV-G or THOV-G gene sequences are not present in infected target cells.

Possibility of homologous recombination in the mouse:
- a) HIV-1 does not infect mice;
- b) there is no HIV-1 present in Animal Facility, unless the personnel were infected with HIV-1, which would cause greater risk of HIV-1 infection itself than theoretical recombination;
- c) the lentivectors and wt HIV would have to infect the same cells within a given mouse for recombination to occur;
- d) wt HIV-1 cannot infect mouse cells so all wt HIV-1 would be generated only from the cell(s) in which the recombination took place and therefore the amount of wt HIV-1 would be small;
- e) VSV-G or THOV-G gene sequences are not present in infected target cells.

Possibility of homologous recombination in humans accidentally exposed to vectors:
- a) the vectors do not produce infectious progeny particles;
- b) if the wt HIV-1 were present in humans, it would cause the disease without the need for recombination;
- c) VSV-G or THOV-G gene sequences are not present in humans.

Possibility of cross-contamination of vectors with wt HIV-1:
Purified plasmid DNA will be transfected into 293T cells. Contaminating wt HIVV-1 does not infect 293T cells and thus would not be amplified. Theoretically, plasmid DNA might also be contaminated by plasmid carrying a molecular clone of wt HIV-1, but the likelihood is very small as such molecular clones are not used in the laboratory. Vector stocks will be grown exclusively in a LEVEL-2 laboratory, in a dedicated incubator, in which the risk of accidental exposure to HIV-1 is zero.

As the virus integrates into the host genome there is a potential risk should the virus integrate into a region containing a tumour suppressor gene.

Transmission and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
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Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

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</table>
Use of self-inactivating lentiviral vectors for the study of degenerative diseases

The laboratory is interested in understanding the mechanisms underlying neurodegeneration in a wide range of diseases, such as Parkinson's Disease, Alzheimer's Disease, Friedrich's Ataxia, Amyotrophic Lateral Sclerosis and Familial Hypercholesterolaemia. The use of recombinant replication defective lentiviral particles is required due to their ability to infect quiescent cells.

Recipient or parental organism

- E. coli K12 and B derivatives - class 1
- Mammalian cell lines and primary cells - class 1
- Packaging cell lines such as HEK293 cells used for generating replication defective lentiviral particles

Host/vector system

Standard replication defective lentiviral vectors which have the essential genes separated onto different plasmids or stably expressed from the packaging cell line

Origin & function

- Reporter genes (e.g. GFP, Luciferase, B-galactosidase)
- Standard antibiotic resistance (e.g. Neomycin, Hygromycin, Puromycin and ampicillin)
- shRNAs, μRNAs.
- Genome editing tools (e.g. TALENs, Cas9-CRISPR).
- Tet repressor/activator systems.
- Neurotrophic/neuroprotective factors (e.g. BDNF, CNTF, GDNF and NGF).
Factors involved in neural reprogramming (e.g. SOX2, MASH1, GATA3),
- Neurodegenerative susceptibility genes (e.g. SNCA-1, GBA and MAPT).
- Susceptibility genes for familial hypercholesterolaemia (e.g. LDLR)
- Genes involved in the mechanisms of neurodegenerative (e.g. LC3, P62, TFEB, DRP1, DLP1)

Woodchuck post transcriptional regulatory element may be present in the replication defective lentiviral vectors

### Evaluation of foreseeable effects

Reporter genes and standard antibiotic resistance genes have a history of safe use and are not expected to increase the risk of the viral particles.

The protein products of the genes to be studied (over expressed, mutated or knocked down) are not expected to be toxic or oncogenic but some of the genes of interest may modulate immune function.

Initially, the RNAs, TALENs or Cas9-CRISPR systems to be used are not targeting known tumour suppressors. However, there is potential for off-target effects with, which could potentially have tumorigenic effects. This will be minimised by careful design with as little sequence homology as possible to off-target genes, including BLAST searches against genome databases. In the immediate future the experiments will target individual genes, it is possible however that genome-wide screening will be performed using shRNA technology with out iPS derived disease models. In this case the risk of the shRNAs will be greater, as shRNAs against all possible genes would be employed, including tumour suppressors.

Gene transfer is possible, significantly from the transfected producer cell lines, as the resulting Lentiviral/Retroviral vector will be able to infect a wide range of cells, including human cells, as the VSV-G or Rabies-G envelope enable amphotropic infection. However, dissemination in the environment is limited due to the viruses being replication defective.

Some concern has been raised with regards to the potential oncogenic activity of the post-transcriptional regulatory element from the woodchuck hepatitis virus (WPRE) which is present in mainly lentiviral vectors, including the lentiviral backbones that we will use here.

The main risks from in vivo work are associated with the method of viral delivery itself which usually involves localised injections and, therefore, the use of needles and sharps. However, with proper supervision and training of new personnel, ensuring the use of gloves, awareness of the needle position during injection, no re-shafting of needles and proper disposal directly into a sharps bin, the risk is considered to be low. Additionally, to minimise the generation of aerosols during the loading/unloading of the injecting device, viral suspensions will be prepared in dedicated Class 2 Microbiological Safety Cabinets. Once the gene is stably inserted into the target host the risk is minimal due to the replication defective nature of the virus and no additional containment is required compared to other genetically modified animals.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
<thead>
<tr>
<th>N/A</th>
</tr>
</thead>
</table>

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

<table>
<thead>
<tr>
<th>N/A</th>
</tr>
</thead>
</table>

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| N/A |

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to
drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
</tr>
<tr>
<td>Animal Units L2 Yes L3 L4 L2</td>
<td>Large Scale Activities L3 L4 L2</td>
<td>Human Clinical Applications L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 553/14.8

Date Ackn'd 13/06/2014
CU2 Project Title Vaccinia Viruses for cancer gene therapy, enhanced tumour delivery using mechanical
Class
CultureVolClass2 CultureVolumeClass3-4
Vaccinia virus has a natural tropism for tumourous cells and can accommodate large amounts of foreign DNA making them useful tools in the treatment of cancer and other diseases. They also elicit immune responses to the expressed antigens.

**Recipient or parental organism**

Vaccinia Virus, strains such as Copenhagen, Western Reserve, Wyeth

Modified vaccinia ankara

**Host/vector system**

Homologous recombination between shuttle plasmids and the viral genome

**Origin & function**

Reporter genes such as luciferase GFP etc.

Enzymes such as cytosine deaminase-phosphoribosyl transferease fusion enzyme which converts a prodrug to an active drug.

**Evaluation of foreseeable effects**

Humans are not the natural hosts for Vaccinia but injection into the skin leads to a brief and limited subcutaneous infection that stimulates an immune response. It was used world wide to eradicate smallpox and was generally well tolerated.

Inserts are placed into the Thymidine kinase gene resulting in disruption of the gene and reducing virulence in normal tissue. However, they have inherent tumour tropism thought to be due to the overexpression of cellular TK in cancer cells supplying the TK in trans.

Some strains also have ribonucleotide reductase deletions are also significantly attenuated with respect to the wild type as the gene catalyzes the formulation of deoxyribonucleotides from ribonucleotides. Deoxyribonucleotides are required for the synthesis of the vaccinia DNA genome.

Luciferase and other standard reporter genes have a history of safe use.
FUC1 converts a prodrug into an active metabolite and therefore cytotoxicity is dependent on the presence of the prodrug. Other prodrug/enzyme combinations may be used.

Ligand and retargeted and polymer coated viruses are expected to have reduced tropism and therefore less pathogenic to the wild type strains. Initially the tropism will be for cancer cells by the addition of ligands that target specific receptors on cancer cells. Other diseases may be looked at in the future.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding – EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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</table>

**Project Ref** 553/14.9

- **Date Ackn’d**: 16/07/2014
- **CU2 Project Title**: Investigation of signalling protein functions
- **Class**: 2
- **Culture Volume**: ≤ 1 Litre
- **Consent Granted**: Yes
- **Project notified under transitional arrangements**: Yes

**Historical Significant Changes**

<table>
<thead>
<tr>
<th>Significant Change ID</th>
<th>Date of Significant Change</th>
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**Project Additional Information**

**Purposes of the contained use**

Rhomboid proteases play a critical role in regulation of the EGF receptor signalling in the fruit fly Drosophila melanogaster. Notably, deregulated EGF receptor activity contributes to cancer in humans; however the role of Rhomboids in this process has not yet been investigated. Therefore, we propose to investigate the role played by mammalian rhomboid proteases and rhomboid-like proteins in human disease(s), including cancer using replication defective lentivirus.
Recipient or parental organism
E. coli K-12 and B derivatives - Class 1
mammalian cells - Class 1
Packaging cell lines, such as HEK293 cells, used for packaging replication defective lentiviral particles

Host/vector system
Standard replication defective lentiviral vectors which have the essential genes separated onto different plasmids or stably expressed from the packaging cell line.

Origin & function
Standard reporter genes, such as Neomycin, puromycin and kanamycin
shRNA or mir30-based shRNA-mirs
Silencing or overexpression of Rhomboid proteases, related proteins and their putative substrates. Silencing or overexpression of common housekeeping genes, such as GAPDH.
CRISPR or similar technology will be used to allow loss of function mutations at specific site within human and murine genomes.
WPRE may be present in some of the lentiviral vectors.

Evaluation of foreseeable effects
Rhomboids are intramembrane serine proteases that cleave membrane proteins within the bilayer and are involved in a wide range of biological processes. Mammalian rhomboid targets include thrombomodulin, ephrinB4 and EGF but Rhomboids are generally highly specific with a limited number of substrates.

iRhom molecules lack intramembrane protease activity but are required for activation of Tumour Necrosis Factor α Converting Enzyme (TACE) and thus secretion of TNFα and various EGFR ligands.

EGFR ligands, such as EGF, amphiregulin and HB-EGF will lead to activation of downstream signalling cascades such as the MAPK and JNK pathways leading to processes such as migration or proliferation. As such a precautionary approach will be taken.

The diversity of biological functions already known to depend on rhomboids is reflected in evidence that rhomboids play a role in a variety of diseases including cancer, parasite infection, Parkinsons disease and diabetes.

Standard fluorescent protein reporter genes, such as enhanced green fluorescent protein (EGFP), will be transduced into cells using lentiviral vectors, to provide positive controls with which to assess the efficiency of lentiviral transfer. Fluorescent proteins are a common investigative tool and no hazards are associated with their use.

The silencing of rhomboid and rhomboid related genes can be achieved using a Cas9 VRISPR-associated nucleae expressed under a EFS promoter which is guided to specific sites within the host genomes of human and mouse cells by a customizable stRNA (synthetic single-guide RNA), under a U6 promoter. DNA double strand breaks, catalysed by Cas9, create frame shift insertion/deletion (indel) mutations that result in a loss-of-function allele.

Some concern has been raised with regards to the potential oncogenic

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Degree of kill:
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Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

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<td>L3</td>
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<tr>
<td>L4</td>
<td>L2</td>
<td>L4</td>
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</tbody>
</table>

Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
Project Additional Information

Purposes of the contained use

Viruses are intracellular parasites that rely on the cellular resources to complete their biological cycle. In the context of virus infection, RNA represents a key molecule because it can function as a messenger and, in some instances, also as genome. Thus, the interaction between host proteins and viral RNA represents a central theme in modern virology. This project will explore the dynamics of RNA-binding proteins in response to infection as well as the composition and plasticity of viral ribonucleoproteins throughout the infection.

Recipient or parental organism

A number of different viruses will be utilised during this project including both hazard group 2 and 3 viruses. Examples include:
- Hazard group 2
  - Encephalomyocarditis virus
  - Influenza virus
  - Vaccinia virus
  - Sindbis virus
- HIV replicons and single-round replication viruses
- EcoHIV which replicates in mice
- Replication defective lentiviral vectors (3rd or 411 generation)
Hazard group 3
human immunodeficiency virus

Host/vector system

- Standard E. coli K-12 or B derivative
- Standard plasmids
- Standard packaging cell lines
- Flp-in and Flp-oul expression system
- CRISPRiCas 9 cloning systems
- Standard mammalian cell lines including CD4 lymphocytes and monocytes
- Primary murine CD4 cells

Origin & function

- Standard reporter genes, such as eGFP, mCherry, luciferase and standard antibiotic resistance genes are not expected to alter the virulence of the viruses.
- MS2 loops or b-boxes - RNA elements which are recognised by specific RNA binding proteins
- Point mutations within the virus to assess viral interaction with host RNA
- Expression of individual viral genes in cell lines and over-expression/knockdown of host cellular genes has been deemed class 1 and this work is covered by a separate risk assessment. These cell lines will be used in combination with the viruses listed in this risk assessment and handled at a level according to the viral work.

Evaluation of foreseeable effects

- HIV replicons are based on standard HJV molecular clones (e.g. NL4-3), which lack the glycoproteins (env) as well as either of the accessory genes vpr or rev. These constructs can replicate efficiently within the cell but cannot give rise to infectious particles due to the lack of structural proteins which are essential for viral particle formation. Replicons are generated by transfecting constructs into cells which express the env and vpr (or rev) in trans. These viral particles will be handled at containment level 2 as they require 2 competent and independent recombination event to acquire full infectivity. Test for replication competent virus will be done by simple re-infection assays.
- In EcoHIV, the tropism determinant gp120 is replace by the MIV glycoprotein gp80 which directs HIV to its natural range of target cells but restricts the infection to rodents. Although this virus doesn't infect humans only one recombination step with wild type gp is required and as such will be handled at containment level 2 and never used along side wt env expressing cells.
- Encephalomyocarditis virus (ECMV) is a virus that can infect pigs, however the strain to be used has adapted to being grown in cultured cells and is thus attenuated for cultured cells. It will be handled at containment level 2.
- Sindbis virus is passed to humans via a bite from an infected mosquito and is designated hazard group 2 by ADCP. It will be handled at containment level 2.
- Influenza virus (FLU) is generally transmitted through aerosols and vaccinations are available and is designated to hazard group 2 by ADCP. It will be handled at containment level 2.
- Vaccinia Virus (W) is the active constituent of the vaccine used to eradicate smallpox and is assigned to hazard group 2 by ADCP. It will be handled at containment level 2.
- Replication defective lentiviral vectors will be used in some experiments. These will be based on 3rd or 4th generation vectors which have a number of safety feature but which may contain the WPRE fragment and as such may pose a minimal risk. They will be handled at containment level 2.
- Hazard group 2 viruses may be used for in vivo work. Infection with EcoHIV will be through intravenous injection (tail vein). In the case of EMCV, FIU and W the infection can be performed following intravenous or by intranasal
inoculation. HIV is a hazard group 3 virus which causes AIDS and as such work with this virus will require handling in a dedicated containment level 3 suite.

Most of the viruses will just be modified to contain a reporter gene which will allow the tracking of the virus and infective cells. In addition, MS2 loops or b-boxes can be inserted in the viral genome. These RNA elements are recognised by specific RNA-binding proteins (MS2 and lambda, respectively) that will fuse to a fluorescent reporter or to a purification tag to allow RNA localisation assays (fluorescent version) or RNA purification (using the tagged version. e.g MBP, GST, FLAG or His).

Single point mutations of RNA binding sites of host and viral factors will be utilised maintaining amino acid sequence in the glycoproteins but may alter amino acid sequences in accessory proteins.

The lentiviral vectors will be used to employ CRISPR technology and expression of siRNA but may also be used when required for expression of individual genes linked to this project within mammalian cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. No sharps will be used with live hazard group 3 material.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

### Project Containment

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<td>L3 Yes</td>
<td>L4</td>
</tr>
</tbody>
</table>

**Animal Units**

- L2 Yes
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

### Project Ref 553/15.10

- **Date Ackn'd**: 04/11/2015
- **CU2 Project Title**: Use of lentiviruses to study cellular stress in tissue culture

- **Class**: Class 2
- **Culture Volume**: ≤ 1 Litre
- **Non-GMM**: Consent Granted

**Project notified under transitional arrangements**: N

- **Historical Significant Changes**: N
- **Historical Date of Additional Info**: 04/11/2015
- **Significant Change ID**: 04/11/2015
- **Date of Significant Change**: 04/11/2015

### Project Additional Information
**Purposes of the contained use**

This programme of work will use amphotropic lentiviruses to help study the functions of cellular genes that affect the regulation of cellular stress, proliferation and survival, and that may be involved in the generation of phenotypic heterogeneity in cancer. The principal aim of this work is to create human cell lines that either enhance or attenuate an endogenous gene of interest related to cellular stress for subsequent biochemical analyses.

**Recipient or parental organism**

<table>
<thead>
<tr>
<th>E. coli - standard K-12 and B derivatives (class 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packaging cells lines, such as those derived from HEK293 cells</td>
</tr>
<tr>
<td>Mammalian cell lines and primary cells</td>
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</table>

**Host/vector system**

<table>
<thead>
<tr>
<th>Standard E.coli K-12 and B derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard plasmids (non-mobilisable and mobilisation defective)</td>
</tr>
<tr>
<td>Replication defective lentiviral particles some of which may include the WPRE sequence</td>
</tr>
</tbody>
</table>

**Origin & function**

The cDNAs carried by the lentivirus vectors will be used to express genes with roles in maintenance of endoplasmic reticulum (ER) homeostasis. This may include genes linked with: protein folding, protein degradation, lipid biosynthesis, calcium regulation, protein translation, stress response, protein secretion, protein trafficking and any secretory cargo.

Examples of genes involved in cellular stress are Hrd1, SEL 1L, FAM8A1, EMC1-10, UBXD8, HERPUD1

Although not anticipated, some genes may promote cell proliferation and/or decrease apoptosis - such genes are generally, but not exclusively, oncogenes. Conversely, cellular and viral genes encoding proteins that inhibit cell proliferation and/or promote apoptosis - such genes are generally, but not exclusively, tumour suppressor genes.

Also shRNA will be used to knock down expression of gene families listed above.

Standard reporter genes, such as GFP.

Standard antibiotic resistance genes, such as Neomycin

**Evaluation of foreseeable effects**

No risk from E. coli work (class 1)

Executive

Contained Use Notification

Packaging cell lines will produce replication defective viral particles which are capable of a single round of infection in a variety of mammalian cells, including humans. They also have the ability to integrate into the host genome. Genes required for viral production are separated onto 3 plasmids that have minimal homology and as such there is limited potential for recombination to create replication competent viral particles. In some cases, genes such as gag, pol and env are stably integrated into the genome of the packaging cell lines decreasing the risk of RCVs even further.

Once mammalian cells have been transduced and the virus has integrated into the host genome the cells pose minimal risk as the virus is unable to replicate.

The group are not specifically planning to work with oncogenes or knocking out tumour suppressor genes, however as they are looking at a number of genes in pathways involved in proliferation and cell survival there is a potential that they may inadvertently work with such genes. Therefore a precautionary approach is being taken.
WPRE may be present in a number of the lentiviral vectors. As this is a potential oncogene a precautionary approach is being taken. Genes involved in cellular stress are not predicted to be oncogenes or tumour suppressor genes however a number of genes to be looked at have unknown function. It is unlikely that over expression or knock down of a single gene would have major effects on cell growth and survival.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps - Sharps will not be used

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 99.998% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment
Project Ref 553/15.11

Date Ackn’d 18/11/2015

Date Project Ceased

CU2 Project Title Use of genetically modified salmonella typhimurium and escherichia coli expressing antigen in assays assessing functionality of antibodies against the bacterial antigens

Class 2

CultureVol Class 2 < 1 Litre

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Bactericidal assays allow the assessment of functional antibodies against a number of bacteria. In vitro assays developed in-house allow the assessment of functional antibodies against a superfamily of enzymes common to Gram-negative bacteria. One in vitro assay measures susceptibility of bacteria to antibiotics and the other measures enzymatic breakdown of beta-lactams by beta-lactamase enzymes.

Recipient or parental organism

Hazard Group 2 gram negative bacteria such as Salmonella Typhimurium and Escherichia coli.

Host/vector system

Standard E. coli strains such as K-12 and B derivatives
Individual antigens from other bacterial species such as the Vi antigen from Salmonella typhi and the beta-lactamase antigen from Klebsiella pneumoniae or Pseudomonas aeruginosa.

Evaluation of foreseeable effects

Salmonella Typhimurium constitutively expressing the Vi antigen from Salmonella Typhi is not expected to have increased pathogenicity. This modification will allow the group to study the efficacy of vaccines against Salmonella Typhi without having to work with a hazard group 3 pathogen. Beta lactamases are enzymes that produce antibiotic resistance to B-lactam antibiotics (such as penicillin, cephemycins and carbapenems) in gram negative bacteria. There are a number of different types of beta lactamases some of which are specific (i.e. against penicillin) other are have broad spectrum activity. Antibiotic resistant strains of E. coli are present in the population, however most of the strains used in this project will be based on strains with limited pathogenicity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the NHS clinical waste stream for incineration.
Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.
Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the NHS clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

### Project Containment

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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<td></td>
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### Project Ref 553/15.2

- **Date Ackn’d**: 25/02/2015
- **CU2 Project Title**: Investigating Neurological disorders using improved cell and animal models
- **Class**: Class 2
- **CultureVolumeClass2**: < 1 Litre
- **Consen Granted**: Consent Granted
- **Project notified under transitional arrangements**: N

#### Project Additional Information

- **Purposes of the contained use**
  - Use' of Sendai virus to generate induced pluripotent stem cells
  - Use of Replication defective Lentivirus
  - Use of Adenovirus and AdenoAssociated virus
### Recipient or parental organism

- E. coli K12 or B derivatives
- Primary mammalian cells
- Mammalian cell lines

### Host/vector system

- Standard replication defective lentiviral, adenoviral, Adeno associated virus and sendal viral vectors in E. coli cells and packaging cell lines such as HEK293

### Origin & function

**Oct4, Sox2, Klf4, Myc** are 4 genes required for the reprogramming of cells to generate induced pluripotent stem cells.

- Wild-type ion channel and neuronal function genes such as CACNA1A, TRESK, SCN1A, Tau, Cluslerin
- Mutated versions of above with deletions, insertions and/or point mutations.
- Selective modifications of any/all of the above.
- Short hairpin RNA interference sequences against the above mentioned genes (or other genes).
- Standard reporter genes, such as, Beta-galactosidase (lacZ), Jellyfish green fluorescent protein (GFP and derivatives), Td tomato and nerve growth factor.
- Standard antibiotic resistance genes, such as, puromycin and neomycin (puroR and neaR).

**WPRE** present in viral vectors

### Evaluation of foreseeable effects

**Myc** is a known human oncogene, and will be under the control of constitutively active mammalian promotors. As such it poses the risk of cell transformation if it is accidentally administered. Klf4 is also associated with cellular transformation in some scenarios, so should be assigned a similar level of risk.

The four genes (Oct4, Sox2, Klf4 and Mye) together, either in tandem in one vector, or together as pooled virus preps, are capable of reprogramming cells to become pluripotent. As such they pose a greater risk in combination than individually. The risk of induction of pluripotency in the event of ingestion, inhalation or injection is very low, as even if the genetic material is successfully introduced into the genome of cells within the body, reprogramming is an inherently very inefficient process (~0.01% efficiency) even in defined tissue culture conditions, and the environment in which a reprogramming cell would find itself would not be conducive to the reprogramming process (other signalling events induced by the local environment would be likely to override the reprogramming process). If, despite this low level of risk, reprogramming does occur within a laboratory worker, then it is possible that a teratoma could result, as is the case when iPS cells are injected into immunosuppressed mice.

**OCT4, Sox2, Klf4 and Myc** will only be expressed in Sendai virus using a kit available from invitrogen (or other similar available source). The kit uses vectors based on a modified, non-transmissible form of Sendai virus (SeV) to safely and effectively deliver and express key genetic factors necessary for reprogramming somatic cells into iPSCs. This vector is non-integrating and remains in the cytoplasm. The Fusion protein (F gene) has been deleted from the vectors rendering the virus incapable of producing infectious particles from infected cells. In addition, the presence of functional mutations such as temperature sensitivity in the amino acid sequence of several SeV proteins (SeVFTS.6..F, SeVFTS 12..6..F, and SeVrrS15ll.F) renders the vectors easily removable from transduced cells.

Replication defective lentiviral vectors will be employed to overexpress or knock down genes thought to be involved in neuronal function. None of the genes are expected to be hazardous however the lentiviral vectors may contain the...
WPRE gene which may have the potential to be oncogenic. As such, these viruses will be handled at containment level 2.
Replication defective Adenoassociated vectors also contain WPRE and although these vectors (and adenovirus vectors) are non-interacting a precautionary approach is being taken with them being handled the same as replication defective lentiviral vectors.
In addition, these viral particles will be used in vivo however the risks are minimal due to the use of anaesthesia and a stereotaxic frame.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream.
Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.
Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream.
Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121: 1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), dispose via the industrial waste stream OR dispose via clinical waste stream for incineration.
Animal carcasses - dispose via clinical waste stream for incineration.
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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<tr>
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**Project Ref** 553/15.3

- **Date Ackn’d**: 18/03/2015
- **CU2 Project Title**: Cellular response to Eppstein-Barr viral infection
- **Class**: Class 2
- **Culture Volume**: ≤ 1 Litre
- **Class Culture Vol**: Class 2
- **Class Volume Culture**: Class 3-4
- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Project Additional Information**

- **Purposes of the contained use**
  1. to describe and understand basic mechanisms of EBV virology, particularly the mechanisms by which EBV influences cell polarity and cell differentiation status
  2. to investigate whether natural variation in EBV genomes might have a consequence for the basic virology and...
tropism of the virus
3. to examine the impact of Helicobacter p)1ofi infection on the EBV reproductive state and the general influence of both pathogens on the Infectious abilities of one another
4. to investigate the contribution to of EBV to disease

**Recipient or parental organism**

Eppstein Barr Virus· human herpes virus that infects the lymphocytes and some epithelial cens of humans and a few closely related primates to which most of the POPUlation has be exposed to in early childhood.

**Host/vector system**

Addition of reporter gene performed in collaborators lab
HEK293 cells will be used as producer ceNs after transfection with pBZLF1 or pBalF4 which contain the GFP gene

**Origin & function**

GFP 0( other similar reporter genes that have a history of safe use.

**Evaluation of foreseeable effects**

The addition of a reporter gene is not expected to have any effect on the virulence of the virus and will be handled in the same manner as wild type.
Wild type virus often causes asymptomatic infections or are undisguishable from other acute viral infections.
Infections in adolescence causes glandular fever. EBV infections are linked to a number of cancers where It is thought 10 contribute to the diseases by supplementing cell genetic changes.
The group will work with ceDs that are derived from EBV infected cancers where infection is latent and little virus is produced. They will also Infect cells with w~d type and GFP versions of EBV

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plastlcwafe e9 pipettes, flasks. tubes) - autoaave using a make safe cycle as specified In B5 2646, Part 3, 1993leither 121-1250C for alleast 15 minutes or 12fli.13OoC for at least 10 minutes or 134-138oC for at least 3 minutes). discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.
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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for Incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Animal Units

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Project Ref 553/15.4

Date Ackn'd 17/04/2015
CU2 Project Title Use of self-inactivating lentiviral vectors for the study of degenerative diseases
Date Project Ceased

Class CultureVol Class 2 CultureVolume Class 3-4
Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes

02/03/2022 Page 9171 of 15326
Replication defective lentiviral vectors are a well-established system for the long term and stable expression of a gene of interest which is routinely used in laboratories world-wide. These vectors are an excellent way to introduce fluorescent markers and genetic modifications and have significant advantages over other delivery methods such as transfection. Transient transfection is inefficient, especially in terminally differentiated cells such as those derived from IPS cells and primary cultures. Lentiviral vectors provide the opportunity to transduce high percentages of cells in these models and have the added advantage that they can be used to produce long-term and stable expression due to their ability to integrate into the target genome.

Recipient or parental organism

Mammalian cell lines, such as primary cells, induced pluripotent stem cells and tissue samples.

Host/vector system

HEK293 cells (and derivatives) are used as a producer cell line. Second and third generation lentiviral vectors will be used where the gene of interest, the Gag-Pol genes and envelope genes are on separate plasmids such as pHR-SIN and lentilox.

Origin & function

- Standard Reporter genes (e.g. GFP, YFP, RFP)
- Standard antibiotic resistance (e.g. Neomycin, Hygromycin, Puromycin and ampicillin).
- shRNAs, siRNAs.
- Genome editing tools (e.g. TALENs, Cas9-CRISPR).
- Tet repressor/activator systems.
- Neurotrophic/neuroprotective factors (e.g. BDNF, CNTF, GDNF and NGF).
- Neurodegenerative susceptibility genes (e.g. C9orf72, TOP-43).
- Optogenetics/calcium sensors (e.g. ChR2, ChR1, GaMP)
- pH sensitive vesicular endo/exocytosis fluorescent reporters (e.g. Synapto-pHluorin, SynpH)
- Ral GTPases (e.g. RALA, RALB)
- Factors involved in neuronal differentiation (e.g. Bm3a, Ngn1, Ngn2)
- Designer receptors exclusively activated by designer drugs (DREAODs) for specific neuronal silencing (e.g. GluC1, hM4D)

Evaluation of foreseeable effects

The protein products of the genes to be studied are not expected to be toxic or cancer inducing in any way. However, the H1V receptors are also receptors for chemokines and as such the genes could modulate immune function, especially chemotaxis, in target cells. This could provide a growth/invasion advantage to cells. Initially, the shRNAs, TALENs or Cas9-CRISPR systems to be used are not targeting known tumour suppressors.
However, there is potential for off-target effects with, which could potentially have tumorigenic effects. This will be
minimised by careful design with as little sequence homology as possible to off-target genes, including BLAST
searches against genome databases.
Gene transfer is possible, significantly from the transfected producer cell lines, as the resulting LentiviralRetroviral
vector will be able to infect a wide range of cells, including human cells, as the VSV-G or Rabies-G envelope enable
amphotropic infection. However dissemination in the environment is limited due to the viruses being replication
defective.
Some of the vectors may contain the woodchuck hepatitis virus regulatory element (WPRE) and there is some limited
evidence of its oncogenic potential.
Some of the genes of interest may modulate immune function.
Once the virus has integrated into the genome of the host cell it poses minimal risk and the cells can be handled at
containment level 1 after a given period of time.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS
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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines) |

### Is an emergency plan required according to regulation 20?

| N |

### If yes, tick to confirm that it is attached to this form

| N |

### Tick to confirm that you have attached a risk assessment to this form

| Y |

### Tick if you are claiming exemption from disclosure for section of the risk assessment

| N |
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Project Ref** 553/15.5

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<td>Use of retro- and lentiviral vectors for functional genomics to identify biomarkers and novel drug target candidates in tissue culture</td>
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<td>&lt; 1 Litre</td>
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| Class CultureVolClass2 CultureVolumeClass3-4 |
|----------------------------------------------|-----------------------------------------------|
| Non-GMM Consent Granted | Project notified under transitional arrangements |

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

The group develops new approaches to study signalling networks in cancer cells and to uncover specific weaknesses, particularly in breast and lung cancer and other tumour types that can be used for developing more effective targeted drugs and for guiding treatment decisions. We develop and employ high-throughput chemical and genetic screening.
approaches and haploid genetics to engineer isogenic human cancer cells and tease out drug-gene interactions that could be therapeutically exploited.

**Recipient or parental organism**
- E. coli K12 or B derivatives
- Mammalian cell lines
- Primary mammalian cells

**Host/vector system**
- Standard plasmids
- Standard non-replicating retro and lentiviruses

**Origin & function**
- Standard reporter genes, such as GFP
- Standard antibiotic resistance genes, such as ampicillin
- shRNA to knock down a variety of genes involved in signalling pathways
- cDNA libraries involved in signalling pathways
- Genes from oncogenic viruses, such as Adenovirus E1A gene or SV40 large T antigen
- Element designed to modify the host DNA sequence, such as CRISPR, TALENS and genetrap

**Evaluation of foreseeable effects**

No increased risk is expected from genes in E. coli as they will be driven by mammalian promoters. Class 1.

No increased risk from cell lines and primary cells is expected other than those covered under CoSHH (presence of adventitious agents) as the viral vectors are non-replicating and not infectious once integrated into the host genome. Cells are likely to be detected as foreign and removed by the immune system and have strict nutritional requirements which renders their persistence in the environment negligible. Class 1.

Viral producer cells will be handled at containment level 2 as will all procedures with infectious virus. Class 2.

Standard antibiotic resistance genes and reporter genes have a history of safe use and are not expected to increase the risk.

shRNA libraries will knock down gene expression. As these are libraries of genes there is the potential to knock down the expression of tumour suppressor genes especially given that some of the libraries are related to tumourogenesis there is potentially an increased risk.

cDNA libraries will over express genes. As these libraries are related to tumourogenesis there is potentially an increased risk.

Genes from oncogenic viruses are often used to immortalise cells and are therefore potentially there is an increased risk.

The use of CRISPR targets modification to specific sites and therefore minimises random integration effects.

The inserted material may intentionally or accidentally affect the host DNA and/or gene expression and thereby stimulate oncogenic transformation of eukaryotic cells. Likewise, the random insertion of lentiviral or retroviral vectors (which may be used to transfer the sequences above into the host cells) can result in the altered expression of cancer driver genes. As such, these vectors and inserts pose the risk of cell transformation when accidentally administered to the scientists working with these vectors and this represents the greatest health and safety hazard. However, these risks are strongly mitigated by the Biosafety Features of the Vectors (see below) as well as the Risk Reduction for lenti- and retroviral transductions.

The viral vectors used are second or third generation and create non-replicating viruses which integrate into the host
genome. There are a number of different safety features depending on the system used. Only the minimal number of viral genes are used, genes are often separated onto 3 of 4 different plasmids and they contain L TR regions which self inactivate the virus.

In the event that a person will be in direct contact with a lentivirus or retrovirus carrying an oncogene the chance that this will result in transduction of cells and induction of a tumor is very small. The infection efficiency of these viruses into primary human cells is very low, particularly in those terminally differentiated and not proliferating cells lining the skin and mucosa or present in the bloodstream. Furthermore, even if such cells would be transduced they would be shed/disposed from the body within days and thus could not result in tumorigenesis. Furthermore, tumorigenesis is a multi step process, requiring several genetic events and these viruses would only carry single cancer genes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. Sharps will not be used for viral work.

Degree of kill:

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022

Page 9176 of 15326
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4 L2 L4</td>
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**Animal Units**

<table>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2</td>
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**Project Ref** 553/15.6

**Date Ackn'd** 27/08/2015

**CU2 Project Title** Targeted gene modification of human antigen-specific T cells

**Date Project Ceased**

**Class** Class 2

**CultureVolClass2** < 1 Litre

**CultureVolumeClass3-4** Non-GMM Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

This project involves modulating (overexpress or down-regulate) the expression of genes involved in the promotion or inhibition of T cell activation, proliferation, differentiation and polyfunction survival, genes encoding transcription factors directly or indirectly mediating their expression, and genes encoding their interacting proteins in human cell lines and primary cells. In the course of the study, other molecules such as cell cycle or gene expression regulators.
Recipient or parental organism

Primary mammalian cells, including those from HIV patients and other patients

Host/vector system

Standard replication defective retro and lentivirus systems
E-coli K-12 and B derivatives and standard vectors will be used for cloning purposes

Origin & function

Genes involved in:
- T cell activation, such as CD28 and CD69
- T cell proliferation and inhibition, such as MAL and DNMT1
- T cell differentiation, such as CD28 and CD69
- Polyfunction survival, such as BCL2 and PRF1
- Transcription factors, such as NFkB 1 and ST AT3
- Cell cycle regulators, such as MCM2 and E2F1
- Immune regulators, such as TYROBP and FCRL3
- WPRE is present in numerous lentiviral systems and some studies have shown this to be a potential oncogene
- Genes in which expression is expected to be dependent upon the genome editing technology
- Genes such as those listed above that are already expressed in the genome editing technology

Evaluation of foreseeable effects

CRISPR/Cas system (and similar systems) allows targeted gene editing on endogenous genes. It has been isolated from the bacterium Streptococcus pyogenes. The SpCas9 has been human codon-optimized. Although it has been isolated from a hazard group 2 micro-organism the risks are limited due to only the necessary genes being used, the fact that these genes have been codon optimised for expression in human cells and the fact that the editing is limited to specific genes within the genome rather than random integration.

Standard replication defective lentiviral vector systems can integrate into the genome

Before infecting recipient cells with replication defective lentivirus, all the cells isolated from HIV-1 infected patients are tested by nested PCR and confirmed free of HIV-1 genes (especially viral packaging genes: gag-capsid and polreverse transcriptase-integrase genes) within genome. In this case, there is also minimal possibility of undesirable recombination events occurring which could contribute to the generation of replication-competent virus.

In addition work with primary cells from patients positive for HIV, HepB and HepC will be carried out in a containment level 3 laboratory due to the requirements of the CoSHH regulations.

The ability of the virus to infect human cells, the potentially oncogenic nature of some of the inserted genetic material, and the likelihood that expression will lead to changes in the proliferation of infected cells and in some cases possibly contribute to oncogenic transformation, increases the risk to human health. However the use of sharps is minimal and the likelihood of exposure is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Project Containment

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</table>

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Ref 553/15.7
The objective of this project is to determine the role of autophagy in the mechanism behind artemisinin-resistant Plasmodium falciparum.

Recipient or parental organism

Plasmodium falciparum - hazard group 3

Host/vector system

replication defective retro / lentiviral vectors

Origin & function

Reporter genes, such as eGFP or m-cherry.

Executive

Knock down I knock out or specific mutations of parasite genes thought to be involved in autophagy such as the Atg genes.

Evaluation of foreseeable effects

Plasmodium is a protozoa which is transmitted to humans via mosquitos and may cause serious disease. The Plasmodium which is modified to express reporter genes is not expected to be any more hazardous than the wild type versions. There is no report in the literature of increased virulence following addition of reporter genes. The Plasmodium in which certain proteins have been edited (either knocked down or SNP inserted) are not expected to have increased virulence however they may be involved with the resistance to artemisinin. In the event of accidental exposure any infection would be treated with a partner drug such as chloroquine which have different
mechanisms of action and are therefore unlikely to share the same resistance mechanisms. This work does not involve mosquitos and these insects are not present in the UK therefore the risk to the environment is minimal as without a host I vector the protozoa have limited ability to survive. The only sharps present are glass slides necessary for the creation of blood smears which will be disposed of immediately into a sharps bin. Replication defective Lentiviral particles will be used to modify the plasmodium, some of which may contain the WPRE sequence which is a potential oncogene and are therefore considered to be class 2. The addition of reporter genes and plasmodium genes is not expected to increase the hazard of the lentiviral particles. However should accidental exposure occur there is the potential for integration into the genome at random sites which may alter expression of genes at that site.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Laboratory not sealed for fumigation. There is no foreseeable need to fumigate the laboratory. In the event of a major spillage, the bulk of material would be soaked up on absorbent paper towels then autoclaved, killing any parasites. The area would be wiped down with disinfectant. Any aerosols created that were not cleaned in this initial effort would dry out, killing both host cell and parasite. The area of the laboratory would be left isolated for a period to ensure effective drying. Effective parasite transmission is only possible via direct inoculation of viable parasites. No negative pressure, HEPA filtered extract, microbiological safety cabinet or specified measures to control aerosol dissemination. The organism presents no hazard of infection by the airborne route. Class II microbiological safety cabinets are in use in the dedicated laboratory, they are necessary for sterile culture of the organism; they are not required for operator safety. Activities involve small scale, standard laboratory techniques with no aerosol generating procedures. Autoclave and some other equipment not within the laboratory

Although the laboratory contains most of the necessary equipment for the work, some activities use specialist equipment that cannot reasonably be accommodated within the dedicated facility. In all cases a safe system of transport involving secondary containment is in use, any equipment used is cleaned and disinfected immediately on completion of the work and any contaminated items either returned to the dedicated laboratory or removed for disposal as waste. The following activities are undertaken outside the dedicated laboratory:

i) Fluorescence microscopy: a fluorescent microscope is located within the same building; there is a second fluorescent microscope in the Weatherall Institute of Molecular Medicine, which is connected

ii) Storage: transfected parasite stocks are frozen and stored at -800C or in liquid nitrogen within the same building. The freezers are locked and accessed only by authorised personnel.

ii i) Autoclaving: an autoclave validated for disposal of waste is located within the same building.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (e.g. plasticware) All plastic ware used in the procedure is treated with 2-4% Virkon before being disposed of according to the CL3 CoP. All waste from the Containment Level 3 laboratory must be immediately placed in a suitable receptacle, e.g. sharps into a sharps box; plastic, gloves and tissues into a hazard waste bag. Plastic ware or human tissue that has been in contact with hazard group 3 organisms must first be disinfected in 2% Virkon. They are then treated as normal clinical waste. Disinfected items must immediately be placed in a yellow clinical waste burn bin. When each bin is either full or filled to the line indicated on the side, seal the lid down. Ensure all
the locator tabs are secure. The person locking the bin must label it with a waste reconciliation sticker and complete waste reconciliation form accordingly. Sealed and labelled bins are placed in corridor outside the lab for removal by cleaning staff in Facilities.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Project Ref 553/15.8

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
The purpose of this project is to genetically modify yeast genes involved in chromosome segregation to understand their function.

Yeast strains such as Candida glabrata

Standard E coli K-12 or B derivatives will be used for the cloning stages of this project.

Standard antibiotic resistance genes such as ampicillin, gentomycin and hygromycin

Auxotrophic marker genes such as ADE, HIS, LEU, LYS, MET, TRP, URA

Epitope tags such as Flag, HA, His, Myc, PK

Marker genes such as kan, nat and hph

Cohesin complex subunits: such as Smc1, Smc3, Scc1, Scc3, Pds5

Cohesin complex regulatory proteins: such as Scc2, Scc4, Wapi, Eco1

Cohesin confers both intrachromatid and interchromatid cohesion through formation of a tripartite ring within which DNA is thought to be entrapped. Cohesin loading onto chromosomes is catalysed by the Scc2/Scc4 complex, whereas dissociation is catalysed by Wapi and several other cohesin subunits like Scc3 and Pds5. Establishment of cohesion during S phase involves neutralization of the dissociation activity through Eco1-mediated acetylation of Smc3. This locks rings shut until opened irreversibly by Scc1 cleavage through the action of separase, an event that triggers the metaphase to anaphase transition.
The anticipated effect of the changes are chromosome segregation defects, cell cycle arrest and changes in cohesin ring formation. Addition of standard reporter genes and epitope tags is not expected to increase the hazard of this micro-organism. Switching the promoter sequences with those from other yeast species to alter the expression level is also not expected to have an adverse effect.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. |
| Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. |
| Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. |
| Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. |

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment
The overall aim of work under this risk assessment is to better understand bacteriocin uptake pathways leading to their antibiotic activity, allowing their exploitation to produce novel antibiotics. Bacteriocins are potent narrow spectrum protein antibiotics, produced by Gram negative bacteria to kill closely related bacteria. Many Gram negative bacteria produce bacteriocins, but with the exception of the colicins of Escherichia coli, little is known about their receptors and uptakes routes.

Recipient or parental organism

Hazard group 2 bacteria such as Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia

E. coli K-12 or B derivatives

Host/vector system

**Project Additional Information**
Standard plasmids

Origin & function

Bacteriocins, bacteriocin immunity proteins, bacteriocin receptors or proteins involved in bacteriocin uptake from hazard group 2 bacteria such as Escherichia coli, Pseudomonas spp. or Klebsiella spp., along with standard resistance markers which are not generally used for treatment.

Evaluation of foreseeable effects

Bacteriocins, bacteriocin immunity proteins, bacteriocin receptors or proteins involved in bacteriocin uptake from hazard group 2 bacteria, along with resistance markers, will be individually cloned into standard plasmids and expressed in E. coli K-12 or B derivatives such as BL-21. Whilst bacteriocins are potent toxins, their activity is narrow-spectrum, acting against their specific target bacterial species. Bacteriocin receptors are often nutrient receptors sometimes classified as virulence factors, analogous pathways exist in the host strain and without other components of the uptake pathway the inserted genetic material should not increase the level of risk for the GMMs. Class 1.

Addition of WbbL to E. coli K-12 strains. The inability of K-12 to colonise the GI tract in animal models is likely due to their phenotypically rough LPS, lacking O-antigen due to disruption of the wbbL gene by an insertion sequence. Restoration of the wbbL gene, and hence the rhamnose transferase it encodes, resulting in the production of 016 serotype LPS. Whilst the ability of O-antigen restored K-12 to colonise the GI tract has not been studied in animal models it is likely to be greater than that of K-12 carrying the wbbL gene disruption, most likely comparable to the ancestral commensal E. coli K-12. Class 2.

Work with Hazard group 2 bacteria, such as Uropathogenic E. coli, Pseudomonas aeruginosa, and Klebsiella pneumonia. The inserted genetic material should not increase the level of risk for the GMMs. The insertions will be (i) chromosomal and lead to gene inactivation and are likely to attenuate the host, (ii) plasmids allowing the expression of wild-type or mutated components of the bacteriocin uptake pathways from hazard group 2 bacteria such as E. coli, P. aeruginosa or K. pneumonia or (iii) plasmids encoding bacteriocin immunity proteins and should not promote the survivability of the GMM. The inserted material will contain resistance markers for agents not usually used to treat humans with infections with these bacteria. Class 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

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Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed Incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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Animal Units

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Large Scale Activities

Human Clinical Applications

**Project Ref** 553/16.2

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<th>CultureVolumeClass3-4</th>
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<td>28/01/2016</td>
<td>Use of replication defective viruses to investigate cerebral cortical development</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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</table>

Date Project Ceased

Project notified under transitional arrangements

02/03/2022
The main focus of this research is on cerebral cortical development. Topics covered range from neural progenitor fate specification to early thalamocortical and corticothalamic anatomy and connectivity. Replication defective viruses will be used to assist in this research.

Recipient or parental organism
- Mammalian cells
- In vivo neurons

Host/vector system
- Replication defective lentiviruses second or third generation
- Replication defective adenoviruses, such as E1 or E1 and E3 deleted
- Producer cell lines such as HEK 293 cells
- E. coli K-12 or B derivatives

Origin & function
- Standard reporter genes such as GFP, Luciferase and B-gal
- Standard antibiotic resistance genes
- WPRE element from Woodchuck Hepatitis virus
- Mammalian genes thought to be involved in cortical development or neuroprotective roles, such as Serpini1 gene

Evaluation of foreseeable effects
- No adverse effect are expected with the generation of plasmids in E. coli as the genes are driven by mammalian promoter and as such the work is considered to be class 1.
- Standard reporter genes and standard antibiotic resistance genes have a history of safe use and are not expected to increase the hazard of the viruses. Overexpression of fluorescent proteins in transgenic mice had shown no adverse effect.
- Genes which are involved in cortical development or neuroprotective roles are not expected to increase the hazard of the virus. Overexpression of serpini in transgenic mice shown slight anxiety
- The replication defective viruses are standardly used in research, however some versions of both the lentivirus and the adenovirus contain the WPRE element that has been shown to be a potential oncogene and a such a precautionary approach is being taken.
- In vivo work will involve sharps however the risks will be minimised by using anaesthetic or stereotaxic equipment and...
the sharps will be very fine pulled glass capillaries. Sharps will be disposed of immediately. Due to the location of innoculation and the replication defective nature of the viruses there is minimal risk of transmission post innoculation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.
Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.
Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.
Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.
Animal carcasses - dispose via clinical waste stream for incineration.
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment
Project Ref 553/16.3

Date Ackn'd 17/02/2016

date Project Ceased

CU2 Project Title Exosomes as RNA delivery platforms

Class Class 2

Culture Vol

Non-GMM Consent Granted

Under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Use of replication defective Lentiviral vectors to study cellular communication.

Recipient or parental organism

Standard mammalian cells

HEK293 derivatives for lentivirus production

Standard E. coli K-12 or B derivatives for plasmid creation

Host/vector system

Standard plasmids
Replication defective lentiviral vectors

**Origin & function**

- Standard reporter genes, such as GFP, luciferase
- Standard antibiotic resistance genes such as puromycin, hygromycin and neomycin
- Health and Safety
  - Executive
  - Contained Use Notification
- CRISPR (Clustered regularly interspaced short palindromic repeats) system which is an RNA guided endonuclease technology for genome editing
- Cre recombinase - Lox P system
- Extracellular vesicle markers, such as CD9, CD63 and CD81 of the Tetraspanin family
- Extracellular vesicle targeting sequence
- Transcriptional activators such as VP64, VP160 and VPR
- Transcription of RNA to induce or abolish expression of reporter genes
- WPRE element

**Evaluation of foreseeable effects**

Second and third generation lentiviral vectors will be used which means that all the components required for lentiviral replication (e.g. gag, pol, rev, env, vif, vpr, tat, vpu and nef) are on two separate plasmids and do not contain the packaging sequences. A third plasmid supplies the transgene and a packaging signal. This generates viral particles that are able to enter cells and integrate within the host genome but are unable to replicate.

- WPRE is present in many of the lentiviral vectors and is a potential oncogene. As such a precautionary approach is being taken with the viral work being done at Class 2.
- Standard reporter genes and antibiotic resistance genes have a history of safe use and are not expected to increase the hazard of the lentiviral particles.
- Cre recombinase - Lox P system allows for either the inversion, deletion or translocation of genes. This system has been in use for a number of years without any obvious adverse effects. The genes to be targeted are standard reporter genes which are not normally present in mammalian cells nor are the Lox P sites.
- Although CRISPR and Cas9 are originally of bacterial origin they are not expected to increase the hazard of the E. coli due to the fact that they are driven by mammalian promoters which are not active in bacterial cells. Therefore this work can remain at class 1. In addition the guide RNA is targeted to a specific genomic sequence in mammalian cells and would thus limit off target effects.
- Extracellular vesicle markers have not been noted to cause any harmful effects and tagging with reporter genes is not expected to alter that.
- Transcriptional activators when fused to an inactive Cas9 are capable of up-regulating gene transcription of targeted genes to enhance expression.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

<table>
<thead>
<tr>
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<tbody>
<tr>
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<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
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<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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Project Ref 553/16.4

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<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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### Project Additional Information

**Purposes of the contained use**

The project will utilise lentiviral vectors expressing shRNA, or marker proteins, and host proteins to transfect immortalised cell lines. By transfecting relevant cells with lentiviral vectors expressing host restriction factors and/or their variants, the effect of their genetic variation on protein expression can also be investigated.

**Recipient or parental organism**

Mammalian cell lines such as C8166, U937 etc
Mammalian primary cells

**Host/vector system**

HEK293 cells or similar virus producer cells
Second and third generation replication defective retro/lentiviral systems derived from HIV, SIV, FIV, EIAV or similar VSV-G pseudotyped viruses

**Origin & function**

- shRNA to knock down the expression of genes of host proteins
- Standard antibiotic resistance genes such as carbencillin and puromycin
- Standard reporter genes such as GFP
- Woodchuck hepatitis post transcriptional regulatory element (WPRE)
- Host proteins such as restriction factors from the TRIM family of proteins, and eventually other restriction factors such as SAMHD1, APOBEC, and Tetherin,

**Evaluation of foreseeable effects**

Standard antibiotic resistance genes and standard reporter genes have a history of safe use and are not expected to pose an issue over that posed by replication defective lentiviral particles.
Replication defective retro/lentiviruses have non-essential genes removed and the remaining genes split onto separate plasmids with minimal homology to reduce the risk of homologous recombination. As the gag, pol, and env plasmids do not contain the packaging sequence none of the genes are present in the package viral genome. The likelihood of these viral particles obtaining the missing genes is very low and therefore the risk of replication competent viruses being created is minimal.

WPRE may be present in the vectors and a precautionary approach is taken with this potential oncogene. One of the hallmarks of restriction factors is a significant decrease in retroviral activity during their overexpression, or a significant increase in retroviral activity during their attenuation. However it is highly unlikely that there would be adverse effects in the event of accidental exposure due to the low tranfection efficiency of viral vectors and their inability to replicate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Degree of kill:

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

### Project Containment

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<td>L4</td>
</tr>
</tbody>
</table>

- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

### Project Ref 553/16.5

- **Date Ackn'd**: 05/05/2016
- **CU2 Project Title**: Use of replication impaired lentiviral vectors to stably transfec human and mouse cells in vitro systems
- **Class**: Class 2
- **Culture Vol**: ≤ 1 Litre
- **Non-GMM Consent Granted**: Yes

- **Project notified under transitional arrangements**: Yes

- **Withdrawn**: Yes

<table>
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<tr>
<th>Significant Change ID</th>
<th>Date of Significant Change</th>
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</table>

### Project Additional Information

**Purposes of the contained use**

To deliver genes in cell system using replication defective lentiviral particles to either over express protein or silence gene to reduce expression of proteins. It will therefore allow us to study particular proteins functions in vitro.

**Recipient or parental organism**
<table>
<thead>
<tr>
<th>E. coli K12 and B derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>mammalian cells lines, such as Jurkat and THP-1</td>
</tr>
<tr>
<td>Primary mammalian cells</td>
</tr>
<tr>
<td>Virus producer cell lines, such as HEK293 (and derivatives)</td>
</tr>
</tbody>
</table>

### Host/vector system

Standard replication defective lentiviral vectors

### Origin & function

- Standard reporter genes such as Green Fluorescent Protein (GFP)
- Standard antibiotic resistance genes such as ampicillin

**Health and Safety**

- Executive signalling protein, cell surface protein, and other proteins, such as APRATAxin, CRN1 and DRP from mammalian sources such as human and mice
- CRISPR/Cas9 system
- shRNA
- Woodchuck hepatitis virus regulatory element (WPRE)

### Evaluation of foreseeable effects

Standard reporter genes and standard antibiotic resistance genes have a history of safe use and are not expected to alter the hazard of the viral particles.

- Cas9 has been codon optimised for expression in mammalian genes and is not expected to alter the hazard when expressed in E. coli. Use in the viral particles is expected to increase specificity in the targeting of gene mutation and therefore can be considered the same as non CAS viral particles.
- The genes to be either over-expressed or knocked down are signalling proteins, cell surface proteins and other proteins. None of the genes are expected to be oncogenic when over expressed, knocked down or mutated.

- Standard replication defective lentiviral particles can infect mammalian cells however they lack the essential genes required for replication. Lentiviral particles integrate into the host cell genome and this integration could in theory knock down a tumour suppressor gene. Most modern lentiviral systems contain self inactivating features which reduce the likelihood of activating genes within the host genome. Minimising the use of sharps will reduce the likelihood of accidental exposure.
- The lentiviral particles may contain a version of the WPRE which may have oncogenic potential therefore a precautionary approach will be taken with these viral particles.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The waste disposal routes vary for the locations as one is embedded within the hospital and follows their waste stream.
On WIMM site

i) Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes).

ii) Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge to drains.

iii) Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes).

iv) Sharps (eg needles, syringes, scalpels) - No sharps should be used in the course of the work. However, if applicable for any reason they will be disposed via clinical waste stream for incineration.

On John Radcliffe hospital site

i) Consumables (mainly plastic ware eg pipettes, flasks, tubes)

All disposables will go into the Clinical Waste Stream. Disposables will be placed in clinical waste limb bins designated for incineration.

ii) Liquids (eg samples, culture supernatants, tissue culture media)

Liquid waste will be subjected to Virkon or Chemgene-decontamination or incinerated. A prolonged exposure to 1% Virkon or Chemgene will be utilised after which waste can be discharged to drains. For any applicable liquid waste, it will be placed in clinical waste limb bins designated for incineration.

iii) Agar plates

Agar plates will be disposed of in clinical waste limb bins designated for incineration.

iv) Sharps (eg needles, syringes, scalpels)

No sharps should be used in the course of this work. However, if applicable for any reason, they will be disposed of via clinical waste stream for incineration.

Degree of kill:

- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

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Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment
Project Ref 553/16.6

Date Ackn'd 30/06/2016

CU2 Project Title Interaction between the immune system and bacteria within the gastrointestinal tract

Class Class 2 CultureVolClass2 < 1 Litre CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To study the effect of bacterial fermentation products, such as short chain fatty acids, on the regulation of the immune system within the gastrointestinal tract and prevention of inappropriate immune responses.

Recipient or parental organism
Hazard group 2 bacteria involved in gastrointestinal illnesses such as Salmonella typhimurium

Host/vector system
Standard vector systems

Origin & function
Standard reporter genes such as GFP and Luciferase
Standard antibiotic resistance genes such as ampicillin and Kanamycin.
Deletion of genes or insertion of antibiotic resistance into gene to knock them out which may attenuate the bacteria

Evaluation of foreseeable effects
Salmonella typhimurium can cause diarrhoea, vomiting and gastrointestinal symptoms, recovery is usually within a week. It is potentially more hazardous to pregnant women due to complications being more common in pregnant women and the antibiotic of choice for treatment being associated with birth defects. The route of infection is generally oral and therefore good hygiene must be adhered to.
Addition of standard reporter genes and standard antibiotic resistance genes is unlikely to have any adverse effects.
Deletion of aroA attenuates the Salmonella as strains with mutations in the aromatic pathway require p-aminobenzoic acid (PABA), which is lacking in mammalian tissues. However they will be handled the same at the parental strain.
Other similarly attenuated strains may be modified. Again they will be handled the same as the parental strain.
Bacteria will be used in in-vivo experiments however as this is via oral gavage there is minimal risk from sharps.
Other strains of bacteria which cause gastrointestinal illness, such as Helicobacter pylori, Campylobacter and E. coli may be modified by either attenuation and I or addition of reporter genes. These bacteria have similar risks and routes of exposure to Salmonella and will be handled in the same manner.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
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Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.
Animal carcasses - dispose via clinical waste stream for incineration.
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)
Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Animal Units**

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**Project Ref**  553/16.7

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<tr>
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<td>Modification and regulation of gene expression in tumour cell lines with lentiviral particles to study their metastatic behaviour</td>
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Date Project Ceased

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<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
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<td>≤ 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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Project notified under transitional arrangements  N

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The overall aim of our research is to determine the steps at which specific gene products influence metastasis. Replication defective lentiviral particles will be used to alter expression of genes affecting survival, angiogenesis, and some proteases that have previously been shown to alter metastatic behaviour.

#### Recipient or parental organism

- E. coli K12 or B derivatives: class 1
- Hek293 cells (or similar) lentiviral producer cell lines
- Mammalian cell lines including tumour cells

#### Host/vector system

- Standard replication defective lentiviral systems

#### Origin & function

- Genes such as mcp-1, pn1, mmp9, FGF-2, PAD4, STING, Interferon V etc that are involved in tumour cell metastatic behaviour, including cell survival, proliferation and vascular remodelling, will be either knockdown by Lentiviral particles expressing shRNA, overexpressed or mutated. Some of these genes may have oncogenic potential or be tumour supressor genes.
- WPRE - Woodchuck Hepatitis Virus Post-translational regulatory element. Is commonly found in replication defective lentiviral systems but may be a potential oncogene.
- Standard reporter genes such as GFP, DsRed and Luciferase
- Standard antibiotic resistance genes such as hygromycin and puromycin

#### Evaluation of foreseeable effects

Standard replication defective lentiviral particles are infectious for mammalian cells however their replication defective nature means that they are only capable of a single round of infection. As they integrate into the genome in a random manner there is a potential for adverse effects should accidental exposure occur. However, laboratory procedures which minimise the use of sharps reduce the likelihood of this happening.

Standard reporter genes and standard antibiotic resistance genes have a history of safe use and are not expected to increase the risk over that posed by empty lentiviral particles.

Some of the lentiviral particles may contain WPRE which may be a potential oncogene.

Some of the genes being overexpressed, mutated or knockdown may be involved in pathways regulating cell survival, cell proliferation, vascular remodelling and metastasis and as such may have detrimental effects should accidental exposure occur.

Cells transduced with replication defective virus may be used invivo, however as the virus is replication defective these cells are deemed to be of no greater risk that the untransformed cells after 2 days post transfection and multiple washing / media changes and as such are assigned to class 1.

Individual plasmids transfected into E. coli are not expected to pose a risk as the genes are driven by mammalian promotors and will not be expressed within E. coli.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:

- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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02/03/2022
Project Ref 553/16.8

Date Ackn’d 23/11/2016

Date Project Ceased

CU2 Project Title
Protein secretion in porphyromonas gingivalis

Class CultureVol
Class 2 1-50 Litres

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To study the structure and mechanism of secretion systems within Porphyromonas gingivalis or other similar microorganisms within the Bacteroides-Cytophaga-Flavobacterium phylum.

Recipient or parental organism
Hazard group 2 micro-organisms from the Bacteroides-Cytophaga-Flavobacterium phylum such as Porphyromonas gingivalis.

Host/vector system
Standard plasmids or shuttle vectors derived from other BCF group micro-organisms such as Capnocytophaga canimorsus.
Standard plasmids will be constructed in E. coli for recombination in P. gingivalis by electroporation.
Conjugation from E. coli may be required if electroporation fails.

Origin & function
Most of the work will involve knocking down, over expressing or mutation of normal P. gingivalis genes. Overexpression will be achieved using plasmids with an origin of replication that is functional in P. gingivalis. Affinity tagging will involve standardly available affinity tags. Expression will be driven by either native P. gingivalis promoters, consensus P. gingivalis promoters or promoters from antibiotic resistance genes that express in P. gingivalis. Antibiotic resistance genes, such as erythromycin, tetracyclin, chloramphenicol and cephalaxin. Counterselectable marker genes, such as sacB, codA, upp and pyre.

**Evaluation of foreseeable effects**

P. gingivalis can cause periodontal disease. Where possible work will be done using a strain that is considered less virulent, however on occasion other strains may be required for comparative studies. The P. gingivalis host organism already possesses functional copies of all genes to be introduced or modified in this study and the T9SS in the host organism is already evolutionarily optimised for function. Our genetic modifications of T9SS or substrate genes (e.g. affinity tagging or fusion to reporter proteins) are expected to reduce the functionality of the T9SS and therefore reduce the fitness and potential pathogenicity of the recombinant strains. Some of the T9SS proteins contribute to P. gingivalis pathogenicity however it is not expected that expression of single components of the T9SS pathway in E. coli K-12 will increase the hazard or overcome the disabled nature of the strain. The antibiotic resistance genes to be used are widely distributed in the BCF phylum and so are unlikely to increase the pathogenicity above that of natural populations. We will not be introducing resistance genes against the antibiotics that are most commonly used in the treatment of advanced P. gingivalis-associated periodontal disease: metronidazole, doxycycline, amoxicillin, and azithromycin. Reporter genes, affinity tags and counter selectable markers are all standardly used in biotechnological systems and have a history of safe use.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill: Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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Animal Units

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Project Ref 553/17.1

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<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>08/03/2017</td>
<td>Production and use of replication incompetent (SIN) lentiviral particles to evaluate the function of lysosomal proteins</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
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Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
### Project Additional Information

**Purposes of the contained use**

Use of replication defective lentiviral particles to investigate lysosomal storage and related diseases.

**Recipient or parental organism**

- **E. coli - Class 1**
- Hek293 (and derivatives) - viral production cell lines
- Mammalian cell lines
- Primary cells

**Host/vector system**

- Standard replication defective lentiviral systems
- Hek 293 (and derivatives) producer cells

**Origin & function**

- Standard reporter genes, such as GFP.
- Standard antibiotic resistance genes, such as amp.
- Health and Safety Executive
- Proteins thought to be involved in normal lysosomal function or implicated in lysosomal disease such as Cathpsin D, NPC1, Prosaposin, ATPB1-B4, and TFEB.
- siRNA / shRNA for achieving knock out of genes implicated in lysosomal disease.
- CRiPSR / CAS 9 gene elements to allow targeted gene mutation.
- WPRE is standardly used in lentiviral vectors.

**Evaluation of foreseeable effects**

Standard replication defective lentiviral systems are commonly used in molecular biology. Second and third generation systems have a number of safety features which prevent the generation of replication competent viruses.
but they do still pose a risk of insertional mutagenesis. In-vitro work will limit the risk by reducing the use of sharps
however for in-vivo work sharps will be required. Risks will be mitigated by training and procedures including no
resheathing of sharps and immediate disposal into a sharps bin.
Over-expression or knock-out of genes implicated in lysosomal disease is not expected to pose an increased risk over
and above that posed by lentiviral vectors.
Expression of standard antibiotic resistance genes and reporter genes is not expected to pose an increased risk over
and above that posed by the lentiviral vectors.
Use of the CRISPR system allows for targetted mutation or knocking out of genes and as such will reduce the risk of
off target integration.
WPRE is commonly used in replication defective lentiviral particles however there has been some concern raised with
regards the potential oncogenic nature of this gene. As such a precautionary approach will be taken with these
particles.
Once the viral particles have integrated into the host genome the host cells pose minimal risk and therefore can be
handled at Class 1.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS
2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10m inutes or 134-1380C for at
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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-
1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to
drains, dispose of solids via the industrial (black bag) waste stream for landfill.
Animal carcasses - dispose via clinical waste stream for incineration.
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Project Ref**  553/17.10

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<td>06/09/2017</td>
<td>Elucidating Immunological Pathways In Vitro and In Vivo</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022


**Project Additional Information**

**Purposes of the contained use**

The project aims to use replication defective lentiviral particles to help elucidate immunological pathways in response to infections and tumour development.

**Recipient or parental organism**

Mammalian cell lines: such as THP1, C1 Rand Hap1 cell line.  
Cell lines, such as HEK293 (or derivatives of) for the production of replication defective lentivirus.

**Host/vector system**

Disabled E. coli, K12: such as DH10b, DH5a, XL 10, Stabl2, Stabl3, TOP10, as well as commercially available class I - approved dam-/dcm- strains. Additionally, Pir+ expressing strains for propagation of pir-based replication origins may be used.  
Standard non-mobilisable or mobilisation defective vectors will be used.

**Origin & function**

Mammalian genes implicated in immune responses will be over-expressed, knocked down or mutated.  
CRISPR/CAS 9 (or similar) and guide RNA will be used for mutation of genes.  
Standard antibiotic resistance genes, such as Puromycin and Neomycin.  
Standard reporter genes, such as GFP and Luciferase.  
shRNA for knock down of genes.  
WPRE may be present in some of the vectors.

**Evaluation of foreseeable effects**

Inserts code for either normal mammalian genes, selective alterations of those genes, codon-optimized Cas9 and a single guide RNA targeting a mammalian gene, or an shRNA targeting a mammalian RNA. The target genes do not include known oncogenes but may be antigens expressed by tumour and/or normal cells, genes involved in the processing and presentation of antigens, genes involved in immune responses, genes involved in intracellular trafficking and genes involved in the unfolded protein response. Some genes have been found to be associated with hereditary diseases, expressed on tumour cells or involved with immune response. Since human sequences are targeted, modification of cellular DNA is theoretically possible upon gene transfer, but risk of gene transfer is minimal and unlikely to be hazardous.

Standard marker genes such as lacZ, eGFP, dsRed, or mCherry, and standard antibiotic resistance genes have a history of safe use and are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of the cloning host.

WPRE is commonly present in lentiviral vector systems and could be potentially oncogenic.  
Where possible sharps will not be used with lentiviruses. Where required e.g. for in vivo work, adequate training will be provided and training records maintained. Sharps will be disposed of immediately, not resheathed and the needle will not be removed from the syringe prior to disposal.

As the viruses are replication defective, shedding is not expected to be an issue and therefore minimal risk from in vivo work subsequent to inoculation. Inoculation of transduced cells is also not expected to pose a risk as the cells
should be recognised as foreign and cleared by the immune system.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| N/A |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - Dispose via the industrial waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - Dispose via clinical waste stream for incineration.

Degree of kill

- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical disinfection, used according to manufacturers instructions under standard conditions, manufacturers validation [eg4.79] log reduction ([eg99.998]% kill).

| Is an emergency plan required according to regulation 20? N |

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by SACGM.
Collective T cell differentiation and T cell responses

Use of hazard group 2 micro-organisms to study the signals controlling T cell immune responses to pathogens. Generating the right balance between effector and memory subsets is crucial to insure both short- and long-term protection, and we are studying how this balance is determined. In particular, we are studying:

- The signals regulating T cell fate
- The spatio-temporal T cell response and commitment
- T cell collective responses

Recipient or parental organism

- Hazard group 2 micro-organisms, such as Listeria monocytogenes.
- Influenza strains, such as A/HKx31
Host/vector system

Immunogens have been fused to signal sequence and promoter of hly gene and reintroduced into listeria. The bacteria are created elsewhere.
Immunogens have been inserted after the HA gene in influenza. These stains have been created elsewhere.

Origin & function

Immunogens, such as Ovalbumin, introduced into micro-organisms.

Evaluation of foreseeable effects

Wild type listeria is a hazaard group 2 micro-organism and the introduction of immunogens is not expected to increase or decrease the risks from this pathogen.
Strains of influenza used are attenuated strains however X31 still has low pathogenicity in humans although it cannot invade lung epithelium. Due to this a precautionary approach is being taken and containment level 2 requirements will be used. PR8 strain has been shown to be non infectious to humans however it causes illness in mice and as such containment level 2 will be required in BMS facilities.
Ovalbumin is the main immunogen in eggs but has been used in research for many years without any reports of induction of egg allergy.
These engineered strains of Listeria and influenza allow us to study, for example, the function of T cell affinity for the peptide or the spatio-temporal T cell response following infection.
Infection of mice and analysis post infection by immunological assay, imaging or phenotyping. Infection of mice will be done by intravenous injection for Listeria and intranasal inoculation for influenza. Needles will be single used and disposed of directly in sharps bin. Strictly no re-sheathing of needles. The volume will most commonly be 100-200jll. Infected mice will be handled in a Class II micro-biological safety cabinet and housed in IVCs. Generation of single cell suspensions from tissues will be done in an MSC. Cages will be clearly labelled.
Listeria monocytogenes cannot naturally infect mice, as it is unable to interact with mouse E-Cadherin and therefore invade the gut. Therefore, natural Listeria transmission to other mice is limited. Listeria will be cleared in mice after around 10 days post infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

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Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

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### Project Ref  553/17.3

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<td>15/03/2017</td>
<td>Production of recombinant replication-defective lentivirus as part of the WIMM virus production facility (VPF)</td>
<td>Class 2</td>
<td>&lt; 1 L</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Project Additional Information

**Purposes of the contained use**
The design and generation of lentiviral particles, in particular siRNA, shRNA and guide RNA for customised library screens but also viral particles for over expression of genes.

**Recipient or parental organism**
- E. coli K12 and B derivatives - class 1
- Viral producer cells, such as HEK293 cells or derivatives
- Mammlian cells

**Host/vector system**
Standard replication defective retro- or lenti-viral particles based on viruses such as MoMLV, HIV, EIAV
- Viral producer cells, such as HEK293 cells or derivatives.

**Origin & function**
As the nature of the work is generating library screens it is difficult to predict what the intended functions will be and there is the potential to express oncogenes or to knock-down tumour supressor genes. As such all viral particles will be handled with a precautionary approach.
WPRE may be present in some of the viral vector plasmids.

**Evaluation of foreseeable effects**
As the overall aims of the service is to generate sgRNA and shRNA libraries for customised library screens. Therefore there is the potential that the sgRNA I shRNA could target tumour supressor genes or other essential genes which could have a deleterious effect. The control measures in place should reduce the potential for exposure to occur. Occasionally genes may be expressed from the lentivirus however these are not expected to increase the hazard over that posed by the presence of WPRE, which is potentially an oncogene.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

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Animal Units

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This aim of this project is to assess mouse cytomegalovirus as a vaccine vector for its ability to induce long and durable immune responses, both cell mediated and humoral, against an antigen of interest.

Recipient or parental organism

Producer cell lines, such as NIH 3T3
Standard mammalian cells lines
Primary cells
Mice

Host/vector system

Standard E. coli transfected with a bacterial artificial chromosome (BAC) containing the whole MCMV genome

Origin & function

Inserted genetic material will be a gene of interest from numerous infectious pathogens with the aim of triggering an immune response to the pathogen. Examples include antigens from Plasmodium, Influenza and Mycobacterium etc
Other inserts will be model antigens such as Ovalbumin or Hen Egg Lysozyme
Standard reporter genes such as GFP, mCherry or Luciferase

contained
**Evaluation of foreseeable effects**

MCMV has been shown to infect human cells in vitro, but infection leads to an abortive cycle and is not thought to induce any disease. It can however infect murine samples and the prevalence in the wild population is estimated to be 65-90%. It leads to an asymptomatic infection and is likely to persist in the environment however control measures are in place to prevent accidental release into the environment. MCMV is an enveloped virus and therefore has poor survival in the environment. MCMV transmission is thought to require direct contact with contaminated excretion/secretions. Cross protection between wild type and recombinant strains is expected.

The inserts are not expected to alter the pathogenicity of the virus nor is it expected to alter the host range. It is expected that exposure to the virus would induce immunity to the transgenes and subsequently the pathogens from which they are obtained.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| N/A |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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| Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. | N/A |
| Sharps (eg needles, syringes, scalpels) - autoclave then dispose via clinical waste stream for incineration. | N/A |

**Degree of kill:**

- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed inCinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

**Is an emergency plan required according to regulation 20?**

| N |

**If yes, tick to confirm that it is attached to this form**

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**Tick to confirm that you have attached a risk assessment to this form**

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<td>L3 L4 L2 L3</td>
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</table>

**Project Ref** 553/17.5

- **Date Ackn'd**: 10/07/2017
- **CU2 Project Title**: Studying Pseudomonas Biofilms
- **Class**: Class 2
- **Culture Volume**: < 1 Litre
- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Project Additional Information**

- **Purposes of the contained use**
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Animal Units

Large Scale Activities

Human Clinical Applications
The aim of the research is to study how changes in intracellular and extracellular pH affect cellular or tissue physiology and pathophysiology.

Recipient or parental organism

Primary mammalian cells
Mammalian cell lines
Lentiviral producer cell lines such as HEK293

Host/vector system

Standard replication defective lentiviral systems

Origin & function

Enzymes that synthesise and degrade mobile H+ carriers (such as CARN1, CARNMT1, CDNP).
Proteins that form cell-to-cell connexin channels (such as mainly Cx43 isoform).
Genes involved in the regulation of extracellular acidity (such as SLC4A2 (coding for AE2)).
Genes coding for membrane transporters, such as SLC superfamily members coding for NHE and NBC proteins. Genes involved in changes to the pH regulatory apparatus, including nuclear NHE1, as well as genes coding for proteins that synthesise and breakdown histidyl-based mobile buffers. Standard reporter genes such as GFP and luciferase. Standard antibiotic resistance genes such as puromycin and neomycin. Woodchuck hepatitis virus post transcriptional regulatory element. CRISPR / Cas 9 and sgRNA system.

**Evaluation of foreseeable effects**

Lentiviral particles can enter mammalian cells and, in case of successful transduction, integrate into the genome. There is therefore potential for the inadvertent disruption of tumour supressor genes, however this will be mitigated by not using sharps. The lentiviral particles are replication defective and should they enter the cell they will not be able to generate more infectious virus. The WPRE gene fragment is present, which may have oncogenic potential. Genes will be knocked down using lentiviral delivery of shRNA, overexpressed by lentiviral delivery of genes or mutated using CRISPR / Cas 9. CRISPR sgRNA and the Cas9 genes are on separate lentiviral plamids and therefore the possibility of gene drive is minimised. Knock down or over expression of the genes is likely to affect the pH of the intra / extracellular environment resulting in a change of cellular or tissue physiology and pathophysiology.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Consumables (mainly plasticware eg pipettes, flasks, tubes) | - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. 
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Degree of kill: 
Autoclaving, effectively 100% kill (annual validation) 
Incineration, effectively 100% kill (licensed incinerator) 
Chemical, effectively 100% kill (following manufacturers guidelines) |
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM. Contained

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<tr>
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<td>L3 L4 L2 L3</td>
<td>L2 L3 L4 L2</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2 L3 L4</td>
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### Project Ref 553/17.7

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<tr>
<td>28/06/2017</td>
<td>Production of recombinant proteins for structural and functional studies</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Consent Granted</td>
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Non-GMM

Historical Significant Changes

<table>
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<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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<tr>
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</table>

Historical Date of Additional Info

Significant Change ID

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

A replication defective lenti-viral system will be used to produce large quantities of both secreted and membranebound recombinant proteins in order to determine their structure and function.

**Recipient or parental organism**

- E. coli K-12 - class 1
  - Standard eukaryotic cell lines, primary cells, iPS cells, immortalized cells and secondary cells.

**Host/vector system**

- Standard replication defective Lentivirus
  - HEK293 (or derivatives / similar) producer cells

**Origin & function**

This program of work will investigate proteins involved in development and neurology. Typically neuronal proteins required for signal transduction and cell adhesion and also proteins involved in cell adhesion and signalling that are important in development and disease. we plan to express peptides, proteins, naturally occurring genes (or mutants thereof), synthetic oligonucleotides (designed to create mRNA in eukaryotic cells for recombinant protein expression) and short synthetic genes (for example, codon optimised genes). Examples include the Wnt signalling pathway, FLRT family, semaphorins and nanobodies.

Standard antibiotic resistance genes and standard reporter genes, such as GFP, Luc, amp and Kan.

Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE) is also present in many of the lentiviral constructs.

The constructs may also include genes to allow for an inducible system of gene expression, such as Tet-on.

**Evaluation of foreseeable effects**

The lentivirus to be used are replication defective however they do have the ability to integrate into the host genome and pose a risk of insertional mutagenesis. The worst-case scenarios are: i) expression of oncogenes, ii) the chance integration of the virus into a tumour suppressor disrupting that gene’s function and/or iii) reprogramming of cells and subsequent teratoma formation. However, in all cases, the virus particle would be incapable of infecting more than one cell and would not self-replicate. In-vitro work will limit the risk by reducing the use of sharps. Risks will be mitigated by training and procedures.

Many of the inserts are not expected to pose a hazard however a number of them are linked to stem-cell replication and are implicated in some cancers. As such a precautionary approach will be taken with these genes.

WPRE is commonly used in replication defective lentiviral particles however there has been some concern raised with regards the potential oncogenic nature of this gene. As such a precautionary approach will be taken with these particles.

Expression of standard antibiotic resistance genes and reporter genes is not expected to pose an increased risk over and above that posed by the lentiviral vectors.

Once the viral particles have integrated into the host genome the host cells pose minimal risk and therefore can be handled at Class 1.
### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Consumables (mainly plasticware eg pipettes, flasks, tubes) | Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. |
|------------------------------------------------------------|
| Liquids (eg samples, culture supernatants, tissue culture media) | Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. |
| Agar plates | Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. |

#### Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

### Project Containment

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#### Animal Units

### Large Scale Activities

### Human Clinical Applications

#### Is an emergency plan required according to regulation 20? N

#### If yes, tick to confirm that it is attached to this form N

#### Tick to confirm that you have attached a risk assessment to this form Y

#### Tick if you are claiming exemption from disclosure for section of the risk assessment N

---

**Please enter comments on the GM safety committee on the risk assessment**

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.
Project Ref 553/17.8

Working with HIV-1 Infectious Molecular Clones expressing reporter proteins.

Class 3

Class Culture Vol Class 2 Culture Vol Class 3-4
Not Applicable 10 mls multiples of

Non-GMM Consent Granted
Yes

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Project Additional Information

Purposes of the contained use
Use of HIV infectious molecular clones to look at antigen processing and presentation in HIV-1 infected cells and to develop assays used to determine efficacy of vaccines.

Recipient or parental organism
Human immunodeficiency virus (HIV)

Host/vector system
The viruses are created elsewhere however they will be expanded in vitro within the department to generate viral stocks using human PBMC.

Origin & function
Standard reporter genes such as those based on Luciferase, Renilla and GFP,
Various strains of HIV isolates will be used which originate in different parts of the world and have different clades.

Evaluation of foreseeable effects
The LucR, GFP and RFP inserts are commonly used reporter genes and are not expected to alter the virulence of the
viruses.
The HIV isolates are all found in the natural population with different strains being more prevalent in different parts of the world.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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<tr>
<th>Consumables (mainly plasticware eg pipettes, flasks, tubes)</th>
<th>Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.</th>
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<tbody>
<tr>
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<td>- Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.</td>
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<td>Agar plates</td>
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</tr>
<tr>
<td>Sharps (eg needles, syringes, scalpels)</td>
<td>- No sharps to be used</td>
</tr>
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</table>

**Degree of kill:**
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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02/03/2022
### Project Ref 553/17.9

<table>
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<th>CultureVolumeClass3-4</th>
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<td>16/08/2017</td>
<td>Use of bacteria to investigate mechanisms of biofilm formation and implications for infection</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Consent Granted</td>
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#### Project notifed under transitional arrangements

<table>
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</thead>
<tbody>
<tr>
<td>N</td>
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</tbody>
</table>

#### Historical Significant Changes
- Notification transferred to GM20

### Project Additional Information

#### Purposes of the contained use
The aims of the project are to understand how adhesion of bacteria to surfaces, followed by defined cell-cell interactions between bacteria, lead to the formation of macroscopic three-dimensional bacterial communities called biofilms.

#### Recipient or parental organism
- Hazard Group 2 bacteria such as Pseudomonas aeruginosa
- Hazard group 1 bacteria such as Pseudomonas fluorescens and disabled strains of E. coli (K-12 and B derivatives)

#### Host/vector system
- E. coli K-12 or B derivatives and non-mobilisable vectors

#### Origin & function
Standard reporter genes such as GFP and Luciferase
Standard antibiotic resistance genes such as Amp and Kan
Over expression or knockdown of genes proposed to be involved in the bacterial adhesion process, such as fimbriae genes, Ag43, Pel and PsI.
Over expression or knockdown of genes identified from bacterial samples from Cystic fibrosis patients to be involved in biofilm formation.

Evaluation of foreseeable effects

Addition of standard reporter genes has a history of safe use and is not expected to alter the pathogenicity of the host.
Standard antibiotic resistance genes are not used to treat the disease and therefore will not alter the ability to treat infections.
Over expression of proteins in disabled E. coli K-12 or B derivatives to enable production of proteins for study is not expected to overcome the disabled nature of the strains.
Overexpression of genes involved in biofilm formation in P. aeruginosa is not expected to increase virulence. Where there may be potential for affecting virulence the work will be done in strains which are Type IV pili negative.
Deletion mutants are likely to have a reduced ability to form biofilms and consequently are less likely to cause infection.
Parallel work in P. fluorescens and E. coli will be done to the same standards as P. aeruginosa to ensure consistency.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps (eg needles, syringes, scalpels) - sharps are not required for this project
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Is an emergency plan required according to regulation 20?  

Tick to confirm that it is attached to this form

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

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### Project Containment

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

### Project Ref 553/18.1

<table>
<thead>
<tr>
<th>Date Ackn’d</th>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tr>
<td>18/01/2018</td>
<td>Rabies vaccinology and structural biology</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
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</table>

- Non-GMM
- Consent Granted

Project notified under transitional arrangements
Purposes of the contained use

We wish to use a replication-incompetent 'deltaG' rabies virus system as a laboratory tool. This will be used for in vitro studies of the structure of the rabies glycoprotein and for virological assays (such as antibody neutralization assays). Plasmids encoding a rabies genome lacking the essential gene for the virion glycoprotein (G), and helper plasmids will be transfected into cell lines which express the rabies G protein or an alternative G protein which results in rabies virions that cannot propagate due to lack of G protein in their genome. This system has been used in the USA, Switzerland and other UK Universities.

In addition as a connected part of this work we wish to use standard replication defective lentiviral systems.

Recipient or parental organism

SAD B19 strain of rabies (vaccine strain used to immunize wild animals in Europe and other locations).

Also standard replication defective strains of Lentivirus

Host/vector system

E. coli K12 and B derivatives will be used to propagate plasmids.

Plasmids used are non-mobilisable.

Plasmids containing the deltaG rabies genome will be transfected into packaging cell lines such as HEK and BHK.

Plasmids containing the genes for replication defective lentivirus will be transfected into packaging cell lines such as HEK.

Origin & function

TEV protease, rabies glycoprotein, modified rabies glycoproteins or other modified glycoproteins. The modifications to the rabies glycoprotein typically entail introduction of affinity purification and protease cleavage tags within the glycoprotein sequence. The genes are to be introduced into the packaging cells lines using replication defective lentivirus.

delta G rabies sequence may also contain standard reporter genes such as mCherry and GFP.

CRISPR guide RNAs and Cas 9. The primary genes of interest for the work to be undertaken here will be those encoding cell-surface receptors. There are however thousands of such genes and it is often more economical/practical to use a pre-existing genome wide gRNA library than to design and synthesize a custom gRNA library targeting a subset of genes. This risk assessment thus includes and addresses the likelihood of using gRNAs targeting any gene in the human or mouse genome.

In the case of CRISPR-Cas9 work, cell lines will then be screened for a phenotype of interest, for example flow cytometric assays of binding of a protein.

Selectable drug resistance markers (such as puromycin and Olasticidin resistance)

Evaluation of foreseeable effects

Wild type Rabies is a group A pathogen on schedule 5, is a hazard group 3 pathogen under ADCP and is a specified animal pathogen. However no wild type will be handled, the work is restricted to a replication defect version of the vaccine strain used widely in Europe to control rabies in wild animals but not in the UK.

The strain of Rabies Virus that has been modified is the vaccine strain SAD 819 which has been routinely distributed.
within the environment of Europe in an effort to vaccinate wild animals and has contributed to a reduction in wild type rabies cases across Europe. SAD 819 is a highly attenuated rabies virus derivative, generated by serial passage of an original 1935 field isolate first in mouse brain cells then in two in vitro cell lines. SAD 819 is licensed in Europe as a live-attenuated veterinary vaccine against rabies ("Fuchsoral", IDT Biologika, Germany; License Number 479/a/91). Millions of doses have been distributed in the environment for wildlife vaccination; there has never been a report of human disease due to SAD 819. Safety studies have found that SAD 819 is incapable of causing disease in any of 16 tested species including baboons and chimpanzees, with the exception of highly attenuated pathogenicity (without further transmission) in certain experimental rodents.

Key biosafety features of this system are as follows:

1. The principal safeguard is that the nucleic acid genome carried by ~GR virions lacks the essential glycoprotein gene and are thus unable to replicate in cells other than the packaging cell line. The risk of recombination reconstituting a G-containing genome is generally regarded negligible due to the absence of any regions of homology between the backbone of the glycoprotein expression vectors and the ~GR genomic sequence. The system has been used in multiple laboratories since 1996 and recombination has never been reported, despite the fact that it would be very readily detected in the course of routine experiments in vitro or in vivo.

2. The G-deleted genome of the ~GR virions is that of the Street Alabama Dufferin 819 strain (SAD 819) of rabies. SAD 819 is highly attenuated, to the extent that it has been extensively and deliberately released into the environment as live vaccine for immunisation of foxes in Europe and has been shown not to be transmitted between wildlife.

3. Where possible, we will use a recently described ~GR-variant known as ‘self-inactivating rabies’ (SiR). In addition to lacking the gene for G, the viral nucleocapsid (N) protein-coding gene in the SiR genome carries a proteasome-targeting tag. This results in destabilisation of the essential N protein in any cells other than the packaging cell line (which expresses a protease mediating removal of the tag). An additional recombination event would thus be required to reconstitute replication-competent virus from the SiR genome.

Reconstitution of an infective virion would require recombination of the ~GR genome with the G protein gene present in the packaging cell line; the risk of recombination reconstituting a fully infection-competent virus is widely regarded as negligible for the following two reasons:

- Firstly, there are no regions of homology between the backbone of Glycoprotein expression vectors and the genomic sequence of the ~GR, making RNA recombination extremely improbable.
- Secondly, rabies is a negative strand RNA virus replicating in the cytoplasm. It does not enter the nucleus or have a DNA intermediate stage, let alone one which integrates in the genome. There is therefore effectively no chance of recombination between the ~GR rabies genome and the genomically-integrated G-coding DNA present in the packaging cell lines.

Accordingly, in more than 300 rounds of production in other laboratories over the last decade not a single case of recombination has been detected (Marco Tripodi, personal communication). Had they occurred, these events would have been readily identifiable during routine studies as they would confer a large selective advantage to those particles, dramatically changing their behaviour.

The risk of recombination of the Gvar-coding nucleic acid in the pseudotype packaging cell lines with the ~GR genome is negligible for the same reasons outlined above.

Infection of humans with wild-type rabies requires percutaneous or mucosal contact with the virus. Containment Level 2 measures and the prohibition of use of any sharps with ~GR virus will reduce the opportunity for such contact to a minimum.

The SAD B19 vaccine strain of rabies safety has been extensively evaluated in multiple animal species; it does not cause disease in any of 16 tested species (except after parenteral administration in mice). Potential for transmission between animals has been specifically examined and ruled out. The risk of environmental harm would therefore be
negligible even if intact SAD B19 was used; risk pertaining to this work is even lower because no intact SAD B19
genomes will be involved. Risk of environmental harm is therefore assessed as effectively zero.
Addition of standard reporter genes is not expected to increase the hazard of the replication defective virus.
The packaging cells lines are generally based on HEK and BHK cells which are both widely used laboratory cells
lines. Expression of the TEV or the glycoproteins is not expected to increase the risks from the cells. Replication
defective lentiviruses will be used that may contain WPRE and as such these will also be handled at containment
level 2.
Both the lentivirus itself and the lipofectamine-plasmid mixtures used in lentiviral production have the potential to
transduce mammalian cells. The following events of concern might occur after such transduction events:
1. Insertional mutagenesis
2. Oncogenesis as a result of the use of the WPRE post-transcriptional regulatory element.
3. Mutagenesis as a result of the CRISPR-Cas9 system.
Insertional mutagenesis and WPRE-driven oncogenesis are considered to be improbable, even should skin contact
with lentivirus occur. Use of gloves and standard Containment Level 2 measures is assessed to reduce these risks to
a negligible level, as has been widely accepted for such work elsewhere.
Mutagenesis in a worker's cells by the CRISPR-Cas9 system could potentially lead to activation, inactivation or
mutation of any gene (in the case of use of a genome-wide gRNA library, and dependent upon the Cas9 variant in use
[nuclease active, trans-activating or transcriptionally-repressing]). However, any such effects would require
transduction of the same cell with both a guide RNA and the Cas9 nuclease; either constituent on its own is inactive.
Potential for such effects will thus be avoided by the use of systems in which these elements are supplied by different
vectors during different cell-transduction steps. This work will not use bicistronic vectors carrying both Cas9 and
gRNA, and nor will these be handled simultaneously at any point. The combination of this measure with the use of
gloves and standard Containment Level 2 measures is thus assessed to reduce to a negligible level the risk to the
worker of CRISPR-Cas9-driven mutagenesis.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS
2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at
least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream
for landfill.
Liquids (eg samples, culture supernatants, tissue culture media) -autoclave using a make safe cycle as specified in
BS 2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C
for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.
Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-1250C for at least
15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to
drains, dispose of solids via the industrial (black bag) waste stream for landfill.
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4 L2</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
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Project Ref 553/18.10

Date Ackn’d 20/12/2018
CU2 Project Title Understanding the role of chromatin in the heritable maintenance of chromosome structure and gene expression

Class CultureVol Class CultureVolume
Class 2 < 1 Litre
Non-GMM Consent Granted

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.
Consideration has been given to the appropriateness of mandatory pre-exposure rabies vaccination of workers using t.GR virus. It has been decided that this would not be appropriate because the work will not involve any replication-competent rabies virus and additional safeguards against harmful exposure are in place (use of the SAD 819 backbone, containment level 2 measures and prohibition of sharps).
The delta G strains has already been approved for work at class 2 (GM53/13.4) in another Department within the University and is also worked on as a class 2 at another UK University. Advice from DEFRA at that time was that it would fall outside the scope of SAPO due to the replication defective nature. We also feel it falls outside of ATCSA.
**Project Additional Information**

**Purposes of the contained use**

Use of replication defective retrovirus and lentivirus to study how chromatin structure is established and how it is stably inherited epigenetically.

**Recipient or parental organism**

E. coli K-12 and B derivatives
Mammalian cells lines including human primary cells, immortalized cells and cancer cell lines

**Host/vector system**

Non-mobilisable plasmids
Standard replication defective retrovirus and lentivirus systems

**Origin & function**

Standard reporter genes such as SNAP, HALO, other fluorescent proteins.
Standard epitope tags such as HA, FLAG and Myc.
CRISPR-Cas9 system using guide RNA to knock out genes mentioned below.
Housekeeping genes involved in the functioning of the centromere such as CENP-A, CENP-C and CENP-T.
Genes involved in interferon signalling such as STAT and IRF transcription factors.
WPRE - Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element.

**Evaluation of foreseeable effects**

Viral particles are non-replicating and non-lytic, however they do have the capacity to enter human cells and integrate within the host DNA. This could potentially cause random insertional mutagenesis.
Standard reporter genes and epitope tags have a history of safe use and are not likely to increase the risk over that posed by the viral vectors themselves.
Cas 9 nuclease along with its guide RNA will be delivered into target cells via lipofectamine or viral delivery. The purpose is to create targetted double stranded breaks to disrupt the gene of interest or to insert a reporter gene at a specific location. The genes to be disrupted are not associated with major pathologies and are not known as major cancer drivers.
WPRE is commonly found in lentiviral systems and may be a potential oncogene. As such a precautionary approach is being taken and work with viral particles will be done in a micro-biological safety cabinet.
Genes will not be expressed in E. coli as they are driven by mammalian promoters, therefore this work can be assigned to class 1. Mammalian cells transduced with the virus pose minimal risk as the viral particles are non-replicating, therefore this work can be assigned to class 1 after the media has been changed. Producer cells will produce viral particles capable of one round of infection once they have been transfected with the plasmids and will therefore be handled at containment level 2 along with viral preparation for subsequent transductions.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| N/A |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. **Degree of kill:** Autoclaving, effectively 100% kill (annual validation) Incineration, effectively 100% kill (licensed incinerator) Chemical, effectively 100% kill (following manufacturers guidelines) |

**Is an emergency plan required according to regulation 20?**

| N |

**If yes, tick to confirm that it is attached to this form**

| N |

**Tick to confirm that you have attached a risk assessment to this form**

| Y |

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

| N |

**Please enter comments on the GM safety committee on the risk assessment**

| Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM. |

### Project Containment
**Project Ref** 553/18.2

<table>
<thead>
<tr>
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<td>01/02/2018</td>
<td>Transfection of mammalian cells using replication defective lentiviral vectors</td>
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**Class** | **CultureVol** | **Class2** | **CultureVolumeClass3-4** |
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**Non-GMM** | **Consent Granted** |
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<td>Not Applicable</td>
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</table>

**Project notified under transitional arrangements** | **N**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Use of replication defective lentivirus to assist in the studies performed in this department. Examples of the studies include cancer metabolism and hypoxia, the role of mitochondria in ME/CFS, investigating the role of mitophagy in patients with mitochondrial diseases and also investigative studies on pre-eclampsia and endometriosis.

**Recipient or parental organism**

Standard E. coli K-12 and B derivatives.
Standard mammalian cells including primary and immortalized cells.
Standard viral producer cells, such as HEK293 derivatives.

**Host/vector system**

02/03/2022
Standard replication defective lentivirus, second generation or higher, where the genes required are separated on to at least 2 different plasmids.

**Origin & function**

cDNA sequences of mammalian origin and/or shRNA sequences.
Standard reporter genes (eg GFP, mCherry) and standard antibiotic resistance genes (eg Amp, Kan, Neo).
Genes / shRNA will be cloned into the lentiviral vectors including partial/entire gene sequences or mutant forms of genes under the control of constitutive promoters.
Some of the genes will have unknown function and as a result may include oncogenes or tumour suppressors.
WPRESS may be present in some of the vectors used. As this may have a potential oncogenic effect a precautionary approach will be taken.

**Evaluation of foreseeable effects**

Standard reporter genes and standard antibiotic resistance genes have been used for many years with no adverse effects being reported.
Replication defective lentiviral particles have the potential to infect cells and integrate into the host cell DNA. As this integration is random there is the potential for insertional mutagenesis. The risks of this occurring are minimised by the fact that sharps will not be require for the experimental procedures.
WPRESS may be a potential oncogene and as such a precautionary approach is being taken.
Some of the projects may involve work with tumour supressors or genes involved in oncogenesis. In addition many of the genes have an unknown function as such a precautionary approach is being taken.
Work with lentiviral particles will be considered Class 2.
Once cell lines have been transfected and the viral particles integrated (approx 5 media changes) cells will be considered low risk (excluding any CoSHH issues) and may be handled at containment level 1.
Genes are not expected to increase the risk of E. coli K-12 I B derivatives and can be considered class 1.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - into yellow (limb) bins which go for incineration via the NHS system.
Liquids (eg samples, culture supernatants, tissue culture media) - inactivate by chemical means (following manufacturers guidelines), discharge to drains.
Agar plates - into yellow (limb) bins which go for incineration via the NHS system.
Sharps (eg needles, syringes, scalpels) - dispose via NHS clinical waste stream for incineration.
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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<th>Laboratory Activities</th>
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**Project Ref** 553/18.3

- **Date Ackn’d**: 08/02/2018
- **CU2 Project Title**: Molecular engineering for bacterial vaccine development
- **Class CultureVolClass2 CultureVolumeClass3-4**: Class 2 ≤ 1 Litre
- **Historical Significant Changes**: N
- **Historical Date of Additional Info**: N
- **Significant Change ID**: N
Purposes of the contained use

The project will exploit the ease with which bacteria can be genetically manipulated to advance vaccine development. Bacterial diseases are responsible for a huge burden of morbidity and mortality and with increasing global levels of antimicrobial resistance, vaccination is an attractive option for combatting the public health problem presented by such diseases. Bacteria will be genetically modified to engineer candidate vaccines. Vaccines will consist of whole cell killed vaccines, live attenuated vaccines, vesicle vaccines and subunit vaccines.

Recipient or parental organism

Hazard group 2 bacteria, such as Neisseria and Salmonella (nontyphoidal) or similar bacteria.

Host/vector system

Standard E.coli K-12 and B derivatives for manipulation of standard plasmids (e.g. pUC based), Use of lambda-red recombinase to delete and insert targeted genes into the bacterial chromosome.

Origin & function

In the main, genes will be from the hazard group 2 bacteria being assessed. Standard reporter genes and standard antibiotic resistance genes may also be utilized.

Health and Safety

Executive

Examples of genes are included below but other genes, which have potential to be good antigens will be assessed as the research progresses.

Genes targeted for deletion will include those required for essential metabolic process e.g. aroA, aroD, htrA - mutations likely to render bacteria non-pathogenic for synthesis of lipopolysaccharide and e.g. msbB, htrB, pagP, galE, wbaP - mutation markedly reduces reactogenicity of bacteria maintaining integrity of linkage between inner and outer membrane e.g. Rmp, tolR, gna33 - mutation upregulates release of vesicles from the bacterial surface

Genes targeted for upregulation will include those encoding key antigens that are potential targets of protective immunity e.g. fHbp, nadA* mtrE, sseB, ompD.

Upregulation will enhance the immune response directed against such molecules.

Evaluation of foreseeable effects

Alteration of individual or multiple genes is not expected to increase the pathogenicity above that of the parental wild type. Most alterations are expected to attenuate the micro-organism in respect to the wild type to allow for suitable vaccine development.

Genetically inserted material will be bacterial surface antigens that have been identified as potential targets of the protective immune response. Increased expression of these antigens will permit an enhanced immune response to be made against them, while not increasing the risk associated with each organism.

Addition of standard reporter genes is not expected to alter the pathogenicity of the bacteria and have a history of safe use.
Addition of standard antibiotic resistance genes is not expected to be of concern as these antibiotics are not used to treat infections.

In vivo work may include the use of needles. Risks will be minimised by initial training using non-infectious material, non-resheathing of sharps, immediate disposal into a sharps bin and where possible anaesthesia or restraining to minimise movement during inoculations.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - Chemical method using Chemgene to a final concentration of 2% incubate 30 mins and discard to drains. For larger scale CL2 work autoclave in the Jenner CL3 facility autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose as infectious clinical waste stream for incineration.

Degree of kill:

- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Project Ref** 553/18.4

- **Date Ackn'd**: 29/03/2018
- **CU2 Project Title**: Understanding DNA Repair Mechanisms
- **Class**: Class 2
- **Culture Vol**: ≤ 1 Litre
- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Project Additional Information**

- **Purposes of the contained use**: Use of replication defective lentiviral particles to study the DNA damage response pathways.
- **Recipient or parental organism**: E. coli K12 derivatives (class 1)
Mammalian cell lines
Replication defective lentiviral particles

**Host/vector system**

E. coli K12 derivatives (class 1)
Mammalian cell lines
Replication defective lentiviral particles

**Origin & function**

Genes to be studied are mammalian in origin and predicted to be involved in the DNA damage response pathway. Examples of such genes include PARP gene family, genes involved in DNA repair (such as Ku70, BRCA1, XRCC1) and other DNA damage response genes (such as ATM, ATR, Chk1). Genes may be over-expressed, knocked down or contain specific mutations.

Other genes are standard antibiotic resistance genes and standard reporter genes.

CRISPR/Cas9 system and guide RNA will be used for genome editing to target genes creating specific mutations.

WPRE is frequently used to enhance viral titres.

**Evaluation of foreseeable effects**

The lentiviral particles generated will be able to infect a cell but unable to replicate. As the genes being looked at are involved in the DNA damage response pathway there is the potential that some of the modifications could affect tumour suppressor genes or other systems which could lead to proliferation. Risks will be controlled by the use of MSCs and not using sharps.

CRISPR/Cas9 system will be used to create specific mutations to genes. As above, some of the genes to be targeted may be tumour suppressor genes or mutations lead to proliferation. There is some evidence that use of lipid based transfection methods with CRISPR/Cas9 can lead to modification of epidermal cells. As such it is a requirement that gloves are worn.

WPRE may have potential oncogenic properties.

Standard antibiotic resistance genes and reporter genes have a history of safe use and are not expected to alter the overall risks.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.
Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. Sharps (eg needles, syringes, scalpels) - No sharps will be used.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM. All members of the committee approved the classification of the work.

Project Containment

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Project Ref 553/18.6

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<td>07/06/2018</td>
<td>Use of retroviral and lentiviral vectors for expression studies in mammalian cell lines and primary cells</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Date Project Ceased

02/03/2022
This project aims to study the role of genes in regions associated with risk of developing type 1 and type 2 diabetes using replication defective retro and lentivirus.

Standard mammalian cell lines from human and rodents including podocytes, myocytes, neuronal and induced pluripotent stem cells.

Pancreatic islets from human donors and other human primary cells.

Pancreatic islets from other species.

Standard commercially available replication defective retro and lentiviral systems.

Virus producer cells, such as HEK293 cells.

Mammalian genes and their regulatory elements associated with the signalling pathways and biological processes linked to diabetes. Examples of genes include cytokines (such as IL-2 and IL-21), splice factors (such as RBM17) and kinases (such as PFKFB3). shRNA and sgRNA targeted to these genes.

Standard reporter genes such as LacZ, luciferace and fluorescent genes.

Standard antibiotic resistance genes.

CRISPRiCas9 genes for targeted gene editing.

WPRE may be present in the lentiviral systems.

Retro/lentiviral vectors are replication defective and therefore pose minimal risk to the environment however they could pose a risk of insertional mutagenesis. This is managed by minimising use of sharps. WPRE is present in many lentiviral systems and could potentially be an oncogene.

The genes under investigation are associated with the risk of developing diabetes. Overexpression, knocking out, selective mutations or alterations to regulatory elements are not expected to be hazardous if accidental exposure were to occur.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Sharps (eg needles, syringes, scalpels) - No sharps to be used for virus work

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
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</table>
Correlative electron microscopy studies of HIV pseudoviruses and host factors

This project aims to define the effects of host factors on the function of capsid and viral infectivity in the context of native host cells using non-infectious virus-like particles and non-replicating pseudoviruses.

Recipient or parental organism

- E. coli K-12 or B derivatives
- Mammalian cells

Host/vector system

- Retroviral packaging plasmids, such as p8.91 and p8.2
- Envelope plasmids, such as pMDG
- Plasmid carrying the transgene, such as pHRSINcSGW (CSGW)
- Standard packaging cell lines, such as HEK293 cells

Origin & function

Plasmids are based on standardly available backbones but contain various combinations of HIV genes to create 2nd generation replication defective lentiviral particles.

- p8.2 encodes HIV-1 Gag-pol, tat, rev, vpr, vpu, vif, and nef genes and is based on the HIV-1 provirus with a 1.3Kb
deletion of the env gene and deletions of the L TRs, whereas pS.91 contains only gag-pol, tat and rev genes from HIV-1 and has a more greater section of the genome deleted. The transgene is solely a standard reporter gene to allow for visualisation of the virus like particles once inside the target cells. WPRE may also be present in some of the plasmids.

Standard antibiotic resistance genes.

Evaluation of foreseeable effects

To generate replication defective virus like particles, either pS.2 or pS.91 will be transfected along with the plasmids supplying the envelope gene and the transgene into a standard producer cell line, such as HEK293 cells. Work will initially be with pS.91 and then the effect of the accessory proteins and their interactions with the host factors will be studied using pS.2. Neither pS.2 nor pS.91 contain the env gene, which is responsible for engaging the CD4 receptor in the surface of the target cell, therefore they cannot generate infectious particles once inside the target cell. The risk of recombination between the plasmids is low as there are no significant regions of homology between them (except pS.2 and pS.91 which aren't transfected together). Therefore the likelihood of generating replication competent HIV is very low. Accidental exposure could lead to insertional mutagenesis however the likelihood of this occurring is also low as no sharps are used in the process.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Degree of kill:

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical, effectively 100% kill (following manufacturers guidelines)
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form Y

Is an emergency plan required according to regulation 20? N

Tick if notifying a connected programme of work N

Project Containment

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Animal Units

L2 L3 L4 L2

Large Scale Activities

L3 L4 L2

Human Clinical Applications

L3 L4

Project Ref 553/18.8

Date Ackn'd 20/09/2018

CU2 Project Title Mechanisms of tumorigenesis resulting from mutations in various cellular pathways

Class 2

CultureVol Class 2 CultureVolume Class 3-4

≤ 1 Litre

Consent Granted Not Applicable

Non-GMM

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**

Use of replication defective lentiviral systems to modulate expression of candidate genes to study the role played in tumorigenesis. Also the use of CRISPR to create point mutations, additions and deletions, in candidate genes.

**Recipient or parental organism**

Mammalian cell lines and primary cells - especially disabled hosts which should be recognised as foreign by the human immune system. Due to the potential presence of adventitious agents certain cell lines will be handled at containment level 2.

**Host/vector system**

Standard replication defective lentiviral vectors where the number of HIV genes have been reduced to gag, pol, rev, tat and vpr. The envelope protein is VSV-G which allows amphotrophic infection. The gene are split onto different plasm ids so this risk of replication competent virus being produced is minimal. Virus producer cells, such as HEK 293 cells (and derivatives of)

**Origin & function**

- mammalian cDNA for genes involved in cellular pathways such as fumerate hydratase, succinate dehydrogenase and G-protein coupled receptors, BMP2, GREM1, p16, p21, SOX9
- shRNA to knock down expression of genes involved in cellular pathways
- Standard reporter genes, such as GFP
- Standard antibiotic resistance genes, such as amp, kan and Neo.
- Cre recombinase
- WPRE
- methylation or histone modification factors, such as DNMT3A
- Other genes identified during the course of the project may also be expressed / knocked down.
- CRISPRi/Cas 9 gene editing technologies (including guide RNA) will be used to make deletion, insertion or point mutations.

**Evaluation of foreseeable effects**

E. coli K12 or B derivatives are disabled hosts which cannot colonise the human gut and have a history of safe use. Genes are not expected to be expressed in these systems due to the lack of bacterial promoter. Mammalian cell cultures are especially disabled hosts which should be recognised as foreign by the human immune system. Due to the presence of adventitious agents certain cell lines will be handled at Containment Level 2. As lentivirus integrates into the host DNA and lacks essential genes for replication, mammalian cells, which have been infected pose minimal risk.

293 cells (and various derivatives) are used to produce viral particles which are capable of one round of infection. These cells will be handled at Containment Level 2 with the use of sharps minimised. Lentiviral particles are infectious but replication defective however they are capable of integrating into the host genome where they may cause insertional mutagenesis. Control measures are in place to minimise the likelihood of exposure however needles are required for inoculations.
Innoculations using transduced cells pose minimal risk to the worker as the lentivirus will be integrated into the host genome and replication defective. The cells should be recognised as foreign by the human immune system.

Innoculations using lentivirus capable of one round of infection poses the greatest risk due to the use of needles. Workers are trained in these procedures including the policy on not re-sheathing needles and disposing of intact needle-syringe assembly into sharps bins immediately after use.

shRNA constructs will be used to deplete the target endogenous mRNA and therefore restrict gene function. The constructs carrying a target cDNA will be used to express or overexpress a specific gene and thus to study its function.

The inserts primarily have a role in regulating cellular pathways and are not considered to be inherently toxic, to have harmful physiological or pharmacological properties or to affect the pathogenicity of the host. Although loss of activity or overexpression of some of the genes is seen in certain tumors it is likely that other mutations are required for the progression of the disease. However as some of the genes could be potential oncogenes (or tumor suppressor genes) a precautionary approach with these genes in combination with lentivirus will be taken and handled at containment level 2.

CRISPR Cas 9 technologies are used in conjunction with lentivirus or standard plasmid systems to make deletion, insertion or point mutations to specific genes. Nickase Cas9 and dead Cas9 will be utilised where necessary. Some concerns have been raised with regards to CRISPR technology in conjunction with lipofectamine and as such a precautionary approach will be taken with gloves being worn to prevent exposure to the skin.

Methylation or histone modification factors can be used to activate or suppress the transcription of endogenous genes. Accidental exposure to a single gene is considered to be low risk.

WPRE is commonly present in lentiviral vector systems and may be a potential oncogene. As such a precautionary approach will be taken.

Some concerns have been raised internally with regards the use of CRISPR systems in conjunction with lipid based delivery systems. As such the use of gloves is required and the work assigned to class 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| N/A |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.**

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**Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.**

**Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.**
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Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Project Ref** 553/18.9

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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<td>Control of leukocyte responses by surface receptors</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
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</table>

Date Project Ceased

02/03/2022
## Project Additional Information

### Purposes of the contained use

Use of replication defective retro / lentiviral vectors to study how ligand binding to receptors triggers intracellular signalling and how signals are integrated by different receptors to shape cellular activation.

### Recipient or parental organism

Mammalian cell lines and primary cells - especially disabled hosts

### Host/vector system

- Standard replication defective retro / lentiviral systems (2nd and 3rd generation)
- Viral producer cell lines, such as HEK293 cells (and derivatives of)

### Origin & function

- Surface receptors, such as T-cell and B-cell receptors, collagen and OSCAR
- Signalling adaptor molecules, such as GADS, LAT and DAP
- Phoshatases, such as CD45, SHP and PTPN
- Kinases, such as ZAP, LCK and CSK
- shRNA to knock down the expression of endogenous surface receptors and molecules in their signalling pathway.
- Standard protein tools, such as Biotin, Streptactin and Spy Catcher
- Standard reporter genes, such as GFP, Luciferase, and Flag
- Standard antibiotic resistance genes, such as amp, kan, and neo.
- Woodchuck hepatitis posttranslational regulatory element (WPRE) to enhance transgene expression.

### Evaluation of foreseeable effects

The inserts express a protein or modify the expression of an endogenous protein and these are not known to be oncogenes. Individually the genes are not expected to have any adverse effects.

The viruses are replication defective and easily inactive in the environment.

The protein tags, reporter genes and antibiotic resistance genes are standardly used and have a history of safe use.

WPRE may be present in some of the constructs and as this may be a potential oncogene a precautionary approach is being taken.

Sharps are not required for this work and therefore this potential route for exposure is minimised.

Once the virus has integrated into the host genome the cells pose no greater risk than the untransformed version.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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Animal Units | Large Scale Activities | Human Clinical Applications
### Project Additional Information

**Purposes of the contained use**

We wish to investigate several key questions about the evolution of social traits (cell-cell interactions) using hazard group 2 human pathogens, including Salmonella Typhimurium and Pseudomonas aeruginosa and go on to use our understanding of social evolution theory and ecological theory to understand bacterial behaviour in infections. Specifically, we will investigate the extent to which social behaviour of bacteria affects their ability to infect hosts, cause disease and compete with other bacteria. We will focus on the use of molecular weapons as competitive traits and ask how human gut bacteria can resist invasion by a pathogenic competitor. We will measure the extent to which the natural microbiome can give us insights into how social behaviour can mediate colonisation resistance (prevention of a pathogen taking hold) within a host.

**Recipient or parental organism**

Hazard group 2 non-aerosol born enteric and environmental bacterial pathogens such as:
- Salmonella typhimurium
- Pseudomonas aeruginosa

**Host/vector system**

- Standard K-12F-, coliand B derivatives
- Standard non-mobilisable and mobilisation defective plasmids
Origin & function

Standard reporter genes such as GFP and other fluorophores
Standard antibiotic resistance genes
Colicin, pyocins, T655 effectors and prophage knock-outs and knock-ins. These are toxins or other competition mechanisms that the bacteria use to target and kill other bacteria (akin to antibiotics). They are not known to have harmful effects on humans.
Transposon (Tn) library mutants and evolved bacteria have mutations of random genes

Evaluation of foreseeable effects

Standard reporter genes have a history of safe use and are not expected to alter the characteristics of the GMO with respect to the wild type.
Standard antibiotic resistace genes are those commonly used in biotechnology. None of the antibiotics are used in a clinical setting to treat infections involving these pathogens.
Gene deletions of Colicin, pyocin, T655 effectors and prophage. Bacteria with knockouts of these regulatory genes are less able to fight against other bacteria or more vulnerable to the attack of their own kind due to the lack of the immunity genes. Therefore they are either likely to have or have been demonstrated to have reduced fitness and virulence relative to wild type strains. Tissue tropism and host range are unlikely to be affected.
Tn libraries. Bacteria with a randomly inserted transposon likely carry a disruption in a specific gene, resulting in a multitude of possible effects.
Consequently, harmful properties associated with the GMMs are at the most comparable to those of the wild type organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. Use of sharps will be avoided where possible but scalpels are required for in vivo work.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.
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Degree of kill:
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Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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Project Ref 553/19.10

Date Ackn’d 04/09/2019
CU2 Project Title In-vitro, In-vivo and Ex-vivo studies using Hepatitis B virus
Class 3
Culture Vol Class 2 < 1 Litre
Non-GMM Yes
Consent Granted

Project notified under transitional arrangements N
Withdrawn N
Tick if notifying a connected programme of work N
The overall aim of the work is to study Hepatitis B Virus through in-vitro cell culture methods, in-vivo animal models and ex-vivo human HBV samples.

Recipient or parental organism

Hepatitis B virus - Hazard Group 3

Host/vector system

Cell lines capable of generating Hepatitis B and supporting HBV infection, such as HepG2.2.15 and HepG2.A64 and na"ive HepG2, Huh 7, and HepaRG.
HBV- replicon plasm ids
Replication deficient Adeno associated virus to transfer HBV genome into cells
Replication deficient Adenovirus to transfer HBV gennome into cells

Origin & function

HepG2 was transfected with a plasmid that contains four 5' to 3' tandem copies of the HBV genome and resistance to G418 to create cell line HepG2.2.15. The DNA is found to be chromosomally integrated and also episomally as relaxed circular, covalently closed DNA. Viral DNA is also found in growth media.
HepG2 was transfected with a plasmid (pTriexHBV1.1) which contains 1.1 copies of the HBV DNA to create cell line HepG2.A64.
Other plasmids containing copies (multiple or overlength) of HBV DNA of various specific genotypes will be utilized. CRIPSR-Cas9 will be used to create mutations of HBV genes.
Standard reporter genes such as GFP and Luciferase.

Evaluation of foreseeable effects

The HBV particles isolated from human blood, serum, culture supernatants of HepG2.2.15, HepG2.64 and cells transfected or infected with HBV replicon plasm ids or Adeno-HBV/AAV-HBV are infectious in nature. HBV is transmitted through percutaneous or permucosal exposure to infectious bodily fluids (or infectious cell media).
Addition of standard reporter genes is not expected to increase the hazard of HBV over that with the wild type version poses. Neither are standard antibiotic resistance genes.
Mutation of individual genes is expected to have a negative consequence for the fitness of the virus however they will still handled as class 3.

AAV and replication defective adenovirus transfer of HBV genome. These viruses are replication defective and nonintegrating and therefore pose minimal risk to the environment.

In vivo studies pose the greatest risk due to the use of needles. This will be minimised by the work being carried out by suitably trained individuals. The animals will be restrained in a smooth tunnel which allows efficient access to the tail vein. All needles will be disposed of immediately and not resheathed.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

As Hepatitis B is a blood borne pathogen which is not infectious via the aerosol route it is highly unlikely that the laboratories would be fumigated in the event of a spill.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10m inutes or 1 34-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. |  |
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| Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. |  |
| Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or dispose via clinical waste stream for incineration. |  |
| Animal carcasses - autoclave then dispose via clinical waste stream for incineration. |  |
| Degree of kill: |  |
| Autoclaving, effectively 100% kill (annual validation) |  |
| Incineration, effectively 100% kill (licensed incinerator) |  |
| Chemical, effectively 100% kill (following manufacturers guidelines) |  |

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 (Yes)</td>
<td>L4</td>
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<td>L2</td>
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<th>Animal Units</th>
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<tr>
<td>L2</td>
<td>L3 (Yes)</td>
<td>L4</td>
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**Project Ref** 553/19.11

<table>
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<tr>
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<th>Class</th>
<th>CultureVolClass2</th>
<th>Class</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<tbody>
<tr>
<td>11/10/2019</td>
<td>Imaging of herpesvirus infection process in their host cells</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
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<tbody>
<tr>
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</table>

**Project Additional Information**

**Purposes of the contained use**

The aim of the project is the study of the infection process of herpesvirus infections.

**Recipient or parental organism**
Mammalian cells such as African green monkey kidney cells and baby hamster kidney cells
Human cell lines such as foreskin fibroblasts and retinal pigment epithelial cells

**Host/vector system**

BACS containing complete viral genome of Human Herpesvirus transfected into mammalian cells to generate replication competent infectious viral particles. Modification of the BACS will be done in E. coli using standard molecular biology techniques. This part of the work does not involve infectious viruses and following the guidance in section 2 of SACGM Compendium of guidance has been assigned to class 1.

**Origin & function**

Standard reporter genes such as GFP, YFP, mCherry, CFP and other small molecule tags, such as His, IHF, streptavidin, HA, snap, halo, clip.
Genes involved in the process of viral entry, such as gB, gH/gL, gM, gN and gO may be modified or knocked out to assess their involvement in the infection process.

**Evaluation of foreseeable effects**

Herpesviruses (HSV 1 and 2 and Human herpesvirus 5) are hazard group 2 micro-organisms and the addition of standard reporter genes and small molecular tags are not expected to alter the characteristics of the parental virus.
Tagging or knocking out entry associated gene are expected to result in equal or reduced infectivity compared to wild type virus.
HSV-1 can cause cold sores and related skin lesions in healthy individuals. HSV-2 is generally sexually transmitted. Human herpesvirus 5 is also known as human cytomegalovirus. The viruses become latent after the primary infection and can cause re-occurring infections. Approximately two thirds of the population have latent HSV-1 infections and approximately 70% of the population have had human CMV. Transmission is via contact either direct or indirect (eg kissing and sharing toothbrushes) for HSV-1, sexual contact for HSV-2, aerosols may also pose a risk from these viruses in the laboratory setting, and via contact and aerosols for Human herpesvirus 5.

For production of virions, mammalian cells will be transfected with BACS containing viral genome. The extracellular virus is then purified from the cell culture supernatant by ultracentrifugation and the purified virus used for infection, structural and biochemical studies, including cryo electron microscopy and mass spectrometry.
Work will be carried out in a class II micro-biological safety cabinet. Centrifuge rotors will be loaded and unloaded in a class II cabinet and ultracentrifugation will be done with aerosol-tight buckets. Plunge freezing is also done in an MSC. Gloves must be worn and care must be taken to ensure gloves are not contaminated.
Acyclovir, Gancyclovir or Foscarnet may be prescribed in the event of accidental exposure.

**N/A**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

WASTE MANAGEMENT MEASURES
All waste will be disposed of in accordance with local WTCHG Waste Disinfection policy, and using practice
consistent with the University of Oxford BioRisk Management policy.
Consumables (mainly plasticware e.g. pipettes, flasks, tubes) - will be rinsed/soaked in 1% Virkon solution in a
dispojar contained within the MSC. At the end of the session, plastic disposables will be transferred to an autoclave
bag, which will then be sealed and autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (134C
for 5 minutes / WHG discard autoclaves), with discharge of any excess liquids to drains, and disposal of solids via the
industrial (black bag) waste stream for landfill.
Liquids (e.g. samples, culture supernatants, tissue culture media) will be collected within the MSC, disinfected with
1% Virkon solution, prior to being autoclaved out of the lab. There are no drains available for liquid disposal in the
facility (OPIC yellow suite). Should the work move to a different containment level 2 laboratory 1% virkon overnight
will be sufficient without additional autoclaving.
Sharps - No sharps will be used.
Degree of kill:
Autoclaving, effectively 100% kill (annual validation);
Incineration, effectively 100% kill (licensed incinerator);
Chemical disinfection with 1% Virkon, effectively 99.998% kill.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
Harvesting of gradient bands using gradient fractionator - Does this do away with the use of needles to pierce the tube to collect the band? They don't mention the use of needles in the risk assessment so I presume so. Use of sharp needles would be one of the biggest risk when working with this virus so if they are using them this would need to be addressed.

The use of the gradient fractionator avoids the need for using needles, and thus eliminates the risk associated with sharp needles.

Virus purification/dialysis - I presume this is done to concentrate the virus? Are there any risks to this step? The virus is more concentrated but it is also in smaller volumes. The use of PPE (gown, gloves and glasses) and biosafety cabinet (TriMat 2) minimise the splash risk/generation of aerosols which constitutes the main potential route of transmission, thus virus handling is the same both before and after concentration.

Total volume of cell culture supernatant may be up to 800ml - this seems a rather large volume, is it made up of multiple smaller ones?

This is made up of multiples of less than 50mL aliquots, which combined make a total volume of up to 800mL.

It might be worth considering whether there are any differences in the pathogenicity / attenuation of the various laboratory strains available - is it possible to do the work using a relatively highly attenuated strain, or not, or are the strains which are already proposed highly attenuated?

Where possible attenuated versions of these viral strains are used, however, to answer questions regarding viral entry and replication attenuated viruses are not appropriate.

My 'index of concern' about HSV is a bit higher than for some other things which are handled at CL2 (eg 3rd gen lentiviruses). Although common it is a genuine pathogen & I wouldn't want to splash it in my face - as well as encephalitis, it can cause nasty corneal ulcers, and any infection which is acquired will establish lifelong latency.

It might be worth ensuring that users are aware that aciclovir might be felt to be worthwhile / beneficial for some exposures & so it is certainly worth contacting occupational health in the event of an exposure (more so than for some other organisms for which occupational health can't offer much!).

Is there any need to avoid sharps when handling purified HSV? It's not mentioned but presumably it's desirable, as with most other Class 2 assessments?

Discuss and accurately define waste parameters.

They could better specify the disinfecting agent(s) that will be used to treat the ultracentrifuge buckets. Virkon is corrosive, so it would damage the seals etc. EtOH would dry out any virions, thus inactivating them, but it would need to be applied thoroughly and left to dry properly.

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**Project Ref** 553/19.12
### Purposes of the contained use

Use of attenuated Maraba virus to deliver HIV antigens to promote an immune response to HIV either individually or in combination with other vaccines.

### Recipient or parental organism

Maraba virus - Hazard Group 2

### Host/vector system

Virus stocks will be imported ready made

### Origin & function

The Maraba virus has been modified to have mutations in the L 123W and the Q242R genes. The transgenes to be delivered are synthetic genes coding for chimeric proteins derived from parts of HIV-1 and filovirus genes such as tHIVconscX and FILOcepX. These chimeric proteins are based on conserved regions to give an increased depth of T-cell responses. These artificial proteins have no known enzymatic or biological activity or significant homology to human proteins. They are not expected to increase virulence or decrease the level of attenuation or change the host range of the virus. Standard reporter genes such as GFP or luciferease may also be present in the virus.

### Evaluation of foreseeable effects

Maraba virus is a single stranded RNA virus of the vesiculovirus genus of the Rhabdoviridae family which was isolated from the Brazilian sand fly. It has been genetically modified by mutations L 123W in the gene for the Matrix protein and Q242R in the gene for the glycoprotein. The mutations profoundly affects the ability of the virus to...
replicate in normal cells however it demonstrated a faster replication, a larger burst size, and an increased killing potency in tumor cells, in comparison to the wild-type. Infection of immune cells is abortive. Human phase 1 trials are underway and no adverse effects have been reported other than 'flu like' symptoms with high intravenous doses. Two work place exposures from needlestick injuries lead to milder symptoms. Humans are not a natural host for the virus but tissue tropism has been influenced to improve replication in cancer cells.

The transgenes to be delivered are for artificial proteins and have no known enzymatic or biological activity or significant homology to human proteins. They are not expected to increase virulence or decrease the level of attenuation or change the host range of the virus. Transgene product will be expressed in the cytoplasm of the infected cells before the normal cell shuts down the virus replication.

Standard reporter genes have a history of safe use.

The greatest risk will be from the in-vivo work due to the presence of sharps. These risks will be mitigated by ensuring that workers are suitably trained in procedures before using this virus. The animals will be restrained during inoculations into the tail vein. Sharps will be disposed of immediately and not resheathed. There is published data that reported that no live virus was detected in secretions / excretion and therefore this is an unlikely route of transmission to other animals or workers however standard procedures will be followed and animals and bedding will be treated as infectious.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Sharps (eg needles, syringes, scalpels) - autoclave then dispose via clinical waste stream for incineration.

Animal bedding - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), or via clinical waste stream for incineration.

Animal carcasses - autoclave and dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed inCinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Animal Units

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<td>L2 L3 L4 L2 L3 L4</td>
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Project Ref 553/19.13

Date Ackn'd 20/12/2019

CU2 Project Title Investigation into the interactions between plants and plant pathogens

Date Project Ceased

Class 2

CultureVol 1 Litre

Consent Granted

Non-GMM

Project notified under transitional arrangements N

Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
### Purposes of the contained use

Plant pathogens are major causes of crop losses around the world. This work aims to understand plant immunity against various types of plant pathogens.

#### Recipient or parental organism

- **a.** Bacterial plant pathogens such as *Pseudomonas syringae*, *Acidovorax avenae* and similar pathogens.
- **b.** Filamentous plant pathogens such as *Phytophthora infestans*, *Phytophthora capsici*, *Cladosporium fulvum* and similar pathogens.
- **c.** Viral plant pathogens such as tobacco mosaic virus, tobacco rattle virus, potato virus X and similar pathogens.

#### Host/vector system

- E. coli K-12 or B derivatives
- A. tumefaciens (disarmed strains)
- Standard plasmids

#### Origin & function

- **a.** The genomes of *P. syringae* and *A. avenae* will be modified (gene deletion, modification or overexpression) to generate knock out strains, strains with altered expression of endogenous genes as well as strains able to express foreign DNA and standard reporter genes. Target bacterial genes will be selected based on their involvement in disease such as pathogen associated molecular patterns (plant defence elicitors) and putative inhibitors of plant enzymes.

- **b.** The genomes of the plant pathogenic fungus *Cladosporium fulvum* and the plant pathogenic oomycetes *Phytophthora infestans* and *P. capsici* will be modified to generate deletion strains, strains with modified endogenous genes as well as strains able to express standard reporter genes. Target genes in each species will be selected based on their involvement in disease such as effector proteins (pathogen derived modulator of the plant immune system) and will be deleted or modified using gene targeting or CRISPR-Cas9 derived techniques.

- **c.** The gene material inserted in the viral vectors used for protein expression include fluorescent markers (e.g. GFP, RFP); fragments of genes from plants (for VIGS) or effectors (mostly hydrolase inhibitors) from pathogens that we hold under the DEFRA licence (for transient expression). For gene silencing, the viral vectors will carry a 200-400 bp fragment of a plant (*N. benthamiana* or *S. lycopersicum*) gene of interest. This fragment will not encode a full-length protein and it is usually not translated. The fragment triggers the degradation of the targeted plant gene. The overexpression or downregulation of genes in these plants is likely to modify aspects of the physiology of the intervened plants associated to the plant immune system.

### Evaluation of foreseeable effects

A number of the pathogens to be genetically modified are pathogens of crops and require a licence from the relevant regulatory authority to comply with the Plant Health Order. A licence is already in place and the group have extensive experience of working with wild type pathogens.

The modifications to the plant pathogens are not expected to make them more hazardous than the wild type strains. Where they have any observable effect, mutations are likely to reduce plant pathogenesis.

Expression of standard reporter genes has a history of safe use and is not expected to alter the pathogenicity.

Proteins are not expected to be expressed in cloning strains of *E. coli* and *A. tumefaciens* due to promoter specificity.

The pathogens are not known to pose a hazard to humans and the modification are not expected to alter this.

All pathogens will be used for in-vivo inoculations of plants (*N. benthamiana*, *A. thaliana*, tomato and bean). This
poses the greatest risk of loss of containment. Therefore any pathogens transmissible by aerosols will be handled in a Class II micro-biological safety cabinet and will be transported between rooms in sealed containers. Standard laboratory practice should minimise loss of containment for those pathogens requiring direct contact. Plants infected with sporulating plant pathogens will be grown in sealed boxes within growth cabinets. In-vivo inoculations are via syringe inoculation, dip inoculation, spray infection or directly inoculated to plants by rubbing with an abrasive. Infected plants are housed separately to non-infected in either growth chambers or a growth room.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial waste stream for landfill. Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. 2% virkon for at least 30 mins is effective against the pathogens being used.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - sharps are not used but syringes are dispose via clinical waste stream for incineration. Sharps bins are autoclaved prior to disposal.

Plant material and soil - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge via the industrial waste stream for landfill.

Degree of kill:

- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GMSC had comments on:-
1) movement between locations.
   we use sealed propagation boxes to move inoculated plants between CL2 rooms on the 2nd and 3rd floor;
2) containment for spray inoculations and volumes.
   we use the Class-II safety cabinet in the inoculation room for spray inoculations;
3) Clarify how inoculations are done.
   we will use the CL2 inoculation room and laboratory for droplet inoculations, infiltrations and dip inoculations when the
   risk of aerosols are negligible;
4) Containment of spores from sporulating pathogens.
   we will only open sealed boxes with sporulating GM pathogens in the Class-II safety cabinet;
5) Did access to the room containing growth cabinets need to be restricted.
   no door lock is needed for room S210 since there is restricted access to the building and a clear sign on the door and
   on the relevant growth cabinets that it is a CL2 lab containing licenced pathogens. Sporulating plant pathogens will
   only be in sealed boxes inside one of these cabinets;
6) Transport of waste to the autoclave.
   waste bags containing plant pathogens will be sealed before taken to the autoclave in the basement.

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Project Ref 553/19.2

Date Ackn'd 22/02/2019

CU2 Project Title Cloning and expression of eukaryotic membrane proteins in mammalian cells using an
attenuated Lentivirus system

Class Culture Vol Class 2 Culture Volume Class 3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N
The aim of this research is to determine the structure and biochemically characterise clinically relevant membrane proteins. In order to generate large quantities, we wish to use lentivirus to stably integrate the gene into mammalian cells.

Recipient or parental organism

Standard mammalian cell lines
Replication defective lentiviral systems such as a 3 plasmid system.

Host/vector system

Standard E. coli K-12 and B deriviatives and non-mobilisable or mobilisation defective plasmids.

Origin & function

Peptide transporters, organic anion transporting polypeptide transporters, nucleotide sugar exchangers, amino acid transporters, such as
SLC15 Peptide transporters
SLC19 Vitamin transporters
SLC35 nucleotide sugar transporters
SLC6, 7, 36, 38 amino acid transporters
SLC22 organic anion transporter
SLC16 monocarboxylate transporters
Inducible promoters e.g. Tet on / off.
WPRE element
di and tri peptides
thiamine, folic acid
nucleotide sugars
amino acids
monoamine neurotransmitter, carnitine, cAMP, urate
lactate, pyruvate
Inducible promoters e.g. Tet on / off.
WPRE element

**Evaluation of foreseeable effects**

Replication defective lentiviral particles can enter mammalian cells and integrate randomly into the host genome however they cannot replicate and spread. The genes required for viral particles are split onto multiple plasmids significantly reducing the likelihood of generating replication competent virus. There is the potential for the random integration to knock out a tumour suppressor gene however the likelihood of this occurring is minimal.

None of the inserted genetic material is expected to pose a risk. in addition the genes are often driven by an inducible promoter and as such wouldn’t be expressed outside of laboratory conditions. The lentiviral vectors contain the WPRE element which may have potential oncogenic activity and therefore a precautionary approach is taken.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

13. * Is an emergency plan required according to regulation 21?
DYes [ ] No

D If
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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**Animal Units**

- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

**Project Ref** 553/19.4

**Identification and genetic modelling of leukaemic and pre-leukaemic stem cells in myeloid malignancies**

**CU2 Project Title**

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<tr>
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<th>Culture Vol</th>
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<td>Identification and genetic modelling of leukaemic and pre-leukaemic stem cells in myeloid malignancies</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
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**Date Project Ceased**

Date Ackn'd

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
**Project Additional Information**

**Purposes of the contained use**
The aim of the project is to characterise the cellular and molecular biology of haematopoietic stem and progenitor cells (HSPCs) and how these become disrupted during the development of myeloid malignancies. Molecular targets identified in our studies will be mechanistically validated using a combination of retroviral or lentiviral overexpression/knockdown or CRISPR/CAS9 mediated genome editing.

**Recipient or parental organism**
- Standard E. coli K-12 and / or B derivatives - class 1
- Mammalian cells, such as TF1, HL60, K562 etc
- Primary haematopoietic cells, murine and human
- Viral producer cells, such as HEK293 derivatives or NIH3T3 derivatives

**Host/vector system**
- Standard replication defective lenti and retroviral particles generated by transfection of producer cells with multiple vectors providing necessary genes, such as Gag, pol, env and gene of interest.

**Origin & function**
The proteins under study, e.g. MPL, NF1, KRAS, G6B are expected to influence aspects of lineage choice, selfrenewal and/or differentiation in the final haematopoietic recipients, reflecting what is known or suspected of their normal biological function.
- Standard reporter genes, such as GFP
- Standard antibiotic resistance genes, such as ampicillin,
- Many lentiviral vectors also contain WPRE which is potentially an oncogene.
- CRISPR / Cas 9 and guide RNA

**Evaluation of foreseeable effects**
Some of the gene products are potentially oncogenic, for example, through modification of self-renewal or proliferation pathways. We are interested in targeting both tumour suppressor and oncogenes known to be associated with the development of haematological malignancies. As such they could have potentially harmful consequences.
- Standard reporter genes have a history of safe use.
- Standard antibiotic resistance genes have a history of safe use.
- Amphotropic retroviruses pose minimal risk to human health as they cannot enter human cells and being replication defective pose minimal risk to the environment.
- CRISPR/CAS9 allow targeted mutation of specific genes. Off target mutations will be minimised by blast searches comparing guide RNA with the genome.
- Mammalian cells which have been transduced by the virus will be used in vivo. As the viruses are replication defective
they pose minimal risk once they have integrated into the cellular DNA and the cells can be handled at the same containment as the unmodified ones.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware e.g. pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (e.g. needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

**Degree of kill:**

- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

The risk assessment has been amended to take into account the comments of the GMSC. Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

| Laboratory Activities | Glass Houses | Growth Rooms |
We aim to understand basic principles of host recognition and control of virus infections and of mechanisms by which immune responses are regulated including understanding viral strategies for evading immune control. Replication-defective lentivirus or other retroviruses will be used to modulate or edit gene expression.

Recipient or parental organism

- Standard E. coli K-12 for cloning (class 1)
- Mammalian cells, such as HEK293, Jurkat, Hela and Vero
- Primary cells, including human PBMCs and isolated T cells

Host/vector system

- Standard systems for generation of replication defective Lentivirus and retrovirus where genes required for virion production are split onto multiple plasm ids.
**Standard virus produced cells, typically based on HEK293 cells**

**Origin & function**

Mammalian genes or viral genes (fragments, mutants or tag versions) typically encoding viral receptors or proteins with roles in the host immune system, such as MHC molecules, T cell and NK cell receptors and co-stimulatory molecules, cellular ligands for NK receptors and costimulatory molecules, cytokines/chemokines, antiviral proteins. Genes derived from viruses, particularly herpesviruses

- shRNA or RNAi
- CRISPR I Cas 9 gene editing system including guide RNA
- Standard reporter genes, such as GFP
- Standard antibiotic resistance genes, such as geneticin
- WPRE gene

**Evaluation of foreseeable effects**

Replication defective lentivirus and retroviruses have had all non-essential genes removed from the vectors and the remaining genes split onto separate plasmids with minimal homology to reduce the risk of homologous recombination. As they are replication defective they are unable to disseminate within the host or environment. They do however integrate into the host genome and as such there is the potential for insertional mutagenesis. Also many lentiviral systems contain the post-transcriptional regulatory element from the woodchuck hepatitis virus, which may potentially have oncogenic activity.

The genes to be expressed, knocked down or edited are not expected to be hazardous. They are not known to increase the survival of the GMO, nor increase their ability to evade the immune system. None of the genes to be expressed or modified are known oncogenes.

CRISPR/Cas 9 has become a common tool for targeted gene modification. The guide RNA targets the Cas9 nuclease to introduce a break at a specific site in the genome, repair of which introduces mutations/indels into the targeted gene.

Standard reporter genes and antibiotic resistance genes have a history of safe use.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream.
Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. No sharps will be using in conjunction with the replication defective viruses.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<td>Animal Units</td>
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### Project Ref 553/19.6

<table>
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<tr>
<th>Date Ackn'd</th>
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<tr>
<td>23/05/2019</td>
<td>Functional and structural analysis of genes implicated across multiple autoimmune diseases by electroporation, transfection and transduction</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Not Applicable</td>
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Date Project Ceased: 02/03/2022

Project notified under transitional arrangements [N]
### Project Additional Information

#### Purposes of the contained use

This project aims to use lentivirus to study the role of genes in regions associated with the risk of developing multiple autoimmune diseases. Related genes acting in the same signalling pathways or biological processes may also be investigated.

#### Recipient or parental organism

- Standard mammalian cells, such as HEK293, Jukat and THP-1
- Primary mammalian cells

#### Host/vector system

- Standard replication defective lentiviral systems where the genes, such as gag-pol and env are split on to separate plasmids. All non-essential genes are deleted.
- Viral producer cells, such as HEK293 derivatives

#### Origin & function

- Overexpression, knockdown or mutation of genes associated with the risk of developing multiple different autoimmune diseases or related genes acting in the same signalling pathways, such as Tyrosine kinase 2 (TYK2), Interleukin-12 receptor B1 and B2 (IL 12RB1) and (IL 12RB2), Interleukin-23 receptor (IL23R), Interferon alpha and beta receptor subunit 1 and 2 (IFNAR1 and IFNAR2). TYK2 is a member of the Janus-activated kinase family and plays a key role in the signal transduction pathway of many cytokines, including type 1 interferons, IL-12, and IL-23. IL 12RB1 and B2 are a cytokine receptor chain shared by the IL-12 and IL-23 cytokine receptor. IL23R is a cytokine receptor chain of the IL-23 cytokine receptor. IFNAR1 and 2 are a cytokine receptor chain of the IFN-alpha and IFN-beta receptor.
- CRISPR Cas 9/ guideRNA and TALENS used to create specific mutations to genes
- Standard marker or reporter genes such as LacZ, fluorescent proteins, luciferase etc
- Standard antibiotic resistance genes, such as amp, kan, hygro etc
- WPRE - Woodchuck post regulatory element.

#### Evaluation of foreseeable effects

- Replication defective lentiviral particles are capable of integrating into the host genome and as such have the potential for insertional mutagenesis. This risk is minimised as no sharps are used in conjunction with the virus. Once integrated the particles are incapable of producing more viral particles as the necessary genes are not present. Many lentiviral systems contain the post-transcriptional regulatory element from the woodchuck hepatitis virus (WPRE) which could potentially have oncogenic activity.
The inserts are not expected to have harmful physiological or pharmacological properties. All genes to be investigated have only small individual effects on overall disease risk as the genes are non-HLA genes and are candidates for common (not monogenic) autoimmune diseases. Thus no single gene studied would be capable of causing autoimmune disease upon manipulation. Modification of the genes is not expected to increase their hazard. TALENS and CRIPSR-Cas9 will be used to modify the genes at specific locations. Off target results will be minimised by using highly specific guides and checking specificity bioinformatically. Standard reporter genes and antibiotic resistance genes have a history of safe use.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
<thead>
<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
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<tr>
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</table>

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill. Liquids (eg samples, culture supernatants, tissue culture media) -autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains. Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. NO SHARPS USED FOR VIRUS WORK |
| Degree of kill: Autoclaving, effectively 100% kill (annual validation) Incineration, effectively 100% kill (licensed incinerator) Chemical, effectively 100% kill (following manufacturers guidelines) |

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.
This project aims to explore the mucosal immune responses to bacterial infection in airways disease and their modulation by macrolides and immune cell ligands.

Recipient or parental organism

Hazard Group 2 bacteria such as Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus.

Standard laboratory strains of E. coli K-12.
**Host/vector system**

Mobilisation defective plasm ids such as PLS88 and PeJ18

**Origin & function**

Antibiotic resistance genes, such as those resistant to azithromycin.
In addition the plasm ids will contain standard antibiotic resistance genes, such as Kanamycin for cloning purposes and selection of resistant clones.

**Evaluation of foreseeable effects**

Respiratory pathogens used in this work will be hazard group 2 pathogens. These pathogens are opportunistic and generally only cause disease in compromised hosts.
The introduction of a single antibiotic resistance gene will confer resistance to that antibiotic. However, the bacteria will still be treatable with other classes of antibiotics.
Procedures that generate aerosols will be carried out within a class II micro-biological safety cabinet. Where FACS is carried out the samples will be fixed prior to analysis. If sorting is required it will be done in a system with aerosol management in place.
Mice will be intranasally infected with small volumes of bacteria. These infections will be carried out within a class II micro-biological safety cabinet. After a designated period of time samples will be homogenised and CFU counts obtained. All work will be done in a class II MSC.
As the antibiotic resistance gene is on a mobilisation defective plasmid, it's introduction into a standard laboratory strain of E. coli ·K-12 is not expected to overcome the attenuations of the strain and the work can be carried out at Class 1.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.
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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.
Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.
Animal bedding - dispose via clinical waste stream for incineration.
Animal carcasses - dispose via clinical waste stream for incineration.
Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

<table>
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<th>Laboratory Activities</th>
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Project Ref 553/19.8

Date Ackn'd 19/07/2019

Date Project Ceased

CU2 Project Title Correlative electron microscopy studies of Human Immunodeficiency Virus (HIV) egress

Class Class 3

CultureVolClass2 Non-GMM Consent Granted

ClassVolumeClass3-4 < 1 Litre

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
## Project Additional Information

### Purposes of the contained use

This project aims to study the HIV budding process in the context of native host cells, using cellular cryo-electron tomography (cryoET) and correlative live-cell and cryoET imaging in super-resolution.

### Recipient or parental organism

Mammalian cell lines such as U2OS, COS? and U3?3-MAGI

Human Immunodeficiency Virus

### Host/vector system

- Non-mobilisable plasmids encoding full molecular sequence of HIV.
- E. coli K-12 or B derivatives

### Origin & function

Standard marker genes such as GFP and other fluorescent tags.

Various mutants of HIV typically those of genes in the late domain which are involved in subverting the cellular machinery that regulates cargo sorting, membrane remodelling and fission processes, the Endosomal Sorting Complex Required for Transport (ESCRT) machinery. For example PTAP late domain, YPxL late domain and YP late domain.

### Evaluation of foreseeable effects

E. coli K-12 and B derivatives are disabled hosts which are used for cloning purposes. Protein expression does not occur due to the lack of bacterial promoters. Protein expression only occurs in eukaryotic cells. Accidental exposure poses minimal risk as the bacteria are non-colonizable, the amount of plasmid DNA present is low and the transfection efficiency of naked DNA is insignificant. Good Microbiological Practice will minimise the risk of exposure.

Work assigned to class 1

Transfection of mammalian cells with the plasmid will lead to the production of HIV.

The late-domain mutants are being compared with wild type to assess the effect on localisation in the cell, gag lattice morphology, presence of lattice gaps, evidence of maturation and other structural features. It is not expected that these mutants will present any greater hazard than the wild type.

Addition of standard reporter genes is not expected to alter the hazard from that of the wild type.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - inactivate by chemical means then autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - inactivated by chemical means then autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

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Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM. Discussions have been held with regard training for working with HIV and the need to validate inactivation with paraformaldehyde.

**Project Containment**

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</table>
Research into the molecular cell biology of various kinetoplastid parasites, primarily the generation and function of cell organisation to address questions about the function of cell shape and structure on motility.

Kinetoplastids such as
Hazard group 3 - Leishmania donovani, Leishmania braziliensis, Trypanosoma brucei rhodesiense
Hazard group 2 - Leishmania mexicana, Leishmania major, Trypanosoma brucei brucei, Trypanosoma brucei gambiense
Hazard group 1 - Leishmania Tarentolae, Crithidia (fasciculata, oncopelti, deanei) and Herpetomonas (megaseliae and roitman)
Trypanosoma brucei are specified animal pathogens and will also comply with those regulations.

E. coli K-12 and standard plasmids or
Homologous recombination of linear DNA

The main class of genes which will be subjected to targeted mutations or expressed exogenously are proteins involved in the core biochemistry or molecular biology of the cells. This will primarily involve: Cytoskeletal and flagellar
genes, such as microtubule associated proteins, microtubule motor proteins and regulatory secondary messenger binding proteins, membrane (particularly flagellar) proteins; housekeeping genes involved in the generation of the cytoskeleton and flagellum, such as intraflagellar transport proteins, cargo adapter proteins and enzymes regulating their function or sensing the environment; genes involved in the targeting of proteins to particular cellular compartments or structures, such as RAB/RAN GTPases potentially regulating organelle targeting, structural components like transition zone proteins and transmembrane protein transporters; and genes involved in the regulation of the expression of these proteins, such as mRNA binding and degrading proteins, nuclear compartment proteins and transcription regulatory factors.

Also RNA to interfere with expression of exogenous genes
Standard antibiotic resistance genes
Standard reporter genes
CRISPRI Cas 9 (or similar) technology

**Evaluation of foreseeable effects**

Genetic modifications will involve genes expected to be involved in the fundamental cell biology or biochemistry of the cell and are expected to either reduce cell viability and attenuate virulence or to be neutral for use as a molecular cell biology tool. It is extremely unlikely that these genetic modifications will increase virulence as the core biochemistry of the cell has been heavily selected through evolution for cell viability therefore any change is likely to be detrimental. It is not plausible that these changes will affect host range, widen tissue tropism or reduce sensitivity of the parasite to host immune defences. It is also considered that no new risks could be created through the transfer of any cryptic gene to a potentially pathogenic species background.

The mutants generated will be phenotypically and genetically stable with negligible potential for unintended transfer of genetic material. Mutants are likely to be of reduced fitness to wild-type organisms (as outlined in the project overview) with no possibility of increasing the vector or host range, enhance or widen issue tropism or decreasing susceptibility to destruction by the immune system.

Drug selectable markers used are unrelated to the primary therapeutic agents for the parasites.

Standard reporter genes are not expected to increase the pathogenicity and have a history of safe use.

CRISPRiCas 9 technology will be used for directed modification of the endogenous loci. Expression of the Cas 9 protein is not expected to increase the pathogenicity of the parasites.

In vivo work poses a risk due to the use of needles. A standard operating procedure detailing use of sharps will be produced for the work and individuals will be well trained on procedures using less hazardous micro-organisms in the first instance. Parasites are not transmissible via saliva or excreta but a bite could generate an open wound which provides a site for potential exposure. Experimental infection of the insect vector (sandflies or tsetse flies) involves feeding a blood meal (or analog) mixed with parasites. Insects are maintained in sealed and/or netted containers within a dedicated insectarium with a standard operating procedure detailing monitoring of insects and dealing with escapees.

Live cell microscopy will require the use of glass slides and coverslips and specialised glass bottomed fluidic chambers. A standard operating procedure for the handling of this will be produced.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Laboratory sealable for fumigation - As these parasites are not normally infectious by the airborne route and are rapidly killed outside of a host or controlled culture conditions by dessication, osmotic shock, and detergents,
fumigation would not be required. Use of micro-biological safety cabinet - parasites are not transmitted via aerosols (although in reality most work will be done in an MSC for sterility reasons)

Specific measures to control aerosol dissemination - parasites are not transmitted via aerosols and are rapidly killed outside of the host or controlled culture conditions by dessication, osmotic shock and detergents.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - Where possible sharps will be avoided however some use is essential and they will be disposed via clinical waste stream for incineration.

Animal bedding - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration.

Solid insect food and dead insects - EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Degree of kill:

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

02/03/2022

Page 9286 of 15326
Project Additional Information

**Purposes of the contained use**

The purpose of this research is to use lentiviruses to deliver genetic elements to specific brain regions or mammalian cell lines to test the genes’ role in sleep homeostasis. In doing so, this project will provide evidence for or against the hypothesis that these genes are crucial to the regulation of mammalian sleep in the regions that have been targeted.

**Recipient or parental organism**

| E. coli K-12 or B derivatives |
| Mammalian cells |
Host/vector system

Plasmids are standard non-mobilisable or mobilisation defective. The viral genes, generally gag, pol and env, derived from lentiviruses such as HIV, MoMLV and EIAV, are divided onto different plasmids, with the gene of interest also being on a separate plasmid. Producer cell lines, such as HEK293 cells or derivatives of.

Origin & function

shRNAs or microRNAs targeting ion channels and related subunits
Genes involved in function of neurons or processes within such as Kcnab1-3
Reporter genes (e.g. GFP, luc etc)
Standard antibiotic resistance (e.g. chloramphenicol, ampicillin, etc)

Health and Safety

Normal mammalian (e.g. TRPV1, P2X2) or algal (e.g. channelrhodopsin-2) ion channels or selective alterations of those channels
Genome editing tools (e.g. TALENs, Cas9-CRISPR)
Mitochondrial electron transport chain elements (e.g. SOG1, AOX)
Producers of oxidative agents (e.g. miniSOG)
Redox sensors (e.g. MitoTimer)
Oxidative stress-reducing agents (e.g. Glutathione peroxidase)
Woodchuck hepatitis virus post transcriptional regulatory element

Evaluation of foreseeable effects

Genes are not expected to be expressed in E. coli - therefore cloning in E. coli is class 1

Contained Use Notification

The lentiviral particles are able to transduce a wide variety of cells however due to their replication defective nature they are only capable of a single round of infection. Replication defective lentiviruses have key genes (gag, pol, rev and env) separated onto separate plasmids with minimal homology. Separating the DNA required for virion production onto 3 plasmids with minimal homology means there is minimal risk of homologous recombination. A third plasmid contains the gene of interest along with cis-acting signals required for reverse transcription and integration. As the gag, pol, rev and env plasmids do not contain packaging sequences none of the genes are present in the packaged viral genome.

The packaged sequence may contain the post-transcriptional regulatory element from the woodchuck hepatitis virus (WPRE) which may have oncogenic potential.

The main risk with lentivirus would be the random integration into the host genome resulting in a disruption of a gene function. However control measures reduce the likelihood of that occurring. As the virus particles are replication defective dissemination into the environment is unlikely.

The inserts to be delivered to the target neurons and cell lines are not expected to be toxic or oncogenic. The inserts are all deemed not to increase the pathogenicity or alter the tropism of the virus.

The greatest risk comes from the use of sharps for the in vivo work. Injections are done using stereotaxic equipment and fine glass 'needles' which minimises contact.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - autoclave then dispose via clinical waste stream for incineration.

Animal bedding - autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
 Autoclaving, effectively 100% kill (annual validation)
 Incineration, effectively 100% kill (licensed incinerator)
 Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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02/03/2022

Page 9289 of 15326
### Project Ref 553/20.2

**Date Ackn’ed**: 15/01/2020

**CU2 Project Title**: Molecular genetics of interorganelle communication

**Class**: Class 2

**CultureVolClass2**: < 1 Litre

**CultureVolumeClass3-4**: Non-GMM

**Consent Granted**: Yes

**Withdrawn**: No

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

Use of replication defective lentivirus to study interorganelle communication

**Recipient or parental organism**

E. coli K-12 and B derivatives

Mammalian cell lines

**Host/vector system**

Plasmids are standard non-mobilisable or mobilisation defective.

Health and Safety

Executive

Activity Notification

Standard replication defective lentiviral vectors where the gag, pol, env and the gene of interest are split across 3
plasm ids
Producer cell lines, such as HEK293 and their derivatives.

Origin & function
Proteins involved in organelle tethering, lipid transport or organelle fission, such as cytoskeleton associated proteins (Miro, Cenp-f), Fission machinery (MFF, DRP1, MID49, MID51), lipid modifying proteins (cyclopropane fatty acid synthase, PE methyl transferase, PS decarboxylase)
Standard selection markers (auxotrophies, resistance to chemicals), detectable markers (fluorescent, luminescent proteins, epitope tags), lipid modifying and membrane targeting proteins from non-pathogenic organisms (E. coli, Acetobacter aceti), or enzymes necessary for genomic modifications (Cre recombinase, flp recombinase, Ac transposase, Cas9 nuclease)
Some of the genes are from pathogens however at no point will the pathogen be handled. Genes will be obtained in plasmids. e.g. TEV protease from tomato etch virus, HA from H. influenzae, Cas9 nuclease from S. pyogenes

Evaluation of foreseeable effects
Genes are not expected to be expressed in E. coli - therefore cloning in E. coli is class 1.

Executive
Activity Notification
The lentiviral particles are able to transduce a wide variety of cells however due to their replication defective nature they are only capable of a single round of 'infection'. Replication defective lentiviruses have key genes (gag, pol and env) separated onto different plasm ids with minimal homology. Separating the DNA required for virion production onto 3 plasm ids with minimal homology means there is minimal risk of homologous recombination. The plasmid with the gene of interest has the cis-acting signals required for reverse transcription and integration. As the gag, pol and env plasm ids do not contain packaging sequences none of the genes are present in the packaged viral genome. The packaged sequence may contain the post-transcriptional regulatory element from the woodchuck hepatitis virus (WPRE) which may have oncogenic potential.
The main risk with lentivirus would be the random integration into the host genome resulting in a disruption of a genes function. However control measures reduce the likelihood of that occurring. As the virus particles are replication defective desemmination into the environment is unlikely.
The inserts to be delivered are not expected to increase the hazard of the lentiviral particles.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.
Liquids (eg samples, culture supernatants, tissue culture media) -autoclave using a make safe cycle as specified in
BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (e.g., needles, syringes, scalpels) - dispose via clinical waste stream for incineration. However not used in conjunction with lentivirus particles.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM,

**Project Containment**

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**Project Ref 553/20.3**

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<th>Date Ackn'd</th>
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<th>CultureVolumeClass3-4</th>
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<tr>
<td>16/01/2020</td>
<td>Generation, preparation and use of a MVA adapted to grow in Vero cells</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</tr>
</tbody>
</table>
**Project Additional Information**

**Purposes of the contained use**

Use of a modified MVA to produce vaccines against many pathogens

**Recipient or parental organism**

Modified Vaccinia Ankara is a replication deficient viral vector (due to abortive infection) that was derived from the vaccine used to eradicate smallpox. It is employed widely in the development of vaccines and has a history of safe use.

**Host/vector system**

- E. coli and BAC-MVA for modifying the genome
- Vero (African green monkey) cells for production of the virus
- other mammalian cell line and primary cells

**Origin & function**

- Deletion of a single gene in left terminal region
- Standard marker genes such as GFP etc.
- Genes encoding proteins expressed either for the purpose of generating an immune response (as an antigen) or to enhance the immune response to the vector (as an adjuvant) may be inserted.
- Some inserted genes may express pathogen glycoproteins. These could potentially be incorporated into the MVA membrane and change the cell tropism of MVA VerOX. Genetic material will be synthetically synthesised and not obtained from micro-organisms.
- Vaccines against many diseases are being investigated but examples include Nucleoprotein (NP) and Matrixprotein (M1) from influenza, antigens from Mycobacterium tuberculosis, HIV and malaria.

**Evaluation of foreseeable effects**

- MVA-BAC - the genome of MVA and its derivatives in DNA form is non-infectious and cannot generate virus without the aid of a helper virus (generally attenuated fowlpox, FP9)
MVA is a naturally attenuated virus which is severely host restricted and can't replicate in human cells. A single deletion was created in the left terminal region leading to a strain which showed limited replication in Vero cells. This strain was then passaged more than 40 times in Vero cells which resulted in a strain that could replicate to significantly greater levels than the original making it practical for vaccine manufacturing. This passaging lead to other natural deletions within the MVA genome. When tested in human cell lines (HEK293 and Huh7.5) the mutant strain showed the ability to replicate and show cytopathic effects. Therefore the work is being reassigned to class 2 while further work is done to assess replication in other human cells (both cell lines and primary cells). As a vaccine strain this will be used to express standard marker genes such as GFP etc and the expression of these proteins should not increase the risk. Also genes encoding proteins expressed either for the purpose of generating an immune response (an antigen) or to enhance the immune response (an adjuvant). Inserts will not be oncogenic, allergenic or toxic. Some inserted genes may express pathogen glycoproteins. These could potentially be incorporated into the MVA membrane and change the cell tropism of MVA VerOX. These proteins will be modified to ensure either secretion of the glycoprotein or mutations in the receptor binding domain of the glycoproteins to block cell tropism. Genetic material will be synthetically synthesised and not obtained from micro-organisms. The inserts are expected to generate an immune response to provide protection against the pathogen. Vaccines will be used for in-vivo work. Inoculations will take place in a class II micro-biological safety cabinet. Accidental needlestick injuries are a potential route of exposure however all staff are well trained and competent in the procedures using hazard group 1 / class 1 viruses. Animal bites could also be a potential route of infection and all staff are aware of first aid procedures and symptoms of infection.

N/A

Containing and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - inactivate by chemical means (following manufacturers guidelines), discharge to drains. 5% chemgene or 1% virkon for at least 30 mins.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - sharps bins autoclaved prior to disposal via clinical waste stream for incineration.

Animal bedding - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), dispose of as offensive waste.
Animal carcasses - autoclave and then dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM

Project Containment

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</table>

Project Ref 553/20.4

Date Ackn'd: 05/03/2020

CU2 Project Title: The role of the host AHR in sensing bacterial infection

Class: Class 2

Consent Granted: Not Applicable

Project notified under transitional arrangements [N]

Tick if notifying a connected programme of work [N]
**Project Additional Information**

**Purposes of the contained use**
To study the communication system between bacterial pathogens and their hosts.

**Recipient or parental organism**
Hazard groups 2 bacteria such as Pseudomonas aeruginosa

**Host/vector system**
The majority of the modified P. aeruginosa have been generated elsewhere although some may be created in house in the future.
E. coli K-12 or B derivatives, such as DHSalpha and Top10 and standard plasmids such as those based on pUC backbone. Cell lines and organoid model systems Zebrafish larvae.

**Origin & function**
Modification of the genes involved in quorum sensing such as phz, lasR, rhlR
Standard reporter genes such as fluorescent (GFP) and luminescence (Lux) genes

**Evaluation of foreseeable effects**
Quorum sensing (OS) plays a major role in biofilm formation and virulence. Modification to the OS associated pathway genes are expected to decrease biofilm forming potential and reduce potential pathogenicity of the resulting strains.
Addition of standard reporter genes is not expected to alter the ability to form biofilms or the virulence.
Standard work with cell lines or organoids exposed to bacteria will be done in a class 2 MSC.
Injections into zebrafish embryos will involve 5nl and will be done using a micromanipulator so there is no direct handling of the needle once it has been loaded with minimal likelihood of aerosols being generated.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:

- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

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02/03/2022
Investigating malnutrition-associated intestinal inflammation and impact on cryptosporidium infection

Study using genetically modified Cryptosporidium in-vitro and in-vivo to assess effects of modulating intestinal immune regulatory function, modulating diet and host-microbiota cross-talk.

Recipient or parental organism
Cryptosporidium tyzzeri

Host/vector system
The genetic modification involves CRISPR-Cas driven homologous recombination.

Origin & function
Standard reporter genes such as luciferase and mCherry
Standard antibiotic resistance genes such as Neomycin
Expression of model antigen / peptide e.g. ovalbumin epitope SIINFEKL integrated into a parasite protein that is ordinarily exported into infected epithelial cells e.g. MEDLE2 in orer to explore the generation of antigen specific immune responses.

Evaluation of foreseeable effects
Cryptosporidium tyzzeri is a apicomplexan protozoal parasite that was isolated from a wild mouse. It is generally considered to be a mouse pathogen however infections
have been detected in humans and as such shows zoonotic potential. Route of infection is oral - faecal

In immuocompetent hosts infection is generally self limiting. Immuno compromised individuals are at risk of prolonged infection and complications.

The addition of standard reporter genes and standard antibiotic resistance genes is not expected to alter the pathogenicity of the parasite nor alter its host range.

The addition of model antigen / peptide is not expected to alter the pathogenicity of the parasite nor alter its host range.

Suspensions of oocysts will be used for infection studies via oral gavage using blunt needles.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
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### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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</tr>
<tr>
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</tr>
<tr>
<td>Decontamination of equipment and surfaces: Equipment and surfaces that might come into contact should be treated with 6% hydrogen peroxide (prepared in water), with a contact/wet time of at least 20 minutes.</td>
</tr>
</tbody>
</table>

### Degree of kill:

- **Autoclaving**, effectively 100% kill (annual validation)
- **Incineration**, effectively 100% kill (licensed incinerator)
- **Chemical**, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Project Ref 553/20.9

Date Ackn’d 31/12/2020

CU2 Project Title Use of replication-defective lentiviral and other retroviral vectors for transduction of mammalian cells

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022
### Purposes of the contained use

Use of replication defective lentiviral particles to study hypoxia processes and hypoxia related signalling pathways to provide a fundamental overview of the epigenetic modification change in the whole genome. This will help us to better understand these basic biological processes, like hypoxia and related tumour development.

### Recipient or parental organism

<table>
<thead>
<tr>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli K-12 or B derivatives such as DH5α, JM109 etc</td>
</tr>
<tr>
<td>Mammalian cell lines such as HepG2, THP1 etc</td>
</tr>
<tr>
<td>Standard viral producer cell lines such as those based on HEK293 etc</td>
</tr>
</tbody>
</table>

### Host/vector system

<table>
<thead>
<tr>
<th>System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard plasmids for cloning and expression</td>
</tr>
<tr>
<td>Standard replication defective lentiviral plasmids or other replication defective retroviral systems where the genes require are split onto multiple plasmids. Where possible third generation systems will be employed however occasionally second generation systems may be used.</td>
</tr>
</tbody>
</table>

### Origin & function

<table>
<thead>
<tr>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRISPR / Cas9 technology - this technology allow targeted alterations to specific genes</td>
</tr>
<tr>
<td>Standard reporter genes and antibiotic resistance genes such as GFP, Luciferase, ampicilin, gentamycin etc</td>
</tr>
<tr>
<td>shRNA and iRNA technology</td>
</tr>
<tr>
<td>Overexpression, knockdown expression and mutation of genes thought to be involved in hypoxia and hypoxia related signalling pathways such as HIF1α, HIF2α, YAP, TAZ. Some of these genes may have oncogenic potential</td>
</tr>
<tr>
<td>Woodchuck hepatitis virus post-transcriptional regulatory element - some concern has been raised about the potential oncogenic nature of this gene</td>
</tr>
</tbody>
</table>

### Evaluation of foreseeable effects

<table>
<thead>
<tr>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli work - class 1 Genes are driven by mammalian promotors and therefore are not expected to be expressed in E. coli</td>
</tr>
<tr>
<td>Lentiviral vectors provide a means of transducing otherwise hard to transfect cell types (e.g. monocyte cell lines), and they also enable stable expression of genes within cells. These systems split essential genes (Gag, Pol, Rev and Env) along with the transgene of interest onto mutiple plasmids. Within the cells the vector sequence is generated and is packaged into infectious lentiviral particles that are released into the supernatant. These can then be harvested and used to infect cell lines of interest. However as these vector particles do not encode all the genes necessary for viral replication, the cells infected will not produce viral particles, and there will be no further viral spread. Lentivirus has the potential to cause insertional mutagenesis and as such the use of sharps will be minimised. Once the mammalian cells have been transduced and viral media removed they are safe to be handled as class 1 due to the replication defective nature of the virus.</td>
</tr>
<tr>
<td>Standard reporter genes and antibiotic resistance genes have a history of safe use and are not expected to increase the hazard posed. Some of the genes whose expression is being modulated could potentially be oncogenes or affect proliferation as disruptions to the genes either overexpression, decreased expression or mutations are often linked to various cancers. However it is likely that other factors also contribute to formation of cancers. To minimise potential exposure sharps will not be used in conjunction with the viral particles and an MSC will be used. CRISPR/Cas 9 is now a frequently used tool to create specific mutations in genes. As with the lentivirus, modulation of the expression of genes of interest could alter the proliferation of transduced cells. As such the use of an MSC and no sharps policy will be implemented.</td>
</tr>
</tbody>
</table>

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
<thead>
<tr>
<th>Measures &amp; Justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
</tr>
</tbody>
</table>

### For only GMMs - application for any derogation from full containment for the Class of activity

<table>
<thead>
<tr>
<th>Measures &amp; Justification</th>
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</thead>
<tbody>
<tr>
<td>N/A</td>
</tr>
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</table>
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) –autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - Not used

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

Project Containment

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<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
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<tr>
<td>L2 L3 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 553/21.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
Antigen processing and presentation of viral epitopes to T cells

T cells are activated following recognition of viral epitopes on HLA molecules on the cell surface of, mostly, antigen presenting cells. Our aim is to identify and investigate the viral epitopes presented to T cells and determine how these epitopes are generated through antigen processing. By using replication impaired lentiviral vectors, target genes will be delivered to cell lines for high-level and long-term expression. We will then investigate the T cell response to these viral proteins as well as manipulating components of the antigen processing pathway to determine how each viral protein is processed within the cell.

Recipient or parental organism

- mammalian cells
- HEK293 cells (and derivatives)
- Primary human cells

Host/vector system

- E. coli K-12 or B derivatives
- Standard non-mobilisable or mobilisation defective plasmids.
- Standard replication defective lentivirus systems

Origin & function

Our initial focus for these studies will be SARS-CoV-2 but this work is likely to expand to other viral infections in the future but any risks will be similar.

1. Generate B cell lines with stable expression of SARS-CoV-2 proteins:
   a. Individual expression of Spike, Nucleoprotein, Membrane protein, ORF3a, ORF7a, ORF7b, ORF8
   b. Tandem expression of Spike + Nucleoprotein, Nucleoprotein + ORF3a, Nucleoprotein & ORF7a, Nucleoprotein + ORF7b, Nucleoprotein + ORF8
2. Generate B cell lines with natural mutations within SARS-CoV-2 proteins:
   a. Mutations within ORF3a and Nucleoprotein
3. Generate B cell lines with stable expression of the human receptor ACE2 for live infection assays of these cells to confirm data seen in cell lines with individual SARS-CoV-2 proteins.

Standard reporter genes such as GFP (and derivatives), luciferase and epitope tags
Standard Antibiotic resistance genes such as Amp, Kan, Gen

WPRE - Woodchuck hepatitis virus post-transcriptional regulatory element

### Evaluation of foreseeable effects

Standard replication defective lenti (or retro) viral particles will be used. The genetic material is split onto a number of separate plasmids with a number of genes from the original virus omitted. These insert their genetic material into the host genome and as such could potentially cause insertional mutagenesis.

Individual expression of viral proteins holds little risk as no viral particles could be generated in these cells.

It has been shown that spike alone can induce a neutralising antibody response in murine studies. However, it has also been shown that expression of spike protein alone is sufficient to induce syncytia formation of ACE2 expressing cells. This was only true for some cell lines and was shown to be inhibited by the presence of IFITM1 protein at the cell surface which is naturally expressed by B Cell Lines. Additionally, B cell lines do not naturally express ACE2 which should prevent syncytia formation. ORF3a alone has been shown to induce apoptosis of cell lines when expressed individually.

Tandem expression of two proteins should still prevent the propagation of viral particles due to a lack of genes necessary for propagation.

Addition of ACE2 to B cells will increase their infection by SARS-CoV-2 but this tool is essential for these experiments and labs across the world are using ACE2-transduced cell lines for these experiments. A number of human cell types already express the ACE2 receptor. Potential exposure to the virus containing this insert is minimised by the use of a Class II MSC and not using sharps.

Standard reporter genes are not expected to be hazardous and have a history of safe use.
Standard antibiotic resistance genes are not expected to be hazardous and have a history of safe use.

Some concern has been raised with regards to the potential oncogenic activity of the post-transcriptional regulatory element from the woodchuck hepatitis virus (WPRE), which is frequently included to increase transgene expression in lentiviral systems.

Sharps will not be used in preparations containing virus particles or transfected packaging cells. Thus, reducing the chances of such exposure

FACS analysis may be utilised however once the replication defective lentivirus has integrated into the hose genome it poses minimal risk. Cells will be washed to remove any unbound viral particles.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15
minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) –autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. Sharps are not used with the viral particles.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 99.99% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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<td>L3 L4 L2</td>
<td>L3 L4</td>
</tr>
<tr>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
<td></td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
<td></td>
</tr>
</tbody>
</table>

Project Ref 553/21.10
The project will assess the live/dead populations of bacteria in the enterobacteriaceae family when exposed to various concentrations of either single or combinations of antibiotics.

**Recipient or parental organism**

Enterobacteriaceae such as *E. coli* and *Klebsiella pneumoniae*

**Host/vector system**

Non-mobilizable vectors, such as
- pBAD26
- pUC18/19
- pHSG398
- pHSG299
- pAw8-mCherry

**Origin & function**

Standard reporter genes, such as mCherry, GFP and RFP.

- mcr-1 - ‘mobilised colistin resistance’ gene – confers resistance to the antibiotic colistin only on vectors pHSG398 and pHSG299.
- mgrB - mediates susceptibility to colistin - only on vectors pHSG398 and pHSG299.

**Evaluation of foreseeable effects**

*E. coli* is a common inhabitant of the human gastrointestinal tract. Pathogenic strains can cause mild disease in most humans; it is the most common cause of UTIs, and is...
also known to cause enteritis, sepsis and neonatal meningitis.

K. pneumoniae is a normal commensal living in the human mouth and gut, and is ubiquitously found in nature, including soil, water and animals. It is an opportunistic pathogen and is associated with hospital-acquired infections (pneumonia, UTI, meningitis, sepsis and infections around indwelling medical devices), mainly in immunocompromised individuals.

The colistin resistance gene on pHSG398/pHSG299 plasmids does not increase the virulence of the strains. Additionally, there is a wide variety of alternative antimicrobial agents that can be used to successfully treat infections cause by E. coli, as colistin is used as a last-resort antibiotic. Handled strains are not multi-drug resistant and hence are susceptible to a range of other antibiotics.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) – inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 99.99% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.
We aim to understand how cells of the body produce and detoxify endogenous toxins, such as reactive aldehydes, and what processes are required to prevent, or repair DNA lesions induced by those toxic metabolites in the context of tissue homeostasis, cancer and aging. To this end, we plan to combine different cellular systems and genome editing tools to (1) search for genes that mediate abovementioned processes, (2) to validate candidate factors and (3) to understand the molecular mechanisms that govern cellular and systemic responses to genotoxic stress.

Several converging lines of evidence implicate elevated levels of endogenous aldehydes might alter in the immune responses to viral and pathogen infections in humans. We can model this by infecting aldehyde-accumulating and control mice with various pathogens expressing fluorescent or bioluminescent reporters or epitope tags which facilitate monitoring of viral infection and immune responses.
<table>
<thead>
<tr>
<th>Recipient or parental organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian cell lines</td>
</tr>
<tr>
<td>Mammlian primary cells including mouse oocytes</td>
</tr>
<tr>
<td>Insect and avian cell lines</td>
</tr>
</tbody>
</table>

Standard Yeast (Saccharomyces cerevisae, Schizosaccharomyces pombe, Kluyveromyces lactis, Pichia pastoris.) and bacteria (K12 and B derivative strains) will be used for expression studies of individual genes.

Mice
Pathogens such as adenovirus type 1 (MAV-1), rotavirus, influenza A (mouse-adapted PR8 strain), salmonella typhimurium, or citrobacter rodentium.

<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Bacterial (K-12 or B derivatives) and vector (non-mobilisable or mobilisation defective)</td>
</tr>
<tr>
<td>Standard replication defective lentiviral, retroviral, adenoviral and adeno-associated systems</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>genome-wide collections of gRNAs for CRISPR-Cas9 editing to generate large populations of single knock out clones.</td>
</tr>
<tr>
<td>Inactivation of gene expression using CRISPR-Cas9</td>
</tr>
<tr>
<td>Modulation of gene expression using siRNA or shRNA</td>
</tr>
<tr>
<td>Overexpression of genes using cDNA</td>
</tr>
</tbody>
</table>

Genes of interest will likely include known enzymes that carry out detoxification of toxic metabolites (e.g. Adh5 or Aldh2 that detoxify aldehydes), genes involved in metabolic processes that directly lead to production of toxic by-products (such as histone demethylation which generates formaldehyde), DNA repair factors (such as members of the Fanconi Anaemia pathway, nucleotide-excision repair pathway and other major repair mechanisms) as well as gene identified by screens (1) or relevant scientific literature searches.

Woodchuck hepatitis virus post-transcription regulatory element (WPRE)

Standard marker proteins - fluorescent or bioluminescent reporters or epitope tags
Standard antibiotic resistance genes such as amp, kan, gen

<table>
<thead>
<tr>
<th>Evaluation of foreseeable effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>The proteins to be expressed, modulated or knocked down will generally not pose any hazard, but some of the mammalian proteins could be oncogenic and some may be tumour suppressors (5% of human genes). Modulating expression of the genes may lead to an alteration of the proliferation/differentiation balance or induction of apoptosis of the transduced cells and/or their progeny. Other possible effects include altered drug response, increased/decreased tolerance to genotoxic stress, changes in cellular metabolism and homeostasis. The possibility of inadvertently generating novel biological activities cannot be excluded.</td>
</tr>
</tbody>
</table>

WPRE is a common component of replication defective viral systems but concerns have been raised about the oncogenic potential.

Lentiviral and amphotropic retroviral particles integrate into the host genome which could potential lead to insertional mutagenesis. Sharps will not be used except where necessary for in vivo work.
AAV and adenoviral particles generally remain episomal and the expression of the genes of interest is transient.

Expression of standard marker proteins is not expected to alter the pathogenicity of the viruses / bacteria.

In some cases, lenti-/retro-/AAV- or adenoviral preps will also be directly delivered to mice by injection.

In addition, mice will be exposed to certain pathogens as below. These pathogens will be modified to contain standard reporter genes (e.g. GFP derivatives and luciferase) or epitope tags. This is not expected to alter the pathogenicity.

While adenoviridae in general are classified as hazard group 2 by ACDP, there is no report of MAV-1 causing human infection. The genome is quite similar to that of human adenoviruses but MAV-1 does not encode virus-associated RNAs (which are expressed by human adenovirus to suppress innate immune recognition). MAV-1 has a tropism for endothelial cells and cells of the monocyte/macrophage lineage, and it also infects astrocytes. MAV-1 infects tissues throughout the mouse, and the highest levels of virus are found in the brain, spinal cord, and spleen after intraperitoneal infection. Mice infected with MAV-1 develop adaptive immune responses, both cell mediated and humoral.

Human rotavirus is classified as hazard group 2. However, rotaviruses are usually species-specific, and only SA11 and derivatives will be used, a simian strain that is well-adapted to tissue culture and propagated in MA104 cells. No cases of human infection by SA11 have been reported, and it is unlikely to cause human disease.

Influenza A is classified as a hazard group 2 pathogen. However, Influenza A (mouse-adapted PR8 strain) is an H1N1 virus based on a human isolate from Puerto Rico in 1934. The virus used is highly attenuated, non-virulent and non-infectious in immunocompetent humans but is mouse-adapted and highly infectious in mice. This is a commonly used and well-studied model of influenza infection.

Salmonella typhimurium is classified as a hazard group 2 pathogen. It is a gram-negative bacterium causing disease in a broad range of hosts. In human the disease is frequently limited to the gastrointestinal tract. Enterocolitis from S. Typhimurium manifests within 72 hours and features acute inflammation of the intestinal mucosa and Peyer’s patches with a polymorphonuclear leukocyte infiltration, causing secretory diarrhoea. In the absence of invasive disease, infection does not proceed beyond the mesenteric lymph nodes and there is no bacteraemia. Antibiotics are not indicated in uncomplicated S. Typhimurium infection, management is conservative only.

Citrobacter rodentium is a gram-negative enteric pathogen causing gastrointestinal disease in mice but there are no reports of pathogenicity to humans. Hazard Group 1 but will be handled in a manner to limit spread to other mice.

Mice will be inoculated with pathogens in a class II MSC. The inoculation will take place using oral gavage, intranasal delivery, or by injection subcutaneously, intravenously, or intraperitoneally. Subcutaneous and intraperitoneal injections should be performed using short needles and sufficient distance between injection site and restraining hand that injection through the animal into the operator is physically impossible. For intravenous injections, mice should be restrained in a mechanical restrainer so that injection can be performed with a single hand. Syringes with needles attached are to be disposed of into sharps bins immediately after use without resheathing. In case of large-volume injections (95 ml per kg body weight), mice will be anaesthetised during the injection to minimize the stress to the animals and enabling easy administration of the virus/DNA by a trained operator.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

N/A
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Sharps (eg needles, syringes, scalpels) - autoclave then dispose via clinical waste stream for incineration.

Animal bedding – EITHER autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill OR dispose via clinical waste stream for incineration.

Animal carcasses - dispose via clinical waste stream for incineration. Those which may contain replication competent pathogens will be autoclave prior to disposal via the clinical waste stream.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Please enter comments on the GM safety committee on the risk assessment

The committee had a number of comments on the initial draft of the risk assessment which were dealt with in subsequent drafts.
1. FACS sorting is not mentioned in the work section. Please can this be addressed, including whether or not the FACS sorter needs to be within an MSC and a justification as to why not if that is the case.
2. Are any of the pathogens listed GMOs?
3. Have you discussed with BMS whether these pathogens can be used in the facility?
4. Injection of mice with virus: You will need to provide a lot more information on the inoculations as this is one of the biggest risks. So, how are the mice restrained, what type of inoculations, how needles are disposed of, no resheathing of needles etc.

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.
## Project Containment

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<tr>
<td>L2 L3 L4</td>
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### Project Ref 553/21.3

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<td>Use of self-inactivating lentiviral vectors for the study of neurodegenerative and neurogenetic diseases</td>
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Non-GMM  
Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

### Project Additional Information

**Purposes of the contained use**

- Replication defective lentiviral vectors will be used to facilitate research in understanding the mechanisms underlying neurodegeneration in a range of diseases

**Recipient or parental organism**

- Mammalian cell lines, such as primary cells, induced pluripotent stem cells and tissue samples

**Host/vector system**

02/03/2022
E. coli K12 derivatives - class 1
Second and third generation lentiviral vectors will be used where the gene of interest, the gag-pol genes and envelope genes are on separate plasmids
Virus producer cell lines, such as HEK293 and derivatives

### Origin & function

- Reporter genes (e.g. GFP, YFP, RFP, mCherry)
- Standard antibiotic resistance (e.g. Neomycin, Hygromycin, Puromycin and ampicillin).
- shRNAs, sgRNAs.
- Genome editing tools (e.g. TALENs, Cas9-CRISPR).
- Tet repressor/activator systems.
- Neurotrophic / neuroprotective factors (e.g. BDNF, CNTF, GDNF and NGF).
- Inflammatory mediators (e.g. TREM2)
- Protein homeostasis pathways (e.g. NEDD4, USP30)
- Neurodegenerative susceptibility genes (e.g. SNCA, DJ1, OPA1).
- Optogenetics/calcium sensors (e.g. ChR2, ChR1, GCaMP)
- pH sensitive vesicular endo/exocytosis fluorescent reporters (e.g. Synapto-pHluorin, SypH)
- Protein trafficking (e.g. RAB7, autophagy genes)
- Designer receptors exclusively activated by designer drugs (DREADDs) for specific neuronal silencing (e.g. GluC1, hM4D)

### Evaluation of foreseeable effects

Replication defective Lentiviral particles can enter numerous host cells depending on the envelope protein. They integrate into the host genome and as such have the potential to cause insertional mutagenesis if there was accidental exposure to them. The use of sharps is minimised when handling lentiviral particle however their use in necessary for in-vivo work.

Animals with be anaesthetised and lentiviral constructs expressing the gene of interest will be stereotaxically injected into the substantia nigra using a microinfusion pump. To prevent needlestick injuries during these experiments, needles will be disposed immediately after the procedure in appropriate sharps-bin.

The protein products of the genes to be studied are not expected to be toxic or cancer inducing in any way. However, the HIV receptors are also receptors for chemokines and as such the genes could modulate immune function, especially chemotaxis, in target cells. This could provide a growth/ invasion advantage to cells. Initially, the shRNAs, TALENs or Cas9-CRISPR systems to be used are not targeting known tumour suppressors. However, there is potential for off-target effects with, which could potentially have tumorigenic effects. This will be minimised by careful design with as little sequence homology as possible to off-target genes, including BLAST searches against genome databases.

Gene transfer is possible, significantly from the transfected producer cell lines, as the resulting Lentiviral/Retroviral vector will be able to infect a wide range of cells, including human cells, as the VSV-G or Rabies-G envelope enable amphotropic infection. However dissemination in the environment is limited due to the viruses being replication defective.

Some of the vectors may contain the woodchuck hepatitis virus regulatory element (WPRE) and there is some limited evidence of its oncogenic potential. Some of the genes of interest may modulate immune function.

Once the virus has integrated into the genome of the host cell it poses minimal risk and the cells can be handled at containment level 1 after a given period of time. Viral concentration is achieved by centrifugation and ultracentrifugation in air-tight containers and rotors which are loaded and unloaded within an MSC.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
**SELECT STANDARD PARAGRAPHS FROM THE FOLLOWING – ALTER FOR ON HOSPITAL OR SCIENCE AREA SITE**

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Animal bedding – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), dispose via the industrial (black bag) waste stream for landfill.

Animal carcasses - dispose via clinical waste stream for incineration.

Degree of kill:
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 100% kill (following manufacturers guidelines)

---

**Is an emergency plan required according to regulation 20?**

- **If yes, tick to confirm that it is attached to this form**

- **Tick to confirm that you have attached a risk assessment to this form**

- **Tick if you are claiming exemption from disclosure for section of the risk assessment**

---

**Please enter comments on the GM safety committee on the risk assessment**

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

---

**Project Containment**

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<tr>
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**Project Ref** 553/21.4
The aim of the project is to analyse the genes and regulatory regions involved in extreme immune responses. Generation of replication defective lentivirus for modulation of genes implicated in or linked to the immune response. Plasmids and lentiviral particles containing sgRNAs (for CRISPR/CAS9 gene-editing) will be generated with the aim of disrupting and/or editing genes and regulatory regions in order to assess their impact on extreme immune responses.

Recipient or parental organism

Mammalian cell lines, such as HPA1 and K562
Primary human cells, such as monocytes and macrophages

Host/vector system

E. coli K12 derivatives
Second and third generation lentiviral vectors will be used where the gene of interest, the gag-pol genes and envelope genes are on separate plasmids
Virus producer cell lines, such as HEK293 and derivatives

Origin & function

Initial genes of interest will include TLR4, Myd88 and Zfp36 (genes involved in immune response) but other genes involved in the immune response will be looked at.

TLR4 is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity. TLRs are highly conserved from Drosophila to humans and share structural and functional similarities. They recognize pathogen-associated molecular patterns that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity. This
The receptor has been implicated in signal transduction events induced by lipopolysaccharide (LPS) found in most gram-negative bacteria.

Myd88 is a gene that encodes a cytosolic adapter protein that plays a central role in the innate and adaptive immune response. This protein functions as an essential signal transducer in the interleukin-1 and Toll-like receptor signalling pathways. These pathways regulate the activation of numerous proinflammatory genes.

Zfp36 plays a role in anti-inflammatory responses; suppresses tumour necrosis factor (TNF)-alpha production by stimulating ARE (AU-rich element)-mediated TNF-alpha mRNA decay and several other inflammatory ARE-containing mRNAs in interferon (IFN)- and/or lipopolysaccharide (LPS)-induced macrophages. It plays also a role in the regulation of dendritic cell maturation at the post-transcriptional level, and hence operates as part of a negative feedback loop to limit the inflammatory response.

Other genes will be included, for example gene hits from iPS-macrophage CRISPR screen (work previously carried out at the TDI).

Other targets involving putative regulatory regions relevant to the induced innate immune response based on previous and ongoing work in the lab will also be designed.

The sgRNAs used will be chosen mainly from the published TKOv3 CRISPR library (Hart et al., 2017), which has been designed to minimize off-target effects, or from custom design for specific target regions.

Standard reporter genes, such as GFP

CRISPR/Cas9

Standard antibiotic resistance genes, such as puromycin

Evaluation of foreseeable effects

Replication defective Lentiviral particles can enter numerous host cells depending on the envelope protein. They integrate into the host genome and as such have the potential to cause insertional mutagenesis if there was accidental exposure to them. The use of sharps is minimised when handling lentiviral particles.

The shRNAs Cas9-CRISPR systems to be used are not targeting known tumour suppressors. However, there is potential for off-target effects with, which could potentially have tumorigenic effects. This will be minimised by careful design with as little sequence homology as possible to off-target genes, including BLAST searches against genome databases.

Some of the vectors may contain the woodchuck hepatitis virus regulatory element (WPRE) and there is some limited evidence of its oncogenic potential.

Some of the genes of interest may modulate immune function.

Standard reporter genes and antibiotic resistance genes have a history of safe use.

Once the virus has integrated into the genome of the host cell it poses minimal risk and the cells can be handled at containment level 1 after a given period of time.

Most of the genetic inserts are not likely to cause significant effect on an individual. It is theoretically possible that upon accidental exposure, the lentivirus could infect the cells of an individual, integrate into the genome and
disrupt the genes that it is designed to target (for example TLR4, Myd88 and Zfp36), thus causing impaired immune
response in the infected cells of the exposed individual. However, as described previously, these viruses are self-inactivating, they are not capable of activating gene
transcription from the inactivated viral LTR and they are
replication incompetent. Thus, in this extremely unlikely event, the gene modification would only occur in the
infected cells and would not spread to other cells.

The worst-case scenarios could be 1) expression of oncogenic genes and/or 2) the chance integration of the virus into a tumour suppressor, thus disrupting that gene's
function, or 3) reprogramming of cells of the exposed individual and subsequent teratoma formation. However, in all cases, the virus particle would be incapable of infecting
more than one cell and would not be self-replicating. In terms of immune response to infection, the body would employ several methods to target and eliminate any cells
infected with the lentivirus (first with the innate immune response via IFN gamma secretion and then with adaptive immune response via development of transgene-specific
T cell-mediated and antibody-mediated responses).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware e.g. pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15
minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag)
waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15
minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to
drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or
134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form
N

Tick to confirm that you have attached a risk assessment to this form
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment
N
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

### Project Containment

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

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### Project Additional Information

**Purposes of the contained use**

Study of viral and cellular modifications during infection. Ubiquitylation is one of the most commonly utilized cellular post-translational modifications (PTM), routinely targeted by pathogens to deregulate and hijack host intracellular pathways. Viruses utilize ubiquitylation to target central signalling pathways such as NFκB and MAPK that are crucial for their replication, propagation and immune evasion.

Other viruses reorganize the host secretory pathway to undergo cell-to-cell transmission via secretory autophagosomes or exosomes, while evading immune detection.

**Recipient or parental organism**
Influenza Virus - 8-Plasmid-based influenza-virus production from HEK293T cells to rescue recombinant virus.

Zika Virus such as MR766 or PRVABC59

Replication defective Lentivirus

Host/vector system

Mammalian cells such as HEK293T, A549, Calu-3 or HeLa cells (wild type or modified versions of)

Viral producer cells such as MDCK, HEK293, Vero (and variants of)

Primary cells such as CD14+ monocytes or iPS cells

Origin & function

Influenza virus

Recombinant H1N1 with loss-of function mutants:

A/WSN/33 (H1N1) deltaNS1 (non structural protein 1)

A/PR8 (H1N1) deltaNS1

The other inserted genetic sequences into recombinant influenza (into the non-structural protein NS1 or matrix protein M2 lacking the LC3 interaction domain) are loss-of-function mutations and makes the viruses less competent in replication, assembly and budding.

Zika Virus

venus-tag version of of Zika strains such as MR766

Replication defective lentivirus

Use of shRNA to silence expression of specific genes of the autophagy and lipid droplet pathways in mammalian cells.

Expression of cDNA of wild type or modified genes in deficient backgroud.

Use of CRIPSR/Cas9 systems to modify genes of interest

Genes of interest are autophagy pathway (e.g. Ulk1, Atg16L, LC3, Beclin-1), lysosomal degradation (Snap25, Syntaxin-17, Vamp8) or ER quality control processes (Hrd1, Ube2g2, Aup1).

Generation of subviral particles

Expression of recombinant flavivirus prME and that of coronavirus N, E, M and S from pCS-lentiviral vectors are expected to form subviral particles (non-replicative) that are constitutively secreted from cells and express a fluorescent tag for visualisation. These are structural proteins of these viruses and will not carry any genetic material within them. Structural proteins that carry mutations which result in a loss of their ability to form subviral particles will also be used in parallel.

Evaluation of foreseeable effects

Influenza is a Hazard group 2 respiratory pathogen. All strains to be used are either lab adapted versions or currently circulating strains. No variant of high risk will be used.

The modifications to the viruses are loss of function mutations and makes the viruses less competent in replication, assembly and budding than the wild type version. All vial work will be done in Class II MSC, and centrifugation will be done in sealed rotors.

Zika virus is a hazard group 2 pathogen and is primarily transmitted to humans through the bite of an infected Aedes species mosquito. Evidence suggests that transmission may also occur from mother to child in utero, through sexual contact, or through blood transfusion. All aerosol generating procedures will be done in a class II MSC and centrifugation will be done in sealed rotors. No sharps will be used in the processes. The addition of standard reporter genes is not expected to increase the risk from the genetically modified versions over the wild type.

Replication defective lentivirus can stably integrate into the host cell genome however no further viral particles can be produced. The modification are to either over-express, knock down or mutate expression of genes involved in autophagy pathway, lysosomal degradation or ER quality control processes. Modification of these genes is not expected to pose a risk however lentiviral integration can potentially cause insertional mutagenesis. As such no sharps will be used.
Replication defective lentiviruses will also be used to produce subviral particles. The normal function of these subviral particles is to reorganise host secretory pathways – ER, ERGIC and Golgi membranes, as well as autophagic compartments to generate unique secretory vesicles for transport. However they contain no genetic material and are not capable of replication. Expression of the subviral particles is not expected to pose a risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Liquids (eg samples, culture supernatants, tissue culture media) – inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. Not used for Zika work

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Appropriate containment and control safety measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The focus of the research conducted by our group is to better understand protein glycosylation, and the roles that glycans play in health and disease. To help achieve these research aims, we will use the lentivirus expression system.

**Recipient or parental organism**

Mammalian cells such as CHO (and derivatives of)

**Host/vector system**

Standard replication defective lentiviral systems (2nd or 3rd generation)

Lentiviral producer cells such as HEK293 cells (and derivatives of)
Standard reporter genes such as GFP, Emerald, Luc, Cherry etc.
Standard antibiotic resistance genes such as puro etc.

Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE)

Genes of glycoproteins such as ion channels (e.g. acetylcholine receptor subunits), transporters (e.g. amino acid transporters like SLC1A2) receptors (e.g. MUSK, LRP4), and excreted proteins (e.g. antibodies and AGRN).
Genes of proteins known to interact with glycoproteins such as RAPSN, DOK7, DMD.
Genes of proteins involved in protein glycosylation and dolichol synthesis pathways, such as DPAGT1, ALG13, ALG14, ALG1, ALG2, ALG11, PMM2, GMPPB, OGT and SRD5A3.

In addition, modified versions of the GOI will also be used.

**Evaluation of foreseeable effects**

Standard replication defective lentiviral HIV based systems use only essential genes - tat, rev, gag, pol. They lack the accessory genes such as vif, vpr, vpu and nef, which are essential for viral replication. The likelihood of generating replication competent lentivirus is therefore minimal.

Lentiviral particles integrate into the host genome as such have the potential to cause insertional mutagenesis. Use of sharps is limited when handling lentiviral particles.

Lentiviral systems often contain WPRE which may have oncogenic properties. Exposure is limited due to control measures such as use of MSCs to control aerosols and no sharps.

Standard reporter genes have a history of safe use and are not expected to increase the hazard of the lentiviral particles.

The genes of interest are not expected to increase the hazard. In many cases gene expression is controlled by Tet and therefore only occurs in the presence of doxycycline.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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Liquids (eg samples, culture supernatants, tissue culture media) – inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - No sharps are used in the procedures; however, 0.45µM filters are used in combination with syringes, to filter the lentivirus. These will be disposed of in a sharps bin and disposed of via the clinical waste stream for incineration.
Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 99.99% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Comments from the WIMM GM Safety Committee
Reviewer 1.
1. Will the work be done solely in the WIMM? This is important because if it will be done in other Departments the GMRA will have to be approved by their committees as well.
2. Where will the EM grid screening be done?
3. Cell line post transduction are class 1 as they are especially disabled hosts. They may need handling at CL2 under CoSHH but that is separate legislation.
Reviewer 2
1. In the UK it is A&E not ER. – Done
2. Can you put in the link to the IRIS accident reporting? – Done
Reviewer 3
1. You have a list of plasmids for the Lentiviral work. What we also need is a list of the genes you will be expressing or manipulating, and some brief details about them.
Other reviewers: Typos only and addition of wording.

Project Containment

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02/03/2022  Page 9323 of 15326
Purposes of the contained use

The aim of this project is to characterize the function of known and candidate tumour modifying genes by CRISPR-mediated knock-out (CRISPR-KO), knock-in (CRISPR-KI), interference (CRISPRi), and/or activation (CRISPRa).

Recipient or parental organism

E. coli K-12 - class 1

Mammalian cell lines such as human cell lines and human primary cells

Viral producer cell lines such as HEK293 cells (and derivatives of)

Host/vector system

Standard replication defective lentiviral systems

Origin & function

Standard reporter genes such as GFP etc

Protein degradation moieties such as an auxin-induced degron and/or small epitopes

Standard antibiotic resistance genes such as puromycin
CRISPR Cas 9 or other derivatives

guide RNAs to target Cas9 modification of:-
Cancer promoting genes and tumour supressor genes for knock-in, knock-out, interference and / or activation.

Evaluation of foreseeable effects

Replication defective lentivirus particles are derived from HIV however only essential genes are still present such as gag, pol, rev and env (generally from VSV-G). The plasmids containing these genes do not contain LTRs or packaging signals and therefore none of the structural genes are present in the packaged viral genome. The packaging plasmid containing the Cas9 associated RNAs contains a packaging signal and the LTRs. This is self inactivating as it carries a deletion in the 3'LTR. Upon integration into the host genome the 3' LTR is copied to the 5' LTR rendering it transcriptionally inactive. As these can integrate into the human genome there is potential for insertional mutagenesis.

CRISPR-Cas9 is derived from S. pyogenes and can be programmed to introduce DNA double-strand breaks at specific loci specified by a sgRNA. When targetted to coding regions of genes Cas9 can create a frame shift insertion / deletion mutation that results in a loss of function (knock-out).

When a template DNA, containing tag-encoding DNA and homology sequences encompassing the Cas9-targetting site, are separately provided with Cas9 and sgRNA, the tag can be incorporated at the site of Cas9-induced DNA damage through homology-directed repair mechanisms (knock-in).

An engineered catalytically dead Cas9 (dCas9) mutant, typically containing two silencing mutations (D10A + H841A) of the nuclease domains, can also be used to effectively modify gene expression, known as CRISPR interference (CRISPRi) or CRISPR activation (CRISPRa). Functional CRISPRi machinery requires co-expression of sgRNA and a dCas9 mutant gene. The sgRNA allows for highly specific binding to a desired position in the genome, and coupled with dCas9, the complex provides sterical hindrance or activation of RNA polymerase at the site, leading to efficient transcriptional modification.

An efficient transcriptional repression can be accomplished by fusing dCas9 to an effector domain, i.e. Krüppel associated box (KRAB). The dCas9-KRAB complex recruits co-repressors, such as KRAB-box-associated protein-1 (KAP-1), at a sgRNA-defined site, and in this way, suppresses transcription (CRISPRi). Conversely, when the dCas9-targeting platform is used to recruit strong transcriptional activators, by fusing dCas9 to a tripartite transactivation complex (VPR), which is composed of VP64 (i.e. four copies of the transactivation domain (VP16) of the Herpes simplex virus), P65, and Rta proteins, at a sgRNA-defined site, gene expression can be induced (CRISPRa). Both CRISPRi and CRISPRa have successfully been used for genome-wide studies.

Expression of known cancer causing (oncogenes) and supressing genes (tumour suppressor genes) can promote and inhibit cell growth, respectively. The manipulation of cancer modifying genes may affect cell growth in culture. Whilst there is potential risk that these genes may promote cancer, there is no evidence that these genes acting alone have any cancer initiating activity, and their activity in cancer is in the context of modulating the phenotype of existing activated oncogenic mutations.

A worst-case scenario would be the chance integration of virus into tumour suppressor gene, thus disrupting its function. Even so, this single event is unlikely to cause any tumour formation and so the overall risk is still low.

Fluorescent proteins are common investigative tools and no hazards are associated with their use.

Blasticidin and puromycin genes are widely used for antibiotic selection and no hazards have been identified with its expression in mammalian cells.

WPRE has been linked to liver tumours in mice and therefore could have oncogenic potential.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) –autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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<tr>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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</table>

Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
Project Additional Information

Purposes of the contained use
We aim to develop microfluidic devices for imaging gene expression dynamics of Salmonella at a single-cell level. GMMs engineered in this work will include deletions, transcriptional and translational fusions to DNA repair and regulatory genes.

Recipient or parental organism
Salmonella typhimurium

Host/vector system
Standard E. coli K-12 and B derivatives
Standard non-mobilisable and mobilisation defective plasmids.

Origin & function
Standard marker genes such as gfp, cfp, mCherry and mYPet
Other reporter genes such as halotag
Standard antibiotic resistance genes such as ampicillin, Kanamycin and Chloramphenicol

02/03/2022
<table>
<thead>
<tr>
<th>Perturbation of the DNA damage stress response such as:</th>
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</thead>
<tbody>
<tr>
<td>mutation of DNA repair genes e.g. DNA glycosylases, DNA methyltransferases, DNA ligases, DNA polymerases</td>
</tr>
<tr>
<td>mutation of regulatory genes e.g. sigma factors or transcriptional activators (rpoS, recA, ada)</td>
</tr>
<tr>
<td>mutation of stress response genes e.g. SOS regulon (lexA, dinB) or the OxyR regulon (katG, grxA)</td>
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<tr>
<td>mutation of genes involved in motility</td>
</tr>
</tbody>
</table>

### Evaluation of foreseeable effects

The modified versions are expected to display either comparable properties to the wild type strains or reduced fitness and virulence.

Marker proteins have a history of safe use and expected not to alter the harmful properties associated with the wild type.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. Use of sharps will be avoided where possible.

Glass ware - autoclave using make safe cycle, then clean for reuse

### Degree of kill:

- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical, effectively 99.99% kill (following manufacturers guidelines)

### Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022  Page 9328 of 15326
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment

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Animal Units

<table>
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<th>Large Scale Activities</th>
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Project Ref 553/22.2

Date Ackn’d: 19/01/2022

CU2 Project Title: Profiling the pharmacokinetics of lentivirus gene therapy vectors in normoperfused organs

Class: Class 2
CultureVol: 1-50 Litres
Consent Granted

Historical Significant Changes

Withdrawn: N

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: N

Project Additional Information

Purposes of the contained use

Use of replication defective lentiviral vectors to assess its pharmacokinetic profile in a closed loop system.
Recipient or parental organism

Mammalian cells and organs.

Host/vector system

Standard replication defective lentiviral vectors

Origin & function

Replication defective lentivirus system is based on HIV-1 however only 3 genes from HIV are required to generate high titre virus. These are gag/pol and rev. The genes are codon optimised to minimise regions of homology. The mutated form of WPRE is used preventing the expression of the pro-proliferative X-protein. VSV-G env is expressed to coat the vector particle and allows entry into human cells.

Standard reporter genes such as GFP

Evaluation of foreseeable effects

The viral vectors are replication defective but can enter human cells and integrate into the host genome potentially causing insertional mutagenesis.

GFP is a standard marker protein which allows for visualisation of cells containing the virus.

The virus will be dosed into a closed system containing an organ and approx 1.6L of blood. Regular samples can be taken without the need for needles.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) –autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines), discharge to drains or if blood sent for incineration as clinical waste.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration. Use of sharps in minimised

Organs - sent for incineration as clinical waste.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturers guidelines)
Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

**Project Containment**

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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref** 553/94.1

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<tr>
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<td>CONSTRUCTION AND USE OF VACCINIA VIRUS RECOMBINANTS AS VECTORS TO EXPRESS POTENTIAL T CELL ANTIGENS AND OTHER VIRAL PROTEINS.</td>
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<th>Significant Date of Additional Info</th>
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<tr>
<th>Date of Significant Change</th>
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</table>
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
### Project Ref: 553/94.2

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<tr>
<td>11/01/1994</td>
<td>THE EFFECTS OF GENETIC MODIFICATION OF HIV VIRUS ON ITS REPLICATION WITHIN HUMAN CELLS</td>
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- **Date Project Ceased**: 19/04/2004
- **Non-GMM**: yes
- **Project notified under transitional arrangements**: yes
- **Withdrawn**: no

#### History

- **Historical Significant Changes**: GM553/00.5
- **Historical Date of Additional Info**: 01/03/2000

#### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 553/94.3

Date Ackn'd 26/03/2001  CU2 Project Title INVESTIGATION OF NEISSERIA MENINGITIDIS, NEISSERIA GONORRHOEAE Class 2  Class CultureVolClass2 CultureVolumeClass3-4
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref 553/94.4**

- **Date Ackn’d**: 12/01/1994
- **Project Title**: CONTROL OF BETA LACTAMASE SYNTHESIS IN STAPHYLOCOCCUS AUREUS
- **Date Project Ceased**: 11/12/2003
- **Class**: Class 2
- **CultureVolClass2**: Non-GMM
- **CultureVolumeClass3-4**: Class 2
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y
- **Historical Significant Changes**: GM553/96.A, GM553/99.6
- **Historical Date of Additional Info**: 09/05/1996, 19/07/1999

Tick if notifying a connected programme of work [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref: 553/98.12

Date Ackn'd: 18/11/1998

CU2 Project Title: MOLECULAR MECHANISMS MEDIATING ANTIGENIC VARIATION IN THE TRYPANOSOME, TRYPANOSOMA BRUCEI

Class: Class 2

Consent Granted: not applicable

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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<td>L2 L3 L4 L2</td>
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Project Ref  553/98.3

Date Ackn’d  25/08/1998  Project Title  INVESTIGATING MECHANISMS OF PATHOGENESIS OF STAPHYLOCCUS  Class 2

Class  CultureVolClass2  CultureVolumeClass3-4

25/08/1998  INVESTIGATING MECHANISMS OF PATHOGENESIS OF STAPHYLOCCUS  Class 2
**Project Additional Information**

- Purposes of the contained use

- Recipient or parental organism

- Host/vector system

- Origin & function

- Evaluation of foreseeable effects

- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Laboratory Activities**

- L2
- L3
- L4

**Glass Houses**

- L2
- L3
- L4

**Growth Rooms**

- L2
- L3
- L4

**Animal Units**

- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

**Project Ref**  553/98.6

**Date Ackn’d**  18/09/1998

**CU2 Project Title**  THE USE OF TRANSGENIC PLASMODIUM BERGHEI SPOROZOITES TO STUDY IMMUNE RESPONSES AND PROTECTION IN MICE

**Class**  Class 2

**CultureVolClass2**  

**CultureVolumeClass3-4**  

**Non-GMM**  

**Consent Granted**  not applicable

**Project notified under transitional arrangements**  Y

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<th>Laboratory Activities</th>
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02/03/2022
EVALUATION OF SHIGELLA FLEXNERI 2A VACCINE CANDIDATE S TO ACT AS CARRIERS OF FOREIGN ANTIGENS

21/05/1999

Consent Granted

Project notified under transitional arrangements

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref**  553/99.9

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<td>GENETIC MODIFICATION OF PLASMODIUM FALCIPARUM BLOODSTREAM</td>
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Date Project Ceased
30/05/2003

Non-GMM Consent Granted
yes

Project notified under transitional arrangements

Withdrawn
N

Tick if notifying a connected programme of work
N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

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Project Ref 553/trans1

Date Ackn’d 04/01/2001

CU2 Project Title

THE ATTENUATION OF VACCINIA VIRUS AND EXPRESSION OF FOREIGN GENES IN VACCINIA VIRUS; INTERACTION OF VACCINIA VIRUS WITH ANTIGEN PRESENTING CELLS

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements Y

With withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

GM553/00.11, GM553/00.12, GM553/95.8, GM553/95.3

Historical Date of Additional Info

### Project Additional Information

#### Purposes of the contained use

The aim is to study the detailed function of the type III secretion system of Shigella flexneri serotype 5a in relation to this organism's ability to invade eukaryotic cells in culture (as simplified model system for how it causes disease). Type III secretion systems (secretons) are widely distributed, essential virulence determinants of Gram-negative bacteria. They are encoded by approximately 25 genes, which share homology with those encoding flagellar basal bodies. Type III secretion systems serve to translocate, upon contact with eukaryotic host cells, proteins from the bacterial cytoplasm into the host cell cytoplasm. The protein effectors of virulence are thought to be moved directly from the bacterial cytoplasm through the secreton (embedded in both bacterial membranes) to insert within Shigella flexneri serotype such as 5a and 2a, wild-type and mutants.

#### Recipient or parental organism

Shigella flexneri serotype such as 5a and 2a, wild-type and mutants.

#### Host/vector system

Non-mobilisable plasmid vectors.

#### Origin & function

DNA from pWR100 (S. flexneri 5a large virulence plasmid) carrying genes or fragments of genes from the ipa, mxi/or spa operons.

Normal/expected biological action of inserted DNA/RNA or transcribed/translated gene product: formulation of a part of the type III secretion machinery (each gene product alone cannot lead to biologically active structure in terms of secretion). Localisation in either the bacterial cytoplasm, inner or outer membrane of bacterial surface. Alternatively generation of a complete or a fragment of the known translocated effector proteins (IpaA-D and IgD) in the bacterial cytoplasm.

#### Evaluation of foreseeable effects

The modifications to the Shigella flexneri are likely either not to affect the organism's fitness/virulence or to actually decrease it. Since the mutations made or planned expression of genes resulting in the loss of a normal function, in no instance would the work to be undertaken be expected to increase fitness or virulence. Antibiotic markers will be used that are not the primary therapeutic agents. The reporter genes will be either neutral or lead to a reduction in bacterial fitness. Gene transfer in this species does not necessitate the use of vectors that will increase the subsequent movement of altered genes. In each instance the donor and recipient species are the same except during gene construction and manipulations in disabled E.coli. Therefore, no new or additional potential risks will be created through the movement of any virulence gene to a new potentially pathogenic species background. For these reasons none of these manipulations will affect the host range, enhance or broaden tissue tropism, or decrease susceptibility to host defence mechanisms.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware e.g. pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) - optionally pre-treat using 2% Virkon then always autoclave using a make safe cycle as specified in BS...
2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (e.g. needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill:

autoclaving, effectively 100% kill (animal validation)
incineration, effectively 100% (licensed incinerator).

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

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Lentiviral-mediated expression of proteins involved in membrane trafficking and cytokinesis in mammalian cells.

Project Ref 554/08.1

Date Ackn'd 27/07/2011

Date Project 02/03/2022

CU2 Project Title Lentiviral-mediated expression of proteins involved in membrane trafficking and cytokinesis in mammalian cells.

Class 2 Consent Granted

Culture Vol Class 2 < 1 L

Non-GMM
The aims of this work is the quantitative transduction of gene products in mammalian cell lines (human telomerase immortalized retinal pigment epithelium — hTERT-RPE1, mouse NIH3T3 fibroblasts, and dog kidney polarized epithelial cells — MUCK) with the purpose of studying the effects of protein expression resulting from these gene products on membrane trafficking and cytokinesis in these cells. Protein expression will be achieved via lentivirus transduction of gene products encoding the proteins of interest (some of which may be oncogenes).

Recipient or parental organism

Mammalian cell lines (eg human telomerase immortalized retinal pigment epithelium — hTERT-RPE1, mouse NIH3T3 fibroblasts, and dog kidney polarized epithelial cells — MDCK). They are auxotrophic and require growth conditions that would not be satisfied outside the laboratory cell culture facility and thus have extremely limited survival in the environment.

Host/vector system

We will use the ViraPower T-Rex Lentiviral Expression System (Invitrogen). This system facilitates highly efficient delivery of a target gene or RNA to dividing and non-dividing mammalian cells using a replication-incompetent lentivirus. Based on the lentikat system developed by Cell Genesys (Dull et al. J. Virol. 1998 72:8463-8471), the ViraPower Lentiviral Technology possesses features which enhance its biosafety while allowing high levels of expression in a wider range of cell types than traditional retroviral systems. See attached assessment for details.

Origin & function

The inserted DNA sequences code for proteins involved in the regulation of membrane trafficking and cytokinesis. The genes to be investigated fall into two categories: (i) membrane traffic, the 63 Rab GTPases and the family of 40 GTPase-activating protein regulators; (ii) cytokinesis, the Aurora BINCENP/Borealin/Survivin, Cit, and Plk1 mitotic kinases, protein phosphatases Cdc4, PP1 and PP2A, the microtubule binding protein PRC1, anillin and septin scaffolding molecules, RhoA, the centralspindlin Rho regulator complex, microtubule severing enzymes of the katanin family, and the kinesins KIF2OA, K1F23, KIF4A, and KIF14. While some of these DNA sequences may code either for oncogenes, or modulators of cell growth, none of the DNA sequences code for toxins.

Evaluation of foreseeable effects

Insertion of the gene into the target cell lines will be accomplished using lentiviral-mediated transduction. With this procedure accidental exposure as a result of needle sticks will be minimal because sharps will not be used. However, in the case of accidental exposure it is unlikely that the inserted gene would result in the formation of a neoplasm. Although some of the genes used may code for oncogenes, carcinogenesis is a multifaceted process that requires change both within the cell and to the cell microenvironment. Therefore, it is unlikely that the inserted gene would satisfy these requirements. Moreover, immune surveillance will likely remove any cells which begin...
To express high levels of the gene product. In end effect, the chance for harmful biological effect to manifest itself as a result of accidental exposure is minimal and likely equivalent to other transfection techniques.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated with a final concentration of 0.1% Virkon S overnight prior to disposal down the sink. Solid waste will be autoclaved and then sent for incineration. The autoclave dedicated to waste disposal is routinely serviced and runs will be logged. Liquid spills will be treated with 1% Virkon S prior to clean up and disposal as solid waste. Surfaces will be wiped with 10% ethanol in liquid form. Virus inactivation by 0.09% Virkon S solutions has been demonstrated to result in a 6-log decrease in titres of purified and unpurified virus within 5 minutes. The use of 0.1% Virkon S with overnight incubation is therefore an effective means to inactivate viruses.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Committee expressed agreement with the assessment as Class 2.

Project Containment

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Project Ref 62/94.3

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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**Glass Houses**
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**Growth Rooms**
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**Project Ref** 628/03.2

**Date Ackn’d** 10/08/2011

**CU2 Project Title**

IN VITRO ANALYSIS OF THE INTERACTION OF HEPATITIS C VIRUS PSEUDOPARTICLES (HCV PP) WITH CELL LINES AND HUMAN PERIPHERAL BLOOD MONOCUCLAR CELLS (PBMC) SUBSETS.

**Class** Class 2

**CultureVol** < 1 Litre

**Class CultureVol** Class 2 < 1 Litre

**Consent Granted**

**Non-GMM** Consent Granted

**Project notified under transitional arrangements**

**Withdrawn**

**Tick if notifying a connected programme of work**

**Historical Significant Changes**

Transferred from GM97 10/08/2011

**Historical Date of Additional Info**

25/07/2007

**Significant Change ID**

628/03.2a

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02/03/2022
### Project Additional Information

#### Purposes of the contained use

In order to aid our previous studies of the interaction of the hepatitis C (HCV) E2 glycoprotein with different PBMC subsets and the possible immunomodulatory consequences of this interaction, we would like to carry out studies with the HCV pp, which express heterodimers of E1 and E2 on their surface. The proposed work would include in vitro analysis of interaction of HCV pp with cell lines and PBMC subsets and investigation of whether HCV pp are able to modulate the activation/functions of different immune system cell types (derived from human peripheral blood), using a variety of in vitro immunological assays.

#### Recipient or parental organism

Murine leukemia virus (MLV), which is a murine retrovirus classified as a category 1 pathogen.

#### Host/vector system

The vector packaged into the pseudotyped MLV particles encodes the marker protein green fluorescent protein (GFP). This is not thought to have any harmful properties. The particles will be pseudotyped with the HCV E1 and E2 proteins. These are not thought to be toxic or oncogenic, but there is some evidence to suggest that the E2 protein may be able to modulate the activation/functions of human lymphocyte subsets. However, it is very unlikely that accidental exposure to a small quantity of HCV pp (which are not able to replicate) would have any harmful immunomodulatory effects.

#### Origin & function

The HCV pp will be provided by our collaborators, the Institute Pasteur in Lille, France; they will not be produced here.

The method by which the HCV pp are generated is described in Bartosch et al, HJournal of Experimental Medicine, 197:633-642 (2003).

In vitro experimental use at containment level 2.

#### Evaluation of foreseeable effects

Pseudotyping of the MLV-based particles with the HCV E1 and E2 proteins results in the particles having a tropism different to that of ecotropic MLV. HCV pp have been shown to bind to and enter human cells expressing what are thought to be HCV receptors (CD81 and other proteins) [Bartosch et al, Journal of Experimental Medicine, 197:633-642, 2003].

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All equipment and waste that has been in contact with HCV pp will be disinfected using procedures approved for destroying the infectivity of lipid-enveloped viruses.

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Tick to confirm that you have attached a risk assessment to this form Y
The committee recommended that each batch of HCVpp should be tested for infectivity on a murine cell line, and with this provisionally approved the work to be done at containment level 2.

**Project Containment**

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**Project Ref** 77/02.1

Date Ackn'd 28/09/2011

CU2 Project Title INVESTIGATION OF BACTERIAL PATHOGENESIS

Class 2

Consent Granted Not Applicable

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes Project transferred from GM8 28/09/2011

**Project Additional Information**

The aim of this project is to 1) construct strains of these bacteria lacking specific genes, 2) establish whether gene inactivation has affected their ability to cause disease.
and 3) further characterise the proteins encoded by these virulence genes.

Recipient or parental organism

*Escherichia coli*, *Shigella* spp., *Neisseria meningitidis*, *Neisseria gonorrhoeae* and *Haemophilus influenzae* are ACDP Hazard Group 2, as they are human pathogens. The organisms will have the equivalent or less potential to cause human disease as the wild-type bacterium. The insertions will interrupt gene function and are likely to attenuate the host.

Host/vector system

- *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Haemophilus influenzae* (Hosts)/pMID216 and derivatives (vector)
- *Shigella* and *E. coli* (Host)/pCR2.1, pUC and pET derivatives, pACYC184, pSTM115, pYH204, pMID216 (vectors)

Origin & function

Source of genetic material (inserts): transposons and antibiotic resistance markers.

Source of genetic material (vectors): pCR 2.1 topo, pACYC184, pUC and pET derivatives: commercially available

- pMID216 from collaborator
- pYH204, pSTM115: generated in own laboratory

Evaluation of foreseeable effects

All the GMMs are ACDP Hazard Group 2 except the disabled *E. coli* strain.

The modification of the host bacteria is in the form of insertions into the chromosome that inactivate gene function. The insertions carry antibiotic resistance markers that encode for resistance against antibiotics not used for treatment of infections caused by these micro-organisms. These modifications should not pose any increased risk to human health. The environmental survivability of the GMM will be similar to the parental wild-type strains.

For disabled *E. coli*, the modifications is in the form of a self replicating plasmid which may contain cloned genes from the pathogenic bacteria. The plasmids confer resistance to ampicillin, kanamycin, and erythromycin. There is a wide variety of alternative agents that can be used to treat successfully infections cause by *E. coli*. The environmental survivability of the GMM will be similar to the parental strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Chemical disinfection of GMM waste except *N. meningitidis***:
1% virkon as per manufacturers instructions, at least 1 hour exposure. Plates and plasticare sterilised by autoclaving.
Discard cycle 134 degrees C for 30 min. 100% kill. Machine and cycle validated after each cycle by thermograph; autoclaves thermocouple tested regularly (6 monthly) qualified engineer.

**Autoclaving of waste contaminated by *Neisseria meningitidis***: Discard cycle 134 degrees C for 30 min. 100% kill. Machine and cycle validated after each cycle by thermograph; autoclaves tested regularly qualified engineer.
It was considered that work with disabled E. coli strains could be conducted in Containment Level 1 facilities. However, this work will be done in a Containment Level 2 laboratory along with the work on Neisseria gonorrhoeae, Haemophilus influenzae, Shigella flexneri, and E. coli K1. The importance of prompt reporting any incidents and episodes of diarrhoeal disease among researchers working with Shigella spp. was highlighted.

People working with N. meningitidis must complete a health questionnaire; any persons with suspected immunodeficiency must be seen by the Occupational Health doctor before working with the bacterium. It was recommended that work with N. meningitidis is carried out in a dedicated (Containment Level 3) room. Only individuals who have undergone a locally approved training programme would be allowed to work with live N. meningitidis. All person must be aware of the signs and symptoms of meningococcal infection, and should seek early medical advice should they become unwell, informing medical staff of their potential exposure to N. meningitidis at the work place. All accidents/spillages to be reported promptly to the Occupational Health Department and Department Safety Officer. A list of individuals allowed to work with the meningococcus, details of the clinical presentation of N. meningitidis infection, and guidelines for safe working is to be kept at the entrance to the designated facility at all times.

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Project Additional Information

Purposes of the contained use

Human immunodeficiency virus type 1 (HIV-1) is a lentivirus that causes a persistent infection in humans associated with the development of an acquired immunodeficiency syndrome (AIDS) which, if untreated, is ultimately fatal. We are studying innate and T cell responses in individuals who have repeatedly been exposed to HIV but have no developed fully-seropositive infection (and unexposed control individuals), and in patients recently infected with HIV who naturally contained viral replication with differing efficiency. Our objective is to identify viral and immune correlates of good control of virus replication, to inform HIV vaccine design. As part of these studies, we propose to compare the relative susceptibility of PDMCs from different individuals to in vitro infection with different HIV isolates, and then go on to dissect mechanisms contributing to differences in in vitro viral replication (focusing particularly on the role of host innate effector mechanisms in inhibiting viral replication).

Some experiments will be carried out with uncloned HIV stocks (including primary virus isolates derived from different infected individuals); but we would also like to work with viral stocks prepared from proviral DNA clones, both for consistency, and to enable identification of viral genetic determinants of differences in in vitro replication capacity.

Recipient or parental organism

Plasmids containing infectious molecular clones of HIV will be grown up in disabled host bacteria such as the E.coli K12 derivatise DH5α; and then transfected into a eukaryotic cell line such as 293T (a human kidney cell line).

The bacteria to be used are non-pathogenic and unlikely to survive outside culture media or disseminate themselves in the environment. There should be no expression of HIV gene products with these bacteria; but should the plasmid DNA somehow gain entry into dividing eukaryotic cells, there would be potential for HIV production from these cells (HIV RNA production being driven from the LTRs).

293T cells are a highly transfecatable derivatise of the 293 cell line (an adherent cell line of epithelial morphology derived from human kidney, which contains some adenovirus 5 DNA sequences) into which the temperature sensitive gene for SV40T antigen was inserted. Due to the presence of adenoviral and SV-40 T sequences, which confer the potential to form tumours, this cell line should be handled at containment level 2.

Transfection of these cells with molecular clones of HIV will result in production of HIV.

HIV is a lentivirus that is known to infect humans (via routes including the blood and via mucosal surfaces), and establishes a lifelong persistent infection ultimately associated with the development of AIDS. HIV -1 is classified as an ACDP category 3 pathogen. Many of the infectious molecular HIV clones to be used will be composed of sequences derived from different HIV isolates - ie will result in the generation of genetically modified (chimeric) virus.

Host/vector system

We initially plan to work with plasmid pNL4.3, which contains an infectious molecular clone derived from the NY5 and LAV HIV isolates (Adachi et al, J Virol 59 284-291).
1986) and generates a CXCR4-utilising virus; and a matched clone (pNL4.3BaL ecto) in which the gp160 ectodomain has been replaced with that of HIV BaL (a CCR5-utilising virus isolate). Details of these HIV molecular clones (both of which are contained in the plasmid pUC18) are provided in the two accompanying pdf files. In future, infectious clones of other HIV isolates and chimeras containing other gp160s or gp 160 fragments may also be used. Some clones may also include marker proteins such as GFP. All the viruses generated from these clones will be of similar or lower pathogenicity to those detailed above. These clones will be obtained from repositories or external collaborators; we do not propose to generate or modify infectious molecular clones of HIV ourselves.

Origin & function

As detailed above, we plan to work with infectious molecular clones derived from CXCR4 and CCR5-utilising HIV isolates. These will include chimeric clones, in which the env gene (or fragments thereof) may be derived from different HIV isolates. Some clones may also contain the marker protein GFP. Infectious HIV will be derived from these clones. Viruses expressing different envelope glycoproteins (the virion surface protein that mediates attachment and entry into host cells) will be generated from different chimeric clones.

Evaluation of foreseeable effects

1. Growth of plasmids containing infectious molecular clones of HIV in E.coli. Bacteria
The plasmids we will work with will contain full-length molecular clones of HIV. Plasmid stocks will be prepared by growth in disabled host bacteria such as the E.coli K12 derivative DH5α.
The pathogenicity of the host bacteria in which the plasmids are to be grown is very unlikely to be affected by the presence of the plasmids. There should not be any expression of HIV genes within the bacteria.
The HIV molecular clones will be contained within the pUC18 plasmid, which is a non-mobilisable vector. Plasmid sequences are thus unlikely to be transferred to other bacteria.
E.coli bacteria containing HIV molecular clones are very unlikely to cause harm to human health. They should not express any HIV gene products, and the presence of the plasmid DNA is not expected to affect their pathogenicity.
It is very unlikely that E.coli bacteria transfected with HIV molecular clones would survive in the environment; and since the plasmid DNA is non-mobilisable, it would also be very unlikely that this would be transferred to other bacteria. These GMOs thus pose an extremely low risk to the environment.

It is unlikely that plasmid DNA contained within the bacteria would be transferred into human cells and initiate HIV production. Nonetheless, this could potentially occur, and is a risk that should be considered. Given the (extremely low) risk that plasmid DNA encoding full-length molecular clones of HIV could conceivably enter replication-competent human cells and initiate production of infectious HIV, it is proposed that plasmid DNA and bacteria containing it should be handled under containment level 2 conditions, to minimise the risk of an infectious exposure incident occurring.
Plasmids containing HIV molecular clones and bacteria containing these plasmids will thus be worked with in category 2 containment laboratories. Gloves and eye protection will be worn at all times when handling these reagents (to minimise the risk of contact with skin or mucosal surfaces). The use of glass and of sharps (eg needles) will be avoided where possible, to minimise the risk of sharps injuries that may result in exposure to plasmid DNA. Equipment and work-surfaces will be decontaminated after use using a suitable a chlorine-containing disinfectant, and all waste will also be treated with disinfectant and/or autoclaved.

2. Transfection of eukaryotic cells with full-length molecular clones of HIV to generate infectious virus
Plasmids containing full-length molecular clones of HIV will be transfected into eukaryotic cells (eg 293T cells), which will then produce infectious HIV particles. Upon transfection of the plasmids into 293T cells, the pathogenicity of the cells themselves is unlikely to change. There is also very little risk of plasmid sequences being transferred from transfected 293T cells to other cell lines.
293T cells transfected with HIV molecular clones would also be very unlikely to survive in the environment.
However 293T cells transfected with HIV molecular clones will produce infectious HIV, which could initiate human HIV infection. These cells and the viruses derived from them thus present a high risk to human health - equal to that of naturally-occurring HIV isolates.
Transfection of plasmids into eukaryotic cells and all subsequent work with transfected cells with thus be performed under in category 3 containment laboratories, following the category 3 laboratory code of practice.
3. Use of HIV viruses derived from infectious molecular clones
HIV is a lentivirus that is known to infect humans (via routes including blood and via mucosal surfaces), and establishes a lifelong persistent infection ultimately associated with the development of AIDS. HIV-1 is classified as an ACIP category 3 pathogen.

Some of the HIV clones we propose to work with are chimeric clones containing partial/entire env genes from one HIV isolate in the backbone of another. As gp160 is the virion envelope glycoprotein, its sequence does affect virus interaction with host cell receptors and hence viral tropism and pathogenicity. However it is not expected that any of the chimeric viruses we will generate will exhibit a level of pathogenicity greater than that of the group of primary HIV isolates from which their component sequences were derived.

HIV sequence could potentially be transferred from the chimeric viruses that we generate from molecular clones to other HIV viruses, if replication competent cells were to become co-infected with the chimeric virus and another HIV isolate. However this should not result in the generation of recombinant viruses any more pathogenic than those that could be generated naturally by recombination between the parental virus from which the chimeric virus was derived and the HIV isolate concerned.

The HIV virions we generate would pose a similar risk to the environment as that posed by natural HIV isolates. HIV virions are extremely labile- but if laboratory staff were to become infected, they could potentially transmit virus to other people. To minimise this risk, in the event of any potential HIV exposure incident, the staff involved would be tested for HIV infection, and also receive antiretroviral therapy to try and prevent full-blown infection from occurring.

HIV viruses derived from infectious molecular clones will only be used for in vitro experimental work. All work involving these viruses will be performed in category 3 containment laboratories, following the category 3 laboratory code of practice.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from bacterial cultures will be treated with 1% bleach for > 30 minutes prior to discarding (100% kill). Solid waste will be disinfected by autoclaving or incinerated (100% kill). Equipment and work-surfaces will be decontaminated after use using a suitable chlorine disinfectant.

Liquid waste from the category 3 containment labs will be treated with a suitable disinfectant (eg.1% Virkon for > 30 minutes) prior to discarding (100% kill). Solid waste will be disinfected by autoclaving (100% kill). Equipment and work surfaces will be decontaminated after using a suitable disinfectant (eg 70% ethanol).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The Institute for Animal Health's Genetic Modification Safety Committee has reviewed and approved this work and associated risk assessment.

Project Containment
### Project Ref 97/09.1

**Date Ackn’ed**
10/08/2011

**CU2 Project Title**
GENERATION OF HCVcc FROM INFECTIOUS MOLECULAR CLONES, AND ITS IN VITRO USE

**Class**
Class 3

**Culture Volume**
60ml

**Non-GMM Consent Granted**
Yes

**Project notified under transitional arrangements**
N

#### Historical Significant Changes
Transferred from GM97 10/08/2011

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### Purposes of the contained use

Hepatitis C Virus (HCV) is a Hepacivirus belonging to the family of the Flaviviridae that causes a persistent infection in about 80% of infected individuals. The primary site of infection is the liver, where chronic infection can lead to liver cirrhosis and hepatocellular carcinoma (HCC). The virus has also been found in peripheral blood mononuclear cells (PBMCs) and the central nervous system (CNS).

We are studying the modulation of immune functions and infection of different PBMC subsets by the virus, to understand how HCV establishes and maintains a persistent infection.

As HCV isolates cannot be grown in cell culture, these experiments will be carried out with the HCVcc generated from different molecular clones (as detailed below).

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### Recipient or parental organism

The E coli K12 derivative will be DH5α will be transduced with the plasmids containing the HCV molecular clones and cultured. The isolated plasmid DNA from these...
bacteria such as the E. coli K12 derivative DH5α will be used to produce plasmids containing infectious molecular clones of HCV; these will then be transfected into a hepatocyte cell line such as Huh-7.5. The bacteria to be used are non-pathogenic and unlikely to survive outside culture media or disseminate themselves in the environment. The Huh-7 cell line and its derivatives (Huh-7-Lunet) are derived from a hepatocellular carcinoma (HCC). The Huh-7.5 cell line (used for production of HCVcc) is derived from the Huh-7 cell line but contains an inactivating mutation in the RIG-1 gene. RIG-1 is the intracellular sensor for dsRNA and its inactivation makes the Huh-7.5 cell line highly permissive to HCVcc replication. When uninfected, this cell line should be handled at containment level 1. Many of the infectious molecular HCV clones to be used will be composed of sequences derived from different HCV isolates - i.e. will result in the generation of genetically modified (chimeric) virus.

HCV is a Hepacivirus that is known to infect humans, via routes including the blood and via mucosal surfaces. It establishes a lifelong persistent infection in about 80% of infected individuals that is ultimately associated with diseases such as liver cirrhosis and HCC. HCV is classified as an ACDP hazard group 3 pathogen.

Until recently, HCV could not be grown in cell culture, so the use of HCV replicons, HCV proteins or HCV pseudoparticles (HCVpp) was necessary. The discovery of a clone called JFH-1, isolated from a Japanese patient with fulminant hepatitis, revolutionised the field, because it was the first clone that could replicate and produce infectious virus in cell culture and led to the production of what is called HCVcc (cell-culture derived HCV). Currently, there are only a few HCV clones that can replicate and produce infectious virus. These include H77 (genotype 1a), Con1 and HCV-CG1b (genotype 1b), JFH-1 (genotype 2a) and a few structural chimeras of JFH-1 (H77-JFH-1, Con1-JFH-1, 452-JFH-1 and J6-JFH-1). We initially plan to work with the HCVcc chimera J6-JFH-1, which generates higher infectious titres compared to the JFH-1 clone, but also plan to use the original JFH-1 clone, the chimeras listed above and other structural chimeras (JFH-1 viruses containing glycoproteins from other viruses) in the future. Details of these HCVcc constructs are provided in the attached table.

We do not propose to generate or modify infectious molecular clones of HCV ourselves. However, we will grow up bacteria (E. coli derivatives) that harbour plasmids containing HCVcc molecular clones supplied to us by other investigators (Ralf Bartenschlage, Arvind Patel). There should be no expression of HCV gene products within these bacteria; but should the plasmid DNA somehow gain entry into dividing eukaryotic cells, there would be potential for HCV production from these cells. Viral stocks will be generated by transfecting plasmids containing proviral DNA clones into Huh-7.5 cells or other hepatocyte cell lines, collecting the supernatant, and finally concentrating the virus. These viral stocks will then be used in in vitro assays.

### Origin & function

As detailed above, we plan to work with different infectious HCV molecular clones. These will include chimeric clones, in which the structural proteins and the non-structural proteins may be derived from different HCV clones. Infectious HCV will be derived from all these clones.

### Evaluation of foreseeable effects

1. Growth of plasmids containing infectious molecular clones of HCV in E. coli. Bacteria

Disabled host bacteria such as the E. coli K12 derivative DH5α will be used to isolate plasmids containing infectious molecular clones of HCV; these will then be transfected into a hepatocyte cell line such as Huh-7.5 for productuon of HCVcc.

The bacteria to be used are non-pathogenic and unlikely to survive outside culture media or disseminate themselves in the environment. E. coli bacteria containing HCV molecular clones are very unlikely to cause harm to human health. There should be no expression of HCV genes within the bacteria. The pathogenicity of the host bacteria in which the plasmids are to be grown up is very unlikely to be affected by the presence of plasmids.

It is very unlikely that E. coli bacteria transfected with HCV molecular clones would survive in the environment; and since the plasmid DNA is non-mobilisable, it would also be very unlikely that this would be transferred to other bacteria. These GMOs thus pose an extremely low risk to the environment.

It is unlikely that plasmid DNA contained within the bacteria would be transferred into human cells and initiate HCV production. Nonetheless, this could potentially occur, and is a risk that should be considered.

Given the (extremely low) risk that plasmid DNA encoding full-length molecular clones of HCV would conceivably enter replication-competent human cells and initiate production of infectious HCV, it is proposed that plasmid DNA and bacteria containing it should be handled under containment level 2 conditions and in a microbiology safety cabinet, to minimise the risk of infectious exposure incident occurring.
Plasmids containing HCV molecular clones and bacteria containing these plasmids will thus be worked with in category 2 containment laboratories. Gloves and eye protection will be worn at all times when handling these reagents (to minimise the risk of contact with skin or mucosal surfaces). The use of glass and of sharps (e.g. needles) will be avoided where possible, to minimise the risk of sharps injuries that may result in exposure to plasmid DNA. Equipment and work-surfaces will be decontaminated after use using 70% Ethanol and all waste will also be treated with 5% Virkon over night and/or autoclaved (see attached reference for minimal contact times in order to inactivate HCV and general disinfection procedures used in our containment level 3 laboratory).

2. Transfection of liver cell with full-length molecular clones of HCV to generate infectious virus
Plasmids containing full-length molecular clones of HCV will be transfected into liver cell lines (Huh-7.5 cells), which will then produce infectious HCV particles. Upon transfection of the plasmids into Huh-7.5 cells, the pathogenicity of the cells themselves is unlikely to change. There is also very little risk of plasmid sequences being transferred from transfected Huh-7.5 cells to other cell lines. Huh-7.5 cells transfected with HCV molecular clones would also be very unlikely to survive in the environment. However Huh-7.5 cells transfected with HCV molecular clones will produce infectious HCV, which could initiate human HCV infection. These cells and the viruses derived from them thus present a high risk to human health - equal to that of naturally-occurring HCV isolates.
Transfection of plasmids into liver cell lines and all subsequent work with transfected cells will thus be performed under in containment level 3 laboratories, following the category 3 laboratory code of practice.

3. Use of HCV viruses derived from infectious molecular clones
Many of the infectious molecular HCV clones to be used will be composed of sequences derived from different HCV isolates - i.e. will result in the generation of genetically modified (chimeric) virus. HCV is a Hepacivirus that is known to infect humans, via routes including the blood and via mucosal surfaces. It establishes a lifelong persistent infection in about 80% of infected individuals that is ultimately associated with diseases such as liver cirrhosis and HCC. HCV is classified as an ACDP hazard group 3 pathogen. Some of the HCV clones we propose to work with are chimeric clones containing structural genes from one HCV isolate in the backbone of another. As E1 and E2 are the virion envelope glycoproteins, their sequences affect virus interaction with host cell receptors and hence viral tropism and pathogenicity. However it is not expected that any of the chimeric viruses we will generate will exhibit a level of pathogenicity greater than that of the group of primary HCV isolates from which their component sequences were derived.
The viruses derived from them would pose a similar risk to the environment as that posed by natural HCV isolates. Being enveloped viruses, HCV virions are extremely labile - but if the laboratory staff were to become infected, they could potentially transmit virus to other people. To minimise this risk, in the event of any potential HCV exposure incident, the staff involved would be tested for HCV infection, and also receive antiviral therapy to try and prevent full-blown infection from occurring. HCV viruses derived from infectious molecular clones will only be used for in vitro experimental work. All work involving these viruses will be performed in containment level 3 laboratories, following the category 3 laboratory code of practice.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from bacterial cultures will be treated with 5% Virkon for over night prior to discarding. Solid waste will be disinfected by autoclaving or incinerated. Equipment and work-surfaces will be decontaminated after use using a suitable chlorine-containing disinfectant.

Liquid waste from the category 3 containment labs will be treated with a suitable disinfectant (e.g. 5% Virkon over night) prior to discarding. Solid waste will be disinfected by autoclaving. Equipment and work-surfaces will be decontaminated after use using a suitable disinfectant (e.g. 70% ethanol)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The Institute for Animal Health's Genetic Modification Safety Committee has reviewed and approved this work and associated risk assessment.

Please enter comments on the GM safety committee on the risk assessment

The Institute for Animal Health's Genetic Modification Safety Committee has reviewed and approved this work and associated risk assessment.

**Project Containment**

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**Name**

UNIVERSITY OF LIVERPOOL

**Name 2**

SAFETY ADVISORY SERVICE

**Campus Estate or Research Centre**

**Road Name**

24 OXFORD STREET

**Town**

LIVERPOOL

**District**

MERSEYSIDE

**County**

ENGLAND

**Postcode**

L69 7ZX

**Tel Number**

0151 794 3042

**Fax Number**

0151 794 3191

**E-mail**


**HSE Division**

NORTH WEST

**Comments**

**Date at Which Additional Info Submitted**

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Precautions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment 

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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Significant Change ID
Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment
Project Containment

Laboratory Activities

L2 L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4

Glass Houses

Animal Units

Large Scale Activities

Growth Rooms

Human Clinical Applications

Project Ref 118/01.2

Cloning and identification of mRNAs differentially expressed in breast lesions

Class CultureVol

Class 2

Consent Granted

Not Applicable

Project notified under transitional arrangements Y

Historical Significant Changes

TRANSFERRED FROM GM 118 - 15/1/07

Date of Significant Change

02/03/2022

02/03/2022
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref  118/01.3

Date Ackn'd  02/03/2022

CU2 Project Title

Class  

CultureVolClass2  

CultureVolumeClass3-4
GENETIC CHANGES IN A TRANSGENIC MOUSE MODEL OF HUMAN BREAST CANCER

Date Project Ceased: 01/06/2011

Historical Significant Changes: TRANSFERRED FROM GM118 - 15/1/07. PROJECT CLOSED 01/06/2011

Withdrawn: N

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 118/04.1

Date Ackn’d 15/01/2007

CU2 Project Title
IDENTIFICATION AND CHARACTERISATION OF BIOFILM-REGULATED FACTORS PRESENT IN MEMBERS OF THE ORAL MICROBIAL FLORA

Class 2
Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes
TRANSFERRED FROM GM 118 - 15/1/07
Cells grown in biofilms behave and respond differently to cells grown in liquid media suspensions. Understanding how these changes affect the phenotype of a biofilm-grown cell is critical to our ability to control and manipulate bacterial biofilms, which play a major role in beneficial applications like water purification and detrimental scenarios like infections from implanted devices and bio-fouling of air-conditioning systems and water distribution networks. Members of the oral bacterial flora have been extensively studied and cultured in mono- and mixed- species culture, and thus lend themselves to more sophisticated studies involving the up-regulation and down-regulation of factors that direct their adaptation to the biofilm lifestyle. In the main, the biofilm-adaptive factors identified in different bacterial strains have been associated with stress responses as well as activation of mobile genetic elements, but the regulatory factors are still not well understood, nor have all the biofilm adaptive factors been identified. The goals of the work covered by this application are to examine in detail these biofilm adaptive responses in members of a well characterised mixed oral bacterial consortium both in single and mixed culture conditions.

1. Porphyromonas gingivalis, 2. Prevotella nigrescens, 3. Fusobacterium nucleatum, 4. Neisseria subflava, 5. Streptococcus mutans, 6. Streptococcus oralis, 7. Streptococcus sanguis, 8. Veillonella dispar, 9. Actinomyces naeslundii, 10. Lactobacillus rhamnosus. 1, 2, 3, 8 are Gram negative anaerobes, 4 is a Gram negative aerobe, 5,6,7,9 and 10 are Gram positive facultative anaerobes. All species are members of the normal human oral flora and all have been on occasion reported as opportunistic pathogens. The strains used will initially be 1. W50, 2. T588, 3. ATCC10953, 4. A1078, 5. R9 and UA159, 6. EF186, 7. 209, 8. ATCC17745, 9. WVU627 and 10. AC413.

Recipient organism: K12 laboratory strains of Escherichia coli will be used for all cloning work. K12 strains are considered to be non-colonising.

E. coli strains MC1061 or DH5 - a will be used to propagate shuttle and suicide vectors. E.coli strain S17 will be used to mobilise the mobilisable (but not conjugative) shuttle vectors. Various shuttle vectors proposed for use are pVA838, pDL278 and pFX3 which are nonmobilisable Streptococcal/E.coli shuttle vectors; pT-COW a mobilisable but cnonconjugative E.coli/Prevotella-Porphyromonas shuttle vector; and pBR322 a non mobilisable E.coli plasmid. His-tag vectors like the pQE derivatives from Clontech for specific use in E.coli.

Various biofilm adaptive factors from these oral strains will be cloned and identified. Not overt attempt to express these elements will be made until they have been suitably characterised by sequencing. Identified factors that have been determined not to be toxins may be cloned into vectors for the express purposes of recombinant protein purification or expression for future studies on their role in biofilm adaptation.

In all cases the aim of this work is to identify factors present in ACDP cat2 organisms that are activated under biofilm growth. No overt attempts will be made to express any of these factors in E.coli until they have been identified. Although increased toxin production has been associated with other biofilm-forming organisms like Pseudomonas aeruginosa, the purpose of these studies will not be to study toxin expression, so if toxins are identified as being biofilm adaptive factors in these oral organisms they will not be targets for future study. Instead, this proposed work involves identifying other genetic elements that are strictly involved in the cells ability to adapt to the biofilm environment (i.e. global regulatory factors, mobile genetic elements, stress response facorsnonovel membrane proteins, and other elements not directly capable of conferring virulence factors to the ACDP category 1 E.coli K12 species beyond that of the original ACDP category 2 oral organism. Therefore handling recombinant E.coli at containment level 2 conditions obviates any foreseeable effects. In the case where vectors are introduced into the oral bacteria this will be done either to mark them with an antibiotic resistance marker found on vectors suitable to those organisms or to reintroduce regulatory sequences for reporter gene analysis. In either case, the virulence of the oral bacteria will not be enhanced so work done under containment level 2 conditions, again, obviates any foreseeable effects.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All biological waste will be handled according to the School's strictest guidelines. All waste, with the exception of glass pipettes will be bagged or boxed in metal tin cans and be subjected to autoclaving according to the British Laboratory Standards for biological waste. These autoclaves are regularly serviced and tested by the School. This will result in a 100% kill with all non-glass waste ultimately subjected to incineration and glass waste being washed (post autoclaving) with recirculation into the general glassware pool. Glass pipettes will be neutralised in fresh bleach-based disinfectant before being washed and autoclaved as is the standard practice within the School.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

At the meeting of the safety committee on 10th July 2003 it was agreed that the project was class 2.

## Project Containment

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### Project Ref  118/04.2

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<td>15/01/2007</td>
<td>ANALYSIS OF SIGNALLING AND GENE REGULATION IN ASPERGILLUS FUMIGATUS</td>
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Date Project Ceased  02/03/2022
The objective is to develop our understanding of key regulatory and signalling systems such as those influencing nitrogen metabolism and the regulatory response to ambient pH in A. fumigatus. This will utilise approaches that will have successful employed with A. nidulans. In response to comparative genomic analysis as well as proteomic and transcriptomic data, molecular genetic analysis will be undertaken to investigate the function of key regulons and the putative regulatory and signalling components. An initial step will be to construct strains disrupted for specific genes identified by the upstream analysis. This will be achieved by homologous integration of deletion constructs bearing selectable markers such as Neurospora crassa pyr-4 (encoding pyrophosphorylase orotidine MP decarboxylase). Additionally we will construct epitope tagged versions of key genes to identify interacting proteins. This work involves standard cloning procedures in laboratory strains of E. coli, followed by transformation into A. fumigatus. Assessment of the effect of mutations will be through plate tests, northern and proteomic analyses of wild-type and mutant strains. The identity of transformed A. fumigatus strains will be confirmed by PCR, sequencing and Southern, as appropriate.

A. fumigatus is harmless to normal healthy people, but it is listed in ACDP hazard group 2. It can cause postoperative infections, aspergilloma (particularly in patients with cystic fibrosis, post-TB, asthmatics), sinusitis in normal people and eye infections. Immunosuppressed individuals are the most at risk of developing pneumonia, disseminatin to other organs and serious illness. Conidia (spores can cause an allergic response in sensitive individuals. It is usually transmitted by inhalation of airborne condia which are widely distributed in nature. Conidia can be readily isolated from most environments including air, human homes, offices, work-places and soil. A. fumigatus is sensitive to amphotericin B, itracomazole and voriconazole. It is also susceptible to 1% sodium hypochlorite and 70% ethanol.

Standard cloning procedures will be carried out in laboratory strains of E. coli to produce deletion constructs and epitope tagged versions of regulatory genes. Standard pUC based vectors will be used for this work. These constructs or PCR amplified and gene disruption in A. fumigatus utilises cosmids (e.g. from pWe15 cosmid libraries) bearing the sequence of the gene of interest will be prepared for transformation into A. fumigatus in E. coli strains (e.g. KS272) expressing the phage Reda and Redb recombination functions. This will involve replacement of a region of the gene of interest with the sequence of a marker gene to facilitate selecion of recombinants in both E. coli and A. fumigatus. Purified cosm id DNS will be transformed into A. fumigatus.

The genetic material will originate from A. fumigatus and will have a role in regulation or signalling in nitrogen metabolism or in response to pH.

Virulence is multifactorial, and so far discrete virulence determinants in A. fumigatus have not been identified. However, nitrogen and pH regulatory mechanisms have been implicated in the pathogenicity of other pathogenic fungi. Strains deleted are AfareA which have been tested for pathogenicity have reduced pathogenicity compared with...
the parental strain. One study has reported that a revertant from AfareA deletion, where an uncharacterised mutation led to increased expression of specific nitrogen regulated genes, had increased pathogenicity. We do not intend to create revertants in this project, and we anticipate that this project will therefore produce strains with reduced pathogenicity. The regulatory genes and signalling genes produce regulatory proteins or enzymes, which are unlikely to have a harmful biological activity alone. Experiments using isolated DNA, RNA or protein should therefore involve little risk. Cloning within laboratory strains of E.coli will not involve expression, and should also be low risk. Vectors will be capable of expression in Afumigatus and Anidulans, but not in E.coli.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Conidia: Measures to control the spread of conidia by handling plate cultures in a Class 2 cabinet. The caninet to be sprayed with 70% ethanol before and after use, and between strains. Plates to be taped closed and exterior decontaminated (70% ethanol) for transport between cabinet and incubator in case of dropping a plate. Use scatter plates before/after work to test that conidia are contained by these measures. Mycelium: Mycelium to be ground to a powder in cabinet. Waste mycelium will be autoclaved to ensure that it is inactivated if not previously killed by extraction procedures. Any contamination incidents decontaminated with 1% sodium hypochlorite for 30 min. Spent growth medium: Spent medium, and washes from liquid cultures to be incubated with bleach prior to discard down drain. Inform Occupational Health so they can record work with this allergenic sensitiser (if deemed appropriate).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

At the meeting of the safety committee on 10th July 2002 it was commented that the work should take place in a pathogen handling room. The containment should be checked with a non-pathogenic or non-GM strain before worked commenced.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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STUDIES ON THE ROLE AND REGULATION OF SECRETED PROTEINS OF STAPHYLOCOCCUS AUREUS AND ENTEROCOCCUS FAECALIS

Purpose of the contained use

Contained use is required to prevent dissemination of genetically altered commensal bacteria that have the capacity to cause disease.

Recipient or parental organism

The parental strains of the two organisms that will be used are for Staphylococcus aureus strains SH1000 (8325/4) and Newman and for Enterococcus faecalis strains OG1RF and JH2-2. These are opportunistic pathogenic bacteria. These are not attenuated strains but are the commonly used strains for genetic manipulation. Staphylococcus aureus SH1000 (8325/4), Newman in conjunction with the following plasmids: pAZ106, pLTV1, pMUTIN4, pCL84, (all integrative) pCU1, pSB2035 (both replicative).

Enterococcus faecalis OG1RF, JH2-2 with/without the following plasmids: pAZ106, pLTV1, pMUTIN4, (all integrative), pSB2035, pCU1, pTCVlac, pAT28 (replicative).

Escherichia coli BL21 (DE3) in conjunction with pETderivative vectors (T7 promoter)

Host/vector system

S. aureus and E. faecalis genes will be amplified and cloned into the vectors listed in section 7 using E. coli. The plasmids produced and containing the correct inserts will be purified and used to transform E. faecalis and S. aureus. The plasmids used are for two separate types: 1) replicative and maintained extrachromosomally for functions such as gene reporters and gene complementation; 2) integrative for generating insertional replacement mutations and maintained due to recombination with chromosome.

Overexpression of proteins will only be done in E.coli BL21 (DE3) using the pET system. No overexpression of proteins will be done with either S. aureus or E. faecalis as host.

Origin & function
The S. aureus and E. faecalis strains that will be used for the proposed study are laboratory isolates that have been obtained from laboratories that are currently researching the area of host/pathogen biology. Originally, these were isolated from humans.


The intended functions are to study the role and regulation of surface and secreted proteins of these two commensal bacteria to investigate their potential roles during opportunistic infection. This will mainly be done by in vitro analysis of gene and protein expression and this will be done in coordination with a diversity of characterised mutant strains of these organisms to determine the hierarchy of gene expression for virulence and secreted protein production. Murine abscess models of infection will be used for study of staphylococcal mutants that are predicted by virtue of in vitro observations to have a role in virulence. These models of infection will be studied outwith the University of Liverpool in collaboration with others. E. faecalis mutants will be tested in invertebrate models of infection at Liverpool using the worm Caenorhabditis elegans, a well established model of infection.

Evaluation of foreseeable effects

S. aureus is a category 2 microorganism and the mutations that will be made are likely to reduce virulence of the organism compared to the parent strain. This drop in virulence may even reduce the S. aureus strain to category 1, however, due to the ability of S. aureus to colonise humans all of the work will be done in category 2 condition.

E. faecalis is a category 2 microorganism and the mutations that will be made are likely to reduce virulence of the organism compared to the parent strain. This drop in virulence may even reduce the E. faecalis strain to category 1, however, due to the ability of E. faecalis to colonise the digestive tract of human the work will be done in category 2 conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be placed in labelled, capped glass bottles and autoclaved, which results in total kill. Any spillages will be cleaned up with 2% w/v stericol and waste will be autoclaved, again resulting in a total kill. Lab coats will be worn at all times and after work is completed hands will be vigorously washed prior to exiting the category 2 laboratory.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No specific comments were made.
ANTIGENIC VARIATION IN PILI OF NEISSERIA MENINGITIDIS AND COMMENSAL NEISSERIA SPP

TRANSFERRED FROM GM 118 - 15/1/07

Historical Significant Changes

TRANSFERRED FROM GM 118 - 15/1/07

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

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Project Ref 14/11.1

Date Ackn'd 02/03/2022
Hepatitis B virus (HBV) genome cloning and phenotypic studies

02/12/2011

Date Project Ceased

Class 3 < 1 Litre < 1 Litre

Non-GMM Consent Granted

Yes

Project notified under transitional arrangements

N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Project transferred from GM14

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

We are conducting studies on the impact of Human Immunodeficiency Virus (HIV) and its treatment among people who are co-infected with the hepatitis B virus (HBV). For this purpose we are collecting blood samples from two cohorts of patients in Ghana and Malawi. In these regions antiretroviral therapy has recently become available to treat HIV infection. The first-line combination includes three drugs active against HIV, typically stavudine, lamivudine (LMV) and nevirapine. Of these drugs, LMV also has activity against HBV. Thus patients who are HIV/HBV co-infected receive three drugs for HIV but only one for HBV. There are two problems associated with the use of LMV as the sole agent against HBV:

1) Control of HBV replication is usually suboptimal, leading to the development of HBV drug resistant variants carrying mutations in the polymerase (pol) gene targeted by LMV. Ongoing HBV replication leads to progressive liver damage and a risk of liver cancer. Thus, as survival increases in these communities due to control of HIV, a potential is emerging for significant HBV-related liver disease to become apparent.

2) As the HBV surface (S) and pol genes are expressed in alternative reading frames from the same region of the viral genome (Figure 1), selective pressure exerted by LMV on pol can cause mutations in both pol and S. Mutations in the HBV surface antigen (HBsAg) can affect key antigenic determinants, creating the potential for immune and vaccine escape. In addition mutations in HBsAg can lead to loss of recognition in diagnostic assays.

We have preliminary data that show high rates of emerging HBV LMV resistance in the two African cohorts and have also identified virus variants that carry highly mutated HBsAg, including some diagnostic escape mutants. We now wish to extend these studies by constructing a system for HBV that will allow us to characterize the viral phenotype, including drug resistance, viral fitness, and HBsAg expression and immune recognition in vitro. We have extensive experience with similar systems applied to the study of HIV phenotypes, where we use both replicative and non-replicate recombinant vectors containing virus gene segments of interest inserted as "cassettes". The vector may also be modified by site directed mutagenesis (SDM) to study mutations of interest. We plan to follow a similar approach with HBV.

It has long been established that certain circular DNA viruses replicate through a rolling circle mechanism. By mimicking this mechanism, a construct that contains a full length viral genome plus a partial repeat ("full-length plus") can direct the production of virus particles upon transfection into cell lines in vitro. HBV clones have been traditionally produced in this way. We will amplify the whole HBV genome from one or a few interesting samples and clone it into standard cloning vectors (pUC or pCR based vectors). Full-length plus HBV clones will be generated by adding a partial repeat to the cloned full genome. Individual gene segments will be sub-cloned into generic expression vectors (pCMV or pSV40 expression vectors). In some cases, gene segments derived from certain patient viruses will be exchanged with those of the reference clone to study their effect on phenotype in the context of a common backbone. In addition, mutations of interest will be introduced by SDM. We will then transfect the viral...
construct in vitro into cell lines with the purpose of studying replication capacity, drug susceptibility and immune recognition of the various viruses.

To facilitate the interpretation of the proposed experiments, we summarise below the main characteristics of the HBV replication cycle (Figure 2). One key feature is that the virus replicates through an RNA intermediate form by reverse transcription, and in this respect it is similar to HIV.

UNABLE TO INCLUDE DIAGRAM (Figure 1)
Figure 1. Schematic representation of the structure of the HBV genome with the four opening reading frames (ORFs) P, S, C, and X. The ORFs encode seven different proteins through use of varying in-frame start codons. ORF P encodes for the polymerase protein; ORF S encodes the three surface proteins; ORF C encodes both the e and core protein; ORF X encodes the X protein. The genome also contains genetic elements which regulate levels of transcription, determine the site of polyadenylation, and mark a specific transcript for encapsidation into the nucleocapsid. The four ORFs transcription are controlled by four promoter elements (preS1, preS2, core and X).

HBV regions contain a partially double stranded 3.2 kb genome termed the relaxed circle DNA (rcDNA). The negative sense strand is complete while the positive sense strand is only partial. Upon infection of cells, the genome is transported into the nucleus where it is converted into a fully double stranded, covalently closed circular DNA (cccDNA). This persists as an epiphasis in the nucleus, a sort of minichromosome that serves as a template for the replication of viral RNAs by the cellular RNA polymerase II. Several transcripts are produced, which are classed as genomic and sub-genomic RNAs. Subgenomic transcripts serve as mRNA for the X and surface proteins. The larger genomic transcripts are longer than one genome in length and serve to produce e, core and polymerase proteins. A particular genomic transcript, lacking the ATG start codon for the e protein, is designated as pregenomic RNA (pgRNA). The pgRNA, in addition to serving as mRNA for the core antigen and the viral polymerase, is selectively packaged into immature viral capsids in the cytoplasm, and reverse transcribed by the viral polymerase to produce the rcDNA genome for progeny virions.

UNABLE TO INCLUDE DIAGRAM (Figure 2)
Figure 2. Schematic representation of the HBV life cycle. The virus particle attaches to the hepatocyte through cellular receptor (1). After translocation to the cytoplasm (2), the nucleocapsid releases the viral genome to the nucleus (3) where it is converted into a covalently-closed circular DNA (cccDNA) to serve as template for viral RNA transcription (4). The viral mRNAs are translated into viral proteins in the cytoplasm (5), and the pgRNA (6) is encapsidated together with the viral polymerase into a capsid made of core protein (7). Upon maturation of the virus particle, DNA is synthesized by the viral polymerase using pgRNA as template (8-9). The newly synthesized nucleocapsid can either initiate a new round of cccDNA synthesis in the nucleus (10), or acquire the viral envelope in the ER/Golgi apparatus with release of mature virions by exocytosis (11).

This mode of virus production does not result in cell death.

Recipient or parental organism

E.coli strains K12 (for example DH5 alpha, HB101, TOP10 and Mach1)
Cell lines - Epithelial cell lines such a HepG2, HepaRG cells, HepG2.2.15, HeLa; T-cell lines such as Jurkat; Embryonic cell lines such as HEK293.

Host/vector system

Cloning vectors: pUC and pCR vectors.
Mammalian expression vectors: pCMV and pSV vectors.

The cloning vectors used for this study will be pUC or pCR vectors. They contain a T7 promoter/priming site as well as f1 and co/E1 origin of replication. They also contain genes that confer resistance to the antibiotics Kanamycin and Ampicillin. They are not known to cause harm to humans. If cloning vectors escaped into environment, their antibiotic resistance gene may potentially be taken up by bacteria making them less susceptible to the antibiotics.

The expression vectors used will be pCMV and pSV vectors. They contain promoters/replication origin sites from cytomegalovirus and SV40 respectively, which are needed to maintain plasmids in mammalian cells and transcribe the inserted genes. They also contain genes that confer resistance to Kanamycin and Ampicillin to allow selection in E. coli and to Gentamicin or Puromycin for selection in mammalian cells. They are potentially able to replicate in mammalian cells and cause the cells to become resistant to the specific antibiotics. They may potentially cause drug resistance if they are taken up by and maintained in bacteria.
Two full-length and full-length plus constructs will be produced for each sample in cloning vectors (pUC or pCR) (Figure 3).

The first two products, pF1 and pF2, corresponding to the full-length 3.2kb HBV DNA genome, are produced with two sets of PCR primers targeting different sites of the HBV genome. The third construct, pHB-1, contains full-length HBV DNA plus a partially repeated sequence ("full-length plus"). Only this full-length plus plasmid is capable in vitro of producing all the viral proteins and RNAs required for assembly of HBV virions.

Individual viral genes (i.e. surface antigen, e antigen, core antigen, x protein, polymerase) will be subcloned into expression vectors. The proteins will be expressed in cell lines.

Some of the products will be modified by SDM.

HBV production does not cause major pathogenic effects on the cells. If samples are not handled appropriately, virus proteins may potentially cause immune reactions.

All plasmid production and purification will occur in the containment level 2 molecular laboratory. All bacterial cultures will be grown in plastic flasks and laboratory health and safety procedures will be followed. Plasmids will be stored in clearly labelled boxes at -20°C.

Hepatitis B Virus

The cloned full-length plus HBV genome will be capable of expressing the proteins and the nucleic acid required for the assembly of HBV particles. The individual gene clones will be able to produce individual proteins, such as HBsAg, e antigen, core antigen, polymerase, x protein. Samples may harbour mutations in certain regions of the genome, either occurring in vivo in the clinical sample under study or introduced SDM. Some of the mutations are expected to modify the susceptibility of HBV to antiviral drugs or immune responses, while others are expected to modify the levels of HBsAg expression and recognition. Some mutations are expected to affect virus fitness i.e. the virus ability to replicate and infect. The mutations that will be studied are those found to occur naturally in infected individuals. In SDM experiments involving the pol gene, it is anticipated that mutations will also indirectly be introduced to the S gene due to overlapping structure of the ORFs.

The likelihood of adverse events after accidental exposure is estimated to be low.

Accidental transfer may occur through percutaneous exposure (e.g., sharp injuries) or membrane exposure (e.g., splash to the eyes) of infectious material. HBV virus particles are quite stable and remain viable for 7 or more days on environmental surfaces at room temperature. The full-length plus HBV sequences and HBV particles are potentially harmful to humans. The outcomes may include acute infection resulting in hepatitis; in a subset of individuals a chronic carrier state may be established. Over several years, a subset of chronically infected individuals will develop complications such as liver cirrhosis and hepatocellular carcinoma.

A number of factors attenuate the risk of adverse outcomes.

Production of infectious HBV by the in vitro system described in this proposal has proved difficult. To date only non-productive infections have been achieved. However, transfection of full-length plus genomes has been shown to produce virions capable of infecting transgenic mice. Thus the possibility of human infection should be taken into account. Clinical samples from HBV infected patients represent a further and more significant potential source of infection.

In the healthcare setting we are very familiar with the management of occupational exposure to HBV and clear protocols are in place. These include minimising the risk of accidental exposure through good laboratory practice (e.g., no sharps, use of personal protective equipment, prompt disinfection of spillages), pre-exposure HBV vaccination, and post-exposure HBV prophylaxis. All people performing the laboratory work have been vaccinated against HBV as per national regulations. Should an exposure occur the laboratory worker has received instructions on how to manage exposed sites by careful washing and first aid kits are available in the laboratory for this purpose. The laboratory worker will then contact the responsible virology consultant immediately (or deputy) for deciding upon post-exposure prophylaxis. This may include
further HBV vaccine dose, HBV Immunoglobulin or antiviral therapy as deemed appropriate by the consultant, who will take into account the circumstances of the exposure, the source of the exposure, and the vaccination history of the recipient to guide the decision. Should infection occur despite these pre- and post-exposure interventions, acute hepatitis may result which may require clinical management but very rarely will result in a risk of mortality. Pre-exposure vaccination and post-exposure prophylaxis, even in the unlikely scenario that they are not sufficient to prevent infection, will attenuate the risk of severe acute hepatitis. In immune competent adults, acute hepatitis B rarely (<1-5%) results in a chronic HBV infection, and the risk is further reduced by the use of pre-exposure vaccination and post-exposure prophylaxis.

Thus, the overall risk of exposure to infectious material is considered to be low and the clinical risk associated to an exposure is considered to be minimal.

As a further potential hazard, individual proteins may cause immune reactions and trigger antibody production.

The full-length plus sequences and virus particles are unlikely to cause harm to species in the environment as HBV has a narrow host range which is limited to humans and chimpanzees; in these species it may cause acute and chronic hepatitis. Humans are the main natural host for HBV. As noted above, HBV virus particles are quite stable in the environment and remains viable for 7 or more days on environmental surfaces at room temperature. The individual proteins have the capacity to cause immune reactions if accidentally transferred to species in the environment.

These risks will be addressed by HBV vaccination of laboratory workers as per national guidelines, good laboratory practice (e.g., no sharps; use of personal protective equipment including laboratory coats, eye protection and gloved, and prompt disinfection of spillages with either 5% Trigene or 70% ethanol and proper disposal of waste). These procedures are covered in the laboratory Health & Safety manual and Standard Operating Procedure (SOP). HBV particles produced in vitro as part of this study will be maintained in the containment level 3 laboratory. It should be noted that the HBV constructs have no or limited ability to establish productive infections (see above).

**Evaluation of foreseeable effects**

The HBV sequences to be used in this study will be derived from plasma and serum of HBV infected patients. The donor organism is pathogenic if not handled appropriately and may cause infection as described above.

The risk of infection and related adverse clinical events is considered to be low.

The HBV sequences that we aim to amplify and express are the cause of HBV infection and mediate disease pathogenesis.

The full-length plus HBV clone is capable of producing virions when expressed in HepG2 cells; therefore the most hazardous step is transfection of the construct into the cell line and transduction with HBV viral supernatants.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste in the Department of Virology is disposed of by autoclaving (Standard autoclave cycle is 135 degrees C, 3150 mBar, 10 minutes). Liquid waste is collected in glass waste bottles and autoclaved. Solid waste is placed in double autoclave bags, autoclaved and then disposed of through approved routes. Details of these procedures can be obtained in the department Safety Manual. Spillages should be absorbed onto paper towels which will be autoclaved and the surface should be decontaminated and cleaned with 70% ethanol.

Waste from the CL3 laboratory (GF478) is additionally treated with the following measures. Virus, infected cells, pipette tips, flasks and all other plastics used will be inactivated in 10% TriGene overnight in disposable jars. Liquid waste, in 105 trigene, is solidified with vernagel prior to removal from the CL3 laboratory. Sealed containers...
and disinfected bags are placed inside metal tins which will be closed and disinfected prior to transport to the autoclave. The autoclaving conditions are checked with 12 thermocouples. The expected degree of kill is 100%

1. Liquid waste including viruses, infected cells, pipette tips, flasks and all other plastics used will be inactivated by Trigene 5% followed by autoclaving.

Bacterial culture liquid waste and contaminated plastics will be autoclaved. Bacterial cultures will be grown in disposable plastic flasks that will be autoclaved after use.

2. The degree of kill for these procedures is 100%

3. The autoclaving conditions follow those applied to the NHS diagnostic guidelines according to the requirements of Clinical Pathologic Accreditation. Standard autoclave cycle is 135 degrees C, 3150 mBar, 10 minutes. The autoclaving conditions are checked with 12 thermocouples.

4. Sealed and disinfected bags placed inside metal tins which will be closed and disinfected prior to transport to the autoclave.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

In consultation with virology experts at the Royal Free, the GMSC approved this work as an Activity Class 3 project

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Project Ref 272/01.1

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Historical Significant Changes
TRANSFERRED FROM GM 272 - 15/1/07

Project Additional Information

Purposes of the contained use

Generate improved Avian Pneumovirus (APV) vaccines by introducing rational changes using infectious clone technology.

Current commercial vaccines have been created randomly by serial passage of field virus isolates in cell culture. The mutations generated appear to be reversible. These vaccines have been shown to lead to disease in commercial poultry, almost certainly as a result of their reversion back to the parent type virus.

The aim of this project is to introduce attenuating mutations using reverse genetics/infectious clone technology. It is anticipated that stable vaccines, incapable of reversion will be generated. This will be by either:

- incorporation of several attenuating motifs (identified from studying existing vaccines and their progenitors)
- deletion of genes demonstrated to attenuate another similar pneumovirus (Respiratory syncytial virus).

Candidate vaccines will be tested in turkeys in the Leahurst Poultry Isolation Unit.

Recipient or parental organism

Avian pneumovirus is in common circulation in commercial poultry, game birds and probably other unidentified avian species. In experimental conditions, APV has been shown to infect only a minority or avian species. Infection of a range of birds (turkeys, chickene, ducks, geese, pheasants, guineafowl and pigeons) resulted in virus only being isolated from the first two (Gough 1988). Experimental infection of the most susceptible avian species known (turkeys) causes mild, non-fatal respiratory disease. Infection in chickens is frequently asymptomatic and game birds infected with APV do not show disease. Wild seagulls have also been found to be seropositive for the virus but disease has not been observed. It is thought likely that other wild birds may be infected but no significant studies have been carried out.

Because of its low pathogenicity, APV is considered to be a category 1 organism (though not yet formally classified). Field strains are treated at this level of containment in UK laboratories (IAH Compton, CVL Weybridge, Intervet Cambs. Vet. Path Liverpool University) and similar laboratories abroad.

With APV infection in commercial turkeys farms, the virus can predispose to secondary pathogens and there can be serious economic losses. With commercial chickens farms disease is rarely seen but reduced weight gain and egg laying statistics suggest that a sub clinical infection probably occurs.

APV is a labile enveloped virus which has a short life in the environment outside of an avian host. Turkeys placed in a room vacated of infected birds did not become infected. It can be demonstrated to transmit to other birds in close contact in experimental conditions. Bird to bird contact has been shown to be the most successful method of virus transfer between birds but aerosol transmission by infected respiratory droplets probably also plays a part. The initial 1980's APV outbreak which spread...
rapidly from East Anglia to Wales, against the prevailing wind suggests that direct contact with personnel and vehicles to be the main mode of transmission. This is supported by the fact that a key hatchery in southern Scotland which has avoided such contact still remains free of the disease, 15 years later.

### Host/vector system

The virus itself is being attenuated hence no host is involved.

### Origin & function

**Origin:** APV field virus.

**Function of modified organism:** Vaccination of turkeys and chickens against disease caused by APV.

### Evaluation of foreseeable effects

The initial work will produce a virus of identical sequence to the field strain and it will therefore have exactly the characteristics of that strain and hence present identical risks.

The modified viruses (the candidate vaccines) would either show reduced pathogenicity or remain unaltered. This has proved the case with similar reverse genetics work with other members of the Paramyxoviridae (e.g. RSV, NDV, Rinderpest). Any viable viruses resulting have all shown reduced or unaltered pathogenicity. Tropism stayed the same or become more restricted.

The risks to the environment would be expected to be the same or less than those encountered when making and testing conventional live attenuated APV vaccines and less than when using field strains (as used routinely in all vaccine challenge models). The changes introduced will either remove virulence factors or introduce attenuating mutations (as identified by sequencing existing conventional live vaccines and their progenitors). In contrast the conventional live vaccines in general use and produced by random mutations, would be likely to introduce larger unknown changes. However such changes have never been observed.

APV causes an acute respiratory infection and has never been shown to or suspected of causing persistent infections. In experimental conditions turkeys remain infected for a short period (approximately 1 week) and it appears likely to be of advantage to the virus to produce maximal disease and virus shedding within that period. Rapid generation of high viral titres and maximum virulence are likely to be the infection characteristics most likely to give the virus a selection advantage. Hence it is likely that current field strains are close to the maximum potential virulence of the organism. Certainly viruses randomly mutated during conventional vaccine development have never been found to increase virulence - rather they have become less virulent or virulence has remained unchanged.

It is likely that vaccines are even less fit to survive in the environment than wild type strains. Conventional vaccine studies employing molecular biological diagnostics have indicated that live vaccines do not persist in the environment. During long term studies on UK turkeys farms, vaccine was only detected after it has been applied to the actual groups of birds, whereas field strains were found to cycle routinely. It is assumed that this reflects the vaccine's reduced fitness compared to better adapted field strains, in general circulation in the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

AVP is labile envelope virus which is not stable in the environment outside of a host. Mild agents such as 70% ethanol readily inactive the virus.

LABORATORY
All manipulations and culture work will be contained within laboratory 1.10. Generated viruses may be moved in sealed containers to en suite freezer facilities for storage.

Manipulations will be performed in purpose installed class 2 cabinet. Areas within the cabinet will be wiped down routinely with Virkon after use.

All infected materials will be transferred to autoclave bags which will be contained within lock top polyethylene bins. The combination will prevent spillage or penetration in the event collision. The combination will be moved to the en-suite autoclave where bags will be transferred and treated.

**ISOLATION UNIT**

Birds will be vaccinated with the GMO then 3 weeks later challenged with field virus to test vaccine efficacy.

The unit contains 8 poultry rooms. Entry to the unit is via a locked door into a robing area where full overalls, head covering and gloves are put on. Boots are dipped in FAM disinfectant. After robing a central corridor is entered and from here there is access to each poultry room via its own anti room. Exhaust air is from each poultry room is HEPA filtered. Footbaths containing FAM disinfectant for boot dipping are in the corridor and inside the entrance of each anti room. Overalls, head coverings and gloves are discarded into FAM disinfectant after use, while still within the outer secure area.

Rooms and anti rooms will be disinfected by Virkon spay before and after each experiment.

Poultry and litter will be bagged and incinerated after each experiment. Both are almost certainly NOT infectious by the experiment finish but, in any event, persisting virus would be wild type field virus (not the GMO).

Drains within poultry areas are sealed for the duration of the experiment and until disinfection has occurred.

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**Project Containment**

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<tr>
<th>Laboratory Activities</th>
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<td>L3 L4 L2 L3 L4 L4 L3 L4</td>
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**Project Ref**: 272/01.2

**Date Ackn'd**: 15/01/2007

**CU2 Project Title**: GENERATION OF FELINE HERPESVIRUS (FHV) DELETION MUTANTS. DELETION OF PUTATIVE VIRULENCE HOMOLOGUES AND IDENTIFICATION OF SUCH BY INSERTION OF A GREEN FLUORESCENT PROTEIN (GFP) EXPRESSION CASSETTE

**Class**: Class 2

**CultureVolClass2**: ≤ 1 Litre

**Class CultureVolClass3-4**: Not Applicable

**Non-GMM Consent Granted**: Not Applicable

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**: TRANSFERRED FROM GM 272 - 15/1/07

**Historical Date of Additional Info**: N

**Significant Change ID**: N

**Date of Significant Change**: N

**Project Additional Information**

**Purposes of the contained use**
To investigate putative virulence homologues of FHV with a view to improve the safety and efficacy of vaccines

**Recipient or parental organism**
FHV is a respiratory pathogen of cats not known to infect non-felids. FHV in which putative virulence homologues have been deleted is expected to show an attenuated phenotype.

**Host/vector system**
FHV/gfp

**Origin & function**
The gfp expression cassette to be inserted has been obtained by PCR from the commercially available vector pEGFP-N1 (Clontech UK Ltd). GFP is a marker protein that fluoresces green and is being used to allow the identification and selection of recombinant FHVs.

**Evaluation of foreseeable effects**
Recipient micro-organism FHV is not listed in ACDP hazard groups 2, 3, or 4. Any recombination (unlikely) would only result in reversion to wild type phenotype. There are no known hazards associated with the inserted gene product and its expression (personal communication, Clontech, attached). GFP has been widely used as a marker in many infectious systems and no harmful effect has ever been demonstrated following insertion of this gene. Deletions of putative virulence homologues or other alpha-herpesviruses have resulted in attenuation of the virus and as FHV is not infective for humans it is highly unlikely that the GMM would be infective for humans. No aspect of the proposed work require specific control measures to safeguard human health. FHV only survives up to 24 hours in the environment and is highly...
susceptable to standard disinfectants. FHV with specific deletions in putative virulence homologues would be expected to be attenuated relative to wild type virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All laboratory waste is placed in autoclave bags and transported to the autoclave in lidded buckets. Waste is autoclaved at a temperature of 121C for 20 minutes. The autoclave is routinely monitored and maintained according to the guidelines laid down by the insurers. After autoclaving, the waste bags are secured in yellow clinical waste sacks and placed in the refrigerated container designated for such waste awaiting collection by the University contractor, for ultimate incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The local genetic modification safety committee has ratified this risk assessment without comment

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 485/02.1

Date Ackn'd 15/01/2007

CU2 Project Title SEMLIKI FOREST VIRUS MEDIATED IL12 IMMUNOGENE THERAPY FOR

Class 2

CultureVolClass2 1-50 Litres

ClassVolumeClass3-4
The aim of the project is to evaluate in malignant human glioma cell lines cytotoxicity, dose response effect, and IL12 secretion of a recombinant Semliki Forest Virus vector (SFV) expressing transgene human IL12.

The recipient micro-organism are immortalised human malignant brain tumour cells (cell lines U87MG, LNZ308, T98).

The vector is based on SFV, an insect alphavirus naturally infecting and replicating in subtropical mosquito species only. The human IL-12 gene has been inserted downstream from non-structural genes of SFV, whose structural genes encoding essential capsid and membrane proteins have been deleted and are provided by a helper vector. Recombinant SFV vectors are produced by recombination in the packaging cell line HEK293.

The inserted cDNA encodes human IL-12, an immunostimulatory cytokine identified as an activator of the cytolytic function of NK cells, antigen-specified cytotoxic T cells, and a potent inducer of IFNy production.

Natural SFV infection only occurs when infected mosquitoes take a blood meal from viraemic vertebrate hosts. There is virtually no risk of natural spread of SFV from one human to another or from an animal to a human in the absence of the competent mosquito vector. There are no known mosquito species susceptible to SFV in the UK and therefore the risk of virus transmission to wildlife is considered negligible. Humans infected with SFV by mosquitoes may develop mild symptoms with uncomplicated and complete recovery. The present SFV virus is not mobilisable because it is replication incompetent. Replication disabled SFV mutants have been produced by deleting structural virus genes, which are provided in trans by a helper vector for recombination in a suitable cell line. This ensures that the recombinant SFV undergoes only one cycle of infection without further replication. In theory, replication competent virus (RCV) may be produced by recombination in the packaging cell line, but the real chance of such recombination is virtually nil because of the multiple deletions in the virus genome. In addition, the SFV vector does not encode resistance to any drug or antibiotic. The human IL-12 gene cannot be incorporated into the virus envelope, and thus the possibility of alteration of tissue tropism or host range of SFV is considered negligible. Furthermore, liposomal encapsulation further reduces natural SFV tropism. All virus preparations will be produced at the facilities of Regulon, Inc., in Palo Alto, CA, USA. Sterile recombinant SFV in suspension is delivered ready to use.
The inserted human IL12 gene does not encode a pathogenic protein as proven by prior in vitro and in vivo data. No harmful biological activity of hIL12 is known.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste which may contain viable SFV vectors, such as used medium, SFV infected cells, and plastic disposable objects (flasks, pipettes, etc.) will be collected and autoclaved. The autoclaved waste will be then subjected to the normal waste management cycle in the laboratory. The autoclave is managed by a contractor company (Priorclave Tactrol). Autoclave testing and quality assurance are performed and recorded yearly. The autoclave is located in the laboratory tract immediately across the GM rooms and run by lab staff.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Reviewed by the University of Liverpool Department of Medicine Biosafety Committee.

Project Containment

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Project Ref 485/03.1

Date Ackn'd 15/01/2007

CU2 Project Title STUDIES OF PKC DYNAMICS (INVOLVING EXPRESSION OF PKC CONSTRUCTS)

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

02/03/2022
Project Additional Information

Purposes of the contained use
PKC has been shown to be a key signalling molecule in pathophysiology of the pancreas. The aim of this study is to examine Ca (2+) dependent/independent translocation of GFP tagged PKC isoforms in living mouse pancreatic acinar cells. A variety of PKC isoforms will be expressed in pancreatic acinar cells using replication deficient adenoviruses.

Recipient or parental organism
During production of replication deficient adenoviruses following Host organisms will be used: E.coli derivatives: XL1 BLUE, DH10B and a cells line QB1-HEK-293 (supplied by Obliogen, Nottingham, UK). All hosts are well characterised, non-pathogenic and are included by Obliogen (Nottingham, UK) in a standard kit for production of replication deficient adenoviruses. Finally replication deficient adenoviruses will be used to transfect freshly isolated pancreatic acinar cells maintained in primary culture. These cells are short lived, and therefore will not be able to survive outside tissue culture conditions and do not present any danger to environment.

Host/vector system
Vestors to be used in this project pEGFP (Clontech, Oxford, UK), pCDNA 3 (Invitrogen, Paisley, UK), pCR259 and pCR276 (Qbiogene, Nottingham, UK) pGEM, pSHUTTLE (Promega, Southampton, UK)
Recombinant PKC - GFP constructs will by produced and incorporated into virus transfer vectors. The production of replication deficient adenoviruses will require standard E. coli derivatives (XL1 Blue, DH10B) and HEK-293 cell lines.
All viral vectors contain necessary components for Tn7 transposition events. Viral vectors: pCR259, a 4488bp adenoviral transfer vector containing coding sequences for Ampicillin resistance, Tn7 transposition CMV promoter, origin of replication and a multiple cloning site. Transpose-Ad 294 vector is a 38275 bp vector containing the adenoviral genome (minus regions E1-3 needed for replication) and coding sequences for Tn7 transposition.

Origin & function
E. coli derivatives (XL1 Blue (Stratagene California, USA), DH10B (Qbiogene, Nottingham, UK) and QB1-HEK-293 cell lines will be purchased from Obliogen (Nottingham, UK) other vectors (see above) will be purchased from Clontech (Oxford UK), Invitrogen (Paisley, UK) and Promega (Southampton, UK). PKC encoding vectors, PKC-GFP encoding vectors and/or virus transfer vectors of these constructs and/or E1-E3 deficient recombinant viral plasmids will be received from collaborating laboratories. There are a few laboratories that developed PKC-GFP constructs (eg laboratory of Professor Saito in Tohoku University in Japan, Laboratory of Professor R. Rizzulto in University of Ferrara in Italy, Laboratory of Professor T Mayer in Stanford University, USA). We will receive constructs from one or more of these highly reputable laboratories. The overall aim of the project is to elucidate the role of PKC activation in pathophysiology of acute pancreatitis. A variety of PKC isoforms from different sources will be...
expressed in pancreatic acinar cells using replication deficient adenoviruses.

The cells will be stimulated by substances that could potentially be responsible for triggering acute pancreatitis (bile acids, ethanol and high doses of calcium releasing secretagogues). The activation/translocation of PKC isoforms will be monitored using fluorescence microscopy.

**Evaluation of foreseeable effects**

The PKC constructs encode functional regulatory proteins. This necessitates containment level 2. The constructs are linked to non-hazardous reporter proteins (eg GFP). Viruses containing constructs of interest could potentially be able to infect human cells. However these viruses would not be able to replicate in human cells. The recombinant adenoviruses made with Transpose-Ad expression system kits are replication deficient viruses that have deletions in the E1 and E3 regions; they will not replicate in cells other than complementing cells (QB1-HEK-293).

The E.coli derivatives to be used are disabled, non-pathogenic in man and considered ACDP group 1. The DNA inserted plasmid is eukaryotic in origin, contains a promoter which does not encode for a protein and hence is not hazardous, the marker proteins (eg green fluorescent protein) have no known hazardous effects. Eukaryotic genes delivered are unlikely to give a selective advantage to the E. coli host and it is unlikely that they would be processed in bacteria correctly as they contain eukaryotic signals eg for mRNA processing.

Established mammalian cell lines require defined media and conditions for growth, and therefore are unable to survive outside these conditions.

Isolated pancreatic acinar cells are short living cells, and unable to survive more than 24 hours after isolation.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Both liquid and solid GM waste produced during the stages of virus production including and following transfection of QB1-HEK293 cells will be autoclaved to achieve 100% kill prior to disposal. Autoclaving will be done at the end of each working day when contained work has been carried out. The autoclave is managed by laboratory staff and maintained by Priorclave engineers. The autoclave is services and calibrated every half year by Priorclave engineers. The autoclave is located in the laboratory suite (room 1.05a in Henry Wellcome Gastro Laboratories). Both liquid and solid waste will then be sent for incineration.

Solid waste produced at earlier stages of the virus production (including bacterial culture plates, disposable plastic pipettes, pipette tips and microfuge tubes) will be disposed of into autoclave bags in dedicated bins for regular autoclaving. Autoclaved waste will be later incinerated. Disposable culture vessels and other contaminated glass and plastic-ware will be soaked in disinfectant overnight, then sent for incineration. Waste media produced before stages of virus production (before transfection of QB1-HEK 293 cells) will be chemically disinfected (1% Vircon overnight) prior to disposal in dedicated sinks. Re-useable glass and plasticware will be disinfected overnight or autoclaved. Benches will be disinfected routinely before and after procedures and at the end of each day.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

02/03/2022
On its meeting held on 27 January 2003 the Genetic Manipulation Committee of Departments of Physiology and Human Anatomy and Cell Biology of The University of Liverpool considered application for contained use of PKC constructs and replication deficient adenoviruses containing PKC constructs. The Committee considered that containment level 2 is appropriate for stages of laboratory work involving transfection of HEK 293 cells, maintenance of these cells in culture, isolation of viruses and transfection of pancreatic acinar cells. These procedures will be conducted in Henry Wellcome Gastro Laboratories facilities shared by Department of Medicine and Department of Physiology, located on the premises of Department of Medicine. Other stages of experimentation will require containment level 1. The Genetic Manipulation Committee considered and approved Risk Assessment and waste disposal procedures for this project.

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**Project Ref** 554/00.1

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<td>TYPE III SECRETION KNOCK-OUT MUTANTS IN BURKHOLDERIA CEPACIA</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Tick if you are claiming exemption from disclosure for section of the risk assessment Y
### Project Additional Information

#### Purposes of the contained use

The level of containment use is required for the handling of B. cepacia. In this proposal, the GM B. cepacia strains represent no greater risk to human health or the environment than do the parent strains.

#### Recipient or parental organism

The recipient organism is a ubiquitous environmental bacterium. It is also an opportunistic pathogen, particularly important with patients with cystic fibrosis. The GMOs produced in this project will not represent any greater threat than the parental organism.

#### Host/vector system

The vectors to be used are pKNOCK vectors, pDM4 and pCVD442, all plasmids incapable of replication in B. cepacia. If the experiments are successful, sequences introduced into these plasmids will be incorporated into the B. cepacia genome by homologous recombination. The plasmid pBBR1Tp will be used to re-introduce functional genes in order to complement mutations.

#### Origin & function

The intended functions of the introduced genetic material is to replace functional genes with genes disrupted or "knocked out" by the insertion of antibiotic resistance markers. By knocking-out genes required for functional type III secretion it will be possible to further elucidate the role of such a secretion system in the pathogenicity of B. cepacia. The antibiotic genes chosen do not confer resistance to antibiotics normally used for the treatment of B. cepacia infections. In the B. cepacia strains to be used, there are levels of intrinsic resistance to these antibiotics that would render them useless for therapeutic purposes. The experiments will use selection for high-level resistance.

#### Evaluation of foreseeable effects

The GMOs will probably not be as capable of causing disease as the parental organisms, since our purpose is to knock-out possible virulent genes. However, this has yet to be demonstrated experimentally. The GMOs do not represent a greater hazard than the parental organisms and will be treated in the same way, at containment level 2. B. cepacia is already widespread in the environment and would therefore not represent an environmental threat. Careful handling in the laboratory should ensure that there is no risk of infection to laboratory workers.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures of GM B. cepacia, and all consumables used in the handling of the GMOs will be subjected to autoclaving and incineration following the normal procedures for disposal of clinical waste. The effective degree of kill is 100%. The efficiency of the autoclaving is monitored annually by the multi-point thermocouple checks during an annual service and inspection. External validation of the pressure vessel certificate also occurs annually. In addition, a chart recorder produces a record of each run to confirm effective killing.

---

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
The genetic modification safety committee met on 3rd November 2000 and considered that the work proposed represented no greater hazard than working with wild-type B. cepacia. It was agreed that permission should be sought for work to go ahead at containment level 2 (activity class 2).

Project Containment

Laboratory Activities

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Human Clinical Applications

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Project Ref 554/01.1

Date Ackn'd 11/06/2001

GM VIRUSES EXPRESSING HERPESVIRUS GENE PRODUCTS

Class 2

Culture Vol Class 2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

The aim of this experimentation is to transfer the genes encoding herpesvirus gene products to cells in tissue culture, including professional antigen presenting cells, to investigate the immune response to such antigens and their potential immune evasion properties. Transduced target cells will be used in cytotoxicity assays with epitope-specific cytotoxic T lymphocytes (CTL). It is planned to utilise two different GM viral vector systems to express the same panel of foreign genes. This will enable specific target cells to be infected with the optimal vector system and allow the research to be carried out in a comprehensive manner. The two vector/host systems are (1) Replication-defective genetically modified adenovirus (Ad5) vectors and (2) vaccinia viruses, based on the western reserve (WR) strain. These will be used to achieve specific and efficient gene transfer of the inserted herpesvirus coding sequences.

Recipient or parental organism

Adenoviruses

The vectors will be constructed based on adenovirus serotype 5 (Ad5) which is an ACDP Hazard Group 2 pathogen. Ad5 infects humans, in healthy adults it does not normally cause overt disease, and most adults will have been exposed to one of the common subgroup C viruses (includes Ad5) by adolescence. Ad5 primarily infects the respiratory tract, although it is shed via the gut and can probably replicate therein. Live enteric vaccines (for serotypes 2, 4, 5 and 7) have been used successfully in adults. The vectors used in this work are replication-defective by virtue of E1-deletion, and so are regarded as safer than the wild-type virus. The genetically modified adenoviral vectors are designed to result in efficient expression of the herpesvirus genes in transduced cells, in the absence of expression of adenovirus gene products. Adenoviruses are relatively stable at room temperature and are naturally infectious to the respiratory tract as aerosols.

Vaccinia viruses

The vectors will be constructed based on vaccinia virus western reserve (WR) strain, which is an ACDP Hazard Group 2 pathogen. Vaccinia virus is a large DNA virus, with a coding capacity of over 175kb and contains approximately 150-200 genes, many of which are essential for productive virus infection. Routes of natural infection with wild type vaccinia virus include vaccination (application of freeze dried virus preparation to abraded skin area) and contact with skin lesions produced by vaccination. In the laboratory, vaccinia may be transmitted to workers by ingestion, injury with contaminated sharps, and droplet or aerosol exposure of mucus membranes or broken skin. Vaccinia infection results in local pustular lesions at site of infection. It may cause severe disease in individuals with active skin disorders such as eczeme or psoriasis, or in immunocomprised individuals. Acute conjunctivitis may occur after inoculation into the eye but permanent eye damage is rare. Eczema vaccinatum may occur in people suffering from eczema. Infection may spread rapidly producing deep seated pustular lesions in affected skin. Disseminated vaccinia necrosum can occur in immunosuppressed or immunodeficient individuals.

Host/vector system

Adenovirus vectors

The vectors to be used in this work will be replication-defective, genetically modified human adenovirus type 5 (E1-deleted) viruses. They are regarded as safer than the wild-type virus. If accidental inoculation were to occur, the viral DNA would be introduced into a number of cells giving transient, high level expression of the transgene over a period of a few days to weeks (possibly months). However, the GM virus will be replication-defective and following an accidental inoculation, further spread would not occur. The recombinant virus will not be capable of autonomous spread in the event of accidental operator infection. In the unlikely event that an operator were to receive an 'infectious dose' of virus via the respiratory tract, it would only lead to replication of the GM virus if they had a concurrent infection with wild type virus, and the same cells took up both the wild type and GM viruses. The risk of double infection with recombinant and wild type virus is considered to be very low.

Vaccinia vectors

Vaccinia virus is a large DNA virus, with a coding capacity of over 175kb and contains approximately 150-200 genes, many of which are essential for productive virus infection. However, a number of genes are dispensable for growth, in vitro, and are suitable insertion sites for foreign genes. Vaccinia virus WR will be the host strain used either a the wild type strain for insertion of foreign genes into the thymidine kinase (TK) locus or a strain deleted for the VP37 gene (designated vRB12). Insertion of
foreign genes in to the TK locus results in the generation of viruses with a TK-minus phenotype, which have been shown to be attenuated compared to wild type virus in mice. However, this should not be taken to imply a lower virulence in man. The VP37 gene encodes an envelope protein which is essential for the envelopment and egress of extracellular virus particles, thus deletion of this gene prevents production of extracellular virus and blocks plaque formation in standard viral plaque assays. Using the vRB12 virus (VP37-deleted) as the host vector foreign genes are inserted into the VP37 locus in conjunction with a wild type VP37 gene, thus rescuing the VP37 deletion and restoring a wild type (WR) phenotype.

Origin & function

Epstein-Barr virus

Epstein-Barr virus (EBV) is a B-lymphotropic y-herpesvirus that is carried as a life-long infection by the vast majority of individuals in all communities. Despite the asymptomatic nature of EBV infection, the virus has potent cell growth transforming potential for its principal target cells, the B lymphocyte, and experimental infection of B cells in vitro leads to the establishment of permanently growing B lymphoblastoid cell lines (LCLs) which carry EBV as a latent infection. In healthy EBV-positive individuals the persistent viral infection is controlled by immune mechanisms, in particular cytotoxic T cell surveillance. All of the eight EBV proteins expressed in a latently infected B cell (EBNAs 1, 2, 3A, 3B, 3C, -LP and LMPs 1 and 2) elicit CTL responses, as do all of the lytic antigens studied to date (eg BMLF1, BMRF1, BRLF1, BZLF1). EBV induced transformation requires the co-operative action of at least five of the latent antigens (EBNAs 1, 2, 3A, 3C and LMP 1).

Latent antigens:

EBNA 1: exhibits sequence specific DNA binding activity, binds to the origin of plasmid replication on the viral genome and is essential for the cell cycle-depenent replication of the viral episome in the transformed cell: EBNA 1 may act as a transcriptional activator in addition to its genetic maintenance function.

EBNA 2: essential for transformation, a transcriptional transactivator with no demonstrable sequence-specific DNA binding activity. EBNA2 has the capacity to transactivate both viral and cellular gene expression.

EBNA3A: essential for transformation, a presumed transcriptional transactivator with no demonstrable sequence-specific DNA binding activity.

EBNA3B: no known function, no known activity in in vitro assays, and is dispensible for B cell transformation.

EBNA3C: essential for transformation, a presumed transcriptional transactivator with no demonstrable sequence-specific DNA binding activity.

EBNA-LP: required for optimal transformation. EBNA-LP deleted viruses still have transforming capacity for resting human B cells but their efficiency of transformation is drastically reduced. It enhances the transcriptional transactivator function of EBNA2.

LMP1: the major transforming protein of the virus with multiple phenotypic effects in human cells. LMP1 has transformin function in the conventional Rat 1 assay system, and in certain 3T3 cell assays. However, expression of LMP1 in human cells (eg the SCC 12F epithelial cell assay) induces phenotypic changes but does not convert the cells to malignancy.

LMP2: dispensable for B cell transformation, with no known function in conventional rodent cell transformation assays. This protein is thought to interfere with virus reactivation from latency to lytic cycle in B cells by blocking signalling events at the latently infected cell membrane.

Lytic antigens: EBV lytic cycle genes are expressed at the onset of EBV lytic cycle and expression follows a temporal and sequential order. Lytic gene products are required for full activation of the lytic cycle and are required for lytic gene expression, DNA replication, morphogenesis and egress of virus particles. The following are examples of the lytic genes to be expressed.

BMLF1: a post-transcriptional activator of gene expression.
BZLF1: transactivator of lytic gene expression, DNA binding protein.

BRLF1: a post-transcriptional activator of gene expression.

gp350: expressed during the lytic cycle, it is of the most abundant glycoprotein present on the viral envelope. Mediates viral infection of B cells through binding to the viral receptor CD21.

gp85: expressed during the lytic cycle, has significant homology to the HSV-1 glycoprotein gH. It is a relatively minor component of the envelope which is important in fusion of virus and cell membranes.

Lymphocryptoviruses (LCV)

Lymphocryptoviruses are γ-herpesviruses closely related to EBV, which naturally infect Old World Primates. In vivo LCV infection in non-human primates resembles EBV infection in humans. Sequence analysis of the genomes of both H. Rhesus (Macaques) and H Papio (baboons) has identified close homologues of virtually every EBV gene, both latent and lytic antigens.

EBNA1 homologues: EBNA1 proteins are very well conserved in both H. Rhesus and H Papio. Episomal maintenance and transcriptional transactivation functions are conserved, as are the presence of internal repeat domains.

Human herpesvirus 8 (or KSHV)

Human herpesvirus 8 (or KSHV), is a γ-herpesvirus initially identified in Kaposi's sarcoma tumor cells. The target cells of HHV8 have not been clearly identified, although B-lymphocytes and endothelial cells can be infected with low efficiency in vitro.

LNA-1: HHV8 latency associated nuclear antigen 1 (LNA-1) is involved in maintaining the HHV8 episome in infected cells, where it is thought to tether the viral genome to chromatin during mitosis.

Murine herpesvirus 68

Murine herpesvirus 68 (MHV-68), is a γ-herpesvirus which is a natural pathogen of small rodents. Primary infection occurs in lung epithelial cells, after which a latent infection is established in B lymphocytes.

ORF73: No function has yet been identified for the ORF73 gene product.

Herpesvirus saimiri

Herpesvirus saimiri (HSV) is a γ-herpesviruses which can establish specific replicative and persistent infections in primate hosts. In the natural host, squirrel monkeys, HVS infection causes an asymptomatic infection.

ORF73: No function yet identified for the ORF73 gene product. Shown to encode a 64kDa nuclear protein.

Herpes simplex virus
Herpes simplex virus (HSV), a member of the herpesvirus family, is a common human pathogen which first infects mucosa, before spreading via sensory neurons into ganglia where a life-long latent infection is established. During periods of latency, HSV is relatively resistant to host immune responses. This can be attributed to the fact that during latency HSV infects neuronal cells which lack MHC class I molecules and to limited viral gene expression. Upon viral reactivation/lytic cycle HSV infects epithelial cells and may come under immune surveillance.

ICP46: encoded by an immediate early gene, ICP47 is a small protein which binds to the cytosolic face of the transporter associated with antigen processing (TAP), thereby preventing translocation of antigenic peptides from the cytosol into the ER.

**Evaluation of foreseeable effects**

**Adenovirus**

Ad5 infects humans and is classified as an ACDP group 2 pathogen. In healthy adults it does not normally cause overt disease, and most adults will have been exposed to one of the common subgroup C viruses (includes Ad5) by adolescence. The vectors used in this work are replication-defective by virtue of E1-deletion, and so are regarded as safer than the wild-type virus. The adenovirus vectors are able to enter human cells, which may lead to the expression of the reporter genes. Integration of the viral DNA into the chromosomes of the host cell is not a part of the normal life cycle of the virus, and would be a very rare event. The viral DNA is likely to be lost from the infected cells over a period of days to a few weeks; this may be faster in replicating cells, or in vivo where immune responses against the foreign gene products or against viral proteins expressed at a low level may eliminate the infected cells. The recombinant virus will not be capable of autonomous spread in the event of accidental operator infection. Any exposure to recombinant virus would be likely to elicit an immune response to the incoming virus, and as the major capsid protein (hexon) is the major neutralising antigen, this would be primarily a type 5 response. Many individuals have pre-existing immunity to Ad5 and a secondary immune response to the recombinant virus would be expected.

It is not thought that the recombinant viruses would pose a serious risk to plants or animals. There is no evidence that human adenoviruses cross species barriers and so a replication defective virus could not be maintained in non-human hosts. It is not anticipated that insertion of foreign genes into the adenoviral vector would broaden the range of host species, nor increase the stability of the viruses in the environment.

**Vaccinia virus**

The GM modified viruses are replication competent and the host virus is classified as an ACDP Hazard Group 2 pathogen. There is no reason to suspect that the inserted genes will increase the potential hazards, nor will have any attenuation effect. However, it must be considered that vaccinia virus may cause severe disease to humans who have skin disorders or to immunocompromised individuals.

Vaccinia virus has a broad host range which includes animal species such as cattle, cats, rodents, pigs and rabbits, therefore presents the possibility of infection of these animals if accidentally released. However, there is no evidence that vaccinia can become established in the wild and there appears to be no natural animal reservoir of the virus.

It is not thought that the recombinant viruses would pose a serious risk to plants.

It is not anticipated that insertion of foreign genes into the vaccinia virus would broaden the range of host species, nor alter it pathogenic properties.

**Inserted genes:**

EBV induced transformation requires the co-operative action of at least five latent antigens (EBNAs 1, 2, 3A, 3C and LMP1). However, when expressed individually only two have transforming potential.

The EBV LMP1 gene when expressed alone may pose an oncogenic risk to human cells. However, even in the most sensitive assay of human epithelial cell transformation...
available in vitro (where for instance activated ras molecules induce efficient malignancy), LMP1 alters the cellular phenotype but does not confer a malignant phenotype on the cells. The EBV EBNA1 gene has been shown in one report to induce lymphomas in transgenic mice when expression was directed to the B cell compartment. This has not been confirmed in further independent studies. Importantly, vaccinia virus infection of cells ultimately leads to cell death and lysis of infected cells. Therefore, transformation of the infected cell is unlikely to occur. Furthermore, Ad5-based vectors are unable to infect B lymphocytes to any significant level in vitro and thus potential infection of B lymphocytes in vivo as a consequence of accidental exposure of the operator to the GM virus would appear very low. It is also worth noting that in healthy EBV-positive individuals the persistent viral infection is controlled by immune mechanisms, in particular cytotoxic T cell surveillance. All of the above EBV latent proteins can elicit CD8+ CTL responses. With the notable exception of EBNA1, all of the latent antigens are efficiently processed by the MHC Class I antigen processing pathway. We therefore consider it likely that cells expressing individual EBV latent proteins from a recombinant viral vector would be immunogenic to the T cell system in vivo. The specific issue of T cell recognition of EBNA 1 is one of the questions which the present experimental protocol is designed to address. Immunity to vaccinia virus per se (both humoral and cell mediated) will also play a role in the immune control of any accidental infection of laboratory workers. Thus cell infected with a vaccinia expressing EBNA1 will be subject to immune control by anti-vaccinia immunity.

LCV EBNA1-homologues are reported to be close homologues to EBV EBNA1 and thus for the reasons stated above are unlikely to alter the existing traits of the GM viruses.

HHV8 LNA-1 has been reported to interact with p53 and pRB, thus may have a potential role in transformation, although this is not yet been shown. In the case of these GM viruses for the same reasons as stated for the EBV potential transforming proteins, it is not expected LNA-1 will alter the pathogenic traits of either Ad5 or vaccinia.

It is not known if the ORF73 proteins from MHV-68 or HVS have any transforming potential. It is not expected that expression of these gene products will lead to additional hazards.

The question of whether the ORF73 homologues can be targets for CD8+ CTL is one of the aspects being investigated.

HSV ICP47 is expressed as an immediate early gene product, which can bind to the cytosolic portion of human TAPs thus preventing transport of antigenic peptides. This block in the normal MHC class I antigen processing pathway will lead to reduced surface expression of MHC class I molecules at the cell surface. This will ultimately prevent recognition of infected cells by CTL. However, it should be noted that HSV-specific CD8+ CTL can be generated from PBMC of HSV infected individuals and that, target cells infected with ICP47 +ve recombinant HSV vectors expressing a foreign antigen can be recognised by antigen specific CD8+ CTL in cytotoxicity assays. However, as the level of expression of ICP47 from the GM viruses may be somewhat higher than from the HSV, it may be expected that a CTL response to the GM virus may be reduced as a result of the ICP47 protein. Nevertheless, the recombinant virus should still be controlled by the host's humoral immune response, including antibody-dependent cellular cytotoxicity (ADCC). Furthermore, an MHC class I negative cell is likely to be targeted by natural killer (NK) cells, which are known to control HSV infection in vivo. It has also been shown that production of IFN and the consequent up regulation of TAP genes can overcome the ICP47 mediated block. This may be relevant in vivo.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For both types of GM viruses 1% Virkon has been tested and shown to be effective. Additionally 1% SDS have been tested on adenovirus and 70% ethanol on vaccinia virus and shown to be effective. Solid virus containing waste is bagged and placed in a designated metal container which is removed to the autoclave. Liquid virus waste is discarded to bottles which are placed in a designated metal container for autoclaving. Any sharps and pipettes are steeped in disinfectant (1% Virkon) for a minimum of 20 minutes before disposal in an approved sharps box. The disinfectant is discarded down the sink. Any glassware used in the virus room is steeped overnight in 1% Virkon.
The genetic modification safety committee met on 17th May 2001 and discussed this proposal. There was some discussion whether it was appropriate to notify two vector systems using the same form but the committee decided that this was a valid approach. The committee considered that the work proposed was no greater than class 2 activity.

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee met on 17th May 2001 and discussed this proposal. There was some discussion whether it was appropriate to notify two vector systems using the same form but the committee decided that this was a valid approach. The committee considered that the work proposed was no greater than class 2 activity.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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Project Ref 554/01.2

Date Ackn'd 11/06/2001

Date Project Ceased

GM EPSTEIN-BARR VIRUSES AND EBV+VE HUMAN CELL LINES

Class 2

Consent Granted not applicable

Project notified under transitional arrangements N

Historical Significant Changes
### Project Additional Information

#### Purposes of the contained use

To generate recombinant Epstein-Barr virus genomes (R-EBV) which contain variants of the Epstein-Barr Nuclear Antigen (EBNA) 1 gene. The R-EBVs will be based on the B95.8 prototypic laboratory strain of EBV. EBV transformed human B lymphocytes (lymphoblastoid cell lines, LCLs) will be generated using both R-EBVs and the B95.8 prototype strain. The EBNA1 protein from the prototypic laboratory strain (B95.8) contains an internal repeat of approx. 250 amino acids, comprising of exclusively glycine and alanine residues (the GAR domain). EBNA1 variants containing different sized GAR domains have been generated in plasmid constructs. The aim of this proposal is to transfer these EBNA1 constructs into the EBV genome for analysis of immune recognition of R-EBV transformed human B lymphocytes (LCLs) by EBNA1-specific CD8+ cytotoxic T lymphocytes (CTL). These R-EBVs will also express a reporter gene (E-GFP) and the selectable marker neomycin. Wild type B95.8 strain EBV transformed human B cells (LCLs) will also be generated as controls for the above experiments.

#### Host/vector system

The R-EBVs will be based on the B95.8 prototypic laboratory strain of EBV.
The neomycin resistance is gene from the Tn5 transposon and confers resistance to the drug G418, allowing positive selection of cells containing the EBV episome. Green fluorescent protein (GFP) is a naturally fluorescent protein from the jellyfish Aequorea victoria which fluoresces bright green upon exposure to UV light. GFPs are becoming widely used as reporter genes.

**Evaluation of foreseeable effects**

EBV is widespread in human populations with up to 90% of adults carrying the virus as a persistent infection. EBV is naturally transmitted by the oral route via salivary contact. There is no evidence of aerosol spread. In the laboratory there is the risk of direct infection of blood lymphocytes in the event of accidental inoculation of virus-producing cells or of virus containing culture supernatant into the bloodstream of the operator via a cut or wound. However, the potential for growth of EBV-positive cells in vivo following accidental self-inoculation is extremely unlikely, further such an occurrence has never been reported in the literature.

Innoculation of cells from in vitro maintained EBV-positive human cells lines into the operator would almost certainly elicit a vigorous cytotoxic T cell response against allogeneic HLA class I antigens expressed on the cell surface. In the case of B cell lines, an additional response would also be generated against HLA class II antigens since such lines express class II as well as class I antigens. Such allogeneic responses are quantitatively much stronger (i.e. involve a greater frequency of responder cells within the circulating T cell pool) than responses against conventional foreign antigens such as virus-encoded proteins. Hence the allogeneic cell population would almost certainly be destroyed in vivo.

There is no difference expected in terms of pathogenic potential between wild type EBV and R-EBV. The presence of reporter genes or of drug resistance genes in the EBV genome is very unlikely to influence the fate of viral infection.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

1% Virkon has been tested on EBV and shown to be effective. Solid virus containing waste is bagged and placed in a designated metal container which is removed to the autoclave. Liquid virus waste is discarded to bottles, which are placed in a designated metal container for autoclaving. Sharps and pipettes are steeped in disinfectant (1% Virkon) for a minimum of 20 minutes before disposal in an approved sharps box. The disinfectant is discarded down the sink. Any glassware used in the virus room is steeped overnight in 1% Virkon before removal for normal tissue culture wash up processing.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee met on 17th May 2001 and considered that the work proposed was no greater than class 2 activity.

**Project Containment**
Project Ref 554/02.1

Date Ackn'd 18/12/2002

CU2 Project Title KNOCK-OUT MUTATION OF POTENTIAL VIRULENCE GENES IN PSEUDOMONAS AERUGINOSA

Class 2

CultureVolClass2 < 1 litre

CultureVolumeClass3-4

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
The contained use is required for handling P. aeruginosa regardless of whether the bacterium is genetically-modified or not.

Recipient or parental organism
The GMO and the wild-type parent are opportunistic pathogens that do not represent a significant risk to healthy individuals so long as they are handled properly, under containment level 2. There would be a greater risk associated with anybody who has cystic fibrosis. The GMOs do not represent any greater risk than the parental organisms.

Host/vector system
Vectors to be tried include the pKNOCK system, and various plasmids that carry the sacB counter-selectable marker. All of these plasmids are incapable of replication in...
P. aeruginosa. Plasmid pBBR1Tp would be used to complement mutations by re-introducing cloned genes into P. aeruginosa. A plasposon-based system for the introduction of transposon-based DNA to create random mutants in P. aeruginosa may also be used. The integration is stably maintained because the transposon lacks the necessary transposase after integration has occurred and cannot therefore transpose again. In each case antibiotic resistance markers for chloramphenicol, kanamycin, tetracycline or trimethoprim will be introduced into P. aeruginosa.

Origin & function

The intended function of the introduced genetic material is to replace functional genes with genes disrupted or “knocked out” by the insertion of antibiotic resistance markers. By knocking out functional genes we will be able to study the role of such genes in virulence-related functions such as adherence or cytotoxicity. The DNA will be introduced from disabled E. coli strains using the vector systems described. Cloned P. aeruginosa DNA will come either from a cosmid library or be synthesised using PCR amplification.

Evaluation of foreseeable effects

The GMOs will represent no greater hazard than the parent organism. Indeed, if we have knocked-out genes that are important in virulence, they may actually be less hazardous. The P. aeruginosa parent will acquire antibiotic resistances against antibiotics that are not of therapeutic use. P. aeruginosa occurs naturally in environments such as soil and water, but the clinically-adapted strains that we are using are likely to be less able to compete in such environments than those occurring there naturally. There are no foreseeable effects that suggest any GMO requires any greater care in handling than the parent organism, which requires containment level 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures of GM P. aeruginosa, and all consumables used in the handling of the GMOs, will be subjected to autoclaving and incineration following the normal procedures for disposal of clinical waste. The effective degree of kill is 100%. The efficacy of the autoclave is monitored annually by multi-point thermocouple checks during an annual service and inspection. External validation of the pressure vessel leading to the issue of a pressure validation certificate also occurs annually. In addition, a chart recorder produces a record of each run to confirm effective killing.

Is an emergency plan required according to regulation 20?  
Y

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The Departmental Genetic Modification Safety Committee met on 27 September 2002 and considered that the work proposed represented no greater hazard than working with the wild-type organism. It was noted that the proposal was very similar to a previously notified activity using Burkholderia cepacia.

Project Containment
### Project Additional Information

**Purposes of the contained use**

Biomedical Research. The contained use is required for handling parental viruses regardless of whether they are genetically-modified or not.

**Recipient or parental organism**

Murine herpesvirus type 4 (MHV-4); Herpes simplex virus type 1 (HSV-1); Varicella-Zoster Virus (VZV); vaccinia virus; disabled murine retrovirus.

**Host/vector system**

Recombinant herpesviruses (MHV4, HSV1, VZV) and replication defective derivatives.

Vaccinia virus WR strain.

Disabled murine retroviruses based upon either murine stem cell retrovirus or Maloney murine leukaemia virus.
For recombinant herpesviruses, the recombinants will be based upon laboratory strains of the viruses contained in bacterial artificial chromosome clones. The intended function of the introduced genetic material is to replace functional genes with genes disrupted or "knocked out" either by point mutation or by the insertion of marker genes such as the jelly fish green fluorescent protein. This enables the study of the functions of the disrupted genes.

For recombinant vaccinia and defective retroviruses the viruses are based on laboratory strains and the inserts will be genes derived from the above herpesviruses. The aim is to study the functions of the proteins by expression in mammalian cell culture systems.

**Evaluation of foreseeable effects**

For the recombinant herpesviruses and vaccinia virus, the GMOs will represent no greater hazard than the parent organism. Indeed, if we have knocked-out genes that are important in virulence, they may actually be less hazardous. There are no foreseeable effects that suggest any GMO requires any greater care in handling than the parent organisms, which require containment level 2.

For recombinant disabled retroviruses, some of the inserts derived from herpesviruses will be transforming to cells in culture and are therefore potential oncogenes. The recombinants are replication defective and are therefore unable to multiply and spread after initial infection. In addition the likelihood of infection is low, being however, these vectors will be infectious to human cells and the direct introduction of viruses containing potential oncogenes represents a potential hazard greater than the parental organism. Retroviruses containing such inserts therefore require containment level 2.

**Origin & function**

For recombinant herpesviruses and vaccinia virus, the GMOs will represent no greater hazard than the parent organism. Indeed, if we have knocked-out genes that are important in virulence, they may actually be less hazardous. There are no foreseeable effects that suggest any GMO requires any greater care in handling than the parent organisms, which require containment level 2.

For recombinant disabled retroviruses, some of the inserts derived from herpesviruses will be transforming to cells in culture and are therefore potential oncogenes. The recombinants are replication defective and are therefore unable to multiply and spread after initial infection. In addition the likelihood of infection is low, being however, these vectors will be infectious to human cells and the direct introduction of viruses containing potential oncogenes represents a potential hazard greater than the parental organism. Retroviruses containing such inserts therefore require containment level 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures of GM viruses, and all consumables used in the handling of the GMOs, will be subjected to autoclaving and incineration following the normal procedures for disposal of clinical waste. The effective degree of kill is 100%. The efficacy of the autoclave is monitored annually by multi-point thermocouple checks during an annual service and inspection. External validation of the pressure vessel leading to the issue of a pressure validation certificate also occurs annually. In addition, a chart recorder produces a record of each run to confirm effective killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Departmental Genetic Modification Safety Committee met on 27 September 2002 and considered that, for herpesvirus and retrovirus recombinants the work proposed represented no greater hazard than working with the wild-type organism and that for retrovirus recombinants the proposed level of containment was reasonable.
Project Additional Information

Purposes of the contained use

The contained use is required for handling wild-type E. coli regardless of whether the bacterium is genetically-modified or not.

Recipient or parental organism

The work is part of an investigation into the interactions between the colonic epithelial cell and mucosa-associated Escherichia coli isolated from Crohn's disease and colon cancer (Martin et al 2004). The work is a collaboration between the Medical Microbiology Department at Duncan Building and the Gastroenterology Group, School of Clinical Sciences, Henry Wellcome Laboratories [GM485]. The mucosa-associated E. coli isolates lack conventional virulence genes but have adhesive/invasive properties. The GMOs do not represent any greater risk than the parental organisms. Indeed the purpose of the GM work is to make mutations in genes associated with adhesive/invasive properties or cytotoxicity. The role of the mucosal E. coli in disease, if any, remains unclear, but they will be treated as recommended for non-VTEC E. coli (ACDP hazard groups 2).
Host/vector system

We will use the E. coli strain SM10 (pir) as a source of the mini-Tn5phoA donor plasmid pUTKml. We also propose to keep the option of using a similar plasposon based random mutagenesis system. In addition, we propose to make a number of specific mutations in genes with possible roles in adhesion/invasion, or cytotoxicity. For this we will use the method described by Datsenko et al (2000) as used by Barnich et al (2004) in which a targeted sequence containing the gene of interest is replaced with a selectable antibiotic resistance gene (kanamycin) generated by PCR using primers with 50-nucleotide extensions that are homologous to regions adjacent to the gene of interest. The inactivated DNA will be introduced by electroporation into target E.coli strains (colonic epithelial cell and mucosa-associated Escherichia coli isolates) transformed previously with plasmid pKOBEG (or a similar vector), encoding red proteins from bacteriophage lambda. An alternative strategy will involve using pKNOCK vectors carrying resistance genes for chloramphenicol, tetracycline and kanamycin (Alexeyev 1999). The mutants will be complemented to demonstrate that any phenotypic variation is due to the specific gene inactivation. This will involve reintroducing intact cloned genes into the mutants using standard plasmid cloning vectors. The antibiotic resistance markers will be restricted to chloramphenicol, tetracycline, kanamycin or ampicillin.

Origin & function

Mutants will be screened for loss of the ability to cause haemagglutination of human group O red blood cells. In addition, we propose to make a number of specific mutations in genes with possible roles in adhesion/invasion, or cytotoxicity. The effect of these inactivations will then be assessed using as endpoints red cell haemagglutination, adherence to, and invasion of, intestinal epithelial cells, intracellular localisation and multiplication within epithelial cells, expression of flagellae and pili. In addition, we will study the action of mutant E. coli strains on surrogate markers for inflammation and development of colonic malignancy including COX2 activation (overexpressed in malignancy), NFkB activation and IKK degradation (tumour promotion), disruption of apoptosis (malignant cell survival), VEGF expression (angiogenic potential) and proinflammatory cytokine expression.

These will be introduced into the chromosome. The intended function is to cause mutation of activities that may have a role to play in virulence.

Evaluations of foreseeable effects

Random mutagenesis can lead to inadvertent up-regulation of genes. As stated previously, these strains appear to lack the conventional virulence genes carried by EPECs and other pathogenic groups. However, there is a small risk that an unknown virulence-related gene may be up-regulated. Such mutants will not be retained following the screen for reduced haemagglutination and/or adherence/invasion/cytotoxicity. Any alteration of pathogenic traits in the specific knock-08tmmutations is likely to be making the strain less pathogenic since the aim is to "knock-out" genes that might contribute to pathogenicity. There are no foreseeable effects that suggest GMOs would require any greater care in handling than the parent organism, which requires containment level 2.

The organism may be hazardous but lacks known virulence genes. Antibiotic resistance genes may be introduced into the organism and it is theoretically possible that they might be passed on to other bacteria. Antibiotics unsuitable for therapeutic use will be used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Duncan Building:
All cultures of GM E. coli, and all consumables used in the handling of the GMOs, will be subjected to autoclaving and incineration following the normal procedures for disposal of clinical waste. The effective degree of kill is 100%. The efficacy of the autoclave is monitored annually by multi-point thermocouple checks during an annual service and inspection. In addition, a chart recorder produces a record of each run to confirm effective killing.

Henry Wellcome Laboratories:
Solid waste (tips, tubes etc.) are placed in a pot with a sealable lid and taken for autoclaving and incineration. Disposable cell-culture plastic vessels will be soaked in...
disinfectant (1% Virkon) overnight, autoclaved and then sent for incineration. Waste media will be chemically disinfected (1% Virkon) overnight prior to disposal in disinfectant. Benches will be disinfected routinely before and after experimental procedures and at the end of each working day. Autoclaving (to achieve 100% kill) will be performed within the working laboratory (NUF1.05A) at the end of each working day when contained work has been carried out. The autoclave is managed by laboratory staff and maintained by Priorclave engineers (calibrated and serviced on a 6 month basis).

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<tr>
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<th>Glass Houses</th>
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**Project Containment**

Project Ref 554/07.1

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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>Culture Volume Class 2</th>
<th>Culture Volume Class 3-4</th>
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<td>15/05/2007</td>
<td>Over-Expression and Knockdown Studies of Key Genes Implicated in Repair And Regeneration of Orthopaedic Tissues.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Non-GMM Consent Granted: Not Applicable
**Project Additional Information**

**Purposes of the contained use**

We are interested in the processes involved in tissue regeneration and repair, and the mechanisms by which we can manipulate the healing process to enhance repair. This work involved characterising and manipulating various aspects of cell biochemistry in order to understand the mechanisms of these processes. We will use commercially available lentiviral and retroviral systems to mediate stable over-expression and knock down of key genes that are implicated in the processes that occur subsequent to tissue injury, as well as those involved in the repair process.

**Recipient or parental organism**

Packaging cells will be used to produce both lentiviral and retroviral particles. These particles will then be used to transduce our cell line of interest (The immortalised human juvenile costal chondrocytes (T/C-28a2) and primary cultures of a variety of mesenchymal cells, in particular chondrocytes). These pseudotyped viruses are both replication defective and the probability of reversion to wild-type is negligible. For more information please refer to the attached risk assessment form.

**Host/vector system**

Retroviral system: The plasmids used for retroviral gene delivery and expression are derived from the Moloney murine leukemia virus (MMLV). To generate infectious virus particles the retroviral transfer vector will be transfected into commercially available packaging cell lines containing chromosomally integrated expression cassettes for viral proteins Gag, Pol, and Env, which would result in the formation of pseudotyped virus particles.

Lentiviral system: pKLO.1-puro vector transfer plasmids will be co-transfected into packaging cells along with the pCMV-VSV-G envelope vector, and a packaging vector (MISSION Lentiviral Packaging Mix, Sigma), the identity of which is proprietary. For more information of host cells please refer to the attached risk assessment form.

**Origin & function**

Small interfering RNAs (siRNAs) expressed from short hairpin RNAs (shRNAs) are used to mediate gene specific RNA interference (RNAi) in mammalian cells. shRNAs are encoded by short sequences (~20-60 bp) specific to genes of interest and are supplied in commercially available lentiviral or retroviral transfer vectors. Gene over-expression will be mediated by full length gene sequences amplified by PCR and cloned into the multiple cloning sites of commercially available viral transfer vectors. Lentiviral system: The lentiviral packaging plasmid retains the Gag, Pol, and Rev protein encoding sequences from HIV-1. The VSV-G sequence in the envelope vector derived from the vesicular stomatitis virus. The pKLO.1-puro plasmid only retains the HIV-1 viral sequences necessary for reverse transcription, RNA packaging, RNA transport (RRE), and cDNA integration into the hosts chromosome.

Retroviral system: A third generation retroviral system will be utilised in this study. The plasmid vectors used for retroviral gene delivery and expression are derived from MMLV. Vectors contain the bacterial origin of replication and ampicillin-resistance gene, an extended MMLV packaging signal, and a multiple cloning site (MCS) located between the MMLV 5’ and 3’ long terminal repeat sequences (LTRs).

In both systems, once packaging cell line has been transfected with the necessary vectors, pseudotyped viral particles are produced. The supernatant is then used to infect target cells. Upon infection, the viral RNA molecule is reverse transcribed by reverse transcriptase (which is present in the virion particle), and the gene of interest, flanked by the LTRs, is integrated into the host DNA. Because the vector itself does not express viral proteins, once a target cell is infected, the LTR expression cassette is incapable of another round of virus production.

02/03/2022
Three systems utilise pseudotyped viruses, which increases the possibility of transduction of human cells at high titre. Utilisation of SIN vectors attenuates the potential risk of insertional mutagenesis that would otherwise exist for these vectors. The specific safety features of third generation retroviral systems significantly reduces the likelihood of replication competent viral (RCV) generation, by increasing the number of recombination events that are required to reconstitute a competent viral genome. For additional information please refer to attached risk assessment form.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be aspirated into Virkon and disposed of in an appropriate manner. Used plastic and glassware will be treated with Virkon and autoclaved promptly.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The LGMSC were in agreement with the proposed Activity class/risk assessment.

Project Containment

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<tr>
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<td>Large Scale Activities</td>
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<td>L2 L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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</table>

Project Ref 554/08.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
Lentiviral-mediated expression of proteins involved in membrane trafficking and cytokinesis in mammalian cells.

The aim of this work is the quantitative transduction of gene products in mammalian cell lines (human telomerase immortalized retinal pigmented epithelium — hTERT-RPE1, mouse NIH3T3 fibroblasts, and dog kidney polarized epithelial cells - MUCK) with the purpose of studying the effects of protein expression resulting from these gene products on membrane trafficking and cytokinesis in these cells. Protein expression will be achieved via lentivirus transduction of gene products encoding the proteins of interest (some of which may be oncogenes).

Recipient or parental organism

Mammalian cell lines (eg human telomerase immortalized retinal pigmented epithelium — hTERT-RPE1, mouse NIH3T3 fibroblasts, and dog kidney polarized epithelial cells - MDCK). They are auxotrophic and require growth conditions that would not be satsisfied outside the laboratory cell culture facility and thus have extremely limited survival in the environment.

Host/vector system

We will use the ViraPower T-Rex Lentiviral Expression System (InVitrogen). This system facilitates highly efficient delivery of a target gene or RNA to dividing and non-dividing mammalian cells using a replication-incompetent lentivirus. Based on the lentikat system developed by Cell Genesys (Dull et al. J. Virol. 1998 72:8463-8471), the ViraPower Lentiviral Technology possesses features which enhance its biosafety while allowing high levels of expression in a wider range of cell types than traditional retroviral systems. See attached assessment for details.

Origin & function

The inserted DNA sequences code for proteins involved in the regulation of membrane trafficking and cytokinesis. The genes to be investigated fall into two categories: (i) membrane traffic, the 63 Rab GTPases and the family of 40 GTPase-activating protein regulators; (ii) cytokinesis, the Aurora B, INCENP/Borealin/Survivin, Cit, and Piki mitotic kinases, protein phosphatases Cdc4, PP1 and PP2A, the microtubule binding protein PRCI, anillin and septin scaffolding molecules, RhoA, the centralspindlin Rho regulator complex, microtubule severing enzymes of the katanin family, and the kinesins KIF2OA, K1F23, KIF4A, and KIF14. While some of these DNA sequences may code either for oncogenes, or modulators of cell growth, none of the DNA sequences code for toxins.
Insertion of the gene into the target ceN lines will be accomplished using lentiviral-mediated transduction. With this procedure accidental exposure as a result of needle sticks will be minimal because sharps will not be used. However, in the case of accidental exposure it is unlikely that the inserted gene would result in the formation of a neoplasm. Although some of the genes used may code for oncogenes, carcinogenesis is a multifaceted process that requires change both within the cell and to the cell microenvironment. Therefore, it is unlikely that the inserted gene would satisfy these requirements. Moreover, immune surveillance will likely remove any cells which begin to express high levels of the gene product. In end effect, the chance for harmful biological effect to manifest itself as a result of accidental exposure is minimal and likely equivalent to other transfection techniques.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste will be treated with a final concentration of 0.1% Virkon S overnight prior to disposal down the sink. Solid waste will be autoclaved and then sent for incineration. The autoclave dedicated to waste disposal is routinely serviced and runs will be logged. Liquid spills will be treated with 1% Virkon S prior to clean up and disposal as solid waste. Surfaces will be wiped with 10% ethanol in liquid form. Virus inactivation by 0.09% Virkon S solutions has been demonstrated to result in a 6-log decrease in titres of purified and unpurified virus within 5 minutes. The use of 0.1% Virkon S with overnight incubation is therefore an effective means to inactivate viruses.

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<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
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<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
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### Project Containment

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<th>Growth Rooms</th>
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**Project Ref** 554/08.2

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<td>13/03/2008</td>
<td>Novel renal proximal tubular pathways of injury: Angiotensin II induces fibrosis via Angiotensin II</td>
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<th>CultureVolClass2</th>
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<tr>
<td></td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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**Non-GMM**

Consent Granted

Not Applicable

Project notified under transitional arrangements

- N

**Historical Significant Changes**

Withdrawn

- N

Tick if notifying a connected programme of work

- N

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

This work will investigate the pathological effects and functions of Angiotensin type 1 and type 2 receptors, Ang 4 and Mas receptors in promoting fibrosis in response to stimulation by AngiotensinII. The project involves the use of short hairpin RNAs (shRNAs) to prevent the mRNA expression of RAS target genes. In primary human renal proximal tubular cells (hPTC; Cambrex) and immortalised hPTC line (HK-2 cells; ATTC). The cells will be transfected with the respective shRNAs using lentivirus plasmid vector pLKO. 1-Puro alone or packed as lentiviral transduction particles.

**Recipient or parental organism**

DH5 and HB101 bacteria, HK-2 cells and primary hPTC are recognised as non-colonising and disabled and are equivalent to ACDP hazard group 1. The likelihood of the GMMs actively causing harm is negligible. The non-pathogenic and the inserted gene products are not expected to increase the fitness of the organism. The potential to cause harm to animals and plants is negligible. The GMMs are unlikely to survive in the environment due to the requirement for auxotrophs for survival which are unlikely to be satisfied in the environment.

**Host/vector system**

The pLKO.1 vector is a lentiviral (HIV)-based plasmid which integrates into the host genome. The vector is regarded as a Risk Group Level 2 (RGL-2) 1 material and safe to use due to its modified features (deletion of a number of accessory genes implicated in the virulence of HIV, minimal genome of the viral particles, non-replicating and self-inactivation features) making it incapable of producing virus once infected into the host cell. Potentially, the lentiviral particles are capable of infecting human cells.

**Origin & function**

For the purpose of this project, specific shRNAs are designed to bind in a complementary way mRNA of target members of the human Renin-Angiotensin System (RAS). This will lead to the degradation of the target mRNAs and will abolish the protein expression of these genes.
The shRNA sequences are cloned into pLKO.1 vector to facilitate the transfection of the cells and stable expression of the shRNAs. The vector is a lentiviral (HIV) based plasmid which integrates into the host genome. As stated above, the vector safe to use due to its modified features (deletion of a number of accessory genes implicated in the virulence of HIV, minimal genome of the viral particles, non-replicating and self-inactivation features), making it incapable of producing virus once infected into the host cell.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The procedure outlined for level 2 containment will be sufficient and so no unusual procedures should be necessary. It is standard school of clinical science practice that all tissue culture work is carried out at biological safety level 2. No sharps will be used during transformation/ transfection procedures, nor in the subsequent culturing of the cells.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The waste material containing viable GMMs including spent culture fluid and other media will be chemically inactivated (Virkon) as per manufacturer's specifications. The hard waste will be packed accordingly and autoclaved.

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

The GMSC were in agreement with the proposed activity classifications.

Project Containment

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02/03/2022
Knockout mutation of putative virulence genes in Escherichia coli associated with urinary infections, and a study of the effects on ureteric contractility

The work is part of an investigation to study the interaction of bacteria with the surface channels and receptors of human and rat ureters. The work is a collaboration between Prof Susan Wray and Dr Theodor Burdyga (School of Biomedical Sciences, Division of Physiology) and Dr Craig Winstanley (School of Infection and Host Defence, Division of Medical Microbiology). This will be achieved by making mutations in urinary pathogenic E.coli (UPEC)-specific genes and studying the effects of such mutations on ureteric contractility. The genetic manipulation of E.coli strains will take place within the School of Infection & Host Defence, with the contractility assays taking place in Physiology. The recipient bacteria are the most widely studied UPEC strains J96, 536 and CFT073, each of which were isolated from a patient with pyelonephritis. The GMM's do not represent any greater risk than the parental organisms and therefore will be treated as ACDP hazard group 2.

We will make the knockout mutants by using the method described by Datsenko & Wanner (2000) (PNAS USA 97:6640) as used by Barnich et al (2004) (Infect Immun 72:2484) in which a targeted sequence containing the gene of interest is replaced with a selectable antibiotic resistance gene (kanamycin or possibly tetracycline) generated by PCR using primers with 50-nucleotide extensions that are homologous to regions adjacent to the gene of interest. The inactivated DNA will be introduced by electroporation into target E.coli strains transformed previously with plasmid pKOBEG encoding red proteins from bacteriophage lambda. The genes to be targeted for mutation include genes encoding CNF1 and LPS production. The effect of gene inactivation will then be assessed using incubation with rat or human ureter tissue and isolated cells followed by contractility measurements and confocal imaging. Mutants will be complemented by cloning gene back using the plasmid vector pHSG575 (carries chloramphenicol resistance).

In addition, we propose to introduce a green fluorescent protein expressing plasmid vector (pBRD940; Wigley et al. 2001 (Infect Immun 69:7873): carrying ampicillin resistance) into a UPEC strain (J96,536 and CFT073) in order to monitor the location of bacteria following incubation with ureter samples.
The GMMs will be uropathogenic E.coli with additional kanamycin resistance. These will be knockout mutants, and may be less pathogenic than wild-type UPEC, but they should be considered as potentially pathogenic (in the same way as the wild-type strains). Other GMM's will be UPECs containing low copy number plasmids (pHSG575) for complementation, or GFP-containing plasmids (pBRD940). The added genetic material will be either antibiotic resistance genes or a green fluorescent protein expressing plasmid. The antibiotic resistance genes chosen are not themselves harmful. GFP is not harmful and will be introduced in order to monitor the location of bacteria following incubation with ureter samples.

Evaluation of foreseeable effects

The recipient bacteria are the most widely studied UPEC strains J96, 536 and CFT073, each of which was isolated from a patient with pyelonephritis. The antibiotic resistance genes are not themselves harmful and GFP is not known to be harmful. Tetracycline, chloramphenicol and kanamycin are not antibiotics generally used in the treatment of E.coli infections.

The risk for human health is the same as with wild-type UPEC. Any alteration of pathogenic traits in the specific knock-out mutations is likely to be making the strain less pathogenic since the aim is to "knock-out" genes that might contribute to pathogenicity. The most likely infection route is gastrointestinal however, they lack the necessary virulence genes to cause enteropathogenic infections, but they could conceivably become part of the normal flora. There are no foreseeable events that suggest GMMs would require any greater care in handling than the parent organism which are very well characterised UPEC that require containment level 2 handling. The infectivity and host-range of the GMM can be assumed to be comparable to that of wild-type UPECs.

Origin & function

The GMMs will be uropathogenic E.coli with additional kanamycin resistance. These will be knockout mutants, and may be less pathogenic than wild-type UPEC, but they should be considered as potentially pathogenic (in the same way as the wild-type strains). Other GMM's will be UPECs containing low copy number plasmids (pHSG575) for complementation, or GFP-containing plasmids (pBRD940). The added genetic material will be either antibiotic resistance genes or a green fluorescent protein expressing plasmid. The antibiotic resistance genes chosen are not themselves harmful. GFP is not harmful and will be introduced in order to monitor the location of bacteria following incubation with ureter samples.

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Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Duncan Building:
All cultures of GM E.coli and all consumables used in the handling of the GMMs will be subjected to autoclaving and incineration following the normal procedures for disposal of clinical waste. The effective degree of kill is 100%. The efficacy of the autoclaves is monitored quarterly by multi-point thermocouple checks. Autoclaves are subjected to an annual service and inspection. External validation of the pressure vessel leading to the tissue of a pressure validation certificate also occurs annually. In addition, a chart recorder produces a record of each run to confirm effective killing.

Physiology laboratories:
Small solid waste (tips, tubes etc) will be collected in an autoclave biohazard bin and taken for autoclaving and incineration. Disposable cell-culture consumables will be soaked in 1% Virkon disinfectant overnight and autoclaved prior to incineration. Waste media will be chemically inactivated in 1% Virkon overnight prior to disposal. Cell culture hoods and benches will be disinfected routinely before and after experimental procedures and at the end of each working day. Autoclaving will be monitored using TST (Time, steam, temperature) test strips in each run (Albert Browne Ltd, TST class 6 emulating indicator 121 degrees C for 20 mminutes). The autoclave is validated by annual thermocouple mapping and maintained by Priorclave engineers annually.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
The Division of Medical Microbiology and GU medicine Genetic Modification Advisory Committee met on 13th November 2008 and considered that the work proposed represented no greater hazard than working with the wild-type organism. The Department of Physiology Genetic Modification Advisory Committee met on 3rd February 2009 and approved the risk assessment.

**Project Containment**

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Animal Units

Laboratory Activities

Animal Units

Large Scale Activities

Human Clinical Applications

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<td>Class 2 &lt; 1 Litre</td>
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Induction of pluripotent stem cells from primary human keratinocytes using nucleofection to deliver reprogramming genes

Project Ref 554/09.2

Date Ackn'd 13/10/2009

CU2 Project Title

Induction of pluripotent stem cells from primary human keratinocytes using nucleofection to deliver reprogramming genes

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
**Purposes of the contained use**

The generation of induced pluripotent stem (iPS) cells from adult somatic cells may potentially provide a patient-specific source of pluripotent stem cells creating significant clinical potential. The use of iPS cells for therapeutic purposes is at present hampered due to the use of potentially harmful retroviral vectors involved in their production.

The aim of this study is to investigate non-viral methods of producing iPS cells, namely nucleofection. The cells of interest will be keratinocytes isolated from normal adult humans and the study will involve cotransfecting these cells with plasmid DNA using a nucleofector device, manufactured by Amaxa, in order to stimulate iPS cell production.

**Recipient or parental organism**

Human epidermal keratinocytes isolated from normal human adult donors, which have been tested for mycoplasma, Hepatitis B, Hepatitis C and HIV-1 viruses and do not pose any risk to human health and safety.

**Host/vector system**

Plasmid DNA, which will be constructed commercially and arrive ready for transfection. Each vector will contain the open reading frame of the desired genetic material within a PCMV6 vector. This vector contains a CMV promoter for mammalian expression of the inserted genetic material, the human growth hormone polyA signal is located downstream of the insert and the SV40 ori for replication in mammalian cells. It also contains the ColE1 ori, which is the bacterial origin of replication and the f1 ori, which is the filamentous phage origin or replication allowing the recovery of single stranded plasmids. Each vector also contains an antibiotic resistance gene for isolation of successfully transfected cells and fluorescent tag.

**Origin & function**

The following genes are involved in regulating the undifferentiated state of embryonic stem cells. Through transferring these genes to somatic cells the cells intrinsic regulatory network can be overcome allowing the cells to revert back to a pluripotent, undifferentiated state.

- **Oct4**, transcript variant 1. Accession number: NM_002701.4
  This transcription factor is essential to maintain the self renewal of undifferentiated embryonic stem cells and is associated with a pluripotent phenotype.

- **Sox2**. Accession number: NM_003106.2.
  This is another transcription factor that works in conjunction with Oct4 in maintaining the self renewal of undifferentiated embryonic stem cells and is also associated with a pluripotent phenotype.

- **Kruppel-like factor 4 (Klf4)**. Accession number: NM_004235.3
  This transcription factor normally functions in differentiating epithelial cells but can act as both an oncogene by repressing p53 and also as a tumour suppressor.

**Evaluation of foreseeable effects**

The nucleofector device will be used to deliver each of the three genes to the nucleus of human epidermal keratinocytes. Cells that are successfully co-transfected with each of these genes will undergo reprogrmming and revert to an undifferentiated phenotype. Even in combination with the nucleofector solution and optimised nucleofection program, which aid entry of these genes to the nucleus it is predicted that the likelihood that these cells will receive all three reprogramming genes will be around 1% using optimised conditions, therefore when considering the risks associated with humans being exposed to each of these three genes the likelihood that they would receive all three is extremely unlikely and coupled with the stringent containment measures that will be put in place we feel the risks to human health and safety are minimal.

Along with the direct effects of reprogramming and maintaining an undifferentiated state these genes may interact with a large number of other genes affecting a wide range of cellular functions, previous studies have used the same combination of genes and have not shown any unusual effects therefore it is unlikely they pose any additional risks should humans be exposed to these genes. It should also be noted that whilst absorption of plasmid DNA through intact skin is unlikely it is possible [Kang.

The majority of the inserted genetic material will pose no risk to human health and safety however one of the proposed genes, Klf4 is capable of producing oncogenic proteins and must be given due consideration. This investigation utilises an optimised electroporation method, which transfers the genetic material to the cell nucleus however the efficiency of this gene transfer is likely to be low. When considering the risks to human health and safety and the likelihood of this gene transfer is likely to be low. When considering the risks to human health and safety and the likelihood of this genetic material being transferred to human cells through direct contact we have found this to be extremely unlikely due to the poor mobilisation of the proposed vector. As we are aware of the potential effects of gene transfer containment measures will be put in place to minimise any risk to human health and safety.

There are also significant barriers that will prevent the transfer of inserted genetic material to the environment. In order for transfer to occur there has to be a release of functional DNA, persistence of the DNA then uptake by competent organisms. The lack of homology between eukaryotic and prokaryotic DNA makes transfer of genetic material to bacteria highly unlikely. Most bacteria in the environment are not normally in a competent state and those that are have low transformation efficiencies. The possibility that there would be an adverse environmental impact is even more remote since assuming that this highly unlikely transformation even did occur there would have to be expression of the protein and the expression would have to confer a selective advantage otherwise the genetic material would be lost due to random mutation or deletion. In combination with the stringent containment measures that will be followed I do not foresee any potential hazards.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste, such as plastic disposables, will be placed in leak proof container containing 1% Virkon solution for 24 hours then autoclaved on cycle 1, which reaches 134 degrees celcius and lasts for 2 hours. Once autoclaved all waste will be disposed of in a yellow clinical waste incinerator bag and incinerated.

All fluid waste, such as cell culture medium, will be emptied into Virkon 1% solution and also autoclaved on cycle 1. Once work has been carried out using the designated class II safety cabinet this cabinet will be dismantled and disinfected with Virkon 1% solution then washed down twice with 70% ethanol and subjected to formaldehyde bomb fumigation to remove any waste.

Virkon manufacturer's data states it is a bactericidal and fungicidal disinfectant suitable for killing bacteria, spores and viruses from hard surfaces such as an experimental equipment and safety cabinets.

The autoclave manufacturer states that all autoclaved material will be sterilised and free from bacteria and viruses giving 100% kill.

The formaldehyde bomb manufacturer states all microorganisms will be killed post fumigation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form N
Feedback has been incorporated into this final risk assessment and accepted by the committee.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<td>L2 Yes</td>
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<td>L3 L4 L2 L3</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
</tr>
</tbody>
</table>

**Project Ref**  554/12.1

- **CU2 Project Title**: Production and use of replication-incompetent adeno-associated viruses (AAV) containing human genomic DNA as a method of altering endogenous gene expression

- **Class**: Class 2
- **Culture Volume**: < 1 Litre
- **Consent Granted**: Non-GMM
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

<table>
<thead>
<tr>
<th>Significant Change ID</th>
<th>Date of Significant Change</th>
</tr>
</thead>
</table>

**Project Additional Information**

**Purposes of the contained use**

We will clone fragments of human genomic DNA encompassing specific exons of our genes of interest or a protein tag, along with a selection marker, into the pAAV vector. The plasmid will then be transfected into HEK293T cell line along with the pHelper packaging and pAAV-RC envelope vectors. The transfected cells will release...
replication-incompetent AAV particles into the supernatant and this virus will be used to infect human cell lines in order to generate recombinant cell lines. In these cell lines, the endogenous exons of our genes of interest will be replaced by the ones encoded in the designed pAAV vector resulting in either

- exon loss to knock-out gene function
- introduction of a SNP/small deletion/small insertion into the endogenous gene or
- a tagged version of the endogenous gene using a reporter gene e.g. luciferase.

**Recipient or parental organism**

AAV vector plasmid will be propagated in bacterial strains of E. coli, such as:
- XL-1 blue (Invitrogen)
- DH5alpha (Invitrogen)
- SCS110 (Stratagene)
- JM109, JM110 (Promega)

Viral vectors will be propagated in mammalian cell lines from the following list; all cell lines are from ATCC/CRUK mammalian cell collections unless otherwise stated.

- Only fully authenticated, long-established cell-lines posing minimal risk will be used.
- HeLa Human cervical adenocarcinoma cells
- AAV-293 cells Human embryonic kidney cells optimised for producing AAV vector
- HEK293T Human embryonic kidney cells
- K562 Human chronic myeloid leukemia cells
- HepG2 Human Hepatocellular Carcinoma cells
- DU145 Human Prostate carcinoma cells
- LNCap Human Prostate carcinoma cells
- HT29 Human Colonic Epithelial cells
- PANC1 Human Pancreatic carcinoma cells
- SUIT-2 Human Pancreatic carcinoma cells (Mark Boyd laboratory, Liverpool)
- MCF-7 Human Breast Carcinoma
- MCF-10 Human Breast Carcinoma
- H1975 human lung adenocarcinoma cells
- H1680 human lung adenocarcinoma cells
- A549 human lung adenocarcinoma cells
- SW48 human colonic epithelial cells
- MCF10a human breast cancer cells
- NIH 3T3 NIH Mouse embryonic fibroblasts
- MIA Paca-2 Pancreatic carcinoma cells
- BxPC-3 Ovarian carcinoma cells
- AsPC-1 Pancreatic carcinoma cells
- Capan-1 Pancreatic carcinoma cells
- Capan-2 Pancreatic carcinoma cells
- FAMPAC Pancreatic carcinoma cells
- SW837 Rectal cell line
- SW480 Colonic cell line
- HPDE6-E6E7 pancreatic ductal cells

The following isogenic cell lines expressing an oncogene or with loss of tumour suppressor may be subject to further modifications:

- X-Man (Horizon Discovery; http://www.horizondiscovery.com/) isogenic MCF 10a human breast cancer cells and SW48 human colonic epithelial cancer cells expressing wither a single mutant PI3Kalpa (1047R), PI3Kalpa (E545K), EGFR (deleted E746-A570) or K-Ras (G12V), a double mutant of PI3Kalpa (H1047R) & EGFR
(delE746-A750) or finally MCF10a cells null for PTEN.

SW837 Rectal cell line
SW480 Colonic cell line
HPDE6-E6E7 pancreatic ductal cells

The following isogenic cell lines expressing an oncogene or with loss of a tumour suppressor may be subject to further modifications:
X-Man (Horizon Discovery; http://www.horizondiscovery.com/) isogenic MCF10a human breast cancer cells and SW48 human colonic epithelial cancer cells expressing
either a single mutant P13Kalpha (H1047R), P13Kalpha (E545K), EGFR (deleted E746-A750) or K-Ras (G12V), a double mutant of P13Kalpha (H1047R) & EGFR (del
E746-A750) or finally MCF10a cells null for PTEN.

Host/vector system

pAAV-MCS, pHelper and pAAV-RC vectors from the Stratagene Helper-Free AAV system will be used to make AAV-2 viral particles.

A synthetic plasmid equivalent to pAAV-MCS but containing the homology arms of the target genomic DNA surrounding a Neomycin resistance selection cassette will be
made for transfection into the AAV-293 cells

Origin & function

PCR-generated protein-coding regions from mammalian genomic DNA for intracellular proteins involved in endocytosis, signalling, oncogenesis, tumour suppression, and
the Kennedy pathway. These include kinases and phosphatases that respectively add or remove phosphate groups from other proteins or lipids. GTPases that are
GDP/GTP binding molecular switches acting as adaptors for the recruitment of other signalling proteins. Ubiquitin modifying enzymes that add or remove ubiquitin - a major
post-translational modification in all signalling networks. Enzymes involved in lipid biosynthesis and lipid modifications . Cytoskeletal proteins important for regulating cell
organisation, cell division and membrane trafficking. Transcription factors involved gene expression and other DNA binding proteins. None of the DNA sequences code for
toxins.

Evaluation of foreseeable effects

Viral vector and host bacteria:
Wild type AAVs are not categorised by the Advisory Committee on Dangerous Pathogens (ACDP). AAVs are defective by nature (i.e. need a helper virus to replicate). They
are not associated with human illnesses, although a possible link between AAV and spontaneous abortion has been made. The hazards to human health can be expected
to be low. The AAV system we are using (Stratagene Helper-Free AAV system) is a 'gutless' system which does NOT require wild-type adenovirus to supply helper
functions.
The AAV system that will be used is AAV serotype 2 virus, over 80% of the human population is serotype positive illness. Transmission may be via direct needle stick,
aerosol, faecal-oral route or direct conjunctival injection.
The main hazard associated with AAV vectors arises from the inserted genetic material.
The pAAV-MCS plasmid containing the two homology arms each targetting approx 2kb of genomic DNA and transfected into AAV-293 cells together with helper plasmids
(pHelper and pAAV-RC) to enable replication and packaging of AAV-2 viral particles.
Bacterial strains, such as DH5alpha E. coli will be used for DNA propagation.

Mammalian cells that will be modified:
We will be modifying endogenous genes cloned from mammals encoding signalling, transcription, tumour suppressors, oncoproteins and trafficking proteins. Some of our
proteins of interest are known proto-oncogenes (theGTPase Ras and the protein and lipid kinases Raf, EGFR, Met, PI3K, Akt) or tumour suppressors (p53, APC, Rb,
PTEN). NB. The majority of proteins that we study have no known oncogenic potential however they are subject to the same precautions in our laboratory as the known
proto-oncogenes.
The genetic material inserted into the AAV vector will be of 3 types:
1. For exon loss to knock-out functions. Genetic material: Genetic DNA surrounding the region to be targetted and a selection cassette to knock-out gene function.

2. Introduction of a SNP/small deletion/small insertion into the endogenous gene. Genetic material: Genomic DNA encompassing the region to introduce the SNP/small deletion/small insertion of interest and a selection cassette.

3. For a tagged version of the endogenous gene using a reporter gene eg. Luciferase, GFP, HaloTag. Genetic material DNA encompassing the region to be targetted, a reporter gene and a selection cassette.

In every case we will not be inserting the complete gene from the endogenous locus. This technology targets the endogenous gene locus and therefore we only need to target the region of interest.

It is possible that the AAV will be able to exist episomally within the cell, this is a short lived phenomenon typically lost within the first 2 passages of cell culture. Drug selection for positive clones is only initiated after the first passages to prevent selection for false positives derived from episomally expressed Neomycin resistance. No cDNA will be used and the AAV2 viruses will be incapable of expressing any part of the genomic DNA that they carry. The human pGK promoter is 3’ to the LH arm targeting the endogenous gene. The promoter is only there to enable expression of the drug resistance cassette eg. For Neomycin resistance and will not drive expression of any part of the target gene.

**Direct effects:**

Insertion of a gene into the target cells will be accomplished via AAV-mediated transduction. With this procedure accidental exposure as a result of needle sticks will be minimal because sharps will not be used. However in the case of accidental exposure it is unlikely that the inserted gene will result in the formation of a neoplasm for two reasons:

1. We are using fragments of DNA to target parts of exons within endogenous genes via targeted homologous recombination. This is an inefficient process with >0.01% successful targeting per virion therefore the vast majority of infections result in insertions outside the target area generating non-functional/non-coding DNA Integration.

2. Although some genes that we will target may code for oncogenes or tumour suppressor genes (TSGs) carcinogenesis is a multifaceted process that requires changes both within the cell and to the cell microenvironment. Therefore it is unlikely that the inserted genes would satisfy these requirements. The chance for harmful biological effect to manifest itself as a result of accidental exposure is minimal and likely equivalent to other transfection techniques.

**Effects of gene transfer:**

For each of the 3 types of inserted genetic material the hazards are as follows:

1. Exon-loss to knock out functions.

This will have very few hazards with it as we typically removing gene function. If the gene targetted is a TSG (eg. PTEN or p53) there may be an effect on the cell characteristics depending on the particular gene.

Worst case scenario: if an end-user were to infect themselves it is possible that the targeting construct could remove the function of the endogenous gene in the infected cells. However it would only target one allele of the gene rendering the cell heterozygous. For TSGs the tumourigenic effect is only seen when both alleles are removed. Since cancer is a multistep process, the removal of gene function is very unlikely to lead to complete cancer. At worst it would make the few targetted cells one step along the path to tumourigenesis.

2. Introduction of a SNP/small deletion/small insertion.

This is the procedure that has the most risk. For example we may want to introduce a mutaton known to be oncogenic (eg. Changing codon 12 from Glycine to Valine in Ras). Transformation of cells has been shown to be a multi-step process requiring mutation in more than one gene therefore introduction of a single mutation provides a low risk of transforming any cell line. There is a potential for it to be oncogenic when recombined in the genes endogenous locus. However expression levels of this altered version of the gene will be generally lower as it is expressed under the control of its endogenous promoter, this is in comparison to the effects on cells of the same mutation under the control of a high level exogenous (eg. Viral) promoter which has been typically shown to be transforming in cell lines.

Worst case scenario: If an end user were to infect themselves with this type of recombinant AAV vector it is possible that the targeting construct could change the function of the endogenous gene in the infected cells. It would only target on a allele of the gene rendering the infected cell heterozygous but has been shown to be associated with progression along the tumourigenic process. Since cancer is a multistep process this gain of gene function is very unlikely to lead to complete cancer. At very worst it would make the few targetted cells one step along the path to tumourigenesis. The degree of infection is likely to be small in the end user because the targeting process is very inefficient so even if cells are infected with recombinant AAV virus very few of these would have the DNA corrected inserted into the target gene. In summary, the highest
level of risk comes from this class of gene manipulations however even if an end-user were to be infected there would be a very low risk of the infected cells being correctly
targetted. If the cells are correctly targetted these cells are unlikely to be cancerous - at worst they will be one step along the path to cancer.

This will have very low hazards associated with it as we are not changing the endogenous expression or characteristics of the targetted gene. Reporter genes such as GFP
or luciferase have no hazards associated with them.
Worst case scenario: if an end-user were to infect themselves with this type of recombinant AAV vector it is possible that the targetting construct could make a fusion
protein with the reporter gene. However since the reporter gene has no hazards associated with it the only result would be that the infected cells would express the reporter
gene.
There is no evidence that when the inserted genetic material recombines with the host cell lines genomic DNA that any sequences of the AAV viral genome are
incorporated. In the recombinant AAV viral particles the only AAV genomic sequences present are the LTRs (for specifying integration sites) and there is no evidence that
these are incorporated into the host genome during homologous recombination.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste (Plastic dishes, pipettes and tubes used for cell, bacterial and viral culture and handling)
Disposal procedure: Waste double bagged and autoclaved on standard cycle. Pipettes decontaminated via soaking in holder with 2% Trigene Advance or 5% Rely+On
Virkon advance before disposal in Cin-bin for incineration.

Waste will be transported to the autoclave double bagged in autoclave bags and placed in a lidded container.

Autoclaves: Boxer 200/200L, LTE Scientific series 280 and F03 LTE osprey. Validation: Annual service and callibration carried out by engineer. Boxer 200 gives printout
our individual runs to confirm temperature and time.

Liquid waste (Waste media from viral, bacterial and cell cultures)
Disposal procedure: Trigene Advance at a final concentration of >2% or Rely+On Virkon (final concentration 5%). Overnight prior to disposal in dedicated sinks.

Good occupational hygiene practice will be used throughout.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The genetic modification safety committee agreed that the project is assessed as containment level 2.

A summary of comments was tabled and the following items discussed.

- E. coli cloning was not adequately represented/included in the risk assessment. This was corrected.
- The start date was incorrect and should be changed
- Section 2.1 - Discussion was held about whether the cell lines which would be modified by AAV in this project were previously purposefully infected with viruses, for the purposes of transforming them. Following discussion, it was agreed that the cancer cell lines in this study were transformed in nature.

The committee pointed out that although the AAV are not categorised by the Advisory Committee on Dangerous Pathogens (ACDP), it does not naturally follow that they are Containment Level 1.

- Section 4.6.1 - The committee advised that 'recent publications have looked for a possible link between AAV and spontaneous abortion'. This is a tenuous risk but needs to be included on the risk assessment as a duty of care to staff.

### Project Containment

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<td>L2 L3 L4 L2 L4</td>
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</table>

- Animal Units
  - L2
  - L3 L4

- Large Scale Activities
  - L2 L3 L4 L2

- Human Clinical Applications
  - L2 L3 L4

### Project Ref 554/12.2

**Date Ackn’d** 31/01/2012

**CU2 Project Title**

- Molecular microbiology and physiology of the human commensals and opportunistic pathogenic bacteria Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis and Enterococcus faecium

**Class**

- Class 2

**CultureVolClass2** ≤ 1 Litre

**Non-GMM Consent Granted**

- Consent Granted

**Project notified under transitional arrangements**

- **N**

**Tick if notifying a connected programme of work**

- **N**
The research aims are to determine the virulence and environmental survival determinants that enable these bacteria to cause disease and survive in the environment and therefore to provide theories regarding their transmission and ability to cause pathology during infection. The use of recombinant strains will allow the individual genetic contributions of determinants.

Recipient or parental organism

(see attached documentation)
Staphylococcus aureus strains: SH1000 (and isogenic variants 8325-4, RN4220); Newman; UAMS-1.
Staphylococcus epidermidis strains: RP62a, 1457, Tu3298
Enterococcus faecalis: OG1RF; JH2-2; V583, CG110
Enterococcus faecalis: TX0104
Escherichia coli: (K12 strains: DH5alpha,Top10,), BL21(lambda DE3)

Host/vector system

(see attached documentation)
For S. aureus and epidermidis strains:
pAZ106, pMUTIN4, pSK5632, pCU1, pMK4, pLTV1, pSB2035

Plasmids for proposed work:
1. General cloning plasmids (pBluescriptSK, pUC18, pSL1190, pAZ106) colE1 based plasmids for cloning S. epidermidis genes without attempting to express encoded products.
3. pSB2035 RNAIII-gfp-luxABCDE, this is a pMK4 based plasmid that allows promoter fusions that expresses the gfp and lux operons for assays of gene expression in S. epidermidis (with cloning in E. coli strains listed) Cm [PMID: 11598083]
4. pLTV1 21kb colE1, pE194ts replicons, shuttle plasmid that contains spoVG-lacZ translational fusion for generating lacZ fusions during rounds of mutagenesis using the transposon Tn917 mutations in S. aureus. Amp, Cm, Tet, Erm [PMID: 2163385]
5. pBT2, pE194ts replicon containing the Mariner transposon, with similar properties to Tn917, except it has a more random integration pattern. Amp, Tet, Erm [PMID:21173311]

For E. faecalis and faecium work:
1. General cloning plasmids (pBluescriptSK, pUC18, pSL1190, pAZ106) colE1 based plasmids for cloning S. epidermidis genes without attempting to express encoded products.
products.

2. pTCVlac  12kb pAMB1, pACYC184 replicons, shuttle plasmid that contains spoVG-lacZ translational fusion for generating lacZ reporters in E. faecalis (FEMS Micro Lett 156:193 [1997]) Ern Kan


4. pTCVlac(gfp)(lux) this is a plasmid that will be generated to allow translational fusions to gfp gene or lux operons for assays of gene expression in E. faecalis (with cloning in E. coli strains listed)  Erm Kan

5. pAT18 pAMB1, colE1 replicons, universal shuttle vector with multiple cloning site from pUC18 (Nucleic Acids Res 18:4296 [1990])Amp, Spc

6. pCU1 pE194, colE1 replicons, universal shuttle vector with multiple cloning site from pUC19 (Nucl eic Acids Res 18:4296 [1990]), Amp,Cm

7. pLTV1  21kb colE1, pE194ts replicons, shuttle plasmid that contains spoVG-lacZ translational fusion for generating lacZ fusions during rounds of mutagenesis using the transposon Tn917 mutations in E. faecalis. Amp, Cm, Tet, Ern (PMID: 2854092, PMID: 8763945)

8. pAZ106 8.3kb colE1 replicon based vector that contains spoVG-lacZ translational fusion for generating reporter fusions in E. faecalis.  Amp, Ern (PMID: 9846745)

9. pET family plasmids (pET24d+, pET21a) and pTB361/375 colE1 replicon based vector for T7 promoter driven overexpression of proteins.

Origin & function

The genetic material will be derived from Staphylococcus or Enterococcus strains, either those listed above or from clinical isolates. The selection of DNA will NOT include known toxins and correspondingly there will be no attempt to increase expression of toxins by their cloning with promoters for overexpression. These toxins are very well characterised and not the subject of investigation.

The derived strains are for use with models of survival, growth and bacterium-bacterium interaction.

Evaluation of foreseeable effects

The four bacteria that will be genetically manipulated are commensals that cause opportunist disease. The manipulations that will be undertaken are aimed at generating mutants that have reduced fitness and should thus be attenuated for infection or of equal virulence to their isogenic parent. Studies are NOT aimed at increasing virulence. Infections with GMM will be prevented using the standard microbiological containment procedures used with expertise in the laboratory. The GMM will remain sensitive to clinical antibiotics prescribed for disease and will not be endowed with increased pathogenicity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Containment level 2 only

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

(information also on risk assessment forms attached)
Waste will be collected in bags (solid waste e.g. tips, wipes, agar plates) and transported, double bagged in sealed metal cannisters from Room 302 to the autoclaves, which are located in the wash up facility in room 101 of the Life Sciences Building. There are two cycles in use; Sterilisation cycle 121 degrees C for 15 minutes; Discard cycle 131 degrees C for 15 minutes. These machines are serviced, validated by thermocouple testing by contractor and calibrated every 6 months. In event of an autoclave breakdown waste will be stored at 4°C until autoclave repaired.

Spills will be wiped with solid waste treated as above. The area contaminated will then be sterilised using chemical treatment with Virkon or Trigene using the described procedures for these chemicals; Information on efficacy can be found at http://www2.dupont.com/RelyOn/en_US/assets/downloads/europe/Virkon_efficacy_data.pdf Trigene; Basic information of efficacy is available at the website below and a full list of organisms against which it can be used is available from the manufacturer or the Safety Office in the Life Sciences Building room G31.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
Studies on the growth, physiology and virulence of Staphylococcus epidermidis: major commensal of human skin and opportunist pathogen.

14/12/10 Considered by the Biological Safety Committee. It was requested that a number of minor alterations be made to sections 2.1, 4.1, 4.3, 4.4, 4.4.1, 4.4.2 and 4.6.1.

15/6/11 Reconsidered by the Biological Safety Committee. Alterations made satisfactorily. Approved at Class 2.

Studies on the growth, physiology and virulence of Enterococcus faecium: gut commensal of humans and opportunist pathogen.

14/12/10 Considered by the Biological Safety Committee. It was requested that a number of minor alterations be made to sections 2.1, 4.1, 4.3, 4.4, 4.4.1, 4.4.2 and 4.6.1.

15/6/11 Reconsidered by the Biological Safety Committee. Alterations made satisfactorily. Approved at Class 2.

Studies on the growth, physiology and virulence of E. faecalis: gut commensal of humans and opportunist pathogen.

Periodic up-date of Class 2 risk assessment 2003-08. The following minor changes have been made from the original proposal:

The title has been changed from 'Studies on the role and regulation of Enterococcus faecalis surface protein'.

Additionally use commonly used laboratory strain Enterococcus faecalis V583.

Additionally use cloning plasmid pAZ106 (A promoterless lacZ erm insertion vector).

Additionally use pCJK72 Bursa aurealis mariner transposon delivery plasmid, Cm, Erm. There is published work describing the use of this plasmid.

Updated risk assessment considered by the Institute Biological Safety Officer and the University Biological Safety Advisor, July 2011 and circulated to the Biological Safety Committee August 2011. It was agreed that the proposal is Class 2.
Studies on the growth, physiology and virulence of Staphylococcus aureus

Periodic up-date of risk assessment 2003-07. The following minor changes have been made from the original proposal:

The title has been changed from: Studies on the role and regulation of Staphylococcus aureus surface proteins.

There has been up-dating of S. aureus strains by including Newman (a Staphylococcus aureus strain that maintains the agr phenotype and UAMS-1 (a rsbU-positive clinical isolate).

Additionally use cloning vector pSK5632, a low copy S. aureus shuttle vector, pBT2 and pE194ts containing the Mariner transposon.

Updated risk assessment considered by the Institute Biological Safety Officer and the University Biological Safety Advisor, July 2011 and circulated to the Biological Safety Committee August 2011. It was agreed that this proposal is Class 2.

### Project Containment

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### Project Ref 554/12.3

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<td>24/08/2012</td>
<td>Environmental adaptation, gene regulation and virulence of Salmonella</td>
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<td>&lt; 1 Litre</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**

The research aims to determine virulence and environmental survival determinants of Salmonella Typhimurium. Analysis of Salmonella Typhimurium transcriptomes (using the next generation sequencing-based RNA-Seq approach) from different infection-relevant environmental conditions will identify candidate genes involved in virulence and environmental survival. The use of recombinant strains will allow the individual genetic contributions of these different determinants to be elucidated. We hope that the identification of genes responsible for virulence and environmental survival will lead to novel prophylactic or therapeutic approaches to combat Salmonella infection.

**Recipient or parental organism**

Salmonella enterica serovar Typhimurium, strains: ST4/74 (and derivative SL1344), LT2, D23580
Escherichia coli K12, strains: DH5alpha, Top10

**Host/vector system**

Plasmids:
- pKD46, pKD20 - which encode the Lambda Red system for making chromosomal mutations (Datsenko and Wanner, PNAS 2000).
- pCP20 - encodes Flp recombinase gene for removal of cassettes inserted by Lambda Red, and flanked by Flp sites (Cherepanov et al., Gene 1995)
- pDIGc and pDIGi - these plasmids each carry a fluorescence marker. These will be used to perform a newly developed technique called Fluorescence Dilution that measures intracellular survival and replication of bacteria (Helaine et al., PNAS 2009)
- Plasmids expressing GFP and RFP to be used for competition experiments (Segura et al., J. Microb. Meth. 2003)
- Standard cloning vectors: pBAD (Invitrogen), pUC (Vieira et al., Gene 1982)
Generalised transduction with bacteriophage P22.

**Origin & function**

The genetic material will be derived from the Salmonella enterica serovar Typhimurium strains, listed above. The genes that will be analysed are potentially involved in environmental survival and virulence. The derived strains are for use with models of bacterial infection: in vitro growth on solid and in liquid media mimicking infection-relevant conditions, as well as infection models involving cultured cell lines (RAW264.7 macrophages, and HeLa and Caco-2 epithelial cells).

**Evaluation of foreseeable effects**

One of the Salmonella Typhimurium strains we will manipulate genetically is non-virulent (LT2), and the other three cause disease (ST4/74, SL1344, and D23580). The manipulations that will be undertaken are aimed at generating mutants that have single, or sets of inactivated genes. None of the resulting GMO will be more virulent than the D23580 strain. Studies are NOT aimed at increasing virulence.
Any effect of infections with GMM will be prevented using the standard microbiological containment procedures that are carefully applied in the laboratory. The GMM will remain sensitive to clinically-relevant antibiotics.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Containment level 2 only.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Waste will be collected in bags (solid waste e.g. tips, wipes, agar plates) and transported, double bagged in sealed metal canisters from Room 302 to the autoclaves, which are located in the wash-up facility in Room 101 of the Life Sciences Building. There are two cycles in use; Sterilisation cycle 121 degrees C for 15 minutes; Discard cycle 131 degrees C for 15 minutes. These machines are serviced, validated by thermocouple testing by contractor and calibrated every 6 months. In event of an autoclave breakdown (highly unlikely as there are 5 autoclaves in the facility), waste will be stored at 4°C until autoclave repaired. Spills will be wiped with solid waste treated as above. The area contaminated will then be sterilised using chemical treatment with Virkon or Trigene using the described procedures for these chemicals; Information on efficacy of Virkon can be found at http://www2.dupont.com/RelyOn/en_US/assets/downloads/europe/Virkon_efficacy_data.pdf Trigene; Basic information of efficacy is available at the website below and a full list of organisms against which it can be used is available from the manufacturer or the Safety Office in the Life Sciences Building room G31. http://www.medi-mark.co.uk/images/uploads/TriGene_2p_lab.pdf |

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This risk assessment was considered by a meeting of the Biological Safety Committee on 26th June 2012. The following changes were requested before final approval could be given.

1. In the summary, please include something about the genetic modification risks.
The genetic modifications planned for this project do not aim to, and are highly unlikely to make a more virulent strain than ST313. 

2.1 Please provide more detail of the strains. Is STM SL1344 correct?
The full names have now been provided.

3.1 b. It is not clear from the text the reason for the plasmids being listed under Inserted genetic material.
We have now specified how the plasmids will be used, and why.

3.2 a. Human health is again considered here. This section is about the environment. Should consider any possible effects on animal health.
STM LT2 is not virulent, and does therefore not impose a risk on the environment. SL1344 and ST4/74 are known to cause disease in humans (self-limited gastroenteritis), as well as in calf, pig and chicken. Salmonella Typhimurium ST313 only causes serious disease in the at-risk patient group (see 4.6.1).

The level 2 containment and control measures should prevent the GM organisms from reaching the environment and causing harm to humans or animals outside the laboratory.

4.1 It was not felt that delayed opening would reduce the risk of aerosols. At least a validated time delay should be stated. State what the standard protective measures are.
Centrifugation and vortexing will be carried out in sealed tubes. Potential risk will be minimised by delaying opening for a minimum of 30 seconds, opening and transferring in a safety cabinet and the wearing of appropriate personal protective clothing.

4.4 Give effective contact time for Trigene. Can give the Trigene web-site, but should also give the appropriate details on the form. The safety office is 3.19 not G31.
For every-day use, a 1:100 dilution of Trigene advance will be used, and contact time for disinfection will be at least one minute. In the case of destruction of liquid waste, or in case of a larger spill (e.g. bacteria culture, max volume 50 ml) a 1:50 solution of Trigene will be used, and the contact time for disinfection will be at least 10 minutes before rinsing or soaking up with blue wipes.

4.4.1. Five autoclaves are available so no need to store. OK
4.4.2 Give details specific for Salmonella and correct location of safety office 3.19 OK

4.6.1. The effect on immuno-compromised individuals should be considered in the risks to human health section. With the level 2 containment and good laboratory practice, the risk of Salmonella infection should be negligible. However, any immuno-compromised individual working on this project in the lab must have permission from relevant consultant, as there is a potential risk that these individuals, if infected, can aquire the more severe form of disease associated with ST313.
Corrections were made and the risk assessment was approved on 28th June 2012.

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### Project Containment

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<tr>
<td>L2 Yes</td>
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02/03/2022
### Project Additional Information

#### Purposes of the contained use

Mutation of Salmonella and Campylobacter isolates by transduction using bacteriophages or by insertion mutagenesis using plasmids enables us to study the roles of certain genes during infection.

#### Recipient or parental organism

Salmonella enterica. To include serovars: Typhimurium, Pullorum, Derby, Virchow, Gallinarum, Enteritidis

Campylobacter jejuni

#### Host/vector system

- **pUT mini-Tn5 plasmids** (R6K-based suicide delivery plasmids)
- **EZ-Tn5 transposome <KAN-2>** (R6K deleted)
- **P22 temperate bacteriophage** (infects Salmonella Typhimurium). A P22 mutant, P22 HT105/1 int-201 will be used as it has a high transducing frequency.

#### Origin & function

Transposons (Mini-Tn5 transposons) encoding antibiotic resistance genes (kanamycin, ampicillin, nalidixic acid, tetracycline) and/or green fluorescent protein (Mini-Tn5TcGFP transposons). EZ-Tn5 transposons containing kanamycin selectable marker (<KAN-2>). The function of the transposons is to induce insertional
mutagenesis in the host organism. The function of the antibiotic resistance genes and green fluorescent protein is as selection markers. The Tn5 system is a naturally occurring transposition system found in gram-negative bacteria. EZ-Tn5 is a hyperactive Tn5 transposition system.

Bacteriophages P22 containing Salmonella Typhimurium chromosomal DNA fragments (transducing particles) and antibiotic resistance genes. P22 is related to bacteriophage λ and is used for generalized transduction of host organisms whereby genes of interest can be inserted into the host genome.

Evaluation of foreseeable effects

The GMOs will not pose any greater risk than wild-type Salmonella spp or Campylobacter jejuni. The purpose of transduction of the isolates and/or the insertional mutagenesis is to disrupt the function of certain genes. This disruption will either attenuate the organism's survival and/or virulence or the organism will continue to display wild-type phenotype.

An indirect effect of the mutagenesis is the acquirement of antibiotic resistance. However, the antibiotic resistance genes used as markers are not routinely used in the treatment of enteric infections. Theoretically it would be possible for antibiotic resistance genes to be transferred from mutant isolates to other bacteria, however this would be unlikely to occur in a laboratory situation. For gene transfer to occur, a series of very low frequency events would have to take place.

There is a very small risk that unknown virulence-related genes may be up-regulated with introduction of GFP. However, this has not been observed for GFP-expressing strains already constructed including Salmonella Pullorum 449/87 pBRD940.

There may be a theoretical risk to animals (which is low because clinical disease is not usually seen in infected animals). There is no known potential for harm to plants. These isolates are unlikely to survive well in the environment, although this has not been specifically tested. The likelihood of the GMM actively causing additional harm is very low and may be less fit than the unmodified organism.

Transposase activity of the insert is not maintained in the target cells so gene transfer/recombination would occur only at the normal, random rate. Plasmids used are designed to have a restricted host range and will not be conjugative (i.e. plasmids will not carry genes necessary for the ability to transfer themselves to other bacteria). As such these sequences cannot readily be transferred to other organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste including contaminated agar plates, cell culture plastic ware, pipette tips, microplates and tubes will be autoclaved at 126 degrees Celcius for 15 minutes and then incinerated. Pipette tips and innoculating loops will also be soaked in 1% Virkon before incineration. Liquid waste including cell growth media and bacterial cultures will be autoclaved at 12 degrees Celcius for 15 minutes and then incinerated. Small volumes of liquid waste will be disinfected in 1% Virkon and then washed down the sink with copius amounts of water. Contaminated surfaces will be disinfected with 1% Trigene.

The autoclave is monitored using a thermocouple and there is a regular testing schedule. It is serviced biannually.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Risk assessment was presented to GMSC meeting on 12 December 2012 after external expert review. Several minor comments were raised that required revision primarily the addition of extra detail such as room numbers. The revised version was circulated January 2013 and approved at the GMSC meeting 5th February 2013

**Project Containment**

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**Project Ref** 554/13.2

- **Date Ackn'd**: 03/04/2013
- **CU2 Project Title**: GM Adenovirus expressing CD40 Ligand or TRAIL
- **Class**: Class 2
- **Culture Volume**: ≤ 1 Litre
- **Class Culture**: Consensus Granted
- **Non-GMM**: Consent Granted
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

- **Significant Change ID**: 554/13.2a
- **Date of Significant Change**: 19/12/2013

**Project Additional Information**

**Purposes of the contained use**

The tumour necrosis factor (TNF) cytokine superfamily plays a crucial role in immune regulation by modulating lymphocyte proliferation and apoptosis. CD40 ligand (CD40-L) and TRAIL both belong to this family. Expression of CD40-L and TRAIL have been reported to induce cell death and apoptosis in carcinomas. The aim of the study is to assess whether membrane bound mutant of CD40-L and a
CD40-TRAIL chimera (composed of CD40-L N-terminal domain and the TRAIL C-terminus domain) which resist cleavage from the membrane would be more potent in cell death induction in CD40 or TRAIL-positive E1-negative established carcinomas, also the mechanism by which these ligands induce cell death.

The effect on growth, survival and cell signalling of these established mammalian cell lines will be monitored following expression of CD40-L, CD40-L-TRAIL chimera and mutant sequences (which resist cleavage from membrane) by replication deficient recombinant adenovirus (RAD) or replication competent recombinant adenovirus infection. The virus (dl922-947) has a mutation within the CR2 domain of the E1A region, this virus would only be tested with carcinoma cell lines with a defect in the Rb pathway. Taking into account the widespread expression of CD40 and TRAIL receptors in carcinomas and the growth inhibitory effects of CD40-L and TRAIL in CD40-and TRAIL-positive tumour cell lines respectively, these studies may provide novel therapeutic approaches for the treatment of carcinoma.

Recipient or parental organism

For work related to replication deficient adenovirus expressing CD40L, only E1-negative established CD40-positive tumour cell lines would be used (Pancreatic cell lines including Panc1, Suit2, Bladder carcinoma cell line EJ, Liver cell line HepG2, other CD40-positive or negative (as a negative control) may be used as well, as long as these cell lines are E1-negative to avoid any possible recombination with the replication deficient adenoviral vector. As a safety measure all carcinoma cell lines (either CD40 positive or negative) intended to be tested with the Adenoviral vectors would be tested firstly by PCR for any possible contamination with E1a and E1b DNA.

Host/vector system

For work related to replication efficient adenovirus (dl922-947, within the CR2 domain of the E1A region) expressing CD40L only carcinoma cell lines that either CD40-positive or negative and are E1-negative, in addition have established defect in Rb pathway would be tested. This virus in no way would replicate in normal cells or carcinoma cell lines that have a normal pRb pathway and are E1-negative due to the mutation within the CR2 domain of the viral E1A.

For work related to replication deficient adenovirus expressing TRAIL, only E1-negative carcinoma cell lines that either TRAIL receptor-positive or negative (as a negative control) would be tested to avoid any possible recombination. These viruses have already been constructed and so no bacterial cell work is required.

Although we already have enough stock from these viruses, but possible bulking up of these viruses may be needed, for bulking up replication deficient adenovirus expressing either CD40L or TRAIL, HEK 293 cells would be used, although this cell line is E1-positive, and there is a possibility of contamination of the produced stocks from this cell line with the wild-type adeno vector due to possible recombination, the contaminating wild-type
adenoviruses would be incapable of expressing the transgene as the transgene sequence would be lost during the recombination process, also the resultant wild-type viral DNA sequence would be missing the E3 region required for suppression of host immunity. To minimize the probability of recombination, propagation of these viruses in HEK 293 cells would be carried out for a minimal period (around 3 days), also produced stocks would be routinely checked by PCR technique (we will check the presence of deleted E1 sequence in the viral DNA) for any possible contamination with wild-type adenovirus, any contaminated viral stocks with wild-type adenovirus would be destroyed.

For bulking up replication efficient adenovirus expressing CD40L, the alveolar basal epithelial A549 cell line would be used, this cell line has a well-established defect in pRb pathway which would result in replication of our replication efficient adenovirus. However, it is E1 negative, therefore, there is no possibility of recombination occurring, for additional safety viral stocks produced would be checked for any contamination with the wild-type adenovirus by PCR using specific primers to test the presence of CR2-deleted sequence (d922-947), positive and negative controls would be tested within the PCR test.

Origin & function

1. CD40L, wild-type and non-cleavable mutant,
2. Wild type CD40L and non-cleavable CD40L mutant (lacking cleavage site, 324-348bp) are expressed under the CMV promoter as membrane bound structures, where only wild-type CD40L would be subject to cleavage from membrane into soluble ligand by the action of MMPs enzymes
3. TRAIL wild-type, expressed under CMV promoter as membrane bound, also subject to cleavage into soluble ligand by the action of MMPs enzymes
4. CD40L-TRAIL fusion, composed of the intercellular domain of CD40L (1-324bp) fused to extracellular domain of TRAIL (340-846bp), expressed under the CMV promoter as a membrane bound molecules not subject to cleavage from membrane as it lacks both CD40L and TRAIL cleavage sites

Evaluation of foreseeable effects

Theoretically expression of transgene is possible in airway epithelium if exposed to vector within aerosols. However, the membrane expression resultant from these vectors is such that this would be very localised. Furthermore, target receptor expression is low/absent in normal compared with malignant epithelium such that these agents are likely to have a differentially greater effect on cancer cells compared with normal cells such that the risk of adverse effects is likely to be minimal.

CD40 ligation has the potential to stimulate inflammation in exposed airway. However, again, the membrane expression of ligands is such that this would be very localised. Ligation of TRAIL receptors would not induce inflammatory effects.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

I would not like to request derogation from full containment measures

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For solid waste: 1% Virkon used for most surface decontamination. Due to corrosion potential of Virkon, 0.5% SDS is used if decontamination of centrifuge buckets is required. We have validated both of these against adenovirus. We have also validated Klercide 5 against adenovirus.

All liquid waste is treated by autoclaving at 130°C for 60 mins, before disposal to drains. 1% Virkon used for most surface decontamination.

Disposable solid waste which is or may be contaminated with GMMs is also inactivated by autoclaving at 130°C for 30 mins, before removal as "clinical waste". Autoclaving achieves effectively 100% kill of all GMMs.

There is also possible risk of accidental spillage in the process of centrifugations, however if this is the case we would immediately decontaminate all the affected areas with enough 1% Virkon solution.

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GSC confirmed the opinion of Dr Elmitwalli that this project does represent class 2 activity, and that it can safely be conducted in the existing facilities within the Department of Molecular and Clinical Cancer Medicine (subject to notification to the HSE)

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Animal Units

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02/03/2022
### Project Ref 554/13.3

<table>
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<th>CU2 Project Title</th>
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<tr>
<td>19/04/2013</td>
<td>Production of lentiviral particles using 293K packaging cells. Use of lentivirus to transfect immortalized cell lines and primary cells from orthopaedic tissues</td>
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<tr>
<td>Non-GMM</td>
<td>Consent Granted</td>
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| Project notified under transitional arrangements | N |

#### Significant Change ID

#### Date of Significant Change

#### Project Additional Information

**Purposes of the contained use**

Knockdown of gene expression and expression of decoy RNA molecules in primary human cell types

**Recipient or parental organism**

The pLKO.1 pLenti6/V5-DEST lentiviral vectors. Mammalian cell lines and primary cells including 293FT packaging cell line.

**Host/vector system**

The pLKO.1 & pLenti6/V5-DEST

**Origin & function**

shRNA targeting RNA binding proteins (KSRP, HuR and TTP), green fluorescent protein, non-coding cDNA sequences designed to be expressed as “RNA decoys” (with the aim of promoting upregulation of SOX9 mRNA)

**Evaluation of foreseeable effects**

Lentiviral particles have the capacity to infect mammalian cells, including those of the laboratory worker. Upon infection the lentivirus will incorporate its genetic material into the host cell's genome. This has the potential to result in insertional mutagenesis. This is minimized in each of the lentiviral systems employed through self-inactivating systems where the virus is deleted in 3'LTR.

Lentiviral particles produced and /or used in this study will be replication defective and the probability of reversion to wild-type is negligible. Viral particles have the capacity to infect human cells and will insert genetic material into the cells’ genomes, which carries the risk of insertional mutagenesis. The specific safety features of the vectors...
carried by these viral particles allow these microorganisms as to be handled safely at containment level 2 in accordance with ACDP class 2 guidelines. The major risk in this work is through sharps injuries and aerosol generation. Sharps will not be used in the experiments under any circumstances, whilst exposure to aerosols will be minimilised by working in a class II laminar flow hood and not centrifuging open tubes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste including contaminated cell culture plastic ware, pipette tips, microplates and tubes will be autoclaved at 126 degrees Celcius for 15 minutes and then incinerated. Liquid waste will be disinfected in 2% Virkon and then washed down the sink with copius amounts of water. Contaminated surfaces will be disinfected with 1% Trigene. The autoclave is monitored using a thermocouple and there is a regular testing schedule. It is serviced biannually.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The need for greater description of the genes targeted by the GM strategy was discussed and has been amended now on the form. Disposal of waste material was discussed. The use of dedicated bins for rooms G23 and G26 will be instigated. These bins will have sealable lids for transportation of the waste to the autoclave. This system will be rolled out across other containment level II projects within the Leahurst main building. There was some debate about whether this work requires level II containment given that the lentiviral systems used are of the latest generation and are replication incompetent, which gives the potential to handle them under level I containment. As a result of these discussions it has been decided that because (i) genes targeted have been linked to tumor formation in some studies and (ii) much of the work will be conducted on primary human cells (requiring level II containment anyway) that level II containment would be the most appropriate.

Project Containment

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Animal Units Large Scale Activities Human Clinical Applications
**Project Additional Information**

**Purposes of the contained use**

The research aims to understand the molecular mechanisms by which viruses cause disease in their natural environment, hence providing information on how effective anti-viral therapies may be designed and validated.

**Recipient or parental organism**

Mouse cells will be used and will include: mouse embryonic fibroblasts from different mouse strains: wild type 129/Sv; IFNalpha/beta reector KO; RNaseL KO; PKR KP; and established cell lines NIH3T; BHK21. Canine cell line, MDCK, will be used in vitro for virus plaque assays to measure virus infectivity and fitness.

**Host/vector system**


**Origin & function**

For MHV-68 and SFV vioruses where deletion mutants may be used, these constructs have been shown to have similar growth properties to their parental (wild type) viruses and in some cases grow even less well in comparison to their parental viruses. Therefore, there is no increased hazard associated with these constructs.
References to back up above statement include:
No genetic manipulation will be carried out on the mouse-adapted flu virus.

Evaluation of foreseeable effects

Studies are not aimed at increasing virulence. No virus infection in mice will be carried out at the Biosciences Building. Only mouse cells and canine cell line, MDCK will be infected with these viruses for in vitro work.

Mouse cell cultures: Cell lines BHK 21 (ATCC No. CCL-10), C127 (ATCC No. CRL-1804), L929 (ATCC No. CCL-1) and NIH3T3 (ATCC No. CRL-1658) will be used. We will also use a number of mouse embryo fibroblast (MEF) cell cultures to assess the growth characteristics of MHV-68 and SFV-derived viruses. These primary cells are obtained from healthy mice and will be provided from our collaborators (Edinburgh and Institute of Animal Health). These include 129/SvEv and IFNa/bRKO129/SvEv C57BL/6x129/SvEv C57BL/6x129/SvEv PKRKO.

MDCK is a canine epithelial cell line (ATCC No. CCl34).

Viruses: MHV-68 BAC pHA3 (Adler et al., 2000) is the parental BAC construct which was used to generate MHV-68 Viruses: MHV-68 mutants. Our interest is in the non-structural genes of MHV-68 which may play a role in RNA degradation. Currently, only one gene, ORF37, has been linked to RNA degradation. The virus constructs MHV-68ORF37 Stop and its corresponding revertant MHV-68ORF37Rev will be used in our in vitro studies. The growth of MHV-68ORF37 is highly attenuated in immunocompetent cells and the growth of MHV-68ORF37Rev is similar to the parental virus. SP6-SFV4 is the parental infectious clone used to generate SFV mutants (Liljestrom and Garoff, 1991). The constructs are SFV4(3H)-RLuc (Attarzadeh-Yazdi et al., 2009), SFV4-SteGFP (Tanberg et al., 2007), SFV4-RDR (Fazakerley et al., 2002).

Fazakerley et al. 2002: A single amino acid change in the nuclear localization sequence of the nsP2 protein affects the neurovirulence of Semliki Forest virus. J. Virol. 76:392-6

Widely used mouse-adapted laboratory flu A strains A/WSN/33 (H1N1) and A/PR/8/34 (H1N1) will be used for in vitro assays.

Fazakerley et al. 2002: A single amino acid change in the nuclear localization sequence of the nsP2 protein affects the neurovirulence of Semliki Forest virus. J. Virol. 76:392-6

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Evaluation of foreseeable effects

No glass or sharps will be used. Working surfaces e.g. insides of containment cabinets shall be cleaned first with 1% Virkon solution followed by 70% ethanol both before and after operations. In the event of a significant spillage (when working with high titre stocks), the class 2 cabinet will be fumigated with formaldehyde and also prior to maintenance work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Understanding the molecular basis of mRNA degradation in viral infections.

Reviewed by the Biological Safety Committee on 26th June 2012. It was requested that a number of alterations be made to sections 1, 2.2, 2.3, 3.1b, 3.1.1, 4.3, 4.4 and 4.4.2.

Approved 9th January 2013. Additional change to section 4.4.1 made, June 2013.

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Project Ref 554/14.1

Date Ackn'd 24/03/2014

CU2 Project Title Knockout mutation of virulence genes in Streptococcus pneumoniae

Class 2

Culture Volume Class2 < 1 Litre

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes Withdrawn N

Tick if notifying a connected programme of work N

Historical Date of Additional Info
Project Additional Information

Purposes of the contained use

Types of activities: Bacterial and cell culture, Electroporation of plasmids into bacteria, use of In vivo mouse models

The aim of this project is to create mutations in genes encoding for such virulence factors and determine the effect of the mutations on various phenotypic properties including host tissue adhesion and invasion properties, growth characteristics in artificial sputum medium and other biofilm models, and virulence properties in vitro as well as in vivo using previously established mouse models (carriage, invasive pneumonia, sepsis, meningitis). The proposed research involves the introduction of antibiotic resistance cassettes (i.e., ampicillin, chloramphenicol, erythromycin, penicillin, spectinomycin) into target genes in the pneumococcal chromosome or complete deletion of target genes. Such

7. Characteristics of the GMO(s) including the evaluation of foreseeable effects - Public Register experiments have been previously carried out for this organism in the AK research group (University of Leicester), and the systems used for the creation of "knock-out" mutants have therefore already been optimized and validated.

Gene-deficient mutants (e.g., NanA−) will be made by insertion-duplication mutagenesis. For example, part of the nanA gene (HindIII nucleotide [nt] 2449 to SphI nt 3090 fragment) will be cloned into pVA891, and the construct transformed into the laboratory strain D39. A single crossover mutation occurs which incorporates the entire plasmid, thereby disrupting the NanA gene and rendering the recombinant erythromycin resistant (Manco et al. (2006) Infect Immun. 74(7): 4014–4020). Analysis of NanA− by Southern blotting will confirm that there is an insertion mutation in the nanA gene. Other S. pneumoniae mutants (e.g., pneumolysin, NanB) will be constructed using insertion-duplication, point, or deletion mutations, as described previously (Berry et al. (1999) Infect Immun. 67(2): 981–985). D39 and other strains in which ply has been disrupted by insertion-duplication mutagenesis or in which the wildtype ply has been replaced by a mutated gene encoding pneumolysin with two or three amino acid substitutions - Asp385→Asn, Cys428→Gly and Trp433→Phe - will be tested for their complement activation and cytolitic activities. All transformations will be carried out into Escherichia coli DH5α.

Complementation of mutants will be carried out by using pBBR1Tp or similar plasmid vectors to introduce cloned genes back into pneumococcal mutants. In addition, we propose to keep the option of
using a plasposon based random mutagenesis system with the same antibiotic resistance markers plus a tetracycline resistance cassette.

Molecular marking of relevant S. pneumoniae strains:
S. pneumoniae strains including the laboratory strain D39 will be labelled with an erythromycin resistance cassette and fluorescence markers including green fluorescent protein or GFP, using a previously described procedure (Kadioglu et al. 2001 FEMS Microbiol Lett., 194 (1): 105-110). The plasmid pGFP1 has gfp in the shuttle vector pVA838. The gene, for the mut 3 variant of GFP, is excised as a BamH1–Sph1 fragment from pKEN2, and ligated into pVA838, previously cut with BamH1 and Sph1, to generate pST1. Expression of gfp is from an uncharacterised pneumococcal promoter, isolated from a library of pneumococcal DNA in pST1. Chromosomal DNA is isolated from pneumococcal clinical isolate CCUG10175 (University of Göteborg Culture Collection). DNA is digested with Sau3A and fragments between 0.2 and 2 kb ligated into dephosphorylated BamH1-digested pST1. To accumulate stocks of recombinant plasmids, the library is first electroporated into Escherichia coli DH5a. Plasmid DNA will be isolated from all transformants and then the pooled DNA used to transform wild-type pneumococcus, using competence-stimulating peptide. Pneumococcal transformants will be selected on Brain–Heart Infusion agar containing erythromycin (1 mg/ml). Pneumococcal transformants will be examined for fluorescence under UV microscopy. Labelled strains will be used in vitro as well as in vivo (risk assessments specific to experimentations in mice have been reviewed and approved by the Biomedical Services Unit committee of the University of Liverpool) and will be visualised using epifluorescence and confocal microscopy.

Recipient or parental organism

Streptococcus pneumoniae. Its serotypes of importance to human infection such as serotypes 1, 2, 3, 4, 6B, 19F etc.

Host/vector system

plasmid pMSH14, reporter plasmid pGFP1, plasmid pVA838, pVA891, pALC2073, pKEN2, pST1

Origin & function


S. pneumoniae mutant strains with penicillin resistance. Penicillin and its derivatives are the most commonly used antibiotics in humans for the treatment of pneumococcal infections. However, the mutants we create will be gene mutants of major virulence factors, hence the resulting mutants will be attenuated in virulence and will not present a hazard.

Evaluation of foreseeable effects

The antibiotic resistance genes chosen are not themselves harmful. In addition, selection during our experiments will require antibiotic levels in excess of 50 μg/ml in order to overcome the inherent resistance of the strains. These are levels in excess of any that would be of therapeutic use.
Inserting fluorescent markers does not affect fitness or confer any additional virulence. For knockout mutations the vectors are designed to be incapable of replication in Streptococcus pneumoniae (suicide vectors). The insertionally inactivated or mutated genes are introduced into the chromosome via homologous recombination. The vector is lost because of inability to replicate. Any alteration of pathogenic traits will make the host strain substantially less pathogenic (in most cases avirulent) since the aim is to "knock-out" genes that contribute to pathogenicity and bacterial virulence.

<table>
<thead>
<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not applicable</td>
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</table>

<table>
<thead>
<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not applicable</td>
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</table>

<table>
<thead>
<tr>
<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The following procedures will be implemented:</td>
</tr>
<tr>
<td>For solid waste e.g. Tips, tubes, pipettes and agar plates: Agar plates containing cultures are discarded to a designated metal container for autoclaving. Solid waste (tips, tubes etc.) are placed in a sealed &quot;sweetie jar&quot; and taken for autoclaving and incineration. Pipettes are steeped in disinfectant (1% Virkon) for a minimum of 20 minutes before disposal. The disinfectant is discarded down the sink. Any spillage is disinfected using 1% Virkon. For liquid waste: Liquid cultures are autoclaved. Any spillage is disinfected using 1% Virkon. Autoclaves in Ronald Ross Building are operated by trained laboratory staff. A record is produced for each decontamination run to confirm, by means of a temperature probe placed within the load, attainment of suitable temperatures for 100% kill of viable organisms. Validation of autoclave cycles is maintained by 6-monthly servicing and yearly thermocouple testing carried out by BMM Weston engineers.</td>
</tr>
</tbody>
</table>

| Is an emergency plan required according to regulation 20? N |

| If yes, tick to confirm that it is attached to this form N |

| Tick to confirm that you have attached a risk assessment to this form Y |

| Tick if you are claiming exemption from disclosure for section of the risk assessment N |

Please enter comments on the GM safety committee on the risk assessment
The original proposal was circulated a week in advance of the GMSC meeting of 12th December 2012. Following the meeting a number of amendments were requested. These were mostly typing errors, but some more detail on vectors and inserted DNA was required. These amendments have now been completed.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 Yes L3 L4</td>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
<tr>
<td>L2 Yes L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

**Project Ref 554/14.2**

- **Date Ackn’d**: 24/03/2014
- **CU2 Project Title**: Identification and characterisation of virulence factors of Salmonella enterica and Campylobacter jejuni isolates during experimental infection of chickens
- **Class**: Class 2
- **Culture Vol**: ≤ 1 Litre
- **Consent Granted**: Non-GMM

**Project notified under transitional arrangements**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Mutation of Salmonella and Campylobacter isolates by transduction using bacteriophages or by insertion mutagenesis using plasmids enables us to study the roles of certain genes during infection.
Bacteria expressing GFP allow imaging of infection sites by confocal microscopy to better understand the infection process.

Recipient or parental organism

Salmonella enterica, including serovars Typhimurium, Pullorum, Derby, Virchow, Gallinarum, Enteritidis, Hadar and Infantis
Campylobacter jejuni and coli

Host/vector system

pUT mini-Tn5 plasmids (R6K-based suicide delivery plasmids)
EZ-Tn5 transposome <KAN-2> (R6K deleted)
P22 temperate bacteriophage (injects Salmonella Typhimurium). A P22 mutant, P22 HT105/1 int-201 will be used as it has a high transducing frequency.

Origin & function

Transposons (Mini-Tn5 transposons) encoding antibiotic resistance genes (kanamycin, ampicillin, nalidixic acid, tetracycline) and/or green fluorescent protein (Mini-Tn5TcGFP transposons). EZ-Tn5 transposons containing kanamycin selectable marker (<KAN-2>). The function of the transposons is to induce insertional mutagenesis in the host organism. The function of the antibiotic resistance genes and green fluorescent protein is as selection markers. The Tn5 system is a naturally occurring transposition system found in gram-negative bacteria. EZ-Tn5 is a hyperactive Tn5 transposition system.

Bacteriophages P22 containing Salmonella Typhimurium chromosomal DNA fragments (transducing particles) and antibiotic resistance genes. P22 is related to bacteriophage λ and is used for generalized transduction of host organisms whereby genes of interest can be inserted into the host genome.

Evaluation of foreseeable effects

The GMOs will not pose any greater risk than wild-type Salmonella spp or Campylobacter jejuni. The purpose of transduction of the isolates and/or the insertional mutagenesis is to disrupt the function of certain genes. This disruption will either attenuate the organism's survival and/or virulence or the organism will continue to display wild-type phenotype.

An indirect effect of the mutagenesis is the acquirement of antibiotic resistance. However, the antibiotic resistance genes used as markers are not routinely used in the treatment of enteric infections. Theoretically it would be possible for antibiotic resistance genes to be transferred from mutant isolates to other bacteria, however this would be unlikely to occur in a laboratory situation. For gene transfer to occur, a series of very low frequency events would have to take place.

There is a very small risk that unknown virulence-related genes may be up-regulated with introduction of GFP. However, this has not been observed for GFP-expressing strains already constructed including Salmonella Pullorum 449/87 pBRD940.

There may be a theoretical risk to animals (which is low because clinical disease is not usually seen in infected animals). There is no known potential for harm to plants. These isolates are unlikely to survive well in the environment, although this has not been specifically tested. The likelihood of the GMM actively causing additional harm is very low and may be less fit than the unmodified organism.

Tranposase activity of the insert is not maintained in the target cells so gene transfer/recombination would occur only at the normal, random rate. Plasmids used are designed to have a restricted host range and will not be conjugative (i.e. plasmids will not carry genes necessary for the ability to transfer themselves to other bacteria). As such these sequences cannot readily be transferred to other organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Laboratory Waste**
Solid waste including contaminated agar plates, cell culture plastic ware, pipette tips, microplates and tubes will be autoclaved at 126 degrees Celsius for 15 minutes and then incinerated. Pipette tips and inoculating loops will also be soaked in 1% Virkon before incineration.

Liquid waste including cell growth media and bacterial cultures will be autoclaved at 12 degrees Celcius for 15minutes and then incinerated. Small volumes of liquid waste will be disinfected in 1% Virkon and then washed down the sink with copius amounts of water.

Contaminated surfaces will be disinfected with 1% Trigene or Distel.

The autoclave is monitored using a thermocouple and there is a regular testing schedule. It is serviced biannually.

**Animal Unit Waste**
Solid waste including animal bedding, uneaten feed, animal bodies (all are eviscerated as part of necropsy), used protective clothing, paper, wipes and swabs will be autoclaved at 126 degrees Celsius for 20 minutes and then incinerated.

Liquid waste including cell growth media and bacterial cultures will be autoclaved at 12 degrees Celsius for 15 minutes and then incinerated. Small volumes of liquid waste will be disinfected in 1% Virkon and then washed down the sink with copius amounts of water.

Contaminated surfaces will be disinfected with 2% Trigene or Distel following Standard Operating Procedures of the unit.

The autoclave is newly installed and is monitored using a thermocouple and there is a regular testing schedule. It will be serviced biannually.

Is an emergency plan required according to regulation 20?  
Y

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

GMSC on 11/2/2014 recomended minor changes to the risk assessment. The amended assessment was approved by Chair’s action after circulation to Chair, Biological Safety Officer and University Biological Safety advisor on 13/2/2014

Project Containment
### Project Ref 554/14.3

**Date Ackn'd** 09/04/2014  
**CU2 Project Title** Modulation of adhesion signalling in cell culture by expressing or suppressing adhesion signalling-associated proteins via lentiviral infection  
**Class** Class 2  
**Culture Vol** < 1 Litre  
**Consent Granted** Non-GMM  
**Project notified under transitional arrangements** N

#### Project Additional Information

**Purposes of the contained use**

Proteins identified as components or regulators of adhesion-signalling complexes (adhesome) will be transiently or stably over-expressed in mammalian and human primary and established cell lines using a lentiviral transduction system. For some of the signalling molecules, mutated constructs will be lentivirally-transduced to modulate functional activity eg dominant-negative or constitutively active constructs. Lentivirus-mediated delivery of shRNA will be used to suppress expression of adhesome components in the same cell lines. Also, lentivirus-mediated delivery of Tetracycline (Tet)-inducible shRNA will be used to suppress expression of adhesome components in the same cell lines.

The categories of molecules to be expressed or suppressed (including some key examples) are:

- **Transmembrane adhesion receptors** e.g. Integrins, syndecans, CD98, Cadherins, Nephrin
- **Receptor tyrosine kinases** e.g. EGFR, PDGFRa, HER2, FGFR
- **Cytoskeletal and focal adhesion proteins** e.g. filamin A, talin, kindlin, vinculin, EB1, ACF7, APC, Afadin, WASP family, LifeAct
- **GTPases and GTPase regulators** e.g. Rac1, RhoA, Cdc42, Arf6, p190RhoGAP, IQGAP, Ras suppressor 1, p115RhoGEF, RacGAP1
- **Phosphorylation regulators** e.g. PKC, Src, RACK1, ILK, FAK, MAPKs, PTPN13, PPP2a/1a
- **Matrix proteinase regulators** e.g. MT1-MMP, MMP9, MMP2, UPAR, TIMP-1
Intracellular trafficking regulators e.g. AP2, flotillin, clathrin, caveolin, dynamin

Intramolecular and intermolecular FRET biosensors e.g. Raichu-Rac, Lyn-Src biosensor, Arf6-CyPET/YPET-GGA3

Following lentiviral expression or shRNA transduction, the function of these molecules will be assessed by proteomic analysis of adhesion-signalling complexes, co-immunoprecipitation, assessment of signalling molecule activity/phosphorylation and analysis of cell migration and adhesion. Fluorescently labelled proteins will be expressed for imaging applications.

In section 7, consideration is given separately to 2 stages of the lentiviral transduction process:

Work Package A) Co-transfection of lentiviral packaging vectors and lentiviral expression plasmids into packaging cells and subsequent harvesting of gene of interest-encoding or shRNA encoding lentiviruses

Work Package B) Infection of recipient cells with lentiviruses encoding gene of interest or shRNA oligonucleotide sequences targeting gene of interest

Recipient or parental organism

Work Package A)
293T and 293FT Human renal epithelial cell line. Stably transfected with SV40 large T-antigen.
293T and 293FT are well-characterised human cell lines that are unable to colonise the worker and contains no known adventitious agents. However it is stably transfected with the SV40 large T-antigen to allow episomal amplification of vectors that contain the SV40 origin. Therefore it is classified as specially-disabled (SACGM guidance notes, part 2, section 2.5, table 2.5.1)

Work Package B)
Immortal established cell lines:
Mouse embryonic fibroblasts (MEF) – Immortalised with Large T-antigen
T-antigen Immortalised Fibroblasts (TIF) – Human fibroblasts immortalised with Large T-antigen
CA1, H357, C1, VB6, VB6Δ11aa - established human oral squamous cell carcinoma cell lines
BT20, MCF7, MDA-MB-231, MDA-MB-468, BT-474, SKBR3 - established human breast carcinoma cell lines
MCF10a - established human breast epithelial cell line
H441, DMS 114, DMS 53, DMS 153, NCI-H1299, CaLu - established human lung carcinoma and adenoacarcinoma cell lines
Capan-1, PaCa-2, PaCa-3, BxPC-3, PANC-1, - established human pancreatic carcinoma and adenocarcinoma cell lines
AsPC-1 - established human pancreatic adenocarcinoma cell lines derived from metastatic site (ascites)
A375 – established human malignant melanoma cell line -
B16-F10 – established mouse melanoma cell line
HT1080 – human fibrosarcoma cell line
A2780 - established human ovarian carcinoma cell lines
K562 – established human erythroleukaemia cell line
MRC-5, MRC-9 - human lung fibroblast cells (+/- hTert immortalisation)
PS-1 – pancreatic stellate cells (+/- hTert immortalisation)
hTERT-HPNE – established pancreatic ductal cells (hTert immortalised)

Primary cell lines:
Primary human umbilical artery endothelial cells (HUAEC)
Primary human umbilical vein endothelial cells (HUVEC)
Primary mouse mammary epithelial and pancreatic ductal adenocarcinoma cells
Human foreskin fibroblasts (HFF) – Primary normal dermal fibroblasts from foreskin
Mesenchymal stem cells (MSC) – derived from human bone marrow
Human embryonic stem cells (huESC) – derived from human blastocyst
Mouse embryonic stem cells (muESC) – derived from mouse blastocyst

Adventitious agents are not present in established immortalised cell lines. Mouse primary cell lines will not contain human adventitious agents. Primary human cells have been screened for basic microbiological adventitious agents but should be treated as potentially infectious and contaminated biological specimens and handled at Biosafety Level-2.

Host/vector system

Work Package Ai) Lentiviral protein or shRNA Expression System:
Lentiviral Packaging Vectors (pMD2.G, psPAX2)
Lentiviral expression Vectors (pWPXld, pWPi, PLVTHM) encoding:
Human or Mouse adhesion signalling-associated proteins or shRNAs (detailed in Section 6):  
- Transmembrane adhesion receptors  
- Receptor tyrosine kinases  
- Cytoskeletal and focal adhesion proteins  
- GTPases and GTPase regulators  
- Phosphorylation regulators  
- Matrix proteinase regulators  
- Intracellular trafficking regulators  
- Intramolecular and intermolecular FRET biosensors

Work Package Aii) Tet-inducible Lentiviral shRNA System
Lentiviral Packaging Vectors (pLP1, pLP2, pLP/VSVG), pENTR/H1/TO Entry construct
pLenti4/BLOCK-iT Vector encoding:
shRNA oligonucleotides to suppress expression of Human or Mouse adhesion signalling-associated proteins:  
- Transmembrane adhesion receptors  
- Receptor tyrosine kinases  
- Cytoskeletal and focal adhesion proteins  
- GTPases and GTPase regulators  
- Phosphorylation regulators  
- Matrix proteinase regulators  
- Intracellular trafficking regulators  
- Intramolecular and intermolecular FRET biosensors

Work Package B) Lentiviruses produced as a result of transfection of the lentiviral expression and packaging vectors described in Section 7Ai and 7Aii will be used to infect the immortal established cell lines and primary cell lines described above.

Origin & function

Work Package Ai) Lentiviral protein or shRNA Expression System
Lentiviral Packaging Vectors (pMD2.G, psPAX2):
Origins:
- HIV1 (gag, pol, rev and tat)
- Vesicular Stomatitis Virus (VSVg envelope protein)

Biological activities/functions:
- gag – structural capsid protein from HIV1 required for generating the viral core.
- pol – reverse transcriptase from HIV1 required for reverse transcription of the RNA genome prior to pro-viral integration.
- rev – accessory protein from HIV1 required for the nuclear export of the full length viral genome to promote packaging.
- tat – accessory protein from HIV1 required for extension of transcription from the HIV1 LTR promoter.
- VSVg – amphotropic viral envelope protein permitting viral infection into most vertebrate cell types.

Lentiviral expression Vectors (pWPXLD, pWPI, PLVTHM):

Human or Mouse adhesion signalling-associated proteins or shRNAs:
- Transmembrane adhesion receptors
- Receptor tyrosine kinases
- Cytoskeletal and focal adhesion proteins
- GTPases and GTPase regulators
- Phosphorylation regulators
- Matrix proteinase regulators
- Intracellular trafficking regulators
- Intramolecular and intermolecular FRET biosensors

Biological activities:
Adhesion signalling pathways can affect many cellular properties including cell fate, proliferation, differentiation, apoptosis and migration, and can be associated with tumourigenesis and immune response. The exact effects will, however, be cell type specific.

Work Package Aii) Tet-inducible Lentiviral shRNA System

Lentiviral Packaging Vectors (pLP1, pLP2, pLP/VSVG)

Origins:
- HIV1 (gag, pol, rev and tat)
- Vesicular Stomatitis Virus (VSVg envelope protein)

Biological activities/functions:
- gag – structural capsid protein from HIV1 required for generating the viral core.
- pol – reverse transcriptase from HIV1 required for reverse transcription of the RNA genome prior to pro-viral integration.
- rev – accessory protein from HIV1 required for the nuclear export of the full length viral genome to promote packaging.
- tat – accessory protein from HIV1 required for extension of transcription from the HIV1 LTR promoter.
- VSVg – amphotropic viral envelope protein permitting viral infection into most vertebrate cell types.
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<td>• Intracellular trafficking regulators</td>
</tr>
<tr>
<td>• Intramolecular and intermolecular FRET biosensors</td>
</tr>
</tbody>
</table>

### Biological activities:
Adhesion signalling pathways can affect many cellular properties including cell fate, proliferation, differentiation, apoptosis and migration, and can be associated with tumourigenesis and immune response. The exact effects will, however, be cell type specific.

#### Work Package B)

Lentiviral transduction will be used to express or suppress the following classes of Human or Mouse adhesion signalling-associated proteins:

|• Transmembrane adhesion receptors |
|• Receptor tyrosine kinases         |
|• Cytoskeletal and focal adhesion proteins |
|• GTPases and GTPase regulators    |
|• Phosphorylation regulators       |
|• Matrix proteinase regulators     |
|• Intracellular trafficking regulators |
|• Intramolecular and intermolecular FRET biosensors |

Adhesion signalling pathways can affect many cellular properties including cell fate, proliferation, differentiation, apoptosis and migration, and can be associated with tumourigenesis. The exact effects will, however, be cell type specific. Use of lentiviral vectors for the expression of these proteins or shRNA oligonucleotides will ensure their long-term expression in infected cell lines.

As the generated lentiviruses are replication-incompetent, upon exposure to human tissue, infection would be restricted to the site of exposure (see below). Experimental procedures will be conducted without the use of sharps preventing the possibility of needlestick injuries. Furthermore, the generated lentiviruses will not survive outside of its specific culture medium.

### Evaluation of foreseeable effects

#### Work Package A) Mechanisms of gene product expression control:

**Lentiviral Expression System:**

| Lentiviral Packaging Vectors (pMD2.G, psPAX2), gag, pol and rev – CAG promoter which is a strong constitutively active eukaryotic promoter comprising the CMV enhancer, and chicken beta-actin promoter and intron. |
| VSVg – CMV promoter which is a strong constitutively active eukaryotic promoter |
| Lentiviral Expression Vector (pWPXLd, pWPI, PLVTHM) |
Human or Mouse adhesion signalling-associated proteins or shRNAs (classes listed above):

- Eukaryotic promoter comprising elongation factor 1 alpha (EF1A) promoter.

Tet-inducible Lentiviral shRNA System:

Lentiviral Packaging Vectors (pLP1, pLP2, pLP/VSVG),
gag and pol – eukaryotic promoter comprising the CMV promoter and human beta-globin intron
rev – RSV enhancer/promoter

VSVg – CMV promoter which is a strong constitutively active eukaryotic promoter and human beta-globin intron

pLenti4/BLOCK-iT Vector:

shRNA oligonucleotides targeting Human or Mouse adhesion signalling-associated proteins (classes listed above) – Rous Sarcoma Virus (RSV) enhancer/promoter for Tat-independent production of viral mRNA in eukaryotic cells. Modified human H1 promoter containing 2 prokaryotic tet operator 2 (TetO2) sequences induces shRNA expression only in presence of tetracycline.

Potential for recombination and reversion to wild type:

The pWPXLd, pWPI, PLVTHM and pLenti4/BLOCK-iT-DEST vector systems are engineered to prevent generation of replication competent viruses.

The pWPXLd, pWPI, PLVTHM and pLenti4/BLOCK-iT-DEST vectors contain the LTRs and packaging sequences from HIV1 but they lack the gag, pol, env, rev and tat genes. Therefore these vectors cannot be packaged successfully in the absence of an external source of these proteins.

In addition, the 3' LTR contains a mutation within the promoter sequence. During reverse transcription and genomic integration of the provirus, this sequence will be copied to the 5' LTR. As a consequence, transcription of the complete viral genome from the 5' LTR of the provirus will not be possible preventing the generation of new viral particles even in the presence of viral proteins.

Mutation of the 3' LTR promoter also ensures that transcription of downstream genomic sequences following proviral integration cannot occur.

The different proposed inserts will not alter the stability of the recombinant viruses compared to the parental virus. It is very unlikely that the minimum of four independent recombination events required to produce a replication competent virus will occur. In addition, the vif, vpr, vpu and nef HIV1 virulence genes have been deleted. Therefore the likelihood of reversion to wild type virus is negligible.

The pWPXLd, pWPI, PLVTHM and pLenti4/BLOCK-iT-DEST vectors also contain an RRE element to enhance the nuclear export of the genomic transcript (but only in the presence of rev expression), and hence viral packaging, and a WPRE sequence to improve transcript stability. The WPRE, however, does not encode protein X as the codon encoding the initiating methionine has been mutated.

The VSV-G from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope

The pWPXLd system is a 2nd generation lentiviral system and the pLenti4/BLOCK-iT lentiviral shRNA system is a 3rd generation (gag/pol, rev/tat and VSVg are separated on different vectors).
For the pLenti4/BLOCK-iT Lentiviral shRNA System, expression of the gag and pol genes from pLP1 has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev

WP A Lentiviral Systems References:


Work Package A Direct Effects:

Transfection of all of the vectors in either the Lentiviral Expression Systems, or Tet-inducible Lentiviral shRNA System, into the eukaryotic host cells will initiate production of replication-incompetent and self-inactivating lentiviruses encoding adhesion signalling-associated proteins or shRNA oligonucleotides to suppress expression of adhesion signalling-associated proteins. Pseudotyped viruses will be produced carrying the VSVg coat protein. As a consequence the recombinant viruses will be able to infect most vertebrate cell lines and tissues, including humans.

The host cells will produce lentiviruses that will either express or suppress the following classes of Human or Mouse adhesion signalling-associated proteins:

- Transmembrane adhesion receptors
- Receptor tyrosine kinases
- Cytoskeletal and focal adhesion proteins
- GTPases and GTPase regulators
- Phosphorylation regulators
- Matrix metalloproteinase regulators
- Intracellular trafficking regulators
- Intramolecular and intermolecular FRET biosensors

Exposure of humans to these viruses would cause the localised expression or suppression of components of adhesion-mediated signalling pathways. Adhesion signalling pathways can affect many cellular properties including cell fate, proliferation, differentiation, apoptosis and migration, and can be associated with tumorigenesis, angiogenesis, inflammation and immunological responses. Adhesion signalling mechanisms are also targeted by pathogens, to promote cellular uptake, so it is conceivable that the viruses could modulate the susceptibility of cells to infection. Thus, it is possible that exposure to the generated viruses may regulate these processes in humans. However, as described in 3.1b and “Assessment Form 4”, the viral delivery systems have been engineered to ensure that generated viruses are replication-competent and self-inactivating.
However the likelihood of harmful effects to humans caused by exposure to the transfected mammalian cells (293T/293FT) is minimised as the gene products will be expressed in a well characterised cell line that has been screened for adventitious agents, is unable to colonise the worker, will not survive outside of its specific culture medium, and will be immune rejected if accidental exposure occurs. Moreover, as detailed in “Assessment Form 4”, the lentiviral vector systems are engineered to prevent generation of replication competent viruses. Moreover, the VSVg coat protein will activate complement unlike the HIV1 coat protein. As a consequence, a stronger immunological reaction will be provoked following infection with the recombinant pseudotyped viruses improving viral clearance.

WP A Direct Effects References:


Work Package A Indirect Effects:

The VSVg coat protein will activate complement unlike the HIV1 coat protein. As a consequence, a stronger immunological reaction will be provoked following infection with recombinant pseudotyped viruses improving viral clearance.

Individually, expression of the HIV1 gag and pol proteins is cytotoxic, whilst expression of VSVg can cause cell fusion.

Work Package A Effect of Gene Transfer:

The vectors are mobilisable, but only once as they are self-inactivating. The encoded cDNAs are incapable of integrating into the eukaryotic genome of the host cells.

As discussed above, using these vector systems, it is very unlikely that the minimum of four independent recombination events required to produce a replication competent virus will occur. In addition, the vif, vpr, vpu and nef HIV1 virulence genes have been deleted. Therefore the likelihood of reversion to wild type virus is negligible.

If accidental release was to occur, host cells or viruses encoding the genetic material will not survive outside of their specific culture medium. 293T and 293FT cells are an...
established human cell line that are multiple auxotrophs, and are very sensitive to both dehydration and UV exposure. They will not survive beyond a few minutes outside of their normal growth medium.

Work Package A Summary of Risks:

Viruses encoding Adhesion Signalling-associated molecules may have the potential to regulate processes associated with tumourigenesis and immune function. However, the selected viral delivery systems are engineered to eliminate viral replication in infected cells. The primary risks are associated with exposure to the viruses rather than the virally-transduced cell lines

Laboratory use and decontamination procedures are in place to prevent exposure of workers and co-workers to both viruses and infected cells during handling

Work Package B) Mechanisms of gene expression control

The pWPXLd, pWPI, PLVTHM and pLenti4/BLOCK-iT-DEST vector systems are engineered to prevent generation of replication competent viruses.

The pWPXLd, pWPI, PLVTHM and pLenti4/BLOCK-iT-DEST vectors contain the LTRs and packaging sequences from HIV-1 but they lack the gag, pol, env, rev and tat genes. Therefore these vectors cannot be packaged successfully in the absence of an external source of these proteins.

In addition, the 3’ LTR contains a mutation within the promoter sequence. During reverse transcription and genomic integration of the provirus, this sequence will be copied to the 5’ LTR. As a consequence, transcription of the complete viral genome from the 5’ LTR of the provirus will not be possible preventing the generation of new viral particles even in the presence of viral proteins.

Mutation of the 3’ LTR promoter also ensures that transcription of downstream genomic sequences following proviral integration cannot occur.

The different proposed inserts will not alter the stability of the recombinant viruses compared to the parental virus. It is very unlikely that the minimum of four independent recombination events required to produce a replication competent virus will occur. In addition, the vif, vpr, vpu and nef HIV-1 virulence genes have been deleted. Therefore the likelihood of reversion to wild type virus is negligible.

The pWPXLd, pWPI, PLVTHM and pLenti4/BLOCK-iT-DEST vectors also contain an RRE element to enhance the nuclear export of the genomic transcript (but only in the presence of rev expression), and hence viral packaging, and a WPRE sequence to improve transcript stability. The WPRE, however, does not encode protein X as the codon encoding the initiating methionine has been mutated.

The VSV-G from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope

The pWPXLd system is a 2nd generation lentiviral system and the pLenti4/BLOCK-iT lentiviral shRNA system is a 3rd generation (gag/pol, rev/tat and VSVg are separated on different vectors).

For the pLenti4/BLOCK-iT Lentiviral shRNA System, expression of the gag and pol genes from pLP1 has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev

WP B Lentiviral Systems References:
Pseudotyped viruses will be produced carrying the VSVg coat protein. As a consequence the recombinant viruses will be able to infect most vertebrate cell lines and tissues, including humans. Exposure to the viruses would cause the localised expression or suppression of components of adhesion-mediated signalling pathways. The viruses would modulate adhesion signalling pathways which can affect many cellular properties including cell fate, proliferation, differentiation, apoptosis and migration, and can be associated with tumourigenesis, angiogenesis, inflammation and immunological responses. Adhesion signalling mechanisms are also targeted by pathogens, to promote cellular uptake, so it is conceivable that the viruses could modulate the susceptibility of cells to infection. The exact effects will, however, be cell type specific.

The viral vector has the potential to deliver genetic material into humans, as the VSVg env protein permits infection of most vertebrate cells, including humans. However, as discussed in 3.1.b, the lentiviral vector systems are engineered to prevent generation of replication competent viruses. Moreover, the VSVg coat protein will activate complement unlike the HIV1 coat protein. As a consequence, a stronger immunological reaction will be provoked following infection with the recombinant pseudotyped viruses improving viral clearance.

WP B Direct Effects References:


Work package B Indirect Effects:

Some of the encoded "adhesion signalling-associated" genetic material may alter the tumourigenicity or invasion of infected cancer cell lines. However, these cells are likely be immune rejected upon accidental exposure.

The VSVg coat protein will activate complement unlike the HIV1 coat protein. As a consequence, a stronger immunological reaction will be provoked following infection with recombinant pseudotyped viruses improving viral clearance.

Individually, expression of the HIV1 gag and pol proteins is cytotoxic, whilst expression of VSVg can cause cell fusion.

Work package B Effect of Gene Transfer:

The vectors are mobilisable, but only once as they are self-inactivating. The encoded cDNAs are incapable of integrating into the eukaryotic genome of the host cells.

As discussed above, using these vector systems, it is very unlikely that the minimum of four independent recombination events required to produce a replication competent virus will occur. In addition, the vif, vpr, vpu and nef HIV1 virulence genes have been deleted. Therefore the likelihood of reversion to wild type virus is negligible.

If accidental release was to occur, host cells or viruses encoding the genetic material will not survive outside of its specific culture medium.

Work Package B Summary of Risks:

Viruses encoding Adhesion Signalling-associated molecules may have the potential to regulate processes associated with tumourgenesis and immune function. However, the selected viral delivery systems are engineered to eliminate viral replication in infected cells. The primary risks are associated with exposure to the viruses rather than the virally-transduced cell lines. Laboratory use and decontamination procedures are in place to prevent exposure of workers and co-workers to both viruses and infected cells during handling.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste: Pipettes, culture flasks, centrifuge tubes, filters, syringes
Solid waste will be autoclaved (124°C for 15mins). All autoclaved waste will be subsequently incinerated.
Degree of kill: 100%

Liquid Waste:
Virus-containing medium from transfected 293T/293FT and medium from lentivirally-transduced recipient cells (which will not be generating virus)
Liquid waste will be autoclaved (124°C for 15mins) followed by disposal down a sink with copious amounts of water.
Degree of kill: 100%
Primary Autoclave: 200L Boxer Autoclave.
Room 3.17 Nuffield Building

Alternative Autoclave: Astell Scientific AA091.
Room 1.19 Nuffield Building

Autoclave testing/monitoring procedures:
200L Boxer autoclave: Automated report printout system
200L Boxer/Astell Scientific AA091: Annual Thermocouple test and calibration by service engineer

Committee asked that it was ensured that any areas where virus was stored was secure. It was agreed that the building security systems offered suitable security.

Details of how liquid waste will be transported to autoclave to be added

Add that "any cuts to the skin will be covered"

Recommendation to liase with technical staff so that waste transported to the autoclave is processed immediately, any storage of waste should be in the laboratory until the autoclave is available.

Committee agreed with Class 2 designation. Lesley Andrews (UoL Biological Safety Advisor) will undertake notification to HSE once relevant forms are completed.

**Project Containment**

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</tr>
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<td>Human Clinical Applications</td>
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02/03/2022
Examining the role of ROR2 in the cartilage differentiation pathway of mesenchymal stem cells

Project Additional Information

Purposes of the contained use

Mesenchymal stem cells (MSCs) from bone marrow can be used to generate cartilage cells for the use in treatment of cartilage diseases such as osteoarthritis. This research aims to examine the mechanisms by which stem cells produce cartilage and to determine whether the amount and quality of cartilage produced can be maximised.

Recipient or parental organism

The recipient cells will be primary human bone marrow-derived mesenchymal stem cells (MSCs). Bone marrow is obtained from human patients and MSCs are isolated and grown for up to 8 passages in vitro.

Host/vector system

The initial aim will be to transfect plasmids into human MSCs using nucleofection (electroporation). If this method proves inefficient, Integration-Deficient Lentiviral particles will be used to deliver DNA and/or mRNA into cells. These are third-generation disabled, self-inactivating and replication incompetent lentiviruses (Dull, T. et al. A third generation lentivirus vector with a conditional packaging system. J. Virol. 72, 8463-8471 1998).

Origin & function

The inserted genetic material will be:

a) The gene for receptor tyrosine kinase-like orphan receptor 2 (ROR2). ROR2 is a transmembrane receptor protein
for Wnt5a which plays a key role in cartilage and growth plate development. The aim is to examine the role of ROR2 in the differentiation of stem cells into cartilage.
b) Reporter genes such as luciferase or fluorescent proteins will be inserted for promoter-reporter screening studies. Both ROR2 and the reporter genes will be generated artificially for incorporation into plasmids.

Evaluation of foreseeable effects

The genetically modified human MSCs will only be used for in vitro mechanistic studies on cells grown in the laboratory.

The most hazardous GMO which could potentially be constructed during this work would be a recombinant lentivirus containing the ROR2 gene.

ROR2 is known to be overexpressed in a number of solid tumours. However it is a receptor protein within the complex Wnt signalling pathway and has not been shown to be oncogenic alone. As the ligand for ROR2, namely Wnt5a, has been shown to be oncogenic in some settings, there is the potential that ROR2 itself could be oncogenic despite the lack of specific information on this point. However, where ROR2 will be over-expressed it will be under the control of an inducible promoter.

As described above, the lentivirus particles are third-generation disabled, self-inactivating and replication incompetent lentiviruses and although the viruses could infect human cells if internalised, they would not spread.

Reporter genes such as luciferase or fluorescent proteins have no known effects which are harmful to human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any solid waste (tissue culture flasks, serological pipettes, plastic tubes etc) will be autoclaved (131°C for 15 mins) prior to incineration. Any liquid waste will be inactivated by incubating with 1% Virkon for 24 hours before being autoclaved and discarded.

No sharps will be used during any of the procedures. Working surfaces e.g. insides of Class II Biological Safety Cabinets will be cleaned with 1% Virkon solution before and after operations. In the event of a significant spillage, the cabinet will be fumigated with formaldehyde and also prior to any maintenance work.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This risk assessment was considered at the meeting of the IIB/School of Life Sciences Biological Safety Committee on 12th December 2014. The Committee raised the following points.

1. The committee noted the potential hazard of the inserted DNA and considered it appropriate that the risk assessment be Class 2 as proposed.
2. Under 3.1, in the list of features of the third generation packaging system, in b), please include a statement that the system is self inactivating. Deletion of the 3’ untranslated region reduces the potential for transactivating cellular genes due to insertion.
3. Under 4.1, please indicate how/whether the procedures will reduce aerosols.

The above points should be addressed on this version of the risk assessment and the changes made highlighted on the form.

The required changes to the risk assessment have been made and the risk assessment is approved, 16th December 2014.

Project Containment

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Project Ref 554/15.2

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<td>02/02/2015</td>
<td>Analysis of respiratory virus pathogenesis</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
### Purposes of the contained use

Biomedical Research. The contained use is required for handling parental viruses regardless of whether they are genetically-modified or not.

### Recipient or parental organism

Influenza A virus

### Host/vector system

Recombinant influenza A virus

### Origin & function

Influenza A recombinants will be based upon laboratory strains of the viruses or low pathogenicity human strains contained in plasmid vectors. The intended function of the genetic material is disrupt/"knock out" or alter function by mutation or add fluorescent marker genes. This enables the study of the functions of the disrupted genes.

### Evaluation of foreseeable effects

The experiments proposed involve gene deletion, mutation or replacement of specific genes with the corresponding gene from another strain. For reassortants between the human-derived or laboratory adapted viruses, this is not predicted to create viruses with novel tropism or pathogenicity as they either already share similar characteristics (the recent human isolates) or are highly attenuated in humans (PR8, WSN). Whilst we cannot exclude the possibility that changing the internal genes might increase the pathogenicity of the virus, the fact that the tropism remains the same, there is widespread immunity to the human strains proposed and the use of attenuated strains limits the risk.

All influenza virus strains used are classified by ACDP as Hazard Group 2. Since none of the currently proposed modifications is expected to make the virus more hazardous than the wild-type parent, the work will be assigned to ACGM class 2.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures of GM viruses, and all consumables used in the handling of the GMOs, will be subjected to autoclaving and incineration following the normal procedures for disposal of clinical waste. The effective degree of kill is 100%. The efficacy of the autoclave is monitored annually by multi-point thermocouple checks during an annual service and inspection. External validation of the pressure vessel leading to the issue of a pressure validation certificate also occurs annually. In addition, a chart recorder produces a record of each run to confirm effective killing.

Is an emergency plan required according to regulation 20?  N  

If yes, tick to confirm that it is attached to this form  N  

Tick to confirm that you have attached a risk assessment to this form  Y  

02/03/2022
The Departmental Genetic Modification Safety Committee met on 18th November 2014 and considered that the work proposed presented no greater hazard than working with the wild-type organism and that the proposed level of containment was reasonable.

### Project Containment

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#### Project Ref 554/15.3

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<td>26/08/2015</td>
<td>Understanding the pathogenic mechanism of bovine digital dermatitis treponemes</td>
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information
Purposes of the contained use

Treponema species, specifically Treponema medium-like, Treponema phagedenis-like and Treponema pedis digital dermatitis (DD) associated spirochetes, are thought to be the aetiological agents of DD. Digital dermatitis (DD) is an endemic, global disease which results in severe lameness in agricultural ruminants such as cows, sheep and goats. As such, the disease causes significant welfare and economic concerns. No effective vaccine is available and treatment options are limited and generally ineffective.

Physical characteristics of DD treponemes are poorly characterized. Due to their anaerobic and fastidious nature, growth of DD treponemes is difficult in the laboratory setting. However, our laboratory has been able to successfully isolate and grow DD treponemes for a number of years now and have recently obtained genome sequences for several isolates. The ability to generate targeted gene knockouts these DD treponemes will enable our laboratory to identify possible transmission and virulence factors, such as cell surface antigens and define novel functions. This will ultimately aid in the development of potential treatments, vaccines and diagnostics for DD.

Recipient or parental organism

The recipient organisms are proposed to be: Treponema medium-like DD spirochete T19, Treponema phagedenis-like DD spirochete T320A and Treponema pedis T3552B. There are currently no attenuated strains available and all are considered ACDP hazard group 2 ("Treponema spp."). These organisms are anaerobic and fastidious in nature. They are not a known zoonosis agent. They are related distantly at a genus level to the human syphilis pathogens (Treponema pallidum subspecies pallidum) although demonstrate most similarity to treponemes associated with human periodontal disease (Treponema medium, Treponema denticola), a polymicrobial disease of humans considered to involve both environmental factors as well as said infectious agents. Therefore there could be considered a relatively limited potential risk to humans. Not known to produce toxins or agents which can cause harm to humans.

Host/vector system

We plan to perform this work by transformation of a knockout cassette (for details of genetic material involved, see next section) directly into the recipient DD treponemes. The knockout cassette functions via replacement of the target gene of interest with an antibiotic resistance marker, through the process of recombination. The cassette will be transformed into the cells on a linearized pGEM®-T vector (distributed by Promega. Catalog number: A1360). The vector will be linearized, non-mobilisable and has no protein expression capability (except for the erythromycin resistance determinant). Upon successful recombination, the vector will be will be genomically located, further reducing risk of horizontal transfer. The knockout cassette will be generated, maintained and propagated in Escherichia coli, for which a separate GM Class 1 risk assessment has been performed.

Origin & function

Putative virulence factors targeted for deletion will be identified by bioinformatics analysis of DD treponeme genomes. Genes will be deleted by allelic replacement of the target gene with an erythromycin resistance deletion cassette constructed in E. coli. Successful replacement will therefore provide the recipient organisms with resistance to erythromycin.

The artificially generated deletion cassette will consist of flanking upstream and downstream regions of the gene targeted for deletion, which will be amplified using genomic DD Treponema DNA as PCR template. The flanking upstream and downstream regions will be approximately 500 bp in size. These fragments will consist of either non-protein coding regions or fragments of protein coding regions. In the cases where protein coding regions will be included, care will be taken to ensure resulting fragments will not have the capacity to encode for a functional protein. A target T19 gene will not be considered if are adjacent to an entire small protein coding region (<500 bp) which would inadvertently be contained within a resulting up or downstream amplicon.

Generated up and downstream regions will be artificially fused by SOEing PCR (Synthesis by Overlap Extension PCR) to an Erythromycin resistance cassette (EryC).
including promoter and gene encoding erythromycin resistance determinant (eryC). The eryC resistance cassette will be amplified from plasmid pLN164 (a commercial Staphylococcus expression vector: distributed by Sigma Aldrich, Catalog number T6701-2UG) by PCR. The pLN164 plasmid is for PCR template only and will not be transformed into any organism.

The three generated PCR amplicons will be synthesized into a single fragment by SOEing PCR. The resulting artificial fragment will be cloned into a pGEM®-T vector (distributed by Promega. Catalog number: A1360) and resulting recombinant plasmids transformed, maintained and propagated in the laboratory strain E. coli TOP10 (distributed by Invitrogen. Catalog number: C4040-10). For transformation, the plasmid harbouring the resistance cassette will be harvested from E. coli and linerized using a suitable restriction enzyme prior to transformation into the recipient host.

**Evaluation of foreseeable effects**

The function of the inserted material is to replace a gene encoding a potential virulence factor with an erythromycin resistance gene, thereby deleting the gene encoding the potential virulence factor from the recipient microorganism. The inserted material will only code for erythromycin resistance and will not code for any other proteins. Successful recipients will display erythromycin resistance through constituent expression of EryC, a ribosomal methylase. EryC methylates erythromycin ribosomal binding sites, rendering the cells immune to erythromycin action. No direct (e.g. toxin, oncogenic, etc), known harmful effects to humans or wildlife are known regarding EryC. Insertion of the deletion cassette is intended to delete putative virulence factors. As such it may alter pathogenicity and immunogenicity. It is predicted that as potential virulence factors are deleted, virulence and viability would be reduced. Deletion of potential virulence factors in Treponema denticola have demonstrated reduced pathogenicity however, increased biofilm formation was observed in when a hypothetical protein, TDE2508, was deleted in T. denticola ATCC 35405. As such there is a potential for an indirect increase in factors involved in virulence.

In the event of a breach in bio containment resulting in a highly unlikely human infection, the effect of the presence of eryC would presumably prevent the use of Erythromycin as a treatment option. However, other effective antibiotics, such as penicillin, are available for treatment. As the inserted material encodes for erythromycin resistance an indirect environmental hazard is the potential for wild type DD treponemes harboring erythromycin resistance. Erythromycin is not used to treat cases of DD, so development of erythromycin resistance amongst environmental treponemes in the wild, although possible, is unlikely. Erythromycin is used to treat other, mainly Gram positive bovine infections, such as mastitis.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste will be placed in loosely sealed autoclave bags and autoclaved at 121°C, 15psi, 15min. Autoclaved waste will then be incinerated externally. Liquid waste will either be managed with Chemical inactivation with 2% Virkon for 24 hours or, Non – virkon treated liquid waste will be placed in loosely sealed pyrex duran bottles and autoclaved at 121°C, 15psi, 15min.

In house autoclaves are validated annually by a United Kingdom Accreditation Service (UKAS) calibration test. Degree of kill is 100%. Process is monitored by use of autoclave tape*

Virkon is a powerful bacteriocidal disinfectant that has been shown to provide 100% kill against many microorganisms when used at 1% concentration for 1 hour. We have validated virkon effectiveness against all three groups of DD treponemes when used at 2% for 24h and routinely use this method with wild type treponemes, including the strains proposed for genetic modification.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
The Departmental Genetic Modification Safety Committee met on 12th May 2015 and considered that the work proposed presented no greater hazard than working with the wild-type organism and that the proposed level of containment was reasonable.

**Project Containment**

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**Project Ref** 554/16.1

- Date Ackn'd: 06/04/2016
- CU2 Project Title: Interactions between Toxoplasma gondii and the host immune system
- Class: Class 2
- CultureVolClass2: Non-GMM
- CultureVolumeClass3-4: Consent Granted

**Project Additional Information**

- Purposes of the contained use:
  The aim of this project is to study interactions between Toxoplasma gondii and the host immune system, both in vitro and in mouse models. We aim to understand how the
parasites manipulates cells of the host immune system to enhance its spread. To identify cells and tissues containing parasites we will utilise parasites engineered to express fluorescent proteins, and to secrete cre recombinase into target cells. To study T cell responses to the parasite, we will use parasites engineered to express fluorescent proteins and the model antigen, ovalbumin (OVA). To investigate the role of parasite factors in manipulation of host cell behaviour, we will use gene knockout parasites.

Recipient or parental organism

Toxoplasma gondii is a parasitic protozoan of the phylum apicomplexa. It is an ACDP HG2 pathogen. T. gondii establishes chronic infection, and is highly prevalent in human populations, livestock, and the environment. 30% of all people are thought to have been infected with T. gondii at some point in their lives. Infection in immune competent people is usually asymptomatic, but may present as a mild flu-like illness or, in a minority of cases, ocular disease. However, encephalitis can occur following reactivation of the parasite in immune-compromised individuals, and infection during pregnancy can result in damage to the developing fetus. Natural infection most commonly occurs through the oral route. The parasite stages used are susceptible to most disinfectants and there is a very low risk of released material remaining infectious in the environment. Prophylactic treatment is available following accidental exposure.

Host/vector system

These parasites have already been generated at other institutions. They are described in:

Coombes et al, PNAS, 2013, Epub PMID: 23650399

Td-tomato, OVA and Cre-expressing lines:
T. gondii of the Prugniaud strain and lacking the endogenous gene for hypoxanthine guanine phosphoribosyl transferase (HPT) were used. To generate parasites expressing tandem dimeric tomato red fluorescent protein, parasites were electroporated with the pCTR2t vector which expresses td-tomato under the constitutive alpha-tubulin promoter, and the selectable marker chloramphenicol acetyl transferase (CAT). Stable lines expressing tdTomato were generated by chloramphenicol selection, and cloned by limiting dilution.

The parental Prugniaud-tdTomato parasites were electroporated with the pBT/P30OVA vector, which expresses secreted P30-OVA from the alpha-tubulin promoter and the selectable marker Ble. Stable lines expressing OVA were generated by phleomycin selection, and cloned by limiting dilution.

tdtomato and OVA expressing parasites were then engineered to secrete Cre recombinase into host cells. Parasites were electroporated with the toxofilin-Cre vector, which expresses the selectable marker HPT, and the epitope tagged rhoptry protein, toxofilin, fused to Cre. Stable lines expressing Cre were generated by mycophenolic acid selection, and cloned by limiting dilution.

Similar lines containing proteins fluorescing at different wavelengths may also be used (for example, Green fluorescent protein (GFP) in place of Td-tomato). Different background strains of T. gondii expressing these same proteins, and generated using the same methods, may also be employed (for example RH instead of Prugniaud). Risks are identical to as described for the Prugniaud Td-tomato lines.

GAD (glutamate decarboxylase) deficient line:
A cassette encoding CAT or a phleomycin resistance cassette and a gentamycin resistance marker was amplified by PCR, adding 50 bp matching the immediate 5’ and 3’ flanks of the TGME49_080700 (GAD) locus. These cassettes were introduced into EL250 bacteria carrying cosmid TOXP947 and recombination was induced by heat shock. Clones carrying recombinant cosmids were isolated by kanamycin/gentamycin double selection and replacement was verified by restriction analysis. The modified cosmids were transfected into a ∆Ku80 Prugniaud background (deficient in non-homologous end joining) and chloramphenicol or phleomycin resistant parasites were established and cloned. Parental ∆Ku80 Prugniaud parasites will also be used in this study.
**Origin & function**

Td tomato is a fluorescent protein. It will be used to identify parasites in infected cells and tissues.

Ovalbumin is used as a model T cell antigen and is the most abundant protein in egg white.

Cre recombinase, derived from the bacteriophage P1, is an enzyme that catalyzes site specific DNA recombination. It is used to determine when host cells have been actively invaded by the parasite.

CAT, HPT and Ble are selectable marker genes. CAT is a bacterial enzyme responsible for chloramphenicol resistance. HPT (T. gondii) is a non-essential nucleotide salvage pathway enzyme. Reintroduction of this gene into HPT mutants confers resistance to culture in mycophenolic acid. Ble, derived from the bacterial transposon, Tn5, confers resistance to phleomycin.

**Evaluation of foreseeable effects**

There is no evidence to suggest that expression of Tdtomato, OVA, or Cre-recombinase changes the pathogenicity, immunogenicity, host range, tissue tropism or means of transmission of the parasite.

TdTomato and ovalbumin can be considered inert markers, and there is no evidence to suggest any harmful effects. Cre recombinase is only functional in other hosts if exogenous LoxP sites have been introduced. There is an extremely low chance of an endogenous LoxP site occurring in a mammalian genome. Phleomycin and Chloramphenicol are not effective treatments for Toxoplasmosis in humans, so the presence of the selectable marker genes should not pose an additional risk. GAD deficient parasites exhibit a modest reduction in virulence in mice compared to the parental strain based on survival, time to death, or weight loss.

We will be working with the tachyzoite and bradyzoite/tissue cyst forms of the parasite. These are susceptible to most disinfectants and are unlikely to survive or persist if accidentally released. Laboratory exposures are most often associated with the use of sharps. Splashing of concentrated parasite preparations onto mucous membranes may also result in infection. Prophylactic chemotherapy is available following known exposures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

We will be working with the tachyzoite and bradyzoite/tissue cyst forms of the parasite. Infectivity is lost following exposure to most disinfectants and to heat.

Solid waste will be autoclaved (discard cycle - 126 degrees for 30 minutes), followed by incineration by an external contractor. Transport to autoclave is in autoclave bags contained within plastic, lidded bins.

Liquid waste will be autoclaved (discard cycle - 126 degrees for 30 mins). Transport to autoclave is in bottles sealed with a screw cap and placed within plastic bins.

The autoclave undergoes annual service and thermocouple validation by a priorclave engineer. Prints of runs are available. In the event of autoclave failure, chemical disinfection may be used. The ability of 2% virkon to inactivate parasites has been validated in-house.
Assessment circulated in May 2013
Committee met on 21st May 2013
28th June comments sent by UBSA who also sits on IB GMSC but had not been able to attend the meeting

1.2 Information on the strains used was requested to be added - completed
4.1 'Class 2 Microbiological Safety Cabinet' - altered to ClassII
Transport between buildings to be following triple packaging principles –clarified
4.2 RA/SOP requested for intraperitoneal infection of mice and Vibratome sectioning –provided
4.4 Discard cycle must be defined - completed
4.4.1 Complete means of transport to autoclave - completed
Alternative arrangements in the event of autoclave failure will be by chemical inactivation not autoclave in Ronald Ross building.
4.4.2 In house testing of 2% Virkon for inactivation completed by Senior Technician
4.5.2 More detail required for spillage information - completed
4.6.1 All workers will be notified of risks to immunocompromised/pregnant individuals - notices are posted on laboratory door when work is taking place with T.gondii

Please enter comments on the GM safety committee on the risk assessment

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### Project Ref 554/16.2

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<td>Determining how genome variability of enterovirus 71 affects the biological and clinical</td>
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<td>≤ 1 Litre</td>
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Enterovirus 71 (EV71) is an emerging cause of mass outbreaks of hand-foot-and-mouth disease, with neurological complications, in Asia which threatens the UK. Using virus isolates, reverse genetics and clinical material collected already from our studies in Asia (Solomon et al, Lancet, 2010), plus tissue culture models, and in house ‘Omics approaches (Isabel García-Dorival et al, JProteome, 2014) RNA-Seq will be used to identify and quantify the host cell transcriptome response to infection, conduct a bioinformatics comparison for severe versus mild disease, and validate the findings using molecular/cell biology approaches. The ultimate aim is to determine avenues for treatment of this potentially devastating infection.

To achieve this aim, recombinant EV71 virus will be produced from the transfection of plasmids containing the genome of EV71 into mammalian cell culture, resulting in the expression of virus that is antigenically homologous to that of the native virus originally isolated. The plasmid contains a RNA Pol I promoter at the beginning of the EV71 genome which, when transfected into the human RD cell line, will facilitate transcription of mRNA which will translated and form infectious virus.

Using site directed mutagenesis, the region of the genome that produces the immunogenic capsid protein of the virus will be mutated and a substituted with a triple codon sequence of another EV71 that has been associated with a differing severity of disease. The plasmid can then be used to express the recombinant virus and assessment of virulence change can be measured via cell culture methods and changes in host transcriptome can be measured by transcriptomic and proteomic methods.

Recipient or parental organism
Recipients of the specialized expression plasmids are common laboratory authenticated commercial mammalian cell lines Human Rhabdomyosarcoma (RD) cells, Vero and A549 Cells purchased from ECCAC.

Host/vector system
ThermoFisher Scientific generic bacterial plasmid with Kanamycin resistance, RNA Pol I promoter, HDV Ribozyme Cleavage site and murine terminator sequence to be transfected into Human Rhabdomyosarcoma (RD) cells, Vero cells or A549 cells for virus production.

Origin & function
Three synthetically generated EV71 genomes originally isolated from patients in Malaysia with varying degrees of severity. The strains to be inserted are similar to Human Enterovirus 71 isolate S19841-SAR-03. They are B5 genotypes.
The inserted genome will result in the expression of fully formed infectious virus, identical to the native virus originally isolated.

The inserted material will produce infectious EV71 when transfected into cell culture, this is potentially hazardous to humans. It is known to cause Hand, Foot and Mouth Disease (HFMD) and in severe cases neurological and cardiac complications, it is transmitted via faecal-oral route.

Site directed mutagenesis on the antigenic viral epitope may have a positive or negative effect on the pathogenicity of the virus depending on the sequence used to mutate the gene. However often the results of mutations results in an attenuating affect.

Given the linear, non-segmented genome of enteroviruses, the likelihood, in the event of a containment breach, of it being able to result in any gene transfer or recombination is low. Recombination could only occur if the plasmid was in cell culture alongside another EV71 strain. No wild type or other recombinant variants will ever be in the same vicinity as another. Therefore gene transfer cannot occur. In the event that gene transfer did occur, the resultant GMOs will be no more harmful than the original recipient.

**Evaluation of foreseeable effects**

There is a potential risk to human health in the event that there is a containment breach however as long as the general laboratory safety procedures and control measures stated in risk assessment are followed there is no greater risk to human health than that of any other hazard group 2 pathogen.

While the most severe EV71 genome isolate inserted may have caused neurological or cardiac complications, it cannot be solely assumed that this was as a result of the virus; complications with the hosts’ response may have been a factor in the development of a more severe illness.

Resultant GMOs of culture origins are unlikely to survive in the environment, as their optimal growth conditions are not commonly found outside of the laboratory. EV71 is does not code for an animal or insect toxin or another product that could silence a gene encoding any crucial metabolic enzyme in susceptible hosts.

Enterovirus 71 genome does not encode any material for drug or antibiotic resistance, including those currently used for medical treatment, including animals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Used cell culture flasks directly to autoclaved at a minimum of 121°C (load temperature) for 15 minutes before being destroyed as clinical waste.

Stripettes to be soaked overnight in 2% Virkon* then incinerated.

Solid waste will be autoclaved at a minimum of 121°C (load temperature for 15 minutes and may then be disposed of via the normal industrial waste skip).

Any cell culture media will be collected in reinforced glass bottles (as per standard practice in IC2) and then autoclaved at a minimum of 121°C (load temperature) for 15 minutes.

Process testing will be thermocouple testing schedule

*See manufacture’s data: https://www.chemours.com/RelyOn/en_US/assets/downloads/europe/Virkon_Lit_No_C-1.pdf
Poliovirus belongs to the same genus and species as EV71, however is a different serotype. It has been demonstrated by the manufacturer that virkon decontaminates poliovirus, therefore it can be inferred that virkon should also disinfect EV71

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

CU2 Form on HSE website to be completed and signed. The final document to be sent to Lesley Andrews. Lesley will send to the HSE with details of payment, (the correct method to be discussed and arranged with Steph Sanford, IGH Finance Team). Lesley will advise on the start date of the project.

Start date on the risk assessment form will need amending.

Infectious dose is not known.

Section 2.1 – Cells lines – phrase is ‘authenticated commercial cells lines’ and to include where purchased from.

3.1a Further ID of cells needed, also mention virus here.

3.1b – Elaborate on how pathogenicity of viruses increases or decreases – route of infection needs to be included

4.1 – now Class II cabinet. Virus pipetting mentioned – all tissue work to be carried out in cabinet

4.3 – Scale of the project is good – add number of replicates – plan for standard experiment of 6 or 9 flasks

4.4 – Remove plant treatment of solid waste, tick autoclave box for solid waste. Glassware – to be reinforced – no breakable glass to be used

4. In the event of autoclave break-down – correct transportation SOP has already been completed. Transport left blank, means of validation to be supplied by Debby Sales. Correct link to Virkon to be used. Carry out some validation experiments on Virkon, send validation data. Carry out in-activation experiments and record outcomes.

Project Containment

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

The spirochetes include the aetiological agents of ruminant digital dermatitis (DD), human syphilis and leptospirosis. Digital dermatitis and leptospirosis are endemic, global diseases which results in severe disease in agricultural ruminants such as cows, sheep and goats. As such, these diseases causes significant welfare and economic concerns. Physical characteristics of pathogenic spirochetes are poorly characterized largely due to their fastidious nature. The aim of this study is to validate and use a novel expression vector which will enable expression of bacterial and specifically spirochetal outer membrane proteins in *Escherichia coli* to define novel functions. This will ultimately should aid in the development of potential treatments, vaccines and diagnostics.

Initial validation of plasmid will include construction of recombinant plasmids containing both spirochetal (*Treponema spp.* and *Leptospira spp.*) and non spirochetal bacterial (*Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Wolbachia*) genes encoding outer membrane proteins (OMPs) whilst subsequent use of plasmid to study bacterial function will be limited to spirochete genes only.

Transformation of the recombinant plasmids into the commercially available protein expression host *E. coli* BL21 AI or BL21 DE3.

Heterologous expression of these bacterial outer membrane proteins in the outer membrane of transformed *E. coli*.

Demonstrate functionality of OMPs heterologously expressed in *E. coli*.

Demonstrate proteins can be purified from *E. coli* outer membranes.
Escherichia coli TOP10 and BL21-Al or BL21 DE3. Escherichia coli with the exception of non-pathogenic strains are HG2 biological agents (http://www.hse.gov.uk/pubns/misc208.pdf). E. coli TOP10 is a lab strain disabled to such an extent that it is unable to cause harm to human health and therefore is considered as HG1 (http://www.hse.gov.uk/biosafety/gmo/notifications/publicregister.pdf). Specifically E. coli TOP10 is a laboratory strain which contains recA and endA mutations that prevent undesirable recombination events and carryover nuclease in miniprep DNA, respectively. E. coli BL21-Al or BL21 DE3 is/are lab strain routinely used as an expression host. BL21 have been shown to be non pathogenic (Chart et al., Journal of Applied Microbiology (2000) 89: 1048-1058) and therefore can be classified as HGI. For BL21-Al and BL21 DE3 the expression of the recombinant proteins is dependent of a supply of the inducing agent, Arabinose and/or IPTG respectively. The use of an inducible expression system, as opposed to a constitutive expression system, is a deliberate choice to add an extra layer of control over protein expression. In the event of a containment breach, protein expression will be diminished. Maintenance of the recombinant plasmid (within either host) on which the protein is encoded is dependent on the presence of ampicillin. Removal of ampicillin will quickly result in the loss of the recombinant plasmid due to its metabolic burden on the host. Comparison between these lab adapted strains and pathogenic strains have typically identified around 20 virulence genes difference between commensal and pathogenic E. coli (Lu S, et al., Emerg Microbes Infect. 2016 Dec 7,5(12):e122. Bekal et al., J Clin Microbiol. 2003 May;41(5):2113-25. Chapman TA, et al., Appl Environ Microbiol. 2006 Jul;72(7):4782-95,) and have a long established history of safe use. Given here we are only inserting a single gene under tight regulation the rule of producing a pathogen should be considered negligible. Indeed it is widely considered that a single gene is insufficient to convert commensal E. coli pathogenic E. coli, and that instead a combination of genes encoding toxins, colonization factors and other functions are required to make a commensal E. coli pathogenic (Kaper JB et al., Nat Rev Microbiol. 2004 Feb;2(2):123-40).

**Host/vector system**

- Pent-D-TOPO, a gateway entry vector
- pDESpMOMP, a modified Invitrogen pDEST17 expression vector.
- All pDEST and pENT vectors are non-mobile
- pENT vectors do not have expression functionality

**Origin & function**

- porA from Neisseria meningitidis
- wsp from Wolbachia pipientis
- ompL 1, from Leptospira interrogans
- TP0326 from Treponema pallidum
- + 12 Further predicted adhesins from Treponema spp. and Leptospira spp.

The genes of interest are Leptospira/Treponema-specific genes that encode surface-exposed proteins. We already have data that these genes encode cell surface proteins with functions of binding to the host extra-cellular matrix or wound healing proteins.

**Evaluation of foreseeable effects**

The generated E. coli strains are predicted, upon induction, to be heterologously expressing a recombinant protein in their outer membrane. (Details of the proposed proteins are below). All of the described proteins below are considered putative virulence factors through the mechanism of adhesion with the exception of PorA which is a porin.
However, the addition of the above proteins to E. coli BL21 AI or BL21 DE3 is unlikely to cause the strain to become virulent in human or animals. This is thought for several reasons:

Several mechanisms are required for E. coli to become pathogenic. E. coli BL21 does not carry the well-recognized pathogenic mechanisms required to cause the majority of enteric infections and are unlikely to survive in host tissues and cause disease (for example, BL21 primary outer membrane protease, OmpT, is disabled). Addition of a single virulence factor, in the absence of other virulence factors, is unlikely to cause the strain to regain its ancestral pathogenic potential.

The expression of the recombinant proteins is dependent of a supply of the inducing agent, Arabinose or IPTG.

The use of an inducible expression system, as opposed to a constitutive expression system, is a deliberate choice to add an extra layer of control over protein expression. In the event of a containment breach, protein expression will be diminished.

Maintenance of the recombinant plasmid on which the protein is encoded is dependent on the presence of ampicillin. Removal of ampicillin will quickly result in the loss of the recombinant plasmid due to its metabolic burden on the host.

Additionally observation of good laboratory practices should minimize any risk. In the event of a breach in containment, absence of the plasmid maintenance and protein expression dependent features described above (Arabinose and IPTG and Ampicillin) will render the strain unable to express the recombinant proteins.

Further detail on proteins encoded by cloned genes:

- **PorA**: N. meningitidis PorA is a major outer membrane porin [1]. It is a major component of the meningitis B recombinant vaccine. Additionally, it is speculated to have some pathogenic potential. PorA was assumed to have function in antimicrobial resistance; however, this was recently disproven [2].

- **TP0326**: The Treponema pallidum TP0326 protein is a BamA ortholog [3]. These proteins are conserved in all Gram-negative bacteria, both pathogenic and non-pathogenic. Its function is involved in the maintenance of the outer membrane. As such, it is not considered a virulence factor. Previous heterologous expression of TP0326 in E. coli did not cause any observable phenotype.

- **WSP**: Wsp is a major outer membrane protein of the arthropod endosymbiont Wolbachia pipientis [4]. Wsp function is not well understood, but is thought that it may play some role in adhesion to endosymbiont host [5]. Therefore it could be argued that this protein has the ability to provide E. coli with increased adhesin to cellular structures. While this is a possibility, it is unlikely that this will cause the strain to become virulent in humans or animals.

- **OmpL 1**: Leptospira interrogans porin L 1 is an outer membrane protein with the ability to adhere to specific ECM components [6].

- **12 Further predicted adhesins from Treponema spp. (n=6) and Leptospira spp. (n=6)**. The genes of interest are Leptospira/Treponema-specific genes that encode surface-exposed beta barrel proteins. We have produced data that demonstrate these genes as encoding cell surface proteins with functions of binding to the host extra-cellular matrix or wound healing proteins. In each case only one gene will be added for expression and functional study. The addition of single adhesins to E. coli in the absence of other virulence factors, is unlikely to cause the strain to regain its ancestral pathogenic potential.

Given the bacterial strains are lab attenuated the addition of a single gene with adhesive or porin ability is unlikely to enable the production of a pathogen. However it could be considered that there is some limited risk given the outer membrane proteins (OMPs) from Treponema spp. or Leptospira interrogans could enhance virulence of the E. coli host strains, enabling invasiveness and colonization of the human gastrointestinal tract (if accidental exposure/infection of a person were to occur). Furthermore there may also be potential for extra-intestinal infection and the virulence determinants required to enable extra intestinal infection are less clear. Taken together these
Concerns we consider this contained use activity is considered to be class 2.

References


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM solid waste material including contaminated agar plates, cell culture plastic ware, pipette tips, microplates and tubes etc are to be autoclaved (waste transferred either weekly or when bin full for autoclaving) with autoclave at 126 degrees C for 30 minutes. Primary machine is a Priorclave which runs this program and back up machine is a Rodwell autoclave. Autoclaved waste will then be incinerated externally. Liquid GM waste including cell growth media will be placed in loosely sealed pyrex duran bottles and autoclaved at 126°C, 30 min. Liquid waste will then be washed down the sink with copious amounts of water. Contaminated surfaces will be disinfected with 1 % Distel. In house autoclaves are validated annually by a United Kingdom Accreditation Service (UKAS) calibration test. Degree of kill is 100%. Process is monitored by use of autoclave tape* Distel has been validated for deactivation of E. coli at 1 % concentration (https://www.starlabgroup.com/GBen/ sidelcumentldownload/186645_123)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
Circulated 5/9/18. The Leahurst Departmental Genetic Modification Safety Committee met on 12th September 2018 and it was suggested that the work proposed did present a greater hazard than working with the wild-type organism and that the proposed level of containment potentially needed to be amended to category 2. Amendments on containment and waste disposal streams were suggested. After additional review the project was deemed Class 2. Agreed approval on chair’s action subject to amendments. Approval 21/9/18 subject to HSE notification.

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Project Ref 554/19.1

Date Ackn’d 29/11/2019  CU2 Project Title Connected programme of work on the functional and immunological characterisation of virulence determinants from select human microbial pathogens in one laboratory

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Non-GMM Consent Granted

Tick if notifying a connected programme of work Y

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

(Scientific purpose, aim and Justification)

Our research group focuses on the scientific understanding of the pathogenesis and genomics of human pathogens from ACDP category 2, with a particular emphasis on identifying and characterising virulence factors, immune resistance determinants and potential vaccine and drug targets from bacterial pathogens. Our work involves genetic manipulation of lab-adapted and commensal strains that colonise or infect humans and the cloning e.g., knockout or insertion, expression and characterisation of virulence determinants from these strains into class 1 disabled laboratory strains of bacteria such as Escherichia coli or food-grade Lactobacillus Lactis for heterologous expression.

The following is an overview of the GM research activities that form our connected programme of work:

Activity 1. Creation of knock-out and insertion mutations in genes encoding for virulence factors in Streptococcus pneumoniae, S. agalactiae, and S. pyogenes - and determine the effect of these mutations on various phenotypic properties including adhesion and invasion properties, growth characteristics in vitro, e.g., cell lines and biofilm models, and virulence properties in vivo using previously established mouse models e.g., carriage, invasive pneumonia, sepsis, septic arthritis.

We will clone DNA fragments from the Streptococcal species (listed above) into non-mobilisable vectors in laboratory strains respective of each bacterial species. It is often necessary to clone genes or DNA fragments of interest from class 2 pathogens into class 1 disabled laboratory strains of bacteria such as E. coli to enable subsequent analysis such as DNA sequencing or to enable further constructs to be engineered. The results obtained in Activity 1 will inform part of the work carried out in Activity 2.

Activity 2. Expression of vaccine candidate genes (identified in Activity 1 or elsewhere) in a disabled bacterial host for the purpose of assessing their immunogenicity and protective efficacy in murine models. We will exclude from this assessment deliberate cloning of intact soluble toxin genes, hence toxin will only be expressed in their non-lytic variant form i.e., toxoid.

Within the last 20 years the concept of bacterial vector-based (BVB) vaccines has gained renewed interest due to increased immunological understanding and availability of molecular techniques making possible the construction of safer bacterial vector-based vaccine formulations. BVB vaccines include genetically modified and attenuated strains of human commensal bacteria whereby high levels of expression can be achieved hence facilitating evaluation of the immunogenicity and protective efficacy of vaccine gene candidates. The following class 1 microorganisms will be used as vaccine antigen-BVB formulations:

1. food-grade Lactococcus lactis strains
2. derivatives of the Escherichia coli K-12 strain which have been demonstrated to be avirulent, and have a long history of safe use in humans
3. auxotrophic aroA-deleted virulence attenuated Salmonella typhimurium

These 3 BVB systems were selected on the basis of recent WHO literature reporting their safe use in humans for oral (i.e., nasopharynx or gut by oral gavage) and parenteral (e.g., intramuscular, intravenous, intraperitoneal) delivery routes. (WHO meeting report 2003, Informal consultation on characterisation and quality aspect of vaccines based on live vectors, Lin, et al. (2015). Live-Attenuated Bacterial Vectors: Tools for Vaccine and Therapeutic Agent Delivery. Vaccines, 3(4), 940–972).

Work carried out in activity 2 strictly pertains to the in vitro and in vivo handling of the BVBs described within this document, but does not pertain to any of the mutagenesis or cloning experimental procedures that have been/will be used to generate the BVBs. The vaccine-antigen BVB formulations will strictly be outsourced from commercial sources e.g., Thermo Fisher Gene Art.

Recipient or parental organism

Activity 1:
- Streptococcus pneumoniae
- Streptococcus agalactiae
- Streptococcus pyogenes

Activity 2:
- Food-grade Lactobacillus Lactis
- Escherichia Coli K12 and derivatives
- AroA-deficient attenuated Salmonella Tiphymurium*

Host/vector system

Activity 1:
Any of the following expression vectors (i.e., plasmids) may be used for transformation into the 3 streptococcal species of interest:
1. Suicide vectors for Site directed mutagenesis (pucMUT, pACH74 and pAS1TAG)
2. Transposon mutagenesis (pR412, pJR233 and pJDM-STM)
4. GFP expressing integrative plasmids (pBaSysBioll, pBSU101, pGFP, YFP, pCFP, pLS1GFP, pMV158GFP, pLS70GFPcat, pMV158GFP)
5. pLS758, pMSP3535, pCEM, pCTet, Janus are used for tagging or replacement of genes with resistance genes against tetracycline, erythromycin and Kanamycin.
6. pET is an expression vector for protein overproduction.
7. pHY304 is a temperature-sensitive plasmid for insertional mutagenesis.
8. pSIV is an inducible shuttle vector system.

Activity 2:
Any of the following plasmid vectors may be used:
Lactobacillus Lactis: pTRKH3-slpGFP, pTRKH3-ermGFP, pT1NX, pPA32, pVE5547
Escherichia Coli K12: pBR322, OPPF-UK pOPIN vectors, PCDNA3.1
Salmonella Tiphymurium: pBR322, pSC101, pmQ117 and derivatives.

In this coordinated programme of work, the vectors that will be used are well-characterised vectors that (i) are either non-mobilisable or mobilisation-defective in bacteria; and (ii) contain only selective markers that are already in routine use in standard cloning vectors; and (iii) contain no recognisable harmful sequences e.g., Schedule 5 of the ACS ACT 2001 or substances prohibited by the Chemical Weapons Convention.

Origin & function

All genetic materials will be derived synthetically or via production of genetic material in E. coli using standard molecular biology approaches.

Activity 1:
The genes studied in this work will derive from streptococcal species: S. pneumoniae, S. agalactiae, S. pyogenes. The genes may be involved in nutrient uptake, metabolism, signaling, host immune responses, in vitro growth and cell wall biosynthesis and assembly. The aim of this work is to establish the functions of genes involved in these essential processes through gene deletion and observing/characterizing the phenotypes of these mutant strains in murine models, in comparison to their wild-type counterparts.

Activity 2:
The vaccine candidate genes studied in this work may derive from any of the family of proteins listed below, which will be expressed in one or more of the bacterial hosts listed in section 2 of this document:
Bacteria: Various cell-wall associated proteins (e.g., choline-binding proteins), surface-exposed proteins involved in complement activation as well as various neuraminidases, IgA proteases, adhesins and metalloproteases, and pore-forming toxins e.g., pneumolysin, streptolysin, β-hemolysin/cytolysin. Other virulence factors are those that promote immune evasion and resistance to host defences.
Viruses: hemagglutinin, genes of the polymerase complex, neuraminidases, non-structural glycoproteins, envelop proteins.
Parasites: proteases, surface antigen e.g., gp46/M2 or parasitesurface antigen 2 (PSA-2), kinases, ribosomal proteins, flagellar antigens.

The risk assessment for Activity 2 does not pertain to any of the mutagenesis or cloning experimental procedures that will be used to generate the genetically modified bacterial hosts – as these will be outsourced commercially or via the University of Liverpool Technology Directorate - but only to the handling and evaluation of their immunological properties in vitro and in vivo. In other words, the risk assessment for Activity 2 does NOT pertain to the handling or genetic modification of any ADCP 2 pathogens. In all cases, the intent is to assess the immunogenicity and protective efficacy of food-grade or disabled microorganisms class 1 genetically modified to express a non-functional subunit protein, i.e., a truncated protein variant containing immunodominant epitopes only and no biological function.

Evaluation of foreseeable effects

RISKS TO HUMANS:
• Exposure of humans to the bacterial species listed in section 5, by inhalation or contact with contaminated aerosols could occur in the event of a breach of containment such as the spillage of viable culture. Sepsis, respiratory or skin inflammatory reactions could result. However, this is highly unlikely as the strains used in this project are either lab-adapted or disabled strains, hence represents a very low risk to human health. Streptococcus agalactiae will infect cattle but is only of risk to severely immunocompromised human individuals.
• All of the vectors are in common usage for bacterial research. None of the vectors are likely to transfer genetic material to any other environmental microorganisms. The
genes which will be transferred or deleted in this investigation belong to specific targeted functional classes the expression or loss of expression of which is highly unlikely to influence the pathogenesis of the hosts. Expression of antibiotic resistance cassettes for selection use resistance to antibiotics (e.g. ampicillin, kanamycin, gentamicin or streptomycin), which will confer resistance to antibiotics well below the therapeutically used levels.

- The selection strategy will specifically exclude the use of genes such as toxins or known allergens.
- For the reasons outlined above the risk to humans from exposure to the GMMs is highly unlikely to be greater than exposure to the original parental strains. None of the deletion/insertion mutants, or other manipulated strains are likely to be any more or any less pathogenic than the parental strains. The GMMs are expected to exhibit the same level of pathogenicity as the parental strains and will therefore be handled under the same level of containment (class 2) and with the same stringent handling and disposal procedures as the unaltered parental strains as described in the available associated local risk assessment, SOPs and COPs.

**ENVIRONMENTAL RISKS:**

- The foreseeable effects include the introduction of antibiotic resistance genes which could represent a hazard if released into the environment and transferred to other organisms. All GMOs and GM material will be decontaminated and sterilized before disposal and operational protocols described herein, hence make release unlikely. Therefore transfer of genetic material to other environmental microorganisms remains extremely unlikely.
- Other than those hazards intrinsic to the vectors named above, there is no risk to the environment. All necessary safety precautions will be taken so that there is sufficient containment with no environmental release i.e., bagging, autoclaving and/or incineration of all disposed biological materials.
- The environmental survivability of our GMO is envisaged to be the same as their wild type, with the advantage that without appropriate selection pressure reversion to the wild type is likely, hence there is no increased risk to the environment posed by the GMM compared to the wild type.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For preparation of plasmid DNA/protein, bacteria will be collected by centrifugation and lysed. Liquid waste material will be rendered non-viable by decontamination overnight with Virkon (1%) and autoclaving prior to sink disposal. Any contaminated solid waste (e.g. contaminated disposable plasticware, agar plates, DNA) will be either bagged or placed in lidded Disposafe jars, and autoclaved (121 degrees C for 30 minutes) prior to disposal.

Virkon is routinely used as per the manufacturers recommendations:

- Solid surfaces are disinfected with 1% Virkon solution.
- Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.
- Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation).
- Accidental spillages of liquid waste will be inactivated with 2% Virkon

The category II laboratory will be regularly monitored by members of staff and laboratory manager to ensure good housekeeping and hygiene is regularly maintained with particular emphasis on treatment and disposal of infectious biological waste.

Monitoring:

- Disinfection: The disinfectant of choice (Virkon) is used in strict accordance with the manufacturer’s (Antec’s) guidelines.
- Autoclaving: To ensure 100% efficacy, testing of the autoclave is carried out annually, by the manufacturer, to demonstrate, using a 12 point test with independent thermocouples, that the correct temperature and pressure have been reached for the required time. On subsequent runs verification that the correct conditions were reached is obtained through use of a temperature probe in the centre of the load. Testing is arranged and test reports are kept by the Health and Safety Co-ordinator.
- Inspections: Safety inspections are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health and Safety Co-ordinator.
- Microbiological Safety Cabinets: Tested on a six monthly basis with annual KI test. Certificates of conformity are displayed on each cabinet and copies kept by the Health
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<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
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</tr>
<tr>
<td>Tick to confirm that you have attached a risk assessment</td>
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<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
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Please enter comments on the GM safety committee on the risk assessment
In relation to Activity 1 risk assessment:
Assessment circulated 06/06/19, meeting held 27/06/19, revised RA received 16/07/19 and approved 17/07/19.

Panel comments on original submission:
Panel agreed with proposed class II designation but felt that elements of the RA required clarification. More detail needed on at-risk groups (e.g. pregnant women for GBS).
Could get potential virulent strains
Home office license number missing
Pregnant women should not work with Strep B
Section 1.1: Project title - need to change title to include 'Knockout and Insertion'
Section 1.1: Location of work – need to add BSU to this section
Section 1.2: HSE notification number missing
Section 2.1: ‘routine basis’ – needs to be more specific.
Section 3.1b, Inserted genetic material: needs to be re written
Section 3.1b, Direct effects: Info needed
Section 4.2: May use sharps at BSU?
Section 4.3: More detail required. How often? Scale? How much?
Section 4.4: Plastic container, not a metal container. Swap Sweetie jar to Disposafe jar. Overnight soaking in 1% Virkon, not 20 minutes. For liquid waste, place Duran bottle and/or plastic tubes in to appropriate plastic container for autoclaving.
Section 4.4.1: Include room numbers for autoclave rooms on 1st floor and 2nd floor. Replace ‘BMM Weston engineers’ with ‘Autoclave engineers’

UPDATED 17/07/19: Revised RA received and reviewed. All suggested changes have been made and a class II designation confirmed.

UPDATE 24/07/19 – from University of Liverpool Biological Safety Officer:
The UBSA was provided with the signed off risk assessment on 18 July 2019. She had not been invited to the GMSC meeting and provided feedback on the RA on 19 July 2019.
A general comment is lack of justification of statements- you must justify every statement and give details as prompted by the notes- read the notes please.
1.1 location of work requires more details, room numbers and space refs if possible for all areas. In vivo work presumably in BSU not TC rooms as stated. Start date needs amending.
1.2 1.2 page 3 there is a reference to HSE notification xxxxx?
It's good to future proof by including the Tn option.
The animal work does not appear to be described? This is very important- to describe the work procedures, hazards to human health and the environment and the control measures- HSE scrutinise this very carefully.
2.1 state hazard groups of recipients, state properties of the vectors, are they mobilisable, transmissible? State origins of the inserts- where is this genetic material from?
2.2 is there a possibility that with virulence genes knocked out the organism may become less pathogenic? This would be worth mentioning although of course it's unlikely to lose its pathogenicity altogether as multiple factors are involved.
3.1b- what they need to address is whether the antibiotics used for selection are used therapeutically? Consider if someone became infected could they be treated effectively with another antibiotic?
Effect of gene transfer contains some good reasoning but must consider IF the antibiotic resistance genes were transferred to other bacteria what could be outcome?

3.1.1 consult the notes on what is required- this doesn’t answer the question. – consequence of exposure- likelihood of exposure, any uncertainties must be recorded.

3.2 must focus on environmental risk- not repeat what has been said for human health 3.2a I think there are other Streps that can infect animals but these three can’t? 3.2.b – concentrate on environment and give justification in the Summary

4.1 tissue homogenisation suddenly appears with no indication of how we got there! This work flow needs to be included in the RA. I would put it into section 1.2

4.2 the information provided seems to be focused on sharps bins and disposal but information is required about use of sharps and control measures- HSE have asked for more information on this in the past and will scrutinise it in detail. This is one of their “things”

4.3 not sure what Committee is referred to here but there is no BSU GMSC.

4.4 no contact times stated for Virkon, is 1% correct- most people say 2% or 1% final concentration. Animals?

4.4.1 trolley used to move waste?

4.4.2- do you really have in house test data- if you state it HSE may ask to see this.

In relation to Activity 2 risk assessment:

Assessment circulated 06/06/19, meeting held 27/06/19, revised RA received 16/07/19 and approved 17/07/19

The original RA contained the work proposed here together with some proposed work on viral vaccine vectors. Panel advised splitting into two separate RAs with a viral vector one designated class I and a bacterial vector one designated class II.

Additional suggestions made by the panel were:

- Need to be more specific about storage
- RA is very broad. It needs to be project specific – i.e. which viruses using?
- Aims need to be more specific.
- Could split in to 2 – viruses Class I and bacteria Class II

Section 3: More references are required.

Section 3.1b, Inserted genetic material: needs to be re written

Section 4.2: Sharps will be used. What would you do if there was a needle stick injury?

Section 4.3: How much bacteria will be used? How frequent?

Section 4.4: Autoclaves bags are not used in the main lab for autoclaving biological waste. Small plastics are put in a Disposafe jar and then transferred to an autoclave bin. Bacterial plates and large plastics (flasks, plates etc), are placed directly in to an autoclave bin. Stripettes soaked in 1% Virkon overnight.

Liquid waste – need to re-write this section. Glassware is not decontaminated with 5% Virkon prior to autoclaving. Virkon should not be autoclaved.

Replace ‘BMM Weston engineers’ with ‘Autoclave engineers’

Section 4.6: Ticked yes for OH. Please confirm.

UPDATE 17/07/19 – Revised RA reviewed and designated class II. All panel comments have been addressed.

UPDATE 24/07/19 – from, University of Liverpool Biological Safety Officer:

The UBSA was provided with the signed off risk assessment on 18 July 2019. She had not been invited to the GMSC meeting and provided feedback on the RA on 19 July 2019.

1.1- Don’t use acronyms, include details of rooms including space refs, start date amendment
1.2 - There is some confusion in the middle of second paragraph around the word order
Safe use/intended use- do you mean all recipients (not vectors?)
Again very little information on mouse work- this is essential to describe the work procedures, hazards to human health and the environment and the control measures-
HSE scrutinise this very carefully.
2.1- hazard groups of recipients? This may be 1? Properties of vectors- mobilisable, transmissible, do they express?
2.3 and 2.4 both answered no- would this not be 2.3 yes, and 2.4 not sure? As it stands it would be class 1, but class 2 selected (correctly but not justified)
31. focuses on the antibiotic resistance genes and fluorescence markers but the virulence genes are not mentioned, not considered. The work is class 2 largely because of
the virulence genes being cloned? I don’t understand how class 2 provisional is reached from the assessment provided. Summary of risks to human health states “None”
but class 2 is chosen?
3.2 does not adequately consider and justify risk to the environment, you must do this and justify statements
4.1 sudden mention of animal tissue homogenisation with no lead up to this- we don’t know what this is or how it has been generated.
4.3 scale- I think they mean bacterial cultures (not vectors)- serum? What serum- how are the hazards if any from this controlled?
This section does include some information on animal work which is helpful. However a more detailed consideration of the risk to human health and the environment is
required.
4.4 solid waste – animal carcasses? Isn’t incineration used for final disposal? Sealed containers would explode in the autoclave, is 1% Virkon correct? No contact time
stated, how long is autoclave cycle at 130?

**Project Containment**

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**Project Ref** 554/20.1

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**Project Additional Information**

**Purposes of the contained use**

We want to propagate class 2 GM respiratory viruses in the laboratory for use in infection studies. This includes rhinovirus (RV) and respiratory syncytial virus (RSV).

**Propagation of RV in cell lines**
RVC was recently identified (2006) by PCR based methods. Few studies have used RVC in infection studies as it could not be propagated in the laboratory like RVA and RVB (propagation in HeLa cells). This is due to the fact RVC uses a different receptor for host cell entry (CDHR3) than RVA and RVB, which is not expressed on HeLa cells or other tissue culture cell lines. Reverse genetics methods have now been utilised to propagate RVC in the laboratory by transfecting standard cell lines (HeLa/ WI-38/ MRC5) with viral RNA. Rhinovirus has a positive-sense SS-RNA genome, the naked RNA is infectious, therefore, it can initiate a complete viral reproduction cycle that produces infectious progeny viruses when it is transfected into a permissive cell. Using this method RVC cannot propagate between cells but can replicate within the transfected cells to produce viable virus that can be isolated and purified for further infection studies.

We will use plasmids already constructed by collaborators and construct our own plasmids that contain a whole genome cDNA copy of RVC, RVA and RVB genomes. The majority of the strains propagated are wild type (WT), however, some of these plasmids will also contain a eGFP gene (fluorescent virus) which will allow us to visualize viral infection during infection studies. RNA generated from these plasmids when transfected into permissive cell lines will allow us to propagate these viruses and purify them for use in infection studies in primary human airway epithelial cells (can be naturally infected by all RV strains).

**Propagation of RV in HeLa-E8 cell line**
RVC uses a different receptor (human Cadherin Related Family Member 3 (CDHR3)) for host cell entry, than RVA and RVB, which is not expressed on HeLa cells or other cells lines normally used to propagate RV strains in the laboratory. Current methods used to propagate RVC in the laboratory for infection studies are by transfecting standard cell lines (HeLa/ WI-38/ MRC5) with infectious viral RNA. However, this virus cannot propagate between cells. As the virus cannot spread to other adjacent cells the amount of virus produced is limited, limiting the scope and number of experiment that can be performed.

CDHR3 has multiple alleles, one of these has been associated with severe asthma exacerbations (GG>AA). This AA allele has been shown to cause increased RVC binding and replication as it results in increased CDHR3 expression on the cell surface. So in order to get round the fact RVC doesn’t naturally infect HeLa cell line, which are used for RVA and RVB propagation, these cells have been engineered to express the CDHR3 asthma allele (AA). This allows RVC to naturally infect cells and spread to adjacent cells during infection. Negating the need for the long winded reverse genetics cloning approaches that are currently required to work on RVC strains. We will use this cell line, which has been engineered and will be provided by collaborators, to propagate RVC viral strains via natural infection (clinical isolates) or through transfection of a whole genome copy of viral RNA into the GM cell line.

**Propagation of fluorescent tagged RSV strains**
Fluorescent tagged RSV will be propagated in Hep2 cell line, with infection occurring naturally. Infection in these cells will allow viral propagation and subsequent purification for future infection studies in primary human airway epithelial cells (can be natural infected by all RSV strains). The RSV fluorescent tagged virus will be purchased commercially from ViraTree (https://www.viratree.com), RSV-GFP5/ RSV-DsRed5/ RSV-FarRed5, which all grow similarly to the parent virus.
GM virus will be propagated in:

- Commercially available cell lines (HeLa/ WI-38/ MRC5/ Hep2)
- GM modified cell line (HeLa-E8)
- Primary human airway epithelial cells (nasal/ bronchial brushings)

Cells infected with virus

HeLa - HeLa cells are the oldest and most commonly used cell line and are derived from human cervical cancer. These are widely used in the virology research field and are used for propagation of rhinovirus in the laboratory.

WI-38 - is an immortalised human cell line composed of fibroblasts derived from lung tissue of a 3-month-gestation aborted female fetus. These are widely used in the virology research field and are used for propagation of rhinovirus in the laboratory.

MRC5 - is an immortalised human cell line composed of fibroblasts derived from lung tissue of a 14 week old aborted caucasian male fetus. These are widely used in the virology research field and are used for propagation of rhinovirus in the laboratory.

Hep2 - This line was originally thought to be derived from an epidermoid carcinoma of the larynx, but was subsequently found to have been established via HeLa cell contamination. These are widely used in the virology research field and are used for propagation of RSV in the laboratory.

Primary human airway epithelial cells - obtained from nasal or bronchial brushings. Cells are widely used in the respiratory research field as they are the main target of a number of respiratory viruses, including RV and RSV. RVC can infect these cells naturally.

HeLa-E8 - GM modified version of HeLa cell line, which expresses the CDHR3 RVC receptor.

Production of the HeLa-E8 cell line is described in Bochkov et al, 2015 (1) and in patent US20190055521A1. Briefly, to make this HeLa-E8 cell line, the mutation in domain 5 (C529Y) of CDHR3, which is a naturally occurring mutation (AA allele) seen in around 3% of the population, was engineered into a lentiviral vector containing wild-type CDHR3 sequence (GG allele) under the control of a CMV promoter to express the gene of interest. Causing high level expression of CDHR3 on the HeLa cell surface, and the resultant transduced cells were susceptible to RVC infection. In nature people with the AA allele express CDHR3 protein at higher levels on the cell surface compared to the GG allele. This allele is associated with childhood asthma with severe exacerbations, and has been shown to result in increased binding, infection and viral replication during RVC infection.

Adventitious agents are not present in established immortalised cell lines. Primary human cells should be treated as potentially infectious and contaminated biological specimens and handled at Biosafety Level 2.


Host/vector system

Construction of GM viruses using reverse genetics

Reverse genetics methods are required to propagate RVC in the laboratory by transfecting standard cell lines with viral RNA. Rhinovirus has a positive-sense SS-RNA genome, the naked RNA is infectious, therefore, it can initiate a complete viral reproduction cycle that produces infectious progeny viruses when it is transfected into a permissive cell. The virus can then be purified and use in infection studies in primary airway epithelial cells.
The RNA for transfection is amplified from plasmids in which a full length cDNA copy of the viral genome has been cloned (7.2kb). RNA is generated from these plasmids using the Promega RibomAX Large Scale RNA Production Systems, generating a RNA copy of the viral genome via T7 RNA polymerase. Construction of the plasmids used for RNA generation is described in our class 1 bacteria GM risk assessment. This procedure is also described in detail in Rhinoviruses Methods and Protocols, chapter 12 and Bochkov et al 2011 and 2015 1,2.

Some of these plasmids have been provided to us by our collaborators Professor Gern (University of Wisconsin), who developed the methodology for RVC viral replication (2). We will also generate plasmid containing a cDNA copy of the RV genome from clinical isolates. Some of these will also contain a GFP tag. Therefore, viruses generated using this methodology will be wild type viruses (cDNA cloned from clinical isolates) or GFP tagged viruses. If GFP tagged the eGFP gene sequence will be inserted in-frame between proteins VP1 and 2A protease (as described in Bochkov et al, 2015 (1). This gene is inserted between the two 2A protease recognition sites (LISSA/GPS) to release the GFP from viral polyprotein upon translation. Virus generated from this plasmid will fluoresce during infection and this will allow us to visualise viral infection in tissue culture and primary cells.

Rhinovirus GM virus

For fluorescence microscopy studies we will also use a fluorescent tagged RSV, which are commercially available and have been constructed as described on the ViraTree website (https://www.viratree.com), RSV-GFP5/ RSV-DsRed5/ RSV-FarRed5. These GM viruses generated in wild-type parent RSV strain A2 are modified to express the fluorescence proteins from a gene cassette placed between the P and M genes.


HeLa-E8

HeLa-E8 cells have already been constructed and will not be modified further. Production of the HeLa-E8 cell line is described in Bochkov et al, 2015 (1) and in patent US20190055521A1.

CDHR3

pLX304 expressing wild-type CDHR3 was purchased from TransOmic. The pLX304 vector provides a high level expression of CDHR3, and the resultant transduced cells were susceptible to RVC infection. The vector is publicly available. The mutation in domain 5 (C529Y) of CDHR3 was engineered into this vector containing wild-type CDHR3 sequence. This genetic change causes increased expression of CDHR3 on the HeLa cell surface, making them susceptible to RVC infection. Both alleles of this gene are naturally present in the human population.

The exact role of CDHR3 in the host is not 100% clear but the protein once produced is found within the cell or cell surface associated. It is a member of the Cadherin Related Family Member proteins, cadherins are important for the formation of adherence junctions so it is hypothesised to be involved in cell to cell adhesion.

GM viruses

For production of viable virus A RNA copy of the viral genome is generated from plasmids provided to us by collaborators or constructed by ourselves (described in detail In our GM bacterial class 1 RA). RNA is generated from cDNA using the Promega RibomAX Large Scale RNA Production Systems, generating a RNA copy of the viral genome via T7 RNA polymerase. Viruses generated in this manner are wild type but still classed as GM, except the strains where a eGFP gene has been incorporated into the genome creating a fluorescent virus, these are not wild type. cDNA that is cloned into the plasmids is generated from patient sample (such as nasopharyngeal aspirates) that have been infected with RV naturally. The eGFP gene which encodes a green fluorescent protein will be amplified from the pC15-GFP, which was constructed as described in Bochkov et al 2015 (2). Enhanced green fluorescent protein was originally isolated from Aequorea Victoria, and produces bright green fluorescence when exposed to blue light (395nm). This gene and is widely used in the research field as a reporter gene.

RSV strains, for fluorescence microscopy studies, are commercially available and have been constructed as described on the ViraTree website (https://www.viratree.com), RSV-GFP5/ RSV-DsRed5/ RSV-FarRed5. Infectious viral virions will be provided to us to propagate in the laboratory. In these strains the fluorescent genes are cloned on an expression cassette between viral genes P and M. This is inserted into parent RSV strain A2. Genes cloned into these strains to make them fluorescent include eGFP (RSV-GFP5) as noted above. RSV-DsRed5 contains a variant of Discosoma sp. red fluorescent protein which has faster maturation and lower non-specific aggregation. It fluoresces red/orange when excited with light at 488/ 532nm. RSV-FarRed5 expresses super bright far-red fluorescent protein mKate2, an improved version of a monomeric far-red fluorescent protein from sea anemone Entacmaea quadricolor (excitation/emission maxima at 588 and 633 nm). All proteins are widely used in the research field.

1- Bochkov, Y. A. et al. Cadherin-related family member 3, a childhood asthma susceptibility gene product, mediates rhinovirus C binding and replication. Proc Natl Acad
Overview

Viral GM

Reverse genetics methods are required to propagate RVC in the laboratory by transfecting standard cell lines with viral RNA. Viral RNA used for cell transfection is amplified from plasmids in which a full length cDNA copy of the viral genome has been cloned (7.2kb). These have been provided to us by our collaborators Professor Gern (University of Wisconsin), who developed the methodology for RVC viral replication. Plasmids provided to us contain a full length cDNA copy of the viral RNA genome from RVA (pA16-R16.939), RVB (pB52-Rz_V.2), RVC (pC15-Rz) and RVC GFP tagged (pC15-GFP). The cDNA genome has been cloned downstream of a T7 promoter. RNA is generated from these plasmids using the Promega RiboMAX Large Scale RNA Production Systems, generating a RNA copy of the viral genome via T7 RNA polymerase. We will use the same methodology to create plasmids that contain a cDNA copy of the viral RNA genome of clinical RVC strains. Some will also be GFP tagged using the same methodology as that used to generate pC15-GFP.

RSV strains

For fluorescence microscopy studies using RSV, we will use fluorescent tagged RSV strains, which are commercially available and have been constructed as described on the ViraTree website (https://www.viratree.com), RSV-GFP5/ RSV-DsRed5/ RSV-FarRed5. These GM viruses generated in wild-type parent RSV strain A2 are modified to express the fluorescence proteins from a gene cassette placed between the P and M genes. Using these strains we can visualise viral infection of cells.

HeLa-E8 cell line GM

HeLa-E8 cells have been produced using a lentiviral vector systems as described above. They have been provided to us by collaborators. HeLa-E8 cells express the CDHR3 viral receptor for RVC on their cell surface, and are used for RVC propagation in the laboratory as RVC can naturally infect these cells. These cells will be used to propagate RV through natural infection and also by transfection of a whole RNA viral genome into cells. This Includes WT RV clinical isolates and GFP tagged RV clinical isolates.

Direct effects

HeLa-E8

The cell line HeLa-E8 is not infectious. For the construction of this cell line lentiviral vectors were used, these are self inactivating and replication incompetent so would not still be present. The transfer plamid (pLX304) selected for in the HeLa-E8 clone contains no genetic material that could result in lentiviral replication and progeny production. Further, once we receive the cell line the cells will not contain any plasmid DNA.

If the cells were released into the environment they would not survive as they require media enrichment to survive. The CDHR3 gene transfected into the cells is naturally occurring, this transfected Y529 allele that is present in the human population causes increased cell surface expression of CDHR3 on the HeLa cells resulting in increased viral binding and replication. This phenomenon is also the same in the cells of people who carry this allele (Y529). The single amino acid change in the protein does not change the total cellular expression of the CDHR3 protein but increases the levels present at the cell surface. The exact role of CDHR3 in the host is not 100% clear but the protein once produced is found within the cell or cell surface associated, it would not be release into the tissue culture media or be toxic to humans if we encountered it. These cells are unable to colonise workers and contains no adventitious agents.

Environmentally these cells are human derived and would be unable to colonise plants or animals, and contains no adventitious agents. The CDHR3 protein is human derived and it would not have any effects on plants or animals if released. There are animal homologues of this protein but as RVC is solely a human pathogen, genetic changes in this protein would mean it is different enough to not function the same as the human CDHR3. Therefore, it would not be toxic to animals or plants in the unlikely event it was released.

Propagation of GM virus

Transfection of a complete viral RNA genome into tissue culture cells results in production of live infective virions, which can be isolated and purified for infection studies. All viral strains used in this study originally came from clinical isolates and are therefore pathogenic. Except the fluorescent tagged viruses, all tagged strains have been shown to grow similarly to the wild type strain and addition of these genes gives no advantage to the virus. Propagation of all viruses noted in the RA pose potential hazard to health, they are class 2 respiratory pathogens. Both viruses in healthy individuals cause mild cold/ flu like symptoms. Disease can be more severe in children, the elderly, and people with underlying health conditions.

These viruses are solely human pathogens and they can spread when an infected person coughs or sneezes. You can get infected if you get droplets from a cough or...
sneeze in your eyes, nose, or mouth, or if you touch a surface that has the virus on it, like a doorknob, and then touch your face before washing your hands. These viruses can survive for several hours on hard surfaces, for shorter periods on fabrics. 40% of rhinoviruses are still infectious on hands after an hour.

These viruses are solely human pathogens. There are animal infection models for both of these pathogens (RV/ RSV) but these require large doses of virus for infection and often don’t recapitulate the symptoms of human disease. Therefore, these viruses don’t pose a direct infectious threat to any plant or animal present in the external environment, bar humans. The viral infected cells (cell lines/ primary cells) cannot survive outside of cell culture as they require media supplement to survive so pose no threat to the environment.

Indirect effects
HeLa-E8 cells

The CDHR3 receptor is expressed on human cells and is the receptor for RVC. The CDHR3 allele cloned into the HeLa cells is naturally occurring. In these cells the CDHR3 protein is expressed to higher levels on the cell surface. Its role in the host is not fully clear but it hypothesised to be involved in cell to cell adhesion. Overexpression of this protein doesn’t affect the cells in any way that would make them hazardous to work with.

These HeLa-E8 cells are used for propagation of RVC, which uses this CDHR3 receptor in nature to infect host cells. Genetic mutation have been noted in the viral strains after multiple passaged in HeLa-E8 cells, but these changes made the virus more infectious for these cells and less infectious in primary human cells and was therefore less virulent in humans.

There are animal homologues of CDHR3, but as RVC is solely a human pathogen, genetic differences in this protein mean it is different enough to not function the same as the human CDHR3. Therefore, any genetic changes that occur in the infecting virus would not make them more virulent for animals or plants. To prevent the chance of genetic changes occurring in viral progeny a maximum passage number of 5 will be used when creating viral stocks.

Propagation of GM virus

Infection of the cell lines/ primary cells should not be effected by the virus. These viruses have no DNA intermediates so there is no potential for viral genetic material to integrate into the cells genetic material. All cells lines and primary cells are widely used in the virology field. These cells are unable to colonise workers and contains no adventitious agents. Therefore, the cells do not pose a hazard to human health. If the cells were released into the environment they would not survive as they require media enrichment to survive.

There is the potential for genetic changes to occur in the viral pathogens. This occurs naturally in these viruses which are generally very genetically variable, especially rhinovirus. Infection of these cell lines with virus could lead to the virus adapting to growth in these cell and uncontrolled genetic changes occurring in the virus. Genetic changes have been noted after multiple passaged of the virus in cell lines. However, this often results in better growth of the virus in the cell line used for propagation rather than increased infectivity and growth in primary cells. So, would reduce its infectivity in primary human cells. Infection of primary human cells will only be for infection studies (virus not passaged) reducing the chance of viral mutations occurring that would make them better at growth in human cells. Further the primary AEC donor will never be that of the person growing the cells, preventing the potential for the viruses to develop a tropism for growth in that donors cells, and become more virulent.

The infected cells pose no hazard to the environment. If the cells were released into the environment they would not survive as they require media enrichment to survive. Genetic changes that could occur after multiple passages would not make them more infective for animals or plants as all cell lines are all human derived and viruses solely human pathogens. To reduce the chance of any genetic mutations occurring in the viruses a maximum passage number of 5 will be used to make viral stocks using all cell lines.

Gene transfer
HeLa-E8 cells

CDHR3 is stably integrated into the HeLa-E8 genome and cannot be transferred between this cell line and others. The lentiviral vectors used for gene delivery are self inactivating and replication incompetent, therefore no lentiviral particles would remain in the cells once received from collaborators.

RV has no DNA intermediates during its replication cycle. Therefore, viral genetic material would not be incorporated into the genetic material of these cells. If the cells were released into the environment they would not survive as they require media supplement to survive. The viruses themselves if released into the environment could survive for a short period outside the host.

Propagation of GM virus

These viruses can survive for several hours on hard surfaces, for shorter periods on fabrics. 40% of rhinoviruses are still infectious on hands after an hour. Both viruses have no DNA intermediates during their replication cycle. Therefore, we would not expect viral genetic material to be incorporated into the genetic material of the cells in which the viruses are propagated/ infected. If the cells were released into the environment they would not survive as they require media supplement to survive. The viruses themselves if released into the environment could survive for a short period outside the host, we would not expect the viruses if coming into contact with other
similar viruses to exchange genetic material. There is a small chance if the virus was to infect the same cells of the same type of virus that genetic material could be exchanged. Horizontal gene transfer in this manner does not commonly occur in these types of virus. Further, the majority of the viruses used are wild type and are circulating in the human population, the exception being the fluorescent tagged viruses. The addition of the fluorescent tags to these viruses has not changed the virulence of these strains as shown by similar growth characteristics of the parent strain and would not be more hazardous to humans. These viruses do have the potential if infecting a human to be transferred person to person as this is the natural route of infection of these viruses.

Summary
HeLa-E8 cell line
The constructed cell line poses no threat to human health and the environment, it is not infectious and would be unable to survive if not supplemented with tissue culture media. The cell line contains a gene that is already present in nature. This cell line is solely used for propagation of RV strains and therefore all work will be performed in a class II microbiological safety cabinet (MSCs), therefore risk of release of the cell line or propagating virus is extremely low. Expression of the CDHR3 gene in this cell line does not increase the virulence of viral strains we are infecting them with. No sharps will be involved in the process outlined. Transfer of material outside of the class II microbiological safety cabinets (MSCs) will be in sealed containers.

Propagation of GM virus
The main hazard is release of the GM respiratory viruses and potential for infection of lab members, as these viruses can survive for a short period on inanimate objects outside of the host. The majority of viruses used are not GM and the ones that are tagged with fluorescent markers are not more pathogenic than the WT strain. If infection did occur we would expect this to result in mild cold symptoms as seen in the majority of the human population, as all lab members are healthy adults. If the GM virus infects the same cell of someone already infected with that virus there is potential for viruses to exchange genetic material. However, the majority of the viruses used are wild type and already circulate in nature, bar the fluorescent tagged viruses. The addition of the fluorescent tags to these viruses has not changed the virulence of these viruses.

There is a small chance if the virus was to infect the same cells of the same type of virus that genetic material could be exchanged. Horizontal gene transfer in this manner does not commonly occur in these types of virus. Further, the majority of the viruses used are wild type and are circulating in the human population, the exception being the fluorescent tagged viruses. The addition of the fluorescent tags to these viruses has not changed the virulence of these strains as shown by similar growth characteristics of the parent strain and would not be more hazardous to humans. These viruses do have the potential if infecting a human to be transferred person to person as this is the natural route of infection of these viruses.

Summary
HeLa-E8 cell line
The constructed cell line poses no threat to human health and the environment, it is not infectious and would be unable to survive if not supplemented with tissue culture media. The cell line contains a gene that is already present in nature.
This cell line is solely used for propagation of RV strains and therefore all work will be performed in a class II microbiological safety cabinet (MSCs), therefore risk of release of the cell line or propagating virus is extremely low. Expression of the CDHR3 gene in this cell line does not increase the virulence of viral strains we are infecting them with. No sharps will be involved in the process outlined. Transfer of material outside of the class II microbiological safety cabinets (MSCs) will be in sealed containers. Transfer of material between laboratories will also be done in accordance with the Carriage of Dangerous Goods Regulations if live virus is being transferred. Members working with class 2 agents will be appropriately trained. We have an appropriate disinfection procedure in the event of a spillage/accidental release.

Propagation of GM virus

The main hazard is release of the GM respiratory viruses and potential for infection of lab members, as these viruses can survive for a short period on inanimate objects outside of the host. The majority of viruses used are not GM and the ones that are tagged with fluorescent markers are not more pathogenic than the WT strain. If infection did occur we would expect this to result in mild cold symptoms as seen in the majority of the human population, as all lab members are healthy adults. If the GM virus infects the same cell of someone already infected with that virus there is potential for viruses to exchange genetic material. However, the majority of the viruses used are wild type and already circulate in nature, bar the fluorescent tagged viruses. This gene does not alter viral virulence. There is the possibility of viral strains acquiring genetic changes which could alter their virulence, however this is no more likely than the chance of this occurring in nature. The most likely outcome is the pathogens change to adapt to become less virulent due to their passage in cell lines. Passage of virus will not be performed in primary human cells, these will only be used for infection studies. A limited passage number of 5 will be used when propagating viruses in cell lines. The likelihood of infection is extremely low as all work will be performed in a class 2 microbiology cabinet. No sharps will be involved in the process outlined. Transfer of material outside of the class 2 biosafety cabinet will be in sealed containers. Transfer of material between laboratories will also be done in accordance with the Carriage of Dangerous Goods Regulations if live virus is being transferred. Members working with class 2 agents will be appropriately trained. We have an appropriate disinfection procedure in the event of a spillage/accidental release. If plates/tubes need to be opened at sites other than IITP, this will be performed in a class II microbiological safety cabinet (MSCs).

The viral GM strains pose a negligible hazard to the environment. These viruses are solely human pathogens and are not infectious in animals or plants

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste: Pipettes, culture flasks, centrifuge tubes, filters, syringes, infected cultures on transwells
Solid waste will be autoclaved (126°C for 45mins free steam cycle). All autoclaved waste will be subsequently incinerated.
Degree of kill: 100%

Liquid Waste:
Virus containing medium from viral propagation and viral infection studies and medium from growth of HeLa-E8 cells will be disposed of as below.
Liquid waste will be autoclaved (126°C for 45mins free steam cycle) followed by disposal down a sink with copious amounts of water.
Degree of kill: 100%

Viral infected cultures can also be chemically inactivated using 2% Virkon, mixed 1:1 with liquid waste (final concentration 1%). Waste can be disposed of down the sink after 1h with copious amounts of water. Autoclave disposal will be used preferentially over chemical disinfection.
Degree of kill: 100%

Primary Autoclave: Astell Scientific Autoclave Model AMA250BT65.
Room laboratory 1st floor, Institute in the Park, Alder Hey
Alternative Autoclave: Astell Scientific Autoclave Model AMA340POJ.
Room 0624 Centre for Women's Health Research

Autoclave testing/monitoring procedures:
Both Astell Scientific Models: Annual Thermocouple test and calibration by service engineer.
Committee suggested splitting RA into multiple RA, so class 1 is included in one RA and all class 2 work on a separate RA.

Committee asked to include an extra site so that we have a back up autoclave in the event of a failure of the one in the IITP.

Recommendation to avoid transferring waste/GM cultures between laboratories where possible trial alternative viral purification methods to ultracentrifugation.

Stipulated before undertaking experiments evidence must be provided to show no viral aerosols are created when adding neutrophils to live infective culture during microscopy (see GM RA 2).

Committee agreed with Class 1 designation for RA 1 and Class 2 designation for RA 2 and 3. Lesley Andrews (UoL Biological Safety Advisor) will undertake notification to HSE once relevant forms are completed.

Project Containment

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</tbody>
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Animal Units

- L2
- L3
- L4

Large Scale Activities

- L2
- L3
- L4

Human Clinical Applications

- L2
- L3
- L4

Project Ref 554/20.2

- Date Ackn’d: 17/06/2020
- CU2 Project Title: Understanding patterns and drivers of evolution in microbial communities using labelled strains and targeted knockouts
- Class: Class 2
- CultureVolClass2: 1-50 Litres
- Non-GMM: Consent Granted
- Date Project Ceased: 02/03/2022

Project notified under transitional arrangements: N
Bacterial evolution plays an important role in ecosystem functioning, and has a significant effect on human health and disease, food security, and industrial output. Interactions between species play a key role in determining the trajectory and tempo of evolution. Species interactions can involve competitive and facilitative interactions between organisms, and can also involve horizontal gene transfer. Characterising the interactions between bacteria is a necessary step in understanding the patterns and drivers of microbial evolution. To achieve this, genetic tools are routinely used to track different members of the community, and to generate targeted gene knockouts to assess the causal contribution of different alleles to fitness. Genetic markers are usually resistance genes or encode fluorescent proteins. Generating knockouts also routinely involves the use of selectable resistance markers.

Recipient or parental organism

- The mini-Tn7 system, used extensively in Pseudomonas species (Bao et al. 1991 [Gene 109 (1): 167–68], Choi et al. 2005 [Nature Methods 2 (6): 443–48], Koch et al. 2001 [J. Microbiol. Methods 45 (3): 187–95], Lambertsen et al. 2004 [Env Microbiol. 6 (7): 726–32]) inserts DNA into a single, known, non-coding attTn7 site in the genome. For Pseudomonas aeruginosa, selectable markers and reporters, with or without the lacIq repressor, will be introduced into the delivery vector pUC18T-mini-Tn7 which is unable to reproduce in Pseudomonas. The construct will then be inserted into the recipient Pseudomonas by transformation alongside a helper suicide plasmid pTNS2 which carries the Tn7 transposase genes in trans. Selection for the mini-Tn7 cargo (i.e. lacIq alongside selectable markers) results in the outgrowth of clones which have acquired the mini-Tn7 cassette into the attTn7 site. Candidates will be screened by PCR, targeted sequencing, and phenotypic assays to ensure they are of the expected genotype.
- A homologous recombination method which replaces an allele at a single known genomic site (Court et al. 2002 [Ann. Rev. Gen. 36:361-388]). The replacement allele and ~2 kb of flanking region is cloned into the multiple cloning site of the suicide vector pTS-1, which cannot replicate in Pseudomonas. Selection for the genes carried by pTS-1 results in the outgrowth of merodiploid (single crossover) integrants. Selection against pTS-1 integration using sucrose (pTS-1 encodes SacB which renders carriers sensitive to sucrose) results on double crossovers whereby in some cases the resident allele has been replaced.
- Introduction of the plasmid pRL27 which harbours an active kanamycin resistance Tn5 derivative (Larsen et al. 2002 [Arch. Microbiol. 178.3: 193-201]). Introduction of pRL27 is likely to result in the insertion of the Tn5 derivative into a resident replicon, potentially causing gene disruption.
- Plasmid curing will be achieved by one of two routes. (i) Introduction of the sacB gene into the plasmid by pTS-1-mediated homologous recombination as described above. Selection on sucrose favours strains which have lost the plasmid. (ii) Introduction of the CRISPR-encoding vector pCAS9 derivative pNAW136 (Jiang et al. 2013 [Nat. Biotech. 31 (3): 233-239]). This plasmid encodes the Cas9 nuclease and a locus into which CRISPR spacers can be introduced by PCR. Plasmids can be cured by
introducing anti-plasmid spacers, which cause Cas9 to degrade the plasmid DNA. pNAW136 is unstable due to a low copy number origin of replication (RK2) and thus is quickly lost in the absence of selection.

Candidates will be screened by PCR, targeted sequencing, and phenotypic assays to ensure they are of the expected genotype. Generated strains will be used in growth experiments, including long-term culture, fitness assays, and gene expression assays.

### Origin & function

<table>
<thead>
<tr>
<th>Selectable and reporter markers:</th>
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</thead>
<tbody>
<tr>
<td>- <code>aacC1</code> (encodes gentamicin acetyltransferase), confers resistance to the antibiotic gentamicin.</td>
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<tr>
<td>- <code>strpAB</code> (encodes aminoglycoside 3-phosphotransferase), confers resistance to the antibiotic streptomycin.</td>
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<tr>
<td>- <code>aphA1</code> (encodes aminoglycoside 3-phosphotransferase II), confers resistance to the antibiotic kanamycin.</td>
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<tr>
<td>- <code>cat</code> (encodes chloramphenicol acetyltransferase), confers chloramphenicol resistance.</td>
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<tr>
<td>- <code>teiAB</code> (and similar), confers tellurite resistance.</td>
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<tr>
<td>- <code>teiAR</code> genes, confer tetracycline resistance.</td>
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<tr>
<td>- <code>sacB</code> gene, confers sucrose sensitivity.</td>
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<tr>
<td>- <code>lacZ</code> (encodes beta-galactosidase), results in production of blue pigment when grown on X-gal.</td>
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<tr>
<td>- The <code>lux</code> reporter operon, which encodes luciferase.</td>
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<tr>
<td>- Fluorescent proteins, including eYFP, eGFP, eCFP, tdTomato, mCherry, tagBFP, and similar and derived genes.</td>
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</table>

Insertion cassettes will express fluorescent proteins under the control of the lac derivative promoter PA1/03/04 or Pτac which are constitutively expressed in Pseudomonas. Where a non-lac-repressible promoter is required the rmB1 promoter or the pLpp promoters will alternatively be used.

Selectable markers are expressed from either their own native promoter, or the Pc promoter.

### Evaluation of foreseeable effects

1. The GMOs will be modified to carry selectable markers and reporter genes, including antibiotic resistance genes and fluorescent protein-encoding genes outlined above. These modifications will modulate specific resistance to particular antimicrobials and other chemicals (outlined above) and cause the modified strains to exhibit particular fluorescence or luminescence properties. The biggest hazard comes from the introduction of antibiotic resistance genes, as accidental release could accelerate the spread of antimicrobial resistance and raise infection risk, particularly for those who are immunocompromised e.g. with cystic fibrosis.

2. The GMOs will be modified to delete endogenous genes, in particular to generate strains that fail to produce metabolites and secretions that mediate cell-cell interactions, and/or virulence factors such as pyocyanin and lasR protease. Many of these and similar knockout mutants generated previously have reduced growth and virulence properties relative to wild-type, so their release would be unlikely to pose any additional hazard.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not applicable.**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Not applicable.**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

1. Solid waste, including: agar plates and spent solid culture media; glass media bottles; microtitre plates; liquid handling tips.

Waste is inactivated by autoclaving before disposal. Plastic waste will be placed in sealed boxes and transported to the linked Life Sciences building for autoclaving at...
126°C for 20 minutes.

Contaminated single use pipette tips are placed into dedicated yellow “Bio-Bins” for incineration. These are labelled and sealed before they leave the laboratory. Contaminated plates and single use tubes are disposed of via yellow incineration bags, labelled and sealed with a cable tie. These are transported to the appropriate clinical waste bins outside Life Sciences Building Stores in a clearly marked UN-compliant CL2 transport container.

2. Spent culture media.

Autoclaving is the preferred route for inactivation of GMMs. Cultures will be autoclaved in the Life Sciences building at 126°C for 20 minutes. These autoclaves have a computerised log of all runs, which can be downloaded. The autoclaves are serviced twice a year and validated/calibrated once yearly by Select Engineering Services, 13 Hurst Close, Over Hulton, Bolton BL5 1DT. Inactivated cultures will be poured down the laboratory sink and flushed copiously with water.

Where autoclaving is not possible, Virkon disinfectant will be added to final concentration of 1% w/v and left for at least 8 hours before being poured down the laboratory sink and flushing copiously with water. Virkon is an effective agent for >5-log reduction of Pseudomonas aeruginosa according to the manufacturer's guidelines, which have been supported by in-house tests.

Sample for analytical flow cytometry will be inactivated by fixation with formaldehyde/glutaraldehyde. Each batch of fixative will be validated by spreading samples of fixed cells on solid media and inspecting for growth.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment
The IIB/SoLS BSSC met on 30th January and reviewed this GMM CL2 risk assessment. The committee requested the following modifications.

1. This RA is a Class 2 and must be notified to HSE.
2. The university safety officer advised Dr Hall there is a process before this work can be carried out:
   • Jamie to complete an HSE form (JB to email the form to Jamie).
   • Fee approx. £1,000.00
   • Jamie to provide contact details of person who administers the finance.
   • Only when Jamie receives an acknowledgement receipt, and payment (preferably by BACS) has been cleared can the work be carried out.
3. Section 2.1 Hazard Group 2 needs to be stated.
4. Section 2.1 Clinical plasmids needs more detail.
5. Section 2.2. Queried does this increase organism’s virulence? Advised that detail in this section should state “does not make more virulent”.
6. Section 3 Need to state which antibiotics are effective treatments.
7. Section 3.1.1 Need to state no anticipated additional risk as mentioned in previous section.
8. Section 4.3 Scale of activity needs to state frequency, volume, and quantity.
9. Section 4.4 “Solid Waste” should state Bio bins are dedicated for contaminated single use pipette tips.
10. Section 4.4.1 - Need to state Autoclave cycle.
11. Section 4.4.2 - The Virkon link appears to be broken.
12. Section 4.6.1 – Warning signs to be also put on main doors.

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Animal Units

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Project Ref 554/21.1
The aim of this project is to test the protective properties of select recombinant parasite proteins when used to vaccinate against African trypanosomes (Trypanosoma vivax) in mouse models. We will vaccinate mice with multiple recombinant antigens and challenge vaccinated and control mice via subcutaneous injection of trypanosomes. We will compare patent infections in vaccinated and control mice after needle challenge to quantify the protective effect of vaccination by measuring parasitaemia with a bioluminescent assay that detects parasites anywhere within the host animal. A positive result will be complete protection against infection in vaccinated mice, that is, no pathology and no observable parasites in bioluminescent screens. The outcome of the project will be such assessment of metacyclic-stage proteins discovered by our labs in previous work. Positive demonstration of protection will lead to clinical trials of the vaccine candidate in livestock.

Trypanosoma vivax strain Y486 is a unicellular eukaryotic flagellate. It is a potent pathogen of livestock, capable of inducing life-threatening, acute infections and thereafter cyclical, chronic infections that may cause death in animals that do not self-cure. The organism requires inoculation by blood-feeding flies (Glossina spp.) to effect transmission in the natural system. The organism is incapable of infecting humans and its natural insect vector is not found outside of Africa. T. vivax is a SAPO2 organism and therefore requires containment level 2 (License SAPO/554/2016/1a).

Trypanosoma vivax strain Y486 is genetically modified to introduce specific reporter genes that will facilitate parasite imaging in the mouse. These reporter genes, which naturally provide either fluorescence or bioluminescence to many organisms, include the green fluorescent protein mNeonGreen, the red fluorescent protein mCherry, and the bioluminescence-generating firefly luciferase. Reporter genes are accompanied by selection marker genes used for the selection of trypanosomes which have acquired the reporter genes (selection drugs not used in the mouse). These genes, which naturally confer on to bacteria resistance to specific antibiotics, include hyg, neo, pac and bsr. All transgenic inserts will contain the bla gene which is used for the selection of bacteria during the molecular cloning phase of the transgene creation (not used when in mice).
The mNeonGreen gene is an engineered version (21 coding substitutions) of the Branchiostoma lanceolatum green fluorescent protein LanYFP.
The mCherry gene derives from the DsRed fluorescent protein found in Discosoma spp.
The luciferase gene is found in the Renilla spp. firefly.
The hyg gene (kinase from Streptomyces hygroscopicus), confers resistance to hygromycin B through phosphorylation.
The neo gene (aminoglycoside phosphotransferase from Streptomyces fradiae), confers resistance to neomycin.
The pac gene (puromycin N-acetyltransferase from Streptomyces alboniger), confers resistance to puromycin.
The bsr gene (deaminase from Aspergillus terreus), confers resistance to blasticidin resistance gene.
The bla gene (beta-lactamase from Escherichia coli), confers resistance to ampicillin.

Evaluation of foreseeable effects

Both reporter and drug selection genes (described above) can be considered inert markers, and there is no evidence to suggest any harmful effects. Fluorescent proteins and luciferases have been extensively used in molecular and cellular biology to make whole cells fluoresce, including pathogenic parasites such as trypanosomes, and are known to have no effects in cell physiology and virulence.
Drug selection markers (hyg, neo, pac, bsr, bla) have also been broadly used in multiple organisms to positively select for genetically modified individuals. Drug selection is only carried out in vitro so the selection drugs will not be administered to infected mice. The presence of these resistance markers in trypanosomes does not pose and additional risk since these drugs are not treated for trypanosomiasis in the host.
We will be inoculating mice with infected blood, either from frozen stabilate or existing infections. Bloodstream forms of the parasite are susceptible to most disinfectants and are unlikely to survive or persist more than a few minutes if accidentally spilt. The greatest risk to livestock is the accidental release of an infected mouse, which is subsequently bitten by a blood-feeding fly that also feeds on livestock. There is no risk to human health. Prophylactic chemotherapy is available for animals following known exposures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Infecctivity of trypanosome cells is lost following exposure to most disinfectants and to heat.
Solid waste will be autoclaved (discard cycle - 126 degrees for 30 minutes), followed by incineration by an external contractor. Transport to autoclave is in autoclave bags contained within plastic, lidded bins.
Liquid waste will be autoclaved (discard cycle - 126 degrees for 30 mins). Transport to autoclave is in bottles sealed with a screw cap and placed within plastic bins.
The autoclave undergoes annual service and thermocouple validation by a priorclave engineer. Prints of runs are available. In the event of autoclave failure, chemical disinfection may be used. The ability of 2% virkon to inactivate parasites has been validated in-house.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Assessment circulated in 4th March 2020
Committee met on 28th October 2020
The committee was content to approve the proposed work dependent upon satisfactory clarification of the following points:
• Section 1.1 room numbers/space refs and specific contained use activities must be explicitly stated - Completed
• Section 3.1 state these are SAPO 2 organisms- as this is why it’s class 2 - Stated, with licence number
• 3.1b can the GMM RA for bacterial cloning be cross referenced - RA provided and cross-referenced
• 3.2a I would add that these parasites are not found in UK livestock - Statement added.
• 3.2.1 what if an infected mouse escaped? - Careful consideration of the risk and impact added.
• 4.1 flow cytometry- is this the first mention? Where is this done? Need a proper Sop/diagram for the packaging and transport - Need for flow cytometry removed.
• What about the containment and control measures in the BSU and CPI? Remember need to comply with containment measures in Table 1c (GMMs used in animal facility) of regs in addition to Table 1 for lab containment measures. - Details of animal containment measures added.
• 4.4, Is some waste --cadavers?-- to be taken to BSU? - Waste disposal routes clarified and justified in the GMM; SOP for transport added.
• 4.4.1 virkon treatment- but not for cadavers- what if BSU autoclave broke down? - Contingency for broken autoclave stated.

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Project Ref 554/21.2

Connected programme of work on the generation of strains of A. baumannii containing defined resistance genes for use in anti-microbial discovery

<table>
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<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<td>22/12/2021</td>
<td>Connected programme of work on the generation of strains of A. baumannii containing defined resistance genes for use in anti-microbial discovery</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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Project notified under transitional arrangements N
Our research group focuses on the understanding of the pathogenesis of human pathogens from ADCP category 2 with an emphasis on anti-microbial resistance mechanisms. This work will focus on genetically manipulating a "wild-type" A. baumannii strain (ATCC17978) to introduce/knockout resistance genes, with the aim of creating model pathogens with distinctly defined resistance genotypes/phenotypes.

What follows is an overview of the GM activities pertaining to this programme of work, the technologies are similar and include a hybrid CRISPR/Cas9 and recombinase approach.

Activity 1: Generation of mutations and insertion of genes that encode for resistance factors in A. baumannii and determination of the effect of these mutations on resistance to anti-microbials.

The genes inserted and/or mutated will encode for common class of anti-microbial resistance genes including:

- **β-lactamases:**
  - Class 1: TEM, SHV, CTX-M-15, VEB-3, ACD-30
  - Class 2: IMP-1, VIM-1, NDM-1
  - Class 3: Oxa-23, Oxa-40, Oxa-51, Oxa-58, Oxa-66, Oxa-72

- **Aminoglycosides:**
  - ArmA, AAC(3)-IIa, ANT(2")-Ia, ANT(3")-Ia, APH(3")-Ia, APH(3")-Vla

- **Ade Family:** AdeR D20N, AdeR A91V, AdeR P116L, AdeR E219A, AdeR G30D, AdeR R152K, AdeR T153M

- **Tetracycline resistance:**
  - TetA, TetB, TetM

- **Fluoroquinolone resistance:**
  - gyrA: S83L, D87N, E84K
  - ParC: G78C, S80R/L/W

Activity 2: Using CRISPR-Cas9 "base-editing" to knock-out resistance genes in A. baumannii. This activity will utilise a Cas9-fused "base editor" to introduce premature stop codons into certain A. baumannii genes effectively knocking out protein expression. Guide RNAs targeting early glutamate residues (CAG and CAA) in the genes below will result in gene disruption.

- **LpxCAD operon:** LpxA, LpxC, LpxD
- **PmrAB:** PmrA, PmrB,
- **Ade family:** AdeA, AdeB, AdeG, AdeH, AdeJ, AdeK, AdeL, AdeN, AdeS
- **Omp family:** OmpA, OmpD, Omp25, Omp33
- **Porins:** CarO

Work carried out in activities 1 and 2 pertains to the handling and mutagenesis of A. baumannii. Synthesis of the tools to enable this is contained in a separate Class I activity that has been passed by the Departmental GM committee at Liverpool.
2. Acinetobacter baumannii (ATTC17978)

**Host/vector system**

**Activity 1:**
The following plasmids will be used for modification of A. baumannii:
1. **pCasAb-apr_121998**
   - Ampicillin resistance cassette, S. pyogenes Cas9, A. baumannii RecAb recombinase system.
   - Contains the broad-host range plasmid RSF1010 replication of origin (allows replication in E. coli and A. baumannii).
2. **pSGAb-km_121999**
   - Contains WH1266 and ColE1/pUC replication origin (replication in A. baumannii and E. coli respectively). SacB counter-selection marker, Kanamycin resistance cassette and an S. pyogenes guide RNA cassette.
3. **pSGAb-spe_122000**
   - Contains WH1266 and ColE1/pUC replication origin (replication in A. and E. coli respectively). SacB counter-selection marker, Spectinomycin resistance cassette and an S. pyogenes guide RNA cassette.
   - In addition a DNA template containing the gene to be inserted flanked by regions of homology to the A. baumannii genome to facilitate homology-dependent repair.
4. **pAB1 expression plasmid based on the pSGAb-km plasmid backbone**
   - generated from pSGAb-km following KpnI and XbaI digestion and polylinker insertion.
   - Contains WH1266 and ColE1/pUC replication origin (replication in A. baumannii and E. coli respectively). Kanamycin resistance cassette and polylinker.
5. **pAB2 expression plasmid based on the pSGAb-spe plasmid backbone**
   - generated from pSGAb-spe following KpnI and XbaI digestion and polylinker insertion.
   - Contains WH1266 and ColE1/pUC replication origin (replication in A. baumannii and E. coli respectively). Spectinomycin resistance cassette and polylinker.

**Activity 2:**
1. **pBECAB-apr_122001**
   - Contains WH1266 and ColE1/pUC replication origins (replication in A. baumannii and E. coli respectively). Ampicillin resistance cassette for selection in E. coli, S. pyrogenes nCas9 (D10A) fused to Apobec-1 (cytosine deaminase), SacB counter-selection marker and a S. pyogenes guide RNA cassette.
   - The vectors are low copy and contain common selective markers. The Cas9 protein and base-editor are under a control of a lac operon which is only active following derepression with IPTG (pAB2 expression plasmid based on the pSGAb-spe plasmid backbone generated from pSGAb-spe following KpnI and XbaI digestion and polylinker insertion contains WH1266 and ColE1/pUC replication origin (replication in A. baumannii and E. coli respectively). Spectinomycin resistance cassette and polylinker.) an alolactose mimic which activates transcription from the lac operon, so no editing will occur in the absence of IPTG. The plasmids also contain a sacB counterselection marker for “curing” the plasmids following editing. The sacB gene encodes a levansucrae which when transformed bacteria are cultured in the presence of sucrose produces the cytotoxic metabolite levan effectively selecting for clones that have lost the editing plasmids.

**Origin & function**

All inserted genetic materials will be derived synthetically then cloned using standard molecular biology approaches.

**Activity 1:**
- **β-lactamases:**
  - Resistance to beta-lactam antibiotics is primarily through expression of β-lactamases which hydrolyse β-lactam rings resulting in inactivation of β-lactam antibiotics. There are several beta-lactamases associated with resistant A. baumannii strains.
  - **Class 1:** TEM, SHV, CTX-M-15, VEB-3, ACD-30
  - **Class 2:** IMP-1, VIM-1, NDM-1
  - **Class 3:** Oxa-23, Oxa-40, Oxa-51, Oxa-58, Oxa-66, Oxa-72
  - Strains expressing these proteins will retain sensitivity to aminoglycosides, tetracyclines, fluoroquinolones, and colistins.
  - Amino glycoside resistance:
    - ArmA, AAC(3)-Ila, ANT(2’)-Ia, ANT(3’)-Ia, APH(3’’)-Ia, APH(3’’)-Vla
  - Ade Family:
Strains expressing mutations in these proteins will retain sensitivity to beta-lactams, tetracyclines, fluoroquinolones and colistins.

Tetracycline resistance:
TetA, TetB, TetM
Strains expressing these proteins will retain sensitivity to β-lactams, aminoglycosides, fluoroquinolones, and colistins.

Fluoroquinolone resistance:
Mutations in the DNA gyrase subunit A (GyrA) and the topoisomerase IV subunit C (ParC) have been identified in clinical isolates and are associated with resistance to fluoroquinolones.
GyrA: S83L, D87N, E84K
ParC: G78C, S80R/L/W
Strains containing these mutations will retain sensitivity to β-lactams, aminoglycosides, tetracyclines and colistins.

Activity 2:
Guide RNA targeting early glutamines (CGA, CAA) in the following genes will be targeted to introduce premature STOP codons (conversion to TGA, TAA).
LpxCAD operon: LpxA, LpxC, LpxD
PmrAB: PmrA, PmrB,
Omp family: OmpA, OmpD, Omp25, Omp33
Porins: CarO

Evaluation of foreseeable effects

The vectors are low copy and contain common selective markers. The Cas9 protein and base-editor are under a control of a lac operon which is only active following derepression with IPTG (Isopropyl beta-D-1-thiogalactopyranoside) an alolactose mimic which activates transcription from the lac operon, so no editing will occur in the absence of IPTG. The plasmids also contain a sacB counterselection marker for "curing" the plasmids following editing. The sacB gene encodes a levansucrase which when transformed bacteria are cultured in the presence of sucrose produces the cytotoxic metabolite levan effectively selecting for clones that have lost the editing plasmids.

Risk to Humans:
Yes, but as an opportunistic pathogen, infection is nosocomial and then, only causing symptoms in immunocompromised individuals, after surgery, or those with complex comorbidities. There is a possibility of hazard to employees. Immunocompromised individuals are at a greater risk of infection and therefore should not be handling this organism. Employees should be trained in handling HG 2 pathogens and established disinfection protocols before work starts.

Risk of community spread is unlikely. It will be handled in a laboratory that deals with other HG 2 organisms and has comprehensive disinfection and waste management protocols in place. Staff are trained in handling such pathogens and new employees receive extensive training before any work starts. Laboratory access is also restricted to trained individuals.

There are many clinical options for treatment. Patients would be monitored and only treated if symptoms develop. The treatment strategy is then empiric and guided by the anti-biogram. So there is no one strategy, but many options which would depend on the anti-biogram.

Exposure to humans of A. baumani and the modified strains could occur in the event of breach of containment through aerosols or spillage. Risk of aerosol transmission will be minimised by the use of a Class 2 biological safety cabinet. There is no risk of A. baumannii infection from aerosols in a healthy, non-immunocompromised individual. Anti-biograms will be performed on the modified strains and clinical options will be available, if required.

None of the genes we intend to modify are recognised virulence genes so modified strains will not pose a greater risk of infection than the wild-type strain (ATCC17978).

Risk to the Environment:
The genes are genomically encoded and not within any mobilizable genomic element so the risk of horizontal transfer is low.
The Class 2 laboratory in which this work will be carried out, personnel regularly work with hazard group 2 micro-organisms and their AMR variants procedures are in place for containment which are covered by a specific Group 2 organisms General Risk Assessment.

Protocols include:
Restricting laboratory access to authorized individuals
Risk assessment-specified disinfection procedures.
Safe storage for organisms and cell lines.
Laboratory procedures which give rise to infectious aerosols are conducted in a microbiological safety cabinet. Bench surfaces are decontaminated at the end of the day and following a spillage. Protocols have been in place for many years regarding waste management and disinfection protocols to mitigate against environmental transmission. Risk of community spread is unlikely. It will be handled in a laboratory that deals with HG 2 organisms and has comprehensive disinfection and waste management protocols in place. Staff are trained in handling such pathogens and new employees receive extensive training before any work starts. Laboratory access is also restricted to trained individuals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

E. coli:
For preparation of plasmid DNA and basic cloning/manipulation, bacteria will be collected by centrifugation and lysed according to the plasmid purification kit instructions. Liquid waste will be disinfected using Virkon (1%) prior to sink disposal.

NEB Stable E. coli (recA1): From NEB MSDS: Biological Safety Level One (BSL-1) containment, using standard microbiological practices, is suitable for work involving well-characterized microbiological organisms not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. Centers for Disease Control and Prevention (CDC) Guidelines (Biosafety in Microbiological and Biomedical Laboratories, 5th Edition).

A. baumannii:
Virkon is a broad spectrum disinfectant, which when used at a concentration of 1% has been shown to cause a 5-log reduction in A. baumannii counts. It has also been demonstrated to have activity against MDR A. baumannii and has been used to control outbreaks in healthcare settings. Doidge M, Allworth AM, Woods M, Marshall P, Terry M, O'Brien K, Goh HM, George N, Nimmo GR, Schembri MA, Lipman J, Paterson DL. Control of an outbreak of carbapenem-resistant Acinetobacter baumannii in Australia after introduction of environmental cleaning with a commercial oxidizing disinfectant. Infect Control Hosp Epidemiol. 2010 Apr;31(4):418-20. doi: 10.1086/651312. PMID: 20175684.

Monitoring:
The laboratory in which this work will be carried out already handles several hazard group 2 MDR organisms and has already specific containment and disinfection procedures in place. Agar plates with bacterial cultures will be autoclaved (121°C for at least 60 min) prior to disposal and incineration. Tubes contaminated with bacterial medium will be autoclaved (121°C for at least 60min) prior to disposal and incineration. For larger spills contaminated tissue paper will be transferred to a biohazard bag and autoclaved. Smaller spills will be wiped with 1% Virkon solution followed by 70% Ethanol and contaminated wipes disposed as solid waste in biohazard bags (autoclaving cannot be used when using Virkon). Autoclaving will be the preferred method of decontamination of solid waste but in the event disinfection is required vessels will be completely immersed in 1% Virkon overnight (according to the Manufacturer's instructions).

Inspections:
Safety inspectors are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are up to date. Reports are kept by the Health and Safety Coordinator.

Biological Safety Cabinets:
Cabinets are tested every 6 months with and annual KI test. Certificates of conformity are displayed on each cabinet and copies kept by the Health and Safety Coordinator.
A. baumannii is currently unclassified and therefore not listed on the HSE’s approved list of biological agents (ACDP), although it is listed on the PHE website as a Class 2 pathogen. A justification was therefore written for the Biological Safety Officer who agreed to locally classify it as Class 2.

Regarding comments of the local GM safety committee:

Activity 1: Recombinase-Aided CRISPR-Cas9 Insertion of Resistance Genes into Acinetobacter baumannii

Researchers applying for local classification as Hazard group 2 because unclassified in ACDP.

Risk assessment received 03/09/2021
Circulated to Committee 11/09/2021
Discussed at committee meeting 16/09/2021 with researcher

“Recombinase-Aided CRISPR-Cas9 Insertion of Resistance Genes into Acinetobacter baumannii” was discussed. The committee requested the following amendments:

- Please address comments from above consistent with all 3 GMRAs
- In Section 4.6.1, remove section on “severe burns”

Pending amendments the proposal was formally approved as Class 2 and will be submitted for HSE approval by UBSA
- In Section 1.1, revise start and end dates
- In Section 2.2, indicate the most hazardous GMM to be constructed
- In Section 4.3, provide more detail regarding the scale of activity
- In Section 4.4, replace bacterial with bacteriocidal
- In Section 4.4.1, remove reference to liquid waste transportation
- In Section 4.4.1, correct references to ITM

Activity 2: Inactivation/Mutation of antimicrobial resistance genes using Base-Editing in Acinetobacter baumannii

“Inactivation/Mutation of antimicrobial resistance genes using Base-Editing in Acinetobacter baumannii” was discussed. A baumanii is currently unclassified in ACDP and the research group is seeking approval from HSE to classify the bacteria locally as hazard group 2. The committee requested the following amendments:

- Please address comments from above consistent with all 3 GMRAs
- Add a table of abbreviations
- Indicate / acknowledge that the plasmids are transmissible
- In Section 4.4, rewrite section on inactivation including amend/correct “hood surface”, remove mention of liquid waste in solid waste section (Section 4.4.1), remove wording of “out of my control” (Section 4.4.1)
- In Section 4.4.2, provide information and not just a link
- Move information from Section 4.5.3 to Section 4.6
- In Section 4.6, provide details

Pending amendments the proposal was formally approved as Class 2 and will be submitted for HSE approval by UBSA

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**Project Containment**

<table>
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02/03/2022
Our project aims to understand how the immune systems of calves respond to Cryptosporidium parvum infections. C. parvum is a parasite that infects the gastrointestinal tract of many animals. In cattle, infection is most pronounced in very young calves of less than six weeks old, where it often results in diarrhoea. C. parvum negatively impacts both the animals' welfare and productivity by compromising gut function during infection and longer-term.

We are interested in using B-cells isolated from C. parvum-infected calves to produce anti-Cryptosporidial monoclonal antibodies. It has been demonstrated that the co-expression of human B-cell lymphoma 6 (BCL-6) and human B-cell lymphoma extra-large (BCL-XL) causes the immortalisation of B-cells. The immortalisation of B-cells using this method allows for the continuous production of monoclonal antibodies. The continuous supply will in turn allow us to assess the neutralising effects the antibodies could have on C. parvum. We hypothesise that supplementing milk replacer with antibodies against C. parvum will enable farmers to protect their calves from this parasitic infection, particularly where natural passive transfer has failed.

We will transfect pMIEG3-BCL6 and pMIG-BCL-XL plasmids into HEK293T cells (a Human Embryo Kidney epithelial-derived cell line), along with human immunodeficiency virus (HIV) or murine leukaemia virus (MLV) gag/pol, and VSVG packaging vectors to produce pseudoviruses that are capable of delivering the human BCL-6 and human BCL-XL genes. We also propose to use reporter genes (luciferase and/or GFP) as controls for the production of the pseudovirus. We will use both MLV derived vectors as well as the 2nd Generation HIV packaging / reporter vectors in order to maximise the prospect of successfully immortalising primary bovine B-cells.
Recipient of the plasmids is a common laboratory authenticated commercial mammalian cell line Human Embryo Kidney (HEK)293T cells, purchased from ECCAC. Primary B cells from calves with Cryptosporidium parvum infection

Pseudotypes containing either BCL-6 or BCL-XL will be produced from HEK293T cells after they have been transfected with a packaging vector (murine leukaemia virus, MLV; MLV-CMVpr-gag-pol [aka pHCMV5349] OR a 2nd Generation HIV self-inactivating plasmid pCMV D R8.91 OR psPax2) AND pMD2.G VSVG

Origin & function

The pseudotypes will deliver BCL-6 and BCL-XL to isolated bovine B cells to immortalise them. Onward transmission of the oncogenes is not possible as it is delivered by a single round infectious pseudovirus. The pseudovirus is a potential hazard to humans, as it could deliver the oncogenes through direct contact with skin.

Evaluation of foreseeable effects

There is a potential risk to human health in the event that there is a containment breach however as long as the general laboratory safety procedures and control measures stated in the risk assessment are followed there is no greater risk to human health than that of any other hazard group 2 pathogen. BCL-6 and BCL-XL are considered oncogenes as they lead to cell immortalization when expressed together. The viral pseudotype produced after transfection of HEK 293T cells can potentially be transmitted to workers through direct contact with skin and also with the mucosa e.g., through a percutaneous injury or a splash in the eye. This direct contact between the workers cells and the pseudovirus will lead to cell immortalisation (cell will become cancerous) through the forced expression of oncogenes. Therefore, long gloves will be worn as an extra precaution to ensure there is no uncovered skin while experiments are carried. All experiments will also be performed inside an ACDP Category/Class II Microbiological safety cabinet which will again help to mitigate the risk of contact between the pseudovirus and worker. However, given that the pseudovirus is replication-defective, it cannot propagate in humans and therefore will not cause further damage.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For both bacterial and tissue culture work, the solid waste produced will be autoclaved at a minimum temperature of 126°C (load temp) for 50 minutes before being destroyed as clinical waste. Bacterial liquid waste will be treated with an equal volume of 2% Virkon* and left overnight before disposal down the drain with copious water. Cell culture liquid waste from tissue culture experiments will be collected in reinforced glass bottles (as per standard practice in IC2) and then autoclaved at a minimum temperature of 126°C (load temp) for 50 minutes before being destroyed as clinical waste. In the event that the autoclave in IC2 breaks, solid and liquid waste from pseudovirus experiments and also solid waste from bacterial work will be decontaminated using 2% Virkon and left overnight. The waste is then rinsed and disposed of in yellow clinical waste bags to be incinerated. Autoclaves in IC2 Building are operated by trained laboratory staff. A chart record is produced for each decontamination run to confirm, by means of a temperature probe placed within the load, attainment of suitable temperatures for 100% kill of viable organisms. Validation of autoclave cycles is maintained by 6-monthly servicing and yearly thermocouple testing carried out by an external engineer. *2% Virkon is a well characterized disinfectant known to completely inactivate many viruses including HIV-1 virus and poxviruses, which are much "hardier" than the pseudotypes being produced.

https://www.fishersci.co.uk/webfiles/uk/web-docs/SLSGD05.PDF
Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

CU2 Form on HSE website to be completed and signed. The final document to be sent to the BSO. The BSO will send to the HSE with details of payment, (the correct method to be discussed and arranged with XXX, IVES Finance Team). The BSO will advise on the start date of the project.

Start date on the RA will need amending

Assessment submitted to GMSC 03/08/21 and circulated to GMSC members 12/8/21.

Comments were received by email and discussed at GMSC meeting 09/9/21.

GMSC agreed that this was a Class 2 activity. A number of minor amendments were made that required to be addressed. Upon addressing these comments it was agreed to be pass the assessment by Chair’s Action.

Amended assessment received 23/09/21 and approved by Chair 28/09/21

Project Containment

<table>
<thead>
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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref  554/99.1

Date Ackn'd  10/08/1999

Date Project Ceased  05/04/2003

Project notified under transitional arrangements Y

With withdrawn N

Tick if notifying a connected programme of work N

Date

Project Title

INDUCTION OF CHROMOSOMAL ABNORMALITIES BY HUMAN PAPILLOMAVIRUSES (HPVS) (PROJECT TRANSFERRED TO GM 317)

Class 2

Consent Granted

not applicable

Project notified under transitional arrangements

Page 9517 of 15326
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref 601/06.1**

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<td>Transfection of mammalian genes into human cell lines using plasmid vectors.</td>
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**Class**

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**Non-GMM Consent Granted**

**Withdrawn**

| N |

**Historical Significant Changes**

TRANSFERRED TO GM554 ON CLOSURE OF GM601

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

<table>
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**Project Additional Information**

02/03/2022
**Purposes of the contained use**

This research is to be notified as a connected programme of work utilising transfection of cDNAs into human cell-lines in order to induce expression of said gene products under the control of known promoters provided by plasmid vectors. It contributes to the research goal of understanding the contribution of genes to hormone signalling pathways, especially in relation to breast cancer.

**Recipient or parental organism**

Recipients are established human cell lines (eg breast cancer cell lines) and an immortalised normal breast epithelial cell line hTERT-HME1. Epithelial tumour cells can cause tumours when injected subcutaneously in immuno-compromised mice. However, the potential for harm is much less in immuno-competent humans, where the primary potential for harm comes from possible adventitious pathogens (viruses). Cell lines are expected to have limited survivability in the environment and modifications are not expected to increase their pathogenicity.

**Host/vector system**

The plasmid vectors are mobilisation deficient, devoid of significant known toxic or pathogenic potential and would be classified as class 1 were it not for their use in mammalian cell-lines. The design of the RheoSwitch Mammalian Inducible Expression System is such that the insert DNA is only expressed in the presence of RSL1 ligand, which is a synthetic non-steroidal compound and therefore extremely unlikely to be found in the environment. Any other vectors will be limited to similarly well characterised plasmids.

**Origin & function**

The primary inserts will be full length cDNAs encoding estrogen receptors Eralpha and Erbeta, derived from human normal or tumour tissues or cell line material. In addition cDNAs resulting from alternative splicing of the ER mRNA (for both Eralpha and Erbeta) will also be used. The cDNAs resulting from alternative splicing of the ER mRNA (for both Eralpha and Erbeta) will also be used. The cDNAs to be used are unlikely to include a toxic or pathogenic determinant although due to the unknown nature of the estrogen receptor variants, the deletion variants may have a deleterious effects on the recipient cell line.

Alternative gene products (eg X-box binding protein 1) will be limited to those unlikely to increase the toxic or pathogenic potential for harm, even when constitutively expressed or modified by addition of reporter proteins. Most inserts will be transcription factors, or genes known to modulate transcription (eg co-activators of co-repressors).

Some inserts may have been previously mutated by site-directed mutagenesis to pinpoint specific residues/amino acids involved in protein function. It is envisaged that insert modification (eg fluorescent labelling or tagging) is more likely be detrimental to gene function than enhance any latent pathogenicity or toxicity.

Initial expression of the insert DNA will be induced only by addition of RSL1 synthetic ligand (requiring additional transfection with plasmid pNEBR-R1), subsequently a strong constitutive promotor may be used (eg CMV). Plasmid vectors additionally contain selective markers (eg G418 resistance).

**Evaluation of foreseeable effects**

Recipients are established human cell lines eg breast cancer cell lines: KPL-1, MCF7, ZR75, MDA-MB-231 and an immortalised normal breast epithelial cell line hTERT-HME1. These cell lines are biological agents for which containment level 2 is appropriate, owing largely to the fact that they are long-term cultures of human origin and are not fully characterised. The primary potential for harm comes from possible adventitious pathogens (viruses). Cell lines are expected to have limited survivability in the environment.

The primary inserts will be full length cDNAs encoding estrogen receptors (ER), cDNAs resulting from alternative splicing of the ER mRNA or alternative gene products, largely constituting related transcription factors. The cDNAs to be used are unlikely to include a toxic or pathogenic determinant although due to the unknown nature of the estrogen receptor variants, the deletion variants may have a deleterious effects on the growth properties of the recipient cell lines. Most insert DNA is naturally occurring in these cell lines, the experiments will merely control the level of expression of individual variants.

The plasmid vectors when used are mobilisation deficient, devoid of significant known toxic or pathogenic potential and would be classified as class 1 were it not for their
use in mammalian cell-lines. All sequences cloned will be of mammalian origin and unlikely to contain promoters active in non-mammalian organisms. Expression in mammalian cells is controlled by previously validated promoter elements (very strictly inducible in the case of the Rheoswitch system) and is unlikely to be observed outside of a controlled laboratory environment. Potential for transfer to and gene expression in other organisms is negligible and therefore negligible hazard to human health. Vectors are unlikely to alter pathogenic traits of the host.

There is little potential for alteration of pathogenic traits and no obvious significant increase on the hazards of the non-transfected cell lines.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- This work involves standard laboratory protocols, and there are no unusual procedures that require additional containment measures. Standards of work and containment are specified in the CCRT Code of Practice, with reference to the governing regulations and guidelines. All personnel should be familiar with the relevant safety documentation and have received training specifically for the use of GMM.
- Both unmodified and GM modified cells will be grown in small scale closed containers in a category 2 containment area. A class II safety cabinet will be used for all manipulations and both sharps glass containers will be avoided. As specified in the governing code of practice for biological agents.
- Solid waste will be bagged, sterilised by autoclaving and later incinerated as clinical waste. Liquid waste in disposable culture flasks will be sterilised by autoclaving and incinerated as clinical waste. Other liquid waste will be sterilised by autoclaving and disposed of via the drains. The autoclave is situated within the laboratory suite and is managed by Wirral NHS Trust engineers. A programme of testing and quality assurance is carried out on a quarterly and annual basis and records kept.
- It is appropriate for all manipulations to be undertaken within the specified GM area in a laboratory where access is restricted to authorised personnel only. Regular safety checks are performed by safety officers and matters arising reported to safety committees; safety records will be kept.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Solid waste will be bagged, sterilised by autoclaving and later incinerated as clinical waste. Liquid waste in disposable culture flasks will be sterilised by autoclaving and incinerated as clinical waste. Other liquid waste will be sterilised by autoclaving and disposed of via the drains. The autoclave is situated within the laboratory suite and is managed by Wirral NHS Trust engineers. A programme of testing and quality assurance is carried out on a quarterly and annual basis and records kept.

**Is an emergency plan required according to regulation 20?**

- N

**If yes, tick to confirm that it is attached to this form**

- N

**Tick to confirm that you have attached a risk assessment to this form**

- Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- N

**Please enter comments on the GM safety committee on the risk assessment**

The CCRT GM safety committee is satisfied that the risk assessment is suitable for the purpose stated, providing suitable records are kept and any significant variation notified.

The assessment has been reviewed by the University Biological Safety Adviser who made a number of suggestions regarding the scope of the work, in particular that the work should be notified as connected programme, given the number of different phases that are proposed. These suggestions were incorporated in the final assessment.

**Project Containment**
Project Additional Information

**Purposes of the contained use**

To study the role of the p53 pathway in cancer. Genes such as MDM2 have been implicated as critical molecules in this pathway and to study this we need to be able to efficiently deliver these genes (also non-oncogenic genes such as p53, MTBP, E-MTBP and Lac-Z will be expressed from similar viruses) to a range of cell types - particularly had to transfect cells such as mouse embryo fibroblasts (MEFs) and a range of pancreatic cancer derived cell lines.

**Recipient or parental organism**

E. coli XL-Blue are a K12 derivative strain and are not expected to survive outside of the laboratory. There is no evidence that these bacteria are pathogenic. 293T, MEFs and other cell lines would not be able to survive outside of the laboratory environment. Retroviral vectors have generally been designated as ACGM category 1 except where the gene to be inserted is pathogenic or where the envelope is human tropic. We propose to create replication defective retroviruses with either rodent or human tropic envelopes. Retroviral vectors with these modifications are ACGM2.
Host/vector system

The three-plasmid system would require at least 2 recombination events to produce a single fragment of DNA that could encode replication competent retrovirus (RCR). Since there is little or no homology between the three plasmids a single recombination event is unlikely.

Origin & function

MDM2 (for example) is known to be an oncogenic protein. Retroviruses are also inherently potentially oncogenic because they are obligate mutagens: when the provirus is integrated into the host genome, it may at random be integrated into a site that causes inactivation of a tumour suppression gene. MTBP, E-MTBP and p53 induce cell cycle arrest (Boyd et al., JBC 2000). We will be inserting wild-type p53. As yet the roles of MTBP and E-MTBP, a deletion mutant of MTBP, are not completely known, although it has been demonstrated that MTBP causes G1 arrest and E-MTBP inhibits tumour cell growth more effectively in the cell lines tested than either MTBP or p53. Retroviruses are obligate mutagens: when the provirus is integrated into the host genome this might occur at random into, for example, a tumour suppression gene.

Evaluation of foreseeable effects

Bacterial transformation - the transformed strain will be no more virulent or pathogenic than the original.

The three plasmid system used should ensure that the virus is not mobilisable.

We expect moderately high levels of expression of the transduced genes driven by the MDMV LTR.

These replication defective viruses could infect human cells.

Retroviral production - a VSV-G envelope will be used to improve infectivity of the virus but only to produce replication defective viruses containing a non-oncogenic insert. We will test supernatants for evidence of RCR by infecting permissive cells with viral supernatant and then passing cells. Supernatant from these cells will be screened at weekly intervals by re-inoculating these at \( \geq 1/10 \) dilution onto permissive cells. MDM2 can readily be detected by western blot. By inoculating double null (MDM2/p53 null) MEFs we can readily detect even very low levels of MDM2.

MTBP and E-MTBP can readily be detected by western blot. By inoculating p53 null cells (H1299) p53 can also be detected by western blot. Lac-Z can be screened by in-situ Beta Gal assay.

Retroviral production - we will use either an ecotropic or a VSV-G envelope. These do not by themselves increase the pathogenic traits of the retrovirus although the VSV-G envelope which will be used to improve infectivity of the virus (it is monomeric and therefore higher tires of more stable virus can be produced c.f. eco/ampho-tropic retroviral envelopes) does alter the virus host range to include humans and other mammals.

The retroviral vector will also contain the neomycin gene, which will result in resistance to G418. This would not be used as a treatment for laboratory-acquired infection.

The major alteration affecting likely harm will be the inclusion of a known oncogene (eg MDM2) into the retrovirus. This will create the risk of transfer of an oncogene into mammalian in particular human cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

After bacterial transformation, flasks will be decontaminated by the addition of fresh 10% bleach.

During retroviral production, once the producer cells have been transfected with the 3 plasmid components they will be considered potentially infectious. BSL2 precautions will be required. Experiments will take place in a laminar flow hood with the operator wearing a disposable surgical gown (near opening) with snug fitting surgical latex gloves worn over the elasticated wrists of the gown. Care will be taken so as not to produce aerosolisation of viral containing supernatants. Items of equipment exposed to virus will be soaked in 1% Virkon or 1% sodium hypochlorite or 10% bleach for 15 mins before removal from the laminar flow hood and then disposed of in the usual manner: ie all solid waste will be incinerated and all inactivated liquid waste will be disposed of down the sink. Once cells are no longer required for producing virus, they will be destroyed by adding fresh 10% bleach and leaving for 24 hours before disposal as liquid or solid waste whichever is most appropriate. All solid waste will be transferred to yellow clinical waste bins and sent to the RLBHU for incineration.
The GMSC considered the risk assessment and decided that the use of potentially oncogenic inserts means that this project is Class 2. The GMSC were content that the workers involved were aware of the risks and were technically capable of working at Class 2 level. The laboratories to be used are adequate for this work. As an additional observation it was noted that Class 1 work would take place alongside this Class 2 project. The GMSC requested that all work to be carried out in the laboratories alongside this class 2 activity should be carried out to CL2 standards.

### Project Containment

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### Project Ref  99/09.1

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<td>GENERATION OF RECOMBINANT HIV-1 TO STUDY THE EFFECTS ON VIRUS PHENOTYPE OF VARIATIONS WITHIN VIRAL GENES ENCODING PROTEINS TARGETED BY ANTIRETOVIRAL THERAPY (POL, GAG AND ENV)</td>
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Consent Granted: Yes

Project notified under transitional arrangements: No

Withdrawn: No

Tick if notifying a connected programme of work: No

Historical Significant Changes: Project transferred from GM99

Historical Date of Additional Info: 02/03/2022
## Project Additional Information

### Purposes of the contained use

To study the effect of mutations encoding antiretroviral resistance upon replicative function and drug susceptibility

### Recipient or parental organism

#### Introduction

Wild type HIV-1 (wt HIV-1): Wild type HIV-1 describes infectious HIV-1 viruses that do not exhibit resistance to therapeutic drugs and lack the according resistance mutations. These are produced by eukaryotic cells transfected with plasmids such as pHXB2D, pNL4-3 and pMJ4. All plasmids contain single retroviral genomes (proviruses) derived from patient isolates or laboratory strains and produce infectious viruses when transfected into permissive cells.

HIV-1 vectors: HIV-1 vectors are plasmid clones of HIV-1 proviruses derived from lab strains which are NOT replication-competent. Those vectors used within this project have deletions within their genes to prevent production of infectious virus. Such deletions will be contained within either the gag (protease), pol (RT) or env genetic regions. Such vectors also carry a resistance gene e.g. ampicillin, in order to enable the selection of vector containing prokaryotic cells. The risk from these vectors is minimal in prokaryotic cells e.g. E. coli, as the HIV-1 genes are unable to be expressed. As a result work involving these vectors may be carried out under ACGM class 1 conditions as the clone itself is non-infectious. Once transfected into an appropriate cell line in the presence of full length genes (for which the vector is defective) infectious virions are able to be produced. Vectors used within this investigation will be defective due to deletions in the gag, pol and env genetic regions.

Drug resistant HIV-1: Genes of interest encoding mutations will be amplified from clinical material derived from patients exhibiting resistance to drugs after therapy, or generated by site specific mutagenesis upon wt genetic material. These mutations within the gene of interest will encode for an HIV-1 with a phenotype of resistance to a particular, or a combination of, antiretroviral drugs.

Recombinant viruses: Recombinant viruses will be constructed by homologous recombinations between the HIV vectors (with deletions in the appropriate genes) and genetic material derived from either laboratory strains (simulating wild type virus) or 'normal' genes in the context of the same viral background. The presence of both components is required within the appropriate cells before recombination can take place.

#### Risk of infection

Levels of infectious recombinant virus produced (under containment conditions) during this investigation are anticipated to fall within the ranges measured in the blood of persons infected with HIV-1, although many viruses with drug resistance mutations replicate at reduced rates compares to wt virus. The risk of HIV-1 infection following exposure to infected blood through sharp injury is estimated to be around 0.3% , whereas that associated with a splash exposure with blood is in the region of 0.09%. This in absence of Post - Exposure Prophylaxis (PEP).

#### Management of Infection

If exposure does occur, it is managed through the administration of PEP constituting a four week course of antiretroviral drugs . Although the success rate of this course is high, it is not 100%. Some recombinant and wild-type viruses contain mutations which render them of increased susceptibility to particular antiretroviral drugs. Some resistance mutations may affect more than one drug; however there are sufficient drug combinations currently available against drug resistant HIV-1.

#### Other risks
The potential integration of a complete retroviral vector into the host genome may also have the potential to affect host cellular function i.e. insertional mutagenesis, leading to the possibility of tumour formation, subsequent to exposure to wt lentiviral vectors and HIV-1 lab strains. Integration of complete lentiviral vectors into host cell genomes has been recently described however the overall likelihood of such events is thought to be low (J. Virol. 2008 Oct 22). Additionally, it may be speculated that the transduction of eukaryotic cells with expression vectors, or infection of such cells with recombinant HIV-1 or retroviral vectors may also result in the immortalisation of the cell line with altered properties. Such cells may retain the ability to establish tumour formation within exposed laboratory workers following a significant exposure although the likelihood of such an event is considered extremely remote. Even in such a rare occurrence, the chances of such cells becoming established and productively proliferating within the worker would be further limited by immune defence mechanisms such as MHC mismatching. Furthermore, mutations within the env region may result in the altered tropism of viruses. Such events as these however would be unlikely to influence the susceptibility of the virus for particular drug therapies in the event of exposure.

Environmentally, these viruses pose a minimal risk. HIV-1 is an enveloped particle and is consequently labile. There is no reason to suspect that mutations will result in structural changes to progeny virions, thus it is unlikely that any changes to viral genes will affect its interactions within the physical environment. Infectivity of wt HIV-1 is completely destroyed by desiccation or disinfection and waste disposal protocols implemented within the containment level 3 (CL3) laboratory, the chance of residual virus remaining infectious is unlikely.

Virus producing cell lines

The cell lines, MT4 and C8166 that will be used to propagate the virus in vitro are persistently infected with HTLV-I (although C8166 cells carry, but do not express the HTLV-I genome). C8166 is derived from umbilical cord blood cells (Virology. 1983; 129:51) whereas MT4 is a human T cell lymphoma cell line (Acta Microbiol Hung. 1992; 39(3-4):271-9). Both have been immortalised by HTLV-I virus. Cell lines were chosen as they exhibit characteristic morphologies when infected by HIV-1. There is a risk of exposure to HTLV-I when working with MT4 cells, although the virus is known to be highly cell-associated and the chance of infection is low. Additionally, the risk of HTLV-I virus particles recombining with lentiviral vectors is minimal, as is the likelihood of recombination with recombinant HIV.

In summary: the main consequence of exposure to vectors engineered to express viral genes is the potential transfer of GMM to the laboratory worker. Laboratory associated exposure to the recombinant virus would carry similar implications as exposure to the 'non-resistant' wild type HIV-1 (grown from clones in cells) and drug resistant HIV-1 (derived from patient material). In spite of any such mutations effective drugs remain available to treat infection with recombinant viruses. Additionally, such risks working with such GM organisms are further reduced by good working practice and adherence to SOPs under CL3 conditions. The availability of PEP further reduces the risk of infection after a significant exposure. Therefore the overall likelihood of infection is very low. Should infection occur despite PEP then consequently the health risks are those associated with wt HIV-1 infection.

Characteristics of the GMO

Recombinant viruses will fall into three groups:

i) Those in which inserted regions, cloned directly from patient samples, contain mutations which encode for resistance to particular (or a combination of) antiretroviral drugs.
ii) Those in which inserted regions are cloned directly from wild type HIV-1 laboratory strains such as pHXB2.
iii) Those in which inserted regions contain mutations generated in vitro which represent intermediate 'halfway' stages between drug resistance and wild type

Forseeable effects

1) Health
There is no reason to expect that viruses generated by this work will be more pathogenic than 'non-resistant' wt HIV-1 virus. There is also the possibility that exposed laboratory workers may acquire a mixed infection (of both resistant and non-resistant HIV-1) although this risk is reduced as the viruses will be cultured under CL3
containment conditions however any such exposure to mixed viral populations is considered to pose no greater danger than infection by either virus individually. Although resistance mutations may affect more than one drug there are considered to be sufficient numbers of drugs, with different mechanisms of action, currently available for application against drug-resistant virus.

2) Environment
The presence and persistence of recombinant viruses within the environment is extremely limited and therefore adherence to disinfection and waste disposal protocols implemented within the CL3 laboratory further reduces the chances of viruses' surviving within the environment.

Cell lines
Cells proposed for use within this project will fall into 2 classes:

i) Prokaryotic cells required for the propagation of HIV-1 vectors. These include E. coli DH5α, TOP10 and equivalent cells.

ii) Eukaryotic cells required for construction and propagation of recombinant HIV-1. These include 293T, MT4, C8166 and equivalent cells.

Origin & function
Origin of viral genomic genetic material
Several HIV-1 vectors are intended for use within this investigation. Genetic regions of these vectors will be replaced with regions of interest in order to study the effects regarding susceptibility to certain antiretroviral drugs and on viral replication.

The pHIVΔRTStEII vector contains the complete provirus of the HIV-1 laboratory strain HXB2D with a 1.4kb deletion within the RT region. The clone is non-infectious but when transfected into an appropriate cell line with full length RT coding sequence provided in trans infectious virus is produced by homologous recombination. All procedures within the protocol prior to transfection are carried out in E. coli and present no infection risk. This vector is unable to initiate virus production when introduced into a target cell in the absence of a full length RT coding sequence and therefore is unlikely to regain wild type phenotype in the absence of RT. This minimises the level of risk to the laboratory worker to the vector during laboratory manipulations. Sharps are not used and good laboratory practice means the risk to workers is negligible.

This plasmid and its equivalents [containing deletions/mutations within the gag (protease), pol (Integrase) and env] will be used to determine the impact of mutations within these regions upon resistance

All molecular clones used within this investigation will be obtained from the National Institute of Health AIDS Research and Reference Reagent program (www.aidsreagent.org)

Origins of the genetic material to be inserted into replication-deficient vectors
Inserts encoding for drug resistance: Genes of interest encoding mutations will be amplified from clinical material derived from patients exhibiting resistance to drugs after therapy. These mutated genes of interest will encode for an HIV-1 with a phenotype of resistance to a particular, or a combination of, antiretroviral drugs.

Inserts coding for 'non-resistant' wild type HIV-1: Genes of interest (gag, pol and env) will be cloned from the HIV-1 laboratory strains pHXB2D, pNL4-3 and pMJ4. All three plasmids contain proviruses derived from patient isolated and laboratory strains which produce infectious virus when transfected into appropriate cells. Such inserts will encode for an HIV-1 virus without a resistance phenotype.

Inserts containing intermediate mutations for HIV-1 drug resistance: Genes of interest will be cloned from the HIV-1 laboratory strains and isolates. Sequences will then be altered using in-vitro mutagenesis to induce partial sequence identity to drug resistance motifs, simulating intermediate stages in the development of drug resistance to particular (or a combination of) antiretroviral drugs.
Cloning vectors: These are commercial vectors used for the purpose of cloning and amplifying regions of interest and include pCR2.1TOPO derivatives, pUC vectors, pBLUESCRIPT vectors, pcDNA3.1, pCMV/Zeo and pEGFP-1.

Evaluation of foreseeable effects

These are detailed in the above sections.

To summarise: The main risk is accidental exposure of laboratory workers to infectious recombinant and wild-type HIV-1.

The handling of bacterial cultures and plasmids for cloning is carried out under ACGM class I conditions and therefore poses little risk, if any. At this stage of the work the risk of exposure of workers to infectious virus is minimal.

The HIV-1 vectors are derived from well characterised laboratory strains of HIV-1, Theses are not able to form infectious particles unless in the presence of a full length RT region in trans (either wild type or mutated). Such an event would ONLY occur within the contained environment of the CL3 suite. An additional foreseeable risk is the oncogenic potential of the vector to the host genome upon potential exposure. The probability of such an event is extremely low and in this respect these vectors pose a minimal risk.

The defective HIV-1 vectors can ONLY form replication-competent viruses when both the HIV-1 vector and genetic region of interest are present within the cell. Recombination in the absence of either is extremely unlikely, as is the chance of spontaneous recombination with retrovirus genomes within, or associated with the aforementioned cell lines. The presence of replication competent recombinant HIV-1 poses the main risk to laboratory workers, although the risk is no more significant than that posed by handling wild type HIV-1 virus. The main risk of exposure in the laboratory is via cuts and abrasions with contaminated sharp instruments, or splash exposures as HIV-1 is not readily transmitted by aerosol. Through adherence to CL3 working guidelines and good laboratory practice, this risk is significantly reduced. The use of PEP after accidental exposure makes the risk of an established infection very low. Should infection occur, the recombinant viruses are unlikely to be any more pathogenic than those circulating in infected patients. No genes of the source strain of HIV-1, from which the lentiviral vector has been derived, have been shown to be oncogenic or teratogenic.

The potential risk of exposure to HTLV-I during this work has been discussed previously, however as work with this cell line is confined to the CL3 suite; the risk of exposure to this virus is minimal.

Environmentally, providing correct guidelines upon disinfection and waste are followed there are no foreseeable risks posed by either the wild type or recombinant viruses due to their nature as enveloped viruses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratory workers are responsible for the following procedures:

Class 1 GMM products include HIV-1 genetic regions of interest derived from clinical samples or laboratory strains which have been cloned into plasmid vectors and the production of replication defective viral vectors.

Method of Class I GMM waste disposal
All GMM waste both solid (e.g. flasks, tubes and plates) and liquid (e.g. culture medium) is double bagged into clear autoclave bags and placed into metal autoclave tins for autoclaving. Autoclaving conditions follow the NHS diagnostic guidelines and adhere strictly to the requirements of the Clinical Pathology Accreditation. Standard autoclave cycle conditions are 135°C at 3150 mBar for 10 minutes. Temperature and pressure parameters of each autoclave cycle are recorded and reviewed. Additionally, Browns tubes and autoclave tape are used to assess each run.

Spillages are dealt with using KLORSEPT granules. Disinfected spillages are then absorbed onto paper towels which are then autoclaved.

Methods of Class 3 GMM waste disposal

Class 3 waste products will include cell culture medium containing antiretroviral drugs, cells and wt/recombinant HIV-1 virus.

All CL3 GMM liquid waste is emptied from plastic culture flasks into a sweetie jar within the MSC containing 2x6g sachets if Vernagel gelling agent. All CL3 GMM solid waste (e.g. flasks, tubes, tips and plates) are then immersed in a container of 10% Trigene Advance located within the microbiology safety cabinet and allowed to disinfect for at least 16 hours. Lids are sealed upon 2L lidded containers and liquid contents are allowed to gel before being double baged, and loosely swan-necked prior to disinfection and being placed within a sealable plastic autoclave bucket.

After disinfection of solid waste has been allowed to take place the container is removed from the hood. Disinfected solid waste is poured into a sieve placed over a sink whilst disinfected liquid waste is poured down the sink and washed down the mains drainage with copious amounts of water. All disposable solid waste from the sieve is placed into double autoclave bags which are disinfected and swan-necked loosely. Laboratory plasticware including jugs are double bagged and loosely sealed by swan-necking prior to further disinfection. Bagged and sealed plastic ware (i.e. beakers and jugs) are then placed in a sealable metal tin for autoclaving. The remaining disinfected solid waste is placed (using the sieve) into double autoclave bags. These are loosely sealed prior to disinfection and placed into sealed yellow autoclave bins. After each work session all surfaces are disinfected with 5% Trigene Advance in order to eliminate any residual virus. At the end of each work day, autoclave bins and sealed metal tins are surface disinfected with 5% Trigene Advance before they are placed in a lockable trolley by a trained member of the CL3 team, and transported directly to the local autoclave (within the department) for immediate incineration under standard autoclaving cycles. No GMOs will remain viable after treatment.

After autoclaving, sealed plastic autoclave bins will be labelled and sent offsite for incineration. Metal transport tins and any plastic ware from the CL3 are returned directly to the CL3 suite in order to prevent them entering general laboratory circulation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Part of this work have been approved by the Royal Free & University College Medical School (Hampstead) GM99 Committee on the 28th November, 2007. Document references include HC40/07 and HC37/07.

Project Containment
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<td>EAST AND SOUTH EAST</td>
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Date at Which Additional Info Submitted: 02/03/2022
### Premises Addresses

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<th>Building</th>
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<th>County</th>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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Tick if confidential

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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste autoclaved at 121deg C for 15 minutes.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
<tr>
<th>Data Premises Notified</th>
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### Name

IMUTRAN LTD

### Data Premises Closed

**31/03/2001**

### Emergency Plan Required?

**N**

### HSE Division

EAST AND SOUTH EAST

### Comments

**Date at Which Additional Info Submitted**

09/02/2001

IMUTRAN LTD

### Campus Estate or Research Centre

P O BOX 399

### Building

P O BOX 399

### Town

CAMBRIDGE

### County

CAMBRIDGESHIRE

### Postcode

CB2 2YP

### Country

ENGLAND

**Tel Number**

01223 840874

**Fax Number**

01223 500153

E-mail
## Premises Addresses

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## Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: [ ]
- **Give brief details of the genetic modification safety committee**

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- **Other (please specify)**: [ ]

<table>
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<th>Transgenic Fish</th>
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</tr>
<tr>
<td>Other(s)</td>
<td></td>
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</tr>
</tbody>
</table>

- Liquid waste is deactivated by bleach or Virkon. Verified by culturing deactivated cells on agar plates for 24 hrs, and looking for any bacteria growth.
- Solid waste is autoclaved. Each run is verified by indicator paper and autoclaves are regularly validated.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
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</table>

**Name**

BIORELIANCE LTD

**Campus Estate or Research Centre**

TODD CAMPUS

**Road Name**

WEST OF SCOTLAND SCIENCE PARK

**Town**

GLASGOW

**County**

RENFREWSHIRE

**Postcode**

G20 OXA

**Country**

SCOTLAND

**Tel Number**

0141 946 9999

**Fax Number**

0141 946 0000

**HSE Division**

SCOTLAND

**Comments**

MERGED WITH GM562 AND CHANGED NAME TO BIORELIANCE BIOTECH LTD AS OF 8/10/2003

**Date at Which Additional Info Submitted**

31/05/2001
### Premises Addresses

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<td>Plants</td>
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**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 557/01.1**

**Date Ackn'd** 29/08/2001

**CU2 Project Title**

TO PRODUCE A STOCK OF RECOMBINANT VACCINIA FOR USE AS AN ANTIGEN

**Date Project Ceased** 02/03/2022

**Class** Class 2

**CultureVolClass2** < 1 litre

**Consent Granted**

Non-GMM not applicable

**CultureVolumeClass3-4**
### Project Additional Information

#### Purposes of the contained use

The sponsor will use the material as a positive control antigen in an in vitro immunological study. Typically as ELISA spot test and antigen-specific intracellular cytokine assay for measurement of antigen-specific T lymphocytes.

#### Recipient or parental organism

The parental organism is the Vaccinia WR strain (American Type Culture Collection [ATCC] # VR-1354). It is thymidine kinase negative. The virus has been used as a vaccine strain in humans.

#### Host/vector system

Three constructs of the vaccinia will harbour the nucleic acids encoding the following:
- Hepatitis B core antigen
- Hepatitis B surface antigen
- Influenza matrix protein M1.

#### Origin & function

The material will be used to provide viral antigens for in vitro immunological studies. The source of the material is as follows:
- Hepatitis B core antigen: Virology Department of GlaxoSmithKline Biologicals
- Hepatitis B surface antigen: Virology Department of GlaxoSmithKline Biologicals (originally from ATCC VR# 2046)
- Influenza matrix protein M1: Virology Department of GlaxoSmithKline Biologicals

#### Evaluation of foreseeable effects

The GMMs consist of the WR strain of vaccinia with one of each of the viral genes listed above inserted into the thymidine kinase locus of the parental vaccinia. There are no foreseeable increased adverse properties of the GMMs compared to the parental vaccine backbone.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste consisting of disposable pipettes will be submerged overnight (16h minimum) in 2% Virkon, removed and double bagged into autoclave bags. The bags will be autoclaved at 131 C for 30 minutes, bagged and finally removed from the facility and incinerated as clinical waste. Disposable plastic disposable culture vessels will be treated as above. Liquid waste will be mixed with an equal volume of 2% Virkon in a sealed vessel, left overnight and treated as above. The inactivation is 100%. The waste autoclave is validated and maintained. The sterilising run is completed before bagging for incineration.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Committee required that the work be re-notified to the HSE. The work was to be undertaken in a designated room with designated personnel. There was to be limited access to personnel while the work was underway. Fumigation of the Biocontainment hood was to be done at the cessation of work for the day. All waste disposal and laboratory cleaning was to be done by the designated operator.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
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Project Ref  557/03.1

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<tr>
<td>24/06/2003</td>
<td>TO PRODUCE A STOCK OF ATTENUATED VENEZUELAN EQUINE ENCEPHALITIS VIRUS (VEEV) VACCINE CANDIDATE STRAIN V3526</td>
<td>Class 2</td>
<td>1-50 litres</td>
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Date Project Ceased

02/03/2022
Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The purpose of the work is to manufacture a master virus seed stock of an attenuated VEEV human vaccine candidate, followed by the manufacture of a Phase 1 clinical lot of the attenuated VEEV vaccine candidate strain V3526. The attenuated virus will be cultured in MRC5 cells, following electroporation of the RNA, and purified using sucrose gradient centrifugation. All procedures will be conducted under full GMP compliance.

Recipient or parental organism

The parental organism is VEEV. The candidate strain is an attenuated VEEV human vaccine candidate.

Host/vector system

Escherichia voli vector pBR322.

Origin & function

The alteration of the macromolecular structure in V3526 through site-directed mutagenesis reduces infectivity associated with wild-type virulent VEE virus, while at the same time retaining its immunogenic potential. As these intrinsic differences between the origin and molecular structure of wild-type VEE virus and the V3526 vaccine candidate are such a critical element of the hazard assessment, the construction and selection of V3526 are described below:

Wild-type clone V3000 is a full-length RNA genome clone of VEE 1A/B (Trinidad donkey strain, VEE TrD) in the Escherichia coli vector pBR322 (Davis, et al., 1991; Davis et al., 1989b). It was constructed from four complementary DNA (cDNA) fragments in a series of cloning steps, including the placement of a T7 promoter at the 5’-end of the VEE genomic clone, for in-vitro run-off transcription of viral genomic replicas (Figure 1) (Davis, et al., 1991; Davies et al., 1989a; Johnston, R.E. and J.F. Smith 1988). The resulting viral RNA can be transfected into cell cultures (eg chicken embryo fibroblasts) to generate virus progeny (Davis, et al., 1991; Davis, et al., 1989a).

Site-directed mutagenesis of V3000 resulted in a set of 15 full-length viral cDNA clones with single mutations. These 15 full-length clones were constructed based on mutations originally identified by either comparative sequence analysis of attenuated VEE virus strains or by targeted mutagenesis of replication-critical domains of the VEE genome (Davis, et al., 1984; Grieder, et al., 1995). The most attractive single mutations were then combined in various permutations resulting in vaccine candidates with multiple mutations (Davis, et al., 1995). Fourteen of these multiple-mutation candidates were again evaluated in murine models for safety and immunogenicity. Based on these results, V3526 was chosen.

Evaluation of foreseeable effects

There are no foreseeable increased adverse properties of the GMM compared to the parental VEE virus. Please refer to the hazard assessment enclosed with this application.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
The GMM derogation has been downgraded to category level 2. Please refer to the hazard assessment enclosed with this application for justification.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste consisting of disposable pipettes will be submerged overnight (16 hours minimum) in 2% Virkon, removed and double bagged into autoclave bags. The bags will be autoclaved at 131 deg C for 30 minutes, bagged and finally removed from the facility and utilising a "non-Burn" heat disinfection process will be rendered non-hazardous.

Liquid waste will be mixed with an equal volume of 2% Virkon in a sealed vessel, left overnight and treated as above. The inactivation is 100%.

The waste autoclave is validated and maintained. The sterilising run is completed before bagging for incineration.

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee required that the work be notified to the HSE.

The work is to be undertaken in a designated room by designated personnel. There is to be limited access to personnel while the work is underway.

Fumigation of the Biocontainment hood will be performed and the room will be cleaned by a 30 minute contact time with 2% Virkon at the cessation of work for the day.

All waste disposal and laboratory cleaning will be performed by the designated operator.

Project Containment

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<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Project Ref 557/03.2

Date Ackn'd 02/03/2022
## Project Additional Information

### Purposes of the contained use

The GMO under consideration is a replication competent Herpes Simplex virus Type 1 containing a gene encoding for GM-CSF, a cytokine involved in the stimulation of T cells. The tests to be undertaken are routine safety testing for the presence of viral contaminants.

### Recipient or parental organism

The parental organism is HSV type 1.

### Host/vector system

ICP34.5 deleted HSV1 can replicate in dividing cells but cannot replicate in non-dividing cells.

### Origin & function

JSV1 deleted for the ICP34.5 gene will only replicate in dividing cells including cancerous cells. The insertion of the gene encoding for GM-CSF which produces a cytokine encoding for T-cells enables the vector to be utilised in forms of cancer therapy.

### Evaluation of foreseeable effects

There are no foreseeable increased adverse properties of the GMM compared to the parental Herpes Simplex virus. Please refer to the hazard assessment enclosed with this application.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Not applicable.
Solid waste consisting of disposable pipettes will be submerged overnight (16 hours minimum) in 2% Virkon, removed and double bagged into autoclave bags. The bags will be autoclaved in 131 deg C for 30 minutes, bagged and finally removed from the facility and utilising a "non-burn" heat disinfection process will be rendered non-hazardous.

Liquid waste will be mixed with an equal volume of 2% Virkon in a sealed vessel, left overnight and treated as above.

The waste autoclave is validated and maintained. The sterilising run is completed before utilising a "non-burn" heat disinfection process.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The committee required that the work be notified to the HSE.
The work is to be undertaken in designated rooms by designated personnel.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Project Ref 557/04.1

Date Ackn'd 07/05/2004

Date Project Ceased

CU2 Project Title

To test a recombinant Vaccinia (rVV) containing tumour antigens for cancer therapy by in vitro methods.

Class 2

CultureVolClass2 Not Applicable

Consent Granted

Non-GMM Not Applicable
The recombinant Vaccinia virus will be used in tests undertaken as part of routine safety testing for the presence of viral contaminants or to investigate integration and expression of the vector construct in the germ line, target tissue or non-target tissue as part of a biodistribution study. Nucleic acid will be extracted from viral seed, viral vaccine or tissues harvested from animals inoculated with the recombinant vector, and detection of the viral vector assessed in each sample by Polymerase Chain Reaction.
Extracted viral nucleic acid will also be used as a positive control during the study.

**Evaluation of foreseeable effects**

As the insertion of the tumour antigen genes into the vaccinia viral vector does not alter the tropism of the virus or its interactions with host defences it is considered to be no more pathogenic than the parental virus, which is categorised by ACDP as a hazard group 2 biological agent (ACDP Categorization of biological agents according to hazard and categories of containment, 4th edition, 1995 [second supplement]).

Please refer to the GMO Risk Assessment enclosed with this application.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The ACGM and ACDP level of containment would be category 2. All manipulations using infected tissue or live virus will be performed in a class 2 safety cabinet within a level 2 containment facility. Handling of extracted DNA will be performed within a level 2 laboratory.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste consisting of disposable pipettes will be submerged overnight (16 hours minimum) in 2% Virkon, removed and double bagged into autoclave bags. The bags will be autoclaved at 121 deg C for 15 mins, bagged and finally removed from the facility and utilising a “non-burn” heat treatment/disinfection process will be rendered non-hazardous.

Liquid waste will be mixed with an equal volume of 2% Virkon in a sealed vessel, left overnight and treated as above.

The waste autoclave is validated and maintained. The sterilising run is completed before bagging for incineration.

The combination of autoclaving and non-burn heat treatment/disinfection will result in an inactivation of 100%.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

The committee required that the work be notified to the HSE.
The work is to be undertaken in a designated room by designated personnel.
Fumigation of the biocontainment hood will be performed.
All waste disposal and laboratory cleaning will be performed by the designated operator.

**Project Containment**
The sample will be clarified to remove cellular debris, ultracentrifuged to pellet any viral particles present and resuspended in disruption buffer containing detergents, followed by Reverse Transcriptase Polymerase Chain Reaction.
considered one of the safest and effective live attenuated vaccines ever developed. According to current biosafety guidelines, the ChimeriVax - JE vaccine virus is officially BSL2.

**Origin & function**

The infectious clone (cDNA copy) of 17D virus, which was subsequently used to create ChimeriVax - JE, was constructed starting from a sample of the virus obtained from ATCC at passage 2347-8. Since ATCC is not currently distributing passage 234 sample, a Safety Data Sheet for this virus is not available. However, this virus is virtually identical to all other 17D vaccines manufactured worldwide, because its passage history differs from the commercial vaccines only at a few terminal passages. All 17D vaccines, including the ATCC passage 234 sample, have originated from one sample, Colombia 88 vaccine at passage 228. All current commercial 17D vaccines are produced at passage levels between 233 and 239 to ensure phenotypic and genetic similarity. The 187D virus is a BSL - 2 agent.

The Chimerivax will be used in tests undertaken as part of routine safety testing for the presence of viral contaminants, especially reverse transcriptase activity. The sample will be clarified to remove cellular debris, ultracentrifuged to pellet any viral particles present and resuspended in disruption buffer containing detergent, followed by an RT-PCR. The sample consists of ChimeriVax TM-JE Bulk Harvest cultured in Vero cells.

During the procedure the virus will be inactivated by disruptin buffer containing the following detergents, Igepal and DTT. Handling of the sample will be performed in a Class II hood. The process in which the recombinant virus will be used does not increase the risk associated with the virus.

**Evaluation of foreseeable effects**

Insertion of the envelope protein genes into the viral vector does not alter the tropism of the virus or its interactions with host defences. In selecting the most successful YF 17D chimeras containing the prM-E genes, two strains were examined, Nakayama, a virulent JE strain and the attenuated JE Vaccine strain SA14-14-2. Both chimeras replicated efficiently in several simian, human, mouse and mosquito cell cultures. It was concluded that the SA-14-14-2 envelope protein provided an additional degree of attenuation, therefore this was selected as the candidate chimeric JE vaccine.

For wild type yellow fever virus, antural hosts are Aedes mosquitoes. However, as a result of attenuation, the 17D vaccine strain lost its ability to replicate in mosquitoes, and replicates to extremely low levels in humans and non-human primates, which precludes infection of mosquitoes feeding on vaccinees and thus uncontrolled spread in nature. The ChimeriVax - JE vaccine had been demonstrated to possess the same restricted replication characteristics.

If introduced to humans it is possible that Chimerivax - JE would replicate to low levels, but cannot replicate in mosquitoes. However, due to the nature of the work to be performed and the containment procedures in place within the laboratory, the overall risk to the environment is considered negligible.

Chimerivax -JE can replicate in humans and non-human promates to extremely low levels. As the virus cannot replicate in mosquitoes there is no vector for transmission. The risk to human health and safety is negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

In conclusion the recombinant Chimerivax-JE virus is considered as a Hazard Group 2 biological agent, this conclusion arises due to the fact that JEV and YF vaccine strains belong to Hazard Group 2 (ACDP Categorization of biological agents according to hazard and categories of containment, 4th edition, 1995).

The ACGM level of containment would be class 2 and ACDP level of containment is level 2. All manipulations will be performed in a level 2 containment facility.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste consisting of disposable pipettes will be submerged overnight (16 hours minimum) in 2% Virkon, removed and double bagged into autoclave bags. The bags will be autoclaved at a minimum of 121 deg C for 15 minutes, bagged and finally removed from the facility and utilising a "Non-Burn" heat treatment/disinfection process will be rendered non-hazardous.
Liquid waste will be mixed with an equal volume of 2% Virkon in a sealed vessel, left overnight and treated as above.

The waste autoclave is validated and maintained. The sterilising run is completed before bagging for incineration.

The combination of autoclaving and non-burn heat treatment/disinfection will result in an inactivation of 100%.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

The committee required that the work be notified to the HSE

The work is to be undertaken in a designated rooms by designated personnel.

Fumigation of the Biocontainment hood will be performed.

All waste disposal and laboratory cleaning will be performed by the designated operator.

Project Containment

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<td>L3 L4 L2 L3</td>
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Project Ref 557/05.1

<table>
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<th>CultureVolumeClass3-4</th>
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<td>07/02/2005</td>
<td>Venezuelan Equine Encephalitis (VEE) Replicon Vector Expressing HSV gD2</td>
<td>Class 2</td>
<td>Not Applicable</td>
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</tbody>
</table>
Purposes of the contained use

The sponsor's material will be tested using a number of different assays for the detection of adventitious agents. This will include in vitro and in vivo tests for growth of infectious agents in various cell lines or animal systems, as well as reverse-transcriptase assays and possibly nucleic acid testing.

Recipient or parental organism

The replicon vaccine vector system was developed from an attenuated strain of Venezuelan equine encephalitis virus (VEE). The replicon RNA consists of the cis-acting 5' and 3' ends of the VEE genome, the complete non-structural protein gene region, and the subgenomic 26S promoter. The genes encoding the VEE structural proteins were replaced with the gene encoding gD2.

Host/vector system

The replicon construct is a single cycle vector. It contains the 5' region of the virus genome encoding the non-structural protein genes, which express replicase and transcriptase functions, the cis-acting sequences required for replication and packaging of the genome, and the promoter sequence for transcription of the subgenomic mRNA, upstream of the inserted gD2 gene. It is therefore capable of RNA replication and transcription, and high level expression of the inserted gene, within the cell cytoplasm. It lacks the sequences for the structural protein genes, and therefore cannot induce particle assembly and spread.

Origin & function

Sequences encoding the HSV-2 gD gene were inserted. High level expression of HSV-2 gD would be expected in infected cells.

Herpes simplex virus is a hazard group 2 human pathogen. Glycoprotein D is an envelope glycoprotein, involved in receptor-recognition and entry into cells. The protein is not known to be toxic, and a number of subunit HSV-2 vaccines incorporating gD-2 have been shown to be safe in humans.

Evaluation of foreseeable effects

The replicon RNA is packaged into VRPs for use of a bipartite helper system. Two separate RNA constructs, encoding the capsid and envelope genes respectively, are co-transfected into Vero cells with the replicon RNA, to provide the structural proteins in trans. The helper RNA constructs retain the cis-acting elements required for genome replication, and the promoter for transcription of subgenomic RNA, but lack the packaging signals.

Although VRPs will not produce infectious virus and spread, there is a small but finite risk that replication competent virus could be produced either by co-packaging of...
Replication competent virus was detected following co-transfection of a VEE replicon and a number of different monopartite (single) helper plasmids. Partial sequence analysis of three independent isolates suggested that these arose from non-homologous recombination, at different sites within the heterologous gene insert. The use of a bipartite helper system was introduced to significantly reduce the risk of replication competent virus, by increasing the number of recombination events required to generate an infectious virus.

At least two independent recombination events would be required to generate a recombinant, replication competent virus. The Sponsor estimates the frequency of such events to be in the order of 1 in 10e12. In addition, as these are likely to be non-homologous recombination events, the frequency of generating a replication competent virus may be considerably lower, as most such events might be expected to result in deletion/truncation of functional sequence elements, and generate non-viable recombinants.

No replication competent virus was detected in VRP stocks generated using the bipartite helper system, either in plaque assays, following blind passage on BHK cells, or following intracranial inoculation of suckling mice. A number of studies have subsequently been published using this system for different VEE replicon vaccine constructs, reporting no detection of replication competent virus. It was also noted that over 1000 rodents, a very sensitive host for VEE, and 64 macaques have been given primary doses as high as 10e8 of VRP, with no detectable clinical signs.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The vector is based on an attenuated vaccine-candidate strain, V3014. The replicon construct is a single-cycle vector, which will not produce infectious virus progeny, and will not spread to uninfected cells. Replication of the virus RNA takes place in the cytoplasm, and there is no DNA intermediate, minimising the risk of integration into the host cell genome. The inserted gD sequence would not be expected to increase the risk of the construct. The vector can therefore be considered to be of significantly lower risk than the parent strain, and classed as a class 2 GM activity.

The ACGM level of containment would be class 2 and the ACDP level of containment is level 2. All manipulations will be performed in a minimum of level 2 containment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

VEE is an enveloped virus, of low-medium resistance to physico-chemical inactivation. It is sensitive to solvents and detergents, and to disinfectants including 1% sodium hypochlorite, 70% glutaraldehyde, and formaldehyde.2.3.

All liquid waste will either be autoclaved, or discarded into Virkon at a final concentration of at least 1%, and allowed to soak overnight prior to discard. Solid waste will either be soaked overnight in * 1% Virkon, and then autoclaved, or autoclaved directly at a minimum of 120 degrees C, 15 lbs pressure for 15 minutes. Autoclaved waste is then uplifted by a contractor and subsequently processed through a heat treatment process, prior to landfill.

In-house studies have shown that 1% Virkon is effective for the inactivation of a broad range of viruses. The performance of the autoclave is validated on an annual basis.

Solid (non-carcass) animal waste will be collected, placed in an incinerator bag in a locked bin, and then sent for incineration off-site. All liquid waste will be discarded into Virkon (final concentration 1%), for a minimum of 16 hours prior to discard. Carcasses will be placed in an incinerator bag in a locked bin, and incinerated on-site.
The committee required that the work be notified to the HSE. The work is to be undertaken in designated rooms by designated personnel. Fumigation of the biocontainment hood will be performed. All waste disposal and laboratory cleaning will be performed by the designated operator.

**Project Containment**

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</tbody>
</table>

- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 557/06.1

- **Date Ackn'd**: 17/05/2006
- **CU2 Project Title**: BIOLOGICAL SAFETY TESTING OF AN ONCOLYTIC ADENOVIRUS VECTOR, BASED ON HUMAN ADENOVIRUS TYPE 5, AND EXPRESSING RELAXIN.
- **Class**: Class 2
- **CultureVolClass2**: Not Applicable
- **CultureVolumeClass3-4**: Not Applicable
- **Non-GMM**: Consent Granted
- **Consent Granted**: Not Applicable
- **Date Project Ceased**: 10/01/2007
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Historical Significant Changes**: 
- **Historical Date of Additional Info**: 
- **Significant Change ID**: 

**Additional Information**

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee required that the work be notified to the HSE. The work is to be undertaken in designated rooms by designated personnel. Fumigation of the biocontainment hood will be performed. All waste disposal and laboratory cleaning will be performed by the designated operator.
### Date of Significant Change

#### Project Additional Information

#### Purposes of the contained use

To perform routine biological safety testing on materials intended for human use. Materials will be tested using a number of different assays for the detection of adventitious agents. This may include tests for growth of infectious agents, as well as reverse-transcriptase assays and nucleic acid testing.

#### Recipient or parental organism

The parental virus for this vector is Human adenovirus type 5 (a group C adenovirus). Human adenoviruses are widespread, and are associated with a number of clinical syndromes, including acute febrile respiratory illness, epidemic keratoconjunctivitis and gastroenteritis.

Human adenoviruses are classed as hazard group 2 agents, by ACDP.

#### Host/vector system

The vector is a conditional replication competent adenovirus. The main features include (i) replacement of the Ad5 E1 promoter with a modified human telomerase reverse transcriptase (mTERT) promoter, to allow preferential expression of E1A in tumour cells, (ii) insertion of a stop codon at the start of the E1B 19K open reading frame, and (iii) insertion of the gene for human relaxin within a non-essential region of the virus genome.

#### Origin & function

The vector system is based on human Ad5. The construct is a conditional replication competent virus, designed to replicate efficiently within tumour cells, but not in most normal cell types. There is no known toxicity associated with the inserted gene, and expression of the gene would not be expected to alter the tropism of the virus. Based on its host range restriction, the vector would be expected to be no more hazardous, or possibly less so, than its parent virus.

Vector stocks provided for testing are grown on HEK 293 cells, a complementing cell line containing the E1 region of Human Ad5. There is therefore a risk that high titre stocks could contain replication competent adenovirus, containing a wild-type E1 region, and the inserted relaxin gene. Such recombinants would not be expected to be significantly more pathogenic than the parent virus.

#### Evaluation of foreseeable effects

The vector is based on human Ad5, and is host-range restricted, growing efficiently only in cells expressing active telomerase. The inclusion of relaxin is not expected to increase the risk of the construct. The construct is not considered to be of higher risk than its wild-type parent. Wil-type Ad5 is a class 2 human pathogen.

Due to the containment procedures in place within laboratory areas, the procedures governing working practices, and the fact that human adenovirus would not be considered pathogenic for animals following natural infection, the overall risk to the environment is considered low.

Therefore, the adenovirus vector described above is classed as ACGM class 2 and ACDP class 2.

All work with infectious materials will be performed within containment level 2 laboratories, with all manipulations performed in a biosafety cabinet. Use of sharps, and
procedures which may generate aerosols, will be avoided wherever possible. Operators handling infectious materials will wear appropriate protective clothing (gown/lab coat or equivalent, gloves, eye protection).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be inactivated by either autoclaving (with or without prior chemical inactivation), or by incubation overnight in the presence of Virkon at a final concentration of at least 1%, prior to discard.

Solid waste will be collected in suitable clinical waste packaging, appropriately sealed, then placed and stored in designated containers within a secure area until collected for off-site incineration.

In the event of a spill, the area will be cleaned using 2% Virkon, and 70% isopropanol, and mopped up with disposable wipes, which are then discarded as for solid waste. Larger spills may be disinfected using solid Virkon, and hoods may be fumigated with formaldehyde vapour if necessary.

In-house studies show that 1% Virkon, and formaldehyde fumigation, are effective for the inactivation of a broad range of viruses, including Ad5. The performance of the autoclave is validated on an annual basis. All disinfectants have a defined expiry date and a documented procedure is in place for preparation of the reagents.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

The GMO committee agreed with the classification, and the content of the risk assessment. Minor amendments were requested and incorporated, to add clarification concerning the risk to the environment (animal health) in the event of escape.

The committee required the work to be notified to the HSE.

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02/03/2022
### Project Additional Information

**Purposes of the contained use**

To manufacture a pre-master virus seed stock, master virus seed stock and clinical batches of reassortment H5N1 vaccine material under good manufacturing practice.

**Recipient or parental organism**

Human influenza virus strain A/PR/8/34 (PR8) (H1N1).

**Host/vector system**

The reassortments will be produced by reverse genetics using either an 8 plasmid or 12 plasmid strategy.

**Origin & function**

The reassortments are intended for use in the production of inactivated human influenza pandemic vaccines.

The parental virus is the human influenza virus strain A/PR/8/34 and is a member of the influenza A genera of the orthomyxoviridae family. PR8 has over 100 passages in each of mice, ferrets and embryonated eggs. The result of such a passage history is almost complete inability to replicate in man and complete attenuation in man.

The inserted genes are the haemagglutination (HA) and neuraminidase (NA) gene segments of a highly pathogenic H5N1 avian influenza virus. The HA gene is modified to reduce pathogenicity; the multiple basic amino acids at the HA connecting peptide (cleavage site), which are associated with high pathogenicity, will be greatly reduced to a single basic amino acid. Further detailed information is provided in the attached risk assessment and the WHO publication "WHO (2005) annex 5. WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines".
The reassortment virus is derived using reverse genetics and contains 6 internal gene segments of PR8 and the NA and modified HA segment of highly pathogenic H5N1 avian influenza virus isolates. It is envisaged that such reassortments derived in this way would be attenuated for humans and would be of low pathogenicity for chickens and animals compared to the H5 wild type. An indirect hazard may exist through secondary reassortment with a human or animal influenza virus. Several events would need to occur for this to happen and the probability that a PR8 reassortment strain will replicate and combine with another influenza virus(es) in human cells is considered to be minimal. The reassortment virus has avian receptor specificity and the contribution of the PR8 genes to replication and virulence in birds is unknown. However, experimental evidence has shown that not only is the virus attenuated for humans but also for chickens, whereby removal of the multi-basic amino acids from the H5 x PR8 reassortments is likely to have a major part to play in reducing the risk for chickens. Although pigs are not susceptible to infection with PR8, a reassortment containing a single gene (HA) from an H1N1 isolate infected pigs. It is therefore possible that pigs could be susceptible to a H5N1 reassortment and that these species would also be susceptible to secondary reassortments between H5N1 reassortment and a pig virus. Furthermore, there is evidence in the USA that triple reassortments between avian, pig and human influenza viruses can circulate in pigs. Further detailed information is provided in the attached risk assessment and the WHO publication "WHO (2005) annex 5. WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines".

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated waste from the cleanrooms is sealed in autoclave bags which are then sprayed liberally with 2% Virkon and 70% IPA. The sealed bag is placed inside a second autoclave bag which is sprayed with 2% Virkon and 70% IPA and sealed. Disposable pipettes, which have been in contact with viral material, are disposed of (in biosafety hood) into a roller bottle or other container, which is at least a third full of 2% Virkon. When this container is full the lid is replaced, and the whole container sprayed with 2% Virkon and 70% IPA prior to placing in the autoclave bag.

Autoclave waste is held in the cleanroom, where possible, until all processing is complete for that day. After processing all waste is transported to the waste autoclave room.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The risk assessment attached has been revised to take account of the comments provided by the GMSC, and the content therefore reflects the consensus view of the committee.

The question was raised of how long a quarantine period should be applied for laboratory workers coming in contact with porcine species; it was agreed that a period of 48 hours should be recommended and this was duly noted in the risk assessment.

The matter of avoidance of any work performed with other influenza viruses while work on the H5N1 reassortment virus is being performed was discussed. It was agreed that on the whole the standard working procedures and policies governing work practices at BioReliance would preclude work being performed on influenza at the same time and within close proximity to the H5N1 work. In addition, pipettes should be decontaminated prior to being removed from the biological safety cabinet by soaking in 2% Virkon overnight.

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**Project Ref** 557/08.1

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<td>03/09/2008</td>
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<td>Class 2</td>
<td>1-50 Litres</td>
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**Historical Significant Changes**

- Withdrawn [N]
- Not Applicable

**Project notified under transitional arrangements** [N]
Project Additional Information

Purposes of the contained use
To manufacture adenovirus vector material, including pre-master virus seed stock, master virus seed stock and clinical batches, in compliance with Good Manufacturing Practice.

Recipient or parental organism
Human and Simian adenoviruses

Host/vector system
Adenovirus vectors provided for manufacturing are typically replication incompetent, deleted in the E1 region of the genome. A region essential for adenovirus replication. The E1-deleted vectors can therefore only be propagated in specifically designed complementing cell lines expressing the adenovirus E1 region (e.g. 293/PER.C6 cells). These vectors would not replicate and spread within an infected host, and therefore can be considered non-pathogenic in humans and animals. (continued on page 10 *)

Origin & function
Manufacturing
Adenovirus vectors provided to BioReliance for manufacturing are designed for use as vaccines, or for use in gene therapy, or the treatment of the cancer. Examples of genes expressed by these vectors are shown in table 2, Appendix I of GMO # 190. The genes are stably inserted within the virus genome, most commonly under promoters designed for high level expression in the infected cells. They include vaccine antigens from structural or surface proteins of the target pathogen, hormones or cytokines.

Evaluation of foreseeable effects
Vaccine antigens are typically structural or surface proteins derived from the target pathogen, and not themselves biologically active, and are therefore generally unlikely to be directly toxic or harmful. There may, however, be exceptions. For example, the Ebola virus glycoprotein, which is involved in receptor binding and virus entry, is also thought to contribute to haemorrhage during infection, and can induce a cytopathic effect in transfected cells in vitro. Tumour-specific antigens may be expressed by vectors designed for use in the treatment of cancer, and again these are typically not biologically active, and are unlikely to be directly toxic or harmful.

As adenoviruses are non-enveloped viruses, with a tightly regulated capsid structure, it is considered unlikely that the expressed antigens could become incorporated into the virus capsid, and alter the host range or tropism of the vector particle.

Therapeutic products expressed by the gene therapy vectors may be biologically active, e.g. hormones or cytokines. Although typically intended to replace normal human proteins deficient in the relevant individuals, some of these products may potentially be harmful to a host if expressed at high levels or within certain tissues, or be of higher risk for certain individuals (e.g. pregnant women, immuno-compromised host) Growth factors or cytokines, for example, may be teratogenic in an unborn foetus, or potentially provide a growth advantage to a developing tumour.

In the majority of cases, it is considered unlikely that expression of therapeutic gene product or vaccine antigen would increase the host range or pathogenicity of the adenovirus vector. However, vectors used as vaccines or for cancer therapy may also express immuno-modulatory genes, designed to enhance the immune response to tumour cells or target antigens. In the absence of direct data, it may be difficult to predict the effect such genes might have on the normal immune response to the virus, and hence the effect on the pathogenicity or risk of the recombinant adenovirus. (continued on page 10 ##).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
N
If yes, tick to confirm that it is attached to this form  
N
Tick to confirm that you have attached a risk assessment to this form  
Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

A comment was made that clarification of the following terms used within the manufacturing environment at BioReliance should be made within the risk assessment:

1. Clarification on the zones referenced with respect to clean rooms and biosafety cabinet (section 6.2)
2. Clarification on the name of disinfectant referenced (LpH, section 7.0)

Both of these comments were addressed within the drafting of the GMO risk assessment.

Project Containment

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Project Ref 557/10.1

Date Ackn’d 22/01/2010

Date Project Ceased

Biological safety testing of lymphocytes isolated from HIV patients and transduced with retrovirus vector

Class 3

Consent Granted Yes

Project notified under transitional arrangements N
### Project Additional Information

**Purposes of the contained use**

To perform biological safety testing of transduced lymphocytes intended for use in anti-retroviral therapy. Tests may include biochemical, immune-based or molecular assays, such as ELISA or PCR assays, as well as inoculation of the materials in vitro. The assays are intended for detection of potential containments (e.g. sterility, mycoplasma, in vitro and PCR assays for viral containments including replication competent retrovirus).

**Recipient or parental organism**

Human lymphocytes isolated from patients positive for Human Immunodeficiency virus.

**Host/vector system**

The human lymphocytes are transduced with a retrovirus vector whose expression inhibits infection with Human Immunodeficiency virus type 1 (HIV-1). The vector, M87o, codes for a protein that consist of the following modules: a signal peptide (SP) to direct translocation in the cell, the antiviral C peptide (C46), a hinge and a membrane spanning domain (MSD). M87o was obtained by insertion of this cassette into the optimized retroviral vector, MP71, derived from Myeloproliferative sarcoma virus (MPSV). The vector expresses a membrane-anchored peptide derived from the HIV-1 gp41 transmembrane glycoprotein which blocks membrane fusion and hence entry of the HIV virus. Transcription of the inserted genes is regulated by the LTR promoter of MPSV. The SP comprises the sequence dLNGFR, the low affinity nerve growth factor receptor, to direct translocation into the endoplasmic reticulum. The antiviral C peptide (C46) interacts with the trimeric coiled coil of HIV-1 gp41 which is consequently locked in a fusion incompetent state and entry of HIV-1 into the target cell is inhibited. The hinge sequence is derived from the human IgG2 to reduce potential immunogenicity and the MSD from human CD34 T cells which enables more stable anchoring in the membrane in the target cell. At the 3' UTR, there are regulatory elements, WPRE (from woodchuck hepatitis virus, without X protein) and RRE (Rev-responsive element).

**Origin & function**

The GMO under review is cultured human lymphocytes isolated from HIV patients which utilise membrane-bound T20 expressed, via a retroviral vector (M87o), on the surface of HIV target cells to act as an effective inhibitor of HIV replication. The planned procedure includes that patients may receive a transplant of autologous CD34+ cells transduced with M87o or allogenic m87o-modified CD34+ cells as part of an anti-retroviral therapy. Highly active antiretroviral therapy (HAART) can effectively suppress human immunodeficiency virus (HIV) replication and thereby slow progression of immunodeficiency but cannot eradicate the virus and must be administered to the patient continuously to prevent viral rebound. Extended treatment with antiretroviral drugs is invariably accompanied with problems such as toxicity and viral resistance, hence, complementary antiviral therapies, such as gene therapy could improve efficacy of treatment.

**Evaluation of foreseeable effects**

Primary human cells are extremely unlikely to survive in the environment, or following accidental inoculation of an operator. Retroviral vectors are unlikely to survive in vivo as they are susceptible to complement-mediated killing. Further, independently of transduction, the patient's lymphocytes would be recognised as allogeneic (non-self).
cells, and destroyed by an immune competent operator's immune system.

The vector is stably integrated into the host cell genome. There are no human retroviral sequences or infectious human retroviruses able to mobilise an MLV retroviral sequence, such as this vector. Therefore, the risk of mobilization/transfer is low. The expressed proteins are not known to be toxic or harmful, and the level of protein expression is unlikely to increase the risk of the materials.

The vector is replication defective. It does not encode capsid or envelope proteins, or the proteins required for replication, mobilization or integration of the retrovirus genome. The vector is integrated in the host cell, and therefore should represent a low risk of transfer. The vector contains promoters and enhancers for expression of the transgene and is not expected to be toxic or harmful as it is present in a multitude of clinically used retroviral vectors.

Testing for replication competent retroviruses (RCR) is to be performed and although no RCR formation has been described to date, there is the possibility of RCR presence.

Since the GMO has been derived from HIV+ patient cells, the presence of wild-type HIV in the test item cannot be excluded and constitutes a significant risk in handling. The transduced lymphocytes must be considered a GM class 3 activity due to the risk of infection with the ACDP category 3 virus, HIV-. Furthermore, operators should be aware that in the event of accidental exposure, a serological response to HIV may occur due to the possible expression of gp41 HIV derived gene insert (independent of any extraneous HIV being present in the test material).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In accordance with Bio Reliance standard operating procedures (SOPs), all waste will be inactivated by autoclaving (with or without prior chemical inactivation), prior to collection by an external contractor. The waste will then be subject to further heat treatment.

All solid waste will be collected in suitable clinical waste packaging i.e. double seamless, tear-resistant autoclave bags, appropriately sealed, placed and stored in designated containers within a secure area until autoclaved and then collected for off-site inactivation.

All plasticware and solid waste will be rinsed and/or immersed in 2% Virkon prior to being placed in autoclave bags in the Class II hood which will be closed with autoclave tape and placed in the large double autoclave bags for autoclaving and subsequent removal as above.

Disposal of potentially infectious material such as the test article or supernatant from infected cell cultures should be discarded according to the relevant SOP and discarded into 10% Virkon or equivalent by an operator wearing appropriate PPE. Following overnight decontamination in the Class II hood under UV illumination, the sealed container should be autoclaved at a minimum of 121°C for 20 minutes.

In-house studies have shown 1% Virkon to be effective in the inactivation of a broad range of viruses, including HIV, a retrovirus model (CP39222).

The performance of the autoclaves is validated in accordance with a pre-determined schedule.
1. Sections 4.0 and 5.0; include justification for stating primary human cells are unlikely to survive in the environment.

2. Add as per SOP # KPSP2010, lab must be evacuated immediately for all spills occurring outside the biological safety cabinet.

**Project Containment**

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**Project Ref 557/12.1**

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<td>Production of a stock recombinant live attenuated Measles virus expressing Chikungunya genes on behalf of client</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Consent Granted</td>
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</tbody>
</table>

- Non-GMM
- Consent Granted

**Project notified under transitional arrangements**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**
**Project Additional Information**

**Purposes of the contained use**

To produce a stock of recombinant live attenuated Measles virus expressing Chikungunya genes. The manufacture will be performed in a containment level 2 cleanroom within our GMP manufacturing facility.

**Recipient or parental organism**

The recipient organism is the Schwartz strain of the Measles virus.

The measles virion is an enveloped, non-segmented spherical particle of diameter 120-150nm, classified as a species of the genus Morbillivirus, in the family Paramyxoviridae containing a helical nucleocapsid of negative stranded RNA which encodes nine known proteins in the order 3' -N-PVCR-M-F-H-L-5'. Proteins P, V, C and R are in overlapping reading frames. Viral proteins have been purified from these products as the haemagglutinin (H), matrix (M), nuclecapsid (N), fusion (F) and phosphoproteins (P).

Measles virus can be grouped into a large number of genotypes based on the sequences of the NP or the H gene, enabling epidemiological study and this is the basis for global vaccination programmes. Measles Virus haemagglutinates primate erythrocytes only and lacks neuraminidase.

The Measles Virus can be adapted for growth in a number of cell lines or in eggs.

Replication of virus is cytoplasmic and syncytial formation of multinuclear giant cells occurs followed by gradual cell death. Progeny virions exit the cell by budding at the plasma membrane.

The Edmonston strain of MV was isolated in 1954 by cultivation on primary human kidney and primary human amnion cells. It was subsequently adapted to propagation in chicken embryos and to chicken embryo fibroblast cells, to produce the attenuated Edmonston A and B seeds, The Edmonston B vaccine was licensed in 1963 as the first Measles vaccine and was widely used until 1975. This vaccine strain was efficacious, but extremely reactogenic producing fever and rash. Further passages of Edmonston A and B on chicken embryo fibroblast cells produced the more attenuated Schwarts and Moraten strains, whose sequences have been shown to be identical. The Schwartz reversion of vaccine strains to those causing pathogenicity has not been demonstrated. Recombination between paramyxoviruses is rare and does not occur naturally. Measles virus replication occurs solely in the cytoplasm which excludes risk of integration of genetic material. The Measles vaccine virus infects only human or primate cells expressing the CD46 receptor. Following inoculation of humans, its replication is transitory and viraemia is very low, therefore the risk of transmission is negligible.

Wild-type Measles Virus is an ACPD hazard group 2 pathogen.

**Host/vector system**

The GMO described is a recombinant MV vector containing a Chikungunya virus (CHIKV) construct composed of envelope protein. There are many instances in the literature which cite the use of CHIKV E1, and E2 envelope proteins as CHIKV-specific serodiagnostic for Chikungunya fever.

The MV vector was derived from one of the safest and most widely used Measles vaccines, the live, attenuated Schwartz strain vaccine virus, by cloning cDNA from viral particles purified from an industrial production batch using procedures designed to optimise cloning fidelity. The vector sequence, present in the construct, is the same as that if the Schwartz vaccinal strain. Investigators demonstrated that the growth kinetics and production yield of the molecular clone and the original virus were identical.

The plasmid was transfected into the helper cell line 293-3-46 to enable rescue of the recombinant virus and co-cultivated with primate Vero cells. Recombinant virus rapidly adapted to growth in Vero cells. Construction of the original cloned Schwartz strain included sequencing of the rescued virus following co-culture in CEF cells.
co-culture in Vero cells was accompanied by mutation in 2 out of 15,894 nucleotides which were widespread throughout all clones representing the viral population. Thus, it was suggested that adaptation of the host cell of Schwartz virus might affect the properties of the virus. However, safety appears to be retained, as since 2003, the date of the original cloning of the Schwartz strain, the recombinant virus has been developed for use as a vaccine for West Nile Virus.

Origin & function

The GMO under consideration is a replication competent live attenuated recombinant Measles Virus that contains the Chikungunya envelope protein. The recombinant virus was derived from the widely used Schwartz strain Measles vaccine. The Sponsor has indicated that the recombinant virus is intended to be used as a vaccine against Chikungunya fever. From information provided by the Sponsor, the GMO is unlikely to cause disease. The client has stated that infection by the virus in humans is via CD4 receptor, but entry into host cell is also reported to be mediated by CD46 for the Edmondson strain of the virus.

Chikungunya virus (CHIKV) is an insect-borne Togavirus, of the genus Alphavirus. Chikungunya (CHIK) fever, the disease caused by CHIKV, was first recognised in epidemic form in East Africa during 1952-1953. The virus is indigenous to tropical Africa and Asia, where it is transmitted to humans by the bite of infected mosquitoes, usually of the genus Aedes aegypti salivary glands is asymptomatic and lifelong, CHIK fever is transmitted by Aedes aegypti to humans, but it should be noted that there is no human to human transmission. The main symptoms of Chikungunya infection are sudden onset of chills, fever, headache, rash and debilitating arthralgia causes fever and severe joint pain. Other symptoms include muscle pain, headache, nausea, fatigue and rash. The word Chikungunya is thought to derive from description in local dialect of the contorted posture of patients addlicted with the severe joint pain associated with this disease. The disease shares some clinical signs with Dengue, and can be misdiagnosed in areas where Dengue is common.

There have been recent breakouts of Chikungunya in Africa, Asia and the Indian subcontinent. In recent decades mosquito vectors of Chikungunya have spread to Europe and the Americas. In 2007, disease transmission was reported for the first time in Europe. Since the beginning of 2006, there has also been an explosive emergence of the disease in nations in the Indian Ocean area. By March 7, 2006, 157,000 people had been infected in the French island La Reunion, and the disease had spread to the islands of Seychelles, Mauritius, and Mayotte. Subsequently, the disease has appeared in India, China and European countries.

Evaluation of foreseeable effects

Expression of the Chikungunya product of the inserted gene results in an antigen designed to produce antibody to Chikungunya fever. In the absence of any information being provided by the Sponsor, toxicity is assumed and as a result, the material should be handled in line with standard COSHH level 2 safety procedures. Efficacy and safety results have similarly not been disclosed by the Sponsor.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste from the manufacturing pilot labs and cleanrooms must be disposed of in accordance with current SOP's. The current procedures for inactivating viral waste in manufacturing pilot labs and cleanrooms are as outlined below:

Liquid waste is treated with LpH solution (at a concentration of 16ml LpH per 2L of liquid waste) and left for at least 30 minutes contact time. Pipettes are disinfected by repeated aspirating (3 times) with LpH solution (at a concentration of 16ml LpH per 2L of water for irrigation) prior to placing back into the pipette wrapper which is subsequently sprayed with Klercide B and 70% IPA prior. Flasks and other vessels are wiped down with wipes sprayed with LpH solution, Klercide B and 70% IPA prior to removal from the biosafety cabinet. Gilson pipette tips are discarded into an appropriate vessel which is closed and cleaned prior to removal from the biosafety cabinet. All viral waste is placed into separate autoclave bags which are sanitised with LpH (at a concentration of 16ml LpH per 2L of water for irrigation) prior to exiting the cleanroom/pilot lab. All waste should be double bagged in autoclave bags prior to removal from the pilot lab/cleanroom. The double bagged waste is placed in yellow UN...
approved outer disposal bags and placed in yellow bins for transfer to the secure containers for waste uplift for off site inactivation.

In the event of a spillage the procedures are summarised below:

1. For a small scale spill within the biosafety cabinet (<500ml) and low concentration of material (diluted virus stock or bulk harvest materials) the spillage is contained with cleanroom wipes. The affected area is treated with LpH solution (at a concentration of 16ml LpH per 2L of water for irrigation) and left for at least 30 minutes contact time before wiping dry and spraying with 70% IPA and wiping dry again.

2. For a larger volume spill (>500ml) or high concentration of virus (final product or concentrated virus) within the biosafety cabinet work will stop and the spillage will be contained by wiping up with cleanroom wipes. The affected area is treated with LpH solution (at a concentration of 16ml LpH per 2L of water for irrigation) and left for at least 30 minutes contact time before wiping dry and spraying with 70% IPA and wiping dry again. A senior member of staff is contacted for further advice and the biosafety cabinet formaldehyde fumigated at the end of the processing day.

3. For any size of spill out with the biosafety cabinet but within the cleanroom operators should leave the cleanroom immediately to contain aerosols and notify senior manufacturing staff. After clearance from senior manufacturing staff and after at least 30 minutes the spillage will be contained with cleanroom wipes treated with LpH solution left for a 30 minutes contact period. The area once treated with LpH solution is wiped dry and sprayed with 70% IPA and wiped dry again. All actions taken will be recorded in an incident/accident report.

The risk assessment attached has been revised to take account of the comments provided by the GMSC, and the content therefore reflects the consensus view of the committee.

Note that the committee endorses the view that a cautionary approach to classification be adopted. The attached risk assessment reflects the planned manufacturing activities at BioReliance, taking account of manufacturing processes and scale.

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Purposes of the contained use

Biosafety testing of recombinant lentiviral vector (pCCL-INVO-SP) virus-containing supernatant and associated materials. Testing will compromise sterility testing, mycoplasma detection assays, in vitro cell culture assays, molecular-based nucleic acid detection assays, biochemical and immunological based detection assays. All assays are designed to detect adventitious agents.

The GMO under consideration is a recombinant lentiviral vector, derived from HIV-1, expressing a therapeutic gene SPINK5 product which performs a critical role in the maintenance of a healthy skin barrier. The gene, SPINK5, is under the control of a 500kb element cloned from the human involucrin promoter which restricts transgene expression to the site of the skin pathology. The recombinant lentivirus has been pseudotyped with an alternative coat protein, the vesicular stomatitis virus glycoprotein (VSV) G protein, to increase the tissue-tropism range of the vector. The vector is intended for ex vivo clinical use in humans. The vector will be used to transduce keratinocyte stem cells (KSC) from patients with the skin disease Netherton syndrome (NS). The gene-modified stem cells will be propagated and used as autografts in NS patients in a Phase I clinical trial.

Recipient or parental organism

The parental virus is Human immunodeficiency virus type 1 (HIV-1), a species in the genus Lentivirus (family Retroviridae), which is the primary aetiologic agent of Aquired Immunodeficiency Syndrome (AIDS), a disease that results in the gradual destruction of the helper-T cell population in infected individuals, leading to immunosuppression.

Lentiviruses are large single-stranded, positive sense, enveloped RNA viruses which contain an RNA-dependent DNA polymerase. Following entry into the host cell, viral RNA is reverse-transcribed into a covalently linked circle of double-stranded DNA (provirus) which is integrated into the cellular DNA by action of the virus-encoded integrase and other cellular co-factors. The integrated proviral DNA thereafter remains latent or is transcribed by cellular transcriptional activators and is replicated in tandem with the host cell DNA. All lentiviruses contain several genes in addition to the three major genes encoding the main retrovirus structural proteins Gag and Env and the main enzymic protein Pol.
Several factors make retroviral vectors attractive for gene therapy including their relatively large cloning capacity and their ability to stably integrate into the host cell genome, which is a likely requisite for long-term expression. Further, they do not transfer viral genes and avoid transduced cells that are destroyed by virus-specific cytotoxic T cells. In addition to these characteristics that are common to all retroviral vectors, lentiviral vectors can transduce non-dividing cells, a crucial asset for genetically modifying the main target tissues of gene therapy such as the brain, soft-tissue organs and the haematopoietic system, and enable efficient in vivo delivery, integration and stable expression of transgenes. However, biosafety of HIV-based vectors requires most careful evaluation, taking into account the significant pathogenicity of the parent virus.

HIV-1, a category 3 pathogen can infect man and some non-human primates.

The HIV-1 genome is complex and attenuation of the recombinant virus described here has been achieved by removal, modification or replacement of viral genes encoding factors necessary for virulence and replication competence.

**Host/vector system**

The lentiviral vector is derived from HIV-1. Wild type HIV-1 has been assigned to ACDP hazard group 3 however the recombinant HIV-1 vector has been modified extensively as detailed below, such that it can be derogated and re-classified as suitable for handling under ACGM level 2 conditions in BioReliance.

The HIV-1 based lentiviral vector gene-delivery system comprises two elements, the packaging system, which includes structural proteins and enzymes for the generation of infectious particles, and the transfer vector, itself, intended to deliver the inserted therapeutic gene to the host cell. The GMO comprising this submission is a “third generation” HIV-1-derived vector containing only three of the nine HIV-1 parental genes, gag, pol and rev, rendering it clinically acceptable. The extensive deletions have also eliminated the possibility of generation of wild-type virus through recombination.

This 3rd generation, replication-defective, self-inactivating (SIN) vector has undergone further modification by introduction of a deletion in the U3 region of the 3’ long terminal repeat (LTR) of the DNA used to produce the vector RNA. During reverse transcription, this deletion transferred to the 5’ LTR of the proviral DNA, the implication being that sufficient viral sequence can be eliminated to abolish transcriptional activity of the LTR thus, abolishing the ability to produce full length vector RNA in transduced cells which minimizes the risk of emergence of replication competent retroviruses (RCR). The SIN lentiviral particle enables production of infective particles only in the first round of infection. The risk associated with the expressed product(s) of the SIN-LTR element is expected to be low. Self inactivation minimizes the risk that RCR will be produced. Further, it reduces the likelihood that cellular coding sequences located adjacent to the vector integration site will be aberrantly expressed, either due to the promoter activity of the 3’ LTR or through an enhancer effect. Finally, the SIN design prevents potential transcriptional interference between the LTR and the internal promoter driving the transgene.

**Origin & function**

The inserted genes comprise a codon-optimised human SPINK5/LEKTI gene which functions as a serine protease inhibitor and the regulatory elements (involucrin promoter and the mutated WPRE intended to increase transgene expression).

The expressed product of the therapeutic gene, SPINK5 is not expected to be toxic or harmful. The gene product is a protein named LEKTI, a serine protease inhibitor expressed in the uppermost compartments of the skin epidermis, which plays a critical role in the regulation of proteases involved in production of cellular components necessary to the maintenance of a healthy skin barrier. The natural gene is expressed in healthy individuals. The SPINK5 gene is under control of a 572bp enhancer derived from the human involucrin promoter which mediates compartment specific gene expression restricted to the supra-basal layers of the epidermis and is resistant to methylation mediated silencing effects. The natural gene is also expressed in healthy individuals and there is confirmatory evidence of in vitro expression of the gene being restricted to the outer layers of the skin. In addition there is evidence of efficacy and safety of this vector use in a mouse model.

The lentiviral vector was produced following transient transfection of human 293T cells by four plasmids using the calcium phosphate precipitation technique. One plasmid encoded the therapeutic transgene. The 3 accessory plasmids encoded separately, the structural proteins and enzymes required for replication, the surface glycoprotein (env coat protein of vesicular stomatitis virus [VSV-G]) and the regulatory elements required for packaging the recombinant. The final product is illustrated in Figure 2 and...
shows the lentivirus 5' and 3' SIN long terminal repeat (LTR) flanking the rev responsive element (RRE), central polypurine tract (cPPT), the transgene expression cassette including the internal promoter INVO, the therapeutic gene SPINK5 and the post-transcriptional regulatory element (WPREmt). Please see attached Risk Assessment for vector maps.

**Evaluation of foreseeable effects**

The GMO under consideration is a lentiviral vector derived from HIV-1. Wild type HIV-1 has been assigned to ACDP hazard group 3 however the recombinant HIV-1 vector has been modified extensively. The GMO is a replication defective, self-inactivating virus vector incapable of stable integration in target cells.

There is only a theoretical risk of generation of RCR following recombination events between the transfer and packaging plasmids during production. The material has not yet been tested for the presence of RCR; however, testing is planned.

Expression of the vector gene product (LEKTI protein) is unlikely to represent a significant risk to the operator as the natural gene product is expressed in healthy individuals. Expression of the therapeutic gene will be limited to the outer layers of the skin. This specificity of site of the target cells and the likelihood of complement inactivation of the VSV-G attachment protein will likely mitigate adverse effects of a possible needle stick injury.

The risk of accidental inoculation by sharp or needlestick injury is low if safety procedures for ACDP class 2 organisms are followed, however, there remains a low risk of seroconversion to the vector product or the vector itself as a universal consequence of introduction of foreign protein into an immunocompetent host.

Based on the above considerations it is judged by BioReliance that the GMO as a replication defective, self-inactivating virus vector, can be derogated from the ACGM classification of the wild type virus and re-classified as suitable for handling under ACGM level 2 conditions.

The intended purpose of the recombinant virus is to transduce stem cells which will be expanded in vitro and used as autograft material in specified patients. It has been judged that risks associated with handling of the recombinant virus and virus-infected cells etc will be appropriately contained using COSHH level 2 safety procedures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Lentivirus vectors (including HIV vectors) are enveloped viruses, which are generally sensitive to disinfectants including 1% sodium hyperchlorite, 2% glutaraldehyde and formaldehyde. Inhouse studies suggest that 2% Virkon and formaldehyde fumigation are effective for the inactivation of HIV viruses, under the conditions used at BioReliance UK.

All waste must be disposed of in accordance with current Standard Operating Procedure. Current requirements are as follows:

- Liquid waste will be inactivated by either autoclaving (with or without prior chemical inactivation), or by incubation overnight in the presence of Virkon at a final concentration of at least 1% (v/v), prior to discard.
- Solid waste will be collected in suitable clinical waste packaging, appropriately sealed, placed and stored in designated containers within a secure area until collected for off-site incineration. In addition, in Pentlands, animal carcasses will be placed in an incinerator bag in a locked bin and incinerated on-site.
- In the event of a spill, the area will be cleaned using 2% (w/v) Virkon, and 70% (v/v) isopropanol, and mopped up with disposable wipes, which are placed in an autoclave bag, for incineration. Larger spills may be disinfected using solid Virkon, and hoods may be fumigated with formaldehyde vapour if necessary.

In house studies have shown that 1% Virkon is effective for the inactivation for the inactivation of a broad range of viruses, including HIV (Validation Report # 39222).

**MONITORING**
BioReliance works to the quality standards of Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP). These quality standards require that the performance of all equipment is validated before use, and that equipment is re-validated to a pre-determined schedule. The monitoring and validation is overseen by the Equipment Support Department. In addition, all equipment is required to be maintained and checked for function on a regular basis. The equipment includes all air handling, biosafety cabinets, autoclaves etc.

All disinfectants have a defined expiry date and a documented procedure is in place for preparation of the reagents.

All work with the genetically modified organism will be documented, and will be performed in accordance with standard protocols and/or standard operating procedures.

ENVIRONMENTAL PROTECTION

Humans and some non-human primates are the only natural hosts for HIV-1. All biosafety testing will be performed on this recombinant lentivirus vector, in BioReliance, using class 2 biological safety cabinets in ACGM class 2 containment laboratories.

All work with the recombinant lentivirus vector will be performed at a minimum of containment level 2 with all waste decontaminated appropriately. In order to protect the samples during testing, work will generally be performed at containment level 2, and manipulation of the samples will be performed within a class 2 biosafety cabinet, or negative pressure isolator.

Due to the low risk of spread of the vector, the containment procedures in place within the laboratory areas, and the procedures covering working practices, the overall risk to the environment is considered negligible.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment (GMO#228) attached has been submitted to HSE as the organism assessed therein does not conform absolutely to the conditions set for classification of activities already notified under BioReliance Risk Assessment for Connected Programme of Work (CPW#02). Specifically, the GMO was assessed as a 3rd generation, self inactivating lentiviral vector based on HIV-1 and as such considered highly attenuated and replication deficient. RCR testing is requested but has not been performed as yet. The material was assigned to ACGM and COSHH class 2.

The risk assessment attached has been revised to take account of the comments provided by the GMSC, and the content therefore reflects the consensus view of the committee.

Project Containment

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</tr>
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Project Additional Information

Purposes of the contained use
To produce a stock of recombinant live attenuated Measles virus expressing Dengue genes. The manufacture will be performed in a containment level 2 cleanroom within our GMP manufacturing facility

Recipient or parental organism
The recipient organism is the schwartz strain of the Measles virus.

Measles Virus (MV) is an enveloped, spherical particle of diameter 120-150nm, containing a helical nucleocapsid of negative stranded RNA which encodes eight known proteins in the order 3'-N-PCV-M-F-H-L-5'. Proteins P,C and V are in overlapping reading frames. Viral proteins have been purified from these products as the haemagglutinin (H), matrix (M), nucleocapsid (N), fusion (F) and phosphoproteins (P). Measles virus can be grouped into a large number of genotypes based on the sequences of the NP or the H gene enabling epidemiological study and is the basis for global vaccination programmes. Virus haemagglutinates primate erythrocytes only and lacks neuraminidase. Virus can be adapted for growth in a number of cell lines or in eggs. Entry into the host cell is via a cell surface receptor (CD46 for Edmonston strain), replication is cytoplasmic and syncytial formation of multinuclear giant cells occurs followed by gradual cell death. Progeny virions exit by budding at the plasma membrane.

The Edmonston strain of MV was isolated in 1954 by cultivation on primary human kidney and primary human amnion cells. It was subsequently adapted to chicken...
embryos and to chicken embryo fibroblast cells to produce the attenuated Edmonston A and B seeds. The Edmonston B vaccine was licensed in 1963 as the first Measles vaccine and was widely used until 1975. The vaccine strain was efficacious but reactogenic, producing fever and rash. Further passages of Edmonston A and B on chicken embryo fibroblast cells produced the more attenuated Schwarz and Moraten viruses, whose sequences have been shown to be identical. The Schwarz vaccine has a history of safe and effective use. Measles virus is very stable (only phenotype exists) and reversion of vaccine strains to those causing pathogenicity has not been demonstrated. Recombination between paramyxoviruses is rare and does not occur naturally. Measles vaccine virus infects only human or primate cells expressing the CD46 receptor. Following inoculation of humans, its replication is transitory and viraemia is very low, therefore the risk of transmission is negligible.

Wild-type Measles virus is an ACDP hazard group 2 pathogen.

Host/vector system

The GMO described here is a recombinant MV vector containing a Dengue virus (DV) construct composed of four envelope domain III (EdIII), each taken from one of the four DV serotypes, fused with the ectodomain of the DV membrane protein (ectoM). Dengue virus is pathogenic flavivirus (mosquito-borne), endemic in vast tropical and sub-tropical areas, which infects 50-100 million humans annually with 25,000 deaths. Infection with one serotype of DV does not confer protection to infection from the other three. In fact DV, in the presence of non-neutralising antibody, can produce disease of increased severity. An effective vaccine against DV must induce antibody against all four serotypes.

The MV vector was derived from one of the safest and most widely used Measles vaccines, the live, attenuated Schwarz strain vaccine virus, by cloning cDNA from viral particles purified from an industrial production batch using procedures designed to optimize cloning fidelity. The vector sequence, present in the construct, is the same as that if the Schwarz vaccinial strain. Investigators demonstrated that the growth kinetics and production yield of the molecular clone and the original virus were identical.

An additional transcription unit (ATU) was inserted into the plasmid (pTM-MVSchw) to enable expression of the transgene product(s). Sequences coding for the tetravalent DV antigen, incorporating the EdIII of DV serotypes 1-4 in combination with the pro-apoptotic ectoM sequence was cloned into the plasmid. The tetravalent DV antigen is designed to induce neutralising and non-facilitating antibodies against all four DV serotypes.

The C-terminal immunoglobulin-like EdIII, a 100 amino-acid sequence stabilized by a disulphide bridge, is involved in receptor binding and contains critical epitopes that elicit both type-specific antibodies (i.e., that cross-neutralise DV but not other Flaviviruses), and serotype-specific antibodies (i.e., that neutralise one DV serotype but not another), thus is the antigen of choice to develop a tetravalent DV vaccine.

The plasmid was transfected into the helper cell line 293-3-46 to enable rescue of the recombinant virus and co-cultivated with primate Vero cells. Recombinant virus rapidly adapted to growth in Vero cells. Construction of the original cloned Schwarz strain included sequencing of the rescued virus following co-culture in CEF and in Verocells. It was found that while the MV sequence remained unchanged following co-culture in CEF cells, co-culture in Vero cell was accompanied by mutation in 2 out of 15,894 nucleotides which were widespread throughout all clones representing the viral population. Thus, it was posited that adaptation of the host cell of Schwarz virus might affect the properties of the virus. However, safety appears to be retained as since 2003, the date of the original cloning of the Schwarz strain, the recombinant virus has been used safely as a vaccine for West Nile Virus.

Origin & function

The GMO under consideration is a replication competent, live attenuated recombinant Measles Virus that contains genetic elements from Dengue Virus (DV). The recombinant virus was derived from the widely used "Schwarz strain" Measles vaccine. The Sponsor has indicated that the recombinant virus is intended to be used as a vaccine against all 4 strains of DV. From information provided by the Sponsor the GMO is unlikely to cause disease. The client has stated that infection by the virus in humans is via the CD4 receptor, but entry into the host cell is also reported to be mediated by CD46 for the Edmonston strain of the virus.

Dengue is a mosquito-borne infection which is found in tropical and sub-tropical climates that causes a severe flu-like illness (dengue fever) and can cause a potentially lethal complication called dengue haemorrhagic fever. Aedes aegypti or Aedes albopictus mosquitoes which feed during the day transmit Dengue to humans. In some Asian countries dengue haemorrhagic fever is a leading cause of serious illness and death among children.
**Evaluation of foreseeable effects**

Expression of the Dengue product of the inserted gene results in an antigen to produce antibodies to Dengue types 1-4. In the absence of any information being provided by the Sponsor, toxicity is assumed and as a result, the material should be handled in line with standard COSHH level 2 safety procedures. Efficacy and safety results have similarly not been disclosed by the Sponsor.

For in vivo work the assays would be performed in suckling mice, adult mice and embryonated chicken eggs.

- 20 suckling mice will be inoculated via the intraperitoneal and intracerebral routes and observed for evidence of viral contaminants for 14 days.
- 10 adult mice will be inoculated via the intraperitoneal and intracerebral routes and observed for evidence of viral contaminants for 21 days.
- Eggs will be inoculated via the allantoic or yolk sac route and incubated for 7 days at the end of which the embryos will be examined for abnormalities/mortality and the allantoic and yolk sac fluids analysed for the presence of haemagglutinating viruses using a haemagglutination assay.

Control measures which would be used for in vivo activities are:

- All work will be performed within containment level 2 laboratories
- Animals will be accommodated in microfiltration cages
- Inoculation of eggs and animals will be performed in class II biological safety cabinets
- Post mortem examination and egg fluid harvests and analysis will be performed in class II biological safety cabinets
- Rodent barriers will be in place at all external exit doors
- Needles will not be re-sheathed after use but will be placed in sharps bin immediately after use.

For in vivo work the assays would be performed

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste from the manufacturing pilot labs and cleanrooms must be disposed of in accordance with current SOP's. The current procedures for inactivating viral waste in manufacturing pilot labs and cleanrooms are as outlined below:

Liquid waste is treated with LpH solution (at a concentration of 16ml LpH per 2L of liquid waste) and left for at least 30 minutes contact time. Pipettes are disinfected by repeated aspirating (3 times) with LpH solution (at a concentration of 16ml LpH per 2L of water for irrigation) prior to placing back into the pipette wrapper which is subsequently sprayed with Klercide B and 70% IPA. Flasks and other vessels are wiped down with wipes sprayed with LpH solution, Klercide B and 70% IPA prior to removal from the biosafety cabinet. Gilson pipette tips are discarded into an appropriate vessel which is closed and cleaned prior to removal from the biosafety cabinet. All viral waste is placed into separate autoclave bags which are sanitised with LpH (at a concentration of 16ml LpH per 2L if water for irrigation) prior to exiting the cleanroom/pilot lab. All waste should be double bagged in autoclave bags prior to removal from the pilot lab/cleanroom. The double bagged waste is placed in yellow UN approved outer disposal bags and placed in yellow bins for transfer to the secure containers for waste uplift for off site incineration.

In the event of a spillage the procedures are summarized below:
1. For a small scale spill within the biosafety cabinet (<500ml) and low concentration of material (diluted virus stock or bulk harvest material) the spillage is contained with cleanroom wipes. The affected area is treated with LpH solution (at a concentration of 16ml LpH per 2L of water for irrigation) and left for at least 30 minutes contact time before wiping dry and spraying with 70% IPA and wiping dry again.

2. For a larger volume spill (>500ml) or high concentration of virus (final product or concentrated virus) within the biosafety cabinet work will stop and the spillage will be contained by wiping up with cleanroom wipes. The affected area is treated with LpH solution (at a concentration of 16ml LpH per 2L of water for irrigation) and left for at least 30 minutes contact time before wiping dry and spraying 70% IPA and wiping dry again. A senior member of staff is contacted for further advice and the biosafety cabinet formaldehyde fumigated at the end of the processing day.

3. For any size of spill out with the biosafety cabinet but within the cleanroom operators should leave the cleanroom immediately to contain aerosols and notify senior manufacturing staff. After clearance from senior manufacturing staff and after at least 30 minutes the spillage will be contained with clean room wipes treated with LpH solution left for a 30 minutes contact period. The area once treated with LpH solution is wiped dry and sprayed with 70% IPA and wiped dry again. All actions taken will be recorded in an incident/accident report.

For in vivo work waste bedding and carcasses will be incinerated on site.

Is an emergency plan required according to regulation 20?  N

Tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The risk assessment has been revised to take account of the comments provided by the GMSC, and the content therefore reflects the consensus view of the committee.

Note that the committee endorses the view that a cautionary approach to classification be adopted. The attached risk assessment reflects the planned manufacturing activities at BioReliance, taking into account of manufacturing processes and scale.

Project Containment

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To perform a range of Biosafety tests on the material(s) [replication competent, attenuated recombinant Dengue Virus] as requested by the Sponsor (Bioreliance client)

Purposes of the contained use

Biosafety testing of replication competent, attenuated recombinant Dengue Virus (virus-containing supernatant and associated materials). Information from the Sponsor indicates that testing comprises only molecular-based nucleic acid detection assays designed to detect adventitious agents. The risk assessment will therefore be restricted to handling of the GMO under conditions which will not amplify infectious virus.

The GMO under consideration is a replication competent, attenuated recombinant Dengue Virus. The recombinant virus was derived from Dengue virus serotype 4 into which the M and E sequences of Dengue virus serotype 2 have been inserted. The Sponsor has indicated that the recombinant viruses intended to be used as a vaccine against Dengue Virus in humans and is unlikely to cause disease having been shown to be attenuated in healthy dengue- naive adults.

Recipient or parental organism

Dengue virus (DENV) is a pathogenic flavivirus (mosquito-borne), endemic in vast tropical and sub-tropical areas, which infects 50-100 million humans annually with 500,000 cases of severe disease and up to 21,000 deaths. Natural hosts for dengue infection are humans and mosquitoes but the virus can infect several monkey species. DENVs are most commonly transmitted to humans by infected Aedes aegypti mosquitoes and infection is maintained in a human to mosquito to human cycle. The drastic increase in the incidence of DENV infection (approximately 30 fold) over the last 50 years is primarily due to the geographical spread of Ae. Aegypti to new countries and from urban to rural settings Dengue represents a major public health problem throughout the tropical areas of the world and there is an urgent need to develop a protective vaccine.

The causative dengue viruses are members of the genus Flavivirus within the family Flaviviridae. There are four serotypes termed DENV1 to DENV4 and at least three genotypes within each serotype. All flaviviruses are lipid-enveloped, positive-sense, single stranded RNA viruses approximately 55nm in diameter. The genome of approximately 11000 nucleotides is capped at the 5’ terminus but has no 3’ terminal poly A tract. It encodes a single open reading frame, flanked by untranslated regions at each end, which is translated into a polyprotein that is further cleaved into at least ten proteins. The main structural proteins are the capsid which forms a nucleocapsid complex with virion RNA, a non-glycosylated membrane (M) protein derived from a precursor (prM) and an envelope (E) protein which are both embedded in the lipid envelope and displayed on the virion surface. The E protein plays a role in receptor binding and membrane fusion and is known to constitute a major immunogen during flavivirus infection. E protein contains most of the sites that react with neutralising antibodies and with many protective epitopes. There are seven non-structural proteins,
including an RNA-dependent RNA polymerase, a helicase and a protease, which act as enzymes enabling various stages of the virus replication cycle. The viral proteins required for inducing protective immunity are the prM, E and/or NS1 genes and have been used in the development of a number of genetically engineered dengue vaccines.

Following infection the virus is thought to replicate in local dendritic cells, infect macrophages and lymphocytes then enter the bloodstream from where it spreads to the peripheral organs and central nervous system. Most DENV infections are non or mildly symptomatic. The incubation period is generally 4 to 7 days and most symptomatic infections display some or all of the symptoms including sudden onset fever, headache, muscle and joint pain anorexia, abdominal pain and nausea. Rash is common in dengue as are reactions in numbers of white blood cells and platelets circulating in the bloodstream. However, dengue illness can be life threatening when there is evidence of clinically significant bleeding or of severe organ involvement or of severe plasma leakage leading to shock or respiratory compromise. Without proper treatment, the case-fatality rate of the severe form of dengue can reach 20% and most of the hospitalized patients are children.

Infection with one serotype of DENV does not confer protection to infection from the other three. In fact DENV, in the presence of non-neutralising antibody, can produce disease of increased severity. It is likely that an effective vaccine against DENV must induce antibody protective against all four serotypes. There are currently no commercially available DENV vaccines but several candidate vaccines exist at pre-clinical and clinical trial stages.

Wild-type Dengue virus is an ACDP hazard group 3 pathogen.

Host/vector system

The GMO described here is a recombinant serotype 4 dengue virus where the M and E sequences have been replaced with the M and E sequences of Dengue virus serotype 2.1. The recombinant virus is replication competent but has been shown to be attenuated in healthy dengue-naïve adults.

The GMO is a live virus, rDEN2/DEN4Δ30 (ME) and was developed using a chimerization approach. The coding sequences of the prM (membrane precursor) and the E structural proteins of a recombinant DEN4 virus were replaced by those of a prototypic DEN2 strain (New Guinea C) and further attenuated by the introduction of a 30-nucleotide deletion (Δ30) into the untranslated region of the genome. 1.10. This deletion had been previously identified as attenuating in the candidate vaccine rDEN4Δ30, developed at the NIH institute of health, which was found to be safe and immunogenic in healthy adult volunteers.

In preclinical studies, rDEN2/DEN4Δ30(ME) was found to be attenuated in two dengue animal models (SCID-HuH7 mice and rhesus macaques)12. In addition, the virus was found to be poorly infectious for mosquitos by both oral and intrathoracic routes 12. Evaluation of the vaccines in humans was therefore conducted in a double-blind placebo controlled phase I clinical trial and was found to be safe and highly immunogenic. 10 The recombinant virus was found to be highly infectious with 100% of vaccines having developed viraemia and/or seroconverted following administration of a dose of 3log10 pfu virus. The study authors noted four points that indicated safety of the candidate vaccine:

● No symptoms of dengue illness was noted in the vaccinees.

● The level of viraemia noted was very low. Only 11% vaccines inoculated with 3log10 pfu virus showed viraemia with a mean peak titre of 6log 10pfu/ml which is very low compared to the 5 to 9log 101U/ml viraemia levels noted in patients infected with wild type virus. This indicates significant restriction of replication of the vaccine virus in humans.

● Each of the individual isolates in the study cohort that were evaluated (18) retained the delusion mutation and nucleotide changes in the vicinity of the deletion were not observed. Thus the attenuating mutation was highly stable after a period of replication of over two weeks in the vaccines.

● The above properties were displayed by all the dengue species (rDEN4Δ30, rDEN1Δ30and rDEN2/DEN4Δ30(ME) used as vaccines in other clinical trials giving confidence that the attenuating deletion is stable and effective in a range of viruses.

Thus the GMO under consideration shows evidence of safe use in humans at the vaccine dose. However, there remains concern regarding handling of large amounts of virus. The virus is infectious and can replicate to high titre in Vero cells (>7log10 pfu/ml)10 which must be considered when handling the virus in vitro cell culture-based
assays. In addition, there is a potential concern that immunocompromised individuals could experience higher levels of viraemia associated with the vaccine dose.

The recombinant virus has been sequenced and only DENV sequences are present. Similarly, the small vaccine trial showed genetic stability for the virus over the short term of viraemia in the vaccines. The engineered mutation of a 30 nucleotide deletion makes reversion to the wild type phenotype by mutation highly unlikely, however, there is a risk of mutation following subculture in vitro which is pertinent in this GMO risk assessment. Nucleotide and amino acid changes were noted following extended (10 passages) subculture in MRC-5 and Vero cell lines. These changes correlated with a small increase in virus titre and higher pathogenicity (neurovirulence/DENV-induced haemorrhage) in newborn mouse models using inocula containing virus doses higher than those used for human vaccination.

There remains a theoretical risk that recombination might occur with wild-type or other virulent dengue viruses and produce a less attenuated virus. Such a risk is almost negligible in a non-endemic area like the United Kingdom and the risk applies to people that have been previously infected.

**Origin & function**

Expression of the DENV products of the inserted genes results in an antigen designed to produce antibody to DENV serotype 2. There is no additional risk associated with expression of the serotype 2 M and E proteins compared to those of other dengue serotypes. DENV2 proteins have been used as components of live attenuated dengue vaccines which have shown safe use in Phase I clinical trials and are currently under investigation in larger clinical studies in Brazil. In addition, recombinant yellow fever virus-based vaccine expressing tetravalent dengue antigens has shown both safety and efficacy in humans and has progressed to Phase III clinical trials in Australia. The recombinant virus under review is a chimera of DENV 4 and DENV 2 sequences which has produced a replication competent virus. Safety of this recombinant virus rests on evaluation of the extent of attenuation of the virus. As described above, the recombinant virus has shown favorable levels of safety, viraemia and immunogenicity in human clinical trials.

**Evaluation of foreseeable effects**

The recombinant virus is replication competent but has shown evidence of attenuation in mice, rhesus monkeys and mosquitos. In addition, the vaccine virus has been used safely in a Phase I clinical trial in humans and has progressed to further clinical development. The virus is intended for use as a vaccine in humans.

The risk of reversion to a more virulent virus by mutation or recombination is very low. The vaccine virus is a positive sense, single stranded RNA virus for which class of viruses there is little or no evidence of recombination. Deliberate laboratory manipulation of various flavivirus species designed to select and amplify the products of rare recombination events has obtained recombinant viruses only from Japanese encephalitis virus which, moreover, exhibited impaired growth properties. Thus the risk of reversion to wild-type characteristics through either recombination or mutation which would necessarily be required to counteract the effects of a deletion of 30 nucleotides in the genome in this virus is considered to be very low.

Since handling of the GMO will be restricted to laboratory manipulations which will not amplify infectious virus, the risk is judged negligible.

Based on the above considerations, the recombinant virus is judged to be ACGM level 2.

As per BioReliance CPW#2 section 4, the GMO is considered a replication competent virus based on an attenuated strain of a hazard group 3 pathogen. There is a negligible risk of reversion to the wild type phenotype and ACGM level 2 is judged appropriate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Virus is susceptible to several common disinfectants including 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde and detergents. Reminders should be given to laboratory personnel that gloved hands should be disinfected prior to withdrawal from the category II safety cabinet.

All waste must be disposed of in accordance with current Standard Operating Procedure. Current requirements are as follows:

Liquid waste will be inactivated by either autoclaving (with or without prior chemical inactivation), or by incubation overnight in the presence of Virkon at a final concentration of at least 1% (w/v), prior to discard.

Solid waste will be collected in suitable clinical waste packaging, appropriately sealed, placed and stored in designated containers within a secure area until collected for off-site incineration. In addition, in Pentlands, animal carcasses will be placed in an incinerator bag in a locked bin, and incinerated on-site.

In the event of a spill, the area will be cleaned using 2% (w/v) Virkon, and 70% (v/v) isopropanol, and mopped up with disposable wipes, which are placed in an autoclave bag, for incineration. Larger spills may be disinfected using solid Virkon, and hoods may be fumigated with formaldehyde vapour if necessary.

In house studies have shown that 1% Virkon is effective for the inactivation of a broad range of viruses.

**MONITORING**

BioReliance works to the quality standards of Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP). These quality standards require that the performance of all equipment is validated before use, and that equipment is re-validated to a pre-determined schedule. The monitoring and validation is overseen by the Equipment Support Department. In addition, all equipment is required to be maintained and checked for function on a regular basis. The equipment includes all air handling, biosafety cabinets, autoclaves etc.

All disinfectants have a defined expiry date and a documented procedure is in place for preparation of the reagents.

All work with the genetically modified organism will be documented, and will be performed in accordance with standard protocols and/or standard operating procedures.

**ENVIRONMENTAL PROTECTION**

It is expected that conditions appropriate for the parental dengue virus would apply to the recombinant virus. Dengue virus is a lipid-enveloped virus, sensitive to physio-chemical inactivation. It is relatively labile to heat and can be inactivated by exposure to ultra violet and visible light and steam sterilization.

Dengue virus is susceptible to several common disinfectants including 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde and detergents.

Humans, some non-human primates and some laboratory strains of mice would be expected to be susceptible to infection with the recombinant virus, however it is significantly attenuated for pathogenicity. The transmissibility of the recombinant virus has been characterized by the Sponsor as very low due to the absence of the mosquito vectors in the United Kingdom.

Humans and mosquitoes are the only natural hosts of dengue virus and infection is not transmitted from human to human.

All work with the recombinant virus will be performed at a minimum of containment level 2 with the additional requirement that no operators who are immunocompromised in any way should handle the recombinant virus. This would include operators who are pregnant or are at risk of pregnancy.

Since the risk assessment will be restricted to handling of the GMO under conditions which will not amplify infectious virus and with consideration of the containment procedures in place within the laboratory areas including the procedures governing working practices, the overall risk to the environment is considered negligible.

Wild-type dengue virus is an ACDP hazard group 3 human pathogen with the potential for serious disease. There is a correlation with the presence of non-neutralising
antibody to dengue virus in an infected person and the likelihood of developing severe disease. The likelihood of developing severe disease is linked to the age and immune status of the infected person.

The recombinant dengue virus under review is replication competent but is attenuated and has shown safe usage in mice, rhesus monkey and mosquito models. There is evidence of safe use of the virus as a vaccine in humans in a small phase I clinical trial. The recombinant virus should therefore present no significant risk to human health and, if handled appropriately with regard to the risk of spread, to the environment.

However, infection with the live recombinant virus could represent a risk to pregnant women and a significant risk to immunocompromised individuals. The risk is unknown as neither of these patient demographics is well-represented in clinical trials involving safety of therapeutics. The risk assessment should be read and understood by all operators and any operator who might be at increased risk for virus infection should not handle the recombinant virus.

The risk, albeit low, to immunocompetent persons nevertheless requires strict adherence to laboratory health and safety rules for containment in order to limit the chance of laboratory acquired infection and subsequent spread to more susceptible individuals.

Due to the presumed attenuated nature of the virus, the containment procedures in place within the laboratory areas, and the procedures governing working practices, the overall risk to human health and safety is considered low.

Due to the potential for severe disease additional advice has been requested from the HSE which will be included in the final version of the risk assessment.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The risk assessment (GMO#235_Draft) attached has been submitted to HSE as the organism assessed therein does not conform absolutely to the conditions set for classification of activities already notified under BioReliance Risk Assessment for Connected Programme of Work (CPW#02). Specifically, the GMO was assessed as a replication competent virus based on an attenuated strain of hazard group 3 pathogen. There is a negligible/very low risk of reversion to the wild type phenotype and ACGM level 2 is judged appropriate, however, given the serious pathogenic potential of the parent virus, additional discussion with the HSE is required before a final decision is reached on the classification. The material was assigned to ACGM and COSHH class 2.

The risk assessment attached has been revised to take account of the comments provided by the GMSC, and the content therefore reflects the consensus view of the committee, however the GMO risk assessment will not be finalised until a response from the HSE has been received.

Project Containment

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## Project Additional Information

### Purposes of the contained use

Biosafety testing of recombinant lentivirus virus-containing supernatant and associated materials. Testing will comprise sterility testing in the first instance as this is the only test requested by the Sponsor but the GMO risk assessment has included the possibility that other in vitro Bio Safety tests might be performed including, in vitro cell culture assays, molecular-based nucleic acid detection assays, biochemical and immunological based detection assays. All assays are designed to detect adventitious agents.

The GMO under consideration is a recombinant lentivirus derived from a HIV-1 plasmid and has been engineered to be replication competent but attenuated by deletion of the nef element. The virus is intended for therapeutic use in patients infected with wild type HIV-1.

### Recipient or parental organism

The parental virus is Human Immunodeficiency virus type (HIV-1), a species in the genus Lentivirus (family Tetroviridae), which is the primary aetiologic agent of Acquired Immunodeficiency Syndrome (AIDS), a disease that results in the gradual destruction of the helper-T cell population in infected individuals, leading to immunosuppression.

Biosafety of HIV-like viruses/vectors requires most careful evaluation, taking into account the significant pathogenicity of the parent virus.

HIV-1, a category 3 pathogenic can infect man and some non-human primates.
The HIV-1 genome is complex and attenuation of the recombinant virus described here has been achieved by the modification of the nef viral gene encoding factors necessary for virulence. Note that the virus remains replication competent.

**Host/vector system**

The GMO is produced following transfection of Jurkat cells by the HIV-1 plasmid (pELI-71-91). The cells are stably transfected.

**Origin & function**

The virus is intended as a therapeutic in HIV-infected patients. The rationale behind the use of the GMO is that continuous exposure of symptomatic HIV infected patients to an HIV-1 immunogen (nef-deleted but retaining the elements tat, pol, gag, env and vpr will result in a decrease in HIV viral load due to one or more immunological mechanisms including chemokine-mediated restoration of specific cytotoxic T lymphocytes against HIV antigen-expressing cells and activation of B lymphocytes.

**Evaluation of foreseeable effects**

The GMO under consideration is an attenuated replication competent lentivirus derived from HIV-1. Wild type HIV-1 has been assigned to ACDP hazard group 3. Like the wild-type virus, the GMO has been assigned to ACDP hazard group 3 and must be handled at a minimum of BioSafety Level 3. Although the recombinant virus has been modified by deletion of the nef gene which confers significant attenuation of the virus the virus remains replication competent and is therefore classified as suitable for handling only under ACGM level 3 conditions in BioReliance.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Lentiviruses (including HIV vectors) are enveloped viruses, which are generally sensitive to disinfectants including 1% sodium hypochlorite, 2% glutaraldehyde and formaldehyde. In house studies suggest that 2% Virkon and formaldehyde fumigation are effective for the inactivation of HIV viruses, under the conditions used at BioReliance UK. All waste must be disposed of in accordance with current Standard Operating Procedures. Current requirements are as follows:

- Liquid waste will be inactivated by autoclaving in the Category 3 facility, following incubation overnight in the presence of Virkon at a final concentration of at least 1% (w/v).
- Solid waste will be collected in suitable clinical waste packaging, autoclaved in the Category 3 facility, appropriately sealed, placed and stored in designated containers within a secure area until collected for off-site incineration.

In the event of a spill, the area will be cleaned using 2% (w/v) Virkon, and 70% (v/v) isopropanol, and mopped up with disposable wipes, which are placed in an autoclave bag, for incineration. Larger spills may be disinfected using solid Virkon, and hoods may be fumigated with formaldehyde vapour if necessary. In house studies have shown that 1% Virkon is effective for the inactivation of a broad range of viruses, including HIV (Validation Report #39222).

The GMO under consideration is a replication competent infectious Category 3 virus and as such must be handled only under ACGM level 3 conditions. BioReliance standard operating procedures apply: KPSP 3006, KPSP 1901, KPSP2003.

**MONITORING**

BioReliance works to the quality standards of Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP). These quality standards require that the
performance of all equipment is validated before use, and that equipment is re-validated on a pre-determined schedule. The monitoring and validation is overseen by the Equipment Support Department. In addition, all equipment is required to be maintained and checked for function on a regular basis. The equipment includes all air handling, biosafety cabinets, autoclaves etc.

All disinfectants have a defined expiry date and a documented procedure is in place for preparation of the reagents.

All work with the genetically modified organism will be documented, and will be performed in accordance with standard protocols and/or standard operating procedures.

ENVIRONMENTAL PROTECTION

Humans and some non-human primates are the only natural hosts for HIV-1. All work with the recombinant lentivirus vector will be performed at a minimum containment level 3 with all waste decontaminated appropriately by autoclaving prior to discard and eventual incineration.

Due to the low risk of spread of the virus, the containment procedures in place within the Category 3 laboratory and the procedures governing working practices, the overall risk to the environment is considered low.

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment (GMO#251) attached has been submitted to the HSE as the organism assessed therein does not conform absolutely to the conditions set for classification of activities already notified under BioReliance Risk Assessment for Connected Programme of Work (CPW#02)

Specifically, the GMO was assessed as a Class 3 virus.

Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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Production of GMP seed stocks of replication competent Respiratory syncytial viruses (RSV) on behalf of client

Purposes of the contained use

To produce seed stocks of replication competent genetically modified RSV based upon a clinical isolate previously adapted to tissue culture.

Recipient or parental organism

The recipient organism is chimeric A2 strain of RSV.

Human respiratory syncytial virus (RSV) is an enveloped. Non segmented negative strand RNA virus classified as a member of the Pneumovirinae, a subfamily of the Paramyxoviridae. RSV is ubiquitous, existing as a single serotype but as two antigenic subgroups which co-circulate globally. Infection is spread by respiratory secretions and almost all children are infected by age four years. Outbreaks occur annually in winter or early spring. The immune response to RSV does not protect against reinfection long term (protective immunity is induced but recedes with time) and the infectious rate is about 40% for all exposed people. Antibody to RSV generally decreases illness severity, although cases of inflammatory disease have been observed in vulnerable hosts with low level antibody. Disease may be asymptomatic, mild or severe, including bronchiolitis and pneumonia. RSV represents a particular risk to immune-compromised individuals including the elderly.

The RSV virion consists of a lipid-enveloped, RNAse resistant helical nucleocapsid containing the viral polymerase. Entry into the host cell is by cell-surface fusion; viral replication is cytoplasmic and progeny virions exit by budding at the plasma membrane. The genome is a single strand of negative sense RNA which is transcribed in a sequential stop-restart mode, producing subgenomic mRNAs in which eleven genes and their encoded proteins have been identified. The virus grows in a variety of human and animal cells, notably Hep-2 and A549 cell lines and less efficiently in some bovine cells, however serial propagation of virus infected cultures can result in reduced yields of virus.

All members of the Paramyxoviridae have six essential genes in common, encoding the following:- the RNA -binding N protein of the nucleocapsid; the nucleocapsid phosphoprotein P; the major polymerase subunit L; the nonglycosylated matrix protein M; an attachment glycoprotein G, H or HN; the fusion glycoprotein F. Individual virus...
species have one to five additional genes. Manipulation of these genes has produced alteration in the encoded proteins which, under selection pressure has produced phenotypes useful for vaccine development.

For some years vaccine research has centred on live cold-passaged (cp), temperature-sensitive (ts) RSV vaccines (denoted cpts vaccines) which are thought to be most useful in young infants. The availability of cDNA technology has allowed further refinement of existing live attenuated cpts candidate vaccines to produce engineered vaccines that are satisfactorily attenuated, immunogenic, and phenotypically stable.

Wild-type RSV is an ACDP hazard group 2 pathogen.

Host/vector system

The recombinant viruses described here have been produced by synthetic generation of wild type viral nucleic acid sequence.

No new insets are present and the viruses were recovered from Vero cells.

The GMO's are replication competent but are host range restricted, replicating in Vero cells and Hep-2 (human epithelial) cells. The GMO's have not been attenuated in any way therefore as infectious viruses there is a risk of RSV infection to any non-immune host. Infection with RSV is not expected to be clinically significant in healthy, immunocompetent adults but there is a risk to immunocompromised individuals. Risk is likely to be minimal when handled appropriately under Category 2 containment.

Origin & function

The risk assessment considers risk from the perspective of the virus as synthetically-produced wild type virus which for RSV is ACDP Class 2.

The genetic manipulations have not altered the risk for the synthetic RSV, which is assumed to not be significantly changed from that of the wild type virus. There is no additional risk associated with the insert since no new genes have been inserted into the recombinant viruses. All wild type RSV strains are ACDP class 2 pathogens.

Evaluation of foreseeable effects

Wild type RSV - all strains are ACDP class 2 pathogens.

The recombinant RSV's are replication competent and will be propagated in vitro in a designated cell lines. The Sponsor states that the viruses are host range restricted and can grow only in Vero and HEP2 cell lines but there is a significant amount of literature reporting growth of wild type RSV in other cell lines e.g. primary human respiratory epithelia and the A549 cell line. It should be noted therefore that in vitro propagation of these viruses can result in high titres of virus for which there is a significant risk of aerosolisation. All handlers will be made aware of this risk and the requirement for strict adherence to aseptic technique and good laboratory safety practices appropriate to ACDP class 2. For this purpose a "read and understand" log is part of BioReliance's risk assessment and should be signed by all personnel prior to handling the GMOs.

The vaccine viruses are negative sense RNA viruses for which class of viruses, recombination has not been observed, thus the risks considered for these GMO's are those of the wild type virus.

Based on the considerations, the recombinant respiratory syncytial viruses are judged to be ACGM level 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Disposal of all waste from the manufacturing cleanrooms must be in accordance with current SOPs. Procedures currently in place for inactivating viral waste in manufacturing pilot labs and cleanrooms are as outlined below:

Liquid waste is treated with LpH solution (at a concentration of 16ml LpH per 2 litres(L) of liquid waste) and left for at least 30 minutes contact time. Pipettes are disinfected by repeated aspiration (3 times) with LpH solution (at a concentration of 16m; LpH per 2L of ‘water for irrigation’) before being replaced in the pipette wrapper which is then sprayed with Klercide B and 70% IPA. Flasks and other vessels are wiped down with the wipes sprayed with LpH solution, Klercide B and 70% IPA prior to their removal from the biosafety cabinet. Gilson pipette tips are discarded into an appropriate decontamination vessel which is closed and cleaned prior to its removal from the biosafety cabinet. All viral waste is placed into separate autoclave bags which are sanitised with LpH (at a concentration of 16ml LpH per 2L of 'Water for irrigation') prior to exiting the cleanroom/pilot lab. All waste should be double bagged in autoclave bags prior to removal from the pilot lab/cleanroom. The double bagged waste is then placed in yellow UN approved outer disposal bags and palced in the yellow bins for transfer to the secure containers intended for waste uplift for offsite inactivation.

In the even of a spillage the procedures are summarized below:

1. For a small scale spill within the biosafety cabinet (<500ml) and low concentration of material (Diluted virus stock or bulk harvest material), the spillage is contained with cleanroom wipes. The affected area is treated with LpH solution (at a concentration of 16 ml LpH per 2L of 'Water for irrigation') and left for at least 20 minutes contact time before wiping dry and re-spraying with 70% IPA followed by a second wipe clean.

2. For a larger volume spill (>500ml) or high concentration of virus (final product or concentrated virus) within the biosafety cabinet, work will stop and the spillage contained by wiping up with cleanroom wipes. The affected area is treated with LpH solution (at a concentration of 16ml LpH per 2L of 'Water for irrigation') and left for at least 30 minutes contact time before wiping dry and re-spraying with 70% IPA followed by a second wipe clean. A senior member of staff should be contacted for further advice and the biosafety cabinet fumigated with formaldehyde at the end of the processing day. There would be no strict requirement for the cabinet to be "out of commission" and not used between spillage/clean and fumigation, however, since use of the cleanroom is limited to one product at a time, there would be no risk of contamination to other test samples.

3. For any size of spill out with the biosafety cabinet but contained within the cleanroom, operators should leave the cleanroom immediately to mitigate risk from aerosols and notify senior manufacturing staff. Following a waiting period of at least 30 minutes and when permission for re-entry has been given by senior manufacturing staff, the spillage will be contained with cleanroom wipes treated with LpH solution with a minimum 30 minutes contact period. The area once treated with LpH solution is wiped dry and re-sprayed with 70% IPA followed by a second wipe clean. All actions taken will be recorded in an incident/accident report.

The risk assessment attached has been revised to take into account of the comments provided by the GMSC, and the content therefore reflects the consensus of the committee.

Note that the committee endorses the view that a cautionary approach to classification be adopted. The attached risk assesment (BioReliance GMO#261) reflects the planned manufacturing activities at BioReliance, taking into account of manufacturing processes and scale.

Project Containment
## Project Ref 557/15.1

**Date Ackn'd**
07/01/2015

**CU2 Project Title**
A replication competent attenuated vif, vpr, vpu nef-deleted human immunodeficiency virus (HIV 1) intended for use as a therapeutic vaccine

**Class**
Class 3

**CultureVolClass2**
< 1 litre

**Consent Granted**
Yes

**Project notified under transitional arrangements**
N

**Withdrawn**
N

**Tick if notifying a connected programme of work**
N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

The proposed use is two-fold: firstly establish ing virus batch titre of the recombinant lentivirus and secondly the subsequent use of the GMO recombinant lentivirus as a positive control in BioSafety testing designed to supply system suitability information during testing of the Sponsor's test articles to be submitted for future testing. Testing will therefore compromise a basic virus titration assay (as described above) and routine assays for the detection of replication competent lentiviruses (RCL). The titration assay is deSigned to quantify the level of viral infectivity in the current GMO and the RCL assay is designed to detect replication competent lentivirus in the Sponsor's future test article in assays which will include a positive control culture inoculated with a dilution of the GMO. All handling, inoculation, culture maintenance and passage will be performed in the Category 3 laboratory (for initial titration and subsequent use in routine testing).

**Recipient or parental organism**
The parental virus is Human immunodeficiency virus type 1 (HIV-1), a species in the genus Lenlivirus (family Retroviridae), which is the primary aetologic agent of Acquired Immunodeficiency Syndrome (AIDS), a disease that results in the gradual destruction of the helper-T cell population in infected individuals, leading to immunosuppression. Assessment of the biosafety of HIV-like viruses/vectors requires most careful evaluation, taking into account the significant pathogenicity of the parent virus.

HIV-1, a category 3 pathogen can infect man and some non-human primates. It is infectious to a range of cell lines in vitro.

The HIV-1 genome is complex and attenuation of the recombinant virus described here has been achieved by the deletion of the nef, vpr, vif and vpu viral genes encoding factors necessary for virulence. Note that the virus remains replication competent necessitating strict adherence to handling procedures appropriate to Category 3 pathogens. All handling, inoculation, culture maintenance and passage will be performed in the Category 3 laboratory (for initial titration and subsequent use in routine testing).

Handling of this GMO in BioReliance should present no hazards additional to those routinely encountered in the Category 3 laboratory where other Category 3 pathogens including HIV are handled.

Host/vector system

The GMO is produced following transfection of an unspecified producer cell line by the HIV-1 plasmid (HXB2-del-acccUCS?). The cells are stably transfected.

The virus containing supernatant is inoculated and amplified in C8166 lymphoblastoid cells.

Origin & function

The Sponsor's future test articles are (a range of) replication incompetent lentiviral vectors based on H1V-1 . This material, although Class 2 pathogens, would be handled under Class 3 containment conditions due to the requirement for the use of a Class 3 pathogen as a positive control in the routine assay.

The GMO under review is intended as a positive control for use in in vitro assay systems, specifically related to detection of RCl as described in Sections 5 and 6 above. The rationale behind the use of the GMO is to provide a system suitability control ie a positive control virus that is also a genetically modified virus based on HIV-1 in order to better mimic the test article and achieve the optimum sensitivity and accuracy of the test system.

Evaluation of foreseeable effects

The GMO under consideration is an attenuated replication competent lentivirus derived from HIV-1. Wild type HIV-1 has been assigned to ACDP hazard group 3. Like the wild-type virus, the GMO has been assigned to ACDP hazard group 3 and must be handled at a minimum of BioSafety level 3. Although the recombinant virus has been modified by deletion of the nef, vif, vpr and vpu gene which confers significant attenuation of the virus the virus remains replication competent; therefore, the modified virus is classified as suitable for handling only under ACGM level 3 conditions in BioReliance.

All handling, inoculation, culture maintenance and passage will be performed in the Category 3 laboratory (for initial titration and subsequent use in routine testing).

Handling of this GMO in BioReliance should present no hazards additional to those routinely encountered in the Category 3 laboratory where other Category 3 pathogens including HIV are handled.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable
lentiviruses (including HIV vectors) are enveloped viruses, which are generally sensitive to disinfectants including 1% sodium hypochlorite, 2% glutaraldehyde and formaldehyde. In house studies suggest that 2% Virkon and formaldehyde fumigation are effective for the inactivation of HIV viruses, under the conditions used at BioReliance UK.

All waste must be disposed of in accordance with current Standard Operating Procedures. Current requirements are as follows:
- Liquid waste will be inactivated by autoclaving in the Category 3 facility, following incubation overnight in the presence of Virkon at a final concentration of at least 1% (w/v).
- Solid waste will be collected in suitable clinical waste packaging, autoclaved in the Category 3 facility, appropriately sealed, placed and stored in designated containers within a secure area until collected for off-site incineration.
- In the event of a spill, the area will be cleaned using 2% (w/v) Virkon, and 70% (v/v) isopropanol, and mopped up with disposable wipes, which are placed in an autoclave bag, for incineration. Larger spills may be disinfected using solid Virkon, and hoods may be fumigated with formaldehyde vapour if necessary.
- In house studies have shown that 1% Virkon is effective for the inactivation of a broad range of viruses, including HIV (Validation Report # 39222).

The GMO under consideration is a replication competent infectious Category 3 virus and as such must be handled only under ACGM level 3 conditions. BioReliance standard operating procedures apply: KPSP 3006, KPSP 1901, KPSP2003.

**MONITORING**
BioReliance works to the quality standards of Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP). These quality standards require that the performance of all equipment is validated before use, and that equipment is re-validated to a pre-determined schedule. The monitoring and validation is overseen by the Equipment Support Department. In addition, all equipment is required to be maintained and checked for function on a regular basis.

The equipment includes all air handling, biosafety cabinets, autoclaves etc.

All disinfectants have a defined expiry date and a documented procedure is in place for preparation of the reagents.

All work with the genetically modified organism will be documented, and will be performed in accordance with standard protocols and/or standard operating procedures.

**ENVIRONMENTAL PROTECTION**
Humans and some non-human primates are the only natural hosts for HIV-1. All biosafety testing will be performed on this recombinant lentivirus, in BioReliance in at least ACGM class 3 containment laboratories.

All work with the recombinant lentivirus vector will be performed at a minimum of containment level 3 with all waste decontaminated appropriately by autoclaving prior to discard and eventual incineration. Due to the low risk of spread of the virus, the containment procedures in place within the Category 3 laboratory and the procedures governing working practices, the overall risk to the environment is considered low.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The risk assessment (GMO#262) attached has been submitted to HSE as the organism assessed therein does not conform absolutely to the conditions set for classification of activities already notified under BioReliance Risk Assessment for Connected Programme of Work (CPW # 02).
Specifically, the GMO was assessed as a Class 3 virus.

Project Containment

<table>
<thead>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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Project Ref 557/18.1

Date Ackn'd: 05/09/2018

CU2 Project Title: Human Mesenchymal stem cell line containing a self-inactivating Lentiviral Vector

Class: Class 2

Culture Volume: < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

To perform one or more BioSafety tests to exclude the presence of adventitious agents in the Sponsor's GMO material. All handling, inoculation, culture maintenance, passage and storage of test materials will be performed under ACDP BioSafety Level 2 conditions. Additional endpoint testing (e.g. by Molecular methods such as QPCR, QPERT etc), if such is required, will be performed under ACDP BioSafety Level 2 conditions.

Recipient or parental organism

The host cell line is a human bone marrow Mesenchymal Stem Cell (MSC) line from a healthy donor, produced by single clonal selection of cells transduced by a replication incompetent lentiviral vector containing one or more therapeutic genes. MSCs are multipotent stromal cells capable of producing a variety of specialised differentiated cell types including oestoblasts, adipocytes, chondrocytes and myocytes. They contribute to the maintenance and regeneration of diverse tissues after injury and during chronic inflammation. The damaged tissue releases signals which result in the mobilization of MSCs and their subsequent recruitment to the site of injury. Tumours are seen by the body as something akin to a chronic wound, and as a result, MSCs are actively recruited to the tumour environment, resulting in a potential to exploit MSCs as targeted vectors in anti-tumour therapy.

Mammalian cells are not themselves considered pathogenic to animals or humans, and are viewed as especially disabled hosts, as they are considered incapable of survival outwith their highly specific tissue culture environment, and would be rapidly cleared by the host immune response in the event of accidental exposure of an operator (Ref.: ACGM Compendium of Guidance 2016). Many cell cultures have a long history of safe use, and are considered unlikely to cause harm to humans, animals or plants and are viewed as presenting minimal risk to operators and the environment (in the absence of adventitious agents).

MSCs are considered safe (Ref.: Zhao Q. et al, J Cellular Immunotherapy (2016) 2:3-20)- they do not form teratoma and can be used for tissue regeneration and repair. On 19 June 2018, 850 clinical trials were identified as extant in "ClinicalTrials.gov". If this safe description applies in a clinical setting with parenteral inoculation of MSCs in patients, in vitro use i.e. handling of limited volumes of MSCs in a laboratory setting with no expected direct contact with operators should be considered safe.

However, as all cell lines have the potential to harbour or amplify adventitious agents, and since testing for presence of such agents will be performed in the test facility, all procedures involving the culture of the cell line will be performed under containment level 2 (for COSHH purposes).

Host/vector system

The GM cell line has been transduced by a replication incompetent second generation lentiviral vector based on HIV. The parental virus of this type of lentiviral vector is Human immunodeficiency virus type 1 (HIV-1), a species in the genus Lentivirus (family Retroviridae) and the primary aetiologic agent of Acquired Immunodeficiency Syndrome (AIDS), a disease that results in the gradual destruction of the helper-T cell population in infected individuals leading to immunosuppression. Lentiviruses are large single-stranded, positive sense, enveloped RNA viruses which contain an RNA-dependent DNA polymerase. Following entry into the host cell, viral RNA is reverse-transcribed into a covalently linked circle of double-stranded DNA (provirus) which is integrated into the cellular DNA by action of the virus-encoded integrase and other cellular co-factors. The integrated proviral DNA thereafter remains latent or is transcribed by cellular transcriptional activators and is replicated in tandem with the host cell DNA. All lentiviruses contain several genes in addition to the three major genes encoding the main retrovirus structural proteins Gag and Env and the main enzymatic protein Pol.

Several factors make retroviral vectors attractive tools for gene therapy including their relatively large cloning capacity
and their ability to stably integrate into the host cell genome, which is a likely requisite for long-term expression. Further, they do not transfer viral genes and avoid transduced cells that are destroyed by virus-specific cytotoxic T cells. In addition to these characteristics that are common to all retroviral vectors, lentiviral vectors can transduce non-dividing cells, a crucial asset for genetically modifying the main target tissues of gene therapy such as the brain, soft-tissue organs and the haematopoetic system, and enable efficient in vivo delivery, integration and stable expression of transgenes. However, biosafety of HIV-based vectors requires most careful evaluation, taking into account the significant pathogenicity of the parent virus.

The HIV-1 genome is complex, comprising the standard retroviral elements that are each synthesized as three polyproteins that produce either, the inner virion structure-Gag (cleaved into matrix, capsid and nucleocapsid proteins), the viral polymerase enzyme-Pol (producing reverse transcriptase and endonuclease/integrase enzymes) or the glycoprotein of the virion envelope-Env. The HIV 1 genome also encodes proteins with regulatory and auxiliary functions. These comprise two essential regulatory elements: Tat, which activates transcription and Rev, which modulates viral RNA transport. In contrast, the elements, Nef, Vpr, Vif and Vpu are non-essential for replication in certain tissues and are termed accessory proteins. These small proteins are multifunctional and in general, Vif and Nef function as infectivity enhancers by suppression of cellular enzymes and down regulation of host cell surface receptors. Vpr is associated with intracellular virus transport and cell cycle arrest and Vpu augments the release of progeny virus.

The recombinant lentiviral vector described here has been modified extensively as detailed below, such that it can be derogated and re-classified as suitable for handling under ACGM level 2 containment conditions in BioReliance. Production of the lentiviral vector encoding the therapeutic transgene has been accomplished by transient cotransfection of the human epithelial cell, HEK 293 with a combination of three plasmids (transfer, packaging and envelope vectors). The Sponsor has stated that the transfer vector contains the viral L TRs, psi (41) packaging signal sequence and therapeutic gene. The packaging vector encodes the gag, pol, Rev and Tat genes. The envelope vector encodes Env (VSV-G) gene. On transfection of the producer cell line (HEK 293), this split packaging system enables production of lentiviral particles that contain only the genetic elements contained in the transfer vector. Four virulence genes, negative factor (nef), virion infectivity factor (vif), viral protein U (vpu) and viral protein R (vpr) have been deleted from the viral packaging construct. Additionally, the wild type envelope protein has also been deleted from the construct.

The GMO under evaluation is a 2nd generation replication-defective, self-inactivating (SIN) vector and has undergone further modification by introduction of a deletion in the U3 region of the 3’ long terminal repeat (L TR) of the DNA used to produce the vector RNA. During reverse transcription, this deletion is transferred to the 5’ L TR of the proviral DNA, the implication being that sufficient viral sequence can be eliminated to abolish transcriptional activity of the L TR (i.e. no transcription of the full length virus is possible after it has been incorporated into the host cell) thereby abolishing the ability to produce full length vector RNA in transduced cells which minimizes the risk of emergence of replication competent retroviruses.

The Sponsor has stated that the lentiviral vector used to transduce the MSC cells is non-mobilisable and nonreplicative, as it lacks the required genes for assembly of the virus. Since only the transfer vector contains L TRs and a psi sequence, only it can integrate into the host cell chromosome and such a minimal genome cannot produce replication competent virus.

Origin & function

The Sponsor's test article described here and related test articles (same cell line transduced using the same lentiviral vector platform but expressing different therapeutic genes) are cell lines transduced with second generation, self-inactivating lentiviral vectors expressing human proteins intended for therapeutic use in humans. These materials would be handled in the test facility during the performance of a range of biosafety testing at a
Evaluation of foreseeable effects

The Sponsor has indicated the use of Addgene vector pWPT1 to produce the transfer plasmid containing the L TR with SIN element and encoding the cytidine deaminase gene and Woodchuck Hepatitis virus Post-translational Regulatory Element; two separate accessory plasmids encoding CMV, Gag, Pol, RRE, Rev and Tat (Packaging Vector) which will produce the surface glycoprotein, the structural proteins, nucleic-acid polymerases/integrase, reverse transcriptase and regulatory elements required for packaging a recombinant lentiviral particle and VSV-G, PolyA and CMV promoter (Envelope Vector) which will produce the envelope protein and enable infectivity of the final packaged vector. The system is based on 3 non-overlapping expression constructs in order to increase the segregation of cis and trans acting functions and has been engineered such that minimal homology exists between packaging and transfer vectors, thereby reducing the likelihood of homologous recombination and the generation of replication competent lentiviruses (RCLs). In addition, the packaging construct is deleted of four additional HIV accessory proteins; vpu, vpr, nef, vif. The Rev responsive element (RRE) maintained in the gag/pol plasmid makes the gag/pol gene expression rev dependent. The transfer vector encodes the transgene and sequences necessary for expression, encapsidation, reverse transcription and integration of the viral genome. In order to minimize the risk of RCL generation, the 3’ L TR of the transfer vector has been deleted in the U3 region by the Sponsor. The introduction of this 254 bp deletion is designed to abolish the production of full-length vector RNA in transduced cells by exploiting the reverse transcriptase mechanism which generates both U3 regions from the 3’ of the viral genome, thus transferring the deletion to the 5’ L TR of the proviral DNA. The lentiviral particle is therefore conceived with a self-inactivating mechanism that enables the production of infective particle only in the first run of infection. A Woodchuck Hepatitis Virus Post-transcriptional Regulatory Element (WPRE) sequence is also included in the transfer vector, which has the effect of increasing transgene expression by transcript stabilisation. Routinely mutated WPRE sequences are designed lacking a transcription fragment of the potentially oncogenic woodchuck hepatitis virus-X protein, however, the Sponsor has not indicated that is the case in this submission. The likelihood of reversion to wild type virus by recombination events is effectively zero in a virus with such extensive deletions. Further, due to deletions in the U3 region of the 5’ and 3’ L TRs, the vector is self-inactivating, and therefore represents minimal risk of generation of RCR.

The recombinant lentivirus vector will integrate into target cells, thus there is a theoretical risk of insertional mutagenesis, however the nature of self-inactivation, where both L TRs are inactivated upon integration into the target cell genome, makes it unlikely that cellular coding sequences located adjacent to the vector integration site would be aberrantly expressed due to genetic elements within the vector. In addition, the self-inactivated vector will replicate only once following initial infection. This very limited replication will occur only in the transduced cells that come into contact with the vector with no spread to adjacent cells further limiting the likelihood of significant effects on cellular genes. Thus, in the very unlikely event of transfer of the viral vector to a handler or other mammalian hosts, the extent of spread would be limited to the cells initially transduced.

The recombinant lentivirus has been pseudotyped with an alternative coat protein, the vesicular stomatitis virus glycoprotein (VSV) G protein, with consequent alteration to the tissue-tropism range of the vector to include human, mouse and bovine cell types. Biosafety testing will involve handling of the vector in a variety of cell types however, there is published evidence that lentiviruses pseudotyped with VSV-G are rapidly inactivated by human complement which would reduce the potential of infection from a needle stick injury.

As described above, the risk associated with the GMO is expected to be low due to the presence of the SIN-L TR element. Self inactivation minimizes the likelihood that RCR will be produced. Further, it reduces the likelihood that cellular coding sequences located adjacent to the vector integration site will be aberrantly expressed, either due to the promoter activity of the 3’L TR or through an enhancer effect. Finally, the SIN design prevents potential transcriptional interference between the L TR and the internal promoter driving the transgene.
The Sponsor has stated that the entire genome of the lentiviral vector has been sequenced and a conclusion reached that no components enabling replication competent lentivirus were present. Testing for Replication competent lentivirus has been performed on the GMO cell seed stock has been performed by the Sponsor but the RCL test status of this GMO material is not known. The material has been submitted to the test facility and the sister test facility (BioReliance US) for RCL testing.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The GMO is a replication defective, self-inactivating virus vector incapable of stable integration in target cells. There is a theoretical risk of generation of RCR following recombination events between the transfer and packaging plasmids during production. The material has not yet been tested for the presence of RCL, however, testing is planned. Expression of the vector gene product (cytidine deaminase) is unlikely to represent a significant risk to the operator. Further, it is unlikely that an isolated contamination incident would enable expression of sufficient quantities of the protein to cause a clinical effect. The likelihood of complement inactivation of the VSV-G attachment protein would also likely mitigate adverse effects of a possible needle stick injury. The risk of accidental inoculation by sharp or needlestick injury is low if safety procedures for ACDP class 2 organisms are followed; however, there remains a low risk of seroconversion to the vector product or the vector itself as a universal consequence of introduction of foreign protein into an immunocompetent host. It is expected that exposure to the test article would not be harmful in an immunocompetent individual, however the effects of the antibody or an unborn foetus are not known. Therefore COSHH assessment recommends that this material be handled under COSHH level 2 containment and that pregnant women avoid contact with large volumes of the test article. Based on the above considerations, it is judged that the GMO as a replication defective, self-inactivating virus vector, can be derogated from the ACGM classification of the wild type virus and re-classified as suitable for handling under ACGM level 2 conditions.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The main risks associated with the processes to be used are where infectious agents are handled at high titre or under conditions, which may result in amplification of the virus (i.e. cell culture assays). While the wild-type virus is restricted to infection of CD4+ cells, this recombinant virus vector has been pseudotyped with VSV-G env so that infection of a broad range of mammalian cell types is possible in a laboratory setting. However, that the recombinant virus is replication incompetent mitigates this risk significantly. The major risk of operator exposure is via direct inoculation (e.g. needlestick injury), ingestion, or droplet or aerosol exposure of mucous membranes or broken skin. Operators should be aware that eye infections can have serious consequences, and so the use of safety glasses is particularly important. Care should be taken at all times to ensure that all operators wear appropriate protective clothing that is correctly fitted and fastened appropriately. All biosafety testing of this recombinant lentivirus vector should be performed in at least ACGM class 2 containment laboratories. All work with the recombinant lentivirus vector will be performed at a minimum of containment level 2. In order to protect both the samples during testing, and the operator, all handling of this GMO will be performed at containment level 2 within a class 2 biosafety cabinet or negative pressure isolator. Lentivirus vectors are enveloped viruses, which are generally sensitive to disinfectants including 1 % sodium
hyperchlorite, 2% glutaraldehyde and formaldehyde. In house studies suggest that 2% Virkon and formaldehyde fumigation are effective for the inactivation of HIV viruses, under the conditions used at BioReliance UK. All waste must be disposed of in accordance with current Standard Operating Procedure. Current requirements are as follows:

Liquid waste will be inactivated by either autoclaving (with or without prior chemical inactivation), or by incubation overnight in the presence of Virkon at a final concentration of at least 1% (w/v), prior to discard.

Solid waste will be collected in suitable clinical waste packaging, appropriately sealed, placed and stored in designated containers within a secure area until collected for off-site incineration. In addition, in Pentlands, animal carcasses will be placed in an incinerator bag in a locked bin, and incinerated on-site.

In the event of a spill, the area will be cleaned using 2% (w/v) Virkon, and 70% (v/v) isopropanol, and mopped up with disposable wipes, which are placed in an autoclave bag, for incineration. Larger spills may be disinfected using solid Virkon, and hoods may be fumigated with formaldehyde vapour if necessary.

In house studies have shown that 1% Virkon is effective for the inactivation of a broad range of viruses, including HIV (Validation Report # 39222).

BioReliance works to the quality standards of Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP). These quality standards require that the performance of all equipment is validated before use, and that equipment is re-validated to a pre-determined schedule. The monitoring and validation is overseen by the Equipment Support Department. In addition, all equipment is required to be maintained and checked for function on a regular basis. The equipment includes all air handling, biosafety cabinets, autoclaves etc.

All disinfectants have a defined expiry date and a documented procedure is in place for preparation of the reagents. All work with the genetically modified organism will be documented, and will be performed in accordance with standard protocols and/or standard operating procedures.

Humans and some non-human primates are the only natural hosts for HIV-1. All biosafety testing will be performed on this recombinant lentivirus vector, in BioReliance, using class 2 biological safety cabinets in ACGM class 2 containment laboratories.

All work with the recombinant lentivirus vector will be performed at a minimum of containment level 2 with all waste decontaminated appropriately. In order to protect the samples during testing, work will generally be performed at containment level 2, and manipulation of the samples will be performed within a class 2 biosafety cabinet, or negative pressure isolator.

Due to the low risk of spread of the vector, the containment procedures in place within the laboratory areas, and the procedures governing working practices, the overall risk to the environment is considered negligible.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Testing for RCL is planned
**Project Containment**

**Laboratory Activities**
- L2
- Yes
- L3
- L4
- L2

**Glass Houses**
- L3
- L4
- L2

**Growth Rooms**
- L3
- L4
- L2

**Animal Units**
- L2
- L3
- L4
- L2

**Large Scale Activities**
- L3
- L4
- L2

**Human Clinical Applications**
- L3
- L4

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**Project Ref** 557/21.1

**Date Ackn'd** 08/01/2021

**CU2 Project Title**
To perform a range of biosafety testing on an attenuated replication competent Equine Herpes Virus derived from the RacH strain, expressing a codon optimised version of the rabies virus G (glycoprotein)

**Class**
- Class 2

**CultureVol**
- Class 2
- < 1 Litre

**Consent Granted**
- Non-GMM
- Consent Granted

**Project notified under transitional arrangements**
- N

---

**Historical Significant Changes**

**Historical Date of Additional Info**

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**Project Additional Information**

**Purposes of the contained use**
To perform one or more Biosafety tests to exclude the presence of adventitious agents in the Sponsor's GMO material.
All handling, inoculation, culture maintenance, passage and storage of test materials will be performed under ACDP Biosafety Level 2 conditions.

**Recipient or parental organism**
Herpesviruses are relatively large enveloped double-stranded DNA viruses in the family of Herpesviridae in order of...
Herpesvirales. They have large genomes and virions are generally in the size of 180-225 nm in diameter.
Equid herpesvirus-1 is of a genus of Varicelloviruses that causes wide-spread infection in horses. Primary infection
by EHV-1 is characterized by upper respiratory tract disease of varying severity that is related to the age and
immunological status of the infected animal.
The RacH strain was derived from EHV-1 and was attenuated by repeated passage in cell culture. Wild-type EHV-1
and the RacH strain are considered to not pose any health risks to humans, however, the RacH EHV-1 can replicate
in vitro in various cell type including MDBK and Vero cells.

Host/vector system
The RacH EHV-1 genome was originally cloned as a bacmid that included a CMV-driven GFP gene as a marker. An
expression cassette encoding the gene of interest (Rabies G protein) was then cloned into the RacH EHV-1 backbone
using a two-step recombination process. The donor DNA was inserted into an existing ORF1/2 deletion present in the
RacH genome. This deletion has been shown to not negatively impact RacH replication in vitro or change its
immunogenicity.
A codon-optimized gene encoding the Rabies G (glycoprotein) from Rhabdovirus strain SADB19 is inserted in the
RacH backbone. The insert is under the control of the CMV5 mammalian promotor with a polyadenylation signal
inserted in the ORF1/2 region described above. SADB19 is the standard vaccine strain used for vaccinating animals
globally and has a safe history of use.
The safety of RacH-based products in equines has been demonstrated for several modified live vaccines. For
example, the Rhinopneumonitis Modified Live Virus vaccine, Rhinomune® is an example of a licensed vaccine
derived from RacH. In addition to horses, RacH based vectors have been tested in other species including cattle,
pigs, dogs, cats and mice without any serious side effects.

Origin & function
The recombinant virus is intended as a veterinary vaccine. The recombinant virus has been submitted for Biosafety
testing in the Merck BioReliance Services facility.
In vitro assays for detection of adventitious agents will be performed.

Evaluation of foreseeable effects
The genetic modification itself poses no additional risk to the health of the operator, the wider community or the
environment.
The recombinant vector is a replication-competent EHV-1, expressing a single Rabies virus G protein. Although the
vector is replication-competent it is not expected to be pathogenic to humans or animals. The construct is known to
replicate in a variety of vertebrate cells, including some commonly used for in vitro biosafety assays (e.g. Vero,
MDBK). The G protein expressed from the inserted gene is not known to have any associated toxicity, although the
protein may elicit an antigenic response if in contact with an individual’s immune system.
The risk of accidental inoculation by sharp or needlestick injury is low if safety procedures are followed; it is expected
that exposure to the test article would not be harmful in an immune competent individual, however the effects on an
unborn foetus are not known.
The recombinant virus should present no significant risk to human health, or to the environment; however after
assessment it is recommended that this material be handled under a minimum of containment level 21ACGM Class 2
containment and that pregnant women avoid contact with large volumes of the test article.
The recombinant virus is a live, attenuated vaccine strain which has not undergone inactivation but has been
attenuated, although the attenuation factor has not been determined. A* work using the recombinant virus, including
disposal of waste materials, will be performed at a minimum of containment level 2.

02/03/2022
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be inactivated by either; autoclaving (with or without prior chemical inactivation), or by incubation overnight in the presence of Virkon at a final concentration of at least 1% (w/v), prior to discard. Solid waste will be collected in suitable clinical waste packaging, appropriately sealed, placed and stored in designated containers within a secure area until collected for off-site incineration. In the event of a spill, the area will be cleaned using 2% (w/v) Virkon, and 70% (v/v) isopropanol, and mopped up with disposable wipes, which are placed in an autoclave bag, for incineration. Larger spills may be disinfected using solid Virkon, and hoods may be fumigated with formaldehyde vapor if necessary.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Approved and reviewed.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tbody>
<tr>
<td>L2 Yes</td>
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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 557/21.3

Date Ackn’d CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
A Simian Retrovirus Type 3 (SRV-3) viral seed stock synthetically produced by the transfection of 293T cells

As a retrovirus, the SRV-3 is an enveloped single stranded RNA virus. It has an icosahedral capsid, and its genome contains four genes: gag, pro, pol and env. It transcribes its RNA genome into double-stranded DNA by using a Mg2+ dependent reverse transcriptase enzyme.

SRV-3, formerly known as Mason-Pfizer Monkey Virus (M-PMV) is a beta retrovirus, i.e., simian type D retrovirus and is enzootic in many populations of Asian monkeys of the genus Macaca and is associated with immunodeficiency diseases in these animals. In infected macaques, SRV-3 may be present in blood, saliva, urine, and other body fluids.

SRV-3 is not classified as a Specified Animal Pathogen nor cited on The Advisory Committee on Dangerous Pathogens (ACDP) "Approved List". The United States Centers for Disease Control and Prevention (CDC) and National Institutes for Health (NIH) classify SRV as a Hazard Group 2 pathogen.

The recombinant SRV-3 virus stock will be used to generate a further virus bank at Merck BioReliance® Services (UK) which will serve as a positive control for specific (non-routine) replication-competent retrovirus (RCR) testing.

The virus is synthetically produced by the transfection of 293T cells. The virus has a wild-type-like genome and is not attenuated or deliberately modified. The virus therefore poses no greater risk than the wild-type virus. The 293T cells are transiently transfected using a single plasmid expressing the full-length SRV-3 genome. The plasmid, a standard cloning vector (pIC119H) is of pUC origin. Additional to the SRV-3 genome it encodes standard selective markers beta-lactamase (ampicillin resistance). A full sequence and annotation is included in the risk assessment.

The recombinant virus will be used as a positive control specific (non-routine) replication-competent retrovirus (RCR)
Testing conducted at Merck BioReliance Services.

**Evaluation of foreseeable effects**

The virus will be cultured to high titres for the preparation of the virus bank in cell culture, and then used in multipassage culture assays with the potential to grow to high titres in those assays.

Although the recombinant virus is not known to be zoonotic and is considered of low risk to the operators themselves where manipulation of the virus occurs, this should be conducted at Containment Level 2 [CU] and any open container work only be performed in a Class 2 Biological Safety Cabinet with operators wearing gloves, sleeve covers and a disposable gown.

Primary routes of infection with the virus is via injection, due to the natural route of transmission of the virus therefore, the use of sharps is strictly prohibited. Accidental splashing and exposure to mucosal membranes is a secondary risk factor therefore, all open container work will be performed within a Class 2 Biological Safety Cabinet and centrifugation of preparations with infectious potential must be performed using sealed centrifuge rotors or sample cups which are loaded and unloaded in the Class 2 Biological Safety Cabinet.

Due to the containment procedures in place within the laboratory areas, the intrinsic design of the C12 laboratory, decontamination of waste, the procedures governing working practices, the overall risk to escape to the environment is considered low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Liquid waste as per Section 12 will be subject to chemical inactivation prior to disposal. Therefore a derogation to Part 2, Table la 8 "Autoclave required in the building" of the Regulations is requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste is treated with Virkon prior to disposal to drain. Virkon is used at a minimum final concentration of 2% for the purpose of decontaminating liquid waste. Liquid waste is left to decontaminate for at least 16 hours. Merck BioReliance Services have conducted in house studies to determine the viricidal effect of Virkon, with data demonstrating that enveloped viruses are inactivated at a concentration of 2% Virkon following a 16hr incubation with a minimum reduction of at least 5.9 logs. The studies were conducted using 10% FBS to mimic a protein load. The studies on these enveloped viruses demonstrate that rSRV-3 being used would be inactivated in the same manner based on the biological and physical properties of the wild type virus.

Solid waste will be collected in suitable clinical waste packaging, appropriately sealed, placed and stored in designated containers within a secure area until collected for off-site incineration.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
Please enter comments on the GM safety committee on the risk assessment

Approved and reviewed.

Project Containment

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<tr>
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<td>L3</td>
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Project Ref 562/01.1

Date Ackn'd 25/11/2004

Date Project Ceased

CU2 Project Title PRODUCTION OF GENETICALLY MODIFIED HERPES SIMPLEX TYPE-1 VIRUS IN SMALL-SCALE TISSUE CULTURE ON FULLY CHARACTERISED MAMMALIAN CELLS

Class Class 2

CultureVolClass2 1-50 Litres

CultureVolumeClass3-4

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N


Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Small scale production of Herpes Simplex type-1 virus for further biologic al testing with the aim of optimising production methods.

Recipient or parental organism

Herpes Simplex type-1 virus (strain JS-1 ECACC Accession Number 01010209)
**Host/vector system**

Herpes Simplex type-1 virus deleted for neurovirulence factor ICP34.5 and culture in BHK cells in vitro. GM-CSF, a cytokine involved in the stimulation of T-cells, is under control of the human cytomegalvirus immediate early promotor (HCMV IE).

**Origin & function**


**Evaluation of foreseeable effects**

The design of the facility and the category 2 handling and waste inactivation procedures utilised mean that release of vector outside of contained use is highly unlikely. If such release was to occur then it is unlikely that the vector will be harmful to humans, animals or plants. The vector is modified so that replication only occurs in rapidly dividing cells and not non-dividing cells. Considerable literature shows that HSV 1 deleted for ICP34.5 is non-pathogenic in animals and humans. Approximately 80% of the human population are seropositive for HSV-1. Infection is by contact and transmission by the aerosol route is not though to occur. The therapeutic gene delivered, human GM-CSF is present in normal, healthy humans. The virus is fragile, and is rapidly inactivated by desiccation, lipid solvents and mild detergents. The fact that contact is required for transmission attests to the instability of the virus outside the host.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste materials (eg. liquid waste, flasks and pipettes) are inactivated by autoclaving (126 degrees C for 45 minutes) before disposal to effectively give 100% kill of the GMM. The autoclaving procedure is performed according to formal written standard operating procedures (KPBT0161 and KPBT0162) and has been validated. The inactivation of waste by autoclaving is monitored/validated by the following means:

* chemical indicators (eg autoclave tape) are used on all autoclave runs and the temperature and pressure for each run is recorded and verified against requirements.

* Once a week correct autoclave operation is verified by the use of chemical indicators.

* Once every two months correct autoclave operation is verified by the use of biological indicators.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

Please enter comments on the GM safety committee on the risk assessment

Standard level 2 containment procedures should be employed for this work.

**Project Containment**

02/03/2022
The sample will be cleared to remove cellular debris, ultracentrifuged to pellet any viral particles present and resuspended in disruption buffer containing detergents, followed by Reverse Transcriptase Polymerase Chain Reaction.
The yellow fever live attenuated vaccine virus strain 17D replicates to low levels in vaccinees, and cannot infect mosquitoes, which precludes its spread in nature. It is considered one of the safest and effective live attenuated vaccines ever developed. According to current biosafety guidelines, the ChimeriVax - JE vaccine is officially BSL2.

**Origin & function**

The infectious clone (cDNA copy) of 17D virus, which was subsequently used to create ChimeriVax - JE, was constructed starting from a sample of the virus from ATCC at passage 2347-8. Since ATCC is not currently distributing passage 234 sample, a Safety Data Sheet for this virus is not available. However, this virus is virtually identical to all other 17D vaccines manufactured worldwide, because its passage history differs from the commercial vaccines only at a few terminal passages. All 17D vaccines, including the ATCC passage 234 sample, have originated from one sample, Colombia 88 vaccine at passage 228. All current commercial 17D vaccines are produced at passage levels between 233 and 239 to ensure phenotypic and genetic similarity. The 17D virus is a BSL-2 agent.

The ChimeriVax will be used in tests undertaken as part of routine safety testing for the presence of viral contaminants, especially reverse transcriptase activity. The sample will be clarified to remove cellular debris, ultracentrifuged to pellet any viral particles present and resuspended in disruption buffer containing detergent, followed by an RT-PCR. The sample consists of ChimeriVax TM-JE Bulk Harvest cultured in Vero cells. During the procedure the virus will be inactivated by disruption buffer containing the following detergents, Igepal and DTT. Handling of the sample will be performed in a Class II hood. The process in which the recombinant virus will be used does not increase the risk associated with the virus.

**Evaluation of foreseeable effects**

In sertion of the envelope protein genes into the viral vector does not alter the tropism of the virus or its interactions with host defences. In selecting the most successful YF 17D chimeras containing the prM-E genes, two strains were examined, Nakayama, a virulent JE strain and the attenuated JE vaccine strain, SA14-14-2. Both chimeras replicated efficiently in several simian, human, mouse and mosquito cell cultures. It was concluded that the SA-14-14-2 envelope protein provided an additional degree of attenuation, therefore this was selected as the candidate chimeric JE vaccine.

For wild type yellow fever virus, natural hosts are Aedes mosquitoes. However, as a result of attenuation, the 17D vaccine strain lost its ability to replicate in mosquitoes, and replicates to extremely low levels in humans and non-human primates, which precludes infection of mosquitoes feeding on vaccinees and thus uncontrolled spread in nature. The ChimeriVax - JE vaccine has been demonstrated to possess the same restricted replication characteristics.

If introduced to humans it is possible that Chimerivax-JE would replicate to low levels, but cannot replicate in mosquitoes. However, due to the nature of the work to be preformed and the contamination procedures in place within the laboratory, the overall risk to the environment is considered negligible.

Chimerivax -JE can replicate in humans and non-human primates to extremely low levels. As the virus cannot replicate in mosquitoes there is no vector for transmission. The risks to human health and safety is negligible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

In conclusion the recombinant Chimerivax-JE virus is considered as a Hazard Group 2 biological agent, this conclusion arises due to the fact that JEV and YF vaccine strains belong to Hazard Group 2 (ACDP Categorization of biological agents according to hazard and categories of containment, 4th edition, 1995).

The ACGM level of containment would be class 2 and the ACDP level of containment is level 2. All manipulations will be performed in a level 2 containment facility.

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Animal Units**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
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### Project Ref 315/01.1

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<td>Class 2</td>
<td>&lt; 1 litre</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity:

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
Date Project Ceased

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20? 

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form


Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 315/01.2

Date Ackn’d

11/10/2002

Date Project Ceased

Class

Class 2

CultureVolClass2

Class

CultureVolumeClass3-4

Class

Consent Granted

not applicable

Project notified under transitional arrangements

N

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
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**Date Ackn'd:** 11/10/2002

**CU2 Project Title:** EXPRESSION OF INTRACELLULAR PROTEIN KINASES AND ANALOGUES IN ANIMAL CELLS

**Class:** Class 2

**Consent Granted:** not applicable

**Tick if notifying a connected programme of work:** N

**Project notified under transitional arrangements:** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

## Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref  315/01.4

Date Ackn’d  11/10/2002

CU2 Project Title  EXPRESSION OF RECEPTOR KINASE LIGANDS IN E COLI AND ANIMAL CELL

Class  Class 2

CultureVolClass2  Class CultureVolumeClass3-4
Date Project Ceased

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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Animal Units

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Project Ref 315/01.5

Date Ackn’d 11/10/2002

CU2 Project Title EXPRESSION OF INTRACELLULAR PROTEIN KINASES AND ANALOGUES IN ANIMAL CELLS

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
N

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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02/03/2022
In the search for new and effective pharmaceuticals with minimal side effects, GSK intends to express known and novel membrane receptors in mammalian cells in order to obtain functional data and to determine the specificity of closely related receptors. Regulatory proteins such as accessory G proteins which modulate the function of the receptor, and may be essential for activity, will also be expressed and evaluated. To avoid the generation of large numbers of stable recombinant cell lines, we intend to use a recombinant baculovirus system which will allow transient expression in mammalian cell lines. Baculoviruses are a well studied system for the production of recombinant proteins in insect cells. They are unable to replicate in mammalian cells, and the usual insect-specific promotors are ineffective in mammalian cells. By replacing the standard polyhedrin gene promoter sequence with that of an efficient mammalian promoter (such as the CMV IE promoter/enhancer) baculoviruses are able to express recombinant products in mammalian cells, though they remain incapable of replication in these cells. (Condreay et al 1998). Baculovirus particles containing the product gene under CMV promoter control will be generated in insect cell cultures using standard procedures, and used to transfect mammalian cell lines. The transient gene expression, generally at low levels compared to stable cell lines, can be increased ten-fold or more by addition of a histone deacetylase inhibitor such as sodium butyrate which inhibits the normal silencing of transfected DNA.

Ref: Condreay et al (1998) PNAS 96 127
**Baculovirus CL1**
These organisms fall into ACDP hazard group 1

**Host/vector system**
For generation of recombinant baculovirus, shuttle vectors containing the product gene under CMV promoter control are transfected into insect cells (e.g. Sf-9) using standard procedures such as the Bac to Bac expression system (Life Technologies).

(Recombinant cell lines expressing receptors and G-proteins: CL 1)

**Origin & function**

**Evaluation of foreseeable effects**
The baculovirus is able to transfect mammalian cells, but is unable to replicate in them. Efficient expression of the gene products under control of the CMV promoter is achieved only by addition of an inhibitor of cellular histone deacetylase. It is possible that inappropriate expression of a transmembrane receptor or regulatory protein could have detrimental effects. However, the only likely routes of infection would be mouth, nose or eyes, and the amounts which might enter by such routes (via aerosols or splashes) would be small (microlitres). In addition, baculoviruses are rapidly inactivated (>99% in 30 minutes) by the complement system of normal human serum (Hofmann and Strauss '98). The work will use standard laboratory and bioreactor methods and does not involve processes which would generate significant quantities of aerosols. Needles (sharps) will not be used. Laboratory operations involving production of baculoviruses and transfection of cell lines will be carried out in a microbiologic safety cabinet, which will be sufficient to reduce the likelihood of infection to effectively zero. Bioreactor operations will be carried out under CL2 conditions as defined in table 2 of the GMO Regulations 2000. Typically, the baculovirus will be used to transfect mammalian cells either in multi-well plates (eg 96-well) for assay, or in bioreactors for preparation of batches of cells. Batches of cells will be collected (centrifugation) and washed under CL2 conditions. The transient recombinant cell lines expressing receptors generated by this system, including low levels of residual virus, are unlikely to pose a risk to health, and may be handled under conditions of CL1 (e.g for analysis). Similar stable cell lines have previously been categorised as CL1.


**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Waste cultures, supernatants, and cell debris of up to 1 litre will be inactivated by autoclaving in validated autoclaves (>120 deg C for > 15 min); larger volumes will be heat-treated in fermenters (>120 deg C for > 15 min); plastic and other solid waste will be autoclaved as described and/or incinerated.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N
The Committee approved the Risk assessment for production of baculovirus and transfection of cell lines at CL2.

**Project Containment**

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**Project Ref** 315/01.7

- **Date Ackn’d**: 11/10/2002
- **CU2 Project Title**: UTILISATION OF STREPTOMYCES SPECIES SUCH AS STREPTOMYCES LIVIDANS AS AN EXPRESSION SYSTEM FOR THE PRODUCTION OF CYTOKINES
- **Class**: Class 2
- **CultureVolClass2**: < 1 litre
- **Consent Granted**: Non-GMM
- **Consent Granted**: not applicable
- **Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The aim is evaluate and utilise Streptomyces lividans for production of heterologous proteins of biopharmaceutical relevance. Initially murine TNFa. will be overexpressed and secreted by Streptomyces lividans to evaluate the conditions required for high level protein production. If successful the work may be extended to use of other Streptomyces species and expression of other cytokines, including novel cytokines. Laboratory work with Streptomyces will be carried out at up to 0.5L volumes. If required, larger volumes of crude product (up to 15L) will be produced from bioreactor cultures.
**Recipient or parental organism**

Streptomyces species such as Streptomyces lividans. These organisms fall into the lowest ACDP hazard group 1.

**Host/vector system**

Non-pathogenic Streptomyces species transfected with non-conjugative plasmids (unable to transfer to other species).

**Origin & function**

GSK proprietary libraries.

**Evaluation of foreseeable effects**

Human and non-human cytokines may have biological activity if delivered to a site of action (eg if ingested or inhaled). The use of Class 2 biological safety cabinets and gloves will reduce the likelihood of harm from laboratory work to effectively zero. For larger scale work, the operation of bioreactors is designed to minimise release through exhaust gases or during sampling of cultures, and the work will be effectively contained at CL2 according to Schedule 8 of the GMO (Contained Use) Regulations 2000.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste cultures, supernatants, and cell debris of up to 0.5 litre will be inactivated by autoclaving in validated autoclaves (>120 deg C for >15 min); larger volumes will be heat-treated in fermenters (>120 deg C for >15 min); plastic and other solid waste will be autoclaved as described and/or incinerated.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The Committee approved the risk assessment at CL2.

**Project Containment**

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<thead>
<tr>
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</table>
**Project Additional Information**

**Purposes of the contained use**

In the search for new and effective pharmaceuticals with minimal side effects, GSK intends to express known and novel kinases in mammalian cells in order to obtain functional data and to determine the specificity of closely related kinases. Kinases are a large class of enzymes found in all cell types which activate their target proteins by phosphorylation. Frequently, the target molecule itself will be a kinase, which when activated can progress the signal by phosphorylation of the next enzyme in the signal cascade. The kinases may be modified to modulate their activity and to evaluate the mechanism of action, for example by analysing the effect of single amino acid substitutions. To avoid the generation of large numbers of stable recombinant cell lines, we intend to use a recombinant baculovirus system which will allow transient expression in mammalian cell lines.

Baculoviruses are a well studied system for the production of recombinant proteins in insect cells. They are unable to replicate in mammalian cells, and the usual insect-specific promoters are ineffective in mammalian cells. By replacing the standard polyhedrin gene promoter sequence with that of an efficient mammalian promoter (such as the CMV IE promoter/enhancer) baculoviruses are able to express recombinant products in mammalian cells, though they remain incapable of replication in these cells. (Condreay et al 1998). Baculovirus particles containing the product gene under CMV promoter control will be generated in insect cell cultures using standard procedures, and used to transfect mammalian cell lines. The transient gene expression, generally at low levels compared to stable cell lines, can be increased ten-fold or more by addition of a histone deacetylase inhibitor such as sodium butyrate which inhibits the normal silencing of transfected DNA.
Baculovirus CL1
These organisms fall into ACDP hazard group 1.

Host/vector system
For generation of recombinant baculovirus, shuttle vectors containing the product gene under CMV promoter control are transfected into insect cells (eg Sf-9) using standard procedures such as the Bac to Bac expression system (Life Technologies).

(Recombinant cell lines expressing kinases: CL1)

Origin & function
Libraries of human DNA from academic collaborators.

Evaluation of foreseeable effects
The baculovirus is able to transfect mammalian cells, but is unable to replicate in them. Efficient expression of the gene products under control of the CMV promoter is achieved only by addition of an inhibitor of cellular histone deacetylase. It is possible that inappropriate expression of a kinase could have detrimental effects. However, the only likely routes of infection would be mouth, nose or eyes, and the amounts which might enter by such routes (via aerosols or splashes) would be small (microlitres). In addition, baculoviruses are rapidly inactivated (>99% in 30 minutes) by the complement system of normal human serum (Hofmann and Strauss ‘98)*. The work will use standard laboratory and bioreactor methods and does not involve processes which would generate significant quantities of aerosols. Needles (sharps) will not be used. Laboratory operations involving production of baculoviruses and transfection of cell lines will be carried out in a microbiological safety cabinet, which will be sufficient to reduce the likelihood of infection to effectively zero. Bioreactor operations will be carried out under CL2 conditions as defined in table 2 of the GMO Regulations 2000. Typically, the baculovirus will be used to transfect mammalian cells either in multi-well plates (eg. 96-well) for assay, or in bioreactors for preparation of batches of cells. Batches of cells will be collected (centrifugation) and washed under CL2 conditions. The transient recombinant cell lines expressing kinases generated by this system, including low levels of residual virus, are unlikely to pose a risk to health, and may be handled under conditions of CL1 (eg. for analysis). Similar stable cell lines have previously been categorised as CL1.


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste culture, supernatants, and cell debris of up to 1 litre will be inactivated by autoclaving in validated autoclaves (>120 deg C for >15 min); larger volumes will be heat-treated in fermenters (>120 deg C for >15min); plastic and other solid waste will be autoclaved as described and/or incinerated. These treatments will give complete killing of the cells and baculovirus.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
The Committee approved the Risk assessment for production of baculovirus and transfection of cell lines at CL2.

### Project Containment

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**Project Ref**: 315/01.9

**Date Ackn’d**: 11/10/2002

**CU2 Project Title**: EXPRESSION OF NUCLEAR RECEPTORS IN MAMMALIAN CELLS USING THE BACULOVIRUS SYSTEM

**Class**: Class 2

**Culture Vol Class**: 1-50 litres

**Project notified under transitional arrangements**: N

**Non-GMM**: not applicable

**Consent Granted**: not applicable

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

In the search for new and effective pharmaceuticals with minimal side effects, GSK intends to express known and novel nuclear receptors in mammalian cells in order to obtain functional data and to determine the specificity of closely related receptors. Nuclear receptors are ligand regulated transcription factors which mediate their physiological effects by modulating the expression of target genes. The nuclear receptors may be modified to modulate their activity and to evaluate the mechanism of
action, for example by analysis the effect of single amino acid substitutions. To avoid the generation of large numbers of stable recombinant cell lines, we intend to use a recombinant baculovirus system which will allow transient expression in mammalian cell lines.

Baculoviruses are a well studied system for the production of recombinant proteins in insect cells. They are unable to replicate in mammalian cells, and the usual insect-specific promoters are ineffective in mammalian cells. By replacing the standard polyhedrin gene promoter sequence with that of an efficient mammalian promoter (such as the CMV IE promoter/enhancer) baculoviruses are able to express recombinant products in mammalian cells, though they remain incapable of replication in these cells. (Condreay et al 1998). Baculovirus particles containing the product gene under CMV promoter control will be generated in insect cell cultures using standard procedures, and used to transfect mammalian cell lines. The transient gene expression, generally at low levels compared to stable cell lines, can be increased ten-fold or more by addition of a histone deacetylase inhibitor such as sodium butyrate which inhibits the normal silencing of transfected DNA.


Recipient or parental organism

Baculovirus CL1.
These organisms fall into ACDP hazard group 1.

Host/vector system

For generation of recombinant baculovirus, shuttle vectors containing the product gene under CMV promoter control are transfected into insect cells (eg Sf-9) using standard procedures such as the Bac to Bac expression system (Life Technologies).

(Recombinant cell lines expressing nuclear receptors: CL1).

Origin & function

Libraries of human DNA commercially available or from academic collaborators.

Evaluation of foreseeable effects

The baculovirus is able to transfect mammalian cells, but is unable to replicate in them. Efficient expression of the gene products under control of the CMV promoter is achieved only by addition of an inhibitor of cellular histone deacetylase. It is possible that inappropriate expression of a nuclear receptor could have detrimental effects. However, the only likely routes of infection would be mouth, nose or eyes, and the amounts which might enter by such routes (via aerosols or splashes) would be small (microlitres). In addition, baculoviruses are rapidly inactivated (>99% in 30 minutes) by the complement system of normal human serum (Hofmann and Strauss ‘98)*. The work will use standard laboratory and bioreactor methods and does not involve processes which would generate significant quantities of aerosols. Needles (sharps) will not be used. Laboratory operations involving production of baculoviruses and transfection of cell lines will be carried out in a microbiological safety cabinet, which will be sufficient to reduce the likelihood of infection to effectively zero. Bioreactor operations will be carried out under CL2 conditions as defined in table 2 of the GMO Regulations 2000. Typically, the baculovirus will be used to transfect mammalian cells either in multi-well plates (eg 96-well) for assay, or in bioreactors for preparation of batches of cells. Batches of cells will be collected (centrifugation) and washed under CL2 conditions.

The transient recombinant cell lines expressing nuclear receptors generated by this system, including low levels of residual virus, are unlikely to pose a risk to health, and may be handled under conditions of CL1 (eg for analysis).


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste culture supernatants, and cell debris of up to 1 litre will be inactivated by autoclaving in validated autoclaves (>120 deg C for >15 min); larger volumes will be heat-treated in fermenters (>120 deg C for >15min); plastic and other solid waste will be autoclaved as described and/or incinerated. These treatments will give complete killing of the cells and baculovirus.

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Project Ref 52/93.1

Date Ackn’d 06/05/1993

Date Project Ceased

INTRODUCTION OF GENES INTO INFLUENZA VIRUS

Class 2 Culture Vol Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**  N

**If yes, tick to confirm that it is attached to this form**  N
**Project Containment**

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**Project Ref** 52/93.2

**Date Ackn'd**
- 15/06/1993

**CU2 Project Title**
- EXPRESSION OF FOREIGN GENES IN MAMMALIAN CELLS USING RETROVIRAL VECTORS

**Class**
- Class 2

**CultureVol**
- not applicable

**CultureVolume**
- not applicable

**Non-GMM Consent Granted**
- not applicable

**Project notified under transitional arrangements**
- Y

**Historical Significant Changes**
- Withdrawn
- N

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

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For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref  52/trans2**

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Project notified under transitional arrangements [Y]
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form
Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

| L2 L3 L4 L2 L3 L4 | L2 L3 L4 | L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 | L2 L3 L4 | L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 |

Project Ref 52/trans3

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Date Project Ceased 27/08/2008

Non-GMM Consent Granted yes

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
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- **Non-GMM Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**
- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Animal Units
| L2 | L3  | L4 | L2 | L3  | L4 |

Large Scale Activities | Human Clinical Applications
| L2 | L3  | L4 | L2 | L3  | L4 |

Project Ref  52/trans5

Date Ackn’d  15/01/2001

CU2 Project Title  INTRODUCTION OF HUMAN HERPES VIRUS GENES INTO MAMMALIAN CELLS BY DNA TRANSFECTION

Class  Class 2

Non-GMM  Consent Granted  not applicable

Project notified under transitional arrangements  [Y]

Withdrawn  [N]  Tick if notifying a connected programme of work  [N]
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

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Project Ref 558/00.5

Date Ackn'd 28/04/2000

CU2 Project Title GENERATION OF BVDV/HCV IRES CHIMERIC VIRUS

Class 2

CultureVolClass2

CultureVolumeClass3-4

Non-GMM Consent Granted not applicable

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
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**Project Ref**  558/00.8

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<tr>
<td>CU2 Project Title</td>
<td>REPLICATION OF GBV-B AGENT IN HEPATIC CELLS DERIVED FROM PRIMATES</td>
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<tr>
<td>Class</td>
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<td>Project notified under transitional arrangements</td>
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**Withdrawn**  N  

**Tick if notifying a connected programme of work**  N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>Project Ref 558/01.4</td>
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Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Date Ackn’d 18/05/2001

Date Project Ceased

Expression of Human Epithelial Cell Membrane Glycoprotein in Vaccinia Virus Strain WR

Class CultureVolClass2 CultureVolumeClass3-4

Consent Granted

Non-GMM

not applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work
### Project Additional Information

<table>
<thead>
<tr>
<th>Purpose of the contained use</th>
<th>Pharmaceutical Research and Development</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recipient or parental organism</strong></td>
<td>Vaccinia virus WR strain, ACDP Hazard Group 2.</td>
</tr>
<tr>
<td><strong>Host/vector system</strong></td>
<td>Vaccinia vector system pSC11, using the thymidine kinase gene as an insertion site to create a TK-recombinant virus.</td>
</tr>
<tr>
<td><strong>Origin &amp; function</strong></td>
<td>A human epithelial cell membrane glycoprotein whose exact function is unknown.</td>
</tr>
<tr>
<td><strong>Evaluation of foreseeable effects</strong></td>
<td>The exact function of the human epithelial cell membrane glycoprotein is unknown but it is not considered to present a serious hazard. The only theoretical hazard that can be postulated would be induction of auto-immunity to the protein expressing cells should infection with vaccinia occur. This is considered unlikely as many hundreds of cancer patients have been intentionally immunised against this protein without serious adverse effects. Insertion of this into the vaccinia TK gene should not increase the virulence of the virus and is believed to reduce virulence in mice. The chances of environmental consequences are considered to be effectively zero. Experience gained from widespread use of vaccinia over many years during the smallpox eradication campaign indicates it is unlikely to survive or infect wild mammals.</td>
</tr>
<tr>
<td><strong>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</strong></td>
<td>Not applicable.</td>
</tr>
<tr>
<td><strong>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</strong></td>
<td>Not applicable.</td>
</tr>
<tr>
<td><strong>Describe the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</strong></td>
<td>Not applicable.</td>
</tr>
</tbody>
</table>
All virus containing liquids will be treated with Virkon disinfectant in accordance with manufacturer’s recommendations, left overnight. All potentially contaminated materials, including solids and used disinfectant, will be sent for autoclaving at 134°C for at least 3 minutes or 121°C for at least 15 minutes.

The relevant appointed officers of the GMSC have considered this risk assessment and consider ACGM laboratory containment level 2 and our standard waste disposal techniques appropriate for this work. A segregated laboratory to which access by only authorised persons is allowed will be used for work with vaccinia. A dedicated class II microbiological safety cabinet will be used for handling vaccinia.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
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<td>L2 L3 L4</td>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
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<td>L2 L3 L4 L2</td>
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**Project Ref** 558/01.5

<table>
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<tr>
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<th>Consent Granted</th>
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<tr>
<td>27/11/2001</td>
<td>EXPRESSION OF A HUMAN PROSTATE SPECIFIC MEMBRANE PROTEIN IN THE VACCINIA VIRUS STRAIN WR</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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Withdrawn N  Tick if notifying a connected programme of work N

Historical Significant Changes
**Project Additional Information**

**Purposes of the contained use**
Pharmaceutical Research & Development

**Recipient or parental organism**
Vaccinia virus WR strain, ACDP Hazard Group 2

**Host/vector system**
Vaccinia vector system pSC11, using the thymidine kinase gene as an insertion site to create a TK- recombinant virus

**Origin & function**
A human prostate specific membrane protein whose exact function is unknown but is naturally expressed at high level in normal prostate tissue.

**Evaluation of foreseeable effects**
The exact function of the human prostate-specific membrane protein is unknown but it is not considered to present a serious hazard as it is naturally expressed at high level in normal prostate tissue. The only theoretical hazard that can be postulated would be the induction of a transient immune response to the human prostate specific membrane protein should infection with vaccinia occur.

Insertion of this gene into the vaccinia TK gene should not increase the virulence of the virus and is believed to reduce the virulence in mice.

The chances of environmental consequences are considered to be effectively zero. Experience gained from widespread use of the vaccinia over many years during the smallpox eradication campaign indicates it is unlikely to survive or infect wild mammals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All virus containing liquids will be treated with Virkon disinfectant in accordance with the manufacturer's recommendations and left overnight. All potentially contaminated materials, including solids and used disinfectant, will be autoclaved at 134C for at least 3 minutes or 121C for at least 15 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The relevant appointed officers of the GMSC have considered this risk assessment and consider the ACGM laboratory containment level 2+ and our standard waste disposal techniques appropriate for this work. A segregated laboratory to which access by only authorised persons is allowed will be used for work with vaccinia. A dedicated class II microbiological safety cabinet will be used for handling vaccinia. All authorised workers will be screened and counselled by Employee Health Management.

Project Containment

**Laboratory Activities**
- L2
- L3
- L4
- L2
- L3
- L4
- L2
- L3
- L4

**Glass Houses**
- L2
- L3
- L4
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**Growth Rooms**
- L2
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**Animal Units**
- L2
- L3
- L4
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- L3
- L4

**Large Scale Activities**
- L2
- L3
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- L2
- L3
- L4

**Human Clinical Applications**
- L2
- L3
- L4
- L2
- L3
- L4

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**Project Ref** 558/01.6

**Date Ackn’ed** 17/12/2001

**CU2 Project Title** USE OF ADENO-ASSOCIATED VIRUS (AAV TYPE 2) FOR EXPRESSION OF HUMAN LIPOPROTEIN ASSOCIATED PHOSPHOLIPASE A2 (LP-PLA2).  

**Class** Class 2

**Consent Granted** not applicable

**Project notified under transitional arrangements** N

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**Project Additional Information**
<table>
<thead>
<tr>
<th>Purpose of the contained use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical Research &amp; Development</td>
</tr>
</tbody>
</table>

**Recipient or parental organism**

- Adeno-associated virus type 2

**Host/vector system**

- Replication defective Adeno-associated virus (AAV) vectors encoding human lipoprotein associated phospholipase A2 (Lp-PLA2) and a control inactive Lp-PLA2

**Origin & function**

- Human lipoprotein associated phospholipase A2

**Evaluation of foreseeable effects**

Increased Lp-PLA2 in the blood may be associated with an increased risk for the development of atherosclerosis, and therefore might be detrimental to health if delivered to the target tissue.

The work with AAV does not involve procedures, which would create a significant risk, such as generation of aerosols, and we calculate that if the total virus stock were delivered to a single adult, it would be sufficient only to increase active Lp-PLA2 levels by approximately 1% of normal levels. Therefore initial laboratory work would be highly unlikely to be deleterious to humans, and would be categorised as Class 1.

However, future work may involve increased amounts of virus (up to 10 x 10^10 particles) and will involve the use of sharps, which could deliver the virus to the site of action (blood). Therefore work with AAV will be carried out in microbiological safety cabinets and access to the laboratories will be restricted as a precaution. This indicates containment level 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All virus containing liquids will be treated with Virkon disinfectant in accordance with the manufacturer's recommendations. All potentially contaminated materials, including solids and used disinfectant, will be sent for autoclaving at 134°C for at least 3 minutes or 121°C for at least 15 minutes.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
The relevant appointed officers of the GMSC have considered this risk assessment and consider ACGM laboratory containment level 2 and our standard waste disposal techniques appropriate for this work.

## Project Containment

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## Project Ref  558/02.1

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<tr>
<td>20/12/2001</td>
<td>TRANSIENT EXPRESSION OF ION CHANNELS IN CULTURED MAMMALIAN CELLS USING A RECOMBINANT BACULOVIRUS VECTOR</td>
<td>Class 2</td>
<td>1-50 litres</td>
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<td>Historical Date of Additional Info</td>
<td>02/08/2002</td>
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</table>

## Project Additional Information

**Purposes of the contained use**
- Pharmaceutical Research & Development

**Recipient or parental organism**
- Baculovirus
Host/vector system

Sf9 Insect cells / pBacMaM-1 (based on pFastBac-1 shuttle vector (Invitrogen) whose baculovirus polyhedrin promoter has been removed and replaced with that of the CMV IE promoter/enhancer).

Origin & function

Ion channel sub-units, ion channel modulator proteins from human, rat and mouse which control entry/exit of ions across the cell membrane.

Evaluation of foreseeable effects

The expression of ion channels in Baculovirus is highly unlikely to increase the pathogenicity of the host or its ability to survive outside the culture medium. The pFastBacMam series of vectors and the derivative pBacMam-1 are non-pathogenic and non-replicative in mammalian cells. In the unlikely event of virus entering the body, transient expression of gene products is possible. It is therefore considered that generation of viral stock in Sf9 insect cells should be carried out under ACGM CL2 conditions.

The initial transduction of cultured mammalian cells with pBacMam-1 will be carried out at ACGM CL2 until all residual viral particles not taken up by the cells have been washed away. The effectiveness of this washing step in removing residual viral particles has been proven experimentally.

The other steps in the experiment, namely growing stocks of the vector in E. coli and culture of the modified mammalian cells is considered to entail minimal risk and will be conducted at ACGM CL1.

The expression of ion channels in Baculovirus is not expected to alter its host range or survivability in the environment. The host Sf9 insect cells are disabled and unlikely to persist in the environment. The budable form of the Baculovirus vectors being used is disabled and cannot infect hosts outside the laboratory - the normal insect host is only infected by the occluded form of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All virus containing liquids will be treated with suitable disinfectant eg Virkon or Hycolin in accordance with the manufacturer's recommendations & left overnight. All potentially contaminated materials, including solids and used disinfectant, will be autoclaved either at 134C for at least 3 minutes or 121C for at least 15 minutes.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The GMSC has considered this risk assessment and deemed ACGM laboratory containment level 2 appropriate for the Sf9 insect cell and mammalian cell transformation parts of this work with ACGM laboratory containment level 1 appropriate for the other parts. All work will use our standard waste treatment methods.
### Project Containment

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### Project Ref 558/02.2

**Date Ackn'd** 07/08/2002  
**CU2 Project Title** PRODUCTION OF RECOMBINANT HUMAN CELL LINES EXPRESSING THE MOUSE ECOTROPIC RECEPTOR  
**Class** Class 2  
**Culture Vol Class** 1-50 litres  
**Class Culture Volume**  
**Non-GMM** not applicable  
**Consent Granted**  
**Project notified under transitional arrangements** N

### Project Additional Information

**Purposes of the contained use**  
Pharmaceutical research and development

**Recipient or parental organism**  
Standard human cell lines including HEK293T, Jurkat, K562, Hel, Cos, RAMOS, RPM 18226, Saos2, IMR-32, U20S, A549, H292, T84, Hela, SKN-SH-Sy5y, SKN-SH-MC, N132, NG108, Ntera2, U373MG, HepG2 which are all considered to be well characterised and having a long history of safe use.

**Host/vector system**
Modified human (FLYA13) "split genome" packaging cell line and/or Orbigen Phoenix Ampho Human HEK293 packaging cell line, and/or Clontech Ampho-pack HEK293 packaging cell line, using a murine amphotrophic virus (pWZLneo) vector based upon the Murine) MuLV) group of retroviruses, having the ecotropic receptor inserted downstream of the LTR promoter in order to generate replication incompetent viral particles.

**Origin & function**

Mouse cationic amino acid transporter protein (Mouse Ecotropic Receptor).

**Evaluation of foreseeable effects**

The aim of the work is to produce a set of standard human cell lines expressing the Mouse Ecotropic Receptor at the cell surface and thereby enable various other human proteins to be introduced into those lines via a modified virus which would be unable to infect human cells lacking the mouse ecotropic receptor.

The nature of the expressed insert, the mouse ecotropic receptor, is considered to pose no risk to humans.

The use of split human cell line packaging systems for the pWZLneo vector that bear the Gag, Env and Pol genes on separate plasmid systems within the line will minimise the risk of recombination occurring when producing the modified viral particles to give replication competent virus.

Human packaging cell lines also reduce the risk, compared to murine packaging cell lines, of the cell line bearing endogenous retroviral sequences that can recombine to produce competent virus.

The murine amphotrophic virus is capable of infecting human cells and the properties conveyed by the ecotropic receptor might permit any cells carrying this receptor to be infected by viruses that would otherwise not be capable of infecting a human cell. For this reason the GMSC consider use of a Class II microbiologic al safety cabinet is essential for work involving infecting the human cell lines with pWZLNeo carrying the mouse ecotropic receptor. The cells that result can safely be handled at containment level 1.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not required

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All contaminated liquids will be treated with appropriate disinfectant (Virkon or Hycolin) in accordance with the manufacturer's recommendations and left overnight. All potentially contaminated materials will also be subject to steam treatment using validated autoclaves to maintain the full load either at 134 degrees C for at least 3 minutes or 121 degrees C for at least 15 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
The GSK R&D GMSC have considered this risk assessment and consider the use of a class II MSC for infecting human cells with the modified virus essential.

### Project Containment

<table>
<thead>
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</table>

- Animal Units
- Large Scale Activities
- Human Clinical Applications

### Project Ref 558/02.4

<table>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<td>TRANSIENT EXPRESSION OF NUCLEAR RECEPTORS AND CO-REGULATORS IN CULTURED MAMMALIAN CELLS USING A RECOMBINANT BACULOVIRUS VECTOR.</td>
<td>Class 2</td>
<td>1-50 litres</td>
<td>not applicable</td>
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</table>

- Withdrawn: N
- Project notified under transitional arrangements: N

### Project Additional Information

- **Purposes of the contained use**: Pharmaceutical research and development.
- **Recipient or parental organism**: Immortalised mammalian cell line such as T47D and CV1.
Host/vector system

Immortalised mammalian cell line such as T47D and CV1/pBacMam-1 (based on pFastBac-1 shuttle vector (Invitrogen) whose baculovirus polyhedrin promoter has been removed and replaced with that of the CMV 1E promoter/enhancer).

Origin & function

cDNA encoding nuclear receptors and co-regulators from human, rat and mouse. Nuclear receptors are ligand regulated transcription factors which mediate their physiological effects through modulation of the expression levels of target genes. Nuclear co-regulators modulate the effects of nuclear receptors by promoting or suppressing their transcriptional activity.

Evaluation of foreseeable effects

The expression of nuclear receptors and co-regulators is highly unlikely to increase the pathogenicity of the host or its ability to survive outside of the culture medium. The pFastBacMam series of vectors and the derivative pBacMam-1 are non-pathogenic and non-replicative in mammalian cells. In the unlikely event of virus entering the body, transient expression of gene products is possible. It is therefore considered that generation of viral stock in Sf9 insect cells should be carried out under ACGM CL2 conditions.

The initial transduction of the cultured mammalian cells with pBacMam-1 will be carried out at ACGM CL2 until all residual viral particles not taken up by the cells have been washed away. The effectiveness of this washing step in removing residual viral particles has been proven experimentally.

The expression of nuclear receptors and co-regulators is not expected to alter host range or survivability in the environment. The host Sf9 insect cells are disabled and unlikely to persist in the environment. The budable form of the Baculovirus vectors being used is disabled and cannot infect hosts outside the laboratory - the normal insect host is only infected by the occluded form of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All virus containing liquids will be treated with suitable disinfectant eg Virkon or Hycolin in accordance with the manufacturer's recommendations and left overnight. All other potentially contaminated materials will be autoclaved either at 134 degrees C for at least 3 minutes or 121 degrees C for at least 15 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The GMSC has considered the risk assessment and deemed ACGM laboratory containment level 2 appropriate for the Sf9 insect cell and mammalian cell transformation parts of this work with ACGM laboratory containment level 1 appropriate for the other parts. All work will use our standard waste treatment methods.

02/03/2022
Project Containment

Laboratory Activities

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<thead>
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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

<table>
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Human Clinical Applications

<table>
<thead>
<tr>
<th>Class</th>
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</table>

Project Ref 558/02.5

Date Ackn'd 17/10/2002

CU2 Project Title

EXPRESSION OF NEUROTRANSMITTER TRANSPORTERS IN CULTURED MAMMALIAN CELLS USING A RECOMBINANT BACULOVIRUS VECTOR.

Class CultureVolClass2 CultureVolumeClass3-4

<table>
<thead>
<tr>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>Class 2</td>
<td>1-50 litres</td>
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</tr>
</tbody>
</table>

Non-GMM

Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Pharmaceutical research and development

Recipient or parental organism

Mammalian cell lines such as HEK293, CV1, SHSY5Y, SKNMC, CHO, CV1, HeLa, primary cell lines from human and animal tissues.

Host/vector system
Mammalian cell lines/ pBacMaM-1 (based on pFastBac-1 shuttle vector (Invitrogen) whose baculovirus polyhedrin promoter has been removed and replaced with that of the CMV IE promoter/enhancer).

Origin & function

cDNA encoding neurotransmitter transporters from human. Neurotransmitter transporters are transmembrane proteins responsible for the transport of neurotransmitters into and out of cells, they fall into three main classes, Na/Cl-dependent plasma membrane transporters, Na-dependent plasma membrane transporters and vesicular transporters.

Evaluation of foreseeable effects

The expression of neurotransmitter transporters is highly unlikely to increase the pathogenicity of the host or its ability to survive outside of the culture medium. The pFastBacMam series of vectors and the derivative pBacMam-1 are non-pathogenic and non-replicative in mammalian cells. In the unlikely event of virus entering the body, transient expression of gene products is possible.

The initial transduction of the cultured mammalian cells with pBacMam-1 will be carried out at ACGM CL2 until all residual viral particles not taken up by the cells have been washed away. The effectiveness of this washing step in removing residual viral particles has been proven experimentally.

The expression of neurotransmitter transporters is not expected to alter host range or survivability in the environment. The budable form of the Baculovirus vectors being used is disabled and cannot infect hosts outside the laboratory - the normal insect host is only infected by the occluded form of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All virus containing liquids will be treated with suitable disinfectant eg Virkon or Bycolin in accordance with the manufacturer's recommendations and left overnight. All other potentially contaminated materials will be autoclaved either at 134 degrees C for at least 3 minutes or 121 degrees C for at least 15 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC has considered this risk assessment and deemed ACGM laboratory containment level 2 appropriate for the Sf9 insect cell and mammalian cell transformation parts of this work with ACGM laboratory containment level 1 appropriate for the other parts. All work will use our standard waste treatment methods.

Project Containment
Project Ref 558/02.6

Date Ackn’d 03/12/2002
CU2 Project Title USE OF SEMLIKI FOREST VIRUS TO EXPRESS 6-PROTEIN COUPLED RECEPTORS

Class 2
CultureVolClass2 1-50 litres
CultureVolumeClass3-4

Non-GMM
Consent Granted not applicable

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Pharmaceutical Research and Development

Recipient or parental organism
Immortalised mammalian cell line such as HEK293 and CHO cells.

Host/vector system
The vector system contains only the non-structural SFV genes and a conditionally lethal mutation in the spike gene of the helper construct which means that virus is non-infectious until activated by protease.

Origin & function
All GPCRs and accessory protein (e.g., RAMPS, G-proteins, peptide and protein (GPCR fusions)). GPCRs are cell associated proteins with seven transmembrane domains linking the outer and inner cell surfaces. They are responsible for passing signals (usually in the form of an extracellular ligand) to the intracellular signalling apparatus via various accessory proteins such as G-proteins.

**Evaluation of foreseeable effects**

The expression of GPCRs is highly unlikely to increase the pathogenicity of the host or its ability to survive outside of the culture medium. The gene products are all membrane associated. Function requires the presence of a specific ligand and the presence of intact intracellular signalling apparatus. The gene may only function in particular cell types and requires expression in appropriate cell types. The expression of GPCRs is not expected to alter host range or survivability in the environment.

Semliki forest virus can be classified as a mild human pathogen. It uses a blood-borne transmission pathway and is normally spread by mosquitoes among rodents and birds in central Africa. In humans, mild flu symptoms lasting 7 days may occur. The vector system used in the lab is not capable of replication as only the RNA containing the non-structural genes and insert gene is packaged into the viral particle. Additional safety measures include a conditionally lethal mutation in the spike gene of the helper construct which means that virus is non-infectious until activated by protease.

**Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All virus containing liquids will be treated with suitable disinfectant e.g., Virkon or Hycolin in accordance with the manufacturer's recommendations and left overnight. All other potentially contaminated materials will be autoclaved either at 134 degrees C for at least 3 minutes or 121 degrees C for at least 15 minutes.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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</tbody>
</table>

Animal Units  | Large Scale Activities  | Human Clinical Applications

02/03/2022
## Project Additional Information

### Purposes of the contained use

Pharmaceutical Research and Development.

### Recipient or parental organism

Immortalised mammalian cell lines such as HEK293 and CHO cells.

### Host/vector system

Immortalised mammalian cell lines such as HEK293 and CHO cells pBacMam-1 (based on pFastBac-1 shuttle vector (invitrogen) whose baculovirus polyhedrin promoter has been removed and replaced with that of the CMV IE promoter/enhancer).

### Origin & function

All GPCRs and accessory protein (e.g., RAMPS, G-proteins, peptide and protein GPCR fusions). GPCRs are cell-associated proteins with seven transmembrane domains linking the outer and inner cell surfaces. They are responsible for passing signals (usually in the form of an extracellular ligand) to the intracellular signalling apparatus via various accessory proteins such as G-proteins.

### Evaluation of foreseeable effects

The expression of GPCRs is highly unlikely to increase the pathogenicity of the host or its ability to survive outside of the culture medium. The gene products are all...
membrane associated. Function requires the presence of a specific ligand and the presence of intact intracellular signalling apparatus. The Gene may only function in particular cell types and requires expression in appropriate cell types. The pFastBacMam series of vectors and the derivative pBacMam-1 are non-pathogenic and non-replicative in mammalian cells. In the unlikely event of virus entering the body, transient expression of gene products is possible. It is therefore considered that generation of viral stock in Sf9 insect cells should be carried out under ACGM CL2 conditions.

The initial transduction of the cultured mammalian cells with pBacMam-1 will be carried out at ACGM CL2 until all residual viral particles not taken up by the cells have been washed away. The effectiveness of this washing step in removing residual viral particles has been proven experimentally.

The expression of GPCRs is not expected to alter host range or survivability in the environment. The host Sf9 insect cells are disabled and unlikely to persist in the environment. The budable form of the Baculovirus vectors being used is disabled and cannot infect hosts outside the laboratory - the normal insect host is only infected by the occluded form of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All virus containing liquids will be treated with suitable disinfectant eg Virkon or Hycolin in accordance with the manufacturer's recommendations and left overnight. All other potentially contaminated materials will be autoclaved either at 134 degrees C for at least 3 minutes or 121 degrees C for at least 15 minutes.

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Project Containment

<table>
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<th>Laboratory Activities</th>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>

The GMSC has considered this risk assessment and deemed ACGM laboratory containment level 2 appropriate for the Sf9 insect cell and mammalian cell transformation parts of this work with ACGM laboratory containment level 1 appropriate for the other parts. All work will use our standard waste treatment methods.
MANIPULATION OF FULL LENGTH CDNA CLONES OF HUMAN PAPILLOMA VIRUS.

<table>
<thead>
<tr>
<th>Purposes of the contained use</th>
</tr>
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<tbody>
<tr>
<td>Pharmaceutical research and development.</td>
</tr>
</tbody>
</table>

**Recipient or parental organism**

auxotrophic strains of E. coli, HB101 and DH5alpha.

**Host/vector system**

Non-mobilisable plasmids, Puc19, pBR322 & pBluescript SK containing full length clones of HPV-6, HPV-11, HPV-16 & HPV-18

**Origin & function**

Full length clones of Human papilloma virus will be cloned into E.coli, maintained and amplified. The resultant plasmids will purified for use in the development of DNA vaccines.

**Evaluation of foreseeable effects**

The amplification of the HPV plasmid is very unlikely to increase the pathogenicity of the E coli host or increase its ability to survive outside of the culture medium. In the unlikely event of the E. coli being ingested there is an effectively zero risk of infection from the HPV clones as the virus is not a mucosal pathogen of the gut.

The HPV clones will be re-ligated and digested as part of the preparation for some experiments. These manipulations will generate HPV, which may be infectious if
accidentally inoculated into human skin unprotected by gloves.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquids contaminated with GMM will be treated with suitable disinfectant eg Virkon or Hycolin in accordance with the manufacturer's recommendations and left overnight. All other potentially contaminated materials will be autoclaved either at 134 degrees C for at least 3 minutes or 121 degrees C for at least 15 minutes.

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The GMSC has considered this risk assessment and deemed ACGM laboratory containment level 2 appropriate for this activity. All work will use our standard waste treatment methods.

Project Containment

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Animal Units

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Large Scale Activities

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Human Clinical Applications

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<tr>
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<tr>
<td>CU2 Project Title</td>
<td>USE OF VACCINIA VIRUS RECOMBINANT EXPRESSING OVALBUMIN IN ANIMAL</td>
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Project Ref 558/03.2

Date Ackn'd 28/10/2003

CU2 Project Title USE OF VACCINIA VIRUS RECOMBINANT EXPRESSING OVALBUMIN IN ANIMAL

Class Class 2

CultureVolClass2 < 1 litre

CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Pharmaceutical research and development.

Recipient or parental organism

WR strain of vaccinia virus, a tissue culture adapted virus from a vaccine strain used as part of the smallpox eradication programme and is designated ACDP hazard group 2.

Host/vector system

Recombinant vaccinia viruses encoding ovalbumin.

Origin & function

Inserts coding for Ovalbumin will be expressed in vaccinia, recombinants will then be used as a challenge virus in vaccine efficacy studies in mice. It should be noted that recombinant vaccinia virus in this model is being used as a challenge virus rather than a vector for the delivery of heterologous antigen.

Evaluation of foreseeable effects

The WR strain of vaccinia virus does not occur naturally in humans and there is no animal reservoir. The rate of severe adverse reactions post vaccination is 1 in 50,000. It may cause particularly severe disease in people with active skin disorders or the immune compromised, therefore staff will be counselled for their suitability to work with vaccinia virus. Infection with vaccinia is generally self-limiting and therefore, any side-effects of foreign gene expression is likely to be transient.


The work will involve inoculation of mice with infectious vaccinia virus and the subsequent handling of infected tissues from the animals. There is a risk of accidental infection when carrying out these procedures. The use of a TK-vaccinia recombinant limits the spread of the virus within an infected animal and reduces the likelihood of virus being secreted therefore reducing the risk of transmission to the laboratory worker. Administration by the i.p and i.v. routes means that no infectious skin lesions are formed which also reduces possible accidental transmission.
All work involving infectious vaccinia virus will be carried out in a separate designated animal facility. Infected animals are to be housed in cages within negative pressure isolators. All procedural work will be carried out using a class 1 microbiological safety cabinet. The room used for housing mice is maintained under negative pressure relative to the immediate surroundings. All waste (food, bedding carcasses) will be removed from the isolator in triple heat sealed portex tubing. All waste will be autoclaved (134 degrees C for a minimum of 30 minutes).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All liquids contaminated with GMMs will be treated with suitable disinfectant eg Virkon or Hycolin in accordance with the manufacturer's recommendations and left overnight. All other potentially contaminated materials will be autoclaved either at 134 degrees C for at least 3 minutes or 121 degrees C for at least 15 minutes.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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Animal Units

<table>
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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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</table>

Project Ref 558/03.3

Date Ackn'd CU2 Project Title

02/03/2022
GENERATION AND USE OF VACCINIA VIRUS RECOMBINANTS EXPRESSING HCV ANTIGENS AND OVALBUMIN.

Project Additional Information

Purposes of the contained use
Pharmaceutical research and development.

Recipient or parental organism
WR strain of vaccinia virus, a tissue culture adapted virus from a vaccine strain used as part of the smallpox eradication programme and is designated ACDP hazard group 2.

Host/vector system
Recombinant vaccinia viruses will be generated by homologous recombination between sequences within the vaccinia expression vector pSC11 and the vaccinia virus genome.

Origin & function
Inserts coding for Ovalbumin and HCV antigens will be expressed in vaccinia, recombinants will then be used in immunological assay.

Evaluation of foreseeable effects
The WR strain of vaccinia virus does not occur naturally in humans and there is no animal reservoir. The rate of severe adverse reactions post vaccination is 1 in 50,000. It may cause particularly severe disease in people with active skin disorders or the immune compromised, therefore staff will be counselled for their suitability to work with vaccinia virus. Infection with vaccinia is generally self limiting and, therefore, any side-effects of foreign gene expression is likely to be transient. The thymidine kinase (TK) gene is used as the insertion site creating a TK-recombinant virus which is believed to reduce the virulence of the virus in mice, but does not necessarily imply lower virulence in man.

Vaccinia recombinants expressing various HCV antigens under the control of a bacteriophage T7 promoter, have been used in-house. No increase in the virulence of these recombinants above the wild type virus has been observed. In addition our proposed recombinants will contain gene inserts with inactivated enzymes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquids contaminated with GMMs will be treated with suitable disinfectant eg Virkon or Hycolin in accordance with the manufacturer's recommendations and left overnight. All other potentially contaminated materials will be autoclaved either at 134 degrees C for at least 3 minutes or 121 degrees C for at least 15 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The GMSC has considered this risk assessment and deemed ACGM laboratory containment level 2 appropriate for this activity. All work involving infectious vaccinia virus will be carried out in a designated vaccinia virus handling lab. All individuals involved in this work should attend EHM for baseline health surveillance prior to commencing work and an entry made in their COSHH record.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
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<td>L4</td>
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</tbody>
</table>

Animal Units

<table>
<thead>
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<th>Large Scale Activities</th>
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</tr>
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<td>L2</td>
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Project Ref 558/04.1

Date Ackn’d 09/09/2004
CU2 Project Title Cloning of shRNA expression cassettes into adeno-associated virus (AAV) vectors
Class 2
CultureVolClass2 1-50 Litres
CultureVolumeClass3-4
and their use to suppress the expression of specific genes in cultured mammalian cells by RNA interference

Pharmaceutical research and development.

Auxotrophic strain of E. coli, DH5alpha and HEK293 cells.

Recombinant plasmids encoding shRNAs will be amplified in the E. coli host. These will be transfected into HEK293 cells together with the plasmid encoding the AAV replication and virion proteins (rep, cap) and the adenoviral 'helper' proteins (VA, E2a, E4). Recombinant AAV vectors will be harvested.

Chemically synthesized, double-stranded oligonucleotides, of sequence corresponding to siRNAs are to be ligated downstream of either the H1 or U6 pol III promoter in AAV vector shuttle plasmid. These will then be packaged into infectious AAV vectors and inoculated into cell cultures (primary cell types from human and rodent origin including neurons, microglia, monocytes, and macrophages as well as continuous cell lines such as Ramos and Jurkat) in an attempt to suppress the expression of genes of interest.

The amplification of the AAV plasmid is very unlikely to increase the pathogenicity of the E. coli host or increase its ability to survive outside of the culture medium. In the unlikely event of the E. coli being ingested there is an effectively zero risk of infection from the HPV clones as the virus is not a mucosal pathogen of the gut.

The viral vectors are replication-defective and as such can transduce cells but are unable to replicate or be transmitted to other cells or hosts. The viral vectors will be inoculated into primary human and rodent cells and continuous cell lines such as Jurkat and Ramos and the effects upon target gene expression evaluated. The parental viral vectors have been well characterised and the expression of the shRNA cassette is not predicted to be harmful, but since the shRNA expression constructs they encode are designed to target novel target genes, downstream effects of gene suppression may not easily be predicted.

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquids contaminated with GMM will be treated with suitable disinfectant eg Virkon or Hycolin in accordance with the manufacturer's recommendations and left overnight. All other potentially contaminated materials will be autoclaved either at 134 degrees C for at least 3 minutes or 121 degrees C for at least 15 minutes.

Is an emergency plan required according to regulation 20?  

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If yes, tick to confirm that it is attached to this form

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Tick to confirm that you have attached a risk assessment to this form

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Tick if you are claiming exemption from disclosure for section of the risk assessment

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Since the scope of this activity covers a wide range of shRNA expression constructs which are designed to target novel genes, and hence downstream effects of gene suppression may not easily be predicted. ACGM laboratory containment level 2 is considered appropriate for this activity.

All work will use our standard waste treatment methods.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref** 558/04.2

**Date Ackn’d** 09/09/2004

**Date Project Ceased**

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<td>Class 2</td>
<td>1-50 Litres</td>
<td>Non-GMM</td>
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Project notified under transitional arrangements

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form
Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

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| CU2 Project Title | Cloning of shRNA expression cassettes into adenoviral vectors and their use to suppress the expression of genes in cultured mammalian cells by RNA interference |

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| Project notified under transitional arrangements | N |

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| Tick if notifying a connected programme of work | N |

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Project Additional Information

<table>
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<th>Purposes of the contained use</th>
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</table>

| Pharmaceutical research and development |

02/03/2022
Recipient or parental organism
Auxotrophic stains of E.coli, TOP10 & GT116, continuous & primary mammalian cell lines eg HEK 293A, Ramos & Jurkat

Host/vector system
Recombinant plasmids encoding shRNAs will be amplified in the E.coli host. These will then be packaged into infectious replication-defective adenoviral vectors using a commercially available kit (the BLOCK-iT Adenoviral Expression System, Invirogen Inc.)

Origin & function
Chemically synthesized, double-stranded oligonucleotides, of sequences corresponding to siRNAs, will be incorporated into infectious, replication-defective adenoviral vectors using the BLOCK-iT Adenoviral Expression System (invitrogen Inc.). Prepared virus will be inoculated into cell cultures (primary cell types from human and rodent origin including neurons, microglia, monocytes, and macrophages as well as continuous cell lines such as Ramos and Jurkat), in an attempt to suppress the expression of genes of interest.

Evaluation of foreseeable effects
The amplification of the plasmid is very unlikely to increase the pathogenicity of the E.coli hosts or increase their ability to survive outside of the culture medium. In the unlikely of the E.coli being ingested there is an effectively zero risk of infection from the clones as the virus is not a mucosal pathogen of the gut.

Since the entire E1 region of the viral genome has been deleted the resulting adenoviral vectors are replication-defective, unable to establish a productive infection and thus amplify itself in infected cells. Viral replication can only occur in cells that express the E1a and E1b proteins such as the 293A cell line used to generate the vector. Adenovirus does not integrate into the host genome upon transduction. The adenoviral vectors, however, are unable to infect a wide range of different cell types and express the shRNA in those infected cells.

The precise effects of expression of the shRNA cassette when delivered to a target cell, are difficult to predict as their purpose is the partial suppression of novel genes. Although in the worse case suppression may lead to cell death or an increase in cell proliferation, this would not be expected to have any adverse affect on an exposed individual unless the exposuree affected a very large number of cells and this is extremely unlikely at the viral titres involved. Additionally, since the virus is replication-competent, the viral genome is present only transiently and will eventually be diluted out as cell division occurs.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All liquids contaminated with GMM will be treated with suitable disinfectant eg Virkon or Hycolin in accordance with the manufacturer's recommendations & left overnight. All other potentially contaminated materials will be autoclaved either at 134 degrees for at least 3 minutes or 121 degrees for at least 15 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
Since the scope of this activity covers a wide range of shRNA expression constructs which are designed to target novel genes, and hence downstream effects of gene suppression may not easily be predicted, ACGM Laboratory containment level 2 is considered appropriate for this activity. All work will use our standard waste treatment methods.

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms

L2  Yes  L3  L4  L2  L3  L4  L2  L3  L4

Animal Units  Large Scale Activities  Human Clinical Applications

L2  L3  L4  L2  L3  L4  L2  L3  L4

Project Ref  558/07.1

Date Ackn'd  17/04/2008

CU2 Project Title  Cloning of protein expression cassettes into adeno-associated virus (AAV) vectors and their use to express specific genes in cultured mammalian cells and animal models for target validation.

Date Project Ceased

Class  CultureVolClass2  CultureVolumeClass3-4

Class 2  1-50 Litres

Non-GMM  Consent Granted

Not Applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
### Purposes of the contained use

Pharmaceutical Research and Development.

### Recipient or parental organism

Replication defective Adeno Associated virus, Auxotrophic stains of E.coli, TOP10 & GT116, continuous & primary mammalian cell lines eg HEK 293A, Ramos & Jurkat.

### Host/vector system

Open reading frames encoding potential target proteins, will be ligated downstream of the CMV IE mammalian pol II promoter in a AVV vector shuttle plasmid and packaged into infectious replication defective AAV vectors.

### Origin & function

Replication-defective AAV vectors encoding the genes of interest (GPCR, IC, NR and Protease, obtained from commercially available cDNA libraries), will be used to transfect cell cultures (primary cell types of rodent origin such as neurons as well as continuous cell lines such as HEK293A cells) and also be administered to laboratory rodents (by the intravenous, intraspinal or intracranial routes) in order to express the genes of interest and validated them as potential drug targets.

### Evaluation of foreseeable effects

The amplification of the plasmids is very unlikely to increase the pathogenicity of the E.coli hosts or increase their ability to survive outside of the medium. In the unlikely event of the E.coli being ingested there is an effectively zero risk of infection from the clones as the virus is not a mucosal pathogen of the gut.

The viral vectors are replication-defective and as such can infect cells but are unable to replicate or be transmitted to other cells or hosts. Proteins representing potential drug targets will be over-expressed, ie they are naturally expressed in the cells and rodent tissues to which the viral vectors will be administered. Over-expression of target genes is not expected to have any toxic or oncogenic effects.

Some of plasmids involved encode the Woodchuck hepatitis virus post transcriptional regulatory element (WPRE). There are reports describing the development of tumours in a small number of mice inoculated in utero with lentiviral vectors containing the WPRE sequence and of the possibility that sequences within the WPRE may possess oncogenic properties.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquids contaminated with GMM will be treated with suitable disinfectant eg Virkon in accordance with the manufacturer's recommendations & left overnight. All other potentially contaminated materials will be autoclaved either at 134°C for at least 3 minutes or 121°C for at least 15 minutes. Autoclaves are monitored on each run and validated annually.

### Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

02/03/2022
Since the scope of this activity include plasmids encoding the WPRE sequence containment level 2 is appropriate for this work. Employees performing this work will be informed of the risk associated with this sequence and the precautions to be followed particularly with regard to the avoidance of inoculation injury.

All work will use our standard waste treatment methods.

Project Containment

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Project Ref 558/08.1

Date Ackn’d 04/01/2008

CU2 Project Title
Expression of ion-channel blocking peptides as long half-life biopharmaceuticals.

Date Project Ceased

Class 2
Culture Vol 1-50 Litres

Non-GMM Consent Granted
Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

The purpose of the work is to produce long half-life forms of venom peptides which block ion channels. These may have pharmacological utility in regulation of immune activation.

**Recipient or parental organism**

3 expression systems will be used, as described below, under a, b and c:

a) mammalian systems: human epithelial kidney cell line HEK293E, an immortal suspension adapted variant of the parental line transfected with a plasmid encoding the Epstein-Barr virus nuclear antigen.

b) Pichia pastoris expression: using Pichia pastoris KM71H or X33 strains.

c) E. coli expression: using the K12 strain TOP10.

**Host/vector system**

a) The pTT5 vector will be used.

b) The commercially available plasmid pPICZalpha will be used.

c) The pUC based plasmid pDOM5 will be used.

**Origin & function**

The genetic material to be used is fully synthetic in origin. The amino acid sequences on which it was based are derived from a human immunoglobulin variable domain joined to the channel blocking peptide by a synthetic flexible linker region. The channel blocking peptide is derived from a venom toxin peptide. Based on toxicology data from publications, the expressed protein will be a highly active potassium and calcium channel blocker, with a long serum half-life. The effects of prolonging the serum half-life of the toxin are not known. Based on steric effects, it would be likely to decrease the potency of the toxin, but the prolonged half-life would likely result in an enhancement of toxic effects. The biodistribution of the fusion protein would be altered relative to the unfused peptide, but the effect of any breakdown products could be similar to the native peptide. For the purposes of risk assessment, it is assumed that the biological effects would be similar, but more prolonged.

**Evaluation of foreseeable effects**

It is considered unlikely that the GMO will present any environmental risks. There are possible human health effects as detailed below.

**E coli** - The toxic effects by ingestion or expression in the gut are not known. Presumably, as a peptide toxin, a significant portion may be degraded, although due to the highly disulphide crosslinked nature of the peptide, it may be protease resistant. The toxic effects by this route would be potentially immunosuppressive at mucosal surfaces. Systemic exposure to the toxin via gut absorption may result in systemic exposure to the protein. The biological effects of this would be possibly to be toxic, with the possibility of blocking potassium channels in the heart for example. The threshold for this effect is considered higher than could be achieved using the strains used.

**Pichia** - Pichia has been chosen as an expression host, as it is non-colonising, the vectors used have promoters that are non-functional in E. coli, and are integrated and tightly repressed in Pichia culture before induction, thus giving maximal containment of the expression construct. Additionally, conditions for induction of expression in this vector require low glucose levels in the presence of methanol, conditions which are considered highly unlikely to be met in the human gut. The integrated nature of the vector means that transfer to other organisms will be minimised.

**Mammalian expression** - The pTT vector is designed for high level transient expression of cloned genes in the suspension cell line HEK293-E. These cells are a derivative of HEK293-T that have been adapted to suspension growth and to grow in serum-free conditions. The HEK293-E cells are stably transfected with a gene encoding the Epstein-Barr virus nuclear antigen, a protein that promotes nuclear localization of DNA5 that contain its’ binding sequence. The pTT vector contains 1 g tandem repeats of the appropriate sequence, with the aim of maximizing the nuclear localization of the plasmid. Expression of the constructs in these cells may act as an immunosuppressive, and could potentially enhance the ability of the cells to engraft, despite their highly disabled nature although this is considered unlikely.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will take the form of cell pellets, cuire supernatants, and contaminated consumables. All wastes will be disposed of by autoclaving followed by incineration. The autoclaving is carried out in a autoclave that has been temperature tested, and a load thermocouple is used to ensure target times and temperatures are met. This is backed up by disposal by incineration in a licensed contractor's clinical waste incinerator, which is the route for all of our GM waste. This route will give 100% kill by combination of two validated methods.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC have reviewed this assessment and consider containment level 2 precautions adequate for this work. Accidental injection of the purified protein or supernants presents the highest hazard and particular attention to avoidance of the use sharps will be employed.

**Project Containment**

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Project Ref 558/08.2

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022  Page 9673 of 15326
Production and Propagation of Recombinant and/or Chimeric hepatitis C Virus (HCV).

Date Project Ceased: 27/08/2008

Non-GMM Consent Granted: Yes

Project notified under transitional arrangements: N

Purposes of the contained use:
To produce infectious particles of recombinant and/or chimeric HCV by transfection of viral RNA into cells, followed by the subsequent culture of such viruses by standard virological techniques. (As described in Nature Methods 2, 565 (2005).

Recipient or parental organism:
Hepatocyte cell lines, mostly derivatives of the Huh7 human hepatoma laboratory cell line.

Host/vector system:
Plasmids will be generated containing thU length HCV sequences from different genotypes. Full length RNA will be transcribed from these plasmids and the resulting RNAs will be transfected into hepatocyte cell lines.

Origin & function:
HCV sequences of various genotypes originate from Ralf Bartenschlager (University of Heidelberg), NIH, Rockefeller, clinical isolates and chimeric sequences generated in-house by cloning from the above. Full length viral RNA containing the aforementioned HCV sequences will be generated by in vitro transcription. The RNA will be transfected into hepatocyte cell lines, usually by electroporation but could be by another transfection method (eg lipofection). The cells will produce infectious virus particles which will be harvested, concentrated by centrifugation and used to infect naive cells to further propagate the virus as described in Nature Methods 2, 565 (2005). These viruses will then be used in conventional virus assays to test for efficacy of anti-viral agents and to understand mechanism of action of these agents.

Evaluation of foreseeable effects:
The recombinant chimeric viruses produced will be designed to replicate efficiently in cell culture to produce titres of approximately 1 x 10^6 infectious particles per nil (as measured by fluorescence foci assay). HCV is classified as a ACDP3 pathogen and all the chimeric viruses produced would fall within the same risk category. The work will not affect the host cell tropism range but the viruses may be expected to have increased replication capacity in vitro. However, studies with chimeric HCV have shown that sequences which have adapted to be able to replicate with high efficiency in vitro culture do not show a similar increase in replication capacity in vivo in chimpanzees (Bukh et al 2002, PNAS)
The HCV core (C) protein and non-structural proteins, NS3 and NS5A, are potentially hepatocarcinogenic [reviewed in Hepatology research (2008) 38:1-26]. There are several reports detailing the oncogenic potential of NS3 [eg. World J. Gastroenterol (2003) 9 (3):474-478, 3. Virol. (95) 69, (6), 3893-96]. There is no known hazard for any of the remaining structural or non-structural genes. Any virus accidentally released into the environment is not likely to survive as HCV is restricted to replication in human and chimpanzee cells only.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquids contaminated with GMM will be treated with suitable disinfectant eg Virkon in accordance with the manufacturer's recommendations & left overnight. All other potentially contaminated materials will be autoclaved either at 134°C for at least 3 minutes or 121°C for at least 15 minutes. Autoclaves are monitored on each run and validated annually.

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

As this work will generate thil length viral RNA HCV, the GMSC considers that CL3 containment will be required, and this is therefore a Class 3 activity.

Project Containment

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Project Ref 558/08.3
**Assessing the binding and other properties of polyclonal and monoclonal antibodies to Pseudomonas aeruginosa OprM protein.**

The aim of this work is to show binding of polyclonal and monoclonal antibodies to bacteria including Pseudomonas aeruginosa strain PA01 and derivatives. The bacteria would be required to grow in culture in small volumes (up to 250ml). Cells would then be harvested and used in cell-based assays.

**Recipient or parental organism**

Pseudomonas aeruginosa PA01 and derivatives (\(\Delta\)mexAB-oprM, \(\Delta\)mex CD-oprJ, \(\Delta\)mexEFoprN), Genes deleted from part of the RND-type multidrug efflux pump family. Deletion of these genes is likely to make the host strain more susceptible to antibiotics. Various disables commercially purchased E.coli strains. These will be mainly K-12 derivatives (eg DH5a) and CHO-K1/HEK293 cells and derivatives eg DG44.

**Host/vector system**

Expression vectors typically RSV promoter driven. Resistance marker amipicillin, dihydrofolate reductase. All are non-mobilisable plasmids. Also, episomal expression vectors. CMV5 promoter driven. All are non-mobilisable plasmids.

**Origin & function**

Any genetic material used will originate from various disabled commercially purchased E.coli strains (in the case of DNA). These will be mainly K-12 derivatives (eg DH5a). Any protein material will originate from CHO-K1 and HEK293 cells and derivatives eg DG44 or HEK293 cells.

**Evaluation of foreseeable effects**

The aim of this work is to identify polyclonal and monoclonal antibodies to a Pseudomonas aeruginosa membrane protein. The Pseudomonas aeruginosa PA01 and derivatives strains (\(\Delta\)mexAB-oprM, \(\Delta\)mexCD-oprJ, \(\Delta\)mex EFoprN) used have genes deleted that form part of the RND-type multidrug efflux pump family. Deletion of these genes is likely to make the host strain more susceptible to antibodies and is not expected to increase the pathogenicity of the strains.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Autoclaving followed by on-site incineration.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

This work involves the use of modified Ps.aerguinosa, which is categorised as an ACDP hazard group 2 organism, containment level 2 controls must be applied to this work.

Project Containment

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<tr>
<td>L2</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 558/09.1

Date Ackn'd 06/05/2009

CU2 Project Title

Cloning of protein expression cassettes into self-inactivating human immunodeficiency virus-1 (HIV-1) vectors and their use to express specific genes in cultured mammalian cells and animal models for target validation.

Date Project Ceased

Class 2

Consent Granted

Class CultureVol Class CultureVolume

Class 2 1-50 Litres Non-GMM

02/03/2022 Page 9677 of 15326
### Project Additional Information

#### Purposes of the contained use

Open reading frames encoding potential target proteins will be ligated downstream of pol II promoters in a HIV-1 vector transfer plasmid. These will then be packaged into infectious self-inactivating HIV-1 vectors using a commercially available kit ("Block-it Lentiviral RNAi Expression System", Invitrogen Inc.) or similar, replication-defective self-inactivating lentiviral packaging systems and inoculated into cell cultures (primary cell types from human and rodent origin including neurons, microglia, monocytes, and macrophages as well as continuous cell lines such as Ramos and Jurkat), and/or administered to laboratory rodents (by the intravenous, intraspinal or intracranial routes) in order to express the genes of interest and validate them as potential drug targets.

#### Recipient or parental organism

The commercially available kit from Invitrogen is a third generation system based on HIV-1 (see Dull et al., 1998: J. Virol. 72, 8463-71) which allows the construction of a recombinant replication-incompetent, HIV-1-based lentivirus. There are a number of biosafety features which have been introduced to minimise risks. These are as follows:

1. All HIV sequences required for encapsidation and reverse transcription are absent.
2. There is a deletion in the 3' UTR resulting in self-inactivation of virus genome.
3. None of the HIV-1 structural genes contain LTRs and are thus never expressed in the target cell.
4. It contains the minimal number of HIV-1 genes required to generate an efficient vector (only three of the nine are used: gag, pol, and rev).
5. The removal genes are those which are pathogenic therefore no recombinant vector can acquire the pathogenetic features of the parental virus.
6. The tat gene, which is important for replication, has been removed.
7. The packaging genes are contained on 4 separate plasmids and thus the system relies on 4 separate plasmids for the production of transducing particles.
8. These plasmids contain no regions of homology thus preventing any recombination between plasmids.
9. The virus is replication incompetent and as a replication-deficient vector it poses significantly reduced risks. Thus these viruses may infect cells and integrate DNA into the target cell but they are incapable of further replication.

#### Host/vector system

293T cells will be transfected with a lentiviral expression vector (pLenti6, into which the gene of interest will be cloned) and the required packaging plasmids pLP1, pLP2 and pLP3 which supply the structural and replication proteins required for generation of lentiviral vectors.

#### Origin & function

Proteins representing potential drug targets will be over-expressed, i.e. they are naturally expressed in the rodent tissues to which the viral vectors will be administered. Although over-expression of target genes is not expected to have any toxic or oncogenic effects, the precise outcome is difficult to predict. The worse possible scenario for each target gene family is given below:

GPCRs have a role in cell signalling and typically have functions associated with smell sensing, feeding, behaviour, metabolism & homeostasis. Modulating expression of...
GPCRs could therefore be associated with changes in smell sensing, behaviour and the balance of salts, fats and carbohydrates within cells. This could potentially result in decreased cell viability and possibly cell death.

NRs have a role in cell signalling and typically have functions associated with homeostasis, inflammation & regulation of gene expression. Modulating expression of NRs could therefore be associated with changes in the balance of salts, fats and carbohydrates within cells. In addition, modulation of NR expression could lead to the stimulation of, or modulation of, inflammatory responses. This could potentially result in decreased cell viability and possibly cell death.

Ion Channels role is in the movement of ions across cell membranes, they typically have functions associated with neurotransmission and homeostasis. Modulating neurotransmission could thus lead to changes in sensory, motor or cognitive functions. Modulating homeostasis could lead to changes in salt balance which could, in turn, lead to a decreased cell viability and possibly cell death.

Proteases have a role in protein degradation and functional control they are typically associated with tissue remodelling. Modulating the expression of proteases could affect functions such as wound healing or lead to cell death due to the simulation of apoptosis.

Known oncogenes will not be expressed.

**Evaluation of foreseeable effects**

The viral vectors will be inoculated into primary human and rodent cells and continuous cell lines such as HEK293T and Neuro2a and the effects upon target gene expression evaluated. The parental viral vectors have been well characterised and the expression of the cDNA cassette by the transfected cells is not predicted to be harmful.

The viral vector is able to infect a wide range of different cell types, however, since it is replication defective it is unable to establish a productive infection and thus amplify itself in infected cells. Adverse effects following administration of lentivirus vectors have been reported only very rarely and are considered extremely unlikely following accidental exposure to these vectors at the titres to be used.

The viral vector only contains elements required for genome packaging, integration and transgene expression. Furthermore, natural elements that control virus expression have been modified to minimise homology with other retroviral sequences. These features not only prevent viral vector replication but also minimise the potential for the production of replication competent virus by homologous recombination with endogenous retroviral sequences. The viral vector is designed to bestow blasticidin-resistance upon infected cells, however blasticidin is not used for drug therapy in humans and thus would not compromise treatment.

Lentiviral vectors integrate into the host chromosomes and can therefore result in long-term expression. The precise effects of expression of the cDNA cassette when delivered to target cells are difficult to predict as their purpose is the overexpression of novel genes. Although in the worst case, overexpression may lead to cell death or an increase in cell proliferation although this would not be expected as manipulation of genes expected to lead to such a phenotype will not be generated. Any other adverse effects are extremely unlikely at the viral titres to be used.

Designed vectors might, on occasion, contain the Woodchuck Post-transcriptional Regulatory Element (WPRE). Vectors containing the WPRE have been widely used in the past, however one study has reported an association between the use of WPRE and tumour development. Thus the risk of this occurring with the use of our vectors cannot be completely ruled out. In addition, although over-expression of target proteins is not expected to have any toxic or oncogenic effects, the precise outcome is difficult to predict.

All staff generating and testing these vectors will be alerted to the potential oncogenic hazard associated with the use of WPRE-containing vectors. Host E.coli will not be propagated in volumes greater than 400 ml. HIV vectors will be generated in cell cultures of less than 1000ml volumes using CL2 procedures. Syringes will be loaded with viral vectors under CL2 conditions prior to administration to rodents. Volumes injected will be less than 250 ul per injection site. Total dose of viral vector will be between 10e9 and 10e10 transducing units per injection. Animals will be injected by staff trained in the administration of viral vectors to rodents, using standard techniques taking precautions to minimize the chances of inoculating personnel and paying particular attention to the safe disposal of needles. Where possible, animals will be restrained in stereotaxic frames prior to injection. Numbers of animals injected will be no greater than 20 per day. Following injection, the virus is fully contained within the animal. All in vivo work will be done according to specified containment measures.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N / A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N / A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated plasticware, glassware and cell media etc will be decontaminated using a 1% Virkon solution followed by incineration on-site.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Although this work involves the use of a disabled vector the involvement of the WRPE sequence and the need to take particular precautions to avoid shaps injuries the committee considers CL2 precautions are appropriate for this work.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2  Yes</td>
<td>L3</td>
<td>L2 L4</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

**Project Ref**  558/09.2

Date Ackn'd  18/05/2009  CU2 Project Title  The use of adeno-associated virus (AAV) to express specific genes in cultured mammalian cells and animal models

Date Project Ceased  02/03/2022  Class  2  CultureVolClass2  < 1 Litre  Consent Granted

Non-GMM  Consent Granted

Class CultureVolClass2 CultureVolumeClass3-4
**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

#### Purposes of the contained use

AAV vectors have great utility in providing the possibility high level sustained expression of recombinant proteins in a variety of tissues in vivo. The recombinant AAV vectors contain no AAV replication proteins, and so are entirely replication deficient, minimising any risk of transmission. The vectors covered in this proposal will be used to express a variety of recombinant gene products known to have low or no inherent toxicity oncogenicity or potential for harm. However, the genes may have the potential to produce a biological effect that may result in a reduction in the viability of the infected cells, so the precise outcome is difficult to predict. Known oncogenes will not be expressed.

#### Recipient or parental organism

E.coli, standard lab strains eg. DH5alpha, JM109, DH10Bac. No expression expected to arise from mammalian promoters in E. coli.

Mammalian cells (CHO K1, HEK 293) are established cell lines.

AAV is not associated with any disease in humans.

#### Host/vector system

AAV vectors have a long history of safe use. The recombinant vectors will be replication defective and are most unlikely to show altered tropism, pathogenicity or immune response. Designed vectors might, on occasion, contain the Woodchuck Post-transcriptional Regulatory Element (WPRE). Vectors containing the WPRE have been widely used in the past, however one study has reported an association between the use of WPRE and tumour development. Thus the risk of this occurring with the use of our vectors cannot be completely ruled out.

#### Origin & function

Many of the target proteins to be expressed using the AAV system are likely to have already been expressed in other mammalian expression systems based on plasmids or Baculoviruses. Genes selected for use will have no or low known toxicity, oncogenicity or potential for harm. Genes will be of human or animal origin, and may be isolated from tissues by PCR or assembled from synthetic oligonucleotides.

#### Evaluation of foreseeable effects

Recombinant AAV's have a long history of safe clinical use. The viral vector is able to infect a wide range of different cell types. However, since it is replication defective it is unable to establish a productive infection and thus amplify itself in infected cells.

The effect of the GMO is therefore most likely to be dependant on the effect of the expressed protein. Genes selected for use will have no or low known toxicity.
oncogenicity or potential for harm. However, the genes may have the potential to produce a biological effect that may result in a reduction in the viability of the infected cells so the precise outcome is difficult to predict. Known oncogenes will not be expressed.

One specific example, considered to be the greatest likely risk is the cytokine, IL-23.

IL-23 is a member of a small family of pro-inflammatory heterodimeric cytokines. The cytokine is naturally produced by activated dendritic cells and phagocytic cells. The receptors for IL-23 are expressed by T cells, natural killer cells and monocytes and DCs. IL-23 is believed to be a critical sign that drives the newly identified T-helper 17 pathway. This can lead to the recruitment of a range of inflammatory cells, in addition to Th17 T cells, and has been shown to be crucial to the pathogenesis of a number of immune-mediated inflammatory diseases (e.g., Psoriasis).

Murine models of chemical carcinogenesis using either IL-23 gene knockout animals or treated with anti-IL-23 neutralising mAbs demonstrate that ablation of the IL-23 gene product allows for a more robust adaptive immune response within the tumour - rendering a protective effect against the chemically induced tumour. An increase in IL-23 expression may be associated with an increased risk of development of cancers.

The work will involve inoculation of mice with recombinant non-replicating AAV and the subsequent handling of tissues from the animals. There is a risk of accidental needlestick injuries when carrying out these procedures. The use of a replication deficient AAV minimises the spread of the virus within an infected animal and minimises the likelihood of virus being secreted therefore reducing the risk of transmission to the laboratory worker.

The work will be carried out as follows:

Procedure for Immunisation with AAV.
Mice will be inoculated inside a class I microbiological safety cabinet for the containment of any aerosols. Virus will be administered by the intradermal, intraperitoneal or intravenous routes using a needle and syringe. Used needles and syringes will be disposed of in the appropriate sharps bin inside the class I cabinet. Any potential contamination of the operator via aerosols should be minimised by the wearing of eye protection, gloves, hat, mask. At the end of the immunisation procedure gloves, hat, mask should be disposed of in the appropriate waste bags.

Animal Containment
Infected animals are to be housed in cages.
All procedural work will be carried out using a class I microbiological safety cabinet.
The room used for housing mice is maintained under negative pressure relative to the immediate surroundings.
All waste (food, bedding carcasses) will be removed from the unit and incinerated.

Procedure for Isolation of Tissues
Tissue isolation will take place in a class I microbiological safety cabinet. The operator should wear the appropriate protective clothing as described above. Mice will be euthanased with an overdose of barbiturate drug (Sagatal). Sharps disposed of in the bin inside of the cabinet. Tissue samples will be removed into either:
(i) a plastic disposable tissue grinder, complete with plastic sheath and screw cap lid to minimise aerosol contamination. Or collected into fixative for histology. These can then be transported to the lab for subsequent processing.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated plasticware, glassware and cell media etc will be decontaminated using a 1% Virkon solution followed by incineration on-site. Animal waste is sent direct to

02/03/2022  Page 9682 of 15326
Although this work involves the use of a disabled vector the involvement of the WRPE sequence, the expression of cytokines and the need to take particular precautions to avoid sharps injuries the committee considers CL2 precautions to be appropriate for this work.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
</tr>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2</td>
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</table>

**Project Ref** 558/16.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Date Project Ceased</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/09/2016</td>
<td>Transduction of primary cells/generation of stable cell lines using lentiviral vectors containing wild type fragments of the Woodchuck Hepatitis Virus. Posttranscriptional regulatory region.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
<td></td>
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<table>
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<tr>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
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<tbody>
<tr>
<td>N</td>
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</table>

**Historical Significant Changes**

- 15/09/2016
- Transduction of primary cells/generation of stable cell lines using lentiviral vectors containing wild type fragments of the Woodchuck Hepatitis Virus. Posttranscriptional regulatory region.

<table>
<thead>
<tr>
<th>Project notified under transitional arrangements</th>
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<tbody>
<tr>
<td>N</td>
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</tbody>
</table>
### Project Additional Information

**Purposes of the contained use**

Use of lentiviral vectors containing wild type fragments of the Woodchuck Hepatitis Virus Posttranscriptional regulatory region.

**Recipient or parental organism**

HIV-1 based lentiviral vectors

**Host/vector system**

Lentiviral vector system

**Origin & function**

The WPRE region is included in lentiviral vectors to increase transgene expression by improving the processing of the 3' end of mRNA transcripts.

**Evaluation of foreseeable effects**

The wild type version of the WPRE element contains an open reading frame that codes for a C-terminal fragment of the Woodchuck hepatitis virus X protein. The C-terminal fragment has been indirectly linked with tumour formation, by a possible cooperation with ras and myc oncogenes (1,2); however, mice treated with LVs containing the wild type WPRE or a mutated version did not develop tumours (14).


**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be decontaminated using Virkon at 1% in a 12h exposure. Solid waste will be double-bagged and autoclaved before disposal. Sharps will be disposed of in sharp bins that will be autoclaved before disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Although the vector contains the WPRE sequence it is not capable of expressing the X protein of WHV and so Class 1 categorisation is consistent with the SACGM guidance on lentiviral vectors. March 2016

Change of classification due to using LV-GFP, since this vector has a wild type version of the WPRE element. Also because of the introduction of using Rapid Evaporative Ionization Mass Spectrometry which will create a small amount of aerosol.

Project Containment

<table>
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<tr>
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<td><strong>L2</strong> L3 L4 L2 L3 L4 L2 L3 L4</td>
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</table>

Animal Units

| L2 Yes L3 L4 L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 L2 L3 L4 |

Project Ref 558/16.2

Date Ackn'd 28/12/2016

CU2 Project Title Generation of lentivirus coding for an oncogene or cytokine

Class 2

CultureVolClass2 1-50 Litres

Consent Granted

Project notified under transitional arrangements N

02/03/2022
## Project Additional Information

### Purposes of the contained use

Use of Lentiviral vectors with the WPRE region coding for Oncogene or Cytokine.

### Recipient or parental organism

E.Coli, CHO, Primary Cells

### Host/vector system

Lentivirus Vectors.

### Origin & function

The WPRE region is included in the lentiviral vectors to increase transgene expression by improving the process of the 3'end of the mRNA transcripts.

### Evaluation of foreseeable effects

High likelihood of harm arising from GMM given the delivery is of an oncogene known to be involved in carcinogenesis, further exacerbated by the use of a delivery vector that will integrate into genomes and the use of a pseudotype that enables transduction of a wide variety of cell types.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This is not applicable to this notification.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Autoclaved waste destined for incineration will be transported off site by a registered and competent waste contractor. The Waste will be appropriately contained and labelled up for transportation to an incinerator.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be decontaminated using Virkon at 1 % in a 12h exposure. Solid waste will be double-bagged and autoclaved before disposal. Sharps will be disposed of in sharp bins that will be autoclaved before disposal.

---

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N
The risk assessment attached has been reviewed by the GMSC and it has been agreed that this risk assessment should be notified to the HSE as a class 2 GMO.

### Project Containment

<table>
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<th>Laboratory Activities</th>
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<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units L2 L3 L4 L2</td>
<td>Large Scale Activities L3 L4 L2</td>
<td>Human Clinical Applications L3 L4</td>
</tr>
</tbody>
</table>

### Project Ref 558/16.3

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>Culture Vol</th>
<th>Culture Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>28/12/2016</td>
<td>Production, storage use and distribution of lentiviral vector based genome engineering tools utilising CRISPR Cas9 system</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Class 3-4</td>
</tr>
</tbody>
</table>

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Withdrawn

Tick if notifying a connected programme of work

Project Additional Information
<table>
<thead>
<tr>
<th><strong>Purposes of the contained use</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of Lentiviral vectors with the WPRE region. There is a risk of expression of genes with oncogenic potential.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Recipient or parental organism</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>E.Coli, HEK cells</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Host/vector system</strong></th>
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</thead>
<tbody>
<tr>
<td>Lentivirus Vectors.</td>
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</table>

<table>
<thead>
<tr>
<th><strong>Origin &amp; function</strong></th>
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<tr>
<td>The WPRE region is included in the lentiviral vectors to increase transgene expression by improving the process of the 3'end of the mRNA transcripts.</td>
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<tr>
<th><strong>Evaluation of foreseeable effects</strong></th>
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<tbody>
<tr>
<td>The knock out genes could have oncogenic potential. The off target effect of CAS 9 could affect the expression of genes with oncogenic potential. This will be reduced by avoiding concentration of viral supernatants when known tumor suppressor genes are targeted as single gene targets.</td>
</tr>
</tbody>
</table>

<table>
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<th><strong>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</strong></th>
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<th><strong>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</strong></th>
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<td>Autoclaved waste destined for incineration will be transported off site by a registered and competent waste contractor. The Waste will be appropriately contained and labelled up for transportation to an incinerator.</td>
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<th><strong>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</strong></th>
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<tr>
<td>All liquid waste will be decontaminated using Virkon at 1% in a 12h exposure. Solid waste will be collected in an autoclave bag and autoclaved on site before sending for incineration.</td>
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<tr>
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<tbody>
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<tr>
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<th><strong>Tick if you are claiming exemption from disclosure for section of the risk assessment</strong></th>
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<th><strong>Please enter comments on the GM safety committee on the risk assessment</strong></th>
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<td>The risk assessment attached has been reviewed by the GMSC and it has been agreed that this risk assessment should be notified to the HSE as a class 2 GMO</td>
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</table>
Project Containment

Laboratory Activities  Glass Houses  Growth Rooms
L2  Yes  L3  L4  L2  L3  L4  L2  L3  L4
Animal Units  Large Scale Activities  Human Clinical Applications
L2  L3  L4  L2  L3  L4  L2  L3  L4

Project Ref  558/17.1

Date Ackn'd  06/04/2017
CU2 Project Title  Generation of lentivirus coding for an immune checkpoint or growth factor inhibitor

Class  CultureVolClass2  CultureVolumeClass3-4
Class 2  1-50 Litres

Non-GMM  Consent Granted

Tick if notifying a connected programme of work  N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
High likelihood of harm arising from GMO could potentiate an immune response.

Recipient or parental organism
E. Coli and Continuous Cell Lines

Host/vector system
**Lentiviral Vectors**

**Origin & function**
Viral vectors produced in Continuous Cell lines or sourced from a commercial supplier. Lentivirus coding for an immune checkpoint or growth factor inhibitor.

**Evaluation of foreseeable effects**
High likelihood of harm arising from GMM could potentiate an immune response. This is further exacerbated by the use of a delivery vector that will integrate into genomes and the use of a pseudotype that enables transduction of a wide variety of cell types.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not relevant to this application

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste to be autoclaved prior to incineration. Liquid waste to be treated with 1% Virkon disinfectant to inactivate lentivirus.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment
This risk assessment has been reviewed by the GMSC and it has been decided this risk assessment falls into the Class 2 controls and therefore will need to be notified to the HSE.

## Project Containment

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>

02/03/2022
Cloning of protein expressing cassettes into human immunodeficiency virus -1 (HIV-1) lentiviral vectors containing the endogenous long terminal repeat (LTR) and their use to express specific genes in cultured mammalian cells and subsequent to transduction with lentiviral vectors.

Recipient or parental organism

E.Coli, CHO, HEK, Jurkat cells, Hepatocytes, Primary Cells

Host/vector system

Lentiviral vectors

Origin & function

Peripheral blood mononuclear cells (PBMCs) will be isolated from peripheral blood (obtained from commercial supplier) and activated with TransAct beads (obtained from a commercial supplier) prior to transduction with LTR and SIN GFP encoding lentiviral vectors, or CAR encoding LTRISIN lentiviral vectors.

The chimeric antigen receptor single chain variable fragment (scFv) sequences will be taken from monoclonal antibodies specific for targets including Human Epidermal Growth Factor 2 (HER2/ErbB2), epidermal growth factor receptor variant III (EGFRvIII) and Carcinoembryonic antigen (CEA) - all of which are antigens commonly expressed.
CAR constructs will be designed to include C028 or 4-1 BB costimulatory domains and different spacer domains (i.e. C08 spacer, C04 spacer or TNFR2 spacer) - to enable determination of whether different CAR constructs components can lead to increased risk of insertional mutagenesis. In addition to this, the phosphoglycerate kinase (pGK) promoter commonly used within third generation (SIN) lentiviral vectors may be exchanged for the stronger Spleen Focus-Forming virus (SFFV) promoter within future experiments. The use of this stronger promoter within SIN third generation lentiviral vectors may increase the cis activation and genotoxicity of the viral vector insertion, without increasing the risk of recombination with wild-type HIV-1 virus. This may provide an improved positive control for genotoxicity, compared to 2nd generation L TR lentiviral vectors, by increasing genotoxicity whilst maintaining the SIN lentiviral vector format.

Evaluation of foreseeable effects

If the second generation L TR containing lentiviral vectors were to come into contact with individuals or cells infected with a wild type HIV-1 infection, there would be an increased risk of recombination with wildtype HIV-1 viruses and the formation of replication competent lentiviral vectors compared to when using third generation SIN lentiviral vectors. However, the risk of recombination of second generation lentiviruses with wildtype HIV-1 viruses remains low due to the low homology that the lentiviral vectors have with endogenous HIV-1 viruses. This risk can be further reduced by ensuring that all blood donors are screened for HIV-1 infection. In addition to this, extra pre-cautions would be taken if a lab user suffers from a HIV-1 infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This is not applicable to this notification.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste, such as media taken from transduced cells, will be inactivated with 1 % virkon solution for 24 hours prior to pouring down the sink with plenty of water. Autoclaved waste destined for incineration will be transported off site by a registered and competent waste contractor. The Waste will be appropriately contained and labelled up for transportation to an incinerator.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The risk assessment attached has been reviewed by the GMSC and it has been agreed that this risk assessment should be notified to the HSE as a class 2 GMO.
Project Title: Conditionally self-inactivating lentiviral vector CRISPR-sgRNA libraries

Class 2

Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Purposes of the contained use

Conditionally self-inactivating lentiviral vector CRISPR-sgRNA libraries

Recipient or parental organism

E.coli K12 derivatives

Host/vector system
Lentiviral Vectors

Origin & function

Lentiviral Vector - sourced from an external supplier

CRISPR Cas9 from Streptococcus Pyrogenes, function - Cas9 introduces a double strand DNA break in cells, targeted by sgRNA, dCas9-VP64 and MS2-P65-HSF1 activate transcription of promoters adjacent to genome location targeted by sgRNA

Evaluation of foreseeable effects

According to the American Biological Safety Association, "the two major risks of lentiviral vectors are: a) the potential generation of replication competent virus; and b) the potential for oncogenesis through insertional mutagenesis."

a) Due to the usage of 3rd generation packaging systems, the lentiviral particles that are produced are replicationdeficient and the virus cannot propagate itself.
b) Following accidental exposure with lentiviral particles, it cannot be excluded that the lentivirus could integrate randomly into the host's genome, and this could increase the risk for oncogenesis when regulatory genes or regions are targeted.

The CRISPR/Cas9 knockout and gene activation libraries have the potential to knock out or activate transcription of all human genes through either on- or off-target activity. If introduced into an operator's cells, knockout or activation of tumour suppressor genes or proto-oncogenes could lead to oncogenesis. Disruption of genes involved in innate or humoral immunity could lead to dysregulation of the immune system. The risk of these events during this work is expected to be extremely low as expressing Cas9 or Cas9 derivatives and sgRNA from separate lentiviral transfer vectors means that vectors encoding Cas9 protein and sgRNA must both enter the same cell to be functionally active against the DNA target site. If target cells are transduced with a Cas9 lentiviral vector and a separate sgRNA lentiviral vector at different timepoints (e.g. 2 weeks apart) this further reduces the risk that both vectors could enter the same cell and carry out gene editing as two separate operator exposures resulting in transduction of the same cell(s) would need to occur.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not relevant to this application.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be decontaminated by using a 1 % Virkon solution, allowing over night decontamination before disposal.
All plastic ware and gloves are collected in an autoclavable plastic bag and autoclaved on site before incineration.
Surface decontamination will be with 1 % Virkon solution.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N
The general opinion from the GMSC is that the assessment being submitted is a class 2 GMO and as such requires notification to the HSE.

Please enter comments on the GM safety committee on the risk assessment

The general opinion from the GMSC is that the assessment being submitted is a class 2 GMO and as such requires notification to the HSE.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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</tr>
</thead>
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<tr>
<td>L2 Yes</td>
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<td>L4</td>
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Animal Units

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</thead>
<tbody>
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</table>

Project Ref 558/19.1

Date Ackn'd: 10/01/2019

CU2 Project Title: Infection of cultured human cells with mutant strains of Salmonella enterica serovar Typhimurium lacking the prgJ, fltC and fljB genes

Class: Class 2

Culture Vol: < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Date of Significant Change

Project Additional Information
### Purposes of the contained use

Use of *Salmonella enteritica* serovar *typhimurium* Organisms with knock out genes which are still capable of replicating and potentially causing infection.

### Recipient or parental organism

*Salmonella enteritica* serovar *typhimurium*

### Host/vector system

*Salmonella enteritica* serovar *typhimurium*

### Origin & function

The *LlfIC L’fIjB, L’prgJ* and *L’fIiC L’fIjB L’prgJ* strains described in this document carry mutations that result in a degree of attenuation in mouse models of colitis, characterised mainly by reduced intestinal inflammation.

### Evaluation of foreseeable effects

The wild-type *S. Typhimurium* parent strain of these mutants is a Biosafety level 2 organism that can cause human disease, in particular, gastroenteritis. This is usually characterised by vomiting, abdominal cramps, headache, chills and fever up to 39°C. The document 'Standard Risk Assessment for Work with *Salmonella enterica* serovar *Typhimurium'* should be consulted for details on how to work safely with this strain. Details of these controls can be found below:

**Engineering Controls:**
- Work is conducted in laboratories designed to BSL 2 standards. Class 2 Ducted Microbiological Safety Cabinets are used for work which may generate infectious aerosols.
- Sealed centrifuge buckets or rotors are used to contain aerosols during centrifugation of infectious material.

**Administrative Controls:**
- Access to the laboratory is restricted to authorised persons.
- Work is carried out only by suitably trained persons and is conducted following the principles of good microbiological practice and occupational hygiene as described in the Local R&D Biosafety Manual and regulatory guidance for work at BSL 2. The work area will be thoroughly cleaned following completion of the work, and all surfaces disinfected with 70% ethanol or a 1:1 00 Virkon solution to prevent cross contamination.

**Personal Protective Equipment:**
- Laboratory coats and safety glasses are mandatory. Appropriate gloves (from local glove selection guide) are worn when handling hazardous material.

**Signage:**
- A Hazard Card system will be used during all work with *S. Typhimurium* and will clearly display the nature of the hazards to any nearby individuals.

The *b.fIiC b.fIjB, b.prgJ* and *b.fIiC b.fIjB b.prgJ* strains described in this document carry mutations that result in a degree of attenuation in mouse models of colitis, characterised mainly by reduced intestinal inflammation. Although none of these strains have been tested in humans, extrapolation from the mouse data suggests that although they may be partially attenuated, they are still likely to colonise human hosts and have the potential to cause some degree of disease. It is highly likely that they would elicit less inflammation and pathology than the wild-type parent, SL 1344, but they should still be considered virulent.

---

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be decontaminated by using a 1% Virkon solution, allowing overnight decontamination before disposal.
All plastic ware and gloves are collected in an autoclavable plastic bag and autoclaved on site before incineration.
Surface decontamination will be with 1% Virkon solution.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The general opinion from the GMSC is that the assessment being submitted is a class 2 GMO and as such requires a notification to the HSE.

Project Containment

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Date Ackn'd: 31/01/2019
CU2 Project Title: Use of KILR® Retroparticles as a vehicle for the stable delivery of the KILR reporter construct into human cell lines

Class: Class 2
Culture Volume Class 2: < 1 Litre
Non-GMM: Consent Granted

02/03/2022
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

1 Litre

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Spillages will be inactivated and killed by 1 % Virkon and waste will be inactivated and killed with 1 % Virkon solution prior to disposal down the sink with plenty of water. Solid consumable waste contaminated with particles will be double-bagged to prevent piercing of the bag by pipette tips for example, and autoclaved.
The consensus of the GMSC is that although this GMO risk assessment could be considered to be on the border line it would be beneficial to air on the side of caution especially as it can not be ruled out what the house keeping gene is. Therefore this risk assessment is being notified to the HSE.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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</table>

Project Ref 558/19.3

Date Ackn'd 28/02/2019
CU2 Project Title Expression of BMI-1 in mammalian expression systems
Date Project Ceased

Class 2 CultureVol 1-50 Litres Consent Granted
Non-GMM

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID

Tick if notifying a connected programme of work
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Plasticware will be disposed of via incineration after autoclaving. Liquids will be autoclaved under appropriate conditions for inactivation of virus

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

The GMSC agree that the comments on the Risk Assessment have been addressed and the notification can be sent to the HSE.
Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 Yes | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4

Project Ref 558/19.4

Date Ackn'd 28/03/2019
Date Project Ceased

CU2 Project Title
Development of mobilizable transfer elements supporting lentiviral vector manufacturing developability

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Recipient or parental organism
Host/vector system
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable for this application.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be decontaminated by using a 1 % Virkon solution, allowing overnight decontamination before disposal.
All plastic ware and gloves are collected in an autoclavable plastic bag and autoclaved on site before incineration.
Surface decontamination will be with 1 % Virkon solution.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC met to discuss this risk assessment and felt the risk assessment had been well written. It was requested that there was a little more clarity added around the cleaning between experiments. Add in a reference to the SACGM guidance. Please provide a little more clarity on the use of 2nd generation L TR and 3rd generation packing components. It was agreed by the GMSC this was a class 2 GMO risk assessment and therefore requires a notification to the HSE.

Project Containment

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Animal Units          | Large Scale Activities | Human Clinical Applications

**Project Ref 558/20.1**

**Use of a replication defective Adenovirus Type 5 viral vector (AdV5) expressing cytokine gene for generation of appropriate translational disease models**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
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<td>Use of a replication defective Adenovirus Type 5 viral vector (AdV5) expressing cytokine gene for generation of appropriate translational disease models</td>
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Project notified under transitional arrangements N

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste to be decontaminated with approved disinfectant decontaminant prior to disposal via appropriate waste streams.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The consensus of the GMSC is that although this GMO risk assessment could be considered to be on the border line it would be beneficial to air on the side of caution especially as it can not be ruled out what the house keeping gene is. Therefore this risk assessment is being notified to the HSE.

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Animal Units

<table>
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<tr>
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<th>Human Clinical Applications</th>
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<tbody>
<tr>
<td>L2 Yes</td>
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Project Ref 558/95.1

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Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**

**If yes, tick to confirm that it is attached to this form**

**Tick to confirm that you have attached a risk assessment to this form**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
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**Laboratory Activities**
- Glass Houses
- Growth Rooms

**Historical Significant Changes**

**Project Ref** 558/95.2B

<table>
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**Class**
- Class 2

**Culture**
- CultureVol

**Class Volume**
- Class 2 Culture Volume Class 3-4

Non-GMM
- Consent Granted
  - not applicable

Project notified under transitional arrangements
- Y

Withdrawn
- N

Tick if notifying a connected programme of work
- N

Project notified under transitional arrangements
- Y

**Project Additional Information**

Purposes of the contained use
Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
USE OF BIOLUMINESCENCE TO DETERMINE INTRACELLULAR VIABILITY IN MYCOBACTERIUM MICROTI

 containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 558/97.1

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<td>CONSTRUCTION OF FUCOSYLTRANSFERASE KNOCKOUT STRAINS OF HELICOBACTER PYLORI</td>
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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N
Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  558/97.2

Date Ackn'd  30/06/1997

CU2 Project Title  USE OF RECOMBINANT SINBIS VIRUS EXPRESSING THE HEPATITAS C VIRUS NON-STRUCTURAL (NS) 3/4A PROTEASE AND A PROTEASE SUBSTRATE SEQUENCE WITHIN THE GENOME CODING REGION

Class  Class 2

Consent Granted  not applicable

Project notified under transitional arrangements  Y

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

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Please enter comments on the GM safety committee on the risk assessment

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Project Ref 558/98.3

Date Ackn'd 21/03/1998

CU2 Project Title IMMORTILISATION OF PRIMARY HUMAN FIBROBLASTS USING HUMAN PAPILLOMA VIRUS GENES E6 AND E7

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

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Project Ref  558/98.8

Date Ackn'd  07/09/1998

Date Project Ceased

CU2 Project Title  CLONING & EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTORS (VEGFS) & TRANSCRIPTION FACTORS THAT INDUCE VEGFS IN ADENOVIRAL VECTORS

Class  Class 2

Consent Granted  not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Withdrawn N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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### Project Ref 558/99.1

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**Historical Significant Changes**

GM558/01.3, GM558/01.2, GM558/00.3, GM558/00.1, GM558/99.2,

**Historical Date of Additional Info**

01/02/2001, 01/02/2001,

28/01/2000, 28/01/2000, 08/02/1999

**Date Ackn'd**

**Date Project Ceased**

**Project notified under transitional arrangements**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

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02/03/2022
IMPLEMENTATION OF A TRANSIENT TRANSFECTION ASSAY FOR INVESTIGATING CYTOCHROME P450 3A INDUCTION

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**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
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Animal Units
Large Scale Activities
Human Clinical Applications

Project Ref 558/99.8

Date Ackn'd 02/06/1999

Date Project Ceased

Withdrawn

Tick if notifying a connected programme of work

Consent Granted

Project notified under transitional arrangements
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

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Project Ref 558/99.9

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Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

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Project Ref 798/02.1

Date Ackn'd 22/06/2007

CU2 Project Title

GENERATION, EXPRESSION AND PURIFICATION OF HUMAN TUMOUR NECROSIS FACTOR ALPHA (TNF-ALPHA) IN E.COLI

Date Project Ceased

Consent Granted

Non-GMM

Consent Granted

Project notified under transitional arrangements ❏

Tick if notifying a connected programme of work ❏

Withdrawn ❏
Project Additional Information

Purposes of the contained use

The purpose of the contained use is to generate recombinant human TNF-alpha for purification and subsequent use as reagent in in vitro assays.

Recipient or parental organism

E. coli JM105 (thi, rpsL(Str), endA, sbcB15, hsdR4, supE_(lac-proAB), f'[traD36,proAB+, lacI, LacZ_M15]), supplied by Amersham Biotech, Amersham, UK).

E.coli NovaBlue (endA1, hsdR17(rk12-,Mk12+), supE44, thi-1, recA1, gyrA96, relA1, lac[f'proA+B+, lacQZ_M15_Tn10(tet)], supplied by Novagen, Madison, USA).

E.coli XL1 blue (recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [FcpROAB lacIq ZDM15 Tn10 (Tet)] (Stratagene).

The host E coli strains NovaBlue, XL1blue and JM105 are E. coli K12 derivatives and have a long history of safe use. They are non-colonising and multiple disabled. In terms of environmental safety, the host strains are multiply disabled, and are unlikely to survive in the environment, and considered non-pathogenic to humans or animals.

Host/vector system

The plasmids are well characterised, non-mobilisable, derivatives of commercially supplied plasmids with an established history of safe laboratory use.

Origin & function

The cDNA encoding human TNF-alpha will be obtained from the Medical Research Council Laboratory of Molecular Biology, in either the native human form, or the E. coli codeon optimised form. Native TNF is expressed in the human as an inactive membrane-bound 233 amino acid precursor, which becomes active upon cleavage between residues 76-77. We will clone the truncated cDNA, encoding the active portion of the molecule in-frame with the N-terminal epitope tags His6 and HA or FLAG. Induction of expression with IPTG (isopropylthiogalactoside) will result in the production of a soluble TNF-alpha fusion protein, which is likely to be biologically active. This fusion protein will be affinity purified on nickel based resin, and eluted with imidazole to produce epitope-tagged TNF-alpha, which may be further purified.

The intended function is therefore to produce active soluble TNF-alpha.

Evaluation of foreseeable effects

TNF-alpha is a pro-inflammatory cytokine, produced mainly by activated monocytes and macrophages, and appears to be a key mediator in the induction of cytokine cascades associated with inflammatory responses. Recombinant human TNF-alpha has been used in a number of clinical trials as an anti-cancer therapy with intravenous doses of up to 300mg being tolerated (eg F Lejeune et al (1994) Journal of Cellular Biochemistry 56:52-61).
The most likely effect of accidental ingestion or injection of recombinant TNF-alpha would be localised inflammation and pyrexia. It is extremely unlikely that a person could be exposed to TNF-alpha levels exceeding the levels used clinically through the experimental work above. Even if all native gut flora were replaced with E. coli harbouring the plasmid encoding the TNF-alpha fusion protein, in the absence of induction protein expression levels are low, and unlikely to exceed this level. There is also a small risk of anaphylaxis, although this risk is common to many proteins, and the Class 2 precautions that this work will be done under will reduce this risk to effectively zero.

The planned experiments utilise disabled host strains that are unlikely to survive in the environment. In terms of environmental safety, the host strains are multiply disabled, and are unable to survive in the environment, and considered non-pathogenic to humans or animals. The expression of a pro-inflammatory cytokine is unlikely to enhance the pathogenic potential of the final GMO, and may decrease any such potential, by inducing a more potent immune response.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

*not applicable*

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

*No containment derogations are being applied for.*

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid waste contaminated by GMMs and bacterial cell pellets to be disposed of by autoclaving at a load temperature of 121 degrees C for 20 minutes. This disposal route is considered to give essentially 100% kill of E. coli. The site autoclave is routinely tested for temperature and pressure attainment, and verification of waste inactivation.

All GMM liquid wastes to be inactivated by addition of Virkon to >0.25% final concentration, allowed to stand for 30 min and then disposed of down sinks, with additional running water to dilute the waste.

Contaminated re-useable glass and plastic ware will be similarly disinfected with 0.25% Virkon prior to washing. Virkon has been shown to give >6 logs of kill of E. coli under these conditions (source: Antec International). This will be verified by streaking of a sample of the inactivated fluid onto Luria agar plates, which should show no growth if inactivation is effective.

These procedures will reduce any risk to humans or the environment to effectively zero.

**Is an emergency plan required according to regulation 20?**

*No*

**If yes, tick to confirm that it is attached to this form**

*No*

**Tick to confirm that you have attached a risk assessment to this form**

*Yes*

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

*No*

**Please enter comments on the GM safety committee on the risk assessment**

The application was considered by the GMSC to be at class 2. The host/vector system used is of minimal hazard, but hearing in mind the potential biological activity of the cytokine produced, class 2 working was considered appropriate to contain all potential hazards.

**Project Containment**
**Project Ref:** 798/05.1

**Date Ackn'd:** 22/06/2007

**CU2 Project Title:** Expression of Peptostreptococcus magnus Protein L domains B1-4 in E. coli under shake flask and fermentation conditions.

**Class:** Class 2  
**CultureVolClass:** 100-1000 mL  
**Consent Granted:** Not Applicable

**Recipient or parental organism:** RV308 ( (lac)X74 galPO-308::IS2 rpsL). Source ATCC. An E. coli K12 derivative, non-colonising.

**Host/vector system:** pAL15M. Kanamycin resistant, toluic acid inducible expression of insert protein. Mobilisable, OriV and OriT wild type from RK2 are present. Expression is induced in this system using the specific inducer toluic acid. Expression levels in the absence of inducer are low.

---

**Purposes of the contained use:**

Protein L is a cell wall protein of Peptostreptococcus magnus. It has a multiple domain structure, consisting of an A domain, and five B domains, termed B1-B5 which are homologous IgG binding domains. The remainder of the protein anchors it to the peptostreptococcal cell wall.
Origin & function

Insert DNA originally from Peptostreptococcus magnus. Contains the B1-B4 domains of protein L, giving a high affinity IgG binding protein. This will be produced as soluble functional intracellular protein once the culture has been induced with toluic acid.

Evaluation of foreseeable effects

The full length protein is a bacterial superantigen. Expression of the protein in Peptostreptococcus sp. Correlates with virulence. Expression as a cytoplasmic protein would not be predicted based on the available evidence to make the E. coli pathogenic, as the proteins role in pathogenesis correlates with cell surface expression in the wild-type organism.

The B1-B4 domain shown here will also act as a superantigen, and has been shown to stimulate B cell proliferation in a polyclonal fashion, and to cause depletion of subsets of B-cells via apoptosis. Thus the protein may be expected to have immunological effects.


The protein has also been shown to trigger histamine and other cytokine release from mast cells and basophils via cross linking to surface IgEs. Therefore it has the potential to cause anaphylaxis on inhalation or absorption.


The protein may be a sensitiser, and thus skin contact or inhalation must be avoided.

The use of mobilisation competent vector makes transfer to related species more likely. However, expression from a mobilisation defective vector has been shown to be lower, therefore requiring larger culture volumes and increased risks (Affitech data not shown).

Various Peptostreptococci are already found in the environment, some of which express Protein L as a superantigen. Therefore, with the containment used within the lab, together with the inactivation methods used, makes the risk of any additional environmental hazards effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Spills will be disinfected with solid Virkon powder. This has been tested by plating experiments on shake flask cultures and low cell density fermentation to give essentially
100% inactivation of organisms in split media. Fermenters will be sterilised post fermentation at 121 degrees C for 60 minutes. This has been shown to give 100% inactivation of organisms. Liquid waste and spent culture medium will be sterilised by autoclaving at 121 degrees C for 60 minutes. This has been shown to give 100% inactivation of viable organisms. Sterile liquid waste will then be offsite incinerated. Centrifuge bottles and solid wastes will be sterilised by autoclaving as above. This has been shown to give 100% inactivation of viable organism. Solid waste will then be offsite incinerated with the clinical waste stream. Re-usable lab items will be disinfected in >2% Virkon for >30 minutes. This has been demonstrated to give essentially 100% kill of viable organisms. They will then be washed. Additional testing of inactivation will be performed, where necessary, by streaking samples onto 2xTY plus kanamycin plates. All items to be cleaned will be kept wet to avoid the production of dry material containing Protein L with dust hazard potential and cleaned promptly.

The application of the expression of Protein L using the Affitech HCD fermentation protocol was approved at class 2 as DM38, and will be notified to the HSE. Emphasis must be placed on the potential for sensitisation and anaphylaxis for the protein, under COSHH. All work with this system must be covered by SOPs for exposure control and cleanup. These will be drawn up and approved before work can start.

Project Containment

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Please enter comments on the GM safety committee on the risk assessment

The application of the expression of Protein L using the Affitech HCD fermentation protocol was approved at class 2 as DM38, and will be notified to the HSE. Emphasis must be placed on the potential for sensitisation and anaphylaxis for the protein, under COSHH. All work with this system must be covered by SOPs for exposure control and cleanup. These will be drawn up and approved before work can start.

Project Ref 798/06.1

<table>
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<td>22/06/2007</td>
<td>Expression of a human cytokine genetically fused to a protein moiety capable of</td>
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extending serum half-life in E. coli and P. pastoris.

E. coli will be transformed with an expression construct with the intention of maximising expression levels of the fused protein. It has been shown that periplasmic/soluble expression of this cytokine in E. coli results in active protein. In vivo, the protein is known to affect cell growth and development and in clinical trials has been shown to cause a range of effects, both local and systemic. The Domantis GMSC considered the possibility of deleterious effects due to this GMO warranted classification to CL2.

Likewise P. pastoris will, in all probability, secrete active protein. It is considered that the recipient organism in this instance is less liable to colonise or persist in the environment, and this work is therefore classed at CL1.

E. coli stains based upon the genetic backgrounds K12 (TOP10f', JM83, HB2151), W (Mach I) and B (BL21) will be used for cloning and expression work. Vectors used will be based upon the commercially available vectors pET21 and pET23, and the Domantis vector pDOM5. The pET vectors contain the T7 promoter under IPTG control; pDOM5 contains the lac promoter under IPTG control. None of the vectors are considered mobilizable.

The cDNA for cytokine will be created synthetically under contract. The genetic material for the half-life extending moiety derives from Domantis phage libraries. In these constructs this functionality will be inactivated.

The expression constructs generated will be used to direct the production of a recombinant fusion protein, different strategies will be employed based upon refolded inclusion bodies as well as soluble secreted protein. It has been assessed that the fusion expressed in inclusion bodies results in a less hazardous GMM that can be manipulated under CL1. Soluble material will also be produced in mammalian cell culture. Due to the nature of this expression system, it is also considered to be suitable for manipulation at CL1, although for sample protection it will be handled at CL2.

The cytokine is a potent growth factor, with a pleiotropic range of biological effects. Two distinct receptors exist though the specific roles of these distinct molecules has yet to be fully elucidated. The cytokine has been shown to be mitogenic for certain cancer types including breast cancer, and mutations in one of the receptors are associated...
with a range of adverse effects in affected individuals. The production of a fully functional cytokine from genetically modified E. coli has the potential to, in a worst-case setting, expose workers to the effects of the cytokine, which are reportedly related to various types of pain. The formatting with a half-life extending moiety has the likely effect of extending the residence time in the circulation of anyone that may become exposed to the cytokine. This will be unlikely to occur in these compounds where the function will be inactivated; however the larger molecule may still have some minor half-life extension. Although E. coli strains being used are disabled, it is the recommendation of the GMSC that additional containment in line with CL2 regulations are applied for this work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste contaminated by GMMs and bacterial cell pellets to be disposed of by autoclaving at a load temperature of 121°C for 20 minutes. This disposal route is considered to give essentially 100% kill of E.coli. The site autoclave is routinely tested for temperature and pressure attainment, and verification of waste inactivation. All GMM liquid wastes to be inactivated by addition of Virkon to >0.5% final concentration & allowed to stand for 30 min. Waste collected in autoclavable carboys and autoclaved prior to disposal via sink with copious amounts of water. Contaminated re-useable glass and plastic ware will be similarly disinfected with 0.5% Virkon prior to washing. Virkon has been shown to give >6 logs of kill of E.coli under these conditions (source: Antec International). This will be verified by streaking of a sample of the inactivated fluid onto Luria agar plates, which should show no growth if inactivation is effective. These procedures will reduce any risk to humans or the environment to effectively zero.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The application was considered by the GMSC to be at class 2. The host/vector system used was considered to be of low hazard, but attention was paid to the potential biological activity of the cytokine produced and the likely extended half-life in the event of serum exposure. Therefore class 2 working was considered appropriate to contain all potential hazards.

Project Containment

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<th>Laboratory Activities</th>
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02/03/2022
Project Ref 90/93.1

Date Ackn’d 25/11/1993

CU2 Project Title INVESTIGATION OF THE IN VIVO RELEVANCE OF THE HERPES SIMPLEX RIBONUCLEOTIDE REDUCTASE GENE

Class 2

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Ref** 90/93.3

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<td>12/08/1993</td>
<td>INVESTIGATION OF THE POTENTIAL OF BETA-GALACTOSIDASE EXPRESS HERPES SIMPLEX VIRUS (RH1 16) IN ANIMAL MODELS</td>
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Tick if notifying a connected programme of work

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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### Project Ref 90/93.4

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<td>GENERATION OF MUTANT HUMAN CYTOMEGALOVIRUS (HCMV)</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

### Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
Project Ref 90/94.1

Date Ackn’d 16/06/1994

CU2 Project Title EXPRESSION OF HUMAN BRADYKININ RECEPTOR IN MAMMALIAN CELLS USING SEMLIKI FOREST VIRUS (SFV)

Class 2

CultureVolClass2 ClassCultureVolumeClass3-4

Non-GMM not applicable

Consent Granted

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form
N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
N

Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 90/94.2

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Withdrawn  N

Tick if notifying a connected programme of work  N
Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref** 90/94.3

- **Date Ackn'd**: 20/07/1994
- **CU2 Project Title**: EXPRESSION OF HUMAN 5HT (SEROTONIN) RECEPTOR GENES IN MAMMALIAN CELLS USING SEMLIKI FOREST VIRUS (SFV)
- **Class**: Class 2
- **CultureVolClass2**: not applicable
- **CultureVolumeClass3-4**: not applicable
- **Non-GMM Consent Granted**: not applicable
- **Project notified under transitional arrangements**: Y

**Historical Significant Changes**

- GM90/95.2, GM90/96.1, GM90/96.2, GM90/96.3, GM558/96.4

**Historical Date of Additional Info**


**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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**Project Ref**: 90/trans1

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<tr>
<td>15/01/2001</td>
<td>HBV TRANSFECTED HUMAN HEPATOMA CELLS (HEPG2 CLONE 2.2.15)</td>
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**Class**: Class 3

**Non-GMM Consent Granted**: Yes

**Project notified under transitional arrangements**: Yes

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
Tick if yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<th>Laboratory Activities</th>
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<td>Date Premises Closed</td>
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<td>01/09/2008</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<tr>
<td>Level 2 (GMMs)</td>
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<td>Level 3 (GMMs)</td>
<td>Yes</td>
<td>Yes</td>
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</table>
The liquid waste (red cells, parasitised red cells) is inactivated with PreSept (according to the manufacturer's directions) and flushed down the sink. The solid waste is autoclaved and incinerated.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 505/97.3

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>13/08/1997</td>
<td>EXPRESSION OF TUMOUR SUPPRESSION GENES</td>
<td>Class 2</td>
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Date Project Ceased

Non-GMM

Consent Granted

not applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
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<td>L2 L3 L4 L2 L3 L4</td>
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Animal Units

<table>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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Project Ref 559/00.1

Date Ackn’d 20/03/2000

CU2 Project Title

STUDIES ON THE REPLICATION OF THE HEPATITIS C VIRUS IN MAMMALIAN CELLS

Class CultureVolClass2 CultureVolumeClass3-4

Class 3

Non-GMM Consent Granted yes

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022 Page 9749 of 15326
Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
- Host/vector system
- Origin & function
- Evaluation of foreseeable effects
- Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Growth Rooms</th>
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02/03/2022
Project Ref 559/00.2

Date Ackn'd 19/01/2001

CU2 Project Title INVESTIGATION OF THE THERAPEUTIC POTENTIAL OF CYTOKINE GENE TRANSFER TO TUMOURS

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>L2 L3 L4</td>
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<td>L3 L2 L3 L4</td>
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Animal Units

L2 L3 L4 L2

Large Scale Activities

L2 L3 L4 L2

Human Clinical Applications

L2 L3 L4 L2

Project Ref 559/00.3

Date Ackn'd 19/01/2001

CU2 Project Title ANTI TUMOUR IMMUNOTHERAPY USING GENE TRANSFER

Class CultureVolClass2 CultureVolumeClass3-4

Class 2
Date Project Ceased: 01/12/2005

Non-GMM: consent not applicable

Tick if notifying a connected programme of work: N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<td>L2 L3 L4</td>
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**Project Ref** 559/01.1

<table>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>Culture Vol</th>
<th>Consent Granted</th>
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<tr>
<td>30/01/2001</td>
<td>ENGINEERING MYCOBACTERIA TO EXPRESS HUMAN AND AND MURINE CYTOKINE AND TUMOUR ASSOCIATED ANTIGEN GENES</td>
<td>Class 3</td>
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<table>
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<th>Non-GMM</th>
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<tbody>
<tr>
<td>30/06/2003</td>
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历史显著变化

- Neutral

- Neutral

- Neutral

- Neutral

- Neutral

- Neutral

- Neutral
Project Additional Information

Purposes of the contained use

Minimise risk to human health that may occur should infection of mycobacterium bovis (BCG) with antigens/cytokines modify the BCG to cause a pathological response.

Recipient or parental organism

Mycobacterium Bovis (BCG)
Mycobacterium Smegmatis
Mycobacterium Vaccai
E. coli (DH 5 degrees C)

Host/vector system

Non-mobilisable shuttle-vectors, including pMOD-8, pMOD-12
Expression regulated by mycobacterial heat shock protein.

Origin & function

- Characterised cytokines and antigens.
- The aim is to a) induce an immune response; b) modify the immune response to mycobacteria by expressing cytokines and antigens.

Evaluation of foreseeable effects

Expressions of cytokines and antigens in mycobacteria is likely to improve the immune response. These mycobacteria may be useful for the treatment of cancer by
- generating systemic specific immune responses
- delivering cytokines locally.

No known risk is presented to the environment; The mycobacteria will not obtain a survival advantage when expressing cytokines/antigens.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Work with BCG expressing antigen genes that are not allergens and murine cytokines that are known not to act on human cells will be carried out at containment 2 (Class 2 activity), as these antigens and mouse cytokines have no known biological effect on human cells and consequently will not cause increased pathogenicity of BCG.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All mycobacteria waste generated in Class 1, 2 or 3 activities is to be autoclaved to ensure 100% kill. (Autoclave efficiency checked weekly).

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
The unit Biological Safety Committee felt it was highly unlikely expression of proposed cytokines and antigens would modify the pathogenicity of BCG, however in the absence of data to support this, work with BCG will be performed at Class 3 if using human cytokines.

### Project Containment

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### Project Ref  559/01.2

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<td>19/02/2001</td>
<td>TRANSFER OF STAPHYLOCOCCUS EPIDERMIS LIPASE GENES TO E COLI XL-BLUE</td>
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### Project Additional Information

- Purposes of the contained use
- Recipient or parental organism
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

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Please enter comments on the GM safety committee on the risk assessment

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CONSTRUCTION OF A LUCIFERARE REPORTER SYSTEM USING THE REPORTER PLASMID PSB330 CONTAINING PROMOTER REGIONS FROM STAPHYLOCOCCUS EPIDERMIS GEHC AND GEHD LIPASE GENES

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<tr>
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Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref  559/01.4

Date Ackn'd  19/02/2001

CU2 Project Title  CONSTRUCTION OF LUCIFERASE REPORTER SYSTEM USING THE REPORTER PLASMID PSB330 CONTAINING PROMOTER REGIONS FROM STAPHYLOCOCCUS AUREA TST GENES

Class  Class 2

CultureVolClass2  Class 2

CultureVolumeClass3-4  not applicable

Non-GMM Consent Granted

Historical Significant Changes

Historical Date of Additional Info

02/03/2022  Page 9759 of 15326
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**  
**N**

**If yes, tick to confirm that it is attached to this form**  
**N**

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
**N**

**Please enter comments on the GM safety committee on the risk assessment**

**Project Containment**
Project Ref  559/01.5

Date Ackn'd  19/02/2001

CU2 Project Title

CLONING AND EXPRESSION OF STAPHYLOCOCCUS AGPRC USING NON-MOBILISABLE EXPRESSION VECTORS, DISABLED HOSTS AND S AUREUS

Class  Class 2

Consent Granted

not applicable

Project notified under transitional arrangements  Y

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

|            | L2 L3 L4 L2 L3 L4 L2 L3 L4 |              |              |

Large Scale Activities

|            | L2 L3 L4 L2 L3 L4 L2 L3 L4 |              |              |

Human Clinical Applications

|            | L2 L3 L4 L2 L3 L4 L2 L3 L4 |              |              |

Project Ref  559/01.6

Date Ackn’d  19/02/2001  
CU2 Project Title  TRANSFER OF STAPHYLOCOCCUS AUREAU AND PROPIONIBACTERIUM  
Class  Class 2  
CultureVolClass2  
CultureVolumeClass3-4  

02/03/2022  
Page 9762 of 15326
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 559/01.7

Date Ackn’d 20/04/2001

CU2 Project Title RANDOM AND SITE DIRECTED TN5 MEDIATED MUTAGENESIS OF BURKHOLDERIA CEPACIA WITH MARKER RESCUE ANALYSIS, TO IDENTIFY HOW ENVIRONMENTAL FACTORS AFFECT GENE EXPRESSION AND RESISTANCE

Class 2  Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**
To facilitate an MRC/DERA research programme to identify genes that may be involved in resistance to antimicrobial peptides. Additionally this research will examine how the expression of these genes are affected by culture environment.

**Recipient or parental organism**
*Burkholderia cepacia* is a ubiquitous Class ACDP category 2, environmental organism which can cause lower respiratory tract infection in individuals with cystic fibrosis and systemic sepsis in immunocompromised patients especially those with chronic granulomatous disease. GMOs generated during this project are likely to be no more harmful than the unmodified host.

**Host/vector system**
*Burkholderia cepacia* will be the host for insertion of plasmid mediated transposons (Tn5) for insertional inactivation and marker rescue. Initially pOT182 will be used for this purpose as it has been fully characterised and shown to be effective for transposon mediated mutagenesis in *Burkholderia cepacia*.

**Origin & function**
*Burkholderia cepacia* strains will be provided by Dr. K Kerr of the Microbiology Department of the University of Leeds. Dr Kerr has a great deal of experience of working with this organism. The Tn5 transposon containing plasmid pOT182 is a pBR325 based plasmid containing the Tn5-B21 cassette from pSUP 102(Gm) and was provided by Professor I Lamont. The construction of this vector has been described (Merriman and Lamont, 1993, Gene, 17-23). The Tn5 will be used to mutagenise *burkholderia cepacia* and to identify genes that alter susceptibility to antimicrobial peptides. The genes will be identified by sequencing the DNA adjacent to the site of transposition.

**Evaluation of foreseeable effects**
It is expected that recombinant isolates of *burkholderia cepacia* containing the Tn5 cassette are likely to be no more hazardous than the parental organism. The rationale for this is that no new genes (other than those contained within the transposons) will be inserted into the host. The reason for classification of this as a class 2 project is that *burkholderia cepacia* is listed as an ACDP category 2 pathogen.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Solid laboratory waste such as pipette tips and disposable loops will be soaked overnight in discard pots containing 1% Virkon and autoclaved. All glassware that comes into contact with *burkholderia cepacia* will be autoclaved. All liquid and solid cultures will be autoclaved. Autoclaving will be carried out at 121°C for 15 minutes. Disposable autoclave waste will be removed for incineration. It is envisaged that these proposals will ensure a 100% kill of all GMOs.

**Is an emergency plan required according to regulation 20?**
- **N**

**If yes, tick to confirm that it is attached to this form**
- **N**

**Tick to confirm that you have attached a risk assessment to this form**
- **Y**
The following statement is taken from the minutes of the Worley Building GM Committee meeting held on February 9th 2001.

"Two projects from the Dental School were considered; one involving cDNA cloning was approved as Class I. The second one falls under category two in view of the organism involved, the organism being burkholderia cepacia. Dr Bonass is to notify the HSE for approval".

**Project Containment**

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

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<td>TRANSFORMATION OF ASPERGILLUS FUMIGATUS TO ENABLE: DISRUPTION OF GENES ENCODING CHITINASES AND THEIR TRANSCRIPTIONAL REGULATORS; REPORTER GENE STUDIES WITH GENES ENCODING CHITINASES</td>
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<td>Withdrawn</td>
<td>N</td>
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- **Project notified under transitional arrangements**

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**Project Additional Information**
Purposes of the contained use

The phenotypes of gene disruptants will indicate whether the gene products have essential roles in A. fumigatus. This, in turn, will allow us to evaluate these proteins as potential targets for antifungal agents. Reporter gene studies will enable us to monitor the expression of individual chitinases and their regulators in the intact organism during key morphogenetic events including spore germination, hyphal side branch formation and sporulation. Tandem affinity purification will enable us to purify proteins of interest along with associated components. The co-purifying proteins/other components will be identified by mass spectrometry. Novel regulatory mechanisms identified by this approach may present new targets for antifungal agents.

Recipient or parental organism

Aspergillus fumigatus strain ATCC 13073

Host/vector system

A. fumigatus/all vectors are derived from plasmids of the pUC series and are considered to be non-mobilisable.

Origin & function

For gene disruption, gene(s) encoding enzymes that confer resistance to antibacterial antibiotics (hygromycin and/or phleomycin) will be used to disrupt genes encoding A. fumigatus chitinases or transcriptional regulators by insertion of the resistance genes into the A. fumigatus genome. For reporter gene studies, an Escherichia coli B-galactosidase gene will be inserted into the A. fumigatus genome so that this gene is expressed under the control of specific A. fumigatus promoters. For TAP-tagging, genes encoding a calmodulin binding protein and protein A will be inserted at the C-terminus of chitinase genes to enable expression of tagged chitinase proteins.

Evaluation of foreseeable effects

Most humans inhale several hundred A. fumigatus conidia per day and inhalation of conidia by immunocompetent individuals rarely has any adverse effect. A. fumigatus may provoke severe allergic reactions such as farmer's lung in individuals exposed repeatedly to large numbers of conidia and may cause aspergilloma, an outgrowth of fungus on the surface of preexisting cavities in the lungs of patients treated successfully for TB. A. fumigatus can cause serious, often fatal, infections in immunocompromised hosts. Gene disruption may reduce the virulence of the host while reporter gene studies and TAP-tagging are unlikely to affect the pathogenesis of A. fumigatus.

It should be emphasised that A. fumigatus will be cultured in a sealed incubator and if breakage of a culture vessel occurred within the incubator this could be dealt with readily, using Hycolin, with negligible risk to the operator. If breakage of a 400 ml culture occurred in the laboratory any spores present would, in the first instance, be contained within small drops of the culture medium which could be dealt with, using Hycolin, immediately ie the situation would not arise where large numbers of spores became desiccated and airborne.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Vessels used for the culture and disruption of genetically-modified A. fumigatus will be de-contaminated by autoclaving (departmental facility). Contaminated surfaces will be disinfected using Hycolin and materials used for disinfection will be autoclaved (departmental facility). Culture stocks will be disposed of by autoclaving (departmental facility). It is anticipated that these procedures will result in 100% kill. Autoclaved material will be removed from the building as clinical waste.
At its meeting of 1/3/01 the GM Safety Committee recommended minor modifications to the risk assessment and notification form. These modifications have now been made to the satisfaction of the Committee. D G Adams 27/3/01.

Please enter comments on the GM safety committee on the risk assessment

At its meeting of 1/3/01 the GM Safety Committee recommended minor modifications to the risk assessment and notification form. These modifications have now been made to the satisfaction of the Committee. D G Adams 27/3/01.

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**Project Ref** 559/01.9

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<tr>
<td>16/08/2001</td>
<td>TRANSFECTION OF PLASMODIUM FALCIPARUM WITH GENES ENCODING METABOLIC ENZYMES (&quot;HOUSEKEEPING GENES&quot;)</td>
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<td>10ml maximum</td>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Purposes of the contained use**

To investigate the functions of gene products in the blood stages of the malaria parasite for the discovery of new targets for treatment. Proliferation of the blood stages are responsible for the mortality although these stages are innocuous outside of the bloodstream.

**Recipient or parental organism**

Strains of *P.falciparum* susceptible to several antimalarial drugs will be used. Most commonly, strain 3D7 which is susceptible to all known antimalarial drugs, particularly chloroquine. Only the blood stages will be transfected. High numbers of these parasites (>10^4) injected I.V. would be required to cause infection.

**Host/vector system**

The plasmid vectors for transfection are 1) the published plasmid which encodes resistance to pyrimethamine and 2) a plasmid containing a bacterial resistance gene for the antifungal agent blasticidin. The standard disabled cloning strains of *E.coli* are used for amplification of the plasmid vector.

**Origin & function**

The genetic material consists of genes encoding metabolic enzymes from *P.falciparum* or related protozoa (*Toxoplasma gondii, Eimeria tenella*). There are no foreseeable risks for transfection of housekeeping genes. The genes may integrate into the *P.falciparum* genome, disrupting the endogenous gene, and decreasing the viability of the organism.

**Evaluation of foreseeable effects**

One of the vectors will induce resistance to the antimalarial drug pyrimethamine but pyrimethamine is not commonly used because of the high amount resistance in the field. All the transfected parasites will remain sensitive to other antimalarial drugs (eg chloroquine). There are no foreseeable effects on the risks for the GMMs with the genes transfected except the possible decrease in parasite viability. The blood stages of the parasites are only dangerous if injected into the bloodstream. Injection is impossible as no sharps are permitted in the cultivation facility. Hence the risk is essentially zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

I request a derogation to Class 2 for the following activities: movement of material to autoclave; performing manipulations outside the Class 2 cabinet; and aerosol minimisation. I request derogation of the requirement for autoclaving of solid waste to take place within the Class 3 facility to supervised movement of the solid waste to the autoclave 10 meters down the hall. I request permission to perform manipulations of the transfected *P.falciparum* on the benchtop in the Class 3 facility in accordance with Class 2 regulations (Under schedule 8, GM-Contained Use 2000 Regulations). Lastly, all procedures performed will minimise aerosols in accordance with Class 2 regulations rather than prevent aerosols. These derogations are requested because the assessed risk of infection or transmission is negligible for the blood stages of the parasite and infections are treatable with common antimalarial drugs.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is treated with 2.5% Precept for at least 12 hours, then rinsed down the drain with copious quantities of water.

Solid waste is autoclaved in isolated runs, then incinerated via the University system. Validation of autoclaving via Brown’s TST Control Integrator strips. Validation for incineration via the University of Leeds tagging system.

---

02/03/2022
Approved as appropriate with commendation for the disposal procedure.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Is an emergency plan required according to regulation 20? N

Tick if yes, tick to confirm that it is attached to this form N

Tick if notifying a connected programme of work N

Class 2 Consent Granted

Class CultureVol

< 1 litre

Non-GMM

not applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Containment

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Project Ref 559/02.1

Date Ackn'd 14/08/2002

CU2 Project Title REPORTER AND SPECIFIC/RANDOM GENE KNOCK-OUT STUDIES OF ENTEROCOCCUS FAECALIS. (A) REPORTER GENES BASED ON (GFP) WILL BE USED TO MONITOR PROMOTOR ACTITY OF GENES OF INTEREST (B) ...

Class 2 Non-GMM

< 1 litre

Consent Granted

not applicable

Withdrawn N

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

Research work to investigate regulation of cyl (haemolysin) and other virulence factor gene expression in Enterococcus faecalis

Recipient or parental organism

Enterococcus faecalis laboratory and environmental/clinical strains

Host/vector system

Host:: (E. faecalis NCTC775 and environmental isolates (strain types 1, 2 and 3)). E.faecalis is a class 2 bacterium.NCTC775 has been in laboratory culture for many years; it has lost the cyl haemolysis virulence genes essential for infection. The strain types 2 and 3 are also spontaneously attenuated, since they too have lost these cyl genes. Strain 1 (of strain type 1) possesses cyl, but exhibits high sensitivity to vancomycin and was isolated as a contaminant from the environment, rather than a focus of infection. No connection with an infection has been established for this strain. E.coli strains involved in plasmid construct preparation will be laboratory-adapted auxotrophs that are unlikely to survive in the environment, and are therefore considered to be class 1 and hence containment level 1 is sufficient. Vectors. Plasmids pUC, pBluescript; pVA838; pLTV1, pVT1-OK.

Origin & function

Plasmids pUC, pBluescript will be used for specific knock-outs; report studies use pVA838 with gfp gene inserted; random mutagenesis involves pLTV1, pLTV-OK. These are on-mobilisable plasmid vectors that do not possess mob sites; pUC and pBluescript cannot replicate in E. Faecalis. pLTV1 and pTV1-OK both possess Tn917 for insertionional inactivation; these plasmids possess a temperature-sensitive locus that ensures that the plasmid is lost from Enterococcus strains at 42-45OC. There are no genes that might increase the level of risk. the knock-out plasmids also possess erm and tet resistance markers; these are not of clinical significance. Reporter plasmids are based on pVA838 that possesses a streptococcal origin of replication, gfp reporter gene and erm resistance marker. This too is a low risk plasmid since it is unlikely to encounter a suitable host, and possesses no harm or clinically-significant genes.

Evaluation of foreseeable effects

Reporter constructs: GFP reporters have no known toxic properties and there is no known hazard to the envirnment. These genes will be linked to the promoters of specific E faecalis genes such as the cyl (haemolysin) genes; these are short DNA sequences that do not encode proteins.

Mutant construction: no known harmful effect of the Tn917 transposon.

Modified E faecalis likely to be of similar harm as the unmodified host; insertion of Tn917 is expected to insertionally - inactivate genes on the chromosome of E faecalis; therefore, most likely to make the organism less harmful.

Reporters/KOs: The presence of TN917-possessing, or GFP-expressing E faecalis is likely to be no greater risk to humans etc or the environment than the risk of the unmodified organism. Antibiotic resistance markers on the plasmids are either of no clinical significance (Cm) in relation to E faecalis infection, or resistance is found in wild type strains anyway (Em: 50% of isolated strains) and alternative therapies are available. NCTC775, strain types 2 and 3 lack the essential haemolysin virulence factor, hence less harmful than environmental/WT strains. Strain 2 strains are wild type.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Cultures will be decontaminated by autoclaving (121°C/15 min); expected survival is 0%. Regular screening to ensure proper functioning of autoclaves is routinely carried out. Contaminated lab areas can be disinfected effectively; swabs of bench areas can be taken to assess survival; expected survival is 0%. The laboratories (3-04, 3-09) are located in the Old Medical School; the wider environment is unlikely to become contaminated.

In the unlikely event of escape:

E. faecalis: Survival in the environment and ability to colonise are possible, but there are effective containment and disinfection procedures in place, minimising the chances of contact with suitable hosts.

E. coli: these are laboratory-attenuated auxotrophs and therefore are very unlikely to survive in the environment or to colonise.

No significantly increased risk to lab workers and other health individuals in the community at large.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

The GM Safety Committee recommended changes to the original risk assessment and notification form. These modifications have now been made to the satisfaction of the BSO and GMSC chair.

**Project Containment**

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**Project Ref** 559/02.2

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RANDOM AND SITE DIRECTED TRANSPOSON MEDIATED MUTAGENESIS OF ORAL BACTERIA TO IDENTIFY HOW ENVIRONMENTAL FACTORS AFFECT GENE EXPRESSION AND RESISTANCE TO ANTIMICROBIAL COMPOUNDS.

Purposes of the contained use

To facilitate an ongoing research project involving microbial communities and factors affecting gene expression. One aim of the research is to identify genes that may be involved in resistance to antimicrobial peptides or other stress inducing factors eg temperature, oxidation. Additionally this research will examine how the expression of these genes is affected by culture conditions including growth as a complex biofilm.

Recipient or parental organism

P. gingivalis, P. intermedia, P. nigrescens, S. mutans, S. Oralis, S. sobrinus, S. Gordonii, A. naeslundii, C. rectus, T. denticola, A. actinomycetemcomitans are ACDP category 2 organisms which commonly inhabit the oral cavity but are not considered separately to be pathogenic. GMOs generated during this project will have genes inactivated and are therefore likely to be no more harmful than the unmodified host.

Origin & function

Common laboratory strains of the bacteria listed will be used. Many of these bacteria have been the subject of genome sequencing projects and all are well characterised. It is not the aim of this project to transfer genes between the listed species but to inactivate individual genes in a single species and to determine the effects on survival in the community. The aim is to identify genes that may give a selective advantage to a particular species under a defined set of laboratory culture conditions. All cultures will be destroyed subsequent to each experiment. Genetic material to be transferred will include well characterised transposons such as Tn5 and will be delivered via well characterised laboratory strains of E. coli. The Tn5 transposon containing plasmid pOT182 is a pBR325 based plasmid containing the Tn5-B21 cassette from pSUP 102(Gm) and was provided by Professor I Lamont. The construction of this vector has been described (Merriman and Lamont, 1993, Gene, 17-23). These procedures are in routine use in our laboratories.

Recipient or parental organism

P. gingivalis, P. intermedia, P. nigrescens, S. mutans, S. Oralis, S. sobrinus, S. Gordonii, A. naeslundii, C. rectus, T. denticola, A. actinomycetemcomitans are ACDP category 2 organisms which commonly inhabit the oral cavity but are not considered separately to be pathogenic. GMOs generated during this project will have genes inactivated and are therefore likely to be no more harmful than the unmodified host.

Host/vector system

Commercially available (or other well characterised) transposon containing vectors (eg pOT182, pVA2198) grown in suitable laboratory strains of E. coli (eg S17-1, SM10) will be used to inactivate specific, or randomly targeted genes in the oral bacteria P. gingivalis, P. intermedia, P. nigrescens, S. mutans, S. Oralis, S. sobrinus, S. Gordonii, A. naeslundii, C. rectus, T. denticola, A. actinomycetemcomitans. These procedures are in routine use in our laboratories for other projects.

Origin & function

Common laboratory strains of the bacteria listed will be used. Many of these bacteria have been the subject of genome sequencing projects and all are well characterised. It is not the aim of this project to transfer genes between the listed species but to inactivate individual genes in a single species and to determine the effects on survival in the community. The aim is to identify genes that may give a selective advantage to a particular species under a defined set of laboratory culture conditions. All cultures will be destroyed subsequent to each experiment. Genetic material to be transferred will include well characterised transposons such as Tn5 and will be delivered via well characterised laboratory strains of E. coli. The Tn5 transposon containing plasmid pOT182 is a pBR325 based plasmid containing the Tn5-B21 cassette from pSUP 102(Gm) and was provided by Professor I Lamont. The construction of this vector has been described (Merriman and Lamont, 1993, Gene, 17-23). These procedures are in routine use in our laboratories.
Evaluation of foreseeable effects

It is expected that recombinant isolates of P. gingivalis, P. intermedia, P. nigrescens, S. mutans, S. Oralis, S. sobrinus, S. Gordonii, A. Naislundii, C. rectus, T. denticola, A. actinomycetemcomitans containing the transposons are likely to be no more hazardous than the parental organism. The rationale for this is that no new genes (other than those contained within the transposon) will be inserted into the host. The reason for classification of this as a class 2 project is that each of these organisms is listed as an ACDP category 2 microorganism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid laboratory waste such as pipette tips and disposable loops will be soaked overnight in discard pots containing 1% Virkon and autoclaved. All classware that comes into contact with the listed bacterial species will be autoclaved. All liquid and solid cultures will be autoclaved. Autoclaving will be carried out at 121 degrees C for 15 minutes. Disposable autoclave waste will be removed for incineration. It is envisaged that these proposals will ensure a 100% kill of all GMOs.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The following statement is taken from the minutes of the Worsley Building GM Committee meeting held on 17 April 2002.

1. "One project (DS18) was submitted by the Dental School. Since the organisms to be used in this study were classified as Class II in ACGM, although they are known not to be harmful, the project needs to be notified to HSE".

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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02/03/2022
TO CLONE THE CDNA(S) FOR THE CELL SURFACE RECEPTOR FOR HUMAN ADENOVIRUS TYPE 3 (AD3). WE AIM TO ADOPT A MAMMALIAN CDNA EXPRESSION STRATEGY WHEREBY A CDNA LIBRARY IS INTRODUCED.

**Project Additional Information**

**Purposes of the contained use**

Fundamental studies on virus receptors and mechanisms of uptake into human cells.

**Recipient or parental organism**

Human 293 cells (to generate replication-defective retroviruses and adenoviruses) and hamster CHO cells for expression screening. No foreseeable effect.

**Host/vector system**

**Host:** See above.

**Vector:** Murine Leukaemia Virus-based vector for expression

Plasmid containing the MuLV LTR (pFB retroviral vector); pVPack; pVpack VSV-G

Adenovirus vector (AdEasy, Qiogene Inc.)

**Origin & function**

The cDNA Library is derived from HeLa cells, which we have shown to be receptor-positive.

The function of the receptor will convert the cell lines from receptor-negative to receptor-positive.

**Evaluation of foreseeable effects**

As most humans already have long-lasting immunity to adenoviruses and already possess the Ad 3 cellular receptor, we do not anticipate that expression of the adenovirus...
3 cellular receptor will confer increased susceptibility to humans of adenovirus or other virus infections.

For both categories of recombinant viruses, the risk of expression of a viral receptor is unlikely to be hazardous to humans as expression of this receptor is ubiquitous.

Receptor-expression human and hamster cells will not colonize the human host.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

We will be performing the production of replication defective retroviruses and screening of CHO cells under containment level 2. Recombinant adenoviruses will also be produced and used under containment level 2.

Virus-containing cells will be treated with Virkon to kill any remaining virus.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Receptor-expressing and transfected cells will be killed by treatment with Virkon. Liquid waste will also be treated with Virkon. Solid waste will be autoclaved, securely packaged and incinerated. Quantities of cells and medium to be used will not generate major spillages, however, in the event of such an incident, we would treat the spillage with granular hypochlorite.

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **N**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

Please enter comments on the GM safety committee on the risk assessment

The project was discussed at the local ACGM committee and it was decided that, although relatively low risk, the committee considered it notifiable at Class 2 due to the remote possibility of producing replication competent adenoviruses (RCA) or retroviruses (RCR). However, we will monitor for the generation of RCA and RCR and keep this project under assessment in the light of such findings.

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Animal Units | Large Scale Activities | Human Clinical Applications
CLONING OF CDNA COPY OF THE MOUSE INTERLEUKIN-2 (MUIL2) INTO THE XYLANASE OPERON OF BACTEROIDES OVATUS IN E.COLI DH5A. TRANSFER OF CONSTRUCT TO E.COLI J53/R751 AND THEN VIA CONJUGATION TO B. OVATUS

The modified B. ovatus will be used to assess xylan-inducible expression of MuIL2 in vitro. This organism will then be used in experiments as a novel immunotherapy to try and treat and prevent disease.

B. ovatus is ACDP Hazard Group II. The final modified organism will be tetracycline resistant and will be xylanase negative. Although a gut commensal, the likelihood of a GMM colonizing humans or persisting in the environment is minimal. Expression of MuIL2 by this organism should not pose a hazard.

E. coli vector pGEM is non-mobilisable. Vector pBT-2 is an E. coli-Bacteroides mobilisation-defective shuttle vector requiring E. coli strain J53/R751 for transfer. This is a suicide vector in Bacteroides, requiring integration into the genome for maintenance.

The xylanase operon originates from B. ovatus. The MuIL2 gene is a cDNA copy which will be transcribed in a xylan-inducible manner in the final GMM.
The final GMM will be tetracycline resistant and xylanase negative. Expression of MuIL2 should be regulated and the modified organism must compete with resident microflora to colonise. Release of the number of viable organisms required to colonise other animals or humans is unlikely. Survival in the environment is likely to be limited. MuIL2 is unlikely to be produced in large enough quantities to have a significant effect on humans. The modified organism should pose no greater hazard than the wild type.

**Evaluation of foreseeable effects**

Standard containment level 2.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- All pipette tips, slides, and small disposable plasticware will be disposed of in discard pots containing 2% (v/v) Tego prior to being autoclaved and discarded.
- All waste cultures, media and large plasticware will be autoclaved prior to discarding.
- All glassware will be autoclaved before being washed and returned for use.
- Sharps will be disposed of in a cin bin which will then be autoclaved and incinerated.
- All culture spills will be mopped up with stericol and tissue which will then be autoclaved prior to discarding.
- No viable GMMs will be left after treatment.

**Is an emergency plan required according to regulation 20?**

**If yes, tick to confirm that it is attached to this form**

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

The GM safety committee requested some minor changes to the risk assessment. These changes have been completed to the satisfaction of the BSO.

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We aim to use a replication defective human adenoviral vector to introduce the following genes into primary cultures of human cardiac myocytes, vascular smooth muscle and endothelial cells:

(i) the angiotensin type 1 receptor (AT1)
(ii) matrix metalloprotease 9 (MMP9) in both sense and antisense orientations, and also dominant negative mutants of MMP9 may be expressed.
(iii) peroxisome proliferation activation receptor (PPAR) gamma isoform 1 in both sense and antisense orientations.
(iv) Reporter expression cassettes consisting of the bacterial beta-lactamase gene (obtained from Aurora Biosystems) under the control of either the human AP-1, CRE or NF-AT response elements.
(v) A reporter expression cassette in which expression of either the enhanced yellow (EYFP) or enhanced green fluorescent protein (EGFP) (Available from Clontech) is driven by the upstream regulatory region of human angiotensin receptor type 1 or type 2.

By placing genes (i-iii) under the control of the tetracycline response element, the levels of expression of recombinant proteins can be regulated enabling a better understanding of the role of signal transduction pathways in cardiac pathologies.
All transgenes are integrated into an adenoviral genome (using the AdEasy system from Stratagene) from which the early genes essential for replication, contained in the E1 region of the viral genome, have been deleted, to generate replication defective viruses which can only be propagated in human 293 cells. This is a helper cell line (human embryonic kidney cells) which express the leftmost 11% of the Ad5 genome containing the E1 region in trans thus complementing the deleted genes in the viral vector. In addition E3 gene has also been deleted from this particular recombinant virus, significantly reducing the ability of the virus to evade a host immune response.

Origin & function

pAdEasy-1 vector
The pAdEasy-1 vector (Stratagene) is a modified version of human Adenovirus 5 in which the genes in the E1 region of the viral genome, essential for replication, are deleted to generate a replication defective virus. In addition the E3 gene is deleted which significantly reduces the ability of the virus to evade detection by the host immune system.

Human angiotensin receptor.
The peptide agonist angiotensin II activates this receptor to produce a variety of regulatory actions on the cardiovascular, renal, endocrine and neural systems including vasoconstriction, hypertension and aldosterone release. It is not envisaged that expression of this gene will be oncogenic or have adventitious effects in host cells at the levels of expression used. Expression of the gene will be placed under the control of a tetracycline inducible promoter system that levels of expression of the transgene can be finely regulated by the addition of tetracycline. The full coding sequence of the angiotensin type 1 receptor is available as a cDNA in our laboratory.

Peroxisome Proliferation Activation Receptor (PPAR) Gamma
PPARgamma is a transcription factor which is expressed in all of the major tissues of the vasculature including endothelial cells, vascular smooth muscle cells (VSMCs) and monocytes and macrophages. Drugs which activate PPARgamma have been shown to inhibit the proliferation and migration of VSMCs in cell culture. Current data suggests that the antiproliferative effect of PPARgamma on the cell cycle is due to an increase in the levels of cyclin-dependent kinase inhibitors such as p27. Again, as stated in the case for the angiotensin receptor, it must be borne in mind that in all cases, PPARgamma transcripts whether sense or antisense will be under the strict control of a Tetracycline-inducible promoter. A cDNA encoding full length human PPARgamma isoform 1 was obtained from Laboratory of Molecular Hematopoiesis, Sloan-Kettering Institute, New York.

Matrix Metalloprotease 9
MMP9 (also known as Gelatinase B) is responsible for basement membrane degradation essential for tissue remodelling in a number of key developmental and growth processes. MMP9 is found to be up-regulated in many forms of cancer, as its role in basement membrane degradation facilitates malignant cell invasion and metastasis. Transformation of a cell to a full malignant phenotype however, is dependent on a number of changes in expression in genes controlling signal transduction/gene expression cascades within the cell or genes involved in regulation of the cell cycle. It is highly unlikely therefore that alteration of levels of MMP9 expression alone would confer an oncogenic phenotype upon a mammalian cell and to the best of my knowledge there is no case of this being documented in the research literature. In addition, as has been stated for both the case of the angiotensin receptor and PPARgamma above, all transcripts whether sense, antisense or dominant negative mutant will be under the control of a Tetracycline-inducible promoter. A cDNA encoding full length human MMP9 was obtained from Department of Immunology and Oncology, Universidad Autonoma de Madrid.

AP-1/CRE/NF-AT Reporter Constructs
These constructs consist of the bacterial beta-lactamase gene under the control of either the AP-1, CRE or NF-AT response element. The activity of each response element can be measured by assaying the activity of the product protein of the beta-lactamase gene through a simple optically based assay. It is not envisaged that introduction of the beta-lactamase gene into mammalian cells should pose a significant safety risk as mammalian cells are routinely exposed to commercially available ampicillin resistant plasmids containing the beta-lactamase gene with no adverse effects.

Upstream Regulatory Region of Human Angiotensin Type 1 and Type 2 Receptor
The reporter cassette in this virus will contain either a fragment of DNA encompassing approximately 1300bp immediately upstream of the transcriptional start site of human angiotensin II type 2 receptor (GenBank Acc No. X87722) or a fragment encompassing approximately 2700bp upstream of the initiation ATG of human angiotensin II type 1 receptor (GenBank Acc No U07144 - detailed in 'Molecular cloning and expression of the gene encoding human angiotensin II type 2 receptor' Guo et al. Biochem
Reverse Tetracycline Transcription Activator (rtTA)

The rtTA gene was obtained from the plasmid pTet-On which is commercially available from Clontech. It is necessary to make a recombinant virus which expresses this protein so that expression of genes under the control of the Tetracycline response element can be activated when a co-activator ligand such as Doxorubicin is added.

Evaluation of foreseeable effects

All strains of Ad5 are non-oncogenic. Wild-type Ad5 may cause mild respiratory diseases in children. Because the recombinant virus will lack the E3 gene this may produce a virus that will evoke a stronger inflammatory response in a host, but this should also lead to more rapid clearance of the virus from a host system. Primary infection of adenovirus is thought to generate life-long immunity and it is thought that the majority of the adult population are likely to have antibodies to the wild-type virus.

Although replication has been shown to occur in the lungs of experimentally infected cotton rats administered with high doses of virus, there is no evidence that adenoviruses can naturally colonise non-human hosts and therefore pose no significant harm to animals, plants or ecosystems.

Human angiotensin receptor.

The peptide agonist angiotensin II activates this receptor to produce a variety of regulatory actions on the cardiovascular, renal, endocrine and neural systems including vasoconstriction, hypertension and aldosterone release. It is not envisaged that expression of this gene will be oncogenic or have adventitious effects in host cells at the levels of expression used. Expression of the gene will be placed under the control of a tetracycline inducible promoter system that levels of expression of the transgene can be finely regulated by the addition of tetracycline.

Peroxisome Proliferation Activation Receptor (PPAR) Gamma

PPARgamma is a transcription factor which was initially characterised as an adipose-tissue specific factor that played a role in the differentiation of this tissue and regulated a number of adipose specific genes. Drugs which activate PPARgamma have been shown to inhibit the proliferation and migration of VSMCs in cell culture. Current data suggests that the antiproliferative effect of PPARgamma on the cell cycle is due to an increase in the levels of cyclin-dependent kinase inhibitors such as p27. Again, as stated in the case for the angiotensin receptor, it must be borne in mind that in all cases, PPARgamma transcripts whether sense, antisense or dominant negative mutant will be under the strict control of a Tetracycline-inducible promoter.

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AP-1/CRE/NF-AT Reporter Constructs

These constructs consist of the bacterial beta-lactamase gene under the control of either the AP-1, CRE or NF-AT response element. The activity of each response element can be measured by assaying the activity of the protein product of the beta-lactamase gene through a simple optically based assay. It is not envisaged that introduction of the beta-lactamase gene into mammalian cells should pose a significant safety risk as mammalian cells are routinely exposed to commercially available ampicillin resistant plasmids containing the beta-lactamase gene with no adverse effects.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
All work will be performed in tissue culture (Category II) facilities with restricted access. The flow cabinet in which work will be performed is equipped with a UV light source which enables sterilisation of the work area upon completion of work. Liquid waste will be virkon treated. Plasticware will be disinfected prior to autoclaving and then incinerated. All work surfaces will be treated with 70% ethanol and 1% virkon spray prior to and upon completion of work. Kill rate by virkon or autoclave treatments is 100%.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Since replication deficient recombinant adenoviruses are normally considered to be class I GMAG work, the risk assessments concerning this work were initially submitted to our local GMAG committee as a class 1 project. Concern was expressed, however, that because of the biological potency of some of the human genes which we intended to express in our adenoviral vector system, such as the angiotensin II type 1 receptor, that this work might fall into the remot of a class 2 project. Because of these reservations, the local GMAG committee suggested that the project be provisionally rejected until we advice had been sought from the HSE on whether the project was class 1 or class 2. After correspondence with HSE we were informed that the proposed project was class 2 and so the risk assessments and HSE notification were amended accordingly.

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Project Ref 559/02.6

Date Ackn'd: 24/10/2002
CU2 Project Title: EXPLOITING HERPESVIRUS SAIMIRI AS A GENE KNOCK-IN OR KNOCK-OUT
Class: 2
CultureVolClass2: < 1 litre
CultureVolumeClass3-4:
SYSTEM IN TUMOUR CELLS, PRIMARILY TUMOURS OF THE EWING'S SARCOMA FAMILY (ESFT)

Minimise risk to human health that may occur should people become infected with virus expressing tumour suppressor genes or oncogenes.

Recipient or parental organism
Herpesvirus saimiri (HVS)

Host/vector system
Non-mobilisable expression vectors
Ribozymes

Characterised cytokines, antigens, tumour suppressor genes, oncogenes will be over expressed or expression will be knocked out to investigate their effect on cell behaviour and phenotype using model systems.

The aim of the research is to decrease the growth and induce cell death in the cells by modulating gene expression.

HSV is to be exploited as a delivery system as 1. it has high rates of infectivity and 2. the promoters used are not heavily methylated (hence maximising gene expression/knock-out).

Expression of knock out of cytokines, antigens, tumour suppressor gene or oncogenes will be maximised to decrease the malignant phenotype; characterised by features including cell proliferation, apoptosis and invasion assays. This may lead to the identification of new targets for the development of novel therapeutic strategies.

No known risk is presented to the environment; the HVS will not obtain a survival advantage allowing growth outside the proposed culture conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Effective kill for liquid and solid waste is 100%.
Solid waste (flasks and pipettes): autoclaved and subsequently incinerated. Autoclave efficiency is tested weekly.
Liquid waste: treated with Virkon at a final concentration of 2% for 30 minutes to ensure 100% kill (according to manufacturers instructions). Dispose to drain. Virkon is prepared fresh each week.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Reviewed at Cancer Research UK Clinical Unit GM safety meeting on Monday 23 September 2002. The assessment of risk was accepted and approved.
The University Biological Safety Office has been informed of the assessment of risk and notification.

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Project Ref  559/04.1

Date Ackn’d  16/06/2004

CU2 Project Title  Replication of recombinant picornaviruses

Date Project Ceased

Class  Class 2
Culture Vol  < 1 Litre
Non-GMM  Consent Granted  Not Applicable

Project notified under transitional arrangements N

02/03/2022
Purposes of the contained use

This submission covers the construction and use of recombinant forms of the picornaviruses Human Rhinovirus (HRV), Poliovirus (PV), Equine Rhinitis A virus (ERAV), Coxackie A virus (CAV). Such recombinant viruses are intended to facilitate the determination of viral replication efficiency in cell culture, by the expression of a reporter gene from the recombinant genome.

Recipient or parental organism

The picornavirus family are non-enveloped, single stranded, positive sense RNA viruses which includes a number of important human and animal pathogens. The picornavirus genome is approximately 7 Kb and contains a single large ORF encoding a polypeptide of approximately 2,000 amino acids which is processed by host and viral encoded proteases into mature proteins. The life-cycle of these viruses is well characterised. The proposed picornaviruses (section 6) are classified by the ACDP as class II or below.

Host/vector system

Host: Viral RNA transcribed in vitro in the form of sub-genomic replicons will not encode capsid proteins, will not form virus and can not colonise further cells. RNA in the form of a modified full-length genome will give rise to replication competent virus.

Vector: Modifications will be made to viral cDNA contained in standard non-mobilisable DNA plasmids, grown in laboratory adapted strains of Escherichia coli that are unlikely to survive in the human gut (ACGM/HSE./DOE Note 7, annex 1).

Origin & function

* Well documented ‘reporter’ genes expressing proteins such as Green Fluorescent Protein, B-Galactosidase or Luciferase. Such proteins are non-toxic and are not predicted to alter the nature of the virus.
* Rescue of replication-defective mutations by expression of the p7 protein of hepatitis C virus (HCV): The picornavirus 2B protein is thought to have ion channel activity that is required for replication and mutations in this gene are therefore expected to prevent viral replication. We propose to attempt the partial rescue of such mutations by engineering recombinant picornavirus genomes (as described in Sections A and B) to express HCV p7, a 7kD ion channel protein that has been characterised in the laboratory at Leeds. It plays no part in the transmission or host tropism of HCV and as an isolated gene is not toxic when expressed in mammalian cell culture. It is not thought to contribute to the pathogenesis of HCV, beyond its role as an ion-channel required for HCV replication.

Evaluation of foreseeable effects

Viral RNA transcribed in vitro in the form of sub-genomic replicons will not encode capsid proteins, will not form virus and can not colonise further cells. RNA in the form of a modified full-length genome may give rise to replication competent virus. The pathogenic properties of any modified form of the genome are likely to be reduced relative to wild type virus.

Modified viruses present no more human hazard than wild type virus. The infection resulting from such an exposure is predicted to be significantly reduced relative to wild
type virus, due to the reduced replication efficiency of the modified virus. The effect of expression of a non toxic reporter gene product in infected cells would be negligible relative to the effect of normal wild-type virus replication which destroys the cell. The growth of replication-defective mutants is likely to be very inefficient.

Furthermore, in the context of a natural infection, the genetic instability of the inserted gene combined with normal in vivo selection pressures is predicted to cause rapid reversion to a genotype effectively identical to wild-type.

Infection with ERAV causes a mild respiratory disease in horses.

Picornaviruses present no hazard to any other animals, plants or ecosystems.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste and contaminated glassware is inactivated by overnight treatment with chloros (10% final concentration) or virkon (2% final concentration). Plasticware is autoclaved (130 degrees C, 20 minutes). Such chemical or heat treatment results in 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Following its meeting on 18 December 2003, the Microbiology GM Safety Committee requested some minor changes to the wording of the risk assessment to clarify which picornaviruses were to be used for the project. These changes have been completed to the satisfaction of the BSO.

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</table>

Animal Units

| L2 L3 L4 L2 |
| L3 L4 L2 |

Large Scale Activities

| L2 L3 L4 L2 |
| L3 L4 L2 |

Human Clinical Applications

| L2 L3 L4 L2 |
| L3 L4 L2 |
The aim of the research is to identify how the gut protects the body from invasion and disease caused by bacteria that either normally live in the gut or those that gain entry via contaminated food. Specifically, we will determine the role intestinal epithelial cells play in preventing microbes from invading the body by determining what the consequences of interfering with the expression of genes known to be important in protecting other cells of the body from invasion are on the ability of intestinal epithelial cells to prevent infection by different types of bacteria. A type of virus, Lentivirus, will be used to deliver toxic molecules to epithelial cells that will interfere with the expression of genes involved in microbial defence.

Lentivirus based vectors have been chosen for this study since of all available gene delivery vehicles they are most suited to the delivery of genes into primary, non-dividing, cells. The vectors to be used are replication defective, HIV-1 based, recombinant lentiviral vectors that can only be generated using a transient triple transfection system. Since all HIV coding sequences have been deleted no viral genes can be transferred to the target cells. Due to the transient and reversible effects of the Lentivirus delivered genes, the final modified cells are expected to be more harmful than the unmodified cells.

Recipient or parental organism

Lentivirus has been significantly modified for biosafety. Use is made of self-inactivating (SIN) vectors, which prevent promoter activity in the viral 5' LTR in the integrated provirus from in target cells, reducing the probability of insertional activation. To minimise the possibility for homologous recombination, the HIV genome is divided into three parts, cloned into separate expression plasmids and extensively modified. The following modifications have been made to prevent viral replication.

1. Packaging vector lacks both LTRs and has no viral packaging signal (y).
2. The env, tat, rev, vpr, vpu, vif and nef viral enes have been deleted from the packaging vector.
3. Rev is supplied in trans on a different vector.
4. The vector expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion) and expresses no viral gene product.
5. Envelope (VSVG) is expressed on a separate vector.

Relevant references:
Packaging vectors:

Self inactivating LTR:

Origin & function
The inserted synthetic sequences will encode interfering RNA (RNAi) or short hairpin RNA (shRNA) species that will result in reduced or no expression of the target genes (eg NOD2, alkaline phosphatase, B-galactosidase) in intestinal epithelial cells. The effect of the NRAi/shRNA is expected to be transient and non-lethal and will only interfere with the cells ability to produce certain cytokines in response to challenge with infectious and non-infectious stimuli.

Evaluation of foreseeable effects
Since all HIV coding sequences have been deleted from the Lentivirus vectors to be used no viral genes can be transferred to the target cells. Due to the transient and reversible effects of the Lentivirus delivered genes, the final modified cells are expected to be no more harmful than the unmodified cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
There is very little risk associated with the "escape" of the Lentivirus vectors which alone or in combination cannot produce replication competent virus. Cultured primary intestinal epithelial cells cannot survive outside the culture medium and have a finite life span (≤2 weeks) in vitro. It is expected that Lentivirus-transduced cells will not survive any longer than non-transduced cells (≤2 weeks).

The likelihood of the escape of treated cells is remote. Virus-transduced cells are kept in tissue culture incubators in a category 2 designated tissue culture room within a research laboratory to which only authorised laboratory personnel have access. Access to this laboratory is via locked doors that have a coded keypad locking system and access to the tissue culture room within is restricted to designated personnel and is locked when not in use. A Class 2 cabinet to BSEN 12469 specification will be used to contain virus-producing cell lines and vector-transduced cells. Sharps will be eliminated from all aspects of the laboratory work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Routine disinfection will be carried out using 1% Virkon spray.
Liquid waste will be diluted with an equal volume of 2% Virkon and left for a minimum of 16h prior to drain discard.
Solid waste will be disposed of by autoclaving at 121 degrees C followed by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
Approved by chairmans action in consultation with local BSO, Faculty Safety Manager and an experienced virologist.
To be ratified by the full committee at the next meeting.

6/7/2004 - School of Biology GMAG Committee Chairman.

Project Containment

<table>
<thead>
<tr>
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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref   559/04.3

Date Ackn’d 12/08/2004

CU2 Project Title Investigation of the cellular effects of oncolytic reovirus

Class 2 CultureVolClass2 < 1 Litre

Consent Granted

Non-GMM

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022
Purposes of the contained use

To minimise risk of human infection by wild type reovirus.

Recipient or parental organism

Wild type reovirus is replication competent. However, its replication is selective to cells which have an activated Ras signalling pathway. Most adults possess antibodies to reovirus and administration of the virus to healthy volunteers only has mild toxicity, including minor respiratory or enteric symptoms (Rosen et al, American Journal of Hygiene) 77:29-37. (Norman and Lee, The journal of Clinical Investigation) (2000): 105, pg 1035-38.

Host/vector system

Wild type reovirus will be used to infect a range of established murine and human tumour cell lines, these are hazard Group 1. Reovirus may also be used to infect normal and tumour liver tissue, this is considered hazard Group 2 due to the potential risk of infection from endogenous pathogens.

Origin & function

Reovirus will be provided by Oncolytics Biotech Inc, Calgary, Canada (Reolysin). This product is currently being used in clinical trials and we wish to investigate whether oncolytic killing caused by reovirus infection can stimulate the immune system and release tumour associated antigens for T cell priming.

Evaluation of foreseeable effects

Reovirus will infect all human cells however, it is only replication competent in cells with activated ras signalling pathways therefore, the risk to human health is minimised. Ras mutations and over expression of the ras signalling pathway are most frequently associated with a transformed phenotype and the development of malignancy. However, there is a theoretical risk that ras signalling may be activated in growth factor stimulated cells, cells that proliferate (wound healing) or in people with cellular proliferative disorders. Therefore, we cannot exclude the possibility that reovirus (Reolysin) might infect and replicate in apparently normal cells, causing lysis. Furthermore, the release of tumour associated antigens following lysis may modify the host immune system to infected cells. However, this is unlikely to result in inadvertent disease. Reovirus will also be readily transmitted via aerosols. Therefore, to protect people from the potential risk of infection all work associated with the virus will be carried out at Class 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All reovirus work will be carried out at containment level 2 (Class 2 activity). Virkon is the disinfectant of choice and when used according to the manufacturer's instructions gives 100% kill. Unit procedures for disinfection under category 2 will be followed (see attached sheet). Briefly, all surfaces will be wiped with 2% Virkon and 70% ethanol. All plastics and infected material will be disinfected with 2% Virkon overnight. Gloves and disinfected waste will be autoclaved and incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
This project was reviewed by the GMO safety committee of the CR-UK Clinical Centrem STUH on Thursday 15 July 2004. Although reolysin will only replicate in cells with activated attenuated ras signalling pathways the potential for replicating during wound-healing, cellular proliferative disorders and the aerosol transmission of the reovirus warrant classification at Class 2.

### Project Containment

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### Project Ref 559/04.4

**Date Ackn'd**: 23/09/2004

**CU2 Project Title**: Evaluation of intracellular inhibitors of the HIV-1 Nef protein.

**Class**: Class 3

**CultureVolClass2**: 250 ml

**Non-GMM Consent Granted**: Yes

**Project notified under transitional arrangements**: N

### Project Additional Information
**Purposes of the contained use**

RNA aptamers targeted to the HIV-1 Nef protein will be generated and subsequently tested for the ability to inhibit HIV-1 replication using established cell culture assays. HIV-1 will be produced by transfection of Cos-7 or 293T cells with a proviral clone and subsequent harvesting of culture supernatants. This virus will be used to infect susceptible cells in the presence or absence of aptamers and virus production analysed.

**Recipient or parental organism**

The GMO will be propagated in E. coli strain DH5α as a proviral plasmid clone (DNA copy of the viral RNA genome). Purified DNA will be transfected into either Cos-7 (monkey kidney) or 293-T (human embryonic kidney) cells. Virus will be harvested from these transfected cells and used to infect either Hela-CD4 (human cervical carcinoma) or T-cell lines (Jurkat, CEM) for subsequent experiments.

**Host/vector system**

Plasmids containing a DNA copy of the complete genome of HIV-1 (termed the provirus) will be obtained from University of Heidelberg. The vector backbone is pUC18 - non-mobilisable.

**Origin & function**

Inserted Sequence.

The original pNL4-3 plasmid was generated by cloning a provirus from a bacteriophage library. The library was generated from EcoRI restricted DNA isolated from peripheral blood mononuclear cells (PBMCs) from North American HIV-1 positive individuals. (Genbank accession number M19921). The Nef coding sequence of pNL4-3 was replaced by that of the HIV-1 SF2 isolate (Genbank accession number K02007). The resulting chimeric virus is indistinguishable from pNL4-3 in terms of replication in tissue culture - both Nef isolates have similar functional characteristics.

**Evaluation of foreseeable effects**

The E. coli strains used are laboratory adapted multiple auxotrophs and are unlikely to survive in the environment (ACGM/HSE.DOE Note 7, annex 1). HIV-1 particles are labile, additionally human cell lines containing HIV-1 would be unable to survive outwith tissue culture medium and so present no risk either to the environment or to human health and safety. HIV-1 is not considered to be infectious by any route other than parenteral. The risk of aerosol or water-borne infection of humans is effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Propagation of proviral plasmids in E. coli. The E. coli strain used (DH5α) is a laboratory adapted multiple auxotroph and is unlikely to survive in the human gut (ACGM/HSE.DOE Note 7, annex 1). Growth of plasmids can therefore be carried out at Level 2. Purification of plasmids from E. coli and their subsequent use in transfections of mammalian cells will be carried out at Level 3.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Spent culture medium from growth of E. coli containing proviral plasmids will be inactivated by the addition of Virkon (2% s/v - equivalent to 2000 ppm available chlorine) prior to disposal to the sink.

All work involving introduction of proviral plasmid clones into mammalian cells will be carried out in a Class II cabinet. Waste will be inactivated by addition of Virkon to a final concentration of 1% (w/v - equivalent to 1000 ppm available chlorine) for a minimum period of 2 hours prior to removal from the cabinet and disposal to the sink. Used tissue culture pasticware and other solid waste material will be sterilised by autoclaving (121 degrees C for 20 minutes) using the autoclave located within the Level 3 containment facility.
The proposed waste inactivation measures are appropriate for HIV. As an enveloped virus it will be 100% inactivated by either autoclaving or Virkon treatment.

The GMSC was happy to accept the risk assessment. There was a general feeling that it would be better to grow the E. coli carrying the provirus-containing plasmid in the CL3 room 4.15 in Garstang South, since this would then limit all of the work to the CL3 room. This was only a suggestion rather than a requirement and the committee understood and accepted the rationale for conducting this part of the work under Level 2 conditions.

Please enter comments on the GM safety committee on the risk assessment

The GMSC was happy to accept the risk assessment. There was a general feeling that it would be better to grow the E. coli carrying the provirus-containing plasmid in the CL3 room 4.15 in Garstang South, since this would then limit all of the work to the CL3 room. This was only a suggestion rather than a requirement and the committee understood and accepted the rationale for conducting this part of the work under Level 2 conditions.

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 559/04.5

Date Ackn’d: 22/10/2004

CU2 Project Title: COMPARATIVE ANALYSIS OF PROTEIN EXPORT BY SEC TRANSLOCASES

Class: Class 2

CultureVolClass2: < 1 Litre

Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Withdrawn: N

02/03/2022 Page 9793 of 15326
## Project Additional Information

### Purposes of the contained use

The genetically modified strains will be used for a comparative analysis of proteins exported by the SecA1 and SecA2 translocases of S. pneumoniae, S. aureus, E. faecalis, B. cereus and C. difficile. It is anticipated that the knowledge gained from the comparative analysis of these processes in two pathogens will ultimately lead to the development of novel antimicrobial agents to control infection.

### Host/vector system

The widely used allele replacement vector pG+Host will be used for mutagenesis and gene inactivation of the secA1 and secA2 genes of S. pneumoniae, S. aureus, E. faecalis, B. cereus and C. difficile. The secA1 and secA2 genes will be deleted and mutated in their natural host and also cloned into E. coli for expression and protein purification.

### Origin & function

The comparative nature of the proposed project dictates that the genetic material will originate from S. pneumoniae, S. aureus, E. faecalis, B. cereus and C. difficile. The secA1 and secA2 genes will be deleted and mutated in their natural host and also cloned into E. coli for expression and protein purification.

### Evaluation of foreseeable effects

The release of numbers of viable organisms required to colonise or infect animals or humans is highly unlikely. The genetically modified organisms are unlikely to pose any greater hazard to health than the respective unmodified wild types. The current containment and working procedures in the laboratory are more than sufficient to contain the organisms and prevent a major escape. If any spillages occur the organisms will be inactivated with a disinfectant such as Stericol, then absorbed with paper towels that will be autoclaved prior to disposal. All cultures, media, contaminated glassware and contaminated plasticware will also be autoclaved prior to disposal or reuse, as per School of Biochemistry and Microbiology standard operating procedures.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Prior to autoclaving all small items of glassware and plasticware will be immersed in 0.5% TEGO in specified discard pots. All waste cultures, media and large plastic items...
will be autoclaved prior to discarding. Contaminated reusable glassware will be autoclaved before being washed. Sharps will be disposed of in cin-bins that will be subsequently autoclaved and incinerated. Spillages of microorganisms will be absorbed with absorbant towels, all organisms killed with Stericol and the towels autoclaved prior to disposal. No genetically modified organisms will be viable after the measures described above have been carried out.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Microbiology GM Safety Committee requested some minor changes to be made to the Risk Assessment and notification form. These changes have been completed to the satisfaction of the BSO.

Project Containment

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<td>Human Clinical Applications</td>
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Project Ref  559/05.1

Date Ackn’d  03/02/2005
CU2 Project Title  Use of recombinant herpes simplex helper vectors to express variants of human RYRI gene in HEK293 cells and murine dyspedic myoblasts.

Date Project Ceased
Withdrawn  N

Tick if notifying a connected programme of work  N
Purposes of the contained use

The aim of the research is to understand if and how sequence variations in the human RYRI gene implicated in susceptibility to malignant hyperthermia (MH), affect calcium release in skeletal muscle cells.

Recipient or parental organism

The work will be carried out exclusively using cell lines; no GMOs are involved.

Host/vector system

HSV-based vectors have been chosen since they are the only ones currently available that are capable of delivering the 15.5 kb RYRI cDNA into HEK293 cells, dyspedic myoblasts and differentiated myotubes. The expression system has been significantly modified for bio-safety. The cosmid set C6 a48 a consists of overlapping clones that are able to produce all components essential for HSV-amplicon packaging and assembly, but which lack any packaging signal themselves. No recombination is possible between C6 a48 a and the amplicon and no infectious viral particles can be generated.

Reference:

Origin & function

The inserted sequences will encode the human skeletal muscle protein, RyR1, and variations thereof engineered into the RYRI coding sequence by site-directed mutagenesis. The RyR1 protein is responsible for regulating calcium release from intracellular stores during muscle contraction.

Evaluation of foreseeable effects

No organisms will be produced. The modified muscle cell and HEK293 cells are no more harmful than unmodified cells. Cultured 2-2 cells, HEK293 cells and dyspedic myoblasts are unable to exist without culture medium. The likelihood therefore of any of these cells being able to accidentally infect or colonise laboratory staff or any vermin vectors is virtually non-existent.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals or plants are involved. Amplicon-transfected cells will be maintained in tissue culture incubators in a category 2 designated tissue culture room within a research laboratory to which only authorised laboratory personnel have access. Access to this laboratory is via doors that are locked when not in use. There is very little risk associated with the “escape” of the HSV-packaged amplicons and levels of replication competent virus are virtually non-detectable. Cultured myoblasts, myotubes and HEK293 cells cannot survive without culture medium and have a finite life span (<3 weeks) in vitro. Transfected cells will not survive any longer than non-transfected cells (<3 weeks).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Routine disinfection will be carried out using 1% Virkon spray.
Liquid waste will be diluted with an equal volume of 2% Virkon and left for a minimum of 16 h prior to drain discard.
Solid waste will be disposed of by autoclaving at 121 degrees C followed by incineration.

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]  

Approved by Chairman's action, in consultation with local BSO and Faculty Safety Manager. To be ratified by the full committee at the next meeting.

### Project Containment

- **Laboratory Activities**
  - L2: Yes
  - L3
  - L4

- **Glass Houses**
  - L2
  - L3
  - L4

- **Growth Rooms**
  - L2
  - L3
  - L4

- **Animal Units**
  - L2
  - L3
  - L4

- **Large Scale Activities**
  - L2
  - L3
  - L4

- **Human Clinical Applications**
  - L2
  - L3
  - L4

### Project Ref 559/05.2

**Cloning and characterisation of the zylanase operon promoter of Bacteroides ovatus.**  
Expression of genes encoding immunomodulatory proteins, growth factors and cytokine/growth factor antagonists by B ovatus under control of the zylanase promoter and secretion of recombinant proteins.

- **Date Ackn'd:** 14/04/2005
- **Date Project Ceased:** 09/10/2008
- **Consent Granted:** Not Applicable
- **Class:** Class 2
  - CultureForClass2: < 1 Litre
- **Non-GMM:** Not Applicable
- **Project notified under transitional arrangements:** [N]

**Tick if notifying a connected programme of work:** [N]
This project has transferred to GM

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The modified B. ovatus will be used to assess zylan-inducible expression of the genes described in Section 5. Recombinant organisms will then be used in experiments as novel immunotherapies to try and treat and prevent disease.

Recipient or parental organism

B. ovatus is ACDP Hazard Group 2. The final modified organism will be tetracycline resistant. Although a gut commensal, the likelihood of the GMM colonising humans or persisting in the environment is minimal. Expression of the genes described in Section 5 by this organism should not pose a hazard.

Host/vector system

E coli vector pGEM is non-mobilisable. Vector pBT-2 is an E. coli-Bacteroides mobilisation-defective shuttle vector requiring E. coli strain J53/R751 for transfer. This is a suicide vector in Bacteroides, requiring integration into the genome for maintenance.

E. coli DH5α and J53/R751 are ACDP Hazard Group 1.

B. ovatus is Hazard Group 2.

Origin & function

The zylanase promoter originates from B. ovatus. They cytokine/growth factor and antagonist genes will be cDNA copies that will be transcribed in a zylan-inducible manner in the final GMM.

Evaluation of foreseeable effects

The final GMM will be tetracycline resistant. Expression of genes described in Section 5 should be regulated and the modified organism must compete with resident microflora to colonise. Release of the number of viable organisms required to colonise other animals or humans is unlikely. Survival in the environment is likely to be limited. Expressed gene products are unlikely to be produced in large enough quantities to have a significant effect on humans. The modified organism should pose no greater hazard than the wild type.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All pipette tips, slides and small disposable plasticware will be disposed of in discard pots containing 2% (v/v) Tego prior to being autoclaved and discarded.

All waste cultures, media and larger plasticware will be autoclaved prior to discarding.

All glassware will be autoclaved before being washed and returned for use.

Sharps will be disposed of in a cin bin which will then be autoclaved and incinerated.

All culture spills will be mopped up with Trigene and tissue that will then be autoclaved prior to discarding.
No viable GMMs will be left after treatment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Following consideration of this application by the Microbiology GM Safety Committee at its meeting on 16 August 2004, the Committee requested some very minor, mostly typographical changes to be made. These changes have been completed to the satisfaction of the BSO.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<thead>
<tr>
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<th>Growth Rooms</th>
</tr>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 559/06.1

Date Ackn'd 19/12/2006

CU2 Project Title Development of plasmid vectors for human skin commensal bacteria, namely propionibacteria and corynebacteria.

Class 2 CultureVolClass2 1-50 Litres

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info
## Project Additional Information

### Purposes of the contained use

To develop plasmid vectors for the introduction of foreign DNA into propionibacteria and corynebacteria. Such vectors will enable the generation of mutants and use of reporter genes to study gene function/regulation in these organisms, metabolism and their role as commensals and potential pathogens.

### Recipient or parental organism

Propionibacterium acnes, P. granulosum and P. avidum are Group 1 organisms and commensals of human skin. Corynebacterium jeikeium is Group 2 and is a human commensal and pathogen. Corynebacterium glutamicum is Group 2 but is not considered pathogenic. Also used will be E.coli K-12 based cloning hosts (e.g. DH5α) that are especially disabled; E.Coli S17-1, a K-12 derivative capable of mobilising plasmids containing the RP4 oriT region; E.coli BL21 and derivatives, hosts for recombinant protein production that are considered equivalent to K-12 strains.

### Host/vector system

- pBluescript, pGEM, pCR and similar pUC-based, non-mobilisable E.coli cloning vectors. pGEM and pET series plasmids, non-mobilisable and mobilisable defective respectively; used for recombinant protein expression. pK18mob2, a pUC-derivative capable of being mobilised from E.coli S-17 but not self transmissible. None of these plasmids encode toxic or otherwise dangerous products. All derive from standard cloning replicons and have a history of safe use in other laboratories.

### Origin & function

Replicons from endogenous propionibacterial and corynebacterial plasmids that will be tested for function in the hosts listed above. Antibiotic resistance markers to allow for selection of recombinant plasmids including hygB from Streptomyces hygroscopicus that confers resistance to hygromycin B, cmx from C.jeikeium conferring resistance gene of transposon Tn5. A fragment of any gene from propionibacteria or corynebacteria used to mediate homologous recombination and inactivation of that gene by plasmid insertion. A promoter of any gene from propionibacteria or corynebacteria ligated to a reporter gene (see below) to allow promoter to be measured. Commonly used reporter genes such as GFP and colour variants, β-galactosidase, bacterial luciferase. Genes from propionibacterial and corynebacterial plasmids encoding proteins potentially involved in replication and transfer functions will be cloned into E.coli for production of recombinant proteins for in vitro functional studies.

### Evaluation of foreseeable effects

The GMOs will contain genes encoding antibiotic resistance but this is not expected to have a harmful effect upon humans, plants or animals as they will not produce potentially harmful products. It is extremely unlikely that GMOs would be able to survive outside culture in the external environment, infect local vermin vectors, or survive as a commensal of animals and plants. These organisms are human commensals (except C.glutamicum) and if they were to escape from containment they must compete with the resident microflora in order to colonise. However, containment measures will ensure that the likelihood of escape of sufficient numbers of organisms for establishment on a human host is effectively zero. The modified organism should pose no greater hazard than the wild-type.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste is treated with an equal volume of 2% Virkon and left overnight before drain disposal with 100 volumes of water. Solid waste (culture or cells) must be autoclaved at 121°C (load temperature) for 20 minutes before being destroyed as clinical waste. All glassware will be autoclaved before being washed and returned for use. Sharps will be disposed of in a cin bin which will then be autoclaved and incinerated. All culture spills will be mopped up with 2% Virkon and tissue which will then be autoclaved prior to discarding. No viable GMMs will be left after treatment.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Ticket to confirm that you have attached a risk assessment to this form Y
Ticket if you are claiming exemption from disclosure for section of the risk assessment N

The committee considered the status of Corynebacterium Jeikeium as a host organism in particular because, although it is generally regarded as a commensal, there are references to it being an opportunistic pathogen of humans and it has occasionally been known to cause serious disease in vulnerable individuals. Hence we concur that the correct classification of this project is 2.

AP Kelly - Committee Chair Nov 2006

Project Containment

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Project Ref 559/08.1

<table>
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<td>Immune consequences of oncolytic virus tumour cell infection.</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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</table>

Date Project Ceased

02/03/2022
**Project Additional Information**

**Purposes of the contained use**
To investigate in 'in vitro' systems the direct adjuvant properties of replication competent wildtype (wt) and recombinant attenuated oncolytic viruses on innate and adaptive immune effector cells. Also, to investigate the ability of wt and recombinant attenuated oncolytic virus infected cells to prime innate and adaptive anti-tumour immunity. Furthermore, since the use of oncolytic viruses may be limited due to the induction of host anti-viral immunity the efficacy of combined oncolytic virotherapy may be investigated.

**Recipient or parental organism**
Wildtype and genetically modified forms of the oncolytic viruses Adenovirus type 5 (Ad5), Measles virus Edmonston B (MV-Edm), vaccinia virus Western Reserve (WR) and specifically wtAd5, Ad5d1309, Ad5d1922-947, Ad5 CMV GFP (expressing GFP), wtMV-Edm, MV-GFP (expressing GFP), wtWR, ddW, vvGMCSF (JX-963) (expressing GMCSF).

**Host/vector system**
A range of established murine and human cancer cell lines will be infected with each of the oncolytic virus strains. These are biological hazard group 1. In addition, primary normal and transformed cells/tissue from cancer patients and allogeneic immune effector cells isolated from the blood of healthy donors will be infected. These are considered to be biological hazard group 2 due to the potential risk of infection from endogenous pathogens.

**Origin & function**
All Ad5 vectors will be provided by (Baits and the London School of Medicine and Dentistry). All MV- Edm vectors will be supplied by (Mayo Clinic College of Medicine, Rochester, Minnesota, USA) The vaccinia virus vectors WtWR and ddW-GFP will be provided by (Stanford University School of Medicine, California, USA) and the vaccina virus vector w-GMCSF (JX-963) will be provided by (JENNEREX Biotherapeutics, Inc., San Francisco, California, USA). The oncolytic viruses Adenovirus type 5 (Ad5), Measles virus Edmonston B, vaccinia virus have all shown promise as therapeutic oncolytic agents. Recombinant oncolytic virus induced-tumour regression generates long term anti-tumour immunity: however the mechanisms by which this occurs remain largely undefined. The aim of this project is to investigate the ability of oncolytic viruses to activate human innate and adaptive anti-tumour immunity. Furthermore, a direct comparison between the wildtype and attenuated recombinant strains of these oncolytic viruses will determine which strain is most effective with regards to tumour selectivity, cytotoxicity, and activating immune effector cells to prime anti-tumour immunity.

**Evaluation of foreseeable effects**
Wildtype Adenovirus type 5 (wtAds) is capable of replication in both normal and transformed cells. The genetically modified variants Ad5d1309 & Ad5d1922-947 can only replicate in tumour cells and are more immunogenic and less hazardous than wt4d5. Ad5-CMV-GFP is completely incapable of replication thus is of negligible hazard. The
natural host for Ad5 is humans, although it can infect a range of other mammalian cells including rodent, swine and rabbit and transduce both dividing and non-dividing cells. It is transmitted via aerosol. Adenoviruses do not integrate into the host genome and therefore the risk of mutagenesis is extremely low. Ad5 has a long history of use as a therapeutic oncolytic agent both as wildtype virus and as a gene therapy vector and has an excellent safety record. Infection in humans usually causes mild, self-limiting disease (mild respiratory disease, gastroenteritis, conjunctivitis) which is well-characterized. Very rarely in infants and children, pertussis-like syndrome, acute febrile pharyngitis and hepatitis has been seen. Exposure to wtAd5, Ad5d1309, Ad5d1922-947 or Ad5-CMV-GFP is highly unlikely to result in toxicity as immune recognition and activation would control viral pathogenesis. All work with these viruses will be carried out in containment level II facilities to minimise exposure to people and the environment. Please see ADDITIONAL COMMENTS SECTION for information on Measles virus Edmonston 8, Vaccinia virus ;Western Reserve.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon disinfectant is effective against Adenovirus, Measles virus, Vaccinia virus at concentrations of 1%, 0. 4%, 1% and 0.16% respectively. The minimum contact time for 100% efficacy is 10 minutes. Virkon at 2% concentration will be used to decontaminate all virally contaminated waste liquids and plasticware and any surfaces and equipment which have been exposed to virus. Following decontamination solid waste will be autoclaved and then sent for incineration. Decontaminated liquid waste will be disposed of via the sink, rinsing with copious water. See also attached risk assessment, sections 7.4 & 7.5.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Chairman, GMAG Local Safety Committee), (LIMM Safety Officer), and ‘University 850) had reviewed the proposal in detail prior to the meeting of the LIMM. The amended proposal was then submitted to the remaining members of the Committee prior to the meeting.

The main issues for discussion and clarification at the meeting centred on where the projects were to be undertaken, the siting of equipment, and routes of disposal. had inspected the proposed work area prior to the meeting. Upon recommendation, the original siting of an EL.ISA plate washer proposed for the study was to be relocated into a Class 2 biological safety cabinet. For centrifugation of samples, the opening of all tubes would be performed in Class 2 biological safety cabinets. For flow cytometry studies, all cells would be fixed with paraformaldehyde before transferring for analysis. asked for the details of the fixation procedure to be included in the submission to HSE. Discussion then centred on procedures for handling and disposing of waste. The committee came to the conclusion that well implemented safety procedures were in place to prevent potential problems of cross contamination of cultures, and safe disposal of contaminated plasticware and solutions. The Committee therefore approved the procedures. The Committee also stressed that all workers entering the lab must be classified as Class 2 workers and register with University Occupational Health.

Permission was given to send off the application to HSE after final approval by on behalf of the University.

02/03/2022  Page 9803 of 15326
We are in the process of characterising three strains of Staphylococcus aureus recovered from patients in Denmark. These strains have been found to possess an antibiotic resistance gene (fusB) which encodes resistance to fusidic acid, but the strains are not phenotypically fusidic acid resistant. To establish how this resistance determinant has been ‘silenced’ in these strains, we wish to introduce several previously-generated plasmid-based constructs into these strains. The constructs are as follows and the rationale for their use is indicated.

Plasmid pCUI carrying the resistance gene fusB expressing from its native promoter will be used to determine whether the gene is being silenced at its promoter. Plasmid pCU1 carrying the fus8 gene fused to a reporter gene, iacZ, will also be used to determine if the fus8 gene is being silenced at its natural promoter. The fusion of iacZ to fusB enables the production of beta-galactosidase which can be easily monitored.
Plasmid pAJ96 carries fusB under the control of an artificial promoter that can be controlled by the antibiotic tetracycline. The construct will enable us to determine if the resistance is silenced post-translationally rather than at the level of gene expression. These plasmid constructs have been used previously in Class I work with the especially disabled host S. aureus RN4220. This application is simply to enable to use of these reporter constructs in non-disabled (clinical) S. aureus host strains to examine their unique properties regarding antibiotic resistance.

Recipient or parental organism

Recipients:
- S. aurous 15522 is a carriage isolate recovered from a human nasal swab.
- S. aureus 2329 was isolated from a patient that developed an abscess with community onset.
- S. aureus 43941 was isolated from a community acquired otitis infection.
All of the above have an inherent potential to cause human infection, be carried by humans and by animal vectors but the modification will not increase the virulence or pathogenicity of the GMMO. Treatment is available for infection and the GMMO is unlikely to cause environmental damage.
The GMMO’s will be modified by the introduction of plasmid vectors containing a resistance gene (fusE) already present but not expressed in these strains, but places it under the control of a native and a non-native promoter and also introduce fusB fused to the reporter gene lacZ.
None of the genes used contain harmful adventitious sequences or oncogenic properties and are not likely to promote allergen/toxin production. The reporter gene has been used previously in laboratory strains of E. coli and S. aureus RN4220.

Host/vector system

Host:
- S. aureus RN4220: restriction-deficient derivative of 8325-4 (which is itself listed as a disabled laboratory strain in the HSE compendium). It accepts foreign (unmodified) DNA.
- E. coli K-12-based cloning hosts (e.g. JM109, XL1 Blue): these are listed in the 1-ISE compendium as especially disabled and are multiple auxotrophs

Vectors:
- pCU1: standard cloning shuffle vector for E. coli/S. aureus (Augustin at at, European Journal of Biochemistry, 1992; 204; 1149-1154)
None of these plasmids encode toxic or otherwise dangerous products.
All these plasmids derive from standard cloning replicons or transposons, have a history of safe use and/or are already in use in other laboratories for similar purposes to those described here, are not self-mobilisable mobilisable in trans.

Origin & function

The inserted sequence, the gene fusB, is present within the recipient organisms and encoded the fusidic acid resistance protein FusB. The vector systems have been described previously and have a safe history of use, the constructs have also been described previously (O’Neill et at, Antimicrobial Agents and Chemotherapy 2007; 51:1731-1740 and O’Neill and Chopra, Molecular Microbiology, 2006; 59:664-676).
The GMMO’s will be used to examine silencing of expression of fusidic acid resistance1 it will determine whether silencing occurs at the gene or protein level. The recipient strains possess the resistance gene fusB but do not have a fusidic acid resistant phenotype, and the constructs when introduced allow the monitoring of gene and protein expression.

Evaluation of foreseeable effects

The final GMMO is highly unlikely to constitute a hazard to the environment or community in the unlikely event of...
maximum scale escape from containment. It is unlikely that it would be able to survive outside culture in the external environment1 infect local vermin vectors, or survive as either a minor pathogen, or a commensal of plants. It is possible that the GMMO may be able to survive as a commensal or as a minor pathogen in animals and humans. However the GMMOs would not be more virulent than the original host strains and no dangerous staphylococcal genes or resistance determinants would be introduced that are not already present in the staphylococci.

The GMMO would not be predicted to cause significant economic or ecological consequences in the case of a major escape.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste (culture or cells) will be autoclaved at 121°C (load temperature) for 20 minutes to give 100% kill before being destroyed as clinical waste. Liquid waste will be similarly autoclaved at 121°C (load temperature) for 20 minutes to give 100% kill before being destroyed as clinical waste. For discarding pipette tips, other small pieces of disposable plastic-ware and associated small volumes of liquid culture (<1 ml) 1% TEGO 2000 disinfectant is used. This is autoclaved at least weekly to give 100% kill. Emergency spill procedure: Liquid spill — treat with Virkon granules until deep pink, or add an equivalent volume of 2% Virkon — will be left for at least half an hour before being mopped up with plenty of water. Wearing strong gloves of the “Marigold” or similar type, solid spills will be gathered into an autoclave bag, the area affected will be wiped liberally with 2% Virkon. The treated area will be left for half an hour for the disinfectant to have time to act and the area will then be mopped up with plenty of water. Immediately after it has been collected and the affected area has been treated with Virkon, the solid waste will be taken for autoclaving, as above.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

**Please enter comments on the GM safety committee on the risk assessment**

The Faculty of Biological Sciences Genetic Modification Safety Committee approved the proposed project at containment level 2 unanimously and without additional comment.

**Project Containment**

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02/03/2022
Project Ref 559/08.3

Date Ackn’d 21/08/2008

CU2 Project Title INHIBITION AND OVEREXPRESSION OF GENES RELATING TO EXTRACELLULAR MATRIX REMODELLING.

Date Project Ceased

Class 2

Consent Granted Not Applicable

Class CultureVol

Volume Class 2

< 1 Litre

Non-GMM

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

We aim to use replication deficient recombinant human adenovirus (rAd) to introduce the following genes and short-hairpin RNAs (shRNA) into primary cultures of human cardiac fibroblasts, vascular smooth muscle cells and endothelial cells, the intact human saphenous vein organ culture (vein graft stenosis model: Porter et al., J Vasc, Surg, 36, 150-157), and commercially available cell lines such as HCAEC's (human coronary artery endothelial cells, Clonetics) and HT-1080s (human fibrosarcoma cells, ECACC):

i) Matrix Metallproteinases (MMP) in both sense and anti-sense orientations and also dominant negative forms may be expressed.

ii) Tissue inhibitors of Matrix Metallproteinases (TIMPs) in both sense and anti-sense orientations and also dominant negative forms may be expressed.

iii) p38 Mitogen-activated protein kinases (p38 MAP kinases) in both sense and anti-sense orientations and also dominant negative forms may be expressed.

iv) Heme-oxygenases (HOs) in both sense and anti-sense orientations and also dominant negative forms may be expressed.

v) Short-hairpin RNAs (shRNA used in RNA interference) targeted against endogenous genes (i-iv)

vi) Coral green fluorescent protein (cGFP)

By placing genes (i-iv) under the control of the tetracycline response element (TRE), the levels of expression of recombinant proteins can be tightly regulated thereby enabling a better understanding of their specific role extracellular matrix (ECM) remodelling in cardiovascular disease.
shRNA – targeted against endogenous genes (i-v) are under the control of the human H1 promoter, an RNA polymerase III (Pol III) promoter, ideally suited to the expression of RNA stem-loops (ie shRNA) in a variety of human cells. cGFP is a fluorescent marker protein, simultaneously expressed by shRNA containing recombinant adenoviruses, and under the control of a cytomegalovirus (CMV) promoter for tracking transfection.

Recipient or parental organism

The recombinant adenoviruses will be introduced into primary cultures of human cardiac fibroblasts, vascular smooth muscle cells and endothelial cells, and into the intact human saphenous vein organ culture (vein graft stenosis model; Porter et al., J, Vasc.36, 150-157.

Host/vector system

Short-hairpin RNA (shRNA used in RNA interference)

shRNA targeted against endogenous genes of stress-induced pathways associated with extracellular matrix (ECM) physiology and regulations (i.e. MMPs, TIMPs, HOs, p38 MAP kinases etc), and therefore essential to cardiovascular remodelling. Custom-designed oligonucleotides (~75bp in length) will be purchased from Invitrogen and cloned into the pRNAT-H1.1/Adeno Shuttle vector.

pRNAT-H1.1/Adeno Shuttle vector (commercially available from www.genscript.com): For use in shRNA expression studies. This is an adenoviral shRNA shuttle vector. It is compatible with Stratagene AdEasy Adenoviral Vector System. The vector is designed for transfection in mammalian cells. An H1 promoter is used to drive siRNA expression. cGFP marker is under the control of cytomegalovirus (CMV) promoter for tracking transfection efficiency. The vector also contains a Kanamycin resistance gene for transformant selection. LITR and RITR regions are available in this vector as a function for recombinant viral DNA replication. Vector is mobilisation defective; capable of replicating plasmid DNA only in bacterial cells under selecting conditions. This plasmid is already available in our laboratory.


This plasmid contains a modified version of the human adenovirus serotype 5 (Ad5 genome in which the E1 and E3 genes have been deleted. The E1 deletion renders the virus replication deficient and incapable of producing infectious viral particles in target cells (provided there is no complementation by the host cells); the E3 deletion significantly reduces the ability of the virus to evade detection by the host immune system. The removal of these two genes not only render the virus incapable of replicating itself, but they also create space for up to 7.5kb of foreign DNA.

Vector is mobilisation defective; capable of replicating plasmid DNA only in bacterial cells under selecting conditions. This plasmid is already available in our laboratory.

Origin & function

To generate the recombination adenovirus (rAd), using the method of T.C. He and colleagues, involves cloning the gene of interest (ie shRNA into a shuttle vector and transferring the gene into the adenovirus genome by means of homologous recombination in an adenovirus packaging cell line. Employing the efficient homologous recombination machinery in E.coli, a rAdS is produced by a double-recombination event between the co-transformed adenoviral backbone plasmid vector and a shuttle vector carrying the gene of interest. Transfect low-passage HEK293 cells with linearised recombinant adenoviral DNA to generate recombinant adenovirus.

pTRE-Shuttle2 (commercially available from www.clontech.com)

pTRE-Shuttle2 is a cloning vector designed for the use with the Adeno-X Tet-on expression system. pTRE-Shuttle2 is used to clone the gene of interest into a tetracycline (Tet)-responsive expression cassette. The expression cassette consists of a Tet-responsive promoter (Phcmv*-1), a multiple cloning site (MCS), and two SV40 polyadenylation signals. The Phcmv*-1 promoter contains the Tet-responsive element (TRE). The TRE element is located just upstream of the minimal cytomegalovirus promoter (PminCMV). Because it lacks the enhancer that is part of the complete CMV promoter, PhCMV*-1 is silent in the absence of binding of the reverse Tet-controlled transactivator (rtTA). Consequently, genes inserted in the MCS are actively transcribed only when rtTA binds the TRE element. Vector is mobilisation defective; capable of
replicating plasmid DNA only in bacterial cells under selecting conditions. This plasmid will be purchased from Clontech.

**Adeno-X System 1 Viral DNA**

To produce recombinant adenovirus transfer the (Tet)-responsive expression cassette containing the gene of interest (from pTRE-Shuttle2) into the ligation-ready Adeno-X System 1 Viral DNA (linearised plasmid containing the recombination deficient Ad5 gene). Transform an E.coli strain with the ligation mixture and identify recombinant clones. Transfect low passage HEK293 cells with linearised recombinant adenoviral DNA to generate recombinant adenovirus. This linearised plasmid will be purchased from Clontech.

**Adeno-X ™Tet-On virus**

trTA is encoded by the Adeno-X Tet-On Virus supplied by Clontech. To establish the Adeno-X Tet-On expression system, target cells are co-infected with the recombinant virus containing the gene of interest together with the Adeno-X Tet-On Virus. The virus will be purchased from Clontech.

**Matrix Metalloproteinases (MMPs)**

Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodelling, as well as in disease processes, such as arthritis and cancer and cardiovascular pathologies. Most MMPs are secreted as inactive proproteins which are activated when cleaved by extracellular proteinases. MMP genes will be cloned into the pTRE Shuttle2 cloning vector and subsequently used to establish an Adeno-X Tet-On expression system. cDNAs encoding full length human MMPs (MMP-2, MMP-3, MMP-9 and MMP-14 in the first instance will be generated in our laboratory.

**Tissue Inhibitors of Matrix Metalloproteinases (TIMPs)**

TIMPs are endogenous regulators of MMPs and adamalysin metalloproteinase activity, and therefore play important roles in regulating cellular functions that are dependant on matrix composition such as invasion migration, differentiation and proliferation. In addition, TIMP-2 has been shown to be required for MMP-2 activation, while TIMP-3 overexpression induces apoptosis. TIMP genes will be cloned into the pTRE-Shuttle2 cloning vector and subsequently used to establish an Adeno-X Tet-On expression system. cDNAs encoding full length human TIMPs (ie TIMP-1, TIMP-2 and TIMP-4) will be generated in our laboratory. cDNA encoding full length human TIMP-3 is already available in our laboratory (a kind gift from Dr Sarah J George (Bristol Heart Institute, University of Bristol).

**p38 MAP Kinases**

Mitogen-activated protein kinases (MAP kinases) are proteins that control cell responses to inflammation and growth signals. p38 MAP kinases, a member of this protein family, controls the production of growth factors and inflammatory cytokines, the molecules produced by the immune system that cause inflammation. p38 genes will be cloned into the pTRE-Shuttle2 cloning vector and subsequently used to establish an Adeno-X Tet-On expression system. cDNA encoding full length human p38 MAP kinase (α,β,δand y) are already available in our laboratory.

**Heme-oxygenases (HOs)**

Endogenous heme catabolism by the heme oxygenase (HO) family of enzymes, which exist in constitutive (HO-2 and HO-3) and inducible (HO-1) isoform, liberate CO, biliverdin and Fe in stoichiometric amounts. HO exists as a macromolecular complex in the endoplasmic reticulum together with cytochrome c reductase and biliverdin reductase. CO has been established as an important, protective signalling molecule in both the heart and vasculature. HO genes will be cloned into the pTRE-Shuttle2 cloning vector and subsequently used to establish an Adeno-X Tet-On expression system. cDNAs encoding full length human HO-1, HO-2 and HO-3 will be generated in our laboratory.
Coral Green Fluorescent Protein (cGFP)

cGFP is a fluorescent marker protein used to assess transfection efficiency in host cells. A cDNA encoding cGFP is already available in the laboratory. cDNA encoding full length cGFP is already available in our laboratory as part of the pRNAT-H1.1/Adeno Shuttle vector (www.genscript.com).

Evaluation of foreseeable effects

All adenovirus serotypes from subclass C, which include the well studied Ad2 and Ad5 serotypes, are non-oncogenic. Wild-type Ad5 infections involve either the respiratory or gastrointestinal tracts (infections are generally mild in children), or the eye (all persons). Because the rAd will lack the E3 gene this may produce a virus that will evoke a stronger inflammatory response in the host, but this should also lead to a more rapid clearance of the virus from the host system. Adenovirus infections are very common, most are asymptomatic. Primary infection of adenovirus is thought to generate life-long immunity and it is thought that the majority of the adult population is likely to have antibodies to the wild-type virus (most people have been infected with at least one type at age 15). Further, whilst the risk is minute the fear is that the defective virus may regain some of its ability to replicate - this is known to occur with adenovirus systems but to date they have always reverted to wild type adenovirus of one sort or another, i.e. never shed genetically modified virus. Although replication has been shown to occur in the lungs of experimentally infected cotton rats administered with high doses of virus, there is no evidence the adenoviruses can naturally colonise non-human hosts and therefore pose no significant harm to animals, plants or ecosystems.

Short-hairpin RNA (shRNA used in RNA interference)

Not being oncogenic themselves, shRNA targeted against endogenous genes (ie MMPs, TIMPs, Hos) associated with extracellular matrix (ECM) degradation (which facilitates malignant cell invasion and metastasis), will not have adventitious effects or confer an oncogenic phenotype upon the host cells at the level of expression used. After conducting a thorough literature review, I am confident there are no additional risks above those already referred to.

Matrix Metalloproteinases (MMPs)

Most of all MMPs have a role in cancer, although not being oncogenic themselves, since their role in extracellular matrix (ECM) degradation, facilitates malignant cell invasion and metastasis. Transformation of a cell to a full malignant phenotype however, is dependent on a number of changes in expression in genes controlling signal transduction/gene expression cascades with the cell or genes involved in regulation of the cell cycle. It is not envisaged that expression of these transcripts whether Sense, Antisense or Dominant negative mutant will have adventitious effects or confer an oncogenic phenotype upon the host cells at the level of expression used. After conducting a thorough literature review, I am confident there are no additional risks above those already referred to. Expression of the gene will be placed under the tight control of a tetracycline-inducible promoter system. Transgene expression can only occur in response to tetracycline exposure, the levels of which can be finely regulated in this system.

Tissue Inhibitors of Matrix Metalloproteinases (TIMPs)

TIMPs are endogenous regulators of MMPs and adamalysin metalloproteinase activity, although not being oncogenic themselves; nevertheless most if not all MMPs have a role in cancer, since their role in ECM degradation facilitates malignant cell invasion and metastasis. However, like the MMPs and after conducting a thorough literature review, it is not envisaged that expression of these transcripts whether Sense, Antisense or Dominant negative mutant will be oncogenic or have adventitious effects in host cells at the level of expression used. Furthermore, expression of the gene will be placed under the control of a tetracycline-inducible promoter.

P38 MAP kinases

Activation of p38 MAP kinase results in increased production of molecules that cause inflammation such as interleukin-1 (IL-1), tumor necrosis factor (TNF), and cyclooxygenase-2 (COX-2). P38 MAP kinase also regulates the expression of a number of other proteins, including interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF), a regulator of new blood vessel formation that can affect cancer cell growth. Inhibition of p38 MAP kinase could reduce the expression of these and other proteins important in the development and progression of inflammatory disease and cancer. Again, after conducting a thorough literature review it is not envisaged that expression of these transcripts whether Sense, Antisense or Dominant negative mutant will be oncogenic or have adventitious effects in host cells at the level of expression used. Furthermore, expression of the gene will be placed under the control of a tetracycline-inducible promoter.
Heme-oxygenases (Hos)

Cardiac atrial and ventricular myocytes express HO-1 and HO-2, and HO-1 levels can be increased by various stress factors including myocardial infarction. CO limits the cellular damage of ischemia/reperfusion injury in the heart. Indeed, greater cardiac damage is seen following ischemia/reperfusion injury in HO-1 knockout mice. Conversely, HO-1 over-expression in the heart reduces infarct size and other marker of damage following ischemia/reperfusion injury. CO also improves cardiac blood supply through dilation of coronary vessels, and reduces cardiac contractility. In the vasculature, CO also exerts numerous beneficial effects. Its ability to dilate blood vessels is long established, endothelium independent and not due to development of hypoxia through displacement of $O_2$. CO has clear, protective effects in various vascular diseases, such as systemic and pulmonary hypertension, development of atherosclerosis and neointimal hyperplasia due to profiliation of vascular smooth muscle cells following vascular injury. However, several human tumours, including renal cell and prostate cancer, express high levels of HO-1. HO-1 may promote tumour cell survival, hindering anti-cancer therapy effectiveness. In contrast, HO inhibition has been shown to enhance tumour regression in some animal models. After conducting a thorough literature review, it is not envisaged that expression of these transcripts whether Sense, Antisense or Dominant negative mutant will be oncogenic or have adventitious effects in host cells at the level of expression used. Furthermore, expression of the gene will be placed under the control of a tetracycline-inducible promoter.

Coral Green Flourescent Protein (cGFP)

Again, it is not envisaged that expression of this transcript will be oncogenic or have adventitious effects in host cells at the level of expression used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All control measures specified as requirements for a Containment Level 2 project will be adhered too (See accompanying Biological Safety Handbook for further details).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Effective operating procedures (including waste management) as outlined by the accompanying Biological Safety Handbook, together with Good Microbiological Practice (GMP) and Good Occupational Safety and Hygiene (GOSH) will be strictly adhered to. All work will be carried out in the Class II Microbiological Safety Cabinet Hood (manufactured to BS EN 12469:2000).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022

Page 9811 of 15326
The Genetic Modification Safety Committee requested the following:
Correction of a number of typos in the risk assessment and accompanying handbook.

Full reference
Reference to workers histo-compatibility (page 6) to be reworded to allow clearer understanding.

All changes have been completed in accordance with the GMSC's wishes. Documents were resubmitted to the committee and approved with no further comment.

The committee has therefore approved the project at Containment Level 2.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### Project Ref 559/08.4

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<td>Assembly of non-infectious Bunyamwera virus RNA segments within mammalian cells.</td>
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### Project Additional Information
Purposes of the contained use

The micro-organism to be contained is a modified version of vaccinia virus called vTF7-3, which expresses the DNA-dependant RNA polymerase of bacteriophage T7. This organism was previously constructed by others (Fuerst, Niles, Studier and Moss. 1986. PNAS 83, p8122), and now is a widely-used laboratory reagent. This organism will not be modified further, and so this application is for the use of this organism, rather than for its modification. In general terms, the intended use of vTF7-3 is to express 17 RNA polymerase within the cytoplasm of mammalian cells. These cells can then be transiently transfected with plasmids containing specific cDNA sequences under the control of 17 RNA polymerase promoters. In the specific experimental system described here, these cDNAs encode BtJNV N and L proteins, as well as the sequence of a BUNV RNA strand. Expression of these components leads to assembly of non-infectious BUNV RNA segments that mimic the gene expression characteristics of authentic BUNV. By removing BUNV genes expressing proteins involved in both cell entry and exit, these segments are unable to spread from the cells in which they were assembled.

Recipient or parental organism

The recipient organism is vaccinia virus, which is a member of the poxviridae family of enveloped DNA viruses. It was used from 1798 as a vaccine against smallpox but is no longer used for inoculation of the general population due to the worldwide eradication of smallpox, although it is still used as a vaccine to inoculate members of armed forces against intentional exposure. However, vaccinia virus is categorized by the Advisory Committee for Dangerous Pathogens as a hazard group 2 biological agent due to its ability to cause disease in healthy adults.

Host/vector system

The modified vaccinia virus vTF7-3 will be used to inject baby hamster kidney cells (BHK) cells.

Origin & function

The foreign genetic material inserted into vaccinia virus to generate the vTF7-3 version originated from bacteriophage T7. The inserted sequence was a single cDNA that comprised the open reading frame encoding 17 RNA polymerase, under the control of the vaccinia virus p7.5 promoter. Details of this construction are contained within the original scientific article (Fuerst, Niles, Studier and Moss. 1986. PNAS 83, p8122). The intention of inserting this sequence was to cause the abundant expression of T7 RNA polymerase within the cytoplasm of vaccinia virus vTF7-3 infected cells.

Evaluation of foreseeable effects

No foreseeable change in the life cycle or growth characteristics of vTF7-3 compared to the parental vaccinia virus. The T7 polymerase expressed from this recombinant vaccinia virus will be inactive within mammalian cells. This is because there are no DNA templates within the cytoplasm of mammalian cells, thus infection of vTF7-3 will result in no change of gene expression characteristics of infected host cells. If the 17 RNA polymerase should enter the nucleus of infected cells, this polymerase should also remain inactive, as mammalian genes do not contain the specific 17 RNA polymerase promoter sequences required for gene expression. Therefore, to the best of my knowledge, modification of vaccinia virus to generate vTF7-3 should result in no change to the hazard group classification of this organism, as such it should remain as class 2.

Hazards to human health.

Routes of exposure for laboratory workers include ingestion, inoculation via needles or sharps, and droplet or aerosol exposure of mucous membranes or broken skin. The clinical disease of the parental vaccinia virus is as follows:

Day 3-4 post-inoculation: Formation of papule.
Day 5-6 post inoculation: Formation of pustule.
Day 8-9 post inoculation: Well-formed pustule.
Day 12+ post inoculation: Formation of a scab
Day 17-21 post inoculation: Scar formation.
Mild systemic illness can accompany the localised reaction, including fever and malaise. VACV may cause particularly severe disease during pregnancy, in people with active skin disorders such as significant clinical eczema or psoriasis, or in immune-compromised individuals.

No foreseeable hazards outside of the laboratory, due to the low amount of vTF7-3 to be used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The faculty standard procedure for biological waste disposal will be followed, which results in 100% kill of viruses. Liquid waste will be treated with an equal volume of 2% Viricon and left overnight before disposal down the drain with 100 volumes of water. Solid waste (culture flasks, dishes or other contaminated plastic ware) v.111 be autoclaved at 121°C (load temperature) for 20 minutes before being destroyed as clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Comments from GM committee -

1. It is not entirely clear if the recombinant vaccinia virus is also part of this assessment or whether this has been constructed previously under another assessment - clarification is required.
2. I'm not sure I like the final 0MM being described as 'less harmful' - no more harmful than the wild type would be better.
3. The proposal OK as class 1 although I have a couple of comments. Firstly, although there is some good background detail in the description, it would be good to know what the host and pathogenicity is for the Sunyamwera virus. Secondly, reference is made, at the bottom of page 3, to the use of a laminar flow cabinet, which doesn't protect the worker; so this should be a safety cabinet, presumably class II.
4. Could some evidence (ret?) be provided that work with vaccinia virus vTF7-3 can be regarded as a class I activity?

Following the comments of the committee the project (originally submitted as a Containment Level 1 project) was reclassified as Containment Level 2 project. The committee had no further comments.

Project Containment

| Laboratory Activities | Glass Houses | Growth Rooms |

02/03/2022
**Project Ref** 559/08.5

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<th>CU2 Project Title</th>
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<td>26/09/2008</td>
<td>Immortalisation of human bronchial epithelial cells with hTERT, ID-1 and CDK4 genes using viral gene delivery systems.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consistent</td>
<td>Non-GMM Consent Granted</td>
<td>Project notified under transitional arrangements</td>
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Immortalisation of human bronchial epithelial cells to produce cell lines for future study. We aim to culture samples of normal or abnormal bronchial tissue in conditions favouring the appearance of outgrowths from the tissue pieces. We will then culture these cells and, using viral gene delivery, express hTERT, ID-1 and/or CDK4R24C genes in order to immortalise the cells and produce stable, cloned cell lines based on the original tissue. We hope to produce cell lines with sufficient genetic and phenotypic similarity to the primary material to be useful models of pre-cancer in the lung.

**Recipient or parental organism**

The primary material for our research will be human bronchial epithelial tissue at a range of histological grades, from normal to cancerous. Abnormal tissue of low histological grade is expected to be available only in extremely low quantities, typically as material obtained from endoscope biopsies. The natural limits of population doubling will be bypassed by expressing specified genes associated with the cell cycle control.

**Host/vector system**

Third generation amphotropic retroviral expression system, such as Phoenix packaging cell line based on the Moloney Murine Leukaemia Virus (MoMLV) will be used.
Replication-defective virions are produced in the Phoenix amphotropic producer cell-line 1.2.3 (derived from 293T human embryonic kidney cell-line). This is a third generation system: the cells contain the viral em, gene driven by a CMV promoter in one construct and poi and gag genes in a separate construct driven by an RSV promoter. They are then transfected with a third construct containing the gene of interest plus retroviral packaging signal to allow them to produce particles capable of infecting human cells. Amphotropic retroviral systems have come into wide-scale use due to the ability of the viruses to infect a wide range of primary cells with high efficiency. Once infected, the virus integrates into the host genome, and the expression of the cloned gene of interest is initiated. The retroviruses used in gene delivery systems are simple enveloped RNA virus vectors that generally consist of three genes: gag (coding for core proteins), pol (coding for the viral RNA reverse transcriptase) and env (responsible for generating the viral envelope). Long terminal repeats (LTR5) are found at the end of the genome; these sequences aid viral genome integration and transcription of the inserted genes. A final stretch of the sequence is present to aid packaging of the virus. Their transforming potential comes from their ability to integrate into a cell’s genome.

Where mitosis-independent infection is required, third generation amphotropic lentiviral systems, such as that described by Dull et al. (1998). These systems are based on I-IV-1 but lack tat, vpr, vif, vpu and nef genes, making production of wild-type virus impossible. HIV envelope gene is replaced with Vesicular stomatitis virus G protein (VSV-G). Replication-defective virions are produced by 293T cells containing three separate, non-overlapping packaging constructs. The first contains VSV-G while the other two contain HIV-1 psi and gag genes and HIV-1 rev gene driven by CMV and RSV promoters respectively. The cells are transfected with a fourth construct, the transgene vector. This contains the gene of interest along with its promoter and a chimaeric 5 LTR containing a constitutive CMV promoter to allow efficient transcription of the gene of interest and its promoter in the absence of Tat. The 3’ LTR has a 133bp deletion to allow self-inactivation of the vector whereby the vector inserts the gene of interest into the target genome without the LTR promoter sequence.

Origin & function

We aim to infect primary cultures of human epithelial cells in order to express hTERT, ID-I and mutant CDK4 genes and cause transformation and immortalisation of the target host cells. The inserts are intended to immortalise human cells by overriding elements of cell-cycle control.

hTERT is a human gene encoding telomerase reverse transcriptase, activity of which is known to allow cells to escape from replicative senescence.

CDK4R24C is a mutant of the human cyclin-dependent kinase 4 gene that is insensitive to p16/INK4a inhibition and can therefore override this arm of cell-cycle regulation.

I-IIV-1 but lack tat, vpr, vif, vpu and nef genes, making production of wild-type virus impossible. HIV envelope gene is replaced with Vesicular stomatitis virus G protein (VSV-G). Replication-defective virions are produced by 293T cells containing three separate, non-overlapping packaging constructs. The first contains VSV-G while the other two contain HIV-1 psi and gag genes and HIV-1 rev gene driven by CMV and RSV promoters respectively. The cells are transfected with a fourth construct, the transgene vector. This contains the gene of interest along with its promoter and a chimaeric 5 LTR containing a constitutive CMV promoter to allow efficient transcription of the gene of interest and its promoter in the absence of Tat. The 3’ LTR has a 133bp deletion to allow self-inactivation of the vector whereby the vector inserts the gene of interest into the target genome without the LTR promoter sequence.

Evaluation of foreseeable effects

All plasmids will be amplified in standard E.coli K12 laboratory strains such as DH5α or DH10. Laboratory strain Escherichia coli K-12 and its derivatives are recognised as non-colonising and disabled! have an extensive history of safe use and the genetic lesions are well understood. They have been categorised as belonging to Hazard Group 1, according to the definitions given by the Scientific Advisory Committee for Genetic Modification (SACGM) in the compendium of guidance. They are not considered harmful to humans.

Both the prokaryotic and eukaryotic cell lines, even when the latter are transformed, are highly disabled and unable to propagate in the environment. The plasmid vectors are non-mobilisable and the retrovirus lentivirus are replication incompetent, so further spread is highly unlikely.

The retroviral system is well-characterised, widely-used and non-replicating. Occasionally a sequence will integrate in an adjacent position to oncogenes, leading to an increase in their transcription driven by enhancer/promoter elements found in the LTR. Alternatively, integration may cause disruption of a tumour suppressing gene (TSG) leading to a loss of their activity.

Their safe application as cloning and expression vehicles was based on the observation that the various functional aspects of the viral sequence could be separated. The gene (cDNA) of interest is inserted into a plasmid vector between the LTR sequences. This vector also includes the psi (w) packaging sequence. This plasmid is then transfected into a viral packaging cell line, characterised by the expression of the gag, poi, rev and env sequences.

In this way, three separate recombination events would be required to produce wild-type virus.

The lentiviral system is also well-characterised, widely-used and non-replicating. As with the retroviral system described, a sequence will occasionally integrate in an adjacent position to oncogenes, leading to an increase in their transcription or, alternatively! integration may cause disruption of a TSG leading to a loss of their activity. The cis and trans functions of the system are separated as far as possible. The sequences that encode the packaging components are on constructs lacking cis-acting sequences and so are unable to package themselves. The gene (cDNA) of interest is inserted into a plasmid vector between the LTR sequences. This vector also includes the psi (w) packaging sequence. This plasmid is then transfected into a viral packaging cell line, characterised by the expression of the gag, poi, rev and env sequences.
We will use a third generation system that has the viral packaging sequences divided among three separate constructs. The q and greatly modified LTR sequences will be within the transfer construct. Wild-type virus cannot be created as key components such as tat and much of the U3 region in the LTRs are absent. The production of replication-competent virus is unlikely due to the requirement of several recombination events.

The infection of a worker with replication-defective retrovirus resulting in expression of one of the genes used for immortalisation is possible. The standard procedure produces up to 10^7 viral particles/ml; as these cannot replicate, only a finite number of cells could be infected. Were these inserts to be expressed in a worker's cells, via direct infection by the retrovirus, they have the potential to cause one of the cell-cycle control mechanisms to be overridden and thus the removal of one barrier to cell proliferation. This is undesirable as anything that could lead to mechanisms of cell-cycle control being circumvented theoretically increases risk of cancer, albeit to a very small degree.

Accidental exposure to cultured human cells that express these inserts poses no significant risk to workers as their human leukocyte antigen (HLA) system would recognise cells from culture as foreign and destroy them. In this way, the cultured cells, whether primary or transformed, have the same level of hazard as any other cell culture. Risk can be reduced to negligible levels by use of a class II hood which is cleaned after each use, prompt decontamination and disposal of waste, use of lids on centrifuge rotors, and by wearing gloves for all manipulations. In addition, having easily identifiable lab-coats (e.g. blue-coloured) and equipment such as centrifuges, pipettors, incubators and class II hoods designated for use in retroviral work will minimise the risk of cross-contamination.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation is not being applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All plasticware that has come into direct contact with viral supernatant or cell material that has been transfected or infected, e.g. pipettes, tubes etc., will be decontaminated either by immersing in 1% virkon or trigene prior to autoclaving or by taking directly for autoclaving in a sealed, watertight container. All other consumables will be autoclaved. Serological pipettes will be rinsed briefly by aspirating from a beaker of 1% virkon or trigene in the hood before being placed in an autoclave bag.

Tips and tubes will be placed in a plastic, sealable disposable jar in the hood that will be sealed when not in use and will be autoclaved once filled. The tips/tubes will not be soaked in virkon or trigene as the disposable jar provides an extra layer within the autoclave bag separating workers from the potentially hazardous material.

Liquid waste will be rendered biologically inactive by adding virkon or trigene to give a final concentration of 1% and leaving overnight before discarding down the sink. It can either be aspirated into a beaker of concentrated virkon or trigene in the hood or into a reservoir of concentrated virkon or trigene using a vacuum aspirator. Should the latter option be employed, the vacuum pump system will be fitted with the appropriate filters to prevent aerosol formation (changed once a month) and the nozzle and hose will be rinsed with 1% virkon or trigene at the end of each day.

In case of spillage, aerosols will be allowed to settle before decontaminating the area with 10% virkon or trigene. Any contaminated clothing will be removed and autoclaved.

In the event of an injury, wounds will be thoroughly washed with soap and warm water before being bandaged. If material has entered the eye, it will be washed thoroughly from the eye-wash stations. Where any personal contact has occurred the local Biological Safety Officer (Paul Beal) will be informed.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The main issues for discussion and clarification at the meeting were:
(i) where the project would be undertaken;
(ii) routes of waste disposal; and
(iii) whether the project should be expanded to include a Lentiviral delivery system to increase the chance of success for infection of primary cultures.

It was established that the work would be undertaken in the tissue culture facility on level 7 Wellcome Trust Brenner Building (WTBB).

Procedures were already in place to prevent potential problems of cross contamination of cultures, and safe disposal of contaminated plastic-ware and solutions. It was stressed that workers entering the lab must be classified as Class 2 workers and register with University Occupational Health. Safety procedures).

In the original proposal, only a (third generation) retroviral delivery system had been proposed. After discussion, the recommendation from the Committee was that a (third generation) lentiviral delivery system should also be included in the application. It was felt that such a system may increase the chances of a successful infection of differentiated primary epithelial cells. Consequently, the number of primary cells required would be decreased, and potentially reduce the number of experiments needed to achieve a successful outcome.

The Committee therefore approved the application with a recommendation that a lentiviral delivery system should also be included.

\[\text{Project Containment}\]

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| Date Project Ceased | | | |
|---------------------| | | |

Withdrawn N  
Tick if notifying a connected programme of work N
### Project Additional Information

#### Purposes of the contained use

To better understand metabolic pathways in the obligate intracellular parasite *T. gondii* to identify and valid targets for drug development. These studies will help us understand how the parasite manipulates its host for supply of nutrients. Fluorescent markers (e.g., RFP) will monitor protein expression and localisation and gene knockouts will be generated to examine the requirements for genes encoding enzymes in intermediary metabolism. There is hope of generating attenuated strains through this research will be a safer alternative for future research.

#### Recipient or parental organism

*Toxoplasma gondii* is a single-celled protozoan that is an obligate intracellular ubiquitous parasite that can infect a range of warm blooded animals. Most infections are minor and self-limiting but it can be serious for a developing foetus whose mother contracts the disease during pregnancy or immunocompromised and rare immunocompetent persons. (see [http://www.phac-aspc.gc.ca/msds-ftss/msds 153e-eng.php](http://www.phac-aspc.gc.ca/msds-ftss/msds 153e-eng.php)) hence classification as a Hazard Group 2 pathogen in the ACDP. In the laboratory only the intracellular stages are maintained.

#### Host/vector system

The vector is based on the standard pUC based non-mobilisable cloning vector pKS+ with *T. gondii* sequences for expression in parasites and chloramphenicol acetyl transferase as a selectable marker. There are no several hundred publications using these vectors for *T. gondii* transfections including ones from UK institutions. It does not contain elements for propagation in *T. gondii* (e.g. origins of replication, retrotransposons) and is not expected to be mobilisable.

#### Origin & function

Initial gene disruptions will target an enzyme in amino acid conversion (GenBank No. ACB99414 and ACB99413). Genes encoding enzymes for other metabolic intermediary products (i.e. pyrimidine biosynthetic enzymes, biotin and coenzyme A biosynthesis) may be targeted in future experiments. There are no known hazards associated with these classes of enzymes. Most experiments will only utilise a gene fragment so that the chromosomal copy of the targeted gene is inactivated. The intended function is to disrupt metabolic genes by homologous recombination so that the effect on parasite growth and development can be monitored. The fluorescent marker GFP and RFP, well-described in the literature with no known toxicities, will be fused to genes encoding metabolic enzymes (those described above) to monitor their localisation. There are no foreseeable dangers of non-homologous (i.e. illegitimate) recombination with these gene fragments or markers. No toxins, dangerous enzymes (e.g. proteases), cell surface proteins, or virulence factor (vir) encoding genes will be involved in these studies.

#### Evaluation of foreseeable effects

Infection is possible by ingestion or inoculation of parasites. This will be prevented by following standards of good laboratory practice (e.g. lab coats, disposable gloves, work in Class 2 cabinet, decontamination procedures, handwashing). *Toxoplasma* is not airborne preventing infection by inhalation and all manipulations are conducted in
Microbiology Safety Cabinets to prevent contamination by aerosols. All toxoplasms work is conducted in a CL2 facility devoted to Toxoplasma. Contamination is controlled by using a suitable laboratory environment and decontamination measures, appropriate equipment, safe systems of work, personal protective equipment and by providing the individual worker with suitable information, instruction, training and supervision. The final GMM is likely to be the same risk of harm as the un-modified host. It is possible that the genetic lesions in metabolic pathways will lower fitness. It is plausible that an increase in growth rate may occur in some GMMS but this will not affect virulence. Although Toxoplasma could survive and colonise in animals and humans in the environment in escape is unlikely as this intracellular parasite is dependant upon laboratory culture conditions (media, 37°C, 5% CO2) to survive for any period outside the laboratory. It is sensitive to temperature, dryness, nutrients, and CO2 concentration. In the unlikely event of escape, it would compete poorly with wildtype strains common in the environment (indeed 20-25% of the UK population is seropositive for Toxoplasma). The containment measures used to protect workers health and safety will be sufficient to protect the environment. Even in the event of complete breach of containment, Toxoplasma is normally self-resolving and can be treated with sulfa-pyr and hence there will be little or no risk to the environment. The applicant serves as manager responsible for the CL facility, although safety inspections are undertaken by the Faculty Safety Manager. The CL2 facility only allows authorised users. All users are trained in safety risks and control measures used to mitigate any risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquids containing parasites will be decontaminated with PreSept. PreSept is an efficient method of disinfection against all bacteria, viruses, fungi, algae and protozoa containing sodium dichloroisocyanurate (NaDCC) and effective through the entire biocidal spectrum as well as highly resistant to inactivation by organic soilage. Efficacy is believed to be 100%. Following treatment overnight, liquids are washed down waste with at least 100 volumes of water.

Solid waste (culture or cells) will be autoclaved with minimum cycle parameters of 121°C (load temperature) for 20 minutes. Autoclave function is monitored by indicator strips. No parasite can survive autoclave conditions.

All material coming into contact with viable organisms will be rendered safe by the use of Virkon, used in accordance with the manufacturer's instructions.

Emergency spill procedure:

Liquid spill - treat with Virkon granules until deep pink or add an equivalent volume of 2% Virkon – will be left for at least half an hour before being mopped up with plenty of water.

Wearing strong gloves of the “Marigold” or similar type, solid spills will be gathered into an autoclave bag, the area affected will be wiped liberally with 2% Virkon. The treated area will be left for half an hour for the disinfectant to have time to act and the area will then be mopped up with plenty of water. Immediately after it has been collected and the affected area has been treated with Virkon, the solid waste will be taken for autoclaving, as above.

All spills of genetically modified microorganisms (GMMO) will be reported immediately to the local Biological Safety Officer, or local Safety Supervisor in the absence of the BSO: all spills and their mode of treatment will be recorded. Any personal injury where an infection by a GMMO may be involved will be reported immediately to one of the local BSO's or Safety Supervisors and the immediate advice of Occupational Health will be sought.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N
Please enter comments on the GM safety committee on the risk assessment

Review of GM risk assessment/10.07.08/Fbs8011 Comments:
*Note: All issues below have been addressed in approved Risk Assessment*
See attached document for comments of the GMSC and corrections made to address those comments.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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**Animal Units**

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**Project Ref 559/09.1**

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<th>Date Project Ceased</th>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
<th>Historical Significant Changes</th>
<th>Historical Date of Additional Info</th>
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<td>10/02/2009</td>
<td>MANIPULATION OF THE REGULATORY STATE OF HAEMATOPOIETIC CELLS</td>
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<td>1-50 Litres</td>
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<td>05/11/2012</td>
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<td>N</td>
<td>Project transferred to GM116</td>
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**Project Additional Information**

**Purposes of the contained use**

To infect human or murine cells with retro- or lenti-viral gene delivery systems. As the result, cells will permanently harbour genes encoding (1) transcription factors,
epigenetic regulatory molecules, cell cycle regulators or oncogenes, (2) small hairpin RNA, (3) RNA aptamers or (4) genes encoding peptide aptamers without any replicating viruses. These genes are continuously expressed or can be induced by addition of chemicals such as 17β-estradiol, Tamoxifen, or Tetracycline. Transduced cells will be used for DNA and RNA analysis, cultured in vitro or transplanted into immuno-compromised mice.

Recipient or parental organism

MMLV (Moloney murine leukaemia virus) or MSCV (murine stem cell virus) based replication defective, non-mobilisable retroviral vectors e.g. pBabe. These are well-established and widely used retrovirus based expression vectors with a history of safe use.

The third generation of lentiviral vectors, e.g. pHIV7, HR’CS-G and their derivatives, Drug inducible expression vectors, pLVX-Tight-Puro (Clontech) co-transfected with the regulator pLVX-Tet-On Advanced or pLVX-Tet-Off Advanced. Virus particles in this system are produced by the Lenti-X HT Packaging Mix which utilises a trans-lenti viral vector system.

Lentiviral vector systems in which shRNA constructs can be directly cloned, e.g. pKLO.1.

MSCV-pmiR30, pPRIME-CMV-GFP-FF3, pPRIME-CMV-dsRed-FF3 or pPRIME-TET will be provided by Dr Stephen Elledge (Department of Genetics Center for Genetics and Genomics Harvard Medical School).

MSCV-MigR1 will be provided by Dr. Warren Pear (Abramson Family Cancer Research Institute University of Pennsylvania).

MSCV-Ires-hCD2t will be provided by professor Meinrad Bussligner (IMP, Vienna).

pHIV7-GFP will be provided by professor John J. Rossi (Beckman Institute, CA, USA).

pLL3.7 will be provided by Tyler Jacks (MIT).

A full list of components (inserts, vectors and eukaryotic cell lines) can be found as a separate attached document (Manipulation of the regulatory state of haematopoietic cells COMPONENTS.doc).

Host/vector system

Human and mouse cell lines including haematopoietic, fibroblastic, hepatic cells. Mouse primary cells including embryonic stem cells, haematopoietic cells and embryonic fibroblasts from wild type transgenic or knock out animals. These are categorised as belonging to biological hazard group 1.

Primary human cells such as CD34+ cells, leukaemic blast cells, myeloid cells and lymphoid cells from normal donors and patients. These are biological hazard group 2 due to the potential risk of infection from endogenous pathogens.

Origin & function

Generation, propagation and use of these retro- or lenti-viral expression vectors aim to introduce the coding sequence of transcription factors, oncoproteins, inhibitory RNA molecules or peptide aptamers into murine or human primary cells or established cell lines. The objective is to over-express or knock down the expression of targeted molecules or to interfere with their function into cells, and analyse alterations in the expression and structure of the target genes and cellular phenotypes.

Evaluation of foreseeable effects

The E.coli bacteria used are not pathogenic to humans or animals and are not expected to survive in the environment, they therefore belong to hazard group 1.

The cells which will be transduced in the project are either established cell lines, mouse primary cells or human haematopoietic cells. They are highly unlikely to be able to survive outside the culture vessel and are non-transplantable due to immuno-incompatibility. The use of Class 1 containment with good laboratory practice will be sufficient to limit hazards to human health and the environment from these cell lines. Primary cell lines are categorised as requiring containment level 2 due to the potential for adventitious agents to be present.

Packaging cells for retroviral production are either HEK 293T or NIH-3T3 derivatives and HEK 293T for lentiviruses. Prior to the induction of viral vectors or packaging
vectors, they do not produce any viral products and are considered in the same way as an established cell line. Once virus vectors and packaging vectors are transfected into packaging cells, these cells are capable of producing viral particle which can infect human cells and have to be used at containment level 2.

Once transduced, viral sequences are integrated into the host genome, and occasionally integrate in a position adjacent to oncogenes which thereby leads to a constitutive increase in the level of expression of these oncogenes due to the promoter/enhancer in the Long Terminal Repeat (LTR). Depending on the position, virus integration can cause the aberrant silencing or disruption of the endogenous genes. Lentiviral systems described in this assessment have a self-inactivating (SIN) mutation which abolishes LTR promoter activity so as to prevent the aberrant activation of endogenous genes. With third generation retrovirus systems, the retroviral structural genes are encoded on two different plasmids and contain additional proteins, and at least 3 non-homologous recombination events are necessary to generate replication competent virus. Consequently, they are highly unlikely to acquire a replicative ability in cells. Lenti-vector particles are generated by co-expressing the virion packaging elements and the vector genome in a cell used as a producer. In the case of HIV-1-based vectors, the core and enzymatic components of the virion come from HIV-1, while the envelope is derived from a heterologous virus. To decrease the probability of generating replication competent recombinant (RCR) virus a second generation vector was created which has deletions in its crucial virulence factors. In order to further improve their safety a third generation of lentiviral vectors was developed in which the transgene vector contains a deletion in the 3' LTR U3 region making it self-inactivating in target cells and the packaging vector is a split genome construct with the tat gene deleted and the rev gene expressed by a separate non-overlapping construct. This vector achieves transduction of target cells as well as that observed with the previous generation. To date, neither second nor third generation lentiviral vectors have been found to generate RC virus.

Some of the inserts described in the risk assessment have been shown to have transforming activities when overexpressed. The major risk of generating viral vectors expressing normal transcriptional regulatory proteins therefore comes from either introducing abnormally high levels of a normal or mutated factor into normal cells or from the downregulation of endogenous proteins by RNAi. In some cases, this could lead to aberrant cell differentiation, proliferation, apoptosis or in rare cases, oncogenic transformation. However, the expression of these proteins is highly unlikely to change the natural immunogenic characteristics of the cells they are expressed in, which are therefore very unlikely to colonize humans due to immuno-incompatibility. Consequently, the transfection of these cells and expression of the transfected genes will not significantly alter the capacity of these cells to persist in the environment or cause harm to either humans, animals or other aspects of the environment.

The work will be undertaken in accordance with containment level 2. This includes culture of primary cells, retroviral infection protocols and subsequent culture of any cell-lines produced that have not been screened for the presence of retrovirus. Screening of the cell-lines will be by assay of reverse transcriptase activity. The assay will be carried out within the laboratory designated for Class 2 work until cell culture-derived material has been dissociated using lysis buffer (50mM Tris, 80mM KCl, 2.5mM DTT, 0.75mM EDTA, 0.5% Triton-x-100), after which time it can be continued outside of the facility providing the principles of Good Laboratory Practice are followed. Cell-lines in which no retroviral activity has been found can be de-rated to Class 1. Access to cell culture facilities such as incubators or safety cabinets in which Class 2 work is being performed will be restricted to users registered for Class 2 work. The University Occupational Health Department will be given a list of names of all workers with access to the facility and will give these workers appropriate health surveillance.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All plastic ware that has come into direct contact with viral supernatants or cell material that has been transfected or infected, e.g., pipettes, tubes etc., will be decontaminated either by immersing in 1% Virkon or trypsin prior to autoclaving, or be taken directly for autoclaving in a sealed, watertight container. All other consumables will be autoclaved.

Tips and tubes will be placed in a plastic, sealable disposable jar in the hood that will be sealed immediately after use. The outside will be sprayed with a disinfectant, put into an autoclave bag, closed, then removed from safety cabinet, put into another autoclave bag and subsequently autoclaved. The tips/tubes will not be soaked in virkon or trypsin as the disposable jar provides an extra layer within the autoclave bag separating workers from the potentially hazardous material.

Serological pipettes will be rinsed with disinfectant by aspirating the solution a few times before disposal into a two-layered autoclave bags. Immediately after the
procedure, the autoclave bag will be sealed and subsequently autoclaved. Used culture flasks, plates, and dishes will be put into an autoclave bag, closed, the outside of the autoclave bag will be sprayed with a disinfectant, then put in another autoclave bag and subsequently autoclaved. The final route for all solid material that has been contaminated is incineration.

Liquid waste will be rendered biologically inactive by adding virkon or trigene to give a final concentration of 1% and leaving overnight before discarding down the sink. It can either be emptied aspirated in a beaker of concentrated virkon or trigene in the hood or by using a vacuum aspirator directly into a reservoir of concentrated virkon or trigene. Should the latter option be employed, the vacuum pump system must be fitted with the appropriate filters (changed once a month) and the nozzle and hose will be rinsed with 1% virkon or trigene at the end of each day.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The application for this connected program of work was considered by the LIMM Local GM Safety Committee at its 6 monthly meeting on the 14th July 2008. Copies of the risk assessment had been sent to the members of the Committee approximately 10 days in advance of the meeting. The Committee felt that the project had been well set out with a clear description of the (second and third) generation retro and lentiviral vector and host systems presented, along with a description of the potential risks associated with their proposed use. Furthermore, there was a very detailed description of the inserts proposed to be investigated.

Discussion mainly centered on where the work would be undertaken and the routes of waste disposal. The bulk of the work would be undertaken in the tissue culture room on Level 6 Wellcome Trust Brenner building. However, viral stocks would sometimes require centrifugation in an ultracentrifuge based in a different ancillary room. It was felt that so long as the centrifuge tubes containing the viral cultures were both sealed and opened in the tissue culture hoods then there was not a problem with this procedure. However, emergency decontamination provision must be put in place in the event of a tube failure. The main topic of conversation regarding waste disposal related to tip disposal. It was decided that the safest route of disposal of the tips would be via collection in Dispo-SAFE P.E.T. jars (Microbiological Supply Company) that would then be (double) bagged in autoclave bags by workers registered at Class 2 and then transported to Level 3 WTBB for autoclaving. After autoclaving this waste is sent for incineration as a matter of course. The consensus of the Committee was that the work described was a Class 2 project and that the group of individual projects merited the title ‘a connected program of work’. They felt that the project could now be submitted to HSE for approval.

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**Project Ref** 559/09.2

**Date Ackn'd**
21/07/2009

**CU2 Project Title**
Replication of the FMDV replicon

**Class**
Class 2

**CultureVolClass2**
< 1 Litre

**Class Culture Vol Class 2**
Non-GMM

**Culture Volume Class 3-4**
Consent Granted

**Date Project Ceased**

**Project notified under transitional arrangements**
N

**Withdrawn**
N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The work involves the use of constructs called sub-genomic replicons derived from a cDNA copy of the picornaviral genome. These constructs lack the coding sequence for the structural proteins and thus are unable to undergo a complete viral lifecycle. Viral particles are therefore not produced. Permission has been granted previously to use a variety of different picornaviral sub-genomic replicons to study viral genomic replication under category 2 containment and we would like to extend this to include studies with another picornavirus, foot-and-mouth disease virus (FMDV). It is only recently that FMDV replicons have been available, allowing academics to study the genome replication of this important pathogen.

We wish to use the replicon to study the biochemistry of FMDV genome replication within cells. We will also undertake a cell biological approach by using indirect fluorescence microscopy to localise viral proteins in fixed cells.

We expect that this work will lead to a better understanding of FMDV genome replication and may lead to the development of novel therapeutic approaches in the future.

**Recipient or parental organism**

Sub-genomic replicon RNA or cDNA will be introduced into two immortalised cell lines of non-human origin - BHK21 (hamster embryonic fibroblasts) and EBL (an embryonic bovine lung cell line). Both cell lines have been shown to support the genome replication of FMDV derived sub-genomic replicons [McInerney et al. (2000) Replication-competent foot-and-mouth disease virus RNAs lacking capsid coding sequences. J. Gen. Virol. 81, 1699-1702].

**Host/vector system**

The sub-genomic replicon cDNA is cloned into a standard non-mobilisable DNA plasmid (pUC based), and will be grown in laboratory-adapted strains of Escherichia coli that do not survive in the human gut (ACGM/HSE/DOE Note 7, annex 1). Standard in vitro mutagenesis protocols (e.g. Quikchange) will be used to introduce defined
mutations into the replicon.

**Origin & function**

The replicon includes sequences derived from foot-and-mouth disease virus genome, however, NO structural protein sequences are included. The replicon is thus unable to generate virus particles or mediate cell-to-cell spread. It is limited to the cell into which it is introduced by transfection (electroporation) and maintained episomally.

Three forms of reporter/selection protein will be employed. Such proteins are non-toxic and are not predicted to alter the nature of the construct.

(i) a fluorescent protein (green fluorescent protein, GFP and different colour derivatives thereof) will enable the identification of cells in which RNA replication is occurring and measurement of replication.

(ii) luciferase proteins (Luc; Renilla or firefly) will provide easy, highly sensitive, biochemical assays for RNA replication and


Since Lpro plays important roles in replication (e.g. ‘shut-off’ of cap-dependent translation, we will also re-insert the deleted Lpro sequences restoring the full complement of genome replication proteins. As none of these modifications will restore the structural proteins they will not enable the replicon to generate infectious virus.

**Evaluation of foreseeable effects**

The replicon is non-hazardous for the following reasons.

i) It does not encode any structural proteins and therefore lacks the ability for replicon-derived RNA to be packaged. It is therefore impossible to produce FMDV particles from this construct. Naked replicon RNA is highly unstable and cannot survive in the extracellular environment. Furthermore, it can only be transmitted to naïve cells using sophisticated laboratory-based techniques.

ii) Trans-encapsidation with structural proteins from other viruses would be extremely unlikely as this would need to have arisen from a) the presence of both the replicon and a replicating virus within the same cell and b) the ability of the coat proteins produced by the other virus to recognise and package FMDV subgenomic replicon-derived RNA. Even if both of these events were to occur, the resulting particles could not set up an infection as they would lack any genes encoding structural proteins and thus would only be able to undertake cell entry and replication of non-structural proteins. Furthermore, the absence of the structural protein coding sequences would prevent packaging and cell-cell spread.

iii) A recombination event between the replicon and another virus (termed X) could only theoretically occur if these were very closely related (e.g. another picornavirus) and if both were present in the same cell. The lack of any other picornavirus research in the laboratory, together with the containment precautions proposed for this work, mean that this event is extremely unlikely to occur. However, even if this highly unlikely event were to occur, recombination involving incorporation of viral structural genes into the replicon would result in the presence of structural proteins from virus X, not from FMDV. Even if the resulting construct was able to make RNA and proteins successfully, the capsid proteins of virus X are highly unlikely to be competent to package the RNA. But even if this was to occur, any particles would have the capsid of virus X (not FMDV) and therefore, due to inability to bind to appropriate receptors, would not duplicate the pathogenic profile of FMDV. Furthermore, as picornaviruses do not replicate via a DNA intermediate and replicate entirely in the cytoplasm, there could be no host cell mediated homologous DNA recombination. Recombination between host RNA molecules is not a possible route for the provision of structural protein sequences.

There is no impact on antibody testing for FMDV due to the absence of FMDV structural proteins.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a
Our colleague, Professor Martin Ryan (St Andrews) has been involved in extensive discussions with HSE and The Scottish Executive (Scottish equivalent of DEFRA) concerning the safe use of the FMDV construct. These regulatory bodies concluded that level 2 containment would be appropriate providing that studies on the replicons are carried out in a different location to any work with picornaviral structural proteins.

For only GMM - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All FMDV capsid sequences will be removed from Leeds (they will be transferred to IAH, Pirbright).

Work will be carried out in laboratory Garstang 8.54d in the University of Leeds. This will allow complete segregation from all other Picornaviridae experiments. The room has recently been refurbished to a high standard.

The use of a dedicated class II cabinet for all cell culture manipulations and measures to prevent formation or escape of aerosols from samples taken outside of the cabinet (e.g. samples contained in plastic screw-capped vials, centrifugation carried out in sealed rotors etc.) will be implemented. All liquid waste will be discarded directly into Virkon (2%) within the Class II cabinet and inactivated by overnight treatment with Virkon prior to autoclaving. Spent plastic-ware will also be autoclaved (130°C, 20 minutes). Class II cabinets are tested every 6 months, as recommended by HSE guidelines.

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Tick if you are claiming exemption from disclosure for section of the risk assessment

The GMSC was happy to accept the risk assessment and the project was approved.

Project Ref 559/09.3

Date Ackn'd CU2 Project Title

Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
We are engaged in studies to understand the development of antibiotic resistance in Pseudomonas aeruginosa, and in particular, the role played in this process by mutation. Specifically, we will examine the genetic and environmental conditions which are conducive to the rise of hyper-mutator variants during growth in biofilm, since such isolates have been implicated in the development of antibiotic resistance and persistence during in vivo infections (Oliver et al. [2000], Science, 288, 1251-1253).

Recent work from this laboratory demonstrated increased mutability (as determined by increased frequency of antibiotic-resistance) in biofilms compared to planktonic cultures of Ps. aeruginosa PAO1, correlative with a decrease in the expression of key oxidase-type enzymes which offer protection to the organism from oxidative stress (Driffield et al. [2008] Journal of Antimicrobial Chemotherapy, 61, 1053-1056). Thus, the proposed work is a natural extension of the previous study and aims to establish the role of oxidase-type enzymes in mutability in biofilms, as well as whether the inactivation of one or more of the genes encoding oxidase-type enzymes would lead to the rise of hyper-mutators.

Ps. aeruginosa PAO1: ACDP hazard group opportunistic human pathogen which is widely used for research, and has a history of safe use in research laboratories. Strain PAO1 is a strain of choice in Ps. aeruginosa research, with in excess of 1000 Pubmed basic search hits (citations and/pr publications) at the time of writing this application.

Recipient or parental organism
Ps. aeruginosa PAO1: ACDP hazard group opportunistic human pathogen which is widely used for research, and has a history of safe use in research laboratories. Strain PAO1 is a strain of choice in Ps. aeruginosa research, with in excess of 1000 Pubmed basic search hits (citations and/pr publications) at the time of writing this application.

Host/vector system
E. coli K-12-based cloning hosts (e.g. DH5α, JM109, XL1 Blue) will be used to carry out gene manipulations and vector construction in the first instance, before introduction into Ps. aeruginosa. These E. coli host strains are commercially available and the most widely used hosts for gene cloning and/or expression.

Vectors to be used in this study include standard cloning vectors (e.g. pUC19 and comparable available vectors) in standard E. coli cloning hosts (e.g. DH5-alpha, JM109, XL1 Blue, and comparable cloning hosts), as well as shuttle vectors (e.g. pUCP19) which can be maintained in either E. coli or Ps. aeruginosa PAO1 hosts (e.g. pUCP19).

Origin & function
The genetic material involved in the proposed study includes:

1. Vectors/plasmids
1.1. pUC19 (and other standard, commercially-available E. coli cloning vectors; New England Biolabs, UK)
1.2. pUCP19: a shuttle vector for E. coli/Ps. aeruginosa (West et al. [1994] Gene, 128, 81-86.
1.3. Other comparable shuttle E. coli/Ps. aeruginosa vectors as may be necessary in the course of the work.
1.5. Plasmid pMDGF carrying a non-functional GFP (+1 frameshift mutation) to be used for the isolation of potential hyper-mutators in Ps. aeruginosa biofilms (Conibear et al. [2009] PloS One, 4, e6289), and parent vector pMF36 (Franklin and Ohman [1993] Journal of Bacteriology, 175, 5057-5065).

2. Inserted sequences
2.1. Frameshifted and point lacZ mutants will be amplified from E. coli strains CC101-CC111 (Cupples et al. [1990] Genetics, 125, 275-280), and cloned in E. coli/Ps. aeruginosa shuttle vectors, and subsequently introduced into Ps. aeruginosa.
2.2. Insertional inactivations in Ps. aeruginosa PAO1 oxidase-type genes katA, ahpC, tsaA, sodA, and in relevant genes bfrA (encodes bacterioferritin and a positive regulator of katA) and acnA (encodes aconitate hydratase and is up-regulated during growth in biofilm; Driffield et al., 2008). These mutant strains already exist and are available from the Department of Genome Sciences at the University of Washington (Seattle) as part of a transposon mutant collection (containing either a lacZ and/or a phoA insertion linked to the gene encoding tetracycline-resistance; Jacob et al. [2003] PNAS, 100, 14339-14344).
2.3. Insertional inactivation of DNA repair genes (e.g. rec and mut genes), or acquisition of such mutant strains to work with from other laboratories.
2.4. Re-introduction and overexpression in strain PAO1 endogenous genes encoding oxidase-type enzymes (katA, ahpC, tsaA, sodA), and other relevant genes (e.g. bfrA, acnA, rec, and mut genes).

Evaluation of foreseeable effects

None of the genes used in this study contain harmful adventitious sequences or oncogenic properties, and are not likely to promote allergen/toxin production or increase host pathogenic/infective ability.

None of plasmids proposed for use encode toxins or otherwise dangerous products, and have a history of safe use and/or are already in use in other laboratories for similar purposes to those described here, and will not be used in strains containing conjugative plasmids/transposons that may accidentally mobilise them in trans.

The final GMMOs are unlikely to be more harmful than the un-modified host. Inserted and/or modified sequences should not increase the ability of the host to colonise humans, plants or animals and should not increase the mobility of the vector. The modified host, in the event of escape from containment, is not expected to have any advantage over parent strain which exists naturally in the environment. In the highly unlikely event of escape from containment, the small volume of cultures used and the modifications introduced are unlikely to support the modified host to establish itself in the environment. The GMMOs would not be predicted to cause significant economic or ecological consequences in the case of a major escape.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated liquid waste is autoclaved under high pressure steam at 121C (or more) for 20 min, ensuring complete killing of all living agents. Solid waste is similarly treated before disposal as clinical waste.

Emergency spill procedure:

Liquid spill – treat with Virkon granules until deep pink, or add an equivalent volume of 2% Virkon – will be left for at least half an hour before being mopped up with plenty of water.
Wearing strong gloves of the “Marigold” or similar type, solid spills will be gathered into an autoclave bag, the area affected will be wiped liberally with 2% Virkon. The treated area will be left for half an hour for the disinfectant to have time to act and the area will then be mopped up with plenty of water. Immediately after it has been collected and the affected area has been treated with Virkon, the solid waste will be taken for autoclaving, as above.

For spills inside centrifuges or incubators the local Standard Operating Procedure as indicated on the equipment will be followed.

All spills of genetically modified microorganisms (GMMOs) will be reported immediately to the local Biological Safety Officer, or local Safety Supervisor in the absence of the BSO: all spills and their mode of treatment will be recorded.

Any personal injury where an infection by a GMMO may be involved will be reported immediately to one of the local BSO’s or Safety supervisors and the immediate advice of Occupational Health will be sought.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The Committee were happy that the controls identified in the risk assessment adequately addressed the risk

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4 L2</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

Project Ref 559/09.4

Date Ackn’d 19/11/2009

CU2 Project Title Manipulation and expression of mouse and human gene sequences using lentiviral and adenoviral vectors, to alter gene function and cellular responses in primary and transformed cells in order to study effects on healthy and diseased states

Date Project Ceased

Class 2

Consent Granted

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

The aim of this project is to investigate gene function and cellular responses in healthy and diseased states of cancer and atherosclerosis specifically in relation to intracellular signalling.

#### Recipient or parental organism

This project is essentially split into two connected programmes of work, the first part being a standard molecular biology approach using tissue culture and molecular biology techniques, and the second part involving in vivo studies using mice. See attached assessment which give complete details of the contents of the project.

#### Host/vector system

<table>
<thead>
<tr>
<th>Host</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Third / fourth generation amphotropic lentiviral, self-inactivating (SIN) lentiviral vectors expression systems and high capacity adenoviral vector systems:</td>
</tr>
<tr>
<td>Mammalian</td>
<td>- 3rd generation lentiviral systems (Naldini et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>- commercial lentiviral systems (Addgene, Sigma-Aldrich, Open Biosystems, Invitrogen, Cell Biolabs)</td>
</tr>
<tr>
<td></td>
<td>- Inducible Tet-on Tet-Off Lenti-X system (Clontech)</td>
</tr>
<tr>
<td></td>
<td>First generation and High-Capacity Adenoviral vectors (Volpers and Kochanek, 2004).</td>
</tr>
</tbody>
</table>

#### Origin & function

<table>
<thead>
<tr>
<th>Inserted sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small hairpin RNAs (shRNAs) targeting transcripts of mammalian genes that regulate cellular responses in vascular and non-vascular cells will be used. For example, the LOX-1 scavenger receptor, VEGF receptors, insulin and insulin-like growth factor receptors, p53, FAK, PI3-kinase-AKT signalling pathways are relevant to our current research. In addition, the phospholipases (cPLA2alpha, iPLA2) as well as cyclooxygenase enzymes that are downstream of growth factor (e.g. VEGF) stimulation will also be analysed (see Appendix 2). cDNAs encoding the above as well as point and deletion mutants thereof will also be cloned in the vectors (see Appendix 2).</td>
</tr>
<tr>
<td>Briefly, the genes to be targeted/expressed are as follows; LOX-1, VEGFR1-3, cPLA2α, iPLA2, p53, p14/ARF, p19/ARF, FAK, AKT, PI-3-Kinase subunits, RHEB, IGF-1, IGFBPs, iASPP, LC3, zyxin, Insulin receptor, IGF-1R, and linked signalling gene products. Constructs expressing constitutively active mutants of AKT, PI-3-kinase and</td>
</tr>
</tbody>
</table>
RHEB and those directing knock-down of p53, p14/19/Arf are likely to contribute to immortalisation and transformation of the target host cell. Another question is the role of different factors in regulating blood clotting and we can test the role FXIII-A, transglutaminase 2 (TG2) or GLO-II gene products using viral-based transgene expression to manipulate synthesis, secretion, localisation and biological activity in cultured cells.

**Evaluation of foreseeable effects**

Bacterial E.coli strains used for standard molecular biology have a history of safe use, they are unable to establish and colonise the gut, have auxotrophic requirement that will not be met in the environment, the genetic lesions are well documented. Even in the event of failure of containment it is highly unlikely that harm would occur to humans or the environment.

Vectors

The viral vectors used will be previously described replication defective lentiviruses or adenoviruses engineered to contain mouse shRNA sequences to inhibit gene expression. These will be checked for effectiveness in immortalised mouse cells in culture (e.g. RAW647 mouse macrophage line) before delivery into mice using approved procedures.

Lentiviral vectors:

These 3rd and 4th generation viral gene transfer system are well-characterised, have a history of safe use, are widely-used, non-replicating and are unlikely to generate replication competent virus. The SIN versions of these vectors have higher biosafety when compared to the standard versions.

Adenoviral vectors:

The non-replicating first generation and high capacity adenoviral vectors have been well-characterised. The wildtype adenovirus itself is not associated with any tumour formation in humans. The vector system is very well-characterised and cannot replicate in the absence of the adenoviral E1 gene products.

Virus-transformed cells:

Although some of the virus-transformed mammalian cells created in this project will likely have altered growth properties, eukaryotic cells, even when transformed, are highly disabled and unable to propagate in the environment. Thus the likelihood of harm to humans, animals or the environment is considered to be low.

Mice: Mice injected with the viral vectors are not expected to be any more harmful to humans or the environment than the host mice. Wild-type, ApoE -/- and LDL-R -/- knockout mice cannot support replication of infectious lentivirus (based on HIV-1 genes). As a result, the potential for shedding of recombinant virus from such animals is very low. We are using viral vectors with a negligible risk of becoming replication-competent viruses.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- Rodent control external to the building to prevent ingress of wild type mice
- Use of door barriers to prevent escape of mice from holding rooms
- Mice housed in home office approved caging system which is resistant to escape
- Animal technicians are trained in the correct technique for animal handling
- Only approved SOPs are used for cage changing tasks
- All waste is autoclaved prior to leaving the animal facility, final disposal of waste from the facility is via incineration
- Access to the facility is restricted via an electronic access system to approved personnel

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

To minimise any residual risk to workers or the environment procedures adhering to a GM Class 2 safety manual for recombinant viral work will be followed. This includes the use of a tissue culture class II hood that is cleaned after each use, prompt decontamination and disposal of biological waste, use of lids on centrifuge rotors and
wearing gloves for all gene manipulations. In addition, easily identifiable colour coded labcoats (e.g. blue coloured) and equipment such as centrifuges, pipettors, incubators and tissue culture class II hoods designated for use in viral work will minimise biosafety risks. Routine cleaning and waste management will be carried out by the named person (SLS) and additional trained people working to Class 2 standard.

Facilities to be used:
All work with cell culture and viral particles will be done in the designated GMO Class 2 room and all equipment will be designated as available for GMO class 2 work only.

Safety precautions:
The work will be undertaken in accordance with the requirements for GMO containment level 2. This includes culture of primary cells, viral infection protocols and subsequent culture of any cell lines produced that have not been screened for the presence of virus. Screening of the cell lines will be by assay of reverse transcriptase activity, for example, by using the Roche reverse transcriptase assay, colorimetric (catalogue number 11 468 120 910). The assay will be carried out within the Class 2 facility until cell culture derived material has been put into the lysis buffer (isotonic buffers containing non-ionic detergents (0.5-1% TX-100 or NP- 40) or ionic detergents (2% SDS)), after which time it can be continued outside of the facility providing the principles of Good Laboratory Practice are followed.

Access:
Only named persons will have access to the facility. Viral vector particles will be stored in a locked freezer. Prior to being given access, any named staff must receive training via local induction involving the experienced virologist (SLS) and the local safety officer in LIGHT (Dr. S. Futers). Thorough decontamination of any potentially affected areas will be completed before maintenance staff are granted access.

All contaminated liquid waste is autoclaved under high pressure steam with a temperature of at least 121C for 20 min, ensuring complete killing of all living agents. Solid waste is similarly treated before disposal as clinical waste.

Emergency spill procedure:
Liquid spill – treat with Virkon granules until deep pink, or add an equivalent volume of 2% Virkon – will be left for at least half an hour before being mopped up with plenty of water.

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All spills of genetically modified microorganisms (GMMOs) will be reported immediately to the local Biological Safety Officer, or local Safety Supervisor in the absence of the BSO: all spills and their mode of treatment will be recorded.

Any personal injury where an infection by a GMMO may be involved will be reported immediately to one of the local BSO’s or Safety supervisors and the immediate advice of Occupational Health will be sought.
1. This one is a very well written and appropriately detailed assessment.

2. P2, under Host(s) – class II ACDP oppo paths… should read ACDP hazard group 2 opp paths.

3. P4, Final GMM, can you make this plural as you have two hosts i.e. GMMs, also in text can you make GMMO plural. Same again under next section, make GMM plural.

4. Under section on “environmental and activity” level 1 containment facilities should read level 2. Also GMMO should be plural.

5. P8, under training & authorisation please describe your training in GM work, can be experience and/or formal courses. Have you registered for the faculty’s on-line Gm training course?

6. I’m ok about FBS9024aon – Although Ps aeruginosa is a mild pathogen they seem to have all safety considerations in place and it seems quite well thought out.

7. This project looks OK to me. I presume it is Category II on the basis of the recipient organism?

8. OK by me.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to use gene delivery technology to characterise the roles of both viral and cellular proteins in viral replication, pathogenesis and immune evasion.

These genes (viral and host) will be expressed, or their expression reduced with shRNA, (delivered by non-replication competent retroviral and lentiviral vectors). The modified cells will then be tested for changes in various signalling pathways associated with growth, stress and the immune response. Once changes have been identified, the mechanism of action of the viral or host protein will be characterised. Mutants of the expressed proteins will be made to inform which parts of the protein are important for activity. Understanding the mechanisms by which viruses cause pathogenesis will enable us to block these responses and may alleviate human diseases.

In addition, this technology will be used to extend the lifespan of primary cells in culture for biochemical studies.

**Recipient or parental organism**

**Host(s)**

Bacteria

E. coli K12 strains such as XL-1 blue, DH5 alpha and similar commercially available GM strains for plasmid propagation and gene manipulation.

Mammalian

Mammalian primary cells and immortalised cell lines (such as HeLa, SiHa, Caski, HEK-293, HEK-293T, HEK-293 derivatives, NIH-3T3, HaCaT, Vero, Cos-7, Huh-7, Huh-7.5, Hep-G2, CHO and RAW264.7).
Vector

Retroviral

In the first instance we will be using pBabe-Puro retroviral vector (commercially available from Cell Biolabs Inc.), derived from Moloney Murine Leukaemia Virus (MMLV). This vector provides a viral packaging signal, transcription and processing elements and a multiple cloning sequence for cloning of the gene of interest. The vector also contains a bacterial origin of replication, ampicillin resistance gene and a puromycin resistance gene for the growth of infected mammalian cells to select stable cell lines. Other 2nd (and later) generations of safety modified retroviral vectors may be used in this project.

Lentiviral

Amphotropic lentiviral self-inactivating (SIN) lentiviral vector expression system. This system includes p8.91 (pCMVD8.91) which is a HIV-1 plasmid encoding gag-pol tat and rev and deleted for the other accessory gene, pCSGW (pHRSIN-cPPT-CEW) which is a HIV-1 vector encoding green fluorescent protein, which will encode the gene of interest pMDG, which encodes VSV-G envelope protein.

Host/vector system

Retroviral packaging system

In the first instance we will utilize the well characterised Phoenix packaging cell line. Phoenix is a second-generation retrovirus producer cell line for the generation of helper virus free amphotropic retroviruses. These lines, generated in the Nolan laboratory (Stanford University, USA) and now available through the ATCC, are in routine use in over 2500 laboratories worldwide. They are based on the HEK293 cell line (a human embryonic kidney cell line, transformed with adenovirus E1A carrying a temperature sensitive T antigen). For biosafety purposes both the gag pol and envelope constructs utilize non-Moloney promoters to minimize recombination potential with introduced retroviral constructs, and different promoters for gag-pol and envelope are used to minimize their inter-recombination potential. Importantly, this cell line has been tested extensively by the Nolan laboratory for helper virus production and has been established as being helper virus free. The viruses do not contain DNA that encodes virus proteins and so this system cannot produce replication competent virus. Other packaging cell lines compatible with 2nd and later generation retroviral gene delivery technology may also be considered.

Lentiviral delivery system

SIN vectors have a deletion in viral promoter region (U3) of the long terminal repeat (LTR) and following reverse transcription have no viral promoter in the provirus and so are safer than non-SIN vectors. The other gene products (Gag-pol and the envelope) are provided in trans from different plasmids so as to minimise any risk of replication competent lentiviruses. In this system replication-competent viruses can be produced only after three separate recombination events. Co-transfection of the plasmids including one carrying a viral glycoprotein (VSV-G) into a designated human packaging cell line (e.g. HEK293T) enables production of replication-incompetent viral-like articles that retain the ability to deliver the viral genome to a broad range of host cells.

The accessory genes essential for wild-type lentivirus replication are absent in this system. Additionally, the vesicular stomatitis virus envelope glycoprotein (VSV-G) is encoded by a separate plasmid that enables coating of the replication-defective particles with VSV-G, enabling delivery of virions to a wide-range of host cells in culture. For biosafety purposes, the integrated lentiviral construct within the genome of the host lacks the capability of excision or VSV-G gene sequence to enable packaging and export of infective virions.

Purified plasmids will be transfected into Phoenix or HEK293T cells, media collected and retroviral / lentiviral virus-like particles purified by high-speed centrifugation in sealed tubes. Alternatively, commercial kits are available for viral purification (e.g. Invitrogen, Clontech)

Origin & function

Inserted sequences.

This project is concerned with the expression of four kinds of inserted sequences, which in combination will be used to dissect viral pathogenesis and cellular processes. These inserts include; viral genes cellular genes, shRNAs and libraries created from cell lines and each will be discussed in more detail below

Viral sequences.
The inserted sequences consist of both structural and non-structural proteins, some of which are potential transforming proteins, derived from:

- human adenoviruses (E1A, E1B, E3-14.7K, fibre)
- human papillomavirus (HPV) (E5, E6, E7)
- hepatitis C virus (HCV) (NS2, NS3/4A, NS4B, NS5A).

We will PCR amplify the sequences for these cDNAs and clone wild-type, point and deletion mutants thereof into the viral expression plasmids.

Cellular sequences.

We are interested in proteins associated with signalling. Briefly, the genes to be targeted/expressed include protein kinases, ubiquitin ligases, phosphatases and adaptor proteins. In particular we will work with proteins associated with nuclear factor kappa B (NFkB) signalling (e.g. NEMO, optineurin and TRAF ligases), interferon (IFN) signalling (e.g. TBK1 and IKK epsilon), mitogen activated protein kinases (MAPks) (e.g. ERK and p38 MAPK), pattern recognition receptor signalling (e.g. RIG-1 and Mda-5), energy balance detection (e.g. AMPK) and beta catenin signalling (e.g. GSK3 beta and beta-catenin). Introduction of some of these constructs is expected to change the growth properties of the cell lines, increasing or decreasing growth rate and ability to survive indefinitely in culture, or abrogation of this ability, depending on the host genotype and the particular construct.

shRNA sequences.

We will also utilise the Phoenix delivery system to introduce small hairpin RNAs (shRNAs) targeting transcripts of the above viral and cellular targets.

In addition, we will extend the lifespan of primary cells in culture by the expression of the HPV E6 and E7 proteins using viral delivery. Over-expression of HPV E6/E7 has been demonstrated to be effective as a means of prolonging growth in the laboratory of different primary cells (Maeda et al., 2005).


Evaluation of foreseeable effects

The main environmental and safety hazard from these inserts would be the transmission of oncogenes to a wild-type animal or person. This is an extremely unlikely event. However, if this occurred then the worst-case scenario (transmission of an oncogene), would be limited to primary contacts in a wild-type population. However, any viral vector would have to evade the host immune system; the human oncogene would have to be transcribed correctly, and the resultant oncogenic event would also need to evade the host immune system. The likelihood of harm to the environment and safety of workers from the inserts is therefore assessed as negligible. Individuals involved with project are instructed to declare changes to their immune status (caused by e.g. antirejection drugs, chemotherapy, radiotherapy etc), where this is identified then individuals will have a personal risk assessment.

Final GMM

Virus: This project specifically excludes the use of viable Wild-type viruses. All viral vectors used are replication deficient and cannot propagate in any organism except in the producer cell line used for packaging and production of virus-like particles that are replication-defective.

Cells: Virally infected cells are not expected to be more harmful to humans or the environment than the untransduced host cells. They will still be highly unlikely to survive outside tissue culture conditions as eukaryotic cells, even when transformed, are highly disabled and unable to propagate in the environment.

There is no cancer risk associated with human adenoviruses; high risk HPV (such as HPV16 and 18) is associated with cervical cancer and ano-genital cancers with HCV is associated with hepatocellular carcinoma; however transformation frequencies of human cells are very low and multiple open reading frames are often required to stably transform primary human cells. All experiments utilising the recombinant replication defective viruses will be performed under containment level 2 conditions (cell culture laboratories 8.51b, 8.54d and 8.61j). The Phoenix cell lines used to produce recombinant viruses are currently employed in at least 2500 laboratories world-wide for the delivery genes into cells for biomedical research. Additionally, we have consulted with colleagues in Belfast (who provided the pBabe-Puro plasmid) and University College London (who provided the lentiviral vectors) who use this delivery system for similar purposes and they work at Containment Level 2. Similarly, consideratons apply to the use of cells infected with viruses expressing oncogenes. For example, studies on colon cancer have documented that many oncogenes, tumour suppressors and
mis-match repair enzymes must be mutated or inactivated in order to generate a malignant phenotype in vivo. Therefore, cell lines generated are unlikely to be harmful to the operator. Furthermore, accidental infection of the operator with retrovirus expressing viral oncogenes is very unlikely to cause harm.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

To minimise an residual risk to workers or the environment procedures adhering to a GM Containment Level 2 safety manual for recombinant viral work will be followed. This includes the use of a tissue culture class two hood that is cleaned after each use, prompt decontamination and disposal of biological waste, use of aerosol tight lids on centrifuge rotors and wearing gloves for all gene manipulations. In addition, easily identifiable colour coded lab coats and equipment such as centrifuges, pipettors, incubators and tissue culture class two hoods designated for use in viral work will minimise biosafety risks. Routine cleaning and waste management will be carried out by the named person (KHR) and additional trained people working to Class Two standard.

Facilities to be used.
All work will be performed in the laboratories in FBS, level 8. All work with cell culture and viral particles will be done in designated GMO Containment Level 2 rooms and all equipment will be designated as available for GMO Class two work only.

Safety precautions.
This work will be performed in accordance with requirements for GMO containment level two. This includes culture of primary cells, viral infection protocols and subsequent culture of any cell lines. Cells cultured for biochemical experimentation will be lysed within the GMO class two facility, after which time it can be continued outside the facility providing the principles of Good Laboratory Practice are followed. The major risk of working with retrovirus vectors is associated with needlestick injuries. To prevent contamination of workers by virus, no glass or sharps will be used in the cell culture facilities and a lab coat and nitrile gloves will protect workers. Skin lesions will be covered with a bandage in addition to the protective wear described in this application. People with severe skin conditions or wounds will be prevented from working with infectious virus.

Access
Only named persons will have access to the cell culture facility. Viral vectors will be stored in a freezer. Prior to being given access to cell culture staff must receive training via local induction involving the experienced virologist (KHR). Thorough decontamination of any potentially affected areas will be completed before maintenance staff are granted access.

Spillage/accidents.
All spills of genetically modified microorganisms (GMO) will be reported immediately to the local Biological Safety Officer, or local Safety Supervisor in the absence of the BSO: all spills and their mode of treatment will be recorded. In case of spillage, aerosols will be allowed to settle for 30-60 mins before decontaminating the area with 10% Virkon or Trigene. Any contaminated clothing should be removed and autoclaved. Any personal injury where an infection by a GMMO may be involved will be reported immediately to one of the local BSO's or Safety supervisors and the immediate advice of Occupational Health will be sought. In the event of an injury, wounds should be thoroughly washed with soap and warm water before being bandaged. If material has entered the eye, it should be washed thoroughly from the eye wash stations.

Training
An experienced retrovirologist (KHR) will train others in appropriate use of viral gene delivery techniques.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not reequired

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste generated as part of standard procedures containing viable organisms will be inactivated prior to removal from site by a licenced clinical waste contractor. Inactivation will either be the use of a validated biocidal agent (Virkon or Trigene) or autoclaving using a cycle with sufficient load heat and dwell time to achieve sterilisation.
Members of the Faculty of Biological Sciences, Biological Safety Committee discussed the technical aspects of A M's risk assessment. Two questions were raised: one was the need to test for replication competent viruses in the lentiviral vector preparations. It was thought that the safety features built into the systems being used were well tried and tested so there was no need to test for RCVs unless primary cell lines were used that could possibly be infected with retrovirus. Secondly, could transmission of oncogenes by lentivirus vectors into workers be described as low risk? The committee thought this was the case and agreed with the risk assessment on this (to include non-use of sharps).
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to understand the role of oncogenes, tumour suppressor, developmental and cellular signalling genes and pathways in the development and progression of cancer. This includes construction of viral vectors to introduce gene sequences in human cell lines or primary cultures to induce over-expression or knock-down of target genes.

Three expression cassettes will be used:

1. Inserts coding for reporter genes including eGFP and derivatives, dsRed and derivatives.
2. cDNAs encoded by wild-type or mutant known or potential oncogenes, tumour suppressor genes and other genes involved in cell signalling and/or development under the control of cloned promotor fragments. These cDNAs may be unmodified or expressed as fusions with a fluorescent dye such as eGFP or dsRed for identification of cells expressing constructs.
3. Cassettes containing and expressing sequences to effect gene knockdown using RNA interference under the control of human or viral promoters as above. RNA interference would be targeted primarily but not exclusively to genes involved, or thought to be involved, in development and progression of tumours. These may be transient or stable knockdowns.

Any of the above (cassettes 1, 2 and 3) may also include transfer of a drug resistance marker to select recombinant cells.

**Recipient or parental organism**

For propagation of plasmid DNA

E.coli K-12 derived plasmids because of their safe history of use

E. coli XL-Blue (recA1 enA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F' proAB lacIqZΔM15 Tn10 (Tetr)]) because of its safe history of use and efficient cloning of methylated DNA. Although XL1-Blue is able to mobilise plasmids, none of the vectors to be used are mobilisable therefore minimizing risk.

Retrovirus packaging cell lines

e.g. Phoenix amrho, derived from human embryonic kidney cell line HEK293T by introduction of separate gag-pol and env constructs [two plasmids with different selectable markers] designed to minimise potential recombination.
Established human cell lines

Primary human cells

Human cell lines, primary cells and packaging cell lines are not capable of survival outside the laboratory and are non-transplantable due to immunoincompatibility. They are therefore considered to be highly disabled hosts. Established cell lines with a history of safe use may be considered hazard Group 1, however primary cells may contain endogenous pathogens and must be treated as Group 2.


Host/vector system

Retrovirus (including MMTV, MoMLV) - ACDP1

Lentivirus (including safety modified HIV-1)

No work is proposed which would involve using intact wild-type retrovirus, HIV-1 or HVS. Lentivirus backbones to be used are considered safer than wildtype HIV-1 because of modifications which reduce probability of live virus production significantly. The non-human adenoviral vectors (Herpesvirus saimiri - HVS) to be used are non-pathogenic, deletion of the transforming genes Stp and Tip removing any oncogenic potential of the virus.

1) Viral vectors from collaborators and commercial sources -

a. Retrovirus - vectors include FB series (Stratgene), RETROSUPER (Netherlands Cancer Institute), RetroTET system (Clontech). Retroviruses infect dividing cells and integrate into the host cell genome. Integration is random but preferentially into transcriptionally active chromatin and thus carries a risk of insertional mutagenesis. The retroviruses used for vector construction are based on murine retroviruses MMTV, MoMLV and MSCV in ACDP hazard Group 1, however amphotropic virus is produced which can infect human cells, potentially increasing the hazard. The retroviral vectors to be used are replication-defective, requiring transfection into special packaging cell lines to produce infectious virus and have a long history of safe use. Viruses are capable of infecting cells but once integrated, virus genomes lack the sequences necessary for production of infectious virions. Stable retrovirus producer cells cannot be made. Packaging cell lines (Phoenix Ampho) have been developed which carry two plasmids encoding the viral gag-pol and env genes separately to minimise the possibility of multiple recombination events regenerating wild type virus. Vectors carry drug resistance markers for selection of transduced cells - these genes are non-hazardous to human helath.


b. Lentivirus (see Appendix for examples)

Lentiviruses are a sub-group of retroviruses that can infect both dividing and quiescent cells. The lentiviral vectors to be used in the proposed study are based on HIV-1 (e.g. Invitrogen pLenti6 series, Clontech pLVX). However these 3rd generation self-inactivating vectors are multiply-deleted and lack the ability to replicate or re-create an intact virus genome once integrated into the host cell genome, and would thus pose a much reduced risk compared to the parent genome once integrated into the host cell genome, and would thus pose a much reduced risk compared to the parent virus. The vectors typically contain less than 30% of the wild type HIV-1 genome. Safety is increased by the use of commercial packaging systems such as the Clontech Lenti-X or Open Biosystems Trans-Lentiviral Packaging System in which the functions required for virion production are separated and carried on 4 or 5 co-transduced plasmids. These contain minimal virus sequence to minimise the potential for recombination. Stable producer cell lines cannot be made with the modified vectors to be used. Virions produced from lentivirus vectors will be pseudotyped with VSV-G structural proteins to allow entry into multiple cell types, however recent work has shown that amphotropic lentiviruses rapidly lose infectivity at 37°C. Commercially available 3rd generation lentiviruses with ORF or siRNA inserts ay also be used. The vectors carry drug resistance markers for selection of transduced cells; these are non-hazardous to humans.

SACGM Compendium of Guidance Jan 2007 pp116-125
2) Plasmid vectors obtained from collaborators or commercial sources, including but not limited to pFB series. All are mobilisation-defective. All contain antibiotic resistance genes for selection with ampicillin, kanamycin or chloramphenicol in bacteria. Resistance to these antibiotics is not clinically relevant due to widespread resistance or antibiotic toxicity.

All vectors and viruses received from external sources (commercial or from collaborators) will be handled in a designated labelled restricted access class 2 biological safety cabinet by experienced trained laboratory personnel, and checked for contamination with bacteria, yeast or mycoplasma prior to preparation of stocks and use. Receipt of vectors and viruses, source and details of subsequent handling will be recorded and dated (written record).

Origin & function

ORIGINS:

i) Human DNA, including cloned cDNA, shRNA, siRNA prepared in-house, from collaborators or commercial sources.

ii) Low hazard for plasmid DNA or human cell line DNA

Risk of infection in preparation of DNA from primary cell cultures; if donor known to be HIV or HBV +ve on basis of medical history they are excluded to minimise this hazard.

FUNCTIONS:

1. Inserted sequences coding for reporter genes including eGFP and derivatives, dsRed and derivatives, luciferase, beta-galactosidase.

2. cDNAs encoding wild-type or mutant known and candidate oncogenes, tumour suppressor genes and other genes involved in cell signalling and development. Expression is under the control of cloned tissue-specific huma promoter sequences, constitutive viral promoters (e.g. MoMV 5’ LTR) or inducible promoters (e.g. TET ON or TET OFF system, inducible lacZ promoter). cDNAs may be unmodified or expressed as fusions with eGFP or similar for fluorescent detection of protein expression.

3. Expression cassettes encoding shRNA or siRNA molecules under the control of human or viral promoter. shRNAs or siRNAs will be targeted primarily but not exclusively to genes that may be, or are known to be, involved in cancer development and progression. Stable expression of shRNA has the potential for off-target effects on other genes that can deliver an antiviral response resulting in altered cellular metabolism and local inflammation following accidental exposure to RNAi-expressing GMOs. This will be minimised by careful selection of target sequences using sequence databases.

Evaluation of foreseeable effects

Retroviruses are hazard group 1 and are les hazardous than the parent organism. The replication defective system consists of a packaging cell line and the vector; structural genes are removed from the vector to prevent it replicating once it has entered the cell. Infection with retroviruses can lead to insertional mutagenesis adjacent to the site of insertion, although it is only capable of one round of infection. Infection with retrovirus has the potential to transcriptionally activate cellular oncogenes. However, this is partly titre-dependent and unlikely to occur with the small amount of virus handled within this laboratory assessment. The inserted genes code for known or potential oncogenes, tumour suppressor or developmental genes that may be under the control of strong promoters. Theoretically these changes may contribute to cellular transformation if expressed in the cells of the operator, although several further genetic changes would be required for progression to malignancy. Furthermore removal of structural genes from the vector prevents it from replicating after it has entered the cell.

Lentiviruses are no more harmful than the parent virus, and frequently pose a lower risk reflecting deletions in the virus backbone and inability to replicate or re-create an intact virus genome when integrated into the host cell genome. In the proposed study the high infectivity rates and long-term persistence of vectors, combined with the ORF 73 regulatory region to overcome promoter silencing, may be utilised. Additional vectors are HIV-1 based but multiply-deleted and unable to replicate or cause disease. The
main theoretical risk, as for retroviruses, is from insertional deleted and unable to replicate or cause disease. The main theoretical risk, as for retroviruses, is from insertional mutagenesis and expression of inserts in the host cell leading to cellular transformation. Lentiviral vector systems that include specific genes (gag, pol, env) for virulence or pathogenicity on 2, 3 or 4 plasmids lower the risk for recombinaton and the production of replication-competent HIV, thereby providing greater safety to the researcher.

E. coli strains and human primary and established cell lines are disabled and cannot survive outside laboratory growth conditions.

Retroviruses and lentiviruses are enveloped and could not survive in environment and no modifications of the viruses will be made that would be likely to alter this property. All virus vectors contain multiple deletions and can only be propagated in specialised laboratory cell lines. Any infected cells would be targeted and destroyed by the immune system. Furthermore the retrovirus genomes lack the sequences necessary for production of infectious virions limiting infectivity following cellular integration.

Lentivirus based on HIV: In theory it would be possible for lentivirus to recombine with wild type HIV in a person infected with HIV, however this would require that infectious virus reaches and infects CD4 cells where HIV exists. Since the lentivirus-recipient cells are non-permissive for wild type HIV maintenance and replication, this should not arise.

There are no economic or ecological consequences of major release of any of the GMOs; the work is small-scale and non-hazardous to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work to be carried out in accordance with LIMM safety manual rules for work with Biological Agents and GMOs.

All work with cell lines, virus production and handling of virus to be performed in designated Class 2 biological safety cabinets using designated, labelled equipment to minimise aerosols and risk of accidental infection. Centrifugation to be carried out in closed buckets opened only inside Class 2 safety cabinet.

Minimise risk of ingestion by good laboratory practice, hygiene procedures, hand washing, wearing of gloves and laboratory coat (see LIMM safety manual).

No use of sharps with virus or virus-infected cell cultures or naked DNA (gel excision tips to cut DNA bands form gels).

Immuno-compromised individuals or those with skin conditions such as eczema will be advised to consult occupational health before working with live virus.

All waste to be disposed of according to LIMM safety manual procedures.

Liquid waste will be treated with Virkon granules or an equal volume of 20% Trigene Advance or 10% Virusolve+ and left for at least 30 minutes before disposal down the drain with copious amounts of water.

Solid waste (culture or cells) will be autoclaved at a minimum of 121°C (load temperature) for 15 minutes before being disposed as clinical waste.

Emergency spill procedure:

Liquid spill - Wearing gloves treat with Virkon granules until deep pink, or add an equivalent volume of 20% Trigene Advance or 10% Virusolve+; leave for at least 30 minutes before mopping up with paper towels and wash area with plenty of water.
Solid spill - Wearing appropriate gloves, solid spills will be gathered into an autoclave bag, the area affected will be wiped liberally with 2% Virkon or 5% Trigene Advance. The disinfectant to remain in contact with the treated area for at least 30 minutes to allow the disinfectant to act; the area will then be mopped up with paper towels and washed with plenty of water. Immediately after it has been collected and the affected area has been treated with Virkon, the solid waste will be taken for autoclaving, as above.

For spills inside centrifuges or incubators the local Standard Operating Procedure is indicated on the equipment will be followed.

All spills of genetically modified microorganisms (GMMOs) will be reported immediately to a safety supervisor: all spills and the mode of decontamination and disposal will be recorded in the GMO incident book.

Any personal injury where an infection by a GMMO may be involved will be reported immediately to a safety supervisor and the advice of Occupational Health will be sought. Such incidents will be recorded in the accident book.

The identity of any vectors and viruses received will be confirmed by restriction enzyme digest to authenticate the suitability of clones received from external sources for work under the current licence application.

Please enter comments on the GM safety committee on the risk assessment

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
I have passed the application around members of the LIMM GMAG Safety Committee asking for any comments. A few responses were received. The only negative comment related to the generic feel of the application re the inserts to be employed.

1. Section 12 - There was concern over the phrase 'cDNAs encoding wild-type or mutant known and candidate oncogenes, tumour suppressor genes and other genes involved in cell signalling and development'. It was felt that this phrase was very generic and could encompass half the genes in the genome. For previous applications, details of specific genes and the consequences of over-expressing or knocking them out were asked for. Are we now going back down the generic route? Will the HSE accept this? Would it be wise to include one or two examples as worse case scenarios to compare the rest with e.g. The over-expression of mutant RAS may be more hazardous than the over-expression of polo kinase’

2. Section 11 - For lentiviral production: are third generation packaging systems always going to be used? Are the ones in the attachment the chosen systems? Additional systems could be included at a later date if required.

3. Perhaps a brief comment re the process of accepting viruses from collaborators may be in order with respect to evaluation of the hazards of the system.

Comments?

Section of Opthalmology and Neuroscience
Leeds Institute of Molecular Medicine
Level 8, Wellcome Trust Brenner Building
St James University Hospital
Leeds LS9 7TF
West Yorkshire
UK

In response to comments
1. Generic nature of the application - I have added some examples of potential effect of some specific inserts. Most importantly though the constructs are not anticipated to change the host range for the viruses. (Section 12)

2. These are examples as stated in text (Section 11).

3. I have added a comment about handling viruses from collaborators. (Section 12)

Are you both happy that we submit this now?
Do you have any additional comments?

I had not noticed before that HVS (I assume this is herpesvirus saimiri) appears inappropriately under the heading of Lentivirus. This monkey gamma herpes virus does not appear to be described in the vectors to be used. Is this the AW's vector that has had the transforming oncogene bits removed (perhaps including one of A's appropriate references). On its own, I assume this is classified as a Class 1 vector anyway as its host is monkey? I seem to remember that it infects some (primary) human cells but does not replicate?

I also cannot see where a comment is made re analysis of the safety of vectors being imported from collaborators. Can you add a sentence after the comment about logging in clones something along the lines that 'The potential hazards of clones from external sources will be evaluated to check for their suitability under the licence being applied for' This is one of the points I wanted to be addressed and commented upon in the application as it was raised by the Safety Committee. The other point was the request for examples of the potentially most damaging inserts (worse case scenario) that was addressed.

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Leeds Institute of Molecular Medicine,
Level 8, Wellcome Trust Brenner Building
St James University Hospital
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West Yorkshire
UK

Tel 0113 3438419
Fax 0113 3438603

Thank you for these helpful comments.
Yes the HVS non-human viral vectors are especially disabled by deletion of Stp and Tip to remove the oncogenic potential of the virus. We have previously used Herpesvirus saimiri (from A) in the lab- that was Class 1 (low risk). We are likely to use this again and potentially other similar class 1 vectors that A is developing that have modifications to the promoters to prevent methylation. However as this is class 1 I have removed this from the text. Have inserted additional text on receipt from collaborators as you requested.

Please can you insert the information for section 2, 6 and 16, and any information that might be needed in Section 15 from the genetic modification safety committee.

Please can we submit this to HSE now?
Professor of adolescent and paediatric cancer research
Leeds Institute of Molecular Medicine
St James University Hospital
Beckett Street
Leeds LS9 7TF

The local biological safety committee approved the project at containment level 2 without further comment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022
V(D)J recombination generates diversity of immunoglobulin and T cell receptor genes, a reaction that is catalysed by the recombination activation gene (RAG) proteins, RAG1 and 2. During this reaction, the DNA that lies between the recombinating V, D and J gene segments is excised out of the genome. Previously, this excised DNA was thought to be lost during cell division but recent evidence indicates that RAG1 and RAG2 can catalyse an aberrant V(D)J recombination reaction in which these by-products are reinserted back into the genome. This re-integration reaction is thought to be a very frequent reaction during lymphocyte development and has been proposed to have pathogenic effects by triggering genome instability. The non-core regions of RAG proteins have been shown to inhibit the re-integration reaction in vivo. The aim of the activity described here is to purify full-length wtRAG1 and wtRAG2, and mutant form(s) of these RAG proteins. These will be used in subsequent in vitro experiments to further analyse the biochemistry of the reintegration reaction to thus better characterise how the re-integration reactions are inhibited. The longer term goal of this work is to develop specific small molecule inhibitors of these potentially pathogenic reactions.

It is not possible to purify active forms of full-length RAG1 and RAG2 when expressed in E. coli or insect cells, so early biochemical studies of these proteins made use of truncated versions, where residues non-essential for the basic V(D)J recombination reaction are removed to give "core" RAGs. However, the "non-core" regions are now known to be essential for the correct regulation of recombination as well as re-integration reactions. Previous studies (e.g. Elkin et al., 2003, EMBO J 22, p1931 and McBlane et al., 1995, Cell 83, p387) indicate that expression by vaccinia virus in HeLa cells is the only method of expressing and purifying catalytically-active full-length
RAG1 and RAG2 proteins. These full-length proteins are thus essential to characterise the mechanism by which the inhibitory non-core regions regulate re-integration.

Protein expression using the vaccinia virus/T7 RNA polymerase hybrid system

The most efficient procedures for expression of genes in mammalian cells utilize a recombinant vaccinia virus encoding the bacteriophage T7 RNA polymerase (Fuerst et al 1986 PNAS 83, 8122). Two main variations of this system exist. In the first, cells are infected with a recombinant vaccinia virus expressing the T7 RNA polymerase (vTF7-3), and then transiently transfected with a plasmid construct containing the gene of interest situated 3’ to a T7 promoter. However, according to the developers of the system, the level of expression achieved using this method is not high enough to make a large stock of purified protein, that we require for our subsequent biochemical analyses (Moss and Elroy-Stein, 1998, Curr Prot Prot Sci, Unit 5.15; Elroy-Stein et al 1989, PNAS p87). To achieve the level of expression required for mass protein production, the preferred method is to make a second recombinant virus containing the gene of interest situated 3’ to the T7 promoter (Fuerst et al 1987, Mol Cell Biol 7, p2583; Moss et al 1990, Nature 348, P91; Barrett et al 1989, AIDS res. Human Retroviruses 5, 159-171). This is because the efficiency of viral infection is much greater than the efficiency of transfection by the calcium phosphate of liposome methods. In a typical experiment, 99% of infected cells are observed to express the desired protein, compared with as little as 60% in transfected cells (Moss and Elroy-Stein, 1998, Curr Prot Prot Sci, Unit 5.15).

Recipient or parental organism

HeLa S3 cells will be used to produce wild-type vaccinia stocks and also in the expression and purification of RAG1 and RAG2. These cells are non-pathogenic to humans or animals, non-toxic and non-allergic. BSC-1 cells will be used for the production and screening of recombinant vaccinia virus. HeLa S3 and BSC-1 cells are of human and primate origin respectively, and both are well characterised, authenticated continuous cell lines that have essentially zero risk of endogenous infection and as such are both categorised as ACDP Hazard level 1.

Host/vector system

The hybrid vaccinia/T7 RNA polymerase expression has been described above. Full-length RAG genes will be subcloned into the plasmid transfer vector pTM11, which is non-mobilisable and poses no hazard in itself (Moss et al 1990, Nature 348, p91). pTM1 is then inserted into the genome of WR vaccinia virus via homologous recombination in BSC-1 cells. This recombinant virus is then used as the vector for transfer of RAG genes in HeLa S3 cells. Transfer of genetic material is achieved by infection of mammalian cell culture. The Western Reserve strain of vaccinia virus in not attenuated, and is replication competent in human cells. However, the risk posed by vaccinia infection is low, and level 2 containment procedures are sufficient to minimise the risk posed to workers (see “Evaluation of foreseeable effects” below).

Origin & function

The full length RAG1 and Rag2 cDNAs will be subcloned from plasmid vectors available in the lab.

The DNA sequence will not be isolated from a donor organism, therefore there are no associated hazards.

Two recombinant vaccinia viruses will be made, one containing the mammalian RAG1 gene, and the other containing the mammalian RAG2 gene, as well as mutant derivatives of these proteins. Both wild type and mutant proteins will be cloned 3’ to a T7 promoter and these vectors will be used to individually infect HeLa cells to produce high levels of RAG proteins. Rag1 and RAG2 must work as a complex to trigger recombination or re-integration and thus these proteins pose no hazard when expressed on their own. Likewise, the mutant derivatives also pose no hazard when expressed as individual proteins. Thus, the risk to personnel should they become infected, is negligible and if infection with both viruses should occur, the risk is still very small, such that it is close to negligible (see “evaluation of foreseeable effects”)

Evaluation of foreseeable effects

Production of recombinant vaccinia virus involved the use of the Western reserve (WR) strain of vaccinia virus. To healthy individuals this virus poses no possible threat of serious disease, with typical symptoms being the development of a small pustule on the skin at the site of infection. Mild systemic disease may accompany the skin lesion, such as mild fever and general malaise. The low risk posed by vaccinia virus infection is demonstrated by the fact that this virus has been used in vaccination protocols against smallpox virus since the late 18th century. Furthermore, all individuals who grew up in the UK and are now over the age of 40 are likely immune due to previous
Immunization with vaccinia virus to protect against smallpox. However, vaccinia virus has been known to cause skin irritation and lesions in individuals with severe eczema and psoriasis, or individuals who are severely immune deficient or pregnant. For this reason, class 2 containment practices will be employed at all times, including the use of class 2 microbiology safety cabinets within a laboratory designed specifically for class 2 activities. A fraction (10-15%) of vaccinia virus can survive for up to 24 hours in the environment. However, handling of, or work with, virus will be performed in a Class II safety cabinet where the surfaces will be wiped with 2% Virkon after virus use, thereby minimising the risk of virus escape. Nucleic acid from the recombinant vaccinia virus will be extracted and stored at -20°C. This DNA will be analysed to confirm the production and isolation of recombinant virus, and will then be disposed of. The DNA in isolation in non-mobilisable and poses no risk. The RAG1 and RAG2 proteins are non-toxic to humans. Furthermore, expression of the RAG proteins in human cells would also require concomitant infection with vTF7-3, as the RAG gene is under the control of a T7 promoter. As explained above, the RAG1 and 2 proteins are responsible for cutting DNA during V(D)J recombination. Since they are both required for cutting, infection with all three recombinant viruses would be necessary to pose a risk of DNA cutting. The RAG1 and RAG2 infections will be carried out separately, so the risk of infection with all three viruses is reduced to close to zero. In the event of a triple infection, the antigen receptor loci where cutting occurs are not accessible to the RAGs in any cells except B and T cells, giving a very small chance of any potentially harmful DNA cutting. Moreover, in the absence of recombination, the potentially pathogenic re-integration reaction does not occur. Therefore, the modified vaccinia viruses would be no more harmful than the unmodified recipient.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Liquid waste will be treated with an equal volume of 2% Virkon and left overnight to give 100% inactivation of vaccinia viruses before disposal down the drain with copious water. Solid waste (culture or cells) will be autoclaved at a minimum of 121°C (load temperature) for 15 minutes to give 100% kill before being destroyed as clinical waste. Emergency spill procedure: Liquid spill - Wearing appropriate gloves treat with Virkon granules until deep pink, or add an equivalent volume of 2% Virkon - leave for at least half an hour before mopping up with plenty of water. Solid spill - Wearing appropriate gloves, solid spills will be gathered into an autoclave bag, the area affected will be wiped liberally with 2% Virkon. The treated area will be left for half an hour for the disinfectant to have time to act and the area will then be mopped with plenty of water. Immediately after is has been collected and the affected area has been treated with Virkon, the solid waste will be taken for autoclaving, as above. For spills inside centrifuges or incubators the Standard Operating Procedure as indicated on the equipment will be followed. All spills of pathogens or genetically modified microorganisms (GMMOs) will be reported immediately to a safety supervisor: all spills and their mode of treatment will be recorded. Any personal injury where an infection by a pathogen or GMMO may be involved will be reported immediately to a safety supervisor and the immediate advice of Occupational Health will be sought. |

02/03/2022
Dear S,

Thank you for forwarding the risk assessment for work with vaccinia by Dr B. I confirm that the Biological Safety Committee is happy for this to be submitted to the HSE.

Chair, Biological Safety Committee

Professor M H
Institute of Molecular and Cellular Biology
Faculty of Biological Sciences
University of Leeds

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**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

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<th>Project Ref</th>
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**Date Ackn’d** | **CU2 Project Title** | **Class** | **CultureVol** |
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<tr>
<td>25/11/2011</td>
<td>Gene transfer into mammalian cells for study of renal disease</td>
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**Date Project Ceased** | **Consent Granted**
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<td>Non-GMM Consent Granted</td>
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The aim of the project is to understand the role of a variety of genes including oncogenes, tumour suppressor genes and genes encoding components of cellular signalling pathways in renal disease (renal cancer, renal injury and renal failure).

This involves the construction and use of viral vectors to allow targeted over-expression or knock-down of genes of interest in human and mammalian cell lines. Viral vectors will contain expression cassettes that harbour:
- Reporter genes or
- cDNA or
- Interfering RNA

These vectors carry drug resistance markers to allow selection in E. coli and human/mammalian cells.

**Recipient or parental organism**

E. coli K12 strains such as:
- XL-1Blue (recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F’ proAB lacQZΔM15 Tn10 (Tetr)])
- DH5α (F− Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK−, mK+) phoA supE44 λ− thi-1 gyrA96 relA1)

will be used for propagation of plasmid DNA.

E. coli K12 strains are attenuated, non-colonising, non-pathogenic strains, unlikely to survive outside the laboratory and have a long history of safe use, and are regarded as non-hazardous.

**Cell lines**

Retrovirus packaging cell lines such as Phoenix Ampho (Orbigen; www.stanford.edu/group/nolan) which are derived from the human embryonic kidney cell line HEK293T by introduction of separate gag-pol and env constructs [two plasmids with different selectable markers] designed to minimise potential for recombination will be used for virus production.

Lentivirus packaging systems such as the HEK293T-based Lenti-X™ HTX (Clontech; www.clontech.com) or Open Biosystems Trans-Lentiviral™ GIPZ (Thermo Scientific; www.openbiosystems.com) systems in which the functions required for virion production are separated and carried on multiple co-transfected plasmids will be used for virus production.

Primary and established human cell lines and mammalian cell lines.
The cell lines used are not capable of survival outside the laboratory and are non-transplantable due to immuno-incompatibility. They are therefore considered to be highly disabled hosts. Established cell lines with a history of safe use may be considered hazard group 1, however primary cell lines may contain endogenous pathogens and are treated as hazard Group 2.

## Host/vector system

Viral vectors to be used are from collaborators and commercial sources:

a. **Retrovirus** – vectors such as pFB-Neo (Stratagene), pPRETROSUPER (Netherlands Cancer Institute), pBABE-puro (addgene) and pMSCVhyg (Clontech)

Gamma-retroviruses (such as MMLV) only infect actively dividing cells and integrate into the host cell genome. Integration is random but preferentially into transcriptionally active chromatin and thus carries a risk of insertional mutagenesis. The retroviruses used for vector construction are based on murine retroviruses Moloney Murine Leukemia virus (MMLV) and Murine Embryonic Stem Cell virus (MSCV) in ACDP hazard Group 1; however amphotropic virus is produced which can infect human cells, potentially increasing the hazard. The retroviral vectors to be used are replication-defective, requiring transfection into special packaging cell lines for production of infectious virus and have a long history of safe use. Once integrated, virus genomes lack the sequences necessary for production of infectious virions; stable retrovirus producer cells cannot be made. Packaging cell lines (such as Phoenix Ampo; www.stanford.edu/group/nolan) have been developed which carry two plasmids encoding the viral gag-pol and env genes separately to minimise the possibility of multiple recombination events regenerating wild type replication competent virus.


b. **Lentivirus** – vectors such as pLOC/pGIPZ (Thermo Scientific), pLenti6 series (Invitrogen), pLVX-Puro (Clontech)

Lentiviruses are a sub-group of retroviruses that can infect both dividing and quiescent cells. The lentivirus vectors to be used are based on HIV-1 which is listed in ACDP Hazard group 3. However these 3rd and 4th generation self-inactivating vectors are multiply-deleted and lack the ability to replicate or re-create an intact virus genome once integrated into the host cell genome and thus pose a much reduced risk. The vectors typically contain less than 30% of the wild type HIV-1 genome. Safety is increased by the use of commercial packaging systems such as the Lenti-X HTX (Clontech; www.clontech.com/products) or Open Biosystems Trans-Lentiviral GIPZ (Thermo Scientific; www.openbiosystems.com) packaging systems in which the functions required for virion production are separated and carried on multiple co-transfected plasmids. These contain minimal virus sequence in order to minimise the potential for recombination. Stable producer cell lines cannot be made with the modified vectors to be used. Virions produced from lentivirus vectors are usually pseudotyped with VSV-G structural proteins to allow entry into multiple cell types (VSV-G allows entry in to a very broad host range, i.e. most mammalian cells).

Recent work has shown that amphotropic lentiviruses rapidly lose infectivity at 37oC. Commercially available 3rd generation lentiviruses with ORF or siRNA inserts may also be used.

References:


All contain drug resistance markers to allow selection in E. coli and human / mammalian cells. Resistance to these antibiotics is not clinically relevant due to widespread resistance or antibiotic toxicity.

## Origin & function

The following will be delivered to target mammalian (including human) cell lines predominantly by viral vectors:

1. Reportor genes including GFP and derivatives, luciferase and β- galactosidase.
2. cDNAs encoding wild-type or mutant known and candidate oncogenes, tumour suppressor genes and other genes involved in cell signalling, apoptosis and/or development of renal diseases including cancer. Expression is under the control of cloned human promoter sequences, constitutive viral promoters (such as CMV or SV40 early promoters, MoMLV 5’ LTR) or inducible promoters (such as TET ON/OFF system, inducible lacZ promoter). cDNAs may also be expressed as fusions with GFP and derivatives to allow fluorescent detection or with epitope tags such as haemagglutinin (HA) or FLAG or with poly-histidine tags to facilitate protein detection and purification.
3. shRNA or siRNA (interfering RNA) molecules under the control of human or viral promoters targeted to genes thought to be involved in renal disease.

In terms of hazard, loss of tumour suppressor or gain of oncogene function may contribute to the development of a malignant cell phenotype and loss of normal growth and survival controls. Certain inserts may immortalise cells. It is highly unlikely that inserts will alter the host range of the virus or result in a GMO capable of replicating outside the laboratory.

High levels of interfering RNAs can trigger the antiviral response, resulting in altered cellular metabolism and local inflammation following accidental exposure to interfering RNA-expressing GMOs. Expression of interfering RNAs has the potential for off-target effects on other genes. This will be minimised by careful selection of target sequences using sequence databases.

In E. coli, Inserts will be under the control of eukaryotic promoter sequences and would not be expressed in bacteria and thus are of no or low hazard.

DNA
Human/mammalian DNA isolated from tissues, primary or established cell lines; cloned cDNA or shRNA/siRNA from collaborators or commercial sources.
Plasmid DNA and DNA isolated from established cell lines and mammalian primary cell lines and tissues is low hazard.

There is a risk of infection in preparation of DNA from human tissue samples and primary cell lines, however samples are not collected from patients known to be high risk or positive for HIV or HBV on basis of medical history to minimise this hazard.

All personnel handling human tissue must show hepatitis B virus (HBV) immunity.

**Evaluation of foreseeable effects**

Modified retrovirus – is less harmful than the parent organism. The replication defective system consists of a packaging cell line and a vector; the vector is not capable of replicating once it has entered a cell thus virus is only capable of one round of infection. Infection with retroviruses can lead to insertional mutagenesis adjacent to the site of insertion. This has the potential to transcriptionally activate cellular oncogenes. However, this is partly titre-dependent and unlikely to occur with the amount of virus handled within this assessment (<50ml supernatant at any one time). The inserted genes/interference RNAs include those that code for/target known or potential oncogenes or tumour suppressor genes and could potentially lead to cellular transformation if expressed in an operator's cells although several further genetic changes would be required for progression to malignancy.

Lentivirus – less harmful than the parent organism. The vector is HIV-1-based but multiply-deleted and unable to replicate or cause disease. It would in theory be possible for lentivirus to recombine with wild type HIV in a person infected with HIV, however this would require that infectious virus reaches and infects CD4 cells where HIV exists, followed by multiple recombination events. The lentivirus-recipient cells (human and rodent cell lines derived from epithelium or stroma) are non-permissive for wild type HIV maintenance and replication. The main risk, as for retroviruses, is from insertional mutagenesis and expression of inserts in the host cell leading to cellular transformation.

Hazard identification in respect of the environment:
E. coli strains and human primary and established cell lines are disabled and cannot survive outside the laboratory.

Retro- and lentiviruses are enveloped and could not survive in the environment and no modifications of the viruses will be made that would be likely to alter this property. All virus vectors contain multiple deletions and can only be propagated in specialised laboratory cell lines.

Infected cells would be destroyed by immune system.

Work is small-scale thus there is no chance of major release of any GMO, and the work is non-hazardous to the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
All work to be carried out in accordance with LIMM safety manual rules for work with Biological Agents and GMOs.

All work with cell lines, virus production and handling of virus to be performed in designated Class 2 biological safety cabinets using designated, labelled equipment to minimise aerosols and risk of accidental infection. Centrifugation to be carried out in closed buckets opened only inside Class 2 safety cabinet.

Minimise risk of ingestion by good laboratory practice, hygiene procedures, hand washing, wearing of gloves and laboratory coat (see LIMM safety manual).

No use of sharps with virus or virus-infected cell cultures or naked DNA (gel excision tips to cut DNA bands from gels).

Immuno-compromised individuals or those with skin conditions such as eczema will be advised to consult occupational health before working with live virus.

All waste to be disposed of according to LIMM safety manual procedures.

Liquid waste will be treated with Virkon granules or an equal volume of 20% Trigene Advance or 10% Virusolve+ and left for at least 30 minutes before disposal down the drain with copious amounts of water.

Solid waste (culture or cells) will be autoclaved at a minimum of 121°C (load temperature) for 15 minutes before being disposed as clinical waste.

Emergency spill procedure:
Liquid spill – Wearing gloves treat with Virkon granules until deep pink, or add an equivalent volume of 20% Trigene Advance or 10% Virusolve+ ; leave for at least 30 minutes before mopping up with paper towels and wash area with plenty of water.

Solid spill – Wearing appropriate gloves, solid spills will be gathered into an autoclave bag, the area affected will be wiped liberally with 2% Virkon or 5% Trigene Advance. The disinfectant to remain in contact with the treated area for at least 30 minutes to allow the disinfectant to act; the area will then be mopped up paper towels and washed with plenty of water. Immediately after it has been collected and the affected area has been treated with Virkon, the solid waste will be taken for autoclaving, as above.

For spills inside centrifuges or incubators the local Standard Operating Procedure as indicated on the equipment will be followed.

All spills of genetically modified microorganisms (GMMOs) will be reported immediately to a safety supervisor: all spills and the mode of decontamination and disposal will be recorded in the GMO incident book.

Any personal injury where an infection by a G MMO may be involved will be reported immediately to a safety supervisor and the advice of Occupational Health will be sought. Such incidents will be recorded in the accident book.

The identify of any vectors and viruses received will be confirmed by restriction enzyme digest to authenticate the suitability of clones received from external sources for work under the current licence application.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
The committee were happy that the hazards of the project have been correctly identified, and that appropriate safe systems of work will be used to control those risks identified. The project was approved by the committee without further comment.

### Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<th>Human Clinical Applications</th>
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### Project Ref 559/12.1

- **Date Ackn'd**: 14/02/2012
- **CU2 Project Title**: Use of viral gene delivery systems for the study of angiogenesis and cancer
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **CultureVolumeClass3-4**: Non-GMM
- **Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

### Project Additional Information

**Purposes of the contained use**

In order to understand tumour, endothelial and stem cell processes that contribute to cancer it is necessary to perform functional analyses by overexpression and knockdown of genes of interest identified in genetic screens. The groups aim to use viral gene delivery systems to achieve high efficiency of transduction and stable expression in cell types of interest. The following inserts will be used:
1) Reporter genes.
2) shRNAs and micro-RNA derivatives to knockdown of genes involved in cell signalling, angiogenesis, immunity, cancer or normal development.
3) cDNAs encoded by wild-type or mutant genes involved in cell signalling, angiogenesis, immunity, cancer or normal development.
4) Antibody fragments, function-blocking peptides and peptide aptamer sequences to inhibit the function of proteins involved in cell signalling, angiogenesis, immune responses, cancer or normal development.

Recipient or parental organism

Prokaryotic host:
Well established laboratory E. coli K-12 derivatives strain will be used for propagation of plasmid DNA. These are recognised as non-colonising and disabled, have an extensive history of safe use and the genetic mutations are well understood. They have been categorised as belonging to ACDP Hazard Group 1 and are not considered harmful to humans.

Eukaryotic host:
Host cells will be tumour cell lines, mouse and human primary cells. Human cell lines, primary cells and packaging cell lines (see Section 11) cannot survive away from laboratory culture conditions, they are allogeneic and therefore non transplantable. For these reasons they are categorized as belonging to ACDP Hazard Group 1. Unscreened primary human cells would be handled in tissue culture hoods at ACDP Hazard Group 2.

Lentivirus packaging systems such as the HEK293T-based Lenti-X™ HTX (Clontech; www.clontech.com) or Open Biosystems Trans-Lentiviral™ GIPZ (Thermo Scientific; www.openbiosystems.com) systems in which the functions required for virion production are separated and carried on multiple co-transfected plasmids will be used for virus production.

Primary and established human cell lines and mammalian cell lines.

The cells lines used are not capable of survival outside the laboratory and are non-transplantable due to immuno-incompatibility. They are therefore considered to be highly disabled hosts. Established cell lines with a history of safe use may be considered hazard group 1, however primary cell lines may contain endogenous pathogens and are treated as hazard Group 2.

Host/vector system

Replication-defective retroviral vector system:
The Phoenix ecotropic and amphotropic retroviral transduction systems (packaging producer line is derived from 293T human embryonic kidney cell-line), or equivalent, 3 plasmid packaging system will used. The transducing particles produced are simple enveloped RNA virus vectors that consist of gag (coding for the core proteins), pol (coding for the viral RNA reverse transcriptase) and env (coding for the viral envelope). Long terminal repeats (LTRs) harbour sequences that aid viral genome integration and transcription of the inserted genes. The ecotropic particles cannot transduce human cells and are therefore categorized as ACDP Hazard group 1. The amphotropic particles can transduce human cells and therefore are categorized and Hazard group 2. In the 3 plasmid packaging system one construct harbour gags and pol and a separate construct harbour env. A third construct is then transfected into the producer cells that contains: (a) the gene of interest plus the retroviral packaging signal to allow production of particles capable of transducing human target cells; and (b) sequences within the Long Terminal Repeats (LTRs) that aid viral genome integration and transcription of the inserted genes. Only sequences within the LTRs are integrated in the genome of target cells. In this packaging system, the functions required for production of transducing units are separated in three different plasmids making the potential for recombination and virus production in the target cells virtually impossible.

The retroviral vectors to be used with this system are pBABE and pBabe derivatives or equivalent MLV-derived retroviral vectors. The plasmids are non-mobilisable but are designed to be integrated into the host genome. Vectors carry drug resistance markers or reporter genes that are non hazardous to humans.

Replication-defective lentiviral vector system:
Where mitosis-independent transduction is required lentiviral systems will be used. These systems are based on HIV-1. However, viral genes such as vpr, vif, vpu and nef are deleted and the HIV envelope gene is replaced with Vesicular stomatitis virus G protein (VSV-G) to increase the host range of the virus. Replication defective transducing units are produced by co-transfection of a transgene vector harbouring the gene of interest together with at least two separate non-overlapping packaging constructs containing gag, pol and env making the potential for recombination virtually impossible. Biosafety is further increased by the use of self-inactivating vectors. The particles can transduce human cells therefore they are categorized and Hazard group 2 and will be handled under Containment level 2 conditions.

The plasmids to be used with the lentiviral system pLKO.1 (addgene), pGIPZ, pLEX (Open Biosystems)5 or equivalent self-inactivating (SIN) lentiviral vectors due to deletion of part of the HIV-1 3' LTR – upon integration the provirus lacks a promoter so full-length vector sequence can no longer be transcribed. Vectors carry drug resistance markers or reporter genes that are non hazardous to humans.

Ori9n & function

Retroviral and / or lentiviral vectors will harbour the following sequences:

1) Inserts encoding reporter genes such as EGFP and derivatives, dsRed and derivatives, Firefly luciferase (Fluc), β-galactosidase under the control of mammalian or viral promoters (eg CMV or MLV 5' LTR) to enable tracking of cells.
2) Inserts encoding wild-type or mutant genes involved in normal development, cell signalling, angiogenesis or cancer. cDNAs may be expressed as fusions with a fluorescent molecules EGFP or dsRed; in non tagged or tagged form (N- or C-terminal MYC, HA, FLAG, or HIS-tag); cDNA libraries may be used. Expression will be constitutive via mammalian or viral promoters (e.g. CMV or MLV LTR) or inducible (e.g. TET ON or TET OFF systems).
3) Small hairpin RNA molecules (shRNAs) or derivatives of microRNAs to knockdown genes involved in normal development, cell signalling, angiogenesis or cancer under the control of U6 or H1 promoters. shRNA and miRNA libraries may be used.
4) Inserts encoding function-blocking peptides, antibody fragments or aptamers to inhibit the function of proteins involved in normal development, cell signalling, angiogenesis or cancer.

Small hairpin RNAs (shRNAs) and micro-RNA derivatives, function blocking antibody sequences, and aptamer sequences will be synthesized commercially or obtained from collaborators. Mammalian expression vectors harbouring cDNAs and reporter genes will be obtained from commercial sources and collaborators. Retroviral and lentiviral vectors will be obtained from commercial sources and collaborators (lentiviral vectors will be SIN vectors. Plasmids will be propagated in E. coli K-12 derivatives

Evaluation of foreseeable effects

Occasionally a sequence may integrate in an adjacent position to oncogenes, leading to an increase in their transcription driven by enhancer/promoter elements found in the LTR. Alternatively, integration may cause disruption of a TSG leading to a loss of their activity. Loss of tumour suppressor or gain of oncogene function as result of random integration or over-expression or knockdown of potential oncogenes or tumour suppressor genes may contribute to the development of a malignant cell phenotype. However, target cells will allogeneic and therefore not immunologically viable.

The infection of a worker with replication-defective transducing particles resulting in expression of an oncogene or tumour suppressor gene is possible but highly unlikely. Since viral particles cannot replicate, only a finite number of cells could be infected. This is undesirable since theoretically it increases the risk of cancer albeit to a very small degree. The workers’ immune system in healthy workers provides protection against this risk.

Hazard identification in respect of the environment:

E. coli strains and human primary and established cell lines are disabled and cannot survive outside the laboratory.

Retro- and lentiviruses are enveloped and could not survive in the environment and no modifications of the viruses will be made that would be likely to alter this property. All virus vectors contain multiple deletions and can only be propagated in specialised laboratory cell lines.

Infected cells would be destroyed by immune system.

Work is small-scale thus there is no chance of major release of any GMO. and the work is non-hazardous to the environment.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work to be carried out in accordance with LIMM safety manual rules for work with Biological Agents and GMOs.

All work with cell lines, virus production and handling of virus to be performed in designated Class 2 biological safety cabinets using designated, labelled equipment to minimise aerosols and risk of accidental infection.

Minimise risk of ingestion by good laboratory practice, hygiene procedures, hand washing, wearing of gloves and laboratory coat (see LIMM safety manual).

Immu-no-compromised individuals or those with skin conditions such as eczema will be advised to consult occupational health before working with live virus.

All waste to be disposed of according to LIMM safety manual procedures.

Liquid waste will be treated with Virkon granules or an equal volume of 2% Virkon solution, 20% Trigene Advance or 10% Virusolve+ and left for at least 30 minutes before disposal down the drain with copious amounts of water.

Solid waste (culture or cells) will be autoclaved at a minimum of 121°C (load temperature) for 15 minutes before being destroyed as clinical waste.

Emergency spill procedure:
Liquid spill – Wearing gloves treat with Virkon granules until deep pink, or add an equivalent volume of 20% Trigene Advance or 10% Virusolve+; leave for at least 30 minutes before mopping up with paper towels and wash area with plenty of water.

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For spills inside centrifuges or incubators the local Standard Operating Procedure as indicated on the equipment will be followed.

All spills of genetically modified microorganisms (GMMOs) will be reported immediately to a safety supervisor: all spills and the mode of decontamination and disposal will be recorded in the GMO incident book.

Any personal injury where an infection by a GMMO may be involved will be reported immediately to a safety supervisor and the advice of Occupational Health will be sought. Such incidents will be recorded in the accident book.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
The committee were happy that the hazards of the project have been correctly identified, and that appropriate safe systems of work will be used to control those risks identified. The project was approved by the committee without further comment.

## Project Containment

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### Project Ref 559/13.1

**Date Ackn'd:** 06/03/2013

**CU2 Project Title:** Functional analysis of proteins of Human Respiratory Syncytial Virus (HRSV) using a variety of methods including gene deletion viruses, and viruses containing reporter genes (e.g. GFP) to infect both primary and continuous cells in culture

**Class:** Class 2

**Culture Volume:** < 1 Litre

**Non-GMM Consent Granted:** Consent Granted

**Project notified under transitional arrangements:** N

**Withdrawn:** N

**Tick if notifying a connected programme of work:** N

### Project Additional Information

**Purposes of the contained use:**

The aim of the project is to functionally analyse the proteins of the human respiratory syncytial virus (HRSV), the genome of which contains 10 genes encoding 11 proteins.

We will examine how protein expression during virus infection affects key cellular activities, as well as taking a proteomics approach to study the changes in total cell
proteins in response to infection with both wild-type and specific gene deletion viruses. The strategy of using a genetically modified HRSV will allow us to examine the effects of protein expression in a system that is highly relevant in terms of virus biology, as all other virus-encoded gene products will be expressed at appropriate times, locations and abundances within infected cells.

Alternative methods that do not involve the use of genetically modified HRSV do exist, however, these methods are flawed, as they often result in overexpression of viral proteins within inappropriate cellular locations, which are likely to lead to the generation of cellular artefacts.

The initial focus of the project will be to investigate the function of the small hydrophobic (SH) protein, which forms part of the viral coat. Previous work has shown that HRSV lacking the SH gene is attenuated in both cell culture and in small animal models, indicating the SH protein contributes to virus growth and overall pathogenicity. The underlying mechanism behind the altered phenotype is not understood, although there is increasing evidence to suggest that SH acts as a membrane channel, resulting in alteration of the ion balance within HRSV infected cells. The proposed membrane channel activities of SH suggest that it may be involved in multiple cellular processes including innate immune signalling, apoptosis, and cell cycle regulation.

Recipient or parental organism

The recipients of the genetically modified HRSV will be mammalian cells. These will be either from continuous cell lines such as A549 or BHK-21, or alternatively primary cells derived from human lung or tracheal tissue. These cells do not belong to a hazard group. These cells require specialized culture conditions and growth supplements, and these conditions are not present outside of the laboratory. Therefore, accidental release of the recipient will pose no threat to the environment. HRSV infected cells do release infectious virus particles, although the majority of released virus remains cell-associated. Any released virus that is not cell-associated will be contained within the nutrient media that covers the cells, and thus is highly unlikely to be released as an aerosol. HRSV infected cells will be grown and maintained in dedicated incubators within the CL-2 laboratory space. Cross infection of cells contained within a incubator is possible, although unlikely due to the presence of liquid media covering all cells, and the use of vented cell culture flasks.

Host/vector system

The wild type vector is human respiratory syncytial virus (HRSV), laboratory strain A2. HRSV is a ubiquitous human pathogen that is responsible for a generally mild respiratory infection. Most humans are infected at least once with HRSV by the age of 2 years. The consequences of HRSV infection are a generally mild and localized upper respiratory tract infection, with associated headache and rhinitis, and infected individuals generally recover within 1-2 weeks. Infection by HRSV is protective to subsequent exposure, although repeat infections are possible due to variation in circulating strains in different geographic locations and in different years. In the context of the proposed work, all individuals handling the virus will have encountered HRSV multiple times, and no contact with infants is anticipated. The accidental release of wild-type HRSV will have negligible impact due to the massive scale of natural transmissions. Despite this, laboratory acquired infection of HRSV is undesirable, and provided all the CL-2 procedures are adhered to, will be extremely unlikely.

HRSV is classified as a hazard level 2 pathogen.

Genetic modification of the HRSV genome will be achieved by PCR-mediated mutagenesis of a cDNA encoding the entire HRSV genome sequence. The resultant cDNA can then be transfected into mammalian cell culture systems along with supporting cDNAs expressing specific HRSV structural proteins, resulting in the generation of infectious virus. This strategy can be used to engineer nucleotide insertions, deletions or modifications into the HRSV genome.

The three modified viruses initially required for this research project have previously been generated from altered cDNAs by collaborators Dr. Michael Teng and Dr. Peter Collins, who have kindly agreed to supply these altered viruses.

In the eventuality that other gene deletions within the HRSV genome are required for completion of this project, a cDNA encoding the entire HRSV genome will be modified using PCR-based mutagenesis. Infectious viruses will be generated from this cDNA as described above.
We will be using the HRSV genome which is responsible for encoding the virus, the genome is made up of 10 open reading frames (encoding 11 proteins). The work will involve creating deletion mutants as well as mutants containing reporter genes such as GFP.

The work will primarily focus on the SH protein, in order to do this we will initially use three genetically modified HRSV variants:

1) Delta-SH HRSV, in which the SH gene has been completely removed from the wild type HRSV genome.
2) A derivative of the Delta-SH virus, in which a gene expressing enhanced green fluorescent protein (eGFP) has been inserted in place of the SH gene.
3) A derivative of the wild-type HRSV, in which the eGFP gene has been inserted.

These viruses have previously been generated by others and have been shown to be attenuated in cell culture and small animal models (Bukreyev A., Whitehead S. S., Murphy B. R., Collins P. L. (1997) Recombinant respiratory syncytial virus from which the entire SH gene has been deleted grows efficiently in cell culture and exhibits site-specific attenuation in the respiratory tract of the mouse. J. Virol. 71:8973–8982).

**Evaluation of foreseeable effects**

For the initial work on the SH gene, deletion of the gene from the HRSV genome is known to result in attenuation of HRSV growth in cell culture systems, and reduced symptoms in small animal models (see above). A consequence of these reduced growth characteristics is that the titre of released altered virus is lower than wild-type. Smaller deletions within the SH gene will be predicted to reduce SH protein function, and so will also be predicted to result in reduced growth and disease compared to the unmodified virus. Taken together, these data strongly suggest the resulting SH-deleted and SH-modified viruses would be less harmful to humans than the wild-type.

The insertion of reporter gene coding sequence into the HRSV genome is predicted to attenuate virus growth due to reduced expression of viral gene products. Therefore the resulting GFP-expressing virus is predicted to be attenuated compared to the wild-type. In addition, the titre of released altered virus will be lower than wild-type due to its reduced growth characteristics, which further reduces potential harm.

Transfer of reporter gene transgenes to other genetic systems is extremely unlikely. HRSV possesses a negative sense RNA genome, and such viruses are widely regarded as being resistant to recombination with other nucleic acid sources within cells. Furthermore, as described above, no adverse consequences of reporter gene expression have been identified, and so even if transfer did occur, no risk is currently associated.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No application is sought.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Treatment of HRSV containing liquid waste fluids will be performed under the Faculty of Biological Sciences SOP for CL-2 waste treatment, which are summarized as follows: HRSV-infected liquids from cell culture flasks and plates will be aspirated using both vacuum lines and manual pipettes. All liquid handling procedures involving infectious HRSV will be performed inside a laminar flow hood to prevent risk of aerosolized virus spread outside the work area. All aspirated liquids containing virus will be immediately treated with 2% virkon, and incubated overnight to allow complete virus killing.

Plastic ware exposed to HRSV, including pipette tips, plastic universal bottles, cell culture flasks and plates, will be treated with 2% virkon, and incubated overnight. Following this treatment, plastic ware will be autoclaved to ensure complete virus killing.

Infected cells and cell lysates required for further analysis are rendered non-infectious by denaturation by detergents of chaotropic agents prior to removal from laminar flow hoods within the CL-2 containment facility.
All surfaces potentially in contact with HRSV droplets and aerosols are decontaminated by contact with 2% virkon and, where possible, UV treatment for 30 minutes.

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Project Ref 559/14.1

Date Ackn'd 09/06/2014

CU2 Project Title Structure and function analysis of genes and proteins of members of the Polyomaviridae family of viruses.

Class 2

Culture Vol Class 2 < 1 Litre

Non-GMM Not Applicable

Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

The purpose of the contained use is to allow the study of the polyomaviridae family of virus, in both wild type and genetically modified forms in a variety of research conditions including cell based as well as for study in model host organisms. Members of the polyomaviridae family are able to infect humans (although for the vast majority of the population the affects are sub-clinical) and have therefore been categorised as hazard group 2 agents. Containment level 2 laboratories and animal facilities will be used in order to control the risk of infection to workers and the wider community.

A) General description of polyomavirus

Polyomaviruses are ubiquitous, species-specific family of viruses that infect mammals and birds with varying clinical significance. The family was named from the founding virus, polyoma virus, meaning “many tumors,” which was discovered in mice, followed later by the prototypical primate polyomavirus, simian virus 40 (SV40), from the rhesus monkey.

In 2010, the polyomaviridae study group of the International Committee on Taxonomy of Viruses recommended revision of the polyomaviridae family by the division of the polyomavirus genus into three genera:

• Orthopolyomavirus, containing the “classic” mammalian polyomaviruses (e.g., JCPyV, BKPyV, SV40, mouse polyomavirus, etc.);
• Wukipolyomavirus, containing the novel human polyomaviruses including the Karolinska Institute polyomavirus (KIPyV) and the Washington University polyomavirus (WUPyV);
• Avipolyomavirus containing the avian polyomaviruses.

Individual species making up each genera are usually named after either the patient they were first isolated from (e.g. the human polyomaviruses BK and JC) or the host species in which they were first identified (e.g. mouse MPyV). High throughput DNA sequencing technology is continuing to identify new family members, resulting in a number of viruses classified as Polyomaviruses that are yet to be categorised into one of the three new genera. Appendix 3 (attached) shows the phylogenetic relationships between the members of the polyomaviridae family.

B) Distribution

Members of the polyomaviridae family have been isolated from a broad range of mammals (humans, primates, elephants, horses, bats, rodents etc) and birds. There are currently 12 identified human polyomaviruses, five viruses that are associated with disease in birds, and many others that infect mammals other than humans (Van Ghelue et al, 2012). Most species of polyomavirus have very narrow host ranges, infecting only a small number of closely related hosts. The exception to this is avian polyomavirus which has a much broader host range within birds.

C) Structure

Polyomaviruses virions are non-enveloped, approximately 45nm diameter icosahedral particles. There are 3 capsid proteins, VP1-3, which form 72 pentameric capsomers, 60 hexagonally co-ordinated and 12 pentamERICally co-ordinated (at the vertices). Each virion contains 360 copies of VP1 (i.e. 72 x 5) and 30-60 copies each of VP2 and
VP3, i.e. approximately 1 copy per pentamer. Each copy of VP1 has a sialic acid binding site on the surface and these form the receptor-binding site for the virus; hence the particles have haemagglutinating properties. VP2/3 have overlapping sequences with VP2 containing the entire sequence of VP3 at its C-terminus, and 115 amino acids at the N-terminus.

Polyomavirus genomes are double stranded, circular DNA molecules, ~5kb in size. The entire nucleotide sequence of all the viruses so far discovered in the family is known and the architecture of their genomes (i.e. number and arrangement of genes and regulatory signals and systems) has been studied in detail. Within the particles, the DNA assumes a supercoiled form (like plasmid DNA), and four cellular histones H2A, H2B, H3 and H4 are associated with it.

Polyomavirus genomic organisation is designed to pack maximal information (6 genes) into minimal space (5kbp), which is achieved by the use of both strands of the genome DNA and overlapping genes.

The genome is divided into three regions:

Early: encodes non-structural proteins (i.e. not present in virus particles), these are expressed early in virus infection, i.e. before genome replication and continues into the late stage of infection.

Late: encodes structural proteins which are expressed later in virus infection, i.e. during and after genome replication.

Regulatory region: contains transcriptional promoters and enhancers as well as the unique origin of DNA replication.

D) Replication

On encountering a host cell, a number of distinct steps are seen:

1. Attachment of the viral proteins to host receptors mediates endocytosis of the virus into the host cell.
2. Virion transits through endoplasmic reticulum where host protein disulfide isomerases rearrange its capsid structure.
3. Export of misfolded virion to the cytoplasm (possibly through host endoplasmic-reticulum-associated protein degradation pathway).
4. Loss of VP1 in the low-calcium conditions of the cytosol
5. Import of genomic DNA into host nucleus.
6. Transcription of early genes (large Tumour and small Tumour antigens, and middle T antigen for mouse virus)
7. Replication of the DNA genome in the nucleus.
8. Transcription of late genes encoding for structural proteins (VP1, VP2 and VP3).
10. Virions are released by lysis of the cell.

The polyomavirus non-structural proteins, Tumour antigens (T-antigens), expressed by the early coding region are of interest because of their role in modulating host cell function to facilitate efficient genome replication. The early region of viral cDNA is transcribed/translated by host cell apparatus to express a number of T-antigens variants through alternative splicing. The number of different polyomavirus T-antigens varies between species but their function is similar, that is to facilitate viral replication and ultimate lytic release. T-antigens exert influence over host cells through interactions with a variety of cellular proteins; in particular, the large T-antigen has been implicated as the master regulator in influencing host cell processes for the benefit of viral proliferation and is absolutely required for viral genome replication as well as regulation of viral gene expression. This protein is expressed by all Polyomaviridae family members identified to date with key DNA and protein binding domains clearly identifiable through sequence conservation across the family.

A consequence of the action of large T-antigen is the potential for cellular transformation because of the modulation of cellular function to drive the cell into S-Phase promoting viral DNA replication and inhibiting apoptosis thus ensuring efficient viral replication and assembly of progeny. As their name suggests, expression of these proteins has a long association with oncogenesis; however, expression does not guarantee transformation. The particular actions that lend themselves to transformation to an oncogenic state (e.g. attachment independent growth) include binding and inhibition of p53 which results in proliferation of DNA errors (the cell cycle would ordinarily be paused to facilitate repair) and sequestering of Retinoblastoma, resulting in uncontrolled transcription of cell cycle promoters.

Additional non-structural proteins expressed by polyomaviruses include:

- small T-antigen which is ubiquitous in family members identified to date,
- middle T-antigen which appear to be specific to mouse polyomavirus, and has a strong oncogenic potential
- and agnoprotein which is expressed in some species.

The exact function of these and other non-structural proteins is has not been elucidated but it is probable that these have an accessory function in virally mediated host cell modulation.
E) Pathogenesis
Polyomavirus is highly infectious, and is likely to have a number of modes of transmission:

- Body fluids (saliva, blood etc)
- Aerosol route (coughs)
- Faecal-oral route
- Surface contact
- Transplacental transmission (mother to foetus)
- Consumption of contaminate food or water
- The virus has been found to survive for up to a month in sewage, and the live virus has been found in shell fish

Infection of host cells by polyomavirus can result in two outcomes:

- Productive (lytic) infection
- Non-productive (abortive) infection

The outcome appears to be determined primarily by the cell type infected. However, after infection, some (unknown) determinant in the intracellular environment determines the outcome of the infection. The factors responsible for the transformation of latent polyomavirus infection to a lytic infection are not well understood. The intensity of immunosuppression is widely believed to be an important risk factor for viral reactivation.

F) Role of polyomavirus in disease

Humans

Polyomaviruses are not associated with clinically significant disease in healthy individuals. The general population exhibits high seroprevalence for each of the human polyomaviruses identified to date. There is variation depending on species, with approximately 70% of people over the age of 10 years being seropositive for each of the human polyomavirus species.

However, human associated polyomavirus are the causative agents of significant diseases in immunocompromised individuals (e.g. organ transplant patients, people with active HIV/AIDS) as viral replication and dissemination in the body is permitted by an altered immune system. Polyomaviruses have been shown to be the causative agents of at least two major diseases in immunosuppressed individuals through reactivation from a persistant subclinical state to a lytic infection. Polyomavirus mediated disease is typically associated with individuals who are HIV positive or those undergoing organ transplantation. Any condition where treatment is through medical disruption of the immune system can make individuals vulnerable including Multiple Sclerosis, Psoriasis and Crohn’s Disease. Reactivation of virus is detectable through an increase in viral loading in many individuals with a compromised immune system but progresses to serious disease in only a small percentage of cases. This process stimulates significant viral replication and lytic release followed by translocation to many different tissues in the body which can result in serious illness and often death.

The human polyomavirus JC virus discovered in 1971 has been linked with Progressive Multifocal Leukoencephalopathy (PML) which is a viral infection of the CNS resulting in cell death and cerebral lesions and is frequently fatal. PML is very rare despite the high frequency of JCPyV infection indicated by seroepidemiological studies, however, the incidence of PML caused by JC virus has risen significantly as the global HIV-AIDS epidemic has spread.

BK virus is the causative agent of Polyomavirus-Associated Nephropathy (PVAN) which is a condition resulting in necrosis and cell damage in the key structures in the kidney; typically encountered in kidney transplant patients.

A common feature of human polyomavirus is that the occurrence of viral-associated disease in the population is very rare and yet antibodies to virus can be detected in a large percentage of people, indicating that infection is widespread. For example, most people have antibodies to BKPyV and JCPyV by the second decade of life, but viremia is rarely detected (except in patients with PVAN and PML).

With the increased use of Biological drugs (also called “Biologics”) as immunomodulators in the treatment of conditions related to an abnormal immune response it has been found that patients are becoming more susceptible to polyomavirus-associated disease because of increasing efficiency of medication in suppressing the immune system. Examples include reactivation of JC polyomaviruses and subsequent onset of PML in Multiple Sclerosis or Crohns Disease sufferers being treated with the immune modulators Natalizumab or Psoriasis patients undergoing treatment with Adalimumab or similar biological drugs.

Despite the clear potential for cellular transformation and therefore a link with the development of human cancers there has not been a clear association made with oncogenesis in otherwise healthy individuals. The International Agency for Research on Cancer classify BK and JC Virus as category 2B carcinogens (possibly cause cancer, see IARC). Expression of BK virus in human fibroblasts has been shown to cause cellular transformation. Experimental work where polyomaviruses have been inoculated to animals (or expressed in cells in tissue culture) which are outside of the normal host range (so-called non-permissive cells) results in a stronger tumorigenic
The recently discovered Merkel Cell Polyomavirus has been associated with tumours in humans, a very rare but aggressive form of Skin Cancer (Merkel Cell Carcinoma). Epidemiological studies have revealed a striking correlation with immunocompromised individuals, older people or those who expose their skin to significant levels of UV light. However, Merkel Cell Polyomavirus does not appear to be a causative agent of cancer in otherwise healthy people. Researchers have found that otherwise healthy women in the second and third trimester of pregnancy can undergo an asymptomatic viral reactivation which results in detectable levels of polyomavirus within urine (viruria). A small number develop a clinically significant disease, however, it has been postulated that this is due to the hyperoestrogenic state during pregnancy and the subsequent alterations to the immune system allowing polyomavirus reactivation rather than from the effects of primary viral infection after conception. Despite the clear link with disease in immunocompromised individuals and a high seroprevalence in the general human population, the mode of transmission remains unconfirmed. This has clear implications on the protection of laboratory workers using polyomaviruses as all exposure routes must be assumed to pose a risk of exposure.

Avians
There are at least 5 species in the avipolyomavirus genus including the archetypal Avian Polyomavirus (formally called Budgerigar Fledgling Disease Virus) which has a surprisingly broad host range infecting a wide range of bird species. Other distinct polyomaviruses have been isolated from geese, crows and finches. The mode of transmission to birds remains unknown (suspected to be via shed virus in dander and droppings). Infection in fledglings can be detrimental to brood sizes and has significant impact in commercial bird flocks. None of the avian polyomaviruses has so far been shown to have tumourogenic activity.

F) Research aims
The aim of our research is to further our understanding of the role of polyomavirus in disease, and in particular oncogenesis:

a) Initially, greater emphasis will be placed on the mechanism of action and role in pathogenesis (and potential oncogenesis) of the non-structural proteins expressed by the polyomavirus early coding region. Research will focus on structure and function studies of these non-structural proteins (e.g. Large T-Antigen) to elucidate their role and mechanism of host cell interactions and cell modulation, viral genome replication and facilitation of viral gene expression. The means by which structure and function of non-structural proteins will be investigated is through the generation of modified virus particles carrying specifically targeted mutations, deletions or insertions. Mutant viruses will be subjected to detailed in vitro characterisation in eukaryotic cells.

b) By analysing genetically modified viruses insight will be gained into the function of the wild type pathogen (e.g. binding to key cellular partners to modify host cell function) and therefore potential vulnerabilities that could be targeted for therapeutics. A primary objective would then be to exploit those therapeutic targets to reduce disease in immunocompromised individuals as well as gaining an insight into the oncogenic action in case a stronger link is identified in the future.

c) Because polyomaviruses have very narrow host ranges and only small differences in the non-structural proteins expressed by different viral species (e.g. variations on the Large T-Antigen structure and function), we will engineer host cells to express the receptor required to allow infection by a virus that would not normally infect that species (e.g. expression of a human polyomavirus in Chinese Hamster Ovary (CHO) cells). This technique is a safer alternative to engineering viruses (i.e. pseudotyping to alter host tropism) to express in a different range of cells because of the increased risk of infection.

d) Other components of the virus will also be subject to detailed study with respect to their involvement in disease, the virus’s ability to evade the immune system, and its ability to become dormant.

e) A further research aim will be the co-expression of reporter genes (e.g. Green Fluorescent Protein) with wild type and modified viruses to allow study of cellular location during the viral infection-replication-release cycle.

f) The research will also include the infection of rodents with either wild type or genetically modified viruses, and will extend to the use of transgenic mice (e.g. expression of a human cell surface marker to allow viral infection). The work will be carried out in the University’s new (construction completed 2011) dedicated animal unit which as well as being built for containment of transgenic animals, has purpose built containment level 2 facilities to support the safe use of infectious material in animal studies, including provision for where material is shed by the animal after initial inoculation (e.g. in skin, urine or faeces into bedding).

Recipient or parental organism

<table>
<thead>
<tr>
<th>Bacterial cells</th>
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<tbody>
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<td>Laboratory strains of E.coli such as XL1-Blue and BL21 will be used to maintained, propagated and manipulated polyomavirus cDNA.</td>
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<table>
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<tr>
<th>Mammalian cells (un-infected)</th>
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<tbody>
<tr>
<td>Permissive cell lines (i.e. mammalian and avian) both primary and established will be grown in nutrient media in temperature controlled incubators within a tissue culture suite.</td>
</tr>
</tbody>
</table>
Permissive cell lines will be genetically modified to facilitate infection by polyomaviruses where the wild type cell line would not normally be infected (e.g. modification of mouse cells with human cell surface receptor genes to facilitate infection by human polyomavirus).

Murine Host for study of infection
Murine host organisms (mice) will be used in this research.

Host/vector system

Cloning vector system
Viral DNA will be maintained, manipulated and propagated in simple cloning vectors such as the pUC series or pBR322.
Viral DNA cloned into these vectors will be manipulated using molecular biological techniques (e.g. PCR using mutagenic primers) to generate cDNA encoding modified viruses to facilitate structure and function studies as detailed above. Mutagenesis will be site directed to introduce either precise point mutations or targeted deletions or insertions or to generate fragments for recombinant expression.
For transfection into permissive cells, the cDNA fragment encoding polyomavirus will be excised and recircularised using restriction and ligation enzymes before being transfected using a lipid-based transfection reagent (e.g. Lipofectamine) into cells in tissue culture.
Mammalian Cells containing polyomavirus
Permissive cells (mammalian and avian), primary and established, modified and unmodified will be transfected / infected with polyomavirus (GM and WT).
Murine host containing polyomavirus
Murine host organisms (mice) will be infected with Polyomavirus (GM and WT).

Origin & function

Polyomavirus
Double stranded viral DNA (usually isolated from respiratory tract samples and faeces/urine samples) will be obtained from collaborators and cloned into a simple expression vectors as detailed previously. The following techniques will be used:
a) Mutations will be introduced (e.g. point mutations and larger scale deletions) to systematically investigate the structure and function of elements of the polyomaviridae family.
b) cDNA encoding non-structural proteins from selected members of the polyomavirus family will be substituted for cDNA expressing equivalent proteins in other family members to generate hybrid polyomaviruses to allow the study of differences between different viral species. For example, useful insight would be gained in understanding non-structural protein function through expression of hybrid mutants of the Large T-antigens expressed by human and mouse polyomavirus species.
c) Insertion of reporter genes (e.g. GFP) to allow tracking of proteins during infection.
d) The virus particles produced by infected cells will be isolated from tissue culture media, concentrated using centrifugation before use for further studies such as reinfection of naïve cells or lysis for protein or DNA extraction for further analysis.

Evaluation of foreseeable effects

Risks associated with manipulation of viral cDNA
As polyomaviruses use host transcription and translation apparatus to replicate themselves it is not possible to generate a live virus during propagation of plasmid DNA in bacteria or during molecular manipulation to generate recircularised DNA for transfection.
Viral cDNA will be manipulated (e.g. deletions, point mutations, insertions) using techniques such as PCR to generate cDNA to produce mutant virus genomes. This work poses no risk of viral expression as the cellular apparatus required to make virus particles are not available.
There is a very low probability of polyomavirus infection if viral cDNA is inoculated into a worker, e.g. by a needlestick, during molecular biological work with DNA. However naked DNA is likely to be detected by the immune system and destroyed as part of the bodily response to an injury.
Polyomavirus T-Antigen expression has been shown to generate oncogenic effects without viral replication, especially through recombinant expression in non-permissive cells (species outside of the host range e.g. SV40 infection to human cells has been shown to cause transformation in tissue culture although SV40 is not thought to be an oncogenic virus in humans) and therefore accidental introduction of viral protein must be considered in the risk assessment process. While it is theoretically possible for a worker to accidentally inoculate themselves with cDNA encoding a polyomavirus or viral protein (e.g. through a needle stick) the likelihood of expression of viral protein or an introduced protein causing an oncogenic effect is unlikely because of the immune response triggered by the body.
As viral expression is not possible in a bacterial host and the potential for harm following inoculation to a researcher is negligible, this element of the work has been
assessed as being suitable for work at containment level 1.

Cloning vector system
Viral DNA will be maintained, manipulated and propagated in simple cloning vectors such as the pUC series or pBR322. Viral DNA requires host transcription and DNA replication apparatus that are not available in bacteria to generate infectious material so there is no risk at this stage, and this work is therefore assessed as being suitable for containment level 1.

Viral DNA cloned into these vectors will be manipulated using molecular biological techniques (e.g. PCR using mutagenic primers) to generate cDNA encoding GM viruses to facilitate structure and function studies. Mutagenesis will be site directed to introduce either precise point mutations, or targeted deletions or insertions or to generate fragments for recombinant expression. Any modified mutant virus is expected to be less able to infect and modulate cell function and so therefore is expected to present less of a hazard than wild type viruses.

There is a remote risk of mobilisation of vectors containing viral DNA from lab strains to other microbes such as commensal microbiota or soil bacteria in the external environment. However, the use of containment level 1 laboratory procedures (such as no eating or drinking, good hand hygiene, disinfection of live cultures prior to disposal, e.t.c.) reduces this risk to a negligible level. It is expected that even if mobilisation of polyomavirus in a vector to other families/species of bacteria were to occur the likelihood of harm (e.g. production of infectious virus or a recombination event leading to pathogenic bacteria) is assessed as being highly unlikely.

For transfection, the cDNA fragment encoding polyomavirus will be excised and recircularised using restriction and ligation enzymes before being transfected using a lipid-based transfection reagent (e.g. Lipofectamine) into cells in tissue culture. In this form (viral DNA in lipid vesicles) there is a risk of infection (albeit with lower infectivity than the wild type); this procedure therefore requires containment at an appropriate level, in this case containment level 2.

Risks associated with production of virus in tissue culture
Following transfection with viral cDNA, cells grown in tissue culture will produce live polyomavirus (wild type or genetically modified) that will be shed into the tissue culture following lytic release from infected cells. Because the media will become enriched with infectious material this may be a source of infection; especially during cell manipulations (e.g. passage of cells). The use of Containment Level 2 procedures are considered suitable for reducing this risk. In particular, application of the principles of good lab practice, the confinement of high risk procedures (e.g. where aerosols could be produced) to microbiological safety cabinets, disinfection of liquid cultures prior to disposal and treatment of consumables (e.g. cell plates) prior to consignment to the waste processing system will reduce the risk of infection to staff or students who enter the facility or transfer of infectious material outside of the containment area.

Live wild type and modified virus particles shed into tissue culture media will be collected and concentrated using techniques such as centrifugation. Centrifugation poses a risk from aerosol production and will be considered as a high risk procedure and therefore key steps of the process will be carried out within a microbiological safety cabinet (i.e. opening and closing tubes within the cabinet) so that potential aerosols are controlled.

Typically, live virus that has been concentrated will be lysed for protein and DNA extraction for further studies (e.g. PCR or Western blot). The chemicals/enzymes used for this will inactivate the virus and so subsequent analyses can be carried out in a standard Containment Level 1 laboratory.

Viral isolates will also be used (following concentration to increase titre) for live infection of naive cells for further studies. This will require inoculation of virus isolates into tissue culture media to allow cells to become infected and subsequently produce virus by lytic release. However, this process will be controlled using the same Containment Level 2 control measures which will control the risk.

Some immortalised cell lines that could be used for research contain (or may contain) fully integrated viral sequences (e.g. human papillomavirus found in HeLa Cells) which could in theory mobilise to generate infectious material. However, after many years of use (over 60 years in the case of HeLa), there have been no documented cases of harm occurring to researchers using these stable cell lines.

Due to the fact that only a small number of members of the polyomaviridae family are listed on the Approved List of Dangerous Pathogens as belonging to Hazard Group 2; we will be taking a precautionary approach and will require that work with any infectious polyomavirus material will be handled under Containment Level 2 conditions.

Research with live virus will therefore be carried out in a dedicated Containment Level 2 laboratory with established local procedures and safe working practices as detailed in the attached appendix.

Host cell lines will be genetically modified to facilitate infection by polyomaviruses where the wild type cell line would not normally be infected (e.g. modification of mouse cells to facilitate infection by human polyomavirus). In this example the modifications required will allow those cells to express a human cell surface receptor which will not present any hazard to laboratory workers (unless those cells are subsequently infected with human polyomavirus).

Work with recipient organism (i.e. cell lines) in their uninfected state is assessed as being low risk and the likelihood of harm to workers or the environment is negligible.

Effects of genetic modification
Modified viruses designed to facilitate investigations into the structure and function of non-structural proteins expressed within the host cell (e.g. T-antigens) will be
engineered with mutations that are designed to disable a specific function. Because mutations will be designed to disrupt the action of non-structural proteins (e.g. investigate key protein residues in a binding site by systematically altering them) it is anticipated that mutant viruses will be less able to infect cells and modulate host cell function. This will therefore reduce the likelihood of viral replication/lytic release and lower the probability of cellular transformation (e.g. attachment independent growth) of cells expressing mutant viruses; these factors therefore reduce the risk in the event of a failure of control measures resulting in exposure to live material. It should be noted that there is no intention to attempt to engineer viruses with increased pathogenesis.

Point mutations will be introduced into viral cDNA based on careful analysis of the protein sequence e.g. to identify key protein or DNA binding motifs that are likely to be associated with virus activity. Specific mutations will be introduce using site directed mutagenesis to obtain very specific alterations which rather than random mutagenesis (e.g. with chemicals), the latter which would generate unknown mutants where the phenotype cannot be predicted. Insertions or deletions will be engineered into cDNA using standard molecular biological techniques such as PCR and ligation of DNA fragments. It is in the interests of the research to ensure the fragment of interest has been inserted/deleted properly (e.g. no frame shifts resulting in truncated protein) and so DNA Sequencing will be used to confirm that the expected DNA construct is being used to express a GM virus therefore avoiding expression of modified viruses with unexpected characteristics.

The co-expression of reporter proteins (such as GFP) in place of a deleted non-structural protein is similarly not predicted to have an adverse effect on human or animal health in the event of accidental exposure compared to the wild type virus. Reporter genes have a long history of safe use as a co-location marker and therefore level 2 containment measures in place to protect workers from the virus itself will be sufficient. Cells expressing reporter gene-labelled viruses will only be removed from the containment facility (e.g. for observation by microscopy) once they have been inactivated. For example, Cells will be fixed with Formaldehyde (or suitable alternative) to inactivate viruses before removal from containment. There is no intention to carry out live imaging studies without fixation of cells before removal from containment.

Murine host containing polyomavirus

Both wild type and modified viruses will be inoculated into rodents as part of this research to assess in vivo effects and to further aid structure and function studies. For the containment of infectious material and animals inoculated with infectious/GM material all such work will be conducted in the dedicated animal unit facility. Handling of infectious/GM material within the animal unit will be carried out under the same strict containment conditions as detailed above for laboratory work with equivalent control measures (e.g. disinfection, waste streaming and personal/workplace hygiene measures).

Animals will be infected with virus in a Microbiological Safety Cabinet within the animal unit’s CL2 facility before transfer to containment in Individually Ventilated Cages (IVCs; e.g. Techniplast system). Animals are housed within IVCs set to negative pressure in relation to the holding room which provides protection for both workers and other animals as well as containing GM material. Polyomavirus will be shed in rodent urine and onto the skin (as well as being present in blood and other body fluids); cage bedding will therefore pose a risk to workers. Husbandry of animals housed within the Category 2 animal suite is carried out under strict containment measures to protect researchers, husbandry technicians and facility technicians (e.g. cage cleaning staff). Briefly, cage changes are carried out within Microbiological Safety Cabinets and all contaminated material such as cages and bedding is autoclaved to inactivate infectious material prior to handling to consign to the healthcare waste route.

The programme will additionally involve generation of transgenic animals to facilitate studies of altered viral tropism (e.g. expression of a human cell surface protein in rodents to allow expression of a human virus in rodents). Animals expressing the necessary cell surface protein to facilitate viral infection (e.g. an integrin or glycoprotein) will be highly unlikely to cause additional harm to workers than wild type animals (e.g. through a bite) when not infected with Polyomavirus. Following infection it is probable that Polyomavirus will be shed as detailed above. However, the precautions noted above for use of animals will be applied equally to transgene animals for the protection of workers.

Research animals are housed in a state of the art modern animal facility such that escape (and therefore loss of containment of GM material) would be highly unlikely. Animals are maintained within IVCs within climate controlled holding rooms within a secure unit with only 3 exits to the outside. Escapees from holding cages would be detected very quickly and are unlikely to get to the outside. However, while the environmental risks from production of transgenic animals in this work are negligible because alterations are unlikely to confer any benefit there may be risks to the local rodent population should a rodent infected with GM polyomavirus escape to the environment. GM polyomavirus are likely to be less able to express and replicate and so will represent a reduced hazard compared to wild type polyomaviruses.

Polyomavirus work that is specifically excluded

• It should be noted that there is no intention to attempt to engineer viruses with increased pathogenesis.
• There is no intention to increase the lethality of the virus e.g. by cloning interleukin-4 into polyomavirus genome.
• Insertion of genes coding for toxins is specifically excluded
• There is no intention to carry out live imaging studies without fixation of cells before removal from containment.

Function of the Biological Safety Committee

Researchers wanting to carry out research under this broad notification will be required to obtain consent from the University biological safety committee using the
University biological risk assessment process. If in the committees opinion the research falls within the remit of this notification and there is no increase to the overall risk associated with the work then the work will be approved. The biological safety committee has been given executive authority by senior managers at the University to grant or deny approval for a project to begin.

In applying to the biological safety committee the researcher are expected to have determined the risks associated with any new Polyomavirus research with particular attention given to factors such as potential links with human disease, percentage of the population that are seropositive by adulthood (for human viruses) and the proximity and possibility of transmission to an animal host. Should the use of a virus increase the level of risk to a level greater than that indicated for this notification then a new notification will be made to the HSE.

It is probable that new Polyomavirus species or additional serotypes of known species will be identified in the future and cDNA encoding them will become available from our collaborators. Should the research programme require the use of a Polyomavirus species that is not included in Appendix 1 then the risks associated with the virus will be carefully defined and our internal process to obtain approval for the research from the biological safety committee will be followed. Where there is an increased potential for harm to human or animal health or damage to the environment then the committee will not grant approval unless a new notification is made the HSE.

All infectious material containing polyomavirus will be handled under the Containment Level 2 Standard Operating Procedure for polyomavirus detailed in Appendix 2. Appendix 1 shows an example internal application for work with BK, JC and Merkel Cell Polyomaviruses.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

See the attached Standard Operating Procedure for polyomavirus work at containment level 2 appendix 2 (sections 9 to 13) for details on waste treatment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The internal assessment (FBS13078) was reviewed by the local committee (25th October 2013) and approved as appropriate for work at containment level 2. The committee noted that a notification to the HSE would be needed.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3</td>
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<td>L3</td>
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02/03/2022
This application seeks to establish virus-encoded epitope libraries (VEEL) as a research platform to investigate potential therapeutic benefit and associated immune responses to VEEL-derived tumour vaccines. These will be utilised both in vitro and in pre-clinical animal models of human/murine/hamster cancer.

Specifically, we will utilise the following platforms:

- Wild-type adenovirus or replication competent, attenuated Adenovirus of various serotypes obtained by collaboration (Dr Iain McNeish, University of Glasgow);
- Replication-defective Adenovirus (various serotypes), obtained commercially (Clontech/Invitrogen) or in collaboration (Dr Iain McNeish, University of Glasgow; Dr Eric Blair University of Leeds);
- Attenuated Maraba Virus (Vesiculovirus genus) obtained by collaboration (Dr David Stojdl, CHEO Research Institute, Ottawa); replication-defective VSV (VSV-∆G);
- Replication competent, attenuated vaccinia virus.

Virus platforms will be used to harbour fragment cDNA libraries, generated via random hexa-nucleotide priming in an unbiased fashion or via oligo-dT primers. Libraries will be sourced from either human/murine tumour cell lines or ex-vivo normal/cancerous human tissue, obtained with ethical approval and appropriate consent. This has been formerly demonstrated in the VSV system*. Resultant expression therefore comprises proteins derived from each source.

The primary objective of research projects carried out under this assessment is to use VEEL, both in vitro (immune priming assays) and in murine/hamster models (syngeneic models for e.g. Prostate cancer: C57BL6/TC2), to stimulate anti-tumour immunity. This will aid one of the principal research aims, namely “defining the molecules involved in human diseases and using this knowledge to develop novel therapies and new drugs”.

Generation of VEEL from various cell line/primary cell sources will be followed up using a series of investigative PCRs to confirm the presence of pre-determined “indicator”
cDNA fragments pertaining to their origin. Stocks of VEEL encoding libraries will be deliberately maintained as heterogeneous mixed populations, without the purification/expansion of individual clones, although PCR and immune-cell expansion will be used to identify antigens selected by the induction of immunity*.


Recipient or parental organism

Bacterial cells
Laboratory strains of E.coli such as XL1-Blue and BL21 will be used to maintained, propagated and manipulated polyomavirus cDNA. Laboratory strains of E.coli have a long history of safe use. Growth of plasmid vectors is assessed as harmful neither to the environment nor people and can be carried out at containment level 1.

Mammalian cells (un-infected)
Mammalian cell lines both primary/tumour and established will be grown in nutrient media in temperature controlled incubators within a tissue culture suite.

Murine Host for study of infection
Murine host organisms (mice) will be used in this research.

Host/vector system

All proposed viral vectors have either a long history of safe clinical use, or gene deletions and/or attenuation mutations that render them either tumour cell specific in terms if replication, or dependent on (helper) cell lines for propagation. cDNA libraries will be directly transferred into viral vectors for the most part, but may also be cloned via “shuttle” plasmid vectors where appropriate – these are non-mobilisable.

Animals vaccinated using VEEL will be kept in appropriate isolation conditions as standard for level 2 animal procedures.

Maraba virus is a Rhabdovirus originally isolated from south American sandflies. It is a negative strand, ssRNA virus comprising a “bullet-shaped” nucleocapsid and a lipid envelope, populated by the viral glycoprotein (G).

Maraba virus is listed as a distinct species (ICTV: http://ictvdb.bio-mirror.cn/ictv/fs_rhabd.htm) within the Rhabdoviridae and, whilst closely related to strains of VSV such as Indiana, does not cause vesicular disease with symptoms overlapping with FMDV in cattle or other ruminants*.

Toxicity experiments conducted by colleagues in Canada demonstrate that systemically delivered MG1 displays no overt dose-limiting toxicity in immune-competent mice, with minimal associated symptoms. Direct cranial injection does result in neurotoxicity and fatal encephalitis, but this is only occurs via this invasive route of delivery shedding and spreading of MG1 from infected Macaques is minimal, viral genomes only being detectable in tissues via PCR, not by the less sensitive IHC or plaque assay (personal communication D. Stojdl, CHEO Research Institute, Ottawa).

Maraba virus has been shown to have limited spread outside of sandflies* and no natural mammalian host has been identified, with only one documented example of human seropositivity. Vectors based on Maraba virus contain two attenuating mutations in the glycoprotein (G) and matrix (M) proteins. The M mutation limits evasion of innate antiviral immunity but the mechanism of action of the G-protein mutation is not known+. Genetic modification of MG1 comprises an insertion site between the G and M ORFS, resulting in expression from an internal transcription initiation site. Attenuations mean that high titre growth of MG1 and derived VEEL is only possible in tumour cell lines. This property has resulted in MG1 being proposed for use as a highly selective oncolytic virus.

between the M and the deleted G ORFs. G-deleted VSV is commonly licensed by the HSE in the UK for work under a class 2 bio-containment.

Adenoviruses (Ad)
Ad systems to be used under this notification will be:

- Wild-type Ad
- Replication competent, attenuated Ad
- Replication-defective Ad, deleted in the E1 ORF; unable to replicate except in specialised, E1-expressing cell lines (e.g. HEK293).

Wild-type adenoviral vectors are replication competent and could infect humans. Wild-type virus infection in humans with an intact immune system leads to a mild and self-limiting flu-like illness.

The replication competent adenoviruses contain mutations within the viral genome that attenuates replication in normal cells and tissues. The deletions occur in regions of the viral genome that are necessary for replication in normal cells but are complemented by the altered gene expression in cancer cells. The tumour selective mutants can infect normal cells but the ability to replicate is highly attenuated.

Replication-deficient viruses are incapable of replication in cells lacking adenovirus E1 function. Infection of normal human cells as well as malignant cells results in no infectious virion production.

In the absence of replication, long-term viral gene expression can occur only following integration into the host cell genome which is extremely rare – this can occur with a frequency of 1 in 105 in exponentially growing human cells and thus presents a minimal risk.

Vaccinia Virus
Various replication competent forms of the Western Reserve, Wyeth and Lister strains of vaccinia virus.

Insertion of the cDNA libraries leads to disruption of the viral thymidine kinase (tk) gene which results in attenuation of the virus as it is then dependent on tk expression in the host cell. Tk is transiently expressed during S-phase in proliferating normal cells but is constitutively expressed at high levels in most cancer cells, hence the attenuation limits vaccinia virus infection of normal tissue.

Like Adenovirus, vaccinia virus does not integrate into the host genome and hence risk of mutagenesis is very low.

Origin & function
Sequences selected for virus-mediated expression in either cell culture or pre-clinical murine models will derive from both cancerous and "normal" human/murine tissue. Libraries will be sourced from either human/murine tumour cell lines or ex-vivo normal/cancerous human tissue.

Fragment cDNA libraries will be generated via random hexa-nucleotide priming in an unbiased fashion or via oligo-dT primers. However, sequences are unlikely to comprise complete ORFs due to viral coding capacities.

Evaluation of foreseeable effects
GM viral particles or transduced mammalian cells are expected to pose no additional risk to safety. Production of replication-competent VSV-∆G by transduced cells is highly unlikely.

Helper Cell Lines
The mammalian cells produced after transfection for production and packaging of replication-defective virus particles are not expected to be more harmful than the unmodified cell line, with the exception of risks from production of virus covered elsewhere in this assessment.

Generation of a cell that is capable of producing wild type, replication-competent VSV-∆G is highly unlikely due to the safety features incorporated into the design of the systems as described in Section 3.2.

Transduced cell lines and primary cells
Following viral transduction, cell lines and primary cells will not be considered any more hazardous than the parental helper lines in which vectors are expressed/pseudotyped. Monitoring for the generation of replication-competent VSV-∆G will be carried out periodically, and initially for each cell combination employed, using either PCR for viral genome components or direct assay for live virus (e.g. plaque assay). Expression of virus from transduced cells or the generation of replication-competent VSV-∆G is highly unlikely (as described in Section 3.2).

The exception to this concerns work with cells derived from clinical samples as these may harbour existing viruses such as HIV or hepatitis viruses. The main risk to operators handling such cells would be infection with the adventitious agent via needle-stick injury. To reduce the risk, such samples will only be taken from low risk groups or patients screened by physicians with knowledge and experience of the patients to exclude high risk samples. Workers handling clinical material should be vaccinated against HBV. Samples later found to be from high risk individuals must be destroyed or re-evaluated to determine whether any adventitious agent can be inactivated.

02/03/2022
Human health hazards

• Replication-competent Ad vectors have a long history of use as a therapeutic oncolytic agent both as wild-type virus and as a gene therapy vector. They have an excellent safety record with many cancer patients having been treated at high doses without adverse effects3-5

• Replication-defective Ad vectors contain deletions in their E1 region, thus requiring helper cell lines which are modified to encode these regions (e.g. HEK293) in order to complete a full infectious cycle. In all other cells, these vectors are limited to single-round infections.

• MaraV MG1 vectors contain two stable attenuations6, one in the glycoprotein (G) ORF, and one in matrix (M). These have been demonstrated to render MG1 capable of replicating only in tumour cell lines.

• VSV-∆G vectors have had their glycoprotein (G) ORF deleted and so are also dependent on modified helper cell lines for replication. Thus, these are limited to single-round infections in all other cell types.

• Replication-competent vaccinia virus has a long history of safe use as a live vaccine in human smallpox vaccination programs7, and in addition a good safety record in clinical immunotherapy trials8-11. The level of vaccinia-induced human toxicity is strain-dependent7. Usually: minimal grade 3 and no grade 4 toxicity (observed across immunotherapy trials in a wide variety of cancers with administration via intramuscular, intratumoural, intravesical and subcutaneous routes).

The risk to human health arising from the use of any of the above vectors is assessed as being very low. There is a risk that mammalian cells may carry adventitious agents such as human immunodeficiency virus (HIV), or hepatitis B/C viruses (HBV, HCV). To reduce this risk, patient samples will only be taken from low risk groups or patients screened by physicians with knowledge and experience of the patients to exclude high risk samples. Workers handling clinical material will be vaccinated against HBV. Samples later found to be from high risk or infected individuals must be destroyed or re-evaluated to determine whether any adventitious agent can be inactivated.

Immunocompromised individuals should be identified and assessed individually in conjunction with UoL Occupational Health to determine whether it is safe to undertake work.

Hazard identification in respect of the environment

Mammalian cells producing viral components (producer/packaging cell lines) or those that have been stably transduced (target cell lines) are highly unlikely to survive outside a tissue culture laboratory due to rapid dehydration and specialised growth and nutritional requirements.

Primary cells derived from human blood (e.g. PBMC) have extremely limited viability and would not survive environmental exposure outside of laboratory conditions. It is highly unlikely that cultured mammalian cells would survive in a “host” organism unless the host was severely immunocompromised and therefore unlikely itself to survive in the environment. Immunocompromised individuals working in LICAP will be individually risk-assessed before undertaking work with viruses.

(Pseudo)-infectious viral particles:

• Engineered viruses are unable to replicate outside of helper cell lines (VSV-∆G, Ad-∆E1) so infections would be self-limiting.

• Attenuated viruses are able to infect normal cells but do not replicate in them, they replicate efficiently only in tumour cells.

• Rhabdovirus particles are membrane coated and would be quickly degraded in the environment through UV exposure and dehydration.

• Both vaccinia and Ad wild type particles are already present in the environment. Accidental release would therefore increase the risk of exposure to potential hosts (rabbits, rodents, cattle (vaccinia), rodents, rabbits, pigs (adenovirus)), but immune recognition and activation would control viral pathogenesis in all cases.

• The host range for all viruses is well defined.

The hazard to the environment from accidental release of cells or virus is assessed as negligible. As MaraV does not cause vesicular disease in cattle, it does not require SAPO classification.

The worst case scenario of an individual worker being exposed to replication-competent virus via e.g. needle-stick injury would result in a mild febrile illness which would be self-limiting as a result of activation of the immune system. Even less likely would be the transfer of MG1 or VSV-∆G via an insect vector as insect tropism is highly limited and would not include hosts present in Northern Europe.

This work does not alter the overall risk to workers and/or the environment in our laboratory since:

• Work will only be carried out in laboratory facilities meeting requirements for containment level 2

• All work is managed to comply with the requirements for containment level 2

• Standard operating procedures for this type of work are in place in LICAP (see attached). These are the minimum requirement within the Institutes: local rules may be more stringent.
• Animal experiments are conducted in a highly regulated and well established level 2 animal facility.
• The scope of the notified activity is restricted to the connected buildings Wellcome Trust Brenner Building, Clinical Sciences Building, Cancer Research Building and Robert Ogden Building on the University of Leeds St James’s University Hospital site.

Activities specifically excluded from this assessment
Projects that will be specifically excluded from this system of work will be those for which the risk to worker and/or the environment is increased over that posed by the work outlined here.

Inserts that could increase pathogenicity compared to wild type.
Deliberate infection of humans with viruses. Such applications e.g. for gene therapy purposes or clinical trials, are outside the scope of this assessment and would require separate notification.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Infected murine hosts (including GM variants) will be housed the purpose built (2006) animal unit located on site. The animals are housed in IVC's (mainly Techniplast but also Optimice housing system).

A variety of standard operating procedures are used within the unit and govern how work is carried out.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Submitted assessment discussed at GM and Biological Safety Committee meeting 21.7.2014. Committee requested more information on methodology and clarification of various points but agreed that assessment should be approved subject to these changes being made. This revised assessment incorporates these changes

Project Containment

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Animal Units
Large Scale Activities
Human Clinical Applications

02/03/2022
### Project Additional Information

**Purposes of the contained use**

We seek to establish a research portfolio to investigate aspects of the molecular mechanisms of virus genome replication and assembly of the arthropod-borne viruses (also known as Arboviruses) classified in the two virus genera - Flaviviruses and Alphaviruses. Flavivirus work will be limited to the hazard group 3 pathogen Dengue virus (DENV). Alphavirus work will include the re-emerging hazard group 3 pathogen chikungunya virus (CHIKV), as well as Sindbis virus (SINV) and Semliki Forest Virus (SFV); two viruses that are less pathogenic than CHIKV and thus classified at hazard group 2. A number of viruses are considered within the non-taxonomic group Arboviruses as they are transmitted by mosquitoes, midges, ticks and fleas to susceptible vertebrates including humans and other mammals.

Work will be performed by two research groups involving three PIs (Harris, Tuplin and Stockley), all of which have extensive experience of research involving infectious viruses both within the Faculty of Biological Sciences at University of Leeds but also drawing on previous experience at other Institutions (Tuplin joined the University of Leeds in 2013 having previously worked in the Universities of Edinburgh and Warwick as well as the NERC Institute of Virology, Oxford).

DENV is a member of the genus Flavivirus (Family Flaviviridae) that includes a number of important human pathogens including Yellow fever virus, West Nile virus and tick-borne encephalitis virus. DENV is the aetiological agent of dengue fever and dengue shock syndrome and is considered the most important arbovirus. Four serotypes have been described (DENV 1-4), these emerged separately from a sylvatic ancestor (infecting primates) and the virus is now transmitted in a human urban transmission cycle. An estimated 2.5 billion people are considered to be at risk from infection, and annually 50-100 million cases are infected with 500,000 hospital admissions in some years.

Dengue-associated disease manifests as a number of syndromes, often as fever, headache, muscle and joint pain but in the most severe cases as dengue haemorrhagic
fever (DHF)/Dengue shock syndrome (DSS) which is characterised by vascular leakage; a range of intermediate syndromes are also possible. Cases of severe dengue often increase following exposure to different DENV serotypes as a result of infection-enhancing antibodies.

DENV is principally transmitted by Aedes aegypti mosquitoes but A. albopictus can also act as vector. This is becoming an issue due to the widespread distribution of A. albopictus, including in Europe. Indeed, autochthonous transmission of DENV has been described in the South of France.

DENV has a positive sense single stranded RNA genome of approximately 11 kb in length, containing one open reading frame (ORF) that encodes a single polyprotein (see Figure 1 below). This polyprotein is cleaved by viral and cellular proteases into three structural proteins (C, prM, E) located at the N terminus, and seven non-structural proteins at the C terminus (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The non-structural proteins are involved in virus replication and also antagonism of host responses. The 5’ end of the DENV genome is capped and highly structured, and the 3’ end is also highly structured but not polyadenylated.

CHIKV is a member of the genus Alphavirus (Family Togaviridae) which also includes a number of highly pathogenic human and animal pathogens (eg Venezuelan and Eastern equine encephalitis viruses). Infection with CHIKV can cause fever, headache, myalgia, rash, as well as acute and persistent arthralgia. CHIKV disease can cause severe morbidity and, as more recently described, fatality. The virus is vectored by Aedine mosquitoes, mainly A. aegypti in Africa, but both A. aegypti and A. albopictus in Asia. Urban cycles occur in Asia and the virus has been imported into many countries by returning travellers, including Europe. Several large outbreaks have occurred in recent times, especially in the Indian Ocean (Reunion Island) (Morrison TE (2014) J.Virol 88:11644-11647). Most interestingly, the outbreaks in the Indian Ocean were caused by an African strain with a mutation (A226V) in the E1 glycoprotein, which results in better dissemination in A. albopictus. This mutation is also found in CHIKV in India. Pertinently, outbreaks of CHIKV in Southern Europe (Italy) where the virus was presumably imported by travellers and amplified by A. albopictus (this mosquito is also now present in parts of S. Europe) have shown the same mutation in E1. CHIKV outbreaks have also been reported in France.

A significant development in the re-emergence of CHIKV was the report in Dec 2013 of the first case in the Americas - on the Caribbean island of Saint Martin. Some 7 months later there were 440,000 cases of CHIKV infection reported across the Caribbean and Central/South America and a few cases in Florida. Given the scale and geographical distribution of these outbreaks, more research on CHIKV is urgently needed.

CHIKV also has a positive sense single stranded RNA genome which is 12 kb in length and encodes two ORFs which are translated from 5’ capped mRNAs (Figure 1). The first ORF encodes the virus non-structural proteins that are co- and post-translational cleaved into mature proteins. The second ORF is translated from a sub-genomic RNA molecule and encodes the virus structural proteins.

In addition to work with CHIKV we also plan to work with less pathogenic Alphaviruses - Semliki Forest virus (SFV) and Sindbis (SINV). Although both of these viruses can infect humans they are classified as hazard group 2 pathogens by the ACDP. SINV has a broad distribution, although originally isolated in Egypt it has been found as far afield as Scandinavia and Australia. It is generally asymptomatic in humans but can cause rash and arthritis. It is transmitted from the natural host (wild birds) via both Aedes and Culex species of mosquitoes. SFV is naturally found in Central and Southern Africa and is transmitted between animals and birds mainly by Aedes aegypti and Aedes africanus. SFV has been linked to human disease on only two occasions but serological surveys present evidence for widespread human infection.

There are three specific reasons for experiments with these CL2 viruses in addition to our work with CHIKV and DENV at CL3. Firstly, as part of the training programme for work at CL3; the procedures for the propagation and titration of all Alphaviruses will be very similar (CHIKV, SINV and SFV), it is logical for new members of staff to be initially trained by working with SINV or SFV at CL2. This will allow staff to become familiar and competent with these techniques, prior to being trained for work at CL3 and applying those protocols under those more stringent containment conditions. We have a rigorous training regime for work at CL3 and this would form part of that process for those staff involved in Alphavirus work.
Secondly, it will be important to extrapolate results obtained with CHIKV to other Alphaviruses to determine if the molecular mechanisms under study are conserved across the genus. This would provide valuable information about the processes of genome replication and pathogenesis. For example, if we identify specific RNA structures in the CHIKV genome, or amino acid residues in CHIKV proteins, that are required for virus genome replication, homologous sites could be mutated in SINV or SFV to determine if the phenotype of the mutant was the same.

Thirdly, Stockley's studies seek to understand the genome-nucleocapsid organisation of Alphaviruses, building on his recent identification of putative packaging signals (PSs - short regions of secondary structure in the virus genome that are involved in packaging of virus genomes into infectious particles) in other positive strand RNA viruses (Borodavka et al (2012) PNAS 109:15769; Dykeman et al (2014) PNAS 111:5261; Patel et al (2015) PNAS 112:2227). This will involve technically challenging biochemical approaches such as CLIP-SEQ which will be impractical to perform at CL3. Thus these experiments will be conducted with SINV or SFV. These techniques will be complemented with in vitro approaches such as SELEX to identify PSs, and preparation of nucleocapsids for analysis of molecular mechanisms of RNA release. Mutation of PSs in infectious cDNA clones and observation of the phenotype will validate the existence of PSs. An additional strand will be to screen libraries of small molecular weight clinically approved drugs that bind in vitro to PSs for effects on virus assembly.

Studies will focus on two broad aspects of virus biology:

1) The role of RNA structures within the genomes in controlling virus replication, translation and assembly and interactions with host cell factors.
2) The role of the viral proteins in virus RNA replication, translation, assembly and interactions with the host cell.

Details of the GM approach are discussed in the following sections. Briefly, cDNA clones of infectious viruses will be manipulated to generate mutants in which either the conformation of essential RNA structures have been destroyed or specific mutations are introduced into the coding sequence of the viral proteins. The effect of these mutations will be assayed by translation of marker genes incorporated into the virus construct (eg luciferase or GFP), or virus titration. Mutations will be incorporated by conventional site directed mutagenesis. Downstream a range of biochemical and cell biological approaches will be used to understand the phenotypes of these mutations and determine the role(s) of both viral RNA structures and proteins in the lifecycle. These will include imaging procedures such as confocal microscopy (for CHIKV or DENV this would involve utilising the unique facilities available within our CL3 laboratory) but also proteomic analysis and the study of protein-protein and protein–RNA interactions by immunoprecipitation and affinity purification.

Recipient or parental organism

Viruses of the Flaviviridae family:
- Dengue virus (serotypes 1-4), for example DENV2 New Guinea C. ACDP3.

Viruses of the Togaviridae family:
- Chikungunya virus (strain RL2006 OPY1 [ICRES clone], other strains). ACDP3.

Complementary DNA encoding DENV (termed an infectious clones) principally the DENV-2 New Guinea C strain, will be obtained from Dr Andrew Davidson at the University of Bristol and the CHIKV infectious clones from Dr Alain Kohl at the University of Glasgow Centre for Virus Research and Dr Andres Merits, University of Tartu, Estonia. The CHIKV infectious clone is based on strain RL2006 OPY1 (constructed by Dr Merits laboratory as part of an EU FP7 consortium). This clone and derived viruses are based on CHIKV sequences from the East/Central/South African (ECSA) genotype and contain the E1-A226V mutation.

DENV and CHIKV listed additionally under Anti-Terrorism Crime and Security Act.

Plasmids containing either SINV or SFV cDNAs will be obtained from either Dr Alain Kohl (CVR, Glasgow), Dr Andres Merits (University of Tartu, Estonia) or Professor William Gelbart (UCLA).

Host/vector system

Reverse genetics systems for both DENV and the alphaviruses (specifically CHIKV, SINV and SFV) are well established in many laboratories. In these systems,
complementary DNAs (cDNA) of the complete genomes of both viruses have been cloned into plasmids which can be amplified and manipulated in standard bacterial systems. Following linearisation of the plasmid at the 3' end of the inserted viral sequences in vitro transcription of the plasmid cDNA using either T7 or SP6 bacteriophage RNA polymerase produces RNA which, when transfected into eukaryotic cells can give rise to infectious virus. Viral RNA must be capped at the 5' end, thus it is necessary to use a commercial in vitro transcription kit (Life Technologies mMessage mMachine) to ensure efficient capping of in vitro transcripts. Plasmids contain an origin of replication followed by a prokaryotic selectable marker (ampicillin or kanamycin resistance) and are non-mobilisable vectors in a disabled prokaryotic host. No live virus is produced during this process.

RNA generated from these constructs in vitro will be transfected into a range of transformed mammalian cell lines (including HEK293, A549 and Huh7 human cells, C2C12 murine muscle cells and BHK21) and Aedes mosquito cell lines (including A20 and Aag2 from A. aegypti and C6/36 from A. albopictus) by lipofection or electroporation for analysis of virus replication. No work with live vectors (i.e. mosquitos) will be undertaken as part of these projects.

The established protocol for this work (as determined by consultation with other investigators) is that cells will be transfected with RNA encoding CHIKV or DENV at CL* and then immediately transferred to the CL3 lab. From discussions the consensus in the field is that no virus is released from transfected cells for at least 4 hours after transfection. We propose that the first experiment once approval is granted will be to test this statement. Susceptible cells will be transfected with either CHIKV or DENV RNA, transferred to CL3 and supernatant harvested every hour from 1h post-transfection for virus titration. This will allow us to accurately determine the early time-scale of virus infection and validate the experimental procedure. Cells transfected with RNA encoding SFV or SINV will be maintained within our CL2 lab.

Our approach is to generate defined site directed mutants of the target before expressing and characterising mutant viruses along with reporter genes in mammalian or invertebrate cells in tissue culture.

DNA constructs to facilitate the production of viral cDNA will be constructed and/or manipulated to generate mutant viruses using standard molecular biological techniques. In prokaryotic cells, only selectable (e.g. antibiotic resistance) genes will be translated; prokaryotic cells may also be used for protein expression and subsequent purification.

Transcription of cDNA to capped RNA will be carried out using a commercial kit (Life Technologies mMessage mMachine), which will then be transfected into transformed mammalian or invertebrate cell lines using electroporation or commercially available reagents such as Lipofectamine or FuGene.

Following transfection, where necessary, cells will be transferred to the CL3 laboratory. Once viral production is established mutant viruses will be characterised. Research will focus on key areas detailed here:

Research thread 1: Analysis of RNA structures:

Our strategy will involve generating specific mutations (generally synonymous in the protein coding regions), which will disrupt RNA secondary structures within both protein coding and non-translated regions of the genomes. Synonymous – or silent - mutations do not change the amino acid sequence encoded by the virus and as such viral proteins will not be mutated. Although it is known that both viruses encode RNA structures vital for translating and replicating their genomes their mechanisms of action and interaction with host cell factors is not understood.

In preliminary studies utilising subgenomic replicons a number of RNA stem-loops in the open-reading frame of CHIKV have been shown to act as host dependent cis-replicating elements (CREs) during early stages of virus genome replication (manuscript in preparation). Disrupting the CREs inhibited replication in human cells and stimulated it in mosquito cells. Utilising mutant infectious virus systems and intracellular RNA structure mapping we will define host-specific genetic and structural determinants of CRE function, precise roles during early virus replication and specific protein trans-activator interactions. Comparison of results between in human and mosquito cell systems will enhance detailed dissection of mechanisms involved, which we expect to have direct application in identification of novel therapeutic targets. Furthermore, mechanistic understanding of CRE-mutant attenuation will have direct application in studies towards a genetically stable attenuated vaccine.

Similar approaches will be used in SINV/SFV to examine the roles of putative PS sites in viral assembly. The two known clades of alphaviruses contain distinct, although interchangeable, single-copy, high affinity PS sites (Kim et al (2011) J. Virol. 85, 8022–8036), but the mechanism by which they regulate nucleocapsid assembly is
unknown. We will determine if there are secondary PS sites and what their roles are in assembly.

Research thread 2: Analysis of protein function:

Mutations will be introduced into the infectious clones to change specific amino acids. These will be predicted to disrupt specific functions or protein-protein interactions, for example by alanine substitution of charged amino acids or potential phosphorylated residues (serine/threonine/tyrosine). Mutations will potentially be generated in the coding sequences of all the viral proteins however initially a particular focus will be the CHIKV non-structural protein nsp3.

This protein contains an N-terminal macro-domain which has been described to possess ADP-ribose binding and ADP-ribose-1"-phosphate phosphatase activity (Malet et al (2009) J.Virol. 83:6534), however the role of these functions in genome replication has not been determined. Specific mutations that are predicted to ablate these functions will be generated and the replication of the resulting viruses will be analysed in both mammalian and mosquito cell lines. As the macro-domain is conserved in all Alphaviruses (as well as Coronaviruses and hepatitis E virus) it is highly likely that it has a functional role in the virus lifecycle, and therefore unlikely that putative inactivating mutants would enhance either replication or pathogenicity of the virus. Indeed, this statement is supported by the recent observation by our collaborator Dr Andres Merits that mutations in one of the other viral non-structural proteins, nsp2, that reduce one of its enzymatic activities (RNA helicase) result in a non-cytopathic phenotype and allow persistence of subgenomic replicons (Utt et al (2015) J. Virol. 89:3145).

We will also generate mutations in other regions of nsp3, for example the C-terminal polyproline motif which has been shown by our collaborator Prof Kalle Saksela (University of Helsinki) to interact with the SH3 domain of amphiphysin (Neuvonen et al (2011) PLoS Pathogens e1002383), again the details of how this interaction (and interactions with other cellular SH3 domains) is involved in genome replication remains elusive. The functions of nsp3 are poorly understood and we plan to complement our mutagenic studies by introducing either fluorescent (eg GFP or mCherry) or affinity (eg One-Strep or FLAG) tags into the C-terminus of nsp3 to facilitate downstream microscopic or proteomic approaches to study nsp3 function. The viability of this strategy has previously been demonstrated by other laboratories, including that of our collaborator, Dr Andres Merits (University of Tartu).

As described previously, findings from work with CHIKV will be replicated in work with SFV or SINV to provide valuable information about the processes of genome replication and pathogenesis. This will require the generation of mutations in SFV or SINV cDNA followed by expression and characterisation under Category 2 containment.

Evaluation of foreseeable effects

Cloning into bacterial vectors and growth in Escherichia coli: the E.coli strain used (DH5a) is a laboratory adapted multiple auxotroph and is unlikely to survive in the human gut (ACGM/HSE/DOE Note 7, annex 1). All plasmids will be non-mobilisable – derived from the pUC or pBluescript series. These procedures can therefore be carried out at CL2 within standard research laboratories.

Production of viral RNA: Viral RNA is transcribed in vitro using a commercial kit (Life Technologies mMessage mMachine). This procedure will generate RNA which is capped at the 5' end and will result in the generation of live virus when transfected into susceptible cells. A standard in vitro transcription produces approximately 20 ug of RNA at a concentration of approximately 100ng/ul. It is extremely unlikely that handling this material could result in uptake into susceptible cells and production of infectious virus because the naked RNA is labile and highly susceptible to degradation by serum nucleases. An additional safeguard is that there is no requirement to purify the RNA before transfection, thus limiting the downstream processing and handling. This procedure will therefore be carried out at CL2.

Transformation of viral RNA into susceptible cells: appropriate susceptible cells (see list above) will be propagated under standard tissue culture conditions at CL2 and transfected with viral RNA using reagents such as lipofectamine or FuGene or by electroporation. Transfected cells will be placed into complete media (therefore containing serum and nucleases) following treatment cells will be either transferred immediately to the CL3 laboratory for studies using DENV or CHIKV or maintained at CL2 for work with SFV or SINV. Control experiments using subgenomic replicons of CHIKV have shown that viral genome replication (as measured by detection of firefly luciferase expressed from the subgenomic RNA) is not apparent until 4 hours post transfection in either mammalian or mosquito cells and then continues to increase by 1000-fold up to 24 hours post transfection. Thus no infectious virus will be produced until after 4 hours.

Expression in mammalian or invertebrate cells: mammalian or invertebrate cell lines transfected with infectious DENV or CHIKV RNA, or infected with DENV or CHIKV, will
be confined to our CL3 facility and processed according to the Standard Operating Procedures (SOPs) for that laboratory. This SOP has been updated to reflect the use of DENV and CHIKV to account for control of insects in particular. Briefly, the lab is sealable to enable fumigation and is leak tested every 12 months. Penetration to the lab to allow inward air flow contain a course filter. The lab is in a large research building in an urban environment. The lab is not open to the outside and there are 2 doors between the corridor immediately adjacent to the lab and the outside external environment (i.e. 4 doors between work area and the outside). Therefore the potential for a vector (e.g. mosquito) entering the laboratory and moreover becoming infected with live virus is very small. However, an insect control device will be placed in the corridor outside the lab and periodically monitored to check insect activity in the area.

SINV and SFV will be used under category 2 containment. The containment labs within the Virology group is a very well established lab for the study of viruses at Containment Level 2 and work is carried out under a Faculty-based protocol which establishes requirements for the lab design and operation and is translated into local requirements by a strict SOP and supervision by academic members of staff and a Laboratory Manager. Work with SINV will additionally be carried out in a newly established laboratory in an adjoining building but procedures have been established which mirror those in place in the established virology area. The Laboratory Coordinator has indeed spent time working within the established virology lab.

Within the CL2 containment labs strict practices are enforced on users to ensure containment of infectious material and to protect users themselves from infection. Access is limited to those who are suitably trained and all new users are given an initial induction and training. All processes involving the generation of aerosols are contained within safety cabinets which are serviced and tested in accordance with COSHH requirements for Local Exhaust Ventilation. Agents are grown in specific incubators and are inactivated using suitable disinfectants. Waste is processed through the faculty autoclave system before removal by our waste contractor. University cleaning staff are forbidden from entering the area and so cleaning is carried out by users on a rota basis. Spill procedures are in operation, although volumes are kept to a minimum where possible.

Removal of samples from CL3: Samples that require CL3 containment that need to be taken out of the containment laboratory will be inactivated by detergent treatment or fixation (paraformaldehyde). The only exception to this is the temporary removal of samples for virus concentration by ultra-centrifugation. In this case, as outlined in the SOPs, virus is contained within a sealed centrifuge rotor which is only re-opened inside the safety cabinet to control aerosols. A spillage procedure is in place within the laboratory SOP. However, the cell lines containing infectious virus would be unable to survive outwith tissue culture medium and so present no risk either to the environment or to human health and safety.

It is theoretically possible for members of viral families to undergo recombination events to produce a hybrid virus. Therefore, work in the CL2 laboratory with cDNA encoding CHIKV and SINV/SFV will be kept entirely separate. Subsequently, culture of cells infected with virus will be carried out in laboratories that are several floors apart where strict containment procedures are in place. This spatial separation will exclude any possibility of recombination.

Generation of mutant viruses: as discussed above any GMO generated by mutagenesis would most likely be less harmful than the wildtype viruses from which they were derived. Although it is formally possible that some mutations might enhance in vitro infectivity, as all viruses are treated as potentially infectious and handled at CL2 or 3 respectively, the resulting standard safety protocols will ensure that any viruses with enhanced infectivity will not represent an increased risk.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

For in vitro transcription of viral RNA and initial transfection into mammalian or invertebrate cells we are seeking to undertake this element of the work at Containment Level 2.

This is in line with the SOP (attached) for the CL3 facility – these have been reviewed recently by an HSE Biological Agents Specialist Inspector. Transfected cells will be immediately transferred to the CL3 facility.

Previous control experiments using subgenomic replicons have shown that virus genome replication cannot be detected before 4 hours post transfection and therefore no
infectious virus can be produced until this time at the earliest. As discussed previously, a revalidation experiment will be conducted once approval is granted – cells transfected with full length virus genomes will be transferred to the CL3 facility and sampled at 1 hour intervals for virus titration to demonstrate that virus is not detectable within the first four hours of transfection.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For work at Containment Level 3:
Routine waste disposal: Both DENV and CHIKV are susceptible to a wide range of disinfectants as well as moist heat.
Liquid waste will be treated with an equal volume of 2% Virkon and left overnight before disposal down the drain with copious water.

Solid waste (culture or cells) will be autoclaved at a minimum of 121°C (load temperature) for 15 minutes within the CL3 laboratory before being processed again in the Faculty autoclave waste system and discharged from the Faculty as Healthcare Waste (Contractor is GW Bulter, Bradford).

Emergency spill procedure:
The CL3 laboratory SOP has detailed systems for spillages. The laboratory can be made ready for fumigation within a few minutes in the event of a spill outside of a safety cabinet and an emergency egress procedure is in place.

For work at Containment Level 2:
The Faculty of Biological Sciences has documented standard procedures for working with ACDP Hazard Group 2 materials. This stipulates that local procedures must be in place to deal with the waste generated by a particular project.

Briefly, the following procedures are in place.
All solid waste (e.g. plastic-ware, tubes, tips and gloves) from Category 2 projects is collected in autoclave bags which are packed into tins with a close fitting lid. These are collected from labs by a waste processing technician and taken to a dedicated facility for treatment. The waste discard autoclaves undergo regular servicing and validation using the 12-point thermocouple test to ensure a holding temperature of 121°C for 20 minutes to achieve a suitable kill.

Liquid cultures are treated with a suitable disinfectant (e.g. 2% final volume Virkon) and left in situ (such as in a tissue culture hood) for at least 30 minutes before disposal to the drain.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Comments attached and highlighted.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022

Page 9882 of 15326
The aim of the project will be to analyse the interactions of arthropod-borne viruses (so-called arboviruses) with the mammalian immune system. As model systems, we study positive-stranded RNA viruses of the Togaviridae (genus Alphavirus), and Flaviviridae families, and negative-stranded RNA viruses of the Bunyaviridae. These viruses include; Semliki Forest virus (SFV), Ross River virus (RRV), o’nyong-nyong virus (ONNV); Bunyamwera virus (BUNV), Schmallenberg virus (SBV); and the Langat virus (LGTV). We also plan to use replicons derived from these viruses or related alpha- and flaviviruses. The response to virus infection by mammalian immune responses will be studied by infecting mice (including genetically modified mice). The use of genetically modified mice will enable hypothesis testing and will provide an important route by which mechanisms of disease can be determined. The project covered by this notification will use cell lines derived from vertebrates and arthropods (e.g. mosquitoes) as well as mice (including genetically modified mice).
systems are commonly used at ADCP level 1, but to avoid confusion we will use all material, both replicons and viruses as if they are all ADCP2. See below for a full description of these systems and further justification for the use ADCP level 2. The use of replicons is also discussed in section 3.2.

The following models will be used in these studies:

**Arboviruses and derived replicons used will be of the Flaviviridae, Bunyaviridae and Togaviridae (genus Alphavirus) virus families.**

**Model for Togaviridae:**
- Semliki Forest virus prototype (SFV4), SFV6, A7 and A7(74) strains.
- Semliki Forest virus-derived replicons and virus-replicon particles.
- O’nyong-nyong virus (ONNV).
- Ross River virus (RRV).

**Other arthropod-borne alphavirus replicons.**

**Model for Flaviviridae:**
- Langat virus (strain TP21, the most commonly used lab strain, or others).
- Langat virus-derived replicons.
- Other arthropod-borne flavivirus replicons (for example derived from tick-borne encephalitis virus or dengue virus).

**Model for Bunyaviridae:**
- Bunyamwera virus (genus Orthobunyavirus), and derived recombinant viruses.
- Schmallenberg virus (genus Orthobunyavirus), and derived recombinant viruses.

**Health hazards associated with virus and replicon infection**

**SFV:**
In Africa, natural infections have been associated with mild febrile illness (Mathiot et al., 1990). Various laboratory strains of SFV including prototype-SFV4, L10, A7 and A7(74) have been used extensively in many laboratories and even (in the past) for student practical classes. There is one report of laboratory based human disease. Death of a laboratory worker in Germany was associated with infection by the Osterrieth strain of SFV (Willems et al., 1979). This (Osterrieth) strain of SFV is no longer used experimentally. Whilst the above case report is noted, it dates back over 30 years and is difficult to reconcile with the extensive use of other strains of SFV with no reported adverse effects over a 50-year period. This case report makes the unpublished observation that antibodies against SFV can be demonstrated in the serum of many laboratory personnel working with it. It is generally considered that the individual who died was probably immunosuppressed and therefore highly susceptible to infection. Anecdotal reports and reported laboratory incidents indicate no human clinical signs after accidental inoculation with various laboratory strains of SFV. The classification into ACDP hazard group 2 indicates current expert opinion that containment level 2 precautions are considered adequate for controlling the risks associated with working with SFV.

**ONNV:**
This virus is classified as ACDP hazard group 2. In endemic areas of Africa, Anopheles species mosquitoes spread ONNV. Outbreaks of this virus are infrequent, although they can be large in scale (Uganda 1959-1962, spreading to Tanzania and Zaire; Uganda 1996-1997). Person to person transmission is not documented and by 1980 only two laboratory-acquired infections had been described. Symptoms include polyarthritis, rash and fever, occasionally eye pain, chest pain, lymphadenitis and lethargy. No fatalities have been recorded, and three strains of the virus are known (Braault et al, 2004).

**RRV:**
Although classified as an ADCP group 2 pathogen, there have been continuing outbreaks of infection in Australia that have caused disease in humans. RRV is spread to humans by infected Aedes aegypti mosquitoes and can cause a febrile illness. In some cases virus disseminates to the joints where it causes arthralgia. Symptoms can include polyarthritis, rash and fever, occasionally eye pain and lethargy. No fatalities have been recorded (Jacups et al., 2008).

**LGTV:**
LGTV is a tick-borne flavivirus of the Flaviviridae family. It was first isolated from Ixodes granulatus ticks in 1956 in Malaysia (Smith, 1956). The natural transmission cycle
of the virus is believed to involve rats in areas such as Thailand, Malaysia and Russia. LGTV can cause encephalitis in experimentally infected mice (Webb et al., 1968) but is not very neuroinvasive compared to other more virulent members of the tick-borne flavivirus group (Thind & Price, 1966) and is considered the non-pathogenic member of the Tick-borne encephalitis complex. Indeed, under normal conditions LGTV is only very rarely pathogenic to humans. The naturally attenuated Malaysian strain TP21 (the most common laboratory strain), is not associated with human disease (Rumyantsev et al., 2006) and LGTV has been used as a live attenuated vaccine in humans (Dubov et al., 1972). Development of this vaccine was stopped in the 1980s because of rare (1 in 20,000) occurrences of post-vaccinal neurological disease (Rumyantsev et al., 2006). LGTV is not listed on the ACDP list but should be classified as hazard group 2 (current use in the UK and internationally, see http://www.healthsafe.uab.edu/pages/biosafety/biosafetymanual.pdf)

BUNV:
Bunyamwera virus (BUNV) is the prototype virus of the family. Originally isolated in Uganda from infected mosquitoes, most of our understanding of bunyavirus biology is due to studies on this model bunyavirus (Elliott, 1989). Infection with this virus can cause a febrile illness with headache, arthralgias, rash and infrequent central nervous system involvement (Gonzalez and Georges, 1988). The classification into ACDP hazard group 2 indicates current expert opinion that containment level 2 precautions are considered adequate for controlling the risks associated with working with BUNV.

SBV:
This an animal pathogen that is widely distributed in Northern Europe (Gibbens, 2012), including the UK, and not likely to be a risk to human health by current guidelines (http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=795).
SBV was recently isolated from cattle in Germany and leads to disease (fever, diarrhoea, malformations, abortion) in cattle and sheep. SBV is not listed on the ACDP or SAPO list but has been recommended to be classified as hazard group animal pathogen 2. We propose to treat SBV like BUNV in terms of risk for human infections and health unless other regulation is in place for a particular pathogen. In addition, advice will be given to lab personnel that they should not be in contact with susceptible animals or visit farms/zoos where they could potentially come into contact with susceptible species for a period of 48 hours following work with SBV.

Genetic modification of all these viruses as proposed here (e.g. addition of reporter genes) is highly unlikely to add or increase the risk to human health. The viral envelope glycoproteins and capsid protein sequences are the major determinants of host range and cell tropism will not be changed beyond the addition of marker genes such as GFP (as fusion or cleavable inserts into this coding region) into the structural open reading frame; host range and cell tropism are therefore unlikely to be altered.

Personnel working with these viruses must be registered with Occupational Health and are expected to inform Occupational Heath should they become pregnant or immunosuppressed.

Virus-derived replicons and virus-like particles
In addition to infection with virus, it is possible to use virus-derived replicons (VRPs). The system exploits the wide cell tropism of e.g. SFV, but has no potential for virulence since genes for the viral structural proteins are prevented from being incorporated into new virus, or are simply deleted and replaced with marker genes e.g. GFP. This system is deemed extremely safe and can be used at ADCP2 level containment (Smerdou and Liljestrom, 1999). In VRPs, the structural genes can be replaced by a polylinker which allows insertion of foreign sequences. In the absence of genes encoding the viral structural proteins in replicons, new infectious virus particles capable of initiating a propagating infection cannot be regenerated in VRP-infected cells; VRPs are thus also referred to as ‘suicide particles’. Replicons can be used to express reporter genes or other genes of interest. See Atkins et al 2008 for a recent review of Alphavirus vectors and their replicons.
VRPs can be generated by transfecting host mammalian cells with either two, or potentially three, distinct plasmids. Each plasmid contains a different section of the viral genome. These additional plasmids are referred to as helper plasmids. Most commonly one plasmid will express the replicase genes, whilst the second and third will express either structural genes or a foreign gene that has been inserted. Transfection and translation of these plasmids results in the production of ‘suicide’ VRPs that are able to infect susceptible cells, but are limited to only one round of multiplication because they lack the viral structural protein genes that have been supplied by the helper plasmids (Atkins et al, 2008). This system has been specially engineered to prevent the possibility of plasmid recombination and has been well tested. This includes the use of two helper plasmids to decrease the probability of a successful recombination, and the inclusion of a mutated capsid protein cleavage site e.g. in SFV, that in the unlikely event of recombination would result in a defective virus.
In addition, we plan to carry out comparative studies with arthropod-borne alphavirus replicons such as those derived from chikungunya virus; those replicons are not covered by schedule 5 and no sequences for the complete genome (i.e. sequences that encode structural genes that complement the viral protein set to potentially give infectious virions) will be stored in-house. Those replicons will not be packaged into VRPs, only RNA will be introduced into cells to study replication; replicons will not be mixed in the same cells (either by co-infection/transfection, or by superinfecting spreading virus) with related viruses from within the same genus to avoid recombination events during co-infection.

Host/vector system

Plasmid vectors and bacteria:
All viruses and plasmids will be sourced from Dr Alain Kohl (Centre for Virus Research, University of Glasgow), Prof John Fazakerley (Pirbright Institute) and Prof Andres Merits (University of Tartu). Viral sequences (including full length clones or partial sequences of SFV, ONNV, RRV, LGTV, BUNV and SBV) and other genes (cellular, viral or reporter) are available in plasmid-encoded cDNA form. Plasmid vectors are based on bacterial plasmids and contain an origin of replication for prokaryotic cells and selectable antibiotic/drug resistance (pro- or eukaryotic) markers (Atkins et al 2008).

By themselves these vectors present no risk to human health. Plasmid vectors have no selective advantage and would soon be lost from the bacterial population. The bacterial systems are non-mobilisable vectors in a disabled prokaryotic host. Transcription of even complete viral cDNA will result in no infectious RNA since the promoters are not active in prokaryotic hosts. Systems for prokaryotic gene expression are under control of an inducible prokaryotic promoter. Systems for eukaryotic RNA or protein expression are under the control of promoters of viral (such as CMV or SV40), eukaryotic (eg actin promoter, pathway inducible promoter such as STAT) or prokaryotic (such as T7 or SP6 promoter for in vitro transcription) origin. Plasmids with eukaryotic promoters such as the CMV promoter can be transcribed in human and rodent cells. None of the genes used in these studies are oncogenes. The hazard of expressing ‘foreign’ genes from these eukaryotic promoter plasmids would depend on the expected physiological effects of the foreign gene product and is discussed in more detail below.

Hosts

Prokaryotic organisms: All strains will be disabled, commercially available E. coli derivatives classified as ‘especially disabled hosts’ by ACDP. These strains cannot colonise humans and are known not to cause harm to humans.

Eukaryotic cells: Cell lines to be used would not survive inside the human body (immune rejection) and are not known to carry harmful pathogens. Addition of DNA or RNA will confer no growth or survival advantage in or outside the laboratory to cell lines of vertebrate or arthropod origin.

Mice (including genetically modified mice): These will be sourced from commercial suppliers (Charles River and Harlan) and collaborators. Mice (including genetically modified mice) will be housed and handled in designated areas approved by Biomedical Services and the Home office. Upon infection with viruses, mice will be housed in filter cages that keep the mice in a sealed environment. Work with virus replicons and VRPs will be done under the same procedures and containment as work with infectious virus (wild type or genetically modified). Mice act as dead-end hosts and cannot actively infect humans, although biting arthropods that take a blood meal from a viraemic mouse can theoretically become infected. Measures to prevent human or accidental arthropod infection include; use of filter cages to house infected mice; work in designated rooms with restricted access; and wearing of gloves. Once housed at biomedical services in filter cages, an encounter with a biting arthropod is highly unlikely. Live mice and samples (dead mice or material from these) will be transported to biomedical services or our CL2 laboratory according to standard regulations.

Animal work utilising class 2 organisms will be carried out following established, approved level 2 protocols at SBS

The use of genetically modified mice does not pose any additional risks to human health above and beyond that associated with infection of non-genetically modified mice

Origin & function

Sequences to be inserted into arboviruses genomes:

Genes: Reporter genes of prokaryotic or eukaryotic origin (e.g. luciferase, fluorescent proteins etc.) and antigenic peptides e.g. ovalbumin (OVA).

Non-coding RNA sequences: non-viral RNA (e.g. mammalian miRNA recognition elements), or viral RNA sequences (e.g. flavivirus 3' untranslated regions) to study
mechanisms of roles of non-coding sequences in replicative processes, immune responses and dissemination of virus in vivo.

Sequence changes in viral sequences: These (through mutation or deletion) will target disruption of non-structural protein functions or non-coding viral sequences.

All of these sequences have no inherent hazard and it is very unlikely that these RNA or proteins would have any harmful toxin-like effect outside cells.

Evaluation of foreseeable effects

Hazard associated with receiving genetic material

Reporter genes: No harmful properties have been attributed to reporter genes of prokaryotic or eukaryotic origin (e.g. luciferase, fluorescent proteins etc.). There would be no altered physiology expected if any of these genes were expressed; eGFP for example is a spontaneously fluorescent protein isolated from Aequorea victoria a coelenterate and has no known detrimental effects. Luciferase is another commonly used reporter gene and is derived from the firefly Photinus pyralis or the jellyfish Renilla reniformis. It is an enzyme which catalyses production of light from luciferin and ATP; the light produced is used as a measure of gene activity. The enzyme has no other biological function.

Antigenic peptides: Most individually expressed proteins (e.g. OVA) are unlikely to have harmful effects in eukaryotic cells, however some could perturb cellular metabolism, predispose or protect against cell death or render cells more or less susceptible to other infections.

Non-coding, non-viral RNA sequences: Individually expressed RNA sequences to be used as described are unlikely to pose a risk. They may suppress gene function or have effect on replication in individual cells but are unlikely to lead to whole body physiological effects in humans as they do not code for the production of secreted bioactive molecules. Viral non-coding RNA sequences too are likely to have minimal effects on virus replication, and do not encode biologically active molecules that are likely to induce physiological effects in humans.

Sequence changes in viral sequences: Mutation or deletion will target disruption of non-structural protein functions or non-coding viral sequences are likely to have no or deleterious effects on viral replication. Thus there is no increased risk or hazards associated with viruses modified as described.

Genetic changes that will be introduced into the virus sequences will be targeted at disrupting or changing specific functions of virus RNA or virus proteins and can be expected to attenuate virus fitness; such changes are highly unlikely to increase virus virulence as they modify virus sequences, or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication. The viral glycoprotein amino acid sequences that are the major determinants of host range and cell tropism will not be changed beyond the addition of marker genes as fusion or cleavable markers.

In summary, genetic changes that will be introduced into the arbovirus nucleic acid sequences as described above can be expected to attenuate virus fitness; such changes are highly unlikely to increase virus virulence as they modify virus sequences (which are optimized for replication), or change genome length, or lead to expression of additional sequences such as reporter genes which confer no advantage to virus replication.
This work does not alter the overall risk to workers and/or the environment in our laboratory since:

- Work will only be carried out in laboratory facilities meeting requirements for containment level 2
- All work is managed to comply with the requirements for containment level 2
- Standard operating procedures for this type of work are in place in LICAP (see attached). These are the minimum requirement within the Institutes: local rules may be more stringent.
- Animal experiments are conducted in a highly regulated and well established level 2 animal facility.
- The scope of the notified activity is restricted to the connected buildings Wellcome Trust Brenner Building, Clinical Sciences Building, Cancer Research Building and Robert Ogden Building on the University of Leeds St James’s University Hospital site.

Projects that will be specifically excluded from this system of work will be those for which the risk to worker and/or the environment is increased over that posed by the work outlined here.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Work with infected mice:**

- The SBS animal unit is relatively new (extensive refurb completed 2007) and is built to a high standard, with all animal holding or procedure rooms meeting the requirements for containment level 2 (although the vast majority of work is level 1).
- Mice are housed in independently ventilated cages (IVC’s) which are HEPA filtered to prevent movement of particles (including viruses) between IVC’s.
- Infected mice are further isolated from uninfected mice in a quarantine/holding room that is physically isolated from other animal holding rooms.
- Routine husbandry with infected mice outside of IVC’s (e.g. cage changing) is carried out in Class 2 cage change cabinets.
- All task are assessed and animal workers follow standard operating procedures which are designed to maintain consistency and minimise risk.
- Animal workers receive specific training and are supervised until such a time that they are identified as competent.

### Waste disposal

In addition to standard default procedures described below:

- **Liquids** (e.g. samples, culture supernatants, tissue culture media) – add Virkon or other validated disinfectant to final concentration of 1% (w/v) for a minimum of 12 hours, then discharge to drains.
- **Liquids** (E. coli culture medium) - add Virkon to final concentration of 1% (w/v) for a minimum of 12 h, then discharge to drains.
- **Sharps** (in sharps bin, e.g. needles, syringes, scalpels) – autoclave by using a make safe cycle of 121°C for a minimum of 15 minutes.
- **Mouse cadavers, tissue samples and other solids** will be disposed of by Biomedical services.
- **Mouse cadavers** are bagged, and frozen (-20degC) before collection for incineration.
- **Tissue samples and other solids waste samples** will be placed in sealed autoclave bags, disposed of by autoclaving using a make safe cycle of 121-123°C for a minimum of 15 minutes and then incinerated.

**Degree of kill:**

Chemical Sterilization by Virkon, or other validated disinfectant - effectively 100% kill.

Autoclaving - effectively 100% kill (annual validation)

---

**Is an emergency plan required according to regulation 20?**

- **N**

**If yes, tick to confirm that it is attached to this form**

- **N**

**Tick to confirm that you have attached a risk assessment to this form**

- **Y**

---

02/03/2022
Comments from the local Biological Safety Committee (20/07/2015)

- McKimmie Project: the Committee discussed the form submitted and various points were raised, as follows:
  - Information provided needs to be expanded.
  - Animal work will require a project license – discussions should take place with [the unit manager] in SBS.
  - It is important to provide more details on the vectors being used.
  - Line management details need to be amended on the form.
  - Section 5 should be completed.
  - Some of the viruses [Dengue replicon] involved will attract the attention of counter terrorism and therefore notification needs to be made to the CTSA.

It was agreed that the form should be circulated to the Committee after amendments have been made.

[The committee agreed the classification of the project, Class 2, and that notification to the HSE should be made once the above points had been addressed].

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3 L4 L4</td>
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</tr>
</tbody>
</table>

Project Ref 559/16.1

<table>
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<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
</tr>
</thead>
<tbody>
<tr>
<td>30/06/2016</td>
<td>Study of the replication cycle of Bunyamwera virus, and other hazard level 2 bunyaviruses</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
<td></td>
<td></td>
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<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Withdrawn</th>
<th>Tick if notifying a connected programme of work</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
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</tbody>
</table>

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info
Project Additional Information

Purposes of the contained use

The principle aim of this project is to study Bunyamwera virus (BUNV) inside infected host cells as it progresses through its replication cycle.

BUNV is the prototypic member of the Bunyaviridae family, which comprises over 300 related viruses that infect a broad range of hosts including humans, animals, insects and plants. Several of these viruses are classified by ACDP as hazard level 3 and 4 pathogens, however BUNV is classified as a hazard level 2 pathogen.

BUNV is known to share many fundamental aspects of its molecular and cellular biology with other Bunyaviridae family members, and thus is a well-established model system with which to study the more dangerous pathogens within the family. The BUNV genome comprises three strands of negative sense RNA, which each encode a single mRNA that are translated to yield a total of 6 proteins. Four of these are structural proteins that are found in the virus particle, namely N, L, Gn and Gc, whereas NSs and NSm proteins are non-structural and are only present within the cytosol of the infected cell. These six virus proteins are also expressed by most other bunyaviruses.

Very little is known about how BUNV and other related bunyaviruses gain entry into cells and then alter the host cell environment to favour their own multiplication. Being able to visualize individual virus particles as well as sub-viral components will provide us a tool kit with which to dissect many virus activities that together make up the replication cycle. A better understanding of these processes will aid in the development of anti-viral therapies to prevent bunyavirus-mediated human and animal disease.

This project focuses on BUNV for three reasons:
1) highly-efficient 'reverse-genetics' systems are currently available for rescue of infectious wild-type BUNV from cDNAs, which also permits the site-specific manipulation of the BUNV RNA genome, described below;
2) BUNV replication cycle is the best characterised of any bunyavirus and many reagents are already available that will facilitate our studies;
3) BUNV is a hazard level 2 pathogen, which allows the manipulation of its genome and subsequent analysis within the relatively amenable confines of containment level 2 facilities.

Similar 'reverse genetics' systems have also recently become available for the manipulation of two other hazard group 2 bunyaviruses, namely Uukuniemi virus (UUKV; Rezelj et al, 2015, J. Virol. 89(9) 4849-4856) and Hazara virus (HAZV; unpublished). We are aiming to generate modified viruses based on these and other hazard level 2 bunyaviruses in order to better understand the replication cycle of the Bunyaviridae family as a whole.

Therefore, our work will be restricted to the modification of members of the Bunyaviridae family which:
• are classified as hazard level 2 pathogens (i.e. able to infect humans)
• are not identified by DEFRA as a specified animal pathogen
• and specifically no work will be performed on viruses classified as ACDP hazard level 3 or 4 pathogens.

One of the key methodologies we will be using to study bunyaviruses replication in live cells will be to generate infectious viruses that have been modified such that their proteins or genomes are appended to fluorescent molecules that can be detected using confocal microscopy. These fluorescent molecules can be incorporated into the virus in one of three ways:
1. by genetically altering the open reading frames (ORFs) of viral proteins to include the ORF of a fluorescent protein such as eGFP, or one of its derivatives.
2. by incorporating a specific amino acid sequence into a viral protein ORF that has high affinity for a fluorescent molecule, which can be introduced into the infected cell.
3. by altering a non-translated region of the bunyavirus genome to incorporate RNA structures that have high affinity for a fluorescent molecule, as above, that can be introduced into the infected cell.

In addition to adding heterologous sequences, we will also be modifying sequences within the bunyavirus genome by deleting or altering nucleotides within regions of
defined functions.

All of these genetic manipulations will involve site-specific modification of the bunyavirus RNA genome, and this is achieved by first manipulating corresponding plasmid DNAs that encode its genome. This will be achieved according to procedures described in relevant publications. For BUNV these are Bridgen and Elliott, 1996, PNAS 93(26) 15400-4; Lowen et al, 2004, Virology, 330(2) 493-500, and for UUKV these are (Rezelj et al, 2015, J. Virol. 89(9) 4849-4856).

Procedures for HAZV are currently unpublished, but follow the same general principles as the BUNV systems. The basis for these systems is a set of cDNA plasmids that encode the virus genome. As the bunyavirus genome is separated into three individual RNA strands called segments, the complete BUNV genome can be encoded by three separate plasmids. These plasmids are designed such that they possess promoter sequences that drive transcription of the viral RNAs using a variety of DNA-dependant RNA polymerases, and popular choices include bacteriophage T7 RNA polymerase (T7-pol), and cellular DNA polymerase I (Pol-I). The three plasmids are transfected into cells in culture that express T7-pol or Pol-I, and the viral genome is transcribed. If additional plasmids expressing virus structural proteins N, L, Gn and Gc are also supplied to these cells, infectious virus particles can form, which are independent of further plasmid-derived components. These viruses can then be purified and used to infect further cells for the intended analysis.

Recipient or parental organism

The recipients of the genetically modified virus will be mammalian or insect cells. These will be continuous cells lines such as A549, BHK, SW13, Vero or C6-36. There are no hazards currently associated with these cell types. These cells do not belong to a hazard group.

Host/vector system

The vector will be the bunyavirus genome, primarily BUNV, UUKV or HAZV. All modifications to the bunyavirus genome will be designed using high-resolution structural models of viral proteins or their homologues (eg. Ariza et al, 2013, Nucleic Acids Research, 41(11) 5912-26; Gerlach et al, 2015, Cell, 161(6) 1267-79), or previous functional analysis of cis- and trans-acting viral components (reviewed in: Walter and Barr, 2011, J. Gen. Virol, 92(11) 2467-84). In addition to constructing new recombinant viruses, we also have access to a variety of previously generated genetically modified BUNV variants from the laboratory of Professor Richard Elliott. One of these possesses a V5 epitope within the L ORF (Shi et al 2009, J. Gen. Virol, 90(2) 297-306), and the other expresses a modified version of Gc, which is fused to either eGFP or mCherry (Shi et al, 2010, J. Virol, 84(17) 8470-9).

The growth characteristics of these viruses have been characterised, which revealed they all exhibited approximately 100-fold reduced virus titres compared to the wild-type. This is not surprising given that RNA viruses such as BUNV are able to rapidly evolve in order to perform their replication cycles with optimal efficiency; extraneous and non-advantageous protein or RNA-encoding elements reduce this efficiency, and have not been documented to enhance virus growth. Consequently, we anticipate that all modifications we make to the genome of BUNV, or other bunyaviruses, will either result in a reduced or unchanged virus growth and infectivity.

BUNV, UUKV and HAZV are arthropod-borne pathogens classified by ACDP within hazard level 2. The principle arthropod hosts of BUNV are midges of the Culicoides species, whereas both UUK and HAZV are associated with ticks of the Ixodes species. These viruses can be transmitted to humans by the bite from an infected arthropod host.

Infected domestic mammals can sustain a viremia, and exhibit neurological symptoms (Chung et al, 1990, Vet. Micro, 21, 297-307). No definitive association between BUNV, UUK or HAZV infection and human disease has been made. In the case of BUNV, closely related viruses within the Bunyamwera serogroup, such as Cache Valley virus, have been linked to a febrile illness that is known collectively as Bunyamwera fever (Tauro et al, 2012, J. Clin Virol, 54, 98-99).

Origin & function

The genetic material involved has several sources. As described above, various genetic modifications to the bunyavirus genome will be made, most of which will comprise sequence insertions. One of these inserted sequences will be the gene encoding enhanced green fluorescent protein (eGFP) derived from the jellyfish Aequorea Victoria. Another will be the TR sequence derived from bacteriophage MS2, as well as the translational operator sequence from bacteriophage PP7, both of which binds their respective viral coat proteins. Other inserted sequences we may use include V5 and FLAG epitopes, tetracysteine motifs, or ACP and MCP-tags that are based on the E. coli Acyl Carrier Protein. It is highly likely that other molecules with more favourable characteristics will in future supersede these sequences and associated ligands, and if
so we will adopt the best possible system for our intended applications.

**Evaluation of foreseeable effects**

The insertion of eGFP ORF and V5 epitope sequences into the BUNV genome is known to attenuate virus growth by approximately 100-fold, as described in the previously cited references (Shi et al 2009, J. Gen. Virol, 90(2) 297-306; Shi et al, 2010, J. Virol, 84(17) 8470-9). Similarly, we predict that all other nucleotide insertions that we make to the BUNV, UUKV or HAZV genomes will reduce or have no effect on resultant virus growth and infectivity compared to the unmodified parental wild-type virus.

In addition, the titre of released altered virus will be lower than wild-type due to its reduced growth characteristics, which further reduces potential harm.

Transfer of the transgenes to other genetic systems is extremely unlikely. Bunyaviruses possess a negative sense RNA genome, and such viruses are widely regarded as being resistant to recombination with other nucleic acid sources within cells. Furthermore, as described above, no adverse consequences of inserts such as eGFP expression have been identified, and so even if transfer did occur, no risk is currently associated.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No application is sought

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Treatment of bunyavirus containing liquid waste fluids will be performed under the Faculty of Biological Sciences SOP for CL-2 waste treatment, which are summarized as follows:

Bunyavirus-infected liquids from cell culture flasks and plates will be aspirated using both vacuum lines and manual pipettes. All liquid handling procedures involving infectious virus will be performed inside a biological containment cabinet to prevent risk of aerosolized virus spread outside the work area. All aspirated liquids containing virus will be immediately treated with 2% virkon, and incubated overnight to allow complete virus killing.

Plastic ware exposed to bunyaviruses, including pipette tips, plastic universal bottles, cell culture flasks and plates, will be treated with 2% virkon, and incubated overnight. Following this treatment, plastic ware will be autoclaved to ensure complete virus killing.

Infected cells and cell lysates required for further analysis are rendered non-infectious by denaturation by detergents of chaotropic agents prior to removal from laminar flow hoods within the CL-2 containment facility.

All surfaces potentially in contact with virus-containing droplets and aerosols are decontaminated by contact with 2% virkon and, where possible, UV treatment for 30 minutes.

**Is an emergency plan required according to regulation 20?**

Yes

**If yes, tick to confirm that it is attached to this form**

Yes

**Tick to confirm that you have attached a risk assessment to this form**

Yes

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

No
The attached version of the risk assessment form was passed by the local safety committee of the Faculty of Biological Sciences, University of Leeds.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
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</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

### Project Ref 559/16.2

- **Date Ackn’ed**: 03/11/2016
- **CU2 Project Title**: Characterising interactions between Hazard Group 2 members of Herpesviridae family of viruses and host cells
- **Class**: Class 2
- **Culture Volume**: < 1 L
- **Non-GMM Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

### Project Additional Information

**Purposes of the contained use**

The aim of this project is to gain a deeper understanding of how individual herpesvirus-encoded proteins enhance virus replication and also how they modulate host cellular processes to produce an environment conducive for virus replication.

To investigate the role of herpesvirus-encoded proteins we will compare the molecular and cellular biology of wild type viruses with genetically modified virus recombinants.
which lack herpesvirus-encoded proteins or contain site-directed mutations with specific regions of the herpesviral proteins. We aim to assess the contribution of each herpesvirus-encoded protein on virus replication using various techniques such as:

- qRT-PCR to assess virus gene expression,
- qPCR to assess viral DNA load,
- immunoblotting to assess viral protein production
- western blotting for virus structural proteins

Other laboratory techniques will be used to examine genomes and proteomes.

We will be exploring how herpesvirus-encoded proteins manipulate cellular processes to produce a cellular environment to enhance their own replication. These host cell pathways include cell signalling, cell proliferation and transformation, immune evasion and intracellular trafficking. We will examine how virus-encoded proteins, which function in regulating viral gene expression and genome replication, affect these cellular pathways using various techniques such as high-throughput Next Generation Sequencing (NGS) and quantitative proteomic approaches to analyse changes in host cell gene expression profiles and protein expression levels in response to virus infection (both wild type and genetically modified members of the Herpesviridae family).

Analysis will be performed on various Hazard Group 2 members of the 3 sub-families of Herpesviridae, which are:

- Alpha-herpesvirinae (e.g. Herpes Simplex Virus type I)
- Beta-herpesvirinae (e.g. Human cytomegalovirus, Human Herpesvirus 6 etc.)
- Gamma-herpesvirinae herpesviruses (e.g. Epstein Barr virus, Kaposi's sarcoma-associated herpesvirus, etc).

For these studies we will engineer genetically modified herpesvirus recombinants which lack specific herpesvirus-encoded proteins or contain site-directed mutations with specific functional regions of the viral proteins.

Recipient or parental organism

Recipients of both wild type and genetically modified members of the Herpesviridae family of viruses will be various mammalian cell types. These will either be from continuous cell lines such as Vero, HEK 293, HEK293T, or alternatively commercially sourced primary cells derived from kidney, skin, blood or endothelial tissue. These require specialised culture conditions and growth supplements, and these are not readily available outside of the laboratory. Therefore, accidental release of the recipient will pose no significant threat to the environment.

Infected cells do release infectious virus particles, although the titre of virus from these cells is low (Virus titres of <1x10^8 pfu/ml are expected). Any released viruses will be contained within the nutrient media that covers the cells, and thus will not be released as droplets. Cross infection of cells contained within an incubator is has been considered, but is unlikely due to the presence of liquid media covering all cells, and the use of filter-lidded cell culture flasks. Any laboratory procedures that may cause droplets or aerosol generation (such as use of vortex or pipette) will be performed inside a safety cabinet to prevent risk of infection. Following use or spillage of media containing herpesviruses, all surfaces will be decontaminated with both 2% virkon washing and where possible, exposure to UV light.

In addition, cloning will be performed in the E. coli laboratory strains such as DH5α; which are non-pathogenic disabled bacterial hosts that cannot survive in the human gut, belonging to hazard group 1.

Host/vector system

Vectors will be various Herpesviridae member genomes representing the three sub-families, such as:

- Herpes Simplex Virus type 1 (HSV-1);
- Human Cytomegalovirus (hCMV);
- Human Herpesvirus 6 (HHV-6A);
- Epstein Barr Virus (EBV);
- Kaposi's sarcoma-associated herpesvirus (KSHV).

Modifications will be performed on the viral genome prior to infection of host mammalian cell lines. Genetic modifications that will be used include the deletion or site-directed mutation of specific regions of the viral genome to enable studies of the interaction between viral proteins and host cell proteins.
Mutations to be generated are intended to disrupt the function of viral proteins and in the majority of cases are therefore likely to reduce the effectiveness of the virus to replicate.

The deletion of herpesvirus non-structural genes results in attenuation of virus growth in cell culture systems. Moreover, mutation of non-structural encoded viral proteins will not affect host or tissue tropism of modified viruses. In addition the titre of released altered virus will be lower than wild type due to its reduced growth characteristics. Similarly, smaller deletions and site-directed modifications within non-structural proteins will also reduce its function, and so will also be predicted to result in reduced growth and disease compared to the unmodified viruses. Taken together, these data strongly suggest the resulting virus bearing deletions and other modified viruses would be less harmful to humans than the wild type.

Knock outs or deletions in those genes involved with viral latency may cause an increase in pathogenicity of herpesviruses, for example deletion of the latency-associated nuclear antigen (in Kaposi's sarcoma-associated herpesvirus) leads to an increase in viral titre (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2626151/). Increase in viral titre is an expected outcome following this type of modification as cells will no longer be able to harbour latent viruses and instead all virus infected cells will produce virus particles (this effect is also seen when treating infected cells with sodium butyrate). In terms of risk, this is seen as a slight increase in overall risk, but still falls within the requirements for hazard group 2 categorisation. Controls identified for working at containment level 2 are sufficient to mitigate the small increase in risk.

Deletions or modifications to structural genes are expected to result in reduced viral viability and infectivity.

Origin & function

The various genetic modifications to members of Herpesviridae family genomes will be made, mostly comprising mutations to disrupt the function of viral proteins. Genetic modifications that will be used include the deletion or site-directed mutation of specific regions of the viral genome to enable studies of the interaction between viral proteins and host cell proteins. These will focus on non-structural encoded viral proteins which will not affect host or tissue tropism of modified viruses, specifically non-structural proteins which function in regulating viral gene expression and genome replication.

Evaluation of foreseeable effects

Herpesviruses are spread by close contact, saliva or a sexual route. Therefore, potential routes of transmission include inoculation by sharps, contact with open wounds and exposure to droplets. Local rules of Category II work preclude the use of sharps and the compulsory wearing of gloves, and protection of droplets will be controlled by the use of a Class II biological safety cabinet. Furthermore, infection which subsequently results in clinical disease is more unlikely, firstly because the human immune system restricts the ability of the herpesvirus to generate a lytic infection; herpesviruses establish a persistent, latent infection and secondly because a high percentage of the population is seropositive for these human herpesviruses and so a second exposure to a seropositive individual is not likely to establish a persistent infection.

However, a key hazard that will be addressed through procedures associated with the work is the risk of infection in immunocompromised individuals. Staff, students or lab visitors (including contractors and cleaners) who have an immunocompromised condition (e.g. HIV or treatment with immunosuppressive drugs) are no less likely to be seropositive from an early age than an immunocompetent individual and so are vulnerable to viral reactivation that is independent of exposures through research activity. However, a primary infection stemming from research samples may trigger a lytic infection which leads to disease. Such individuals will therefore be managed under a specific risk assessment.

The Herpesviridae family cause a range of diseases, which in the vast majority of cases are mild (e.g. HSV-1 causing localised lesions). In rare cases members of the Herpesviridae can cause significant infections, potentially with severe consequences for the infected individual (e.g. CMV linked to Guillain–Barré syndrome). These extreme cases are very rare and often arise as a result of the immune system being compromised. People involved with work on herpesviruses undergo University Occupational Health screening, and are asked to inform the University if they become aware that their immune status has changed (i.e. that they are immunocompromised).

A potential hazard associated with contained use of these viruses is accidental release outside of the containment facility. However, infection of persons either through entering the containment facility or by contact with infectious material (e.g. waste being removed from the lab) is unlikely.

Some members of the Herpesviridae family, Gamma-herpesviruses, are specifically known to be oncogenic (e.g. EBV and KSHV), and in general members of this sub-family have the potential to cause host cells to evade the immune system and elements of cellular control (e.g. apoptosis and proliferation). Therefore one worst case scenario for this virus family is accidental inoculation leading to infection followed by an oncogenic event. However, these cancers are restricted to those with particular risk factors such as significant UV exposure or an immunocompromising condition and therefore exposure from research activity is unlikely to cause ill health. Standard
CL2 controls will prevent accidental inoculation.

We predict that the proposed mutations which disrupt the function of viral proteins will reduce the effectiveness of the virus to replicate, a deletion of herpesvirus non-structural genes results in attenuation of virus growth in cell culture systems. Importantly, mutation of non-structural encoded viral proteins will not affect host or tissue tropism of modified viruses. In addition the titre of released altered virus will be lower than wild type due to its reduced growth characteristics. Similarly, smaller deletions and site-directed modifications within non-structural proteins will also reduce its function, and so will also be predicted to result in reduced growth titre and disease compared to the unmodified viruses. Taken together, these data strongly suggest the resulting virus bearing non-structural deletions and other modified viruses would be less harmful to humans than the wild type.

Final GMO:
The genetically modified viruses we intend to use are able to infect cell culture systems, although at a substantially reduced rate compared to wild type. Therefore, it is likely that they will also be able to infect humans. Cells infected with modified viruses will also release clonal progeny viruses. However, as described above, the titre of altered viruses, with exceptions (alteration to latent genes), is expected to be lower than wild type. Therefore, we acknowledge that there is risk that accidental release may result in human infections. However, as most intended modified viruses described exhibit attenuated growth, and will be present with lower titres, these viruses pose less risk than the unmodified form and will be subject to containment under category 2 conditions.

Controls
Work with Human herpesviruses will only be performed within a CL-2 laboratory with restricted access and virus samples will be stored in a lockable freezer. All manipulations will be performed by staff wearing disposable gloves. No sharps will be permitted in the virus handling area and all unwanted infected media will be aspirated by vacuum directly into 2% Virkon and stored overnight before disposal. Viruses will be grown in tissue culture vessels provided with filters to prevent escape of aerosols. All pipette tips will be disposed of vial plastic ‘sweetie jars’. All materials (e.g. pipettes and culture flasks) that have potentially been in contact with live viruses will be treated with Virkon before being placed in disposable autoclave plastic bags and placed within metal autoclave tins before being passed on to cleaning/waste disposal staff. These measures will be applied at the end of each session of handling live virus. Standard BCL2 waste disposal and Faculty Cat 2 spill containment procedures will minimise the chance of virus release.

Herpesviridae that are covered by this notification must be classified as belonging to ACDP hazard group 2 and will be used under a containment level 2 standard operating procedure.

Herpesviridae belonging to higher hazard groups (e.g. Herpes B virus, also known as Macacine herpesvirus 1) are specifically excluded from this notification. No members of Herpesviridae currently fall under SAPO.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application is sought

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Treatment of herpesvirus containing liquid waste fluids will be performed under the Faculty of Biological Sciences SOP for CL-2 waste treatment, which are summarized as follows:

Herpesvirus-infected liquids from cell culture flasks and plates will be aspirated using both vacuum lines and manual pipettes. All liquid handling procedures involving infectious virus will be performed inside a biological containment cabinet to prevent risk of aerosolized virus spread outside the work area. All aspirated liquids containing virus will be immediately treated with 2% virkon, and incubated overnight to allow complete virus killing.

Plastic ware exposed to herpesviruses, including pipette tips, plastic universal bottles, cell culture flasks and plates, will be treated with 2% virkon, and incubated overnight. Following this treatment, plastic ware will be autoclaved to ensure complete virus killing.
Infected cells and cell lysates required for further analysis are rendered non-infectious by denaturation by detergents of chaotropic agents prior to removal from laminar flow hoods within the CL-2 containment facility.

All surfaces potentially in contact with virus-containing droplets and aerosols are decontaminated by contact with 2% virkon and, where possible, UV treatment for 30 minutes.

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<tr>
<td>Project notified under transitional arrangements</td>
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<td></td>
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</tbody>
</table>

Investigation of interactions within gut microflora including those identified as belonging to Hazard Group 2

Date Ackn'd: 15/02/2018

Date Project Ceased: 15/02/2018

Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Tick if notifying a connected programme of work: N
The aim of this work is to improve our overall understanding of how microbial ecosystems in a host gut function, particularly within the human gut. The study of isolated discrete components of gut ecosystems is unlikely to yield comprehensive results, hence the need to work with complex systems in their entirety. The human gut microbiota consists of a complex mutualistic community of microorganisms. Bacterial species within the gut microbiota communicate amongst themselves to coordinate various processes, such as biofilm formation, and exchange of genetic material e.g. antibiotic resistance determinants.

Areas of research will include:
- microbe-to-microbe interactions within the gut and gut models during healthy and diseased states
- investigating the natural transfer of genetic material between bacterial communities by horizontal gene transfer.
- interaction of gut pathogens within the residual bacterial community during disease
- host-cell-to-microbiota interactions,
- host-to-microbiota interactions (e.g. effect of the host immune system on the microbiota and vice versa)
- effect of bacteriophages on a microbial gut ecosystem,
- quorum sensing and microbiota coordination,
- effect of external factors such as diet on microbiota,
- identifying targets for therapeutic intervention,
- studying the effect of gut microbiota on therapeutic interventions including those interventions unconnected to the gut microbiota (e.g. cancer chemotherapy or radiotherapy treatment).

There are numerous genetic manipulation techniques that can be used to do this, including:
1. Allelic exchange to construct defined gene deletions & complementation to probe the role of specific genes.
2. Targeted intron insertion to construct gene disruptions to probe the role of specific genes where allelic exchange is not possible.
3. Random transposon mutagenesis to construct libraries for high-throughput analysis to identify genetic pathways involved in a biological process, such as colonisation.
4. Plasmid manipulation techniques for studying expression, such as complementation or interference RNA, or genetic transfer studies, such as the addition of reporter genes e.g. fluorescent markers.
5. Use of naturally occurring plasmids harbouring antibiotic-resistance determinants, such as carbapenemases. Once isolated and sequenced, a fluorescent reporter can be inserted within plasmids to aid in investigating the dissemination of such plasmids to other micro-organisms in the absence or presence of antibiotic-selective pressure.
6. Studying the effect of the host genetic background on microbiota or specific bacteria in the gut. This would involve use of host organisms (e.g. mice) with specific genetic modifications using conventional GM techniques.

Recipient or parental organism

Recipient/Parental organisms will be members of normal and disease associated gut microflora such as:
<table>
<thead>
<tr>
<th>Organism</th>
<th>Hazard Group</th>
<th>Prevalence in the population (%)</th>
<th>Main Mode of Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides fragilis</td>
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<tr>
<td>Bacteroides melaninogenicus</td>
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<td>Clostridium perfringens</td>
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<td>Person-to-person</td>
</tr>
</tbody>
</table>

All the hazard group 2 organisms listed above are capable of causing disease in their host, usually as self limiting opportunistic infections often where the immune system has been compromised. For example Staphylococcus aureus is able to cause a number of conditions including:

- food poisoning resulting from the consumption of food contaminated with enterotoxins,
- local infections from bites leading to cellulitis, erythema, tenderness, mild fever, adenopathy, and lymphangitis
- Scalded skin syndrome is caused by exfoliative toxins secreted on the epidermis
- necrotizing fasciitis in immunocompromised individuals
- superantigen TSST-1 producers are responsible for 75% of toxic shock syndrome cases

Whilst Clostridium difficile is the main cause of nosocomial antibiotic-associated diarrhea. C. difficile can also cause; bacteremia, intra-abdominal abscess, osteomyelitis, visceral abscess, empyema, toxic megacolon, colonic perforation, and reactive arthritis.

All of these bacteria have been identified as naturally occurring in and on humans, with worldwide carriage rates ranging from a few percent to 100% for bacteria such as Bacteroides fragilis and Escherichia coli. For the most part they coexist as part of a wider bacterial ecosystem within the host without causing harm, infact research clearly shows that removal or limiting diversity of the gut microbiota is damaging to the human (or animal) host.

Genetic modifications will include:

For complementation studies, deleted genes can be complemented back on the bacterial chromosome. These may be tagged e.g., a watermark codon may be used to denote complemented allele. The nature of these genes will vary, however, as they will be restored under their own promotor no enhanced virulence is expected. In certain cases, the transgenic sequence for genes to be complemented will come from wild-type donor strains isolated from human faeces.

2. Generation of single insertion sites using targeted introns
For example the insertion of the Group 2 intron from the ltrB of Lactococcus lactis may be used. Owing to the addition of an ermB cassette (a selectable marker), the insertion of this intron in the genome confers resistance to marcolide antibiotics (namely erythromycin, clindamycin and lincomycin).

3. Generation of random insertion immobilised transposon libraries
For example the Mariner-family transposable elements are active in a wide variety of organisms with the himar1 transposon, harbouring an ermB cassette, being able to randomly insert into the chromosome of a number of bacteria. The insertion sequence for this transposon occurs at any AT sequence. This transposon system is under the control of a tetracycline promoter and derepression of this system occurs in the presence of tetracycline or homologues of tetracycline.

4. Addition of reporter proteins
For example GFP and LOV derivatives or SNAP tags (based on O6-alkylguanine-DNA alkyltransferase). Once expressed these proteins confer fluorescent properties in the recipient.

Where bacterial genes are disrupted (targeted insertions, transposon mutagenesis, etc.) it is expected that in the vast majority of cases the disruption will either be of no effect on the recipient or will result in reduced survivability or attenuation of virulence properties.

However, there are a number of examples where disruption of a single gene can increase virulence, e.g. disruption of the tcdC gene in C. difficile causes loss of regulation of the tcdA and tcdB, two genes involved in the production of cytotoxins.

For gene tagging using a variety of reporters - these genes have previously been expressed in a range of bacterial species without any enhanced virulence being observed. In fact, many reports have shown that pathogens harbouring some of these genes have attenuated virulence properties. Although considered unlikely, the addition of a fluorescent protein to candidate proteins could alter the cellular location or enzymatic properties of the candidate gene potentially causing increased virulence in recipient strains.

We will also be making use of in vivo animal models which will include colonising the guts of both wild type and genetically modified animals with WT and genetically modified bacteria. Animals will be small mammals (e.g. mice, rats, hamsters, etc.) which can be housed, isolated, and maintained in specialised animal caging (such as the Tecniplast isocage system). Genetically modified small mammals (on their own) are unlikely to be of increased risk to humans or the environment as most modifications tend to reduce viability or have no phenotypic effect. However, there are a small number of modifications which can increase the potential for harm:

- Modifications to genes associated with behaviour resulting in increased aggression
- Modifications that allow the animal to act as a novel reservoir for human (or other) pathogens

**Host/vector system**

A variety of vector systems will be used including:

- Plasmid based (e.g. pMLT series of Clostridium shuttle vectors) mutated alleles, or alleles containing fluorescent / metabolic proteins. Allele exchange plasmids are pseudo-suicide plasmids meaning they have a poor replication efficiency, and harbours ermB, an antibiotic resistance marker. Mutations created in this way are stable chromosomal mutations.
- Transposon based shuttle vectors e.g. mariner-like elements (such as the himar1 transposon) harbouraged on suicide shuttle plasmids such as pRPF215 and pYAA023.
- Targeted introns to create single insertion sites, e.g. pMLT007C contains the re-targeted L. lactis ltrB intron with ermB resistance cassette along with catP gene, conferring resistance to chloramphenicol/thiamphenicol.
- Manipulation of naturally occurring plasmids harbouring antibiotic resistance determinants isolated from wild-type strains (e.g. plasmids harbouring blaKPC resistance determinant). Plasmids will be genetically manipulated with the inclusion of reporter genes (e.g. GFP) and inserted in to the original strain using an appropriate method. This will enable the examination of horizontal gene transfer to other microbial species.
- Constitutive or inducible expression constructs (e.g. tetracycline inducible systems).

Manipulation of vector systems will be carried out using laboratory strains of E.coli, this element of the work is categorised as requiring containment level 1. Most of these vector systems will be transferred to target bacterial populations by conjugation with E. coli (lab adapted strain containing the F plasmid). Conjugation in this way requires deliberate co-culturing of donor and recipient organisms. However, other methods may also be used to transfer vectors (e.g. electroporation, chemical...
Many (or most) of the organisms found in the gut microflora are identified as hazard group 2 pathogenic agents able to cause disease (usually opportunistic infections). The host vector systems described above are generally unlikely to increase the pathogenicity of the potential hosts described in the previous section. However, there are a small number of exceptions where inactivation or induction of already present genes can result in more harmful phenotype. Even with a more harmful phenotype these organisms are still expected to be categorised within hazard group 2 (i.e. they can cause human disease and may be a hazard to employees; they are unlikely to spread to the community and there is usually effective prophylaxis or treatment available).

Genetic material will be derived from a variety of gut microflora (described previously) and will include all coding and non-coding regions of their chromosomes as well as other genetic elements (such as plasmids and transposons). These genes will have an extensive range of functions including:

- DNA metabolism
- RNA metabolism
- Protein processing, folding, and secretion
- Cellular processes
- Energetic and intermediary metabolism
- Signalling
- Transport
- Chemotaxis
- Housekeeping
- Pathogenicity (colonisation, adhesion, cytotoxins etc.)
- Resistance (antibiotic, restriction endonucleases, sporulation)

Alterations (inactivation or activation) to the vast majority of these genes will result in either no change to the phenotype or a reduction in the organisms viability. However, in a limited number cases inactivation or activation of particular genes can increase the potential for harm by some, but not all, of these bacteria.

The overall aim of this research is to gain a better understanding of the gut ecosystem, there is no deliberate intention to specifically engineer enhanced pathogenic phenotypes or to increase virulence of any of the bacteria forming gut microbiotas.

Hazard to humans
Many species form part of a humans/animals gut flora and are usually beneficial to the host, however, given the right set of conditions many of these bacteria can become pathogenic, for example, disease casing infection by C. difficile usually occurs following perturbation of the gut flora by antibotic exposure.
Given that healthy individuals have a natural barrier to colonising microflora, in general, the vast majority of GM isolates are not expected to overcome this barrier, thus the risk factors associated with wild-type strains and GM strains are expected to be the same, and is not expected to change the overall hazard rating of this research.

For the most part, genetic manipulation will result in a loss of an important phenotype, either cell-cell communication, environmental signalling or other infection-related processes.

There are examples where modification of a gene (inactivation or activation) can enhance a pathogenic trait (e.g. inactivation of tcdC of C.difficile results in unregulated cytotoxin production), however, for harm to occur there would also need to be an accidental inoculation event:
- for the percutaneous route this would always be hazardous for an individual worker almost regardless of whether the bacteria was GM or not.
- for the oral route, the modified bacteria would need to become established within the natural barrier of the already present host gut bacteria.

For the vast majority of bacteria identified previously the main routes for transmission are oral (e.g. hand-to-mouth) or infection of wounds (i.e. contact with pre-existing wounds or innoculating wounds such as needlestick). The use of physical and management controls identified at CL2 (e.g. wearing gloves, hand washing etc.) will reduce the potential for infection to a very low level (i.e. negligible).

The use of physical and management controls identified for GM work at containment level 2 is sufficient to prevent accidental inoculation.
With regard to specific modifications:

• Alterations to microbiota genes (i.e. inactivation or activation) – it is expected that in the vast majority of cases these alterations will either have no effect on the gut bacteria or will result in reduced survivability or attenuation of any pathogenic/virulance properties.

• Use of reporter genes – The production of fluorescent proteins in the resultant GMOs is not expected to alter virulence properties of the microorganisms, in fact several studies have shown that expression of fluorescent proteins can attenuate the host, potentially through steric hindrance of associated protein. Although the risk is considered low, fusion of a fluorescent protein could potentially change the cellular location of the fusion leading to enhanced virulence.

• Use of transposons – In a worst case scenario, the construction of transposon libraries has the potential to enhance the virulence of target bacteria potentially altering virulence determinant expression (through mutation of regulator genes) or cell tropism.

• Use of antibiotic resistance genes – these may make it harder to treat an infection, however, most laboratory vector based resistance markers are for antibiotics that are generally not clinically useful. These resistance genes are already present in the environment and readily move between bacteria (horizontal gene transfer).

• Construction of vector systems – E.coli strains used for plasmid propagation/conjugation are lab-adapted strains unable to cause disease in humans & animals, this work is categorised at containment level 1.

It should be noted that workers undergoing certain types of pharmacological interventions (e.g. taking a course of antibiotics) are likely to have altered susceptibility to some of the organisms listed previously. People involved with this research will be instructed to inform senior researchers if they are affected, an individual assessment of risk will then be undertaken. The same principal will be applied to workers that become significantly immunocompromised, or have conditions that have a major impact on innate defences such as the skin or mucus membranes (e.g. psoriasis or eczema resulting in broken skin).

Combined animal and bacterial work

The combination of a modified host animal and modified bacterial population in general is not expected to increase the risk of the activity, most modifications will result in either no phenotypic alteration, or reduced viability of the host and or the bacteria. However, there are a small number of examples where there is a potential increase in risk to workers, these are:

• Increased shedding of modified bacteria through alterations to the host
• Increased overall bacterial load in the host
• Generation of more aggressive animals which could increase the likelihood of being bitten (and introduction of modified bacteria to a worker via the wound).

The increased risk of the first two examples is mitigated by the use of good laboratory practices and decontamination procedures (as is already required for work at containment level 2). For the third example, increased risk of being bitten is mitigated by: animal handling is reduced to the minimum possible, remaining handling is carried out by experienced workers, keeping infected animals alive only for the duration of the associated experiment, using incapacitation techniques that reduce the potential for being bitten (e.g. restraining devices, anaesthetics, etc.).

Hazard to the Environment

The gut microbiota identified above can be described as ubiquitous within the environment, some are spore formers, and most are able to survive in the environment. With the exception of vector systems all the genetic material is derived from the natural environment, if a laboratory release were to occur it would be of material that was already present in the environment.

With respect to accidental release of GM gut bacteria, these are expected to have the same or reduced viability when compared to the wild type, i.e. modifications are not expected to confer any selective advantage for survival outside of the host.

Use of animal gut models – potential for hazard to humans or the environment

The potential for hazards to arise from the use of animal models such as mice and rats are:

• Percutaneous route (animal bites) and direct transmission of microorganisms into the wound – animal workers receive extensive training on the correct handling of animals and wear surgical gloves (these will not provide protection for anything other than a minor nip). Various restraining devices (e.g. tube restrainers) will be used for higher hazard activities (e.g. tail injection).

• Faecal oral route – animal workers follow good animal husbandry techniques as well as good occupational hygiene techniques which will limit the potential for accidental transmission to virtually zero. Animal workers wear surgical gloves which are routinely changed between study group animals and holding rooms.

• Transmission of microorganisms to other animals in the animal facility – animals are housed in independently ventilated cages (currently techniplast), facilities operate a "clean-to-dirty” regime with “dirty” animals being separated (different holding rooms) from “clean” animals. Staff will follow a clean to dirty regime (i.e. only move from clean
to dirty and not the other way around). These measures as well as measures identified above reduce the potential for transmission to other animals to virtually zero. In addition, animals are regularly screened for opportunistic infections using accredited testing companies (e.g. Charles River).

- Escape of GM pathogen to the environment e.g. incorrect disposal of soiled bedding. Facilities work to strict SOPs, all waste is inactivated by autoclaving, and autoclaved waste is sent for incineration. The potential risk to the environment is assessed as negligible. Work with animals (mainly mice and possibly rats) will only take place in home office approved facilities which also meet the requirements containment level 2 work. The risk to the environment is reduced by using either IVCs or filter top cages to prevent the escape of contaminated dust/aerosols. The escape of an infected animal is considered unlikely as these cages are sealed when the animals are housed. In addition, all rooms are fitted with rodent barriers to prevent animals escaping from the room. All procedure/husbandry work will be done in a class 2 safety cabinet with a HEPA2 filter. This helps to reduce environmental exposure, protecting the immediate area and human workers from GMO contamination. Any staff handling animals will be required to wear PPE, such as, the use of autoclaved lab coats and double gloves. This will minimise the risk of scratches and bites, and potential transmission of the GMO.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste is disposed of in accordance with local procedures to insure inactivation of biologically hazardous waste. In general this involves a minimum of:

Liquid waste is treated either by:
- for low concentration and low organic load, mixing with an approved biocidal product (e.g. virkon, sodium hypochlorite etc.) at a concentration that is able to achieve a 5 to 6 log reduction may be used, or
- for high concentration or high organic load contaminated liquid waste (waste collection bottles/sample pots/falcon tubes/tissue culture flasks) are placed in autoclave bags. These are then autoclaved (with minimum parameters being identified as 121DegC and 15psi (1Bar) for at least 15 minutes). Autoclaved waste is then collected by an authorised healthcare waste contractor for final offsite disposal by approved methodology (e.g. incineration, rotoclave, etc.).

Solid waste (agar plates, used plastic consumables etc.) is treated by:
- Contaminated materials are placed in autoclave bags, these are then autoclaved (with minimum parameters being identified as 121DegC and 15psi (1Bar) for at least 15 minutes). Autoclaved waste is then collected by an authorised healthcare waste contractor for final offsite disposal by approved methodology (e.g. incineration, rotoclave, etc.).

Disinfectants are used as per the manufacturers’ instruction and are selected in accordance with their documented efficacy. Autoclaves are validated on a minimum of a yearly basis by 12 point thermocouple test under load conditions by an approved validator.

Is an emergency plan required according to regulation 20? No

If yes, tick to confirm that it is attached to this form No

Tick to confirm that you have attached a risk assessment to this form Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment No

Please enter comments on the GM safety committee on the risk assessment
Campus BioSafety Committee 24th October 2017
FBS17138 Cell-cell interactions of human gut microbiota – A. Buckley
NC reported that he advised that the SoP be incorporated into plans, which SB had chased up. Draft SoPs have been provided and another meeting will be arranged in order to finalise details. Final details will be checked with PB and MB. 
PB reported on a risk of exposure to anyone on antibiotics. 
NC asked clarification regarding carcass disposal. Should carcasses be double bagged and frozen or autoclaved? 
MH confirmed that the project will need to be submitted to HSE. 
Action: NC to confirm details and report back to Committee.

Campus BioSafety Committee 16th January 2018
FBS17138. Cell-cell interactions of human gut microbiota – A. Buckley – Class 2 submission
Various concerns had been raised regarding adequacy of SoPs and the animal element [these have now been addressed and the project approved] 
Action: MB to send complete broad application for HSE. This should be successful in future-proofing work as similar projects will be covered by the same notification.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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### Project Ref 559/20.1

<table>
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<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
<th>Class2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>30/01/2020</td>
<td>Study of virus-host interactions during the influenza virus replication cycle</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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</tbody>
</table>

- **Historical Significant Changes**: N
- **Historical Date of Additional Info**: N
- **Significant Change ID**: N
Influenza virus is a negative sense RNA virus within the Orthomyxoviridae family and its genome comprises eight separate RNA strands known as segments. Influenza viruses are classified within four subtypes A, B, C and D, with all the human pathogen strains falling within the A and B groups.

Influenza A virus (IAV) is an important pathogen of humans, birds and several other mammalian species, causing widespread seasonal infections, as well as occasional pandemics within the human population. Repeat infections of IAV are common, as a result of the high mutation rates of the virus, and consequently many strains of IAV exist.

Influenza B virus (IBV) has a limited host range and is only known to infect humans and seals, and has not been implicated in pandemics. Whilst IBV mutates at a rate 2 to 3 times slower than type A, it is accepted that Influenza B virus causes significant morbidity and mortality worldwide.

The overarching aim of this research is to identify and understand the role of virus and host interactions during the various stages of the influenza virus infection and replication cycle.

The planned work will use strains of IAV as well as strains belonging to the IBV group classified within hazard group 2. Strains of virus will be studied at the containment level appropriate to the hazard group of the virus (i.e. HG2 at CL2). In particular, much of our focus will be on the well-studied and laboratory cell culture adapted WSN strain of IAV, A/WS/33 (H1N1; ATCC VR-1520).

The scope of our planned experiments is very broad and may include:

- analysis of the overall effect of IAV or IBV multiplication on host cell processes
- the interaction between host and viral proteins
- location of IAV or IBV proteins within the cell during various stages of the replication cycle
- and also the dependence of IAV or IBV on various cellular processes for efficient virus growth.

To this end, we will infect cells in culture with infectious and replication-competent IAV or IBV, and examine the subsequent viral and cellular processes that occur using biochemical and biophysical techniques.

While much of our work can be performed using wild type strains of IAV or IBV, we also wish to generate genetically-modified IAV or IBV, in which specific genetic changes have been engineered. The roles of the genetic changes that we are planning fall into two categories:

1. Genetic changes that result in alteration of specific amino acid or nucleotide sequences, with the intention of causing a corresponding loss of viral function. This is the principle of reverse genetics and it will allow us to define and assign functions to any genetic element within the IAV/IBV genome. The major benefit of using reverse genetics in the context of infectious IAV/IBV is that it will allow us to determine the role of specific sequences and corresponding functions in the context of the full IAV/IBV replication cycle. Design of such changes will be made by rational methods including the use of structural models of viral proteins, sequence alignments followed by identification of regions of nucleotide and amino acid conservation, as well as previously identified amino acid or nucleotide sequence motifs.

2. Genetic changes that result in the addition of sequences to the IAV/IBV genome, specifically to facilitate the visualization of IAV/IBV particles or components as they are generated within infected cells. A typical example of this activity is the modification of an IAV/IBV gene segment by the addition of the coding sequence for a fluorescent protein such as eGFP, allowing its fusion to a native viral protein. On infection of new cells, the resulting IAV/IBV particles will express the viral protein fused to eGFP, which will thus be made visible within the infected cell. An example of such a modification is the fusion of eGFP to the viral protein PA (Lakdawala et al, 2014; doi: 10.1371/journal.ppat.1003971), allowing detection of IAV PA gene expression and polymerase location within infected cells, and many similar insertions have been reported (Reviewed in Breen et al, 2016; doi: 10.3390/v8070179).

All of these genetic manipulations will involve generating infectious IAV or IBV using plasmid-based reverse genetics systems.

For IAV the most efficient of these comprises eight individual plasmids that each encode one of the eight IAV segments. These segment sequences are flanked at one end by the promoter sequence for cellular DNA-polymerase-I (Pol-I), and at the other end by cellular DNA polymerase II (Pol-II). Pol-I drives the transcription of a negative sense copy of each IAV segment, thus generating the complete IAV RNA genome, whereas Pol-II transcribes each IAV segment as a positive sense mRNA molecule, which subsequently is translated to yield the IAV proteomes. Transfection of these eight plasmids into suitable mammalian cells (eg MDCK and HEK293T cells) leads to co-expression of the eight IAV segment RNAs along with the corresponding viral proteins, and subsequently allows assembly of IAV particles that can autonomously infect new cells. To generate a IAV bearing a specific genetic change, the modification is engineered into the corresponding plasmid cDNA, so as to result in the transcription of a
modified RNA bearing the desired nucleotide change. The complete reverse genetics system and the associated protocols are described in Hoffmann et al (2000) PNAS May 23;97(11):6108-13 (DOI: 10.1073/pnas.100133697).

The following Influenza viruses fall under SAPO or are Pandemic and are outside the scope of this notification:

A. We will not work with any avian influenza that are:
   o uncharacterised
   o Type A viruses which have an intravenous pathogenicity index in six week old chickens of greater than 1.2
   o Type A viruses H5 or H7 subtype for which nucleotide sequencing has demonstrated multiple basic amino acids at the cleavage site of haemagglutinin.

B. Influenza viruses identified as newly emergent pandemic strains as per ACDP’s Advice on Experimental working with Influenza Viruses of Pandemic Potential section 10 a. and b. (www.hse.gov.uk/biosafety/diseases/acdpflu.pdf)

There is no intention to work with any of the viruses listed (A and B) above.

Recipient or parental organism

The recipients of the genetically modified virus will be mammalian cells. These will be either from continuous cells lines such as MDCK, A549, BHK, SW13, Calu-3 human lung carcinoma cells. In addition, we will use primary lung cells as well as cells derived from human bronchial progenitor cell lines (e.g. BCi-NS1 and NHBEC-BMI-1) to provide a more authentic cell system. The cells identified above are not identified as hazardous, do not belong to a hazard group, and therefore are estimated to be of negligible or no risk.

Host/vector system

The vector will be the influenza virus genome. As described above, this will primarily be IAV, but also possibly IBV if working with this virus is deemed beneficial to our research aims. As described above, the design of such changes will be made by rational approaches such as using structural models of viral proteins, sequence alignments to identify regions of nucleotide and amino acid conservation, as well as searching influenza virus sequences for previously identified functional motifs.

Foreseeable effects from the host vector system:

Viral infection of staff or others: The consequence of infection with IAV and IBV to human health is strain-dependant. For example, the main laboratory cell culture adapted WSN strain of IAV that we intend to use, A/WS/33 (H1N1; ATCC VR-1520), is not associated with severe human disease. In general, infections with seasonal influenza is characterized by a sudden onset of fever, cough (usually dry), headache, muscle and joint pain, general malaise, sore throat and a runny nose. Most people recover from fever and other symptoms within 7-10 days without requiring medical attention. A vaccine based on the circulating strains of seasonal IAV and IBV is made available each year and is broadly effective in preventing disease.

Origin & function

The genetic material involved will originate from several sources. As described above, various genetic modifications to the influenza virus genome will be made, most of which will comprise sequence insertions. One of these inserted sequences will be the gene encoding enhanced green fluorescent protein (eGFP) derived from the jellyfish Aequorea Victoria. Another will be the TR sequence derived from bacteriophage MS2, as well as the translational operator sequence from bacteriophage PP7, both of which binds their respective viral coat proteins. Other inserted sequences we may use include V5 and FLAG epitopes, tetracysteine motifs, or ACP and MCP-tags that are based on the E. coli Acyl Carrier Protein. It is highly likely that other molecules with more favourable characteristics will in future supersede these sequences and associated ligands, and if so we will adopt the best possible system for our intended applications.

Evaluation of foreseeable effects

The insertion of the eGFP ORF and other exogeneous sequences into the IAV genome is known to result in minor attenuation of virus growth as described in previous work (Reviewed in Breen et al, 2016; doi: 10.3390/v8070179). Similarly, we predict that all other nucleotide insertions that we make to the IAV or IBV genomes will reduce or have no effect on resultant virus growth and infectivity compared to the unmodified parental wild-type virus.

In addition, the titre of released altered virus will be lower than wild-type due to its reduced growth characteristics, which further reduces potential harm.
Transfer of the transgenes to other genetic systems is extremely unlikely. Influenza viruses possesses a negative sense RNA genome, and such viruses are widely regarded as being resistant to recombination with DNA sources within cells. Furthermore, as described above, no adverse consequences of inserts such as eGFP expression have been identified, and so even if transfer did occur, no risk is currently associated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation is sought

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Treatment of influenza virus containing liquid waste fluids will be performed under the Faculty of Biological Sciences SOP for CL-2 waste treatment, which are summarized as follows:

Influenza virus-infected liquids from cell culture flasks and plates will be aspirated using both vacuum lines and manual pipettes. All liquid handling procedures involving infectious virus will be performed inside a biological containment cabinet to prevent risk of aerosolized virus spread outside the work area. All aspirated liquids containing virus will be immediately treated with 2% virkon, and incubated overnight to allow complete virus killing.

Plastic ware exposed to influenza viruses, including pipette tips, plastic universal bottles, cell culture flasks and plates, will be treated with 2% virkon, and incubated overnight. Following this treatment, plastic ware will be autoclaved to ensure complete virus killing.

Infected cells and cell lysates required for further analysis are rendered non-infectious by denaturation by detergents of chaotropic agents prior to removal from laminar flow hoods within the CL2 containment facility.

All surfaces potentially in contact with virus-containing droplets and aerosols are decontaminated by contact with 2% virkon and, where possible, UV treatment for 30 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The attached version of the risk assessment form was passed by the local safety committee of the Faculty of Biological Sciences, University of Leeds (10/09/2019). Comments were made with respect to future proofing the project and the potential for work at CL3, however for the purposes of the current work CL2 and the use of HG2 influenza virus is currently sufficient.

Project Containment
We aim to use a replication defective human adenoviral vector to introduce the following genes into primary cultures of human cardiac myocytes, vascular smooth muscle and endothelial cells:

(i) the angiotensin type 1 receptor (AT1)  
(ii) matrix metalloprotease 9 (MMP9) in both sense and antisense orientations, and also dominant negative mutants of MMP9 may be expressed.  
(iii) peroxisome proliferation activation receptor (PPAR) gamma isoform 1 in both sense and antisense orientations.  
(iv) Reporter expression cassettes consisting of the bacterial beta-lactamase gene (obtained from Aurora Biosystems) under the control of either the human AP-1, CRE or NF-AT response elements.  
(v) A reporter expression cassette in which expression of either the enhanced yellow (EYFP) or enhanced green fluorescent protein (EGFP) (available from Clontech) is driven by the upstream regulatory region of human angiotensin receptor type 1 or type 2.
The bacterial Reverse Tetracycline Transcription Activator (rtTA) under the control of the human Cytomegalovirus (CMV) immediate early promoter.

By placing genes (i-iii) under the control of the tetracycline response element, the levels of expression of recombinant proteins can be regulated enabling a better understanding of the role of signal transduction pathways in cardiac pathologies.

Recipient or parental organism

The recombinant adenoviral vector will be introduced into primary cultures of human cardiac myocytes, vascular smooth muscle and endothelial cells.

Host/vector system

All transgenes are integrated into an adenoviral genome (using the AdEasy system from Stratagene) from which the early genes essential for replication, contained in the E1 region of the viral genome, have been deleted, to generate replication defective viruses which can only be propagated in human 293 cells. This is a helper cell line (human embryonic kidney cells) which express the leftmost 11% of the Ad5 genome containing the E1 region in trans thus reducing the ability of the virus to evade a host immune response.

Origin & function

**pAdEasy-1 vector**

The pAdEasy-1 vector (Stratagene) is a modified versions of human Adenovirus 5 in which the genes in the E1 region of the viral genome, essential for replication, are deleted to generate a replication defective virus. In addition the E3 gene is deleted which significantly reduces the ability of the virus to evade detection by the host immune system.

**Human angiotensin receptor.**

The peptide agonist angiotensin II activates this receptor to produce a variety of regulatory actions on the cardiovascular, renal, endocrine and neural systems including vasoconstriction, hypertension and aldosterone release. It is not envisaged that expression of this gene will be oncogenic or have adventitious effects in host cells at the levels of expression used. Expression of the gene will be placed under the control of a tetracycline inducible promoter system that levels of expression of the transgene can be finely regulated by the addition of tetracycline. The full coding sequence of the angiotensin type 1 receptor is available as a cDNA in our laboratory.

**Peroxisome Proliferation Activation Receptor (PPAR) Gamma**

PPARgamma is a transcription factor which is expressed in all of the major tissues of the vasculature including endothelial cells, vascular smooth muscle cells (VSMCs) and monocytes and macrophages. Drugs which activate PPARgamma have been shown to inhibit the proliferation and migration of VSMCs in cell culture. Current data suggests that the antiproliferative effect of PPARgamma on the cell cycle is due to an increase in the levels of cyclin-dependent kinase inhibitors such as p27. Again, as stated in the case for the angiotensin receptor, it must be borne in mind that in all cases, PPARgamma transcripts whether sense or antisense will be under the strict control of a Tetracycline-inducible promoter. A cDNA encoding full length human PPAR gamma isoform 1 was obtained from Laboratory of Molecular Hematopoiesis, Sloan-Kettering Institute, New York.

**Matrix Metalloprotease 9**

MMP9 (also known as Gelatinase B) is responsible for basement membrane degradation essential for tissue remodelling in a number of key developmental and growth processes. MMP9 is found to be up-regulated in many forms of cancer, as its role in basement membrane degradation facilitates malignant cell invasion and metastasis. Transformation of a cell to a full malignant phenotype however, is dependent on a number of changes in expression in genes controlling signal transduction/gene expression cascades within the cell or genes involved in regulation of the cell cycle. It is highly unlikely therefore that alteration of levels of MMP9 expression alone would confer an oncogenic phenotype upon a mammalian cell and to the best of my knowledge there is no case of this being documented in the research literature. In addition, as has been stated for both the case of the angiotensin receptor and PPARgamma above, all transcripts whether sense, antisense or dominant negative mutant will be under the strict control of a Tetracycline-inducible promoter. A cDNA encoding full length human MMP9 was obtained from Department of Immunology and Oncology, Universidad Autonoma de Madrid).
AP-1/CRE/NF-AT Reporter Constructs

These constructs consist of the bacterial beta-lactamase gene under the control of either the AP-1, CRE or NF-AT response element. The activity of each response element can be measured by assaying the activity of the protein product of the beta-lactamase gene through a simple optically based assay. It is not envisaged that introduction of the beta-lactamase gene into mammalian cells should pose a significant safety risk as mammalian cells are routinely exposed to commercially available ampicillin resistant plasmids containing the beta-lactamase gene with no adverse effects.

Upstream Regulatory Region of Human Angiotensin Type 1 and Type 2 Receptor

The reporter cassette in this virus will contain either a fragment of DNA encompassing approximately 1300bp immediately upstream of the transcriptional start site of human angiotensin II type 2 receptor (GenBank Acc No X87722) or a fragment encompassing approximately 2700bp upstream of the initiation ATG of human angiotensin II type 1 receptor (GenBank Acc No. U07144 - detailed in 'Molecular cloning and expression of the gene encoding human angiotensin II type 2 receptor' Guo et al. Biochem Biophy Res Commun. 1994 May 16; 200(3):1449-54).

Reverse Tetracycline Transcription Activator (rtTA)

The rtTA gene was obtained from the plasmid pTet-On which is commercially available from Clontech. It is necessary to make a recombinant virus which express this protein so that expression of genes under the control of the Tetracycline response element can be activated when a co-activator ligand such as Doxorubicin is added.

Evaluation of foreseeable effects

All strains of AD5 are non-oncogenic. Wild type AD5 may cause mild respiratory diseases in children. Because the recombinant virus will lack the E3 gene this may produce a virus that will evoke a stronger inflammatory response in a host, but this should also lead to more rapid clearance of the virus from a host system. Primary infection of adenovirus is thought to generate life-long immunity and it is thought that the majority of the adult population are likely to have antibodies to the wild-type virus.

Although replication has been shown to occur in the lungs of experimentally infected cotton rats administered with high doses of virus, there is no evidence that adenoviruses can naturally colonise non-human hosts and therefore pose no significant harm to animals, plants or ecosystems.

Human angiotensin receptor.

The peptide agonist angiotensin II activates this receptor to produce a variety of regulatory actions on the cardiovascular, renal, endocrine and neural systems including vasoconstriction, hypertension and aldosterone release. It is not envisaged that expression of this gene will be oncogenic or have adventitious effects in host cells at the levels of expression used. Expression of the gene will be placed under the control of a tetracycline inducible promoter system that levels of expression of the transgene can be finely reguylated by the addition of tetracycline.

Peroxisome Proliferation Activation Receptor (PPAR) Gamma

PPARgamma is a transcription factor which was initially characterised as an adipose-tissue specific facgtor that played a role in the differentiation of this tissue and regulated a number of adipose specific genes. PPAR gamma is also expressed in all of the major tissues of the vasculature including endothelial cells, vascular smooth muscle cells (VSMCs) and monocytes and macrophages. Drugs which activate PPARgamma have been shown to inhibit the proliferation and migration of VSMCs in cell culture. Current data suggests that the antiproliferative effect of PPARgamma on the cell cycle is due to an increase in the levels of cyclin-dependent kinase inhibitors such as p27. Again, as stated in the case for the angiotensin receptor, it must be borne in mind that in all cases, PPARgamma transcripts whether sense, antisense or dominant negative mutant will be under the strict control of a Tetracycline-inducible promoter.

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Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All work will be performed in tissue culture (Category II) facilities with restricted access. The flow cabinet in which work will be performed is equipped with a UV light source which enables sterilisation of the work area upon completion of work. Liquid waste will be virkon treated. Plasticware will be disinfected prior to autoclaving and then incinerated. All work surfaces will be treated with 70% ethanol and 1% Virkon spray prior to and upon completion of work. Kill rate by virkon or autoclave treatments is 100%.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
### Project Additional Information

#### Purposes of the contained use

- 

#### Recipient or parental organism

- 

#### Host/vector system

- 

#### Origin & function

- 

#### Evaluation of foreseeable effects

- 

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- 

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- 

---

**Historical Significant Changes**

- GM554/99.3

**Historical Date of Additional Info**

- 08/06/1999

**Significant Change ID**

- 559/98.2b

**Date of Significant Change**

- 28/05/2014
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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- **Animal Units**
  - L2 L3 L4 L2 L3 L4 L2 L3 L4

- **Large Scale Activities**
  - L2 L3 L4 L2 L3 L4 L2 L3 L4

- **Human Clinical Applications**
  - L2 L3 L4 L2 L3 L4

**Project Ref**  559/98.4

- **Date Ackn'd**  19/01/2001
- **CU2 Project Title**  TRANSENT AND STABLE EXPRESSION OF THE WILD TYPE AND MODIFIED MURINE PRION PROTEIN IN MAMMALIAN CELL LINES
- **Class**  Class 2
- **CultureVolClass2**  Class 2
- **CultureVolumeClass3-4**  Class 2
- **Non-GMM**  Consent Granted
- **Withdrawn**  N
- **Tick if notifying a connected programme of work**  N

**Historical Significant Changes**

**Historical Date of Additional Info**

02/03/2022  Page 9913 of 15326
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
Project Ref 559/98.5

Date Ackn'd 19/01/2001

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 559/99.1

Date Ackn’d 28/06/1999

CU2 Project Title ANALYSIS OF A NOVEL GENE THERAPY VECTOR BASED ON HERPESVIRUS

Class 2

CultureVolClass2

CultureVolumeClass3-4
Date Project Ceased
SAMIRI

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements

Withdrawn
N
Tick if notifying a connected programme of work
N

Historical Significant Changes

Historical Date of Additional Info
1/2/01

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Withdrawn | N | Tick if notifying a connected programme of work | N |
**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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02/03/2022
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**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Please enter comments on the GM safety committee on the risk assessment

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### GM Centre Number: 560

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#### Name

**ORGANON LABORATORIES LIMITED**

#### Name 2

**Department**

PHARMACOLOGY

#### Campus Estate or Research Centre

#### Road Name

#### Town

MOTHERWELL

#### District

NEWHOUSE

#### County

Lanarkshire

#### Postcode

ML1 5SH

#### Country

SCOTLAND

#### Tel Number

01698 732600

#### Fax Number

01698 736336

#### E-mail

#### HSE Division

SCOTLAND

#### Comments

GM CENTRE CLOSED 22/12/2010

#### Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
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Tick if confidential

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02/03/2022
Maximum volume of culture is 500 ml.

All liquid cultures are deactivated by addition of Virkon, or Presept according to manufacturers instructions. Following deactivation, liquid is disposed by washing down sink.

All solid waste is decontaminated by autoclaving. Laboratory autoclaves are serviced and calibrated once a year.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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<th>HSE Division</th>
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<table>
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<tr>
<td>GM562 merged with GM557 on 25/11/2004</td>
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Premises Addresses

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<th>Name</th>
<th>Department</th>
<th>Name 2</th>
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<th>Building</th>
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<th>District</th>
<th>Town</th>
<th>County</th>
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<tr>
<td>25/11/2004</td>
<td>BIORELIANCE LTD</td>
<td>STIRLING UNIVERSITY INNOVATION PARK</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
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<tr>
<td>Non-microbial</td>
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Other (please specify) | Tick if confidential

<table>
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<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
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<th>Microbiology Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref 562/01.1**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
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<tbody>
<tr>
<td>30/03/2001</td>
<td>PRODUCTION OF GENETICALLY MODIFIED HERPES SIMPLEX TYPE-1 VIRUS IN SMALL-SCALE TISSUE CULTURE ON FULLY CHARACTERISED MAMMALIAN CELLS.</td>
<td>Class 2</td>
<td>1-50 litres</td>
<td>not applicable</td>
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Date Project Ceased: 25/11/2004

Withdrawn: N

Tick if notifying a connected programme of work: N


Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
**Purposes of the contained use**
Small scale production of Herpes Simplex type-1 virus for further biologic testing with the aim of optimising production methods.

**Recipient or parental organism**
Herpes Simplex type-1 virus (strain JS-1 ECACC Accession Number 01010209)

**Host/vector system**
Herpes Simplex type-1 virus deleted for neurovirulence factor ICP34.5 and culture in BHK cells in vitro. GM-CSF, a cytokine involved in the stimulation of T-cells, is under control of the human cytomegalvirus immediate early promotor (HCMV IE).

**Origin & function**

**Evaluation of foreseeable effects**
The design of the facility and the category 2 handling and waste inactivation procedures utilised mean that release of vector outside of contained use is highly unlikely. If such release was to occur then it is unlikely that the vector will be harmful to humans, animals or plants. The vector is modified so that replication only occurs in rapidly dividing cells and not non-dividing cells. Considerable literature shows that HSV 1 deleted for ICP34.5 is non-pathogenic in animals and humans. Approximately 80% of the human population are seropositive for HSV-1. Infection is by contact and transmission by the aerosol route is not though to occur. The therapeutic gene delivered, human GM-CSF is present in normal, healthy humans. The virus is fragile, and is rapidly inactivated by desiccation, lipid solvents and mild detergents. The fact that contact is required for transmission attests to the instability of the virus outside the host.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Not applied for.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
All waste materials (eg. liquid waste, flasks and pipettes) are inactivated by autoclaving (126 degrees C for 45 minutes) before disposal to effectively give 100% kill of the GMM. The autoclaving procedure is performed according to formal written standard operating procedures (KPBT0161 and KPBT0162) and has been validated. The inactivation of waste by autoclaving is monitored/validated by the following means:
* chemical indicators (eg autoclave tape) are used on all autoclave runs and the temperature and pressure for each run is recorded and verified against requirements.
* Once a week correct autoclave operation is verified by the use of chemical indicators.
* Once every two months correct autoclave operation is verified by the use of biological indicators.

**Is an emergency plan required according to regulation 20?**
N

**If yes, tick to confirm that it is attached to this form**
N

**Tick to confirm that you have attached a risk assessment to this form**
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**
N
Standard level 2 containment procedures should be employed for this work.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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GM Centre Number: 564

Data Premises Notified (Originally) 12/07/1994

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises

Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Name

NAPIER UNIVERSITY

Department

SCHOOL OF LIFE, SPORT & SOCIAL SCIENCES

Campus Estate or Research Centre

Building

Road Name SIGHTHILL COURT

District

Town EDINBURGH

County RENFREWSHIRE

Postcode EH11 4BN

Country SCOTLAND

Tel Number 0131 455 2235

Fax Number 0131 455 2291

E-mail

HSE Division SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>SIGHTHILL COURT</td>
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<td>RENFREWSHIRE</td>
<td>EH11 4BN</td>
<td>SCOTLAND</td>
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### Premises Conditions

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</tbody>
</table>

02/03/2022  Page 9931 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity:

- Plates and broths of GMMO are placed in the autoclave (120 degrees C for 15 mins).
- Any paper waste etc. is bagged and autoclaved as normal.
- Immobilised films and 96 well plates (liquid culture) are completely submerged in a strong (10%) solution of Hibitane and left for at least 24 hours to kill the bacteria.
- The 96 well plates are then emptied and put into general waste.
- Films are removed still attached to the glass slide and placed in a bag for autoclaving and disposal whilst the containers are washed and recycled.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
**Project Additional Information**

### Purposes of the contained use

- The lux operon will be delivered to the recipient chromosome by mating, and the recipients will be used as bioluminescent biosensors for toxicity evaluation or pollutant detection.

### Recipient or parental organism

- Recipient organisms are isolates from industrial wastewater treatment plants, and clean and contaminated soils.

### Host/vector system

- **pUTmini-Tn5 luxCDABEKm2**: Carries an unpromoted luxCDABE operon from the terrestrial bacterium Photorhabdus luminescens, located within the 1 and O elements of a disarmed min-Tn5 transposon, together with a Km resistance gene for insertional selection into the recipient chromosome. The disarmed vector is maintained in E. coli S17/1pir. Transfer is via conjugation.

### Origin & function

- **pUTmini-Tn5 luxCDABEKm2**: Carries an unpromoted luxCDABE operon from the terrestrial bacterium Photorhabdus luminescens. The vector is a suicide vector as it needs the R6K-specified p protein for replication. Thus, it has to be propagated in E. coli lpir strains, and the recipients will be lpir … negative. The pUT plasmid carries the tnp* gene which allows the transposon to jump from the plasmid to the chromosome of the recipient, but as the transposon itself is disarmed, and the recipients will be lpir … negative, the plasmid cannot replicate in the recipient and the kanamycin selection will select strains in which the transposon has integrated with the chromosome.

- An alternative is the use of the transposome technology of Epicentre (Goryshin & Reznikoff, 1998), which would involve the introduction of lux genes to the transposon, and electroporation of the transposome system. This contains the Tn903 derived kanamycin resistance gene. The introduced DNA is linear and non-replicative.


### Evaluation of foreseeable effects

- It is possible that the insertion of the DNA in this random mutagenesis strategy could alter the expression of flanking sequences either up- or down-regulating expression and it is a theoretical possibility that such an insertion could result in the increased expression of a virulence factor. However, such an insertion is unlikely to generate a mutant of increased virulence since pathogenicity is multifactorial.

- Kanamycin resistance…... Kanamycin is used in the treatment E. coli, Proteus (both indole-positive and indole-negative). Enterobacter aerogenes, Klebsiella pneumoniae, Serratia marcescens, Acinetobacter species. However, chromosomal integration reduced the risk of transfer to negligible.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- **N/A**

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All class 2 waste (liquid and solid) will be autoclaved - 100% kill. A brownes tube is included in each load to indicate correct operation ensuring a temperature of 121°C for 15 min. The autoclaves are serviced annually.

<table>
<thead>
<tr>
<th>Project Containment</th>
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<tbody>
<tr>
<td><strong>Laboratory Activities</strong></td>
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**Project Ref** 564/08.1

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<td>20/05/2008</td>
<td>The use of recombinant Salmonella to examine gene function and expression.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM: Not Applicable</td>
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<th>Class CultureVolume</th>
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<th>Tick if notifying a connected programme of work</th>
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<tbody>
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Historical Significant Changes

Historical Date of Additional Info
The objectives of this project are to understand the biology of Salmonella spp. and gain insights into virulence and disease pathogenesis to identify potential antimicrobial targets. This will involve cloning, expression and disruption of Salmonella genes. This research will be conducted using plasmids in disabled E. coli K12 hosts, and also reintroducing cloned DNA sequences back into strains of Salmonella.

Escherichia coli K12 strains (md. DH5alpha, JM101, JM1O & S17-1 lambda-pir), in the ACGM 2000 disabled hosts category, part 2A, annex II.

Well characterised attenuated Salmonella: S. Typhimurium (5L3261) attenuated by lesions in genes of the aromatic amino acid biosynthetic pathway. This strain carries the same attenuating mutations as the strains listed in the ACGM 2000, part 2A, annex II as disabled hosts. The only wild type hosts to be used are S. Typhimurium SL1 344 & WG49 which have both been used extensively and are well-characterised in the literature.

Cloning & expression vectors: All derived from those listed in the ACGM 2000, part 2A, annex II as ‘nonimobilisable’ (eg pUC series, pGEM series & derivatives) or ‘mobilisation defective’ (pBR322 & derivatives).

Only DNA sequences constituting genes or parts of genes from Salmonella or E. coli that encode non-toxic products of known function will be inserted into the vectors for introduction into Salmonella. These products will contribute to basic cellular physiology such as essential metal ion homeostasis (including metal-transporting P-type ATPases and metal-sensing proteins). Introduced DNA will be expressed either individually as full-length proteins or sub-regions, and/or as fusions to other genes (within the vector) encoding non-toxic products, including the reporter genes lacZ (encoding beta-galactosidase), gfp (encoding green fluorescent protein from jelly fish), luxCDABE (encoding luciferase from Vibrio fischeri).

All sequences will be well characterised prior to introduction into Salmonella strains and will be generated by PCR using specific primers designed to amplify known DNA sequences (no random sequences will be introduced).

Introduced DNA sequences will either (i) remain on self-replicating plasmids to drive reporter gene expression or for expression of genes from their natural promoter or from vector promoters, or (ii) be integrated into the genome to generate gene disruption mutants of Salmonella.

Human health:
The attenuated Salmonella strains employed are specifically designed to be given as oral vaccines. They can survive in and colonise the host gut sufficiently to induce an immune response however the ability to colonise the host is very dose dependent, and requires large numbers. There are no recorded instances of aro mutants spreading between hosts. The wild-type S. Typhimurium strains to be used are only likely to cause food poisoning if ingested in a large dose. These strains have been safely used for 20 years and the likelihood of hazard can therefore be considered low. We will only introduce very short (<4 kb) DNA sequences into Salmonella using well characterised harmless vectors. Introduced DNA will be well defined genes (or parts of genes) that encode non-toxic products that contribute to basic physiology (such as the sensing and transport of essential metal ions).

Environment:
The disabled host strains will not replicate in the environment. Infectious doses for man and animals are high and
the probability of accidental infection low. The host strains are not known to be infectious for plants. The wild-type S Typhimurium strains may be able to replicate in the environment. The chance of transfer of the vectors to other organisms in the environment can be considered low. It is difficult to envisage how any of the genetic manipulations proposed in this project could confer a harmful phenotype or competitive advantage to the host strains. The environmental hazards associated with the project are therefore considered no greater than those associated with handling non-GM Salmonella. The project involves small-scale work for research purposes and all contaminated material will be completely inactivated by autoclaving prior to disposal. In the highly unlikely event of release into the environment no risks are envisaged.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste materials (bacterial liquid cultures/agar plates) will be completely inactivated (100% kill) by autoclaving: All autoclave runs are validated by monitoring with a chart recorder and the autoclaves are validated annually by thermocouple testing. All solid waste will subsequently be incinerated.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The risk assessment has been approved by Napier University School of Life Sciences GM Safety Committee.

Project Containment

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<tr>
<td>L4</td>
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Animal Units

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Large Scale Activities

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<td>L4</td>
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Human Clinical Applications

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<tr>
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</table>

Project Ref  564/09.1

02/03/2022  Page 9936 of 15326
The objectives of this project are to understand the biology of L. monocytogenes and gain insights into virulence and disease pathogenesis to identify potential antimicrobial targets. This will involve cloning, expression and disruption of Listeria genes. This research will be conducted using plasmids in disabled E. coli K12 hosts, and also re-introducing cloned DNA sequences back into strains of L. monocytogenes.

Recipient or parental organism

Escherichia coli K12 strains (incl. DH5alpha, JM101, JM109 & S17-1 lambda-pir), in the ACGM 2000 disabled hosts category, part 2A, annex II. The L. monocytogenes strain EGD-e has been used extensively by a number of research groups and is well-characterised in the literature.

Host/vector system

Cloning & expression vectors: All derived from those listed in the ACGM 2000, part 2A, annex II as 'non-mobilisable' (eg pUC series, pGEM series & derivatives) or ‘mobilisation defective’ (pBR322 & derivatives). The Gram positive/negative shuttle vectors pAUL-A and pUNK1 possess tempertaure sensitive origins of replication to facilitate insertion into the L. monocytogenes chromosome.

Origin & function

Only DNA sequences constituting genes or parts of genes from L. monocytogenes that encode non-toxic products of known function will be inserted into the vectors for introduction into L. monocytogenes. These products will contribute to basic cellular physiology such as essential metal ion homeostasis (including metal-transporting P-type ATPases and metal-sensing proteins) and vitamin B12 synthesis. Introduced DNA will be expressed either individually as full-length proteins or sub-regions, and/or as fusions to other genes (within the vector) encoding non-toxic products, including the reporter genes lacZ (encoding beta-galactosidase), gfp (encoding green fluorescent protein from jelly fish), luxCDABE (encoding luciferase from Vibrio fischeri).

All sequences will be well characterised prior to introduction into L. monocytogenes and will be generated by PCR using specific primers designed to amplify known DNA sequences (no random sequences will be introduced).
Introduced DNA sequences will either (i) remain on self-replicating plasmids to drive reporter gene expression or for expression of genes from their natural promoter or from vector promoters, or (ii) be integrated into the genome to generate gene disruption mutants of L. monocytogenes. Plasmid vectors used in L. monocytogenes do not encode resistance to ampicillin or penicillin which are often the therapeutic antibiotics of choice for L. monocytogenes infection.

**Evaluation of foreseeable effects**

**Human health:**
L. monocytogenes is capable of causing infection in humans although infection of healthy individuals is rare. The infectious dose remains unknown and may be lower in immunocompromised individuals. Thirteen serovars are associated with L. monocytogenes however serovar 4b is more commonly associated with invasive disease. L. monocytogenes EGD-e to be employed in this work is a serovar 1/2a strain.

Strain EGD-e is the sequenced reference strain and has been used in laboratories worldwide safely used for over 20 years and the likelihood of hazard can therefore be considered low. We will only introduce very short (<4 kb) DNA sequences into L. monocytogenes using well characterised harmless vectors. Introduced DNA will be well defined genes (or parts of genes) that encode non-toxic products that contribute to basic physiology (such as the sensing and transport of essential metal ions).

For reasons outlined above, the likelihood of harm in the event of exposure would be no greater than that with non-GM L. monocytogenes and can be considered negligible.

**Environment:**
L. monocytogenes is not a respiratory pathogen hence accidental infection via aerosol contamination is considered to be negligible. The host strains are not known to be infectious for plants. The wild-type L. monocytogenes strains may be able to replicate in the environment. The chance of transfer of the vectors to other organisms in the environment can be considered low. It is difficult to envisage how any of the genetic manipulations proposed in this project could confer a harmful phenotype or competitive advantage to the host strains. The environmental hazards associated with the project are therefore considered no greater than those associated with handling non-GM L. monocytogenes. The project involves small-scale work for research purposes and all contaminated material will be completely inactivated by autoclaving prior to disposal. In the highly unlikely event of release into the environment no risks are envisaged.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All contaminated waste materials (bacterial liquid cultures/agar plates) will be completely inactivated (100% kill) by autoclaving: All autoclave runs are validated by monitoring with a chart recorder and the autoclaves are validated annually by thermocouple testing. All solid waste will subsequently be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The risk assessment has been approved by the School of Life Sciences GM Safety Committee

### Project Containment

<table>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**GM Centre Number: 565**

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**Name**

SHEFFIELD HALLAM UNIVERSITY

**Name 2**

**Department**

BIOMEDICAL SCIENCES

**Campus Estate or Research Centre**

CITY CAMPUS

**Road Name**

38-40 HOWARD STREET

**Town**

SHEFFIELD

**County**

YORKSHIRE

**Postcode**

S1 1WB

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ENGLAND

**Tel Number**

0114 225 5555

**Fax Number**

0114 225 3066

**E-mail**

**HSE Division**

YORKSHIRE AND NORTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
**Premises Addresses**

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<td>DEPARTMENT OF SCIENCE</td>
<td></td>
<td>38-40 HOWARD STREET</td>
<td>SHEFFIELD</td>
<td>CHESHIRE</td>
<td>S1 1WB</td>
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology | Parasitology | Transgenic | Microbiology | Research
---|---|---|---|---

Virology | Transgenic | Transgenic | Gene Therapy
---|---|---|---

Animals | Birds | Fish
---|---|---
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid culture will be inactivated by adding one half of a bleach tablet per container. Pipette tips, Solid (agar) cultures and any other solid waste will be autoclaved. The autoclave conditions will be set to 130 degrees C for 30 minutes. A printout from the autoclave run will be kept as evidence of autoclave conditions during the run.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 565/09.1

Evaluation of the effects of lentiviral vectors encoding shRNA for knockdown of ADAM17 on primary murine astrocytes and microglia ...........

Date Ackn'd 16/01/2009

Date Project Ceased

Withdrawn N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Tick if notifying a connected programme of work N

Class 2 CultureVol

< 1 Litre Class3-4
Project Additional Information

Purposes of the contained use

ADAM17 (a disintegrin and metalloproteinase) is a sheddase, a membrane bound enzyme involved in the cleavage of proteins from cell surfaces. Some of the proteins shed, such as TNF, are implicated in the disease process of multiple sclerosis (MS). Our work has also shown that ADAM17 is increased in active MS lesions suggesting a role in MS (Plumb et al (2006) Multiple Sclerosis 12, 375-385).

The purpose of this project is to determine the functional role of ADAM17 in CR-EAE, an animal model of MS, to gain further insight into its role in MS pathogenesis and as a potential therapeutic target. The project involves using lentiviral vectors expressing shRNA targeted against ADAM17 to knockdown ADAM17. The lentiviral vectors will be developed in the laboratory of Prof Mimoun Azzouz at the University of Sheffield. The effectiveness of the developed vectors to knockdown ADAM17 at the mRNA and protein level will be studied in the Biomedical Research Centre (BMRC) at Sheffield Hallam University, initially in a murine brain endothelial cell line, bEND3, then in primary murine astrocytes and microglia.

The vector which produces maximal knockdown will be used to treat mice with CR-EAE to see if their symptoms are ameliorated. This animal work will be done in the laboratory of Dr Chris Bolton, the William Harvey Research Institute, St Bartholomews Hospital London and he will obtain the relevant permissions for this. Tissues from these mice will be analysed for ADAM17 knockdown and inflammatory changes at the BMRC, Sheffield Hallam University.

Recipient or parental organism

Lentiviral vectors produced in Prof Azzouz's laboratory will be used to infect the bEND3 cell line and primary murine CNS derived cells including astrocytes and microglia. In vitro studies will be carried out at containment level 2. Snap frozen tissues from Biozzi mice with CR-EAE, treated with lentiviral vectors, will be analysed at the BMRC, Sheffield Hallam University.

Host/vector system

We intend to use both primate and non primate derived replication defective lentiviral vectors. We will be testing HIV-1 based vectors, the self inactivating (SIN) HIV-based lentiviral system which will be provided by Dr Nicole Deglon (France). We also propose to use Equine infectious anaemia virus (EIAV)-based lentiviral vectors which will be provided by Oxford BioMedica Ltd (Oxford, UK)

HIV-derived lentiviral vectors:

We propose to use the self inactivating (SIN) HIV-based lentiviral system which will be provided by Dr Nicole Deglon under MTA from Commissariat a L'Energie Atomique (CEA) CNRS URA2210, Service Hospitalier Frederic Joliot and ImaGene Program Orsay Cedex, France. Vector-based on lentiviruses have been refined to a very high safety and efficiency levels. Any system used will incorporate the safety features outlined below.

SIN-lentiviral vector system (from Nicole Deglon)

Viral vectors will be generated with a four-plasmid system ("fourth generation" HIV-based vectors (Zufferey et al. (1997) Nat Biotechnol 17:871-875; Hottinger et al. (2000) J Neurosci 20:5587-5593). The four plasmids are as follows:

1) SIN-cPPT-PGK-W, gene transfer vector.
2)pCMVΔR-8.92, packaging construct
3)pRSV-Rev, the accessory protein rev plasmid
4) pMD.G, Vesicular stomatitis virus-G envelope protein. It may be necessary to use other envelope proteins such as rabies-G glycoprotein to achieve specific tropism.

Features that prevent generation of live virus, recombination with endogenous retroviruses and viable HIV.

1) Production of the viral vectors form the promoter cell line requires the transfection of 4 plasmids (SIN-CpPT-PGK-W, pCMVΔR-8.92, pRSV-Rev, pMD.G) in a transient transfection. All four plasmids have been designed, through lack of common sequences, to avoid the possibility of recombination with each other. The plasmids expressing the structural and packaging genes are not packaged with the produced virus, since none of them contain LTRs or the packaging RNA sequence. Thus, replication-competent viruses cannot be produced, nor can the packaging.

02/03/2022
Vector genome SIN-cPPT-PGK-WPRE: A 400-bp fragment of the U3 region of the 3' LTR was deleted to obtain self-inactivating (SIN) transfer vector. The mouse phosphoglycerate kinase 1 (PGK) promoter was used as internal promoter. This plasmid was further modified by insertion of the posttranscriptional 1cis-acting regulatory element WHV.

pRSV-Rev is rev cDNA-expressing plasmid in which the joined second and third exons of HIV-1 rev are under the transcriptional control of the RSV U3 promoter. The HIV promoter has been deleted and replaced by the RSV U3 promoter for safety.

pCMVAR8:92 (packaging construct): The Vpu, Vpr, Nef and Rev coding sequences, required for viral packaging etc have been deleted. The accessory protein Rev, which is required for the transport of RNA from the nucleus to the cytoplasm, is expressed on a separate plasmid. Expression of gag/pol is under the control of the CMV promoter rather than the viral promoter for safety.

EIAV- based lentiviral vector system (generated by Oxford BioMedica Ltd)

The EIAV minimal vector system

Equine infectious anaemia virus (EIAV) is a non-primate lentivirus that causes a self-limiting, lifelong but rarely fatal infection of all Equidae, and is a world-wide disease of horses, most prevalent in warmer climates. The aim when designing an EIAV-based lentiviral vector system is three-fold. The first is to minimize the amount of viral sequence in the transfer vector, thus increasing the capacity for transgenes and associated regulatory elements. The second is to eliminate the expression of viral sequences in the target cells. Viral genes may elicit immune responses that result in destruction of transduced cells and may have undesirable effects per se. The third is to express, in the production system, only those viral proteins required for efficient production of transduction competent vector. All three components, vector genome, Gag/Pol and envelope necessary to generate EIAV-base lentiviral system are being optimized by Oxford BioMedica Lts and the current status is summerised below.

The safety profile of the EIAV vector system

EIAV vector system was modified with safety in mind making use of the strategies used in the murine leukaemia virus (MLV) vector field to avoid the production of a replication competent retrovirus (RCR). The most important features for safety are:

The partition of the components of the vector system in three independent expression cassettes: vector genome, gag/pol and envelope plasmids.

The minimal sequence homology between the vector, gag/pol and env components.

The use of human cell lines with low levels of engogenous retroviral sequences as the basis for producer cell lines.

Some additional features that will reduce the potential for recombinant formation and the potential pathological consequences arising from the presence of such recombinants in the EIAV vector system are: These features provide a high safety margin.

The use of a heterologous envelope component. Recombination events can not generate wild type EIAV;

The use of vectors which have SIN or conditional SIN configurations and therefore are almost transcriptionally silent in transduced cells;

The elimination of accessory proteins from the system. Tat, Rev and S2 can be eliminated from the system without affecting transduction efficiency; however, Rev is maintained in the producer system since it improves titre by increasing cytoplasmic levels of vector RNA. Expression of Rev is from an independent expression cassette, in which rev is 'codon-optimised' to minimize the chances of its involvement in recombination reactions.

Transcription of the vector genome is driven by the human CMV enhancer/promoter fused to the R region of EIAV so that the first base of the transcript is the same as that formed as a result of transcription from the EIAV U3 region. This configuration allows high titres to be obtained in the absence of Tat protein which would normally serve this function.

The codon-optimised gag/pol gene (pESYNGP) is now being utilized for construction of EIAV packaging and producer cell lines. The coden-optimisation process increases the safety of the system in two ways. First, gag/pol/mRNA is unlikely to be packaged as efficiently as the wild type gag/pol RNA. Secondly, due to the lack of significant homology with the vector in the region of the packaging signal, it is unlikely to be involved in recombination reactions which might result in generation of an RCR, even if it was incorporated into the vector particle. The optimization process makes the expression of gag/pol Rev-independent, possibly as a result of removing RNA instability sequences.

Vectors will be produced in the laboratory of Prof Azzouz who has the appropriate permissions. Viral stocks will be received as aliquots in PBS which are stored frozen at -70C.

Origin & function

Vectors will express shRNA targeting the expression of ADAM17 or a missence sequence as a control. DNA sites for RNAi targeting will be determined using online algorithms (Dharmacon). The viral vector will also contain the RNA Pol III H-1 promoter, derived from human embryonic kidney 293T cell line and cloned into the lentiviral
vector. They will also contain a reporter gene for green fluorescent protein (GFP) or luciferase.
The vectors will be used to knockdown ADAM17 in murine bEND3 cells and primary murine CNS-derived cells in vitro. They will also be used in vivo to treat Bioxxi mice with CR-EAE in Dr Bolton's laboratory in London.

Evaluation of foreseeable effects

Environmental assessment:
The replication defective viruses, as stocks or in cultured cells, culture media or tissues would be inactivated prior to disposal and given their inability to replicate, they have no significant additional environmental hazard.

Worst case scenarios:
The above protocols should minimize any risk of exposure to personnel using, or in the vicinity of, work using these lentiviral vector systems. The worst scenario would be exposure to a main stock of virus, resulting from the centrifuge failure or spillage. Risks of centrifuge failure-associated contamination (the only readily conceivable mechanism likely to generate a substantial aerosol of many infective particles) should be minimized by good microbiological practice and steps outlined in section 12. The major exposure routes would be cutaneous, mucocutaneous and respiratory. Effective infection of intact skin is unlikely as skin is a very effective barrier and would be minimized by good laboratory safety and hygiene. The viruses may infect mucocutaneous surfaces and respiratory epithelia and macrophages with which they came into contact. Again this would be minimized by strict adherence by trained personnel to proper laboratory procedures e.g. use of microbiological safety cabinet class II, masks, eyes protection and gloves will be observed. However the particles are replication defective and have many safety features preventing generation of replication competent viral particles. The vectors are replication defective hence spread from an initially infected cell is very unlikely. Some vectors to be used for this project may contain wild type or truncated forms of woodchuck post-transcriptional regulatory element (WPRE) sequences. Following the recommendation from HSE and the SACGM measures will be taken to minimize the risks when using these sequences:
- Good microbiological practice by using safety cabinet class II
- Adopt procedures that reduce the likelihood of exposure, such as wearing gloves, mask and eye protection, etc.
- Accidents would be minimized by strict adherence by well trained personnel to good standards of safety and hygiene. Professor Azzouz has successfully used this technique in the past, and will provide training for staff using lentiviral vectors.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Anti-viral disinfectants (virusolve, virkon, bleach) will always be available prior to starting work involving handling of viral particles, in identified containers easily to hand (e.g. spraygun)
- All culture will be performed using a Class 2 hood, in a designed Class 2 laboratory. All cells exposed to the virus will be handled in the category 2 hood. After infection, viral supernatant will be removed and the cells washed. Once the media has been changed on at least one further occasion (after which active viral particles should not be present), then functional assays may involve handling transfected cells for brief periods on the bench using acceptable good practice but any cells taken out of the laboratory for analysis (e.g. flow cytometry) will be fixed or rendered fully non-viable (e.g. treated with formaldehyde solutions prior to flow cytometry).
- Where large volumes of supernatants containing, or potentially containing, viral particles require centrifugation, individually sealable buckets will be used in the relevant centrifuge. The buckets and contents will be inspected before opening (in the Class 2 hood). Buckets would be cleaned appropriately using virusolve, virkon or bleach after use. Supernatants from virally-transfected cells which had been cultured for more than one media change over several days would not be regarded as being likely to contain significant numbers of active virus, but would be handled according to accepted good practice and neutralized prior to final disposal.
- Hoods will be carefully cleaned after each session in which culture with virus particles takes place and after each session, when cells that have been transduced by virus on a previous, occasion are handled.
- Viral particles and supernatants from infected cells will be handled using filtered pipettes and pipette tips.
- All murine tissues, supernatants, plastic ware and cells that have come into contact with virus particles or viral infected cells will be treated with virusolve, virkon or
bleach, at concentrations recommended by the manufacturer, for 20-30 minutes prior to discard.
- Solid waste from this project will be segregated from containment level 1 waste in appropriate leak-proof containers and inactivated on-site by autoclaving or off-site by an appropriately licensed contractor.
- Safe practice will be rigorously enforced (lab coats, eye protection, gloves, etc).
- Other lab personnel will be made aware of the use of lentiviral systems in the laboratory.

Containment management
1. Where spills occur in contained environments such as Class 2 hoods, neat virusolve, bleach or virkon will be added and left for 20-30 minutes to neutralize viral particles prior to wiping, cleansing and disposal.
2. If a small contained spill occurs outside of Class 2 hoods, management will be as above. Any spills outside of Cat 2 hood will be reported to a principal investigator, and assessment made to determine whether avoidable features are present to prevent future accidents.
3. Where aerosolized contamination is suspected to have occurred outside of Class 2 hoods (e.g. as a result of centrifuge failure), the lab will be cleaned immediately to prevent aerosol inhalation. The air conditioning system will replace the air within the room within 10 min and will serve to desiccate and dilute the hazard. A single designated worker will return to the lab after 15 min and apply appropriate disinfectant to liquid traces.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment for class 2 activity was considered by the Genetic Modification Safety Committee on 3/11/2008. This was approved subject to minor amendments which have now been made.

Project Containment

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Project Ref 565/10.1

Date Ackn'd 08/07/2010
CU2 Project Title Expression of functional recombinant peptides found naturally in scorpion venom
Class Class 2
CultureVolClass2 1-50 Litres
CultureVolumeClass3-4

Since these peptides are typically found in small quantities as part of a complex venom mixture, the ability to produce a pure peptide in sufficient quantities to enable its full characterisation is essential.

The aim of the project is to express functional recombinant peptides found naturally in scorpion venom. These peptides can be broadly divided into four groups:

i) Sodium channel toxins
ii) Potassium channel toxins
iii) Chloride channel toxins
iv) Other peptides

We propose to express these peptides in E.coli as indicated in Table 1 below:

Table 1 Vector combinations for the expression of recombinant peptides in E. coli

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<td>Non-mobilisable Vector</td>
<td>Mobilisation defective Vector</td>
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<td>Secreted protein expression</td>
<td>Secreted protein expression</td>
<td>Cyto- or periplasmic expression</td>
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<tr>
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<td>----------</td>
</tr>
<tr>
<td>i) Sodium channel toxins</td>
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<td>N</td>
</tr>
<tr>
<td>ii) Potassium channel toxins</td>
<td>Y</td>
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<td>Y</td>
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<tr>
<td>iii) Chloride channel toxins</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>iv) Other peptides</td>
<td>Y</td>
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</table>

This proposal also covers the expression of the above peptides in Pichia pastoris.

Sequences will either be amplified directly from scorpion genetic material or synthetic sequences will be generated by back-translation of known peptide sequences. These sequences will be inserted into E. coli maintenance vectors and P. pastoris expression vectors. After transformation of the host, proteins will be expressed in <5L per culture.

**Recipient or parental organism**

Non-pathogenic E. coli K-12 or B derivatives (e.g. DH5, JM109, TB1); Top 10; DH5alpha and BL21 (DE3) derivatives (e.g. NEB express, origami-2, origami B, Rosetta-gami-2, Rosetta-gami-B) or laboratory strains of yeast Pichia pastoris will be used.

E. coli K-12 or B derivatives are unable to colonise humans and are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside laboratory culture. E. coli DH5 alpha and BL21 (DE3) derivatives lack necessary pathogenic mechanisms required by strains of E. coli to cause enteric infections (Chart et al. (2000) J. Applied Microbiol. 89, 1048-1058 and can be considered broadly equivalent to E. coli K12 strains.

Laboratory strains of yeast Pichia pastoris will be used. This is a non-pathogenic disabled host which is unlikely to persist in the gut, lung or survive outside the culture media. It is not normally infectious nor able to colonise humans and the main biological characteristics are known.

**Host/vector system**

For expression in E. coli, standard commercially available, well-characterised vectors that are neither non-mobilisable (e.g. pUC series and their derivatives) or mobilisation-defective (e.g. pBR322 and its derivatives) in bacteria will be used. They will contain only selective markers that are already in routine use in standard cloning vectors. All expression systems will be induced by IPTG.

Example vectors include:

- **pMal**: E. coli plasmid cloning vectors designed for recombinant protein expression and purification using the pMal protein fusion and purification system. pMal vectors (e.g. pMAL-c5X, pMALp5X, pMALp5G, pMALp5E, pMALp2X) are derived from PBR322 (a mobilisation defective bacterial plasmid vector) and expression is inducible and directed either to the cytoplasm or the periplasm.

- **pGEX**: E. coli plasmid expression vectors designed for cytoplasmic expression of glutathione S-transferase (GST) fusion proteins. pGEX vectors (e.g. pGEX-P series, pGEX-T series, pGEX-X series, pGEX-2TK) are non-mobilisable bacterial plasmid vectors. Expression is inducible and catabolite repression possible.

- **pET SUMO**: Inducible expression vectors designed to express small ubiquitin-like modifier (SUMO) fusion proteins in the cytoplasm, pET vectors are mobilisation defective bacterial plasmid vectors.


For expression in P. pastoris, standard commercially available, non-conjugative, non-mobilisable or mobilisation defective expression vectors which utilise the AOX1 gene promoter will be employed (e.g. pPICZ series vectors, pPink series vectors). These vectors exhibit tight regulation by induction mechanisms and repression/derepression (Daly & Hearn (2005) J. Mol. Recognit 18, 119-138) for tight cytoplasmic or secreted expression of proteins in methanol containing media.

Sodium channel toxins will only be cloned using non-mobilisable vectors.
There inserted gene products will be derived from scorpion DNA/cDNA. Sequences will either be amplified directly from scorpion genetic material or synthetic sequences will be generated by back-translation of known peptide sequences. The expressed peptides will be purified and used in structure/function studies.

The gene products can be divided into four broad groups:

i) Sodium channel toxins:
ii) Potassium channel toxins:
iii) Chloride channel toxins:
iv) Other peptides:

Evaluation of foreseeable effects

Toxicity of product:
All peptides discussed below are unlikely to have significant oral toxicity because of their inability to escape the lining of the stomach. Ion channel toxins derived from venom have a high density of positive charges and are therefore extremely unlikely to cross cell membranes. As peptides, they will be susceptible to proteases in the gut.

The four groups of gene products will be considered separately:

i) Sodium channel toxins:
This group of toxins is primarily responsible for the clinical effects of scorpion venoms when individuals are stung and the venom enters the bloodstream. The most toxic scorpion venom (Leirus quinquestriatus has LD50 0.25mg/kg via subcutaneous injection (Simard & Watt (1984) J. Physiol (Paris) 79, 185-91). The most toxic sodium channel toxins have approx 10 fold higher toxicities when injected directly into the brain (e.g. LD50 10μg/kg intracebroventricular, toxin AaHI vs 25μg/kg subcutaneous toxin Cs11 (Kharrat et al (1989) European Journal of Biochemistry 181, 381-90, Martin (1987) Journal of Biological Chemistry 262, 4452-9). However, the likelihood of administering a significant dose subcutaneously during our use in the lab is extremely low. Extrapolation by weight from the subcutaneous LD50 for Cs11 in mice would suggest a subcutaneous LD50 of 1.75 mg for a 70 kg human (equivalent to 1.1L of E.coli culture or 17.5 ml Pichia pastoris culture (see below). Although the risk of colonisation is low, the toxicity of this group of toxins has influenced our restricted use of mobilisation-defective vectors for this particular class of peptides and the decision not to express these toxins in E.coli via secretory pathways (see Table 1). Native sodium channel toxins are used routinely in our laboratory.

ii) Potassium channel toxins:
Compared to sodium channel toxins these toxins are essentially non-toxic via intraperitoneal injection (LD50 30mg/kg, (Harvey & Karlsson (1980) Naun-Schmied. Arch Pharmacol. 312, 1-6)). They are only lethal via direct injection into the brain (LD50 4μg/kg (Schweitz et al (1989) Biochemistry 28, 9708-14)). The likelihood of administering these toxins directly into the brain is negligible. Native potassium channel toxins are used routinely in our laboratory.

iii) Chloride channel toxins:
Although a recombinant chloride toxin from a Chinese scorpion venom has extremely surprisingly been quoted to have a mouse LD50 of 4.3mg/kg (Fu et al (2005) Biotechnol Letts. 27, 1597-1603), the overwhelming majority of papers contradict this finding and indicate that this group of toxins are not toxic to mammals, including chlorotoxin from Leiurus quinquestriatus (Soreceanu et al (1998) Cancer Research 58, 4871-9) and chloride channel toxins from Mesobuthus tamulus (Dhawan et al (2002) FEBS Letts 528, 261-66; Wudyagiri et al (2001) BMC Biochemistry 2, 16 (Epub)). Indeed, synthetic chlorotoxin is now in clinical trials (Mumelak et al (2006) J. Clin. Oncol. 24, 3644-3650). As with the sodium channel toxins, the likelihood of administering a significant dose subcutaneously during our use in the lab is extremely low. Native chloride channel toxins are used routinely in our laboratory.

iv) Other peptides:
Molecules from scorpion venoms are primarily characterised based on toxicity assays. We can therefore confidently predict that the molecules with the highest toxicity that are present in our venoms of interest have been characterised (as sodium channel toxins). Native scorpion venom is used routinely in our laboratory (Newton et al (2007) Rapid Commun. Mass Spectrom. 21, 3467-76).
Expression levels of scorpion venom peptides obtained previously are typically 1-10 mg/L culture (1mg/L for a chloride channel toxin expressed in P. pastoris (Trung et al (2006) BMC Biotechnology 6, 18); 1.6mg/L for a sodium channel toxin expressed in E.coli (Fu et al (2004) Protein Expression and Purification 38, 45-50); 8-10mg/L for a potassium channel toxin expressed in Saccharomyces cerevisiae (Wu et al (2002) Biochemistry 41, 2844-2849); 5mg/L for a sodium channel toxin expressed in Saccharomyces cerevisiae (Shao et al (1999) Protein Expression and Purification 17, 358-365), although expression of 100-120mg/L culture has been reported for a sodium channel toxin expressed in P. pastori (Wang et al (2006) Biotechnol Lett 28, 1767-1772). These expression levels suggest that accidental intoxication to the level of the lethal dose via these cultures is highly unlikely.

It is difficult to envisage how the cloned sequences described above could enhance the ability of the disabled E. coli K12 strains or the yeast strains to colonise humans or otherwise cause disease.

The specific function of any individual scorpion toxin is highly dependent on its sequence and these sequences have evolved and been selected for during the evolutionary process. It is therefore probable that this sequence has been optimised for biological activity and any unintentional sequence alteration is likely to reduce activity.

Environmental assessment:
Use of disabled E. coli or yeast strains which have very limited ability to survive outside the laboratory, with non-mobilisable or mobilisation-defective vectors, will ensure that in the very unlikely event of escape the GMM will be unable to survive, disseminate with and/or displace other organisms and the vectors will be unlikely to transfer to related microorganisms.

The GMM will be unable to colonise or otherwise cause harm to animals. The expressed product is unlikely to cause a problem to human health or harm other mammals. The greatest risk will be posed by subcutaneous injection of a sodium channel toxin. The published expression levels for similar peptides suggest that the likelihood of sufficient toxin entering the blood stream to cause harm to human health is extremely low.

Some scorpion toxins exhibit toxicity to invertebrates. An insect-specific chloride channel toxin from Mesobuthus tamulus is toxic toward insect larvae (LD50 5μh/100mg, Dhawan et al (2002) FEBS Letts 528, 261-66) However, these toxins are "normally effective when injected into insects and are typically ineffective when administered orally because they are unable to access the central nervous system or other target sites where they are active" (Fitches et al (2010) Pest Manag Sci. 66, 74-83). A second insect-specific chloride channel toxin from Mesobuthus tamulus has been characterised (Wudyagiri et al (2001) BMC Biochemistry 2, 16 (Epub)) and although it has been reported to account for almost all the insect lethality of the crude venom toward some invertebrates when directly injected, Fitches et al (2010) (Pest Manag Sci. 66, 74-83) demonstrated that this toxin exhibits no oral activity in these species. The most toxic insect-selective scorpion venom toxins is the sodium channel toxin AaIT (LD50 of 10μg/100 mg body weight (Zlotkin et al (1992) Arch Insect Biochem Physiol. 21, 41-52), however, the toxin is inaccessible by oral and topical applications (Zlotkin et al (2000) Biochimie 82, 869-81). Since all insect toxicity is obtained by direct injection of toxins, it is extremely unlikely that this situation will be replicated outside the laboratory.

There is no evidence to suggest that scorpion venom proteins are able to cause harm to plants. Use of only disabled host strains which have very limited ability to survive outside the laboratory, combined with cloned sequences that will not express a product that could have toxic or other detrimental effects if delivered to plants, will ensure that in the very unlikely event of escape, the GMM will be unable to cause harm to plants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

• All manipulations involving GMC/O will be undertaken within a level 2 containment laboratory where access is restricted to authorised personnel/students using good laboratory practice. Only staff/students trained in the safety aspect of this work will be involved.
• Workers will wear dedicated laboratory coats that will remain in the laboratory at all times These coats will be laundered "in house".
• All GM solid waste material will be disposed of on-site by autoclaving or by incineration off-site by a contractor licensed for disposal of GM waste. Before inactivation, solid GM waste will be bagged and then stored or transported in leak-proof containers. Liquid GMO waste will be inactivated by adding bleach tablets according to the manufacturer's instructions to give an available chlorine concentration of at least 1,000 ppm and left to stand for 30 minutes before disposal; alternatively, liquid GM waste will be sterilised by autoclaving. GMC/O waste is autoclaved on a sterilising cycle for which a printed record of the conditions is logged after each run. Twice yearly, a temperature indicator strip, a Browne's tube and a spore tube are incorporated into the autoclave run to comply with HSE regulations. Following this run, the spore tube is incubated at 55°C for 48 hours with a non-autoclaved control tube. The autoclaved tube should remain yellow in colour indicating a 100% kill rate and the control tube will turn purple indicating spore growth.
• In the event of a significant spillage of GMC/O the area concerned will be flooded with disinfectant and left for at least 10 minutes. The spillage will then be mopped up using disposable paper towels which will be placed into and autoclave bag for sterilisation by autoclaving. The incident will be recorded on the GM accident notification form AND in the departmental accident book and the Faculty Biological Safety Officer will be notified.

The risk assessment for class 2 activity was considered by the genetic modification safety committee on 05/02/10. This was approved subject to amendments which have now been made.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Project Ref 565/15.1

Date Ackn'd 21/10/2015

CU2 Project Title Periodontal pathogens and interactions with human epithelial cells

Class 2 CultureVolClass2 1-50 Litres

Non-GMM Consent Granted

Date Project 02/03/2022

Page 9951 of 15326
The study aims at elucidating the mechanisms underlying cellular responses triggered in oral epithelial cells following infection with the periodontal pathogens, Tannerella forsythia and/or Porphyromonas gingivalis.

The methods used in this study include growth of bacteria under anaerobic conditions, culture of immortalised human cell lines, infection of these human cell lines with the pathogens, protein extraction and analysis using a range of techniques (including western blotting, ELISA, protein assays/mass spectrometry) as well as DNA and total RNA (RNA, miRNA, LnRNAs) extraction for analysis (e.g. PCR and qPCR). Infected cells will also be analysed by immunofluorescence methods (e.g. immunofluorescence microscopy, confocal microscopy or flow cytometry).

The studies described here do not aim to generate or introduce new genetic modification within the pathogens and cell lines used, rather they will involve the use of genetically modified oral anaerobic bacteria: Porphyromonas gingivalis and Tannerella forsythia that have been generated by collaborators in Sheffield and Buffalo, NY. All of these mutations reduce the virulence of the pathogens as they lack specific virulence factors.

Recipient or parental organism

(1) Tannerella forsythia is a Gram-negative, anaerobic bacterium, which is frequently isolated from patients with periodontal disease. It was formerly known as Bacteroides forsythus and in some literature is referred to as T. forsythis. It has extremely fastidious growth requirements and requires N-acetyl neuramic acid supplementation in media to enable growth. It falls into Hazard Group 2 with other Bacteroides spp. The strains used in this study will be the type strain Tannerella forsythia ATCC 43037 and the following mutants:
   - Tannerella forsythia nanH (lacking sialidase)
   - Tannerella forsythia ed1 (aberrant surface glycosylation)

(2) Porphyromonas gingivalis is a Gram-negative, anaerobic black-pigmented bacterium that is frequently isolated from patients with periodontal disease. Hazard Group 2. The strains used in this study will be NCTC 11834, W50 and 381 containing null mutation.
   - Porphyromonas gingivalis rgpA, rgpB, kgp (containing null mutations in arginine and lysine proteases)
   - Porphyromonas gingivalis PG0352 (lacking sialidase)
   - Porphyromonas gingivalis ompA (lacking a surface protein)

Host/vector system
On occasion, complemented Porphyromonas strains transformed with the plasmid pT-COW will be used. It is a non-transferrable shuttle vector for expression in E.coli and Porphyromonas and carries a tetracycline resistance cassette- again these strains will be generated by our collaborators at TuOS (The University of Sheffield).

**Origin & function**

The bacterial pathogens Porphyromonas gingivalis and Tannerella forsythia will be used to infect oral epithelial cell lines (H357 or OK-F6 cells; see below) to study host-cell interactions. These will include determining which cellular pathways are being triggered following infection (e.g. apoptosis, autophagy) as well as validating proteins which were found to be dysregulated in previous proteomic studies.

**Evaluation of foreseeable effects**

Porphyromonas gingivalis and Tannerella forsythia can cause periodontal disease in certain individuals, but the site of infection is limited to periodontal pockets. The organisms are strictly anaerobic, nutritionally fastidious and unable to survive for longer than a few hours in air. They are ACDP Hazard Group 2 biological agents.

Complemented Porphyromonas strains transformed with the narrow host-range plasmid pTCOW will be used. It is a non-transferrable shuttle vector for expression in E.coli and Porphyromonas and carries a tetracycline resistance cassette- again these strains will be generated by our collaborators at TuOS.

Gene products inserted in pTCOW will be complementation constructs of the genes that have been deleted, they are expressed from natural promoters and only restore gene expression to wild-type levels, thus not increasing virulence of strains.

The strains will either have reduced (mutant strains) or wild-type traits (complementations), in no cases do we intend to increase pathogenic potential.

Oral anaerobes are strictly anaerobic, nutritionally fastidious organisms that do not survive well in the environment and potential hazards of sequences within the GMM being transferred to related micro-organisms are negligible.

The likelihood that the GMM could cause harm to human health in the event of exposure is negligible. It is not likely to be released under the requirements of the containment level to protect human health.

These pathogens are strict anaerobes with fastidious growth requirements (such as Hemin, cysteine and menadione). Whilst both P. gingivalis and T. forsythia have been linked to the oral disease of periodontitis, this condition has a complex, multi-factorial aetiology and acquisition of single species is not thought to predispose to disease. Additionally, host response, risk factors (including smoking, immune health) as well as the oral microbial community present all contribute to disease. As the pathogens are strict anaerobes, they are unlikely to survive in the aerobic atmosphere of the buccal cavity; they normally survive in periodontal pockets.

The likelihood that the GMM will be released into the environment under the requirements of the containment level to protect human health is negligible. The consequences of GMM dissemination in the environment are negligible since the aerobic survival time of the organisms is short. Due to their short survival time, the GMM are not likely to displace other organisms. These pathogens are also strict anaerobes with fastidious growth requirements (such as hemin, cysteine and menadione).

As the pathogens will not survive in the environment, they will not cause any harm to animals.

No harm will be caused to plants by these strictly anaerobic and nutritiouslly fastidious pathogens, so will not survive in the environment.

They are unlikely to cause harm to other cells/organisms.

Risk of transfer of genetic material between the GMC/O and other cells/organisms are negligible.

These pathogens have been in use for a while with no known risks of phenotypic or genetic instability.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- All manipulations involving GMC/O will be undertaken within a level 2 containment laboratory where access is restricted to authorised personnel/students only using good experimental practice and hygiene. Only staff/students trained in the safety aspect of this work will be involved.
- Workers will wear dedicated laboratory coats that will remain in the laboratory at all times. These coats will be laundered "in house."

All GM solid waste material will be disposed of off-site by a contractor licensed for disposal of GM waste. Solid GM waste is stored and transported in leak-proof containers (biohazardous waste bins) before being taken to the outside store holding area ready for collection by the contractor. Liquid GMO waste will be inactivated by adding bleach tablets according to the manufacturer’s instructions to give an available chlorine concentration of at least 1000 ppm and left to stand for 30 minutes before disposal; alternatively, liquid GM waste will be sterilised by autoclaving. All microbial waste is autoclaved on a sterilising cycle for which a printed record of the conditions is logged after each run. Twice yearly, a temperature indicator strip, a Browne’s tube and a spore tube are incorporated into the autoclave run to comply with HSE regulations. Following this run, the spore tube is incubated at 55°C for 48 hours with a non-autoclaved control tube. The autoclaved tube should remain yellow in colour indicating a 100% kill rate and the control tube will turn purple indicating spore growth.

Outline any additional measures regarding disposal route:
- Liquid Waste from GM mammalian cell culture should only contain culture media plus expressed secreted proteins and possibly cell waste.

Outline any additional control measures:
- Personal protective equipment in the form of gloves and lab coats should be worn at all times.

Liquid Waste:
Cell culture liquid waste under 50 ml is disposed of in a sealed falcon tube and then placed directly into yellow clinical waste bins. Waste over 50 ml is emptied into an old media bottle containing half a pre-sept tablet. This is left overnight and then emptied down the sink and the bottle disposed of in the black bin.

Vacuum pumps can be used but these must be cleaned and maintained by the user. There is only one pump available for use.

Any flasks that are infected with fungi/bacteria or mycoplasma are sealed in a bag and placed in an almost full yellow bin, so that sealing of the yellow bin and removal from the lab is imminent.

Solid waste:
Any solid waste will be disposed of in the yellow clinical waste bin along with used pipettes, tips, gloves and other consumable items.
- Liquid waste can be directly aspirated into the waste reservoir from culture dishes or flasks using the vacuum pump.
- Liquid waste collected in a beaker should be carefully aspirated into the waste reservoir using the vacuum pump.
- Do not fill the reservoir past the marked fill line.
- The liquid waste must be inactivated for 30 minutes using bleach tablets according to standard operating procedures for disposal of cell culture waste before disposal down the sink in the Tissue Preparation room.

If the reservoir is full to the fill line, excess waste must be inactivated with presept (bleach tablets) for 30 minutes and disposed of down the sink in the Tissue Preparation room.

Outline any additional control measures:

Personal protective equipment in the form of gloves and lab coats should be worn at all times.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
This project has been reviewed by the genetic modification safety committee, and was approved following minor amendments concerning clarification of specific details of the host organisms and likelihood of interacting with humans.

Project Containment

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<th>Growth Rooms</th>
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Animal Units

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**Name**

COBRA BIOLOGICS LIMITED

**Campus Estate or Research Centre**

THE SCIENCE PARK

**Town**

KEELE

**District**

STAFFORDSHIRE

**County**

ST5 5SP

**Country**

ENGLAND

**Tel Number**

01782 714181

**Fax Number**

01782 714167

**HSE Division**

MIDLANDS

**Comments**

FORMERLY COBRA THERAPEUTICS LTD UP TO 25/06/2002 & COBRA BIOMANUFACTURING UNTIL 22/04/2010. PREVIOUSLY RECIPHARMCOBRA BIO

**Date at Which Additional Info Submitted**

15/05/2001  18/09/2002
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
<th>Level 4 (GMMs)</th>
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02/03/2022
### Project Ref 566/01.1

<table>
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<td>17/09/2001</td>
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- **Non-GMM**: not applicable
- **Consent Granted**: not applicable

- **Project notified under transitional arrangements**: N
- **Historical Significant Changes**: N

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

- **Tick to confirm that you are attaching a summary of the risk assessment**: □
- **Tick if you are claiming exemption from disclosure for sections of the risk assessment**: □

Please enter comments of the GM safety committee on the risk assessment
**Project Additional Information**

**Purposes of the contained use**

To rescue recombinant ovine adenovirus (OAV) from a plasmid in an ovine cell line and to propagate OAV in this cell line for human therapeutic use.

**Recipient or parental organism**

OAV623 is an OAV that contains the full viral genome and a prokaryotic transgene. OAVs are capable of infecting human cells but have been shown to unable to complete their full cycle of replication (Khatri, A et al [1997]: Virology 239 226-237), resulting in an abortive infection. Infection is still abortive in the presence of co-infected human adenovirus suggesting minimal risk to the worker when propagating this virus. It is expected however that there will be an immune response in humans on exposure to this virus.

**Host/vector system**

A plasmid that contains the whole OAV623 viral genome and is therefore a GMM, though of similar or lower risk to the parental virus. The host cells and viral vector are both ovine and the virus is incapable of effective replication in humans. In addition, the expression of the transgene is under the control of a human tissue specific promoter.

**Origin & function**

Virus and cell line are ovine. The cell line is to allow efficient propagation of the recombinant ovine virus. The virus carries a prokaryotic transgene and is intended to deliver the transgene to human cells via an infective but non-replicative action. Expression of the transgene is under the control of a human tissue specific promoter. The gene product is a human enzyme that will only be expressed in target tissues and will be capable of generating a toxic product from a prodrug that is administered separately.

**Evaluation of foreseeable effects**

OAV623 is a recombinant adenovirus, which contains the full viral genome and a transgene containing a human tissue-specific promoter and a gene encoding an enzyme that can act on a pro-drug to produce a toxic agent. OAV is capable of infecting human cells, but it has been shown to be unable to complete its full replication cycle (Khatri, A et al [1997]: Virology 239 p.226-237), resulting in an abortive infection. OAV has also been shown to replicate abortively in human cells, which are co-infected with human adenoviruses. The abortive replication cycle of these viruses suggests that there is minimal risk to the worker when propagating these viruses. However, due to the lack of pre-existing immunity of the worker to OAV, there will be limited immunity on first exposure to the virus. Subsequent exposure would result in an unknown immune response to the virus.

The plasmid DNA containing the full genome of OAV623 will be used to rescue the virus under segregated conditions. As the plasmid contains the same genetic material as the intact virus, it is highly unlikely that the plasmid would be of greater risk to the worker than the virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The virus will only be handled in class II microbiological safety cabinets that will be swabbed with a viricide (e.g. Virkon or Klearcide) after use. All contaminated materials will be treated by chemical means (e.g. 1% Virkon solution, which is proven to destroy both Mastadenoviruses and Aviadenoviruses) and/or autoclaving at 121°C for a minimum of 15 minutes prior to removal by a contractor for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC agree that class 1 would normally have been appropriate for the protection of human health, but as this virus can cause mild infection in sheep it becomes a class 2 activity to ensure protection of sheep in the environment.

**Project Containment**

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**Project Ref** 566/02.1

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Date Project Ceased 13/09/2002

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The aim of this project is to evaluate the immune-therapeutic benefit of expressing immune-stimulating proteins in murine tumour cells killed in vivo by enzyme/prodrug combinations, namely NTR/CB1954 or Cyp1A2/acetaminophen. Immun-therapeutic effects will be studied using the mouse 4T1 breast cancer cell line in the host BALB/c mice in the first instance. The mouse TRAMP spontaneous prostate tumour model will subsequently be used.

#### Recipient or parental organism

The parental organism is a replication-defective, human Adenovirus (Ad) type 5. Human Ad are found worldwide, and over 40 different serotypes have been isolated. Ad5 is associated with mild upper respiratory tract infections in young children. Immunity to Ad infection is thought to be life-long following primary infection. Although some serotypes are closely related they nevertheless remain distinct entities suggesting that the potential for recombination in vivo is not high. Human serotypes do not naturally infect other animal species and there are relatively few reports of virus replication following inoculation of Ad into experimental animals. There is no evidence that human Ad cross species and so a replication defective virus could not be maintained in non-human host. The replication-defective vector is not expected to be harmful. The potential hazard to the environment, from inadvertent release of the quantities of virus expected to be handled, is extremely low. The recipient cell line used to produce the virus is the human PER.C6 cell line (Fallaux et al., Human Gene Therapy, 1998, 9: 1910-1917). This E1-containing cell line is engineered to avoid recombination between the vector genome and the viral sequences present in the genome of the producer cells and thereby RCAs (Replication-Component Adenviruses) should not be generated at significant levels.

#### Host/vector system

The vector system is a replication-defective human adenovirus type 5 (E1 and E3 deleted). The host cell line is PER.C6 (Fallaux et al., Human Gene Therapy, 1998, 9: 1910-1917).

#### Origin & function

Murine immune stimulatory genes will be generated by RT-PCR from mouse splenocytes or from commercially available sources with immune modulating activity in humans.

#### Evaluation of foreseeable effects

Ad expressing xenogeneic proteins such as a bacterial nitroreductase or a mouse cytokine that is inactive in humans may be immunogenic. It is possible that the Ads expressing a mouse cytokine that is active in man (ie that can bind the equivalent human cytokine receptor) could give additional immunogenicity, eg a local inflammatory response. However, this would be very limited and cleared rapidly in most humans, since the great majority of people have antibodies to Ad coat proteins through exposure to the virus in early childhood. Human cytokines have been evaluated in clinical studies and only show toxicity with systemic administration at high levels.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Local contamination of the safety cabinets and other control and containment equipment and devices will be carried out by swabbing with 1% Virkon (as recommended by the manufacturer to achieve most effective killing) followed by 70% ethanol. Cell culture media will be immediately treated with Virkon as recommended by the manufacturer.

All waste materials will be treated with Virkon or autoclaved and/or incinerated.

In the case of an accidental puncture of the skin by a contaminated sharp, the procedure is to wipe the wound with 70% ethanol and report immediately to the Medical Centre.

Standard operating procedure for injection of GM adenoviruses into tumours.
1. All viral vectors have to be handled within class II containment cabinets.
2. Viruses must be carried in a tube enclosed within a sealed container (e.g. cyrovial inside a 50 ml Falcon tube)
3. Operator must wear suitable protective clothing (gown) and latex gloves.
4. If virus leaks from the injection site, the site is wiped with paper tissue soaked in 1% Virkon. Any spillages are wiped up with tissues soaked in Virkon, all tissues must be discarded into an autoclave bag.
5. Contaminated syringes and tips must be soaked in 1% Virkon for at least 30 minutes before being discarded into the sharps bin.
6. Following all work, all surfaces must be decontaminated by wiping with 1% Virkon followed by 70% ethanol, tissues solid waste, gloves etc must be discarded into an autoclave bag.

Standard operating procedure for husbandry of mice injected with recombinant adenoviral vectors.
1. Wear a dedicated lab coat/gown and gloves for handling the animals.
2. Keep a supply of 1% Virkon solution in the room for decontamination purposes.
3. After transferring the animals to clean cages, dirty cages must be put in bags and autoclaved with their contents.
4. The box used for weighing the animals must be disinfected at the end of each session.
5. Routine cleaning of cage racks and filters should be preceded by spraying with 1% Virkon and allowing to stand for 1 hour.
6. All animal carcases etc. are disposed of by incineration off site.
7. All protective clothing is to be autoclaved prior to laundering.
8. All solid waste, disposable gloves etc. is to be autoclaved prior to incineration.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

02/03/2022
The Genetic Modification and Biological Safety Committee (GMBSC) have received and considered this proposal and are in agreement with the classification suggested. Two example cytokines have been described in the risk assessment but the GMBSC will require to review any other cytokines on a case-by-case basis and notify HSE if there is any resulting alteration to the classification.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Conf L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Conf L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 566/05.1

Date Ackn'd: 01/03/2005

CU2 Project Title: Production of modified forms of herpes simplex virus for cancer therapy.

Class: Class 2

Culture Vol: < 1 Litre

Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements

Historical Significant Changes

Tick if notifying a connected programme of work

Withdrawn

Project Additional Information

Purposes of the contained use

Contained manufacture for human clinical trials.
HSV1 vectors deleted for the neurovirulence factor ICP34.5 have been engineered to express human and mouse forms of GM-CSF and other immune modulators such as RANTES, B7.1, and CD40L. These viruses are designed to replicate in tumours but not surrounding tissue (considerable literature demonstrates such a phenotype for HSV1 and 2 ICP34.5 deleted viruses) with the additional delivery of immuno-stimulatory factors. Some viruses will be additionally modified such as HSV genes that usually minimise immune responses to HSV infection are also deleted. Such genes are UL43, vhs and ICP47. Each of the deletions in ICP34.5, vhs or ICP47 has been shown individually to generate a non-pathogenic, though still replication competent virus. Deletion of UL43 alone probably does not significantly reduce pathogenicity, but this deletion will only be included in viruses already deleted for the other gene(s) above.

### Host/vector system

Vero cells/Herpes Simplex Virus 1 or 2.

### Origin & function

Inserted genetic material is of either human or rodent origin and has been cloned by PCR or obtained from collaborators in plasmid form. Genes to be inserted include GM-CSF, RANTES, B7.1, and CD40L.

### Evaluation of foreseeable effects

None of the genes to be inserted are anticipated to result in harmful effects.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Derogation re: autoclave on site.**

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste generated during the activity that has been in contact with the GMM will be treated with a Virkon solution. This broad-spectrum disinfectant is effective against herpes simplex virus at a 0.5% (w/v) concentration giving terminal disinfection after 10 minutes of contact. Normal contact time is about 16 hr (overnight) prior to disposal to drain.

Solid (mainly plastic disposables) waste will be double bagged and placed in Weaver bins then transported as clinical waste by a registered carrier for incineration offsite.

### Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

### Please enter comments on the GM safety committee on the risk assessment

The GMBSC opinion is that the viruses to be used are Class 2 GMOs requiring Containment level 2.
### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tbody>
<tr>
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<td>L3</td>
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<table>
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<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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</tbody>
</table>

### Project Ref 566/05.2

#### Date Ackn'd
14/11/2005

#### CU2 Project Title
PRODUCTION OF MODIFIED FORMS OF HERPES SIMPLEX VIRUS FOR CANCER THERAPY

#### Class
Class 2

#### CultureVolClass2
1-50 Litres

#### Non-GMM Consent Granted
Not Applicable

#### Project notified under transitional arrangements
N

#### Withdrew
N

#### Date Project Ceased

#### Tick if notifying a connected programme of work
N

#### Historical Significant Changes

#### Historical Date of Additional Info

#### Significant Change ID

#### Date of Significant Change

### Project Additional Information

#### Purposes of the contained use
Prevention of class 2 GMOs being a hazard to workers

#### Recipient or parental organism

#### The HSV2 vectors are deleted for the neurovirulence factor ICP34.5 as well as vhs, ICP47 and UL43. These viruses are modified so that the HSV genes that usually minimise immune responses to HSV infection are deleted. No transgenes are present.

#### Host/vector system

02/03/2022 Page 9965 of 15326
Herpes simplex virus 2.

Origin & function
No genes are being inserted. Only deletions of the HSV genes are being carried out.

Evaluation of foreseeable effects
No harmful effects are anticipated due to the increased immuno-stimulatory status of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not required.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste generated during the activity that has been in contact with the GMO will be treated with a 1% Virkon solution. This broad spectrum disinfectant is effective against herpes simplex virus at a 0.5% (w/v) concentration giving terminal disinfection after 10 minutes of contact.

All disposable items will be placed into Weaver bins and sealed within the cleanrooms. These are then sprayed with Biocide B, with a contact time of 5 minutes before leaving the room. These are then removed to an external compound where they are placed in clinical waste bins. A registered contractor (White Rose Environmental) then removes the bins for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The GMSC opinion is that the virus to be used is probably a class 2, although the risks are low.

Project Containment

<table>
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<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Yes</td>
<td>L2</td>
<td>L3</td>
</tr>
</tbody>
</table>

Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
Deletion of two genes from the chromosome of a Gram-positive bacterium.

Purposes of the contained use
To create a strain of a Gram-positive bacterium that does not produce two proteins which have therapeutic applications. This will be used as a control strain in assays allowing all the other proteins produced by the bacterium to be detected.

Recipient or parental organism
A Gram-positive bacterium.

Host/vector system
A plasmid possessing the pMB1 origin of replication and an antibiotic resistance gene flanked by dif sites, flanked by regions homologous to the target genetic loci. This will replicate in E. coli only, not in the target organism. The vector system is for gene deletion only, not gene expression.

Origin & function
A gene cassette will be synthesised (de novo and in E. coli) consisting of an antibiotic resistance gene flanked by the recognition sites for the native Xer site-specific recombinases (dif sites). This will in turn be flanked by regions of chromosomal homology upstream and downstream of the gene to be deleted. Competent cells of the target Gram-positive bacterium will be made and transformed with the linearised gene cassette, with deletion mutants identified by survival on the selective antibiotic. The deletion mutant will be cultured in the absence of the antibiotic, allowing cells to undergo site-specific recombination to delete the antibiotic resistance gene. These recombinants will be identified by antibiotic sensitivity and by PCR. The process will then be repeated for the second gene to be deleted. To determine transformation efficiencies, plasmids possessing the pMB1 and pUB110 origins of replication will be transformed into the Gram-positive bacterium and colony counts performed, but these...
will not be used for further culture.

Evaluation of foreseeable effects

The deletion of two genes is not predicted to have any adverse effects on the biosafety of this organism. Indeed, the proposed modifications are likely to reduce the fitness and potential pathogenicity of this organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste generated will be inactivated by disinfection overnight with 2.5 % (v/v) Chloros (hypochlorite). Solid waste (mainly plastic disposables) will be double-bagged and placed in Weaver bins, then transported as clinical waste by a registered carrier to incineration off-site.

Project Containment

- **Laboratory Activities**
  - L2: Yes
  - L3: L4
  - L4: L2

- **Glass Houses**
  - L3: L4
  - L2: L3
  - L4: L2

- **Growth Rooms**
  - L3: L4
  - L2: L3
  - L4: L2

- **Animal Units**
  - L2: L3
  - L4: L2

- **Large Scale Activities**
  - L3: L4
  - L2: L3
  - L4: L2

- **Human Clinical Applications**
  - L3: L4
  - L2: L3
  - L4: L2

Project Ref: 566/07.1

Date Ackn'd: 02/03/2022

CU2 Project Title: Class Culture Vol Class 2 Culture Volume Class 3-4

Tick if you are claiming exemption from disclosure for section of the risk assessment: Y

Tick if you are claiming exemption from disclosure for section of the risk assessment: N
Production of a hybrid AAV2/AAV1 vector expressing human alpha1-antitrypsin (hAAT) by the infection of human HEK-293 cells with two recombinant HSV-1 helper vectors.

Overview: A recombinant AAV1-hAAT (hybrid) vector is produced by co-infection of HEK-293 cells with two HSV-1 based vectors: rHSV-rep2/cap1 and rHSV-rAAV2-AAT. rHSV-rep2/cap1 expresses the AAV Rep and Cap proteins. Rep proteins are derived from serotype AAV-2 and Cap proteins from serotype AAV-1. rHSV-AAV2-AAT contains the recombinant AAV-2 vector, comprising the ITRs (Inverted Terminal Repeats) from serotype AAV-2 and the expression cassette for human alpha1-antitrypsin (hAAT). This is the AAV genome, which will be packaged into the AAV virions. The producer cell line 293 is an embryonic human kidney cell line that has been transformed by human Ad5 E1 gene sequences (Cobra BRA0015) and it is a well characterised cell line that has been used for many years for the production of biopharmaceuticals.

The recombinant AAV is produced after co-infection of HEK-293 cells with both HSV helper viruses. GMO evaluations: 1. Recombinant HSV vectors: The two HSV vectors in question are deleted for the viral gene ICP27 thereby, in theory, disabling the replication capacity of the virus. In both vectors the TK gene is disrupted by insertion of either the Rep2/Cap1 expression cassette (rHSV-rep2/cap1) or the recombinant AAV2-hAAT cassette (rHSV-AAV2-AAT). The potential for recombination with wild-type HSV-1 in already infected humans is very low. The inserted gene sequences could potentially recombine with wild-type HSV-1 if the vectors are present within the same cell. The vectors would then be converted to wt HSV-1 by acquiring the TK and ICP27 genes, vice versa, the wt HSV-1 would be inactivated by deletion of ICP27 and TK genes. The two HSV helper viruses are produced on the complementing cell line V27 (containing the ICP27 gene to allow amplification of the two HSV vectors), and the virus preparations are contaminated with recombinant, replication-competent HSV vector (rHSV). Contamination is normally in the range of <10pfu rHSV/1xE+08 pfu of rHSV. Also, some amplification of the contaminating rHSV is expected after infection of the HEK-293 cells with the two recombinant HSV vectors (the ratio of rHSV/recombinant HSV increases up to 1000-fold). The replication-competent vectors will be wild-type for ICP27 and will have either the Rep/Cap cassette or the AAV genome with the hAAT expression cassette at the TK gene position, respectively. They will not contain the TK gene, as this gene is not present in the V27 producer cell line and cannot be acquired by recombination (TK is not needed for in-vitro cell culture replication). Therefore the rHSV viruses would still be resistant to antiviral drugs like acyclovir. Also, the rHSV viruses would be still sensitive to other anti-HSV drugs like vidarabine and foscarnet (Martuza RL et al., Science 1991: 252, 854-856). The TKminus rHSV viruses are attenuated in-vivo especially for the neurovirulent phenotype (Martuza RL et al., Science 1991: 252: 854-856). Also, this Tkminus virus can replicate in dividing cells but is severely impaired for replication in non-dividing cells. Recombination of these replication-competent HSV-1 contaminations (rHSV) with wild-type HSV-1 present in the same cell could cause the exchange of the TK gene and the recombinant cassette present in the rHSV vectors (either the AAV2-AAT cassette or the Rep2/Cap1 expression cassette). This would not change the type of vectors being present in the cell. Interaction of the two recombinant HSV vectors or the contaminating rHSV with
the HEK-293 cell host or subsequent processing media should not change/increase the hazard and/or classification. 2. Recombinant AAV

Adeno-Associated Virus (AAV) belongs to the Parvoviridae family and here to the subfamily Parvovirinae and here to the Genus Dependovirus. The name Dependovirus represents the fact that this virus cannot induce a lytic cycle after infection if a Helper Virus is not present. Helper Virus can be Adenovirus, HSV, Vaccinia Virus, CMV or HPV. In case of the production the recombinant rAAV-AAT, the helper functions are supplied by the HSV vectors. AAV is normally transmitted via respiratory and fecal/oral routes. AAV particles are very robust and can survive outside the host for several weeks. Generally AAV is not associated with human disease. Recombinant AAV vectors are not pathogenic, have comparatively low intrinsic immunogenicity, can transduce non-proliferating cells and are capable of long-term transgene expression. Serotype AAV-1 is most effective in infecting skeletal muscle cells. The AAV virus produced within this procedure is completely defective for Rep and Cap genes and is therefore not able to replicate even in the presence of an Helper Virus in the target cell. After infection of HEK-293 cells with the two rHSV, the HEK-293 cells will produce recombinant AAV particles containing the hAAT expression cassette. The tropism of this rAAV is identical to that of AAV1 as Capsid proteins are derived from serotype AAV-1. Rep proteins are derived from AAV-2 but do not influence the tropism of the virus particle. It is not thought that the recombinant AAV would pose any serious risk to the environment. It might survive at maximum a few weeks outside the host cell. Infection is restricted to humans. Interaction of the recombinant AAV virus with the HEK-293 cell host or subsequent processing media should not change/increase the hazard and/or classification.

Host/vector system

Host: The producer cell line HEK-293 is an embryonic human kidney cell line that has been transformed by human Ad5 E1 gene sequences (Cobra Biological Risk Assessment 0015). The recombinant AAV will be produced after co-infection of HEK-293 cells with both rHSV vectors. Vector: Recombinant HSV-1 and recombinant, hybrid AAV1/AAV2 with AAV-1 tropism.

Origin & function

The expression cassette comprises the human CMV enhancer in combination with the chicken C-actin promoter, an intron, the cDNA for human alpha1-antitrypsin and the SV40 poly(A) signal. Therefore expression is constitutively switched on in mammalian cells. Alpha 1-antitrypsin (AAT) is a 52 kDa serine proteinase inhibitor that is normally secreted from hepatocytes and circulates in the plasma, protecting lung elastin from degradation by neutrophil elastase and related proteases. Neutrophil elastase is released from white blood cells to fight infection but if not properly controlled can attack normal cells and lead to e.g. emphysema in lung alveoli. For therapeutic applications expression of 800 mg/ml AAT seems necessary to avoid the development of clinically significant lung disease, much higher levels (8 mg/ml or more) are observed without obvious side effect. Earlier studies of protein replacement indicated that no adverse effects were seen with serum hAAT levels greater than 100-fold above the therapeutic threshold (i.e. > 80 mg/ml).

Evaluation of foreseeable effects

1. Vectors Both recombinant HSV vectors are based on the parental virus HSV-1 (strain Kos). Wild-type HSV-1 is classified as ACDP 2 biological material. HSV-1 belongs to the Alphaherpesvirinae subfamily. HSV-1 has got a wide host range, multiplies efficiently and rapidly destroys the infected host cell. HSV-1 is more frequently associated with non-genital infection (infection above the belt, e.g. skin lesions: Herpes labialis, Keratoconjunctivitis). Within Europe, about 70-80% of the human population are sero-positive for HSV-1. HSV-1 is a universally prevalent virus, which, even in its wild-type form, rarely causes severe problems unless the virus somehow manages to get to the brain or the eye where it could cause encephalitis and retinopathy, respectively. HSV-1 typically exists in a latent form (for life time; primarily but not exclusively in sensory ganglia) after primary infection, and outbreaks of subsequent active viral replication ("cold sores") are associated with episodes of lowered host cell immunity allowing low levels of virus to evade the immune system and re-infect. Reactivation of latent HSV is mainly caused by UV light, stress and fever. Transmission between human hosts requires contact, and transmission by aerosols is not thought to occur. Infection would require direct contact between culture or process media containing virus and process operators via oral, eye or broken skin routes. The contaminating ICP27plus/Tkminus replication-competent viruses (rHSV) would still be significantly attenuated in vivo especially for neurovirulence. Also, it can replicate in dividing cells but is severely impaired for replication in non-dividing cells. The rHSV viruses would be resistant to acyclovir but are still sensitive (like wild-type HSV-1) to alternative drugs like vidarabine and foscarnet (Martuza RL et al., Science 1991: 252, 854-856). HSV-1 causes mainly infection above the belt (skin and eye). In more severe cases HSV-1 infects the brain or the eye and leads to encephalitis and retinopathy, respectively. About 70-80% of people (Europe) are expected to be sero-positive for HSV-1. For those people, the presence of neutralising antibodies should protect against infection with HSV-1. Immunosuppressed persons are at higher risk. We conclude that accidental infection with the low contaminant replication-deficient viruses (ICP+/TK-) should result in a significantly attenuated infection compared to infection with wild-type HSV-1 infection especially for neuropsychogenicity. 2. Transgene Because excess levels of hAAT in serum (100-fold) do not indicate any adverse effects, no harmful consequences after accidental inoculation of the recombinant AAV virus or the recombinant rHSV-Rep2/Cap1 vector by an operator are expected.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation re: autoclave on site.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Environmental evaluation: HSV-1 is species-specific in its infectivity, and thus there is negligible potential for harm to non-human species in the environment. The virus/vector is fragile and rapidly inactivated by desiccation, lipids solvents and mild detergents. The fact that contact is required for transmission attests to the instability of the virus outside the host. Operational evaluation: - All operations will be performed under GMO class II containment levels. - A detailed inactivation protocol for inactivation of HSV and AAV has been supplied by the Client (see inactivation data as performed by the client attached with the GM Risk Assessment). Small volume spills will be treated with excess volume of 0.525% (v/v) Sodium Hypochlorite (1:20 dilution of 10-11% Sodium Hypochlorite stock giving a final conc. of 0.525%) for at least 10 minutes, which should give complete inactivation. The contaminated surface area will then be wiped with tissues wetted with 0.525% Sodium Hypochlorite solution. Larger volume spills of up to 5L will be treated with the emergency procedure for virus or animal cells (addition of equal volume of 1.05% Sodium Hypochlorite stock solution: 1:10 dilution of 10-11% Sodium Hypochlorite stock to give the same final concentration of 0.525%; incubation for at least 10 minutes). Once chemical inactivation is complete liquids will be mopped up with spill granules (Spill-kit available for the Lab where the work will take place) and disposed of in weaver bins by approved disposal route for GMO class II only. - Access to the facility is restricted and appropriate disinfection and waste management procedures for class II waste are in place (e.g. weaver bins for solid waste (double bagged); contaminated consumables will be removed from site by accredited sub-contractor and destroyed by incineration). - Handling will be done by trained operators wearing appropriate PPE (labcoat, gloves and safety goggles). - Arm wrists should be covered at all times when working with virus: this must be done either by coverage with gloves or by the usage of disposable arm sleeves. - If clothing is wet call for immediate assistance and change of clothing. - If accidental contact with gloves happened, these should be changed immediately.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

Please enter comments on the GM safety committee on the risk assessment

The GMBSC opinion is that because of the contamination of the culture with replication-competent HSV-1 vector the recombinant HSV viruses to be used are Class 2 GMOs requiring Containment level 2.
**Project Ref:** 566/08.1

**Date Ackn'd:** 05/03/2008  
**CU2 Project Title:** Propagation and purification of replication incompetent adenoviral vector for use as an investigational drug for pancreatic cancer.

<table>
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<tbody>
<tr>
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**Non-GMM Consent Granted:** Not Applicable

**Project notified under transitional arrangements:** N

**Historical Significant Changes**

**Historical Date of Additional Info:**

**Historical Date of Additional Info:**

**Significant Change ID:**

**Date of Significant Change:**

**Project Additional Information**

**Purposes of the contained use:**

Production of an investigational drug for use in clinical trials.

**Recipient or parental organism:**

The work that will be performed using this GMO covers the technical transfer of a 20L manufacturing process of the biologic TNFerade and the scale up of this process to 100L manufacture. TNFerade is a replication incompetent human Adenovirus (serotype 5) Vector containing a Tumour Necrosis Factor-a (TNF-a) transgene. The virus will be propagated in the 293-ORF6 human cell line which was generated by stably transfecting 293 cells with an inducible E4-ORF6 expression cassette (Brough D, Lizonova A, Hsu C, Kulesa V, and Kovesdi I, J. Virol Sept 1996. 70(9): 6497-6501). The cells will be grown up in a combination of shaker flasks, disposable bag cell culture systems and stainless steel fermentors to a volume of between 20-100L. When an optimal cell density is achieved the cells will be infected with the seed virus. Subsequently the propagated virus will be harvested and purified using column chromatography.

The modified virus is replication incompetent due to the complete deletions of the E1 and E4 regions. There is also a partial deletion of the E3 region. The virus therefore needs a genetically modified cell line that can supply E1 and E4 elements in trans in order to replicate. The inserted TNF-a expression cassette replaces the deleted E1 region. Tumour necrosis factor-alpha(TNF-a) is a pleiotrophic inflammatory cytokine with biological activity that includes immunomodulation, oncostatic, osteolytic proinflammatory amongst other effects.

The virus described is replication incompetent and alone would not therefore be able to replicate in the environment if it were to be accidentally released. There is a theoretical possibility that the vector could become replication competent via recombination or complementation events. Because the TNF-a cassette replaces the deleted E1 region in the viral genome recombination with a wild type Ad5 at this site would result in a disabled virus with no overall gain in pathogenicity.
Host/vector system

The host cell line is 293-ORF6, which was produced from 293HEK cells by stably transfecting them with an inducible E4-ORF6 expression cassette.

Origin & function

The modified adenovirus is replication incompetent due to the complete deletions of the E1 and E2 regions. There is also a partial deletion of the E3 region. The inserted TNF-a expression cassette replaces the deleted E1 region, this is controlled by a chemoradiation sensitive promoter. The purified GM virus produced will be used as a commercial treatment for pancreatic cancer. The virus will be administered to the patient via intramoral injection. Targeted radiotherapy will then be used to switch on the TNF-a gene where it will have multiple anti-tumour effects.

Evaluation of foreseeable effects

Vector: The virus may be transmitted by inhalation, ingestion, skin adsorption or via needle stick injury. The health effects in humans are not well characterised but based on the limited clinical trial data available administration of TNFerade via intramoral injection in combination with radiation or chemoradiation therapy caused side effects of flu-like symptoms, abdominal pain, anaemia, anorexia, dehydration, diarrhoea, esophagitis, hypotension, headache and tachycardia.

Transgene: Any GM that were to enter a human cell has the potential to express biologically active TNF-a, however the virus cannot replicate in human cells and therefore the transient expression of the TNF-a should not pose a hazard to human health. Furthermore the transgene is controlled by a promoter that is switched on by radiotherapy lessening the expression levels that would be achieved in a person who was accidently inoculated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Part II, Table 1a, Containment Measures, Equipment:

8. Autoclave required on site.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All product contacting disposable materials (solid waste) will be handled as class 2 GMO waste and disposed of in weaver bins, this waste will be removed from site for incineration by an approved contractor. In house studies have shown that cultures of liquid human adenovirus type 5 can be effectively inactivated by 1% (final concentration) Sodium Hypochlorite solution for a contact time of 5 minutes (refer to attached risk assessment) this can be applied to either spillages of Adenovirus or unwanted cultures, any spill lit materials that are used to mop up a treated adenovirus spill will be disposed of as solid waste as described above. The virus will only be handled by appropriately trained personnel.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMBSC have agreed that this organism should be handled as a class 2 GMO and therefore requires level 2 containment measures.
Project Containment

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**Project Ref** 566/09.1

- **Date Ackn’d**: 04/03/2009
- **CU2 Project Title**: Deletion of combinations of the genes guaBA, thyA, dapD, set and sen from the Shigella flexneri chromosome.
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Consent Granted**: Not Applicable

---

**Project Additional Information**

- **Purposes of the contained use**: To create an attenuated strain of Shigella flexneri suitable for plasmid maintenance that is free of antibiotic resistance genes.
- **Recipient or parental organism**: Shigella flexneri 2a.
- **Host/vector system**
The host is Shigella flexneri 2a. There is no associated expression vector system.

Origin & function

Gene cassettes will be synthesised consisting of an antibiotic resistance gene flanked by the recognition sites for the native Xer site-specific recombinases (dif sites). This will in turn be flanked by regions of chromosomal homology upstream and downstream of the gene to be deleted. Competent S.flexneri cells will be prepared that contain a helper plasmid transiently expressing the lambda Red recombination functions from genes bet, exo and gam. These will be transformed with the linearised gene cassette, with deletion mutants identified by survival on the selective antibiotic. The deletion mutant will be cultured in the absence of the antibiotic, allowing cells to undergo site-specific recombination to delete the antibiotic resistance gene, in addition to the loss of the helper plasmid. These recombinants will be identified by antibiotic sensitivity and by PCR. The process will then be repeated for the other genes to be deleted. A maximum of three genes will be deleted per strain: set, sen and one of the following: guaBA thyA or dapD.

Evaluation of foreseeable effects

The proposed modifications are designed to significantly reduce the pathogenicity of Shigella flexneri. These mutations have been carried out by others and have resulted in strains that are safe and well-tolerated in mice and humans following oral or mucosal inoculation (see Risk Assessment).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste generated will be inactivated by disinfection overnight with a solution of sodium hypochlorite shown to reduce viability by greater than five orders of magnitude (S.flexneri is known to be sensitive to 1% sodium hypochlorite). Solid waste (mainly plastic disposables) will be double-bagged and placed in Wiva bins, then transported as clinical waste by a registered carrier for incineration off-site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMBSC opinion is that Shigella flexneri 2a is a Class 2 GMO requiring containment level 2.

Project Containment

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02/03/2022
Project Additional Information

Purposes of the contained use
To create a strain of BCG suitable for plasmid maintenance that is free of antibiotic resistance genes.

Recipient or parental organism
BCG.

Host/vector system
The host is BCG, with the vector system consisting of plasmids possessing the pAL5000 origin of replication for secreted expression of recombinant antigens.

Origin & function
Gene cassettes will be synthesised consisting of a hygromycin resistance gene flanked by the recognition sites for the native Xer site-specific recombinases (dif sites). Adjacent to this will be the tetracycline (tet) repressor gene, and the tet operator/promoter region. This will in turn be flanked by regions of chromosomal homology upstream and downstream of the essential chromosomesal genes to be targeted. Competent BCG cells will be prepared that contain a helper plasmid transiently expressing bacteriophage recombination functions. These will be transformed with the linearised gene cassette, with ORT strains identified by growth dependant on a sub-lethal
Evaluation of foreseeable effects

BCG has been used to safely immunise over three billion people worldwide. The proposed modifications will reduce the already minimal pathogenic effects of BCG through the metabolic burden of plasmid maintenance and recombinant protein expression. Additionally, the ORT strains will have expression of an essential gene made conditional on the presence of a sub-lethal concentration of tetracycline or of a plasmid possessing the tet operator sequence, thus increasing their biosafety. The HIVCONSV gene is a synthetic construct with no biochemical activity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste generated will be inactivated by disinfectant overnight with a solution of sodium hypochlorite shown to reduce viability by greater than five orders of magnitude (BCG is known to be sensitive to 1% sodium hypochlorite). Solid waste (mainly plastic disposable) will be double-bagged and placed in Wiva bins, then transported as clinical waste by a registered carrier for incineration off-site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMBSC opinion is the BCG is a Class 2 GMO requiring containment level 2.

Project Containment

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Project Ref 566/11.1

Date Ackn’d 25/02/2011

Process development and cGMP manufacture of a Conditionally-Replicating Adenovirus (CRAd) serotype-5-based vector for use in preclinical toxicity and Phase I/II clinical trial (solid tumours).

Class 2 1-50 Litres

Non-GMM Consent Granted

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Withdrawn N

Project notified under transitional arrangements N

Historical Significant Changes

Project Additional Information

Purpose of the contained use
Production of an investigational drug for use in clinical trials (solid tumors).

Recipient or parental organism
This risk assessment covers the assay/process development and cGMP manufacturing process of a biological oncolytic virus up to a scale of 20L. The oncolytic virus is a CRAd (serotype 5) vector containing three distinct mutations in the adenovirus genome, through which infection of and replication in tumour cells is enhanced.

Host/vector system
The virus is based on human Adenovirus serotype 5 (Ad5 is transmissible via the mouth, nasopharynx or ocular conjunctiva and uniquely via the airborne route in a military setting. Ad5 is most commonly spread in children by the faecal/oral route although other strains may largely spread via contaminated water (Fields, Virology (1996): page 2153). Whilst adenoviruses can transform rodent cells numerous screens have failed to correlate the presence of adenoviruses with oncogenesis in humans. Willd-type Ad5 is classified as ACDP class 2 biologic according to UK HSE standards. The infection with Ad5 is very common within populations and within the Western world over
90% of individuals are sero-positive for Ad5 resulting in lifelong immunity.

The aim of Conditionally-Replicating Adenoviruses (CRAds) is to destroy tumour cells by selective virus replication and associated oncolysis and to spare healthy, non-tumour cells. The CRAd will be produced on A549 human lung cancer cells, which are well characterised and have been used in the laboratory since 1972. A549 cells do not contain adenoviral sequences excluding the possibility of homologous recombination between virus and host genome but they do support the replication and progeny production of the CRAd, thus identifying the producer cell line also as a permissive tumour cell type.

**Evaluation of foreseeable effects**

The virus will be propagated on human lung cancer cells A549. Uncontaminated cell lines serving as host for virus propagation pose little hazard to healthy individuals. Even if direct dermal inoculation were to occur it would likely result only in localised inflammation.

The virus may be transmitted by inhalation, ingestion, skin absorption or via needle stick injury. The determination of toxicity of replicating adenovirus in animal models is difficult as human Ad5 does not replicate in mice. Semi-permissive animal models for replication of human Ads are the syrian hamster and the cotton rat.

In case of accidental inoculation of an operator with the CRAd, it is expected that the infection is cleared efficiently in sero-positive individuals. Additionally, the capacity to replicate in normal, non-dividing tissue should be decreased compared to wild-type Ad5. However, it is expected that the level of cancer-specific virus replication is not complete and some leaky, residual replication in normal cells might be present. The level of residual replication in normal cells seems to be dependent on the cell type used. Virus replication/propagation could happen if the CRAd does infect cells that are actively dividing. However, again this event should be controlled by the operator's immunity against Ad5.

Further, several clinical studies with comparable oncolytic viruses have been done or are being conducted at the moment.

**Consideration of recombination events:**

As the production host A549 does not contain any adenoviral DNA sequence, homologous recombination can not happen. In case of infecting HEK293 or HER911 cells with the CRAd for assay purposs (small scale work only), recombination between adenoviral DNA within the host genome and DNA sequences within the CRAd can theoretically happen. However, this recombination happens at low frequency and several rounds of amplification are normally required to achieve detectable amounts of recombinant virus. As the virus will not be propagated for more than one round of infection on HEK293 or HER911 cells for assay purposes, the risk of accumulation of any reasonable amount of such a recombinant virus is very low.

**Consideration of interaction with other organisms and GMOs:**

When performing infectivity and titre assays the virus might be handled with other adenoviral vectors including E1-deleted, replication defective vectors. Even theoretical recombination between such vectors e.g. within a host cell or within an operator after accidental inoculation should not generate any more hazardous recombinant than already discussed above. The parallel handling with other viruses is not planned or expected. Working with the CRAd will be performed uner segregated conditions (spatiotemporal)

In summary, it is expected that the CRAd is less cytotoxic after accidental inoculation than wild-type Ad5.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Treatment where cell line only is present
Where the cell line alone is being handled small scale spills and liquid waste can be treated with 1% solution (final concentration) of sodium hypochlorite solution (0.12% available chlorine) for at least 5 minutes or 1% Virkon for least an hour. Large spills may also be treated with 1% (final concentration) Sodium Hypochlorite solution for 5 minutes or 1% Virkon for at least 1 hour. Inactivated spills may be mopped up with spill kits and disposed of as solid clinical waste. Contaminated cultures in bioreactors will be killed by heat sterilisation or via the following disinfectants: 2% TEGO, 0.5M NaOH or 10% sodium hypochlorite (1.2% final active chloride). The usage of 1% Virkon is also possible in non-cGMP labs. All these methods will result in a greater than 5-log kill.

2. Treatment of virus and infected cell cultures

a. Fluids of virus and/or infected cells

Virus containing fluids can be inactivated with either 0.5M NaOH or 10% sodium hypochlorite (1.2% active chloride) for a minimum of 1 hour to give a minimum 5-log kill.

B. Routine surface cleaning of stainless steel, bench surface and vinyl

Routine surface cleaning should be carried out with Biocide B spray for a minimum of 30 min contact time followed by wipe over with 70% IPA wpes. Stainless steel and bench surfaces are easily cleaned by this method whereby vinyl surface is more resistant to cleaning. Accordingly, surfaces in class II MSCs should be cleaned after each usage with Biocide B spray followed by a final wipe over with 70% IPA impregnated wipes. A full MSC clean (trays to be taken out) is performed according to a cleaning rota every month using Biocide B followed by 70% IPA cleaning. Theses methods will result in a greater than 5-log kill.

C. Fresh virus/infected cell spills (small and large)

Small-scale spills of the GMO (up to 50ml) will be inactivated by treatment with 10% sodium hypochlorite solution (1.2% available chloride) for a minimum of 5 minutes, the inactivated spill may then be absorbed with spill kits and disposed of as class II solid waste. Where the work performed presents the potential for a large spillage to occur spill kits should be kept in the immediate vicinity of the work such that they could be employed to stem the flow of virus as the room is vacated. Sufficient time should be allowed before re-entering the room to ensure there is no risk of inhaling any aerosols generated. The spill should then be treated with 10% (1.2% active chloride) sodium hypochlorite solution for 5 minutes, after which time the spill should be mopped up and any solid waste disposed of as Class II waste via wiva bins.

General handling:

All product contacting disposable materials (solid waste) will be handled as class II GMO waste and disposed of in wiva bins according to the local rules, waste will be removed from site for incineration by an approved contractor. Non-disposable product contacting items will be kept to an absolute minimum; glassware and any stainless steel items will be disinfected with 1% Sodium hypochlorite solution for 5 minutes prior to washing.

Storage:

The purified virus product and any associated samples will be double contained and stored at -80°C using a biotainer or other suitable, sealable, 'unbreakable' container as the secondary containment vessel. These containers will be labelled with a class 2 GMO label accordingly.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |
The GMBSC have agreed that this organism should be handled as a Class II GMO and therefore required level 2 containment measures.

**Project Containment**

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**Project Ref** 566/15.1

**Date Ackn’d** 03/06/2015

**CU2 Project Title** The production and purification of 3rd generation lentiviral vector at a scale of 2L using 4 plasmid transient

**Class** Class 2

**Culture Vol** 1-50 Litres

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Production of a modellentivirus to assess Cobras’ ability to produce high quality lentiviral vector.

**Recipient or parental organism**

The production process will utilise a four plasmid vector system to generate a third generation lentiviral vector. By splitting the vector system into 4 plasmids (3 helper plasm ids and 1 containing the vector genome plus transgene), the
The number of recombination events required to form a complete replication-competent vector increases, thereby reducing the possibility of making a replication-competent vector particle. The system is designed to produce 'self inactivating' lentiviral vector.

Replication-defective lentiviral vectors are not known to cause any diseases in humans or animals. The risks associated with working with lentiviral vectors are:

1) Lentiviral vectors produce 2 proteins common to HIV: If the lentiviral vector accidentally enters the human body there is a possibility that seroconversion towards HIV-1 viral proteins could result. The antibodies raised to the lentiviral vector proteins could result in a partially positive HIV test result.

2) Possibility of recombination event producing a replication competent lentiviral vector (RCL): If there was an extremely unlikely recombination event a replication competent vector could result which could infect and replicate in humans (Cancer/AIDS may result). Recombination would only pose a significant risk if the operator was HIV positive.

3) Possibility of oncogenesis via random chromosomal integration: Lentiviral vector transgene could integrate into chromosomal DNA. If the vector enters the body the transgene could integrate into the cells chromosome. If it were to integrate by an active gene, it could cause cancer.

Host/vector system

Transient transfection of HEK293T cells will be performed. HEK293T cells are a well characterised producer cell line for the production of lentiviral vector and are considered to be non-hazardous to humans.

Origin & function

The lentiviral vector will be produced using 4 separate plasmids co-transfected. The plasmids were produced in E.coli and purified by column chromatography. The lentiviral vector produced will be assessed for titre and quality by the customer to assess Cobra's ability to produce lentiviral vector. Successful completion of this task would lead to larger scale production which would be addressed in a future risk assessment and CU2 form.

Evaluation of foreseeable effects

The risks associated with working with lentiviral vector are:

1) Lentiviral vector produces 2 proteins common to HIV: If the lentiviral vector accidentally enters the human body there is a possibility that seroconversion towards HIV-1 viral proteins could result. The antibodies raised to the lentiviral vector proteins could result in a partially positive HIV test result. All open manipulations will be performed inside a class II microbial safety cabinet, (MSC); the 2L production upstream processing will be performed within the MSC as a closed system.

2) Possibility of recombination event producing a replication competent lentiviral vector (RCL): If there was an extremely unlikely recombination event a replication competent vector could result which could infect and replicate in humans (Cancer/AIDS may result). Recombination would only pose a significant risk if the operator was HIV positive. The 3rd generation 4 plasmid split gene transfection process has been proposed to perform this work, which is designed to prevent the formation of replicational competent lentiviral vector and offers the maximal biosafety.

3) Possibility of oncogenesis via random chromosomal integration: Lentiviral vector transgene could integrate into chromosomal DNA. If the vector enters the body the transgene could integrate into the cells chromosome. If it were to integrate by an active gene, it could cause cancer.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The most probable route of exposure for this work would be dermal via sharps (needle-sticks), absorption through exposed scratches or abrasions on the skin, or mucous membrane exposure of the eyes, nose and mouth. Another route would be inhalation via aerosols. Given the containment measures that will be used whilst handling the lentiviral
The likelihood of accidental inoculation is low. The following measures must be adhered to whilst handling lentiviral vector:

1. Physical Containment: All work will be performed in a room with negative pressure (not essential), a dedicated Class II MSC (left on continuously) and a dedicated tissue culture incubator. A warning sign must be posted on the door alerting personnel of the presence of lentiviral vector particles.

2. Personal Protective Equipment (PPE): A disposable non-sterile Tyvex suit or lab coat should be worn at all times. A fresh suit must be worn for each processing session. A full face shield will be worn. Double gloves and disposable oversleeves must be worn paying particular attention to cover bare skin at the wrists. Potentially contaminated gloves must be removed and replaced with new gloves before touching anything outside the MSC, such as the incubator.

3. Spill kit: The lab must have a spill kit comprising easy-to-read outline spill response procedures, gloves, masks, goggles, clean lab coat, paper towels to absorb contaminated liquids; disinfectant and a biohazardous waste bag.

4. Sharps: Sharps and glassware cannot be used.

5. Solid Waste: Everything that contacts lentiviral vector-containing solutions or vessels must be decontaminated or contained before leaving the MSC. Solid waste can be collected in a biohazard bag inside the MSC. Pipette tips must be collected in a dedicated, labelled sharps bin.

6. Liquid Waste: Must be decontaminated using the sodium hyperchlorite at > 5000ppm free chlorine for 1 hour before drain disposal.

7. Centrifugation: Centrifuge tubes must be prepared and sealed in the MSC. The centrifuge tubes must then be sealed into lidded centrifuge rotor buckets prior to removal from the MSC. Once centrifugation is complete the sealed centrifuge buckets must be returned to the MSC prior to opening.

8. Filtration: Ensure the filter is vented away from the MSC opening I operator.

With all of these precautions the possibility that an operator will be exposed to the organism is extremely unlikely. Dehydration alone reduces the titre by 90% within several hours, which means that with the prescribed cleaning procedures of 70%IPA and hyperchlorite (Biocide E), the possibility of any organisms escaping the class II containment is highly unlikely.

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For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste: Everything that contacts lentiviral vector-containing solutions or vessels must be decontaminated or contained before leaving the MSC. Solid waste can be collected in a biohazard bag inside the MSC. Pipette tips must be collected in a dedicated, labelled sharps bin.

Liquid Waste: Must be decontaminated using the sodium hyperchlorite at > 5000ppm free chlorine for 1 hour before drain disposal.

Inactivation protocols:

1) Small spills in the MSC (<25mL) can be decontaminated by covering the spill with a dry wipe and applying Biocide E. The dry wipes should be removed to a biohazard bag/container within the MSC. Residual Biocide E can be removed by spraying with IPA and wiped using sterile IPA wipes. Replace any contaminated PPE.

2) Large spills (>25mL) in the MSC. The MSC must be left running and personnel removed from the room. A warning sign must be placed on the door advising personnel not to enter. Allow 30mins for possible aerosols to settle. Cover the spill with dry wipes, apply Biocide E and allow 20 minutes to inactivate the virus. Deposit soaked wipes in a biohazard bag in the MSC. Decontaminate the interior of the MSC by wiping down with IPA. Replace any contaminated PPE.

3) Spills outside the MSC: Spills generate aerosols therefore alert all personnel present in the area. Remove...
contaminated PPE. Exit the room and place a warning sign advising personnel not to enter. Allow 30 minutes for aerosols to settle. On re-entry to the room, wear fresh PPE and cover the spill with dry wipes. Soak with Biocide E and allow 20 minutes for the virus to be inactivated. Transfer soaked wipes to a biohazard bag. Wipe up any residual spill with dry wipes and then wipe-down with IPA. These inactivation methods will result in a minimum of a 5-log kill. All product-contacting disposable material (solid waste), will be treated as class II GMO waste and disposed of using WIVA bins.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

The GMBSC have agreed that this organism should be handled as a class II GMO and therefore requires level 2 containment measures.

Project Containment

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Project Ref 566/15.2

Date Ackn'd 04/11/2015

CU2 Project Title The production and purification of 3rd generation lentiviral vector to a scale up to 10L using 4 plasmid transient transfection

Class 2

CultureVolClass2 < 1 Litre

Consent Granted

Project notified under transitional arrangements
Project Additional Information

Purposes of the contained use

To develop a production and purification process for the supply of Lentiviral Vector for client's clinical needs.

Recipient or parental organism

The production process will utilise a four plasmid vector system to generate a third generation lentiviral vector. By splitting the vector system into 4 plasmids (3 helper plasmids and 1 containing the vector genome plus transgene), the number of recombination events required to form a complete replication-competent vector increases, thereby reducing the possibility of making a replication-competent vector particle. The system is designed to produce 'self inactivating' lentiviral vector.

Additionally a commercial GFP lentiviral vector will be used as reference material for lentiviral titre assays. This virus has been produced using the third generation plasmid system and contains an inactivated 3' L TR.

Replication-defective lentiviral vectors are not known to cause any diseases in humans or animals.

The risks associated with working with lentiviral vectors are:
1) Lentiviral vectors produce 2 proteins common to HIV: If the lentiviral vector accidentally enters the human body there is a possibility that seroconversion towards HIV-1 viral proteins could result. The antibodies raised to the lentiviral vector proteins could result in a partially positive HIV test result.
2) Possibility of recombination event producing a replication competent lentiviral vector (RCL): If there was an extremely unlikely recombination event a replication competent vector could result which could infect and replicate in humans (Cancer/AIDS may result). Recombination would only pose a significant risk if the operator was HIV positive.
3) Possibility of oncogenesis via random chromosomal integration: Lentiviral vector transgene could integrate into chromosomal DNA. If the vector enters the body the transgene could integrate into the cells chromosome. If it were to integrate by an active gene, it could cause cancer.

Host/vector system

Transient transfection of HEK293FT cells will be performed. HEK293FT cells are a well characterised producer cell line for the production of lentiviral vector and are considered to be non-hazardous to humans.

Origin & function

The lentiviral vector will be produced using 4 separate plasmids co-transfected. The plasmids were produced in E.coli and purified by column chromatography. The final purified Lenti vector material will be delivered to the client to enable them to perform clinical trials.
### Evaluation of foreseeable effects

The risks associated with working with lentiviral vector are:

1. **Lentiviral vector produces 2 proteins common to HIV**: If the lentiviral vector accidentally enters the human body there is a possibility that seroconversion towards HIV-1 viral proteins could result. The antibodies raised to the lentiviral vector proteins could result in a partially positive HIV test result.

   All open manipulations will be performed inside a class 2 microbial safety cabinet, (MSC); the 2 x 3.9L production upstream processing will be performed within the MSC as a closed system.

2. **Possibility of recombination event producing a replication competent lentiviral vector (RCL)**: If there was an extremely unlikely recombination event a replication competent vector could result which could infect and replicate in humans (Cancer/AIDS may result). Recombination would only pose a significant risk if the operator was HIV positive. The 3rd generation 4 plasmid split gene transfection process has been proposed to perform this work, which is designed to prevent the formation of replicational competent lentiviral vector and offers the maximal biosafety.

3. **Possibility of oncogenesis via random chromosomal integration**: Lentiviral vector transgene could integrate into chromosomal DNA. If the vector enters the body the transgene could integrate into the cells chromosome. If it were to integrate by an active gene, it could cause cancer.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The most probable route of exposure for this work would be dermal via sharps (needle-sticks), absorption through exposed scratches or abrasions on the skin, or mucous membrane exposure of the eyes, nose and mouth. Another route would be inhalation via aerosols. Given the containment measures that will be used whilst handling the lentiviral vector the likelihood of accidental inoculation is low. The following measures must be adhered to whilst handling lentiviral vector:

1. **Physical Containment**: All open work will be performed in Class 2 MSCs (left on continuously). A warning sign must be posted on the door to the laboratory alerting personnel of the presence of lentiviral vector particles.

2. **Personal Protective Equipment (PPE)**: A disposable non-sterile Tyvex suit or lab coat should be worn at all times. A fresh suit must be worn for each processing session. A full face shield will be worn during operations that could result in aerosols if there was a system failure ie the use of peristaltic pumps. Double gloves and disposable oversleeves must be worn paying particular attention to cover bare skin at the wrists. Potentially contaminated gloves must be removed and replaced with new gloves before touching anything outside the MSC, such as the incubator.

3. **Spill kit**: The lab must have a spill kit comprising easy-to-read outline spill response procedures, gloves, masks, goggles, clean lab coat, paper towels to absorb contaminated liquids; disinfectant and a biohazardous waste bag.

4. **Sharps**: Sharps i.e. needles cannot be used.

5. **Solid Waste**: Everything that contacts lentiviral vector-containing solutions or vessels must be decontaminated or contained before leaving the MSC. Solid waste can be collected in a biohazard bag inside the MSC. Pipette tips must be collected in a dedicated, labelled container.

6. **Liquid Waste**: Must be decontaminated using sodium hypochlorite (> 5000ppm free chlorine) for 1 hour or 0.5M sodium hydroxide before drain disposal.

7. **Centrifugation**: Centrifuge tubes must be prepared and sealed in the MSC. The centrifuge tubes must then be sealed into lidded centrifuge rotor buckets prior to removal from the MSC. Once centrifugation is complete the sealed centrifuge buckets must be returned to the MSC prior to opening.

8. **Filtration**: Ensure the filter is vented away from the MSC opening I operator.

With all of these precautions the possibility that an operator will be exposed to the organism is extremely unlikely. Dehydration alone reduces the titre by 90% within several hours, which means that with the prescribed cleaning procedures of 70%IPA and hypochlorite (Biocide E), the possibility of any organisms escaping the class 2 containment is highly unlikely.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste: Everything that contacts lentiviral vector-containing solutions or vessels must be decontaminated or contained before leaving the MSC. Solid waste can be collected in a biohazard bag inside the MSC. Pipette tips must be collected in a dedicated, labelled container.

All waste is treated with disinfectants for the stipulated period of time and solid waste is disposed in WIVA bins which are then bagged. Bags are then stored in a designated Class 2 storage container on the premises, this container is then taken by the contractor off site for incineration of the contents.

Liquid Waste: Must be decontaminated using sodium hypochlorite (> 2000 ppm free chlorine) or 0.5M sodium hydroxide before drain disposal.

Inactivation protocols:

1. Small spills in the MSC (< 2SmL) can be decontaminated by covering the spill with a dry wipe and applying Biocide E. The dry wipes should be removed to a biohazard bag/container within the MSC. Residual Biocide E can be removed by spraying with IPA and wiped using sterile IPA wipes. Replace any contaminated PPE.

2. Large spills (> 2SmL) in the MSC. The MSC must be left running and personnel removed from the room. A warning sign must be placed on the door advising personnel not to enter. Allow 30 mins for possible aerosols to settle. Cover the spill with dry wipes, apply Biocide E and allow 20 minutes to inactivate the virus. Deposit soaked wipes in a biohazard bag in the MSC. Decontaminate the interior of the MSC by wiping down with IPA. Replace any contaminated PPE.

3. Spills outside the MSC: Spills generate aerosols therefore alert all personnel present in the area. Remove contaminated PPE. Exit the room and place a warning sign advising personnel not to enter. Allow 30 minutes for aerosols to settle. On re-entry to the room, wear fresh PPE and cover the spill with dry wipes. Soak with Biocide E and allow 20 minutes for the virus to be inactivated. Transfer soaked wipes to a biohazard bag. Wipe up any residual spill with dry wipes and then wipe-down with IPA.

These inactivation methods will result in a minimum of a 5-log kill.

All product-contacting disposable material (solid waste), will be treated as class 2 GMO waste and disposed of using WIVA bins.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The GMBSC have agreed that this organism should be handled as a class 2 GMO and therefore requires level 2 containment measures.
### Project Additional Information

**Purposes of the contained use**

To develop a production and purification process for the supply of Lentiviral Vector for client's clinical needs.

**Recipient or parental organism**

The production process will utilise a four plasmid vector system to generate a third generation lentiviral vector. By splitting the vector system into 4 plasmids (3 helper plasmids and 1 containing the vector genome plus transgene), the number of recombination events required to form a complete replication-competent vector increases, thereby reducing the possibility of making a replication-competent vector particle. The system is designed to produce 'self inactivating'
lentiviral vector. Additionally a commercial GFP lentiviral vector will be used as reference material for lentiviral titre assays. This virus has been produced using the third generation plasmid system and contains an inactivated 3’ L TR. Replication-defective lentiviral vectors are not known to cause any diseases in humans or animals.

The risks associated with working with lentiviral vectors are:
1) Lentiviral vectors produce 2 proteins common to HIV: If the lentiviral vector accidentally enters the human body there is a possibility that seroconversion towards HIV-1 viral proteins could result. The antibodies raised to the lentiviral vector proteins could result in a partially positive HIV test result.
2) Possibility of recombination event producing a replication competent lentiviral vector (RCL): If there was an extremely unlikely recombination event a replication competent vector could result which could infect and replicate in humans (Cancer/AIDS may result). Recombination would only pose a significant risk if the operator was HIV positive.
3) Possibility of oncogenesis via random chromosomal integration: Lentiviral vector transgene could integrate into chromosomal DNA. If the vector enters the body the transgene could integrate into the cells chromosome. If it were to integrate by an active gene, it could cause cancer.

Host/vector system

Transient transfection of HEK293FT cells will be performed. HEK293FT cells are a well characterised producer cell line for the production of lentiviral vector and are considered to be non-hazardous to humans.

Origin & function

The lentiviral vector will be produced using 4 separate plasmids co-transfected. The plasmids were produced in E.coli and purified by column chromatography. The final purified lentiviral vector material will be used for process development or delivered to the client for their own purposes.

Evaluation of foreseeable effects

The risks associated with working with lentiviral vector are:
1) Lentiviral vector produces 2 proteins common to HIV: If the lentiviral vector accidentally enters the human body there is a possibility that seroconversion towards HIV-1 viral proteins could result. The antibodies raised to the lentiviral vector proteins could result in a partially positive HIV test result.
2) Possibility of recombination event producing a replication competent lentiviral vector (RCL): If there was an extremely unlikely recombination event a replication competent vector could result which could infect and replicate in humans (Cancer/AIDS may result). Recombination would only pose a significant risk if the operator was HIV positive.
3) Possibility of oncogenesis via random chromosomal integration: Lentiviral vector transgene could integrate into chromosomal DNA. If the vector enters the body the transgene could integrate into the cells chromosome. If it were to integrate by an active gene, it could cause cancer.

Possible Effects of Infection of a Person with, or Exposure of a Person to, the Recombinant Vector:
The lentiviral vector transgene is intended for use in a CAR T cell treatment of Multiple Myeloma.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
The most probable route of exposure for this work would be dermal via sharps (needle-sticks), absorption through exposed scratches or abrasions on the skin, or mucous membrane exposure of the eyes, nose and mouth. Another
route would be inhalation via aerosols. Given the containment measures that will be used whilst handling the lentiviral vector the likelihood of accidental inoculation is low. The following measures must be adhered to whilst handling lentiviral vector:

1. Physical Containment: All open work will be performed in Class 2 MSCs (left on continuously). A warning sign must be posted on the door to the laboratory alerting personnel of the presence of lentiviral vector particles.
2. Personal Protective Equipment (PPE): A disposable non-sterile Tyvex suit or lab coat should be worn at all times. A fresh suit must be worn for each processing session. A full face shield will be worn during operations that could result in aerosols if there was a system failure ie the use of peristaltic pumps. Double gloves and disposable oversleeves must be worn paying particular attention to cover bare skin at the wrists. Potentially contaminated gloves must be removed and replaced with new gloves before touching anything outside the MSC, such as the incubator.
3. Spill kit: The lab must have a spill kit comprising easy-to-read outline spill response procedures, gloves, masks, goggles, clean lab coat, paper towels to absorb contaminated liquids; disinfectant and a biohazardous waste bag.
4. Sharps: Sharps (ie needles) cannot be used.
5. Solid Waste: Everything that contacts lentiviral vector-containing solutions or vessels must be decontaminated or contained before leaving the MSC. Solid waste can be collected in a biohazard bag inside the MSC. Pipette tips must be collected in a dedicated, labelled container.
6. Liquid Waste: Must be decontaminated using sodium hypochlorite (> 5000ppm free chlorine) for 1 hour or 0.5M sodium hydroxide before drain disposal.
7. Centrifugation: Centrifuge tubes must be prepared and sealed in the MSC. The centrifuge tubes must then be sealed into lidded centrifuge rotor buckets prior to removal from the MSC. Once centrifugation is complete the sealed centrifuge buckets must be returned to the MSC prior to opening.
8. Filtration: Ensure the filter is vented away from the MSC opening / operator.

With all of these precautions the possibility that an operator will be exposed to the organism is extremely unlikely. Dehydration alone reduces the titre by 90% within several hours, which means that with the prescribed cleaning procedures of 70%IPA and hypochlorite (Biocide E), the possibility of any organisms escaping the class 2 containment is highly unlikely.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Application for a derogation regarding the treatment of Class 2 waste:
All waste is treated with disinfectants for the stipulated period of time and solid waste is disposed in WIVA bins which are then bagged. Bags are then stored in a designated Class 2 storage container on the premises, this container is then taken by the contractor off site for incineration of the contents.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste: Everything that contacts lentiviral vector-containing solutions or vessels must be decontaminated or contained before leaving the MSC. Solid waste can be collected in a biohazard bag inside the MSC. Pipette tips must be collected in a dedicated, labelled container.
All waste is treated with disinfectants for the stipulated period of time and solid waste is disposed in WIVA bins which are then bagged. Bags are then stored in a designated Class 2 storage container on the premises, this container is then taken by the contractor off site for incineration of the contents.
Liquid Waste: Must be decontaminated using sodium hypochlorite (> 5000ppm free chlorine) or 0.5M sodium hydroxide before drain disposal.
Inactivation protocols:
1) Small spills in the MSC (<25mL) can be decontaminated by covering the spill with a dry wipe and applying Biocide E. The dry wipes should be removed to a biohazard bag/container within the MSC. Residual Biocide E can be
removed by spraying with IPA and wiped using sterile IPA wipes. Replace any contaminated PPE.

2) Large spills (>25mL) in the MSC. The MSC must be left running and personnel removed from the room. A warning sign must be placed on the door advising personnel not to enter. Allow 30 mins for possible aerosols to settle. Cover the spill with dry wipes, apply Biocide E and allow 20 minutes to inactivate the virus. Deposit soaked wipes in a biohazard bag in the MSC. Decontaminate the interior of the MSC by wiping down with IPA. Replace any contaminated PPE.

3) Spills outside the MSC: Spills generate aerosols therefore alert all personnel present in the area. Remove contaminated PPE. Exit the room and place a warning sign advising personnel not to enter. Allow 30 minutes for aerosols to settle. On re-entry to the room, wear fresh PPE and cover the spill with dry wipes. Soak with Biocide E and allow 20 minutes for the virus to be inactivated. Transfer soaked wipes to a biohazard bag. Wipe up any residual spill with dry wipes and then wipe-down with IPA.

These inactivation methods will result in a minimum of a 5-log kill.

All product-contacting disposable material (solid waste), will be treated as class 2 GMO waste and disposed of using WIVA bins.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMBSC have agreed that this organism should be handled as a Class 2 GMO and therefore requires level 2 containment measures.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4 L2</td>
</tr>
<tr>
<td>L3</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>L4</td>
<td>L2 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2</td>
<td>L2 L3 L4 L2</td>
<td>L2 L3 L4</td>
</tr>
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Project Ref 566/16.2

Project Title Class CultureVolClass2 CultureVolumeClass3-4

Date Ackn'd CU2 Project Title
The production and handling of 3rd generation lentiviral vector at GMP production vector at 5 x HyperStack scale (up to 25L)

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To develop a production and purification process for the supply of Lentiviral Vector for client's clinical needs.

Recipient or parental organism

The production process will utilise a four plasmid vector system to generate a third generation lentiviral vector. By splitting the vector system into 4 plasmids (3 helper plasmids and 1 containing the vector genome plus transgene), the number of recombination events required to form a complete replication-competent vector increases, thereby reducing the possibility of making a replication-competent vector particle. The system is designed to produce 'self inactivating' lentiviral vector.

Additionally a commercial GFP lentiviral vector will be used as reference material for lentiviral titre assays. This virus has been produced using the third generation plasmid system and contains an inactivated 3' LTR.

Replication-defective lentiviral vectors are not known to cause any diseases in humans or animals. The risks associated with working with lentiviral vectors are:

1} Lentiviral vectors produce 2 proteins common to HIV: If the lentiviral vector accidentally enters the human body there is a possibility that seroconversion towards HIV-1 viral proteins could result. The antibodies raised to the lentiviral vector proteins could result in a partially positive HIV test result.

2} Possibility of recombination event producing a replication competent lentiviral vector (RCL): If there was an extremely unlikely recombination event a replication competent vector could result which could infect and replicate in humans (Cancer/AIDS may result). Recombination would only pose a significant risk if the operator was HIV positive.

3} Possibility of oncogenesis via random chromosomal integration: Lentiviral vector transgene could integrate into chromosomal DNA. If the vector enters the body the transgene could integrate into the cells chromosome. If it were to integrate by an active gene, it could cause cancer.

Host/vector system

Transient transfection of HEK293FT cells will be performed. HEK293FT cells are a well characterised producer cell
Origin & function

The lentiviral vector will be produced using 4 separate plasmids co-transfected. The plasmids were produced in E.coli and purified by column chromatography. The final purified Lentiviral vector material will be delivered to the client to enable them to perform clinical trials.

Evaluation of foreseeable effects

The risks associated with working with lentiviral vector are:
1) Lentiviral vector produces 2 proteins common to HIV: If the lentiviral vector accidentally enters the human body there is a possibility that seroconversion towards HIV-1 viral proteins could result. The antibodies raised to the lentiviral vector proteins could result in a partially positive HIV test result.
   All open manipulations will be performed inside a class 2 microbial safety cabinet, (MSC); the 5 x 3.9L production upstream processing as a closed system. Down stream processing will be performed as a closed system.
2) Possibility of recombination event producing a replication competent lentiviral vector (RCL): If there was an extremely unlikely recombination event a replication competent vector could result which could infect and replicate in humans (Cancer/AIDS may result). Recombination would only pose a significant risk if the operator was HIV positive.
   The 3rd generation 4 plasmid split gene transfection process has been proposed to perform this work, which is designed to prevent the formation of replicational competent lentiviral vector and offers the maximal biosafety.
3) Possibility of oncogenesis via random chromosomal integration: Lentiviral vector transgene could integrate into chromosomal DNA. If the vector enters the body the transgene could integrate into the cells chromosome. If it were to integrate by an active gene, it could cause cancer.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The most probable route of exposure for this work would be dermal via sharps (needle-sticks), absorption through exposed scratches or abrasions on the skin, or mucous membrane exposure of the eyes, nose and mouth. Another route would be inhalation via aerosols. Given the containment measures that will be used whilst handling the lentiviral vector the likelihood of accidental inoculation is low. The following measures must be adhered to whilst handling lentiviral vector:
1. Physical Containment: All open work will be performed in Class 2 MSCs (left on continuously). A warning sign must be posted on the; door to the laboratory alerting personnel of the presence of lentiviral vector particles.
2. Personal protective Equipment (PPE): Operators must change into appropriate PPE consisting of sterile Tyvek, overboots, facemask, safety glasses and double gloves. When working in the MSC, operators must wear two pairs of gloves and over-sleeves. Potentially contaminated gloves (outer-layer) must be removed to a Dispo-Safe container or waste bag inside the MSC and replaced before touching anything outside the MSC.
   Operators will wear a full face shield during pumping operations and safety glasses at all other times.
3. Spill Kit: The processing room must have a spill kit available. Procedures for dealing with spills must be displayed in the processing room. In addition all operators must undergo training including a mock spill event prior to the commencement of any work with LV.
4. Sharps: The use of sharps is forbidden when working with LV.
5. Solid Waste Disposal: Pipettes must be re sheathed post use and placed into a outer bag, the outer bag must be sealed using a cable tie, wiped with 70% IPA and immediately placed into a Wiva Bin. Waste bags must be sealed using cable ties and Dispo-Safe containers closed tightly prior to removal from the MSC. Dispo-Safe containers and yellow waste bags must be transferred to a Wiva Bin after sealing. When the Wiva Bins are full of processing has ended, bins must be sealed using the lids provided. Wiva Bins must be sprayed with Biocide E, left for 20 minutes and
then wiped with 70% IPA so that it can be transferred from the processing room. The bins are then handed over to Technical Support Services for disposal by incineration using external contractors.  

6. Liquid Waste Disposal: Liquid waste containing LV must be decontaminated either inside the MSC or by welding on a bag containing Sodium Hypochlorite to a final concentration of ≥ 1%. The waste must be decontaminated for at least 1 hour prior to disposal to the drain. Hypochlorite can cause a thermal reaction, care must be taken at all times.  

7. Centrifugation: Centrifuge tubes must be prepared and sealed in the MSC. The centrifuge tubes must then be sealed into lidded centrifuge rotor buckets prior to removal from the MSC. Once centrifugation is complete the sealed centrifuge buckets must be returned to the MSC prior to opening  

9. Filtration: Ensure the filter is vented away from the MSC opening / operator.  

With all of these precautions the possibility that an operator will be exposed to the organism is extremely unlikely. Dehydration alone reduces the titre by 90% within several hours, which means that with the prescribed cleaning procedures of 70%IPA and hypochlorite (Biocide E), the possibility of any organisms escaping the class 2 containment is highly unlikely.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Application for a derogation regarding the treatment of Class 2 waste:  
All waste is treated with disinfectants for the stipulated period of time and solid waste is disposed in red plastic bags or WIVA bins which are then bagged. Bags are then stored in a designated Class 2 storage container on the premises, this container is then taken by the contractor off site for incineration of the contents. Red

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Solid Waste: Everything that contacts lentiviral vector-containing solutions or vessels must be decontaminated or contained before leaving the MSC. Solid waste can be collected in a biohazard bag inside the MSC. Pipette tips must be collected in a dedicated, labelled container.  

All waste is treated with disinfectants for the stipulated period of time and solid waste is disposed in WIVA bins which are then bagged. Bags are then stored in a designated Class 2 storage container on the premises, this container is then taken by the contractor off site for incineration of the contents.  

2. Liquid Waste: Must be decontaminated using sodium hypochlorite (> 5000 ppm free chlorine) or 0.5M sodium hydroxide before drain disposal.  

3. General handling of HyperStacks. During incubation (post seeding) HyperStack vessels must be removed from the incubator by two operators and placed into a container suitable to hold the vessel and the capacity to hold up to 4L liquid volume in the event of spillages from the HyperStack. The vessel must be transported inside the aforementioned secondary container using a trolley. This applies to transport between the bench and incubator and rooms within the same building. These will be disposed of into the Class II waste disposal bags.  

HyperStack Decontamination and Disposal: Following harvest of the cells from the HyperStack, the vessel must be decontaminated by filling with Sodium Hypochlorite solution (1% final concentration) and leaving for >1 hour. This is to be drained into a sink and the vessel disposed of in a yellow biohazardous waste bag which are sealed with cable ties, sprayed with Biocide E, left for 20 minutes and then wiped with 70% IPA so that it can be transferred from the processing room. The bags are then handed over to Technical Support Services for disposal by incineration using external contractors.  

4. Storage of LV: LV product is stored at ≤-70°C in a controlled storage area with restricted personnel access. The material must be double contained and enclosed in a labelled, sealable GMO Class II storage bag.  

5. Use of peristaltic pumps. The operators must inspect all pump tubing to ensure integrity and that all connections are properly secured with cable ties. Once the tubing has been connected, the operator must ensure that no clamps are closed that will prevent pumping or cause a build up of pressure.

02/03/2022 Page 9994 of 15326
When pumping, the operators must initially run the pump at a low setting (-10% of maximum) and observe to ensure the tubing is running correctly through the pump head. The pump speed can then be gradually increased.

6. Use of Biowelder. Post welding, discarded tubing must be removed from the welder carefully so as not to open the sealed tubing and transferred immediately to the MSC where the tubing will be decontaminated in sodium hypochlorite to 1% for v/v ≥ 1 hour.

7. Seeding cells in HyperStacks: Pumping media containing HEK293T cells. Pumping: Refer to activity: Use of peristaltic pumps. Handling of HyperStacks: Refer to Activity: General handling of HyperStacks.

Decontamination of liquid waste: Waste medium, assumed to contain HEK293T (but no LV), will be pumped into a flexboy bag. Liquid waste containing LV must be decontaminated inside the MSC with 0.5M NaOH for a minimum of one hour prior to disposal to the drain. Spillages: All spillages of HEK293T cells (BRA 0136[2]) inside the MSC must be mopped up using helapets and decontaminated

8. Transfection of cells in HyperStacks: Pumping media containing DNA:PEI complexes.

Pumping: Refer to activity: Use of peristaltic pumps.
Welding: Refer to activity: Use of Biowelders.

• Handling of HyperStacks: Refer to Activity: General handling of HyperStacks.

Spillages: All spillages of medium containing DNA:PEI complexes must be mopped up using helapets and the area cleaned using 70% IPA spray (5 minutes contact), followed by a final wipe using dry wipes.

9. Media exchange post-transfection/ final harvest of supernatant containing LV: Pumping media containing LV.

Pumping: Refer to activity: Use of peristaltic pumps.
Welding: Refer to activity: Use of Biowelders.

• Handling of HyperStacks: Refer to Activity: General handling of HyperStacks.

Decontamination of liquid waste: Waste medium, assumed to contain LV particles, will be pumped into a flexboy bag. This must, be decontaminated in the MSC by pumping into the bag a volume of 12% sodium hypochlorite sufficient to make a final concentration of 1% v/v sodium hypochlorite. Decontamination of LV-containing solutions must be performed for ≥ 1 hour prior to disposal.

Spillages: Decontamination of spillages of materials containing LV must be performed by addition of sodium hypochlorite to 1% v/v for ≥ 1 hour.

10. Harvest of HYPERStack vessels. Welding: Refer to activity: Use of Biowelders. HYPERStacks shall be welded onto recovery line(s) of the first closed system assembly. HYPERStacks will be tilted so that supernatant drains into central pooling bag. Two HYPERStacks can be drained at the same time before being sealed and replaced with next vessel.

11. Clarification of Lentiviral vector containing Culture Supernatant.

• Welding: Refer to activity: Use of Biowelders

Inspect tubing to be used for signs of damage prior to use. Perform a finger trace of the filtration flow path prior to filtration to ensure that no blockages exist and that all valves are opened/closed as appropriate. Full face visors must be worn during all pumping activities. Start pumping at a low speed, observing for signs of high pressure build up prior to increasing pump speed. Filtration pressure shall be monitored throughout filtration and shall be terminated if the inlet pressure exceeds 14psi. When venting filters, the vent port shall be directed away from the operator/ working hatch. The vent port shall be opened/closed using an ethanol soaked wipe. Should PPE become soiled during venting process, this should be replaced with clean PPE (i.e. gloves/oversleeves).

Sampling shall be performed using appropriate sized pipettes. Pipettes must be re sheathed post use and placed into a second labelled bag inside the MSC, the outer bag must be sealed using a cable tie, sprayed with Biocide E, left for 20 minutes and then wiped with 70% IPA transferred out of the MSC and immediately placed into a Wiva Bin.
12. Benzoase® digestion of Lentiviral vector containing cell culture supernatant.
Welding: Refer to activity: Use of Biowelders
Sampling shall be performed using appropriate sized pipettes. Pipettes must be re sheathed post use and placed into a outer bag, the outer bag must be sealed using a cable tie, wiped with 70% IPA and immediately placed into a Wiva Bin. Prior to removal from the MSC for temperature controlled incubation, all vessels containing Lentiviral vector material must be sealed. Vessels must remain sealed throughout any period of time that the vessel is outside of the MSC environment.
During temperature controlled incubation the collection bag will be placed onto the WAVE rocking platform, this will be heated to 37°C, care must be taken to avoid burns and prevent crush events from moving parts during mixing.
13. TFF of Lentiviral vector containing cell culture supernatant.
Welding: Refer to activity: Use of Biowelders
Inspect tubing to be used for signs of damage prior to use. Perform a finger trace of the TFF flow path prior to filtration to ensure that no blockages exist and that all valves are opened/ closed as appropriate. Pressure sensors shall be used throughout processing to monitor for signs of system over pressure. Filter leak test will be performed prior to TFF this involves monitoring a higher pressure over the Filter and observing for any reduction in pressure over time. Clamps must be securely in place on the tubing post filter and formlation buffer used for priming pumped at low speeds to increase the pressure to a maximum of 14psi (1 bar), clamps will then be securely closed pre filter to maintain pressure. Pressure monitors will be used to see any reduction in pressure over time. Start pumping at a low speed, observing for signs of high pressure buildup prior to increasing pump speed. Decontamination of LV-containing tubing rig must be performed for ≥ 1 hour prior to disposal. This must be decontaminated by pumping into the TFF rig a volume of 12% sodium hypochlorite sufficient to make a final concentration of 1 % v/v sodium hypochlorite.
14. Final fill of Lentiviral vector. Sampling will performed using a appropriate sized pipette.
Pipettes must be re sheathed post use and placed into a outer bag, the outer bag must be sealed using a cable tie, wiped with 70% IPA and immediately placed into a Wiva Bin ..
All pipetting into appropriately sized containers must be performed within the MSC.
15. Transfer and Storage. All samples shall be labelled with preprinted labels detailing that the product is a Class II organism and transferred out of the MSC prior to any decontamination methods. All sample containment vessels must be suitable for storage ≤-650C. All sample containment must be stored within a secondary containment vessel, ideally a plastic box with lid. All samples will be stored in controlled storage with restricted personnel access.
16. Cleaning of spillages. Full details regarding spillage handling are found in GMO Risk assessment GM/2015/04. Spill procedure: Procedures for dealing with spills must be displayed in the room. In addition all operators should undergo training including a mock spill event prior to the commencement of any work with LV.
Inactivation protocols: Lentiviral Vector Spill procedures
Lentiviral Vector Spill Procedures
1. Small spills (<2ML) in the MSC.
Cover spill with a fresh Helapet.
Once aliquotting complete remove Helapet to a biohazard bag
Spray the MSC with IPA and wipe with a sterile IPA wipes
2. Small spills (<25ML)) in the MSC
Cover spill with a dry wipe and apply Biocide E (20 mins contact time)
Remove to a biohazard bag/ container within the MSC
Remove residual Biocide E by spraying with IPA and wiping with sterile IPA wipes

Replace and contaminated PPE
3. Large spills (>25mL) in the MSC
Ensure MSC is running and remove personnel from the room.

For very large spills which may spread beneath the metal of the MSC (200mL), use barriers to contain the liquid

Place a warning sign on the door advising personnel not to enter

Allow 30 minutes for possible aerosols to settle

Cover the spill with dry wipes, apply Biocide E and allow 20 minutes to inactivate the virus

Deposit soaked wipes in a biohazard bag/container within the MSC and seal
Decontaminate the interior of the MSC by wiping down with IPA

Replace any contaminated PPE
4. Spills outside the MSC
Spills generate aerosols therefore alert all personnel present in the area

For very large spills (>100mL), use absorbent barriers to contain the spill

Exit the room and place a warning sign on the door advising personnel not to enter

Allow 30 minutes for the aerosols to settle

On re-entry to the room, wear fresh PPE and cover the spill with dry wipes or absorbent mats in a large spill

Soak with Biocide E and allow 20 minutes for the virus to be inactivated

Transfer soaked wipes to a biohazard bag/container and seal
Wipe up any residual spill with dry wipes
Wipe down with IPA

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GMSSC have agreed that this organism should be handled as a class 2 GMO and therefore requires level 2 containment measures.

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
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Animal Units

| L2 L3 L4 L2 L3 L4 |

Large Scale Activities

| L2 Yes |

| L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 |

Project Ref 566/19.1

Date Ackn'd 17/01/2019

CU2 Project Title The production and purification of 3" generation lentiviral vector to a scale up to 40L using 4 plasmid transient transfection by polyethylenimine (PEI) or calcium phosphate.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

| Class 2 1-50 Litres |

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To develop a platform production and purification method as a part of the process and research development activities; to use the established method and/or transferred method of production for the supply of the lentiviral vector for client's clinical requirements.
The production process will utilise a four plasmid vector system to generate a third generation lentiviral vector. By splitting the vector system into 4 plasmids (3 helper plasmids and 1 containing the vector genome plus transgene), the number of recombination events required to form a complete replication-competent vector increases, thereby reducing the possibility of making a replication-competent vector particle. The system is designed to produce 'self inactivating' lentiviral vector.

The components encoded by the plasmids are:
- Plasmid 1 - encodes gag (matrix and core proteins) and pol (polymerase, encoding protease, integrase and reverse transcriptase) proteins required for viral vector assembly;
- Plasmid 2 - encodes the Rev protein under a ubiquitous promoter (e.g. RSV, CMV), which acts on the rev responsive element (RRE) sequence to assist in the transportation of the viral mRNA into the cytoplasm;
- Plasmid 3 - provides the envelope function. Commonly, a broad-specificity VSV-G (vesicular stomatitis virus glycoprotein G) is used as the envelope. Any other common envelope whether with broad specificity or tissue-specific tropism can be used in this system, provided it is non-toxic and has been separately evaluated in a risk-assessment;
- Plasmid 4 - transgene encoded between 2 LTRs under control of any tissue-specific mammalian or any other non-specific promoter, depending on the disease indication for LV use. LTRs in 3rd generation lentivirus system are self-inactivating (SIN) and transcription is regulated by a mammalian promoter at the 5’ LTR.

Any common reporter transgene constructs, likely to be green fluorescent protein (GFP), Luciferase or LacZ will be used as a transgene. These transgenes are not known to be associated with any hazards for human health. Other transgenes will be determined by the client’s indication/target and will be non-oncogenic.

Recipient or parental organism

The production process will utilise a four plasmid vector system to generate a third generation lentiviral vector. By splitting the vector system into 4 plasmids (3 helper plasmids and 1 containing the vector genome plus transgene), the number of recombination events required to form a complete replication-competent vector increases, thereby reducing the possibility of making a replication-competent vector particle. The system is designed to produce 'self inactivating' lentiviral vector.

The components encoded by the plasmids are:
- Plasmid 1 - encodes gag (matrix and core proteins) and pol (polymerase, encoding protease, integrase and reverse transcriptase) proteins required for viral vector assembly;
- Plasmid 2 - encodes the Rev protein under a ubiquitous promoter (e.g. RSV, CMV), which acts on the rev responsive element (RRE) sequence to assist in the transportation of the viral mRNA into the cytoplasm;
- Plasmid 3 - provides the envelope function. Commonly, a broad-specificity VSV-G (vesicular stomatitis virus glycoprotein G) is used as the envelope. Any other common envelope whether with broad specificity or tissue-specific tropism can be used in this system, provided it is non-toxic and has been separately evaluated in a risk-assessment;
- Plasmid 4 - transgene encoded between 2 LTRs under control of any tissue-specific mammalian or any other non-specific promoter, depending on the disease indication for LV use. LTRs in 3rd generation lentivirus system are self-inactivating (SIN) and transcription is regulated by a mammalian promoter at the 5’ LTR.

Any common reporter transgene constructs, likely to be green fluorescent protein (GFP), Luciferase or LacZ will be used as a transgene. These transgenes are not known to be associated with any hazards for human health. Other transgenes will be determined by the client’s indication/target and will be non-oncogenic.

Origin & function

The lentiviral vector will be produced using 4 separate plasmids co-transfected into mammalian packaging cell line using PEI or Calcium phosphate reagents. The plasmids encode for the essential structural and genome components of the lentivirus including gene of interest to be delivered to the target cells. The plasmids are produced in E.coli, under appropriate antibiotic selection.

Intended uses of the final purified Lentiviral vector material will include: process development work, assay development work for internal use at Cobra Biologics as a part of platform process development; material supply to client and research collaborators as research or GMP grade material.

Evaluation of foreseeable effects

The risks associated with working with lentiviral vector are:
1) Lentiviral vector produces 2 proteins common to HIV: If the lentiviral vector accidentally enters the human body there is a possibility that seroconversion towards HIV-1 viral proteins could result. The antibodies raised to the lentiviral vector proteins could result in a partially positive HIV test result.

All open manipulations will be performed inside a class 2 microbial safety cabinet, (MSC); otherwise all processes will be performed within a closed system. This will virtually eliminate the risk of exposure to any lentivirus containing aerosols. PPE worn by the operator will protect from accidental dermal exposure.

2) Possibility of recombination event producing a replication competent lentiviral vector (RCL). If there was an
extremely unlikely recombination event a replication competent vector could result which could infect and replicate in humans (Cancer/AIDS may result). Recombination would only pose a significant risk if the operator was HIV positive.

The 3rd generation lentiviral vector system only will be used. This system relies on the use of 4 plasmids split gene transfection process, which is designed to prevent the formation of replication competent lentiviral vector and offers the maximal biosafety.

3) Possibility of oncogenesis via random chromosomal integration: Lentiviral vector transgene could integrate into chromosomal DNA. If the vector enters the body the transgene could integrate into the cell's chromosome. If it were to integrate within an active gene, it could cause cancer. The risk of introducing sufficient amount of lentivirus vector via accidental exposure route (e.g. inhalation of aerosols, skin exposure) is extremely low.

The most probable route of exposure for this work would be dermal via sharps (needle-sticks), absorption through exposed scratches or abrasions on the skin, or mucous membrane exposure of the eyes, nose and mouth. Another route would be inhalation via aerosols. Given the containment measures that will be used whilst handling the lentiviral vector the likelihood of accidental inoculation is low. The following measures will be adhered to by the trained operator whilst handling lentiviral vector:

1. Physical Containment: All open work will be performed in Class 2 MSCs (left in continuously). A warning sign must be posted on the door to the laboratory alerting personnel of the presence of lentiviral vector particles.
2. Personal Protective Equipment (PPE): A disposable non-sterile Tyvex suit or lab coat should be worn at all times. A fresh suit must be worn for each processing session. A full face shield will be available to be worn during operations that could result in aerosols if there was a system failure i.e. the use of peristaltic pumps. Double gloves and disposable oversleeves must be worn paying particular attention to cover bare skin at the wrists. Potentially contaminated gloves must be removed and replaced with new gloves before touching anything outside the MSC, such as the incubator.
3. Spill kit: The lab must have a spill kit comprising easy-to-read outline spill response procedures, gloves, masks, goggles, clean lab coat, paper towels to absorb contaminated liquids; disinfectant and a biohazardous waste bag.
4. Sharps: Sharps i.e. needles cannot be used.
5. Solid Waste: Everything that contacts lentiviral vector-containing solutions or vessels must be decontaminated or contained before leaving the MSC. Solid waste can be collected in a biohazard bag inside the MSC. Pipette tips must be collected in a dedicated, labelled container.
6. Liquid Waste: Must be decontaminated using sodium hypochlorite (> 5000ppm free chlorine) for 1 hour or 0.5M sodium hydroxide before drain disposal.
7. Centrifugation: Centrifuge tubes must be prepared and sealed in the MSC. The centrifuge tubes must then be sealed into lidded centrifuge rotor buckets prior to removal from the MSC. Once centrifugation is complete the sealed centrifuge buckets must be returned to the MSC prior to opening.
8. Filtration: The filter must be vented away from the MSC opening / operator.
9. All tangential flow filtration (TFF) operations will be performed within a MSC.

With all of these precautions the possibility that an operator will be exposed to the organism is extremely unlikely. Dehydration alone reduces the titre by 90% within several hours, which means that with the prescribed cleaning procedures of 70% IPA and hypochlorite (Biocide E), the possibility of any organisms escaping the class 2 containment is highly unlikely.

Risks to the environment: The lentiviral vector will only be manipulated inside an MSC. Transfer of the lentiviral vector from the MSC to storage will be performed in sealed containers with secondary containment in place. Lentiviral vectors are fragile and would not survive long in the environment; therefore the risk to the environment is negligible and classed as Class 1. All waste will be inactivated following an approved appropriate procedure.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| None |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Solid Waste: Everything that contacts lentiviral vector-containing solutions or vessels must be decontaminated or contained before leaving the MSC. Solid waste can be collected in a biohazard bag inside the MSC. Pipette tips must be collected in a dedicated, labelled container. All waste is treated with disinfectants for the stipulated period of time and solid waste is disposed in WIVA bins which are then bagged. Bags are then stored in a designated Class 2 storage container on the premises, this container is then taken by the contractor off site for incineration of the contents. Liquid Waste: Must be decontaminated using sodium hypochlorite (> 5000ppm free chlorine) or 0.5M sodium hydroxide before drain disposal. Inactivation protocols: 1) Small spills in the MSC (525mL) can be decontaminated by covering the spill with a dry wipe and applying Biocide E. The dry wipes should be removed to a biohazard bag/container within the MSC. Residual Biocide E can be removed by spraying with IPA and wiped using sterile IPA wipes. Replace any contaminated PPE. 2) Large spills (>25mL) in the MSC. The MSC must be left running and personnel removed from the room. A warning sign must be placed on the door advising personnel not to enter. At least 30 mins will be allowed for possible aerosols to settle. The spills will be covered with dry wipes, Biocide E will be applied and allowed to work for at least 20 minutes in order to inactivate the virus. Soaked wipes will be disposed off into a biohazard bag in the MSC. The interior of the MSC will be decontaminated by wiping down with IPA. Any contaminated PPE will be immediately replaced. 3) Spills outside the MSC: Spills generate aerosols therefore all personnel present in the area will be alerted of any spills. The following steps for managing spills will be adopted: 1. Remove contaminated PPE; 2. Exit the room and place a warning sign advising personnel not to enter; 3. Allow 30 minutes for aerosols to settle; 4. On re-entry to the room, wear fresh PPE and cover the spill with dry wipes; 5. Soak with Biocide E and allow 20 minutes for the virus to be inactivated; 6. Transfer soaked wipes to a biohazard bag; 7. Wipe up any residual spill with dry wipes and then wipe-down with IPA. These inactivation methods will result in a minimum of a 5-log kill. All product-contacting disposable material (solid waste), will be treated as Class 2 GMO waste and disposed of using clearly labelled WIVA bins. |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |
The Genetically Modified Biological Safety Committee at Cobra Biologics have agreed that this organism should be handled as a Class 2 GMO and therefore requires level 2 containment measures.

**Project Containment**

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**Project Ref 566/19.2**

- **Date Ackn'd**: 31/01/2019
- **CU2 Project Title**: This risk assessment covers the storage and handling of stable cell lines generated for the production of retroviral vectors. The cell lines express Retroviral vector Moloney murine leukemia virus (MoMLV)
- **Class**: Class 2
- **Culture Volume Class 2**: ≤ 1 Litre
- **Non-GMM**: Not Applicable
- **Consent Granted**: Project notified under transitional arrangements

**Project Additional Information**

- **Purposes of the contained use**: Generation of HEK293 stable producer Master Cell Bank.
- **Recipient or parental organism**: The cell lines express one or more of retroviral vector plasmids (MLV gagpol under heterologous promoter, lacking...
full packaging signal, RD114 heterologous envelope under heterologous promoter and MLV genome plasmid containing CAR proteins and/or suicide genes and/or selection markers and/or surface tags) These risk assessment will cover two CAR's targeting two different target molecules I receptors.

Host/vector system

Transient transfection of immortalised human embryonic kidney (HEK)293-related cells including but not limited to HEK293T) will be performed. HEK293 cells are a well characterised producer cell line for the production of lentivirus and other gene therapy vectors and are considered to be non-hazardous to humans. The stable producer cells are based on the HEK293 standard human cell line; cells have been genetically modified to produce retroviral vectors. The cells are unable to exist for long periods of time outside the body and are extremely sensitive to temperature, pH, osmolality and radiation. They will not replicate under normal environmental conditions and therefore do not represent an adverse risk to the environment. The retroviral vector could theoretically transduce a broad range of cell types of multiple species and theoretically express the CAR and/or suicide gene within those cells. Expression of these proteins is unlikely to cause harm if accidentally transferred to humans. Due to the inherent instability of the retroviral vector outside of the cell, the risk of this is extremely low.

The retroviral vector contains only the minimal elements required for genome packaging, integration and transgene expression and is replication deficient, in the unlikely event of accidental transfer to human, it will not propagate. The separation of the 3 components and the absence of significant regions of homology between components (gagpol, envelope and genome) minimises the chance of production of replication-competent virus due to recombination events. Retroviral transduced cells are not expected to be of any greater hazard than the original unmodified cells.

Origin & function

Evaluation of foreseeable effects

1) There are only two theoretical scenarios for in which the cells could pose a risk to human health; accidental release during transport or transfer via needle stick injury. Thus the risk to human health takes precedence.
2) There are only two theoretical scenarios for AUTO2, AUTO3 and AUTO4 transmission; namely accidental release during transport or transfer via needle stick injury. Accidental release would result in environmental exposure, but with no adverse effects expected due to the nonpersistence or replication of cells.
   Needle stick injury could introduce modified cells into the exposed person. Under these circumstances, the cells would be recognised as non-self cells (allo-reaction) and be eliminated through the individual's innate and adaptive immune system. Given the small magnitude of possible dose, adverse event would likely be limited to a normal immune reaction to non-self cells.
3) The retroviral vectors produced will be replication deficient vectors derived from either MoMLV or HIV. The vectors are replication deficient by design since the viral proteins that confer infectivity and replication are not present within the viral RNA packaged into vector particles. The vector can thus undergo one round of transduction only. The only way for the retroviral vector to become replication competent would be by a series of recombination events. In order to minimise this possibility, the genes encoding viral proteins have been separated onto different plasmids using the so-called split packaging design. Furthermore, the plasmids used in the production of vectors do not contain homology with each other and thus the generation of replication competent retroviral vectors by homologous recombination is extremely unlikely.
Accidental insertion of the vector genome into human cells could theoretically lead to insertional mutagenesis in the transduced cells; the likelihood of the latter is extremely low. Any vector that may enter the body, either by needle stick injury or other means, would be inactivated by human serum. RD114 pseudotyped retroviral vectors have a half-life of 4-8 hours at 37°C, and although no conclusive data is available on the half-life of RD114 envelope in the presence of sera, VSV-G pseudotyped retroviral vectors have an in vivo half-life in the order of a few minutes (DePolo et al. 2000). Furthermore, cell transduction by retroviral vectors in the absence of facilitating agents, such as RetroNectin®️, is very inefficient. It is unlikely a clinical event would be detected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The following measures detailed in points 1–5 must be adhered to whilst handling the stable producer cell line.

Inactivation protocols:
1) Solid Waste: Everything that contacts the stable producer cell line must be decontaminated or contained before leaving the Class II MSC. Solid waste must be collected in a biohazard bag inside the Class II MSC. Pipette tips must be collected in a dedicated labelled container.
2) Liquid Waste: Liquid waste must be decontaminated using 1% final concentration sodium hypochlorite (>5000ppm free chlorine) for 1 hour before drain disposal.
3) Small spills in the Class II MSC <25mL can be decontaminated by covering the spill with a dry wipe and applying Klercide Sporicidal Active Chlorine. The dry wipes must be removed to a biohazard waste bag within the Class II MSC. Residual Klercide Sporicidal Active Chlorine can be removed by spraying with IPA and wiping using a sterile IPA wipe. Replace any contaminated PPE.
4) Large spills in the MSC >25mL. The MSC must be left running and personnel evacuated from the room. A warning sign must be placed on the door instructing personnel not to enter. Allow 30 minutes for any possible aerosols to settle. Return to the spill and cover with dry wipes, apply Klercide Sporicidal Active Chlorine and allow 20 minutes to inactivate the retroviral vector. Deposit soaked wipes in a biohazard bag within the Class II MSC. Decontaminate the interior of the Class II MSC by wiping with a sterile IPA wipe. Replace any contaminated PPE.
5) Spill outside the MSC generate aerosols therefore alert all personnel present in the area. Remove contaminated PPE and exit the room, place a warning sign instructing personnel not to enter. Allow 30 minutes for any potential aerosols to settle. On re-entry to the room wear fresh PPE and cover the spill with dry wipes. Soak with Klercide Sporicidal Active Chlorine and allow 20 minutes for the retroviral vector to be inactivated. Transfer soaked wipes to a biohazard bag. Wipe any residual spill with sterile IPA wipes.

All product contacting disposable material (solid waste), will be treated as Class 2 GMO waste and disposed of using WIVA bins or red plastic hazardous waste bags as appropriate.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The Genetically Modified Biological Safety Committee at Cobra Biologics have agreed that this organism should be handled as a Class 2 GMO and therefore requires level 2 containment measures.

Project Containment

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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 566/20.1

This risk assessment covers the receipt, storage, and handling of stable cell lines generated for the production of retroviral vectors. The cell lines express retroviral vector Moloney murine leukemia virus (MoMLV).
Purposes of the contained use

Generation of H EK293T stable producer Master Cell Bank.

Recipient or parental organism

The cell line expresses one or more of retroviral vector plasmids (MLV gagpol under heterologous promoter, lacking full packaging signal, RD114 heterologous envelope under heterologous promoter and MLV genome plasmid containing CAR proteins and/or suicide genes and/or selection markers and/or surface tags).

Host/vector system

HEK293T cells are a well characterised producer cell line for the production of lentivirus and other gene therapy vectors. These cell lines are considered to be non-hazardous to humans. The stable producer cells are based on the HEK293T standard human cell line; cells have been genetically modified to produce retroviral vectors. The cells are unable to exist for long periods of time outside the body and are extremely sensitive to temperature, pH, osmolality and radiation. They will not replicate under normal environmental conditions and therefore do not represent an adverse risk to the environment. The retroviral vector could theoretically transduce a broad range of cell types of multiple species and theoretically express the CAR, co-receptors, and/or suicide gene within those cells. Expression of these proteins is unlikely to cause harm if accidentally transferred to humans. Due to the inherent instability of the retroviral vector outside of the cell, the risk of this is extremely low. The retroviral vector contains only the minimal elements required for genome packaging, integration and transgene expression and is replication deficient, in the unlikely event of accidental transfer to human, it will not propagate. The separation of the 3 components and the absence of significant regions of homology between components (gagpol, envelope and genome) minimises the chance of production of replication-competent virus due to recombination events. Retroviral transduced cells are not expected to be of any greater hazard than the original unmodified cells.

Origin & function

Evaluation of foreseeable effects

1) There are only two theoretical scenarios in which unmodified HEK293T cells could pose a risk to human health; accidental release during transport or transfer via needle stick injury. Thus the risk to human health takes precedence.
2) There are only two theoretical scenarios for transmission; namely accidental release during transport or transfer via needle stick injury. Accidental release would result in environmental exposure, but with no adverse effects expected due to the nonpersistence or replication of cells.
   Needle stick injury could introduce modified cells into the exposed person. Under these circumstances, the cells would be recognised as non-self cells (allo-reaction) and be eliminated through the individual's innate and adaptive immune system. Given the small magnitude of possible dose, adverse event would likely be limited to a normal immune reaction to non-self cells.
3) The retroviral vector is spontaneously expressed into supernatant by the cell line during growth. The MoM LV-derived vector is replication deficient by design since the viral proteins that confer infectivity and replication are not present within the viral RNA packaged into vector particles. The vector can thus undergo one round of transduction only. The only way for the retroviral vector to become replication competent would be by a series of
recombination events. In order to minimise this possibility, the genes encoding viral proteins have been separated onto different plasmids using the so-called split packaging design. Furthermore, the plasmids used in the production of vectors do not contain homology with each other and thus the generation of replication competent retroviral vectors by homologous recombination is extremely unlikely.

Accidental insertion of the vector genome into human cells could theoretically lead to insertional mutagenesis in the transduced cells; the likelihood of the latter is extremely low.

Any vector that may enter the body, either by needle stick injury or other means, would be inactivated by human serum. RD114 pseudotyped retroviral vectors have a half-life of 4-8 hours at 3°C, and although no conclusive data is available on the half-life of RD114 envelope in the presence of sera, VSV-G pseudotyped retroviral vectors have an in vivo half-life in the order of a few minutes (DePolo et al. 2000). Furthermore, cell transduction by retroviral vectors in the absence of facilitating agents, such as RetroNectin®, is very inefficient. It is unlikely a clinical event would be detected.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The following measures detailed in points 1-5 must be adhered to whilst handling the stable producer cell line.

**Inactivation protocols:**

1) **Solid Waste:** Everything that contacts the stable producer cell line must be contained before leaving the Class II MSC. Solid waste, such as cell culture flasks, media bottles, and reagent or waste vessels must have lids tightly secured inside the Class II MSC, ready to be collected in a biohazard bag immediately upon removal. Stripettes must be re-sheathed, and pipette tips must be collected in a dedicated labelled container.

2) **Liquid Waste:** Liquid waste must be decontaminated using 1% final concentration sodium hypochlorite (>5000ppm free chlorine) or sodium hydroxide (1 M) for a minimum of 1 hour before drain disposal.

3) **Small spills in the Class II MSC <25mL** can be decontaminated by covering the spill with a dry wipe and applying an appropriate volume of Klercide Sporicidal Active Chlorine. Cell culture flasks and/or vessels containing cell suspension must be wiped with 70/130 IPA and removed from the Class II MSC before application of Klercide Sporicidal Active Chlorine. The contaminated dry wipes must be removed to a biohazard waste bag within the Class II MSC. Residual Klercide Sporicidal Active Chlorine can be removed by spraying with IPA and wiping using a sterile IPA wipe. Replace any contaminated PPE.

4) **Large spills in the MSC >25mL.** The MSC must be left running and personnel evacuated from the room. A warning sign must be placed on the door instructing personnel not to enter. Allow 30 minutes for any possible aerosols to settle. Return to the spill and cover with dry wipes, apply Klercide Sporicidal Active Chlorine and allow 20 minutes to inactivate the retroviral vector. Deposit soaked wipes in a biohazard bag within the Class II MSC. Decontaminate the interior of the Class II MSC by wiping with a sterile IPA wipe. Replace any contaminated PPE.

5) **Spill outside the MSC generate aerosols therefore alert all personnel present in the area.** Remove contaminated PPE and exit the room, place a warning sign instructing personnel not to enter. Allow 30 minutes for any potential aerosols to settle. On re-entry to the room wear fresh PPE and cover the spill with dry wipes. Soak with Klercide Sporicidal Active Chlorine and allow 20 minutes for the retroviral vector to be inactivated. Transfer soaked wipes to a biohazard bag. Wipe any residual spill with sterile IPA wipes.
All product contacting disposable material (solid waste), will be treated as Class 2 GMO waste and disposed of using WIVA bins or red plastic hazardous waste bags as appropriate.

The Genetically Modified Biological Safety Committee at Cobra Biologics have agreed that this organism should be handled as Class 2 GMO and therefore requires level 2 containment measures.

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Project Ref 566/20.2

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<td>1-50 Litres</td>
<td>Non-GMM Consen granted</td>
<td>N</td>
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</tbody>
</table>

Historical Significant Changes

Tick if notifying a connected programme of work N
### Project Additional Information

#### Purposes of the contained use

| Generation of HEK293T stable producer Master Cell Bank |

#### Recipient or parental organism

| HEK293T cells are a well characterised producer cell line for the production of lentivirus and other gene therapy vectors. These celllinse are considered to be non-hazardous to humans. The stable producer cells are based on the HEK293T standard human cell line; cells have been genetically modified to produce retroviral vectors. The cells are unable to exist for long periods of time outside the body and are extremely sensitive to temperature, pH, osmolality and radiation. They will not replicate under normal environmental conditions and therefore do not represent an adverse risk to the environment. The retroviral vector could theoretically transduce a broad range of cell types of multiple species and theoretically express the CAR, co-receptors, and/or suicide gene within those cells. Expression of these proteins is unlikely to cause harm if accidentally transferred to humans. Due to the inherent instability of the retroviral vector outside of the cell, the risk of this is extremely low. The retroviral vector contains only the minimal elements required for genome packaging, integration and transgene expression and is replication deficient, in the unlikely event of accidental transfer to human, it will not propagate. The separation of the 3 components and the absence of significant regions of homology between components (gagpol, envelope and genome) minimises the chance of production of replication-competent virus due to recombination events. Retroviral transduced cells are not expected to be of any greater hazard than the original unmodified cells. |

#### Host/vector system

| Origin & function |

---

02/03/2022
Evaluation of foreseeable effects

1) There are only two theoretical scenarios in which unmodified HEK293T cells could pose a risk to human health; accidental release during transport or transfer via needle stick injury. Thus the risk to human health takes precedence.
2) There are only two theoretical scenarios for AUT04 transmission; namely accidental release during transport or transfer via needle stick injury.

Accidental release would result in environmental exposure, but with no adverse effects expected due to the nonpersistence or replication of cells.

Needle stick injury could introduce modified cells into the exposed person. Under these circumstances, the cells would be recognised as non-self cells (allo-reaction) and be eliminated through the individual’s innate and adaptive immune system. Given the small magnitude of possible dose, adverse event would likely be limited to a normal immune reaction to non-self cells.

3) The retroviral vector is spontaneously expressed into supernatant by the AUT04 cell line during growth. The MoM LV-derived vector is replication deficient by design since the viral proteins that confer infectivity and replication are not present within the viral RNA packaged into vector particles. The vector can thus undergo one round of transduction only. The only way for the retroviral vector to become replication competent would be by a series of recombination events. In order to minimise this possibility, the genes encoding viral proteins have been separated onto different plasmids using the so-called split packaging design. Furthermore, the plasmids used in the production of vectors do not contain homology with each other and thus the generation of replication competent retroviral vectors by homologous recombination is extremely unlikely.

Accidental insertion of the vector genome into human cells could theoretically lead to insertional mutagenesis in the transduced cells; the likelihood of the latter is extremely low.

Any vector that may enter the body, either by needle stick injury or other means, would be inactivated by human serum. RD114 pseudotyped retroviral vectors have a half-life of 4-8 hours at 3rC, and although no conclusive data is available on the half-life of RD114 envelope in the presence of sera, VSV-G pseudotyped retroviral vectors have an in vivo half-life in the order of a few minutes (DePolo et al. 2000). Furthermore, cell transduction by retroviral vectors in the absence of facilitating agents, such as RetroNectin®, is very inefficient. It is unlikely a clinical event would be detected.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The following measures detailed in points 1-5 must be adhered to whilst handling the stable producer cell line.

Inactivation protocols:

1) Solid Waste: Everything that contacts the stable producer cell line must be contained before leaving the Class II MSC. Solid waste, such as cell culture flasks, media bottles, and reagent or waste vessels must have lids tightly secured inside the Class II MSC, ready to be collected in a biohazard bag immediately upon removal. Stripettes must be re-sheathed, and pipette tips must be collected in a dedicated labelled container.

2) Liquid Waste: Liquid waste must be decontaminated using 1 % final concentration sodium hypochlorite (>5000ppm free chlorine) or sodium hydroxide (1 M) for a minimum of 1 hour before drain disposal.

3) Small spills in the Class II MSC <25mL can be decontaminated by covering the spill with a dry wipe and applying...
an appropriate volume of Klercide Sporicidal Active Chlorine. Cell culture flasks and/or vessels containing cell suspension must be wiped with 70/30 IPA and removed from the Class II MSC before application of Klercide Sporicidal Active Chlorine. The contaminated dry wipes must be removed to a biohazard waste bag within the Class II MSC. Residual Klercide Sporicidal Active Chlorine can be removed by spraying with IPA and wiping using a sterile IPA wipe. Replace any contaminated PPE.

4) Large spills in the MSC >25mL. The MSC must be left running and personnel evacuated from the room. A warning sign must be placed on the door instructing personnel not to enter. Allow 30 minutes for any possible aerosols to settle. Return to the spill and cover with dry wipes, apply Klercide Sporicidal Active Chlorine and allow 20 minutes to inactivate the retroviral vector. Deposit soaked wipes in a biohazard bag within the Class II MSC. Decontaminate the interior of the Class II MSC by wiping with a sterile IPA wipe. Replace any contaminated PPE.

5) Spill outside the MSC generate aerosols therefore alert all personnel present in the area. Remove contaminated PPE and exit the room, place a warning sign instructing personnel not to enter. Allow 30 minutes for any potential aerosols to settle. On re-entry to the room wear fresh PPE and cover the spill with dry wipes. Soak with Klercide Sporicidal Active Chlorine and allow 20 minutes for the retroviral vector to be inactivated. Transfer soaked wipes to a biohazard bag. Wipe any residual spill with sterile IPA wipes.

All product contacting disposable material (solid waste), will be treated as Class 2 GMO waste and disposed of using WIVA bins or red plastic hazardous waste bags as appropriate.

The Genetically Modified Biological Safety Committee at Cobra Biologics have agreed that this organism should be handled as Class 2 GMO and therefore requires level 2 containment measures.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if an emergency plan required according to regulation 20?

Tick to confirm that it is attached to this form

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>
This risk assessment covers the receipt, storage, and handling of stable cell lines generated for the production of retroviral vectors. The cell lines express retroviral vector Moloney murine leukemia virus (MoMLV).

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Generation of HEK293T stable producer Master Cell Bank.

**Recipient or parental organism**

**Host/vector system**

HEK293T cells are a well characterised producer cell line for the production of lentivirus and other gene therapy vectors. These cell lines are considered to be non-hazardous to humans.

The stable producer cells are based on the HEK293T standard human cell line; cells have been genetically modified to produce retroviral vectors. The cells are unable to exist for long periods of time outside the body and are extremely sensitive to temperature, pH, osmolality and radiation. They will not replicate under normal environmental conditions and therefore do not represent an adverse risk to the environment.

The retroviral vector contains only the minimal elements required for genome packaging, integration and transgene expression and is replication deficient, in the unlikely event of accidental transfer to human, it will not propagate.

The separation of the 3 components and the absence of significant regions of homology between components (gagpol, envelope and genome) minimises the chance of production of replication-competent virus due to recombination events.

Retroviral transduced cells are not expected to be of any greater hazard than the original unmodified cells.
Evaluation of foreseeable effects

1) There are only two theoretical scenarios in which unmodified HEK293T cells could pose a risk to human health; accidental release during transport or transfer via needle stick injury. Thus the risk to human health takes precedence.
2) There are only two theoretical scenarios for HEK293T transmission; namely accidental release during transport or transfer via needle stick injury.
   Accidental release would result in environmental exposure, but with no adverse effects expected due to the nonpersistence or replication of cells.
   Needle stick injury could introduce modified cells into the exposed person. Under these circumstances, the cells would be recognised as non-self cells (allo-reaction) and be eliminated through the individual's innate and adaptive immune system. Given the small magnitude of possible dose, adverse event would likely be limited to a normal immune reaction to non-self cells.
3) The retroviral vector is spontaneously expressed into supernatant by the HEK293T cell line during growth. The MoMLV-derived vector is replication deficient by design since the viral proteins that confer infectivity and replication are not present within the viral RNA packaged into vector particles. The vector can thus undergo one round of transduction only. The only way for the retroviral vector to become replication competent would be by a series of recombination events. In order to minimise this possibility, the genes encoding viral proteins have been separated onto different plasmids using the so-called split packaging design. Furthermore, the plasmids used in the production of vectors do not contain homology with each other and thus the generation of replication competent retroviral vectors by homologous recombination is extremely unlikely.
   Accidental insertion of the vector genome into human cells could theoretically lead to insertional mutagenesis in the transduced cells; the likelihood of the latter is extremely low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The following measures detailed in points 1-5 must be adhered to whilst handling the stable producer cell line.

Inactivation protocols:
1) Solid Waste: Everything that contacts the stable producer cell line must be contained before leaving the Class II MSC. Solid waste, such as cell culture flasks, media bottles, and reagent or waste vessels must have lids tightly secured inside the Class II MSC, ready to be collected in a biohazard bag immediately upon removal. Stripettes must be re-sheathed, and pipette tips must be collected in a dedicated labelled container.
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MSC. Residual Klercide Sporicidal Active Chlorine can be removed by spraying with IPA and wiping using a sterile IPA wipe. Replace any contaminated PPE.

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Decontaminate the interior of the Class II MSC by wiping with a sterile IPA wipe. Replace any contaminated PPE.

5) Spill outside the MSC generate aerosols therefore alert all personnel present in the area. Remove contaminated PPE and exit the room, place a warning sign instructing personnel not to enter. Allow 30 minutes for any potential aerosols to settle. On re-entry to the room wear fresh PPE and cover the spill with dry wipes. Soak with Klercide Sporicidal Active Chlorine and allow 20 minutes for the retroviral vector to be inactivated. Transfer soaked wipes to a biohazard bag. Wipe any residual spill with sterile IPA wipes.

All product contacting disposable material (solid waste), will be treated as Class 2 GMO waste and disposed of using VVIVA bins or red plastic hazardous waste bags as appropriate.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The Genetically Modified Biological Safety Committee at Cobra Biologics have agreed that this organism should be handled as Class 2 GMO and therefore requires level 2 containment measures. Previous HEK293T stable producer cell lines authorised for use related to this Connected Program of Work have been classified as Class II GMOs. This Connected Program of Work will cover prospective HEK293T cell lines, which are stable producers of Moloney murine leukemia virus (MoMLV). Assessment will be made by the Cobra Biologics GMBSC on a case-by-case basis to determine whether this Connected Program of Work is applicable.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</table>
CONSTRUCTION OF AN ADENOVIRUS VECTOR FOR GENE THERAPY

Class 2
1-50 litres

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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Animal Units

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**Name**

MANCHESTER METROPOLITAN UNIVERSITY

**Name 2**

**Department**

BIOLOGICAL SCIENCES

**Building**

JOHN DALTON BUILDING

**District**

**Road Name**

CHESTER ST

**Town**

MANCHESTER

**County**

GREATER MANCHESTER

**Postcode**

M1 5GD

**Country**

ENGLAND

**Tel Number**

0161 247 1234

**Fax Number**

0161 247 6325

**E-mail**

**HSE Division**

NORTH WEST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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<th>Department</th>
<th>Name 2</th>
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<th>Building</th>
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<td>BIOLOGICAL SCIENCES</td>
<td>CHESTER STREET</td>
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<td>CHESHIRE</td>
<td>M1 5GD</td>
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<tr>
<td>MANCHESTER METROPOLITAN UNIVERSITY</td>
<td>FACULTY OF SCIENCE &amp; ENGINEERING</td>
<td>JOHN DALTON BUILDING</td>
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<td>M1 5GD</td>
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</tbody>
</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The committee consists of 8 members:

- GMO is a senior lecturer with over 15 yrs experience in cloning at the Max-Planck Institute and Newcastle University, GMO is a senior lecturer with over 15 years experience in cloning at the Max-Planck Institute and Newcastle University, involving various cell systems and constructs.
- Head technician of the 1st & 3rd floor research labs who has prior experience in GM at Manchester University.
- Head technician of the teaching labs on the 4th floor who has experience in cloning and microbiology.
- Health and Safety trade union representative.
- Professor with prior experience as a GMO and BSO at Manchester University. Also has experience with lenti-viruses.
- Senior research fellow with experience in cloning and lenti-viruses and being part of a GMO committee at Manchester University.
- Postdoctoral Research fellow with experience in GM work at Christies.
- Senior lecturer with experience in yeast mutagenesis.
- Head of division of Biology & Conservation Ecology with expertise in microbial ecology and bacterial transformation.

The committee intends to meet 4 times a year. Appraisals will be assessed in the form of an internal document detailing the project, vectors and cells being used and possible hazards - these will be reviewed by the committee to determine which class the project will fall under. Modifications will also be reviewed.

Training will also be given and reviewed and signed documentations gathered

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
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<td>Level 1 (GMMs)</td>
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<tr>
<td>Level 2 (GMMs)</td>
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</tbody>
</table>
Waste Disposal
Solid waste (plastics): 2% Virkon overnight. The manufacturer specifies that this will give 99.9999% kill. The decontaminated waste will be then autoclaved prior to disposal. After autoclaving solid waste is disposed of as normal refuse.

Liquid waste: will be autoclaved, 134 degrees centigrade applied including air extraction, heating and steaming for 30 minutes. The manufacturer specifies that this will give 100% kill. After autoclaving liquid waste will be disposed down a designated sink in a GM area with copious amounts of water.

Monitoring
Disinfection: The disinfectant of choice (Virkon) is used in strict accordance with the manufacturer's (Antec's) guidelines.

Autoclaving: To ensure 100% efficacy, testing of the autoclave is carried out annually, by the manufacturer, to demonstrate, using a 12 point test with independent thermocouples, that the correct temperature and pressure have been reached for the required time. On subsequent runs verification that the correct conditions were reached is obtained through use of a temperature probe in the centre of the load. Testing is arranged and test reports are kept by the Health and Safety Co-ordinator.

Inspections: Safety inspections are carried out annually to ensure Local Rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health & Safety Co-ordinator. Microbiological Safety Cabinets: Services and KI tested on annual basis. Certificates of conformity are displayed on each cabinet and copies kept by the Health and Safety Information Officer.
The GMC have in place an "acknowledgement of training" form that those listed in the risk assessment must sign. Those listed in the risk assessment must add their names to the list of GMO workers kept by the GMC.

The risk assessment is valid for 5 years. Any use of organisms and genetic material and work spaces not covered in the risk assessment is valid for 5 years. Any use of organisms and genetic material and work spaces not covered in the risk assessment must be notified to the GMC through filling in the modifications section.

Any Class II activities are not covered by this form and a separate CU2 must be prepared and submitted (the GMC can advise and support completion and submission).

---

**Project Ref** 567/16.1

**Date Ackn'd** 24/02/2016

**CU2 Project Title** Genetic manipulation of mammalian cells in culture to study cellular signaling mechanisms in normal development and disease

**Class** Class 2

**CultureVol** < 1 Litre

**CultureVolumeClass**

---

**Non-GMM Consent Granted**

---

**Project notified under transitional arrangements**

---

**Historical Significant Changes**

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**Historical Date of Additional Info**

---

**Date of Significant Change**

---

**Project Additional Information**

**Purposes of the contained use**

Development of vectors (viral and plasmid) and molecules for the study of cellular signaling processes in normal development and disease. This will involve transgene over-expression, gene knockdown, cellular reprogramming and directed differentiation. All gene modifier vectors are disabled and lack essential genes which prevent their autonomous replication. All require specific packaging cell types to produce a viral particle which is replication deficient and cannot multiply or produce progeny. All vectors will have no effect to the environment as they will be maintained and contained under strict laboratory conditions.

**Recipient or parental organism**

**Recipient Cells**

Mammalian cell lines (for example 293T, 3T3, CHO, HepG2, Huh7, U2OS, HeLa, PC3, DU145).

Primary human and mammalian cells (for example fetal and adult fibroblasts, blood cells, and vascular endothelial cells).
Human and mouse embryonic stem cells and adult stem cells either obtained commercially or from collaborators and repositories or from biopsy material (for example; mesenchymal stem cells, cardiac and vascular stem cells, pancreatic/hepatobiliary progenitors).

Human embryonic cells and primary cells or tissues are obtained by donation after pathogen screening (HIV, Hepb/c). Human primary cells are donated from unscreened patients and are quaranteened pending the pathogen screening as described prior to genetic modification.

Viral Vectors
Retroviral vectors used are derived from Moloney murine leukaemia virus (MMLV), a mouse virus therefore chances of mobilisation by recombination with endogenous viruses in a human are practically impossible. Furthermore, the vectors contain no MMLV protein coding sequences and so are completely replication disabled and the chances of recombination are minimised.

Lentiviral vectors derived from HIV1, SIV, EIAV and FIV are based on genome integrating viruses. Like retroviruses, the severely deleted genomes contain no endogenous protein coding sequences and the 3'-Long Terminal Repeat (LTR) contains a self-inactivating mutation that prevents the replication cycle initiating after reverse transcription of the RNA viral genome.

Adenoviral vectors (serotypes Ad2/5) are disabled by mutations to the E1a and E3 genes that are fundamental to the early stages of viral replication. Although adenoviruses are human pathogens infection does not result in serious illness and as such are a low risk to humans. The viral genome is transient in infected cells (does not naturally integrate into the host genome) and so there is minimal risk of insertional mutagenesis.

Adeno-associated Viral vectors (AAV) are disabled by mutations to the Rep gene which is necessary and fundamental to replication. Furthermore, AAV requires the expression of adenoviral genes to facilitate its replication. Recombinant forms of AAV will integrate into the host cell genome at very low frequency and so the chances of insertional mutagenesis are low.

Host/vector system

Origin & function
Transgenic material - Human and mammalian cDNAs of transcribed genes involved in aspects of cellular transformation, self-renewal, differentiation, proliferation, senescence, apoptosis and metabolism will be expressed from pol II promoters. These genes would include transcription factors, signaling molecules, enzymes, growth factors and cytokines.

Such genes would include but are not limited to:
- Transformation; hTERT, BMI-1, C-MYC
- Self-renewal; OCT4, Nanog, SOX2, LIN28, KLF4, STAT3
- Differentiation: Wnt, PI3K, Notch, Hedgehog, TGFbeta, BMP, MAPK, ERK, JNK, RAS, NFkB
- Cell adhesion, migration & communication: ICAM-1, VCAM-1, Selectins, RhoGTPases, Ezrin, PDK1, Cadherins, B-Catenin, HGF, eNOS.
- Hypoxic Responses: HIF, ARNT, C-MET
- Innate Immunity: TLRs, RLRs, TBK, NFkB, IRF3, IRF7

The above gene targets will also be subject to gene knockdown with siRNA and miRNA expressed from pol II/III promoters.

Bacterial Cre-recombinase for removal of LoxP flanked gene sequences.

Reporter genes will also be expressed from endogenous and viral promoters with or without additional enhancer elements either constitutively or conditionally to mark cells or quantify surrogate gene expression. These will include;
Flourescent- e.g. GFP, YFP, CFP, dsRed, mCherry.
Luminescent- e.g. firefly and renilla, gaussia, vargula luciferases.
Biochemical marker genes e.g. CAT, AFP.

**Evaluation of foreseeable effects**

The recombinant viral vectors are all highly disabled vectors.

**Lentivirus/retrovirus:** These self-inactivating vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/ infection cycles. Whilst the envelope pseudotyping extends the cellular tropism and confers greater stability the viral vector will always be contained within a class II safety cabinet thus user exposure to liquid aerosol is minimised.

The exposure of non-human hosts to pseudotyped vector could result in initial infection but, again seroconversion is highly unlikely.

Adenoviral vectors are deleted of essential replication/packaging genes that are supplemented by an established packaging cell line (293T).

Adeno-associated Virus is defective by nature and has a replication disabled genome and is incapable of replication without the presence of helper adenovirus proteins. In this instance the vectors used would require the provision of cap and rep genes in trans in order to replicate and disseminate. There is no significant chance of reversion to the wild-type. Furthermore, the AAV8 & 5 subtypes derived from humans are not able to replicate or cause disease in any animal species therefore it highly unlikely that such a virus, if released into the environment, would represent a significant risk. Furthermore, most AAVs proposed for use constitute AAV5 genomes pseudotyped with other AAV envelopes so would never occur in nature and thus would be incapable of reversion.

The majority of the gene products expressed are expected to affect cellular properties such as proliferation, apoptosis, migration, adhesion and fate. Also, some have known or suspected oncogenic or tumour suppressor properties.

Gene products in plasmid form exist in non-mobilisable constructs and are therefore of minimal environmental risk. Viral vector expressing potential oncogenes are a potential environmental risk as host infection could elicit a transformation event. All work is carried out in a class II cabinet and all liquid waste inactivated by virkon treatment and solid waste autoclaved.

Primary cells infected with viral vector containing potential oncogene would pose minimal threat as expression would most likely be restricted to the infected cell, which in itself would be rejected by the host immune system.

Sharps will not be used in the production or handling of any of the viral vectors described in this project.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not Applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All viral production, handling and experimental procedures will occur in containment level 2 laboratories (designated cell culture and tissue culture on the 1st and 3rd Floor John Dalton Building). The laboratories have restricted access, through a card key and number pad only accessible by staff and authorised personnel trained in containment level 2 GM use.

Non-viral work will occur in Class 1 conditions.
Liquid waste will be treated with freshly prepared 1% Virkon or 10% hypochlorite solution for at least one hour prior to disposal via the sink. Solid waste will be autoclaved at 134°C on a destruct cycle prior to be placed in Tiger striped bags for disposal. Sharps will be placed in yellow sharps boxes that will be first autoclaved then removed by the waste contractor for incineration. Cryovials containing virus will be stored in specified cryofreezer and opened only in Class II microbiological safety cabinets. Spillages and working surfaces will be cleaned with 2% Distel or 1% Virkon and with 70% ethanol.

Solid waste that has not been autoclaved will be placed in clearly labelled biohazard double bags will be incinerated as clinical waste.

Hazard conditions consist of possible spillages, aerosol spray when opening cryovials.

Work is restricted to determined areas (containment level 2 laboratories), disinfecting solutions will always be available, working guidelines are clearly posted, unauthorised personnel is not permitted. Strict adherence to Category II working practices. Reporting in case of accident is according to College procedures using the online or paper accident / incident forms or by contacting the Safety, Health and Environment Office directly on extn. 5365.

Training to work with class II GMM's will include Instruction of the guidelines regarding dressing code, how to treat spillages and disinfection with 2% Distel, 1% Virkon and 70% ethanol.

Documented spillage procedures are clearly established. In case of accident with sharps, College procedure with blood borne viruses will be followed.

Autoclaves are regularly serviced and checked. Autoclaved material is checked using autoclaving tape and spore strips to assess has reached appropriate temperature for disinfection.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification and Biological Agents Committee at the School of Healthcare Science, Manchester Metropolitan University met on the 17th December 2015 and have considered the risk assessment for this project and consider it to be suitable and appropriate and agree with the allocation of the final containment level 2.

The committee agreed the following:
Brief reports will be made and documented of GM2 agents produced and disposed of will be made.
Acknowledgement of training for all individuals engaged in GM2 activities.
All staff are made aware of GM2 activities within the School and their location within specific units.
All GM2 activities will be carried out in designated tissue culture facilities.

Project Containment

| Laboratory Activities | Glass Houses | Growth Rooms |

02/03/2022
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Name
MACAULAY LAND USE RESEARCH INSTITUTE

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

District
CRAIGIEBUCKLER

Town
ABERDEEN

County
ABERDEENSHIRE

Postcode
AB15 8QH

Country
SCOTLAND

Tel Number
01224 318611

Fax Number
01224 311556

E-mail

HSE Division
SCOTLAND

Comments
MERGED WITH GM 250 SCOTTISH CROP RESEARCH INSTITUTE 01/04/2011 NOW CALLED JAMES HUTTON RESEARCH INSTITUTE

Date at Which Additional Info Submitted
02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All GMO materials are autoclaved at 121 degrees C at 15 psi for 20 minutes before disposal in three successive cycles. The activity of the autoclave is automatically logged.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Level 1 (GMMs)

Level 2 (GMMs)  Yes

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  Tick if confidential

Bacteriology  Parasitology  Transgenic  Microbiology

Virology  Transgenic Animals  Transgenic Fish  Gene Therapy

02/03/2022  Page 10029 of 15326
Mycology | Transgenic | Transgenic | Other (please specify below)
---|---|---|---
Invertebrates | Plants |  |

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref** 570/00.1

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Withdrawn  N  

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022  Page 10030 of 15326
### Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

### Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [N]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]  

Please enter comments on the GM safety committee on the risk assessment

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## Project Containment

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Measurement of survival of Pseudomonas aeruginosa following bacteriophage infection.

Pseudomonas aeruginosa expressing green fluorescent protein will be co-cultured with an established and well characterised human bronchial epithelial cell line to mimic an infection. Wild-type Pseudomonas-specific bacteriophage will be introduced and their ability to kill the bacteria determined. The eventual aim of this project is to determine if such bacteriophage could be useful as a novel treatment for chest infections in patients with cystic fibrosis.

PAO1 is an ACDP Hazard Group 2 organism which only presents a risk to cystic fibrosis patients, immunocompromised individuals or those with severe burns. Thus the containment level for the GMO can be no lower than Category 2.

Recipient or parental organism

PAO1 is an ACDP Hazard Group 2 organism which only presents a risk to cystic fibrosis patients, immunocompromised individuals or those with severe burns. Thus the containment level for the GMO can be no lower than Category 2.

Host/vector system

The core construct is a standard pUCP18 vector incorporating a fragment of the lacZ gene for blue-white selection. pUCP18 is an E. coli-P. aeruginosa shuttle vector carrying a 1.8kb fragment of pRO1614 which confers stability in Pseudomonas and is inserted into a non-essential region of pUC1 8. The gene coding for green fluorescent protein was inserted into the MCS as a 700bp HindIII/BamHI fragment to form vector pLS10. This vector would be assigned to Class 1 if it were carried in a species with a lower ACDP hazard categorisation.
Both construction of the recombinant plasmid and transformation of host bacteria were performed by others (Feldman et al. 1998, Cormack et al., 1996). pLS1 0 does not carry sequences which are predicted to alter the pathogenic traits of P. aeruginosa with respect to virulence, infectivity, immunogenicity, or host range (SACGM compendium of Guidance Part 2, page 15). pLS1O carries an ampicillin resistance gene (bla) as a selectable marker in E. coli. However as P. aeruginosa possesses its own endogenous beta-lactamases, treatment with most beta-lactam antibiotics would not be attempted, thus prophylactic options are unaffected. This additional bla gene increases the MIC (minimum inhibitory concentration) of beta-lactam antibiotics for P. aeruginosa e.g. the MIC of Carbenicillin for wild type PAO1 is 64 microg/ml (Kwon etal., 2006) ,with the addition of the bla gene on pLS1O this increases to >300 microg/mL The gene for green fluorescent protein is expressed from this construct allowing visualisation of the GMM under ultraviolet light.

References

Evaluation of foreseeable effects
Pseudomonas aeruginosa is widespread throughout the environment and poses a low risk to animals (Rhame, 1980). The primary risk is therefore to human health. P.aeruginosa is an ACDP Hazard Group 2 organism which only presents a risk to cystic fibrosis patients, immunocompromised individuals or those with severe burns (Hatchette et al., 2000, Driscoll et al., 2007). Thus with proper control measures the risk of the GMM to human health is negligible. Transfer of the antibiotic resistance gene to other micro-organisms is unlikely as the plasmid is only suitable for P. aeruginosa and E. coli and cannot be mobilised between micro-organisms (Schweizer, 1991).

References
SchweizerH. rr9z EschechiaPseudomonas shuttle vectors derived from pUCI8f 9. Gene97: 109-112-

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The waste generated will consist of limited numbers of agar petri dishes with the GMM growing on them as colonies, spent liquid bacterial cultures & broths (less then 2L at any one time) and a small number of 6 well tissue culture dishes. All solid waste is autoclaved using an autoclave in the building. Solid waste is autoclaved at 121 degrees centigrade for 1 hour at 20 p.s.i.. This gives a chance of 1 in 1 to the power 34 of an individual organism surviving and represents the highest level of killing currently possible for any organism. Thus there is an utterly negligible chance of organismal survival. All liquid waste is subjected to the same autoclave treatment cycle. The autoclave killing is validated on each run by multiple independent thermocouples producing a print out which is archived for reference purposes.
The risk assessment associated with this notice of intent has been read and approved by the BSC/GMO, the microbiological safety representative and the Chair of the Safety Committee.

Project Containment

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Project Ref 570/09.1

Date Ackn'd 24/03/2009

CU2 Project Title Investigation of virulence factors, biofilm formation, and antimicrobial resistance in proteus mirabilis, pseudomonas aeruginosa and staphylococcus aureus

Class 2 Culture Volume Class2 < 1 litre

Non-GMM Consent Granted Yes

Project notified under transitional arrangements No

Withdrawn No

Tick if notifying a connected programme of work No

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to utilise molecular genetic approaches to investigate the genetic basis of virulence, biofilm formation and antimicrobial resistance in three bacterial opportunistic pathogens (two Gram-negative and one Gram-positive). The interaction of these organisms with bacteriophage (viruses who infect and kill host bacteria) will also be studied on a genetic level, with the view to generating novel bacteriophage based antimicrobials. A greater understanding of the virulence and antimicrobial resistance will lead to novel strategies to treat these infections.

**Recipient or parental organism**

E. coli hosts: Standard, disabled E. coli cloning strains such as EPI300, JM109, S17.1, SM10, HB101, DH5alpha, XL1-Blue etc. All strains to be used are non-hazardous and non-pathogenic. Hazard group 1. L. Lactic hosts: L. lactis cloning strains as used by other researchers, such as NZ9000, H61 etc. L. lactic is non-hazardous, non-colonising, non-invasive, and non-pathogenic. Hazard group 1. Self cloning strategies will also be employed with P. Mirabilis, Ps. Aeruginosa and S. aureus (all Hazard group 2).

**Host/vector system**

No vectors capable of self-transfer between bacterial species will be used. All vectors to be used require introduction through laboratory manipulation. The vector systems to be used include standard narrow host range E. coli and L. lactis cloning vectors, broad host range vectors for self cloning strategies, and E. coli or L. lactis expression vectors. All selectable markers present are naturally occurring, and already present in the wider environment. The majority of vectors to be used are commercially available and all have a history of safe use. For full details see risk assessment.

**Origin & function**

Genetic material will originate from P. mirabilis, Ps. aeruginosa or S. aureus, which are all opportunistic pathogens capable of causing disease in susceptible, compromised individuals, and do not normally constitute a risk for health individuals. Effective treatment exists for healthy individuals and as such the overall level of risk to human health and the environment associated with these pathogens is low. Ps. Aeruginosa may also cause opportunistic infections in plants, but no species or strain known to be pathogenic to plants will be used in these studies. P. mirabilis and S. aureus have no known ability to cause infection in plants. All GMMs created will be the result of mutagenesis procedures, self-cloning or transfer of DNA from hazard group 2 pathogens (P. mirabilis, Ps. Aeruginosa and S. aureus) into hazard group 1 disabled cloning strains (E. coli and L. lactis). The primary molecular genetic approaches employed will be the generation of attenuated mutants and identification of disrupted genes. Genes identified in this way will subsequently be cloned and maintained in standard E. coli or L. lactis disabled cloning host for further study and characterisation. Clones derived from P. mirabilis, Ps. Aeruginosa or S. aureus will encode genes related to virulence attributes, biofilm formation and antimicrobial resistance.

**Evaluation of foreseeable effects**

The primary approach used to study these organisms will be the generation of mutants attenuated in virulence mechanisms, biofilm formation, general growth in laboratory media, or antimicrobial resistance, using site-directed gene knockouts or random transposon mutagenesis. As mutants will be attenuated in comparison to the wild type organisms, no additional risk is associated with this procedure above that of the original hazard group assigned to P. mirabilis, Ps. Aeruginosa and S. aureus (Hazard group 2). Cloned DNA derived from P. mirabilis, Ps. Aeruginosa and S. aureus may contain virulence or antimicrobial resistance genes that are expressed in the E. coli or L. lactis hosts, and as such there is potential to increase the pathogenicity and resistance of these organisms. However these genes will be cloned in disabled level 1 receipt organisms (E. coli and L. lactis standard cloning strains), which are unlikely to survive outside the laboratory, and are sensitive to a wide range of antimicrobial agents. As such the risk associated with expression of virulence genes and antimicrobial resistance genes in the disabled E. coli or L. lactis cloning strains is negligible. The resulting GMMs will not possess an ability to displace environmental strains or survive in the environment above that of the original parental species, which are all Hazard Group 2 organisms, and already widely distributed in the environment, and th risk to the environment is low.
Complementation studies which involve the transfer of DNA between E. coli cloning strains and the bacterial species from which cloned DNA originated (self-cloning) will also be undertaken. As this is a self-cloning only procedure (e.g. P. mirabilis DNA cloned in P. mirabilis host only) there is no additional risk above that originally assigned to donor organisms (P. mirabilis, P. aeruginosa and S. aureus), and the overall risk is negligible. Overall inserted and donated genetic material is highly unlikely to increase the pathogenicity or environmental impact of recipient organisms when using the proposed molecular genetic approaches, and the resulting GMMs will not constitute a hazard above that of the original parental species, which are all Hazard Group 2 organisms. The overall risk to the environment and human health is low, and all GMMs will be handled at containment level 2 to reduce any associated risk to effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be sterilised by autoclaving including GMMs. Liquid waste will be collected in robust, sealed vessels in leak proof secondary containers, and all other waste collected in robust leak proof autoclavable boxes or containers with lids. Waste will then be sterilised by autoclaving. Decontamination of laboratory surfaces will be carried out with broad spectrum disinfectants. In accordance with advice offered in the SACGM compendium for guidance (Part 3, section 3.5, clauses 8-22) hypochlorite (bleach) disinfectants will be used for standard decontamination of laboratory surfaces.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 570/10.1

Genetic Modification of mammalian cells in order to understand pathways controlling replicative lifespan and senescence

Class 2

< 1 Litre

Non-GMM

Consent Granted

Date Project Ceased

25/06/2010

Tick if notifying a connected programme of work

N

Withdrawn

Project notified under transitional arrangements

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The aim of this project is to employ replication defective retroviral vectors harbouring inserts coding for proteins that ablate or modify replicative senescence in mammalian cells (typically primary human strains). The objective of this project is to gain a fundamental understanding of the mechanisms underlying cellular ageing. This risk assessment relates to an existing class 2 notification (Ref No: 570/00.1) covering producer cell lines generating amphotrophic retroviral vectors carrying telomerase (hTERT), and Dominant Negative-hTERT.

Recipient or parental organism

E. coli hosts: The standard, disabled E. coli cloning strain TOP10 will be used. These are non-hazardous and non-pathogenic with a long history of safe use. Hazard Group 1.

Mammalian cells: NIH3T3 and other primary cell types to be used in this project are Hazard Group 1. Primary human cell lines which are not fully characterised but low risk for endogenous biological agents will also be used, which are Hazard Group 2. Under no circumstances will operator derived cells be used.

Host/vector system

The vector systems to be employed are third generation retroviral vectors from the pBABE family of retroviral vector systems (e.g. pBABEpuro, pBABEneo, pBABEhygrom, pBABEpuro(LOX)). These are derived from the Moloney Murine Leukemia Virus which is classified as Hazard Group 1. These will be used to maintain the genetic material specified below in E. coli TOP10 (Hazard Group 1), and primary mammalian cells, including those of human origin. Hazard group 1, and Hazard Group 2 primary cells will be used.

Origin & function

The psiCRIP producer cell lines contain 2 mutant Moloney murine leukemia virus-derived proviral genomes with frame shift mutations in the gag-pol or env regions. In both
viral genomes the "psi" sequence has been deleted, which is required for the efficient packaging of retroviral genomes.

The pBABE series of vectors are derived from Moloney murine leukemia virus (Hazard Group 1). These are designed to express an inserted gene and a selectable marker. Viral particles are produced in disabled packaging systems which render the production of replication competent virus highly statistically improbable.

The genetic material to be cloned into pBABE vectors and transferred to E. coli TOP10 or primary mammalian cells. Includes sequences encoding for proteins considered to be oncogenes, originating from human papilloma virus (HPV E6 and E7) and SV40 virus (Large T antigen). This also subsumes telomerase (hTERT), and an inactive form of telomerase (DN-hTERT). Telomerase is a specialised reverse transcriptase which acts to balance the attrition of chromosomal ends and is not generally considered oncogenic HPV E6 and E7 target the p53 and pRB tumour suppressor genes respectively and can immortalise a wide variety of celltypes. SV40 Large T antigen binds both p53 and pRB and thus produces extended lifespan and spontaneous immortalisation at low frequency (1 per million per cell generation). The selectable marker to be used encodes resistance to the antibiotic puromycin.

**Evaluation of foreseeable effects**

E. coli TOP10 harbouring pBABE with cloned genetic material: Genes of human or mammalian origin are not properly expressed or translated into functional proteins by prokaryotic cells, which lack the cellular machinery required to process mammalian genes. E. coli TOP10 strains are hazard group 1 and it is highly unlikely that inserts cloned on pBABE vectors harboured by E. coli TOP10 will increase the hazard above this level. E. coli TOP10 is unlikely to survive outside the laboratory and cloned genes will not provide any survival advantage in the environment, and the resulting GMMs will thus pose no risk to the environment, and no risk to human health beyond the original hazard classification assigned to the parental strain (Hazard Group 1).

The mammalian cells to be used are either Hazard group 1 or Hazard group 2. The most probable foreseeable effect of transduction of the specified sequences growth requirements and no operator derived cells will be used there will be no increased risk to human health or the environment from these above that assigned to untransduced cells.

Forseeable hazards posed by amphotrophic vectors include: i) Generation of wild type viruses, ii) Insertional mutagenesis and iii) any hazard arising from transgenes carried by the vector. pBABE vectors are replication defective and it is highly unlikely that the psiCRIP producer cell lines will generate wild-type virus because the required viral genes (gag-pol and env) are encoded on two distinct viral genomes (which contain mutations in other key viral genes). The insertional mutagenesis and immortalisation hazards (ii and iii) are low because retroviruses can only infect cells which are traversing the cell cycle (an event which occurs - once 2-5 years in human fibroblasts). Nonetheless, possible routes of infection by inhalation of aerosols and direct inoculation will be blocked by avoidance necessary, and barring operators from work if they have open skin lesions. The overall risk to human health is thus low, and all work will be conducted at containment level 2 to further reduce the risk to effective zero. Liquid waste will be decontaminated at point of use using 5%-10% (w/v) Virkon and all solid waste will be sterilised by autoclaving. P

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be sterilised by autoclaving including GMMs. Wastes containing GMMs will be double bagged, and transported to adjacent autoclaves in leak proof secondary containers with lids. Waste will then be sterilised by autoclaving. The autoclaves used achieve terminal sterilisation (i.e complete killing) by steam heating above 121 degrees centigrade for 15 minutes. Temperature and run times are automatically recorded using thermocouples which actively monitor in-chamber temperature. Sterilised waste is then disposed of as standard clinical waste by our contractors.

Liquid waste arising from cell culture experiments (small volumes) will be sterilised by 5-10% (w/v) Virkon for 12 hours. This is an extremely robust killing schedule (typically 0.5% Virkon achieves full bacterial killing within 5 minutes and the potency of adenovirus types 5 and 6 can be reduced by greater risk than six logs when subjected to 0.9% Virkon for 12 hours).
Virkon for five minutes under tissue culture conditions). Liquid waste can then be disposed of by standard routes. Validation of Virkon kill is technically impractical on a routine basis (due to the difficulty of removal of the agent to conduct viability screening). However the SACGM compendium of guidance recognises this and considers it an acceptable means of inactivation for this class of procedure.

Decontamination of laboratory surfaces will be carried out with broad spectrum disinfectants (typically 5% virkon). In accordance with advice offered in the SACGM compendium (Part 3, section 3, 5, clauses 8-22). 70% industrial methylated spirits will be used for routine surface sterilisation and 5% (w/v) Virkon for decontamination in the event of spillage.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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GM Centre Number: 572

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Name

MRC CAMBRIDGE CENTRE FOR BRAIN REPAIR

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Campus Estate or Research Centre

FORVIE SITE

Road Name

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E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee
Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)  
Tick if confidential  

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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The Study of diseases of the brain and nervous system, neurodegeneration and repair, using GM microorganisms

**Project Additional Information**

**Purposes of the contained use**

Human and rodent primary and cell line cultures will be transfected by various viral vectors (incl. lenti-, adeno- & adeno-associated virus), to introduce genes or RNA species associated with neurodegenerative disease and/or repair processes. Patient-specific iPSCs will be generated using lentiviral vectors. Viral transduction will be used in vivo to create or repair models of neurodegenerative disease. Containment will be required during the transduction phase of cell culture until stable cell lines are generated, and these will normally be considered class 1 GMOs unless the risk assessment states otherwise.

**Recipient or parental organism**

Well characterised commercial bacteria, e.g. AADH1alpha or One Shot Top 10 Chemically Competent E. coli - disabled, non colonising, non-pathogenic.

Well characterised viral expression systems, e.g. HIV-1 derived 3rd and 4th generation replication defective, lentiviral vectors, and replication defective AAV vectors.

Adenoviral vectors AdEasy and Adtrack, replication defective, deleted E1 and E3 genes.

Commercial packaging cell lines, e.g. HEK293T human cell line, a highly transfectable derivative of human embryonic kidney 293 cells, containing the SV40 T-antigen. This cell line is competent to replicate vectors carrying the SV40 region of replication. It gives high titers when used to produce retroviruses. It has been widely used for retroviral production, gene expression and protein production.

Human and rodent primary neural cultures, somatic neural stem/precursor cells (NPCs), embryonic stem cells, skin fibroblasts and induced pluripotent stem cells.

Characteristics of cell cultures are never totally understood, so primary human cultures will be handled at containment level 2 considering the possibility of adventitious blood borne viruses, rodent cultures will normally be handled at containment level 1 unless the risk assessment advises.

**Host/vector system**

Only well characterised, replication deficient viral vector systems with a history of safe use will be used, for example:

1) Dh1alpha or One Shot Top 10 E. coli/pLVX-EF1a-IRES-Puro or /pLVX-TRE3G plasmids, introduced by heat-shock transformation.

2) HEK-293T/pLVX-EF1a-IRES-Puro or /pLVX-TRE3G plasmids, transient co-transfection of viral gene and insert expression (4th generation system/5 plasmids) production of amphotropic replication deficient HIV-1 derived lentiviral particles using calcium phosphate precipitation.

3) Human and rodent cells/HIV-1 vectors - replication defective lentiviruses, with stable genomic integration.
Inserts are arbitrarily grouped as follows:

Type 1. DNA expression constructs, for example: Human cDNA (+/-TAG sequences) inserts will code for transcription factors, Ascl1, Brn2, Myt11, NeuroD, ngn2, lmx1a, lmx1b, foxA2, Nurr1 and Otx2. These genes are expected to influence cell fate decision and differentiation of fibroblast cells, iPSCs, neural/precursor stem cells, or hES cells towards a neuronal lineage, but are not expected to have an effect on cell proliferation. Other genes may code for growth factors, disease associate proteins such as mutant tau, huntingtin or alpha synuclein, or regrowth-enhancing agents such as chondroitinase.

Type 2. RNA constructs, for example: miRNA sequences and miRNA target sequences (miR-124) will produce non-coding miRNA sequences targeting cell endogenous mRNA or exogenous miRNA target sequences on miRNA regulated vectors -miRNA-124 is thought to affect the differentiation of cells to a neuronal lineage.

Type 3. Reporter genes: (enhanced GFP (eGFP) and destabilized GFP (dGFP) at different cellular locations, firefly luciferase (fLuc) and mCherry, will code for proteins with fluorescent or enzymatic properties to allow identification of genetically modified cells.

Type 4. Selection genes: (bacterial resistance - Ampicillin, untransduced fibroblasts - Puromycin), will express proteins with enzymatic activity to allow selection of bacterial or GM cell cultures.

Type 5. Functional non-coding sequences will act as promoters of transcription (promoter, enhancer), local chromatin structure modifiers (enhancers, insulators), transcription regulators (IRES), or RNA transcript stabilizers (WPRE), of mammalian or viral origin). They will be used individually or in combination as required in viral expression cassettes to achieve best control of transgene expression according to the target cell type.

**Evaluation of foreseeable effects**

- **Human health considerations** -
  1. The most obvious risk to human health is from the recombinant viral particles, via accidental infection of the researcher.
  2. The cells from human samples also carry the possibility of adventitious agents as they are uncharacterised, they will be processed at Containment Level 2 from collection. Because only replication defective viral particles (non-infectious) will be used throughout this project, and inserts will not produce compounds with acute toxicity (such as bacterial toxins), no additional risk will arise from the final genetically modified cells, compared to the initial cell source.

  1. The risk associated with the nature and design of recombinant viral particles - lentiviral recombinant particles (HIV-1 derived) will be packaged in protein G from the vesicular stomatitis virus (VSV-G) to alter the tropism of the retroviral particles, as it only requires interaction with the plasma membrane of cells rather than a specific receptor, allowing increased stability of the viral particles associated with the VSV-G envelope which may add to the aerosol risk in addition to the percutaneous risk. Retroviruses stably integrate into the host cell genome, and there may be a risk of insertional mutagenesis, with subsequent ectopic inhibition/activation of host gene expression. Subsequent de-regulation of endogene expression could theoretically lead to transduction associated detrimental effects such as initiation of oncogenic processes. Moreover the addition of the Woodchuck hepatitis Post transcriptional Regulatory Element (WPRE) to the expression cassette in several retroviral backbones could also enhance the oncogenic potential of such vectors (SACGM 2004). However, the use of a lentiviral backbones is thought to be safer, as the genomic integration profile of lentiviral derived vectors do not show an integration bias towards the transcriptional start site region of host cell genes.
  Risk is also associated with inserted coding sequences. cDNA or shRNA/miRNA inserts carried by viral vectors will direct ectopic expression or inhibition of transcription factors, and strong promoter/enhancer effects from non-coding sequences could lead to ectopic activation of neighbour endogene expression.
  2. Risks are theoretically higher where multiple genes are expressed in the same experiment - researchers will be informed of the additional risk if using polycistronic vectors.
  The risks described above will be moderated by the fact that we will use the strictly replication defective viral vectors such as the Clontech Lenti-X HTX Lentiviral Expression System, a 4th generation 4 plasmid system by separating the gag from the gag-pro-pol sequences normally found on 3rd generation vectors making the production of recombinant lentivirus (RCL) evenmore unlikely, as extra recombination events would be needed. In fact, the emergence of RCL is undetectable from
systems using this approach. (Wu, X. et al (2000) Mol. Ther. 2(1): 47-55). AAVs have no known link to human illness and are commonly used as vectors for the introduction of genes. In most cases Containment Level 1 will be sufficient, unless there are any additional risks due to the biological activity of the insert, identified by risk assessment. For all viral vector types, in the event of an accidental contamination, (percutaneous or inhalation) the amount of viral vector able to effectively contaminate a worker's cell will be minimal, the transduction for cells in vitro needing a specialized protocol for efficient uptake. The VSV-G pseudotyping results in complement sensitivity, increasing the likelihood of immunological neutralization in human hosts. Cells of the cornea, and lung, are terminally differentiated cells with a limited lifespan and high turnover, which greatly diminishes the chance of effective transformation.

- Environmental considerations -
VSV-G pseudotyping makes the recombinant viral particles amphotropic, able to infect a larger range of animal and cell types, however the viral particles are still sensitive to air, temperature and pH, and will have a short lifespan in the open environment. Importantly, they will be replication defective and so will not produce progeny able to spread from an infected host.

Transgene mobilization is theoretically possible if infection occurs in a host cell which is also infected by a wild-type retrovirus - if the wild-type virus is compatible with the recombinant lentivirus, it may provide encapsidation to allow production of a replication competent viral particle carrying the transgene. The likelihood of this mobilization is very low, and replacement of the VSV-G envelope would remove the amphotropism of the resulting viral particle. Generation of replication competent viral particles (RCV) is associated with the highest risk factor for human health and the environment, therefore production steps will be carried out under Containment level 2 conditions. This involves packaging of the separate constructs, concentration of the resultant viral supernatant, transduction of the target cells, and culture/analysis of the resultant GM cell populations. All researchers will be trained in the use of hazards involved in working with recombinant viruses. To decrease the likelihood of infection, standard measures to decrease percutaneous as well as aerolised transmission of viral particles will be adopted, such as no use of sharps and all work to be carried out within a Class II microbiological safety cabinet. All waste will be sterilised by validated means via immediate immersion disinfection and local autoclaving.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMNs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All highly contaminated waste solid material, such as pipette tips, petri dishes, will be immersed in 2% Virkon solution for 24 hours, within the microbiological safety cabinet, effectively 100% kill.

All solid waste will be discarded to autoclave bags and autoclaved locally, at 121 degrees C for at least 45 minutes, before discard in clinical waste bag for incineration, 100% kill.

Small surface spills will be immediately soaked with Distel, followed by wiping with 70% Ethanol, effectively 100% kill.

Large spills will be sprinkled with Virkon powder before cleaning as above, effectively 100% kill.

Safety cabinet surfaces will be wiped down with Distel before and after working, effectively 100% kill.

All discarded GM modified cell cultures will be destroyed by soaking in 2% Virkon solution or 24 hours followed by autoclaving at 121 degrees C for at least 45 minutes and subsequent discard in clinical waste bag for incineration 100% kill.

Autoclave is temperature validated annually.

All mammalian tissue which has been infected with virus will either be fixed with 4% paraformaldehyde or 2% glutaraldehyde, or if fresh will be subject to risk assessment of the possibility of infectious virus still being present, and handled accordingly.
This work was discussed on 30th September 2013 at the Brain repair Centre Biological and Genetic Modification Safety committee meeting. It was agreed that the work could go ahead subject to appropriate controls and conditions. Some Class 1 work was approved and the whole project was written into this notification and the attached risk assessment over the intervening months.

The risk assessment was revised and reviewed after a meeting of the committee on 14th August 2014

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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GM Centre Number: 573

Data Premises Notified (Originally) 13/09/1994

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed 26/04/2005

Transitional Premises Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Name

UNIVERSITY OF WALES COLLEGE OF MEDICINE

Name 2

Department

CARDIOLOGY

Campus Estate or Research Centre

Building

Road Name

HEATH PARK

District

Town

CARDIFF

County

CARDIFF

Postcode

CF4 4XN

Country

WALES

Tel Number 029 2074 2903

Fax Number 029 2074 4869

E-mail

HSE Division WALES AND SOUTH WEST

Comments

GM573 closed and merged with GM130 on 26/04/2005

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
<th>Level 1 (GMMs)</th>
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- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

All liquid waste from GM work is treated with Chloros in accordance with college policy and then disposed of by flushing down the sinks. Maximum culture volumes are 250 mls for a maxiprep at any one time. Solid waste is placed in designated containers in the prokaryotic room and then the bags removed and autoclaved prior to disposal. When autoclaving, monitoring tape is always used and in addition our autoclave is regularly serviced and tested.

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Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 575

Data Premises Notified
(Originally) 03/10/1994

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

CENTRE FOR ENVIRONMENT FISHERIES & AQUACULTURE SCIENCE (CEFAS)

Department

AQUACULTURE & HEALTH GROUP

Building

WEYMOUTH LABORATORY

Road Name

BARRACK ROAD

Town

WEYMOUTH

County

DORSET

Postcode

DT4 8UB

Country

ENGLAND

Tel Number

01305 206600

Fax Number

01305 206601

E-mail

none

HSE Division

WALES AND SOUTH WEST

Date at Which Additional Info Submitted

27/07/2001
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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02/03/2022
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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 575/01.1

<table>
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**Non-GMM**

Consent Granted: not applicable

**Project notified under transitional arrangements** N

Withdrawn: N

Tick if notifying a connected programme of work: Y

Historical Significant Changes
Viral haemorrhagic septicaemia (VHS) is generally considered to be a disease of farmed salmonid fish, and as such poses a serious threat to the farmed rainbow trout (Oncorhynchus mykiss) populations in the UK which have not been exposed to it. In recent years however, the VHS virus (VHSV) has been isolated from an increasing number of marine fish species, including Atlantic herring (Clupea harengus harengus), cod (Gadus morhua), haddock (Melanogrammus aeglefinus), sprat (Sprattus sprattus), and rockling species, which suggests that a natural reservoir of the VHSV exists among marine species. Having already determined the complete genome sequence of pathogenic and non-pathogenic strains, the gene(s) involved in conferring virulence to VHSV will be identified by generating recombinant viruses using sequences amplified from both the marine and fresh water isolates. Virulence data on the recombinant virus, obtained by challenging rainbow trout with the recombinant and non-modified viruses under controlled conditions, will be used to predict the likelihood that, under the selective pressures attributed to the farming practices, more pathogenic strains would be generated following the introduction of marine isolates on to a farm site.

Plasmids containing the VHSV/IHNV cDNAs will be replicated in E.coli strain JM 109. The reporter gene, green fluorescent protein (GFP) from Aequorea victoria will be inserted into the full length cDNA of the fish pathogen VHSV. Recovery of recombinant VHSV and/or IHNV will require the use of either a T7 RNA polymerase expression system based on the Modified Virus Ankora (MVA), a multiply attenuated vaccinia virus, or a fowlpox T7 RNA expression system. VHSV, the MVA and the FP expression vectors will be grown in established fish cell lines (CHSE-214, BF-2- and EPC-cells).

VHSV and IHNV derived cDNA will be inserted into pcDNA3.1 and pCI which are pUC-based vectors considered to be non-mobilisable. These will be used in conjunction with E.coli JM109 which is disabled. They are non-pathogenic to humans and animals and are unable to survive in the environment. The MVA expression vector is a multiply attenuate variant of the vaccinia virus which is unable to produce infectious virus in humans. Fowlpox virus is also unable to replicate in mammalian cells.
The N, P, M, G, Nv and L genes and the 5' and 3' non-coding sequences from VHSV and IHNV will be inserted into the eukaryotic expression vector pCI (Promega) to create a positive sense (antigenome) copy of the complete virus genome under the control of a T7 promoter. A T7 terminator sequence will be inserted downstream of the virus sequences and hepatitis delta virus (HDV) ribozyme sequence will be inserted between the terminator and the 3' trailer to ensure that virus transcripts have the authentic terminal ends required for replication.

The N, P and L genes of VHSV and IHNV will be inserted into the expression vector pcDNA3.1 under the control of a T7 promoter. Infectious virus will be recovered by co-transfection of fish cell lines (EPC, BF-2 or CHSE-214 cells) with the full length cDNA and the N, P and L gene constructs following infection with either the MVA-T7 expression vector (Sutter et al., 1995) or the FP-T7 expression vector (Britton et al., 1996). As a positive control for the expression system, the full length cDNA will be replaced with a minigenome in which the N, P, M, G, Nv and L gene sequences are exchanged for the reporter gene, chloramphenicol acetyl-transferase (CAT).

Single and multiple nucleotide substitutions will be introduced into the VHSV and IHNV cDNA clones at positions thought to play a role in virulence of VHSV and IHNV for rainbow trout. Changes in the replication and tissue tropism of site-directed mutants will be monitored by inserting the gene for Green fluorescent protein (GFP) upstream of the first open reading frame.

HAZARDS TO HUMAN HEALTH
Recombinant DNA will be grown in JM109, a strain of E.coli which is disabled and unable to survive in the environment and is non-pathogenic to humans. The fish rhabdovirus genes, the reporter genes CAT and GFP, and the T7RNA polymerase are considered to be non-toxic and these are unlikely to alter the pathogenicity of the cloning host.

The viral proteins will be expressed using the mammalian expression vectors pcDNA3.1 and pCI which are pUC based and when used in conjunction with the fish cell lines are considered to be non-mobilised.

HAZARDS TO THE ENVIRONMENT
VHSV and IHNV are list II fish viral pathogens and are notifiable both to the Office International des Epizooties (OIE) and under local Fish Health Regulations. Monitoring for VHSV and IHNV currently involves the isolation of the VHS or IHN viruses. In the future surveillance for VHSV could rely more heavily on an assessment of antibody or the presence of virus nucleic acid. Since the proposed work uses disabled hosts and mobilised defective vectors which will not survive in the environment the risk of interference to future diagnostic tests is negligible.

The MVA is a multiply attenuated vaccinia virus containing six major deletions and will not replicate in human cells.

The FP will also not replicate in humans.

Although expression is sought in the fish cells the VHSV and IHNV genes, CAT, GFP and T7RNA polymerase are all considered to be non-toxic.

Insertion of GFP in to the VHSV and/or the IHNV genome is unlikely to alter the pathogenic traits of the recipient viruses.

Both VHSV and IHNV are highly pathogenic for farmed salmonid fish species and the consequences of their release are severe; particularly the potential loss of the "approved zone" status afforded to the UK by the European Union under Council Directive 91/67/EEC.

Therefore, in line with the Department of Environment Food and Rural Affairs (DEFRA) recommendations all work on VHSV and IHNV will be performed at containment level 2.

Evaluation of foreseeable effects

n/a
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

INACTIVATION AND DISPOSAL
All material for disposal (pipette tips, microcentrifuge tubes, Petri dishes, pipettes etc) is placed in biohazard autoclave bags and transported to the autoclave by authorised personnel in robust metal bins. Recyclable items (glassware etc.) are transported to the autoclave in robust metal bins; the latter is done on a daily basis. Clinical waste (infected fish) will only be generated on one or two occasions per year. It will be placed in biohazard autoclave bags, transported to the autoclave by authorised personnel in robust metal bins and autoclaved immediately.

Inactivation of GMMs is performed by autoclaving at 126 degrees C for 30 minutes using an LTE Scientific series 400 autoclave. Autoclaved recyclable items are washed and returned to the clean store. Autoclaved disposable material is discarded with normal laboratory waste. The degree of kill by autoclaving is effectively 100%.

Inactivation of GMMs in water by ozone treatment. The water is saturated with ozone for 30 minutes and the residual ozone above saturation point is always >0.02 ppm. The degree of kill is effectively 100%. Some disinfection of equipment, surfaces etc is by treatment with sodium hypochlorite (200 ppm for 24 hours). The waste liquids generated are further treated with ozone before discharge from the site. The degree of kill is effectively 100%.

VALIDATION AND MONITORING OF CONTROL METHODS
The autoclaves are serviced every three months, and at the annual service independent thermocouples are used to demonstrate that the correct temperature and pressure have been reached for the required time. On subsequent runs, a chart recorder is used to verify that the correct conditions were reached. Should those conditions not be reached, the load will be retained until the autoclave has been serviced/mended.

Validation tests on the ozone disinfection system using spores of Bacillus cereus have shown complete inactivation is effected when the water is saturated with ozone.
Saturation can be demonstrated by measurement of residual ozone; any residual detected is indicative of water saturation by ozone. The ozone plant runs with an ozone residual of greater than 0.02 ppm, which is continuously monitored, and should the residual fall below that level, there are automatic systems in place to prevent discharge of untreated water. The water is not discharged until a residual of >0.02 ppm ozone is obtained, or the water has been treated with sodium hypochlorite.


Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form Y
Tick to confirm that you have attached a risk assessment to this form N
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The proposal was reviewed by the CEFAS Weymouth Laboratory Genetic Modification Safety Committee and approval given for the work to proceed subject to endorsement by the HSE.

Project Containment

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02/03/2022
Prion Proteins in Fish

The presence of the normal cellular isoform of the prion protein is an absolute requirement for prion replication. Recently the first data on homologues to tetrapod prion proteins (PrP) were reported in fish (Oidtmann et al. 2003, Rivera-Milla et al. 2003). Therefore fish fill the basic precondition to contract a TSE (Transmissible Spongiform Encephalopathy).

The aims of this work are to
1. Identify further PrP gene sequences in various fish species.
2. Develop diagnostic tools to detect PrP expression.
3. Develop diagnostic tools to detect possible formation of PrP-res (the protease resistant form of the prion protein). These are required for our planned experiments infecting fish with BSE or Scrapie agent and in vitro conversion to detect fish PrPres formation.
4. Further characterisation of the fish prion protein (e.g. 3D structure).
5. Investigate, using in vitro conversion, whether recombinant fish PrP can be converted into a PrP res form.

For sequencing purposes DNA and cDNA of the fish prion protein gene will be cloned into non-mobilisable plasmid vectors. The fish prion protein cDNAs/DNAs will be replicated in a disabled E.coli strain for production of sequencing quality DNA.

For fish prion protein expression in prokaryotic hosts, cDNAs of full or partial length of the translated region of the fish PrP gene will be cloned into a vector host system expressing the inserted sequence only after induction, and in which expression is otherwise suppressed. The hosts used for expression are E.coli K12 strains or strains derived from E.coli BL21, which are disabled. Alternatively, E.coli BL21 strains or strains derived from E. coli BL21, will be used, BL21 strains being considered broadly...
equivalent to E. coli K12 strains.

For prion protein expression in eukaryotic expression systems, cDNAs of full or partial length of the translated region of the fish PrP gene will be cloned into Pichia pastoris, which is disabled. Vectors that will be used in combination with Pichia pastoris are pUC or pBR322 derived vectors.

Constructs that will be generated for sequencing purposes:
are for example:
non-mobilisable plasmid vectors containing fish PrP DNA or cDNAs replicated in the E. coli K12 strain JM109 for production of sequencing quality DNA.
Examples of constructs that will be generated for expression in prokaryotic hosts:

1. Using pET vectors
For fish prion protein expression, cDNAs of full or partial length of the translated region of the PrP gene will first be established in a non-expression host (some E. coli K12 strain, e.g. JM109). Thereafter, they will be transformed into a host bearing the polymerase genes required for expression of the target protein inserted in the retrospective vector. For this purpose cDNAs of full or partial length of translated region of the PrP gene will be cloned into vector pET15b or another vector from the pET system (Novagen). Inserts cloned into vectors of the pET series will only be expressed when the vector is transferred into a host cell supplying the T7 RNA polymerase gene for expression. The hosts that are intended to be used for this purpose are: E. coli BL21, Rosetta series (derived from Tuner, which are BL21 lacZY derivates), Rosetta Blue (derived from Nova Blue, which is a K12 strain), Rosetta gami (derived from Origami, which is a K12 derivate). In the mentioned hosts, DE3 strains will be used, which means that the host is a lysogen of ADE3, and therefore carries a chromosomal copy of the T7 RNA Polymerase gene under control of the lacUV5 promoter, making this strain suitable for protein expression using vectors containing the T7 or T7 lac promoter.

2. Using pGEX vectors
Fish Prion protein expression will also be attempted using pGEX vectors. Expression of the inserted sequence is induced by IPTG, thus expression of the insert is tightly controlled. We would intend to use the same strains as mentioned above, which are either E. coli K12 or BL21 derivates.

Examples of constructs that will be generated for expression in eukaryotic hosts:
For Prion protein expression in eukaryotic expression systems, cDNAs will be cloned into vector pPIC 3.5 K or pPIC 9 (which are pBR322 derived) for expression in Pichia pastoris GS115 and KM 71 or an equivalent Pichia pastoris strain.

Example of a construct that will be generated for sequencing purposes:
DNA/cDNA of the complete or partial fish prion protein genes from various fish species will be inserted into vector pGEM-T-easy, pCR (latter being a pUC based vector), or an equivalent non-mobilisable vector. These will be used in conjunction with E. coli JM109, or another disabled E. coli K12 strain. Plasmids will be replicated in E. coli K12 JM109 (or another disabled E. coli K12 strain) for production of sequencing quality DNA.

Examples of constructs that will be generated for expression in prokaryotic hosts:

1. Using pET vectors
For fish Prion protein expression, cDNAs of full or partial length of the translated region of the PrP gene will first be established in a non-expression host (some E. coli K12 strain, e.g JM109). Thereafter, they will be transformed into a host bearing the polymerase genes required for expression of the target protein inserted in the respective vector. For this purpose cDNAs of full or partial length of translated region of the PrP gene will be cloned into vector pET15b or another vector from the pET system (Novagen). Inserts cloned into vectors of the pET series will only be expressed when the vector is transferred into a host cell supplying the T7 RNA polymerase gene for expression. The hosts that are intended to be used for this purpose are: E. coli BL21, Rosetta series (derived from Tuner, which are BL21 lacZY derivates), Rosetta Blue (derived from Nova Blue, which is a K12 strain), Rosetta gami (derived from Origami, which is a K12 derivate). In the mentioned hosts, DE3 strains will be used, which means that the host is a lysogen of ADE3, and therefore carries a chromosomal copy of the T7 RNA Polymerase gene under control of the lacUV5 promoter, making this strain suitable for protein expression using vectors containing the T7 or T7 lac promoter.
expression. The hosts that are intended to be used for this purpose are E. coli BL21, Rosetta series (derived from Tuner, which are BL21 lacZY derivates), Rosetta Blue (derived from Nova Blue, which is a K12 strain), Rosetta gami (derived from Origami, which is a K12 derivate). In the mentioned hosts, DE3 strains will be used, which means that the host is a lysogen of DE3, and therefore carries a chromosomal copy of the T7 RNA Polymerase gene under control of the lacUV5 promoter, making this strain suitable for protein expression using vectors containing the T7 or T7/lac promoter.

2. Using pGEX vectors

Expression of inserts cloned into a pGEX vector (which is a pBR322 derived vector) is under the control of the IPTG-inducible tac promoter. All pGEX vectors are also engineered with an internal LacIq gene. The lacIq gene product is a repressor protein that binds to the operator region of the tac promoter preventing expression until induction by IPTG, thus maintaining tight control over expression of the insert. Expression can be achieved in any E. coli strain. We intend to use the same strains as mentioned above, which are either E. coli K12 or BL21 derivatives.

Examples of constructs that will be generated for expression in eukaryotic hosts:

For Prion protein expression in eukaryotic expression systems, cDNAs will be cloned into vector pPIC 3.5 K or pPIC 9 (which are pBR322 derived and therefore mobilisation defective vectors) for expression in Pichia pastoris GS115 and KM 71 or an equivalent Pichia pastoris strain.

Origin & function

From fish species in which the PrP gene sequences are still not identified, the sequence will be identified by use of degenerate primers followed by RACE PCR. The PCR-products will be transferred into a suitable vector for sequencing purposes (see under 7 and 8)

Partial or full length PrP cDNA from fish species from which the sequences have already been identified (eg rainbow trout and Atlantic Salmon), or may be identified in the future, will be cloned into an expression vector in order to obtain full or partial length Prion Protein. The obtained protein(s) will be used for the following purposes:

Firstly, in order to have an antigen available, which can be used for the generation of polyclonal antisera or monoclonal antibodies. Those polyclonal antisera or monoclonal antibodies can then be used for several purposes: a) detection of expression of the normal isoform of fish prion protein in various fish tissues; b) detection of potential formation of misfolded Prion Protein after in vitro conversion experiments; c) detection of potential formation of misfolded Prion Protein in fish infected with PrP (Sc) originating from a mammalian source.

Secondly, in order to generate correctly folded fish Prion Protein, which can be analysed for its 3D structure.

Thirdly, in order to perform in vitro conversion experiments.

Evaluation of foreseeable effects

Hazard to human health

For sequencing purposes, the complete or partial DNA/cDNA of PrP genes from various fish species will be inserted into a non-mobilisable vector. These constructs will be used in conjunction with a disabled E. coli strain.

Disabled E. coli strains are non-pathogenic to humans and animals and are unable to survive in the environment. The fish prion protein DNA. cDNA sequences that will be amplified, are coding for the normal prion protein gene of fish and not for a form which is thought to be associated with human disease or disease in mammals.

The fish prion proteins will be expressed using a vector described above (pET series or pGEX series or an equivalent to those) and used in conjunction with Escherichia coli BL21 or BL21 derived strains or with E. coli K12 or K12 derived strains. E. coli BL21 is considered unlikely to colonise and establish a persistent infection in the gut of a healthy individual. A study (Chart et al. 2000. An investigation into the pathogenic properties of Escherichia coli strains BLR, BL21, DH5a and EQ1. Journal of Applied Microbiology, 89, p1048-1058) has shown that BL21 is unlikely to be pathogenic, lacking any of the pathogenic mechanisms associated with E. coli strains involved in enteric infections. Therefore BL21 strains are now considered broadly equivalent to E. coli K12 (“Guidance on BL21”; paper relating to Newsletter 30, HSE).

For expression in an eukaryotic system, Pichia pastoris will be used, which is disabled and therefore non-pathogenic to humans and animals and unable to survive in the
environment.

The fish Prion protein genes have no association with any known hereditary or acquired animal disease and have not been shown to convert human forms of PrP into pathogenic isoforms.

Hazards to the environment
The proposed work uses disabled hosts and mobilisation defective or non-mobilisable vectors, which will not survive in the environment. The fish prion protein genes have no association with animal disease.

All laboratory waste GMMs and proteins expressed by the GMMs will be autoclaved on site before disposal.

According to the Advisory Committee on Genetic Modification Compendium of Guidance, non-expression activities in disabled hosts as well as expression of normal human or animal PrP genes are both required to be performed at containment level 2. This work will be done in a containment level 2 laboratory using the safety procedures appropriate for that level.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Inactivation and Disposal:-
All material for disposal (pipette tips, microcentrifuge tubes, Petri dishes, pipettes etc) is placed in biohazard autoclave bags and transported to the autoclave by authorised personnel in robust metal bins; the latter is done on a daily basis. Inactivation of GMMs is performed by autoclaving at 126°C for 30 minutes using an LTE scientific series 400 autoclave. Autoclaved recyclable items are washed and returned to the clean store. Autoclaved disposable material is discarded with normal laboratory waste. The degree of kill by autoclaving is effectively 100%. Generated fish Prion Protein (full and partial length) will also be autoclaved before disposal as described above.

Validation and Monitoring of Control Methods:-
The autoclaves are serviced every 3 months, and at each service independent thermocouples are used to demonstrate that the correct temperature and pressure have been reached for the required time. On subsequent runs, a chart recorder is used to verify that the correct conditions were reached. Should those conditions not be reached, the load will be retained until the autoclave has been serviced/mended.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee approved the submission of this application.
## Project Containment

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**Name**

ACCURO BIOLOGICS LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

ABERDEEN SCIENCE PARK

**Road Name**

BALGOWNIE DRIVE

**Building**

CROMBIE LODGE

**District**

**Town**

ABERDEEN

**County**

ABERDEENSHIRE

**Postcode**

AB22 8GU

**Country**

SCOTLAND

**Tel Number**

01224 707 337

**Fax Number**

01224 708816

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

Company change of ownership and change of name from Biovation to Accuro Biologics Limited on 1/9/2005

**Date at Which Additional Info Submitted**

19/01/2005
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify) □

Tick if confidential □

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum culture volume that could be released at one time is 5 L. Waste is deactivated by autoclaving (121 degrees C, 15-20 lb pressure).

 Tick to confirm that you are attaching a summary of the risk assessment

 Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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<tr>
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**Name**

UNIVERSITY OF DERBY

**Name 2**

BIOLOGICAL SCIENCES

**Department**

**Campus Estate or Research Centre Building**

KEDLESTON ROAD

**Road Name**

DERBY

**Town**

DERBYSHIRE

**County**

DE22 1GB

**Postcode**

ENGLAND

**Country**

**Tel Number**

01332 591748

**Fax Number**

01332622747

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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- Level 1 (GMMs)
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- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)

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<tr>
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<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</table>

Tick if confidential
A maximum of 101 of culture medium may occasionally be disposed of at one time. Contaminated material is placed in biohazard bags or dedicated steel bins prior to autoclaving. The autoclave is situated within the containment suite. Culture media and contaminated equipment is sterilised by autoclaving (121 degrees C, 15 psi, for 20-30 minutes depending on the load size and characteristics) prior to disposal. After autoclaving liquid waste is poured down the drains with copious amounts of water and solid waste is disposed of with the University's general rubbish. Records are kept of all autoclave runs, including the name of the operator, the type of material and the cycle characteristics. The chamber temperature for each run is recorded on a chart recorder and the effectiveness of the cycle checked using a sterilization indicator (Propper Manufacture Inc.). Each month the effectiveness of the autoclave is checked using spore strips (Propper Manufacture Inc.). Equipment which cannot be autoclaved, bench services etc. are disinfected using an amphoteric detergent Tego (ASAB). Swabs are taken around the containment laboratories on a monthly basis to confirm the effectiveness of the disinfection procedures.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 578

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Name
HEALTH & SAFETY LABORATORY

Name 2

Department
BIOMEDICAL SCIENCES

Campus Estate or Research Centre

Building

Road Name
HARPUR HILL

Town
BUXTON

County
DERBYSHIRE

Postcode
SK17 9JN

Country
ENGLAND

Tel Number
01298 218000

Fax Number
01298 218590

E-mail

HSE Division
MIDLANDS

Comments
HSL Moved to a new site in Buxton on 1/11/2004

Date at Which Additional Info Submitted
02/03/2022

Page 10068 of 15326
### Premises Addresses

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### Premises Conditions

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Give brief details of the genetic modification safety committee

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- **Bacteriology**
  - Parasitology
- **Virology**
  - Transgenic Animals
  - Transgenic Fish
- **Microbiology Research**
- **Gene Therapy**
- **Gene Therapy**
Maximum culture volume 100 ml.
All waste is autoclaved at 121 degrees C for 15 minutes.
This method of deactivation was validated by plating out for growth on autoclaved culture - there was no growth. The autoclave is calibrated to NAMAS/UHAS/ISO9001 specifications and the temperature and time for each run is recorded.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Emergency Plan Required?** Y

**Transferred from 1992 Regs?** Y

**Transitional Premises Class** 1

**Transitional Premises Withdrawn** N

**Non-GMMs** Y

**Date at Which Additional Info Submitted**

02/03/2022
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Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum culture volumes are 250 ml. Typically culture is on a small scale in broths (10 ml) or agar plates. Waste is transported in sealed autoclave bags within autoclavable containers to the

Autoclaving uses 121 °C for 30 min.

Performance is monitored by the use of Browns tubes, chart recording, visual monitoring of temperature and pressure dials. Additionally the autoclaves are service on at least an annual basis.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 579/03.1

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Project notified under transitional arrangements: N

Historical Significant Changes

Historical Date of Additional Info
**Project Additional Information**

**Purposes of the contained use**

The purpose of the contained use is to minimise the risk to personnel of infection from the organisms used and genetically modified during the proposed programme. The proposed work will involve ADCP group 2 organisms, particularly Salmonella sp. Salmonella infection can result in a self-limiting gastrointestinal illness in healthy individuals. There is therefore a risk of infectious disease from this work and the purpose of the containment is primarily to minimise this risk. Additionally we consider that there is a low risk of transfer of uncharacterised genetic material within Salmonella sp. during experimentation.

It is possible that this work will be extended in the future to include other ADCP 2 organisms eg Campylobacter sp. and Listeria sp. requiring similar contained use.

**Recipient or parental organism**

Recipient strains of Salmonella may include S. Typhimurium and S. Enteritidis as well as other Salmonella species, and may include those previously isolated from foods. The resulting Salmonellae may be modified to contain reporter genes (eg genes for bioluminescence) and antibiotic resistance markers typically used in cloning vectors (eg ampicillin, kanamycin). This work will not include recognised more pathogenic species such as Salmonella typhi or S. paratyphi categorised as ADPC 3 pathogens.

It is possible in the future that this programme of work will be extended to include similar studies with other ADCP 2 organisms eg Campylobacter sp. and Listeria sp. (It is likely that related class 1 containment level 1 experiments to support this work will also be required eg the growth and isolation of plasmids from disabled E. coli host strains or and transfer of plasmids from disabled hosts to ADPC 2 pathogens).

**Host/vector system**

Vectors will be pUC derivatives in the first instance. pUC derivatives are of limited host range and considered as non-mobilisable. Genetic experiments may be required to transfer reporter genes within Salmonella either through the use of broad host range vectors or the transduction of reporter genes using phage such as P22 or wild type phages isolated from Salmonella sp.

The host systems will be derived from strains of obtained from recognised culture collections, academic institutions or those isolated in this laboratory from food samples but specifically not Salmonella typhor S. paratyphi.

**Origin & function**

Genetic constructs will be obtained from academic establishments or may be constructed within this laboratory. The intended function of the genetic constructs is to act as reporter systems. The reporter genes may be used as a measure of gene expression in an infected cell eg as part of a detection mechanism or for the detection of cell signalling responses during enrichment culture. An example of this is the plasmid pSB367 * This is a pUC18 construct and confers resistance to ampicillin. It contains a fusion of the Salmonella dublin spvRA promoter with the luxCDABE from Photorhabdus luminescens. The expression of the spv operon requires functional RpoS and results in bioluminescence when the spvRA promoter is induced.


**Evaluation of foreseeable effects**

It is possible that co-transfer of pathogenic determinants between the pathogens used may occur during genetic experiments eg transformation and transduction.
experiments. In this instance we are primarily concerned with the transfer of plasmid sequences containing reporter genes or construction of transducing phage carrying fragments of cloned reporter gene/vector sequences. However generalised transduction could potentially transfer large fragments of DNA and pathogenicity islands within strains. Any expression of co-transferred DNA in the absence of selective pressure is dependent on overcoming restriction and recombination barriers in the recipient cell.

The primary aim of transduction experiments will be to establish a lytic cycle in the recipient cell resulting in expression of the reporter gene and subsequent death of the recipient cell. It is unlikely that the proposed programme will result in the construction of more virulent Salmonella strains. The use of reporter genes is well established and not associated with any other activities.

A worst case scenario could envisage the co-transfer of a pathogenicity island during transduction experiments with a vector associated antibiotic resistance, followed by infection of laboratory personnel. Transfer of pathogenicity traits within Salmonella already could conceivably occur as a rare event during generalised transduction, however the resulting strain is not anticipated to be more pathogenic than existing Salmonellae. Antibiotic treatment is not used clinically for the treatment of Salmonellosis however in rare cases of septicaemia treatment with guinolone antibiotics can be used. Antibiotics associated with resistant markers commonly used in vectors eg for ampicillin and kanamycin are not routinely used to treat Salmonella infection. There are increasing concerns regarding the emergence of multiple antibiotic resistant Salmonella eg Salmonella enteritidis DT104.

Avoidance of infection of laboratory personnel can be achieved by Good Microbiological Practice, compliance with COSHH, adequate training of staff in the handling and safe disposal of microorganisms and making staff aware of hazards that may arise due to particular circumstances, eg increased risks for immuno-compromised or pregnant staff.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste materials will be autoclaved. These will include solid agar plates. 100ml liquid cultures, and plastic tips. Waste treatment will be carried out using LFI's waste autoclave operating at 121 C for 30 minutes. No viable cells of the GMM will survive the process. Waste treatment is monitored by a trained technician with records kept for every autoclave run, indicating temperature and time operated.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
The genetic modification safety committee reviewed example risk assessments associated with this work. The committee recommended that the proposed studies should use recognised disabled host strains and characterised strains as detailed in the risk assessment. It was recognised that the intended approaches used in the programme could eventually be applied to wild type strains of Salmonella eg the use of phage for detection of Salmonella sp. in enrichment culture.

It was noted that transfer between genera can be permitted from recognised disabled donor strains and would be carried out in the programme. It was agreed that such experiments would not include transfer between wild type strains of ACDP 2 pathogens eg between Salmonella and Campylobacter). Work with Salmonella typhi or S. paratyphi is not permitted since these are ACDP 3 pathogens. The proposed work on Salmonella sp was classified as class 2 containment level 2.

It was noted that experiments would be performed to examine the influence of signalling molecules from ACDP 2 organisms. It was noted that the proposed host strain Vibrio harveyi was a relatively safe Vibrio sp. However Vibrio sp are ACDP 2 pathogens and since this work involves other ACDP 2 organisms then the work should be considered class 1 containment level 2.

Any future studies other than those directly proposed within the risk assessments cannot be carried out without risk assessment and the approval of the GMSC.

### Project Containment

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**Name**

UNIVERSITY OF LIVERPOOL

**Name 2**

ROYAL LIVERPOOL UNIVERSITY HOSPITAL

**Department**

PATHOLOGY

**Campus Estate or Research Centre**

5TH & 6TH FLOOR DUNCAN BUILDING

**Road Name**

DAULBY STREET

**Town**

LIVERPOOL

**County**

MERSEYSIDE

**Postcode**

L69 3GA

**Country**

ENGLAND

**Tel Number**

0151 706 4483

**Fax Number**

0151 706 5859

**E-mail**

**HSE Division**

NORTH WEST

**Comments**

Date at Which Additional Info Submitted
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Glass House</th>
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Non-microbial

Other (please specify) Tick if confidential

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<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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A. Tissue culture.

All work involving genetically modified mammalian cells utilises established well-characterised cell lines of rat or human origin or primary cultures of animal cells as the host ie. they are especially disabled. The cells are unable to survive outside of the controlled tissue culture environment. Nevertheless, tissue culture fluids from these cultures are routinely aspirated off with disposable Pasteur pipettes and collected in a flask containing hypochlorous acid (4 'Presept' tablets per litre of medium). After a minimum of 4 hours, the inactivated waste is poured down a sink. Pasteur pipettes are immersed in a similar solution for at least 24 hours before being consigned to a glass-disposal bucket.

The maximum volume of culture fluid that may be released at any one time is 800 ml.

Any culture plates bearing viable cells that need to be disposed of are inactivated with 70% ethanol or bleach before being consigned to a waste bag for incineration.

B. Bacterial culture.

The following procedures conform to the manufacturers recommendations which guarantee inactivation of all mammalian cells and bacteria and validation is not required.

Bacteria used are commercially available disabled strains of E. coli with non mobilisable or poorly mobilisable vectors. Culture dishes containing agar and bacteria for disposal are placed in an autoclave bag and autoclaved prior to bagging for incineration.

Flasks containing media broth and bacteria are submerged in a bucket of sodium hypochlorite (5% commercial bleach) for 24 hours prior to washing and autoclaving.

Tubes containing bacterial residue are soaked for 24 hours in 1% "Virkon" disinfectant before washing and autoclaving.

That all users of transfected cells comply with the above procedure is regularly confirmed by the designated laboratory supervisors.
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**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities
  - [ ]
- Give brief details of the genetic modification safety committee

#### Laboratory

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

#### Animal Unit

- Other (please specify) [ ]

#### Growth Room

- Transgenic Animals
- Transgenic Fish
- Transgenic Birds

#### Glass House

- Parasitology
- Transgenic

#### Large Scale

- Microbiology Research
- Gene Therapy
The maximum culture volume that could be released at any one time would be 10 L.

Waste is deactivated by autoclaving or by soaking in Virkon solution (1% w/v) for 20 mins.

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For activities involving GMMs, describe the waste management measures which will apply to the activity:

The maximum culture volume that could be released at any one time would be 10 L.

Waste is deactivated by autoclaving or by soaking in Virkon solution (1% w/v) for 20 mins.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 585**

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Page 1083 of 15326
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
Our maximum bacterial volume at any time is 1.5 litres.

Cell Culture: we estimate approximately 2 litres of cell culture media used for GM cells.

Waste Deactivation:
- All liquid bacterial waste is collected in the flasks used to grow the E.coli. This is then autoclaved at 132 degrees C, 1.9 psi for 30 minutes. The liquid waste is then considered safe to dispose of to the drains with excess water.
- All liquid cell culture waste is added to Hycolin reagent for decontamination, (obtained from William Pearson Chemicals). Hycolin reagent conforms to BS6905:1987. One litre waste bottles have 20 ml of Hycolin reagent added to them prior to any routine culture work. All waste media is then added to this such that the final concentration of Hycolin, after the bottle is full, is 2%. Therefore, GM cell culture waste is never treated with less than the recommended 2%. This is then disposed of to the drains with excess water.
- All contaminated plasticware is autoclaved.
- All contaminated glassware is autoclaved.

Validation of the Deactivation Method
The Hycolin reagent is bought from a reputable source and is routinely used at the recommended concentration.
Our autoclave is serviced and performance tested by engineers from the manufacturers (Astell Scientific) every six months.

Monitoring of the Deactivation Method.
Hycolin disinfectant is a broad-spectrum disinfectant used at the recommended dilution for full effectiveness. Indeed, since we routinely add the waste to the disinfectant, there is no chance of inefficient deactivation due to poor mixing of waste and disinfectant. Given the severity of the treatment, and the fragility of mammalian cells, monitoring of the deactivation was felt to be unnecessary.

The efficiency of autoclaving is routinely monitored using sterilisation indicator tape. Bio-Hazard bags are not completely sealed in order to allow efficient steam sterilisation of the contents. Only if the indicator tape shows that sterilisation has taken place is the waste discarded.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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| Comments                           | CLOSED & MERGED WITH GM731  |

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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste used in genetic modification work is sterilised by autoclaving prior to disposal.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 587

Data Premises Notified (Originally) 16/03/1995

Data Premises Closed

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Transitional Premises

Emergency Plan Required? N

Name

INSTITUTE OF TERRESTRIAL ECOLOGY (MONKSWOOD)

Name 2

Department POLLUTION & ECOTOXICOLOGY

Campus Estate or Research Centre

Building

Road Name

District ABBOTS RIPTON

Town HUNTINGDON

County CAMBRIDGESHIRE

Postcode PE28 2LS

Country ENGLAND

Tel Number 01487 773381

Fax Number 01487 773467

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

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Give brief details of the genetic modification safety committee

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
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Tick if confidential

- **Bacteriology**
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- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

02/03/2022
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Please enter comments of the GM safety committee on the risk assessment
| Data Premises Notified (Originally) | 27/04/1995 | Transferred from 1992 Regs? | Y |
| Data Premises Closed | N |
| Transitional Premises Class | 1 |
| Emergency Plan Required? | N |
| Non-GMMs | N |
| Withdrawn | N |

Name

UK RESEARCH & INNOVATION

Name 2

Department

SYNCHROTRON RADIATION

Campus Estate or Research Centre

Building

Road Name

KECKWICK LANE

District

DARESBURY

Town

WARRINGTON

County

CHESHIRE

Postcode

WA4 4AD

Country

ENGLAND

Tel Number

01925 603107

Fax Number

01925 603124

E-mail

HSE Division

NORTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Cultures (no greater than 100 ml in 5 ml aliquots) and contaminated laboratory ware will be autoclaved at 121 degrees C for 15 minutes at 15 psi.

The autoclave undergoes an annual technical inspection by the insurers. Brownes indicator tubes will be included in the load to ensure that the correct conditions have been reached.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref  589/09.1

Date Ackn'd  29/04/2009  CU2 Project Title  Genetic Analysis of Campolybacter jejuni (C.jejun) and Yersinia pseudotuberculosis (Y.pstb)

Class  2  CultureVolClass  2  CultureVolumeClass  < 1 Litre  Non-GMM  Consent Granted

Withdrawn  N  Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022  Page 10097 of 15326
Project Additional Information

Purposes of the contained use

The purpose of contained use is to reduce risk of contamination of the environment and infection. E. coli strains (commercially available) and their derivatives are unlikely to pose any health risk, don't have ability to survive in the environment due to the presence of various mutations. Campylobacter and Yersinia pseudotuberculosis strains are classified as Class 2 microorganisms. The GM strains of these bacteria (mutants) are likely to have decreased survival/virulence. Therefore, CL2 will provide adequate measure for containment of all strains and derivatives to be used in this study.

Recipient or parental organism

- E. coli - Class 1
- C. jejuni - Class 2
- Y. Pseudotuberculosis - Class 2

Host/vector system

Delivery vector for making C. jejuni mutants is plasmid pUC18, which cannot be maintained or replicate in this host unless it contains a fragment complementary to a gene target, in which case recombination will result in allelic replacement of the target gene with its modified copy.

In case of Y. pstb, a PCR product is used for gene delivery/recombination, which is assisted by the presence of lambda-red recombination system present in vector pAJD434. This helper plasmid carries trimethoprim antibiotic resistance marker used for selection. The vector is temperature sensitive and is lost at 42°C.

The mutants and complemented strains will carry a kanamycin or chloramphenical antibiotic resistance marker, or both.

E. coli host used for cloning will contain vectors, or plasmids carrying individual genes (or their fragments) from C. jejuni and Y. pstb, and will carry an ampicilling resistance marker.

Origin & function

The genes to be mutated in both C. jejuni and Y. pstb are potentially (by similarity to other bacteria) involved in stress response, virulence or survival in the environment. One set of genes encodes putative proteases playing a variety of functions in bacteria. Some of these enzymes are required for processing of other proteins, others - for digestion of bacteria survival in stress conditions, and in reduced virulence. Another set of genes to be targeted are those potentially involved in interaction with host cell receptors. Elimination of these gene products by mutagenesis is expected to reduced virulence properties of the recipient strains.

The insertion of antibiotic resistance markers does not increase bacterial virulence or ability to survive in the environment and is a standard genetic manipulation procedure.

Evaluation of foreseeable effects

Class 1 microorganisms (CL1 required)

- E. coli K12 strains to be used are designed for cloning experiments. They are non-pathogenic and unable to survive in the environment and are therefore classified as Class 1 organisms. Attenuated E. coli strains carrying fragments or full copies of genes (e.g. those encoding proteases or adhesins) originated from C. jejuni and Y. pstb.

Class 2 microorganisms (CL2 required)

- Wild types, mutants and complemented derivatives of C. jejuni and Y. pstb. Although mutations are most likely to result in reduction of virulence, a possibility of at least partial retention of virulence properties will remain, so that CL2 will be required. Confirmation of phenotypic changes will also require complementation studies, in which an exogenous copy of the inactivated gene will be introduced into the mutant. Since this gene will 1) only mimic the wild type copy 2) be expressed at suboptimal level from an artificial promoter 3) integrated as a single copy into bacterial chromosome, it will most likely not restore the full phenotype of the wild type strain and will not result in any increase in pathogenicity. Therefore, these construction and investigation of partially complemented strains not require containment level higher than CL2.
Recombinant E. coli strains

It is highly unlikely that cloning of individual genes and their fragments into attenuated E. coli will make the latter virulent. None of the genes cloned into these strains encode putative toxins and are highly unlikely to be able to convert a non-pathogenic recipient E. coli strain into a pathogenic organism. This is because (with some exception, e.g. as with cloning toxins or invasions) virulence normally requires expression of more than one virulence-related genes. We therefore consider that cloning/expression of genes in this host can be conducted under CL2.

The likelihood that the GMM will be released into the environment under the requirements of the containment level to protect human health is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No experiments with plants or animals will be conducted

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The project will generate the following waste: liquid (up to 100ml) and agar plate cultures, contaminated pipettes, pipette tips and glassware. All contaminated waste, will be autoclaved on a “kill cycle” ensuring that the waste is held at a holding temperature of 121°C (15psi) for 15 minutes. This will kill all GMM's. Autoclaving efficiency will be monitored using thermocouple recorder, and Browne's indicator tubes (Type 1, Black spot) according to manufacturers protocol.

VIRKON (Antec International) will be used for all disinfection work involving wild type C. jejuni and Y. ptsb strains, their mutants and derivatives. 1% (v/v) use-dilution will be prepared fresh for us each day by adding the appropriate volume of concentrate to cold tap water, following the manufacturers instructions. The use-dilution may be stored for 24 hours only.

1% VIRKON is shown to be an effective disinfectant killing many bacteria including C. jejuni and Y.ptsb (see reference below). Any spillage will first be wiped out with dry tissue paper, then treated with tissue paper soaked with 1% VIRKON. The used contaminated tissue paper will be collected in bags/containers and autoclaved. Where appropriate (e.g. small bench areas) will be treated with 70% ethanol.

New staff will undergo appropriate training in order to minimise risk when working with these bacteria.

The work will be conducted in CL2 environment in a lab suite designed to the current safety standards and having all required equipment, including a Class 1 safety cabinet, which can be used if required. However, according to the current risk assessment the use of Class 1 safety cabinet is not required for this project because no experiments producing substantial aerosols (homogenisation and sonication) will be conducted, and due to low risk of infection associated with work involving these enteric pathogens, which can only cause infection when transmitted via oral route (usually with contaminated food/drinks). Therefore, the risk of infection with aerosol is low.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Project Containment

Laboratory Activities  | Glass Houses  | Growth Rooms
---|---|---
L2 Yes | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4
Animal Units | Large Scale Activities | Human Clinical Applications

Project Ref 589/13.1

Date Ackn’d 13/08/2013

CU2 Project Title Generation of recombinant bacteria to analyse the function of genes

Class 2

Culture Vol

Class 2 < 1 Litre

Consent Granted

Non-GMM

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The purpose of the contained use is to prevent laboratory required infections and any contamination to the environment. Regarding all 3 activities, the commercially available laboratory cloning strains of E. coli that will be used are highly attenuated due to their multiple mutations and cannot survive in the environment. They are Class 1 microorganisms which are unlikely to pose any health risk. The expression of a single gene from the above organisms in E. coli will not render these organisms virulent. Nevertheless all work with recombinant E. coli will be conducted under CL2 conditions (in a class 1 safety cabinet in a category 2 laboratory).

For activities 1-3 the mutant strains of the above listed organisms are likely to have a marginal decrease in both virulence and survival and the complemented versions of these strains will express the relevant intact gene at suboptimal levels and will thus not be more virulent than the parent strain. All work will be conducted under CL2 conditions which will provide an adequate measure for containment for all strains and their derivatives.
For activities 1-3, Class 1 recipient strains will be attenuated, laboratory cloning strains of E. coli such as JM109 and DH5 alpha and class 2 recipient strains will include Neisseria spp., Helicobacter spp., Staphylococcus spp., and P. aeruginosa

Host/vector system

For activities 1-3, PCR cloning vectors such as pCR2.1, pJET and pCR-II-TOPO will be used to clone genomic DNA from the above listed organisms in a laboratory cloning strain of E. coli. Appropriate DNA fragments will be excised and cloned into the following vectors:

- Activity 1 For generating targeted mutant strains in Neisseria spp., Helicobacter spp., and Staphylococcus spp. plasmid pUC18/19 carrying the relevant gene disrupted by an antibiotic resistance gene such as the kanamycin resistance gene will be used for delivery of the disrupted gene to the microorganism for homologous recombination in the chromosome to take place. The pUC18/19 plasmid vectors will also be used for generating constructs for complementing the mutant strains and will carry the wild type gene and a different antibiotic resistance gene such as the chloramphenicol resistance gene.
- Activity 2 For generating un-marked, deletion mutant strains of P. aeruginosa, plasmid vector pMQ30 (Robert et al., 2006) carrying DNA upstream and downstream of the gene of interest will be used to permit homologous recombination in the recipient organism. The pBroadgate plasmid vector (Kovach et al., 1995) carrying the relevant wild-type gene will be used to complement mutant strains.
- Activity 3 For constructing the transposon library of mutants in N. meningitidis and N. gonorrhoeae the EZ:: TN<KAN-2> transposome kit (Epicentre will be used that permits the random integraton of the Tn903 kanamycin resistance gene into the chromosome. Plasmid vector pMR33 (Ramsey et al., 2012) will be used to complement selected mutant strains and will carry the appropriate intact gene from the wild type strain and the ermC gene which confers resistance to erythromycin. The pMR33 construct designed specifically for complementation in N. meningitidis and N. gonorrhoeae contains regions of homology with iga and neighbouring trpB and directs the insertion of the gene of interest and ermC into the intergenic region between these two genes by homologous recombination leaving iga and Neisseria and permits controlled expression of the relevant gene.

In activities 1-3, the antibiotic resistance markers that will be used are not the antibiotics that would be used to treat infection caused by any of these organisms.

References


Origin & function

For activities 1-3, the function of the PCR cloning vectors is to allow cloning of PCR products of the genes of interest form bacterial genomic DNA as well as antibiotic resistance genes for the purpose of creating plasmid constructs in E. coli. These constructs will then be used for generating targeted knock outs in Neisseria spp., Helicobacter spp., Staphylococcus spp and P. aeruginosa and for complementing the mutant strains of these organisms.

Specifically the function of the antibiotic resistance gene in the constructs designed to generate targeted gene knock outs or the Tn903-kanamycin resistance gene in the transposon for generating random mutants is 2 fold. Firstly the insertion of the antibiotic resistance gene disrupts the target gene or random gene respectively in the recipient strain and secondly it enables the selection of successful mutated cells following growth of the cells on the appropriate antibiotic. Like wise the second antibiotic resistance gene in complementation constructs enables the selection of successfully complemented strains by growth on the relevant antibiotic. Improtantly, the insertion of antibiotic resistance genes will not increase the virulence of the bacterium or its ability to survive in the environment and is a standard genetic manipulation used in microbiology.

Evaluation of foreseeable effects

For activities 1-3;
Recombinant attenuated E. coli cloning strains.
The attenuated laboratory strains of E. coli harbouring PCR cloning vectors or plasmid constructs for generating targeted knock out mutant strains or plasmid constructs for complementary mutant strains will merely carry an antibiotic resistance gene and a single gene or equivalent sized fragment of genomic DNA from Neiseria spp., Helicobacter spp., Staphylococcus spp. or P. aeruginosa. Since virulence requires the expression of multiple virulence-associated genes, the expression of one gene and at a low level in E. coli will not render this organism more pathogenic. The antibiotic resistance genes expressed by the GM strains are not the antibiotics that would be used to clinically treat infection caused by that organism. The likelihood that the GMM will be released into the environment under the requirements of the CL2 is extremely low. All construction and analysis of recombinant E. coli will be conducted under CL2 conditions.


The mutant strains are likely to be marginally less virulent than the parent strains.

The complemented strains carrying an intact copy of the inactivated gene will not be more virulent than the parent strain due to the inserted gene being integrated as a single copy into the chromosome and expressed at suboptimal levels. Thus the complemented strains are expected to show only partial restoration of the relevant wild type phenotype. Thus these strains will not require containment label higher than CL2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No experiments with plants or animals will be conducted

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The project will generate the following waste: liquid (up to 100 ml) and agar plate cultures, contaminated pipettes, pipette tips and glassware. All contaminated waste, will be autoclaved on a "kill cycle" ensuring that the waste is held at a holding temperature of 134° (15 psi) for 45 minutes. This will kill all GMMs. Autoclaving efficiency will be monitored using thermocouple recorder, and Browne's indicator tubes (Type 1, Black spot) according to manufacturer's protocol.

VIRKON (Antec International) will be used for all disinfection work involving wild type E. coli and N. meningitidis strains and all recombinant strains of these organisms 1% (v/v) use-dilution will be prepared fresh for use each day by adding the appropriate volume of concentrate to cold tap water, following the manufacturer's instructions. The use-dilution may be store for 24 hours only.

1% VIRKON is shown to be an effective disinfectant killing many bacteria including N. meningitidis. Any spillage will first be wiped out with dry tissue paper, then treated with tissue paper soaked with 1% VIRKON. The used contaminated tissue paper will be collected in bags/containers and autoclaved. Where appropriate (e.g. small bench areas) will be treated with 70% ethanol.

New staff will undergo appropriate training. New staff working with N. meningitidis will be vaccinated in order to minimise risk when working with this organism.

The work will be conducted in CL2 environment in a lab suite designed to the current safety standards and having all required equipment, including a Class 1 safety cabinet, which will be used for all work.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The Kingston University GM committee agreed at a meeting held on the 18th June 2013 that all proposed activities (i.e. activities 1-3) can be carried out safely under the safety measures outlined in this document.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
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<td>L2</td>
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**Project Ref** 589/17.1

- **Date Ackn'd** 17/02/2017
- **CU2 Project Title** Regulation of protein phosphorylation in cardiomyocyte studies in vitro, pertaining to the development of heart failure
- **Class** Class 2
- **Culture Volume Class** < 1 Litre
- **Consent** Consent Granted

**Project Additional Information**

**Purposes of the contained use**

The level of protein phosphorylation in the individual cardiomyocyte of the heart has been identified to have a causative role in the development of heart failure. The level of phosphorylation is controlled by the balance in activity.
of a superfamilies of protein kinases and protein phosphatases. Therefore it is important to better understand how these proteins are regulated in heart failure and which of these protein may play a causative role in developing heart failure. The work outlined in this notification will use adenoviral (serotype 5) vectors to genetically modify cardiomyocytes in culture. The vectors to be constructed during these studies will allow us to express or silence the expression of wild type or mutated type 2A protein phosphatase catalytic subunits (PP2AC, PP4C and PP6C) and their associated regulatory subunits to delineate their role in the pathogenesis of heart failure.

Recipient or parental organism

Recipient will be primary (adult rat ventricular myocytes) or cell line (H9c2) cardiomyocytes both of which will be maintained/grown in cell culture

Host/vector system

The vectors to be used are attenuated adenoviral vectors. These will be designed to express wild type proteins, constitutively active or inactive mutants, or to produce siRNA to knock-down native protein levels. We wish to characterise the effects of these alterations on protein phosphorylation in cardiomyocytes in in vitro experiments. The adenoviral vector to be used is serotype 5 with deletions of the E1 and E3 regions. The probability of reversion to wild type is very low, as the deletions are non-contiguous, making rescue by homologous recombination with wild type adenovirus very unlikely.

Origin & function

| PP2A catalytic subunit and associated regulatory proteins of the scaffold "A" and regulatory "B" classes. Elevated activity and expression of these proteins are thought to contribute to the development of heart failure. | Adenovirus. |
| PP6 catalytic subunit and associated regulatory proteins from the sit4 associated proteins (SAPI -3) and ankyrin repeat domain (ANKRD) proteins ANKRD28/44/52. Unknown function in cardiomyocytes. | Adenovirus. |
| Type 2A protein phosphatase regulatory protein immunoglobulin binding protein 1 (IGBP1) also known as alpha4 is known to be antiapoptotic in mu-iple cell types. Unknown function in cardiomyocytes. | Adenovirus. |
| Type 2A protein phosphatase regulatory protein TIPRL(Tip41) thought to inhibit activity of type 2A protein phosphatases and promotes cell death in response to genotoxic stress. Unknown function in cardiomyocytes. | Adenovirus. |
| PKB rNT, constitutively active, kinase-dead and inhibitor-resistant forms) Kinase, implicated in diverse signalling events. Our recent evidence suggests that activation of PKB has an inhibitory effect on NHE activity. Intend to create inhibitor-resistant form to determine role in NHE regulation. Constitutively active form is oncogenic (ie. v-Akt: inhibits apoptosis, promotes constitutive activity of ERK pathway). | Adenovirus. |
| PKCalpha, PKCdelta, PKCepsilon kinases (GFP fusion or kinase-dead forms): Implicated in diverse signalling events in the cytoplasm and nucleus. Suggested to phosphorylate many ion channels, cytoskeletal proteins, receptors and transcription factors. Some isotypes, ie. PKCalpha are highly active in transformed cells and are growth promoters. | Adenovirus. |
| ERK1 , ERK2 rNT only) Extracellular-signal regulated kinases downstream of MEK and upstream of RSK. Protooncogene, but overexpression of wild type forms may promote mrosis . | Adenovirus. |
| MEK rytT, constitutively active or kinase-dead forms) Kinase, implicated in diverse signalling events in the cytoplasm and nucleus. Various isoforms to be used as upstream activators of MAPKs. Phosphorylates downstream MAPKs. MEKs exhibit high activity in transformed cells and are growth promoters. | Adenovirus. |
| Ras (NI? inactive and V12 constitutively active forms) Ras regulates downstream Signalling such as MAPK activation. | Adenovirus. |
WT and active mutants of Ras are growth promoters/oncogenes. Active Ras will not be used in primary human cells or in vivo. Adenovirus.


Gbetagamma: G-protein beta and gamma subunits. Regulates phospholipase Cgamma activation and downstream signalling. Adenovirus (bicistronic expression of both proteins from one mRNA)


Rac (N? inactive and V12 constitutively active forms) : Rac is a small GTP-binding protein that regulates downstream signalling such as MAPKisAPK activation. WT and active mutants of Rac are growth promoters/oncogenes. Adenovirus.


PKA-Rl ryvT and cysteine mutants) Regulatory domain of protein kinase A; binds cAMP and the catalytic domain; no known hazards. Adenovirus

PKD rNT, kinase-dead, Silencing) Protein kinase D; activated by PKC; phosphorylates several substrates such as cMyoBPC, troponin I, histone deacetylases; promotes increased MEF2 activity and hypertrophy in cardiac myocytes; no known hazards. Adenovirus.

MKK3 rNT, Dominant- negative, Constitutively active and truncated MKK3[3] form) : MKK3 dual-specificity protein kinase that activates p38-MAPK. Activated by different forms of cellular stress and inflammatory cytokines; no known hazards. Adenovirus.

Health and Safety Executive

MKK6 (WT and Dominant- negative form): MKK6 dual-specificity protein kinase that activates p38-MAPK. Activated by different forms of cellular stress and inflammatory cytokines; no known hazards. Adenovirus

G5K3-a and 13 (Wt)Glycogen synthase kinase, regulator of numerous proteins. Involved in a variety of different pathways and implicated in a number of diseases, including Type II diabetes, Alzheimer's disease, inflammation and cancer. Adenovirus

Calcineurin A (constitutively active): Calcium-dependent protein phosphatase; activates NFAT-dependent transcription; expression of constitutively active calcineurin A in mice resulted in diabetes. Adenovirus

CAIN: Calcineurin inhibitor protein; no known hazards. Adenovirus.

856alpha (full length and N-terminal domain): Targeting subunit of protein phosphatase 2a; determines localisation of phosphatase activity in cardiac myocytes; no known hazards. Adenovirus.

HDAC4, 5 & 7 Class 2a histone deacetylase isoforms; sequester pro-hypertrophic transcription factors in cardiac myocyte nucleus, which are released when HDAC is phosphorylated by PKD or CaMK; no known hazards. Adenovirus.

NHE1 (WT & drug-resistant or C-terminal domain fragments) Na+IH+ exchanger regulatory domain; wild type and various 5er:Ala mutants; target for several kinases; no known enzyme activity; unlikely to be hazardous. Adenovirus.

14-3-3 proteins binds to phosphorylated proteins, including NHE1 ; also mutant variant which cannot bind; no enzyme activity; not known to be hazardous. Adenovirus.

p90 ribosomal S6 kinase (isoform 1-4): Downstream target of ERK pathway, regulates a subset of growth factor-induced transcription factors and other cellular targets such as NHE1 . Intend to create silencing construct to decrease R5K expression. No known hazards. Adenovirus.

phospholemman: Regulator of NaK-ATPase activity in cardiac myocytes. Alters NaK-ATPase activity in response to
intracellular signalling via PKA, PKC; no known hazards. WT and 5er-Ala mutants. Adenovirus.
p38 MAPKL: p38 MAPK, all 4 isoforms (α, θ, V, ~); stress/cytokine-activated kinase, phosphorylates MAPKAPK2, ATF2, MEF2, heat shock proteins; no known hazards. Adenovirus.
Glutathione-S-transferase (GST): full length GST; involved in glutathiolation of proteins and lipids; active enzyme; not known to be hazardous. Adenovirus.
Luciferase: Firefly protein used as a reporter of eg MEF2 promoter activity; no known hazards. Adenovirus.
EGFP & ECFP Green fluorescent protein from jellyfish Aequorea victoria (and cyan fluorescent protein [CFP]); used as a reporter; no known hazards. Adenovirus.
beta-galactosidase full length beta-galactosidase; involved in metabolism of galactose; active enzyme; not known to be hazardous. Adenovirus.

Evaluation of foreseeable effects

All of the viral vectors to be used are attenuated and unable to reproduce without laboratory-specific helper cells to provide essential deleted genes in trans. In the intended recipient cells, the vectors are expected to express the specific proteins cloned into the vector, sometimes together with marker proteins such as green fluorescent protein, or to suppress the expression of native proteins. The expression or loss of the various proteins is anticipated to modulate the response of cardiomyocytes to various added factors, which will be tested in in vitro experiments. The modification of human cardiomyocytes in a contained manner is not likely to present a hazard.
The accidental inoculation of personnel with modified cardiomyocytes should also not be a hazard, as the cells should be rapidly cleared by the immune system.
Exposure of workers to viral vectors should also result in rapid clearance by the immune system. Any vectors which manage to infect cells will be unable to reproduce, so any effect of transgene expression should be minimal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None under the current project

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste (tips, serological pipettes, tissue culture plastic, vials and other containers) disinfected by immersion in 10% Microsol3+ overnight. After rinsing with water, plastic waste is disposed of via clinical waste stream (orange bags).
For liquid waste, Microsol3+ is added to 10 % and left overnight. Waste is then flushed down drain with lots of water.
Surfaces are wiped down with 10 % Microsol3+.
Microsol3+ is effective against bacteria, fungi and viruses, and overnight incubation at the stated dilution should result in 100 % kill.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y
The Kingston University Viral Genetic Modification Advisory Group (vGMAG) reviewed the assessment on 16th August 2016 and approved it at GM Class 2 with no amendments required.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment N

### Project Containment

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GM Centre Number: 590

Data Premises Notified (Originally) 07/07/1995

Transferred from 1992 Regs? Y

Transitional Premises Class none

Data Premises Closed 12/09/2001

Transitional Premises N

Emergency Plan Required? N

Non-GMMs Y

Withdrawn N

Name
MARINE HARVEST MCCONNELL

Name 2

Department
HEALTH DEVELOPMENT

Campus Estate or Research Centre

Building

Road Name

District
LOCHAILORT

Town
INVERNESS

County
HIGHLAND

Postcode
PH38 4LZ

Country
SCOTLAND

Tel Number 0131 336 1777

Fax Number 0131 336 1199

E-mail

HSE Division SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee

<table>
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- Other (please specify)  
  - Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Name

UNIVERSITY OF STAFFORDSHIRE

Name 2

Department

DIVISION OF BIOLOGY

Campus Estate or Research Centre

Building

Road Name

COLLEGE ROAD

District

Town

STOKE ON TRENT

County

STAFFORDSHIRE

Postcode

ST4 2DE

Country

ENGLAND

Tel Number

01782 294000

Fax Number

01782 745506

E-mail

HSE Division

MIDLANDS

Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs) | | | | |
Level 2 (GMMs) | | | | |
Level 3 (GMMs) | | | | |
Level 4 (GMMs) | | | | |
Non-microbial | | | | |
Other (please specify) | | | | | Tick if confidential |

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research
---|---|---|---
Virology | Transgenic Animals | Transgenic Fish | Gene Therapy
Mycology Transgenic Invertebrates Transgenic Plants Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 591/95.1

CU2 Project Title

DEVELOPMENT OF TRANSFORMATION SYSTEMS FOR GRAM POSITIVE BACTERIA

Class CultureVolClass2 CultureVolumeClass3-4

Class 2

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ] N

If yes, tick to confirm that it is attached to this form [ ] N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ] N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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GM Centre Number: 594

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Name

UNIVERSITY OF NOTTINGHAM

Name 2

SCHOOL OF CLINICAL LABORATORY SCIENCES

Campus Estate or Research Centre

QUEENS MEDICAL CENTRE

Building

UNIVERSITY HOSPITAL

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County

NG7 2UH

Postcode

ENGLAND

Country

Tel Number

0115 970 9924

Fax Number

0115 970 9233

E-mail

HSE Division

MIDLANDS

Comments

GM594 closed and transferred to GM470 on 18/02/2005

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

  - [ ]

- Give brief details of the genetic modification safety committee

  - Laboratory
  - Animal Unit
  - Growth Room
  - Glass House
  - Large Scale

### Level 1 (GMMs)

### Level 2 (GMMs)

### Level 3 (GMMs)

### Level 4 (GMMs)

### Non-microbial

### Other (please specify)

  - [ ] Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

02/03/2022

Page 10117 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 594/01.1**

**CU2 Project Title**

PATHOGENESIS OF , AND HUMAN IMMUNE RESPONSE TO, ACUTE BACTERIA INFECTIONS: IDENTIFICATION, CLONING, EXPRESSION AND MUTAGENESIS OF GENES FROM CLASS 2 BACTERIA, INCLUDING NEISSERIA

**Date Ackn'd**

11/06/2001

**Date Project Ceased**

18/02/2005

Non-GMM Consent Granted

Project notified under transitional arrangements

---

### Project Additional Information

GM594/01.1 TRANSFERRED TO GM470 ON 18/02/2005
Purposes of the contained use

We are interested in studying the role of individual bacterial genes in (a) the pathogenesis of bacterial infection (b) the host immune response to products of these genes, and (c) killing by colicins. The studies are designed to increase our understanding of host-pathogen interaction and develop new preventative and therapeutic methods against invasive bacterial disease. We attempt to detect proteins of interest (usually vaccine candidates or antimicrobials) from sequence data or genomic expression libraries (eg in the phage based x-ZapII) and identify their encoding genes. We then amplify the genes by PCR, clone them in plasmid vectors and express them under the influence of IPTG. These will be used to carry out studies on the characterisation of the molecular, immunochemical and functional properties of the antigens of interest.

Recipient or parental organism

Neisseria: including Neisseria meningitidis, N. gonorrhoea and commensal Neisseria (eg N. lactamica)
Campylobacter: Campylobacter jejuni and C. coli.
Klebsiella: K. pneumoniae ssp and K. oxytoca.

Host/vector system

Hosts:
All are E. coli, derivatives of strain K12: Examples:

JM109 (recA1, endA1, gyrA96, thi, hsdR17 (rK-,mK+), relA1, supE44, (lac-proAB), [F’, traD36, proAB, lacIqZM15].
XL10-Gold (Tetr, (mcrA) 183, (mcrCB-hsdSMR-mrr) 173, endA1, SupE44, thi-1, recA1, gyrA96, relA1, lac hte, [F’proAB lacIqZ M15 Tn10 (Tet) Amy Camr].
XL-1 Blue (endA1, hsdR17 (rK-, mK+) relA1, supE44, thi-1, gyrA96, (lac-) [F’ proAB lacIqZ M15 Tn1].

Vectors (examples)
1. Plasmid vectors that will be used for studies of protein over-expression from native and inducible promoters (native constructs - pGEM-T, pBr4, pBR322, pUC18/19; inducible vectors - pBAD, pET etc) are considered non-Mobil sable or mobilisation defective. These plasmids will remain in the transformed host and will confer antibiotic resistance genes to the host. Examples of antibiotics to be used are tetracycline, kanamycin, chloramphenicol or ampicillin.

pBluescript: pUC19 derivative, which is non-mobilisable. It contains a colE1 origin of replication, T3 and T7 promoters, and an ampicillin resistance gene. It has an F1 origin that contains an initiator and terminator for M13 DNA replication and the lacZx peptide for blue/white selection.

pGEM: pUC19 derivative, which is non-mobilisable. It contains the colE1 origin of replication, SP6 and T7 promoters, ampicillin resistance marker and the lacZx peptide for blue/white selection.

pREP4: pACYC-184 derivative, which is non-mobilisable. It contains a kanamycin resistance gene and lacIq for constitutive expression of the lac repressor.

pQE30: pDS-56 derivative, which is non-mobilisable. It contains an ampicillin resistance gene and a 6x Histidine tag for Ni/NTA purification of expressed proteins.

PET and pBAD: non-mobilisable expression vectors that contain; ampicillin or kanamycin resistance genes and a 6x Histidine tag for Ni/NTA purification of expressed proteins (pET); or an arabinose inducible promoter for regulated, inducible expression (pBAD).

PCRT7/NT-TOPO and its related plasmids: pUC derivative, which is non-mobilisable. It contains an ampicillin resistance gene and a 6x Histidine tag for Ni/NTA purification of expressed proteins.
2. Phages:

zapII: a based expressed phagemid useful for cloning genomic DNA in the range of 2-10 kb. zapII contains the pBluescript replicon. At the junction between n and the pBluescript DNA sequences there is an initiator and terminator for M13 replication.

Transposons: Tn5 derivatives (eg EZ::TN transposomes from Cambio) which have been constructed to transpose once. Plasmid vectors that will be used for studies of protein over-expression from native and inducible promoters (native constructs - pGEM-T, pBR322, pUC18/19; inducible vectors- pBAD, pET etc) are considered non-Mobil sable or mobilisation defective. These plasmids will remain in the transformed host and will confer antibiotic resistance genes to the host. Examples of antibiotics to be used are tetracycline, kanamycin, chloramphenicol or ampicillin.

Origin & function

Origins of the geneic material are the above mentioned bacterial pathogens. They include:
Neisseria: including Neisseria meningitdis, N. gonorrhoea and commensal Neisseria (eg N. lactamica)
Campylobacter: Campylobacter jejuni and C. coli. Helicobacter pylori
Klebsiella: K. oxytoca and K. pneumoniae ssp.

Evaluation of foreseeable effects

All of the E.coli host strains that we propose to use are K12 derivatives, which are recognised to be non-colonising and are considered to be non-pathogenic to humans or animals. In addition, they have multiple auxotrophic requirements which are unlikely to be met in the environment and so they are assumed to have very limited survivability in the environment.

The pathogens are mostly human commensal and/or opportunistic bacteria. They are common colonisers of skin and mucosal surfaces. Normal healthy individuals, when exposed, usually become transiently colonised with no consequences. However, in susceptible individuals, the pathogenic bacteria can cause invasive disease and in some cases (particularly N. meningitidis) they are capable of causing life threatening diseases.

The plasmid vectors used are all non-mobilisable and are, therefore, unlikely to be transferred to other environmental bacteria. The genes cloned from these bacteria will initially be of unknown function. It is unlikely, however, that any DNA sequence will confer on the host a significant increase in fitness or pathogenicity to the host due to the multiple disablements present in the host and the multifactorial nature of pathogenicity.

The antibiotic cassettes that we propose to use as selectable markers to facilitate inactivation of genes in the bacteria will confer resistance to antibiotics that are not in use in either the treatment or prophylaxis of disease. Furthermore, the mutants can be constructed in such a way that the selectable marker can be subsequently removed where appropriate.

The proposed E. coli host strains are all disabled E. coli K12 derivatives are are in the biological agents hazard group 1. Although the introduction of cloned genes might conceivably increase the fitness or survivability of these organisms it is assumed to be very unlikely. However, because of the small potential increase in perceived hazard the recombinant organisms will be treated as biological agents hazard group 2 and, therefore, containment level 2 precautions will be applied. The organisms will, therefore, be provisionally classified as hazard group 2.

All the bacteria included in the study are in the biological agents hazard group 2. After inactivation of the genes of interest, it is highly unlikely (although theoretically possible) that there will be an increase in the virulence or fitness of the organism.

All manipulations of live bacteria (including host E. coli in which genes of interest are overexpressed) will be contained using containment level 2. Genetically modified bacteria and, where appropriate, host E coli in which genes of interest are overexpressed will be handled in a class 1 cabinet.
The containment precautions to be used are considered sufficient to reduce the risk of accidental infection, or release to the environment, to negligible or zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All bacteria will be handled and disposed of using rigorous procedures, including autoclave of all cultures and contaminated materials. All genetically modified E. coli will be cultured in volumes of less than 100 ml (except for large scale production in an E. coli expression host) in the Microbiology laboratory. Genetically modified bacteria will always be contained and handled in a class 1 microbiological safety cabinet within a category 2 laboratory. Genetically modified pathogenic bacteria will be cultured in volumes of less than 100 ml in sealed containers that can be autoclaved. All liquid cultures will be transferred in sealed containers to be autoclaved before disposal via the sink. Solid media on which modified bacteria have been cultured will also be transferred in sealed containers to be autoclaved before disposal. All liquid non-recombinant E. coli cultures will be sterilised in a 2% (v/v) phenolic disinfectant (Hycolin) for 24 hours before disposal down the sink waste. Solid media on which genetically modified E. coli have been grown will be autoclaved before disposal. If, in the unlikely event the genetically modified E. coli should escape the risk will still be negligible, as the host E. coli are disabled and unable to colonise a human host or persist in the environment. The risk of escape of genetically modified test pathogens will be negligible because of the level of containment that we propose to use. In the unlikely event of an escape of these bacteria the genetically modified organism is unlikely to survive for more than a very short time in the environment. The risk of transfer of vectors from genetically modified E. coli to environmental organisms is also low due to the non-mobilisable nature of the vectors to be used.

To reduce the likelihood of transmission of GMMs to people in the laboratory, in addition to the precautions detailed above (recombinant test bacteria and, where appropriate, recombinant E. coli to be contained and handled in class 1 cabinet) laboratory personnel will wear gloves to reduce the likelihood of contamination of hands. All waste produced while handling genetically modified test bacteria will be transferred immediately by the person handling the organism to be autoclaved. Contaminated laboratory coats will also be autoclaved.

These measures are considered to reduce the likelihood of any potential hazard effectively to zero.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment were discussed thoroughly, and passed, at the appropriate committee.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
CD95 is a cell surface protein that transduces apoptotic signals critical to B cell development. Loss of CD95 function may also be important in the genesis of B cell lymphoma. We have made the novel observation that expression of CD95 in certain B cell lymphomas appears to be regulated by membrane trafficking (1). This project will investigate the mechanisms controlling trafficking of the CD95 molecule. The project is based on the creation of cell lines expressing tagged recombinant wild type or mutated CD95. Retroviral vectors are the most efficient tools for creating such cell lines.

The commercially available retroviral packaging cell line PT67 (expressing viral proteins gag, pol and env in trans) will initially be used to facilitate the production of replication incompetent retrovirus that can be used to introduce CD95 into other mammalian cells lines which will be used for trafficking studies.

Recombinant virus will be used to transduce the Burkitt's lymphoma - like cell lines Mutu I and Mutu III will also be transferred into immortalised (virus-negative) human breast cancer cell lines MCF-7.

Future studies may require introduction of constructs into additional, known virus-negative human cell lines.
All cell lines to be used are considered to be equivalent to ACDP hazard Group I and are routinely handled using Class I containment precautions.

Host/vector system

pRevTet off and pRevTre are commercially available retroviral vectors derived from moloney murine leukaemia virus and moloney murine sarcoma viruses respectively. The expression of cloned inserts within pRevTet off is tightly controlled using tetracycline. Both retroviruses are defective, that is they lack the genes necessary (gag, pol and env) to undergo a full found of replication outside their packaging cell line PT67N.

The cell line PT67N provides the genes in trans that are missing in the retroviral vector. This is a commercially available standard packaging cell line.

Virus containing the cloned insert is produced transiently in the packaging cell line and used to transduce the target cell lines Mutu I, Mutu III or MCF-7.

Origin & function

Human CD95 is readily available as a cloned reagent from investigators. A CD95/CFP (cyan fluorescent protein) fusion protein will be generated as well as fusion proteins containing mutations within CD95 itself. These constructs will be cloned into the pRevTet off vector under the tight control of the tetracycline operator and used to transduce the target cell lines. Trafficking of the wild type fusion protein and the effect of mutations on trafficking will then be observed.

Evaluation of foreseeable effects

Modification of the retroviral packaging cell line PT67 or the Burkitt's lymphoma - like cell lines Mutu I and Mutu III by introduction of recombinant CD95 is considered highly unlikely to affect the ability of these cell lines to establish themselves in a mammalian host. These GMOs are therefore considered to pose no significant risk to human or animal health or the environment.

The effects of expressing mutant forms of CD95 in cells already expressing wild type protein are unknown, however, the possibility of generating a trans-dominant mutant within an amphotropic retrovirus must not be discounted. CD95 is not an oncogene but deletion of CD95 has been linked to development of lymphoma. This situation could potentially arise if workers were accidentally exposed to packaged recombinant retrovirus and the virus became integrated into mammalian cells. This likelihood will be minimised by use of a Class 1 Microbiological safety cabinet for all manipulations involving packaged recombinant retrovirus and derivation of stable cell lines. The risk from accidental exposure of workers to recombinant retrovirus is also likely to be further reduced by the nature of the defective retrovirus which dictates that replication is only possible within the packaging cell. Once integrated into mammalian cells the retrovirus is unable to replicate and produce further virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Once the cell lines have been established they are considered to pose no threat to human or animal health or the environment as the defective retrovirus can not undergo replication and viral particles are not produced. It is therefore requested that to make effective use of these reagents, once the cell lines have been established they are classified as equivalent to ACDP hazard Group 1 organisms allowing their use under containment procedures, appropriate for Class 1 activities.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste will be autoclaved to achieve a 100% kill. Autoclave waste will then be removed from the site by licensed disposal contractors prior to disposal to landfill.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N
The initial assessment was modified to recognise the potential risk to human health of packaged retrovirus to be used for transfection into cell lines for trafficking studies and this part of the work was considered to require the additional containment precautions of a Class 2 activity plus the use of a Class 1 microbiological safety cabinet.

Project Containment

<table>
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Project Ref 594/03.1

Date Ackn'd: 13/08/2003

CU2 Project Title: GENERATION OF PSEUDOTYPE VIRUSES CARRYING HEPATITIS C VIRUS OR HUMAN IMMUNODEFICIENCY VIRUS GLYCOPEPTIDES ON THEIR SURFACE

Class: Class 3
Culture Vol: Class 2 Culture 1-50 litres
Culture Volume: Class 3-4 Culture 500 ml

Non-GMM Consent Granted: yes

Project notified under transitional arrangements: N

Historical Significant Changes: GM594/03.1 TRANSFERRED TO GM470 ON 18/02/2005

Project Additional Information
Purposes of the contained use

The aim of this project is to develop a single cycle infection system to study the role of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) glycoproteins in cell attachment and entry. The unavailability of an efficient culture system for HCV has restricted the study of the mechanisms of HCV infection and development of effective antiviral agents. Several research groups have developed systems to generate infectious pseudoparticles displaying heterologous viral glycoproteins in their functional form onto retroviral core particles. Recently, Bartosch et al. (2003) described such a system for producing pseudotype particles carrying functional HCV glycoproteins on their surface.

The pseudotype retrovirus systems utilise replication-defective packaging and transfer vectors. The transfer vectors containing the transgene are rendered replication-defective by inactivating critical genes involved in viral replication (eg the env, nef and vpr genes), which still retain the viral cis-acting sequences such as LTRs, packaging sequences and regions involved in reverse transcription. The transfer vectors are propagated by trans-complementing constructs (packaging vectors) which supply in trans the packaging proteins whose genes have been deleted in the transfer vector. The packaging vectors are also replication defective as they lack cis-acting sequences such as LTRs, packaging sequences, some structural genes (eg gag and pol) and regions involved in reverse transcription. The trans-complementing factors on the packaging vectors can be segregated and supplied as two separate complementing genomes, the first one for the Gag-pol proteins and the second one for the viral glycoproteins. For the generation of pseudotype particles, the glycoproteins derived from the virus of interest are used.

The project will utilise existing retroviral transfer/packaging vectors and vectors encoding HCV or HIV glycoproteins supplied in trans to produce infectious replication-incompetent pseudotype viruses. Three packaging/backbone vectors will be used: two derived from HIV and the other from murine leukaemia virus (MLV). Three systems will be used because they have been shown to have different efficiencies in different cell types.

HCV pseudotype particles generated in mammalian cells will carry recombinant RNA genome and are capable of infecting human (and possible animal) cells. The particles are likely to be hepatotropic due to the presence of HCV glycoproteins on their surface and could infect the target cells if accidentally injected into humans. Alternative routes of transmission are unlikely to be successful since the HCV envelope proteins will determine the tropism, and route of entry. Upon cell entry and uncoating the genome will be transcribed and proteins synthesized. However, the packaged RNA genome is incapable of replication and therefore no virus progeny will be produced.

HIV pseudotype particles generated in mammalian cells will carry recombinant RNA genome and are capable of infecting human (and possible animal) cells. The HIV envelope proteins will therefore confer the ability to infect cells expressing CD4 plus one of the known co-receptor molecules (eg CCR5 and CXCR4), which are predominantly, but not exclusively, expressed on cells of the immune system. The particles could therefore infect the target cells if accidentally injected into humans. Upon cell entry and uncoating the genome will be transcribed and proteins synthesized. However, the packaged RNA genome is incapable of replication and therefore no virus progeny will be produced.

Recipient or parental organism

HCV pseudotype particles generated in mammalian cells will carry recombinant RNA genome and are capable of infecting human (and possible animal) cells. The particles are likely to be hepatotropic due to the presence of HCV glycoproteins on their surface and could infect the target cells if accidentally injected into humans. Alternative routes of transmission are unlikely to be successful since the HCV envelope proteins will determine the tropism, and route of entry. Upon cell entry and uncoating the genome will be transcribed and proteins synthesized. However, the packaged RNA genome is incapable of replication and therefore no virus progeny will be produced.

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Host/vector system

1st generation HIV vector (pNL4-3.Luc.R.E-): HIV is a category 3 human pathogen that is predominantly transmitted via sexual contact, vertical transmission (from infected mother to infant) and also via contaminated blood and blood products (eg needlestick injury). The transfer vector, pNL4-3.Luc.R.E-, is a disabled (ie replication-incompetent) retroviral vector carrying the provirus sequences derived from HIV isolate NL4-3. The construct has been rendered replication defective by introducing two frameshift mutations into the vpr and env genes. In addition, a reporter gene has been introduced into the nef gene, rendering the vector nef-. Two versions of the vector will be used. One has the reporter gene firefly luciferase (lux), whilst the other contains green fluorescent protein (GFP). This vector retains all the cis-acting elements (such as LTRs, packaging sequences and regions involved in reverse transcription) and encodes functional Gag-pol proteins necessary for particle assembly and secretion. In addition, it also encodes all the remaining viral proteins.

The pseudotype virus particles will be re covered from the vector backbone by co-transfecting mammalian cell lines with vector expressing CMV promoter-driven HCV envelope proteins E1 and E2 or HIV gp120/41 (see below). These pseudotype particles will package the original defective backbone pNL4-3-derived RNA.
2nd Generation HIV packaging/reporter vectors (Packaging vector pCMV D R8.91, lentiviral reporter vector pWPT-GFP; pCMV D R8.91 is a disabled (ie replication-incompetent) HIV-1 retroviral vector that has been rendered replication defective by removing the accessory genes vpr, vif, vpu and nef as well as the env gene. This acts as a source of reverse transcriptase and gag proteins, which assemble and package transcripts produced from the pWPT-GFP vector. This vector is the only genetic material transferred to the target cells. It comprises the transgene cassette (GFP) flanked by cis-acting elements necessary for its encapsidation, reverse transcription and integration. The vector is a self-inactivating (SIN) HIV-1-derived vector, which lose the transcriptional capacity of the viral long terminal repeat (LTR) once transferred to target cells. This minimises the risk of emergence of replication competent recombinants (RCR) and avoids problems linked to promoter interference. The post-transcriptional regulatory element of woodchuck hepatitis virus has been inserted to enhance transgene expression.

MLV (MLV-CMC pr-gag-pol and MLV- CMVpr-GFP): MLV is an amphotropic virus but is non-pathogenic to humans. Vectors derived from MLV are frequently used in gene transfer/therapy experiments. Here we propose to use a three systems for generating HCV pseudotype particles. The three vectors are: the MLV-CMVpr-gag-pol packaging vector (which serves as a source of retroviral packaging proteins and is transcribed via a CMV promoter), a transfer vector, pMLV-CMVpr-GFP (carrying CMV promoter-driven GFP transcriptional unit flanked by MLV LTRs and a packaging signal), and a plasmid expressing HCV glycoproteins (see below). Complete pseudotype particles will be produced from mammalian cells co-transfected with the three plasmids.

Vectors containing the HCV glycoprotein (envelope) genes: The HCV envelope genes E1 and E2 derived from various genotypes have been amplified via PCR and directly cloned into pcDNA3 based vectors that contain the CMV early promoter to facilitate mammalian protein expression.

E.coli host. The vectors will be maintain in the TOP10F strain of E. coli (F mcrA [mrr-hsdRMS-mcrBC], 80lacZ M15, lacX74, deoR, recA1, araD139 [ara-leu]7697, galU, galK, rpsL, endA1, nupG) or other K-12 derived laboratory strains that are non-colonising and disabled. The survival of these bacteria in the environment is expected to be low as they have multiple auxotrophic requirements that are unlikely to be met outside of laboratory culture: the recA1 disablement of TOP10F means that it is unable to repair DNA by homologous recombination and is UV sensitive, and the [ara-leu] 7697 mutation shows that it is auxotrophic for leucine, and unable to grow in the presence of arabinose.

pNL4-3.Luc.R.-E: The transfer vector, pNL4-3.Luc.R.-E, is a disabled (ie replication-incompetent) retroviral vector carrying the provirus sequences derived from HIV isolate NL4-3. The construct has been rendered replication defective by introducing two frameshift mutations into the vpr and env genes. In addition, a reporter gene has been introduced into the nef gene, rendering the vector nef. The original pNL4-3 clone was derived from NYS (5') and LAV (3') cloned directly from genomic DNA into pUC18.

This vector retains all the cis-acting elements (such as LTRs, packaging sequences and regions involved in reverse transcription) and encodes functional Gag-pol proteins necessary for particle assembly and secretion. In addition, it also encodes all the remaining viral proteins, apart from vpr, as well as a reporter gene. Production of pseudotype viruses occurs when this plasmid is co-transfected with the viral glycoprotein-carrying pcDNA3 vectors.

MLV-CMVP-pr-gag-pol: the MLV-CMVP-pr-gag-pol packaging vector serves as a source of retroviral packaging proteins and is transcribed via a CMV promoter. Production of pseudotype viruses occurs when this plasmid is co-transfected with the viral glycoprotein-carrying pcDNA3 and the MLV-CMVP-pr-gag-pol vectors.

MLV-CMVP-pr-GFP: pMLV-CMVP-pr-GFP ( carrying CMV promoter-driven GFP transcriptional unit flanked by MLV LTRs and a packaging signal), acts as a transfer vector. Production of pseudotype viruses occurs when this plasmid is co-transfected with the viral glycoprotein-carrying pcDNA3 and the MLV-CMVP pr-gag-pol packaging vector.

Vectors containing the HCV glycoprotein (envelope) genes: The HCV envelope genes E1 and E2 derived from various genotypes have been amplified via PCR and directly cloned into pcDNA3 based vectors that contain the CMV early promoter to facilitate mammalian protein expression. This vector acts as a source of viral glycoproteins for pseudotype formation.

Evaluation of foreseeable effects

The vectors may be able to undergo recombination with endogenous retroviruses present in the culture cells (or accidentally infected host), or package transcripts from
endogenous retroviral sequences. Also, the vectors could undergo recombination with wild-type virus. However the resulting recombinant would not be more pathogenic than the wild-type virus.

Pseudotype viruses could in principle cause infection, but would not replicate. The potential target cell would be dictated by the envelope proteins incorporated into the pseudotype virus (ie HCV envelope proteins). Infection by pseudotype virus would result in reverse transcription then possible integration of the packaged genome. This in turn might lead to the production of viral/reporter proteins, but as the genome is defective, new infectious virus would not be produced. The effect of GFP or luciferase expression on the host cell is unknown, whilst some of the HIV gene products have been shown to have some detrimental effects (eg apoptosis, toxicity etc), at least in vitro. Whilst, cells producing viral proteins would be targeted by the host immune response (eg cytotoxic T-cells), it can not be assumed that exposure to the GMM will not cause harm.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

HCV E1E2 pseudotypes produced using the pNL4-3 system: The parental virus for the pNL4-3 vector is a category III organism. Infection by the pseudotype virus would lead to the production of viral proteins and reporter proteins. The pathogenic potential of these proteins is largely unknown, although some effects have been reported in in vitro studies. Whilst the resulting pseudotype virus produced is infectious, it is not able to replicate. Infection could lead to insertion of the packaged genome into the host chromosome. However, it lacks known transforming sequences. The risk of recombination between the HCV E1E2 containing vector and the pNL4-3 is low. As the inactivating mutations within the pNL4 backbone are small deletions there is a small risk of reversion and subsequent production of wild type virus. However, the cell lines used to produce the pseudoviruses are incapable of supporting HIV replication. Even so, the viruses are infectious and are capable of integration and subsequent protein production. There is a small but real risk of the mobilisation of endogenous retroviral sequences and the formation of replicative competent retroviruses. Therefore we propose that the containment level for this aspect of work is Level 2, with the following additional precautions.

All work involving the pseudotype viruses to be carried out within a Class 1 or Class II safety cabinet located in a room where access is restricted at times when manipulations involving these viruses are taking place. The work will involve standard laboratory procedures, however, considering the routes of infection of the viruses (parenteral), no sharps or glassware will be used when handling the pseudotype viruses. All solid waste materials will be autoclaved and liquid waste disinfected with gluteraldehyde. Training records for all new staff using these methods will be kept.

HCV and HIV pseudotypes produced using the 2nd Generation HIV packaging/reporter vectors (Packaging vector pCMV DR8.91, lentiviral reporter vector pWPT-GFP): pCMV D R8.91 is a disabled (ie replication-incompetent) HIV-1 retroviral vector that has been rendered replication defective by removing the accessory genes vpr, vif, vpu and nef as well as the env gene. This acts as a source of reverse transcriptase and gag proteins, which assemble and package transcripts produced from the pWPT-GFP vector. This vector is the only genetic material transferred to the target cells. It comprises the transgene cassette (GFP) flanked by cis-acting elements necessary for its encapsidation, reverse transcription and integration. The vector is a self inactivating (SIN) HIV-1 derived vector, which lose the transcriptional capacity of the viral long terminal repeat (LTR) once transferred to target cells. This minimises the risk of emergence of replication competent recombinants (RCR) and avoids problems linked to promoter interference. The post-transcriptional regulatory element of woodchuck hepatitis virus has been inserted to enhance transgene expression. Complete retroviral particles are produced in the presence of a third vector containing the viral glycoproteins under study. Infection of actively replicating cells allows reverse transcription of the RNA genome and subsequent integration of the GFP marker protein. Upon accidental transmission, the transcribed GFP cDNA may become integrated into recipient cells. The transduced cells will contain viral genes on split plasmids. Details of the constructs and the demonstration that they do not give rise to replication competent viruses are available in Zufferey et al Nat Biotechnol. 15: 871-875. There is evidence that such replication defective retroviruses are not pathogenic when tested in mice. Therefore we propose that the containment level for this aspect of work is Level 2, with the following additional precautions:

All work involving the pseudotype viruses to be carried out within a Class 1 or Class II safety cabinet located in a room where access is restricted at times when manipulations involving these viruses are taking place. The work will involve standard laboratory procedures, however, considering the routes of infection of the viruses (parenteral), no sharps or glassware will be used when handling the pseudotype viruses. All solid waste materials will be autoclaved and liquid waste disinfected with gluteraldehyde. Training records for all new staff using these methods will be kept.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2: All work involving the pseudotype viruses to be carried out within a Class 1 or safety cabinet located in a room where access is restricted at times when manipulations involving these viruses are taking place. All solid waste materials will be autoclaved at 134-137 degrees C, 21.2-2.2 bar pressure with a holding time of 5 minutes. A chart record is maintained for each run. Autoclaves are also subject to quarterly thermocouple testing. All liquid waste known to be, or potentially infected with category 2 viruses will be discarded into a 500 ml polyethylene discard pot containing 50 ml of 100% Gagasept, so that when full with liquid waste the final concentration of Gagasept will be 10%. An enquiry date for the gagasept, which should be two weeks from the date of first use, will be written on the surface of the discard pot. If the discard pot is not full before this expiry date then the discard pot will be filled to 500 ml with water and left for 24 hours to inactivate virus. Preparation of working dilutions of Gagasept will be carried out within the class 1 cabinet.

Containment level 3: All liquid waste known to be, or potentially infected with category 3 viruses will be discarded into a 500 ml polyethylene discard pot containing 50 ml of Gagasept, so that when full with liquid waste the final concentration of Gagasept will be 10%. An enquiry date for the gagasept, which should be two weeks from the date of first use, will be written on the surface of the discard pot. If the discard pot is not full before this expiry date then the discard pot will be filled to 500 ml with water and left for 24 hours to inactivate virus. Preparation of working dilutions of Gagasept will be carried out within the class 1 cabinet. All solid waste will be autoclaved prior to disposal. Autoclaving of waste will take place using a destructive cycle of 126 degrees C for 45 minutes. The maximum load will be one autoclave bag (containing a maximum of 2 laboratory gowns) together with 4 discard pots. The autoclave is checked on a monthly basis by independent thermocouple testing using typical loads. Cabinet generated solid waste will be accumulated in paper bags placed within the cabinet. At the end of each working period the waste will be placed into an autoclave bag and transferred to the autoclave. Solid waste bags will be left open and screw-capped lids loosen prior to autoclaving to allow steam penetration. Discard of all Gigasept solutions will take place using the designated sink within the main virology laboratory.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

RA approved by the local GMSC on 7 July 2003.

Project Containment

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**Name**

SIGMA ALDRICH COMPANY LTD

**Name 2**

Department

PRODUCTION DIVISION - POOLE

**Campus Estate or Research Centre**

**Road Name**

FANCY ROAD

**Town**

POOLE

**District**

**County**

DORSET

**Postcode**

BH12 4NZ

**Country**

ENGLAND

**Tel Number**

01202 733114

**Fax Number**

01202 742575

**E-mail**


**HSE Division**

WALES AND SOUTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Laboratory</th>
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Bacteriology       Parasitology       Transgenic Birds       Microbiology Research       Gene Therapy
Virology            Transgenic Animals   Transgenic Fish       

02/03/2022
No genetic manipulation work is carried out; operations confined to shake flask cultures (vol 50 to 800 mls). Solid waste (plate cultures) is autoclaved (15 psi/15 mins) then sent for incineration. Liquid waste is either autoclaved (15 psi/15 mins) or deactivated with Virkon (1% solution, min contact time 10 mins, following manufacturers instructions).

The maximum culture volume that could be released at any one time would be about 800 mls (contents of one shake flask).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 596**

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**EASTMAN DENTAL INSTITUTE**

**Department**

MICROBIOLOGY

**Campus Estate or Research Centre**

**Road Name**

256 GRAYS INN ROAD

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

WC1X 8LD

**Country**

ENGLAND

**Tel Number**

0207 915 1000

**Fax Number**

0207 915 1039

**HSE Division**

LONDON

**Comments**

GM CENTRE CLOSED & ALL WORK TRANSFERRED TO GM14

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Animals
- Transgenic Birds
- Gene Therapy
- Microbiology Research
- Transgenic Fish
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.

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**Project Ref 596/00.1**

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Withdrawn N

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Historical Significant Changes

PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED

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**Project Additional Information**
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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AN INVESTIGATION OF THE ROLE PLAYED BY INTEGRINS AND CELL SURFACE RECEPTORS IN TISSUE REPAIR AND PATHOLOGY.

Purposes of the contained use

The purpose of this programme of work is to examine the role of integrins and cell surface receptors in tissue repair and pathology. The genes for integrins/cell receptors will be cloned and expressed or knocked out in human cell lines and primary cultures of human cells.

Recipient or parental organism

The recipient cells will either be human cell lines of mesenchymal origin (eg epithelial/keratinocyte cell lines) or primary cultures of human mesenchymal cells (eg muscle myoblasts).

The human cell lines that will be used in this programme of work are cells which have been in safe use for over ten years in a number of laboratories. The primary human cells on the other hand represent a greater inherent risk as they may contain adventitious agents.

Host/vector system

The hosts will be either human primary mesenchymal cells or human cell lines with a safe record of use such as H357. A number of viral vector systems will be used which lack the structural genes for particle formation and replication. In particular PRcCMV, PBaPuro, pLXIN and pLXS will be used in conjunction with the packaging cell lines AM12, E86 and PT67.

Origin & function
The genetic material will be obtained from human cells and will code for integrins/cell surface receptors. These genes will be inserted into host cells which lack the proteins they encode so as to determine the role played by these molecules in cell-cell and cell-extracellular matrix interaction.

**Evaluation of foreseeable effects**

Parental and recipient cells: The cell lines to be used in this programme of work have been in safe use for over ten years and are unlikely to cause harm to humans or the environment. The human primary cell could contain adventitious agents which may cause harm to humans.

Viral vector systems: The viral vector systems to be used are disabled and as such to not represent a risk to humans or the environment.

GMMs: The GMMs constructed in this programme of work will express integrin/cell surface receptors that may alter their tissue tropism. Thus while these GMMs represent no risk to the environment it is possible that they may cause harm to humans.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Conditioned media from GMMs and contaminated cell culture plasticware will be treated with a 1% solution of Virkon (final concentration) prior to disposal as clinical waste for incineration. Virkon treatment has been shown to rapidly produce 99.9999% virucidal, bactericidal and fungicidal activity. Contaminated serological pipettes and pipette tips will be autoclaved at 121 degrees C for 20 minutes prior to disposal as clinical waste for incineration. The autoclave to be used is regularly validated and services.

**Is an emergency plan required according to regulation 20?**

<table>
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If yes, tick to confirm that it is attached to this form

| Y |

Tick to confirm that you have attached a risk assessment to this form

| Y |

Tick if you are claiming exemption from disclosure for section of the risk assessment

| N |

**Project Containment**

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**Project Ref 596/06.1**

**Date Ackn'd**: 06/02/2006  
**Date Project Ceased**: 24/04/2012

**CU2 Project Title**: The molecular basis of resuscitation of Campylobacter jejuni from a viable but not-culturable (VBNC) state.

**Class Culture Vol Class**:  
**Class 2**:  
**< 1 Litre**:  

**Non-GMM**: Not Applicable  
**Consent Granted**: Not Applicable

**Project notified under transitional arrangements**: N

**Historical Significant Changes**: PROJECT TRANSFERRED TO GM 14 AND GM CENTRE CLOSED

**Recipient or parental organism**
- Campylobacter jejuni NCTC 11168
- Campylobacter jejuni 81-176

**Host/vector system**
- Genes encoding putative resuscitation factors will be cloned in E. coli using the plasmid pGEM-T-Easy (Promega UK Ltd., classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM). These genes will be inactivated by insertion of a gene encoding kanamycin resistance (aphA3) and re-introduced into C. jejuni NCTC 11168 and 81-176. As pGEM-T-Easy is not able to replicate in C. jejuni, double cross-over homologous recombination events will direct allelic replacement of the chromosomal wild-type copy of the gene with the mutated allele present in the vector. pGEM-T-Easy carries an ampicillin resistance gene.

- Genetic complementation will be done using an E. coli vector (pRRC) that delivers, by homologous recombination, cloned genes (under the control of a Campylobacter-derived promoter) into the 16S-28S rRNA spacer regions on the C. jejuni chromosome (Karlyshev and Wren 2005. Appl. Environ. Microbiol. 71:4004).

**Project Additional Information**

**Purposes of the contained use**
- Construction and complementation of defined Campylobacter jejuni mutants to investigate the function of genes potentially involved in resuscitation from the viable but non-culturable state.
This vector is not able to replicate in C. jejuni, is classified as non-mobiliseable (Bom-, Mob- and Tra-minus) by the ACGM and carries a chloramphenicol resistance gene.

Origin & function

C. jejuni genes encoding potential resuscitation factors (Genome sequence Genbank NC_002163; gene ID 904972, possible secreted transglycosylase; gene ID 904469, unknown function; gene ID 905140, possible secreted transglycosylase) will be amplified by PCR from genomic DNA of strain 11168.

PGEM-T-Easy is supplied by Promega UK Ltd.; pRRC will be obtained from London School of Hygiene and Tropical Medicine.

The gene encoding kanamycin resistance, aphA3, will be obtained from plasmid pLP1433 of Campylobacter Coli (Genbank M26832)

Evaluation of foreseeable effects

Trains will be disposed of by autoclaving on a kill cycle.

Although the genetically modified organisms produced in this project will be resistant to kanamycin or to both kanamycin and chloramphenicol, neither are antibiotics of choice for treatment of Campylobacter infections. Therefore the potential risks associated with the genetically modified organisms are the same as those of the parental strains and the likelihood of these risks being realised is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The project will generate the following waste: broth- (of 100 ml maximum volume) and agar plate cultures of the GMOs, contaminated pipettes, pipette tips and glassware. All contaminated waste, will be autoclaved on a "kill cycle" ensuring that the waste is held at a holding temperature of 121 degrees C for at least 15 minutes. This will kill all GMOs. Autoclaves contain temperature probes to check the heat penetration and sterilisation indicator strips are also included in each run. Autoclaved liquid waste will be disposed down sinks. Autoclaved solid waste will be sent for incineration.

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

Part 1

The committee noted that this project was similar in nature to a programme of work previously approved by the HSE (GM596/99.1) but had the safety advantage over that programme of work in that the vector systems to be used could not replicate in the host organisms under any conditions. The committee also recommended that the inexperienced research on the grant be sent on a training course in good microbiological practice which has now been done.
### Project Containment

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### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 596/99.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
**Please enter comments on the GM safety committee on the risk assessment**

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**Name**

UNIVERSITY OF EXETER

**Name 2**

**Department**

PATHOLOGY

**Campus Estate or Research Centre**

ST. LUKE'S CAMPUS

**Road Name**

MAGDALEN ROAD

**Town**

EXETER

**County**

DEVON

**Postcode**

EX1 2LU

**Country**

ENGLAND

**Tel Number**

01392 403043

**Fax Number**

01392 403051

**E-mail**

**HSE Division**

WALES AND SOUTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Laboratory</th>
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Tick if confidential

02/03/2022
### Bacteriology
- E.Coli

### Parasitology
- Transgenic Birds

### Transgenic
- Birds
- Fish
- Plants

### Microbiology
- Research

### Virology
- Transgenic Animals

### Transgenic
- Animals
- Invertebrates

### Mycology
- Transgenic
- Invertebrates

### Transgenic
- Invertebrates
- Plants

### Other (please specify below)

### Transgenic
- Birds
- Fish
- Plants

### Microbiology
- Research

### Gene Therapy

---

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

- Solid, i.e. plates with E.Coli - Autoclave 126C 10 minutes.
- Liquid - Broths etc - Sodium hypochlorite added to 2% available Chlorine final concentration.

---

**Please enter comments of the GM safety committee on the risk assessment**
**GM Centre Number: 599**

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**Name**

RAFT INSTITUTE FOR PLASTIC SURGERY

**Name 2**

MOUNT VERNON HOSPITAL

**Department**

MOLECULAR AND CELL BIOLOGY

**Campus Estate or Research Centre**

LEOPOD MULLER BUILDING

**Town**

NORTHWOOD

**District**

MIDDLESEX

**Road Name**

LEOPOD MULLER BUILDING

**Postcode**

HA6 2RN

**Country**

ENGLAND

**Tel Number**

01923 844 212

**Fax Number**

01923 844 031

**E-mail**

**HSE Division**

LONDON

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

1. Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

2. Give brief details of the genetic modification safety committee

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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste Treatment:
Solid - all incinerated.
Liquid - treated overnight with bleach then flushed down sink.
> one can tell if bleach was added from swell, change of colour and froth. Maximum volume is approximately 2 L.
same for bacterial pellets
> one can see that they are lysed.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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| Comments                          | CLOSED 27/06/2011 PROJECT TRANSFERRED TO GM554 |

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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Other (please specify) Tick if confidential

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<td>Transgenic Fish</td>
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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 601/06.1

<table>
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Withdrawn N

Historical Significant Changes

TRANSFERRED TO GM554 ON CLOSURE OF GM601

Project Additional Information

02/03/2022
Purposes of the contained use

This research is to be notified as a connected programme of work utilising transfection of cDNAs into human cell-lines in order to induce expression of said gene products under the control of known promoters provided by plasmid vectors. It contributes to the research goal of understanding the contribution of genes to hormone signalling pathways, especially in relation to breast cancer.

Recipient or parental organism

Recipients are established human cell lines (eg breast cancer cell lines) and an immortalised normal breast epithelial cell line hTERT-HME1. Epithelial tumour cells can cause tumours when injected subcutaneously in immuno-compromised mice. However, the potential for harm is much less in immuno-competent humans, where the primary potential for harm comes from possible adventitious pathogens (viruses). Cell lines are expected to have limited survivability in the environment and modifications are not expected to increase their pathogenicity.

Host/vector system

The plasmid vectors are mobilisation deficient, devoid of significant known toxic or pathogenic potential and would be classified as class 1 were it not for their use in mammalian cell-lines. The design of the RheoSwitch Mammalian Inducible Expression System is such that the insert DNA is only expressed in the presence of RSL1 ligand, which is a synthetic non-steroidal compound and therefore extremely unlikely to be found in the environment. Any other vectors will be limited to similarly well characterised plasmids.

Origin & function

The primary inserts will be full length cDNAs encoding estrogen receptors Eralpha and Erbeta, derived from human normal or tumour tissues or cell line material. In addition cDNAs resulting from alternative splicing of the ER mRNA (for both Eralpha and Erbeta) will also be used. The cDNAs resulting from alternative splicing of the ER mRNA (for both Eralpha and Erbeta) will also be used. The cDNAs to be used are unlikely to include a toxic or pathogenic determinant although due to the unknown nature of the estrogen receptor variants, the deletion variants may have a deleterious effects on the recipient cell line.

Alternative gene products (eg X-box binding protein 1) will be limited to those unlikely to increase the toxic or pathogenic potential for harm, even when constitutively expressed or modified by addition of reporter proteins. Most inserts will be transcription factors, or genes known to modulate transcription (eg co-activators of co-repressors).

Some inserts may have been previously mutated by site-directed mutagenesis to pinpoint specific residues/amino acids involved in protein function. It is envisaged that insert modification (eg fluorescent labelling or tagging) is more likely be detrimental to gene function than enhance any latent pathogenicity or toxicity. Initial expression of the insert DNA will be induced only by addition of RSL1 synthetic ligand (requiring additional transfection with plasmid pNEBR-R1), subsequently a strong constitutive promotor may be used (eg CMV). Plasmid vectors additionally contain selective markers (eg G418 resistance).

Evaluation of foreseeable effects

Recipients are established human cell lines eg breast cancer cell lines: KPL-1, MCF7, ZR75, MDA-MB-231 and an immortalised normal breast epithelial cell line hTERT-HME1. These cell lines are biological agents for which containment level 2 is appropriate, owing largely to the fact that they are long-term cultures of human origin and are not fully characterised. The primary potential for harm comes from possible adventitious pathogens (viruses). Cell lines are expected to have limited survivability in the environment.

The primary inserts will be full length cDNAs encoding estrogen receptors (ER), cDNAs resulting from alternative splicing of the ER mRNA or alternative gene products, largely constituting related transcription factors. The cDNAs to be used are unlikely to include a toxic or pathogenic determinant although due to the unknown nature of the estrogen receptor variants, the deletion variants may have a deleterious effects on the growth properties of the recipient cell lines. Most insert DNA is naturally occurring in these cell lines, the experiments will merely control the level of expression of individual variants.

The plasmid vectors when used are mobilisation deficient, devoid of significant known toxic or pathogenic potential and would be classified as class 1 were it not for their
use in mammalian cell-lines. All sequences cloned will be of mammalian origin and unlikely to contain promoters active in non-mammalian organisms. Expression in mammalian cells is controlled by previously validated promoter elements (very strictly inducible in the case of the Rheoswitch system) and is unlikely to be observed outside of a controlled laboratory environment. Potential for transfer to and gene expression in other organisms is negligible and therefore negligible hazard to human health. Vectors are unlikely to alter pathogenic traits of the host.

There is little potential for alteration of pathogenic traits and no obvious significant increase on the hazards of the non-transfected cell lines.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This work involves standard laboratory protocols, and there are no unusual procedures that require additional containment measures. Standards of work and containment are specified in the CCRT Code of Practice, with reference to the governing regulations and guidelines. All personnel should be familiar with the relevant safety documentation and have received training specifically for the use of GMM. Both unmodified and GM modified cells will be grown in small scale closed containers in a category 2 containment area. A class II safety cabinet will be used for all manipulations and both sharps glass containers will be avoided. As specified in the governing code of practice for biological agents.

Solid waste will be bagged, sterilised by autoclaving and later incinerated as clinical waste. Liquid waste in disposable culture flasks will be sterilised by autoclaving and incinerated as clinical waste. Other liquid waste will be sterilised by autoclaving and disposed of via the drains. The autoclave is situated within the laboratory suite and is managed by Wirral NHS Trust engineers. A programme of testing and quality assurance is carried out on a quarterly and annual basis and records kept.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be bagged, sterilised by autoclaving and later incinerated as clinical waste. Liquid waste in disposable culture flasks will be sterilised by autoclaving and incinerated as clinical waste. Other liquid waste will be sterilised by autoclaving and disposed of via the drains. The autoclave is situated within the laboratory suite and is managed by Wirral NHS Trust engineers. A programme of testing and quality assurance is carried out on a quarterly and annual basis and records kept.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The CCRT GM safety committee is satisfied that the risk assessment is suitable for the purpose stated, providing suitable records are kept and any significant variation notified. The assessment has been reviewed by the University Biological Safety Adviser who made a number of suggestions regarding the scope of the work, in particular that the work should be notified as connected programme, given the number of different phases that are proposed. These suggestions were incorporated in the final assessment.

Project Containment
<table>
<thead>
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<th>Glass Houses</th>
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**Project Ref 601/96.1**

**CU2 Project Title**
CONSTRUCTION OF CDNA LIBRARIES CORRESPONDING TO DIFFERENTIALLY EXPRESSED GENES IN HUMAN TUMOURS AND CELL LINES

**Class**
Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM**
not applicable

**Consent Granted**

**Project notified under transitional arrangements**
Y

**Withdrawn**
N

**Tick if notifying a connected programme of work**
N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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Name

ACAMBIS RESEARCH LIMITED

Name 2

Department

Campus Estate or Research Centre

PETERHOUSE TECHNOLOGY PARK

Road Name

100 FULBOURN ROAD

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB1 9PT

Country

ENGLAND

Tel Number

01223 275 300

Fax Number

01223 416 300

E-mail

www.peptide.co.uk

HSE Division

EAST AND SOUTH EAST

Comments

FORMERLY KNOWN AS PEPTIDE THERAPEUTICS BEFORE DECEMBER 2000  Closed down 19/01/2005

Date at Which Additional Info Submitted

19/01/2005
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 603/01.1**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<th>Project notified under transitional arrangements</th>
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Withdrawn N  

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
**Project Additional Information**

**Purposes of the contained use**
- To generate sufficient hepatitis B core antigen constructs with the potential to block the allergic response.

**Recipient or parental organism**
- The E.coli bacterial hosts are engineered to express recombinant hepatitis B core antigens (HBcAgs) that display short peptide sequences derived from human IgE or peptide mimics of these sequences. Recombinant HBcAg is a non-infectious capsid particle - (it forms the inner capsid in wild type virus) - composed of a single protein species that does not contain any hepatitis B virus genomic material.

**Host/vector system**
- **Hosts:** Escherichia coli K12 strains.
- **Vector to be used:** pPN1 (Brown, A. L. et al, Vaccine, 1991, 9, 595-601) - pAT153 based (Bom-/Nic-, Mob-, Tra-) that encodes the HBcAg.

**Origin & function**
- The HBcAg itself is expressed from a cloned copy of the HBcAg gene that has been engineered to allow the insertion of the peptide encoding sequence of choice (Brown et al, 1991, as above). Only low levels of expression are expected prior to the appropriate conditions such as the addition of an inducing agent to stimulate expression. The intended use of these constructs is to evaluate their potential as vaccine candidates against IgE mediated allergic disease. However, the effect of such a vaccine in vivo is not known.

**Evaluation of foreseeable effects**
- **Likelihood of hazard:** All procedures will be carried out according to good microbiological practice in a separate, controlled access laboratory. All residues and waste will be sterilised before leaving the laboratory. The chance of release to the environment is negligible.
- **Consequence of hazard:** Plasmid DNA is non-mobilisable and E.coli K12 hosts are used. K12 host cells are crippled for survival outside of a laboratory environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
  - N/A.
  - **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
  - All waste plasticware autoclaved at 130°C for 40 minutes and liquid culture and glassware treated with 30g/l Virkon for at least 30 minutes. Spillage of E.coli cells treated with Virkon powder to soak up liquid and powder removed with tissue wipes, 30 g/l Virkon solution used to clean area than 70% ethanol wipes. Tissue wipes autoclaved.
The GM Safety Committee had requested that an earlier draft risk assessment be split up. From the meeting minutes:

'Section A of the assessment covered expression of proteins resulting in both Cat 1 and 2 GMOs, whereas the Project Description stated that:

‘In circumstances where such a project will involve expression of a heterologous antigen which cannot be considered "highly unlikely to cause any harmful biological effect because the function of the protein is well understood or because it is encountered in high concentrations in nature" a separate and specific risk assessment will be carried out.

It was felt that these should be separated out so that there was a clear delineation between those projects which, as Cat 2, should be carried out in the side labs’.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Conf</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref 603/01.2**

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<tbody>
<tr>
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<td></td>
<td>N</td>
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</table>
**Project Additional Information**

**Purposes of the contained use**

To generate small quantities of N. meningitidis proteins, to develop an assay system for the proteins raised in more useful carriers.

**Recipient or parental organism**

The E. coli bacterial hosts are engineered to express recombinant N. meningitidis proteins. The BL21 (DE3) hosts have not been crippled to prevent survival in the harsh conditions of the external environment (unlike K12 host cells). However, the antigens derived from N. meningitidis have no known toxicity. GNA1220 shows some homology to the human SLP2 integral membrane protein but a cross-reactive response in humans to SLP2 is unlikely since it is localised on the cytoplasmic surface and membrane. The BL21 (DE3), BLR (DE3) and HMS174 (DE3) strains and derivatives thereof, are F and so are incapable of conjugation.

**Host/vector system**

Hosts: E. coli K12 or BL21 (DE3) and derivative strains. BL21 (DE3) derivative strains express proteins to repress basal levels of expression from the T7 promoter via degradation of T7 polymerase.

Vector to be used: pET17b or similar. Expression is driven by the T7 promoter.

**Origin & function**

The DNA sequences encoding the N. meningitidis antigens are derived by amplification reactions using genomic DNA as template. Genomic DNA purified from heat killed bacteria supplied by Dr Ray Borrows at the PHLS, Manchester.

**Evaluation of foreseeable effects**

i. Likelihood of hazard: All procedures will be carried out according to good microbiological practice in a separate, controlled access laboratory. All residues and waste will be sterilised before leaving the laboratory. The chance of release to the environment is negligible.

ii. Consequence of hazard: Plasmid DNA is mobilisation defective. The K12 host cells are crippled for survival outside of a laboratory environment. The BL21 (DE3) and K12 hosts to be used are both F and so are non-conjugative, further reducing the risk of transfer of genetic material. Antigens to be expressed are not known to be toxic.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
All waste plasticware autoclaved at 130°C for 40 minutes and liquid culture and glassware treated with 30g/l Virkon for at least 30 minutes. Spillage of E. coli cells treated with Virkon powder to soak up liquid and powder removed with tissue wipes, 30 g/l Virkon solution used to clean area then 70% ethanol wipes. Tissue wipes autoclaved.

The GM Safety Committee had requested that an earlier draft risk assessment be split up as it consisted of two different cell lines (HMS 174 and BL21) which resulted in different Containment Levels. We felt it was important to make it clear which was the Containment Level 2.

From the meeting minutes:

"It was decided to split ACGM076 into two separate risk assessments as BL21's result in a GMO Cat 2 and HMS174, a GMO Cat 1."

Project Containment

<table>
<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 603/01.3

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Non-GMM

Consent Granted: not applicable

Project notified under transitional arrangements: N
### Project Additional Information

#### Purposes of the contained use

To safely generate small amounts of haemoglobinases for our drug discovery programme.

#### Recipient or parental organism

Haemoglobinases are biologically inactive in E. coli due to expression as insoluble protein within inclusion bodies.

#### Host/vector system

- **Hosts:** Escherichia coli BL21.
- **Vectors to be used:** pUC-based.

#### Origin & function

Haemoglobinases are present in the digestive vacuole of the human malarial parasite Plasmodium falciparum and involved in the degradation of erythrocyte’s haemoglobin.

#### Evaluation of foreseeable effects

- **Likelihood of hazard:** All glass cultureware soaked in Virkon (30 g/l) then autoclaved, all waste and plastic will be autoclaved at 126°C for 15 minutes. All E. coli will be performed in negative pressure genetics rooms and large cultures placed in Class II hood when opened.
- **Consequence of hazard:** Plasmid DNA is non-mobilisable and transformed E. coli are a B strain.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A.

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A.

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid clinical waste must be autoclaved at 130°C for 40 minutes prior to being placed in the large yellow clinical waste safe in the external compound. The Clinical waste safe is collected regularly and the waste disposed of by incineration.

Liquid clinical waste and glassware must be treated with 30 g/l Virkon for at least 30 minutes. Gloves must always be worn when dealing with spills and breakages.
Minor spills:
Either cover the spill with Virkon disinfectant granules, leave for not less than 10 minutes and scoop up into a dustpan,
or, using double gloves, mop up the spill with a pad soaked in a 30 g/l Virkon solution.

Larger spills:
Either cover the spill with Virkon disinfectant granules as above,
or cover the spill with paper towels, moisten with 30 g/l Virkon solution and leave for at least 10 minutes before scooping up into a dustpan. Do NOT use a brush.

The scientists are referred to has017, the Code of Practice for Biological Hazards for more details.

The original risk assessment had given an Access figure (under the Brenner Scheme) of 10-3/1. On further investigation, we found that the ACGM guidance notes (Part 2A, Annex II, Part iii) say that BL21 cells may be given a value of 10-3. The risk assessment was altered and a Containment Level of 2 was given to the project. It was agreed that all work would be carried out in the Genetics Lab and the project was signed off.

Project Containment

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Animal Units

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Project Ref 603/02.1

Date Ackn'd CU2 Project Title
Project Additional Information

Purposes of the contained use

Libraries of Chlamydia trachomatis DNA will be created by cloning fragments of genomic DNA into expression vectors. The libraries will be used to transform attenuated strains of Salmonella typhi and the expressed peptides will be screened in a variety of assays.

Recipient or parental organism

Salmonella typhi CVD908 (aroC, aroD) and ACAM948 (aroC, aroD, htrA).

In a letter dated 25 November 1993 from Dr Stuart Smith, then ACGM secretary at the HSE, to Dr Stephen Chatfield, the developer of CVD908 and CVD908HtrA (same genotype as ACAM948) at Medeva, both strains fulfil the criteria of schedule 2 and qualify as Group 1 organisms.

Host/vector system

The vectors used will be non-mobilisable (eg pTrcHis, Invitrogen) which, when combined with the S. typhi strains described above, have been assigned an 'Access factor' (Brenner Scheme) of 10 (-6) (See Dr Smith's letter, above). The promoters used will be trc (high expression in the presence of IPTG) or htrA or nirB (induced in vivo by low oxygen tension or entry into macrophages).

Origin & function

The genetic material will be derived from Chlamydia trachomatis genomic DNA that is partially digested with restriction endonucleases. Fragments of approximately 1 kb will be cloned into the vectors. The vectors will provide an ATG start codon adjacent to the promoter and 5' of the cloning site, and stop codons downstream of the cloning site, such that in frame fusions can be created. In this system some inserts will be out of frame and no protein will be expressed whereas in other constructs fragments of C. trachomatis proteins will be produced and occasionally full-length proteins may be expressed.

The immunological characteristics of these expressed proteins and the resultant recombinant Salmonella strains will be assessed using a range of assays.

Evaluation of foreseeable effects

The majority of the polypeptides expressed will be fragments of C. trachomatis proteins and are unlikely to be biologically active since the library is designed for cloning of...
partial gene sequences. However, it is possible that a few of the expressed polypeptides may be biologically active or full-length proteins. If this were the case, only strains harboring plasmids where the promoter is htrA or nirB would be expected to synthesize the protein under natural conditions. This promoter has only limited activity outside animal tissue. The host range of S. typhi strains is limited to human and certain primates. In the event of colonization (which, given the phenotype of the strains, would be self limiting), the levels of protein expression will be highly regulated by the host strain and confined to certain tissues (e.g. the gut and reticuloendothelial system), therefore the likelihood of harmful effects is extremely low.

These strains cannot survive in the environment due to the presence of aro mutations. The possibility of complementation by the C. trachomatis aro or htrA genes exists. However this is highly unlikely given that full-length gene sequences of at least two separate genes would need to be expressed in the same bacterial host cell for this to occur. The aroA, aroC and htrA genes are located far apart in the bacterial chromosome.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid clinical waste must be autoclaved at 130 degrees C for 40 minutes prior to being placed in the large yellow clinical waste safe in the external compound. The Clinical waste safe is collected regularly and the waste disposed of by incineration. Liquid clinical waste and glassware must be treated with 30 g/l Virkon for at least 30 minutes. Gloves must always be worn when dealing with spills and breakages.

Minor spills:
* Either cover the spill with Virkon disinfectant granules, leave for not less than 10 minutes and scoop up into a dustpan.
* or, using double gloves, mop up the spill with a pad soaked in a 30 g/l Virkon solution.

Larger spills:
* Either cover the spill with Virkon disinfectant granules as above
* or cover the spill with paper towels, moisten with a 30 g/l Virkon solution and leave for at least 10 minutes before scooping up into a dustpan. Do NOT use a brush.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Excerpt from meeting minutes:

"This project is similar to the previous except the work is carried out in S. typhi rather than xxx. As a consequence, the Access figure is higher at 10 (-3), which takes the work into a Category 2. Work cannot start until the ACGM has reviewed the project".
Project Additional Information

**Purposes of the contained use**

Libraries of Chlamydia trachomatis DNA will be created by cloning fragments of genomic DNA into retroviral vectors. Replication-incompetent virus will then be generated using packaging cell lines, and used to infect antigen-presenting cells with subsequent expression of proteins. These APCs will be screened in a variety of immunological assays. Known epitopes will be expressed in order to validate the system.

**Recipient or parental organism**

Containment categorisation for production of the Chlamydia trachomatis expression library using retroviral vectors was assigned using the HSC's ACGM Compendium of Guidance (Part 2B Annex III) "Guidance for commonly used viral vectors - March 2000".
Disabled retrovirus vectors
Section 18, 19, 20, 23, 28 and 30.

The retroviruses generated cannot replicate in the environment because they lack functional gag, pol and env genes. They could, theoretically, infect a single cell but could not then produce more virus. The viruses themselves are not robust in the environment. Protein products are very unlikely to be produced at levels that would have a deleterious effect on other organisms.

The work was considered to be Cat. 2.

Host/vector system

The retroviral vectors initially used to generate the Chlamydia trachomatis expression library are mobilisation defective in lab strains of disabled E. coli (pFB, pCFB series from Stratagene, pL, pQ vector series from BD Biosciences). The packaging cell line (Amphopack 293 - BD Biosciences) has been tested to ensure that replication-competent viruses are not present in the culture medium. The indicator cells (HT-1080, ATCC CCL-121), used to demonstrate that the packaged library does not contain replication competent virus, are Biosafety level 1. When the library is transformed into the packaging cell line, replication-incompetent infectious virus (RIV) is produced and the categorisation changes from cat 1 to cat 2.

Origin & function

The genetic material will be derived from Chlamydia trachomatis genomic DNA that is partially digested with restriction endonucleases. Fragments of approximately 2kb will be cloned into retroviral vectors. The vectors provide a packaging signal for virus assembly, long terminal repeats (LTRs) to allow for integration into the target cell DNA, a promoter sequence, an ATG start codon, a multiple cloning site 3' of the ATG and stop codons downstream of the cloning site. The vector is provided in three frames such that in-frame fusions can be created. The vector does not contain functional gag, pol or env genes that are essential for production of replication competent virus. In this system some inserts will be out of frame in the vector and no protein will be expressed when the virus infects a target cell, whereas in other constructs fragments of C. trachomatis proteins will be produced and occasionally full-length proteins may be expressed.

Production of replication-incompetent virus (RIV) will occur only when vectors are transfected into a packaging cell line, which stably expresses gag, pol and env genes from different loci on the chromosome. Packaged RIV will be used to infect target cells, the immunological characteristics of these expressed proteins will be assessed using a range of assays.

Evaluation of foreseeable effects

The retroviruses generated cannot replicate in the environment due to lack of functional gag, pol and env genes. They could theoretically infect a single cell but could not then produce more virus. The viruses themselves are not robust in the environment. Protein products are very unlikely to be produced at levels that would have a deleterious effect on other organisms.

To demonstrate the absence of replication-competent viruses in vector stocks, direct plating of vector supernatant onto mitotically active indicator cells will be carried out (HT 1080 cells).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

N/A
All solid clinical waste must be autoclaved at 130 degrees C for 40 minutes prior to being placed in the large yellow clinical waste safe in the external compound. The clinical waste safe is collected regularly and the waste disposed of by incineration.

Liquid clinical waste and glassware must be treated with freshly made Precept solution (10g/500ml) and left for at least 20 minutes. Gloves must always be worn when dealing with spills and breakages.

Spills:
* Cover the spill with paper towels, moisten the Precept solution, leave for not less than 20 minutes and then remove to double autoclave bags and autoclave.
* Do NOT use a brush, and avoid creating aerosols.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

AM felt that he was erring on the side of caution by categorising this as 2, but supported this decision by stating this was standard throughout the industry as there is the potential for localised infection of a worker’s cells, albeit a very low risk.

**Project Containment**

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02/03/2022
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**Name**

| J E & V M DALTON LTD |

**Campus Estate or Research Centre**  

**Road Name**

**Town**

| PETERBOROUGH |

**District**

| EYE |

**County**

| NORTHUMBERLAND |

**Postcode**

| PE6 7UD |

**Country**

| ENGLAND |

**Tel Number**

| 01733 222391 |

**Fax Number**

| 01733 223246 |

**HSE Division**

| EAST AND SOUTH EAST |

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
<table>
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**Premises Addresses**

**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 605

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Name

UNIVERSITY OF NOTTINGHAM

Name 2

CITY HOSPITAL

Department

CRC DEPARTMENT OF CLINICAL ONCOLOGY

Campus Estate or Research Centre

Building

Road Name

HUCKNALL ROAD

Town

NOTTINGHAM

County

NOTTINGHAMSHIRE

Postcode

NG5 1PB

Country

ENGLAND

Tel Number

0115 962 8035

Fax Number

0115 962 7923

E-mail

HSE Division

MIDLANDS

Comments

GM605 was merged with GM470 and closed down on 18/02/2005

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee

### Laboratory

- Animal Unit

### Growth Room

- Glass House

### Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

### Other (please specify)

- Tick if confidential

### Microbiology

- Bacteriology
- Parasitology
- Transgenic Birds
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

- Virology
- Transgenic Birds
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

**Give brief details of the genetic modification safety committee**

- **HMR GMSC**
  - Core members (all studies)
  - BSO (chair)
  - Managing Director
  - Medical Director
  - Chair of the HMR Health and Safety committee
  - Ward Manager

- Core members (staff allocated to the specific trial being considered by the GMSC)
  - Principal Investigator
  - Research Physician
  - Clinical Project Manager
  - Representatives of relevant departments (such as ward, pharmacy or laboratory).

- Optional members (if required)
  - Chief Medical Adviser
  - Director of Scientific Services
  - Expert advisor from outside of HMR

---

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale
Disinfect all waste that has come into contact with GMM by soaking in freshly prepared Virkon solution (1%) for 10 mins.

Soak all waste material in a solution of freshly prepared Virkon solution (10%), for 10 mins and dispose of down a designated GMM sink.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Disinfect all waste that has come into contact with GMM by soaking in freshly prepared Virkon solution (1%) for 10 mins.

Soak all waste material in a solution of freshly prepared Virkon solution (10%), for 10 mins and dispose of down a designated GMM sink.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 608**

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**Department**

**Campus Estate or Research Centre**

**Road Name**

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**Date at Which Additional Info Submitted**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  

Tick if confidential

Bacteriology  
Parasitology  
Transgenic Birds  
Microbiology Research

Virology  
Transgenic Animals  
Transgenic Fish  
Gene Therapy
The Gm work produces a maximum of 10 litres of liquid waste in a day, but the average is usually much less. All liquid waste is autoclaved as it is produced and is not stored. Solid waste is autoclaved. Each load is checked with indicators included in the material being autoclaved. The autoclave is on an annual maintenance contract with an external company and their work is checked form our insurance company.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Please enter comments of the GM safety committee on the risk assessment
Data Premises Notified (Originally) 05/06/1996

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

VANTIA LIMITED

Name 2

Department

BIOLOGY

Building

CHILWORTH RESEARCH CENTRE

Campus Estate or Research Centre

SOUTHAMPTON SCIENCE PARK

Road Name

1 VENTURE ROAD

District

CHILWORTH

Town

SOUTHAMPTON

County

HAMPShIRE

Postcode

SO16 7NP

Country

ENGLAND

Tel Number 02380 763400

Fax Number 02380 766755

E-mail None

HSE Division EAST AND SOUTH EAST

Comments

Changed from Ferring Research Institute to Vantia Limited as of 06/03/2008.

Date at Which Additional Info Submitted

06/03/2008
### Premises Addresses

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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: [ ]

- **Give brief details of the genetic modification safety committee**

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
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- **Tick if confidential**: [ ]

- **Bacteriology**
  - Parasitology
  - Transgenic Birds
- **Microbiology Research**
For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Virology</th>
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<td>Mycology</td>
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Other(s)

The maximum volume of waste that could be released at any one time is 3 litres.
* All waste is treated by sterilisation in an autoclave prior to disposal via the sewer or as normal solid waste.
* The autoclave is regularly serviced and validated and used according to the manufacturer's instructions.
* The waste is monitored regularly - growth on agar during a 24 hour incubation period.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 610

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- **Date at Which Additional Info Submitted:** 31/01/2003

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**02/03/2022**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

Virology Transgenic Animals Transgenic Fish Gene Therapy

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste Management

The maximum culture volume that could be released at any one time is 10 litres. All solid (e.g., bacterial cell pellets) and liquid (e.g., cell-conditioned culture medium) waste is treated with Virkon before disposal. When used according to the manufacturer's instructions, Virkon achieves 100% disinfection. Equipment (e.g., shaker flasks) used for culturing GMOs is disinfected with Virkon and then sterilised by autoclaving. Disposable cell cultureware is autoclaved and then incinerated.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 611**

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**Name**

CENTRE FOR ECOLOGY AND HYDROLOGY - MERLEWOOD

**Name 2**

MERLEWOOD RESEARCH STATION

**Department**

SOIL ECOLOGY

**Campus Estate or Research Centre**

MERLEWOOD RESEARCH STATION

**Building**

MERLEWOOD RESEARCH STATION

**Road Name**

WINDERMERE ROAD

**District**

**Town**

GRANGE OVER SANDS

**County**

CUMBRIA

**Postcode**

LA11 6JU

**Country**

ENGLAND

**Tel Number**

015395 32264

**Fax Number**

015395 34705

**E-mail**

**HSE Division**

NORTH WEST

**Comments**

CLOSED ON 21/5/2003. WORK TRANSFERRED TO GM169 ON 21/5/2003

**Date at Which Additional Info Submitted**

19/11/2002
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
Cultures of bacterial clones will be inactivated by autoclaving. Maximum volume of cultures will be 100 ml. The autoclaving process is monitored by temperature and pressure gauges and indicator tape is used to verify successful autoclaving (121 degrees C for 15-30 mins as appropriate).

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

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Name

ENVIGO RMS (UK) LTD

Name 2

Department

Campus Estate or Research Centre

SHAWS FARM

Road Name

STATION RD

District

BLACKTHORN

Town

BICESTER

County

OXFORDSHIRE

Postcode

OX6 OTP

Country

ENGLAND

Tel Number

01869 243241

Fax Number

01869 246759

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Name change from Harlan UK Ltd notified 24/01/2017

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

| Date Premises Closed | Name                          | Department | Name 2 | Campus Estate or Research Centre | Building     | Road Name     | District     | Town      | County   | Post-code | Country    | Withdrawn |
|----------------------|-------------------------------|------------|--------|---------------------------------|--------------|---------------|--------------|-----------|----------|-----------|------------|------------|-----------|
| ENVIGO RMS (UK) LTD  |                               |            |        |                                 | SHAWS FARM   | STATION ROAD  | BLACKTHORN  | BICESTER | OXON     | OX6 0TP   | ENGLAND    | N         |

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic**
- **Birds**
- **Microbiology**
- **Research**
- **Virology**
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- **Transgenic**
- **Fish**
- **Gene Therapy**

02/03/2022
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

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**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022 |
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Tick if confidential

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Virology  Transgenic Animals  Transgenic Fish  Gene Therapy
Mycology  Transgenic Invertebrates  Transgenic Plants  Other (please specify below)
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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<table>
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**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**
**Project Ref** 613/97.1

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

| L2 | L3 | L4 | L2 |

Large Scale Activities

| L2 | L3 | L4 | L2 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 613/97.2

Date Ackn’d 26/03/1997

CU2 Project Title MOLECULAR CHARACTERISATION OF THE MECHANISMS OF ENTEROVIRAL

Class 2

CultureVolClass2

CultureVolumeClass3-4
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick to confirm that it is attached to this form

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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**Name**

UNIVERSITY OF SUNDERLAND

**Name 2**

SCHOOL OF SCIENCES

**Campus Estate or Research Centre**

FLEMING BUILDING

**Road Name**

WHARNCLIFFE STREET

**Town**

SUNDERLAND

**County**

TYNE AND WEAR

**Postcode**

SR2 3SD

**Country**

ENGLAND

**Tel Number**

0191 515 2504

**Fax Number**

0191 515 2502

**E-mail**

**HSE Division**

YORKSHIRE AND NORTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
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- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Treatment with Presept Hypochlorite solution followed by autoclaving. Incineration of solid waste.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 614/02.1

**CU2 Project Title**
IDENTIFICATION OF POTENTIAL ANTIMICROBIAL DRUG TARGETS THROUGH INVESTIGATION OF THE MOLECULAR BASIS OF STREPTOCOCCAL VIRULENCE

**Date Ackn'd**
23/09/2002

**Date Project Ceased**
10/09/2020

**Consent Granted**
Not applicable

**Non-GMM**

**Class**
Class 2

**CultureVolume Class 2**
< 1 litre

**Project notified under transitional arrangements**
N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

02/03/2022
Project Additional Information

Purposes of the contained use

The aim of this programme of study is to investigate the role of specific genes in the virulence of pathogens of the genus Streptococcus. It is anticipated that the identification of genes involved in streptococcal virulence will reveal proteins that may be suitable as targets for antimicrobial therapies or as vaccine candidates. Genes encoding various streptococcal proteins post-translationally modified with lipids (lipoproteins) and the genes involved in lipoprotein biosynthesis will be permanently inactivated in the host streptococci by means of either insertional inactivation or allelic replacement.

Recipient or parental organism

Various strains of Streptococcus agalactiae (Group B streptococcus), Streptococcus equi (subspecies equi and subspecies zooepidemicus) and Streptococcus suis will be modified by insertional inactivation or allelic replacement to inactivate specific genes. These streptococcal host strains are either human or animal pathogens and are within ACDP hazard group 2. It is anticipated that the inactivation of streptococcal genes encoding putative virulence factors will generate mutant strains that are less virulent than the parent strains from which they are derived. Indeed the demonstration of a role in virulence for any individual protein usually requires experimental evidence of attenuated virulence in mutant strains lacking the protein of interest.

Host/vector system

For insertional inactivation an internal fragment of the gene of interest will be cloned into an appropriate vector to generate a recombinant plasmid. In this work plasmids that cannot replicate in streptococci, such as the plasmid pVA891 (Macrina et al., 1983), will be used as vectors. When the recombinant plasmid is introduced into a suitable streptococcal host strain specific, homologous recombination between the cloned gene fragment and the endogenous gene in the host results in insertion of the entire recombinant plasmid into the gene of interest. Insertion of vector sequences into an open reading frame results in inactivation of the gene. The recombination event that leads to insertional inactivation can result in the expression of truncated forms of the proteins of interest but these truncated forms are likely to be non-functional.

In allelic replacement an in-frame deletion is made in a copy of the gene of interest. The gene harbouring the deletion is introduced into an integration vector to generate a recombinant plasmid. Vectors of the pG+host range (Biswas et al., 1993) will be used in these studies. These vectors have temperature sensitive origins of replication allowing replication at 28 degrees C but not 37 degrees C. Once the recombinant plasmid is introduced into a suitable streptococcal strain at 27 degrees C, increasing the temperature to 37 degrees C results in recombination between the cloned, deletion derivative of the gene and the endogenous gene in the host strain. This results in replacement of the endogenous gene with the deletion derivative. The deletion is targeted to regions of the gene that encode functional regions of the protein, in order to generate a mutant strain that lacks the activity of the protein of interest.


Origin & function

All target genetic material will originate from a parental streptococcal strain (see list of species above).

Evaluation of foreseeable effects

Any protein products arising from these manipulations are likely to be non-functional (and thus less hazardous than the corresponding product of the wild type).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated liquid waste will be disinfected by autoclaving or treatment with Virkon, which have 100% kill rates. The disinfectant activity of Virkon has been validated for the streptococcal species being studied. Likewise all solid waste contaminated with GMMs will be autoclaved prior to final disposal. All autoclaving of GMM waste will be carried out in a validated autoclave. Autoclaved waste will then be disposed of as clinical waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

This application has been approved by the local genetic modification safety committee.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Project Ref 614/15.1

Date Ackn'd 22/07/2015

CU2 Project Title Knockout mutation and plasmid-based complementation of genes encoding AmpC beta-lactamases from clinical isolates of Enterobacteriaceae

Class 2 CultureVolClass2 < 1 Litre

Non-GMM Consent Granted

Date Project Ceased 10/09/2020

Project notified under transitional arrangements N
We will be analysing the parental, knockout and complemented strains using MALDI-TOF mass-spectrometry in order to develop a clinically-useful rapid diagnostic method testing method for AmpC bacteria.

Clinical isolates of Enterobacteriaceae: E.coli or Enterobacter cloacae. These are all designated as ACDP2

Host/vector system

The knockout mutagenesis will be carried out using the lambda Red based GeneDoctoring method (Lee et al. 2009 BMC Microbiol. 9: 252) followed by excision of the kanamycin cassette using the FLP recombinase expressed from pCP20 (Cherepanov & Wackernagel 1995 Gene 158: 9). The cloned beta-lactamase (blaAmpC) gene (with cognate promoter) for complementation of the knockout will be carried on a low-copy-number non-mobilisable plasmid (pACYC184). Plasmid construction will be carried out in standard K-12 lab strains of E.coli i.e. ACDP1 disabled or non-colonising hosts.

Origin & function

The proposed modification does not involve permanent introduction of foreign genetic material except for plasmid vectors. The blaAmpC knockout mutation will remove an antibiotic resistance from the modified strain but is not predicted to affect pathogenicity in any way. The complementation will replace this same antibiotic resistance gene (probably at a somewhat higher copy number). The major concern here would seem to centre on any potential for mobilisation of these antibiotic resistance genes, which are already naturally plasmid-borne in many clinical isolates (Phillipon et al. 2002 Antimicrob. Agents Chemother. 46: 1). We are not using any conjugative vectors and to the best of our knowledge the plasmids involved are all non-mobilisable, so we feel that the risks of significant spread of blaAmpC genes in the community, if any strains escape containment, are low and certainly insignificant compared to the existing natural background of blaAmpC horizontal genetic exchange. The selective markers carried on the plasmid vectors to be used here are either not clinically relevant for treatment of potential UTI or bacteraemias (e.g. those conferring resistance to kanamycin or chloramphenicol) or confer resistance to antibiotics which these AmpC bacteria are already resistant to (ampicillin).
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Standard disposal route for contaminated plastic items (spreaders, loops) or small volumes of liquid is to disinfectant (1% Trigene / Distel) pots; larger volumes of liquid or larger plasticware / tubes are disposed of via autoclaving. Distel / Trigene is known to be effective against most non-sporulating bacteria and our decontamination protocol has been tested in use-dilution tests and shown to be 100% effective against various strains of E. coli, but will be re-validated against cultures of any host bacteria (Enterobacter cloacae) to be used during this project. Autoclaving at 121 °C for 15 minutes is well-established as an effective sterilisation method and can be assumed to result in 100% kill. Our autoclaves are serviced annually and physical parameters for each cycle are logged and checked. |

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Comments on a draft version of the RA were received from three members of the panel. No significant objections were raised, but several suggestions, for example about appropriate PPE, were incorporated in the final version of the RA as attached here. All comments can be supplied on request.

Project Containment

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02/03/2022
# SCOTTISH ASSOCIATION FOR MARINE SCIENCE

## DUNSTAFFNAGE MARINE LABORATORY

**Emergency Plan Required?**

**Non-GMMs**

**Transitional Premises**

**Transferred from 1992 Regs?**

---

**GM Centre Number:** 616

**Data Premises Notified**

22/07/1996

(Originally)

**Transitional Premises Class**

1

**Data Premises Closed**

**Transitional Premises**

**Emergency Plan Required?**

**Non-GMMs**

**Withdrawn**

N

---

**Name**

SCOTTISH ASSOCIATION FOR MARINE SCIENCE

---

**Name 2**

DUNSTAFFNAGE MARINE LABORATORY

---

**Department**

BIOTECHNOLOGY

---

**Campus Estate or Research Centre**

---

**Building**

---

**Road Name**

DUNBEG

---

**District**

OBAN

---

**Town**

ARGYLL

---

**County**

ARGYLL

---

**Postcode**

PA37 1QA

---

**Country**

SCOTLAND

---

**Tel Number**

01631 567829

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**Fax Number**

01631 571150

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**E-mail**

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**HSE Division**

SCOTLAND

---

**Comments**

---

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<th>Level 1 (GMMs)</th>
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Tick if confidential

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<th>Microbiology Research</th>
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<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 617**

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**Name**

WESTLAKES SCIENTIFIC CONSULTING LIMITED

**Name 2**

PRINCESS ROYAL BUILDING

**Campus Estate or Research Centre**

WESTLAKES SCIENCE AND TECHNOLOGY PK

**Road Name**

MOOR ROW

**Town**

WHITEHAVEN

**County**

CUMBRIA

**Postcode**

CA24 3LN

**Country**

ENGLAND

**Tel Number**

01946 514000

**Fax Number**

01946514040

**E-mail**

**HSE Division**

NORTH WEST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

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Give brief details of the genetic modification safety committee

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial
Other (please specify)

Tick if confidential

Bacteriology  Parasitology  Transgenic Birds  Microbiology Research
Virology  Transgenic Animals  Transgenic Fish  Gene Therapy
All material will be sterilised by autoclaving at 121 degrees C for 15 mins before disposal. Maximum volume will not exceed 5 L. Autoclave performance is monitored by the inclusion of indicator discs with every cycle. These discs change colour after the autoclave has reached 121 degrees C for 15 mins.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Date at Which Additional Info Submitted: 02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity

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The maximum culture volume does not exceed one litre and this is then inactivated by treatment with presept tablets. These are used at one 5 g tablet per litre and the resulting solution left to stand for at least one hour before disposal down the sink. Solid waste material is disposed of by autoclaving prior to incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Name

LONZA BIOLOGICS PLC

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

228 BATH ROAD

District

Town

SLOUGH

County

BERKSHIRE

Postcode

SL1 4DY

Country

ENGLAND

Tel Number

01753 777000

Fax Number

01753 777001

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

GMSC ESTABLISHED IN NOVEMBER 2010

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<thead>
<tr>
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Non-microbial

Other (please specify)  
Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
- Mycology
- Transgenic Invertebrates
- Transgenic Plants
- Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
### Project Ref 619/96.1

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#### Project Additional Information

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Date Ackn’d**: 02/09/1996

- **Date Project Ceased**: 

- **Withdrawn**: N

- **Tick if notifying a connected programme of work**: Y

- **Historical Significant Changes**

- **Historical Date of Additional Info**

- **Significant Change ID**

- **Date of Significant Change**

02/03/2022
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Name
PARKE-DAVIS NEUROSCIENCE RESEARCH CENTRE

Name 2

Department

Campus Estate or Research Centre
CAMBRIDGE UNIVERSITY FORVIE SITE

Road Name
ROBINSON WAY

District

Town
CAMBRIDGE

County
CAMBRIDGESHIRE

Postcode
CB2 2QB

Country
ENGLAND

Tel Number
01223 210 929

Fax Number
01223

E-mail

HSE Division
EAST AND SOUTH EAST

Comments
AQUIRED BY PFIZER. ALL WORK TRANSFERED TO GM 331

Date at Which Additional Info Submitted
02/03/2022
### Premises Addresses

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<td>CB2 2QB</td>
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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities
- Give brief details of the genetic modification safety committee

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<thead>
<tr>
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<th>Animal Unit</th>
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- Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 621

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Name
UNIVERSITY OF LIVERPOOL

Name 2

Department
PHARMACOLOGY & THERAPEUTICS

Building
OLD ROYAL INFIRMARY BUILDING

Road Name
70 PEMBROKE PLACE

Town
LIVERPOOL

Country
ENGLAND

County
MERSEYSIDE

Postcode
L69 3GF

Tel Number
0151 794 8219

Fax Number
0151 794 8217

E-mail
none

HSE Division
NORTH WEST

Comments

Date at Which Additional Info Submitted
02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**:
- **Animal Unit**:  
- **Growth Room**:  
- **Glass House**:  
- **Large Scale**:  

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**

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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 622

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#### Name

**EISAI LIMITED**

**Department**

**BIOLOGY AND BIOCHEMISTRY**

#### Campus Estate or Research Centre

**EUROPEAN KNOWLEDGE CENTRE**

#### Road Name

**MOSQUITO WAY**

#### Town

**HERTFORDSHIRE**

#### District

**HATFIELD**

#### County

**ENGLAND**

#### Postcode

**AL10 9SN**

<table>
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<tr>
<th>Tel Number</th>
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<th>Fax Number</th>
<th>0207 413 1121</th>
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#### HSE Division

**LONDON**

#### Comments

previously Eisai London Research Laboratories Ltd name change 01/10/2009

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
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<th>Laboratory</th>
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Non-microbial

Other (please specify)

Tick if confidential

Bacteriology       Parasitology       Transgenic Birds       Microbiology Research

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 622/03.1

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### Project Additional Information

#### Purposes of the contained use

To investigate the effect of kinases and proteases in APP processing and Amyloid beta production

#### Recipient or parental organism

The HEK 293 cell line is a permanent line of primary human embryonic kidney transformed by sheared adenovirus type 5 (Ad5) DNA. The HEK 293FT cell line in addition to being transformed by Ad5 DNA dos stably and constitutively express the SV40 large T-Antigen to facilitate optimal lentivirus production (Naldini et al. 1996). HEK 293 and HEK293FT cell lines have been established in cell culture for many years and are well characterised. Being of human origin they may harbour adventitious agents that could harm humans, however, there are no reports of such occurrences. Primary rat cortical neurons are non-dividing cells which require special medium to grow. Both HEK293 cells and cortical neurons will be unable to survive outside the lab.

#### Host/vector system

The lentiviral system that we will be using is produced by Invitrogen and it has been widely tested in a variety of cell types. The system is derived from the Human Immunodeficiency Virus type 1 (HIV-1). Its genome has been divided into four distinct plasmids; a pLenti6 expression vector into which the gene of interest is cloned and three packaging plasmids which supply the helper functions and the structural and replication proteins in trans required to produce the Lentivirus.

To generate the lentiviral vector of interest, the pLenti6-expression construct containing the gene of interest is transfected into the 293FT producer cell line together with a mix of the three packaging plasmids. The 293FT producer cell line stably and constitutively expresses the SV40 large T antigen under the control of a CMV promoter. Studies have demonstrated maximal virus production in human 293 cells expressing large SV40 large T antigen (Naldini et al. 1996) making the 293FT cell line a particularly suitable host for generating lentiviral constructs. 48 to 72 hours after transfection, the viral supernatant is harvested and its titre determined. The viral supernatant can then be added to our mammalian cells of interest (HEK293 cells and rat cortical neurons).

The expression vector (pLenti6) contains a deletion in the 3' LTR (U3) that does not affect generation of the viral genome in the producer cell line, but results in self-inactivation of the lentivirus after transduction of the target cell through the inactivation of the viral genome promoter (Yee et al. 1987; Yu et al. 1986; Zufferey et al 1998). No recombinant viral particles containing our genes of interest can be produced even in the occurrence of co-infection with WT HIV-1(Zufferey et al. 1998).

Additional safety features are: the number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol and rev) out of the nine genes present in the genome of the parental virus. Furthermore, the VSV-G gene from Vesicular Stomatitis Virus is used in place of of the HIV-1 envelope (Burns et al. 1993; Emi et al 1991; Yee et al 1994).

Genes encoding the structural and other components required for packaging the pLenti vector RNA are on three separate plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent recombination events that could lead to the generation of a replication-competent virus (Dull et al 1998).

Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, VSV-G_ in the 293FT producer cell line, none of them contain LTRs or the 0 packaging sequence. This means that none of the these HIV-1 structural genes are ever present in any infectious viruses. Expression of the gag and pol genes from one of the helper plasmids has been rendered Rev-dependent by virute of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al. 1998).

#### Origin & function

APP(WT and APP processing mutants) as well as all other constructs were made in house.

Our aim is to make lentiviruses that express WT or APP processing mutants to produce a measurable amount of Amyloid-beta. We will then look at the effects of co-expressing various kinases or proteases on Amyloid-beta production. The expression of all genes will be under the control of the CMV promoter.

We would also like to establish the expression of RNAi using Lentivirus here in the lab. To do that we will transduce HEK293 cells and rat cortical neurons with a GFP or lacZ lentiviral vector and later transduce the same cells with a RNAi GFP or lacZ lentiviral vector and see whether we can block GFP or lacZ expression from those cells. The RNAi expression will be under the control of the U6 promoter. Similar experiments have been previously carried out in other labs (Haibin Xia et al 2002, Nature Biotech, 20, 1006-1010).
The mammalian cells are unlikely to survive outside the laboratory as they require special medium to grow. Therefore, it is unlikely that any harm would occur to the environment if any were released. As the GMMs are unlikely to survive outside laboratory, the consequences of release are negligible. Gene transfer from transfected mammalian cells is very unlikely. The inserted genes of interest are unlikely to provide a selective advantage to the host. The lentiviral-based vectors used are replication incompetent and have the additional safety features described above. The major risks of working with lentiviral vectors are associated with needle stick injury (however no needles will be used at any stage during the handling of the viruses), exposure to open wounds (gloves and disposable lab coat will be worn at all times and any open wounds will be covered), and inhalation (aerosolisation of viral stocks will be minimised; screw cap tubes will be used to centrifuge and to store the virus and sonication will not be used). Because the vector uses a VSV-Glycoprotein it is able to infect a wide range of mammalian cells including human cells. However, for the reasons given above the risk from infection of the user by the viruses is very low. No new replication-competent virus can be produced and therefore the viruses will not be able to propagate after infection. Although escaped virus might infect other mammals, the same considerations apply to human exposure and so are minimal. If humans or environment were to receive the virus the effects will be mainly dependent on the type of proteins that the infected cells would express. Below is a summary of the effect of overexpression of those proteins in mammalian cells:

- LacZ and GFP are considered non-pathogenic in the host cell lines.
- The recombinant APP proteins are considered non-pathogenic in HEK293 cells, however, overexpression in rat cortical neurons was shown to induce apoptosis (Lienlen-Campard et al 2002 JBC, 277, 15666-70) and mice overexpressing SW APP show degeneration of choline acetyl transferase-immunoreactive fibres in the environment of beta-amyloid plaques and activated glial cells suggesting a role of beta-amyloid and/or inflammation in specific degeneration of cholinergic synaptic structures (Luth HJ. et al, 2003 Brain Res, 977, 16-22). However the cells most at risk from infection are the lung and gut epithelium for which APP is non-pathogenic. Infection of neuronal cells is a highly unlikely event, and it is not clear that infection of a few neurones would be pathogenic to any other cells. The kinases and proteases to be studied are likewise not considered a hazard upon expression in mammalian cells.

To summarise expression of these recombinant proteins will not alter the pathogenicity and or oncogenicity of mammalian cells nor will they alter the viral characteristics.

Infection of neuronal cells is a highly unlikely event, and it is not clear that infection of a few neurones would be pathogenic to any other cells. The kinases and proteases to be studied are likewise not considered a hazard upon expression in mammalian cells.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All paper and plastic waste (tubes, tissue culture flasks, dishes, pipettes and tips) as well as small volumes of liquid waste, which will be collected in plates or flasks, will be collected in autoclavable bags. These will be sealed and autoclaved at 134 degrees celsius prior to disposal by inactivation.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
Sharps are to be banned from the virus room, any procedures that involve sharps will have to be put before the Committee for specific approval. No such procedure is necessary for the present project. Security on the room was questioned and it was confirmed that the room is only accessible by those with specific card keys and these will only be sanctioned upon approval of a specific application to the Committee. Communication with others outside the room in the event of a spill was also considered. In the event of an accident the experimenter may not be able to easily leave the room to seek help. A telephone will now be installed in the room at all times during use. Waste handling and disposal was discussed and approved. The possibility of an escape of in the event of an accident was considered and it was felt that given the standard of the equipment and room, the small scale of virus production that will occur, an escape would not occur.

**Project Containment**

<table>
<thead>
<tr>
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<th>Growth Rooms</th>
</tr>
</thead>
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**Project Ref** 622/04.1

**CU2 Project Title**

FURTHER INVESTIGATION INTO KINASE AND PROTEASE REGULATION OF AMYLOID-BETA (A) PRODUCTION. PRODUCTION OF SECOND GENERATION LENTIVIRUSES.

**Class**

Class 2

**CultureVolClass2**

1-50 litres

**Consent Granted**

not applicable

**Project notified under transitional arrangements**

N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

To investigate the effect of kinases and proteases in APP processing and Amyloid beta (AB) production.

Recipient or parental organism

The HEK 293 cell line is a permanent line of primary human embryonic kidney transformed by sheared adenovirus type 5 (Ad 5) DNA. HEK293T cell line stably and constitutively express the SV40 large T-antigen to facilitate optimal lentivirus production (Naidini et al., 1996). HEK 293 and HEK293T cell lines have been established in cell culture for many years and are well characterised. Being of human origin they may harbour adventitious agents that could harm humans, however, there are no reports of such occurrences. Primary rat cells require special medium to grow. HEK 293 cells and rat primary cells will be unable to survive outside the lab.

Host/vector system

We will be using a second generation lentiviral system (Zuffery et al., 1996). This system has been widely tested in a variety of cell types. It is derived from the Human immunodeficiency Virus type 1 (HIV-1).

For the second generation virus, the HIV-1 genome has been divided into three distinct plasmids: an expression vector into which the gene of interest is cloned (pHR-SIN-CSGW) and two packaging plasmids (pCMVR8.91 and pMO-G) which supply the helper functions and the structural and replication proteins in trans required to produce the Lentivirus.

To generate the Lentiviral vector of interest, the expression construct containing the gene of interest is transfected into the 293T producer cell line together with a mix of the two packaging plasmids. The 293T producer cell line stably and constitutively expresses the SV40 large T antigen under the control of a CMV promoter. Studies have demonstrated maximal virus production in human 293 cells expressing SV40 large T antigen (Naidini et al., 1996) making the 293T cell line a particular suitable host for generating lentiviral constructs. Forty-eight to 72 hours after transfection, the viral supernatant is harvested and its titre determined. The viral supernatant can then be added to our mammalian cells of interest (HEK 293 cells and rat primary cells). Both expression vectors contain a deletion in the 3’ LTR (U3) that does not affect generation of the viral genome in the producer cell line, but results in self-inactivation of the lentivirus after transduction of the target cell through the inactivation of the viral genome promoter (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). No recombinant viral particles containing our genes of interest can be produced even in the occurrence of co-infection with WT HIV-1 (Zufferey et al., 1998).

Additional safety features are: the number of genes from HIV-1 that are used in the system has been reduced to three (ie gag, pol, and rev) out of the nine genes present in the genome of the parental virus. Furthermore, the VSV-Gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns et al., 1993 Emi et al., 1991; Yee et al., 1994)

Genes encoding the structural and other components required for packaging the lentivirus vector RNA are on two separate plasmids. All plasmids have been engineered not to contain any regions of homology with each other to prevent recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998). Although the packaging plasmids allow expression in trans of proteins required to produce viral progeny (eg gal, pol, rev, VSV-G) in the 293T producer cell line, none of them contain LTRs or the O packaging sequence. This means that none of these HIV-1 structural genes are ever present in any infectious viruses.

Expression of the gag and pol genes from one of the helper plasmids has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al., 1998)

Origin & function

APP (WT and APP processing mutants) as well as all other constructs were made in house.

Our aim is to make lentiviruses that express WT or APP processing mutants to produce a measurable amount of AB. We will then look at the effects of co-expressing various kinases or proteases on AB production. The expression of all genes will be under the control of the SFFV promoter (Spleen Focus Forming Virus). We will also make lentiviruses expressing LacZ, nisLacZ GFP to use as sonctols of infection and markers for infected cells.

We would also like to establish the expression of RNAi using Lentivirus here in the lab. To do that we will transduce HEK293 cells and rat primary cells with a GFP or LacZ lentiviral vector and later transduce the same cells with a RNAi GFP or LacZ lentiviral vector and see whether we can block GFP or LacZ expression from those cells. The RNAi expression will be under the control of the U6 promoter. Similar experiments have been previously carried out in other labs (Haibin Xia et al., 2002 Nature Biotech, 20, 1006-1010).
The mammalian cells are unlikely to survive outside the laboratory as they require special medium to grow. Therefore, it is unlikely that any harm would occur to the environment if any were released. As the GMMs are unlikely to survive outside laboratory, the consequences of release are negligible.

Gene transfer from transfected mammalian cells is very unlikely. The inserted genes of interest are unlikely to provide a selective advantage to the host. The lentiviral-based vectors used are replication incompetent and have the additional safety features described above. The major risks of working with lentiviral vectors are associated with needle stick injury (however no needles will be used at any stage during the handling of the viruses), exposure to open wounds (gloves and disposable lab coat will be worn at all times and any open wounds will be covered) and inhalation (aerosolisation of viral stocks will be minimised: screw cap tubes will be used to centrifuge and to store the virus and sonication will not be used).

Because the vector uses a VSV-Glycoprotein it is able to infect a wide range of mammalian cells including human cells. However for the reasons given above the risk from infection of the user by the viruses is very low.

No replication-competent virus can be produced and therefore the viruses will not be able to propagate after infection. Although escaped virus might infect other mammals, the same considerations apply to human exposure and so are minimal.

If humans or environment were to receive the virus the effects will be mainly dependent on the type of proteins that the infected cells would express. Below is a summary of the effect of overexpression of those proteins in mammalian cells:

- **BACE, LacZ and GFP** are considered non-pathogenic in the host cell lines.
- The recombinant APP proteins are considered non-pathogenic in the HEK 293 cells, however overexpression in rat cortical neurons was shown to induce apoptosis (Kienlen-Campart et al., 2002 JBC, 277, 15666-70) and miceoverexpressing SW App show degeneration of choline acetyl transferase-immunoreactive fibres in the environment of beta-amyloid plaques and activated gial cells suggesting a role of beta-amyloid and/or inflammation in specific degeneration of cholinergic synaptic structures (Luch HJ et al., 2003 Brain Res, 977, 16-22). However, the cells most at risk of infection are the lung and gut epithelium for which APP is non-pathogenic.
- Infection of neuronal cells is a highly unlikely event, and it is not clear that infection of a few neurones would be pathogenic to any other cells. The kinases and proteases to be studied are likewise not considered a hazard upon expression in mammalian cells.

To summarise expression of these recombinant proteins will not alter the pathogenicity and/or oncogenicity of mammalian cells nor will they alter the viral characterists. Furthermore, the lentiviral particles used in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced even in the occurrence of co-infection with WT HIV-1. There is therefore negligible risk to humans in the event of exposure.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All paper and plastic waste (tubes, tissue culture flasks, dishes, pipettes and tips) as well as small volumes of liquid waste, which will be collected in plates or flasks, will be collected in autoclavable bags. These will be sealed and autoclaved at 134 degrees C prior to disposal by incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form
The safety committee discussed this project and agreed that the differences between this project and the previous project were only in the details of the viral production system. Since the safety aspects of the viral production system are identical to that in the previous project no changes to procedures were required. The safety committee therefore approved this project.

**Project Containment**

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**Project Ref** 622/05.1

- **CU2 Project Title**: Investigation of oligodendrocyte differentiation
- **Class CultureVolClass2 CultureVolumeClass3-4**: Class 2 1-50 Litres
- **Non-GMM Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N

**Project Additional Information**
**Purposes of the contained use**

To investigate the effect of various transcription factors on oligodendrocyte differentiation.

**Recipient or parental organism**

The HEK 293 cell line is a permanent line of primary human embryonic kidney transformed by sheared adenovirus type 5 (Ad5) DNA. The HEK 293FT cell line in addition to being transformed by Ad5 DNA does stably and constitutively express the SV40 large T-Antigen to facilitate optimal lentivirus production (Naldini et al., 1996). HEK 293 and HEK 293FT cell lines have been established in cell culture for many years and are well characterised. Being of human origin they may harbour adventitious agents that could harm humans, however, there are no reports of such occurrences. Neural precursor cells (NPC's) are derived from embryonic, postnatal and adult murine tissue and required special medium to grow. Both HEK 293 cells and NPC's will be unable to survive outside the lab.

**Host/vector system**

The Retroviral system that we will be using is produced by Imgenex and it has been widely tested in a variety of cell types. RetroMaxTM retrovirus vector system is based on the pCL vector system developed by Naviaux et al. (1996). The system is derived from the moloney murine leukaemia virus (MMLV). Its genome has been divided into two distinct plasmids: a retroviral expression vector (pMX vector) in which the gene of interest is cloned and a packaging plasmid (pCL-Eco vector) which supplies the helper functions and the structural and replication proteins in trans required to produce the Retrovirus.

To generate the Retroviral vector of interest, the pMX-construct containing the gene of interest is transfected into the HEK293FT producer cell line together with the packaging vector. 24 hours after transfection the medium is changed to media suitable for growth of neutral precursor cells. Forty-eight to 96 hours after transfection, the viral supernatant is harvested and its titre determined. The viral supernatant can then be added to our mammalian cells of interest (HEK 293 cells and NPC's).

The expression vector (pMX) contains the 5' and 3' LTR derived from the MMLV. Although the vector contains the 5 prime half of the gag coding sequence, the initiation codon of this gag gene is deleted in the pMX vector. So, even in the event of recombination, the risk that replication competent virus will be produced is very low.

The pCL-ECO packaging vector has also been safety modified by deleting the packaging signal and the 3'LTR enhancer. This makes the RNAs of the helper genome un-packageable. The advantage of these pCL packaging plasmids is a high level expression of gag, pol, and env proteins with a balanced stoichiometry that is not achieved with either transiently or stably expressed split-genome packaging constructs. Furthermore, the packaging system pCL-Eco generates viral particles that will infect mouse and rat cells, but not human cells. This is due to a lack of tropism for human cells with the retroviral vectors. The viral envelope protein, gp70, determines which cells the virus will enter. The vectors expressing the mouse ecotropic envelope infect mouse and rat cells only.

Although the packaging plasmid allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, env) in the 293 producer cell lines, it does not contain the Ø packaging sequence. This means that none of these retroviral structural genes are ever present in any infectious viruses.

The expression of all inserted genes will be under the control of the PGK promoter.

**Origin & function**

All constructs will be made in house. Our aim is to make retroviruses that express Olig1, Olig2, the bHLH domain of Olig1, ID4, E47 and combinations of such. The constructs will be used to study Oligodendrocyte differentiation in neural progenitor cells.

**Evaluation of foreseeable effects**

The mammalian cells are unlikely to survive outside the laboratory as they require special medium to grow. Therefore, it is unlikely that any harm would occur to the environment if any were released. The release of GMM's or viral particles into the environment is very unlikely since all waste as well as any instruments that were in contact with viruses will be autoclaved prior to disposal by incineration. In addition all experiments will be in a small scale and even in the event of an accident there will be unlikely a release.

Gene transfer from transfected mammalian cells is very unlikely. The inserted genes of interest are unlikely to provide a selective advantage to the host.

The retroviral-based vectors used are replication incompetent and have the additional safety features described above. The major risks of working with retroviral vectors are replication incompetent and have additional safety features described above. The major risks of working with retroviral vectors are associated with needle stick injury.
(however no needles will be used at any stage during the handling of the viruses), exposure to open wounds (gloves and disposable lab coat will be worn at all times and any open wounds will be covered) and inhalation (aerosolisation of viral stocks will be minimised: screw cap tubes will be used to centrifuge and to store the virus and sonication will not be used). Otherwise, the cells most at risk are epithelial cells which are rapidly replaced and therefore unlikely to pose a hazard even upon receiving these constructs. Since viral work will carried out according to guidelines in a Biosafety Level 2 facility, it is very unlikely that any virus can infect mammals outside the laboratory. In addition, it is unlikely that the replication-deficient virus will be able to propagate after such an infection. Although escaped virus might infect other mammals, these viruses cannot infect human described above. The vectors can only become potentially infectious for human cells by recombination with mouse endogenous retrovirus genes encoding amphotrophic or polytropic envelopes.

If the environment (e.g. endogenous populations) were to receive the virus the effects will be mainly dependent on the type of proteins that the infected cells would express. Below is a summary of the effect of overexpression of those proteins in mammalian cells:

- **EGFP, bHLH domain of Olig1, hygromycin B phosphotransferase gene (hydro) and puromycin (puro) resistance gene (puro)** are considered non-pathogenic. Olig1 and Olig2 are basic helix-loop transcription factors and are expressed specifically in cells of the oligodendrocyte lineage (Zhou et al., 2000). Olig1 genes are strongly expressed in oligodendroglioma (Lu QR, 2001) and astrocytoma (Bouvier C, 2003). Immunoreactivity for Olig1 was also found in dysembryoplastic neuroepithelial tumors (Azzarelli B, 2004). Olig2 is also universally expressed in glioma (Ligon KL, 2004) and brain tumors (Lu QR 2001). However, no direct evidence exists that overexpression of Olig1 causes such glioma. Nevertheless, there is a slim possibility that overexpression of Olig1 and forms of Olig1 carrying the nuclear localisation signal could be oncogenic.

- **ID4** is a member of the helix-loop transcription factors and plays important roles throughout gastrulation, neurogenesis, cellular differentiation, proliferation and apoptosis. Due to its pivotal role in cell cycle control, Dysregulation might lead to cancer, e.g. bladder cancer, breast cancer and leukemia (de Candia, 2004, Wu Q, 2005). E47 is a member of the class 1 helix-loop-helix transcription factors that are expressed ubiquitously and are able to dimerise with tissue-specific class B bHLH factors to activate gene expression (Massari and Murre, 200). E47 plays a role in B-cell development and ectopic expression of E47 or E12 promotes the death of E2A-deficient lymphomas (Engel 1999).

Although mammals might be infected by escaped virus, the virus cannot propagate in infected animals. Therefore, spreading of virus to other animals cannot occur and the anticipated effects on endogenous populations are insignificant.

For the reasons given above (negligible risk of release of GMM, safety features of the vector system, limited tropism of the vector for mouse and rat cells), the potential risk arising from release of GMM for users and environment is very low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All paper and plastic waste (tubes, tissue culture flasks, dishes, pipettes and tips) as well as small volumes of liquid waste, which will be collected in plates or flasks, will be collected in autoclavable bags. These will be sealed and autoclaved at 134°C prior to disposal by incineration.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y
The safety committee discussed this project and agreed that the differences between this project and the previous projects were only in the details of the viral vectors used. Since the safety aspects of the viral production system are identical to that in the previous project no changes to procedures were required. The safety committee therefore approved this project.

**Project Containment**

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**Project Ref** 622/06.1

Investigation of oligodendrocyte differentiation.

**Project Additional Information**

To investigate the effect of various transcription factors on oligodendrocyte differentiation.
The HEK 293 cell line is a permanent line of primary human embryonic kidney transformed by sheared adenovirus type 5 (Ad5) DNA. The HEK 294 FT cell line in addition to being transformed by Ad5 DNA does stably and constitutively express the SV40 large T-antigen to facilitate optimal lentivirus production (Naldini et al., 1996). HEK 293 and HEK293FT cell lines have been established in cell culture for many years and are well characterised. Being of human origin they may harbour adventitious agents that could harm humans, however, there are no reports of such occurrences. The P19 cell line was isolated by implanting a 7 day old mouse embryo under the testis capsule of an adult which resulted in formation of a tumor (McBurney and Rogers, 1982). Tumor cells were grown in culture and one clone, P19, established as a line. P19 cells have a normal karyotype and reproduce rapidly and without apparent limit in culture; they express several early embryonic markers. P19 cells have been established in cell culture for many years and are well characterised. There are no reports that P19 cells may harbour adventitious agents that could harm humans. Neural precursor cells (NPC's) are derived from embryonic, postnatal and adult rodent tissue and require special medium to grow. HEK 293 cells, P19 cells and NPC's will be unable to survive outside the lab.

Recipient or parental organism

The HEK 293 cell line is a permanent line of primary human embryonic kidney transformed by sheared adenovirus type 5 (Ad5) DNA. The HEK 294 FT cell line in addition to being transformed by Ad5 DNA does stably and constitutively express the SV40 large T-antigen to facilitate optimal lentivirus production (Naldini et al., 1996). HEK 293 and HEK293FT cell lines have been established in cell culture for many years and are well characterised. Being of human origin they may harbour adventitious agents that could harm humans, however, there are no reports of such occurrences. The P19 cell line was isolated by implanting a 7 day old mouse embryo under the testis capsule of an adult which resulted in formation of a tumor (McBurney and Rogers, 1982). Tumor cells were grown in culture and one clone, P19, established as a line. P19 cells have a normal karyotype and reproduce rapidly and without apparent limit in culture; they express several early embryonic markers. P19 cells have been established in cell culture for many years and are well characterised. There are no reports that P19 cells may harbour adventitious agents that could harm humans. Neural precursor cells (NPC's) are derived from embryonic, postnatal and adult rodent tissue and require special medium to grow. HEK 293 cells, P19 cells and NPC's will be unable to survive outside the lab.

Host/vector system

The lentiviral system that we will be using is produced by Invitrogen and it has been widely tested in a variety of cell types. The system is derived from the Human Immunodeficiency Virus type 1 (HIV-1). Its genome has been divided into four distinct plasmids: pLenti6 expression vector into which the gene of interest is cloned and three packaging plasmids which supply the helper functions and the structural and replication proteins in transrequired to produce the Lentivirus.

To generate the Lentiviral vector of interest, the pLenti6-expression construct containing the gene of interest is transfected into the 193FT producer cell line together with a mix of the three packaging plasmids. The 293FT producer cell line stably and constitutively expresses the SV40 large T antigen under the control of a CMV promoter. Studies have demonstrated maximal viral production in human 293 cells expressing SV40 large T antigen (Naldini et al., 1996), making the 293FT cell line a particularly suitable host for generating lentiviral constructs. Forty-eight to 72 hours after transfection, the viral supernatant is harvested and its titre determined. The viral supernatant can then be added to our mammalian cells of interest (HEK 293 cells and rodent neural precursor cells).

The expression vector (pLenti6) contains a deletion in the 3' LTR (U3) that does not affect generation of the viral genome in the producer cell line, but results in self-inactivation of the lentivirus after transduction of the target cell through the inactivation of the viral genome promoter (Yee et al., 1987; Ue et al., 1986; Sufferey et al., 1998). No recombinant viral particles containing our genes of interest can be produced even in the occurrence of co-infection with WT HIV-1 (Zufferey et al., 1998).

Additional safety features are: the number of genes from HIV-1 that are used in the system has been reduced to three (ie gag, pol, and rev) out of the nine genes present in the genome of the parental virus. Furthermore, the VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns et al., 1993; Emi et al., 1991; Yee et al., 1994).

Genes encoding the structural and other components required for packaging the pLenti6 vector RNA are on three separate plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).

Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (eg gal, pol, rev, VSV-G) in the 293FT producer cell line, none of them contain LTRs or the 0 packaging sequence. This means that none of these HIV-1 structural genes are event present in any infectious viruses.

Expression of the gag and pol genes from one of the helper plasmids has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al., 1998).

Origin & function

All constructs will be made in house.

Our aim is to make lentiviruses that express Olig1, Olig2, the basic helix-loop-helix domain of Olig1, ID4, E47 and combinations of such. The constructs will be used to study Oligodendrocyte differentiation in neural progenitor cells.
Evaluation of foreseeable effects

The mammalian cells are unlikely to survive outside the laboratory as they require special medium to grow. Therefore, it is unlikely that any harm would occur to the environment if any were released. As the GMMs are unlikely to survive outside laboratory, the consequences of release are negligible.

Gene transfer from transfected mammalian cells is very unlikely. The inserted genes of interest are unlikely to provide a selective advantage to the host.

The lentiviral-based vectors used are replication incompetent and have the additional safety features described above. The major risks of working with lentiviral vectors are associated with needle stick injury (however no needles will be used at any stage during the handling of the viruses), exposure to open wounds (gloves and disposable lab coat will be worn at all times and any open wounds will be covered) and inhalation (aerosolisation of viral stocks will be minimised; screw cap tubes will be used to centrifuge and to store the virus and sonication will not be used).

Because the vector uses a VSV-Glycoprotein it is able to infect a wide range of mammalian cells including human cells. However for the reasons given above the risk from infection of the user by the viruses is very low.

No new replication-competent virus can be produced and therefore the viruses will not be able to propagate after infection. Although escaped virus might infect other mammals, the same considerations apply to human exposure and so are minimal.

If the environment (eg endogenous populations) were to receive the virus the effects will be mainly dependent on the type of proteins that the infected cells would express. Below is a summary of the effect of overexpression of those proteins in mammalian cells:

- EGFP, bHLH domain of Olig1, hygromycin B phosphotransferase gene (hygro) and puromycin (puro) resistance gene (puro) are considered non-pathogenic. Olig 1 and Olig2 are basic helix-loop-helix transcription factors and are expressed specifically in cells of the ologodendrocyte lineage (Zhou et al., 2000). Olig1 genes are strongly expressed in oligodendroglioma (Lu QR, 2001) and astrocytoma (Bouvier C, 2003). Immunoactivity for Olig 1 was also found in dysembryioplastic neuroepethelial tumors (Azzarelli B, 2004). Olig2 is also universally expressed in glioma (Ligon KL, 2004) and brain tumors (Lu QR 2001). However, no direct evidence exists that overexpression of Olig 1 causes such glioma. Nevertheless, there is a slim possibility that overexpression of Olig1 and forms of Olig1 carrying the nuclear localisation signal could be oncogenic.

- ID4 is a member of the helix-loop-helix transcription factors and plays important roles throughout gastrulation, neurogenesis, cellular differentiation, proliferation and apoptosis. Due to its pivotal role in cell cycle control, dysregulation might lead to cancer, eg bladder cancer, breast cancer, and leukemia (de Candia, 2004, Wu Q, 2005).

- E47 is a member of the class 1 helix-loop-helix transcription factors that are expressed ubiquitously and are able to dimerize with tissue-specific class B bHLH factors to activate gene expression (Massari and Murre, 2000). E47 plays a role in B-cell development and ectopic expression of E47 and E12 promotes the death of E2A-deficient lymphomas (Engel 1999).

Although mammals might be infected by escaped virus, the virus cannot propagate in infected animals. Therefore, spreading of virus to other animals cannot occur and the anticipated effects on endogenous populations are insignificant.

For the reasons given above (negligible risk of release of GMM, safety features of the vector system), the potential risk arising from release of GMM for users and environment is very low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This application was discussed at the GMSC on 21st November 2005.

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<td>L2</td>
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Human Clinical Applications

<table>
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Project Ref 622/07.1

In vitro analysis of differentiation of an oligodendrocyte precursor cell line.

Date Ackn'd 22/08/2007

CU2 Project Title

Class 2 CultureVolClass2 1-50 Litres

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
Project Additional Information

**Purposes of the contained use**

To develop analysis tools for investigating oligodendrocyte differentiation.

**Recipient or parental organism**

The Oli-neu cell line (Jung et. Al, (1995) Eur. J. Neurosci. 7:1245-1265) was established by immortalising enriched primary shake-off cultures of oligodendrocyte precursors from E15-mouse brains by expression of the oncogene t-neu. This oncogene has constitutively active tyrosine kinase activity, which induces unlimited expansion of these cells. Oli-neu cells have a normal karyotype and replicate rapidly and without apparent limit in culture; they express several early oligodendrocyte markers. Oli-neu cells have been established in cell culture for many years and are well characterised. There are no reports that Oli-neu cells may harbour adventitious agents that could harm humans.

**Host/vector system**

Introduction of the additional DNA constructs is not foreseen to constitute any additional hazard from the vector view point.

**Origin & function**

Oligodendrocyte specific promoter sequences upstream of lacZ and PLAP reporter constructs in pCDNA3.1 derived or pSEAP1-basic (Clontech) vectors. pTK-Hygromycin was purchased from Takara Europe/Clontech (Cat number 631750).

**Evaluation of foreseeable effects**

Oli-neu cell line was established by immortalising enriched primary shake-off cultures of oligodendrocyte precursors from E15-mouse brains by expression of the oncogene t-neu. This oncogene has constitutively active tyrosine kinase activity, which induces unlimited expansion of these cells. The disabled retroviral based vector used to introduce the oncogene is not able to repackage due to the lack of any encoded retroviral proteins. However it is possible (due to the presence of LTR sequences) that the retroviral/oncogene RNA could be package in the event of a co-infection with a WT retrovirus of a type that was able to interact with the Moloney Murine Leukemia Virus (MoMLV) packaging sequence. If this virus were able to infect a human cell this could result in the oncogene being introduced into human cells. Consultation with the ELL named vet and the vet at our animal supplier indicated that they thought this extremely unlikely since the neu cell line was established by immortalising enriched primary shake off cultures of oligodendrocyte precursors from E15 conceive a means for virus to be passed from an animal to the cells. In addition the ELL vet was of the opinion that retrovirus able to infect both mouse and human cells were very rare. This leaves the possibility that the cells were already infected when
we obtained them, however the supplier of these cells stated that they did not treat them as a risk and that there were no known risks associated with these cells. Arguing for a more rigorous containment is the fact that viruses do exist that can infect both murine and human cells and so could precipitate this hazard. In addition we have been informed that on rare occasions animal cell lines and primary cultures spontaneously produce infectious gammararetroviruses. Gammararetroviruses are able to package the MoMLV type packaging sequences.

The introduction of a t-neu expression construct into human cells could result in tumour induction, so is extremely undesirable. Release of the virus packaged t-neu expression construct into the environment is less of a hazard as these viruses are extremely labile and unless directly introduced into an animal that the virus can infect the hazard is low.

Gene transfer from transfected mammalian cells other than by the reconstituted virus detailed above is very unlikely. The mammalian cells are unlikely to survive outside the laboratory as they require special medium to grow. Therefore, it is unlikely that any harm would occur to the environment if any were released.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All paper and plastic waste (tubes, tissue culture flasks, dishes, pipettes and tips) as well as small volumes of liquid waste, which will be collected in plates or flasks, will be collected in autoclavable bags. These will be sealed and autoclaved at 134°C prior to disposal by incineration.

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<tbody>
<tr>
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<td>N</td>
</tr>
<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
</tr>
<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>N</td>
</tr>
</tbody>
</table>

Please enter comments on the GM safety committee on the risk assessment

Containment is warranted. The safety committee therefore approved this project.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>

02/03/2022
# Project Title

**Transduction of ReNcells with GFP or LRRK2 containing constructs using BacMam system**

## Purposes of the contained use

Investigate the effect of over-expression of mutant vs WT LRRK2 constructs on ReNcells viability/functions.

## Recipient or parental organism

ReNcell VM is an immortalized human neural stem cell line (ReNeuron Ltd., Guildford, Surrey, UK) derived from the ventral mesencephalon from 10-week fetal neural tissue. ReNcell VM cells were infected with high titer amphotropic retrovirus (TEFLY-A) carrying the immortalizing transgene v-myc plus a selection marker (Hoffrogge et al., 2006). This recently derived human cell line requires containment at level 2.

## Host/vector system

The BacMam 2.0 system uses a modified insect cell virus (baculovirus) as a vehicle to efficiently deliver and express genes in mammalian cells. The transgene encodes WT or mutant LRRK2 (Leucine-rich repeat kinase 2 (LRRK2), an enzyme with a kinase and GTPase domains).

## Origin & function

BacMam 2.0 Reagents, Life Technologies: Research purpose only.

## Evaluation of foreseeable effects

ReNcell VM cell line was derived from the ventral mesencephalon of 10-week fetal tissue. Maternal blood was negative for HIV, HTLV, HepB&C. ReNcells were transformed using the oncogene v-myc (Donato et al. 2007). In the event of gene transfer of this to human or other animal cells there is a serious risk of oncogenesis.
however this is extremely unlikely to happen since the gene is integrated into the ReNcell genome and the RNA encoding the v-Myc is unlikely to be packaged into an infections viral particle. To minimise the risk of transfer no sharps will be used in any procedure and the cells will be cultured under containment level 2 to avoid exposure to WT retrovirus.

BacMam 2.0 vectors can transduce human cells but in the absence of sharps and the use of limited volumes of virus preparation this is unlikely to occur to a human.

Gene transfer of the baculovirus based constructs from transfected mammalian cells is unlikely. In the event of a transfer of these constructs into a human it is not likely that they will be maintained as they will not integrate into the mammalian genome. The transient expression of LRRK2, mutants of LRRK2 or GFP is not thought to pose a risk to human helath.

BacMam vectors contain a post transcriptional response lement derived from the Woodchuck Hepatitus virus genome (WPRE) that has been shown to increase transgene expression by many folds. There is tenuous evidence linking WPRE to cancer induction. Since the baculovirus based vector DMA, even if delivered into a cell, is extremely unlikely to integrate into the genome its transient nature ensures the WPRE poses minimal risk (see HSE Compendium of guidance paragrap 13, page 120, section 2).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All paper and plastic waste (tubes, tissue culture flasks, dishes, pipettes and tips) as well as small volumes of liquid waste, which will be sealed in tubes or flasks, will be collected in autoclavable bags. These will be sealed, double bagged and autoclaved at 134°C prior to disposal by incineration. If imaging of live cells is required, plates will be securely sealed with adhesive membranes that allow only gaseous exchange before transfer of the plates from the containment level 2 suite to imaging facilities. Once imaging is completed, plates will be returned to the containment level 2 suite and disposed off as described above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The safety committee agreed with the assessment and approved this project. Level 2 containment is warranted

Project Containment

<table>
<thead>
<tr>
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<td>L3 L4</td>
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</tr>
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</table>
### Investigating the function of genes implicated in Parkinson's Disease in cultured cells and primary neurons using HSV viruses to express full length wild type and mutant protein

#### Purposes of the contained use

To investigate the biological effects of expressing wild type (WT) and mutant proteins of interest in neuronal systems. Biological endpoints will include measurement of neurotoxicity, neurite shortening, mitochondrial homeostasis, and protein clearance.

**Experimental outline:**

1. Express target protein in primary neurons or cell lines using HSV viruses containing full length wild type and mutant protein or Azami Green separated by a 2A peptide sequence. Determine expression by western blotting and cell staining.
2. Determine the effects of expressing the different viruses mentioned above on cells using a number of fixed cell and live cell imaging assays. Assays will include mitochondrial integrity, neurite shortening, autophagy assays, and cell toxicity using the relevant cell dyes, imaging markers, and western blotting reagents.

#### Recipient or parental organism

**BE(2)-C** cells are derived from a human neuroblastoma. HEK293, SH-SY5Y, and NT2 are also human derived cell lines. All have been established in cell culture for many years and are well characterised. Being of human origin they may harbour adventitious agents that could harm humans, however, there are no reports of such occurrences. These cells will not be able to survive outside the laboratory.

The rat PC12 cell line is a permanent line derived from a transplantable rat adrenal phaeochromocytoma.
The mouse Neuro2A cell line is a permanent line derived from a spontaneous tumour in an albino strain A mouse. PC12 and Neuro2A cells have been established in cell culture for many years and are well characterised. These cells will not be able to survive outside the laboratory.

Rat primary neurons are cultured in Neurobasal media with B27 supplement. This media does not support dividing cells and the useful lifespan of cultures is approximately 30 days. These cells will not be able to survive outside the laboratory.

**Host/vector system**

The work will use HSV-1 strain 1764. All viruses will be disabled by deletion of ICP4 and Vmw65/VP16. As these genes are essential for virus replication and cytotoxicity, the resulting vectors will be replication incompetent and will only be able to replicate in complementing cells that provide these genes in trans (Fields Virology 4th edition (2001), ed Knipe DM et al., pub Lippincott, Williams and Wilkins). These vectors will therefore be non pathogenic and unable to productively infect any cell type other than the complementing cell line. The vectors will also have deletions in the latency associated (LAT) region. Insertion of gene expression cassettes will be into the LAT region.

**Origin & function**

Live disabled viruses produced by Biovex, Oxford (Amgen, UK; reference Anesti. A.M., P. J. Peeters, I. Royaux, and R.S Coffin. 2008. Efficient delivery of RNA interference to peripheral neurons in vivo using herpes simplex virus. Nucleic Acids Res. Vol 36:e86.) HSV viruses will be deficient in ICP4 and VP16 genes which are required for wild type HSV-1 replication and cytotoxicity respectively. The genes implicated in Parkinson's Disease will be inserted into the LAT region of the virus backbone.

**Evaluation of foreseeable effects**

The HSV viruses that will be used are disabled by deletion of ICP4 and VP16 genes. Although wild type HSV-1 is considered pathogenic the disabled vector backbone however will not cause harm to humans in the event of gene transfer.

The foreseeable effects of gene transfer from the genes implicated in Parkinson's Disease cannot be disclosed for business reasons.

It is not considered that expression of the Azami Green or 2A polypeptides would be in any way hazardous.

Whilst it is theoretically possible that the vector could recombine with a wild type HSV virus to generate a replication-competent wild type backbone virus that can express the transgenes, it is highly unlikely (given that the stock virus will only be used in a class II hood) as the two viruses would have to be replicating in the same cell(s) at the same time. However, as a precaution we will not allow any work with the modified viruses if the researcher has symptoms of an active HSV infection. It has also been shown experimentally that these vectors can not reactivate latent wild type virus to produce replication competent recombinant virus at detectable levels in either in vitro culture systems or in vivo in mice (Smith et al., 2003, Gene Therapy and Regulation, Vol 2 (1), p29-47). This suggests any person with a latent HSV infection is not at risk from any recombination events between vector and wild type viruses.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All manipulation of high titre virus will be in class II containment flow-hood. All paper and plastic waste (tubes, tissue culture flasks, dishes, pipettes and tips) as well as small volumes of liquid waste, which will be collected in plates or flasks, will be removed from the hood and placed in autoclavable bags. Any equipment for reuse will be bagged separately. These will be sealed and then double bagged prior to removal from the containment level 2 room. The bags will be directly placed in a leakproof crate which will be autoclaved at 134°C on site prior to the waste bags being taken off site for incineration.
The Safety Committee discussed this project and agreed that it required containment level 2. It was requested that the risk assessment form was modified to include explicit comments that the virus stocks would only be manipulated within the containment level 2 flow hood. Waste GMO inactivation and disposal were discussed and the Safety Committee agreed that the procedures described in the risk assessment form were appropriate. A question was raised as to the best course of action if a human were to become exposed to these virus constructs. It was thought that there was negligible risk unless the virus was inhaled, swallowed or applied to a wound (which will always be covered prior to commencement of any work). In these cases however little could be done except copious washing of the exposed tissues and long term medical follow up. It was emphasised however that it was not thought likely that the expression constructs would be a serious threat unless introduced into large numbers of neurons, which was thought extremely unlikely.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
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<tr>
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<td>L2</td>
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### Project Ref 622/18.1

- **Date Ackn'd**: 31/08/2018
- **CU2 Project Title**: Investigating abnormal protein spreading and aggregation using proteinopathy patient-derived brain samples in a mammalian expression system
- **Class**: Class 2
- **Culture Vol**: < 1 Litre
- **Culture Volume Class**: Consent Granted

- **Date Project Ceased**:  
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

- **Project notified under transitional arrangements**: N
### Project Additional Information

#### Purposes of the contained use

To measure abnormal protein spreading and aggregation using proteinopathy patient-derived brain samples in a cell system, which are transiently overexpressing genes implicated in dementia.

**Experimental outline:**
- Cells will be transfected with expression plasmids encoding disease related mutant genes of interest.
- Proteinopathy patient-derived brain samples will be added to the cell culture medium.
- Abnormal protein spreading and aggregation will be measured by fluorescent based imaging system. Agents that can potentially inhibit the spreading and aggregation of abnormal protein will be evaluated.

#### Recipient or parental organism

Lenti-X 293T cells are clonally selected from HEK293T cells, which are human derived cell lines and expressed SV40 large T antigen.

It has been established in cell culture for many years and is well characterised. Being of human origin it may harbour adventitious agents that could harm humans, however, there are no reports of such occurrences. This cell will not be able to survive outside the laboratory.

#### Host/vector system

The pcDNA3.1(+) vector is Bom/Nic negative, so is unable to undergo plasmid nicking and relaxing neccessary for conjugalional transfer, and is unlikely to be transferred to WT E.coli. In the event of a transfer to WT E.coli these constructs do not contain prokaryotic ribosome binding sites proximal to the inserted genes therefore expression of the encoded proteins will be minimal. Any protein expressed in E.coli would not constitute a threat to human health. The constructs are not thought to be able to alter the pathogenicity of E.coli.

It is considered that ingestion of bacterial culture or DNA could result in take up of small amounts of DNA into cells, which in the case of pcDNA3.1(+) vector would result in expression of the encoded proteins. However, cells at risk such as the gut epithelium are replaced on a regular basis and the vectors involved cannot replicate in mammalian cells in the absence of the SV40 large T antigen.

#### Origin & function

The gene implicated in demetia was inserted into pcDNA3.1(+) based expression vector. It was produced and provided by ThermoFischer scientific.
### Evaluation of foreseeable effects

It is considered that ingestion of a GMM culture or of DNA in sufficient quantity could result in take up of small amounts of DNA into eukaryotic cells however this is unlikely to occur due to laboratory practices and would only involve cells such as the gut epithelium that are replaced on a regular basis so would not result in harm to humans. Sharps will not be used so material is unlikely to be introduced into other tissues. It is not thought that any mutations would alter the risk of harm posed by these constructs. The foreseeable effects of gene transfer of the gene of interest cannot be made public for business reasons. Virus screening of the dementia patient-derived brain materials is not routinely performed. Therefore there is a small risk of these materials containing human virus. While it is highly likely that any virus will be inactivated during the extraction of the test samples, if virus were to survive they may propagate when incubated with the GMO. It is also unlikely but possible that rearrangement may occur to integrate the over expression construct into a human infective virus. Both of these scenarios pose a hazard to human health.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not applicable**

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All manipulations using patient brain materials with GMO cells will be in class II containment flow-hood. All paper and plastic waste (tubes, tissue culture flasks, dishes, pipettes and tips) as well as small volumes of liquid waste, which will be collected in plates or flasks, will be removed from the hood and placed in autoclavable bags. Any equipment for reuse will be bagged separately. These bags will be sealed and then double bagged and placed in a leak proof crate prior to removal from the containment level 2 room. The bags leakproof crate and contents will be autoclaved at 134°C on site prior to the waste bags being taken off site for incineration.

### Is an emergency plan required according to regulation 20?

Y

### If yes, tick to confirm that it is attached to this form

Y

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

---

**Please enter comments on the GM safety committee on the risk assessment**

On 27th June 2018 the Genetic Modification Safety Committee discussed this project and agreed that it required containment at level 2 due to the treatment of a GMO with human brain samples that pose risk to humans and themselves require containment at level 2. In view of the length of time since Eisai last considered a containment level 2 project it was considered important that the procedures in place be checked. In particular the GMO Work Emergency Arrangements to ensure they are still applicable.
### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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- **Animal Units**: L2 L3 L4 L2
- **Large Scale Activities**: L3 L4 L2
- **Human Clinical Applications**: L3 L4

02/03/2022

Page 10255 of 15326
### GM Centre Number: 623

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**Name**

| INCYTE GENOMICS LTD |

**Name 2**

**Department**

**Campus Estate or Research Centre**

| 214 CAMBRIDGE SCIENCE PARK |

**Road Name**

| MILTON ROAD |

**District**

**Town**

| CAMBRIDGE |

**County**

| CAMBRIDGESHIRE |

**Postcode**

| CB4 0WA |

**Country**

| ENGLAND |

**Tel Number**

| 01223 424 877 |

**Fax Number**

| 01223 424 855 |

**E-mail**

**HSE Division**

| EAST AND SOUTH EAST |

**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022 |
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity

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</thead>
<tbody>
<tr>
<td>Other(s)</td>
<td></td>
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</tbody>
</table>

The maximum culture would not exceed 500 mls. Waste is deactivated by mixing with Chloros or other sterilising agent, this is accepted standard laboratory practice. Disposals are recorded in a log book which is monitored by the Biological Safety Officer.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 624

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#### Name 2

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#### Tel Number

| 029 2061 5888 |

#### Fax Number

| 029 2052 2694 |

#### HSE Division

| WALES AND SOUTH WEST |

#### Comments

Transferred and Merged with GM130 on 15/09/2005.

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Animals
- Transgenic Birds
- Transgenic Fish
- Microbiology Research
- Gene Therapy

Page 10260 of 15326

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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Project Additional Information
Purposes of the contained use

Recombinant vaccinia virus will be used to infect target cells in vitro, normally human B-lymphocytes derived from patients with cancer of the cervix. The recombinant (TA-HPV) is derived from the Wyeth strain of vaccinia and expresses human papilloma virus 16 and 18 fusion protein E6/E7. The virus is replication-competent and is not attenuated. Infected B cells will be used as in vitro targets to monitor the success of a vaccination schedule carried out using dendritic cells loaded with E6/E7 in vivo. The purpose of the vaccination is to induce an immune response to cancer cells expressing E6/E7 in vivo. The recombinant will be obtained from the University of Wales College of Medicine and no further genetic modification of the virus will be carried out at Velindre. Patients will not be exposed to vaccinia.

Recipient or parental organism

The recombinant (vaccinia TA-HPV) expresses an HPV16 and 18 E6/E7 fusion protein which is produced from the two separate sequences by a modification of the E6 termination codon. It is known that expression of E6 and E7 in mammalian cells is able to induce both immortalisation and a transformed phenotype so that infection of an individual with TA-HPV must be considered to increase the risk of carcinogenicity. However, vaccinia is a lytic virus, so that all infected cells die. In addition, the E7 sequence has been modified to abolish its ability to bind Rb and hence to cause immortalisation. Both these factors are considered to reduce the risk of carcinogenicity. Vaccinia itself is a pathogen and is in ACDP Hazard Group 2. The recombinant is considered to pose an elevated risk compared to wild-type Wyth strain vaccinia.

Host/vector system

Vector: Wyeth strain vaccinia virus (TA-HPV) expressing human papilloma virus fusion protein E6/E7. The sequence has been modified to reduce Rb binding and hence the risk of immortalisation of the host cell. The virus is replication competent, unattenuated and lytic.

Host: Mammalian cells in vitro. These will normally be human B-lymphocytes derived from patients with cancer of the uterine cervix.

Origin & function

The vaccinia recombinant TA-HPV has been constructed elsewhere and will be obtained from the University of Wales College of Medicine, Cardiff. Mammalian cells infected in vitro with TA-HPV will express the human papilloma virus 16 and 18 fusion protein E6/E7. These cells will then be used as targets for T-cells derived from vaccinated patients before vaccinia-induced lysis occurs.

Evaluation of foreseeable effects

Pathogenicity of vaccinia: Vaccinia is in ACDP Hazard Group 2. The Wyeth strain has been widely used in vaccination programmes and its effects are well characterised. Infection can lead to a vesicular lesion usually accompanied by a generalised infection. The lesion generally heals in about 10 days. In a recent case involving the accidental infection of a laboratory worker, lesions appeared on the finger and on a recently-pierced eyebrow. However, it is considered unlikely that infection will lead to a serious long-term sequelae. Skin penetration or mucosal contact is required for infection to occur.

Pathogenicity of TA-HPV recombinant: The recombinant expresses the human papilloma virus 16 and 18 fusion protein E6/E7. These proteins are known to have immortalisation and transforming potential; however the E7 sequence has been modified to reduce binding to Rb which will minimise the probability of immortalisation. Also, the fusion of E6 and E7 reduces the risk of transformation. Nevertheless the E6/E7 sequence must be considered to be potentially oncogenic so that pathogenicity of the recombinant is greater than that of the wild-type. However, the probability of carcinogenesis in vivo is reduced by cell lysis caused by vaccinia.

Hazard to the environment: Wyeth strain vaccinia has been widely used in wild animal vaccination programmes involving the release of genetically modified virus into the environment. This work has demonstrated that there is a low risk of environmental damage due to vaccinia. It is unlikely that this risk will be significantly greater in TA-HPV than in the wild type.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Small-scale solid and liquid waste will be generated (less than 100gm). All waste will be inactivated by autoclaving, and chemical disinfectants will not be permitted unless they are compatible with subsequent autoclaving.

Autoclaving will be carried out using a sterilizing criterion of 126°C held for 10 minutes. These conditions within the load are verified annually using positioned thermocouples. Autoclaved waste will be sealed in a yellow bag for incineration off-site as clinical waste.

**Project Containment**

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<tr>
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<th>Growth Rooms</th>
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**Project Ref** 624/02.1

**Date Ackn’ed** 18/06/2002

**CU2 Project Title**

CANCER GENE THERAPY USING THE CONVERSION OF ETHANOL TO ACETALDEHYDE MEDIATED BY ADENOVIRUS DELIVERED HUMAN ENZYME ALCOHOL DEHYDROGENASE (ADH).

**Class** Class 2

**CultureVolClass2** < 1 litre

**Consent Granted** not applicable
**Project Additional Information**

**Purposes of the contained use**
To examine the ability of cells over expressing the ADH gene to be damaged by excess acetaldehyde production following exposure to ethanol.

**Recipient or parental organism**
The adenovirus employed in these experiments is adenovirus serotype 5 (ad5). This virus is associated with childhood respiratory infections and most adults will have antibodies to the wild type virus. The virus has had the genes for the E1 gene (nucleotides 1-3533) completely removed. This gene is an absolute requirement for replication and so its removal makes it unable to replicate and so incapable of making infectious viral particles. The removal of the E1 gene has the effect of minimising the pathogenicity of the virus to human contacts. This removal places the virus in the ACGM Hazard group 1 'unlikely to cause human disease'.
The gene of interest is inserted into the site of the disabling mutation. This should minimise the theoretical possibility of recombination with wild type adenovirus.

**Host/vector system**
Cancer cell lines including:
- HBL 100
- Hela
- Jurkat
- Daudi

**Origin & function**
- *Adenovirus serotype 5*
The virus is made replication deficient due to deletion of E1 locus.
(ref He et al; PNAS 95; 2509-2514).

- *Human alcohol dehydrogenase beta 2 (ADH b2) enzyme cDNA.* Obtained from human liver cDNA (ref Ikuta et al PNAS 83; 634-638)

**Evaluation of foreseeable effects**
The risk to human health is low as; The virus has very low infectivity for human cells outside of the in vitro experimental setting. The virus is non-replicating. In infected cells the gene is expressed for 3-4 days only.
The gene encoded is a normal human gene expressed in many cells. The virus and the cDNA are non-oncogenic, non-toxic and harmless to human health. The modification to the virus will not expand the host range of the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All the cells, containers, pipettes etc used in the project using the adenovirus will be disposed of via the on site autoclave. This is validated annually and has an assumed 100% kill. Laboratory surfaces/hoods etc will be disinfected with sodium hypochlorite solution.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The committee are happy that this project poses a negligible risk to both human health and the environment. Purely for GM purposes it would be allocated to containment level 1, but as the work will involve primary human cells the project will need to be performed a category 2.

Project Containment

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| Date at Which Additional Info Submitted | 02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Other (please specify)

Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 625/98.2

Date Ackn'd 11/09/1998

CU2 Project Title HIV PSEUDOTYPE ASSAY

Class 2

CultureVolClass2

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Significant Change ID 331/05.1

Date of Significant Change 09/02/2005

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Non-GMM Consent Granted  yes

Project notified under transitional arrangements  Y

Withdrawn  N

Tick if notifying a connected programme of work  N

**Historical Significant Changes**

**Historical Date of Additional Info**

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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| Comments | |

| Date at Which Additional Info Submitted | 19/08/2003 |

02/03/2022
Premises Addresses

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<td>KT19 9AP</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)  
Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
The maximum culture volume is 8 litres, which is later destroyed by autoclaving the isolated cells and equipment used in an industrial autoclave. Validation is carried out by post-autoclaving plate culture and cell counts made. It is monitored by sequential dilution of used bacterium and controlled culture in the same format. No survival has been detected to date. All surfaces are treated with Chloros.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
EUROFINS PHARMA BIOANALYSIS SERVICES UK LIMITED

Name
EUROFINS PHARMA BIOANALYSIS SERVICES UK LIMITED

Name 2

Department

Campus Estate or Research Centre

Building
6-7 TECHNO PARK

Road Name
NEWMARKET ROAD

District

Town
CAMBRIDGE

County
CAMBRIDGESHIRE

Postcode
CB5 8PB

Country
ENGLAND

Tel Number
01223 508191

Fax Number
01223 508198

E-mail

HSE Division
EAST AND SOUTH EAST

Comments

notified 1/08/08 that the GMSC committee representation has changed - letter in file.

Date at Which Additional Info Submitted

15/05/2001

13/07/2001
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
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<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Bacterial cultures derived from our activities is inactivated in chlorine solution using 'HazTab' tablets at > 2500 ppm/litre overnight and disposed. Solid bacterial waste is autoclaved before disposed of.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 628

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Name

**EDWARD JENNER INSTITUTE**

Name 2

**INSTITUTE FOR ANIMAL HEALTH**

Campus Estate or Research Centre

Road Name

District

**COMPTON**

Town

**NEWBURY**

County

**BERKSHIRE**

Postcode

**RG20 7NN**

Country

**ENGLAND**

Tel Number

01635 577900

Fax Number

01635 577901

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.

Project Ref 628/01.1

Date Ackn'd 15/02/2001

Date Project Ceased 20/09/2006

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

CU2 Project Title PRODUCTION OF REPLICATION DEFECTIVE RECOMBINANT RETROVIRUSES AND THEIR USE FOR EXPRESSION OF GENES IN EUKARYOTIC CELLS

Class 2

Consent Granted not applicable

Non-GMM

Class CultureVolClass2 CultureVolumeClass3-4

Historical Date of Additional Info TRANSFERRED TO GM 97 - 22/9/06.

Historical Significant Changes

Significant Change ID

Date of Significant Change
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
**Project Additional Information**

- **Purposes of the contained use**

- **Recipient or parental organism**

- **Host/vector system**

- **Origin & function**

- **Evaluation of foreseeable effects**

- **Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 628/01.3

Date Ack'n'd   26/11/2001

Date Project Ceased 20/09/2006

CU2 Project Title

IMMORTALISATION OF MURINE CHONDROCYTES USING SV40 LARGE T-ANTIGEN-ENCODING RETROVIRUS.

Class CultureVol Class2 CultureVolume Class3-4

Class 2 < 1 litre

Non-GMM Consent Granted not applicable

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Withdrawn N
### Project Additional Information

#### Purposes of the contained use

A murine chondrocyte cell line would provide a source of joint specific antigens including the model antigen Flu nucleoprotein (NP) that is expressed in the chondrocytes of CIINP14 mice. As reactive arthritis is likely to result from activation of MHC class 1 and class II-restricted T-cell responses (as well as possibly antibody responses), an immunogen that includes multiple relevant antigens may enhance the development of joint-specific disease. Immunisation with NP +ve chondrocytes would provide a known antigen, the response to which could be followed with tetramers etc., as well as uncharacterised antigens that may be more potent targets in the joint.

#### Recipient or parental organism

Murine chondrocytes expressing either wild-type or a temperature sensitive mutant of SV40 Large T-antigen. As chondrocytes will not produce recombinant virus or survive outside tissue culture the foreseeable effect on human health/safety or the environment is negligible.

2 cells containing pZipNeoSV(X)1 and producing helper-free recombinant retrovirus expressing either wild-type or a temperature sensitive mutant of the SV40 Large T-antigen. The effects described above apply here also with the exception that 2 cells will produce recombinant retrovirus. These viruses however, will only infect murine cells so it is still unlikely that Large T-antigen will gain access to human cells. However, in the unlikely event that SV40 Large T-antigen gains access to human cells it has the capacity to immortalise them.

#### Host/vector system

2 cells to be used in this study are transfected with pZipNeo SV(X) vectors. These contain the sequence required for viral packaging deleted from PMOV- but replace the viral gag, pol and env genes with an inserted transgene (see section C for description of cloning vector). These transfected cells are capable of producing helper-free, recombinant retrovirus (Cepko et al., 1984. Cell, 37:1053-1062). In this study, pZipNeo SV(X)1 vectors incorporate either Large T-antigen or a temperature-sensitive version of this gene which is derived from Simian Virus (SV)40.

#### Origin & function

SV40 Large T-antigen (from the DNA tumour virus simian virus 40), tsTAg (temperature sensitive mutant of the SV40 Large T-antigen gene).

The SV40 large T-antigen is found in early region of the virus together with the small T-antigen which together are sufficient for transformation of cell lines. Studies have shown that the large T-antigen alone is sufficient for establishment of primary cells but that these cells do not display a transformed phenotype hence the intention in this study to transform chondrocytes.

#### Evaluation of foreseeable effects

As mentioned immortalised chondrocytes and 2 producer cells will not survive outside tissue culture. They are unlikely to encounter murine hosts other than in experimental conditions in the case of chondrocytes.
Recombinant retrovirus will not infect human cells and is helper-free therefore can not replicate in murine hosts. However infection of mice could theoretically result in transformation of cells although this is unlikely as SV40 Large T-antigen transduced cells are non-tumourigenic in murine hosts. The cells will be handled as category 2 agents for all in vitro experiments, limiting the potential of exposure to the environment to a negligible level.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solutions that have been in contact with retrovirus, 2 cells or transformed chondrocytes will be treated with virkon disinfectant. All exposed materials will be autoclaved before disposal.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The committee wish to bring to the proposer's attention paragraph 20 in AnnexIII "Guidance on commonly used viral vectors" to demonstrate the absence of PCRs in vector stocks from the HSE's Compendium of Guidance. Also proposer's attention is requested to observe precautions for handling oncogenic DNA (mandatory wearing of gloves, avoidance of sharps).

Project Containment

<table>
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<tr>
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<th>Growth Rooms</th>
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Animal Units

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Project Ref 628/02.1

Date Ackn'd 02/03/2022  
CU2 Project Title  
Class  
CultureVolClass2  
CultureVolumeClass3-4
In order to aid our studies of the human disease reactive arthritis which may be induced as a result of salmonella infection, we need to study the interaction of salmonella infected cells with antigen specific T cells. Model antigens, to which we have specific T cells for in the laboratory, will be expressed in salmonella. T cell responses to salmonella infected cells can then be assessed.

Recipient or parental organism

Strains of Salmonella typhimurium, some of which are attenuated.

Host/vector system

Non-mobilisable or mobilisation defective plasmid vector systems, using prokaryotic promoter system for expression of recombinant antigens.

Origin & function

In vitro and in vivo experimental use at containment level 2, for induction of immune responses and models of human disease.

Evaluation of foreseeable effects

Transformation of Salmonella with recombinant DNA plasmid vectors is not expected to increase virulence or infectivity of Salmonella. The organisms will be handled as category 2 organisms for all in vitro and in vivo experiments, limiting the potential of exposure to the environment to a negligible level.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solutions that have been in contact with Salmonella will be treated with bactericidal disinfectant. All infected materials will be autoclaved before disposal.

Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 628/03.1

Date Ackn'd

28/10/2003

CU2 Project Title

USE OF RECOMBINANT ADENOVIRUSES TO STUDY THE EFFECT OF THE S AND N PROTEINS OF THE SARS VIRUS IN AN IN VIVO MOUSE MODEL.

Date Project Ceased

20/09/2006

Class

Class 2

Consent Granted

not applicable

Historical Significant Changes

TRANSFERRED TO GM 97 - 22/9/06
Project Additional Information

Purposes of the contained use

Recombinant adenovirus vectors encoding the S and N genes of the SARS virus under the control of the CMV promoter will be used to study the effect of these proteins in vivo. The recombinant adenovirus vectors will be administered to mice and tissues from treated mice will then be removed to examine the host immune response. In some experiments mice treated with one of these vectors will then be infected with virus or bacteria, for example influenza virus or salmonella. The aim is to be able to examine the effect of the S and N genes from SARS on the host immune response.

Recipient or parental organism

The recombinant adenoviruses will be obtained from another investigator Dr Tripp, in the Centres for Disease Control and Prevention in Atlanta, GA. The recombinants were made in his laboratory using the commercial AdEasy vector system from Invitrogen.

The SARS proteins S (spike) and N (nucleocapsid) are from the Urbani strain of SARS isolated by the CDC in Hong Kong. This strain was sequenced by the CDC and the S and N genes were then inserted into the adenovirus vectors in the CDC in Atlanta.

It is presently unknown whether the S or N proteins have any transforming potential or any harmful sequences. None have been reported.

Host/vector system

Adenovirus vector system

The recombinants are derived from the adenovirus serotype 5 genome and have deletions in the E1 and E3 regions of the virus genome. The E1 deletion prevents the recombinant virus from replicating and these viruses are therefore replication incompetent. Virus stocks can be prepared by growing in 293 cells where virus replication is made possible due to the expression in that cell line of an E1 helper function in trans. Viral stocks will be titred by plaque assay on 293 cells. The E3 region which is not essential for virus growth is also deleted.

Adenoviruses are associated with a number of mild disorders. The pathology results primarily from inflammation and loss of infected epithelial cells. Serotype 5 which will be used here commonly causes upper respiratory infections in the elderly and in young children. Adenoviruses are Biosafety Level II pathogens.

The adenovirus vectors do not express any harmful agents. The E3 region which encodes proteins involved in modulating the immune response of infected cells has been deleted. The recombinant adenoviruses are replication deficient due to a large deletion in the E1 gene.
Origin & function

The recombinant adenoviruses will be obtained from another investigator Dr Tripp, in the Centres for Disease Control and Prevention in Atlanta, GA. The recombinants were made in his laboratory using the commercial AdEasy vector system from Invitrogen.

The SARS proteins S and N are from the Urbani strain of SARS isolated by the CDC in Hong Kong. This strain was sequenced by the CDC and the S and N genes were then inserted into the adenovirus vectors in the CDC in Atlanta.

These constructs will be used to examine the effects of the S and N proteins of SARS on the host immune response. The vectors will be administered to mice and at certain time-points thereafter the mice will be sacrificed and tissues will be extracted.

Evaluation of foreseeable effects

The possibility that replication competence could be restored by rescue of the E1 deletion from the helper cell line or from wild-type adenovirus cannot be excluded. However even if this were the case, it is unlikely that the virus would be more pathogenic than the wild type adenovirus.

The inserted genes would not be expected to render the recombinant viruses able to replicate or to affect the tropism of the virus. Variations of S protein among strains of coronaviruses are responsible for host range and tissue tropism. However, as adenoviruses are not enveloped it is unlikely that the S protein of SARS will affect the tropism of the recombinant adenoviruses. It is unknown how the expression of the N and S genes of SARS will affect the host cells, as no work has been carried out examining this. So far there are no reports that either S or N protein is associated with toxicity or oncogenesis. Also neither is known to be closely homologous to human proteins against which autoimmune responses would be likely to be induced.

The possibility that the inserted sequences may be transferred to other related organisms is remote. The only potential risk may occur if the S or N genes of SARS were transferred to another corona virus. In that case potential problems may occur but the resulting virulence or pathogenicity if such an event occurs is not known.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the animal, culture and tissues culture waste will be disposed of by incineration according to the normal rules for Biosafety level II biological waste disposal. Liquid waste will be added to Virkon (final concentration 1%) and left for 24 hours. No microorganisms will be viable afterwards. Solid waste will be put in a metal box, sealed with autoclave tape, autoclaved at 134 degrees C for 30 minutes, before being sent to an on-site clinical waste incinerator. The degree of kill is 100%.

Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
### Project Containment

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<th>Human Clinical Applications</th>
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### Project Ref 628/03.2

**Date Ackn'd:** 07/11/2003  
**CU2 Project Title:** IN VITRO ANALYSIS OF THE INTERACTION OF HEPATITIS C VIRUS PSEUDOPARTICLES (HCV PP) WITH CELL LINES AND HUMAN PERIPHERAL BLOOD MONOCULEAR CELLS (PBMC) SUBSETS.  
**Class:** Class 2  
**Culture Volume:** ≤ 1 litre  
**Non-GMM Consent Granted:** not applicable  

**Project notified under transitional arrangements:** N  
**Withdrawn:** N  

**Historical Significant Changes:** TRANSFERRED TO GM 97 - 22/9/06  
**Historical Date of Additional Info:**  

**Significant Change ID:** 628/03.2a  
**Date of Significant Change:** 25/07/2007

### Project Additional Information

**Purposes of the contain use:**

In order to aid our previous studies of the interaction of the hepatitis C (HCV) E2 glycoprotein with different PBMC subsets and the possible immunomodulatory consequences of this interaction, we would like to carry out studies the HCV pp, which express heterodimers of E1 and E2 on their surface. The proposed work would include in vitro analysis of interaction of HCVpp with cell lines and PBMC subsets and investigation of whether HCVpp are able to modulate the activation/functions of different immune system cell types (derived from human peripheral blood), using a variety of in vitro immunological assays.

**Recipient or parental organism:**
Murine leukemia virus (MLV), which is a murine retrovirus classified as a category 1 pathogen.

**Host/vector system**

The vector packaged into the pseudotyped MLV particles encodes the marker protein green fluorescent protein (GFP). This is not thought to have any harmful properties. The particles will be pseudotyped with the HCV E1 and E2 proteins. These are not thought to be toxic or oncogenic, but there is some evidence to suggest that the E2 protein may be able to modulate the activation/functions of human lymphocyte subsets. However, it is very unlikely that accidental exposure to a small quantity of HCV pp (which are not able to replicate) would have any harmful immunomodulatory effects.

**Origin & function**

The HCVpp will be provided by our collaborators, the Institute Pasteur in Lille, France; they will not be produced here. The method by which the HCVpp are generated is described in Bartosch et al, *Journal of Experimental Medicine, 197:633-642 (2003)*.

In vitro experimental use at containment level 2.

**Evaluation of foreseeable effects**

Pseudotyping of the MLV-based particles with the HCV E1 and E2 proteins results in the particles having a tropism different to that of ecotropic MLV. HCVpp have been shown to bind to and enter human cells expressing what are thought to be HCV receptors (CD81 and other proteins) [Bartosch et al, *Journal of Experimental Medicine, 197:633-642, 2003*].

This alteration in tropism increases the potential for disease to be induced in humans, although the risk of this still remains very low. Although able to infect human cells, the pseudoparticles would not replicate therein, and expression of GFP within the transduced cells would be unlikely to have deleterious effects. However, it is possible that integration of the MLV vector could lead to modulation of host cell gene expression, and potentially to transformation of the cell.

It is very unlikely that sequences from the GFP-expressing MLV vector within the HCVpp would be transferred to another micro-organism during the course of the planned in vitro experimental work; and even should this occur, it is unlikely to be hazardous, as this vector only encodes GFP.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All equipment and waste that has been in contact with HCVpp will be disinfected using procedures approved for destroying the infectivity of lipid-enveloped viruses.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y
The committee recommended that each batch of HCVpp should be tested for infectivity on a murine cell line, and with this provisionally approved the work to be done at containment level 2.

### Project Containment

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#### Project Ref 628/04.1

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<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>22/01/2004</td>
<td>USE OF A SERIES OF DERIVATIVE INFLUENZA VIRUSES TO INVESTIGATE THE EFFECTS ON VIRULENCE IN MICE.</td>
<td>20/09/2006</td>
<td>Class 2</td>
<td>≤ 1 litre</td>
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- Withdrawn: N
- Tick if notifying a connected programme of work: N
- Historical Significant Changes: transferred to gm 97 - 22/9/06

### Project Additional Information

**Purposes of the contained use**

A recombinant influenza virus of subtype A/Victoria/3/75 (H3N2) has been generated by reverse genetics at the University of Reading. In addition, a series of derivative viruses based on A/Victoria/3/75 have been generated in which the open reading frame of the protein PB1-F2 has been deleted, truncated or extended. PB1-F2 is believed to induce apoptosis in immune cells (Chen et al., 2001). We aim to carry out apoptosis studies and mouse model studies to determine whether alterations in PB1-affect virus virulence.
A recombinant influenza virus of subtype B/Beijing/87 has also been generated at the University of Reading, as well as an isogenic virus in which the NB protein has been deleted. The NB protein is believed to function as an ion channel and, although dispensable in vitro, is believed to promote efficient viral growth in mice (Hatta and Kawaoka, 2003). We would like to confirm and extend these observations.

**Recipient or parental organism**

Influenza viruses infect humans and avians and cause a respiratory infection. Transmission is by respiratory infection via aerosols. The recombinant influenza viruses are unlikely to cause harm greater than their parental strains, which are themselves laboratory passaged and thus attenuated versions of naturally occurring influenza viruses, classified as class 2 pathogens. Both A/Victoria/3/75 and B/Beijing/87 have been used extensively in laboratories worldwide with no history of infection of workers or adverse effects as detailed in the following publications and citations therein:


In the event that the A/Victoria/3/75 virus does not produce obvious symptoms in mice, a virus with A/Panama/99 antigens and the same internal genetic backbone will be used. A/Panama/99 surface antigens were used to generate large quantities of vaccines throughout the world in recent years and no adverse affect are associated.

**Host/vector system**

Not applicable.

**Origin & function**

A recombinant influenza virus of subtype A/Victoria/3/75 (H3N2) has been generated by reverse genetics at the University of Reading. In addition, a series of derivative viruses based on A/Victoria/3/75 have been generated in which the open reading frame of the protein PB1-F2 has been deleted, truncated or extended. PB1-F2 is believed to induce apoptosis in immune cells (Chen et al., 2001). We aim to carry out apoptosis studies and mouse model studies to determine whether alterations in PB1-affect virus virulence.

A recombinant influenza virus of subtype B/Beijing/87 has also been generated at the University of Reading, as well as an isogenic virus in which the NB protein has been deleted. The NB protein is believed to function as an ion channel and, although dispensable in vitro, is believed to promote efficient viral growth in mice (Hatta and Kawaoka, 2003). We would like to confirm and extend these observations.

**Evaluation of foreseeable effects**

All mutations in influenza viruses to be used in this study will be attenuating for replication and therefore the hazards will be reduced. This includes the virus strain in which the PB1-F2 gene will be extended by 3 amino acids in order to retain the length of the wild-type gene. There is no expected alteration in host range since neither of these genes is associated with that trait. It is possible that the types of cells affected following infection with the PB1-F2 deletant will be reduced rather than extended.

Since influenza virus has a segmented genome, there is a possibility that reassortant viruses containing genes from the mutants might be generated within a laboratory worker who was coinfected with a wild type strain. We consider this unlikely, but advise all workers to refrain from experiments if influenza infection is suspected.

If reassortment did occur, the PB1-F2 deletion introduced into A/Victoria/75 is not associated with virulence and is likely to be an attenuating mutation. The virus harbouring deletion of NB from B/Beijing/87 is reported to be highly attenuated and cannot replicate efficiently in vivo (Hatta and Kawaoka). Therefore regardless of the genetic background of the virus harbouring these changes, we expect them to be attenuating.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the animal, culture and tissues culture waste will be disposed of by incineration according to the normal rules for Biosafety Level II biological waste disposal. Liquid waste will be added to Virkon (final concentration 1%), and left for 24 hours. No microorganisms will be viable afterwards. Solid waste will be put in a metal box, sealed with autoclave tape, autoclaved at 134 degrees C for 30 minutes, before being sent to an on-site clinical waste incinerator. The degree of kill is 100%.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The local GMSC are happy with this risk assessment (Ref 2003/018) provided the requirements in the associated risk assessment for influenza A and B viruses are adhered to.

### Project Containment

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<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L2 L3 L4</td>
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### Animal Units

| L2 Yes | L3 L4 L2 |

### Large Scale Activities

| L2 Yes | L3 L4 L2 |

### Human Clinical Applications

| L2 Yes | L3 L4 L2 |

### Project Ref 628/04.2

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<th>Culture Volume Class 3-4</th>
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<tr>
<td>22/01/2004</td>
<td>USE OF NOVEL REVERSE GENETICS-GENERATED INFLUENZA VIRUS (RG-X31-SWINE PB1) TO INVESTIGATE THE ROLE OF VIRUS-INDUCED APOPTOSIS</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td></td>
<td>not applicable</td>
<td></td>
</tr>
<tr>
<td>Date Project Ceased</td>
<td>20/09/2006</td>
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</table>

Project notified under transitional arrangements  N
Purposes of the contained use

Influenza viruses induce apoptosis in cultured cells and in vivo. We have recently shown that influenza virus detrimentally affects early lymphoid progenitor cells by inducing apoptosis mediated by TNFalpha/LTalpha. An alternative reading frame of the PB1 gene called PB1-F2 of the influenza virus has been implicated in inducing apoptosis in vitro. We want to determine whether PB1-F2 is responsible for the apoptosis that occurs in the bone marrow (or other tissues) following infection of mice with influenza virus. We therefore wish to use a novel influenza virus (RG-X31-swine PB1) that has been generated by reverse genetics (RG) to examine these questions. The PB1-F2 open reading frame (ORF) is present in nearly all human influenza A strains but is absent from swine influenza isolates. Therefore by replacing the PB1 of the wild-type x 31 (A/H3N2) virus which contains the PB1-F2 ORF with the PB1 from a swine influenza virus (A/swine/NorthCarolina/44173/00) which does not, we will be able to address these questions. Mice will be infected with the wild type/recombinant virus and sacrificed at certain time-points to assess the degree of cell death in various organs and any other differences in pathogenicity caused by the recombinant virus.

The RG influenza virus RG-x31-swine PB1 will be obtained from St Jude Children's Research Hospital, Memphis TN, USA. The viruses are being made in the laboratory for our use.

Recipient or parental organism

Influenza viruses infect humans and avians and cause a respiratory infection. Transmission is by respiratory infection via aerosols. The strain of influenza virus is a reassortant which has the surface proteins of A/Aichi/68 and the internal genes of A/PR/8/34. It is a laboratory strain which is commonly used in immunological studies and is BioSafety Level 2 pathogen. The recombinant influenza virus is unlikely to cause harm greater than the parental strain, which is itself laboratory passaged and thus an attenuated version of a naturally occurring influenza virus.

Origin & function

We have recently shown that influenza virus detrimentally affects early lymphoid progenitor cells by inducing apoptosis mediated by TNFalpha/LTalpha. An alternative reading frame of the PB1 gene called PB1-F2 of the influenza virus has been implicated in inducing apoptosis in vitro. We want to determine whether PB1-F2 is responsible for the apoptosis that occurs in the bone marrow (or other tissues) following infection of mice with influenza virus. We therefore wish to use a novel influenza virus (RG-X31-swine PB1) that has been generated by reverse genetics (RG) to examine these questions. The PB1-F2 open reading frame (ORF) is present in nearly all human influenza A strains but is absent from swine influenza isolates. Therefore by replacing the PB1 of the wild-type x 31 (A/H3N2) virus which contains the PB1-F2 ORF with the PB1 from a swine influenza virus (A/swine/NorthCarolina/44173/00) which does not, we will be able to address the degree of cell death in various organs and any other differences in pathogenicity caused by the recombinant virus.

Evaluation of foreseeable effects

No hazards should arise from the alteration of wild-type x 31 to RG-x31-swine PB1. Neither virus growth nor tropism should be affected. The surface proteins HA and NA...
have not been altered. Reverse genetics enables a recombinant virus to be produced in a much safer way than previously because one has complete control of the genes being transfected to produce the recombinant virus. Therefore there is no possibility that other influenza virus genes can be introduced. It is expected that the inclusion of the swine PB1 gene would either not change the pathogenicity/virulence of x31 or make it less so.

Little is known about the new PB1-F2 protein as it has only recently been identified. It is not essential for viral replication in vitro. The evidence to date suggests its involved in modulating the host immune response to influenza A by caused death of immune cells. A synthetic version of PB1-F2 induces apoptosis in vitro and targeted mutations which interfere with expression of PB1-F2 induce less apoptosis than those that express PB1-F2.

There is no evidence that the PB1-F2 gene causes toxicity or has oncogenic properties.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the animal, culture and tissues culture waste will be disposed of by incineration according to the normal rules for Biosafety Level II biological waste disposal. Liquid waste will be added to Virkon (final concentration 1%), and left for 24 hours. No microorganisms will be viable afterwards. Solid waste will be put in a metal box, sealed with autoclave tape, autoclaved at 134 degrees C for 30 minutes, before being sent to an on-site clinical waste incinerator. The degree of kill is 100%.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The local GMSC are happy with this risk assessment (Ref 2003/017) provided the requirements in the associated risk assessment for influenza A and B viruses are adhered to.

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02/03/2022
USE OF A NOVEL REVERSE GENETICS-GENERATED INFLUENZA VIRUS (RG-X31-156NS1) TO INVESTIGATE THE ROLE OF VIRUS-INDUCED APOPTOSIS

Influenza viruses induce apoptosis in cultured cells and in vivo. We have recently shown that influenza virus detrimentally affects early lymphoid progenitor cells by inducing apoptosis mediated by TNFalpha/LTalpha. The NS1 gene of influenza virus has been implicated in inducing apoptosis in vitro. We want to determine whether the NS1 gene is responsible for the apoptosis that occurs in the bone marrow (or other tissues) following infection of mice with influenza virus. We therefore wish to use a novel influenza virus that has been generated by reverse genetics (RG) to examine these questions. The NS1 gene in wild-type x31 will be replaced by the NS1 from the H5N1/97 virus, in order to assess whether a greater degree of cell death in bone marrow, thymus and other organs occurs as a result. Mice will be infected with the wild type/recombinant virus and sacrificed at certain time-points to assess the degree of cell death in various organs and any other differences in pathogenicity caused by the viruses.

The strain of influenza virus that will be the recipient of the foreign NS1 gene is x31 (H3N2). This strain of influenza virus is a reassortant which has the surface proteins of A/Aichi/68 and the internal genes of A/PR/8/34. It is a laboratory strain which is commonly used in immunological studies and is a BioSafety Level 2 pathogen. Research to date suggests NS1 is a virulence factor which plays a major role in inhibiting the IFN-mediated antiviral responses of the host. NS1 is a non-structural protein of influenza virus. It is an RNA binding protein which has been implicated in several regulatory functions during the influenza virus replication cycle:

1. It inhibits host mRNA polyadenylation.
2. It inhibits the nuclear export of mRNAs by binding to their poly (A) tail.
3. It inhibits pre-mRNA splicing.
4. It prevents IFN-mediated antiviral responses by binding to ds RNA.
5. It has been suggested to play a role in regulation of viral RNApolymerase activity.
6. It is able to stimulate the translation of specific viral mRNAs.

There is no evidence that the NS1 gene causes toxicity or has oncogenic properties.
Recombinant virus will be produced that replaces the NS gene or x31 with the NS gene of H5N1/97. Background: The H5N1/97 viruses transmitted to humans in 1997 and were highly virulent. Reactive hemophagocytic syndrome with elevated concentrations of proinflammatory cytokines were reported. Lethal H5N1 viruses (unlike other human, avian and swine viruses) are resistant to the antiviral effects of IFNs and TNFalpha. This resistance is associated with NS1 and is thought to be the mechanism of the high virulence of H5N1 viruses in humans. RE enables a recombinant influenza virus to be produced in a much safer way than previously (reassortment) because one has complete control of the genes being transfected to produce the recombinant virus. Therefore there is no possibility that the HA or other genes from the H5N1 can be included in the recombinant virus. The tropism or host range of RG-x31-156NS1 should not therefore be different from wild type x31. It would be expected that the inclusion of the H5N1, NS1 gene into x31 would make the resulting RG-x31-156NS1 virus more pathogenic (perhaps greater loss of body weight, more prolonged virus shedding) as a result of increased virus replication due to the resistance of the NS1 to aniviral cytokines.

Host/vector system

Not applicable.

Origin & function

The RG influenza virus will be obtained from Dr Richard Webby, St Jude Children’s Research Hospital, Memphis TN, USA. The viruses are being made in his laboratory for our use. Influenza viruses induce apoptosis in cultured cells and in vivo. We have recently shown that influenza virus detrimentally affects early lymphoid progenitor cells by inducing apoptosis mediated by TNFalpha/TLalpha. The NS1 gene of influenza virus has been implicated in inducing apoptosis in vitro. We want to determine whether the NS1 gene is responsible for the apoptosis that occurs in the bone marrow (or other tissues) following infection of mice with influenza virus. We therefore wish to use a novel influenza virus that has been generated by reverse genetics (RG) to examine these questions. The NS1 gene in wild-type x31 will be replaced by the NS1 from the H5N1/97 virus, in order to assess whether a greater degree of cell death in bone marrow, thymus and other organs occurs as a result. Mice will be infected with the wild type/recombinant virus, in order to assess whether a greater degree of cell death in bone marrow, thymus and other organs occurs as a result. Mice will be infected with the wild type/recombinant virus, in order to assess whether a greater degree of cell death in bone marrow, thymus and other organs occurs as a result. Mice will be infected with the wild type/recombinant virus, in order to assess whether a greater degree of cell death in bone marrow, thymus and other organs occurs as a result.

Evaluation of foreseeable effects

The possibility that the inserted sequences may be transferred to other influenza viruses is remote. The only potential risk may occur if the RG-x31-156NS1 virus was grown in eggs or tissue culture at the same time as other influenza viruses so that reassortment occurs. The possibility of this is remote. In the event of exposure the virus may cause infection in humans. One may expect the infection to be more virulent than wild type x31, due to the expected increase in resistance to anti viral cytokines. In te event that any person is exposed to the virus a prearranged protocol will be followed (see attached protocol called The Edward Jenner Institute for Vaccine Research: Protocol for staff working with influenza A viruses shown to have a higher risk of transmission to humans). All work with the recombinant influenza RG-x31-156S will be carried out in a Biosafety Level 3 facility. (An almost identical virus has recently been made in Dr Webby's department in St.Jude CRH but on the backbone of A/PR8/34 which is more virulent than x31. The work is carried out in Biosafety Level 3 facilities, Nat.Med. 8:950-954, Sept 2002). Immunosuppressed individuals or those with chronic respiratory problems will not work with these recombinants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Culture volumes approximately 100ml

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid animal, culture and tissues culture waste will be disposed of by autoclaving followed by incineration according to the normal rules for Biosafety Level III biological waste disposal. Liquid waste will be added to Virkon Final concentration 1% for 24 hours) or chlorine compounds (0.2-3 for 10-30 minutes). No micro-organisms will be viable afterwards. Solid waste will be put in a metal box, sealed with autoclave tape, autoclaved at 134 degrees for 30 minutes, before being sent to an on-site clinical facility.
waste incinerator. The degree of kill is 100%

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The local GMSC agree with this risk assessment Ref 2003/016/

Project Containment

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</tr>
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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 628/96.1

Date Ackn'd: 09/12/1996

CU2 Project Title: DELIVERY OF IMMUNOGENS INTO DENDRITIC CELLS USING RETROVIRUS VECTOR

Class: Class 2

CultureVol: Class 2

Consent Granted: not applicable

Date Project Ceased: 20/09/2006

Withdrawn: [N]

Tick if notifying a connected programme of work [N]

Historical Significant Changes: TRANSFERRED TO GM 97 - 22/9/06.

Historical Date of Additional Info

Significant Change ID

02/03/2022

Page 10299 of 15326
Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment
Project Ref 628/97.1

Date Ackn'd 21/07/1997

Date Project Ceased 20/09/2006

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
TRANSFERRED TO GM 97 - 22/9/06.

Project notified under transitional arrangements Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [N]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 628/99.1

Date Ackn’d | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4 |
-------------|-------------------|-------|------------------|-----------------------|
08/02/1999   | PRODUCTION AND USE OF REPLICATION DEFECTIVE RECOMBINANT | Class 2 | | |
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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**Name**

UNIVERSITY OF EDINBURGH

**Name 2**

WESTERN GENERAL HOSPITAL

**Department**

MOLECULAR MEDICINE CENTRE

**Campus Estate or Research Centre**

**Building**

**Road Name**

CREWE RD

**District**

**Town**

EDINBURGH

**County**

EAST LOTHIAN

**Postcode**

EH4 2XU

**Country**

SCOTLAND

**Tel Number** 0131 651 1000

**Fax Number** 0131 651 1085

**E-mail**

health.safety@ed.ac.uk

**HSE Division**

SCOTLAND

**Comments**

Merged with GM207 on 17/02/2004

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify)  

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 630/01.1

Date Ackn'd 12/02/2001

Date Project Ceased 17/02/2004

Withdrawn N

Historical Significant Changes
Transferred to GM 207 on 17/02/2004

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Name**

ST BARTHOLOMEWS & THE ROYAL LONDON SCHOOL OF MEDICINE & DENTISTRY

**Department**

Endocrine and Chemical Endocrinology

**Campus Estate or Research Centre**

QUEEN MARY & WESTFIELD COLLEGE

**Building**

Endocrine and Chemical Endocrinology

**Road Name**

ST BARTHOLOMEW'S HOSPITAL

**District**

WEST SMITHFIELD

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

EC1A 7BE

**Country**

ENGLAND

**Tel Number**

0207 601 7445

**Fax Number**

0207 601 8468

**E-mail**

**HSE Division**

LONDON

**Comments**

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

**Level 1 (GMMs)**

**Level 2 (GMMs)**

**Level 3 (GMMs)**

**Level 4 (GMMs)**

**Non-microbial**

**Other (please specify)**

Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

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Other(s)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

SCOTTISH AGRICULTURAL COLLEGE

**Name 2**

MACROBERT FARM LABORATORIES

**Campus Estate or Research Centre**

AGRONOMY

**Building**

CRAIBSTONE ESTATE

**District**

BUCKSBURN

**Town**

ABERDEEN

**County**

ABERDEENSHIRE

**Postcode**

AB2 9TQ

**Country**

SCOTLAND

**Tel Number**

01224 711143

**Fax Number**

01224 714591

**HSE Division**

SCOTLAND

**Comments**

PREMISES CLOSED 08/03/2010

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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- Laboratory
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- Growth Room
- Glass House
- Large Scale
- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

**Give brief details of the genetic modification safety committee**

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity.

- Tick to confirm that you are attaching a summary of the risk assessment [☐]
- Tick if you are claiming exemption from disclosure for sections of the risk assessment [☐]

Please enter comments of the GM safety committee on the risk assessment.
GM Centre Number: 635

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Name

PROVALIS PLC

Name 2

Department

Campus Estate or Research Centre

DEESIDE INDUSTRIAL PARK

Road Name

NEWTECH SQUARE

District

Town

DEESIDE

County

FLINTSHIRE

Postcode

CH5 2NT

Country

WALES

Tel Number

01244 288888

Fax Number

01244 280117

E-mail

HSE Division

WALES AND SOUTH WEST

Comments

PREMISES PRESUMED CLOSED - CONTACTED 6 FEB 2003

Date at Which Additional Info Submitted

03/02/2005
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

### Level 1 (GMMs)

### Level 2 (GMMs)

### Level 3 (GMMs)

### Level 4 (GMMs)

### Non-microbial

Other (please specify)  
Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

OXFORD BIOMEDICA (UK) LTD

**Name 2**

Department

**Campus Estate or Research Centre**

WINDRUSH COURT

**Road Name**

TRANSPORT WAY

**District**

COWLEY

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX4 6LT

**Country**

ENGLAND

**Tel Number**

01865 783000

**Fax Number**

01865 783001

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

19/08/2003
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<td>Hammersmith Hospital, Du Cane Road</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Laboratory</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref  636/01.1**

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**Project Additional Information**

**Purposes of the contained use**

To construct recombinant paroviruses for cancer immunotherapy applications. Recombinant paroviruses expressing human and murine forms of oncofoetal antigen 5T4, single-chain antibody to 5T4, cytokines and immune co-factors such as B7-1.

**Recipient or parental organism**

Human cell lines such as 293T cells will be used to generate recombinant paroviral particles. 293T cells contain the SV40 large T antigen allowing expression of the capsid genes in the helper DNA plasmid. Paroviral particles (which are defective and cannot propagate themselves) are produced from separate DNA components; the recombinant paroviral molecular clone and a helper plasmid (which provides the capsid proteins in trans). Packaging cell lines stably expressing capsid proteins can also be used. (Mammalian cells eg. COS cells will also be used.)

**Host/vector system**

Disabled E. coli strains such as: JM109, XL1-blue SURE cells.

The rodent parovirus vectors based on MVM (minute virus of mice) and H-1 (Rat H-1) will be generated in 293T cells which being defective cannot propagate themselves in human cells.

**Origin & function**

Reporter genes such as β-galactosidase (lac-Z) or green fluorescent protein (GFP) will be used. Human and murine forms of the oncofoetal antigen 5T4 and its single-chain antibody will be expressed. Additional genes to express include immune co-stimulatory genes (B7-1) and cytokines.

Successful tumour suppression and tumour lysis in mouse models may lead to clinical trials.

**Evaluation of foreseeable effects**

Recombinant rodent parovirus particles will be generated using 293T cells. Viral titres ranging from 1-3 x 10^7 units/ml should be achieved. The recombinant paroviruses will be defective since they lack the functional capsid genes and cannot therefore undergo secondary infection.

Recombination events producing replication competent paroviruses (RCPs) are predicted to be low and can be further minimised by decreasing the homology between the two molecules. The use of packaging cell lines in which a number of copies of the helper construct are stably integrated into chromosomal DNA. RCPs will be tested for by plaque assay.
All of the recombinant parvoviruses generated lack the functional capsid genes and will only achieve a one hit transduction in target tumour cells. Only when such cells are in S phase will the NS gene product cause oncolysis. These defective parvoviral recombinants cannot undergo secondary infection and enter neighbouring cells.

Additionally, the recombinant viral stocks will be tested for the generation of contaminating replication competent viruses (RCVs) will be tested using established plaque-assay based standard operating procedures.

Contamination and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All cancer immunotherapy animal experimentation will be performed by animal license holders at the Institute of Virology, Mansfield Road, Oxford (GM No. 151).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All work will be carried out at containment level 2. The ACGM Classifies Parvoviruses as containment level 1. However highly trained scientists will be carrying out all the laboratory activities in our containment level 2 facilities as recommended by ACGM for mammalian cell work.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All biological waste (liquid and solid) will be disposed of: liquid waste will be soaked in 1% Virkon for 2 hours: Solid waste will be autoclaved at 136°C for 15 minutes. Following autoclave treatment, this solid waste will be collected for incineration by a registered company.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The ACGM compendium of guidance classifies parovirus work as containment level 1. However, the research will be performed in our purpose built containment level 2 laboratories at the premises of Oxford BioMedica (also in accordance with ACGM guidelines).

All of the recombinant parvoviruses generated lack the functional capsid genes and will only achieve a one hit transduction in target tumour cells. Only when such cells are in S phase will the NS gene product cause oncolysis. These defective parvoviral recombinants cannot undergo secondary infection and enter neighbouring cells. Additionally, the recombinant viral stocks will be tested for the generation of contaminating replication competent viruses (RCVs) will be tested using established plaque-assay based standard operating procedures.

Project Containment

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02/03/2022
The construction (cloning) and manufacture of recombinant AAV vectors with different serotypes (e.g. Serotypes 1-9) expressing various genes via different promoters.

Recipient or parental organism
Since AAV vectors are defective in nature (e.g. require co-infection with a helper virus in order to replicate) and are not associated with any human disease the hazards posed to human health associated with the recipient/parental virus are expected to be low. The main hazards are likely to arise from the properties of any inserted gene and will be risk assessed on an individual basis.

Host/vector system
Disabled E.coli strains such as: JM1 09, XL1 blue, SURE cells. The recombinant AAV vectors will be generated using helper+ virus — free system using human HEK293T cells. The AAV vectors are replication defective and cannot propagate themselves in human cells. Other cells lines may be used to evaluate the AAV vectors, refer to attached risk assessments. Any in vivo work will be carried out off-site.

Origin & function
The genetic inserts will include but are not limited to reporter genes (GFP, LacZ), cytokines, growth factors, angiogenic and angiostatic factors, enzymes, receptors, structural proteins, secreted factors each of which will be risk assessed on an individual basis. The promoters will include constitutive promoters such as CMV, tissue specific and regulated. The genetic material ( promoters and/or genes) may be of human or non-human origin. It is not expected that the genetic material will alter the phenotype or pathogenic traits of the AAV vector although each new recombinant AAV vector will be risk assessed on an individual basis.
Recombinant AAV vector particles will be generated in HEK293T cells. Viral particles up to $1 \times 10^{13}$ genome particles/ml could be achieved. The recombinant MV vectors (like the parental virus) are expected to be non-pathogenic to humans or other organisms, and will be replication defective since they lack the functional rep and cap genes, therefore there can be no secondary infection and transduction of neighbouring cells. Recombination events to produce a replication competent AAV (RCA) are predicted to be low, the only remaining MV sequence in the recombinant genome will be the inverted terminal repeats (ITR5). The possibility of the recombinant AAV recombining with a resident AAV-2 virus via the ITRs is theoretically possible (80% of human population have been exposed to MV-2), however the likelihood of generating a novel functional recombinant AAV capable of being packaged and able to replicate is low. The likely impact of the recombinant AAV on the environment is expected to be low, please refer to the individual risk assessments. Although non-targeted proviral insertions is seen with AAV vectors at low level it is expected to happen at passively low level (<10% genomes) and the likelihood of insertional mutagenesis is low, it has never been observed in numerous human clinical trials.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No plans for derogation from full containment for the determined class of activity.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Current version of Code of Practice 004: Disinfection and Decontamination will apply.
In brief the following procedures are routinely employed;
Liquids
All liquids are disinfected in 2% Virkon solution with the final concentration reaching no less than 1%. After a minimum of 60 minutes disinfected liquids are discarded to drains.
70% Ethanol/IMS are used for general cleaning of benches and minimisation of contamination.
Solid
All solid waste is autoclaved at 134°C for 10 minutes. The autoclaved waste is then collected for incineration by a licensed contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The vectors have been assessed and reviewed by the genetic modification safety committee (GMSC). The risk assessments have also been reviewed by the external biological safety officer.

Project Containment
Project Ref 636/12.1

Date Ackn’d 05/07/2012

CU2 Project Title Development and subsequent use of replication-competent retrovirus (RCR) or lentivirus (RCL) assays utilising replicating proficient positive controls based on HIV-1 or MLV

Date Project Ceased

Class 3

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Yes

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

HIV-1 is a class 3, containment level 3 biological.

Envelope-replaced MLV-4070A will be contained at level 3 for precautionary reasons but has been risk assessed as a class 2 operation.

Recipient or parental organism

1) Lab strains of Human Immunodeficiency Virus-1 (HIV-1)

2) MLV-4070A (MoMLV-4070A Hybrid virus).

Host/vector system

Plasmid DNA encoding modified provirus will be propagated in K12 disabled nonpathogenic strains of E. coli such as TURBO (NEB), JM109 (Stratagene), DH5alpha (Invitrogen), Stbl2 (Invitrogen), ‘Clean genome’ (Scarab). Hazard group 1.
1) Mammalian cell lines permissive for HIV-1 infection such as C8166.

2) Mammalian cell lines permissive for MLV-4070A and Envelope-replaced MLV-4070A such as Hela and mammalian cell lines stably expressing the appropriate receptor to the respective heterologous envelope cloned into MLV.

Origin & function

1) Lab strains of HIV-1 such as NL4-3 will be modified. These strains will be attenuated in accessory genes vif, vpr, vpu and nef, and also optionally mad integration defective. These viruses will be evaluated for potential use as surrogate RCL positive control virus in RCL assay development.

2) MLV-4070A will be modified such that the amphotropic 4070A envelope has been replaced with a heterologous envelope with restricted tropism, such as those based on modified Sindbis virus envelope, and optionally made integration defective. These viruses will be evaluated for potential use as surrogate RCL positive control virus in RCL assay development. Modified MLV stably expressing the heterologous envelope will model a putative RCL derived from clinical retroviral/lentiviral vectors that utilise said heterologous envelope.

RCL assay development:
Both types of the above modified viruses will be evaluated for replication kinetics within tissue cultures of permissive cell types from low multiplicities of infection. Virus detection/quantification will be performed by quantitative RT-PCR of associated reverse transcriptase activity within virus-containing culture supernatants or qPCR of DNA from infected cells, wherein virus infectivity has been abolished by approved methods of inactivation stated within Oxford Biomedica Ltd’s Containment Level 3 codes of practice.

Evaluation of foreseeable effects

1) Lab strains of HIV-1 such as NL4-3 will be modified. These strains will be attenuated by mutation of accessory genes vif, vpr, vpu and nef such that no functional protein will be expressed, and optionally made integration defective by mutation of integrase and/or LTR sequences. These attenuated strains are expected to be less virulent than either parental or ‘field’ strains of HIV-1.

2) MLV-4070a will be modified such that the amphotropic 4070a envelope has been replaced with a heterologous envelope with restricted tropism, such as those based on modified Sindbis virus envelope. For example, derivatives have been engineered such that the broad tropism of wild type Sindbis virus envelope has been substantially reduced, and secondary mutations increase the efficiency of modified Sindbis entry into Dentric cells (dC). Whilst the modified Sindbis envelope may be more cytotoxic than the 4070a envelope, expression in DCs (Antigen presenting cells) would likely result in Sindbis modified envelope antigen presentation in vivo, resulting in immune repression and subsequent clearance. Overall, the proposed modified MLVs have been risk assessed and are not expected to pose greater risks to humans or the environment than MLV-4070A

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Envelope-replaced MLV-4070a-based viruses have been rated class 2 organisms but will be contained at level 3 fr precautionary measures. Oxford Biomendica Ltd would like to retain the option for derogation of a specific Envelope-replaced MLV-4070A-based viruses from containment level 3 to containment level 2 once a virus has been well characterised.

Overall, whilst the heterologous envelope replacing 4070A may be rated as potentially more cytotoxic than 4070A, the substantially reduced tropism compared to 4070A and the fact that no human infection of MLV has been reported to date, justifies the potential for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
For containment level 2 activities, the current version of OXB's Codes of Practice 004: Disinfection and Decontamination will apply.

For containment level 3 activities, the current version of OXB's 'Containment Level 3 Codes of Practice' will apply

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The CU2 notification and attached risk assessments were reviewed and discussed by the genetic modification safety committee (GMSC). The GMSC approved the risk assessments and agreed that the assessments are suitable and sufficient.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 636/13.1

Date Ackn'd 01/07/2013

CU2 Project Title Manufacture of OXB's proprietary Lentiviral Vector platform for the delivery of therapeutic genes

Class 2

CultureVolClass2 51-500 Litres

Consent Granted

Project notified under transitional arrangements [N]

Withdrawn [N]

Tick if notifying a connected programme of work [N]
**Purposes of the contained use**

OXB has developed minimal lentiviral vector systems for the delivery of therapeutic genes based on either Equine Infectious Anaemia virus (EIAV) or Human Immunodeficiency Virus (HIV).

The therapeutic genes utilised include but are not restricted to dopamine biosynthetic enzymes, angiostatic proteins, growth factors or chimeric antigen receptor (CAR), OXB has previously notified the development of lentiviral vectors as a delivery system for laboratory scale activities. We are now in a position where we want to manufacture up to 100L of therapeutic vector.

**Recipient or parental organism**

Manual Equine Infectious Anaemia viral vectors (min.EIAV)

Minimal Human Immunodeficiency viral vectors (min.HIV).

The vectors are replication defective, self-inactivating and minimal to prevent replication competent lentivirus production. The vectors are replication defective, self-inactivating and minimal to prevent replication competent lentivirus production. The vectors are pseudotyped with Vesicular Stomatitis virus envelope proteins.

**Host/vector system**

The plasmids that will be used for the production of the minimal HIV and EIAV vectors will be manufactured to GMP standard by Contract Manufacturing Facility. The plasmids are propagated in K12 disabled non-pathogenic strains of E.coli.

Mammalian cell lines: the well characterised mammalian cell line 293T cells will be used. The cells require specialised growth media and as a result will not survive outside the laboratory environment.

**Origin & function**

The final product will be used in human clinical trials. The lentiviral vectors are all designed to be minimal and are replication defective. Their intended purpose is to deliver a functional version of the therapeutic gene to treat patients in human clinical trials; e.g. treatment of Parkinson's or B cell lymphoma. Please refer to the accompanying risk assessments for an example of OXB therapeutic products.

Each new GMO containing therapeutic genes will be risk assessed separately.

**Evaluation of foreseeable effects**

The vectors are pseudotyped with the Vesicular Stomatitis Virus envelope protein and are replication defective, self- inactivating and minimal to prevent replication competent lentivirus production. Therefore transduction with the lentiviral based GMO's will be limited to the intital target cell e.g. there is no intended cell-to-cell spread of the GMO.

Mammalian cell lines: the well characterised mammalian cell line 293T cells will be used. The cells require specialised growth media and as a result will not survive outside the laboratory environment.
Please refer to the accompanying risk assessments.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| No derogation required |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Sodium hydroxide at the following concentrations 0.2M, 0.5M and 1M will be used to decontaminate tubing, cartridges etc. The antiviral effects of different concentrations of Sodium hydroxide has previously been evaluated (OXB report number LV-study-08). Standard Operating Procedure SOP30003 will apply. In addition all plastic ware that has been in contact with biological material and/or after Sodium hydroxide treatment is placed in sealable containers in preparation for collection by a licenced contractor for incineration. |

Is an emergency plan required according to regulation 20?  

| N |

If yes, tick to confirm that it is attached to this form  

| N |

Tick to confirm that you have attached a risk assessment to this form  

| Y |

Tick if you are claiming exemption from disclosure for section of the risk assessment  

| Y |

Please enter comments on the GM safety committee on the risk assessment

The CU2 notification and attached risk assessments have been reviewed and approved by the GMSC. In addition OXB's external Biological Safety Officer has reviewed and approved risk assessment project 121.

**Project Containment**

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<tr>
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<th>Human Clinical Applications</th>
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</table>

**Project Ref** 636/14.1
Equine Infectious Anaemia Virus (EIAV) or sequence-optimised EIAV that expresses heterologous viral envelopes

The aims of the research are 2-fold:

1. To identify EIAV variants with improved replication kinetics in human cell lines in order to test potentially beneficial mutations to Company's EIAV-based lentiviral vector system and
2. To develop an improved EIAV-based replication competent lentivirus (RCL) assay for testing clinical material

EIAV is a class 1 micro-organism but is contained at level 3 in order to protect naive equine populations in the UK

The envelope gene of EIAV will be replaced in this project. The modification of virus genomes will be based on EIAVΔ wherein one or more of the accessory genes have been functionally deleted. The accessory genes contribute to the pathogenic state in vivo but are not necessarily required for replication in vitro, so this attenuation is considered to be a significant safety feature.

Plasmid DNA encoding modified provirus will be propagated in K12 disabled nonpathogenic strains of E.coli such as TURBO (NEB), JM109 (Stratagene), DH5alpha (Invitrogen, Stbl2 (Invitrogen), 'Clean genome' (Scarab). Hazard group 1.

Immortalised human cell lines (e.g. HEK293, HeLa) stably expressing EIAC fusion protein

The envelope gene of EIAV will be replaced in this project. The modification of virus genomes will be based on replication competent EIAVΔ, wherein one or more accessory genes have been functionally deleted. The accessory genes contribute to the pathogenic state in vivo but is not required for replication in vitro, so this attenuation is considered to be a significant safety feature.
The basic viral genome will be modified such that heterologous viral envelopes will be inserted replacing the natural EIAV envelope.

The use of heterologous viral envelopes will be passaged through immortalised human cell lines expressing equine fusion proteins.

**Evaluation of foreseeable effects**

The envelope-modified EIAV variants will have altered tropism compared to wild type EIAV. It is likely that use of heterologous envelopes will greatly broaden the tropism of EIAV. However, it is expected that replication of these modified viruses will be restricted to cells wherein the EIAV U3 is active; this excludes human cells as huCT1 cannot participate with eTat and eTAR in activating the EIAV LTR. Moreover, the use of the background Δ accessory gene mutation in these viruses will make them highly attenuated in Equines. Therefore, it is expected that these modified viruses will only replicate in mammalian cells in vitro wherein the appropriate receptor is expressed and the EIAV U3 is active.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No plans to derogate GMMs

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

For containment level 2 activities, the current version of OXB's 'Codes of Practice 004: Disinfection and Decontamination' will apply.

In brief all liquid waste is treated with 4% Virkon to give a final concentration of 1%, allowed to inactivate for minimum of 60 minutes before discarding to drains. All solid waste is autoclaved before collection by licensed contractor for incineration.

For containment level 3 activities, the current version of OXB's 'Containment Level 3 Codes of Practice' will apply.

In brief all liquid is treated with 4% Virkon to give a final concentration of 1% and allowed to inactivate for a minimum of 60 minutes before being autoclaved. The autoclaved liquid waste is removed from the CL3 and discarded to drains. All solid waste which has been in contact with biohazard is treated with Virkon allowed to decontaminate for 60 mins. The Virkon is removed and the solid waste autoclaved before collection by licensed contractor for incineration.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment
Comments from the GMSC: The risk assessment was approved on the proviso that all recommendations and amendments are completed. As per protocol the GMSC approved risk assessment is forwarded to the External Biological Safety Officer for final review and comment.

Comments from External BSO: Project 122 is the latest effort to make the OXB EIAV-based vectors acceptably efficient and to provide the basis for RCR detection. Working under CL3 is entirely appropriate. I am comfortable with the risk assessment and its conclusions, although I suggest the text "EIAV is the simplest retrovirus known" is replaced with "EIAV is the simplest lentivirus known".

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
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**Animal Units**

| L2 | L3 | L4 |

**Large Scale Activities**

| L2 | L3 | L4 |

**Human Clinical Applications**

| L2 | L3 | L4 |

**Project Ref** 636/17.1

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Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

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<td>31/08/2018</td>
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**Project Additional Information**
Purposes of the contained use

The aim is to utilise lentiviral vectors that express genes which are useful in the treatment of human diseases. The vectors will be tested for their ability to transfer desirable characteristics to target cells in vitro and in vivo. It is anticipated we will be able to modify the properties of target cells such that they express the desired characteristics e.g. expression of innocuous reporter genes and therapeutic genes.

The initial study is to test the feasibility of the manufacture of the lentiviral vector encoding the therapeutic gene. If the initial study is successful than large-scale vector production will be undertaken to provide collaborators with sufficient material for clinical trials.

If large scale vector production is to be initiated, a manufacturing risk assessment will be drafted and submitted for approval by our GMSC.

As other projects arise i.e. the evaluation of different therapeutic genes; each body of work will be assessed by our GMSC separately.

Recipient or parental organism

The engineered lentiviral vector is a self-inactivating minimal lentiviral vector capable of delivering heterologous nucleic acid to dividing and non-dividing cells in vitro and in vivo. Tropism of the vector may vary depending on envelope pseudotype employed.

Host/vector system

Plasmids will be manufactured by a Contract Manufacturing Facility to a suitable grade necessary for vector production. The plasmids are propagated in K12 disabled non-pathogenic strains of E.coli.

Mammalian cell lines: the well characterised mammalian cell lines.

Vector System

The engineered vector is a self-inactivating minimal lentiviral vector capable of delivering heterologous nucleic acid to dividing and non-dividing cells in vitro and in vivo. Tropism of the vector may vary depending on envelope pseudotype employed.

Each new GMO containing novel therapeutic genes and/or envelope will be risk assessed separately.

Origin & function

The lentiviral vectors are all designed to be minimal and are replication defective. Their intended purpose is to deliver a functional version of the therapeutic gene to treat patients in human clinical trials. Please refer to the accompanying risk assessments for an example of therapeutic products.

Evaluation of foreseeable effects

The vectors are replication defective, self-inactivating and minimal to prevent replication competent lentivirus production. The vectors are pseudotyped with paramyxovirus envelope proteins. Therefore transduction with the lentiviral based GMOs will be limited to the initial target cell i.e. there is no intended cell-to-cell spread of the GMO.

Mammalian cell lines: the well characterised mammalian cell lines will be used. The cells require specialised growth media and conditions and as a result will not survive outside the laboratory environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For general laboratory activities the current version of Code of Practice 004: Disinfection and decontamination will apply. In brief the following procedures are routinely employed:

Liquids
All liquids are disinfected with 2% Virkon solution with the final concentration reaching no less than 1%. After a minimum of 60 minutes disinfected liquids are discarded to the drains.

Solid
All contaminated solid waste is autoclaved at 132°C for 10mins. The autoclaved waste is then collected for incineration by a licensed contractor.

Non infectious, non-GM, non-contacted biomaterial solid waste is segregated into yellow and black waste bags (provided by licensed waste contractor). This waste is collected by a licensed contractor for incineration.

For suspension vessels; Sodium hydroxide at the following concentrations 0.2M, 0.5M and 1M will be used to decontaminate tubing, cartridges etc. In addition all plastic ware that has been in contact with biological material will after sodium hydroxide treatment is placed in sealable containers in preparation for collection by a licensed contractor for incineration. The antiviral effects of different concentrations of Sodium hydroxide has been evaluated by OXB.

For suspension vessel related laboratory activities Standard Operating Procedures PR&DSOP 027 to 032 will apply.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The attached GM risk assessments (GMRA162 & 163) have been reviewed and approved by the GMSC. In addition OXB’s external Biological Safety Officer has reviewed and approved the assessments. The risk assessments are associated with laboratory scale activities for a feasibility study. If the feasibility study activities are successful additional work packages will be considered which includes scaling up to 200l scale. The increase in scale will be risk assessed as and when the work packages have been agreed.

Project Containment

<table>
<thead>
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02/03/2022
**Project Ref** 636/19.1

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<th>Consent Granted</th>
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**Withdrawn** N  
**Tick if notifying a connected programme of work** N

### Historical Significant Changes

- **Historical Date of Additional Info**:  

- **Significant Change ID**:  

- **Date of Significant Change**:  

### Project Additional Information

**Purposes of the contained use**

Development (from wild type) and subsequent use of attenuated replication-competent lentiviral (RCL) in assays utilising replication proficient positive controls based on SIVagm (SIV strain found in African Green Monkeys).

**Recipient or parental organism**

Wild Type Simian Immunodeficiency Virus (SIV) - Classed as SCDP Hazard Group 3.

Creation of attenuated lab strains of this virus - Assessed as GM Class 3.

Both wild type and attenuated SIV viruses are anticipated to be replication competent within CD4+CCR5+ T-cells (primary or cell lines).

**Host/vector system**

1. Plasmid DNA encoding modified provirus will be propagated in K12 disabled strains of E. coli (e.g. TOP10, TURBO, JM109, DH5alpha and others) - ACDP Hazard Group 1*
2. Mammalian cell lines (e.g. lentiviral vector production cell lines such as HEK293/HEK293T, Tcell lines such as C8166-45 and Molt4-8) - ACDP Hazard Group 1)

Please note all our laboratories are designed to CL2 and we work to a minimum of CL2 even with HG1 organisms.
Origin & function

Origins of genetic material is wild type SIVagm virus. Attenuation will be achieved by removing pathogenic factors potentially without inhibiting replication competence.

RCL assay development:
Attenuated SIV virus will be evaluated for replication kinetics within tissue cultures of permissive cell types from low multiplicities of infection. Virus detection/quantification will be performed by quantitative RT-PCR of associated reverse transcriptase activity within virus-containing culture supernatants or qPCR of DNA from infected cells, wherein virus infectivity has been abolished by approved methods of inactivation stated within Oxford Biomedica UK Ltd's Containment Level 3 Codes of Practice.

Evaluation of foreseeable effects

Potential hazard to human health:
SIV is not a human pathogen, but is classed as ACDP Hazard Group 3 because of its ancestral link with HIV-1/-2, and therefore in theory may be able to cause pathogenic disease in humans should exposure be significant. SIV is capable of transferring its genome into dividing and non-dividing cells, though it is restricted to CD4+CCR5+ T cells.

Given the presence of human antiviral mechanisms, such as TRIM5α and APOBEC3G, accidental exposure to SIV is anticipated to be asymptomatic.

SIV is capable of infecting CD4+ T cells and integrates into the host cell DNA. There is a theoretical risk of insertional mutagenesis (and therefore potential oncogenesis) but since infection at high viral load is lytic in vivo there has been no report of this type of pathogenicity in T cells or from latent reservoir cells (e.g. macrophage).

Prokaryotes: Whilst the genes encoding SIV proteins will not be inserted immediately downstream of a bacterial promoter, it is expected that leaky expression of these genes may be disadvantageous for bacteria. It is not expected that any of these proteins will increase the tropism or pathogenicity of bacteria in humans.

Eukaryotes: All cell lines used in this project are classed as hazard group 1 but will be maintained at containment level 2 using microbiological safety cabinets at OXB. Pathogenicity of the mammalian cell lines for use in this study is not expected to be altered by SIV components. It is not expected that the SIV viruses will be capable of mobilising other sequences during production in HEK293(T) based cells or within T-cell lines.

Potential hazard to Environment:
WT SIV can be pathogenic to certain simian species, targeting CD4+CCR5+ T-cells. The properties of ‘wild type’ SIVagm are not expected to be altered; however, the GMM strain is essentially ‘lab adapted’ by passing in T-cell lines within a lab environment. For this reason, the strain is likely to be less ‘fit’ that field strains of SIVagm. The attenuated SIVagm strain is likely to be incapable of sustaining productive infection in monkeys.

The viruses do not harbour transgenic inserts. Plasmids will not encode known bacterial promoters directly upstream of Eukaryotic transgenes. However, viral promoters such as CMVp have been shown to have significant promoter activity in E.coli. High-copy number origin of replication plasmids will typically be utilised. Antibiotic-resistance gene markers: e.g. Ampicillin, Kanamycin, Blasticidin. The plasmid/E.coli host combinations will ensure that none of the plasmids will be mobilisable, hence maintenance in the human gut and transfer of antibiotic resistance to endogenous E.coli/gut bacteria is unlikely.

Most hazardous GMM (both to human health and environment): Attenuated SIV virus, although replication competent, will not harbour transgenic sequences but will be capable of infecting CD4+CCR5+ T cells. They are designed to be less hazardous than the wild type and to make the virus much less able to cause infections in both human and non-human primates. However, we intend to handle all such virus in the same way as wild type under CL3 conditions.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste procedures are all validated and described in the CL3 Codes of Practice 21:

COP021.4 Containment Level 3 (CL3) - Standard Operating Procedures for the Inactivation of Samples Generated in the CL3 Laboratory.
COP021.5 Containment Level 3 (CL3) Standard Operating Procedures for Decontamination and Disinfection of Waste Material.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

The GMRA was introduced by the PI to the members of the GMSC and the committee had the chance to ask questions and make comments.

A recommendation was made to review the control measures required and to expand on in particular separation measures to ensure that during handling, equipment use and storage of the proviral WT SIV DNA the likelihood of inadvertent mixing and recombination with other pathogens used in the labs will be minimised.

This recommendation was addressed (see 'additional measures' in the GMRA) and the reviewed GMRA176 was approved by both the GMSC and our external consultant BSO.

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<table>
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**Project Ref 636/97.1**

Date Ackn'd  CU2 Project Title  Class  Culture Vol Class 2  Culture Volume Class 3-4
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<tbody>
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<td>L2</td>
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Animal Units

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tbody>
<tr>
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**Project Ref 636/97.2**

Date Ackn’d 03/07/1997

Date Project Ceased

CU2 Project Title THE USE OF RECOMBINANT ADENOVIRUS VECTORS FOR GENE DELIVERY TO PRIMARY HUMAN CELLS

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes GM636/99.1, GM636/98.1

Historical Date of Additional Info 11/03/1999, 13/03/1998,

Significant Change ID 636/97.2a

Date of Significant Change 25/11/2020
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Growth Rooms</th>
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02/03/2022
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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Project Ref 636/97.4

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**Withdrawn** | N

**Tick if notifying a connected programme of work** | Y

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## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment.

**Project Containment**

<table>
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<tr>
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Animal Units

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<th>Animal Units</th>
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**Project Ref 636/98.5**

- **Date Ackn’d**: 17/06/1998
- **CU2 Project Title**: THE DEVELOPMENT OF RETROVIRAL VECTOR SYSTEMS TO INHIBIT THE ACTION OF REPLICATION COMPETENT RETROVIRUSES.
- **Class**: Class 3
- **Consent Granted**: Yes
- **Project notified under transitional arrangements**: Yes

Withdrawn: N

Tick if notifying a connected programme of work: N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory       Animal Unit       Growth Room     Glass House     Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)

Non-microbial

Other (please specify)   Tick if confidential

Bacteriology  Parasitology  Transgenic Birds  Microbiology Research

Virology  Transgenic Animals  Transgenic Fish  Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity:

| Maximum volume of one litre.
| Waste is deactivated by addition of Lysol as per manufacturers instructions.
| Lysol waste checked by culture on agar plates.
| Contaminated plastics autoclaved at 121 degrees C for 30 minutes. Process validated by TST indicator.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 642**

<table>
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<tr>
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**Name**

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**Name 2**

**Department**

**Campus Estate or Research Centre**

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**Country**

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**Comments**

**Date at Which Additional Info Submitted**

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## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Level 2 (GMMs)</td>
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<td>Level 3 (GMMs)</td>
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Tick if confidential

<table>
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<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Transgenic Animals</th>
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</table>
The maximum culture volume that could be released at one time is 1 L. Waste is deactivated by autoclaving (15 minutes at 121 degrees C). All autoclaved waste is then incinerated. Effective functioning of the autoclave is assessed under a service contract.

<table>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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## Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

[ ]

Give brief details of the genetic modification safety committee

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</table>
All work involves ACGM class 1 organisms, so risk is minimal. Maximum individual culture volumes do not exceed 1 litre (routinely 500 ml). Liquid waste is deactivated overnight by addition of 10% Chloros (1 x 10,000 ppm available chlorine). It is then disposed of to drain with copious amounts of water. The decontamination is validated by the absence of viable cells in 3 x 100 ul samples of the decontaminated culture. This validation to be performed at approximately 6 monthly intervals and records retained by the GMSC secretary. Solid waste is disposed of by incineration at a validated facility.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

OXAGEN LIMITED

**Name 2**

RECEPTION

**Campus Estate or Research Centre**

**Road Name**

91 MILTON PARK

**District**

**Town**

ABINGDON

**County**

OXFORDSHIRE

**Postcode**

OX14 4RY

**Country**

ENGLAND

**Tel Number**

01235443300

**Fax Number**

01235 443301

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

21/11/2003  19/05/2005
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)  
Tick if confidential

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Virology

- Transgenic Animals
- Transgenic Fish
For activities involving GMMs, describe the waste management measures which will apply to the activity

The machine autoclaves at 136 degrees C for 30 minutes followed by 12 minutes free steam. The pressure is a maximum of 1.08 bar and a minimum of 1.06 bar.
In addition to the autoclave tape there is a temperature probe in the machine.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

02/03/2022
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**Name**

**UNIVERSITY OF WALES COLLEGE OF MEDICINE**

**Name 2**

**LLANDOUGH HOSPITAL**

**Campus Estate or Research Centre**

**ACADEMIC CENTRE**

**Road Name**

**PENLAN ROAD**

**Town**

**PENARTH**

**County**

**CARDIFF**

**Postcode**

**CF64 2**

**Country**

**WALES**

**Tel Number**

**029 20716998**

**Fax Number**

**029 20711267**

**HSE Division**

**WALES AND SOUTH WEST**

**Comments**

GM645 closed and merged with GM130 on 26/04/2005

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

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Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity.

A small volume (100 ml) of culture media will be used 2/3 times a year, waste media will be treated with a dichloroisocyanurate based disinfectant before disposal as clinical waste as per UWCM/Trust policy. All plastics and glassware used during the procedure will also be disinfected before disposal or reuse.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

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**Name**

ROYAL MARSDEN NHS TRUST

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

DOWNS ROAD

**District**

**Town**

SUTTON

**County**

SURREY

**Postcode**

SM2 5PT

**Country**

ENGLAND

**Tel Number**

0208 642 6011

**Fax Number**

NONE

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

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02/03/2022
**Premises Conditions**

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Give brief details of the genetic modification safety committee

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Other (please specify)  
Tick if confidential

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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

All waste generated will be autoclaved prior to off site incineration. The autoclave process will be undertaken on site and will be validated in accordance with the requirements of HTM 2010. Standard disinfection procedures will apply to all areas involved in gene therapy studies. The exact chemical disinfectant required will be specified in the risk assessment documentation and will be appropriately validated.
**Project Ref 646/09.1**

**CU2 Project Title**
A randomized Phase 3 Clinical Trial to evaluate the efficacy and safety of treatment with oncoVEXGM-CSF compared to subcutaneously administered GM-CSF in melanoma patients with unresectable stage IIib, IIic and IV disease

**Class**
Class 2

**CultureVolClass2**
< 1 Litre

**Class CultureVolClass3-4**

**Non-GMM Consent Granted**

**Date Ackn'd**
03/09/2009

**Date Project Ceased**
15/01/2013

**Tick if notifying a connected programme of work**
N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The purpose of the trial is to evaluate the safety and the risks of using OncoVEXGM-CSF to treat patients with melanoma and to see if OncoVEXGM-CSF can destroy these tumours as it destroyed tumours in animal test and human studies. This study may provide information on the usefulness of OncoVEXGM-CSF as a future treatment for melanoma and the safety and usefulness of OncoVEXGM-CSF as a treatment for melanoma.

Patients in this trial will be given either OncoVEXGM-CSF or GM-CSF (there is twice the likelihood of receiving OncoVEXGM-CSF) The Onco VEXGM-CSF will be directly injected into skin lesions and the dose will depend upon the number and size of lesions being injected, up to a total volume of 4.0mL in any one visit.

**Recipient or parental organism**
The target recipients are melanoma patients with unresectable stage IIIb, IIIc and IV melanoma that have volunteered for the clinical trial. These patients will be carefully selected based on the inclusion and exclusion criteria defined in the study protocol. The GMO will be administered by direct injection into the melanoma lesions.

Host/vector system

HSV-1 clinical isolate strain JS-1 (ECACC Accession Number 01010209) deleted for the genes encoding ICP34.5 and ICP47.

Origin & function

The WHO Vero cell line ATCC No X38 (Kidney, African green Monkey: cercopithecus aethiops) is used as the producer cell line for OncoVEXGM-CSF. The gene of interest is human GM-CSF. GM-CSF acts as a stimulator for dendritic cells. These cells are the most potent antigen presenting cells which can take up cell fragments and incoming pathogens, then present these antigens to the immune system inducing an immune response. Tumour cells typically fail to stimulate optimal antigen presentation and do not generate immune reactions that are sufficiently potent to prevent lethal tumour progression. The intended function of GM-CSF is to enhance functions of cells critical for immune activation against tumour cells.

Evaluation of foreseeable effects

It is unlikely that the modified viruses would pose a serious risk to humans, animals or plants. As the GMO is a virus, it requires host cells to replicate and does not form survival structures, such as seeds, spores and sclerotia. The GMO would not be expected to be able to survive in the environment if released. HSV is an enveloped virus that has been shown to be fragile and rapidly inactivated by dessiccation, lipid solvents and mild detergents. The fact that direct contact is required for transmission attests to the instability of the virus outside the host. The modifications made to the GMO would not be expected to have any effect on the ability of the virus to survive outside of its host since they do not affect or stabilise the lipid envelope of the virus.

The GMO contains deletions for the ICP34.5 and ICP47 genes. The deletion of ICP34.5 severely reduces the pathogenicity of the GMO whilst still allowing it to replicate in rapidly dividing cells, such as in tumours, and in culture. The GMO would be expected to be less pathogenic in humans compared to wild type virus. The therapeutic genes to be delivered are present in normal, healthy humans.

Infection is by contact and transmission by the aerosol route is not thought to occur. The GMO is being administered by direct injection into tumours and therefore most of the GMO will be in a localised area. The injection site will be covered by an occlusive dressing to prevent any potentially shed GMO from coming into contact with the environment. The most likely route of contamination of hospital staff handling the GMO is by needle stick, splashing of virus onto the skin or into the eye, or by contact with other contaminated material. The most likely route of contamination of others who come into contact with the patient is by contact with the injection site. In order to prevent transmission to third parties involved in the clinical trial, relevant precautions will be taken.

Low levels of virus shedding into urine have been detected in a very small number of samples tested in previous clinical trials of the GMO. Potential shedding of the GMO from urine is not thought to present a hazard as there is data to demonstrate that household disinfectants destroy the GMO. Experiments have shown that the risk of transmission of wild-type HSV from wastewater is minimal. The deletion of the ICP34.5 and ICP47 genes and the insertion of the hGM-CSF gene would not increase the ability of the GMO to survive in wastewater compared to wild type HSV.

It is not known wether the GMO can establish latency in man and if so, wether the virus would be able to reactivate. However, the human experience with the GMO to date has not shown this to be a risk as none of the dosed patients have developed lesions at the injection site or other symptoms suggestive of virus reactivation. Additionally, there are no reported incidences of establishment and/or reactivation from latency of other HSV-based oncolytic viruses.

Humans are the only natural host species for HSV and it is universally prevalent with approximately 80% of the human population being seropositive. Although other species can be infected experimentally, these appear to be "dead end" hosts. It is therefore unlikely that there would be gene transfer from the GMO to man or any other species.

Homologous recombination between different HSV strains can occur if the two viruses are undergoing replication in the same cell at the same time. However, it has been
shown that recombination between the GMO and a wild type virus cannot result in the generation of a wild type virus carrying the hGM-CSF gene.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All needles and syringes will be discarded into a designated sharps bin which will be sealed and then collected by the relevant contractor.

Used vials will be retained at the pharmacy until inventoried by the study monitor. They will then be removed and destroyed per hospital specific procedures. If the hospital does not allow the storage of used vials until they are inventoried by the study monitor, detailed records of the return or destruction of the vials will be maintained.

All other materials will be treated as clinical waste and disposed of in yellow clinical waste bags.

| N/A |

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

The GMSC questioned the safety aspects of intra tumoural injections and whether injections will be split between lesions. The research nurse confirmed these will be split by the investigator and that a maximum of 4 mls is given. Discrepancies on the needlestick and waste SOPs were noted, which required updating. There were queries on whether a negative pressure room was required, which is not needed for this trial, although it may be used for convenience purposes. It was confirmed that it was important for this to be included in the study paperwork. It was noted that contact is a main issue, and there was discussion on the protective clothing used and that it should all be disposable and single use only.

It was questioned if the disposal of PPE is treated as GM waste and it was confirmed by that this would go in yellow bags or in the red lidded bins.

It was confirmed that all patients connected with this study will be attending at the Chelsea site only but suggested that something should be put in place in case attendance in Sutton is required for any reason and that a contingency plan be added to the paperwork.

The chairman then confirmed that the Committee agreed that this study could go ahead, with relevant amendments being made as follows:

- Contingency plan to include Sutton if required.
- Clarify that the side room used does not need to be negative pressure.
- Reference to Virkon to be removed and replaced with Haz Tab solution or Milton.
- Waste disposal SOP to be updated.
- Needlestick SOP to be updated.
- Appendix C of the policy including staff list to be submitted to risk management prior to the trial commencing as per GM policy.

**Project Containment**

| Laboratory Activities | Glass Houses | Growth Rooms |

02/03/2022
Project Additional Information

Purposes of the contained use

RP2 will be administered to subjects as an anti-tumour therapy, as part of a Phase I clinical trial to treat a wide range of solid tumour types. The initial clinical trial intends to test RP2 as a single agent and in combination with PD1 blockade in patients with solid tumours.

Recipient or parental organism

RP2 (rHSV-1 hGM-CSF/GALV-GP-R-/ahCTLA-4) is a selectively replication competent Herpes Simplex Virus-1 (HSV-1). The virus contains a codon-optimised sequence for GALV-GP-R-, a codon-optimised sequence for hGM-CSF, and the sequence for a single chain antibody-like molecule to human cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). GALV-GP-R- expression leads to cell to cell fusion (syncytial) formation in infected tumour cells through binding to the constitutively expressed PIT-1 receptor for GAL V. This results in the death of the cells by membrane fusion. Since the RP2 selectively replicates in tumour cells, the expression of the GALV-GP-R- is minimised in normal tissues. The oncolytic destruction of tumour cells leads to the release of tumour associated antigens that are intended...
To engender an anti-tumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further enhanced through GALV-GP-R- mediated fusion associated cell death, which is also expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, un.injected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. The anti-human CTLA-4 antibody-like molecule which interferes with the interaction of CTLA-4 with B7 molecules on professional antigen presenting cells. This is intended to result in enhanced local T-cell activation due to blockade of the inhibition otherwise mediated by the CTLA-4/B7 interaction. The resulting enhanced anti-tumour T-cell activation, proliferation, and lymphocyte infiltration into tumours is intended to lead to improved local and systemic anti-tumour effect. RP2 is intended for direct injection into solid tumours.

**Host/vector system**

RP2 (HSV-1 hGM-CSF/GALV-GP-R-/ahCTLA-4) is derived from the RH018 strain of Herpes Simplex Virus-1. RP2 is produced in the Vero cell and released into the culture media during cell lysis, prior to purification.

**Origin & function**

RP2 was constructed using a new strain of HSV-1 (strain RH018). For the development of RP2, 29 new clinical strains of HSV-1 were obtained for comparison to identify more effective strains for development. Of the strains tested, strain RH018 was the most effective at killing a panel of human tumour cell lines, and selected for use in the construction of RP2.

RP2 expresses the immune stimulatory protein GM-CSF, which augments therapeutic activity. GALV-GP-R- binds to the Pit1 receptor, which is widely expressed on mammalian cells including human tumour cells. Pit1 is also critical for cell proliferation, and its expression is therefore unlikely to be lost or down-regulated in response to cancer treatment. The truncated R- version of the protein provides constitutive fusion activity without GALV (i.e. the virus) itself. Expressing GALV-GP-R- together with GM-CSF is expected to increase clinical activity as compared to only expressing GM-CSF. As well as causing direct tumour cell death by cell to cell fusion, cell to cell fusion followed by death is highly immunogenic and includes the release of immunogenic tumour antigen-containing exosomes. Expression of GALV-GP-R- from an oncolytic virus is therefore expected to improve systemic, immune mediated, effects, as well as effects in the directly treated tumour thereby increasing synergy with other immunemediated approaches to cancer therapy such as immune co-inhibitory pathway blockade.

The addition of the expression of an anti-humanCTLA-4 antibody-like molecule (ahCTLA-4) interferes with the interaction of CTLA-4 (expressed on a subset of activated T cells) with B7 (CD80/CD86) molecules on professional antigen presenting cells. This is intended to result in enhanced T-cell activation due to blockade of the inhibition otherwise mediated by the CTLA-4/B7 interaction. The resulting enhanced local anti-tumour T-cell activation, proliferation, and lymphocyte infiltration into tumours, is intended to lead to improved local and systemic anti-tumour effects, including in combination with PD-1 or PD-L1 blockade. Intratumoural expression of the anti-human CTLA-4 antibody-like molecule would also be expected to reduce toxicity as compared to systemic administration of an antihuman CTLA-4 antibody, including in combination with antibodies targeting PD-1 or PD-L1.

**Evaluation of foreseeable effects**


The oncolytic destruction of tumour cells leads to the release of tumour associated antigens that are intended to engender an anti-tumour immune response, enhanced by the local expression of GM-CSF.

This is intended to be further amplified through GALV-GP-R- mediated killing, fusion associated cell death which results in the production of the highly immunogenic exosomes and is expected to contribute to this immune effect.
immune response generated may then lead to immune destruction of distant, uninjected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. The anti-human CTLA-4 antibody-like molecule which interferes with the interaction of CTLA-4 with B7 molecules on professional antigen presenting cells. This is intended to result in enhanced local T-cell activation due to blockade of the inhibition otherwise mediated by the CTLA-4/B7 interaction. The resulting enhanced anti-tumour T-cell activation, proliferation, and lymphocyte infiltration into tumours is intended to lead to improved local and systemic anti-tumour effects. RP2 is intended for direct injection into solid tumours.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| No derogation is requested. |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| All used and unused RP2 and diluent vials used in preparation and syringes will be destroyed per institutional policy. As per the wild-type HSV-1 virus, the recombinant HSV-1 vector that represents RP2 are highly susceptible to dehydration, rapidly inactivated outside the host and easily inactivated (for example with 1 % sodium hypochlorite). As part of phase I of the clinical trial, biodistribution and shedding will be monitored. RP2 DNA levels in blood and urine will be determined at time-points outlined in the Schedule of Assessments of the clinical protocol (day 1, days 2 and 3, day 15, days 16 and 17, day 29, days 30 and 31, day 43, day 57 and as part of the follow up, 30 days and 60 days after the last dose of RP2). These samples will be collected prior to dosing on treatment visits. In addition, for the first three doses, samples will be collected after dosing at 6 (±2hr) hours. Swabs of saliva/oral mucosa, injection sites and exterior of occlusive dressings will also be obtained at these timepoints |

**Is an emergency plan required according to regulation 20?**

| N |

**If yes, tick to confirm that it is attached to this form**

| N |

**Tick to confirm that you have attached a risk assessment to this form**

| Y |

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

| Y |
Shipment of RP2
1. RP2 and diluent will be shipped on dry ice, and should not be used if the dry ice is not present upon receipt at the clinical site. A temperature monitor will also be included with the shipment to assure shipment remained within temperature.
2. RP2 will be provided as a sterile frozen liquid in a single-use 3-cc glass vial with a gray Fluortec®-coated bromobutyl stopper, aluminum seal and a polypropylene cap. Each vial will contain a minimum of 1.0 mL RP2 in a buffer containing sodium phosphate, pH 7.4, sodium chloride, myo-inositol, sorbitol, human serum albumin (HSA) in water for injection (WFI). RP2 vials will be provided as concentrations of 10^6 PFU/mL, 10^7 PFU/mL or 10^8 PFU/mL.
3. Upon receipt of the study drug shipment, the pharmacist or study coordinator/research nurse will verify the condition of the study supplies, register the study drug via Interactive Response Technology (IRT), and document per instructions in the Pharmacy Manual and the IRT Manual.
4. The site must store the study drug in a limited access area at -70 DC or below. Only authorized site study personnel may access study drug.

All handling and management of IMP upon receipt in the pharmacy department to collection by research nurse will be as pharmacy SOP1.15, "Safe handling, spillage and destruction of GMO and trial specific dispensing procedures".

Administration of RP2 (NB First in Human)
1. This Phase 1 study is a multicentre, open-label, single agent dose escalation and combination treatment study of RP2. The study will be conducted in two parts.
   The first part of the study is an open-label, dose escalation first in human Phase 1 study to assess the safety and tolerability of RP2 and to determine the recommended Phase 2 dose (RP2D) to be used in the second part of the study.
2. The second part of the study is an open label design to further investigate safety of RP2 in combination with nivolumab. It will also assess the biological activity of multiple doses of RP2 in combination with nivolumab.
3. RP2 will be administered intra-tumourally in up to 36 participants in dosing cohorts. The appropriate amount of RP2 will be injected into the tumour and a record of the volume administered kept.
4. Please refer to section 6.2 of the protocol (v1.0 07Feb19) for further detail. Please refer to section 6.2.2 of the protocol (v1.0 07Feb19) for combination dosing with Nivolumab
5. The injection site should be swabbed with alcohol following injection, and then covered with a dry occlusive dressing for up to one week. If the dressing comes off before 1 week, it should be replaced if the injection site is oozing or weeping. Waste dressings should be disposed of according to institutional guidelines, e.g. by incineration.

Clean up and Disposal Following Treatment
1. All personnel should be advised that RP2 is classified as a level 2 biosafety hazard and therefore universal precautions must be observed by appropriately trained personnel when handling the IMP or contaminated equipment.
2. The sponsor recommends that during the study all staff involved in the administration of IMP and sample
3. Waste should be disposed of as per GM trust policy. All waste generated during this activity (including sharps, needle sets, vials and disposable safety clothing) that has been in contact with the IMP will be placed in a 5 litre yellow sharps bin, with a yellow lid. This will then be sealed at the patients' point of care with all standard information accurately completed on the side of the bin including study specific information and relevant contact details. A GM label must also be completed with the relevant department, date, and a signature and then fixed to the sharps bin.

4. The research personnel will then contact the waste department via extension 4591 (morning) or 4619 (afternoon) to dispose of the material.

5. Systematic decontamination of treatment area and surfaces must be undertaken with Antichlor following RP2 administration.

Patient Management Following Treatment and Discharge Advice

Patients are informed of common side effects. (PIS V1 25Feb19).

Treated patients may excrete the virus from the injected lesions. Close contacts are advised to wear protective gloves if they will be touching the lesions or dressing. Those who are immunosuppressed should avoid direct contact with the patient.

In the event of exposure to RP2, individuals are advised to clean the site with soap and water. RP2 is responsive to acyclovir or anti-viral drugs activated by the viral thymidine kinase gene.

Handling of Spills

All spillages must be dealt with immediately by the specialist nurse in charge.

Accidental exposures to Biohazard agents must be recorded on an Accident/Incident Form and reported to Occupational Health on the same working day as the incident.

A spillage kit should be available wherever RP2 is handled. If items are used from the spillage kit they must be replaced immediately after the spillage has been dealt with.

A spillage kit consists of the following items:

A laminated copy of this procedure
A 'Kimal' pre packaged spill kit containing:
- 2 pairs of sterile nitrile gloves (medium)
- 1 plastic apron
- 1 pair blue shoe covers
- 1 blue tray wrap
- 1 disposable gown (large)
- 1 fluid shield face mask
- 1 paper towel
1 yellow adhesive waste bag
1 x 5 litre sharps bin and lid
1 pack of lint free wipes
1 x 500ml bottle of Sterile Sodium Chloride 0.9% for Irrigation BP (for eye washing)
1 x tablet of Actichlor in 1 litre of water (Chlorine 100,000 ppm) or a virucidal agent such as 1% sodium hypochlorite or Virkon.

**Spillages**

1. Put on an apron, gown, fluid shield mask, shoe covers and 2 pairs of gloves.
2. Allow aerosols to settle; gently cover spill with paper towel and apply Actichlor or a virucidal agent such as 1% sodium hypochlorite or Virkon, starting at the perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up. Put all used wipes into the 5 litre sharps bin.
3. When all the liquid has been mopped up, wash and dry the area twice using Actichlor solution and lint free wipes. Place used wipes into the 5 litre sharps bin.
4. Remove outer pair of gloves, overshoes and apron, dispose of in the 5 litre sharps bin.
5. Clean goggles and return them to the spillage kit.
6. Seal the 5 litre sharps bin and label with a Biohazard GM Medicines Waste sticker.
7. Ensure that the spillage kit is replaced.

**In the event of spillage onto clothing**

1. Staff involved in handling IMP should wear standard RM uniform. Patients should be supplied with hospital clothing during administration. In the event of any spillage onto protective clothing, the contaminated garment should be removed and placed into a yellow sharps bin. This should then be sealed with biohazard tape.

**In the event of spillage onto the skin**

1. Any spillage on to the skin should be washed off immediately with soap and water or the skin decontaminated using alcohol hand rub.

**Action in Case of Inoculation/Eye Splash Injury**

Where there is an additional risk of exposure to blood-borne viruses, this SOP should be used in conjunction with the Trust policy 181 Blood-Borne Virus Prevention of Infection (Needlestick Procedures).

In the case of needle stick injury, spillage on broken skin or splash onto mucous membranes (eyes or mouth), use the following procedure:

1. Discard the needle and/or syringe into a designated sharps bin.
2. In the event of exposure to broken skin or needle stick, clean the site thoroughly with soap and water or a skin disinfectant. See a physician for monitoring for signs of infection. Acyclovir or other anti-viral drugs may be administered prophylactically.
3. In the event of accidental exposure through a splash to the eyes or mucous membranes, flush with sterile sodium chloride 0.9% or clean running water for at least 15 minutes.
4. Contact the relevant personnel to inform them of the injury (the Principal Investigator who will inform the GM Safety Committee) and inform Occupational Health.
5. Complete an Accident/Incident Form.
6. Occupational Health will assess the exposed individual with regard to blood-borne virus (BBV) and determine whether any further medical surveillance and/or treatment are indicated.
7. In the event of inoculation/eye splash injury out of hours, if there is a risk of BBV in addition to risk of exposure to RP1, Trust policy 181 Blood-Borne Virus Prevention of Infection (Needlestick Procedures) should be followed. The physician responsible for the care of the patient receiving the drug, or whoever is on call for the unit, will assess the exposed individual. Occupational health should be informed as soon as they are open.
Gene therapy product RP3 (Common name rHSV-1/GALV-GP-R-/ahCTLA-4/hCD40L/h4-1BBL) is an oncolytic immunotherapy product that is developed for the treatment of solid tumours.

RP3 is a selectively replication competent herpes simplex type-1 virus which expresses a truncated version of the gibbon ape leukaemia virus fusogenic glycoprotein (GALV-GP-R-), an anti-human CTLA-4 antibody-like molecule (ahCTLA-4), human CD40 ligand (hCD40L), and a human 4-1BB ligand (h4-1BBL).

The genes coding for neurovirulence factor ICP34.5 and the HSV immunomodulatory protein ICP47 are deleted. The ICP34.5 deletion allows the virus to replicate as a single agent and in combination with anti-PD1 therapy in patients with solid tumours as part of a Phase I, multicentre, open label clinical trial to investigate the safety and tolerability.
Host/vector system

RP3 (rHSV-1/GALV-GP-R-/ahCTLA-4/hCD40L/h4-1BBL) is derived from the RH018 strain of Herpes Simplex Virus-1 (HSV-1). RP3 is produced in Vero cells and virus is released into the culture media by budding from the cells and during cell lysis, prior to purification.

Origin & function

RP3 was constructed to enhance anti-tumour activity by using a new isolate of HSV-1 (strain RH018). The RP3 virus is armed with coding sequences to express a truncated fusogenic protein from gibbon ape leukaemia virus, GALV-GP-R-, an anti-human CTLA-4 antibody-like molecule (ahCTLA-4), human cluster of differentiation 40 ligand (hCD40L), and human 4-1BB ligand (h4-1BBL).

The neurovirulence factor, ICP34.5-encoding genes, and the ICP47-encoding gene are deleted from the virus. The ICP34.5 deletion allowed the virus to replicate selectively in tumours. ICP47 is a protein that inhibits the major histocompatibility complex (MHC) class 1 antigen presentation pathway by binding to the transporter associated with antigen presentation (TAP). The ICP47 deletion also results in the increased and earlier expression of the HSV-1 US11 gene. ICP34.5 deletion reduces virus replication somewhat in tumours, as well as preventing efficient replication in normal tissue. The increased and earlier expression of US11 partially overcomes the reduced replication in tumours, but without decreasing tumour selectivity.

RP3 was engineered with the following exogeneous genes:

- Truncated fusogenic envelope glycoprotein from gibbon ape leukaemia virus (GALV-GP-R-) with the R-sequence deleted (R-). GALV-GP R- causes cell to cell fusion resulting in cell death. The truncated R- version provides constitutive fusion activity, which is intended to be beneficial for tumour treatment. GALV-GP-R- expression is under the control of the RSV LTR promoter.

- Human CTLA-4 antibody-like molecule, under the control of human cytomegalovirus (hCMV) promoter. This is intended to block the interaction of CTLA-4 with B7, a negative T cell immune checkpoint, which otherwise inhibits the potency of the immune responses that are induced.

- Human CD40 ligand (CD154 or hCD40L) under the control of Moloney murine leukaemia virus (MMLV) LTR promoter. CD40L binds to the CD40 receptor present on antigen presenting cells (APCs) such as dendritic cells and macrophages. CD40L-CD40 interaction results in the maturation of dendritic cells, intended to result in increased cross presentation of tumour antigens to CD8+ T cells. Mature dendritic cells further activate CD8+ T cells by increased expression of immune co-stimulatory molecules, intended to lead to enhanced antitumour immunity and immunological memory formation.

- Human 4-1 BBL, under the control of mouse cytomegalovirus promoter (mCMV). The 4-1 BBL binds to the 4-1BB (CD137) co-stimulatory signalling receptor present on CD8+ T cells. The 4 1 BBL binds to the 4-1BB (CD137) co-stimulatory signalling receptor present on CD8+ T cells. The 4-1BBL-4-1BB engagement results in activation NF KB, AKT, p38 MAPK, and ERK pathways. These signalling pathways induce the expression of survival genes and decrease the expression of pro-apoptotic genes thus resulting enhanced anti-tumour T-cell activation, proliferation, and lymphocyte infiltration into tumours, is intended to lead to improved local and systemic anti-tumour effects.

- CD40L is a protein that is primarily expressed on activated T cells and is a member of the TNF superfamily of molecules. It binds to cluster of differentiation 40 (CD40) on antigen-presenting cells. CD40L binds to the CD40 receptor present on antigen presenting cells (APCs) such as dendritic cells and macrophages. CD40L-CD40 interaction results in the maturation of dendritic cells, intended to result in increased cross presentation of tumour antigens to CD8+ T cells. Mature dendritic cells further activate CD8+ T cells by increased expression of immune co-stimulatory molecules, intended to lead to enhanced antitumour immunity and immunological memory formation.

- Human 4-1 BBL is also found on antigen presenting cells and binds to h4-1BB, a type 2 transmembrane glycoprotein receptor belonging to the TNF superfamily, expressed on activated T lymphocytes particularly CD8+ T cells, and induces secretion of high levels of IFN-γ by binding to 4-1BB protein. The 4-1BBL binds to the 4-1BB (CD137) co-stimulatory signalling receptor present on CD8+ T cells. The 4-1BBL-4-1BB engagement results in activation NF-KB, AKT, p38 MAPK, and ERK pathways. These signalling pathways induce the expression of survival genes and decrease the expression of pro-apoptotic genes thus intended to promote CD8+ T cell activation, survival, and increased cytokine production leading to augmented antitumour immunity.
intended to promote CD8+ T cell activation, survival, and increased cytokine production leading to augmented antitumour immunity. The initial clinical trial protocol intends to test RP3 in several tumour indications as a monotherapy and in combination with anti-PD-1 therapy.

**Evaluation of foreseeable effects**

No studies have yet been conducted with RP3 and therefore no data is available. HSV-1 is a proven virus for development into a safe and effective oncolytic therapy. Beneficial characteristics include:

- The large 152 Kb genome has non-essential genes that can be replaced with therapeutic genes or removed;
- Infection of many cell types, including tumour cells across species followed by the destruction of the cell by viral replication;
- The availability of effective anti-viral drugs to ameliorate any non-therapeutic viremia;
- The viral genome does not integrate into the host genome. From a safety perspective, wild type HSV-1 only rarely produces severe illness in immune competent adults.

The therapeutic strategy is to induce direct viral "oncolytic" destruction of tumours that is enhanced compared to previous oncolytic agents and combined with the enhanced induction of a systemic antitumour immune response mediated by the inclusion of anti-CTLA-4 antibody-like molecule and the immune co-stimulatory ligands hCD40L and h4-1BBL. This is intended to provide overall and long-term clinical benefit. The broad antitumour response that is anticipated, including to tumour neo-antigens, is expected to be synergistic with immune checkpoint blockade agents such as those targeting PD-1/programmed cell death ligand (PD-L1).

RP3 is intended for direct injection into non-neurological solid tumours.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation is requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All used and unused vials of RP3 will be disposed of per institutional policies and procedures and in line with the RP3 IB. Spills should be treated with virucidal agents, such as, e.g., 70% isopropanol, 1% sodium hypochlorite or Virkon solution. All materials contaminated with RP3 will be disposed of in compliance with local institutional polices and SOPs. Incineration is appropriate.

In the event of exposure to broken skin or needle stick, the site will be cleaned thoroughly with soap and water or a skin disinfectant. A physician for monitoring for signs of infection and occupational health will be contacted for support. Acyclovir or other anti-viral drugs may be administered prophylactically.

Patients will be provided full instructions and support from the Principal and co-investigators which will include care of the lesions and advice on shedding. Patients will be provided with spare dressings, instructions for use and safe disposal equipment which will be returned to the clinical site. In addition, the patients are given out of hours contact numbers and advice will be available from the clinical site team, if needed. All advice given will be in line with the sponsor instruction and the study protocol.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Please enter comments on the GM safety committee on the risk assessment**

The Royal Marsden NHS Foundation Trust GMSC classified as Class 2.
A Phase 1/2a, Multicenter, Open-label Trial of TBio-6517, an Oncolytic Vaccinia Virus, Administered Alone and in Combination with Pembrolizumab, in Patients with Advanced Solid Tumors
The GMO (TBio-6517) is an oncolytic vaccine virus and is released in accordance with a clinical Trial Protocol Number TBio-6517-TUu001. The study is a Phase 1/2a, Multicenter, Open label Trial of TBio-6517, an Oncolytic Vaccinia Virus. Administered alone and in Combination with Pembrolizumab, in Patients with Advanced Solid Tumors.

It consists of a novel backbone named SKV built on clinical experience with first generation oncolybc vaccinia viruses. SKV was isolated from naturally-occurring variants of the parental Copenhagen stain of Vaccinia Virus. Compared to the parent vaccinia virus, TBio-6517 has several of the stamina genes removed, leading to tumor cell selectivity.

TBio-6517 expresses synthetically-driven transgenes encoding human anti-CTLA-4 antibody (ipilimumab), human FLT3L and human membrane bound IL-12p35. Details on the nature and Source of inserted genetic material are provided below.

**Upend**: FLT3L:
- Expends intratumoural CD141+ BatF3 DC, a potent cross presiding DC to facilitate epitope spreading
- Reduces T-cell attracting chemokines to 'inflame' turnout
- Associated with anti-PD1 responsiveness

**Antibody Anti-CTLA-4**
- Lowers threshold for T-cell activation
- May deplete Tregs

**• Targets subsets of exhausted CD8**

**Synergizes with anti-PD-1/PD-L1 immunotherapy**

**Cytokine: Membrane-bound IL-12p35:**
- Promotes Th1 and GD8+ cytotoxic T cell responses
- Activates innate immunity, including NK cells
- Reverses immune suppression of dysfunctional myeloid cells
- Abrogates suppressive activity of Treg cells
- Promotes priming via IFN-g mediated increase in MHC I processing and presentation

Encoding of these 3 transgenes in TBio-6517 restricts expression in tumor cells and thereby facilitates the creation of high local concentrations of these immune modulatory agents (IMAs) in the tumor microenvironment.

The modifications result in an attenuated, selective, replication competent organism which results in immunodulation of tumor cells.

The recipient of the GMO are adults over the age of 18 years who have existing advanced solid tumors. There are specific inclusion and exclusion criteria (identified in the protocol) which identifies a patient's suitability to fit in with the study population.

**DC** = Dendritic cell;  **NK** = Natural killer;  **Treg** = Regulatory T Cell

### Evaluation of foreseeable effects

There are no pathogenic properties in TBio-6517 that are not found in wild-type vaccinia virus. The genetic modifications in TBio-6517 does not replicate within the cell nucleus and as a result there is no integration into the host genome.

TBio-6517 selectively replicates within tumors.

Shedding and pustule formation will be monitored.

Horizontal transmission is not expected, but will also be monitored.

Replication and survival in the environment is not expected.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Because TBio-43517 is easily inactivated (as wild-type vaccinia is), testing/monitoring of the inactivation process has not been performed
Infectivity of the wild-type Vaccine virus, and thus TBio-6517, is reliant in part to its intact envelope. therefore, any chemical agents (liquid solvents and mild detergents) or physical inactivation (dehydration, heat, low pH) that disrupts the envelope reduces the infectivity.
All disposable materials which come into direct contact with TBio-6517 should be disposed of in standard biohazardous or sharps waste. This includes any syringes, needles, bandages etc. Non-disposable items such as medical equipment, linens etc. should be sterilized or laundered per hospital policy.
TBio-6517 can be inactivated through a variety of physical and chemical means. The US Centers for Disease Control (CDC) recommends that standard universal precautions and institutional policies be followed for cleaning and decontamination whilst handling Vaccinia virus based products.
Examples of chemical agents that have demonstrated inactivation properties include:
• At least 60% alcohol
• Bleach solution (with at least 0.6% of active chlorine) In the context of BSL-2 hood or demo hood use. it is recommended to follow with alcohol to prevent corrosion
• Other institutional recommended agents, hospital-grade disinfectants. or virocidals, such as but not limited to 1% cresylic acid, phenolics. chlonnated phenol. 2 5% phenol, 0.4% HCl, 7% sodium orthophenylphenate. and sodium hypochlorate
• The manufacturer's instructions should be followed to ensure adequate contact time and confirm the ability of the equipment to withstand the disinfectant used. All contaminated material should be disposed of in a clearly-marked biomedical waste container and discarded according to regular institution procedure for infectious waste.

Examples of physical inactivation methods include:
• Autoclave
• Hot soapy water (>71 degs C). Textiles and fabrics can be laundered in hot water (>71 degs C) with detergent and hot air drying. TBio-6517 should be transported in an appropriate sealed container. preferably shielded from light and labelled as a biohazardous material.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Recipient or parental organism:
Replication competent herpes simplex type-1 virus that is derived from HSV1 strain JS-1 (ECACC No. 01010209). The OncoVEX(GM-CSF) vector is deleted for the neurovirulence factor, ICP34.5. Deletion of this gene provides a virus that is non-pathogenic while providing the property of tumour selective replication. The vector is also deleted for ICP47, which blocks antigen presentation by blocking the transporter associated with antigen processing (TAP1 and TAP2). The vector also contains the...
coding sequence for human GM-CSF, a cytokine involved in the stimulation of T-cells. Human GM-CSF gene was cloned from an IMAGE clone 2340997/5808-K14 (UK HGMP Resource Centre). GM-CSF expression is under the control of the human cytomegalovirus immediate early promoter (HCMV IE). GM-CSF occurs normally in the human body. This cytokine is a licensed pharmaceutical product that is used to aid myeloid recovery following bone marrow transplant and chemotherapy, GM-CSF rarely causes anaphylaxis and cardiovascular disorders. Patients with congestive cardiac failure and renal insufficiency will be excluded from the clinical trial. However, only very low-level systemic levels of GM-CSF are expected following intra-tumoural inoculation of the virus where GM-CSF will be locally produced. Thus toxic effects associated with high levels of systemic GM-CSF are not anticipated.

The deletion of ICP47 and the expression of GM-CSF should enhance the immune response towards tumour cells. GM-CSF expression is under the control of the human cytomegalovirus immediate early promoter (HCMV IE). This is an infectious gene therapy product that replicates selectively in rapidly dividing eukaryotic cells. The parent vector is ACDP category 2 and ACGM level 2 containment is appropriate for OncoVEX(GM-CSF) vector due to the conditional replication competence of the vector, and the expression of a biologically active cytokine.

Host/vector system

Replication competent herpes simplex type-1 virus that is derived from HSV1 strain JS-1 (ECACC No. 01010209). The OncoVEX(GM-CSF) vector is deleted for the neurovirulence factor, ICP34.5. Deletion of this gene provides a virus that is non-pathogenic while providing the property of tumour selective replication. The vector is also deleted for ICP47, which blocks antigen presentation by blocking the transporter associated with antigen processing (TAP1 and TAP2). The vector also contains the coding sequence for human GM-CSF, a cytokine involved in the stimulation of T-cells. Human GM-CSF gene was cloned from an IMAGE clone 2340997/5808-K14 (UK HGMP Resource Centre). GM-CSF expression is under the control of the human cytomegalovirus immediate early promoter (HCMV IE). GM-CSF occurs normally in the human body. This cytokine is a licensed pharmaceutical product that is used to aid myeloid recovery following bone marrow transplant and chemotherapy, GM-CSF rarely causes anaphylaxis and cardiovascular disorders. Patients with congestive cardiac failure and renal insufficiency will be excluded from the clinical trial. However, only very low-level systemic levels of GM-CSF are expected following intra-tumoural inoculation of the virus where GM-CSF will be locally produced. Thus toxic effects associated with high levels of systemic GM-CSF are not anticipated.

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As regards the safety of this vector, there are a number of specific relevant points:
* The vector is deleted for ICP34.5 & ICP47, deletion of both of which renders the virus non-pathogenic.
* HSV and HSV vectors do not to integrate into the host DNA.
* The HSV thymidine kinase (TK) gene is intact in OncoVEX (GM-CSF) and expressed from the vector. This renders the viruses sensitive to anti-viral agents such as acyclovir, which can be used to block virus replication in the unlikely event that this is required.

There is no anticipated hazards should release of OncoVEX(GM-CSF) into the environment occur. However, procedures will be used to prevent contamination of the environment with OncoVEX(GM-CSF) even though (i)there is no anticipated hazard of OncoVEX(GM-CSF) to the environment and (ii) OncoVEX(GM-CSF) would not be expected to be able to survive in the environment if release occurred.

Origin & function

Replication competent herpes simplex type-1 virus that is derived from HSV1 strain JS-1 (ECACC No. 01010209). The OncoVEX(GM-CSF) vector is deleted for the neurovirulence factor, ICP34.5. Deletion of this gene provides a virus that is non-pathogenic while providing the property of tumour selective replication. The vector is also deleted for ICP47, which blocks antigen presentation by blocking the transporter associated with antigen processing (TAP1 and TAP2). The vector also contains the coding sequence for human GM-CSF, a cytokine involved in the stimulation of T-cells. Human GM-CSF gene was cloned from an IMAGE clone 2340997/5808-K14 (UK HGMP Resource Centre). GM-CSF expression is under the control of the human cytomegalovirus immediate early promoter (HCMV IE). GM-CSF occurs normally in the human body. This cytokine is a licensed pharmaceutical product that is used to aid myeloid recovery following bone marrow transplant and chemotherapy, GM-CSF rarely causes anaphylaxis and cardiovascular disorders. Patients with congestive cardiac failure and renal insufficiency will be excluded from the clinical trial. However, only very low-level systemic levels of GM-CSF are expected following intra-tumoural inoculation of the virus where GM-CSF will be locally produced. Thus toxic effects associated with high
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The study will be a single arm open label upward titration of up to three dose levels of OncoVEX(GM-CSF) in combination with radiotherapy and concomitant cisplatin in the treatment of patients with locally advanced stage III and IV head and neck cancer with one or more nodal metastasis in the neck (N3-N3). Treatment with OncoVEX(GM-CSF) will be scheduled to commence on the day on which chemoradiotherapy begins. Patients will be admitted to the outpatients department or a day ward for all injections of virus. The injections will always be performed in a side room where the trial subjects can be segregated from other patients for safety reasons. Groups of four evaluable patients will be treated with dose levels of 10 (to the power of 6) pfu/mL on four occasions, 10 (to the power of 6) pfu/mL on one occasion followed by 10 (to the power of 7) pfu/mL on three occasions and 10 (to the power of 6) pfu/mL on one occasion followed by 10 (to the power of 8) pfu/mL on three occasions. The first dose of virus will be up to 2 mL per tumour (4 mL in total) of the dosage strength 10 (to the power of 6) pfu/mL given on the same day that treatment with cisplatin and radiation begins. Subsequent doses of virus (which will always be administered to the same nodes) will consist of a volume of up to 4 mL in weeks 3 (day 21), 6 (day 42) and 9 at a dosage strength of either 10 (to the power of 6), 10 (to the power of 7), or 10 (to the power of 8) pfu/mL depending on the dose level to which the patient is allocated. The timing of these doses is designed to avoid chemotheraphy-induced nadirs in the white cell count. The initial dose strength of 10 (to the power of 6) pfu/mL will be used in order to allow the patient to become tolerant to HSV1. Each injection will then be made, under local anaesthetic, along multiple tracks in order to disperse the vector throughout the tumour. Following injection, the injected tumour will be covered with a double occlusive dressing (Tegaderm) that must be kept in place for 24 hours. The injected tumour(s) will be swabbed 24 and 48 hours after injection in order to ensure that there is no shedding of virus from the injection site. If shedding or weeping is detected, the dressing must be replaced and changed every 24 hours, with the swabs repeated every 24 hours until shedding has ceased for at least 72 hours.

During the chemoradiotherapy the following clinical safety laboratory tests will be obtained at weekly intervals (including those tests taken prior to OncoVEX(GM-CSF) injection): Haematology (full blood count), clinical chemistry (including urea, creatinine and electrolytes, liver function tests). The following immunology laboratory tests will be obtained before the second, third and fourth injections of OncoVEX(GM-CSF): HSV-antibodies, antinuclear antibody (autoantibody). There will be a final follow-up visit one month after the week 10 investigations or one month after surgery. At this time, a physical examination will be conducted and blood samples will be obtained for haematology (full blood count), clinical chemistry (including urea, creatinine and electrolytes, liver function tests) and HSV-1 antibodies, antinuclear antibody (autoantibody). Adverse events and concomitant medications will be documented from the time of screening until the patient comes off study.

**Evaluation of foreseeable effects**

Treatment with OncoVEX(GM-CSF) will be scheduled to commence on the day on which chemoradiotherapy begins. Patients will be admitted to the outpatients department or a day ward for all injections of virus. The injections will always be performed in a side room where the trial subjects can be segregated from other patients for safety reasons. Before each injection, the ECOG score will be recorded. Vital signs (core temperature, heart rate, blood pressure) will be measured and recorded. The patients will be examined for the presence of active herpes labialis. If this is present, the injection must be delayed until healing occurs. Blood will be obtained for clinical chemistry and haematology. The patients will also be questioned about any symptoms that they are experiencing at the time and any medications they may be taking. Before the first injection, the node(s) that will be injected will be identified and allocated a tumour number. Where appropriate, the node(s) that will not be injected will also be identified and numbered. All nodes will be palpated and evaluated for injection site reaction prior to injection.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| N/A |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste generated by trial will be autoclaved prior to being incinerated as clinical waste by the Trust waste contractor White Rose. All small materials (eg syringes, sharps)
will be soaked in a virucidal agent (such as Virkon 10%) for 30 minutes prior to autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

GSCM - Action points

HSV-GMCSF
* HSE also aware of trial. Would like a full report to the HSE - Actioned
* Dressing type to be confirmed on patient information letter - actioned
* Final version of patient letter to be confirmed - actioned
* BG discussed concerns over blood samples taken for staff from Occ Health and where these may be stored. Also how staff with needlestick injuries will be monitored KH felt that the risk is minimal, however will confirm action. MG felt that a swab should be taken if an abnormal lesion should develop on a staff member - actioned
* KH to amend paragraph two on the first page of GMCSF study relation to deletions - actioned
* SOP's to be made available on Weston Ward, Labs and Radiotherapy - actioned

Project Containment

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GM Centre Number: 649

Data Premises Notified (Originally) 22/08/1997

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed 28/02/2011

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

TAUNTON AND SOMERSET FOUNDATION TRUST

Name 2

MUSGROVE PARK HOSPITAL

Department

PATHOLOGY

Campus Estate or Research Centre

Building

Road Name

District

Town

TAUNTON

County

SOMERSET

Postcode

TA1 5DA

Country

ENGLAND

Tel Number 01823 342298

Fax Number 01823 271023

E-mail

HSE Division WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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Tick if confidential

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<td>Gene Therapy</td>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum culture volume that could be released at one time is 100 ml. All waste (solid and liquid) is put through a standard steam pressure autoclave on site. The autoclave is maintained and monitored by Public Health Laboratory Services.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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| **Tel Number** | **0207 942 4000** | **Fax Number** | **0207 942 4202** | **E-mail** | **HSE Division** | **LONDON** |

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
### Mycology

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Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 651**

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**Name**

TOPO TARGET UK LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

87A MILTON PARK

**Town**

ABINGDON

**District**

OXFORDSHIRE

**Country**

ENGLAND

**Postcode**

OX14 4RY

**Tel Number**

01235 443700

**Fax Number**

01235 835557

**HSE Division**

EAST AND SOUTH EAST

**Comments**

CHANGED COMPANY NAME FROM PROLIFIX TO TOPO TARGET ON 11/07/2003 - MOVED PREMISES ON 13/1/2003

**Date at Which Additional Info Submitted**

22/05/2002 10/12/2002
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- **Give brief details of the genetic modification safety committee**

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

- **Tick if confidential**

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
For activities involving GMMs, describe the waste management measures which will apply to the activity

**Tick to confirm that you are attaching a summary of the risk assessment**

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 651/01.1**

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**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The identification of proteins essential for cell cycle regulation.

**Recipient or parental organism**

- DH5x - Non-colonising disabled E. coli K12 derivative - Considered to be containment level 1.
- JM109 - Non-colonising disabled E. coli K12 derivative - Considered to be containment level 2.
- BLR (DE3)pLysS - Disabled BL21 derivative - Considered to be containment level 1.
- Rosetta - Disabled Turner derived strain - Considered to be containment level 1.

**Host/vector system**

Each of the DH5x, JM109, BLR (DE3)pLysS or Rosetta hosts will be transformed with a single vector contained within the lists below:

- Non-mobilisable vector series: pGEX, pGEM, pcDNA3.1, Bluescript, pACT, pBind, pGAD424, pGBT9, pFastBac, pYX or pRS.
- Mobilisation defective vector series: pET or pMAL.

**Origin & function**

DNA used in this project will be from human, mouse and yeast sources.

Products from such genes are expected to regulate the cell cycle.

**Evaluation of foreseeable effects**

The usage of non-pathogenic disabled and/or non-colonising bacteria in accordance with good microbiological practice, will limit human and environmental contamination, and as such pose a negligible risk to both. The provisional requirement for containment is therefore level 1.

The majority of genetic material used in this study has not been reported or believed to be oncogenic in nature, however, due to the cell cycle regulatory nature of such proteins, Prolifix wishes to assign containment levels relevant to oncogenes for all genes currently used within the company. Inclusion of such genes into the listed host/vector systems would not be expected to effect the host pathogenicity. However, classifying such genes as oncogenic, should raise the level of containment to level 2. All work will therefore be carried out in accordance with Schedule (8), table 1a, outlined in ‘A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000’.

Worst case scenario - gut infestation of transformed bacteria.

The maximal viability of disabled bacteria in the gut is around seven days. During this period it is possible that the bacteria may express the cloned gene as a fusion protein. It is however unlikely that the proteins will be pharmacologically active, due to the inclusion of a fusion protein and/or the lack of post-translational modification in the chosen bacteria. It is also envisaged that the bulk of expressed protein will be present as insoluble inclusion bodies.
The choice of non-mobilisational and mobilisation defective vectors will make the risk of vector transfer to normal gut flora negligible. No genetic modifications undertaken will result in increased mobilisation status of the vector carrying the insert.

There is a theoretical risk of plasmid transfer to a contaminated human host, however the frequency of such an occurrence has been shown to be extremely low. Such a transfer would constitute a very low risk of human cell transformation, should the gene be expressed in the contaminated individual. However, since tumour formation arises as a result of a number of genetic modifications and gene deletions, the chance of tumour development in an infected host is negligible.

Fragments of genes will also be inserted into the same host/vector systems as detailed for whole genes. However, in these cases the risks involved would be expected to be that of the host (level 1), as the fragments will be less pharmacologically active and not capable of altering the pathogenicity of the host. However, such host/vector systems will be confined to containment level 2.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All bacterial pellets and bacterial contaminated material will initially be treated with Virkon at 1:200, which has been validated to give 100% kill against 22 E. coli strains when used in accordance with the manufacturers instructions. All waste will then be autoclaved in a routinely calibrated autoclave.

Bacterial supernatant resulting from bacterial culture centrifugation will be treated with Virkon at 1:200 in accordance with the manufacturers instructions, which will then be disposed of via the laboratory sink.

**Is an emergency plan required according to regulation 20?**

- [ ] Y

If yes, tick to confirm that it is attached to this form

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] N

### Please enter comments on the GM safety committee on the risk assessment

The Safety Committee were satisfied that the proposed level 2 containment procedures are suitable for the notified GMM work to be safely conducted at Prolifix.

### Project Containment

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02/03/2022
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GM Centre Number: 652

Data Premises Notified (Originally) 08/09/1997

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises N

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

NORTH BRISTOL NHS TRUST

Name 2

SOUTHMEAD HOSPITAL

Department

CYTOGENETICS CLINICAL CHEMISTRY

Campus Estate or Research Centre

REGIONAL CYTOGENETICS CENTRE

Building

Road Name

District

Town

BRISTOL

County

AVON

Postcode

BS10 5NB

Country

ENGLAND

Tel Number 0117 950 5050

Fax Number 0117 959 5572

E-mail

HSE Division WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Level 4 (GMMs)

Non-microbial

Other (please specify)

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<td>Gene Therapy</td>
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<td>Mycology</td>
<td>Transgenic Invertebrates</td>
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<td>Other (please specify below)</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum culture volume released at one time = 100 mls.
Waste deactivated in 10% hypochlorite for 24 hours, then autoclaved within Cytogenetics Centre.
Validation and monitoring of deactivation: For each autoclave run, the type of waste is recorded together with temperature and time of that particular run (by chart recorder).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Comments**
Bayer was bought out by Microbial Developments Ltd in 2004

**Date at Which Additional Info Submitted**
02/03/2022
### Premises Addresses

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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 654

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#### Name

THE LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

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#### Comments

Date at Which Additional Info Submitted

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**Significant Change**

654/01.3a

**Date of Additional Information (significant change only)**

01/02/2022

**Premises Addresses**

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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Give brief details of the genetic modification safety committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity

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Tick to confirm that you are attaching a summary of the risk assessment

02/03/2022
### Project Additional Information

**Purposes of the contained use**

The purpose of this work is to study the biological function of genes expressed in the human malaria parasite *Plasmodium falciparum*. Work is primarily focused on genes involved in development of the sexual stage (transmitted by mosquito) of the parasite, but also aspects of the biology of the asexual forms are of interest. Our main approach is the use of gene deletion but also tagging of certain genes is used to study their localisation. The long term objectives of the study are to develop new effective anti-malarial drugs to prevent human disease. Work may also be carried out on a rodent malaria species (*Plasmodium berghei*) which does not infect humans.

**Recipient or parental organism**

Our aim is to produce mutant malaria parasites which are deficient in specific signal transduction molecules and are consequently unable to develop into specific life cycle stages. These organisms will therefore be attenuated to a degree and less viable.
Plasmodium falciparum is the most serious form of the malaria parasites to infect humans. In non-immune individuals, infection with the parasite can be fatal if not treated with drugs. The organism is therefore used under ACDP category 3 conditions at the LSHTM. There are several effective, safe drugs available which clear infection rapidly. Should an increase in resistance to a particular drug occur, alternative drugs with different mechanisms of action could be used. The chances of infection arising from the laboratory, however, are extremely low and would only result from injection to the organism directly into the blood stream.

Our experiments involve deletion or tagging of specified genes which usually results in decreased viability (or no change in viability) of the organism. It is therefore highly likely that infection with the unmodified organism would be the worst possible scenario. In other words, the modified organism is unlikely to be more hazardous than the wild type. Person to person infection does not occur. Transmission can only occur via an infected Anopheles mosquito which is maintained under strict laboratory conditions (controlled temperature, humidity and feeding) for 7-10 days. Infective malaria parasites can only survive in the blood stream or in sealed flasks containing human blood under strict conditions. It would therefore be impossible for the parasite cultures to come into contact with a mosquito.

**Host/vector system**

The plasmid vectors we use in these experiments which result in gene deletion or tagging are maintained by selective drug pressure, but are quickly lost on removal of the drug. Integration events maintain parts of the plasmid backbone which are responsible for propagating the plasmid in attenuated laboratory bacteria (under containment level one conditions).

**Origin & function**

Genes encoding green fluorescent protein used to study localisation of plasmodial proteins are obtained from commercial sources. Drug selectable marker genes are from heterologous sources for example the rodent malaria parasite species P. berghei.

The majority of the studies in our lab focus on enzymatic components of signal transduction pathways to gain an understanding of what controls progression of the parasite from one stage of its life cycle to another. Genes encoding these enzymes are amplified from P.falciparum itself by PCR.

**Evaluation of foreseeable effects**

The experiments that we will perform will attempt to produce forms which cannot develop into specific life cycle stages. In this way we hope to discover the genes which control the processes. Production of such forms would clearly reduce virulence.

It is highly likely that experiments will result in genetically modified organisms that are less virulent than the unmodified forms. In the extremely unlikely event that modified organisms had unexpected effects on drug sensitivity, several different types of malaria drugs are available which have different mechanisms of action (and targets in the cell). It is only in malarious areas of the world with high levels of malaria transmission, where a patient could be infected with multiple different clones (with resistance to more than 1 drug) of the organism, resistance to drugs is a problem. In the lab, infection with even 1 type of malaria parasite is an unlikely event.

When working with P. falciparum all necessary precautions are taken to avoid becoming infected with the organism. This is the case whether or not it has been genetically modified. It is extremely difficult to get infected in the lab because sharps objects are not used in a malaria culture lab. If suspected infection did occur then diagnosis could be quickly carried out and the necessary drug or drug combination prescribed. Infection would be quickly cleared and would not recur. The specified of human malaria parasite which recur cannot be cultured in the laboratory.

Since sharps are excluded from malaria culture labs, stab wounds should not occur. If they were to occur whilst using the organisms, diagnosis and appropriate drug treatment would be applied. The LSHTM is the home of the malaria reference centre where advice is given to people all over the world on which drugs to take and so local expertise is available.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste containing the organisms is decanted directly into a 10% solution of chloros and left there until the next morning before autoclaving even though contact with the solution will kill the organisms instantly. Malaria parasites are extremely sensitive to culture conditions. Even a slight rise in pH of the culture medium will kill them. Spillage is treated with a 70% ethanol spray which kills the organisms immediately. Solid waste is placed directly into autoclavable bins lined with bags. These are sealed and autoclaved following recommended procedures for ACDP category 3 containment. Autoclave tape indicates that the process has occurred. In all cases 100% killing is achieved.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All procedures have been approved by the appropriate safety committees.

Project Containment

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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 654/01.3

Date Ackn'd 21/02/2001

Date Project Ceased

CU2 Project Title GENETIC TRANSFORMATION OF TRYPANOSOMA CRUZI AND LEISHMANIA

Class 3

Non-GMM Consent Granted yes

Culture Volume Class 3-4 upto 100 ml
The purpose of this programme of work is to study gene function in the related insect transmitted protozoan parasites Trypanosoma cruzi and Leishmania, particularly in relation to drug-resistance and infectivity. GM techniques are used to investigate genes encoding components of the oxidative defence system, the major surface glycoproteins, components of intracellular signalling pathways, and the major cysteine proteinase. Other experiments involve tagging parasites with marker genes to study subcellular localisation, immunological processes and mechanisms of sexual reproduction. The long term aim of this work is to develop better approaches to chemotherapy and vaccine design by gaining a greater understanding of parasite biology at the molecular level.

T. cruzi (the causative agent of Chagas' disease) is designated ACDP category 3 and Leishmania sp. (causative agents of visceral or cutaneous leishmaniasis) as category 2 or 3 depending on the sub-species. At LSHTM we use category 3 facilities for all Leishmania work. The experiments undertaken fall into 3 main types; those that could potentially result in gain of function (over-expression of endogenous genes or complementation) or a loss of function (gene knockout) or those that are likely to be neutral (expression of marker genes such as those encoding as firefly luciferase or green fluorescent protein). In gene knockout experiments the resulting recombinant organisms are likely to be deficient in some aspect of infectivity. Phenotypes associated with overexpression of components of the oxidative defence system (eg peroxidases) could include increased drug resistance, although this has not been observed. Complementation experiments are carried out on organisms that are attenuated or otherwise deficient in a defined function. In this case GM procedures are unlikely to produce an organism more hazardous than the wild-type.

The major risk associated with this programme, irrespective of the GM procedures, is infection of the worker. This risk is minimal if category 3 guidelines are followed. Infection with these parasites can only occur via broken skin or mucosal membranes. In addition parasites do not survive desiccation, are not free living and are not transmissible by any known insect vector in the UK. For these reasons the chances of environmental damage arising from this work can be regarded as remote or non-existent.

The overexpression experiments are performed with specific episomal vectors (pTEX, pcosTL) that are not maintained in T. cruzi or Leishmania in the prolonged absence of selective drug pressure. Gene deletion experiments are performed with linear DNA molecules that only function after integration into the parasite genome.

The genes used as drug-selectable markers are those that confer resistance to G418, hygromycin, phleomycin and puromycin. These bacterial genes were obtained from commercial sources.

Genes encoding red and green fluorescent proteins are used to investigate subcellular localisation in T. cruzi and Leishmania. Those encoding firefly luciferase and bacterial chloramphenicol acetyl transferase (CAT) are used for markers of gene expression in transfected parasites. Expression of mammalian ovalbumin in Leishmania
Donovani and T. cruzi is used as a marker for antigen processing.

Studies of oxidative defence in Trypanosoma cruzi involve modulating the expression of superoxide dismutase and thiol dependent peroxidases. The role of these enzymes in protecting the parasite from reactive oxygen species is being studied. Components of the adenylyl cyclase signal transduction pathways and members of the WD40 family are being investigated to determine their function in life-cycle regulation. Cruzipain, the major cysteine proteinase on the surface of T. cruzi has been postulated to have a role in host cell invasion and differentiation. This is being investigated by gene-knockout.

**Evaluation of foreseeable effects**

Most experiments (eg gene deletion) will result in genetically modified organisms that are less virulent or infectious than wild-type. Other procedures involving the expression of marker genes, as outlined above, are unlikely to affect the virulence phenotype. Some procedures, such as those involving expression of components of the T. cruzi oxidative defence system, may result in parasites with increased resistance to the drugs nifurtimox and benznidazole. However this outcome can also be achieved using more classical methods such as growing parasites in increasing concentrations of the corresponding drug.

Over-expression of proteins that have a role in regulatory events could enhance the ability of culture from T. cruzi epimastigotes (the non-infectious insect form of the parasite on which most GM experiments are performed) to develop an infectious metacyclic phenotype. In non-transfected cells of the X10/6 or CL Brener strains with which we work, typically 5-10% of the cells will develop into metacyclics after 12 days in culture. With other strains however, values of 70-80% are observed, a figure unlikely to be exceeded by recombinants of the X10/6 or CL Brener strains.

The control measures used when handling these organisms are in accordance with ACDP guidelines. All work is carried out in a category 3 containment laboratory with a class II microbiology safety cabinet. To prevent contamination stringent requirements including the wearing of goggles, gloves and face masks are implemented. As a matter of course, each worker is serologically tested every six months and advised that they should not act as a blood donor. These measures are designed to eliminate, as far as possible, the chance of accidental infection of laboratory workers. The chance of further spread can be regarded as extremely remote.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All cultures and waste materials are treated in situ by immersion in chloros (a powerful bleach), and then autoclaved. Culture flasks/vessels are treated with 70% ethanol, then chloros, then autoclaved. Each of these steps results in 100% killing. Autoclaves are monitored for temperature and pressure. Autoclave tape confirms treatment.

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**Please enter comments on the GM safety committee on the risk assessment**

All procedures have been approved by the appropriate safety committees.

02/03/2022
Purposes of the contained use

The purpose of this is to generate or use available reporter gene mutants of Streptococcus pneumoniae, Pseudomonas aeruginosa and other respiratory pathogens to follow their uptake and handling by the innate immune system. In other experiments the genes that encode proteins on the bacterial surface which interact with innate immune proteins will be deleted. The long term aim is to understand of mechanisms of early resistance to infection.

Recipient or parental organism

S pneumoniae, Burkholderia cepacia, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae are category 2 organisms. They will be derived from antibiotic susceptible isolates. For example the S pneumoniae strains 3 and 6B used in experiments will be penicillin susceptible. Experiments involving reporter genes
are extremely unlikely to alter virulence. In all cases so far reported this has provided to be the case. In gene knock-out experiments the resulting organisms are likely to be deficient in some aspect of infectivity. Complementation experiments in which the deleted gene is added back will be required if a "knock-out" phenotype is obtained. This is unlikely to generate an organism more hazardous than the wild-type.

Host/vector system

Deletion experiments will be accomplished with vectors incapable of autonomous replication in S pneumoniae or similar recipient organisms. Transformation results from integration by homologous recombination and selection is based on chloramphenicol resistance. Standard treatments of pneumonia have to assume that S pneumoniae is a potential cause and recommendations are a macrolide or doxycycline, or a newer fluoroquinolone for mild disease with little risk. A macrolide or extended spectrum cephalosporin or coamoxiclav is recommended for more severe disease or risk (Infectious Disease Society of America (2000) [ Bartlett et al (2000) Clin Inf Dis., 31, 347].

Origin & function

The aim of this project is to study the immune response to S pneumoniae, Ps aeruginosa, B cepacia, K. pneumoniae and H. influenzae which are respiratory pathogens. The reasons for examining this range of organisms is that they are bound by proteins of the innate immune proteins. It is the function of these proteins that we wish to test. This will be aided by the generation of a modified form of these bacteria expressing reporter genes green or red fluorescent protein or luciferase.

Deletions of genes that code for bacterial surface proteins or genes that code for enzymes that synthesise bacterial surface carbohydrate will generate bacteria which would typically have altered adherence properties or capacity to evade immune systems such as complement. In specific cases mutants would be generated that no longer interact with innate immune proteins such as C-reactive protein. There is no evidence that this or other such changes makes bacteria more virulent. Examples of this might be genes responsible for capsule formation in S pneumoniae. In the case of Ps aeruginosa an as yet unidentified gene that generates a protein of 35 kD would be deleted to generate a mutant that did not interact with innate immune proteins. These can be used to confirm the role of specific components of the innate immune response. Mutants will be used in vitro and in vivo experiments of resolving infection to examine immune response.

Evaluation of foreseeable effects

Reporter genes, GFP, RFP and luciferase have been used in many other systems. They have not been found to have toxic effects either on the modified organism or following introduction into a mammalian system. Examples include GFP in S pneumoniae [Kadioglu et al, (2001) FEMS Microbiol Lett 194, 105-10; ]; Luciferase in S pneumoniae [Francis et al., (2001) Infect and Immunity, 69, 3350] or GFP in Pseudomonas aeruginosa [Bloemberg et al., (1997) App and Env Microbiology, 63, 4543]. Gene deletion will be expected to have a neutral or negative impact on virulence. This should be reversed to wild type virulence on complementation (eg pneumolysis or autolysin in S pneumoniae; Berry et al., (1992) Microb Pathog 12:87-93). In another example of 126 deletion mutants of S pneumoniae, 36 were as virulent as the wild type and all others showed reduced virulence [Polissi et al., (1998) Infection and Immunity, 66, 5620]. Resistance is conferred by antibody to capsular types in normal individuals and absence of capsule renders the organism avirulent. K pneumoniae deletion mutants of surface structures [Regue et al (2001) J Bacteriol. 193, 3564-73] show reduced virulence. Examples of this are also available for H influenzae [Humphries and High, (2002) FEMS Immunol Med Microbiol 34;221-30]. Genes knocked out will be surface proteins and enzymes involved in synthesis of capsid structures.

Genetically modified organism will be grown at small scale always less than 1 litre. The organisms will be harvested by centrifugation in sealed buckets. All containers and other materials will be cleaned with ethanol and chloros and autoclaved. Both the laboratory for in vitro work and in vivo work are suitable for category 2 ACDP work as defined in that criteria. All waste is disinfected and autoclaved.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All cultures and waste are treated with chloros at the work bench and then autoclaved. Culture flasks will be treated with chloros and then autoclaved. All waste from mouse work will be autoclaved or incinerated. Autoclaved tape checks for the treatment and autoclaves are regularly monitored for efficiency.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All procedures have been approved by the appropriate safety committees.

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Ref 654/06.1

Date Ackn'd 06/03/2006

CU2 Project Title Transmission of genetically modified Plasmodium to Anopheles mosquitoes.

Date Project Ceased

Class 3

Class CultureVolClass2 CultureVolumeClass3-4

Non-GMM Consent Granted Yes

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info 08/05/2006 - centre also carrying
This proposal is an extension of a project for which we already have HSE permission to genetically modify the malaria parasite Plasmodium falciparum and the rodent malaria P. berghei (GM654/01.2). Here we propose to transmit genetically modified Plasmodium to Anopheles mosquitoes in the laboratory. Our main areas of interest are those genes involved in signal transduction and metabolism. To determine whether a specific Plasmodium gene of interest is essential for transmission to mosquitoes, it is necessary to delete this gene from the parasite genome and test for infectivity. Parasites are transfected with plasmid vectors containing a drug selectable marker by electroporation and genetically modified parasites are selected in the presence of the corresponding drug. Gametocytes (the transmissible sexual blood forms of the Plasmodium life cycle) are cultivated in vitro before feeding to female Anopheles mosquitoes to establish infection. The mosquitoes are then maintained at 24 degrees C and 75% relative humidity for ten 10 days and dissected to assess oocyst numbers in their midguts. Alternatively, after 14 days their salivary glands are examined for the presence of sporozoites. These experiments will allow us to determine which Plasmodium genes are essential for transmission to mosquitoes. This will define and validate new targets for transmission blocking drugs and vaccines.

P. falciparum causes the most serious form of malaria in humans that can lead to cerebral malaria or severe anaemia and can be fatal if left untreated. The organism is therefore maintained under ACDP category 3 containment at the LSHTM. The P. falciparum strain (3DT) that we routinely modify genetically is sensitive to all currently used anti-malarial drugs. If in the unlikely event that infection of laboratory personnel occurred or was suspected, drug treatment would be quick and effective. The 3D7 strain is currently being used worldwide to infect large numbers of volunteers in vaccine trials. Those not protected by experimental vaccines are readily treated and make a full recovery. P. berghei infects only certain rodents and cannot infect humans and is covered by local ACGM guidelines and does not require HSE notification. Therefore the majority of this document will deal with the human pathogen P. falciparum.

To our knowledge there is no instance of accidental P. falciparum infection in a UK laboratory. However, GM parasites causing an accidental infection in humans would be resistant only to the compound used for drug selection in vitro. This is usually pyrimethamine or an analogue (WR99210) but also neomycin and blasticidin can be used. Drug treatment of accidental infections would be quick and effective due to the availability of a range of antimalarial drugs (with distinct molecular targets) such as chloroquine, mefloquine, quinine, artesunate/artemether, and atovaquone/proguanil. Drug resistance in the modified parasites would not be a problem because of the drugs already available that could be used to treat accidental laboratory infections.

We propose to infect Anopheles mosquitoes with genetically modified P. falciparum parasites that have had a gene deleted. The genes of interest are thought to play an essential role in transmission to mosquitoes. If this is the case, then they would be expected to be less viable than unmodified parasites and attenuated to a degree. It is extremely unlikely that deletion of a gene would result in increased infectivity or virulence. It is more likely that infection with the unmodified organism would be a worst scenario.

Plasmid vectors are first constructed in various commercially available non-colonising E. coli strains (JM109, XL1Blue and XL10Gold, Stratagene) under ACGM containment level 1. These strains are considered as non-pathogenic for humans and other animals. The plasmids are based on the pUC series and are non-mobilisable. The plasmid vectors contain a drug selectable marker (usually a pyrimethamine-resistant human dihydrofolate reductase gene, but also neomycin phosphotransferase and blasticidin deacetylase genes can be utilised) whose expression is controlled by flanking P. falciparum sequences (usually calmodulin and heat shock protein 86). The plasmids are introduced into malaria parasites by electroporation and genetically modified parasites are selected under drug pressure. Integration by single crossover recombination results in incorporation of the plasmid backbone into the genome of the GM parasites. Integration by double crossover results in incorporation of only the drug selectable marker expression cassette. This part of the work has previously been given HSE permission (GM654/01.2). In experiments involving P. berghei,
Transfections can be carried out using linear DNA fragments comprising only the drug selection cassette flanked by around 0.5kb of the gene of interest.

### Origin & function

Genes encoding drug selectable markers are from heterologous sources such as human and the rodent malaria parasite P. berghei. Promoter and termination sequences are derived from P. falciparum and P. berghei. Reporter gene 'tags' such as Green Fluorescent Protein are obtained from commercial sources. The majority of work will be carried out on P. falciparum genes involved in signal transduction and metabolism, particularly in relation to development of the sexual (transmissible) stages of the parasite life cycle.

### Evaluation of foreseeable effects

These experiments are designed to produce GM P. falciparum that are unable to infect Anopheles mosquitoes. In this way we hope to identify genes that play an essential role in transmission of malaria parasites to mosquitoes. In the majority of cases, GM parasites will have no change or reduced capacity to infect mosquitoes. It is highly unlikely that the GM parasites will be more hazardous than the unmodified organism. Any accidental infection could be rapidly diagnosed and drug treated with one of several anti-malarial drugs available.

When working with P. falciparum in the laboratory, all necessary precautions are taken to avoid becoming infected with the organism. It is extremely difficult to get infected in the lab because sharp objects are not used in the culture lab. In this proposal we intend to infect mosquitoes with these GM organisms and so there is an additional risk of infection to laboratory workers. The safety precautions are dealt with below. It should be re-emphasised however that there is no reason to suspect that the GM parasites will be more hazardous than the unmodified organism. Neither modified nor unmodified P. berghei will have any affect on humans.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

A new Specific Code of Practice is in the process of being implemented relating to the handling of Anopheles mosquitoes infected with human malaria in the newly refurbished secure insectories and associated ACDP category 3 laboratory at the LSHTM. Relevant components are detailed below and will be rigorously applied to mosquitoes infected with genetically modified P. falciparum. Mosquito colonies of several genera, including Anopheles vectors of malaria, are held at LSHTM. Only guaranteed pathogen free stocks are routinely held. Any infected insects are always maintained under high security conditions in dedicated insectories. Facilities and working practices in the secure insectories are designed to meet containment level 3 equivalent guidelines to ensure safety of both workers and the public. The bite of a sporozoite positive infected mosquito could result in transmission of malaria. A female mosquito having taken an infective feed requires a minimum of 10-15 days at tropical temperatures before oocysts rupture and sporozoites migrate to the salivary gland. The relatively short longevity of female mosquitoes makes the percentage living long enough to become infective relatively low, and life expectancy post-infective short. Moreover, the developing malaria parasite within the mosquito is more sensitive to sub-optimal temperature and humidity than the mosquito itself. Unless multi-drug resistant strains are involved, rapid diagnosis, successful treatment and recovery are normally assured.

Risk of Infection

Risk to secure insectory users:

The bite of a sporozoite-positive infected mosquito could result in transmission of malaria. Prevention of mosquito release is a high priority and strictly regulated. Diagnosis and treatment is relatively straightforward. Use of multi-drug resistant strains of malaria is restricted. All users of the facility are suitably trained in entomological and parasitological techniques.

Risk to external individuals:

Whilst the tropical malaria vectors used are unable to establish populations under normal British environmental conditions, anyone bitten by an infective mosquito escaping from the secure insectory could develop malaria. To reduce this risk, the secure insectories have an interlocking double-door system. The doors to the insectory anti-room will only open singly, only one of the 3 at any one time. There is an "insect-o-cutor" flying insect killer installed within the anti-room. In reality, the risks are higher in individuals in close proximity to the secure insectory, in particular, the associated malaria culture laboratory which has restricted category 3 access. An infective mosquito escaping both the secure insectory and the associated culture lab would theoretically be able to bite any other member of school staff/student or visitor in the vicinity. The likelihood of an infected mosquito leaving the building and biting a member of the public is very low as environmental conditions in all but the hottest/most humid British summer are unfavourable to the Tropical mosquito species used. Risk of prolonged survival (more than a few days) is very low. Colonisation in the UK is not possible with
the species used. Malaria would be easy to treat but diagnosis may be less rapid in those not directly connected to the secure insectory and associated malaria culture laboratories.

Procedures for use of secure insectory

Access to the outer malaria culture laboratory is containment level 3 with restricted access. All procedures in the culture laboratory to be in accordance with the Specific Code of Practice for Plasmodium falciparum.

Access to the inner secure insectories is restricted. Only approved and suitably trained individuals have access through the interlocked double-entry doors which are controlled by key-code. The entry code will be changed regularly. The facility is fully sealed and insect-proof with double insect-proof mesh on all vents and sealed door.

Mosquitoes are only permitted entry into the secure insectory in either "infection pots" (cardboard pots and sealed netting covers) or netting cages within secondary sealed containers. The exact number of insects taken into the room will be recorded in the designated experimental log along with name of the Principal Investigator of the project and signature of the responsible person handling them. The log will hold details of the procedures taking place along with exact numbers of insects killed during, or on completion, of each experiment. The number of dead insects in the final log entry must correspond with the number originally taken into the facility as a further check to monitor accidental release. Any discrepancy between log total and actual number of insects in experimental pots must be immediately reported to the Departmental Safety Supervisor (or their deputy) and both doors to the insectory to remain sealed until a detailed check has been made. All inner surfaces of the secure insectory are white and unobstructed to facilitate easy detection of mosquitoes. Should a mosquito be released without verification of its subsequent capture/destruction, the Departmental Safety Supervisor will arrange for the facility to be sealed for a period of 30 days, or the use of a fast-acting non-residual pyrethrum insecticide to disinfect the facility.

No viable insects are permitted to be removed from the secure insectory. Before removal all mosquitoes will be disabled by either a) removal of both wings or b) total immersion in PBS or 70% alcohol. Chilling or anaesthetising are NOT suitable means of disabling insects as they may subsequently recover, although they may be used prior to other methods such as wing removal.

All dead insects, culture media and contaminated waste shall be treated as Cat 3 and disposed of as per code of practice for P. falciparum (10% Chloros overnight followed by autoclave). Items for re-use, such as glass feeding chambers, shall be left overnight in 10% Chloros followed by rinsing with 70% alcohol or autoclave to sterilise.

All users of the secure insectory in possible contact with insects must be under health surveillance with Occupational Health due to risk of sensitisation/allergy.

In conclusion, transmission of both GM and non-GM Plasmodium falciparum is routine in several laboratories worldwide and is already carried out in central London by our colleagues at Imperial College. We intend to follow the above stringent safety procedures to avoid release or infection with modified or unmodified P. falciparum.

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Protocol for working with Plasmodium falciparum.
Malaria parasites are extremely sensitive to changes in culture conditions; a slight rise in pH of the culture medium will kill them. All work with P. falciparum will be conducted in a designated containment level 3 laboratory. Actual manipulation of the organism within such labs is to full Cat 3 level although as there is no risk of airborne infection with this parasite. Consequently, there is a reduced necessity for airflow regulation in such areas. As the most significant risk of infection is via inoculation, the use of sharps and glassware is restricted wherever possible.

Waste disposal
Liquid waste containing P. falciparum is decanted directly into a 10% solution of chloros (10,000 ppm available chlorine) in autoclavable pots in class II cabinets. These are stored overnight before autoclaving even though contact with this solution would kill the organisms instantly. Solid waste is placed directly in autoclavable bins which are sealed and autoclaved in a newly built adjoining facility unit following recommended procedures for ACDP category 3 containment.
Decontamination and disinfection
The disinfectant of choice for P. falciparum is 10% chloros. Ethanol can also be used to decontaminate surfaces. All infective material, cultures and plasticware must be inactivated in 10% Chloros overnight before being transported in Cat 3 boxes to be autoclaved. Surfaces will be routinely cleaned with 70% ethanol after use. As there is no airborne risk from this parasite, laboratory fumigation is not considered necessary. Fumigation of safety cabinets would not inactivate parasites in culture spills so much be supplemented or replaced by surface decontamination with Chloros or ethanol.

Spillages and accidents
Spillages of infective material shall be contained to prevent their spread then treated with sufficient 10% Chloros to inactivate the material. Larger spills should be treated with undiluted Chloros and left overnight. All materials used to clean up a spillage will be autoclaved. All spillages and accidents must be reported to the Departmental Safety Supervisor as soon as possible. There must always be a fresh supply of 10% Chloros readily available in case of spillage or accidents.

Disposal of mosquitoes infected with P. falciparum
Mosquitoes that have been exposed to infectious blood containing P. falciparum mosquitoes are stunned immediately after feeding by chilling or ethyl acetate followed by physical immobilisation to render them non-viable (wings removed). After counts (of oocysts on day 10 or sporozoites on day 14) have been made, the mosquitoes are killed by prolonged freezing and placed in sealed containers within autoclavable bins. The remainder of the infectious blood is placed immediately into a pot 10% chloros overnight prior to autoclaving.

Health surveillance
All workers using P. falciparum must be registered with the Occupational Health Advisor. All individuals will be familiarised with the symptoms of malaria infection and a ready supply of suitable drugs for treatment will be available. Health records of personnel involved in this work will kept by the LSHTM for 40 years.

Project Containment

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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This submission has been prepared in consultation with LSHTM Biological Safety Officer for work involving genetic modification. He agrees that the GM parasite work should be category 3.

The submission has also been prepared in consultation with the Departmental Safety Supervisor for the Department of Infectious and Tropical Diseases. Information and advice on general safety procedures, but specifically relating to infected mosquitoes has been given.
The purpose of this work is to study the biological function of genes expressed in the human/simian malaria parasite Plasmodium knowlesi. We plan to transfet P. knowlesi parasites with plasmid DNA (vectors) containing all or part of selected P. knowlesi genes as well as plasmids to facilitate Cas9 nuclease mediated genome editing. The products of these genes are important in the maturation of the parasite, the invasion process and/or development within the invaded erythrocyte. Our main approach is the use of gene deletion or tagging of genes to determine protein localisation and function. The target genes will be those that encode proteins located on the surface of mature parasites or the infective stage of the parasite (the merozoite), proteases or signalling proteins important in parasite development and merozoite release, organelle specific proteins and factors involved in the parasite's metabolism. All of these are involved in maturation of the parasite, the invasion process or developmental events in the invaded erythrocyte. Understanding the role and function of these genes will contribute towards new intervention methods to treat or prevent human disease, including both vaccines and anti-malarial drugs.

P. knowlesi is a protozoan parasite of the phylum Apicomplexa, and is one of the agents responsible for causing human malaria. It is normally found south-east Asia where it is transmitted as a zoonosis from macaques via
mosquitoes of the Anopheles spp. It is possible to cultivate the asexual and sexual erythrocytic stages in tissue culture in vitro in donated human or macaque red blood cells. Whilst P. knowlesi can cause severe disease in humans (up to 7% of hospitalised cases develop severe disease) there is currently no evidence of drug resistance, in either field or laboratory strains, and there are several safe and effective drugs available which clear infection rapidly. Should an increase in resistance to a particular drug occur, alternative drugs with different mechanisms of action could be used. The chances of infection arising from the laboratory, however, are extremely low and would only result from the injection of the organism into the blood stream. The majority of our experiments involve deletion or tagging of specified genes which usually results in decreased viability (or no change in viability) of the organism. It is therefore highly likely that infection with the unmodified organism would be the worst possible scenario. In other words, the modified organism is unlikely to be more hazardous than the wild type.

Some experiments such as the introduction of nucleotide polymorphisms found in field isolates or replacement of endogenous genes with alleles from the closely related parasite P. vivax, may result in an increase in resistance to particular drugs or an increase in invasion efficiency in certain blood types. It is however important to note that malaria parasites maintained in vitro over extended time periods, such as those used in these studies, have a reduced virulence level when reintroduced to a host. Indeed, in recent work with collaborators we have demonstrated that these lines are initially avirulent when injected into rhesus macaques (a highly permissive host) and require multiple passages to regain wild type virulence levels. Thus, it is extremely unlikely that the parasites produced will be more hazardous than field isolates.

Person to person infection does not occur. Transmission can only occur via an infected Anopheles mosquito which is maintained under strict laboratory conditions (controlled temperature, humidity and feeding) for 7-10 days. Infective malaria parasites can only survive in the blood stream or in sealed flasks containing human/macaque blood under strict conditions. It would therefore be extremely unlikely for the parasite cultures to come into contact with a mosquito.

<table>
<thead>
<tr>
<th>Host/vector system</th>
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</thead>
<tbody>
<tr>
<td><strong>Host:</strong></td>
</tr>
<tr>
<td>The host sequence is derived from genes encoding proteins involved in the development and maturation of the parasite, parasite metabolism, or recognition and invasion of erythrocytes by the merozoite.</td>
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<tr>
<td><strong>Vector:</strong></td>
</tr>
<tr>
<td>The vectors are of the pH1, pARL, pLN, pSSPF, pXL-BACII-hDHFR and pH1 series or their derivatives, which are derived from pUC series plasmids of E. coli. These have been modified to include drug resistance cassettes, 5’ and 3’ malarial untranslated regulatory sequences (UTR) and appropriate multiple cloning sites. We will also use derivatives of the pUF1 and pL7 plasmids for Cas9 nuclease mediated genome editing. The vectors may also contain sequences encoding reporter proteins: these include widely used enzyme reporters such as firefly luciferase and chloramphenicol acetyl transferase (CAT), fluorescent proteins (e.g. GFP, OsRed, RFP varieties) or epitope tags (e.g. cmyc, HA3, FLAG or Ty1).</td>
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<table>
<thead>
<tr>
<th>Origin &amp; function</th>
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<tbody>
<tr>
<td><strong>Origin of coding sequence:</strong></td>
</tr>
<tr>
<td>Culture adapted cloned P. knowlesi; parasite lines:</td>
</tr>
<tr>
<td>A1, Nuri, Washington and H strain</td>
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<tr>
<td>Parasite lines cloned and adapted from Malaysian clinical isolates</td>
</tr>
<tr>
<td>Orthologous genes encoded by closely related parasites P. falciparum, P. vivax, P. malariae or P. ovale</td>
</tr>
<tr>
<td><strong>Intended function:</strong></td>
</tr>
<tr>
<td>a) To disrupt the gene of interest in order to ascertain its function (i.e. perform a gene knock out).</td>
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</tbody>
</table>
b) To 'tag' the gene with a marker in order to detect and study the function and activity of the gene product.
   1) This can be done using sequences for GFP or related proteins cloned into a part of the target sequence in order to produce a fusion protein that can be detected by optical techniques such as fluorescence.
   2) Similarly this can be done using short peptide epitope tags (e.g. HA, cmyc, FLAG, Ty1 tags). Gene products modified with these tags can be readily detected using immunochemical techniques.

   c) Further functional studies on the protein by gene modification or truncation, or replacement with orthologous genes of other species of malaria parasites. This will help to determine the parts of the molecule that are required for its biological function. These truncated genes can also be tagged as in b).

   d) To overexpress genes of interest which are otherwise of low abundance (as episomes or integrated into a nonessential site of the parasite genome)

Origin of vector: pHH, pARL, pLN, pSSPF, pXL-BACII-hDHFR and pHTH plasmid series. These contain selectable markers (drug-resistance) and regulatory or mRNA stabilising elements (5' and 3' malaria UTRs).

Evaluation of foreseeable effects

The main concern when considering this project is that P. knowlesi is pathogenic to man. A code of practice (COP) is already in place (attached) to minimise accidental infection of laboratory workers. The parasite can only be transmitted through the mosquito secondary host or by direct contact with infected blood by injection or through cuts. No P. knowlesi work on these premises involves exposure to sharps such as needles or broken glass. The one exception to this rule is the use of glass slides for microscopic examination of parasite cultures, as no suitable alternative is currently available. All parent parasite lines have been screened for drug susceptibility and are known to be sensitive to chloroquine. Where this characteristic is likely to be altered by experiments, several other malaria drugs are available with different mechanism of action (and targets within the cell). In the unlikely event that a person is believed to have been accidentally infected, treatment with the appropriate anti-malarial drug will commence immediately. Infection would be quickly cleared and would not recur. P. knowlesi infections, unlike other species, cannot result in recurrent cases of malaria. The LSHTM is the home of the Malaria Reference Laboratory where advice is given to people all over the world on which drugs to take and so local expertise is available.

All used equipment and reagents are decontaminated before leaving the containment 2 tissue culture room (see COP) and thus no live modified parasite will be discarded and transported out of the room. If it is necessary to transport live parasites to another facility, e.g. for centrifugation or microscopy, the parasites are sealed (in a centrifuge bottle or sealed coverslip on a microscope slide) and transported in a sealed container. Work with P. knowlesi is undertaken under containment 2 guidelines and modification of the parasite does not pose greater risks of infection to humans. These modified paraSites will not be put through the mosquito cycle and thus there is no risk that they will enter the wild population. Most of the genes under study are unique to P. knowlesi and thus it would be difficult to transfer these genes to another organism.

The parasite will only be cultivated in vitro; no insect transmission will be undertaken. The work with live parasites is carried out in a sealed room so there will be no possibility of mosquitoes being infected and infecting the wider populace. The parasite is not viable ex vivo except in the special in vitro culture conditions used.

All parasite material is rendered non-infectious with reagents such as phenol or detergents before further analysis is carried out in non-category 2 containment facilities. Safety protocols for such reagents will be adhered to.

The vectors contain drug-resistance cassettes. The drugs most used in the parasite selection process (WR99210, neomycin, blasticidin and puromycin) are not in general use for the clinical treatment of humans or animals and thus the problem of drug resistance appearing in the human/animal population is not an issue. Less often, pyrimethamine is also used in selection of transgenics. Resistance to this drug in wild parasite populations is widespread, and it is not recommended for use alone for either prophylaxis or treatment of acute malaria. Ampicillin is used in the cloning of the vector in the bacterial host. This antibiotic has been in widespread use in standard laboratory molecular cloning protocols for a number of years and there have been no recorded cases of drug resistance linked to using these
vectors in bacteria.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The work with live parasites is required to be performed under Containment 2 facilities. However, once the parasite has been treated with denaturing reagents, such as phenol or detergents, the parasites are non-infectious and can be worked with under Containment 1 facilities.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Refer to Code of Practice, attached. All lab ware (pipettes, flasks, tips and tubes) and culture waste (spent growth medium) are treated by immersion in 10% chloros and left there until the next morning, even though contact with the solution will kill the organisms instantly. The medium is rendered colourless, or dark brown precipitates (parasitised erythrocytes) are observed as the killing process occurs. Solid waste is placed directly into autoclavable bins lined with bags. These are sealed and autoclaved at 121°C for 15 minutes before being discarded. Autoclave tape indicates that the process has occurred and there is 100% destruction of infectious material after these treatments. Spillages within the safety hood are treated with 70% Ethanol, which kills the organisms immediately. In the unlikely event of a large spillage outside of the safety hood, the spillage is contained with tissue paper and left overnight in 100% chloros. Small spillages are cleaned with 10% chloros and tissue paper which is disposed of in autoclave bins.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

This proposal and associated risk assessment and codes of practice were reviewed by the committee (by email circulation) and the committee is content with the submission.

Project Containment

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02/03/2022
Investigation into the effects of disruption/inhibition of energy metabolism on Mycobacteria tuberculosis and related organisms

Mycobacterium tuberculosis (Mtb) still claims more lives each year than any other bacterial infection and with the sharp increase in drug resistance in recent years, global tuberculosis (TB) control programs are under threat. The discovery of bedaquiline (BDQ) revolutionized the treatment paradigm for patients suffering from multi- and extremely drug resistant TB (MDR/XDR-TB). BDQ inhibits the ATP synthase of Mtb and has also brought mycobacterial bioenergetics and energy metabolism to the forefront of TB research, as well as new anti-TB drug discovery efforts.

Combination targeting of Mtb's energy metabolism is not completely understood. There is, however, a growing body of literature showing the significant rearrangement of Mtb's central carbon metabolism under BDQ treatment, to compensate for the rapid decrease in ATP levels. Less is known about the rearrangements induced by other energy metabolism targeting compounds, eg. Q203 and CFZ which especially target oxidative phosphorylation (OXPHOS). Better insight into these metabolic rearrangements driven by BDQ and other compounds would allow us to design drug regimens for rapid and synergistic killing of Mtb, via the complete shutdown of Mtb energy metabolism.

The aim of this project is to develop new research tools and methodologies to finely dissect Mtb energy metabolism, to identify metabolic vulnerabilities and aid in the development of new therapies for TB.

Our goal is to accelerate the development of novel anti-TB drug regimens via the establishment of in vitro/in vivo omic approaches to Mtb's energy metabolism pathways, and thereby determine the optimal targets which will help us in developing potential drug combinations for rapid and synergistic killing of Mtb. This will also help us to understand aspects of target-based drug antagonisms that might arise. It is also important to determine the effect of metabolic target inhibitors that have on the host metabolome to mitigate possible host side effects and to explain possible in vitro vs. in vivo regimen efficacy differences.
**Mycobacterium tuberculosis** (strains; H37Rv, H37Ra, Erdman) and Clinical Isolates (1st line drug susceptible only)
- Mycobacterium bovis BCG
- Mycobacterium kansaii
- Mycobacterium avium complex
- Mycobacterium marinum
- Mycobacterium smegmatis MC2-155
- E.coli lab adapted strains (for instance JM109, DH5α)

### Host/vector system

Vectors include:
1. pRH2502
2. pRH2521
3. pTIGC
4. pUC
5. pSMT3
6. pCRISpathbrick.
7. pTigC

**CRISPR-Cas and CRISPRi vectors** express an inducible Cas9/dCAS9 enzyme and a scaffold RNA sequence into which a small guide RNAs (sgRNA) can be inserted.

pTigC is a dual reporter fluorescent construct to enable GFP and TurboRed expression of viable and metabolically active bacteria.

Plasmids and similar constructs will contain origin of replication (COIEI, Ori E and pAL500), promoter for the gene of interest and an antibiotic resistance marker. No resistance marker will be used that could potentially alter the susceptibility of the GMM to 1st line antibiotics against infectious disease. These vectors will be non-mobilisable.

### Origin & function

The following list of genes is indicative of those to be investigated. Related genes may also be targeted.

#### Energy Metabolism pathways:
1. ctaE-qcrCAB – cytochrome bc1-aa3 (terminal oxidase)
2. cydABCD – cytochrome bd (terminal oxidase)
3. ndh-ndhA – NADH dehydrogenase (type II)
4. nuoA-J - NADH dehydrogenase (type I)
5. atpACDEFHG – ATP synthase
6. sdhABCD – succinate dehydrogenase
7. menABCDEGH – menaquinone biosynthesis
8. glcB - malate synthase
9. icl1/2 - isocitrate lyase (1 and 2)
10. pca - pyruvate carboxylase
11. pykA - pyruvate kinase
12. aceE - pyruvate dehydrogenase (E1 component)

#### Efflux pathways:
13. mmpL/S5 – efflux pump (multi-drug efflux, contributes to bacterial tolerance)
14. Rv0678 – mmpL/S5 regulator (efflux regulator)

**Luminescent reporter genes:** e.g. lux AB (from Firefly or Gaussia princeps), lux ABCDE (full operon) from Photobacterium luminescens or Vibrio harveyi

**Fluorescent reporter genes** - e.g. green fluorescent protein (GFP), red fluorescent protein (RFP), mCherry

**Selectable markers:** hygromycin (hyg) (not clinically used), kanamycin, spectinomycin, zeocin (not part of first line therapy), sometimes in combination with the sucrose and
the SacB enzyme as a counterselectable marker.

**Evaluation of foreseeable effects**

All strains with modified energy metabolism are expected to have moderate or severe growth defects. Gene inserts are restricted to reporter genes and are not expected to alter or contribute strain virulence or fitness. Efflux KO strains are expected to be more susceptible to anti-infective treatment. All selective antibiotic resistance markers will not contribute to front line antibiotic treatment resistance. There is no evidence that any of the GMM used in this project will be any more hazardous than parental strains.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Class 3 GMO:
All liquid waste infected with mycobacteria are discarded by adding surfanios disinfectant (minimum final concentration of 5%) and leaving overnight before placing in a clear autoclave bag and yellow rigid bins for autoclaving at a temperature of 134°C for 30 mins or 126°C for 20 mins depending on load. Autoclaved waste is then disposed of at a licensed facility.

All solid waste infected with mycobacteria will be placed in autoclave bags and rigid yellow bins, autoclaved and disposed of as above.

Class 2 GMO:
All waste is placed in clear autoclave bags and autoclaved, the waste is then placed in yelow/tiger striped bags and disposed of at a licenced facility.

Spillages are decontaminated with 70% ethanol.

Autoclaves are serviced bi-annually and validated and insurance inspected annually in accordance with LSHTM and manufacturers schedules.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The GM Safety Committee discussed your new GM Class 3 Risk Assessment: LSHTM-0036.01: Investigation into the effects of disruption/inhibition of energy metabolism on Mycobacteria tuberculosis and related organisms.

The following discussion was recorded at the meeting:
* and * gave a brief description of the experiment.
* asked if they were planning to use MDR strains in the short term
* confirmed, not in the short term.
* requested this was removed from the GM sections of the Risk Assessment and can be added as a scientific change to the HSE if used in the future.
* questioned the Vacuuming filtration Technique in Section 1.2 and if this would release aerosols?
* explained this is being developed by *, it is in the very early stages and may not be used.
* queried with reference to comments submitted by * (section 1.3: rooms) if the Flow Cytometry is fixed in the Imaging/FACs room or in the CL3 for CAT3 use only.
* explained further discussions on how that is used in the CL3 are required and * is updating this information for lab users.
* pointed out that if the Flow Cytometry 1.2. is fixed in the CL3 Lab we need to consider if anything be moved between CL2 and CL3
* suggested the risk assessment needed to be clear what is done in CL2 and CL3 and aerosol risk in CL2
* added the COP and SOPs will help clarify this in more details.
* asked about if only lab strains mentioned in section 2.2.3 and is there would be a potential issue with complementation and over expression.
* explained that they would not be working with CRISPR-1 that affect complementation
* suggested they added this information in section 2.31 to make it clear.
* queried how will you contain work in CL2?
* answered there will be done in separate labs and work done separately
* asked about their use of sharps in section 2.3.8
* answered there were no plan to use them, but a handy resource.
* suggested to move to 5.8, and asked if sharps were used to dissect, in the BSF or main CL3 lab
* answered this is always done in the BSF
* added all infectious work is done in the MSC
* asked * how is the SOP managed
* answered they will be shared with GMSC. All pre-existing SOPs are being used and updated for this project.
* would like what disinfectants and deactivation, concentrations details to be included in the SOP also.
* queried if Class 1 is appropriate for this work (5.1)
* answered that although both give good operator protection, class 1 is less easily disturbed, the airflow is cleaner in a class 2 for sample protection.
* asked on behalf of * whether optimised fluorescent and bioluminescent reporter Mtb strains at LSHTM from the Gates imaging project will be used
* answered that they would use these as a handy resource and they fell within the remit of this GM project.

Please address the following amendments required:

After Section 1.3: The following declaration must be completed by the PI responsible for this project- ensure this section is completed.

Section 2: Ethics approval required
2.2:* needs spelling out in the first use
2.4.1: Remove the Downdraft tables technique from the risk assessment
4.11: most hazardous Procedure, performed in cat3 then on benches in CL2 (separate the work) also separate the Class 2 and 3 work throughout the form.
4.3: Units of the volumes should be added and checked throughout the form.
4.2-4.9: Routes of infection for all infectious agents should be included here.

Section 10: emergency procedures: Should be Bio Risk Manager, not BSO.
11.3: Electronic inventories samples needs to be mentioned here.

GM Classification: 3; Containment Level: 3
Consent: Approval from the HSE is required
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### Project Ref 654/98.1

<table>
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<td>TRANSCRIPTION CONTROL IN TREPANOSOMA BRUCEI BRUCEI.</td>
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Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

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Please enter comments on the GM safety committee on the risk assessment

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Project Ref 654/99.1

Date Ackn'd 02/03/2022  CU2 Project Title
GENETIC TRANSFORMATION OF ENTAMOEBA HISTOLYTICA

Date Project Ceased: 25/06/1999

Consent Granted: Non-GMM

Historical Significant Changes

Project notified under transitional arrangements: Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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**Project Ref** 654/99.2

**Date Ackn’d** 13/08/1999

**CU2 Project Title**
CHARACTERISATION OF VIRULENCE DETERMINANTS FROM ENTEROPATHOGENIC BACTERIA A CONNECTED PROGRAMME

**Consent Granted**
Non-GMM: not applicable

**Tick if notifying a connected programme of work** N

**Project notified under transitional arrangements** Y
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**

Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste which may contain GMOs is segregated from general laboratory waste and is transported to an autoclave within the same building in leakproof plastic containers. Solid waste is autoclaved for 30 minutes at 121 degrees C and then incinerated. Validation of autoclave function is provided by heat-sensitive detection strips, and by an internal pressure and temperature monitor.

Liquid waste (maximum 5L) is inactivated either by autoclaving for 30 minutes at 121 degrees C or using recommended disinfectants eg. Virkon (Antec International) at manufacturer's recommended dilution for a minimum of 24 hours, which has been validated by the manufacturer to achieve complete eradication of microorganisms. After sterilisation, liquid waste is released into public drainage system.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 656**

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**Name**

ARCINOVA

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

TAYLOR DRIVE

**District**

**Town**

ALNWICK

**County**

NORTHUMBERLAND

**Postcode**

NE66 2DH

**Country**

ENGLAND

**Tel Number**

01665 608300

**Fax Number**

01665 608315

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

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Maximum culture volume: 10 litres.

Waste deactivation
1. All culture media residues are autoclaved.
2. Surface decontamination and sterilisation of non autoclaved items with a 2% Virkon solution.
3. All dry wastes are disposed of as biological waste (in double yellow bags) and incinerated.

Validation of Method:
1. The autoclave is controlled via a load probe to run at 121 degrees C for 30 minutes (overkill cycle), the largest volume of waste ie. slowest to heat up, is probed, to ensure correct temperature for all vessels is achieved.
2. Contact culture tests have previously been performed (over 5 processes) on surfaces to confirm decontamination - no positive results.
3. Routine audits of the waste disposal route and contractor.

Monitoring of Method:
1. At the end of each autoclave cycle a chart is produced, which is checked and entered into a log book. The probe is calibrated in the autoclave every 6 months to ensure correct temperatures are being achieved.
2. Contact culture tests will be repeated every 3 months.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

VISIBLE GENETICS UK LIMITED

**Campus Estate or Research Centre**

184 CAMBRIDGE SCIENCE PARK

**Road Name**

MILTON ROAD

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 OGA

**Country**

ENGLAND

**Tel Number**

01223 728 800

**Fax Number**

01223 728 801

**HSE Division**

EAST AND SOUTH EAST

**Comments**

FORMERLY VIRCO LTD

**Date at Which Additional Info Submitted**

03/10/2001
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Chair
- Management representative
- Employee representative

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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

02/03/2022  Page 10444 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All work and risk assessments will be reviewed by the local GM safety committee and any significant changes in the work or personnel will be subject to further review by the committee.

---

Project Ref 657/01.1

Date Ackn'd 19/02/2001

Date Project Ceased 06/12/2002

CU2 Project Title DEVELOPMENT OF PHENOTYPIC RESISTANCE ASSAYS FOR HIV-1, HEPATITIS B AND HEPATITIS C VIRUSES.

Class 3

CultureVolClass2 not exceeding 500ml

Consent Granted yes

Project notified under transitional arrangements N

Historical Significant Changes

Withdrawn N

Tick if notifying a connected programme of work N
### Project Additional Information

#### Purposes of the contained use

HIV-1, HBV and HCV virus gene sequences will be cloned and manipulated (typically by site directed mutagenesis) in order to express wild-type and mutant recombinant proteins in E. coli and baculovirus. These proteins will be used to obtain crystal structure information and to develop biochemical assays. Non-infectious molecular clones of HCV will be manipulated in plasmid vectors for the development of new phenotypic drug resistance assays. In addition, full length HIV-1 and HBV clones will be manipulated to produce specific mutations that confer drug resistance (ref. Schinazi, RF, Larder, BA, Mellors, JW. Mutations in retroviral genes associated with drug resistance: 2000-2001 update. Int. Antiviral News (2000) 6, 65-91) and these will subsequently be either transfected into human cell lines to generate recombinant virus. For HBV specifically the state of the art is the use of recombinant baculovirus vector (rather than transfection) whereupon productive viral replication ensues. Full-length drug resistant or wild-type forms of HBV genome will therefore be inserted into baculovirus transfer vectors prior to the construction of recombinant baculovirus expressing infectious HBV genomes. ALL FULL LENGTH HIV/HBV TRANSFECTION/INFECTION EXPERIMENTS WILL BE CARRIED OUT IN A BIOSAFETY CATEGORY 3 FACILITY.

#### Recipient or parental organism

Disabled E. coli hosts (JM109, XL-1 Blue, DH5a, TOP10), spodoptera cells (Sf9, Sf21) and human hepatoma cells (HepG2, Huh7).

#### Host/vector system

Non-mobilisable plasmid vectors based around pUC, pGEM will be used for prokaryotic work. Standard baculovirus transfer vectors eg. pBacPAK8 and pBR322 based eukaryotic vectors eg. ptetHBV for insect and mammalian cell expression respectively.

#### Origin & function

Pre-existing full-length HIV-1 and HBV clones will be used for site-directed mutagenesis to enable replacement of specific viral sequences from drug and antibody-resistant clinical isolates. These will be used to express variant forms of specific viral enzymes and for generation of phenotypically resistant virus. All cell culture related virus work will be carried out in a biosafety category 3 facility.

Pre-existing HCV clones containing 90% of the genome (ie. non-infectious) will be used to isolate and express specific viral enzyme functions and to generate artificial chimeric replicons. The system used involves transcription of recombinant clones and direct transfection of RNA into recipient cells. Chimeric replicons will represent replicons in which the wild-type sequence is replaced by the homologous region from a drug-resistant isolate of the virus. At no stage will any clones be used which have potential for infectivity and there will be no possibility of recombination since multiple replicons will not, at any stage, be transfected into the same cell.

#### Evaluation of foreseeable effects

All experiments using HCV vectors will be undertaken with incomplete genomic clones which are incapable of generating infectious virus. Expression of HCV viral gene products in both prokaryotic and eukaryotic systems is well-documented and is not associated with hazardous consequences.

Full length HIV-1 and HBV clones used in these studies could be accidentally administered, although no evidence exists to suggest that this would cause infection. All staff handling these clones will be vaccinated against HBV and ensured to have protective levels of antibody prior to initiation of experiments. In addition, all handling of live HIV-1 and HBV infectious material will be carried out in a designated Category 3 laboratory under strict operating procedures.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be de-contaminated by treatment with 10,000 ppm chloros (100% kill) and all solid waste will be autoclaved (100% kill).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was reviewed by the genetic modifications safety committee and was accepted as appropriate.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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02/03/2022
**GM Centre Number: 658**

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**Name**

PUBLIC HEALTH ENGLAND

**Name 2**

PHLS HEARTLANDS & SOLIHULL NHS TRUST (TEACHING)

**Department**

ANTI VIRAL SUSCEPTIBILITY REFERENCE UNIT

**Campus Estate or Research Centre**

BORDESLEY GREEN EAST

**Road Name**

BORDESLEY GREEN EAST

**District**

**Town**

BIRMINGHAM

**County**

MIDLANDS

**Postcode**

B9 5SS

**Country**

ENGLAND

**Tel Number**

0121 772 3009

**Fax Number**

0121 772 6229

**HSE Division**

MIDLANDS

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  

Tick if confidential

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Laboratory Activities</th>
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**Historical Significant Changes**
GM658/03.2

**Historical Date of Additional Info**
25/09/2003

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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GM Centre Number: 659

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Name

VERNALIS RESEARCH LTD

Name 2

Department

MOLECULAR PHARMACOLOGY

Building

GRANTA PARK

Road Name

613 READING ROAD

District

ABINGDON

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB21 6GB

Country

ENGLAND

Tel Number

000000000000

Fax Number

000000000000

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

FORMERLY CEREBRUS - GM730 (Ribotargets) merged with Vernalis wef 1/12/2003

Date at Which Additional Info Submitted

02/03/2022

Page 10454 of 15326
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial
Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
All liquid waste including growth media that has come into contact with GMOs is decontaminated according to local rules, using either 1% Virkon (final concentration, according to manufacturers instructions) or a concentrated perchlorite solution, prior to disposal down the drain. All culture procedures are considered small scale in that less than 10 L volumes are disposed of at any one time. All solid waste is placed in yellow clinical waste bins, which are sealed and incinerated within 24 hours of collection by an external waste disposal company.

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 660

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Comments

GM660 TRANSFERRED TO GM697 ON 22/9/2003

Date at Which Additional Info Submitted

02/03/2022
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

Bacteriology           Parasitology       Transgenic Birds       Microbiology Research
Virology               Transgenic Animals Transgenic Fish       Gene Therapy
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 662

Data Premises Notified (Originally) 28/01/1998

Data Premises Closed

Transferred from 1992 Regs? Y

Emergency Plan Required? N

Transitional Premises

Non-GMMs N

Withdrawn N

Name

MFM LABORATORIES LTD

Department

PRODUCTION

Campus Estate or Research Centre

SPRINGWOOD IND ESTATE

Road Name

4 WARNER DRIVE RAYNE RD

Town

BRAINTREE

County

ESSEX

Postcode

CM7 2YW

Country

ENGLAND

Tel Number 01376 551222

Fax Number 01376 552612

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted 02/03/2022
## Premises Addresses

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<td>CM7 7YW</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify) ________________

Tick if confidential ________________

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 663**

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**Comments**

THIS CENTRE MERGED WITH GM207 ON 17/02/2004

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Level 1 (GMMs)</th>
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Other (please specify) | Tick if confidential [ ]

- **Bacteriology**
  - Parasitology
  - Transgenic Birds

- **Virology**
  - Transgenic Animals
  - Transgenic Fish

- **Mycology**
  - Transgenic Invertebrates
  - Transgenic Plants

- **Transgenic**
  - Animals
  - Fish
  - Invertebrates
  - Plants

**Microbiology Research**

**Gene Therapy**

**Other (please specify below)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

02/03/2022

Page 10465 of 15326
INVESTIGATION OF THE THERAPEUTIC POTENTIAL OF CYTOKINE GENE TRANSFER TO TUMOURS
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
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GM Centre Number: 665

Data Premises Notified (Originally) 06/02/1998
Transferred from 1992 Regs? Y
Transitional Premises Class 1

Data Premises Closed Transitional Premises Emergency Plan Required? N
Non-GMMs Y Withdrawn N

Name

G R MICRO LTD

Name 2 Department

Campus Estate or Research Centre Building

Road Name District
7-9 WILLIAM ROAD

Town County Postcode Country
LONDON GREATER LONDON NW1 3ER ENGLAND

Tel Number Fax Number
0207 388 7320 0207 388 7324

E-mail

HSE Division

LONDON

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

| Date Premises Closed | Name               | Department | Name 2 | Campus Estate or Research Centre | Building     | Road Name       | District | Town       | County         | Post-code | Country      | Withdrawn |  |
|----------------------|--------------------|------------|--------|----------------------------------|--------------|----------------|----------|------------|----------------|-----------|--------------|-----------|
|                      | G R MICRO LTD      |             |        |                                  | 7-9 WILLIAM ROAD | LONDON        | GREATER LONDON | NW13ER    | ENGLAND   | N             |          |             |           |

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

### Laborator
y Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential

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<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
<th>Virology</th>
<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
<th>Gene Therapy</th>
</tr>
</thead>
</table>

02/03/2022
All contaminated laboratory waste is sealed in labelled 60 L plastic containers (Griff bins, supplied by Griffiths & Neilson Ltd). Of this volume the maximum culture volume containing genetically modified organisms is expected not to exceed 2.25 L. Disposal by incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
Mycology Transgenic Invertebrates

Transgenic Plants

Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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Project Ref 666/04.1

Date Ackn'd 17/03/2004

CU2 Project Title CONSTRUCTION OF A REPLICATION DEFECTIVE RETROVIRAL VECTORS TO EXPRESS ONCOGENES (EG MDM2) OR OTHER NON-TOXIC GENES SUCH AS TUMOUR SUPPRESSOR GENES (EG P53, MTBP, E-MTBP OR LAC-Z)

Date Project Ceased 15/01/2007

Class 2

CultureVolClass2 < 1 litre

Class CultureVolumeClass3-4

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements

Withdrawn

N

Tick if notifying a connected programme of work

N

Historical Significant Changes TRANSFERRED TO GM 554 - 15/1/07

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

To study the role of the p53 pathway in cancer. Genes such as MDM2 have been implicated as critical molecules in this pathway and to study this we need to be able to efficiently deliver these genes (also non-oncogenic genes such as p53, MTBP, E-MTBP and Lac-Z will be expressed from similar viruses) to a range of cell types - particularly had to transfect cells such as mouse embryo fibroblasts (MEFs) and a range of pancreatic cancer derived cell lines.

Recipient or parental organism

E. coli XL-Blue are a K12 derivative strain and are not expected to survive outside of the laboratory. There is no evidence that these bacteria are pathogenic.

MEFs and other cell lines would not be able to survive outside of the laboratory environment. Retroviral vectors have generally been designated as ACGM category 1 except where the gene to be inserted is pathogenic or where the envelope is human tropic. We propose to create replication defective retroviruses with either rodent or human tropic envelopes. Retroviral vectors with these modifications are ACGM2.

Host/vector system

The three-plasmid system would require at least 2 recombination events to produce a single fragment of DNA that could encode replication competent retrovirus (RCR). Since there is little or no homology between the three plasmids a single recombination event is unlikely.

Origin & function

MDM2 (for example) is known to be an oncogenic protein. Retroviruses are also inherently potentially oncogenic because they are obligate mutagens: when the provirus is integrated into the host genome, it may at random be integrated into a site that causes inactivation of a tumour suppression gene. MTBP, E-MTBP and p53 induce cell cycle arrest (Boyd et al, JBC 2000). We will be inserting wild-type p53. As yet the roles of MTBP and E-MTBP, a deletion mutant of MTBP, are not completely known, although it has been demonstrated that MTBP causes G1 arrest and E-MTBP inhibits tumour cell growth more effectively in the cell lines tested than either MTBP or p53.

Retroviruses are obligate mutagens: when the provirus is integrated into the host genome this might occur at random into, for example, a tumour suppression gene.

Evaluation of foreseeable effects

Bacterial transformation - the transformed strain will be no more virulent or pathogenic than the original.

The three plasmid system used should ensure that the virus is not mobilisable.

We expect moderately high levels of expression of the transduced genes driven by the MuMLV LTR.

These replication defective viruses could infect human cells.

Retroviral production - a VSV-G envelope will be used to improve infectivity of the virus but only to produce replication defective viruses containing a non-oncogenic insert. We will test supernatants for evidence of RCR by infecting permissive cells with viral supernatant and then passaging cells. Supernatant from these cells will be screened at weekly intervals by re-inoculating these at >/= 1/10 dilution onto permissive cells. MDM2 can readily be detected by western blot. By inoculating double null (MDM2/p53 null) MEFs we can readily detect even very low levels of MDM2.

MTBP and E-MTBP can readily be detected by western blot. By inoculating p53 null cells (H1299) p53 can also be detected by western blot. Lac-Z can be screened by in-situ Beta Gal assay.

Retroviral production - we will use either an ecotropic or a VSV-G envelope. These do not by themselves increase the pathogenic traits of the retrovirus although the VSV-G envelope which will be used to improve infectivity of the virus (it is monomeric and therefore higher tides of more stable virus can be produced c.f. eco/ampho-tropic retroviral envelopes) does alter the virus host range to include humans and other mammals.

The retroviral vector will also contain the neomycin gene, which will result in resistance to G418. This would not be used as a treatment for laboratory-acquired infection.

The major alteration affecting likely harm will be the inclusion of a known oncogene (eg MDM2) into the retrovirus. This will create the risk of transfer of an oncogene into mammalian in particular human cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
After bacterial transformation, flasks will be decontaminated by the addition of fresh 10% bleach.

During retroviral production, once the producer cells have been transfected with the 3 plasmid components they will be considered potentially infectious. BSL2 precautions will be required. Experiments will take place in a laminar flow hood with the operator wearing a disposable surgical gown (near opening) with snug fitting surgical latex gloves worn over the elasticated wrists of the gown. Care will be taken so as not to produce aerosolisation of viral containing supernatants. Items of equipment exposed to virus will be soaked in 1% Virkon or 1% sodium hypochlorite or 10% bleach for 15 mins before removal from the laminar flow hood and then disposed of in the usual manner: ie all solid waste will be incinerated and all inactivated liquid waste will be disposed of down the sink. Once cells are no longer required for producing virus, they will be destroyed by adding fresh 10% bleach and leaving for 24 hours before disposal as liquid or solid waste whichever is most appropriate. All solid waste will be transferred to yellow clinical waste bins and sent to the RLBUH for incineration.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered the risk assessment and decided that the use of potentially oncogenic inserts means that this project is Class 2. The GMSC were content that the workers involved were aware of the risks and were technically capable of working at Class 2 level. The laboratories to be used are adequate for this work. As an additional observation it was noted that Class 1 work would take place alongside this Class 2 project. The GMSC requested that all work to be carried out in the laboratories alongside this class 2 activity should be carried out to CL2 standards.

Project Containment

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Name

CANCER RESEARCH UK

Name 2

THE UNIVERSITY OF EDINBURGH

Department

EDINBURGH ONCOLOGY UNIT

Campus Estate or Research Centre

WESTERN GENERAL HOSPITAL

Building

District

CREWE ROAD SOUTH

Town

EDINBURGH

County

EAST LOTHIAN

Postcode

EH4 2XU

Country

SCOTLAND

Tel Number

0131 332 2471

Fax Number

0131 332 8494

E-mail

HSE Division

SCOTLAND

Comments

FORMERLY IMPERIAL CANCER RESEARCH FUND UNTIL 4/2/2002

Date at Which Additional Info Submitted

02/03/2022

Page 10476 of 15326
Premises Addresses

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02/03/2022
All laboratory waste is disinfected or autoclaved prior to disposal from the Unit. General, non-hazardous waste, such as paper towels, are placed in normal waste bags for disposal.

**Solid Waste:**
All solid waste is autoclaved prior to disposal. Waste is placed in an autoclave bag, which is then transported in metal buckets to the autoclave. Following autoclaving at 121°C for 15 minutes, the waste is collected and removed from site by a specialist waste contractor.

**Agar Waste:**
All agar-containing waste e.g plates, are placed in a separate autoclave bag from other solid waste which is double-bagged prior to transporting in a metal bucket to the autoclave. Autoclaving is carried out as above.

**Liquid Waste:**
All liquid waste containing biological agents is disinfected overnight in a hypochlorite solution by the addition of Haz-Tabs to a chlorine concentration of >500ppm, prior to disposal down the drains, with copious water flow to allow adequate dilution.

**Sharps:**
The use of sharps e.g. hypodermic needles, scalpels, etc. is minimised. If they are used they are disposed of in rigid plastic ‘Sharpsafe’ containers. When full, these are collected and removed from site by a specialist waste contractor.

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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

<table>
<thead>
<tr>
<th>Virology</th>
<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
<th>Gene Therapy</th>
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<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
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<td>Other(s)</td>
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</table>

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

02/03/2022
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<tr>
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<td>Emergency Plan Required?</td>
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<td>Non-GMMs</td>
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<tr>
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<td>N</td>
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**Name**

MEDICAL RESEARCH COUNCIL PRION UNIT

**Name 2**

INSTITUTE OF NEUROLOGY

**Department**

NEURODEGENERATIVE DISEASES

**Campus Estate or Research Centre**

UNIVERSITY COLLEGE LONDON

**Building**

QUEENS SQUARE HOUSE

**Road Name**

QUEENS SQUARE

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

WC1 3BG

**Country**

ENGLAND

**Tel Number**

0208 869 3265

**Fax Number**

0208 869 3270

**E-mail**

**HSE Division**

LONDON

**Comments**

**Date at Which Additional Info Submitted**

05/09/2001 10/10/2001
### Premises Addresses

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<th>Town</th>
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<td>WATFORD ROAD</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Parasitology
Transgenic
Birds
Microbiology
Research

Virology
Transgenic
Animals
Transgenic
Fish
Gene Therapy

Mycology
Transgenic
Invertebrates
Transgenic
Plants

Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 669/05.1

Date Ackn'd
07/09/2005

Date Project Ceased
31/10/2017

CU2 Project Title
Production of a knock-out model of PINK-1 in human cell lines.

Consent Granted
Not Applicable

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

Generation of PINK-1 gene knockout human cell lines in order to create a model for the study of Parkinson's Disease.

#### Recipient or parental organism

Human cell lines.

#### Host/vector system

- Human cell lines such as HEK293, SOAS2, human neuroblastoma cell lines and 197VM human neural stem cells (ReNeuron).
- JS4, a recA-derivative of E. coli MC1061, a disabled K12 strain.
- Phoenix ampho cells, a non-mobilisable derivative of HEK293, free of adventitious agents.
- Vectors: PSUPERretro-puro, PWZLneo EcoR(non-mobilisable), PWZLBlastF(non-mobilisable) and PWZLBlastFEcoR

#### Origin & function

64i nucleotide RNAi constructs designed to knockout PINK-1 expression (Sigma Genesis Ltd). EcoRI fragment of the murine cationic transporter from Julian Downward as pWZLneoEcoR, originally made by D. Conklin.

#### Evaluation of foreseeable effects

Human cell lines will have ability to be infected by murine ecotropic viruses. The gene function of PINK-1 could be knocked out if the PINK-1 RNAi sequences were transferred to a human organism. The consequences of this are not clearly known, although loss of PINK-1 function is thought to be the underlying mechanism in autosomal recessive Parkinson's disease and is therefore associated with neurodegeneration in humans.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- No GM animals or plants will be produced.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- No derogation required.

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Liquid waste and plastic disposable pipettes are chemically decontaminated with either 10,000ppm Chloros or 1% Virkon, followed by incineration (solid material).
- E. coli contaminated solid waste (agar plates, tips, pipettes is autoclaved at 121 C for 20 mins, followed by incineration. Solid waste from tissue culture such as dishes, tubes and flasks are also autoclaved before incineration.
The risk assessment has been approved by the local GM Safety Committee and accepted at Class 2.

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been approved by the local GM Safety Committee and accepted at Class 2.

Project Containment

<table>
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<tr>
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Project Ref 669/06.1

Date Ackn'd 21/02/2006

CU2 Project Title Construction and use of defective helper free amphotropic retroviruses for immortisation of primary human cells.

Class 2 CultureVolClass2 < 1 Litre

Date Project Ceased 31/10/2017

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Withdrawn N

Tick if notifying a connected programme of work N

Project transferred to GM14 on closure of GM669

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

To generate and use stable producer cell lines that produce high titre replication defective amphotropic retroviruses to be used for the immortilisation of freshly isolated primary human cells.

Recipient or parental organism

JS4, a recA-derivative of E.coli MC1061 (a disabled K12 strain). PA371, a non-mobilisable second generation mouse amphotropic producer cell line; TEFLYA and RD cells, both derivatived from TE671 cells, non-mobilisable. Phoenix ampho cells, non-mobilisable derivatives of HEK293, and primary human somatic cells.

Host/vector system

SV40 large T antigen and mutants.
Human catalytic subunit of telomerase and human papilloma virus 16/18.
E7 and E6 gene; Polyoma virus T antigens and mutants; Adenovirus E1A and mutants.
P53 GSE fragment and full length ORF for cellular genes.

Origin & function

SV40 T antigen, the catalytic of human telomerase, HPV E7 and E6 proteins, Polyoma virus T antigens, Adenovirus E1A and p53GSE are all immortalising genes that have the potential to extend the proliferative life span of normal somatic cells.

Evaluation of foreseeable effects

No GM animals or plants will be produced.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No derogation required.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste such as dishes and flasks will be imersed in 1% Virkon for >10 minutes, followed by autoclaving and then inceration.
E.coli liquid waste is inactivated by treatment with 1% Virkon for >10 minutes.
Liquid tissue culture medium is collected into containers containing sufficient Virkon for final concentration of not less than 1% and left for >10 minutes.
E.coli solid waste (agar plates, tips and pipettes) is autoclaved followed by incineration.
Solid waste from tissue culture (tubes, dishes, flasks and pipettes) is autoclaved followed by incineration.

After autoclaving or chemical disinfection, waste is put into yellow clinical waste bags or containers, collected centrally and transported by an approved waste route for incineration.

Established inactivation procedures in molecular biology laboratories, giving effectively 100% kill.
Records of each autoclave run are made and held by the Safety Officer.
The risk assessment has been approved by the local GM Safety Committee and accepted as Class 2.

### Project Containment

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### Project Ref 669/11.1

- **Date Ackn'd**: 20/12/2011
- **CU2 Project Title**: Full-length HTT gene expression in human cells using amphotropic retroviral constructs
- **Class**: Class 2
- **CultureVol**: < 1 Litre
- **Non-GMM**: Consent Granted
- **Historical Significant Changes**: Project transferred to GM14 on closure of GM669
- **Date Project Ceased**: 31/10/2017
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

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*Page 10485 of 15326*
### Project Additional Information

#### Purposes of the contained use

Human neurons expressing either full-length normal or mutant Htt will be generated using STROC05 and 197VM neural stem cell lines transduced with pMSCV-based retroviral constructs. They will then be differentiated into neurons by the removal of key growth factors, which can then be used to set up co-cultures of primary human monocytes and macrophages isolated from Huntington's disease patients and control subjects to investigate neuronal-myeloid cell interactions.

The retroviral vectors to be used contain the full-length sequence of the HTT with either a normal (15Q) or mutant (138Q) CAG repeat length encoding the wild-type and mutant Htt proteins respectively. DNA prepared from E. coli bacterial cultures is packaged as a retrovirus by co-transfection into either HEK293 or BOSC23 cells with separate gag/pol and env packaging co-factor plasmids.

#### Recipient or parental organism

- E.coli K12 strains (eg DH5α, TOP10)
- HEK293T cells (human cell line for retroviral packaging)
- BOSC23 cells (human cell line for retroviral packaging)
- U937 cells (human lymphoma cell line)
- STROC05 cells (human striatal neural stem cell line)
- ReNcell 197VM cells (human neural stem cell line)

#### Host/vector system

- **pMSCVpuro-10366-Htt15Q**
- **pMSCVhyg-10366-Htt138Q**
- gag/pol+ & env plasmids

#### Origin & function

The inserted genetic material will encode the full-length human HTT (huntingtin) sequence (with either 15 or 138 glutamine repeats in exon 1 of the gene). Expression of Htt with an expansion in the number of glutamine residues in its exon 1 (>36Q; mHtt) is thought to be the key pathogenic determinant of Huntington's disease, an inherited neurodegenerative disorder in humans. Htt is widely expressed in human tissue, but its function is unclear. The protein has no sequence homology to other proteins, but it is known to regulate gene expression and may have a functional role in cytoskeletal anchoring or transport of mitochondria or vesicle trafficking. We intend to study the cellular effects of expression of htt in human neural stem cells.

#### Evaluation of foreseeable effects

mHtt expression in neurons is thought to be the underlying cause of Huntington’s disease in humans; the inserted gene product could be harmful to health in the event of its expression in the CNS. Viruses packaged from the pMSCV vector are capable of infecting human cells itself, but are unable to replicate because the viral structural genes are absent. Integrated proviruses are capable of being mobilized by superinfection with helper virus, but the likelihood is low because the cells to be used are not known to produce helper viruses and transduced cells will be cultured in isolation from other cell cultures.

The GMOs themselves are not able to establish infection and maintain propagation in humans or other species. Cultured mammalian cells, transduced or otherwise, are unable to survive outside of the laboratory environment and...
are not infectious. The E. coli bacterial strains are disabled, lack the necessary pathogenic mechanisms required to cause infection and will only have limited survival in the environment. Retroviral particles could conceivably infect cells of other species, resulting in the transfer of genetic material. Effects due to expression of target genes would be unlikely, but they could result in disruption of host genes depending on the site of insertion. Such effects would be limited by the inability of the virus to subsequently replicate. The absence of any potential of direct contact and the lack of survival of any of the proposed GMOs limits the potential effects on other species or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be decontaminated with 1% Virkon for a minimum of two hours, prior to disposal with plenty of running water down a suitable designated sink. The degree of kill using 1% Virkon is 100%, as per supplier's information, and this procedure is well established in molecular/cell biology laboratories. Solid waste such as culture dishes and flasks will be decontaminated with 1% Virkon overnight. Solid waste from mammalian cell culture is autoclaved prior to incineration; this is a standard Departmental procedure for those areas in which Containment level 2 work is carried out. Following chemical disinfection (and autoclaving), all solid waste waste is put into yellow clinical waste bags or containers for collection in designated yellow bins and transport by an approved waste disposal route for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project was approved by the GM 669 committee. This work poses no risks to personnel or the environment when local rules and decontamination procedures are practised in conjunction with the engineering and administrative control measures for work at containment level 2.

Project Containment

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02/03/2022
Expression of mouse prion protein in eukaryotic systems

To employ eukaryotic expression systems to produce the normal cellular form of the murine prion protein. The advantage of using such systems is they enable proper protein folding, disulphide bond formation, and posttranslational modifications such as signal cleavage, proteolytic cleavage, or/and glycosylation that we cannot obtain when using prokaryotic systems.

Recipient or parental organism

Insect cells: Spodoptera frugiperda 9 (Sf9)
Mammalian cells: Chinese hamster ovary (CHO), human embryonic kidney cells (HEK.293)

Host/vector system

pTriEx, pFastBac HT and other commercially available vectors to be used as necessary for baculovirus expression
PTriEx, pSV and pCMV series of vectors and other commercially available vectors to be used for expression in mammalian cells.
**Origin & function**

The vectors used are standard commercially available ones and are designed to allow the expression of the gene of interest. The mouse prion gene may be cloned from mouse cDNA or synthesised by commercial gene synthesis.

**Evaluation of foreseeable effects**

None of the strains of GMMs used are able to survive outside of a laboratory. The baculoviruses used are polyhedrin negative and have a much reduced survival time owing to increased susceptibility to dessication and ultraviolet light. The vectors used will confer antibiotic resistance to antibiotics commonly used in the laboratory, namely ampicillin, blasticidin, zeocin or hygromycin. Although it is theoretically possible that the prion protein could misfold from the cellular form to the disease associated form that could be harmful to health, even were this highly unlikely event to occur, the very low infectious titre of any material in combination with the transmission barrier for prions between mouse and human makes detrimental impact on human health extremely unlikely. The prion protein has been expressed by other laboratories with no reports of changes to the pathogenicity of the host.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| Solid waste will be treated with 20,000 ppm sodium hypochlorite for one hour, or where this is not suitable then sodium hydroxide to a final concentration of 1 M will be used. GMM material will then by autoclaved prior to disposal by incineration. | Is an emergency plan required according to regulation 20? N |
| Liquid waste will be treated with 20,000 ppm sodium hypochlorite for one hour, or where this is not suitable then sodium hydroxide to a final concentration of 1 M will be used prior to disposal down the drain. | If yes, tick to confirm that it is attached to this form N |
| These represent established protocols for the inactivation of prions and should achieve a 100% "kill rate" | Tick to confirm that you have attached a risk assessment to this form Y |
| | Tick if you are claiming exemption from disclosure for section of the risk assessment N |

**Project Containment**

The project was approved by the GM committee. This work poses no risks to personnel or the environment when local rules and decontamination procedures are practiced in conjunction with the engineering and administrative control measures for work at containment level 2.
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Name**

ACS DOBFAR UK LIMITED

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

**Town**

BEDLINGTON

**County**

NORTHUMBERLAND

**Postcode**

NE22 7DB

**Country**

ENGLAND

**Tel Number**

01670 565575

**Fax Number**

01670 810041

**E-mail**

**HSE Division**

YORKSHIRE AND NORTH EAST

**Comments**

FORMERLY SYNPAC PHARMACEUTICALS LIMITED UP UNTIL 31/12/2000.

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Animal Unit</th>
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<th>Glass House</th>
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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

The maximum volume to be cultured will be 50 litres. Waste is deactivated by heating to 121 degrees C, either in an autoclave or in the germination vessel. Smaller quantities (including spills) are deactivated by addition of a suitable disinfectant and/or autoclaving. All methods have been validated. Methods are monitored by sampling post sterilisation.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 671

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Name

**TCS CELLWORKS LTD**

Name 2

Department

RESEARCH & DEVELOPMENT

Campus Estate or Research Centre

WHITELEAF BUSINESS CENTRE

Road Name

11, LITTLE BALMER

Town

BUCKINGHAM

County

BUCKINGHAMSHIRE

Postcode

MK18 1TF

Country

ENGLAND

Tel Number

0128 082 7475

Fax Number

0128 082 7477

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
Deactivation of waste is achieved through addition of a 1% solution of Virkon (Antec International, Sudbury). This solution is active against all bacterial, viral and fungal agents. Following addition of Virkon, liquid waste is flushed down the sink with copious amounts of water. The maximum culture volume to be disposed of at any one time would be 100 ml.

Validation of the deactivation method has been carried out by incubation of the cells with and without added Virkon followed by incubation at 37 degrees C. No cells were apparent upon microscopic examination when Virkon had been added.

Monitoring of the deactivation process is by microscopic examination following Virkon addition.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 672**

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**Name**

ROYAL MANCHESTER CHILDRENS HOSPITAL

**Name 2**

WILLINK BIOCHEMICAL GENETICS UNIT

**Campus Estate or Research Centre**

RESEARCH & POSTGRADUATE CENTRE

**Road Name**

HOSPITAL ROAD

**Town**

MANCHESTER

**District**

PENDLEBURY

**County**

GREATER MANCHESTER

**Postcode**

M27 4HA

**Country**

ENGLAND

**Tel Number**

0161 727 2137/8

**Fax Number**

0161 727 2137

**E-mail**

**HSE Division**

NORTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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| Campus Estate or Research Centre    |                       |
|                                     |                       |

| Road Name                          | EAST DULWICH GROVE    |
|                                     |                       |
| District                            | DULWICH               |

| Town                                | LONDON                |
|                                     |                       |
| County                              | GREATER LONDON        |
| Postcode                            | SE22 8QF              |
| Country                             | ENGLAND               |

| Tel Number                          | 0208 693 2830         |
|                                     |                       |
| Fax Number                          | 0207 346 6477         |
| E-mail                              |                       |

| HSE Division                        | LONDON                |
|                                     |                       |

| Comments                             | FORMERLY KNOWN AS PUBLIC HEALTH LABORATORY SERVICE |
|                                     |                                                   |

<table>
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02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Animal Unit</th>
<th>Growth Room</th>
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<th>Large Scale</th>
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Other (please specify) | Tick if confidential

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All activities are class 1. Liquid waste is treated with 1% chloros and autoclaved prior to disposal as effluent. Maximum volumes involved are 500 ml at any one time. All glassware and plastics are autoclaved and subsequently incinerated. Autoclave is fully monitored.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

- **Other(s)**

<table>
<thead>
<tr>
<th>Mycology</th>
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Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

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02/03/2022

Page 10503 of 15326
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Parasitology</th>
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</table>
Likely maximum culture volume - 2 litres, in 4 x 500 ml batches.

Deactivation method -
1. Culture media collected into single flask.
2. Bleach (15% sodium hypochlorite, 5% anionic detergents) added to final concentration of 1% v/v
3. Culture media incubated at room temperature for 10 minutes to allow deactivation
4. Media disposed in normal waste water, with 10 volumes of water added.

Validation of deactivation method - before disposal of inactivated culture media, 100 ul sample taken and spread on LB Agar, and incubated overnight at 37 degrees C. No colonies should be present.

Monitoring of deactivation method - visual checks to be performed by workers, with double-checking performed by Biological Safety Officer. Disposal events to be recorded in Bacterial Culture log book, and countersigned by Biological Safety Officer.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 674/05.1

Date Ackn'd 04/10/2005

CU2 Project Title Small scale culture and genetic manipulation of mammalian and insect cell lines for the development of molecular biology kits.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 1-50 Litres

Non-GMM Consent Granted Not Applicable
**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**

**Purposes of the contained use**

To culture established containment level 2 cell lines (from the ATCC global bioresource centre). Insect and mammalian expression of exogenous non-hazardous genes for expression analysis and purification. Non-hazardous reporter genes will be used to test new transfection reagents. RNAi will be transfected into cell lines to downregulate target genes. Nucleic acid will be extracted from the cells. All contained experiments will involve research and development leading to the production of molecular biology research kits and reagents.

**Recipient or parental organism**

The following ATCC cell lines and Invitrogen cell lines will be used (ACTT number in brackets). Containment level 2 cells include 2983 (CRL-1573), COS-1 (CRL-1650), Hela (CCL-2). Containment level 1 cells include MCF7 (HTB-22), NIH/3T3 (CRL-1658), CHO-K1 (CCL-61), Jurkat (TIB-152), HepG2 (HB-8065), NIH/3T6 (CCL-96) and H15 (Invitrogen, UK) and SF9 (Invitrogen, UK).

**Host/vector system**

Not applicable, we will not be creating genetically modified whole organisms. Only using established cell lines grown in a monolayer and/or in suspension culture. We will transiently transfecting exogenous genetic material to these cell lines.

**Origin & function**

Green fluorescent protein, in the pACGFP1-N1 (BD Biosciences, UK) plasmid, Luciferase in the pGL4 vector (Promega, UK) and possibly other non-toxic reporter constructs will be transiently and stably transfected into cell lines including those mentioned in paragraph 1 of section 7. Expression levels will be manipulated using RNAi. Non-infectious chemical based transfection reagents will be analysed using these reagents. Green fluorescent protein, in the pACGFP1-N1 (BD Biosciences, UK) plasmid, Luciferase in the pGL4 vector (Promega, UK) and possibly other non-toxic reporter constructs will be transiently and stably transfected into cell lines including those mentioned in paragraph 1 of section 7. Expression levels will be manipulated using RNAi. Non-infectious chemical based transfection reagents will be analysed using these reagents.

The human gene Dicer (Accession NP 085124), the Celery gene Cell (Accession AF237958), the human gene DCP2 (Accession AY135173), RNAse inhibitor (human) will be expressed in bacterial and baculovirus expression systems. The recombinant proteins will be purified using FPLC.

Mouse hybridoma cell lines expressing non-hazardous immunoglobulin proteins will be grown and immunoglobulin purified by FPLC.

**Evaluation of foreseeable effects**

RNAi technology will be used to temporarily downregulate several non-essential cellular genes in the cell lines mentioned in section 7.

All of the work mentioned above will be carried out in a class 2 safety cabinet with appropriate waste disposal methods.
Potential hazards to human health are minimal. No hazards to human and/or environmental health have been published for the genes mentioned above.

RNAi transfection will be transient and specific to non essential genes, eliminating health risks for accidental human administration.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

During the maintenance and manipulation of cell lines waste will include live cells in culture media. The waste cells will be treated with Virkon (Anachem, UK) disinfectant following manufacturers protocols, autoclaved and then discarded into sewage system. This method of disposal has been validated to shown no viable organisms survive. The validation is on going to show no viable organisms survive the disinfection treatment.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

Satisfied that the work proposed is of low risk to humans or the environment and that the containment and disposal procedures outlined are sufficient for the work described above.

The work is not involved in creating organisms which are viable outside the laboratory. No genes toxic to humans will be expressed. The majority of the work involved in this project is routine cell culture and does not involve genetic modification.

T

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022

Page 10508 of 15326
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Name

ANTISOMA RESEARCH LABORATORIES LTD

Name 2

Department

Campus Estate or Research Centre

BIOPARK HERTFORDSHIRE

Road Name

BROADWATER ROAD

District

WELWYN GARDEN CITY

Town

County

HERTFORDSHIRE

Postcode

AL7 3AX

Country

ENGLAND

Tel Number

0208 672 7200

Fax Number

0208 767 1809

E-mail

HSE Division

LONDON

Date at Which Additional Info Submitted

GM Centre closed 27/08/2010
### Premises Addresses

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<td>CRANMER TERRACE</td>
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

02/03/2022 | 02/03/2022 | 10511 of 15326 |
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For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
| Data Premises Notified (Originally) | 10/08/1998 | Transferred from 1992 Regs? | Y | Transitional Premises Class | 2 | Data Premises Closed | 30/03/2006 | Transitional Premises | N | Non-GMMs | N | Withdrawn | N |

**Name**

OXFORD GLYCOSCIENCES PLC

**Name 2**

OXFORD GLYCOSCIENCES UK LTD

**Department**

BIOLOGY

**Campus Estate or Research Centre**

ABINGDON SCIENCE PARK

**Building**

10 THE QUADRANT

**Town**

ABINGDON

**County**

OXFORDSHIRE

**Postcode**

OX14 3YS

**Country**

ENGLAND

**Tel Number**

01235 543200

**Fax Number**

01235 543 299

**E-mail**

none

**HSE Division**

EAST AND SOUTH EAST

**Comments**

INFORMED BY EMAIL (GM82 TOOK THEM OVER) THAT SITE HAD BEEN CLOSED AS OF DECEMBER 2004.

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

<table>
<thead>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

Other (please specify): 

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 676/03.1

**Date Ackn'd** 13/05/2003

**CU2 Project Title**

GENETIC MODIFICATION OF CANDKIDA ALBICANS FOR VALIDATION OF ANTFUNGAL TARGETS

**Class**

Class 2

**CultureVol**

Class2 Volume

< 1 litre

**Non-GMM**

Consent Granted

not applicable

**Project notified under transitional arrangements**

N

**Withdrawn**

N

Tick if notifying a connected programme of work

N

---

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**
purposes of the contained use

The purpose of this work is in the in vitro and in vivo validation of potential antifungal targets through the production of either deletion mutants or conditional expression strains. Additionally, techniques for epitope tagging and the construction of promotor reporter constructs will be used, both for target validation purposes and in the evaluation of potential drug effects.

Recipient or parental organism

The ACDP class Candida albicans in hazard group 2. The organism may be considered a commensal of the normal flora of the digestive and genital tracts. Pathogenicity is essentially a result of changes in the hosts defence mechanisms; thus Candida can be termed an opportunistic pathogen. Candida infections (Candidiasis) range from superficial mucosal infections to serious life threatening systemic infections in the severely immunocompromised host.

Host/vector system

Vectors for transformation into C. albicans will have been constructed in E. coli. In all cases the vectors transformed into C. albicans will be integrated into the hosts genome via homologous recombination.

Origin & function

The majority of the work proposed involves the manipulation and alteration of existing C. albicans genes (for example gene deletions or promotor replacements). This will involve the targeted insertion of DNA into specific locations in the C. albicans genome. The inserted DNA will contain an auxotrophic marker for the selection of transformed cells (eg the C. albicans URA3, ARG4, HIS1 OR ADE2 genes). Foreign DNA present in the inserted DNA will include:

1. S. typhimurium hisG - present as a direct repeat around the C. albicans URA3 gene for gene deletions and subsequent recycling of the marker.
2. Tetracycline transactivator (itself a fusion between E. coli Tn10 tetR and viral VP16 activation domain) to regulate expression of C. albicans genes.
3. Reporter genes such as B-galactosidase, luciferase and yeGFP.
4. The E. coli plasmid backbones (pUC and pBR based) that the insert is contained on will in some cases become inserted into the genome.

Evaluation of foreseeable effects

The genetic modifications proposed would not be expected to increase the virulence of the GMO above that of the parental organism. Therefore the risk to human health can be considered equivalent to that of dealing with the parental organism. The only potential hazard, in healthy individuals, of handling Candida albicans would be from exposure resulting in a mucosal infection. However the risk of this hazard being realised, even if exposure occurred, would be low.

All the genetic modifications proposed would be non mobilisable therefore the risk of transfer of sequences to other micro-organisms is remote. The GMO’s produced would be potentially as fit as the parental organism and may therefore be able to persist in the environment if a breach in containment occurred. However, the GMOs would have no selective advantage. The risk to the environment is therefore very low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste is double bagged in autoclave bags and collected daily. These bags are stored in 60L plastic boxes for a maximum of 2 days before autoclaving. Waste is autoclaved for 15 minutes at 134 degrees C. The autoclave is serviced and calibrated every 3 months to ensure continued effectiveness.

Liquid waste is inactivated by chemical treatment. Culture vessels and supernatants are decontaminated with a 1:50 dilution of TRIGENE (Medichem International) and are...
left for 16 hours prior to disposal down laboratory sink. Glassware is then rinsed and washed by dishwasher, plastic ware is further autoclaved prior to disposal. This method has been validated to give 100% kill.

The major point to emphasize from the risk assessment is that none of the modifications proposed would be expected to increase the virulence of the GMO above that of wild type Candida albicans. Nor would the modifications impact on the sensitivity of the strain to currently used anti-fungal therapies. The assignment of the activities to class 2 is due to the risk posed by the recipient organism itself.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**Project Ref** 676/98.1

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<th>Project notified under transitional arrangements</th>
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Tick if notifying a connected programme of work

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The major point to emphasize from the risk assessment is that none of the modifications proposed would be expected to increase the virulence of the GMO above that of wild type Candida albicans. Nor would the modifications impact on the sensitivity of the strain to currently used anti-fungal therapies. The assignment of the activities to class 2 is due to the risk posed by the recipient organism itself.
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment
### Project Containment

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#### Name

**SMITH & NEPHEW GROUP RESEARCH CENTRE**

#### Name 2

**Department**

**BIOLOGICAL SCIENCES**

#### Campus Estate or Research Centre

**Building**

#### Road Name

**YORK SCIENCE PARK**

#### District

**HESLINGTON**

#### Town

**YORK**

#### County

**YORKSHIRE**

#### Postcode

**YO10 5DF**

#### Country

**ENGLAND**

#### Tel Number

01904 824000

#### Fax Number

01904 824004

#### E-mail

#### HSE Division

**YORKSHIRE AND NORTH EAST**

#### Comments

Date at Which Additional Info Submitted

02/03/2022

Page 10520 of 15326
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Transgenic Transgenic Microbiology Research Gene Therapy

Virology Transgenic Animals Birds Fish
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<th>Transgenic Plants</th>
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### UNIVERSITY OF CAMBRIDGE

#### Data Premises Notified
- **GM Centre Number:** 678
- **Data Premises Notified:** 03/09/1998
- **(Originally) Transferred from:** 1992 Regs?
- **Transitional Premises:**
  - **Class:** 2
  - **Emergency Plan Required:** N
  - **Non-GMMs:** Y
  - **Withdrawn:** N

#### Name
- **UNIVERSITY OF CAMBRIDGE

#### Name 2
- **ADDENBROOKES HOSPITAL

#### Campus Estate or Research Centre
- **Department:**
  - **CAMPBELL INSTITUTE FOR MEDICAL RESEARCH

#### Road Name
- **HILLS ROAD

#### Town
- **CAMBRIDGE

#### District
- **CAMBRIDGESHIRE

#### Postcode
- **CB2 0XY

#### Country
- **ENGLAND

#### Tel Number
- **01223762322

#### Fax Number
- **01223762323

#### E-mail
- **HSE Division:**
  - **EAST AND SOUTH EAST

#### Comments

#### Date at Which Additional Info Submitted
- **02/03/2022**
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

| Laboratory | Animal Unit | Growth Room | Glass House | Large Scale |
|------------|-------------|-------------|-------------|-------------|-------------|

02/03/2022
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Protein misfolding in cells contributes to important diseases ranging from neurodegenerative disorders, such as Parkinson's Disease, to metabolic diseases such as type II diabetes mellitus. A common theme of these conditions is the accumulation, or the threat of accumulation, of misfolded and unfolded proteins that contribute to attrition of key cellular functions. Cells have an elaborate apparatus for recognizing and responding to the threat of protein misfolding, known as the Unfolded Protein Response (UPR). This apparatus has numerous components that promote fitness in the face of the aforementioned threat. Surprisingly, however, the machinery of the unfolded protein response also has components whose activity, in certain contexts appear to enhance the deleterious effects of misfolding [Ron, 2007 #3066].

Rational therapeutic interventions aimed at the pathophysiology of protein misfolding requires the establishment of experimental systems in which the threat of protein misfolding is imposed and the activity of various components of the response to misfolded proteins (the Unfolded Protein Response) are either inactivated or enhanced. Because the clinically-relevant burden of protein misfolding is experienced disproportionately by terminally-differentiated, non-dividing cells, the tools for constructing the aforementioned experimental system must be geared to manipulating gene expression in non-dividing cells.

HIV1-based lentiviruses, modified for safety and gene delivery have emerged as powerful tools for effecting changes in gene expression in terminally-differentiated cultured cells. Therefore, authorization is sought to use such vectors to enforce expression of genes encoding known misfolding-prone proteins in cultured mammalian cells and at the same time alter the cellular response to the misfolded proteins by introducing activated alleles, dominant loss-of-function alleles or shRNA inactivating alleles of genes encoding components of the unfolded protein response. By comparing the response of cells with diverse perturbations in the unfolded protein response to comparable burdens of protein misfolding we can begin to identify components of the unfolded protein response whose activation or inactivation (by pharmacological means) may be of therapeutic benefit in combating diseases of protein misfolding.

7.
Recipient or parental organism

The lentivirus is produced by co-transfecting the packaging plasmid mix and the transfer vector into human HEK293T cells.

The recipient cells will be various murine and human cells in culture.

Both the recipient cells and the producing cells are fastidious and non harmful. They cannot colonise the environment nor cause disease by colonizing human or animal hosts and thus are classified as Hazard Group 1.

Cells from human volunteers, which may be used in this project are uncharacterised and although themselves present no greater risk, may contain adventitious infectious agents and therefore will be handled at CL2 (risk assessment under COSHH regs).

Host/vector system

The pGIPZ and pTRIPZ lentiviral package systems and derivatives thereof like the pLenti6_V5 of invitrogen or the delivery vehicle for Sigman mission shRNA (pLKO.1–Puro) to be used were described by Kappes et al. 2001; Kappes et al. 2003; Wu et al. 2000; Wu et al. 2001 and developed by Open Biosystems. This new generation of lentiviral systems has been used extensively throughout the world and includes important features designed to enhance its biosafety:

The reverse transcriptase (RT) and integrase (IN) genes were split from the Gag-pol polyprotein-encoding gene and are provided in trans from two separate plasmid. The ENV function, provided by the VSV glycoprotein, is introduced in trans by a third plasmid and the only packaging signal is on the transfer plasmid that lacks important HIV genes. Thus, the probability of a series of recombination events that produce a replication-competent virus that can spread in and between hosts is vanishingly small. In the context of this project, the main risk is the consequence of primary infection of humans by high titre viral stocks (see below).

The lentivirus is produced by co-transfecting the packaging plasmid mix (four plasmids for gag, pro, RT, IN, VSV-G, rev and tat expression) and the transfer vector into the HEK293T cells. Particles are harvested over a period of 48 to 72 hours after transfection and are stored at -80C. If titres are low, the virus can be concentrated by ultracentrifugation. Transduction of recipient cells follows. These will be tissue cultures of primary human or other mammalian cell lines.


Origin & function

The shRNA sequences to inactivate genes in the host are derived from the host cell genome. They will be directed to genes that function in protein folding in the endoplasmic reticulum and the response to protein misfolding (examples include: chaperone-encoding genes like Erdj3, enzymes involved in oxidative protein folding like ErO11 and ErO11b, components of the unfolded protein response like Em1 or Eif2aK3). These genes are neither oncogenes nor tumor suppressor genes and thus accidental infection of a human host by these viruses is likely to have no greater consequence than infection by an "empty" virus.

To activate components of the unfolded protein response we will use cloned cDNA that will be over-expressed. As the genes in question are neither oncogenes nor tumour suppressor genes and thus accidental infection of a human host
by these viruses is likely to have no greater consequence than infection by an “empty” virus. To promote misfolded protein stress we will express known misfolding-prone mutants versions of secreted proteins, for example, Ins2 with a mutation that prevents proper disulfide bond formation. As the secreted proteins are not oncogenic and in fact harbour mutations that promote endoplasmic reticulum retention the are either inert or enfeeble the targeted cell, the risk of oncogenic transformation by a virus harbouring a misfolding prone protein are diminished compared to the empty virus. A theoretical exception to this principle is a retrovirus encoding a prion-forming protein, which could in an extremely fanciful scenario set up a prion infection in the host. Therefore, proteins with a potential to undergo prionization (e.g. mutant PrPsc) will not be used.

Following is a table of the specific genes:

- **List of mutant genes encoding proteins that misfold in the endoplasmic reticulum and that will be expressed from lentiviral vectors:**
  - Mouse Ins2C96Y: Encodes insulin, a hormone normally present in serum
  - Human PLAPC121S: Encodes placental alkaline phosphatase, an enzyme normally present in tissue
  - Human SERPINA1PiZ: Encodes a misfold serum protease inhibitor

- **List of genes encoding proteins that function in the processing of protein folding in endoplasmic reticulum or in the response to the stress of protein misfolding in the endoplasmic reticulum.** The expression of these genes will be inhibited by shRNA-containing lentiviral vectors or the genes will be over-expressed by lentiviral vectors:
  - Mouse or human Hspa5, Hsp90b1, ERDJ (1-6): Encode chaperones that folds proteins in the ER
  - Mouse or human PDI1, Erp44, Erp57, Erp72: Encode disulfide isomerases/transfereases than function in oxidative protein folding in the secretory pathway
  - Mouse or Human Ero1l, Ero1lb, Qsox1, Qsox2: Encode protein disulfide oxidases involved in protein folding
  - Mouse or human EIF2AK3, ERN1, ATF6: Encode detectors of unfolded protein stress in the endoplasmic reticulum

**Evaluation of foreseeable effects**

RNAi inactivation of most genes involved in protein handling in the endoplasmic reticulum or in the response to misfolded proteins, will enfeeble cells and render them more sensitive to misfolded protein load. Even those genes that encode proteins that are known to reduce the fitness of cells experiencing high levels of misfolded protein stress are not known oncogenes (example CHOP/Ddit3) Thus the risk associated with viruses encoding such genetic elements is predicted to be less than that or empty lentviruses. Gain-of-function of unfolded protein response components, which may have a benefit in the special circumstances of severe protein misfolding is unlikely to translate to a survival benefit for accidentally-transduced cells, let alone serve as an oncogenic event. Thus the risk posed by gain-of-function vectors is likewise less than that of empty viruses. Lastly, as noted above, the expression of a misfolding-prone protein is severely enfeebling to cells and part from the theoretical establishment of a new strain of prions the risk posed by such vectors is less than that of the empty ones.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none applied for
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture/lab ware will be autoclaved and incinerated. Culture fluids will be treated with 1% Virkon for 0.5 hours, autoclaved and disposed to drains. Recyclable lab ware will be soaked in 1% Virkon for 16 hours. Bench/cabinet surfaces will be wiped down with 10% trigene and 70% ethanol. Spills will be sprinkled with Virkon powder/trigene and vernagel to solidify. Alternatively disinfected liquid waste will be gelled with vernagel in sealed containers labelled with autoclave tape and taken to the autoclave room for transfer to the incinerator. All solid waste will be collected in double autoclave bags, sealed with autoclave tape, labelled with the users name and the room number and will be autoclaved and incinerated.

13. Is an emergency plan required according to regulation 20?

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
Project Additional Information

Purposes of the contained use

Retroviral and lentiviral delivery of a selection of human genes to cytotoxic T lymphocytes (CTLs) to assess their role in regulating secretion of stored proteins required for the immune effector functions.

Recipient or parental organism

FOR CONSTRUCTION OF RETROVIRAL VECTORS
K12 or B derivatives of E. coli will be used as bacterial cloning hosts to generate plasmid expression clones in readiness for expression within specified packaging cell lines. These are disabled hosts that cannot colonise the human gut and have a history of safe use. These hosts may be considered equivalent to ADCP hazard group 1.

FOR PACKAGING RECOMBINANT RETROVIRAL PARTICLES
The packaging cell lines are of human or murine origin, which are well characterised and authenticated and are obtained from commercial sources. Examples of packaging cell lines likely to be used are HEK293T cells (amphotropic) to generate lentivirus or PT67 to produce retrovirus. They can be regarded as low hazard for GM activities and as hosts are suitable for containment level 1 precaution.

FOR RECOMBINANT RETROVIRAL INFECTION:
Human primary lymphoid cells (eg CD8+ blasts or CTL) can be considered as especially disabled hosts and as such may be considered to be equivalent to ADCP hazard group 1 however the cells have the potential to contain adventitious agents and as such must be handled at containment level 2 under COSHH.

Host/vector system

Lentiviral expression plasmid pHRSINcPPT-SGVC containing 5' and 3' LTRs, packaging signal and Rev response element from HIV-1
p8.91 packaging plasmid containing Gag, pol, rev, tat from HIV-1
pMD-G envelope plasmid containing VSV-G envelope protein.

These three vectors are co-transfected into a packaging cell line to generate replication defective lentivirus. This lentivirus expression vector is self-activating as it carries a deletion in the U3 region of the 3'-LTR. Upon integration into the host genome, the 3'-LTR is copied to the 5'LTR, rendering it transcriptionally inactive and therefore unable to generate a functional retrovirus. The HIV-1 Lentivirus based expression vector lacks any of the packaging genes (gag, pro, pol, vif, vpr, tat, rev, nef, eng), and requires the use of a packaging cell line based on HEK293 (ie HEK293T) and 2 other plasmids which provide gag, pol, rev, tat and VSVG. The HIV-1 elements in the lentivirus expression vectors (ie 5' and 3' LTRs and the psi packaging signal) allow packaging of the transgene itself. Therefore the number of genes from HIV-1 has been reduced to 4, ie gag, pol, rev and tat. Genes encoding the structural and other components required for packaging the viral genome are separated onto three plasmids. All three plasmids have been engineered not to contain any regions of homology and each other to prevent undesirable
recombination events that could lead to the generation of a replication-competent virus.

The packaging genes are provided by co-transfection of packaging vectors p8.91 (provides gag/pol, rev and tat) and pMD-G (provides VSV-G envelope) into the HEK293T cell line along with the expression vector. By using this enhanced 3-plasmid system, virus particles are produced that can infect human and mouse cells, but are replication deficient and therefore cannot be transmitted from one cell to another. Although the two packaging plasmids allow expression in trans of proteins required to produce viral progeny (eg gal, pol, rev, env) in the 293T producer cell line, none of them contain LTRs or the psi packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced. The rev response element allows for rev-dependant nuclear export of unspliced viral mRNA. The HIV-1 env gene is replaced with a Vesicular Stomatitis Virus envelope.

Overall then, only Lentiviral particles, which are unable to replicate, but which can deliver the gene of interest, are produced. Therefore the main risk is in their ability to deliver genetic material into a host genome and as described above, this is unlikely to pose any significant risk.

Retroviral expression plasmid pMSCV containing 5’ and 3’ LTR and packaging signals. The LTR is from murine stem cell viral and prevents transcriptional suppression in embryonic stem and embryonal carcinoma cells. Therefore driving high levels of constitutive expression. Retroviral expression plasmid pLEGFP-C1 containing 5’ and 3’ LTR and packaging signals from Moloney murine leukemia virus.

Either of the above 2 plasmids will be transfected into PT67 packaging cells to generate replication defective retrovirus. PT67 packaging cell line is derived from NIH3T3 cells, which have subsequently been stably transfected with 2 plasmids that contain the gag/pol and env gene. This cell line produces viruses, which are dualtropic and have a broad mammalian host range. Split genome design lowers the possibility of generating replication-competent virus. Only the expression plasmids contain the LTRs and the packaging signals and therefore the generated virus contains one of the structural genes rendering it replication defective.

As with the lentivirus the main risk with retroviruses is their ability to deliver genetic material into the host genome. This is unlikely to pose any significant risk.

Origin & function

Mammalian proteins implicated in a specialised lymphoid secretory pathway such as:
- Rab27a (studies have shown that transgenic mice expressing Rab27aGFP are healthy)
- Rab geranylgeranyltransferase: a rab modifying enzyme that adds a geranyl geranyl group to Rab27a and 4 other Rab proteins.
- Rab7 and RILP: Both involved in moving lysosomes along microtubules.
- Tubulin-GFP: forms microtubules along which lysosome move
- Actin-GFP: forms the sub-cortical cytoskeleton.
- LYST: a protein required for the final step of secretion of secretory lysosome.
- AP-3: a clathrin binding adaptor protein involved in sorting proteins to the lysosome.
- Perforin: a pore forming protein use by lymphocytes to form pores in target cells.

Other good candidate genes such as the microtubule motors kinesins or clip proteins that attach lysosomes to microtubules.

All of the above and other candidate proteins will be involved in the secretion of lysosomes in immune cells. None of the available literature indicates any oncogenic or harmful effect of these proteins.

Standard reporter genes such as GFP (Green fluorescent protein) from jelly fish, luciferase from firefly, and B-galactosidase from E. coli.

None of the above proteins are known to have harmful properties.

Standard antibiotic resistance genes such as Neomycin, hygromycin, puromycin and ampicillin.
Evaluation of foreseeable effects

No significant hazards have been identified from insertion of the foreign sequences into E. coli K12 or B derivatives. The vectors are based on the pUC backbone and are non-mobilisable. They are not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms. Gene transfer is possible, but unlikely to be hazardous. The resulting GMOs are not expected to carry any additional risks compared to that of the un-modified recipients and as such can be handled at containment level 1.

Transfection of the HEK 293T cell line with the 3 plasmids is expected to result in the generation of a Lentivirus that is capable of infecting most mammalian cells and integrating into the host DNA but is incapable of replication. As stated above none of the inserted DNA is expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. Therefore all work, which involves the handling of the virus, or cell lines and tissues infected with the virus, will be done at containment level 2, with the use of a microbiological safety cabinet for procedures which generate aerosols.

Transfection of the pT67 cell line with the retroviral expression plasmids is expected to result in the generation of a retrovirus that is capable of infecting most mammalian cells and integrating into the host DNA but is incapable of replication. As stated above none of the inserted DNA is expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. Therefore all work, which involves the handling of the virus, or cell lines and tissues infected with the virus, will be done at containment level 2, with the use of a microbiological safety cabinet for procedures which generate aerosols.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees C for at least 15 minutes or 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Appropriate containment and control measures have been assigned in accordance with the latest guidance issued by ACGM.

Project Containment
Project Ref 678/00.1

Date Ackn'd 27/03/2000

Tick if notifying a connected programme of work N

Project notified under transitional arrangements Y

CU2 Project Title ADENOVIRAL EXPRESSION OF MUTANT & WILD TYPE PPP IR3 & LAMIN A IN CELL CULTURE

Class 2

Class Culture Vol Class 2

withdraw

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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### Project Ref 678/01.1

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**OUTPUT OF THE LEISHMANIA GENOME PROJECT AND EXTENDING HOST/PATHOGEN INTERACTION STUDIES**

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**Historical Significant Changes**

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**Project Additional Information**

**Purposes of the contained use**

To identify new candidate genes for development of drugs and vaccines against Leishmania. Specifically, Leishmania genes will be cloned into E. coli expression vectors to isolate protein, into eukaryotic expression vectors as DNA vaccines, and into Salmonella expression vectors to boost immune response following straight DNA vaccination. Gene function will be assessed by knock-out or over expression in vivo. Pathogens will be labelled using reporter genes to facilitate study of endosome/lysosome fusion events in infection studies.

**Recipient or parental organism**

Tagged GMOs will be readily visualised eg fluorescent E. coli transformed with Leishmania genes will be handled at containment level 1 except where the pBR322 based expression vectors are used which require containment level 2 conditions. Leishmania genes will be introduced into attenuated strains of Salmonella. The effect of this is unpredictable therefore resulting transfectants will be manipulated at containment level 2. The effect of knocking-out or over expression of Leishmania genes is unpredictable but will not increase containment level beyond 3, the level we use for all Leishmania work.

**Host/vector system**

The following host // vector systems will be used:

- E.coli-XL1-Blue DH5 alpha // pUC derivatives eg KSneopt'pALT-neo, pcDNA3;LaRP+cat; pBR322 derivatives eg pET, pKK233-2 pTECH; cLHYG cosmid containing Leishmania splice-acceptor site.
- Salmonella typhimurium wildtype, (triangle) aro A, (triangle) aro A/D // pBR322 derivatives eg pKK233-2 pTECH-2, pGRPnir B; pUC derivatives eg pcDNA3, pGFPmut3.1
- Leishmania major/mexicana/donovan/brazilienisis (lab strains) // pUC and pBR322 derivatives eg cLHYG, KSneopt', pALT-neo, LaRP + cat; pSP72-aNEOaGFP, pCRTM 2.1-aNEOaGFP
- Mycobacterium avium/ bovis (BCG) // p16R1; pKINTA

**Origin & function**

**Reporter genes**: to tag the pathogens for detection in in vitro infections studies.

Leishmania derived cDNAs (known + unknown): to express as proteins in E. coli; for mouse vaccination trials both a direct DNA vaccine and via transfected Salmonella.
To study gene function in Leishmania by knocking-out or over-expressing selected Leishmania genes.

**Evaluation of foreseeable effects**

The pathogenicity of Leishmania species and the hazard this imposes to experimental workers is already covered by Codes of Practice for cultivation in culture (June 1992) and in animals (January 1993). Genetically-modified Leishmania will be subject to the same safety protocols; the environmental risk is therefore no greater than with unmodified parasites.

The lifecycle of Leishmania involves transmission of extracellular parasite stages between hosts by a sandfly vector, followed by intracellular maintenance within macrophages in the mammalian host. No other organism is known to be at risk from infection. There is no sandfly colony in Cambridge, so transmission of the parasite, by inoculation of infective parasites into susceptible mammalian hosts, is impossible. The likelihood of hazard is therefore effectively zero.

The consequence of hazard would be the transmission of leishmaniasis to susceptible mammals, including man. Field studies in endemic areas show that <10% of people infected with the parasite get clinical disease. In susceptible individuals, the disease is treatable (as detailed in the Code of Practice, June 1992). Therefore, the consequence of hazard is low. Overall, the risk is estimated to be effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation applied for.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid waste is treated using 5% chloros for 24 hours prior to disposal. Animal waste is double-bagged within the containment facility and transported direct to the incinerator within the animal unit.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N
tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project (copy attached) has been fully reviewed by the CIMR GMSC and we are satisfied that the risk assessment is accurate and that the work will be carried out under appropriate conditions.

**Project Containment**

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<th>Growth Rooms</th>
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02/03/2022 Page 10536 of 15326
Project Ref 678/01.2

Purposes of the contained use

Dendritic cells are the professional antigen presenting cells of the immune system. We wish to examine the function of genes involved in antigen presentation, and genes that inhibit antigen presentation in dendritic cells. Primary human dendritic cells are non-dividing and therefore not readily transfectable using standard transfection technology. This necessitates the use of replication defective lentiviral vectors based on human immunodeficiency virus type 1 (HIV-1).

Recipient or parental organism

E. Coli transformed with pUC based vectors will be handled at containment level 1. We will use a three plasmid transient transfection system to generate recombinant, replication defective retroviruses. The lentiviral work will be performed at containment level 2. 293T cells will be transfected with: (i) pHRSin - an HIV-1 ‘self inactivating vector’ (SIN). cDNAs encoding the antigen presentation genes will be cloned into the HIV-1 derived SIN vector, which has a deletion in the U3 region of the 3’ long terminal repeat (LTR) of the DNA used to produce the vector RNA. during reverse transcription this deletion is transferred to the 5’ LTR and abolishes the transcriptional activity of the LTR, and as a result the production of full-length vector RNA is transduced cell is abolished. (ii) pCMVR8.0 - a vector containing the HIV-1 derived retroviral sequence encoding viral gag/pol tat and rev genes, from which the virulence genes vif, vpr, vpu and nef have been deleted. (iii) A plasmid encoding the envelope plasmid derived from vesicular stomatitis virus (VSV) G protein which is permissive for human cells.
Host/vector system

The following host // vector system will be used:
E. coli; K12//pUC derivatives carrying the ampicillin resistance genes driven by a bacterial promoter and containing (i) human immunodeficiency virus type 1 (HIV-1) -derived retroviral sequences encoding viral gag/pol and tat and rev sequences. (ii) Viral sequences encoding vesicular stomatitis virus (VSV) G protein. (iii) self-inacting retroviral vectors derived from HIV-1 with retroviral coding sequences eliminated, carrying eukaryotic promoters and marker genes (GFP) and antigen presentation genes. None of these plasmids contain bacterial promoters driving expression of the gene to be expressed.

Origin & function

To study the function of antigen presentation genes in dendritic cells. All genes are of known function and do not act as oncogenes. Human cDNAs encoding the transporter associated with antigen processing (TAP1 and TAP2, tapasin and class 1 genes. (ii) cDNAs encoding viral proteins from human cytomegalovirus US6, UL18 and the K3 gene from human herpes virus 8. (iii) Mutant forms of the above genes which will include point mutants and deletion mutants of putative functional domains. (iv) The reporter genes His, FLAS, GFP, and lacZ.

Evaluation of foreseeable effects

The risk of infection with retroviruses is through direct inoculation and this is unlikely to occur as there is no animal experimentation involved and needles are not being used. There is evidence that these replication defective retroviruses are not pathogenic when tested in mice. Further details of the constructs and the demonstration that they do not give rise to replication competent viruses are available in Zufferey et al Nature Biotechnology 15:871-5. Since the packaging unit of the lentivirus vector expresses only 4 HIV-1 genes (gag, pol, tat and rev) and the crucial virulence factors, Vpr, Vif, Vpu and Nef have all been deleted, this eliminates the possibility of a wild-type virus being reconstituted through recombination. In addition the use of a 'self-inactivation vector' further improves the safety as the transcriptional elements of HIV have been removed from the vector. Since the viral promoter has been abolished, synthesis of full-length vector RNA in target cells is prevented, minimizing the risk that a replication competent virus can occur, or that a cellular gene located downstream of the 3’ LTR will be aberrantly expressed. It would require two separate recombination events to generate replication competent virus - involving (i) recombination between the env gene and the packaging construct and (ii) a recombination event between the packaging construct (which lacks its own packaging signals) and the coding vector. The vector encoding the envelope gene has no homologous region with the packaging construct, thus the likelihood of such recombination occurring is extremely low and has never been recorded. Again, there is the theoretical risk of a recombination event with an endogenous human retrovirus, but no human endogenous lentivirus has been detected. However to ensure that no recombination event has occurred, supernatant from the transduced cell line will be checked rigorously for the absence of replication competent helper virus using assays for reverse transcriptase. In addition the retroviral expression vectors encode green fluorescent protein (GFP) in an IRES vector. Supernatant taken from the transduced cells will be incubated with NIH3T3 cells and these cells examined for expression of the GFP protein and tested by rtPCR for the presence of the inserted gene.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is treated using 5% chloros for 24 hours prior to disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The project has been reviewed by the CIMR GMSC and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

**Project Containment**

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**Animal Units**

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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L3</td>
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**Project Ref** 678/02.1

**CU2 Project Title**

TRANSGENE DELIVERY TO ES, YOLK SAC AND HAEMOPOIETIC CELLS AND MOUSE EMBRYOS USING RETROVIRAL VECTORS

**Date Ackn'd** 08/05/2002

**Date Project Ceased** 16/09/2019

**Class** Class 2

**Culture Volume** 1-50 litres

**Non-GMM**

**Consent Granted** not applicable

**Project notified under transitional arrangements**

**Historical Significant Changes**

Project transferred to GM3071

**Purposes of the contained use**

The purpose of this project is to study the biology of haematopoietic stem cells using the experimental approach of transgene delivery by means of retroviral vector.
systems. The biological questions we aim to address include the molecular controls regulating stem cell formation and stem cell plasticity as well as the molecular defects in stem cells that lead to leukaemia. The proposed work has therefore the potential to open up new areas for both fundamental and applied biomedical research with potential clinical relevance for the treatment of leukaemia.

Recipient or parental organism

E. coli transformed with pUCbased retroviral vectors will be handled at containment level 1. The retroviral work will be performed at containment level 2. The proposed retroviral systems incorporate the following safety modifications to prevent viral replication:
(1) The packaging genes are separated into two plasmids, which lack both LTRs and have no viral packaging signal.
(2) The following viral genes have been deleted from the packaging vector: env, vpr, vpu, vif and nef.
(3) The vectors expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion) and expresses no viral gene products.
(4) Envelope is expressed on a separate vector.

Host/vector system

The following host/vector system will be used:
E. coli; K12/pUC derivatives carrying the ampicillin gene driven by a bacterial promoter together with viral genes and cDNAs driven by eukaryotic promoters that do not express in bacteria.

Origin & function

The purpose of the project is studying both normal and malignant haemopoietic stem cell biology. All the genes we want to deliver to cells are of known function. We will use disabled retro and lenti vectors and virus pseudotypes that only infect mouse cells whenever the inserts are potential oncogenes.

Insert DNA categories:
(1) Reporter genes (lacZ, GFP + variants, luciferase, PLAP) together with regulatory regions enhancers, promoters, silencers
(2) Cre recombinase gene, self-exising with regulatory regions
(3) SV40 large T antigen, HoxB4, Hox11, Notch1: potential oncogenic function (only to be used with ecotropic envelopes)
(4) SCL, Myod, myf5, pax3, pax7, pdx1, ngn3: transcription factors (only to be used with ecotropic envelopes)

Evaluation of foreseeable effects

We propose to generate transgenic mice using the lentiviruses. These mice will be housed in CBS (Addenbrooke's site Cambridge) together with all our other transgenic mice. Transgenic mice will only by produced for the inserts of category 1 (ie. inert reporter genes). Due to the design of the proposed experiments (see above) and the containment procedures of CBS, the environmental risk after introduction of the transgene is negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

We propose to generate transgenic mice using the lentiviruses. These mice will be housed in CBS (Addenbrooke's site Cambridge) together with all our other transgenic mice. Transgenic mice will only by produced for the inserts of category 1 (ie. inert reporter genes). Due to the design of the proposed experiments (see above) and the containment procedures of CBS, the environmental risk after introduction of the transgene is negligible.
None applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is treated using 5% chloros for 24 hours prior to disposal.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The project has been reviewed by the CIMR Biological & Genetic Modification Safety Committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

**Project Containment**

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<td>L4</td>
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Animal Units

| L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

**Project Ref** 678/02.2

<table>
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<th>CU2 Project Title</th>
<th>Class</th>
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<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<td>FUNCTIONAL PROTEIN EXPRESSION IN MAMMALIAN CELLS USING A LENTIVIRAL GENE DELIVERY SYSTEM</td>
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<td>&lt; 1 litre</td>
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Withdrawn

Tick if notifying a connected programme of work

02/03/2022
The purpose of the project is to study the biology of the polycystin protein family in transfected cultured mammalian cells. One of the major blocks to the success of studies in cultured cells has been the difficulty in generating stable cell lines expressing the PKD1 gene product, polycystin-1. The PKD1 gene produces a ~14 kb mRNA with polycystin-1 having a predicted mass of >460 kDa. Standard cell transfection approaches have been unsuccessful in expressing such a large gene therefore the use of a lentiviral gene delivery system has the potential to allow new experimental approaches to study of polycystin to be developed.

Recipient or parental organism
E. coli transformed with the pUC based lentiviral vectors will be handled at containment level 1. The lentiviral work will be performed at containment level 2. The lentiviral system incorporates the following safety modifications to prevent viral replication:
1). PLENT16 contains a deletion of the 3’ LTR that does not affect generation of the viral genome in a producer cell line, but results in inactivation of the lentivirus after transduction into the target cell;
2). The number of genes from HIV-1 has been reduced to three (gag, pol and rev)
3). The VSV-G gene is used in place of HIV-1 envelop
4). Genes encoding the structural and other components required for packaging the viral genome are separated into four plasmids with no homologous regions to prevent undesirable recombination.
5). The lentiviral particles are replication incompetent and only carry the gene of interest.

Host/vector system
The following host/vector system will be used:
E. coli; pUC derivatives carrying the ampicillin gene driven by a bacterial promoter together with viral genes and cDNAs driven by eukaryotic promoters that do not express in bacteria.

Origin & function
The purpose of this project is to study the function of the polycystins in transfected mammalian cells in culture. The polycystins are involved in the regulation of normal kidney epithelial cell function that when disordered through gene mutation leads to an inherited disease in man called autosomal dominant polycystic kidney disease. This disease has no malignant potential. However the precise function of the genes encoding the polycystins, in particular, the PKD1 gene is not known. The disabled lentiviral gene expression system will be used to infect mammalian cells in culture with the PKD1 gene, functional mutants and reporter genes.

Insert DNA categories:
1) Reporter genes (GFP and variants; FLAG, His and V5)
2) PKD1 gene cDNAs and functional mutants.

**Evaluation of foreseeable effects**

The risk of infection with retroviruses is through direct inoculation, which is extremely unlikely as no needles are used in any of the experimental procedures involved in this project. In addition the lentiviruses used are replication defective as previously described and therefore not pathogenic.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None applied for.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste will be treated for 24 hrs with 2% Virkon or Presept tablets before disposal into the laboratory drainage system.
Alternatively disinfected waste will be gelled with Vernagel in sealed containers and removed for incineration.
Solid waste such as plastics will be double bagged before transfer to a waste autoclave within the building, and then disposed of by incineration on site.

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Animal Units
Large Scale Activities
Human Clinical Applications

02/03/2022

Page 10543 of 15326
The nuclear hormone receptor PPARg (peroxisome proliferator-activated receptor gamma) is a ligand inducible transcription factor involved in a number of biological processes, including fat cell differentiation, glucose homeostasis and atherogenesis. A number of PPAR-specific agonists, including naturally occurring fatty acids and prostaglandin J2 and synthetic anti diabetic thiazolidinediones have been identified and shown to be useful tools for investigating the effects of PPARg stimulation in vivo. For example, thiazolidinediones accelerate differentiation of cultured preadipocytes into adipocytes, activate monocytes and increase leptin secretion from cultured trophoblasts. The effects of PPARg inactivation are poorly understood however. Animal studies using PPARg knockout mouse has been hindered by lethal phenotype and no antagonist ligand have been described. Patients harbouring loss-of-function PPARg mutations exhibit varying degrees of lipodystrophy and insulin resistance. In order to study the function of such mutant PPARg, they will be expressed in mammalian cells using a lentiviral expression system. A major advantage of the lentiviral system is the possibility to infect most cells from different species.

**Recipient or parental organism**

A 293 producer cell line will be used to generate the virus particles. Initially, we will analyse the effect of the mutants on the differentiation of murine 3T3-L1 preadipocytes in response to PPARg ligands. Subsequent experiments will address the effects on human preadipocyte and other relevant cells.

**Host/vector system**

A 293 producer cell line will be transfected with the gene of interest constructs and three plasmids each containing different and necessary proteins for virus formation will result in the generation of the viral particles. These three different plasmids used are CMV 8.9 (gag, pol and tat); pRSVrev (rev); pCMV-VSV-G (VSV-G). Only the pHR'CMV-GFP vector containing the gene of interest contains packaging signals and this will result in lentiviral particles which are replication incompetent and only carry the gene of interest. All cloning and lentiviral vectors lack sequences for prokaryotic expression.
Expression of PPARg and GFP are driven by the CMV promoter which is active in most mammalian cell types. The lentiviral particles are replication incompetent and only carry the gene of interest and GFP.

Origin & function

The human nuclear hormone receptor PPARg is a ligand inducible transcription factor involved in a number of biological processes, including fat cell differentiation, glucose homeostasis and atherogenesis which can be analysed in the appropriate cell lines.

Evaluation of foreseeable effects

Neither GFP nor wild type or mutant PPARg are recognised oncogenes, so work with these lentiviral constructs is not expected to pose a cancer risk.

Expression of PPARg and mutants will lead to differences in gene expression in mammalian cells. This will be evaluated by analysing differences in preadipocyte differentiation and gene expression patterns.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated for 24 hrs with 2% Virkon or Presept tablets before disposal into the laboratory drainage system. Alternatively disinfected waste will be gelled with Vernagel in sealed containers and removed for incineration. Solid waste such as plastics will be double bagged before transfer to a waste autoclave within the building, and then disposed of by incineration on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project has been reviewed by the CIMR Biological and Genetic Modification Safety Committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

Project Containment

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02/03/2022

Page 10545 of 15326
Project Ref 678/03.2

Date Ackn’d 06/03/2003

CU2 Project Title ADENOVIRAL EXPRESSION OF NOVEL TIMP MUTANTS AND CHIMERAS IN MAMMALIAN CELLS

Date Project Ceased 23/05/2007

Class CultureVol Class 2 CultureVolume Class 3-4

Class 2 1-50 litres

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes TRANSFERRED TO GM 973 - 23/5/07.

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Characterisation of novel Tissue inhibitor of metalloproteinase (TIMP) 1, 2, 3 and 4 mutants and chimeras and their comparison with wild-type TIMPs in a variety of mammalian cell models to assess their affects on cellular processes dependent on metalloproteinase activity.

Recipient or parental organism

The TIMP molecules are to be expressed from Ad5 adenovirally infected cells expressed from a CMV promotor. The expression of these proteins from Ad5 is not expected to change the properties of the Ad5 adenovirus in terms of increasing its pathogenicity. The recombinant TIMP-Ad5 are not expected to be more virulent in terms of infectivity or replication and host genome integration than wild type Ad5 adenovirus which is designated a Class ll pathogen by ACDP.

Host/vector system

Plasmid DNA will be produced in E. coli K12 strains which are class 1 pathogens and are assessed to be containment level ll microorganisms.

TIMP-Ad5 adenovirus are to be made by Flp dependent recombination by co-transfection of plasmid vectors pDC515 or pDC516 with pBHGFrt(triangle)E1E3, 3flp in HEK293T cells. Vectors pDC515/6 are non mobilisable and contain the TIMP gene and CMV promotor. pBHGFrt(triangle)E1E3,3flp encodes the Ad5 genome and can only produce adenovirus in mammalian cells which express adenoviral proteins E1 and E3, such as HEK293T after transfection. Further information can be obtained from www.Microbix.com who are the manufacturers of the vector system.

TIMP-Ad5 will be used to infect a variety of mammalian cell lines and primary cells. These are not expected to contain the adenoviral genes E1 and E3 and should not
produce viral particles after infection.

TIMPs 1, 2, 3 and 4 are from human cDNA and are inhibitors of metalloproteinases. Chimeras are made from the cDNAs encoding parts of human Furin, murine TACE and GFP, or the polypeptides KDEL, [Leu]17, V5 epitope and [His]6. These are to target TIMPs to particular cellular organelles (Fur, TACE, KDEL, [Leu]17, or to aid detection (GFP, V5, [His]6). Plasmid vectors pDC515/6 and pBHGfrt(triangle)E1E3,3flp are derived from vector pUC18 and recombinant adenovirus Ad5.

Evaluation of foreseeable effects

Replication deficient Ad5 are unlikely to cause disease for purpose of group II classification. However the nature of the inserted gene should be considered in the context of the recombinant virus. Work with adenovirus carries risk of allergy after repeated exposure. This effect is idiosyncratic and may manifest as conjunctivitis and rhino-tracheo-bronchitis. Ad5-CMV based virus are not known to cause these symptoms. However although the virions lack E3 protein and are compromised in their ability to establish and maintain an infection in human cells, should infection occur, the effect of elevated TIMP expression is difficult to predict due to the varied functions of these proteases they inhibit. However, decreases in ECM turnover through MMP inhibition, or inhibition of shedding of growth factors, receptors and cytokines, on balance, is likely to decrease cell survival. All TIMPs have been shown to decrease tumour survival in murine models when expressed by adenoviral infection (variety of transformed cells including epithelial derived cells). The Ad5 adenovirus is non-oncogenic and TIMPs, GFP, ADAM17 and Furin are not recognised as oncogenes. The oncogenic potential of short peptide sequences consisting of the V5 epitope, KDEL and the 17 amino acid Leucine rich peptide is not known.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated for 24hrs with 2% Virkon or Presept tablets before disposal into the laboratory drainage system. Alternatively disinfected waste will be gelled with Vernagel in sealed containers and removed for incineration. Solid waste such as plastics will be double bagged before transfer to a waste autoclave within the building, and then disposed of by incineration on site.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The project has been reviewed by the CIMR Biological and Genetic Modification Safety Committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

Project Containment

02/03/2022  Page 10547 of 15326
Project Ref: 678/04.1

Date Ackn’ed: 24/02/2004

CU2 Project Title: CO-CULTURE OF CONDITIONALLY IMMORTALISED HUMAN MAMMARY FIBROBLASTS AND ENDOTHELIAL CELLS WITH HUMAN CANCER CELL LINES

Date Project Ceased: 23/05/2007

Class: Class 2
CultureVolClass2: 1-50 litres

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: N

Historical Significant Changes: TRANSFERRED TO GM 973 - 23/5/07.

Project Additional Information

**Purposes of the contained use**

The use of co-culture of tumour epithelial cells with tumour stroma (fibroblasts and endothelial cells) to mimic aspects of tumour cell-cell interactions in vitro.

**Recipient or parental organism**

Human Mammary Fibroblasts (HMF clones A to D) and Human Mammary Microvascular Endothelial Cells (HMME clones 2 and 7) were obtained from Ludwig Institute for Cancer Research, London. The characteristics of the cells are described in the publication by O’Hare et al, PNAS, 2001, 98 p646-51. In brief, primary cells derived from breast tissue were immortalised by separate transduction with helper-free amphotropic murine-lukaemia-virus (MLV) encoding the catalytic subunit of human telomerase (hTERT) and a heat labile mutant of the simian virus 40 large tumour antigen (U19tsA58). The resulting cells are immortal, yet retain most genetic and phenotypic characteristics of the primary parental cells. The cells only retain immortality when cultured at the permissive temperature of 34 degrees C, and senesce after 15 passages at 37 degrees C. These cells have the hTERT and U19tsA58 genes stably integrated into the host cell genome by MLV retroviral transduction. They have been tested negative for the presence of LMV and are unlikely to produce LMV containing hTERT and U19tsA58 in the future. However as hTERT and U19tsA58 genes are potentially...
oncogenic, it is advised that the cells are cultured under containment level II conditions and the cell lines are classified as Class II organisms.

### Host/vector system

Cells are derived from primary fibroblasts and microvascular endothelial cells obtained from breast tissue explants.

The vector system used originally used to transform these cells is described in the publication by O'Hare et al, PNAS, 2001, 98p646-51. In brief the vectors used were helper-free amphotropic murine-lukaemia-retrovirus (MLV) containing resistance genes for antibiotics hygromycin and G418 to aid selection of clones which had integrated MLV stably into their genome.

NB. Only the cells derived from this vector system are to be used by the applicants. NOT the vector system itself.

### Origin & function

The human telomerase catalytic domain (hTERT) is derived by removal of the regulatory domains of human telomerase. Human telomerase maintains telomere length which is a means of regulating cell life-span. HTERT prevents telomere shortening and can lead to immortalisation of cells which express it.

The simian virus 40 large tumour antigen is a known oncogene and induces DNA synthesis by by-passing key cell cycle check points through interacting with regulatory proteins (c-myc, Ras). It also blocks p53 dependent apoptosis. U19tsA58 is a temperature sensitive mutant of SV40LTA which is inactive at 37 degrees C yet is active at the permissive temperature of 34 degrees C.

Expression of these constructs individually does not always lead to cell immortalisation, although expression of both will usually lead to complete transformation and immortalisation.

### Evaluation of foreseeable effects

Due to the nature of the hTERT and U19tsA58 genes stably inserted into the genome by MLV transduction there exists a very small but finite risk of a recombination event which could lead to production of amphotropic replication competent MLV containing either the hTERT or U19tsA58 genes should the cells come into contact with MLV or a derivative thereof. To further reduce the risk of recombination, the following will be adhered to.

1. Culture in Containment Category II facility following CIMR Containment II Code of Practice.
2. The cells are not to be cultured in the presence of murine cells.
3. All human cell lines which will be co-cultured with the immortalised fibroblasts or endothelial cells will be tested for the presence of MLV by PCR.
4. The fibroblast and endothelial cells will be tested monthly for the presence of MLV by PCR.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated for 24 hrs with 2% Virkon or Presept tablets before disposal into the laboratory drainage system. Alternatively disinfected waste will be gelled with Vernagel in sealed containers and removed for incineration. Solid waste such as plastics will be double bagged before transfer to a waste autoclave within the building, and then disposed of by incineration on site.
The project has been reviewed by the CIMR Biological and Genetic Modification Safety Committee. We are satisfied that the risks have been properly assessed and that the work will be carried out under the appropriate conditions and controls.

Please enter comments on the GM safety committee on the risk assessment:
The project has been reviewed by the CIMR Biological and Genetic Modification Safety Committee. We are satisfied that the risks have been properly assessed and that the work will be carried out under the appropriate conditions and controls.

Project Containment

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Project Ref 678/05.1

Date Ackn'd: 05/05/2005
Date Project Ceased: 26/08/2016
Withdrawn: N

CU2 Project Title: A study of inhibitory receptors and viral gene products using lentiviral expression vectors.

Class: Class 2
CultureVolClass2: 1-50 Litres
Non-GMM Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Transfered to GM353 on 26/08/2016
# Project Additional Information

## Purposes of the contained use

1. Understanding the biochemical and molecular basis for the function of receptors for the constant (Fc) region of IgG, FcγRs and
2. Dissecting the role of viral gene products in lytic replication, the establishment of latency and immune evasion.

## Recipient or parental organism

The cell lines we wish to transfect are murine B-cells lines and primary human dendritic cells and macrophages. None of these cell types are readily transfectable using standard transfection technology, necessitating the use of lentiviral vectors based on human immunodeficiency virus type 1 (HIV-1).

## Host/vector system

A three plasmid transient transfection system will be used to generate recombinant, replication-defective retroviruses. 293T cells, a human cell line carrying SV40 T antigen will be transfected with:
- (a) An HIV-1 'self-inactivating vector' (SIN).
- (b) An HIV-1 derived retroviral sequence © a plasmid encoding the envelope plasmid derived from vesicular stomatitis virus (VSV) G protein.

## Origin & function

1. Human and mouse cDNAs encoding Fcγ receptors and their gamma chain subunits.
2. Human and mouse cDNAs encoding the soluble tyrosine kinase lyn.
3. MHV68 cDNA encoding M2 gene products.
4. Mutant forms of the above genes which will include point mutants and deletion mutants of putative functional domains.
5. The reporters His, FLAG, GFP, and lacZ.

Cells transduced with these genes will be used for the study of the effect of wild type and mutant Fcγ receptors on the response of cells to immune complexes and opsonised bacteria. We hope to examine their role on phagocytosis, endocytosis and intracellular trafficking of immune complexes, cytokine production and antigen presentation using techniques established within this laboratory.

## Evaluation of foreseeable effects

The genes to be expressed are not known to be harmful and so the classification of the work is determined largely by the nature of the host/vector systems. The lentiviral (HIV) vectors are disabled, self-inactivating (they are unable to propagate following transfection) and multi-component. Hence the risk of generating replication competent viruses (bearing the transgenes) is extremely low.

The lentiviral binding protein has been replaced by VSV G protein which confers potentially a much broader host range/tissue tropism. In response to this, these vectors will be at class 2. Furthermore, the VSV protein pseudotyping represents an aerosol/ingestion opportunity for infection in addition to the percutaneous risk associated with HIV. Therefore the risk assessment indicates the use of a microbiological safety cabinet for operator protection. Although complement sensitive, vectors bearing the VSV glycoprotein are reported to have increased environmental stability emphasizing the importance of paying due regard to principles of GMP/GOSH.

While there is a theoretical potential for animal infection with these virus vectors, all lentivirus work will be carried out in a building where no live animal work is undertaken thus minimizing the risk. Furthermore, as with inadvertent human contact, the viral vectors are self-inactivating and the expressed proteins are not known oncogenes. Thus the consequences of expression in a few cells would be minimal.

The worst case scenario therefore for an exposed individual would be infection of pre-cancerous cell by viral vector expressing immune evasion protein perhaps allowing tumour progression. The most accessible tissue is lungs, but likelihood is small (pre-cancerous tissue would be very much a minority tissue in healthy persons) and virus is
always handled in a safety cabinet. This scenario is considered highly unlikely to occur, as all evidence to date suggests that insertion of the M2 protein into other cells would not enhance proliferation or permit immune evasion. Moreover, there is no evidence that pre-malignant bronchial epithelium is surveyed by the immune system, and there is no data to suggest that a protein stimulating B cells could enhance immune evasion by epithelial cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste will be treated for 24 hours with 2% Virkon or Presept tablets, then gelled if necessary with Vernagel in a sealable container. This is double bagged and transferred to a waste autoclave, and then incinerated on site.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This project has been reviewed by the Institutes Biological and Genetic Modification Safety Committee. This is satisfied that the risks have been properly assessed and the work will be carried out under the appropriate conditions and controls.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Project Ref 678/05.2

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4
02/03/2022
Adenoviral over-expression and siRNA knock-down of metalloproteinases of the MMP and ADAM families of proteinases

Date Project Ceased
23/05/2007

Withdrawn

Historical Significant Changes
TRANSFERRED TO GM 973. (23/5/07)

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Project Additional Information

Purposes of the contained use

We will produce viral particles using the FLP recombinase kit from Microbix. The gene of interest is cloned into pDC515 or pDC516, which contains a CMV promotor or pDC511 and 512 containing the SV40 promotor, allowing expression to be checked under Containment Level 1 conditions prior to generation of virus. Virus is made by co-transfection of the pDC vector with pBHgfrDE1E3, 3FLP in NautCell 293T cells, where FLP recombination occurs producing viral particles. This will be performed under Containment Level 2 conditions in compliance with the institute code of practice.

Recipient or parental organism

E. coli: All strains are K12 derived thus disabled and non-colonising. Vectors are mobilisation defective. Mammalian cells: pDC515/6. Vector is non-mobilisable.
Adenovirus: Microbix adenovirus and all commercially prepared adenoviral genomes are based on Ad5 which is replication deficient but can enter a variety of human cells.

Host/vector system

E. coli, K12 pBr322 based
Mammalian Cell pBR322 based
Mammalian cell Ad5 based virion post infection (E1 deficient).
NautCell293T Ad5 based virion.

Origin & function

Human origin
SiRNA is synthetically derived- used to inhibit expression
cDNAs - used for over expression
Evaluation of foreseeable effects

Replication deficient Ad5 are unlikely to cause disease for purpose of group II classification. However the nature of the inserted gene should be considered in the context of the recombinant virus. Work with adenovirus carries risk of allergy after repeated exposure. However although the virions lack E1/E3 proteins and are compromised in their ability to establish and maintain and infection inhuman cells.

Over expression of MMPs through inadvertent infection by RAd5 virus expressing the enzymes via a CMV or SV40 promotor may result in increases in ECM degradation and hence increases in cellular proliferation and migration. High levels of over expression of most MMPs in tumour cell lines in mouse models of subcutaneous xenotransplation results in increased tumour growth. It is possible therefore that increased rates of cell growth may occur on adenoviral expression. Although increased MMP expression is increased on neoplastic transformation in most tumour types, increased MMP expression itself is not thought to cause cellular transformation at the genetic level, hence MMPs are not considered oncogenes. Effects of ADAM knock down are even harder to predict in vivo through inadvertent infection of sirRNA expressing adenovirus. With the exception of ADAM10 (embryonic lethal due to lack of Notch/Delta processing) or TACE (EGF like knockout phenotype due to poor shedding of EGF ligands) all other mouse knockouts made so far as ADAMs are viable and have no severe phenotypic differences from their wild type litter mates which have been defined as yet.

The virus is physically relatively stable, so need good practice to ensure it isn't spilled and left to present a hazard at some future point; the host range of Ad5 is naturally limited to man and therefore the virus will not infect animals or plants nor, due to its disablement, spread to the wider human community. Bacteria and mammalian cells are unable to survive outside of laboratory culture conditions.

See attached protocol for further details.

In summary, there is little evidence that either knock down of MPs or their over expression would have long term effects, should any pathology result from inadvertent infection by adenovirus. Adherence to the code of practice for containment 2 facilities should be adequate for preventing inadvertent infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be treated with either 2% virkon overnight in labelled containers. Liquid post disinfection will be gelled with Vernagel and placed in sealed containers and taken to room 1.23 for incineration. Solid waste will be placed in double autoclave bags, secured with autoclave tape labelled with room no and user name, followed by autoclaving and incineration. Liquid spills will be treated with 2% Virkon (non metallic surfaces) or 10% Trigene (metallic surfaces), absorbed with paper towels which will then be autoclaved. Surfaces will be routinely cleaned with 10% Trigene.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This project has been reviewed by the Institutes Biological and Genetic Safety Committee. This is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.

**Project Containment**

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**Project Ref** 678/06.1

**Date Ackn’d** 01/02/2006

**CU2 Project Title** Effects of leukaemia-associated oncogenes in the generation and maintenance of leukaemia stem cell populations.

**Date Project Ceased** 17/09/2019

**Class** 2

**CultureVol** 1-50 Litres

**Class Culture** Vol

**Class 2** Culture

**Volume Class 3-4**

**Non-GMM** Consent Granted

**Consent Granted** Not Applicable

**Project notified under transitional arrangements**

**Historical Significant Changes** Transferred to GM678

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

We will produce viral particles which have both ecotropic and amphotropic ranges of tropism, using replication defective MSCV v2.1 based retroviral vectors. The gene of interest is cloned into these vectors which concomitantly either express GFP or an antibiotic resistance marker. Viral particles are produced following transient co-transfection of 294T cells with the retro/lentiviral vector and one (ecotropic) or 2 (amphotropic) packaging constructs. This will be performed under containment level 2 conditions in compliance with institute practice.
Recipient or parental organism

E Coli - All strains are K12 derived thus disablinn and non-colonising. Vectors are non-mobilisable. Mammalian cells - MSCV vector is non-mobilisable. Retrovirus - MSCV vectors are based on replication defective retroviral genomes and FUGW vectors on replication defective lentiviral genomes.

Host/vector system

Host - 293T cells/vector - MSCV retrovirus or FUGW lentivirus
Host - Mammalian cell, post infection/vector - MSCV retrovirus or FUGW lentivirus (replication incompetent).

Origin & function

Human origin
cDNA - for overexpression.
siRNA - synthetically derived to be complimentary to human sequence, used to inhibit protein expression

See attached project application for details.

Evaluation of foreseeable effects

Risk Assessment for human health:
The most obvious potential risk to human health is the expression of oncogenes within accidentally infected human cells. This would be impossible for exotropically packaged retrovirus or lentivirus, but is theoretically possible for amphotropically packaged retrovirus or lentivirus. Theoretically these viruses can infect human cells and lead to integration and expression of the insert in dividing cells. In turn, expression of oncogenes or potential oncogenes or of endogenous genes through insertional mutagensis could potentially be harmful to human health. There is also the theoretical potential for propagation of the virus through recombination and the generation of replication competent retrovirus (RCR). The MSCV vector is replication defective and the production of RCR will be minimised by the use of a 3 plasmid transfection system to prevent recombination. However, testing for RCR will be performed for each batch of amphotropic virus using a marker recovery assay (each virus also expresses either a GFP marker or an antibiotic resistance marker) and only batches negative for RCR will be utilised further.

For lentiviruses, theoretically these viruses can also infect human cells and lead to integration and expression of the insert in dividing and non-dividing cells. In turn, expression of oncogenes or potential oncogenes or of endogenous genes through insertional mutagensis could potentially be harmful to human health. There is also the theoretical potential for propagation of the virus through recombination and the generation of replication competent retrovirus (RCR). The FUGW vector is replication defective and the production of RCR will be minimised by the use of a 3 plasmid transfection system to prevent recombination. However, testing for RCR will be performed for each batch of amphotropic virus using a marker recovery assay (each virus also expresses either a GFP marker or an antibiotic resistance marker), and only batches negative for RCR will be utilised further. In addition, although the lentivirus has modifications to increase transcription and nuclear import (the WRE and HIV-1 flap element respectively) which might theoretically increase the risk of expression in infected human cells, it is replication defective and the SIN modification of the 3'LTR is duplicated in the 5LTR upon reverse transcription. This results in the transcriptional inactivation of the provirus and decreases the likelihood of insertional mutagensis. These vectors also have a 5'LTR modification (U3 region replaced with CMV promoter) which along with the use of a 3 plasmid transfection system reduces the possibility of recombination and minimises the risk of production of RCR. Recently concern has been raised with regard to the WRE element, as mice transplanted with control vectors containing this element have developed tumors. Therefore, in keeping with GTAC and SACGM recommendations these vectors will be handled at containment level 2.

To decrease the likelihood of infection standard measures to decrease percutaneous as well as aerosolised transmission of the virus, such as no use of sharps ans work to be performed within a class II microbiological safety cabinet will be adopted. Aerosolised virus is theoretical risk to infect the respiratory epithelium, but its production would be minimised as above. In addition the respiratory epithelium is terminally differentiated, has a high turn-over and it is unlikely that the haematopoietic specific oncogenes involved in this proposal would have any effects on this tissue in the unlikely event of infection. Moreover, malignancy is a multistep process and it is unlikely that any single "hit" would initiate transformation and in occassional experiments where oncogenes were coexpressed this would be by cotransduction making dual infection of the same human cell highly unlikely. Also VSVg pseudotyping results in complement sensitivity increasing the likelihood of immunological neutralisation in human hosts. However, during the production and concentration of retrovirus, the transduction of cells and the subsequent analysis of these cells, CL2 conditions will be strictly adhered to.
Both viral particles are relatively unstable and standard practices will ensure adequate decontamination of facilities. Bacterial and mammalian cells are unable to survive outside of laboratory culture conditions.

See attached protocol for further details.

In summary, there is little evidence for the likelihood of hazardous long-term effects from accidental infection and overexpression of an oncogene. Adherence to the code of practice for containment 2 facilities should be adequate for prevention of inadvertent infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Waste will be treated with 2% Virkon overnight in labelled containers. Liquid post disinfection will be gelled with vernagel, placed in sealed containers and taken to room 1.23 for incineration. Solid waste will be placed in double autoclave bags, secured with autoclave tape and labelled with the room number and user name, followed by autoclaving and incineration. Liquid spills will be treated with 2% Virkon (non-metallic surface) or 10% trigene (metallic surfaces) and absorbed with paper towels which will then be autoclaved. Surfaces will be routinely cleaned with 10% trigene.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
This project has been reviewed by the institute's biological and genetic safety committee. This is satisfied that the risks have been properly addressed and that the work will be carried out under the appropriate controls and conditions.

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

To identify novel functional roles of MHC and leukocyte receptor complex encoded immunoreceptors through expression analysis and proteomics techniques. Primary human dendritic cells are non-dividing and are not readily transfecatable using standard techniques. This project will therefore require the use of replication defective lentiviral vectors based on human immunodeficiency virus type 1 (HIV-1).

**Recipient or parental organism**

1. E. coli K12 disabled/non-colonising.
2. 293T (human epithelial kidney) especially disabled cells.
3. An HIV-1 "self inactivating" vector (SIN) which has a deletion in the U3 region of the 3' longterminal repeat (LTR) of the DNA used to produce the vector RNA; has sequence encoding virulence genes vif,vpr and nef deleted; bears envelope protein VSVG (the vector thus has altered hostage/tropism) The vector is split over 3 plasmids and is therefore replication disabled.
4. Primary human dendritic and other cells.

**Host/vector system**

E.coli K12-pUC plasmid derivatives, which are non-mobilisable and bearampicillin resistance, bearing- a ) HIV-1 derived retroviral sequences encoding viral gag/pol and tat and rev sequences, b) viral sequences encoding VSVG protein, c) HIV-1 vector bearing eukaryotic promoters, markers and antigen presentation genes. None of these plasmids possess promoters capable of driving expression of the genes to be expressed in bacteria. Any background expression will not result in active proteins being produced. None of the proteins are intrinsically toxic.
293T cells - plasmids bearing the HIV vector components and the genes of interest will be transfected to produce infections vector particles which are harvested for expression work. Human cells of interest - HIV viral vector particles will be used to infect these for further analysis.

**Origin & function**

To study antigen presentation genes using retroviral expression vectors in mammalian cells. All of these genes have functions in the interaction between immune cells.

i) ULBP/RAET molecules; these proteins are structurally related to MHC class 1 molecules and bind to the activating immunoreceptor NKG2D.

ii) HLA-F and TAPBPR to examine trafficking tagged constructs

iii) B7 related BTN ann BTN2L to examine cellular localisation and interacting proteins

iv) mutant forms of the above genes which will include point mutants and deletion mutants of putative functional domains

v) reporter genes FLAG, myc, GFP and Protein A

**Evaluation of foreseeable effects**

None of the bacterial or cellular GMMs produced will have characteristics significantly different from the hosts; many of the genes (eukaryotic) will be in the wrong context to exert any effect on bacteria (prokaryotic) and the eukaryotic cells will be especially disabled. The products are not intrinsically toxic and cannot exert effects on other cells upon release from producer cells-most require membrane insertion for functionality.

The HIV vector is replication disabled and is unable to produce infections progeny from the initial round of infection. Potentially replication competent retrovirus (RCR) could be generated from the producer cells but as the vector is split over a number of plasmids and is self inactivating, the number of recombination events required to restore a functional genome make the probability extremely low. The vector has an altered host range/tropism: usually retroviruses are spread by percutaneous introduction. The VSVg protein potentially allows this as well as transmission via contact and aerosol. Although the virus is unable to infect beyond the initially infected cells, there is potential that the virus could insert into host DNA, thereby causing insertional mutagenesis and possibly adverse effects, but this is also of low probability.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated for 24 hrs with 2% Virkon (effectively 100% kill) before disposal into the laboratory drainage system. Alternatively disinfected waste will be gelled with Vernagel in sealed containers and removed for incineration, 100% kill. Solid waste such as plastics will be double bagged before transfer to a validated waste autoclave within the building. (100% kill) and then disposed of by incineration on site (Addenbrooke's Hospital is GM registered for this purpose)

**Is an emergency plan required according to regulation 20?**

- [ ] N

**If yes, tick to confirm that it is attached to this form**

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
This project has been reviewed by the CIMR Biological and Genetic Modification Safety Committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

**Project Containment**

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**Project Ref** 678/08.1

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<td>Investigating the role of genes KIAA0350, IL2RA and RBM17 in human immunology.</td>
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**Project Additional Information**

**Purposes of the contained use**

To investigate the genetic causes of Type 1 Diabetes (T1D) using lentiviral transfection of RNA interference (RNAi) to knockdown KIAA0350, RBM17, and IL2RA in human cell lines.

**Recipient or parental organism**
### E. Coli K12
- Jurkat cell line
- Human CD4+ and CD8+ T cells
- These are all disabled, non-pathogenic to man and cannot survive in the environment.

### Host/vector system
- Lentiviral pLBm (HIV-1 based vector with vsvg envelop)
- Plasmid + gag/pol genes
- Plasmid + rev gene
- Plasmid + vsvg gene
- 239 cell line

### Origin & function
- RNAi corresponding to
  - KIAAO35O (uncharacterised, associated with TI D)
  - IL2RA, (IL2 receptor associated with TI D)
  - RBM17, (splicing regulatory gene expressed in T cells)
- Expression of RNAi in cells will result on knockdown of the corresponding gene in a sequence specific manner

### Evaluation of foreseeable effects
- If a human is infected by the RNAi, the genes will be knocked down in the infected cells only, as the virus is non replicating. Knock down of the genes in a limited set of cells may not be lethal and is not expected to result in any aberrant immune response. The effect of knocking down RBM17 and IL2RA would lead to less proliferation of the infected T cells.
- The genes are not oncogenes, nor known to be involved in tumour suppression.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
- n/a

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
- n/a

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
- Liquid waste- disinfected with 2%trigene or 2% virkon, left overnight before disposal in sink (effectively 100% kill)
- Dry waste will be double bagged, autoclaved within the building and incinerated on site (Addenbrookes Hospital is GM registered for this purpose (100% kill))

Is an emergency plan required according to regulation 20?  
- N

If yes, tick to confirm that it is attached to this form  
- N

Tick to confirm that you have attached a risk assessment to this form  
- Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
- N
This project has been reviewed by the CIMR Biological and Genetic Modification Safety Committee. We are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

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<td>Use of luminescently-tagged and fluorescently-tagged bacteria to study pathogen-phagocyte interactions</td>
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| Date Project Ceased | 24/02/2016 |

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Project notified under transitional arrangements N

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**Project Additional Information**

**Purposes of the contained use**

The project aims are to understand how non-tuberculous mycobacteria (NTM), specific types of gram negative bacteria (E coli (non-verotoxigenic strains isolated from sputum), P. aeruginosa) and gram positive bacteria (S pneumoniae, S aureus) interact with macrophages to avoid intracellular killing and how pharmacological manipulation of cellular processes (such as the phagosomal ionic environment and macro-autophagy) might enhance intracellular bacterial killing and/or modulate release of
inflammatory mediators.

Recipient or parental organism

NTM (excepting M. bovis BCG) are ubiquitous organisms occurring naturally in the environment (in domestic water, soil and houses). While they can cause lung infections in people with pre-existing structural lung disease (such as Cystic Fibrosis and Chronic Obstructive Pulmonary Disease), they are not pathogenic in healthy people and are not transmissible from person to person. Untransfected, it is usually handled in CL2 areas. M. bovis BCG is still in current world-wide use as a live vaccine against M. tuberculosis and is not pathogenic in health.

Host/vector system

pSMT1 plasmid carrying the luxAB genes from Vibrio harveyi and green fluorescent protein (GFP), Cyan FP, Yellow FP or Red FP; under the control of the constitutively active mycobacterial GroEL (hsp60) promoter; mycobacterial and E. coli origins of replication and a hygromycin resistance cassette. The vectors will not be translated by eukaryotic cells.

Origin & function

pSMT1 plasmid carrying the luxAB genes from Vibrio harveyi and green fluorescent protein (GFP), Cyan FP, Yellow FP or Red FP; under the control of the constitutively active mycobacterial GroEL (hsp60) promoter; mycobacterial and E. coli origins of replication and a hygromycin resistance cassette.

luxAB genes from Vibrio harveyi and green fluorescent protein (GFP) purchased from Clontech


Episomal insertion of plasmid DNA will result in generation of cytoplasmic green fluorescent protein (GFP) and luciferase (lux) as previously utilised in M. bovis and M. tuberculosis (Kampmann et al 2000). No selection advantage is anticipated for LUX-tagged or GFP tagged mycobacteria or bacteria.

Evaluation of foreseeable effects

NTM (excepting M. bovis BCG) are ubiquitous organisms occurring naturally in the environment (in domestic water, soil and houses). While they can cause lung infections in people with pre-existing structural lung disease (such as Cystic Fibrosis and Chronic Obstructive Pulmonary Disease), they are not pathogenic in healthy people and are not transmissible from person to person. Untransfected, it is usually handled in CL2 areas. M. bovis BCG is still in current world-wide use as a live vaccine against M. tuberculosis and is not pathogenic in healthy people. Untransfected and luminescently tagged M. bovis BCG are currently handled in CL2 facilities.

Untransfected and luminescently tagged S. pneumoniae, S aureus, P aeruginosa and E. coli are currently handled in CL2 facilities. While all can cause infections in man, the risk to healthy laboratory staff of these bacteria is extremely low. They will be handled in the tissue culture hoods and no procedures have the potential to generate aerosolised bacteria. The risk of inhalation therefore is extremely low.

ii) the inserted genetic material

The inserted plasmid has no eukaryotic promoter, encodes a luciferase gene and green fluorescent protein, which, in all mycobacteria examined to date, is retained intracellularly. The encoded proteins, lux and fluorescent proteins, have benign metabolic type activities (light production, fluorescence) which would have no significant effect on bacterial or cellular gene expression, though products may be toxic to cells expressing high levels. Extracellularly they have no signalling effects and are of very low toxicity. There is no effective way to deliver genetic material to human cells in these conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Bench tops and spills will be wiped with a disinfectant (1% sodium hypochlorite, or 70% alcohol). Spills will be covered with disinfectant soaked towels for at least 15 min and then wiped. GMM will be killed by addition to 1% sodium hypochlorite for at least 15 min. (as recommended in ‘Standard safety practices in the microbiology laboratory: www.cdc.gov’).

Liquid and solid waste will be double bagged, autoclaved and then incinerated.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

No comments made

**Project Containment**

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**Project Ref 678/09.2**

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<td>Lentiviral over-expression and siRNA knock-down of myosin motor proteins in tissue culture cells</td>
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Date Project Ceased |

02/03/2022
Purposes of the contained use

Lentiviral vectors based on replication disabled HIV. We will use the Lenti-X Expression system developed by Clontech. Our gene of interest will be cloned into one of the Lenti-X fluorescent expression vectors or the Lenti-X Tet-On and Tet-Off advanced inducible expression systems. The proteins required for replication and packaging of the recombinant viral genome are produced from four different, separate vectors for added safety. In this new quadruple, split-gene packaging: gag, pol, VSV-G (env), and rev are expressed from four separate vectors. This further reduces the incidence of replication-competent lentivirus (RCL) to even less than that of other commonly used third-generation systems. The lentiviral expression system will be used under Containment Level 2 conditions in compliance with the institute code of practice.

Recipient or parental organism

E. coli: Strains of bacteria used for initial construction and plasmid amplification are derived from E.coli K12 and are therefore disabled and non-colonising. Packaging vectors of Clontech’s Lenti-X Packaging Systems are the derivates of pUC, which are non-mobilisable and therefore the possibility of transfer of inserted genetic material enabling for example drug resistance to other organisms is negligible.

Mammalian cells: Mammalian primary cells or cell lines are especially disabled and unable to survive or propagate outside of laboratory culture.

Lentivirus: The vectors are part of the Lenti-X Lentiviral Expression system by Clontech. Although these vectors are based on the lentivirus human immunodeficiency virus (HIV) and this is a HG 3 pathogen, vectors are all third generation replication defective and considered class 1. In addition the Clontech’s Lenti-X Vectors contain less than one-third of the wild-type HIV-1 genome. These wild-type sequences mainly consist of the viral LTRs and packaging signal. All essential replication genes have been completely removed and are instead supplied as separate DNA entities in the Lenti-X HT Packaging Mix. Clontech’s Lenti-X HT Packaging System also uses a split-gene packaging strategy to provide the viral packaging elements on individual plasmids that physically separate the viral envelope, env (usually VSV-G), and sequence from the gag-pro-pol sequences.

Host/vector system

Host: Standard mammalian tissue culture cell lines including HeLa cells, fibroblasts, MDCK cells and prostate cancer cell lines such as LNCaP and PC3. In addition we will use primary fibroblasts isolated from the Snell’s waltzer (myosin VI KO) mouse.

Vector: Lentiviral vectors based on replication disabled HIV

Origin & function

The proteins we are working with are either known cytoskeletal, structural proteins ubiquitously expressed in mammalian cells or other known mammalian proteins with no identified toxicity. Therefore a low damage factor is to be expected from their aberrant expression. Recent publications, however, show that myosin VI is dramatically overexpressed in prostate cancer tissues. At present it is not known whether myosin VI is the causal factor for malignant transformation and therefore it will be treated as a
potential oncogene until more information becomes available.

**Evaluation of foreseeable effects**

The vectors are part of the Lenti-X Lentiviral Expression system by Clontech. Although these vectors are based on the lentivirus human immunodeficiency virus (HIV) and this is a HG 3 pathogen, vectors are all third generation replication defective and considered class 1. The vectors used will be self inactivating (SIN). A deletion inactivates transcription form the proviral LTR, so reducing the potential for transcriptional activation of cellular genes and also prevents mobilization of any RCR. However as the virus integrates into the host cell DNA there is still a potential for insertional mutagenesis to occur.

A plasmid encoding the envelope protein from vesicular stomatitis virus envelope protein G (VSV-G) increases the host cell range/tissue tropism and makes it permissive for human cells, including quiescent cells. This feature means that there is a risk of infection through contact/aerosol transmission in addition to the normal HIV percutaneous route. Such particles are also physically more stable.

All Clontech lenti-X expression vectors contain a woodchuck hepatitis virus posttranslational regulatory element (WPRE). Vectors carrying the WPRE element are possibly able to promote tumours in animals and therefore should be handled at CL2.

The proteins we are planning to overexpress or downregulate are either known cytoskeletal proteins ubiquitously expressed in mammalian cells or other known mammalian proteins with no identified toxicity. Therefore a low damage factor is to be expected. Recent publications show that myosin VI is dramatically overexpressed in prostate cancer tissues. At present it is not known whether myosin VI is the causal factor for malignant transformation and therefore it will be treated as a potential oncogene until more information becomes available.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

none applied for

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All disposable tissue culture plastics will be autoclaved and incinerated. Culture medium will be treated with 2 % Virkon for 16 h and disposed to drains. Liquid spills will be treated with 2% Virkon (non metallic surfaces) or 10 % Trigene (metallic surfaces), absorbed with paper towels, which will then be autoclaved. Benches are cleaned with 10 % Trigene.

**Is an emergency plan required according to regulation 20?**  

- [ ] N

**If yes, tick to confirm that it is attached to this form**  

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**  

- [ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  

- [ ] N

This project has been reviewed by the Institutes Biological Genetic Safety. The Committee is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.
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**Project Ref** 678/09.3

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<td>Adenoviral and/or Lentiviral over-expression and siRNA knock-down of reporter constructs and genes to investigate stimulus secretion coupling in enteroendocrine cells in tissue culture</td>
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*Project notified under transitional arrangements*

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**Historical Significant Changes**

Project transferred to GM170 on 28/07/2014

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Hormones such as glucagon-like peptide 1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP) play key roles in postprandial nutrient homeostasis, but little is known of the molecular mechanisms underlying and regulating their release from enteroendocrine cells. Enteroendocrine cells lie scattered throughout the gut epithelium and at least 16 different types, classified by the secreted hormones, have been defined, amounting together to about 1% of the gut epithelium. While they are not readily distinguishable from the surrounding enterocytes (nor from each other) by live cell microscopy, we have recently successfully labeled enteroendocrine cells by expressing fluorescent protein markers under the control of the relevant hormone promoter either delivered as a stable transgene in mice or after adenoviral transfection of mixed gut epithelial cells in primary culture.

We now wish to further investigate the stimulus secretion coupling in these cells using:
which has resulted in increased tumor frequencies in mice, these viruses should be considered class 2. The lenti viral particles in this project will be 'pseudotyped' with the  hepatitis B virus post-transcriptional regulatory element) to boost viral titres and gene expression, and as this element can express an oncogene (part of the X-protein), administered accidentally and the defective replication and self-inactivation of the virus. As some of the commercially available vectors contain the WPRE (woodchuck posttranscriptional regulatory element) to boost viral titres and gene expression, and as this element can express an oncogene (part of the X-protein), of nearby genes due to integration within these genes be excluded. However, as problems arising from the insertion site have only been reported in studies involving third generation (distinguished in the UK by deletion of the tat-gene and replacement of the tat-responsive promoter in the 5'LTR by heterologous alternatives). The theoretical potential for re-emergence of replication competent virus through recombination has been significantly reduced in these vectors, by splitting the replicative, (a) Replication disbaled adenovirus vector, based on Ad5. This virus is deleted for essential replication functions (E1A region) and can only replicate in cells complementing this function (HEK 293 cell lin). Naturally the virus is transmitted by aerosol to respiratory tissues from where it may infect adenoid tissue. There are no reports of other infections such as enteric/kidney. However as the vector is incapable of completing a single round of replication, no progeny virus can be produced and infection would be (self) limited to any initial cells exposed. The number if cells affected would be related to the amount of virus encountered but effects would not progress to any extent approaching natural disease. The vector is considered HG 1.

Recipient or parental organism

Recipients:

a) E.coli: Strains of bacteria used for initial construction and plasmid amplification are derived from E.coli K12 and are therefore disabled and non-colonising.
b) Mammalian cells: Recipients will be various mammalian cells in culture. Most are moderately well characterised (eg HEK293 cell, GLUTag cells) and considered fastidious and non harmful, cannot colonise/cause disease, (ACDP hazard group 1). Primary epithelial cultures established from unscreaned human intestinal tissue could harbour infectious agents and should therefore be handled at containment level 2.

Host/vector system

(1) Replication disbaled adenovirus vector, based on Ad5. This virus is deleted for essential replication functions (E1A region) and can only replicate in cells complementing this function (HEK 293 cell lin). Naturally the virus is transmitted by aerosol to respiratory tissues from where it may infect adenoid tissue. There are no reports of other infections such as enteric/kidney. However as the vector is incapable of completing a single round of replication, no progeny virus can be produced and infection would be (self) limited to any initial cells exposed. The number if cells affected would be related to the amount of virus encountered but effects would not progress to any extent approaching natural disease. The vector is considered HG 1.

(2) Replication disabled lentivirus. Although the starting viruses like HIV themselves are hazard group 3 pathogens, vectors considered for his proposal are all second or third generation (distinguished in the UK by deletion of the tat-gene and replacement of the tat-responsive promoter in the 5'LTR by heterologous alternatives). The theoretical potential for re-emergence of replication competent virus through recombination has been significantly reduced in these vectors, by splitting the replicative, packaging and envelope functions and the desried transgene over ≥ three independent plasmids, therefore requiring multiple recombination events to generate replication competent virus. The emergence of replication competent virus is therefore unlikely and such viruses would be expected to have lost the transgene. In addition, many accessory functions/pathogenicity determinants such as vif have been deleted and they are all self-inactivating, carrying the SIN-deletion in the 3'LTR, including re-activation after integration into the host genome and as the U3 promoter is thereby compromised reducing the risk of transactivation of genes near the integration site. But, as the constructs all carry other (usually constitutively active (eg CMV)) promoters to drive expression of the transgene this can not be excluded, nor can the inactivaton of nearby genes due to integration within these genes be excluded. However, as problems arising from the insertion site have only been reported in studies involving administration of very high viral titers, the likelihood of similar problems in this study is considered low, taking into account the comparitively low viral titers likely to be administered accidentally and the defective replication and self-inactivation of the virus. As some of the commercially available vectors contain the WPRE (woodchuck hepatitis B virus post-transcriptional regulatory element) to boost viral titres and gene expression, and as this element can express an oncogene (part of the X-protein), which has resulted in increased tumor frequencies in mice, these viruses should be considered class 2. The lenti viral particles in this project will be 'pseudotyped' with...
vesicular stomatitis virus envelope protein G (VSVG); this increases host cell range/tropism and such vectors may represent an aerosol means of transmission in addition to the expected percutaneous risk from lentiviruses. Such viruses are more stable and can be purified to high titre. However, they are rendered sensitive to complement.

Origin & function

The expressed proteins range from fluorescent markers, such as GFP, which are unlikely to be harmful, and fusion proteins of fluorescent proteins with activity retaining protein kinase A catalytic subunit (overexpression of which might alter cell physiology, although the probe has successfully been used to investigate cAMP responses in pancreatic beta-cells, without altering their normal Ca²⁺-homeostasis or stimulant-secretion coupling) to other proteins involved in enteroendocrine physiology. Similarly the knock down targets are genes involved in enteroendocrine physiology, including, but not restricted to, glucokinase, SGLT-1 and Kir6.2. As overexpression or knock-down of such genes is unlikely to transform infected cells and is not expected to be toxic, the risk to human health or the environment from accidental infection is considered low.

Evaluation of foreseeable effects

As constitutively active promotors are used, virtually every transfected cell will be expected to express the inserted cDNA or shRNA. The expressed proteins range from fluorescent proteins with activity retaining protein kinase A catalytic subunit (overexpression of which might alter cell physiology, although the probe has successfully been used to investigate cAMP responses in pancreatic beta-cells, without altering their normal Ca²⁺-homeostasis or stimulant-secretion coupling) to other proteins involved in enteroendocrine physiology. Similarly the knock down infected cells and is not expected to be toxic in context of the transfected cells nor after release of proteins from lysed cells the risk to the environment due to accidental infection is considered low. Cells will remain especially disabled and unable to survive outside of laboratory culture.

1) Adenovirus: Adenoviruses are generally species specific; Ad5 infects humans and does not naturally infect other animals (but can experimentally infect cotton tail rats, which are not endogenous in the UK). Therefore there is no risk to other organisms. The virus is replication incompetent therefore could not spread to the wider human population from any infected individual.

2) Lentivirus: VSVG enables these vectors to infect a wide variety of animal cell types including those of different mammalian species. There may be enhanced environmental stability. However the viruses are replication disabled; they cannot produce progeny virus and so cannot spread to the wider human population or other animals.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable tissue culture plastics will be autoclaved and incinerated. Culture medium will be treated 2% Virkon for 16 h and solidified prior to incineration. Liquid spills will be treated with 2% Virkon (non metallic surfaces) or 10% Trigene (metallic surfaces). Absorbed with paper towels, which will then be autoclaved/incinerated. Benches are cleaned with 10% Trigene.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N
This project has been reviewed by the Institutes Biological Genetic Safety. The Committee is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.

### Project Containment

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### Project Ref 678/11.1

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### Project Additional Information

**Purposes of the contained use**

For a viral infection to be successful the virus must survive in the presence of the host immune response. Viral survival strategies have evolved to modify or evade these host immune responses including the modification of host immunoreceptors. This project addresses the modification of host immunoreceptors by the human herpesvirus and lentivirus families.
Human herpesviruses are a family of enveloped double-stranded DNA viruses. There are three subfamilies: alphaherpesvirinae [herpes simplex viruses 1 and 2 (HSV-1, 2); varicella zoster virus (VZV)], betaherpesvirinae [human cytomegalovirus (HCMV), human herpesvirus-6 (HHV-6) and human herpesvirus -7 (HHV-7)] and gammaherpesvirinae [Epstein-Barr virus (EBV) and Kaposi's sarcoma herpesvirus (KSHV)]. The herpesviruses are ubiquitous pathogens of all vertebrates, although infections are usually highly species specific. All herpesviruses apart from KSHV and HSV-2 infect the majority of the human population (seroprevalence of HSV-1, VZV, HCMV, HHV-6 and -7, EBV is 50-100% depending on the population studied, whereas HSV-2 approximately 20%, KSHV 1-50% depending on the population studied). One common feature of all herpesvirus infections is lifelong viral persistence after primary infection despite a strong cell-mediated immune response. Each virus can generate a latent infection in certain cell types. In immunocompromised individuals, such as recipients of organ transplants and patients with advanced HIV infection, reactivation of herpesviruses such as VZV, HCMV, EBV and KSHV, has the potential to cause severe disease.

Human immunodeficiency virus (HIV) is the lentivirus that causes AIDS. This condition is characterised by progressive failure of the immune system, which allows opportunistic infections and cancers to thrive. HIV-1 establishes chronic infections, affecting approximately 33 million people worldwide. Despite considerable advances in HIV science, the goal of eradicating HIV- remains elusive.

There is already considerable evidence of immunoreceptor modulation by herpesviruses and lentiviruses; for example, MHC class I is removed from the cell surface by HSV, HCMV, EBV and KSHV as well as HIV. This proposal aims to investigate novel mechanisms and consequences of viral manipulation of host immunoreceptors during infection. We aim to investigate both (a) the role of individual viral genes on host immunoreceptors (herpesviruses and lentiviruses), as well as (b) whole viral infection in both lytic and latent phases (herpesviruses only). (a) We will clone individual viral genes into delivery vectors, and deliver them to relevant cultured host cells. We will then characterise he effects of theses modifications on the host cell. (b) We will generate herpesviral and disabled HIV-1 stocks in established human cell lines from viral seed stocks in established human cell lines from viral seed stocks or bacterial artificial chromosomes encoding the viral genome. Clinical isolates and their genetically modified derivatives will be used as well as laboratory adapted strains. In vitro infection of human cell lines and primary cell lines will then be carried out to model both lytic and latent viral infections. We will then characterise the effects of these viral modifications on the host cell. Viruses with deletions of, or tags attached to individual genes will be utilised in order to further characterise cellular effects.

Recipient or parental organism

Recipients will be various mammalian cells in culture. Most are well characterised and considered ACDP hazard group 1. Cells from volunteers are uncharacterised and although themselves present no greater risk, may contain adventitious infectious agents and therefore will be handled at CL2 (risk assessment under COSHH regs). Mammalian primary cells or cell lines are especially disabled and unable to survive or propagate outside of laboratory culture. Culture cells expressing the viral genes may be altered in particular surface proteins they express but those changes will not over ride the especially disabled nature of the cells.

The herpesviruses to be studied as detailed above are all ACDP hazard group 2 and will therefore be handled at CL2 within a class II microbiological safety cabinet (MSC). Pregnant or known immunocompromised individuals will not perform any of this work. The modified herpesviruses should be no more pathogenic than wild type. Although there are virus deletion mutants with increased virulence over their parent (for example deletion of A41L in vaccinia virus), to our knowledge, herpesvirus deletion mutants that show increase in virulence have not been described.

Viral gene delivery vectors (retroviruses, lentiviruses) to be used are those typically considered class 1 GMMs, and will all be self-inactivating, and therefore once packaged, are unable to propagate. A three plasmid transient transfection system will be used to generate the recombinant retroviruses and lentiviruses, thereby decreasing the likelihood of recombination within the packaging cell lines to generate replication competent virus. The retroviral vectors are based on the Moloney murine leukaemia virus in which the gag, pol and env genes have been deleted. Gag and pol are supplied in trans on a packaging plasmid. We will generate VSV-G pseudotyped virus in order to deliver the virus particles to human cells. The lentiviral vectors are based on an HIV-1 self inactivating vector (SIN) containing a deletion in the U3 region of the 3’LTR of the virus. In addition, the gag, pol, env, nef, vpu and vpr genes have been deleted. Again, a second packaging plasmid encoding gag, pol, rev and tat will be used along with the plasmid encoding VSV-G to make pseudotyped viruses that will enter human cells. A packaging plasmid encoding nef, vif, vpu and vpr may also be used, in order to improve transduction efficiency of primary human cells. While VSV-G increases host range and their tropism and mode of transmission may also be altered from that exhibited by wild-type retroviruses, aerosol risk to the airway epithelium appears limited but the inability to infect the apical cell surface. The AdZ adeno viral vector system will be used to generate recombinant adenoviruses. These vectors carry a deletion of the E1 gene region rendering them replication incompetent in non-complementing cell lines. In addition, the E3 region (important for viral pathogenesis and immune evasion) has also been deleted making them less pathogenic in...
humans. They are those typically classed as class 2 GMMs and remain so as the inserts are placed within the deleted regions. All three types of viral vectors will be harvested and used in a CL2 laboratory.

The molecular clone that encodes the disabled HIV-1 to be used in this project is derived from the HIV-1 backbone and is currently approved for use when working under CL2 conditions in the Department of Medicine at Addenbrooke's Hospital, Cambridge. To increase the safety of the system, the vector components are segregated onto 2 plasmids: an HIV-1 molecular clone with a critical env deletion and a second plasmid expressing the VSV-G envelope protein. None of the individual plasmids is capable of generating infectious viral particles and, to avoid the generation of replication competent virus via homologous recombination, the constructs do not contain overlapping homologous sequences. Since none of the cell lines or vectors carry intact env-encoding sequences, infectious HIV particles cannot be produced.

The viruses and viral delivery vectors we will use are ACDP hazard group 2/class 1 GM. The viruses are deactivated by treatment with trigene, virkon or 70% EtOH and autoclaving. Therefore following the waste procedures outlined below should eliminate risk of spread to the environments. Many of the herpesviruses are also strictly human specific, therefore there is no risk of spread to other animals or plants. The viral vectors are all replication disabled so will be unable to disseminate in the wider animal or human populations. Modifications will be to add a tag such as a fluorescent protein to viral proteins, or to delete or disrupt viral genes and subsequently restore them. Expression of tags is not expected to be detrimental to human health or the environment. Deletions/disruptions to viral genes are not expected to increase infectivity/pathogenicity. Herpesvirus genes cloned into viral gene delivery vectors may confer an ability to alter immune responses to infected cells. However, the large number of viral immune evasion genes encoded by the human herpesviruses demonstrates the need for a virus to encode more than one immunoevasin in order to effectively evade human immune responses.

We will only express a single herpesviral gene in each gene delivery vector. As the vectors are disabled, any accidental infection of the epithelial cells of the researcher would be localised. Transduced cells would not be able to produce viral particles. Due to the high turnover rate of epithelial cells, any localised infection would not be expected to be long lasting.

Pseudotyped env-deleted HIV-1 viral particles are replication deficient, but theoretically able to transduce epithelial cells of the researcher. If this occurred, all HIV-1 proteins except env would be expressed, but no replication competent virus would be formed. This protein expression would not be expected to cause harm, and the Mammalian tissue culture cell lines (eg HFF2, THP-1, KG-1, HeLa, U373, U937, Huh7, HCT116, Jurkat, Daudi, NK-92, NK-L, HL60, 220, T1, 293, Ramos). Primary human cells (eg monocytes, dendritic cells, T cells, B cells, NK cells). Human herpesviruses including HSV-1 (eg strains: F, KOS, 17, SC16), HSV-2 (eg strains: 333, HVD), VZV, EBV, HCMV (eg strains: Toledo, Merlin, TB40/E, VR1814/FIX-BAC and laboratory adapted strains AD169 and Towne), HHV-6, HHV-7 and KSHV.

A disabled HIV-1 virus with a critical env deletion will also be used.

Bacteria: Top10, Sure 2, alpha-select, clean genome.

Viral gene delivery vectors including retroviruses, lentiviruses and adenoviruses.

Bacterial artificial chromosomes (BACs) that cover the genomes of the herpesviruses and adenovirus serotype 5 so that insertion/deletions in the viral genome can be generated in vitro prior to the generation of recombinant viruses.

Standard cloning vectors (such as TOPO and pcDNA3) and BAC shuttle vectors will be used to clone viral gene disruptions (such as truncations) and to add inserts-tags onto viral genes or to rescue deletion viruses. In addition, these cloning vectors will be used to express individual viral genes to allow their study in host cells.

The HIV-1 molecular clone with the env deletion will be used for targeted gene disruption in vitro prior to the generation of lentiviral particles.

Origin & function

Inserts:
Inserts will be used to label herpesviral and lentiviral genes to enable their visualisation during viral infection (such as the YFP, GFP or RFP fluorescent tags) or to simplify
biochemical analyses of viral genes (such as HA, Strep, Myc and Flag tags). To examine the biological role of individual herpesviral and lentiviral genes, gene deletions/disruptions and subsequent reconstitutions will also be used.

Expected biological action of inserted DNA/RNA or transcribed/translated gene product:
The inserts will be commonly used markers/tags as detailed above. Apart from fluorescence, these tags are biologically inert; their expression should not increase viral virulence or fitness.

Deletion or disruption of viral genes is not expected to increase virulence. For reconstitution of deletion viruses, only the original gene will be re-inserted and therefore no increase in virulence over the original strain is expected. No modifications will be made that are designed to introduce new biological activities into viral proteins. Over-expression of viral genes in viral gene delivery vectors should not pose any risk additional to use of the viruses themselves.

Evaluation of foreseeable effects

Pseudotyped particles have previously been classified as class 2.

Bacterial artificial chromosomes and plasmids will be used to generate recombinant virus. These contain antibiotic resistance markers which could in theory present a problem to the user if they become infected with the bacteria in which the plasmids/BACs were grown. However, in practice the bacterial strains we use are highly attenuated and unlikely to cause disease, while the antibiotic resistance markers encoded by these plasmids/ BACs (Ampicillin, kanamycin and chloramphenicol) are active against antibiotics which are no longer in general use in clinical practice. So whilst the plasmids/BACs pose no threat in a bacterial culture, once transfected into eukaryotic cells the recombinant DNA can be packaged into infectious virions and transfection will therefore be carried out in a CL2 laboratory and subsequent cultures treated as live virus.

The disabled nature of the bacterial hosts will not be altered by the plasmids or BACs in use - no expression is anticipated and would not in any case be compatible with bacterial systems. The bacterial strains we use are highly disabled and unable to replicate to any extent outside the lab. Never-the-less, appropriate control measures, including disinfection (with virkon) of liquid cultures and autoclaving of bacterial plates, will be taken.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Conventional molecular biology techniques will be used for cloning in a CL1 laboratory. Bacterial artificial chromosomes and viral gene delivery vectors will be transfected into mammalian cell lines in a CL1 laboratory then transferred to the CL2 laboratory within 24 hours of transfection (to allow us to evaluate transfection efficiency with a fluorescence microscope). All manipulation of liquid will be carried out in a class II Microbiological safety cabinet (MSC) to reduce the risk of aerosol dissemination. Tubes for ultracentrifugation of virus for the production of concentrated virus stocks will be loaded in MSC into buckets that will be sealed and transported to the ultracentrifuge in secondary containers. Similarly, once the ultracentrifugation is complete, the tubes will be transported back to the CL2 laboratory and the buckets opened in the MSC to allow the virus to be resuspended and aliquoted. Since there is no -80° storage facility in our CL2 laboratory, small aliquots of virus will be transported to 5.29 in secondary containment for storage. Fluorometric and microscopic analysis will be conducted on fixed samples, and protein and nucleic acid analysis will be conducted on material which has been lysed in the CL2 facility prior to transport to the laboratory.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The ultracentrifuges available for concentrating virus preparations are outside of CL2 labs. Virus preparations will be sealed within the centrifuge tubes and buckets within the MSC in CL2 and transported in a shatterproof container. The surfaces of centrifuge tubes and transport containers will be decontaminated with detergent prior to removal from CL2 and only be re-opened once returned to the MSC in CL2. The 'comments' section of the ultracentrifuge logbook will be used to warn other users that the centrifuge contains a CL2 organism. Following centrifugation, the buckets will be returned to the MSC in the CL2 for processing. The buckets will then be immersed in detergent in the MSC prior to removal back to 5.14 for cleaning, rinsing and drying.

The -80 degree freezer for storing virus preparations and supernatant is also outside of the CL2 labs. Vessels containing supernatant and concentrated virus preparations will be decontaminated with Trigene and put into a shatterproof container for transport to and storage at -80 in Lab 5.19.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All liquid waste will be disinfected with Virkon overnight, gelled using Vernagel, double bagged, sealed and labelled for autoclave then incineration according to the Department CL2 procedures. All solid waste will be double bagged and labelled for autoclave then incineration (pipettes will be disinfected in 1% trigene before removal from MSCs to prevent drips). Work surfaces will be wiped down with trigene and 70% ethanol. Small spillages will be mopped up immediately with tissue and disinfected with 1% trigene or 2% virkon. Large spills will be covered with absorbent paper and then sprayed with 10% trigene or 2% virkon, and then mopped up after 30 minutes. The absorbent material will then be disposed of in the solid autoclave waste.

This project has been reviewed by the Institutes Biological & Genetic Modification Safety Committee. The Committee is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.

**Project Containment**

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<th>Large Scale Activities</th>
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**Project Ref** 678/12.1

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<th>Culture Volume Class 3-4</th>
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<td>T-cell signalling mechanisms. Over-expression or down-regulation of the target genes in cell lines and primary cells using transfection</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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**Project Additional Information**

**Purposes of the contained use**

The nature of T-cell surface receptors (TcR, costimulators CD28, CTLA-4, ICOS) signalling pathways responsible for regulation of T-cell activation and adhesion and mobility. Our objective is to study the role of costimulators CD28, CTLA-4, ICOS, PD-1 as well as adaptor proteins SLP-76, ADAP and SKAP-55 and the kinases including ZAP70, LCK FYN and GSK3. Over-expression or down regulation of the target genes in cell lines and primary cells using transfection. T-cell function in vitro including activation, proliferation, and adhesion will be examined.

**Recipient or parental organism**

Mammalian cells: Mammalian primary cells or cell lines are especially disabled and unable to survive or propagate outside of laboratory culture.

**Host/vector system**

- Host Human or murine cell lines, such as Jurkat DC27, T8.1, A20, 3A9 or primary cells and other cell lines such as 3T3, 293T, phoenix cells.
- Retroviral vectors (pinco, MigR1, pMX, pMDG, pMLV-GP, p8.91, pGIPZ)
- Lenti-viral vector (HIV-1 self-inactivating vector SIN, pFIP)
- pLKO.1 vector (lentiviral (HIV)-based plasmid)
- plasmid encoding the envelope plasmid derived from vesicular stomatitis virus (VSV) G protein

Cells will be transfected with retroviral or lentiviral plasmids and viral supernatant harvested (max vol 30ml/vector/transfection) and used to infect target T-cell lines or primary T-cells. After infection twice target cells are harvested and used for functional assessments. Max culture vol of target cells is 100ml/cell type, if viral supernatant is frozen for stock, viral supernatant is aliquot in small vial (~2ml/vials) and stored in allocated areas or boxes in -80 degree freezer. 200 µl will contain approximately 1 x 10^6 TU/ml

For example, shRNAs will be cloned into the pLKO, 1 transfer vector which is compatible with standard two plasmid (packaging vector with rev gene and envelope vector) or a three plasmid (packaging vector without rev gene, envelope vector, and rev expression vector) packaging systems. In the case of MISSION shRNA, the viral particles cannot be propagated and are replication incompetent.

A three plasmid transient transfection system will be used to generate recombinant replication-defective retroviruses 293T cells, a human cell line carrying SV40 T cell antigen will be transfected with (a) An HIV-1 self inactivating vector (SIN) cDNAs encoding the genes of interest will be cloned into the HIV-1 derived SIN vectors, which have a deletion in the U3 region of the 3' long terminal repeat (LTR) of the DNA used to produce the vector RNA. During reverse transcription this deletion is transferred to the 5' LTR and abolishes the transcriptional activity of the LTR and as a result the production of full-length vector RNA in transduced cells is abolished (b) An HIV-1 derived retroviral sequence encoding viral gag/pol tat and rev genes, from which the virulence genes vif, vpr, vpu and nef have been deleted and (c) a plasmid encoding the envelope plasmid derived from vesicular stomatitis virus (VSV) G protein. This is a well defined lentivirus system that is in use under our GM centre No 678.

**Origin & function**
The genes targeted are involved in aspects of cell signalling, activation and proliferation and are not expected to be toxic. None of the proteins are known oncogenes and are therefore highly unlikely to foster tumour progression.

**Evaluation of foreseeable effects**

Mammalian primary cells or cell lines are unable to survive or propagate outside of laboratory culture.

The genes targeted are involved in aspects of cell signalling, activation and proliferation and are not expected to be toxic. None of the proteins are known oncogenes and are therefore highly unlikely to foster tumour progression.

The vectors used will be self inactivating (SIN). A deletion inactivates transcription from the proviral LTR, so reducing the potential for transcriptional activation of cellular genes and also prevents mobilization of any RCL. However as the virus integrates into the host cell DNA there is still a potential for insertional mutagenesis to occur.

A plasmid encoding the envelope protein from vesicular stomatitis virus envelope protein G (VSV-G) increases the host cell range/tissue tropism and makes it permissive for human cells, including quiescent cells. This feature means that there is a risk of infection through contact/aerosol transmission in addition to the normal HIV percutaneous route. Such particles are also physically more stable.

The genes or other sequences of interest will be delivered by an integrative process leading to potential long-term expression for the life of the target cell. The results of such expression for most of the sequences will not be severe and will be limited to the cells targeted and no further.

VSV-G enables the vector virus to infect a wide variety of mammalian cells. There may also be enhanced environmental stability. However, the viruses are replication disabled, they cannot produce progeny virus and so cannot spread to the wider human population or other animals. The possibility of RCL being generated and released is negligible.

The MISSION TRC lentiviral particles are replication incompetent.

None of the modifications will alter the broad properties of the vectors. They will remain replication disabled and so are biologically contained.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| All disposable tissue culture plastics will be autoclaved and incinerated. Culture medium will be treated using vernagel and disposed of via Containment Level II disposal route. Benches are cleaned with 10% trigene and 70% ethanol. |

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
This project has been reviewed by the Institutes Biological & Genetic Modification Safety Committee. The Committee is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.

Project Containment

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Project Ref 678/12.2

Date Ackn'd: 21/02/2012

CU2 Project Title: Viral delivery of modifiers of autophagy and neurodegenerative diseases

Class: Class 2
Culture Vol: < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Withdrawn

Tick if notifying a connected programme of work

Project Additional Information

Purposes of the contained use

Intracellular protein misfolding/aggregation are features of many late-onset neurodegenerative diseases, called proteinopathies. These include Alzheimer's disease,
Parkinson's disease, tauopathies, and ten known neurodegenerative diseases caused by (CAG) in trinucleotide tract expansions that encode abnormally long polyglutamine (polyQ) tracts. These polyQ diseases include Huntington's disease (HD), Huntington's disease-like 2, spinocerebellar ataxias (SCA) types 1,2,3,6,7 and 17, spinobulbar muscular atrophy (SBMA) and dentatorubral-pallidoluysian atrophy (DRPLA). Currently, there are no effective strategies that slow/prevent the neurodegeneration resulting from these proteinopathies. Huntington's disease (HD) is a devastating autosomal dominant neurodegenerative condition that manifests with abnormal movements, psychiatric symptoms and dementia. The HD gene encodes a large protein called huntingtin that contains more than 3000 residues. Exon 1 of the wild-type gene contains the stretch of uninterrupted CAG trinucleotide repeat, which is translated into a polyglutamine (polyQ) tract. Asymptomatic individuals have 35 or fewer CAG repeats and HD is caused by 36 or more repeats. CAG repeat number inversely correlates with age-of-onset of symptoms; the greater the number of CAG repeats, the earlier the age-of-onset. Genetic and transgenic experiments argue that HD and related polyQ diseases are caused predominantly by toxic gain-of-function mechanisms. One of the pathological hallmarks shared by all the known polyQ diseases is the presence of aggregates (also known as inclusions) comprising the mutant protein in affected neurons. The toxicity of polyglutamine-expanded proteins may be attenuated if one can enhance their clearance. We have shown that one way of doing this, at least for mutant huntingtin and ataxin 3 is to enhance macroautophagy, which we will call autophagy. Autophagy is a bulk degradation process that mediates the clearance of long-lived cytoplasmic proteins and organelles. Autophagosomes are formed by double-membraned structures, which engulf portions of cytoplasm. Autophagosomes ultimately fuse with lysosomes, where their contents are degraded. If one blocks autophagy, then one slows the clearance of intracytoplasmic aggregate-prone proteins that cause many neurodegenerative diseases, including Huntington's disease, SCA3, point mutations in alpha-synuclein (causing forms of Parkinson's disease), and wild-type and mutant forms of tau (causing various dementias). This increases aggregation and toxicity. Conversely, our data suggest that autophagy upregulation may be a rational therapeutic strategy for such diseases, as drugs that upregulate this pathway via both mTOR-dependent and independent pathways enhance mutant huntingtin clearance in an autophagy-dependent fashion in cell-culture and in vivo, leading to attenuated disease phenotype in animal models of HD and SCA3.

Our laboratory aims to understand the biology of Huntington's disease and related conditions. To this end we need to be able to study potential modifier genes for this disease and related diseases by knockdown and overexpression. Ideally, we need to do some studies in primary neurons, where viral delivery is preferable to standard transfection. We also study the biology of autophagy both at basic level (where we are seeking to understand the machinery and signalling processes), as well as at the applied level where we study possible drug targets with therapeutic potential. Again, viral delivery of constructs or RNAi is a powerful to enable more effective transduction or efficient delivery into primary neurons.

Recipient or parental organism

Recipients will be various mammalian cells in culture and primary murine cells. Most are well characterised and considered ACDP HG1.

Host/vector system

We propose using a system with lentiviral vectors based on HIV-1. We will use a three plasmid transient transfection system to generate recombinant, replication defective retroviruses in 293T cells. In this system virus replicative, packaging and envelope vectors and the transgene vector are split over three plasmids, requiring multiple recombination events to generate replication competent retrovirus (RCR). Any RCR generated would lose the transgene during recombination events. Many accessory functions/pathogenicity determinants such as vif have been deleted. Vectors insert into the host cell DNA after infection leading to retention of the transgene for the life of the cell. These viruses may transduce non-dividing e.g. (neurons) as well as dividing cells. The transfected vectors are as follows: (i) An HIV-1 ‘self inactivating vector’ (SIN). CDNAs encoding the genes will be cloned into these vectors which have a deletion in the U3 region of the 3' long terminal repeat (LTR) of the DNA used to produce the vector RNA. During reverse transcription this deletion is transferred to the 5' LTR and abolishes the transcriptional activity of the LTR, and as a result the production of full-length vector RNA in transduced cells is abolished. (ii) An HIV-1 derived retroviral sequence encoding viral gag/pol tat and rev genes, from which virulence genes vif, vpr, vpu and nef have been deleted (iii) A plasmid encoding the envelope plasmid derived from vedoric stomatitis virus (VSV-G) protein which is permissive for human cells. This is a well defined lentivirus system that is in use under our GM centre No 678. In addition to this standard protocol replication deficient lentiviral particles may be obtained from commercial sources, such as Dharmacon SMART vector 2.0 Lentiviral shRNA particles or Sigma MISSION shRNA. Or commercially available lentiviral production systems such as Block-IT shRNA vector systems (Invitrogen) may be used . The latter is also based on a multi-plasmid strategy whereby a pLenti based expression vector containing the DNA sequence of interest is transfected along with 3 packaging plasmids. These plasmids supply the helper functions as well as structural and replication proteins in trans required to produce the lentivirus, also using a (VSV-G) as a pseudotyping envelope. As some commercially available vectors contain the WPRE (woodchuck hepatitis B virus post-transcriptional regulatory element) to boost viral titres and gene expression and as this element can express and oncogene, which has resulted in increased tumour frequencies in mice these viruses should be considered class 2. During production and concentration of retrovirus the transduction of cells and the subsequent analysis of these cells level 2 containment will be strictly adhered to. This will involve standard measures to decrease percutaneous as well as aerosolised transmission of the virus i.e. by avoiding sharps use and working in a class II MSC.
Origin & function

a) cDNA or shRNA for genes encoding potential modifiers of Huntington's disease and related conditions, including forms of Parkinson's disease and other dementias and mutant forms of these gene b) cDNA or shRNA for genes encoding modifiers of autophagy c) markers and reporters derived from fluorescent proteins.

For a) the wild-type function of genes involved in Huntington's disease and related conditions is not fully understood however it is thought that the disease-causing genes posses a toxic-gain of function. For b) either an increase or decrease in cellular autophagy would be expected. For c) the markers and reporters would not be expected to have any cellular effect.

Evaluation of foreseeable effects

The expression of genes involved in Huntington's disease and other related conditions may be cytotoxic in neuronal cells as these genes cause late onset neurodegenerative diseases, however the likelihood of exposure of this tissue type to the viral vectors is extremely low. The action of the expressed proteins or shRNAs which modulates autophagy is unlikely to be cytotoxic and the fluorescent reporter constructs which are expected to be biologically inert. None of the genes to be used are known oncogenes and are therefore unlikely to foster tumour progression. The expression of any of these genes, even in the long term, in a minor population of cells at the likely sites of exposure is unlikely to be harmful and expression will not spread beyond exposed tissues. Additional risk comes from insertional mutagenesis following exposure. In the main this risk comes from the possibility that the arising mutation would initiate oncogenesis. Whilst this cannot be ruled out it is unlikely and by handling these constructs at CL2 we aim to reduce this risk to acceptable levels.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Standard bacterial cloning (CL-1) Generation of high titre virus stocks with established cell lines: tissue culture with primary cells and immortalised cell lines, infection of these cells with lentiviral vectors (CL-2). Characterisation of autophagy or fluorescent properties of transfected cells: No work with active viral particles will be performed outside containment level 2. Cultures will be washed with viral free media and either used at least 48h after viral transfection or fixed before being transferred to designated microscopic setups, flow cytometry or bench areas at CL-1. Cells used at containment level 1 or 2 will be autoclaved/incinerated at the end of the procedure. All media exposed to the cells will be treated with Virkon and solidified prior to incineration. All spills will be treated with Virkon. All sharps will be avoided.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture/lab ware will be autoclaved and incinerated. Culture fluids will be treated with 2% Virkon for 16 hours and disposed to drains. Recyclable lab ware will be soaked in 1% Virkon for 16 hours. Bench/cabinet surfaces will be wiped down with 70% ethanol. Spills will be sprinkled with Virkon powder.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project has been reviewed by the institutes Biological Genetic Safety. The Committee is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.
The defining feature of eukaryotes like ourselves, compared with our prokaryotic ancestors, is the presence of membrane-bound subcellular compartments (the endomembrane system), each of which has a distinct protein composition. This means that our cells need to direct >500 different membrane proteins to the correct location, and then either to keep them there or ensure they move about in an orderly fashion via vesicle-mediated intracellular trafficking. There are many examples of genetic disorders that affect trafficking, either because of mutations in cargo proteins or because of mutations in the trafficking machinery itself. The current project is focused on identifying and characterising gene products underlying the formation, function and maintenance of the endomembrane system and understanding the pathophysiological consequences of defects in this system. In particular, we are planning to investigate adaptations of this system supporting tissue-specific functions including, but not limited to, neurotransmitter release, glycolipid metabolism and melanosome biogenesis. This requires efficient manipulation of...
Specifically we intend to alter the expression and/or function of candidate genes and gene products in various tissues and cell lines using Knock-down and/or overexpression of candidate genes, including modified versions, for example harbouring specific mutations and/or detectable epitopes/domains. The function of the endomembrane system will be monitored through expression of reporter constructs bearing detectable biologic tags. To introduce these synthetic constructs to many culture adapted cell lines lipofection based approaches will be sufficient, additionally all constructs will be tested in this manner prior to viral vector construction. Where it is necessary to introduce synthetic constructs into either hard to transfect cell lines or isolated primary cells/tissues in culture adenoviral and/or lentiviral approaches will be used as appropriate.

Recipient or parental organism

a) E. coli: Strains of bacteria used for initial construction and plasmid amplification are derived from E. coli K12 and are therefore disabled and non-colonising.
b) Mammalian cells: Recipients will be various mammalian cells in culture. Most are moderately well characterised (eg HEK293 cells, HeLa cells) and considered fastidious and non harmful, cannot colonise/cause disease (ACDP hazard group 1).
c) Primary cultures established from mouse tissue explants are fastidious and non-harmful (ACDP hazard group 1).
d) Primary tissues and cells isolated from humans are themselves no more hazardous but are unscreened and therefore may harbour infectious agents, therefore all work using human primary cells/tissues will be undertaken at containment level 2

Host/vector system

Replication disabled adenovirus vector, based on Ad5. This virus is deleted for essential replication functions (E1A region) and can only replicate in cells complementing this function (HEK293 cell line). Naturally the virus is transmitted by aerosol to respiratory tissues from where it may infect adenoid tissue. There are reports of other infections such as enteric/kidney. However as the vector is incapable of completing a single round of replication, no progeny virus can be produced and infection would be (self) limited to any initial cells exposed. The numbers of cells affected would be related to the amount of virus encountered but effects would not progress to any extent approaching natural disease. The vector is considered GM class 1.

Replication disabled lentivirus. Although the starting viruses like HIV themselves are hazard group 3 pathogens, vectors considered for this proposal are all second or third generation (distinguished in the UK by deletion of the tat gene and replacement of the tat-responsive promoter in the 5'LTR by heterologous alternatives). The theoretical potential for re-emergence of replication competent virus through recombination has been significantly reduced in these vectors, by splitting the replicative, packaging and envelope functions and the desired transgene over ≥ three independent plasmids, therefore requiring multiple recombination events to generate replication competent virus. The emergence of replication competent virus is therefore unlikely and such viruses would be expected to have lost the transgene. In addition, many accessory functions/pathogenicity determinants such as vif have been deleted and they are self-inactivating, carrying the SIN-deletion in the 3'LTR, inhibiting re-activation after integration into the host genome and, as the U3 promoter is thereby compromised reducing the risk of transactivation of genes near the integration site. But, as the constructs all carry other (usually constitutively active (eg CMV)) promoters to drive expression of the transgene this can not be excluded, nor can the inactivation nearby genes due to integration within these genes be excluded. However, as problems arising from the insertion site have only been reported in studies involving administration of very high viral titers, the likelihood of similar problems in this study is considered low, taking into account the comparitively low viral titers likely to be administered accidentally and the defective replication and self-inactivation of the virus. As some of the commercially available vectors contain the WPRE (woodchuck hepatitis B virus post-transcriptional regulatory element) to boost viral titres and gene expression, and as this element can express an oncogene (part of the X-protein), which has resulted in increased tumour frequencies in mice, these viruses should be considered class 2. The lentiviral particles in this project will be 'pseudotyped' with vesicular stomatitis virus envelope protein G(VSVG); this increases host cell range/tropism and such vectors may represent an aerosol means of transmission in addition to the expected percutaneous risk from lentiviruses. Such viruses are more stable and can be purified to high titre. However, they are rendered sensitive to complement.

Origin & function

The inserted genetic material will encode cDNA and/or ShRNA of/for genes involved in the formation, function and maintenance of the endomembrane system. Marker/Reporter constructs bearing detectable tags (including but not limited to fluorescent proteins such as GFP and short peptide tags such as C-myc). Specific gene families targeted include, but are not limited to, those responsible for forming vesicle coats, including adaptor proteins (i.e. AP complexes 1-5) and structural coat proteins.
such as clathrin as well as gene families underlying enzymatic activity with the lumen of the endomembrane system such as galactocerebrosidase (GALC). Additionally we will target newly identified gene products performing accessory functions to endomembrane system formation and specific vesicular protein cargoes. Inserted genetic material will specifically target the function of the endomembrane system. These manipulations are not expected to alter oncogene expression or transform the target cells. Additionally, inserted genetic material and products thereof will have restricted localisation to expressing cells. The risk to human health from accidental exposure is therefore low.

**Evaluation of foreseeable effects**

The majority of gene products will have actions within the cytoplasm of the cell and as such are unlikely to be secreted or have actions beyond the expression cell. Where secreted gene products are introduced to act as markers for secretory function they will carry biologically inert, but detectable, epitope tags and as such are again unlikely to have actions beyond the expressing cell. As overexpression or knock-down of such genes is unlikely to transform infected cells and is not expected to be toxic in context of the transfected cells nor after release of proteins from lysed cells the risk to the environment due to accidental infections is considered low. Cells will remain especially disabled and unable to survive outside of laboratory culture.

1) Adenovirus: Adenoviruses are generally species specific; Ad5 infects humans and does not naturally infect other animals (but can experimentally infect cotton tail rats, which are not endogenous in the UK). Therefore there is no risk to other organisms. The virus is replication incompetent therefore could not spread to the wider human population from any infected individual.

2) Lentivirus; VSVG enables these vectors to infect a wide variety of animal cell types including those of different mammalian species. There may be enhanced environmental stability. However the viruses are replication disabled; they cannot produce progeny virus and so cannot spread to the wider human population or other animals.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

none applied for

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All disposable tissue culture plastics will be autoclaved and incinerated. Culture medium will be treated with 2% Virkon for 16h and solidified prior to incineration. Liquid spills will be treated with 2% Virkon (non metallic surfaces) or 10% Trigene (metallic surfaces), absorbed with paper towels, which will then be autoclaved /incinerated. Benches are cleaned with 10% Trigene

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

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This project has been reviewed by the Institute’s Biological & Genetic Modification Safety Committee. The Committee is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.

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02/03/2022
Mechanisms of protein degradation - using (i) overexpression or depletion of tagged or untagged genes involved in protein breakdown in lentiviral and retroviral systems

Purposes of the contained use

All mammalian cells have to control their protein content to remove damaged proteins and regulate cell growth. Any disruption in cellular protein levels can cause uncontrolled cell turnover or the accumulation of misfolded proteins that occur in neurodegenerative conditions. The major mechanisms for controlling intracellular protein levels are through ubiquitination, proteasomal degradation and autophagy. This project aims to address how ubiquitinated proteins are differentially selected and efficiently delivered to the proteasome for degradation. The research encompasses several main themes:
1) How different polyubiquitin chains are decoded.
Specificity in the ubiquitin system is generated by the ability of ubiquitin to form eight different polyubiquitin chain linkages. Each type of ubiquitin linkage must be correctly interpreted to facilitate the desired outcome, and ubiquitin binding proteins (UBPs) provide this critical link between chain recognition and cellular fate. For example, we showed chain-specific UBPs facilitate lysine-48 chains to bind the proteasome but block lysine 63-chains from binding this degradative complex. UBPs for other polyubiquitin chain types were unknown, but we have identified UBPs that selectively recognize lysine-11 polyubiquitin chains, an abundant linkage with a critical role in regulating mitosis. Using biochemical and cell biology approaches we aim to investigate the function and physiological importance of these lysine-11 specific UBPs.

2) How ubiquitin binding proteins regulate protein turnover.
We will use forward genetic screens in near-haploid human cells (KBM7) to identify genes required to regulate the degradation of proteins by the proteasome. By the insertion of random mutations into cells expressing fluorescent proteasome reporters, we can identify genes required for the efficient degradation of these ubiquitinated substrates. Retroviral gene traps and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology will be used in the screens. As ubiquitination is also involved in non-proteasomal degradation, we will examine how ubiquitinated proteins are recruited to autophagy and lysosomal pathways.

3) Regulation of the hypoxia response pathway.
We will use forward genetic screens and proteomics to determine novel genes in the regulation of hypoxia inducible transcription factors (HIFs, a pathway critically regulated by ubiquitination). Genes identified as regulating HIFs will be characterized in cell line based assays and primary cells (macrophages and fibroblasts) for their importance in the hypoxia response. Other oxygen-dependent regulatory mechanisms, for example oxidative phosphorylation and protein hydroxylation, will also be studied.

To investigate these aims we will use lentiviruses and retroviruses to:

a) Generate stable mammalian cell lines encoding UBPs, ubiquitin enzymes, genes regulating protein breakdown or oxygen consumption of cells using pHRSIN vectors.

b) Use shRNA with pSIREN vectors to confirm the function of genes identified in the mutagenesis screens.

c) Generate stable knock-out or knock-in (tagged) cell lines of genes identified in the genetic screens using CRISPR technology in lentiviruses encoding the Cas9 nuclease and guide RNAs (sgRNA) (pLKO-TRC).

d) Use stable and tetracyclin-inducible shRNA and sgRNAs.

These stable cell lines will be utilised to further characterise the cellular effects of genes required in the regulation of the ubiquitin proteasome system.
Viral gene delivery vectors including retroviruses, lentiviruses, and adenoviruses.
The lentivirus and retrovirus plasmids contain the WPRE-X
2nd generation retrovirus and lentivirus packaging systems will be used.

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
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<tbody>
<tr>
<td>Plasmids and other nucleic acid vectors:</td>
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<td>Standard cloning vectors (such as TOPO and pcDNA3) and BAC shuttle vectors will be used to clone gene disruptions (such as truncations) and to add inserts/tags onto genes or to rescue deletions. In addition, these cloning vectors will be used to express individual genes to allow their study in host cells.</td>
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<tr>
<td>Inserts:</td>
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<tr>
<td>Inserts will be used to (i) label genes to enable their visualisation during physiological and biological studies of protein degradation (such as the YFP, GFP or RFP fluorescent tags), (ii) encode proteins involved in the ubiquitin proteasome system, autophagy or the hypoxia response, with no known toxicity or oncogenicity, or (iii) to simplify biochemical analyses of genes of interest (such as HA, Strep, Myc and Flag tags). To examine the biological role of individual genes, gene deletions/disruptions and subsequent reconstitutions in the ubiquitin proteasome pathway, autophagy, lysosomal function and the hypoxia response pathway.</td>
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<td>The inserts will be commonly used markers/tags as detailed above. Apart from fluorescence, these tags are biologically inert; their expression should not increase activity of the target genes.</td>
</tr>
<tr>
<td>No modifications will be made that are designed to introduce new biological activities into the proteins. Over-expression of genes involved in the ubiquitin proteasome system, autophagy and hypoxia response should not pose any additional risks.</td>
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<tr>
<td>Uncharacterised genes will only be introduced into viruses when they have been better characterised using standard mammalian expression vectors and shown to function in the recognition of ubiquitin chains, protein degradation, autophagy or hypoxia regulation, and therefore unlikely to be either toxic or oncogenic.</td>
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<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
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</table>
All liquid waste will be disinfected with Virkon overnight, gelled using Vernagel, double bagged and sealed and labelled for autoclave then incineration according to the Department CL2 procedures. All solid waste will be double bagged and labelled for autoclave then incineration (pipettes will be disinfected in 1% Distel before removal from MSCs to prevent drips). Work surfaces will be wiped down with Distel and 70% ethanol. Small spillages will be mopped up immediately with tissue and disinfected with 1% Distel or 2% virkon. Large spills will be covered with absorbent paper and then sprayed with 10% Distel or 2% virkon, and then mopped up after 30 minutes. The absorbent material will then be disposed of in the autoclave waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

None

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
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<td>L3 L4 L2 L2</td>
<td>L3 L4 L2 Yes L3 L4</td>
</tr>
<tr>
<td>Animal Units L2 L3 L4</td>
<td>Large Scale Activities L2 L3 L4</td>
<td>Human Clinical Applications L2 L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 678/16.1

Date Ackn'd 10/02/2016

Date Project Ceased

Modulation of innate immunity by viral infections

Class 2

CultureVolClass2 1-50 Litres

Non-GMM

Consent Granted

Project notified under transitional arrangements N
Human herpesviruses are a family of enveloped double-stranded DNA viruses. There are three subfamilies: alphaherpesvirinae [herpes simplex viruses 1 and 2 (HSV-1, 2); varicella zoster virus (VZV)], betaherpesvirinae [human cytomegalovirus (HCMV), human herpesvirus-6 (HHV-6) and human herpesvirus-7 (HHV-7)] and gammaherpesvirinae [Epstein-Barr virus (EBV) and Kaposi's sarcoma herpesvirus (KSHV)]. The herpesviruses are ubiquitous pathogens of all vertebrates, although infections are usually highly species specific. All herpesviruses apart from KSHV and HSV-2 infect the majority of the human population (seroprevalence of HSV-1, VZV, HCMV, HHV-6 and -7, EBV is 50-100% depending on the population studied, whereas HSV-2 approximately 20%, KSHV 1-50% depending on the population studied). One common feature of all herpesvirus infections is lifelong viral persistence after primary infection despite a strong cell-mediated immune response. Each virus can generate a latent infection in certain cell types. In immunocompromised individuals, such as recipients of organ transplants and patients with advanced HIV infection, reactivation of herpesviruses such as VZV, HCMV, EBV and KSHV, has the potential to cause severe disease.

Human immunodeficiency virus (HIV) is the lentivirus that causes AIDS. This condition is characterised by progressive failure of the immune system, which allows opportunistic infections and cancers to thrive. HIV-1 establishes chronic infections, affecting approximately 33 million people worldwide. Despite considerable advances in HIV science, the goal of eradicating HIV-1 remains elusive.

Human herpes- and lenti-viruses encode multiple factors that modulate innate and adaptive immune defences, however viral subversion of interferon (IFN)-mediated innate immunity is at best partly understood. One survival strategy exemplified by HIV has included the destruction or modification of host antiviral restriction factors, for example Tetherin, APOBEC proteins and the SERINCs. Existing data suggests that there may nevertheless be a number of unidentified factors that act against HIV. The evasion of restriction factors by herpesviruses remains relatively poorly characterised, although PML nuclear body proteins including Sp100 and Daxx have activity against several viruses.

This project aims to characterise novel mechanisms of viral subversion of the IFN response, focusing on new restriction factors that act against herpesviruses and HIV. Therapeutic inhibition of the interaction between these antiviral factors and their viral antagonists could lead to novel antiviral strategies in infected individuals. We have recently conducted proteomic screens of herpesviral infections that have identified candidate novel antiviral restriction factors.

We aim to investigate both (a) the role of individual viral genes on host antiviral proteins (herpesviruses and lentiviruses), as well as (b) whole viral infection in both lytic and latent phases (herpesviruses only). (a) We will clone individual viral genes into delivery vectors, and deliver them to relevant cultured host cells. We will then characterise the effects of these modifications on the host cell. (b) We will generate herpes viral stocks in established human cell
lines from viral seed stocks or bacterial artificial chromosomes encoding the viral genome. Clinical isolates and their genetically modified derivatives will be used as well as laboratory adapted strains. In vitro infection of human cell lines and primary cell lines will then be carried out to model both lytic and latent viral infections. We will then characterise the effects of these viral modifications on the host cell. Viruses with deletions of, or tags attached to individual genes will be utilised in order to further characterise cellular effects.

Recipient or parental organism

Mammalian tissue culture cell lines (eg HFF2, HFF-TERT, THP-1, KG-1, HeLa, U373, U937, Huh?, HCT116, Jurkat, Daudi, NK-92, NK-L, HL60, 220, T1, 293, 293T, Ramos).
Primary human cells (eg monocytes, dendritic cells, red blood cells, skin fibroblasts, endothelial cells, T cells, B cells, NK cells).
Human herpesviruses including HSV-1 (eg strains: F, KOS, 17, SC16), HSV-2 (eg strains: 333, HVD), VZV, EBV, HCMV (eg strains: Toledo, Merlin, TB40/E, VR1814/FIX-BAC and laboratory adapted strains AD169 and Towne), HHV-6, HHV-7 and KSHV.
An HIV-1 viral vector with a critical env deletion will also be used.
Bacteria: Top10, Sure 2, alpha-select, clean genome, DH5a, ccdB SurvivalT1R Chemically Competent E. coli

Host/vector system

Viral /Cell Vectors
Viral gene delivery vectors including retroviruses, lentiviruses, and adenoviruses.
Plasmids and other nucleic acid vectors
Bacterial artificial chromosomes (BACs) that cover the genomes of the herpesviruses and adenovirus serotype 5 so that insertion/deletions in the viral genome can be generated in vitro prior to the generation of recombinant viruses.
Standard cloning vectors (such as TOPO, pcDNA3, Gateway pDONR221, pDONR223, and Gateway pDEST derivative destination vectors) and BAC shuttle vectors will be used to clone gene disruptions (such as truncations) and to add inserts/tags onto genes or to rescue deletions. In addition, these cloning vectors will be used to express individual genes to allow their study in host cells.
The HIV-1 viral vector will be used for targeted gene disruption in vitro prior to the generation of lentiviral particles.
Inserts
Inserts will be used to (i) label genes to enable their visualisation during physiological and biological studies of intracellular infection (such as the YFP, GFP or RFP fluorescent tags) and protein function, (ii) encode mammalian proteins that have already been characterised and are thought to be involved in restriction of viral infection with no known toxicity or oncogenicity (for example Sp100 and Daxx in addition to other characterised proteins that may function as restriction factors), or (iii) to simplify biochemical analyses of genes of interest (such as HA, Strep, Myc and Flag tags). To examine the biological role of individual genes, gene deletions/disruptions and subsequent reconstitutions will also be used.

Origin & function

Expected biological action of inserted DNNRNA or transcribed/translated gene product:
The inserts will be commonly used markers/tags as detailed above. Apart from fluorescence, these tags are biologically inert; their expression should not increase viral virulence or fitness, or activity of the target genes. Deletion or disruption of viral genes is not expected to increase virulence. For reconstitution of deletion viruses, only the original gene will be re-inserted and therefore no increase in virulence over the original strain is expected.
modifications will be made that are designed to introduce new biological activities into proteins. Over-expression of viral genes in viral gene delivery vectors should not pose any risk additional to use of the viruses themselves. Overexpression of already-characterised human genes such as Sp1 OO/Daxx should not pose any additional risks.

Technique used to introduce insert or vector into host:
Standard molecular biology cloning techniques will be used to delete/disrupt and add tags to viral genes within BACs or viral vectors (detailed above). Vectors will then be introduced into mammalian cell lines by transfection for generation of recombinant virus. The resultant recombinant virus will then be harvested and used directly for infection of cells, or will be concentrated and stored at -80°C for subsequent infection of host cells. The infection protocol varies depending on the virus. For example, VZV remains cell associated and therefore must be introduced by cocultivation of infected cells with the target cell. We will use a centrifugal enhancement of viral infectivity to introduce lentiviruses, while HCMV will be allowed to bind to cells on a rocking platform in concentrated form before being transferred to 37°C which will allow virus entry.

Evaluation of foreseeable effects

(i) the host! recipient micro-organism including viral/cell vectors
Health and Safety
Executive

Recipients will be various mammalian cells in culture. Most are well characterised and considered ACDP hazard group 1. Cells from volunteers are uncharacterised and although themselves present no greater risk, may contain adventitious infectious agents and therefore will be handled at CL2 (risk assessment under COSHH regs).

The herpesviruses to be studied as detailed above are all ACDP hazard group 2 and will therefore be handled at CL2 within a class II microbiological safety cabinet (MSC). Pregnant or known immunocompromised individuals will not perform any of this work.

Viral gene delivery vectors (retroviruses, lentiviruses) to be used are those typically considered class 1 GMMs, and will all be self-inactivating, and therefore once packaged, are unable to propagate. A three- or four-plasmid transient transfection system will be used to generate the recombinant retroviruses and lentiviruses, thereby decreasing the likelihood of recombination within the packaging cell lines to generate replication competent virus. The retroviral vectors are based on the Moloney murine leukaemia virus in which the gag, pol and env genes have been deleted. Gag and pol are supplied in trans on a packaging plasmid. We will generate VSV-G pseudotyped virus in order to deliver the virus particles to human cells. The lentiviral vectors are either based on an HIV-1 self-inactivating vector (SIN) containing a deletion in the U3 region of the 3` LTR of the virus, or based on a Gateway pDEST vector. In addition, the gag, pol, env, nef, vif, vpu and vpr genes have been deleted. Again, additional packaging plasmids encoding gag, pol, rev and tat will be used along with the plasmid encoding VSV-G to make pseudotyped viruses that will enter human cells. A packaging plasmid encoding nef, vif, vpu and vpr may also be used, in order to improve transduction efficiency of primary human cells. While VSV-G increases host range and their tropism and mode of transmission may also be altered from that exhibited by wild-type retroviruses, aerosol risk to the airway epithelium appears limited by the inability to infect the apical cell surface.

The AdZ adenoviral vector system will be used to generate recombinant adenoviruses. These vectors carry a deletion of the E1 gene region rendering them replication incompetent in non-complementing cell lines. In addition, the E3 region (important for viral pathogenesis and immune evasion) has also been deleted making them less pathogenic in humans. They are those typically classed as class 1 GMMs and remain so as the inserts are placed within the deleted regions. All three types of viral vectors will be harvested and used in a CL2 laboratory.

The HIV-1 viral vector to be used in this project is derived from the HIV-1 backbone. To increase the safety of the system, the vector components are segregated on to 2 plasmids: an HIV-1 molecular clone with a critical env deletion and a second plasmid expressing the VSV-G envelope protein. None of the individual plasmids is capable of generating infectious viral particles and, to avoid the generation of replication competent virus via homologous
recombination, the constructs do not contain overlapping homologous sequences. Like the retroviral and lentiviral vector systems described, the use of this system has previously been approved when working under CL2 conditions. Since none of the cell lines or vectors carry intact env-encoding sequences, infectious HIV particles cannot be produced.

(ii) the inserted genetic material
Modifications will be to add a tag such as a fluorescent protein to viral proteins, or to delete or disrupt human or viral genes and subsequently restore them. Expression of tags is not expected to be detrimental to human health. Deletions/disruptions to viral genes are not expected to increase infectivity/pathogenicity. Herpesvirus genes cloned into viral gene delivery vectors may confer an ability to alter immune responses to infected cells. However, the large number of viral immune evasion genes encoded by the human herpesviruses demonstrates the need for a virus to encode more than one immunoevasin in order to effectively evade human immune responses. We will only express a single herpesviral gene in each gene delivery vector.

(iii) nucleic acid vectors
Bacterial artificial chromosomes and plasmids will be used to generate recombinant virus. These contain antibiotic resistance markers which could in theory present a problem to the user if they became infected with the bacteria in which the plasmids/BACs were grown. However, in practice the bacterial strains we use are highly attenuated and unlikely to cause disease, while the antibiotic resistance markers encoded by these plasmids/BACs (Ampicillin, kanamycin, streptomycin and chloramphenicol) are active against antibiotics which are no longer in general use in clinical practice. So whilst the plasmids/BACs pose no threat in a bacterial culture, once transfected into eukaryotic cells the recombinant DNA can be packaged into infectious virions and transfection will therefore be carried out in a CL2 laboratory and subsequent cultures treated as live virus.

(iv) the resulting genetically modified micro-organism
The disabled nature of the bacterial hosts will not be altered by the plasmids or BACs in use - no expression is anticipated and would not in any case be compatible with bacterial systems. Cultured cells expressing the viral genes may be altered in particular surface or intracellular proteins they express but these changes will not over ride the especially disabled nature of the cells. Cultured cells expressing the genes functioning in viral restriction may additionally alter intracellular pathways (for example, Sp100 and Daxx have additional roles in regulation of immunity and apoptosis but these changes will not over ride the especially disabled nature of the cells. Viral vectors containing the viral genes may be able to alter the surface expression of cells they infect thereby altering the immune response. As the vectors are disabled, any accidental infection of the epithelial cells of the researcher would be localised. Transduced cells would not be able to produce viral particles. Due to the high turnover rate of epithelial cells, any localised infection would not be expected 10 be long lasting. Pseudotyped env-deleted HIV-1 viral particles are replication deficient, but theoretically able to transduce epithelial cells of the researcher. If this occurred, all HIV-1 proteins except env would be expressed, but no replication competent virus would be formed. This protein expression would not be expected to cause harm, and the pseudotyped particles have previously been classified as class 2. The modified herpesviruses should be no more pathogenic than wild type. Although there are virus deletion mutants with increased virulence over their parent (for example deletion of A41L in vaccinia virus), to our knowledge, herpesvirus deletion mutants that show an increase in virulence have not been described.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Brief description of types of laboratory procedures:

Conventional molecular biology techniques will be used for cloning in a CL 1 laboratory. Bacterial artificial chromosomes and viral gene delivery vectors will be transfected into mammalian cell lines in a CL 1 laboratory then transferred to the CL2 laboratory within 24 hours of transfection (to allow us to evaluate transfection efficiency with a fluorescence microscope). Generation of virus stocks from seed stocks and BACs involving standard tissue culture with established cell lines. Ultracentrifugation of virus. Tissue culture and subsequent infection of established cell lines and primary human cells with virus stocks by co-culture. All manipulation of liquid will be carried out in a class II Microbiological safety cabinet (MSC) to reduce the risk of aerosol dissemination. Tubes for ultracentrifugation of virus for the production of concentrated virus stocks will be loaded in MSC into buckets that will be sealed and transported to the ultracentrifuge in secondary containers. Similarly, once the ultracentrifugation is complete, the tubes will be transported back to the CL2 laboratory and the buckets opened in the MSC to allow the virus to be resuspended and aliquoted. Since there is no -80’ storage facility in our CL2 laboratory, small aliquots of virus will be transported to 6.2 in secondary containment for storage. Fluorometric and microscopic analysis will be either be conducted in the CL2, or on fixed samples, and protein and nucleic acid analysis will be conducted on material which has been lysed or fixed in the CL2 facility prior to transport to the laboratory.

For proteomics analysis, the cells will be lysed in relatively harsh conditions and subsequently centrifuged, as detailed and justified below.

All primary cell lines are screened (e.g. commercially available cell lines from Lonza screened for HIV, HBV, HCV and mycoplasma), and are immortalised and passaged (e.g. human skin fibroblasts). Regardless of the screening results, the blood-derived primary cell lines will permanently remain in the CL2 laboratory.

Health and Safety

Executive

Additional control measures (i.e. safety procedures and devices) required for specific activities: Standard CL2 procedures including wearing double gloves, lab coats and closed shoes will be adhered to. Waste liquid will be decontaminated within the MSC with virkon prior to solidification with Vernagel and autoclaving. All other non-liquid contaminated waste material will be autoclaved. Aerosol resistant pipets and pipet tips will be used to pipet virus. Any spillage can be adequately decontaminated with trigene detergent. These procedures are described in greater detail below.

If other facilities are to be used, what are the risks to others and how will they be conveyed?

The ultracentrifuges available for concentrating virus preparations are outside of CL2 labs. Virus preparations will be sealed within the centrifuge tubes and buckets within the MSC in CL2 and transported in a shatterproof container. The surfaces of centrifuge tubes and transport containers will be decontaminated with detergent prior to removal from CL2 and only be re-opened once returned to the MSC in CL2. The ‘comments’ section of the ultracentrifuge logbook will be used to warn other users that the centrifuge contains a CL2 organism. Following centrifugation, the buckets will be returned to the MSC in the CL2 for processing. The buckets will then be immersed in detergent in the MSC prior to removal back to 5.14 for cleaning, rinsing and drying.

The -80 degree freezer for storing virus preparations and supernatant is also outside of the CL2 labs. Vessels containing supernatant and concentrated virus preparations will be decontaminated with Trigene and put into a shatterproof container for transport to and storage at -80 in Lab 6.2.

Justification for this:

1. Following transduction of cell lines all material containing viral particles is removed and disposed of as described below. At this point it is no longer classified as containment 2 and is moved to containment level 1.

2. For proteomics analysis, cells will be lysed as described below. Post lysis, cells and any associated virus are no
longer biologically hazardous. The remaining downstream processing requires equipment and chemicals unsuitable for our CL2 facility, e.g. specialised centrifuge-ware, chemicals that must be weighed inside a fume hood, and other items that cannot be transferred to CL2.

3. For proteomics experiments involving biotinylation, cells will be briefly cultured in the cold room. The protocol requires incubation at 4 degrees with either gentle rocking or end-over-end rotation. This is currently not possible within CL II facilities. The cells will be transported in sealed contained and waste/surfaces will be cleaned as described below. There will be no manipulation of CL II material within the CL I labs.

Protocol for Lysis

Cells will be washed three times in PBS (by centrifugation and re-suspension in the case of suspension cells and by aspiration in the case of adherent cells). Adherent cells will be recovered by scraping and then pelleted after washing, or directly lysed on the plate after washing.

Cell lysis:

a. Cell pellets re-suspended in 8M Urea/TRIS 1.25units/uL Benzonase and vortexed until pellet is solubilized and incubated at room temperature for 15 minutes.

OR

b. Cell pellets re-suspended in 2% SDS/TRIS and vortexed until pellet is solubilized

OR

c. Cell pellets re-suspended in 6M Guanidine / HEPES and vortexed until pellet is solubilised.

OR

d. Cell pellets are re-suspended in 1% Triton X-100, TBS, 1.25units/uL Benzonase and protease inhibitors and incubated for 15 minutes at room temperature with occasional shaking. Lysed cells are centrifuged to removed debris and supernatants taken for further processing.

Protocol for Biotinylation

In the case of suspension cells, after pelleting in an Eppendorf tube by centrifugation the pellet is resuspended in biotinylation mixture and the entire Eppendorf sealed into a 50mL Falcon Tube. For adherent cells, the biotinylation mixture is added to the culture dish and the dish placed inside a sealed box.

The outer surface of the 50mL falcon tubes or sealed box will be disinfected with Trigene before removal of from CL II to end-over-end rotator or platform rocker in the cold room. The period of incubation is 30 minutes. The cold room doors will be clearly labelled that CL II material can be found within, likewise on the respective shakers. Aftenward the tubes/box wi ll be returned to CL II microbiological safety cabinet before proceeding with the protocol.

Hazards relating to biotinylation mix and other chemicals are covered in risk assessment MPW001 and require no especial modification for CL II.

Control measures to be used when removing CL2 samples from the Containment 2 suites.

Users should use common sense and take all reasonable precautions to avoid the risk of accidents including spills. Biological or GM material should be sufficiently contained in the event of a foreseeable incident.

All liquid material MUST be in a sealed liquid tight container which must be placed in a water-tight plastic or metal outer container, lined with absorbent material to soak up any leakage of liquids.

In the event of an EMERGENCY material MUST be returned to a Containment level 2 suite and left in the MSC Class 2 hood for at least 30 mins before the container is opened and dealt with appropriately.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

(i) the recipient micro-organism including viral/cell vectors (Give ACDP hazard group or appropriate containment level for handling)

The viruses and viral delivery vectors we will use are ACDP hazard group 2/class 1 GM. The viruses are deactivated by treatment with trigene, virkon or 70% EtOH and autoclaving. Therefore following the waste procedures outlined
below should eliminate risk of spread to the environment. Many of the herpesviruses are also strictly human specific, therefore there is no risk of spread to other animals or plants. The viral vectors are all replication disabled so will be unable to disseminate in the wider animal or human populations.
Mammalian primary cells or cell lines are especially disabled and unable to survive or propagate outside of laboratory culture.
Bacteria: the bacterial strains we use are highly disabled and unable to replicate to any extent outside the lab. Nevertheless, appropriate control measures, including disinfection (with virkon) of liquid cultures and autoclaving of bacterial plates, will be taken.
(iii) the inserted (donated) genetic material
Fluorescent proteins are not toxic, nor reported to have deleterious effects on exposure. Deletion/disruption and subsequent reconstitution of viral genes will not introduce any new translated genetic material into the parental viruses, and will therefore pose no extra risk.
(iv) nucleic acid vectors
BACs and cloning vectors and easily destroyed in the environment and therefore pose no risk on their own.
(iv) the resulting genetically modified micro-organism
None of the inserts or deletions in cells, vectors or viruses will be able to alter the non hazardous status of the recipients with respect to environmental risk.
Waste procedures and inactivation methods for the GMM:
All liquid waste will be disinfected with Virkon overnight, gelled using Vernagel, double bagged and sealed and labelled for autoclave then incineration according to the Department CL2 procedures. All solid waste will be double bagged and labelled for autoclave then incineration (pipettes will be disinfected in 1% trigen before removal from MSCs to prevent drips). Work surfaces will be wiped down with trigen and 70% ethanol. Small spillages will be mopped up immediately with tissue and disinfected with 1% trigen or 2% virkon. Large spills will be covered with absorbent paper and then sprayed with 10% trigen or 2% virkon, and then mopped up after 30 minutes. The absorbent material will then be disposed of in the autoclave waste.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

No comments

Project Containment

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<tr>
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<th>Glass Houses</th>
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02/03/2022
The reversible phosphorylation of tyrosine residues on proteins serves as a critical switch in the regulation of fundamental cellular processes including growth, adhesion and movement, and is control led by the antagonistic actions of protein tyrosine kinases and protein tyrosine phosphatases (PTPs). PTPs function as critical "off" switches, antagonising oncogenic kinase signaling, and can initiate signaling cascades in their own right. The molecular mechanism of tyrosine phosphatase catalysis has been well characterised, however, the extracellular regulation and relevant substrates of many receptor tyrosine phosphatases have yet to be discovered. Recent technical developments in areas such as gene editing, organoid culturing, proteomics, and next generation sequencing mean that now is the perfect time to reveal physiological roles and regulatory mechanisms of this important protein family. We aim to understand the functional role of PTPs (specifically PTPRL) in the modulation of signal transduction, protein function and cellular/tissue responses; and their impact on physiological or disease states.

In order to ascertain such information, we will need to alter the expression of candidate genes involved in such processes, using knock-down or overexpression approaches; in addition, to manipulating their function using knock-in constructs, which harbour specific mutations, deletions and/or fusions. Although such manipulation (at least transiently) of cellular proteins structure or function in culture-adapted cell lines can be achieved using standard transfection protocols (lipofection-based, PEI or electro po ration), lentiviral methods will allow long-term (stable)
expression in difficult to transfect cell lines, and isolated primary cells and/or tissues.

Recipient or parental organism
Various established mammalian and human cell lines.
Various primary mammalian and human cell lines.
Murine tissues/explants cultured ex vivo.

Host/vector system
Lentiviral vectors based on replication-disabled HIV.

Origin & function
cDNA and/or shRNA off for genes involved in regulating the phosphorylation state of various proteins involved in signal transduction and/or the modulation of cellular function (including but not limited to members of the PTP (protein tyrosine phosphatase) family of proteins, specifically PTPR). Marker and reporter constructs that bear detectable tags for our gene of interest, such tags include but are not limited to GFP, c-myc, BirA and FLAG. Plasmids involved in CRISPR-Cas9-mediated gene editing, which encode Cas9 variants, guide RNAs targeting our gene of interest and donor template DNA (containing a desired mutation, fusion or marker/reporter). Finally, we will target newly identified gene products that modulate or are a downstream target of protein tyrosine phosphatase activity.

Evaluation of foreseeable effects
We expect the majority of our constructs to increase decrease or otherwise alter protein phosphorylation, signal transduction and protein, cellular or tissue function; however, as the specific role of PTPs is ill-defined the exact outcome is uncertain. Despite this, PTPs are linked to regulating kinase signal transduction and therefore could possibly act as tumour suppressors. Conversely, we would not expect any cellular effects from the expression of markers or reporters.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Culture media will be disinfected with virkon or trigene
Leftover packaging cells and contaminated plasticware will be discarded in a double bagged clear biohazard bag, sealed with red and white tape and placed in a Cfl2 waste bins for autoclaving

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022 Page 10595 of 15326
Please enter comments on the GM safety committee on the risk assessment

None

Project Containment

Laboratory Activities

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Glass Houses

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Large Scale Activities

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Human Clinical Applications

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Project Ref 678/17.1

Investigating the molecular cell pathology of axonopathies.

Class 2

< 1 Litre

Non-GMM

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Our work is focused on study of neurodegenerative genetic conditions, such as hereditary spastic paraplegias (HSPs) and hereditary motor and sensory neuropathies (HMSNs), in which long axons degenerate. Study of these conditions
provides an opportunity to understand molecular mechanisms that are crucial for axonal function. The aims of this project are to understand the normal and pathological functions of proteins encoded by genes mutated in these conditions. To do this we will generate cell models that have i) undergone genome editing by CRISPR-Cas9 methodologies to generate knock-outs, knock-ins, or endogenously tagged (e.g. GFP-tagged) forms of the protein or, ii) over-express wild-type or mutant forms of the protein, including epitope-tagged forms. As we have a special interest in developing neuronal cell models, we will use lentivirus to provide effective delivery in these non-dividing cells. In most cases we will produce the lentiviral particles ourselves, but we may opt to purchase commercially prepared lentiviral particles (e.g. selected from the Sanger Whole Genome CRISPR arrayed library, via Sigma Aldrich). The functions of the genes/protein that we will target tend to concentrate in certain pathways, namely, membrane traffic pathways, lipid metabolic pathways, mitochondrial function, nucleotide metabolism, axon guidance and myelination.

Recipient or parental organism

Mouse primary neurons/fibroblasts or human induced pluripotent stem cells and cell lines differentiated from them, including neurons.

Immortalised mouse or human cell lines, including fibroblasts, HeLa cells, MRC5 cells, PC12 cells, SHSY-5Y, HEK293T cells and other well characterised cell lines.

These are all regarded as especially disabled.

Host/vector system

2nd or 3rd generation lentiviral systems. These vectors may incorporate the WPRE.

Origin & function

Mutation of all of the genes/proteins that we will work on is associated with human neurogenetic disease in which long axons degenerate. The associated clinical picture is of progressive limb weakness and in some cases loss of sensation. A subset of the proteins are associated with additional neurological features, such as developmental learning problems or cognitive impairment. The encoding genes are not known oncogenes or tumour suppressors, nor are the proteins growth factors or growth factor receptors. Inserts may also contain CRISPR-Cas9 reagents, such as guides to target genes for knock-out/knock-in or sequences encoding the Cas9 protein. Cas9 has no known deleterious functions when expressed on its own. In addition, inserts may also encode commonly used tags, such as GFP. Apart from fluorescence, these tags are biologically inert.

Evaluation of foreseeable effects

Generation, purification and use of lentiviruses in cell culture: The systems used will be second or third generation lentiviral systems. These systems enhance biosafety by splitting the components necessary for virus production across multiple plasmids (3 for 2nd-generation systems, 4 for 3rd-generation systems). The components of both systems are i) a lentiviral transfer plasmid encoding the insert of interest. The transgene sequence is flanked by long terminal repeat (LTR) sequences, which facilitate integration of the transfer plasmid sequences into the host genome. Many lentiviral transfer plasmids are based on the HIV-1 virus. For safety reasons, transfer plasmids are all replication incompetent and may contain an additional deletion in the 3’LTR, rendering the virus “self-inactivating” (SIN) after integration, ii) packaging plasmid(s) and iii) envelope plasmid.

Safety Features of the Lentiviral Systems. The 2nd-generation lentiviral system contains a single packaging plasmid encoding the Gag, Pol, Rev, and Tat genes. The transfer plasmid contains the viral LTRs and psi packaging signal. The envelope protein Env (usually VSVG due to its wide infectivity) is encoded on a third, separate, envelope plasmid. The 3rd generation system further improves on the safety of the 2nd generation. First, the packaging system is split
into two plasmids: one encoding Rev and one encoding Gag and Pol. Although safer, this system can be more cumbersome to use and result in lower viral titers due to the addition of one additional plasmid. Second, Tat is eliminated from the 3rd generation system through the addition of a chimeric 5’ LTR fused to a heterologous promoter on the transfer plasmid. Expression of the transgene from this promoter is no longer dependent on Tat transactivation. Use of these systems means that the probability of a series of recombination events that produce a replication-competent virus that can spread within and between hosts is very small.

Risks associated with lentiviral systems. The viral vector would be able to transduce many tissues should it come in contact with them. Aside from any risks associated with the inserted DNA or CRISPR guide sequences (which are likely very small, see below), the main theoretical risk is through random insertional mutagenesis. The true risk of this is unclear. The major hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of infected cells. The two potential transmission routes are by external exposure (either skin lesions or mucous membranes; only in the case of very high titres and aerosol production) and by accidental injection/inoculation using sharps. In the context of this project, the main risk is of primary infection of humans by high titre viral stocks that have been concentrated by ultracentrifugation.

Risks associated with the modified cells. Lack of the proteins (as associated with CRISPR-mediated knockout) is likely to reduce cellular health, perhaps even reducing the oncogenic risks posed by the lentiviral vector. Expression of altered versions of the proteins under investigation is also likely to reduce the biological fitness of the modified cells. The genetically altered cultured cells produced (especially neurons) will be unable to survive outside of laboratory culture.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture/lab ware will be autoclaved and incinerated which eliminates 100% of the infectious material. Culture fluids will be treated with 2% Virkon for 16 hours (which eliminates 100% of infectious material) and absorbed with Vernagel. Recyclable lab ware will be soaked in 1% Virkon for 16 hours. Bench/cabinet surfaces will be wiped down with 70% ethanol. Spills will be sprinkled with Vernagel powder, then transferred to autoclave bags and the area wiped down with distal or similar detergent.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Our local Biological and Genetic Modification Safety Committee has approved this project.

**Project Containment**

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**Laboratory Activities**

- Glass Houses
- Growth Rooms

**Project Ref** 678/19.1

- *Date Ackn'd*: 18/04/2019
- *CU2 Project Title*: Investigating the molecular cell biology of human pathophysiology using viral vector systems
- *Class*: Class 2
- *CultureVol*: 1-50 Litres
- *Consent Granted*: Non-GMM

**Historical Significant Changes**

- *Date of Additional Info*: 02/03/2022

**Project Additional Information**

- *Purposes of the contained use*:

  Our work is focused on study of the molecular and cellular mechanisms of human pathophysiology. This includes study of membrane and lipid traffic pathways, immune function, host-pathogen interaction, protein folding, homeostasis and degradation, oxygen and metabolite sensing, neurodegeneration, cell signalling and ribosome assembly. Study of these cellular processes provides important insights into normal human physiology, as well as an understanding of the molecular mechanisms by which normal physiology can be disrupted to cause human disease. To achieve our aims we require to generate cell models using viral systems.

- *Recipient or parental organism*:

  02/03/2022
Rodent primary cells (e.g. immune cells, fibroblasts, neurons, glia), human primary cells (e.g. skin fibroblasts, muscle cells, immune cells or glia), or human induced pluripotent stem cells and cell lines differentiated from them, including neurons. Immortalised rodent, monkey, canine, porcine, bovine and human cell lines, including fibroblasts, HeLa cells, MRC5 cells, PC12 cells, SHSY-5Y, HEK293T, H35, AR42J, GH3, INS1, CHO, MBDK, MDCK, Vero, COS cells and any other well-establisehd and characterised especially disabled mammalian cell lines. These are all regarded as especially disabled.

### Host/vector system

**Retroviruses:** Retroviral transfer vectors, such as pLXIN or pQCXIP are based on the Murine Moloney Leukemia Virus backbone but without the structural genes needed for virus production and replication. These are provided by independent packaging vectors incorporated into packaging cells.

Lentiviruses: 2nd or 3rd generation lentiviral systems.

The above vector classes may incorporate the woodchuck hepatitis virus postranscriptional regulatory element (WPRE).

**Adenovirus:** The AdEasy adenoviral vector system is used to generate recombinant adenoviruses. These vectors carry a deletion of the E1 gene region rendering them replication incompetent in non-complementing cell lines. In addition, the E3 region (important for viral pathogenesis and immune evasion) has also been deleted making them less pathogenic in humans.

### Origin & function

**Origins:** Adenoviral, Retroviral and Lentiviral system vectors are obtained commercially, from bona fide academic laboratories or from vector repositories (e.g. Addgene).

Gene inserts will be cloned from standard cloning vectors (such as TOPO or pcDNA3). Guide RNAs and short hairpin RNAs will be generated commercially.

**Intended Functions:**

- We will use viral delivery to undertake:
  - i) genome editing by CRISPR-Cas9 methodologies to generate gene knock-outs, knock-ins, endogenous tagging (e.g. incorporating GFP-tag sequences), or inhibition/enhancement of expression. These studies may include genome-wide screens.
  - ii) over-expression of wild-type or mutant forms of proteins under study, including epitope-tagged forms.
  - iii) expression of guide RNAs for CRISPR-Cas9 targeting and short hairpin RNA (shRNA) for protein knock-down, including in genome-wide library screens.

### Evaluation of foreseeable effects

Generation, purification and use of lenti- and retro- viruses in cell culture.

The retroviral vector system used is a 3rd generation system based on the Murine Moloney Leukemia Virus. Replication defective retroviruses will be made by transient transfection of retroviral transfer plasmids (such as pBMN, pLXIN and pQXCI series) into packaging cells lines such as Phoenix (ampho or ecotropic). These packing cells line express gag, pol and env genes separately, thus it would require multiple recombination events to generate replication competent viruses (likelihood extremely low).

For lentiviral work, second or third generation systems will be used. These systems enhance biosafety by splitting the components necessary for virus production across multiple plasmids (3 for 2nd-generation systems, 4 for 3rd-generation systems). The components of both systems are i) a lentiviral transfer plasmid encoding the insert of interest. The transgene sequence is flanked by long terminal repeat (LTR) sequences, which facilitate integration of the transfer plasmid sequences into the host genome. Many lentiviral transfer plasmids are based on the HIV-1 virus. For safety reasons, transfer plasmids are all replication incompetent and may contain an additional deletion in the 3'LTR, rendering the virus "self-inactivating" (SIN) after integration, ii) packaging plasmid(s) and iii) envelope plasmid.

Safety features of specific lentiviral systems.

The 2nd-generation lentiviral system contains a single packaging plasmid encoding the Gag, Pol, Rev, and Tat genes. The transfer plasmid contains the viral LTRs and psi packaging signal. The envelope protein Env (usually VSVG due to its wide infectivity) is encoded on a third, separate, envelope plasmid. The 3rd generation system further improves on the safety of the 2nd generation. First, the packaging system is split into two plasmids: one encoding Rev and one encoding Gag and Pol. Although safer, this system can be more cumbersome to use and result in lower viral titers due to the addition of one additional plasmid. Second, Tat is eliminated from the 3rd generation system through the addition of a chimeric 5' LTR fused to a heterologous promoter on the transfer plasmid. Expression of the transgene from this promoter is no longer dependent on Tat transactivation. Use of these systems means that the probability of a series of recombination events that produce a replication-competent virus that can spread within and between hosts is very small.

**Risks of operator infection with retro- and lenti-viral systems.**

Retroviruses generated using the ecotropic packaging cells are capable of infecting and stably integrating DNA into murine cells, while viruses generated using the
amp phototropic packaging cells are capable of infecting and stably integrating DNA into a broad range of cells, including human cells. However, the risk of human or rodent infection using the above viral system is extremely low as the virus can only infect via direct inoculation. Thus, if no sharps are used in combination with good laboratory practice the risk of infection is extremely low.

VSVG pseudotyped lentivirus can transduce many tissues should it come in contact with them. Here, the two potential transmission routes are by external exposure (either skin lesions or mucous membranes; only in the case of very high titres and aerosol production) and by accidental injection/inoculation using sharps. The most significant hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of infected cells. In the context of this project, the main risk for primary infection of humans is by high titre viral stocks that have been concentrated by ultracentrifugation.

Aside from any risks associated with the inserted DNA or CRISPR guide sequences (which are likely very small, see below), the main theoretical health risk of infection in most cases is the risk of random insertional mutagenesis. The true risk of this is unclear, although the risk of transduction leading to tumourigenesis or other untoward harm following exposure is related in part to the titre of the viral vector and exposure of the operator to quantities of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of retro- or lenti- viral vectors.

Vectors bearing the WPRE insert have been reported to increase tumours in experimental animals and interim advice from SACGM is to handle any such vectors at CL2 minimum. The classification of insert-bearing vectors may remain at class 2 but is subject to additional risk mitigation (measures to guard against needle stick, careful handling of high titre stocks).

Adenoviral systems:

Although most adenoviral work is assessed at CL1, this system is included here to take into account the possibility of targeting for manipulation oncogenes, tumour suppressor genes or other genes that could theoretically pose a human health risk.

Generation, purification and use of adenoviruses in cell culture.

The adenoviral vector system used is the AdEasy vector system. Replication deficient adenoviruses are made by cloning genes of interest or versions of those genes (dominant negative constructs, or fusion proteins) into a transfer vector containing the cytomegalovirus promoter and an IRES-GFP (internal ribosome entry site with Green fluorescent protein) sequence. Transfer vectors are then cloned into the p-AdEasy-1 packaging plasmid that lacks E1 and E3 sequences, which is then transfected into the packaging cell line HEK293 (containing the E1 replication sequence) to generate recombinant replication-deficient adenovirus.

Safety features of adenoviral systems

The AdEasy vector system carries a deletion of the E1 gene region rendering them replication incompetent in non-complementing cell lines. In addition, the E3 region (important for viral pathogenesis and immune evasion) has also been deleted making them less pathogenic in humans. The chances of a recombination event within the packaging cells rendering replication competent adenovirus is very low and is further minimized by not serially propagating the adenovirus in the packaging cells. Additionally, adenoviral DNA integrate into the genome with very low efficiency and so is not usually transferred to daughter cells during cell division, significantly reducing the likelihood of any prolonged in vivo expression, for example of oncogenes.

Risks of operator infection with adenoviral systems

Adenovirus can transduce mammalian tissues by external exposure or by accidental inoculation using sharps. Adenoviral vectors carry the E1 gene deletion rendering them replication incompetent and the E3 gene deletion making them less pathogenic in humans. Since adenoviruses do not usually integrate their DNA into their host there is little chance of prolonged expression of transgenes within human cells.

Risks to the operator associated with biological activity of inserted DNA/RNA delivered by viral systems.

In some cases, particularly with retro- or lenti- viral systems, the inserted DNA may encode an oncogene that could become integrated into the genome of infected cells, and tumour suppressor genes may be targeted with CRISPR-Cas9 or shRNA. This could theoretically be harmful to human health - the true health risks of this are unclear, but are likely to be relatively small as malignancy is a multistep process and it unlikely that any single “hit” would initiate transformation. Inserts may also contain DNA encoding known Mendelian disease genes other than oncogenes/tumour suppressor genes, or reagents that would silence such genes. Again the true risks of this are unknown, but the risk to the operator is likely to be minimal, as in most cases it is unlikely that disease target tissues would be accessible to viral particles, and infection of a very small proportion of accessible bodily cells is very unlikely to be harmful in most cases. As above, the major hazard is presented by the packaged virus, particularly when concentrated.

Inserts may contain CRISPR-Cas9 reagents, such as guides to target genes for manipulation, sequences encoding the Cas9 protein, or Cre-recombinase or the tetracycline-controlled transactivator (TTA). Cas9, Cre-recombinase and TTA have no known deleterious functions when expressed on their own. In addition, inserts may also encode commonly used tags, such as GFP. Apart from fluorescence, these tags are biologically inert.
Risks associated with the modified cells. Lack of the proteins (as associated with CRISPR-mediated knockout) is likely to reduce cellular health, perhaps even reducing the oncogenic risks posed by the lentiviral vector. Expression of altered versions of the proteins under investigation is also likely in many cases to reduce the biological fitness of the modified cells. The genetically altered cultured cells produced will be unable to survive outside of laboratory culture. In the highly unlikely event that cells are inoculated into an operator, they would be expected to be recognised as non-self and destroyed by the immune system. The risk of such modified immortalised cells is unlikely to be greater than the equivalent risk posed by immortalised cell lines in common use. Primary cells may pose a risk of adventitious infection, which is minimised by the mitigation procedures in place.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture/lab ware will be autoclaved and incinerated which eliminates 100% of the infectious material. Culture fluids will be treated with 2% Virkon for 16 hours (which eliminates 100% of infectious material) then disposed of down drains. Recyclable lab ware will be soaked in 1% Virkon for 16 hours. Bench/cabinet surfaces will be wiped down with 70% ethanol. Spills will be sprinkled with Vernagel powder, then transferred to autoclave bags and the area wiped down with distal or similar detergent.

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

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Please enter comments on the GM safety committee on the risk assessment

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Animal Units

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Project Ref 678/19.2
Our goal is to develop and apply molecular and cellular methods approaches to understand host-parasite interactions during the erythrocyte stages of the malaria parasite life cycle, and use this data to identify and prioritise drug and vaccine targets. A key technique in this work is the experimental modification of the sequence or expression levels of specific Plasmodium genes. This enables us to generate and test hypotheses about the role of these genes in general parasite viability and fitness; as well as investigate specific phenotypes that are necessary for erythrocyte invasion, drug resistance, and development transitions. A better understanding of these critical interactions may lead directly to the development of new prevention and control measures such as drugs or vaccines.

**Recipient or parental organism**

*Plasmodium falciparum*

*Plasmodium knowlesi*

**Host/vector system**

**Hosts:**

*Plasmodium falciparum* wild type parasite strains. Standard lab-adapted strains will be used, primarily 307, as that is the strain from which the *P. falciparum* reference genome sequence was generated, or NF54, the parental strain of 307. In certain circumstances other lab-adapted strains will be used in place of 307, such as 7G8, HB3, Od2, or strains recently adapted from clinical isolates. In some cases these strains may be resistant to one of more currently used antimalarial drugs, but none are resistant to all frontline drugs so clinical treatment options are always available.

*Plasmodium knowlesi* parasite strains. Currently only two *P. knowlesi* lines are available for in vitro culture in human erythrocytes (PMIO: 23267069, 24506567); if others become available they will be tested and used if they have advantages, such as increased transfection efficiency. There is currently no known drug resistance in *P. knowlesi*
parasites, and the in vitro cultured line is susceptible to all current frontline antimalarials.

Vectors:
Plasmodium transfection vectors are not commercially available, although some previously produced vectors are available from ATCC through the Malaria Research and Reference Reagent Resource Center (www.mr4.org). Vectors to be used in this study will be obtained from this source, or from research colleagues, then adapted to contain the gene or gene fragments of interest. Essentially all vectors consist of three elements:
1. antibiotic resistance gene with associated expression elements to allow propagation in E. coli (usually encoding Ampicillin or Kanamycin resistance);
2. drug resistance cassette with associated Plasmodium expression elements to allow positive selection in P. falciparum and P. knowlesi (usually anti-folate drug resistance encoded by T. gondii or H. sapiens DHFR, or genes that enable resistance to Blasticidin or Gentamicin);
3. an expression or gene targeting cassette to either express the gene of interest in Plasmodium falciparum or Plasmodium knowlesi or direct insertion of the construct to a specific genomic location in order to ablate or modify the endogenous gene, for example by adding an epitope or fluorescent tag to the encoded protein.
The vectors may be based on a number of scaffolds, most commonly on a pUC type backbone, but also including the pJazz linear vector system (Lucigen) that can accommodate large genomic inserts (on average ~8 kb).
For CRISPR/Cas mediated genome editing, two additional components will be included - a cassette expressing Cas9 endonuclease and a cassette expressing target-specific gRNA. These may be either included on a single vector that will be co-transfected into parasites together with a "donor homology" region supplied on a second vector that facilitates homology repair and editing of the target gene, or as a PCR product, which will direct the desired genome modification. However, alternative designs incorporating the gRNA into the donor homology construct are also possible.

Constructs containing modified Plasmodium genes or fragments of such genes will be generated and propagated in E. coli. Constructs will then be introduced into Plasmodium strains by transfection and selected for using drugs. Constructs will either be maintained as episomes, or will integrate in a targeted manner into the Plasmodium genome.
For CRISPR/Cas genome editing, parasites will be co-transfected with a plasmid to express the Cas9 endonuclease and specific guide RNA (gRNA), as well as a homology repair vector to modify the targeted locus. Note that Plasmodium parasites lack the non-homologous end-joining (NHEJ) DNA repair pathway, meaning that all repair happens via homologous recombination, necessitating the presence of the homology repair vector.
Genetic modifications are aimed at dissecting the fundamental biology of human-infective malaria parasites. Gene inserts and transfection vectors are expected to affect parasite biological function through:
• The placement of epitope tags or fluorescent proteins (GFP or others) to investigate protein localisation
• Disruption of genes either alone or in combination, and assessment of the impact on parasite fitness/viability (e.g. how long genetically modified parasites take to go through their life cycle compared to wild type), or on specific developmental stages (e.g. the ability to invade human erythrocytes).
• Allelic replacement of individual genes with modified versions, either altering individual amino acids/regions in the encoded protein (e.g. to probe protein function), or replacing a gene from one species with that of another (a particularly useful approach in P. knowlesi, which can express genes from P. vivax, which is the major cause of malaria outside Africa, but cannot itself be cultured in vitro).
• Testing the impact of a mutation on resistance to one or more drugs, including those used for positive selection (typically the antifolate WR99210, Blasticidin, or G418), or experimental antimalarial compounds developed by colleagues and collaborators; or to antibodies to prove the vaccine potential of specific targets.
Evaluation of foreseeable effects

Plasmodium falciparum is classified by the ACDP as a Hazard Group 3 human pathogen. Plasmodium knowlesi is classified as Hazard Group 2. Both species are capable of causing human malaria and as such we culture both organisms within the same facility and under the same control measures. A range of symptoms can arise from malaria infection, including general symptoms such as fit-like illness and, in severe cases, coma and death. No vaccine is available.

Parasites are naturally transmitted via a mosquito vector. In the laboratory, we culture both the parasite stage that infects red blood cells as well as the stage that is able to infect mosquitoes, but do not carry out any mosquito infection experiments. Infection of humans in the lab would therefore require direct injection into human blood circulation. There is a LOW risk of blood stage infection of a worker where a penetrating wound occurs. This risk is mitigated by a strict "no-sharps" policy in the derogated CL3 suite. If such an injury were to occur, the injured worker would be referred to an infectious diseases specialist at the nearest hospital (Addenbrookes) and presumptive antimalarials prescribed if deemed necessary. Any onward human transmission is extremely unlikely as it would require a mosquito vector to be available in the local environment with appropriate temperature and humidity conditions (26°C and 80% respectively). These conditions are almost never seen in England. There is no evidence for aerosol-based transmission.

GM Plasmodium strains will have no increased ability to infect humans relative to non-GM strains. In some GM parasites a product will be expressed, for example fluorescent or luciferase-based reporters, either individually or fused to parasite proteins. These products are unlikely to be biologically active in humans. There is no evidence that P. falciparum or P. knowlesi proteins can act as toxins or allergens in humans or can act as oncogenes. Gene inserts could theoretically alter the pathogenicity of the modified line relative to the parental parasite line. However in the majority of cases these modifications will generate GM parasites that are essentially equivalent to non-GM lab or field strains or are less fit than their wild-type counterparts. Introduction of the transfection vectors will make the GM parasite resistant to one or more drugs used for positive selection (typically the antifolate WR99210, Blastiaidin, or G418). However, none of these drugs used for plasmid selection in Plasmodium parasites are in wide use as frontline anti-malarials.

P. falciparum and P. knowlesi are only able to infect humans and certain species of non-human primates. Given the inability of free-living parasites to survive in the environment, and the absence of non-human primates from the Institute or its surroundings, there is no risk of release of genetically modified parasites to the environment. GM parasite stocks held in liquid nitrogen are not infectious in that state.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We request derogation from the requirement to carry out this activity in a laboratory that:
- Is sealable for fumigation
- is maintained at negative air pressure relative to the pressure of the immediate surroundings
- has an autoclave inside the laboratory suite

This request is made in accordance with advise provided in "Biological agents: Managing the risks in laboratories and healthcare premises"; Appendix 3.2 Work with Hazard Group 3 parasites. Justification for these derogations is based on the route of infection for malaria parasites which is direct injection into the blood. There is no evidence of aerosol-related infection.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Any waste in contact with *P. falciparum* or *P. knowlesi* will be disinfected via approved methods and disposed of by incineration following autoclaving. Liquid waste disinfection is carried out with 1 % (w/v) Virkon (final concentration) for 20 minutes, or 5 minutes exposure to 5g/L Klorsept (final concentration) is used as an alternative. This is then disposed of via the drains after dilution with 5 times volume of water. Both treatments have been validated, and specifics are listed in the CL3 handbook; they give effectively 100% kill.

All biological waste that cannot be disinfected (e.g. blood bags) and all solid waste from the CL3* suite will be autoclaved prior to final disposal. Once a biohazard bag is full, the neck of the bag is folded over and the bag placed in an approved rigid sealable container to contain any leaks. All workers wear appropriate PPE when handling waste. Sealed waste containers should be kept inside the CL3* laboratory suite until ready to be collected in the designated waste area by the CIMR custodians.

On arrival of the custodians, the CL3 waste is passed to them, and is taken for immediate autoclaving in the main waste autoclaves. The trolley used in this operation will not contain any other forms of waste. Autoclaved waste is destroyed by incineration following examination of the autoclave run report.

All autoclaves are subjected to an annual inspection to written schemes, annual validation reports, and quarterly servicing. Waste runs are validated to achieve a minimum hold time of 3 minutes at 134°C, equivalent to a 30-log reduction in viability of indicator spores.

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>Animal Units</td>
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**Project Ref** 678/19.3

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Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

No comments

---

Project Ref 678/19.3
Our work is aimed at the generation of drug-like substances (mainly small molecules) for the treatment of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease and other neurodegenerative diseases. This is carried out over several stages all of which may involve use of virally mediated nucleic acid transfer. To achieve our aims we require to generate cell models using viral systems.

Recipient or parental organism

Mouse primary cells (e.g. neurons, glia), human primary cells or human induced pluripotent stem cells and cell lines differentiated from them, including neurons. Immortalised human, monkey, canine, porcine, bovine and rodent cell lines, including fibroblasts, HeLa, MRCS, PC12, NRK, SHSY-5Y, HEK293T, H35, AR42J, GH3, INS1, CHO, MBDK, MDCK, Vero, COS cells and other well characterised mammalian cell lines. These are all regarded as especially disabled.

Host/vector system

Lentiviruses: 2nd or 3rd generation lentiviral systems. Vectors may incorporate the WPRE. Adeno-associated virus (AAV): “gutless” pAAV ITR vector containing inserted gene of interest; AAV vector containing Rep and Cap coding sequences; Adenovirus helper vector required for replication.

Origin & function

Intended Functions:
Inserts may contain the following:
• siRNA to knock down expression of an endogenous gene of interest.
• Wild type human or rodent genes, of interest due to their involvement in a physiological or pathological pathway, but which do not cause disease on their own.
Evaluation of foreseeable effects

For transfer of viral plasmids into mammalian cultured cells (e.g. HEK293T cells) for viral production, standard chemical transfection reagents will be used (e.g. Lipofectamine).

Viral particles will transduce host cells through natural tropism.

Amplification of transfer plasmids and helper plasmids: Plasmids will be propagated and purified using standard E.coli strains (e.g. DH5alpha, STABLE2) and standard techniques of recombinant DNA technology (CL1).

Generation, purification and use of lentiviruses and AAV in cell culture.

Lentivirus:

For lentiviral work, second or third generation lentiviral systems will be used. These systems enhance biosafety by splitting the components necessary for virus production across multiple plasmids (3 for 2nd-generation systems, 4 for 3rd-generation systems). The components of both systems are i) a lentiviral transfer plasmid encoding the insert of interest. The transgene sequence is flanked by long terminal repeat (LTR) sequences, which facilitate integration of the transfer plasmid sequences into the host genome. Many lentiviral transfer plasmids are based on the HIV-1 virus. For safety reasons, transfer plasmids are all replication incompetent and may contain an additional deletion in the 3'LTR, rendering the virus “self-inactivating” (SIN) after integration, ii) packaging plasmid(s) and iii) envelope plasmid.

The 2nd-generation lentiviral system contains a single packaging plasmid encoding the Gag, Pol, Rev, and Tat genes. The transfer plasmid contains the viral LTRs and psi packaging signal. The envelope protein Env (usually VSVG due to its wide infectivity) is encoded on a third, separate, envelope plasmid. The 3rd generation system further improves on the safety of the 2nd generation. First, the packaging system is split into two plasmids: one encoding Rev and one encoding Gag and Pol. Although safer, this system can be more cumbersome to use and result in lower viral titres due to the addition of one additional plasmid. Second, Tat is eliminated from the 3rd generation system through the addition of a chimeric 5' LTR fused to a heterologous promoter on the transfer plasmid. Expression of the transgene from this promoter is no longer dependent on Tat transactivation. Use of these systems means that the probability of a series of recombination events that produce a replication-competent virus that can spread within and between hosts is very small.

Lentivirus can transduce many tissues should it come in contact with them. Here, the two potential transmission routes are by external exposure (either skin lesions or mucous membranes; only in the case of very high titres and aerosol production) and by accidental injection/inoculation using sharps. The most significant hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of infected cells. In the context of this project, the main

Adeno-associated virus:

AAV is non-pathogenic in humans and is only able to replicate in the presence of adenovirus. AAV systems for production of viral particles for gene transfer employ replication defective “gutless” AAV vectors which have the Rep and Cap genes required for the viral life cycle replaced by the inserted gene of interest and expressed using a separate vector. An additional vector contains adenoaviral genes required for replication. Once in a host cell AAV will replicate if a helper virus is present, or otherwise be incorporated in the host genome at a specific location or in the absence of the Rep gene, form episomal concatamers.

AAV can transduce many tissues should it come in contact with them. Here, the two potential transmission routes are by external exposure (either skin lesions or mucous membranes; only in the case of very high titres and aerosol production) and by accidental injection/inoculation using sharps. The most significant hazard is therefore represented by the packaged virus prior to infection of the target cells or organism and residual virus in the medium of infected cells. In the context of this project, the main
risk for primary infection of humans is by high titre viral stocks that have been concentrated by ultracentrifugation. There is negligible risk of random insertional mutagenesis. The defective virus is not incorporated into the host chromosome, so in dividing cells the virus is diluted and degraded over time, but it does persist in non-dividing cells. The main risk is therefore from the inserted DNA sequence. Inserted DNA may contain mutant genes responsible for neurodegenerative diseases in humans. The potential for accidental inoculation of an operator to result in manifestation of disease is very low (lower than for example the risk from oncogenes) as widespread transduction in the CNS from peripheral exposure would be extremely unlikely. 

Inserts may contain CRISPR-Cas9 reagents, such as guides to target genes for manipulation or sequences encoding the Cas9 protein. Inserts may contain Cre-recombinase or the tetracycline-controlled transactivator (tTA). None of these inserts have known deleterious functions when expressed on their own. In addition, inserts may also encode commonly used tags, such as GFP. Apart from fluorescence, these tags are biologically inert. Lack of the proteins (as associated with CRISPR-mediated knockout) is likely to reduce cellular health, perhaps even reducing the oncogenic risks posed by the lentiviral vector. Expression of altered versions of the proteins under investigation is also likely in many cases to reduce the biological fitness of the modified cells. The genetically altered cultured cells produced (especially neurons) will be unable to survive outside of laboratory culture. In the highly unlikely event that cells are inoculated into an operator, they would be expected to be recognised as non-self and destroyed by the immune system. The risk of such modified immortalised cells is unlikely to be greater than the equivalent risk posed by immortalised cell lines in common use. Primary cells may pose a risk of adventitious infection, which is minimised by the mitigation procedures in place.

Lentivirus: 

Production of lentivirus: Whenever possible, we will use 3rd (or subsequent) generation lentiviral systems. The lentivirus is produced by co-transfecting the packaging plasmid mix and the transfer vector into HEK293T packaging cells. A typical scale of viral production might include 1-10 10 cm dishes producing a total of up to 300 mL of viral supernatant. Particles are harvested over a period of 48 to 72 hours after transfection and are stored at -80C. If titres are low, the virus can be concentrated by ultracentrifugation. During this process, the potential hazard from aerosolization of the virus will be mitigated by the requirement that buckets will be sealed and unsealed in a biosafety cabinet and that the outside of the buckets be decontaminated before they leave the biosafety cabinet (see below). Transduction of recipient cells: These will be tissue cultures of human or other mammalian cells (potentially including human and mouse primary cultured cells) and the scale of the transduced sample will be up to 40 single or multiwell plates or flasks, with volumes of 0.05-150 ml per well/flask, depending on plate format, with a total volume of up to 1200 ml (typical volumes will be around 250ml). The recipient cells will be incubated with the virus for 4-24hours after which the viral supernatant will be removed and inactivated. The cells will be washed 3 times in PBS and new virus free medium added. Following 48 hours of virus free culture, the cells can be removed to CL1 as any residual viral particles should no longer be active at that point, so the cells do not pose a biosafety risk. Analysis and selection of transduced (recipient cells): Western blot, plate reader assays, FACS or fluorescence microscopy to analyse protein expression can be used to estimate transduction and transfection efficiency of the viral constructs. If microscopic analysis is required before the 48 hr post viral clearing period, samples will be fixed in 4% formaldehyde for 10-20min prior to removal from CL2, which should inactivate any virus. FACS analysis samples may be fixed as above, or living cells may be transported to the CIMR FACS facility (which is approved for CL2 samples) in leak- and shatter-proof containers. Recipient cells may be treated with antibiotic selection (puromycin or blasticidin or other selective agent). Adeno-associated virus: 

Production of adenovirus: Genes of interest are cloned into AAV genome plasmid containing EGFP driven by the strong mammalian CMV promoter, between two ITRs. This is co-transfected into HEK293 cells along with a helper plasmid containing all the serotype functions required for AAV production in a transient system and an AAV packaging plasmid containing the Rep and Cap coding sequences. Transduction of recipient cells: As with lentivirus. Analysis and selection of transduced (recipient cells): As with lentivirus AAV virus particles may be used for CNS-targeted gene transfer in vivo in mice. This may be done either systemically through tail vein injection into young adult mice, or through intracranial injection carried out stereotactically under general anaesthesia. In both cases, the number of viral particles and the time required for expression of the virally transferred DNA will need to be determined empirically. Expression will be confirmed by immunofluorescence in brain sections and by direct detection of mRNA or protein products of the gene of interest. One aim of this work is to inject AAV into mice, either systemically or intra-cranially. This work will be carried out in CL2 facilities in either CBS or the Ann McLaren building.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture/lab ware will be autoclaved and incinerated which eliminates 100% of the infectious material. Culture fluids will be treated with 2% Virkon for 16 hours (which eliminates 100% of infectious material) then disposed of down drains. Recyclable lab ware will be soaked in 1% Virkon for 16 hours. Bench/cabinet surfaces will be wiped down with 70% ethanol. Spills will be sprinkled with Vernagel powder, then transferred to autoclave bags and the area wiped down with distal or similar detergent.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

No comments

Project Containment

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Project Ref 678/99.2

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31/01/2008

Withdrawn: N

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?: N

If yes, tick to confirm that it is attached to this form: N

Tick to confirm that you have attached a risk assessment to this form
Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

**Project Containment**

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**Name**

| QUOTIENT BIORESEARCH GROUP LTD |

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

| NEWMARKET ROAD |

**District**

| CAMBRIDGESHIRE |

**Town**

| FORDHAM |

| COUNTY |

| CAMBRIDGESHIRE |

| POSTCODE |

| CB7 5WW |

| COUNTRY |

| ENGLAND |

**Tel Number**

| 01638 720 500 |

**Fax Number**

| 01638 724202 |

**E-mail**

**HSE Division**

| EAST AND SOUTH EAST |

**Comments**

| CLOSED 03/02/2011 |

**Date at Which Additional Info Submitted**

| 02/03/2022 |
### Premises Addresses

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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**
  - [ ]

- **Give brief details of the genetic modification safety committee**

  - Laboratory
  - Animal Unit
  - Growth Room
  - Glass House
  - Large Scale

  **Level 1 (GMMs)**

  **Level 2 (GMMs)**

  **Level 3 (GMMs)**

  **Level 4 (GMMs)**

  **Non-microbial**

  **Other (please specify)**
  - [ ]

  **Tick if confidential**

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum culture volume that could be released by a single spill or breakage is 3L.

Deactivation Procedures
* Disinfectant (2% w/v Virkon) is used for spills and cleaning surfaces.
* In the event of spillage, the area can be effectively disinfected and the wider environment is unlikely to become contaminated.
* Liquid biohazard waste is inactivated with 2% w/v Virkon (1% final concentration) for at least 16 hours prior to disposal to the drainage system.
* Pipettes and Class II cabinets are decontaminated using Microsol3 (distributors: Anachem).
* Waste contaminated with biologicals is autoclaved at 131 degrees C for 30 minutes. It is then placed in yellow plastic clinical waste bins that are sealed before removal from the site for incineration.
* Waste contaminated with chemicals only (no biologicals) is placed in yellow plastic clinical waste bins that are sealed before removal from the site for incineration.
* Lab coats are autoclaved at 131 degrees C for 30 minutes before laundering.

Use of Virkon validated and monitored by viability testing.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 680

Data Premises Notified (Originally) 29/09/1998

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

CHARLES RIVER RESEARCH SERVICES UK LTD

Name 2

Department

Campus Estate or Research Centre

CHESTERFORD RESEARCH PARK

Building

THE ROBINSON BUILDING

Road Name

District

Town

SAFFRON WALDEN

County

ESSEX

Postcode

CB10 1XL

Country

ENGLAND

Tel Number 01799 533500

Fax Number 01799 531590

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Name changed from BioFocus Discovery Ltd to BioFocus DPI Ltd - 24/7/07.

Date at Which Additional Info Submitted

25/07/2001  04/02/2003
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</tbody>
</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)  

Tick if confidential

Bacteriology
Parasitology
Transgenic
Birds

Microbiology
Research

Virology
Transgenic
Animals
Transgenic
Fish

Gene Therapy

Mycology
Transgenic
Invertebrates
Transgenic
Plants

Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 680/01.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
USE OF NON-REPLICATING MURINE LEUKAEMIA VIRUS (MLV) FOR THE DISPLAY OF PROTEINS ON ENV (MLV ENVELOPE PROTEIN), INCLUDING TOXIN PROTEINS, IN SYSTEMS INCLUDING ENVELOPE PACKAGING VECTORS

30/07/2001

Date Project Ceased

06/04/2009

Withdrawn

Tick if notifying a connected programme of work

Y

Historical Significant Changes

Historical Date of Additional Info

Project notified under transitional arrangements

N

Class 2

Consent Granted

not applicable

06/04/2009

Project notified under transitional arrangements

N

Tick if notifying a connected programme of work

Y

Project Additional Information

Purposes of the contained use

Initially, for feasibility studies involving the infection of target cell lines with MLV (expressed in producer cell lines), leading eventually to screens for biologically active compounds.

Recipient or parental organism

MLV genome is stably incorporated into the genome of producer cell lines in such a way that the packaging signal required for viral genomic packaging into viral particles has been removed rendering the virus replication incompetent. In addition components of the viral genome (gag/pol, and env) have been separated. In some procedures, however, producer cell lines with the ability to produce viral particles containing packaged viral genomic env gene only (or chimeric forms thereof) will be constructed.

Amphotrophic (4070A) MLV is being used for the feasibility studies, it has a broader host range than ecotropic (Molony) MLV and is able to infect human cells in culture. There is some research that indicates that the replication competent form of this virus is able to survive in non-human primates (Characterization of replication-competent retroviruses from nonhuman primates with virus-induced T-cell lymphomas and observations regarding the mechanism of oncogenesis. Vanin EF, Kaloos M, Broschi C, Nienhuis AW.; J Virol 1994 Jul;68(7):4241-50). There are, however, no examples of this virus infecting humans.

In order for replication competent virus to arise with the fully replication incompetent system a double recombination event would have to arise. However, for the envelope packaging system only a single recombination event would be required to generate replication competent virus.

Host/vector system

Host System: Parental human cell lines able to produce part of all of the MLV proteins, which auto-assemble and bud from the cell surface to form either capsid particles (product of gag/pol) or full virus particles (product of gag/pol and env) containing no genome. In addition any DNA contained between a 5 and 3' LTR with a packaging signal will be processed into the viral particles.

Vector System: Amphotrophic MLV particles containing packaged DNA. This DNA is able to stably insert into the genome of actively dividing target cells.

Target Cell Lines: Normal tissue culture cell lines (including murine, canine, bovine and human types).
### Origin & function

**MLV origin:** Plasmid vectors containing elements of MLV genome transfected into producer cells.

The function of modified MLV is to deliver genetic material to target cell lines. Specific modifications to env (i.e. the display of toxin proteins, such that they are incorporated on the viral surface) effectively limit the ability of the virus to infect target cells by sequestering the viral particles away from the viral receptor proteins.

### Evaluation of foreseeable effects

**Foreseeable Effects**

The use of an envelope packaging system makes the likelihood of replication competent MLV arising as the result of recombination more of a possibility, when compared to systems where none of the retroviral genetic elements are packaged (i.e. fully disabled retroviral vectors). This factor and the display of proteins on env will be the major considerations when evaluating risk to human health and the environment.

**Effects on human health**

With regard to hazards arising from the inserted gene(s) and potential protein products, the following should be considered (it should be stated that these genes are linked to env to produce chimeric envelope):

- **Toxic** - e.g. toxin genes: the display of toxin proteins on env (at approximately 1010-12 molecules per ml) results in a potential dose (during virus production) of only a few picograms of toxin per ml which is several thousand times below the level required to produce any affects for protein toxins, it is therefore unlikely that any transfer of genetic material would result in production of toxic proteins, by the host cells, that would pose a risk.

- **Allergenic** - e.g. from viral proteins or other expressed proteins: there is no data that would indicate the potential allergenicity of MLV proteins or chimeric env proteins.

- **Oncogenic** - e.g. growth factors: the display of growth factor proteins on env (at approximately 1010-12 molecules per ml) results in a potential dose of only a few picograms of growth factor per ml which is below the level required to produce any physiological effect, it is therefore unlikely that any transfer of genetic material would result in production of growth factors, by the host cells, that would pose a risk.

An infection involving MLV is only likely to occur by direct fluid transfer i.e. needle-stick injury or transmission via a wound. However, the virus would have to be introduced in a fairly large quantity (several ml) it would have to survive in the blood until it was able to infect actively dividing cells and it would also have to survive the immune system. The probability of modified MLV causing harm to humans is negligible.

**Effects on the Environment**

MLV occurs naturally in the environment and is a well characterised mouse pathogen, any modifications should place the virus at a selective disadvantage when compared to wild type virus. The probability of release into the environment and harm arising from this release is very low.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not applicable**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- **Waste generated as a result of tissue culture activities:**

  a) All contaminated solid waste, plastics etc., (not including liquid waste) is placed into double autoclave bags housed in a leak-proof stainless steel autoclave box (with lid). This autoclave using a standard cycle 121C, at 2 bar for 30 minutes, the autoclave generates a fully audited, printed record for each run, these are stored in a company.
archive. The autoclave is under a maintenance contract by the manufacturer and is regularly serviced. Waste Material from the autoclave is discarded into permanently sealable leak-proof plastic bins destined for incineration by an approved contractor. This can therefore be considered as 100% kill.

b) Liquid waste (i.e. generated by removal of media from cultures) is disinfected (Tegodyne, 50m/L) overnight before discarding. Tegodyne has been tested by the manufacture and is known to be 100% effective in the activation of retroviruses, when used at the correct concentration.

Spills will be mopped up with suitable qabsorbent material (for example Whatmans spill containment granules), this will then be treated as solid waste, surface will then be treated with a suitable disinfectant (Tegoydne or virokon). 

Monitoring: Viral stocks generated will be tested for the presence of replication competent MLV, please see attached SOP. If as a result of testing RCR's are detected then all experimental material relating to the tested viral stock will e destroyed by chemical inactivation (Tegodyne) and autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
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<table>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<tbody>
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<td>L2</td>
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Project Ref 680/06.1

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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</thead>
<tbody>
<tr>
<td>22/08/2006</td>
<td>Use of mammalian cells transfected with attenuated adenoviral vectors.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Not Applicable</td>
<td></td>
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</tbody>
</table>
### Purposes of the contained use

Use of adenovirally transfected mammalian cells for the purpose of drug discovery, including human ion channels, GPCR'S or kinases. Various experimental techniques may be used including automated electrophysiology, radiochemical and biochemical assays using live cells or cell lysates.

### Recipient or parental organism

Mammalian cell lines including but not limited to: HEK 293, CHO KI and human cystic fibrosis bronchial epithelial cells.

### Host/vector system

**Adenovirus 5 vector system:**

The gene of interest is incorporated into the attenuated adenoviral adaptor plasmid which does not contain the E1 region. This vector is co-transfected with a helper plasmid which contains the remaining coding sequence of the adenovirus, without the E2A region. When the adaptor and helper vectors are transfected into the host cell line, per C6-2A, a human retinal cell line engineered to produce the E1 & E2A gene products replication incompetent adenovirus is produced. The virus is purified and used to transfect host cells which then express the inserted gene. These cells are then used in experiments.

### Origin & function

This is a replication deficient E1 and E2 deleted, type 5 attenuated adenoviral vectors carrying RNAI or CDNA sequences that have been generated at BIOFOCUS (a Galapagos company) in Leiden. The virus is produced in per C6 cell line from Crucell, Leiden. The viruses are laboratory reagents for research, testing and evaluation purposes only, they are not for diagnostic or therapeutic use. The goods are classified as biological specimens category B (UN 3373) and are generated using cell culture techniques (they are not antiserum, blood fractions, nor a modified immunological product. They are not a vaccine for human nor veterinary use. They are not a ferment).

### Evaluation of foreseeable effects

The virus is non-replicative human virus that can only propagate within the packaging cell line.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

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For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Liquid waste collected from flasks & plastics is decontaminated overnight using Phoraid disinfectant. This is left in a sealed container overnight to ensure 100% kill.
2. Solid waste is placed in a yellow biohazard bin. The bin is lined with two biohazard autoclave bags which at the end of the working day are sealed and the lid locked in position so that the container cannot leak or the lid removed. The bins are labelled with a biohazard sign and carries the UN 3291 number. Bins are stored in a locked, brick store with concrete floor until collection by Grundons Waste Ltd. Grundons Waste Ltd are certified to handle and dispose of level 2 waste, which once collected is incinerated ensuring 100% kill. Grundons Ref: BIOF001.
3. Tips and other plasticware which may contain a small amount of liquid after use, will be placed in Phoraid disinfectant overnight before being treated as solid waste.
4. A biohazard spill kit will be available close to hand.

Is an emergency plan required according to regulation 20?  

Tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

After discussion, the GMSC felt that a thorough risk assessment had been carried out and were happy that the personal and environmental hazards identified were adequately addressed.

In addition to the GMSC members, staff working on the project were invited to attend and make comments at the meeting.

Project Containment

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Project Ref  680/07.1

Date Ackn'd  23/04/2007  
CU2 Project Title  1) BV-2 Cell based Luciferase assay 2) BV-2 Cell based TNF (Tumour Necrosis  
Class  2  
CultureVolClass2  < 1 Litre  
CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Recipient or parental organism

BV-2 cells (Mouse Microglial cell line)

Host/vector system

Replication defective J2 retrovirus (recombinant murine sarcoma virus 3611 containing viral oncogenes Myc and Raf).

Origin & function

This work was generated by Prof Rosario Donasto at the University of Perugia in Italy. BV-2 cells were originally transformed by infecting the primary cells with the J2 retrovirus. MSV-3611 (closely related to the M-Mulv) was originally isolated from chemically transformed mouse cells. MSV-3611 is replication defective due to deletion of the gag-pol replication genes and has acquired acute transforming properties due to incorporation of the oncogene v-raf into the proving genome (ref(1) PNAS.RAPP et al (1983) 80: 4218-4222

J2 was derived from MSV-3611 by incorporating a 2nd viral oncogene, V-MYC, into the viral genome (Ref (2) J virology (1985) SS (1): 23-33 Rapp et al) The function of J2 was to transform primary cells to obtain established cell lines. The presence of V-MYC in addition to V-raf removes1L-3 dependant for growth maintenance in culture (ref 2)

This transformed cell line and retrovirus are laboratory reagents for research, testing and evaluation purposes only. They are not for diagnostic or therapeutic use. The goods are classified as biological specimens category B (UN 3373) and are generated using cell culture techniques (they are not anti serum, blood fractions, nor a modified immunological product. They are not vaccine for human or veterinary use)

Evaluation of foreseeable effects

BV-2 cells transformed by replication defective J2 retrovirus (recombinant murine sarcoma virus 3611 containing viral oncogenes Myc & Raf). Other lymphoid cell lines transformed with J2 such as GG2EE have shown to produce viral particles due to the presence of endogenous virus.
Transformed cells and J2 virus have been shown to induce tumours when injected into mice, although the transforming efficiency of J2 is dependant to the pseudotype. Risk to the environment is minimal. Please see enclosed risk assessment. Bio-RA-0081

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste collected from flasks & plastics is decontaminated for a minimum of 12 hours using phoraid disinfectant. This is left in a sealed container during this time to ensure 100% kill.

2. Solid waste is placed in a biohazard bin. The bin is lined with two biohazard autoclave bags which, at the end of the working day are sealed and the lid locked into position so that the container cannot leak or the lid removed. The bins are labelled with the biohazard sign and carries the UN 3291 number. Bins are stored in a locked brick store with concrete floor until collection by Grundons Waste Ltd. Grundons Waste Ltd are certified to handle and dispose of level 2 waste, which once collected is incinerated ensuring 100% kill. Grundons ref= Biofool

3. Tips and other plastic ware which may contain small amounts of liquid after use will be placed in phoraid disinfectant for a minimum of 12 hours before being treated as solid waste.

4. A biohazard spill kit is available in the area.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

After lengthy discussion, the GMSC felt that all work undertaken for this project should be carried out in a Class 2 safety cabinet. That a thorough decontamination procedure should be strictly adhered to. Due to the space constraints in the level 2 laboratory, no other work should be carried out in the area during the running of this project. No other cell lines will be used or grown in the level 2 laboratory while working on this project. Staff working on this project should be thoroughly trained and have a full understanding of the possible risks involved.

**Project Containment**
Project Ref 680/08.1

Date Ackn'd 19/06/2008
CU2 Project Title Use of replication deficient adenovirus to transfect mammalian cells.

Class 2
Non-GMM Not Applicable

CultureVolumeClass3-4

Consent Granted

< 1 Litre

Project notified under transitional arrangements N

Withdrawn N
Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To transfect mammalian cells for the purpose of drug discovery, including human ion channels, GPCR's or kinases. Various experimental techniques may be used including automated electrophysiology, radiochemical and biochemical assays using live transfected cells or cell lysates.

Recipient or parental organism
Mammalian cell lines including, but not limited to: HEK 293, CHO K1

Host/vector system
E1 replacement adenovirus serotypes vector with E2A gene deleted. Replication incompetent. Fiber protein pseudotypes include Ad5 and Ad50.

Origin & function
Recombinant adenovirus containing genes of interest will be produced in the PERC 6 E2A packaging cell line and purified by Biofocus DPI, Leiden, The Netherlands. The purified virus will be shipped to Biofocus DPI UK and used to overexpress the inserted genes in mammalian cells for the purpose of drug discovery research.

The fiber protein that interacts with the cell surface receptors and determines tissue tropism be either wild type Ad5 (coxsackie and adenovirus receptor dependent) or Ad50 pseudotype (SC46 dependent) which will be used to infect cell lines that are refractory to Ad5, e.g. colon tumor cell lines.

**Evaluation of foreseeable effects**

Due to the deletion of the E1 and E2A genes the virus is non-replicative human virus that can only propagate within the packaging cell line. Recombinant adenovirus may mimic wild type adenovirus with exposed individuals experiencing mild respiratory illness. The Ad50 pseudotype may also infect cells/tissues that are not normally infected by wild type Ad5, such as leukemic cells. The initial infection is unlikely to be sustained or spread via viral shedding since it is not replicative. Even if replication competent viruses were generated the risk is low since human adenovirus infection is very common and the majority of adults are immunised. Furthermore the inserted genes will not alter the pathogenicity, stability or immunotoxicity compared to wild type adenovirus.

Live adenovirus is relatively stable and may persist for long periods if released into the environment and may pose a low risk to animals/plants/humans. However, cross species transmission of human adenovirus is generally thought not to occur, so the greatest risk is to young children and immunocompromised patients. Transport of virus and waste will only be undertaken in appropriate UN certified containers.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste collected from flasks and plastics is decontaminated overnight using phoraid disinfectant (validated). This is left in a sealed container overnight to ensure 100% kill. Solid waste is placed in a yellow biohazard bin. The bin is lined with two biohazard autoclave bags which, at the end of the working day, are sealed and the lid locked in position that the container cannot leak or the lid be removed. The bins are labelled with a biohazard sign and carries the UN 3291 number. Full bins are stored in a locked brick store with concrete floor until collection by Grundens Waste Ltd. Grundens Waste Ltd are certified to handle and dispose of level 2 waste, which once collected is incinerated ensuring 100% kill. Grundens ref:Bio001. Bins used are UN approved for hazard packing group 2.

Tips and other plasticware which may contain a small amount of liquid after use is placed in phoraid disinfectant (validated) overnight before being treated as solid waste. Level 2 waste bins are segregated from level 1 waste bins when stored. Disinfected liquid waste is removed from site for incineration by an approved contractor. Spill kits are available in all biology labs.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
Members of the GMSC had access to written papers regarding the origins of the adenovirus and its modifications in advance of the meeting. They were given both risk assessments which accompany this form. After the meeting initial concerns were raised regarding the possible infection of other cell lines being cultured in the tissue culture laboratories and also the possible risk to the environment. Should there be an accidental spill or leak. Our molecular biologist gave a clear and informative presentation explaining how the virus is unable to replicate either in vitro or in vivo due to the deletion of both the replication incompetent E1 gene and the deletion of the E2 gene.

The GMSC were also informed that cells transfected with the adenovirus would be handled in isolation and away from wildtype or susceptible cell lines. The meeting was closed with all members happy that the risk assessments were thorough and that they had a full understanding of the theory behind the replication deficient adenovirus and that the risks are adequately controlled.

### Project Containment

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<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

### Project Ref 680/09.1

**Date Ackn'd**
26/02/2009

**CU2 Project Title**
To determine the effects of compounds on pyruvate dehydrogenase kinase activity in cells as potential drug candidates

**Date Project Ceased**

**Consent Granted**
Not Applicable

**Class CultureVolClass2 CultureVolumeClass3-4**
Class 2 < 1 Litre

**Project notified under transitional arrangements**
N

**Historical Significant Changes**
Withdrawn N

**Date of Significant Change**

**Project Additional Information**

02/03/2022
<table>
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<tr>
<th><strong>Purposes of the contained use</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>The stdH cell line used for this assay originates from a transgenic mouse containing mutated huntingtin gene. This cell line has been immortalized using a retrovirus containing SV40 large T antigen. The SV40 large T antigen is capable of binding to tumor suppressor genes and may have the potential to cause tumors.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Recipient or parental organism</strong></th>
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</thead>
<tbody>
<tr>
<td>E14 striatal precursor cell lines from mouse E14 wild type.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Host/vector system</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Express knockout-in human (Exon 1)/mouse huntingtin gene (homozygous) with varying poly-glutamine lengths (Q7-CAG repeat of N-7 glutamines; Q111 - 111 glutamines)</td>
</tr>
<tr>
<td>Vector: tsA58/U19 large T antigen.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Origin &amp; function</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>STHdh cells will be maintained as with any adherent cell line. They will be differentiated by temperature change to neurons and possibly with the addition of the factors xFGF, IBMx, TPA, Forskolin and dopamine. Cells will be treated with chemical compounds, homogenized and then snap frozen. Once resuspended they will be permeabilized in 0.1% Triton containing buffer. Absorbance is then monitored to determine PDC activity.</td>
</tr>
<tr>
<td>Note: The differentiation protocol (heating cells to 39°C) should destroy the SV40 particles.</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th><strong>Evaluation of foreseeable effects</strong></th>
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<tbody>
<tr>
<td>The differentiation protocol should destroy the SV40 particles. The cells were immortalized with a retrovirus encoding the tsA58 large T-antigen (a transcription factor found in SV40 virus) the retrovirus is replication-defective and so no infectious virus particles can be generated.</td>
</tr>
</tbody>
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<thead>
<tr>
<th><strong>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</strong></th>
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<tbody>
<tr>
<td>N/A</td>
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<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
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<tr>
<th><strong>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</strong></th>
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</table>
| > Liquid waste collected from flasks and plastics is decontaminated overnight using a validated disinfectant. This is left in a sealed container overnight to ensure 100% kill.  
> Solid waste is placed in plastic bins (UN approved for hazard packing group 2). Bins are lined with two biohazard autoclave bags which at the end of the working day are sealed so that the bin cannot leak or the lid be removed. The bins are labelled with a biohazard sign and carries the UN 3291 number. Full bins are stored in a locked brick store with concrete floor until collection (weekly) by Grundons Waste Ltd. Grundons Waste Ltd are certified to handle and dispose of level 2 waste, which one collected is incinerated, ensuring 100% kill. > Tips and other plastic ware which may contain a small volume of liquid after use is placed in a validated disinfectant overnight before being treated as solid waste. > Level 1 & 2 waste bins are segregated when stored in the brick store. > Disinfected liquid waste is removed from site for incineration by an approved contractor. > Biological spill kits are readily available in all labs. |
The article "Dominant phenotypes produced by the HD mutation in STHdh striatal cells" was given to all members of the GMSC 24 hrs before the meeting along with the risk assessment for the project.

Generally, the GMSC was happy that the risk assessment had highlighted areas of higher risk and steps were taken to try to reduce that risk. Unanimously, the GMSC felt that a commercially available kit (MSP18 Mitoprogile Rapid microplate assay kit for PDH activity - by Mitosciences) should be tried as this would eliminate the homogenization steps of freeze/thawing and high speed centrifugation, enabling the whole protocol to be carried out in one tissue culture laboratory and inside a class II cabinet. It will also require less protein and therefore less cells.

### Project Containment

<table>
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<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2</td>
<td>L3</td>
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### Project Ref 680/10.1

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<tr>
<td>11/03/2010</td>
<td>Single channel patch clamp electrophysiology evaluation of adenoviral shRNA corrected Delta F508-CFTR</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Project notified under transitional arrangements</td>
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<th>Date Project Ceased</th>
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<tbody>
<tr>
<td>11/03/2010</td>
<td>N</td>
<td>N</td>
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</table>
### Project Additional Information

**Purposes of the contained use**

Evaluation of 19 adenoviral shRNA-corrected Delta F508-CFTR variants for the purpose of drug discovery, techniques to include routine culturing of cells, transfection and conventional patch clamping.

**Recipient or parental organism**

Bronchial Epithelial cells

**Host/vector system**

pSVori-plasmid (replication defective SV40 virus)

**Origin & function**

The immortalized CFTR cell line originates from Dr. *****. California Pacific Medical Centre Research Institute. San Fransisco, CA, USA

CFBE41 cells are immortalized using the pSVori plasmid (replication defective SV40 virus). These cells will be transfected using 19 Adenoviral targets (see GM notification 680/08.1 entitled "Use of replication deficient adenovirus to transfect mammalian cells") previously shown to promote activity of the delta F508-CFTR. The transfected cells will then be evaluated by conventional patch clamp.

**Evaluation of foreseeable effects**

Exposure to the immortalized cell line has a low risk of foreseeable effects. No infective viral particles can be produced by pSVori. Co-infection of the cell line with another SV40 virus will not rescue pSVori because the origin of replication is non-functional. There may be a risk of rescue by recombination with replication competent SV40 virus but since the viral genome encoded by pSVori does not contain any heterologous genes the virulence or pathogenecity of the rescued virus would not differ from wild type SV40 virus.

If released into the environment, the cell line is not viable without the defined cell media. The cell line is transformed and therefore has the capacity to form tumors but would require oral or subcutaneous exposure.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste collected from flasks and plastics is decontaminated overnight using a validated disinfectant. 12 hour contact time ensures 100% kill. Liquid is held in sealed containers until discarded. Disinfected liquid waste is removed from site for incineration by an approved contractor.

Flasks, tips and other plastic consumables which may retain small levels of liquid are immersed in validated disinfectant overnight before discarding as solid waste.

Glass pipettes and coverslips are placed in a biohazard burn bin. This is disposed of as solid waste.

Solid waste is placed in biohazard bins which have been lined with two autoclave bags. At the end of the working day the bins are sealed and the lid locked in the position so that the container cannot leak or the lid be removed. The Bins are UN approved for the transport of packing group 2 substances and are labelled with a biohazard sign which carries the UN3291 number. Full bins are stored in a lockable brick store with concrete floor and are segregated from level 1 and other waste until collection by Grundons waste limited. Grundons Waste LTD are certified to handle and dispose of (by incineration) level 2 waste. Grundons GM centre number is 782.

Is an emergency plan required according to regulation 20? N

Tick to confirm that you have attached a risk assessment to this form N

If yes, tick to confirm that it is attached to this form

Members of the GMSC were given the following papers to read for background information.

1. Established cell lines used in Cystic Fibrosis Research
2. Culture and Transformation of Human Airway Epithelial cells.

All were happy that they had understood the procedure and that both the risk to human health and exposure to the environment were very low and that the risk assessments were thoroughly written and covered all aspects of the procedure.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Project Containment

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<td>Yes</td>
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Animal Units  Large Scale Activities  Human Clinical Applications

02/03/2022  Page 10632 of 15326
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

Uropathogen E coli wild type. Strain J96

**Host/vector system**

Luminescence genes from LuxCDABE from Potorhabdus luminescence

**Origin & function**

Antibacterial research and drug discovery

**Evaluation of foreseeable effects**

The wild type J96 strain is a known uropathogen. The insertion of the luxCDBE gene is unlikely to render this strain as non replicating and non infectious. With this in mind, all work will be carried out at BSL 2 and all contaminated wasle will be rendered inactive before leaving the premises (refer to waste risk assessment). Only trained and well informed personnel will be permitted to work on this project and occupational screening will be offered both at the start and end of the project. Therefore it is felt that the risk to both staff and the environment is low.
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste collected from flasks and plastics is decontaminated overnight using a validated disinfectant. 12 hour contact time ensures 100% kill. Liquid is held in sealed containers until discarded. Disinfected liquid waste is removed from site for incineration by an approved contractor.

Flasks, tips and other plastic consumables which may retain small levels of liquid are emmersed in validated disinfectant overnight before discarding as solid waste.

Solid waste is placed in biohazard bins which have been lined with two autoclave bags. At the end of the working day the bins are sealed and the lid locked in the position so that the container cannot leak or the lid be removed. The bins are labelled with a biohazard sign, carries the UN3291 number and are UN approved for packing group 2. Full bins are stored in a lockable brick store with concrete floor and are segregated from level 1 and other waste until collection by Grundons waste limited. Grundons Waste LTD are certified to handle and dispose of (by incineration) level 2 waste. Grundons GM centre number is 782.

This uropathogen can sometimes be asymptomatic and therefore does not always show signs of urinary tract infection before causing infection to the kidneys. With this in mind the GMSC felt that screening should be offered to people working with this strain at both start and end point of the project. It was felt after much discussion that the screening should be made compulsory and that staff who were not happy to take the test should not be permitted to work with the organism.

Please enter comments on the GM safety committee on the risk assessment

This uropathogen can sometimes be asymptomatic and therefore does not always show signs of urinary tract infection before causing infection to the kidneys. With this in mind the GMSC felt that screening should be offered to people working with this strain at both start and end point of the project. It was felt after much discussion that the screening should be made compulsory and that staff who were not happy to take the test should not be permitted to work with the organism.

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Animal Units | Large Scale Activities | Human Clinical Applications

02/03/2022
To develop an assay to screen compounds for the purpose of lowering mutant Huntingtin in Huntingdon’s disease patients.

**Cells have been established using the Epstien Barr Virus and also have the potential for carrying viruses, latent viral genomes and other infectious agents in an inapparent state.**

**Recipient or parental organism**

Huntingdon’s disease patient derived B-Lymphocyte

**Host/vector system**

N/A

**Origin & function**

Cells have been established and immortalized by Epstien Barr Virus transformation of peripheral blood mononuclear cells using Phytohemaglutinin as a mitogen.

**Evaluation of foreseeable effects**

At assay end point, the cells are lysed which should be sufficient to destroy any infectious material including EBV particles.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All level 2 waste is chemically treated using validated disinfectants and ensuring 100% kill. All solid and liquid waste is housed in sealed containers and collected for incineration by approved contractor. Derogation requested for omission to autoclave waste.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste collected from flasks and plastics is decontaminated overnight using a validated disinfectant. 12 hour contact time ensures 100% kill. Liquid is held in sealed containers until discarded. Disinfected liquid waste is removed from site for incineration by an approved contractor. Flasks, tips and other plastic consumables which may retain small levels of liquid are immersed in validated disinfectant overnight before discarding as solid waste. Solid waste is placed in biohazard bins which have been lined with two autoclave bags. At the end of the working day, the bins are sealed and the lid locked into position so that the container cannot leak or the lid be removed. The bins are UN approved for the transport of packing group 2 substances and are labelled with a biohazard sign which carries the UN3291 number. Full bins are stored in a lockable brick store with concrete floor and are segregated from level 1 and other waste until collection by Grundons waste limited. Grundons waste Ltd are certified to handle and dispose of (by incineration) level 2 waste. Grundons GM centre number is 782.

Final assay plates will contain lysed cells, but will still be treated with disinfectant to ensure 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Generally the members of the GMSC were satisfied with the current risk assessment in place, however it was decided that due to the fact that the supplier of the cells could not guarantee the cells did not carry other infectious material, that this should be better highlighted on the risk assessment (now in place) and should also be taken into account when training staff on the project. It was also acknowledged that the risk assessment may change slightly as the work develops and that the risk assessments would be updated accordingly, although the actual risk to staff should not be higher than currently declared.
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### Project Ref 680/14.1

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<tr>
<td>11/02/2014</td>
<td>In vitro assays designed to identify potential control agents for Pseudomonas aeruginosa</td>
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<th>Project notified under transitional arrangements</th>
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### Project Additional Information

**Purposes of the contained use**

Section 17 refers

**Recipient or parental organism**

Opportunistic pathogenic Pseudomonas aeruginosa wild type strain PA14

**Host/vector system**
Section 17 refers

Origin & function

The recombinant derivative and wildtype strain of Pseudomonas aeruginosa (PA14) are to be supplied by our US Client to enable us to perform the services described in Section 5. Strain PA14 is a human clinical isolate strain that displays a highly virulent phenotype against broad spectrum of organisms.

The "Purpose of the contained use" is research (biological screening) aimed at identifying new chemicals that control the virulence of P. aeruginosa and thus have the potential to act as therapeutic agents.

Section 17 refers with respect to non-disclosure

Evaluation of foreseeable effects

The wild type PA14 strain is a known opportunistic pathogen: it is present in the environment.

The modified strain of the pathogen is not expected to be more virulent than the wild type strain; however due to it being resistant to tetracycline, additional vigilance would be assigned to working with this strain. As first aid treatments for Pseudomonas infections include third generation cephalosporins, aminoglycosides and carbapenems, then resistance to tetracycline is not expected to incur further hurdles for its treatment should a worker become infected (ie. It is unlikely that the modified strain would pose a greater threat to human health or the environment than the wild type strain).

All open work will be carried out at BSL 2 and all contaminated waste will be rendered inactive before leaving the premises (refer to waste risk assessment). Only trained, competent and well informed personnel will be permitted to work on this project and health screening for P. aeruginosa infection would be undertaken should workers show signs of chest, eye, intestinal, or urinary tract infections.

Any employees who are assigned to the following categories would not be allowed to work with this organism: immunocompromised, diabetics, anyone with cuts and/or burns, anyone with an existing illness/infection and/or are known to have an allergy to antibiotics (see risk assessments).

By ensuring that we work within the guidelines described in our risk assessment we believe that the risk to both staff and the environment is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste collected from plastic flasks and other open plastics is decontaminated overnight using a validated disinfectant. 12 hour contact time ensures 100% kill. Liquid is held in sealed containers until discarded. Disinfected liquid waste is removed from site for incineration by an approved contractor.

Flasks, tips and other open plastic consumables which may retain small levels of liquid are immersed in validated disinfectant overnight before discarding as solid waste.

Solid waste is placed in biohazard bins which are lined with two autoclave bags. All solid waste will be autoclaved and then placed back into the burn bins which are then sealed and the lid locked in the position so that the container cannot leak or the lid be removed. The bins are labelled with a biohazard sign, carries the UN3291 number and are UN approved for packing group 2. Full bins are stored in a lockable brick store with concrete floor and are segregated from level 1 and other waste until collection.
by Grundons Waste Limited. Grundons Waste LTD are certified to handle and dispose of (by incineration) level 2 waste. Grundons GM centre number is 782.

The GMSC were given procedure protocols and risk assessments prior to the meeting.

The following points were raised:

1. Investigations to be made to determine if the absorbance read could be done using sealed plates as opposed to lidded plates, thus reducing the risk of spill. This investigation was carried out and it was concluded that this indeed could be done. The protocol has been changed accordingly.

2. Concerns raised about the amount of equipment the protocol was suggesting to be placed in the class 2 safety cabinet at any one time. This would affect the flow and reduce the safety efficiency of the MBSC. The first point now means that there is no need for the reader to be placed in the MBSC. The protocol has been changed accordingly.

3. The GMSC also discussed the number of plates to be run at any one time. It was decided that the work should be comfortably managed in one day, that the MBSC should not be overfilled and that movement around the MBSC should be minimal so as not to disrupt airflow. Batches of 5-10 plates seemed to be the ideal.

4. It was decided that emergency clothing should be available inside the ante-room. Should clothing become contaminated it would be autoclaved to ensure contamination did not infect the environment.

5. It was suggested that swabbing was carried out on door handles, taps and benches to ensure there was no breach of containment. This should be done at the end of each phase of the project. As this would be easy to carry out, there seemed no good reason not to do it as a "belt and braces" exercise.

The GMSC were comfortable in assigning the strain of P. aeruginosa to Biohazard Level 2.

Although P. aeruginosa is an opportunistic pathogen (i.e. more likely to infect individuals who are sick or immunocompromised, as opposed to healthy individuals), it can cause a wide range of infections, particularly among immunocompromised people (HIV or cancer patients) and persons with severe burns, diabetes mellitus or cystic fibrosis. Consequently, only trained, competent and well informed personnel will be permitted to work on this project. Any employees who are in the following categories would not be allowed to work with this organism: immunocompromised, diabetics, have cuts and/or burns, have an existing illness/infection and/or are known to have an allergy to treatment antibiotics. Health screening for P. aeruginosa infection would be undertaken should workers show signs of chest, eye, intestinal, or urinary tract infections.

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Animal Units  Large Scale Activities  Human Clinical Applications

02/03/2022  Page 10639 of 15326
The aim of the project is to use small molecule libraries and RNAi using non viral transfection vectors, for functional screening of Pu.1 activity.

Non-viral the pu.1 containing cDNA expression vector comprises an expression plasmid (pGL4.23) with a 5X lambdaB reporter. A neomycin resistance cassette is included in the targeting vector.

Viral: the murine cell line (BV-2) was generated by infecting primary microglial cell cultures with a v-raf/v-myc oncogene carrying retrovirus (J2) described by Blasi et al, in J Neuroimmunol. 1990 May;27(2-3):229-37.
Pu.1 transcription factor. Luciferase is a naturally occurring oxidative enzyme that catalyses the conversion of luciferin to oxyluciferin and light.

**Evaluation of foreseeable effects**

The BV-2 cell line produces an enveloped recombinant ecotropic murine retrovirus such virus is known for its in vitro transforming ability and in vivo tumorigenic potential. It should be capable of infecting murine cells only and therefore presents a risk from accidental release into the environment.

There is also a minor risk of the J2 virus infecting human lymphocytes following prolonged (24 hour) exposure as described by Peppoloni et al (Experientia. 1988 Dec 1;44(11-12):1013-5) which should be evaluated as a potential risk to the operator.

BSL 2 containment and control measures as described in the risk assessment, handling within class II biological safety cabinet, wearing personal protective equipment and treating all contaminated waste with validated disinfectant will minimise both of these risks to very low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

All BV-2 cells are lysed as part of the luciferase assay protocol. All category 2 waste from both the screening assay and tissue culture activities is first treated with validated disinfectant overnight to ensure no viable material remains, then double biohazard bagged and disposed for incineration by an approved contractor. Transport to the incinerator is undertaken in UN approved packaging.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All BV-2 cells are lysed as part of the luciferase assay protocol. All category 2 waste from both the screening assay and tissue culture activities is first treated with validated disinfectant overnight to ensure no viable material remains, then double biohazard bagged and disposed for incineration by an approved contractor. Transport to the incinerator is undertaken in UN approved packaging.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

Please enter comments on the GM safety committee on the risk assessment
The prolonged exposure required for infection of human lymphocytes is not tenable under the conditions of use.
We have sufficient pest control measures in place on site so that even in the case of an unexpected failure of containment during the experiment there would be no murine exposure to the wild population.
Following disinfection, the packaging used in transport to the incinerator is designed to prevent any escape to the environment, even though what is being transported is not viable.

Project Containment

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Project Ref 680/17.1

Date Ackn'd: 05/05/2017
Date Project Ceased:

Characterization of the biological effects of Huntington's Disease (HD)-causative mutation

Class 2  ≤ 1 Litre
Non-GMM  Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
### Purposes of the contained use

Cell lines containing either wild-type or gene-edited versions of the human Huntingtin (HTT) gene will be supplied by the Rockefeller University, New York. Cell lines will be grown and differentiated into neuronal cells via characterized small-molecule induction protocols. Samples will be taken at defined points in the process in order to examine differences among the lines (which contain gene-edited alterations of the human HTT gene), using immunohistochemistry and quantitative methods of mRNA determination (e.g. branched DNA assay or qPCR). No further genetic modification is anticipated with these cell lines. Both of these techniques result in the chemical destruction of the cells and removing the potential biological hazard. Identification of differences in the lines may lead to potential new HD therapies or biomarkers to assess the effects of HD treatments.

### Recipient or parental organism

Human embryonic stem cells (RUES2) were derived from a de-identified frozen embryo that was originally generated for reproductive purposes and later donated with informed consent for research purposes. RUES2 cells are sourced from The Rockefeller University, New York. These cells are unscreened for adventitious pathogenic viruses—therefore they are treated as potentially infectious and handled at BioSafety Level 2. In addition, RUES2 cells are totipotent with the potential to form teratomas in immunosuppressed individuals.

### Host/vector system

Non-mobilizable, non-episomal, pUC based plasmids were introduced into the supplied cells by electroporation.

### Origin & function

1. **Homologous Recombination (HR) Targeting Vector.** The vector comprised a synthetic left and right homology arm against the human HTT gene that flank a donor sequence. The donor sequence contained the first exon of the human HTT gene with either normal or expanded lengths of CAG repeats and viral Inverted Tandem Repeats (ITRs) flanking a Puromycin-Thymidine Kinase (TK) positive-negative selection cassette; the HTT exon1 was obtained by PCR from genomic DNA extracted from fibroblasts or iPSCs derived from human HD patients.

2. **Cas9 plasmid contained a synthetic Cas9 endonuclease; codon-optimized for human expression, and a synthetic sequence for the guide RNA targeting the human HTT gene.**

3. **Transposase Plasmid expressing integration-deficient PiggyBac transposase.**

How the supplied cells were generated

The HR targeting vector and Cas9 plasmids were designed to replace the parental HTT exon 1 CAG repeat with a normal or expanded length of CAG repeats to create either a null hESC cell line or a mutant HTT hESC cell line. The inserted Puro-TK selection marker was used to isolate cells that have undergone homologous recombination by positive selection with puromycin. The Transposase plasmid was then used to excise the Puro-TK selection marker without leaving behind any residual residues of the targeting vector. Cells that did not have the Puro-TK marker excised were killed by negative selection with ganciclovir (a pro-drug that is converted into a DNA synthesis inhibitor by TK).

### Evaluation of foreseeable effects

The supplied GMM will possess either a wild type and/or a mutant HTT gene. The presence of an extended CAG repeat in the mutant HTT gene results in mHTT protein aggregates which are toxic to cells, particularly neuronal cells. Plasmid reagents used for the gene editing (Cas9 & Transposase) are not transmissible, do not integrate and are not maintained episomally; they will have been removed from the supplied cell lines through prolonged culturing. The
completed gene editing process will also leave no extraneous residues, such as DNA regulatory sequences or antibiotic selection markers, present in the vectors.

Foreseeable effects from GM.

RUES2 cells are unscreened for adventitious I pathogenic viruses and are therefore treated as being potentially infectious. The supplied genetically modified cells will be handled under BSL2 conditions. As RUES2 cells are totipotent with the potential to form teratomas in immunosuppressed individuals no such individual will be allowed to culture these cells. Non-immunosuppressed individuals would clear the cells rapidly and as such they are not at risk.

Very low risk to human health from the genetic modification. WtHTT/mHTT is not an oncogene, tumor suppressor, hormone, cytokine or involved in the immune system. The genetic modification was conducted in the suppliers laboratories and no further modification is anticipated. Although mHTT protein aggregates are toxic within a cellular context they are non-transmissible and will therefore pose negligible risk.

Very low risk to environment since there are no antibiotic resistant genes or viral/plasmid sequences present and the cells cannot survive outside of defined culture media used within a laboratory environment. Although cells can form teratomas when injected in nude mice there is no chance that this can happen.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We wish to request derogation from the need to autoclave solid waste material. All material (solid and liquid) will be chemically inactivated for 24hrs prior to it being stored securely before being taken away for incineration by an authorised waste disposal contractor. This is sufficient for the level of hazard posed by the materials in question.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste is placed in biohazard bins which are lined with two autoclave bags. Flasks, tips and other plastic consumables which may retain small levels of liquid are immersed in validated disinfectant for 24 hrs before being discarded as solid waste.

At the end of the working day solid waste is sealed in double bags which are then placed in lockable wheelie bins (supplied by Grundons, an external waste disposal contractor (GM Centre number 782)). These are stored in a locked brick waste store with concrete floor until collection by an authorised contractor. Waste is then incinerated ensuring 100% kill.

Only containers certified to UN packing group* or better are used. Solid waste is sealed and the contents and bags incinerated without being opened.

Liquid waste is chemically inactivated for 24hrs with a validated disinfectant prior to disposal. Chemically inactivated liquid waste is decanted into 10 litre waste containers where it remains until removal from site for incineration by an authorised contractor, ensuring 100% kill. Due to site rules, there is no disposal to drains.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The RUES2 embryonic cell line was initially derived from unscreened human material. As such it is treated as potentially infectious. These cells have been genetically modified in the laboratories of the Rockefeller University, New York, to introduce gene sequences encoding mHTT via homologous recombination. The techniques used result in the removal of any extraneous regulatory sequences and selectable markers. The genetic modification does not increase the risk posed by the cells to humans or the environment. Cells cannot survive outside of the laboratory. It is noted that there is a small risk of tetraomas forming in immunosuppressed individuals if injected with RUES2 cells. Such individuals will not work with this material and no sharps will be used.

The main risk comes from the presence of adventitious human pathogens and it is appropriate for the cells to be classified as level 2 and handled as such.

Project Containment

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<tr>
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<td>Human Clinical Applications</td>
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Project Ref 680/17.2

Date Ackn'd 19/07/2017

Date Project Ceased

CU2 Project Title Use of genetic modification to investigate potential therapeutic targets which modify phenotypes of Huntington's Disease

Class 2

Culture Vol < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
**Project Additional Information**

### Purposes of the contained use

Human Embryonic Stem Cells containing either wild-type/endogenous or gene-edited versions of the human Huntingtin (HTT) gene will be transfected or transduced in order to identify or validate gene targets which will modify or counteract the effects of the CAG expansion in the HTT gene. This may lead to the identification of druggable targets which could be used as potential HD therapies.

### Recipient or parental organism

Human embryonic stem cells (RUES2) were derived from a de-identified frozen embryo that was originally generated for reproductive purposes and later donated with informed consent for research purposes. RUES2 cells are sourced from The Rockefeller University, New York. These cells are unscreened for adventitious / pathogenic viruses - therefore they are treated as potentially infectious and handled at BioSafety Level 2. In addition, RUES2 cells are totipotent with the potential to form teratomas in immunosuppressed individuals.

Human Embryonic Stem Cells (hES cells) (either with a normal HTT gene or with a CAG expansion in the HTT gene in chromosome 4 (mHTT) that is associated with HD disease) will also be used. These cells are ethically sourced from donated human embryos resultant from IVF treatment. There is no information available about whether the parental hES cells have been screened for any pathogens. It is assumed however that there is no previous known history of infectious disease and donors are from epidemiologically low-risk populations. Cells may still contain adventitious / pathogenic viruses and will be treated as potentially infectious. Parental hES cells are totipotent with the potential to form teratomas in immunosuppressed individuals.

### Host/vector system

**Generation of RUES2 Cells**

Non-mobilizable, non-episomal, pUC based plasmids were used in the generation of the supplied RUES2 cells by electroporation.

**Expression of Exogenous Genes**

Commercially available expression vectors will be used to introduce genes to act as reporters or involved in differentiation and/or disease pathogenesis. Random stable integration may be desirable and vectors may contain a selectable marker to enable this activity. The use of these sorts of vectors represents a minimal hazard because they are non-mobilisable, lack a eukaryotic origin of replication and can not enter cells easily.

In some cases very efficient delivery of a gene is required. In this case viral delivery methodologies (e.g. lentivirus) could be used. Viral systems represent a higher risk due to their ability to transduce quiescent cells and subsequently integrate into the host genome. This integration may potentially inadvertently activate or inactivate genes with deleterious consequences. The risk of this happening will be mitigated by obtaining small aliquots of pre-prepared packaged viral particles that lack the necessary genes for replication. Any transduction or subsequent transduction will only occur once the GMSC has given approval and is satisfied that in the case of an additional transduction there is a lack of sequence homology between the viral systems being used to decrease the chance of recombination.

**Knock-down of Gene Expression with shRNA**

Lentiviral particles used for either delivery of shRNA constructs for 'knock down' of target gene expression or overexpression of a particular gene will be obtained from commercial sources. Viral particles will be generated using third or fourth generation viral packaging systems. These systems have the following safety features:
1. Viruses produced using third or fourth generation systems are unable to replicate. They have been attenuated in several ways. The gene required to produce viral packaging proteins (en v) is absent from the packaged viral genome. The genes required for viral replication gag, pol, and accessory genes, tat, rev, vif, vpr, vpu, and nef, are also absent from the packaged genome. The env, gag and pol genes (which are sufficient and necessary for viral production) are provided transiently on three separate expression vectors that lack homology during viral particle production. The genetic material to be packaged is supplied on a forth plasmid that is the only vector to contain a packaging motif. The lack of homology essentially prevents recombination in the packaging cell line and hence the chance that genes required for virus replication are packaged into lentiviral particles is minimal.

2. The vectors are self-inactivating. This means that upon integration into the genome of a target cell the Long Terminal Repeats (LTRs) are truncated and this prevents mobilisation of the introduced genetic material.

3. It is noted that nearly all viral delivery systems contain the Woodchuck Hepatitis Post-Transcriptional Regulatory Element (WPRE) to enhance transgene expression. It is known that the WPRE contains an oncogenic sequence unless it has been specifically deleted. The increase in risk due to the ability to infect a wide variety of cell types, including quiescent cells, and the potential presence of an oncogenic sequence in the WPRE is mitigated by using small aliquots of virus. There will be no in-house production of lentiviral particles and the use of sharps is prohibited when using this material to prevent inadvertent percutaneous inoculation.

4. There remain the risk of insertional mutagenesis/inappropriate (in)-activation of a gene. This is reduced by the use of small, single use aliquots and that the material will be handled in a Class II MBSC. Sharps are banned in the tissue culture labs which will prevent the risk of sharps injuries during routine work.

To increase tropism these viruses contain the protein product of the VSV-G gene from Vesicular Stomatitis Virus in their envelope. This increases the hazard but not significantly as a worker is unlikely to be exposed to a sufficient dose to lead to a transformation of cells.

When using lentivirus oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the programmable nucleases and shRNA that will be expressed or targeted will not be designed to disrupt any growth factors, trophic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus or with human ES cells will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by not using sharps in the lab and adhering to other appropriate risk management measures.

Targetted Gene Modification by CRISPAI-Cas9

hES cells will be transiently transfected (e.g. lipofected or nucleofected) with plasmids D010400-hCas9 v1 or D010400-hCas9_T2A_ZsGreen1. Nucleofection is an electroporation-based transfection method that enables the DNA to enter directly the nucleus. Vectors have a pcDNA3.1 backbone and express codon optimized RNA-guided DNA endonuclease enzyme Cas9 from Streptococcus pyogenes under the control of the CMV promotor. The Neomycin mammalian selectable maker is included. The D010400-hCas9_T2A_ZsGreen1 vector additionally expresses the GFP. In order to deliver the guide RNA (gRNA) the plasmid G150300 will be transiently nucleofected into the cells. G150300 is based on the gWIZ vector with Kanamycin resistance. The gRNA is expressed under the
control of the RNA polymerase III promoter U6. The vector additionally encodes a gRNA scaffold necessary for correct functioning of Cas9. Cells will be cloned by FACS sorting individual cells into separate wells of a 96-well plate. The plan is to knock out a gene in hES cells by inducing two Cas9 mediated DNA double-strand breaks by transfecting Simultaneously with two gRNAs targeting the same gene. The endogenous non-homologous end joining (NHEJ) pathway will repair the DNA double-strand breaks and destroy the mRNA integrity and therefore the protein production.

To deliver the guide RNA (gRNA) the plasmid G150300 will be nucleofected into the cells. Additionally cells will be nucleofected with another commercial available vector in order to provide the template for the homology directed repair. The plan here is to introduce one gRNA directed Cas9 mediated DNA double-strand break but this time we will provide a DNA template encoding our tags and on both sides homology arms for the homology directed repair (HDR) to introduce well-characterised protein tags such as, but not limited to, GFP, FLAG or luciferase.

It is not expected that this work will increase the hazard already associated with the human derived ES cells.

Origin & function

RUES2 Cells

1) Homologous Recombination (HR) Targeting Vector. The vector comprised a synthetic left and right homology arm against the human HTT gene that flank a donor sequence. The donor sequence contained the first exon of the human HTT gene with either normal or expanded lengths of CAG repeats and viral Inverted Tandem Repeats (ITRs) flanking a Puromycin-Thymidine Kinase (TK) positive-negative selection cassette; the HTT exon1 was obtained by PCR from genomic DNA extracted from fibroblasts or iPSCs derived from human HD patients.

2) Cas9 plasmid contained a synthetic Cas9 endonuclease; codon-optimized for human expression, and a synthetic sequence for the guide RNA targeting the human HTT gene.

3) Transposase Plasmid expressing integration-deficient PiggyBac transposase.

How the RUES2 cells were generated

The HR targeting vector and Cas9 plasmids were designed to replace the parental HTT exon 1 CAG repeat with a normal or expanded length of CAG repeats to create either a null hESC cell line or a mutant HTT hESC cell line. The inserted Puro-TK selection marker was used to isolate cells that have undergone homologous recombination by positive selection with puromycin. The Transposase plasmid was then used to excise the Puro-TK selection marker without leaving behind any residual residues of the targeting vector. Cells that did not have the Puro-TK marker excised were killed by negative selection with ganciclovir (a pro-drug that is converted into a DNA synthesis inhibitor by TK).

Expression of Exogenous Genes

For systems to monitor the promoter activity of genes involved in differentiation and/or the causative disease gene; two options will be used:

- Direct expression of well characterised reporter genes such as but limited to luciferase or GFP.
- Expression of the gene fused with a reporter gene.

Genes which may be involved in the pathogenesis of HD may be over-expressed either as full length or truncated proteins. Fusions with epitope tags or reporter proteins will also be viewed as an option to enable monitoring of the location and effect of the protein in a cellular environment.

Commonly used reporter genes represent a minimal hazard. Expression of genes involved in disease pathogenesis are unlikely to pose a significant risk because expression would need to be in the brain and in normal epidemiology is present from birth. If the expression of confirmed oncogenes, growth factors, tropic factors or immunomodulators is required this will be via non-viral methodologies. The expression of other genes will have the oversight and require approval from the GMSC.

Knock-down of Gene Expression with shRNA
shRNA constructs will be selected based on project needs to 'knock-down' gene expression of a target gene. This will be subject to oversight by the GMSC. Particular attention will be made to avoid the use of shRNA towards known tumour suppressors, trophic factors and growth factors. Targetted Gene Modification by CRISPAICas9
gRNA sequences will be designed to drive specific targetted modifications in response to project requirement. In the case of knock-outs and fusion proteins the GMSC will have oversight to check that there is no inadvertant introduction of an oncogene or suppression of a tumour suppressor.

**Evaluation of foreseeable effects**

**Foreseeable Effects on Human Health**
The cells to be used are deemed to have been unscreened for adventitious / pathogenic viruses and will be treated as being potentially infectious and handled BSL2 conditions. In deed this is the biggest risk associated with the proposed work. Workers are immunised against HepB. The cells are totipotent with the potential to form teratomas in immunosuppressed individuals; no such individual will be allowed to culture these cells. Non-immunosuppressed individuals would clear the cells rapidly and as such they are not at risk.
The cells possess either a wild type and/or a mutant HTT gene. The presence of an extended CAG repeat in the mutant HTT gene results in mHTT protein aggregates which are toxic to cells, particularly neuronal cells.
For RUES2 cells the plasmid reagents used for the gene editing (Cas9 & Transposase) are not transmissible, do not integrate and are not maintained episomally; they will have been removed from the supplied cell lines through prolonged culturing. The completed gene editing process will also leave no extraneous residues, such as DNA regulatory sequences or antibiotic selection markers, present in the vectors.
In addition cells may also express exogenous proteins, shRNA or additional targetted gene modifications to allow evaluation of effects of interesting genes upon disease pathogenesis.
There is a very low risk to human health from genetic modifications in the WtHTT/mHTT gene. HTT is not an oncogene, tumor suppressor, hormone, cytokine or involved in the immune system. Although mHTT protein aggregates are toxic within a cellular context they are non-transmissible and will therefore pose negligible risk.
Subsequent modifications will be unlikely to increase the hazard associated with working with the cells. Increases in viability or fitness will not be an issue for non-immunosuppressed individuals.

**Environmental Considerations**
Very low risk to environment since plasmid sequences are non-mobilisable. Gene sequences required for viral replication will not be present. All cell lines cannot survive outside of defined culture media used within a laboratory environment. Although cells can form teratomas when injected in nude mice these are only present within a laboratory environment and there is no chance that this can happen.
In the case of viral particles: the chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to. In the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
We wish to request derogation from the need to autoclave solid waste material. All material (solid and liquid) will be...
Chemically inactivated for 24hrs prior to it being stored securely before being taken away for incineration by an authorised waste disposal contractor. This is sufficient for the level of hazard posed by the materials in question.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste is placed in biohazard bins which are lined with two autoclave bags. Flasks, tips and other plastic consumables which may retain small levels of liquid are exposed to validated disinfectant for 24hrs before being discarded as solid waste.

At the end of the working day solid waste is sealed in double bags which are then placed in lockable wheelie bins (supplied by Grundons, an external waste disposal contractor (GM Centre number 782). These are stored in a locked brick waste store with concrete floor until collection by an authorised contractor. Waste is then incinerated ensuring 100% kill.

Only containers certified to UN packing group II or better are used. Solid waste is sealed and the contents and bags incinerated without being opened.

Liquid waste is chemically inactivated for 24hrs with a validated disinfectant prior to disposal. Chemically inactivated liquid waste is decanted into 10 litre waste containers where it remains until removal from site for incineration by an authorised contractor, ensuring 100% kill. Due to site rules, there is no disposal to drains.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The GMSC has considered the above proposal. The work focuses on efforts to understand the molecular basis for HD pathogenesis with an aim to identify potentially new targets for therapeutic intervention. The cell lines are derived from human materials from reputable sources and as such will be regarded as medium risk requiring containment level 2. The introduction of genetic material, either with the aim to over-express or suppress gene expression, by physical methodologies does not increase the risk associated with the original cell lines. In a similar way the editing of genes by CRISPRiCas9 techniques will not increase the hazardous nature of the original material. The use of lentiviral delivery systems is a convenient means to efficiently deliver genetic material to cells. The commercial products being considered predominantly contain WRPE which contains an oncogenic sequence unless specifically deleted. This represents a hazard to workers which is controlled by the use of small aliquots of virus sufficient for one transduction at a time. To increase tropism the VSV-g envelope protein is used. As a result the deliberate introduction of oncogenes, hormones, hormone receptors, inhibition of tumour suppressor genes or modulation of immune regulators should not be conducted by this methodology if deemed essential to the project. Any use of viral material runs the risk of inadvertent recombination events occurring. There is an assumption that donors have no known medical history of infectious disease which would significantly reduce the chance of recombination leading to the generation of active viral particles. The risk is further reduced because the viral particles lack any of the essential and necessary genes for viral replication and are self-inactivating. So even if the cells did contain an adventitious virus the chance that the introduced material could be mobilised and packaged into active virus is minimal. It is the view of the GMSC that this work can be safely conducted at containment level 2. Proposed genes covered under this RA will be submitted to the GMSC for approval first.

Project Containment

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<th>Growth Rooms</th>
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Animal Units

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Human Clinical Applications

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Project Ref 680/18.1

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<td>Development of a CRISPR-based transcription repression system in Staphylococcus aureus</td>
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Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

molecular genetic study of S. aureus to gain an understanding of its virulence, pathogenesis, & drug resistance leading to the discovery of new therapies for treatment of S. aureus infections.

#### Recipient or parental organism

The parental host strain Staph. Aureus ATCC 25923 is well characterised, and the culture conditions coupled with good microbial practise will prevent any release of either recombinant or host Staph aureus, the host is susceptible to most antibiotics, the Psd1 shuttle plasmid confers constitutive expression of the guide RNA (SgRNA) which is specific to the target gene & only in the presence of tetracycline is there expression of the recombinant Cas9 protein which forms a complex with the SgRNA, physically blocking the RNA polymerase from binding to the promotor (resulting in highly selective reversible transcriptional repression). Due to the small scale of the experimental processes & existing good microbial practises. It is highly unlikely that there would be any significant release of viable recombinant Staph aureous into the environment

#### Host/vector system

This work is to form part of a feasibility study to determine if it is possible to knock-down gene expression in Staphylococcus aureus. It is proposed to use a CRISPR-based transcription repression system that has been modified from the pSD1 CRISPR plasmid described in a publication by Zhao et al (https://www.ncbi.nlm.nih.gov/pubmed/28411216). The CRISPR sequence has been modified to incorporate a ccdB cassette between the SapI restriction sites to enable easier cloning of gRNAs into the vector.

#### Origin & function

pSD1 was constructed in-house in accordance with Zhao et al final construct was sequence verified and published on Pub Med

#### Evaluation of foreseeable effects

determine if it is possible to knock-down gene expression in Staphylococcus
aureus. It is proposed to use a CRISPR-based transcription repression system that has been modified from the pSD1 CRISPR plasmid described in a publication by Zhao et al (https://www.ncbi.nlm.nih.gov/pubmed/28411216). The CRISPR sequence has been modified to incorporate a ccdB cassette between the Sapi restriction sites to enable easier cloning of gRNAs into the vector.

Expression of the guide RNA (SgRNA) which is specific to the target gene & only in the presence of tetracycline is there expression of the recombinant Cas9 protein which forms a complex with the SgRNA, physically blocking the RNA polymerase from binding to the promoter (resulting in highly selective reversible transcriptional repression). Due to the small scale of the experimental processes & existing good microbial practises, it is highly unlikely that there would be any significant release of viable recombinant Staph aureous into the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All Staphylococcus aureus work will be done in a BSL2 designated laboratory. Cultures will be treated to create electrocompetent cells and then transformed with the pSD1 CRISPR plasmid. Any unused cultures or waste material generated during these experiments will be disposed of via approved disposal methods for BSL 2. Specifically, bacterial cultures will be destroyed by autoclaving at 103KPa for 15 mins; equipment contaminated with S. aureus will be soaked in 1% w/v Vircon for 30mins prior to autoclaving at 103KPa for 15 mins. Disposables items will be removed from the site by a reputable waste disposal contractor (Grundons) materials will then be destroyed by incineration. Exposure to any aerosols will be prevented by containment in certified biological level 2 safety cabinets e.g. during centrifugation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

'collective agreement of Level 2 containment'
Project Containment

Laboratory Activities

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 680/18.2

Identification of Sting dimerization modulators

Date Ackn’d: 10/08/2018

Date Project Ceased:

Class 2

Cultures Volume Class 2: < 1 Litre

Non-GMM Consent Granted

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Stable expression of stimulator of interferon genes (STING) -232R & ISRE driven luciferase in

Recipient or parental organism

HEX293T cells

Host/vector system
STING: pMSCVpuro (Clontech, cat# PT3303-5)
LUCIFERASE: pGL4.45[luc2P/ISRE/HYGRO] (Promega, cat# E414A)

Origin & function
STING 232R variant derived from human origin (verified by sequencing)

Evaluation of foreseeable effects
stable expression of STING & luciferase (STING will be measured by luminescence) expression products have been assessed to be non-hazardous to human health or the environment

greatest theoretical risk is from the HEX293T cells which because of the SV40 large antigen warrant BSL2 containment

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

recombinant cell will be inactivated with 10% vircon & then destroyed by incineration by a registered contractor (Grundons)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The presence of the SV40 large antigen in the HEK293T cells, does present a very low theoretical risk to human health & as such warrants BSL2 containment

Project Containment

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<th>Growth Rooms</th>
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Project Ref 680/18.3

Date Ackn'd 01/11/2018

CU2 Project Title Recombinant inducible expression of orphan nuclear receptor (NR2F2) in HEK293T

Date Project Ceased

Class 2

Culture Vol Class 2 ≤ 1 Litre

Non-GMM Consent Granted

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
Identify inhibitors or activators of an orphan nuclear receptor (NR2F2)

Recipient or parental organism
HEK293T

Host/vector system
HEK293T pLVX-TETOne-Puro plasmid expressing Dox inducible NR2F2

Origin & function
Sequence verified by client ChromaCure

Evaluation of foreseeable effects
The expression product is a transcriptional activator of gene expression & might play a role in angiogenesis & might trigger tumour growth, the expression host HEK293T warrants BSL2 containment because of the SV40-
T antigen, which according to The National Academy of Sciences has a moderate association of causing cancer in humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Biological cultures & contaminated consumables will be treated with 10% vircon prior to destruction by incineration by licenced contractors (Grundons).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Collective agreement of BSL2

**Project Containment**

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Animal Units

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**Project Ref** 680/18.4

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<tr>
<th>Date Ackn’d</th>
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<tr>
<td>23/11/2018</td>
<td>Stable expression of SLC6A8 P544L mutant in KO HEK 293T cell line</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>

02/03/2022 Page 10657 of 15326
Project Additional Information

Purposes of the contained use

Stable expression of SLC6A8 P544L mutant in KO HEK 293T cell line

Recipient or parental organism

HEK293T

Host/vector system

The human SLC6A8 gene encodes the sodium- and chloride-dependent creatine transporter 1. This is a HEK 293T cell line in which endogenous SLC6A8 expression has been knocked out using the ThermoFisher Cas9 Blast gene editing system in conjunction with CRISPR targeting SLC6A8 gene following expression of SLC6A8 P544L mutant within a pcDNA6.2 vector.

Origin & function

The human SLC6A8 gene encodes the sodium- and chloride-dependent creatine transporter 1. to identify correctors of SLC6A8 trafficking function.

Evaluation of foreseeable effects

HEK-293T cell line is a highly transfectable derivative of HEK293 cells and contains the SV40-T antigen. The hypothesis that SV40 might cause cancer in humans has been a particularly controversial area of research, notably, a 2002 study performed by The National Academy of Sciences Immunization Safety Review committee that stated, "The committee concluded that the biological evidence is moderate that SV40 exposure could lead to cancer in humans under natural conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated waste is treated with 10% Virkon prior to destruction by incineration by a certified waste contractor (Grundons)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Collective agreement of BSL2 containment

Project Containment

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Project Ref 680/18.5

Date Ackn'd 20/12/2018

CU2 Project Title

Transient expression of zinc finger transcription factors (ZFPs) have been designed to specifically repress huntingtin HTT gene expression

Date Project Ceased

Class 2 CultureVolClass2 < 1 Litre

Class CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use
To investigate the use of targeted ZFPs as a therapeutic tool for the study and treatment of Huntingdon's disease

Recipient or parental organism
RUES CAG56 cell line

Host/vector system
pAAV-6P-SEWB

Origin & function
Rockefeller University Institute for Stem cells

Evaluation of foreseeable effects
The parental UES cells are unscreened for adventitious/ pathogenic viruses, & should be treated as potentially infectious, the cells are totipotent with the potential to form teratomas in immunosuppressed individuals, the transient expression of the targeted ZFPs, have been assessed to be of low risk

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
All level 2 waste is chemically treated using validated disinfectants and ensuring 100% kill. All solid and liquid waste is housed in sealed containers and collected for incineration by approved contractor(Grundons). The building does have an autoclave, due to the bulky nature of the waste. Derogation requested for omission to autoclave waste. The chemical disinfection regime has been assessed to be sufficient to prevent any exposure to infectious biological waste

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All liquid waste collected from flasks & plastics is decontaminated overnight using a validated disinfectant. 12 hr contact time ensures 100% kill. Liquid is held in sealed containers mixed with a validated disinfectant disinfected liquid waste is removed from site for destruction by incineration by an approved contractor (Grundons). Contaminated plastic consumables are immersed in validated disinfectant overnight before discarding as solid waste. Solid waste is placed in dedicated biohazard bins which have been lined with two autoclave bags at the end of the working day, the bags are sealed and then placed into lockable biohazard waste bins (UN approved for the transport of packaging group 2 substances with a biohazard sign which carries the UN3291 number. Full bins are stored in a secure brick store with bunded concrete floor. And then collected for incineration by approved contactor (Grundons).

<table>
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<th>Project Ref</th>
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<td>Date Ackn'd</td>
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<tr>
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<td>Generation and use of Lentiviruses for cell line generation and assay development for</td>
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<td>Class</td>
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<td>&lt; 1 Litre</td>
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<td>CultureVolumeClass3-4</td>
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</table>
### Project Additional Information

**Purposes of the contained use**

The aim of the project is to identify compounds that would inhibit biological activity of ADAR1 in cells. The potential ADAR1 inhibitors would be identified in the cell line overexpressing ADAR1. The mechanism of action for any active compounds is expected to be direct inhibition of editing activity of cellular ADAR1 protein.

**Recipient or parental organism**

HEK298T A549 cells

**Host/vector system**

The client, Agios have cloned ADAR1 variants in lentiviral vector system (commercially available from Addgene https://www.addgene.org/guides/lentivirus/) At CRL the vectors carrying gene of interests will be transfected in the 293T (packaging cells) along with packaging and envelope plasmids. The Lentiviruses produced from this experiment will be used to generate stable A549 cells. In A549 cells, the modifications include stable expression of ADAR1 variants and in addition cell line will also expresses reporter constructs carrying ADAR1 substrate to be edited and hence allowing expression of Nanoluciferase as a reporter gene. The cell line clone showing optimal results will be chosen for screening compounds and potential HTS in agreement with the client.

**Origin & function**

The client, Agios have cloned ADAR1 variants in lentiviral vector system (commercially available from Addgene https://www.addgene.org/guides/lentivirus/)
expression studies will be done using HEK293T cells. HEK-293T cell line is a highly transfectable derivative of HEK293 cells and contains the SV40-T antigen. The hypothesis that SV40 might cause cancer in humans has been a particularly controversial area of research, notably, a 2002 study performed by The National Academy of Sciences Immunization Safety Review committee that stated, "The committee concluded that the biological evidence is moderate that SV40 exposure could lead to cancer in humans under natural conditions. It is for this reason that the GMSC has assessed this program of work as BSL2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All level 2 waste is chemically treated using validated disinfectants and ensuring 100% kill. All solid and liquid waste is housed in sealed containers and collected for incineration by approved contractor (Grundons). The building does have an autoclave, due to the bulky nature of the waste derogation is requested for omission to autoclave waste. The chemical disinfection regime has been assessed to be sufficient to prevent any exposure to infectious biological waste.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste from flasks & plastics is decontaminated overnight using a validated disinfectant. 12hr contact time ensures 100% kill. Liquid is held in sealed containers mixed with a validated disinfectant. Disinfected liquid waste is removed from site for destruction by an approved contractor (Grundons). Contaminated plastic consumables are immersed in validated disinfectant overnight before discarding as solid waste. Solid waste is placed in dedicated biohazard bins which have been lined with two autoclave bags at the end of the working day, the bags are sealed and then placed into lockable biohazard waste bins (UN approved for the transport of packaging group 2 substances with a biohazard sign which carries the UN3291 number. Full bins are stored in a secure brick store with bunded concrete floor, and then collected for incineration by approved contractor (Grundons).

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
collective agreement due to the use of HEK293T that this work requires a BSL2 classification

Project Containment

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Project Ref 680/20.1

Date Ackn'd 17/06/2020

CU2 Project Title Development of cannabidiol (CBD) analogues for treatment of epilepsy

Class 2

CultureVolClass2 < 1 Litre

Consent Granted

Non-GMM

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements N

Project Additional Information

Purposes of the contained use

Multispan HEK293T cells expressing human GPR55

Recipient or parental organism
proprietary expression system (Multispan) the present invention provides a vector for facilitating high levels of expression of GPCR proteins in a cell line. The vector includes components such as a cytomegalovirus (CMV) promoter, a signal peptide, and epitope tag, a Kozak sequence, a poly-A site, and a viral origin of replication.

Full-length Human GPR55 cDNA (GenBank Accession Number NM_005683.3) with FLAG-tag sequence at the N-terminus is inserted into the HEK293T host cells and is stably expressed with selection by puromycin. The cloning vector used is the proprietary Multispan vector and the cell line is commercially available only from Multispan.

the overexpression of the intended GPCR would not present a significant risk to human health or the environment however the expression host HEK293T does present a risk HEK-293T cell line is a highly transfecatable derivative of HEK293 cells and contains the SV40-T antigen. The hypothesis that SV40 might cause cancer in humans has been a particularly controversial area of research, notably, a 2002 study performed by The National Academy of Sciences Immunization Safety Review committee that stated, "The committee concluded that the biological evidence is moderate that SV40 exposure could lead to cancer in humans under natural conditions.

All level 2 waste is chemically treated using validated disinfectants and ensuring 100% kill. All solid and liquid waste is housed in sealed containers and collected for incineration by approved contractor (Grundons). The building does have an autoclave, due to bulky nature of the waste. derogation is requested for omission to autoclave waste. The chemical disinfection regime has been assessed to be sufficient to prevent any exposure to infectious biological waste.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid was collected from flasks &plastics is decontaminated overnight using a validated disinfectant. 12hr contact time ensures 100% kill. Liquid is held in sealed containers mixed with a validated disinfectant.
disinfectant disinfected liquid waste is removed from site for destruction by incineration by an approved contractor (Grundon)
Contaminated plastic consumables are immersed in validated disinfectant overnight before discarding as solid waste solid waste is placed in dedicated biohazard bins which hav biohazara waste binse been lined with two autoclave bags at the end of the working day, the bags are sealed and then placed into lockable biohazard waste bins )UN approved for the transport of packaging group 2 substances with a biohazard sign which carries the UN3291 number full bins are stored in a secure brick store with a bunded concrete floor. and then collected for incineration by an approved contractor (Grundons)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
HEK-293T cell line is a highly transfectable derivative of HEK293 cells and contains the SV40-T antigen. The hypothesis that SV40 might cause cancer in humans has been a particularly controversial area of research, notably, a 2002 study performed by The National Academy of Sciences Immunization Safety Review committee that stated, *The committee concluded that the biological evidence is moderate that SV40 exposure could lead to cancer in humans under natural conditions. the primary reason why warrants this CU2 notification*

Project Containment

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Animal Units

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Project Ref 680/20.2

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<td>05/11/2020</td>
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<td>Class 2</td>
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</table>
Purposes of the contained use

To study the role of MAPT in frontotemporal dementia and parkinsonism

Recipient or parental organism

axolGEM iPSC-Derived Neural Stem Cells MAPT P301L HOM (Product Code: ax0324)

Host/vector system

axolGEM iPSC-Derived Neural Stem Cells MAPT P301L HOM (Product Code: ax0324)

Origin & function

The cell line cannot survive outside of the laboratory environment and therefore poses no hazard to the wider environment. Small spills will be treated with Virkon and absorbed onto paper. The area will then be treated with 70% IMS.

Evaluation of foreseeable effects

The cell line cannot survive outside of the laboratory environment and therefore poses no hazard to the wider environment. Small spills will be treated with Virkon and absorbed onto paper. The area will then be treated with 70% IMS.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

axolGEM iPSC-Derived Neural Stem Cells MAPT P301L HOM (Product Code: ax0324) are commercially available from Axol Bioscience. The human iPSCs used to derive this cell line were generated by reprogramming of dermal fibroblasts from a female donor and present normal karyotype. Reprogramming was performed using episomal vectors, which are non-integrating vectors used to introduce reprogramming factor into somatic cells. Using a chemically defined neural induction medium, these iPSCs have been used to generate iPSC-Derived Neural Stem Cells.

Human iPSC-Derived Neural Stem Cells that have been genetically edited using CRISPR-Cas9 technology to introduce the P301L mutation (CCG>CTG) into the MAPT gene. This line is homozygous for the P301L mutation so both alleles contain the mutation.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All level2 waste is chemically treated using validated disinfectants and ensuring 100% kill. all solid and liquid waste is housed in sealed containers and collected for
incineration by approved contractor (Grundons) the building does have an autoclave, due to the bulky nature of the waste. derogation is requested for omission to 
autoclave waste. the chemical disinfection regime has been Assessed to be sufficient to prevent any exposure to infectious biological waste

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

all liquid waste collected from flasks & plastics is decontaminated overnight using a validated disinfectant.

12hr contact time ensures 100% kill. liquid is held in sealed containers, mixed with a validated disinfectant disinfecte liquid waste is removed from site for destruction by incineration by an approved contractor (Grundons)

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Full bins are stored in a secure brick store with bunded concrete floor, anfd then collected for incineration by approved contractor (Grundons)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Collective agreement of BSL2 & a CU2 submission be made

Use of sharps to be avoided, and immunocompromised personnel must NOT use these cells

Associated GM risk assessment sent spiderately to notifications officer.

Project Containment

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02/03/2022
The cells will be used to identify compounds which inhibit the VPS4A gene. Inhibition of VPS4a will cause apoptosis of the cells which will be quantified using cell viability assays.

Recipient or parental organism

ATCC CT 1® CCL-247™

Host/vector system

Vector: Edit-R All-in-one lentiviral (knockout) Human VPS4B sgRNA-mCMVCas9-Puro (Horizon VSGH11937-248432319)

The vector above was delivered by lentiviral transduction and cells were selected with puromycin. The puromycin resistant population was subsequently single-cloned and the VPS4B KO clone was identified.

Origin & function

The cells will be used to identify compounds which inhibit the VPS4A gene. Inhibition of VPS4a will cause apoptosis of the cells which will be quantified using cell viability assays.
Evaluation of foreseeable effects

Based on supplied information this cell line has been categorised as BSL level 2 (i.e. UN3291) and will be disposed of in that waste stream. And treated with Virkon prior to disposal. The genes affected by the genetic manipulation are to our knowledge not proto-oncogenes or tumour suppressor genes. Cells can only exist in a laboratory environment and require continued selective pressure to maintain the genetic manipulation. There is negligible risk to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All liquid waste collected from flasks & plastics is decontaminated overnight using a validated disinfectant. 12 hr contact time ensures 100% kill. Liquid is held in sealed containers mixed with a validated disinfectant, disinfected waste is removed from site for destruction by incineration by an approved contractor (Grundons). Contaminated plastic consumables are immersed in validated disinfectant overnight before being discarded as solid waste. Solid waste is placed in dedicated biohazard bins and then placed into lockable biohazard waste bins (UN approved for the transport of packaging group 2 substances with a biohazard sign which carries the UN3291 number. Full bins are stored in a secure brick store with bunded floor. and then collected for incineration by approved contractor (Grundon’s).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste collected from flasks & plastics is decontaminated overnight using a validated disinfectant. 12 hr contact time ensures 100% kill. Liquid is held in sealed containers mixed with a validated disinfectant, disinfected waste is removed from site for destruction by incineration by an approved contractor (Grundons).

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Collective agreement of BSL2
It is critical that these modified cells will never be exposed to any further rounds of LV. To ensure that viable LV particles are inadvertently created.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
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**Animal Units**

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**Project Ref** 680/21.2

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<th>CU2 Project Title</th>
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<td>20/05/2021</td>
<td>CHOZN cells expressing the A2a receptor</td>
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**Non-GMM Consent Granted**

**Project notified under transitional arrangements**

**Withdrawn**

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

A2a (Adenosine receptor 2a) is a G-protein coupled receptor (GPCR) implicated in a large number of disorders including cancer and neurodegenerative diseases.

**Recipient or parental organism**
Parental Cell Line: CHOZN (CHO K1 adapted to chemically defined suspension culture)

**Host/vector system**

Produced with pLenti viral vector from Origene. The pLenti expression vector is replication deficient as it contains SIN (Self Inactivation), a deletion in the 3' LTR (ΔU3). This SIN deletion does not affect lentiviral packaging, yet results in "self-inactivation" after integration into the transduced cell. The integrated lentiviral genome is no longer capable of self replication. Expression of the transduced target gene is driven either by a cytomegalovirus (CMV) promoter, vector contains blasticidin and ampicillin antibiotic-resistance genes.

**Origin & function**

A2a (Adenosine receptor 2a) is a G-protein coupled receptor (GPCR) implicated in a large number of disorders including cancer and neurodegenerative diseases.

**Evaluation of foreseeable effects**

The foreign DNA (A2a) and antibiotic-resistance genes represent a very low risk to environment since the vector is non-mobilizable. The CHOZN cells are expected to have limited survivability in the environment as the cells only survive in defined cell culture media in a laboratory environment and they are non-colonizing.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- All level2 waste is chemically treated using validated disinfectants and ensuring 100% kill. All soil and liquid waste is housed in sealed containers and collected for incineration by approved contractor (Grundons). The building does have an autoclave, due to the bulky nature of the waste. The chemical disinfection regime has been assessed to be sufficient to prevent any exposure to infectious biological waste.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- All liquid waste collected from flasks & plastics is decontaminated overnight using a validated disinfectant.
- 12hr contact time ensures 100% kill. Liquid is held in sealed containers mixed with a validated disinfectant. Disinfected waste is removed from site for destruction by incineration by an approved contractor (Grundons).
- Contaminated plastic consumables are immersed in validated disinfectant overnight before discarding as solid waste. Solid waste is placed in dedicated biohazard bins which have been lined with two...
autoclave bags at the end of the working day, the bags are sealed and then placed into lockable biohazard waste bins (UN approved for the transport of packaging group 2 substances with a biohazard sign which carries the UN3291 number. Full bins are stored in a secure brick store with bunded concrete floor, and then collected for incineration by approved contractor (Grundons).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Collective agreement of BSL2 to prevent any possibility of creating replicative competent lentivirus this recombinant cell line must NOT be exposed to any further modification with Lentiviral vectors.

Project Containment

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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 680/21.3

Date Ackn'd 30/07/2021

CU2 Project Title Identification of compounds modulating PKD2 for the treatment of Autosomal dominant polycystic kidney disease (ADPKD)

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

Identification of compounds modulating PKD2 for the treatment of Autosomal dominant polycystic kidney disease (ADPKD)

**Recipient or parental organism**

Human Embryonic Kidney 293T cells (HEK293T), originally from American Tissue and Cell Culture Inc.

**Host/vector system**

Hemagglutinin (HA) tagged full length PKD1 (PKD1-HA) and FLAG-tagged full length PKD2 (PKD2-FLAG) transgenes stably overexpressed using third generation lentivirus expression systems in the HEK293T cells. Packaging plasmids were made with one encoding Gag and Pol and another encoding Rev. Envelope plasmid contained VSV-G. Cell lines are replication incompetent after deletion in the 3'LTR, rendering the virus self-inactivating after integration. Expression of the PKD1 and PKD2 genes is induced by the addition of doxycycline to the growth media.

**Origin & function**

There is no serious hazard to the environment as this cell line would not survive outside of a lab environment. Small spills will be treated with validated disinfectant & absorbed onto paper then discarded as UN3291 BSL2 waste.

HEK-293T cell line is a highly transfectable derivative of HEK293 cells and contains the SV40-T antigen. The hypothesis that SV40 might cause cancer in humans has been a particularly controversial area of research, notably, a 2002 study performed by The National Academy of Sciences Immunization Safety Review committee that stated, "The committee concluded that the biological evidence is moderate that SV40 exposure could lead to cancer in humans under natural conditions.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All level 2 waste is chemically treated using validated disinfectants and ensuring 100% kill, All solid and liquid waste
is housed in sealed containers and collected for destruction by incineration by an approved contractor (Grundons).

The building does have an autoclave, due to the bulky nature of the waste.

Derogation is requested for omission to autoclave waste, the chemical disinfection regime has been assessed to be sufficient to prevent any exposure to infectious biological waste

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste collected from flasks & plastics is decontaminated overnight using a validated disinfectant.

12hr contact time ensures 100% kill. liquid is held in sealed containers mixed with a validated disinfectant disinfected

Liquid waste is removed from site for destruction by incineration by an approved contractor (Grundons)

Contaminated plastic consumables are immersed in validated disinfectant overnight before disacarding as solid waste. Solid waste is placed in dedicated biohazard bins which have been lined with two autoclave bags, at the end of the working day the bags are sealed and then placed into lockable biohazard waste bins (UN approved for the transport of packaging group 2 substances with a biohazard sign which carries the UN3291 number).

Full bins are stored in a secure brick store with bunded concrete floor, and then collected for destruction by incineration by approved contractor (Grundons)

HEK-293T cell line is a highly transfectable derivative of HEK293 cells and contains the SV40-T antigen. The hypothesis that SV40 might cause cancer in humans has been a particularly controversial area of research, notably, a 2002 study performed by The National Academy of Sciences Immunization Safety Review committee that stated, “The committee concluded that the biological evidence is moderate that SV40 exposure could lead to cancer in humans under natural conditions.

The primary reason for a CU2 application

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Animal Units

Large Scale Activities

Human Clinical Applications

Project Containment

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
Project Ref 680/21.4

Date Ackn'd 12/08/2021
CU2 Project Title HEK-293T cells (ATCC) expressing pLVX-KCNQ2/3, maintained using puromycin and hygromycin selection

Date Project Ceased

Class 2
CultureVolClass2 < 1 Litre
CultureVolumeClass3-4
Non-GMM Consent Granted

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Cells will be routinely cultured and harvested for use in Qube- or FLIPR-based assays, in order to detect KCNQ2/3 agonists.

Recipient or parental organism
HEK-293T cells

HEK-293T cell line is a highly transfectable derivative of HEK293 cells and contains the SV40-T antigen. The hypothesis that SV40 might cause cancer in humans has been a particularly controversial area of research, notably, a 2002 study performed by The National Academy of Sciences Immunization Safety Review committee that stated, "The committee concluded that the biological evidence is moderate that SV40 exposure could lead to cancer in humans under natural conditions. CU2 submission required.

Host/vector system
pLVX-KCNQ2/3, maintained using puromycin and hygromycin selection.

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All level 2 waste is chemically treated using validated disinfectants and ensuring 100% kill. All solid and liquid waste is housed in sealed containers and collected for incineration by approved contractor (Grundons). The building does have an autoclave, due to the bulky nature of the waste. Derogation is requested for omission to autoclave waste. The chemical disinfection regime has been assessed to be sufficient to prevent any exposure to infectious biological waste.

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

HEK-293T cell line is a highly transfectable derivative of HEK293 cells and contains the SV40-T antigen. The hypothesis that SV40 might cause cancer in humans has been a particularly controversial area of research, notably, a 2002 study performed by The National Academy of Sciences Immunization Safety Review committee that stated, “The committee concluded that the biological evidence is moderate that SV40 exposure could lead to cancer in humans under natural conditions. CU2 submission required.

Project Containment

02/03/2022
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Animal Units

Large Scale Activities

Human Clinical Applications
GM Centre Number: 681

Data Premises Notified (Originally) 09/10/1998  
Transferred from 1992 Regs? Y  
Transitional Premises Class 1  
Data Premises Closed N  
Transitional Premises Emergency Plan Required? N  
Non-GMMs N  
Withdrawn N  

Name  
ANACHEM LTD  

Name 2  

Department  

Campus Estate or Research Centre  

Building  
ANACHEM HOUSE  

Road Name  
CHARLES STREET  

District  

Town  
LUTON  

County  
BEDFORDSHIRE  

Postcode  
LU2 0EB  

Country  
ENGLAND  

Tel Number 01582 745000  
Fax Number 01582 391768  

E-mail  

HSE Division EAST AND SOUTH EAST  

Comments  

Date at Which Additional Info Submitted  
02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Other (please specify)  
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<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

The small amounts of material will be either lysed in the equipment or autoclaved.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
| Data Premises Notified (Originally) | 26/10/1998 | Transferred from 1992 Regs? | Y | Transitional Premises Class | 1 |
| Data Premises Closed | N | Transitional Premises Emergency Plan Required? | N | Non-GMMs | Y | Withdrawn | N |

**Name**

BECKMAN COULTER GENOMICS

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

HOPE END

**District**

**Town**

TAKELEY

**County**

ESSEX

**Postcode**

CM22 6TA

**Country**

ENGLAND

**Tel Number**

01799 503123

**Fax Number**

01799 503124

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Name change from Cogenics Inc to above. Company changed its name from Lark Technologies Inc on 13/5/2004 to the above.

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

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Give brief details of the genetic modification safety committee

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum culture volume that could be released at any one time (breakage of flasks or shaking incubator) would be 250 ml. Surface inactivation of such spillages would involve use of 1% Virkon medical disinfectant.

All other waste both liquid and solid is made safe by autoclaving at 136.4 degrees C for 106 minutes at 2.37 bar. Chart recorder records all autoclave runs.

Validation of autoclaving is carried out using DRG test packs. Autoclave is under a standard manufacturer's maintenance and calibration regime.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 683

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Name

METRIS THERAPEUTICS LTD

Name 2

Department

MOLECULAR BIOLOGY

Campus Estate or Research Centre

Building

Road Name

400 THAMES VALLEY PARK DRIVE

Town

READING

County

BERKSHIRE

Postcode

RG6 1PT

Country

ENGLAND

Tel Number

0118 963 7477

Fax Number

0188 963 7577

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

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<td>RG41 5TU</td>
<td>ENGLAND</td>
<td>N</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Level 2 (GMMs)</td>
<td>Level 3 (GMMs)</td>
<td>Level 4 (GMMs)</td>
<td>Non-microbial</td>
</tr>
</tbody>
</table>

Other (please specify) Tick if confidential

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic</th>
<th>Microbiology</th>
<th>Virology</th>
<th>Transgenic</th>
<th>Transgenic</th>
<th>Gene Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birds</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Research</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Autoclave 1: Sterilisation of raw materials can be GMP validated.
Autoclave 2: Decontamination of potentially contaminated glassware/plasticware, glass fermentor vessels (max volume 5 litres). Decontamination cycle ranges from 121 - 134 degrees C for 15 - 30 minutes. Temperature readout to validate the cycle.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
<tr>
<th>Name</th>
<th>NEUROPA LTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campus Estate or Research Centre</td>
<td></td>
</tr>
<tr>
<td>Road Name</td>
<td>54 DUMBARTON ROAD</td>
</tr>
<tr>
<td>Town</td>
<td>GLASGOW</td>
</tr>
<tr>
<td>County</td>
<td>EAST RENFREWSHIRE</td>
</tr>
<tr>
<td>Postcode</td>
<td>G11 6NU</td>
</tr>
<tr>
<td>Country</td>
<td>SCOTLAND</td>
</tr>
<tr>
<td>Tel Number</td>
<td>0141 330 5264</td>
</tr>
<tr>
<td>Fax Number</td>
<td>0141 330 5910</td>
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<td>E-mail</td>
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<tr>
<td>HSE Division</td>
<td>SCOTLAND</td>
</tr>
<tr>
<td>Comments</td>
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<td>Date at Which Additional Info Submitted</td>
<td>02/03/2022</td>
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## Premises Addresses

<table>
<thead>
<tr>
<th>Date Premises Closed</th>
<th>Name</th>
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<th>Building</th>
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<th>Town</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<td>Level 4 (GMMs)</td>
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<tr>
<td>Other (please specify)</td>
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<td>Tick if confidential</td>
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</tbody>
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- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research

- Virology
- Transgenic
- Animals
- Gene Therapy
- Transgenic
- Fish
All bacterial biohazardous waste is removed by autoclaving. All contaminated/sharps are stored in labelled appropriate containers and taken for disposal/incineration by GU. Liquid bacterial cultures are inactivated for 24 hours in presence of bactericidal detergents. The maximum culture volume of 3 litres will be released at any one time.

<table>
<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  
Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 686

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#### Name

- **UNIVERSITY OF EDINBURGH**

#### Name 2

- **JOHN HUGHES BENNETT LABORATORIES**

#### Campus Estate or Research Centre

- **CREWE ROAD SOUTH**

#### Road Name

- **CREWE ROAD SOUTH**

#### Town

- **EDINBURGH**

#### District

- **EAST LOTHIAN**

#### County

- **EH4 2XU**

#### Country

- **SCOTLAND**

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<td>0131 537 3160</td>
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#### HSE Division

- **SCOTLAND**

#### Comments

- Merged with GM207 on 17/02/2004

#### Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>JOHN HUGHES BENNETT LABORATORIES</td>
<td>WESTERN GENERAL HOSPITAL</td>
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<td>EDINBURGH BREAST UNIT RESEARCH GRO</td>
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<td>PADEREWSKI BUILDING</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<td>Level 4 (GMMs)</td>
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<tr>
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</tr>
<tr>
<td>Other (please specify)</td>
<td></td>
<td></td>
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</table>

Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 686/01.1**

**CU2 Project Title:** AN INVESTIGATION INTO THE ROLE OF DNA METHYLATION IN MURINE EMBRYONIC STEM CELLS VIA INDUCIBLE EXPRESSION OF RELEVANT GENES

**Date Ackn'd:** 30/04/2001

**Date Project Ceased:** 17/02/2004

**Withdrawn:** N

**Historical Significant Changes:** Transferred to GM207 on 17/02/2004

**Class:**
- CultureVolClass2
  - Class 2
  - < 1 litre

**Consent Granted:**
- Non-GMM: not applicable

**Project notified under transitional arrangements:** N
**Project Additional Information**

**Purposes of the contained use**

Introduction of methyl transferase genes, via retrouiral infection, into murine ES cells.

**Recipient or parental organism**

Murine embryonic stem cells, E14 line.

**Host/vector system**

DH5x: Host

Non mobilisable plasmids, pLNCX-Derives: vector.

Replication - incompetent retrouirus production in PT67 cells.

**Origin & function**

Genetic material derived from non-mobilisable pLNCX plasmids and containing commonly used reporter and marker genes together with one of the following three genes.

1. De Novo Methyltransferase 3A.
2. De Novo Methyltransferase 3B.
3. Methyl Binding Domain 2B.

The vectors enable study of these gene functions in cell culture.

**Evaluation of foreseeable effects**

The genetic elements and vector system are well characterised and frequently employed. The proximal aim is to generate replication. Incompetent viral particles for the purpose of infection of murine ES cells and the study of the phenotypic effects of the above-named genes. Since the viral particles will be incapable of autonomous propagation (due to the absence of structural genes) and since the plasmids themselves are non-mobilisable, there are no significant foreseeable environmental or health concerns arising from this experiment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Effective containment and control measures:

1. Non-mobilisable plasmids.
2. Replication - incompetent viral particles.
3. Use of a Level 2 containment facility.
4. Use of a Level 2 tissue culture hood.
5. Chemical and heat inactivation of waste material.
6. Confirmation of inactivation using appropriate methods.
7. Autoclaving of all equipment before removal from facility.
9. Regular inspection of facility and hood.
10. Regular confirmation of satisfactory autoclaving.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management measures:
1. Pipette aspiration of biological cell culture material.
2. Chemical and heat neutralisation of cells and viral particles.
3. Chemical and heat neutralisation of bacterial cultures.
4. Confirmation of 100% neutralisation by demonstration of absolute sterility post treatment.
5. Monitoring of methods using Browne's steriliser tubes.
6. Removal from facility in appropriate labelled bags for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Discussed fully.
Satisfactory risk assessment.
Recorded in safety committee minutes.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>Yes</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>L3</td>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
</tr>
<tr>
<td>L4</td>
<td>L3 L4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2</td>
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**Project Ref** 686/01.2

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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<tr>
<td>17/08/2001</td>
<td>ADOPTIVE IMMUNOTHERAPY FOR CANCER USING VACCINIA VIRUS AND RNA-CONTAINING LIQUID VESICLE VECTORS FOR INDUCING IMMUNITY TO EPSTEIN-BARR VIRUS INFECTED CELLS</td>
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<table>
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<tr>
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<td>17/02/2004</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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Non-GMM: Consent Granted: not applicable

Project notified under transitional arrangements

Withdrawn: N
Tick if notifying a connected programme of work: N

**Historical Significant Changes**
Transferred to GM207 on 17/02/2004

**Project Additional Information**

**Purposes of the contained use**
Introduction of Epsien-Barr virus genes into human antigen presenting cells using Vaccinia virus as a vector.
### Recipient or parental organism

Recombinant Vaccinia viruses are thymidine kinase negative and consequently less virulent than wild type Vaccinia. Manipulated human antigen presenting cells are not known or thought to be any risk to the operator of the environment.

### Host/vector system

<table>
<thead>
<tr>
<th>DH 5a Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vectors will be non-mobilisable plasmids.</td>
</tr>
<tr>
<td>Vaccinia virus will be propagated in TK-143 cells.</td>
</tr>
</tbody>
</table>

### Origin & function

All Epstein-Barr virus genes are derived from the phototype B95.8 strain of EBD. The gene products will be used to stimulate an immune response against cells latently infected with EBV, as with some tumour cells.

### Evaluation of foreseeable effects

EBV is carried out by nearly all individuals in any community, therefore, the effect of any exposure to a single EBV gene product is likely to be effective zero. Vaccinia virus has been used on a wide scale to immunise people. Vaccinia virus Recombinant strains carrying Rabies Virus genes have also been used in non laboratory settings, notably in Belgium where virus infected meat was used to immunise foxes in the wild. Plasmid vectors are of the non-mobilisable type. Thus, these should be incapable of passing genetic material to wild-type bacteria.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- All cultural aspirates pass into concentrated Presept. Aerosols will be contained with an 'in line' 0.22mu filter.
- All solid and liquid waste will be autoclaved after chemical inactivation.
- All bacterial cultures will be chemically treated and its effectiveness monitored by regular culturing to test for viability.
- The autoclave is regularly monitored by use of Browne's tubes and a temperature recorder.
- Removal of waste after treatment to the clinical waste stream.

### Is an emergency plan required according to regulation 20? [N]

### If yes, tick to confirm that it is attached to this form [N]

### Tick to confirm that you have attached a risk assessment to this form [Y]

### Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

### Please enter comments on the GM safety committee on the risk assessment
The Oncology Genetic Modification Safety Committee discussed the Risk Assessment and several minor amendments were requested. It was accepted that the amended Risk Assessment be approved by a senior committee member. The Risk Assessment was approved on the basis of this.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4 L2 L3 L4</td>
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<th>Human Clinical Applications</th>
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</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4</td>
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### Project Ref 686/99.3

- **Date Ackn'd**: 24/08/1999
- **CU2 Project Title**: DEVELOPMENT OF GENE DELIVERY VIRAL & NON-VIRAL METHODS FOR THE GM OF NORMAL & NEOPLASTIC HAEMOTOLOGICAL CELL TYPE, LAB DEVELOPMENT NOVEL APPROACHES TO CELLULAR THERAPY

<table>
<thead>
<tr>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>Class 2</td>
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</table>

- **Non-GMM**: Consent Granted: not applicable
- **Project notified under transitional arrangements**: Yes
- **Historical Significant Changes**: Transferred to GM 207 as of 17/02/2004

### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<thead>
<tr>
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<th>156 FAREHAM ROAD</th>
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<table>
<thead>
<tr>
<th>Town</th>
<th>GOSPORT</th>
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<table>
<thead>
<tr>
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| 01329 224 229 | 01329 224 350 |

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<td>ENGLAND</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

#### Laboratory

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

#### Other (please specify)

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<thead>
<tr>
<th>Bacteriology</th>
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<th>Microbiology Research</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>

Tick if confidential

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum volume which could be released at any one time is 250 mls. The waste is deactivated by autoclaving, which ensures that all the material is microbiologically inactivated. The autoclave is calibrated and procedures are in place to ensure that autoclave cycles are monitored for their correct performance.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 688

Data Premises Notified: 03/03/1999 (Originally)
Transferred from 1992 Regs?: Y
Transitional Premises Class: 1
Data Premises Closed: N
Transitional Premises: N
Emergency Plan Required?: Y
Non-GMMs: Y
Withdrawn: N

Name:
DR REDDY'S LABORATORIES (EU) LTD

Name 2:
A SUBSIDIARY OF THE DOW CHEMICAL COMPANY

Department:

Campus Estate or Research Centre:
DR REDDY'S

Building:
410 CAMBRIDGE SCIENCE PARK

Road Name:
MILTON ROAD

District:

Town:
CAMBRIDGE

County:
CAMBRIDGE

Postcode:
CB4 0PE

Country:
ENGLAND

Tel Number: 01223 728 010
Fax Number: 01223 506 701

E-mail:

HSE Division:
EAST AND SOUTH EAST

Comments:

Date at Which Additional Info Submitted:
02/03/2022
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

**Give brief details of the genetic modification safety committee**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
Genetically modified clones, class 1, are grown at < 31 scale in fermenters and at < 11 scale in flasks. Cultures and waste material are killed by sterilisation at 121 degrees C for 20 minutes for all flasks and for 40 minutes for fermenters. The autoclave is routinely serviced by the manufacturer. Alternatively cultures and waste material may be killed by use of a disinfectant (Virkon from Antec International). All killed liquid waste is then disposed of down the drains.
### GM Centre Number: 689

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#### Name

**UNIVERSITY OF EDINBURGH**

**Name 2**

**ROYAL INFIRMARY**

**Department**

**MEDICAL & RADIOLOGICAL SCIENCES**

**Campus Estate or Research Centre**

**Building**

**Road Name**

**District**

**Town**

**EDINBURGH**

**County**

**EAST LOTHIAN**

**Postcode**

**EH3 9YW**

**Country**

**SCOTLAND**

**Tel Number**

0131 536 2234

**Fax Number**

0131 229 2948

**E-mail**

**HSE Division**

**SCOTLAND**

**Comments**

GM689 MERGED WITH GM663 ON 04/06/2003

**Date at Which Additional Info Submitted**

21/08/2001
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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02/03/2022
Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)  

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<td>Mycology</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 690**

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**UNIVERSITY OF LIVERPOOL**

**Name**

**UNIVERSITY OF LIVERPOOL**

**Name 2**

**CANCER RESEARCH CENTRE**

**Department**

**SCHOOL OF DENTAL STUDIES**

**Campus Estate or Research Centre**

**ROY CASTLE BUILDING**

**Building**

**200 LONDON ROAD**

**District**

**Town**

**LIVERPOOL**

**County**

**MERSEYSIDE**

**Postcode**

**L3 9TA**

**Country**

**ENGLAND**

**Tel Number**

**0151 794 8920**

**Fax Number**

**0151 794 8989**

**E-mail**

**NONE**

**HSE Division**

**NORTH WEST**

**Comments**

This centre now includes details also for GM700 from 15/04/2005

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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### Virology
- Transgenic Animals

### Mycology
- Transgenic Invertebrates

### Transgenic
- Fish
- Plants

### Gene Therapy

### Other (please specify below)

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For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
**GM Centre Number: 691**

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**Name**

RENEURON LTD

**Department**

**Campus Estate or Research Centre**

**Road Name**

PENCOED BUSINESS PARK

**District**

PENCOED

**Town**

BRIDGEN

**County**

GWYNNDD

**Postcode**

CF35 5HY

**Country**

WALES

**Tel Number**

01483 302560

**Fax Number**

01483 534864

**E-mail**

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**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

11/05/2001
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 691/05.1

**Date Ackn'd** 13/04/2005

**CU2 Project Title** Introduction of a cloned modified oncogene into primary cultures of human cells using replication defective retroviruses for the purposes of cell immortalisation

**Class** Class 2

**Culture Volume Class 2** < 1 Litre

**Non-GMM Consent Granted** Not Applicable

**Date Project Ceased**
### Project Additional Information

**Purposes of the contained use**

The generation of replication incompetent retroviruses carrying an immortalising gene including variants of the myc oncogenes. The packaging line will be the TeFLY A-(amphotrophic) and TeFLY-RD cell lines. Harvested virus will be used to transduce primary cultures of human cells for the purpose of immortalisation and cell line generation.

**Recipient or parental organism**

1. TeFLY packaging cells. These are a third generation, multiply replication-disabled packaging line containing Moloney leukaemia virus-encoded retroviral gag/pol proteins from one plasmid and viral envelope genes (MLV-A (amphotropic and feline virus from a second plasmid). Details of the constructs and evidence that they do not give rise to replication competent viruses have been published (Cossett et al., J. Virol., 69:7430-7436, 1995). The original Te671 line (Takeuchi et al., J Virol., 67: 8001-8007, 1994) from which the lines were derived are a human rhabdomyosarcoma line. TeFly packaging cells are obtained from Genethon 11, Paris. These lines were realised under GMP conditions and are clean. 2. Human neural, retinal, pancreatic or hepatic cells in primary culture will be infected with retrovirus harvested from TeFLYA producers.

**Host/vector system**

The cMyc/mycER constructs are cloned into retroviral plasmids including pLNCX-cmycER, pBabe-puro and pLHCX and used to generate amphotrophic, replication incompetent retroviruses using TeFLY producer cells. Cell lines infected with these retroviruses will be screened for the absence of replication-competent retroviruses by transfer of neomycin resistance to human TE671 cells following 3 to 5 passages; infection of susceptible cells then selecting for transfer of G418 resistance.

**Origin & function**

The cMyc/mycER is a recombinant fusion protein of human cMyc and the binding domain of the mouse estradiol receptor. The plasmids carrying the cMyc or cMycER have been prepared by Dr. Z Dong (formerly of ReNeuron). These will be used to transfect TeFLY-RD packaging cells. A transient viral harvest will be collected to infect TeFLY-A cells. Producer lines will be selected and those producing stable, full insert viruses and free of replication-competent retroviruses will be expanded. Retroviral vectors from these lines will be used to infect and immortalise primary cultures of human cells.

**Evaluation of foreseeable effects**

This project involves the infection of human foetal cells in primary culture using a retroviral vector system with amphotropic potential to introduce the cMyc construct. Transfected TeFLY lines will produce retrovirus carrying the cMyc construct capable of transducing human cells in culture. The cMyc gene in viral state is infectious but has a very short half life, is not replication competent and is used in ACGM containment level 2. Following infection of human cells, the cMyc construct is inserted in the eukaryotic genome and so may be considered non-mobilisable. cMyc transfection ensures genetically stable long term proliferation of infected cells. Following transduction with pLNC-cmycER or pBabe-puro-cmycER, immortalisation is inducible by the presence of tamoxifen. The cells stably expressing it have no mechanism to release it into the environment. Myc is a normally expressed protein in human cells although it is
upregulated in cancers.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Oncogenic DNA will be treated overnight with quaternary ammonium compound Barrycidal 36 before being autoclaved and discarded down the drains (all within CL2 laboratory).
2. Liquid waste (viral supernatants and culture media) will be treated with >1% Virkon solution (Antec International) overnight then discarded down the drains.
3. Solid waste is treated with a 1% Virkon solution overnight then sent for incineration where there has been contact with culture media (e.g. pipettes, culture flasks, media bottles). Other solid waste is inactivated with heat treatment (decontaminating autoclave) to give 100% kill and then sent for incineration. The decontaminating autoclave includes a high temperature step of 126.5 Celsius held for 15 minutes. The temperature of the autoclave cycle is printed out in real time.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project approved.

Project Containment

<table>
<thead>
<tr>
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<th>Growth Rooms</th>
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Project Ref 691/99.1
INTRODUCTION OF CLONED MUTANT VIRAL ONCOGENE INTO PRIMARY CULTURES OF HUMAN NEURAL CELLS WITH RECOMBINANT, REPLICATION-DEFECTIVE RETROVIRUSES FOR THE PURPOSE OF CELL IMMORTALISATION.

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
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EMERGENT EUROPE LTD TO EMERGENT BIOSOLUTIONS 1/6/06.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Give brief details of the genetic modification safety committee

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Other (please specify)

Tick if confidential

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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Please enter comments of the GM safety committee on the risk assessment

---

### Project Ref 692/00.2

<table>
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Withdrawn | N | Tick if notifying a connected programme of work | N |

**Historical Significant Changes**

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Glass Houses</th>
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Animal Units

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Project Ref 692/01.1

Date Ackn'd: 19/02/2001
Project Title: CONSTRUCTION OF DEFINED SALMONELLA TYPHI MUTANT WITH NULL

Class 2

Class CultureVolClass2 CultureVolumeClass3-4

Class 2
DELETIONS IN THE ARO C AND SSAV GENES

Date Project Ceased
01/04/2011

Tick if notifying a connected programme of work
N

Project notified under transitional arrangements
Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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Historical Significant Changes

- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
Project Ref 692/01.3

Date Ack'n'd 19/02/2001

CU2 Project Title
EXPRESSION OF RECOMBINANT ANTIGENS IN A LIVE SALMONELLA TYPHIMURIUM VACCINE STRAIN

Class 2

CultureVolClass2

Consent Granted not applicable

Project notified under transitional arrangements Y

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref 692/02.1

Date Ackn'd 25/07/2002
CU2 Project Title GENERATION OF DEFINED DELETIONS IN PASTURELLA MULTOCIDA
Class 2
CultureVolClass2 < 1 litre
CultureVolumeClass3-4

02/03/2022 Page 10729 of 15326
Purposes of the contained use
Construction of a live attenuated Pasteurella vaccine strains for the prevention of disease in meat turkey and chicken layers and breeders.

Recipient or parental organism
Pasteurella multocida.

Host/vector system
Disabled E. coli K12 strains will be used for intermediate cloning steps.

The vector PCVD442 will be used to transfer the genetic material to the Pasteurella multocida chromosome.

The vector PCVD442 is mobilisation defective and unable to replicate in Pasteurella and is therefore integrated into the chromosome along with the genetic material suitable to generate defined deletions by homologous recombination.

Following homologous recombination, there will only be a small fragment of additional DNA sufficient to allow diagnostic restriction enzyme digests.

Origin & function
The material originates from commercial sources or collaboration laboratories and will function to generate defined deletions in certain genes, which will be tested as live attenuated vaccines.

Evaluation of foreseeable effects
The intermediate GMMs will be disabled E.coli K12 strains containing genetic material from P. multocida.

The plasmid vector is unstable in the absence of selective pressure and the recombinant genetic material would be rapidly lost.

It is unlikely that the cloned DNA would offer any advantage to the survival of the E. coli strains in the environment.
The final resulting GMMs will represent vaccine strains of P. multocida. Genes required for virulence will have been deleted and steps will have been taken to prevent any alteration of gene expression.

No genetic material will be included that would provide the recombinant Pasteurella with a selective or pathogenic advantage.

The mutants are likely to be attenuated in humans as well as in animals. Any hazard that is likely to occur in the unlikely event that these mutants become established in the environment is likely to be much less that wild-type strains. The consequences of the hazard is therefore considered low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid cultures are chemically inactivated prior to disposal. Chemical inactivation is performed using Virkon which has been validate for treatment of E. coli and Pasteurella bacteria. Following treatment at 3% for 14 hours, liquid waste is disposed of by dilution via the normal sewage waste drain.

All other contaminated materials are inactivated via autoclaving prior to disposal. A dedicated waste autoclave is used for these activities. This autoclave is serviced and validated annually to ensure proper function. Following inactivation by autoclaving, all waste is then removed by a private contractor for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

This risk assessment has been carried out by the personnel who will carry out the work and reviewed by the Microscience GMSC. It has been given a class II, containment level 2 classification.

Project Containment

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to delete the gene encoding the heat stable toxin from Enterotoxigenic Escherchia coli (ETEC) for use as a challenge strain in clinical trials.

**Recipient or parental organism**

Escherchia coli E24377A which is a Clinical ETEC isolate and has been used previously as a clinical trial challenge strain. It is serogroup 0139:H28 and it expresses LT, ST, CS1 and CS3.

**Host/vector system**

Initial manipulations will be carried out in pUC or similar non-mobilisable vectors in E. coli Top10. The suicide vectors for introducing the deletion region to the chromosome will be pCVD442. These vectors will be manipulated in E. coli PIR1 which does not apply any plasmid transfer machinery, rendering the host vector combination mobilisation defective.

The suicide vector will be delivered by E. coli S17.1 Apir which supplies the Tra functions required to mobilise the plasmid into the ETEC recipient.

**Origin & function**

The material originates from commercial sources or collaboration laboratories and will function to generate defined deletions in certain genes.

**Evaluation of foreseeable effects**

The intermediate GMMs will be disabled E. coli K12 strains containing genetic material from ETEC.
The plasmid vector is unstable in the absence of selective pressure and the recombinant genetic material would be rapidly lost.

It is unlikely that the cloned DNA would offer any advantage to the survival of the E. coli strains in the environment.

No genetic material will be included in the final strain that would provide the recombinant ETEC with a selective or pathogenic advantage.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid cultures of small volumes less than 1L are chemically inactivated prior to disposal. Chemical inactivation is performed using Virkon which has been validate for treatment of E. coli. Following treatment at 3% for 14 hours, liquid waste is disposed of by dilution via the normal sewage waste drain. Liquid cultures of more than 1L are inactivated by autoclaving prior to disposal via the normal sewage waste drain.

All other contaminated materials are inactivated via autoclaving prior to disposal. A dedicated waste autoclave is used for these activities. This autoclave is serviced and validated annually to ensure proper function. Following inactivation by autoclaving, all waste is then removed by a private contractor for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment was reviewed by Microscience's GMSC and given a classification as containment level 2.

Project Containment

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Animal Units

| L2 | L3 | L4 | L2 |

Large Scale Activities

| L3 | L4 | L2 | L3 |

Human Clinical Applications

| L4 | L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |
Expression of Neisseria meningitidis serogroup B genes in Escherichia coli.

Disabled non-colonising strains of E. coli will be used for all cloning and expression steps. It is intended to use the following strains; TOP10, HMS174(DE3), BL21(DE3), Tuner(DE3), Rosetta(DE3), Origami2(DE3), HMS174(DE3) pLysS and BL21(DE3)pLysS.

Cloning vectors pCR2.1-TOPO and pCRXL-TOPO from Invitrogen will be used for initial cloning. The genes will then be transferred to pET26b (Novagen) or pNET(E1) (derivative of pET27b) for expression (T7 expression system).

The N meningitidis genes will be PCR amplified from N. meningitidis genomic DNA or alternatively may be chemically synthesised. The genes are to be expressed in E. coli with the aim of purifying the resultant proteins. The list of genes and their putative functions are included in Section 17.

The host strains to be used are deficient in a number of traits required for pathogenicity and it is therefore unlikely that a single gene would compensate for this. However, a number of the genes are predicted to encode proteins with outer membrane location. Their expression may therefore increase the pathoenicity of the E. coli host strain eg by altering tissue tropism.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be autoclaved or alternatively small volumes (<1L) may be inactivated overnight by addition of hycolin to 3%. Chemical inactivation with hycolin has been test in our laboratory and shown to result in a greater than 7 log reduction in viable E. coli (no viable organisms were detected after overnight inactivation). Liquid waste will then be diluted and disposed of via the normal sewage system. Solid waste will be autoclaved and then removed from site for incineration by a commercial contractor. A dedicated waste autoclave is used for these purposes. The autoclave is serviced and validated annually to ensure correct function.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This risk assessment has been reviewed by the Emergent Europe GMSC and classified as class 2.

Project Containment

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Project Ref 692/08.1

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<td>EXPRESSION AND PURIFICATION OF FULL-LENGTH FUNCTIONAL TOXINS A</td>
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**Project Additional Information**

**Purposes of the contained use**

Preparation of full-length functional C. difficile toxins for use as reagents in various immunoassays.

**Recipient or parental organism**

Disabled non-colonising strains of E. coli will be used for all cloning and expression steps. It is intended to use E. coli TOP10 during the cloning stages. E. coli BL21 (DE3) and HMS174(DE3) will initially be used for protein expression. Other E. coli expression strains may also be used. A study of the pathogenicity of laboratory-adapted E. coli showed that these organisms did not contain the well recognised pathogenic mechanisms required by strains of E.coli causing the majority of enteric infections (Chart et al., 2000. J Appl. Microbiol. 89: 1048-1058).

**Host/vector system**

The C. difficile toxin genes will be amplified by PCR in two halves, cloned separately into pCRXL-TOPO (Invitrogen), and used to transform E. coli TOP10 (Invitrogen). Full length genes or gene halves may also be cloned directly into pET26b (Novagen) without the intermediate pCRXL-TOPO steps. These steps will add on a 6 x histidine tag but not incorporate the pelB leader which is also possible with this vector. The toxins will be under the control of T7lac promoter and expressed in E. coli (DE3) hosts. Other host/vector systems, with the same characteristics may also be used.

**Origin & function**

The C. difficile toxin genes will be PCR amplified from C. difficile genomic DNA. The genes will be expressed in E. coli (DE3) hosts with the aim of purifying full-length functional toxin. Purified toxin will be used in various immunoassays to test sera generated from immunisation of animals/humans with an experimental vaccine.

C. difficile toxins A and B are high molecular weight glucosyltransferases that inhibit members of the Rho family of GTPases. Toxin A, historically referred to as the enterotoxin, has a molecular weight of 300kDa and an isoelectric point (pI) of 5.3. Toxin B, known as the cytotoxin, has a molecular weight of 270 kDa and pI of 4.1. These toxins are produced by C. difficile, the causative organism in antibiotic-associated diarrhoea and pseudomembranous colitis and the toxins play a key role in the pathogenesis.

**Evaluation of foreseeable effects**

The most hazardous GMMs to be constructed will be DE3-containing expression hosts with either toxin gene cloned in the pET26b(+) expression plasmid. The host strains to be used are deficient in a number of traits required for pathogenicity (Chart et al., 2000) and it is unlikely that the expression of a single C. difficile toxin gene would...
compensate for this. The toxins are secreted in C. difficile by an unknown mechanism though it may be mediated by the action of a holin-like protein. They are unlikely to be secreted through the Gram negative E. coli membrane. In the event of a laboratory-acquired infection occurring any unregulated expression of the toxin could lead to increased toxin effects compared to the parent strain. Theses strains have therefore been designated as Class 2; this is consistent with C. difficile and clinical isolates of E. coli.

All other strains constructed are considered as Class 1, as they will lack the ability to express the full length toxins. F strains expressing these toxins were to be ingested this could give rise to biological effects such as diarrhoea. Both toxins are also known to cause irritation following skin contact. It is unlikely that these strains would show altered host range, tissue tropism mode of transmission or resistance to killing by the immune system. However, expression of the toxins would be expected to increase the ability of the host E. coli strains to cause disease compared to the E. coli host strains used.

The following antibiotic resistances will be conferred, Zeocin (to E. coli TOP10 only) and kanamycin (to all E. coli strains used). Zeocin is never used to treat human infections and kanamycin is very rarely used to treat humans. As the plasmids used are all mobilisation defective it is highly unlikely that they would be transferred. If they were to be transferred then dissemination of the antibiotic resistance determinants would be of concern.

It is possible that aerosols could be generated by pipetting.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be disposed of according to the Emergent BioSolutions Laboratory Code of Practice. Briefly, all solid waste and large batches of liquid waste (maximum 2L) will be autoclaved and disposed of appropriately. A serviced and validated autoclave is used at all times. Alternatively small volumes of liquid waste are treated with 1% FAM30 or 1% virkon prior to disposal to drain. 70% ethanol, 1% FAM30 and 1% virkon have been tested on site against representative strains of Streptococcus agalactiae, Neisseria meningitidis, E. coli and Salmonella spp. and were found to be effective.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

This risk assessment has been reviewed by the Emergent GMSC and classified as Class 2.

Project Containment

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02/03/2022
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GM Centre Number: 693

Data Premises Notified (Originally) 04/03/99

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed 26/04/2005

Emergency Plan Required? N

Transitional Premises  N

Non-GMMs N

Withdrawn N

Name

UNIVERSITY OF WALES COLLEGE OF MEDICINE

Name 2

Department

SURGERY

Campus Estate or Research Centre

Building

Road Name

HEATH PARK

District

Town

CARDIFF

County

CARDIFF

Postcode

CF4 4XN

Country

WALES

Tel Number 029 2074 2903

Fax Number 029 2074 4869

E-mail

HSE Division WALES AND SOUTH WEST

Comments

GM693 Closed and merged with GM130 on 26/04/2005.

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Transgenic Animals
- Transgenic Fish
- Microbiology Research
- Virology
- Transgenic Birds
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 693/00.1

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Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Date at Which Additional Info Submitted**: 02/03/2022
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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities
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- Give brief details of the genetic modification safety committee

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- Other (please specify)
  - [ ]

- Tick if confidential
  - [ ]

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<td>Gene Therapy</td>
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02/03/2022
The largest single culture which could be released is 400 ml. Biological waste generated in the lab can be treated in one of two ways dependent on the physical nature of the waste. Liquid waste is treated with freshly prepared Virkon solution and left overnight prior to being washed down the sink with copious amounts of water. Solid waste (Agar plates) as autoclaved at 136 degrees C for 75 minutes.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
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<th><strong>GM Centre Number:</strong> 695</th>
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<td><strong>Data Premises Closed</strong></td>
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<th><strong>Name</strong></th>
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<tr>
<td><strong>Name 2</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Department</strong></td>
<td><strong>PROCESS INSTRUMENTATION DIVISION</strong></td>
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<tr>
<td><strong>Building</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Campus Estate or Research Centre</strong></td>
<td><strong>MELBOURN SCIENCE PARK</strong></td>
</tr>
<tr>
<td><strong>Road Name</strong></td>
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<tr>
<td><strong>Town</strong></td>
<td><strong>ROYSTON</strong></td>
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| **Tel Number**                   | **01763 262626**            |
| **Fax Number**                   | **01763 261964**            |
| **E-mail**                       |                              |
| **HSE Division**                 | **EAST AND SOUTH EAST**      |

**Comments**

CHANGED FROM THE (THE TECHNOLOGY PARTNERSHIP PLC) ON 1 APRIL 2002

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name 2**

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**Town**

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**Fax Number**

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**E-mail**

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**HSE Division**

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**Comments**

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**Date at Which Additional Info Submitted**

| 02/03/2022 |
### Premises Addresses

| Date Premises Closed | Name              | Department | Name 2 | Campus Estate or Research Centre | Building            | Road Name       | District       | Town         | County            | Post-code | Country        | Withdrawn |
|----------------------|-------------------|------------|--------|----------------------------------|---------------------|----------------|----------------|--------------|----------------|----------------|---------------|--------------|-----------|
|                      | UNIVERSITY OF LUTON |            |        | THE RESEARCH CENTRE              | THE SPIRES          | 2 ADELAIDE STREET | LUTON          | BEDFORDSHIRE | LU1 5DU         | ENGLAND | N             |          |

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
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<tbody>
<tr>
<td>Other(s)</td>
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</tr>
</tbody>
</table>

The maximum culture volume that could be released at any one time is 50 ml.
Any waste generated is disposed into Dispo Safe containers containing Virkon. The containers are then autoclaved in a Denley BA852 Sovereign Portable Autoclave for 15 minutes at 121 degrees C. The autoclaves are regularly serviced. Successful decontamination is monitored using autoclave marker tape.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 697

<table>
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<td>Building</td>
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**Comments**

GM660 TRANSFERRED TO GM697 ON 22/9/2003

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
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<tr>
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<td>Growth Room</td>
<td>Glass House</td>
<td>Large Scale</td>
<td>Tick if confidential</td>
</tr>
<tr>
<td>Bacteriology</td>
<td>Parasitology</td>
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<td>Microbiology</td>
<td>Birds</td>
<td>Research</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
Project Ref: 697/01.2

Date Ackn’d: 15/02/2001

CU2 Project Title:
EXPRESSION IN MAMMALIAN CELLS OF GENES ASSOCIATED WITH BREAST CANCER - USING RETROVIRAL VECTORS

Class: 2

CultureVolClass2: Class 2
CultureVolumeClass3-4: Class 2

Non-GMM: not applicable

Consent Granted: not applicable

Project notified under transitional arrangements: Yes

Withdrawn: No

Tick if notifying a connected programme of work: No

Historical Significant Changes:
TRANSFERRED TO GM 386 - 21/9/07

Historical Date of Additional Info: 21/9/07

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 697/01.3

Date Ackn'd 15/02/2001

CU2 Project Title ANALYSIS OF IMMUNE RESPONSE TO MUC1 ANTIGEN IN BREAST CANCER

Class 2

CultureVolClass2 CultureVolumeClass3-4
PATIENTS AND HEALTHY VOLUNTEER DONORS

Non-GMM

Consent Granted

not applicable

Date Project Ceased

21/09/2007

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Non-GMM

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Project notified under transitional arrangements

Tick if notifying a connected programme of work

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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GM Centre Number: 699

Data Premises Notified (Originally) 29/04/1999

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed 06/09/2002

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name
GENESCREEN LTD

Name 2

Department
LABORATORY

Campus Estate or Research Centre

Building

Road Name
QUEENSWAY

District

Town
NEW MILFTON

County
HAMPSHIRE

Postcode
BH24 5NN

Country
ENGLAND

Tel Number 01425 624788

Fax Number 01425 624787

E-mail

HSE Division EAST AND SOUTH EAST

Comments
WORK TRANSFERED TO GM705 ON 6/9/2002

Date at Which Additional Info Submitted 06/09/2002
## Premises Addresses

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<th>Name 2</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<td>Level 1 (GMMs)</td>
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Other (please specify)  

Tick if confidential

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<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum culture volume which could be released in any one incident is one litre.
All microbiological waste is autoclaved at 121 degrees C for 15 minutes before disposal by the waste contractors.
The satisfactory completion of the autoclave cycle is validated by the use of autoclave tape on all items or bags of waste autoclaved.
The autoclave is maintained under a service contract from the autoclave manufacturer and the service records are available for inspection as required.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
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**Name**

UNIVERSITY OF LIVERPOOL

**Name 2**

SCHOOL OF DENTAL STUDIES

**Campus Estate or Research Centre**

EDWARDS BUILDING

**Road Name**

DAULBY STREET

**Town**

LIVERPOOL

**County**

MERSEYSIDE

**Postcode**

L69 3GN

**Country**

ENGLAND

**Tel Number**

0151 706 5268

**Fax Number**

0151 706 5809

**E-mail**

**HSE Division**

NORTH WEST

**Comments**

GM700 closed down and merged with GM690 as of 15/04/2005

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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</tr>
<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
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</table>
The maximum culture volume that may be released at one time is 500-1000 ml. All liquid waste is deactivated using an approved method (1% Virkon for at least 2 hours) prior to disposal. Solid waste is deactivated by autoclaving prior to disposal or washing. This is monitored by a probe in the autoclave.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
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<th>Section</th>
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<td>Department</td>
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Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<th>Microbiology Research</th>
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02/03/2022
All containers which have been in contact with GMM are immersed in Presept at 4.5g/L for a minimum of 1 hour. Available chlorine is 2,500ppm and % kill is 99.999 (data from manufacturer). Alternatively waste is autoclaved for 15 minutes at 121°C. After treatment, materials going to waste are consigned to yellow bags or sharps boxes for incineration.

Spent tissue culture medium is either treated with a 1:1 volume of Presept at 9g/L for volumes up to 100ml or Presept tablet(s) are added directly to the media for volumes over 100ml to give a final concentration of 4.5g/L. Available chlorine is 2,500ppm and the % kill with 10% serum is 99.999 (data from manufacturer). After a minimum of 1 hr the liquid waste is disposed of down the sink. A maximum of 1L of culture medium will be released at any one time.

All Presept solutions are made fresh daily.

Used bacteriological plates and spent bacterial broth are autoclaved for 15 minutes at 121°C before being consigned to yellow bags for incineration (plates) or disposed of down the sink (broth).

The autoclave is serviced twice a year at which a NAMAS one point calibration is carried out.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Name

KUDOS PHARMACEUTICALS LTD

Name 2

Department

Campus Estate or Research Centre

327 CAMBRIDGE SCIENCE PARK

Road Name

MILTON ROAD

Building

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB4 4WG

Country

ENGLAND

Tel Number

01223 719719

Fax Number

01223 719720

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

<table>
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## Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

  - Give brief details of the genetic modification safety committee

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

- Other (please specify)

  - Tick if confidential

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
Virology
Mycology

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 704/09.1**

**Date Ackn'd** 11/03/2009

**CU2 Project Title** Use of EBV transformed human B-Lympholastoid cell lines to determine mechanism of action of small molecule inhibitors of DNA repair pathways.

**Date Project Ceased** 06/04/2009

**Class** Class 2

**CultureVolClass2** < 1 Litre

**CultureVolumeClass3-4**

**Non-GMM Consent Granted** No (Refused)

**Consent Granted**

**Tick if notifying a connected programme of work** N

**Project notified under transitional arrangements** N

**Historical Significant Changes** Y

**Historical Date of Additional Info** Application refused 01/04/2009

**Significant Change ID**

**Date of Significant Change**

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The cell lines are infected with Epstein - Barr virus so therefore work using the cell lines should be carried out under level 2 conditions.

### Recipient or parental organism

Mammalian cells: B-Lymphoblastoid cell lines such as DK0064, GM14680, GM01526. These cell lines are well characterised mammalian cells which have been immortalised through infection with EBV. The cells will not be modified by KuDOS in anyway.

### Host/vector system

N/A

### Origin & function

DK-0064 cells have been derived from a Parkistani family (family 1) with Seckel syndrome (ref Goodship). The FO2-98 cells are primary fibroblasts from this family (ref Stiff). The DK-0064 LBL cells have been derived from the same patient and infected with EBV to immortalise them.

- Stiff et al EMBO 2005
  - "DK0064 is an ATR defective LBL derived from the same patient as FO2-98 cells"

- O'Driscoll et al Nature Genetics 2003
  - "FO2-98 is a fibroblast line established from an individual from family 1 affected with Seckel syndrome". Ref Goodship et al Am J Hum Genet 2000.

The cells will be used in growth inhibition and cytotoxicity assays as well as in western blotting, flow cytometry and FACs to look for ART signalling and identify possible biomarkers.

### Evaluation of foreseeable effects

The cell lines are well characterised mammalian cells which contain EBV. KuDOS has not modified the cell lines in anyway and they are the wild type. The cell lines could survive in the environment however there is a low risk of any adverse consequences to the environment arising from their release.

- Possible routes of transmission to humans would be via exposure to culture medium. The likely routes of transmission would be inoculation by contaminated sharps, contact with open wounds. Therefore no sharps or glass should be used in conjunction with this project, work should be carried out in the designated class II biological safety cabinet and the general rules and procedures outlined in the local GMO rules adhered to.
- Possible route of transmission to the environment would be through the waste disposal. All waste will be processed under level 2 conditions and decontaminated prior to leaving the company by a validated means so therefore the risk to the environment will also below.
- The cells have been classified as containment level 1 however as they are infected with EBV the overall containment level for the project is classified as 2. Therefore the project will be carried out under class 2 containment conditions.
- All users are aware of the risks and are competent at working at a class 2 level.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All infected waste material is disposed in a safe manner. All level 2 GMO waste is required to be inactivated by validated means prior to disposal (Virkon and autoclaving). The autoclave is situated in the same laboratory suite and suitable containers are used to transport the waste material to the autoclaves so ensuring that the waste is contained. Any waste that cannot be autoclaved on site (waste that is too large for the autoclave, such as pipettes) is disinfected using Virkon and then sent for incineration in UN approved containers using an approved external waste management company. The autoclaves are serviced on a 6 monthly basis and inspected annually.

Is an emergency plan required according to regulation 20?  N

Tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification sub-committee requested justification for the use of the EBV infected cell lines.

Response of user: The DK64 Seckel (ATR mutant) cell lines are the most relevant cell lines for the work that needs to be undertaken. All other Seckel cell lines are either fibroblasts or poorly characterised in terms of protein levels and functional assays. Other cell lines have been investigated but there are no other cell lines with the same characteristics which are not EBV transformed. Similarly, B-cell lines such as GMO 1526 which harbour ATM.

Committee response: Justification accepted.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 704/99.1

Date Ackn'd 02/03/2022
IDENTIFICATION OF POTENTIAL CANCER THERAPY DRUGS: INTRODUCTION 
OF DNA REPAIR FACTORS, SELECTABLE MARKERS, APOPTOTIC INDUCING 
FACTORS AND VIRAL ONCOPROTEINS INTO MAMMALIAN CELLS

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
GM Centre Number: 705

Data Premises Notified (Originally) 02/07/1999

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Emergency Plan Required? N

Transitional Premises

Non-GMMs N

Withdrawn N

Name

GENETIX LTD

Name 2

Department

LABORATORY

Campus Estate or Research Centre

Building

Road Name

QUEENSWAY

District

Town

NEW MILTON

County

HAMPSHIRE

Postcode

BH25 5NN

Country

ENGLAND

Tel Number 01425 624788

Fax Number 01425 624787

E-mail

Comments

INCLUDES WORK FROM FORMER GM699

Date at Which Additional Info Submitted

06/09/2002
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

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<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
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</table>
The maximum culture volume that could be released at any one time is less than 1 litre. All waste is deactivated in an autoclave, with a 15 minute cycle at 121 degrees C. Each load has autoclave tape on it, which turns black after 15 minutes at 121 degrees C. The autoclave is under a service contract and is thoroughly checked/serviced every 6 months. Documented record of service contract and each service is available.
# Project Additional Information

## Purposes of the contained use

To culture and propagate the above class II cell lines and carry out various cell biological assay procedures on these lines. Such studies will include microscopic imaging, fluorescent immunoassay development for researching cellular processes (e.g. secretion of an endogenous/transfected protein of interest, expression of endogenous/transfected receptors or bio-markers of interest, etc.), expression and reporter gene function. The GMOs will only be used for research and cell based assay validation purposes.

## Recipient or parental organism

Mammalian cell lines including Jurkat, Human leukaemic T-cell (SupT1), A431, Calu-6, B13 Chinese Hamster Ovary cells, HEK293 cells.

These are immortalised cell lines maintained in sterile cell culture. They are handled by authorised, trained operators only in a contained environment. Gloves, howie-style lab coats and shoes are worn at all times within the lab and are kept in a separate area.

## Host/vector system

HEK293 cell have been stably transfected with a multi-vector non-replicative lentiviral vector system.

DNA plasmid (E.coli based origin) purified and transfected into cells to express human proteins and selectable resistance gene.

B13 CHO cells are transfected with plasmids containing both cytomegalovirus (CMV) and SV40 viral sequences.

## Origin & function

In the case of antibody production, the DNA was generated by taking mouse B cells and fusing them with myeloma cells to immortalise them, selecting the specific clones required and isolating the DNA. The gene of interest is then integrated into a plasmid as described above and transfected into the cell. Other proteins can be isolated from RT-PCR from Human RNA/cDNA samples.

The function of the genetic material in the cells can be to generate sufficient quantities of a therapeutic protein.

Many of the cell lines in question are used for analysis of samples for cell surface markers for identification of genotypes. These have not had any DNA inserted into them.

In the lentiviral system, cells are transfected with multiple vectors expressing components of a lentivirus, including a gene-of-interest. This system is used to produce non-replicative viral particles able to infect cell lines, which then express the gene-of-interest.

## Evaluation of foreseeable effects

Cells stably transfected with lentiviral vector system produce viral particles able to infect human cells and therefore could integrate into the host cell genome, potentially leading to insertion of oncogenic sequences or inactivation of host-cell tumour suppressor function. However, exposure of the operator to quantities of virus high enough to cause such effects would be very unlikely during standard laboratory-based manipulations.

Protective clothing is worn at all times within the lab. Apart from cells transfected with the lentiviral system, the agent is not considered contagious and even though the cancer cells are human, they are still 'non-self'. The only potentially dangerous route of exposure is via injection or sharps injury (broken contaminated cover slips etc) and the effect to the lab staff would at worst be a strong immune response/inflammation to the Cat II agent. Although no infection/tumour would be able to take hold, a sufficiently high 'dose' could trigger an immune response as the cells/virus like particles are rejected and cleared.
Since both cells and sub-genomic VLPs are not infectious there would be no risk to the wider population of the business or to the general public if a lab worker was exposed to these materials.

Measures are in place to ensure that with good laboratory practice this does not happen. It should not be the case that syringe needles are being used with these cell lines, but they may be used by others in the lab for different cell lines. These are deposited in sharps bins immediately after use.

Since the GMOs are considered incapable of survival in the environment no effects on the physical environment are expected

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Mammalian cell lines are maintained in sterile culture on a small scale (no more than 50ml).

The lab is a contained single purpose environment separated from the rest of the building by a semi-sterile lobby area and two sets of doors.

Access is by authorised, trained users only.

All new users are introduced to the safety measures in place in the lab and supervised initially.

Howie style lab coats, protective shoes and gloves are used at all times by anybody entering the lab. These garments are used only for this lab and are kept in the lobby area. At no time are they used for any other purpose.

All lab coats and shoes are changed weekly and subjected to a hot wash to kill any remaining material.

Cell culture work is carried out in laminar flow hoods with all waste disposed of in biohazard bins. Liquid waste is treated with Virkon to destroy any live material. Waste is double bagged in red biohazard bags before removal for autoclaving on site and incineration off site.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposal of any waste which has come into contact with the cells is disposed of in biohazard bins. Waste is double bagged before removing from the lab for autoclaving. Waste is then incinerated off site by Veolia Environmental Services Birmingham Ltd, James Road, Tyseley, Birmingham, B11 2BA. This method gives 100% kill. Liquid waste is treated with Virkon and left for >8 hours. This method is sufficient to kill any living material left in contact with it (100% kill).

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form 

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

02/03/2022
## Project Containment

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- **Animal Units**
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  - L3
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- **Large Scale Activities**
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- **Human Clinical Applications**
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Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

EHS Officer, R&D manager (chair of GM committee), R&D supervisor & Senior Scientist in Molecular Biology

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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Tick if confidential

Bacteriology

Parasitology

Transgenic Birds

Microbiology Research

Transgenic Animals

Transgenic Fish

Gene Therapy
Other(s) Generate recombinant proteins which are used for catalogue sales and for screening potential

For activities involving GMMs, describe the waste management measures which will apply to the activity

i) solid material - double bagged including an outer yellow bag marked with biohazard symbol that is held in rigid plastic theatre bins. When full, the bags are sealed, removed from the bins and collected by a waste management company for incineration.

ii) Contaminated glasswear/lab equipment and liquids - these are cleaned/disposed of in one of 2 ways:
1. Soaking in a 1% Virkon solution for a minimum of 2 hrs, after which the equipment can be rinsed and where appropriate transferred to dishwasher and then drying oven.
2. Autoclave at 121 degrees C for 20 minutes to remove the biohazard risk. When cool, the equipment is washed by hand or in the dishwasher, then placed in the drying oven.

Tick to confirm that you are attaching a summary of the risk assessment □

Tick if you are claiming exemption from disclosure for sections of the risk assessment □

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 707**

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### Premises Conditions

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Give brief details of the genetic modification safety committee

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<th>Transgenic Fish</th>
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<th>Gene Therapy</th>
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**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum culture volume that could be released at any one time is 6 litres (from 12 one-litre flasks half filled with culture medium).

Waste is deactivated either by autoclave (plates etc.) or by treatment with strong hypochlorite (media etc.), 2500 ppm using presept tablets.

1 x 5g Presept tablet in 1 litre water  
1 x 2.5g Presept tablet in 500 ml water  
1 x 0.5g Presept tablet in 100 ml water

Surfaces are treated with hypochlorite (1000 ppm) 1 x 2.5g tablet in 1 litre water.
In the case of autoclaving, the deactivation is validated and monitored by internal temperature probes.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 709**

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**Name**

BRIXHAM ENVIRONMENTAL LABORATORY

**Name 2**

ATRAZENECA

**Department**

Campus Estate or Research Centre

**Building**

Road Name

FRESHWATER QUARRY

**District**

Town

BRIXHAM

**County**

DEVON

**Postcode**

TQ5 8BA

**Country**

ENGLAND

**Tel Number** 01803 882 882

**Fax Number** 01803 882 974

**E-mail**

HSE Division

WALES AND SOUTH WEST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

#### Laboratory

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)

#### Animal Unit

- Non-microbial

#### Growth Room

Other (please specify)

Tick if confidential

#### Glass House

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

- Transgenic Fish

02/03/2022
Largest culture volume will be 5 litres. All solid and liquid GMM waste will be sterilised prior to disposal, by autoclave, or use of a suitable chemical disinfectant. Any spillages will be treated by neat Virkon powder. All autoclaves are serviced at regular intervals.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 711

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**Name**

CENES PHARMACEUTICALS PLC

**Department**

RESEARCH & DEVELOPMENT

**Campus Estate or Research Centre**

VISION PARK

**Building**

COMPASS HOUSE

**Road Name**

CHIVERS WAY

**District**

HISTON

**Town**

HISTON

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 9ZR

**Country**

ENGLAND

**Tel Number**

01223 266 466

**Fax Number**

01223 266 467

**E-mail**

HSE Division

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Tick if confidential

02/03/2022

Page 10795 of 15326
The maximum active culture volume that could be released at one time shall not exceed 20 litres. All cell culture liquid waste is autoclaved using a set autoclave programme giving a holding temperature of 125 degrees C for 15 mins. Bottles containing bacterial liquid waste additionally receive Virkon powder to generate a 1% Virkon solution. Solid waste is placed into autoclavable bags and autoclaved using the same conditions as above. Reusable glassware is treated by pouring 1% Virkon solution into the glassware, rinsing and pouring into an autoclavable waste bottle. Waste bottled are autoclaved at 125 degrees C for 15 mins.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

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**Date at Which Additional Info Submitted**

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| OXFORD RESEARCH UNIT | THE OPEN UNIVERSITY       |                     |                      |                                 |                |               |               |               |              |             |            | N          |

### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

**Give brief details of the genetic modification safety committee**

**Laboratory**

**Animal Unit**

**Growth Room**

**Glass House**

**Large Scale**

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

**Other (please specify)**

**Tick if confidential**

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
The maximum culture volume will be approximately 5 litres.

1. Liquid Waste: All liquid culture waste is disinfected using by adding VIRKON powder to a final concentration of 1% followed by standing for 20 minutes before being disposed to drains.

2. Solid Waste: All solid waste is autoclaved at 121 degrees C for 15 minutes and then disposed by incineration through a clinical waste stream using off-site contractors.

Autoclave activity is monitored using "Sterikon Bioindicators" (Merck) in which the growth of spores from Bacillus stearothermophilus after autoclaving is monitored. This effectively monitors bacterial activity after sterilisation. In addition, all waste is incinerated through a clinical waste stream.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 712/11.1

Date Ackn’d 06/06/2011

Date Project Ceased

CU2 Project Title In vitro investigation of the mechanisms of CNS pathology in autoimmune and degenerative disease

Class 2 CultureVolClass2 < 1 Litre CultureVolumeClass3-4

Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The scientific aim is to investigate in vitro the mechanisms of CNS pathology in autoimmune disorders such as multiple sclerosis or in conditions with an inflammatory component such as Alzheimer's disease. In this context, cultures of three types of brain resident cells, namely microglia, astrocytes and brain endothelial cells, will be transduced with replication-deficient lentiviruses containing specific genes or small RNAs which are thought to be involved in the control of inflammatory processes within the CNS.

The genes under investigation will be amplified in bacteria (step 1)

Lentiviral particles containing the genes will be assembled in a packaging cell line (HEK 293). (step 2)

The human cell lines will be transfected with the genes under investigation using the lentiviral particles. (step 3)

The vectors will be generated using a Lenti virus kit available from Systems Biosciences

#### Recipient or parental organism

Step 1: Standard laboratory K12 derivatives of E. coli (DH5alpha, MC1061/P3) will be used to produce plasmids carrying genes that allow expression of small RNAs. The cloned DNA fragments will be used as reagents for introduction into mammalian cells (see below).

Step 2: A packaging cell line HEK293 will be transiently transfected with plasmids. Since the DNA introduced in the transfection process is usually not integrated into the nuclear genome, the foreign DNA will be diluted through mitosis or degraded and hence transfected HEK293 will not itself be permanently modified.

Step 3: Recipient cells for transduction with lentiviral particles will be primary human cells and human cell lines originating from: 1) human brain tissue: primary brain astrocytes, microglia, neurons, ependymal cells, and endothelial cells, and established glioma, microglial and endothelial cell lines (U373-MG, CHME3, hCMEC/D3); 2) blood and lymphoid tissue: PBMCs, human lymphoblastoid T cell lines (CEM, Jurkat, H9) and monocytic cell lines (U937, THP-1).

#### Host/vector system

Steps 1-3

We will use two commercially available lentiviral systems provided by Thermo Scientific and SBI that will allow us to produce lentiviral particles in-house by transfection of packaging cell lines. This will require transformation of bacteria with the same vectors for amplification (step 1). Lentiviral particles will then be used as vehicles for transducing and expressing different effector molecules or reporter constructs into cells (step 3). Below is a list of all vectors necessary to produce viable lentiviral particles and these have been separated into: A) transducing lentiviral vectors whose genomic sequence contained within the LTR will be integrated into human cell lines; and B) Lentiviral packaging vectors necessary for lentiviral production by the packaging cell lines. Frozen stocks of lentiviral particles containing reporter genes with the 3'UTR of
the gene of interest are also available commercially (Sigma-Aldrich; MISSION® 3’UTR Lenti GoClone). Although in this case, the original vectors will be neither amplified in bacteria nor transfected into packaging cells, the description of the original transducing lentiviral vector has been induced below in the transducing lentiviral vector category as it is relevant for step 3 (transduction of human cell lines).

A) Transducing lentiviral vectors:
1) pTLA1-Pak: Components of the plasmid are: Human cytomegalovirus (Tre-CMV) RNA polymerase ll promoter that permits high-level expression of the lentiviral gag and pro genes in mammalian cells; gag, virion structural proteins for forming the virion core and ribonucleoprotein complex within the core; pro, aspartyl-protease that cleaves the Gag, Gag-Pro-Pol polyproteins to produce viral proteins in their mature forms; rev, viral protein that binds to the RRE and facilitates the transport of unspliced and incompletely spliced mRNAs to the cytoplasm; tat, transcriptional activator of lentiviral gene expression, enhancing LTR-directed transcription; polyA, allows transcription termination and polyadenylation of the mRNA; Ori, permits high-copy replication and maintenance in E. coli; Amp, allows selection of the plasmid in E. coli.

2) pGIPZ (Thermo-Scientific): Lentiviral vector mainly used for transducing small RNAs (microRNAs, shRNAs) that contains; CMV RNA polymerase ll promoter that permit high-level expression of the lentiviral gag and pro genes in mammalian cells; Ψ (Psi), region of viral RNA responsible for directing packaging; PPT, purine-rich sequence cleaved during reverse transcription to produce the RNA primer for synthesis of viral DNA; wpre, post-transcriptional regulatory element derived from the woodchuck hepatitis virus to increase vector independent expression levels of the transgene; 3’ & 5’ LTR with deletion of the transcriptional enhancers & promoter in the U3 region of the 3’ LTR; GFP Green fluorescent protein utilized to track shRNAmir expression; Puro, puromycin-N-acetyl transferase, mammalian drug selectable marker; shRNAmir, microRNA flanking a stem-loop-stem structure; polyA, allows transcription termination and polyadenylation of the mRNA; Ori, permits high-copy replication and maintenance in E. coli.

3) SBI Lentiviral expression vectors that contain Hybrid RSV-5’LTR promoter; cPPT, GAG. LTRs which are genetic elements necessary for the packaging, transduction, and stable integration of the viral expression construct into genomic DNA, the 3’ LTR is a self-inactivating long terminal repeat that results in replication-incompetent particles; SV40 origin, provides stable propagation of the lentiviral plasmid in 293 producer cells; pUC origin, ensures high copy replication and maintenance of the plasmid in E. coli cells; Ampicillin resistance, used for selection in E. coli cells; WPRE element, enhances stability and translation of the lentivector-driven transcripts; SV40 polyadenylation signal. These plasmids also contain a promoter that drives the expression of the gene (CMV, EF1alpha) or small RNA (H1) and reporter genes such as GFP, RFP, Puromycin, Hygromycin, Neomycin or Zeocin selection.

4) pLSG_UTR_RenSP (Sigma-Aldrich) contains Constitutive human RPL10 promoter; RenSP Optimized Renilla luciferase gene; XbaI, NheI, AvrII, Xhol, FseI, Multiple Cloning Site for human 3’UTR insertion; PGKprom Human phosphoglycerate kinase eukaryotic promoter; Puro Puromycin resistance gene for mammalian selection; LTRs Long terminal repeats; Amp, Ampicillin resistance gene for bacterial selection; ori, Origin of replication; RRE, Rev response element.

B) Trans-Lentiviral Packaging Vectors: are an optimized mixture of packaging plasmids to facilitate viral packaging of the transfer vector following co-transfection into HEK293T producer cells. These plasmids supply the helper functions as well as structural and enzymatic proteins in trans required to produce the lentivirus. The plasmids are:

Thermo-Scientific Open Biosystems:
1) pTLA1-Pak: Components of the plasmid are: Human cytomegalovirus (Tre-CMV) RNA polymerase ll promoter that permits high-level expression of the lentiviral gag and pro genes in mammalian cells; gag, virion structural proteins for forming the virion core and ribonucleoprotein complex within the core; pro, aspartyl-protease that cleaves the Gag, Gag-Pro-Pol polyproteins to produce viral proteins in their mature forms; rev, viral protein that binds to the RRE and facilitates the transport of unspliced and incompletely spliced mRNAs to the cytoplasm; tat, transcriptional activator of lentiviral gene expression, enhancing LTR-directed transcription; polyA, allows transcription termination and polyadenylation of the mRNA; Ori, permits high-copy replication and maintenance in E. coli.

2) pTLA1-Enz: Human cytomegalovirus (CMV), RNA polymerase ll promoter that permits high-level expression of the lentiviral RT and IN genes in mammalian cells; Vpr Viral protein that shuttles the RT and IN into the viral particle; RT Reverse Transcriptase, DNA polymerase that copies RNA and DNA; IN Integrase, Enzyme responsible for inserting he linear double-stranded DNA copy of the lentiviral genome into host cell DNA; RRE, binding site for the Rev protein that aids in the transport of unspliced and singly-spliced RNAs from the nucleus to cytoplasm; polyA, as above; Ori, as above.

3) pTLA1-Env: In addition to CMV, polyA and Ori, as above, it contains VSV-G Envelope G glycoprotein from Vesicular Stomatitis Virus to allow production of a pseudotyped lentivirus.

4) pTLA1-Rev: In addition to CMV, polyA and Ori, as above, it contains tat and rev, as above.

5) pTLA1-TOFF: In addition to CMV, polyA and Ori, as above, it contains tTA, a transactivator protein that binds to the TRE in the absence of doxycycline and allows
transcription through the minimal CMV promoter.

System Biosciences (SBI):
1) In addition to CMV, polyA and Ori, as above, the pPACKH1-GAG plasmid (SBI) contains the structural (gag), and replication (pol) genes which code for some of the proteins required to produce the lentivirus. It also encodes the viral env gene, which encodes the envelope protein that defines the tropism (i.e. the range of infectable cells).
2) In addition to CMV, polyA and Ori, as above, the pPACKH1-REV plasmid (SBI) contains the regulatory protein rev that is required for HIV replication.
3) In addition to CMV, polyA and Ori, as above, the pVSV-G plasmid (SBI) expresses the envelope glycoprotein of vesicular stomatitis virus (VSV-G) from the CMV promoter.

The lentiviral particles will be used to directly transfect the primary cells and cell lines (see above), to stably integrate the genes of interest and allow their expression.

Origin & function

Genes to be transduced will be
a) microRNAs or shRNAs under an H1 promoter, in order to knockdown expression of cellular genes which have appropriate microRNA targetting sequences
b) reporter genes such as luciferase with the 3' UTR of the target gene to test microRNA target specificity,

Evaluation of foreseeable effects

Step 1: DNA amplification in bacteria.
E. coli derivatives are non-colonising and disabled, considered non-pathogenic to humans (ACDP hazard group 1). Transformed bacteria will be grown at a scale of a maximum 2 litres/amplification step. The vectors used are well characterised standard non-mobilisable plasmids. Although the cloned fragments may have biological effects as explained above, they have been cloned into sites which are not engineered to drive expression in prokaryotic cells. For the purposes of risk assessment, the cloned gene products may be considered non-harmful. No significant hazards have been identified above. Therefore, the assigned provisional containment level is 1.

Step 2: Transient transfection of vectors into packaging cell lines to produce viable lentiviral particles
Vectors: All plasmid vectors are based upon bacterial replicons and hence cannot be stably maintained as extra chromosomal elements in mammalian cells. Thus, the risk of transfer to humans is extremely low. Stably integrated plasmids are very unlikely to excise from their integration site. Therefore, the potential for horizontal gene transfer is negligible.
Expression: In packaging cells, constructs will drive the expression of pseudoviral RNA by LTR or hybrid RSV/LTR and enzymatically active mRNA or small RNA by the CMV, EF1 or H1 promoters. It is anticipated that the expression of these will be high enough to produce high pseudoviral titres in the supernatant. In packaging cells, constructs will also drive expression of an enzymatically active mRNA or small RNA. The CMV, EF1alpha and H1 promoters driving expression are strong promoters. It is therefore expected expression levels to be high.
Product: The expression of RNA and protein products such as VSV-G and accessory viral proteins from the different plasmids in the packaging cells will result in the production of viable pseudoviral particles which could potentially infect the worker and the potential hazard to human health is high. However, these particles are replication-defective as they do not carry the env gene necessary for viral replication and cannot propagate within the newly infected cell. In target cells, RNA gene products alone will be very unstable outside the cell and are very unlikely to have any biological effect. The protein products, if released outside cells, will be subject to protease degradation, which will greatly reduce the likelihood of hazard.
Recipient cells: The packaging cell line like all human non-tumorigenic cells can be considered as especially disabled host as they are unlikely to colonize the worker and do not contain any adventitious agents. Increased infectivity or pathogenicity of the GMMs is extremely unlikely, especially following transient transfections. Expression lentivectors together with the packaging plasmids from both companies comprise the third-generation lentiviral expression system. The SBI HIV-based lentivectors are based on the vectors developed for gene therapy applications by Dr. J (US patent #5,665,577 and #5,981,276). The Trans-LentiviralTM GIPZ Packaging System uses a replication-incompetent lentivirus based on the trans-lentiviral system developed by Kappes et al. (2001). However, both SBI's and Thermo-Scientific HIV-based lentivector systems are designed to maximize their biosafety features, which include:
• A deletion in the enhancer of the U3 region of 3′LTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.

02/03/2022 Page 10802 of 15326
In the SBI system, the CMV and RSV promoters upstream of 5'LTR in the lentivector allow efficient Tat-independent production of viral RNA, reducing the number of genes from HIV-1 that are used in this system.

In both systems, the number of lentiviral genes are necessary for packaging, replication and transduction; corresponding proteins are expressed from different plasmids lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSVG expression vector, or any other vector, to prevent generation of recombinant replication-competent virus.

In both systems, none of the HIV-1 genes (gag, pol, rev) will be present in the packaged viral genome, as they are expressed from packaging plasmids lacking packaging signal therefore, the lentiviral particles generated are replication-incompetent.

Pseudoviral particles will carry only one copy of the expression construct.

Therefore, even though the genetic manipulation of packaging cell lines in step 2 may be considered as level 1, and despite the above safety features, the production of HIV-based particles falls within Containment Level 2 criteria due to the potential biohazard risk of infection of the worker and hence the potential hazard to human health being high.

Step 3: Transduction of target cells with lentiviral particles
Vectors: Replication-defective lentiviruses will be able to transduce and integrate the genomic sequence flanked by the LTR sequences into most eukaryotic cells.
Expression: In transduced cells, constructs will drive the expression of enzymatically active mRNA or small RNA by promoters are strong and it is therefore expected expression levels of transcripts to be high.
Product: In target cells, transcribed small RNAs are biologically active and will be able to modulate the expression of not only a single gene but also many other putative target genes. Although the transgenes in themselves will not be harmful to the host cells, they may regulate their proliferative ability or induce apoptosis as a consequence of endogenous gene regulation. Outside the cell, RNA gene products alone will be very unstable and are very unlikely to have any biological effect. The protein products, if released outside cells, will be subject to protease degradation which will greatly reduce the likelihood of hazard.
Recipient cells: All established human cell lines can be considered as especially disabled hosts as they are unlikely to colonize the worker and do not contain any adventitious agents. Increased infectivity or pathogenicity of the GMMs is extremely unlikely. Primary human cells are unscreened and can potentially contain adventitious agents.

Recombinations between integrated sequences with endogenous viral sequences resulting in more pathogenic or replication-competent viral particles is extremely unlikely especially due to the transient nature of experiments. In the context of insertional mutagenesis, all transducing lentiviral vectors used are self-inactivating systems where the virus is deleted in the 3' LTR thereby reducing the potential for transactivation of cellular genes as a result of insertion. Also the risk of rescue of such vectors by wild type HIV is significantly reduced. However, the risk of insertional mutagenesis from disruption of genes following insertion although remote must still be considered.

Therefore, the genetic manipulation in step 3 may be considered as level 2, due to the potential remote biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. Measurement of virus titre (e.g. p24 levels by ELISA) should be monitored if any Class 1 GM activity is performed subsequently with the GMM.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be autoclaved in the LTE Autoclave followed by disposal as clinical waste for incineration. All liquid waste will be inactivated in 1% Virkon overnight and flushed away down the sink.
The autoclaves are tested and validated twice a year.
The committee was concerned that the original application included potential oncogenes and the combination of potential oncogenes with the host/vector systems given confused the risk assessment. The committee requested that these inserts should be deleted or submitted as a separate more closely defined project with a more limited range of host cells. This has been done.

**Project Containment**

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### Premises Conditions

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Give brief details of the genetic modification safety committee

The GMSC is a subgroup of our Safety Committee that meets once a month. The Committee is currently, M G PhD (Chair), R B PhD, J J PhD, M L PhD, M W and MM

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For activities involving GMMs, describe the waste management measures which will apply to the activity

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<td>Other(s)</td>
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Maximum culture volume released at any one time = 5 litres.
All solid wastes will be collected in special bags and autoclaved before disposal.
All liquid wastes will be soaked in 1% final Virkon solution for 2 hours before disposing through the sink.
Working space together with equipment will be decontaminated using 1% Virkon solution and IMS.
Spillage will be wiped with 1% Virkon solution or with Virkon powder depending on the quantity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 713/06.1**

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Withdrawn | N |

Tick if notifying a connected programme of work | N |

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The goal of this work is to validate PKCq as a drug discovery target by investigating in vitro the effects of the disruption of PKCq signalling on the function of cultured primary human T Cells. The aim is to transiently transfect primary T cells with a DNA construct expressing kinase-inactive human PKCq in order to interfere with PKCq-dependent signalling (transfecting wild-type human PKCq as a control), and to assess the consequences using standard measures of T cell function such as inflammatory cytokine production and T cell adhesion.

**Recipient or parental organism**

The ultimate recipient strain will be primary human T cells (routinely purified in the laboratory according to standard procedures). Cultures of these cells will be transduced with lentiviruses carrying the pLenti4/TO/V5-DEST, pLenti4/TO/V5-DEST-PKCq, pLenti4/TO/V5-DEST-PKCqK409R or pLenti4/TO?V5-GW/ICNe expression vectors, which is predicted to induce constitutive transient expression of no protein (negative control), PKCq, PKCqK409R or b-galactosidase (positive control), respectively. Full details of all recipients are included in the attached RA.

**Host/vector system**

The ViraPower Lentiviral Expression System will be used. The supplied human 293FT packaging cell line will be transfected with both the Packaging Mix (containing the pLP1, pLP2 and pLP/SVG packaging vectors) and one of each of five expression vectors: pLenti4/TO/V5-DEST, pLenti4/TO/V5-DEST-PKCq, pLenti4/TO/V5-DEST-PKCqK409R, pLenti4/TO/V5-GW/ICNe and pLenti6/TR. This will generate five different replication-incompetent lentiviruses, carrying one of each of the expression vectors. The numerous safety features of this system are detailed in the RA.

**Origin & function**

The pENTR™ 221-PKCq vector is an entry vector containing the PKCq cDNA sequence and will be purchased from Invitrogen Life Technologies. This vector will be mutated (using a standard commercially available kit) at a single codon in the PKCq cDNA to generate pENTR9™ 221-PKCqK409R, which is an identical entry vector containing the cDNA sequence for kinase-inactive (K409R) PKCq.

The PKCq and PKCqK409R cDNAs will then be cloned (using standard techniques) into the pLenti4/TO/V5-DEST lentiviral destination vector (suppliedwith the kit), to generate pLenti5/TO/V5-DEST-PKCq (encodes wild-type human PLCq) and pLenti/TO/V5-DEST-PKCqK409R (encodes kinase-inactive human PKCq). A control destination vector encoding bacterial b-galactosidase, pLenti4/TO/V5-GW/ICNe is also supplied with the kit.

The supplied human 293FT packaging cell line will then be transfected with both the (supplied) ViraPower Packaging Mix (containing the pLP1, pLP2 and pLP/SVG packaging vectors) and one of each of five expression vectors: pLenti4/TO/V5-DEST, pLenti4/TO/V5-DEST-PKCq, pLenti4/TO/V5-DEST-PKCqK409R, pLenti4/TO/V5-GW/ICNe and pLenti6/TR. pLenti6/TR is a vector that encodes the bacterial Tet repressor in mammalian cells and provides the option of Tetracycline-regulated expression of the gene of interest (PKCq) if co-transduced with the pLenti4/TO/V5-DEST expression vectors. This will generate five different replication incompetent lentiviruses, carrying one of each of the expression vectors: pLenti4/TO/V5-DEST, pLenti4/TO/V5-DEST-PKCq, pLenti4/TO/V5-DEST-PKCqK409R, pLenti4/TO/V5-GW/ICNe and pLenti6/TR.

The final recipient strains will be primary human T Cells (routinely purified in the laboratory according to standard procedures). Cultures of these cells will be transduced with lentiviruses carrying the pLenti4/TO/V5-DEST, pLenti4/TO/V5-DEST-PKCq, pLenti4/TO/V5-DEST-PKCq K409R, pLenti4/TO/V5-GW/ICNe expression vectors, which is predicted to induce constitutive transient expression of no protein (negative control), PKCq, PKCqK409R or b-galactosidase (positive control), respectively. Co-transduction of cells with lentiviruses carrying any of these vectors plus lentiviruses carrying the pLenti6/TR plasmid provides the additional option of tetracycline-regulated expression of the gene of interest from the expression vectors. This is because the pLenti6/TR plasmid encodes the bacterial Tet repressor, which inhibits gene expression from pLenti4/TO/V5-DEST derived vectors due to the presence of Tet operator (TetO2) sequences in the pLenti4/TO/V5-DEST promoter.

Finally, the transduced cells would be assayed for PKCq expression and the effects of transduction with the various constructs on T cell function.
Evaluation of foreseeable effects

The most noteworthy GMMs resulting from this work are lentiviruses harbouring the pLenti4/TO/V5-DEST-PKCq and pLenti4/TO/V5-DEST-PKCqK409R vectors generated using the ViraPower lentiviral expression system. This lentiviral system includes a substantial number of safety features designed to enhance its biosafety and minimize its relation to wild-type human retroviruses. It is clear from the safety features incorporated into this lentiviral system (see attached RA) that in this case the recipient microorganism (the lentivirus generated) is extremely unlikely to cause disease and could not spread to the human community or organisms in the environment. We are confident that there are no harmful properties associated with the (third generation) vectors to be utilised in this work, with the inserted material (the PKCq and the PKCqK409R genes) or with the resultant pLenti4/TO/V5-DEST-PKCq and pLenti4/TO/V5-DEST-PKCqK409R expression vectors. There is no evidence indicating that these sequences are inherently biohazardous i.e. there is no evidence that expression of PLCq or the disruption of PKCq signalling in a small subset of human cells is per se oncogenic. Toxic or in any way harmful, despite intensive worldwide research into PKCq signalling using these sequences. It is clear that none of the vectors to be used in this study encode potential pathogenicity determinants such as surface components nor harbour genes predicted to modulate the resistance of the lentivirus to disinfection procedures. Nor do any of these vectors encode animal toxins or any other products representing a specific risk to any organisms in the environment, and we are confident that there is negligible potential for the alteration of the existing traits or ViraPower lentiviruses by the packaging of any of the vectors used in this study. Similarly, the risks of sequences within the GMMs being transferred to related microorganisms are extremely low. The viruses produced in this work could not survive in the external environment, would be extremely unlikely to come into contact with related viruses and are engineered using vectors designed not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus. Nonetheless, the fact remains that replication-incompetent lentiviruses retain the ability to infect primary mammalian cells (by definition) and therefore pose some (albeit low) biohazardous risk. For this reason alone, we believe that the cautionary approach of the assignment of a provisional containment level of biosafety level 2 is preferable, and consistent with the supplier's recommendations.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This work will only involve the transient modification of cultured human cells; no other non-microorganisms will be modified. Purified primary human T cells will be incubated for 24 hours in an isolated 37°C incubator in sealed tissue culture flasks in the presence of lentiviral stocks titrated in tissue culture medium. Subsequently, the virus-containing supernatants are destroyed and the cells are incubated for a further 48 hours in fresh clean medium. Finally, the cells would be assayed for PKCq expression and the effects of transduction with the various constructs on T cell function. Primary T cells are unable to survive in the absence of specific well-defined growth media and cell culture conditions provided by the researcher. In accordance with the industry standard and the recommendations of the suppliers of the ViraPower Lentiviral Expression System (Invitrogen Life Technologies), all manipulations potentially involving infective (mature) lentivirus will be carried out in a certified class II safety cabinet and all medium and plasticware potentially coming into contact with lentivirus will be treated overnight in undiluted bleach (5.28% Sodium hypochlorite) in a certified class II safety cabinet, before disposal by off-site incineration. Upon completion of the experiment, mammalian cells incubated in medium potentially containing lentivirus will be inactivated and disposed of in an identical manner. Numerous studies, most notably by Robert Heimer, have shown that undiluted bleach is an effective and rapidly acting disinfection solution for retroviruses. For this reason alone, we believe that the cautionary approach of the assignment of a provisional containment level of biosafety level 2 is preferable, and consistent with the supplier's recommendations.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In accordance with the industry standard and the recommendations of the suppliers of the ViraPower Lentiviral Expression System (Invitrogen Life Technologies), all manipulations potentially involving infective (mature) lentivirus will be carried out in a certified class II safety cabinet and all medium and plasticware potentially coming into contact with lentivirus will be treated overnight in undiluted bleach in a certified class II cabinet, before disposal by off-site incineration. Upon completion of the experiment, mammalian cells incubated in medium potentially containing lentivirus will be inactivated and disposed of in an identical manner. Numerous studies, most notably by Robert Heimer, have shown that undiluted bleach (5.28% Sodium hypochlorite) is an effective and rapidly acting disinfection solution for retroviruses. However, upon production of the first lentivirus batch, we will confirm experimentally that this disinfection procedure is effective under the actual conditions of use.
A comprehensive risk assessment. The committee asked for further details to be included regarding the suitability of bleach for inactivation of waste. This has been included in the attached risk assessment. We will conduct in-house experiments to confirm this. The committee also discussed procedures to minimise the creation of aerosols, such as the use of sealed buckets during centrifugation. Again, this has been incorporated into the risk assessment.

**Project Containment**

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
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<td>L2</td>
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**Project Ref 713/12.1**

**CU2 Project Title**
Use of BV-2 cells as a model system to investigate neurodegenerative disorders

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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

To use the mouse micoglial BV-2 cell line as a model system to investigate the role of microglia in neurodegenerative disorders. Conventional laboratory techniques will be used to assess the production of cytokine / chemokines / ROS in response to various stimuli.

**Recipient or parental organism**

Mouse micoglial cells. Primary cells have previously been infected with the J2 virus to generate the BV-2 cell-line. (Blasi et al. J Neuroimmunol. 1990 27 (2-3) p229-37)

**Host/vector system**


**Origin & function**

MSV-3611 (closely related to the Mo-MLV) was originally isolated from chemically transformed mouse cells. MSV-3611 is replication defective due to deletion of the gag-pol replication genes and has acquired acute transforming properties due to incorporation of the oncogene v-raf into the proving genome (Rapp et al Proc Natl Acad Sci U S A. 1983 Jul;80(14):4218-22). The function of J2 was to transform primary cells to obtain established cell lines. The presence of v-myc in addition to v-raf removes the dependence on IL-3 for continued growth in culture (Blasi et al J Neuroimmunol. 1990 27 (2-3) p229-37).

The J2 virus was subsequently reported to be capable of replication and the BV-2 cell line was reclassified as Biosafety Level 2 by the ATCC (http://atcc.custhelp.com/app/answers/detail/a_id/550/~/biosafety-level-change-for-cells-carrying-mlv-or-j2).

**Evaluation of foreseeable effects**

The BV-2 cell line releases an ecotropic oncogenic retrovirus (J2) into the culture media that could potentially infect mice or other murine cell-lines if released into the environment.

The expressed gene products are the oncogenes v-raf and v-myc. v-myc is the viral homolog of c-myc. The v-myc oncogene can transform several lineages of cells either alone or in co-operation with other oncogenes. v-raf is a murine leukemia viral oncogene homolog 1, also capable of transforming cells.

There are no obvious hazards to human health. This cell line has been successfully cultured for over 20 years with no reported incidents of human infection. The use of the cell line for research purposes should not represent a risk to humans as the retrovirus from the BV-2 cells is ecotropic, that is only capable of infecting murine cells (http://bioinformatics.istge.it/cldb/cl7130.html).

If the J2 virus came into contact with other virally infected cells it may become amphotropic (able to infect non-mouse cells). The chance of this happening is negligible since none of the cell-lines currently handled in our laboratories are knowingly infected with viruses capable of infecting humans. However, work with this cell line will be done in a separate quarantined laboratory away from other activities.

In addition, retroviruses are highly susceptible to dehydration and are rapidly inactivated outside of the host, as illustrated by the fact that close contact, e.g. injection, ingestion, etc, is required for successful transmission.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not Applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste (culture media etc.) will be disinfected using >1% solution of Virkon for at least 30 minutes following manufacturers instructions. It will then be poured down the sink and flushed with copious amounts of water.

Virkon is specifically designed for inactivating virus (>99.99% reduction in virus titre) when used following the manufacturers instructions, i.e. soaking in a 1% solution for ≥ 30 minutes (DuPont Technical bulletin K09006-Disinfectant).

Solid waste including culture flasks, tubes, contaminated pipette tips and other lab consumables will be decontaminated by soaking in 1% Virkon for at least 30 minutes before being transferred to BioHazard bins. These bins will be sealed with a self-locking lid at the end of the working day. The bins are labeled with a biohazard sign that displays UN 3291. Bins are stored in a locked brick store with concrete floor until collection by our licensed waste contractor. They are certified to handle and dispose of Class 2 GMO waste, which once collected is incinerated ensuring 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee considered that the risk to HUMAN HEALTH was NEGLIGIBLE, as it is extremely unlikely that the virus could become amphoteric and thus capable of infecting human cells, but that risk to the ENVIRONMENT is LOW and so assignment to Risk Class 2 is appropriate.

Project Containment

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Project Ref 713/17.1
Investigations into the function of genes linked to polycystic kidney disease using lentiviral transfection of a human kidney in vitro model

Polycystic kidney diseases are the most prevalent inherited kidney diseases yet the molecular basis of the role of PKD-associated genes is poorly understood. The aim of this work is to determine how mutations in genes such as Polycystin-1 (PC1) and Polycystin-2 (PC2) contribute to cyst formation in kidney cells. Like most primary cells, primary kidney cells are difficult to transduce with exogenous DNA. To circumvent this problem, replication incompetent lentiviruses will be used to deliver exogenous DNA sequences to mammalian cells. Advantages over other transduction techniques include the ability to transduce a wide-range of different cells types that are both dividing and non-dividing. Primary kidney cells will be transduced with a DNA construct expressing either wild-type or mutant forms of PKD relevant genes e.g. PC1 and PC2 in order to determine their effects on in vitro cystogenesis. As controls, primary kidney cells will also be transduced with constructs encoding no protein product (vector only).

Recipient Organisms and evaluation of foreseeable effects
A. Cloning of DNA using Stbl3 chemically competent E. coli (Designated a Class 1 activity)
Stbl3 chemically competent E. coli with genotype: F−mcrB mrrhsdS20(rB−, mB−) recA13 supE44 ara-14 galK2 lacY1 proA2 rpsL20(StrR) xyl-5 λ−leumt1-1. These cells will be transfected with and used to propagate the pLenti6.3 vectors. The plasmids do not contain bacterial promoters so no expression of target protein will take place. Stbl3 is derived from HB101 which is a K-12 derivative of E. coli. This strain is non-colonizing, disabled and considered class 1, due to the lack of pathogenicity in humans or animals

B. Generation of Lentiviral expressing 293FT cells (Class 2 activity)
This is a clonal isolate derived from primary embryonal kidney cells transformed with sheared human adenovirus type 5 DNA and stably express SV40 large T antigen from pCMVSPORT6Tag.neo plasmid under the control of the human CMV promoter. 293T will be transfected with both the Virapower Packaging Mix (containing the pLP1, pLP2 and pLP/VSVG lentivial packaging vectors) and one of each of the pLenti6.3 plasmids described above. 293FT is a commonly-used cell line with a history of safe use and 293FT cells are unable to survive in the absence of specific well-defined growth media and cell culture conditions provided by the researcher. Consequently, these cells can be considered as especially disabled.

C. Transduction of primary human kidney cells and analysis of cyst formation (Class 2 activity)
The final recipient strains will be primary human kidney cells. These cells have been screened for the presence of HIV, Hep B and C infections. Cultures of these cells will be transduced with lentivirus carrying the pLenti6.3 vectors, which is predicted to induce constitutive expression of no protein (negative control), or wild-type PKD gene
products and mutants thereof. Primary kidney cells are unable to survive in the absence of specific well-defined growth media and cell culture conditions provided by the research. These cells can be viewed as being especially disabled.

Conclusion
We are confident that no hazards to the environment are associated with the recipient Stbl3 (bacterial) strain, 293FT (mammalian) cell line or primary human kidney cells when transformed/transfected/transduced with any of the vectors described below. There are no harmful properties associated with these recipient strains in the absence of the vector sequences. The vectors to be transformed/transfected/transduced are not predicted to give rise to a harmful phenotype either directly (genes themselves do not encode for harmful proteins products (oncogene/toxins)) or when acting in concert with existing recipient characteristics (genes do not encode products predicted to alter pathogenicity in normal cells). These GMMs would not be expected to cause any serious harm to humans and/or the environment in the event of a failure of containment and control measures (regulation 21).

The most hazardous GMMs resulting from this work are lentiviruses harboring the pLenti6.3 vectors generated using the ViraPower lentiviral expression system (Thermo Fisher). This lentiviral system, which is based on the HIV-1 virus, includes a substantial number of safety features designed to enhance its biosafety and minimize its relation to wild-type HIV1. Wild type HIV1 is listed as a Hazard Group 3 agent in the ACDP approved list. However, there are a number of safety features built into this third-generation viral system. These include:

- The pLenti6.3/TO/V5-DEST and pLenti6/TR vectors contain a deletion in the 3'LTR (ΔU3) that does not affect the generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a package-able viral genome.
- The number of HIV-1 genes that are used in the system has been reduced to three (gag, pol, and rev), thus reducing the genetic similarity to HIV-1.
- The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope, thus further reducing the relation of the viral particles to HIV-1.
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids (i.e., three packaging plasmids and pLenti6.3/TO/V5-DEST or pLenti6/TR). All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus.
- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication competent virus can be produced.
- The lentiviral particles produced in this system are replication incompetent and only carry the gene of interest. No other viral species are produced.
- Expression of the gag and pol genes from pLP1 has been rendered Rev dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev.
- A constitutive promoter (RSV promoter) has been placed upstream of the 5’LTR in the pLenti6.3/TO/V5-DEST and pLenti6/TR expression vectors to offset the requirement for Tat in the efficient production of viral RNA.

It is clear from the safety features incorporated into this lentiviral system that in this case the recipient microorganism is “unlikely to cause disease” and thus is best categorized as a Hazard Group 2 agent.

Conclusion
These viruses are “defective retrovirus produced from a packaging line in which the helper genes are located in three separate blocks of DNA (thus eliminating the possibility of a reversion to replication competence by a single recombinant event) and which self-inactivated to prevent ‘insertional activation’”. Thus, these viruses are an example of an “inherently safe recipient microorganisms which, depending on the nature of the insert, would in most cases are expected to form the basis of extremely safe GMMs”. In this way, these viral particles are extremely unlikely to cause disease and could not spread to the community.

Origin & function
The goal of this study is to understand the function of genes involved linked with the development of polycystic kidney disease (e.g. PKD1 and PKD2) as much of their function is unclear. Polycystin-1 (PC1), encoded by PKD1, is a large glycoprotein (4,303aa) with an approximately 3,000aa N terminal ectodomain, anchored to the membrane by 11 transmembrane helices with a short C terminal cytoplasmic tail. Polycystin-2 (PC2) encoded by PKD2 is believed to be a member of the transient receptor potential (TRP) ion channel family, a large group of six-trans membrane cation channels that play crucial roles in sensory physiology. PC1 and PC2 form a receptor/ion channel complex through association of their C-terminal coiled-coil domains. Certain mutations in PC1 and PC2 can cause autosomal dominant polycystic kidney disease
ADPKD. Polycystin proteins may participate in many different signaling pathways and regulatory processes, for instance PC1 shares similarities with both adhesion GPCRs and TRP family ion channels. We are confident that there are no harmful properties associated with the vectors to be utilized in this work. The DNA will be synthesized by Genewiz. The lentiviral and packaging vectors supplied with the ViraPower lentiviral system are third-generation vectors and include a substantial number of safety features designed to enhance their biosafety (see above). Regarding the polycystic kidney disease related DNA sequences e.g. (PC1 and PC2) to be inserted into the vectors, there is no evidence indicating that these sequences are inherently biohazardous. We acknowledge that overexpression of even a normal human gene may be harmful, especially if the overexpression is in tissues that do not normally express the protein. However, there is no evidence, to our knowledge, that over-expression of PC1 or PC2 in cell types other than cells of kidney origin is likely to have an effect on cell physiology. The cellular functions of PC1 and PC2 are detailed above. It is not apparent from these functions how overexpression of PC1 or PC2 or the disruption of PC1/PC2 signaling in this setting could be inherently harmful to human cells if the lentiviruses carrying the pLenti6.3 vectors were to accidentally infect living cells from a human worker.

Evaluation of foreseeable effects

From the list of functions of PC1 and PC2 (listed above), it is not clear that the widespread infection of human beings with the pLenti6.3 vectors (details outlined below) or sequences derived therefrom would present a matter of significant concern. Although loss of PKD1/PKD2 may result in ADPKD, there is no evidence to our knowledge that PKD1/PKD2 or the disruption of PC1/PC2-dependent mechanisms in a subset of human cells is per se oncogenic, toxic, or in any way harmful outside the context of the kidney. Regarding the roles of PC1 or PC2 in normal primary human kidney cells, it is almost inconceivable that PC1/PC2 signaling could be disrupted using lentivirus in a sufficiently large number of human kidney cells to affect human health. There is little evidence of potential harmful effects realized as consequence of exposure to lentivirus from the ViraPower system. However, despite enhanced biosafety using replication incompetent viruses and separating viral proteins across numerous packaging constructs, the lentiviruses still harbor the potential to infect mammalian cells. We acknowledge that overexpression of even a normal human gene may be harmful, especially if the overexpression is in tissues that do not normally express the protein. This risk is associated with lentiviruses in general: there is no evidence to our knowledge that PKD1/PKD2 or the disruption of PC1/PC2-dependent mechanisms in a subset of human cells is per se oncogenic, toxic, or in any way harmful outside the context of the kidney. It is not clear that any one of the specific lentiviruses to be generated in this study will be significantly more hazardous than the others. Although it is presumed that lentiviruses harbouring the pLenti6.3 vectors can be slightly more hazardous since they can induce the ectopic expression of a wild-type, gain-of-function, and loss-of-function protein in human cells. It is for this reason that we have considered the lentiviruses harbouring pLenti6.3 vectors to be slightly more biohazardous than the other control lentiviruses to be generated in this work.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment will be used. No derogation required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In accordance with the industry standard and the recommendations of the suppliers of the ViraPower Lentiviral Expression System (Invitrogen Life Technologies, UK), all contaminated solid waste (e.g. plasticware including pipettes and flasks) and liquid waste that has potentially come into contact with lentivirus will be treated overnight in 2% Virkon (Fisher Scientific, UK) in a certified class II safety cabinet prior to disposal. Solid waste is disposed by off-site incineration and inactivated liquid waste will be disposed via sewerage. Virkon has the widest proven spectrum of any disinfectant - proven effective in independent tests against all 18 virus families affecting man and animals, including AIDS (HIV) and Hepatitis B. Upon completion of the experiment, mammalian cells incubated in medium potentially containing lentivirus will be inactivated, and disposed of, in an identical manner.

None of the work proposed in this study requires the use of sharp materials (metal, glass or otherwise). Only standard blunt plastic ware will be necessary.
There was some discussion over whether this work should be categorized as low risk (requiring level 2 containment); other sites have assessed the use of kit-based lentiviral transduction to be effectively controlled through the use of Level-1 containment (negligible risk). However, the committee was satisfied with the assessment of low risk (class 2) containment considering both the use of lentiviral transduction capable of transducing human cells and the expression of novel proteins (including PC1 and PC2).

The committee also recommended using Virkon instead of 5% bleach in order to decontaminate both plasticware and liquid waste. The assessment has been amended to reflect this.

It was unanimously agreed that the work should be carried out under the Level 2 containment procedures outlined in the risk assessment. The HSE should be notified of our intentions.

Action: CU2 form to be completed and the HSE informed of our intended usage with £981 notification fee (MG).

### Project Containment

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- Animal Units
- Large Scale Activities
- Human Clinical Applications
**GM Centre Number: 714**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Y**

Give brief details of the genetic modification safety committee

We do have an established GM Safety Committee that meets at least once every business quarter and is a sub-group of our H&S Committee

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum culture volume = 8000 litres.
Deactivation methods: Bacteria are retained for further processing which involves breakage at high pressures. Culture broth is filtered away from the bacterial cells through a 0.2 um membrane. Fermentation vessels are sterilised by steam at 121 degrees C. Auxiliary equipment which may have been in contact with bacterial cells are disinfected with hypochlorite. Smaller cultures are autoclaved. Solid waste also autoclaved.
All deactivation methods are standard microbiological methods, no culture is discarded directly to drain.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
| Data Premises Notified (Originally) | 11/08/1999 | Transferred from 1992 Regs? | Y | Transitional Premises Class | 1 |
| Data Premises Closed | 01/08/2004 | Transitional Premises | N | Non-GMMs | N |
| Emergency Plan Required? | | | | Withdrawn | N |

**Name**

CUBIST PHARMACEUTICALS (UK) LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

545 IPSWICH ROAD

**Town**

SLOUGH

**District**

**County**

BERKSHIRE

**Postcode**

SL1 4EQ

**Country**

ENGLAND

**Tel Number**

01753 706800

**Fax Number**

01753 706808

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Centre closed down on 1/8/2004 - all work ceased and company moved to Boston.

**Date at Which Additional Info Submitted**

23/02/2001
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

**Background** - Work involving Class 1 genetically modified bacteria and fungi is conducted at the premises from which waste materials are generated. These materials are decontaminated by autoclaving or by chemical deactivation prior to discard.

**Decontamination procedures - Autoclaving** - Waste fluids and materials are autoclaved inside perforated metal bins at 134°C for 20 minutes, well in excess of the minimum sterilisation conditions. Fermenters and supporting peripherals are autoclaved in situ or in a separate autoclave at 121°C for 20 minutes. The building possesses 2 autoclaves, both of which are equipped with alarms and data log print-out facilities which give details of each autoclave cycle. An audible alarm is activated should an autoclave run fail. Autoclaves are serviced every 3 months and probes calibrated every six months by a competent contractor. 100% Kill

**Chemical Deactivation** - Some waste material may be chemically deactivated prior to discharge if autoclaving is not a practical alternative. This is accomplished using total immersion in Virkon disinfectant at concentrations between 0.4% and 1% for 60 minutes, as per the manufacturers recommendations. An evaluation testing the effectiveness of Virkon for chemical deactivation of microorganisms has been performed and is recorded in lab book KC46, experiment number KC010/29/09/95, pages 168-172. 100% Kill.

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment
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**Comments**

Change of ownership and company name from Quintiles Ltd to Aptuit (Edinburgh) Ltd as of 1/10/2005

**Date at Which Additional Info Submitted**

02/03/2022
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For activities involving GMMs, describe the waste management measures which will apply to the activity

As the activity to be undertaken comprises receipt, storage and distribution of material in pre-packaged vials, the only waste that should be produced will be that arising from any breakages, spillages or reject stock. Since the material is supplied in liquid form contained in unbreakable vials, and will be maintained frozen at all times while on site, it is anticipated that little (if any) waste will be produced.

Any such waste arising from the work will be inactivated by off-site incineration. This will provide 100% inactivation of the GMMs. The waste will be consigned as genetically modified clinical waste for incineration at a site notified to the HSE under the Contained Use Regulations. Such waste will be transported in single-use "one way" UN approved sealed waste units. A copy of the incinerator's HSE authorisation will be held by Quintiles. All clinical wastes will be handled in accordance with the "safe Disposal of Clinical Waste" (HSAC 1999).

Spillage

Any spillage of Ad5/FGF-4 will be inactivated by use of Virkon, used in accordance with the manufacturer's instructions. These will specify the concentration to be used and the acting time. Virkon is a broad-spectrum disinfectant with proven efficacy against adenoviruses, and manufacturer's data on the disinfectant and its method of use will be kept in the area spillage kits.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 718**

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### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify)  
Tick if confidential

- Bacteriology  
- Parasitology  
- Transgenic Birds  
- Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum culture volume that is released at any one time is 10 litres. The waste is deactivated by a Virkon soak and autoclaving. The deactivation method is validated by colour coded tape, i.e. tape changes black when reaches required temperature. The deactivation method is monitored by a computer in the autoclave which warns of a cycle fail (e.g. if the autoclave hasn't reached the required temperature or length of time for deactivation of waste).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
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<th><strong>GM Centre Number:</strong> 719</th>
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**Name**

**GENOMIC SOLUTIONS LTD**

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

8 BLACKSTONE ROAD

**District**

**Town**

HUNTINGDON

**County**

CAMBRIDGESHIRE

**Postcode**

PE18 6EF

**Country**

ENGLAND

**Tel Number**

01480 426 700

**Fax Number**

01480 426 767

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
<th>Tick if confidential</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Tick if confidential</td>
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</table>

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research | Gene Therapy

Virology | Transgenic Animals | Transgenic Fish | | | |
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste generated is deactivated in an autoclave (Astell ASB230 purchased July 1999), using the waste programme 134 degrees C for 20 minutes, 8 minutes free steaming, assisted cooling, waste labelled with autoclave tape. Samples of the deactivated waste are taken for growth testing.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 720

Data Premises Notified: 27/08/1999
(Originally)

Transferred from
1992 Regs?: Y

Transitional Premises
Class: 1

Data Premises Closed: N

Transitional Premises
Emergency Plan Required?: N

Non-GMMs: N

Withdrawn: N

Name
BIOTICA TECHNOLOGY LTD

Name 2

Department

Campus Estate or Research Centre

Road Name: 19 GRANTA PARK

District: GREAT ABINGDON

Town: CAMBRIDGE

County: CAMBRIDGESHIRE

Postcode: CB21 6DF

Country: ENGLAND

Tel Number: 01223 276904

Fax Number: 01223 766091

E-mail

HSE Division: EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted
03/10/2003
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Level 2 (GMMs)</td>
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<tr>
<td>Other (please specify)</td>
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</table>
All liquid and solid waste (maximum total volume 5 litres) will be deactivated by autoclaving at 125 degrees C for 20 minutes. Deactivation will be monitored by using 3M comply indicator tape for steam. In addition, small amounts of liquid waste may be deactivated using Chloros solution (concentrated sodium hypochlorite solution, equal or greater than 10% w/w active chlorine).

### Project Ref 720/01.1

**CU2 Project Title**

CLONING OF GENES INVOLVED IN BIOSYNTHESIS OF POLYKETIDES FROM ACTINOMYCETES INTO E. COLI AND OTHER ACTINOMYCETES TO PRODUCE NOVEL POLYKETIDE DERIVATIVES

**Date Ackn’d**

22/06/2001

**Date Project Ceased**

**Class**

Class 2

**CultureVolClass2 CultureVolumeClass3-4**

1-50 litres

**Non-GMM Consent Granted**

not applicable

**Project notified under transitional arrangements**

N

**Withdrawn**

N

Tick if notifying a connected programme of work

N
Project Additional Information

Purposes of the contained use
biosynthesis of novel polyketides of industrial interest.

Recipient or parental organism
Actinomycetes:-
Streptomyces lividans, albus, cinnamomensis, coelicolor, fradiae, avermitilis, rimosus, rochei Saccharopolyspora erythraea, spinosa.
E. coli:-
K-12 strains TG1 recO, DH1OB, XL1-Blue, S17-1, TB1. ET12567, TOP10.

Host/vector system
E.coli vectors will be derivatives of the following plasmids:
Non-mobilisable bacterial plasmids:-
Mobilisation deficient and integrative bacterial plasmids:-
Actinomycete vectors will be derivatives of the following plasmids:-
Non-mobilisable and self-replicating plasmids:-
pCJR29 (pUC19 based) & pWHM3.
Mobilisation deficient and integrative (all pUC19 based) plasmids:-

Origin & function
The genes that will be expressed encode enzymes that cannot conceivably cause harm or disease or affect the environment adversely.
DNA from various Actinomycetes including Nocardia sp. will be cloned into K-12 strains of E. coli and other Actinomycetes. The DNA will contain polyketide synthase genes or portions thereof, as well as auxiliary genes involved in polyketide synthesis (activator genes, methylases, oxidases, hydroxylases, glycosyltransferases, holosynthases, genes for the production of co-substrates for polyketides synthases, genes for the production of sugars).
Those expressed in E. coli under the Tac, Trc and T7 promoters will be expressed at levels between 0 - 50% of total cell protein.
Those expressed in Streptomycyces or Saccharopolyspora under the ActI, ActIII, ErmE, PTR, Spn and TipA promoters will be expressed at levels between 0 - 2% of total
cell protein.

**Evaluation of foreseeable effects**

New GMMs will be created which will produce novel polyketides. These Actinomycete GMM strains should have the same characteristics as wild-type strains being naturally occurring soil bacteria which are not aggressive colonisers, and are generally found in small patches which do not proliferate. They are not human or animal commensals. They are therefore completely safe for humans, animals or plants. E. coli strains created all contain non-reversible disabling mutations and could not infect animals or humans.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid and solid waste (maximum total volume 20 litres) will be deactivated by autoclaving at 125°C for 20 minutes. Deactivation will be monitored by using 3M comply indicator tape for steam. In addition, small amounts of liquid waste may be deactivated using RBS (final concentration of 5% v/v). There is therefore no possibility that bacteria will escape from the laboratory. Where possible the use of sharps is avoided and plastic pipettes are used. However, any sharps used are always placed in a Cin-Bin and disposed of through licenced contractors according to the Company’s Waste Disposal Procedure. A kill curve disinfection test versus time is performed on all organisms.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

**Project Containment**

<table>
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<tr>
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<th>Glass Houses</th>
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Animal Units  
Large Scale Activities  
Human Clinical Applications
### GM Centre Number: 721

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#### Name

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#### HSE Division

| EAST AND SOUTH EAST |

#### Comments

Company name change notified 13/11/2009 previously Prolysis Ltd

#### Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

Other (please specify) Tick if confidential

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tr>
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<td>Gene Therapy</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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</thead>
<tbody>
<tr>
<td>Other(s)</td>
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</tr>
</tbody>
</table>

Maximum culture volume that could be released at any one time: 100 ml.

All solid waste will be autoclaved, before disposal. All liquid waste will be autoclaved, or if appropriate disinfected with Virkon (peroxyxgen compound) before disposal.

All non disposable items (glassware, non disposable plastic containers and contaminated equipment) used with the GMOs will be autoclaved or disinfected with Virkon as appropriate.

The autoclave will be serviced and the in load probe calibrated regularly (using a representative load). The in load probe temperature will be recorded for each batch of waste autoclaved.

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 722

<table>
<thead>
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<th>Data Premises Notified (Originally)</th>
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**Name**

BRITISH ANTARCTIC SURVEY

**Name 2**

BIODLOGICAL SCIENCES

**Campus Estate or Research Centre**

**Road Name**

MADINGLEY ROAD

**District**

HIGH CROSS

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB3 0ET

**Country**

ENGLAND

**Tel Number**

01223 221400

**Fax Number**

01223 362616

**E-mail**


**HSE Division**

EAST AND SOUTH EAST

**Comments**


**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

**Level 1 (GMMs)**

**Level 2 (GMMs)**

**Level 3 (GMMs)**

**Level 4 (GMMs)**

**Non-microbial**

**Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Transgenic Animals**
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- **Transgenic Gene Therapy**
<table>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

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Tick if you are claiming exemption from disclosure for sections of the risk assessment

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<table>
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<th><strong>Name</strong></th>
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<tr>
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**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
<th>Level 1 (GMMs)</th>
<th>Laboratory</th>
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</table>
The maximum culture volume which could be released at one time is less than 20L. The waste is deactivated by autoclaving for 40 minutes at 121 degrees C or, treated with presept (Dichloroisocyanurate, Sodium Salt 50%), the minimum contact time being 1 hour.

Validation of the deactivation processes are supplied by the manufacturers and the recommendations are adhered to. Monitoring the deactivation by presept is currently being undertaken in-house.

Autoclaving is monitored by timer and pressure regulation. The autoclave is regularly checked under a service contract.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 725

Data Premises Notified (Originally) 05/01/2000  
Transferred from 1992 Regs? Y  
Transitional Premises Class 1  
Data Premises Closed 19/10/2018  
Transitional Premises Emergency Plan Required? N  
Non-GMMs N  
Withdrawn N

Name  
MERCK CHEMICALS LTD

Name 2

Department  
ENVIRONMENTAL & HYGIENE ANALYTICS

Campus Estate or Research Centre

IMPERIAL PARK

Road Name

5 THE COURTYARD

Town

NEWPORT

County

NEWPORT

Postcode

NP10 8UL

Country

WALES

Tel Number 01633 776600  
Fax Number 01633 776601

E-mail

HSE Division

WALES AND SOUTH WEST

Comments  
NAME CHANGE FROM MERCK LTD TO MERCK CHEMICALS LIMITED AS OF 19/12/2003

Date at Which Additional Info Submitted

19/12/2003
## Premises Addresses

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## Premises Conditions

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Give brief details of the genetic modification safety committee

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</table>
Maximum waste volume is 1 litre. In practice waste volumes, at a given time, are likely to be less than this.

* Waste will be deactivated by autoclave processing.
* A dedicated autoclave is reserved for inactivation of microbiological wastes.
* GMMs will be deactivated separately from other micro-organisms.
* A high temperature regime [124 degrees C for at least 30 minutes] is used.
* The deactivation regime is monitored by chart recorder [NAMAS accredited] and also by steam sterilisation integrator indicator [Thermalog S] incorporated into the autoclave load itself.
* The above procedures have been previously shown to achieve deactivation of the GMMs.
* Standard microbiological procedures will also be used to confirm sterilisation.

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<tr>
<th>Mycology</th>
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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Maximum waste volume is 1 litre. In practice waste volumes, at a given time, are likely to be less than this.

* Waste will be deactivated by autoclave processing.
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* The above procedures have been previously shown to achieve deactivation of the GMMs.
* Standard microbiological procedures will also be used to confirm sterilisation.

**Tick to confirm that you are attaching a summary of the risk assessment** ☐

**Tick if you are claiming exemption from disclosure for sections of the risk assessment** ☐

**Please enter comments of the GM safety committee on the risk assessment**
GM Centre Number: 726

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Name

HEALTH PROTECTION AGENCY

Name 2

FOOD MICROBIOLOGY RESEARCH UNIT

Campus Estate or Research Centre

HEAVITREE

Road Name

CHURCH LANE

Town

EXETER

County

DEVON

Postcode

EX2 5AD

Country

ENGLAND

Tel Number

01392 402 953

Fax Number

01392 412 835

E-mail

HSE Division

WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: Y

- **Give brief details of the genetic modification safety committee**

  Chairperson, BSO, Management Representative and 2 Employee Representatives.

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

  - Level 1 (GMMs)
  - Level 2 (GMMs)
  - Level 3 (GMMs)
  - Level 4 (GMMs)
  - Non-microbial

  **Other (please specify)**

  Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

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Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  

Tick if confidential

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<tr>
<th>Bacteriology</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum culture volume that could be released at any one time is 20 cm cu.
Deactivation: All waste cultures and contaminated materials to be deactivated by autoclaving at 121 degrees C for 15 minutes. Benches to be wiped down with 60% propan-2-ol after use.
Validation: Indicator strips to be included in the centre of each load to ensure temperature of 121 degrees C for 15 minutes is achieved.
Monitoring: Disposal record of all cultures kept in log book maintained in the Biotechnology Prep room.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
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| **Tel Number** | 0118 944 8000 |
| **Fax Number** | 0118 944 8001 |

| **E-mail** | |
| **HSE Division** | EAST AND SOUTH EAST |

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### Premises Conditions

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Tick if confidential

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02/03/2022
Waste Disposal:
* The anticipated maximum culture volume is 2 - 3 litres per week.
* Cultures will be inactivated with a concentrated solution of Hibicet or Virkon.
* Liquid will be absorbed onto tissue paper.
* Tissue paper, flasks, stripettes etc. will be double-bagged for collection and incineration.

Validation:
* Because all waste will be incinerated, there is no anticipated need for validation of the deactivation.
* If required, random samples of deactivated cells could be cultured to ensure there are no viable cells present.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### GM Centre Number: 730

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### Comments

Company merged with GM659 Vernalis wef 1/9/2003

### Date at Which Additional Info Submitted

23/01/2002
### Premises Addresses

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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

#### Level 1 (GMMs)

#### Level 2 (GMMs)

#### Level 3 (GMMs)

#### Level 4 (GMMs)

#### Non-microbial

Other (please specify) Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
We grow HeLa cells for our research work; approximately half to one litre per week. These cells are killed by using "Promega lysis buffer" which we understand is a proprietary buffer which will disrupt and render harmless enveloped cells and viruses. Secondly, we either (i) autoclave such material (after treatment with said lysis buffer), or (ii) send this in a sealed container to a specialist contractor for incineration.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 730/02.1**

<table>
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<td>08/05/2002</td>
<td>CELLULAR EXPRESSION OF ONCOGENIC PROTEINS FOR LIGAND DESIGN APPLICATIONS</td>
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<td>&lt; 1 litre</td>
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Non-GMM

Consent Granted: not applicable

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes: Project reclassified to Class 1 - company merged with GM659 Vernalis wef 1/12/2003

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

---
**Project Additional Information**

**Purposes of the contained use**
expression of proteins which may have oncogenic potential. Contained use is therefore required.

**Recipient or parental organism**
Human immortalised cell lines HeLa, HT29, HCT116

**Host/vector system**
Mammalian expression vectors pcDNA3 and pcDNA4

**Origin & function**
Human gene sequences which code for potentially oncogenic proteins have been obtained from various institutional sources and cloned into mammalian expression vectors. The GMM (human immortalised cell lines expressing these gene sequences) will be used in cell-based assays of protein function.

**Evaluation of foreseeable effects**
The proposed modification to the recipient organism is not anticipated to alter infectiousness, allergenicity or host range. The recipient organisms will carry heterologous gene sequences which may confer a growth advantage to that organism when compared to the parental cell line in a laboratory setting. None of the expressed proteins are toxic or pathogenic in the environment. The recipient organism has strict media requirements that cannot be met outside of the laboratory. Survivability is effectively zero.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
The general principles of Good Laboratory Practice and Good Occupational Health and Safety will be applied to this activity. All manipulations involving the modified cell line will take place in a Class II safety cabinet. All GMM material will be autoclaved for 20 minutes at 121°C in a regularly maintained autoclave or will be disinfected using Virkon S (shown to achieve 100% killing of HeLa cells). Disinfected waste will then be disposed of by WasteCare clinical waste management contractors.

**Is an emergency plan required according to regulation 20?**
N

**If yes, tick to confirm that it is attached to this form**
N

**Tick to confirm that you have attached a risk assessment to this form**
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**
Y

**Please enter comments on the GM safety committee on the risk assessment**
Passed by GMSC
## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2</td>
<td>Yes</td>
<td>L3</td>
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**Animal Units**
| L2 | L3 | L4 | L2 |

**Large Scale Activities**
| L2 | L3 | L4 | L2 |

**Human Clinical Applications**
<p>| L2 | L3 | L4 | L2 |</p>
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Data Premises Notified (Originally): 01/12/1999

Transferred from 1992 Regs?: Y

Transitional Premises

Class: 2

Non-GMMs: N

Withdrawn: N

Emergency Plan Required?: N

Transitional Premises Closed: N

Transitional Premises

Emergency Plan Required?:
### Premises Addresses

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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**
  - [ ]

- **Give brief details of the genetic modification safety committee**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

**Project Ref** 731/00.1

<table>
<thead>
<tr>
<th>Date Ackn’d</th>
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<th>CultureVolClass2</th>
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<td>23/08/2000</td>
<td>TRANSFORMATION OF TOMATO FOR THE STUDY OF THE PLANT SIGNALLING EVENTS INDUCED BY THE SYMBIOTIC ARBUSCULAR MYCORRHIZAL (AM) FUNGI</td>
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Consent Granted

Project notified under transitional arrangements
<table>
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<tr>
<th>Project Additional Information</th>
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<tr>
<td>Purposes of the contained use</td>
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<tr>
<td>Recipient or parental organism</td>
</tr>
<tr>
<td>Host/vector system</td>
</tr>
<tr>
<td>Origin &amp; function</td>
</tr>
<tr>
<td>Evaluation of foreseeable effects</td>
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| Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants) |

| For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification) |

| Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate) |

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form |   |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |
**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
</tr>
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<tr>
<td>L2</td>
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<tr>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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**Project Ref**  731/12.1

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<td>30/03/2012</td>
<td>Fluorescent tagging of the nucleus in Ramularia collo-cygni, a fungal pathogen of barley</td>
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<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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- Project notified under transitional arrangements: N

**Project Additional Information**

- **Purposes of the contained use**
  - To visualise nuclei of Ramularia collo-cygni in the investigation of possible sexual reproduction in this fungus

- **Recipient or parental organism**
  - Isolates of Ramularia collo-cygni endemic to Scotland
**Host/vector system**

Bacterial Plasmid pMF357 (Ishikawa et al. 2010), in which the green fluorescent protein gene is expressed from the N. crassa ccg-1 promoter and which contains the hygromycin resistance gene.  

Bacterial plasmid pGR02 (Freitag and Selker, 2005), in which the red fluorescent protein gene is expressed under control of the N. crassa ccg-1 promoter and which contains the phleomycin resistance gene.  

Both plasmids also contain the ampicillin resistance gene for selection and propagation in E. coli.

**Origin & function**

Green fluorescent protein (GFP) gene from jellyfish Aequorea victoria. Function: to confer green fluorescence on transformed nuclei.  

Red fluorescent protein (RFP) gene from the coral Discosoma. Function: to confer red fluorescence on transformed nuclei.

**Evaluation of foreseeable effects**

It is not anticipated that the expression of fluorescent protein genes in Tamularia collo-cygni would result in any change in the host range or aggressiveness of this plant pathogen. However, as barley, the natural host for Ramularia, may be cultivated in the vicinity of the laboratory, it is considered that the activity is Class 2. It is not anticipated that E. coli strains used to propagate the plasmids would present a risk to the environment or to human health, primarily as the fluorescent protein genes will not be expressed in the bacterium.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The waste generated will compromise aqueous fungal spore suspensions and fungal mycelium growing on agar plates. Waste E.coli, both intact and fragmented cells, will be generated during propagation and isolation of the plasmids. All waste will be inactivated by heat treatment at 121 degrees Celsius for 15 minutes in an autoclave to give 100% kill. The autoclave is fitted with a data monitor to verify correct temperature and duration. Any spillage of liquid containing GMMs will be treated immediately with Trigene disinfectant.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

**Project Containment**

02/03/2022
Project Ref 731/99.1

Date Ackn'd 01/12/1999

CU2 Project Title
IDENTIFICATION AND STORAGE OF GENES FOR BIOSURFACTANT PRODUCTION IN THE PLANT-BOURNE BACTERIUM, PSEUDOMONAS FLUORESCENS

Class 2

Consent Granted
not applicable

Project notified under transitional arrangements Y

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Animal Units

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Project Ref 731/99.2

Date Ackn'd                CU2 Project Title
01/12/1999                STORAGE OF GENETICALLY MODIFIED MICRO-ORGANISMS
Class                      CultureVolClass2 CultureVolumeClass3-4
Class 2
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### Historical Significant Changes

### Historical Date of Additional Info

### Significant Change ID

### Date of Significant Change

## Project Additional Information

### Purposes of the contained use

### Recipient or parental organism

### Host/vector system

### Origin & function

### Evaluation of foreseeable effects

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
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**Name**

HORTICULTURE RESEARCH INTERNATIONAL

**Name 2**

HRI EFFORD

**Campus Estate or Research Centre**

SOUTH EFFORD HOUSE

**Road Name**

SOUTH EFFORD HOUSE

**Town**

LYMINGTON

**County**

HAMPshire

**Postcode**

SO41 0LZ

**Country**

ENGLAND

**Tel Number**

01590 673341

**Fax Number**

01590671553

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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<th>Date</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Y

Give brief details of the genetic modification safety committee

Chairman, BSO, Deputy BSO, Secretary & Efford Representative.

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

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Mycology
Transgenic Invertebrates
Transgenic Plants
Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### UNIVERSITY HOSPITAL BIRMINGHAM NHS FINANCIAL TRUST

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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02/03/2022
The maximum liquid volume containing the GMOs to be handled at any one time is 1 litre. Contaminated waste to be decontaminated by autoclaving at 134 degrees C for a minimum of 12 minutes, or by neutralisation with appropriate sterilising agents (e.g., sodium hypochlorite). The autoclave validation is by thermocouples embedded in typical loads; monitoring via integral time/temperature printout, which is checked by laboratory management. Sterilising agents validated by compliance with national NHS standards, and/or in-house testing for neutralisation of the vector. Monitoring via records kept in accordance with procedural documentation.

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick if confidential

Bacteriology
Parasitology
Transgenic
Birds
Microbiology
Research

Virology
Transgenic
Animals
Transgenic
Fish
Gene Therapy

Mycology
Transgenic
Invertebrates
Transgenic
Plants
Other (please specify below)

Other(s)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**Project Additional Information**

**Purposes of the contained use**
Clinical testing of gene therapy agents based on herpes simplex virus, with the aim to develop and validate improved treatments for cancer.

**Recipient or parental organism**
The gene therapy agents are modified versions of herpes simplex virus, which are modified to be non-pathogenic in normal tissues, but retain the ability to undergo lytic replication in cancer cells. Similar viruses have already been tested in human clinical trials and found to be safe, even when administered intracranially.

**Host/vector system**
The ultimate hosts in these clinical trials are human cancer patients. The modified herpes virus vectors used are able to replicate and spread within cancerous tissue, but are unable to replicate in normal tissues.

**Origin & function**
The vectors are intended to replicate in cancer tissue, spreading through the cancer and killing the cancer cells. Because the vectors are unable to replicate in normal tissues, they are non-pathogenic and should not harm normal tissues, and would be unable to spread in the population. In order to increase their anticancer activity, the vectors may contain additional therapeutic genes, such as genes encoding human cytokines. These may enhance the generation of immune responses against the cancer cells, which would help to eliminate cancer cells from the patient.

**Evaluation of foreseeable effects**
The virus vectors are expected to replicate in cancer cells, killing them by lysis and releasing identical progeny virus, which can infect surrounding cells. Thus the vectors should spread progressively through a tumour, killing the cancer cells. Although some normal cells, e.g., at the tumour margins, may become infected, the vectors should be...
unable to replicate in these, and spread of the virus should cease. Transmission from the cancer patient to other humans will be prevented, by covering the injection site with occlusive dressings, and retaining the patient in a containment suite until virus shedding has ceased.

In the unlikely event that persons other than the cancer patient become contaminated with the vector, it will be unable to replicate and so it is most likely that no significant effects would be observed. The vectors are unable to generate the "cold sore" lesions associated with the natural herpes simplex virus, because these result from virus replication in the normal skin, whereas the vectors are unable to replicate in normal tissue. A high proportion of the population has been naturally exposed to herpes simplex virus, and has a pre-existing immune response to the virus. If seronegative individuals are contaminated with high doses of the vector (e.g. through inadvertent self-inoculation at the time of administration), then seroconversion may occur. The production of cytokines by the GM vectors may be expected to cause an increased inflammatory response and enhance the generation of immune responses.

Thus the effects of the vector in normal individuals are expected to be minimal. As an additional safety feature, the vectors retain the viral thymidine kinase gene in the virus, and thus infection could be controlled with acyclovir.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste materials contaminated with the GMM will include the vials in which it is supplied, the syringe and needles used for injection, contaminated dressings, latex/vinyl gloves with a risk of contamination. These will be placed into a sharps bin within the containment facility. These are decontaminated by autoclaving within the Microbiology Department, using fully validated and documented procedures, providing 100% kill. The waste is transported to the Microbiology autoclave by knowledgeable, responsible individuals associated with the trial, e.g. research nurses, using specified, appropriate secondary containers showing specific biological hazard labels. These arrangements have been discussed with the HSE, who advised that this complies with the standard level 2 provisions.

We have also validated disinfection with 1% Virkon, 0.5% Chloroclean, and Klercide B to provide complete inactivation of the vector.

Following autoclaving, the decontaminated waste is disposed as clinical waste, being removed by contractors for high temperature incineration off-site.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
The GMSC agreed that Oncovex-GM-CSF is appropriately classified as Class 2; noted that this is the first class 2 GMM to be used within the Trust, and agreed that the containment facilities and procedures are suitable.

The GMSC recommended that in view of the increased hazard of WT HSV in persons who are immunosuppressed, pregnant, or have active eczema, as a precaution healthcare workers who fall into any of these categories should be excluded from possible contact with Oncovex-GM-CSF, by exclusion from the area when the virus is dispensed or administered, and while the patient is shedding the virus.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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#### Laboratory Activities
- Glass Houses
- Growth Rooms

#### Animal Units
- Large Scale Activities
- Human Clinical Applications

### Project Ref: 734/17.1

- Date Ackn'd: 06/01/2017
- Date Ackn'd: 06/01/2017
- Date Project Ceased: 
- Clinical trials involving Pexa-Vec (Vaccinia GM-CSF/Thymidine kinase-Deactivated Virus)
- Class: Class 2
- Volume: < 1 Litre
- Consent Granted: 
- Project notified under transitional arrangements: N

#### Project Additional Information

- Tick if notifying a connected programme of work: Y

#### Historical Significant Changes

- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change
Purposes of the contained use

In the first instance, Pexa-Vec will be used in the clinical trial, "A Phase 3 Randomized, Open-Label Study Comparing Pexa-Vec (Vaccinia GM CSF I Thymidine Kinase-Deactivated Virus) Followed by Sorafenib Versus Sorafenib in Patients with Advanced Hepatocellular Carcinoma (HCC) Without Prior Systemic Therapy". Pexa-Vec is currently in clinical development for the treatment of Hepatocellular Carcinoma. The proposed contained use will be the administration of the investigational product, in a hospital or clinic setting, by intratumoral (IT) injections to patients as part of an international, multicenter clinical trial. This clinical trial is a Phase III trial in patients with Advanced Hepatocellular Carcinoma (HCC) without prior systemic therapy. Results from this pivotal trial will determine whether Pexa-Vec followed by sorafenib increases survival duration in advanced HCC patients compared to treatment with sorafenib alone, and whether sequential dosing with Pexa-Vec followed by sorafenib has a favourable safety profile.

Approximately 40 clinical sites in the EU will enroll patients in the JX594-HEP024 (PHOCUS) study. Additional clinical sites in Australia, Canada, China, Israel, Korea (Republic of), New Zealand, Singapore, Taiwan, Thailand and the USA will also participate in the study. A total of 600 patients will be recruited in this clinical trial with an expectation to enroll 200 patients in EU countries. In the control arm, the 300 patients will not receive Pexa-Vec. After study completion, all patients will be followed up for survival. Among them, 300 patients (i.e. approximately 100 patients in EU) will receive Pexa-Vec by IT injections.

This is being submitted as a Connected Programme Notification, to facilitate the possibility of conducting other, future clinical trials of the same product, e.g. in patients with other cancers or in combination with other treatments.

Recipient or parental organism

Pexa-Vec is a replicative oncolytic recombinant vaccinia virus (W) derived from the commonly used vaccine Wyeth strain, DryvaxTM. W is a member of the Poxviridae family (genus Orthopoxvirus). Multiple strains of W exist that have different levels of virulence for humans and animals. The New York City Board of Health (NYCBOH) strain, from which the Wyeth strain of the Dryvax® vaccine was derived, has low pathogenicity in humans (Fenner F. et al., 1988). W has a long and extensive history of use in humans. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous (SC) infection. As W contains antigens that stimulate an immune response that are cross-reactive with smallpox antigens, the vaccine thereby confers protection from the human smallpox disease. W may cause local reactions including erythema, edema and systemic reactions such as fever and malaise, as has been observed with conventional vaccination to smallpox. During the smallpox vaccination campaign, serious complications had occurred in less than 1 in 4,000 individuals, mainly in immunosuppressed and extremely young individuals. Pexa-Vec is even further attenuated as the thymidine kinase gene has been disrupted which makes replication in normal cells more difficult than the smallpox vaccine. Rare complications included eczema vaccinatum (patients with eczema), disseminated vaccinia rash, progressive vaccinia (in T-cell-deficient individuals) and encephalitis (1-2 per million vaccinated) (Fields B.N., 1996). Recent studies of smallpox vaccines have identified cardiac injury including pericarditis and myocarditis as a potential risk (Halsell J.S. et al., 2003). W replication exclusively occurs in the cytoplasm thus eliminating any risk of integration of the viral DNA into the host genome (Moss B., 2007).

In terms of classification of hazard, W is considered as a Group 2 biological agent as per Directive (2000/54/EC). W is also classified as a Biosafety Level 2 (BSL-2) infectious substance by the US Centers for Disease Control and Prevention (CDC) (CDC, 2009) and as a risk group 2 organism by the US NIH Guidelines (NIH).

Host/vector system
Pexa-Vec was generated by co-transfection of CV-1 cells (Monkey kidney cells) with W (Wyeth strain obtained from the Center for Disease Control, Atlanta, Georgia) and the plasmid pSC65/hGM-CSF. The vector pSC65/hGM-CSF contains DNA sequences coding for the hGM-CSF and β-galactosidase proteins and for their respective promoters. In addition, the transgene sequences are flanked by two W genomic regions (TKL and TKR) that allow homologous recombination between the transfer plasmid and W.

The plasmid pSC65/hGM-CSF is generated from the plasmid pSC65 which was provided by Dr. B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland (Chakrabarti 1997). The plasmid pSC65/hGM-CSF is inserted into W. The insertion of pSC65/hGM-CSF into W can be detected by using colorimetric identification of plaques containing recombinants expressing β-galactosidase.

The pSC65 vector when provided by Dr. B. Moss contains the LacZ gene. The LacZ gene is a reporter gene, under control of the W p7.5 earlylate promoter. The additional donor gene (i.e. gene coding for hGM-CSF) is inserted in pSC65 as follows.

The plasmid pCSF-1 (No. 39754) was obtained from American Type Culture Collection and comprises the full-length cDNA for hGM-CSF (Wong 1985). The hGM-CSF gene was cloned first into the EcoR1 site of pBLUESCRIPTSK (Stratagene, La Jolla, California), generating plasmid pBLUE/hGM-CSF, and providing restriction enzyme sites to allow cloning of the hGM-CSF gene into the SalI and BgiII sites of pSC65. This positioned the hGM-CSF gene downstream of a synthetic promoter (PsE/L) designed by Dr. Moss’ laboratory to give maximal levels of transcription during both the early and late phases of vaccinia infection (Chakrabarti 1997).

Pexa-Vec contains three genetic modifications compared to the wild type Wyeth strain: 1) disruption of the viral thymidine kinase (TK) gene by 2) insertion of the human granulocyte macrophage-colony stimulating factor (hGMCSF) gene and 3) insertion of the LacZ gene. Due to the insertion of the transgenes, the TK gene is inactivated. This decreases W virulence (Buller R. et al. , 1985) by restricting viral replication to proliferating cells. This also targets dissemination of the virus to tumors (Puhlmann M. et al. , 2000).

The LacZ gene is contained in the pSC65 plasmid which was provided by Dr. B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

The LacZ gene encodes for β-galactosidase which is a hydrolase enzyme that catalyzes the hydrolysis of 13-galactosides into monosaccharides.

Of note, following recombination between the W and pSC65/hGM-CSF, the antibiotic resistance gene contained in pSC65 is not part of the insert. The final GMO does thus not contain any genes conferring resistance to antibiotics.

Origin & function

Pexa-Vec is non-integrative, and replicative and propagative characteristics of W have been attenuated in Pexa-Vec, which makes the virus replication dependent on actively dividing cells such as cancer cells. Therapy with a replicating virus can theoretically lead to shedding of the virus into the environment, and potentially to the public, although controls are used in this trial to minimize this occurrence. The clinical information available to date
suggests that Pexa-Vec is safe at the clinical dose of 1 x 10^9 pfu (10,000-fold higher than smallpox vaccine dose) and has not spread to caregivers in contact with the treated patients. Should shedding occur, the level of exposure would be predicted to be low compared to the doses received by patients in the proposed trial, and extremely low compared to doses of non-attenuated vaccines administered to the public (e.g. vaccines against smallpox). In addition, exposed individuals over the age of 35 will likely have been previously immunized with vaccinia. In the highly unlikely event that an exposed individual were to demonstrate virus-associated toxicity, therapy could be initiated with VIG and/or cidofovir. Therefore, public health risks with this virus are extremely low and in fact should be lower than with standard vaccination procedures. To date, no reports of transmission to health care personnel from vaccinia recipients have been published. Routine barriers nursing approaches should be used per institutional guidelines for infectious organisms (e.g. such as for M. tuberculosis, Pseudomonas).

The information regarding the risk to patient contacts and guidelines for reducing the risk of viral transmission is contained within the Participant Information Sheet and Consent Form (PISCF) and the Pexastimogene Devacirepvec (Pexa-Vec) Guidelines (provided in Appendix B of Pexa-Vec Investigator's Brochure). The PISCF will be reviewed with the patients, and their written consent will be obtained, before they undergo any study-specific procedures. A signed copy of the PISCF will be given to the patients so that they can come back to the guidelines at any time. Pexastimogene Devacirepvec (Pexa-Vec) Guidelines will be given to investigators, pharmacists, and all personnel involved in the handling of the product.

The genetic modification of the virus is not expected to result in any post-release shift in biological interactions or host range or in any known or predictable effects on non-target organisms in the environment. It is also not expected that the release of the recombinant virus would result in any increase in pathogenicity as compared to the parental virus strain and/or in any increase in the capacity to recombine with other related viruses.

Therefore, under the conditions for use in the proposed clinical trial, Pexa-Vec is not considered to represent a risk for the environment and for the public health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Used vials, needles, gauze and syringes will be discarded into a designated, autoclavable sharps bin. This will be closed (but not locked, so as to permit steam penetration), and placed into an autoclave bag. Disposable gowns, gloves and other PPE will be discarded directly into an autoclave bag. These are labelled to indicate GM waste, and placed in a rigid, lockable container for temporary storage and transport. The research nurses associated with the trial transport the waste on a trolley to Clinical Laboratory Services, floor -1, QEHB, at a pre-arranged time, where it is decontaminated by autoclaving. Autoclaving is assumed to provide 100% inactivation of the GMM.

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y
The GMSC discussed the risk assessment at a meeting held on 17th May, 2016. The committee noted that the virus is based on the replication-competent, Wyeth strain of vaccinia, although the thymidine kinase deletion results in restricting virus replication to proliferating cells, such as cancer cells. It additionally expresses the human cytokine GM-CSF, which promotes immune responses, as well as bacterial beta-galactosidase. The proposed use of the virus in the trial was outlined. It was noted there was considerable experience from previous clinical trials with the agent. The virus is not shed orally or in urine. A small proportion of treated patients develop up to about 5 pustular lesions typical of vaccinia, however control procedures are in place, involving covering any lesions with dressings. Although transmission to staff has not been observed, the committee agreed that staff in identified risk groups (including pregnancy, immunosuppression, severe eczema) should be excluded when the virus is handled, and from contact with potentially contaminated materials, e.g. skin pustules. The committee agreed the risk assessment could be approved, subject to provision of some missing (non-critical) information, review and if necessary updating of SOPs, and the agreement of the consultant virologist absent from the meeting. Each of these conditions was subsequently met, and further reassurance was provided regarding exclusion of staff in risk groups when there is a risk of infection, and that the Sponsor has procedures in place to follow up and cases of potential transmission to healthcare personnel or other contacts. The BSO and Chair of the GMSC confirm approval of the risk assessment, subject to completion of Notification requirements.

Project Containment

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- 27/01/2000

### Data Premises Closed
- N

### Transferred from 1992 Regs?
- Y

### Transitional Premises
- Class: 1
- Non-GMMs: N
- Emergency Plan Required?: N
- Withdrawn: N

### Name
- LONZA MICROBIAL CONTROL

### Name 2

### Department
- BIOCIDES BUSINESS CENTRE

### Building
- P O BOX 42

### Campus Estate or Research Centre
- EMH013

### Road Name
- HEXAGAN TOWER

### Town
- Manchester

### County
- MANCHESTER

### Postcode
- M9 8ZS

### Country
- ENGLAND

### Tel Number
- 0161 721 1269

### Fax Number
- 0161 721 4173

### HSE Division
- NORTH WEST

### Comments
- Company name change from Arch UK Biocides Ltd 17/11/2011

### Date at Which Additional Info Submitted
- 02/03/2022
### Premises Addresses

<table>
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<tr>
<th>Date</th>
<th>Premises Closed</th>
<th>Name</th>
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### Premises Conditions

1. Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

2. Give brief details of the genetic modification safety committee

   - **Laboratory**
   - **Animal Unit**
   - **Growth Room**
   - **Glass House**
   - **Large Scale**

   - **Level 1 (GMMs)**
   - **Level 2 (GMMs)**
   - **Level 3 (GMMs)**
   - **Level 4 (GMMs)**

   - **Non-microbial**

3. **Other (please specify)**

4. Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

---

02/03/2022  Page 10888 of 15326
**Class 1 activity:**
Maximum culture volume = 100 ml.
Waste deactivation = autoclaving at 121 degrees C for 20 mins.
Validation of deactivation method = check of viability after autoclaving.
Monitoring of deactivation method = temperature probe to check if autoclave reaches 121 degrees C.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

<table>
<thead>
<tr>
<th>Other(s)</th>
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</thead>
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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 738**

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Name

**UNIVERSITY OF NOTTINGHAM**

Name 2

**UNIVERSITY HOSPITAL**

Department

**MEDICAL & SURGICAL SCIENCES CANCER STUDIES UNIT**

Campus Estate or Research Centre

Building

**QUEENS MEDICAL CENTRE**

Town

**NOTTINGHAM**

County

**NOTTINGHAMSHIRE**

Postcode

**NG7 2UH**

Country

**ENGLAND**

Tel Number

0115 970 9248

Fax Number

0115 970 9902

E-mail

HSE Division

**MIDLANDS**

Comments

GM738 closed and merged with GM470 on 18/02/2005

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
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- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
Mycology Transgenic Invertebrates

Transgenic Plants

Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

Project Ref 738/00.1

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Tick if notifying a connected programme of work

Historical Significant Changes

GM738/00.1 transferred to GM470 on 18/02/2005

Project notified under transitional arrangements Y
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<td>Human Clinical Applications</td>
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## Premises Addresses

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## Premises Conditions

 Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Chairman, Management Representative, BSO, Health & Safety Manager, Medical Officer & Co-opted Member.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify) Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 740

Data Premises Notified (Originally) 11/04/2000

Transferred from 1992 Regs? Y

Transitional Premises Class 1

Data Premises Closed N

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

THE CHRISTIE NHS FOUNDATION TRUST

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

WILMSLOW ROAD

District

Town

MANCHESTER

County

CHESHIRE

Postcode

M20 4BX

Country

ENGLAND

Tel Number 0161 446 3000

Fax Number 0161 446 3109

E-mail

HSE Division NORTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Other (please specify)

Tick if confidential

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<tbody>
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</table>
Soft waste is double bagged as per infectious clinical healthcare waste guidelines, sharps waste is disposed of in plastic sharps containers which are then sealed. All waste is labelled and tagged and held in a temporary segregated waste store until removed from the relevant clinical area to the external waste store to await subsequent removal by a waste contractor. There is full traceability of the waste. For modified vaccinia virus/adenovirus/adeno-associated virus, Virkon is used according to the manufacturers guidelines which for general virus inactivation is 2%w/v. In validation studies two representative examples from the poxviridae have been tested (vaccinia and pseudocowpox virus). For vaccinia, a 10 min exposure to both 0.5% and 1% solutions of Virkon caused a 1000 folds reduction in infectious titre with complete deactivation being demonstrated at 30 minutes. For pseudo cowpox virus a greater dilution range was explored and completely deactivation was demonstrated at 30 mins exposure to 1:300 w/v solution.

Virkon is used following manufacturers guidelines. There is a colour indicator which facilitates monitoring the deactivation efficacy of the solution in use.

For activities involving GMMs, describe the waste management measures which will apply to the activity.
**Project Additional Information**

**Purposes of the contained use**

TK-8001 is a cellular therapy which is able to detect MAGE-A1 on the surface of cancer cells and destroy it. TK-8001 is created from special white blood cells, so called T cells, which are collected from a patient’s own blood and which are then being processed in a laboratory and genetically modified to recognize the tumor protein MAGE-A1. When the T cells have been modified, they will be called TK-8001, which represents the medicine. TK-8001 is a personalized drug and can only be given to the person from which the T cells derived.

The study is a combined Phase I and II clinical trial. The purpose of this trial is to test in the first (Phase I) part of the trial different dose levels of TK-8001 for the treatment of advanced-stage cancer. In the second (Phase II) part of the trial, TK-8001 is then tested at the dose identified during the Phase I part. The overall aim is to test the safety, and efficacy against cancer of TK-8001.

**Recipient or parental organism**

- human T cells

**Host/vector system**

- **Gene insert:** self-inactivated gammaretroviral-derived genome carrying as transgene the MAGE-A1 specific TCR.
- T cells get transduced with a retroviral vector particle composed of Moloney Murine Leukemia Virus (MMLV) structural proteins, pseudotyped with GaLV glycoproteins. The vector genome construct contains MMLV derived self-inactivating long terminal repeats (LTRs), as promoter driving the expression of the encoded TCR the short version of human Elongation factor 1alpha promoter (EFS), the TCR, and the posttranscriptional regulatory element of Woodchuck hepatitis virus with deleted sequence of the viral X-protein.

**Origin & function**

The anticipated mode of action of TK-8001 is to bind to MAGE-A1-expressing tumor cells and, by exploiting their cytotoxic properties as well as their ability to secrete cytokines, lead to tumor cell destruction and reduction and potential elimination of the tumor.

**Evaluation of foreseeable effects**

The expected mode of action of TK-8001 is to bind to MAGE-A1-expressing tumor cells and, by exploiting their cytotoxic properties as well as their ability to secrete cytokines, lead to tumor cell destruction and reduction and potential elimination of the tumor.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- Not Applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- Not Applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

02/03/2022
Inactivation and disposal as potentially infectious medical waste as per hospital bio-hazard disposal procedures.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Not Applicable

**Project Containment**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  

Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research | Gene Therapy |
Virology | Transgenic Animals | Transgenic Fish | |

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
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<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
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<tbody>
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<td>Other(s)</td>
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Culture Volume.
The maximum culture volume that could be released is one litre.

Waste Deactivation
Liquid waste will be deactivated by autoclaving (at 126 degrees C for 20 minutes), in a waste only autoclave situated in the building in which the work is carried out. Reusable materials (ie. glassware) and solid waste (ie. disposable plasticware) will be autoclaved as above.

Validation of Deactivation
After receipt of approval to start work, any liquid waste produced will not be disposed of until it has been shown that the organisms being used, E.coli (which is not genetically modified) and bacteriophage cannot be cultured from a sample of waste that has been deactivated as above.

Monitoring of Deactivation.
At periodic intervals (as deemed appropriate by the GMSC), samples of waste will be checked for deactivation as above.

Additional information.
A solution of Chloros will be kept available for dealing with any liquid spillage that may occur. The solution will be used at the concentration, and left for the time, indicated by the manufacturer, prior to the spillage being cleared up.

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment
Data Premises Notified (Originally) 12/07/2000

Transferred from 1992 Regs? Y

Transitional Premises Class 2

Data Premises Closed Transitional Premises
Emergency Plan Required? N

Non-GMMs N Withdrawn N

Name

IMMUNOCORE LTD

Name 2

Department

Campus Estate or Research Centre

Road Name

92 PARK DRIVE

District

MILTON PARK

Town

ABINGDON

County

OXFORDSHIRE

Postcode

OX14 4RY

Country

ENGLAND

Tel Number 01235 438600 Fax Number 01235 438601

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

10/10/2001
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Chairperson (Biological Safety Officer or BioRisk Advisor)
- Vice Chair (EHS director)
- Biological Safety Officers
- HTA Representative
- Technical representatives from research areas
- CL3 manager / deputy
- Lab services manager
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<thead>
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<th>Growth Room</th>
<th>Glass House</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Project Ref 742/01.1

CU2 Project Title
TRANSFECTION OF HUMAN HELA CELLS WITH PIRES CARRYING GENES ENCODING PROTEINS INVOLVED IN IMMUNE RESPONSE

Class 2

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Withdrawn

Tick if notifying a connected programme of work

Historical Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
Personal protection from contamination of workers handling the transfected cells.

Recipient or parental organism
HeLa cells are derived from a human cervical adenocarcinoma. The cell line is obtainable from cell culture collections such as the American Type Culture Collection (ATCC) who advice the cell line to be handled as potentially biohazardous material under at least Biosafety Level 2 containment.

Host/vector system
The plasmid used is pIRES (described and sold by Clontech). It contains a ColE1 origin of replication for propagation as a low copy number plasmid in E. coli, the B-lactamase gene for ampicillin selection in E. coli, and the f1 origin of replication for production of single stranded DNA in E. coli. It contains the immediate early promoter of Cytomegalovirus (CMV) driving transcription of a bi-cistronic transcription unit that translates into two separate proteins by means of an internal ribosome entry site (IRES) from encephalomyocarditis virus (ECMV). An SV40 polyadenylation site directs proper processing of the resulting mRNA. Flanking the multiple cloning sites of the vector are bacteriophage T7 and T3 promoters. For G418-selection in cell cultures the plasmid holds the Neomycin resistance gene (NEO) expressed from an SV40 promoter/enhancer and 3-processing of this transcription unit is mediated by an artificial polyadenylation signal. The SV40 origin allows replication of the plasmid in mammalian cells expressing the SV40 T antigen.

Origin & function
One insert codes for human beta 2-microglobulin in its native form or in a form mutated to express a protein that will inhibit natural cytotoxic T cell responses. The second insert codes for human HLA-A*02011 in its native form. The genetic material is to be used in experiments intended for proving a principle and for patent exemplification.
The naturally occurring wild type proteins are highly unlikely to have implications for human health. Mutant B2-microglobulin is likely to have an immune suppressive effect by inhibiting CTL activity against human cells expressing the gene.

If transfected HeLa cells infect the worker handling the cells by accidental needle stick, or similar injury, and these cells evade the worker's immune system the infection may develop as a metastatic cancer and be fatal. The workers humoral immune system, however, will in all likelihood pick up and destroy these cells based on differences in the repertoire of surface proteins expressed by the HeLa cells and the cells of the worker. As an additional precaution the worker handling the cells must be tissue typed and have a significantly different tissue type from the HeLa cells being transfected.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid, and in some cases liquid, GMO contaminated waste is collected in special containers and sterilised by autoclaving at 121 degrees C for 40 minutes to give 100% kill. Autoclaved liquid waste is disposed of through the sink to public sewer. Solid waste is hereafter treated as contaminated laboratory waste and disposed through a specialist waste management company (Grundon).

The performance is monitored by labelling material with indicator tape. On a routine basis the general performance of the autoclave is monitored by its ability to sterilise multiple 1 litre batches of media used for bacterial growth. Sterilised batches of media are routinely kept at room temperature to facilitate growth and detection of contaminating microorganisms if any.

As a minimum the autoclave is checked and serviced annually by an authorised service contractor. A temperature mapping analysis of the autoclave is carried out as part of the annual service.

Liquid waste which is not autoclaved is inactivated by addition of Virkon to 1% and incubation at room temperature for at least 1 hour.

The method gives a bacterial degree of kill of 99.999%. The performance is monitored by re-plating bacterial samples and comparing scores against non-treated samples. Inactivated liquid waste is disposed of through the sink to public sewer.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

Please enter comments on the GM safety committee on the risk assessment

Risk assessment approved without additional comments.
Project Containment

Laboratory Activities | Glass Houses | Growth Rooms
---|---|---
L2 Yes | L3 | L4 L2 | L3 | L4 L2 | L3 | L4 L3

Animal Units | Large Scale Activities | Human Clinical Applications
L2 | L3 | L4 L2 | L3 | L4 L2 | L3 | L4

Project Ref 742/01.2

Date Ackn’d 05/02/2001
Date Project Ceased 21/10/2019
Withdrawn N

CU2 Project Title
PRODUCTION OF VACCINIA VIRUS FOR EXPRESSION IN HUMAN CELL LINES OF PROTEINS INVOLVED IN IMMUNE RESPONSES

Class Class 2
Culture Volume Class 2 < 1 litre
Consent Granted not applicable

Non-GMM
Project notified under transitional arrangements N

Project Additional Information

Purposes of the contained use
Personal protection from contamination of workers handling the virus and prevention of spread to the environment.

Recipient or parental organism
In addition to endogenous proteins the parental and recipient human cell lines involved in the study will express viral proteins as well as proteins involved in immune responses.

Host/vector system
Vaccinia virus has been used for many years as a vaccine against smallpox and the possible effects of infection are well documented. The recombinant virus will be propagated in a human cell line.

**Origin & function**

The genetic material involved derive from human cell lines and represent human leukocyte antigens molecules in their native form or in a form mutated so as to have impaired CD8 binding capabilities. Expression of mutated HLA should render the infected cells less sensitive to the effect exerted by CD8-dependent cytotoxic T lymphocytes (CTLs) specific for that particular HLA molecule.

**Evaluation of foreseeable effects**

No additional hazards are envisaged by accidental infection with virus expressing wild type HLA molecules. A slight increase in immunological resistance may be observed for viruses expressing a mutated HLA molecule in individuals expressing the wild type HLA molecule from which the mutant HLA derive. Since each individual express HLA class 1 molecules from three different loci and thus between three and six different class 1 molecules depending on their degree of heterozygosity the virus will eventually be eradicated by CTLs with specificity for alternative HLA molecules and by the humoral branch of the immune system.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid and in some cases liquid, GMO contaminated waste is collected in special containers and sterilised by autoclaving at 121 degrees C for 40 minutes to give 100% kill. Autoclaved liquid waste is disposed of through the sink to public sewer. Solid waste is hereafter treated as contaminated laboratory waste and disposed through a specialist waste management company (Grundon).

The performance is monitored by labelling material with indicator tape. On a routine basis the general performance of the autoclave is monitored by its ability to sterilise multiple 1-litre batches of media used for bacterial growth. Sterilised batches of media are routinely kept at room temperature to facilitate growth and detection of contaminating microorganisms if any.

As a minimum the autoclave is checked and serviced annually by an authorised service contractor. A temperature mapping analysis of the autoclave is carried out as part of the annual service.

Liquid waste which is not autoclaved is inactivated by addition of Virkon to 1% and incubation at room temperature for at least 1 hour.

The method gives a bacterial degree of kill of 99.999%. The performance is monitored by re-plating bacterial samples and comparing scores against non-treated samples. Inactivated liquid waste is disposed of through the sink to public sewer.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

02/03/2022
The risk assessment was approved without further comments.

### Project Containment

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### Project Ref 742/04.1

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Project notified under transitional arrangements N

### Project Additional Information

- **Purposes of the contained use**: Personal protection from contamination of workers handling the transfected cells.

- **Recipient or parental organism**:

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Page 10913 of 15326
The recipient cells to be used in these procedures will be established mammalian cell lines and will have the following characteristics: they will be negative for the SV40 large T antigen, they will be non-EBV transformed, and to the best of our knowledge, they will be free of adventitious agents.

Indirect hazards to human health associated with possible adventitious agents have been considered separately.

### Host/vector system

Gene expression for pEE14, pCDNA, pCI-neo, plRES, plRES-blasticidin, plRNEB, pCDNA6.2/GFP DEST and pGFP2 is controlled from the immediate early Cytomegalovirus (CMV) promoter. In plRES the gene transcript is translated into two distinct proteins by means of an internal ribosomal entry site (IRES) from encephalomyocarditis virus (ECMV). The pGFP2 and pCDNA6.2/GFP DEST vectors contain the green Fluorescent protein which can be fused to the C terminus of the gene to be transfected.

All eight vectors contain the SV40 origin which enables episomal replication in mammalian cells that contain the SV40 large T antigen. The recipient cells to be used in these procedures will be established mammalian cell lines and they will be negative for the SV40 large T antigen.

### Origin & function

The genetic material will be derived from tumour cell lines. The desired genes code for proteins that are expressed in tumour cells but will not include defined oncogenes. It is envisaged that the inserts will enable expression of cancer antigen proteins and result in the transfected cell presenting fragments of antigen on its surface by means of the major histocompatibility complex (MHC). The presented antigen complex would then serve as a target in cellular and/or biochemical assays.

### Evaluation of foreseeable effects

In the extremely unlikely event of the cells gaining entry to the worker via a needle-stick type injury, they would in all likelihood be rapidly destroyed by the workers’ immune system by virtue of the differences in cell surface proteins between those of the worker and the transfected cells. It is extremely unlikely that the inserted genes will endow the cells with the ability to evade the workers’ immune system.

Indirect hazards to human health associated with possible adventitious agents have been considered separately.

Mammalian cells are considered especially disabled and are unlikely to survive in the environment in the absence of strict osmotic, buffering, and nutrient conditions.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid, and in some cases liquid, GMO contaminated waste is collected in designated containers and sterilised by autoclaving at 121 degrees C for 40 minutes to achieve 100% kill. Autoclaved liquid waste will be disposed of down a sink to the public sewer. Autoclaved solid waste is hereafter treated as contaminated laboratory waste and disposed through a specialist waste management company (Grundon).

Autoclave performance is monitored by the use of autoclave indicator tape included in every run. On a routine basis the general performance of the autoclave is monitored by its ability to sterilise multiple 1-litre batches of bacterial growth media. Sterilised batches of media are routinely kept at room temperature to facilitate growth and to detect contaminating microorganisms.

On an annual basis, the autoclave is checked and serviced by an authorised service contractor. A temperature mapping analysis of the autoclave is carried out as part of...
Liquid waste which is not autoclaved is inactivated by the addition of Virkon to a final 1% concentration followed by incubation at room temperature for at least one hour. This treatment gives a bacterial kill of 99.999%. Performance is monitored by replating bacterial samples and comparing scores against non-treated samples. Inactivated waste is disposed of through the sink the public sewer.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Risk assessment approved without additional comments.

Project Containment

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<tr>
<th>Laboratory Activities</th>
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Project Ref 742/08.1

Date Ackn'd 15/05/2008

CU2 Project Title
Retrovirus carrying genes encoding for human telomerase reverse transcriptase (hTERT) and enhanced Green Flourescent Protein (GFP).

Class 2
Consent Granted Not Applicable

Non-GMM

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Withdrawn N

Tick if notifying a connected programme of work N
Project Additional Information

Purposes of the contained use

The purpose of the contained use is to infect human cancer specific T cell clones with retrovirus containing a mini gene construct that encodes for the human telomerase reverse transcriptase protein and enhanced Green Fluorescent Protein (GFP). The introduction of human hTERT cDNA into a human T cell clone extends the life span of the clone in vitro. T cell clones containing the hTERT minigene will be used in cellular assays to test the efficacy of biological reagents.

Recipient or parental organism

The recipient cells will be human cancer-specific T cell clones and as such fall into the “Especially Disabled Hosts” category as defined in Part 2A ANNEX II of the ACGM Compendium of Guidance. These T cell clones will not be derived from the blood of any personnel working in MediGene’s cell biology laboratories. The work involving these cells will be undertaken in a Containment Level 2 facility. The cells used are to the best of our knowledge, free of adventitious agents and will be non-self cell lines that will not be able to colonise those involved with the work. Indirect hazards to human health associated with possible adventitious agents have been considered separately in a general Risk Assessment relating to work with human derived materials. All human derived samples are treated as though capable of causing disease.

Host/vector system

The MMLV vector is based on the moloney murine leukaemia virus. The amphotropic form of the virus will be used, meaning that the virus can infect mammalian cells but is replication defective and lacks more than two thirds of the viral genome. In the absence of wild type virus or gene products that provide the functions of the missing genes, the vector is unable to generate a productive infection once introduced into the cell. In addition the MMLV virions are particularly sensitive to human complement. The vector containing a mini gene construct that encodes for the human telomerase reverse transcriptase protein and enhanced Green Fluorescent Protein (GFP). Any T cell clones that are immortalised using this vector will only present a hazard to the original cell donor. T cell clones will not be derived from the blood of any personnel working in MediGene’s cell biology laboratories thus removing this potential hazard. The hTERT protein produced within infected cells can immortalise those cells. If a worker does accidentally infect themselves with the virus in all likelihood the particles will be rapidly destroyed by the workers’ immune system. Any infected cells will appear altered due to the expression of GFP or will be perceived as virally infected and destroyed by the immune system.
The aim of this work is to infect human cancer specific T cell clones with retrovirus containing a mini gene construct that encodes for the human telomerase reverse transcriptase protein and enhanced Green Fluorescent Protein (GFP). The introduction of human hTERT cDNA into a human T cell clone extends the life span of the clone in vitro, T cell clones containing the hTERT minigene will be used in cellular assays to test the efficacy of biological reagents. The GFP gene assists in the selection of positive transfectants. The inserts will confer the T cells with the ability to produce hTERT protein which can potentially render them immortal.

Evaluation of foreseeable effects

The hazards to workers and the environment posed by the vector with insert are considered negligible due to the containment level II measures employed. Skin contact with the organisms or the transfected cells will be avoided by minimizing aerosols (by working in a Class II biosafety cabinet), and by the use of gloves and safety glasses. If a worker does accidentally infect themselves with the virus, in all likelihood it will be rapidly destroyed by the workers’ immune system. MMLV virions are particularly sensitive to human complement.

Should the virus gain entry to a worker, any infected cells will appear altered due to the expression of GFP or will be perceived as virally infected and rapidly destroyed by the immune system. The routine procedures employed (i.e., autoclaving/chemical inactivation of all cellular material prior to disposal) will ensure an adequate level of safety. In the extremely unlikely event of transfected T cell clones gaining entry to the worker, the cells in all likelihood would be rapidly destroyed by the workers’ own immune system by virtue of being “non-self”. Transfected mammalian cells are considered especially disabled and are unlikely to survive in the environment in the absence of strict osmotic, buffering, and nutrient conditions.

Origin & function

The hazards to workers and the environment posed by the vector with insert are considered negligible due to the containment level II measures employed. Skin contact with the organisms or the transfected cells will be avoided by minimizing aerosols (by working in a Class II biosafety cabinet), and by the use of gloves and safety glasses. If a worker does accidentally infect themselves with the virus, in all likelihood it will be rapidly destroyed by the workers’ immune system. MMLV virions are particularly sensitive to human complement.

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Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The solid waste and the majority of the liquid waste generated during this procedure will be collected in specialised containers and sterilised by autoclaving at 121°C for 25 minutes to give 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste under MediGene Limited’s Environment Agency Waste Registration Number; NAAOSO. The autoclaved solid waste will be disposed of via incineration through an appointed specialist waste management company; Grundon Waste Management Limited. Autoclaved liquid waste will be disposed of through a sink to public sewer. Any remaining liquid waste will be treated with an over-kill concentration of VirkonTM (2% fmal w/vol) according to the manufacturers instructions (DuPont) for which independent validated efficacy against retrovirus exists.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The Genetic Modification Committee discussed the proposed work at a recent GM Safety Committee Meeting. After consultation with three additional MediGene staff: the Senior Vice President of Research, the Project Manager, and the Scientist that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity.

**Project Containment**

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**Project Ref**  742/08.2

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<td>Lentivirus carrying genes encoding T Cell Receptors (TCR) and marker molecules.</td>
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<th>Non-GMM Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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**Project Additional Information**

**Purposes of the contained use**

The aim of the contained work is to generate T cells with specificities to the following cells:
1. Cells expressing cancer antigens such as NY-ESO, gpIOO, MAGE A3, WT-1, p450, p53 & survivin.
2. Cells expressing viral epitopes from viruses such as human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein Barr Virus (EMV) & human papilloma virus.
3 Cells expressing putative-immune disease antigens or in graft rejection antigens. The auto-immune diseases to be studied include but are not limited to diabetes mellitus type 1, rheumatoid arthritis, autoimmune hepatitis, & multiple sclerosis. The T cells will also express reporter genes that allow for the selection of transformants and subsequent detection in immunological assays. These include but are not limited to: Green Fluorescent Protein (GFP), Red Fluorescent Protein (RFP), dsRED and/or Luciferase genes.

The T Cell Receptors (ICR) would be either the wild type form or an isoform with one of various affinities. The Lentivirus system would allow us to derive T cell clones stably expressing the TCR of interest, or T cells transiently expressing these genes. T cell clones expressing ectopic TCR would be used in cellular assays to test antigen specific response.

Recipient or parental organism

The recipient cells will be
- a Human T cells not derived from the blood of the user
- or
- b. Fully characterised cell lines such as HT1080 fibrosarcoma cell line.

The work involving these cells will be undertaken in a Containment Level 2 facility. The cells used are to the best of our knowledge, free of adventitious agents and will be non-self cell lines that will not be able to colonise those involved with the work. Indirect hazards to human health associated with possible adventitious agents have been considered separately in a general Risk Assessment relating to work with human derived materials.

All human derived samples are treated as though capable of causing disease.

Host/vector system

Two types of vectors will be used:

Type A. Commercially acquired vectors, typically third (or even fourth) generation Lentivirus systems

Including but not limited to:
- ViraPower Lentiviral Expression systems sold by Invitrogen Ltd. The vectors are: pLenti6iVs-Directional TOPO, pLenti6.2-GW/EniGFP, pLP1, pLP2, and pLP/VSVG plasmids.
- Lenti-X Lentiviral Expression systems sold by Clontech Laboratories, Inc. The vectors include: pLVX-Puro, pLVXDsRed-Monomer-CI, Lenti-X FIT packaging system

Type B. Vectors constructed by our academic collaborators e.g. Cardiff University & University of Pennsylvania (UPenn).

These will typically be second or third generation type systems and so may lack the additional safety features of the commercially acquired systems.

Including but not limited to:
- Vectors obtained from (CardiffUniversity): pUC19-x-SQSG-2A-y shuttle plasmid, pLenti-SxW, pMD2G (VSVG envelope) and p8.91 (gag/pal/rev/tat) plasmids
- Vectors obtained from (University of Pennsylvania; UPenn): Lentiviral vector derived from the ‘Dull’ vector (Dull ci a?, J Viral 1998), and 3 packaging plasmids (gag/pal, rev, VSVG envelope) similar to that used by Zufferey (Zufferey eta?, Nat Biotech 1997)

These Lentiviral Expression Systems are second, third or fourth generation lentivirus systems. The lentiviruses can infect manirnalian cells but cannot replicate in these cells. These systems include some or all of the following safety features:

1. Expression vectors containing a deletion of the 3’ LTR (AU3) that does not affect generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a viral particle.
2. The number of HIV-1 genes that are used in the system has been reduced to three or four only: gag, pal, tat and rev.
3. The VSV-G gene from Vesicular Stoniatitis Virus is used in place of the HIV-1 envelope protein (env).
4. Genes encoding the structural and other components required for packaging the viral genome are separated onto multiple plasmids. These plasmids have been engineered not to contain any homology regions to prevent recombination events.
5. Although the plasmids allow expression in trans of proteins required to produce lentivirus progeny in producer cell lines, none of them contain LTRs or the ‘1’ packaging sequence. This means that none of the MV-I structural genes are present in the packaged viral genome and are thus never transduced in the target cell. No new replication-competent virus can be produced.
6. The lentiviral particles produced in these systems are replication-incompetent and only carry the genes of interest.
7. Expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-I RRE in the gag/pol mRNA transcript.
Any T cells transduced using these vectors will only present a hazard to the original cell donor. Transduced T cells and derived T cell clones will not be derived from the blood of any personnel working in MediGene’s cell biology laboratories thus removing this potential hazard.

Origin & function

Reporter genes such as GFP, RFP and luciferase reporter genes have been used in various transgenic animal systems and have been shown to have no harmful effects on the organisms.
The TCR gene inserts encode for proteins known to be clonally expressed by T cells only. Expression of TCR by cells other than T cells renders the TCR non-functional. In the case of cancer and viral epitope-specific TCR genes, their expression in T cells would induce a response to cells expressing tumour antigens or viral antigens (including virally infected cells).
TCR genes specific for putative auto-antigens or self antigens do present a hazard to the worker performing the work but only if the worker’s own T cells were transfected to express the genes. This could result in T cells that would target normal tissue and would not be recognised by the worker’s immune system as non-self. For this reason, the use of self cells is strictly prohibited.

Evaluation of foreseeable effects

The hazards to workers and the environment posed by the vector with insert are considered negligible due to the containment level II measures employed. Skin contact with the organisms or the transfected cells will be avoided by minimizing aerosols (by working in a Class II biosafety cabinet), and by the use of gloves and safety glasses. If a worker does accidentally infect themselves with the virus, in all likelihood it will be rapidly destroyed by the worker’s immune system. MMLV virions are particularly sensitive to human complement.
Should the virus gain entry to a worker, any infected cells will appear altered due to the expression of GFP, RFP, OsRed or luciferase, or will be perceived as virally infected and rapidly destroyed by the immune system. The routine procedures employed (i.e., autoclaving/chemical inactivation of all cellular material prior to disposal) will ensure an adequate level of safety.
The extremely unlikely event of transfected T cell clones gaining entry to the worker, the cells in all likelihood would be rapidly destroyed by the worker’s own immune system by virtue of being “non-self”.
Transfected mammalian cells are considered especially disabled and are unlikely to survive in the environment in the absence of strict osmotic, buffering, and nutrient conditions.
Lentiviruses are highly susceptible to dehydration. However, they can survive for long periods in high protein media. Lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. The lentivirus cannot replicate and so the consequences of escape are considered negligible.
Risk is effectively zero. Infected mammalian cells are not able to establish themselves and spread in nature and the vectors selected are either disabled or attenuated.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The solid waste and the majority of the liquid waste generated during this procedure will be collected in specialised containers and sterilised by autoclaving at 121°C for 25 minutes to give 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste under MediGene Limited’s Environment Agency Waste Registration Number; NAAO5O. The autoclaved solid waste will be disposed of via incineration through an appointed specialist waste management company; Grundon Waste Management Limited. Autoclaved liquid waste will be disposed of through a sink to public sewer. Any remaining liquid waste will be treated with an over-kill concentration of Virkon (2% final wt/vol) according to the...
manufacturers instructions (DuPont) for which independent validated efficacy against retrovirus exists.

The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity.

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity.

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Project Ref 742/18.1

Date Ackn'd 04/01/2018

Date Project Ceased

Expression of full length, truncated or modified recombinant protein in mammalian cells.

Class

Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment
**Purpose of the contained use**

Mammalian cells are genetically modified to express full length, truncated or modified proteins of mammalian, viral or bacterial origin for use in cell-based assays or in order to purify recombinant proteins as part of the development and testing of TCR-based therapeutic proteins.

**Recipient or parental organism**

The recipient organisms are mammalian cells that can be subcategorised as either:

1. Well characterised, widely used cell lines of mammalian origin with a low risk of endogenous infection with a biological agent and that present no apparent harm to laboratory workers and which have been tested for the most serious pathogens.
2. Cell lines that harbour endogenous biological agents (including agents classified as hazard group 1 or 2) or cells that have been deliberately infected with hazard group 1 or 2 organisms.
3. Primary cells including cells isolated from blood or lymphoid cells of human origin.

**Host/vector system**

Recombinant proteins will be expressed in mammalian cells from commercially available and non-mobilisable mammalian expression plasmid vectors including but not limited to:

- pIRES/FRT Hyg, pCEP4, pCDNA3.1, pMAX, pEE14, pLRNEB, pGFP2, pEE14, pCI-neo, pIRES, pIRES-blasticidin, pIRES, and pCDNA6.2/GFP DEST.

These vectors typically consist of some or all of the following elements:

1. Antibiotic resistance genes for example genes that confer resistance to zeomycin, hygromycin and puromycin.
2. Sequences derived from viral genomes, for example gene expression may be driven by immediate early Cytomegalovirus (CMV) promoter, the gene transcript may be translated into two distinct proteins by means of an internal ribosomal entry site (IRES) from encephalomyocarditis virus (ECMV). Vectors may contain the SV40 origin to enable episomal replication in mammalian cells that contain the SV40 large T antigen.
3. Peptide or proteins tag for example vectors may contain sequences that encode Fc molecules, Green Fluorescent Protein (GFP) or to antigenic peptide sequences by means of a flexible peptide linker such as FLAG or myc-peptide which can be fused to the N or C terminus of the exogenous gene to be expressed to produce a fusion protein product.

**Origin & function**

Inserted DNA sequences covered by this risk assessment encode proteins that can be functionally categorised as:

1. Cancer antigens
2. Antigens derived from microbes such as bacteria or viruses
3. Normal and variant leucocyte proteins including but not limited to T cell receptors (TCRs), Human leucocyte antigens (HLAs), beta 2 microglobulin (β2m), and other proteins likely to be immunomodulatory.
4. Gene products with oncogenic properties (for example that has been reported in the scientific literature to confer anchorage independent growth) or oncogenes.
5. Label probes such as GFP, RFP or luciferase. Inserts that encode genes expected to confer additional hazardous properties on the GMO will be considered in detail as an additional risk assessment by the GMO committee. Inserts which are deemed to raise the class of the GM activity above 2 will not be permitted.

**Evaluation of foreseeable effects**

Primary T cells will be transfected exclusively with genes encoding TCR alpha and beta chains. These genes are known to be expressed on T cells. Although inserted TCR chains could mis-pair with endogenous TCR chains, the consequence of this are unlikely to endow transfected T cells with harmful properties.

For other cell lines, the inserted gene products also include oncogenic and immunomodulatory proteins and toxins. Although some of the proteins produced have the potential to confer harmful properties to recipient cells, these cells would in all likelihood be rapidly destroyed by the workers’ immune system, in case of accidental exposure. The constructs are incapable of infecting human cells.

The hazards related to the release of toxins and proteins with immunomodulatory properties from transfected cells is considered. Appropriate control measures will minimise the risk of worker exposure to these proteins (containment level 2, good microbiology practice, avoid the use of sharp).

The majority of the recipient mammalian cells used are to the best of our knowledge, free of adventitious agents, are purchased from reputable sources (ATCC and DSMZ). However, some of the cell lines may harbour adventitious agents such as viruses (e.g., EBV, Hazard Group 2) or mycoplasmas (Hazard group 2), resulting in some shedding into the culture medium. Indirect hazards to human health associated with possible adventitious agents are mitigated by work in containment level 2 laboratories with good microbiological safety.

These cells are non-self cell lines and targets of immune rejection of non-self tissue. It is therefore highly improbable that accidental exposure would result in survival and replication in normal healthy individuals.

Primary cell cultures are the greatest risk for human health. The potential for contamination of cells with blood-borne viruses including hepatitis B, hepatitis C, HIV, SIV and HTLV will be considered and screened for, where possible.

Workers will not conduct genetic modification work with their own cells or cells derived from other laboratory workers.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Biologically/GMO contaminated solid waste:
- Waste from class 1 activities (e.g. used agar plates), are collected in designated containers and if possible autoclaved at 121 °C for at least 20 minutes.
- If autoclaving is not possible then solid waste from class 1 activities is collected and sealed in designated bags. These bags are placed in a sealable, designated container on site and collected for proper disposal.
- Waste from class 2 activities is treated with a final concentration of 1-10 % virkon for at least 10 minutes before being drained and placed in designated containers for disposal. Virkon is drained into the public sewer via the sink.
- All solid waste incinerated by validated means through a licensed contractor.

Biologically/GMO contaminated liquid waste:
- All liquid waste (e.g. culture medium, phage supernatants, contaminated assay materials) is deactivated before disposal. This is achieved by autoclaving at 121 °C for at least 1 hour or by treatment with disinfectant.
(including but not limited to Virkon, ChemGene HLDL4, Surfamios) according to the manufacturer’s specifications. Autoclave performance is monitored by the use of autoclave indicator tape included in every run. Autoclaves are serviced annually by an authorised service contractor, as part of this service temperature mapping analysis is carried out.

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**Project Ref** 742/18.2

**Date Ackn’d** 05/01/2018

**CU2 Project Title** Use of lentivirus and lentiviral vectors for expression of novel molecules in both E. coli and Mammalian cells of both primary and established cell lines.

**Class** Class 2

**Culture Vol** 1-50 Litres

**Consent Granted** Not Applicable

**Project notified under transitional arrangements** N

**Withdrawn** N

Tick if notifying a connected programme of work N

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The use of Lentivirus transduction technology to generate cell lines, reagents, and develop assays and tools to help in screening for novel TCR molecules with high-affinity and restricted specificity. Ultimately, this will help in the generation of immunotherapeutic biologicals affecting the fields of oncology, autoimmunity, and infectious diseases.

**Recipient or parental organism**

Non-colonising/disabled *E. coli* K-12, *E. coli* B or k-strain derivatives including and not limited to: DH5alpha (NEB5alpha, Zymo 5-alpha, etc), Stbl3. Various established Mammalian cell lines as well as primary cell lines isolated from whole blood and human tissue either made in-house, or sourced from a commercial approved supplier.

**Host/vector system**

Multiple sources of vectors will be used: purchased commercially, made by collaborators, or made in-house.

Commercially acquired *E. coli*/mammalian shuttle vector plasmids, typically 3rd and 4th generation lentiviral systems, including but not limited to: ViraPower (Invitrogen), MISSION (Sigma).

*E. coli*/mammalian shuttle plasmid vectors constructed in-house or by our academic collaborators.

These will be typically 3rd generation type systems and so may lack some additional safety features of the commercially acquired systems.

Including but not limited to: plasmid vectors obtained from Andy Sewell, Cardiff University and derived from the “Dull” vector (Dull et al, J Virol 1998)

Lentiviral packaging: 3 separate packaging plasmids: (gag/pol, rev, VSVG envelope) similar to that used by (Zufferey et al, Nat Biotech 1997). Packaging envelope plasmid can alternatively express the measles F and H gene products.

In-house vector pCluedo-Lenti which is compatible with all 2nd and 3rd generation packaging vectors

**Origin & function**

The inserts (under constitutive or inducible promoters) fall into various categories and include but are not limited to:

- Native TCR molecules
- Variant TCR molecules (chimeras or containing point mutations that improve the stability and surface expression)
- Marker and reporter molecules. Including but not limited to: fluorescent proteins, luminescent proteins, antibiotic resistance proteins, and inert surface markers
- Genome editing molecules. Including but not limited to: safe integrases and recombinases, CRISPR proteins, TALEs, ZFNs
- Individual or libraries of silencing/upregulating strategies' components (siRNA, shRNA, gRNA)
- Human cancer-associated and lineage-specific genes, collectively referred to as ‘target antigens’. Inserts can include whole genes, regions of a gene, or gene fusion products. Inserts will be deemed suitable for lentiviral transduction by individual consideration.
- Genes from viruses including but not limited to hepatitis B virus, collectively referred to as ‘viral target antigens’. Inserts can include whole genes, regions of a gene or gene fusion products. Inserts will be deemed suitable for lentiviral transduction by individual consideration.
- Genes from bacteria including but not limited to Mycobacterium tuberculosis, collectively referred to as ‘bacterial target antigens’. Inserts can include whole genes, regions of genes or gene fusion products. Inserts will be deemed suitable for lentiviral transduction by individual consideration.
- Human genes associated with immune-modulation, collectively referred to as ‘immunomodulators’. Inserts can include co-stimulatory receptors, inhibitory receptors, receptor agonists, and cytokines. Inserts will be deemed suitable for lentiviral transduction by individual consideration.
- Large scale expression of excreted protein as means for production.
- Native TCR, antibodies, effector molecules; and other membrane-displayed libraries for protein selection and affinity maturation
- Cell-fate genes (aka “suicide genes”).
- Human leukocyte antigens (HLAs). Including but not limited to: classical and non-classical HLA class I, beta-2 microglobulin, HLA class I-like molecules (e.g. CD1 molecules, MR1) and HLA class II molecules. Inserts can include whole genes and gene-fusion products
- Inserts encoding short regions of viral, bacterial and human genes, including genes with oncogenic potential, collectively referred to as ‘immunogenic epitopes’
- The GMO committee will advise on which inserts will require additional risk assessment, considering of all existing control measures and whether any additional control measures are required prior to conducting work. Inserts which are deemed to raise the class of the activity above 2 will not be permitted. These are assessed and monitored on a regular basis.

**Evaluation of foreseeable effects**

**Vectors:**
Lentiviruses can infect human cells but cannot replicate in humans. The lentivirus genome randomly integrates into its host genome. This event could transactivate or alter the gene expression in the host at the site of integration. However a number of disabling mutations in the lentiviral system add to a number of safety features outlined in the section below.

**Recipient cells:**
Mammalian cells in general are considered as especially disabled hosts provided they are unable to colonise the worker (i.e. non-self) and to the best of our knowledge they contain no adventitious agents which are potentially harmful. Mammalian cells that are known to be infected with adventitious agents represent additional safety concerns. In particular the interaction of retrovirus with other members of the lentiviral family of with viruses that have different tropisms of transmission properties will be avoided.

**Marker/reporter inserts:**
Reporter genes such as GFP, have been proven to have no hazardous properties to organisms it is used in.

**Lineage-associated genes:**
Genes associated with distinct cell lineages that are not known to alter the growth status of the cell or confer any cytotoxic property are unlikely to pose a significant hazard to human health. This assertion will be supported by relevant knowledge of the function of the gene, including extensive literature review, and will not be inferred.

**Viral genes:**
Several hazards need to be considered when evaluating the risk of introducing genes from a virus into retroviral vectors. Third generation lentiviral systems are designed to be replication incompetent through the removal of non-structural components of the virus and separation of the material to be integrated from the structural components of the virus. Any gene inserted into a lentiviral system that originates from a retrovirus presents a risk of disrupting this system by effectively removing inbuilt safety features. This would increase the risk of generating replication competent virus (RCV). Genes originating from lentivirus will therefore never be used in this system.
Genes from other viruses that do not use the same mechanisms of host integration can represent significant hazards through the increased risk of altering the phenotype of
In particular, incorporation of envelope glycoproteins associated with viral entry represents an increased risk of generating virus with altered tropism and/or transmission properties and should be avoided. Viral gene base pair sequences can encode regions associated with recombination events and transcriptional control elements. This will be considered when designing viral inserts and in particular wherever possible native viral base pair sequences will be avoided. Alternative codon usage at the stage of synthesis will highlight and remove sequences prone to secondary structures and recombination events.

**Toxins, mitogens and superantigens:**
Insertion of genes that are known to produce toxins, mitogens or superantigens represent a risk to human health. These will be considered on an individual basis by additional (part B) risk assessment, inserts which are deemed to increase the class of the activity above 2 will not be permitted.

**Cancer-associated genes:**
Insertion of human, viral or bacterial genes that are known to be involved in the deregulation of cell growth or survival (proto-oncogenes and/or oncogenes) into broadly tropic retroviral systems represent a significant hazard to human health over and above the hazards associated with the recipient strain. Cancer-associated gene inserts will be carefully assessed on an individual basis, particularly for genes that have demonstrable oncogenic properties, for example src or c-myc. The risks associated with exposure to multiple oncogenes is significantly higher than that associated with a single gene, and so under no circumstances will multiple oncogenes be inserted into one vector, and multiple vectors encoding different oncogenes will not be used at the same time. Many proteins have undefined oncogenic properties and in this case will be assumed to have capacity to act directly as an oncogene.

**Immunomodulators:**
Any gene inserted into broadly tropic retrovirus that is able to modulate the immune system represents an increased risk to human health. Recipient strains that contain immunoinhibitory molecules may be able to circumvent the immune system of someone exposed to them, although the broad nature of immune defense against foreign antigen makes this unlikely. A greater risk lies in exposure of the operator to the system itself, where the likelihood of the insert contributing to systemic toxicity or autoimmune reactivity will be considered carefully. The risks associated with exposure to multiple immunomodulatory genes is likely to be higher than that associated with one gene, and so multiple genes will not be inserted into one vector, and multiple vectors encoding different immunomodulatory genes will be used at the same time with caution.

**TCR gene inserts:**
TCR genes that have been transfected into a donors T cells represent a hazard toward that donor. For this reason, scientists are strictly prohibited from working with their own cells.

**Immunogenic epitopes:**
Immunogenic epitopes do not contain sufficient genetic information to impart and altered phenotype on the recipient strain or represent a significant hazard to human health. Epitopes derived from viruses, and in particular retroviruses, will not use viral base pair sequences where possible, to reduce the risk of altering the safety systems of the lentiviral system in any way.

**Human leukocyte antigens:**
Human leukocyte antigens are essentially polymorphic (classical) and nonpolymorphic (non-classical) variants of genes expressed ubiquitously within the body and as such are unlikely to pose a significant risk to human health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Biologically/GMO contaminated solid waste:

- Waste from class 1 activities (e.g., used agar plates), are collected in designated containers and if possible autoclaved at 121 °C for at least 20 minutes.
- If autoclaving is not possible then solid waste from class 1 activities is collected and sealed in designated bags. These bags are placed in a sealable, designated container on site and collected for proper disposal.
- Waste from class 2 activities is treated with a final concentration of 1-10 % virkon for at least 10 minutes before being drained and placed in designated containers for disposal. Virkon is drained into the public sewer via the sink.
- All solid waste incinerated by validated means through a licensed contractor.

Biologically/GMO contaminated liquid waste:

- All liquid waste (e.g., culture medium, phage supernatants, contaminated assay materials) is deactivated before disposal. This is achieved either by autoclaving at 121 °C for at least 1 hour or by treatment with disinfectant (including but not limited to Virkon, ChemGene HLDL4, Surfanios) according to the manufacturer’s specifications.

Autoclave performance is monitored by the use of autoclave indicator tape included in every run. Autoclaves are serviced annually by an authorised service contractor, as part of this service temperature mapping analysis is carried out.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was reviewed by the GMO committee on 11/10/2017. It considered that the risk assessment contained sufficient risk management to justify classification of the project as class 2. It was therefore approved without additional changes.

Project Containment

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## Project Additional Information

### Purposes of the contained use

To use the cells for cellular and molecular applications to test or carry out research activities on our novel biological molecules.

### Recipient or parental organism

The recipient organisms are mammalian cells that can be subcategorised as either:

1. Well characterised, widely used cell lines of mammalian origin with a low risk of endogenous infection with a biological agent and that present no apparent harm to laboratory workers and which have been tested for the most serious pathogens.
2. Cell lines that harbour endogenous biological agents (including agents classified as hazard group 1 or 2) or cells that have been deliberately infected with hazard group 1 or 2 organisms.
3. Primary cells including cells isolated from blood or lymphoid cells of human origin

### Host/vector system

Vector systems include but are not limited to: plasmids such as pLXSN which encode ampicillin and neomycin resistance markers and allow for stable constitutive expression of retroviral genetic material.

### Origin & function

Inserts used by different suppliers include but are not restricted to: human papillomavirus type E6 gene, HPV-16 E6/E7 or SV40 large T-antigen.

### Evaluation of foreseeable effects

The modification causes the cells acquire immortal properties by modulating a variety of pathways including but not limited to:
1) EBV transduced cells: activation of TERT, binding TNF, upregulation of anti-apoptotic pathways, promotion of cell adhesion,
2) HPV transduced cells: E6 and E7 oncogenes targeting p53 and Rb tumour suppressor proteins respectively,
3) SV40 T antigen transduced cells: inactivation of tumour suppressor genes such as p53 and Rb.

The resulting GMOs are not expected to pose any additional risks compared to wild type, as described above, and may therefore be handled at the same containment level.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Biologically/GMO contaminated solid waste:
- Waste from class 1 activities (e.g. used agar plates), are collected in designated containers and if possible autoclaved at 121 °C for at least 20 minutes.
- If autoclaving is not possible then solid waste from class 1 activities is collected and sealed in designated bags. These bags are placed in a sealable, designated container on site and collected for proper disposal.
- Waste from class 2 activities is treated with a final concentration of 1-10 % virkon for at least 10 minutes before being drained and placed in designated containers for disposal. Virkon is drained into the public sewer via the sink.
- All solid waste incinerated by validated means through a licensed contractor.

Biologically/GMO contaminated liquid waste:
- All liquid waste (e.g. culture medium, phage supernatants, contaminated assay materials) is deactivated before disposal. This is achieved either by autoclaving at 121 °C for at least 1 hour or by treatment with disinfectant (including but not limited to Virkon, ChemGene HLDL4, Surfanios) according to the manufacturer’s specifications.

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

The risk assessment was reviewed by the GMO committee on 11/10/2017. It considered that the risk assessment contained sufficient risk management to justify classification of the project as class 2. It was therefore approved without additional changes.
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to generate Hepatitis B viral stocks which will be used to infect either cell lines or primary hepatocytes, and enable screening of Immune Mobilising Monoclonal T cell receptor against virus (ImmTAV) molecules.

**Recipient or parental organism**

Human hepatocellular carcinoma cell line HEPG2.

**Host/vector system**

Plasmid ptetHBV was created by removing the cytomegalovirus immediate-early (CMV-IE) promoter from pCMVhbv and replacing it with the tetracycline-responsive CMV-IE promoter (CMVtet) from pUHD10-3.
pCMVhbv was created by fusion of the sequence corresponding to the cDNA of pregenomic (pg) RNA of HBV, subtype ayw, to the CMV-IE promoter.

Origin & function

The Hepatitis B virus genome that has been used to stably transfect HepAD38 cells was isolated from patient serum of HBSAg sub type ayw.
Plasmid ptetHBV, which contained this construct, was co-transfected with plasmid pUHD15-1neo, which encodes the trans-acting transcriptional regulator of the tetracycline-responsive promoter and neomycin resistance. The resulting cell line was called HepAD38.

Evaluation of foreseeable effects

Insertion of the HBV genome confers harmful properties to the cell line by allowing for production of infection competent HBV. The resulting viruses are not expected to pose any additional risks over wild type HBV and may therefore be handled at the same containment level.
The tasks that will be undertaken as a part of this project are:
1. Handling of infectious cell cultures: HBV is typically transmitted via infectious fluids. The highest risk route of exposure is via percutaneous injury. The use of sharps, or working with an open wound, could allow exposure directly to the blood. Absorption/ingestion could occur following splash injuries to mouth/nose/eyes; however, infection is less likely via this route. Infection is also less likely via intact skin.
2. Centrifugation of viral material: Collapse of a tube or container during centrifugation could lead to aerosol formation due to the rapid motion inside a centrifuge. Broken plastic resulting from any such collapse may also present a sharps hazard, increasing the risk of percutaneous injury.
3. Movement of infectious material within the laboratory: Samples being moved around the laboratory pose a risk of exposure if they are dropped and their containers break. As with centrifugation, broken plastic resulting from any such collapse may also present a sharps hazard, increasing the risk of percutaneous injury.
4. Storage of infectious material: Viral stocks will be stored at -80 °C in a dedicated freezer within the Containment Level 3 (CL3) lab. Exposure to infectious virus is less likely from frozen stocks as the risk of splashing is negligible. Freezer failure could lead to thawing of the samples, which increases the risk of exposure in the event of a tube failure.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The Control of Substances Hazardous to Health (COSHH) Regulations (2002), require that CL3 laboratories are sealable in order to permit disinfection. The most common method used for such disinfection is fumigation with gaseous formaldehyde. However formaldehyde is flammable, corrosive, carcinogenic, mutagenic, and toxic and is covered by 10 hazard statements. Due to these characteristics, the Workplace Exposure Limit is 2 parts per million (2.5 mg/m3). The reclassification of formaldehyde as a carcinogen has led to its use being discouraged by the Health and Safety Executive (see Biological Agents eBulletin Special Edition, July 2015).
A common alternative to formaldehyde is hydrogen peroxide. Used either as a "wet" or "dry" fumigant, it has been commonly used in pharmaceutical environments for disinfecting clean environments, but little data exist on the efficacy of hydrogen peroxide when disinfecting areas spoiled with contaminating material. Hydrogen peroxide is also corrosive, harmful, and can cause serious eye damage. Further, as a vapour it is potentially explosive. The Workplace
Exposure Limit is 1 part per million. Although the laboratory is sealable for fumigation, the facility is derogated from room fumigation due to the following considerations:

- The inherent risks associated with fumigation using either formaldehyde or hydrogen peroxide
- Infection with HBV is less likely via the aerosol route
- No scenario exists in which routine or emergency fumigation (and the associated risks of exposure to the fumigant) is considered to be safer than appropriate spill treatment and surface disinfection.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste from the CL3 laboratory must be treated prior to final disposal. An autoclave for making waste materials safe is located in the laboratory. Robust leakproof containers will be used for storage and transport of waste for autoclaving. The autoclave is serviced every 6 months, and calibrated annually or following any maintenance work to check performance. The autoclave sterilisation cycle will be validated with biological indicators.

Disinfectants:
Sodium hypochlorite is the only disinfectant approved for use in the CL3 laboratory. Residual disinfectant is cleaned up with 70% ethanol (which is not to be considered a disinfectant). No other disinfectant should be used without seeking approval from the CL3 manager. Validation experiments of viral killing will be performed based on validation described in the literature.

Extensive literature evidence is available to support killing of HBV by ClO2:
HBV and DHBV (Duck HBV - a commonly used substitute virus), in the presence of plasma, were inactivated by 3,600 ppm NaOCl. Tsiquaye, K.N. and Barnard, J., “Chemical Disinfection of Duck Hepatitis B Virus: A Model for Inactivation of Infectivity of Hepatitis B Virus,” J. Antimicrob. Chemother. 32(2), 313–323
HBV-infected plasma samples were treated with 4,700 ppm NaOCl and a 3-4 log10 reduction was seen. Payan, C. et al., “Inactivation of Hepatitis B Virus in Plasma by Hospital In-Use Chemical Disinfectants Assessed by a Modified HepG2 Cell Culture,” J. Hosp. Infect. 47(4), 282–287 (2001).
0.1% NaOCl has been shown to deactivate HBV after 10 minutes. Memarian, M., et al., “Disinfection efficiency of irreversible hydrocolloid impressions using different concentrations of sodium hypochlorite: a pilot study.” J Contemp Dent Pract. 2007 May 1;8(4):27-34.
1% NaOCl was compared to 70% ethanol and no viral DNA was found after NaOCl treatment, but there was viral DNA found after ethanol treatment. Ito, K., et al., “Effect of ethanol on antigenicity of hepatitis B virus envelope proteins” Jpn J Infect Dis. 2002 Aug;55(4):117-21.
The use of a 1:100 and a 1:10 dilution of 5% NaOCl was tested against HBV and it was found that a 1:10 dilution (0.5% final) destroyed all viral DNA. Some viral DNA was found after treatment with the 1:100 dilution. Arami, S., et al., “Evaluation of the effect of three disinfectants on removing HBV contamination” J Dent Med. 2006 Spring;19(1):84-90

Non-contaminated general waste:
A bin or autoclave tin fitted with an autoclave bag must be used for depositing non-contaminated waste (e.g. paper towels, tissues, plastic wrapper etc.). When the bin is two-thirds full, detach the autoclave bag from the rim and place loosely inside an autoclave tin (if using a bin). Ensure that the bag is only loosely closed to admit steam freely during autoclaving. Replace the used bin with an empty one containing an autoclave bag.

Contaminated liquid waste:
Decontaminate liquid waste by adding sodium hypochlorite to a final concentration of 10,000 ppm free chlorine and leave overnight before disposal via the sink within the CL3 facility. Ensure that the solution is equilibrated before leaving it for its contact time. Label all waste with the start date and user initials to ensure all waste is appropriately decontaminated.
Contaminated solid waste:
Solid waste generated inside an MSC (such as tissue culture flasks, centrifuge tubes, microtitre plates, pipette tips, cryovials, stripettes etc.) can be disposed of either in the autoclave bag or solid container within the MSC. Once full (or when work has been completed) close the container, spray with sodium hypochlorite, and leave in the MSC for the specified contact time. Once the disinfectant contact time is over, place the waste in an autoclave tin and dispose of by autoclaving. Clean up any sodium hypochlorite residue with 70% ethanol.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee reviewed the risk assessment and deemed it sufficient for the planned scope of the work in the project. The committee identified no further risks to consider that are not covered by the risk assessment and the control measures detailed by the risk assessment are sufficient to mitigate these risks to a reasonable level. The committee agreed with the designation of the work as requiring containment level 3.

Project Containment

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Project Ref 742/18.5

Date Ackn’d 21/06/2018

Generation of recombinant Human Immunodeficiency Virus (HIV) containing specific mutations to develop and test engineered immune-mobilizing monoclonal T cell receptor against virus (ImmTAV) technology against HIV

Date Project Ceased

Class 3

CultureVolClass2 < 1 Litre

Consent Granted Yes
The aim of this project is to facilitate generation of HIV specific ImmTAV molecules effective against a wide range of potential viral escape variants. This will be achieved in this project by generating tools for characterisation of candidate ImmTAV molecules.

The construction of recombinant HIV variants (pre-existing and/or archived intra-patient viral variants) will allow us to test a wide range of ImmTAV molecules and enable us to investigate their respective selection pressures on virus.

From the data obtained we hope to be able to identify ImmTAV molecules of sufficient efficacy that can specifically target the regions of the viral genome least likely to induce escape mutation variants.

For cloning to generate plasmid expression clones:

Non-pathogenic laboratory E. coli strains such as DH5α (B or K12 strain derivatives) cells will be used as the cloning host to generate plasmid expression clones. These strains are disabled non-invasive bacteria that have a history of safe use and may be considered equivalent to ADCP hazard group 1.

For generation of infectious HIV particles and infection with infectious HIV particles a range of mammalian lymphocytic cells will be used. These include, but are not restricted to:

- CEM4 cells: CEM derived clone with high level of surface CD4 expression, originating from lymphoblasts isolated from peripheral blood of a female child with acute leukemia. These are well characterised and available from a commercial source.
- HEK293 cells: Primary human embryonic kidney cells transformed by sheared human adenovirus type 5 DNA. These are well characterised and available from commercial sources.
- C8166: Clone of C63/CRII-4 derived by fusion of primary umbilical cord blood cells with HTLV-1 producing line from adult T cell leukaemia lymphoma patient. Contain defective HTLV-1 genome. The cells should be handled at laboratory containment level 2. These are well characterised and available from commercial sources.
- Jurkat cells: Human T cell line isolated from an adolescent with T cell leukemia. These are well characterised and available from commercial sources.
- H9: Cutaneous T lymphocyte cell line derived from 53 year old male lymphoma patient. Clonal derivative of Hut78 cells. These are well characterised and available from commercial sources.
MOLT4/CCR5 T lymphoblast line derived from 19 year old male acute lymphoblastic leukemia patient. These are well characterised and available from a commercial source.

Primary cells, such as peripheral blood mononuclear cells (PBMCs) and subsets thereof such as CD4+ T cells and CD8+ T cell depleted PBMC.

**Host/vector system**

Standard expression plasmids will be used such as, but not restricted to, pUC18 and PDR8.

pUC18: E. coli plasmid containing the pMB1 replicon rep and the AmpR gene which confers ampicillin resistance. It also contains a fragment of the LacZ gene.

pDR8: E. coli plasmid containing the pMB1 replicon rep and the AmpR gene which confers ampicillin resistance. pDR8 is derived from pUC19, however the LacZ and LacI genes have been removed.

**Origin & function**

Multiple different plasmids will be used which contain genomic DNA encoding the HIV genome. These plasmids include, but are not restricted to:

- pNL4-3 (termed pRG1): Plasmid backbone = pUC18. Insert is a full length, replication- and infection-competent chimeric DNA derived from NY5 (5’) and LAV (3’) provirus strains. This is a widely used and well characterised molecular clone of B Clade HIV (Adachi et al. 1986). Sourced from NIBSC AIDS reagent repository.

- pNL4-3 Ba-L Env (termed pRG2): Plasmid backbone = pUC18. Plasmid is derived from pNL4-3. The insert is a full length replication- and infection competent molecular clone of HIV-1 NL4-3 with wild type envelope sequence derived from Ba-L HIV (B clade RS-tropic strain). Sourced from NIBSC AIDS reagent repository.

- pRG3: Plasmid backbone = pDR8. Derived from pNL4-3. The insert is a 5747bp fragment consisting of 155bp of cellular flanking DNA and 5592bp of DNA from HIV-1NY5 containing the 5’LTR, gag, pol and vif coding regions, and part of the vpr gene cloned into pDR8 (a derivative of pUC19). These were synthesised as whole plasmids by a commercial supplier.

- pRG4: Plasmid backbone = pDR8. Derived from pNL4-3. The insert contains a 4002bp fragment with the LAV vpr (partial), tat, rev, vpu, env and nef coding regions, 3’ LTR and 34bp of cellular DNA cloned into pDR8 (a derivative of pUC19). Green fluorescent protein (GFP) cloned in frame. These were synthesised as whole plasmids by a commercial supplier.

Transfection with both pRG3 and pRG4 is required to produce infectious virus.

Mutations and insertions will be introduced into these or similar HIV producing plasmids in order to generate infectious virus containing the relevant modifications.

**Evaluation of foreseeable effects**

The inserted genes within the generated plasmids allow the generation of infectious HIV particles when transfected into mammalian cells. The pRG3 and pRG4 plasmids must be transfected together to generate infectious virus and thus, by themselves, do not confer harmful properties. The transformation of the plasmids into E. coli will not convey harmful properties as viral protein expression does not occur in E. coli cells.

The mutations that will be introduced into the plasmids will largely be those that have been found in viral sequences in naturally infected populations and would not be expected to increase viral fitness in a human host. It is highly unlikely that genetic modification would produce variants with a higher viral fitness/replicating capacity in humans than variants from naturally infected populations. The mutation rate of the virus means that the individual changes that are deliberately introduced will almost certainly have already been present in nature. The resulting GMOs are not expected to pose any additional risks compared to wild type HIV and may therefore be handled at the same containment level.
The tasks that will be undertaken as a part of this project are:

1. Handling of infectious cell cultures: HIV is typically transmitted via infectious fluids. The highest risk route of exposure is via percutaneous injury. The use of sharps, or working with an open wound, could allow exposure directly to the blood. Absorption/ingestion could occur following splash injuries to mouth/nose/eyes; however, infection is less likely via this route. Infection is also less likely via intact skin.

2. Centrifugation of viral material: Collapse of a tube or container during centrifugation could lead to aerosol formation due to the rapid motion inside a centrifuge. Broken plastic resulting from any such collapse may also present a sharps hazard, increasing the risk of percutaneous injury.

3. Movement of infectious material within the laboratory: Samples being moved around the laboratory pose a risk of exposure if they are dropped and their containers break. As with centrifugation, broken plastic resulting from any such collapse may also present a sharps hazard, increasing the risk of percutaneous injury.

4. Storage of infectious material: Viral stocks will be stored at -80 °C in a dedicated freezer within the CL3 lab. Exposure to infectious virus is less likely from frozen stocks as the risk of splashing is negligible. Freezer failure could lead to thawing of the samples, which increases the risk of exposure in the event of a tube failure.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The Control of Substances Hazardous to Health (COSHH) Regulations (2002), require that CL3 laboratories are sealable in order to permit disinfection. The most common method used for such disinfection is fumigation with gaseous formaldehyde. However formaldehyde is flammable, corrosive, carcinogenic, mutagenic, and toxic and is covered by 10 hazard statements. Due to these characteristics, the Workplace Exposure Limit is 2 parts per million (2.5 mg/m3). The reclassification of formaldehyde as a carcinogen has led to its use being discouraged by the Health and Safety Executive (see Biological Agents eBulletin Special Edition, July 2015).

A common alternative to formaldehyde is hydrogen peroxide. Used either as a “wet” or “dry” fumigant, it has been commonly used in pharmaceutical environments for disinfecting clean environments, but little data exist on the efficacy of hydrogen peroxide when disinfecting areas spoiled with contaminating material. Hydrogen peroxide is also corrosive, harmful, and can cause serious eye damage. Further, as a vapour it is potentially explosive. The Workplace Exposure Limit is 1 part per million.

Although the laboratory is sealable for fumigation, the facility is derogated from room fumigation due to the following considerations:

• The inherent risks associated with fumigation using either formaldehyde or hydrogen peroxide
• Infection with HIV is less likely via the aerosol route
• No scenario exists in which routine or emergency fumigation (and the associated risks of exposure to the fumigant) is considered to be safer than appropriate spill treatment and surface disinfection.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste from the CL3 laboratory must be treated prior to final disposal. An autoclave for making waste materials safe is located in the laboratory. Robust leakproof containers will be used for storage and transport of waste for autoclaving. The autoclave is serviced every 6 months, and calibrated annually or following any maintenance work to check performance. The autoclave sterilisation cycle will be validated with biological indicators.

Disinfectants:
Sodium hypochlorite is the only disinfectant approved for use in the CL3 laboratory. Residual disinfectant is cleaned up with 70% ethanol (which is not to be considered a disinfectant). No other disinfectant should be used without seeking approval from the CL3 manager. Validation experiments of viral killing will be performed based on methods described in the literature.
Extensive literature evidence is available to support killing of HIV by ClO2:


Cell-free HIV-1 in cell culture medium containing 10% FCS was inactivated in 1 minute by 2,500 ppm NaOCl. No p24 was found in the cell culture supernatant. Druce, J.D. et al., “Susceptibility of HIV to Inactivation by Disinfectants and Ultraviolet Light,” J. Hosp. Infect. 30(3), 167–180

Cell-associated HIV-1 in cell culture medium containing 10% FCS was inactivated in 1 minute by 2,500 ppm NaOCl. Druce, J.D. et al., “Susceptibility of HIV to Inactivation by Disinfectants and Ultraviolet Light,” J. Hosp. Infect. 30(3), 167–180

Cell-free HIV was reduced by 3-4 log10 using 50 ppm NaOCl when no plasma was present, and 2,500 ppm NaOCl in the presence of 10% plasma. Equal volumes of 10,000ppm NaOCl and whole blood produced total killing within 2 minutes. Bloomfield, S. F., et al., “Evaluation of hypochlorite-releasing disinfectants against the human immunodeficiency virus (HIV),” J Hosp Infect. 1990 Apr;15(3):273-8.

Non-contaminated general waste:
A bin or autoclave tin fitted with an autoclave bag must be used for depositing non-contaminated waste (e.g. paper towels, tissues, plastic wrapper etc.). When the bin is two-thirds full, detach the autoclave bag from the rim and place loosely inside an autoclave tin (if using a bin). Ensure that the bag is only loosely closed to admit steam freely during autoclaving. Replace the used bin with an empty one containing an autoclave bag.

Contaminated liquid waste:
Decontaminate liquid waste by adding sodium hypochlorite to a final concentration of 10,000 ppm free chlorine and leave overnight before disposal via the sink within the CL3 facility. Ensure that the solution is equilibrated before leaving it for its contact time. Label all waste with the start date and user initials to ensure all waste is appropriately decontaminated.

Contaminated solid waste:
Solid waste generated inside an MSC (such as tissue culture flasks, centrifuge tubes, microtitre plates, pipette tips, cryovials, stripettes etc.) can be disposed of either in the autoclave bag or solid container within the MSC. Once full (or when work has been completed) close the container, spray with sodium hypochlorite, and leave in the MSC for the specified contact time. Once the disinfectant contact time is over, place the waste in an autoclave tin and dispose of by autoclaving. Clean up any sodium hypochlorite residue with 70% ethanol.

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]  

Please enter comments on the GM safety committee on the risk assessment
The genetic modification safety committee reviewed the risk assessment and deemed it sufficient for the planned scope of the work in the project. The committee identified no further risks to consider that are not covered by the risk assessment and the control measures detailed by the risk assessment are sufficient to mitigate these risks to a reasonable level. The committee agreed with the designation of the work as requiring containment level 3.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
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<td>L3 L4 L2 L3 L4 L4</td>
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**Project Ref** 742/21.1

- **Date Ackn’d**: 09/07/2021
- **CU2 Project Title**: Use of mycobacterium bovis BCG-GFP to assess TCR potency against infected monocytic cells

**Class Culture**

- **Class**: 2
- **Culture Volume**
  - **Class 2**: ≤ 1 Litre
  - **Class 3-4**: Non-GMM

**Consent**

- **Consent Granted**: Yes

**Historical Significant Changes**

- **Historical Date of Additional Info**: N

**Project Additional Information**

**Purposes of the contained use**

Mycobacterium bovis BCG-GFP will be used to infect monocytic cells and test whether TCRs recognise these antigen presenting cells. A strain of the mycobacterium bovis BCG with a GFP reporter will be used to measure uptake of the organism by monocytic cells and evaluate BCG infection efficiency.

**Recipient or parental organism**
Mycobacterium bovis BCG vaccine strain.

Host/vector system

Plasmid pSMT1 carrying the gfp gene of jellyfish, cloned under the control of the BCG heat shock protein-60 (hsp60) promoter on a mycobacterial shuttle vector (pOLYG) BCG that has had HspR gene, or other genes, replaced with a defective copy of the gene using a non-replicating “suicide” vector. Selection of mutants is using the hygromycin gene from Streptomyces hygroscopicus that replaces the HspR gene and allows selection of recombinants in which a double cross over event has resulted in exchange with the wild type gene (Husson et al 1990, J. Bacteriol 172: 519-24: Garbe et al Microbiology 140: 133-8).

Origin & function

M. bovis BCG is the vaccine strain that has been used extensively in humans. This BCG-GFP strain we intend on using was developed by Professor Young (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC96782/). We will be using it as received and not be modifying the strain any further. Inclusion of GFP into M. bovis-BCG has been shown not to increase their virulence or effects on how they effect cytokine production or surface molecule expression by antigen presenting cells in vitro (Luo et al 1996 Clin Diag Lab Immunol 3: 761-8).

Evaluation of foreseeable effects

BCG as the established vaccine strain for human use is avirulent and no effect on virulence is foreseen.

The production of BCG-GFP will be at other establishments as detailed by Professor Young (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC96782/). Inclusion of GFP into M. bovis-BCG has been shown not to increase their virulence or effects on how they effect cytokine production or surface molecule expression by antigen presenting cells in vitro (Luo et al 1996 Clin Diag Lab Immunol 3: 761-8).

The organisms will be handled as ACDP category 2 pathogens for all experiments thus reducing the potential of environmental exposure to negligible.

They will be handled in the tissue culture hoods and no procedures have the potential to generate aerosolised bacteria. The risk of inhalation therefore is extremely low.

A Code of Practice for working within the CL2 laboratory at IMC has been written. It details protective and containment measures, including general working arrangements, access controls, training procedures, emergency procedures, health surveillance, disinfection and waste management and decontamination processes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste (mainly plastic disposable) will be placed in burn bins and incinerated using a licensed incineration facility.

All liquid waste resulting from bacteriological procedures involving the propagation, disruption and analysis of the GMO will be treated with Virkon tablets to a final concentration of greater than 1% (weight/volume). Virkon is recommended by the manufacturer for the treatment of liquid waste. Detailed information is available at www.antechh.com. Laboratory benches and equipment will be wiped down with 1% (weight/volume) Virkon solution or where this is inappropriate a solution of 70% ethanol in water on absorbent paper and then paper will be disposed of as solid waste. Spills will be treated with Virkon as above and soaked up on absorbent paper, and treated as solid waste.
Pipettes and similar objects, as well as work surfaces will be disinfected, and surfaces wiped, with virkon disinfectant and ethanol.

The reporter gene (GFP) is well-characterised with no known associated health hazards.

M. bovis BCG is a difficult strain to maintain in the laboratory, with very specific culture methods. Mycobacteria are also sensitive to UV light. If inadvertently M. bovis BCG is introduced to the environment, it is unlikely that the organism will survive.

<table>
<thead>
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</thead>
<tbody>
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<td>L3</td>
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**Name**

CELLDEX THERAPEUTICS LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

410 CAMBRIDGE SCIENCE PARK

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 0PE

**Country**

ENGLAND

**Tel Number**

01223 496 130

**Fax Number**

01223 496 010

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>DEPARTMENT OF VETERINARY MEDICINE</td>
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- **Give brief details of the genetic modification safety committee**

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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<tr>
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</table>

- **Tick if confidential**
Liquid waste is inactivated by Precept or Lifeguard diluted and used in accordance with the manufacturer's instructions. Information indicates that these treatments result in a kill of greater than 5 log10 for bacteria and greater than 99% kill for mammalian viruses. Taking into account the disabled nature of the hosts and vectors used, it is considered that these treatments are effective in providing a high level of protection to humans and the environment. The maximum culture volume used at any time is normally < 1 litre but will not exceed 10 litres. Solid waste is autoclaved at 121°C, 1 bar for 30 mins. The autoclave is serviced and validated at least once per year. Equipment and benches are decontaminated with Precept, Lifeguard or 70% ethanol.
Replication-defective adenovirus type 5 (Ad5) based vectors will be used to deliver genes of interest to mammalian cells in tissue culture and their expression will be studied in order to determine their possible roles in immune cell regulation.

The inclusion of marker genes (such as GFP or lacZ) in the constructs will principally permit the efficiency of virus infection to be evaluated, and enable the sorting of populations of cells expressing the immune regulatory genes.

Gene sequences from both human and murine sources will be cloned into the virus vectors.

Recipient or parental organism

Recipient or parental organism

Host/vector system

Human adenovirus type 5 vectors, constructed in *Escherichia coli* K12 using the commercially available AdEasy system, will contain the gene sequences of interest cloned behind the cytomegalovirus (CMV) or Ubiquitin (UB-C) promoters. These promoters are not active in bacterial hosts. Adenoviral vector DNA will be isolated and used to transfect mammalian 293 cells (human embryonic keratinocytes which have been transformed with the adenoviral E1 gene). Virus plaques will be picked and virus released into tissue culture media by repeated freeze/thawing. Virus titres will then be amplified by re-infection of 293 cells and purified before being used to infect a number of both primary and established mammalian cell lines. Gene expression will be transient since the vector is replication-defective. The effect the gene of interest has on cell function and the expression of cellular genes will be evaluated by analysis of cellular protein and RNA.

The adenovirus vectors to be used are replication-defective by virtue of the deletion of E1 and E3 genes. The replication of such adenovirus vectors is supported only by certain specifically engineered cells lines, such as 293 cells (the propagating cell line), which have been transformed to express the E1 gene product, and thus complement the virus replication in trans. However, other cell lines do not express this E1 gene product naturally, and thus do not support the replication of the virus. This substantially reduces any risk of infection posed by this virus, and thus will limit any potential damage that could be caused to human health by this virus. Additionally, should the adenovirus vectors enter the body, a high percentage of the population have a pre-existing anti-adenovirus humoral response which would prevent the virus from infecting cells, but should cell infection occur, an immune response would be mounted against viral-encoded proteins that should eliminate the infected cells.

It is known that the viral DNA cannot integrate into the genome of human cells.

Human adenovirus type 5 does not naturally infect wild animals or plants. It is pathogenic to cotton rats and mice only when delivered at very high doses via the intravenous, intraperitoneal or intranasal route. The inclusion of either the reporter gene or the immune regulatory genes is not expected to alter the tropism or infectivity of the virus.

Origin & function

Genes encoding Notch receptors, ligands and other related genes of interest will be derived from human and murine genomic DNA or CDNA sources. These genes are
widely expressed in mammals and are involved in cell signaling pathways, particularly in embryonic and adult immune tissues, such as thymus and cellular components of bone marrow. The exact roles of these genes in immunological tolerance remain to be elucidated. We therefore intend to express these genes in mammalian tissue culture systems with the aim of investigating their function within immune cell regulation, with a specific interest in understanding the induction of the state of tolerance.

We initially intend also use the marker gene encoding green-fluorescent protein (GFP), originally derived from the anthazoan Renilla reniformis, and sub-cloned from a commercially available source (pIRES-hr-GFP-1a, Stratagene). This reporter gene will be co-expressed with the immunological regulatory genes of interest during transfection, and will be used as an indicator of transfection efficiency. Additionally, under some circumstances, the GFP construct may be used as a reporter gene to quantify the effect of the test gene product on certain target sequences. Other marker genes, such as beta-galactosidase (lacZ) or luciferase, may also be used.

**Evaluation of foreseeable effects**

Cells expressing full-length Notch ligands could potentially deliver a tolerogenic signal to the immune system, particularly if MHC molecules are being expressed by the same cells and are thus capable of antigen presentation. Transplantation models have shown that the development of tolerance to foreign cells requires the processing of target cells and indirect antigen presentation, as opposed to direct presentation of antigen from transplanted cells. Therefore, since cells used for in vitro infection experiments will not be histocompatible with the operator, it is very unlikely that the tolerant phenotype would develop without the processing of cellular antigens and a high dose of soluble Notch ligand. This risk is further reduced by the use of replication-deficient adenovirus vectors, which are considered to be unable to deliver a pharmacologically relevant dose, thereby further minimising the risk to human health.

Notch and its ligands are known to be important in embryonic development and thus consideration must be given with relation to the potential effects on the developing foetus. A limited amount of transgenic data has shown that the phenotypes of Notch and Notch-ligand-deficient mice are characterised by abnormal embryo development and death in utero. However, despite this, the moderate level of expression generated by the adenovirus vector systems described here, are considered to be unable to deliver a pharmacologically active dose, and no significant hazard is anticipated.

There is a minimal risk that the replication-deficiency could be complemented in vivo, should an accidental infection of the test Ad5 virus particles coincide in the same cell with a natural infection of wild-type adenovirus. As no wild-type adenovirus will be used or cultured in the laboratories, the changes of this occurring are considered to be very low. However, giving consideration to the fact that the exact function of some of the genes of interest is not fully understood, steps will be taken to minimise the exposure of operators to aerosols of Ad5 virus particles. This will entail concentrated suspensions of virus particles being contained within sealed tubes, which will be opened only inside a Class II Biological Safety cabinet, by using centrifuge rotors with sealed lids to contain any possible aerosols, and by limiting the personnel involved in the project only to those with relevant experience. When using diluted virus stocks, the risk of such coincident infect diminishes, and thus it is considered that work with diluted virus, such as analysis by ELISA, FACS or western blotting, could be carried out under Level 1 containment.

Marker/reporter genes will be moderately expressed and are not expected to be a hazard to health. If accidental user infection occurs, the virus would be unable to replicate unless the user is co infected with wild type virus. As wild-type virus is not used in the building the likelihood of co-infection is minimal, and would only occur from the wild-type virus present naturally in the atmosphere. The tropism of the virus will not be altered by inclusion of the reporter gene and it is not anticipated to affect the ability of the virus to infect cells. The reporter gene, is therefore not anticipated to pose any significant risk to human health.

Consequently, in terms of the general risk to human health, the use of replication-defective adenovirus vectors is considered to substantially reduce any significant risk, but that this will be further reduced by the containment measures proposed.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Prior to disposal, all liquid waste, including mammalian cell culture media, will be decontaminated using a proprietary liquid anti-viral agent, such as Virkon or Trigene, used in accordance with the manufacturers instructions. Such products have been proven by independent studies to be highly virucidal against adenovirus, and are widely used in laboratories and health-care settings.

Small liquid spillages will be treated with a liquid anti-viral disinfect; larger spillages will be treated by applying solid and anti-viral disinfectant, in accordance with the manufacturers instructions, before cleaning.

Solid waste, including contaminated plastics, will be double-bagged in strong autoclave bags, placed in a leak-proof container with a lid and autoclaved at 121°C for 30 min. The autoclaved waste will then be sealed into a strong plastic bag and disposed of by incineration.

Re-usable items, such as glassware or centrifuge tubes and certain small equipment, will be swabbed down or soaked in a proprietary liquid anti-viral agent, as appropriate.

Should the use of any sharps by necessary, these will be placed directly in a sharps bin. Full bins will be sealed into a strong plastic bag prior to disposal by incineration.

Inner surfaces of safety cabinet will be cleaned with a proprietary liquid anti-viral agent after each use, and before commencing any servicing to the interior of the unit, the cabinet will be disinfected by formaldehyde vapour treatment.

No virus particles or cells are expected to remain viable after these treatments.

The circumstances under which replication-competent adenovirus might become infectious by fortuitous co-infection with wild-type adenovirus were discussed. Although considered to be highly unlikely, it may be possible that a pharmacologically-active dose of Notch or Notch-ligand could be delivered should such an infection occur. Since such co-infection would be most likely to result from inhalation of aerosols of concentrated virus suspensions, it is recommended that safety cabinets be used when handling open containers of concentrated virus samples, and that additional measures be taken to minimise aerosol generation during centrifugation. However, since the likelihood of co-infection will decrease with virus dilution, protocols such as ELISAs, FACS, Westerns etc using diluted or denatured virus may take place on the open bench, provided that appropriate disinfection measures are taken.

Where Level 2 containment is appropriate, the existing labs were confirmed as suitable for this level of containment, with the following actions: Access to be restricted to lab personnel, and a statement to be made to all staff making them aware that Level 2 work may be carried out in the laboratories; Existing disease control measures were considered adequate, as long as windows are kept shut - heat extraction for the FACS machine to be fitted; Written specific disinfection procedures to be drawn up and displayed in the lab.

ACGM training records should be established for all lab personnel, and that for new starters this should be included in the Starter pack. General Level 2 training will be given at a lab meeting. Individual personnel will be trained regarding the individual Risk Assessments are required when starting work on the individual projects.
<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<tbody>
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| **Comments** | |

| **Date at Which Additional Info Submitted** | 02/03/2022 |
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)

Tick if confidential
**The Role of Microbial Autoinducer in Periodontal Diseases**

**Project Ref:** 744/00.1

**Date Ackn’d:** 12/07/2000

**Date Project Ceased:**

**CU2 Project Title:** THE ROLE OF MICROBIAL AUTOINDUCER IN PERIODONTAL DISEASES

**Class:** Class 2

**Culture Vol Class 2:**

**Culture Volume Class 3-4:**

**Non-GMM Consent Granted:** not applicable

**Consent Granted:**

**Project notified under transitional arrangements:** Y

**Withdrawn:** N

**Tick if notifying a connected programme of work:** N

**Historical Significant Changes:**

**Historical Date of Additional Info:**

---

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Other(s)**
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
## Project Containment

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**Comments**

**Date at Which Additional Info Submitted**

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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee

- **Laboratory**, **Animal Unit**, **Growth Room**, **Glass House**, **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

- **Other (please specify)**

- Tick if confidential

- **Bacteriology**, **Parasitology**, **Transgenic Birds**, **Microbiology Research**
- **Virology**, **Transgenic Animals**, **Transgenic Fish**, **Gene Therapy**

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 745/00.1

Date Ackn'd 12/07/2000

Date Project Ceased 17/11/2008

CU2 Project Title GENERATION OF RECOMBINANT ADENOVIRUS FOR ESTROGEN RECEPTOR ALPHA & BETA EXPRESSION IN MAMMALIAN CELLS

Class 2

Consent Granted not applicable

Project notified under transitional arrangements Y

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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| Comments               |                 |

| Date at Which Additional Info Submitted | 02/03/2022      |
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

Other (please specify)  

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 746/00.1

Date Ackn'd 19/12/2000

CU2 Project Title

AGROBACTERIUM TUMEFACIENS MEDIATED TRANSFORMATION OF BRASSICA SPECIES WITH THE GENES ENCODING FOR ENZYMES IN THE PATHWAY OF HISTIDINE BIOSYNTHESIS IN PLANTS. GROWING & TESTING

Class 2

CultureVolClass2

Consent Granted

ClassVolumeClass3-4

not applicable

Project notified under transitional arrangements Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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**Name**

UNIVERSITY OF CAMBRIDGE

**Name 2**

**Department**

DEPARTMENT OF CHEMICAL ENGINEERING

**Building**

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 3RA

**Country**

ENGLAND

**Tel Number**

01223 334777

**Fax Number**

01223 334796

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- **Give brief details of the genetic modification safety committee**

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 747/01.1

**Date Ackn’ed** 19/02/2001

**CU2 Project Title** IMMUNO-ADSORPTIVE RECOVERY AND PURIFICATION OF RETROVIRAL AGENTS FOR GENE THERAPY

**Class** Class 2

**CultureVol**

**CultureVolume**

**Class 3-4**

**Non-GMM**

**Consent Granted** not applicable

**Project notified under transitional arrangements** Y

**Withdrawn** N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<td>L2 L3 L4 L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
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**Project Ref** 747/06.1

**Date Ackn'd** 13/04/2006

**CU2 Project Title**

Developing and characterising chromatography based purification technology for use with retroviral gene therapy agents. The work will include cultivation of the gene therapy vector, purification and subsequent infectivity testing in mammalian cell cultures.

**Date Project Ceased**

**Class** Class 2

**CultureVol** < 1 Litre

**Consent Granted** Not Applicable

**Project notified under transitional arrangements**

**Tick if notifying a connected programme of work** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

**Tick to confirm that you have attached a risk assessment to this form**
<table>
<thead>
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<th>Purposes of the contained use</th>
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</thead>
<tbody>
<tr>
<td>To develop new purification strategies for retroviral gene therapy vectors.</td>
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</table>

**Recipient or parental organism**

- BL15 cell line: expressing non-viral protein LNGFR
- Bio-lentivir a second generation lentiviral packaging cell line
- K562 and TE671 cell lines (not GMOs)

**Host/vector system**

- Packaging plasmid (pCMV-deltaR8.91) which encodes structural proteins (not env gene)
- Envelope plasmid (pMD.G) which encodes envelope gene VSVg
- Replication deficient lentiviral expression vector with a GFP gene (pHR'SINctwSVGFP/LV.gfp)

**Origin & function**

**Vectors**

- Packaging plasmid (pCMV-deltaR8.91) encodes the genes for HIV structural proteins involved in vector packaging (except the env gene which encodes for the infection of T cells).
- Envelope plasmid (pMD.G) which encodes envelope gene VSVg. This enables the replication defective lentiviral vector to infect a range of cell lines beyond the capabilities of the HIV parent which is limited to T cells.
- Replication deficient lentiviral expression vector with a GFP gene (pHR'SINctwSVGFP/LV.gfp) does not contain the genes for HIV structural proteins and is unable to make virus particles without the packaging cell line.

**Recipients**

- K562 and TE671 cell lines are for infectivity testing but are not able to synthesise virus particles when infected with the replication deficient lentiviral expression vector.

**Packaging cell lines**

- BL15 cell line: expressing non-viral protein LNGFR encodes the non-viral protein LNGFR to assist co-pckaging lentiviral vectors. For use in synthesising the replication deficient lentiviral expression vector.
- Bio-lentivir a second generation lentiviral packaging cell line which contains no auxiliary genes. For use in synthesising the replication deficient lentiviral expression vector.

**Evaluation of foreseeable effects**

- Infection of a laboratory worker with the replication deficient lentiviral expression vector may result in incorporation of the green fluorescent protein (GFP) into the cells of the worker. This may cause localised fluorescence. If the insertion is into a part of the chromosome which encodes genes that control cancer cell proliferation it could feasibly lead to the formation of a tumor though the probability of this occurring is very low.

- There is a very low probability that the replication deficient lentiviral expression vector could incorporate the genetic material present in the packaging and envelope plasmids creating a replication competent lentivirus.

- Both of these low probability foreseen events require containment by the use of containment level 2 procedures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials are steam sterilised in a calibrated autoclave for 20 minutes at 121 degrees C. Solid waste is then incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

It was noted that the lentivirus carries a slightly higher risk than the retroviruses used previously. Philip Oliver advised the committee that this is a standard vector not thought to present any unusual problems, but that work should always need to be carried out in a microbiological safety cabinet as specified in the assessment documentation. The project was accepted by the committee as class 2.

**Project Containment**

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<tr>
<td>L2 L3 L4</td>
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**Project Ref** 747/08.1

**Date Ackn’d** 31/01/2008

**CU2 Project Title** Enhancing and scaling-up chromatography based purification technology and showing its utility for the manufacture of clinical lentivirus for gene therapy. The work will include cultivation of gene therapy vector, purification and subsequent infectivity testing in mammalian cell cultures.

**Class** Class 2

**CultureVolClass2** < 1 Litre

**Non-GMM** Consent Granted: Not Applicable

**Project notified under transitional arrangements** N
To develop new purification strategies for lentiviral gene therapy vectors by removing the biotin requirement as a tag, and instead using a precursor in E.coli 7,8-diaminopelargonate (7-DAPA) that does not bind streptavidin, but can be synthesised into a low-affinity desthiobiotin.

Recipient or parental organism

1. BL15 cell line: modified 293T (human embryonic kidney cell line)-derived cell line which expresses non-viral protein LNGFR-BAP.
3. Human chronic myeloid leukaemia cell line (K562) and human rhabdomyosarcoma cell line (TE671) (not GMOs).

Host/vector system

Lentiviral vectors based on replication disabled HIV.
1. Packaging plasmid (pCMV-deltaR8.91) which encodes structural proteins (not env gene).
2. Envelope plasmid (pMD.G) which encodes envelope gene VSVg.
3. Replication defective lentiviral expression vector with a GFP gene (pHR'SINctwSVGFP/LV.gfp.)

Origin & function

Vectors:
Packaging plasmid (pCMV-deltaR8.91) provides the elements required for vector packaging such as structural proteins, HIV genes (except the gene env which encodes for infection of T cells), and the enzymes that generate vector particles. Envelope plasmid (pMD.G) encodes the envelope gene VSVg. This increases the range of cells the replication-defective lentiviral vector can infect, beyond the capabilities of the HIV parent which is limited to T cells. Replication defective lentiviral expression vector with a GFP gene (pHR'SINctwSVGFP/LV.gfp) does not contain the genes for HIV structural proteins and is unable to make virus particles without the packaging cell line.

Recipient:
K562 and TE671 cell lines are for infectivity testing but are not able to synthesise virus particles when infected with the replication deficient lentiviral expression vector.

Packaging cell lines:
BL15 cell line: expressing non-viral protein LNGFR-BAP and metabolically producing ligand-tagged replication deficient virus that can be efficiently captured and eluted from an affinity absorbent in a concentrated, purified form. BL15 based lentiviral packaging cell line: constructed by insertion of a gene to express bacterial bioD into BL15. The desthiobiotin synthase bioD is naturally present in E.coli and simply allows a more practical route to the incorporation of desthiobiotin into the virus envelope than that permitted by BL15.
Infection of a laboratory worker with the replication deficient lentiviral expression vector may result in incorporation of the green fluorescent protein (GFP) into the cells of the worker. This may cause localised fluorescence. If the insertion is into a part of the chromosome which encodes genes that control cancer cell proliferation it could feasibly lead to the formation of a tumour though the probability of this occurring is very low.

There is a very low probability that the replication deficient lentiviral expression vector could incorporate the genetic material present in the packaging and envelope plasmids creating a replication competent lentivirus.

Both of these low probability forseen events require containment by the use of containment level 2 procedures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All materials are steam sterilised in a calibrated autoclave for 20 minutes at 121°C. Solid waste is then incinerated.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [N]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

This project involves a new cell line currently being constructed at Kings College Hospital. The application papers for the project were discussed at a meeting of the Biological Safety Committee for the Department of Chemical Engineering. It was agreed that, although the disables virus has a negligibly small chance of re-acquiring replication competence by recombination with endogenous lentiviral elements, it might nevertheless be a sensible precaution to additionally ban the use of sharps in the CL2 laboratory. This measure will ensure that needle-stick injuries to any workers who may harbour lentiviral elements in their genome will be completely avoided. The Committee agreed to accept the project at GM Class 2, with the provision that sharps should not be used in the CL2 lab.

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02/03/2022

Page 10972 of 15326
Formulation of live bacterial vaccines for storage and delivery, using human live bacterial vaccine strains that have been previously tested in humans and shown to be safe, such as Salmonella Typhi CVD908-htrA and Shigella dysentariae SC599.

The attenuated bacterial vaccines to be used (e.g. Typhoid fever and Dysentery vaccines) are derived from parental organisms that were human pathogens. However, these parental organisms have already been disabled and attenuated by stable removal of two or more essential genes. Because the essential genes have been stably removed, there is no possibility of workers being exposed to the fully pathogenic parental organisms. Note that because stably attenuated non-pathogenic strains are to be used, Schedule 5 of the Anti-terrorism, Crime and Security Act 2001 does not apply.

No further genetic modification will be conducted in this work- only existing stably attenuated vaccine strains will be used. In cases where non-pathogenic experimental antigens are included, they will be contained within conventional plasmid systems such as the pUC bacterial plasmid vector.

Recipient or parental organism

The attenuated bacterial vaccines to be used (e.g. Typhoid fever and Dysentery vaccines) are derived from parental organisms that were human pathogens. However, these parental organisms have already been disabled and attenuated by stable removal of two or more essential genes. Because the essential genes have been stably removed, there is no possibility of workers being exposed to the fully pathogenic parental organisms. Note that because stably attenuated non-pathogenic strains are to be used, Schedule 5 of the Anti-terrorism, Crime and Security Act 2001 does not apply.

Host/vector system

No further genetic modification will be conducted in this work- only existing stably attenuated vaccine strains will be used. In cases where non-pathogenic experimental antigens are included, they will be contained within conventional plasmid systems such as the pUC bacterial plasmid vector.
No further genetic modification will be conducted in this work—only existing vaccine strains will be used. The host organisms will have genes deleted permanently to render them attenuated. Polypeptides may be included within some vaccine strains as experimental antigens, e.g. non-pathogenic protective antigens from pathogens. Only antigens already tested independently in humans and shown to be safe will be used.

**Evaluation of foreseeable effects**

The vaccine strains used will have been permanently genetically modified and attenuated by stable mutation of disease-causing genes. Subsequently, the strains have been tested in clinical trials in human volunteers and shown to be safe for oral infection as vaccines. Since there is no risk of reversion of the mutations to a pathogenic strain it is not possible for workers to be exposed to a disease causing strain. Vaccine strain samples will be obtained from reliable sources where the strain is stored in strictly defined and controlled stocks, and kept separately from the original pathogenic strain, to eliminate any possibility of obtaining a pathogenic sample.

One potential hazard would be accidental infection of workers; bacterial vaccine strains are infectious by the oral route. This is unlikely, since good microbiological practice and observation of containment level 2 procedures will prevent the exposure of workers to the live organism in any form. No work with sharps is permitted in the facility. Furthermore, the consequences of accidental infection are known to be low, since the strains have been tested previously in human volunteers and deliberate infection by oral administration of live vaccine to volunteers has been shown independently not to result in significant disease. Furthermore, should any unforeseen symptoms develop following accidental infection, bacterial vaccine infection can be treated with antibiotics.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste materials will be steam sterilised within the facility in a calibrated waste autoclave for at least 20 minutes at 121 degrees centigrade. This will achieve effectively 100% kill. Solid waste is then incinerated. Liquid waste will additionally be inactivated using the disinfectant Virkon-S in accordance with the approved written protocol within the facility.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All work approved subject to the following condition:

All work with dried vaccine samples must be conducted within the dedicated vaccine suite of the containment level 2 facility in the CUBE laboratory.

**Project Containment**
Transduction of mammalian neuronal cell line cultures (SH-SY5Y) and rat primary neuronal cultures (hippocampal, dopaminergic neurons by 3rd generation lentiviral vectors

Neuronal cells are difficult to transfect successfully, the lentiviral delivery system will increase transfection efficiency. In this work a 3rd generation lentiviral system will be used to deliver nucleic acids into cells for the expression of fluorescent probes for measuring intracellular molecules (ATP, H2O2, Ca2+ etc.) or processes (synaptic vesicle rupture/fusion), as well as expression of proteins involved in Alzheimers’ and Parkinsons’ diseases which include, but are not limited to, α-synuclein, tau, amyloid-β and their mutants. The transfected cells will be imaged for analysis of molecular pathways of disease.

E. coli Stbl3 (NEB) - for cloning genes of interest into transfer plasmids and plasmid purification.
Human embryonic kidney cells HEK-293T - cells to produce virus
Mammalian neuronal cell line SH-SY5Y - cells to transfect for experimental analysis
Primary neuronal cultures of hippocampal and dopaminergic cells from wildtype Sprague-Dawley rats - cells to transfect for experimental analysis
3rd generation lentivirus plasmids: Contain three of the nine essential genes for the replication of HIV-1 viruses in vivo split across three plasmids, therefore making the 3rd generation of lentivirus replication defective and self inactivating (SIN).


pMDLg/pRRE - contains gag and pol genes as well as the RRE recognised by the Rev protein. The gag genes encode proteins to form the matrix, capsid and nucleocapsid of the virus. The pol genes encode reverse transcriptase and integrase proteins.

pMD.2 - contains the genes encoding proteins to form the glycoprotein envelope of the virus.

Transfer plasmid based on pLJM1-EGFP containing the gene of interest.

All purchased from Addgene.

Genes of interest cloned into the transfer plasmid include:

- Fluorescent proteins (FP), including probes to measure intracellular molecules such as ATP, H2O2, Ca2+ or changes in cellular environments, such as pH.
- Genes involved in Alzheimers' and Parkinsons' including, but not limited to, α-synuclein, tau, amyloid-β and their mutants which are involved in disease.
- Optogenetic genes, such as channelrhodopsin, to allow light controlled activation of cellular processes in neurons.

No genes will be used that are oncogenic or can cause advantageous modifications to the transfected neuronal cells.

Evaluation of foreseeable effects

Risks include the potential to generate replication competent lentiviruses (RCL), however the genes required to make the viruses are split into four plasmids, greatly reducing the possibility of RCL occurring. To date no RCL have been reported for 3rd generation lentivirus vectors (Cornetta, et al., Mol Ther. 2011). To test for replication incompetent lentivirus, supernatant from the transduced cells will be taken and incubated with other non-virus infected cell lines to determine whether the lentiviruses are replication incompetent. The non-virus cell lines should not be transduced with the supernatant from the transduced cells.

No oncogenic genes will be inserted or used in the production of the lentiviruses.

The viruses formed using the 3rd generation lentivirus vector system are capable of infecting human cells, however with proper handling and correct PPE infection should not occur. The gene of interest delivered by the lentivirus would not have an advantageous effect on any infected cell. The viruses are not capable of replication therefore can not spread from person to person. No patient derived cells will be used, excluding the risk of vector recombination in cells of HIV-positive, virus-negative patients.

With correct disposal the lentiviruses pose no risk to the environment.

Evaluation of foreseeable effects

Origin & function

Contents include the potential to generate replication competent lentiviruses (RCL), however the genes required to make the viruses are split into four plasmids, greatly reducing the possibility of RCL occurring. To date no RCL have been reported for 3rd generation lentivirus vectors (Cornetta, et al., Mol Ther. 2011). To test for replication incompetent lentivirus, supernatant from the transduced cells will be taken and incubated with other non-virus infected cell lines to determine whether the lentiviruses are replication incompetent. The non-virus cell lines should not be transduced with the supernatant from the transduced cells.

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With correct disposal the lentiviruses pose no risk to the environment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste and solid waste will be decontaminated with 1% Virkon for at least 12 hours and liquid waste disposed of down the drain. 1% Virkon is commonly used as an effective disinfectant to inactivate viruses and bacteria (Hernandez, et al., J. Hosp. Inf. 2000). Solid waste will subsequently be autoclaved (126 degrees celsius, 30 minutes, >2 bars pressure) and disposed of. 70% ethanol will be used to clean metallic surfaces and and imaging equipment in contact with lentiviral contaminated supernatant. Both 1% Virkon and 70% ethanol have a 100% kill expectancy rate.
Project borderline GM Class 1/2, safer to be designated GM Class 2.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that it is attached to this form

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Tick if you are claiming exemption from disclosure for section of the risk assessment

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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
**GM Centre Number: 748**

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**Name**

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**Name 2**

**Department**

**Campus Estate or Research Centre**

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**E-mail**

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**Comments**

Changed company name on 11/7/2005 from Astex Technology Limited to Astex Therapeutics Limited.

**Date at Which Additional Info Submitted**

| Date at Which Additional Info Submitted | 02/03/2022 |
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</table>

### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee

#### Laboratory

- Animal Unit

- Growth Room

- Glass House

- Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial
- Other (please specify)

#### Other (please specify)

- Tick if confidential

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste Management: All waste is class 1. Media and plates containing GMM are inactivated by autoclaving in open bags at 123°C, 1 hour. This process has been validated by monitoring the penetration of steam using ‘safety strips’ and by taking autoclaved cultures and streaking onto non-selective plates to look for any surviving GMM. The kill rate for this process is 100%. Autoclaved waste is removed, in sealed bags, by S.Grundon (Waste) Ltd (Lakeside Road, Colnbrook, Berks SL3 0EG) and incinerated. Waste media, from which GMMs have been removed, is treated either by autoclaving as above, or by treatment with 2% w/v Virkon followed by overnight incubation, before disposal down the sink.

Culture volumes: Cultures are grown on a litre scale, with a maximum capacity of 30 litres. Release into laboratory areas is considered highly unlikely as the incubator is equipped with a drip tray to effectively contain spillages internally. Centrifugation is carried out in sealed bottles and again any spillages are effectively contained within the closed system. The most likely cause of release would be the dropping of flasks by personnel so making the maximum release likely to be one litre culture. This volume of culture would be decontaminated using 2% w/v Virkon followed by autoclaving all contaminated paperware. Personnel are required to wear a lab coat and gloves at all times which provides adequate protection against class 1 GMM. In addition a class 2 cabinet has been provided for additional safety. Release into the environment is considered to be highly unlikely when considering the procedure outlined above. In addition non mobilisable vectors and disabled host cells are used with expressed products not conferring a pathogenic phenotype, so accidental release would be unlikely to pose any environmental risk or risk to human health.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 748/05.1

Date Ackn’d 22/06/2005
CU2 Project Title The aim of the project is to engineer a fibroblast cell line that expresses FGFR3 and
Class 2
CultureVolClass2 < 1 Litre
Class 3-4

Class
CultureVolClass3-4

< 1 Litre

2022/06/22  10980 of 15326
so can be used to characterise FGF-induced effects and the modulation of these by potential FGFR inhibitors.

**Project Additional Information**

**Purposes of the contained use**
The constructs are oncogenes under control of mammalian promoters and will be stably expressed in NIH 3T3 lines. The cell line will be used to assess compound activity and mechanism of action.

**Recipient or parental organism**
Develop FGFR3 dependency, and analyse survival in the presence of FGFR3 inhibitors.
Activated fibroblast growth factor receptor 3 is an oncogene that contributes to tumour progression in multiple myeloma. Blood. 1 February 2001 729-736.

**Host/vector system**
PCI Neo is the mammalian expression vector.
The cells to be used for construct generation are XL1-Blue.
The NIH 3T3 cells are to be used for stable expression.

**Origin & function**
IMAGE clone of human origin provides the genetic material.
The function is to activate the ligand mediated and ligand independent activation of the MAP kinase pathway.

**Evaluation of foreseeable effects**
FGFR3 should lead to MAP Kinase pathway activation leading to increased proliferation.
DNA is a carcinogen under COSHH Regulations, therefore the DNA will be treated as a carcinogen. All work will be carried out by competent staff to class 2 regulations.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
DA is a carcinogen under COSHH Regulations, therefore the DNA will be treated as a carcinogen. All work will be carried out by competent staff to class 2 regulations.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be inactivated by chemical means, with 2% virkon. Validation of method will be continually assessed.
All solid waste will be autoclaved. Following sterilisation all waste will be disposed of in a sealed incineration bin to S. Grundon (Waste) Ltd.

<table>
<thead>
<tr>
<th>Project Containment</th>
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</thead>
<tbody>
<tr>
<td>Laboratory Activities</td>
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**Project Additional Information**

### Purposes of the contained use

The purpose of generating cell lines which down-regulate the expression of genes linked to oncogenesis is for validating these gene products as potential targets for cancer therapeutics. Stable cell lines which express may also be used in screening assays to test the selectivity of small molecular weight compounds for a specific target.

### Recipient or parental organism

The lentiviral packaging cell line (293FT) distributed by Invitrogen will be used for the initial generation of Lentiviral pseudovirus particles. These particles will then be used to infect a variety of target relevant transformed mammalian cell lines e.g., A549, PANC1, BAF/3, Hek293, HCT-116, etc.

### Host/vector system

Generation of pseudovirus particles for the purpose of expressing gene fragments or shRNAs will be carried out using the pLenti (Invitrogen) vector system. Alternatively recombinant pseudovirus particles which code for specific gene products or shRNAs may be acquired commercially from Sigma-Aldrich, Invitrogen or Santa Cruz Biotechnology. Commercially available Lentiviral pseudovirus particles are derived from 3rd generation lentiviral vectors which cannot replicate within the target cells because the viral structural genes are absent and the LTRs are designed to be self-inactivating upon transduction. The expression of flag tagged gene products for reliable detection in mammalian cells as well as for functional analyses will be performed using the pLenti vector.

### Origin & function

The genetic material involved will be obtained through commercially available shRNA expressing clones from Sigma-Aldrich, Invitrogen or Santa Cruz Biotechnology. These clones should act to specifically down-regulate the gene transcripts to which they were designed. Alternatively gene fragments will be amplified from mammalian cDNA library I.M.A.G.E. clones, including clones for regulators of cellular proliferation and cell death. These gene fragments will be cloned and expressed using the pLenti vector.

### Evaluation of foreseeable effects

All mammalian cells transduced with the described lentiviral particles would not be immunologically compatible with a human host and as such there is no perceived threat in those cells colonizing a human host. Despite the inclusion of the safety features discussed above for the lentivirus particles, the lentivirus produced with these systems can still pose some biohazardous risk since it can transduce primary human cells. For this reason, lentiviral stocks generated will be used at Biosafety Level 2 (BL-2). Furthermore, we do not intend this risk assessment to cover the use of lentiviruses that express shRNA targeting human genes involved in negatively regulating cell division (e.g., tumour suppressor genes) Particular caution will be exercised with expression of gene fragments which are known to function in the regulation of cell proliferation or cell survival (e.g., cellular kinases activated through point mutations that stimulate pro-growth or pro-survival signal transduction pathways) using lentiviral vectors. While there are no known cases of cancer deriving from the usage of 3rd generation Lentiviral vectors, these agents will be treated as potentially oncogenic with regards to all handling and containment procedures.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures are grown in appropriately stoppered flasks to minimize the risk of aerosols and are contained within a closed incubator. In the unlikely event that a spill should occur, or a flask break, it would be effectively controlled within the incubator. Local safety rules are in place for the safe handling and disposal of GMMs. When growing GMMs in culture eye protection is worn, lab coats and gloves are worn and for the disposal of GMMs and culture media either autoclaving at 123°C for 1hr or swabbing/soaking with Virkon (1%w/v) is carried out. No sharps or glassware will be used when working with these agents. If centrifugation of manipulated cell lines is required then sealed buckets will be used to avoid aerosols and allowing for appropriate decontamination. The decontamination methods will be validated by taking swabs of the treated material, followed by transfer to cells in culture. The ability to PCR amplify a lentiviral vector specific gene fragment will then be determined relative to a positive control over 40 PCR cycles.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

An enquiry was made concerning the negative pressure capabilities of the tissue culture suites. The capability exists for the rooms, but it was deemed unnecessary to bring this online for a class II GMO activity, since this is normally only a requirement for class III GMO activities, and there was little opportunity for the lentiviral particles to become aerosolized.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 748/15.1

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**Project Additional Information**

**Purposes of the contained use**
Generation of isogenic lines for mechanism of action studies

**Recipient or parental organism**
Mammalian cell lines e.g., A375, HCT116, SW48, etc.

**Host/vector system**
Mammalian cell lines e.g., A375, HCT116, SW48, etc./ geneart crispr vector

**Origin & function**
Geneart Crispr vectors are commercially available from Invitrogen. The isogenic cell lines derived from the use of this system will be used as screening tools to interrogate pathway dependence for cell survival and signalling

**Evaluation of foreseeable effects**
The system as described by Invitrogen allows for the inactivation or mutation of a specifically targeted gene.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
All handling of modified Crispr Vector DNA will occur in Class II laminar flow hoods with appropriate PPE protection. The ability for human cells to take up the modified DNA vectors will be negligible in the absence of specific transfection reagents which will only be mixed with the vector. All transfected cell cultures are grown in appropriately stoppered flasks to minimize the risk of aerosols and are contained within a closed incubator. In the unlikely event that a spill should occur, or a flask break, it would be effectively controlled within the incubator. Local safety rules are in place for the safe handling and disposal of GMMs. When growing GMMs in culture eye protection is worn, lab coats and gloves are worn.
worn and for the disposal of GMMs and culture media either autoclaving at 123^\circ C for 1hr or swabbing/soaking with Virkon (1%w/v) is carried out. No sharps or glassware will be used when working with these agents.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In the unlikely event that a spill should occur, or a flask break, it would be effectively controlled within the incubator. Local safety rules are in place for the safe handling and disposal of GMMs. When growing GMMs in culture the disposal of GMMs and culture media will be accomplished either by autoclaving at 123^\circ C for 1hr or swabbing/soaking with Virkon (1%w/v). Either method should give a 100% kill rate.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMO committee were in agreement that the work entailed here using commercially available vectors posed no abnormal risks provided Class II GMO procedures were followed including posting signs that this work was ongoing.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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02/03/2022
**GM Centre Number: 749**

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**Name**

SENSE PROTEOMIC LIMITED

**Name 2**

DAILY LABORATORIES

**Department**

BABRAHAM BIOSCIENCE INCUBATOR

**Campus Estate or Research Centre**

BABRAHAM HALL

**Building**

BABRAHAM

**Road Name**

BABRAHAM HALL

**District**

BABRAHAM

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 4AT

**Country**

ENGLAND

**Tel Number**

01223 496702

**Fax Number**

01223 496703

**E-mail**

none

**HSE Division**

EAST AND SOUTH EAST

**Comments**

FORMERLY SENSE THERAPEUTIC LIMITED

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Bacteriology  Parasitology  Transgenic Birds  Microbiology Research
Virolgy  Transgenic Animals  Transgenic Fish  Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<th>Mycology</th>
<th>Transgenic Invertebrates</th>
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</tbody>
</table>

Solid bacterial waste is autoclaved at 120°C 1lb/in for 40 min. The process is checked using autoclave tape.

Liquid bacterial waste is heated with hypochloride under standard, established conditions.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
| Data Premises Notified (Originally) | 21/09/2000 |
| Transferred from 1992 Regs? | Y |
| Transitional Premises Class | 1 |
| Data Premises Closed | |
| Transitional Premises Emergency Plan Required? | N |
| Non-GMMs | Y |
| Withdrawn | N |

**Name**

CATELENT CTS (EDINBURGH) LIMITED

**Campus Estate or Research Centre**

**Building**

UNIT 107

**District**

DEESIDE INDUSTRIAL PARK

**Town**

DEESIDE

**County**

FLINTSHIRE

**Postcode**

CH5 2UA

**Country**

WALES

**Tel Number**

01244 845700

**Fax Number**

01244 845701

**E-mail**

none

**HSE Division**

WALES AND SOUTH WEST

**Comments**

Change of name and ownership from Almedica Europe Ltd to Aptuit (Deeside) Ltd as of 10/10/2005, Name change from Aptuit.

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

#### Levels

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**

#### Other (please specify)

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

Tick if confidential

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

The Almedica site at Unit 103 and Unit 107 on the Deeside Industrial Park are being used for receiving pre-packaged product containing genetically modified organisms for storage and distribution only. There will be no contact with the vials, and no waste will be generated except in the event of accidental damage resulting in a broken vial or vials. Each vial contains less than 1ml of vaccine in dry powder form, and a spillage situation would therefore only involve very small quantities of the product. Our emergency disinfection procedure requires the use of Hypochlorite in the form of Domestos on the recommendation of the HSE on the basis of the recognised efficiency of Hypochlorites in the deactivation of viruses. All disinfected materials will be sent for destruction, clearly marked: "Deactivated Genetically Modified Organisms - for destruction by high temperature incineration". As an emergency procedure, it is felt that monitoring of the disinfection procedure is not applicable. When the distribution process has been completed, any unused stock will be returned to the manufacturer.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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<thead>
<tr>
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**Name**

CHELSEA & WESTMINSTER HOSPITAL NHS FOUNDATION TRUST

**Department**

KOBLER CENTRE ST STEPHENS CLINIC

**Building**

CHELSEA & WESTMINSTER HOSPITAL

**Road Name**

369 FULHAM ROAD

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

SW10 9NH

**Country**

ENGLAND

**Tel Number**

0208 746 8000

**Fax Number**

0208 746 5628

**Date at Which Additional Info Submitted**

05/10/2001

Name changed from Chelsea & Westminster Hospital Healthcare NHS Trust on 27/08/2008.
### Premises Addresses

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<td>ST STEPHENS CLINIC</td>
<td>369 FULHAM ROAD</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
Disposal of Genetically Modified Organisms - Contaminated waste generated will include syringes, needles and empty glass vials, there will be no significant liquid waste. All GM-contaminated material will be placed in a burn bin specifically designed for this purpose. When half full the slide lid will be pulled across leaving a small gap for steam penetration. The bin will be autoclaved and subsequently disposed of by incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

BRIGHTON HEALTHCARE NHS TRUST

**Department**

HIV/GU RESEARCH DEPT

**Building**

RM118 SUSSEX HOUSE

**District**

1 ABBEY ROAD

**Town**

BRIGHTON

**County**

SUSSEX

**Postcode**

BN2 1ES

**Country**

ENGLAND

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<tr>
<th><strong>Tel Number</strong></th>
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**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Give brief details of the genetic modification safety committee

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- **Non-microbial**

- **Other (please specify)**

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
The used vial and syringe/needle will be deactivated prior to removal from the site for incineration. Deactivation will be carried out as follows:

1) Approximately 1ml of concentrated (neat) bleach (Domestos brand) will be injected into the vial to deactivate any residual GMO-containing material. The bleach will be left in the vial.
2) The syringe and needle used for the patient's injection will be placed in a small sharps container. Sufficient Domestos bleach will be poured into the container to cover the syringe and needle. The used vial (containing bleach) will then be placed in this same container.
3) The sharps bin will be sealed with adhesive tape and will be disposed of via our clinical waste contractors.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
<thead>
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<th>Name</th>
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Tick if confidential

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<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

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Other(s)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 754/03.1

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02/03/2022
In the proposed clinical trial programme, angiogenic gene therapy product, consisting of a recombinant adenovirus (human serotype 5) containing the human gene for the fibroblast growth factor 4, and referred to as Ad5FGF-4, will be investigated as a treatment for patients with chronic stable angina due to coronary artery disease (CAD). Generation of new blood supply in the diseased heart by intracoronary administration of angiogenic gene therapy product represents a potential new therapeutic approach to relieve this condition.

Recipient or parental organism

see box below.

Host/vector system

The Ad5FGF-4 gene therapy product consists of a recombinant adenovirus vector (human serotype 5, Ad5) with a deletion in the E1 region; from map until 1.3 to 8.7 of wild-type virus (entire E1A and most of E1B are eliminated). The FGF-4 transgene is inserted, driven by CMV promoter.

Origin & function

The FGF-4 gene was originally isolated from a cDNA library which was constructed from mRNA of Kaposi's sarcoma DNA transformed NIH3T3 cells. The intended function is angiogenesis, the formation of new blood vessels.

Evaluation of foreseeable effects

The probability of adverse consequences resulting from deliberate or accidental release of the gene therapy product Ad5FGF-4 are minimal to nonexistent.

Hazards resulting from environmental release (viral shedding from treated persons, inadvertent contamination of the product prior to administration) are negligible or nonexistent for the following reasons: infection requires large numbers of infectious vectors, and transfection (expression of the inserted gene) requires a multitude of infectious particles.

Hazards associated with the adenoviral vector can be described as having low potential of adverse environmental consequences in humans or animals. The theoretical consequences to humans of several of the hazards associated with ectopic transgene expression, if they actually occurred, could be considered moderately severe (eg promotion of existent malignancy, unknown risk to foetus). However, since any unintended or accidental exposure would most likely be a fraction of the total dose being
administered to patients for therapeutic purposes, the relative risk of the occurrence of these types of adverse effects should be very low.

The possible risks to the environment could be assessed as low to effectively zero. This is based on the low probability of infectious adenoviral particles escaping into the environment either through viral shedding by patients that have received the product, or by incidental exposure during administration procedures. Even if viruses were shed or product spillage occurred the number of infectious viral particles would be too small to result in infection of exposed tissues. The risk to the non-human environment is extremely low to effectively zero because of the species specificity of adenovirus 5, which by natural exposure is only known to infect humans.

The above indicates that the product could be classified as Class 1. However, as a precautionary measure, due to the limited experience available, the product is currently being classified as Class 2. Reclassification into Class 1 may be considered if based on increased data and experience.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Inactivation by autoclaving and effectively 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Committee agreed with the assessment and the assignment of work to Class 2 and requiring the equivalent of Containment Level 2 facilities.

Project Containment

<table>
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<tr>
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<td>020 8258 2200</td>
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Comments

Date at Which Additional Info Submitted

02/03/2022
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</table>

- Bacteriology
- Virology
- Parasitology
- Transgenic Birds
- Transgenic Animals
- Transgenic Fish
- Microbiology Research
- Gene Therapy
At maximum scale, there would be around 1x10 genetically modified Chinese Hamster Ovary cells in the extracapillary space of a single hollow-fibre culture system flowpath, in a volume of approximately 10 litres. In the event of spillage from the culture system, the area can be effectively disinfected. The culture system is contained within a process building, and the wider environment is unlikely to become contaminated.

The waste generated by the work in question falls into 3 categories:

A. Solid waste (which may also contain absorbed or residual levels of liquid) All material falling into this category (including used culture flasks, pipettes, and hollow-fibre flowpaths) are discarded into yellow hard plastic "Biohazard" bins identified as containing GMO waste which, when full, are closed using their dedicated lid and are collected by Site Services and stored in an identified locked bin until sent for incineration. Incineration has been acknowledged (ACGM Newsletter No. 29) as giving effectively 100% kill.

B. Liquid waste from all sources except that in category c (below) All material falling into this category will be chemically inactivated. Specifically, one-quarter volume of Haychlor Industrial (Hays Chemical Distribution Ltd.) - a sodium hypochlorite solution containing >5% available chlorine - will be added to every volume of liquid to be discarded, mixed thoroughly, and left at room temperature for at least 10 minutes before discarding to drain with copious quantities of water. This procedure has been validated with transfected CHO cells, under both the conditions normally expected during the majority of the work (c.1.5x10^5 viable cells/ml, c. 460g/ml protein) and under conditions beyond (i.e. more demanding than) those we would expect to encounter (c.1x10^7 viable cells/ml, > 8300g/ml protein). Under both sets of conditions, the viability of the cells was reduced below the level of detection, i.e. less than approximately 1 remaining viable cell per 1000 viable cells prior to treatment (see BPL R&D Lab. Book 642, pages 53-61). Due to the negligible environmental risk posed by the cells (see Risk Assessment section (v)) this level of inactivation is deemed sufficient.

The inactivation process will be routinely monitored to ensure the presence of free chlorine at the end of the inactivation period. Re-validation of the inactivation will not be performed, unless the conditions of use change from those under which the method was validated. In particular, the method will be revalidated if: i.) a different host cell line is used, or ii.) higher protein concentrations are used in the cell suspension to be inactivated, or iii.) the activity classification changes.

C. Liquid waste from the intracapillary circuit of hollow-fibre culture systems. The cell culture medium in the intracapillary circuit of our hollow-fibre system is separated by an ultra-filtration membrane with a 10kD pore size from the genetically-modified cells in the extracapillary space. Only molecules small enough to pass through these pores will move from the extracapillary space to the intracapillary circuit. These pores have a cross-sectional area which is around 8 orders of magnitude too small to allow passage of the cells, and thus cells could only escape into the intracapillary space if there were a catastrophic loss of membrane integrity. However recovery of our product, (Immunoglobulin G, molecular weight 150 - 160kD) which we harvest from the extracapillary space, is dependant on the integrity of the ultrafiltration membrane, so we would be rapidly aware of even a much smaller loss of membrane integrity. Thus this waste is considered not to contain GMMs, and is released to drain without further treatment.

Monitoring - Compliance with the principles of Good Microbiological Practice and Good Occupational Safety & Hygiene, as well as specific measures outlined above, will be monitored by regular internal and external safety audits.
GM Centre Number: 756

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**Name**

OXFORDSHIRE BIOTECHNET LTD

**Campus Estate or Research Centre**

THE OXFORD BIOBUSINESS CENTRE

**Road Name**

ARMSTRONG ROAD

**Town**

LITTLEMORE

**County**

OXFORDSHIRE

**Postcode**

OX4 4SS

**Country**

ENGLAND

**Tel Number**

01865 405100

**Fax Number**

01865 405101

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

premises closed

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th></th>
<th>Laboratory</th>
<th>Animal Unit</th>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
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<thead>
<tr>
<th>Mycology</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment
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**Name**

OXFORD GENE TECHNOLOGY (OPERATIONS) LIMITED

**Campus Estate or Research Centre**

OXFORD BUSINESS CENTRE

**Road Name**

LITTLEMORE PARK

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX4 4SS

**Country**

ENGLAND

**Tel Number**

01865 405130

**Fax Number**

01865 405120

**HSE Division**

EAST AND SOUTH EAST

**Comments**

MERGED WITH GM 804 ON 04/1/2000

**Date at Which Additional Info Submitted**

27/06/2003
Premises Addresses

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<td>ABINGDON</td>
<td>OXFORDSHIRE</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial
Other (please specify)  
Tick if confidential

Bacteriology  Parasitology  Transgenic Birds  Microbiology Research
Virology  Transgenic Animals  Transgenic Fish  Gene Therapy
The maximum culture volumes of bacterial cells is 250ml at any one time. Waste from such cultures is treated, following manufacturer's instructions, with 1% Virkon solution (Antec International Limited, UK). Efficacy of this treatment is tested every six months by treating a volume of viable culture in this matter and establishing percentage kill by subsequent growth on an agar plate. Records of testing are maintained by the Biological Safety Officer of OGT. Glassware used for bacterial culturing is thoroughly washed, after Virkon treatment, using an acid wash cycle in a Lancer 1300E industrial glass cleaner (designated for used glassware cleaning). Disposable laboratory solid waste is autoclaved at 121C for 20 minutes before removal to clinical waste storage. This is collected on a regular basis and incinerated by the Oxfordshire NHS Ambulance Trust. The autoclave (LTE Falcon 200L MC) is serviced and validated annually by LTE Scientific, Oldham, UK. Regular In-House testing of the autoclave is carried out using a duo-spore biological safety indicator (Propper Manufacturing Co, USA). Records of these procedures are maintained by the OGT Biological Safety Officer.

For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
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Tick if you are claiming exemption from disclosure for sections of the risk assessment

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**Name**

PROIMMUNE LIMITED

**Campus Estate or Research Centre**

**Road Name**

154AH BROOK DRIVE

**Town**

ABINGDON

**District**

MILTON PARK

**County**

OXFORDSHIRE

**Postcode**

OX14 4SD

**Country**

ENGLAND

**Tel Number**

0870042 7279

**Fax Number**

08707120588

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>ENGLAND</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

Non-microbial

Other (please specify) Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**

- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
Mycology
Transgenic
Invertebrates
Transgenic
Plants
Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 758/01.1**

**Date Ackn'd**
19/02/2001

**Date Project Ceased**
30/07/2008

**CU2 Project Title**
THE PROPOGATION OF MAMMALIAN CELL LINES FOR THE PRODUCTION OF CDNA LIBRARIES FOR THE PURPOSE OF OBTAINING GENES ENCODING CELL SURFACE GLYCOPROTEINS OR FOR THE EXPRESSION OF NON-TOXIC GLYCOPROTEINS.

**Class**
Class 2

**CultureVol**

**Class2**
< 1 litre

**Class3-4**

**Non-GMM**
Consent Granted
not applicable

**Project notified under transitional arrangements**
N

**Withdrawn**
N

**Tick if notifying a connected programme of work**
Y

---

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**
### Purposes of the contained use

CDNA libraries will be used as a source of genetic material for molecular cloning of the cell surface glycoproteins. The mammalian cell lines used for expression of the recombinant proteins are required for their ability to express high levels of correctly folded and post-translationally modified mammalian glycoproteins.

### Recipient or parental organism

The mammalian cell lines are sourced from external suppliers and are supplied free from contaminating agents. The cell lines documented here are unable to survive in the environment and have a history of safe use.

### Host/vector system

Transiently transfected cells have a finite life-span of 3-4 days after which they die due to the high number of episomally replicated plasmid molecules present within them and thus are unable to propagate indefinitely. Stably transfected cells are propagated to the desired level and then maintained for a short period to express the desired protein.

The cells eventually exhaust the media nutrients and die, after which the recombinant protein can be harvested from the supernatant.

The plasmids containing the cloned gene are non-mobilisable and therefore cannot transfer into other hosts.

### Origin & function

The cloned glycoprotein genes are of murine or human origin. The constructs to be expressed encode truncated, soluble forms of these cell surface molecules and will be cloned using commercial vector systems with a history of safe use.

The recombinant proteins expressed are non-toxic and will be purified using established methods such as affinity chromatography, ion exchange chromatography and fast protein liquid chromatography.

### Evaluation of foreseeable effects

Non-transfected cell lines are not able to infect healthy humans or animals and will be handled at class 11 containment level. Workers will not be working on any B lymphocytes derived from themselves (see RA 1).

Transiently transfected cells have a finite lifespan, as previously described, and are unable to propagate outside of laboratory conditions.

Stably transfected cells die as a consequence of the culture techniques used for protein expression and harvesting.

Naked DNA vectors are unable to replicate and are non-toxic.

All cell culture is performed in a class 11 biological safety cabinet and waste products are treated as described below. Sufficient precautions are taken to ensure the protection of the health of workers and the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste is disinfected with Virkon solution (Antec International Ltd USA) according to manufacturer's instructions. The treated waste is then disposed of as part of normal laboratory waste. Efficacy of waste management by this method is monitored routinely by treatment of a growing cell culture as detailed above. The effect of Virkon treatment on the cell culture is assessed by staining the cells with a vital dye (trypan blue) and determining the number of live cells by microscopy. Disposal plasticware is disinfected as above and then held in a closed container pending sterilisation by steam (121°C for 20 min). This waste is collected by contracted waste disposal services and incinerated. The autoclave used for this purpose is validated routinely using commercially available testing kits.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GM safety committee have reviewed the risk assessments pertinent to the notification of intention to conduct individual contained use activities (attached) and find them satisfactory for work carried out on these premises.

Proimmune must ensure all staff carrying out these procedures are adequately trained to protect the safety of those working within the premises.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
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<td>Animal Units</td>
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Project Ref 758/13.1

Date Ackn'd 04/11/2013
CU2 Project Title The transient expression of non-toxic, recombinant glycoproteins of human or mammalian origin in HEK293T cells (Human embryonic kidney cells transfected with the SV40 large T antigen) and other mammalian cells
Date Project Ceased

Class CultureVol
Class2 CultureVolume
Class3-4
Class 2 1-50 Litres
Non-GMM Consent Granted
Project Additional Information

Purposes of the contained use

cDNA libraries will be used as a source of genetic material for molecular cloning. The use of HEK293 cells is for their ability to express high levels of non-toxic recombinant proteins. The use of Adenoviral expression system is for its ability to transduce primary human or mammalian cells in order to transiently express non-toxic human or mammalian proteins.

Recipient or parental organism

HEK293T cells are sourced from a commercial supplier
293A cells are sourced from a commercial supplier
Primary human PBMC’s are sourced from the national Blood Service.

Host/vector system

Transiently transfected cells have a finite life-span of 3 – 4 days after which they die due to the high number of episomally replicated plasmid molecules present within them and thus are unable to propagate indefinitely.
Stably transfected cells are propagated to the desired level and then maintained for a short period to express the desired protein. The cells eventually exhaust the media nutrients and die, after which the recombinant protein can be harvested from the supernatant.
The plasmids containing the cloned gene are non-mobilisable and therefore cannot transfer into other hosts.
Replication incompetent Adenovirus infected cells are maintained for a short period to express the desired protein.

Origin & function

1) The HEK293T cells will be used to express cloned genes of mammalian or human origin and are designed express high levels of recombinant human or mammalian antibodies or other recombinant non-toxic proteins. The non-hazardous, soluble proteins will be purified using well documented techniques.
2) The replication deficient adenovirus transduced cells will be used to express non-toxic genes of human or mammalian origin for research purposes.

Evaluation of foreseeable effects

Non-transfected cell lines are not able to infect healthy humans or animals and will be handled at class II containment level. Workers will not be working on any peripheral blood mononuclear cells derived from themselves (see RA 1)

Transiently transfected cells have a finite lifespan, as previously described, and are unable to propagate outside of laboratory conditions.

Stably transfected cells die as a consequence of the culture techniques used for protein expression and harvesting.
Naked DNA vectors are unable to replicate and are non-toxic. The Adenoviral vector is replication incompetent so cannot replicate without specialised culture conditions.

All cell culture is performed in a class I biological safety cabinet and waste products are treated as described below. Sufficient precautions are taken to ensure the protection of the health of workers and the organisms and the genetic material is non-transmissible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Standard laboratory containment level 2. Control measures as stated in the risk assessment table 1.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste is disinfected with Virkon solution (Antec International Ltd. USA) according to manufacturer’s instructions. The treated waste is then disposed of as part of normal laboratory waste.

Efficacy of waste management by this method is monitored routinely by treatment of a growing cell culture as detailed above. The effect of Virkon treatment on the cell culture is assessed by staining the cells with a vital dye (trypan blue) and determining the number of live cells by microscopy.

Disposable plastic ware is disinfected as above and then held in a closed container pending sterilisation by steam (121 degrees C for 20min). This waste is collected by contracted waste disposal services and incinerated.

The autoclave used for this purpose is validated routinely using commercially available testing kits.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GM safety committee have reviewed the risk assessments pertinent to the notification of intention to conduct individual contained use activities (attached) and find them satisfactory for work carried out on these premises.

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### Name

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### Name 2

### Campus Estate or Research Centre

<table>
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### Building

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<td>Fax Number</td>
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### E-mail

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<th>SCOTLAND</th>
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### Comments

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

<table>
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### Premises Conditions

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Give brief details of the genetic modification safety committee

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| Level 1 (GMMs)      |              |             |             |             |
| Level 2 (GMMs)      |              |             |             |             |
| Level 3 (GMMs)      |              |             |             |             |
| Level 4 (GMMs)      |              |             |             |             |
| Non-microbial       |              |             |             |             |

Other (please specify) Tick if confidential

<table>
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<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
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<th>Microbiology Research</th>
<th>Gene Therapy</th>
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<td>Transgenic Fish</td>
<td>Microbiology Research</td>
<td>Gene Therapy</td>
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</table>

02/03/2022
Of all our projects the cloning and protein expression projects using E.coli seems to be most relevant concerning efficient inactivation as mammalian and insect cell cultures are extremely sensitive to decontamination reagents and unlikely to grow outside the incubation units. The maximum culture volumes are normally 500-600mL in 3L Erlenmeyer flasks and fermented in a special E.coli shaking incubator, which is sealed and additionally surrounded by a barrier. After cell harvest by centrifugation the remaining broth is decontaminated over night with hazard tabs and disposed into the sink afterwards. Any other waste including the cell debris from protein extraction are collected in bags and disposed by a professional and licenced company. For validation of the decontamination procedure of liquid bacterial waste we have designed following test, which will be performed in our labs in the week of 22nd to 26th of January 2001; results may certainly be forwarded to the Health & Safety Executive on request. Transformed E.coli cells will be grown overnight in 500ml volumes to maximal OD. To increase the value of the results we will instead of harvesting cells combine the untouched cultures to 2L total volume and decontaminate overnight by a hazard tablet according to our standard protocol. Next day then, samples of the supernatant will be spread on LB-agar plates and eventually surviving cell numbers will be determined. If any germs survive the procedure, I would advise the responsible staff to add an additional autoclaving step or better to increase the incubation time or the concentration of the decontamination reagent. The cell survival assay for process validation would be repeated according to the proposed alteration(s).
Solid waste is inactivated by autoclaving.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

none
### GM Centre Number: 760

<table>
<thead>
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### Comments

CHANGE OF COMPANY NAME AND EMAIL - DETAILS RECEIVED FROM THE BSO ON 30/6/03

### Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Tick Box]

Give brief details of the genetic modification safety committee

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

### Level 1 (GMMs)

### Level 2 (GMMs)

### Level 3 (GMMs)

### Level 4 (GMMs)

### Non-microbial

### Other (please specify)

Tick if confidential

![Tick Box]

### Bacteriology

- Parasitology

### Virology

- Transgenic Animals
- Transgenic Fish

### Transgenic

- Birds
- Fish

### Microbiology Research

- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

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Maximum volumes of 1.5L. Expected volumes usually <50 ml. Cultures in glass or plastic tubes. Agar plates maximum 50 per batch. The waste will be made safe by large laboratory autoclave with a cycle of 126°C for 30 minutes at the standard cycle for waste. The autoclaves have been validated at commissioning by a full "worst case" scenario test under the supervision of the "authorised person" for the hospital autoclave. The autoclaves undergo regular checks at 3 month intervals with multipoint thermocouples carried out by the specialist hospital autoclave engineer. Each individual run is recorded and the cycle checked before the door of the autoclave is released. If there is a run failure the machine is automatically locked and the engineer rectifies the problem and the makesafe cycle is repeated for that load. Records for each cycle are kept and are available for inspection.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**GM Centre Number: 761**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

the regma bio technologies gmsc comprises the director for science and technology and the principal research scientist. meetings of this committee will be convened whenever necessary, either to discuss procedural changes advised by the HSE or to assess new GM proposals/amendments.

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
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Non-microbial

Other (please specify) | Tick if confidential

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Virology

| Transgenic | Gene Therapy |
| Animals    |             |
| Fish       |             |
Liquid waste from cultures will be disinfected with overnight treatment with vircon disinfectant, used in accordance with a manufacturer's instructions. This treatment has been independently assessed for efficacy against a wide range of bacteria, fungi and viruses (details can be found at http://www.antecint.co.uk/) A kill rate of 100% has been claimed against E.Coli and 99.99% against M.smegmatis in 10 minutes exposure test using 1% vircon. Solid waste generated by Regma Bio-Technologies will be disposed of by incineration on site at the DERA incineration facility. Waste will at no stage be transported off site. Liquid waste containing GMOs will be inactivated by treatment with 2% Vircon over a 24 hour period. This inactivation will be carried out within the laboratory before disposal as inactivated waste. At no stage will GMO or unmodified organisms leave the laboratory in a live state.

Solid waste will be collected and inactivated by incineration using existing procedures and equipment at the DERA establishment at Porton Down. Liquid waste will be collected and also disposed of using DERA establishment facilities.

Laboratory surfaces will be disinfected with either vircon on a daily basis. Monitoring for contamination will be carried out on a weekly basis using swab tests to detect the presence of contamination.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The assessment has been agreed and approved for submission

---

**Project Ref 761/01.1**

**CU2 Project Title** DEVELOPMENT OF NOVEL ANTIBIOTIC THERAPEUTIC AGENTS

**Class**

<table>
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**Non-GMM Consent Granted**

not applicable

**Date Ackn'd** 29/05/2001

**Date Project Ceased** 30/06/2003

**Date Project Ceased**

02/03/2022

Page 11031 of 15326
Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Generation and proagation of ACGM class 2 recombinant E.coli and M.smegmatis.

Recipient or parental organism

Non-infectious, non-pathogenic organisms with a history of safe use.

Host/vector system

The host organisms will be either non-infectious lab strains of Escherichia coli (K-12 derivatives) or non-pathogenic Mycobacterium species (eg Mycobacterium smegmatis). Various non-mobilisation or mobilisation defective E.coli and M.smegmatis vectors will be employed.

Origin & function

Many of the genetic elements will be novel in construction, with some sequences based on human protein sequences, but optimised for bacterial expression.

Evaluation of foreseeable effects

The possible effects of the genetic modifications described in detail in the confidential section of this submission have been assessed and minimised wherever possible. Genetic modification is carried out in non-pathogenic and non-infectious hosts. Gene sequence encoding human proteins or sections thereof are optimised for expression in bacterial hosts, and as a result are significantly different to their human counterpart, thus minimising the possibility of human genetic disruption. The vectors involved are not able to be sustained in other organisms in the environment, and therefore carry little risk of environmental damage. Moreover, the majority of the hosts, vectors, genetic elements and expressed proteins have a history of safe use.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste from cultures will be disinfected with overnight treatment with Virkon disinfectant, used in accordance with the manufacturer's instructions. This treatment has been independently assessed for efficacy against a wide range of bacteria, fungi and viruses (details can be found at http://www.antecint.co.uk/). A kill rate of 100% has been claimed against E.coli and 99.99% against M.smegmatis in 10 minute exposure tests using 1% Virkon.

Solid waste will be collected and inactivated by incineration using existing procedures and equipment at the DERA establishment at Porton Down. Liquid waste will be...
collected and also disposed of using DERA establishment facilities.

Laboratory surfaces will be disinfected with either Virkon on a daily basis. Monitoring for contamination will be carried out on a weekly basis using 'swab tests' to detect the presence of contamination.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The assessment has been agreed and approved for submission.

**Project Containment**

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Name

JAMES BLACK FOUNDATION

Name 2

Department

Campus Estate or Research Centre

Building

Road Name 68 HALF MOON LANE

District DULWICH

Town LONDON

County GREATER LONDON

Postcode SE24 9JE

Country ENGLAND

Tel Number 0207 737 8282

Fax Number 0207 274 9687

E-mail

HSE Division LONDON

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

Company Safety Officer (Chairman & Liaison with Safety Committee). Executive Director (Management Representative). Molecular Biology Staff Scientist/Radioligand Binding Staff Scientist - (Staff Representatives). Biological Safety Officer. The committee will meet 6 monthly or as work dictates and will retain an external expert consultant.

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Non-microbial

Other (please specify)  

Tick if confidential

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste cell culture medium will be sterilised in an autoclave on a minimum cycle of 121 for 30min. Expected degree of kill is 100% to be validated by Trypan Blue assay. It will then be disposed of down a designated drain. Waste plastic cell culture plates will be immersed in a 1% solution of commercially available disinfectant "Virkon" for a minimum of 30 mins. Expected degree of kill is 100% to be validated by Trypan Blue assay. Plates will be placed in clinical waste bags for removal and incineration by a licenced contractor.

The Committee have discussed the proposed GM work and have approved the attached Risk Assessment.

Project Ref 762/00.1

CU2 Project Title
CULTURE OF VARIOUS CELL LINES, GENETICALLY MODIFIED TO EXPRESS SPECIFIC RECEPTORS, TO BE USED IN RADIOLIGAND BINDING ASSAYS AND IN FUNCTIONAL BIOASSAYS, TO MEASURE SECOND MESSENGERS RESPONSES.

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 1-50 litres

Non-GMM Consent Granted
not applicable

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Tick if notifying a connected programme of work

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Other(s) Culture of cell lines genetically modified to express various types of receptors

Other (please specify below) Yes

Date Ackn'd 01/12/2000

Date Project Ceased 31/12/2007

Withdrawn

02/03/2022  Page 11036 of 15326
**Project Additional Information**

**Purposes of the contained use**

Assays provide receptor affinity and efficacy estimates for novel, potential drug candidates.

**Recipient or parental organism**

All the cell lines are well characterised and are commercially available from either the American Tissue Collection or the European Culture Collection.

**Host/vector system**

The hosts are all well characterised cell lines. The vectors are all non-viral, synthetic, plasmid constructs.

**Origin & function**

The cell lines used are commercially available and are of human mammalian origin. They are genetically modified using plasmid constructs to express various types of receptors.

**Evaluation of foreseeable effects**

The cell lines are assigned to biosafety level 1 by their commercial supplier. Some have been shown to be tumorigenic in mice but this is not a foreseeable effect in humans. The cell are unable to survive outside the culture medium and have no foreseeable effect on humans or the environment.

Genetic Modification of the cells is unlikely to increase their ability to survive outside the culture, or to increase their pathogenicity. The vectors used are non-mobilisable and prevent no risk to the environment.

The receptor proteins expressed by the cells may be allergenic or immunogenic in humans.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste cell culture medium will be sterilised in an autoclave on a minimum cycle of 121 for 30 min. Expected degree of kill is 100% to be validated by Trypan Blue assay. It will then be disposed of down a designated drain. Waste plastic cell culture plates will be immersed in a 1% solution of commercially available disinfectant "Virkon" for a minimum of 30 mins. Expected degree of kill is 100% to be validated by Trypan Blue assay. Plates will be placed in clinical waste bags for removal and incineration by a licenced contractor.

**Is an emergency plan required according to regulation 20?**

- [ ] N

**If yes, tick to confirm that it is attached to this form**

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] N
The Committee have discussed the proposed GM work and have approved the attached Risk Assessment.

Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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**Name**

CAMBRIDGE UNIVERSITY CHEMICAL LABORATORY

**Name 2**

CHEMISTRY DEPARTMENT

**Campus Estate or Research Centre**

**Road Name**

LENSFIELD ROAD

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 1EW

**Country**

ENGLAND

**Tel Number**

01223 336300

**Fax Number**

01223 336362

**E-mail**

**HSE Division**

LONDON

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Academic staff and Biological Safety Officer:
- Senior Floor Technician:
- Academic staff (biological chemistry):

<table>
<thead>
<tr>
<th>Laboratory</th>
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<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Tick if confidential</td>
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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Waste management</th>
</tr>
</thead>
</table>
| **Solid waste:**  Solid waste will be in the form of agar plates.  
Solid waste will be collected in biohazard bags which will be located by each bench involved in work using GMMs. This material will then be transferred to an autoclave in a nearby room. The material will be inactivated by autoclaving at 121°C for 20 minutes under pressure to give 100% kill. The autoclave is serviced every 6 months and temperature verified using autoclave tape.  
Liquid waste: This will take the form of small scale liquid cultures (overnights), the supernatants of larger scale cultures which have had the cell pellet removed by centrifugation, lysed cell pellet debris which may contain some non-lysed cells.  
For this type of waste disinfectants such as clearsol (1%), or tegodyne (0.5%) will be used. These are commercially available disinfectants and are used in accordance with the manufacturers instructions. These disinfectants have been validated by the manufacturers as 100% effective if used under the recommended conditions. This will yield a 100% kill.  
Spillages: disinfectants will be used at higher doses: clearsol (5%) and tegodyne (3%). 100% kill. Validation see above.  
Ultimate fate: inactivated material will be disposed of by normal methods - liquid waste in the sink, solid waste as non-hazardous waste.  
Monitoring and testing: The Biological Safety Committee will conduct a safety inspection of all laboratories working with GMMs every 6 months. This is to ensure that all the appropriate safety measures are in use and that GMP (good microbiological practice) and GOSH (good operational safety and hygiene). |

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Well-characterised standard laboratory strains of E. coli strains are to be used. These are all disabled and not known to be pathogenic. Inserts are unlikely to increase pathogenicity. The vectors are, at least, mobilisation-defective, if not non-mobilisable. Proteins to be expressed are not known to be toxic, nor are their sequences known to be oncogenic. Use of T7-based promoter systems means that expression levels in anything other than the engineered DE3 strains will be minimal.  
There is a negligible hazard with respect to human health and the environment therefore the assessment of Class 1 (containment level 1) is appropriate for these hosts/vector systems.
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### Name

SCOTTISH BIOMEDICAL FOUNDATION LIMITED

### Name 2

### Department

### Campus Estate or Research Centre

TODD CAMPUS

### Road Name

WEST OF SCOTLAND SCIENCE PARK

### Building

BLK H GROUND FLOOR

### District

### Town

GLASGOW

### County

EAST RENFREWSHIRE

### Postcode

G20 OXA

### Country

SCOTLAND

### Tel Number

141 587 6100

### Fax Number

0141 587 6110

### E-mail

none

### HSE Division

SCOTLAND

### Comments

### Date at Which Additional Info Submitted

21/06/2001
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Yes**

**Laboratory work will commence at the beginning of January. This is when the research staff will arrive.**

At present, the Safety Committee consists of the project leader and the company Chief Executive. In January, a senior scientist with 6 years post-doctorate experience will be appointed as biological safety officer. The committee will spend some days at the beginning of January to consider the vectors to be used in the stated expression systems. The lab operating procedures will also be finalised at this time. After this, the committee will meet on a monthly basis to consider current practices and how to act on any proposed changes to procedures.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Personal - hands will be washed in 'Lab Guard' soap before leaving laboratory. General - work surfaces swabbed with Savlon daily. Spills - Virkon granules onto spills, use tissue to remove to autoclave bag. Liquid - presept 10,000 ppm overnight in container before disposal to drain with copious amounts of water. Glassware - presept 10,000ppm overnight then washed in dishwasher. Waste - solid - dispose as biohazard into autoclave bag for kill run in autoclave. Sharps - into sharps box for incineration. Ugar plates - dispose as biohazard into autoclave for kill run. All autoclaved solid waste sealed in autoclave bags. After kill run sealed into waste bags and disposed in the general bins.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

In the preliminary meeting of the GMSC (team leader and chief executive), it was decided that the above waste management measures were sufficient. Once the staff and work start in January, the biological safety officer and his deputy (to be appointed) will assess teh procedures being used, carry out monitoring of the work area, and set up the inventories of safe storage and records of biological agents in use.

Project Ref 764/07.1

<table>
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<tr>
<th>Date Ackn’d</th>
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<td>25/07/2007</td>
<td>Generation and use of recombinant lentivirus and adenovirus.</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
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We wish to use recombinant disabled adenovirus and lentivirus to transduce a variety of cell lines and primary cell cultures in order to achieve a high level expression of our genes of interest.

We will use the viral vectors to insert genes of interest into several standard mammalian cell lines, including but not exclusively, HEK 293, PC12, RH7777, A549 and primary cells such as myocytes, monocytes, neurites and adult and embryonic stem cells.

We will use the Invitrogen lentivirus and adenovirus systems.

The genes we will subclone to generate our recombinant viral vectors have been isolated from animal and human origin. We will use these genes to investigate their involvement in cellular signalling processes. Using the viral vector protocol we plan to generate a range of cell lines stably expressing our genes of interest. This will provide us with a means of studying the function of our target genes. We wish to use the adenoviral and lentiviral systems as these are the most efficient and in some cases the only method available to insert our target genes into difficult to work with cells.

The adenoviral vectors and virus generated is disabled to prevent replication of live virus outside of the packaging cell lines such as HEK 293 which supply the missing adenoviral genes. The lentiviral vectors and virus are disabled to prevent replication. Generation of live virus can only be achieved by co-transfection with plasmids containing the packaging genes (e.g. gag, pol, rev and env) so that none of the HIV structural genes are present in the packaging viral genome.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Personal - all work involving the use of recombinant viruses will take place in class 2 laminar flow hoods. Labcoats, gloves, mask and safety glasses are worn by all users. Hands are washed in "LabGuard" soap before leaving laboratory.

General - Work surfaces are washed with Virkon then 70% ethanol after each experiment and at the end of the week. All procedures will be performed in class 2 laminar flow cabinets contained in a room designated for this purpose. Spills are absorbed using Virkon granules, removed using tissue and autoclaved.

Waste - Solid waste is disposed as biohazard into autoclave bags for a kill run in the autoclave. All autoclaved solid waste is sealed in autoclave bags. All contaminated plasticware will be soaked in virkon for at least 6 hours before being autoclaved. After the kill run, sealed solid waste is disposed of in the general waste bins. Liquid waste is treated with virkon before disposal.

The autoclave is on a service contract and frequently checked for correct operation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The company Genetic Modification Safety Committee has passed the attached risk assessment and associated plan of work as being suitable for the handling of these biological agents. The proposed procedures were assessed as being class 2 activities. The standard cleaning, waste disposal and decontamination procedures were also assessed as being sufficient for the purposes of this protocol.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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**Name**

ASTERAND UK LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

2A ORCHARD ROAD

**District**

**Town**

ROYSTON

**County**

HERTFORDSHIRE

**Postcode**

SG8 5HD

**Country**

ENGLAND

**Tel Number**

01763 211600

**Fax Number**

01763 211555

**E-mail**

**HSE Division**

LONDON

**Comments**

Company name change from Pharmagene Laboratories to Asterand as of 28/02/2006

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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<td>SG8 5HD</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee and the company safety committee are synonymous. The committee is composed of, chief scientific office, department head supplies - laboratory manager, research assistant, senior scientist, dept. head tissue pharmacology, human resources and facilities officer, therapeutics and information/IT assistant. The Committee meet once a month. The BSO updates the H&S Committee at these meetings.

### Laboratory
- Level 1 (GMMs)
  - Yes
- Level 2 (GMMs)
  - Yes
- Level 3 (GMMs)
- Level 4 (GMMs)

### Animal Unit
- Non-microbial

### Growth Room
- Non-microbial

### Glass House
- Non-microbial

### Large Scale
- Non-microbial

Other (please specify) Tick if confidential

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

All liquid waste is made up to 10% with microsol 3 and left for a minimum of 20 minutes all "contaminated" equipment is either soaked or wiped down with 10% microsol 3 then cleaned with 70% etanol. Disposable plastic and any solid waste are then placed in autoclave bags and sealed in clinical yellow ridged one way burn bins for incineration. Kill rate is 100%

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

A risk assessment has been completed. Any hazards identified containment has been identified and documented with all the safety procedures in place. The work is considered low risk to staff and the environment.

Project Ref 765/01.1

Date Ackn’d 06/02/2001

CU2 Project Title USE OF ESTABLISHED CHARACTERISED CELL LINES (HUMAN OR ANIMAL) WHICH ARE STABLY TRANSFECTED WITH PLASMID VECTORS TO ALLOW THEM TO EXPRESS HUMAN CELL SURFACE RECEPTORS.

Class 2

Culture Vol Class 2 < 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Tick if notifying a connected programme of work
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Screening to measure the affinity of test substances for cell surface human receptors.

**Recipient or parental organism**

Transfected cell lines which propagate under controlled conditions in vitro, but not outside the controlled laboratory environment. Cells are transfected with non-mobilisable vectors. Single cells will be dealt with in Class II cabinets and destroyed prior to disposal there are no foreseeable effect.

**Host/vector system**

Host cells will be CHO-K1 and HEK 293-EBNA supplied commercially by Euroscreen and Invitrogen, respectively.

CHO-K1 cells are transfected with the PC DNA 03 vector (Invitrogen)

HEK 293 EBNA cells are transfected with PCEP4 (Invitrogen) vector.

**Origin & function**

Cell lines (transfected with vectors encoding specific human proteins) will be sourced from commercial suppliers eg. Euroscreen. In general, these companies obtain the CDNA of interest from a human cell or tissue library and then subclone into a suitable vector for expression in a suitable mammalian cell line. Under appropriate conditions, these cell lines then express the target human protein. Pharmagenen intend to purchase these cells and use them to assess the ability of test agents to bind the target human protein of interest.

**Evaluation of foreseeable effects**

This is routine assay work with no foreseeable adverse effect. All cells will be handled in Class II biosafety cabinets and will be destroyed after each assay.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

For studies using whole cells, post-assay, cells are destroyed in situ in assay plates by addition of disinfectant (Virvsol). Plates are then sealed, placed in autoclave bags and incinerated. Therefore, degree of kill = 100%.

Similarly, for studies where membrane preparations have been produced from transfected cells, post-assay, plates or filters containing membranes will be sealed in autoclave bags and incinerated.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N
A risk assessment has been completed. Any hazards have been identified. Containment has been identified and documented.

With all safety procedures in place the work is considered low risk to staff the environment.

### Project Containment

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**Name**

GEN-PROBE CARDIFF LTD

**Campus Estate or Research Centre**

CARDIFF INDUSTRIAL PARK

**Road Name**

5 CHILTERN CLOSE

**Town**

CARDIFF

**Building**


**County**

CEREDIGION

**Postcode**

CF14 5DL

**Country**

WALES

**Tel Number**

029 2074 7033

**Fax Number**

029 2074 7118

**HSE Division**

WALES AND SOUTH WEST

**Comments**

Company name change 17/10/2009 from Molecular Light Technology Research Ltd

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

☐

**Give brief details of the genetic modification safety committee**

The established safety committee of the company will also take on the role of GM safety. Meetings are currently held once per month. The GMSC subgroup consists of the Chief Operations Officer, Quality Health Safety and Environment Officer, Biological Safety Officer (Research scientist), Safety Officer (research scientist), Facilities Manager.

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- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research** Yes
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

02/03/2022
The GMM will only be cultured on a small scale (10-200ml maximum). Work areas will be disinfected after every use with Virkon. Any spill will be treated with disinfection (Virkon) according to manufacturers' instructions immediately after spillage. All waste, solid and liquid, will be autoclaved at 121 degrees C, 15 psi for 20 minutes and then disposed by normal routes. The normal functioning of the autoclave will be monitored to estimate the "degree of kill" on a regular basis (sterilisation indicator strips/temperature recorder). External contractors (S.W. Scientific) service the autoclave annually.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The GMM will only be cultured on a small scale (10-200ml maximum). Work areas will be disinfected after every use with Virkon. Any spill will be treated with disinfection (Virkon) according to manufacturers' instructions immediately after spillage. All waste, solid and liquid, will be autoclaved at 121 degrees C, 15 psi for 20 minutes and then disposed by normal routes. The normal functioning of the autoclave will be monitored to estimate the "degree of kill" on a regular basis (sterilisation indicator strips/temperature recorder). External contractors (S.W. Scientific) service the autoclave annually.

Please enter comments of the GM safety committee on the risk assessment

All members of the GMSC have seen the risk assessment and agree with the provisional classification (Class 1).
LEEDS TEACHING HOSPITALS NHS TRUST

Name

LEEDS TEACHING HOSPITALS NHS TRUST

Name 2

ST JAMES'S UNIVERSITY HOSPITAL

Campus Estate or Research Centre

BECKETT STREET

Road Name

Leeds

Town

YORKSHIRE

County

LS9 7TF

Postcode

ENGLAND

Country

Tel Number

0113 206 4149

Fax Number

0113 206 4099

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

LTHT has a well established multi-disciplinary committee, chaired by a consultant virologist, and representing infection prevention and control, occupational health, pharmacy, oncology, basic science, nursing and medicine.

The committee convenes quarterly, and more recently has introduced responsive review to improve study approval timelines in line with NIHR targets. Formal application forms are used to review risk assessment and standard operating procedures are also scrutinised as part of the authorisation process.

The committee operates within a terms of reference and reports into and is accountable to the organisation's Risk Management Board.

### Level 1 (GMMs)

### Level 2 (GMMs)

### Level 3 (GMMs)

### Level 4 (GMMs)
L2 GMMs prep. for clinical use by pharmacy aseptics and admin. on oncology wards in clin. trials

Waste management is managed through a single process to ensure consistency and compliance irrespective of Cat 1 or Cat 2 status. Specific deactivation of residual agent or delivery equipment, if necessary, is stipulated in the study specific SOP for the trial, which includes compliance with local trust policies and procedures.

All preparation materials and clinical waste derived from the procedure of administration of the agent is contained within a newly configured waste disposal clinical sharps container for each individual procedure carried out. If chemical deactivation is necessary this is achieved by means of a virucidal agent. Biohazard spillage kits are in use and immediately available to staff prior to any procedure. Occlusive dressings are used in some instances where viral shedding is a concern, based on the agent under use, and patients are provided with non-sterile gloves and clinical sharps containers to appropriately dispose of used dressings. These containers are returned to the hospital for safe disposal.

All waste relating to viral or GM activities is labelled appropriately and sent for incineration (external contract with White Rose) as per trust policy for safe disposal of biological and cytotoxic clinical waste.

All staff are trained and assessed in safe and appropriate waste disposal, as well as study specific requirements prior to authorisation to carry out treatment in these studies.

Study specific SOPs include an assessment of risk to patients, staff and the environment and include the process of receipt of the agent, handling, preparation, administration, waste containment and management and incident management (spillage/needlestick injury) of the agent under investigation.

A summary of the agents and clinical trials currently being conducted within LTHT is appended.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The risk assessment for the ONYX-015 virus was duly noted. The safety committee were satisfied that appropriate procedures are in place, in both the pharmacy and ward environments, to minimise the risk to human health and the environment.

**Project Ref 767/01.1**

**Date Ackn’d** 14/02/2001

**CU2 Project Title** A PHASE 1 DOSE-ESCALATION TRIAL OF ONYX-O15 (CI-1042, PFIZER PHARMACEUTICALS), AN E1B-ATTENUATED ADENOVIRUS, AS AN INTRA-VESICAL GENE THERAPY FOR RECURRENT SUPERFICIAL/MUSCLE-INVASIVE BLADDER CANCER

**Class** Class 2

**CultureVolClass2** < 1 litre

**Non-GMM** not applicable

**Consent Granted** Project notified under transitional arrangements

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use** Clinical trial

**Recipient or parental organism**

Human subjects (patients with superficial or muscle-invasive bladder cancer, selected according to strict criteria, as set out in clinical trial protocol). Approval has already been obtained from the Department of Health's Gene Therapy Advisory Committee (GTAC).

**Host/vector system**

ONYX-015 is a chimeric Group C adenovirus (Barker DD and Berk AJ (1987) Virology 156 (1), 107-121), which is selectively replication-competent. It contains no oncogenic nor immune-modifying genes. Rather, as a result of a deletion in the E1B region of the viral genome, it is incapable of replication in normal host cells i.e. those with wild-type p53 gene. In cancer cells with defective p53 function, however, it can undergo replication.

**Origin & function**

Adenovirus is obtained by propagation in 293 human embryonic kidney cells in cell culture. Clinical-grade virus, free of cellular material, will be supplied by Pfizer.
Viral stock solution will be diluted on-site (see Section 12) in sterile saline and instilled into patients' bladders via a urethral catheter on 5 consecutive days, according to a dose-escalation schedule, as set out in the clinical trial protocol, to assess the safety and potential toxicity of intra-vesical administration.

**Evaluation of foreseeable effects**

Extensive previous clinical experience with the ONYX-015 virus has already demonstrated its safety, when administered via a variety of routes, including intravenous and intra-arterial routes. This previous experience suggests that effects of the micro-organism on the individual to whom it is administered are minimal. Toxicities observed have included 'flu-like symptoms, abnormal liver function, mild hypotension, abnormal bleeding and pain at the site of injection. There have been no treatment-related deaths with this virus.

The vast majority of the human population will have been previously exposed to wild-type adenovirus. There is a theoretical possibility that, in individuals still harbouring viable wild-type virus, recombination between ONYX-015 and wild-type viruses may occur. However, the absence of any exogenous genes in the ONYX-015 genome means that the consequences for the individual concerned, those with whom the individual comes in contact, and the environment would be no more than those for wild-type (i.e. possible short-lived, self-limiting upper respiratory tract infection, bronchitis, gastro-enteritis, conjunctivitis or cystitis in humans; the virus has extremely narrow host range, and is extremely unlikely to infect other exposed species).

Wild-type adenoviruses are Hazard Group 2 biological agents (as defined by the Advisory Committee on Dangerous Pathogens), and thus containment level 2 should be adequate for this virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Schedule 8, Part II, Table 1a (8) Autoclave required in the building

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Medium-term storage of viral stocks will be in a dedicated freezer in the hospital pharmacy. Viral stocks will be diluted immediately prior to administration into sterile bags of normal saline within a dedicated vented negative pressure biological safety cabinet with separate air handling plant in a dedicated area of the hospital pharmacy. Waste sharps and other contaminated materials will be autoclaved (124oC, 11 mins) and then incinerated with an anticipated 100% kill. Diluted virus will be transported from Pharmacy to the Oncology Wards in sealed plastic bags inside a sealed plastic box. Short-term storage (no more than a few hours) on the ward will be in a dedicated refrigerator. On the oncology wards, virus will be administered via a urethral catheter in a side-room. Solid waste will be kept separate from other ward waste, then autoclaved and incinerated, with 100% kill expected. Patients will be clinically monitored until 24 hours after final administration of virus, and voided urine will be collected in hypochlorite solution (to a final concentration of 1% for 30 mins), autoclaved (anticipated 100% kill), then disposed of into hospital drains.

Effectiveness of safety cabinets and autoclaves will be monitored by weekly testing of the autoclave and annual testing of safety cabinets in accordance with existing local policy.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y
The risk assessment for the ONYX-015 virus was duly noted. The safety committee were satisfied that appropriate procedures are in place, in both the pharmacy and ward environments, to minimise the risk to human health and the environment.

### Project Containment

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### Project Ref 767/13.1

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Date Project Ceased

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

### Project Additional Information

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<td>Assessment of therapeutic effect within clinical trial</td>
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NHS patients with 'Asymptomatic or Minimally Symptomatic Metastatic, Castrate-Resistant Prostate Cancer

Host/vector system

PROSTVAC-V/F is comprised of two component viral vectors: a recombinant vaccinia virus (PROSTVAC-V) and a recombinant fowlpox virus (PROSTVAC-F) to be used sequentially in a heterologous prime-boost vaccination regimen. Both viruses contain four human genes: prostate-specific antigen (PSA) and three genes encoding human immunological costimulatory molecules: B7.1, intercellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 (LFA-3) (designated TRIad of COstimulatory Molecules or TRICOM). The simultaneous expression of PSA and TRICOM enhances the immune response. The PSA transgene is modified at one amino acid (I155L), to enhance binding and immunogenicity of a particular peptide epitope in HLA-A2-expressing subjects.

PROSTVAC-V: The parental vaccinia virus used for the generation of PROSTVAC-V was derived from a plaque isolate (designated TBC-Wy) from the seed stock of virus used by Wyeth Pharmaceuticals to produce the licensed Dryvax® Smallpox Vaccine. Dryvax was the vaccine used in the USA during the world-wide smallpox eradication campaign, which ended in the early 1970’s. The vaccinia virus is a replicating virus and therefore can shed from the vaccination site.

PROSTVAC-F: The parental fowlpox virus (TBC-FPV) was plaque-purified from a USDA-licensed poultry vaccine, POXVAC-TC, which was manufactured by Schering-Plough Corporation. The fowlpox viral vector is an avipox virus that does not replicate in mammalian cells.

Origin & function

The intent of vaccination with PROSTVAC is to induce an immune response to prostate-specific antigen (PSA), and other prostate- and tumor-specific antigens. This response may provide anti-tumor activity and slow the overall progression of disease.

Evaluation of foreseeable effects

Vaccinia virus (used as a vector in PROSTVAC-V) causes a transient infection, with elimination of viral components over several weeks. Host cells infected with vaccinia virus are short lived (days) and die by a mixed form of apoptosis/necrosis. Vaccinia replicates in the cytoplasm of infected cells, and viral DNA does not integrate into the host cell DNA. Vaccinia virus is known to be shed from the wound site in traditional dermal scarification based vaccination. It is important to note that PROSTVAC-V will be administered via subcutaneous administration in patients who were previously vaccinated with a small-pox vaccine, factors which serve to minimize shedding. Nevertheless, because the vaccinia virus is a replicating virus, there is still a risk for inadvertent inoculation or contact transmission. Thus, there are specific procedures/precautions applied to PROSTVAC-V that may not apply to PROSTVAC-F or the Placebo because the fowlpox virus does not replicate in humans.

Inadvertent sharps injuries (needle-stick, etc.), contact with skin, eyes or genitals inhalation or ingestion.

RISKS FROM EXPOSURE. Adverse reactions attributable to the administration of PROSTVAC-V/F based on the results of the randomized Phase II trial include injection site reactions experienced by 98% of patients (pain, swelling, induration, and redness). More than 10% of patients experienced gastrointestinal disorders (constipation, diarrhea, nausea), arthralgia and dizziness. The majority of events were of Grade 1 and 2 in severity. These side effects are related to both PROSTVAC-V and PROSTVAC-F. As already noted, viral shedding is minimal with PROSTVAC-V, however, there are still risks of inadvertent infection of other body sites (through scratching), or transfer of infection to others. There is no risk with PROSTVAC-F or Placebo for inadvertent infection of other body sites or transfer of infection to others. There are rare risks of severe and possibly life-threatening adverse reactions associated with PROSTVAC-V. These include eczema vaccinatum (~1/25,000, usually with history of underlying skin disorder) or progressive vaccinia (~1/1 000.000, usually with underlying immunodeficiency), or post vaccinial encephalitis (~1/500.000, usually infants or small children). These were observed after the administration of conventional smallpox vaccines, and have not been seen in prior studies of PROSTVAC or related PSA (or other tumor antigen)-containing vaccinia virus vaccines administered to vaccinia pre-immune cancer patients.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Management and containment of agent and potential hazards are detailed in the LTHT SOP for the study and the sponsors Biosafety Process guidance document for Principal Investigators and site personnel. (Attached)

Patients are treated and monitored in restricted access clinical areas. Appropriate use of biohazard signs highlight activity to others in the vicinity. Although PROSTOVAC has been registered with HSE as a deliberate release study, the requirements for handling, treatment delivery and patient management are consistent with our contained

Page 11061 of 15326
use protocols. Therefore this study is highlighted as an example of category 2 activity.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Study registered with HSE by sponsor under deliberate release regulations.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

LTHT BSC risk assessment form attached.

Staff involved in the handling or delivery of the agent, as well as those who may come into contact with patients will be made aware of the risks and necessary precautions required. Staff who may be immunocompromised, pregnant or planning pregnancy will be excluded from drug handling or administration and monitoring activities relating to this study.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

PROSTVAC Trial – Presented by Dr SJ and Research Nurse, JC
SJ presented an overview of this study for castration resistant metastatic prostate cancer patients. She explained the objectives, inclusion/exclusion criteria and method of delivery of the vaccine. In 2 arms of the study patients will have the vaccine with or without GSF. SJ anticipated 1 patient recruited every couple of months. JC or a member of the LCRF team would administer the injection and all would have training to give this type of agent if not done so before. It was being considered that the GSF could be administered at home if patients wish to be taught how to do this. SJ mentioned the expected side effects and the preventative measures they would employ for staff and patients; such as screening patients carefully to find out who they may be in contact with, advising staff of the potential risks should they be pregnant or on immunosuppressant medication.
On discussion ST reported the new biological aseptic rooms are being commissioned in Pharmacy. She stipulated that the injection site should be covered with an occlusive dressing to prevent leakage. This vaccine was in the biosafety level 2 risk group. ST informed the meeting that a different licence would probably be required if patients gave the GSF at home, as the GSF was administered so close to the vaccine injection site and would fall under ‘deliberate release’ guidelines and this could prove extremely difficult for the licence holder to ensure compliance. BSC members and SJ all agreed it would be better for participants to have their treatments at the hospital under the contained use licence and where appropriate disposal of dressings etc can be evidenced. JC explained that they may have difficulties block booking the LCRF beds on Ward 97 for all the days of administering treatment. SJ to confirm if study is to be notified under “deliberate release” regulations (to be consistent with other study sites)
SJ will check with the Sponsor if the blinding code can be broken should there be a needlestick injury. In such cases staff would be referred to Occupational Health. It was agreed that SJ should contact the Infectious Diseases Department to make them aware of this study and so aware of any potential referral. MP had reviewed the study SOP and sent comments back to JC. JC to update the SOP and email back to the BSC Chair.
On review the BSC members agreed this study could go ahead. It was agreed that a letter/email of support from Dr Jane Minton in the Infectious Disease Department should be seen by TH. Corrections to the SOP should be sent back to the Chair. Timing of the start of the study would hinge on the Trust’s application to the HSE for a level 2 licence for these types of vaccines. SJ to be informed when this is available.
Project Ref 767/13.2

A planned programme of Early Clinical Trials using Oncolytic Viruses (OV) in cancer patients. These trials all involve the administration of OV by an intravenous (i.v.) route including genetically modified strains of vaccinia (GL-ONC1 & JX-594) and Herpes Simplex Virus (HSV1716).

Recipient or parental organism

JX-594 & GL-ONC1 are derived from different strains of vaccinia virus derived from the commonly used Wyeth vaccine strain (Dryvax®, Wyeth Laboratories). It was designed to selectively replicate in and destroy cancer cells, while at the same time stimulating a systemic anti-tumour immune response through the expression of its transgene, hGM-CSF, in the context of tumour lysis. Three genetic modifications are included in JX-594: 1) thymidine kinase gene inactivation, 2) GM-CSF gene insertion.
under the control of the synthetic early-late promoter, and 3) lac-Z gene insertion under control of the p7.5 promoter.

GL-ONC1 is a vaccinia virus genetically engineered by Genelux Corporation, designated as GLV-1h68 (laboratory name for GL-ONC1). GLV-1h68 was derived from the LIVP strain (Lister strain obtained from the Institute of Viral Preparations, Moscow) by inserting ruc-gfp (a fusion gene of Renilla luciferase and green fluorescent protein), LacZ (beta-galactosidase), and gusA (beta-glucuronidase) expression cassettes into F14.5L (located between F14L and F15L), thymidine kinase (TK), and hemagglutinin (HA) loci, respectively. Disruption of these nonessential genes and expression of the foregin gene expression cassettes not only attenuated the virus but also enhanced its tumour-specific targeting. GLV-1h68 was used in most of the in vitro cytototoxicity studies and the efficacy studies in animals.

Herpes Simplex Virus (HSV) is a member of the Herpes group of viruses, the Herpesviridae. HSV1716 (JS134.5-/w) is a genetically modified strain of HSV1 incorporating a single deletion of the RL1 gene (ICP34.5).

As a result of the above genetic modifications to the viral backbones, the risk of replication & spread of these viruses in any tissues, other than tumour, is negligible.

Origin & function

Disruption of the Thymidine Kinase (TK) gene in both GL-ONC1 & JX-594 ensures tumour-restrictive replication. GL-ONC1 has also lost several ORFs including genes for virulence (such as the cytokine response modified E protein). These changes also contribute to tumour selectivity as do the engineered deletions of F14.5L and haemagglutinin. Other modifications include insertion of proteins to enable/enhance virus detection & replication in vivo:

- LacZ (encodes β-glucuronidase which allows histological detection of viral replication in tissues & visual detection in cells) - GL-ONC1 & JX-594
- gusA (a reporter gene which results in expression of β-glucuronidase to allow detection of virus) - GL-ONC1
- Green fluorescent protein (GFP) (to enable visualisation of viral infection) - GL-ONC1
- GMCSF (to promote immune cell activation against tumour) - JX-594.

HSV1716 Has only a single deletion of the RL1 gene (ICP34.5), causing a loss of virulence & the ability to establish latency, together with confining viral replication to tumour cells. In a wild-type HSV-1 infection, ICP34.5 plays a key role by mediating escape from a major host defence to mechanism involving the PKR pathway which, if activated, will prevent protein translation and viral replication. In contrast, HSV1716 which lacks functional ICP34.5 proteins, is not able to overcome the host PKR defence and thus HSV1716 is highly attenuated in non-dividing, normal, cells.

Evaluation of foreseeable effects

Risks to human health

All of the above OV are considered a Biosafety Level 2 (BSL-2) infectious substance. The BSL-2 designation and associated guidelines apply to agents of moderate potential hazard to personnel and the environment.

All OV will be administered to patients via an i.v. route, which is low-risk strategy in comparison to other routes, e.g. intralesional.

Vaccinia viruses

Both GL-ONC & JX-594 have been used previously in clinical trials and their safety well-documented. The most extreme risks to human health are the possibility of patients developing infection following treatment which may result in viral spread to others through close contact. Humans are not a natural host of vaccinia virus & exposure of normal, healthy humans to these viruses is unlikely to result in toxicity as immune recognition & activation would control viral pathogenesis. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous infection. However, rescue drugs (e.g. vaccinia immunoglobulin, cidofovir, etc) are available to effectively treat any potential vaccinia infections, which usually manifests itself as a clinically mild rash. Neither GL-ONC1 nor JX-594 are known to produce latent infection.

HSV1716

HSV has been widely investigated for several decades with no instances or evidence of oncogenicity or vertical transmission having been reported. Exposure to HSV1716 is highly unlikely to result in toxicity as it is a non-pathogenic virus.

All OV

The risk of viral shedding has been documented in previous trials to be negligible or non-existent. In addition, to date there have been no reported secondary transmission...
events to healthcare workers or close contacts of treated patients. Despite previous safety data for all the above viruses, containment strategies will minimise the potential risk of infection/transmission. People in at-risk groups (those with skin rashes, the immunocompromised, pregnant women & infants) will be excluded from treatment & advice will be given to patients to avoid direct contact with family members in these groups for duration of treatment. No immunocompromised, pregnant or breastfeeding staff will be involved in pharmacy or patient care.

Environmental Hazards

The environmental impact is likely to be low. All the above GM viruses are not naturally found in the environment. In addition, they are replication attenuated &/or disabled and are unlikely to survive in the environment. Outside of the host, the viruses are fragile & easily inactivated by UV irradiation or with standard hospital disinfectants, e.g. Chloroclean. Shedding of the viruses in treated patients has been shown to be negligible to non-existent, therefore would not pose a risk to anything or anyone. As an additional precaution, the administration site will be swabbed with alcohol and an occlusive dressing will be used following administration if delivered via the subcutaneous route. Due to the nature of the release site, the method of administration and the fact that the viruses are replication attenuated/disabled, no other organisms are likely to be exposed to the GMO.

Risks of recombination.

Vaccinia virus is not naturally found in the environment & has no natural host reservoir. As such, there is no danger of recombination of any of the attenuated vaccinia strains with the wild-type virus. Vaccinia is capable of recombination with homologous DNA sequences. However, there was no specific concern raised during the smallpox vaccination campaigns where hundreds of millions of people were administered with the w/t virus. Despite worldwide use of the live virus vaccine, no reported adverse events due to mutation to a more aggressive phenotype has ever been reported. Various strains of vaccinia virus (such as NYCBH) have been detected in humans shortly after receiving vaccinia-based vaccines. Therefore, to avoid the risk of any potential recombination events, the protocols exclude from the proposed clinical studies patients who have been vaccinated within the past 10 years.

Recombination of orthopoxviruses with other DNA/RNA-viruses have been reported very rarely and appears to be very unusual. In addition, since poxviruses use unique promoters, gene activation by inserted foreign promoters also seems very unlikely.

GL-ONC1 - The risk that GL-ONC1 will revert into more virulent mutants is very low, as the parental LIVP strain, a descendant of the Lister strain, was attenuated by frequent passages on calf skin (more than 500 times). Furthermore, stability testing showed some variation in expression of GFP but consistent expression of the other marker genes 100% of the time. In vivo studies showed equal safety & efficacy in the absence of ruc-gfp expression.

JX-594 - JX-594 could revert its genome to its parental structure by eliminating the expression cassette inserted into the TK gene. Current genetic stability studies have not detected spontaneous revertants. Despite this, even if it was to revert back to wild-type, there would still be no environmental concern, as stated above.

HSV1716 - HSV has been widely investigated for several decades with no instances or evidence of oncogenicity or vertical transmission have been reported.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management is managed through a single process to ensure consistency and compliance irrespective of Cat 1 or Cat 2 status. Specific deactivation of residual agent or delivery equipment, if necessary, is stipulated in the study specific SOP for the trial, which includes compliance with local trust policies & procedures.

All preparation materials and clinical waste derived from the procedure of administration of the the agent is contained within a newly configured waste disposal clinical sharps container for each individual procedure carried out. If chemical deactivation is necessary this is achieved by means of a virucidal agent. Biohazard spillage kits are in use and immediately available to staff prior to any procedure. Occlusive dressings are used in some instances where viral shedding is a concern, based on the agent.
under use, and patients are provided with non-sterile gloves and clinical sharps containers to appropriately dispose of used dressings. These containers are returned to the hospital for safe disposal.

All waste relating to viral or GM activities is labelled appropriately and sent for incineration (external contract with White Rose) as per trust policy for safe disposal of biological & cytotoxic clinical waste.

All staff are trained and assessed in safe & appropriate waste disposal, as well as study-specific requirements prior to authorisation to carry out treatment in these studies.

Study-specific SOPs include an assessment of risk to patients, staff & the environment and include the process of receipt of the agent, handling, preparation, administration, waste containment and management, and incident management (spillage/needlestick injury) of the agent under investigation.

A summary of the agents & clinical trials, either approved or in development, are outlined as follows:

Clinical trials with 2 of these agents already have an approved protocol:
• An open label non-randomised Phase 1b translational study to investigate the effect of the oncolytic virus GL-ONC1 administered intravenously prior to surgery on liver metastases in patients with metastatic colorectal cancer.
  
  PROTOCOL NUMBER: GL-ONC1-008
  
  SPONSOR: Genelux GmbH, Am Neuland1, D-82347 Bernried, Germany.

• A clinical study to evaluate the biological effects of pre-operative intravenous administration of JX-594 (thymidine kinase-deactivated Vaccinia virus plus GM-CSF) prior to planned surgical resection of locally advanced/poor prognosis or metastatic cancers.
  
  PROTOCOL NUMBER: CO12/10151
  
  SPONSOR: University of Leeds, Leeds, West Yorkshire

Future clinical trials in development
• A Phase 1 trial of intravenous herpes HSV1716 in solid tumours - this trial protocol is currently in development & is expected to open within the next year.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
Biological Safety Committee meeting on 11 June 212 - Study discussed at the BSC meeting held on 11 June 2012 and was approved in principle subject to:

i) Finalised SOPs for both studies
ii) An infection lead identified
iii) Ensure staff are informed of the possible risks of exposure before working with this virus.

Biological Safety Committee Minutes - 4 March 2013 - The study involves one dose of the GM with a 28 day follow up.

The virus is attenuated by the TK deactivation.
No toxicities are expected with paracetamol.
Vaccination history of the patients is unknown
Patients will stay overnight in hospital following their drug administration
PI to discuss this study with a specialist infectious diseases consultant to ensure a pathway is in place for advice and referral if required. SOP reviewed and amendments to be incorporated.

HSV1716
The planned HSV1716 clinical trial does not yet have a finalised & approved protocol, but is intended to be presented to the Biological Safety Committee later in the year.
Due to the nature of its genetic modification, i.e. only a single deletion & no insertions, we anticipate the risk assessment & containment strategies to be consistent with the other viral agents previously described.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
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<td>L3</td>
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<td>L2</td>
<td>L3</td>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L2</td>
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Project Ref 767/17.1

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<td>19/04/2017</td>
<td>A phase 3 randomized, open-label study comparing Pexa-Vec (Vaccinia GM CSF/Thymidine kinase-deactivated virus) followed by sorafenib versus sorafenib in patients with advanced hepatocellular carcinoma (HCC) without prior systemic therapy</td>
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<td>Non-GMM  Consent Granted</td>
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Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

Pexa-Vec is currently in clinical development for the treatment of Hepatocellular Carcinoma. The proposed contained use will be the administration of the investigational product, in a hospital or clinic setting, by intratumoral (IT) injections to patients as part of an international, multicenter clinical trial. This clinical trial is a Phase III trial in patients with Advanced Hepatocellular Carcinoma (HCC) without prior systemic therapy. Results from this pivotal trial will determine whether Pexa-Vec followed by sorafenib increases survival duration in advanced HCC patients compared to treatment with sorafenib alone, and whether sequential dosing with Pexa-Vec followed by sorafenib has a favourable safety profile.

Approximately 40 clinical sites in the EU will enroll patients in the JX594-HEP024 (PHOCUS) study. Additional clinical sites in Australia, Canada, China, Israel, Korea (Republic of), New Zealand, Singapore, Taiwan, Thailand and the USA will also participate in the study. A total of 600 patients will be recruited in this clinical trial with an expectation to enroll 200 patients in EU countries. In the control arm, the 300 patients will not receive Pexa-Vec. After study completion, all patients will be followed up for survival. Among them, 300 patients (Le. approximately 100 patients in EU) will receive Pexa-Vec by IT injections.

**Recipient or parental organism**

Pexa-Vec is a replicative oncolytic recombinant vaccinia virus (W) derived from the commonly used vaccine Wyeth strain, DryvaxTM.

W is a member of the Poxviridae family (genus Orthopoxvirus). Multiple strains of W exist that have different levels of virulence for humans and animals. The New York City Board of Health (NYCBOH) strain, from which the Wyeth strain of the Dryvax® vaccine was derived, has low pathogenicity in humans (Fenner F. et al., 1988).

W has a long and extensive history of use in humans. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous (SC) infection. As W contains antigens that stimulate an immune response that are cross-reactive with smallpox antigens, the vaccine thereby confers protection from the human smallpox disease. W may cause local reactions including erythema, edema and systemic reactions such as fever and malaise, as has been observed with conventional vaccination to smallpox. During the smallpox vaccination campaign, serious complications had occurred in less than 1 in 4,000 individuals, mainly in immunosuppressed and extremely young individuals. Pexa-Vec is even further attenuated as the thymidine kinase gene has been disrupted which makes replication in normal cells more difficult than the smallpox vaccine. Rare complications included eczema vaccinatum (patients with eczema), disseminated vaccinia rash, progressive vaccinia (in T-cell-deficient individuals) and encephalitis (1-2 per million vaccinated) (Fields B.N., 1996). Recent studies of smallpox vaccines have identified cardiac injury including pericarditis and myocarditis as a potential risk (Halsell J.S. et al., 2003).

W replication exclusively occurs in the cytoplasm thus eliminating any risk of integration of the viral DNA into the host genome (Moss B., 2007).
In terms of classification of hazard, W is considered as a Group 2 biological agent as per Directive (2000/54/EC). W is also classified as a Biosafety Level 2 (BSL-2) infectious substance by the US Centers for Disease Control and Prevention (CDC) (CDC, 2009) and as a risk group 2 organism by the US NIH Guidelines (NIH).

**Host/vector system**

Pexa-Vec was generated by co-transfection of CV-1 cells (Monkey kidney cells) with W (Wyeth strain obtained from the Center for Disease Control, Atlanta, Georgia) and the plasmid pSC65/hGM-CSF. The vector pSC65/hGM-CSF contains DNA sequences coding for the hGM-CSF and β3-galactosidase proteins and for their respective promoters. In addition, the transgene sequences are flanked by two W genomic regions (TKL and TKR) that allow homologous recombination between the transfer plasmid and W.

The plasmid pSC65/hGM-CSF is generated from the plasmid pSC65 which was provided by Dr. B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland (Chakrabarti 1997).

The plasmid pSC65/hGM-CSF is inserted into W. The insertion of pSC65/hGM-CSF into W can be detected by using colorimetric identification of plaques containing recombinants expressing β3-galactosidase.

The pSC65 vector when provided by Dr. B. Moss contains the LacZ gene. The LacZ gene is a reporter gene, under control of the W p7.5 early/late promoter. The additional donor gene (i.e. gene coding for hGM-CSF) is inserted in pSC65 as follows.

The plasmid pCSF-1 (No. 39754) was obtained from American Type Culture Collection and comprises the full-length cDNA for hGM-CSF (Wong 1985). The hGM-CSF gene was cloned first into the EcoR1 site of pBLUESCRIPTII SK (Stratagene, La Jolla, California), generating plasmid pBLUE/hGM-CSF, and providing restriction enzyme sites to allow cloning of the hGM-CSF gene into the Sail and Bgi II sites of pSC65. This positioned the hGM-CSF gene downstream of a synthetic promoter (PsE/L) designed by Dr Moss' laboratory to give maximal levels of transcription during both the early and late phases of vaccinia infection (Chakrabarti 1997).

**Origin & function**

Pexa-Vec contains three genetic modifications compared to the wild type Wyeth strain: 1) disruption of the viral thymidine kinase (TK) gene by, 2) insertion of the human granulocyte macrophage-colony stimulating factor (hGMCSF) gene and 3) insertion of the LacZ gene.

Due to the insertion of the transgenes, the TK gene is inactivated. This decreases W virulence (Buller R. et al., 1985) by restricting viral replication to proliferating cells. This also targets dissemination of the virus to tumors (Puhlmann M. et al., 2000).

The plasmid pCSF-1 (No. 39754, American Type Tissue Culture Collection, Rockville, Maryland) contains the full-length cDNA for hGM-CSF (Wong G.G. et al., 1985). The cDNA for the hGM-CSF gene was inserted into the TK gene to help elicit an immune response to tumor cells both at the site of viral replication and in distant metastases. The cytokine hGM-CSF was chosen because it was the most potent stimulator of systemic anti-tumor immunity among many tested (Dranoff G. et al., 1993), probably due to its unique ability to promote differentiation of hematopoietic precursors into dendritic cells (Pardoll D.M., 1995). Dendritic cells are professional antigen presenting cells that may take up and present released tumor antigens as the tumor cells are killed by the W.

The LacZ gene is contained in the pSC65 plasmid which was provided by Dr. B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

The LacZ gene encodes for β3-galactosidase which is a hydrolase enzyme that catalyzes the hydrolysis of 13-galactosides into monosaccharides.

Of note, following recombination between the W and pSC65/hGM-CSF, the antibiotic resistance gene contained in pSC65 is not part of the insert. The final GMO does thus not contain any genes conferring resistance to antibiotics.
Evaluation of foreseeable effects

Pexa-Vec is non-integrative, and replicative and propagative characteristics of W have been attenuated in Pexa-Vec, which makes the virus replication dependent on actively dividing cells such as cancer cells. Therapy with a replicating virus can theoretically lead to shedding of the virus into the environment, and potentially to the public, although controls are used in this trial to minimize this occurrence. The clinical information available to date suggests that Pexa-Vec is safe at the clinical dose of 1 x 10^9 pfu (10,000-fold higher than smallpox vaccine dose) and has not spread to caregivers in contact with the treated patients. Should shedding occur, the level of exposure would be predicted to be low compared to the doses received by patients in the proposed trial, and extremely low compared to doses of non-attenuated vaccines administered to the public (e.g. vaccines against smallpox). In addition, exposed individuals over the age of 35 will likely have been previously immunized with vaccinia. In the highly unlikely event that an exposed individual were to demonstrate virus-associated toxicity, therapy could be initiated with VIG and/or cidofovir. Therefore, public health risks with this virus are extremely low and in fact should be lower than with standard vaccination procedures. To date, no reports of transmission to health care personnel from vaccinia recipients have been published. Routine barriers nursing approaches should be used per institutional guidelines for infectious organisms (e.g. such as for M. tuberculosis, Pseudomonas).

The information regarding the risk to patient contacts and guidelines for reducing the risk of viral transmission is contained within the Participant Information Sheet and Consent Form (PISCF) and the Pexastimogene Devacirepvec (Pexa-Vec) Guidelines (provided in Appendix B of Pexa-Vec Investigator's Brochure). The PISCF will be reviewed with the patients, and their written consent will be obtained, before they undergo any study-specific procedures. A signed copy of the PISCF will be given to the patients so that they can come back to the guidelines at any time. Pexastimogene Devacirepvec (Pexa-Vec) Guidelines will be given to investigators, pharmacists, and all personnel involved in the handling of the product.

The genetic modification of the virus is not expected to result in any post-release shift in biological interactions or host range or in any known or predictable effects on non-target organisms in the environment. It is also not expected that the release of the recombinant virus would result in any increase in pathogenicity as compared to the parental virus strain and/or in any increase in the capacity to recombine with other related viruses. Therefore, under the conditions for use in the proposed clinical trial, Pexa-Vec is not considered to represent a risk for the environment and for the public health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials and devices (vials, stoppers, needles, gauze, disposable protective clothing etc) that come into contact with the vaccine will be discarded into a screw-capped jar containing approximately 150 mls Chlorclean solution. The jar will be sealed, rotated to disperse the Chlorclean solution, then placed inside a purple lidded sharps container to be disposed of in the designated Genetically Modified (GM) waste bin which will be provided. This will be disposed of as per L THT Standard Infection Prevention and Control Precautions Policy, L THT Waste Stream)
The GMSC discussed the risk assessment and noted that Pexa-Vec is a replicative oncolytic recombitant vaccinia virus (W) derived from the commonly used vaccine Wyeth strain, DryvaxTM. Pexa-Vec contains three genetic modifications compared to the wild type Wyeth strain, as below.

1. Disruption of the viral thymidine kinase (TK) gene
2. Insertion of the human granulocyte macrophage-colony stimulating factor (hGM-CSF)
3. Insertion of the LacZ gene

Pexa-Vec is designed to selectively replicate in and destroy cancer cells, while at the same time stimulating a systemic anti-tumoural immune response through the expression of its transgene, hGM-CSF, in the context of tumour lysis.

Pexa-Vec is currently in clinical development for the treatment of Hepatocellular carcinoma. The proposed release will be the administration of the investigational product by intratumoural (IT) injections to patients as part of an international, multicentre clinical trial. Results from this pivotal trial will determine whether Pexa-Vec followed by Sorafenib increases survival duration in advanced HCC patients compared to treatment with sorafenib alone and whether sequential dosing with Pexa-Vec followed by sorafenib has a favourable safety profile.

A small proportion of patients develop up to about 5 pustular lesions typical of vaccinia, however control procedures are in place and include covering any lesions with dressings.

The committee also reviewed the Standard Operating Procedure Document for the handling of JX-594 (Vaccinia GM-CSF / TK-deactivated virus) provided by the oncology clinical research department at St James's. Pregnant and breastfeeding women, individuals with exfoliative skin conditions and those who are immunocompromised should avoid direct contact with JX-594 and also patients being treated with such.

It was also acknowledged that the Trust has experience of handling GMO reagents. Dr Alan Anthoney (CoInvestigator) has previous experience ( albeit not handling) of JX-594

Dr Antony Hale, Consultant Virologist, as chair of the biological committee, confirmed on the 26 August 2016 that the required documentation, including GTAC approval had been reviewed and the study could proceed. Regular updates, usually on a quarterly basis, will be required by the committee.

Additional information covering 1) patient management of pustules & safe disposal of dressings, and 2) control of virus shedding is contained in the following three documents accompanying this notification:

ICF v2.5
Management of PV toxicities
Appendix S: Pexa-Vec Guidelines.

Project Containment
Project Additional Information

Purposes of the contained use

RP1 will be administered to subjects as an anti-tumour therapy, as part of a Phase I clinical trial to treat a wide range of solid tumour types. RP1 has a particular utility in combination with immune co-inhibitory pathway blockade. Intended indications to study include soft tissue sarcoma, breast cancer including triple negative breast cancer (TNBC), lung cancer including non-small cell lung cancer (NSCLC), melanoma, non-melanoma skin cancers, head and neck cancer, primary liver and kidney cancer and colorectal cancer. The initial clinical trial protocol intends to test RP1 in several indications as a monotherapy and in combination with anti-PD-1 therapy.

Recipient or parental organism

RP1 (rHSV-1hGM-CSF/GALV-GP) is a selectively replication competent Herpes Simplex Virus-1 (HSV-1). The virus contains a codon-optimised sequence for human granulocyte macrophage colony stimulating factor (hGM-CSF) and a codon optimised sequence for the gibbon ape leukemia virus surface glycoprotein (GALV-GP) with the R- sequence deleted (R- [GALV-GP-R-]. GALV-GP-R- expression leads to cell to cell fusion (syncytial) formation in infected tumour cells.
through binding to the constitutively expressed Pit-1 receptor for GALV. This results in the death of the cells by
membrane fusion and is also intended to enhance the spread of the virus through the tumour. Since the RP1
selectively replicates in tumour cells, the expression of the GALV-GP-R- is minimised in normal tissues. The oncolytic
destruction of tumour cells leads to the release of tumour associated antigens that are intended to engender an antitumour
immune response, enhanced by the local expression of GM-CSF. This is intended to be further enhanced
through GALV-GP-R- mediated killing, fusion associated cell death which also results in the production of the highly
immunogenic exosomes, which is expected to contribute to this immune effect. The immune response generated may
then lead to immune destruction of distant, un injected tumours, and/or delay the progression of distant disease,
and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

### Host/vector system

RP1 (rHSV-1hGM-CSF/ GALV-GP) is derived from the RH018A strain of Herpes Simplex Virus-1. RP1 is produced in the
Vero cell and released into the culture media during cell lysis, prior to purification.

### Origin & function

RP1 was constructed using a new strain of HSV-1 (strain RH018A). Replimune obtained and compared 30 clinical
strains of HSV-1 on a panel of human tumour cell lines and selected the most promising of these (strain RH018A) for
further development.

RP1 expresses the immune stimulatory protein GM-CSF, which augments therapeutic activity.

GALV-GP-R- binds to the Pit1 receptor, which is widely expressed on mammalian cells including human tumour
cells. Pit1 is also critical for cell proliferation, and its expression is therefore unlikely to be lost or down-regulated in
response to cancer treatment. The truncated R- version of the protein provides constitutive fusion activity without
GALV (i.e. the virus) itself. Expressing GALV-GP-R- together with GM-CSF is expected to increase clinical activity as
compared to only expressing GM-CSF. As well as causing direct tumour cell death by cell to cell fusion, cell to cell
fusion followed by death is highly immunogenic and includes the release of immunogenic tumour antigen-containing
exosomes. Expression of GALV-GP-R- from an oncolytic virus is therefore expected to improve systemic, immune
mediated, effects, as well as effects in the directly treated tumour thereby increasing synergy with other immunemediated
approaches to cancer therapy such as immune co-inhibitory pathway blockade.

### Evaluation of foreseeable effects

As described above (under Recipient or Parental Organism), the oncolytic destruction of tumour cells (upon
transduction with RP1) leads to the release of tumour associated antigens that are intended to engender an antitumour
immune response, enhanced by the local expression of GM-CSF. This is intended to be further amplified
through GALV-GP-R- mediated killing, fusion associated cell death which results in the production of the highly
immunogenic exosomes and is expected to contribute to this immune effect. The immune response generated may
then lead to immune destruction of distant, un injected tumours, and/or delay the progression of distant disease,
and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| No derogation is requested |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All used and unused RP1 and diluent vials used in preparation and syringes will be destroyed per institutional policy. As per the wild-type HSV-1 virus, the recombinant HSV-1 vector particles that represent RP1 are highly susceptible to dehydration, rapidly inactivated outside the host and easily inactivated (for example with 1% Virkon solution).

**Phase 1**

As part of phase I of the clinical trial, biodistribution and shedding will be monitored. RP1 DNA levels in blood and urine will be determined at time-points outlined in the Schedule of Assessments of the clinical protocol, (i.e. day 1, day 2, day 3, day 15, day 16, day 17, day 29, day 30, day 31, , day 43, day 57, day 71, day 85, day 99 and as part of the follow up, 30 days and 60 days after the last dose of RP1).

- Blood, saliva/oral mucosa, urine samples and injection site dressing swabs will be collected at the first, second and third RP1 injections at the following time points: pre-dose, 6 (+/-2hr) hours, 21 hours (+/-3hr) and 48 hours (+/-6hr) and also immediately prior to dosing at fourth and fifth dose.
- Specimens (swabs) will be collected at any time there is a suspicion of RP1-related viral infection occur such as vesicular eruptions or other signs of herpes viral infection. Samples should be obtained as soon as possible after symptoms arise to maximize the possibility of detection of virus, optimally within 24 hours, but samples may be collected later if collection within 24hrs is not possible. Subjects will be asked to take swabs at home for the subsequent 7 days after the initial test.

**Phase 2**

As part of phase 2 portion of the clinical trial, biodistribution and shedding will be also monitored.

- RP1 DNA levels in Blood, Saliva/oral mucosa, urine samples and injection site dressing swabs will be collected pre-dose at the first through last RP1 injections (cycles 1 to 8). Additionally, for each of the tumour types enrolled, the first six patients the following additional time points will be required: Doses 1, 2 and 3: 6 (+/-2hr) hours, 21 (+/-3hr) and 48 (+/-6hr) hours and also immediately prior to dosing at the fourth through eighth injections. Samples on injection days will be collected prior to any injections and handling of RP1.
- Samples will also be collected at the 30 and 60 day follow-up visits.
- Specimens (swabs) will also be collected at any time there is a suspicion of RP1-related viral infection occur such as vesicular eruptions or other signs of herpes viral infection.

In phases 1 and 2 of the study, samples on injection days will be done prior to any injections and handling of RP1.

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
Genetically modified vaccinia virus (VV TK-RR-/FCU1) used as advanced therapy medicinal product for the treatment of colorectal cancer

Recipient or parental organism

The recipient is the vaccinia virus (VV) Copenhagen strain, which was selected for TRANSGENE’s oncolytic virus program as one of the most lytic strains. The virus was previously used for smallpox vaccination in Denmark and the Netherlands.

VV is considered a minor pathogen for humans (Dumbell K.R., 1985) and is classified as a biological agent of risk group 2 as per Directive 2000/54/EC. VV has the ability to infect a wide range of human tissues, but do not cause any known human disease. VV replication exclusively occurs in the cytoplasm thus eliminating any risk of integration of the viral DNA into the host genome (Moss B., 2007). VV does not produce a latent infection, so once the infection arises, the virus is rapidly cleared from the host.

Vaccination with VV was associated with mild to severe adverse events. Mild vaccine reactions included skin lesions, fever, myalgia, local lymphadenopathy, fatigue, headache, nausea, rashes, and soreness at the vaccination site (Belongia E.A. and Naleway A.L., 2003). Severe vaccination complications were extremely rare and
included death (1 per million), progressive vaccinia (1.5 per million), postvaccinal encephalitis (12 per million), eczema vaccinatum (39 per million), and generalized vaccinia (241 per million vaccinated) (Lane J.M. et al., 1970). A statistically significant increased risk of myo/pericarditis (1-2 per 10,000 vaccinees) was reported more recently (Arness M.K. et al., 2004). The great majority of the severe AEs occurred in “at risk” subjects including children <12 months of age, severely immunocompromised individuals (e.g. organ transplant recipients, HIV-positive individuals, or those receiving chronic immunosuppressive medication) and people with inflammatory skin conditions (e.g. eczema requiring previous treatment, atopic dermatitis, etc.). In addition, very rare cases of fetal vaccinia were reported during pregnancy and there is theoretical risk of transmission from breastfeeding women to their nursing infant. People from these “at risk” groups will be excluded from study participation as patients and as healthcare and housekeeping workers.

There are 2 deletions in VV genome to produce TG6002 (i.e. deletions of TK and RR genes). The deletion of the TK and RR activities conditions TG6002 replication to highly dividing cells such as cancer cells and considerably reduces the pathogenicity of the recombinant virus compared to its parental virus in mice. Therefore, the use of an attenuated recombinant VV such as TG6002 for treatment of advanced cancer in humans is expected to show an equal or better safety profile than the one of the non-attenuated parental virus during smallpox vaccination campaigns.

No environmental concern was raised during the smallpox eradication program during which hundreds of millions of people were administered with the non-attenuated parental virus of TG6002. The proposed contained use will be the administration of TG6002, in a hospital or clinic setting, by intrahepatic artery infusions to patients with colorectal cancer. Dispositions have been taken in the proposed clinical trial to avoid dissemination and inadvertent transmission of TG6002 and the hospital study staff is experienced in measures/precautions with infectious agents. It is therefore not expected that the use of TG6002 within the proposed clinical trial conditions would result in any environmental effect.

**Host/vector system**

TG6002 dsDNA virus (VV TK-RR-IFCU1) is derived from the VV Copenhagen strain (modifications include the deletions of TK and RR genes as well as the insertion of chimeric FCU1 gene).

**Origin & function**

TG6002 is the vaccinia virus Copenhagen strain in which three genetic modifications have been performed: 1) deletion of the thymidine kinase (TK) gene, 2) deletion of the ribonucleotide reductase (RR) gene, and 3) insertion of the chimeric yeast FCU1 gene in the TK locus. The vaccinia TK and RR genes were deleted to allow selective replication in cancer cells in vivo. The FCU1 gene results from the fusion of two Saccharomyces cerevisiae loci, FCY1 and FUR1 which encode for the cytosine deaminase (CDase) and uracilphosphoribosyltransferase (UPRTase) proteins, respectively. The FCU1 protein expressed by the FCU1 gene is a bifunctional enzyme with CDase and UPRTase activities. FCU1 catalyzes the conversion of the prodrug flucytosine (5-FC) into the toxic chemotherapeutic 5-fluorouracil (5-FU) and 5-fluorouridine monophosphate (5-FUMP) agents.

**Evaluation of foreseeable effects**

TG6002 is designed to have a multi-mechanistic mode of action consisting of infection and selective lysis of tumour cells through direct viral replication (oncolysis), targeted chemotherapy through in situ conversion of 5-FC into 5-FU and 5-FUMP and regression of local and distant tumours by systemic immune response. Some of the VV inherent biological properties (e.g. natural tropism for cancer cells, high efficiency at spreading to distant tumours) have been improved through genetic engineering in TG6002 which makes TG6002 a treatment candidate for tumours with high systemic spread, short doubling time and remote metastases. TG6002 is going to be administered as a viral suspension by a local route (intrahepatic artery (IHA) route) in combination with the oral 5-FC prodrug in patients with advanced and metastatic colorectal cancer refractory to standard treatments.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation is requested

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Disposable material and equipment contaminated by TG6002 (e.g. used and unused vials, injected and non-injected infusion bags/syringes, catheters, needles, tubing, gauze, dressings, gloves) will be discarded into a ‘sweet jar’ containing 150mL of a chlorine releasing solution at a minimum of 6000ppm, sealed and rotated to disperse the chlorine solution and denature the virus. The ‘sweet jar’ is disposed of inside a large, purple lidded sharps container (double seal) and placed in a designated Genetically Modified (GM) waste bin prior to LTHT waste incineration process. NB: All waste arising from the preparation and treatment delivery within this study will be managed as above.

Non-disposable material contaminated by TG6002 (e.g. labcoat, goggles, patient gown, bedding, linens, towels) will be cleaned/treated according to regular hospital procedure for infectious material. All equipment in the pharmacy biosafety cabinet not dedicated for study use will be sterilised or cleaned with an active disinfectant (i.e. a chlorine solution at 0.6% of active chlorine) before using it for other purposes. All non-critical patient-care equipment and medical devices will be cleaned with a hospital-grade disinfectant solution before use on another patient, as per universal precautions/routine practices.

Accidental spillage - In the case of accidental spillage, all liquid will be soaked up using absorbent granules (e.g. Sochlor or equivalent granules) found within the Biohazard Spillage Kits. A chlorine solution at a concentration of 10,000 parts per million (ppm) (e.g. HazTab or Sochlor tab or equivalent) will be used on contaminated surfaces to denature the virus.

Please enter comments on the GM safety committee on the risk assessment

This study has been reviewed by Leeds Teaching Hospitals NHS Trust Biological Safety Group and approved on 22/08/2019. The completed documentation is appended to this form for information and confirmation of review.

Project Containment

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Project Ref 767/21.1

Date Ackn’d 12/11/2021

CU2 Project Title Gene therapy product VCN-01 [genetically modified human adenovirus serotype 5]

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM
Consent Granted
Not Applicable

Project notified under transitional arrangements

Withdrawn
Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purpose of the contained use

The product will be given to subjects as part of a Phase Ib clinical trial evaluating the biological effects of intravenous administration of VCN-01 in patients prior to surgical resection of high-grade brain tumours. Taking into account the naturally lytic replication cycle of adenoviruses together with the genetic modifications inserted in VCN-01 backbone, its biological activity is considered to offer a novel mechanism of action in the field of anticancer therapies, and is claimed to be based on two factors: (1) the selective killing of tumour cells by effect of its replication, which results in a self-amplification of the initial inoculum that is released after cell lysis and spread through the tumour mass, and (2) the expression of hyaluronidase, which degrades partially the extracellular matrix of the tumour facilitating the diffusion of the virus progeny and increasing the accessibility of chemotherapy and other drugs such as therapeutic antibodies.

The primary objectives of the trial are to assess the presence of VCN-01 within the resected surgical specimen and to determine the safety of VCN-01 administration.

Recipient or parental organism

VCN-01 is derived from the wild-type human adenovirus serotype 5 (HAd5). VCN-01 is produced in lung adenocarcinoma cell line A549 and released into the medium by cell lysis after 48h after infection. The cell lysate is purified throughout several steps to obtain VCN-01 at the defined concentration and formulated using a storage buffer (20mM Tris, 25mM NaCl, 2.5% glycerol).

Host/vector system

VCN-01 was constructed using wild type human adenovirus serotype 5 (HAd5).

Origin & function

VCN-01 expresses human sperm hyaluronidase (PH20), a matrix-degrading enzyme that allows reducing interstitial fluid pressure within the tumour mass (thus enabling unique tumour remodelling properties, that improve intratumoural viral spreading, immune system infiltration, and tumour uptake of drugs). The other modifications include:

1) the insertion of a tumour-specific synthetic promoter (corresponding to the E1A endogenous promoter region) that restricts the expression of viral proteins to tumoral
cells;
2) the partial deletion of the E1A gene that inhibits the viral replication in normal cells;
3) modification of adenovirus fiber protein (RGDK substitution of KKTK) that results in improved tumour infectivity and lower liver infection.

**Evaluation of foreseeable effects**

VCN-01 is designed to have a multi-mechanistic mode of action consisting of selective infection and lysis of tumour cells through direct viral replication and expression of PH20 hyaluronidase - the enzyme enabling unique tumour remodelling properties that improve viral spreading, infiltration of immune system and tumour uptake of drugs. Once the virus reaches and infects the tumour cells, its lytic nature enables the efficient generation of tumour neoantigens that can boost the innate immune response. In addition, the capsid of VCN-01 has been modified to ensure the virus evades liver tropism and selectively targets the tumour.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Derogation in respect of initial requirement to apply containment measure pursuant to the Regulation 24 of Genetically Modified Organisms (contained use) Regulations 2014 The request concerns derogation from applying the containment measure: autoclave - required in the building where GMO agents will be administered at LTHT premises.

We are conducting a number of clinical trials with use of viruses classified at level 2 containment, but we do not have direct access to autoclave facilities in our clinical locations. We would like to request an exemption from having the autoclave in the building due to the type of activities performed and the low amount of waste generated. None of the current and future studies would require culturing, concentration or modification of the viruses at our site. We are solely involved in the final preparation and administration of GMO products already pre-packaged by the supplier and the main risk is due to direct contact (vials, needles, study drug bag containing virus, swabs, etc.) following virus administration. We would like to continue to use chemical inactivation methods at Leeds Teaching Hospitals NHS Trust for all the activities involving GMOs classified at level 2 of containment.

The virus inactivation procedure itself does not require the use of, or access to, an autoclave, but does ensure that material contaminated with GM Virus is inactivated in a consistently safe and effective way. All GMO trials conducted at our site are considered carefully, and the appropriate chemical GMO inactivation method is established by ATMP Oversight and Assurance Group based on the available scientific data provided by suppliers. Arrangements for handling and disposal of waste are always clearly defined for each clinical trial in our local standard operating procedures.

Referring to the notification we developed the adenovirus inactivation procedure with use of chlorine releasing solution as per SOP- DH-20.1.0 submitted with notification. We believe this proposal shows methodology, which is safe and adequate from a risk management perspective.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Disposable material and equipment contaminated with VCN-01 agent (e.g. used and unused vials, injected and non-injected infusion bags/syringes, needles, dressings, gloves) will be discarded into a "sweet jar" containing chlorine solution at a minimum of 10000ppm, sealed and rotated to disperse the chlorine solution and denature the virus. The jar is to be disposed of inside a large, purple lidded sharps container (double seal) and to be disposed of according to current LTHT Waste Policy NB: All waste arising from the preparation and treatment delivery within this study will be managed as above (LTHT Standard Infection Prevention and Control Precautions Policy, LTHT Waste Stream).

Non-disposable material contaminated with VCN-01 (e.g. labcoat, googles, patient gown, bedding, towels) will be cleaned/treated according to regular hospital procedure for infectious material. All equipment in the pharmacy biosafety cabinet not dedicated for study use will be sterilised or cleaned with an active disinfectant (i.e. chlorine solution at 0.6% of active chlorine) before using it for other purposes. All non-critical patient care equipment and medical devices will be cleaned with a hospital-grade disinfectant before use on another patient, as per universal precautions/routine practices.
Accidental spillage— in the case of accidental spillage, all liquid will be soaked up using absorbent granules (e.g. Sochlor or equivalent) found in the Biohazard Spillage Kits. A chlorine solution at a concentration of 10000 ppm will be used on contaminated surfaces to denature the virus (e.g. Haz Tabs, Sochlor or equivalent).

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

This study has been reviewed by Leeds Teaching Hospitals NHS Trust Advanced Therapy Medicinal Products ATMP Oversight and Assurance Group on 13th July 2021. The completed documentation is appended to this form for information and confirmation of review.

Project Containment

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Project Ref 767/22.1

Date Ackn’d 16/02/2022

CU2 Project Title

T3P - Y058-739 is a live attenuated bacterium that has been genetically modified. The product is currently being developed as an anticancer therapy in solid tumours as an oncolytic bacteria, to be delivered to cancer patients within a first-in human clinical trial

Date Project Ceased

Consent Granted  
Non-GMM

Class 2

Class CultureVol  
< 1 Litre

Class CultureVolume  
Class 2

Class 3-4

Project notified under transitional arrangements  
N

Tick if notifying a connected programme of work  
N

Historical Significant Changes
Project Additional Information

Purposes of the contained use

T3P-Y058-739 (T3P), a genetically-modified strain of the bacterium Yersinia enterocolitica, will be used to treat patients with cancer (advanced solid tumours) within an open-label, phase I/II clinical trial. T3P is a live attenuated bacterium that has been modified to abrogate pathogenicity and to enable the bacterium to micro-inject surrounding eukaryotic cells with two human immunomodulatory proteins: retinoic acid-inducible gene-1 caspase recruitment domain (RIG-I CARD) and cyclic GMP-AMP synthase (cGAS). These stimulate the production of type I interferons and other cytokines. In addition to delivery of the cargo proteins, the T3P bacteria themselves stimulates the production of potent pro-inflammatory cytokines, especially tumour necrosis factor-α (TNFα). Thus, the presence of T3P in tumours is anticipated to result in stimulation of a strong immune response to the tumour. T3P will be administered either by intratumoural injection or intravenously by infusion over 2 hours.

Recipient or parental organism

T3P-Y058-739 has been derived by genetic engineering from the parental strain Yersinia enterocolitica E40. This strain was isolated in the early 1990s in Belgium from a human patient. Y. enterocolitica E40 belongs to a low-virulent subspecies of Yersinia enterocolitica, called “Palearctica”, which lacks the high pathogenicity island (HPI) coding for the siderophore Yersiniabactin (Ybt).

Host/vector system

The host range is described to be limited to E. coli, selected members of the family Enterobacteriaceae and few other organisms including Serratia marcescens and Yersinia. T3P-Y058-739 has been derived by genetic engineering from the parental strain Yersiniaenterocolitica E40. Y. enterocolitica E40 belongs to a low-virulent subspecies of Y.enterocolitica Palearctica that are characterised by deficient iron acquisition. This deficiency is maintained in T3P-Y058-739. In addition, the following genetic modifications have been introduced to further reduce virulence and to confer the strain with its therapeutic activity.

Due to the deletion of all six effector proteins, the virulence of T3P-Y058-739 is dramatically reduced compared to its parental strain Y. enterocolitica E40.

Evaluation of foreseeable effects

Therapy with T3P is expected to be well tolerated. The main side effects are expected to be local inflammation following intra-tumoural (IT) injection, and transient pyrexia and ‘flu-like’ symptoms related to systemic endotoxin exposure following intravenous (IV) administration.
It is anticipated that T3P will result in anti-tumour activity when administered as monotherapy by IT injection or by IV infusion (IT injection will be evaluated initially). T3P is also predicted to have additivity or synergy with checkpoint inhibitors (CPIs), and evaluation of T3P in combination with the CPI pembrolizumab is also planned.

T3P is a live attenuated bacterium that has been modified to remove pathogenicity and to enable the bacterium to micro-inject surrounding human cells with two human immunomodulatory proteins (RIG-I CARD and cGAS). These proteins stimulate the production of type I interferons and other cytokines which attract and activate cells of the immune system. Microinjection of the cargo proteins by T3P takes place via the naturally occurring bacterial type 3 secretion system (T3SS). The T3SS looks and acts like minute syringes (sometimes called nano-syringes or injectisomes) on the surface of the bacteria. Microinjection of cargo proteins may occur very rapidly (within minutes) of T3P being introduced into a tumour by local intra-tumoural (IT) injection. It can also occur more slowly, as tumours become colonised by T3P following intravenous (IV) administration.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation in respect of initial requirement to apply containment measure pursuant to the Regulation 24 of Genetically Modified Organisms (contained use) Regulations 2014 The request concerns derogation from applying the containment measure: autoclave - required in the building where GMO agents will be administered at LTHT premises.

While we have not conducted studies using a GMO bacteria before, are delivering a number of clinical trials which use viruses classified at level 2 containment. We do not have direct access to autoclave facilities in our clinical locations. We would like to request an exemption from having the autoclave in the building due to the type of activities performed and the low amount of waste generated. None of the current and future studies would require culturing, concentration or modification of the viruses at our site. We are solely involved in the final preparation and administration of GMO products already pre-packaged by the supplier and the main risk is due to direct contact (vials, needles, study drug bag containing virus, swabs, etc.) following virus administration. We would like to continue to use chemical inactivation methods at Leeds Teaching Hospitals NHS Trust for all the activities involving GMOs classified at level 2 of containment.

The virus inactivation procedure itself does not require the use of, or access to, an autoclave, but does ensure that material contaminated with GM Virus is inactivated in a consistently safe and effective way. All GMO trials conducted at our site are considered carefully, and the appropriate chemical GMO inactivation method is established by ATMP Oversight and Assurance Group based on the available scientific data provided by suppliers. Arrangements for handling and disposal of waste are always clearly defined for each clinical trial in our local standard operating procedures.

Referring to the notification we developed the GMO Bacteria inactivation procedure with use of chlorine releasing solution as per the T3P IMP handling SOP submitted with notification. We believe this proposal shows methodology, which is safe and adequate from a risk management perspective.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable material and equipment contaminated with the T3P agent (e.g. used and unused vials, injected and non-injected infusion bags/syringes, needles, dressings, gloves) will be discarded into a “sweet jar” containing chlorine solution at a minimum of 10000ppm, sealed and rotated to disperse the chlorine solution and denature the virus. The jar is to be disposed of inside a large, purple lidded sharps container (double seal) and to be disposed of according to current LTHT Waste Policy NB: All waste arising from the preparation and treatment delivery within this study will be managed as above (LTHT Standard Infection Prevention and Control Precautions Policy, LTHT Waste Stream).

Non-disposable material contaminated with T3P(e.g. labcoat, googles, patient gown, bedding, towels) will be cleaned/treated according to regular hospital procedure for infectious material. All equipment in the pharmacy biosafety cabinet not dedicated for study use will be sterilised or cleaned with an active disinfectant (i.e. chlorine solution at 0.6% of active chlorine) before using it for other purposes. All non-critical patient care equipment and medical devices will be cleaned with a hospital-grade disinfectant before use on another patient, as per universal precautions/routine practices.
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Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick to confirm that it is attached to this form N

Please enter comments on the GM safety committee on the risk assessment

This study has been reviewed by Leeds Teaching Hospitals NHS Trust Advanced Therapy Medicinal Products ATMP Oversight and Assurance Group on 16th
The completed documentation is appended to this form for information and confirmation of review.

Project Containment

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Name

MANCHESTER UNIVERSITY NHS FOUNDATION TRUST

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

OXFORD ROAD

District

Town

MANCHESTER

County

GREATER MANCHESTER

Postcode

M13 9WL

Country

ENGLAND

Tel Number

0161-276-4262

Fax Number

0161-276-4230

E-mail

Comments

Formerley Central Manchester Healthcare NHS Trust

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities
- Y

Give brief details of the genetic modification safety committee

Central Manchester University Hospitals NHS Foundation Trust (CMFT) has incorporated the Genetic Modification Safety Committee into its “Early Phase Safety Committee (EPSC)” co-opting appropriate specialists onto the committee when a GM trial is being reviewed. The risk assessment has been expanded to include a Genetic Modification section encompassing GMM pathway, Patient pathway and Waste pathway according to guidance in the SACGM Compendium:

<table>
<thead>
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<th>Level 1 (GMMs)</th>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Waste will be treated as 'clinical waste' with incineration in a licensed incinerator. Transported using a registered waste carrier.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The local Biological Safety Officer signed that "I certify that the local GM Committee has fully approved the risk assessment and other details submitted on this application."
The sheet is also signed as "I approve the application" by the University BSO and the Chairman of the University GM Safety Sub-committee.
### GM Centre Number: 769

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#### Name

MOBIOUS GENOMICS LTD

#### Name 2

UNIVERSITY OF EXETER

#### Department

THE INNOVATION CENTRE

#### Road Name

RENNES DRIVE

#### Town

EXETER

#### District

DEVON

#### County

EX4 4RN

#### Country

ENGLAND

#### Tel Number

08700 112500

#### Fax Number

08700 112600

#### E-mail


#### HSE Division

WALES AND SOUTH WEST

#### Comments


#### Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Four doctors, 2 from University of Exeter, 1 from Mobious Genomics Ltd and 1 from jointly the University and Genomics

<table>
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<tr>
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Tick if confidential

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</table>
Inactivation of GMMs will be by autoclaving at 121°C for 30 mins, resulting in effectively 100% kill, for all containers, supernatants, tips and contaminated liquid containers for 2 to 5 days prior to autoclaving.

Chemical inactivation of GMMs on surfaces or small scale spills with DEDSAN red label hyperchlorite for greater than 2 minutes according to manufacturers instructions.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

ACDP level of microbial host = 1
Microbial host disabled
Vector mobilisation defective
Access score exponents = -6
Insert DNA from prokaryotes
Expression score exponent = 0
Proteins: DNA polymerases and associated replication factors
Damage score exponent = -9
Overall score exponent with respect to human health = -15
Provisional containment level 1
Likelihood of environmental hazard effectively zero
Consequence of environmental hazard: negligible
Risk to environment effectively zero
Class 1 designation

Molecular resonance sequencing
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**Name**

KS BIOMEDIX HOLDINGS PLC

**Department**

DEPARTMENT OF RESEARCH AND DEVELOPMENT

**Campus Estate or Research Centre**

SURREY RESEARCH PARK

**Road Name**

GROUND FLOOR 1 OCCAM COURT

**Town**

GUILDFORD

**County**

SURREY

**Postcode**

GU2 7HJ

**Country**

ENGLAND

**Tel Number**

01483 307500

**Fax Number**

01483 307501

**E-mail**

info@ksbiomedix.com

**HSE Division**

EAST AND SOUTH EAST

**Comments**

COMPANY CLOSED on 13/7/2004

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

GMSC will meet a minimum of twice per year to review existing risk assessments and arrange for audits of the laboratories. Meetings to approve new risk assessments will be held as required. The GMSC activities are reported to the main Health and Safety Committee.

GMSC made up of:
- Chairman and Health and Safety Committee representative
- Biological Safety Officer (Safety Consultant to company)
- Deputy Biological Safety officer (local)
- Scientific/technical staff representative (Molecular Biologist)
- Management representative (Director of R&D)
- Advisory persons (not committee members)
- Supervisory Medical Officer
- Occupational Health Advisor

<table>
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</table>
Inactivation of GMMs in contaminated material will be as follows:

**Liquid Discharge:** Waste to be treated with 1:1 Trigene (Halogenated tertiary amine compound) to a final concentration of 1% v/v. Trigene is MAFF approved and conforms with British and European Standards (CE93/42/EEC) with a 99.999% degree of kill.

**Solid Waste:** Solid waste will be sealed, retained in Biohazard waste sacks (double bagged) and further contained in UN approved rigid containers. Solid waste will be removed for incineration off-site by a nominated commercial 'clinical waste' carrier in accordance with all relevant legislation and guidelines. Degree of inactivation is 100%.

Please enter comments of the GM safety committee on the risk assessment

The GMSC has reviewed the company's GM procedures and the risk assessments performed. The GMSC confirmed the work to be carried out is Class 1 and were satisfied with the assessment of risks to both human health and the environment. The control and containment measures in place are appropriate for Class 1 activities.
### GM Centre Number: 771

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**Name**

EXCELL BIOTECH LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

PENTLANDS SCIENCE PARK

**District**

PENICUIK

**Town**

EDINBURGH

**County**

**Postcode**

EH26 0PZ

**Country**

SCOTLAND

**Tel Number**

0131-445-6231

**Fax Number**

0131-445-6232

**E-mail**

info@excellbiotech.com

**HSE Division**

WALES AND SOUTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Excell Biotech GMSC meets monthly (or as required) and consists of: Operations Director, Biological Safety Officer, Virology Team Leader, Production Manager, MSL, Extra committee members may be included as required.

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</table>

Tick if confidential

Bacteriology | Yes
Parasitology | |
Transgenic Birds | |
Microbiology Research | |
Adenovirus is very susceptible to the disinfectants which will be used at Excell Biotech (2% virkon, 1% hypochlorite, 2% trigene, keride A or B). As well as widespread use of the above disinfectants, all contaminated or potentially contaminated material will be autoclaved at 121°C for at least 15 minutes prior to disposal. 100% kill expected. All disinfectants used have been validated by the manufacturer against adenovirus (or similar), and the autoclave efficacy is monitored regularly using a variety of biological and chemical integrators.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The GMSC found no real problems with the risk assessment. They asked for more information on the genetic insertion. This has been included in section 17.
**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To produce adenovirus bank to cGMP.

**Recipient or parental organism**

Human adenovirus type S, which has 1 gene deletion and 1 gene insertin. These genetic modifications impair the ability of the adenovirus to infect normal human cells.

**Host/vector system**

Fully validated NCI-HS20 cells and SW620 cells. These cell lines are tumour-forming in nude mice in vivo. They are cells of human origin.

**Origin & function**

There is an insertion of a cell-cycle regulated promoter which renders replication of the virus specific to tumour cells. The material will be used in Phase 1 clinical trials.

**Evaluation of foreseeable effects**

The pathogenicity of this virus has been reduced more than 100x by the genetic modification. It is highly unlikely that this virus could establish a productive in vivo infection in humans. There are no zoonoses for human adenovirus, and no vectors, so environmental spread would be limited in the event of an escape.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

none

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Adenovirus is very susceptible to the disinfectants which will be used at Excell Biotech (2% virkon, 1% hypochlorite, 2% trigene, kericide A or B). As well as widespread use of the above disinfectants, all contaminated or potentially contaminated material will be autoclaved at 121°C for at least 15 minutes prior to disposal. 100% kill expected. All disinfectants used have been validated by the manufacturer against adenovirus (or similar), and the autoclave efficacy is monitored regularly using a variety of biological and chemical integrators.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GMSC found no real problems with the risk assessment. They asked for more information on the genetic insertion. This has been included in section 17.

**Project Containment**

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<td>L2 L3 L4</td>
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**Project Ref** 771/02.1

- **Date Ackn’d**: 29/04/2002
- **CU2 Project Title**: GMP PRODUCTION OF AN ADENOVIRUS VECTOR IN 293 CELLS.
- **Class**: Class 2
- **Culture Volume Class 2**: < 1 litre
- **Non-GMM**: not applicable
- **Consent Granted**: N

- **Date Project Ceased**: 01/08/2006
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**: 29/04/2002

**Project Additional Information**

- **Purposes of the contained use**: Production of material for a clinical trial.

- **Recipient or parental organism**: Adenovirus construct with E1 and E3 region deletions. The adenovirus vector contains an insert coding for the expression of an immuno-conjugate (Icon) molecule. The Icon molecule is composed of a mutated mouse factor VII (mfVII) targeting domain and the Fc domain of a human IgG1 molecule. The vector is constructed using the Q

02/03/2022
biogene Ad-easy system.

**Host/vector system**

Host cell: 293A cells (transformed human embryonic kidney cell line). Vials to be used sourced from ATCC originally and GMP bank prepared by BioReliance, Rockville, Maryland, USA. This bank is a fully validated cell bank and is regarded as being non-pathogenic to humans.

**Origin & function**

Direct injection of the adenoviral vector into tumour causes tumour cells infected by the vector to synthesize and secrete the icon into the blood. The blood-borne icon binds with high affinity to tissue factor expressed on endothelial cells lining the lumen of the tumour vasculature. The Fc domain of the icon activates a cytolytic immune attack against the tumour vasculature causing regression and death of the tumour cells.

The purified replication-deficient adenovirus vector is to be produced as a GMP virus bank and it is intended for use in a clinical trial.

**Evaluation of foreseeable effects**

The virus will be handled under full class II containment in a GMP clean room environment.

No foreseeable effects in case of escape. As the virus is a replication-deficient vector and adenoviruses have no zoonoses or vectors which would limit any environmental spread in the unlikely event of an escape. All contaminated or potentially contaminated material will be autoclaved at 121 degrees C for at least 15 minutes prior to disposal. Adenovirus is shown to be completely inactivated by autoclaving under these conditions. Also, the disinfectants routinely used by Excell laboratory staff during production procedures are all known to be virucidal with respect to adenovirus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Virus will be handled under Class II containment.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Although wild-type adenovirus may be able to survive for sometime in the environment, any reversion of the vector to the wild-type is unlikely. There are no zoonoses for human adenovirus and no vectors which would therefore limit environmental spread in the unlikely event of an escape.

All handling of the adenovirus vector and 293 cells infected with the vector will be performed under Class II containment conditions in a validated GMP clean room.

Adenovirus is susceptible to all disinfectants used routinely by staff during production (e.g. Kericide A or B, 2% Virkon, 2% Trigene, 1% Sodium Hypochlorite followed by 70% IPA). All contaminated and potentially contaminated material will be autoclaved at 121 degrees C for at least 15 minutes prior to disposal. Decontamination procedures are documented in the Company Standard Operating Procedures.

Autoclave efficiency is regularly monitored using a combination of chemical and biological indicators. Each run performed on the autoclave is checked to ensure that the correct temperature has been reached. All disinfectants used have full validation certificates on their action on adenoviruses.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
To follow: Risk assessment forwarded to the Moredun GM Safety Committee. Internal Safety Committee at Excell Biotech see no problem for Excell employees in using this product as a result of their training and the facility they will be working in.

**Project Containment**

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**Project Ref** 771/03.1

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<td>GROWTH OF A VIBRIO SPECIES EXPRESSING THE B SUBUNIT OF E.COLI ENTEROTOXIN INTO CELL CULTURE SUPERNATANT. DEVELOPMENT OF THE CELL CULTURE, PRIMARY RECOVERY AND PURIFICATION PROCESS</td>
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<tbody>
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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**
### Purposes of the contained use

Growth of a non pathogenic vibrio species up to 50L fermentation volume expressing the non toxic B subunit of E. coli enterotoxin and recovery of the expressed protein from the cell culture supernatant.

### Recipient or parental organism

Marine vibrio strain (considered non pathogenic to humans)

### Host/vector system

An IPTG induced expression vector carrying the gene for the non-toxic B subunit of E. coli enterotoxin.

### Origin & function

E. coli Enterotoxin B subunit expressed in a vibrio species.

Therapeutic uses,
- Allergy eg Allergic Rhinitis
- Autoimmune Disease eg Rheumatoid Arthritis
- Vaccine Adjuvant

### Evaluation of foreseeable effects

No hazards have been identified.

The introduction of the B subunit genes of the E. coli enterotoxin into the vibrio organism is unlikely to alter any existing pathogenic traits.

The host cell line is considered non pathogenic to humans.

The B subunit of E. coli enterotoxin has been extensively evaluated in vaccine trials without any adverse effects.

As all vibrio species are assigned as biological hazard class 2 organisms by ACDP all project work (cell culture, primary recovery and purification) with the organism will be carried out at Excell Biotech's category II facility, Antigen Production Unit, Pentlands Science Park.

Although the risk is effectively zero cell culture samples for assay will be manipulated in a Cat II microbiological safety cabinet.

All potentially contaminated waste will be autoclaved prior to disposal.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Following fermentation and primary recovery the culture supernatant will be 0.22um filtered and a sterility assay will be carried out. No viable GMO will be present following this stage.

It is unlikely that the vibrio organism would survive in the environment and as the organism is already present in the wider marine environment the consequence of release of the organism into the environment is negligible.

The fermenter vessel will be cleaned and flushed with 0.5M sodium hydroxide, rinsed and steam sterilised.

Membranes and equipment used for primary recovery will be cleaned with 0.5M sodium hydroxide. Cell paste will be held in 0.5M sodium hydroxide to kill cells. No viable GMO will be present following this treatment. Killed cell paste (liquid waste) will be uplifted from the site for disposal by an approved contractor.
All potentially contaminated waste will be autoclaved prior to disposal and uplifted from the site by an approved contractor.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

No hazard was identified.

Review of the GMO risk assessment reviewed at the monthly meeting and communicated to the staff via the meeting minutes, which are circulated to staff, and made available on the H&S notice board.

The GMO RA was submitted for review to the Moredun Institute, Pentlands Science Park, GMO committee. No comments or response has been received at the time of submission of this form to the HSE.

**Project Containment**

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02/03/2022
**GM Centre Number: 772**

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**Name**

ANTHONY NOLAN BONE MARROW TRUST

**Name 2**

ROYAL FREE HOSPITAL

**Campus Estate or Research Centre**

**Building**

**Road Name**

FLEET STREET

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

NW3 2QG

**Country**

ENGLAND

**Tel Number**

0207 284 8319

**Fax Number**

0207 284 8331

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The Joint Genetic Modification Committee for the Royal Free site meets three times a year to discuss GM matters pertaining to the Royal Free Campus of the Medical School [GM 99], The Royal Free NHS Trust and The Anthony Nolan Research Centre.  
The committee composition is that outlined for the RF and UC Medical School GM99.  
The Anthony Nolan has a representative on the above committee and the ANBMT senior scientists and Research Director meet at similar times following the meetings at the RFH for dissemination of information from the main committee, and for discussion of new matters arising thereof they also meet to discuss new work prior to submission.

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<th>Animal Unit</th>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

"All waste is put into a separate autoclave bag and autoclaved on site. This then goes with clinical waste for incineration by an external contractor. Liquid waste is inactivated by adding sodium hypochlorite. If necessary, the addition of chlorine releasing granules will ensure enough chlorine for disinfection."

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Name
ROYAL FREE HAMPSTEAD NHS TRUST

Name 2  
Department

Campus Estate or Research Centre

Road Name  
POND STREET

District  

Town  
LONDON

County  
GREATER LONDON

Postcode  
NW3 2QG

Country  
ENGLAND

Tel Number  
020 7794 0500

Fax Number  
020 7794 0433

E-mail

HSE Division  
LONDON

Comments

Date at Which Additional Info Submitted  
02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes](Yes)

Give brief details of the genetic modification safety committee

The Joint Genetic Modification Committee for the Royal Free site meets three times a year to discuss GM matters pertaining to the Royal Free Campus of the Medical School [GM 99]. The Royal Free NHS Trust and The Anthony Nolan Research Centre.

The committee composition is that outlined for the RF UC Medical School GM99

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Tick if confidential

![No](No)
For activities involving GMMs, describe the waste management measures which will apply to the activity

"All waste is put into a separate autoclave bag and autoclaved on site. This then goes with clinical waste for incineration by an external contractor. Liquid waste is inactivated by adding sodium hypochlorite. If necessary, the addition of chlorine releasing granules will ensure enough chlorine for disinfection."

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

For the risk assessment example sent in Section 7 the Committee expressed concern with aerosol production resulting from vaccine release during the filling of syringes prior to injection. The workers were required to draw up a Standard Operating Procedure which is attached.

It was also noted that the workers were not experienced with GM work. The committee thought as the workers were only handling a prepared GMM for the purposes of injection inexperience in GM work was not a cause for concern.
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**Name**
QUEEN MARY UNIVERSITY OF LONDON

**Campus Estate or Research Centre**

**Road Name**
MILE END ROAD

**Town**
LONDON

**District**
GREATER LONDON

**County**
ENGLAND

**Postcode**
E1 4NS

**Tel Number**
0207 882 5555

**Fax Number**
0207 882 5556

**Date at Which Additional Info Submitted**
04/06/2001
26/05/2002

**Comments**
GM174 MERGED ON 01/01/2009
**Accident ID**
29.00

**Date Accident Notified**
27/07/2018

### Significant Change

| GM774/08.3a | 11/02/2010 |
| 774/07.2a | 18/02/2011 |
| 774/08.3b | 04/04/2019 |
| 174/07.1a | 15/05/2020 |
| 774/07.1a | 12/10/2021 |

### Date of Additional Information (significant change only)

| GM774/08.3a | 11/02/2010 |
| 774/07.2a | 18/02/2011 |
| 774/08.3b | 04/04/2019 |
| 174/07.1a | 15/05/2020 |
| 774/07.1a | 12/10/2021 |

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Committee comprises a Senior Research Scientist who acts as the Chairperson and a Biological Safety Officer who is an experienced Molecular Biologist. Included on the committee is a non-scientific member of staff, the supervisory Medical Officer, The Assistant College Safety Advisor and up to six other scientists from laboratories some of whom will be working with GMM (at least two people will not be working with GMMs).

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Tick if confidential

Bacteriology       Yes  Parasitology       Yes  Transgenic Birds   Yes  Microbiology Research   Yes

Virology           Yes  Transgenic Animals Yes  Transgenic Fish    Yes  Gene Therapy    Yes

Mycology           Yes  Transgenic Invertebrates Transgenic Plants Other (please specify below)
For activities involving GMMs, describe the waste management measures which will apply to the activity

- with 70% ethanol.
- Waste is clearly labelled biohazard double bags will be incinerated as clinical waste.
- Hazard conditions consist of possible spillages, aerosol spray when opening cryovials or during the use of needles when working with animals.
- Work is restricted to determined areas, disinfecting solutions will always be available, working guidelines are clearly posted, unauthorised personnel is not permitted. Strict adherence to Category II working practices. Reporting in case of accident is according to College procedures.
- Training to work with GMM's category II. Guidelines regarding dressing code, how to treat spillages and disinfection with 1% Virkon and 70% ethanol. Medical records held by occupational health advisor.
- Documented spillage procedures are clearly established. In case of accident with sharps, College procedure with blood borne viruses will be followed.
- Autoclaves are regularly serviced and checked. Autoclaved material is checked using autoclaving tape to assess has reached appropriate temperature for disinfection.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The information enclosed in the risk assessment has been discussed and agreed by our local committee.

---

**Project Ref 14/03.2**

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Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Transferred from GM14

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use

The aim of this project is to use lentiviruses as an expression system for investigating mechanisms of molecular regulation of a family of ion channels expressed in neuronal cells. A commercially available lentivirus expression kit from Invitrogen will be used. We will make recombinant lentiviruses encoding membrane proteins which we are interested in: eg. heteromeric G proteins of the Gi/o family (THESE G PROTEINS ARE NOT MEMBERS OF THE SMALL G PROTEIN FAMILY) and fluorescent reporter proteins such as PLCdelta-PH-CFP and PSD95-GFP. The cDNA inserts themselves are very unlikely to have any harmful effects and they are currently used in our research into mechanisms of cellular signalling. The cDNAs encoding these proteins will be subcloned into a viral expression construct (pLenti6, Invitrogen). This will be transfected into HEK293FT cells (producer cells) to generate recombinant virus. The recombinant virus will then be used to infect cultured rat or mouse central neurones in vitro. The neurones will then be used in electrophysiological studies where the effects of the heterologous membrane proteins on the neuronal ion channels will be assessed. These experiments will allow the investigation of specific single proteins (eg Gi/o proteins) on the activity of ion channels in their native neuronal environment.

Recipient or parental organism

The commercially available kit from Invitrogen is a third generation system based on HIV-1 (see Dull et al., 1998: J. Virol. 72, 8463-71) which allows the construction of a recombinant replication-incompetent, HIV-1-based lentivirus. There are a number of biosafety features which have been introduced to minimise risks. These are as follows: (1) All HIV sequences required for encapsidation and reverse transcription are absent. (2) There is a deletion in the 3' UTR resulting in self-inactivation of viral genome. (3) None of the HIV-1 structural genes contain LTRs and are thus never expressed in the target cell. (4) It contains the minimal number of HIV-1 genes required to generate an efficient vector (only three of the nine are used: gag, pol, and rev). (5) The removal genes are those which are pathogenic therefore no recombinant vector can acquire the pathogenetic features of the parental virus. (6) The tat gene, which is important for replication, has been removed. (7) The packaging genes are contained on 4 separate plasmids and thus the system relies on 4 separate plasmids for the production of transducing particles. (8) These plasmids contain no regions of homology thus preventing any recombination which could occur. (9) The virus is replication incompetent and as a replication-deficient vector it poses significantly reduced risks. Thus these viruses may infect cells and integrate DNA into the target cell but they are incapable of further replication.

Host/vector system

Vectors: HIV-based lentivirus expression vector (pLenti6) into which the gene of interest will be cloned. This vector also contains the elements needed for packaging of the expression construct. Kit also contains viral packaging mix of vectors: this is an optimised mixture of three packaging plasmids which supply the structural and replication proteins required for production of lentivirus.

Hosts: HEK293FT cells will be used to produce the virus. Recombinant proteins will then be expressed in primary neuronal cells.

Origin & function

Recombinant lentivirus will be used to transduce primary central neurones in vitro which have been isolated and cultured from rat or mouse. The intention of this project is to study the effects of the signalling proteins (described in Section 6) on ion channel activity in these neurones by using electrophysiological techniques.

Evaluation of foreseeable effects

Recombinant virus is produced in the HEK293FT producer cells. In these cells the virus can replicate. The potential risk which may arise from this is direct exposure of the viral supernatant produced to the operator. This could arise through the use of sharps and a direct needlestick injury, or if viral supernatant is accidentally splashed onto exposed areas of skin which are cut or scratched. This will be prevented by total avoidance of use of glass and sharps (including needles and forceps) since only sterile, disposable plasticware will be used. The plastic pipettes and pipette tips used are blunt and are also aerosol-resistant and contain a filter thus reducing any aerosol formation. Furthermore labcoats and gloves (and overshoes) are worn at all times. In addition, there is virtually no risk to the environment because retroviruses exhibit poor survival in the general environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All surfaces are swabbed before and after use with 10% Virkon and 70% IMS. Sharps (rarely used) and Pipetman pipettes are placed in specified sharps bins. Waste media, pipette tips and plasticware are doused in Virkon prior to autoclaving in sealed and double-bagged plastic autoclave bags. These procedures will ensure an anticipated 100% kill of virus.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The proposal has been reviewed by a representative panel of the Genetic Modification Safety Committee, which agreed with the proposer’s classification of Class 2. The notification will be submitted to the next meeting of the full Committee on 10 April 2003. The laboratories were last inspected in December 2002 and conditions were found to be satisfactory.

Project Containment

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Project Ref 174/00.1

Date Ackn’d 30/09/2009

CU2 Project Title CHARACTERISATION OF A PUTATIVE HAEMOLYSIN FROM C. DIPHTHERIAE

Class 2

CultureVolClass2

CultureVolumeClass3-4
### Project Additional Information

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Ref 174/01.1**

**Date Ackn’d** 30/09/2009

**CU2 Project Title**

CLONING OF POTENTIAL VIRULENCE DETERMINANTS FROM PORPHYROMONAS GINGIVALIS, IN ESCHERICHIA COLI, AND GENERATION OF ISOGENIC MUTANTS

**Class** Class 2

**CultureVol** 1-50 Litres

**Non-GMM** Not Applicable

**Consent Granted**

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Withdrawn** N

**Tick if notifying a connected programme of work** Y

**Date of Significant Change**
Project Additional Information

Purposes of the contained use
- Molecular cloning and expression, of Porphyromonas gingivalis genes encoding extracellular protease, enzymes involved in protein modifications, lipopolysaccharide biosynthesis, and outer membrane proteins in Escherichia coli.
- Manipulate the above constructs for insertional mutagenesis of genes putatively involved in protease and outer membrane protein expression, and post-translational modification and re-introduce into Porphyromonas gingivalis.

Recipient or parental organism
The origin of DNA will be the oral-gram negative anaerobe, Porphyromonas gingivalis ATCC 53978 (W50). The bacterium is extremely sensitive to oxygen and is unlikely to survive outside the anaerobic cabinet after a prolonged exposure to air. The population of Porphyromonas gingivalis is elevated in periodontal diseases of the teeth supporting tissues but otherwise present in low numbers in healthy sites. The proteases and outer membrane proteins are recognised virulence factors. Additionally, the genome of Porphyromonas gingivalis has been sequenced by TiGR (http://www.tigr.org/).

Host/vector system
Escherichia coli K12 derivitives (XL-1 Blue, XL10 Gold, SCS110, SURE, Stratagene), general cloning hosts, will be used. The strains are attenuated, sensitive to bile salts and are unable to survive in the alimentary canal. Porphyromonas gingivalis (see above) will be used for complementation of mutated genes.

E. coli - Plasmids pUC18 - derived (Amp(r), LacZa - pUC18, pUC18notl, pK18) will be the general cloning and manipulating vectors. For expression of poly his-tagged recombinant proteins JFQ- derivitives (commercially known as pQE80, Qiagen) will be used to direct controlled (inducible by Isopropyl -D thiogalactoside, IPTG) expression under ptac or pT5 promoter, respectively.

Porphyromonas gingivalis - pNJR12 (tet(r) Bacteroides/Porphyromonas - E.coli) shuttle plasmid will be used; the plasmid is known to replicate autonomously as a low copy number (<10 copies per bacterium). Alternatively, manipulated genes will be integrated into the genome via the homologous recombination using erythromycin encoding cassette (from pVA2198).

Origin & function
Chromosomal DNA will originate from Porphyromonas gingivalis and will encode orthologues of: Proteases specific for extracellular arginine - peptide bonds (Lys-gingipain) Proteases specific for extracellular lysine peptide bonds (Arg-gingipain) Outer membrane proteins . Enzymes involved in lipopolysaccharide (LPS) biosynthesis, glycan transferases.

The open reading frames (orfs) will be amplified, cloned and manipulated (by insertion of a specialised macrolide-lincosamide cassette, erm) in E. coli with the main intention of inactivating the corresponding gene in Porphyromonas gingivalis via homologous recombination. These mutants will be assessed, biochemically and genetically, to ascertain their contribution to the metabolism, survival and pathogenicity of Porphyromonas gingivalis.

Some of these orfs will be specially cloned for controlled expression in E. coli for subsequent production of antiserum for studies of protein expression in Porphyromonas gingivalis.

Another set of orfs will be cloned into pNJR12 and introduced into Porphyromonas gingivalis to compliment corresponding mutations to assess interconnecting metabolic pathways.

Evaluation of foreseeable effects
The genome of Porphyromonas gingivalis W83 has been sequenced by The Institute of Genomic Research (http://www.tigr.org/) and annotated (http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gpg) so only known orfs will be cloned. Protease gene constructs have previously been shown not to yield active enzyme in E.coli. However, expression of active enzymes from some constructs cannot be ruled out. Expression of Porphyromonas gingivalis proteins in E. coli is unlikely to make the host more pathogenic than Porphyromonas gingivalis itself. Inactivation of Porphyromonas gingivalis genes is expected to lead to metabolic defects that should result in attenuated and poor viability of the bacterium. Complementation of Porphyromonas gingivalis should lead to restoration of normal activities.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All relevant containments are in place to minimise the potential of any risk. All materials (pipette tips, plates, glassware, tissues, gloves, etc) that are contaminated with bacteria or nucleic acids will be thoroughly soaked in 5% Hycolin then autoclaved, or autoclaved directly, and disposed via the College in accordance with departmental containment level 2 safety regulations.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Suggested comments by the local Genetic Modification Safety Committee have been incorporated in the risk assessment.

Project Containment

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Project Ref 174/01.2

Date Ackn’d 30/09/2009

Date Project Ceased

CU2 Project Title USE OF REVERSE GENETICS TO CREATE AN ATTENUATED INFLUENZA VIRUS

Class 2

Culture Vol Class 2 < 1 Litre

Consent Granted Not Applicable

Date Project Ceased

02/03/2022
Purposes of the contained use

To produce an attenuated influenza virus that could be used as a live attenuated vaccine against current strains of influenza virus. Current influenza virus vaccines are only about 70% effective, live attenuated virus vaccines like those we are trying to develop may be more effective.

Recipient or parental organism

The extensively studied PR8 strain of the influenza virus will be used as the recipient influenza strain. The PR8 strain of influenza virus is highly attenuated and does not cause disease in humans. Influenza viruses are classified as ACDP category 2 pathogens.

Host/vector system

Plasmids containing the cloned genes of influenza virus strain PR8 are being generously donated by Professor Brownlee (Sir William Dunn School of Pathology, University of Oxford). The plasmids are pPOLI-PB2-PR8, pPOLI-PB1-PR8, pPOLI-PA-PR8, pPOLI-HA-PR8, pPOLI-NP-PR8, pPOLI-NA-PR8, pPOLI-M-PR8, pPOLI-NS-PR8, pPOLI-Sapl-Rib, pcDNA-PB1-PR8, pcDNA-PB2-PR8, pcDNA-PA-PR8, pcDNA-NP-PR8. These plasmids are all pUC18 derivatives. These will be transfected into either Vero (African Green Monkey) cells or 293T (Human kidney) cells. Recombinant virus will then be retrieved from these transfected cells (see Fodor E, et al. Rescue of influenza A virus from recombinant DNA, J Virol 1999; 7.3.9679-9682).

Origin & function

The introduced genetic material will be the influenza virus neuraminidase and haemagglutinin genes cloned from recent strains of the influenza virus such as A/New Caledonia/11/99. Mutations will be added to these cloned genes to attenuate the virus. Alterations to the neuraminidase gene have been shown to lead to a reduction of virulence (see Solarzano et al. Reduced levels of neuraminidase of influenza A viruses correlate with attenuated phenotypes in mice, J. Gen Virol 2000: 81 Pt3: 737-742). The recombinant virus will thus contain all the PR8 gene segments except those encoding the neuraminidase and haemagglutinin which will come from a recent strain of the influenza virus. The introduced gene products will be expressed on the surface of the recombinant influenza virus and are intended to act as antigens to provoke a protective immune response to any later challenge by the wild type influenza virus.

Evaluation of foreseeable effects

The influenza virus normally causes an upper respiratory infection in humans, however, the attenuated PR8 strain is non-pathogenic in humans. The mutations we add to the influenza virus genome are intended to be stable and to attenuate the virus. The haemagglutinin and neuraminidase proteins have been shown to be non-toxic to humans and have been used extensively in vaccines. Vaccines are available to protect against influenza virus infection and there are antiflu drugs such as Tamiflu and Relenza.

Influenza viruses pose limited environmental risk as they are degraded quickly in the environment (UV, desiccation), the genetic modifications we perform will not change this.
Work with the mutated influenza virus being conducted in Microflow Biological Safety Cabinets in a category 2 GLP laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste produced containing recombinant influenza virus will be treated with Basol a quaternary ammonia compound based detergent. This detergent is known to very rapidly destroy lipid encapsulated viruses such as influenza. To ensure a one hundred per cent kill the waste containing the recombinant virus will be left in the basol for 24 hours. The waste will then be disposed of down the sink. This is a standard technique for disposing of influenza waste.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Both the parent virus and the neuraminidase and haemagglutinin proteins from recent strains have a history of safe use in humans.

Project Containment

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Project Ref  174/02.1

Date Ackn'd  30/09/2009  CLONING OF POTENTIAL VIRULENCE DETERMINANTS FROM PSEUDOMONAS  Class  Class 2  CultureVolClass2 ≤ 1 Litre  CultureVolumeClass3-4
Project Additional Information

Purposes of the contained use

Molecular cloning of Pseudomonas aeruginosa genes encoding metalloproteases, serine proteases, genes involved in type III secretion systems and genes involved in cobalamin (vitamin B12) biosynthesis in Escherichia coli.

Construction of insertion mutants of the genes mentioned above in Pseudomonas aeruginosa.

Recipient or parental organism

The origin of DNA will be the gram-negative bacterium Pseudomonas aeruginosa.

The genome of P. aeruginosa has been sequenced in a collaboration among the Cystic Fibrosis Foundation, the University of Washington Genome Center and Pathogenesis Corporation (http://www.pseudomonas.com/).

In P. aeruginosa the mutated genes are unlikely to confirm any advantage relative to the wild type strain; complemented strains are expected to restore wild type gene activities.

Host/vector system

Escherichia coli JM109 strain a K12 derivative will be used as a cloning host.

This strain is attenuated, sensitive to bile salts and unable to survive in the gut.

E. coli S17-1 a mobilising strain that carries the transfer function of the plasmid RP4 integrated in its chromosome will be used as the host for the plasmid pEX100T carrying the disrupted P. aeruginosa genes.

The vectors used in this project are:

- pUC18, pUC18NotI. Non-mobilisable vectors
- pUCP18-Escherichia-Pseudomonas shuttle vector. Non-mobilisable
- pEX100T-gene replacement vector, mobilisable. The P. aeruginosa genes cloned in this vector are interrupted by the insertion of a gentamicin resistance cassette.
- pUCGm-source of the gentamicin resistance, GM R cassette.

Origin & function

02/03/2022
Chromosomal DNA will originate from Pseudomonas aeruginosa and will encode orthologues of:

- Metalloproteases
- Serine proteases
- Genes involved in Pseudomonas aeruginosa type III secretion system
- Genes involved in cobalamin (vitamin B12) biosynthesis

The open reading frames will be amplified, cloned and manipulated (by insertion of a gentamicin resistance cassette) in E. coli with the main intention of inactivating the corresponding gene in P. aeruginosa via homologous recombination. These mutants will be assessed, biochemically and genetically, to ascertain their contribution to the survival and pathogenicity of P. aeruginosa.

The above open reading frames will be cloned into pUCP18 and introduced into P. aeruginosa in order to complement the corresponding mutations.

**Evaluation of foreseeable effects**

The E. coli JM109 strains carrying the cloned P. aeruginosa genes should not be more hazardous than the P. aeruginosa host. In P. aeruginosa the mutated genes are unlikely to confer any advantage relative to the wild type strain. Complementation of P. aeruginosa should lead to restoration of normal activities.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All relevant containments are in place to minimise the potential of any risk.

All materials (pipette tips, plates, glassware, tissues, gloves) that are contaminated with bacteria or nucleic acid will be thoroughly soaked in 5% Hycolin autoclaved and disposed via the college in accordance with the safety regulations.

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**Please enter comments on the GM safety committee on the risk assessment**

Suggested comments by the local Genetic Modification Safety Committee have been incorporated in this and the attached document.

**Project Containment**

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02/03/2022
Project Ref 174/02.2

Date Ackn'd 30/09/2009

CU2 Project Title CLONING OF POTENTIAL VIRULENCE FACTORS (PROTEOLYTIC ENZYMES) FROM HELICOBACTER PYLORI, IN E. COLI AND GENERATION OF DEFINED ISOGENIC MUTANTS IN H. PYLORI

Class 2

Consent Granted Not Applicable

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Molecular cloning of Helicobacter pylori genes encoding proteolytic enzymes of H. pylori identified using a number of bioinformatic approaches.

Construction of insertion mutants of the genes mentioned above in Helicobacter pylori.

Recipient or parental organism

a) Escherichia coli DH5a (or similar K12 derivative) (supE44 (lacZYA-argF)U169 (80lacZ M15) hsdR17 (rk-, mk+) recA1 endA1 gyrA96 thi-1) that is recognised as a non-colonising and disabled. It may be considered equivalent to ACDP category 1. It has limited survivability in the environment. Over expression strain: ER2566 Genotype: F- fhuA2 [lon] ompT lacZ::T7 gene1 gal sulA11. (mcrC-mrr)114::IS10 R(mcr-73::miniTn10-TetS)2 R(2gb-210::TN10)(TetS) endA1 [dcm].

E. coli DH5a carrying cloned genes from H. pylori should not be more hazardous than the H. pylori host. The cloned genes will be under controlled promoters (lacZ). This will increase the potential virulence of the E. coli but should not be anymore hazardous than the Wild-type H. pylori. The E. coli strains are attenuated and are unable to survive in the gut.
b) Helicobacter pylori SS1, 26695, J99 and NCTC 11637. All strains carry the Cag pathogenicity island and do not carry any virulence plasmids. 26695 and J99 are the 2 sequenced Helicobacter pylori strains. SS1 is the adapted mouse colonising strain and NCTC 11637 is the adapted Mongolian Gerbil colonising strain. All strains are ACDP category 2 and have very limited survivability in the environment. H. pylori must be ingested in large quantities (>10^8 cfu/mL) with acid suppressive drugs to enable successful colonisation, and infection (Marshall, B., et al (1985). Attempt to fulfil Koch’s postulates for pyloric Campylobacter. Med J Aust 142, 436-439. and Morris, A & Nicholson, G. (1987). Ingestion of Campylobacter pyloridis causes gastritis and raised fasting gastric pH. Am J Gastroenterol 82, 192-194). H. pylori is a micro-aerophile. It has a maximum life span of 1 hr if left in an oxygenic. It has poor survivability in the environment.

Helicobacter pylori: The disruption of a gene with either cat or apahA3 is unlikely to confer any advantage to the wild type strain, apart from the introduction of the antibiotic resistance genes. Neither of these antibiotics have ever been and are never likely to be part of treatment regimens for H. pylori infection. If strain cans be complemented, it will restore the Wild-type activities. Further, Large quantities of H. pylori must be ingested along with acid suppressive drugs to allow colonisation.

**Host/vector system**

- **pUC19**: High copy number cloning vector
- **pGem**: High copy number cloning vector
- **pTyb**: H. pylori is a protein fusion vector with a T7 RNA polymerase expressed off a Lac Promoter
  - Overexpression of recombinant proteins will only occur upon addition of IPTG
- **p KatA**: pGem with katA cloned into lacZ. katA::aphA3 (originating from Campylobacter coli)
- **pucCat20**: puc19 vector containing cat originating from Campylobacter coli

None of the above vectors are replicated in H. pylori and will be used as suicide vectors.

**Hosts**

- *Escherichia coli* DH5α (or similar K12 derivative) (supE44 (lacZYA-argF)U169 (80lacZ M15) hsdR17 (rk-, mk+) recA1 endA1 gyrA96 thi-1 relA1)

**Genotype**

- F- -fhuA2 [lon] ompT lacZ::T7 gene1 gas sulA11 (mcrC-mrr)114::IS10 R(mcr-73::miniTn10-TetS)2 R(zgb-210::Tn10)(TetS) endA1 [dcm].

**Origin & function**

All genes identified as potentially encoding proteolytic enzymes of *H. pylori* will be cloned from the sequenced strain *H. pylori* 26695. These genes will be cloned into a vector and disrupted using an antibiotic resistance cassette, such as aphA3 or cat encoding kanamycin and chloramphenicol resistance, respectively (originating from Campylobacter coli). Once the genes have been disrupted in the vector, they will be introduced to *H. pylori* by transformation and incorporated into the genome by allelic exchange. Kanamycin and Chloramphenicol are not currently used and are unlikely to ever be used in therapeutic regimes for the treatment of *H. pylori* infection.

**Evaluation of foreseeable effects**

Creating protease deficient mutants of *H. pylori* are unlikely to confer any advantage to the organism. It is more likely to compromise the pathogenicity of the bacterium.

Introducing virulence factors into the attenuated derivatives of *E. coli* K12 is likely to increase the potential virulence of the *E. coli*. The potential virulence of the *E. coli* will not exceed that of the Wild-type *H. pylori*.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All relevant containments are in place to minimise the potential of any risk.
All materials (pipette tips, plates, glassware and gloves) that are contaminated with bacteria or nucleic acid will be thoroughly soaked in 5% (v/v) hycolin, autoclaved and disposed by incineration via the college in accordance with safety regulations, currently in use for ACDP hazard group 2 organisms.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form  
Tick to confirm that you have attached a risk assessment to this form  
Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Suggested comments made by local GM safety committee have been incorporated into this and the risk assessment.

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**Project Ref** 174/02.3

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<td>DETERMINATION OF THE AMOUNT OF BACTERIAL ADHESION TO EUKARYOTIC CELLS IN THE PRESENCE OF OLIGOSACCHARIDES KNOWN TO INHIBIT BACTERIAL ADHESION.......</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<tr>
<th>Date Project Ceased</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<tbody>
<tr>
<td></td>
<td>Not Applicable</td>
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Tick if notifying a connected programme of work  
Project notified under transitional arrangements  
Withdrawn  
Tick if notifying a connected programme of work
### Project Additional Information

#### Purposes of the contained use

A marker plasmid will be constructed to allow the monitoring of bacteria either by bioluminescence or fluorescence in the presence of a fluorescent substrate.

#### Recipient or parental organism

Recipient organisms: *Escherichia coli K12, Salmonella typhimurium SAL1, Legionella pneumophila NCTC11191, Bacillus cereus NCT2599, Pseudomonas aeruginosa PAK and Burkholderia cepacia NCIMB9091; all are wild type identified laboratory strains. E. coli is ACDP cat. 1 all other organisms are cat. 2.

#### Host/vector system

Plasmid vector (pBBR1MCS4) confers ampicillin resistance and is only mobilisable when the RK2 transfer functions are provided in trans. Plasmid will be modified to also confer chloramphenicol resistance.

#### Origin & function

Firefly luciferase (luc+) from the commercially available plasmid pSR-luc+ or β-galactosidase (lacZ) amplified from the E. coli K12 chromosome will be cloned into the plasmid pBBR1-MCS4. These will allow us to determine the amount of bacteria adhered to eukaryotic cells either by luminescence (luc+) or fluorescent (lacZ).

#### Evaluation of foreseeable effects

Since the plasmid being constructed is only for use as a marker it is not expected to change the virulence properties of the recipients. Therefore a release of the GM organism would result in the same effect as a release of the unmodified organism.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials contaminated with GM bacteria will be soaked in 5% (w/v) hycolin (if not directly autoclaved), autoclaved and disposed of by incineration via the college in accordance with the safety regulations currently in use for ACDP hazard group 2 organisms.
Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

none

Project Containment

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<tr>
<td>L2</td>
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</tbody>
</table>

Animal Units

| L2 | L3 | L4 | L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 | L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref  174/06.1

Date Ackn'd  30/09/2009

CU2 Project Title  Knockout and over-expression of streptococcal DNA replication and repair genes.

Date Project Ceased

Class  Class 2

Culture Vol  Class 2 < 1 Litre

Consent Granted  Not Applicable

Non-GMM  Not Applicable

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements  N
Project Additional Information

Purposes of the contained use
To investigate function of DNA replication, recombination and repair genes, and their role in antibiotic resistance acquisition.

Recipient or parental organism
Clinical and laboratory isolates of Streptococcus pneumoniae and other mitis group streptococci.

Host/vector system
Genes will either be cloned in E. coli vectors unable to replicate in streptococci in order to mediate incorporation into the pneumococcal/streptococcal genome, or cloned in shuttle vectors such as pVA838 and pVA981 which can replicate in pneumococci/streptococci. In some cases genes will be cloned to allow expression: pMSP3535 allows regulated expression in streptococci.

Origin & function

The intention is either to inactivate or to overexpress the genes in streptococci. In some cases genes will be inactivated by insertion of antibiotic resistance cassettes, and reporter genes will be inserted downstream of promoters to assess regulation of expression.

Evaluation of foreseeable effects
Modified S. pneumoniae and mitis group streptococci may have an increased mutation rate and some will carry additional antibiotic resistance genes. Hypermutable organisms are generally at a disadvantage due to the accumulation of errors. Clinical isolates known to have a wide range of mutation frequencies are not thought to be associated with increased virulence. There is potential for an increase in mutation to antibiotic resistance. Penicillin is usually the drug of choice for treating infection - penicillin resistance cannot be gained by point mutation, and none of the resistance cassettes to be used confer penicillin resistance.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All cultures and contaminated materials are autoclaved (effective 100% kill, monitored by regular servicing of autoclave and integrated chart recorder). After autoclaving, disposable solids are removed as clinical waste for incineration, and liquids are disposed of to drain.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 174/06.2

Date Ackn'd 30/09/2009

CU2 Project Title

Investigation of interactions of enterohaemorrhagic Escherichia coli (EHEC) and enteropathogenic Escherichia coli (EPEC) and cells of the organised lymphoid tissue of the human gut.

Class 2

Consent Granted Not Applicable

Project notified under transitional arrangements N

Purposes of the contained use

Investigation of enterohaemorrhagic Escherichia coli (EHEC) and enteropathogenic Escherichia coli (EPEC) can affect and modulate immune cells of the human gut by injecting proteins via a type III secretion system.
Laboratory derivatives of the E. coli strain EHEC 0157:H7. All strains are Shigatoxin-negative.

Laboratory derivatives of the E. coli strain EPEC 0127:H6. All strains are negative for the EspC enterotoxin.

Host/vector system

The strains to be used carry a plasmid (pFPV25.1) with the gene that encodes for green fluorescent protein (GFP) or the plasmid pCX340 with a gene that encodes with NleD ("Non LEE Encoded effector", an EHEC effector protein) fused to TEM-1 β-lactamase which serves as a reporter protein to directly show that a cell has received a signal via type III secretion.

Origin & function

pFPV25.1 expressing the green fluorescent protein (GFP) allows direct visualisation of EHEC and EPEC by fluorescent microscopy. pCX340 expressing NleD or TccP fused to TEM-1 β-lactamase serves as a fluorescent-based reporter for cells which have received a signal via type III secretion.

Evaluation of foreseeable effects

Modification involves introduction of marker genes. These are expected to have no effect on the virulence of the host.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures and contaminated materials are autoclaved (effective 100% kill, monitored by regular servicing of autoclave and integrated chart recorder). After autoclaving, disposable solids are removed as clinical waste for incineration, and liquids are disposed of through the drain.

Project Containment

The comments were that if a member of staff suffers from diarrhea, the PI and the Health and Safety Officer will be notified. A restriction was made on the volume of media in which the organism was cultured (maximum 20 ml).
Project Ref 174/06.3

Date Ackn'd 30/09/2009

CU2 Project Title To make single base changes by site directed mutagenesis in 10 genes involved in replication of the live attenuated Oka Varicella-zoster vaccine virus.

Class 2

Cultivation Class < 1 Litre

Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To determine the biological significance of wildtype alleles present in genes involved in replication that occur at significantly lower frequency in the original Oka vaccine preparation but appear to have been selected for in Oka Varicella vaccine viruses that cause rashes.

Recipient or parental organism
Attenuated Oka varicella vaccine virus present in Cosmids and in a BAC.

Host/vector system
Cosmids and BAC are already assembled containing the entire Oka varicella vaccine virus and will be transfected into E. coli to obtain sufficient titres.

Origin & function
Genes to be manipulated are involved in either DNA replication, gene expression or are of unknown function but are known to be expressed during latency and may include: Ie10 AND ie62 (transactivator proteins), Membrane protein (ORF1), Stearoylated membrane protein (ORF 9), glycoprotein C (ORF14), latency expressed protein (ORF21), glycoprotein B (ORF31), ORF39 (unknown function), 50 (unknown function), Origin of re0plication binding protein (ORF51), Components of DNA helicase/primase complex (ORFs 6, 52, 55) ORF54 (unknown function), Uracil-DNA glycosylase (ORF59).

Mutated cosmids will be transfected into melanoma cells to produce transmissible recombinant virus.

**Evaluation of foreseeable effects**

Recombinant Oka vaccine virus from cosmids or BAC may have increased virulence compared to original Oka vaccine virus but are highly likely to remain attenuated compared to wildtype virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All cultures and contaminated materials are autoclaved (effective 100% kill, monitored by regular servicing of autoclave and integrated chart recorder). After autoclaving, disposable solids are removed as clinical waste for incineration, and liquids are disposed of to drain.

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This connected programme of work aims to investigate the neutralisation and tropism properties of different retroviruses. Both ACDP 2 and ACDP 3 retroviruses will be used including HIV 1 and 2, HTLV 1 and 2, SIV and MLV. Retroviral genes will be amplified from infected cell cultures or patient DNA and either full length molecular clones or plasmids containing different retroviral genes will be generated. Transfections into mammalian cell lines will result in either fully infectious or defective virus particles that are not transmissible.

Recipient or parental organism

Retroviral full length molecular clones will be propagated in E. coli K12 derivatives and in human cells. E. coli K12 derivatives are disabled and non-colonising. Mammalian cell lines will be used but these will not come from worker. Fully infectious virus will be made following transfection of these plasmids into mammalian cell lines. The foreseeable risk for work with these full length molecular clones is the production of infectious virus. Transfections and all subsequent work with these retroviruses will be done in a CL3 laboratory.

Host/vector system

Host cell lines and primary cells including: T-cells, fibroblasts, epithelial cell lines, primary peripheral blood mononuclear cells and macrophages. All cell lines and primary cells will not be from any authorised workers. The bacterial hosts for expression vectors will be disabled E. coli K12 derivatives. The cloning plasmid vectors will include TOPO vectors, pcDNA3.1, pCAGGS, pNL4.3 delta env, pMDG (VSV-G envelope vector) pCNCG (packaging plasmids) and pHIT60 (MLV gag/pol vectors). Vaccinia virus expressing T7 RNA polymerase: VR2153.

Origin & function

Retroviral genes amplified from DNA from patients will be cloned into mammalian expression vectors following cotransfection into mammalian cell lines either fully
infectious virus will be made of defective, non-transmissible virus capable of only a single round of infection will be made. The intended function is to study the neutralisation and tropism properties of these chimeric infectious or pseudotyped viruses.

The vectors to be used include pCGNCG (Sonoeka et al 1995), PCSGW, pSVIII, pCAGGS, PCR-Blunt II and pCR 2.1. Packaging vectors include p8.3, p8.91. Full length molecular clone vectors include pNL4.3, p89.6 and pHXB2. The envelope deleted plasmid can be pNL4.3 delta env. These retrovirus vector systems are standard laboratory reagents.

The intended functions of the plasmid expression vectors, retroviral expression vectors and the general material inserted in them is to change the level of expression of viral and/or host proteins in the cell. This will result in the production of cells and viruses with altered properties.

Evaluation of foreseeable effects

The bacterial strains used for all plasmid propagations are disabled in key bacterial functions and are unable to survive in the environment or pass genetic material onto host bacterial flora following a breach in containment. Expression of the retroviral full length molecular clones could cause infection if injected intra muscularly in sufficient quantities. These clones will only be grown in a maximum culture volume of 100ml and sharps will be forbidden in this laboratory area in order to minimise the above risk. PPE in the form of Howie lab coats, goggles and disposable gloves will also be worn. The plasmids used for the generation of pseudotyped retroviruses split the retroviral genome into separate plasmids such that virus production is only initiated when all plasmids are present in a mammalian cell line. These plasmids are always propagated separately to minimise the risk. No mammalian cell culture is present in the CL2 molecular biology laboratory where this work is carried out. The risk of naked DNA is considered to be negligible.

The recipient cell lines and primary cells which are not from person who work in the laboratory are known to be free from human pathogens ACDP2-4 groups. These cells have limited survivability in the environment and have serum and nutrient requirements that would not likely be met outside laboratory tissue culture. Transfection of the full length molecular clones results in fully infectious virus that is capable of infecting a worker. Recombination between the plasmids for the pseudotyped viruses could also result in infectious virus though this is very unlikely. The risk is managed by carrying out transfections into mammalian cells and all subsequent work in a CL3 laboratory.

A Code of Practice details all working practices for this laboratory. All staff must be fully trained by an experienced worker and copies of all training records kept. All work with cell cultures is carried out in a Class 1 microbiological safety cabinet which is tested every 6 months. Access to the laboratory is controlled by card. Sharps are forbidden. Vaccinia virus is used to boost the expression of retroviral envelopes. In order to limit the exposure of workers to those only involved in the work directly, it will be handled in the CL3 laboratory where access is controlled and a separate class 1 microbiological safety cabinet and CO2 incubator can be dedicated to this work. Workers with vaccinia virus will be fully apprised of the risks prior to working with the virus as per the attached risk assessment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment Level 2:
Bacterial waste: All GMMs are inactivated in 5% Trigene for at least 16 hours before disposal or autoclaved followed by incineration as per college practice.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GMSC required that laboratory workers would not work with plasmids containing full length viral genomes until containment level 2 training has been completed.

### Project Containment

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### Project Ref 174/08.1

- **Investigation of neutralising antibodies and anti-viral compounds on hepatitis viruses and the effect of chronic infection on the response to treatment.**

- **Date Ackn'd:** 30/09/2009
- **CU2 Project Title:** Investigation of neutralising antibodies and anti-viral compounds on hepatitis viruses and the effect of chronic infection on the response to treatment.
- **Class:** Class 3
- **Culture Vol:** < 1 Litre
- **Consent Granted:** Yes
- **Withdrawn:** N
- **Historical Significant Changes:**
  - N
- **Historical Date of Additional Info:**
- **Significant Change ID:**
- **Date of Significant Change:**

### Project Additional Information

**Purposes of the contained use:**

Chronic infection with the hepatitis viruses is an important cause of morbidity and mortality. This connected programme of work aims to investigate the biology of the viruses and potential agents useful in treatment and prevention of infection. Both HBV and HCV will be investigated as will co-infection of HCV and HIV and the effect of HCV infection on HIV virus production and other properties. Viral genes from infectious clones or patient samples transfected into cell lines will result in either fully infectious virus or non-transmissible defective virus particles.
Recipient or parental organism

Full-length or partial viral DNA will be cloned into Ecoli. Transfection of full-length RNA or plasmid DNA, or partial length DNA co-transfected with a defective retroviral plasmid construct into mammalian cell lines or primary cells will be used. These will not come from any laboratory worker. The foreseeable risk for work with these full length molecular clones is the production of infectious virus. Viral RNA will be produced and stored in the CL3 laboratory as will transfections and all subsequent work with these constructs.

Host/vector system

Host cell lines and primary cells including: fibroblasts, hepatoma cell lines, and primary cell, T-cells, peripheral blood mononuclear cells and macrophages. All cell lines and primary cells will not be from any authorised workers. The bacterial hosts for expression vectors will be disabled E.coli K12 derivatives. The cloning plasmid vectors will include TOPO vectors, pGEM, pcDNA31, pCAGGS, pNL4.3 delta env, and pSVIII.

Origin & function

The intended function of the genetic material is to introduce it into mammalian cells so that it becomes possible to study the neutralisation of the hepatitis viruses and their effect on the functions of the cell. Hepatitis genes will be amplified from infected patients and will be cloned into mammalian expression vectors and following cotransfection into mammalian cells defective, non-transmissible virus capable of only a single round of infection will be made. The vectors to be used include the mammalian expression vectors pSVIII, pCAGGS and pNL 4.3 delta env. Other vectors include pGEM Teasy and the TOPO vectors pCR-Blunt land pCR2.1. Full-length RNA from a plasmid containing cloned JFH1 will be used to transfect mammalian hepatoma cell lines (Wakita et al, 2005). This is known to transfect successfully and produce infectious virus particles. Mutations of this construct or constructs from other genotypes or of mixed genotype will also be used. Further, these HCV expressing cell lines will be infected with HIV and other retroviruses to study the effects on HIV biology. These experiments will result in the production of cells and viruses with altered properties. A well characterised hepatoma cell line which is already transfected with the hepatitis B virus, and produces viral particles into the tissue culture medium, will also be used (Sells et al, 1987)

Evaluation of foreseeable effects

The bacterial strains used for all plasmid propagations are disabled in key bacterial functions and are unable to survive in the environment or pass genetic material onto host bacterial flora following a breach in containment. The plasmids used for the generation of pseudotyped retroviruses split the retroviral genome into separate plasmids such that virus production is only initiated when all plasmids are present in a mammalian cell line. These plasmids are always propagated separately in disabled E. coli K12 derivatives to minimize this risk. No mammalian cell culture is present in the CL2 molecular biology laboratory where this work is carried out. The risk of naked DNA is considered to be negligible.

Co-transfection of the envelope deleted retroviral plasmid, a plasmid containing a marker gene and the cloned envelope genes into a mammalian cell can result in a single cycle infectious virus which is not transmissible. To mitigate the risk of infection co-transfection and all subsequent work will only be carried out in a CL3 laboratory covered by a code of practice which details all working practices for this laboratory. All staff must be fully trained by an experienced worker and copies of all training records kept. All work with cell cultures is carried out in a Class 1 microbiological safety cabinet which is tested every 6 months. Access to the laboratory is controlled by card, Sharps are forbidden.

Cell lines expressing full length clones of the hepatitis viruses produce small quantities of infectious virus. All such culture and the subsequent experiments will be carried out in a dedicated CL3 laboratory under its code of practice as described above. All work with HIV viruses will be carried out in a CL3 laboratory with a COP detailing all work practices.

[The recipient cell lines and primary cells which are not from persons who work in the laboratory are known to be free from human pathogens ACDP2-4 groups. These cells have limited survivability in the environment and have serum nutrient requirements that would not likely be met outside laboratory tissue culture.]

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment Level 2:
Bacterial waste: All GMMs are inactivated in 1% Virkon or 5% Trigene for at least 16 hours before disposal. Plates will be autoclaved followed by disposal in the clinical waste as per college practice.

Containment Level 3:
All fluid waste is inactivated in 1% Virkon or 5% Trigene for 16 hours prior to disposal. All disposable plasticware is placed in double autoclave bags and autoclaved within the CL3 laboratory prior to disposal in the clinical waste as per college practice. The performance of the autoclave is tested every 6 months under a service contract. The autoclave is also monitored for each run through its printout and the inclusion of Thermolog chemical integrator strips (Bennett Scientific) to the load.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The GMSC were satisfied with the classification as Class 2 or as Class 3 for the risk assessments attached.

Project Containment

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Project Ref 174/98.1

Date Ackn'd 30/09/2009
CU2 Project Title CHARACTERISATION OF BACTERIAL VIRULENCE DETERMINANTS - A

Class 2

CultureVolClass2
CultureVolumeClass3-4
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<th>Date Project Ceased</th>
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

02/03/2022
Please enter comments on the GM safety committee on the risk assessment

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Animal Units

| L2 | L3 | L4 |

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Project Ref 424/02.1

TO CONSTRUCT RETROVIRAL VECTORS, CAPABLE OF INFECTING HUMAN CELLS THAT EXPRESS GENES ENCODED BY CULTANEOS AND OTHER HUMAN PAPILLOMAVIRUSES (HPV)

Class 2

Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

transferred from centre 424 on 12/7/06.
## Project Additional Information

### Purposes of the contained use
We aim to investigate the effects of expressing HPV genes in human epidermal keratinocytes. We will investigate cellular responses to viral gene expression in the context of ultra-violet radiation. The main purpose of these experiments is to determine whether the viral encoded activities could account for effects in skin cancer.

### Recipient or parental organism
HPV genes will be propagated in bacteria (E. coli K12 strains HB101 and Sure). DNA will then be introduced into suitable mammalian retroviral packaging cell lines (murine) for virus production. Recombinant viruses will then be used to transduce human cells.

### Host/vector system
- pBabe Puro/pBabe-bsd retroviral vectors, propagated in recombination deficient bacteria (E coli HB101/Sure).
- Phoenix cells: murine retro viral packaging cell line.

### Origin & function
All HPV DNA sequences used are previously cloned in plasmid vectors and freely available.
Likely that the HPV encoded sequences will modify the cell cycle, differentiation status and apoptotic responses of the infected human cell.

### Evaluation of foreseeable effects
Recombinant virus is potentially hazardous. Contact will therefore be minimised. Work will be carried out in a safety cabinet thereby reducing the risk of aerosol. Sharps will be avoided. If contact does occur, recombinant virus is replication defective, effect thereby localised; immune rejection of virally infected cells of human/murine origin; handling only one virus at a time will minimise changes of recombination.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
All work will be carried out in purpose built and equipped laboratory space designated and reserved for such experiments.
All liquid waste will be decontaminated with 1% Virkon or 5% sodium hypochlorite. Solid waste will be autoclaved. A 100% kill will result.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste - decontaminated with 1% Virkon or 5% Sodium Hypochlorite. Then to drains.
Solid waste - autoclaved followed by incineration.
100% kill is expected in both cases.

<table>
<thead>
<tr>
<th>Is an emergency plan required according to regulation 20?</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
<td>N</td>
</tr>
<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
</tr>
</tbody>
</table>
The committee considers this work to be class II and therefore all retroviral work will be carried out in our Cat II suite, Room 1.18.

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2  Yes</td>
<td>L3</td>
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</tr>
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Animal Units

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### Project Ref 424/02.2

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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</thead>
<tbody>
<tr>
<td>12/07/2006</td>
<td>IMMORTALISATION OF PRIMARY CULTURES OF HUMAN EPITHELIAL AND MESENCHYMAL CELLS USING STABLE VIRUS PRODUCING PT67/PBABEHYPROH TERT ......</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>

Non-GMM  Consent Granted

<table>
<thead>
<tr>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Applicable</td>
<td>N</td>
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Withdrawn  N

Tick if notifying a connected programme of work  N

**Historical Significant Changes**

TRANSFERRED FROM GM CENTRE 424 - 12/7/06.

**Project Additional Information**

**Purposes of the contained use**

The purpose of the contained use is to generate immortalised human cell lines derived from the skin that can be used for research and drug screening.
Recipient or parental organism

Recipient cells will include human oral, anogenital and cutaneous keratinocytes. Hair follicle, germinative epithelium, matrix, outer root sheath keratinocytes, dermal papilla and connective tissue sheath fibroblasts as well as sebocytes from sebaceous glands.

Host/vector system

The pBABE retroviral vector consists of the long terminal repeat (LTR) packaging sequence from the Moloney murine leukemia virus (MoMuLV). Because the env and reverse transcriptase genes have been deleted and as there is no origin of replication this vector is considered by the ACGM to be non-mobilisable and incapable of further replication in the target cell.

Origin & function

It is intended that infecting cultured human cells with hTERT and SV40LT inserts will result in the production of immortalised cell lines. Therefore, the foreseeable effects of this activity are the generation of immortalised human cell lines.

Evaluation of foreseeable effects

Foreseeable effects will be the immortalisation of skin derived cell lines.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

PT67 packaging cells are considered to be disabled hosts and are not considered to be pathogenic to humans or animals and would have a very limited ability to survive in the environment.

pBabe will be used to express hTERT and SV40LT in human primary cell lines. The human cell lines in both their primary and transformed state are unable to survive outside of the tissue culture flask and are therefore, unlikely to spread or cause damage to the environment. If a human or animal were to be infected by one of these transformed human cell lines, these cells would be rejected by the normal immune system.

All work will be carried out in purpose built and equipped laboratory space designated and reserved for such experiments.

All liquid waste will be decontaminated with 1% Virkon or 5% Sodium Hypochlorite. Solid waste will be autoclaved. A 100% kill will result.

Retroviral supernatants for infection of cell cultures will be produced in volumes of <(less than) 100 ml.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Retroviral vectors to be used in this study are considered by the ACGM to be non-mobilisable and incapable of further replication in the target cell.

Human cell lines in both their primary and transformed state are unable to survive outside of the tissue culture flask and are therefore, unlikely to spread or cause damage to the environment. If a human or animal were to be infected by one of these transformed human cell lines, these cells would be rejected by the normal immune system.

As sharps will not be used in these studies the risk of inoculation into humans is negligible. All work with retroviral supernatants will be carried out by the worker wearing laboratory coat, facemask and double gloves. The risk of human infection is considered negligible.

All liquid waste will be decontaminated with either 1% Virkon or 5% Sodium Hypochlorite. Solid waste will be autoclaved followed by incineration. Members of the MuLV group of viruses require high titre to establish infection in immunologically competent animals and so the risk of harm to either the environment associated with accidental
release of the vector is low.

The committee considers this work to be class II and therefore all retroviral work will be carried out in our Cat II suite, Rm 1.18.

Please enter comments on the GM safety committee on the risk assessment

The committee considers this work to be class II and therefore all retroviral work will be carried out in our Cat II suite, Rm 1.18.

Project Containment

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<td>L2 Yes L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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Animal Units
Large Scale Activities
Human Clinical Applications

Project Ref 774/01.1

<table>
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<th>CU2 Project Title</th>
<th>Date Project Ceased</th>
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<tr>
<td>15/03/2001</td>
<td>GENE THERAPY OF AUTOIMMUNE/INFLAMMATORY DISEASES AND CANCER</td>
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<thead>
<tr>
<th>Class</th>
<th>CultureVolClass2</th>
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<tbody>
<tr>
<td>Class 2</td>
<td>1-50 litres</td>
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</table>

Non-GMM
Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Development of vectors (viral and plasmid) and molecules for the treatment of autoimmune diseases and cancer by affecting the immune system and enabling restoration of normal tissue function.

**Recipient or parental organism**

All genetically modified vectors (see attachments) are disabled and lack essential genes which prevent their autonomous replication. They need specific packaging cell types to produce a viral particle which is replication deficient and cannot multiply or produce progeny. These vectors will have no effect to the environment as they will not be released, and are maintained under strict laboratory conditions.

**Host/vector system**

Retroviral packaging cells from mouse origin (GP+E86-ecotropic also pseudotyped with VSV-G protein; GPenv-AM12 and PT67 amphotropic), human packaging cells (Bosc-23p-ecotropic; ABOSC-23-amphotropic), human 293 cells to grow adeno-associated vectors, HIV vectors, disabled E.coli such as TOP-10, DH5a, LE392. See attached forms for more details.

**Origin & function**

All GMO's we use are genetically disabled. The E.coli strains cannot survive outside the laboratory and the viruses we use cannot replicate and do not contain any viral encoded genes (retroviruses, HIV vectors and adeno-associated virus).

No culture, container or solution containing GMO's is left untreated by Virkon, autoclave or incineration. All personnel involved have been and will be properly trained in the use of category II GMO's. It is not envisaged that any of the microorganisms used will have ecologically disruptive effects as they are genetically disabled and will be used under strict laboratory conditions only.

**Evaluation of foreseeable effects**

- All GMO's we use are genetically disabled. The E.coli strains cannot survive outside the laboratory and the viruses we use cannot replicate and do not contain any viral encoded genes (retroviruses, HIV vectors and adeno-associated virus).
- No culture, container or solution containing GMO's is left untreated by Virkon, autoclave or incineration. All personnel involved have been and will be properly trained in the use of category II GMO's.
- It is not envisaged that any of the microorganisms used will have ecologically disruptive effects as they are genetically disabled and will be used under strict laboratory conditions only.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

none

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

i. Liquid waste will be treated with Virkon 1% for 2 hours (shown by the manufacturer as appropriate for disinfection) before disposal to main drains. Solid waste will be autoclaved and later incinerated. Sharps will be incinerated. Cryovials containing virus will be stored in specified cryofreezer and opened only in Class II cabinets. Spillages and working surfaces will be cleaned with 1% Virkon and with 70% ethanol.

ii. Waste is clearly labelled biohazard double bags will be incinerated as clinical waste.
I. Hazard conditions consist of possible spillages, aerosol spray when opening cryovials or during the use of needles when working with animals.
ii. Work is restricted to determined areas, disinfecting solutions will always be available, working guidelines are clearly posted, unauthorised personnel is not permitted.
iii. Training to work with GMM's category II. Guidelines regarding dressing code, how to treat spillages and disinfection with 1% Virkon and 70% ethanol. Medical records held by occupational health advisor.
iv. Documented spillage procedures are clearly established. In case of accident with sharps, College procedure with blood borne viruses will be followed.
v. Autoclaves are regularly serviced and checked. Autoclaved material is checked using autoclaving tapeto assess has reached appropriate temperature for disinfection.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N
Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

I confirm that the information enclosed in the Risk Assessment has been discussed and agreed by our local committee.

Project Containment

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<tr>
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<td>L3 L4 L2</td>
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</table>

Project Ref 774/02.1

Date Ackn'd 13/08/2002
Date Project Ceased

CU2 Project Title ANGIOPGENIC GENE THERAPY PRODUCT FOR CORONARY ARTERY DISEASE

Class 2
CultureVolClass2 < 1 litre
CultureVolumeClass3-4

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

In the proposed clinical trial programme, angiogenic gene therapy product, consisting of a recombinant adenovirus (human serotype 5) containing the human gene for the fibroblast growth factor 4, and referred to as Ad5FGF-4, will be investigated as a treatment for patients with chronic stable angina due to coronary artery disease (CAD). Generation of new blood supply in the diseased heart by intracoronary administration of angiogenic gene therapy product represents a potential new therapeutic approach to relieve this condition.

**Recipient or parental organism**

See box below

**Host/vector system**

The Ad5FGF-4 gene therapy product consists of a recombinant adenovirus vector (human serotype 5, Ad5) with a deletion in the E 1 region; from map unit 1.3 to 8.7 of wild-type virus (entire E1A and most of E1B are eliminated). The FGF-4 transgene is inserted, driven by CMV promoter.

**Origin & function**

The FGF-4 gene was originally isolated from a cDNA library which was constructed from nRMA of Kaposi's sarcoma DNA transformed NIH3T3 cells. The intended function is angiogenesis, the formation of new blood vessels.

**Evaluation of foreseeable effects**

The probability of adverse consequences resulting from deliberate or accidental release of the gene therapy product Ad5FGF-4 are minimal to nonexistent.

Hazards resulting from environmental release (viral shedding from treated persons, inadvertent contamination of the product prior to administration) are negligible or nonexistent for the following reason: infection requires large numbers of infectious vectors, and transfection (expression of the inserted gene) requires a multitude of infectious particles.

Hazards associated with the adenoviral vector can be described as having low potential of adverse environmental consequences in humans or animals. The theoretical consequences to humans of several of the hazards associated with ectopic transgene expression, if they actually occurred, could be considered moderately severe (e.g. promotion of existent malignancy, unknown risk to foetus). However, since any unintended or accidental exposure would most likely be a fraction of the total dose being administered to patients for therapeutic purposes, the relative risk of the occurrence of these types of adverse effects should be very low.

The possible risks to the environment could be assessed as low to effectively zero. This is based on the low probability of infectious adenoviral particles escaping into the environment either through viral shedding by patients that have received the product, or by incidental exposure during administration procedures. Even if viruses were shed or product spillage occurred the number of infectious viral particles would be too small to result in infection of exposed tissues. The risk to the non-human environment is extremely low to effectively zero because of the species specificity of adenovirus 5, which by natural exposure is only known to infect humans.
The above indicates that the product could be classified as Class 1. However, as a precautionary measure, due to the limited experience available, the product is currently being classified as Class 2. Reclassification into Class 1 may be considered if based on increased data and experience.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be transferred to the autoclave in the London Chest Hospital in a sealed leakproof autoclavable container. Inactivation of Ad5FGF-4 is by autoclaving at 121°C for 15 minutes. This results in 100% kill of the vector. The material is then transported off site and incinerated. Service contracts are in place for the autoclave. Formal inspections will be conducted with the safety officer.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The GM safety committee met on the 8th July 2002 to discuss this proposal. All the relevant individuals involved in the project were also present to answer any queries. Overall the committee felt that the assessment was well written, with all the key issues well documented.

A number of specific questions were raised which were answered by Melanie Preston. These included:

1. The type of patients to be selected and the criteria for inclusion to the study.
2. How consent was obtained and the information to be given to the patients.
3. Whether on balance this study would be conducted at Class 1. On balance it was agreed that class 2 would be appropriate. As more experience with these types of studies is obtained the containment level would be re-examined.

The committee was informed that a safety audit was conducted which was satisfactory.

The assessment was accepted by the committee with some minor amendments and will be sent to the HSE as soon as possible.
### Project Additional Information

**Purpose of the contained use**

To express Semaphorin 4B in cultured mammalian cells to study the uncharacterised function of this protein.

**Recipient or parental organism**

- Cultured mammalian cells
  - Primary human macrophages

**Host/vector system**
E. coli such as DH5alpha  
Bacterial plasmids including pCMV-XL4 (Origene Technologies) and pCMV-script (Stratagene)  
Adenovirus transfer vector pShuttle-CMV (Qbiogene)  
Adenoviral plasmid AdEasy(Qbiogene)

### Origin & function

Bacterial plasmids and adenoviral plasmid constructs will be obtained from Oxford Biomedica Ltd (Oxford). Cultured mammalian cells will be obtained from Cancer Research UK (London) cell services department. Primary human macrophages obtained from buffy coat samples that have been screened for pathogens and dispatched by the national blood transfusion service.

The plasmid constructs will be used to express the Semaphorin4b gene in cultured mammalian cells by transfection and by adenoviral gene transduction. Various assays for migration, invasion, proliferation and gene expression will then be done.

### Evaluation of foreseeable effects

The bacterial host strains used are disabled and so cannot survive in the gut. Non-mobilisable bacterial plasmids including pCMV-XL4 (Origene Technologies) and pCMV-script (Stratagene) capable of stable integration in eukaryotic cells but not transmissible to natural gut bacteria.

The design and production methods of the adenovirus ensure that only replication defective viruses are produced and hence spread from an initially infected cell line is very unlikely. This is achieved by virtue of the adenoviral plasmid lacking the E1/E3 region of the wildtype adenovirus serotype 5 genome from which it is originally derived.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All apparatus (disposable pipettes, pipette tips, tissue culture flasks) used will be submerged in Virkon overnight before being autoclaved and disposed of by incineration.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment

A safety audit of the laboratory where the work will be carried out has been done. The assessment was accepted by the committee with some minor amendments.

### Project Containment

02/03/2022
<table>
<thead>
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**Project Ref 774/03.2**

**Date Ackn'd** 21/10/2003

**CU2 Project Title** TREATMENT OF CANCER MODELS WITH ADENOVIRAL GENE THERAPY VECTOR SYSTEMS, AND THE USE OF RETROVIRAL SYSTEMS TO IMMORTALISE NORMAL CELLS TO ALLOW FOR TRANSFORMATION

**Class** Class 2

**CultureVolClass2** ≤ 1 litre

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The above systems will be used to deliver reporter genes (such as GFP or LacZ) or therapeutic gene such as cytokine and chemokine receptor genes or pro-drug activation genes such as P450 and P450 reductase and cell cycle control genes.

**Recipient or parental organism**

Generation of vector particles in various widely available cell lines.

**Host/vector system**

Initially incorporation into the adenovirus transfer vector.

Adenoviral genome plasmid lacking the E1/E3 region has been created by homologous recombination in bacteria between the adenoviral genome plasmid (AdEasy1 (Qbiogenelnc, 2251 Rutherford Rd, Carisbad, CA 92008) and the aforementioned transfer vector. This system is not likely to generate replication competent adenovirus.
Retroviral systems such as pBabe-puro and murine virus packaging cell lines such as GP+E and AM12.

**Origin & function**

Reporter genes such as GFP and LacZ.
Therapeutic genes such as cytokine or chemokine genes eg IFN-γ of murine or human origin or chemokine receptor genes.
Prodrug activating genes such as P450 and P450-reductase.
Cell cycle control genes such as hTERT to immortalise normal cells to then study transformation.

**Evaluation of foreseeable effects**

The design and production methods of the adenovirus ensures that only replication defective viruses are produced, and hence spread from an initially infected cell is very unlikely. This is achieved by virtue of the adenoviral plasmid lacking the E1/E3 region of the wild type adenovirus serotype 5 genome from which it is originally derived. During viral production the packaging cell line Perc6 (Introgene) complement the deficiency in the E1/E3 region. Because the adenoviral region contained in Perc6 is designed to minimise the length of similarity with the transfected adenoviral plasmid, the generation of replication competent adenoviral particles, whereby the E1/E3 region becomes incorporated into the adenoviral genome, is greatly reduced.

The retroviral systems used do not contain the protein envelope coding sequence and therefore cannot produce any virus particles.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All reasonable precautions will be taken when working with genetically modified organisms.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All apparatus (disposable pipettes, pipette tips, tissue culture flasks) used will be submerged in 5% Virkon overnight before being autoclaved at 121°C for 20 mins and subsequently disposed of by incineration (QMW contract).

Liquid waste will be diluted in 5% Virkon (minimum 1/1000) for at least 24 hours.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Assessments were discussed at GM meeting held on 24/09/03.
Genes to be investigated were clarified.
The assessment was accepted with minimum amendments.

**Project Containment**

02/03/2022
Project Ref 774/04.1

Date Ackn'd 06/04/2004

CU2 Project Title The construction and use of adenoviral vectors for virally directed cancer gene therapy

Class CultureVol CultureVol
Class 2 < 1 litre

Consent Granted

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
To generate, introduce and express in mammalian cell systems genes which have the potential for cancer therapy

Recipient or parental organism
Standard disabled bacteria strains derived from E.coli such as DH5alpha, DH10B, XL-1 and BJ183 for the 'AdEasy' system.

Host/vector system
Various well characterised non-pathogenic mammalian cell lines
Replication incompetent (E1A deleted) adenoviral vectors ('AdEasy' See He et al, Proc Natl Acad Sol USA 1998; 95: 2509-14) containing inserted transgenes. AAV helper-free system (Stratagene) Wild-type adenoviruses (no inserted transgenes)
Replication-competent adenoviruses - DL1520 and DL922-947 mutants (including some inserted transgenes as detailed).
Replication defective viruses will contain the following types of genes: tumour suppressor and apoptotic genes (PTEN, p53, p16 and members of the bcl-2/bax family, IAP, SMAC families; dominant negative mutants -P13 kinase, RAS, AKT, MAPK) produce molecules that would promote tumour cell death but have no effect on normal cells; genes for prodrug activators (cytosine deaminase, HSV thymidine kinase, CYP1A2, carboxypeptidase G2 and linamarase enzyme) would only have a toxic effect to the cells that they have been introduced to and only in the presence of the appropriate prodrug; chemokines and cytokines (TRAIL, TNFalpha,fas ligand, SF14, Fli-3) should represent no hazard to humans as the viruses that express them will be tumour-specific and should increase tumour cell death in these models either directly or indirectly by enhancing anti-tumour immunity. Reporter genes (GFP,Lac-Z) will also be used and other genes as listed in attached risk assessment.

DL1520 and DL922-947 viruses the transgenes introduced will be: Sodium Iodide Symporter gene (hNIS), the pro-drugactivators (cytosine deaminase, thymidine kinase) and cytokines/chemokines TRAIL, TNF-alpha and fas ligand.

In all viruses used only one transgene will be expressed in one construct at any one time.

Wild type adenoviruses when used will contain no additional genes.

Work with adenoviruses will be separate from work with AAV.

Constructs will be targeted to mammalian cells and models of disease.

Evaluation of foreseeable effects

Most gene products as described previously act within tumour cells to promote cell death. Replication defective viruses (E1A deleted) cannot replicate or produce virus from recombination events in normal cells as the genes would not be present to complement. DL1520 does not replicate in normal cells and DL922-947 only replicates in cells that are cycling, both are attenuated relative to wild-type virus. DL 1520 has the E1B-55k region deleted, the protein that binds and inactivates p53. If the virus infects normal cells the cells will enter apoptosis and do so therefore the virus cannot replicate. Conversely in tumour cells where p53 is inactivated, the virus is able to replicate. DL922-947 the E1A-CR2 region is deleted which binds pRB and therefore replicated only in tumour cells where the pathway is inactivated. Both viral forms are attenuated compared to the wild-type adenovirus, in normal cells the attenuated is such that the yield of the virus is 0.1% of the wild type (see Heise, C et al Nat Med 2000 Oct;6(10):1134-9) in the replication competent viruses the transgenes will be inserted into the missing E3 region, which is separate from the site of disableness. Viruses are verified by PCR to ensure no recombination has occurred. The feasibility of insertion into the deleted E1B-55k (for DL1520) and E1A(for DL922-947) regions will be explored. No transgenes used in the viruses could compliment replication in normal cells. None of the genes expressed can complement for the defective nature of the viruses: inclusion of a gene such as p53 or pRb would actually restore wild type function of the cancer cell (rather than the virus) and make the virus unable to replicate. The cytokines/chemokines used should pose no additional risk. There is an established literature on each of them and we have expert collaborators who are using gene transfer systems for them presently (Andre lieber, University of Washington, Seattle USA; Frances Balkwill, Cancer Research UK) Effects on host immune reactions to viral mutants has been explored (Liu TC et.al: Mol Therapy. Wang YH et al: Nat.Biotech 21:1328-1225). Wild type adenovirus could potentially infect humans leading to mild flu-like symptoms, however their use is contained within a Cat 2 environment and manipulated within a biological safety cabinet and protective clothing used. In case of accidental spillage spills would be immediately inactivated with Virkon powder and a breathing mask used to avoid inhalation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

"All work is carried out in approved Category 2 laboratory facilities with standard containment and control measures. All staff are fully trained and work with appropriate protective clothing. All GMO material (and material exposed to GMOs) will be autoclaved prior to disposal."

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste diluted with 1% Virkon overnight or 10 minutes minimum (which gives complete disinfection according to manufacturers tests) then discarded to the sink. Plasticware soaked in 1% Virkon then inactivated material is autoclaved or incinerated.

Contaminated glass (pasteur pipettes) are saked in 1% Virkon before incineration. Use of sharps is discouraged at all times. However where unavoidable, sharps are
discarded into sharp-bins and incinerated.

Solid waste is autoclaved prior to disposal into clinical waste destined to be incinerated by professional contractors.

No viable GMOs are discarded.

Project reviewed and approved at Local GMSC meeting on 29/03/04

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick if you have attached a risk assessment to this form

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

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Animal Units

| L2 L3 L4 L2 L3 L4 |

Large Scale Activities

| L2 L3 L4 L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 L2 L3 L4 |

Project Ref 774/04.2

Date Ackn'd 06/04/2004

CU2 Project Title

THE USE OF MAMMALIAN AND BACTERIAL EXPRESSION VECTORS IN THE STUDY OF GENES AND PROTEINS INVOLVED IN CELL SURVIVAL SIGNALLING.

Date Project Ceased

Class

Class 2

Consent Granted

Non-GMM < 1 litre

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N
The aim of the project is to express various signalling proteins in mammalian cell culture to assess their effects on cell biology, i.e. apoptosis and cell cycle perturbations.

Recipient or parental organism

XL1-Blue, XL-10 Gold bacterial strains

Host/vector system

pCDNA-3, pEGFP, pBABE, pRETRO-SUPER vectors

Origin & function

p53 and family members - tumour suppressors, involved in sensing DNA damage and other stresses, function as transcription factors.
Yes-Associated Protein (YAP) - function as transcription factor co-activators, pro-apoptotic in certain conditions.
Akt - serine/threonine kinase, pro-survival factor, involved in protection from apoptosis and regulator of metabolism.
AMPK-AMP dependent kinase, senses AMP levels, regulator of metabolism in cells.
14-3-3 - phospho-adaptor protein, binds phosphorylated protein in cell, involved as scaffold for signalling.
Forkhead and family members-transcription factors involved in cell cycle and apoptosis.
Bcl-2 family members-proteins that are both pro- and anti-apoptotic.

Evaluation of foreseeable effects

The retroviral vector system contains inherent safety features to minimise the risks associated with them. The vectors themselves, pRETRO-SUPER(pRS) were generated by creating a self-inactivating murine stem cell virus (pMSCV) plasmid. The 3' LTR of the pMSCV was inactivated by an internal (NheI-XbaI) deletion to generate a self-inactivating virus(-LTR). Upon integration to the genome of the virus produced from this vector, the 3' LTR is duplicated to the 5' LTR to generate a provirus that lacks all of the LTRs enhancer-promoter activities (see Brummerkamp et al, Cancer Cekk,2(3):243-7, 29002) For proper integration, plasmid has to be transiently transfected into packaging cells, i.e: Phoenix. Otherwise plasmids will be generated, but not viruses. Packaging cells have viral protein production machinery for viral proteins (env, gag, pol) necessary for virus production-retroviral plasmid itself does not.

Replication competent virus is unlikely to be generated by recombination, three recombination events would be required for replication competent helper-virus outbreak (http://www.stanford.edu/group/nolan/tutorials/retpkg_6_repcomp.html).
Genes encode human proteins, which are all expressed intracellularly and do not have activity outside the cell. Inserted genes are human, will not affect bacterial pathology. Amphotrophic virus would be able to infect human cells if not handled correctly, downregulation of oncogenes or tumour suppressor genes locally could have deleterious effects. However, all work with amphotrophic virus will take place in a category 2 facility and with the requisite care using biological safety cabinets and personal protective clothing, rendering the risk of exposure to low. Where possible, ecotropic virus will be used. In the event of accidental spillage the spillage would occur within our laboratory would be disinfected with Virkon powder leading to the rapid destruction of live material thus eliminating the risk of genetic transfer to the environment. Waste would be treated as detailed in Section 12 to avoid transfer of genetic material to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste diluted with 1% Virkon overnight or 10 minutes minimum (which gives complete disinfection according to manufacturers tests) then discarded to the sink. Plasticware soaked in 1% Virkon then inactivated material is autoclaved or incinerated. Contaminated glass (pasteur pipettes) are saked in 1% Virkon before incineration. Use of sharps is discouraged at all times. However where unavoidable, sharps are discarded into sharp-bins and incinerated.

No viable GMOs are discarded.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project reviewed and approved at Local GMSC meeting on 29/03/04

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02/03/2022
**Project Ref** 774/05.1

<table>
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<th>Project notified under transitional arrangements</th>
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<td>Study of the biology of adhesion for the purpose of developing novel anti-cancer strategies.</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Not Applicable</td>
<td>N</td>
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<tr>
<th>Date of Significant Change</th>
<th>Project Additional Information</th>
</tr>
</thead>
</table>

**Project Additional Information**

**Purposes of the contained use**

To identify novel therapeutic targets related to cancer cell adhesion, migration and invasion by dissecting the molecular basis of cell adhesion in normal and cancer cells and tissues.

**Recipient or parental organism**


**Host/vector system**

Standard disabled bacteria strains mostly derived from E. coli, K12 eg DH5, TOP10, JM109. Various mammalian cell lines. Replication incompetent (due to lack of env, gag and pol(1)) retroviral plasmids (including pBabe, pRev Tet on/pRev TRE (Clontech's tetracycline/doxycyline inducible system), pLNCX2). E1a-deleted adenoviral vectors, various murine cell line strains.

**Origin & function**

Cell adhesion molecules (eg integrins, cadherins, desmosomal proteins) are not considered oncogenes but their expression varies in disease, including cancer. (In fact E-cadherin is considered a tumour suppressor). By selectively deleting or over-expressing wild-type, mutant or fragments of these molecules we will dissect the role of individual molecules in cancer.
Cell-adhesion molecules modulate cell behaviour via their interaction with both signalling and structural molecules. We shall seek to down-regulate the expression of wild-type, mutant or fragments of these molecules in order to trace the role of individual molecules on the function of cell adhesion molecules. These signalling molecules may include growth factors, tyrosine kinases, serine/threonine kinases, phosphatases, GTPases and the structural molecules may include cytoskeletal associated proteins.

We will also use siRNA to down-regulate the adhesion and adhesion-associated molecules described.

**Evaluation of foreseeable effects**

Modulating the function of adhesion molecules, by mechanisms described above, may affect the adhesive and motile behaviour of cells, which may affect tumourigenicity. Some of the relevant signalling molecules (e.g., src, Ras) have oncogenic mutant forms. Similarly, down-regulation of tumour suppressor genes with siRNA would also likely increase tumourigenic potential of cell lines. However, as all GM-active staff are given training in a strict code of practice and as all infectious vectors are replication incompetent, risk associated with such work is reduced to a minimum as described in the risk assessment.

**Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste diluted with Klorsept (to 10,000 ppm chlorine) or 1% Virkon overnight (which gives complete disinfection according to manufacturers tests) then discarded to sink (containment Level 1 activity) or autoclaved (Level 2). Plasticware is soaked overnight in Chlorox (e.g., pipettes) then autoclaved, or just autoclaved (e.g., tissue culture dishes). Transport to the autoclave is in special autoclave bags held in a leak-proof plastic, wheeled-container. Inactivated material is incinerated.

Contaminated glass is soaked in Klorsept then autoclaved before re-use. Use of sharps is discouraged at all times. However, when unavoidable, sharps are discarded into sharp-pins and incinerated.

No viable G/Ms are discarded.

**Is an emergency plan required according to regulation 20?**  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

This was reviewed by members of the local committee and no adverse comments were received.

**Project Containment**
Project Additional Information

Purposes of the contained use

The purpose of the contained use is to undertake an exploratory phase I/II trial of a replicating herpes simplex virus vector, OncoVEX-GM-CSF, in patients with locally advanced epithelial cancer of the head and neck.

Recipient or parental organism

See box below.

Host/vector system

OncoVEX-GM-CSF is a herpes simplex virus type 1 vector. It is based on the strain JS-1 that has been developed at BioVEX, the company that has generated OncoVEX-GM-CSF, rather than the lab strain 17+ that has been used in previous clinical trials. In the GMP manufacture, the virus was propagated on Vero cells (ATCC catalogue number X38) African green monkey kidney cells.

Origin & function

Not Applicable
OncoVEX-GM-CSF I contains three modifications. It is deleted in the neurovirulence factor ICP34.5. Deletion of this gene dramatically reduces the replication of this virus in normal neurons and many previous publications suggest that IPC34.5- HSV vectors are only capable of replicating within tumour cells. OncoVEX-GM-CSF is also deleted in ICP47. The normal function of ICP47 is to block antigen presentation with MHC class I and II on the cell surface by inhibiting the transporters associated with antigen processing (TAP1 and TAP2). This also allowed upregulation of US11, a gene that promotes viral growth in tumour cells without decreasing tumour selectivity. Finally, an expression cassette consisting of the CMV immediate early promoter driving expression of a human GM-CSF gene is situated in the site of both ICP34.5 genes.

OncoVEX-GM-CSF is believed to function via two separate mechanisms. Firstly, it is oncolytic, thereby destroying tumour cells directly. It is thus able to infect and destroy neighbouring, uninfected tumour cells. Secondly, the destruction of tumour cells leads to the release of tumour-associated antigens that engender an immune response, enhanced by the local expression of GM-CSF. It is hoped that this immune response may lead to further immune-mediated destruction of the injected tumour and distant metastases.

**Evaluation of foreseeable effects**

This virus has completed phase 1 trials in humans, so extensive data are available on the effects of deliberate injection into humans.

All patients in the phase 1 trials were injected with OncoVEX-GM-CSF directly into cutaneous or subcutaneous metastases from a variety of primary malignancies, including breast, head and neck and colon cancers or melanoma. HSV type 1 seronegative individuals appear to react differently to those who are seropositive. Doses of 10^6 pfu/ml are well tolerated by seronegative and positive individuals, although the single seronegative patient developed mild fever (<38°C) five days after injection. However, at doses of 10^7 pfu/ml, both seronegative patients developed fever, that was again transient and mild, and erythema around the injection site along with vesicles that took several weeks to heal. In seropositive patients, doses of 10^7 and 10^8 pfu/ml were tolerated without side effects.

However, it must be stressed that the virus was deliberately injected into tumour nodules (which had been confirmed by biopsy prior to enrolment in the trial), rather than normal tissue. Pre-clinical assessment of OncoVEX-GM-CSF and other ICP34.5 deleted HSV1 vectors has suggested that there is minimal replication of ICP34.5 deficient HSV1 vectors has suggested that there is minimal replication of ICP34.5 deficient HSV1 vectors in normal cells. Similarly, BalbC mice tolerated three subcutaneous doses of 1 x 10^9 pfu OncoVEX-GM-CSF and direct intracerebral doses of 1 x 10^7 pfu without significant toxicity, whereas the LD50 for intracerebral wild-type HSV1 in BalbC mice is <10 pfu.

At no point in the trial was recombination with wild-type virus ever detected, nor reactivation of latent wild-type HSV1 infection.

Finally, this virus remains highly susceptible to acyclovir treatment, as the thymidine kinase gene remains intact. Therefore, in the event of accidental inoculation of a health care worker, treatment with acyclovir will lead to rapid elimination of any infected virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All treatment will take place as in-patient procedures. All injections be performed in the new gene therapy and experimental medicine unit, which is equipped with double HEPA filtration. Any liquid waste will be treated with Virkon. A 1% solution of Virkon has been shown to provide effective disinfection within 10 minutes (see http://www.antechh.com/framset.html)

An autoclave is present within the gene therapy unit. All solid waste potentially contaminated with OncoVEX-GM-CSF will be autoclaved prior to disposal.

Surfaces will routinely be disinfected with 1% Virkon and 70% ethanol.

All patients will wear occlusive dressings over injected tumours: in the phase 1 trial of OncoVEX-GM-CSF, no virus was ever detected penetrating these dressings, suggesting that accidental release into the atmosphere is also unlikely.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
All treatment will take place in a dedicated gene therapy unit equipped for work at containment level 2.

Any liquid waste will be treated with Virkon. A 1% solution of Virkon has been shown to provide effective disinfection within 10 minutes. (see http://www.antechh.com/framset.html)

An autoclave is present within the gene therapy unit. All solid waste potentially contaminated with OncoVEX-GM-CSF will be autoclaved prior to disposal.

Surfaces will routinely be disinfected with 1% Virkon and 70% ethanol.

All patients will wear occlusive dressings over injected tumours: in the phase 1 trial of OncoVEX-GM-CSF, no virus was ever detected penetrating these dressings, suggesting that accidental release into the atmosphere is also unlikely.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Please enter comments on the GM safety committee on the risk assessment

This application was discussed at the local GM safety committee on Monday 14th February 2006. Issues raised and discussed were:

- a) provision of standard operating procedures concerning GM work in the gene therapy and experimental medicine unit, especially relating to disposal of waste.
- B) possibility of using retractable needles to minimise the risk of needle stick injuries.
- C) storage and documentation of use of OncoVEX-GM-CSF, including pharmacy records.

The local GM safety committee was satisfied that all these points were adequately addressed.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

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Project Ref 774/06.2
Use of viral vectors for the transfer of genes for studying the biology of adhesion for the purpose of developing novel and-cancer strategies.

Purposes of the contained use
To use lentiviral and adenoviral vectors to identify novel therapeutic targets related to cancer cell adhesion, migration and invasion of dissecting the molecular basis of cell adhesion in normal and cancer cells and tissues.

Recipient or parental organism
Standard disabled bacteria strains mostly derived from E. coli K12 including DH5a, TOP10, JM109. H293-derived packaging cell lines.

Host/vector system
Standard disabled bacteria strains mostly derived from E.coli, K12 eg DH5a, TOP10, JM109. Various mammalian cell lines. Replication incompetent 2nd generation and additionally self-inactivating 3rd generation lentiviruses. Replication-incompetent adenoviral vectors (AdEasy based Ad5 and Ad2 vectors) and replication competent adenoviruses modified to only replicate in tumour cells but not cells with normal p53 and Rb pathways.

Origin & function
Cell adhesion molecules (eg integrins, cadherins, desmosomal proteins) are not considered oncogenes but their expression varies in disease, including cancer. (in fact E-cadherin is considered a tumour suppressor). By selectively deleting or over-expressing wild-type, mutant or fragments of these molecules we will dissect the role of individual molecules in cancer.

Cell-adhesion molecules modulate cell behaviour via their interaction with both signalling and structural molecules. We shall selectively delete or over-express wild-type, mutant or fragments of these molecules in order to trace the role of individual molecules on the function of cell adhesion molecules. These signalling molecules may include growth factors, tyrosine kinases, serine/threonine kinases, phosphates, GTPases and the structural molecules may include cytoskeletal associated proteins.

Insertion of peptide sequences into coat proteins of adenovirus (particularly knob) to re-direct the adenovirus to more restrictive cancer associated targets, including over-expressed adhesion molecules, growth factor receptors and cytokine receptors. Such modifications will include removal of endogenous CAR and integrin binding site.
making to restrict tropism to specific target cells.

We will also use siRNA to down-regulate the adhesion and adhesion-associated molecules described.

Evaluation of foreseeable effects

Modulating the function of adhesion molecules, by mechanisms described above, may affect the adhesive and motile behaviour of cells, which may affect tumourigenicity. Some of the relevant signalling molecules (eg src, Ras) have oncogenic mutant forms. Similarly down-regulation of tumour suppressor genes with siRNA would also likely increase tumourigenic potential of cell lines. In addition, it is understood that the WRPE region of lentivirus is reported to have oncogenic properties. However, as all GM-active staff are given training in a strict code of practise and as all infectious vectors are replication incompetent, risks associated with such work is reduced to a minimum as described in the risk assessment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All facilities are at category 2 standard, with a strictly controlled environment. All rooms and laboratories are constructed so as to prevent accidental release into the environment. In the unlikely event that an escape/leak should occur the organisms concerned will not be able to survive. All waste generated will be autoclaved prior to incineration.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste diluted with Klorsept (to 10,000 ppm chlorine) or 1% Virkon overnight (which gives complete disinfection according to manufacturer's tests) then discarded to sink (containment level 1 activity) or autoclaved (level 2). Plasticware is soaked overnight in Chloros (eg pipettes) then autoclaved, or just autoclaved (eg tissue culture dishes). Transport to the autoclave is in special autoclave bags held in a leak-proof plastic, wheeled-container. Inactivated material is incinerated.

Contaminated glass is soaked in Lorsept then autoclaved before re-use. Use of sharps is discouraged at all times. However, when unavoidable, sharps are discarded into sharp-bins and incinerated.

No viable GMOs are discarded.

Is an emergency plan required according to regulation 20? [ ]

If yes, tick to confirm that it is attached to this form [ ]

Tick to confirm that you have attached a risk assessment to this form [ ]

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ]

Please enter comments on the GM safety committee on the risk assessment

The GMSC considered this proposal on the 27/10/2005
The following points were considered:
1. Environmental risk assessment was considered (attached)
2. Following current guidance from HSE the lentivirus work is deemed to be class 2.
3. Replication competent work was considered to be Class 2.
4. All other adenoviral work was considered to be Class 1.
**Project Additional Information**

**Purposes of the contained use**

The purpose of this work is to explore the ability of vaccinia virus (VV) to accumulate and preferentially replicate within solid tumours. A variety of replication-selective oncolytic VV will be constructed and their efforts on virul tissue specificity and antitumour ability will be assessed. In addition, vectors carrying therapeutic genes to enhance the antitumour potency and the reporter genes GFP and luciferase will be used to follow the location of the virus within host systems.

**Recipient or parental organism**

Vaccinia strains Western Reserve, Lister and Wyeth
All three strains are commercially available and have been widely used in several laboratories for many years. In addition, strains Lister and Wyeth have been used as smallpox vaccines in millions of people worldwide. They are classified as containment level 2 by the ACDP. In our project, we mostly focus on using lister strain.

**Host/vector system**

Host cells: E.coli DH5α, K12. The E.coli strains are widely used, highly attenuated commercial strains.

Vectors: pUC18, pUC4K, pSJH7 and pNT8, these vectors fulfill a number of criteria that make them safe to use.

**Origin & function**

DNA from wild type strains will be amplified by PCR and cloned first into standard, commercially available plasmids (pUC18, pUC4K) where deletion mutants will be constructed. The modified DNA will then be cloned into the specialist plasmid (pSJH7) for transient selection. These DNA manipulations will take place in commercially available E.coli strains, acting as as a clone vector. The plasmids will be co-transfected into the cell lines TK143 or D980R along with a second vector containing an attenuated version of the wild type VV genome, such that viral replication can only occur following recombination of the two plasmids. Virus can then be picked form plaques and successful recombination checked by PCR and Southern bloting. Genes to be targeted will be involved with crucial genes for VV replication (as described in the risk assessment, such as VGF and TK) and the evasion of the host immune system (IFN binding proteins, anti-apoptotic proteins). Inserted DNA will be heavily disrupted and so unlikely to be active and the disruption of these genes is likely to reduce the pathogenicity of the host organism.

In addition, this same techniques will be used to insert the therapeutic genes into the viral genome. These therapeutic genes have been listed in the risk assessment. Vaccinia virus containing reporter genes such as the sodium iodide Symporter, GFP, β-Galactosidaase and Luciferase will also be used as control vectors. We will inform the HSE whenever any inserts that deflect from this form are chosen to arm the viruses.

**Evaluation of foreseeable effects**

The results of genetic modification will reduce the pathogenicity of the parental organism. No adverse effects are anticipated. The inserted therapeutic genes chosen in this project are no harm to human health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All reasonable precautions will be taken with genetically modified organisms. All facilities are at category 2 standard with a strictly controlled environment. All rooms and laboratories are constructed so as to prevent accidental release into the environment. In the unlikely event that an escape/leak should occur the organisms concerned would not be able to survive. All waste generated will be autoclaved prior to incineration.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid waste is deactivated using 1% Virkon overnight or 10 minutes minimum (which gives complete disinfection according to manufacturers tests, see www.antech.com/virindex) then discarded to the sink. Plasticware soaked in 1% Virkon then inactivated material is autoclaved or incinerated. Contaminated glass (pasteur pipettes) are soaked in 1% Virkon before incineration. Use of sharps is discouraged at all times. However where unavoidable, sharps are discarded into sharps bins and incinerated.

Solid waste is autoclaved prior to disposal into clinical waste destined to be incinerated by professional contractors. No viable GMOs are discarded.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form
Project reviewed and approved at local GMSC meeting on 27/10/05. The committee recommended that this project be classified as class 2 due to the ACDP classification of the wild type vaccina virus as containment level 2, despite the fact that created GMMs will be attenuated for virulence.

Project Containment

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<td>Large Scale Activities L2 L3 L4</td>
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<tr>
<td>Human Clinical Applications L2 L3 L4</td>
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</table>

Project Ref 774/06.4

Date Ackn'd 19/07/2006
Date Project Ceased

CU2 Project Title Use of human stem cells to repair neurological disease.

Class 2
Consent Granted Not Applicable

Withdrawn N

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change
Project Additional Information

Purposes of the contained use

During the use of stem cells containment level 2 will be observed. The room will not be accessible to any persons not fully aware of the risks associated with the use of these cells. This especially relates to the risk of injury from sharps that may have been exposed to the GMO.

Recipient or parental organism

The purpose of this work is to examine the ability of modified Human stem cells to augment/recover functional loss in neurological disease.

Host/vector system

Human neural stem cells derived from the ventral mesencephalon infected with a replication defective amphotropic retrovirus carrying the mycERTAM construct. The cells do not carry replication competent retroviruses. Cells are also transduced with resistance to Neo (G418). The two transgenes, mycERTAM and neo, are cloned into a pSVX plasmid and transmitted to the cells via cell suracereceptor-viral envelope protein interaction. The site of insertion into the vector is at the BamH1 site of the pSVX plasmid. v-myc will result in an immortalization of transduced cells.

Origin & function

To ensure a homogenous and persisting population of neural stem cells in vitro, these cells were transduced with the immortilizing mycERTAM, construct, a chimeric gene encoding the c-myc proto-oncogene fused with a tamoxifen-sensitive mutant of the estrogen receptor.

Evaluation of foreseeable effects

These cells carry an oncogene and theoretically there is potential for tumourigenesis, but this is considered to be effectively zero since the oncogene is constrained entirely within the cells. Thus there is no vector to facilitate transfer of the oncogene out of the cells and into the wider environment. Moreover, in the absence of tamoxifen, the oncoprotein is entirely non-functional, so even within the cells the capacity for expression is extremely limited.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All reasonable precautions will be taken with genetically modified organisms.

All facilities are at category 2 standard within a strictly controlled environment. All rooms and laboratories are constructed so as to prevent accidental release into the environment. In the unlikely event that an escape/leak should occur the organisms concerned would not be able to survive. All waste generated will be autoclaved prior to incineration.

In the absence of a helper virus, the transgenic construct is totally constrained within the cells. The cells have no capacity for survival in the environment. Hence the threat to the environment is very small. This vector system is well established in laboratories and has a good record of environmental safety.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste products will be deactivated by overnight soaking in a 1% Virkon solution before being discarded to the sink. Plasticware will be soaked in 1% Virkon to inactivate and then incinerated or autoclaved.

Contaminated glass will be soaked in 15 virkon prior to incineration. Use of sharps will be minimised, but where used will be discarded in a sharps bin and incinerated.
Solid waste will be autoclaved and then incinerated by professional contractors.

No viable GMOs are discarded.

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment

This project was reviewed 27/10/05  
The work was deemed to be at containment level 2  
More construct details were requested (see attached risk assessment)

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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Animal Units

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Project Ref 774/07.1

Date Ackn’d 13/04/2007

CU2 Project Title

The knockdown of tumour suppressor gene expression by RNA interference using amphotropic retroviral and lentiviral vectors.

Date Project Ceased

Project notified under transitional arrangements  

N

Class 2  

Consent Granted

Not Applicable

Tick if notifying a connected programme of work  

Y
Purposes of the contained use

The aim of the project is to generate replication defective amphotropic retroviruses and lentiviruses that express shRNAs against a range of tumour suppressor genes, including CDKN2A, AFR, TP53, PTCH, the telomerase components hTERC and hTERT, AKAP220, PCDH7/8, SERPINB family, LPHH2, GRM7, PLOD2, RASGRF2, OAT3, PIG38 and WDR4, which under certain circumstances can suppress tumour development. Other genes will include MYC, GL-1, GL-2, WNT-1, WNT-8A, WNT-16, WDR4, S33Y, engrailed, winged helix domain, SMO, RAS-GRF2, FOXM1.

This will allow the knockdown in expression of these genes using short hairpin RNAs that generate the stable expression of small interfering RNAs (RNAis) within the target cell. This will enable evaluation of the role of the above genes in human keratinocyte senescence.

Recipient or parental organism

The cells used will be primary cultures of human epidermal, oral, hair follicle and eccrine sweat gland keratinocytes, sebocytes, prostate epithelial cells, dermal papilla fibroblasts, dermal fibroblasts, lung fibroblasts and their immortal or neoplastic derivaties. The target cells in culture are not considered to posr any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory.

Retroviral or lentiviral infection is only transient in the target cell and it will not be able to produce the virus. The cells will therefore not exert any foreseeable effects on either human health or the environment.

Host/vector system

The system used to allow expression of small interfering RNAs against genes mediating senescence in human keratinocytes involves replication-defective amphotropic retroviruses and lentiviruses.

The retroviral (pRETOSUPER and pRETROSUPERIOR) and lentiviral (pLentLox3.7, pLKO.1 or pHR1U1-CMVGFPSin 18 will be used to transfer the shRNAs into human keratinocytes. The lentiviral vectors have the advantage of being able to infect non-diving keratinocytes. These viruses have the potential to infect human cells only when they have been packagaed in appropriately engineered packaging cells. Once packaged these infective viruses are extremely labile and do not have the capability to replicate or produce infective virus in other non-engineered cells.

In more detail:
The pRETROSUPER and pRETROSUPERIOR vectors are derived from the Murine Embryonic Stem Cell virus (pMSCV). The vector has a specifically designed 3'LTR that has a deletion in the LTR promoter elements. This deletion results in inactivation of the LTR mediated transcription upon retroviral integration. The constructs will be grown in the excision repair-deficient E. Coli strain GT116 to avoid removal of the shRNA sequences. The resulting plasmids will then be transfected into Phoenix-E cells.
To produce ectropic retrovirus and then into PT67 cells to generate high titre amphotropic retroviruses. Alternatively the constructs will be transfected into Phoenix A cells to generate amphotropic virus directly. Each of these cell lines contains gag, pol and env genes encoded by 2 separate plasmid expression vectors both of which lack the retroviral cis-acting packaging signal to minimise the likelihood of replication competent virus arising through recombination.

The first lentiviral vector, pHR1U1-CMVGFPSin18, was created by first sub-cloning inserts into the BglII/SalI site in the pIU1-T7 vector. The PCR fragments containing both IU1 promoter and the insert were sub-cloned into the pHRCMVGFPSWsin18 vector. Lentivirus will be generated by co-transfecting 293T cells with the pMD.G, pCMVDR8.91 and pHR1U1-CMVGFPSin18 using the three plasmid lentiviral packaging system.

The second lentiviral vector to be used, pLL3.7, is the third generation system (self inactivating vectors) derived from HIV-1 incorporating many safety features. These include deletion in the enhancer region of the 3'U3 of the long terminal repeat which results in self inactivation after transduction of the target cell. Genes encoding the structural and other components are split into four plasmids which are engineered not to contain any regions of homology to prevent any recombination and none contain LTRs or psi packaging sequence. Thus none of the HIV-1 structural genes are present on the packaged viral genome and thus are never expressed in the transduced target cell. Short hairpin RNAs (shRNAs) will be cloned into the pLentLox3.7 (pLL3.7) lentiviral transfer vector and delivered to the cells in the form of an amphotropic lentivirus. The resulting infected cells will then generate a continuous supply of RNAi in the target cells. Infected cells can be identified by the co-expression of the GFP FLAG with shRNA. The expression cassette to create a vector that simultaneously produces shRNAs and a reporter gene. To facilitate the introduction of RNAi stem cell loops, a multiple cloning site was placed immediately after the U6 promoter. The pLL3.7 constructs will be grown in either DH5 alpha or the excision repair-deficient E.Coli strain to avoid removal of the shRNA sequences. The resulting plasmids will then be transfected into 293FT cells along with the packaging vectors pMD.Lg/pRRE (gag/pol elements), pRSV-REV and pMD.G (VSVG envelope glycoprotein vector) to produce high titre amphotropic lentiviruses. These are available commercially from Invitrogen as pLP1, pLP2 and pLP/VSVG vectors.

The lentiviruses will then be recovered by ultracentrifugation and resuspended in phosphate-buffered saline before titration on HeLa cells. Concentrated viruses 10 million infectious units per ml will then be used to infect the normal human keratinocytes.

pLKO.1-puro (modified third-generation HIV-derived lentiviruses)
This vector is replication incompetent and self-inactivating, and requires the co-transfection of 2 plasmids carrying the gag, pol and VSV G genes to produce lentiviral particles.

Origin & function
The genetic material to be introduced via the retroviral and lentiviral systems consists of short hairpin RNAs that target a number of genes involved in mediating senescence in human keratinocytes. These include the tumour suppressors, hTERC, hTERT, CDKN2A, ARF, TPS3, PTCH, AKAP220, PCDH7/8, SERPINB family, LPHH2, GRM7, PLOD2, RASGRF2, OAT3, PIG38 and WDR4.

It is anticipated that by knocking down the expression of these genes their role in keratinocyte senescence can be further understood.

In addition, the lentiviral vector has been engineered co-express GFP FLAG with the shRNA constructs to act as markers of infected cells.

Evaluation of foreseeable effects
The final GMO human cells will contain stably integrated DNA copies of the retroviral and lentiviral vectors encoding the transferred shRNAs against the genes listed above. The cells will be free of retrovirus or lentivirus. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside the laboratory culture.

It will however, be important to prevent human exposure or environmental release of the replication defective retroviruses or lentiviruses which are used to transiently effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
To minimise the risk to human health it will be essential to prevent exposure to infectious retroviral or lentiviral particles. All work will therefore be carried out under Class II containment conditions. Following experimental work, all viral supernatants will be autoclaved and spent medium will be inactivated using precept tablets (10,000 ppm chlorine), 1% Virkon or 1000cpm Actichlor™ before removal from the Class II laboratory for disposal. Solid waste will be double -bagged in biohazard bags prior to removal from the Class II laboratory and then autoclaved before final consignment of waste approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus.

Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by both infection of appropriate indicator cells and assays of reverse transcriptase activity. Once these criteria have been met the infected target cells will be removed from Class II containment and handled using standard tissue culture procedures.

Separate lab coats are worn within the Class II laboratory, gloves will be worn, plastic ware will be used and all use of sharps will be avoided.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

The risk assessments were reviewed by the GMSC on 30th August 2006. It was agreed that a number of similar assessments would be grouped together since the basic work in each case is fundamentally the same. The committee asked in some cases for more information regarding inserts and vectors. A sub-group of the committee reviewed again (7th Feb 2007) the local risk assessments prior to sending to HSE. The local assessment by Dr The is included in this application as well as the accompanying one as it includes work using RNA silencing techniques.

(The risk assessment pertaining to a very similar notification was discussed at the CRUK Beatson Laboratories, Glasgow, Safety Committee of the 12th May 2004 and was approved with minor modification.)

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02/03/2022
The aim of the project is to generate replication defective amphotropic retroviruses that express human oncogenes, in particular; RAS, MEK-EE (constitutively active), ERBB1, EPS8, MYC, GLI-1, GLI-2, WNT-FOXM1, AXL-1, Htert, Htetc, AKAP220, PCDH7/8, SERPINB family, LPHH2, GRM7, PLOD2, RASGRF2, OAT3, PIG38, WDR4, Akt, Erk1/2, EPS8. Other tyrosine kinases may be included as appropriate.

Other genes include HaRas V12, ikBaM. Other genes associated with the telomerase complex. This will enable evaluation of the role of the above genes in human cellular senescence, immortalisation transformation and telomerase regulation.

Any significant deviation from this the above will be notified to HSE.

The cells used will be primary cultures of human epidermal, oral, hair follicle and eccrine sweat gland keratinocytes, sebocytes, prostate epithelial cells, dermal papilla fibroblasts, dermal fibroblasts, lung fibroblasts and their immortal or neoplastic derivatives.

Other cells will include EBV-transduced human B cells, primary lymphoid cells, phoenix packaging cell line.

The target cells in culture are not considered to pose any inherent hazard to human health, nor do they have the capacity to survive outside the laboratory.

Retroviral infection is only transient in the target cell and it will not be able to produce the virus. The cells will therefore not exert any foreseeable effects on either human health or the environment.

The system used to allow expression of human oncogenes mediating senescence in human cells involves replication-defective amphotropic retroviruses.
The retroviral vectors pBABE, pWZL, pMNDBanshee, pBloxTSH, pLCRESH, LXN and pSIN will be used to transfer the into human keratinocytes. The lentiviral vectors have the advantage of being able to infect non-dividing keratinocytes. These viruses have the potential to infect human cells only when they have been packaged in appropriately engineered packaging cells. Once packaged these infective viruses are extremely labile and do not have the capability to replicate or produce infective virus in other non-engineered cells.

In more detail: pBabe series which is based on the Moloney murine leukaemia virus, MoMLV and contains the LTR and packaging sequences from the MoMLV. The pBabe vector lack the env and reverse transcriptase genes and there is no eukaryotic origin of replication, hence these vectors are considered to be non-mobilisable. This modified virus will be classified as Class 1. The full length hTERT cDNA was cloned in the EcoRI site of pBabe-Hygro, pBabe-Puro and pBabe-Neo downstream of the LTR promoter to create pBabe-Hygro-hTERT, pBabe-Puro-hTERT and pBabe-Neo-hTERT.

To create pBloxTSH, the 3.5-kb EcoRI-SalI fragment from pCI-Neo-hTERT was first cloned in the EcoRI and SalI sites of pBabe-Puro, to create pBabe-Puro-hTERT. The 1.7-kb HpaI-HindIII L-histidinol resistance gene (HISD) from pLXSHD into the 8.2kb SalI-HindIII fragment from pBabe-Puro-hTERT, creating pBTSH. The 5.2kb EcoRI-HindIII sits in pBabe-Puro, creating pBloxTSH.

pMND was created from Moloney Murine Leukemia Virus. The MND backbone, which has alterations to address three viral elements implicated as repressors of expression by Mo-MuLV: the enhancer, the primer binding site, and the negative-control region. pMND-Banshee was modified from pMND so that it harbours a specialised termination signal to result in non-polyadenylated transcripts. hTERC was cloned downstream of the U1 promoter in the EcoRI and HindIII sites of Bluescript II SK (+). U1-hTERC was cloned in the BglII and HindIII sites of the pMND-Banshee-Neo to create pMND-Banshee-hTERC.

pLXSH is a derivative of pLXSN that carries the hygromycin resistance gene. pLXSN contains eukaryotic expression elements: 5’ Moloney murine sarcoma virus, LTR promoter the hygromycin- resistance gene and Moloney murine leukemia virus promoter3’. To create pLCRESH the PstI-MluI fragment from pACN (Cre) was subcloned into the HpaI site of pLXSH.

PWZL

pSIN is derived from Moloney Murine Leukemia Virus and is ‘replication incompetent’ vectors through loss of the gag, pol and env genes that are provided in trans by the amphotropic Phoenix packaging cell line. In addition, pSIN is a ‘Self-Inactivating’ vector through deletion of the 3’ UTR.

Origin & function

The genetic material to be introduced via the retroviral systems consists of human genes involved in mediating senescence, immortalisation or telomerase activation in human cells. These include the oncogenes, hTERC, hTERT, RAS, MEK-EE (constitutively active) ERBB1, MYC, GLI-1, GLI-2, WNT-1, WNT-16, FOXM1, AXL-1, AKAP220, PCDH7/8, SERPINB family, LPHH2, GRM7, PLOD2, RASGRF2, OAT3, PI3 and WDR4.

It is anticipated that by ectropically expressing these genes their role in keratinocytes senescence can be further understood.

Evaluation of foreseeable effects

The final GMO human cells will contain stably integrated DNA copies of the retroviral vectors encoding the transferred oncogenes listed above.

The cells will be free of retrovirus or lentivirus. As a result they will pose no hazard to human health or the environment, since they are unable to colonise humans or survive outside of laboratory culture.

It will however, be important to prevent human exposure or environmental release of the replication defective retroviruses which are used to transiently to effect gene transfer. This will be achieved through the use of appropriate physical containment and inactivation processes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
To minimise the risk to human health it will be essential to prevent exposure to infectious retrovirus lentiviral particles. All work will therefore be carried out under Class II containment conditions. Following experimental work, all viral supernatants will be autoclaved and spent medium will be inactivated using precept tablets (10,000 ppm chlorine), 1% Virkon or 1000cpm Actichlor™ before removal from the Class II laboratory for disposal. Solid waste will be double-bagged in biohazard bags prior to removal from the Class II laboratory and then autoclaved before final consignment of waste to approved contractor. Autoclaves will be validated by annual thermocouple mapping and each run is monitored using Thermalog-S indicator test strips. These processes will result in 100% inactivation of infectious virus. Infected target cells will be cultured under the above regime until medium supernatants have been shown to be free of replication competent virus as judged by both infection of appropriate indicator cells and assays of reverse transcriptase activity. Once these criteria have been met the infected target cells will be removed from Class II containment and handled using standard tissue culture procedures. Separate lab coats are worn within the Class II laboratory, gloves will be worn, plastic ware will be used and all use of sharps will be avoided.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessments were reviewed by the GMSC on 30th August 2006. It was agreed that a number of similar assessments would be grouped together since the basic work in each case is fundamentally the same. The committee asked in some cases for more information regarding inserts and vectors. Due to the complex application a sub-group of the committee reviewed again (7th Feb 2007) the local risk assessments prior to sending to HSE.

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Project Ref  774/08.1

Date Ackn'd 02/03/2022  CU2 Project Title
Purposes of the contained use

Culturing and genetic manipulation of trypanosomatid parasites.

Recipient or parental organism

Trypanosoma brucei brucei (strain MITat427, clone 221a). This organism is non-pathogenic to humans and used as a model system for human pathogenic Trypanosoma brucei sub-species.

Leishmania major (strain Friedin). This organism causes cutaneous leishmaniasis. However, infections will usually self cure, leaving a scar. This species is a model system for other, more severe forms of leishmaniasis.

Host/vector system

Vectors

The only vectors used during this work are based on standard bacterial cloning vectors (e.g., pBluescript-based plasmids). This is to allow propagation of parasite DNA sequences in E.coli.

 Inserts

The trypanosomatid genes (derived from either genomic DNA or cDNA) under study include nitroreductases, peroxidases and superoxide dismutases. Non-coding and/or partial coding genomic DNA sequences from the parasites will be used to construct gene knockout vectors. All coding and non-coding sequence under study are highly unlikely to be involved in pathogenicity/virulence.

Parasite genomic DNA and cDNA will be used to target specific loci by homologous recombination. Modified coding sequences (e.g., epitope tagged versions) will be used to complement mutant phenotypes. DNA introduced into trypanosomes is propagated by insertion into the parasite nuclear genome of non-therapeutic, drug selectable
markers. The drug selectable markers aph (3')-lib from Tn5, ShBle from Streptalloteichus hindustanus, bsd from Aspergillus terreus, pac from Streptomyces alboniger and hph from E.coli plasmids, will be used to transform T.b.brucei or L.major to G418, bleomycin, blasticidin, puromycin and/or hygromycin resistance respectively.

<table>
<thead>
<tr>
<th>Evaluation of foreseeable effects</th>
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<tbody>
<tr>
<td>E.coli -ampicillin resistance</td>
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<tr>
<td>T.b.brucei, L.major, - G418, bleomycin, blasticidin, puromycin and hygromycin resistance.</td>
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<th>Liquid waste will be adjusted to 1% Virkon and left for a minimum of 24 hours before disposal down the sink.</th>
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<tr>
<th>Solid waste will be treated with 1% Virkon and/or transferred to a bagged autoclave box. The boxes will be sealed and labelled before transfer to an autoclave no more than 15 min prior to activation of the autoclave run. Thermolog indicators must be included in all discard runs. Disposal must be documented.</th>
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</thead>
</table>

Virkon for use with T.brucei has been validated experimentally for three time points. Specifically 1x106 T.brucei in 1ml of culture media were microscopically observed to be “healthy-alive and mobile”. To this culture an equal volume (1ml) of 2% Virkon was added to give a final concentration of 1% Virkon. The culture was microscopically observed (on an inverted light microscope with a 40x objective) 10 minutes later all T.brucei were found to be “totally lysed-dead and morphologically unrecognizable”. Cultures were further observed after 1 hour and overnight with the same result. Images of these were taken but independently observed (3 people) to verify the results. This experiment has been repeated using higher concentrations of T.brucei (5x106ml-1) in larger culture volumes (10ml) with the same results as described above (data provided by Dr E. Hendricks, Imperial college London).

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Please enter comments on the GM safety committee on the risk assessment
The application needed to identify a backup autoclave for waste in the SBCS building.

This issue was addressed as follows:
The autoclave (room 411) in the Fogg building is opposite the containment level 2 laboratory. This autoclave is now working correctly with issues related to its maintenance and repair clarified. If the autoclave in room 411 does break down, the contractor will repair it within the week. Any containment level 2 waste generated during this time will be stored in sealed containers in room 401 until the autoclave is fixed. We anticipate that waste levels will be minimal; we will only generate 2 boxes of waste per week. If for any reason the autoclave is irreparable, work in the containment level 2 lab will cease. SBCS will replace the autoclave. We will only commence work when the new autoclave is in place. In extreme emergencies, containment level 2 waste will be double bagged and sterilized in small lab autoclaves. Thermolog indicators will be included.

Informed that the QMUL GM safety committee approved the risk assessment on 22/02/2008; local reference number 0774/0801/06. An inspection of the facility by the local GMSC was carried out on 25/02/2008.

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<tr>
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<td>L3 L4 L2</td>
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<tr>
<td>Animal Units</td>
<td>L3 L2</td>
<td>L3 L4 L2</td>
</tr>
<tr>
<td>Large Scale Activities</td>
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<tr>
<td>Human Clinical Applications</td>
<td>L2 L3 L4</td>
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#### Project Ref 774/08.2

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<th>Class</th>
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<tr>
<td>08/04/2008</td>
<td>Development and use of lentiviral vectors for the study of PI 3-kinase signalling pathways.</td>
<td>09/10/2013</td>
<td>Class 2</td>
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<td>N</td>
<td>Transfer to GM14 09/10/2013</td>
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<td>Significant Change ID</td>
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</tbody>
</table>
**Purposes of the contained use**

This programme of work will use lentiviruses to deliver cDNA encoding specific genes or RNAi to knock out specific genes into various rodent and human cell lines. Established model systems will then assess the effect of these upregulated or downregulated genes in vitro or in cells.

**Recipient or parental organism**

Replication-defective 2nd and 3rd generation HIV lentiviral vectors and modified vectors constructed in house will be packaged by transient transfection of plasmids into HEK 293T cells.

Rodent and human cells will be infected with the packaged lentiviruses.

Cell lines are free of adventitious agents, and have a history of safe usage in the laboratory, equivalent to containment level 1.

**Host/vector system**

Replication-defective lentiviruses used for gene transfer in this study are 2nd and 3rd generation HIV lentiviral vectors. These lentiviruses have a wider tropism than wild-type HIV because the VSV-G Env permits entry into all cells. The virus produced is replication-defective, and all chances of recombination leading to a replicative virus is considered negligible.

**Origin & function**

We wish to use lentiviruses to express:

1. P13K isoforms and P13K signalling pathway kinases, phosphatases and other pathway components (bovine, human or mouse) and mutant oncogenic forms of these, identified in various cancers. Of the 8 catalytic subunits of the P13K family, only p11 Oalpha has so far been found to be mutated in cancer. These mutations increase the enzymatic activity of p11 Oalpha. p11 Oalpha mutations can be concurrent with mutations in other genes encoding proteins such as Ras, p53, PTEN, etc. Overexpression of p11 Oalpha in cells has recently been shown to induce p53-mediated growth arrest and mutational inactivation of p53 is required in order for cells to become transformed (Kim JS et al. 2007, MCB, 27(2):662). Ectopic overexpression of wild-type forms of the catalytic subunits p11 Obeta, p11 Odelta and p11 Ogamma can transform avian fibroblasts (Kang et al., 2006, PNAS, I 03(5):1 289), but there is no evidence for a similar effect in mammalian cells.

2. We also wish to use RNA1 to target the same classes of molecules described in 1. above.


4. Resistance Genes: Neomycin, hygromycin, zeocin resistance genes used as positive selection markers. No potential hazard, have a well-established record of safe use.

5. Gene Regulation: Proteins required for tetracycline regulated expression of genes from tetracycline responsive promoters. These include tTa, rtTA, rtTA2S-M2 and tetR-KRAB; these molecules bind DNA through variants of the tetracycline repressor and do not bind specific eukaryotic DNA sequences. Gre and FLP recombinase: Used to catalyse the site-specific recombination of DNA between loxP sites (Gre) or between FRT sites (FLP). Gre or FLP-coding lentiviruses will be used for infections of cells derived from mice bearing conditional mutations of the genes described above.

**Evaluation of foreseeable effects**

2nd generation lentiviral vectors have the genes and sequences for lentivirus formation split between 3 vectors. The backbone of the lentiviral vector only retains the packaging signal and the LTRs between which transgenes of interest are inserted (pHR or similar). Other genes required for synthesis of lentiviruses are supplied in trans on plasmid vectors and are encoded from CMV promoters. From one plasmid the VSV-G Env protein is expressed (pVSV-G or similar) and from a second plasmid Gag, Pol, Tat and Rev are encoded (pCMV 8.91 or similar). In 3rd generation lentiviruses vectors, the LTRs are also self-inactivating, such that upon reverse transcription they are also deleted. Most experiments will use the 3rd generation lentiviral vectors. A favourable system is the ViraPower Lentiviral Expression System (Invitrogen) which combines a mixture of 3 plasmids pLPi, pLP2 and pLPIVSVG encoding gag/pol and Rev response element, Rev, VSV-G env respectively. These are co-transfected with the packaging vector (four are available eg pLent64NS-DEST).
For the introduction of RNAi to knockdown specific genes, systems such as the pGIPZ from Open Biosystems will be used (http://www.openbiosystems.com/RNAi/shRNAmirLibraries/c3lpzLentiviralshRNAmir).

Lentiviruses have the capacity to infect human cells so infection of normal and HIV infected cells must be considered. Infection of normal cells will result in promoter/transgene incorporation into the genome. Harmful effects could be caused by transgene expression or due to insertional mutagenesis. The lentivirus vectors are replication defective, but there is a theoretical risk of recombination with wild-type HIV-1 or HIV-2. However, there is no report of this ever happening. In any case, recombination restoring all HIV genes — which is necessary to produce a replication - competent virus - would delete the transgene, converting the recombinant virus to wild-type. In order to reduce the risk further, the use of lentiviruses will be at ACGM Containment Level 2.

The recombinant viruses can only be packaged by cotransfecting the plasmid components of the vector system into 293T cells. Transfected cells producing the recombinant virus must be maintained in tissue culture and cannot survive outside the laboratory. Recombinant lentiviruses, are also unstable and unlikely to survive for long with survival time dependent on factors such as temperature and pH. Although theoretically the lentiviruses would be able to infect other animal species such as rodents, the likelihood of this occurring accidentally is negligible. In the event that this did occur, the recombinant virus would be unable to propagate. This programme of work does not include deliberate infection of animal species with lentivirus vectors. No harm to the environment is envisaged.

<table>
<thead>
<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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<tbody>
<tr>
<td>All basic molecular biology manipulations that do not result in production of virions will be conducted on the bench at Containment Level 1 using GLP. All steps involved in production of infectious virions will be Containment level 2. Experienced staff used to work in a containment level 2 environment will conduct the experiments. Strict local rules describing protective wear, handling and disposing of infectious materials will be adhered to. All liquid waste is decontaminated with 1% Virkon overnight. Any potentially contaminated material eg plastic is also soaked in 1% Virkon overnight, double-bagged, transported in leak proof containers to an autoclave in the same building and autoclaved. All equipment that has come into contact with lentivirus will be autoclaved before cleaning or disposal. Aerosols may be produced during the preparation of lentivirus and infection of HEK 293 cells. To prevent the dispersal of aerosols, preparation and infection of the virus will be carried out in a biological safety cabinet class II. All work surfaces will be thoroughly cleaned with disinfectant solution followed by 70% ethanol. Sharps will be used with special care and disposed of into sharps bins. Needles will not be re-sheated before disposal.</td>
</tr>
</tbody>
</table>

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste will be decontaminated by soaking in 1% virkon, then disposed in sealed double-bags which will be autoclaved and incinerated in accordance with local and country regulations for the disposal of biohazardous waste. Inactivation of lentivirus by autoclaving at 121°C for 15 minutes, will give effectively 100% kill. Using a higher temperature, or a longer time, is permissible. Waste will be transported in leak proof containers to autoclaves by trained staff and immediately placed in the autoclave for discard.

If live cells infected with lentiviruses are to be taken out of the Containment Level 2 lab and cultivated elsewhere, they must be tested for replication-competent virus first. In addition it should be noted that live cells infected with lentivirus that are to be used for experiments outside of the isolation room must be transported inside SEALED containers that are wiped over with microsol prior to leaving the hood and then only opened in areas designated as category 2 and for use with lentiviral infected cells. Otherwise cells should be fixed or lysed prior to removal from the hood in the isolation room.
Amendments required by the GMSC have been addressed in the final version of the risk assessment. The oncogenic nature of P13K genes have been assessed, competency and training of workers has been assessed.

Project Containment

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<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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Project Ref 774/08.3

Date Ackn’d 08/04/2008
CU2 Project Title In vitro transfection of primary cells and cell lines for in vitro experiments to assess suitability for in vivo transplantation studies.
Class 2
Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID GM774/08.3a
**Project Additional Information**

**Purposes of the contained use**

The overall goal of the research is to improve the viability, integration and therapeutic benefits of donor cells used for transplantation into the failing heart. The first step towards this aim is to engineer donor cells (by using a lentiviral transfection system) to express a variety of either structural or secreted proteins which will enable improved connections and communication between donor and host cells as assessed in an in vitro co-culture system prior to in vivo transplantation studies.

**Recipient or parental organism**

The donor cells used will be either established cell lines (mouse skeletal C2C12, rat skeletal L6 and 293T cells) or primary cells isolated from rat or mouse (skeletal myoblasts, bone marrow cells, circulating endothelial progenitor cells, fibroblasts, cardiac myocytes). These target cells are not known to pose any hazard to human health and require precise culture conditions therefore will not survive outside the laboratory.

**Host/vector system**

The lentiviral vector plasmid will be based on pHRSIN-CSGW-dlNotl encoding EGFP from the SFFV promoter. This will be engineered to include the genes of interest and the eGFP may be replaced with luciferase. The vector system requires 3 transfer plasmids to be transiently co-transfected into 293T cells to produce the lentiviral particles to be used for subsequent transfection. This system reduces the risk of productive recombination with the vector genome. The system is also self-inactivating (SIN) which minimizes the risk of the creation of replication competent virus.

**Origin & function**

Once the cells have been transfected using the created replication defective lentiviral vectors and washed clean of free vector, they will be used in in vitro co-culture studies or for cell transplantation studies to the heart. The proposed function of the inserted genes is to improve the integration and survival of the donor cells and improve the regeneration of the damaged heart.

- **Connexins**: Structural protein responsible for cell-cell channel (gap junction) formation. Not secreted. Present in most cell types. Function to improve connectivity/communication between donor and host cells. Sequence derived from mouse or rat. Low hazard.
- **Cadherins**: Structural proteins responsible for cell adhesion. Not secreted. Present in most cell types. Function to improve adhesion between donor and host cells. Sequence derived from mouse or rat. Low hazard.
- **IL-I receptor antagonist**: Competes with IL-I for the IL-i receptor. Function is to reduce IL-i mediated effects such as inflammation. Has been administered to human subjects with no adverse effects (Cytokine 4:353, 1992). Human sequence. Low hazard.
- **Muscle specific IGF1**: Function is to aid regeneration by reducing apoptosis. This form of IGF1 has not been associated with oncogenicity. Mouse sequence. Low hazard.
- **SDF-i**: Brief up-regulation naturally occurs after MI. Prolonged expression may aid the recruitment of stem/progenitor cells to the ischaemic area and also aid cell survival.
- **SuperOxide Dismutase (SOD)**: Scavenger enzyme associated with a reduced risk of DNA damage and hence reduced oncogenesis. Function is to reduce the damage caused during reperfusion of the heart. Low hazard. Reporter genes (beta-Gal, GFP, YFP, CFP, luciferase): Used as markers. Not secreted. Expression of these proteins does not affect normal cellular processes. Low hazard.

**Evaluation of foreseeable effects**

Preparation of the lentiviral vectors will give rise to high concentrations of the final non-replicative vector to be used. As the lentiviral system is infective of non-dividing cells, there is a risk of localised infection to the operator, expression of the inserted genes of interest and possible insertional mutagenesis. This risk is reduced by the system being self-inactivating so the operator will never be exposed to replication competent virus, so if infected will not produce more virus. The genes of interest present are unlikely to be detrimental to human health. All work with the lentivirus will be carried out in a class II safety cabinet, labcoats, gloves and safety specs will be worn no sharps
will be used and all waste inactivated immediately. Hence the risk of harm to the operator will be effectively zero. The final transfected cells present a very low risk since they will be washed clean of free vector. The supernatant from the washes will be tested for infectivity of fresh cells to test for remaining virus particles and to determine if replication competent virus is present. If no infection occurs (tested for by the presence of the marker gene eGFP or luciferase — visually or for the gene of interest by PCR), then the cells will be deemed to be clear and can be transferred out of the containment 2 laboratory for further experimentation. The cells need specific conditions for survival and hence will not survive in the environment if released.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be inactivated immediately using 1% final concentration of virkon (minimum incubation of 20 minutes). Inactivated liquid waste will then be disposed via the sink. Virkon is proven to be effective against a wide range of bacteria and viruses including lentiviruses. All solid waste will be rinsed with 1% virkon and placed into autoclave bags. The waste will be double bagged and placed into a covered trolley for transportation to the autoclave. Waste will then be autoclaved prior to disposal via the clinical waste route (incineration). The autoclave is validated annually and records maintained.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Detail and degree of kill for waste control now included in section 14. Test for remaining virus particles now included in section 11. New laboratory inspected by the GMSC.

Project Containment

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02/03/2022
## Project Additional Information

**Purposes of the contained use**

Development of vectors (viral and plasmid) and molecules for the study of diseases of the endocrine system or lipid metabolism. This will involve transgenic over-expression, gene knockdown, cellular reprogramming and directed differentiation. All genetically modified vectors are disabled and lack essential genes which prevent their autonomous replication. All require specific packaging cell types to produce a viral particle which is replication deficient and cannot multiply or produce progeny. All vectors will have no effect to the environment as they will not be released, and are maintained under strict laboratory conditions.

**Recipient or parental organism**

**Recipient Cells**

Mammalian cell lines (for example 293T, 3T3, CHO, HepG2, Huh7, U2O-S), and primary cells (for example fetal and adult fibroblasts). Human and mouse embryonic stem cells and adult stem cells (for example; mesenchymal stem cells, adrenal stem cells, pancreatic/hepatobiliary progenitors).

Human embryonic cells and primary cells or tissues are obtained by donation after comprehensive pathogen screening at the clinic (HIV, Hepb/c). Cell lines are also subjected to the same screening process once in the laboratory context. Human primary cells are donated from unscreened patients and are quarantined pending the pathogen screening as described prior to genetic modification.

**Viral Vectors**

Retroviral vectors used are derived from Moloney murine leukaemia virus (MMLV), a mouse virus therefore chances of mobilisation by recombination with endogenous viruses in a human are practically impossible. Furthermore, the vectors contain no MoMLV coding sequences and so are completely replication disabled and the chances of recombination are minimised.
Lentiviral vectors derived from HIV1, SIV, EOAV and FIV are based on genome integrating viruses. Like retroviruses, the severely deleted genomes contain no endogenous coding sequences and the 3'-Long Terminal Repeat (LTR) contains a self-inactivating mutation that prevents the replication cycle initiating after reverse transcription of the RNA viral genome.

Adenoviral vectors (serotypes Ad2/5) are disabled by mutations to the E1a and E3 genes that are fundamental to the early stages of viral replication. Although adenoviruses are human pathogens infection does not result in serious illness and as such are a low risk to humans. The viral genome is transient in infected cells (does not naturally integrate into the host genome) and so there is minimal risk of insertional mutagenesis.

Adeno-associated Viral vectors (AAV) are disabled by mutations to the Rep gene which is necessary and fundamental to replication. Furthermore, AAV requires the expression of adenoviral genes to facilitate its replication. Recombinant forms of AAV will integrate into the host cell genome at very low frequency and so the chances of insertional mutagenesis are low.

**Host/vector system**

<table>
<thead>
<tr>
<th>Bacterial hosts derived from E. coli strains DH5α, JM109, INV110. Plasmid vectors derived from pcDNA3, pGL3, pENTR-1A, etc.</th>
<th>Viral vectors - Recombinant lentiviral vectors based on HIV-1, EIAV, SIV, FIV pseudotyped with VSV-G, gp64, RRV, ENTV envelopes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retroviral vectors based on MLV, pseudotyped with MLV/VSV-G will be packaged by transient transfection in 293T cells. Established adenoviral vectors (Ad2/5) will be produced by transient transfection of 293T (or derivative) cells. AAV will be produced in an established transient 293T helper system.</td>
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**Origin & function**

Transgenic material - Human and mouse cDNAs of transcribed genes involved in aspects of transformation, self-renewal or differentiation will be expressed from pol II promoters. These genes would include transcription factors, signaling molecules, enzymes, growth factors and cytokines.

<table>
<thead>
<tr>
<th>Such genes would include:</th>
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<tbody>
<tr>
<td>Transformation; hTERT, Bmi-1, C-MYC</td>
</tr>
<tr>
<td>Self-renewal: Oct4, Nanog, Sox2, Lin28, Klf4</td>
</tr>
<tr>
<td>Differentiation; FoxA2, Hnf4, GATA4, Sox9, FGF-2, FGF-10, TGF-B, Wnt3a, BMP, PI3K</td>
</tr>
<tr>
<td>Hedgehog Signalling; Shh, Ihh, Dhh</td>
</tr>
<tr>
<td>Glucocorticoid Biology; MRAP and related proteins</td>
</tr>
<tr>
<td>Lipid Biology; Sar/Sec family proteins, Apo family proteins</td>
</tr>
<tr>
<td>Molecular Chaperones; Hsp70/90, ER trafficking proteins</td>
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</tbody>
</table>

These gene targets will also be subject to gene knockdown with siRNA and miRNA expressed from pol II/III promoters. Reporter genes include; Fluorescent - e.g. GFP, YFP, CFP, dsRed, Luminescent - e.g. firefly and renilla Luciferase, biochemical e.g. CAT, AFP. Mammalian promoter/enhancer elements will be used to drive reporter genes as previously described.

**Evaluation of foreseeable effects**

The recombinant viral vectors are all highly disabled vectors.

Lentivirus/retrovirus: Theses self inactivating vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal. Whilst the envelope pseudotyping extends the cellular tropism and confers greater stability viral vector will always be contained within a class II safety cabinet thus user exposure to liquid aerosol is impossible.

The exposure of non-human hosts to pseudotyped vector could result in initial infection but, again seroconversion is highly unlikely.
Adenoviral vectors are deleted of essential replication/packaging genes that are supplemented by an established packaging cell line (293T).

Adeno-associated Virus is defective by nature and has a replication disabled genome and is incapable of replication without helper adenovirus. In this instance the vectors used would require the provision of cap and rep genes in trans in order to replicate and disseminate. There is no significant chance of reversion to the wild-type. Furthermore, the AAV8 & 5 subtypes derived from humans are not able to replicate or cause disease in any animal species therefore it is highly unlikely that such a virus, if released into the environment, would represent a significant risk.

The majority of the gene products expressed are expected to affect cellular properties such as proliferation, apoptosis, migration, adhesion and fate. Also, most have known or suspected oncogenic or tumour suppressor properties.

Gene products in plasmid form exist in non-mobilisable constructs and are therefore of minimal environmental risk. Viral vector expressing potential oncogenes are a potential environmental risk as host infection could elicit a transformation event. All work is carried out in a class II cabinet and all liquid waste inactivated by virkon treatment and solid waste autoclaved.

Primary cells infected with viral vector containing potential oncogene would pose minimal threat as expression would most likely be restricted to the infected cell, which in itself would be rejected by the host immune system.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| none |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Liquid waste will be treated with Virkon 2% for 2 hours (shown by manufacturer as appropriate for disinfection) before disposal to main drains. Solid waste will be autoclaved and later incinerated. Sharps will be incinerated. Cryovials containing virus will be sorted in specified cryofreezer and openend only in Class II cabinets. Spillages and working surfaces will be cleaned with 2% Virkon and with 70% ethanol. Waste is clearly labelled biohazard double bags will be incinerated as clinical waste. Hazard conditions consist of possible spillages, aerosol spray when opening cryovials or during the use of needles when working with animals. Work is restricted to determined areas, disinfecting solutions will always be available, working guidelines are clearly posted, unauthorised personnel is not permitted. Strict adherence to Category II working practices. Reporting in case of accident is according to College procedures. Training to work with GMM's category II. Guidelines regarding dressing code, how to treat spillages and disinfection with 2% Virkon and 70% ethanol. Medical records held by occupational health advisor. Documented spillage procedures are clearly established. In case of accident with sharps, College procedure with blood borne viruses will be followed. Autoclaves are regularly serviced and checked. Autoclaved material is checked using autoclaving tape to assess has reached appropriate temperature for disinfection. |

The risk assessment was initially prepared and discussed at the QMUL local GM safety committee meeting on 29/04/2010 followed by a site inspection on the 18/05/2010. At the meeting the committee requested clarifications and amendments to all the assessments, in particular control measures, waste management and the biological activity of the inserted sequences were discussed at length. The risk assessment was finally approved at a supplementary meeting of the local GM committee and signed off on 24/05/2010.

**Project Containment**

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**Project Ref**  774/11.2

- **Date Ackn'd**: 23/05/2011
- **CU2 Project Title**: Genetic Manipulation of cells to study various cancer types, including ovarian, pancreatic and blood
- **Class**: Class 2
- **CultureVolClass2**: ≤ 1 Litre
- **Consent Granted**: Non-GMM

**Historical Significant Changes**

- Withdrawn: N
- Tick if notifying a connected programme of work: N

02/03/2022
### Project Additional Information

#### Purposes of the contained use

Genetically modified vectors and plasmids will be generated in order to study human cancers. Methods involved will include transgenic overexpression and gene-knockdown. Gene manipulation of this kind will allow us to gain an understanding of how the genes of interest affect the development and/or progress of cancer.

#### Recipient or parental organism

**Recipient Cells**

Mammalian cell lines (e.g. HEK293T, PANC02, IGROV, TOV21G) and primary cells (e.g. mouse and human epithelial cells). Established cell lines are obtained from commercial suppliers (e.g. ATCC, DSMZ) and have been screened for pathogens prior to sale. Mouse primary cells are obtained from colonies with full health screen. Human primary cells are obtained from donors after comprehensive pathogen screening at the clinic (HIV, Hep B/C etc).

**Viral Vectors**

Retroviral vectors used are derived from Moloney murine leukaemia virus (MMLV), a mouse virus therefore chances of mobilisation by recombination with endogenous viruses in a human are practically impossible. Furthermore, the vectors contain no MoMLV coding sequences and so are completely replication disabled and the chances of recombination are minimised.

Lentiviral vectors derived from HIV1, SIV, EIAV and FIV are based on genome integrating viruses. Like retroviruses, the severely deleted genomes contain no endogenous coding sequences and the 3'-Long Terminal Repeat (LTR) contains a self-inactivating mutation that prevents the replication cycle initiating after reverse transcription of the RNA viral genome.

Adenoviral vectors (serotypes Ad2/5) are disabled by mutations to the E1a and E3 genes that are fundamental to the early stages of viral replication. Although adenoviruses are human pathogens infection does not result in serious illness and as such are a low risk to humans. The viral genome is transient in infected cells (does not naturally integrate into the host genome) and so there is minimal risk of insertional mutagenesis.

Adeno-associated Viral vectors (AAV) are disabled by mutations to the Rep gene which is necessary and fundamental to replication. Furthermore, AAV requires the expression of adenoviral genes to facilitate its replication. Recombinant forms of AAV will integrate into the host cell genome at very low frequency and so the chances of insertional mutagenesis are low.

**Host/vector system**

Bacterial hosts used are derived from E.coli strains e.g. DH5a and derivatives, JM109, INV110. Plasmid vectors used include derivatives of pcDNA3, pGL3, pGFP etc.

Viral vectors:

- Recombinant lentiviral vectors based on HIV-1, EIAV, SIV, FIV pseudotyped with VSV-G, gp64, RRV, ENTV envelopes, safer versions will be used from 2nd, 3rd or later generations.
- Retroviral vectors based on MLV, pseudotyped with MLV/VS-G will be packaged by transient transfection in 293T cells. Established adenoviral vectors (Ad2/5) will be produced by transient transfection of 293T (or derivative) cells. AAV will be produced in an established transient 293T helper system.

#### Origin & function
Once the cells have been transfected using the created replication defective viral vectors and washed clean of free vector, they will be used in in-vitro experiments in the study of the genetics of cancer.

Some of the genes involved in these studies will include:
-dlg4 and scribble (polarity genes)
-HVCN1 (encodes a voltage-gated proton)
-TNF family members, IL family members (cytokines)

These gene targets will also be subject to gene knockdown with siRNA and shRNA.

**Evaluation of foreseeable effects**

The recombinant viral vectors are all highly disabled vectors.

**Lentivirus:**
HIV-1 based. Replication defective, as five of the nine HIV-1 genes required for replication were eliminated, leaving the gag and pol reading frames, which encode for the structural and enzymatic components of the virion, respectively, and the tat and rev genes, fulfilling transcriptional and post-transcriptional functions (Zufferey et al., 1997). Viral particles express VSV-G (G-protein of vesicular stomatitis virus) as envelope protein to facilitate entry in human and mouse cells/cell lines, since its receptor is ubiquitously expressed by mammalian cells. Generation of GIPZ shRNAmir lentiviral particles requires a packaging step during which the expression construct containing the silencing sequence is enclosed in a viral capsid. Gene functions that facilitate this process (e.g., encoded by the structural genes gag, pol, env, etc.) are distributed amongst multiple helper plasmids, which do not contain significant regions of homology. This tactic further minimizes the probability of recombination events that might otherwise generate viruses capable of autonomous replication. GIPZ shRNAmir constructs contain a WPRE sequence devoid of the X protein and its promoter.

**Retrovirus:**
These self inactivating vectors have had regulatory and accessory genes deleted ensuring that viral particles produced in the packaging cells are replication incompetent. Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/ infection cycles. The probability of seroconversion is minimal. Whilst the envelope pseudotyping extends the cellular tropism and confers greater stability viral vector will always be contained within a class II safety cabinet thus user exposure to liquid aerosol is impossible. The exposure of non-human hosts to pseudotyped vector could result in initial infection but, again seroconversion is highly unlikely.

**Adenovirus:**
The design and production methods of the adenovirus ensures that only replication defective viruses are produced and hence spread from an initially infected cell is very unlikely. This is achieved by virtue of the adenoviral plasmid lacking the E1/E3 region of the wild type adenovirus serotype 5 genome from which it is originally derived. During viral production a packaging cell line is used which complements the deficiency in the E1/E3 region. Due to the design of these plasmids, the generation of replication competent adenoviral particles is greatly reduced.

**AAV:**
Adeno-associated virus is replication deficient by nature due to its replication disabled genome, rendering it incapable of replicating without helper adenovirus. In this instance the vectors used would require the provision of cap and rep genes in trans in order to replicate and disseminate. There is no significant chance of reversion to the wild-type. Furthermore, the AAV8 & 5 subtypes derived from humans are not able to replicate or cause disease in any animal species therefore it highly unlikely that such a virus, if released into the environment, would represent a significant risk.

Gene products in plasmid form exist in non-mobilisable constructs and are therefore of minimal environmental risk. Viral vector expressing potential oncogenes are a potential environmental risk as host infection could elicit a transformation event. All work is carried out in a class II microbiological cabinet within laboratories at Containment Level 2; all liquid waste inactivated by Virkon treatment; solid waste autoclaved and incinerated. Stocks checked for RCV by PCR methods before use.
Sharps use minimised and safe handling practised.

Primary cells infected with viral vector containing potential oncogenes would pose minimal threat as expression would most likely be restricted to the infected cell, which in itself would be rejected by the host immune system.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Plasticware will be soaked in 1% Virkon overnight before disposal and autoclaving. Any supernatant will be aspirated into a liquid waste bottle containing concentrated Virkon. Virkon is a broad spectrum disinfectant and is validated to give 5-6 log kill under typical laboratory experimental conditions by manufacturer.

All waste is then disposed of in sealed autoclave bags, which will be autoclaved and incinerated in accordance with UK regulations for the disposal of biohazardous waste. Inactivation of virus by autoclaving at 121°C for 15 minutes, will give 100% kill. Using a higher temperature or a longer time is permissible. Waste will be transported in leak proof containers to autoclaves by trained staff and immediately placed in the autoclave for discard.

All autoclaves are regularly maintained via a service contract in order to ensure instruments are working to correct temperatures and pressures. Following autoclaving, waste will be uplifted by an authorised waste contactor for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project assessed as GM Class 2 and local risk assessments peer reviewed as suitable by GMSC on 21 April 2011. Existing laboratories and facilities to be used which have been inspected by peer review inspections in the last 2-3 years.

Project Containment
Project Ref 774/11.3

Development of lentiviral vectors for the study of various cancer types, including ovarian, pancreas, prostate and colorectal cancers as well as mesothelioma and endometrial cancer

Purposes of the contained use
Genetically modified vectors and plasmids will be generated in order to study human cancers. Methods involved will include transgenic overexpression and gene-knockdown. Gene manipulation of this kind will allow us to gain an understanding of how the genes of interest affect the development and/or progress of cancer.

Recipient or parental organism
Mammalian cell lines (e.g. HEK293T, PANC02, IGROV) and primary cells (e.g. mouse and human epithelial cells). Established cell lines are obtained from commercial suppliers (e.g. ATCC, DSMZ) and have been screened for pathogens prior to sale. Mouse primary cells are obtained from colonies with full health screen. Human primary cells are obtained from donors after comprehensive pathogen screening at the clinic (HIV, Hep B/C etc).

Lentiviral vectors derived from HIV1, SIV, EIAV and FIV are based on genome integrating viruses. They have severely deleted genomes which contain no endogenous coding sequences and the 3'-Long Terminal Repeat (LTR) contains a self-inactivating mutation that prevents the replication cycle initiating after reverse transcription of the RNA viral genome.

Host/vector system
Plasmid vectors used include derivatives of pcDNA3, pGL3, pGFP etc.
Viral vectors: Recombinant lentiviral vectors based on HIV-1, EIAV, SIV, FIV pseudotyped with VSV-G, gp64, RRV, ENTV envelopes, safer versions will be used from 2nd, 3rd or later
Once the cells have been transfected using the created replication defective viral vectors and washed clean of free vector, they will be used in in-vitro experiments in the study of the genetics of cancer.

Some of the genes involved in these studies will include:
Rad51, BRCA1, BRCA2, P63
L4-100k, L4-33k and L4-22k, E1A (adenoviral genes)
TNF family members, IL family members (cytokines)

These gene targets will also be subject to gene knockdown with siRNA and miRNA expressed from pol II/III promoters. Reporter genes include; Flourescent- e.g. GFP, YFP, CFP, dsRed, Luminescent- e.g. firefly and renilla Luciferase.

Evaluation of foreseeable effects

The recombinant viral vectors are all highly disabled vectors.

Lentivirus:
HIV-1 based. Replication defective, as five of the nine HIV-1 genes required for replication were eliminated, leaving the gag and pol reading frames, which encode for the structural and enzymatic components of the virion, respectively, and the tat and rev genes, fulfilling transcriptional and post-transcriptional functions (Zufferey et al., 1997).
Viral particles express VSV-G (G-protein of vesicular stomatitis virus) as envelope protein to facilitate entry in human and mouse cells/cell lines, since its receptor is ubiquitously expressed by mammalian cells.
Generation of GIPZ shRNAmir lentiviral particles requires a packaging step during which the expression construct containing the silencing sequence is enclosed in a viral capsid. Gene functions that facilitate this process (e.g., encoded by the structural genes gag, pol, env, etc.) are distributed amongst multiple helper plasmids, which do not contain significant regions of homology. This tactic further minimizes the probability of recombination events that might otherwise generate viruses capable of autonomous replication.
GIPZ shRNAmir constructs contain a WPRE sequence devoid of the X protein and its promoter.

Gene products in plasmid form exist in non-mobilisable constructs and are therefore of minimal environmental risk. Viral vector expressing potential oncogenes are a potential health / environmental risk as host infection could elicit a transformation event. All work is carried out in a class II microbiological safety cabinet within laboratories at Containment Level 2; all liquid waste inactivated by Virkon treatment; solid waste autoclaved and incinerated. Sharps will be used with special care and disposed of into sharps bins. Needles will not be re-sheathed before disposal. Cells will be tested for RCV by p24 ELISA.

Primary cells infected with viral vector containing potential oncogenes would pose minimal threat as expression would most likely be restricted to the infected cell, which in itself would be likely to be rejected by the host immune system.

Insertion of an oncogene or knockdown sequence into a human host during handling of viral supernatants can poses a theoretical risk. However, insertion of an oncogene into the human cells is not enough to cause a harmful event because it needs a combination of additional alterations. The risk assessment also puts in place control measures to minimise the risk of administration of the virus to an individual, therefore the event is very unlikely.

The viruses are unlikely to survive outside the laboratory, as suitable growth conditions will not exist. The packaging cells and any transduced cells cannot survive outside the laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Plasticware will be soaked in 1% Virkon overnight before disposal and autoclaving. Any supernatant will be aspirated into a liquid waste bottle containing concentrated Virkon. Virkon is a broad spectrum disinfectant and is validated to give 5-6 log kill under typical laboratory experimental conditions by manufacturer.

All waste is then disposed of in sealed autoclave bags, which will be autoclaved and incinerated in accordance with UK regulations for the disposal of biohazardous waste. Inactivation of virus by autoclaving at 121°C for 15 minutes, will give 100% kill. Using a higher temperature or a longer time is permissible. Waste will be transported in leak proof containers to autoclaves by trained staff and immediately placed in the autoclave for discard.

All autoclaves are regularly maintained and calibrated via a service contract in order to ensure instruments are working to correct temperatures and pressures. Following autoclaving, waste will be uplifted by an authorised waste contractor for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project assessed as GM Class 2 and local risk assessment peer reviewed as suitable by GMSC on 24 April 2011. Existing laboratories and facilities to be used which have been inspected by peer review inspections in the last 2 - 3 years.

Project Containment

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Project Ref  774/12.1

Date Ackn'd CU2 Project Title

Class CultureVolClass2 CultureVolumeClass3-4
The main focus of this project is trying to understand what role mechanical forces (e.g. strain or compression) play in cell fate. We also hope to gain insight into how mechanical forces are processed by both embryonic and adult stem cells, and what role they may play in the (re)organisation of the genetic information inside the nucleus, and how that will thereby result in changes in nuclear architecture and gene regulation.

Adult, semi- or terminally differentiated cells, such as primary mouse embryonic fibroblasts or primary (bovine, murine or human) chondrocytes or primary (adult bovine, murine or human) skin fibroblasts, HeLa cells, for example.

Recipient or parental organism

1. Lentiviruses - use of 3rd generation or higher self-inactivating (SIN) pseudolentiviral transduction particles is an efficient way to introduce genetic modifications in a broad spectrum of mammalian cell types, such as those outlined above.

For example, EF1α-STEMCCA-LoxP (OKSM) Lentivirus: (Merk/Millipore Part number CS204439/SCR531).

A 3rd generation SIN Lentiviral transduction particles, modified to deliver a Cre-Recombinase-excisable expression cassette, containing four Yamanka factors, Oct4, Klf4, Sox2 and cMyc (abbreviated hitherto as OKSM). High OKSM expression facilitated through truncated WPRE element (no X-protein produced).

These pseudolentiviral particles are used to generate induced pluripotency in primary, semi- or terminally differentiated cells are non-pathogenic and non-infectious beyond their initial use. To remove any chance that the lentiviral gene vectors would produce any competent virus, the viral genome was stripped of any packaging and envelope-producing sequences [Miyoshi et al. (1998) J. Virol. 72: 8150-8157]. Thus infectivity of these vectors depends on separate envelope production and packaging steps, which only make the initial round infectious, but leave the viruses otherwise replication-deficient. The vector backbone in the EF1α-STEMCCA-LoxP Lentivirus is the pPACKH1 system (System Biosciences), which is based on HIV and FIV lentiviruses, in order to ensure broad-spectrum mammalian expression. Secondly, SIN mutation was engineered into the long terminal repeat of the lentiviral backbone that prevents the replication cycle initiating after reverse transcription of the RNA viral genome.

2. Adenoviruses
Wild-type adenoviruses, including human adenovirus type 5, are associated with a number of mild disorders, such as respiratory infections in the elderly or children. There are approximately 2 phases of adenovirus transcription, namely early and late phases, which occur before and after replication. The early-transcribed regions are E1, E2, E3 and E4. The E1 gene products, including E1A and E1B, are involved in the replication of the virus. Most of the E3 proteins are involved in modulating the immune response of infected cells.

For example, Human Adenovirus Type 5 (dE1/E3), expressing Cre-Recombinase and Green Fluorescent Protein, (Vector Biolabs Catalogue number 1710) is used in conjunction with cells reprogrammed by the EF1α-STEMCCA-LoxP/OKSM lentiviral vector, to remove all traces of the OKSM gene cassette.

To ensure replication deficiency of adenoviral vectors, the E1 region is deleted allowing it to safely be used as a gene delivery tool. To accommodate larger recombinant genes (up to 8 Kb), 1st generation adenoviruses are both E1 and E3 deleted (E1/E3), since the E3 region is not essential for in vitro viral growth. The adenovirus vector is able to deliver genes with 100% efficiency to a wide selection of cell types including dividing or non-dividing cells, or primary cells or cell lines. This ability far surpasses the gene delivery efficiencies of lipid-based transfection approaches or other viral-based gene delivery systems.

3. Bacterial Plasmid Vectors and E. coli hosts.

Bacterial hosts derived from E. coli strains DH5a, JM109, INV110. Plasmid vectors derived from pcDNA3, pGL3, pENTR-1A, etc.

**Origin & function**

1. Genetic material introduced by lentivirus

   The EF1α-STEMCCA-LoxP/OKSM bears a poly-cistronic cassette that will result in the expression of four genes: Oct-4, Klf-4 and Sox2 are all transcription factors associated with the maintenance of the pluripotent and self-renewal phenotypes associated with embryonic stem cells. The fourth element, c-Myc, is an oncogene whose forced overexpression has lead to tumour growth in SCID mice. The infectious lentiviral particles are purchased "ready to use", and the genetic origin of the transgenes is murine.

2. Genetic material introduced by adenovirus

   Adenovirus Type 5/Cre-recombinase/GFP drives the expression of Cre-recombinase, a Type I topoisomerase from bacteriophage P1 that catalyzes the site-specific recombination of DNA between loxP sites, allowing us to to genetically modify DNA in situ, in living cells. In this case, the OKSM cassette, which is flanked by loxP sites, will be deleted from the reprogrammed cells generated by the EF1α-STEMCCA-LoxP lentivirus, restoring the original genotype of the cells.

   Additionally, adenoviruses will occasionally be used to introduce either knockdown or reporter-fusion constructs into primary cells, known to be difficult to transfect by lipid-based or electroporation methods. To assess the functional significance of certain genetic elements involved in cell fate, nuclear architecture or chromatin modification, certain gene targets will also be subject to gene knockdown with siRNA and miRNA expressed from pol II/III promoters. The functional significance and sub-cellular localisation of certain genetic elements will be determined by the use of reporter genes and reporter-fusion constructs. Reporter genes include including fluorescent proteins—e.g. GFP, YFP, CFP, dsRed, mCherry, mRFP will be introduced either on their own, driven by mammalian promoter/enhancer elements, or fused to functional domains from (mammalian) non-hazardous (GM Class 1) nuclear proteins involved in chromatin organisation—e.g.Histone-H2B-GFP, photoactivatable Histone-H4-GFP, GFP-Heterochromatin Protein 1-beta, nuclear architecture—e.g. truncated (dominant negative) mutant of Sad1p/UNC-84-(SUN1)-fused to YFP, truncated (dominant negative) mutant of Nesprin1-Giant fused to GFP, for example.

3. Genetic material introduced by plasmid

   Plasmids will be used to introduce a similar range of knockdown or reporter-fusion elements as listed in the previous paragraph in established/immortalised cell lines or primary mammalian fibroblasts that are more readily transfectible by lipid-based transfection or electroporation (MEFs, mouse ES cells, mouse iPS cells, HeLa, for
Evaluation of foreseeable effects

1. Lentiviral use

The protocol for lentiviral infection is a mixture of GM Class 1 work and GM Class 2 work. As the viruses have a broad-spectrum tropism, the 24 hour period of infection presents an exposure risk to the workers. After this the cells are washed and cultured in virus-free medium, and detectable titres of free virus are reduced by the end of the second 24-hour period. During this 48-hour exposure window, even though the EF1α-STEMCCA-LoxP is self-inactivating beyond its initial infection, there remains the risk of exposure to worker infecting the cells to be reprogrammed. Though, without broken skin or direct mucous membrane contact, infection is unlikely to happen without the use of sharps, and there are no sharps are used at any point throughout this protocol. The remaining (post-infection) portion of the protocol all falls within GM Class 1 work.

Forseeable effects of infection: c-Myc is considered an oncogene, and its forced overexpression has lead to tumour growth in SCID mice. Primary cells infected with viral vector containing potential oncogene would pose minimal threat as expression of murine proteins would most likely be restricted to the infected cell, which in itself would be rejected by the host immune system.

2. Adenoviral use

Cells infected with adenoviral vectors containing non-hazardous transgenes would pose minimal threat as expression would most likely be restricted to the infected cell, which in itself would be rejected by the host immune system.

Adenovirally-based Cre-exision of from any induced Pluripotent Stem Cells generated in the group will restore the genotype of the iPS cells to that of the parent cell type, by removal of the OSKM expression cassette. The resulting cells bear no viral progenic sequences and have no foreseeable effects.

3. Plasmid and bacteriological work

All E. coli strains used for the propagation of plasmid vectors are standard lab strains, designed to be of none-to-minimal health risk to those using them. Plasmid-transformed strains should have no additional risk associated to their use.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We are not applying for any derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid cell culture waste, plasmid-related waste, waste biological material, especially that involved in Class 2 work is immediately collected in appropriate bags, and autoclaved (sterilised for 30 minutes at 121 degrees C, in pressurised steam) within the laboratory suite.

Autoclaved solid waste, marked as ‘autoclaved clinical waste’ is collected, awaiting disposal by a licensed contractor.

Liquid cell culture waste is treated with a disinfectant (1% of fresh Virkon for 10 minutes, as directed by the manufacturer, Dupont-Antec) prior to disposal down the drain. Information provided by Dupont-Antec ensures a 5-log degree of kill over a broad microbial and viral spectrum.
The main reason for classifying the lentiviral transfection stage as Class 2, is due to transient oncogene expression (Yamanaka factor genes and X-protein WPRE sequence) and the fact that pluripotent stem cell modification is a relatively new technology. A similar project was classified as Class 2 from another College Institute recently. It is noted however that the vector is 3rd gen, replication defective, self inactivating, that sharps are not used and that the hazardous elements are excised following the transfection period.

The labs are to be inspected by the GMSC.
### Project Additional Information

#### Purposes of the contained use

The purpose of producing recombinant retroviruses to use them to increase or suppress the expression of wildtype and mutant keratins, homeobox genes, POU domain containing proteins and wildtype and dominant negative mutant of proto-oncogenes such as c-JUN, c-FOS and other members of this family in keratinocytes in order to determine their role in differentiation, growth, apoptosis, migration and tissue invasion.

#### Recipient or parental organism

- Normal oral keratinocytes, Normal skin keratinocytes, HaCaT (spontaneously immortalised human skin keratinocytes), Neb-1 (HPV immortalised normal skin keratinocytes), T103C (HPV immortalised normal oral keratinocytes), SVK14 (SV40 immortalised normal skin keratinocytes), N-Tert (telomerase/P16 induced immortalised normal human skin keratinocytes), OKT-F6 (telomerase/P16 induced immortalised normal human skin keratinocytes). Human oral cancer cell lines derived from dysplastic, tumour and metastatic tissues.

#### Host/vector system

- pLPC_Cmyc: Derived from Moloney murine leukemia (MoMuLV) Moloney murine sarcoma viruses (MoMuSV).
- pSin-B (self inactivating retroviral vector). This vector is derived from MMLV.
- pRetrosuper-Puro (retroviral vector used to silence a gene expression). pRETRO SUPER series is based on the self inactivating Murine Stem Cell Virus, pMSCV. Bacterial hosts: E.coli K12 strains.

Lentiviral vectors (3rd generation) derived from HIV1, SIV and FIV are based on genome integrating viruses. Like other retroviruses, the severely deleted genomes contain minimal endogenous coding sequences with most harmful sequences (e.g. vpr, nif, rev, tat) removed or truncated (e.g. WPRE sequence) to cause inactivation of harmful gene expression, and the 3’ Long Terminal Repeat (LTR) contains a self-inactivating mutation that prevents the replication cycle initiating after reverse transcription of the RNA viral genome.

#### Origin & function

- HOXA7 and other HOX proteins: These proteins are involved in embryonic development, however, in adult HOXA7 induces cell proliferation a keratinocytes. HOXA7 expression is induced in a variety of human tumours.
- C-JUN and c-FOS and other family members: These are proto-oncogenes which can be converted into oncogenes under certain defined conditions leading to genetic alterations.
- Vimentin: It is intermediate filament protein which is expressed in mesenchymal tissues. However, during cancer metastasis vimentin expression is induced in epithelial...
tissues.
SKN1a: This is a POU domain containing protein and it is known to play a role in keratinocyte differentiation.
Keratins: These are cytoskeletal proteins of keratinocytes and a large number of them are associated with cell proliferation, increased cellular migration and invasion.

Evaluation of foreseeable effects

There are no known diseases that are caused by overexpression or knock-down of keratins and vimentin, however, there are properties exhibited by some members of this superfamily of proteins that are similar to those exhibited by oncogenes.

The POU domain containing protein SKN1a is known to induce differentiation, and there is no disease caused by its overexpression or knockdown in animals. Overexpression of certain homeobox genes e.g. HOXA7, increases cell proliferation, but there is no evidence to date that they act as oncogenes in animal cells. The retroviral constructs will be packaged directly in Phoenix A (amphotropic) or Phoenix E (ecotropic) cells. These viruses will then be used to infect a variety of human cell lines but principally primary keratinocytes and cells derived from primary dysplasias, primary carcinoma and fibroblasts.

The activities relating to virus production and transduction will be carried out in a restricted entry laboratory facility. These viruses are replication deficient and some are self-inactivated, so once they are integrated into cellular genome, they can't produce replication competent virus particles. Nevertheless, each construct will be tested to ensure that the transduced cells are not producing infection competent viruses.

The viruses and the transduced cells cannot survive outside the tissue culture flask or dishes and therefore even if they are accidently released in the environment they are unlikely to spread and cause damage to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be decontaminated with 1% virkon, 1000cpm Actichlor or 5% sodium hypochlorite for at least overnight. Virkon is a broad spectrum disinfectant and is validated to give 5-6 log kill under typical laboratory experimental conditions by manufacturer. Chlorine based disinfectants also give similar kill according to manufacturer information.

All waste is then disposed of in sealed autoclave bags, which will be autoclaved and incinerated in accordance with UK regulations for the disposal of biohazardous waste. Inactivation of virus by autoclaving at 121°C for 15 minutes, will give 100% kill. Using a higher temperature or a longer time is permissible (e.g. 134°C for 5 min).

All autoclaves are regularly maintained via a service contract order to ensure instruments are working to correct temperatures and pressures.

All wastes are kept in leak proof yellow bags clearly labelled with biohazard signs. These bags are transported from labs to autoclave facility on sturdy steel trolleys.

The laboratory facility is managed by a users group, which ensures that users are correctly trained in decontamination procedures. Training on GM and Biosafety is provided by the College to all users

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Demarcation of activities at Class 1 and Class 2 defined in the risk assessment. Risk assessment approved by GMSC on 26 July 2011

### Project Containment

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<th>Laboratory Activities</th>
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### Project Ref 774/12.3

- **Expression of recombinant mammalian prion protein (mouse or hamster) using E coli**

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<th>CultureVolumeClass3-4</th>
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<td>Expression of recombinant mammalian prion protein (mouse or hamster) using E coli</td>
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<td>Non-GMM Consent Granted</td>
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- **Project notified under transitional arrangements** N

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### Project Additional Information

- Historical Significant Changes
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change
<table>
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<tr>
<th>Purposes of the contained use</th>
<th>To study the cellular prion proteins structure, spectroscopic and biophysical properties</th>
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<tr>
<td>Recipient or parental organism</td>
<td>E. coli strain BL21(DE3) Rosetta (Novagen) or commercially available derivatives thereof. This routinely used lab strain of E. coli is incapable of surviving outside the laboratory - Mobilisation defective and similar to E. coli; K12 strain</td>
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<tr>
<td>Host/vector system</td>
<td>The gene encoding mouse or hamster PrP (23-231) cloned in to the pET23 vector (Novagen) with a stop codon before the his-tag. This is a standard vector used for bacterial protein production because of the inducible bacterial T7 promoter.</td>
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<td>Origin &amp; function</td>
<td>Prion protein genes are from mouse or hamster (residues 23-231) or truncated fragments which may also contain a his-tag for purification. This genes will lack GPI anchor and export signal domain, and will not be glycosylated.</td>
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<tr>
<td>Evaluation of foreseeable effects</td>
<td>The hazards to human health are low and containment level 2 is considered appropriate. The host E. coli is disabled and therefore incapable of existing in the human gut or in the environment The vectors selected are mobilisation defective minimizing the possibility of gene transfer. The system described produces the prion protein in cellular non-pathogenic isoform. The prion protein is a naturally occurring protein found express at high levels in all healthy humans and other mammals. Protein material is from the mouse or hamster sequence. A species barrier exists to potential conversion of cellular prion protein to the abnormal isoform. This barrier implies that there is a very low probability of human cellular prion protein being converted to the abnormal isoform by mouse or hamster PrP-Sc. Despite many attempts it has proved very difficult to converted recombinant prion protein into a pathogenic form and will only generate infection after cerebral injection into transgenic mice expressing 20 times the normal levels of prion protein. The recombinant prion protein lacks a GPI anchoring and glycosylation. Therefore recombinant material is probably far less dangerous than prion protein expressed by mammalian cells (present ubiquitously in the environment). There is no naturally occurring mouse or hamster transmissible spongiform encephalopathy</td>
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<td>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</td>
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E. coli is readily destroyed using standard methods. However, with the presence of PrP it is safer to disrupt both the protein and host by treating anything that has come into contact with either in 2M NaOH for 1 hour or 20,000 ppm available chlorine for 1 hour. After this, this waste will be autoclaved at 134°C for 20 minutes before disposal by incineration by an authorised contractor route, achieving a complete kill of recombinant host and expressed protein.

Disposable plastic ware will be used wherever possible, disposable solid waste (including E. coli plates) to be put in biohazard burn bins and sent for incineration (100% kill). Reusable contaminated solids (including E. coli growth flasks) will be soaked in 2M sodium hydroxide for at least 1 hour.

Spills would be cleaned up with paper towels which will be treated as solid waste, contaminated spill areas will be soaked in 20,000 ppm available chlorine of sodium hypochlorite for at least 1 hour, except for contaminated metal which will be soaked in 2M sodium hydroxide for at least 1 hour. Where soaking is not possible, contaminated areas will be wiped down with one of the above disinfectants (as appropriate) at least 3 times allowing drying between applications for at least 1 hour.

Project discussed by College GMASC at the 29 March 2012 meeting and project classified as GM Class 2 for part of the work which involves bacterial expression of the recombinant prion protein, with non-expression cloning and final purification steps work to be at GM Class 1. After amendments, project approved by GMSC 9 July 2012. Laboratory to be inspected by GMSC.

It should be noted that recent research has shown conversion of recombinant murine prions into abnormal pathogenic prions utilising specified biochemical protocols (Fel Wang et al Science (2010) 327;1132-35). This development must be kept under review by the Investigator.

**Project Containment**

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<th>Laboratory Activities</th>
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**Project Ref** 774/12.4

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Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick if you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**
Project Additional Information

**Purposes of the contained use**

Culturing and genetic manipulation of trypanosomatid parasites. Such manipulated lines can then be used to analyse the function and importance of a given gene/protein/enzyme to this parasite. The main goals of these studies are to identify new drug treatments to help combat Chagas Disease Vesceral Leishmaniasis and Mucocutaneous Leishmaniasis, the diseases caused by Trypanosoma cruzi, Leishmania donovani/infantum and Leishmania braziliensis, respectively.

**Recipient or parental organism**

Parental organisms: Trypanosoma cruzi, Leishmania donovani/infantum (synL. Chagas) and Leishmania braziliensis.

These organisms are all pathogenic to humans and designated as ADCP Hazard Group 3 organisms.

Trypanosoma cruzi:
Natural Host - mammals including humans.
Primary route of disease transmission is zoonotic via the faeces of triatomid insects (eg Triatoma infestans, Rhodnius prolixus, Panstrongylus megistus) into bite wounds/mucocutaneous membranes. The insects are only found in the Americas and are not present in the U.K.

Leishmania donovani/infantum and Leishmania braziliensis:
Natural host - mammals, predominantly humans and canines.
Primary route of transmission is zoonotic via the hematophagous feeding habits of female Sand fly insect vectors (Lutzomyia in the Americas; Phlebotomus in the rest of the World). Disease transmitting Sand flies are not present in the U.K.

**Host/vector system**

Host organisms: The pathogens are to be used as the host organisms and source of genomic DNA/gene inserts.

Vectors: The vectors used during this work are based on standard bacterial cloning vectors (eg pBluescript & pUC-based plasmids). This is to allow propagation of parasite
DNA sequences in E. coli. Several trypanosomatid expression vectors (episomal and intergrative) will be used in this study including pTEX, pRiboTEX, pRclNDEX and pTRIX. These are all based in the above bacterial cloning plasmids.

**Origin & function**

Experiments will generate trypanosomes/leishmania with altered levels of expression (over expression and heterozygote/null mutant) of a target gene.

Inserts: The trypanosomatid genes (derived from either genomic DNA or cDNA) under study include type 1 nitoreductases, peroxidases (eg ascorbate peroxidases and tryparedoxin peroxidases), iron superoxide dismutases, thioredoxin-like proteins (eg tryparedoxin, glutaredoxin), melinione sulphoxide reductases, trypanothione reductase, cytochrom F450 reductases, prostglindins F2alpha synthase, galactonolactone dehydrogenase, alternative oxidases and enzymes postulated to be involved in DNA repair (eg Apollo/Artemis homologues, Rad51). Complete coding sequences will be used to construct trypanosomatid expression vectors (episomal and integrative). The vectors used (including pTEX, pRiboTEX, pTcINDEX and pTRIX) are all based on the above bacterial cloning plasmids. Non-coding and/or partial coding genomic DNA sequences from the parasites will be used to construct gene knockout vectors.

Parasite genomic DNA and cDNA will be used to target specific loci by homologous recombination. Modified coding sequences (eg, epitope tagged versions) will be used to complement mutant phenotypes. DNA introduced into trypanosomes/leishmania is propagated by insertion into the parasite nuclear genome of non-therapeutic, drug selectable markers (neomycin, hygromycin, bleomycin, puromycin, and blasticidin)

**Evaluation of foreseeable effects**

E. coli - ampicillin resistance
T. cruzi, L. donovani/infantum and L. braziliensis - G418, bleomycin, blaticidin, puromycin and hygromycin resistance.

The gene manipulations to be performed are unlikely to alter the pathogenicity of the host organisms. However certain gene manipulations to be performed are unlikely to alter the pathogenicity of the host organisms. However, certain gene manipulations will generate parasite lines that have altered susceptibility to existing chemotherapies. Due to the procedures in place (eg no sharps use, staff training and rapid response to an incident) and lack of natural insect vectors in the UK. Containment Level 3 measures are deemed sufficient to protect human health (workers and others who may be affected) and the environment.

Due to the latency of Chagas disease, individuals using the CL3 facility are screened for the presence of T. cruzi before starting work with parasites, every six months, and upon ending the period of work as part of a health surveillance program. As the disease manifestation is more immediate for leishmania infections, regular screening for the presence of the parasite is not conducted.

In the unlikely event of any laboratory accident/incident where T. cruzi infection may thought to have occurred, screening will be conducted as soon as possible and suitable treatment and health monitoring will be conducted for all involved and health monitoring will be conducted as soon as possible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation from full containment not sought

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste (eg spent culture medium) will be treated with an equal volume of 2% Trigene to give a final concentration of 1% (minimum contact time of 10 min). Serological pipettes, pastettes and pipette tips will be immersed in 1% Trigene for a minimum contact time of 10 min. Tissue culture flasks, including the neck and centrifuge tubes will be thoroughly washed with 1% Trigene (minimum contact time of 10 min). A5 minute exposure to 0.2% Trigene has been shown to kill 100% trypanosomes/leishmania (Wang et al 2008 Parasites & Vectors. 1.35). All liquid waste is left in a demarked area in the CL3 suite for a minimum of 24h before disposal.
down a designated sink within the CL3 suite. The worker is responsible for disposing of the waste.

All plasticware will then be treated as hazardous solid waste and inactivated as follows: Pipette tips and serological pipettes will be dispensed into dedicated sharpsafe containers. Once deemed 75% full these will be closed and tagged with a numbered bag-tie specific for CL3 waste. The remaining solid waste will be transferred to an autoclave bag contained inside a labelled autoclave box (situated next to the microbiological safety cabinet). When deemed "3/4 full", the bag will be closed, a thermolog indicator put into the autoclave box and the box lid shut. The bag will then be tagged with a numbered bag-tie specific for room 610 waste. All solid waste (bags and sharpsafe bins) are then autoclaved as soon as possible in the CL3 autoclave (located in the suite lobby). An autoclave cycle with a holding temperature of 121°C for 20 mins will be carried out. This cycle will provide a complete kill of any remaining pathogens. Non hazardous materials are not allowed to be treated or sterilised within the autoclave at the same time. After completion of the autoclave cycle, the thermolog indicator plus the readout from the autoclave will be examined to determine if sterilisation has occurred. If successful, the autoclave bag within the autoclave box or sharpsafe container will then be transferred outside of the CL3 suite via a designated leakproof and robust trolley for final incineration and disposal. The autoclave bag/sharpsafe will be transferred to a clinical waste bag, sealed with a numbered tag specific for CL3 waste then put into the clinical waste bins. Every two/three days, the autoclaved waste is transferred to a larger, locked clinical waste bin ready for collection by PHS group, who hold an Upper Tier Carrier License to transport GM waste. The final disposal step involves its incineration at the Sidcup, Kent Incinerator operated by SRCL (this is validated to incinerate GM waste).

If an autoclave run does not occur correctly, the autoclave cycle is repeated. If the failure is repeated , the tagged, bagged and boxed waste will be transferred using a designated trolley to the back up autoclave external to the CL3 suite, where the waste will be autoclaved as described above.

Any autoclave failure will be reported to the Building & Laboratory Manager, Containment Facility Manager and/or Biological Safety Officer, who will contact the designated technical support.

70% ethanol will be used to clean non-disposable materials and surfaces.

Spills and splashes treated with 1% Trigene

Transfer between CL3 rooms within the suite will be in a leak-proof, impact-resistant container

**Is an emergency plan required according to regulation 20?**

- **Y**

If yes, tick to confirm that it is attached to this form

- **N**

**Tick to confirm that you have attached a risk assessment to this form**

- **Y**

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- **N**

**Please enter comments on the GM safety committee on the risk assessment**

Discussed by the QMUL Genetic Modification Safety Committee at an ad hoc meeting convened on 14/10/2012. Amendments requested to local GM risk assessment forms (detail of gene inserts, pipette disposal arrangements, health surveillance screening frequency). These were made and the final, local GM risk assessment forms approved by the committee, and signed off on behalf of the committee by the College Biological Safety Adviser

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02/03/2022
The overall goal is to understand the mechanisms of chromatin regulation in C. albicans. Specific goals are to characterise:

a). The pathways responsible for centromere establishment and maintenance.
b). Regulation of gene expression by the RNAi machinery.
c). The Biology of DNA repeats

Recipient or parental organism

C. albicans strains: CAI4, ura3: CAIS, ura3, ade2: RM1000, his1 , ura3; BWP17, his1, ura3, arg4 and derivatives of these strains carrying further disabling markers

Host/vector system

Vector system: 1. Non-mobilisable E. coli vectors (including pUC18/19, pBluescript, pGEM-T; lac-based expression
plasmids such as pET vectors). 2. Specific non-mobilisable C. albicans vectors (low copy replicating and integrating vectors [pPB1, Clp10, Clp20, Clp30]; low copy replicating and integrating vectors for ectopic expression [YPB-ADHp, pACT1]; integrating expression vectors regulated by methionine [MET3 promoter], maltose [MAL2 promoter], glucose [PCK1 promoter], or doxycycline [tet ON promoter and tet OFF promoter]. These vectors will be prepared from E. coli cultures. We will always use non-pathogenic E. coli strains such as the multiple disabled K12 derivatives, and the ree deficient BLR strain. The E. coli strain BLR is a disabled (recombination deficient) derivative of the strain BL21.

Origin & function

We are planning to generate null mutants in genes involved in:
Health and Safety

1. Centromere assembly and propagation. Null mutants will be expected to compromise centromere function resulting in lethality.
2. RNAi Null mutant will be expected to impair siRNA production of specific chromosomal loci.
3. Histone modifications. Null Mutants will be expected to alter the chromatin structures of specific chromosomal loci.
In some experiments, we will express specific genes in C. albicans ectopically using a MET3, MAL or tet promoter.

Evaluation of foreseeable effects

Transformations will be performed in multiple disabled C. albicans strains (ura3, ade2, arg4, or his1) to ensure that all transformants are avirulent. In none of the cases this is likely to increase virulence; instead transformants will always carry a disabling mutation, thereby rendering them completely avirulent. No null mutations have been described that increase the virulence of C. albicans.
In some experiments, the CAI4 strain will be transformed with URA3 plasmids. Insertion of the URA3 marker gene will partially restore the virulence of CAI4, but this restoration is not complete because the genes neighbouring URA3 remain inactivated. This is enough to attenuate virulence partially.
In some experiments, we will express specific genes in C. albicans ectopically using a MET3, MAL or tet promoter. In these experiments, the expression level is not maximised. Their ectopic expression levels will be <1% of total cell protein. The ectopic expression of most specific genes is unlikely to affect virulence at all, and in some cases it will be expected to reduce C. albicans virulence by adversely affecting growth. In experiments where we over-express genes, expression levels will be carefully regulated by addition of doxycycline; without doxycycline protein levels will be at or below wild type levels. The key point is that the probability of increasing virulence by ectopic expression of a single gene is very low, because pathogenicity is complex and polygenic trait requiring a high level of fitness of the C. albicans cell (Odds [1994] ASM News, 60, 313). Indeed, there is evidence that ectopic expression of factors involved in virulence traits does not increase virulence (Bailey [1997] PhD thesis, University of Aberdeen; Leng [1999] PhD thesis, University of Aberdeen; Rodaki et al. [2006]).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All yeasts will be killed by either heat treatment (all solid waste is to be autoclaved at 134 degrees C with a holding
time of 3 minutes) or addition of appropriate levels of disinfectant (all liquid medium and used consumables will be
treated with 1000 ppm Sodium hypochlorite for 8 hours - a >4 log reduction in Candida albicans viability is observed
after 15 minutes inactivation) in accordance with standard lab procedure post experimentation. All solid waste is then
collected for final incineration at hightemperature by an authorised healthcare waste contracto off site. All liquid
inactivated waste is disposed down specified laboratory drain with flushing with water.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The QMUL GMSC approved the work at GM Class 2 at its meeting on 27 Jan 2015, after discussion with the Project
Supervisor. It was noted that the partial virulence restoration noted with URA3 still made the strain attenuated below
the level of the wild type, but as the attenuations do not completely abolish virulence, work is classified as Class 2.
The GMSC noted that a transport of samples SOP be drawn up for movement of yeast samples between laboratories.
The project workers are also recommended to to arrange an allergen sensitivity test against Candida albicans. If
anyone displays sensitivity, further individual health and safety measures can be investigated.

Project Containment

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Project Ref 774/17.1

Date Ackn’d 26/10/2017

CU2 Project Title A Phase 3 Randomized, Open-Label Study Comparing Pexa-Vec (Vaccinia GM CSF /Thymidine Kinase-Deactivated Virus) Followed by Sorafenib Versus Sorafenib in Patients with Advanced Hepatocellular Carcinoma (HCC) Without Prior Systemic Therapy.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted
Pexa-Vec is a replicative oncolytic recombinant vaccinia virus (VV) derived from the commonly used vaccine Wyeth strain, Dryvax™. Pexa-Vec contains three genetic modifications compared to the wild type Wyeth strain: 1) disruption of the viral thymidine kinase (TK) gene by, 2) insertion of the human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene and 3) insertion of the LacZ gene. Pexa-Vec is designed to selectively replicate in and destroy cancer cells, while at the same time stimulating a systemic anti-tumoural immune response through the expression of its transgene, hGM-CSF, in the context of tumor lysis.

Pexa-Vec is currently in clinical development for the treatment of Hepatocellular Carcinoma. The proposed use will be the administration of the investigational product, in a hospital or clinic setting, by intratumoral (IT) injections to patients as part of an international, multicenter clinical trial. This clinical trial is a Phase III trial in patients with Advanced Hepatocellular Carcinoma (HCC) without prior systemic therapy. Results from this pivotal trial will determine whether Pexa-Vec followed by sorafenib increases survival duration in advanced HCC patients compared to treatment with sorafenib alone, and whether sequential dosing with Pexa-Vec followed by sorafenib has a favourable safety profile.

Approximately 40 clinical sites in the EU will enroll patients in the JX594-HEP024 (PHOCUS) study. Additional clinical sites in Australia, Canada, China, Israel, Korea (Republic of), New Zealand, Singapore, Taiwan, Thailand and the USA will also participate in the study. A total of 600 patients will be recruited in this clinical trial with an expectation to enroll 200 patients in EU countries. Among them, 300 patients (i.e. approximately 100 patients in EU) will receive Pexa Vec by IT injections. In the control arm, the 300 patients will not receive Pexa-Vec. After study completion, all patients will be followed up for survival.

Recipient or parental organism

Pexa-Vec is a replicative oncolytic recombinant vaccinia virus (VV) derived from the commonly used vaccine Wyeth strain, Dryvax™.

Host/vector system

The transfer plasmid pSC65/hGM-CSF is used to generate Pexa-Vec. The plasmid pSC65/hGM-CSF is generated from the plasmid pSC65 which was provided by Dr. B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA.

Origin & function

Pexa-Vec is manufactured by Transgene (France) and labelled, QP released and shipped to investigational sites from PCI/Biotec (UK). At the investigational site, Pexa-Vec will exclusively be used for administration to patients as part of clinical study JX594-HEP024. Thus, only the GMO (Pexa-Vec), but not the wild-type host organism or the vector used to generate the GMO, will be used at the investigational site.

Pexa-Vec contains three genetic modifications compared to the wild type Wyeth strain: 1) disruption of the viral thymidine kinase (TK) gene by, 2) insertion of the human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene and 3) insertion of the LacZ gene. Pexa-Vec is designed to selectively replicate in and destroy cancer cells, while at the same time stimulating a systemic anti-tumoural immune response through the expression of its transgene, hGM-CSF, in the context of tumor lysis.

02/03/2022
The GMO, Pexa-Vec is non-integrative (cytoplasmic localization), preferentially replicative in actively dividing cells (i.e. tumour cells), oncolytic (lysis of infected tumour cells) and propagative (able to spread locally to adjacent cancer cells and systematically by release into the blood stream and lymphatic system of infectious particles (Smith G.L. et al., 2002).

Over 300 adult and pediatric patients with advanced, treatment refractory cancers have received more than 1,200 doses of up to approximately 1 billion infectious units (1 x 109 pfu) of Pexa Vec via IV and/or IT administration (as of January 2014). Treatment with Pexa-Vec doses of approximately 1 billion pfu (approximately 10,000-fold more than the dose of standardized [non-attenuated] vaccinia delivered with vaccinia vaccine), has been generally well-tolerated with transient (<24 hours) flu-like symptoms (fever, chills, fatigue), nausea, hypotension and injection site pain as the most common adverse events (AEs). The overall risk of the wild type (parental) Wyeth (USA smallpox vaccination) strain indicates that out of 14 million vaccinations, there were 572 hospitalisations, 9 deaths and many less severe complications. The rate of severe adverse reactions for the wild type parental strain is approximately 1 in 50,000 vaccinations. Pexa-Vec has been even further attenuated when compared to the small pox vaccine, and therefore the safety is improved.

Secondary spread could occur by: direct contact (with a pustule, with bandages or other wastes contaminated by pustules, with expelled droplets from wound sites or liquid preparations) and indirect contact via medical devices contaminated through contact with a pustules. VV is not spread through the air (US Department of Health & Human Services, http://www.smallpox.gov/QuestionsAnswers.html). So transmission of the virus by inhalation can be assessed as negligible. The clinical information available to date suggests that Pexa-Vec is safe at the clinical dose of 1 x 109 pfu (10,000-fold higher than smallpox vaccine dose) and has not spread to caregivers in contact with the treated patients. Should shedding occur, the level of exposure would be predicted to be low compared to the doses received by patients in the proposed trial, and extremely low compared to doses of non-attenuated vaccines administered to the public (e.g. vaccines against smallpox). In addition, exposed individuals over the age of 42 will likely have been previously immunized with vaccinia (UK smallpox vaccination ceased in 1974). In the highly unlikely event that an exposed individual were to demonstrate virus-associated toxicity, therapy could be initiated with VIG and/or cidofovir. To date, no reports of transmission from healthcare personnel from vaccinia recipients have been published; however at least 9 reports of infection of laboratory workers exist from the last 25 years (see safety data sheet http://www.phac-aspc.gc.ca/lab-bio/res/psds-fsfs/vaccinia-virus-eng.php). Infection control / Containment Level 2 standards and procedures and related healthcare approaches are to be implemented for worker safety.

Viral shedding data collected in a clinical study with Pexa-Vec administered by the IT route (i.e. JX-594-IT-HEP001 trial) demonstrated that the virus is not shed to the environment via urination. The virus could also not be detected in throat swab samples. There are no known or predicted environmental conditions which may increase survival, multiplication and dissemination of the Pexa-Vec. It is commonly thought that VV is not naturally found in the environment; however human to cattle (and vice versa) transmission have been reported via broken skin.

Recombination events of the wild type VV with the GMO are not thought likely, as current genetic stability studies on Pexa-Vec have not detected spontaneous revertants of Pexa-Vec.
Non–sharp solid clinical waste in an autoclave bag will be autoclaved at 121 degrees C for a holding time of 15 min, followed by high temperature incineration.

In the hospital, occlusive bandages will be used around site of wounds until shedding ceases to collect any shed particles, and such bandages autoclaved and incinerated.

Patients will be supplied with biohazard bags and gloves to secure all the waste from changing their bandages at home. They will be asked to return the sealed biohazard bags to the hospital at their next visit or arrangements will be made to collect if necessary. All biohazard bags will be placed into an autoclave bag, autoclaved and then incinerated.

Liquid hazardous waste will be inactivated by an effective disinfectant (currently, trade name ‘Chlor-Clean’, supplier Guest Medical Ltd, active ingredient dichloroisocyanurate, also known as troclosene sodium (i.e., chlorine-releasing component), at a final concentration of 1000 ppm chlorine for a minimum contact time of 5 min) before disposal down ward or pharmacy sink (subject to Thames Water trade effluent permits for site).

Chlor-clean has been validated by the supplier to pass the BS EN 14476 (virucidal) Standard covering non-enveloped and enveloped virus types as well as murine norovirus with an effective kill of > 5 log.

Any spillage of contaminated liquids will be inactivated with Chlor-Clean disinfectant at 10,000 ppm for a minimum contact time of 5 min, solidified with an inert absorbent, scooped into an autoclave bag and waste autoclaved and then incinerated as noted above.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
1. The QMUL BGMSC peer reviewed the risk assessment at its meeting of 19 Oct 2016 and agreed with the classification of GM Class 2. As the vaccine strain is conditionally replicative and can cause human disease, it cannot be classified any lower in risk level (in line with the SACGM / Approved List of Biological Agents classification criteria).

2. The Committee noted that details on management (sections 1, 2), location/s of work (section 3), and waste (section 14), training (section 15), additional assessments (section 17) must be completed before full approval is given. This has been completed and added to risk assessment 21/09/2017.

3. All GMO work areas (wards / pharmacy / waste areas) must achieve the minimum Containment Level 2 standards required for clinical areas, as per Part 6 of the SACGM Compendium of Guidance – see pp 39-42, and consult with the QMUL Biological Safety Adviser to ensure measures are adequate. An Inspection is to be scheduled for Nov 2017.

4. The BGMSC noted that some information was noted to be patient specific and not relevant to worker and public safety, and should be re-worded or removed. This has been completed.

5. The types and rates of adverse reactions from historical vaccinia virus vaccine administrations are now noted and evaluated in the risk assessment.

6. Training course attendance recommended for GM workers and risk assessment sign off on master version.

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<tr>
<td>CU2 Project Title</td>
<td>A genomic and transcriptomics analysis of the evolution and global spread of Ranaviruses</td>
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<td>Project notified under transitional arrangements</td>
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Withdrawn | N |
Tick if notifying a connected programme of work | N |
Understanding the effects of host jumps and climate change on the spread and virulence of infections is of practical and theoretical interest. Recent results have suggested that some ranavirus strains propagate more rapidly at higher temperatures: both in cell lines, in experiments infecting amphibians in the lab and the field. A major goal of this project will be to establish the changes in the expression of genes and miRNAs associated with temperature, viral genotype, and their interactions.

In detail, the project will involve analysis of transcriptomes and miRNAs expression levels of cell lines, before and after infection with the virus, at different temperatures (and probably in cell lines from different species). Then, the differences in the expression of genes and miRNAs encoded by both host and ranaviruses would be detected to probably explain the change in propagation rate with temperature and why it differs between viral strains.

One GMM is to be used in this project, and the GMM is described as below: a FV3 strain RUK13 was modified by targeting loci considered non-essential since they represent genes where presence/absence varies across the virus phylogeny. Puro-EGFP cassette was inserted into RUK13 to generate a GMM RUK13-KOUS22. Puro-EGFP cassette is a dual selection marker consisting of the puromycin resistance gene fused in frame with the enhanced green fluorescent protein (EGFP) reporter under the control of the FV3 immediate-early (IE) 18k promoter. Therefore this GMM is very important for identifying the result of ranavirus infection.

### Recipient or parental organism

**Fish Cell Line**

### Host/vector system

**Ranavirus RUK13 strain**

### Origin & function

Wild-type ranavirus strains – No members of this virus genus (or family) are known to infect mammals and they cannot grow in cell culture at temperatures above approximately 30°C. Neither the wild type viruses nor the GMMs are therefore considered a threat to human health. Virus is cultured in a fish cell line (EPC). Puromycin (antibiotic) resistance marker forms part of the inserted cassette in addition to green fluorescent protein (GFP, not considered hazardous to health). Puromycin is an antibiotic used in laboratory cell culture and does not have medical uses meaning that any theoretical impact of release into the environment should be small.

### Evaluation of foreseeable effects

Wild type ranaviruses may pose an important risk to amphibian hosts and may also infect reptiles and fish. Amphibian ranavirus infections are notifiable to the OIE. For the
GMM RUK13-KOUS22, no revertants have been observed till now.
Puromycin (antibiotic) resistance marker forms part of the inserted cassette in addition to green fluorescent protein (GFP, not considered hazardous to health). Neither the puromycin resistance marker itself nor the GFP are known to have any toxic effects.
Puromycin is an antibiotic used in laboratory cell culture and does not have medical uses meaning that any theoretical impact of release into the environment should be small.
There is a possibility that the inserted cassette could be disseminated and maintained in the outside environment however the inserted genetic materials is innocuous and the risk of dissemination is very small and can be limited further through standard containment protocols.
Ranaviruses may persist for weeks or months in water and soil (Nazir et al., 2012) but they are obligate parasites of poikilothermic vertebrates that elicit infections in a dose-dependent fashion. Virus inactivation with routinely used disinfectants (bleach, virkon) is highly effective (Bryan et al., 2009) and autoclaving of waste provides a second layer of containment. The importance of adhering thoroughly to aseptic procedures to prevent contamination of virus cultures simultaneously serves as a prerequisite barrier to dissemination of virus to the outside environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

An open-topped container of >1% Virkon (final concentration 1% w/v) would be kept in the safety cabinet when manipulating the virus and infected cells. Pipets would be filled with Virkon before being ejected, then the pipets and pipet tips would be ejected into the container with >1% Virkon for 30 minutes >99.99% viruses would be killed. Wash the disinfected solids using fresh cell culture medium, and the medium would be used to infect the cells to test the disinfection efficiency. Then the solids would be properly labelled, identified and deposited into designated yellow collection bin for offsite high temperature incineration by an authorised hazardous waste contractor (currently PHS) at St Mary’s Hospital Incinerator, Sidcup, Kent.

Solid waste: plastic consumables, gloves. Plastic, gloves and other consumables will undergo one autoclave cycle with 15 min holding time at 121℃, prior to off-site high temperature incineration (yellow bag clinical waste).

Liquid waste: cultures. Final concentration of 1% (w/v) RelyOn+ Virkon solution with minimum contact time of 1 minute before disposal down designated laboratory drain with copious amounts of tap water. Supplier efficacy data notes test pass achieved (>4 log) with a suspension test (EN14476) with a model test virus Adenovirus Type 5 at 1:100 dilution. Efficacy testing noted by Bryan, 2009 achieved 8 log kill by 1 minute inactivation with Virkon S (a similar active ingredient (perxygen) disinfectant to RelyOn+ Virkon).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
This virus is not infectious to humans but is infectious and very severely harmful to amphibians, fish, and reptiles. The genetic modification (non-hazardous gene insert - GFP) to this virus has been already made in the Institute of Zoology so the risk to the environment is not from the expressed non-hazardous gene expression but from the virus itself.

A key question put the researchers was what GM Class / Containment level work this modified virus had been assigned to at the Institute of Zoology. If a precedent was set as Class 2 / Containment Level 2, then this classification may need to be followed. This has been confirmed as Class 2 / Containment Level 2 by the lead researcher.

Further risk assessment detail was required as the assessment was patchy in places, and the disinfection and waste disposal procedures need to be set stringently to ensure 100% kill within the laboratory. These amendments have been completed in the revised document.

This virus is currently not on any UK controlled micro-organism listings (DEFRA or any other).

### Project Containment

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<th>Glass Houses</th>
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### Project Ref  774/21.2

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Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

Biotin metabolism and fatty acid biosynthesis are essential pathways for survival of Mycobacterium tuberculosis (Mtb) and therefore they have been targeted for tuberculosis drug development. Biotin protein ligase (BPL) is the terminal gene in biotin metabolism pathway. My previous research has shown that BPL is essential for survival of Mtb in cultures as well as in mice. Silencing BPL expression in Mtb results in rapid death of bacteria and clearance of bacteria from mouse lungs. We have previously also identified a BPL inhibitor that works synergistically with the first line drugs used for TB treatment in the clinics.

In my proposed research work at Queen Mary, I plan to study the role of BPL silencing using genetic tools and chemical inhibition on Mtb interaction with the host immune system. My work will involve:
1. Infection of murine and human derived macrophages with genetically modified Mtb strains where the chromosomal copy of BPL gene has been replaced with tetracycline regulated BPL. I will follow up the course of infection by plating in 7H10 agar plates to enumerate the number of bacteria and study the impact on macrophage biology by microscopy, ELISA and pathway inhibitor studies.
2. Generation of new Mtb mutants in fatty acid biosynthesis pathway using genetic tools for mechanistic studies.
3. Generation of gentically modified Mtb strains with GFP and luciferase reporter genes to be used in microscopy.
4. Treatment of wild type and genetically modified Mtb strains with novel inhibitors in BPL and fatty acid biosynthesis pathways using first line drugs as experimental controls.
5. Extraction of lipids from wild type and genetically modified Mtb strains using organic solvents for use in lipidomics studies.

Recipient or parental organism

Mycobacterium tuberculosis H37Rv
Mycobacterium tuberculosis Erdman

Host/vector system

pTEMCS, pTEMCK, pTEMCZ, pGMCtK series and other similar plasmid vectors commonly used in tuberculosis research.

Origin & function

Foreign inserts:
Antibiotic resistance cassettes: These plasmids contain Hygromycin, Kanamycin, Streptomycin or Zeocin resistance cassettes for screening transformants.
Reporter genes: m-cherry, GFP and Luciferase reporter genes will be occasionally used,
recombinase expression,
Gene deletions: biotin, fattyacid biosynthesis pathway gene deletions, some under the control of a tetracycline regulated expression plasmid

Evaluation of foreseeable effects

The Genetically modified organisms generated/imported for the proposed study have deletions in genes involved in fatty acid/biotin metabolism and other similar pathways in M. tuberculosis. These pathways have been demonstrated to be essential for survival of M. tuberculosis in vitro and inside the hosts. Therefore, any changes in these pathways is expected to results in a GMM strain that may be attenuated in growth and virulence compared to the parental strain.Indeed, we have tested some of the GMMs for survival in mice and our data (PMID: 29695454; bioRxiv 2021.02.08.430271) indicate that silencing the gene expression of genes in these pathways results in an enhanced clearance of Mtb from the lungs and other organs. We therefore do not anticipate any additional risks posed by the GMM strains we propose to work with.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMMs will be inactivated and disposed using the same CL3 practices as the parent Mtb strain.

1. Liquid waste: Liquid waste generated during the experiments will be deactivated by overnight treatment with freshly prepared 5% surfanios following which it will be drained in the sink with copious amounts of water to dilute the disinfectant (validation 4 log kill achieved with 5% surfanios(EN14348 compliant) treatment for a minimum contact time of 60 min)
In addition, in-house testing performed at Blizard Institute in 2015 indicated that 5% Surfaniös does inactivate more than 5 logs Mtb culture within one hour of exposure. Additionally, to re-confirm the efficacy of Surfaniös against Mtb, an in-house testing will be performed before commencing the research related experiments.

2. Solid waste: All solid waste (biological as well as non-biological) generated will be autoclaved at 134 deg C for a holding time of 40 min before it leaves the CL3 suite.
The efficacy of every autoclave cycle will be confirmed by the use of thermocouple strips. The autoclave will be annually serviced for maintenance.

3. Sharps: Plastic tips will be collected in designated sharps waste container. Plastic pipettes will be rinsed with 5% surfanios for decontamination and stored in a designated sharps storage container. Use of needles will be minimized and if required blunt needles will be used and disposed in specific sharps container. All sharps waste will be autoclaved at 134 deg C for a holding time of 40 min before it leaves the CL3 suite.

4. Emergency spill – fumigation using formaldehyde vapour (details specified within CL3 Code of Practice)

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The Queen Mary BGMSC approved the work at GM Class 3 at the meeting held on 25 Feb 2021.

Residual clarifications required in the risk assessment on (1) Surfanios validation (2) autoclave temperature of cycle / holding time and efficacy (3) occupational health surveillance details including follow up upon an exposure, effect on any exposure to vulnerable persons (4) Summary of exposure outcomes / incidence (5) any foreseeable downstream / off target effects (6) any foreseeable changes in drug or therapeutic agent susceptibility or resistance properties.

01 April 2021- Residual clarifications completed by Lead Researcher. Risk Assessment document completed along with CU2 and sent to HSE with cover letter.

Project Containment

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02/03/2022  Page 11217 of 15326
**Data Premises Notified**  
15/03/2001

**Transferred from 1992 Regs?**  
N

**Transitional Premises Class**

**Data Premises Closed**

**Transitional Premises**

**Emergency Plan Required?**

**Non-GMMs**  
N

**Withdrawn**  
N

**Name**

**INTERCYTEX LIMITED**

**Name 2**

**Department**

**INNOVATION HOUSE**

**Campus Estate or Research Centre**

**Building**

**OAKS BUSINESS PARK**

**Road Name**

**District**

**CREWE ROAD**

**WYTHENSHAWE**

**Town**

**County**

**MANCHESTER**

**GREATER MANCHESTER**

**Postcode**

**M23 9QR**

**Country**

**ENGLAND**

**Tel Number**  
0161 904 4500

**Fax Number**  
0161 904 4510

**E-mail**

**HSE Division**

**NORTH WEST**

**Comments**

COMPANY MOVED PREMISES ON 26/01/2004

**Date at Which Additional Info Submitted**

22/01/2004
## Premises Addresses

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<td>48 GRAFTON STREET</td>
<td>MANCHESTER</td>
<td>CHESHIRE</td>
<td>M13 9XX</td>
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|                     | INTERCYTEX LIMITED            | INNOVATION HOUSE            |                | OAKS BUSINESS PARK        | CREWE ROAD      | WYTHENSHAWE | MANCHESTER      | GREATER MANCHESTER | M23 9QR       | ENGLAND     | N         |

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

University Biological Safety Officer, Health and Safety Services, University of Manchester  
Divisional Biological Safety Officer (Biochemistry), School of Biological Services, University of Manchester  
Two senior scientists, a laboratory manager and personal assistant from Intercytex Limited.

<table>
<thead>
<tr>
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- Bacteriology  
- Parasitology  
- Transgenic Birds  
- Microbiology Research
Solid waste will be autoclaved on a waste-processing cycle that reaches 135°C for 5 mins in a fully maintained and validated autoclave. The autoclave is sited in a building adjacent and connected to the Incubator building with direct internal access via a service corridor. It will be transported in autoclave bags inside closed metal containers, which are placed directly into the autoclave. Medium aspirated from cell cultures will be suctioned directly into a closed container supplied with Virkon to a final concentration of not less than 1% (wt/vol) for a minimum of 8 hours. Residual cells within the waste medium are already defined as especially disabled and genetic modification will not alter this status. In addition, Virkon is a disinfectant with an independent testing programme proven to inactivate known adventitious organisms that could be contained in these cells. In the event of a spillage of a large volume (up to 1 litre) of cultured GMOs a mixture of 50% (vol/vol) Virkon / vermiculite (Labelled "GM spill kit") will be spread onto the spill and left to absorb and disinfect for at least 2 hours. After this time the mixture is scraped into an incineration bag (yellow plastic bag) and sent for supervised incineration.

SINCE MOVING PREMISES WHERE PREVIOUSLY WE TRANSPORTED OUR NOTIFIABLE WASTE TO FACILITIES IN AN ADJOINED BUILDING, WE HAVE CONTRACTED FOR THE REMOVAL OF NOTIFIABLE WASTE BY WHITE ROSE ENVIRONMENTAL LIMITED. IN ACCORDANCE WITH HSE GUIDELINES AND NOTIFICATIONS (GM779) PROCEDURES ENCLOSED WITH CHANGE OF CONTACT DETAILS.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
1/ INTERCYTEX Disinfection SOP specific to GMOs (Level 2) should be appended to the proposal. This was duly appended.
2/ The committee reminded INTERCYTEX that the University Proposal form is no longer acceptable to the HSE in isolation and additional information would often be required (e.g. the waste disposal SOP). Also additional hazard and risk assessment information would be useful (e.g. Use of Human Tissues and Cultured Cells SOP). These were duly noted and included in the proposal.
3/ INTERCYTEX needs to cover all procedures, including transport of waste for autoclaving and contingencies for major spillages. These are covered in INTERCYTEX SOPs and are included in the proposal.
4/ The origin of the cell lines should be stated i.e. "established human cells" or "primary mouse cells". This was duly amended.
5/ Host cells to be listed individually together with the organism of origin. Within the limits of claimed confidentiality, the cell lines proposed for use have been listed.
6/ Vector access factors to be considered in the risk assessment apply only to mammalian cells. Since no use of E. coli hosts is indicated, access factors for this organism are irrelevant. The appropriate amendments to the risk assessment have been made.
7/ For human cell lines, the question of "adventitious agents" must be addressed. The question has been addressed as an issue for GM risk assessment and also with consideration to COSHH guidelines and this is now reflected in the risk assessment and notification documents.
8/ Antibiotic resistances conferred by the vectors proposed for use under "known biological activity" Noted and duly amended.
9/ Under ‘Expression Factor’ specify the “strong promoter” to be used. Noted and duly amended.
10/ The committee carried out an inspection of the proposed laboratories areas and provided INTERCYTEX with a report. The report suggested that specific signage be applied to reflect the use of GM Level 2 organisms. The premises and equipment were deemed to be consistent with Level 2 GMO activities and approval for the proposed use of premises for specified GMO activities was granted. This was duly noted and signage will be applied once GM work is approved.
**GM Centre Number: 776**

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**Date at Which Additional Info Submitted**: 02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Risk assessment for GMM is carried out by a subcommittee of the company Health and Safety Committee, Company's biological safety officer, company health and safety officer, radiation protection advisor, Executive Director Research and Development, Executive Director Business and Development (responsible for company H&S policy). Routine matters covered in regular quarterly H&S meetings. Subcommitte meet as required to discuss GMM and radiation issues.

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### Research level protein production

For activities involving GMMs, describe the waste management measures which will apply to the activity

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<td>Plants</td>
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Solid waste and low volume liquid (< 10ml) autoclaved prior to disposal.
Liquid waste collected in 500 ml bottles and treated with chlorine based disinfectant tablets.
Validation of inactivation will be carried out on site by carrying and process and test plating samples after overnight exposure to chlorine based disinfectant.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Accepted company protocols from Dundee site should be transferred and validated on Babraham site.
| Data Premises Notified (Originally) | 23/04/2001 | Transferred from 1992 Regs? | N |
| Data Premises Closed | | Transitional Premises |
| Emergency Plan Required? | | Class |
| Withdrawn | N |

**Name**

HEALTHCARE ENVIRONMENTAL GROUP LIMITED

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**E-mail**

**HSE Division**

SCOTLAND

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- **Y**

**Give brief details of the genetic modification safety committee**

Senior Lecturer (convener), Professor, Lecturer, 3x Research Scientists & BSO's, Post-Graduate Representative, Technician, MSF Representative, University Safety Advisor & University Biological Safety Adviser.

Procedures:- The university Biological Safety Adviser is responsible for:- 1) organising meetings when required; 2) taking minutes; 3) informing Principal Investigator of committee's decision regarding adequacy of risk assessment. The Convener of this committee reports to the Biological Safety Sub-Committee.

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<td>Growth Room</td>
<td>Glass House</td>
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<td>Large Scale</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

The plants would be uplifted by Healthcare Environmental Services, and delivered to Crosshouse Waste Energy plant by a specialist vehicle. All waste will be packaged in 300 gauge bags inside a lockable wheeled bin/eurocants. Upon arrival at Crosshouse Waste energy plant the material would be lifted by hydraulic lift for tipping directly into the waste incinerator. During discharge the load would be held at negative pressure whilst loading into the incinerator. The incinerator is tested weekly by continual monitoring and is regularly tested by the environment agency.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC considered the risk assessment for the transport and incineration of genetically modified tobacco plants by Healthcare Environmental Services Limited. The committee agreed that:

1. the risks to the environment would be adequately controlled by double bagging the plants in 300 gauge black sacks, placing the full sacks into a lockable bin on wheels and transporting the bin in an enclosed vehicle to the incinerator;
2. in the event of a major accident resulting in a loss of the triple containment the extremely low risk of harm to the environment would be minimised by collecting the released plants and soil into plastic bags.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Chairman, Management Rep & Lab Rep.

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Tick if confidential

Bacteriology: Yes

Parasitology

Transgenic Birds

Microbiology Research

Transgenic Animals

Gene Therapy

Transgenic Fish
E. coli K12 derivatives are killed using virkon at a concentration indicated by the manufacturer. Effectiveness of killing is indicated by overnight culture of treated material. Class 1 facilities have virkon, bleach and 70% ethanol available for cleaning and inactivating GMM. Mammalian cell culture waste is treated immediately with bleach. All cell culture waste is autoclaved on site (BMM Weston autoclaves - fully serviced, calibrated). Autoclaved material is subsequently incinerated off site (Cambridge Pet Crematorium). Effectively 100% kill.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The level 2 containment represents the upper limit of containment for GMO work to be performed. The use of level 2 arises mainly from the possibility of adventitious agents in human primary cell cultures. The introduction of immunoprotective genes will increase the resistance of cells if they enter the body. This is a very unlikely event. The risk to human health is significantly less than the use of oncogenes for example. The risk to the environment is extremely low.

**Project Ref 778/01.1**

**CU2 Project Title**

ANALYSIS OF GENES INVOLVED IN CELL PROLIFERATION AND DIFFERENTIATION IN THE MAMMALIAN PANCREAS....

**Class**

Class 2

**CultureVolClass2**

< 1 litre

**CultureVolumeClass3-4**

**Non-GMM**

Consent Granted

not applicable

**Project notified under transitional arrangements**

N

**Historical Significant Changes**

Withdrawn

Tick if notifying a connected programme of work

N

Historical Date of Additional Info

02/03/2022
**Project Additional Information**

**Purposes of the contained use**
The aim is to develop therapies including cell based therapies for the treatment of diabetes and pancreatic cancers.

**Recipient or parental organism**
GMOs include 1) Replication defective retroviral virions based on murine retrovirus. Standard retroviral vectors to be used with both ecotropic and amphotropic host ranges. 2) Genetically modified mammalian cell lines. 3) Genetically modified mammalian primary cell cultures, including human.

**Host/vector system**
- non mobilisable plasmids containing mammalian expression signals, typically puc-based. Transfected into mammalian cells.
- Replication defective retroviral vectors in primary mammalian cell cultures.

**Origin & function**
Genetic material is either derived from the mammalian genome or from mammalian expressed mRNA. Specific genes may also be derived from mammalian viral genomes, obtained as sub-clones with no viral replication potential.

Intended function is
1) to control the growth and differentiation of pancreatic cells
2) to produce human cell based therapies for diabetes that require reduced immunosuppression by pharmaceuticals.

**Evaluation of foreseeable effects**
Primary human cells will be cultured at containment level 2. To account for the possibility of adventitious agents.
Genetic modification will reduce the immunogenicity of human cells. If introduced into the human body these cells will survive longer than wild type cells. However this event is extremely low probability and no harmful consequences would be expected.

All human tissue is pre-screened for HIV and hepatitis B before entering this facility.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
None used.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Culture media will be treated with bleach. All materials will be autoclaved on site in a BMM Weston that is fully calibrated and serviced. Following autoclaving material will be incinerated at Cambridge Pet Crematorium.
The final material will not contain living organisms.
The level 2 containment represents the upper limit of containment for GMO work to be performed. The use of level 2 arises mainly from the possibility of adventitious agents in human primary cell cultures. The introduction of immunoprotective genes will increase the resistance of cells if they enter the body. This is a very unlikely event. The risk to human health is significantly less than the use of oncogenes for example. The risk to the environment is extremely low.

Project Containment

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Date: 02/03/2022
The notified premises are all operated by SRCL Ltd, as specialist healthcare waste premises. All facilities operated by SRCL are authorised under the Environmental Permitting Regulations 2010. Of these facilities 9 of these are Healthcare waste incinerators capable of incinerating all types of clinical waste and related wastes.

For the incineration facilities the clinical waste is mechanically loaded into the Primary Chamber where it is burned at a temperature of 900 to 1000 degrees centigrade for sufficient time to produce an inert sterile ash. The gases and other combustion products then pass into the Decondary Chamber where a minimum temperature of 1000 degrees centigrade is maintained for a minimum of two seconds in an oxygen-rich atmosphere to completely oxidase the combustion products. The flue gas is then cooled to around 130 degrees Centigrade by means of a steam boiler before being treated with powdered activated carbon and hydrated lime in a bag-house to remove any remaining impurities.

For the remaining facilities, GMO and GMM waste collected will be stored at the facility pending transfer to an incineration site in the same containers as collected in.

All the facilities have a designated waste storage area with separate facilities for the storage of all permitted categories of hazardous and non-hazardous wastes. There are well developed tagging systems, paperwork systems and written procedures for the handling of the different types of waste, including GMM and GMO.

For activities involving GMMs, describe the waste management measures which will apply to the activity
The plants are operated around the clock by teams of Shift Leaders and Shift Operators, who are under the management of experienced and highly competent site management in the form of General and Plant Managers.

To satisfy the CDG regulations, clinical waste has to be packaged in UN approved containers, which include yellow bags, sealed units and sharps bins. The sealed units are specifically made for the containment of higher risk materials such as human tissue and body parts, microbiological cultures and outdated medicines. They come in various sizes typically around 30, 50 and 60 litres - and are fitted with a lid which forms a very strong hermetic seal when pushed into place. The whole bin is designed to be incinerated along with its contents, with no manual handling involved. Sharps bins are similar containers, but designed to contain hypodermic syringes, scalpels etc.

SRCL Ltd specialises in the collection, transport and disposal of clinical waste in 770 litre wheeled bins constructed of high density polyethylene with a lockable plastic lid. The wheeled bins are themselves UN approved as a large package, so that in conjunction with the smaller packages they form a UN approved combination package ideal for the transport of infectious waste.

The wheeled bins are transported in purpose-built, twin deck vehicles fitted with straps and tail-lift barriers to secure the waste during transport and during loading/unloading operations. All the vehicles are driven by ADR trained drivers; the company has its own qualified driver trainer and also has a full time, qualified Dangerous Goods Safety Advisor. The bins are mechanically loaded into the incinerators by means of hydraulic lifts, which eliminate any manual handling of waste containers within the bins. Every bin is washed for 4 minutes in a heated bin-washer after it is emptied, using a sanitiser/detergent to clean both the inside and outside of the bin.

Each bin has a unique bar code attached, which is used to track bin movements from waste producer to final disposal on a computerised system, and therefore to satisfy Duty of Care requirements.

Every facility has a spillage kit containing all the equipment needed to clean up a spillage of clinical waste, and staff who are well trained in spillage procedures. Fortunately, because of the mechanical handling systems used, spillages are very rare occurrences and the kits and training are more of a precautionary measure.

In addition, there is a GMO Safety Committee that meet at least annually.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment was carried out to assess all the types of GM material that could possibly be sent for disposal. The requirements for inactivation or biological barriers are the responsibility of the waste producer(s).
### Project Additional Information

#### Purposes of the contained use
The storage, transport and disposal by incineration of GMOs up to Risk Class 2.

#### Recipient or parental organism
The characteristics and evaluation of foreseeable effects will be determined by the individual waste producers.

#### Host/vector system
Determined by individual waste producers.

#### Origin & function
Determined by individual waste producers.

#### Evaluation of foreseeable effects
Determined by individual waste products.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Waste must be inactivated, or the waste producer must be able to demonstrate that the waste will pose no threat to human health or the environment.

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
The GMM waste will be transported and stored in UN approved hermetically sealed containers used for the transport of infectious clinical waste. The whole sealed container is incinerated along with its contents with no manual handling involved.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
The GMO waste will be packaged in UN approved sealed packages, transported in lockable 820 litre wheeled bins, mechanically loaded into clinical waste incinerators operated at a minimum temperature of 100 centigrade. This will give 100% kill of GMOs. The incinerators are approved for infectious clinical waste (including risk group 4 pathogens; for special waste, radioactive waste and hazardous waste. The final product is a sterile ash which goes to a landfill site.

The risk assessment shows the activities as posing no significant risk to the environment due to the appropriate control measures that are in place.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2</td>
<td>L2</td>
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</table>

Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |
GM Centre Number: 780

Data Premises Notified: 12/04/2001

Transferred from 1992 Regs?: N

Transitional Premises Class: N

Data Premises Closed: N

Transitional Premises Emergency Plan Required?: N

Non-GMMs: N

Withdrawn: N

Name:
GLAXOSMITHKLINE

Name 2:

Department:

Campus Estate or Research Centre:
NEW FRONTIERS SCIENCE PARK

Road Name:
THIRD AVENUE

Building:

District:

Town:
HARLOW

County:
ESSEX

Postcode:
CM19 5AW

Country:
ENGLAND

Tel Number: 01279 622000

Fax Number: 01279 875094

E-mail:

HSE Division:
EAST AND SOUTH EAST

Comments:

Date at Which Additional Info Submitted:
02/03/2022
## Premises Addresses

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<td>CENTRE FOR CLINICAL INVESTIGATION</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Chairman, (MCCS) Secretary/Biopharm, Biopharm/Ges, Biopharm/Genetic Technologies, Biopharm/Mol Cell Biology, Biopharm/Comparative Genetics, 2 x Neuroscience, 3 x Vascular Biology, Path Tox., Fryth, LAS, Fryth.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 1 (GMMs)</td>
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<td>Level 2 (GMMs)</td>
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<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Transgenic Animals</th>
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<tr>
<th>Microbiology Research</th>
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<tr>
<td>Gene Therapy</td>
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<table>
<thead>
<tr>
<th>Virology</th>
<th>Transgenic Fish</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
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<td><strong>Other(s)</strong></td>
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</table>

All wastes will be autoclaved in validated equipment. 
All work will be undertaken in Containment Level 2 facilities.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC has approved this work.
### GM Centre Number: 781

<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
<th>21/04/2001</th>
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<tbody>
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<tr>
<td>Transitional Premises</td>
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<tr>
<td>Emergency Plan Required?</td>
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<td>Non-GMMs</td>
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#### Name

<table>
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<th>Name</th>
<th>SEQIRUS VACCINES LTD</th>
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#### Campus Estate or Research Centre

<table>
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<th>Road Name</th>
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<tr>
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<tr>
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<tr>
<th>Tel Number</th>
<th>0151 705 5000</th>
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<td>Fax Number</td>
<td>0151 705 5553</td>
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#### HSE Division

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<tr>
<th>HSE Division</th>
<th>NORTH WEST</th>
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</table>

#### Comments

COMPANY NAME CHANGE AS OF 26/11/2003 FROM EVANS VACCINES TO THE ABOVE.

#### Date at Which Additional Info Submitted

| 26/11/2003 |
**Accident ID**
6.00  21/11/2007
7.00  28/11/2007

**Date Accident Notified**

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**Premises Addresses**

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<th>Date Premises Closed</th>
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Yes

Give brief details of the genetic modification safety committee

Committee meetings are organised by the BSO or HSE manager as new projects arise and a meeting is deemed appropriate. There is no set frequency. Personnel involved include the following, although others may be invited as appropriate:-

- Development or Operations Manager (Chairperson)
- HSE site manager
- Biological Safety Officer
- Project specific manager
- Employee Representative(s)

<table>
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<td>Level 3 (GMMs)</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

For work involving Class 1 GMM's; all waste is heat or chemically inactivated prior to discharge to drain.
The conditions during inactivation are well in excess of those required to kill the organisms (based upon considerable experience and numerous viability/growth/survival studies on the cell line).
In addition to routine inactivation of waste; process equipment is located within suites which are designed to retain spillages.
All production equipment is also disposable or subjected to validated Steam-in-Place or autoclaving procedures.
All disposables are autoclaved prior to removal from site in validated cycles.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

This project was initially discussed at a meeting of the Speke site GMSC on 11/06/92 at which it was concluded that CL1 along with Good large Scale Practice was appropriate. Subsequent meetings confirmed the controls that were to be routinely employed.
Comments of relevant personnel to the updated risk assessment (attached) include the following:-
The production area can be effectively disinfected and fumigated and is designed to retain spillages.
Equipment waste is routinely autoclaved and waste liquid streams are inactivated prior to discharge to drain.
Containment Level 1 is considered sufficient to control the risks to human health and environment although many of the controls listed for CL2 are routinely applied.
The risk of any environmental hazard is considered effectively zero.
## Project Additional Information

**Purposes of the contained use**
To generate pandemic Influenza vaccine which will contribute to stockpiles of vaccine.

**Recipient or parental organism**
A/Puerto Rico/8/1934 (PR8) Flu virus strain has been routinely used as a basis of flu vaccines with an extensive history of use. This has been combined with A/Vietnam/1203/2004 (H5N1) to produce the reverse genetics seed: rg A/Vietnam/1203/2004 X A/PR/8/34 6:2 will be used in these activities.

**Host/vector system**
See above ... generated by reverse genetics.

**Origin & function**
A/Puerto Rico/8/1934 (PR8) & A/Vietnam/1203/2004 (H5N1)

**Evaluation of foreseeable effects**
Extremely remote possibility of reassortment during simultaneous "infection" with rg A/Vietnam/1203/2004 X A/PR/8/34 6:2 and a circulating human flu virus generating a new pathogenic strain.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation from the following measures specified in Table 2, Schedule 8 is applied for :-

* Items 1, 2, 3, 4, 5 & 7 relate to closed systems, such as fermenters. This process is fundamentally NOT a closed system.
* Item 6 relates to seals (there are no significant seals in the Fluvirin manufacturing process (except post viral inactivation steps when seals are in-place to ensure product integrity).
* Item 13. Negative pressure is not appropriate for cGMP activities. Containment arrangements are by alternative means which includes sealing of entrances/exits and use of pressure cascades within new staff changing rooms.
* Item 21. The final discharge for this process for all bulk egg waste will be at Onyx, Southampton (via incineration). Other waste materials eg disposable PPE and potentially contaminated consumables will be incinerated in sealed drums at Cleanaway, Ellesmere Port, Cheshire.

Note: Items 1, 3, 4, 5 & 6 of the above are the only items listed as being required for CL2 activities.

For QC activities listed as part of this application, derogation from the following measure specified in Table 1a, Schedule 8 is applied for :-

* Item 17, requires validated inactivation of GMMs in waste. Such wastes will be incinerated at Cleanaway, Ellesmere Port, Cheshire within sealed drums (as clinical waste).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Use of downward sealing packaging heads (supplied by Hosokawa Micron Ltd) plus Laminar Flow Extract Booth & HEPA Filters. Sealed loading of bulk waste into IBCs and container sided transport to Onyx incineration facility at Fawley, Southampton (covered by separate licence). Single stacking of IBCs is planned during transit. IBCs will be incinerated (not reused).

Low risk waste (disposable PPE, silicone tubing, used HEPA filters etc) will be incinerated at Cleanaway facility in Ellesmere Port within sealed drums.

Note: An emergency plan is not required due to controls being put in-place throughout the project & the submission of a separate notification from Onyx UK (waste handling contractors).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No concerns have been raised by the local GMSC on this project. Discussed at GMSC on 28th July 2005. Next meeting is scheduled for Dec 12th 2005 when the project will be close to commencement. Issues relating to this project on site have been fully discussed with relevant parties during a series of staff briefing sessions. Trade Union reps have also been fully briefed on the project and have had an opportunity to discuss the project with HSE BAU personnel.
Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 781/17.1

Date Ackn'd 14/07/2017

CU2 Project Title Production of Influenza Virus Seeds and Associated Development Activities

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Consent Granted

Non-GMM

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To fulfill government contract orders (typically US or UK) for Working seeds and bulk material for pandemic stock piling. Working seed production for pandemic preparedness. Candidate viruses received from WHO which have been reassorted via reverse genetics (not generated on site) are used. Testing of Seed and bulk material performed by QC and external labs.

Recipient or parental organism

A/Puerto Rico/8/1934 (PR8) Flu virus strain has been routinely used as a
basis of flu vaccines with an extensive history of use. This has been combined with various pre-pandemic and pandemic strains (H5N1, H7N9, H1N1 etc) to produce the reverse genetics candidate vaccine virus: e.g NIBRG 23 A/TURKEY/TURKEY/1/2005. Next project will be ID CDC RG56B A/Hong Kong/125/2017 H7N9, A/Duck Bangladesh/ Any other viruses will be evaluated by the biosafety committee.

Host/vector system

Candidate vaccine virus generated by reverse genetics at a WHO lab prior to receipt - The haemagglutinin (HA) and neuraminidase (NA) genome segments of the wild-type virus are cloned into ‘rescue’ plasmids under the control of an RNA polymerase I promoter. During cloning, the HA segment is modified to delete the stretch of basic amino acids that confer the highly pathogenic phenotype of the virus. These are transfected into Vero cells together with rescue plasmids containing the remaining six viral genome segments from and four expression plasmids that encode the viral replicase machinery of the PR8 virus. Genetically, the rescued RG virus is a reassortant containing six segments from PR8 and two from the wild-type virus, with the HA segment containing a deletion compared with its parent virus. The phenotype of the RG reassorted virus is expression of the wild type coat proteins, good growth in eggs (a PR8 trait), likelihood of attenuation for humans (another PR8 trait) and lack of pathogenicity (owing to the HA deletion).

Safety testing performed prior to receipt - The tests performed to demonstrate that a virus derived by reverse genetics is no longer pathogenic as per WHO guidelines and include a chick embryo lethality test, a chicken pathogenicity test, a ferret pathogenicity test and sequencing. Hazard classification BSL2 enhanced given.

Viruses prepared and received from WHO laboratory. No bacterial plasmid or vero cells are present or handled on site.

Origin & function

Avian, swine, porcine wild type viruses of pandemic potential are reassorted onto PR8 backbone via reverse genetics (see host/vector system section above). Which is historically well known virus with a strong safety record which grows well in embryonated hens eggs (but does not kill them) but is there is very low infectious risk to humans. The reassorted virus generated, safety tested an supplied by approved WHO laboratories

Evaluation of foreseeable effects

Small theoretical risk of reassortment during simultaneous "infection" with RG reassortant (A/Puerto Rico/8/1934 (PR8)) and a circulating human flu virus generating a new pathogenic strain. Risk is further reduced through application of control measures throughout use on site. Biosafety committee views this as a BSL2 hazard however due to this risk enhanced controls area

Pandemic vaccine reassortants have been produced on the human strain A/PR/8/34 (PR8) as recipient virus. PR8 has had over 100 passages in each of mice, ferrets and embryonated chicken eggs. The result of such a passage history is complete attenuation of the virus and its inability to replicate in humans.

Although it is considered that, for example, an H5N1/PR8 reassortant will be either attenuated or possibly non-infectious to humans, an indirect hazard may exist through secondary reassortment with a human or animal influenza virus as influenza viruses are known to exchange genes by the process of reassortment. For secondary reassortants to be generated, several events need to occur; firstly infection of the production staff with the reassortant strain; secondly, for an infected worker to have a mixed infection with a wild type influenza virus, and thirdly for a reassortment event to take place. In practice, manufacturers have 30 years of experience with large scale production of vaccines based on PR8 reassortants and there have been no reported cases of human illness.

Biological Risk assessments for the live areas handling GMO and strains with hazard group 2 enhanced classification for the proposed HHS contract are attached - Seed Manufacturing and QC bioassay.

Control measures for other areas detailed in section 12. Risk assessment currently under review and update for UM, ETA and TD in preparation for possible bulk order.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable. No larger GMM's used or present on site.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable. Refer to risk assessments included or detail provided below

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Non-Autoclavable waste (including White safety shoes from Upstream Manufacturing Areas) must be surface decontaminated, collected in clinical waste containers placed in the change rooms, and sent for disposal via incineration.

Effluent Treatment Area -
Solid waste treatment from UM harvest:
Macerated egg waste is transferred from the Upstream Manufacturing (UM) area via a holding tank and integral transfer pipework into the Effluent Treatment Area (ETA). The waste is stored when in ETA in 40,000 liter holding vessel. This vessel is HEPA filtered and linked the ETA scrubber system. The waste is continuously feed from the vessel into the Gouda Driers, these are paddle driers that heat the material to above 100c for a total of 7 hours. The Driers are steam heated through the Paddles and jacket, temperature probes throughout the drier monitor the temperature of the material being
processed. All gas generated by the drying process are pulled into the LEV scrubber system.

LEV Scrubber system:
All gas generated from the drying system and the post HEPA filtered vessel gas is treated through the LEV scrubber system. This system consists of an acid scrubber, carbon filter and a Caustic scrubber before the gas can be released through the stack to atmosphere.

Virally active waste liquid:
All waste water and cleaning materials from the Upstream area are treated in the ETA facility. The drains in the UM area direct all waste to the external sump which is sealed and HEPA filtered. This material is transferred into the ETA holding vessel via integral pipework, the vessel is HEPA filtered and the extract air is processed through the LEV scrubber system. The waste liquid is continuously feed from the vessel into the waste liquid treatment skid (Actini). The Actini is a thermal decontamination unit which inactivates the virus at 100c and is held for 1 minute. The Actini unit is controlled by temperature control system which directs the material back to the holding vessel if the temperature drops below the 100c limit. After treatment the waste liquid is transferred to the external sewer sump where it is pH adjusted checked to be within temperature specified for release into the sewer system.
The ETA area is contained and all drains are directed to internal ETA sumps which are then put through the waste treatment processes to prevent any waste material being released without treatment.

All waste treatment temperatures have assessed and justified through technical documents which have been reviewed and approved by the HSE.

During Pandemic processing all ETA staff are vaccinated and in the event of a spillage all staff will wear a 3M face mask or Jupiter hood as outlined by the ETA gowning procedure.

Technical Development - small scale, low volumes of virally active material generated. Waste eggs, gowns and contaminated waste from pilot plant, containment labs and lab consumables are treated with approved disinfectants and sealed contaminated waste bins. Decontaminated egg waste and contaminated waste bins are incinerated at approved off site location. Adherence with APHA import licence. No drains. Negative pressure pilot plant and single HEPA filter. Use of MBSCs with double HEPA filtration of extract air (cabinet and containment labs). The drains in the Pilot plant area direct all waste to the external sump which is sealed and HEPA filtered.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
GMSC initially met and risk assessed the current proposed GMO work 24Apr17. It was concluded current controls and practices are sufficient with some preparation activities to complete including submission of this form and notification to HSE being required. Risk assessment and procedures in place were reviewed by all impacted parties. Following satisfactory completion of the safety testing by CDC of the virus (ID CDC RG-56B A/Hong Kong/125/2017 it was confirmed the site is ready to receive the virus to plan in Seed Manufacture to meet the HHS (Health and Human Services USA) contract. Currently the order is for Seed production only. Control measures and communications captured confirmed appropriate. Further meetings held to review progression of actions as per site procedures and GMSC requirements held. Further meetings will also be held should the contract be granted for bulk manufacturing.

**Project Containment**

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**Name**

GRUNDON WASTE MANAGEMENT LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

LAKESIDE ROAD

**District**

COLNBROOK

**Town**

SLOUGH

**County**

BERKSHIRE

**Postcode**

SL3 0EG

**Country**

ENGLAND

**Tel Number**

01753 686777

**Fax Number**

01753 686002

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Y

**Give brief details of the genetic modification safety committee**

5 Members:
- 1 - Biological Safety Officer for Grundon
- 1 - Dangerous Goods Safety Advisor
- 1 - General Manager for S Grundon (Waste) Limited
- 2 - Co-Opted Technical Advisers.

A meeting is to be held every 6 months, unless special circumstances arise.

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**Other (please specify)**

- Hazardous Waste Incinerator & Hazardous Waste Store

**Tick if confidential**

- ( )
All GM Waste will be destroyed by High Temperature Incineration in temperatures of +1000°C. 100% Kill.

For activities involving GMMs, describe the waste management measures which will apply to the activity

All GM Waste will be destroyed by High Temperature Incineration in temperatures of +1000°C. 100% Kill.

Project Ref 782/01.1

Date Ackn’d 16/05/2001

CU2 Project Title DISPOSAL BY HIGH TEMPERATURE INCINERATION OF GM WASTE

Date Project Ceased

Class 2

Cultures Vol Class 2

Consent Granted not applicable

Non-GMM

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Project Additional Information

**Purposes of the contained use**
To store and transport GM Waste prior to incineration

**Recipient or parental organism**
All characteristics of the GMO will be fully described by the Waste producer

**Host/vector system**
All characteristics of the GMO will be fully described by the Waste producer

**Origin & function**
All GM's will be incinerated

**Evaluation of foreseeable effects**
Forseeable affects will be different depending on the individual GM Waste to be accepted.

All information on effects will be supplied via the waste producer.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
All GM Waste must be contained in either a UN approved clinical waste sack or a one-way burn bin depending on the nature of the waste. It is the waste producers responsibility to adequately package their waste to prevent its escape, under their Duty of Care.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
We request a derogation from everything written in Schedule 8. The reason for this is that we operate a Hazardous Waste Incinerator plant and the control measures listed are not appropriate to our operation. The control measures stated within the notification should be sufficient to control the risks.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
All GM Waste will be destroyed by High Temperature Incineration in temperatures of +1000C. 100% Kill.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
It was deemed that the risk assessments were adequate for collecting, storing and disposal of GM Waste.

**Project Containment**

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### WICKHAM MICRO LIMITED

#### GM Centre Number: 783

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#### Name

WICKHAM MICRO LIMITED

**Name**

WICKHAM MICRO LIMITED

**Department**


#### Campus Estate or Research Centre

HOEFORED POINT

**Road Name**

BARWELL LANE

**Building**


#### Town

GOSPORT

**District**


#### County

HAMPERSHIRE

**Postcode**

PO13 0AU

**Country**

ENGLAND

**Tel Number** 01329 832511

**Fax Number** 01329 834262

**E-mail**


**HSE Division**

EAST AND SOUTH EAST

**Comments**


**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Chairman: Business Manager - Microbiology
Members: Health and Safety Officer,
Laboratory Manager - Pharmaceutical Microbiology
Senior Technician - Microbiology
Senior technician - Virology

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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid biological waste: inactivated by autoclaving prior to removal from premises and incineration.
Liquid biological waste: inactivated with 1% w/v Virkon solution for a minimum of 30 minutes.

Please enter comments of the GM safety committee on the risk assessment

The GM Safety Committee have no comments on this notification.
Project Additional Information

Purposes of the contained use

To determine the sterility status of recombinant ovine adenovirus using the steritest system within a half suit isolator.

Recipient or parental organism

OAV623 is an OAV that contains the full viral genome and a transgene of human origin. OAVs are capable of infecting human cells but have been shown to be unable to complete their full cycle of replication (Khatri, A et al [1997]: Virology 239 226-237), resulting in an abortive infection. Infection is still abortive in the presence of co-infected human adenovirus suggesting minimal risk to the worker when propagating this virus. It is expected however that there will be an immune response in humans on exposure to this virus.

Host/vector system

A plasmid that contains the whole OAV623 viral genome and is therefore a GMM, though of similar or lower risk to the parental virus.

The host cells and viral vector are both ovine and the virus is incapable of effective replication in humans. In addition, the expression of the transgene is under the control of a tissue specific promoter.

Origin & function

Virus and cell line are ovine. The cell line is to allow efficient propagation of the recombinant ovine virus. The virus carried a human transgene and is intended to deliver the transgene to human cells via an infective but non-replicative action. Expression of the transgene is under the control of a tissue specific promoter. The gene product is a human enzyme that will only be expressed in target tissues and will be capable of generating a toxic product from a prodrug that is administered separately.

Evaluation of foreseeable effects

OAV623 is a recombinant adenovirus, which contains the full viral genome and a transgene containing a tissue-specific promoter and a human gene encoding an enzyme that can act on a pro-drug to produce a toxic agent. OAV is capable of infecting human cells, but it has been shown to be unable to complete its full replication cycle (khatri, A et al [1997]: Virology 239 p.226-237), resulting in an abortive infection. OAV has also been shown to replicate abortively in human cells, which are co-infected with human adenoviruses. The abortive replication cycle of these viruses suggests that there is minimal risk to the worker when propagating these viruses. However, due to the lack of pre-existing immunity of the worker to OAV, there will be limited immunity on first exposure to the virus. Subsequent exposure would result in a strong immune response to the virus.
The plasmid DNA containing the full genome of OAV 623 will be used to rescue the virus under segregated conditions. As the plasmid contains the same genetic material as the intact virus, it is highly unlikely that the plasmid would be of greater risk to the worker than the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The virus will only be handled in class II microbiological safety cabinets and half suit isolators that will be swabbed with a viricide (eg Virkon or Klearcide) after use.

All equipment will be swabbed with a viricide (eg Virkon or Klearcide) after use.

All contaminated materials will be treated by chemical means (eg 1% Virkon solution, which is proven to destroy both Mastadenoviruses and Aviadenoviruses) and/or autoclaving at 121 degrees C for a minimum of 15 mins prior to removal by a contractor for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC agree that class 1 would normally have been appropriate for the protection of human health, but as this virus can cause mild infection in sheep it becomes a class 2 activity to ensure protection of sheep in the environment.

Project Containment

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Principal Scientist, Biotechnology
- Senior Manager, Validation Services
- Validation Laboratory Manager
- Senior Laboratory Scientist
- Consultant to Pall Europe on GMM, University of Portsmouth

<table>
<thead>
<tr>
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<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify): Tick if confidential
All GMM waste material will be subject to autoclaving in a 400-litre autoclave at 121 degrees C for 15-30 min dependent upon volume of material. The autoclave is housed in specifically designed facility within an enclosed area. Floors are sealed and the flooring is extended up onto the walls to a height of 10 cm. This provides a high level of containment for any spillage and easy cleaning of spilled material.

The efficiency of the autoclave procedure is monitored using thermal strips and Bacillus spore ampoules on a routine basis. The autoclave is validated annually using multi-probe Kaye-validator, to which the thermal strips and ampoules are also validated. The autoclave also has a load-probe, which is validated annually.

The facility consists os separate laboratories for growth, handling and purification of GMMs or heterologous protein purified from the GMMs. This facility is separated from the rest of the environment by a locked (coded) door with limited access. Each laboratory has been designed to a standard of containment equivalent to class 2. This includes filtered air and negative, or positive, air pressure as appropriate. Transport of waste material to the autoclave takes place entirely within this facility and would include a maximum distance travelled of 10 metres.

_Filtration and separation of micro organisms_
The use of recombinant viral vectors is increasing in scope for clinical gene therapy applications due to the demonstration of long term transgene expression from viral vectors with little associated toxicity and good overall safety profiles in both preclinical and clinical trials. A major requirement for the development and eventual marketing of a gene therapy drug is the ability to produce the gene delivery vector at a sufficient scale. The purpose of the contained use activity is to enable fast track process development to pilot-scale ensuring a robust upstream process with satisfactory virus productivity. Recombinant viral vectors will be produced in ‘development projects’ for a number of Pall’s customers. The work will involve culturing a range of immortalised mammalian cell lines which will be transfected with a replication incompetent viral transfer plasmid encoding the gene insert of interest, plus viral packaging and envelope plasmids. Plasmids and DNA constructs will have been produced in well characterised bacterial expression systems and supplied to Pall. Transfected mammalian cells will produce high-titre recombinant virus particles intended for cell and gene based therapeutic applications.

Vectors based on adeno-associated virus (AAV), adenovirus, lentivirus and v-retrovirus will be used; these have gained importance in somatic gene therapy approaches in recent years, with use of AAV and lentiviral vectors being the most common. Inserted genes will include therapeutic genes, including genes known to be mutated in disease conditions, genes known to ameliorate pathology and genes used to manipulate cells for ex vivo therapies. Recombinant virus particles will be harvested from either the cell culture supernatant or from lysed cells using purification techniques such as chromatography or centrifugation. The viral vector systems to be used have been developed for these applications, are commercially available and/or have a long history of safe use, with good overall safety profiles in trials. Each development project will be specific to each customer in terms of the cell lines/types used, and the viral vector and gene of interest used.

Manipulations will be performed under aseptic conditions, in class II microbiological safety cabinets. Cells will be grown in adherent mode using a range of single use vessels, including T-flasks, cell factories, or in suspension mode using microcarriers in spinner flasks, or shake flasks, or in fixed-bed bioreactors, such as Pall’s iCELLis Nano. Working volumes will range from 10 - 100mL in T-flask to 1 L in the iCELLis Nano, and then up to a surface area of 500m2 in the iCELLis, with a 500L perfusion capability.
Two example risk assessment reports are submitted in support of this notification: MeiraGTx (GMM no. 002); and Adaptimmune (GMM no. 004). These risk assessments are representative of the contained use work to be performed under this notification. Any subsequent projects will be assessed to determine whether they fall within the scope of this notification. In the event that the assessment determines that future projects fall out of scope, subsequent notification to the Competent Authority will be made.

**Recipient or parental organism**

Cell lines will include:
- HEK293/293T - fully characterised cell lines from commercial source (ATCC/ECACC);
- PG13 - derived from NIH3T3
- Other, similarly well characterised cell lines may also be used.

**Host/vector system**

Viral vectors will include:
- Adeno-Associated Virus
- Adenovirus
- Lentivirus
- Gamma retrovirus
- Herpes-Simplex virus

**Origin & function**

Therapeutic genes include genes known to be mutated in disease conditions and genes known to ameliorate pathology.
- Marker genes such as GFP, RFP, LacZ, dsRED, luciferase
- Vision related genes such as Rho, Prph2, Aipl1, Mertk, RPGR, RPGRIP, RPE65
- Neuroprotective genes such as CNTF, GDNF
- Immunomodulatory genes such as IL 1, IL 10, IL 1Ra, IL4, TGF13
- Angiostatic genes such as sFlt, Endostatin, Angiostatin
- Transcription factors (ocular development) such as Crx, Pax6, NeuroD, Nrl, N2E3
- Cell cycle genes: E2F2, YAP1
- T cell receptor genes specific for cancer peptide/HLA antigens including but not limited to peptides of NY-ESO, gp100 and MAGE A3
- TCR genes specific for viral epitopes, including but not limited to human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein Barr virus (EBV) and human papillomavirus (HPV)
- TCR genes specific for putative auto-antigens or self-antigens thought to play a role in auto-immune disease processes or in graft rejection. The auto-immune diseases include but are not linked to diabetes mellitus, type 1, rheumatoid arthritis, autoimmune hepatitis and multiple sclerosis.
- HLA genes for the production of antigen presentation cells (APC)
- AA V viral genes: Rep, Cap
- Lentiviral production genes such as GAG, POL, vesicular stomatitis virus envelope
- Vectors:
  - Cloning vectors such as pGEM-T Easy
  - Expression vectors such as pcDNA3.1, pCMV
  - Viral vector backbones, such as pD10 (AA V), pHRSIN (HIV)
Viral vector helper plasmids such as pHAV7.3 (AAV), p8.91 and pMDG (HIV1) Virapower (Invitrogen Ltd), Lenti-X (Clontech Laboratories Inc).

Recipient (Host) organisms:
Escherichia coli: DH5α, JM109
Mammalian Cells: 293T, ARPE19, HeLa, BHK, human and mouse embryonic stem cells.

Evaluation of foreseeable effects

The viral particles produced in these systems are replication-incompetent, only carrying the genes of interest. Recipient cells fall into the category of 'Especially disabled hosts' as defined by the HSE. Commercially available cloning and expression vectors have a long history of safe use, with no evidence of harm to health or environment. Viral helper plasmids for AAV and lentivirus produce the proteins required for the production of viral vectors. These plasmids have been designed to have minimal homology with wild type and recombinant viral genomes to avoid mobilization. There is no evidence of harm to health or environment. Genes of interest and other components of the viral packaging mix will not be constructed on-site but will be supplied to Pall on plasmids provided by the customer requesting the process development work. Gene sequences will vary and be specific to the customer and the application of the product. AAV: cells with plasmid vector containing the cell cycle gene E2F2 will be the most hazardous GMM produced, as persistent expression of the gene is potentially tumourigenic.

LV: no plasmid contains long terminal repeats (LTRs) or the packaging sequence. This enables the viral genome to be generated in the producer cell line but results in self-inactivation of the lentivirus after transduction into the target cell and so the lentiviral particles produced are replication-incompetent.

The likelihood of exposure or release to the environment is considered low due to the containment procedures that will be employed. Infected mammalian cells will be unable to establish themselves in nature. It is very unlikely that genetic inserts would be transferred in the environment if a breach were to occur. If such a transfer did occur, persistence in the recipient is very unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

A derogation is applied for in respect of containment measure 5 in table 2 of Schedule 8; inactivation of bulk culture fluids before removal from the closed system required by validated means. For the large scale (up to 500 L) element of the contained use activity, bulk culture fluid must be removed from the bioreactor in order to harvest the vector virus products. The justification for the derogation is that recombinant viral vectors will be harvested from the bulk culture fluids using closed systems. Spent growth medium will be transferred aseptically to an intermediate bulk container, which will be sealed and taken for incineration by a licenced waste contractor. Sections D and E of the risk assessments submitted in support of this notification describe these measures.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Small volumes of liquid waste and any solid waste generated in the contained use activities will be autoclaved within the laboratory suite for a minimum of 20 minutes at 134°C in a validated discard cycle.
Small volumes of liquid waste may also be chemically inactivated using 2% (v/v) sodium hypochlorite solution or 1% (w/v) Virkon solution.
An approximate 100% kill will be achieved using either chemical or physical waste treatment methods. The autoclave is subject to quarterly servicing and annual 12-point thermocouple testing.
Large volumes of liquid waste will be transferred aseptically to intermediate bulk containers, sealed and sent for incineration by a licenced waste contractor.

Pall's GMSC has reviewed the risk assessments relating to this notification and agreed a classification of class 2 for the contained use activities.

Please enter comments on the GM safety committee on the risk assessment

Pall's GMSC has reviewed the risk assessments relating to this notification and agreed a classification of class 2 for the contained use activities.

Project Containment

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Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick if yes, tick to confirm that it is attached to this form

Tick if confirmed that it is attached to this form

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Please enter comments on the GM safety committee on the risk assessment

Pall's GMSC has reviewed the risk assessments relating to this notification and agreed a classification of class 2 for the contained use activities.
**GM Centre Number: 785**

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**Date at Which Additional Info Submitted**

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02/03/2022  

Page 11270 of 15326
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

As based within UCL, we will use the established GMSC of the University submitting internal notifications to them for approval.

<table>
<thead>
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Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity:

- **Bacteria** - E.Coli, trigene disinfectant, soaked for at least 30 minutes.
- **Viruses** - HSV, adenovirus, adeno-associated virus - Virkon disinfectant, soaked for at least 10 minutes.

After inactivating GMMs, all disposed as clinical waste and burnt off-site.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

The GMSC opinion is that the viruses to be used are probably Class 2, although as risks are low, Class 1 could be considered appropriate.

---

**Project Ref** 14/01.1

**Date Ackn'd** 12/06/2002

**CU2 Project Title** IMMUNOTHERAPEUTIC APPLICATIONS OF HERPES VIRUS VECTORS (OR PREVIOUS TITLE DENDRITIC CELL MEDIATED IMMUNOTHERAPY OF CANCER)

**Class** Class 2

**CultureVol** < 1 litre

**History of Additional Info**

**Historical Date of Additional Info**

**Historical Significant Changes** PROJECT TITLE AMENDED 9/1/07 (FROM CONSTRUCTION AND PRE-
**Project Additional Information**

**Purposes of the contained use**

Construction and pre-clinical testing of modified forms of herpes simplex virus for vaccination purposes.

**Recipient or parental organism**

Herpes simplex virus strains with attenuating mutations but in which replication competence is retained. Attenuating mutations remove HSV genes, which usually inhibit immune responses and also render the virus non-pathogenic. These genes are VHS and/or ICP47 and may additionally include further mutations in ICP34.5, UL43, U35 and/or vmw65. The modified HSV strains also have genes encoding non-HSV antigens inserted (from infectious disease causing agents or which are selectively expressed in tumour cells) and may additionally have inserted genes encoding immune modulatory molecules such as cytokines and chemokines.

The work aims to include immune responses to the delivered antigens and to untoward effects are anticipated. The mutations to the virus have previously been shown to render the virus non-pathogenic and the genes to be delivered are not anticipated to be harmful according to previous work published in the literature.

**Host/vector system**

Herpes simplex virus 1 or 2

**Origin & function**

Inserted genetic material is of either human or rodent origin and has been cloned by PCR or obtained from collaborators in plasmid form. Genes to be inserted include tumour antigens such as MART-1, MAGE-1, tyrosinase, gp100, Her2neu, Muc-1, PS1, CEA for the development of anit-tumour vaccines. Antigens from a variety of infectious agents for vaccine development purposes will also be used as will human or rodent forms of various immunomodulatory molecules eg GM-CSF, IL12, CD40L, B7.1, RANTES. The immunomodulatory genes are aimed at the enhancement of the immune response to delivered antigens.

**Evaluation of foreseeable effects**

None of the genes to be inserted are anticipated to result in harmful effects.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste generated during the activity that has been in contact with the GMM will be treated with a Virkon solution. This broad spectrum disinfectant is effective against herpes simplex virus at a 0.5% (w/v) concentration giving terminal disinfection after 10 minutes of contact.

After treatment with the above disinfectant, the waste will then be placed into clinical waste bags and burnt offsite.
The GMSC opinion is that the viruses to be used are probably Class 2, although as the risks are low, Class 1 could be considered appropriate.

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Project Ref 785.08.2

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Non-GMM Consent Granted

Historical Significant Changes

Tick if notifying a connected programme of work

Project notified under transitional arrangements
Project Additional Information

Purposes of the contained use

The primary objective of the clinical study is to assess how well the vaccine is tolerated following subcutaneous administration of three doses six weeks apart. It is planned that the vaccine will be given at three dose levels, with a safety review being carried out between each cohort. The secondary objectives of the study are: 1) to assess biodistribution of the virus in blood and urine; 2) to assess the induction of immune responses following administration of the vaccine; and 3) to determine a dose schedule for evaluation in later phase II efficacy trials.

Recipient or parental organism

HSV-2 strain HG52 (GenBank database accession number NC_001798)

Host/vector system

Strain HG52 disabled by the deletion of the vhs, 1CP47, 1CP34.5, UL43 and US5 genes (HG52 vhs AICP47 AUL43 A34.5 AUS5, ImmunoVEX HSV2). The deletions were introduced into the viral genome by homologous recombination using shuttle plasmids containing the gene of interest, which had been appropriately modified to achieve the deletion required.

Origin & function

There are no exogenous non-HSV coding sequences inserted into ImmunoVEXHSV2.

Evaluation of foreseeable effects

HSV-2 is the major cause of genital herpes, which even in its wild type form rarely causes severe systemic problems unless the virus somehow manages to get to the brain or the eye where it causes encephalitis and retinopathy respectively. Usual symptoms are genital lesions, which are routinely treated with acyclovir, famciclovir and valacyclovir. It is only transmissible through direct contact.

ImmunoVEXHSV2 has been disabled by the inclusion of mutations known to prevent pathogenicity. The vaccine contains a combination of deletions in the vhs, 1CP47 and 1CP34.5 genes, each of which are known to reduce the pathogenicity of HSV in animal models (Goldsmith et al., J.Exp.Med 187:341; Strelow and Leib, J. Virol. 69:6779; Rampling et al., Gene Therapy 7:859). ImmunoVEXHSV2 has been shown in several preclinical studies to be non-pathogenic in guinea pigs, mice and rats using doses of up to 1.00E6 pfu (the top planned clinical dose) by the subcutaneous, intranasal and intracranial routes. In addition, ImmunoVEXHSV2 has been shown to be unable to establish detectable levels of latency in mice (as measured by qPCR) following administration by the subcutaneous and intranasal routes.

The deleted gene sequences cannot be repaired during vaccine production, as the deleted genes sequences have not been inserted into the genome of the cell line used for virus growth, and thus there is no potential for recombination of the vaccine virus genome with the DNA of the production cell line. There is a small possibility of reversion of the deleted sequences to a wild-type sequence if the vaccine virus was undergoing replication in the same cell as a Wild-type virus. Under these circumstances it would be possible for recombination between the vaccine and the wild type virus DNA to occur. However, bearing in mind the intended subcutaneous administration route (in the upper arm), this is very unlikely to occur in practice. In the unlikely event that this were to occur, recombination with a wild type virus would result only in a mixture of progeny virions that either contain one or more of the deletions in the vaccine virus (i.e. a disabled virus), or wild type virus. As there are no exogenous genes inserted into the vaccine, a wild type virus containing an exogenous gene sequence could not be generated following recombination with the vaccine virus DNA. Non-homologous recombination (i.e. between different regions of two viral genomes) has not been shown to occur at detectable levels.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Chelsea and Westminster Trust Pharmacy: All plastic ware (syringes and female-to-female Luer lock adaptors) will be disassembled and soaked in a 1% Virkon solution for a minimum of 10 minutes. The plastics will then be placed in a clinical waste bin for incineration. The liquid waste generated during dose preparation will be treated with a 1% solution of Virkon before disposal in the dirty utility room.

St Stephen’s Centre: After use, all sharps will be immediately placed in a specific, clearly labeled sharps bin. The disposable drape used to cover the injection site will also be placed in the sharps bin for disposal. All other clinical waste will be placed in clinical waste bag. These will then be transferred to the St Stephen’s Centre 5th Floor laboratories for autoclaving before being transferred for incineration. All dirty bed linen will be treated in accordance with infected linen procedures and will be washed using a high temperature cycle. If a vaccine or specimen spill occurs, the bed linen will be autoclaved in St Stephen’s Centre 5th Floor laboratories before being sent for washing.

Autoclave: The waste will be autoclaved before disposal as described in HIV/GUM Sop-al 3, ‘Disposal of Clinical Waste’. Any retained samples will be disposed of as described in HIV/GUM SOP-024, ‘Disposal of Retained Samples’. The autoclave is operated as described in NV/GUM SOP-034, ‘Use of the 1SOL Boxer Autoclave’. The autoclave will be pre-booked before it is required to ensure that it is available and that the waste can be autoclaved as soon as possible after each subject visit. The waste will be stored in a defined area (please add) and will be transported to the autoclave area when the autoclave becomes available. The sharps bins used for the study will be clearly labelled to indicate they contain GM waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The draft risk assessment was considered by two independent clinical GMO safety advisors who passed their comments onto the BioVex GMSC. These comments mainly requested clarification on the procedures described in the draft RA and no new procedures or procedural changes were recommended. These comments have been used to modify the RA so that all procedures are clearly explained.

The notification was then considered by the GMSC and the panel agrees that the work should be carried out at containment level 2 with no additional measures required.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
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<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>
## Project Additional Information

### Purposes of the contained use

Prevention of class 2 GMOs being a hazard to workers.

### Recipient or parental organism

HSV1 vectors deleted for the neurovirulence factor ICP34.5 will be engineered to express human and mouse forms of GM-CSF and other immune modulators such as RANTES, B7.1 and CD40L. These viruses are designed to replicate in tumours but not surrounding tissue (considerable literature demonstrates such a phenotype for HSV1 and 2 ICP34.5 deleted viruses) with the additional delivery of immuno-stimulatory factors. Some viruses will be additionally modified such that HSV genes that usually minimise immune responses to HSV infection are also deleted. Such genes are UL43, vhs and ICP47. Each of the deletions in ICP34.5, vhs or ICP47 has been shown individually to generate a non-pathogenic, though still replication competent virus. Deletion of UL43 alone probably does not significantly reduce pathogenicity, but this deletion will only be included in viruses already deleted for the other gene(s) above.

### Host/vector system

Herpes simples virus 1 or 2.

### Origin & function

Inserted genetic material is of either human or rodent origin and has been cloned by pcr or obtained from collaborators in plasmid form. Genes to be inserted include GM-CSF, RANTES, B7.1, and CD40L.

### Evaluation of foreseeable effects

None of the genes to be inserted are anticipated to result in harmful effects.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated during the activity that has been in contact with the GMM will be treated with a Virkon solution. This broad-spectrum disinfectant is effective against herpes simplex virus at a 0.5% (w/v) concentration giving terminal disinfection after 10 minutes of contact.

After treatment with the above disinfectant, the waste will be placed into clinical waste bags and burnt offsite.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The GMSC opinion is that the viruses to be used are probably Class 2, although as risks are low, Class 1 could be considered appropriate.

Project Containment

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<td>Animal Units</td>
<td>L3</td>
<td>L4</td>
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</table>

Project Ref  785/01.2

Date Ackn'd  21/09/2001

CU2 Project Title  ICP34.5 DELETED HERPES SIMPLEX VIRUS 1 EXPRESSING TRUNCATED

Class  2

CultureVolClass2  1-50 litres

CultureVolumeClass3-4

02/03/2022  Page 11278 of 15326
**Project Additional Information**

**Purposes of the contained use**

ICP34.5 deleted herpes simplex virus 1 expressing truncated fusogenic retroviral glycoprotein for cancer treatment.

**Recipient or parental organism**

Herpes simplex viruses deleted for the neurovirulence factor ICP34.5 have been shown in considerable literature to replicate only in dividing cells and not in surrounding tissue. Some viruses will have additional deletions of ICP47, vhs and/or UL43 genes. Deletions in ICP47 and vhs have been shown to further disable HSV.

ICP34.5: Toxicity evaluation of replication competent herpes simplex virus (ICP34.5 null mutant) in patients with malignant melanoma. (2000) Rampling, R et al, Gene Therapy (7), 859-866


**Host/vector system**

HSV1 with deletions as stated above.

**Origin & function**

Genes to be inserted are for retroviral glycoproteins. The genes will come from Moloney leukaemia virus (MLV) and Gibbon ape leukaemia virus (GALV) which are each artificially truncated in the transmembrane protein and Human Endogenous Retrovirus HERV-W which is naturally truncated in the transmembrane protein. When inserted into the ICP34.5- HSV they are expected to give viruses with additional cytotoxic killing of tumour cells due to the truncated retroviral glycoproteins. These are truncated by 16 amino acids in the transmembrane R-peptide of the wild-type protein, which normally serves to restrict fusion of the envelope until it is cleaved during viral infection. This alteration renders the protein constitutively highly fusogenic and therefore cytotoxic to human tumour cells.

The loss or mutation of the R peptide sequence which limits the titre of retroviral production together with the fact that these retroviral glycoproteins have no packaging...
sequences should limit any interaction with other HERVs.


Evaluation of foreseeable effects

HSV1 is a universally prevalent virus that even in its wild type form rarely causes severe problems. Usual symptoms are cold sores. The viruses to be used will be disabled by the inclusion of mutations known to prevent pathogenicity.

HSV is not normally transmitted through the aerosol route. Infection is by contact, which attests to the instability of the virus outside the host. HSV is rapidly inactivated by desiccation, lipid solvents and mild detergents. Humans are the only natural host for HSV. It is unlikely, if working at containment level 2 that the viruses would reach the environment, if it did it could cause no harm to humans or animals that it came into contact with.

Gene sequences will be inserted into the disabling sites so that transfer to non-disabled versions of HSV will not occur. In the unlikely event that transfer were to occur, the resulting virus would then itself be disabled. Genes cannot be repaired, as there is no potential for recombination with DNA in the cells used for growth.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This notification was considered by the GMSC of UCL and the panel of advisors has afforded approval and agree that this work should be carried out at containment level 2.

Project Containment

Laboratory Activities Glass Houses Growth Rooms

02/03/2022 Page 11280 of 15326
### Project Additional Information

**Purposes of the contained use**

ICP34.5 deleted herpes simplex virus 1 expressing enzyme prodrug activator genes for cancer treatment.

**Recipient or parental organism**

Herpes simplex viruses deleted for the neurovirulence factor ICP34.5 have been shown in considerable literature to replicate only in dividing cells and not in surrounding tissue. Some viruses will have additional deletions of ICP47, vhs and/or UL43 genes. Deletions in ICP47 and vhs have been shown to further disable HSV.

ICP34.5: Toxicity evaluation of replication competent herpes simplex virus (ICP34.5 null mutant) in patients with malignant melanoma. (2000) Rampling, R. et al, Gene Therapy (7), 859-866

vhs: Role of the virion host shutoff of HSV1 in latency and pathogenesis (1995) Strelow, L. & Leib, D., J. Virol (69), 6779-6786

ICP47: (1) Construction and properties of a viable herpes simplex virus 1 recombinant lacking coding sequences of the alpha 47 gene. (1991) Mavromara et al., J. Virol

Host/vector system

HSV 1 with deletions as stated above.

Origin & function

Genes to be inserted are for enzyme prodrug activators. A number of different enzyme/prodrug systems has been designed, these include:


These enzyme/prodrug systems may be used in combination with truncated fusogenic retroviral glycoprotein (GM785/01.2)

Evaluation of foreseeable effects

HSV1 is a universally prevalent virus that even in its wild type form rarely causes severe problems. Usual symptoms are cold sores. The viruses to be used will be disabled by the inclusion of mutations known to prevent pathogenicity.

HSV is not normally transmitted through the aerosol route. Infection is by contact, which attests to the instability of the virus outside the host. HSV is rapidly inactivated by desiccation, lipid solvents and mild detergents. Humans are the only natural host for HSV. It is unlikely, if working at containment level 2 that the viruses would reach the environment, if it did it could cause no harm to humans or animals that it came into contact with.

Gene sequences will be inserted into the disabling sites so that transfer to non-disabled versions of HSV will not occur. In the unlikely event that transfer were to occur, the resulting virus would then itself be disabled. Genes cannot be repaired, as there is no potential for recombination with DNA in the cells used for growth.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
This notification was considered by the GMSC of UCL and the panel of advisors has afforded approval and agree that this work should be carried out at containment level 2.

### Project Containment

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<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

*Animal Units*  
L2 Yes L3 L4 L2 L2 L3 L4 L2 L3 L4

### Project Ref 785/02.2

**Date Ackn'd**  27/09/2002  
**CU2 Project Title**  GENERATION OF RECOMBINANT VACCINIA VIRUSES TO MONITOR AND RESTIMULATE CYTOTOXIC T CELL RESPONSES  
**Date Project Ceased**  25/10/2013  
**Class**  Class 2  
**Culture Vol Class**  1-50 litres  
**Non-GMM Consent Granted**  not applicable  
**Project notified under transitional arrangements**  N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

02/03/2022
<table>
<thead>
<tr>
<th>Purposes of the contained use</th>
</tr>
</thead>
<tbody>
<tr>
<td>It is planned to generate recombinant vaccinia viruses which express tumour antigen genes from melanoma (gp100, MART-1 tyrosinase), ovarian/breast cancer (MUC1, HER2, CEA, MAGE), or prostate cancer (PMSA). Controls will also be made of recombinant vaccinia which express proteins from other viruses including influenza (Haemagglutinin, nucleoprotein, matrix protein), HBV (surface antigen and core antigen).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Recipient or parental organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinia virus vector deleted from thymidine kinase.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinia virus as described above.</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Hosts are</th>
</tr>
</thead>
<tbody>
<tr>
<td>human PBMCs and B cell lines, mouse cell lines (EL4, NIH3T3, Ltk-), mouse splenocytes, mouse and human dendritic cells.</td>
</tr>
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</table>

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<th>Origin &amp; function</th>
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<td>It is planned to generate recombinant vaccinia viruses which express tumour antigen genes from human melanoma (gp100, MART-1, tyrosinase), human ovarian/breast cancer (MUC1, HER2, CEA, MAGE), or human prostate cancer (PMSA). Controls will also be made of recombinant vaccinia which express proteins from viruses including influenza (Haemagglutinin, nucleoprotein, matrix protein), HBV (surface antigen and core antigen).</td>
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<tr>
<th>Evaluation of foreseeable effects</th>
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<tbody>
<tr>
<td>Vaccinia virus is an orthopoxvirus that has been extensively used in the past for vaccination leading to immunity to smallpox. Inoculation of non-immune individuals leads to a papule that resolves in 14-21 days. There are only very occasional complications in young children. The vector will be replication competent and accidental infection of non-immune workers with vector also encoding an exogenous protein may induce an immune response to such an encoded protein. There is, however, no evidence that the products of the genes to be inserted could be harmful to human health.</td>
</tr>
</tbody>
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<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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<td>All waste generated during the activity that has been in contact or potential contact with the GMM will be treated with a Virkon solution. This broad-spectrum disinfectant is effective against orthopoxvirus at a 1% (w/v) concentration, giving terminal disinfection after 10 minutes of contact. After treatment with the above disinfectant, the waste will then be placed into clinical waste bags and burnt offsite.</td>
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</table>

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y
The notification was considered by the GMSC and the panel agrees that the work should be carried out at containment level 2.

**Project Containment**

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<td>L4</td>
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</table>

- Animal Units
  - L2
  - L3
  - L4
  - L2

- Large Scale Activities
  - L2
  - L3
  - L4
  - L2

- Human Clinical Applications
  - L2
  - Yes
  - L3
  - L4

**Project Ref** 785/03.1

- **Date Ackn'd**: 13/10/2003
- **CU2 Project Title**: GENERATION OF HSV-1 BASED HIV IN VIVO VACCINES.
- **Class**: Class 2
- **CultureVolClass2**: 1-50 litres
- **Non-GMM**: not applicable

**Project Additional Information**

Purposes of the contained use:

It is planned to generate vectors for the delivery of HIV antigens to antigen presenting cells in vivo using multiple disabled HSV1 vectors deleted for genes involved in the avoidance of host immune responses (ICP47, vhs, UL43) and/or with deletions in genes preventing replication (ICP4, ICP22, vmw65, ICP34.5).
### Recipient or parental organism

HSV1 disabled by deletion of combination of the UL43, ICP4, ICP22, ICP47, vmw65, ICP34.5 and vhs genes.

### Host/vector system

HSV1 as described above.

Hosts are:
- BHK Cells
- Vero Cells

### Origin & function

It is planned to generate HSV1 vectors which express Human Immunodeficiency Virus (HIV) gp120 (env) and a gag-pol or gag-pol-nef fusion protein. Vectors may additionally contain sequences encoding human GM-CSF.

HIV sequences will be inserted into the vhs region of HSV1 either under the control of the hCMV immediate-early promoter or the rous sarcoma virus (RSV) promoter.

Where vectors contain human GM-CSF this will be under the control of the Moloney murine sarcoma virus (MMSV) promoter.

### Evaluation of foreseeable effects

The vectors will contain a combination of disabling deletions of the UL43, ICP4, ICP22, ICP47, vmw65, ICP34.5 and vhs genes. There is a small possibility of reversion of these deleted sequences to wild-type sequence if recombination of these vectors with wild-type virus were to occur. However, the vectors will contain multiple disabling deletions. Furthermore, the transgenes encoding HIV gp120 (env) and the gag-pol-nef fusion will be inserted into the site of the deleted vhs gene. Recombination of the viral vector with wild-type HSV would therefore result in deletion of vhs from the progeny virus. Deletion of vhs has previously been shown to generate a non-pathogenic virus.

Genes to be inserted are aimed at generating immune responses. There is no evidence that the products of these genes could be harmful to human health.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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After treatment with the above disinfectant, the waste will then be placed into clinical waste bags and burnt offsite.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
The notification was considered by the GMSC and the panel agrees that the work should be carried out at containment level 2 with no additional measures required.

### Project Containment

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<td></td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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**Animal Units**
- L2
- L3
- L4

**Large Scale Activities**
- L2
- L3
- L4

**Human Clinical Applications**
- L2
- L3
- L4

### Project Ref 785/04.1

**Date Ackn’d**
- 15/03/2017

**CU2 Project Title**
- THE GENERATION OF HSV2/HPV IN VIVO VACCINES

**Class**
- Class 2

**CultureVol**
- Class 2

**CultureVolume**
- 1-50 litres

**Non-GMM**
- not applicable

**Consent Granted**

**Project notified under transitional arrangements**

### Project Additional Information

**Purposes of the contained use**

Multiply disabled HSV2 vectors deleted for genes involved in the avoidance of hot immune responses (ICP47, vhs, UL43) and/or with deletions in genes preventing replication (ICP4, ICP22, wmw65, ICP34.5) will be used to generate vectors for the delivery of HPV antigens to antigen presenting cells (APC) in vivo.
HSV2 (e.g. strain HG52) disabled by deletion of combinations of the UL43, ICP4, ICP22, ICP47, vwm65, ICP34.5 and vhs genes. Trangenes will only be inserted into viruses that contain at least two disabling mutations.

Host/vector system

HSV2 as described above.

Hosts are:
BHK cells
Vero cells

Origin & function

Sequences encoding varius Human Papillomavirus type 16 (HPV16) genes including those for L1, L2, E2 and E7 will be inserted into either the vhs or ICP34.5 region of HSV2 under the control of the human cytomegalovirus (cmv) immediate-early promoter, the rous sarcoma virus (RSV promoter, anS SV40-HTL (human T-cell lymphotrophic viurs) fusion promoter or the Moloney murine sarcoma virus (MMSV) LTR promoter.

Vectors may additionally contain human GM-CSF under the control of the SV40-HTLV fusion promoter, or the moloney murine sarcoma viruses (MMSV) LTR promoter.

Evaluation of foreseeable effects

HSV2 is the primary cause of genital herpes. It is a prevalent virus, which evin in its wild type form rarely causes severe problems unless the virus somehow manages to get to the brain or the eye causing encephalitis and retinopathy respectively.

The HSV2 vectors will contain a combination of disabling deletions of the UL43, ICP4, ICP22, ICP47, vwm65, ICP34.5 and vhs genes. There is a small possibility of reversion of these deleted sequences to wild-type sequence if recombination of these vectors with wild-type virus were to occur. However, the vectors will contain multiple disabling deletions. Furthermore, the transgenes encoding HPV16 E2, E6 and E7 will be inserted into the site of the deleted vhs or ICP34.5 genes. Recombination of the viral vector with wild-type HSV2 would therefore result in deletion of vhs or ICP34.5 from the progeny virus. Deletion of vhs or ICP34.5 has previously been shown to generate a non-pathogenic virus.

The genes to be inserted are aimed at generating immune responses. The wild type HPV16 E6 and E7 sequences are oncogenic and implicated in cervical tumorigenesis. The sequences for these genes that will be inserted into the HSV2 vector have been modified to ensure that a) they are non-oncogenic and b) there is no possibility of these disabling mutations being repaired following recombination with wild type HPV16 sequences.

The HPV 16 E protein interacts with p53 leading to its degradation. The E6 p53 binding site has been identified and previously modified to eliminate this interaction (Slebos et al. 1995 Virology 208:111-120). This mutation has been incorporated into the E6 sequence to be inserted. The sequence has also been rearranged to prevent repair of this mutation by recombinatin with wild-type HPV16 sequence. In the re-arranged HPV16 E6, the coding sequences for amino acids 73-151 was moved to the 5' terminus followed by coding sequences for amino acids 2-69.

The HPV 16 protein interacts with pRb leading to deregulation of cell cycle control. The E7 pRb binding site has been identified and previously modified to eliminate this interaction (Smahel et al. 1995 Virology 281: 231-238). This mutation has been incorporated into the E7 sequence to be inserted. The sequence has also been rearranged to prevent repair of this mutation by recombinatin with wild type HPV15 sequence. In the rearranged HPV16 E7, the coding sequences for amino acids 27-98 are located at the 5' terminus followed by the coding sequences for amino acids 2-23.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
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If yes, tick to confirm that it is attached to this form  

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Please enter comments on the GM safety committee on the risk assessment

The notification was considered by the GMSC and the panel agrees that the work should be carried out at containment level 2.

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Project Ref 785/05.1

Date Ackn’ed 12/10/2005  
CU2 Project Title Expression of antigens from recombinant lentivirus vectors.

Date Project Ceased  

Date  

Class CultureVolClass2 CultureVolumeClass3-4  
Class 2 < 1 L  
Non-GMM Consent Granted

02/03/2022 Page 11289 of 15326
The project aims to use lentiviral vectors to direct expression of exogenous genes in different cell types. Specifically, expression in EBV transformed B cells, PBMC, dendritic cells and transformed eukaryotic cell lines will be performed. The antigens expressed will include GFP and tumour antigens.

The properties of the lentiviral vectors to be used are reviewed in Buchschacher and Wong-Staal, Blood 95:2499. The specific vector to be used is pHV described in Ikeda et al Nature Biotech 21:569 (2003). The vector is devoid of all accessory and potentially pathogenic genes derived from the parental virus, including vif, vpr, vpu and nef. The only remaining parental viral sequences are the long terminal repeats, the packaging signal and an internal flap incapable of multiplying and spreading to other cells unless certain complementing functions are provided in trans. The assembly events that occur in the packaging cell line results in the vector being contained in a virus containing the VSV-G protein within its envelope. The remaining lentiviral cis-acting sequences in the vector permit only a single round of vector replication in normal cells. The packaged vector can enter certain cells, either dividing or non-dividing, where it can be converted to DNA which can integrate into the host genome.

The pseudotyping of the lentiviral vector with VSV-G glycoprotein results in tropism determined by this molecule. Cellular uptake of vectors containing VSV-G is considered generally to occur by endocytosis, so that they have a very broad tropism. Such vectors have been used to transduce numerous cell types from both man and mouse. In addition, such vectors can also infect non-mammalian cells including those from insects and fish.

The vector will be used experimentally in EBV transformed B cells, PBMC, dendritic cells and transformed eukaryotic cell lines.

The gene encoding Green fluorescent protein is considered non-hazardous. The genes encoding human tumour antigens including gp100, MART-1, tyrosinase, CEA or MUC-1 are also considered non-hazardous. The gene encoding a modified form of Her2, in which the kinase domain has been removed, is also considered non-hazardous.

It is unlikely that the recombinant lentiviral vectors would be harmful to humans, animals, or plants. A potential hazard is due to integration leading to a mutagenic event. This is of low probability as the level of replicating genome within a cell will be very low as the vector is replication-incompetent. It is also relevant that there are no reported
instances of human carrying high levels of wild-type lentiviral loads showing evidence of insertional mutagensis (Buchschacher and Wong-Staal, 2000). The encoded genes have no oncogenic potential. The attenuated lentivirus vectors are expected to have very limited survival potential in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated during the activity that has been in contact or potential contact with the GMM will be treated with a Virkon solution. This broad-spectrum disinfectant is effective against lentivirus at a 1% (w/v) concentration, giving terminal disinfection after 20 minutes of contact.

After treatment with the above disinfectant, the waste will then be placed into double clinical waste bags and burnt offsite.

The notification was considered by the GMSC and the panel agrees that the work should be carried out of containment level 2.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 785/08.1

Date Ackn'd CU2 Project Title

02/03/2022
It is planned to generate vectors for the delivery of simian immunodeficiency virus (SIV) antigens to antigen presenting cells in vivo using multiply disabled herpes simplex viruses type 1 (HSV-1) vectors. The vectors will be deleted for genes involved in the avoidance of host immune responses (1CP47, vhs, UL43, ICP34.5) and/or with deletions in genes preventing replication (1CP22, 1CP34.5).

**Recipient or parental organism**

HSV-1 disabled by the deletion of a combination of the UL43, 1CP22, 1CP47, 1CP34.5 and vhs genes.

**Host/vector system**

HSV-1 as described above.

Host are: BHK cells, and cell lines expressing HSV genes that have been generated using BHK cells

Vero cells, and cell lines expressing HSV genes that have been generated using vero cells.

**Origin & function**

It is planned to generate HSV-1 vectors which express Simian Immunodeficiency Virus (SIV) mac 239 gag, gly6O (env) and a rev/tat/nef fusion protein. It is planned that the gly6O will be a membrane bound version of the protein. However, if there are problems with the level of expression of this form of the protein, the sequence encoding a secreted form may be inserted instead.

SIV sequences will be inserted into the vhs region of HSV-1 either under the control of the hCMV immediate-early promoter or the rous sarcoma virus (RSV) promoter.

**Evaluation of foreseeable effects**

The vectors will contain a combination of disabling deletions of the UL43, 1CP22, 1CP47, ICP34.5 and vhs genes. There is a small possibility of reversion of these deleted sequences to wild-type sequence if recombination of these vectors with wild-type virus were to occur. However, the vectors will contain multiple disabling deletions, Furthermore, the transgenes encoding HIV gly2O (env) and the gag-pol-nef fusion will be inserted either into the site of the deleted vhs or ICP34.5 genes. Recombination of the viral vector with wild-type HSV would therefore result in deletion of vhs from the progeny virus.
Each of the deletions in the vectors other than UL43 (ICP34.5, ICP22, vhs or ICP47) has previously been shown individually to generate viruses with significantly reduced pathogenicity (see references below. Deletion of UL43 alone probably will not significantly reduce pathogenicity, but this deletion will only be included in viruses already deleted for the other gene(s) above.

ICP47: Goldsmith K et al. (1998), J.Exp.Med., 187, 341

The SIV antigen coding sequences are not expected to alter the virulence or tropism of the vectors. All of the homologous HIV genes, as well as most SIV genes, have been added to a variety of viral vectors in the past (i.e. pox viruses, adenoviruses, vesicular stomatitis virus, herpes viruses), without producing unexpected phenotypic properties. The nef gene sequence to be used is nonfunctional as it is made as a fusion protein and the N terminus myristoylation site has been mutated. Myristoylation of the nef protein has been shown to be essential for its cellular localization in HIV (Kaminchik et al. (1991), J Virol. 65:583); nef-mediated down regulation of MHC-1 and CD4 (Harris and Neil (1994), J Mol Biol. 241:136), and nef-mediated impaired thymopoiesis by interaction with PACS-1, SH3 and PAK2 (Stove et al. (2003), Blood. 102:2925).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Most of the waste (small plastic and liquid) generated during the activity that has been in contact or potential contact with the GMM will be treated with a 1% Virkon solution (a broad-spectrum disinfectant) for a minimum of 10 minutes. After treatment with the above disinfectant, the liquid waste will be flushed down the drain. All small plastic waste will then be placed into clinical waste bags and incinerated offsite using a registered company. Large plastic objects (such as roller bottles) will be placed in autoclave bags and autoclaved at 121°C using a standard waste sterilisation programme. The autoclaved plastics will then be placed into clinical waste bags and incinerated offsite using a registered company.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The notification was considered by the GMSC and the panel agrees that the work should be carried out at containment level 2 with no additional measures required.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
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<tbody>
<tr>
<td>L2 Yes</td>
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<td>Large Scale Activities</td>
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**GM Centre Number: 786**

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**Name**

**CENTRE FOR ECOLOGY & HYDROLOGY (CEH DORSET)**

**Department**

WINFRITH TECHNOLOGY CENTRE

**Campus Estate or Research Centre**

**Building**

**Road Name**

**District**

NEWBURGH

**Town**

DORCHESTER

**County**

DORSET

**Postcode**

DT2 8DZ

**Country**

ENGLAND

**Tel Number**

01305 213500

**Fax Number**

01305 213600

**E-mail**

**HSE Division**

WALES AND SOUTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: [Y]  

Give brief details of the genetic modification safety committee:

Director of CEH - Chair, Group Leader - Senior Science Officer, Site Safety Rep, CEH Oxford's Biosafety Officer - advisory role, Biosafety Officer, IPMS Union Safety Rep. 2 post doc lab users, 2 possible contacts, 3 UKAEA reps - own building.

<table>
<thead>
<tr>
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Other (please specify) Tick if confidential: [ ]

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Disposable items (plastic tips, agar plates etc) will be collected in bio-hazard bags. Glassware and other non-disposable items will be collected in a specific area beside the autoclave. All waste will be autoclaved at the end of each working day (because the laboratory and autoclave are quite small). The autoclave is an Astell Scientific Swiftlock autoclave, 5000 series, 35 litre capacity, front loading. The programme used will be 15 mins at 121 degrees C with 1-5 minutes free steam depending on load.

Each disposal run of the autoclave will be recorded and a test strip (which indicates that the correct sterilisation temperature has been obtained) will be placed in the centre of the load. The dated test strips will be kept to form a permanent disposal record. At the annual service of the autoclave, the engineer will test and record the temperature in the centre of the full autoclave.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC agreed that containment level 1 would be appropriate for the proposed work and the following points were raised in discussion:

1. Limiting scale of bacterial cultures to a maximum of 2 litres. This is for the practical reason that our incubator and autoclave are quite small, not out of consideration for the GMM.

2. Confirmation that laboratory coats and gloves would be removed before leaving the laboratory suite. This is our standard laboratory practice and will be continued.

3. Gloves will be worn when handling GMMs to avoid contamination of the products and to protect personnel from ampicillin, not the GMM.

4. In case of injury and accidental introduction of the GMM to a wound, the GMM under consideration should not pose a significant hazard and normal first aid and disinfection are appropriate.

5. Visitors, including students, may enter the laboratory whilst work is in progress but should not startle the staff.

6. Fire. Given the (non-hazardous) nature of the GMM, it is not considered necessary to inform local emergency services or prepare a specific emergency plan.

7. Advice on monitoring and testing of the autoclave; this will be undertaken by an engineer (prior to work commencing - annually thereafter). All disposal runs will be recorded.
GM Centre Number: 787

Data Premises Notified: 27/06/2001
(Originally)

Transferred from 1992 Regs?: N

Transitional Premises

Class: N

Data Premises Closed: N

Emergency Plan Required?: N

Non-GMMs: N

Withdrawn: N

Name

CARDIFF METROPOLITAN UNIVERSITY

Name 2

Department

SCHOOL OF APPLIED SCIENCE

Campus Estate or Research Centre

Building

Road Name

WESTERN AVENUE

District

LLANDAFF

Town

CARDIFF

County

CARDIFF

Postcode

CF5 2YB

Country

WALES

Tel Number

02920 416830

Fax Number

02920 416982

E-mail

Comments

Change of name from University of Wales Institute 08/12/2011

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Four senior lecturers with relevant experience, (one of whom has served on a GM committee in another university), the Chief Technician, (representing the School Health and Safety Committee), a University Safety Adviser.

<table>
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Although assessed as a Class 1 activity, as all our microbiology laboratories are containment level 2, the work will be carried out in a containment level 2 laboratory in accordance with the appropriate level 2 procedures and controls. All experimental/contaminated material is either autoclaved in designated waste autoclaves or collected in sealed containers by a disposal company for incineration. Waste autoclaves are run at 136 degrees C for 30 minutes, there is a temperature probe in each load which gives a printout of the sterilisation cycle, also indicator strips in each load. Spore checks are carried out quarterly and servicing annually. Expected kill is 100%.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The committee has met and discussed the risk assessment for B.Sc. and M.Sc. practical classes in gene cloning at UWIC, and agrees with the assessment as a Class 1 activity. We note that although the practical classes are a Class 1 activity they will be carried out in a containment level two laboratory with level two controls.
GM Centre Number: 788

Data Premises Notified (Originally) 04/07/2001

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed 05/02/2002

Transitional Premises Emergency Plan Required? N

Non-GMMs N Withdrawn N

Name

NOTTINGHAM TRENT UNIVERSITY

Name 2

Department

DEPARTMENT OF LIFE SCIENCES

Campus Estate or Research Centre

Building

ERASMUS DARWIN BUILDING

Road Name

CLIFTON LANE

District

Town

NOTTINGHAM

County

NOTTINGHAMSHIRE

Postcode

NG11 8NS

Country

ENGLAND

Tel Number 0115 941 8418 Fax Number 0115 948 6636

E-mail

HSE Division MIDLANDS

Comments

WORK TRANSFERED TO GM187 AS THEY REGISTERED TWICE BY MISTAKE.

Date at Which Additional Info Submitted

21/01/2002
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Chairman, Senior Research Fellow - Life Sciences, Reader in Life Sciences, Chief Technician in Microbiology - Life Sciences, Safety Officer - Life Sciences, Chief Technician - Life Sciences, Senior Lecturer - Life Sciences, Safety Officer - Microbiology, Occupational Health - City Health Centre, Dean of Research - Nottingham Trent University, Student Health Care - Nottingham Trent University, Principal lecturer - Nottingham Trent University, Head of Department - Life Sciences, Post Doctoral Representative - Life Sciences, UNISON Representative, Safety Officer - Nottingham Trent University & Post Graduate Student Representative - Life Sciences.

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Other (please specify)

Tick if confidential

Bacteriology: Yes

Parasitology

Transgenic Birds

Microbiology Research: Yes

Page 11302 of 15326
### Project Ref 788/01.1

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- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**: 

**Significant Change ID**

**Date of Significant Change**

---

For activities involving GMMs, describe the waste management measures which will apply to the activity

The materials are first autoclaved in the department and then a registered carrier collects them for incineration.

Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: 

Please enter comments of the GM safety committee on the risk assessment:

The safety committee has approved a format for the assessment, but we are in the process of producing a better revised version.
### Project Additional Information

#### Purposes of the contained use

To elucidate the proposed function of tissue transglutaminase (tTG) in the binding to and stabilisation of the extra cellular matrix.

#### Recipient or parental organism

Mammalian cell lines including tumour cell lines of murine and human origin.

#### Host/vector system

For expression of tTG in mammalian cells vector systems will include mammalian expression vectors such as pSG5 under which the enzyme is constitutively expressed. For more controlled expression of the enzyme vectors using the tetracycline regulating promoter will be used. The expression vectors will be produced and purified using disabled E. coli eg. JM109.

#### Origin & function

Cell lines and genetic material is available at The Nottingham Trent University, other vectors will be purchased by from commercial sources. Transfected named cell lines and tumour cells will be observed for any phenotype changes especially those in the extra cellular matrix. Tumour cells are often deficient in tissue transglutaminase. Replacing this deficiency by transfection of the enzyme into the cells may have a number of affects on the malignant phenotype. Tumour cells transfected with tissue transglutaminase will be tested for any effects on tumour growth and progression in murine animal models.

#### Evaluation of foreseeable effects

The E.coli hosts to be used are well established disabled bacteria that are unable to colonise the tumour gut. Cultural transfected mammalian cells will not survive outside the tissue culture environment.

Human tumour cells and those transfected with tissue transglutaminase do have the possibility of surviving in the human body if immunologically compatible but only when injected in large quantities eg. $10^6$ (The six should be superscript) cell quantities.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Any animal studies undertaken with transfected murine cell lines eg. to observe the effects of expression of tissue transglutaminase on tumour cell growth will be undertaken under the required containment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposal of infected material will be supervised by staff specifically trained in the requisite techniques.

As Previous - Virkon and Autoclave (Copy from scripts).

<table>
<thead>
<tr>
<th>Is an emergency plan required according to regulation 20?</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
<td>N</td>
</tr>
</tbody>
</table>
For studies involving the transfection of murine cell lines including murine tumour cell lines these should be undertaken under containment level 1. For studies involving the transfection of human cell lines including tumour cell lines these should be undertaken under containment level 2.

Using these conditions the committee felt there was no risk to the environment and the risk to human health was minimal.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
<td>L2 Yes L3 L4</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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<tr>
<td>L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
<td>L3 L4 L2 L3 L4</td>
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</table>

Project Ref 788/01.2

Date Ack'n'd 04/07/2001

CU2 Project Title

IMMUNO-GENE THERAPY OF CANCER

Class Culture Volume

Class 2 < 1 litre

Non-GMM Consent Granted

not applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Withdrawn N
Project Additional Information

Purposes of the contained use
To enhance anti-tumour immunity and develop strategies for developing cancer vaccines.

Recipient or parental organism
Mammalian cell lines, of murine and human origin.

Host/vector system
Viral vectors, including disabled adeno and Herpes expression vectors.

Origin & function
The vectors have been obtained from commercial sources (either under material transfer agreements or purchased from suppliers). Genes from either antigens (including mini gene sequences encoding immunogenic peptides) and cytokine/immune response genes are incorporated into the vector. Appropriate cell infection leads to gene expression.

Evaluation of foreseeable effects
Expression of genes in appropriate cell systems. Viruses are disabled and therefore the progeny are non-infectious. An extensive literature exists on the safety of disables HSV and Adeno viruses.
Experiments are designed to evaluate the ability of gene products to activate adaptive and innate immunity to tumours.
Expression of genes in the human body is possible, but viruses have limited replicative capacity, and gene products would have a transient effect on the immune system.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
In vivo: use of vectors will be restricted to the appropriate containment facilities (containment 2), and all experiments will conform to Home Office guidelines for the use of animals.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste material will be either inactivated with disinfectant and autoclaving, and animal waste will be prepared for incineration according to appropriate codes of conduct (approved by NTU safety services and the Home Office).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
For studies involving the transfection of murine cell lines including murine tumour cell lines these should be undertaken under containment level 1. For studies involving the transfection of human cell lines including tumour cell lines these should be undertaken under containment level 2. Using these conditions the committee felt there was no risk to the environment and the risk to human health was minimal.

### Project Containment

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- **Animal Units**
  - L2
  - L3
  - L4

- **Large Scale Activities**
  - L2
  - L3
  - L4

- **Human Clinical Applications**
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  - L3
  - L4
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<td>Emergency Plan Required?</td>
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<tr>
<td>Transitional Premises</td>
<td></td>
</tr>
<tr>
<td>Non-GMMs</td>
<td>N</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>N</td>
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</table>

**Name**

VIRAGEN (SCOTLAND) LIMITED

**Campus Estate or Research Centre**

**Road Name**

PENTLANDS SCIENCE PARK

**District**

BUSHLOAN

**Town**

PENICUIK

**County**

MIDLOTHIAN

**Postcode**

EH26 0PZ

**Country**

SCOTLAND

**Tel Number**

0131 445 6268

**Fax Number**

0131 445 6269

**HSE Division**

SCOTLAND

**Date at Which Additional Info Submitted**

18/02/2002
## Premises Addresses

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<tr>
<th>Date Premises Closed</th>
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<th>Department</th>
<th>Name 2</th>
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<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
<th>Withdrawn</th>
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<tr>
<td>04/01/2008</td>
<td>VIRAGEN (SCOTLAND) LIMITED</td>
<td></td>
<td></td>
<td>PENTLANDS SCIENCE PARK</td>
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<td>BUSHLOAN PENICUIK MIDLOTHIAN EH26 0PZ</td>
<td>SCOTLAND</td>
<td>N</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes](false)

Give brief details of the genetic modification safety committee

Viragen's GMSC comprises of the Research and Clinical Directors of Viragen Europe, the BSO, the QC Manager, the Health & Safety Manager, the Regulatory Affairs/QA Manager and the Operations Manager in charge of cell handling. The BSO chairs the committee. The GMSC's role is to consider the risks associated with handling procedures, proposed and actual, for work involving GMO's. Initially the committee will meet twice yearly, or more frequently if required.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
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<td>Level 3 (GMMs)</td>
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<td>Parasitology</td>
<td>Transgenic Birds</td>
<td>Microbiology Research</td>
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<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
<td></td>
</tr>
</tbody>
</table>
Cell suspensions will be treated by chemical means to inactivate cells, for example 1% Virkon solution. This is known to be effective for other mammalian cells and the efficiency of cell killing will be confirmed with the genetically modified cell lines. Concentrated cell masses and disposable materials that have contacted the cells will be decontaminated by autoclaving. Autoclaves are available in the Viragen facility and on the Pentlands Science Park site. Material will be incinerated following autoclaving.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Mammalian cell lines expressing transgenes.
**Project Additional Information**

**Purposes of the contained use**

The short term aim of this work is the generation of packaged lentivectors containing the sequences necessary and sufficient for transgene expression. The long term aim of this work is the generation of transgenic chickens, via lentivector transduction, that express heterologous proteins of interest in a spatio-temporally regulated manner.

**Recipient or parental organism**

This work will involve the transient co-transfection of three or four plasmids into HEK293T cells. All of these plasmids contain short, synthetic sequences derived from the Equine Infectious Anaemia virus (EIAV) genome, but at no point will the entire EIAV genome be present in the transfected cells thereby reducing the risk of any recombination events occurring. The HEK293T cell line is dependent on highly specialised growth conditions found only in the laboratory, it is not anticipated that transfection will significantly alter this characteristic. Furthermore, the cell line will only be transiently transfected and then destroyed within one week, there will be no storage for transfected cells.

**Host/vector system**

The viral vector system used during this project is based on EIAV and was developed by Oxford Biomedica to allow the highly efficient production of replication incompetent lentivectors. Numerous safety systems have been included in the system. For example, all of these sequence components required to make an infectious EIAV clone are never present, individual components are partitioned across multiple independent plasmids, codon optimisation to reduce potential homology and the elimination of accessory proteins.

**Origin & function**

The plasmids co-transfected into the HEK293T producer cells can be divided into the following groups:

1. One or two plasmids to express the essential viral proteins such as gag, pol and/or rev. These virus-related coding sequences will be synthetic, codon optimised versions and not derived directly from an infectious viral genome. Their function is to allow the efficient expression and packaging of RNAs expressed from the viral genome (Group 3 below).
2. A plasmid that expresses an envelope protein, again a synthetic copy of the gene, to allow viral pseudotyping.
3. A plasmid encoding the remainder of the minimal viral genome as well as the target gene to be expressed and associated promoter sequences. The target gene will usually be a reporter gene derived from a commercially available plasmid. The promoter will usually be a commonly used constitutive promoter derived from a commercially available plasmid. Alternatively, promoter fragments derived directly from the chicken genome may also be used.

**Evaluation of foreseeable effects**

The risks associated with the lentivector system can be broken down into the following groups.

The risk of insertional mutagenesis whereby the vector integrates into a host chromosome. This will be minimised by the use of self-inactivating vectors and the use of appropriate containment procedures. Furthermore, in the unlikely event of exposure the most likely outcome would be transient transduction or regularly replaced epithelial tissues such as skin, eye or lung lining. A related risk is that of transgene expression in transduced cells, however only commonly used reporter genes will be used, the expression of which is anticipated to have no significant impact on human health.

The potential of sensitization to lentiviral vector components and/or reagents used in the process is considered extremely unlikely.

Another theoretical risk is the generation of replication competent lentivectors (RCLs). This can occur via recombination between vector system components (minimised by reducing or abolishing any regions of shared sequence homology), or between wild type viral sequences in the producer cells (again a minimal concern as no EIAV-homologous sequences have been found in the human genome so far). Furthermore, in-process testing to allow validation of RCL negative status of both producer
cells and packaged lentivector preparations will be developed and implemented.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No applicable, work to generate transgenic chickens is not covered by this risk assessment and will be carried out by collaborators off site.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

In Part II, Schedule 8 (Table 1a) it is stated that an autoclave is required in the building for work at containment level 2. Viragen (Scotland) Limited is applying for a derogation from this requirement. VSL does operate an autoclave within our premises but it is not currently used for waste treatment. At present, all VSL GMO category 1 waste is disposed of by incineration after autoclaving, with the exception of Category 3 waste, which is disposed of by chemical inactivation. Our proposal is to dispose of Category 2 waste via the same route, and to retain an autoclave on site but to allow Category 2 waste to be disposed of by incineration instead of autoclaving. This will enable us to handle Category 2 and above waste, with no need to establish an in-house autoclave waste disposal system, and allowing it to be handled by operators who are experienced with Category 2 and above waste. Furthermore, attached data also demonstrates that the Virkon treated waste would be 100% inactivated before leaving the building. The waste will be double bagged, transferred into secure, sealable, autoclavable stainless steel containers and transported 200m to the MRI.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This project will generate contaminated liquid waste and plasticware. All waste will be soaked in 2% (w/v) Virkon, a commercially available virucidal disinfectant, for a minimum of 24 hours prior to disposal. Liquid waste will be collected in 500ml Duran bottles pre-filled with 100mls of 10% Virkon. Once full, after a minimum of 24 hour incubation, the sealed and dated bottled will be transferred for autoclaving. After autoclaving the liquid waste will be disposed of via the drains with copious dilution in accordance with the manufacturers guidelines. Virkon product information states clearly that this can be expected to have minimal environmental impact or effects on standard sewage treatment processes as Virkon will decompose into harmless by-products. Used plasticware will also be Virkon soaked for a minimum of 24 hours and then double bagged and transferred for autoclaving in secure, sealable stainless steel autoclave containers. The autoclave is located within a different building (Moredun Research Institute) on the same science park (Pentland Science Park) and is annually validated for waste disposal. Autoclaved waste is then removed for incineration off site.

All waste procedures at Viragen (Scotland) Limited are documented in the Waste Manual (VSM 005) which is available on requires.

Is an emergency plan required according to regulation 20? [Y]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

0

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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</table>

02/03/2022
### Project Additional Information

**Purposes of the contained use**

Bunyamwera virus made by a plasmid based transfection system has been obtained from collaborators at the UNIVERSITY OF Glasgow, an expert on the Bunyaviridae family of viruses. Cells will be infected by virus as a model to analyse the anti-viral activity of several cytokine products, including Multiferon™.

**Recipient or parental organism**

The system utilised for the production of transfectant Bunyamwera virus particles has been published (Bridgen, A. and Elliott, R.M. (1996) Rescue of a segmented negative-strand RNA virus entirely from cloned complementary DNAs. PNAS. 93:15400-4). HeLaT4+ cells are infected with recombinant vaccinia virus vTF7-3 to express bacteriophage T7 RNA polymerase. Cells are subsequently transfected firstly, with a mixture of three plasmids to allow for expression of all the Bunyamwera viral proteins, secondly to introduce the three plasmids containing cDNAs encoding the entisense genomes of the Bunyamwera virus. After 43 hours cell extract/virus is infected onto C6/36 mosquito cells (aedes albopictus to enrich the low yield of Bunyamwera virus particles, after 4-7 days virus in the supernatant can be plaque purified in BHK cells as is commonly performed for a number of viruses.

**Host/vector system**

The system utilised for the production of transfectant Bunyamwera virus particles has been published (Bridgen, A. and Elliott, R.M. (1996) Rescue of a segmented negative-strand RNA virus entirely from cloned complementary DNAs. PNAS. 93:15400-4). HeLaT4+ cells are infected with recombinant vaccinia virus vTF7-3 to express bacteriophage T7 RNA polymerase. Cells are subsequently transfected firstly, with a mixture of three plasmids to allow for expression of all the Bunyamwera viral proteins, secondly to introduce the three plasmids containing cDNAs encoding the entisense genomes of the Bunyamwera virus. After 43 hours cell extract/virus is infected onto C6/36 mosquito cells (aedes albopictus to enrich the low yield of Bunyamwera virus particles, after 4-7 days virus in the supernatant can be plaque purified in BHK cells as is commonly performed for a number of viruses.
Cell lines used included HeLaT4+ (a genetically modified derivative of the HeLa human carcinoma cell line), C6/36 mosquito cells, and BHK hamster kidney cells. C6/36 and BHK cells are not considered hazardous to humans. These cell lines are not used or stored at VSL. Furthermore, there are no HeLaT4+ cells present in the final Bunyamwera virus preparation used and stored at VSL as the final viral preparation has been derived from supernatant media and lysed HeLaT4+ cell extracts. This extract was then enriched for the low number of Bunyamwera virus particles present in the mosquito cell line, then plaque purified in BHK cells, in a two-step process removing any presence of host cells.

Origin & function

The nucleic acids used in these studies for the generation of Bunyamwera virus constructs (for expression of Bunyamwera viral proteins and encoding the antisense genome sequence), were derived by a combination of PCR from existing Bunyamwera virus sequences and reassembling complete sequences within plasmids. These sequences encode functional proteins and antisense genome sequences but have been subcloned into plasmid vectors with T7 promoters that require the infection of a recombinant vaccinia virus expressing T7 RNA polymerase to allow expression of these functional proteins etc. These plasmids will not be used or stored at VSL and at this point there is no proposed work involving the manipulation of these plasmids.

Resultant transfectant Bunyamwera virus produced in the GM system in all ways resembles authentic wild type Bunyamwera virus. This has been verified by comparing plaque formation (size, morphology), growth kinetics and titre, neutralisation by anti-Bunyamwera virus polyclonal antiserum, and viral protein expression profiles (by western blot).

Evaluation of foreseeable effects

Infection with Bunyamwera virus can result in febrile illness with headache, arthralgia, rash, and infrequent nervous system involvement (reviewed in Bowen et al, 2001. Virology. 291:185-90). However, infection normally occurs following arthropod transmission by mosquito bite. There is extremely little chance of this method of transmission occurring in the laboratory, as the General Virology Laboratory is at the end of a sequence of antechamber plus two laboratories specifically designed for tissue culture with double-door access between each laboratory. In the laboratory, infection of humans by the aerosol route is also considered unlikely unless very high concentrations of virus are used to generate the aerosols. The risk of human infection is low if the virus preparation is handled in an appropriate safety cabinet and not injected accidentally. VSL employs a restricted needle use policy, specifically with regards to handling virus.

Therefore, it is extremely unlikely that infection will occur upon the accidental spillage of virus during its handling, usage or storage.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable, no GM animals or plants will be generated during this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

In Part II, Schedule 8 (Table 1a) it is stated that an autoclave is required in the building for work at Containment Level 2. Viragen (Scotland) Limited has an existing derogation from this requirement for other Containment Level 2 work and would like this to be extended to cover the new Bunyaviridae waste. As stated in Section 12 of this form it is our intention to use Virkon for the chemical inactivation of all waste generated by this work. Manufacturers data demonstrates the efficacy of this product against Bunyaviridae therefore the prior inactivated waste will then be double bagged, transferred into sealable, autoclavable stainless steel containers and transported 200m to an autoclave on site (located at the Moredun Research Institute). This autoclave has been validated for waste disposal and supporting documentation is attached. Print outs of each sterilisation run (a minimum of 121 degrees Celsius for 15 minutes) are retained providing a record of time, temperature and pressure. Autoclaved waste is then removed for incineration off site.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This project will generate contaminated liquid waste and plasticware. All waste will be soaked in 1% (w/v) Virkon, a commercially available virucidal disinfectant, for a minimum of 24 hours prior to disposal. Virkon usage follows manufacturers guidelines on bactericidal and virucidal activity of the product. Virkon liquid waste will be disposed of via the drains with copious dilution in accordance with the manufacturers guidelines. Virkon product information states clearly that this can be expected to have minimal environmental impact or effects on standard sewage treatment processes as Virkon will decompose into harmless by-products. Soaked plasticware such as
empty tissue culture flasks will be double bagged before being transferred for autoclaving. To avoid the risk of bag piecing, soaked pipettes will be transferred into sealable, rigid plastic containers before being transferred for autoclaving. The autoclave is located within a different building (Moredun Research Institute) on the same science park (Pentland Science Park). This autoclave has been validated for waste disposal, print out of each sterilisation cycle are retained (time, temperature and pressure). Autoclaved waste is then removed for incineration off site.

All waste procedures at Viragen (Scotland) Limited are documented in the Waste Manual which is available on request.

The GMSC raised no major concerns with the risk assessment however it was felt that a reference to Viragen (Scotland) Limited's needlestick policy in Section 3.2 should be clarified. It has now been established that needles will never be used during any handling of the virus so this reference has been removed from the text to avoid confusion.

Please enter comments on the GM safety committee on the risk assessment

The GMSC raised no major concerns with the risk assessment however it was felt that a reference to Viragen (Scotland) Limited's needlestick policy in Section 3.2 should be clarified. It has now been established that needles will never be used during any handling of the virus so this reference has been removed from the text to avoid confusion.

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</tr>
</thead>
<tbody>
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</table>

- **Animal Units**
  - L2 L3 L4 L2 L3 L4

- **Large Scale Activities**
  - L2 L3 L4 L2 L3 L4

- **Human Clinical Applications**
  - L2 L3 L4 L2 L3 L4

### Project Ref 789/05.1

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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<td>The use of lentiviral vector technology with associated molecular biology and tissue culture.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Project notified under transitional arrangements N

Date Project Ceased 04/01/2008
Project Additional Information

Purposes of the contained use
The short term of this work is the generation of packaged lentiviral vectors containing the sequences necessary and sufficient for transgene expression where such transgenes are clinically relevant and potential human therapeutic proteins. The long term aim of this work is the generation of transgenic chickens, via lentiviral vector transduction, that express heterologous proteins in a spatio-temporally regulated manner.

Recipient or parental organism
This work will involve the transient co-transfection of three or four plasmids into HEK293T cells. All of these plasmids contain short, synthetic sequences based on the Equine Infectious Anaemia Virus (EIAV) genome, but at no point will the entire EIAV genome be present in the transfected cells thereby reducing the risk of any recombination events occurring. The HEK293Y cell line is dependent on highly specialised growth conditions found only in the laboratory, it is not anticipated that transfection will significantly alter this characteristic. Furthermore, the cell line will only be transiently transfected and then destroyed, within one week; there will be no storage of transfected cells.

Host/vector system
The lentiviral vector system used during this project is based on EIAV and was developed by Oxford Biomedica to allow the efficient production of replication incompetent vectors. Numerous safety features have been included in the system. For example, all of the sequence components required to make an infectious EIAV clone are never present, individual components are partitioned across multiple independent plasmids, codon optimisation to reduce potential homology and the elimination of accessory proteins.

Origin & function
The plasmids co-transfected into the HEK293T producer cells can be divided into the following groups:

1) One or two plasmids to express the essential viral proteins such as gag, pol and/or rev. These virus-based coding sequences are synthetic, codon optimised versions and not derived directly from an infectious viral genome. Their function is to allow the efficient expression and packaging of RNAs expressed from the viral genome (Group 3 below).

2) A plasmid that expresses an envelope protein, again a synthetic copy of the gene, to allow viral pseudotyping.

3) A plasmid encoding the remainder of the minimal genome as well as the target gene to be expressed and associated internal promoter sequences. The target gene will usually encode a clinically relevant, potential human therapeutic protein. The promoter will usually be one of several possible promoter fragments derived directly from endogenous chicken genes. No constitutively active internal promoter sequences will be present within the minimal viral genome.

Evaluation of foreseeable effects
The risks associated with the lentiviral vector system can be broken down into the following groups:
The risk of insertional mutagenesis whereby the vector integrates into a host chromosome. This will be minimised by the use of self-inactivating vectors and the implementation of appropriate containment procedures. Furthermore, in the unlikely event of exposure the most likely outcome would be transient transduction of regularly replaced epithelial tissues such as skin, eye or lung lining. A related risk is that of transgene expression in transduced cells, however only non-harmful, potentially therapeutic human proteins would be used the expression of which is not anticipated to have a detrimental impact on human health.

The potential of sensitization to lentiviral vector components and/or reagents used in the process is considered extremely unlikely.

Another theoretical risk is the generation of replication competent lentiviruses (RCLs). This can occur via recombination between vector system components (minimised by reducing or abolishing any regions or shared homology) or between wild type viral sequences in the producer cells (again a minimal concern as no EIAV-homologous sequences have been found in the human genome so far). Furthermore, no RCLs have ever been detected in packaged viral samples based on third generation minimal EIAV vector genomes. It is therefore extremely unlikely that any such RCLs would be present in vectors packaged at VSL. Nonetheless, at an appropriate time material will be made available for RCL testing.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable, work to generate transgenic chickens is not covered by this Risk Assessment and will be carried out by collaborators off site.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

In Part II, Schedule 8 (Table 1a) it is stated that an autoclave is required in the building for work at Containment Level 2. Viragen (Scotland) Limited has an existing derogation from this requirement for other lentiviral vector Containment Level 2 work and would like it extended to cover this closely-related work. As stated in Section 12 of this form it is our intention to use Virkon for the chemical inactivation of all waste generated by this work. Manufacturers' data demonstrates the efficacy of this product against HIV (a closely related lentivirus) therefore the chemically inactivated waste will then be double bagged, transferred into sealable, secure, autoclavable stainless steel containers and transported 200m to an autoclave on site (at Moredun Research Institute). This autoclave has been validated for waste disposal. Print outs of each sterilisation run (a minimum of 121 degrees Celsius for 15 minutes) are retained providing a record of time, temperature and pressure. Autoclave waste is then removed for incineration off site.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This project will generate contaminated liquid waste and plasticware. All waste will be soaked in 2% (w/v) Virkon, a commercially available virucidal disinfectant, for a minimum of 24 hours prior to disposal. Liquid waste will be collected in 500ml Duran bottles pre-filled with 100mls of 10% Virkon. Once full, and after a minimum of 24 hour incubation, the sealed and dated bottles will be transferred for autoclaving. After autoclaving the liquid waste will be disposed of via the drains and copious dilution in accordance with the manufacturers’ guidelines. Virkon product information states clearly that this can be expected to have minimal environmental impact or effects on standard sewage treatment processes as Virkon will decompose into harmless by-products. Used plasticware will also be Virkon soaked for a minimum of 24 hours and then double bagged and transferred for autoclaving in secure, sealable stainless steel autoclave containers. The autoclave is located within a different building (Moredun Research Institute) on the same science park (Pentland Science Park) and is annually validated for waste disposal. Autoclaved waste is then removed for incineration off-site.

All waste procedures are documented in the Waste SOP (SOP/PRO/002 and VL/PRO/001)which are available on request.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GMSC raised no major concerns with the risk assessment, the minor comments or recommendations that were made are listed below:

All lentiviral vector tissue culture will be done within the new Vector Laboratory. The GMSC suggested that SOP references be changed to the Vector Laboratory-specific codes (with a VL prefix) rather than general Viragen SOPs.

Minor text corrections:
The GMSC queried the origin of, and the virus tested status of foetal bovine serum (FBS) used during tissue culture and asked that this be verified prior to submission of this application. Copies of the validated gamma irradiation virus inactivation procedure for FBS have been obtained from supplier (Invitrogen).

All of the above suggestions and recommendations were incorporated into the final version of the risk assessment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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**Project Ref** 789/06.1

**Date Ackn’d**

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**Date Project Ceased**

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**Class**

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**Non-GMM Consent Granted**

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**Project notified under transitional arrangements**

| N |

**Historical Significant Changes**

<table>
<thead>
<tr>
<th>Significant Change ID</th>
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</thead>
</table>

| N |

02/03/2022
The aim of the work is to assess the Interferon-modulatory effects of various viral proteins, namely VP24 and VP35 from both Ebola and Marburg viruses. Stably transfected cell lines expressing these proteins will be generated and analysed via standard antiviral bioassays.

Constructs containing the VP35 and VP24 genes in the commercial vector, pClneo (Promega) (to be constructed at off-site), will be stably transfected into African Green Monkey Kidney Epithelial Vero E6 cells and Human Epithelial Kidney 293T cells. According to the literature, expression of VP35 in 293T cells will change certain characteristics of the cells, rendering them unable to induce type I IFN. VeroE6 cells do not produce IFN, therefore the expression of VP35 is expected to leave this characteristic unchanged. It is possible but unknown whether VP35 from Marburg virus strains will have a similar effect on 293T cells. Furthermore, it is unknown whether VP35 from any strain of Ebola or Marburg to be tested will affect the cell's ability to respond to IFN treatment (applicable to both 293T and VeroE6 cells). Finally, it is unknown whether VP24 will have any effect on IFN induction or response to IFN treatment.

The virally-derived sequences will be present within the commercially available plasmid, pClneo (Promega). This plasmid is based on a pUC backbone and is non-mobilisable.

The coding sequences of both VP24 and 35 will be obtained by PCR cDNA prepared from virus-infected cells. Four types of each protein coding sequence will be analysed; from the Zaire strain of Ebola, the Ci67 strain of Marburg, the Musoke strain of Marburg and the Angola strain of Marburg. The PCR primers used have introduced suitable restriction enzyme sites to the 5' and 3' ends of the genes to facilitate cloning in the expression vector. This work will all been performed off site and not at Viragen (Scotland) Limited. These sequences will encode functional protein domains but will be subcloned into plasmid backbones that lack the control sequences necessary for expression in bacteria.

The pClneo vector contains a human Cytomegalovirus immediate/early promoter/enhancer to control expression of the virus coding sequences, note this promoter can direct heterologous gene expression in human cells. The vector also contains the neomycin resistance gene allowing selection of stably transfected cell clones.

The foreseeable risks associated with this work can be listed as:

- from base cell lines, eg. HEK293T due to either co-cultured adventitious agents or proteins within the cells such as SV40 Tag. For a series of experiments a single vial will be recovered from frozen, after the completion of that series of experiments the cultured cells will be disposed of. Long term culture of the cells will be minimised hence reducing the potential for an adventitious agent to infect the cultures. Considered extremely unlikely and measures in place to minimise contact between operator and cells.

- from plasmid DNA during transient transfection. Exposure of a human to the plasmid DNA constructs used to generate the stably transfected cell lines is considered to be unlikely but if it did occur there is a chance that human health could be harmed. Any gene introduced into cells that can inhibit cellular response to type I IFN could in theory result in that cell(s) demonstrating increased susceptibility to virus infection. This is likely to also be the case with regards to other type of infection. Considered possible but unlikely and this process constitutes a very small part of whole research programme.

- from virally derived protein expressed in the transfected cells or the introduced DNA present within them. The DNA introduced into these cells will be stably integrated at
low copy number. The transfected cells will not be manipulated using sharps so the risk of stab injuries is low. It is considered extremely unlikely that any genetic material from the cells, of which the expression plasmids only contribute a tiny proportion, will be transferred to the operator by this or related routes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

In part II, Schedule 8 (Table 1a) it is stated that an autoclave is required in the building for work at Containment Level 2. Viragen (Scotland) Limited has an existing derogation from this requirement for other lentiviral vector Containment Level 2 work and would like it extended to this work also. As stated in Section 12 of this form it is our intention to use Virkon for the chemical inactivation of all waste generated by this work. Previous submissions to the HSE have included data demonstrating efficient mammalian cell killing by 2% Virkon therefore the chemically inactivated waste will then be double bagged, transferred into sealable, secure, autoclavable stainless steel containers and transported 200m to an autoclave on site (at Moredun Research Institute). This autoclave has been validated for waste disposal. Print outs of each sterilisation run (a minimum of 121 degrees Celsius for 15 minutes) are retained providing a record of time, temperature and pressure. Autoclaved waste is then removed for incineration off-site.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This project will generate contaminated liquid and plasticware. All waste will be soaked in 2% (w/v) Virkon, a commercially available virucidal disinfectant, for a minimum of 24 hours prior to disposal. Liquid waste will be collected in 500ml Duran bottles pre-filled with 100mls of Virkon. Once full, and after a minimum of 24 hours incubation, the sealed and dated bottles will be transferred for autoclaving. After autoclaving the liquid waste will be disposed of via the drains with copious dilution in accordance with manufacturers' guidelines. Virkon product information clearly states that this can be expected to have minimal environmental impact or effects on standard treatment processes as Virkon will decompose into harmless by-products. Used plasticware will also be Virkon soaked for a minimum of 24 hours and then double bagged and transferred for autoclaving in sealable, secure, stainless steel containers. The autoclave is located within a different building (Moredun Research Institute) on the same science park (Pentland Science Park) and is annually validated for waste disposal. Autoclaved waste is then removed for incineration off-site.

All waste procedures at Viragen (Scotland) Limited are documented in the Waste SOP (SOP/PRO/002) which is available on request.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
The GMSC raised no major concerns with the risk assessment, minor comments are listed below:

Request to amend reference that cell lines with a variety of expression levels will be banked (Section 2.2.2).

Suggestion that the theoretical risks from cell line exposure also include consideration of the risks specific to those cell lines, for example SV40 Tag exposure as well as general adventitious agent exposure. RA013 will be amended to this effect.

The reference to GM bacteria in section 3.4 will also be removed as at present no GM bacteria will be created at VSL during the course of this work.

Minor formatting correction in Section 3.1 to be corrected.

Also, to replace the reference in Section 3.3 to a "laminar flow hood" with "Class II Biological Safety Cabinet".

All of the above suggestions and recommendations were incorporated into the final version of the risk assessment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L2</td>
<td>L2</td>
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<tr>
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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

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02/03/2022
### GM Centre Number: 790

| Data Premises Notified (Originally) | 12/07/2001 |
| Data Premises Closed | N |
| Emergency Plan Required? | N |
| Transferred from 1992 Regs? | N |
| Transitional Premises Class | N |
| Non-GMMs | N |
| Withdrawn | N |

#### Name

| ISOGENICA LIMITED |

#### Name 2

#### Department

#### Campus Estate or Research Centre

| THE MANSION |

#### Building

| CHESTERFORD RESEARCH PARK |

#### Road Name

| LITTLE CHESTERFORD |

#### District

#### Town

| ESSEX |

#### County

| CB10 1XL |

#### Postcode

| ENGLAND |

#### Country

#### Tel Number

| 01799 533680 |

#### Fax Number

| 01799 531940 |

#### E-mail

#### HSE Division

| EAST AND SOUTH EAST |

#### Comments

#### Date at Which Additional Info Submitted

02/03/2022
**Premises Addresses**

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<th>Date Premises Closed</th>
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The committee (GMSC) comprises The CEO, The Commercial Director & one senior scientist. The GMSC shall meet quarterly. Risk assessments for new projects involving GMMs shall be fully discussed by the GMSC & no work on such projects shall commence prior to the approval of the GMSC. Approval shall only be given if the project can be safely conducted under containment levels already established & notified to the HSE. Any projects requiring additional levels of containment shall commence only following the authorisation by the HSE.

<table>
<thead>
<tr>
<th>Laboratory (GMMs)</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
<td></td>
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</table>
Yes

GMM Waste will be placed in the designated yellow bags, autoclaved and sent, with similarly processed material generated on The Babraham Institute Campus, for incineration at the Duxford Pet Crematorium. Benches will be regularly cleaned and sterilised following work with GMOs. Where suspected spills have occurred, swabs will be taken and used to inoculate appropriate media containing diagnostic antibiotics.

Other (please specify)  

Tick if confidential  

When ticked, for activities involving GMMs, describe the waste management measures which will apply to the activity.

Since the risk assessment has already been approved by the HSE (Ref: GM702) The GMSC unanimously agreed its content.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

Project Ref  790/20.1

Date Ackn'd  02/03/2022  

CU2 Project Title  

Class  

CultureVolClass2  

CultureVolumeClass3-4  

Page 11324 of 15326
**Date Project Ceased**

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<td>Transfection of animal cell lines (HG2) with plasmid DNA using micelle transformation</td>
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

## Project Additional Information

### Purposes of the contained use

Use of transiently transfected cells for downstream processes such as panning (CIS or phage display), screening (binding assays using in-house generated binders or commercially available antibodies) and production of cell material. Possible fixation of cells prior to analysis.

### Recipient or parental organism

The recipient organism are hazard group 2 cells: HEK293 (T, S, NTR1 transformed, muCD71) and Raji cells (wt and muc1-C transformed), HeLa cells, J.RT3-T3.5 and Jurkat E6.1 cells.

### Host/vector system

Fc1.0 (in-house), pCAG (epoch), pCDNA31 (thermofisher), pCMV3-CGFPSpark (sino biological)

### Origin & function

Cells are from transformed cell culture lines of various commercial sources. The cells Use of transfected cells from part 1 for downstream processes such as panning (CIS or phage display), screening (binding assays using inhouse generated binders or commercially available antibodies) and production of cell material. Possible fixation of cells prior to analysis.

### Evaluation of foreseeable effects

Although these cells contain pathogens that may be hazardous to human health if the correct containment is used there should be no problems. In addition, there is no historical documentation of these cells nor
transformation methods either spreading disease or genetically modified material to direct users or the outside environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

we would like to apply for a derogation to use our disposal company Grundon to incinerate our waste rather than autoclave it before disposal due to having limited facilities for autoclaving waste in the lab.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste is collected in a yellow bin which is sealed before collection by an approved waste disposal contractor who is licensed to remove such waste for incineration. Small spills are absorbed onto paper soaked in disinfectant (distel (starlab) 1/10,) collected in a yellow bins for disposal as above. Liquid waste is collected for autoclaving or incineration. In both cases the waste is poured into the waste bins and disposed of via incineration. Due to the low volumes of cultures being used (no more than 100ml per flask), any spills can be absorbed onto paper soaked in disinfectant as detailed above.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

---

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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</tr>
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<tbody>
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**Name**

THE COLLECTIVE GALLERY

**Name 2 Department**

**Campus Estate or Research Centre**

**Road Name**

22-28 COCKBURN STREET

**District**

**Town**

EDINBURGH

**County**

EAST RENFREWSHIRE

**Postcode**

EH1 1BJ

**Country**

SCOTLAND

**Tel Number**

0131 220 1260

**Fax Number**

NONE

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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<td>EH1 1BJ</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee is composed of two academic members from Heriot Watt University - 2x Scientists. Health & Safety issues have been discussed during two meetings. Another meeting will take place before and during the exhibition.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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<th>Large Scale</th>
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</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Transgenic Plants are to be exposed in an art gallery space (protected be</td>
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<th>Bacteriology</th>
<th>Parasitology</th>
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<th>Microbiology Research</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>
All Transgenic plants are killed by autoclaving before disposal. The autoclave is not on the gallery premises, therefore plants will be returned to Heriot Watt University for disposal. Autoclaving will be carried out at 121°C with a holding time of 25 minutes.

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<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
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**Other(s)**

Display of Transgenic Plants.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The Transgenic Plants are considered to be of no risk, and will be held in a secure environment; cannot be touched or moved by the public. Therefore the genetic safety committee has no reservations about this particular venture.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
### GM Centre Number: 792

<table>
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<th>Data Premises Notified (Originally)</th>
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| Comments              |                        |

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## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The committee comprises a range of academic and medical staff who have complementary areas of expertise. Committee composition:- Chairperson, Secretary and 9 other members from The University of Hull.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

The maximum volume of waste released at any one time - All GMM waste will be treated before use however the maximum volume released after treatment would be no more than 2L per day.

Deactivation of waste - All GMM waste produced can be treated in one of two ways.
1) Solid and liquid media (including disposable plastics and glassware) will be autoclaved at 121°C for a period appropriate to the volume but for a period not less than 15min.
2) Liquid waste up to 500ml (this volume should not exceed the volume of Virkon in the pot) or pipettes can be placed in Virkon (2% w/v). This should be left for at least 1h before discarding the material down the sink. Disposable plasticware should then be rinsed with water before autoclaving.

Validation and monitoring of the deactivation method - When autoclaving GMM materials Sterilisation indicator strip are used to monitor the process. All autoclaves will also be treated every 6 months (during servicing for the larger systems) by thermocouple. The results from these will be notified to the BHO. The Virkon solution (2% w/v) is made up fresh every 2 days, however if the colour fades the solution should be discarded. The results of efficacy testing of Virkon are available from Antec International however it has been shown to produce 100% kill in a wide range of bacteria (350 strains and species including E. coli), viruses and fungi at dilutions down to 1: 200 and over a contact time of 20 min. We have also tested the efficacy of Virkon against Coliphage M13 and E. coli. Cells (10^7) or virus particles (10^6) in solution were exposed to 2% w/v Virkon (final concentration 1% w/v) for periods up to 1hour. Samples were taken and the materials washed by centrifugation to remove excess Virkon. The pelleted material was then plated out on nutrient agar or nutrient agar seeded with E. coli. By 10 min we were unable to culture either E. coli or generate plaques.

Disposal of materials:- After autoclaving material is sent for landfill.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Comments for the committee in relation to the new risk assessments

The committee had previously passed these assessments; these comments directly relate to the new regulations.
1.) Are all the bacterial vectors derived from E. coli K12?
2.) What cell lines will be used for the transient transfection system?
3.) Do we need to outline the disinfection process for the cleaning of spillages levels of kill?
Project Additional Information

Purposes of the contained use

Genes encoding for proteins involved in transport processes or chromosomal organisation and segregation, identified either by homology-based database searches or sequencing by mass spectrometry, will be cloned and manipulated in cultured insect and bloodstream forms of trypanosomes. This will allow the characterisation of gene function and contribute to our understanding of the parasites' strategies to survive in its mammalian host.

Recipient or parental organism

GMO cultured insect or bloodstream forms of the parasite. Insect forms are non-pathogenic, non transmissible. Bloodstream forms require tsetse fly transmission. These are only indigenous in Africa. No tsetse fly colonies are maintained at the University of Manchester.

Trypanosomes do not produce toxins and we expect most genetic manipulation of the parasite to reduce fitness and therefore virulence.

Host/vector system

Parental organism: Trypanosoma brucei spp, blood and insect forms.

Vector system: pGEM or pBluescript based bacterial shuttle vectors engineered to enable parasite gene expression or modulation in the parent organisms using a tetracycline regulated inducible expression system. These are non mobilisable shuttle vectors. Specific vectors are pET series, pGem, pGemT-easy, pLew100, pZJM, pBluescript II, p2T7, pHD451 and pHD430.

Origin & function

1. T.brucie spp. genes or proteins implicated in transport processes (eg chromosomal segregation) and chromatin organisation of the parasite. Their function is expected...
To address issues of gene regulation and genome stability.

2. Reporter gene expression to allow selection of transgenic parasites or detection of the expression pattern or location of those proteins described in (1). This will include genes for resistance to antibiotics used for parasite selection and green fluorescent protein gene fusions for protein localisation.

Evaluation of foreseeable effects

Depending on the specific class of proteins under investigation we are expected to see range of phenotypes. These will include defects in nuclear segregation, chromosome instability, abnormal cell morphology, or even lethality. The under investigation genes will be expressed using a tetracycline inducible expression system. Therefore, in the absence of tetracycline the genes are effectively silenced. A similar inducible system will be used for experiments using RNA interference to ablate specific proteins of the cell.

Antibiotic resistance genes used for selection of transgenic parasites are not those used for clinical treatment of the parasite and will otherwise have no foreseeable effect.

Reporter genes (*eg green fluorescent protein) are for localisation and assay of expression pattern for particular parasite genes or sequences. There is no foreseeable consequence of this.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

T.brucei spp. does not produce spores. They are rapidly killed outside of the culture vessel or blood by desiccation osmotic shock (washing with water, detergents) and cannot penetrate unbroken skin. Parasite genes are expressed or perturbed using a tetracycline inducible system and will be effectively silenced outwith the culture vessel. Tsetse flies are required for transmission and completion of the life cycle; these are indigenous only in sub Saharan Africa. Thus the parasites, whilst mammal infective as the bloodstream forms are effectively deficient in mobilisation capacity. The wild type bloodstream form parasite is mammal infective and virulent. It is highly unlikely that genetic modification will enhance this; rather gene manipulation is likely to reduce virulence and transmissibility.

We request derogation from Containment class III to containment class II for specific containment measures.

CONTAINMENT MEASURE:

1. Isolation. The laboratory is separate from other laboratories in the building being fully contained within a locked laboratory, with access restricted to authorised lab personnel. The laboratory has been designed as a fully functional level 3 facility since a previous project, now discontinued, used this facility to experiment on Mycobacterium tuberculosis.

2. Laboratory sealable for fumigation. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSSH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for sealability for fumigation.

4. Entry via airlock. Trypanosomes can only be transmitted by blood-blood contact or by skin puncture. They cannot survive in air and do not form spores. We request derogation as risk assessment does not indicate a requirement for this measure.

5. Negative pressure. The trypanosome cell line used has a contained use regulation level of 3 but the containment recommended by the COSSH assessment is level 2. Therefore the containment applied will be level 3. However, we request derogation to allow relaxation of the requirement for airflow. However, as stated under 1. the lab does have negative pressure that is maintained at all times.

6. Filtration of extract and input air. Trypanosomes do not form spores and cannot undergo airborne transmission. Handling of bloodstream form parasites is most commonly as in vitro cultured forms. These are opened only in a microbiological safety cabinet with HEPA filtration. We request derogation to level 2. However as stated
under 1 the lab does have filtered extract and input air.

7. Microbiological safety cabinet. Trypanosomes do not form spores and cannot undergo airborne transmission. Handling of bloodstream form parasites is most commonly as in vitro cultured forms although purification of parasites from blood is not practical in a safety cabinet. However, there is no generation of aerosols. The laboratory is self contained providing containment. We request derogation to level 2.

8. Autoclave in the laboratory suite. The laboratory is equipped with a build-in-the-wall, two-sided access autoclave which will be used to sterilise all equipment that has come into contact with parasites. Spillages are rendered non-infective by soaking in 2% Virkon. Transport within the laboratory is in sealed containers. However, we request derogation to level 2.

11. Shower. Trypanosomes are only transmitted by blood:blood contact or by inoculation. Skin penetration of the naked parasite is not possible. Parasites cannot survive in the external environment. Therefore we request derogation to level 2. However, as stated under 1. The lab does have a built-in shower.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

A laboratory scale project employing good microbiological practice and good occupational safety and hygiene.

Disinfection with 2% aqueous Virkon solution according to University Policy and Guidance document. Material that has come into contact with parasites is inactivated by soaking in hypochlorite (1:500) for 24h.

Liquid waste and solid waste (other than sharps) is sterilised in an autoclave maintained and validated by the manufacturer’s service organisation for the destruction of clinical waste prior to transfer by a licensed operator to a commercial incinerator site authorised to treat GM waste. Sharps and animal waste are transferred directly to the incinerator for destruction.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GM committee endorsed the decision of the local GM committee (dated 20/2/2004) that this project requires Containment Level 2 facilities that are available in the Microbiology Containment Laboratory in the Department. No special problems were identified that could not be contained by Good Microbiological Practice. It was noted that Group II organisms were involved and that HSE approval was required before work could start.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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GM Centre Number: 793

Data Premises Notified (Originally) 17/07/2001

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed 21/12/2016

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name
CAMBRIDGE CENTRE FOR BRAIN REPAIR

Name 2

Department

Campus Estate or Research Centre FORVITE SITE

Building E.D.ADRIAN BUILDING

Road Name ROBINSON WAY

District

Town CAMBRIDGE

County CAMBRIDGESHIRE

Postcode CB2 2PY

Country ENGLAND

Tel Number 01223 331160

Fax Number 01223 331174

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted 29/11/2004
# Premises Addresses

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# Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Compostion:
  - Biological Safety Officer (Acedemic Staff), Deputy Biological Safety Officer (Acedemic Staff), Department Safety Officer (Acedemic Staff), Management Representative (Administrator), Staff Representative (Assistant Staff), University Biological Safety Officer (Acedemic Staff), School Safety Officer (Clinical Medicine or Biological Sciences), & Technical Expert (when necessary).
  - Frequency: Monthly (or when required).
  - Procedure: New projects, ongoing projects, facilities and problems.

<table>
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Other (please specify) Tick if confidential

- Bacteriology Yes
- Parasitology
- Transgenic Birds
- Microbiology Research Yes
### Virology
- Yes

### Transgenic Animals
- Yes

### Transgenic Fish
- Yes

### Gene Therapy
- Yes

### Mycology
- Transgenic Invertebrates

### Transgenic Plants
- Other (please specify below)

### Other(s)

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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

<table>
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<tr>
<th>Class 1 activities:</th>
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<tbody>
<tr>
<td>i) Adenovirus based vectors for gene delivery in the mammalian nervous system cells</td>
</tr>
<tr>
<td>ii) Neuroanatomical tracer studies in rats involving vector-derived Green Fluorescent Protein (GFP)</td>
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</table>
| iii) Lentiviral vectors for gene delivery in mammalian precursor cells and CNS* The waste management measures will include:-  
All GMM waste containing and including tissue sample, contaminated plastics will be incinerated in the on site incinerator. This will give 100% degree of kill. For contaminated liquid waste, a 5% solution of Vercon (concentration used validated by manufacturer) will be added to media and liquids overnight and then disposed of down the sink. |
| *iii) Project Title: Regulation of neural precursor behaviour using retroviral plasmid vectors. |
| v) Project Title: Mechanisms of protein aggregation in neurodegenerative diseases. |

---

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

---

**Please enter comments of the GM safety committee on the risk assessment**

The committee meets 4 times per year to review all current and new applications. It is composed of principal investigators, senior laboratory technicians as well as head of department and biological safety officer.
**GM Centre Number: 794**

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**Name**

MEDIVIR UK LTD

**Name 2**

Department

**Campus Estate or Research Centre**

CHESTERFORD RESEARCH PARK

**Road Name**

LITTLE CHESTERFORD

**Town**

ESSEX

**County**

ESSEX

**Postcode**

CB10 1XL

**Country**

ENGLAND

**Tel Number**

01799 532100

**Fax Number**

01799 532101

**E-mail**

HSE Division

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Yes

Give brief details of the genetic modification safety committee

SAME COMMITTEE AS THAT FOR ACAMBIS RESEARCH AS WE ARE USING THE SAME FACILITIES AND SAFETY CODE OF PRACTICE AND WE NEED TO ENSURE THERE IS AN AGREED AND COMMON APPROACH TO SAFETY.

THE COMMITTEE CONSISTS OF A CHAIRMAN, A BIOLOGICAL SAFETY OFFICER, A SECRETARY AND TWO LAB LIAISONS, ONE OF WHOM IS A LAY PERSON.

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Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

THE ORIGINAL RISK ASSESSMENT HAD GIVEN AN ACCESS FIGURE (UNDER THE BRENNER SCHEME) OF 10/1. ON FURTHER INVESTIGATION, WE FOUND THAT THE ACGM GUIDANCE NOTES (PART 2A, ANNEX II, PART III) SAY THAT BL21 CELLS MAY BE GIVEN A VALUE OF 10. THE RISK ASSESSMENT WAS ALTERED AND A CONTAINMENT LEVEL OF 2 WAS GIVEN TO THE PROJECT. IT WAS AGREED THAT ALL WORK WOULD BE CARRIED OUT IN THE GENETIC LAB AND THE PROJECT WAS SIGNED OFF.
### GM Centre Number: 795

<table>
<thead>
<tr>
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<th>Transitional Premises Class</th>
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**Name**

MILLENNIUM PHARMACEUTICALS RESEARCH & DEVELOPMENT LTD

**Campus Estate or Research Centre**

**Name 2**

**Department**

**Road Name**

GRANTA PARK

**District**

GREAT ABINGTON

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB1 6ET

**Country**

ENGLAND

**Tel Number**

01223 722400

**Fax Number**

01223 722401

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

CHANGED NAME FROM MILLENNIUM PHARMACEUTICALS LTD ON 29/10/2002

**Date at Which Additional Info Submitted**

06/11/2002
### Premises Addresses

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<td>CB1 6ET</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Genetic Modification (GM) Safety Committee consists of 5 members - a Chairman, a GM Safety officer and 3 other members representing the Biology Department, the Chemistry Department and non-Scientists. The Committee convenes formal meetings when risk assessment is required and on changes in the HSE advice and regulations in addition.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<td>Level 1 (GMMs)</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

A built-in fully serviced (regularly) autoclave will be used for all heat inactivation. Monitoring tape will be used on waste vessels as a back-up indicator to ensure complete heat inactivation cycles. This autoclave is in the same building as the laboratories used, and waste will be disinfected using 30 g/l to minimise GMO growth while awaiting heat inactivation. All solid waste (plastic ware) will be autoclaved then incinerated as clinical waste. The above processes will ensure 100% kill and limit the risk of escape to the environment to effectively zero. Local rules will be displayed in all GM labs and on the Company Intranet to advise on both safe handling and disposal of GM's. All relevant staff will be trained on GMM handling as part of the Company induction process. Regular audits of GM practice will be performed by members of the GM Committee.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Training required for laboratory technician to operate autoclaves and spillage procedure.

Project Ref 795/01.1

Date Ackn'd 30/11/2001

Date Project Ceased 12/09/2003

Class CultureVol

Class 2 1-50 litres

Consent Granted not applicable

Project notified under transitional arrangements N
The purpose of this work is to allow the pharmacological evaluation of novel chemical entities which may interact with mammalian ion channels and receptors (e.g. HERG channels are the molecular target responsible for the cardiac toxicity of many pharmaceutical agents. Evaluation of the degree of interaction of a novel chemical entity with recombinant HERG channels will therefore allow an assessment of the likely cardiac side-effects of the novel chemical entity).

HEK293 (Human embryonic kidney cells, clone 293) is a cell line with epithelial morphology which was first created in the 1970s by treating human embryonic kidney cells with sheared fragments of adenovirus type 5 (Ad5) DNA (Graham et al, J Gen. Virol., 36, 59-72). The cells contain about 22% of the viral genome, including Ad5 early region E1A. The expression of the E1A has made these cells a popular host cell line, as expression of recombinant proteins driven by an SV40 promoter are maximized (since the SV40 is activated by E1A product). Since the late 1970s, HEK-293s have been commonly used for the cultivation of adenoviruses, and the expression of recombinant mammalian (including human) membrane receptors and ion channels, and numerous other mammalian proteins. There is no evidence for the presence of infectious virus or toxic products in HEK293 cells (see ECACC [European Collection of Cell Cultures] guidelines for these cells [ECACC 85120602]). The oncogenicity of these cells has been evaluated: Injection of 10-20 million cells subcutaneously into 5-6 week-old "nude" mice. After 6-8 weeks no tumours were visible and the animals re-injected. After 15-20 weeks after the first injection tumours became apparent in a small proportion of treated animals (In contrast, Ad5 DNA transformed rat or hamster cells induced tumours in 90-100% of injected mice within 3-6 weeks).

Commercial mammalian expression vectors, such as pcDNA3, or its later derivatives, will be used. Such vectors are considered non-mobilizable.

The ion channel/receptor genes to be expressed will either be obtained commercially, or from academic institutions, or from the USA parent company of Millennium Pharmaceuticals. Genes obtained from these sources will first be sub-cloned into the mammalian expression vectors referred to above prior to any further work. The expressed protein products of the genes expressed in HEK-293 cells will be used in a variety of pharmacological assays designed to assess the interaction of small molecules with these proteins.

HEK293 cells, when injected in large numbers, have been reported to produce tumours in nude mice (see above). The live cells should therefore be considered potentially oncogenic to humans. For this reason alone it is suggested these cells should be handled at containment level 2. Such a classification is consistent with the category 2 containment level recommended by ECACC for these cells.
The expressed proteins are unlikely to alter the pathogenicity of the cloning host, except in so much as indicated below. Furthermore, HEK-293 cell lines expressing a wide variety of receptors and channels are reported to exhibit no significant alterations in growth or morphology characteristics from the parental cell line.

As a protein normally expressed on the extracellular surfaces of cells, recombinant channels and receptors might conceivably present a hazard if personnel were to develop antibodies to these proteins, and thereby develop an autoimmune reaction. However, the expression of similar membrane proteins in HEK-293 cells, and in other mammalian cell hosts, has never given rise to a single reported incidence of this type. The risk must therefore be considered minimal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The HEK-293 cells, and HEK293 cells transfected with mammalian ion channels and receptors would be grown, harvested and processed to membrane preparations using published methods. All plasticware would then be autoclaved and disposed of by incineration. Culture media supernatant will be disposed of by addition of virkon to 30g/litre, and heat inactivation by autoclave. Cells will be pelleted by centrifugation and then processed into membranes by using a tissue homogenizer. Homogenizers and related equipment will be disinfected with virkon and subsequently water. All processes will be designed to minimize exposure to the operator in the form of aerosols (all such activities, such as homogenization, will be carried out in a fume hood). All processes for disinfection and disposal will be validated prior to regular use with HEK293 cells.

In the event of spillage from the flasks either within the incubators or the laboratory, the area can be disinfected, effectively. This will be performed using virkon solution (1%) followed by 70% alcohol wipes. For laboratory operations a standard containment level 2 facility will be used. This, in combination with the use of good cell culture practice will be sufficient to limit contact with humans and the environment.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All plasticware and cells for disposal would then be autoclaved on site, then taken off-site by a licenced clinical waste disposal company and disposed of by incineration. Culture media supernatant will be disposed of by addition of virkon to a final concentration of 1% virkon, left to stand overnight, and then disposed of as aqueous waste in the normal water supply waste system.

Daily, weekly and quarterly inspection and maintenance is carried out and documented on the autoclave, according to the manufacturers instructions.

Virkon used at a final concentration of 1% is a powerful broad spectrum disinfectant, with efficacy against 61 different strains of virus falling into 19 virus families, 397 different strains of bacteria in 42 genera, 102 strains of fungi in 26 genera. Virkon also has a very favourable eco-toxicity and biodegradability profile, and is recommended for use by disposal in the normal water supply system (see www.antechh.com for further details on virkon). The use of virkon to successfully kill HEK293 cells does not appear to have been documented in the literature. For this reason, all disinfection and disposal procedures using virkon to dispose of HEK293 cells will be validated and documented prior to regular use with HEK293 cells.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
GMSC agree with the evaluation of this work, ie; that it should be considered class 2. In connection with the attached Risk Assessment, a concern was raised over documenting the training of lab personnel to work at this level of containment. It was agreed that training should be documented as a separate item on the "COSHH and safety training record" form for each individual worker.

### Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Name**

RENOVO LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

MANCHESTER INCUBATOR BUILDING

**Road Name**

48 GRAFTON STREET

**District**

**Town**

MANCHESTER

**County**

GREATER MANCHESTER

**Postcode**

M13 9XX

**Country**

ENGLAND

**Tel Number**

0161 606 7222

**Fax Number**

0161 606 7333

**E-mail**

**HSE Division**

NORTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

**Y**

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee Members: Research Programme Manager, Research Director, Laboratory Manager, Biological Safety Officer, Administration Representative and the Consultant Biological Safety Officer.

The function of the GMSC: Provide advice on, review and approve risk assessments submitted to it for approval to start GMO activities. Review the progress of GMO activities. The GMSC will meet at least every 3 months or sooner once a risk assessment has been submitted for approval.

<table>
<thead>
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<tr>
<td>Non-microbial</td>
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<td>Yes</td>
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Other (please specify) Tick if confidential

02/03/2022

Page 11351 of 15326
GM laboratory waste will be inactivated by means of autoclaving with validated autoclave cycles to ensure that the load is maintained at a minimum temperature of 121°C for a minimum of 15 minutes to effectively produce 100% kill. The temperature and time of the autoclave cycle is printed out by the autoclave chart recorder which has been calibrated for accuracy.

The autoclave will be re-validated on an annual basis using UKAS certified independent thermocouples placed in the centre of the load.

Liquid waste that has been autoclaved will be emptied to drain.

Solid waste that has been autoclaved will then be transported off site by a registered contractor for incineration.

For activities involving GMMs, describe the waste management measures which will apply to the activity

GM laboratory waste will be inactivated by means of autoclaving with validated autoclave cycles to ensure that the load is maintained at a minimum temperature of 121°C for a minimum of 15 minutes to effectively produce 100% kill. The temperature and time of the autoclave cycle is printed out by the autoclave chart recorder which has been calibrated for accuracy. The autoclave will be re-validated on an annual basis using UKAS certified independent thermocouples placed in the centre of the load. Liquid waste that has been autoclaved will be emptied to drain. Solid waste that has been autoclaved will then be transported off site by a registered contractor for incineration.

<table>
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<tr>
<th>Other(s)</th>
<th>In-Vitro cell culture</th>
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</thead>
</table>

Tick to confirm that you are attaching a summary of the risk assessment

Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Y

Please enter comments of the GM safety committee on the risk assessment

The Genetic Modification Safety Committee has fully reviewed the Risk Assessment and the control measures in place and these are satisfactory to control the level of risk.

Conclusion: The GMSC approved the project.
GM Centre Number: 797

Data Premises Notified (Originally) 29/08/2001

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Emergency Plan Required? N

Transitional Premises

Non-GMMs N

Withdrawn N

Name

REPLIZYME LIMITED

Name 2

Department

Campus Estate or Research Centre

Building

GENESIS 2 BUILDING

District

HESLINGTON

Road Name

YORK SCIENCE PARK

Town

YORK

County

YORKSHIRE

Postcode

YO10 5DQ

Country

ENGLAND

Tel Number 01904 75 11 55

Fax Number 01904 778040

E-mail

HSE Division YORKSHIRE AND NORTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>HESLINGTON</td>
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<td>YORKSHIRE</td>
<td>YO10 5DQ</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Composed of Special Projects Manager, acting as Company Safety Officer, with view to overall safety issues; target development leader, acting as Biological Safety Officer, with a view to laboratory based safety issues; Science Officer who manages all science in the laboratory and the Science Director who determines the nature of the science the company undertakes. The GMSC meets to discuss all new projects and to review current company policy on safety and other GM issues. Meetings are held quarterly and additionally, as required.

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Tick if confidential

Yes

Bacteriology: Yes

Parasitology: Yes

Transgenic Birds: Yes

Microbiology Research: Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Category</th>
<th>Action</th>
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<tbody>
<tr>
<td><strong>LIQUID WASTE</strong></td>
<td>Add an equal volume of 2% VIRKON solution and leave overnight prior to disposal down assigned sinks.</td>
</tr>
<tr>
<td><strong>DISPOSABLE PLASTICS NON-CONTAMINATED</strong></td>
<td>Placed in yellow incinerator bags. Sealed with swan necks and placed in storage container provided by disposal company prior to their removal by our contractors (Johnsons).</td>
</tr>
<tr>
<td><strong>REUSABLE PLASTICS CONTAMINATED</strong></td>
<td>Immerse in 1% VIRKON overnight. Rinse in 5 changes of tap water, fill with tap water and autoclave at 121C/20. Once decontaminated, rinse with diHO and reuse.</td>
</tr>
<tr>
<td><strong>DISPOSABLE PIPETTES</strong></td>
<td>Placed in ? QUIVERS. Removed by contractors.</td>
</tr>
<tr>
<td><strong>BACTERIAL CULTURES (AGAR PLATES)</strong></td>
<td>Discarded in pots of 1% VIRKON. Left overnight. Drain of liquid and place in 60L medibin for disposal by contractors.</td>
</tr>
<tr>
<td><strong>LAB COATS</strong></td>
<td>If contaminated, autoclave. All lab coats cleaned by outside contractors.</td>
</tr>
<tr>
<td><strong>N.B.</strong></td>
<td>Liquid waste i.e. culture solution is periodically checked for efficiency by culture on agar plates. All solutions are made up fresh to avoid inactivation.</td>
</tr>
</tbody>
</table>

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Science Director - Risk assessment for the activities at Replizyme have been carried out and it appears their assignment to class 1 is entirely appropriate.

Biological Safety Officer - I confirm that the risk assessment is an accurate representation of the nature of the GMM work carried out at Replizyme and that level 1 containment facilities are adequate.
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The GMSC will initially consist of three members (of the current seven employees) and will expand as the company expands to incorporate representative of all involved employees. The current makeup is:
- Chairman and Biological Safety Officer
- The Chief Scientific Officer, as a representative of senior management
- Staff representative
- An external specialist advisor will be consulted on an ad hoc basis where necessary to supplement internal expertise
- As new employees are recruited, the following two additional people will be elected to the GMSC:
  - A representative of the Research Scientists (currently only Senior Research Scientists are employed)
  - A representative of the Technical Staff (as above, no technical staff are currently employed).

The Diversys GMSC will meet on a quarterly basis, or whenever necessary for company needs. Its remit is to advise on risk assessments made on new GMO activities, or proposed changes to ongoing activities, under the Genetically Modified Organisms (Contained Use) Regulations 2000.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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All solid waste contaminated by GMMs and bacterial cell pellets to be disposed of by autoclaving, followed by incineration. This disposal route is considered to give essentially 100% kill of E.coli. The site autoclave is routinely tested for temperature and pressure attainment, and verification of waste inactivation.

All GMM liquid wastes to be inactivated by addition of Virkon to >0.25% final concentration, allowed to stand for 30 mins and then disposed of down sinks.

Contaminated reusable glass and plastic ware will be similarly disinfected with 0.25% Virkon prior to washing. Virkon has been shown to give >6 logs of kill of E.coli under these conditions (source: Antec International). This will be verified by streaking of a sample of the inactivated fluid onto Luria agar plates, which should show no growth if inactivation is effective.

These procedures will reduce any risk to humans or the environment to effectively zero.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
This application was considered by circulation. The work involves standard antibody repertoire expression and selection, with subsequent expression of the selected antibody. The feature where this differs from many such projects is in the expression of human cDNA libraries for use in screening. The libraries are from healthy individuals (and anonymous, so there is no ethical problem if anything unexpected is detected) and are only expressed as trace amounts of protein. Moreover, expression is in E. coli and no refolding will be undertaken, rather the cytoplasm used directly so that the protein (or fragments of it) will be denatured and highly unlikely to display any potential activity. The work falls naturally into Class 1, requiring only Containment Level 1.

**Project Ref** 798/02.1

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<td>GENERATION, EXPRESSION AND PURIFICATION OF HUMAN TUMOUR NECROSIS FACTOR ALPHA (TNF-ALPHA) IN E.COLI</td>
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<tr>
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**Recipient or parental organism**

E. coli JM105 (thi, rpsL(Str ), endA, sbcB15, hsdR4, supE_ (lac-proAB), f[traD36,proAB+, lacIq, LacZ_M15]), supplied by Amersham Biotech, Amersham, UK.

E.coli NovaBlue (endA1, hsdR17(rk12-,Mk12+), supE44, thi-1, recA1, gyrA96, relA1, lac[f'proA+B+,lacqZ_M15_Tn10(tet)], supplied by Novagen, Madison, USA).

E.coli XL1 blue (recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [FcproAB lacIq ZDM15 Tn10 (Tet)] (Stratagene).

The host E coli strains NovaBlue, XL1blue and JM105 are E. coli K12 derivatives and have a long history of safe use. They are non-colonising and multiple disabled. In terms of environmental safety, the host strains are multiply disabled, and are unlikely to survive in the environment, and considered non-pathogenic to humans or animals.
The plasmids are well characterised, non-mobilisable, derivatives of commercially supplied plasmids with an established history of safe laboratory use.

**Host/vector system**

The cDNA encoding human TNF-alpha will be obtained from the Medical Research Council Laboratory of Molecular Biology, in either the native human form, or the E. coli codon optimised form. Native TNF is expressed in the human as an inactive membrane-bound 233 amino acid precursor, which becomes active upon cleavage between residues 76-77. We will clone the truncated cDNA, encoding the active portion of the molecule in-frame with the N-terminal epitope tags His6 and HA or FLAG. Induction of expression with IPTG (isopropylthiogalactoside) will result in the production of a soluble TNF-alpha fusion protein, which is likely to be biologically active. This fusion protein will be affinity purified on nickel based resin, and eluted with imidazole to produce epitope-tagged TNF-alpha, which may be further purified.

The intended function is therefore to produce active soluble TNF-alpha.

**Origin & function**

TNF-alpha is a pro-inflammatory cytokine, produced mainly by activated monocytes and macrophages, and appears to be a key mediator in the induction of cytokine cascades associated with inflammatory responses. Recombinant human TNF-alpha has been used in a number of clinical trials as an anti-cancer therapy with intravenous doses of up to 300mg being tolerated (eg F Lejeune et al (1994) Journal of Cellular Biochemistry 56:52-61).

The most likely effect of accidental ingestion or injection of recombinant TNF-alpha would be localised inflammation and pyrexia. It is extremely unlikely that a person could be exposed to TNF-alpha levels exceeding the levels used clinically through the experimental work above. Even if all native gut flora were replaced with E. coli harbouring the plasmid encoding the TNF-alpha fusion protein, in the absence of induction protein expression levels are low, and unlikely to exceed this level. There is also a small risk of anaphylaxis, although this risk is common to many proteins, and the Class 2 precautions that this work will be done under will reduce this risk to effectively zero.

The planned experiments utilise disabled host strains that are unlikely to survive in the environment. In terms of environmental safety, the host strains are multiply disabled, and are unable to survive in the environment, and considered non-pathogenic to humans or animals. The expression of a pro-inflammatory cytokine is unlikely to enhance the pathogenic potential of the final GMO, and may decrease any such potential, by inducing a more potent immune response.

**Evaluation of foreseeable effects**

TNF-alpha is a pro-inflammatory cytokine, produced mainly by activated monocytes and macrophages, and appears to be a key mediator in the induction of cytokine cascades associated with inflammatory responses. Recombinant human TNF-alpha has been used in a number of clinical trials as an anti-cancer therapy with intravenous doses of up to 300mg being tolerated (eg F Lejeune et al (1994) Journal of Cellular Biochemistry 56:52-61).

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**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

*not applicable*

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

*No containment derogations are being applied for.*

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid waste contaminated by GMMs and bacterial cell pellets to be disposed of by autoclaving at a load temperature of 121 degrees C for 20 minutes. This disposal route is considered to give essentially 100% kill of E. coli. The site autoclave is routinely tested for temperature and pressure attainment, and verification of waste inactivation.

All GMM liquid wastes to be inactivated by addition of Virkon to >0.25% final concentration, allowed to stand for 30 min and then disposed of down sinks, with additional running water to dilute the waste.

Contaminated re-useable glass and plastic ware will be similarly disinfected with 0.25% Virkon prior to washing. Virkon has been shown to give >6 logs of kill of E. coli under these conditions (source: Antec International). This will be verified by streaking of a sample of the inactivated fluid onto Luria agar plates, which should show no growth if inactivation is effective.
These procedures will reduce any risk to humans or the environment to effectively zero.

The application was considered by the GMSC to be at class 2. The host/vector system used is of minimal hazard, but hearing in mind the potential biological activity of the cytokine produced, class 2 working was considered appropriate to contain all potential hazards.

Please enter comments on the GM safety committee on the risk assessment

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<tr>
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Project Ref 798/05.1

Date Ackn'd 02/09/2005  CU2 Project Title

Expression of Peptostreptococcus magnus Protein L domains B1-4 in E. coli under shake flask and fermentation conditions.

Date Project Ceased 22/06/2007

Class 2  CultureVol

< 1 Litre  ClassVol

< 1 Litre

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

Protein L is a cell wall protein of *Peptostreptococcus magnus*. It has a multiple domain structure, consisting of an A domain, and five B domains, termed B1-B5 which are homologous IgG binding domains. The remainder of the protein anchors it to the peptostreptococcal cell wall.

Domains B1-B4 have been cloned into a E. coli expression vector, pJB, to give plasmid pAL15M, which will be transformed into E. coli RV308. On induction with toluic acid, expression of intracellular protein consisting of domains B1-4 is induced. The protein is released from the cells by freeze-thaw cycling and further purified.

The resulting protein is a high affinity capture and purification reagent for mammalian immunoglobulins and light chain antibody fragments.

**Recipient or parental organism**

RV308 ( (lac)X74 galPO-308::IS2 rpsL). Source ATCC. An E. coli K12 derivative, non-colonising.

**Host/vector system**

pAL15M. Kanamycin resistant, toluic acid inducible expression of insert protein. Mobilisable, OriV and OriT wild type from RK2 are present.

Expression is induced in this system using the specific inducer toluic acid. Expression levels in the absence of inducer are low.

**Origin & function**

Insert DNA originally from *Peptostreptococcus magnus*. Contains the B1-B4 domains of protein L, giving a high affinity IgG binding protein. This will be produced as soluble functional intracellular protein once the culture has been induced with toluic acid.

**Evaluation of foreseeable effects**

The full length protein is a bacterial superantigen. Expression of the protein in *Peptostreptococci* sp. Correlates with virulence. Expression as a cytoplasmic protein would not be predicted based on the available evidence to make the E. coli pathogenic, as the proteins role in pathogenesis correlates with cell surface expression in the wild-type organism.

The B1-B4 domain shown here will also act as a superantigen, and has been shown to stimulate B cel proliferation in a polyclonal fashion, and to cause depletion of
subsets of B-cells via apoptosis. Thus the protein may be expected to have immunological effects.


The protein has also been shown to trigger histamine and other cytokine release from mast cells and basophils via cross linking to surface IgEs. Therefore it has the potential to cause anaphylaxis on inhalation or absorption.


The protein may be a sensitiser, and thus skin contact or inhalation must be avoided.

The use of mobilisation competent vector makes transfer to related species more likely. However, expression from a mobilisation defective vector has been shown to be lower, therefore requiring larger culture volumes and increased risks (Affitech data not shown).

Various Peptostreptococci are already found in the environment, some of which express Protein L as a superantigen. Therefore, with the containment used within the lab, together with the inactivation methods used, makes the risk of any additional environmental hazards effectively zero.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Spills will be disinfected with solid Virkon powder. This has been tested by plating experiments on shake flask cultures and low cell density fermentation to give assentially 100% inactivation of organisms in split media.

Fermenters will be sterilised post fermentation at 121 degrees C for 60 minutes. This has been shown to give 100% inactivation of organisms.

Liquid waste and spent culture medium will be sterilised by autoclaving at 121 degrees C for 60 minutes. This has been shown to give 100% inactivation of viable organisms. Sterile liquid waste will then be offsite incinerated.

Centrifuge bottles and solid wastes will be sterilised by autoclaving as above. This has been shown to give 100% inactivation of viable organism. Solid waste will then be offsite incinerated with the clinical waste stream.

Re-usable lab items will be disinfected in >2% Virkon for >30 minutes. This has been demonstrated to give essentially 100% kill of viable organisms. They will then be washed.

Additional testing of inactivation will be performed, where necessary, by streaking samples onto 2xTY plus kanamycin plates.

All items to be cleaned will be kept wet to avoid the production of dry material containing Protein L with dust hazard potential and cleaned promptly.
The application of the expression of Protein L using the Affitech HCD fermentation protocol was approved at class 2 as DM38, and will be notified to the HSE.

Emphasis must be placed on the potential for sensitisation and anaphylaxis for the protein, under COSHH. All work with this system must be covered by SOPs for exposure control and cleanup. These will be drawn up and approved before work can start.

Project Containment

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<tr>
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Project Ref 798/06.1

Expression of a human cytokine genetically fused to a protein moiety capable of extending serum half-life in E. coli and P. pastoris.

Date Ackn'd: 13/11/2006
Date Project Ceased: 22/06/2007

Consent Granted: Not Applicable

Historical Significant Changes: TRANSFERRED TO GM 558 (22/6/07).
**Significant Change ID**

**Date of Significant Change**

## Project Additional Information

### Purposes of the contained use

Generation of a potential therapeutic compound with extended in vivo half-life.

### Recipient or parental organism

E. coli will be transformed with an expression construct with the intention of maximising expression levels of the fused protein. It has been shown that periplasmic/soluble expression of this cytokine in E. coli results in active protein. In vivo, the protein is known to affect cell growth and development and in clinical trials has been shown to cause a range of effects, both local and systemic. The Domantis GMSC considered the possibility of deleterious effects due to this GMO warranted classification to CL2.

Likewise P. pastoris will, in all probability, secrete active protein. It is considered that the recipient organism in this instance is less liable to colonise or persist in the environment, and this work is therefore classed at CL1.

### Host/vector system

E. coli stains based upon the genetic backgrounds K12 (TOP10f', JM83, HB2151), W (Mach I) and B (BL21) will be used for cloning and expression work. Vectors used will be based upon the commercially available vectors pET21 and pET23, and the Domantis vector pDOM5. The pET vectors contain the T7 promoter under IPTG control; pDOM5 contains the lac promoter under IPTG control. None of the vectors are considered mobilizable.

### Origin & function

The cDNA for cytokine will be created synthetically under contract. The genetic material for the half-life extending moiety derives from Domantis phage libraries. In these constructs this functionality will be inactivated.

The expression constructs generated will be used to direct the production of a recombinant fusion protein, different strategies will be employed based upon refolded inclusion bodies as well as soluble secreted protein. It has been assessed that the fusion expressed in inclusion bodies results in a less hazardous GMM that can be manipulated under CL1. Soluble material will also be produced in mammalian cell culture. Due to the nature of this expression system, it is also considered to be suitable for manipulation at CL1, although for sample protection it will be handled at CL2.

### Evaluation of foreseeable effects

The cytokine is a potent growth factor, with a pleiotropic range of biological effects. Two distinct receptors exist though the specific roles of these distinct molecules has yet to be fully elucidated. The cytokine has been shown to be mitogenic for certain cancer types including breast cancer, and mutations in one of the receptors are associated with a range of adverse effects in affected individuals.

The production of a fully functional cytokine from genetically modified E. coli has the potential to, in a worst-case setting, expose workers to the effects of the cytokine, which are reportedly related to various types of pain. The formatting with a half-life extending moiety has the likely effect of extending the residence time in the circulation of anyone that may become exposed to the cytokine. This will be unlikely to occur in these compounds where the function will be inactivated; however the larger molecule may still have some minor half-life extension. Although E. coli strains being used are disabled, it is the recommendation of the GMSC that additional containment in line with CL2 regulations are applied for this work.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste contaminated by GMMs and bacterial cell pellets to be disposed of by autoclaving at a load temperature of 121°C for 20 minutes. This disposal route is considered to give essentially 100% kill of E.coli. The site autoclave is routinely tested for temperature and pressure attainment, and verification of waste inactivation. All GMM liquid wastes to be inactivated by addition of Virkon to >0.5% final concentration & allowed to stand for 30 min. Waste collected in autoclavable carboys and autoclaved prior to disposal via sink with copious amounts of water. Contaminated re-useable glass and plastic ware will be similarly disinfected with 0.5% Virkon prior to washing. Virkon has been shown to give >6 logs of kill of E.coli under these conditions (source: Antec International). This will be verified by streaking of a sample of the inactivated fluid onto Luria agar plates, which should show no growth if inactivation is effective. These procedures will reduce any risk to humans or the environment to effectively zero.

Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Tick to confirm that you have attached a risk assessment to this form N

Please enter comments on the GM safety committee on the risk assessment

The application was considered by the GMSC to be at class 2. The host/vector system used was considered to be of low hazard, but attention was paid to the potential biological activity of the cytokine produced and the likely extended half-life in the event of serum exposure. Therefore class 2 working was considered appropriate to contain all potential hazards.

Project Containment

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</tbody>
</table>

### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Y

**Give brief details of the genetic modification safety committee**

The Genetic Modification Safety Committee consists of a Research Director, a Research Scientist employee (who has attended a 2-day course on genetic modification at Leeds University) and a Scientific Consultant from the Department of Genetics, University of Cambridge.

The Committee will meet as and when necessary depending on when there are any proposed changes to working conditions, equipment, level of GMO etc. The committee will review copies of all relevant documents raised within the company regarding safety aspects of genetic modification.

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<tr>
<td>Level 2 (GMMs)</td>
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</table>

02/03/2022
All liquid cultures and plastics and glassware etc. contaminated with bacterial cultures will be stored in the laboratory until they are autoclaved. No more than 1 litre (in 100 ml volumes) will be allowed to accumulate in the laboratory before autoclaving. Similarly, autoclave bags will be treated as soon as they are full.

The autoclave is in a central facility provided by the Babraham Research Institute and its efficacy is monitored regularly. Autoclaved waste is then sent for incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

I confirm that the comments from all members of our GM Safety Committee are that the risk assessment and all other information given in form CU1 is acceptable and correct.
**Project Additional Information**

**Purposes of the contained use**

Contained use will allow the modification of a suitable vector to include a gene which will inhibit the growth of the ACDP hazard group 2 organism S. aureus and for this vector to be maintained in an S. aureus strain.

**Recipient or parental organism**

S. aureus strains are classified as ACDP hazard group 2 organisms, capable of colonisation and infection of humans and animals as well as survival in the general environment.

**Host/vector system**

The host comprises a strain of S. aureus and the principal vectors include non-mobilisable plasmids of both S. aureus and E. coli.

**Origin & function**

The gene to be used and the protein itself, are ubiquitous in the environment. The intended function of the protein is to inhibit growth of pathogenic bacteria. A suitable marker gene will also be included to track the presence of the proposed gene.

**Evaluation of foreseeable effects**

As an ACDP hazard group 2 biological agent, S. aureus is pathogenic to humans and animals, and capable of survival and growth in the environment. The possibility of gene transfer between S. aureus strains is possible. However, expression of the proposed gene in recipient bacterial cells will most likely lead to cell death and the propagation of any natural vector containing the gene would therefore be minimised or inhibited. Thus, whilst a risk to human health has been identified in the pathogenic nature of wild type S. aureus, none would be directly associated with the presence of the proposed gene. In fact, the gene may actually reduce the overall fitness of the GMM, and therefore the risk to both human health and the environment.

The use of the proposed marker genes would not result in the introduction of novel resistance determinants or the production of hazardous compounds in the natural S.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Appropriate disinfectant will be added to spent liquid cultures. This waste will not be stored for more than 3 days and no more than 1 litre combined volume will be allowed to accumulate before autoclaving.

Plates and other contaminated waste including plasticware will be discarded to autoclave bags and autoclaved as soon as the bags are full. The autoclave is in a central facility provided by the Babraham Institute and its efficacy is monitored regularly. Autoclaved waste is then placed into leak-proof containers and collected for incineration by a Babraham Institute appointed organisation.

Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Tick if you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

The Genetic Modification Safety Committee do not have any particular comments.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
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**Name**

STEM CELL SCIENCES (UK) LIMITED

**Name 2**

CENTRE FOR GENOME RESEARCH

**Campus Estate or Research Centre**

**Road Name**

WEST MAINS ROAD

**Town**

EDINBURGH

**County**

EAST LOTHIAN

**Postcode**

EH9 3JQ

**Country**

SCOTLAND

**Tel Number**

0131 650 8587

**Fax Number**

0131 662 9779

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

A local Genetic Modification Safety Committee (LGMSC) has been established consisting of four individuals, two from within the company (Chief Operating Officer and Principal Scientist). The University of Edinburgh staff are both involved with daily operation of the University LGMSC scheme. The Committee reviews risk assessments of proposed projects prior to their commencement and will notify HSE accordingly. Meetings are held on a quarterly basis to review the validity of any current risk assessments. The minutes of these meetings together with copies of all Risk Assessments performed and decisions taken are held by the BSO. Annual returns to the HSE will also be held by the BSE. Copies of the Regulations and The Compendium of Guidance are also held by the BSE. These are available for consultation by all company staff but must be signed out.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

Bacteriology: Yes
Parasitology
Transgenic
Birds
Microbiology: Research
Virology
Transgenic
Animals
Transgenic
Fish
Gene Therapy
Mycology
Transgenic
Invertebrates
Transgenic
Plants
Other (please specify below): Yes

For activities involving GMMs, describe the waste management measures which will apply to the activity:

In vitro generation of pure populations of cell types by genetic selection... Embryonic Stem Cells.
All such laboratory waste will be disposed of by autoclaving and/or incineration. This will be achieved using existing University of Edinburgh procedures and facilities. The efficient operation of autoclaves is ensured by regular servicing by qualified engineers.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The committee were satisfied that the risk assessment SCS001 had been undertaken satisfactorily but felt that the inclusion of the following details would make it easier to understand for the general public.
1. The Encephalo Myocarditus Virus IRES sequence to be used should be referenced
2. The calculation of risk should be explained better
3. The fact that mouse cells are manipulated in class II laminar flow hoods should be included in Section 3 stages II and IV

All of these points have been addressed in the enclosed Risk Assessment.
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<th>Field</th>
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<td>0141 576 5546</td>
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<tr>
<td>E-mail</td>
<td><a href="mailto:info@pantherix.com">info@pantherix.com</a></td>
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<td>HSE Division</td>
<td>SCOTLAND</td>
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<tr>
<td>Comments</td>
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<td>Date at Which Additional Info Submitted</td>
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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<tbody>
<tr>
<td><strong>Company Safety Officer, Biological Safety Officer, Two laboratory scientists (both involved in GM work, one scientist at PhD level).</strong></td>
<td><strong>Safety Committee to meet at least every two months to review risk assessments and procedures.</strong></td>
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**Laboratory** | **Animal Unit** | **Growth Room** | **Glass House** | **Large Scale** |
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**Non-microbial**

Other (please specify)  

Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**
- **Mycology**
- **Transgenic Invertebrates**
- **Transgenic Plants**
- **Other (please specify below)**

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity.
### Waste Management

All waste material is routinely inactivated by sterilisation in an autoclave. This procedure will effectively result in a 100% kill of GMMs.

Validation of the sterilisation procedure is via annual testing of the autoclave using independent thermocouples.

Verification of routine inactivation is by a load probe monitored by chart recorder, and by the inclusion of test indicators.

**Tick to confirm that you are attaching a summary of the risk assessment** Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment** Y

---

### Project Ref 801/01.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
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<tr>
<td>07/09/2001</td>
<td>OVER EXPRESSION OF HOUSEKEEPING GENES IN STAPHYLOCOCCUS AUREUS 8325-4 TO YIELD STRAINS OVERPRODUCING PROTEINS</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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**Class CultureVolClass2 CultureVolumeClass3-4**

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<th>Date Project Ceased</th>
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<td>17/11/2003</td>
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**Tick if notifying a connected programme of work** Y

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### Project Additional Information

- Project notified under transitional arrangements N

---

**Historical Significant Changes**

- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change
### Purposes of the contained use

Creating strains of Staphylococcus aureus to be used in research into the discovery of antibiotics.

### Recipient or parental organism

Staphylococcus aureus 8325-4 and derivatives.

### Host/vector system

Vectors based on the non-mobilisable Bacillus subtilis vector pUB110.

### Origin & function

The origin of the genetic material is chromosomal DNA from various bacteria, including non-pathogenic and pathogenic (human pathogens from ACDP Class 2) bacteria. The genetic material comprises a number of housekeeping genes which are involved in normal metabolic processes in bacteria including the biosynthesis of vitamins, cofactors and amino acids. These genes are obtained by PCR amplification of DNA and sequenced prior to insertion into Staphylococcal cloning vectors such that the inserts are well-characterised. The inserted genetic material is intended to result in the over-expression of housekeeping genes.

### Evaluation of foreseeable effects

The results of the GM work will be to produce strains of Staphylococcus aureus that over-express genes coding for housekeeping proteins. These proteins are not involved in virulence mechanisms or pathogenicity and are therefore very unlikely to increase the pathogenicity of the host bacteria. Staphylococcus aureus 8325-4 is recognised to be a disabled strain and non-colonising towards humans. The possibility that over-expression of housekeeping genes could increase the pathogenicity of Staphylococcus aureus 8325-4 is negligible. Since the work will be carried out in a Level 2 containment laboratory, the level of containment applied to safeguard human health will also be sufficient to protect the environment.

Conclusions. The work is highly unlikely to have any effects on human health or the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Type and form of waste: General laboratory microbiological waste - GMMs in liquid cultures, and on agar plates, contaminated plasticware and glassware. Inactivation: All waste material is inactivated by sterilisation in an autoclave. This procedure will effectively result in a 100% kill of GMMs. Validation of the sterilisation procedure is via annual testing of the autoclave using independent thermocouples. Verification of routine inactivation is by a load probe monitored by chart recorder, and by the inclusion of test indicators.
Fate of waste: Inactivated liquid waste will be discharged into the waste water system. Inactivated solid waste will be disposed of by incineration.

The genetic modification safety committee considered that the control measures to be applied would prevent exposure to the hazard.

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee considered that the control measures to be applied would prevent exposure to the hazard.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 L3 L4 L2</td>
<td>L2 L3 L4 L2</td>
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Animal Units

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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### CONCEPT LIFE SCIENCES LIMITED

**Data Premises Notified (Originally)**: 14/09/2001  
**Transferred from 1992 Regs?**: N  
**Transitional Premises Class**:  
**Data Premises Closed**:  
**Transitional Premises Emergency Plan Required?**: N  
**Non-GMMs**: N  
**Withdrawn**: N

**Campus Estate or Research Centre**:  
**Building**: DUNDEE TECHNOPOLE  
**Road Name**: 2 JAMES LINDSAY PLACE  
**District**:  
**Town**: DUNDEE  
**County**: ANGUS  
**Postcode**: DD1 5JJ  
**Country**: SCOTLAND

**Tel Number**: 01382 432163  
**Fax Number**: 01382 432153

**HSE Division**: SCOTLAND

**Comments**:  
FORMERLY KNOWN AS XENOMICS LIMITED UP UNTIL 15 NOVEMBER 2001, Name change from CXR Bioscience Ltd 26/06/2017

**Date at Which Additional Info Submitted**: 12/12/2001
### Premises Addresses

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<td>JAMES LINDSAY PLACE</td>
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<td>ANGUS</td>
<td>DD1 5JJ</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

**Biological Safety Officer, Deputy Biological Safety Officer/chairperson, Laboratory Operations Manager, Senior Research Scientist and University of Dundee Biological Safety Adviser.**

The committee meets as required at least twice/year. Give advice on risk assessments, Confirm local rules are appropriate and implemented and Review inspection reports and accident reports.

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<td>Non-microbial</td>
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</table>

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

Yes Yes Yes Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity:

- **Solid waste** — Autoclaved, Essentially 100% kill, validated annually & in line temperature probe.
- **Liquid waste** — 1% Virkon; overnight exposure. Essentially 100% kill. Disposal into the drain, with liberal dilution using tapwater.

Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: 

---

**Project Ref**: 802/06.1

- **Date Ackn'd**: 13/04/2006
- **CU2 Project Title**: Generation of mammalian cell lines for use in drug development/toxicological screening.
- **Class**: Class 2
- **Culture Vol**: < 1 Litre
- **Consent Granted**: Not Applicable

---

Project notified under transitional arrangements: N

---

**Virology**

**Transgenic Animals**

**Gene Therapy**

**Mycology**

**Transgenic Invertebrates**

**Other (please specify below)**: Tissue culture

**Transgenic Fish**

**Tissue culture**

**Transgenic Plants**

**Gene Therapy**

**Other (please specify below)**: Yes
### Project Additional Information

**Purposes of the contained use**

To introduce DNA sequences of interest such as drug metabolising genes, reporter genes and inducible regulatory elements into recipient mammalian cell-lines to develop non-animal toxicological screens which facilitate drug-development.

**Recipient or parental organism**

Well characterised packaging cell-lines eg African green monkey kidney (VERO76)  
Commercially available, immortalised mammalian cell-lines such as MCF7, HepG2 and CHO. Primary cell-lines eg mouse, rat, human.

**Host/vector system**

Only well-characterised, replication deficient viral vector systems with a history of safe use will be used eg Virapower derived from the Human Immunodeficiency Virus 1 (HIV-1) genome and iBAC derived from Herpes Simplex Virus-1 (HSV-1) and Epstein-Barr Virus (EBV) genomes.

**Origin & function**

Viral genetic materials will be obtained from the licensed vector systems and used according to the manufacturer's protocols.

DNA sequences to be packaged for the generation of mammalian cell-lines shall be constructed de novo from synthetic oligonucleotides or PCR-amplified from plasmid, cDNA, BAC or genomic sources. Propagation and assembly shall be performed within disabled prokaryotic host strains.

The DNA sequences shall confer upon transduced cells the ability to report on toxicity by the following mechanisms:

1. Since metabolism by phase 1, 2 and 3 drug-metabolising enzymes are a major determinant of drug toxicity, the cell-lines shall be engineered to replicate human drug metabolism and consequent potential for toxicity through expression of these gene products.

2. Increases in the rate of expression of certain gene products eg CYP450s are indicative of toxicity. The intention is to place reporter genes under the direct control of inducible regulatory gene sequences to provide a convenient readout of potential toxicity.

**Evaluation of foreseeable effects**

The viral vectors used may infect a wide range of mammalian cells and though they will retain some of the properties of the wild-type virus, they shall be specially disabled to prevent proliferation outwith special packaging cell lines or in the presence of complimentary plasmids. Retroviral vectors, for example, will retain the ability to integrate into the host cell genome and stable express the gene of interest but shall be unable to replicate in any host. HSV-1 vectors will be maintained episomally and whilst they may replicate, their large size precludes them from being packaged into viable virus. Viral systems used contain additional safety features limiting the risk of the formation of wild-type virus via rare recombination events (1, 2).
The genes of interest are considered non-hazardous and are unlikely to pose a significant hazard to human health or the environment should exposure occur and no attempt shall be made to maximise the expression of a gene product. The inserted genetic materials are not expected to complement the disabled viral components and it is unlikely there will be any effect on the host range of tropism of wild-type virus should recombination occur.

All procedures involving viral vectors shall be performed at containment level 2 to prevent infection of staff or release into the wider environment.

Under ACDP guidelines wild-type HIV is a category 3 pathogen that can cause severe immunologic disease and whilst there is little risk of a wild-type pathogen being produced, the use of sharps will be avoided to eliminate the most likely route of transmission. Wild-type HSV-1 is a category 2 pathogen which, though common in the general population, can in rare cases cause encephalitis. The most likely route of transmission of the wild type virus is by aerosol thus all work shall be carried out in a class II biological safety cabinet.


Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

During the production of replication-defective viruses, all solid waste (plastics etc.) is placed in lined, biohazard, autoclavable bins. Non-sealable solid waste such as pipettes are rinsed in 1% Terminex prior to transfer to the autoclavable bin. Immediately following the completion of work the liners are loosely sealed and the lidded bin transported, on a trolley, directly to the autoclave facility for immediate autoclaving and disposal as controlled waste.

All liquid waste is collected in robust, autoclavable, sealable containers containing sufficient disinfectant (Terminex) to give a 1% final volume. After work the sealed container is transported, on a trolley, directly to the autoclave facility for immediate autoclaving. After autoclaving, liquid waste is disposed of to drain with copious amounts of cold water.

The autoclave is fitted with a thermocouple linked to a chart recorder to monitor the effective completion of the sterilisation cycle. SOPs stipulate that no material should be removed from the autoclave without first checking that the cycle was completed. The degree of kill for autoclaving is effectively 100%. The autoclaves are serviced and calibrated annually by a reputable service provider.

The use of sharps is avoided.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

### Project Containment

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

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**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Chair
- Management Representative
- Employee Representative
- Employee Representative
- Employee Representative

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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Other (please specify)

Tick if confidential

02/03/2022
### For activities involving GMMs, describe the waste management measures which will apply to the activity

All bacterially contaminated waste will be autoclaved or soaked in Virkon prior to disposal. Autoclave programme will be as follows:
- 134°C for 20 minutes, 8 minutes freestream x 2 monitored using autoclave tape or soaking in a 1% Virkon solution for at least 4 hours.
- Once inactivated, waste will be tested by incubation in a non-selective broth or solid media.

---

Tick to confirm that you are attaching a summary of the risk assessment: [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment: [ ]

---

Please enter comments of the GM safety committee on the risk assessment

The Genetic Modification Safety Committee will meet regularly (every quarter) to assess the risk of any new project which may affect the existing risk assessment, and to review existing measures. If any changes are deemed to be necessary, appropriate training or changes to the lab practice will be implemented by the appointed BSO.
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GM Centre Number: 804

| Data Premises Notified (Originally) | 04/10/2001 |
| Transferred from 1992 Regs? | N |
| Emergency Plan Required? | N |
| Data Premises Closed | 31/12/2010 |
| Transitional Premises Class | N |
| Non-GMMs | N |
| Withdrawn | N |

Comments
GM757 MERGED WITH ARROW THERAPEUTICS ON 04/1/2000
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes](Y)

Give brief details of the genetic modification safety committee

- Biological Safety Officer
- Chief Scientific Officer
- Chief Executive Officer
- Senior molecular biologist
- Molecular biologist

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<tr>
<th>Laboratory</th>
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All spent liquid media will be incubated overnight with 1% Virkon for sterilisation. Media will then be disposed of as normal liquid waste. All contaminated (disposable) solid waste ie pipettes, pipette tips, flasks and other plasticware will be double bagged in dedicated waste bins and autoclaved 20 min at 20 psi prior to disposal as solid waste. These procedures should ensure 100% kill of the recombinant bacteria. To test for killing efficacy in liquid media, samples will be removed periodically (3 monthly) for growth testing on L-agar plates.

Please enter comments of the GM safety committee on the risk assessment

The GMSC is satisfied that the cloning and small scale expression of the proteins described, which are mainly E.coli/S.aureus enzymes with no known pathogenic role, represents a minimal danger to Company personnel and to the environment; these proteins are ubiquitous in the environment (the E. coli proteins are present in the normal human gut flora) and have no known deleterious effects. The normal precautions outlined when undertaking the proposed GMO work ie restriction of laboratory access to authorised personnel and sterilisation/autoclaving of waste should minimise any unforeseen risk.

Project Ref 804/01.1

Date Ackn’d 05/10/2001  
Date Project Ceased

CU2 Project Title TRANSPOSON MUTAGENESIS OF STAPHYLOCOCCUS AUREUS

Class 2  
Consent Granted not applicable

CultureVolClass2 < 1 litre

Non-GMM

Page 11391 of 15326
Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

A non-pathogenic laboratory strain of Staphylococcus aureus RN4220 will be initially used to test the capacity of the transposon Tn917, housed in the plasmid pTn917-T7, to generate transposon mutants. Subsequently a second closely related transposon, Tn551, will also be used.

Recipient or parental organism

The recipient organism for transposogenesis, RN4220, is a non-pathogenic laboratory strain of Staphylococcus aureus. This strain is restriction minus, modification plus and possesses a capsule mutation rendering it non-pathogenic. The transposon mutations are likely to have different effects on the recipient depending on the function of the gene transposed and the position where the transposon inserts. If an essential gene is interrupted, the organism won't be viable, if non-essential it is expected that the organism will either be unaffected or may have a reduced viability. It is highly unlikely that the fitness of the organism would be enhanced significantly by any transposition event. Mutants which have taken up a transposon will be grown on erythromycin as a selectable marker.

Host/vector system

The host is the Staphylococcus strain RN4220. The vector is either:

1. The transposon Tn917, modified to incorporate a kanamycin resistance cassette, the pBR322 origin of replication and the erythromycin resistance gene or the backbone of pTn917-T7 contains a chloramphenicol resistance marker and a temperature sensitive origin of replication, allowing selection against the vector at temperatures above 43 degrees C.

2. Tn551 which is 99.8% identical to Tn917, and which will be modified in a similar way. The backbone of the Tn551 containing plasmid contains a Cadmium resistance marker and a temperature sensitive origin of replication, allowing selection against the vector at temperatures above 43 degrees C.

Origin & function

The transposon Tn917 and the closely related transposon Tn551 have been used for genetic studies on Staphylococcus aureus for more than fifteen years. The plasmid pET9d (Novagen) will be used as a source of the pBR322 replication origin and the kanamycin resistance cassette, the plasmid plD408 as a source of Tn917 and the erythromycin resistance gene. The engineered plasmid pTn917 will be electroporated into RN4220 cells using standard methods. Transformed bacteria will be selected on erythromycin plates, incubated at 30 degrees to induce transposition and integration into the S. aureus chromosome. Mutations will be investigated using an inverse PCR strategy to sequence the transposon/gene junctions and identify the targeted S. aureus gene.

Evaluation of foreseeable effects

This strain is restriction minus, modification plus and possesses a capsule mutation rendering it non-pathogenic. The transposon mutations are likely to have different
effects on the recipient depending on the function of the gene transposed and the position where the transposon inserts. If an essential gene is interrupted, the organism won't be viable, if non-essential it is expected that the organism will either be unaffected or may have a reduced viability. It is highly unlikely that the fitness of the organism would be enhanced significantly by any transposition event. Mutants which have taken up a transposon will be grown on erythromycin as a selectable marker.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The Class 2 activity described will generate contaminated plasticware - flasks, pipettes, pipette tips and disposable tubes used in the growth of the microorganisms, and in the manipulation and preparation of plasmid and chromosomal DNA. There will also be spent media, both liquid broth and agar, used to grow the bacteria. Disposable plasticware will be autoclaved at 20 psi after collection in double bagged autoclave bags. Liquid media will be disinfected by incubation overnight in 2% Virkon. Following disinfection the media will be autoclaved as above before disposal via the drains. These disposal procedures should ensure 100% kill of microorganisms and destruction of residual nucleic acids.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The transposons described have a long history of use in the study of Staphylococcus aureus genetics; although the recipient organism falls into ACDP Category 2 the GMOs resulting from these experiments are not anticipated to display any enhanced pathogenicity, and the strain proposed has an additional mutation reducing its pathogenicity - the safe history of use of this system together with the proposed control measures and the fact that these experiments have been approved as Category 2 of the University of Cambridge Veterinary School satisfies the GMSC that the proposed work should proceed under the conditions described.

Project Containment

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02/03/2022
TO GENERATE LIBRARIES OF RANDOM GENE MUTATIONS IN SALMONELLA TYPHIMURIUM USING TWO WELL-CHARACTERISED TRANSPOSONS, TN5 AND TN10, TO INDUCE TRANSPOSITION BY CONJUGATION AND/OR ELECTROPORATION

Project Additional Information

Purposes of the contained use

A pathogenic strain of S. typhimurium will be used to test the capacity of the Tn5 and Tn10 transposons to generate random transposon mutants.

Recipient or parental organism

The recipient organism for transposogenesis is SL 1344, a pathogenic strain of S. typhimurium. The transposon mutations are likely to have different effects on the recipient depending on the function of the gene transposed and the position where the transposon inserts. If an essential gene is interrupted, the organism won't be viable, if non-essential it is expected that the organism will either be unaffected or may have a reduced viability. It is highly unlikely that the fitness of the organism would be enhanced significantly by any transposition event. Mutants which have taken up a transposon will be grown using selectable markers (kanamycin, gentamicin, tetracycline or chloramphenicol - depending on which mini Tn derivative is used successfully in the construction of random mutants).

Host/vector system

The host is Salmonella typhimurium SL 1344.

The vector is either:
1. The transposon mini Tn5 (modified to incorporate T7 and SP6 promoters, 2 rare homing endonuclease sites and a neomycin cassette), R6K ori, RP4 OriT and the ampicillin resistance gene or
2. The transposon mini Tn10 (modified to incorporate T7 and SP6 promoters, 2 rare homing endonuclease sites and either a neomycin, chloramphenicol, tetracycline or gentamicin cassette), R6L y ori, RP4 mob region and the ampicillin resistance gene.

Origin & function
Tn5 is one of the best studied transposons. Tn5, Tn10 and their mini derivatives have been extensively used for genetic studies of Gram negative bacteria. We will engineer the Mini Tn derivatives to contain SP6 and T7 promoters to facilitate mutant analysis on an oligonucleotide array. Mutants will also be investigated using an inverse PCR strategy to sequence the Tn/gene junctions and to identify the disrupted S. typhimurium gene.

**Evaluation of foreseeable effects**

The recipient organism for transposogenesis is SL 1344, a pathogenic strain of S. typhimurium. The transposon mutations are likely to have different effects on the recipient depending on the function of the gene transposed and the position where the transposon inserts. If an essential gene is interrupted, the organism won't be viable, if non-essential it is expected that the organism will either be unaffected or may have a reduced viability. It is highly unlikely that the fitness of the organism would be enhanced significantly by any transposition event. Mutants which have taken up a transposon will be grown using selectable markers (kanamycin, gentamicin, tetracycline or chloramphenicol - depending on which mini Tn derivative is used successfully in the construction of random mutants).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The Class 2 activity described will generate contaminated plasticware - flasks, pipettes, pipette tips and disposable tubes used in the growth of the microorganisms, and in the manipulation and preparation of plasmid and chromosomal DNA. There will also be spent media, both liquid broth and agar, used to grow the bacteria. Disposable plasticware will be autoclaved at 20 psi after collection in double bagged autoclave bags. Media will be disinfected by incubation overnight in 2% Virkon. Following disinfection the media will be autoclaved as above before disposal via the drains. These disposal procedures should ensure 100% kill of microorganisms and destruction of residual nucleic acids.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

The proposed GM experiments using S. typhimurium would normally be done using a more attenuated strain; the reason for using SL 1344 is to ultimately give the investigators the potential to address the question of in vivo attenuation of transposition mutants. Given that mutants are likely to be either unaltered or attenuated in their virulence, doing these experiments in such a strain in Class 2 laboratories should not represent a problem providing the procedures for minimising hazard in the accompanying risk assessment are followed.

**Project Containment**

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02/03/2022
**Project Ref** 804/03.1

**Date Ackn'd** 29/01/2003  
**Date Project Ceased** 31/12/2010

**CU2 Project Title**  
GENERATION OF GENOMIC MARINER TRANSPOSON LIBRARIES IN STAPHYLOCOCCUS AUREUS.

**Class** Class 2  
**CultureVolClass2** < 1 litre  
**CultureVolumeClass3-4**

**Non-GMM Consent Granted** not applicable  
**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To generate transposon libraries in S. aureus using a Mariner transposon derivative; the aim of this work is to obtain maximum coverage of the bacterial genome using this transposon system, which is known to have less propensity for 'hotspot' integration as occurs with other transposons such as Tn5 and Tn10 in E. coli, and Tn917/551 in S. aureus.

**Recipient or parental organism**

The GMOs will be bacterial colonies where an average one gene in the genome has been disrupted by transposon insertion; in nearly every case the insertion is expected to have either no effect or result in slower growth. There should therefore be no increased risk to the operator compared to the parental organism.

**Host/vector system**

The hosts are E. coli PIR1 cells (Invitrogen). These are recA and used for manipulation of the recombinant mini-Mariner transposon. The S. aureus strains are SH1000 (minor derivative of 8325), N315, MW2, Mu50, MSSA and RN4220. N315, MW2, Mu50 and MSSA are well-characterised strains which are used in various sequencing projects. RH4220 is a modified laboratory strain that is restriction minus/modification plus and used for propagating vectors.
Two vectors will be used:
TS1 has a S. aureus ts origin and chloramphenicol resistance marker, and carries a recombinant mini-Mariner transposon which will be exchanged with a second variant. The latter contains a y-ori R6K (to permit replication in E. coli expressing PIR1), a kan resistance marker (to allow vector selection in E. coli and an erythromycin resistance marker (for vector/mutant selection in S. aureus).
pFA is compatible for replication with recombinant TS1 and contains a tetracycline resistance marker and the Mariner transposon gene.

Origin & function
The mariner transposon system is used for transposition into a variety of organisms, the transposon originating from the horn fly. Transposition systems in S. aureus are limited and the mariner system offers a way of generating a random library. The intended function of the libraries thus generated is to provide templates for RNA runoffs to identify the disrupted gene in a similar strategy as in the previous notified S. aureus activity GM 804/01.1

Evaluation of foreseeable effects
The effects of transposition of mariner or any other transposon depend on the gene into which the transposon insets and also the position it inserts within the gene. If the gene is non-essential the organism is likely to be either unaffected or have reduced viability-if essential it won't grow. It is highly unlikely that any significant enhancement of viability would result from a transposition.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The Class 2 activities described will generate contaminated plasticware - pipettes, pipette tips, flasks and disposable tubes used in the growth of the microorganisms, and in the manipulation and preparation of plasmid and chromosomal DNA. Spent liquid and solid (agar) media used to grow the bacteria will also be produced. Disposable plasticware will be autoclaved at 20 psi after collection in double bagged autoclave bags. Autoclaving is validated by printouts from every run, weekly use of indicator strips and monthly by measuring the kill of Bacillus stearothermophilus spores. Liquid media will be disinfected by incubation overnight in 2% Virkon. The disinfection efficacy is periodically validated by plating out Virkon treated and untreated bacterial cultures. The disposal procedures have been demonstrated to give 100% kill of microorganisms and destruction of residual nucleic acids.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
During discussion of this proposal the possibility of enhanced virulence of a microbe if a transposon inserts randomly was raised. The consensus view was that while this is a formal possibility eg insertion resulting in enhanced promoter activity it has never been reported and reduction rather than enhancement of virulence is more likely (and is mentioned in Part 2, p2 of Arrow's Risk Assessment form for this project).

The use of erythromycin as a selective marker was also queried; this antibiotic is used in some cases of human infection. However the strains to be used are all confirmed as being MSSA ie sensitive to penicillin and derivatives; thus there is no danger of non-treatability in the highly unlikely scenario of infection with an erythromycin-resistant Staphylococcus aureus strain. The approach proposed is complementary to the original S. aureus transposon work. In addition, resistance will disappear following plasmid cure at 44 degrees. The GMSC had no further comments and approved the proposal.

**Project Containment**

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**Project Ref 804/09.1**

- **Date Ackn'd**: 22/07/2009
- **CU2 Project Title**: Propagation of infectious hepatitis C virus particles in mammalian cell culture
- **Class**: Class 3
- **Culture Vol Class 2**: 500ml maximum
- **Non-GMM Consent Granted**: Yes
- **Date Project Ceased**: 31/12/2010
- **Withdrawn**: No
- **Tick if notifying a connected programme of work**: No

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**

---
Purposes of the contained use

To study the antiviral activity and mode of action of small molecule compounds against HCV.

Recipient or parental organism

The parental organism is the JFH1 strain of hepatitis C virus, an ACDP class 3 pathogen, derived from a Japanese patient with a fulminant hepatitis C infection. The virus has been shown to replicate in human hepatoma cell lines following transfection with HCV RNA transcribed in vitro. Propagation of virus therefore occurs only under defined culture conditions.

Host/vector system

Viral cDNA is propagated as a recombinant DNA plasmid in a pUC-derived vector, and the plasmid is non-mobilisable. The plasmid is maintained in E.coli strains which do not encode T7 polymerase, the enzyme required for transcription of HCV RNA from the T7 promoter.

Origin & function

The original JFH1 HCV sequence was isolated from a fulminant HCV viral infection in a Japanese patient and is a consensus sequence obtained from sequencining multiple overlapping clones derived from PCR analysis of the HCV cDNA. The virus is genotype 2a. RNA transcribed from the cloned JFH1 sequence was shown to be infectious for the human hepatoma cell line Huh-7 and to produce HCV viral particles (Wakita, T et al., (2005). 'Production of infectious hepatitis C virus in tissue culture from a cloned viral genome' Nat. Med. 11, 791-796; Pietschmann, T et al. (2006). A series of intra and intergenotypic HCV chimeras has also been described based on the JFH1 and J6CF-derived sequences (both genotype 2a). 'Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras' Proc. Natl. Acad. Sci. USA 103, 7408-7413).

A firefly luciferase reporter gene fused to the 5'untranslated region and part of the core gene comprises the first cistron; the second cistron comprisin the HCV RNA is expressed through the encephalomyocarditis virus internal ribosome entry site (IRES) and translated into a polyprotein which is then proteolytically cleaved to produce the individual viral proteins listed below:

- C - virus capsid protein.
- E1, E2 - viral envelope glycoproteins.
- P7 - ion channel.
- NS2 - autoprotease.
- NS3 - protease/helicase.
- NS4A - protease co-factor.
- NS4B - pleiotropic functions including mediation of membranous web formation.
- NS5A - also pleiotropic functions, control of viral replication, cellular interactions/signalling.
- NS5B - RNA polymerase.

Evaluation of foreseeable effects

Transfection of cultured human liver cells with in vitro transcribed HCV RNA will lead to viral protein expression and the assembly of HCV particles. These particles are able to propagate in liver-derived cells and can be assumed to be infectious to man by the percutaneous route, the main route for normal infection. In the context of experimental data from chimpanzees, it is not foreseen that there will be an increase in the pathogenicity of the GMO's generated: productive replication in chimpanzees. Proc. Natl. Acad. Sci. USA 99, 14416-14421.


Engineering variant HCV genomes, where changes are introduced into the parental genome by construction of intra or intergenotypic chimeras or by site-directed mutagenesis, could lead to a change in the replication properties of the virus. HCV replication of any variant sequences will be monitored and recorded.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste generated within the CL3 laboratory will be handled only by trained personnel until decontaminated. Solid waste eg paper gowns, gloves, plastic pipettes, eppendorf tubes, pipette tips, will be disposed in double bagged autocleavable bags in a biohazard waste bin. The bags will be sealed prior to removal to the CL3 lobby room where they will be autoclaved in a cycle held at 134ºC for 15 mins. After autoclaving the bags will be disposed of as clinical waste by an external contractor. All solid waste will be autoclaved at the end of the working day or session. Each autoclave cycle is monitored electronically and validated. A printed record is kept of each cycle. The autoclave will be tested every six months under a maintenance contract. Biological validation of autoclave efficacy will be verified on a regular basis using ampoules of a bacterial spore suspension (Geobacillus stearothermophilus, Raven Laboratories) with the autoclave waste, which give a colorimetric readout of successful killing. This method has been used routinely in the company over the last 8 years to provide a biological validation for our main autoclave in Biology. All liquid waste will be treated with Trigene at a final concentration of 5% for at least 12 hours (in practice overnight). Trigene has been shown to inactivate enveloped viruses, and is widely used as an effective virucidal agent.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment  

The GMSC felt that the risk assessment and containment classification address the key safety issues for handling and propagation in vitro of the HCV infectious particle.

Project Containment

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Safety Committee regularly meets once a month to discuss GMO procedures and approve risk assessments. It is composed of the Director of Biochemistry (Chairman), Section Manager (Advisor) and Molecular Biologist (Secretary).

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Other (please specify) Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research

02/03/2022
All solid waste exposed to viable GMMs will be collected in a double autoclavable biohazard bag left open to allow steam penetration, and autoclaved for 20 minutes at 121 degrees C. The autoclaved waste will subsequently be disposed of by incineration. A correct autoclave procedure is considered to reach 100% kill. The efficiency of the autoclave is validated using sterilization indicator strips placed in the middle of the load and the autoclave itself is serviced and calibrated every 6 months.

All liquid containing viable GMMs, will be inactivated by incubating the sample in at least 1% (w/v) Virkon for a minimum of 12 hours. After this inactivation step, the waste will be disposed of down the sink with copious quantities of water. This chemical inactivation procedure is considered to typically result in virtually 100% kill, as shown in independent testing performed by the Virkon manufacturer (Antec International) on all retroviruses and E.Coli strains tested.

Drug Discovery (expression and purification of recombinant proteins)

For activities involving GMMs, describe the waste management measures which will apply to the activity

All members of the Safety Committee have reviewed the risk assessment and consider it adequate for the type of work proposed.

Please enter comments of the GM safety committee on the risk assessment

All members of the Safety Committee have reviewed the risk assessment and consider it adequate for the type of work proposed.

Project Ref 805/01.1

EXPRESSION OF MURINE ECOTROPIC RECEPTOR IN HUMAN CELL LINES USING AN MULV REPLICATION-DEFECTIVE RETROVIRAL VECTOR.

Class 2 Consent Granted

Consent Granted

Project notified under transitional arrangements

Class 2 non-GMM

< 1 litre
**Project Additional Information**

**Purposes of the contained use**

Drug discovery.

**Recipient or parental organism**

The introduction of the murine ecotropic receptor into human cell lines will not alter the pathogenicity of the recipient cell. They will become infectable with ecotropic retroviruses that cannot normally infect human cells, and are therefore safer to use.

**Host/vector system**

The amphotropic replication-defective retrovirus used can infect human cells but, being replication-defective, cannot propagate. Moreover, the amphotropic retrovirus cannot survive outside laboratory conditions.

**Origin & function**

The ecotropic receptor is a basic amino acid transporter expressed by murine cells and is not inherently harmful. It is also exploited by the MuLV ecotropic retrovirus for entering the target cell. Since it is not expressed by human cells, ecotropic MuLV cannot infect human cells, unless they have been modified to express the ecotropic receptor. This would allow the use of MuLV ecotropic retroviral vectors on human cells that have been modified, reducing the risk to the operator. Indeed, MuLV ecotropic retrovirus-defective work is considered to be a Class 1 activity.

**Evaluation of foreseeable effects**

The risks associated with the modified retroviral vector we intend to use are mainly related to its ability to infect human cells. This means that potentially this virus could infect the operator and for this reason only we propose a containment level of 2. In the unlikely event of such an infection occurring, the infection will not spread to other cells as the virus we intend to use is replication-defective. Therefore, the risks to human health associated with this work are very low.

The risk associated to the environment are very low too, as the MuLV retrovirus we intend to use is unlikely to survive outside laboratory conditions. In addition, as mentioned before, the virus cannot self propagate and therefore cannot proliferate in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All solid waste exposed to the GMM will be collected in a double autoclavable biohazard bag left open to allow steam penetration, and immediately autoclaved for 20 minutes at 121 degrees C. The autoclaved waste will subsequently be disposed of by incineration. A correct autoclave procedure is considered to reach 100% kill. The efficiency of the autoclave is validated using sterilization indicator strips placed in the middle of the load and the autoclave itself is services and calibrated every 6 months. In addition, the TC dishes exposed to the virus will be left to soak in 1% w/v Virkon for at least 12 hours and subsequently autoclaved.

All liquid containing viable GMMs, will be inactivated by incubating the sample in at least 1% w/v Virkon for a minimum of 12 hours. After this inactivation step, the waste will be disposed of down the sink with copious quantities of water. This chemical inactivation procedure is considered to typically result in virtually 100% kill, as shown in independent testing performed by the Virkon manufacturer (Antec International) on all retroviruses and E.Coli strains tested.

The risk assessment was reviewed by all the members of the safety committee and was considered adequate for the type of work proposed.

**Project Containment**

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**Project Ref 805/17.1**

- **Date Ackn'd**: 24/02/2017
- **CU2 Project Title**: Use of Hela GFP-LC3 stable cells to screen for modulators of LC3 lipidation
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Non-GMM Consent Granted**: Consent Granted

**Project notified under transitional arrangements**: N
**Project Additional Information**

**Purposes of the contained use**

*Drug Discovery: Identification of novel compounds, which modulate LC3 lipidation*

The HeLa GFP-LC3 stable cells will be supplied by an external client. This cell line will be employed in a screening assay to identify novel compounds, which modulate LC3 lipidation.

**Recipient or parental organism**

The HeLa GFP-LC3 stable cell line was generated by transfecting HeLa cells (ATCC® CCL-2™) with a pEGFPLC3 plasmid and selecting with geneticin (G418).

**Host/vector system**

The pEGFP-LC3 plasmid used to generate the HeLa GFP-LC3 stable cell line was generated according to the reference: EMBO J. 2000 Nov 1;19(21):5720-8. Briefly, cDNA encoding rat LC3 was obtained by RT-PCR from rat brain total cDNA, which was subcloned into the EcoRI site of the eukaryotic expression vector pCL-neo (Promega, Madison, WI). The pEGFP-LC3 plasmid was generated by inserting the rat LC3 cDNA into the BglII and EcoRI sites of the pEGFP-C1, a GFP fusion protein expression vector (Clontech Laboratories). This plasmid was then employed to generate the HeLa GFP-LC3 stable cell line as described previously.

**Origin & function**

*The parental HeLa cell line contains sequences of the human papilloma virus genome (HPV18), and is therefore Biosafety Level 2, according to the ATCC. The Hela GFP-LC3 cells are therefore GMO Class 2, as they are a Biosafety Level 2 cell line, transfected with the pEGFP-LC3 plasmid. Therefore, all culture and use of the cell line must be carried out in a Class II Containment Laboratory.*

Several specific practices beyond standard aseptic technique are required for handling the Hela LC3-GFP cell line (GMO class 2). The following practices will be adhered to:

- **Signage** will be displayed outside the tissue culture lab, notifying other employees of the usage of the GMO cell line.
- **Materials should always be manipulated within a Class II safety cabinet.** A beaker of 1% Virkon should be placed in the safety cabinet and pipette tips should be decontaminated in this, prior to disposal into bin for autoclaving and incineration.
Pipettes should similarly be immersed immediately in a cylinder of 1% Virkon, prior to autoclaving and incineration.

Cell culture flasks should have the lid closed prior to disposal into biohazard bags for autoclaving.

Generation of aerosols should be minimised by careful pipetting.

Passage of GMO cell lines, should be separated in the safety cabinet from other cell lines, such that flasks of other lines are not present in the cabinet at the same time. This is to prevent any possible cross contamination between cell lines.

Use a dedicated 37°C incubator for the Hela LC3 GFP cells, to prevent cross contamination between cell lines.

Cells must also be inactivated in this laboratory in a Class II safety cabinet (e.g. by addition of detergent to generate a lysate or fixation) prior to transfer to other laboratories for analysis.

Cells should always be centrifuged in sealed buckets.

Safety cabinets must be cleaned with 1% Virkon and 70% IMS before and after use.

Public Register

Health and Safety

Executive

CU 2 2015 (rev 11/15) Page 4 of 10

All solid tissue culture waste will be collected in double autoclavable biohazard bag and autoclaved for 20 minutes at 121°C. The autoclaved waste will subsequently be disposed of by incineration by licenced contractor.

All liquid containing viable GMOs, will be inactivated, by incubating the sample in at least 1% (w/v) Virkon for a minimum of 1 hour. After this inactivation step, the waste will be disposed to drain.

Due to the containment procedures and the waste management systems described above, the risks to laboratory personal and the environment are very low. Good microbiological containment practices will be adhered to and all waste will be either be autoclaved and incinerated OR treated with disinfectant.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid tissue culture waste will be double bagged in autoclavable biohazard bags, and transported to an autoclave via a communal corridor using a leak proof trolley with a lid, which provides secondary containment. The double-bagged waste will then be autoclaved for 20 minutes at 121°C (100% kill). This procedure will be carried out at the end of each day. NB: Autoclaves are serviced twice a year and have a 12-Pen Validation test for Plastic discard waste (121°C for 15 minutes) performed annually to UKAS Quality Procedure UQP.

Following autoclaving, the bagged sterile waste will be placed into a yellow class 6.2 marked sack and placed in UN approved 7701 yellow 'wheelie bins' marked with the appropriate biohazard diamond. Grundons Waste Ltd are certified to handle and dispose Level 2 waste from the Harlow site.

All liquid containing viable GMOs, will be inactivated, by incubating the sample in at least 1% (w/v) Virkon for a minimum of 1 hour (100% kill). After this inactivation step, the waste will be disposed to drain.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The GMO Safety Committee meets once a month to discuss GMO procedures and approve risk assessments. The Committee is composed of the Director of Biology (Chairman), Biology Manager (Advisor) and Molecular Biologist (Secretary).

The risk assessment was reviewed by all members of the GMO Safety Committee and was considered adequate for the proposed work.

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PROSIDION LIMITED

WINDRUSH BUILDING
COWLEY
OXFORD
OXFORDSHIRE
OX4 6LT
ENGLAND

01865 780800
01865 780801

EAST AND SOUTH EAST

OSI PHARMACEUTICALS LIMITED Changed its name to PROSIDION LIMITED on 1/12/2004. CLOSED 20/12/2011

28/09/2004

Page 11409 of 15326
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

The OSI Genetic Modification Safety Committee (GMSC) will be a sub-committee of experienced biologists drawn from the Biological Safety Committee (BSC). The BSC will report to the Main Safety Committee (MSC). The MSC will represent all of the company's major functions and include senior management representation. The GMSC will review all GMO risk assessments. It will be empowered to categorise level 1 and II activities, to approve level 1 activities and to recommend BSC approval of level II; level II activities will be notified to the HSE in parallel with BSC review. The GMSC will comprise the Biological Safety Officer (BSO) (PhD scientist with >15 years GMO experience), a deputy BSO (23 years experience in molecular biology plus experience of the role in a former employment) and at least one other experienced biologist with GMO experience. The BSC will contain additional representation from within the Biology Department. Should the company appoint a Health and Safety Manager, she/he will also sit on the BSC.

Individual scientists will present their written risk assessments to the members of the GMSC prior to review to allow time for due consideration. Review will be conducted on an ad hoc basis in the form of open discussion with the involvement of all interested parties. The BSC will meet bimonthly or more frequently if required.

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Tick if confidential
Contaminated solid waste and glassware will be treated by autoclaving (140min/134 degrees C/3.2bar absolute). The autoclaves are maintained and tested annually. A hard copy output detailing the temperatures/pressures achieved is generated for each run.

Culture medium/liquid waste will be decontaminated by treatment with Trigene prior to disposal via the drain (2% (v/v) final; >1h under high organic load eg blood). Trigene (MediChem International Ltd, PO Box 237, Sevenoaks, Kent, TN15 OZJ) is a non-toxic, non-hazardous, biodegradable agent with broad spectrum activity (bactericidal, fungicidal, virucidal, mycobactericidal and sporidical) which is suitable for use under conditions of high soilage. See http://www.medichem.co.uk/medical_products.html for laboratory test data on this product.

Contaminated, small glass articles eg vials, microscope slides etc will be discarded into sharps containers (Cinbins) destined for incineration by Grundon at their Colnbrook site (Slough, SL3 OEG).

Routine surface disinfection will use 1% Trigene. Any substantial bio-spills will be treated with the Body Fluid Response Kit supplied by the same manufacturer.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  Y

Please enter comments of the GM safety committee on the risk assessment

The work in question involves the expression of a human serine protease in CHO-K1 cells for subsequent biochemical studies. The GMSC has examined the potential impact of this GMM in terms of hazard to human health and damage to the environment. The protease in question is expressed as an inactive zymogen and, particularly at the level of protein expression anticipated, it is unlikely to offer any hazard to human health or the environment.

All E.Coli manipulations will be performed in K12 (DH5a) or K12/B hybrid (DB3.1) strains of ADCP hazard group 1. Furthermore, since the parental CHO-K1 cell line is a commonly used line of hamster origin with a proven track record in safe utility and the introduction of a serine protease is unlikely to alter this fact, the GMSC has recommended this work be carried out under containment level 1 conditions.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  Y

Please enter comments of the GM safety committee on the risk assessment

The work in question involves the expression of a human serine protease in CHO-K1 cells for subsequent biochemical studies. The GMSC has examined the potential impact of this GMM in terms of hazard to human health and damage to the environment. The protease in question is expressed as an inactive zymogen and, particularly at the level of protein expression anticipated, it is unlikely to offer any hazard to human health or the environment.

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**GM Centre Number: 807**

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**Comments**

Date at Which Additional Info Submitted

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Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Hammersmith Hospitals Trust Gene Therapy/Genetic Modification Safety Committee (GT/GMSC) is a sub-committee of the Hammersmith Hospitals Trust Health and Safety Committee.

The GT/GMSC includes representatives from the following:

* Management of the Gene Therapy Centre
* Infection Control
* Facilities - waste disposal, portering and domestic services
* Staff representatives (Chair of Joint Staff Committee)
* GMSC chairs and other representatives of the HHT’s ‘Partner’ organisations - Imperial College (IC) and The Clinical Sciences Centre (CSC?MRC)
* Occupational Health
* Research heads in the Imperial Cancer Research Fund (ICRF)
* The Ethics Committee
* Pharmacy
* The Safety Office
* Department of Infectious Diseases
* Director of Service’s Office

The Committee members experience of genetic modification work varies from nil to many years. Each member is very experienced in their own area of work.

The Committee meets as and when projects are received for approval - but will meet at least quarterly.

The Committee will advise on, and approve risk assessments for genetic modification work (it's statutory role). It will also undertake other roles as described in the ACGM compendium of guidance such as advise on good practice, training, inspections and review accidents/incidents.

The majority of Committee members have managerial responsibility across both campuses of the Trust. Therefore the Trust will have one Committee that approves risk assessments for both Hammersmith and Charing Cross Hospitals.

The GT/GMSC includes representatives from the following:

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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### Non-microbial

**Other (please specify)** Gene therapy suite and associated wards. * Diagnostic laboratories.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

### Project Ref 807/01.1

<table>
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<th>Date Ackn'd</th>
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<td>25/10/2001</td>
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In the proposed clinical trial programme, an angiogenic gene therapy product, consisting of a recombinant adenovirus (human serotype 5) containing the human gene for
the fibroblast growth factor 4, and referred to as Ad5FGF-4, will be investigated as a treatment for patients with chronic stable angina due to coronary artery disease (CAD).

Generation of new blood supply in the diseased heart by intracoronary administration of the angiogenic gene therapy product represents a potential new therapeutic
approach to relieve this condition.

The Ad5FGF-4 gene therapy product consists of a recombinant adenovirus vector (human serotype 5, Ad5) with a deletion in the E1 region; from map unit 1.3 to 8.7 of
wild-type virus (entire E1A and most of E1B are eliminated). The FGF-4 transgene is inserted, driven by CMV promoter.

The FGF-4 gene was originally isolated from a CDNA library which was constructed from mRNA of Kaposi's sarcoma DNA transformed NIH3T3 cells.

The intended function is angiogenesis, the formation of new blood vessels.

The probability of adverse consequences resulting from deliberate or accidental release of the gene therapy product Ad5FGF-4 are minimal to noneexistent.

Hazards resulting from environmental release (viral shedding from treated persons, inadvertent contamination of the product prior to administration) are negligible or
noneexistent for the following reason: infection requires large numbers of infectious vectors, and transfection (expression of the inserted gene) requires a multitude of
infectious particles.

Hazards associated with the adenoviral vector are believed to have a low potential of adverse environmental consequences in humans or animals. The theoretical
consequences to humans of several of the hazards associated with ectopic transgene expression, if they actually occurred, could be considered moderately severe (eg
promotion of existent malignancy, unknown risk to foetus). However, since any unintended or accidental exposure would most likely be a fraction of the total dose being
administered to patients for therapeutic purposes, the relative risk of the occurrence of these types of adverse effects should be very low.
The possible risks to the environment could be assessed as low to effectively zero. This is based on the low probability of infectious adenoviral particles escaping into the environment either through viral shedding by patients that have received the product, or by incidental exposure during administration procedures. Even if viruses were shed or product spillage occurred the number of infectious viral particles would be too small to result in infection of exposed tissues. The risk to the non-human environment is extremely low to effectively zero because of the species specificity of adenovirus5, which by natural exposure is only known to infect humans.

The above indicates that the product could be classified as Class 1. However, as a precautionary measure, due to the limited experience available, the product is currently being classified as Class 2. Reclassification into Class 1 may be considered if based on increased data and experience.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

* All waste will be transferred to the autoclave in Hammersmith Hospital to be autoclaved by Microbiology staff. It will be transported in sealed leakproof autoclavable containers. Inactivation of Ad5FGF-4 is by autoclaving at 134 degrees C for 15 minutes (This is the standard cycle used in the Microbiology Department - it is not changed for GM waste collections). This results in 100% kill of the vector, the material is then transported off site and incinerated. The chemical disinfectant is now Actichlor (10,000 ppm chlorine)

* Service contracts are in place for the autoclaves.

* Formal inspections will be conducted quarterly with the safety officer.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

On original submission the committee asked for more information about the potential transmissibility and survival of the vector based on other previous use in USA.

The Committee also requested a description of the patient pathway.

The Committee was satisfied with the detailed information provided and agreed that the project could be safely conducted as described.

Project Containment
### Project Additional Information

**Purposes of the contained use**

The study has been set up to assess the safety, biodistribution and biological activity of OncoVex GM-CSF. The study involves intratumoural injection into cutaneous or subcutaneous nodules.

**Recipient or parental organism**

The oncovex human GM-CSF vector (Oncovex hGM-CSF) is a replication competent herpes simplex type-1 virus which will be tested in a phase 1 clinical trial for its safety, biodistribution and efficacy in a variety of solid tumours. The strain of HSV which OncoVEX is based on is JS-1 (ECACC no. 85011433). The oncovex GM-CSF vector is deleted for the neurovirulence factor, ICP34.5 which is essential for pathogenicity. This deletion allows the vector to selectively replicate in dividing cells. The vector is also deleted for ICP47, which blocks antigen presentation to MHC class 1 and II molecules by blocking the transporter associated with antigen processing (TAP 1 and TAP 2). The vector also contains the coding sequence for human GM-CSF, a cytokine involved in the stimulation of T-cells. The deletion of ICP47 and the expression of GM-CSF should enhance the immune response towards tumour cells. GM-CSF expression is under the control of the human cytomegalovirus immediate early promoter (HC MV IE). This is an infectious gene therapy product that can only replicate in rapidly dividing eukaryotic cells.
Reference:

Host/vector system
HSV strain JS1/34.5-/47-/CMVGM-CSF (OncoVEX GM-CSF)

Origin & function
The hGM-CSF gene was cloned from an IMAGE clone 2340997/5808-K14 (UK HGMP Resource Centre). GM-CSF is a potent cytokine responsible for the differentiation and proliferation of dendritic cell precursors and therefore is a potent immune stimulator. Thus if the vectors were to enter a human it would be anticipated to give an improved immune response against HSV.

Evaluation of foreseeable effects
GM-CSF should enhance the immune response towards tumour cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste will be placed in a sharps bin or bag. These will then be placed in an external container in order to prevent external spillages and will be identified as potentially containing genetically modified organisms according to Hammersmith Hospital Trust standard operating procedures. They will then be transferred to the pathology department for autoclaving. The degree of kill is effectively 100%. The autoclaves are validated on an ongoing basis. Autoclaves are validated at least once a year and for each cycle autoclave tape and the integral autoclave printer readout will be used to confirm that waste has been autoclaved. After autoclaving, the waste then enters the Trust Clinical Waste route. The waste is then placed into yellow eurocarts which are locked and transported offsite for incineration or microwaving elsewhere. The chemical disinfectant has been changed to Actichlor (10,000 ppm chlorine) and the autoclave cycle is 134C for 30 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The Committee requested:
- more information to support the statement regarding the non pathogenic nature of the organism
- more detail on the time and protocols for screening samples
- Occupational Health information be included
- the timing and management of specimens to be taken be added to the patient pathway.

The Committee was satisfied with the detailed information provided and agreed that the project could be safely conducted as described.

## Project Containment

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**Project Ref 807/06.1**

- **Date Ackn’d**: 01/11/2006
- **CU2 Project Title**: A Phase 2, Randomised, Double-blind, Placebo-controlled, Parallel-group, Multicentre, Dose-selection Study of Adenovirus type2/Hypoxia Inducible Factor (HIF)-1a/VP16 in Patients with Intermittent Claudication.
- **Class**: Class 2
- **Consent Granted**: Not Applicable

**Historical Significant Changes**

- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**
Purposes of the contained use

The purpose of this study is to assess the effect of Ad2/HIF-1a/VP16 as a gene therapy agent to induce angiogenesis and thus improve the clinical function of patients with lower limb ischaemia.

Recipient or parental organism

The agent studied contains Adenovirus type2. However, the Adenovirus used in this study should not be able to reproduce in the body (and even accidental inoculation is unlikely to cause symptoms) due to the deletion of E1 genes and all but one of the E4 genes.

Host/vector system

The hypoxia inducible factor / VP 16 hybrid is comprised of amino acids 1-390 of (HIF)-1a fused to amino acids 413-490 of Herpes simplex virus VP16. This is then recombined with Adenovirus type2 to transport the transcription factor.

Origin & function

HIF-1a (hypoxia inducible factor - 1a) is an inducible transcriptional regulatory factor that, in combination with its constitutively expressed dimerisation partner, HIF-1a, plays a principal role in the cellular response to changes in oxygen tension. Experiments in animals have established that HIF-1a is essential for regulating gene expression in response to changes in oxygen tension both during development and in response to hypoxia in postnatal life. The HIF-1a/VP16 hybrid transcription factor is part of the amino acid chain of HIF-1a fused to that of Herpes Simplex Virus. It is hoped that the administration of the modified HIF-1a/VP16 transcription factor via gene therapy will allow new blood vessels to grow in the ischaemic tissues. In patients suffering from lower limb ischaemia and whose walking is limited by pain, this effect should improve their circulation and in turn their symptoms and allow them to walk further.

Evaluation of foreseeable effects

There is a very minimal risk that the virus could be passed from an individual accidentally exposed to Ad2/HIF-1a/VP16, or the blood/serum of a patient having been treated with Ad2/HIF-1a/VP16 within the previous 7 days, to another person. Replication of Ad2/HIF-1a/VP16 in the patient is extremely unlikely. Deletion of E1 genes and all but one of the E4 genes have rendered the Ad2/HIF-1a/VP16 viral construct defective for autonomous replication. Shedding of the virus from the individual exposed to Ad2/HIF-1a/VP16, or the blood/serum of a patient having been treated with Ad2/HIF-1a/VP16 within the previous 7 days, to someone else is also extremely unlikely. Fertile men and women should follow an approved method of contraception for 60 days after an exposure to Ad2/HIF-1a/VP16 or in the event the exposure is to the blood/serum of a patient having been treated with Ad2/HIF-1a/VP16.In the extremely unlikely event that environmental release was to occur, the most likely virus involved would be wild-type adenovirus that had overgrown Ad2/HIF-1a/VP16 or a recombinant with no biological activity other than that associated with a generalised infection of the wild-type virus. This includes mild cold-like symptoms and more severe respiratory infections like bronchitis, croup and pneumonia.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste is disposed of in labelled autoclave bags or sharps bins, transported in sealed, leak proof containers to the autoclave suite in the Microbiology Department. Waste is autoclaved then placed in solid yellow (UN approved) bins, sealed, tagged and removed for incineration or microwaving off site. The standard autoclave cycle used is 134. C for 30 minutes. The autoclaves are tested weekly, quarterly and annually. A chart recorder is used. Autoclave tape is used to indicate that autoclaving has taken place. Twelve-point thermocouple testing is carried out.
The Hammersmith Hospitals NHS Trust Gene Therapy and Genetic Modification Committee were satisfied with the risk assessment for this project. The committee was assured that all potential risks were identified at storage, preparation, transport, administration and disposal stages of the study. They were also satisfied that all necessary controls have been put in place to ensure minimum risk of exposure for staff, public and the environment.

Please enter comments on the GM safety committee on the risk assessment

The Hammersmith Hospitals NHS Trust Gene Therapy and Genetic Modification Committee were satisfied with the risk assessment for this project. The committee was assured that all potential risks were identified at storage, preparation, transport, administration and disposal stages of the study. They were also satisfied that all necessary controls have been put in place to ensure minimum risk of exposure for staff, public and the environment.

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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- L4

Large Scale Activities

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- L3
- L4

Human Clinical Applications

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- L3
- L4

Project Ref 807/17.1

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<td>19/04/2017</td>
<td>A phase 3 randomized, open-label study comparing Pexa-Vec (Vaccinia GM CSF/Thymidine kinase-deactivated virus) followed by sorafenib versus sorafenib in patients with advanced hepatocellular carcinoma (HCC without prior systemic therapy</td>
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<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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Date Project Ceased

- N

Historical Significant Changes

- N

Tick if notifying a connected programme of work

- N
Pexas-Vec is currently in clinical development for the treatment of Hepatocellular Carcinoma. The proposed contained use will be the administration of the investigational product, in a hospital or clinic setting, by intratumoral (IT) injections to patients as part of an international, multicenter clinical trial. This clinical trial is a Phase III trial in patients with Advanced Hepatocellular Carcinoma (HCC) without prior systemic therapy. Results from this pivotal trial will determine whether Pexas-Vec followed by sorafenib increases survival duration in advanced HCC patients compared to treatment with sorafenib alone, and whether sequential dosing with Pexas-Vec followed by sorafenib has a favourable safety profile.

Approximately 40 clinical sites in the EU will enroll patients in the JX94-HEP024 (PHOCUS) study. Additional clinical sites in Australia, Canada, China, Israel, Korea (Republic of), New Zealand, Singapore, Taiwan, Thailand and the USA will also participate in the study. A total of 600 patients will be recruited in this clinical trial with an expectation to enroll 200 patients in EU countries. In the control arm, the 300 patients will not receive Pexas-Vec. After study completion, all patients will be followed up for survival. Among them, 300 patients (i.e. approximately 100 patients in EU) will receive Pexas-Vec by IT injections.

Pexas-Vec is a replicative oncolytic recombinant vaccinia virus (VV) derived from the commonly used vaccine Wyeth strain, DryvaxTM.

VV is a member of the Poxviridae family (genus Orthopoxvirus). Multiple strains of VV exist that have different levels of virulence for humans and animals. The New York City Board of Health (NYCBOH) strain, from which the Wyeth strain of the Dryvax® vaccine was derived, has low pathogenicity in humans (Fenner F. et al., 1988).

VV has a long and extensive history of use in humans. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous (SC) infection. As VV contains antigens that stimulate an immune response that are cross-reactive with smallpox antigens, the vaccine thereby confers protection from the human smallpox disease. VV may cause local reactions including erythema, edema and systemic reactions such as fever and malaise, as has been observed with conventional vaccination against smallpox. During the smallpox vaccination campaign, serious complications had occurred in less than 1 in 4,000 individuals, mainly in immunosuppressed and extremely young individuals. Pexas-Vec is even further attenuated as the thymidine kinase gene has been disrupted which makes replication in normal cells more difficult than the smallpox vaccine. Rare complications included eczema vaccinatum (patients with eczema), disseminated vaccinia rash, progressive vaccinia (in T-cell-deficient individuals) and encephalitis (1-2 per million vaccinated) (Fields B.N., 1996). Recent studies of smallpox vaccines have identified cardiac injury including pericarditis and myocarditis as a potential risk (Halsell J.S. et al., 2003).

VV replication exclusively occurs in the cytoplasm thus eliminating any risk of integration of the viral DNA into the host genome (Moss B., 2007).

In terms of classification of hazard, VV is considered as a Group 2 biological agent as per Directive (2000/54/EC). VV is also classified as a Biosafety Level 2 (BSL-2) infectious substance by the US Centers for Disease Control and Prevention (CDC) (CDC, 2009) and as a risk group 2 organism by the US NIH Guidelines (NIH).

Pexas-Vec was generated by co-transfection of CV-1 cells (Monkey kidney cells) with VV (Wyeth strain obtained from the Center for Disease Control, Atlanta, Georgia) and the plasmid pSC65/hGM-CSF. The vector pSC65/hGM-CSF contains DNA sequences coding for the hGM-CSF and β-galactosidase proteins and for their respective promoters. In addition, the transgene sequences are flanked by two VV genomic regions (TKL and TKR) that allow homologous recombination between the transfer plasmid and VV. The plasmid pSC65/hGM-CSF is generated from the plasmid pSC65 which was provided by Dr. B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland (Chakrabarti 1997).
The plasmid pSC65/hGM-CSF is inserted into VV. The insertion of pSC65/hGM-CSF into VV can be detected by using colorimetric identification of plaques containing recombinants expressing β-galactosidase. The pSC65 vector when provided by Dr. B. Moss contains the LacZ gene. The LacZ gene is a reporter gene, under control of the VV p7.5 early/late promoter. The additional donor gene (i.e. gene coding for hGM-CSF) is inserted in pSC65 as follows. The plasmid pCSF-1 (No. 39754) was obtained from American Type Culture Collection and comprises the full-length cDNA for hGM-CSF (Wong 1985). The hGM-CSF gene was cloned first into the EcoR1 site of pBLUESCRIPTII SK (Stratagene, La Jolla, California), generating plasmid pBLUE/hGM-CSF, and providing restriction enzyme sites to allow cloning of the hGM-CSF gene into the Sal I and Bgl II sites of pSC65. This positioned the hGM-CSF gene downstream of a synthetic promoter (PsE/L) designed by Dr Moss' laboratory to give maximal levels of transcription during both the early and late phases of vaccinia infection (Chakrabarti 1997).

Origin & function

Pexa-Vec contains three genetic modifications compared to the wild type Wyeth strain: 1) disruption of the viral thymidine kinase (TK) gene by, 2) insertion of the human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene and 3) insertion of the LacZ gene.

Due to the insertion of the transgenes, the TK gene is inactivated. This decreases VV virulence (Buller R. et al., 1985) by restricting viral replication to proliferating cells. This also targets dissemination of the virus to tumors (Puhlmann M. et al., 2000).

The plasmid pCSF-1 (No. 39754, American Type Tissue Culture Collection, Rockville, Maryland) contains the full–length cDNA for hGM-CSF (Wong G.G. et al., 1985). The cDNA for the hGM-CSF gene was inserted into the TK gene to help elicit an immune response to tumor cells both at the site of viral replication and in distant metastases. The cytokine hGM-CSF was chosen because it was the most potent stimulator of systemic anti-tumor immunity among many tested (Dranoff G. et al., 1993), probably due to its unique ability to promote differentiation of hematopoietic precursors into dendritic cells (Pardoll D.M., 1995). Dendritic cells are professional antigen presenting cells that may take up and present released tumor antigens as the tumor cells are killed by the VV.

The LacZ gene is contained in the pSC65 plasmid which was provided by Dr. B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland. The LacZ gene encodes for β-galactosidase which is a hydrolase enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides.

Evaluation of foreseeable effects

Pexa-Vec is non-integrative, and replicative and propagative characteristics of VV have been attenuated in Pexa-Vec, which makes the virus replication dependent on actively dividing cells such as cancer cells. Therapy with a replicating virus can theoretically lead to shedding of the virus into the environment, and potentially to the public, although controls are used in this trial to minimize this occurrence. The clinical information available to date suggests that Pexa-Vec is safe at the clinical dose of 1 x 10^9 pfu (10,000-fold higher than smallpox vaccine dose) and has not spread to caregivers in contact with the treated patients. Should shedding occur, the level of exposure would be predicted to be low compared to the doses received by patients in the proposed trial, and extremely low compared to doses of non-attenuated vaccines administered to the public (e.g. vaccines against smallpox). In addition, exposed individuals over the age of 35 will likely have been previously immunized with vaccinia. In the highly unlikely event that an exposed individual were to demonstrate virus-associated toxicity, therapy could be initiated with VIG and/or cidofovir. Therefore, public health risks with this virus are extremely low and in fact should be lower than with standard vaccination procedures. To date, no reports of transmission to health care personnel from vaccinia recipients have been published. Routine barriers nursing approaches should be used per institutional guidelines for infectious organisms (e.g. such as for M. tuberculosis, Pseudomonas). The information regarding the risk to patient contacts and guidelines for reducing the risk of viral transmission is contained within the Participant Information Sheet and Consent Form (PISCF) and the Pexastimogene Devacirepvec (Pexa-Vec) Guidelines (provided in Appendix B of Pexa-Vec Investigator’s Brochure). The PISCF will be reviewed with the patients, and their written consent will be obtained, before they undergo any study-specific procedures. A signed copy of the PISCF will be given to the patients so that they can come back to the guidelines at any time. Pexastimogene Devacirepvec (Pexa-Vec) Guidelines will be given to investigators, pharmacists, and all personnel involved in the handling of the product.

The genetic modification of the virus is not expected to result in any post-release shift in biological interactions or host range or in any known or predictable effects on.
non-target organisms in the environment. It is also not expected that the release of the recombinant virus would result in any increase in pathogenicity as compared to the parental virus strain and/or in any increase in the capacity to recombine with other related viruses. Therefore, under the conditions for use in the proposed clinical trial, Pexa-Vec is not considered to represent a risk for the environment and for the public health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste, including any dressings returned by patients, will be disposed via hospital waste stream which is treated according to Trust procedures for infectious waste. (The Orange waste stream which is for waste items that are infectious waste, clinical waste contaminated with blood or bodily fluid from patients with transmissible infectious diseases and large volumes of blood, pus and wound exudates). Infectious waste is collected and incinerated/destroyed by a contractor approved by the Trust.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

The Trust Committee is the Joint Clinical Research Committee of Imperial Healthcare NHS Trust and Imperial College London. The committee incorporates the role of GMSC for the Trust.

The committee queried whether this study required Class I or Class II since all the work with GMOs on site is low risk. Agreed to proceed with Class II in line with other centres involved in this study.

The committee requested clarification of the infectious waste stream and Q12 has been amended accordingly.

Pharmacy noted procedures on preparation of the IMP to ensure purity of the production for injection not due to excessive risk of the IMP for workers.

Project Containment

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02/03/2022
**Project Additional Information**

**Purposes of the contained use**

The purpose of the contained use is to manufacture CD19-targeting CART cells for potential immunotherapeutic use. The Lentiviral Vector developed for the ex vivo transduction of T cells in order to confer expression of an anti-CD19 chimeric antigen receptor (CAR).

**Recipient or parental organism**

Lentivirus-modified recipient T-cells

**Host/vector system**

This Lentiviral system is designed for the ex vivo transfer of genes to target cells which can be expanded and delivered back to patients. The vector is pseudotyped containing a heterologous envelope protein, the vesicular stomatitis virus G envelope protein (VSV-G), to confer broad tropism for transduction of a wide variety of mammalian cell types. The particular replication deficient construct is a modified HIV-1 backbone based on the pNL-A-3 molecular clone which possesses the following features:

- A deletion in the enhancer of the U3 region of the 3'LTR to ensure self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of target cells
- Multiple deletions of accessory genes to remove as much of the HIV backbone as possible and still maintain efficient production of lentiviral particles
- The vector and helper constructs contain no significant areas of homology, minimizing possibility for recombination
- Separation of the gag-pol open reading frame (ORF) from the VSV-G ORF on two plasmids, with the transducing...
vector carried on a third plasmid.

**Origin & function**

The intended use is expression of an anti-CD19 chimeric antigen receptor (CAR).

**Evaluation of foreseeable effects**

This construct is replication-defective, therefore poses no risk to humans or animals. Lentiviral vectors can however integrate into host chromatin, and thus pose some risk on insertional mutagenesis. In the the finished CAR-T cell product, free lentiviral particles are significantly diluted and washed during the manufacturing process. The transduction efficacy of these lentiviral particles will be considered inefficient by the end of the manufacturing process and therefore we consider the product to be safe for therapeutic application.

Operators undertaking the manufacturing process will be fully trained and apply use of personal protective equipment.

There is added assurance that the process is designed as a 'closed system' and the lentivirus will be introduced to culture by connecting to kit using a sterile tubing welder.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No autoclave in building and therefore require the services of an established third party provider for waste disposal.

Packaging: Will be as recommended by waste management service provider, with dedicated collection. Waste will be destined for autoclave / high temperature Incineration.

Storage & Collection: Dedicated storage area will be denoted in the laboratory until the waste management company collects for disposal.

Transportation & Disposal: Dedicated collection will be arranged with waste will be destined for autoclave / high temperature Incineration.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste, spills: Allow aerosols to settle; contain spill and decontaminate with 10% chlorine bleach; allow sufficient contact time (30 min) before clean up. Surface is then cleaned with 70% ethanol for residual removal of cleaning agent.

Sharps & other solid waste: Packaged as recommended by waste management service provider with dedicated collection. Waste will be destined for autoclave / high temperature Incineration.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
The safety committee has confirmed the study has undergone review and the Risk Assessment is satisfactory and the activities may proceed as described. The safety committee has asked that all staff are trained on this risk assessment according to their roles, especially the PPE and waste management issues. Yellow-black or orange bags/bins must never be used for waste that may contain GMMs.

### Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Date at Which Additional Info Submitted:
02/03/2022
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Discerna GMSC will meet a minimum of once per year to review existing risk assessments and arrange for an audit of the laboratories. Meetings to approve new risk assessments will be held as required. The Discerna GMSC activities are reported to the Discerna Health & Safety Committee and the GMSC of the Babraham institute who oversees site GM safety.

Discerna GMSC is made up of:
- Chairman and Management Representative
- Biological Safety Officer
- Technical Staff Representative

<table>
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<tr>
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Inactivation of GMMs in contaminated material will be as follows:

**Liquid Discharge:** Waste to be treated with 1:1 Trigene (Halogenated tertiary amine compound) to a final concentration of 1% v/v or with 1% Virkon S. Trigene and Virkon are MAFF approved and conform with British and European Standards (CE93/42/EEC) with a 99.999% degree of kill.

**Solid Waste:** Solid waste will be sealed, retained in Biohazard waste sacks and autoclaved. Clinical waste will be removed for incineration off-site by a nominated commercial 'clinical waste' carrier in accordance with all relevant legislation and guidelines. Degree of inactivation is 100%.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

The GMSC has reviewed the company's GM procedures and the risk assessments performed. The GMSC confirmed the work to be carried out is Class 1 and were satisfied with the assessment of risks to both human health and the environment. The control and containment measures in place are appropriate for Class 1 activities.
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| Comments                           |               |

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## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Biological Safety Officer (Head of Fermentation)
- Manager R&D Administration (Health and Safety Officer)
- Senior Scientist - PhD Microbiology
- Graduate Scientist - MSc Molecular Biology
- Meeting twice yearly (or more frequently if required)

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

Yes

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research
Yes
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For activities involving GMMs, describe the waste management measures which will apply to the activity

All contaminated waste is autoclaved

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Committee is content with the risk assessment, which is an accurate representation of the nature of the GMM work carried out. The Committee considers level 1 containment facilities to be adequate.
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**Name**

UNIVERSITY OF HUDDERSFIELD

**Name 2**

**Department**

DEPARTMENT OF CHEMICAL AND BIOLOGICAL SCIENCES

**Campus Estate or Research Centre**

SCHOOL OF APPLIED SCIENCES

**Building**

JOSEPH PRIESTLEY BUILDING

**Road Name**

QUEENSgate

**District**

**Town**

HUDDERSFIELD

**County**

YORKSHIRE

**Postcode**

HD1 3DH

**Country**

ENGLAND

**Tel Number**

01484 473 142

**Fax Number**

01484 472 182

**E-mail**

**HSE Division**

YORKSHIRE AND NORTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The genetic modifications committee is a sub-committee of the University Health and Safety Committee and has been constituted according to the guidelines in ACGM/HSE and comprises of: Chairperson, School of Applied Sciences Safety Co-ordinator, Biological Safety Officer, University Safety Officer. The GM committee will meet biannually to oversee the running of GM operations at the University of Huddersfield.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)  Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  

Tick if confidential

Bacteriology  Yes  Parasitology  Transgenic Birds  Microbiology Research
All GMMs will be disposed of by autoclaving at 121 degrees C for 15 minutes. This method of killing is 100% effective. Validation of effective killing of GMOs will be based on the annual testing of the autoclave using independent monitors placed at the centre of the load so that it is confirmed that the correct temperature and pressure have been reached for the required time.

As a precautionary measure any bulk liquid left to stand around untreated for any length of time prior to autoclaving will be treated with the appropriate disinfectant.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM safety committee are all concordant with the risk assessment and are happy that the work outlined in the risk assessment should proceed.
### Project Additional Information

**Purposes of the contained use**

Generation of human skin cells by reprogramming of human fibroblasts and keratinocytes to induced pluripotent stem (iPS) cells and differentiation: The project aims at molecular characterisation of monogenic skin diseases and the development of advanced in vitro disease models suitable for drug testing and screening and therapeutic approaches. Primary fibroblasts and keratinocytes are obtained from skin samples and cultivated using standard procedures. Fibroblasts are reprogrammed using 'Yamanaka factors', Oct4, Sox2, Klf4, and cMyc.

In vitro models to study cancer development and cellular responses to pro-apoptotic signals: The aims of the work are to determine how specific molecular/genetic events contribute to the initiation and progression of cancer in human epithelial tissues as well as how they modify the apoptotic susceptibility of epithelial cells to the immune system and its products. ATCC derived cell lines are transduced using viral vectors to express fluorescent protein reporters and small hairpin RNAs (shRNAs) to study pro-apoptotic (cell death) signals.

Development of an iPS cell model system, which can be differentiated into a neuronal model for drug and biomarker development. Fibroblasts are reprogrammed using the "Yamanaka factors", Oct4, Sox2, Klf4, and cMyc. Their genes are contained in a polycistronic plasmid, pHAGE2-EF1aFull-Oct4-F2A-Klf4-IRES-Sox2-E2A-cMyc-LoxP. The genes are transferred into fibroblasts and expressed using a non-integrative sendai virus transduction. Generated iPS cells are thoroughly characterised using morphological and molecular characteristics. iPS cells are cultivated, manually passaged, and differentiated into target cells with selected media. Target cells are further characterised and used to generate in vitro models.

**Recipient or parental organism**

Mammalian cell line commercially available from ATCC, HEK293, is hazard group 1 and not known to cause harm to human health.

PT67 (Clonetech) mouse fibroblast packaging cell line (contains env, gag and pol genes integrated separately) used for retroviral production is Biosafety group 2.

Primary fibroblasts and keratinocytes are not serotyped, however, are non-permissive for HIV, HBV and HCV and therefore not supposed to cause harm to human health.

**Host/vector system**
Viral vectors: pLXSN or pSIREN-RetroQ-based vectors (both Clontech) are replication deficient and do not contain the structural genes (gag, pol, and env) necessary for particle formation and thus pose negligible risk to human health. The structural genes (gag, pol, env) are stably integrated into PT67 packaging mouse derived cell line and subsequent introduction of pLXSN, and the gene of interest produces replication-incompetent infectious virus inside the PT67 cells. Lentiviral vectors used (pHAGE2, pLenti and pLKO) are 2nd/3rd generation and are replication deficient. pHAGE2 is used in combination with pHDM and pRC/CMV-Rev1 b, pLenti (Thermo) with pLP1 , pLP2, pLPIVSVG and pLKO (SigmaAldrich) with pMISSIONgagpol and pMISSIONvsvg helper plasmids for viral packaging. Helper plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus. Helper plasmids are nonmobilisable and not known to cause harm to human health. Co-transfection of mammalian (HEK293T) cells with the pHAGE2/pLenti/pLKO plasmid and helper plasmids results in the packaging of replication deficient lentiviral vectors. Mammalian cells are destroyed by the virus, which is harvested from the media and further used for transduction of target cells (human fibroblasts). The virus is not capable of producing further virus particles in the target cells. The Sendai virus contains a polycistronic vector that has a backbone containing new temperature sensitivity mutations to the polymerase-related genes, and this helps to clear the virus faster after reprogramming and causes less cytotoxicity to the cells. Sendai virus is ACDP hazard group 1.

Origin & function

1) Human genes for Oct4, Sox2, Klf4, cMyc. These genes are known for their role in cell differentiation rather than driving cell proliferation and malignancy. 2) Inserts also include sequences designed to knock-down endogenous mRNAs (siRNAs, shRNAs) encoding for mammalian proteins, and thus reduce encoded protein products. Inserts of mammalian origin used in combination with viral vectors include genes (or parts of) known to promote cell death/apoptosis or attenuate cell proliferation. The inserts have no foreseeable risk to human health or to the environment. 3) Regulatory sequences upstream (EF1a promoter) or downstream (3' regulatory element: woodchuck hepatitis virus post-transcriptional regulatory element) of coding sequence. 4) HIV gag, pol, tat, rev1 b, VSV-G (in helper phage; used only for HEK 293T). 5) Green fluorescent protein from Aequorea victoria or luciferase from firefly.

Evaluation of foreseeable effects

Transduction of the mouse PT67 cell line, which contains stably integrated structural genes (gag, pol, env), with replication-deficient retroviral vectors results in replication and packaging of novel virus particles resulting in breakdown of the parental PT76 cells. Co-transduction of replication deficient pHAGE2/pLenti/pLKO and helper plasmids into HEK293 cells similarly results in replication and packaging of the virus and death of the parental cells. The resulting virus particles are collected into containers with sealed caps whilst working within laminar flow class II safety cabinet. Personal protective equipment (lab coats, gloves and safety goggles) are used and all virus particles are handled in a dedicated laboratory space and in class II safety cabinets with laminar flow using safe operating protocols. No sharps are to be used in the viral work. The viral vectors are replication-deficient and do not contain sequences that would allow the gene of interest to recombine and insert into the parental cell genome. Lentivirus is unstable and non-transferrable by air. The risks to human health or to the environment are thus negligible to effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMMs will be disposed of by autoclaving at 126 degrees Centigrade for 60 minutes. This method of killing is 100% effective. The effectiveness of the autoclave at reaching the correct temperature and pressure is continuously monitored by an electronic monitor that prints out the temperatures and pressure reached at the end of each run. Validation of effective killing of GMMs is monitored by annual testing of the autoclave using independent thermocouples placed at the centre of the load, that the correct time and pressure have been reached for the required time.

As a precautionary measure any bulk liquid left to stand around for any length of time prior to autoclaving will be treated with Distel (formerly Trigene; 10%; by Tristel) or 2-4% Virkon (DuPont) according to the manufacturers recommendations. To minimise the generation of aerosols, safe removal of supernatants from cultured mammalian cells (handled in class II laminar flow cabinets) is performed by aspiration using a vacuum ('trap') system, where liquids are treated in 10% Virkon. All types of culture medium and plastic ware used in mammalian cell culture are treated with 2% Virkon (final concentration) overnight, followed by autoclaving at 126 degrees for 60 minutes for all plastic ware before disposal. All virus-containing culture medium is decontaminated in 10% Virkon, which effectively kills enveloped and non-enveloped viruses.

Any laboratory ware that has been in contact with GMMs will be treated with Distel or 0.5 - 2 % Virkon for a minimum of four hours prior to dish washing. Disposable plastic ware that has been in contact with GMM will be autoclaved at 126 degrees Centigrade for 60 minutes prior to disposal. Random samples of waste will be checked for living GMMs once every two weeks. All discard going out from the School is recorded.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

GM safety committee has been consulted and has found the risk assessment as appropriate.

Project Containment

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Animal Units Large Scale Activities Human Clinical Applications

02/03/2022
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**Name**

MICROSENS BIOPHAGE LIMITED

**Name 2**

**Department**

LONDON BIOSCIENCE INNOVATION CENTRE

**Campus Estate or Research Centre**

**Building**

**Road Name**

2 ROYAL COLLEGE STREET

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

NW1 0TU

**Country**

ENGLAND

**Tel Number**

020 7691 2147

**Fax Number**

NONE

**E-mail**

**HSE Division**

LONDON

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee:

- Safety Officer, Biological Safety Officer, Company CEO, Company Scientific Director. Meeting every six months or as required.
- Outside consultants have also been appointed to review the company's procedures.

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<tr>
<th>Laboratory</th>
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<th>Glass House</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

- All liquid waste treated with 1,000 ppm free chlorine - 100% kill.
- All solid waste double-bagged and incinerated - 100% kill.

Tick to confirm that you are attaching a summary of the risk assessment 🍉

Tick if you are claiming exemption from disclosure for sections of the risk assessment 🍉

Please enter comments of the GM safety committee on the risk assessment

## GM Centre Number: 812

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**Name**

SCHERING HEALTH CARE LIMITED

**Campus Estate or Research Centre**

**Road Name**

THE BROW

**Town**

BURGESS HILL

**District**

WEST SUSSEX

**Country**

ENGLAND

**Tel Number**

01444 232323

**Fax Number**

01444 246613

**E-mail**

none

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

<table>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification committee is composed of the following four members:

- Biological Safety Officer
- Associate Medical Director
- Clinical Pharmacy Manager
- Radiation Protection Supervisor

In addition, there are four co-opted members from Schering affiliated companies in the USA and Germany. They provide, when necessary, additional advice on biotechnology, medical, pre-clinical and toxicological matters respectively.

The committee generally meets on a quarterly basis, although it can convene in the interim should the need arise.

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Warehouse

Tick if confidential
As the site is only for storage and distribution, no waste will be generated.
Spillages that could arise in the event of an unforeseen gross failure of the sealed packaging system will be treated with 5% bleach or 0.5% sodium hydroxide solution (effectively 100% kill).

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The genetic modification safety committee approved the finalised version of the risk assessment without any comment being made.
### GM Centre Number: 813

<table>
<thead>
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**Name**

| CELLZOME UK |

**Name 2**

**Department**

**Campus Estate or Research Centre**

| CHESTERFORD RESEARCH PARK |

**Road Name**

| LITTLE CHESTERFORD |

**Town**

| CAMBRIDGE |

**County**

| CAMBRIDGESHIRE |

**Postcode**

| CB10 1XL |

**Country**

| ENGLAND |

**Tel Number**

| 01799 532 800 |

**Fax Number**

| 01799 532 801 |

**HSE Division**

| EAST AND SOUTH EAST |

**E-mail**

**Comments**

**Date at Which Additional Info Submitted**

| 19/04/2005 |
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The GMSC is a sub-committee of the Cellzome UK Health & Safety Committee. It is comprised of the following:- Chairman; Biological Safety Officer; Secretary and research scientist involved in the GMM work. The committee meets at least every 2 months or more frequently if required during the initial start-up phase. Each meeting has an agenda, recorded minutes and actions. As the company expands, the membership broadens to ensure a fuller representation of scientific expertise.

A risk assessment must be completed and submitted to the GMSC prior to the work commencing. The GMSC must review and approve the risk assessment and assign a final activity class. The approved risk assessments are available electronically to all workers on a shared computer area. Local codes of practice are available for work involving GMMs at both containment levels 1 and 2. The GMSC also ensures good laboratory practice and ensures full discussion with all those concerned, on safety, training and laboratory discipline.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Tick if confidential</td>
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</table>
A number of different routes will be used for category 1 waste disposal.

**SOLID WASTE DISPOSAL:**

1. All waste will be placed in EU designated yellow bags in solid bins. When the bags are 2/3rds full the bags are sealed and placed in a wheelie bin belonging to accredited waste management company, White Rose Environmental. These bins are lockable and are collected by White Rose for incineration.

2. All category 1 biological waste will be placed in autoclave bags inside solid autoclavable bins with lids. These bins will be placed directly into the autoclave which is available in the building. The waste will be autoclaved at 121C for 20 minutes. The autoclave bags will then be placed in yellow bags and placed in the wheelie bins for incineration by White Rose. Laboratory staff are responsible for the waste they produce.

In the event of autoclave malfunction, Category 1 biological waste may be disposed of in hermetically sealed containers provided by White Rose. These containers are then disposed of in the aforementioned wheelie bins for incineration.

The autoclaves are fully compliant with BS 2646:1993 - autoclaves for sterilisation in laboratories. They are commissioned and validated by the manufacturer and independently certified to PD 5500:2000 Cat 3. They are also compliant with European pressure equipment directive 97/23EC. They are regularly serviced by an independent qualified engineer.

**LIQUID WASTE DISPOSAL:**


**SHARPS:**

All sharps must be placed in specially provided sharps bins and discarded in White Rose wheelie bins.

---

For activities involving GMMs, describe the waste management measures which will apply to the activity

---

---

Tick to confirm that you are attaching a summary of the risk assessment

Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

---

---

Please enter comments of the GM safety committee on the risk assessment

This risk assessment was considered at a GMSC meeting conducted on the 4th December 2001 and assigned a containment classification 1 and activity class 1. The committee advised that the vector map must be attached to the risk assessment.
**GM Centre Number: 814**

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**Name**

INPHARMATICA LIMITED

**Name 2**

Department

**Campus Estate or Research Centre**

127 SCIENCE PARK

**Road Name**

MILTON ROAD

**Town**

CAMBRIDGE

**District**

CAMBRIDGESHIRE

**County**

CB4 0GD

**Country**

ENGLAND

**Tel Number**

01223 424825

**Fax Number**

01223 425416

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Moved premises to Cambridge in December 2005

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities Y

Give brief details of the genetic modification safety committee

The genetic modifications safety committee consists of a Chairman - Chief Scientific Officer, Biological Safety Officer, Associate Director Operations, Associate Director Screening, Employee Representative. An outside consultant who we are in the process of appointing. On our previous site formal meetings have taken place every 6 months, with additional meetings occurring as necessary. This schedule is likely to continue.

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Tick if confidential

Bacteriology Yes Parasitology Transgenic Birds Microbiology Research

Virology Transgenic Animals Transgenic Fish Gene Therapy
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid waste will be autoclaved (121 C for 30 minutes) and in each autoclave run, monitors will be included to ensure effective temperature and pressure. The degree of kill will be 100%. The autoclave is tested annually (12 point test) and is serviced twice a year. After autoclaving, solid waste is disposed as clinical waste (for incineration).

Liquid waste will be treated with a laboratory disinfectant such as Virkon following the manufacturers guidelines to achieve 100% kill. After treatment, liquid waste is disposed down the drain.

All workers will be trained to follow local rules.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The genetic modification safety committee approved the forms to be used for risk assessment of projects.
GM Centre Number: 815

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Name

LUCIGEN LIMITED

Name 2

Department

Campus Estate or Research Centre

Building

BUILDING 227

Road Name

PORTON DOWN SCIENCE PARK

District

PORTON DOWN

Town

SALISBURY

County

WILTSHIRE

Postcode

SP4 OJQ

Country

ENGLAND

Tel Number

01980 556 530

Fax Number

01980 591 148

E-mail

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Lucigen has established a GMSC. The Committee is composed of the Biological Safety Officer, two scientists and a scientific consultant and will meet twice yearly, or as necessary, to review all risk assessments.

<table>
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<td>Microbiology Research</td>
<td>Gene Therapy</td>
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</table>

02/03/2022
All liquid and solid waste contaminated with GMMs will be autoclaved prior to disposal by incineration. Autoclaves used in this process will be validated, by qualified persons, on a yearly basis.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Activities involving this low risk GMM require containment at level I. However, process requirements dictate that the activity will be undertaken at a level similar to containment level II.
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Name

PAPWORTH HOSPITAL NHS TRUST

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

District

PAPWORTH EVERARD

Town

CAMBRIDGE

Country

ENGLAND

County

CAMBRIDGESHIRE

Postcode

CB3 8RE

Tel Number

01480 364662

Fax Number

01480 364686

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The local genetic modification safety committee has been established as a subgroup of the Trust's Risk Management Group and includes representatives from various backgrounds within the hospital. It is chaired by the Director of Nursing and Quality. It includes both clinical and non-clinical staff. The committee has reviewed both the risk assessment and the standard operating procedures for the trial and will meet periodically throughout the project to review safety issues. The first review is envisaged when 3-5 patients have been treated but will be sooner if felt appropriate by the risk or biological safety officers.

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Bacteriology  Parasitology  Transgenic  Microbiology

Birds          Research  

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Having reviewed the risk assessments, the Genetic Modifications Safety Committee (GMSC) were satisfied that the arrangements for containment and disposal of the drug containing the GMM, and any associated clinical or patient wastes, are adequate and that suitable facilities are available for segregation of the patients who are included in the trial. The GMSC require that all staff associated with the treatment or care of patients to whom GMM drug therapy is given will receive suitable training and information about the procedures, the safety measures and the nature of the hazards the drug presents.

Project Ref 816/02.1

Date Ackn'd 21/01/2002

CU2 Project Title PART OF A MULTI-CENTRE TRIAL TO LOOK AT THE CLINICAL EFFICACY OF AN ANGIGENIC GENE THERAPY PRODUCT IN THE TREATMENT OF REFRACTORY ANGINA IN PATIENTS WHO ARE UNSUITABLE FOR OR FOR WHOM THERE IS NO ...

Class 2

CultureVol

Class 2

CultureVolumeClass 2

< 1 litre

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

The purpose of this trial is to assess whether the use of angiogenetic growth factors such as FGF-4 will improve symptoms of angina and enhance the quality of life for patients with chronic refractory angina. Previous studies suggest that there is likely to be a positive benefit, however this has not been assessed systematically. This is the first European trial to attempt to do this.

#### Recipient or parental organism

The transgene is a human fibroblast growth factor 4 gene driven by a CMV promoter. The recipients of the GMO will be patients in whom there is no conventional (ie angioplasty or bypass surgery) option. The transgene will be delivered by cardiac catheterisation directly into the coronary circulation.

#### Host/vector system

The vector is based on a serotype 5 adenovirus. Adenovirus type 5 is typically a respiratory pathogen, with 50% of the adult population having antibodies to the wild-type virus. The vector is replication incompetent (the E1 area of the viral genome has been deleted.) It is expected to have the same tissue tropism as the wild type virus.

#### Origin & function

The E1 area of the viral genome is replaced by the FGF-4 transgene. This is a 1.2Kb cDNA for the human FGF-4 gene driven by a Cytomegalovirus promoter. The gene therapy product will be delivered by a single injection at cardiac catheterisation. Induction of angiogenesis is potentially a new therapeutic option. The effect is based on the growth of collateral vessels to ischaemic areas of heart muscle. This effect has been demonstrated in animal models and in small numbers of patients but has not been evaluated in a systematic way.

#### Evaluation of foreseeable effects

Infection of immunocompetent adults with wild type adenovirus would be expected to produce a mild upper respiratory tract infection. The normal method of spread is as an aerosolised droplet infection spread person to person. Measures are to be undertaken to minimise staff contact and to reduce the risk of aerosolisation of the virus. Conjunctivitis and gastroenteritis are also described. Insertion of the FGF-4 or CMV promoter is not expected to alter the tissue topiism or infectivity of the virus. Accidental injection of the study product would be expected to cause local inflammation.

Adenovirus type 5 has not been shown to have any tumorigenicity in vivo. There is a theoretical risk of abnormal angiogenesis and oncogenesis from the transgene. The risk of this is likely to be low for the following reasons;

To become oncogenic the, transgene would have to be incorporated into cellular DNA. Combination with cellular DNA is described in only 0.1 to 0.001% of transfected cells. Transfection efficiency is itself low (circa 25%) and is concentration dependent. Following intracoronary administration, the amount reaching the systemic circulation is 10,000 fold lower than that given locally and is cleared from the bloodstream in minutes.

b) Long term gene expression is necessary for oncogenesis. Following aerosolised administration of an adenovirus type 5 to normal individuals, it was found that vector DNA peaked at day 7 and reached less than 10% of baseline levels by 2 weeks. This effect was again concentration dependent. In animal studies, with an adenovirus-FGF product the longest adenovirus DNA could be detected was 3 months - at this stage no transgene could be detected.

There is not expected to any environmental impact from this project.

---

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No autoclave in building: The method of waste disposal for material used during the preparation and delivery of the gene therapy product will be by autoclaving. The autoclave facility to be used is located in the building used to prepare the product (when concentrations of adenoviral product will be highest). The actual delivery of the product will be in the cardiac catheterisation laboratories which are in a separate building. At the end of the procedure all equipment which has been in contact with the adenoviral product will be placed in sealed autoclave bins for transport to the autoclave - which is approximately 200m away. All waste will be double bagged prior to transportation. The metal boxes are leak proof and are tested for compatibility with the autoclave prior to use.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste containing the adenovirus material will be disposed of as biohazardous. All contaminated material will be put into sealable, leakproof autoclavable containers. Inactivation of the adenovirus product is by autoclaving at 121 degrees C for 15 minutes giving 100% kill. The autoclave cycle will be validated prior to commencement of this project and will be validated on a yearly basis. For each cycle, autoclave indicator tape and the integral autoclave printout will be used to confirm that the waste has been autoclaved. All autoclaved waste will then be incinerated.

Autoclave boxes will be kept at each location that biohazard waste will be generated, namely at product reconstitution and at cardiac catheterisation.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form Y

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee felt that the risks represented by this project were likely to be small and that with the proper conduct of the trial, and adherence to the safety procedures that these risks could be minimised further. It was noted that Papworth Hospital has been involved in randomised controlled trials amongst similar patient groups previously and that these trials had been well conducted and received. However, this is a new technique at Papworth so they were keen that the project is appropriately supervised and managed and that staff will appropriately trained and familiarised with the project. They noted that any member of staff who had the potential (through family disease history or current infection) to be affected by the GMO would not be expected to work with patients receiving the therapy.

Project Containment

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Name

TRANSGENOMIC LIMITED

Name 2

Department

Campus Estate or Research Centre

Road Name

SOUTH NELSON ROAD

District

Town

CRAmlINGTON

County

NORTHUMBERLAND

Postcode

NE23 1WF

Country

ENGLAND

Tel Number

01670 732992

Fax Number

01670 730454

E-mail

HSE Division

YORKSHIRE AND NORTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

Operations Manager, Quality Group Leader, Senior Research Scientist & Research Scientist.
The committee meets on a six monthly basis to review new proposal or changes since the last meeting. Meetings are scheduled for November & May.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
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</table>

Virology

02/03/2022
All biological waste is processed in the following ways:

1. Autoclaved on an automatic waste cycle of a pre-programmed MDH Labclave (122C/2 bar for 40 minutes). Each run has a chart recorder recording the load temperature measured by a wandering probe placed in the load. This generates a trace record of each run to verify that the load has reached the specific temperature for the prescribed time. This is augument by visual checks. The autoclave is serviced on a six monthly basis and calibrated to a NAMAS specified standard.

2. Additional sanitation on equipment that cannot be autoclaved e.g. filtration units is by re-circulation of 1.0M Sodium Hydroxide for 1 hour as described in the standard operating procedure for the unit.

3. Fermentation Equipment is sterilised with steam in situ with the vessel being held at 121C for 40 minutes after each use. Sterility of the vessel is tested by refilling with fresh sterile media and running at 37C for 24 hours. No growth should be observed after this period.

4. Centrifuge tubes and test tubes used in the down stream processing are autoclaved (121C/40 minutes) and then acid washed in a commercial glass washing machine.

5. General work surfaces are frequently decontaminated using either 70% methanol or 2% virkon leaving the area in question to be in contact with the disinfectant for 20 minutes.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The first of 2 standard systems used is:

The standard host vector system used is E coli JM109 host which is a K12 derivative and therefore none colonising with pUC18 which is recognised as a non-mobilisable vector.

This has been assessed as having an overall risk factor of 10- and therefore category 1 is appropriate.

The second typical system used for protein expression.

The host strain is Ecoli Novablue (DE3) which is an E.coli B strain and is recognised as a disabled or non-colonising. The vector generally used is pET 30, which is a mobilisation defective vector. The typical gene product is a DNA modifying enzyme. The expression is inducible from a strong T7 promoter using IPTG and is optimised for the production of the protein. Damage is extremely unlikely that the over expression could occur except in vitro and should the product enter the environment it is unlikely to cause any damage as it is unstable outside of the cell at ambient temperatures and unlikely to survive. The toxicology of the product is unrecorded as it will be naturally present in the environment. The Overall risk for this system is 10-5 . Therefore containment level 1 is appropriate.
### GM Centre Number: 818

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### Name

IONIX PHARMACEUTICALS LIMITED

### Name 2


### Campus Estate or Research Centre

CAMBRIDGE SCIENCE PARK

### Building

UNIT 418

### Road Name

MILTON ROAD

### District


### Town

CAMBRIDGE

### County

CAMBRIDGESHIRE

### Postcode

CB4 0PA

### Country

ENGLAND

### Tel Number

01223 433 777

### Fax Number

01223 433 788

### E-mail

info@ionixpharma.com

### HSE Division

EAST AND SOUTH EAST

### Comments

Premises closed down as of 24/06/2005

### Date at Which Additional Info Submitted

14/06/2005
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Head of Biology (Management representative), Senior Research Scientist Biology, Research Scientist Biology & Biological Safety Officer.

A combination of formal and informal evaluation and approval procedures will be implemented.

Researchers will circulate risk assessments (available on line via local network) to the members of the committee. Individual members of the committee may advise researchers on an informal basis.

A three monthly meeting will be called for formal review of all applications and current issues relating to genetic modification.

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<thead>
<tr>
<th>Laboratory</th>
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Tick if confidential

Yes
Only small scale liquid waste will be generated (< 5 litres at any one time). Liquid waste will be collected and inactivated using chlorine releasing tablets prior to sink disposal. Solid waste will be collected in buckets lined with autoclave bags. Waste will be autoclaved in a cycle of 121°C for 15 minutes. A fully calibrated autoclave will be used for inactivation. Calibration will take place yearly. Waste will be collected as clinical waste by an external contractor (Grundon). Sharp solid waste will be collected separately in sharpsafe bins and autoclaved as above. Chlorination and autoclaving are accepted procedures which will result in complete killing.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment was approved by the committee.
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities:

Y

**Give brief details of the genetic modification safety committee**:

Chairman - Research Director, Secretary - Administrating Research Assistant (BSO), Committee Member - Research Director. Monthly meetings to discuss any matters pertaining to genetic modification procedures.

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Tick if confidential: [ ]

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research
---|---|---|---
Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity:

- Treatment of all consumables and liquid waste that have been, or may have been in contact with GMMs with chloros, vircon or microsol 3 -followed by double-bagging. Alternatively where use of virocides is not practicable double-bagging will be used. All such waste will then be incinerated to give 100% kill. Regular swabbing of work surfaces to check for antibiotic resistant microorganisms.

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Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: __

Please enter comments of the GM safety committee on the risk assessment:

The safety committee believes that the procedures put in place meet the relevant criteria and is seeking advice from the HSE that all appropriate procedures are in place.

---

**Project Ref 819/02.1**

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Non-GMM: Consent Granted: not applicable

- Project notified under transitional arrangements: N

- Withdrawn: N

Tick if notifying a connected programme of work: N
### Project Additional Information

#### Purposes of the contained use

To immortalise human and rat cells, for potential therapeutic use in the treatment of various cytodegenerative disorders.

#### Recipient or parental organism

The recipient cells will be human and rat cells derived from various body tissues.

#### Host/vector system

The retroviral shuttle vector to be used is essentially that reported by Cepko et al (Cell 37 [1984] 1053). The vector is based on the moloney murine leukemia virus (M-MULV) and comprises the following M-MULV sequences: Long terminal repeats (LTRs) necessary for the initiation of viral transcription, the polyadenylation of viral transcripts as well as for genomic integration; sequences necessary for the reverse transcription of the viral genome; sequences for the encapsulation of viral RNA; 5' and 3' splicing signals involved in the generation of genomic viral env RNA. In place of the M-MULV sequences encoding the gag-pol and env polypeptides, BamH I and XhoI restriction sites have been inserted. Within the BamH I restriction site, a temperature-sensitive mutant of the simian virus 40 large tumour antigen (ts)A58SV40T has been placed, allowing expression of the large T mutant A58. In addition, sequences derived from the transposon 6 encoding the geneticin resistance (G418) selection marker, have also been included. The pBR322-derived SV40-derived origins of the replication have been introduced into the XhoI site allowing the construct to act as a shuttle vector, (for review see Noble et al (Brain Pathology 2 (1992) 39).

The aforementioned retroviral vector construct in plasmid form was used to transfect an amphotropic packaging cell line (Dance and Mulligan, PNAS 85 [1988] 6460). The packaging cell line has been designed so the helper functions are encoded by two separate segments of DNA, ensuring the safe generation of recombinant retroviruses. There is no possibility of transduced cells producing infectious virus particles (Stamps et al Eur J Cancer 28A [1982] 1495).

#### Origin & function

The temperature-sensitive form of the simian virus derived large T-antigen binds endogenous p53 at 33 degrees C to inhibit cellular apoptosis.

#### Evaluation of foreseeable effects

The process has been widely practiced in many laboratories throughout Europe and the USA. No untoward effects have been reported.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Treatment of all consumables and liquid waste that have, or may have been in contact with virus with Chloros, Virkon or Microsol 3, followed by double-bagging. Alternatively where use of virocides is not practicable double-bagging will be used. Double-bagged waste will then be autoclaed prior to being sent for incineration to give 100% kill. Mammalian cells are destroyed outside growth medium, however, swabs of work surfaces will be performed and cells screened by bioassay to check for cross-over reactivity.
The Safety Committee believes that the procedures put in place meet the relevant criteria and is seeking advice from the HSE that all appropriate procedures are in place.

Please enter comments on the GM safety committee on the risk assessment

The Safety Committee believes that the procedures put in place meet the relevant criteria and is seeking advice from the HSE that all appropriate procedures are in place.

Project Containment

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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
**ADDENBROOKE'S NHS TRUST**

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Date at Which Additional Info Submitted: 02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Safety Committee advise on risk assessments of contained use activities and check that waste received are from registered centres, and are properly packaged for transport/handling.

Committee comprises:
DGSA & Maintenance Manager - Chairman
Prof of Infectious Diseases & Hon. Consultant Physician
Engineering Services Manager
Co-ordinator Waste Management

Committee meets annually

<table>
<thead>
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<tr>
<td>Level 1 (GMMs)</td>
<td>Level 2 (GMMs)</td>
<td>Level 3 (GMMs)</td>
<td>Level 4 (GMMs)</td>
<td>Non-microbial</td>
</tr>
<tr>
<td>Other (please specify)</td>
<td>Incineration of waste GM materials.</td>
<td>Tick if confidential</td>
<td></td>
<td></td>
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</tbody>
</table>
The registration is specifically for an incinerator facility, receiving GM waste from other registered users.

The waste will be collected or delivered in double packed UN approved leak-proof containers and tipped into a hopper from where it is automatically conveyed into the incinerator.

Incineration achieved 100% destruction of any biological material and therefore will achieve complete inactivation of all GMOs.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The registration is specifically for an incinerator facility, receiving GM waste from other registered users.

The waste will be collected or delivered in double packed UN approved leak-proof containers and tipped into a hopper from where it is automatically conveyed into the incinerator.

Incineration achieved 100% destruction of any biological material and therefore will achieve complete inactivation of all GMOs.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Safety Committee has checked the waste is coming from registered centres (certificate copied), has been assessed as being level 1 (risk assessment copied) and is properly packaged for transport/handling by the incinerator staff.
<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
<th>05/02/2002</th>
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<tbody>
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<td>Transferred from 1992 Regs?</td>
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<tr>
<td>Transitional Premises Class</td>
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<tr>
<td>Data Premises Closed</td>
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<td>Transitional Premises</td>
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</tr>
<tr>
<td>Emergency Plan Required?</td>
<td></td>
</tr>
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<td>Non-GMMs</td>
<td>N</td>
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<tr>
<td>Withdrawn</td>
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</table>

**Name**

MRC TOXICOLOGY UNIT

**Name 2**

UNIVERSITY OF CAMBRIDGE

**Campus Estate or Research Centre**

**Building**

GLEESON BUILDING

**Road Name**

TENNIS COURT ROAD

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 1QR

**Country**

ENGLAND

**Tel Number**

0116 2525611

**Fax Number**

0116 2525599

**HSE Division**

MIDLANDS

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

<table>
<thead>
<tr>
<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
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<td>TOXICOLOGY UNIT</td>
<td></td>
<td></td>
<td>BOX 138</td>
<td>HODGKIN BUILDING</td>
<td>LANCASTER ROAD</td>
<td>LEICESTER</td>
<td>LEICESTERSHIRE</td>
<td>LE1 9HN</td>
<td>ENGLAND</td>
<td>N</td>
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<td>CB2 1QR</td>
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<td>N</td>
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</tr>
</tbody>
</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Yes**

Give brief details of the genetic modification safety committee

Unit Director, Biological Safety Officer, Health & Safety Officer, Leicester University Biological & Chemical Safety Officer

Five members are research team leaders - Doctors with different areas of expertise within toxicology.

<table>
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<td></td>
<td></td>
</tr>
<tr>
<td>Level 2 (GMMs)</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
All liquid waste will be treated with hypochlorite (concentration 10,000ppm available chlorine) once the waste is deactivated it will be run to drains. All plasticware will be treated with hypochlorite (10,000ppm) before being bagged up and autoclaved at 121 degrees C 15 mins, this achieves 100% kill.

All sterile plasticware is then sent for incineration by authorised contractors.

The autoclaves are tested annually for validation purposes.
The aim of the project is to express ion transport systems (as the Na+ -Ca 2+ exchanger, the plasma membrane Ca 2+ pump), the calcium indicator protein aequorin and the green fluorescence protein (GFP) in primary neuronal cultures and neuronal cell lines. We will use a new replication-defective hybrid lentiviral vector originally derived from HIV.

Origin & function

Evaluation of foreseeable effects
The lentivirus vectors have been developed as tools for gene therapy in conditions when known viral vector systems proved ineffective. In addition to their capacity to integrate into the genome in absence of the original viral genes and the lack of typical immunological effects of the parental (HIV) vector, lentiviral vectors possess an enormous capacity to infect non dividing cells. The viral vector particles are replication defective and consist of the core and enzyme of a lentivirus and the envelope of the Vesicular stomatitis virus (VSV). To assemble the lentiviral vector, a third-generation packaging system is used:
> Three plasmids (packaging constructs) for respectively
  * the gag and pol genes,
  * the envelope,
  * the gene for Rev protein

> One transfer construct containing the transgene and sequences for all cis functions allowing efficient encapsidation, reverse transcription, nuclear transport and integration of the vector into the target cells.

Transient cotransfection in 293T cells (with calcium phosphate-DNA precipitation) of these four separate plasmids reduce remarkably the likelihood of replication-competent virus (RCR).

To improve the biosafety of this vector and to reduce the likelihood of recombination between the constructs used to make vectorm, the following measures have been
adopted:
- Elimination all viral genes, that are not essential for gene transfer (vif, vpr, vpu, nef) from the packaging constructs.
- Elimination of tat gene (transcriptional activator, that plays a pivotal role in the exceedingly high replication rates that characterise HIV-imdicted disease).
- Elimination of the gene for Rev protein (implicated in the cytoplasmic export of unspliced and singly-spliced viral transcripts expressing the late viral proteins) from the core packaging construct.
- Deletion of the U3 region of the LTR from the transfer construct (transcriptional inactivation of the LTR > self inactivating transfer vectors > diminished risk of vector mobilisation).

We have chosen this vector system because of the particular ability of HIV-based viral vectors to deliver genes in vivo not only into proliferating cells of cell lines but into non-dividing cells, like primary neurons.

The production of the viral particles is the major hazard in our work, where we have concentrated all our attention to achieve the biosafety...
In the neuronal cultures the viral-derived particles inactivate very quickly. The probability of spontaneous recombination events is extremely low.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
<thead>
<tr>
<th>Waste disposal:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>* All liquid waste will be treated with sodium hypochlorite (10000 ppm active chloride) for 8 hours</td>
<td></td>
</tr>
<tr>
<td>* All waste material containing viable GMM (spent culture fluid and other media) are inactivated by the addition of sodium hypochlorite 10% in robust leak proof container.</td>
<td></td>
</tr>
<tr>
<td>* Pipettes contaminated by the virus will be immersed in sodium hypochlorite, drained and collected in the cabinet where they will be double bagged ready for autoclaving. All contaminated plastic waste is immersed in sodium hypochlorite (10000 ppm active chlorine) for at least 8 hours, drained, double bagged, then autoclaved 121 degrees C 20 minutes. All sterile waste then goes for incineration.</td>
<td></td>
</tr>
</tbody>
</table>

**Proposed testing/monitoring measures:**
- The cells culture infected with the virus are tested, after the change of the medium, with p24 ELISA test and with RT-PCR to see the presence of the replication competent recombinants (RCR).
- Culture are set-up by adding aliquots of the medium to C8166 (or other mammalian cells) to see eventual RCR.
- The cells infected with Lentiviral particles are fixed, washed or lysed in level 2 containment room (513b).

**Is an emergency plan required according to regulation 20?**
- [ ] Y
- [ ] N

**If yes, tick to confirm that it is attached to this form**
- [ ] Y
- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**
- [ ] Y
- [ ] N

**Tick if you are claiming exemption from disclosure for section of the risk assessment**
- [ ] Y
- [ ] N

02/03/2022

Page 11481 of 15326
It is considered that the testing for replication competent recombinants is essential and that approval should be on the condition this is always carried out.

### Project Containment

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<thead>
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<th>Laboratory Activities</th>
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</tr>
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<tbody>
<tr>
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<td>L2 L3 L4 L2</td>
<td>L2 L3 L4</td>
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<td>L2 L3 L4 L2</td>
<td>L2 L3 L4</td>
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</table>

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### Project Ref 821/04.1

**Date Ackn'd**

28/01/2004

**Date Project Ceased**

15/10/2009

**CU2 Project Title**

THE ROLE OF THE PROMYELOCYTIC LEUKAEMIA GENE (PML) DAXX AND C-JUN/C-JUN-N-TERMINAL KINASE 1/2 IN THE RESPONSE TO VARIOUS CELLULAR STRESSES

**Class**

Class 2

**CultureVolClass2**

< 1 litre

**Consent Granted**

not applicable

**Project notified under transitional arrangements**

N

---

**Historical Significant Changes**

This project transfers to GM Centre 14 on 15/10/2009

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**Project Additional Information**

**Purposes of the contained use**

To study the effect of overexpression or down regulation of PML and DAXX on apoptosis and the regulation of the response to irradiation. The c-Jun and June-N-terminal kinase 1/2 pathway regulates apoptosis induced by UV radiation. We wish to test the effect of the siRNA-based downregulation or functional inactivation of various components of this pathway on the response to UV.

**Recipient or parental organism**

---

02/03/2022

Page 11482 of 15326
Mouse and human primary keratinocytes, mouse embryo fibroblasts, mouse bone marrow cells, mouse foetal liver cells, 293 cells (epithelial cell line), primary mouse cerebellum granule cells, neuroblastoma cell lines and adrenal chromaffine cells.

Host/vector system

Retroviral vectors such as MoMLV, MSCV and HIV-derived lentiviral vectors will be used. All the vectors are replication defective. All the trans acting genes (structural, regulatory and accessory proteins) are excluded from the transfer vectors, which encode the genes of interest. The transacting plasmids are provided by the packaging plasmids. The biosafety of these vectors is further advanced by deleting unnecessary genes and the two regulatory genes (tata and rev).

Origin & function

PML and DAXX cDNAs are from human and mouse origin. siRNA oligos for PML, DAXX, c-JUN and JNK1/2 will be designed based on mouse and human sequences. All the genes we intend to study are involved in the regulation of apoptosis.

Evaluation of foreseeable effects

There is a potential risk of infection at the stage of packaging and infection while using the lentiviral vectors, however the inserts we plan to transduce are not a hazard to human health. The host cells and plasmids generated by the parental virus are not harmful. The virus can infect the cells with which it comes into contact but cannot replicate, therefore the hazards to human health are low and containment level 2 is appropriate.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste:
Solid waste will be treated with hypochlorite (10,000ppm) before being autoclaved prior to disposal (100% kill). The sterile waste is then sent for incineration by authorised contractors.

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Liquid waste will be treated with hypochlorite (concentration 10,000ppm available chlorine) overnight and autoclaved before disposal. Any spillages will be cleaned with chlorine disinfectant and the waste paper autoclaved and incinerated.

Autoclave cycle - 121 degrees C for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
It has been agreed by the Genetic Modification Safety Committee that a designation of class 2 is appropriate for this work and that the risk assessment is sound and should be carried out at Containment level 2.

**Project Containment**

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<td>L2 L3 L4 L2</td>
</tr>
</tbody>
</table>

**Project Ref** 821/04.2

**Date Ackn'd**
28/01/2004

**CU2 Project Title**
EXPRESSION BY LENTIVIRAL INFECTION OF THE FOLLOWING CLONED GENES - GFP, CEBP FAMILY, BCL11A AND B, MLT, PRDM6 - IN B CELLS.

**Date Project Ceased**
17/01/2014

**Consent Granted**
not applicable

**Project notified under transitional arrangements**
N

**Historical Significant Changes**
Transferred to GM11 on 17/01/2014

**Project Additional Information**

**Purposes of the contained use**
To clone and express genes that have been identified as having a possible involvement in haematological malignancies, in primary B cells and B cell lines of human origin. The aim in the first instance is to assess whether the genes of interest have an effect on cell growth and/or apoptosis. Primary B cells cannot easily be made to express cloned genes by any other transfection method.

**Recipient or parental organism**
Primary normal human B cells and B cell lines of human origin.

Host/vector system

Pseudolentiviral particle based on HIV is used to infect the primary B cells (Naldini et al., Science. 1996 Apr 12;272 (5259):263-7). The lentiviral transfer vector pRRLsin.PPT.hCMV.pre will contain the gene of interest under the CMV promoter and between the 5' and 3' LTRs of HIV for integration into the cellular genome. It is transfected into 293T cells together with 3 core packaging plasmids and hybrid viral particles are made with the core proteins and enzymes of a lentivirus and the envelope of vesicular stomatitis virus. All structural, regulatory and accessory genes of HIV are excluded from the lentiviral vector and from the packaging plasmids. Virus particles are made which are replication-defective. The procedure involves transient transfection ie a short time for production of particles. The particles are collected from the culture supernatant, concentrated by centrifugation and can be stored frozen before being used to infect the target cells.

Origin & function

GFP - green fluorescent protein. Non-harmful.
Bcl11A and B - zinc finger genes, possible transcription factors. Human genes.
MLT - a paracaspase and may be involved in apoptotic pathways. Human gene.
PRDM6 - SET domain, may be involved in chromatin remodelling. Human gene.
It is likely that we will use mutated versions of all these genes also.
GFOP is only used as a test control gene. All the other genes are possible oncogenes.

Evaluation of foreseeable effects

At the packaging stage the viral particles could infect human cells. Therefore, the production of the virus and infection of host cells with the virus is carried out in containment level 2. Cells infected by the lentiviral particles are not hazardous as the virus cannot replicate. However, they could be overexpressing the gene inserted. The inserted genes are of unknown function and could be oncogenes but it is unlikely they will function on their own without other cellular changes. There is a low possibility of infecting individual cells in vivo. The virus particles cannot replicate and infection would remain localised to the site of entry.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Autoclave cycle - 121 degrees C for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.
It has been agreed by the Genetic Modification Safety Committee that a designation of Class 2 is appropriate for this work and that the risk assessment is sound and should be carried out at Containment level 2.

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**Project Ref**  821/04.3

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<th>CultureVolumeClass3-4</th>
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<tbody>
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<td>28/01/2004</td>
<td>EXPRESSION OF ATAXIN-1 (SCA1) IN PRIMARY NEURONAL CELLS USING LENTIVIRAL VECTORS</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
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<table>
<thead>
<tr>
<th>Non-GMM</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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</thead>
<tbody>
<tr>
<td>not applicable</td>
<td></td>
<td>N</td>
</tr>
</tbody>
</table>

Withdrawn  N  

Tick if notifying a connected programme of work  N  

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Project Additional Information

#### Purposes of the contained use

Spinocerebellar ataxia type 1 (SCA1) is an autosomal-dominant neurological disorder. We wish to understand the role of the calcium extrusion system and its deregulation in this disorder. The aim of this project is to express ataxin-1 wild type and ataxin-1 containing a 92-polyglutamine expansion in primary neuronal cultures.

#### Recipient or parental organism

<table>
<thead>
<tr>
<th>293T cells (epithelial cell line)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary cultures of cerebellum granule cells.</td>
</tr>
</tbody>
</table>

#### Host/vector system

The proposed lentiviral vectors are replication-defective so only the early steps of the life cycle are maintained. The vector to be used is a pseudotype HIV-derived lentiviral vector using a third generation packaging system (pRRLsin.PPT.hCMV.Wpre). The transfer plasmid contains the human cytomegalovirus promoter. It is defective for the expression of the viral proteins required in trans but retains all the cis-acting sequences crucial for packaging, reverse transcription and integration of the transcript derived from the transfer vector. An almost complete deletion in the U3 region of the LTR region has been made thus enabling the generation of self-inactivating virus particles and increasing the biosafety of the system.

#### Origin & function

The Ataxin genes are of human origin. These will be cloned into the Self-inactivating (sin) transfer vector plasmid pRRLsin.PPT.hCMV.Wpre and viral particles prepared by co-transfection of 293T cells with a 3rd generation packaging system (three packaging plasmids). The transducing viral particles obtained by this method will then be added directly to target primary cultures of cerebellum granule cells thus allowing an assessment of the role of Ataxin-1 in neuronal cells.

#### Evaluation of foreseeable effects

There is a potential risk of infection at the stage of packaging and infection while using the lentiviral vectors, however, the vectors are replication defective and the likelihood of recombinant events is low. The hazards to human health are low and containment level 2 is appropriate.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Autoclave cycle - 121 degrees C for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

It has been agreed by the Genetic Modification Safety Committee that a designation of Class 2 is appropriate for this work and that the risk assessment is sound and should be carried out at Containment level 2.

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<table>
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<thead>
<tr>
<th>Date Ackn'd</th>
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</thead>
<tbody>
<tr>
<td>28/01/2004</td>
<td>EXPRESSION OF CALPASTATIN, CALPASTATIN (AA 1-251) CONJUGATED WITH YELLOW FLUORESCENT PROTEIN (YFP), CASPASE-3-AND CALPAIN- SENSIBLE FLUORESCENT......</td>
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<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td></td>
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</tr>
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<tbody>
<tr>
<td></td>
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<table>
<thead>
<tr>
<th>Project notified under transitional arrangements</th>
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Historical Significant Changes

<table>
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<tr>
<th>Withdrawn</th>
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</table>

02/03/2022
Calcium signals govern a wide range of vital cell functions, necessary for cell survival. However, a prolonged perturbation of intracellular calcium homeostasis can trigger cell death. Our aim is to investigate the mechanism whereby changes in calcium are toxic to cells using a variety of probes to assess the role of calcium in primary cells. The aim of this project is to express calpastatin, calpastatin (AA 1-251) conjugated with Yellow Fluorescent Protein (YFP), caspase-3- and calpain-sensitive fluorescent probes (C-devd-Y and C-u calpain site-Y, respectively), cameleon and Ratio-Pericam (2mtRP) in primary cell cultures.

Recipient or parental organism

293 cells (epithelial cell line).
Primary cultures of cerebellum granule cells, cardiac myocytes and skeletal muscle cells.

Host/vector system

The proposed lentiviral vectors are replication-defective so only the early steps of the life cycle are maintained. The vector to be used is a pseudotype HIV-derived lentiviral vector using a third generation packaging system (pRRLsin.PPT.hCMV.Wpre). The transfer plasmid contains the human cytomegalovirus promoter. The biosafety is further advanced by deleting unnecessary genes and the two regulatory genes (tata and rev), so any replication-competent virus that could be generated during vector manufacture will lack the genes necessary for replication. Furthermore, the generation of self-inactivating lentivirus has been obtained by almost complete deletion in the U3 region of the HIV 3’LTR.

Origin & function

The calpastatin genes are of human origin. The caspase-3 and calpain probes and cameleon and Ratio-Pericam are engineered chimeric DNA sequences. These DNA sequences will be cloned into the Self-inactivating (sin) transfer vector plasmid pRRLsin.PPT.hCMV.Wpre and viral particles prepared by co-transfection of 293T cells with a 3rd generation packaging system (using three packaging plasmids). The transducing viral particles obtained by this method will then be added directly to target primary cultures of cerebellum granule cells, cardiac myocytes and skeletal muscle cells thus allowing one to assess the role of calcium signalling in primary cells.

Evaluation of foreseeable effects

There is a potential risk of infection at the stage of packaging and infection while using the lentiviral vectors, however the vectors are replication defective and the likelihood of recombinant events is low. The hazards to human health are low and containment level 2 is appropriate.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste:
Solid waste will be treated with hypochlorite (10,000ppm) before being autoclaved prior to disposal (100% kill). The sterile waste is then sent for incineration by authorised contractors.

Liquid waste:
Liquid waste will be treated with hypochlorite (concentration 10,000ppm available chlorine) overnight and autoclaved before disposal. Any spillages will be cleaned with chlorine disinfectant and the waste paper autoclaved and incinerated.

Autoclave cycle - 121 degrees C for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

It has been agreed by the Genetic Modification Safety Committee that a designation of Class 2 is appropriate for this work and that the risk assessment is sound and should be carried out at Containment Level 2.

Project Containment

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Project Ref 821/04.5
To clone and express genes that may be involved in the pathogenesis of B cell lymphoma in primary B cells and B cell lines of human origin. The aim of the project is to assess whether the genes of interest have an effect on the proliferation and/or apoptosis of primary B cells.

Recipient or parental organism

Primary B cells.
B cell lines of human origin.

Host/vector system

The transfer vector is pBabe puro and consists of portions of the MMLV provirus. The genes of interest will be cloned downstream of the SV40 promotor and between the 3’ and the 5’ LTRs of MMLV (RU5) which are necessary for integration into the cellular genome. The 3’ U3 of the enhancer region of the LTR has a deletion which results in a transcriptionally inactive vector that cannot be converted into full length RNA. The only transcription comes from the SV40 promotor through the gene of interest. Therefore the virus and resulting virus particles are self inactivating.

The final retroviral particle is created by transient transfection of the transfer vector into Phoenix 293T cells. These cells are an amphoteric cell line that contains integrated gag, pol and env genes under separate antibiotic selection markers. The transfection plasmid contains the sequence for part of the gag gene and a puromycin resistance marker. Hybrid virus particles are made with the core proteins and enzymes of a retrovirus and the envelope of a vesicular stomatis virus.

Origin & function

GFP-Green fluorescent protein. Non harmful.
Bc111A and B-zinc finger genes, possibly transcription factors. Human genes.
MLT-a paracaspase and may be involved in apoptotic pathways. Human gene.
PRDM6-SET domain gene. May be involved in chromatin remodelling. Human gene.
BCNP1 - may be involved in cell signalling. Human gene.
SNX25 - could be involved in signal transduction and cell cycle control. Human gene.

Mutated forms of these genes may also be involved.

GFP is a test control gene. Other genes are possible oncogenes.

**Evaluation of foreseeable effects**

The retroviral particles made at the packaging stage can infect human cells but are replication defective.

Cells infected with the retroviral particles are not hazardous as the virus cannot replicate however they could be overexpressing the gene inserted. The inserted genes are of unknown function and could be oncogenes however it is unlikely they will function on their own without other cellular changes.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Solid waste**

Solid waste will be treated with hypochlorite (10,000 ppm) before being autoclaved prior to disposal (100% kill). The sterile waste is then sent for incineration by authorised contractors.

**Liquid waste**

Liquid waste will be treated with hypochlorite overnight (10,000ppm available chlorine) and autoclaved before disposal. Any spillages will be cleaned with chlorine disinfectant and the waste paper autoclaved and incinerated.

Autoclave cycle: 121 degrees C for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.

**Is an emergency plan required according to regulation 20?**  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

**Please enter comments on the GM safety committee on the risk assessment**

It has been agreed by the Genetic Modification Safety Committee that a designation of Class 2 is appropriate for this work and that the risk assessment is sound and should be carried out at Containment Level 2.
Project Additional Information

Purposes of the contained use

The m3-muscarinic receptor is expressed in neurones, salivary cells and smooth muscle. The purpose of this proposal is to investigate the signalling role of this receptor and the processes that regulate the receptor.

Recipient or parental organism

Top 10 E. coli
HEK 293 cells.
Rat and mouse cerebellar, salivary and smooth muscle preparations.
**CHO cells.**

**Host/vector system**

Inserts will be cloned into the pRRLsin transfer vector. This HIV-derived vector lacks the structural, regulatory and accessory proteins required for making replication competent retrovirus. This vector will be co-transfected into HEK293 cells alongside the pMDLg/pRRE, pRSV-Rev and pMD2.VSVG vectors in order to create replication defective lentiviral particles.

**Origin & function**

GFP - Green fluorescent protein. Non harmful. To be used as a test control gene.

M3 - muscarinic receptor
M3 - muscarinic receptors containing point mutations in the phospho-acceptor sites.

The receptors are of mouse origin and are involved in cell signalling.

**Evaluation of foreseeable effects**

At the packaging stage the virus particles could infect human cells. The virus is, however, replication deficient. Transacting structural genes are excluded from the transfer vector, which only encodes the gene of interest. The essential regulatory genes gag and pol are encoded in a separate plasmid, as is rev gene. These constructs have been altered to contain non-overlapping sequences hence minimising the possibility of recombination. In addition non-essential genes have been eliminated, hence any replication-competent virus generated would lack essential factors for replication and virulence in vivo.

The inserts are extremely unlikely to be hazardous. There is no experimental evidence that wild type or mutant receptor proteins act as oncogenes, drive cell growth or induce apoptosis. It cannot, however, be ruled out that the insert in an environment as yet untested may have a deleterious effect on host cells. For this reason the insert is considered here as 'potentially harmful'.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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Liquid waste will be treated with hypochlorite overnight (10,000 ppm available chlorine) and autoclaved before disposal. Any spillages will be cleaned with chlorine disinfectant and the waste paper autoclaved and incinerated.

Autoclave cycle: 121 degrees C for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N
It has been agreed by the Genetic Modification Safety Committee that a designation of Class 2 is appropriate for this work and that the risk assessment is sound and should be carried out at Containment Level 2.

Project Containment

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Project Ref 821/04.7

Date Ackn'd 02/09/2004

CU2 Project Title

Neuropathy Target Esterase and membrane homeostasis in primary cell cultures

Class Culture Vol

Class 2 < 1 Litre

Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
### Purposes of the contained use

To reduce NTE expression in primary cultured cells using lentiviral mediated RNAi technology.

### Recipient or parental organism

Primary cultures of mouse cells including neurons and glia  
293T cell line  
Hela cell line

### Host/vector system

A pLV-THM lentiviral vector will be used. The vector will be co-transfected into 293 T cells alongside pMDLg/pRRE, pRSV-Rev and pMD2G vectors to create replication defective lentiviral particles. Trans-acting element genes are excluded from the transfer vector, which contain only the genes of interest. The essential regulatory genes gag and pol are encoded in a separate plasmid, as are Rev and envelope genes. These vectors have been engineered to contain non-overlapping sequences, thereby minimizing the possibility of recombination. Non-essential genes have also been eliminated so any replication-competent virus generated would lack essential factors for replication and virulence in vivo.

### Origin & function

Short hairpin RNA will be expressed that is homologous to human and mouse NTE genes with the aim of lowering their expression by means of RNAi. The 30 bp insert comprises of a 19bp sense sequence of DNA homologous to NTE cDNAs, a 9bp look and an intensense sequence of the same 19bp DNA to form a hairpin structure when expressed in infected cells. This hairpin RNA will mediate specific degradation of NTE mRNA through RNA interference mechanisms.

### Evaluation of foreseeable effects

At the packaging stage the virus particles could infect human cells. Therefore the production of the virus and the infection of host cells with the virus is carried out at containment level 2.

Cells infected by the virus particles are not hazardous as the virus cannot replicate, however they could lower NTE expression in local infected cells, it is very unlikely that inhibition of NTE expression at the local cell level will be hazardous.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be treated with hypochlorite (10,000 ppm) before being autoclaved prior to disposal (100% kill). The sterile waste is then sent for incineration by authorised contractors.

Liquid waste will be treated with hypochlorite overnight (10,000 ppm available chlorine) and autoclaved before disposal. Any spillages will be cleaned with chlorine disinfectant and the waste paper autoclaved and incinerated.

Autoclave cycle: 121 degrees C for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.
It has been agreed by the Genetic Modification Safety Committee that a designation of Class 2 is appropriate for this work and that the risk assessment is sound and should be carried out at Containment Level 2.

Project Containment

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- Animal Units
- Large Scale Activities
- Human Clinical Applications

Project Ref  821/04.8

- Date Ackn'd: 09/12/2004
- CU2 Project Title: Immortalisation of primary rodent cells with SV40 Large T
- Class: Class 2
- CultureVolClass2: < 1 Litre
- Non-GMM: Not Applicable
- Consent Granted: Non-GMM
- Project notified under transitional arrangements: N

Historical Significant Changes

Tick if notifying a connected programme of work: N
### Project Additional Information

#### Purposes of the contained use

To immortalize rodent cells. SV40 Large T is a transforming oncogene inhibiting both p53 and Rb tumour suppressors.

#### Recipient or parental organism

- Bosc 23 ecotropic packaging cell line
- Ecotropic Phoenix packaging cells
- 293 epithelial cells
- Primary rodent cells

#### Host/vector system

The vectors used will be the retroviral vectors pBabe, pRetrosuper and pSuperior. These vectors are replication defective so only the life cycle of the vectors are maintained. All trans acting genes (structural, regulatory and accessory proteins) are excluded from the transfer vectors which encode the genes of interest. The biosafety of these vectors is further advanced by deleting unnecessary genes and the two regulatory genes tata and rev. The vectors can be transferred to eukaryotic cells but are self inactivating once inserted into the genome.

#### Origin & function

SV-40 Large T of simian virus origin. The function is to immortalize primary rodent cell lines.

#### Evaluation of foreseeable effects

The retroviral vectors to be used can be transferred to eukaryotic cells but are self inactivating once inserted into the genome, however if retroviral viruses are produced in ecotropic packaging cells they can only infect cells from rodents. The transduced molecules are a known hazard to human health. Neither the host rodent cells nor the plasmids generated by the parental virus are harmful. In cell cultures the virus can inactivate very quickly. The possibility of spontaneous recombination events is low. There is a low possibility of infecting individual cells in vivo, however the viral particles inactivate rapidly and the infection remains localised to the site of entry. Care is required in handling this GMM to avoid skin contact.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Solid waste**
  - Solid waste will be treated with hypochlorite (10,000ppm) before being autoclaved prior to disposal (100% kill). The sterile waste is then sent for incineration by authorised contractors.
- **Liquid waste**
  - Liquid waste will be treated with hypochlorite overnight (10,000ppm available chlorine). Any spillages will be cleaned with chlorine disinfectant and the waste paper autoclaved and incinerated.

**Autoclave cycle:** 121°C for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.
Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 821/05.1

Date Ackn'd 23/03/2005

CU2 Project Title

The expression of genes in human and rodent cells using retroviral infection procedures in order to assess the role of specific genes in toxicological signalling pathways.

Class 2

Culture Volume Class

< 1 Litre

Consent Granted

Not Applicable

Non-GMM

Date Project Ceased

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Tick if notifying a connected programme of work Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

It has been agreed by the Genetic Modification Safety Committee that a designation of Class 2 is appropriate for this work and that the risk assessment is sound and should be carried out at Containment level 2.
Purposes of the contained use

To clone and express genes encoding proteins involved in cellular processes such as apoptosis, the cell cycle, cell signalling and neuronal pathology. Genes will be expressed in cell lines of human and rodent origin.

Recipient or parental organism

Human cells including cell lines of human origin and primary cells. Amphotropic packaging cells (for example Phoenix 293 cells) will be used to express the core packaging plasmids, therefore the virus produced could infect human cells. However, the packaged virus cannot replicate so infected target cells would not be hazardous, although they would be overexpressing the gene inserted. To minimise risk sharps will not be used and primary human cells will only be obtained from donors outside the building.

Rodent cells will also be used, both primary and cell lines. In this case ecotropic packaging cells will be used to ensure the viruses produced cannot infect human cells.

Host/vector system

The vectors used will be retroviral vectors pPINCO puro and pXy.puro consisting of portions of the MMLV provirus. The genes of interest will be cloned downstream of the SV40 promoter and between the 3' and the 5' LTRs of MMLV necessary for integration into the genome. The 3' U3 of the enhancer region of the LTR has a deletion which ensures the vector is transcriptionally inactive and cannot be converted into full length RNA. The only transcription comes from the SV40 promoter through the gene of interest. Therefore the vector and resulting viral particles are self-inactivating.

Origin & function

The genetic material to be expressed will include genes encoding proteins known to regulate cellular processes. This will include genes involved in apoptosis, the cell cycle, cell signalling and neuronal pathology. Examples will include ALAS1, CREB, HIF, cJun, CEBP family, BCPN1, MLT, Bcl, m3-muscarinic receptor, PML, DAXX, calpastatin genes and Green fluorescent protein.

Genes will be expressed that are well characterised as well as potential oncogenes or tumour suppressors and also mutated forms of these genes (including the CEBP family, BCNP1, PRDM6, MLT, Bcl11, SNX25 and C2TA). However, it is unlikely these genes would function as potential oncogenes or tumour suppressors on their own in the absence of other additional cellular changes.

Evaluation of foreseeable effects

Amphotropic retroviral particles made at the packaging stage could infect human cells, however the vector and resulting viral particles are self-inactivating (sin). The vector contains the psi-signal for packaging but the envelope gene (dENV) has been deleted. The vector also contains the gag gene necessary for transcribing the helper genes. Cells infected with the retroviral particles would not be hazardous as the virus is replication defective, however the cells could be overexpressing the genes of interest. Some inserted genes would be of unknown function and could be oncogenes; however it is unlikely these inserts could function on their own without other cellular changes occurring.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid waste
Solid waste will be treated with hypochlorite (10,000ppm) before being autoclaved prior to disposal (100% kill). The sterile waste is then sent for incineration by authorised contractors.

Liquid waste
Liquid waste will be treated with hypochlorite overnight (10,000ppm available chlorine). Any spillages will be cleaned with chlorine disinfectant and the waste paper autoclaved and incinerated.

Autoclave cycle: 121 degrees celcius for 15 minutes. The autoclaves are validated annually in the MRC Toxicology Unit.

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Project Ref 821/05.2

Date Ackn’d 23/03/2005

CU2 Project Title
The expression of genes in human and rodent cells using lentiviral infection procedures in order to assess the role of specific genes in toxilogical signalling pathways

Class 2
Culture Volume Class 2 < 1 Litre

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N
**Project Additional Information**

### Purposes of the contained use

To clone and express genes encoding proteins involved in cellular processes such as apoptosis, the cell cycle, cell signalling and neuronal pathology. Genes will be expressed in cell lines of human and rodent origin.

### Recipient or parental organism

Human cells including cell lines of human origin and primary cells. Amphotropic packaging cells (for example Pheonix 293 cells) will be used to express the core packaging plasmids, therefore the virus produced could infect human cells. However, the packaged virus cannot replicate so infected target cells would not be hazardous, although they would be overexpressing the gene inserted. To minimize risk sharps will not be used and primary human cells will only be obtained from donors outside the building.

Rodent cells will also be used, both primary and cell lines. In this case ecotropic packaging cells will be used to ensure the viruses produced cannot infect human cells.

### Host/vector system

Inserts will be cloned into the pRRLsin transfer vector. This HIV-derived vector lacks the structural, regulatory and accessory proteins required for making replication competent retrovirus. This vector will be co-transfected into packaging cells alongside the pMDLg/pRRE, pRSV-Rev and pMD2.VSVG vectors in order to create replication-defective lentiviral particles.

### Origin & function

The genetic material to be expressed will include genes encoding proteins known to regulate cellular processes. This will include genes involved in apoptosis the cell cycle, cell signalling and neuronal pathology. Examples will include ALAS1, CREB, HIF, cJun, CEBP family, BCPN1, MLT, Bcl, m3-muscarinic receptor, PML, DAXX, calpastatin genes and Green fluorescent protein.

Genes will be expressed that are well characterised as well as potential oncogenes or tumour suppressors and also mutated forms of these genes (including the CEBP family, BCNP1, PRDM6, MLT, Bcl11, SNX25 and C2TA). However, it is unlikely these genes would function as potential oncogenes or tumour supressors on their own in the absence of other additional cellular changes.

### Evaluation of foreseeable effects

At the packaging stage the virus particles could infect human cells. The virus is, however, replication deficient. Transacting structural genes are excluded from the transfer vector, which only encodes the gene of interest. The essential regulatory genes gag and pol are encoded in a separate plasmid, as is rev gene. These constructs have been altered to contain non-overlapping sequences hence minimising the possibility of recombination. In addition non-essential genes have been eliminated, hence any replication-competent virus generated would lack essential factors for replication and virulence in vivo.

Some inserted genes would be of unknown function and could be oncogenes, however it is unlikely they could function on their own without other cellular changes occurring. It cannot, however, be ruled out that an insert in an environment as yet untested may have a deleterious effect on host cells. For this reason these inserts are
considered as potentially harmful.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

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Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

It has been agreed by the GMSC that a designation of Class 2 is appropriate for this work and that the risk assessment should be carried out at a containment level 2.

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Animal Units

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Large Scale Activities

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Human Clinical Applications

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Project Ref  821/15.1
Investigation of the signalling properties of protein kinases in Plasmodium falciparum

Plasmodium falciparum is one of the parasites responsible for human malaria. The aim of this study is to establish the role of protein phosphorylation in maintaining the blood stage of the parasite and the essential processes involved in progressing the parasite through the blood stage. For this we will be generating and importing mutant parasite strains where certain protein kinase genes have been mutated as well as other genes involved in invasion and gametogenesis.

General overview of the project:
It is estimated that 300-500 million people world wide suffer from malaria with over 2 million deaths per year, mostly in children under the age of 5 (Breman, 2001). The most severe cases of malaria are caused by the parasite Plasmodium. falciparum. This parasite is transmitted to humans in the saliva of an infected mosquito. Once in the blood stream the parasite makes its way to the liver where it invades liver cells. Inside liver cells the parasite divides and changes into a form that is able to infect red blood cells. The infected liver cells eventually release parasites into the blood stream where they quickly invade red blood cells. Once inside the red blood cells the parasite once again divides filling the red blood cells with new parasites which are released when the cell membrane eventually ruptures. These released parasites invade other red blood cells and the cycle is repeated.

Many studies aimed at developing novel treatments for malaria focus on the blood stage form of P.falciparum (Pleass and Holder, 2005). Hence a detailed understanding of this stage in the life cycle will provide robust targets for further drug discovery.

We will study the biochemical processes of invasion of P.falciparum into human red blood cells, progression of the parasite through the red blood cell cycle and gametogenesis with particular focus on the role of protein phosphorylation. The following will be undertaken:
1. Investigation of novel potential drug targets including protein kinases and signalling molecules involved in parasite development and gametogenesis. Gene knockout experiments have demonstrated that certain proteins are essential for viability of malaria parasites. Among these genes are those that encode for protein kinases. Therefore, replacement of wild-type alleles of protein kinases with alleles carrying a mutation conferring hypersensitivity to inhibitors will allow validation of these proteins as potential drug targets.

2. Localisation and purification of tagged proteins/parasites: Subcellular localisation of Plasmodium proteins is important for determining how they may be pharmacologically targeted. Localisation of potential drug targets to different parts of the cells will be achieved through GFP or epitope-tag gene constructs. The addition of tags (such as 6-His, HA) to proteins such as kinases, cyclins and metabolic enzymes will allow direct purification of these proteins complexed with their natural protein partners, which can then be analysed further. Such purified proteins can also be used to directly screen against potential drug compounds in luminescent assays.

3. Investigation of erythrocyte invasion by malaria parasites. Gene knock-out of Plasmodium genes of the EBA family presumed to be involved in invasion will determine the role of individual proteins in this process.

4. Determination of the phospho-proteome of plasmodium falciparum using wild-type and genetically-modified parasites in comparative studies.

We will be looking at mutants of various protein kinases and signalling proteins including PfCPDK1, PfCLK1, PfCLK3, PfGSK3, PfPK6 and the gatekeeper mutant of PfPKG and NEK family (PfNEK1-4) kinase mutants as well as mutants in invasion related proteins (such as the EBAs) and regulatory proteins including K13-propeller and PfRh protein.

Recipient or parental organism

Plasmodium falciparum -307 and NF54

Host/vector system

DNA vector to be used will be pH1
This vector will confer ampicillin resistance

Origin & function

The genetic material used has its origin in a cDNA library obtained from the reverse transcription of mRNA derived from the schizont stage of P. falciparum.

The specific genetic material is discussed below:

Dominant negative mutants of:
PfGSK3 (+GTP/HA/His tagged versions)
PfPK6 (+GTP/HA/His tagged versions)

We will inhibit the action of specific protein kinases by overexpression of dominant negative mutants. These will include dominant negative mutants to GSK3 and PK6.

PfGSK3 is a protein kinase. The normal function of this kinase in malaria is unknown other than it is an essential kinase to maintain the blood stage of the parasite.

PfPK6 is a protein kinase. This kinase is homologous to the cyclin-dependent kinase and therefore likely to be involved in the cell cycle.

Gatekeeper mutant of:
PfPKG (+GTP/HAIHis tagged versions)

Mutants of the essential protein kinase PKG that render the parasite insensitive to inhibitors of PKG will be used to dissect the role of this kinase in the survival of the parasite.
PIPKG is a protein kinase. It is an essential kinase for parasite survival and is involved in parasite development in the red blood cell, egress and invasion into red blood cells. This kinase is also involved in formation of gametocytes. Protein kinases:

- CDPK1 (HA-tagged)
- NEK 'kinase family (NEK1 -4)(HA and GFP tagged)
- CLK1 and CLK3 (+GTP/HA/His tagged versions)

Wild type and mutants of a number of protein kinases that are essential for the progression of the parasite through the erythrocytic cycle will be investigated to establish the role played by these kinases in maintaining the viability of the parasite. The mutations to be introduced either involve epitope tags or removal of regulatory phosphorylation sites or introducing mutations that change sensitivity to protein kinase inhibitors.

PfCLK1/3 are protein kinases. They are involved in RNA processing in the parasite.

Invasion proteins

- EBA family (e.g. EBA-175)

Wilde type and mutants of key proteins involved in invasion (such as EBA-family) will reveal the role played by these proteins and the mechanisms of regulation such as protein phosphorylation.

EBA family. These are cell surface antigens involved in binding parasites to the surface of red blood cells.

Regulatory proteins

- K13-propeller. This is a transcriptional regulator of unknown function.
- PfRh family (+GTP/HA/His lagged versions)

Drug resistance is a major problem in malaria. It is becoming clear that a number of proteins including K13-propeller proteins are mutated in drug resistant parasites. We will use mutants of these proteins to establish what mechanisms are in play during drug resistance.

The PfRH-family are a group of rhoptry proteins which are released from the parasite during invasion and are essential for the invasion process.

**Evaluation of foreseeable effects**

*P. falciparum* is a serious human pathogen but the parasite is unable to infect any vertebrate species apart from man and certain primates, and can only infect certain species of Anopheles mosquitoes. Natural infection of humans occurs through the bite of an infected mosquito vector of the genus Anopheles. The research programmes that will be implemented in the Cat3 facilities do not involve mosquito vectors, so the risk of insect-borne inoculation is very low. The only way that the GM could enter the environment and survive is if a mosquito able to transmit malaria was to enter the lab, feed on the culture and pick up from the culture gametocytes. This is almost impossible for the following reasons:

Firstly there are only a few species of mosquito in the UK able to act as the vector for *P. falciparum*, of those that do exist they are in very low numbers in Leicestershire.

• The mosquito would then have to enter the closed environment of the malaria lab and gain access to the class 2 hood, or the incubator, which is extremely unlikely.

The mosquito would have to feed on the culture (which it is unlikely since it is only 10% human blood)

In the meal the mosquito would need to pick up gametocytes which are in extremely low numbers in Pf in vitro cultures (less the 0.5% of the infected red blood cells)

After all this it is almost impossible for a single infected mosquito to successful propagate malaria. For this to happen a large number of mosquitoes would have to go through the above process.

In the laboratory environment, infection of humans may take place by direct inoculation of infected blood (cultured material) into the bloodstream of the laboratory worker, e.g. through contaminated hypodermic needle. This risk is minimal since no needles or any glass are used in the dedicated culture room.

Since the parasite may enter through a cut in the skin then any skin abrasions should be cover in a plaster and the
operator where gloves and appropriate PPE. This should reduce any risk of infection to close to zero.
The only parasite lines which will be used in this programme (307, NF54) are all susceptible to chloroquine and,
importantly, the effectiveness of commonly used antimalarial drugs, e.g. chloroquine, is unaffected by the genetic
modification.
The function of the inserted parasite gene product will frequently be unknown, or can only be implied through
homology with other organisms. Knockouts of potential drug targets (e.g. protein kinases) are aimed at reducing the
fitness of the knockout parasites, preferably to produce non-viable or severely functionally-impaired parasites. Allelic
replacement of protein kinase genes with those exhibiting hypersensitivity to inhibitors should result in parasites with
increased sensitivity to these inhibitors. Growth rates are not expected to be affected, other than negatively, in any of
these experiments. The GMM is very unlikely to be more harmful to humans than the parental organism.
Pathogenicity in Plasmodium does not depend on any known toxin or clonable agent. Malaria parasites are extremely
host-specific and there have been no reports of malaria parasites crossing species barriers, with the exception of
some non-human primate malarias that are able to establish very mild infection in humans, and experimental infection
of certain non-human primates with human malarias. It is not possible for the human malarias to establish infection in
any other vertebrates.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

P. falciparum is a parasite responsible for human malaria. As such this organism is categorised by ACOP as a Hazard
group 3 parasite but handling without negative pressure/HEPA filler is permitted (appendix 3.2 Biological agents
managing the risks in laboratories and healthcare premise). This is justified on the following grounds:

- The only way to contract malaria from an in vitro erythrocytic culture of P. falciparum is through a puncture wound
  of the skin. It is not possible, for example, to gain infection via spillage on the skin, touching contaminated cloths or
  by touching or rubbing eyes with contaminated hands or tissues.
- It is not possible to be infected by inhalation of parasites contained in aerosols, for example caused by
  centrifugation or by pipetting, or from evaporation from culture media.
- It is not possible to be infected by contaminated clothing.
- It is not possible to become infected by spillages unless some how the spillage was able to enter the body through
  punctured skin.

Since the parasite may enter through a cut in the skin then any skin abrasions should be cover in a plaster and
the operator where gloves and appropriate PPE. This should reduce any risk of infection to close to zero.
For these reasons it is not necessary to implement full cal 3 containment. Specifically the following key elements of
cat 3 contained are not required for P. falciparum in vitro culturing for the following reasons:

- The laboratory is sealable for fumigation:
  This is not necessary as there is no inherent risk of infection from P. falciparum through inhalation. Hence fumigation
does not present any inherent risk of parasite infection. Thus, it is not necessary to conduct fumigation in a sealed
  room. It would seem that this would in fact seriously increase the risk associated with the fumigation procedure with
  no benefit in decreasing the risk of parasite infection.
- There is an autoclave available within the laboratory suite:
  Health and Safety Executive

Il is not possible to become infected by the parasite culture other than through a puncture wound. Thus, the solid
waste is currently double bagged and autoclaved in the building autoclave facility because it is not possible to contract
an infection through accidental contact of any residual waste culture material with the skin, by inhalation or ingestion.
There is no justification therefore for autoclaving in the malaria lab. In fact having an additional autoclave would increase risk of injury through autoclaving.

Note that all liquid waste is decontaminated in the malaria lab and disposed of in the malaria lab therefore is not autoclaved.

Entry to the lab is via an airlock
This is unnecessary since it is not possible to be infected by the malaria parasite from inhalation or from aerosols or vapour or any airborne source. Hence an airlock is not necessary.

The lab is at negative pressure relative to the pressure of the immediate surroundings
This is not necessary for the same reasons as described above for the airlock.

Extract and input air from the laboratory must be HEPA filtered
This is not necessary for the same reasons as described above for the airlock/negative pressure.

Controls to mitigate risk of infection from in vitro P. falciparum cultures.
To mitigate the risk of infection from a puncture wound the following standard operating procedure for P. falciparum in vitro culture has been in operation for some time in line with the recommendations from the University and MRC safety office and as approved by the HSE.

a) Only the culture of P.falciparum is permitted in this laboratory.
b) Each culture batch will be up to 30ml
c) Only staff that have gone through the appropriate safety training and that have been approved by the University and MRC safety officers will be allowed to work in lab 201.
d) Access to lab 201 will be limited to personnel who have received training and approval by the supervisor/group leader.
e) Only trained personnel will be given the access code to enter the lab that will be locked at all times with a swipe card entry lock.
f) All the equipment required for the culture of P.falciparum will be contained in lab 201. This includes the class 2 microbiological cabinet, incubator, centrifuge and microscope.
g) Liquid tissue culture medium is stored in lab 202/209.
h) The use of sharps is strictly forbidden. This includes in particular glass pipettes and glass bollies. Glass slides will, however, be permitted for the staining of parasites in culture.
i) Liquid waste will be inactivated with presept (1,000ppm equivalent to 4 x 2.5g tablet per 50ml) and disposed of down the designated sink.
j) All solid waste will be double bagged and autoclaved.
k) Gloves will be worn at all times.
l) Safety glasses to be worn at all times.
m) Dedicated blue lab coats will be worn at all times.
n) Buckets in the centrifuge must be capped when spinning tubes containing the parasite cultures.
o) In the event of spillage, liquid waste will be absorbed onto CLAN Unisafe absorbent granules and placed in solid waste and autoclaved. Area will then be washed with presept (10,000ppm equivalent to 4 x 2.5g tablet per 50ml) for disinfecting.
p) In the event of centrifuge disruption or if a spill is suspected then the following procedure must be adhered to:
   1. Leave 20 minutes before opening
   2. If the integrity of the buckets is not comprised, these (with caps) will be moved into the class II microbiological cabinet and opened.
3. Liquid will be disposed of in liquid waste container, treated with presept (1,000ppm equivalent to 4 x 2.5g tablet per 500ml) and disposed of in the designated sink. Solid waste will be disposed of in the solid waste disposal bin and autoclaved.

4. If the integrity of the buckets is comprised, then liquid waste will be absorbed onto CLAN Unisafe absorbent granules and placed in solid waste and autoclaved.

5. The centrifuge should then be thoroughly cleaned with presept (10,000ppm equivalent to 4 x 2.59 tablet per 500ml) and all waste disposed of as solid waste and autoclaved.

Q) Transport of potentially infected material from laboratory 201 (e.g. cell lines, frozen ampoules, serum etc.) will be done in an approved sealed vessel clearly labeled and a trolley will be used.

r) All solid waste is autoclaved.

s) Eating, chewing, drinking, smoking, taking medication, storing food and applying cosmetics is forbidden in the laboratory.

t) Mouth pipetting is forbidden.

u) In case of a spillage of P. falciparum on broken skin or a puncture wound (from for example a contaminated slide) the wound should be washed thoroughly under running water and Occupational Health consulted.

v) When leaving the malaria lab (room 201) with malaria samples gloves and lab coats need to be changed.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be inactivated by addition of Presept (Johnson & Johnson Medical) - 4x2.5g tablet per 500ml (equivalent to 10,000ppm) overnight and disposed of down the designated sink.

In the event of a large spillage, liquid waste will be absorbed onto CLAN Unisafe absorbent granules and placed in solid waste and autoclaved. Area will then be washed with Presept (10000ppm equivalent to 2.59 tablet in 500ml). In the event of small spillages or splashes, the surface should be cleaned with 70% IMS.

All solid waste will be placed in an appropriate autoclave bag, taped closed and autoclaved at 133°C for 12 mins. All pipettes will be placed in a sealed orange topped 7L sharps bin and autoclaved at 133°C for 12 mins.

All waste will then be incinerated by an approved contractor.

*Note:- Presept data - a solution of presept was made up in water where the presept concentration was 10,000ppm. 1ml of this solution was added per 10ml 307 or NF54 P. falciparum culture for 24 hours (final concentration was 1,000ppm). In the control culture parasitemia was 1% and in the presept treated culture, no live parasites were observed. Therefore a final concentration of 10,000ppm is deemed far higher than necessary to kill the parasites.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The genetic modification safety committee have agreed the following:

i. That a designation of Class 2 is appropriate
ii. That the risk assessment is sound and that work should be carried out at Containment Level 3 but can be handled without negative pressure/HEPA filter
iii. That the project will be notified to the HSE,
and, further, they have noted:
iv. That the proposer will contact HSE to notify the work as a new project.

### Project Containment

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The expression of genes in human and rodent cells using lentiviral infection procedures in order to assess the role of specific genes in toxicological signalling pathways, haematopoietic differentiation and malignant transformation

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Tick if notifying a connected programme of work | N |

Project notified under transitional arrangements | N |
Purposes of the contained use

To clone and express genes encoding proteins involved in cellular processes such as apoptosis, the cell cycle, cell signalling, neuronal pathology and the process of haematopoietic lineage commitment and megakaryopoiesis. This project aims to characterise such genes by over-expression and knockout in primary cells or cell lines of human and rodent origin.

Recipient or parental organism

Human cells including cell lines of human origin and primary cells will be used. The unmodified cell lines are nonharmful, unable to colonise or cause disease (hazard group 1). Primary cells are derived from screened healthy donors, however as there is a possibility that material may contain adventitious infectious agents, it will be handled at Containment level 2.

Amphotropic packaging cells will be used to express the core packaging plasmids therefore the virus produced could infect human cells, however the packaged virus cannot replicate so infected target cells would not be hazardous, although they would be overexpressing the gene inserted. To minimise risk sharps will not be used and primary human cells will only be obtained from donors outside the building.

Rodent cells will also be used, both primary and cell lines, including stem cells. In this case ecotropic packaging cells will be used to ensure the viruses produced cannot infect human cells.

Host/vector system

Vectors are defective lentiviruses (i.e. replication incompetent, e.g. second or third generation derivatives of HIV-1) in which the structural and accessory genes necessary to produce lentiviral particles are located on separate blocks of DNA (i.e. separate plasmids). This means that three (for second generation) or four (for third generation) recombination events would have to occur simultaneously to generate replication competent lentivirus. Wildtype virulence and replication genes are deleted, therefore the HIV vectors lack the structural, regulatory and accessory proteins required for making replication competent retroviruses. Such vectors may infect human cells and integrate into the cellular DNA but when unmodified are considered Class 1 GMMs. The SACGM guidance on 'pseudotyped' vectors suggests they should be considered as Class 2 GMMs. When modified with genes of interest they will be treated as Class 2.

Origin & function

The genetic material to be expressed will include genes encoding proteins known to regulate cellular processes. This will include genes involved in apoptosis, the cell cycle, cell signalling, neuronal pathology and haematopoiesis. This will include known human leukaemia genes, including MEIS1 as well as factors involved in transcriptional regulation. Examples will also include ALAS1, CREB, HIF, cJun, CEBP family, BCNP1, MLT, Bcl family, m3-muscarinic receptor, PML, DAXX, calpstatin genes and Green Fluorescent Protein.

Genes will be expressed that are well characterised as well as potential oncogenes or tumour suppressors and also mutated forms of these genes, however it is unlikely these genes would function as potential oncogenes or tumour suppressors on their own in the absence of other additional cell changes.

In addition to ectopically express genes short hairpin RNAs and the genetic editing system CRISPR/CAS9 will be used to knock down/out target genes and modulate their expression. CRISPR/CAS9 will be also used to perform genetic screens.

Evaluation of foreseeable effects

In addition to ectopically express genes, short hairpin RNAs and the genetic editing system CRISPR/CAS9 will be
used to knock down/out target genes and modulate their expression. CRISPR/CAS9 will be also used to perform genetic screens. At the packaging stage the virus particles could infect human cells. The virus is, however, replication deficient. Trans-acting structural genes are excluded from the transfer vector, which only codes the gene of interest. The components required for viral production are present on separate vectors, transfected into the packaging cell line so that newly formed viral particles contain only the transgene and the minimal viral genes required for its genome integration and expression. They do not contain all the factors necessary for replication of the virus. In addition virulence genes have been completely removed from the system. The constructs have been altered to contain non-overlapping sequences, minimising the possibility of recombination. The possibility of sequential recombination events in the packaging cell line giving rise to replication-competent virus is extremely unlikely. Viral stocks will be stored in lockable freezers in areas of restricted access thus the vectors do not present a risk to the wider human population or the environment. The viruses will be VSV-G pseudotyped. This coat protein confers a wider tissue tropism than for the natural virus. The vectors may be transmitted by contact/aerosol as well as the natural percutaneous route. Lentiviruses are able to infect non-dividing cells unlike other retroviruses; the range of cells and modes of transmission are thus significant. Such chimaeric particles exhibit physical stability but are more sensitive to the complement system. Considered class 2. On infection, these viruses insert their DNA into the cellular DNA and so are capable of expression for the life of the cell. Insertion itself could lead to a deleterious mutagenic event but this is of low probability. Some inserted genes would be of unknown function and could be oncogenes, however it is unlikely they could function on their own without other cellular changes occurring and a single expression event is unlikely to result in any clinical manifestation. It cannot however be ruled out that an insert in an environment as yet untested may have a deleterious effect on host cells. For this reason these inserts are considered as potentially harmful. The overall risk may be considered as Class 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be treated with hypochlorite (10,000ppm for at least 16 hours) before being autoclaved prior to disposal or another suitable, validated disinfectant. The Sterile waste is then sent for incineration via authorised waste contractors. (100% kill).
Liquid waste will be treated with hypochlorite (10,000ppm for at least 16 hours before disposal via the sink or another suitable, validated disinfectant. (100% kill).
Any spillages will be cleaned with a chlorine disinfectant or other suitable, validated and any waste tissues autoclaved and sent for incineration.
Autoclave cycle: 121°C for 15 minutes. The autoclaves are validated annually within the MRC Toxicology Unit.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N
The work was discussed by the GMSC on 23/09/2020 and again on 23/06/21. It was agreed that a designation of Class 2 is appropriate for this work and that the Risk Assessment is sound and should be carried out at Containment Level 2.

Please enter comments on the GM safety committee on the risk assessment

The work was discussed by the GMSC on 23/09/2020 and again on 23/06/21. It was agreed that a designation of Class 2 is appropriate for this work and that the Risk Assessment is sound and should be carried out at Containment Level 2.

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**Company no longer exists.**
## Premises Addresses

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<th>Name</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The committee comprises two representatives of ExpressOn BioSystems Ltd. The Technical Director who is ultimately responsible for ExpressOn's GMO operations and represents the management, and the company biological safety officer who additionally represents ExpressOn's employees. Also on the committee are the health and safety officer from the Roslin Institute and an educated lay person with a background in insurance and no vested interest in either ExpressOn or the Roslin Institute. This person represents the wider community. The company is small enough that the committee meets as and when necessary at the start of any new project and to review risk assessments if any new information comes to light.

<table>
<thead>
<tr>
<th>Laboratory</th>
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Other (please specify) Tick if confidential

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<th>Microbiology Research</th>
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<tbody>
<tr>
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</table>
ExpressOn BioSystems Ltd is a sub-tenant of the Roslin BioCentre and as such is expected to comply with waste management systems in place at Roslin.

Solid disposable (plastics, solid media) waste contaminated or potentially contaminated with biological material, whether GMO or not, will be collected in clearly marked HMHD autoclave bags and autoclaved for 15 min at 121°C. The autoclave is within ExpressOn's laboratory and will be tested monthly using TST strips included with a waste load. A test log will be kept. Once made safe autoclaved waste will be disposed of in black bags to general waste.

Liquid waste will have virkon powder added to 1% and will be stored overnight before disposal to drains. This process results in complete kill of micro-organisms of the type in use. Because of the low risk associated with the GMOs in use, degree of kill is not usually verified. If higher risk experiments were to be carried out then degree of kill will be assessed by culturing a sample of liquid waste on appropriate plates prior to disposal.

Non-disposable lab-ware contaminated with biological material will be soaked overnight in a solution of 1% virkon before washing as normal.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The two risk assessments enclosed are derived from projects that were assessed at the University of Edinburgh prior to ExpressOn moving to Roslin and setting up its own GMO committee. The project risk assessments have been re-written as part of this application and have been circulated to ExpressOn's GMO committee. Because the constructs used in the present projects are not designed to express in the host organism and because the cloned sequences are not known to be associated with any pathological condition it was considered that class 1 containing is adequate.

The TSE-related small RNA project is considered low risk but careful monitoring of the literature is necessary. If any nucleic acid component is ever thought to be associated with the TSE agent then this project will be stopped immediately and reassessed. A weekly literature alert will be set up with ExpressOn's information service, NERAC, to draw our attention to any emerging research that we might otherwise miss. A level of containment is afforded by the fact that sequences of interest are maintained and propagated as CDNAs whereas any activity would be associated with the RNA version. The cDNAs are derived from hamster brain RNA. Purified nucleic acid fractions from TSE infected animals are not infections and pose no greater risk than nucleic acids purified from non-infected animals.
**GM Centre Number: 823**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee consists of a group of senior scientists including the Scientific Director, all members are at PhD level and have several years laboratory experience. Meetings are held at the start of new projects to ensure all aspects of risk assessment are considered. Projects are reviewed periodically to ensure that no deviation from the original classification has occurred.

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<td>Transgenic</td>
<td>Fish</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All contaminated waste will be inactivated prior to disposal. Liquid waste (cultures) will be treated with 1% Virkon for a minimum of 30 minutes (according to manufacturer's instructions) to reduce viability to $<10^{-5}$. Solid waste will be autoclaved prior to disposal for incineration using a licensed waste management company. The autoclave will be serviced and include an annual UKAS calibration. Period test swabs from autoclaved waste will be used to ensure that the levels of survival are below detectable levels.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The PLMS project in the Risk Assessment summary represents an acceptably low risk and as such can be classified as Hazard Group I.
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**Name**

COMPLEMENT GENOMICS LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

BUSINESS & INNOVATION CENTRE

**Building**

UNIT 1241

**Road Name**

SUNDERLAND ENTERPRISE PARK EAST

**District**

**Town**

SUNDERLAND

**County**

TYNE AND WEAR

**Postcode**

SR5 2TA

**Country**

ENGLAND

**Tel Number**

0191 516 6500

**Fax Number**

0191 516 6501

**E-mail**

**HSE Division**

YORKSHIRE AND NORTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GMO committee comprises of a Chairman (Chief Operating Officer), a senior management representative (Chief Executive Officer) the laboratory Manager (also designated Health and Safety and ISO/Quality Manager) and a laboratory representative. Where deemed necessary, an independent external advisor from an appropriate scientific background will be invited to attend. Due to the small size of the Company, the role of Biological Safety Officer will be shared between the COO and CEO who are both suitably qualified for this position. In addition to renewing risk assessments for new procedures involving GMOs the Committee will advise on preparation of facilities, training and the development of SOPs to ensure the safety of exposed workers and the environment. Meetings will be called on an ad hoc basis but not less than every six months where a complete review of current practices will be held.

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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Waste disposal/Decontamination</th>
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<tbody>
<tr>
<td>Bacterial cultures no longer in use will be decontaminated by addition of 10% Virkon (bactericidal/virucidal and left overnight).</td>
</tr>
<tr>
<td>Used agar/broth will be sterilised by heat treatment (121 degrees C for 15 minutes). The degree of kill is in excess of 99.99% which is suitable for the Class I activities we propose.</td>
</tr>
</tbody>
</table>

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

We have reviewed the Risk Assessment and would ask that the methods for kill via autoclaving are validated, ie. the service contract for the autoclave is checked and that the autoclave is validated.
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Data Premises Notified (Originally) 11/04/2002

Transferred from 1992 Regs? N

Transitional Premises

Class

Data Premises Closed

Emergency Plan Required?

Transitional Premises

N

Non-GMMs N

Withdrawn N

Name

HAMPShIRE ADVISory AND TECHNICAL SERVICES (H.A.T.S) LIMITED

Name 2

Department

Campus Estate or Research Centre

Building

19 WESSEX TRADE CENTRE

Road Name

OLD WAREHAM ROAD

District

Town

POOLE

County

DORSET

Postcode

BH12 3PF

Country

ENGLAND

Tel Number 01202 747633

Fax Number 01202 747634

E-mail

HSE Division WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>PO BOX 88</td>
<td>MANCHESTER</td>
<td>CHESHIRE</td>
<td>M60 1QD</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Yes

Give brief details of the genetic modification safety committee

- Genetic Modification Safety Committee at UMIST (HSE GM Centre 79)

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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</table>

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

Residual or waste cultures to be autoclaved prior to disposal.
Disposable plastic lab-ware (pipette tip etc.) to be autoclaved prior to disposal.
Contaminated Glassware to be autoclaved prior to washing and re-use

Tick to confirm that you are attaching a summary of the risk assessment  
Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

UMIST GM safety risk assessment:

Autoclave all waste.  Negligible risk to the environment.  Yeast (S. cerevisiae) is non-pathogenic to mammals including man.

Containment level 1
GM Centre Number: 826

<table>
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Name

NORTHUMBRIA UNIVERSITY

Name 2

Department

VICE CHANCELLOR'S OFFICE

Campus Estate or Research Centre

DEPT OF APPLIED SCIENCES

Building

FACULTY OF HEALTH & LIFE SCIENCES

Road Name

ELLISON BUILDING, ELLISON TERRACE

District

Town

NEWCASTLE UPON TYNE

County

NORTHUMBERLAND

Postcode

NE1 8ST

Country

ENGLAND

Tel Number

0191 227 4002

Fax Number

0191 227 4417

E-mail

HSE Division

YORKSHIRE AND NORTH EAST

Comments

Date at Which Additional Info Submitted

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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**  
Y

**Give brief details of the genetic modification safety committee**

- One appointed safety personnel.
- One management representative.
- Three technical representatives
- Three academic staff.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential  

02/03/2022
All used liquid growth media will be sterilised by the addition of 10% (v/v) of 10g/1 Virkon for 60 minutes. The treated waste will then be discarded down a designated sink, accompanied by copious quantities of water, in the containment level 1 laboratory where the work has taken place. All other waste will be sterilised by autoclaving and then incinerated.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

With reference to the analysis outlined in the attached summary, the GMSC recommended that GLP will adhered to, that GMM works should only be conducted in laboratories which are of containment level 1 status within the School, that waste management should be conducted as set out in Section 6, and investigators are especially aware of local procedures for the treatment of inevitable minor biological accidents, such as bacterial culture spills (these will require immediate treatment with a powdered disinfectant - Virkon).

Project Ref 826/17.1

Date Ackn’d 06/07/2017

Date Project Ceased

CU2 Project Title Investigation of the fundamental biology and adaption to virulence of bacterial pathogens using genetic manipulation.

Class Culture Vol

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N
Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The work to be carried out will address the fundamental biology of bacterial pathogens with respect to their:

1. nutritional adaption to colonisation and infection, 2. biosynthesis and construction of envelope layers, 3. trafficking of materials across envelope layers, 4. development of resistance to envelope-targeted antimicrobials and 5. their responses to challenge by the innate immune system.

Recipient or parental organism

Respiratory pathogens, particularly Rhodococcus equi and Streptococcus equi will be the focus of much of the work.

Other ACDP Hazard Group 2 nocardioform actinomycetes maybe used for comparison to Rhodococcus equi, for example Mycobacterium smegmatis.

Some gastrointestinal pathogens may be used for comparison, for example Salmonella enterica. The extent of the modification here will be limited to using reporter constructs to facilitate detection of bacterial growth or gene expression in model infection systems.

Host/vector system

For Nocardioform actinomycetes:

Only vectors that are either non-mobilisable or mobilisation-defective in bacteria as defined in the Compendium of Guidance will be used. These will include the broad host range pNV18 and 19 series (the low copy number variants are most appropriate) and the low copy mycobacterial vectors pMV261 and pMIND, and the integrating pMV306.

For mutagenesis, the anticipated systems are the pSELACT system, which has been developed for constructing unmarked in-frame deletion mutants in rhodococci.

Inducible merodiploid strains may be constructed using the integrating pMV306 vector or similar, if necessary, to explore conditional genetic essentiality in targeted genes.

Streptococcus equi:

Mutagenesis will be carried out in S. equi using homologous recombination using the thermosensitive shuttle vector pG+host9. Complementation analyses will be carried out using non-mobilisable vectors such as pCD123.

Random insertional mutagenesis will use the EZ-Tn5<KAN-2>Tnp Transposome system, which relies on a pre-formed complex of a simple T5 transposon and transposase components to introduce a Kanamycin resistance cassette stably into the bacterial chromosome.

RNA interference: The 'knockdown' of essential genes by RNA interference in nocardioform actinomycetes will be attempted using the tetracycline-inducible mycobacterial shuttle vector pMIND.

It is anticipated that newer systems based around CRISPR-Cas9 will be explored as these are developed. These are often stably incorporated into the bacterial chromosome and would not be transferable to other organisms.

Origin & function

Inserted DNA will be derived from genomic DNA extracts of bacterial pathogens of the respiratory tract, particularly nocardioform actinomycetes and Streptococcus equi.

Where ACDP Hazard group 3 pathogens are used, such as Mycobacterium tuberculosis, sterile DNA extracts will be obtained from other laboratories.
The genetic manipulation involved will include:

(i) the mutagenesis of bacterial pathogens (ACDP hazard groups 1 and 2) in targeted genes relating to the 5 categories described above in section 6. Mutagenesis will be carried out using homologous recombination on a ‘suicide’ plasmid such as pSELACT or a thermosensitive plasmid such as pG+host9 to form unmarked in-frame deletions or by random insertional inactivation using transposons.

(ii) the complementation of such mutants with related sequences of bacterial origin (or synthetic derivatives thereof) to validate gene function.

(iii) RNA interference to transiently silence essential genes

(iv) the generation of harmless reporter functions (e.g. GFP/luciferase/β-galactosidase) to probe bacterial viability and/or gene expression and its regulation.

For activities i-iii, the bacteria used principally will be respiratory tract pathogens of humans and mammals, and their close relatives. These will be limited to:

(a) the nocardioform actinomycetes principally Rhodococcus equi, however, other rhodococci, and selected bacteria from other closely-related genera including Nocardia, Corynebacterium, Gordonia, Segniliparus and Mycobacterium will be used periodically for comparison. Where possible and experimentally valid, ACDP hazard group 1 species will be used. Periodically, genes encoding transporter proteins or enzymes of ACDP hazard group 3 pathogens Mycobacterium tuberculosis and/or Mycobacterium leprae will be used in activity ii.

(b) Streptococcus equi.

The range of pathogens used in activity (iv) will be more diverse to include ACDP hazard group 2 species in general. Our principal interest is in respiratory pathogens, likely subjects will be Haemophilus influenzae and Pseudomonas aeruginosa. Sometimes gastrointestinal tract pathogens such as Salmonella enterica will be used for comparison.

Our interest lies in the characterisation of gene products that are related to the fundamental biological systems of these bacterial pathogens rather than ‘aggressive’ virulence factors such as toxins or super-antigens. Cloning of such sequences is excluded from this proposal. Predominantly, we will focus our investigations on genes that (i) direct the biosynthesis of the bacterial cell envelope (ii) enable the trafficking of proteins across the envelope, (iii) enable the import and exploitation of nutrients or the biosynthesis / export of components of dedicated acquisition systems (e.g. siderophores), (iv) allow the bacterium to adapt to environmental changes such as acidification. (v) In studies of the regulation of these genes or of the persistence of the organism in in vitro infection models, various well-described reporter genes will be used (e.g. β-galactosidase, fluorescent proteins, luciferase).

Our aims will require authentic physiological expression levels to validate gene function. Genes assessed in complementation studies will be expressed from native promoters or well-characterised constitutive or regulated promoters.

**Evaluation of foreseeable effects**

**Potential Hazards:**

(i) Genes that direct the biosynthesis of the bacterial cell envelope.

Genes that will be targeted will include glycolysyltransferases, components of fatty acyl synthases, fatty acid modifying enzymes, and associated genes that provide substrates via reactions such as epimerisation. Principally, these genes will be deleted individually but in some cases in low numbers. Complementation experiments will involve the low level expression of single genes or operons to establish gene function. In almost all situations, modifications will likely merely replace deleted functions that should severely limit the fitness of the microorganism and will generate no greater hazard than the original host. The modification of the biosynthesis of cell wall lipoarabinomannan, a likely immune suppressor, might induce a more aggressive response to the bacterium by macrophage but this would be expected to lead to early clearance by the innate immune system rather than an amplified inflammatory response.

(ii) Genes that enable the trafficking of proteins across the envelope.

Genes that will be targeted will include genes that have been identified by a functional genomic / bioinformatic approach. By definition, these sequences will not be limited to pathogenic species so risks associated with cloning uncharacterised sequences are adequately controlled.

(iii) Genes that enable the import and exploitation of nutrients or biosynthesis / export of components of dedicated acquisition systems.

Genes will be manipulated in isolation or in small numbers. Deletion will be expected to limit the ability of the bacterium to establish an infection through loss of fitness. The
mutation of genes encoding iron-responsive regulatory proteins might de-regulate virulence-associated factors like adhesins and siderophores. These genes would be at least partly deregulated as part of the normal colonisation process; the outcome is likely to be inefficient biosynthesis reducing bacterial fitness. The mutation of such regulatory genes will be only attempted in Rhodococcus equi as the organism produces no known toxins that might be subject to transcriptional control by iron availability and thus overexpressed as a consequence of this manipulation.

(iv) Genes that allow the bacteria to adapt to environmental changes such as acidification. Deletion of such genes is expected to severely limit the fitness of the organism and lead to rapid clearance by the innate immune system. Complementation studies will not seek to over express such systems and thus significantly enhance the tolerance of the bacterium to the killing mechanism applied by the innate immune system. Low gene doses will be maintained throughout to ensure physiological relevance of the observed phenotype and avoid the generation of exceptionally tolerant strains.

(v) Reporter constructs. The purpose of the reporter constructs is to indicate organism viability or differential gene expression. The reporters are all well characterised and accepted as harmless research tools.

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<tr>
<td>The work will generate various wastes that may contain live GMMs, often at low densities. These will include bacterial pastes, bacterial lysates and spent medium. All will be sterilised by autoclaving before disposal as solid or aqueous waste, as appropriate. Additionally, there will be some generation of contaminated plasticware and glassware. Predominantly, these will be sterilised by autoclaving. If the material is to be reused and heat treatment is not compatible with such reuse, the items will be disinfected by treatment with 1% Rely+On Virkon for at least one hour. To avoid corrosive damage, the metal probes used in our ultrasonication equipment will be cleaned after each use by thorough wiping with 70% ethanol. This is a precautionary measure as the ultrasonication in itself will kill the overwhelming majority of the bacteria in the sample. With reference to manufacturers’ data, Hernández et al. 2000 (J Hosp Infection 46: 203–209) and in our own experience, these treatments are adequate to disinfect surfaces contaminated with these vegetative bacteria. Autoclave performance is regularly monitored by technical staff. Spills will be contained by adsorption on tissues or 3M Powersorb spill management kits (SK5), choice will be based on size of the spill. The spill management kits can contain 5L spillages. The contaminated adsorbant will be bagged and sterilised by autoclaving. The area affected by the spill will be mopped/swabbed using 1% virkon which will be allowed to stand for at least one hour. If the surface is metal, Virkon will be applied for 10 minutes and followed up with 70% Ethanol, to avoid corrosive damage. The efficacy of the clean up procedure for spills of &gt;10 ml high density cultures and &gt;100ml of spent media will be monitored by attempting to culture bacteria from the spill site.</td>
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The risk assessment has been reviewed by the University GM committee and found to be satisfactory. The assessment has been approved. Lee Rounds (Chair of GMSC)

### Project Containment

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<th>Growth Rooms</th>
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### Project Ref 826/21.1

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| Project notified under transitional arrangements | N |

### Purposes of the contained use

This project is an investigation into the molecular basis for Bordetella infection and immunity. It attempts to elucidate the processes by which these bacteria colonise the host tissues, multiply within these tissues, cause pathology, transmit to other hosts and how the host immune response reacts to the bacteria. In particular, it studies the genetic and cellular basis for these processes. Data arising from this project will obviously expand understanding of the infection biology of these bacteria. However the
Bordetella are models for numerous processes that are of general interest to the infection and immunity field, including control of gene expression, interactions with ciliated respiratory epithelia, mucosal immunity in the respiratory tract, host-adaptation and the evolution of virulence. This project involves studies at the cellular level using whole bacteria and at the molecular level using purified bacterial components (proteins, nucleic acids, carbohydrates, lipids and glycolipids).

Recipient or parental organism

B.pertussis, B. parapertussis, and B. holmesii are pathogens of the human respiratory tract and the causative agents of whooping cough. Infants who have yet to receive the full schedule of pertussis vaccinations are susceptible to whooping cough. Adolescents and adults for whom vaccine or infection-induced immunity has waned are susceptible to chronic cough and are sources of infection of susceptible infants. Pertussis is naturally endemic in the human population. Little is known about the contribution of B. parapertussis and B. holmesii to the burden of whooping cough disease. The pertussis vaccines do not fully protect against B. parapertussis or B. holmesii but may lessen the severity of parapertussis and holmesii disease. Thus, laboratory workers are at risk from infection but the morbidity from such infection is very low. Appropriate control measures to prevent exposure should be used. B. bronchiseptica infects a very wide range of mammals but is described as a pathogen of just a few (cats, dogs, pigs), causing a relatively mild respiratory tract infection. Human infections are rare and almost always associated with immuno-compromised people or close contact with infected animals. It is not considered a risk to healthy humans.

The other Bordetellae cause sporadic disease in humans including respiratory disease and septicaemia. Risk factors for infection by these organisms are unknown, as is their true pathogenic potential. Although infections by these bacteria are rare, appropriate control measures are sensible. Workers may spread the bacteria, particularly B. bronchiseptica, to other susceptible animals if they are carried out of the lab on clothing or person. All workers will be made explicitly aware of the need to maintain rigorous standards of occupational hygiene to minimize the risk of this. All workers will be required to sign that they understand this risk and the control measures required to prevent it on personal safety training/risk assessment records.

Importantly, Bordetella that are pathogenic to humans, and capable of aerosol transmission, will be handled in a Class II Biological Safety cabinet to minimise the chance of exposure of humans to these bacteria.

Host/vector system

Bordetella DNA will be cloned, maintained and manipulated in lab strains of E. coli. The main purpose for this is to introduce defined mutations into the DNA and then to move the mutated loci back into Bordetella to generate allelic replacement mutants. Mostly, mutations will be constructed by inserting an antibiotic resistance cassette into the Bordetella locus.

Origin & function

The creation of defined bacterial mutants via allelic exchange mutagenesis is a powerful technique for elucidating gene function, is a standard technique in my laboratory and forms the majority of genetic manipulation in this project. The phenotype of the wild-type parental strain is compared to that of the isogenic mutants. Changes in phenotype are correlated to the genotype. This approach is a powerful technique for ascribing gene function, for elucidating the role of that gene in the biology of the host organism and is used widely in studies of bacterial pathogenicity.

Some Bordetella mutants will be used to infect mice to test their phenotypes in an in vivo model of infection and immunity. The mouse model of Bordetella infection is well characterised and has been instrumental in understanding the infection biology of these bacteria, this will be conducted outside of Northumbria University.

In other cases, Bordetella genes will be cloned to express them from the recombinant plasmids and is also used to study gene function. Complementation of mutations is used to confirm that a mutant phenotype is due to the experimentally constructed mutation and not a secondary effect or artifact. Expression of genes in heterologous hosts is used to identify possible functions of the gene by analysing the phenotypes of the recombinant strain. Finally, heterologous gene expression in appropriate E. coli host strains is widely used to generate high levels of recombinant, purifiable protein for in vitro studies of protein function.
Evaluation of foreseeable effects

It is considered very unlikely that using lab strains of E. coli in this way will result in a pathogenic phenotype. A vast majority of DNA constructs used will not encode functional proteins. Bordetella promoters are not well recognised in E. coli. In the very rare instances where a Bordetella encoded protein is expressed in these E. coli hosts, it is highly unlikely to convert them to an infectious phenotype.

Deleterious effects from mutating Bordetella are unlikely. Most mutants are of lower virulence/fitness than the parental wild-type strains (for example see: Pilione, M. R., Pishko, E. J., Preston, A., Maskell, D. J. and Harvill, E. T. 2004. PagP is required for resistance to antibody-mediated complement lysis during Bordetella bronchiseptica respiratory infection. Infect. Immun. 72: 2837-42; Burns, V. C., Pishko, E. J., Preston, A., Maskell, D. J. and Harvill, E. T. 2003. The role of Bordetella O-antigen in respiratory tract infection. Infect. Immun. 71: 86-94; Harvill, E. T., Preston, A., Cotter, P. A., Allen, A. G., Maskell, D. J. and Miller, J. F. 2000. Multiple roles for Bordetella lipopolysaccharide molecules during respiratory tract infection. Infect. Immun. 68:6720-8). To my knowledge, there is no report of a Bordetella mutant showing increased infectivity or causing greater pathology compared to its wild type. There is clear scientific evidence that the host ranges of these bacteria is determined by multiple factors (multiple different adhesins are used, multiple toxins interact with different components of the host immune system, defective metabolic pathways (e.g. glycolysis, cysteine synthesis) contain multiple genetic deficiencies and thus would not be activated by gain of a single gene.

There are no foreseeable adverse effects from performing in vivo work with Bordetella mutants. The bacteria will be handled in containment level two facilities and infected animals housed in isolator facilities (outside of Northumbria University) that will not permit release of bacteria in to the environment or to other animals. Transport of the GMOs will be in accordance with .

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None made

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste-autoclave. Autoclave at 134°C for 10 minutes (+10 minutes free steaming), validated by temperature probe. Liquid cultures- autoclave. Autoclave at 134°C for 10 minutes (+10 minutes free steaming), validated by temperature probe.

Liquid spills will be mopped up by paper towels and these will be autoclaved as above (hazardous cultures will be handled in a safety cabinet which will aid containment of spills).

Autoclave performance is regularly monitored by technical staff.

Contaminated materials and surfaces will be disinfected using 70% ethanol which achieves a 100% kill of Bordetella. This was validated in house by the PI. Suspensions of bacteria were deposited on bench surface, bio-safety cabinet surface and floor. 1x10^3, 1x10^5, 1x10^7, 1 x10^9 and 1x10^10 colony-forming units were deposited in PBS in a 100μl volume and allowed to dry for 1 hour. Each spot was then sprayed with a saturating volume of 70% ethanol (no fixed volume) and allowed to stand for 1 minute. The ethanol was then removed by wiping dry with a paper towel. PBS was then pipetted onto the spot where bacteria were deposited and agitated with a sterile swab. The PBS was then collected by aspiration and plated onto Bordet-Gengou agar supplemented with 15% defibrinated sheep blood (standard Bordetella growth medium) and incubated at 37°C for the appropriate length of time to allow growth. No colonies were recovered from any surface. The experiment was repeated twice with the same result.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N
We have considered the proposed project under the management of Dr Iain MacArthur and are satisfied that the risk assessments made and countermeasures proposed are appropriate. New workers will be directed towards our generic in-house training which will be supplemented with project specific training provided by Dr MacArthur or a trusted delegate. The site intended for in vivo testing currently is at University of Louisiana, USA and we have discussed appropriate packaging for the transfer.

### Project Containment

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Tick to confirm that you have attached a risk assessment to this form: Y

Tick if you are claiming exemption from disclosure for section of the risk assessment: N
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Date at Which Additional Info Submitted
02/03/2022
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<td>CLINICAL IMMUNOLOGY &amp; ALLERGY LABORATORY</td>
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<td>GREATER LONDON</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The GM 386 committee has reviewed this project. This project has also been considered by a working group of the genetic modification safety committee for King’s College Hospital. The hospital is in the process of establishing their own GMSC and a full committee meeting will take place on 29 May 2002.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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Tick if confidential
### Project Ref 827/02.1

**Date Ackn'd**

29/04/2002

**CU2 Project Title**

A EUROPEAN MULTI-CENTRE RANDOMISED DOUBLE-BLIND PLACEBO CONTROLLED STUDY TO EVALUATE EFFICACY AND SAFETY OF AD5 FGF-4 IN PATIENTS WITH STABLE ANGINA.

**Class**

Class 2

**CultureVolClass2**

< 1 litre

**CultureVolumeClass3-4**

not applicable

**Non-GMM Consent Granted**

not applicable

**Consent Granted**

Project notified under transitional arrangements

**Date Project Ceased**

29/04/2002

**Withdrawn**

No

**Tick if notifying a connected programme of work**

No

**Historical Significant Changes**

No

---

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

---

**The GMSC approved the GM RA as a Class 2 activity.**
In the proposed clinical trial programme, angiogenic gene therapy product, consisting of a recombinant adenovirus (human serotype 5) containing the human gene for the fibroblast growth factor 4, and referred to as Ad5FGF-4, will be investigated as a treatment for patients with chronic stable angina due to coronary artery disease (CAD). Generation of new blood supply in the diseased heart by intracoronary administration of angiogenic gene therapy product represents a potential new therapeutic approach to relieve this condition.

The Ad5FGF-4 gene therapy product consists of a recombinant adenovirus vector (human serotype 5, Ad5) with a deletion in the E1 region; from map until 1.3 to 8.7 of wild-type virus (entire E1A and most of E1B are eliminated). The FGF-4 transgene is inserted, driven by CMV promoter.

The FGF-4 gene was originally isolated from a cDNA library which was constructed from mRNA of Kaposi's sarcoma DNA transformed NIH3T3 cells. The intended function is angiogenesis, the formation of new blood vessels.

The probability of adverse consequences resulting from deliberate or accidental release of the gene therapy product Ad5FGF-4 are minimal to non existent. Hazards resulting from environmental release (viral shedding from treated persons, inadvertent contamination of the product prior to administration) are negligible or none existent for the following reason: infection requires large numbers of infectious vectors, and transfection (expression of the inserted gene) requires a multitude of infectious particles.

Hazards associated with the adenoviral vector can be described as having low potential of adverse environmental consequences in humans or animals. The theoretical consequences to humans of several of the hazards associated with ectopic transgene expression, if they actually occurred, could be considered moderately severe (eg promotion of existent malignancy, unknown risk to foetus). However, since any unintended or accidental exposure would most likely be a fraction of the total dose being administered to patients for therapeutic purposes, the relative risk of the occurrence of these types of adverse effects should be very low.

The possible risks to the environment could be assessed as low to effectively zero. This is based on the low probability of infectious adenoviral particles escaping into the environment either through viral shedding by patients that have received the product, or by incidental exposure during administration procedures. Even if viruses were shed or product spillage occurred the number of infectious viral particles would be too small to result in infection of exposed tissues. The risk to the non-human environment is extremely low to effectively zero because of the species specificity of adenovirus 5, which by natural exposure is only known to infect humans.

The above indicates that the product could be classified as Class 1. However, as a precautionary measure, due to the limited experience available, the product is currently being classified as Class 2. Reclassification into Class 1 may be considered if based on increased data and experience.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Inactivation by autoclaving and effectively 100% kill.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The GMSC approved the RA as a Class 2 activity.

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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## Project Ref  827/17.1

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<td>25/08/2017</td>
<td>A Phase 3 Randomized, Open-Label Study Comparing Pexa-Vec (Vaccinia GM CSF I</td>
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<td>&lt; 1 Litre</td>
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<td>Thymidine Kinase-Deactivated Virus) Followed by Sorafenib Versus Sorafenib in Patients with Advanced Hepatocellular Carcinoma (HCC) Without</td>
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Date Project Ceased  25/08/2017

Class Non-GMM Consent Granted
Pexa-Vec is currently in clinical development for the treatment of Hepatocellular Carcinoma. The proposed contained use will be the administration of the investigational product, in a hospital or clinic setting, by intratumoral (IT) injections to patients as part of an international, multicenter clinical trial. This clinical trial is a Phase III trial in patients with Advanced Hepatocellular Carcinoma (HCC) without prior systemic therapy. Results from this pivotal trial will determine whether Pexa-Vec followed by sorafenib increases survival duration in advanced HCC patients compared to treatment with sorafenib alone, and whether sequential dosing with Pexa-Vec followed by sorafenib has a favourable safety profile.

Approximately 40 clinical sites in the EU will enroll patients in the JX594-HEP024 (PHOCUS) study. Additional clinical sites in Australia, Canada, China, Israel, Korea (Republic of), New Zealand, Singapore, Taiwan, Thailand and the USA will also participate in the study. A total of 600 patients will be recruited in this clinical trial with an expectation to enroll 200 patients in EU countries. In the control arm, the 300 patients will not receive Pexa-Vec. After study completion, all patients will be followed up for survival. Among them, 300 patients (i.e. approximately 100 patients in EU) will receive Pexa-Vec by IT injections. This is being submitted as a Connected Programme Notification to facilitate the possibility of conducting other, future clinical trials of the same product, e.g. in patients with other cancers or in combination with other treatments.

Pexa-Vec is a replicative oncolytic recombinant vaccinia virus (VV) derived from the commonly used vaccine Wyeth strain, DryvaxTM. W is a member of the Poxviridae family (genus Orthopoxvirus). Multiple strains of W exist that have different levels of virulence for humans and animals. The New York City Board of Health (NYCBOH) strain, from which the Wyeth strain of the Dryvax® vaccine was derived, has low pathogenicity in humans (Fenner F. et al., 1988). W has a long and extensive history of use in humans. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous (SC) infection. As W contains antigens that stimulate an immune response that are cross-reactive with smallpox antigens, the vaccine thereby confers protection from the human smallpox disease. W may cause local reactions including erythema, edema and systemic reactions such as fever and malaise, as has been observed with conventional vaccinia nation to smallpox. During the smallpox vaccination campaign, serious complications had occurred in less than 1 in 4,000 individuals, mainly in immunosuppressed and extremely young individuals. Pexa-Vec is even further attenuated as the thymidine kinase gene has been disrupted which makes replication in normal cells more difficult than the smallpox vaccine. Rare complications included eczema vaccinatum (patients with eczema), disseminated vaccinia rash, progressive vaccinia (in T-cell-deficient individuals) and...
encephalitis (1-2 per million vaccinated) (Fields B.N., 1996). Recent studies of smallpox vaccines have identified
cardiac injury including pericarditis and myocarditis as a potential risk (Halsell J.S. et al. , 2003).
W replication exclusively occurs in the cytoplasm thus eliminating any risk of integration of the viral DNA into the host
genome (Moss B. , 2007).

In terms of classification of hazard, W is considered as a Group 2 biological agent as per Directive (2000/54/EC). W is
also classified as a Biosafety Level 2 (BSL-2) infectious substance by the US Centers for Disease Control and
Prevention (CDC) (CDC, 2009) and as a risk group 2 organism by the US NIH Guidelines (NIH).

Host/vector system

Pexa-Vec was generated by co-transfection of CV-1 cells (Monkey kidney cells) with W (Wyeth strain obtained from
the Center for Disease Control, Atlanta, Georgia) and the plasmid pSC65/hGM-CSF. The vector pSC65/hGM-CSF
contains DNA sequences coding for the hGM-CSF and ~galactosidase proteins and for their respective promoters. In
addition, the transgene sequences are flanked by two W genomic regions (TKL and TKR) that allow homologous
recombination between the transfer plasmid and W.
The plasmid pSC65/hGM-CSF is generated from the plasmid pSC65 which was provided by Dr. B. Moss, National
Institute of Allergy and Infectious Diseases, Bethesda, Maryland (Chakrabarti 1997).
The plasmid pSC65/hGM-CSF is inserted into VV. The insertion of pSC65/hGM-CSF into VV can be detected by
using colorimetric identification of plaques containing recombinants expressing ~galactosidase.
The pSC65 vector when provided by Dr. B. Moss contains the LacZ gene. The LacZ gene is a reporter gene, under
control of the W p7.5 early/late promoter. The additional donor gene (i.e. gene coding for hGM-CSF) is inserted in
pSC65 as follows.
The plasmid pCSF-1 (No. 39754) was obtained from American Type Tissue Culture Collection and comprises the full-length
cDNA for hGM-CSF (Wong 1985). The hGM-CSF gene was cloned first into the EcoR1 site of pBLUESCRIPTII SK
(Siratagene, La Jolla, California), generating plasmid pBLUEhGM-CSF, and providing restriction enzyme sites to
allow cloning of the hGM-CSF gene into the Sal I and 8g1 II sites of pSC65. This positioned the hGM-CSF gene
downstream of a synthetic promoter (PsE/L) designed by Dr Moss’ laboratory to give maximal levels of transcript when
during both the early and late phases of vaccinia infection (Chakrabarti 1997).

Origin & function

P!/xa-Vec contains three genetic modifications compared to the wild type Wyeth strain: 1) disruption of the viral
thymidine kinase (TK) gene by, 2) insertion of the human granulocyte macrophage-colony stimulating factor (hGMCSF)
gene and 3) insertion of the LacZ gene.

Due to the insertion of the transgenes, the TK gene is inactivated. This decreases W virulence (Buller R. et al., 1985)
by restricting viral replication to proliferating cells. This also targets dissemination of the virus to tumors (Puhlmann M.
01 al., 2000).
The plasmid pCSF-1 (No. 39754, American Type Tissue Culture Collection, Rockville, Maryland) contains the full-length
cDNA for hGM-CSF (Wong G.G. et al., 1985). The cDNA for the hGM-CSF gene was inserted into the TK gene
to help elicit an immune response to tumor cells both at the site of viral replication and in distant metastases.
The cytokine hGM-CSF was chosen because it was the most potent stimulator of systemic anti-tumor immunity
among many tested (Dra noff G. et al., 1993), probably due to its unique ability to promote differentiation of
hematopoietic precursors into dendritic cells (Pardoll D.M., 1995). Dendritic cells are professional antigen presenting
cells that may take up and present released tumor antigens as the tumor cells are killed by the W.
The LacZ gene is contained in the pSC65 plasmid which was provided by Dr. B. Moss, National Institute of Allergy
and Infectious Diseases, Bethesda, Maryland.
The LacZ gene encodes for ~galactosidase which is a hydrolase enzyme that catalyzes the hydrolysis of 13-
galactosides into monosaccharides. Of note, following recombination between the Wand pSC65/hGM-CSF, the antibiotic resistance gene contained in pSC65 is not part of the insert. The final GMO does thus not contain any genes conferring resistance to antibiotics.

**Evaluation of foreseeable effects**

Pexa-Vec is non-integrative, and replicative and propagative characteristics of W have been attenuated in Pexa-Vec, which makes the virus replication dependent on actively dividing cells such as cancer cells. Therapy with a replicating virus can theoretically lead to shedding of the virus into the environment, and potentially to the public, although controls are used in this trial to minimize this occurrence. The clinical information available to date suggests that Pexa-Vec is safe at the clinical dose of 1 x 10^9 pfu (10,000-fold higher than smallpox vaccine dose) and has not spread to caregivers in contact with the treated patients. Should shedding occur, the level of exposure would be predicted to be low compared to the doses received by patients in the proposed trial, and extremely low compared to doses of non-attenuated vaccines administered to the public (e.g. vaccines against smallpox). In addition, exposed individuals over the age of 35 will likely have been previously immunized with vaccinia. In the highly unlikely event that an exposed individual were to demonstrate virus-associated toxicity, therapy could be initiated with VIG and/or cidofovir. Therefore, public health risks with this virus are extremely low and in fact should be lower than with standard vaccination procedures. To date, no reports of transmission to health care personnel from vaccinia recipients have been published. Routine barriers nursing approaches should be used per institutional guidelines for infectious organisms (e.g. such as for M. tuberculosis, Pseudomonas).

The information regarding the risk to patient contacts and guidelines for reducing the risk of viral transmission is contained within the Participant Information Sheet and Consent Form (PISCF) and the Pexastimogene Devacirepvec (Pexa-Vec) Guidelines (provided in Appendix B of Pexa-Vec Investigator's Brochure). The PISCF will be reviewed with the patients, and their written consent will be obtained, before they undergo any study-specific procedures. A signed copy of the PISCF will be given to the patients so that they can come back to the guidelines at any time. Pexastimogene Devacirepvec (Pexa-Vec) Guidelines will be given to investigators, pharmacists, and all personnel involved in the handling of the product.

The genetic modification of the virus is not expected to result in any post-release shift in biological interactions or host range or in any known or predictable effects on non-target organisms in the environment. It is also not expected that the release of the recombinant virus would result in any increase in pathogenicity as compared to the parental virus strain and/or in any increase in the capacity to recombine with other related viruses. Therefore, under the conditions for use in the proposed clinical trial, Pexa-Vec is not considered to represent a risk for the environment and for the public health.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**Not applicable.**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Used vials, needles, syringes and gauze will be discarded into a designated autoclavable sharps bin. This will be closed (but not locked, so as to permit steam penetration), and placed into an autoclave bag. Disposable gowns, gloves and other personal protective equipment will be discarded directly into an autoclave bag. These are labelled to indicate GM waste, and placed in a rigid, lockable container for temporary storage and transportation. The research nurses will follow King's College Hospital's Yellow Waste Stream Standard Operating Procedure and...
will arrange for the designated waste porter to transport the waste to a suitably licensed incineration facility. Any sharps will be safely disposed of according to local institutional policies. All contaminated material, including sharps, should be disposed of in a clearly-marked biomedical waste container and discarded according to regular institution procedure for infectious waste i.e.: autoclaving, incineration, or treatment with sodium hypochlorite solution. Textiles and fabrics can be decontaminated by laundering using routine protocols for healthcare facilities (e.g., hot water washing with detergent and hot air drying).

<table>
<thead>
<tr>
<th>Is an emergency plan required according to regulation 20?</th>
<th>N</th>
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<tbody>
<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
<td>N</td>
</tr>
<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
</tr>
<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>N</td>
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</table>

Please enter comments on the GM safety committee on the risk assessment
The Kings Health Partners Biosafety Committee discussed the risk assessment at a meeting held on the 22 July 2016, with further information discussed on the 1st September 2016. The committee noted that Pexa-Vec is a GM H2 recombinant product which will be given by direct injection into the tumour. It is a vaccinia virus modified by the insertion of two external genes into the viral thymidine kinase gene. Although it retains reproductive potential, PexaVec is active only in tumour cells and not normal cells. The virus remains active for 2 weeks post administration. Approximately 300 people have received Pexa-Vec, with systemic side effects reported with intravenous administration. This study aims to assess the efficacy of intratumoral injection.

The committee identified the following potential risks:
- shedding of the virus from skin lesions (a small proportion of previously treated patients developed up to 5 pustular lesions typical of the vaccinia)
- shedding of the virus from the oral cavity if mouth lesions develop
- accidental inoculation of the IMP (this has previously occurred in 2 members of staff, which one developing a pustule at the site of the injection).

The committee acknowledged that although the risk from intralesional injection would be smaller than IV administration; they cannot be totally negated.

The IMP will be reconstituted at the Guy’s Hospital GM facility and transported by specialist courier to the KCH site. The courier has experience of in the transportation of GM materials.

The committee commented on the suitability of facilities to accommodate these patients and further information was provided as follows:

The IMP will be administered in the angiotherapy room in the Radiology Department; and the patient will be isolated immediately after the procedure for 4 hours before transfer into a single room on the private patients suite. Although there has been no evidence of transmission via urine, patients will use disposable bedpans and urinals which will be appropriately disposed off. There will be no interaction with other patients.

All staff involved will receive appropriate training and education by the biological safety officer to enable provision of safe care, and ensure that universal infection control precautions are maintained. Standard operating procedures have been written to cover training and waste disposal and will be disseminated to all persons involved with the study. The committee has indicated that all staff (nursing, ancillary, catering and housekeeping) who are either pregnant, immunocompromised or have chronic skin conditions, should not be exposed to the IMP.

The committee also raised the issue of the unavailability of immunoglobins within the UK (stored in Singapore); and was satisfied with Sillajen’s response that the response noted in the case of exposure was of a mild nature (flu-like symptoms) and self-limiting. However, should the IG be needed, Sillajen could ensure it was made available within 24-48 hours. Sillajen also has robust plans in place to follow up each case of exposure until resolution.

The biological safety committee was satisfied that all potential risks had been identified and suitable resolutions were in place.

### Project Containment

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02/03/2022
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**Name**

| **GENE EXPRESSION TECHNOLOGIES LIMITED** |

**Name 2**

**Department**

| **Campus Estate or Research Centre** |
| **THE LONDON BIOSC INNOVATION CENTRE** |

**Road Name**

| **ROYAL COLLEGE STREET** |

**District**

| **Town** |
| **LONDON** |

| **County** |
| **GREATER LONDON** |

| **Postcode** |
| **NW1 0TU** |

| **Country** |
| **ENGLAND** |

| **Tel Number** |
| **0207 691 0978** |

| **Fax Number** |
| **0207 681 9129** |

| **E-mail** |

| **HSE Division** |
| **LONDON** |

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Gene Expression Technologies Ltd has formed a Genetic Modification Safety Committee comprising a chair, a biological safety officer and workers representatives. In addition the chair and the biological safety officer of the Genetic Modification Safety Committee of the Royal Veterinary College will be co-opted in an advisory capacity.

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Other (please specify) Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<tr>
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**Solid waste:** Solid GM waste will be stored in an autoclave bag prior to transporting in a labelled steel bucket with lid for autoclaving. The waste will be autoclaved (100% kill), and then disposed of as clinical waste.

**Liquid waste:** Liquid waste will be treated with 1% Virkon overnight in a closed plastic container and disposed of via sink (100% kill).

**Glassware:** Glassware will be treated with 1% Virkon overnight and then washed (100% kill).

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

The advice of the Chairman and the Biological Safety Officer of the Genetic Modification Safety Committee of the Royal Veterinary College is that the project is as described (class 1 activities involving level 1 GMMs).
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**Comments**
PREVIOUSLY ST THOMAS' HOSPITAL CHANGED 30/03/2011 ON MERGER WITH GUYS HOSPITAL

**Date at Which Additional Info Submitted**
02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

This study was reviewed by the safety committee for GM386. Once this study has been approved by the HSE, the hospital trust will set up a GMSC.

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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC approved the GMRA as a class 2 activity.

Project Ref 829/02.1

Date Ackn'd 30/04/2002

CU2 Project Title A EUROPEAN MULTICENTER, RANDOMISED, DOUBLE-BLIND, PLACEBO CONTROLLED STUDY TO EVALUATE THE EFFICACY AND SAFETY OF AD5FGF-4 IN PATIENTS WITH STABLE ANGINA.

Date Project Ceased 13/10/2010

Class 2

CultureVol

Class 2

< 1 litre

Consent Granted not applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
**Project Additional Information**

### Purposes of the contained use

In the proposed clinical trial programme, angiogenic gene therapy product, consisting of a recombinant adenovirus (human serotype 5) containing the human gene for the fibroblast growth factor 4, and referred to as Ad5FGF-4, will be investigated as a treatment for patients with chronic stable angina due to coronary artery disease (CAD). Generation of new blood supply in the diseased heart by intracoronary administration of angiogenic gene therapy product represents a potential new therapeutic approach to relieve this condition.

### Recipient or parental organism

See box below

### Host/vector system

The AD5FGF-4 gene therapy product consists of a recombinant adenovirus vector (human serotype 5, Ad5) with a deletion in the E1 region; from map unit 1.3 to 8.7 of wild-type virus (entire E1A and most of E1B are eliminated). The FGF-4 transgene is inserted, driven by CMV promoter.

### Origin & function

The FGF-4 gene was originally isolated from a cDNA library which was constructed from mRNA of Kaposi’s sarcoma DNA transformed NIH3T3 cells. The intended function is angiogenesis, the formation of new blood vessels.

### Evaluation of foreseeable effects

The probability of adverse consequences resulting from deliberate or accidental release of the gene therapy product Ad5FGF-4 are minimal to none existent.

Hazards resulting from environmental release (viral shedding from treated persons, inadvertent contamination of the product prior to administration) are negligible or none existent for the following reason: infection requires large numbers of infectious vectors, and transfection (expression of the inserted gene) requires a multitude of infectious particles.

Hazards associated with the adenoviral vector can be described as having low potential of adverse environmental consequences in humans or animals. The theoretical consequences to humans of several of the hazards associated with ectopic transgene expression, if they actually occurred, could be considered moderately severe (e.g., promotion of existing malignancy, unknown risk to foetus). However, since any unintended or accidental exposure would most likely be a fraction of the total dose being...
administered to patients for therapeutic purposes, the relative risk of the occurrence of these types of adverse effects should be very low. The possible risks to the environment could be assessed as low to effectively zero. This is based on the low probability of infectious adenoviral particles escaping into the environment either through viral shedding by patients that have received the product, or by incidental exposure during administration procedures. Even if viruses were shed or product spillage occurred the number of infectious viral particles would be too small to result in infection of exposed tissues. The risk to the non-human environment is extremely low to effectively zero because of the species specificity of adenovirus 5, which by natural exposure is only known to infect humans.

The above indicates that the product could be classified as Class 1. However, as a precautionary measure, due to the limited experience available, the product is currently being classified as Class 2. Reclassification into Class 1 may be considered if based on increased data and experience.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Inactivation by autoclaving and effectively 100% kill.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

**Please enter comments on the GM safety committee on the risk assessment**

The GMSC approved the GM RA as a class 2 activity.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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02/03/2022
Project Additional Information

Purposes of the contained use

This notification and risk assessment relates to a planned phase 1 clinical trial of "T4 immunotherapy", involving patients with squamous cell carcinoma of head and neck. T4 immunotherapy refers to a combined gene/ cell therapy, as described below. The trial will be conducted in patients with terminal malignancy, for whom alternative therapeutic options do not exist.

Squamous cell carcinoma of the head and neck (SCCHN) is the sixth most common cancer worldwide, with 600,000 cases diagnosed annually. Despite state of the art multimodal and multidisciplinary therapy incorporating surgery, radiotherapy, chemotherapy and targeted agents, five-year survival remains at only 50%. Indeed there has been little improvement in patient survival over the past 30 years. In patients with recurrent or metastatic disease the median survival time is a mere six months.

In designing this clinical trial, we have identified two key areas of unmet need. The first of these is loco-regional recurrent disease, which accounts for the majority of deaths in patients with SCCHN. Second, about 10% of patients who present to GSTFT have locally advanced tumours that are not amenable to any form of active therapy.

This study aims to investigate intra-tumoral administration of a novel cellular immunotherapy in patients with at least one measurable and accessible site of loco-regional progressive/ advanced disease, with or without concurrent distant metastases.

We will harvest peripheral blood from recruited patients in order to generate an autologous therapeutic cell product. Specifically, peripheral blood T-cells will be genetically engineered using the SFG retroviral vector to co-express two new proteins, named T1E28z and 4 alpha beta respectively. Consequently, this experimental therapeutic approach is referred to as T4 immunotherapy.

(i) T1E28z is an example of a chimeric antigen receptor (CAR). These are bespoke fusion molecules that direct T-cell
activation upon engagement of a chosen target found on tumour cells. In the case of T1E28z, tumour cell recognition is mediated by the ability of this CAR to bind several ErbB heterodimers. ErbB receptors (notably ErbB1) are highly upregulated in SCCHN. T-cell activation ensues because T1E28z contains a fused CD3 zeta + CD28 endodomain. (ii) 4 alpha beta is a chimeric cytokine receptor that allows the selective proliferation and enrichment of gene-modified T-cells using IL-4. This makes the ex-vivo expansion of therapeutic cell products more efficient since gene-modified cells have a selective advantage (compared to non-transduced T-cells) in the presence of IL-4.

Recipient or parental organism

Therapeutic genes will be delivered to activated T-cells for re-infusion into patients from whom they were originally isolated

Host/vector system


Origin & function

Two insert genes will be used, which encode for:
(i) T1E28z is a second-generation chimeric antigen receptor (CAR). Second generation CARs are fusion molecules in which a targeting moiety specific for a tumour-associated molecule is coupled via a hinge and transmembrane segment to a modular signalling domain in which the CD28 endodomain is placed upstream of CD3 zeta. In T1E28z, the targeting moiety is chimeric peptide named T1E which is a promiscuous ligand for the ErbB family of receptor tyrosine kinases. As a result T1E28z can bind to several ErbB receptor dimer species that are expressed at high levels by head and neck cancer cells. Following this binding process, an activating signal is delivered to the T-cell by CD28 (“signal 2”) and CD3 zeta (“signal 1”). This combined signal elicits T-cell activation and tumour cell attack. (Ref: Davies et al. Manuscript in preparation).


Evaluation of foreseeable effects

Potential toxicities related to T4 immunotherapy are classified into five categories:

- Related to the 4 alpha beta transgene
- Related to the T1E28z transgene
- Related to infusion of transduced T-cells
- Delayed inflammatory reactions
- Related to retroviral gene-modification of human cells

1. T-cell proliferation related to the 4 alpha beta transgene:

Use of the 4 alpha beta chimeric cytokine receptor may permit in-vivo exploitation of the elevated level of endogenous IL-4 found in serum and at the invasive tumour margin in patients with SCCHN. At best however, this effect is likely to be operate only in the tumour microenvironment, where small numbers of IL-4-producing leukocytes have been identified. Serum IL-4 is elevated in patients with some cancers (including SCCHN), in addition to atopic subjects. However, circulating concentrations found in these pathological states are uniformly in the low picogram range. Such
levels are 10,000-fold below the saturating concentration of IL-4 required for optimum support of the expansion of 4 alpha beta-expressing T-cells in-vitro. Thus, IL-4 in patients is highly unlikely to promote the uncontrolled proliferation of these cells.

2. ErbB targeting related to the T1E28z transgene:
Members of the ErbB family have been safely targeted using several pharmaco- and immunotherapy approaches to treat diverse solid tumours, including SCCHN. Importantly, toxicity attributed to these approaches has been acceptable, despite the prevalent expression of targeted ErbB receptors at low levels in many normal tissues (eg skin toxicity with ErbB1 targeting). Of concern however, Rosenberg and colleagues have recently treated a patient with widely metastatic ErbB2+ colorectal cancer with autologous T-cells genetically re-targeted with an ErbB2-specific chimeric antigen receptor. The patient succumbed to a fatal serious adverse event which was clearly attributable to the infused T-cells (Morgan et al (2010) Mol Ther. 18: 843-51). The cause of death has variously been ascribed to immune recognition of target antigen (ErbB2) either within the pulmonary parenchyma or microvasculature. This event has important implications for the safe development of T-cell immunotherapy targeted against the ErbB family and requires detailed analysis. We have identified five attributes of the experimental therapy used in that study that we believe are highly relevant to the fatal outcome reported, none of which will apply in our clinical trial.

(i) The patient underwent preparatory lymphodepletion with cyclophosphamide and fludarabine prior to receiving the infused T-cells. Lymphodepletion has also been used in one other patient who died following CAR-based immunotherapy (although the connection with the T-cells was less clear in that case). Furthermore, lymphodepletion was used in a third clinical trial that has recently been terminated owing to pulmonary and other toxicities. By contrast, no comparable toxic event(s) have been reported in any CAR-based clinical study where lymphodepletion was not used. This may reflect the fact that lymphodepletion eliminates buffering cell populations such as regulatory T-cells and some suppressive myeloid cell populations that would be expected to counter the development of cytokine storm. Lymphodepletion will not be used in this study.

(ii) The infused dose in the Rosenberg study was 10,000,000,000 gene modified T-cells of which 79% expressed the CAR transgene. By contrast, in our study it is planned to commence with a T-cell dose that is 1000-fold lower. A group of three patients will be enrolled at this low initial dose. Dose escalation will only proceed in the absence of dose limiting toxicity. Should dose limiting toxicity not occur, the maximum T-cell dose will be 1,000,000,000 cells which is 10-fold less that used by Rosenberg. Taken with the lack of lymphodepletion and cautious dosing regimen, we anticipate that the risk of cytokine storm is rendered remote under these circumstances.

(iii) A rapid ex-vivo T-cell expansion process was used in the Rosenberg study. Gene-modified T-cells were cultured for 14 days using ultra-high dose IL-2 (6,000 IU/ml) with 50ng/ml anti-CD3 mAb OKT3 and 100-fold excess of irradiated allogeneic peripheral blood lymphocytes. The latter served as “feeder cells” and would also have potentiated the action of the OKT3 antibody through Fc receptor immobilisation. This T-cell expansion process generates a highly activated cell product, which in turn would favour delayed transit through the pulmonary circulation. By contrast, we will expand T-cells ex-vivo using cytokine alone (IL-4, used to deliver an IL-2 type signal).

(iv) T-cells were administered by rapid IV infusion. Activated T-cells traverse the pulmonary circulation with a greatly reduced intravascular velocity, compared to non-activated or naïve counterparts. This is T-cells. By contrast, a regional (intratumoral) T-cell delivery system will be used in this clinical trial.

(v) Finally, the endodomain used by Rosenberg et al. consisted of an in-series fusion of CD28, 4-1BB and CD3 zeta. Incorporation of the 4-1BB co-stimulatory domain lowers the threshold for T-cell activation. In fact, some studies have demonstrated that 4-1BB-based CARs may provide constitutive signalling in
activated T-cells the absence of ligand binding. We will not employ a 4-1BB-based CAR in this study.

3. Infusion-related reactions:
Allergic reactions including rash, fever, rigors and bronchospasm are possible after infusion of transduced T-cells, but this is considered unlikely.

4. Delayed inflammatory reactions:
Localised T-cell activation within the tumour microenvironment would be expected to incite a local inflammatory response, particularly as ErbB+ tumour cells are engaged. Efforts to control local inflammation in principle might also reduce therapeutic efficacy. Consequently, we would not routinely propose to treat patients with anti-inflammatory agents unless local reactions were symptomatic. Patients to be enrolled in this study will only be selected if the lesion for injection is not situated near an airway or major artery. Furthermore, a tracheostomy will already be in place in some enrolled patients, thereby mitigating risks further. Nonetheless, it should be appreciated that inflammatory reactions within the oral cavity can track to involve the upper airway. Consequently, patients considered at high risk of airway occlusion will be cared for on an ENT ward on the night after immunotherapy is administered.

5. Related to retroviral gene-modification of human cells:
The ability of retroviruses to integrate into the host cell chromosome raises the possibility of insertional mutagenesis and oncogene activation. This event has been observed in clinical studies involving the use of retrovirus-transduced haemopoietic stem cells to treat primary immunodeficiency disorders. By contrast, this event has never been reported in human studies where T-cells have been genetically modified with similar vectors. Such clinical experience encompasses over 120 patients treated with suicide gene-engineered T-cells and extends back over 20 years to the first clinical study in which T-cells were genetically modified. Consequently, we envision that the likelihood of insertional mutagenesis is very low in this study.

Based upon these considerations, patients will be admitted for the 24 hour period following the administration of T4 immunotherapy. Extensive monitoring tests will be performed to screen for toxicity over the following 6 weeks. These are summarised in the risk assessment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All materials that have been in contact with genetically modified material will be chemically disinfected with Virkon (10g/l overnight). All waste is double bagged and labelled with purple GM waste labels. This material is transported to the autoclave room (Room 5E) on the 15th floor of Guy's tower, nearby to the GMP facility, where it is autoclaved at 134 degrees Centigrade for 20 minutes. This results in effectively 100% kill, a finding that is validated using biological indicators prior to waste disposal. Validation of the autoclave used to HTM 2010 is performed every 3 months. Once waste has been autoclaved, it is bagged into yellow incineration plastic bags and transported to the waste store and placed into the yellow waste stream.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N
Several comments were provided. Details in response to these comments have been added to the amended risk assessment. Other responses are provided in capital text.
- Clarify that full disclosure to public register will occur - YES
- Complete details in response to Q2 - ENTERED WITH DATA PROVIDED BY COMMITTEE
- Clarify waste control. What data supports virucidal activity of virkon (NOW PROVIDED); how will waste control measures be validated (DETAILS NOW PROVIDED).
- Clarify control of PPE (DETAILS NOW PROVIDED).
- Provide names of safety personnel (NOW PROVIDED)
- Outline how blood and tissue will be transported in the hospital (NOW PROVIDED)
- Outline risk associated with use of a therapeutic blood product and how this is mitigated by screening of patients for relevant chronic infective agents (NOW PROVIDED).
- Explain what EUFETS is and provide relevant contact details (NOW PROVIDED).
- Explain dosing regimen in greater clarity (DETAIL NOW PROVIDED IN BACKGROUND SECTION)
- Greek characters not visible in CU2 (FONT ALTERATIONS NOT PERMITTED IN CU2 FORM SO GREEK LETTERS HAVE BEEN SPELT OUT)
- Remove reference to work not to be performed in the GMP facility (MODIFIED WHERE POSSIBLE)
- Outline where patients will physically be during treatment (PATIENT JOURNEY DESCRIPTION ADDED)

**Project Containment**

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**Project Ref 829/18.1**

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Thymidine Kinase-Deactivated Virus) Followed by Sorafenib Versus Sorafenib in Patients with Advanced Hepatocellular Carcinoma (HCC) Without Prior Systemic Therapy

Purposes of the contained use

Pexa-Vec is a replicative oncolytic recombinant vaccinia virus (W) derived from the commonly used vaccine Wyeth strain, Dryvax(TM). Pexa-Vec contains three genetic modifications compared to the wild-type Wyeth strain: 1) disruption of the viral thymidine kinase (TK) gene by; 2) insertion of the human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene and; 3) insertion of the LacZ gene. Pexa-Vec is designed to replicate selectively in and destroy cancer cells, while at the same time stimulating a systemic anti-tumoural immune response immune response through the expression of its transgene, hGM-CSF, in the context of tumour lysis. Pexa-Vec is currently in clinical development for the treatment of Hepatocellular Carcinoma. The proposed use will be the administration of the investigational product, in a hospital or clinic setting, by intratumoural (IT) injections to patients as part of an international, multicentre clinical trial. This clinical trial is a Phase III trial in patients with Advanced Hepatocellular Carcinoma (HCC) without prior systemic therapy. Results from this pivotal trial will determine whether Pexa-Vec followed by sorafenib increases survival duration in advanced HCC patients compared to treatment with sorafenib alone, and whether sequential dosing with Pexa-Vec followed by sorafenib has a favourable safety profile. Approximately 40 clinical sites in the EU will enroll patients in the JX594-HEP024 (PHOCUS) study. Additional clinical sites in Australia, Canada, China, Israel, Korea (Republic of), New Zealand, Singapore, Taiwan, Thailand and the USA will also participate in the study. A total of 600 patients will be recruited in this clinical trial with an expectation to enroll 200 patients in EU countries. Among them, 300 patients (i.e. approximately 100 patients in EU) will receive Pexa-Vec by IT injections. In the control arm, the 300 patients will not receive Pexa-Vec. After study completion, all patients will be followed up for survival.

Recipient or parental organism

Pexa-Vec is a replicative oncolytic recombinant vaccinia virus (W) derived from the commonly used vaccine Wyeth strain, Dryvax(TM).

Host/vector system

The transfer plasmid pSC65/hGM-CSF is used to generate Pexa-Vec. The plasmid pSC65/hGM-CSF is generated from the plasmid pSC65, which was provided by Dr B. Moss, National Institute of Allergy and Infectious Diseases,
Pexa-Vec is manufactured by Transgene (France) and labelled, OP released and shipped to investigational sites from PCIIBiotec (UK). At the investigation site, PexaOVec will exclusively be used for administration to patients as apart of clinical study JX594-HEP024. Thus, only the GMO (Pexa-Vect), but not the wild-type host organism or the vector used to generate the GMO, will be used at the investigational site.

Pexa-Vect contains three modifications compared to the wild-type Wyeth strain: 1) disruption of the viral thymidine kinase (TK) gene by; 2) insertion of the human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene and; 3) insertion of the LacZ gene. Pexa-Vect is designed to replicate selectively in and destroy cancer cells, while at the same time stimulating a systemic anti-tumoural immune response through the expression of its transgene, hGM-CSF, in the context of tumour lysis.

Origin & function

The GMO, Pexa-Vect, is a non-integrative (cytoplasmic localisation), preferentially replicative in actively dividing cells (i.e. tumour cells), oncolytic (lysis of infected tumour cells) and propagative (able to spread locally to adjacent cancer cells and systemically by release into the blood stream and lymphatic system of infectious particles (Smith G.L. et al., 2002).

Over 300 adult and paediatric patients with advanced treatment refractory cancers have received more than 1,200 doses of up to approximately 1 billion infectious units (1 x 10^9) of Pexa-Vect via IV and/or IT administration (as of January 2014). Treatment with Pexa-Vect doses of approximately 1 billion pfu (approximately 10,000-fold more than the dose of standardised [non-attenuated] vaccinia delivered with vaccinia vaccine), has been generally well-tolerated with transient (<24 hours) flu-like symptoms (fever, chills, fatigue), nausea, hypotension and injection site pain as the most common adverse events (AEs). The overall risk of the wild-type (parental) Wyeth (USA smallpox vaccination) strain indicates that out of 14 million vaccinations, there were 572 hospitalisations, 9 deaths and many less severe complications. The rate of severe adverse reactions for the wild-type parental strain is approximately 1 in 50,000 vaccinations. Pexa-Vect has been attenuated when compared to the smallpox vaccine, and therefore the safety is improved.

Secondary spread could occur by direct contact (with a pustule, with bandages or other wastes contaminated by pustules, with expelled droplets from wound sites or liquid preparations) and indirect contact via medical devices contaminated through contact with a pustule. W is not spread through the air (US Department of Health & Human Services, www.smallpox.gov/QuestionsAnswers.html). Transmission of the virus by inhalation can be assessed as negligible. The clinical information available to date suggests that Pexa-Vect is safe at the clinical dose of 1 billion pfu (10,000-fold higher than smallpox vaccine dose) and has not spread to caregivers in contact with the treated patients. Should shedding occur, the level of exposure would be predicted to be low compared to the doses received by patients in the trial, and extremely low compared to doses of non-attenuated vaccines administered to the public (e.g. vaccines against smallpox). In addition, exposed individuals over the age of 43 will likely have been previously immunized with vaccinia (UK smallpox vaccination ceased in 1974). In the highly unlikely event that an exposed individual were to demonstrate virus-associated toxicity, therapy could be initiated with VIG and/or cidofovir. To date, no reports of transmission to health care personnel from vaccinia recipients have been published; however, at least nine reports of infection of laboratory workers exist from the last 25 years (see safety data sheet www.phacaspc.gc.ca/lab-bio/res/psds-ftss/vaccinia-virus-eng.php). Infection control/Containment level 2 standards and procedures and related healthcare approaches are to be implemented for worker safety.

Viral shedding data collected in a clinical study with Pexa-Vect administered by the IT route (i.e. JX-594-IT-HEP001 trial) demonstrated that the virus is not shed to the environment via urination. The virus could also not be detected in
throat-swab samples. There are no known or predicted environmental conditions which may increase survival, multiplication and dissemination of the Pexa-Vec. It is commonly thought that W is not naturally found in the environment; however, human to cattle (and vice versa) transmission has been reported via broken skin. Recombination events of the wild-type W with the GMO are not thought likely, as current genetic stability studies on Pexa-Vec have not detected spontaneous revertants of Pexa-Vec.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

I would not like to request derogation from full containment measures

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid contaminated waste will be bagged and inactivated by autoclave (135°C for 20 min) prior to being placed into clinical waste streams for incineration. Sharps waste will be placed in sharps containers after use and prior to autoclaving and incineration. All open processing of Pexa-Vec will be undertaken in isolators. Any spillage of contaminated liquids in isolators will be inactivated with Klercide biocides and hydrogen peroxide vapour decontamination.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Guy's and St Thomas' Biological Safety Committee reviewed the risk assessment on 24JAN2018 and agreed with the classification of GM class 2. Approval for this study was given by the Committee on the condition the following additional information be added to the risk assessment:
- state the autoclave temperature and hold time
- justify the use of sharps
- state who has responsibility for the product during transportation
These updates were completed on 25JAN2018 and approved on 06FEB2018.

Project Containment

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**Name**

OXXON THERAPEUTICS LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

2ND FLOOR FLOREY HOUSE

**Road Name**

3 ROBERT ROBINSON AVENUE

**District**

THE OXFORD SCIENCE PARK

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX4 4GP

**Country**

ENGLAND

**Tel Number**

01865 398100

**Fax Number**

01865 398 101

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Company changed its name from Oxxon Pharmaccines to Oxxon Therapeutics Ltd on 13/04/2004

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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<td>OXFORDSHIRE</td>
<td>OX4 4GP</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities **Y**

Give brief details of the genetic modification safety committee

Chairman, Secretary, Biological Safety Officer and Deputy Biological Safety Officer, Premises Manager and External Advisor from University of Oxford, also research and development representative.

Meeting every 6 months or when called to consider new risk assessments or GMM related operating issues.

<table>
<thead>
<tr>
<th>Laboratory (GMMs)</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential **N**
For activities involving GMMs, describe the waste management measures which will apply to the activity

Escherichia coli: cultures, glassware and plasticware will be chemically inactivated with Microsol 3 (Anachem) following the manufacturers instructions [1:200 dilution, 10 minutes]

Attenuated poxviruses, (modified vaccinia virus Ankara, fowlpox strain): cultures, glassware and plasticware will be chemically inactivated with Microsol 3 (Anachem) following the manufacturers instructions [1:50 dilution, 10 minutes]

All chemically disinfected material will be autoclaved at 121 degrees C for 20 minutes. Autoclaved waste will be incinerated.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Project Ref 830/04.1

Date Ackn'd 15/09/2004
Date Project Ceased

Characterisation of Ovine Adenovirus Vectors Expressing Marker Genes or Human Immunodeficiency Virus Antigens

Class 2

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Non-GMM Consent Granted

Consent Granted Not Applicable

Page 11566 of 15326
**Project Additional Information**

**Purposes of the contained use**

To assess the ability of ovine adenovirus vectors expressing foreign antigen genes to induce a potent immune response to that foreign antigen.

**Recipient or parental organism**

The parental organism is wild-type ovine adenovirus, strain OAdV7. Ovine adenoviruses are members of the adenoviridae family. As such, the Advisory Committee on Dangerous Pathogens classifies them as Hazard Group 2 pathogens. (Categorisation of biological agents according to hazard and categories of containment, Fourth Edition, 1995. HSE Books).

OAdV7 is known to infect a wide range of human cell types. However, the virus will replicate efficiently only in ovine foetal lung (CSL503) and skin (HVO 156) cell lines, (GW Both 2004 Immunol. Cell Biol. 82: 189-195).

**Host/vector system**

No prokaryote work associated with this project will be performed by or at Oxxon Therapeutics Ltd.

**Origin & function**

The foreign gene to be delivered by the recombinant ovine adenovirus encodes:

1. A marker gene, (either Escherichia coli beta-galactosidase, firefly luciferase, or Aequorea victoria green fluorescent protein), derived from commercially available plasmids.

2. Human immunodeficiency virus type 1 (HIV1) antigens which have been extensively mutated to destroy the activities of the encoded proteins. These mutations allow the proteins to function only as antigens in the recombinant vaccine.

Both marker genes and the HIV1 antigens will be expressed from either the human cytomegalovirus immediate-early promoter or the rous sarcoma promoter.

**Evaluation of foreseeable effects**

Strain OAdV7 was isolated from a sheep in Western Australia. Challenge experiments with this strain in adult sheep have failed to produce noticeable symptoms. However, although the US strain of ovine adenovirus was isolated from three dead lambs, it still remains to be demonstrated whether ovine adenovirus can be harmful in lambs, (GW Both 2004 Immunol. Cell Biol. 82: 189-195).
Ovine adenovirus strain OAdV7 is known to infect a wide range of human cell types. However, the virus will replicate efficiently only in ovine foetal lung (CSL503) and skin (HVO156) cell lines. OAdV7, produces abortive infections in all human cell lines tested; the block in replication occurring at the early or late transcription stages, or during assembly, depending on the cell type, (D. Kumin et al 2002 J. Virol. 76(21): 10882-10893).

OAdV7, like human adenovirus strains, maintains an episomal (non-integrated) state after infection; this precludes insertion event-dependent mutagenesis or activation, such as can occur with retrovirus vectors.

OAdV7 shares little sequence homology with human adenovirus: There is no apparent homologue of the E1a protein and the putative E1b homologue, LH3, has a three amino acid insertion in the p52 oncogene-binding region, (GW Both 2004 Immunol. Cell Biol. 82: 189-195).

The ACGM Compendium of Guidance, Part 2B, Annex III states that genes encoding marker genes, such as beta-galactosidase, luciferase, or green fluorescent protein, do not increase the hazards associated with adenovirus vectors. Expression of either of these marker genes from an ovine adenovirus vector is not expected to increase the risk of harm to human health or the environment.

The synthetic polypeptide encoding disabled HIV 1 antigens will function only as an immunogen and is not expected to increase the risk of harm to human health or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be deactivated in Lifeguard disinfectant used at 5% (v/v) for 30 minutes or Virkon solution at 1% for 10 minutes before disposal down the drain. Plastic waste will be chemically deactivated with Lifeguard used at 5% (v/v) for 30 minutes or Virkon solution used at 1% (w/v) for 10 minutes before being double-bagged, autoclaved and/or incinerated. All contaminated surfaces will be wiped down using Lifeguard and then sprayed with Microzid, which is left to evaporate.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

The safety Committee agreed that recombinant ovine adenoviruses based on strain OAdV7 expressing commonly used marker genes, or inactivated HIV1 antigen genes, pose no risk to either human health or adult sheep, but that there is possible risk to young lambs. Consequently, the recombinant ovine adenoviruses must be stored and handled under Containment Level 2 conditions.
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units
Large Scale Activities
Human Clinical Applications

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02/03/2022
### ONYVAX LIMITED

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### Additional Information

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Onyx has an established health and safety committee which meets regularly on a monthly basis. The committee consists of senior directors within the company (CEO and Finance director), the laboratory manager, the radiation protection supervisor (RPS) and the biological safety officer (BSO). The committee reviews all operational health and safety matters along with new items, which require its consideration.

All on-going projects involving GMMs and risk assessments are reviewed regularly. An agenda of items for discussion is drawn up prior to the health and safety meetings and the minutes of the meeting are open to all staff members for reading, once approved by the committee. The committee has the power to approve, adopt and change company policies and procedure (within the current legislation framework). These changes are then disseminated through the company by an approved route from the safety officers through to the laboratory group leaders to the scientists working in the laboratories/project concerned.

<table>
<thead>
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<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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02/03/2022
Yes All trained operatives will be properly attired in white laboratory coat and surgical gloves. Such attire is only to be used in the level 2 designated area. All surfaces exposed to potential contamination by retrovirus will be sprayed with 70% Ethanol before and after use.

All solid waste (serological pipettes, tissue flasks, used gloves, etc) is first autoclaved and placed in yellow bags and finally incinerated.

All liquid waste is disposed of by inactivation with a 1% Virkon for 12 hrs followed by sink disposal. This product has been tested and proven effective against major pathogens including viruses (HIV, hepatitis B and C, herpes and enteroviruses), bacteria (staphlococcus aureus) and fungi. A 1% solution of ‘Virkon’ has a good safety profile. Precautions for its use: wash splashes from eyes and skin immediately, keep concentrate of skin. Do not mix with other chemicals and keep out of direct sunlight. We have a copy of the validation tests for this product.

Specimens (supernatants and cell lines) testing positive for replication-competent viral particles will be destroyed by disinfection and autoclaving as soon as possible.

Records of destroyed specimens should be kept up to date. All work areas and equipment will be decontaminated with virkon and/or autoclaving.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Following review by the local GMSC, the risk assessment was modified and now has been approved.
In order to provide permanent cell lines, from surgical discarded material, for the development of whole cell vaccines, cells have to be immortal. This can be achieved spontaneously or by the introduction of an immortalising gene. Such genes are the cloned E6 and E7 genes isolated from the wild type human papillomavirus 16.

We propose to use retroviral vectors to immortalise human cells in vitro. The first retroviral construct will contain the cloned subgenomic HPV 16-E6 and -E7 genes in combination in the same construct, pLXSN. This construct has been modified to remove all origins of replication to minimise potential recombination events. The pLXSN vector is a commercially available retroviral vector provided by Clontech Laboratories Inc. Palo Alto, CA. The second construct will contain the HPV16-E6 gene alone in pLXSN. The HPV16-E7 and -E7 genes code for nuclear oncoproteins that have the ability to initiate the transformation of primary human cells to an immortalised state. In doing so they have transformed the recipient human cells into a condition that renders them susceptible to further genetic insults resulting in the full transformation to a malignant state.

Our required use of the constructs is to develop immortalised cell lines, not to fully transform the recipient cells. Due to the small possibility of a recombination event occurring and wild type, replication competent virus being produced and the presence of oncogenic sequences, all cells (producer cell lines and recipient human cells) will need to be cultured under Level 2 containment in accordance with the ACGM Compendium guidelines. Further, Level 2 containment will be required for all cultures whether proven to be free of replication competent virus or not. If replication competent virus is found the stock and recipient cultures will be destroyed.

Recipient or parental organism

The first recipient organism will be the packaging cell line used to generate the retroviral supernatant. This cell line will either be PA317 (a second-generation packaging cell line) or preferably, a third generation packaging cell line (if available). These types of cell lines contain the gag, pol, and env genes necessary for viral production. However due to deletion, they lack the ps1 and 3'LTR sequences, so that two recombination events are necessary to generate a wild-type virus. Third-generation packaging cell lines contain the packaging genes as to separate components, thereby significantly reducing the frequency of recombination between the vector and packaging sequences further.
The second recipient organism(s) will comprise of actively dividing human cells in culture (epithelial, fibroblastic or endothelial). When these human cells are transduced with these oncoproteins they will be transformed into a condition that renders them susceptible to further genetic insults resulting in the full transformation to a malignant state.

A close association between cervical cancer and the HPV's has been established (zur Hausen 1996). The high-risk HPV types are HPV 16 and 18. HPV16 DNA can be found in >50% of cervical cancers. Functional studies of the early region of these high-risk genomes have demonstrated that the two early genes, E7 and E7, are both necessary and sufficient for the efficient immortalisation of primary human keratinocytes in vitro, (Hudson JB 1990). The major mechanism by which E7 and E7 contribute to immortalisation is by targeting two distinct cellular tumour suppressor proteins for inactivation or degradation. E7 binds and inactivates the reinoblastoma tumour suppressor protein (pRB) and two closely related proteins, p107 and p130, leading to the activation of E2F responsive genes and the loss of a G1 checkpoint (Jones and Munger 1996). E6 forms a ternary complex with p53 and the E6AP ubiquitin protein ligase resulting in the ubiquitination and degradation of p53 (Scheffner et al 1990). Loss of p53 results in deregulated cellular growth and genomic instability, both of which are characteristics of immortalised cells (Hartwell 1992).

These recipient human cells are derived from surgical material obtained following informed consent at SGHMS. Normally these cells would be classified as Class 1 material. However, it is possible that these primary cultures may contain adventitious viruses. As these cells are going to be infected with retrovirus and will have not been prescreened for adventitious viruses at this point in the immortalisation process we will treat them as potentially suspect material and use them under Level 2 containment and work with them using Level 2 procedures.

References

Host/vector system
The vector construct into which the HPV16 sub-genomic sequences will be cloned will be pLXSN and the packaging cell line PT67 will be supplied by Clonetics, Palo Alto California, USA.
A more detailed description of this construct is given on the manufacturer's website, www.clontech.com

Origin & function
The HPV E6 and E7 genes were originally cloned as sub-genomic fragments from the wild type HPV 16 virus provided by H zur Hausen at Deutsches Krebsforschungszentrum, Heidelberg, Germany

The supplier of the HPV16 E6 and E7 retroviral producer cell lines will be the Department of Biology, University of York, UK. The provenance of the retrovirus and its producer line will be provided by the supplier. Further, viral titering results and data from helper virus testing of producer clones will also be provided by supplier. These recipient human cells are derived from surgical material obtained following informed consent at SGHMS and grown in our own facilities at SGHMS.

Evaluation of foreseeable effects
The recipient cells or tissues, in culture, will be immortalised and will provide a source of permanent, non-mortal, cell lines. Cell lines in this partially transformed state are rendered susceptible to further modification by either the introduction of additional genes or by mutagenesis with chemical carcinogens resulting in the potential development of fully transformed cells capable of forming tumours in immuno-compromised mice.

Given that these HPV-E6 and E7 sequences can partially transform human cells operatives will at no time introduce the E6 or E7 genes into human cells derived from a member of the research team. Further, all operatives will not use sharps in the containment lab and will be free of sores or open cuts. Also appropriate attire (gowns,
gloves hats and masks) will be worn at all times thereby minimising risk.

Further, operatives will be disqualified from working in the Level 2 containment facility if they have any pre-existing condition that may render them susceptible to cancer-incidence following exposure to the retrovirus containing the E7 and E7 genes. All retroviral work is performed in a designated Class 2 bio-safety cabinet and cultures are grown in an assigned incubator. Both are located in a separate room(s) set aside for human and retroviral work under Level 2 containment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

The retrovirus(s) will be capable of infecting and transforming human cells. As such all retrovirus supernatants and infected recipient cells will be manipulated under Level 2 containment and conditions.

Operatives will at no time introduce the E6 or E7 genes into human cells derived from a member of the research team. Further, all operatives will not use sharps in the containment lab and will be free of sores or open cuts. Also appropriate attire (gowns, gloves hats and masks) will be worn at all times thereby minimising risk.

Further, operatives will be disqualified from working in the Level 2 containment facility if they have any pre-existing condition that may render them susceptible to cancer-incidence following exposure to the retrovirus containing the E7 and E7 genes. All retroviral work is performed in a designated Class 2 bio-safety cabinet and cultures are grown in an assigned incubator. Both are located in a separate room(s) set aside for human and retroviral work under Level 2 containment.

All cultures exposed to and producing wild type virus will be disinfected and disposed of in accordance with ACGM guidelines (Section IV). All aqueous material will be treated with Virkon (1% solution, Manufacturer's recommendation). After 24 hours the disinfected aqueous material will be put down the sink with an excess of running water. All tissue culture plastic will be disinfected, bagged, autoclaved and incinerated.

The hoods in the Level 2 containment facility will then be decontaminated by fumigation prior to and following any program of retroviral work. The level 2 containment facility will be sited separately from any clinical or animal facility so the risk of human or animal infection is minimised.

The hazard in the use of these constructs is the potential for recombination of the HPV E6 and E7 genes with any contaminating wild type retrovirus resulting in the development of a new oncogenic retrovirus which would be infectious. We will test for infectious virus and reverse transcriptase activity on every batch of retrovirus that is produced.

In addition, the same tests will be employed when the virus is used to immortalise the human cells. Once an immortalised cell line has been produced the same test will be used prior to cryo-preservation and storage. We will also regularly test, once a month, our human cell cultures for mycoplasma and bacterial contamination. So the transfer of virus to other organisms is minimised.

As the retroviruses will be supplied by an outside laboratory European and International regulations govern the packaging and shipment of such biological materials. The supplier will be responsible for the shipment and will strictly follow the principle of triple packaging systems. This is absolutely necessary in the case of infectious and non-infectious biological substances. It is the obligation of the supplier to comply with the regulations relating to the biological material which is to be transported.

**Basic triple packaging system**

The system consists of three layers as follows:

1. **Primary receptacle.** A labelled primary watertight, leak-proof receptacle will be used to contain the retrovirus packaging cell lines. The receptacle will be wrapped in enough absorbent material to absorb all fluid in case of breakage.
2. **Secondary receptacle.** A second durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle(s) will be used. Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material will be used to cushion multiple primary receptacles.
3. **Outer shipping package.** The second receptacle will be placed in an outer shipping package which will protect it and its contents from outside influences such as physical damage and water while in transit.
Specimen data forms, letters and other types of information that identify or describe the specimen and also identify the shipper and receiver will be taped to the outside. Accurate and informative signage on the outer shipping package will be used.

Receiving broken or damaged specimen packages. If packages are received from the shipper and the contents are not intact, then the package and contents will be destroyed by autoclaving. The shipper (and) courier will be notified and if necessary amendments made to the packaging. Records of destroyed specimens will be kept.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation from full containment under Containment Level 2 will be required.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All trained operatives will be properly attired in white laboratory coat and surgical gloves. Such attire is only to be used in the level 2 designated area. All surfaces exposed to potential contamination by retrovirus will be sprayed with 70% Ethanol before and after use.

All solid waste (serological pipettes, tissue flasks, used gloves, etc) is first autoclaved and placed in yellow bags and finally incinerated.

All liquid waste is disposed of by inactivation with a 1% Virkon for 12 hrs followed by sink disposal. This product has been tested and proven effective against major pathogens including viruses (HIV, hepatitis B and C, herpes and enteroviruses), bacteria (staphylococcus aureus) and fungi. A 1% solution of ‘Virkon’ has a good safety profile. Precautions for its use: wash splashes from eyes and skin immediately, keep concentrate of skin. Do not mix with other chemicals and keep out of direct sunlight. We have a copy of the validation tests for this product.

Specimens (supernatants and cell lines) testing positive for replication-competent viral particles will be destroyed by disinfection and autoclaving as soon as possible.

Records of destroyed specimens should be kept up to date. All work areas and equipment will be decontaminated with virkon and/or autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Following review by the local GMSC, the risk assessment was modified and now has been approved.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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02/03/2022
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<th>Animal Units</th>
<th>Large Scale Activities</th>
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**Name**

ILLUMINA CAMBRIDGE LIMITED

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

19 GRANTA PARK

**District**

GREAT ABINGDON

**Town**

CAMBRIDGE

**County**

CAMBRIDGEShire

**Postcode**

CB21 6DF

**Country**

ENGLAND

**Tel Number**

01799 532300

**Fax Number**

01799 532301

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The standing committee is composed of four staff members:-
1. The Chief Scientific Officer.
2. Company safety officer.

The committee meets as the situation dictates, eg this application process and the examination and adoption of Standard Operating Procedure (SOP's) appropriate to the facility. Staff safety representatives are invited to attend GMSC meetings.

Minutes are kept as emails between standing committee members and copies are posted onto company shared files space.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
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<td>Level 1 (GMMs)</td>
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<td>Level 3 (GMMs)</td>
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</table>
Yes

Please see attached waste operating procedures document. All laboratory waste will be segregated into chemical and GMO contaminated waste streams, and also segregated by the nature of the hazard ie contaminated sharps, liquids and solids. GMO contaminated materials are autoclaved using a validated autoclave following SOP’s designed to achieve a 100% kill. The inactivated materials are then contracted out for incineration.

The disinfectant Virkon will be used, as the manufacturer direct, for the inactivation of any accidental spills of GMOs. An active solution of Virkon will be present in the laboratory at strategic locations at all times.

Validation of autoclave. This is a new instrument, which is the subject of a yearly service contract and validation testing. The autoclave has a process printer attached for clear automated record keeping producing a hardcopy print out put of each run using a load temperature probe.

The expected degree of kill by all procedures is to below detectable levels.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Please enter comments of the GM safety committee on the risk assessment

The activities proposed are at Class 1 level, ie of negligible risk to humans or the environment.

The GMSC gave advice on the risk assessment which was duly modified.
## ANGEL BIOTECHNOLOGY HOLDINGS PLC

### Data Premises Notified
- **Date:** 02/05/2002
- **Status:** Transferred from 1992 Regs?
- **Emergency Plan Required?**
- **Transitional Premises Class**
- **Non-GMMs**
- **Withdrawn**

### Name
- **Name:** ANGEL BIOTECHNOLOGY HOLDINGS PLC

### Data Premises Closed
- **Transitional Premises**
- **Class**
- **Non-GMMs**
- **Withdrawn**

### Name 2
- **Department**

### Campus Estate or Research Centre
- **Campus Estate or Research Centre:** PENTLANDS SCIENCE PARK

### Road Name
- **District:** BUSH LOAN

### Town
- **Town:** PENICUIK

### Building
- **Building:**

### County
- **County:** EAST LOTHIAN

### Postcode
- **Postcode:** EH26 0PZ

### Country
- **Country:** ENGLAND

### Tel Number
- **Tel Number:** 0131 445 6076

### Fax Number
- **Fax Number:** 0131 445 6071

### E-mail

### HSE Division
- **HSE Division:** SCOTLAND

### Comments
- **Comments:**

### Date at Which Additional Info Submitted
- **Date:** 19/12/2002

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**ANGEL BIOTECHNOLOGY LIMITED INTO ADMINISTRATION 11/7/07 - TAKEN OVER BY THE ABOVE.**
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The GMSC of Angel Biotechnology Ltd. will be composed of representatives from the departments and groups within the company that might be expected to come into contact with GMO’s, as well as a senior manager. All assessments done under the regulations will be distributed to the members for comment, the full committee will meet if there are any concerns or problems. Otherwise, there will be an annual review meeting to ensure that all assessments and legislation are discussed. The senior manager will be the chair and primary contact for the HSE and will distribute information and call meetings when necessary. Any member of the committee will be able to call a meeting at any time.

#### Level 1 (GMMs)

<table>
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<tr>
<td>Yes</td>
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</table>
Laboratory waste (agar, small volumes of liquid, disposable plasticware that has come into contact with GMMs etc) will be autoclaved at 121°C for 20 minutes. This has been validated by looking for surviving organisms. Checks will be made at regular intervals that the inactivation is complete. Separate, labelled, handling facilities (bins etc) are in place to manage the waste prior to sterilisation. Pilot fermentation waste broth is inactivated by heating to 80°C for 30 minutes. This is enough to kill the organism in use beyond detectable levels, even in its spore stage. This has been validated by growth tests. Checks will be made at regular intervals to ensure the validity of these results. Spills and non-autoclavable equipment will be cleaned using standard laboratory disinfectants (e.g. 70% ethanol, cetrimide soaked wipes, etc). These then go to be autoclaved via the standard laboratory waste route. Swabs or contact plates will be used to validate the disinfection. Glassware will be autoclaved before cleaning, or exposed to disinfectants if autoclaving is not possible. In all cases at least 99.999% kill will be looked for, although the preferred situation is that there will be no detectable viable organisms in any waste sent for disposal.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

There were no comments.
Project Ref 833/02.1

TO MODIFY A STRAIN OF STAPHYLOCOCCUS AUREUS BY TARGETED GENE MODIFICATION AND DELETION FOR ENHANCED PRODUCTIVITY OF A TARGET GENE

Class 2 1-50 litres

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: N

Historical Significant Changes

Tick if notifying a connected programme of work: Y

Withdrawn: N

Project Additional Information

Purposes of the contained use

i) To reduce the levels of secretory toxin.
ii) To enhance the levels of target protein secretion.

Recipient or parental organism

A target protein secreting strain of Staphylococcus aureus, A676.

Host/vector system

A common cloning vector for Bacillus Subtilis and Staphylococcus aureus will be used.

Origin & function

A DNA is amplified by PCR from a wild type strain of Staphylococcus aureus.

Evaluation of foreseeable effects

1. The strains produced will exhibit negligible/zero levels of a specific enterotoxin, which may make them less pathogenic than the equivalent wild type strain.
2. The amount of target protein secreted will be increased (by up to 10x) This may increase the infectivity of the organism but the nature of work is such, that the possibility
of infection is low and all work will be contained.
3. The micro-organism will be unlikely to survive outside of the culture vessel due to the growth requirements.
4. No vector DNA will be present in the final GMO as the plasmid is temperature sensitive and can be cured from a bacterial population.
5. No mobilisable elements will be introduced into the micro-organism.
6. No mobilisable elements will be introduced into the micro-organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

* Solid waste eg LB agar plates, contaminated disposable falcon tubes; All waste will be autoclaved at 126 degrees C for 30 minutes, then incinerated before disposal.
* Liquid waste eg waste media, culture; All liquid waste will be autoclaved as above and then pH treated [pH taken to 10.5, then reduced to neutral] before disposal.
* Drain waste; All drain waste will be pH treated as above.
* Sharps; All contaminated sharps will be placed in designated sharps bins and will be autoclaved as above and then incinerated.

Testing: The conditions for all waste management systems will be checked to ensure that they are appropriate for the organism at regular intervals. Samples will be taken at regular intervals (1 month) for plate testing post-autoclaving and waste treating. At regular intervals waste will be tested for viable organisms. We expect no viable GMOs to remain after sterilisation.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
All comments have been made and working procedures have been altered accordingly on the risk assessments.

Project Containment

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<td>Large Scale Activities L3 L4</td>
<td>Human Clinical Applications L2 L3 L4</td>
</tr>
</tbody>
</table>

02/03/2022
### Project Additional Information

**Purposes of the contained use**

To maximise expression of the fusion gene in *E. coli*

**Recipient or parental organism**

*E. coli* K12 and B strain derivatives

**Host/vector system**

- Disabled *E. coli* K12 derivatives JM109(DE3), AD494(DE3), HMS174(DE3), Origami(DE3), E104, TG1, BL21(DE3)
- Disabled *E. coli* B strain (BL21) derivatives BL21(DE3), BL21trx(DE3)
- Disabled *E. coli* B strain (Tuner) derivatives NovaBlue(DE3), Rosetta(DE3), Tuner(DE3)
- Mobilisation defective pBR322 derivative
- Mobilisation defective pET21A derivative

**Origin & function**

The gene encoding the fusion protein is derived from human, synthetic and plant sequences.

**Evaluation of foreseeable effects**

If expressed within the human body the fusion protein has the potential to be toxic to human cells. In the worst case scenario, cells expressing the protein would colonise the human gut and produce toxin at a toxic level. The following factors reduce the possibility of this eventuality:
1) The E. coli strains to be used are disabled strains unlikely to survive outside the culture vessel. It should be noted, however, that the work will involve a number of BL21-derived strains. Though recent research indicates that these strains can now be considered disabled for risk assessment purposes (Chart et al, 2000)), these strains may present a higher colonisation potential (ACGM Newsletter 30).

2) The expression plasmids involved are mobilisation defective and unlikely to transfer to a colonising E. coli strain.

3) The fusion gene will be under the control of tightly regulated promoters:
   * The araB promoter requires arabinose and the product of the araC gene to initiate expression.
   * The T7 promoter requires T7 RNA polymerase to initiate expression, the polymerase being encoded on a lysogenised DE3 phage sequence. The T7 polymerase gene is in turn under the control of the lacUV5 promoter inducible by lactose or IPTG and would be unlikely to express in the gut. Also, in the event of transfer to a colonising E. coli strain, the mechanism for expression would not be present.

4) The plasmid vectors to be used confer resistance to ampicillin and tetracycline. Alternative prophylactic antibiotics are readily available for treatment.

<table>
<thead>
<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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<tbody>
<tr>
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<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
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<tbody>
<tr>
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<thead>
<tr>
<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
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</thead>
<tbody>
<tr>
<td>Disposal. All contaminated solid waste, eg agar plates and contaminated plastics, will be autoclaved prior to disposal. Liquid waste will be autoclaved and pH-treated (kill tank) before disposal. Contaminated 'sharps' such as tips, pipettes, needles and inoculating loops will be discarded into a sharps container and autoclaved prior to collection by a registered specialist disposal company for incineration.</td>
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<table>
<thead>
<tr>
<th>Testing</th>
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<tr>
<td>Post-sterilisation samples will be taken from all waste streams at monthly intervals and plate-tested for viable organisms. We expect no viable organisms to remain after sterilisation.</td>
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<th>Tick if you are claiming exemption from disclosure for section of the risk assessment</th>
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<tbody>
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The attached risk assessments were discussed at a meeting of the GMSC on 9 January 2004. The GMSC agrees with the proposed containment level and with the precautions to be taken to safeguard human health.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Animal Units**

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<th>Human Clinical Applications</th>
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**Project Ref 833/05.1**

<table>
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<tr>
<td>05/09/2005</td>
<td>Culture of c-mycER (TM) transformed human stem cell lines</td>
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<table>
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<tr>
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<tbody>
<tr>
<td>Class 2</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements

**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to develop intermediate-scale methods for the culture and cell banking of human stem cell lines. No GMMs will be constructed by Angel Biotechnology during this project. Instead, the project will involve the culture of GM human stem cell lines that have been produced by a collaborating company in Guildford.

**Recipient or parental organism**
The GM stem cell lines were derived by ReNeuron from a variety of human tissues including brain, pancreas and liver.

**Host/vector system**

The derivation process involved immortalising the cells by infecting them with replication-defective murine leukaemia virus (MLV)-based retrovirus vectors carrying a recombinant 'conditional' oncogene, c-mycER. The retrovirus vectors were pLNC-cmycER and pBABEpuro-cmycER, derived from pLNCX (Clontech) and pBABEpuro respectively.

**Origin & function**

The C-mycER gene is a genetically engineered oncogene, composed of sequences from the human c-myc proto-oncogene and the mouse oestrogen receptor gene. (An oncogene is a gene whose function is to trigger cell growth). The encoded protein is only functional in the presence of the synthetic steroid 4-hydroxy tamoxifen (4-HT).

Stem cells are usually difficult to grow culture and it is usually necessary to add 'feeder cells' to them to support their growth. However, stem cells that carry the c-mycER gene can be stimulated to grow without feeder cells by adding 4-HT to the cell culture medium. As their growth is totally dependent on the continual presence of 4-HT in the medium, these stem cell lines are described as being 'conditionally immortal'.

**Evaluation of foreseeable effects**

The MLV retrovirus vectors that were used to introduce the c-mycER gene into the stem cells are replication-defective because they don't carry any retroviral structural protein genes. However, they do possess retroviral LTR and packaging sequences, so it is possible that they could be mobilised as replication-defective virus particles if the GM stem cells were to become infected with a replication-competent retrovirus that had 'helper' properties. The production of a replication-competent virus carrying c-mycER gene to a full set of retrovirus structural genes would not favour efficient packaging of a recombinant genome. Even if such a recombinant virus were to be created, its pathogenic properties should not be enhanced because of the requirement for 4-HT for c-mycER function. If a breach in containment took place and the GM stem cells were released into the environment they would not survive long enough for infection with potential helper viruses to take place. Retrovirus particles themselves do not survive well in the environment and require close contact between hosts for transmission to occur. Consequently, if a containment breach happened after the stem cells had been accidently infected with a helper virus, it is unlikely that any 'mobilized' c-mycER virus particles would survive long enough or in sufficiently high titres to be transmitted to new hosts in the environment.

Although considered to pose no risk to the environment because of their extreme sensitivity to adverse culture conditions, the GM stem cells may pose a risk to human health because they were derived from primary human tissue, so the possibility exists that the cells carry on as-yet-undiscovered infectious agent. For this reason the work will be carried out at Containment level 2 using a class 2 safety cabinet when necessary.

With regards to risks arising from the genetic modification of the stem cells, the 'conditional' oncogenic property of the c-mycER gene is dependent on 4-HT being added to the cell culture medium. 4-HT is not a natural compound and it is not found in vivo unless it is being deliberately administered for therapeutic reasons. Therefore under normal circumstances (ie in normal, healthy individuals) the c-mycER gene will not make the cells more invasive in vivo than their non-GM equivalents. The stem cells will be unable to colonize laboratory workers who have normal, non-suppressed immune systems because the cells will be recognised as foreign, and rejected.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Disposal.**

All cell culture-contaminated solid waste, and most liquid waste will be autoclaved at 121 degrees C (15lb) for 30 minutes prior to disposal. Some culture supernatants may be mixed with hyperchlorite to a final concentration of 5% and left for a minimum of 1 hour at room temperature before disposal via the waste water drain. Any spillages will...
be treated either with hyperchlorite solution or Virkon. Liquid disposed of via the waste water drain is collected in a kill tank and pH treated prior to release into external drains.

Plastic graduated and Pasteur pipettes, and pipette tips will be soaked in hyperchlorite solution before removal from the class 2 safety cabinet, then autoclaved as above prior to disposal.

Testing
Two internal probes are used to record the chamber temperature and load temperature of the autoclave. Post-sterilisation samples will be taken at three monthly intervals to check for viable organisms. We expect no viable organisms to remain after sterilisation.

The attached risk assessment was discussed at a meeting of the Angel Biotechnology GMSC on 30th June 2005. The GMSC agrees with the proposed containment level and with the precautions to be taken to safeguard human health.

**Project Containment**

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<th>Growth Rooms</th>
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<td>Human Clinical Applications</td>
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**Project Ref** 833/06.1

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<tbody>
<tr>
<td>12/10/2006</td>
<td>CULTURE OF CANCER-SPECIFIC T CELLS AND CANCER CELLS</td>
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**Date Project Ceased** 02/03/2022
### Project Additional Information

**Purposes of the contained use**

The aim of the project is to develop large scale culture processes for a human recombinant T cell line, and to establish bioassays using a recombinant cancer cell line to measure T cell function. No GMOs will be constructed by Angel Biotechnology during this work. Instead, the project will involve the culture of GM cell lines that have been obtained either from a client company, a research institute, or from a national cell culture collection.

**Recipient or parental organism**

- The parental T cell line originated from a biopsy taken from a patient suffering from cutaneous T cell lymphoma.
- The parental cancer cell line originated from a patient suffering a non-lymphoid malignant disease.

**Host/vector system**

- The process of generating the recombinant T cell line involved transducing the parental T cell line with a replication-defective Moloney murine leukemia virus (MMLV) retroviral vector (MMLV phi+packaging signal, mobilisation defective).
- The recombinant cancer cell line was produced by transfecting the parental cancer cell line with the plasmid pcDNA3.

**Origin & function**

- **MMLV vector**
  
  Instead of MMLV structural protein genes, this vector carries a gene for a human T cell receptor (TCR) alpha-chain, and a gene for a human TCR beta-chain. Each gene is under the control of a separate constitutive promoter element to drive gene expression. When expressed in the T cells, the alpha and beta chains associated to form functional TCR proteins on the cell surface. The vector also carries a neomycin resistance gene that gives the transduced cells resistance to the antibiotic G418.

- **pcDNA3 vector**
  
  This vector is a commercially available plasmids expression vector into which MHC class I molecule gene has been cloned. The MHC gene is under the control of a constitutive promoter element derived from human cytomegalovirus (CMV). Cancer cells transfected with this vector express the MHC molecule on their surface. As in MMLV vector above, pcDNA3 also carries a neomycin resistance gene that gives transfected cells resistance to the antibiotic G418.

**Evaluation of foreseeable effects**

The recombinant T cells are CD8+ and have cytotoxic properties. The TCR protein they express (via the MMLV vector) on their surface gives them the ability to recognise...
and kill the recombinant cancer cells which express the human MHC molecule.

The MMLV retrovirus vector that was used to introduce the TCR genes into the T cells is replication-defective because it does not carry any retroviral structural protein genes. However, it does possess retroviral LTR and packaging sequences, so it is possible that it could be mobilized as replication-defective virus particles if the recombinant T cells were to become infected with a replication-competent retrovirus that had ‘helper’ properties. The production of a replication-competent virus carrying the TCR genes in this situation is unlikely because the increase in virus genome size that would be caused by linkage of the TCR genes to a full set of retrovirus structural genes would not favour efficient packaging of a recombinant genome. Even if such a recombinant virus were to be created, its pathogenic properties should not be enhanced because TCR proteins are not toxic per se. They are naturally occurring proteins that all humans possess on their T cells. If a breach in containment took place and the recombinant T cells were released into the environment they would not survive long enough for infection with potential helper viruses (e.g., from mice) to take place. Retrovirus particles themselves do not survive well in the environment and require close contact between hosts for transmission to occur. Consequently, if a containment breach happened after the T cells had been accidentally infected with a helper virus, it is unlikely that any ‘mobilized’ TCR-positive virus particles would survive long enough or in sufficiently high titres to be transmitted to new hosts in the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All work involving these cell lines will be performed in a class II biological safety cabinet and any manipulations (centrifugation etc.) will be sealed containers, sterile-vented tissue culture flasks or sterile-vented bioreactors* (8T-cells only). No sharps will be used and all pipettes will be plastic, single-use disposables. The laboratory suite is Class II and HEPA-filtered, all workers will don laboratory coats, latex gloves, overshoes and mob caps that are to be removed on leaving the room. All intended workers are vaccinated against Hepatitis B and persons with open wounds will not be allowed access to the facility. We have in place SOPs for use and rotation of disinfectants, regular cleaning of the Class II suite and spillage procedures. All waste will be autoclaved on site. Please refer to Angle Biotechnology GM risk assessment 0065 for specifics.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Small volumes of liquid waste (e.g. spent culture medium) and disposable pipettes and pipette tips will be added to a bleach solution so that the final concentration does not fall below 5% v/v. After a 24 hour incubation period the liquid waste will be disposed of via the laboratory sinks and the plastic disposables transferred to an autoclave bag and autoclaved on site.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment
<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
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</tr>
<tr>
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#### Contact Information

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### Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee shall consist of the Principal Scientist and a Director of the Company. It shall meet on a Quarterly basis, or at such times as are necessary to monitor and update internal processes concerning genetic manipulation with reference to health, safety and environmental impact. It shall be responsible for ensuring that the Company and its Personnel comply with all national and local rules, regulations and statutory requirements. It shall ensure that it also liaises with and complies with all rules and regulations established by the Genetic Modification Safety Committee of the University of Wales College of Medicine. The Committee shall be responsible for the assessment of the genetic modification of GMMs requested by its Clients and to maintain all such information in strict confidence, due to commercial sensitivity and issues relating to the intellectual Property Rights of both the Company and its Clients.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>
Management of waste conforms to the essential rules for GM work guidelines as issued by Health & Safety. Liquid waste management involves the use of sodium dichloroisocyanurate (Chloros, Precept)) in the form of tablets or solutions for the disposal of liquid cultures. Solid waste management involves a chloros soak, autoclaving, and/or incineration. Ultimate form of waste would exist as incinerated debris (solid) or chlorinated liquid waste. The expected degree of kill is 100% and will be validated routinely by assessment of growth in appropriate prokaryotic or eukaryotic culture media.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Management of waste conforms to the essential rules for GM work guidelines as issued by Health & Safety. Liquid waste management involves the use of sodium dichloroisocyanurate (Chloros, Precept) in the form of tablets or solutions for the disposal of liquid cultures. Solid waste management involves a chloros soak, autoclaving, and/or incineration. Ultimate form of waste would exist as incinerated debris (solid) or chlorinated liquid waste. The expected degree of kill is 100% and will be validated routinely by assessment of growth in appropriate prokaryotic or eukaryotic culture media.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The idENTIGEN Genetic Modification sub-committee agree to the risk assessment proposed for the programme of work entitled: Construction of eukaryotic expression vectors containing inserted cDNAs encoding intracellular, membrane-bound and extracellular proteins. The recommendations of the Genetic Modifications sub-Committee are in agreement with the proposal for the classification of the work as Group 1, containment level 1, whereby it is noted that all manipulations undertaken with vector/eukaryotic host are performed in a containment level 2 environment.
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Date at Which Additional Info Submitted

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [Y]

Give brief details of the genetic modification safety committee

The F2G Ltd GMSC is a sub-committee of the main H&S committee which reports to the F2G board directly.

1. Composition:- Company Director, BSO, H&S Coordinator (chairperson), H&S Officer (liaises between GMSC and main H&S committee), Office Representative, Technical Representative, Post Doctoral Representative, Fire Officer, First Aid Officer, External Representative (University of Manchester BSO).
2. Operating Procedures:- (a) advise on risk assessments relating to activities involving GMO’s (Regulation 6 - Contained Use Regulations 2000); (b) advise on risk assessments relating to the environment (required under the EPA); (c) review safety, training and lab discipline; (d) review accidents and incidents; (e) all members views to be heard so that advice given is genuinely that of a committee and not a single individual (f) meeting minutes, progress and achievements to be monitored, published and reviewed regularly by senior management at board meetings.
3. Frequency of Meetings:- Meetings to be held every 6 months.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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<td>Other (please specify)</td>
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<td>Tick if confidential</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The F2G Limited GMSC, at a meeting held on 19/2/2002, endorsed the decision that this project requires Containment Level 2 facilities that are available in laboratories 2N09 and 2N07 (second floor, Manchester Incubator Building). No special problems were identified that could not be adequately controlled by level 2 containment methods and by the application of good microbiological practice and good occupational health and hygiene practices. It was noted that Group II organisms were involved and that HSE approval was required before work could start. The forms CU1 2000, CU2 2000 and the attached risk assessment were examined in detail and it was agreed by all members that the application process was to proceed.

Project Ref 836/02.1

Date Ackn'd 17/06/2002

Date Project Ceased

CU2 Project Title INSERTIONAL MUTAGENESIS OF ASPERGILLUS SPECIES (INCLUDING ASPERGILLUS FUMIGATUS)

Class 2

Consent Granted not applicable

Project notified under transitional arrangements N
Aspergillus fumigatus is an opportunistic pathogen of humans. Immunocompromised individuals such as premature babies, cancer sufferers, transplant patients and AIDS sufferers are at risk from invasive aspergillosis which is often fatal. This project aims to aid the drug screening process used to develop novel anti-fungal compounds. A fumigatus will be transformed using an insertional mutagenesis approach to identify essential genes and genes critical for growth and virulence. The genes knocked out of these strains will be identified using the inserted sequence as a tag to identify the flanking gene sequence.

Recipient or parental organism

The parental organism of the host strains to be used in these experiments is the A. fumigatus wild-type clinical isolate AF293 (available to the public from the NCPF repository (Bristol, UK) and the CBS repository (Belgium)) used in the A. fumigatus genome sequencing project at TIGR (see http://www.tigr.org/tdb/e2k1/afu1). This wild-type parental strain will not be used directly in any experiment, only multiple auxotrophic mutants will be used.

Host/vector system

We will use an engineered plasmid pMB3 based on the cloning vector pCR2.1 which contains the A. fumigatus pyrG gene as a selective marker, bacterial antibiotic resistance genes and a bacterial origin of replication. This plasmid will be linearised and used to transform (in a random integration manner) an A. fumigatus AF293 diploid (niaD+/niaD-/cnx+/cnx-/pyrG-/pyrG-/pabaA-/pabaA-) Insertion of this linearised plasmid DNA into the host genome can be screened for by a change in the phenotype from pyrG- to pyrG+. The host gene or non-coding region of DNA which has been disrupted, can then be identified by plasmid rescue and a relationship established between gene and phenotype.

Evaluation of foreseeable effects

Insertion of the foreign DNA into the diploid host genome will allow the diploid transformant to overcome its pyrG auxotrophy. This does not restore full wild-type activity due to a gene dosage effect (only one copy of the functional pyrG gene is active instead of two) and in this case the resultant transformant would be less pathogenic than the parental wild -type clinical isolate. Due to the cripling PABA mutation these diploid transformants would only grow on media supplemented with PABA.

These insertions/modifications are expected to have an adverse effect on the fungus, reducing its fitness. Insertion of a large piece of transforming DNA (in this case 5.8 kb) into an active gene will in most cases lead to the disruption of that gene's function (by halting transcription and therefore stopping expression of the gene product) and
would in turn reduce its survivability/function in the body or the environment. However, as the studies are aimed at elucidating the function of genes, we cannot be absolutely certain that all modification will lead to a less harmful fungus (Smith et al., 1996) although the fitness of these mutants in the environment will be severely reduced by their absolute requirement for PABA in the growth medium.

A. fumigatus is readily isolated from most environmental locations (air, soil and leaf litter) and does not have an adverse effect on the environment. Indeed it has an important role to play in the degradation of environmental wastes ie leaf litter, decaying organic matter etc. Although wild-type A. fumigatus has no adverse effects on plants in the environment it is sometimes found as an opportunistic pathogen of mammals (ie dogs) and birds (ie parrots in captivity and falcons) and causes similar symptoms to those found in humans, affecting ly immunocompromised animals.

In the event of a breach of containment, a transformed diploid GMM would not be viable in the environment or indeed in the human body in the absence of PABA supplementation.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable - no transgenics involved.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

This application covers a laboratory scale project where F2G Ltd applies the principles of good microbiological practice and good occupational health and hygiene practices.

Disinfection with % aqueous Virkon solution is used for spillages, contaminated plasticware and laboratory surface decontamination. Degree of kill (should always be 100%) is measured by plating out samples from known concentrations of fungal inoculum after being subjected to a standard method of Virkon decontamination. This decontaminated plasticware is then autoclaved as detailed in the following section as a double safeguard measure. The ultimate form of this type of waste is decontaminated plasticware which is placed in clinical waste bags, the contents are tagged and recorded in a log book and the bags are then sent to be incinerated by a registered clinical waste disposal service. Full written records regarding autoclave settings, degree of kill and clinical waste disposal via incineration are kept.

Liquid GM waste (culture supernatants containing GM fungi) and solid GM waste (agar plates containing GM fungi) is sterilised in an on-site (same floor of building) autoclave maintained and validated for GM waste sterilisation by a specialist autoclave validation company Lab3 (http://www.lab3.co.uk). The Lab3 Preventive Maintenance Contract allows us to fulfil our legal obligations and includes full servicing, validation and calibration services. All certification is available for viewing. Storage of non-inactivated waste is restricted to within the generating laboratory. The material may not be stored longer than 24 hours prior to inactivation. Degree of kill (should always be 100%) is measured by plating out samples from known concentrations of fungal inoculum after being subjected to a validated GM sterilisation autoclave cycle in different parts of the same autoclave. The ultimate form of this type of waste is decontaminated liquid waste which is cooled to allow solidification and then placed in clinical waste bags to be incinerated by a registered clinical waste disposal service. Full written records regarding autoclave settings, degree of kill and clinical waste disposal via incineration are kept.

All current and new employees who handle GM waste are trained regarding the proper handling of this type of waste.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y
The F2G Ltd Genetic Modification Safety Committee, at a meeting held on 19.02.2002 endorsed the decision that this project requires Containment Level 2 facilities that are available in laboratories 2N09 and 2N07 (second floor, Manchester Incubator Building). No special problems were identified that could not be adequately controlled by level 2 containment methods and by the application of good microbiological practice and good occupational health and hygiene practices. It was noted that Group II organisms were involved and that HSE approval was required before work could start.

**Project Containment**

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<tr>
<td>Animal Units</td>
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<td>Human Clinical Applications</td>
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<td>L2</td>
<td>L3 L4</td>
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**Project Ref 836/16.1**

- **Date Ackn’d** 19/05/2016
- **CU2 Project Title** Validation of bacterial genes essential for pathogenesis
- **Class** Class 2
- **CultureVol** < 1 Litre
- **Class CultureVol** Consent Granted
- **Project notified under transitional arrangements** N

**Project Additional Information**

- **Withdrawn** N
- **Tick if notifying a connected programme of work** N
- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

02/03/2022
Purposes of the contained use

The disruption or deletion of bacterial genes to determine the effect of knocking out specific genes on growth and virulence. Thus, identifying potential gene targets for antibiotic drug development.

Recipient or parental organism

Escherichia coli, Acinetobacter baumannii and Pseudomonas aeruginosa

Host/vector system

Cloning steps will involve laboratory strains of E. coli, such as DH5α and NEB 10β. Genes will be targeted in Escherichia coli ATCC 11775 (NCTC 9001); Acinetobacter baumannii ATCC® 19606, Pseudomonas aeruginosa ATCC® 10145 or similar strains. Vectors will be pGEMTeasy, pACD4K-C (from TargeTron KO system, Sigma), pRED-ET (Red/ET Recombination, GeneBridges), 707-FLPe (Red/ET Recombination, GeneBridges). It is likely that the final construct for transformation will be a linear piece of double stranded DNA containing sequences homologous to the gene to be targeted and an antibiotic -resistant selection marker.

Origin & function

Genes will be cloned from the target organisms: Escherichia coli, Acinetobacter baumannii and Pseudomonas aeruginosa. The genes will be disrupted and used to engineer bacteria with specific genes knocked out. The knockout strains will be tested for changes in growth and virulence.

Evaluation of foreseeable effects

The intention is to create GMMs that will likely be less fit than the parental strain as we are attempting to knockout putative antibiotic target genes. The only situation where the GMMs created could have a selective advantage over the parent organisms would be if the gene targeted was not important for growth and virulence, and at the same time the GMMs are challenged with the antibiotic used as a selection marker (ampicillin, kanamycin, zeocin) which is highly unlikely. As the parent organisms are ACDP hazard group 2 organisms capable of causing human disease the worst case scenario is that they infect a lab worker. This is highly unlikely as good microbiological procedures, personal protective equipment and disinfection, decontamination and disposal protocols are in place. A class II microbiological cabinet will be used for processes involving the possibility of aerosol creation and for large culture volumes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Plasticware contaminated with GMO’s will be decontaminated by soaking in 1% Virkon for a minimum of 24h followed by collection in Wiva bins and disposed via incineration by an approved healthcare waste specialist (currently SRCL Ltd.). Liquid waste will be decontaminated by soaking in 1% (w/v) Virkon for a minimum of 24h, prior to autoclaving or disposal in sealed containers in Wiva bins that are incinerated as above. Glassware contaminated by Class 2 GMMs will be soaked with 1% Virkon for 24h, then autoclaved before washing and re-use. 1% Virkon has been shown to leave no viable cfu after a 5 min exposure of 1-3 x 10^6 of E. coli and P. aeruginosa, a greater than 5-log reduction in cfu (Hernandez et al, J Hosp Infect. 2000, 46: 203-209). This rate of disinfection will be tested in house for these organisms together with A. baumannii. Autoclaving and/or incineration will ensure complete sterilisation. Thus degree of kill will be 100%.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The F2G Ltd Genetic modification safety committee met on 11th February 2016. The project covered in this application (GM risk assessment F2G016) was discussed. The committee endorsed the content of the risk assessment, agreeing to the classification as a Class 2 activity. The safety measures and associated processes (waste disposal etc) were discussed and approved during the meeting. Drafts of the RA have been distributed to the committee at various stages for comment and they approved the final version.

Please enter comments on the GM safety committee on the risk assessment

The F2G Ltd Genetic modification safety committee met on 11th February 2016. The project covered in this application (GM risk assessment F2G016) was discussed. The committee endorsed the content of the risk assessment, agreeing to the classification as a Class 2 activity. The safety measures and associated processes (waste disposal etc) were discussed and approved during the meeting. Drafts of the RA have been distributed to the committee at various stages for comment and they approved the final version.

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GM Centre Number: 838

Data Premises Notified: 26/07/2002
(Originally)

Data Premises Closed: 14/10/2005

Transferred from 1992 Regs?: N

Emergency Plan Required?: N

Non-GMMs: N

Withdrawn: N

Name
INNOVATA PLC

Name 2

Department
MED IC 4

Building

Road Name
THE SCIENCE AND BUSINESS PARK

District

Town
KEELE

County
STAFFORDSHIRE

Postcode
ST5 5NL

Country
ENGLAND

Tel Number
0845 006 0924

Fax Number
0845 006 0921

E-mail

HSE Division
MIDLANDS

Comments
Company name change from ML Laboratories Plc to Innovata Plc on 15/08/2005

Date at Which Additional Info Submitted
15/08/2005
## Premises Addresses

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<th>Name</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee is a joint committee between ML Laboratories and Cobra BioManufacturing and also acts as the biological safety committee. This committee is known as the GMBSC (Genetic Modification and Biological Safety Committee). The committee meets approximately every six weeks and consists of the following posts.

Chairman, Biological Safety Officer, Assistant Biological Safety Officer, Safety Advisor, M L Management representative, Cobra management representative, ML staff representative, Virologist, Pharmacologist.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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<th>Large Scale</th>
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<tr>
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<td>Yes</td>
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</table>

Other (please specify)  

Tick if confidential

02/03/2022
All waste generated from this work will be handled according to the in-house protocols for handling of containment level 2 waste. Liquid waste will chemically inactivated using Virkon, or other appropriate inactivating agent, as recommended by the manufacturer to achieve most effective killing (usually 1% solution). Virkon treatment has been validated by the manufacturer to inactivate all known 18 families of human and animal viruses (including Mastadenoviruses).

Solid waste is collected in a plastic 'Weaver' bin (50L) lined with a plastic bag. When full the bag is tied and the lid secured to seal the box. The box is externally disinfected and then transferred to a designated 820L wheeled waste unit. The wheeled waste unit is collected by White Rose Environmental and handled and incinerated according to their procedures for Class 2 waste. Prior to collection a completed consignment note (detailing the type of waste and the precautions taken) is faxed to the Plant Manager of the receiving incinerator. A copy of the consignment note travels with the waste and the precautions taken) is faxed to the Plant Manager of the receiving incinerator. A copy of the consignment note travels with the waste and the original is kept by the Facilities Manager at Stephenson Building (Keele University Science Park). When disposal of the waste is complete the disposers section of the consignment note is completed by the Plant Manager and faxed to the Facilities Manager. Thus a record of all disposals and confirmation of disposals is kept by us. White Rose's documentation has been audited by our Biological Safety Officer.

Local decontamination of the safety cabinets and other control and containment equipment and devices will be carried out by swabbing with 1% Virkon (as recommended by the manufacturer to achieve most effective killing) followed by 70% ethanol. Cell culture media will be immediately treated with Virkon as recommended by the manufacturer.

In the case of an accidental puncture of the skin by a contaminated sharp, the procedure is to wipe the wound with 70% ethanol and report immediately to the Medical Centre.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Genetic Modification and Biological Safety Committee (GMBSC) have received and considered this proposal and are in agreement with the classification suggested. Two example immunostimulatory genes have been described in the risk assessment but the GMBSC will require to review any other genes on a case-by-case basis and notify HSE if there is any resulting alteration to the classification.
The aim of this project is to evaluate the immune-therapeutic benefit of expressing immune-stimulating proteins in murine tumour cells killed in vivo by enzyme/prodrug combinations, namely NTR/CB1954 or Cyp1A2/acetaminophen. Immune-therapeutic effects will be studied using the mouse 4T1 breast cancer cell line in the host BALB/c mice in the first instance. The mouse TRAMP spontaneous prostate tumour model will subsequently be used.

The parental organism is a replication-defective, human Adenovirus (Ad) type 5. Human Ad are found worldwide, and over 40 different serotypes have been isolated. Ad5 is associated with mild upper respiratory tract infections in young children. Immunity to Ad infection is thought to be life-long following primary infection. Although some serotypes are closely related they nevertheless remain distinct entities suggesting that the potential for recombination in vivo is not high. Human serotypes do not naturally infect other animal species and there are relatively few reports of virus replication following inoculation of Ad into experimental animals. There is no evidence that human Ad cross species and so a replication defective virus could not be maintained in non-human hosts. The replication-defective vector is not expected to be harmful. The potential hazard to the environment, from inadvertent release of the quantities of virus expected to be handled, is extremely low.

The recipient cell line sued to produce the virus is the human PER.C6 cell line (Fallaux et al., Human Gene Therapy, 1998,9: 1910-1917). This E1-containing cell line is engineered to avoid recombination between the vector genome and the viral sequences present in the genome of the producer cells and thereby RCAs (Replication-Component Adenoviruses) should not be generated at significant levels.

The final constructs will express the immune stimulatory molecules along or in combination with either bacterial NTR or rodent/human Cyp1A2. For additional safety reasons, the immunostimulatory gene will be cloned into the E1 region. In a case of recombination in vivo (to generate a replication competent virus) this will result in the...
The NTR or Cyp1A2 cDNA can be inserted into E1 or E3. In case of recombination in vivo, to generate a replication competent virus, by a virus containing NTR or Cyp1A2 in the E3 region the recombinant virus would still contain the NTR cDNA or the Cyp1A2 cDNA but there is no obvious reason that these viruses could be more dangerous than an E1-recombined virus that does not contain one of these two cDNAs in E3. This is a justified assumption because the gene products NTR and Cyp1A2 are not toxic by themselves to the human cells. There are no native substrates so far described in the human organism for NTR. Moreover, Drabek et al. (Gene Therapy, 1997, 4:93-100) showed that transgenic mice expressing NTR in T cells are completely healthy and give no changed phenotype.

Cyp1A2 is constitutively expressed in human liver and is induced to higher levels if exposed to exogenous compounds like aromatic hydrocarbons and halogenated hydrocarbons. Endogenous substrates include steroids, fatty acids, prostaglandins and vitamins (Quattrochi et al., 1994, JBC 269:6949-6954). Therefore if a replication-competent virus expressing Cyp1A2 should infect cells, the Cyp1A2 would only convert its natural occurring substrates which are anyway converted by Cyp1A2 already present in the cell or by redundant CytochromeP450-dependent enzymes.

Finally, as all the prepared viruses are deleted for the E3 proteins all generated viruses are considered to be much more immunogenic as E3 proteins help to escape the host immune response against the infected cell (eg MHC class 1 down regulation, inhibition of TNFalpha-mediated effects). In agreement with this, the likelihood that these viruses will persist in the human population should be much lower, again because they are much more immunogenic and therefore the effective production of viral progeny should be significantly decreased.

**Host/vector system**

The vector system is a replication-defective human adenovirus type 5 (E1 and E3 deleted). The host cell line is PER.C6 (Fallaux et al., Human Gene Therapy, 1998, 9:1910-1917).

**Origin & function**

Murine immune stimulatory genes will be generated by PT-PCR from mouse splenocytes or from commercially available sources with immune modulating activity in humans.

**Evaluation of foreseeable effects**

Ad expressing xenogeneic proteins such as a bacterial nitroreductase or a mouse cytokine that is inactive in humans may be immunogenic. It is possible that the Ads expressing a mouse cytokine that is active in man (ie that can bind the equivalent human cytokine receptor) could give additional immunogenicity, eg a local inflammatory response. However, this would be very limited and cleared rapidly in most humans, since the great majority of people have antibodies to Ad coat proteins through exposure to the virus in early childhood. Human cytokines have been evaluated in clinical studies and only show toxicity with systemic administration of high levels.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated from this work will be handled according to the in-house protocols for handling of containment level 2 waste. Liquid waste will chemically inactivated using Virkon, or other appropriate inactivating agent, as recommended by the manufacturer to achieve most effective killing (usually 1% solution). Virkon treatment has been validated by the manufacturer to inactivate all known 18 families of human and animal viruses (including Mastadenoviruses).

Solid waste is collected in a plastic 'Weaver' bin (50L) lined with a plastic bag. When full the bag is tied and the lid secured to seal the box. The box is externally...
disinfected and then transferred to a designated 820L wheeled waste unit. The wheeled waste unit is collected by White Rose Environmental and handled and incinerated according to their procedures for Class 2 waste. Prior to collection a completed consignment note (detailing the type of waste and the precautions taken) is faxed to the Plant Manager of the receiving incinerator. A copy of the consignment note travels with the waste and the original is kept by the Facilities Manager at Stephenson Building (Keele University Science Park). When disposal of the waste is complete the disposers section of the consignment note is completed by the Plant Manager and faxed to the Facilities Manager.

Thus a record of all disposals and confirmation of disposals is kept by us. White Rose’s documentation has been audited by our Biological Safety Officer.

Local decontamination of the safety cabinets and other control and containment equipment and devices will be carried out by swabbing with 1% Virkon (as recommended by the manufacturer to achieve most effective killing) followed by 70% ethanol. Cell culture media will be immediately treated with Virkon as recommended by the manufacturer.

In the case of an accidental puncture of the skin by a contaminated sharp, the procedure is to wipe the wound with 70% ethanol and report immediately to the Medical Centre.

The Genetic Modification and Biological Safety Committee (GMBSC) have received and considered this proposal and are in agreement with the classification suggested. Two example immunostimulatory genes have been described in the risk assessment but the GMBSC will require to review any other genes on a case-by-case basis and notify HSE if there is any resulting alteration to the classification.

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Project Ref 838/02.2

Date Ackn’d CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
# Rescue and Propagation of Recombinant Ovine Adenovirus

## Project Additional Information

### Purposes of the contained use
To rescue recombinant ovine adenovirus (OAV) from a plasmid in an ovine cell line, and its use to transduce human dendritic cells to achieve transgene expression in vitro for immunological assay only.

### Recipient or parental organism
The recombinant OAV will bear transgenes for either the fluorescent reporter protein, eGFP, or for the human melanoma-associated proteins, MART1 and gp100. In all cases, expression will be governed by the IECMV promoter. OAVs are capable of infecting human cells but have been shown to be unable to complete their full cycle of replication (Khatri, A et al [1997]: Virology 239 p.226-237), resulting in an abortive infection. Infection is still abortive in the presence of co-infected human adenovirus suggesting minimal risk to the worker when propagating this virus. It is expected however that there will be an immune response in humans on exposure to this virus.

### Host/vector system
A plasmid that contains the whole OAV viral genome, and is therefore a GMM once modified, though of similar or lower risk to the parental virus. The host cells and viral vector are both ovine and the virus is incapable of effective replication in humans.

### Origin & function
Virus and cell line are ovine. The cell line is to allow efficient propagation of the recombinant ovine virus. The virus encodes the genes for either a fluorescent reporter protein (eGFP), or a human gene derived from melanocytes, immunity to which is associated with protection from melanoma. It is intended to deliver the transgene via the OAV transduction vector to human dendritic cells, which is an infective but non-replicative action. Dendritic cells so infected will then be evaluated for transgene expression, and for the presentation of the melanoma-associated antigens to T-cells derived from either healthy volunteers, or patients suffering from melanoma.

### Evaluation of foreseeable effects
OAV is capable of infecting human cells, but it has been shown to be unable to complete its full replication cycle (Khatri, A et al [1997]: Virology 239 p.226-237), resulting in an abortive infection. OAV has also been shown to replicate abortively in human cells, which are co-infected with human adenoviruses. The abortive replication cycle of these viruses suggests that there is minimal risk to the worker when propagating these viruses. However, due to the lack of pre-existing immunity of the worker to OAV, there will be limited immunity on first exposure to the virus. Subsequent exposure would result in a strong immune response to the virus.
The plasmid DNA containing the full OAV genome will be used to rescue the virus under segregated conditions. As the plasmid contains the same genetic material as the intact virus, it is highly unlikely that the plasmid would be of greater risk to the worker than the virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The virus will only be handled in class II microbiological safety cabinets that will be swabbed with a viricide (eg Virkon or Klearcide) after use. All contaminated materials will be treated by chemical means (eg 1% Virkon solution, which is proven to destroy both Mastadenoviruses and Aviadenoviruses). Solid waste is collected in a plastic 'Weaver' bin (50L) lined with a plastic bag. When full the bag is tied and the lid secured to seal the box. The box is externally disinfected and then transferred to a designated 820L wheeled waste unit. The wheeled waste unit is collected by White Rose Environmental and handled and incinerated according to their procedures for Class 2 waste. Prior to collection a completed consignment note (detailing the type of waste and the precautions taken) is faxed to the Plant Manager of the receiving incinerator. A copy of the consignment note travels with the waste and the original is kept by the Facilities Manager at Stephenson Building (Keele University Science Park). When disposal of the waste is complete the disposers section of the consignment note is completed by the Plant Manager and faxed to the Facilities Manager. Thus a record of all dispossals and confirmation of disposals is kept by us. White Rose's documentation has been audited by our Biological Safety Officer.

Local decontamination of the safety cabinets and other control and containment equipment and devices will be carried out by swabbing with 1%Virkon (as recommended by the manufacturer to achieve most effective killing) followed by 70% ethanol. Cell culture media will be immediately treated with Virkon as recommended by the manufacturer.

In the case of an accidental puncture of the skin by a contaminated sharp, the procedure is to wipe the wound with 70% ethanol and report immediately to the Medical Centre.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC agree that class 1 would normally have been appropriate for the protection of human health, but as this virus can cause mild infection in sheep it becomes a class 2 activity to ensure protection of sheep in the environment.

Project Containment

| Laboratory Activities | Glass Houses | Growth Rooms |

02/03/2022
Project Additional Information

**Purposes of the contained use**

To produce replication incompetent amphotropic murine leukaemia virus using packaging cell lines which express heterologous envelope proteins that will extend the tropism of the virus to human cells and to a broad range of mammalian cells.

**Recipient or parental organism**

The recombinant virus will be produced using the "Ampho Pack-293" or "Retro Pack PT67" packaging cell lines (www.clontech.com) which express heterologous envelope proteins ("4070A" or "10A1") that will extend the tropism of the virus to human cells. Since MuLV is an integrating virus there is the potential for insertional mutagenesis. The virus will be replication incompetent. There is however a theoretical risk of contamination with low levels of replication competent retrovirus (RCR), generated during the packaging process. The recombinant virus will have a wide tropism and thus presents the same hazards as above to other mammals.

**Host/vector system**

The Retro Pack PT67 packaging cell line is a mouse fibroblast (NIH-3T3-derived line. Ampho Pack-293 is a human embryonic kidney (HEK 293) - derived cell line. The vector is cloned into a non-mobilisable plasmid backbone. Viral components in the plasmid are all under eukaryotic promoters and therefore will not be expressed in...
bacteria. The gag/pol and env genes are not present in the vector and therefore there is no possibility of viral production by transfection of cells with the plasmid alone. The virus produced is murine but will express a heterologous envelope protein that will extend the tropism to a broad range of mammalian cells.

Origin & function

The packaging cell lines will be of mouse or human origin. The cell lines contain stably integrated copies of gag, pol and env genes necessary for particle formation and replication. The lines were created by sequential stable transfection resulting in the integration of gag/pol and env genes at different locations thus minimising the chance of producing replication competent virus.

The vector is cloned into a non-mobilisable plasmid backbone. The vector contains the packaging signal +, transcription and processing elements required for virus production. These components are erived from murine leukaemia virus.

The final GMM will express a reporter gene (β-galactosidase, EGFP or luciferase) from the ubiquitous CMV promoter/enhancer downstream of a DNA fragment encompassing portions of the bidivergently transcribed RNP1A2 and HP1HY genes (“UCOE”). No additional hazard would be predicted due to the expression of these transgenes since they encode innocous proteins that have been widely used to measure promoter activity in both cultured cells and in vivo (expression in all tissues throughout the life of transgenic animals has no detectable pathological effect).

Evaluation of foreseeable effects

Infection of humans could potentially result in an insertional inactivation event:

The vector will be of the self-inactivating variety (“SIN”) preventing activation of genes adjacent to the integration site by the viral LTRs. However, there will be the potential for activation of adjacent genes by the UCOE element. This may occur over a large distance (extent is currently unknown).

Thus the detrimental consequences of provirus insertion would be a contribution to tumorigenesis through either the inactivation of a tumour suppressor gene, such as p53, or the activation of a dominant oncogene by the UCOE element.

However, tumour suppressor gene inactivation would leave one allele intact, except for the minority of individuals carrying a defective allele.

Furthermore, integration will only occur in cycling cells further reducing the chance that exposure will result in an

Finally, infected cells will express a reporter gene encoding a foreign protein and would be expected to be eliminated by the immune system in the majority of cases.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated from this work will be handled according to the in-house protocols for handling of containment level 2 waste.

Liquid waste will chemically inactivated using Virkon, or other appropriate inactivating agent, as recommended by the manufacturer to achieve most effective killing (usually 1% solution). Virkon treatment has been validated by the manufacturer to inactivate all known 18 families of human and animal viruses (including Mastadenoviruses). Solid waste is collected in a plastic ‘Weaver’ bin (50L) lined with a plastic bag. When full the bag is tied and the lid secured to seal the box. The box is externally disinfected and then transferred to a designated 820L wheeled waste unit. The wheeled waste unit is collected by White Rose Environmental and handled and incinerated according to their procedures for Class 2 waste. Prior to collection a completed consignment note (detailing the type of waste and the precautions taken) is faxed to the plant manager.
of the receiving incinerator. A copy of the consignment note travels with the waste and the original is kept by the Facilities Manager at Stephenson Building (Keele University Science Pak). When disposal of the waste is complete the disposers section of the consignment note is completed by the Plant Manager and faxed to the Facilities Manager. Thus a record of all disposals and confirmation of disposals is kept by us. White Rose's documentation has been audited by our Biological Safety Officer.

Local decontamination of the safety cabinets and other control and containment equipment and devices will be carried out by swabbing with 1% Virkon (as recommended by the manufacturer to achieve most effective killing) followed by 70% ethanol. Cell culture media will be immediately treated with Virkon as recommended by the manufacturer.

In the case of an accidental puncture of the skin by a contaminated sharp, the procedure is to wipe the wound with 70% ethanol and report immediately to the Medical Centre.

According to the ACGM compendium of Guidance amphotropic replication defective retrovirus' containing a non-harmful insert should not need additional containment over containment level 1 (Part 2B - Annex III paragraph 30). Thus this risk assessment could be considered to be containment level 1. However, due to the presence of the UCOE and a lack of certain knowledge of the distance at which it may act (although anecdotal data suggests this is not great) the possibility of ongogene activation by insertional mutagenesis MAY be increased). Thus, as a precautionary measure the committee feels containment level 2 is appropriate for this activity.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

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Project Containment

Project Ref 838/02.4

Date Ackn'd 08/10/2002
CU2 Project Title GENERATION OF E1/E3-DELETED, EXCLUSIVE REPPLICATION-DEFICIENT AD5
Class 2
Culture Vol Class 2 < 1 litre

02/03/2022
The aim of this project is to evaluate the therapeutic benefit of expressing a parasite derived immune-stimulating protein in murine tumour cells to enhance anti-tumour immunity, resulting in the in vivo death of the tumour. Immune-therapeutic effects will be studied using the mouse 4T1 breast cancer cell line in the BALB/c mouse in the first instance.

The parental organism is a replication-defective, human adenovirus (Ad) type 5. Human Ad are found worldwide, and over 40 different serotypes have been isolated. Ad5 is associated with mild upper respiratory tract infections in young children. Immunity to Ad infection is thought to be life-long following primary infection. Although some serotypes are closely related they nevertheless remain distinct entities suggesting that the potential for recombination in vivo is not high. Human serotypes do not naturally infect other animal species and there are relatively few reports of virus replication following inoculation of Ad into experimental animals. There is no evidence that human Ad cross species and so a replication defective virus could not be maintained in non-human hosts. The replication-defective vector is not expected to be harmful. The potential hazard to the environment, from inadvertent release of the quantities of virus expected to be handled, is extremely low. The recipient cell line used to produce the virus is the human PER.C6 cell line (Fallaux et al., Human Gene Therapy, 1998, 9: 1910-1917). This E1-containing cell line is engineered to avoid recombination between the vector genome and the viral sequences present in the genome of the producer cells and thereby RCAs (Replication-Component Adenoviruses) should not be generated at significant levels.

The final constructs will express the immune stimulatory molecule from the E1 locus. In a case of recombination in vivo (to generate a replication competent virus) this will result in the deletion of the immunostimulatory gene cassett in E1. finally, as all the prepared viruses are deleted for the E3 proteins, all generated viruses are considered to be much more immunogenic as E3 proteins help to escape the host immune response against the infected cell (e.g. MHC class I down regulation, inhibition of TNFalpha-mediated effects). In agreement with this, the likelihood that these viruses will persist in the human population should be much lower, again because they are much more immunogenic and therefore the effective production of viral progeny should be significantly decreased.

The vector system is a replication-defective human adenovirus type 5 (E1 and E3 deleted). The host cell line is PEr.C6 (Fallaux et al., Human Gene Therapy, 1998, 9: 1910-1917).
Total parasite mRNA will be sourced via an academic collaboration. A cDNA encoding the open reading frame for the immunostimulatory factor will be generated by standard RT-PCR techniques. A cDNA for a protein associated with the induction of apoptosis will be generated by RT-PCR from mouse splenocytes. All expression cassettes will be inserted into the E1 locus of Ad5.

**Evaluation of foreseeable effects**

It cannot be excluded that accidental incorporation of even very small amount of Ad vector expressing immune-modulating proteins could result in a local inflammatory response that is enhanced compared to exposure with an empty virus, or a virus expressing a non-hazardous foreign protein. Local and immediate inflammatory responses should be cleared relatively fast as most humans have been exposed to Ad5 infection in early childhood, and have low-to-high serum antibody levels against Ad5 hexon and fibre protein. The immunostimulating factor is a foreign protein that will invoke a neutralising antibody response, which is capable of inhibiting its pro-inflammatory activity, thus limiting any longterm effect on the host. A fusion construct incorporating elements of a molecule associated with the induction of apoptosis will not contain the native extra-cellular domains associated with the ligation of the protein by its native ligand. Therefore, all biological effects will be dependent upon the behaviour of the parasite derived moiety, and hence the fusion protein does not represent any greater risk than the soluble native parasite material.

As with other replication-defective Ad constructs, any inadvertent generation of replication-competent virus, or helper virus, in the viral stocks would be readily apparent from the appearance of typical cytopathic effects in culture. The effects on the operator of accidental ingestion, absorption or inoculation are, however, difficult to estimate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste generated from this work will be handled according to the in-house protocols for handling of containment level 2 waste.

Liquid waste will chemically inactivated using Virkon, or other appropriate inactivating agent, as recommended by the manufacturer to achieve most effective killing (usually 1% solution). Virkon treatment has been validated by the manufacturer to inactivate all known 18 families of human and animal viruses (including Mastadenoviruses). Solid waste is collected in a plastic 'Weaver' bin (50L) lined with a plastic bag. When full the bag is tied and the lid secured to seal the box. The box is externally disinfected and then transferred to a designated 820L wheeled waste unit. The wheeled waste unit is collected by White Rose Environmental and handled and incinerated according to their procedures for Class 2 waste. Prior to collection a completed consignment note (detailing the type of waste and the precautions taken) is faxed to the Plant Manager of the receiving incinerator. A copy of the consignment note travels with the waste and the original is kept by the Facilities Manager at Stephenson Building (Keele University Science Park). When disposal of the waste is complete the disposers section of the consignment note is completed by the Plant Manager and faxed to the Facilities Manager. Thus a record of all disposals and confirmation of disposals is kept by us. White Rose's documentation has been audited by our Biological Safety Officer.

Local decontamination of the safety cabinets and other control and containment equipment and devices will be carried out by swabbing with 1% Virkon (as recommended by the manufacturer to achieve most effective killing) followed by 70% ethanol. Cell culture media will be immediately treated with Virkon as recommended by the manufacturer.

In the case of an accident puncture of the skin by a contaminated sharp, the procedure is to wipe the wound with 70% ethanol and report immediately to the Medical Centre.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The parasite-derived immunostimulating molecule has been used in vivo as a vaccine candidate without any adverse reactions, however, as its activity is not well characterised and could have unknown functions it is recommended that the work is carried out at containment level 2. Thus the Genetic Modification and Biological Safety Committee (GMBSC) is in agreement with the classification suggested by the risk assessment.

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**Project Ref** 838/02.5

**Date Ackn'd** 13/11/2002

**CU2 Project Title**

MOUSE IMMUNISATION WITH E1/E3-DELETED, REPLICATION-DEFICIENT ADENOVIRUS-5 VECTORS EXPRESSING AN ONCOGENE FROM A CONSTITUTIVELY ACTIVE EUKARYOTIC PROMOTER (EG CMV)

**Class** Class 2

**CultureVolClass2** < 1 litre

**CultureVolumeClass3-4**

**Date Project Ceased**

**Non-GMM** not applicable

**Consent Granted**

**Tick if notifying a connected programme of work** Y

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
### Purposes of the contained use

The aim of this project is to evaluate the immunogenicity of an oncoprotein in vivo. The ability of the resulting anti-oncogene immune responses to have anti-rumour effects in in vivo models will also be evaluated.

### Recipient or parental organism

The parental organism is a replication-defective, human Adenovirus (Ad) type 5. Human Ad are found worldwide, and over 40 different serotypes have been isolated. Ad5 is associated with mild upper respiratory tract infections in young children. Immunity to Ad infection is thought to be life-long following primary infection. Although some serotypes are closely related they nevertheless remain distinct entities suggested that the potential for recombination in vivo is not high. Human serotypes do not naturally infect other animal species and there are relatively few reports of virus replication following inoculation of Ad into experimental animals. There is no evidence that human Ad cross species and so a replication defective virus could not be maintained in non-human hosts. The replication-defective vector is not expected to be harmful. The potential hazard to the environment, from inadvertent release of the quantities of virus expected to be handled, is extremely low.

Any virus produced in-house will be produced in PerC6 cells (Fallaux et al., Human Gene Therapy, 1998, 9: 1910-1917), an E1-containing cell line designed to avoid recombination between the vector and the viral sequence present in the genome of the producer cells, and thereby replication-competent adenovirus (RCA) vectors should not be generated at significant levels. Some virus will be obtained from collaborators and this will be produced in 293 cells and thus will have a higher risk of RCA contamination. As with other replication-defective Ad constructs, any inadvertent generation of replication-competent virus, or helper virus, in the viral stocks would be readily apparent from the appearance of typical cytopathic effects in culture.

The oncogene will be inserted at the E1 locus. In a case of recombination in vivo (to generate a RCA), this will result in the deletion of the oncogene cassette in E1. Finally, as all the prepared viruses will be deleted for the E3 proteins, all generated viruses will be considered as much more immunogenic than non E3-deleted Ad5. Indeed, E3 proteins help to escape the host immune response against the infected cell (eg MHC class I down regulation, inhibition of TNFalpha-mediated effects). In agreement with this, the likelihood that these viruses will persist in the human population should be much lower, again because they are much more immunogenic and therefore the effective production of viral progeny should be significantly decreased, due to clearance of infected cells by the immune system.

### Host/vector system

The vector system is a replication-defective human adenovirus type 5 (E1 and E3 deleted). The host cell lines are PER.C6 (Fallaux et al., Human Gene Therapy, 1998, 9: 1910-1917) for in house viruses, and 293 for viruses obtained from collaborators.

### Origin & function

E1/E3-deleted, replication-deficient Ad-5 vector expressing the oncogene will be sourced via academic collaboration. This construct should temporarily express the oncoprotein within infected cells in vivo. This oncoprotein is intended to raise a tumour-protective, specific immune response in mice.

### Evaluation of foreseeable effects

Oncogene overexpression has been shown to immortalise cells. Thus the major risk to human health is stable insertion of oncogene into an individuals cells resulting in the development of a tumour. For this risk of tumour induction to be realised the following events must take place i) the adenovirus must be accidentally administered to an individual, ii) the administered adenovirus must transduce susceptible cells (avoiding the anti-adenovirus immune response which would already be present in the majority of immunocompetent individuals), iii) a transduced cell must stably express the oncogene, iv) transient expression and stable integration is a rare event, ie the oncogene expressing cell must not be lysed by anti-oncogene or anti-adenovirus immune responses (adenoviral proteins are highly likely to be co-expressed with the oncogene), v) the oncogene must not be silenced, vi) the oncogene expressing cell must have other gene disregulation events such that the cell can become tumourogenic. Thus although there is a risk of tumour formation if there was an inadvertent administration of the virus to an individual, this risk is deemed to be low. The risk assessment also puts in place control measures to minimise the risk of administration of the virus to an individual.

The risk of the virus to the environment is deemed to be negligible since the virus is replication defective (any RCAs are unlikely to carry the oncogene), the virus will be more immunogenic than wild-type virus due to the deletion of E3 and most importantly control measures are in place to ensure that the virus is not released into the environment.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated from this work will be handled according to the in-house protocols for handling of containment level 2 waste.

Liquid waste will be chemically inactivated using Virkon, or other appropriate inactivating agent, as recommended by the manufacturer to achieve most effective killing (usually 1% solution). Virkon treatment has been validated by the manufacturer to inactivate all known 18 families of human and animal viruses (including Mastadenoviruses).

Solid waste is collected in a plastic 'Weaver' bin (50L) lined with a plastic bag. When full the bag is tied and the lid secured to seal the box. The box is externally disinfected and then transferred to a designated 820L wheeled waste unit. The wheeled waste unit is collected by White Rose Environmental and handled and incinerated according to their procedures for Class 2 waste. Prior to collection a completed consignment note (detailing the type of waste and the precautions taken) is faxed to the Plant Manager of the receiving incinerator. A copy of the consignment note travels with the waste and the original is kept by the Facilities Manager at Stephenson Building (Keele University Science Park). When disposal of the waste is complete the disposers section of the consignment note is completed by the Plant Manager and faxed to the Facilities Manager. Thus a record of all disposals and confirmation of disposals is kept by us. White Rose's documentation has been audited by our Biological safety Officer.

Local decontamination of the safety cabinets and other control and containment equipment will be carried out using 1% Virkon (as recommended by the manufacturer to achieve most effective killing) followed by 70% ethanol. Cell culture media will be immediately treated with Virkon as recommended by the manufacturer. No sharp or glassware will be allowed during cell culture involving the construct.

In the case of an accidental puncture of the skin by a contaminated needle during animal injection, the procedure is to apply pressure around the wound to encourage bleeding, resulting in physical expulsion of the accidentally injected product, and report immediately to medical centre.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMBSC felt that the risk assessment clearly identified the hazards and risks and put suitable measures in place to control the risks. It also examined whether any of the containment measures that would be required for an increase to containment level 3 would reduce the risk to the workers or the environment and took the view that this would not be the case. Thus, after much discussion, the GMBSC agreed with the assignment of containment level 2 for this work. As this is the first risk assessment dealing with an oncogene (and especially since the oncogene will be placed in a viral vector) the GMBSC would appreciate some feedback from the HSE on this risk assessment.

Project Containment

Laboratory Activities

Glass Houses

Growth Rooms

02/03/2022
The aim of this project is to evaluate a particular DNA element termed "UCOE" (ubiquitous chromatin opening domain) to prevent the silencing of a eukaryotic promoter-driven marker gene(s) in cells transduced in vitro using an amphotropic (VSV-G pseudotyped) lentiviral vector based on HIV-1.

The recombinant virus is 3rd generation SIN (self inactivating) replication-incompetent lentivirus based on HIV-1 (Human Immunodeficiency Virus-1). The virus is amphotropic and expresses the VSV-G envelope protein which gives the virus a wide tropism including human cells. This type of vector has been used safely and successfully in various laboratories worldwide. Since lentiviruses are integrating vectors there is the potential risk for insertional mutagenesis. The recombinant virus will have a wide tropism and thus presents the same hazards as above to other mammals.

The recombinant virus will be used to transduce category 1 cell lines such as N1H3T3, HT1080, HeLa, CHO and Category II primary cells such as human mesenchymal stem cells (MSCs) and human haematopoietic stem cells (HSCs). These transductions will result in stable integration of the recombinant lentivirus. The final GMM (stably transduced cell line) will express a marker protein (either GFP, luciferase or B-galactosidase) and a gene for antibiotic resistance.
The vector system is a replication-defective 3rd generation SIN lentivirus produced using 293FT cells (obtained from Invitrogen). The vector is cloned into non-mobilisable plasmid backbones based on the on Puc series (obtained from Invitrogen). A total of 4 plasmids is used to transiently transfect the 293FT cells. Viral components in the plasmids are all under eukaryotic promoters and therefore will not be expressed in bacteria in which they are prepared. 3 plasmids contain the packaging and viral genes (gag/pol on one, env on another and rev on the third). These plasmids contain the genes required for production of the lentiviral particles in trans and do not contain a packaging signal, so the final vector is devoid of these viral genes. In addition they do not contain viral LTRs and are designed to have minimal regions of viral homology therefore minimising homologous recombination events. The expression plasmid will encode one of the marker genes under the control of a eukaryotic promoter along with the bidivergently transcribed UCOE under test and an antibiotic resistance gene. This plasmid will encode the RNA transcript that is packaged into the lentiviral vector. The recombinant virus is replication incompetent. However there is the theoretical risk of contamination with very low levels of replication competent virus. However only three of the HIV-1 genes present in wild type virus remain in this vector and upon integration the vector self-inactivates due to a deletion in the U3 region of the 3’LTR and cannot undergo further rounds of replication. The recombinant virus will contain a reporter gene (either LacZ, EGFP or Luc) driven by a eukaryotic promoter downstream of a DNA fragment encompassing the test UCOE. It may also contain mammalian antibiotic resistant genes pac or BSD.

Origin & function

The recombinant virus will be used to transduce and to stably integrate genes into mammalian cells in culture (as detailed above). EGFP is derived from the jellyfish Aequoria Victoria, the Luc from the firefly Photinus Pyralis and LacZ from the bacterium E. coli. Test UCOE elements and a number of eukaryotic promoters may be used. Pac is derived from Streptomyces alboniger and BSD from Aspergillus terreus. EGFP is a fluorescent protein that can be easily detected by fluorescence activated cell scanning (FACS) and acts as a marker protein for detection of gene expression in the final GMM. Similarly Luc encodes for luciferase and LacZ encodes for B-galactosidase, which are enzymes that can be detected by enzyme assay or histochemistry. A eukaryotic promoter is required to drive expression of the marker genes, as the promoter in the integrated LTR is self-inactivated in this vector system. The UCOE is the functional element that will be under test to evaluate its potential to prevent the silencing of the marker gene(s). Pac encodes for N-acetyl-transferase an enzyme that confers resistance to the antibiotic puromycin and similarly BSD encodes for blasticidin-S deaminase that confers resistance to the blasticidin S. Conferring antibiotic resistance allows for in vitro selection of cells containing integrated transgenes. No additional hazards would be predicted due to the expression of these transgenes since they encode proteins that have been widely used to measure promoter activity in both cultured cells and in vivo (expression in all tissues throughout the life of transgenic animals has no detectable pathological effect) or have been widely used for the selection of stable integrants.

Evaluation of foreseeable effects

There is a theoretical possibility of generating RCV either in the packaging cell line or in the final transduced GMM. However this is minimised by design of the vector system and by use of cells or cell lines that do not have known lentiviral sequences. Infection of humans could potentially result in an insertional inactivation event. However the vector will be of the self-inactivating variety ("SIN") preventing activation of genes adjacent to the integration site by the viral LTRs. There may be the potential for activation of adjacent genes by the UCOE element. This may occur over a large distance (extent is currently unknown). Thus the detrimental consequences of provirus insertion would be a contribution to tumorigenesis through either the inactivation of a tumour suppressor gene, such as p53, or the activation of a dominant oncogene by the UCOE element. However, tumour suppressor gene inactivation would leave one allele intact, except for the minority of individuals carrying a defective allele. Any infected cells will express a reporter gene encoding a foreign protein and would be expected to be eliminated by the immune system in the majority of cases.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated from this work will be handled according to the in-house protocols for handling of containment level 2 waste. Liquid waste will chemically inactivated using Virkon, or other appropriate inactivating agent, as recommended by the manufacturer to achieve most effective killing (usually 1% solution). Virkon treatment has been validated by the manufacturer to inactivate all known 18 families of human and animal viruses (including Mastadenoviruses).
Solid waste is collected in a plastic 'Weaver' bin (50L) lined with a plastic bag. When full the bag is tied and the lid secured to seal the box. The box is externally disinfected and then transferred to a designated 820L wheeled waste unit. The wheeled waste unit is collected by White Rose Environmental and handled and incinerated according to their procedures for Class 2 waste. Prior to collection a completed consignment note (detailing the type of waste and the precautions taken) is faxed to the Plant Manager of the receiving incinerator. A copy of the consignment note travels with the waste and the original is kept by the Facilities Manager at Stephenson Building (Keele University Science Park). When disposal of the waste is complete the disposers section of the consignment note is completed by the Plant Manager and faxed to the Facilities Manager. Thus a record of all disposals and confirmation of disposals is kept by us. White Rose's documentation has been audited by our Biological Safety Officer.

Local decontamination of the safety cabinets and other control and containment equipment and devices will be carried out by swabbing with 1% Virkon (as recommended by the manufacturer to achieve most effective killing) followed by 70% ethanol. Cell culture media will be immediately treated with Virkon as recommended by the manufacturer. No sharps or glassware will be allowed during cell culture involving the construct.

The committee felt that all the risks associated with this program of work had been addressed in the risk assessment and agreed with the containment measures and level proposed.

### Project Containment

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<thead>
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<th>Glass Houses</th>
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**Name**

NEWCASTLE UPON TYNE NHS FOUNDATION TRUST

**Name 2**

FREEMAN HOSPITAL

**Department**


**Campus Estate or Research Centre**


**R**oad **N**ame


**T**own

NEWCASTLE UPON TYNE

**C**ounty

NORTHUMBERLAND

**P**ostcode

NE7 7DN

**C**ountry

ENGLAND

**Tel Number** 0191 284 3111

**Fax Number** 0191 213 1968

**E-mail**


**HSE Division** YORKSHIRE AND NORTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)
Waste materials that have contained or otherwise contacted the virus dose will be collected in appropriate rigid container, transported to Microbiology Department by the Urology Research Nurses and autoclaved prior to disposal and incineration as per GMO SOP. Disposal of this decontaminated waste, and all other waste produced within the patient's room, will be in accordance with the Trust Waste Management Policy and GMO SOP. Waste unlikely to be contaminated with the GMO would be processed via the Trust's routine 'infectious waste' stream ie by incineration.

Processing in the autoclave (SAL MC type) would be expected to completely inactivate the adenovirus. The process is monitored by automatic parametric testing and recording and a regular schedule of validation and autoclave testing in accordance with HTM2010.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Waste materials that have contained or otherwise contacted the virus dose will be collected in appropriate rigid container, transported to Microbiology Department by the Urology Research Nurses and autoclaved prior to disposal and incinerated as per GMO SOP. Disposal of this decontaminated waste, and all other waste produced within the patient's room, will be in accordance with the Trust Waste Management Policy and GMO SOP. Waste unlikely to be contaminated with the GMO would be processed via the Trust's routine 'infectious waste' stream ie by incineration.

Processing in the autoclave (SAL MC type) would be expected to completely inactivate the adenovirus. The process is monitored by automatic parametric testing and recording and a regular schedule of validation and autoclave testing in accordance with HTM2010.

**A study of gene directed enzyme prodrug therapy in primary and locally recurrent prostate cancer.**

**Risk assessment accepted by the Genetic Modification Safety Committee.**
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Given brief details of the genetic modification safety committee

Director, National Science Laboratory - Employers representative (Chair).
Section Head, Cell Therapies, Biological Safety Officer.
Project Investigator, Employees representative & Safety Representative.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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<th>Large Scale</th>
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See risk assessment 3.3 Stage III.
The degree of kill will be 100% using presept-tablets tested for by streaking on an agar culture plate with a sample of the waste and noting any colonies growing at 24 hours.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The waste once inactivated will be disposed of down the sink.
Any solid waste will be disposed of by incineration.

Cellular therapies including adoptive immunotherapy
Cultured and subsequent use of a transfected human embryonic kidney cell line (293-F) that over-express normal human cellular prion protein (HuPrPC)

Date Project Ceased
25/09/2014

Tick if notifying a connected programme of work
N

Withdrawn
N

Non-GMM Consent Granted

Project notified under transitional arrangements
N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The realization that the infectious agent responsible for variant Creutzfeldt-Jakob disease (vCJD) can be transmitted by blood transfusion and that effected individuals are infectious during a protected preclinical phase has raised concerns that a pool of potentially infectious asymptomatic individuals could exist in the UK blood donor population. Assays capable of detecting infectivity in blood/plasma could help to prevent further cases of secondary disease transmission. Prion diseases, including cCJD, are closely associated with the conversion of the normal host cellular prion protein (+PrPC) to a misfolded, disease-associated form (PrPSc), which is considered by some to be the major (if not the sole) component of the infectious agent and is the only unambiguous disease marker identified to date. Hence the assays for the detection of vCJD infectivity in blood/plasma have focused on the detection of PrPSc. However, such a screening assay would have to specifically detect minute amounts of PrPSc in a large excess of PrPC.

One such potential plasma screening assay has been developed SNBTS. The assay is based on the amplification of minute amounts of PrPSc to readily detectable levels using a technique called protein misfolding cyclic amplification (PMCA) and its subsequent detection by conformation dependent immunoassay (CDI). In the assay's original format, plasma samples are first treated with NaCl to both precipitate any PrPSc present in the sample and remove the factor/factors found in plasma which inhibit PrPSc amplification, following centrifugation the resulting pellets are resuspended in the PMCA substrate (10% (w/v) human platelet lysate in PMCA conversion buffer) and subjected to 4 rounds of serial PMCA (sPMCA), finally the 4th round sPMCA products are screened for the presence of PrPSc by CDI. In this format the assay could reproducibly detect PrPSc in plasma spiked with a 10-8 dilution of a 10% (w/v) vCJD brain homogenate and no false positive results were detected in the limited number of normal US plasma samples tested. Whilst these initial results were encouraging, in all probability in order to detect the amounts of PrPSc the assay would need to be improved. A 100-fold increase in PrPSc detection sensitivity, resulting in the reproducible detection of PrPSc in plasma spiked with a 10-10 dilution of a 10% (w/v) vCJD brain homogenate, was achieved using a PMCA substrate supplied by Japan Blood Products Organisation (JBPO). This substrate was prepared form a transfected embryonic kidney cell line (293-F) that over-express normal human PrPC of the PRNP methionine codon, 129 genotype. In order to further evaluate and validate the use of this substrate in the assay developed at the transfected 293-F cell line from Japan Blood Products Organisation to SNBTS so that the transfected cell line can be cultured, harvested and used to prepare the PMCA substrate in-house at SNBTS.

Summary of the project

- Import of the transfected 293-F cell line and the expression vector used to transfect the cells from Japan Blood Products to SNBTS.
- The transfected 293-F cell line will initially be grown in suspension cultures and frozen stocks will be laid down.
- For routine preparation of PMCA substrate, suspension cultures will be seeded from the frozen stocks and the cells harvested by centrifugation.
- The resulting cell pellets will be lysed in PMCA conversion buffer to a prepare a 20% (w/v) cell lysate which will be stored, in suitable sized aliquots at -80°C ready for use in subsequent PMCA reactions.

Note that the aims of this project only cover the culture, harvesting and subsequent use of the transfected cells to prepare PMCA substrate. All PMCA reactions using this substrate will be carried out in the dedicated derogated containment level 2 C D Research Laboratory.

Recipient or parental organism

293-F human embryonic kidney cells

The 293 cell line is a commercially available, well characterised cell line established from primary embryonic human kidney cells transformed with sheared human adenovirus type 5 DNA. The EIA adenovirus gene is expressed in these cells and participates in transactivation of some viral promotors, allowing these cells to produce very high levels of protein expression.

The 293-F cell line is a variant of the 293 cell line that has been adapted to grow in suspension culture.

Host/vector system

pIERESneo3 is a mammalian expression vector that contains the internal ribosome entry site (URES) of the encephalomyocarditis virus (ECMV), which permits the translation of two opening reading frames from one messenger RNA, in this case the gene of interest (gene encoding normal human cellular prion proteins) and the gene conferring resistance to neomycin (neomycin phosphotransferase NPT 11 gene). Thus after selection in neomycin nearly all surviving colonies will stably express the gene of interest.

For propagation, selection and expression in mammalian cells the expression cassette of pIRESneo3 contain the human cytomegalovirus (CMV) major immediate early promoter/enhancer followed my a multiple cloning site (MCS) that precedes stop codons in all three reading frames, a synthetic intron known to enhance the stability of the mRNA the EVMNV IRES followed by the neomycin phosphotransferase (NPT II) gene (which confers resistance to neomycin), and the polyadenylation signal from sv40. Ribosomes can enter the bicistronic mRNA at the 5’ end to translate the antibiotic (neomycin) resistance marker. When using the pIRESneo3 vector, the antibiotic exerts selective pressure on the entire expression cassette; thus a high dose of antibiotic will select cells expressing a high level of the gene of interest. This selective pressure also ensures that the expression of the gene of interest will be stable over time in culture.

The vector also contains an ampicillin resistance (b-lactamase) Gene and a ColE1 origin of replication for selection in E. coli, and an f1 origin for single-stranded DNA production.

Origin & function

The gene sequence inserted into the pIRESneo3 expression vector is a chimeric mouse/human DNA sequence which encodes the N-terminal signal sequence from the mouse prion protein gene and the full length human prion protein amino acid sequence with methionine at codon 129. Thus the transfected 293-F cell line will over-express normal cellular prion protein (PrPC) which is homoyzogus for methionin at codon 129 of the human PRNP gene. The normal form of human PrPC is expressed in most cell and tissue types with the highest expression levels found in the brain. The exact role of PrPC is not known, however, it is believed to be involved in signal transduction and cell adhesion

Evaluation of foreseeable effects

The 293-F cell line is a well characterised, commercially available cell line, which should be of no risk to human health and would be incapable of surviving within an accidentally inoculated immunocompetent host. However, the supplier (Invitrogen) recommends that 293-F cells should be handled as potentially biohazardous material under at least Containment Level 2 conditions.
The pIRESnneo3 expression vector is commercially available and has no known associated hazards.

At normal physiological levels human PrPC is not considered a risk to human health. However, Section 34, Page 45 of Part 2 of the SACGM Compendium of Guidance states that GMMs over-expressing normal prion proteins should be handled at Containment Level 2 as they may be pathogenic. It is conceivable that in culture the human PrPC gene sequence could undergo mutation leading to the production of pathogenic forms of human PrPC such as those associated with genetic/familial forms of human prion disease. This potential risk is highlighted even though there is no evidence to suggest that these forms of human prion disease can be transmitted from person-to-person. It has also been reported that cell cultures expressing PrPC can support the propagation of the agents causing a variety of animal and human prion disease. Thus it is conceivable, but highly unlikely, that a cell line over-expressing human PrPC could propagate the infectious agent responsible for variant Creutzfeldt-Jakob disease (vCJD) should it be exposed to the infectious agent responsible for bovine spongiform encephalopathy (BSE) through the use of contaminated bovine-derived products in tissue culture supernatants. This could lead to a potential exposure to the infectious agent that causes cCJD in humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Culture of the transfected 293-F cell line will be carried out at Containment Level 2 and the following additional control measures will be adopted:-

- Cell culture supernatants to be centrifuged in sealed containers which will only be opened in a Biological Safety cabinet.
- All work where aerosols might be generated will be carried out in a Biological Safety Cabinet.
- The use of sharps and glassware will be avoided.
- Transfected 293-F cell to be cultured in serum free medium and bovine-derived tissue culture supplements will not be used.
- All waste generated from the culture of the transfected 293-F cell line to be treated as potentially infectious and will be decontaminated and disposed of as described in SOP LIB 049 CJD RESEARCH LABORATORY: WASTE DISPOSAL.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogations are requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste products generated from the culture of the transfected cell line will be treated as potentially infectious for human prions will be decontaminated and disposed as described below:-

- All liquid waste (spent culture medium) will be decontaminated using NaOH at a final 2M concentration for a minimum of 1 hour (ideally overnight) in sealable buckets, sawdust will then be added to absorb the liquid waste, the buckets will be sealed, double bagged in yellow clinical waste bags and disposed of by incineration.
- Used pipette tips will be disposed of into sharpsafes, when the sharpsafes are full they will be sealed, sprayed with 2M NaOH, left for a minimum of 1 hour (ideally overnight), rinsed with tap water and disposed of by incineration.
- All other waste which has not been exposed to potentially infectious agents will be double bagged in yellow clinical waste bags and disposed of by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Th attached risk assessment was discussed and approved at the SNBTS Biological safety & GMO Committee meeting held on the 11th July 2014

## Project Containment

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#### Name 2

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#### Comments

#### Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Committee members: Chief Scientific Officer UK, Regulome Corporation; Regulome Corporation staff representative; Biological Safety Officer, John Innes Centre; Resources Manager, Norwich Bio-Incubator; external member from John Innes Centre Biological Safety Committee.

The committee will meet annually to review all risk assessments and HSE notifications. Additional meetings will be held to consider new risk assessments and notification requirements as required. All assessments and notifications will be circulated by email and comments from the committee taken into account prior to any new procedures being adopted. Any assessments which fall outside the expertise of more than three of the committee will be sent to external advisors drawn from experienced scientists working in the relevant area on the Norwich Research Park.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

02/03/2022
Bacteriology | Yes | Parasitology | Transgenic | Transgenic | Bacteria | Transgenic | Birds | Bacteria | Microbiology | Yes | Research | Bacteria
Virology | Transgenic | Animals | Transgenic | Fish | Gene Therapy
Mycology | Transgenic | Invertebrates | Transgenic | Plants | Other (please specify below) | Yes
Other(s) | Mammalian cell culture.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid biological waste will be placed in unsealed bags placed in lidded but unsealed plastic boxes and removed from the laboratory to the John Innes Centre central autoclave service where it will be sterilized. The autoclave cycle will include heating the material to at least 120 degrees C for 15 minutes to ensure 100% kill for all material.

Liquid waste and contaminated glassware will be treated with hycolin disinfectant, diluted according to the manufactures instructions, overnight at room temperature. An initial check will be carried out the first time the process is carried out to check that no culturable cells remain before disposal. This check will be repeated periodically depending on classification of risk and whenever waste from different cell types or quantities of culture are produced to ensure that the procedure results in 100% kill.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All members of the genetic modifications safety committee have read the attached risk assessment and have agreed with its conclusions.
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Name

ROYAL BOTANIC GARDENS KEW

Name 2

Department

SEED CONSERVATION

Campus Estate or Research Centre

Building

WAKEHURST PLACE

Road Name

SELSFIELD ROAD

Town

ARDINGLY

County

WEST SUSSEX

Postcode

RH17 6TN

Country

ENGLAND

Tel Number

01444 894100

Fax Number

01444 894110

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Composition
Head of Research Section
Lab Manager
Scientific Officer Diagnostics
PhD student
Senior Scientific Officer Diagnostics, appointed Biological Safety Officer
Meetings take place 2 x per year, or ad hoc if required by circumstances.

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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

**Heat inactivation**
All solid GMM material will be disposed in autoclavable bags placed in clearly labelled 'biological hazard' bins. When half-full bags will be autoclaved (30 min at 130 degrees C) and placed in special bins for incineration. All liquid GMM material will be autoclaved (30 min at 130 degrees C) and subsequently disposed with wastewater.

Degree of kill
Degree of kill with this autoclaving procedure is 100%

Monitoring measures:
A Brown T.S.T Control test strip (Albert Browne Ltd) is included with every run of the autoclave as a control for proper heat-exposure.

**Tick to confirm that you are attaching a summary of the risk assessment** Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

Please enter comments of the GM safety committee on the risk assessment

* A comment was made on the lack of specification of a species under 9i)(b) par.2, with regards to the cloning of anonymous cDNAs. The BSO explained that no other species has been targeted yet, but that a new risk assessment will be performed as soon as this happens.
* Addition of text under (i)(b) par.2: 'for in vitro RNA production'.
* The Standard Operating Procedure (SOP) for cloning of cDNA, of which a draft version is in place, will be merged with a SOP for use of micro-organisms.
* Advise to include an additional reference (HSE compendium).
* Advise to include author, site and date in the footer of the document.
* Some minor editing comments were made.
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| Date at Which Additional Info Submitted | 02/03/2022 |
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

GMSC consists of the Technical Director of DRI, the Quality Management Systems Manager, a Senior Research and Development Scientist, the COSHH Safety Officer and the Biological Safety Officer (BSO). The committee meets monthly to discuss and review GM and Health and Safety issues within the company. All GM work is approved by the BSO/GMSC and a risk assessment carried out to determine the class of activity before commencement.

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All GMM waste will be collected in an autoclave bag, separate from standard laboratory/clinical waste. GMM waste will then be inactivated by autoclaving at 121 degrees C for 20 mins in an autoclave within the same building before being incinerated with other laboratory/clinical waste by a laboratory/clinical waste disposal company (at present the contractor is PHS Allclear Limited).

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Tick to confirm that you are attaching a summary of the risk assessment  
Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

Good microbiological practice should ensure that the GMM is contained and will not cause harm to people or the environment.

The GMSC has agreed that the GM activity in the risk assessment is low risk and agrees that the activity should be categorised as a Class 1 activity.

The waste disposal measures for GMM waste are suitable for treatment of Class 2 waste even though this is not required for Class 1 activities, but this is standard practice for the inactivation of GM waste within DRI Limited.
GM Centre Number: 844

Data Premises Notified (Originally) 03/10/2002

Transferred from 1992 Regs? N

Transitional Premises Closed N

Emergency Plan Required? N

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

PSIOXUS THERAPEUTICS LTD

Name 2

PSIOXUS THERAPEUTICS LTD

Department

Building

UNITS 4-10 THE QUADRANT

District

ABINGDON

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX14 3YS

Country

ENGLAND

Tel Number 01865 224924

Fax Number 01869 238069

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

21/10/2004

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The Oxford BioBusiness centre has an established Genetic modification safety committee comprising of suitably qualified members from some of the companies based there and an outside expert from the University of Oxford. Our risk assessment has been studied and approved by the committee.

<table>
<thead>
<tr>
<th>Laboratory</th>
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<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 4 (GMMs)</td>
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</table>
Solid virus-containing waste is bagged and placed in a designated metal container which is removed to the autoclave. Liquid virus waste is discarded to bottles which are placed in a designated metal container for autoclaving. All liquid waste is treated by autoclaving at 130 degrees C for 60 mins, before disposal to drains.

Disposable solid waste, which is or may be contaminated with GMMs is also inactivated by autoclaving at 130 degrees C for 30 mins, before removal as "clinical waste" by specialist contractors, with final disposal by incineration.

The exceptions to the above are:
Sharps and pipets are steeped in 1% Virkon for a minimum of 30 minutes before disposal in an approved sharps box. The Virkon is discarded down the sink.
Glassware is soaked overnight in 1% Virkon before removal for normal tissue culture wash up processing.
Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above.
The autoclave on site is regularly tested and services.

Expected degree of kill:
Autoclaving achieves effectively 100% kill of all GMMs.
The manufacturer's information indicates efficacy of Virkon against a variety of adenoviruses at 1% concentration, and we have demonstrated that 15 minutes exposure to 1% Virkon achieves >4 log (10) kill for adenovirus-infected cell pellets, and >6 log (10) kill of adenovirus seed stocks.
It was suggested that we test our adenovirus preparations for recombinant adenovirus (RCA). A recombination event would generate a wild-type Ad5. Bulk preparation of virus will not be performed at Littlemore Park, but will be provided by colleagues working in the Department of Clinical Pharmacology at Oxford University. We have arranged for all virus preps to be tested for RCA by performing plaque assays on A549 cells, which support the growth of wild type virus but not replication deficient viruses.

**Project Ref 844/05.1**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol/Class2</th>
<th>Consent Granted</th>
<th>Non-GMM</th>
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<td>07/10/2005</td>
<td>The use of conditionally replicating adenoviral, attenuated vaccinia and attenuated herpes simplex virus 1 vectors for cancer gene therapy.</td>
<td>Class 2</td>
<td>1-50 Litres</td>
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Withdrawn: N

Tick if notifying a connected programme of work: N

**Project Additional Information**

**Purposes of the contained use**

Development of polymer-coated retargeted viral vectors.

**Recipient or parental organism**

The vectors are attenuated and are less pathogenic than their corresponding wild type virus. They have also been engineered to be more active in tumour cells than normal tissues. The inserts encode reporter genes, prodrug activating enzyme genes or anti-angiogenic genes which will not increase the pathogenicity of the viruses. The inserted gene products are expressed at very low levels, however it is possible that they may cause an immune response in healthy subjects. Immunocompromised individuals may be less likely than normal healthy individuals to mount an immune response to incoming viruses, but as the modified viruses are replication defective, this should not lead to replicative spread of the virus. With respect to the environment, the viruses require a host to replicate; it is very unlikely that the GM strains will be able to form a successful infection.

**Host/vector system**
Commercially available human tumour cell lines, 293 and 911 cells, BHK21 fibroblasts, BSC-1 (African green monkey kidney) cells. Attenuated Vaccinia virus, attenuated HSV1, conditionally replicating adenoviral vectors.

**Origin & function**

The viral vectors and inserts will be obtained from collaborators. All the vectors are attenuated and are less pathogenic than the corresponding wild type virus. The inserts encode products which will not increase the pathogenicity of the viruses. We plan to non-genetically modify the viruses using polymer coating and attachment of targeting ligands for use in cancer gene therapy.

**Evaluation of foreseeable effects**

The vectors are conditionally replicating, and the inserts code for innocuous products that will be expressed at very low levels. The recombinant viruses are less pathogenic than their parental wild types. There is little risk to human health or the environment as it is very unlikely that the GM viruses could find a host in which to successfully replicate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Non

Describe the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Virkon is used for soaking pipettes, TC plates and flasks, Virkon powder is added to liquid waste to give a final concentration of 1%.

Virkon is used in accordance with the manufacturer's instructions (ie 1% solution). It has been validated as being effective within 10 minutes at this concentration. See www.antechh.com/virkonapps.html. We routinely decontaminate for at least 1 hour.

Virkon stocks are routinely tested. Viruses are exposed to 1% Virkon for 15 minutes, dialysed and diluted in culture media. This is then put on to cells and transgene expression, which indicates viability of the virus, is assayed after 24 hours.

Decontaminated waste is allowed to dry before being put into clinical waste bags, these are then transferred into large clinical waste bins located approximately 20 metres from the laboratory. The yellow bags are labelled with the date and time of decontamination and a written record is kept. Bags are removed from the site by a commercial company for incineration.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
We should emphasise that we are not genetically modifying any vaccinia, or HSV ourselves, just using GM strains that are commercially available or available through collaborations. That polymer coating and retargeting would further limit the tropism of all viruses and therefore the survival of the GMM in the environment is likely to be less than for wild-type.

### Project Containment

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<td>L3</td>
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**Animal Units**

- L2
- L3
- L4
- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4
- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

### Project Ref 844/14.1

**Date Ackn'd**

29/05/2014

**CU2 Project Title**

Development of armed oncolytic viral vectors

**Class**

Class 2

**CultureVolClass2**

1-50 Litres

**Consent Granted**

Non-GMM

**Project notified under transitional arrangements**

- N

**Withdrawn**

- N

**Tick if notifying a connected programme of work**

- N

### Historical Significant Changes

- Historical Date of Additional Info

### Project Additional Information

**Purposes of the contained use**

Development of conditionally replicating adenovirus vectors expressing therapeutic transgenes for cancer therapy

**Recipient or parental organism**

02/03/2022
ColoAd1 is an Ad11p/Ad3 chimeric group B adenovirus virus that demonstrates selective replication in tumour cells. ColoAd1 is attenuated and is less pathogenic than the corresponding wild type virus. The inserts will encode for tumour associated antigens, cytokines, mammalian receptors and ligands, enzymes, siRNA's and full length antibodies or antibody domains which will not increase the pathogenicity of the viruses. The inserted gene products will be expressed under a range of different promoters including tumour specific promoters and endogenous viral promoters.

It is possible that the gene products may cause an immune response in healthy subjects. Immunocompromised individuals may be less likely than normal healthy individuals to mount an immune response to incoming viruses. However, normal human cells are non-permissive for ColoAd1 replication, which will not lead to replicative spread of the virus.

With respect to the environment, the viruses require a host to replicate and do not replicate in non-human cells, so it is very unlikely that the GM strains will be able to form a successful infection.

### Host/vector system

Commercially available human tumour cell lines, 293 and 911 cells conditionally replicating adenoviral vectors.

### Origin & function

Vectors will be generated in house and will be based on conditionally replicating adenoviruses. All the vectors are attenuated and are less pathogenic than the corresponding wild type virus. The inserts encode mammalian protein products which will not increase the pathogenicity of the viruses, to evaluate their potential function for treatment of cancer when delivered in this form.

### Evaluation of foreseeable effects

The vectors are conditionally replicating, and the inserts code for products that will be expressed at low levels. The recombinant viruses are less pathogenic than their parental wild types. There is little risk to human health or the environment as it is very unlikely that the GM viruses could find a host in which to successfully replicate.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Virkon is used for soaking pipettes, TC plates and flasks, Virkon powder is added to liquid waste to give a final concentration of 1%. Virkon is used in accordance with the manufacturer's instructions (ie 1% solution). It has been validated as being effective within 10 minutes at this concentration. See www.antechh.com/virkonapps.html.

Decontaminated waste is allowed to dry before being put into clinical waste bags, these are then transferred into large clinical waste bins. Bags are removed from the site by a licensed commercial company for incineration.

**Is an emergency plan required according to regulation 20?**

- [ ] Y

- [ ] N

**If yes, tick to confirm that it is attached to this form**

- [ ] Y

- [ ] N

**Tick to confirm that you have attached a risk assessment to this form**

- [ ] Y

- [ ] N

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- [ ] Y

- [ ] N

02/03/2022
The GMSC was consulted during the drafting of the risk assessment. The GMSC recommended further details on environmental hazards and waste disposal. The recommendations of the GMSC were incorporated into the risk assessment and the GMSC approved the risk assessment.

### Project Containment

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**Name**

LOTHIAN NHS BOARD

**Name 2**

ROYAL INFIRMARY

**Department**

**Campus Estate or Research Centre**

WAVERLEY GATE

**Building**

**Road Name**

2-4 WAVERLEY PLACE

**District**

**Town**

EDINBURGH

**County**

MIDLOTHIAN

**Postcode**

EH1 3EG

**Country**

SCOTLAND

**Tel Number**

0131 536 9000

**Fax Number**

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The GMSC has been established as a sub-committee of the Trust R&D Committee with the R&D Director as Chair. The Committee comprises

* Chair of Trust Control of Infection Committee
* Trust Head of Health and Safety
* Assistant Director of Nursing with responsibility for clinical risk
* Academic member of Edinburgh University medical School GM Safety Committee
* Academic representative on GTAG
* R&D Director
* Nominated Biological Safety Advisor
* Staff representative
* Project co-ordinator (study specific)

Level 1 (GMMs)  
Level 2 (GMMs)  
Yes
Level 3 (GMMs)  
Level 4 (GMMs)  
Non-microbial
For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A

Please enter comments of the GM safety committee on the risk assessment

GMSC assessed this project and the risk assessment provided. The Committee felt this was a thorough assessment and approved the activity with the following requirements:

* An SOP or equivalent should be readily available in the event of a needlestick injury
* A return visit by the Chair of Control of Infection Committee must be organised by the principal investigator when the Class II cabinet has been commissioned. Operational procedures will be reviewed at that stage. No patient should be recruited
* Occupational Health should know of the procedures so they can give advice in the unlikely event of an incident
* There should be a record of all staff who handle the product
* It is the responsibility of the principal investigator to ensure all staff working in the Unit are aware of the study and reassured about containment procedures
* A copy of any report submitted must be forwarded to the Chair of Trust GMSC
Project Additional Information

**Purposes of the contained use**

In the proposed clinical trial programme, angiogenic gene therapy product, consisting of a recombinant adenovirus (human serotype 5) containing the human gene for the fibroblast growth factor 4, and referred to as Ad5FGF-4, will be investigated as a treatment for patients with chronic stable angina due to coronary artery disease (CAD). Generation of new blood supply in the diseased heart by intracoronary administration of angiogenic gene therapy product represents a potential new therapeutic approach to relieve this condition.

**Recipient or parental organism**

See box below

**Host/vector system**

The Ad5FGF-4 gene therapy product consists of a recombinant adenovirus vector (human serotype 5, Ad5) with a deletion in the E1 region; from map unit 1.3 to 8.7 of wild-type virus (entire E1A and most of E1B are eliminated). The FGF-4 transgene is inserted, driven by CMV promoter.

**Origin & function**

The FGF-4 gene was originally isolated from a cDNA library which was constructed from mRNA of Kaposi's sarcoma DNA transformed NIH3T3 cells. The intended function is angiogenesis, the formation of new blood vessels.

**Evaluation of foreseeable effects**

The probability of adverse consequences resulting from deliberate or accidental release of the gene therapy product Ad5FGF-4 are minimal to nonexistent.

Risks resulting from environmental release (viral shedding from treated persons, inadvertent contamination of the product prior to administration) are negligible or nonexistent for the following reason: infection requires large numbers of infectious vectors, and transfection (expression of the inserted gene) requires a multitude of infectious particles.

Hazards associated with the adenoviral vector can be described as having low potential of adverse environmental consequences in humans or animals. The theoretical
consequences to humans of several of the hazards associated with ectopic transgene expression, if they actually occurred, could be considered moderately severe (eg promotion of existent malignancy, unknown risk to foetus). However, since any unintended or accidental exposure would most likely be a fraction of the total dose being administered to patients for therapeutic purposes, the relative risk of the occurrence of these types of adverse effects should be very low.

The possible risks to the environment could be assessed as negligible. This is based on the low probability of infectious adenoviral particles escaping into the environment either through viral shedding by patients that have received the product, or by incidental exposure during administration procedures. Even if viruses were shed or product spillage occurred the number of infectious viral particles would be too small to result in infection of exposed tissues. The risk to the non-human environment is extremely low to effectively zero because of the species specificity of adenovirus 5, which by natural exposure is only known to infect humans.

The above indicates that the product could be classified as Class 1. However, as a precautionary measure, due to the limited experience available, the product is currently being classified as Class 2. Reclassification into Class 1 may be considered if based on increased data and experience.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Inactivation by autoclaving and effectively 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Trust has set up a Genetically Modified Safety Committee as a sub-committee of its R&D Committee. GMSC assessed this project and approved the activity with the following requirements:-

* An SOP or equivalent should be readily available in the event of a needlestick injury
* A return visit by the Chair of Control of Infection Committee must be organised by the principal investigator when the Class II cabinet has been commissioned. Operational procedures will be reviewed at that stage. No patient should be recruited
* Occupational Health should know of the procedures so they can give advice in the unlikely event of an incident
* There should be a record of all staff who handle the product
* It is the responsibility of the principal investigator to ensure all staff working in the Unit are aware of the study and reassured about containment procedures
* A copy of any report submitted must be forwarded to the Chair of Trust GMSC

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Name

CENTRE FOR ECOLOGY AND HYDROLOGY

Name 2

CEH

Department

Building

Road Name

BUSH ESTATE

District

Town

PENICUIK

County

Midlothian

Postcode

EH26 0QB

Country

SCOTLAND

Tel Number

0131 445 4343

Fax Number

0131 445 3943

E-mail

HSE Division

SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>MIDLOTHIAN</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

Chair: Head of Biosystems Management Section  
Committee: Local Safety Advisor, Biological Safety Officer, Unionside H&S Representative, project personnel.  
The committee will meet every time a risk assessment is received, or 6 monthly, whichever is more frequent.  
Occupational Health personnel have been informed. Meetings will be minuted and copied to Site Director and Station Secretary and Occupational Health.

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Tick if confidential

Bacteriology  
Parasitology  
Transgenic  
Birds  
Microbiology  
Research  Yes
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**Mycology**

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<tr>
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<th>Other (please specify below)</th>
</tr>
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</table>

**Other(s)**

---

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

All items will be autoclaved before disposal. Autoclaves will be validated by annual thermocouple mapping, and, depending on the autoclave, each run will be monitored by continuous recording of the temperature profile, or time, steam, and temperature test strips. A log book will be kept.

---

Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: 

---

**Please enter comments of the GM safety committee on the risk assessment**

Risk assessment E1 was approved by the committee, subject to registration of the lab with the HSE. The committee noted that the biological materials to be used were well within the containment level 1 category, and requested that the risk assessment should include reference to good laboratory practice with respect to the use of lab books to record all experimental activities, and a log book to record the sterilisation and disposal of waste. Appropriate COSHH forms and Safe systems of work should be completed before work commences.
### GM Centre Number: 847

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### Name

**PROTHERICS MOLECULAR DESIGN LIMITED**

### Name 2

#### Department

#### Campus Estate or Research Centre

#### Road Name

THE HEATH BUSINESS AND TECHNICAL PARK

#### District

THE HEATH BUSINESS AND TECHNICAL PARK

#### Town

RUNCORN

#### County

CHESHIRE

#### Postcode

WA7 4QF

#### Country

ENGLAND

Tel Number | 01928 518000 |
Fax Number | 01928 518002 |

E-mail

HSE Division | NORTH WEST |

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Group leader of R&D Immunosciences, Research Immunologist - Molecular Biologist, Research Immunologist - Protein Biochemist and also the Runcorn site Biological Safety Officer.

Meetings will be carried out once a month to discuss GMO experimental design and risk assessment and review and update GMOs.

<table>
<thead>
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<td>Non-microbial</td>
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</tbody>
</table>

Other (please specify) Tick if confidential
Yes

No mouth pipetting, manipulate potentially infectious materials carefully to avoid spills and minimise production of aerosols. Restrict the use of needles and syringes to those procedures for which there are no alternatives. Use protective lab coats and gloves. Wash hands following all laboratory activities following the removal of gloves and immediately following contact with infectious materials. Decontaminate work surfaces with chloros or virkon before and after use and immediately after spills. The use of sharps in operations involving GMOs (hyperdermic needles, scalpels) should be avoided whenever possible. Where possible the use of glassware for containing GMOs should be minimised in favour of disposable plasticware. Cultures of GMOs in glass flasks should be transported between labs by way of communal throughfares must be carried in a suitable leak-proof container capable of containing any spillage and made from a material permitting subsequent disinfection. Operations involving the potential generation of aerosols (eg tissue homogenisation, sonication of genetically modified organism) must be performed in a Class 1 microbiological safety cabinet. Where the samples have been lysed (ie all cells dead) beforehand by the addition of detergent or strong alkaline denaturant then the operations may be performed in a fume cupboard. Dispose of contaminated GMO waste in biohazard waste bins for incineration by an external waste disposal company. Decontaminate liquid cultures with chloros or 1% virkon for at least 20 minutes before pouring down the sink into the drains.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All experiments involving GMOs must be risk assessed in accordance with procedures laid down by the GMO safety committee. All work must be approved by the biosafety committee before experimental work is begun. A risk assessment system will be stored on the company shared drive electronically, and also a filed paper copy.
### GM Centre Number: 848

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**Name**

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**Campus Estate or Research Centre**

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**Building**

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**Comments**

Date at Which Additional Info Submitted:

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Page 11665 of 15326
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee will consist of the Head of Research, the Laboratory Manager, the Site Safety Manager and an expert Molecular Biologist. The committee will meet as necessary to discuss new work as requested whether it falls within the current containment level or if an increase in containment level is required. The committee will also meet quarterly to review the progress of GMO studies, whether new work has been requested or not.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Tick if confidential</td>
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Bacteriology Yes  Parasitology  Transgenic Birds  Microbiology Research Yes
All work will be conducted with commercially available attenuated strains. All material resulting from studies with GMMs will be autoclaved or treated with a suitable antibacterial detergent (e.g., Triagene) and then transported for incineration by a registered waste carrier and disposer or disposed of to the sewage system respectively. GMM material undergoing inactivation procedures will be periodically tested for viability using standard plate counting methods.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The genetic modification safety committee (GMSC) considers that the work outlined in the risk assessment will be confined to specific laboratories and falls well within the existing safety protocols. As a result the work poses minimal risk to individuals working with the genetically modified micro-organisms. As the work involves commercial attenuated strains there will be minimal risk to the environment following accidental release. Equally, as this work is confined to the laboratory and will be used as a laboratory tool it poses minimal risk to the associates and animals housed on site. Therefore, the GMSC considers that this work requires level 1 containment.

---

Project Ref 848/03.1

Date Ackn'd 04/12/2003

CU2 Project Title THE GENETICALLY MODIFIED MICROORGANISM WILL BE USED IN A COMPARATIVE SCREEN OF ORAL BACTERIAL ISOLATES FOR EXOPOLYSACCHARIDE (EPS) PRODUCTION

Date Project Ceased

Class 2

CultureVolClass2 < 1 litre

Non-GMM Consent Granted not applicable

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Pseudomas aeruginosa strain uu3OR2::CM is a non-EPS producing mutant that will be used as a negative control in screening studies for an oral health research programme.

**Recipient or parental organism**

P. aeruginosa strain 883OR2::CM is a gift from Institute of Infections and Immunity and Inflammation, University of Nottingham. P aeruginosa strain 8830R2::CM is a mutant of P. aerugompsa straom 8830, which is a mucoid cystic fibrosis isolate (Darzins & Chakrabarty, 1994, J. Bact., 159:9-18).

**Host/vector system**

N/A

**Origin & function**

The mutation of P. aeruginosa strain 8830R2::CM is in the algR2 gene, which is a regulator of alginate production. Alginate is a virulence factor in P. aeruginosa pathogenesis. P. aeruginosa strain 8830R2::CM will be used as a negative control in EPS producing oral isolate screening and as such will not be further modified.

**Evaluation of foreseeable effects**

P. aeruginosa is an opportunistic pathogen of humans and animals and is a class 2 pathogen. Although rarely, if ever, infectin healthy tissues, infections in immunocompromised individuals and cystic fibrosis patients may be fatal. The mutation in the algR2 locus of P. aeruginosa strain 8830R2::CM results in a lack of alginate (EPS) production. Key to the pathogenicity of P. aeruginosa, is its ability to form biofilms and hence resist antibiotic therapy. EPS (alginate in P. aeruginosa) is vital for biofilm formation and as such is an important virulence compared to the wild type strain 8830. In accordance with safe microbiological practice the GMO would still be treated as a class 2 pathogen and would be handled under containment level 2 conditions.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
All broths, agar plates, biological waste and laboratory consumables which have been used in experiments with the GMM will be autoclaved. All experimental materials, except glassware, will be disposed of by incineration by Whiterose Environmental. Following autoclaving any glassware will be washed in a suitable detergent and reused as appropriate. A record will be kept of all GMO waste disposals.

The genetic modification safety committee (GMSC) considers that the work outlined in the risk assessments will be confined to specified laboratories and falls well within the existing safety protocols. As a result the work poses minimal risk to individuals working with the genetically modified micro-organisms. As this work is confined to the laboratory and will be used as a laboratory tool it poses minimal risk to the associates and animals housed on site. Equally, by following the Waltham Centre Laboratory standard operating procedures, utilising the appropriate safety equipment (eg class 2 safety cabinets) and undertaking the waste disposal methods outlined previously in this application, there will be minimal risk to the environment as a result of work with this organism. The GMM is derived from a class 2 pathogen, therefore, the GMSC considers that this work requires level 2 containment.

Project Containment

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Project Ref 848/07.1

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<td>23/02/2007</td>
<td>cDNAs encoding a series of proteins (e.g. COX2, haem oxygenase 1) of both canine and feline origin will be amplified by PCR. These will be introduced into the african green monkey cell line, COS1, in order to produce recombinant protein.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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</table>
Expressed recombinant proteins will be used to measure antibody cross reactivity and/or tested for bioactivity in order to produce functional assays for the proteins.

The African green monkey cell line, COS1, was derived from the parental CV1 cell line by transformation using a plasmid containing the SV40 virus sequence containing a deletion in the viral origin of replication (Gluzman et. Al.). The cell line is permissive for growth of SV40 but no free viral DNA was detected in this cell line. As SV40 infection causes a cytopathic effect in COS1 cells and this is not seen during normal culture of these cells it can be assumed that no viable virus is produced by the cells.

cDNAs will be inserted into the commercially available plasmid vector pCDNA3.1. This will be introduced and amplified in E. Coli K12.

Origin & function
DNA encoding a number of canine and/or feline genes will be amplified by polymerase chain reaction and inserted into the plasmid vector. These plasmids will be introduced into the COS1 cell line. Driven by the SV40 origin in the plasmid vector this will amplify to a high number episomally and result in a high level of expression of the protein encoded in the cDNA.

None of the recombinant proteins being expressed will have the same sequence as the orthologous human protein nor will any proteins suggested to be oncogenic be expressed. The COS1 cell line is incapable of producing viable SV40 virus by itself. There is a very slim possibility that the inserted SV40 DNA could recombine with the SV40 origin of the replication contained in the pCDNA3.1 plasmid vector to produce a viable SV40 virus. SV40 virus has not been proven to be pathogenic in humans but viral DNA has been isolated from a number of human tumours. Because of this, this work will be carried out under level II restrictions.

None of the recombinant proteins being expressed will have the same sequence as the orthologous human protein nor will any proteins suggested to be oncogenic be expressed.

SV40 virus has not been proven to be pathogenic in humans but viral DNA has been isolated from a number of human tumours. Because of this, this work will be carried out under level II restrictions.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Transfected COS 1 cells will be killed by a variety of methods including treatment with 100% metanol, 1% paraformaldehyde and/or heating to 95C with 2% SDS, 1% beta-mercaptoethanol. These methods should result in 100% cell death and this will be confirmed via microscopic examination. All supernatant will be treated with a 1:50 dilution of the viricidal disinfectant trigene. Following confirmation that all GMO's have been killed, all products will be transferred offsite for incineration.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

It was recommended that the size and sequence of the insert once cloned was determined (using any of the universal primers spanning the cloning sites - from each direction) prior to commencing transfection experiments in the COS 1 cells. This would ensure the correct insert had in fact been inserted before any protein was produced.

The GMSC were satisfied with the list of genes initially identified for this study. Any intentions to clone and express any additional genes in future would have to be formally reviewed and recorded by the GMSC prior to work commencing.

Project Containment

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02/03/2022
GM Centre Number: 849

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Name

CAMBRIDGE MEDICAL INNOVATIONS LIMITED

Name 2

Department

Campus Estate or Research Centre

ST JOHN'S INNOVATION PARK

Road Name

COWLEY ROAD

Building

EDINBURGH HOUSE

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB4 0DS

Country

ENGLAND

Tel Number

01223 225 333

Fax Number

01223 225 322

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Composition: Lab Manager/HSEQ, Biological Safety Officer/Senior Scientist (Chairperson), Employee Representative/Microbiologist & CEO.

The GMSC meets quarterly whilst Akubio is finalising the scope of the GM work it requires to carry out. Once this is done the GMSC will meet half yearly. The GMSC reviews all GM work to be carried out and ensures safety levels are maintained.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

All disposable material will be autoclaved at 121°C for 20 minutes. Following this process the material will be sent off-site for incineration. The autoclave is a new LTE Osprey 150 which will be maintained to the manufacturers recommendations and validated at least once a year. Autoclave indicator tape will also be used to ensure this process is effective.

Glassware and hard surfaces which have come into contact with any GMM will be cleaned with a 1% solution of Virkon (as per manufacturer’s recommendations) for at least 10 minutes. 1% solution of virkon are stable for 7 days so fresh solution will be made on a weekly basis or sooner if required.

Spillages of solutions containing a GMM will be treated by covering with Virkon powder until the liquid is absorbed. The powder will then be scrapped into a suitable container for disposal.

Laboratory clothing coming into contact with a GMM will be soaked in a 1% solution of Virkon for 10 minutes.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

As both the adenovirus and HSV to be used have been genetically modified to make them safe to use in a class 1 environment and therefore they pose no risk to humans, animals or the environment the GMSC will allow this project to begin subject to the approval of the HSE for the premises notification form CU1 2000 (Notification of intention to use premises for contained use activities).
### GM Centre Number: 850

**Data Premises Notified (Originally)**: 12/12/2002  
**Transferred from 1992 Regs?**: N  
**Transitional Premises Class**:  
**Data Premises Closed**: 01/08/2006  
**Transitional Premises Emergency Plan Required?**:  
**Non-GMMs**: N  
**Withdrawn**: N  

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**Comments**

**Date at Which Additional Info Submitted**: 02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Xcellsyz is a spinout company from the University of Newcastle and its GM work has been assessed by the GM Committee of the University (GM Ref 540). There is a Committee Chair, a Secretary and a Biological Safety Officer.

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<thead>
<tr>
<th>Laboratory</th>
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Bacteriology    Parasitology    Transgenic Birds    Microbiology Research
Virology        Transgenic Animals Transgenic Fish Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste will be treated as clinical waste and, after de-activation by autoclaving, will be removed by a commercial contractor. All work is class 1 and an emergency plan is not applicable.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

(i) Enclosed are details of the application submitted to the University GM Committee.
(ii) The application was approved, subject to notification of the premises to the HSE and to signing of a Memorandum of Understanding between Xcellsyz and the University.
GM Centre Number: 851

Data Premises Notified (Originally) 12/12/2002
Transferred from 1992 Regs? N
Transitional Premises Class

Data Premises Closed 31/08/2018
Transitional Premises Emergency Plan Required? N
Non-GMMs N
Withdrawn N

Name
TWISTDX LTD

Name 2
Department

Campus Estate or Research Centre
UNIT 9 COLDHAM'S LANE BUSINESS PARK

Road Name
NORMAN WAY

District

Town
CAMBRIDGE

County
CAMBRIDGESHIRE

Postcode
CB1 3LH

Country
ENGLAND

Tel Number 01223 496 700
Fax Number 01223 496 038

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted
02/03/2022
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee representative of ASM Scientific Ltd will be the Director of Research. This representative will meet at least twice annually with an on-site safety committee at the BBT (Babraham Bioscience Technologies), within which the company is located. This committee serves to uphold and enforce on-site standards in accordance with a policy and code of practice drawn up for the BBT site. Each on-site company has a representative on this committee.
Class 1 activities at ASM Scientific Ltd will comprise the propagation of recombinant plasmid vectors in standard laboratory strains of E. coli ie strain K12 and its derivatives. Waste management of bacterial cultures, and contaminated materials such as handling gloves and plastic ware will be as follows:

1. Liquid cultures and infected media will be sterilised prior to disposal by treatment with chloros or Virkon. Following complete sterilisation solutions will be disposed of by foul sewer.

2. Contaminated clothing, plasticware, culture dishes and other items will be placed into dedicated autoclave bags and subsequently autoclaved. Disposal of sterile autoclaved rubbish will then occur through on-site laboratory waste disposal service.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
The company is based on a research campus, Babraham Bioincubator, which has its own safety committee and infrastructure to which we must conform. (see letter of file dated 10.12.02)
GM Centre Number: 852

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Name

BIOSYNTHA TECHNOLOGY LIMITED

Name 2

Department

Campus Estate or Research Centre

BIOPARK HERTFORDSHIRE

Road Name

BROADWATER ROAD

Town

WELWYN GARDEN CITY

County

HERTFORDSHIRE

Postcode

AL7 3AX

Country

ENGLAND

Tel Number

01707 284 511

Fax Number

01707 281 059

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

name change from Novacta Biosystems Ltd 13/04/2012

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Committee Members:
From Novacta: Research Director (Company Safety Officer), Fermentation Group Leader (Biological Safety Officer), Molecular Biology 1 Group Leader, Molecular Biology 2 Group Leader and a Molecular Biologist.
From Norwich Bioincubator: The Resources Manager.

The Committee will meet annually to review all risk assessments and HSE notifications. Additional meetings will be held to consider new risk assessments and notification requirements as required. All assessments and notifications will be circulated by email and comments from the committee taken into account prior to any new procedures being adopted. Any assessments which fall outside the expertise of more than three of the committee will be sent to external advisors drawn from experienced scientists working in the relevant area on the Norwich Research Park.

### Laboratory

- Level 1 (GMMs)
  - Yes

- Level 2 (GMMs)

- Level 3 (GMMs)

- Level 4 (GMMs)

- Non-microbial

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02/03/2022

Page 11683 of 15326
Solid biological waste will be placed in unsealed bags placed in lidded but unsealed plastic boxes and removed from the laboratory to the John Innes Centre central autoclave service where it will be sterilized. The autoclave cycle will include heating the material to at least 120 degrees C for 15 minutes to ensure 100% kill for all material.

Liquid waste and contaminated glassware will be treated in one of two ways:
1) Autoclaving: The autoclave cycle will include heating the material to at least 120 degrees C for 15 minutes to ensure 100% kill for all material.
2) Hycolin disinfectant: Hycolin disinfectant, diluted according to the manufactureres instructions, overnight at room temperature.

In either case an initial check will be carried out the first time the process is carried out to check that no culturable cells remain before disposal. This check will be repeated periodically depending on classification of risk and whenever waste from different cell types or quantities of culture are produced to ensure that the procedure results in a 100% kill.

Members of the Genetic Modification Safety Committee have read the attached risk assessment and have agreed with its conclusions.
To produce a long-chain polyunsaturated fatty acid for application as a dietary supplement

The host strain Pythium irregulare is regarded as a plant pathogen. Pythium strains are generally less damaging than Phytophthora, but can cause damping-off of seedlings, attacking the roots and stems of a variety of plants including ornamentals. P. irregulare is widespread globally and is indigenous to the UK. Long-chain polyunsaturated fatty acids do not represent a component of plant pathogenicity or virulence, and the likelihood that increased production of these or any other genetic manipulation involved in fatty acid biosynthesis would increase the virulence of this strain is considered highly unlikely.
The host is Pythium irregularare, an oomycete regarded as a plant pathogen. The vectors to be used will be standard oomycete, fungal or yeast vectors, eg pTOR, widely used for oomycete genetic manipulation. pTOR is selectable with the G418 antibiotic.

Origin & function

Much of the work will involve upregulation or deletion of host genes, but some heterologous genes will be used. These will be derived from fungal, plant or bacterial sources, and be well-characterized genes involved in fatty acid biosynthesis.

Evaluation of foreseeable effects

P. irregularare is classified as ACDP1, having no known pathogenicity to man. The E.coli hosts to be used as intermediates for cloning are non-pathogenic laboratory strains (K12 or BL21) and do not represent a human health problem. In the case of E.coli strains, multiple mutations and nutritional requirements ensure that prolonged survival and propagation in the environment is unlikely. Therefore the overall risk to human health and to the environment of these disabled E.coli strains is low. The use of nonmobilisable vectors minimises the risk of transfer to other microorganisms. Any heterologous genes to be used will represent well-characterised genes for enzymes of known function or homologous to genes of known function, and are highly unlikely to influence the pathogenicity or survival and propagation of the host strains. In the very unlikely event of a construct escaping into the environment and surviving, it is difficult to conceive of any hazards arising that would be greater than those represented by naturally-occurring environmental microorganisms including native Pythium irregularare strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated materials will be autoclaved (124°C/30min) or treated with disinfectants (0.5% Presept, minimum 10 minutes contact time) as appropriate. These procedures have been shown to give 100% kill. In the event of accidental breakage of a culture vessel, escape of the organism will be contained, either by the incubator or secondary containment (i.e. tray, carrying basket). Spillages will be treated with antimicrobial agents (eg 0.5% Presept) as appropriate. Personal contamination (skin & clothes) is the most likely accident to result from such cultures. The use of suitable and appropriate protective clothing (lab coats, gloves etc) should enable effective decontamination. Contaminated lab coats or other clothing must be autoclaved. Gloves and all other disposable items must be double-bagged and autoclaved before disposal. Escapes should be negligible.
Classification Class 2 considered appropriate as the organism is a plant pathogen, and DEFRA require such strains to be handled under category 2 conditions. The genetic modifications planned should not increase pathogenicity or virulence of the strain or represent any threat to human health or the environment.

### Project Containment

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

GMSC composition: Chair, Biological Safety Officer, Head of Research Administration, Secretary, representatives of all groups carrying out GM research.

Meets approximately twice per year, or more frequently if new projects need consideration.

<table>
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<th>Animal Unit</th>
<th>Growth Room</th>
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<th>Large Scale</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste produced falls into either group A, B, or C as defined by the Health Services Advisory Committee.

Liquid waste (maximum culture volumes of 500 ml) will be chemically treated with Virkon powder to a final concentration of 1% solution for at least 30 minutes (the manufacturers specified conditions for inactivation), before disposing of via the drainage system with copious amounts of water.

Solid waste will be chemically treated with a 1% solution of Virkon for 30 minutes (the manufacturers specified conditions for inactivation), then placed into autoclavable waste bags and autoclaved at 123 degrees C for 15 minutes to inactivate. Sharps waste will be placed in containers conforming to BS7320 and will be autoclaved at 123 degrees C for 15 minutes to inactivate. The load temperature of the autoclave will be monitored by means of a UKAS/NAMAS certified internal temperature probe. Additionally the autoclave will be tested and calibrated four times per year.

Carcasses and soiled bedding from transgenic animals will be autoclaved at 123 degrees C for 15 minutes to inactivate. Inactivated waste will then be placed into sealed, yellow, printed pathology waste bins meeting BS381C. Individually numbered ties will be attached.

Sealed bins and bags will be stored in locked clinical waste bins before collection by designated staff for transportation to a registered off site facility for incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment was approved as a Class 1 activity at the local GM committee meeting on 18 December 2001. Minor modifications were made to comply with current policy on GM waste disposal.
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Name

OXFORD CARDIAC PHARMACOLOGY LIMITED

Name 2

Department

Campus Estate or Research Centre

MEDAWAR CENTRE

Road Name

THE OXFORD SCIENCE PARK

District

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX4 4GA

Country

ENGLAND

Tel Number

01865 405158

Fax Number

01865405158

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:
There is a pre-existing GMO committee at the Oxfordshire BiotechNet that comprises representatives from the individual companies and a representative from the University of Oxford.

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<th>Glass House</th>
<th>Large Scale</th>
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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
Liquid waste is disinfected with Virkon solution (Antec International Ltd. USA) the appropriate concentration and time of immersion will be used according to manufacturer's instructions. Treated waste is then disposed of as part of normal laboratory waste. Disposable plasticware and glassware is disinfected with Virkon as above and then held in a closed container pending sterilisation by steam (121°C for 20 min). This waste is then collected by contracted waste disposal services and incinerated. The autoclave used for this purpose is validated routinely by Oxfordshire BiotechNet.

We would expect the level of kill to be high using these procedures with surviving GMOs to be negligible and to be 'below detectable level'.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

GM Committee was happy with the risk assessment made and consequently made no recommendations.
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

COMPOSITION
Vice President Biology (Chair)
Biology Safety Rep
Facilities and Laboratory Manager
Senior Biologist

ROLES
The Chairman makes the final decisions
The Biology Safety Representative deals with the safety aspects of the operation
The Facilities and Laboratory Manager deals with the practical aspects of the safety concerns (air flows, safety cabinet servicing, waste disposal etc)
The Senior Biologist deals with the organisms that need to be discussed and brought in to the company.

OPERATION
The GMS committee meets quarterly (or more often if required)
The results of the meetings are reported at the Company Health, Safety and Environment Committee meetings. The minutes of these meetings are reported to the Board.
The GMS committee decides the risk category of the work involved, and the necessary safety precautions to be carried out.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes
Level 2 (GMMs)
Level 3 (GMMs)
### Level 4 (GMMs)

**Non-microbial**

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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

All waste material will be autoclaved before being disposed of in clinical waste bins which are then collected by an authorised company (at present White Rose Environmental) for incineration.

**Tick to confirm that you are attaching a summary of the risk assessment** Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment** Y

**Please enter comments of the GM safety committee on the risk assessment**

All the organisms to be used in this initial screen are hazard class 1.

All work with these organisms will be carried out by trained staff, in a specified tissue culture laboratory equipped with a double hepa filtered class II biological safety cabinet. All work with exposed cells will be carried out in the cabinet. At all other times cells will be stored in a closed container.

All waste will be treated with the appropriate disinfectant if necessary, autoclaved, and then placed in a clinical waste bin in a clinical waste bag. The bins are collected regularly by an approved waste management company (currently White Rose Environmental) and taken for incineration.

It was concluded that there is no safety risk to either the staff, public or the environment if these precautions are followed at all times.
**GM Centre Number: 857**

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**Department**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Yes

Give brief details of the genetic modification safety committee

- Research Tutor - Chairman, Lecturer/Researcher, 2x Research Assistants/Secretary.

All levels of responsibility and experience have been included on the committee, which will meet twice a year.

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Tick if confidential

- [ ] Yes

- [ ] Yes

- [ ] Yes

- [ ] Yes

- [ ] Yes

- [ ] Yes

- [ ] Yes

- [ ] Yes

- [ ] Yes

- [ ] Yes

- [ ] Yes

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

The waste generated from the production of the recombinant antibodies will be subjected to initial sterilisation with Virkon to prevent both viral and bacterial survival. Subsequently the waste will be autoclaved, this will result in 100% kill of any GMOs and result in destruction of all genetic material. All processes involving GMOs will be logged by Supervisors and the Biological Safety Officer and will include the monitoring of waste disposal. The autoclave used is serviced and certified in good working order every 6 months.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC has reviewed the Risk Assessment submitted to them for the production of recombinant antibodies. They have concluded that the process and resultant GMO pose no danger to human health or the environment.

I have enclosed a copy of the minutes of the first meeting of the GMSC where the Risk Assessment for recombinant antibodies was discussed.
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The GMSC consists of 8 permanent members.

- 2 users of the proposed GMO laboratory (both of whom have previous molecular biology/practical GMO experience)
- The site safety officer
- The local R&D safety advisor
- A representative from the current microbiology team
- A representative user from the current class 2 laboratory
- Two senior managers (including the head of R&D)

In addition to the above we have enlisted the aid of a third party (an external academic with experience of working with GMOs/GMO safety issues) to give general advice to the committee on an ad hoc basis. During this initial set up phase for the laboratory the GMSC will be meeting at least once a month (or more frequently if required) to identify any issues that arise during the course of the notification and risk assessment process. It is anticipated that once appropriate procedures and practices are in place, the group will meet every 2-3 months to review current practices and any new risk assessments that have been submitted. Minutes will be taken at each meeting and specific comments and recommendations from the committee will be recorded therein.

Laboratory

- Level 1 (GMMs): Yes
- Level 2 (GMMs): Yes
- Level 3 (GMMs)
- Level 4 (GMMs)
**Non-microbial**

Other (please specify)  

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Other(s)  

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<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic</th>
<th>Microbiology</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plants</td>
<td></td>
</tr>
</tbody>
</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

NB: The initial programme of work has been assessed as predominantly class 1 with some class 2 measures thus we are simultaneously submitting an appropriate class 2 activity/class 2 risk assessment with this notification. Waste management and disposal measure are outlined in that document.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

N/A

---

**Project Ref 858/03.1**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<tr>
<td>12/03/2003</td>
<td>GENERATION AND EXPRESSION OF ANTIBODY FRAGMENTS IN ESCHERICHIA COLI FROM A PROTEIN EXPRESSION CDNA LIBRARY.</td>
<td>Class 2</td>
<td>&lt; 1 litre</td>
<td></td>
<td>not applicable</td>
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</table>
Purposes of the contained use

The proposed work programme seeks to generate and express single-chain antibody fragments in Escherichia coli (TG1) from a protein expression cDNA library. This will be performed using a phagemid/helper phage expression system. These fusion proteins (consisting of antibody fragments joined to purification tags or standard reporter enzymes) will then be used in proof of principle experiments to examine the binding properties of these fragments in in vitro assay formats. The contained use risk assessments cover the steps required to produce such proteins in solution in sufficient quantities for use in such experiments (i.e., 0.1-10 mgs of protein). Whilst we will seek to maintain the fragments inherent biological activity (i.e., their ability to bind target antigens) the proteins are not intended for use in vivo. A variety of target binding partners are under consideration (said targets are confidential due to commercial sensitivity/IP consideration).

Recipient or parental organism

The recipient/host organism (TG1 strain of E. coli) is generally recognised to be a non-colonising and disabled micro-organism that is unlikely to survive outside of a laboratory environment. It is not considered to be pathogenic to humans, animals or plants. As such it is considered to be equivalent to ACDP hazard group 1. The original genetic material (i.e., coding DNA that is to be expressed in the host via a phagemid/helper phage system) was obtained from a (non-human) mammalian source via a third party organisation. The parental/donor mammals are bred commercially and the genetic material obtained via ethically approved means (by the above-mentioned third party).

Host/vector system

As stated above, the chosen host organism (TG1 strain of E. coli) is a well-characterised laboratory strain commonly used in GMM work. The target genetic material will be expressed in a non-pathogenic phagemid plasmid (derived from pHEN1, containing an antibiotic (ampicillin) resistance gene) and helper phage M13K07 (an M13 variant with a kanamycin resistance gene) will be used to facilitate transfer of the phagemid to the host organism. Whilst the phagemid in itself is considered mobilisation-defective, the presence of the helper phage does increase its potential mobility to the host and other micro-organisms.

Origin & function

The inserted genetic material is derived from a (non-human) mammalian source and expressed a fragment of the antigen-binding site of an antibody. The coding regions, when expressed as fusion-proteins are for in vitro assay use only and are not thought to be toxic or represent a hazard to humans at the anticipated levels produced here (0.1-10 mgs of protein) or through reasonably foreseeable routes of accidental exposure. Further information is given in the confidential section of this document. The exact nature of the antibody fragments (including the original parental animal from which the original mRNA/cDNA was extracted) is being withheld for reasons of commercial sensitivity.

Evaluation of foreseeable effects

The antibody fragments will be expressed as fusion proteins to standard purification tags/reporter enzymes (e.g., hexa-his tags). The fragments are intended for laboratory
scale proof of principle studies in in vitro assay formats. They are not destined for in vivo use nor are the fragments considered to be inherently toxic to humans, animals, insects or plants. As such the hazards associated with the proposed GMM (E. coli TG1/antibody fragment coded by the phagemid) is deemed to be low. However, accidental transfer of the phagemid via the helper phage to other micro-organisms may represent a slightly higher risk to human health (primarily by conferring antibiotic resistance to the new host rather than inherent dangers associated with the expressed fusion protein). As such it is important that phagemid/helper phage be segregated from other procedures within the lab (ie where both the helper phage and the phagemid are present at high titres eg during transformation/infection of the TG1 etc.) The use of microbiological safety cabinet (Class 2) will help limit the inherent risk to an acceptable level.

In terms of environmental impact, the hazards associated with the proposed GMM (ie TG1/fragment insert) are deemed to be low due as (1) the host strain a well characterised/disabled laboratory strain that is unlikely to survive in the environment; (2) the host is non-pathogenic to humans, animals, insects and plants and (3) the expressed antibody fragments are thought to be both non-toxic and non-oncogenic. However there is a potential hazard associated with the accidental transfer of antibiotic resistance genes to other micro-organisms due to the use of the phagemid/helper phage system. The use of Class 2 containment measures (including isolation of the phage work to a dedicated class 2 microbiological safety cabinet, the combined Virkon S treatment/autoclave based inactivation regime that are to be implemented in the waste disposal measures should reduce any environmental risks to acceptable levels.

Based on the above information (given in sections 5-7) the level of hazard associated with this work predominantly falls within the Class 1 activity area. However, additional class 2 measures are being applied to limit hazards associated with the phagemid/helper phage system. These include the use of a microbiological safety cabinet (Class 2) for handling of all solutions that contain high titres of phagemid and/or helper phage enriched cultures. In addition, all work will take place in a suite of rooms that are self-contained, hepa-filtered and under negative pressure. Waste disposal measures entails chemical inactivation of phage followed by autoclaving. We propose that these measures (class 1 with some class 2 measures) classify the overall work programme as Class 2 and we intend to comply with Class 2 requirements throughout.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogations from full containment are being sought for this work.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMM contaminated waste will be dealt with in the following way;

* Cultures/glassware/disposal plastic-ware and consumables that have been exposed to non-transformed micro-organisms and phagemid carrying micro-organisms in the absence of helper phage (ie where the phagemid has limited mobility) will be autoclaved (at 134 degrees C for 30 minutes) within a reasonable time period (ie within 24 hours of the work being completed). The self-contained molecular biology laboratory has autoclave facilities en-suite. Autoclaving efficiency can be monitored by chart-based time and temperature readings. In the event of autoclave failure, additional autoclaves are available outside the molecular biology suite.
* Cultures/glassware used to grow cultures that have been in contact with high titre cultures of phagemid/helper phage will be treated with solutions of Virkon S (a commonly used, well established virucidal agent) and allowed to stand overnight prior to autoclaving as above.
* Disposable plastic-ware/consumables that have been in contact with high titre culture of phagemid/helper phage (including micro-organism cultures that have been infected with phagemid/helper phage combinations) will be discarded into waste containers containing 1% solutions of Virkon S and allowed to stand overnight prior to autoclaving as above.
* After autoclaving, all disposal solid waste (eg disposable plastic ware and consumables) will then be transferred off site. Autoclaved liquid waste will be disposed of via the main drainage system.
* Work areas potentially exposed to high titre phagemid/phage will be cleaned/wiped with 1% Virkon S solutions immediately before and after use.
* Large volume spills of liquids containing high titre cultures of phagemid/helper phage will be treated with undiluted Virkon S powder (to absorb the liquid). This waste will then be treated as above and the area cleaned/wiped with 1% Virkon S solutions immediately following the spill.
The above measures are based upon validated, well-established regimes for handling E. coli based GMMs and bacteriophage based work. As such, full implementation of the above procedures should result in a 100% kill of GMMs and helper phage. As such these measures have been deemed to be sufficient for class 2 work.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

Please enter comments on the GM safety committee on the risk assessment

Having reviewed the risk assessments, the committee (aided by external advice from a consultant academic with experience in the field) was satisfied that considerable effort had been made to identify potential hazards and minimise the likelihood of them occurring. It was noted that the greatest risk appeared to be associated with accidental infection of bacterial cultures (via the phagemid/phage transformation system) rather than being inherent to the planned experimental output (ie the recombinant antibody fragments). They agreed that extra emphasis should be placed upon the safe handling and disposal of phage-based cultures (eg the use of a dedicated class 2 cabinet microbiology cabinet and Virkon S disinfection/autoclaving). It was also agreed that the proposed waste disposal measures represent well-established procedures that should be more than sufficient to inactivate both the GMMs and the phage.

It was pointed out that in the future, other microbiology based studies may occur within the molecular biology suite of rooms (ie work which is unrelated to the GMM studies but that required access to similar equipment eg culture hoods). Providing that the phage work was limited to a dedicated hood (and other users were prohibited from using it for other purposes), it was deemed that accidental infection was unlikely to occur (and thus the level of risk to human health and the environment was low).

As such the GMSC agree with the final classification of Class 1 plus some class 2 measures (equating to class 2 overall). This classification is deemed cautious but warranted under the circumstances (particularly as the proposed experiments are to be the first GMM work to be performed in the refurbished laboratory suite).

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L3 L4 L2 L3 L4 L2</td>
<td>L3 L4 L2 L3 L4</td>
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**GM Centre Number: 859**

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<td>Non-GMMs</td>
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<tr>
<td>Withdrawn</td>
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**Data Premises Closed**

**Emergency Plan Required?**

**Name**

Metrion Biosciences

**Department**

**Campus Estate or Research Centre**

Suite 1, Riverside 3

**Road Name**

**District**

Granta Park

Great Abingdon

**Town**

Cambridge

**County**

Cambridgeshire

**Postcode**

CB21 6GP

**Country**

England

**Tel Number**

0

**Fax Number**

01223 919100

**E-mail**

**HSE Division**

East and South East

**Comments**

Name change from Eention Discovery Ltd 01/08/2015

**Date at Which Additional Info Submitted**

01/05/2004

02/03/2022
## Premises Addresses

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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Positions are altered yearly.
Chairperson, Biological Safety Officer, GM Worker, Non-Scientist.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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<td>Non-microbial</td>
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Other (please specify)

Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research |
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02/03/2022
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<tbody>
<tr>
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</table>

Other(s) 

transfection of mammalian cells with ion channel encoding genes

For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste is treated with 2500ppm chlorine
Solid waste is autoclaved at 121°C for 30 minutes. A record is kept of individual autoclave runs.
All waste is double bagged and disposed of via White Rose Incineration site

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Xention has the reviewed and endorsed classification of the programme (risk assessment attached) to be class 1.
GM Centre Number: 860

Data Premises Notified (Originally) 18/03/2003

Transferred from 1992 Regs? N

Transitional Premises

Class

Emergency Plan Required?

N

Data Premises Closed 03/12/2015

Transitional Premises

Non-GMMs N

Withdrawn N

Name

PARADIGM THERAPEUTICS LIMITED

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

418 CAMBRIDGE SCIENCE PARK

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB4 0GZ

Country

ENGLAND

Tel Number 01223 477943

Fax Number 01223 477911

E-mail

HSE Division EAST AND SOUTH EAST

Comments

GM CENTRE CLOSED AND MERGED WITH GM3043 TAKEDA CAMBRIDGE LTD

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The committee is composed of the laboratory senior scientist and the heads of protein Chemistry, Molecular Biology and Cell Culture. The committee will meet not less than four times per year to review the use of GMOs. However, it is not anticipated that there will not be any major changes in the types and varieties of GMOs in use.

<table>
<thead>
<tr>
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<td>Other (please specify)</td>
<td>Tick if confidential</td>
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</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

The GMMs that are currently made by Paradigm are completely destroyed by standard autoclaving. All laboratory waste (for example gloves, used plasticware, pipettes, and anything else contaminated) will be subject to autoclaving before leaving the premises. In the case of liquids, a strong disinfectant (for example Chloros) will be used to treat samples and regular testing will take place to gauge the level of killing. For minor spillages it is anticipated that a solution of 70% Ethanol or 60% Isopropanol will be used to decontaminate - any solid waste from the decontamination process will be autoclaved before leaving the building. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are deemed inappropriate. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. In accordance with Good Laboratory Practice, Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Hands are washed following removal of gloves.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Paradigm has been working under licence #GM 253 (to JH Rogers of the Physiological Laboratories, University of Cambridge) with the GMMs outlined in Appendix 1. In accordance with this, the GMSC has recommended that Paradigm continue to work only with fully verified commercial sources of Host strains and vectors to maintain all work at Hazard Level 1 and Containment Level 1.
### COMMERCIAL ELISA KITS CONTAINING HUMAN SOURCED MATERIALS, TOTAL RNA EXTRACTION FROM HUMAN TISSUE SAMPLES AND USE OF ATTENUATED ADENOVIRUS IN THE TAG ON DEMAND SYSTEM FOR PROTEIN EXPRESSION STUDIES

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### Project notified under transitional arrangements

**Withdrawn**

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<tbody>
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</table>

**Historical Significant Changes**

- Project transferred to GM3043 on merger with Paradigm Therapeutics

**Historical Date of Additional Info**

**Significant Date of Change**

**Historical Date of Additional Info**

**Date of Significant Change**

### Project Additional Information

#### Purposes of the contained use

1. Measure the concentration of specific materials in samples.
2. Use extracted RNA for cDNA synthesis for expression profiling of human genes.
3. To transiently generate proteins incorporating an antibody detectable tag to confirm protein expression.

####Recipient or parental organism

- Adenovirus - recipient - mammalian cells grown in culture.

####Host/vector system

- Adenovirus
  - attenuated (non-replicative) adenovirus particles supplied as a suspension
  - no production of virus to be carried out

####Origin & function

- Adenovirus
  - Originator: Invitrogen Corporation
  - the virus suspension is supplied frozen by the above company
  - an aliquot of supernatant is added to cultured mammalian cells and allowed to infect them
  - the virus encodes a mutated tRNA that permits read through of the TAG stop codon in the gene sequence
  - mRNA encoding a TAG stop codon is read through and the subsequent nucleotide sequence used to add additional amino acids to the protein being synthesised
  - in recombinant cells the cDNA encoding the gene of interest contains a TAG stop codon followed by gene sequence encoding a number of amino acids that when synthesised generate an epitope that can be recognised by an antibody
  - the addition of the epitope will allow confirmation of protein expression of the gene of interest in the recombinant cell line
Evaluation of foreseeable effects

The adenovirus particles can infect human cells. In the unlikely event of a user being infected the following effects are likely to happen:

1. Viral particles will infect cells
2. The tRNA will be expressed
3. There will be read through of any mRNA which has a TAG stop codon
4. It is estimated that this will be about 33% of all transcribed genes in each cell infected
5. The adenovirus does not replicate in the human cells so any effect will be transient, a maximum of 8 days
6. The resultant change in the proteins is most likely to be toxic to the cells
7. This may result in cell death of those cells infected
8. As a result of cell death in the local area of administration and an inflammatory immune response will be elicited
9. It is not anticipated that there will be any long term effects due to the attenuated nature of the adenovirus

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

ELISA and human tissue
- human sample material and plastics contaminated with material to be treated with strong disinfectant
- human samples processed for RNA will be effectively neutralised in the extraction process
- all disinfected material will then be incinerated
- for spills, solid materials will be collected and put in a container with disinfectant, liquid spills will be treated with disinfectant and then absorbed with paper towels, the area will then be further cleaned with 1% virkon solution. All collected materials will be incinerated.

Adenovirus
- all contaminated materials and plastics to be treated with strong disinfectant
- all disinfected material will then be incinerated
- for spills, solid materials will be collected and put in a container with disinfectant, liquid spills will be treated with disinfectant and then absorbed with paper towels, the area will then be further cleaned with 1% virkon solution. All collected materials will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Paradigm Therapeutics Ltd has been working under license GM 860 with fully verified commercial sources of host strains and vectors at hazard level 1 and containment 1. Due to foreseen expansion of experimentation, the GMSC has identified that Paradigm Therapeutics Ltd applies that the license be modified to include hazard level 2 and containment 1. All ELISA materials will be sourced commercially and have undergone routine screenings for infectious agents. The adenovirus materials will be sourced commercially from a reputable company and human tissue samples if used will be stored in appropriate containers at low temperature prior to extraction in chaotrophic salts and then phenol/chloroform, which will effectively neutralise any biological hazard. The GMSC feels that these measures detailed above reduce the risk significantly.

Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The CEFAS Lowestoft Laboratory Safety Panel functions as the GM Safety Committee for GM operations at the Laboratory.
The panel comprises:
Chairman
Secretary
CEFAS Health Safety and Quality Team Co-ordinator
Managers/representatives of CEFAS Lowestoft Science Areas and Support units
Biological Safety Officer
CEFAS employee representatives
Representative of SMIT, Research Vessel management on behalf of CEFAS
Representatives of Caxton Contractors (CEFAS Facilities managers)
The Panel meets routinely every 6 months to discuss, review and advises on safe conduct of health and safety of operations and procedures including GM activities, undertaken at the laboratory. Special meetings are convened as and when needed. The Panel have access to medical advice and support via CEFAS Occupational Health Provider.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

All equipment used in GM procedures including glassware for re-use, and waste materials are autoclaved on site at 121 degrees C for at least 15 minutes, with full steam penetration. The procedure ensures 100% kill and inactivation of the GMO and sterilisation of equipment.

Validation of autoclave conditions is undertaken yearly. Between checks confirmation of correct functioning is carried out with each load using an appropriate indicator autoclaved with the load. In event of unsatisfactory operation the load is retained pending suitable repair of the autoclave.

After autoclaving waste material is disposed to landfill with consignments of general waste arising from the laboratory.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The proposal and risk assessment were discussed at a meeting of the Committee on 10th October 2002 and again on 13th February 2003. The committee were content with the assessment and endorsed Notification of intention to use premises for contained use activities.
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#### Date at Which Additional Info Submitted

| 02/03/2022          |
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Evotec OAI Genetic Modification Safety Committee (GMSC) exists as a sub-committee of experienced scientists within the Biological Safety Committee (BSC), which reports to the main Safety Committee. The latter has board level representation. The GMSC reviews GMO and Hazard I & II pathogen risk assessments. The GMSC is empowered to categorise containment level I & II activities, to provisionally approve containment level I activities, subject to review by the BSC and to recommend approval for containment level II activities by the BSC. Once approved by the GMSC, level II activities will be notified to the Competent Authority and in parallel will be reviewed by the BSC. The GMSC is chaired by the company Health and Safety Officer (MIOSHG, Dip2.OSH), and also comprises the Biological Safety Office, who has >13 years post-doctoral experience in microbial genetics in Gram-ve and Gram+ve bacteria and molecular biology in yeast and mammalian cells, and experienced scientists representing each laboratory area using GMOs. The BSC comprises these individuals with additional scientists representing each laboratory are using biological materials. The GMSC and BSC invite scientists requesting approval for risk assessments for discussion at meetings and runs with an open structure including all interested parties. Approximately 40% of the Company’s biological scientists are invited to ad hoc BSC meetings.
Contaminated solid waste and glassware is treated by autoclaving at 121 degrees C. The autoclaves are maintained and tested annually. Culture medium and liquid waste is decontaminated by chemical disinfection prior to disposal via the drains. TEGO 2000 is used for the disinfection of E. coli cultures. This is an amphoteric sanitiser recommended for use with these organisms. Waste is treated according to the manufacturer's instructions to give a final concentration of 0.5% (1:200) and left in contact for a minimum of 30 minutes. Under these conditions viability is reduced to below detectable limits.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Over-expression of the gene (def) encoding the various PDFs (Polypeptide Deformylase) is dependent on the addition of IPTG to the culture. def has the function across the range of bacterial species. It should not effect the pathogenicity of the organism, nor should it confer any extra beneficial properties to the E. coli.
Project Additional Information

**Purposes of the contained use**

To establish cell lines expressing human voltage-gated potassium ion channels. The aim is to develop cell-based assays for high-throughput screening of potential inhibitors of the ion channels.

**Recipient or parental organism**

The final recipient organism is a Chinese Hamster cell line; either lung (CHL) or ovarian (CHO) cells. These are well characterised non-human/ primate cells presenting no hazard to laboratory workers or to the environment. The intermediate hosts for production of recombinant viral particles are either BHK-21 cells (a hamster kidney cell line) or HEK293 cells (human embryonal kidney cells). These are both well characterised cell lines with a history of safe use in the laboratory and present no hazard to laboratory workers or to the environment.

**Host/vector system**

The vector systems to be used are both viral-based systems. One expression system to be used is a Moloney Murine Leukaemia Virus (MMLV)-based retroviral expression system. With this system, the production of recombinant viral particles is based on the cotransfection of HEK293 cells with three independent plasmids. The first contains the viral packaging signal and the potassium ion channel under investigation. The other two contains the other elements required for packaging; one contains the gag and pol genes, whilst the third contains the env gene encoding the envelope protein. Replication-deficient virus-containing supernatant from the cells cotransfected with the three plasmids is then used to infect target cells (Chinese Hamster Lung or Ovarian cells (CHL/CHO) for expression of the potassium ion channel. The host range of the recombinant virion is dependent on the specificity of the envelope protein encoded in the particular env-containing plasmid used. The safest vector produces an ecotropic virion capable of infecting only murine or rat cells. Other vectors to be used have a broader host range and will produce replication-deficient virus capable of infecting human cells. Work with these vectors will require handling at containment level II during the viral production stage of the process.
The second system to be used is a Semliki Forest Virus (SFV)-based expression system. Here the production of recombinant viral particles is based on the cotransfection of BHK-21 cells with three independent RNAs. The first contains the replicase gene and the potassium ion channel under investigation. The other two are helper RNAs, containing the genes for the structural capsid and envelope proteins respectively. Only the replicase-containing RNA is packaged, as the packaging signal is absent from the helper plasmids. Again, replication-deficient virus-containing supernatant is used to infect target cells (Chinese Hamster Lung or Ovarian cells (CHL/CHO)) for expression of the potassium ion channel.

Origin & function

The insert DNAs to be expressed are human voltage-gated potassium ion channel genes obtained as cDNAs and cloned into plasmid vectors. The cDNAs are then inserted into the viral expression vectors. The genes, upon expression in a host cell, enable the cells to express the potassium channels within their cell membranes and allow monitoring/screening of drug interactions with the ion channel.

Evaluation of foreseeable effects

With both systems, the production of recombinant viral particles is dependent on the cotransfection of three independent plasmids or RNAs, with the packaging components split between them. The use of these systems is recommended in the AGCM Compendium of Guidance (Part 2B, Annex 111) as it significantly reduces the frequency of recombination between the sequences, eliminating the generation of replication-competent wild-type virus. With the two systems, only the RNA for the potassium ion-channel is packaged and when the recombinant virus particles are used to infect the host cells, only the ion channel gene is expressed. The expression cassette is then unable to proceed through any further rounds of virus production, limiting the expression of the ion channel to within the initial cells infected.

With the two systems, replication-deficient virus is produced that is capable of infecting human cells. Work with these vectors will require handling at containment level II during the viral production stage of the process.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Spent cells/waste media and contaminated plasticware/solid waste will be decontaminated by autoclaving at 121 degrees C for 15 minutes. The autoclave will be maintained and tested annually.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
The vector systems to be used enable production of replication-deficient virions capable of infecting human cells. Handling of cells/viral supernatant at this intermediate stage must be carried out within a Class II MSC. Production of aerosols should be minimised and the use of sharps is prohibited.

The final modified CHL or CHO cell lines are no more hazardous than the parental cells. Incorporation of the potassium ion channel gene in these cells is non-hazardous. It enables the cells to express these potassium channels within their cell membranes and allows monitoring of drug interactions with the ion channel.

**Project Containment**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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</tbody>
</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee:

The committee is chaired by the Group Managing Director and includes the Group Technical Director and General Managers of the incinerator sites. Meetings are held six monthly as sub meetings of the Group Safety Steering Committee meeting.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<tr>
<td>Level 2 (GMMs)</td>
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<tr>
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<td>Other (please specify)</td>
<td>Incineration Disposal Facility</td>
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</tr>
</tbody>
</table>

Tick if confidential
Incineration of waste.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Incineration of waste.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessments have been carried out by the Group Technical Director and Deputy Technical Director and cross referenced to relevant Safety Instructions and Risk Assessments.

Project Ref 863/03.1

Date Ackn'd 09/05/2003

CU2 Project Title RECEIPT OF WASTE GENETICALLY MODIFIED MATERIAL FOR DISPOSAL BY HIGH TEMPERATURE INCINERATION

Class 2

CultureVolClass2 not applicable

CultureVolumeClass3-4

Non-GMM Consent Granted not applicable

Project notified under transitional arrangements N
### Project Additional Information

**Purposes of the contained use**
- Disposal of GMM waste by incineration.

**Recipient or parental organism**
- N/A

**Host/vector system**
- N/A

**Origin & function**
- N/A

**Evaluation of foreseeable effects**
- N/A

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
- N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

The attached procedure for handling genetically modified material together with the risk assessments and safety instructions indicate those measures specified as the requirements for the relevant containment level are unnecessary. We therefore request permission to omit them.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
- Disposal by high temperature incineration.
The risk assessments have been carried out by the Group Technical Director and Deputy Technical Director and cross referenced to relevant safety instruction and risk assessments.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick to confirm that it is attached to this form

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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**Name**

NPL MANAGEMENT LIMITED

**Name 2**

NATIONAL PHYSICAL LABORATORY

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

QUEENS ROAD

**District**

**Town**

TEDDINGTON

**County**

MIDDLESEX

**Postcode**

TW11 OLW

**Country**

ENGLAND

**Tel Number**

020 8943 7102

**Fax Number**

020 8614 0454

**E-mail**

**HSE Division**

LONDON

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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<td>Premises Conditions</td>
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<td>MODULE 7 LABS G7-L12 AND G7-L19</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Genetic modification advice is given by the Laboratory’s Health, Safety and Environment Committee which meets twice a year (additional meetings are convened when necessary).

The Committee members are: Managing Director, Head of the Corporate Assurance Team, Senior Biological Safety Adviser, Radiation Protection Adviser, Senior Laser Safety Adviser, First Aid Officer, Two Science Centre Representatives, Three TU Safety and Staff Representatives (Prospect), Serco FM’s (facility managers) Safety & Quality Manager, Health, Safety and Environment Co-ordinator.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 1 (GMMs)</td>
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<tr>
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</table>
All GMMs and material in contact with recombinant DNA will be autoclaved on site under class 2 guidelines (i.e., in excess of required containment). Liquid waste will be disposed of into the waste water system after autoclaving and appropriate treatment. In agreement with the Laboratory of the Government Chemist (LGC) solid waste will be bagged separately in clinical waste disposal bags and included in the LGC clinical waste disposal route (incineration in compliance with clinical waste disposal regulations).

Explanatory note: LGC and NPL are co-located on site.
### Project Additional Information

#### Purposes of the contained use

Production of recombinant proteins using yeast and bacterial expression systems.

#### Recipient or parental organism

Escherichia coli, Pichia pastoris

#### Host/vector system

Escherichia coli (predominantly Novagen and Qiagen T7 expression systems) using K-12 and B strains (BL21), Pichia pastoralis (Invitrogen expression system).

#### Origin & function

The genetic material will be cDNAs originating from human, mouse, yeast and bacterial cDNA collections that are maintained by the academic community (eg the I.M.A.G.E. consortium) and also donations of non-publicly available from cDNAs from academic groups or companies. The cDNAs are anticipated to encode enzymes, soluble proteins and membrane proteins that are considered to be "druggable targets" ie proteins of commercial interest due to their involvement in disease states. The cDNAs will be cloned into non-mobilisable vectors. No clinical material will be used or cDNA for virulence factors or cDNAs derived from pathogens.

#### Evaluation of foreseeable effects

Under class 1 containment, there are no foreseeable adverse effects. The proteins expressed will be used to underpin pure research and to provide "proof-of-concept" data for technology and intellectual property development. E coli BL21 is a laboratory derivative of an E coli B strain; though B strains are category 2 pathogens the strain in question is laboratory attenuated and is effectively category 1.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMMs and material in contact with recombinant DNA will be autoclaved on site under class 2 guidelines (ie in excess of required containment). Liquid waste will be disposed of into the waste water system after autoclaving and appropriate treatment. In agreement with the Laboratory of the Government Chemist (LGC) solid waste will be bagged separately in clinical waste disposal bags and included in the LGC clinical waste disposal route (incineration in compliance with clinical waste disposal regulations).
Explanatory note: LGC and NPL are co-located on iste.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

None.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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GM Centre Number: 865

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Name

MANCHESTER UNIVERSITY NHS FOUNDATION TRUST

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Comments

Date at Which Additional Info Submitted:

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Gene Therapy Committee established October 2002 with representation from: Trust Director of R&D, HSE Officer, Trust Risk Manager, Trust Infection Control, Trust Pharmacy, Trust Consultant Virologist and Trust Lead Investigators.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

Virology Transgenic Animals Transgenic Fish Gene Therapy Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The first activity which we intend to undertake is in Class 2 and therefore we will submit a separate activity notification form with this premises notification.

---

**Project Ref 865/03.1**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>Culture Vol</th>
<th>Consent Granted</th>
<th>Project notified under transitional arrangements</th>
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<tr>
<td>29/05/2003</td>
<td>INTRA-CORONARY INSTALLATION OF ADENOVIRUS MEDIATED FIBROBLAST GROWTH FACTOR-4 (ANGIOGENIC GROWTH FACTOR THERAPY).</td>
<td>Class 2</td>
<td>1-50 litres</td>
<td>not applicable</td>
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</table>

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
## Project Additional Information

### Purposes of the contained use

Study to evaluate treatment of patients with angina pectoris.

### Recipient or parental organism

Schering AG (subsidiary of Schering Health Care Ltd) hold the IPR rights.

### Host/vector system

Human adenovirus.

### Origin & function

Human gene for the fibroblast growth factor 4 (FGF-4) referred to as Ad5FGF-4. Intended function to stimulate new blood vessel formation.

### Evaluation of foreseeable effects

Improvement in morbidity and mortality in patients with severe coronary artery disease, who are unable to undergo surgery.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

GMMs to be used.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Wastes containing this material should be properly contained, labelled, stored and disposed of as biohazardous waste. Local and country regulations for the disposal of biohazardous wastes will be met. All contaminated waste products will be placed in a box and autoclaved before disposal. Labels will be used on the container with the wording "WARNING - HAZARDOUS WASTE-CONTAGIOUS". Inactivation of Ad5FGF-4 is by autoclaving at 121 degrees C for 15 minutes, giving effectively 100% kill. Using a higher temperature, or a longer time, is permissible. To comply with UK and Irish regulations, the autoclave is in the same building. The autoclaves are validated on at least an annual basis (including the placing of independent thermocouples at the centre of the load. For each cycle of Ad5FGF-4 waste, autoclave indicator tape and the integral autoclave printer readout will be used to confirm that the waste has been autoclaved.

Puncturing - cutting waste should be put in a special box marked externally with labels: "WARNING - HAZARDOUS WASTE - PUNCTURING, CUTTING, CONTAGIOUS". Other waste products should be handled as conventional waste.

### Is an emergency plan required according to regulation 20?

N

### If yes, tick to confirm that it is attached to this form

N

### Tick to confirm that you have attached a risk assessment to this form

Y

### Tick if you are claiming exemption from disclosure for section of the risk assessment

N
This is a Class 2 activity which would require notification and undertaking a risk assessment for the activity. Risk assessment and SOPs reviewed.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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### NOTTINGHAM CITY HOSPITAL NHS TRUST

**Name**

NOTTINGHAM CITY HOSPITAL NHS TRUST

**Name 2**

NOTTINGHAM CITY HOSPITAL NHS TRUST

**Department**

NOTTINGHAM CITY HOSPITAL NHS TRUST

**Campus Estate or Research Centre**

**Building**

**Road Name**

HUCKNALL ROAD

**District**

**Town**

NOTTINGHAM

**County**

NOTTINGHAMSHIRE

**Postcode**

NG 1PB

**Country**

ENGLAND

**Tel Number**

0115 969 1169

**Fax Number**

0115 962 7968

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022

02/06/2003

0115 969 1169

0115 962 7968

02/03/2022

Page 11739 of 15326
## Premises Addresses

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</table>

## Premises Conditions

### Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

| Y |  |

### Give brief details of the genetic modification safety committee

The Nottingham City Hospital Genetically Modified Organism Safety Committee has met on five occasions to discuss this project. It is proposed that further meetings will take place to review progress, to offer advice and to review accidents/incidents as the need arises.

The majority of committee members have managerial responsibilities within the Trust and are very experienced.

The Committee included the following members:-

- Consultant Microbiologist, Nottingham City Hospital.
- Chief Technician, Cardiology Department, Nottingham City Hospital
- Ward Manager, Nightingale Ward, Nottingham City Hospital.
- Consultant Clinical Scientist, Centre for Medical Genetics, Nottingham City Hospital
- Infection Control Specialist Nurse, Nottingham City Hospital
- Manager CSSD, Nottingham City Hospital
- Manager of Clinical Sciences Building
- Senior Technician, Pharmacy Department, Nottingham City Hospital
- Clinical Nurse Manager, Cardiothoracics, Nottingham City Hospital
- Senior Nurse, Cardiology, Nottingham City Hospital.
- Manager, Health and Safety Department, Nottingham City Hospital
- Senior Pharmacist, Nottingham City Hospital.
- Consultant Microbiologist, Nottingham City Hospital.
- Representative from the Health and Safety Department, University of Nottingham
- Radiographer, Nottingham City Hospital.
- Consultant Physician (with special interest in Virology), Nottingham City Hospital.
| Level 1 (GMMs) | Yes |
| Level 2 (GMMs) | Yes |
| Level 3 (GMMs) | Yes |
| Level 4 (GMMs) | Yes |
| Non-microbial | |

**Other (please specify)**

Cardiac Catheter Lab, Nightingale Ward, Clinical Sciences Building, Pharmacy, Nottingham City Hos

**Tick if confidential**

Tick if you are claiming exemption from disclosure for sections of the risk assessment

**Tick to confirm that you are attaching a summary of the risk assessment**

Y

**Please enter comments of the GM safety committee on the risk assessment**

No comments.

---

**Project Ref** 866/03.1
ANGIOGENIC GENE THERAPY PRODUCTS FOR CORONARY ARTERY DISEASE: A RANDOMISED CLINICAL TRIAL.

In the proposed clinical trial programme, angiogenic gene therapy product, consisting of a recombinant adenovirus (human serotype 5) containing the human gene for the fibroblast growth factor 4, and referred to as Ad5FGF-4, will be investigated as a treatment for patients with chronic stable angina due to coronary artery disease (CAD). Generation of new blood supply in the diseased heart by intracoronary administration of angiogenic gene therapy product represents a potential new therapeutic approach to relieve this condition.

Recipient or parental organism
See box below.

Host/vector system
The Ad5FGF-4 gene therapy product consists of a recombinant adenovirus vector (human serotype 5, Ad5) with a deletion in the E1 region; from map unit 1.3 to 8.7 of wild-type virus (entire E1A and most of E1B are eliminated). The FGF-4 transgene is inserted, driven by CMV promoter.

Origin & function
The FGF-4 gene was originally isolated from a cDNA library which was constructed from mRNA of Kaposi's sarcoma DNA transformed NIH3T3 cells.

Evaluation of foreseeable effects
The probability of adverse consequences resulting from deliberate or accidental release of the gene therapy product Ad5FGF-4 are minimal to nonexistent.

Hazards resulting from environmental release (viral shedding from treated persons, inadvertent contamination of the product prior to administration) are negligible or nonexistent for the following reason: infection requires large numbers of infectious vectors, and transfection (expression of the inserted gene) requires a multitude of infectious particles.
Hazards associated with the adenoviral vector can be described as having low potential of adverse environmental consequences in humans or animals. The theoretical consequences to humans of several of the hazards associated with ectopic transgene expression, if they actually occurred, could be considered moderately severe (e.g., promotion of existent malignancy, unknown risk to foetus). However, since any unintended or accidental exposure would most likely be a fraction of the total dose being administered to patients for therapeutic purposes, the relative risk of the occurrence of these types of adverse effects should be very low.

The possible risks to the environment could be assessed as low to effectively zero. This is based on the low probability of infectious adenoviral particles escaping into the environment either through viral shedding by patients that have received the product, or by incidental exposure during administration procedures. Even if viruses were shed or product spillage occurred the number of infectious viral particles would be too small to result in infection of exposed tissues. The risk to the non-human environment is extremely low to effectively zero because of the species specificity of adenovirus 5, which by natural exposure is only known to infect humans.

The above indicates that the product could be classified as Class 1. However, as a precautionary measure, due to the limited experience available, the product is currently being classified as Class 2. Reclassification into Class 1 may be considered if based on increased data and experience.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste that may have been directly exposed to the study product will be placed in double autoclave bags and transferred by Cardiac Catheter Laboratory staff to an autoclave in the Clinical Sciences building to be autoclaved. Inactivation of Ad5FGF-4 is by autoclaving at 121 degrees C for 15 minutes, resulting in 100% kill of the vector. The material is then incinerated on site. Service contracts are in place for the autoclave.

Waste from managing the patient on the ward will be sealed in yellow clinical waste bags and then be taken for incineration.

All procedures will be tested in a dry run.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

---

**Project Containment**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Yes

Give brief details of the genetic modification safety committee

Chair
Biological Safety Officer (Chief Scientific Officer)
Members
Director
Director
GMO project leader

Meets regularly at 3-month intervals, and as and when necessary to review procedures and new risk assessments.

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<th>Glass House</th>
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Tick if confidential [ ]
All liquid waste will be decontaminated in a final concentration of >2% Virkon overnight. All solid waste will be decontaminated in a final concentration of >2% Virkon overnight and then autoclaved in sealed waste bags.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
None

Project Ref 867/03.1

Date Ackn'd 10/06/2003

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
**Project Additional Information**

**Purposes of the contained use**

The specific aims of this project are to:
1) assess the efficacy of EIAV-based lentiviral vectors expressing the human bcl-2 gene to protect cells in models of apoptotic death;
2) assess the efficacy of HIV-based lentiviral vectors expressing marker genes to transduce rodent organotypic brain slices, rodent primary neurons, neuronal cell lines and other mammalian cell lines in vitro.

**Recipient or parental organism**

Viral production will use immortalised mammalian cell lines, such as HEK293Ts.

**Host/vector system**

All manipulations of plasmid DNA will make use of E. coli K-12 derived strains, such as DH5a, which are non-colonising and disabled. The genome plasmids, into which foreign genes are cloned, of both vector systems are derived from pUC18, and are therefore considered to be non-mobilisable.

Viral vector production will use immortalised mammalian cell lines, such as HEK293Ts.

Vectors will be used to transduce rodent organotypic brain slices, rodent primary neurons, neuronal cell lines and other mammalian cell lines in vitro.

**Origin & function**

All manipulations of plasmid DNA will make use of E. coli K-12 derived strains, such as DH5a, which are non-colonising and disabled. The genome plasmids, into which foreign genes are cloned, of both vector systems are derived from pUC18, and are therefore considered to be non-mobilisable.

Viral production will use immortalised mammalian cell lines, such as HEK293Ts.

Vectors will be used to transduce rodent organotypic brain slices, rodent primary neurons, neuronal cell lines and other mammalian cell lines in vitro.

**Evaluation of foreseeable effects**

The risk to humans is due to the possibility of transduction of cells by minimal EIAV-based or HIV-based lentiviral vectors which will be pseudotyped with either VSV-G.
(vesicular stomatitis virus G) or rabies-G envelopes. These envelopes allow vector particles to enter a wide range of mammalian cells; however they have been shown to have a far more restricted range in vivo than in cultured cell lines. Since the vectors are replication defective, the extent of spread would be limited to the cells initially transduced. Furthermore, the vector systems both have a long (>5 years) history of safe use at Oxford BioMedica plc (EIAV) and Cell Genesys Inc (HIV), from whom Invitrogen have licensed the technology.

Bci-2 has been shown to be anti-apoptotic. Although it is potentially tumourigenic, the formation of a cancer requires the activation of oncogens and the activation of tumour suppressor genes. The introduction of one change into a small number of cells is unlikely to cause cancer.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be decontaminated in a final concentration of >2% Virkon overnight. All solid waste will be decontaminated in a final concentration of ≥2% Virkon overnight and then autoclaved in sealed waste bags.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

None

Project Containment

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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Name**

WILLACY GUINARD HOLDINGS LTD TRADING AS TRADEBE FAWLEY

**Campus Estate or Research Centre**

**Department**

**Road Name**

CHARLESTON ROAD

**District**

HARDLEY

**Town**

HYTHE SOUTHAMPTON

**County**

HAMPShIRE

**Postcode**

SO45 3ZA

**Country**

ENGLAND

**Tel Number**

02380 883 050

**Fax Number**

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

COMPANY NAME CHANGE FROM PYROS ENVIRONMENTAL TO WILLACY GUINARD HOLDINGS LTD T/A AS TRADEBE FAWLEY

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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<td>VEOLIA ENVIRONMENTAL SERVICES PLC</td>
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<td>BRIDGES ROAD</td>
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<td>CHESHIRE</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Technical Advisor and Safety, Health & Environment Co-ordinator provide advice to the Plant Manager on the acceptance, handling, storage and processing of this GMO Class 1 waste generated by Avecia, Billingham.

<table>
<thead>
<tr>
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</table>
High Temperature Incinerator, operated under Environment Agency Authorisation AG 8047 and associated Variations, is to be used to inactivate GMO Class 1 waste at the address shown in Section 1.

Inactivation of GMO Class 1 waste to inactivate

Other (please specify)

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
- Virology
- Transgenic
- Animals
- Transgenic
- Fish
- Gene Therapy
- Mycology
- Transgenic
- Invertebrates
- Transgenic
- Plants
- Other (please specify below) Yes

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

This is a notification of premises only as recommended by the paper relating to Newsletter 30, 'Inactivation of Waste Through Off-site Incineration'. The Shanks Risk Assessment attached refers to local arrangements for loss of containment as the only activity with the GMO Class 1 waste will be destruction of customer-packaged material. General procedures exist for handling, storage and processing of hazardous wastes on the Shanks' site. Avecia, Billingham, have previously submitted information to the HSE on their GMS Committee's comments.

Project Ref 868/05.1

Date Ackn'd 23/12/2005

Date Project Ceased

CU2 Project Title

The incineration of waste arising from the Production of Human Influenza Pandemic Vaccines undertaken at Chiron Vaccines, Liverpool (GM 781)

Class

CultureVolClass2

CultureVolumeClass3-4

Class 2 > 500 Litres

Non-GMM Consent Granted Not Applicable
Project Additional Information

Purposes of the contained use
To remove liquid waste from IBC containers and incinerate. All remaining solids and the containers will be shredded immediately prior to incineration.

Recipient or parental organism
A/Puerto Rico/8/1934 (PR8) & A/Vietnam/1203/2004 (H5N1). A/Puerto Rico/8/1934 (PR8) Flu virus strain has been routinely used as a basis of flu vaccines with an extensive history of use. This has been combined with A/Vietnam/1203/2004 (H5N1) to produce the reverse genetics virus: rg A/Vietnam/1203/2004 X A/PR/8/34 6:2 which will be used in the manufacturing activities at Chiron.

The only foreseeable risk arises from dual "infection" of humans or animals with the reverse genetics virus and a normal circulating influenza virus. In order to minimise the risk several containment measures have been implemented. All waste will arrive in sealed IBCs (Intermediate Bulk Containers) which will be incinerated during this process (not re-used).

Host/vector system
See above - generated by reverse genetics.

Origin & function
A/Puerto Rico/8/1934 (PR8) & A/Vietnam/1203/2004 (H5N1).

Evaluation of foreseeable effects
Extremely remote possibility of reassortment during simultaneous "infection" with rg A/Vietnam/1203/2004 X A/PR/8/34 6:2 and a circulating human flu virus generating a new pathogenic strain.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Derogation from the following measures specified in Table 2, Schedule 8 is applied for:
* Items 1, 2, 3, 4, 5 & 7 relate to closed systems, such as fermenters. This process involves extraction from a closed system (ie an IBC) to achieve optimal incineration capacity by injecting liquids into the Incinerator.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
See Operating Procedure and Risk Assessment

Onyx at Fawley does not have its own GMSC, but the site safety representatives, the local unions and H&S management have all been involved and made aware of this waste. This process will be overseen by the Chiron Vaccines GMSC and, discussed at Chiron Vaccines GMSC on 12 December 2005, and with Onyx on 13th December 2005. Planning has been conducted in consultation with Chiron and HSE. Work has been classified as Class 2 on the basis that no measures for Containment Level 3 are required. This is in line with WHO classification, and under advice from the HSE.

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**Name**

LABCORP EARLY DEVELOPMENT LABORATORIES LIMITED

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**Comments**

Name change from SafePharm Laboratories Ltd notified 24/01/2017, name change from Envigo Research Ltd 13/06/2019

**Date at Which Additional Info Submitted**

02/03/2022
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The genetic modification safety committee consists of members of staff taken from Safepharm Laboratories Limited, Departments of Ecotoxicology and Genetic Toxicology, and Shardlow Business Parks Health and Safety Officer. The members of staff from Safepharm Laboratories Ecotoxicology and Genetic Toxicology Departments consist of the Head of each Department and Study Directors responsible for the conduct of studies using genetically modified organisms.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

Bacteriology          | Conf | Parasitology | Transgenic Birds | Microbiology Research | |

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste produced from studies conducted using GMMs will be stored separately from non-GMM contaminated waste in clearly labelled waste containers prior to disposal by a licensed waste disposal company as clinical waste. All clinical waste generated will be incinerated and as such will result in greater than 99.999% kill.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The genetic modifications safety committee has reviewed the risk assessments conducted for the following study types.

i) Assessment of Estrogenic Activity using Saccharomyces cerevisiae expressing the human hER gene.
ii) Assessment of Androgenic Activity using Saccharomyces cerevisiae expressing the human hAR gene.
iii) Assessment of Mutagenic Activity Studies using Salmonella typhimurium strain TA1535 expressing a defective histidine biosynthesis gene and antibiotic resistance genes.
iv) Assessment of Mutagenic Activity Studies using Salmonella typhimurium strain TA102 and TA97 expressing defective histidine biosynthesis genes and antibiotic resistance genes.

The genetic modification safety committee agrees with the activity class assigned to each of the above study types.
### General Information

- **GM Centre Number:** 870
- **Date Premises Notified (Originally):** 01/07/2003
- **Data Premises Closed:** 15/03/2010
- **Emergency Plan Required:** N
- **Non-GMMs Withdrawn:** N
- **Transitional Premises Closed:** N

### Details

- **Name:** SENSE PROTEOMIC LIMITED
- **Campus Estate or Research Centre:** UNIT 4 THE SWITCHBACK
- **Road Name:** GARDNER ROAD
- **Town:** MAIDENHEAD
- **County:** BERKSHIRE
- **Postcode:** SL6 7RJ
- **Country:** ENGLAND
- **Tel Number:** 01628 513 500
- **Fax Number:** 01628 676 791
- **E-mail:**
- **HSE Division:** EAST AND SOUTH EAST

### Additional Information

- **Date at Which Additional Info Submitted:** 02/03/2022
## Premises Addresses

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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

* The GMSC at Procognia comprises four members, one representing operations and three representing R&D.
* Areas of expertise held by the committee include post doctoral level biology with experience of prokaryotic and eukaryotic gene cloning and expression.
* The committee receive pre-circulated assessments of proposed GM work for comment.
* All comments are collated by the secretary for discussion and approval at the committee meetings.
* The newly formed committee is set to meet every 8 weeks to consider the approval of new assessments for GM work.

### Laboratory

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
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### Animal Unit

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<th>Microbiology Research</th>
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<tbody>
<tr>
<td>Yes</td>
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<td>Yes</td>
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</tbody>
</table>
**For activities involving GMMs, describe the waste management measures which will apply to the activity**

- Biological waste will include, disposable plastic ware eg inoculating loops, Petri dishes and tubes), tissues, gloves, liquids and glassware.
- Disposable materials will be collected in plastic bags marked with the biohazard symbol and held in plastic waste bins. The waste will be double bagged prior to placing in a Morrison container for autoclaving.
- All liquid cultures will be sterilised with 1% ethanol.
- Biological waste awaiting sterilisation in the autoclave will be stored in closed plastic bins in the autoclave room.
- All biological waste will be sterilised by autoclaving (132 degrees C for 45 minutes). Waste is only considered to be sterile and ready for disposal if the printed record indicates that the machine was held 132 degrees for 45 minutes.
- Sterilised waste will be removed from the autoclave and stored in sealed plastic drums prior to incineration.
- All biological waste will only be handled by trained scientists up until the time it is in drums awaiting collection for off site incineration.
- Drums of sterilised biological waste will be collected by a waste management company fortnightly for incineration.

Tick to confirm that you are attaching a summary of the risk assessment: ☒

Tick if you are claiming exemption from disclosure for sections of the risk assessment: ☐

Please enter comments of the GM safety committee on the risk assessment:
The risk assessment GMWG(P)001 was approved by the GMSC.
ROBERT JONES & AGNES HUNT ORTHOPAEDIC HOSPITAL

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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Chair, Employee Representatives (2 People), Management Representative.

They meet every Quarter or if there is a problem they will meet more frequently.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 1 (GMMs)</td>
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Other (please specify)

Tick if confidential

Yes

Bacteriology

Parasitology

Transgenic Birds

Microbiology Research
For tranfection studies, the gene is harmless to man and the environment. Any waste will be autoclaved on site and then sent for incineration.

<table>
<thead>
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<th>Virology</th>
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*For activities involving GMMs, describe the waste management measures which will apply to the activity*

For tranfection studies, the gene is harmless to man and the environment. Any waste will be autoclaved on site and then sent for incineration.

Tick to confirm that you are attaching a summary of the risk assessment  Y  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

The safety committee is in agreement with the risk assessment.
# GM Centre Number: 872

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| HSE Division                        | EAST AND SOUTH EAST |

| Comments                             |                        |

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

At present the company consists of only six people - all six are on the GMSC. The GMSC will review and advise on all risk assessments relating to GMM and GMO work. The GMSC will meet every three months or sooner where necessary.

The GMSC comprises:
Chair (company CSO, Biological Safety Officer, Departmental Safety Officer, Management with responsibility for GMO activities, Employees who have access the GMO, Co-opted members to supplement expertise.

<table>
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<th>Large Scale</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Bacteriology - All solid waste will be autoclaved prior to disposal. Chemical indicator strips (eg 3M Comply Thermalog Steam Chemical Integrator) will be used in with the load to ensure that the correct time and temperature conditions have been achieved. Records of the date and volume of waste autoclaved will be maintained. All liquid waste will be treated with 1.5% Virkon solution for at least 10 minutes prior to disposal into the foul sewer. The level of treatment has been proven to be 100% effective in killing bacteria: http://www.biosafetyusa.com/virkon/virkonifu.pdf; http://www.antecint.co.uk.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessments approved. No objections raised.
<table>
<thead>
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<th>Data Premises Notified (Originally)</th>
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**Name**

SIMBEC RESEARCH LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

MERTHYR INDUSTRIAL PARK

**Road Name**

CARDIFF ROAD

**District**

**Town**

MERTHYR TYDFIL

**City**

GLAMORGAN

**County**

**Postcode**

CF48 4DR

**Country**

WALES

**Tel Number**

01443 690977

**Fax Number**

01443 693570

**E-mail**

**HSE Division**

WALES AND SOUTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee (GMSC) is a sub-committee of the Infection Control Team (ICT). The ICT has been established at Simbec to ensure that all aspects of surveillance, prevention and control of infection are performed in accordance with recommendations made by the Infection Control Consultant and Biological Safety Officer (Consultant Microbiologist).

The ICT/GMSC collaborates with Simbec line management for approval, authorisation and implementation of necessary procedures. The ICT/GMSC is composed of the following personnel: Infection Control Consultant/Biological Safety Officer, Simbec Medical Director, Simbec Infection Control Officer (Senior Research Nurse), Health and Safety Committee Chairman, Quality Management Group Representative. Any appointed safety representatives may sit on the GMSC if they wish.

### Laboratory

| Level 1 (GMMs) | Yes |
| Level 2 (GMMs) |
| Level 3 (GMMs) |
| Level 4 (GMMs) |

### Animal Unit

Tick if confidential
The following routine procedures will be followed at all times.
Simbec Standard Operating Procedure BD/324/1/03 - Hazardous Waste Management
Simbec Standard Operating Procedure BD/324/13/17 - Cleaning the Aseptic Unit

All materials that have been in contact with the vaccine or vaccination site will be disposed of into bins for autoclaving. The autoclave will be situated within a restricted laboratory. Autoclaved waste will be disposed of in yellow incineration bins provided in each laboratory/ward area as per routine hazardous waste management procedures. Syringes and needles will be disposed of into sharps bins for autoclaving before disposal by incineration. The remainder of the vaccine and vial will also be disposed of into bins for autoclaving before incineration.

On completion of immunisation and dressing procedures surfaces on which the vaccine vials have been placed will be cleaned with disinfectant.

Copies of SOPs attached.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
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</table>

**Other(s)**

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The Biological Safety Officer, was absent due to annual leave. It was agreed that the minutes and all documentation relating to the HSE notification should be sent to him as soon as possible. Any feedback from him will then be forwarded to the HSE where necessary.

The committee reviewed the correspondence between MRC Human Immunology Clinic, and HSE. As the HSE has already agreed that the modified vaccine is Class 1 the risk assessment classification was approved.

Following review of the study protocol the following points were added to the risk management section of the risk assessment. Volunteers will be advised not to interfere with any dressings or scab formation on the site of inoculation. Concerns were raised regarding the volunteers leaving site without any dressing on the site of inoculation. It was suggested that volunteers be given a plaster to cover the site however it was decided that this would prevent the formation of a scab. Therefore it was agreed that as the risk to the environment is extremely low the site of inoculation should remain uncovered.

The choice of disinfectant to be used in each work area will be discussed further. The chosen disinfectant will be in accordance with routine Simbec Procedures. All staff, including ward orderlies, will undergo a training day to discuss procedures and protocols. This will also be an opportunity for staff to ask questions and raise any concerns they may have. This training session must be recorded in staff training records. All work performed within the Clinical Unit will be performed in a separate area from other studies to remain clear of other volunteers. Minutes of the first meeting of the GMSC enclosed.
<table>
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<tr>
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Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Chairman
Director CEH
Assistant Director CEH
Biological Safety Officer CEH Oxford
Biological Safety Officer Cybersense
Local Safety Adviser
Supervisory medical officer
GMO external expert
Union side representative
Student representative
Secretary
The committee meets on a 3-monthly basis to review risk assessments.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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</table>
The construction of class 1 GMMs will take place in the CEH laboratories in accordance with all CEH Oxford safety procedures. All waste will be autoclaved in central CEH Oxford facility prior to disposal - 100% kill. The autoclave is thermocouple-tested twice a year. Records are taken of each run to ensure sterilisation. All waste from the mobile unit will be disposed of in this facility. Derogation from containment level 1 requirement for autoclave required on site, when GM activities are undertaken off-site.

The GMM assay protocol, containment, waste management, and accidental spillage procedures are described in the supplemental notes attached.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

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Tick to confirm that you are attaching a summary of the risk assessment  
Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Extensive risk assessment produced therefore the GMSC agrees with the Class 1 classification. No outstanding issues.
GM Centre Number: 875

Data Premises Notified (Originally) 14/08/2003

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

AFFINITI RESEARCH PRODUCTS LIMITED

Name 2

Department

Campus Estate or Research Centre

Building

PALATINE HOUSE

Road Name

MATFORD COURT

District

Town

EXETER

County

DEVON

Postcode

EX2 8NL

Country

ENGLAND

Tel Number 0139 282 5900

Fax Number 0139 282 5910

E-mail

HSE Division WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>SL1 4NL</td>
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<td>N</td>
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</table>

**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

- Biological Research Leader/Safety Officer
- Head of Biology Department
- Biology Scientist
- Chemistry Research Leader

Meeting half yearly or more frequently if required for consideration of proposals for work, and of risk assessments.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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Other (please specify)  

Tick if confidential

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Micro organism (cell kill by soaking overnight in Virkon 2% v/v of waste cell culture media) cell suspensions, for not less than 18 hours prior to disposal via drainage.

Disposal of all waste plastic cultureware, pipettes, eppendorf tips etc to double lined autoclave bags and sterilisation by autoclaving to 121 degrees C x 15 minutes.

Virkon 2% v/v has been experimentally determined to result in 100% kill of mammalian cell lines after overnight incubation, in the presence of 10% serum used for standard cell culture conditions. This will be confirmed by microscopy using trypan blue exclusion/haemocytometer count to look for viable cells post treatment and reinoculation of treated waste material into cell growth medium, with examination for viable cells after a suitable growth period.
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</table>

Date at Which Additional Info Submitted

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

A safety committee comprising Quality Manager, Product Manager and Assistant Nutritionist has been formed. The committee is responsible for carrying out a risk assessment and advising on legislation compliance. Meetings are held as required to ensure regulations are met.

<table>
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<th>Laboratory</th>
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Other(s)

| Other(s) | |

For activities involving GMMs, describe the waste management measures which will apply to the activity

The plant material will be rendered inactive by grinding.
No testing or monitoring measures have been identified. Waste management has been reviewed in the risk assessment.

Tick to confirm that you are attaching a summary of the risk assessment

Y

02/03/2022
The safety committee has reviewed and approved the risk assessment and will monitor the implementation of measures identified by it.
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**Name**

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**Campus Estate or Research Centre**

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**Road Name**

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**District**

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**Town**

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**HSE Division**

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**Comments**

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The centre has an established GMO safety committee for all the companies located within the centre.

| Level 1 (GMMs) | Conf |
| Level 2 (GMMs) | |
| Level 3 (GMMs) | |
| Level 4 (GMMs) | |
| Non-microbial | |

Other (please specify) Tick if confidential

| Bacteriology | Conf |
| Parasitology | |
| Transgenic Birds | |
| Microbiology Research | Conf |
| Virology | |
| Transgenic Animals | |
| Transgenic Fish | |
| Gene Therapy | |
**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Good microbiological practice.
Waste bacterial cultures are treated with disinfectant and then autoclaved at 121 degrees C for 20 mins before disposal down the drain. All biological solid waste such as agar plates and plastics are autoclaved at 121 degrees C for 20 mins and then incinerated off site. Contaminated glassware is autoclaved at 121 degrees C for 20 mins and then washed in accordance with normal laboratory practice (industrial dishwasher)

All lab work surfaces are regularly wiped with a disinfectant.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

---

Please enter comments of the GM safety committee on the risk assessment

The risk assessment has been approved by all members of the GMO safety committee and signed.
### GM Centre Number: 879

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02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The modification committee comprises of a minimum of five members consisting of programme managers, project managers, scientists and a representative from the company health and safety organisation. The programme managers, project managers and scientists all possess expertise in the areas of microbiology, molecular biology, genetics, immunology and chemistry. They have combined at least 60 years of experience in these areas. The procedure for operating is as follows: A request for approval of a proposed GMM experiment is made by a proposer. He/She will be expected to complete a risk assessment using a defined template. The assessment will then be circulated to the members of the committee at least 3 days prior to a meeting. During the meeting a rigorous review of the risk assessment, a decision made on the containment category, whether HSE notification is required and if the experiment can go ahead. Comments are added to the risk assessment and the assessment is stored securely.

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Other (please specify) Tick if confidential

02/03/2022

Page 11787 of 15326
A GMM waste standard operating procedure (SOP) for treatment of waste has been written. Staff trained and are expected to adhere to it rigorously.

Liquid waste will be decontaminated by autoclaving at 121 degrees for 15 minutes. No more than 1 litre will be autoclaved in one vessel. Browning tubes will be used every 3 months to ensure sterilisation conditions are reached. The autoclave is serviced annually and a record of each autoclave run made. The decontaminated waste will then be disposed of down the drain.

Solid waste will be autoclaved at 121 degrees for 15 minutes. The autoclave is serviced annually and a record of each autoclave run made. The waste is then sealed within plastic bags and removed for incineration. All sharps waste will be placed in an autoclavable sharps bin. The sharps waste will be autoclaved at 121 degrees for 15 minutes and removed for incineration.

The small quantity of waste that does not withstand autoclaving will be decontaminated by placing in a 1% Virkon S solution with no air bubbles. This will be left overnight before disposal of liquids down the drain and the solids by incineration. Extensive validation of killing of microbes with Virkon S is provided on the Virkon manufacturer’s web site (http://www.atecint.co.uk/main/virkons.htm). 1% Virkon S overnight provides excellent disinfectant capacity.

Other(s) Molecular Biology techniques such as PCR

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
The host strain E.coli JM109 is an E.coli K12 strain and is considered non-pathogenic. The JM109 strain was determined as disabled because it is RecA-and thi-. It was agreed that the pGEM vector should be classified as non-mobilisable, because it is Bom-, Tra-, Mob- and Nic-. It was noted that there were no pathogenic, toxic, oncogenic or cytokine sequences present within the vector. An overall access figure for the host/vector combination was 10e-9 under the Brenner scheme. The insert from Baccilus globigii is only 92 basepairs and is from the ribosomal DNA and is highly unlikely to be toxic. The sequence is short and there are no pathogenic, toxic, oncogenic or cytokine sequences present. Bacillus globigii is a class 1 organism. The expression level of the insert was discussed. There are T7 and SP6 promoters flanking the insert. It was noted that T7 and SP6 polymerases are not normally present in E.coli JM because they are from bacteriophages. Furthermore, there is no deliberately designed start codon, although potentially there could be one there fortuitously. A classification for expression of 10e-6 was considered appropriate. The damage factor for this GMM at 10e was agreed because the insert is derived from a gene sequence, but there is only a very short sequence of this gene present and any expressed protein is unlikely to be biologically active. An overall assignment of containment level 1 was agreed (overall value under the Brenner scheme was 10e-24). The risk to the environment was also determined as very low because 1 litre or less is to be cultured at one time, the disabled nature of the organism, the organism will be treated under good microbial practice and the waste treatment procedures instigated. The work with this GMM was approved at containment level 1. It was noted that GMM risk assessments should be stored as controlled documents, in both paper and electronic format.
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02/03/2022 Page 11790 of 15326
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Yes

Give brief details of the genetic modification safety committee:

- NBS Manchester Management Representative (Chair)
- Health & Safety Advisor NBS Manchester
- Senior Scientist Paterson Institute Union
- External Independent GM Advisor
- Senior Scientist/Biological Safety Officer NBS Manchester
- Safety Officer Paterson Institute
- Safety Representative NBS Manchester

The committee meets on a 6 monthly basis. New submissions or accepted submissions that require a new amendment for consideration are made electronically to the Biological Safety Officer who distributes the submission to the committee for review. Committee members are required to reply within 2 weeks. Any further amendments must be made and returned to the committee within 2 weeks for further review. After acceptance the risk assessment and approval is signed off by the Biological Safety Officer or the GM safety Committee Chair.

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Non-microbial

For activities involving GMMs, describe the waste management measures which will apply to the activity

All liquid and dry waste will be sent for incineration by White Rose Environmental Ltd. All material for disposal will be placed into 30 litre yellow containers which will be sealed prior to removal from the room where the class one material is being used. These will then be placed into wheelie bins for collection. The wheelie bin used to transport the GM waste will be clearly marked and contain only yellow containers with GM waste. Any minor liquid spillages (≤20ml) will be removed by soaking up with an absorbent wipe or for larger liquid spillages by the addition of absorbent granules and transfer into a yellow container. The area will then be sprayed with either Klericide A or B (a rotational disinfectant with demonstrated virucidal activity. Shield Medicare Ltd) for a minimum of five minutes as per the manufacturer’s instructions. After inactivation, the area will be wiped and the wipe placed into a yellow container. The area will then be sprayed and wiped with 70% isopropyl alcohol and the wipe placed into the yellow container. The container will then be sealed and sent for incineration as above.

There is an on site autoclave that can be used as a back up inactivation system in the event of a problem arising with the incineration procedure.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

A number of issues were raised by the GMSC and the risk assessment was modified to take account of the issues raise.
## GM Centre Number: 881

| Data Premises Notified (Originally) | 14/11/2003 |
| Transferred from 1992 Regs? | N |
| Transitional Premises Class | |
| Data Premises Closed | |
| Transitional Premises | N |
| Emergency Plan Required? | |
| Non-GMMs | N |
| Withdrawn | N |

### Name

EUROFINS PHARMA BIOANALYSIS SERVICES UK LTD

### Name 2

Department

### Campus Estate or Research Centre

KIRTON CAMPUS

### Road Name

2-3 FLEMING ROAD

### Town

LIVINGSTON

### County

ABERDEENSHIRE

### Postcode

EH54 7BN

### Country

SCOTLAND

### Tel Number

01506 404 000

### Fax Number

01506 404 001

### HSE Division

SCOTLAND

### Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

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Give brief details of the genetic modification safety committee

The Safety committee is a sub group of the normal company safety committee, and consist of a range of staff considered experienced in biological work, and quality/regulatory issues.

The committee has been set-up specifically to ensure that the GMO work about to be started is carried out in a safe manner. The company previously operated a biologicals safety committee for the cell lines that we routinely cultivate, which are all Hybridoma cells (now removed from the level 1 classification, but controlled as such within the company).

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Yes

All waste from this project is to be contained within plastic waste bags, and then held within a waste container. This is then removed from site by a contracted waste management company (Biffa). The waste is categorised for Biffa as Type A and B clinical waste. This is then transported to a site for incineration.

The route for waste management described above is to be used for all cell material and in-process solutions and containers.

Reuseable culture vessels will be disinfected and cleaned by the companies routine cleaning procedure, once any cell debris is removed for treatment by Biffa.

The fermenters used for large scale cultivation are 200l final volume. During final processing the cell material is removed and then sent for off-site incineration. All cell free supernatants are either chemically disinfected prior to disposal, or are used to manufacture the final finished product.

All surfaces that may contact the GMO are chemically disinfected with proprietary disinfectants, either chlorine, Hydroxide or Propanol based, dependant on the application.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The company has been operating with Hybridoma cells, both murine and human heterohybridomas, since 1985. During this period these cell lines were classified as requiring level 1 containment and control measures. The company's procedures and training have been designed around this classification, and the current Good Manufacturing Practice guidelines.

The GMO being considered as part of this new project is a class switched hybridoma, prepared for Serologicals by Bristol Institute for Transfusion. All genetic manipulation has been completed at Bristol, and Serologicals will then take this modified cell line for evaluation and large scale manufacture through the existing production facility. No genetic manipulation will be carried out at the serologicals facility.

The committee reviewed an assessment provided for the cell line by Bristol which indicated a Level 1 classification, and agreed that this was the correct classification for the material. The level of risk assigned to the GMO was then agreed to be similar to existing products.

The risk assessment was carried out and reviewed against the Guide to Genetically Modified Organisms (contained Use) Regulations 2000. The Committee agreed that the control measures in place meet the requirements of Table 1c and Table 2 for large scale manufacture.
GM Centre Number: 882

Data Premises Notified

(Originally) 20/11/2003

Transferred from

1992 Regs? N

Transitional Premises

Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

THE AUTOMATION PARTNERSHIP

Name 2 Department

Campus Estate or Research Centre Building

Road Name District

YORK WAY

Town County Postcode Country

ROYSTON HERTFORDSHIRE SG8 RWY ENGLAND

Tel Number Fax Number

01763 227 200 01763 227 201

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Automation Partnership (TAP) GMSC includes:
- **TAP Biological Safety Officer.** The BSO has over 15 years experience of working with GMMs.
- **TAP Research and Development Manager,** whose team will be carrying out the processes involving GMMs.
- A representative from the TAP Project Management Group with responsibility for the project using GMMs.
- **TAPs H&S Consultant,** providing an overall H&S opinion and link with the TAP H&S Committee
- **TAP Operations Director,** representing Senior Management
- **TAP contracted Medical Officer.**

The Committee will meet as required by the nature of the work undertaken with GMOs in the company. Following the initial review meeting held 5 November 2003, there will be a 6 month formal review of practices at which further time-tableing of such meetings will occur. The BSO will maintain continuous review of practices at TAP using GMMs and will convene the GMSC for additional meetings as required for review of task assessments.

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Tick if confidential
Liquid culture media:
Liquid media will be collected in a vessel and disinfectant powder eg Virkon added and dissolved in appropriate amount to the volume to provide a disinfecting solution. Other disinfectants may be used that have similar function. Disinfectant culture media will be disposed of through the general waste water system.

Disposable culture vessels/equipment:
Disposable equipment and labware used for culture of micro-organisms will be soaked in a solution of disinfectant for the period specified by the manufacturers. Followed the disinfectant soak, equipment will be rinsed with water, the rinse going to general waste, and the disinfected, rinsed equipment disposed of in the general waste system.

Non-disposable culture equipment:
Non-disposable equipment and labware used for culture of micro-organisms will be soaked in a solution of disinfectant for the period specified by the manufacturers, soaked in 70% ethanol or autoclaved. Following disinfectant/ethanol soak, equipment will be rinsed with water, the rinse going to general waste.

Solid culture media:
Solid culture media (of no more than 1cm thickness) will be either autoclaved or soaked in disinfectant fluid, followed by disposal in the general waste. Any disinfectant fluid will be disposed of in the general waste water system.

Additional items:
Other solid items, for example accidentally contaminated lab coats will be autoclaved or soaked in disinfectant liquid followed by disposal in the general waste. Any disinfectant fluid will be disposed of in the general waste water system. Solid material once disinfected will be disposed of in the general waste.

Monitoring:
Waste disposal processes will be based around standard disinfection processes using commercially available reagents and apparatus. The robustness of these process in relation to the GMO used in combination with the risk associated with the escape of this GMO lead us to consider continued biological monitoring unnecessary. The major risk revolves around the misuse of the reagents and equipment, as such efforts will be focused on training and assessing the execution of these procedures by staff, and review of the execution of these processes.

Tick to confirm that you are attaching a summary of the risk assessment  

For activities involving GMMs, describe the waste management measures which will apply to the activity
<table>
<thead>
<tr>
<th>Please enter comments of the GM safety committee on the risk assessment</th>
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Data Premises Notified: 13/01/2004
Data Premises Closed: 19/12/2011

Transferred from 1992 Regs?: N
Emergency Plan Required?: N
Non-GMMs: N
Withdrawn: N

Name:
WYETH RESEARCH

Name 2

Department

Campus Estate or Research Centre

Building
POLWARTH BUILDING

Road Name

District
FORESTERHILL

Town
ABERDEEN

County
ABERDEENSHIRE

Postcode
AB25 2ZD

Country
SCOTLAND

Tel Number: 01224 555889
Fax Number: 01224 555844

E-mail

HSE Division
SCOTLAND

Comments

Date at Which Additional Info Submitted
02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Haptogen Limited submits details of risk assessments and applications for contained use activities to the University of Aberdeen Foresterhill Genetic Modification Safety Committee.

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Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research | Yes |
Virology     | Yes | Transgenic Animals | Transgenic Fish | Gene Therapy        |     |
No class 1 activity at present - See CU2 Project

For activities involving GMMs, describe the waste management measures which will apply to the activity

No class 1 activity at present - See CU2 Project

Please enter comments of the GM safety committee on the risk assessment

No class 1 activity at present - See CU2 Project

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**Project Ref 883/04.1**

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Date Project Ceased: 14/02/2011

Withdrawn: N

Tick if notifying a connected programme of work: N

Non-GMM Consent Granted: not applicable

Project notified under transitional arrangements: N

Footnotes:

Historical Significant Changes:

Historical Date of Additional Info:

Significant Change ID:

Date of Significant Change:

Project Additional Information:

02/03/2022
Purposes of the contained use

The goals of the project are to use bacterial reporter assays to compare concentrations of signalling molecules in samples treated with various signal-molecule inhibiting compounds. These results will help us to evaluate our compounds as novel treatments for bacterial diseases.

Recipient or parental organism

Chromobacterium violaceum and staphylococcus aureus

Host/vector system

Chromobacterium violaceum strain CV026 contains a double mini-Tn5 transposon insertion. Staphylococcus aureus strains RN6390B, and RN8463 all contain a reporter plasmid, pRN6683

Origin & function

Chromobacterium violaceum strain CV026 contains a double mini-Tn5 transposon insertion which renders it incapable of producing homoserine lactone signalling molecules. As a result, this strain does not produce the purple dye violacein, unless supplied with exogenous homoserine lactone. Therefore it can be used as a reporter strain for samples containing homoserine lactones. The mini-Tn5 transposon confers resistance to kanamycin as a selectable marker.

The pRN6683 reporter plasmid contains the E. coli blaZ (beta-lactamase) gene fused to the S.aureus agrP3 promoter, to allow determination of levels of S. aureus signalling molecules that activate the reporter construct. The plasmid also contains a gene for chloramphenicol resistance as a selectable marker, plus origins of replication for S. aureus and E.coli.

Evaluation of foreseeable effects

Expression of the mini-Tn5 transposon in Chromobacterium violaceum is unlikely to pose any serious hazard to human health; the strain CV026 has been widely used in these assays in other laboratories since 1997 with no reports of any adverse effects. The sites of the insertion have been mapped to a LuxI homologue (preventing synthesis of homoserine lactone) and a putative repressor locus. The transposon itself lacks the gene required for transposition, so it is unable to transpose to other loci in the genome. The likelihood of transfer to other organisms is virtually nil.

Expression of the pRN6683 plasmid in S. aureus is very unlikely to alter the pathogenicity of the organisms, and no change in the pathogenicity of these strains has been reported in the literature despite their widespread use in these assays since 1995. The pRN6683 plasmid is considered to be non-mobilisable. Again, the likelihood of transfer to other organisms is virtually nil.

Chromobacterium violaceum is not listed as Hazard Group II by the ACGM, but it is considered by most scientists as Group II and we intend to treat it as such. S. aureus is listed as Hazard Group II. The modifications contained by the strains we intend to use are not thought to alter the pathogenicity or Hazard Group classification of either organism.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid wastes: treated with 1% Virkon according to manufacturers instructions. Treated liquid waste will be disposed of down the main laboratory sink. We expect that Virkon treatment will be 100% effective, but this will be verified by plating out some of the treated waste to check for any viable bacteria.

Plasticware: will be autoclaved (121 degrees celsius, 15 minutes). Plastics are double-bagged before being taken in a robust plastic box with lids to autoclaves. We expect the autoclaving to be 100% effective in killing all viable bacteria.

After autoclaving, waste goes for Continuous Feed Auger processing.
Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Approved by GM Committee at Activity Class 2

### Project Containment

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<th>Laboratory Activities</th>
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02/03/2022

Page 11805 of 15326
GM Centre Number: 884

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**Name**
PURELY PROTEINS LIMITED

**Campus Estate or Research Centre**
UNIT 254

**Road Name**
MILTON ROAD

**Town**
CAMBRIDGE

**Road Name**
MILTON ROAD

**Town**
CAMBRIDGE

**Tel Number**
01223 426400

**Fax Number**
01223 426003

**E-mail**
info@purelyproteins.com

**HSE Division**
EAST AND SOUTH EAST

**Date at Which Additional Info Submitted**
02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

A genetic modification safety committee has been established composed of the laboratory Senior Scientist, (Biological Safety Officer), the Head of Proteinomics (Health & Safety Officer) and the Chief Operating Officer. The committee will meet not less than four times per year to review the use of GMOs and to advise on risk assessments and contained use activities. Minutes of the meeting will be recorded and circulated to all staff and the Board of Directors for approval; however, it is not anticipated that their will be any major changes in the types and varieties of GMOs in use.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs) | Yes | | | |
Level 2 (GMMs) | | | | |
Level 3 (GMMs) | | | | |
Level 4 (GMMs) | | | | |
Non-microbial | | | | |
Other (please specify) | | | | |
Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research

Tick if confidential

02/03/2022
The GMMs that are currently made by Purely Proteins Ltd are completely destroyed by standard autoclaving. All laboratory waste (e.g. gloves, plasticware, pipette tips) and anything else contaminated will be subject to autoclaving before being discarded in the general refuse. In the case of liquids, a strong disinfectant (e.g. chlorox) will be used to disinfect samples prior to disposal down the drain. Regular testing will be carried out to gauge the level of killing. Work surfaces, such as bench tops, and equipment will be decontaminated with disinfectant or 70% ethanol - any solid waste from the decontamination process will be autoclaved as above.

The laboratory is designed so that it can be easily cleaned. Carpets and rugs are deemed inappropriate and laboratory furniture is capable of being easily cleaned by wiping. Spaces between cabinets and equipment are easily accessible for cleaning.

In accordance with Good Laboratory Practice, protective lab coats designed for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving and entering other non-laboratory areas (e.g. administrative offices, kitchen). All protective clothing is either disposed of in the laboratory or laundered by the institution or appropriate laundry service; and is never taken home by any personnel. Gloves are worn when hands may contact any potentially infectious materials, contaminated surfaces or equipment. Gloves are disposed of when overtly contaminated, and removed when work with infectious material is complete. Disposable gloves are not washed, reused or used for touching "clean" surfaces (e.g. keyboards, telephones etc) and they are not worn outside the lab. Hands are washed following removal of gloves.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The GMSC has recommended that Purely Proteins work only with fully verified commercial sources of host strains and vectors to maintain all work at Hazard Level 1 and Containment Level 1.

Please enter comments of the GM safety committee on the risk assessment

The GMSC has recommended that Purely Proteins work only with fully verified commercial sources of host strains and vectors to maintain all work at Hazard Level 1 and Containment Level 1.
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**Name**

VIRTTU BIOLOGICS LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

BIOCITY SCOTLAND

**Road Name**

BO'NESS ROAD

**Building**

**District**

NORTH LANARKSHIRE

**Town**

NEWHOUSE

**County**

**Postcode**

ML1 5UH

**Country**

SCOTLAND

**Tel Number**

0141 445 1716

**Fax Number**

0141 445 1715

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
<table>
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<td>Neurology</td>
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</table>
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities Y

Give brief details of the genetic modification safety committee

A genetic modification safety committee has been established by Crusade Labs comprising 6 representatives covering management, research and development, technical and administration. The committee includes a University of Glasgow representative. GM safety at Crusade was originally under the auspices of the local University of Glasgow GMSC at the Southern General Hospital and the Crusade committee will continue close contact with this committee.

The Crusade GMSC meets at least twice per year to review GM safety and on an ad hoc basis as the need arises.

<table>
<thead>
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All work involving genetic manipulations of bacteria, yeasts or mammalian cells will be performed aseptically either on the lab bench or in a microbiological safety cabinet. All solid waste including plastics will be autoclaved. Liquid waste will be either autoclaved or immersed in 5% Virkon or Chloros overnight. Non-disposable items will be either immersed in Virkon/Chloros overnight or autoclaved prior to normal cleaning. These methods are commonly used in microbiology and are known to result in complete deactivation. All work involves standard laboratory protocols with no unusual procedures that require additional containment measures.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All work to date at Crusade had been approved by the local University of Glasgow GMSC. A complete list of current bacterial and yeast strains, mammalian cell lines and plasmids with inserted genes of interest was prepared and approved by the GMSC. The risk assessment for Class 1 work was approved by the GMSC.

Project Ref 885/04.1

Date Ackn'd 04/02/2004
CU2 Project Title THE USE OF THE HSV-1 ICP34.5 DELETION MUTANT 1716 AND ITS
Class 2
CultureVol/Class 2 < 1 litre
CultureVolumeClass 3-4

02/03/2022
All Crusade’s activities are based on HSV-1 strains 17+ and 1716 with the emphasis on HSV 1716 as a tumour killing virus or gene therapy vector. Our research is based on an evolution of HSV 1716 from the basic first generation oncolytic virus with deletion of RL1 through second generation viruses either with enhanced tumour killing potential or as gene therapy agents for the expression of therapeutic proteins to a third generation of viruses with restricted tropism mediated by surface expression of targeting proteins.

Recipient or parental organism

Permissive tissue culture cell lines including BHK, 3T6, Vero, SK-N-SH and a range of tumour cell types, both primary and cell lines.

Host/vector system

The vector HSV 1716 and a varient of HSV-1 strain 17+ which has a deletion of the RL1 gene ablating the neurovirulence factor ICP 34.5. The RL1 locus is also the site of the transgene insertion.

Origin & function

The genes of interest include cell cycle proteins, hormones, growth factors, enzymes, transporters, anti-sense RNA and tumour targeting ligands/antibodies. They function either to improve the ability of HSV1716 to kill cells, to overcome genetic defects in cells infected with HSV1716 or to alter/restrict HSV1716 tropism.

Evaluation of foreseeable effects

When using human herpes viruses as vectors, consideration should be given to the nature and expression level of the novel gene insert. The HSV virus is an attenuated form and is selectively replication competent, only replicating in dividing cells, therefore the virus will be unable to replicate in the adult CNS. We do not expect that insertion of the genes of interest will affect the growth properties or pathogenicity of HSV 1716.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Lab coat, gloves and glasses will be worn when handling viral vectors. All work involving manipulation of the virus will be performed in a microbiological safety cabinet. All solid waste includinVirkon or Chloros overnight or autoclaved prior to normal cleaning. The work involves standard laboratory protocols with no unusual procedures that require additional containment measures. plastics will be autoclaved.
Liquid waste will either be autoclaved or inactivated by incubation in 5% Virkon or Chloros overnight. Non-disposable items will either be incubated in Virkon or autoclaved. 

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification) 

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste generated or equipment used will be either sterilised in Virkon or autoclaved. This will completely deactivate any virus and is a method which is commonly practised in virology. Solid waste is disposed of by incineration after autoclaving. All deactivated liquid waste can be disposed of down the sink with copious amounts of water. Sharps will be incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

All class 2 work currently undertaken by Crusade Labs has been approved by the local University of Glasgow GMSC. Following the establishment of its own GMSC, Crusade Labs extensively reviewed all its current risk assessments at a meeting attended by all Crusade personnel. Detailed consideration was given to all aspects of Crusade's research and development including vectors with improved cell killing, gene therapy vectors, variants with altered tropism and the genes of interest used in the production of all HSV1716 variants. The meeting concluded that none of Crusade's work used toxic genes/oncogenes or generated viruses with a greater risk than wild-type HSV-1 and all risk assessments were approved.

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 885/06.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
Clinical trials using oncolytic herpes simplex virus HSV1716

Clinical testing of gene therapy agents based on herpes simplex virus, with the aim to develop and validate improved treatments for cancer.

The gene therapy agent, HSV1716, is a modified version of herpes simplex virus type 1 (Glasgow strain 17), that has been modified to be non-pathogenic in normal tissues. However, it retains the ability to undergo lytic replication in cancer cells. This virus has already been tested in Phase 1 human clinical trials and found to be safe when administered intracranially.

 HSV1716 is intended to replicate in cancer tissue, spreading through the cancer and killing the cancer cells. Because it is unable to replicate in normal tissues, it is deemed non-pathogenic. Therefore, it should not harm normal tissues, and would be unable to spread in the population.

 HSV1716 is expected to replicate in cancer cells, killing them by lysis, releasing identical progeny virus that can then infect surrounding cells. Therefore, the vector should spread progressively through a tumour, killing the cancer cells. Although some normal cells, eg at the tumour margins, may become infected, HSV1716 should be unable to replicate in these, and spread of the virus should cease. For example, in previous clinical trial work, no infection of ependymal tissue was detected and no infectious virus could be recovered. It is also thought unlikely that HSV1716 could reactivate from a latent state.

Transmission from the cancer patient to other humans will be prevented, by covering the injection site with occlusive dressings and the risk of infection is negligible in comparison to that of wild type HSV. HSV1716 does not shed, so no special post operative containment measures are required.
In the unlikely event that persons other than the cancer patient become contaminated with HSV1716, it will be unable to replicate and so it is most likely that no significant effects would be observed. HSV1716 will be unable to generate the "cold sore" lesions associated with the natural herpes simplex virus, because these result from virus replication in the normal skin, whereas HSV1716 is unable to replicate in normal tissue. A high proportion of the population has been naturally exposed to herpes simplex virus type 1, and has pre-existing immunity to the virus (up to 90% of the population). If seronegative individuals are infected with HSV1716, either deliberately (patient in trial) or through inadvertent self-inoculation at the time of administration, then seroconversion may occur.

Thus the effects of the HSV1716 infection in normal individuals are expected to be minimal. As an additional safety feature, HSV1716 retains the viral thymidine kinase gene, and thus infection can be controlled with the drug acyclovir and gancyclovir.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste materials contaminated with the GMM will include the vials in which it is supplied. Any vials that are dispensed will be held in a sharps bin for 8 hours at room temperature, conditions known to deactivate the virus, before following standard hospital disposal policy for such material (incineration). The disposable aspirate needle systems, contaminated dressings, latex/vinyl gloves, disposable aprons and eye protection with a risk of contamination will be placed into a sharps bin at the point of use, prior to disposal following standard hospital policy. The reusable needle systems will be placed in a standard Savlon solution (Clorhexidine 2-4%) for 5 minutes to deactivate the GMM, rinsed and then sterilised by autoclaving on-site, using fully validated and documented procedures, providing 100% kill.

We have also validated disinfection using 1-2% Virkon (a commercial disinfectant), to provide complete inactivation of HSV1716 as an alternative.

Decontaminated waste is disposed of as clinical waste, being removed by contractors for high temperature incineration off-site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC agreed that HSV1716 is appropriately classified as a Class 2 organism and agreed that the containment facilities and procedures proposed here and in the risk assessment are suitable and fit for purpose. The GMSC recommended, as a precaution, that healthcare workers that are immunosuppressed, pregnant, or have active eczema, should take extra care not to come into contact with HSV1716, particularly in areas where the virus is administered.

Project Containment

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Animal Units

Large Scale Activities

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Name

UNIVERSITY OF CAMBRIDGE

Department

DEPARTMENT OF PAEDIATRICS

Campus Estate or Research Centre

ADDENBROOKE'S HOSPITAL

Road Name

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB2 2QQ

Country

ENGLAND

Tel Number

01223 336 948

Fax Number

01223 336 996

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
# Premises Addresses

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# Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Y

**Give brief details of the genetic modification safety committee**

Advice on risk assessment for GM proposals will be sort in the main departmental safety meetings which are held quarterly each year and will be attended by the departmental BSO or another suitably informed person.

<table>
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**Tick if confidential**

- Bacteriology: Yes
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
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For activities involving GMMs, describe the waste management measures which will apply to the activity

| GMm cultures and glassware used for work with GMMs will be treated with presept tablets (Johnson and Johnson) as described in the manufacturer's instructions. |
| Solid waste will be autoclaved then incinerated. |

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

An extraordinary meeting of University of Cambridge Safety Officer for Clinical Medicine and Department of Paediatrics BSO viewed the risk assessment to be accurate and suggestive that the work is unlikely to have a deleterious effect on the health of workers or the environment.
BIOVENTIX LIMITED

7 ROMANS BUSINESS PARK
EAST STREET
FARNHAM
SURREY
GU9 7SX
ENGLAND

01252 728 001
01252 728 002

EAST AND SOUTH EAST

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Safety Officer/Chairman
- Management representative (Managing Director)
- Representative from each department within Bioventix
- Committee meets biannually and performs one audit of laboratory facility per year

<table>
<thead>
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Other (please specify)

Bacteriology: Yes
Parasitology
Transgenic Birds
Microbiology Research

Tick if confidential

02/03/2022

Page 11822 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

The GMSC has reviewed the company’s GM procedures and the risk assessments performed. The GMSC confirmed the work to be carried out is Class 1 and were satisfied with the assessment of risks to both human health and the environment. The control and containment measures in place are appropriate for class 1 activities.
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| Tel Number                       | 01480 410 850            |
| Fax Number                       | 01480 410 858            |

| E-mail                           |                          |
| HSE Division                     | EAST AND SOUTH EAST      |

| Comments                         |                          |

Date at Which Additional Info Submitted

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The committee consists of two senior PhD qualified scientists who meet once every quarter. Both scientists have >10 years experience working within bacteriological, virological and cell maintenance environments. They also have experience in authoring risk assessments. Their role is to:

1. Oversee the design and implementation of all scientific research with NextGen Sciences Ltd with a view on biological safety.
2. Risk assess each specific project to ensure fit between facilities and required containment.
3. Ensure that the Management Committee at NextGen Sciences is fully aware of HSE notification and company infrastructure requirements.
4. Ensure the laboratory applies required biological safety standards.
5. Provide a reference point for all company employees who have concerns or issues regarding biological safety and manipulation.
6. Ensure completion and submission of the relevant Risk Assessments to HSE including any notification of premises required.

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Tick if confidential
**Bacteriology**  Conf  **Parasitology**  
**Transgenic**  **Microbiology**  
**Birds**  **Research**  

**Virology**  Conf  **Transgenic**  
**Animals**  **Gene Therapy**  

**Mycology**  **Transgenic**  
**Invertebrates**  **Plants**  

**Other (please specify below)**  Yes

**Other(s)**  Recombinant protein expression and purification.

For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

There were no comments.

---

**Project Ref**  888/04.1

**Date Ackn'd**  07/04/2004  **CU2 Project Title**

**USE OF COMMERCIALL PURCHASED AND IN-HOUSE GENERATED DNA VECTORS FOR THE CLONING AND EXPRESSION IN BACTERIAL CELLS OF OPEN READING FRAMES CORRESPONDING TO GENES IDENTIFIED AS POTENTIAL ....**

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**Non-GMM**  

Consent Granted  

**Project notified under transitional arrangements**  N

Tick if notifying a connected programme of work  N

**Historical Significant Changes**
### Project Additional Information

#### Purposes of the contained use

To protect the user from exposure to bacterial strains carrying plasmid DNA sequences which encode potentially carcinogenic sequences under transcriptional control of a strong bacterial promoter. To protect the user from exposure to recombinant virus possessing DNA sequences which encode potentially carcinogenic sequences.

#### Recipient or parental organism

Bacterial strain BL21 (DE3) and derivatives of, which will harbour plasmid DNA containing potentially carcinogenic sequences under transcriptional control of a strong inducible promoter. Expression of protein requires the presence of the DE3 lysogen present in the above strain.

Recombinant baculovirus particles containing potentially carcinogenic gene sequences.

Further characteristics of the GMOs have been provided in the attached risk assessment.

#### Host/vector system

The above strain BL21 (DE3) and its derivatives are currently classified as CAT 1 but in conjunction with the plasmid backbone being used for expression (pET derivative) represents a plasmid mobilisation defective environment. Thus an element of risk exists in the potential for gene transfer between bacterial strains.

An element of risk in handling potentially carcinogenic sequences may exist with regards to tumor development, hence CAT II containment is envisaged to negate this risk.

Assessment of the danger involved with this host/vector relationship have been detailed within the attached risk assessment.

#### Origin & function

The genes identified as potential Breast Cancer targets have been listed with Appendix 2 of the attached risk assessment. It is possible that this list will be extended at which point a further risk assessment will be provided to cover these genes.

The genes have been isolated by a partner (Cytomyx Ltd, Cambridge UK) from human tissue using PCR based cloning methodologies. The ORF for each gene is then supplied to NextGen Sciences in a general cloning vector ie not suitable for protein expression purposes.
Expressed proteins will be purified in microgram quantities and used to attach to solid surfaces for testing of commercial antibodies to Breast cancer target proteins.

**Evaluation of foreseeable effects**

Once produced and purified as protein no foreseeable effects are likely.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

1. All biological materials and disposable labware (except pipettes, see 2) associated with handling/manipulating the biological material will be disposed into autoclavable bags, then double bagged, sprayed with 70% alcohol and inactivated by autoclaving for 15 minutes at > 121 degrees C.
2. Within the CAT II laboratory environment, all disposable pipettes eg 5ml, 10ml and 50ml will be held within a plastic pipette container one quarter filled with appropriately diluted Virkon (1%). Attached micro-organisms will be inactivated in this environment for > 12hrs then disposed of as in 1.
3. All sharp material will be sprayed with 70% alcohol before being held within a standard yellow sharps disposal container. Once filled this container will be double bagged, sprayed with 70% alcohol and disposed into a large metal drum prior to collection for incineration.
4. All glassware used in culturing will be immersed in a large vat of fresh appropriately diluted Virkon (1%) and the microbiological material inactivated for > 12 hrs. The vat will then be sprayed with 70% alcohol and removed to wash-up facilities where the glassware will be washed, sterilised and prepared for reuse.
5. Liquid cultures will be inactivated by direct application onto solid Virkon such that within a 12hr period the concentration to Virkon solution will be greater than or equal to 1%.
6. All bagged autoclaved waste will be disposed into large metal drums and removed by an external vendor for incineration.

NB See Appendix 3 to Risk assessment which details operational procedures within the CAT II environment including waste management.

---

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

---

**Please enter comments on the GM safety committee on the risk assessment**

There were no comments.

---

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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02/03/2022
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**Name**

**NOVACTA BIOSYSTEMS LIMITED**

**Name 2**

**Department**

**Campus Estate or Research Centre**

**NORWICH RESEARCHPPARK**

**Building**

**JOHN INNES CENTRE**

**Road Name**

**COLNEY LANE**

**District**

**Town**

**NORWICH**

**County**

**EAST ANGLIA**

**Postcode**

**NR4 7UH**

**Country**

**ENGLAND**

**Tel Number**

01603 450 966

**Fax Number**

01603 450 971

**E-mail**

info@novactablo.com

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Project Withdrawn on 4/5/2004 should not have been notified as already registered under other GM Centre

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Y**

Give brief details of the genetic modification safety committee

Committee Members:
- From Novacta: Research Director (Company Safety Officer)
- Fermentation Group Leader (Biological Safety Officer)
- Molecular Biology 1 Group Leader, Molecular Biology 2 Group Leader and Molecular Biologist.

The committee will meet annually to review all risk assessments and HSE notifications. Additional meetings will be held to consider new risk assessments and notification requirements as required. All assessments and notifications will be circulated by email and comments from the committee taken into account prior to any new procedures being adopted. Any assessments will fall outside the expertise of more than three of the committee will be sent to external advisors.

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Solid biological waste will be placed in unsealed bags and sterilised within the Novacta facility by autoclaving. If the waste is to be deactivated by the University central services, the unsealed bag will be placed in a lidded but unsealed boxes and removed from the laboratory to the central autoclave service where it will be sterilized. The autoclave cycle will include heating the material to at least 120 degrees C for 15 minutes to ensure 100% kill for all material.

Liquid waste and contaminated glassware will be treated in one or two ways:
1) Autoclaving: The autoclave cycle will include heating the material to at least 120 degrees C for 15 minutes to ensure 100% kill for all material.
2) Hycolin disinfectant: Hycolin disinfectant, diluted according to the manufacturers instructions, overnight at room temperature.

In either case an initial check will be carried out the first time the process is carried out to check that no culturable cells remain before disposal. This check will be repeated periodically depending on classification of risk and whenever waste from different cell types or quantities of culture are produced to ensure that the procedure results in a 100% kill.

Members of the Genetic Modification Safety Committee have read the attached risk assessment and have agreed with its conclusions.
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GMM committee is appointed by, and reports to the Chief Executive. It comprises 3 people, two with science PhDs and all three with first-hand experience of working with GMOs.

<table>
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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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| Other(s) | |
|----------||

### For activities involving GMMs, describe the waste management measures which will apply to the activity

For GMMs (disabled strains of E. coli): render non-viable with Virkon or equivalent, dispose as liquid waste or render non-viable by autoclaving, dispose as liquid or solid waste, as appropriate. For GM invertebrates: kill by freezing (< 10 degrees Celsius, >20 hours, or as required to kill), then dispose as solid clinical waste.

### Please enter comments of the GM safety committee on the risk assessment

The GMO Safety Committee has considered all aspects of the Health & Safety Implications of the proposed work. It has not identified any risks that are greater than working with non-GM versions of the species: these risks are primarily the risk of allergy from working with insects and the normal laboratory hazards of using glassware, equipment and reagents. Waste will be inactivated as described in the risk assessment, and disposed of using a professional waste disposal service. We unanimously believe that the research should be classified as Containment Level 1. Virtually identical research has been carried out in the University of Oxford, Department of Zoology, and the Departmental GMO Safety Committee has come to a similar conclusion.
## CHROMA THERAPEUTICS LIMITED

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**E-mail:**

**HSE Division:** EAST AND SOUTH EAST

**Date at Which Additional Info Submitted:** 02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Chief Scientific Officer (Chairman), Senior Molecular Biologist (Biological Safety Officer), Senior Cell Biologist, Operations Manager (Health and Safety Officer). Meets regularly at 3-month intervals, and/or as and when necessary to review procedures and new risk assessments.

<table>
<thead>
<tr>
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<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Non-microbial

Other (please specify)

Tick if confidential

Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research |

Virology | Transgenic Animals | Transgenic Fish | Gene Therapy |

02/03/2022

Page 11837 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

All liquid waste and all plastic disposable solid waste will be decontaminated in a final concentration of >5% Trigene overnight. The manufacturers of Trigene, The Hygiene Corporation Ltd, state that it exhibits a kill rate (virucidal, bactericidal and fungicidal) of >99.999% after contact with 1% Trigene for 5 minutes under clean conditions, and 5% Trigene for 5 minutes in the presence of 25% protein. Decontaminated waste, and paper waste/gloves, will be sealed in autoclave bags and placed in a one-way burn bin for incineration. Incineration will be undertaken by S Grundon (Waste) Ltd (Lakeside Road, Coinbrook, Slough, SL3 OEB). The company has been approved by the HSE for the incineration of GM waste.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

None.
GM Centre Number: 892

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02/03/2022

Date at Which Additional Info Submitted
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The GMSC consists of the following members:
- Biological Safety Officer
- Principle Investigator
- Post-doctoral scientist
- Support Staff

The committee meet as required and obtain additional expert advice from the clinical school safety officer.

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<th>Glass House</th>
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Level 4 (GMMs)
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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Solid waste will be incinerated and cultures will be inactivated with 1000 ppm free chlorine bleach prior to disposal via drains.

**Tick to confirm that you are attaching a summary of the risk assessment**

Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**


**Please enter comments of the GM safety committee on the risk assessment**

The risk assessment was agreed by the GM Safety Committee.

---

**Project Ref** 892/09.1

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02/03/2022
Characterisation of Genes Involved in Haematopoietic Differentiation and Megakaryopoiesis

Date Project Ceased

31/03/2009

Class 2 1-50 Litres

Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Withdrawn N

Tick if notifying a connected programme of work N

Project Additional Information

Purposes of the contained use

The process of haematopoietic lineage commitment, megakaryopoiesis and platelet production is poorly understood, but necessary for improved clinical management and therapy in platelet disorders. A number of candidate genes of potential importance have been identified. This project aims to characterise such genes by over-expression or knock-down/knockout in primary human cells or cell lines, and subsequent biochemical analysis. For studies using primary human cells, retroviral (lentiviral) vectors are needed to obtain significant levels of gene transfer.

Recipient or parental organism

Recipient of genetic material (first GMO) is a replication-incompetent retrovirus (lentivirus; see information below). This will be used to infect (transduce) various murine and human cells in culture, particularly those of haematopoietic origin and including sem cells. The unmodified cells are fastidious and non-harmful, unable to colonise or cause disease (Hazard group 1). Primary human cells are derived from screened healthy donors. However, as there is a possibility that material may contain adventitious infectious agents, it will be handled at Containment Level 2.

Host/vector system

Vectors are defective lentiviruses (i.e. Replication incompetent, 'third gneration' derivatives of HIV-1) in which the helper genes are located on separate blocks of DNA. Such vectors may infect human cells and integrate into the cellular DNA, but when unmodified are considered class 1 GMMs. The SACGM guidance on 'pseudotyped' vectors suggests they should be considered as class 2 GMM's. When modified with genes of interest they will be treated as class 2.

Origin & function

The 'insert', or donated, genetic material consists of human genes proposed to be involved haematopoiesis, particularly platelet/megakaryocyte function. These include known human leukaemia genes including MEIS1, as well as other factors involved in transcriptional regulation. These will be overexpressed in various cell types to observe effects such as altered growth, differentiation, self-renewal and apoptotic response. In addition, short hairpin RNAs that inhibit expression from transcripts pf the above genes (giving 'knock-down') will be inserted.

Evaluation of foreseeable effects

The viruses used for introduction of the transgene are replication-incompetent. The components required for viral production are present on separate vectors, transfected
into the 'packaging' cell line, so that newly formed viral particles contain only the transgene and the minimal viral genes required for its genome integration and expression. They do not contain all the factors necessary for reproduction of that virus. In addition, virulence genes have been completely removed from the system. The possibility of sequential recombination events in the packaging cell line giving rise to replication-competent virus is extremely unlikely, and recombination with wild-type virus is prevented by regular screening of the packaging cell line for wt HIV-1 by PCR. High titre viral stocks will not be produced in-house but commercially from constructs we supply. Stocks will be stored in lockable freezers in areas of restricted access. Thus the vectors do not present a risk to the wider human population or the environment. The viruses will be VSV-G 'pseudotyped'. This coat protein confers a wider tissue tropism than for the natural virus. The vectors may be transmitted by contact/aerosol as well as the natural percutaneous route. Lentiviruses are able to infect non-diving cells unlike other retroviruses; the range of cells and modes of transmission are thus significant. Such chimaeric particles exhibit a greater physical stability but are more sensitive to the complement system. Considered class 2. On infection, these viruses insert their DNA into the cellular DNA and so are capable of expression for the life of the cell. Insertion itself could lead to a deleterious mutagenic event but this is of low probability. Long term expression of some of the genes/inserts of interest could result in cell transformation or oncogenesis. However, it is unlikely that a single expression event would result in any clinical manifestation and VSV-G pseudotyped virions have been shown to be relatively ineffective at entering via the apical surface of cells of the airway epithelium. These factors combine to greatly reduce the risk of harmful effects from such viruses. The risks from the proposed inserted genes is not considered to significantly increase the overall risk therefore vectors with inserts are class 2. The human cells transduced by such viruses may become transformed/oncogenic; however they would remain fastidious and would be incapable of colonising/causing disease; cells could present a genuine risk to corresponding donor, but there will be no opportunity of donors to be so exposed. Class 1

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

A derogation from the requirement for an autoclave in the building is sought. We are embedded within the National Blood Service and our waste is handled as per their national policies; disposal of clinical waste is under contract to Whit Rose (see below)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All medical sold waste is currently destroyes by incineration under site contract (while Rose Environmental (Now SCRL), authorized/validated to carry/destroy hazardous material of GM Class 2). Disposable labware exposed to Viral GMO's will be treated with 1% Virkon for at least 16 hours, then placed in a toughened plastic autoclave bag within 20L, sealable sharps bins used for commercial incineration: 100% kill. Culture fluids will be exposed to 1% Virkon for at least 16 hours before sink disposal: effectively 100% kill. Work surfaces will be wiped with 1% Trigene followed by 70% ethanol: effectively 100% kill Virkon has been extensively validated for viral inactivation by manufacturers(Antec)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

06/01/09: Departmental GMSC met to discuss proposed work
19/01/09: GMSC agreed that the specific work can be conducted subject to terms of the risk assessment, and following the allocated period (45 days) after acknowledgement by HSE of this notification.
Hepatitis B virus (HBV) and hepatitis C (HCV) infections remain a major global public health problem despite the availability of effective antiviral treatments and anti-HBV vaccine. HBV- and HCV-related chronic hepatitis are the main causes of cirrhosis and hepatocellular carcinoma (HCC) that are responsible for a high rate of morbidity and mortality. In addition, despite continuous technical improvement in blood donation testing, hepatitis virus infection is still a major risk of transfusion/transplantation-transmitted viral infection. It is critical to increase our knowledge about the replication properties of these hepatitis viruses.

Recently, a relatively large number of infected, apparently healthy blood donors escaping HBV surface antigen (HBsAg) detection but with so-called occult HBV infection/carriage (OBI) were identified. OBI is characterized by undetectable HBsAg in the presence of very low levels of viral DNA in blood. The mechanism(s) explaining the transient low levels of virus in OBI donors are largely unknown. We have identified mutations affecting different critical regulatory elements in the HBV genome. These
mutations are assumed to negatively impact viral replication possibly explaining low viral load and HBsAg yield below detection threshold. This hypothesis will be explored in vitro by comparing replication ability of HBV variants and wild type strains from naturally infected individuals. OBI variant strains with significant reduced replication abilities will be 'repaired' by site-directed mutagenesis of the genomic position studied, and the corresponding OBI mutations will be introduced in wild type control genomes in order to definitively link OBI-specific mutations to reduced viral replication.

Over the past years, we collected indirect evidences that HCV particles may carry at their surface a particular cellular protein embedded in the viral envelope. If confirmed, the presence of this cellular protein may or not play a role in the virus replication. Our objective is to purified viral particles from culture supernatants to test for the presence of the cellular protein at the virus surface by using electron microscopy and labelling with specific antibodies.


Recipient or parental organism

- PCR- amplified HBV genome of fully characterized OBI strains and wild type HBV controls. HBV virus can cause both acute and chronic liver disease potentially evolving to cirrhosis or liver cancer on the long term in humans. The virus is transmitted through contact with the blood or other body fluids of an infected person, not through casual contact. HBV is classified as hazard group 3. HBV infection is preventable with a safe and effective vaccine and several effective antiviral drugs are available for treatment. However, the infectious viral particles will not be cultured and only non-replicating viral DNA directly extracted from low titre plasmas will be used

- The pJFH1 plasmid (Wakita et al., 2005, Nat Med, 11: 791-96) containing JFH-1 HCV genotype 2a full-length DNA construct will be directly supplied by, Heidelberg University, Heidelberg, Germany. HCV is classified as a hazard group 3 pathogen and HCV infection can lead to chronic liver infection, liver cirrhosis and hepatic carcinoma. HCV is transmitted through contact with the blood or other body fluids of an infected person. Less common but possible sources of HCV infection are needle-stick or injury with other contaminated sharp instruments, contamination of open wounds, contamination of skin lesions (eg eczema), and splashing of the mucous membranes of the eye, nose or mouth. The HCV genome is under T7 promoter control in the plasmid and consequently requires T7 polymerase for transcription. The latter is a bacteriophage polymerase found in bacteria infected with bacteriophages. It is extremely promoter-specific and only transcribes DNA cloned downstream of a T7 promoter. In the DNA form, the HCV replicon in the pJFH1 plasmid is not infectious and requires transcription by the above bacteriophage polymerase to its RNA form. E. coli strains containing T7 promoter will not be transformed.

Overall risk: Effectively zero.
group 3 and is transmissible by direct contact through percutaneous inoculation (from sharps, including needles, scalpels, broken glass, etc including contamination of exposed skin or pre-existing cuts and abrasion etc) contamination of conjunctivae by inadvertent contact with eyes. The amount of potentially infectious virus produced in culture will be limited by (1) the low volumes of cultures (5-10mL), (2) except for wild type controls, the HBV strains studied have impaired replication properties, and (3) viral particles produced in culture supernatants cannot directly infect HUH-7 and Hep G2 cells and no high viral stocks will be produced. Culture supernatants and transfected cells will be inactivated after final testing. All workers are vaccinated against HBV with documented levels of anti-HBV neutralizing antibodies and antiviral drug therapies are available.

The resulting HBV will not differ radically from the parental virus. Mutations introduced by site directed mutagenesis would either restore a wild type sequence in HBV natural variants or introduce mutations present in naturally infected individuals with evidence of impaired viral replication and no clinical symptom. Consequently, the resulting GMM is not expected to have enhanced replication ability or to be more infectious. Site directed mutagenesis should not affect viral genes involved in antiviral drug resistance or vaccine/immune escape.

- The full length HCV RNA will be transcribed in vitro from the pJFH1 plasmid. In vitro transcribed RNA is highly fragile, susceptible to cleavage and destruction by nucleases ubiquitously present in skin, hands and nails. Transfection into mammalian cells needs special procedures using appropriate transfection reagents. Quantity of RNA for transfection of Huh-7 or HepG2 cells will be 5-10 micrograms. HCV RNA transcript is capable of producing infectious HCV particles upon transfection into mammalian hepatocyte cells (Huh-7 cells and HepG2 cells). HCV is ACDP hazard group 3 and its routes of transmission are similar to the ones described above for HBV. One human plasma, the source of the HCV-H77 (genotype la) genomic consensus sequence, had a chimpanzee infectious dose (CID) tier of about 10e6.5/ml. This was in good agreement with results from the branched DNA probe assay, which demonstrated that the ratio of HCV RNA genome equivalents to CID of ,10e2/ml and a genome equivalent CID ratio of greater than 10000:1. Reasons for these differences are not clear, but could reflect a high level of defective genomes or immune complexes in the plasma of this subject (Walker 1997, Springer Seminars in Immunology, 19:85-98).

The sequence of the HCV strain inserted in pJFH1 plasmid will not be changed during the whole process and the virus produced in culture will be identical to the parental strain.

**Evaluation of foreseeable effects**

- The resulting GMMs will not differ radically from the recipients. The sequences inserted in the plasmids are unlikely to be transcribed efficiently in bacterial cells and any background transcripts would lack the translation signals to engage bacterial protein synthetic machinery. Any viral proteins would not be expected to have any effect on the properties of the bacterial host and there is no mechanism to excrete these proteins. Even if human cells were exposed to the viral proteins, they would not be expected to have any effect.

The modification is not expected to overcome disablement of the host organism, nor affect host specificity, tissue tropism or susceptibility to host defence mechanisms.

- The viral genome is disabled in plasmid and cannot replicate in bacteria.
  - HBV: non-replicative naked linear viral DNA with disrupted viral promoter controlling viral polymerase and core protein gene expression as well as synthesis of the pregenomic viral RNA both essential for HBV replication.
  - HCV: viral genome under the control of T7 promoter and needs transcription by T7 polymerase. The latter is a bacteriophage polymerase not present in uninfected bacteria. E. coli strains are available that contain viral T7 polymerase in their genome (egBL21(DE3)), and care will be taken not to transform these strains.

- Stability of the cloned viral genome:
  - HBV: stability of linear non-replicative HBV double stranded DNA ensures by the addition of Dapl sequences of both termini to prevent release and self re-assembly. HCV: without the T7 promoter, the inserted HCV DNA would not be transcribed and generate a coding genomic RNA (+) strand.

- Analytical scale quantities of HBV linear DNA or in vitro transcribed HCV RNA (total yield ,5 microgram DNA/RNA) to be undertaken at containment level 1 with Good Microbiological Practice and Good Occupational Safety and Hygiene. This work will be conducted at the NHS Blood & Transplant Centre, Long Road, Cambridge, Purification of larger quantities of viral DNA or RNA (up to 10 microgram) for transfection of hepatocyte cell lines will be conducted in the Containment Level 3 laboratory at Lab 21 Ltd, Science Park, Cambridge. Only non-infectious plasmids will be transported in sealed adequate containers between NHS Blood & Transplant Centre and Lab 21 Ltd facilities.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Control measures
Control of HBV genome and site directed mutagenes: containment level 1 with Good Microbiologicval Practice and Good Occupational Safety and Hygiene. Disposal of GM waste is into sealed plastic bins for incineration under NHS Blood & Transplant site rules.

Step 2 - HBV replication in hepatoma cell line cultures: All disposable solid culture/lab waste will be treated with 1% Virkon for at least 16 hours, then autoclaved, and incinerated: 100% kill. Culture fluids will be exposed to 1% Virkon for at least 16 hours, then autoclaved, and incinerated: 100% kill. Culture fluids will be exposed to 1% Virkon for at least 16 hours and disposed to drains: effectively 100% kill. Bench/cabinet surfaces will be wiped with 70% ethanol: effectively 100% kill. Spills will be sprinkled with Virkon powder. Virkon has been extensively validated for viral inactivation including HBV and HCV by the manufacturer (Antec). All waste to be disposed of according to Lab 21 site rules.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

On 01/04/2011, members of the safety committee and a representative of the NHSBT visited Lab 21 to discuss the proposed use of the facility under their procedures. We met the Compliance Officer, Biological Safety Officer and the CL3 Supervisor and were satisfied with the operational arrangements, the induction, storage and waste management procedures of Lab 21.

University and NHSBT employees involved in this project are experienced scientists, and have been inducted at Lab 21.

On a review of the original draft of the risk assessment (RA), we sought further clarification about the possible enhanced risk resulting from generation, in vitro, of higher viral titres than encountered in naturally occuring material. This was more fully addressed in the final RA, which was approved by the GMSC on 15/11/2011

Project Containment

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

- Managing Director - GMSC Chairperson
- Scientific Research Associate - key lab user, experienced in up to Class III work
- Media Manager - representative for non-lab users in organisation.

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Tick if confidential:

- Bacteriology
- Parasitology
- Transgenic Birds
- Transgenic Animals
- Microbiology Research
- Transgenic Fish
- Gene Therapy

02/03/2022
### Other(s)

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For activities involving GMMs, describe the waste management measures which will apply to the activity

- Solid media and contaminated plastics will be contained in approved biohazard waste bags until removal and incineration by an approved contractor.
- Used pipette tips, tubes will be totally immersed in a fresh effective 'use'dilution' of Chloros overnight and the disinfected materials transferred safely to an appropriate container as described.
- Contaminated sharps will be disposed of in approved biohazard incinerator cartons until removal and incineration by an approved contractor.
- Liquid media will be treated overnight with a fresh effective 'user-dilution' of Chloros, storage in a leakproof container until removal and disposal by an approved contractor. The premises to be used consist of one purpose-built laboratory within the unit and has no provision for an autoclave.

All volumes of bacterial suspensions will be small and pose no risk outside the appliance of good laboratory practice. In case of spillage, liquid will be mopped up with absorbent paper and chloros and disposed of in a biohazard bag as above.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Accompanying risk assessment approved by members on safety committee.
### Data Premises Notified
- **Date**: 30/07/2004
- **(Originally)**

### Transferred from 1992 Regs?
- **Yes/No**: N

### Transitional Premises Class
- **Withdrawn**: N

### Data Premises Closed
- **Emergency Plan Required?**: N

### Name
- **SAREUM LIMITED**

### Name 2

### Department

### Campus Estate or Research Centre
- **Building**: UNIT 2 PAMPISFORD PARK

### Road Name
- **LONDON ROAD**

### District
- **PAMPISFORD**

### Town
- **CAMBRIDGE**

### County
- **CAMBRIDGESHIRE**

### Postcode
- **CB2 4EE**

### Country
- **ENGLAND**

### Tel Number
- **01223 497700**

### Fax Number
- **01223 497701**

### E-mail

### HSE Division
- **EAST AND SOUTH EAST**

### Comments

### Date at Which Additional Info Submitted
- **02/03/2022**
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Chair
Head of Molecular Biology
Head of Protein Science

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Other (please specify)

Tick if confidential

Bacteriology

Yes

Parasitology

Transgenic

Birds

Microbiology

Research

Virology

Transgenic

Animals

Gene Therapy

Transgenic

Fish
Solid materials for autoclaving are placed in autoclavable disposal bags (double bagged) and transported to the autoclave in a leak-proof container. After autoclaving all solid waste will be taken away by an appropriate waste management company. Disposable pipettes are placed in appropriate plastic bins prior to autoclaving. All liquid waste must be rendered non-infectious before disposal and will be treated with 30gl Virkon for 24 hours.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Generic risk assessment enclosed for expressing proteins in E coli and one for expressing protein kinases in insect cells using baculovirus. Also enclosed two examples of the sort of work we are going to start in September.
GM Centre Number: 895

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Name

ACS DOBFAR UK LIMITED

Name 2

Department

Campus Estate or Research Centre

Road Name

District

CAMBOIS

Town

BEDLINGTON

County

NORTHUMBERLAND

Postcode

NE22 7DB

Country

ENGLAND

Tel Number

01670 565656

Fax Number

01670 850571

E-mail


HSE Division

YORKSHIRE AND NORTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y**

**Give brief details of the genetic modification safety committee:**

GMSC comprises of ACS DOBFAR staff who have direct and indirect involvement with the GMM or process. Also included are site safety/environmental staff, quality assurance staff, trade union safety representative and an external ‘expert’ consultant.

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**Other (please specify):**

Tick if confidential [ ]

Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research |

Virology | Transgenic Animals | Transgenic Fish | Gene Therapy |
For activities involving GMMs, describe the waste management measures which will apply to the activity

1. ALL HARVEST BROTH PASTEURISED AT 80°C FOR 1 HOUR. THESE CONDITIONS HAVE BEEN DEMONSTRATED TO GIVE A TOTAL KILL OF THE RALSTONIA ORGANISM. TESTING IS ON BLOOD AGAR BASE PLATES, INCUBATED AT 25°C AND 37°C FOR 72 HOURS.
2. ALL FERMENTOR DRAINS AND SAMPLE POINTS ROUTE TO COMMON COLLECTION SYSTEM. BOTH FROM THIS SOURCE TREATED WITH HYPOCHLORITE SOLUTION. NO VIABLE ORGANISM FOUND AFTER TREATMENT (USING BLOOD AGAR BASE PLATES).
3. LABORATORY CONSUMABLES EITHER PLACED IN SAVLON SOLUTION OR AUTOCLAVED.

CONCLUSION OF COMMITTEE THAT ORGANISM AND PROCESS PRESENTS NO RISK TO HUMAN HEALTH OR THE ENVIRONMENT AND CAN BE TREATED AS CONTAINMENT LEVEL 1.

NO ADDITIONAL CONTROL MEASURES NECESSARY OTHER THAN THOSE DEFINED IN RISK ASSESSMENT.

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]
GM Centre Number: 896

Data Premises Notified (Originally) 12/08/2004

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

OXFORD GENE TECHNOLOGY

Name 2

INNOVATIONS CENTRE

Department

Campus Estate or Research Centre

BEGBROKE SCIENCE PARK

Building

Road Name

SANDY LANE

District

YARNTON

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX5 1PY

Country

ENGLAND

Tel Number 01865 856352

Fax Number 01865 842116

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted 02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetics modification safety committee consists of the following people:
The Health & Safety coordinator for the company,
Two scientists with at least 10 years each experience in Molecular Biology,
A member of the management committee,
Normally the decision manager.

At least four members of the committee must be present.

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<tr>
<th>Laboratory</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid waste will be treated by autoclaving at 121 degrees, 1 bar pressure per 30 minutes in an autoclave. The waste will then be removed for incineration by specialist in waste disposal contractors. The autoclave is present within the laboratory and is serviced annually by the suppliers.

Sharps waste (including pipette tips) will be collected in specialist 'sharps bin' which will then be treated by autoclaving at 121 degrees, 1 bar pressure for 30 minutes in an autoclave. The waste will be removed for incineration by specialist waste disposal contractors. The autoclave is present within the laboratory and is serviced annually by the suppliers.

Liquid waste will be treated by autoclaving at 121 degrees, 1 bar pressure for 30 minutes in an autoclave. The waste will be removed for incineration by specialist waste disposal contractors. The autoclave is present within the laboratory and is serviced annually by the suppliers.

Alternatively liquid waste will be treated by putting enough Virkon S Powder in the liquid waste to make up 1% Virkon solution and left overnight. According to the manufacturer's website http://www.antecint.co.uk/main/virkons.htm a 100% decontamination of an E. coli culture was observed after 60 seconds.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment 

Please enter comments of the GM safety committee on the risk assessment
Minutes of the meeting.

* The agreed scale of growth of the GMM, is under 1 litre for Research & Development
* The vector, pSP64, has no Bom-, Nic-, Mob- and Tra- and is therefore non-mobilisable.
* The E.coli strain JM 109, because it is recA and needs added thiamin, is unlikely to survive outside of the laboratory.
* Given the above, the access factor for this host / vector combination of 10-9 was considered appropriate.
* The insert, an intergenic sequence from Saccharomyces cerevisiae was reviewed. A BLAST search to determine if any substantial homology to human sequences was requested. This has been carried out and no substantial homology was detected DH.
* The insert is inserted into the Smal site of pSP64. Therefore, the sequence is downstream of a SP 6 promoter, although the host bacteria does not carry this polymerase. Therefore 10-6 for the expression as under the Brenner scheme is considered appropriate.
* The insert does not code for any known proteins, so the Damage figure of 10-9 is appropriate.
* Handling this GMM under containment level 1 was considered safe by the committee.
* Filing of the Risk Assessment will be both electronically and on paper. The Risk Assessments needs to be signed by at least 4 committee members.
Recipient or parental organism

Mouse embryonic stem cells

Host/vector system

Mouse embryonic stem cells
Mammalian expression vectors e.g. pEGFP were used by our collaborators in order to introduce the GFP tag into the mouse ES cell line via homologous recombination.

Origin & function

GFP reporter genes are inserted in the mouse embryonic stem cell line for the four genes of interest for single cell analysis. The cell line to be received has already had the GFP tag introduced into the cell line by our collaborators. No genetic manipulation of this cell line will take place on the premises of Oxford Gene Technology. The cell line has been fully risk assessed by our collaborators and by ourselves.

GFP tagged cells will allow identification of cells positive for the genetic markers of interest and will concentrate our single cell analysis to only cells of interest amongst a largely heterogenous population of cells.

Evaluation of foreseeable effects

The stem cell line carries genetically modified endogenous genes created by homologous recombination.
Manipulated genes are not involved in or anticipated to have any effect on pathogenicity.
The stem cell line is not expected to survive outside of controlled culture conditions.
No micro-organisms used are listed in ACDP hazard groups 2, 3 or 4 or controlled by DEFRA.
pEGFP is not mobilisable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable - All work carried out within the premises of Oxford Gene Technology will be on mouse embryonic stem cell lines that have genes tagged with GFP.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cell culture flasks in which the cell lines are maintained will be exposed to 10% Virkon for a minimum period of 2 hours. Flasks will then be autoclaved and subsequently incinerated. All lab waste is collected as clinical waste by a professional management company (LAB3) and incinerated.
This is expected to cause 100% death to the cells within the flasks.
In order to ensure that all cells have been killed by exposure to 10% Virkon a “kill” control measure will be carried out. This will entail taking a flask that has been exposed to 10% Virkon and refeeding the cells within the flask. It is expected that the Virkon exposure will prevent any cell growth. Upon receipt of cells this will be carried out immediately and will subsequently be carried out once a month to ensure that 10% Virkon continuously and sufficiently causes cell death even before autoclaving and incineration.
A further control measure will entail removing an aliquot of Virkon from the flask and this will be placed in a fresh flask and re-fed with excess media and left in the incubator for 24 hours. The flask will be analysed the following day to ensure there is no cell growth.
cell culture utensils (such as glass pasteur pipettes) will be autoclaved and subsequently incinerated as described above.
All excess cells following experimentation will also be treated in the manner outlined above.
The safety committee has passed the proposed work following the review of the attached risk assessment.

Please enter comments on the GM safety committee on the risk assessment

The safety committee has passed the proposed work following the review of the attached risk assessment.

**Project Containment**

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**Regulation 20**

- Is an emergency plan required according to regulation 20? N
- Tick to confirm that it is attached to this form N
- Tick to confirm that you have attached a risk assessment to this form Y
- Tick if you are claiming exemption from disclosure for section of the risk assessment N
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### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee at IDIS consists of the following committee members:
- **Title:** Financial & Corporate Affairs Director
  **Positions:** Responsible person
  **Representative Functions:** Leader of integrated risk management committee and member of Health & Safety committee, responsible for the overall supervision and safety of GM activities at the premises.

- **Title:** Quality Assurance Specialist
  **Positions:** Quality Assurance representative on risk management committee
  **Functions:** Responsible for ensuring all GM activities are proceduralised and adequately implemented.

- **Title:** Logistics Manager
  **Positions:** Health & Safety Manager at address of premises.
  **Representative Functions:** appointed as IDIS Biological Safety Officer and representative of management with responsibility for the work in genetic modification. Also responsible for ensuring compliance of Safety Representatives and Safety Committee Regulations.

- **Title:** Pharmaceuticals Service Manager
  **Positions:** Qualified Pharmacist
  **Representative Functions:** Management representative and liaison for technical enquiries

- **Title:** New Product introduction (NPI) Co-ordinator
  **Representative Functions:** Employee representation responsible for co-ordinating the internal progress of GMSC and act as liaison between GMSC and internal departments.

IDIS have agreed that the GMSC will initially meet on a monthly basis, however this will be open to review at a later date.
This is a small premises where no autoclave is available, however, a third party contractor Adchem Environmental Services Limited (www.adchem-ltd.co.uk) will remove waste for remote disposal (incineration). Waste will be stored by IDIS in 60 litre sealed plastic One-way burn bins. The bins are removed by the contractor for incineration at 1000°C. This method will guarantee complete destruction. The company Adchem have confirmed that they are compliant with the Contained Use 2000 Regulations and are monitored by the Environment Agency.
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**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The company GMSC will consist of the General Manager, the Incineration manager, the Biological Safety Officer and the representatives of the chief organisations using the company for the incineration of its genetically modified organism waste.

In addition, a company representative will sit on the GMSC of each client company to represent the safety interests of the waste management process at each client customer.

### Laboratory

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

### Animal Unit

- Other (please specify) Waste Reception Areas, No 2. Waste Incinerator

### Growth Room

### Glass House

### Large Scale

### Bacteriology

### Parasitology

### Transgenic Birds

### Microbiology Research
### For activities involving GMMs, describe the waste management measures which will apply to the activity

1. Please refer to the transportation, packaging and labelling statement.
2. Please refer to the disposal method statement attached.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

None

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**Name**

WRC-NSF LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

HENLEY ROAD

**District**

MEDMENHAM

**Town**

MARLOW

**County**

BUCKINGHAMSHIRE

**Postcode**

SL7 2HD

**Country**

ENGLAND

**Tel Number**

01491 636587

**Fax Number**

01491 636501

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The genetic modification committee (GMSC) has been set up to ensure the safe use of the ER-CALUX Bioassay. The ER-CALUX assay uses genetically modified cells. It is the responsibility of the GMSC to ensure that controls on the contained use of the GMO are followed as stated in the Genetically modified organisms (contained use) regulations 2000. The GMSC committee as of 5th July 2004 are composed of:

- Chairman
- Site safety officer
- Laboratory Manager
- Operations Manager
- Biological Safety Officer
- ER-CALUX Project Manager

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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

All material that has come into contact with or may have come into contact with the genetically modified cells either directly or indirectly is to be placed in an autoclave bag. At the end of the working day the bag is to be sealed ready for autoclaving. The bag will be autoclaved and the autoclaved bags will be placed in bin liners, the top secured and stored in a suitable place. When sufficient bags have been collected they will be disposed via a recognised waste disposal company.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The WRc-NSF Genetic Modification Safety Committee (GMSC) have now met on two occasions to consider the risk assessments and safety precautions that have been implemented at WRc-NSF Medmenham to undertake the estrogenic potency testing using the ER-CALUX bioassay. The GMSC is satisfied that all risk assessments needed to carry out the bioassay have been completed and the committee agrees with the conclusions of these documents that the use of ER CALUX meets the category, Provisional Containment 1. The Committee is also satisfied that the safety procedures implemented meet the requirements on risk minimisation of a Class 1 activity.

Chairman, GMSC
### GM Centre Number: 900

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**Name**  
MEDICAL MARKETING INTERNATIONAL GROUP PLC

**Name 2**  
Department

**Campus Estate or Research Centre**  
BIOSCIENCE INNOVATION CENTRE

**Road Name**  
COWLEY ROAD

**Town**  
CAMBRIDGE

**County**  
CAMBRIDGESIRE

**Postcode**  
CB4 0DS

**Country**  
ENGLAND

**Tel Number**  
01223 477 677

**Fax Number**  
01223 477 678

**E-mail**  

**HSE Division**  
EAST AND SOUTH EAST

**Comments**  

**Date at Which Additional Info Submitted**  
02/03/2022
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Yes

Give brief details of the genetic modification safety committee:

- Committee consists of 4 persons with additional staff being co-opted when additional expertise is required
- 1 representative of all persons having access to the facility and group H&S officer
- 1 representative for Laboratory Health and Safety
- 1 representative for Tissue Culture Laboratory
- 1 representative for customer and internal GMO projects

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste - 5% bleach or 1% Vircon, leave for 12 hours at room temperature before disposal via sink drain followed by copious amount of water.
Plastic waste - Disposed of in hazard bins in laboratory prior to autoclaving at 121 degrees C for at least 10 minutes.

Spills - Affected area will be treated with 70% Ethanol or 70% IMS or 1% solution of Vircon.
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**Name**

ENVIRONMENT AGENCY - BIOLOGICAL EFFECTS LABORATORY

**Campus Estate or Research Centre**

**Road Name**

WATERBERRY DRIVE

**Town**

WATERLOOVILLE

**County**

HAMPSHIRE

**Postcode**

PO7 7XX

**Country**

ENGLAND

**Tel Number**

01903 832675

**Fax Number**

02332 233869

**HSE Division**

EAST AND SOUTH EAST

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The duties of the GMSC are fulfilled by the standing Environment Agency, Ecosystems and Human Health Science Group Health & Safety Committee. The committee is comprised from members of the sections that make up the Ecosystems and Human Health Science Group.

H&S Co-ordinator & Chair, Principal Scientist (Chemicals Assessment Unit), Senior Scientist (Chemicals), Senior Scientist (Human Health), Senior Scientist (Biological Effects), Scientist (Ecosystems) and a Technical Assistant (Chemical Assessment Unit).

OPERATING PROCEDURES AND FREQUENCY OF MEETINGS:-

1. Chair invites members to a quarterly meeting to discuss H&S matters and to review either new, or current, risk assessments.
2. Risk Assessments are circulated prior to the meeting.
3. During the meetings the relevant risk assessments will be discussed and any amendments required will be made by the Chair after liaison with the staff involved in drafting the original risk assessment.
4. The amended risk assessment will then be circulated to all staff in EHH for further comments/suggestions.
5. Finalised risk assessments are passed to the Head of the Environment & Human Health Science for formal approval.

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Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial
The GMM proposed for use in this study is an especially-disabled strain of Saccharomyces cerevisiae (FF18984), ACDP Classification - Class 1: Gentronix Greenscreen EM™. The Environment Agency's Biological Effects laboratory is a UKAS accredited ecotoxicological testing facility. Standardised Working Procedures will be adopted when handling Class 1 GMMs, consistent with, and often exceeding, those required for Class 1 containment (See the attached working practices document).

* GMMs will be handled, wherever possible, within a contained working area (re-circulating chemical fume hood) equipped with HEPA exhaust filters.
* Routine inactivation and disposal of items contaminated with Class 1 GMMs.
* All disposable items contaminated with Class 1 GMMs, such as polypropylene pipette tips, sample vials, cells culture plates and gloves will be sealed in small ‘biohazard’ bags containing absorbant material and inactivated via autoclave (see note 2) before appropriate disposal. Final disposal of items after inactivation will be as either general laboratory waste, recyclable waste (pipette tips/cell culture plates), or hazardous waste - depending on chemical contamination of the items by hazardous substances.
* All re-usable items contaminated with Class 1 GMMs, such as laboratory glassware, will be disinfected by total immersion in freshly prepared 1% (w/v) Virkon solution (Antec International) in a sealed container for 24 hours (see note 1). Items will then be washed and autoclaved (if required) following standard laboratory practices.

### Waste Management Measures for Activities Involving GMMs

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For activities involving GMMs, describe the waste management measures which will apply to the activity.
procedures prior to re-use (EA, Biological Effects Laboratory AQC procedures 9 and 5 respectively).
* Laboratory coats that could potentially have been contaminated with class 1 GMMs must be sealed in large 'biohazard' bags and subject to inactivation by autoclave (see note 2), before normal laundering.
* Stock cultures of class 1 GMMs, when no longer needed, will be sealed in small 'biohazard' bags containing absorbent material and inactivated via autoclave before disposal as general laboratory waste (see note 2).
* Work areas (fume hoods, incubators etc) will be disinfected after use using 70% Ethanol solution (see note 3).
* Hands and wrists of laboratory staff using Class 1 GMMs will be washed immediately using Day-Impex LabGuard (see note 4) if contamination by viable Class 1 GMMs is suspected. In addition, hands and wrists will always be washed using Day-Impex LabGuard (see note 4) before leaving the laboratory after performing any procedure involving viable Class 1 GMMs, even if no contamination is suspected.

Spillage Containment
* Liquid spills of class 1 GMMs on the bench or floor will be contained with absorbent paper tissue, or Virkon powder. Absorbent paper and powdered Virkon waste will be inactivated by autoclave before appropriate disposal (see note 1 and 3). The affected floor/bench area will be disinfected with 70% ethanol/water solution (see note 3). Final disposal of inactivated paper tissue/virkon will be as either general laboratory waste, or hazardous waste - depending on chemical contamination of the liquid spill with hazardous substances.
* Contaminated broken glass will be inactivated by autoclave (see note 2) before appropriate disposal. The affected bench/floor area will be disinfected with 70% ethanol/water solution (see note 3). Final disposal of inactivated broken glassware will be as either general laboratory waste, or hazardous waste - depending on any chemical contamination of the glassware by hazardous chemicals.
* Liquid spills >5ml will be recorded in the Laboratory's GMO activities log and reported to line managers. Areas will be swabbed and suspension tests performed to ensure that disinfection was effective.

Note 1 - Virkon is a peroxynitryl system that is widely used for laboratory disinfection, and is effective against bacteria, viruses and yeast. In addition, Virkon was found to be effective against 3 strains of Saccharomyces cerevisiae (the Host organism proposed for use in this study) after 5 minutes contact time at 1% using a suspension test (Alegente et al). 99.999% degree of kill is assumed after 24 hours contact with 1% Virkon solution.

Note 2 - Items inactivated via autoclave (LTE Scientific, Osprey - EA, Biological Effects Laboratory Inventory number E220) are subject to a temperature of 121ºC for a duration of 20 minutes. The temperature probe used in the autoclave, the autoclave cycle chart recorder and the temperature variation within the autoclave chamber are assessed and calibrated annually by a UKAS certified calibration agent. 100% degree of kill would be achieved.

Note 3 - Alcohols give a very rapid kill of micro-organisms, but do not provide sustained microbial action. 99.999% kill would be achieved.

Note 4 - Day-Impex LabGuard is an antimicrobial handsoap containing 0.5% p-Chloro-m-xylene (PCMX), a bacterial and anti-fungal agent that is effective in destroying a broad spectrum of organisms including Saccharomyces spp. (Day-Impex, 2004) 99.999% kill would be achieved.
GM Centre Number: 902

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

1. Director of Research and Development
2. Director of Business Operations
3. Biological Operations Manager/Health and Safety Officer
4. Head of Biological Services Unit
5. Head of in vitro Biology/ Biological Safety Officer

<table>
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<th>Level 1 (GMMs)</th>
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<th>Level 3 (GMMs)</th>
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</table>
We dispose of all relevant waste by incineration using a contract disposal company (Grundons).

For activities involving GMMs, describe the waste management measures which will apply to the activity

We dispose of all relevant waste by incineration using a contract disposal company (Grundons).

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM Safety Committee have reviewed the risk assessment and believe that the activities described present no risk to the environment or to human health.
GM Centre Number: 903

Data Premises Notified (Originally) 23/11/2004

Transferred from 1992 Regs? N

Transitional Premises

Class

Data Premises Closed 21/05/2008

Transitional Premises

Emergency Plan Required?

Non-GMMs N

Withdrawn N

Name

OXONICA LIMITED

Name 2

Department

Campus Estate or Research Centre

UNIT 8 BEGBROKE SCIENCE PARK

Road Name

SANDY LANE

District

YARNTON

Town

Kidlington

County

OXFORDSHIRE

Postcode

OX5 1PF

Country

ENGLAND

Tel Number 01865 856700

Fax Number 01865 856701

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

VP Research
Molecular Biologist
Company HSE Officer
IP Manager
GM Safety Officer

The GMSC operates as part of Oxonica's general HSE committee, which meets monthly, and the GMSC reports directly into the HSE Committee via its minutes. The GMSC meets on a 6 monthly basis, although exceptional meetings are called as necessary, for example when a new or revised Risk Assessment has been submitted.

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Tick if confidential
Oxonica Waste management for class 1 activities.

The principle method of waste disposal is via incineration for solid materials, and chemical treatment for liquid waste. However, solid waste samples are also pre-treated via autoclaving on-site to incineration.

The maximum culture volume of bacterial cells is 2 litres at any one time. Waste from such cultures is treated, following manufacturer's instructions, with Virkon powder or solution (Antec International Limited, UK). Efficacy of this treatment is tested every six months by treating a volume of viable culture in this manner and establishing percent kill by subsequent growth on an agar plate. Records of testing are maintained by the GM Safety Officer.

Disposable, solid waste is autoclaved at 134 degrees C for 15 minutes before removal to clinical waste storage. This is collected and incinerated by Select Environmental Services, Reading.

The autoclave (LTE Osprey 40) is serviced and calibrated annually by LTE Scientific, Oldhal. In addition, efficacy is monitored every three months using a Geobacillus stearothermophilus SporAmpule biological safety indicator (SPS Medical, New York, USA, distributed in the UK by Medical Engineering Technologies, Folkestone). Records of this procedure are maintained by the GM Safety Officer.

For activities involving GMMs, describe the waste management measures which will apply to the activity

GMSC requested that Permit to Work system be extended to the Biological lab and that fridges and freezers be lockable as best preventive measure against accidental out of hours exposure.
**GM Centre Number: 904**

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**Name**

SURFACE THERAPEUTICS LIMITED

**Campus Estate or Research Centre**

**Road Name**

45C MILTON PARK

**District**

ABINGDON

**Town**

OXON

**County**

OXFORDSHIRE

**Postcode**

OX14 4RU

**Country**

ENGLAND

**Tel Number**

01235 832525

**Fax Number**

08707 064178

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

**Tick: Yes**

Give brief details of the genetic modification safety committee

VP Research, Director Discovery Sciences, Director Biochemistry (Biological Safety Officer) and Senior Scientist.

Individual scientists will present written risk assessments TO THE MEMBERS OF THE gmsc FOR PRIOR EVALUATION. The GMSC will meet on a quarterly basis and/or whenever necessary to review risk assessments and procedures. The GMSC will be empowered to approve level 1 activities and will identify any activities that are deemed to warrant level 2 categorisation and which will therefore require advance notification to the HSE.

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Other (please specify)  
Tick if confidential

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Culture medium/liquid waste will be decontaminated by treatment with Trigene prior to disposal via the drain (2% (v/v) final for a minimum of 1h for routine use; 10% (v/v) final for a minimum of 1h under high organic load eg serum). Any re-usable glassware will be soaked in 2% Trigene for a minimum of 1h prior to washing.

Trigene (Medichem International Limited, PO Box 237, Sevenoaks, Kent, TN15 0ZJ; www.medichem.co.uk) is a non-toxic, non-hazardous, biodegradable agent with broad spectrum activity (bacterial, fungicidal, virucidal, mycobactericidal and sporicidal) recommended for medical, veterinary and environmental use at 2% under soiled/ high risk conditions and at 10% under conditions of very high soilage (blood, faeces etc).

Disposable solid waste (eg plasticware, gloves) will be discarded into 60 litre one-way burn bins, sealed and sent for incineration by S Grundon Waste Management Limited. The company has been approved by the HSE for incineration of GM Waste.

Routine disinfection will use 1% Trigene. Any substantial bio-spills will be treated with the Trigene Body Fluid Response Kit.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The programme of work involves the expression of a human protease for functional and structural studies. The GMSC has examined the potential impact of the GMMs that will be generated in terms of hazard to human health and potential damage to the environment.

The protease itself is considered to represent no greater risk than that of many other proteases commonly used in a laboratory environment eg trypsin and is considered to offer negligible risk of increasing the survivability or fitness of any cell that is induced to express it. All bacterial manipulations will be performed in K12 strains of E.coli or (for expression purposes) within strains that have a long history of safe use in a laboratory environment. The insect and mammalian cells used are considered non-pathogenic and will show a negligible ability to survive outside the cell culture environment.

All vector/host combinations are considered to show negligible likelihood of transfer to other hosts. The GMSC has recommended that this work be carried out under containment level 1 conditions.
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Name

AUVATION LTD

Name 2

Department

Campus Estate or Research Centre

ABERDEEN SCIENCE AND TECHNOLOGY PAR

Road Name

BALGOWNIE DRIVE

Building

District

Town

ABERDEEN

County

ABERDEENSHIRE

Postcode

AB22 8GU

Country

SCOTLAND

Tel Number

01224 355205

Fax Number

01224 708163

E-mail

HSE Division

SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The genetic modification safety committee will meet every three months or before commencement of new projects, and is made up of:-

- Convenors - Managing Director
- Biological Safety Advisor - Research and Development Manager
- Safety Advisor - Laboratory Manager
- Staff Representative - Research Scientist

Set up for the purpose of implementing risk assessments and health and safety issues regarding GM procedures.

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Tick if confidential

02/03/2022
All waste material and contaminated plastic ware are autoclaved at 121 degrees for 15 minutes. This will have an efficiency of 100% kill. Autoclaves are tested with indicator strips to validate the process.

Virkon is used to decontaminate liquid waste. This is a broad spectrum virucidal and bactericidal disinfectant cleaner routinely used to inactivate biological material in liquid cultures. Adding Virkon to liquid waste at a concentration of greater than 1% for two hours will give 100% kill. To confirm decontamination, Virkon is added to a concentration of 1% to a flask containing cells in culture medium. Incubation at 37 degrees for an overnight period confirms 100% kill.

1% Virkon solution and 70% industrial methylated spirit are readily available for disinfection of areas of work and for the event of any spillages.

The safety committee confirm that the risk assessment is a fair and accurate description of the work to be carried out.
## BUTTERWORTH LABORATORIES LIMITED

### Name 2

#### Campus Estate or Research Centre

- **Road Name**: 54-56 WALDEGRAVE ROAD
- **Town**: Teddington

#### Contact Information

- **Tel Number**: 020 8977 0750
- **Fax Number**: 020 8943 2624

#### Other Details

- **E-mail**:  
- **HSE Division**: EAST AND SOUTH EAST

### Comments

#### Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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<td>MIDDLESEX</td>
<td>TW11 8NY</td>
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## Premises Conditions

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Team comprises Laboratory Manager and A N Other, who form part of Butterworth Laboratories Ltd, Health and Safety Committee.
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Other(s)

Analysis of prosavin final product for residual butyrate by chromatography

For activities involving GMMs, describe the waste management measures which will apply to the activity

All simple solutions and artefacts used during analysis to be disinfected with 1% Virkon solution for 60 minutes. Disinfected solutions and artefacts to be removed and incinerated by licensed contract disposal company.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM Safety Committee have concluded that the risk associated with this activity is 'low', as determined by a formal risk assessment.
### DEPARTMENT OF ARCHAEOLOGY, UNIVERSITY OF CAMBRIDGE

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Faculty of Archaeology and Anthropology Safety Policy Committee is comprised of faculty and academic staff and researchers, and meets once every academic term to discuss issues related to University, Departmental and Faculty safety including GMM safety.

Each of the laboratories where GM work will be carried out (see 2) is represented on the Safety Policy Committee by a tenured member of staff from each department responsible for that laboratory. GM Safety and GMM issues are on the agenda as a standing item.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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02/03/2022
Yes

All solid and liquid waste and contaminated disposable plastics ware generated is placed in autoclavable biohazard bags, sealed and autoclaved using a Prestige Omega 121 degrees C laboratory autoclave.

Once autoclaving is complete, solid waste is sealed inside waste bag and placed in the general waste bin. Disposal of liquid waste subsequent to autoclaving is via a dedicated sink to the drains.

Contaminated glass and plasticware are either autoclaved or decontaminated by soaking over night in a 1-3% Virkon solution.

For activities involving GMMs, describe the waste management measures which will apply to the activity

All solid and liquid waste and contaminated disposable plastics ware generated is placed in autoclavable biohazard bags, sealed and autoclaved using a Prestige Omega 121 degrees C laboratory autoclave.

Once autoclaving is complete, solid waste is sealed inside waste bag and placed in the general waste bin. Disposal of liquid waste subsequent to autoclaving is via a dedicated sink to the drains.

Contaminated glass and plasticware are either autoclaved or decontaminated by soaking over night in a 1-3% Virkon solution.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

A faculty-wide standard operating procedure and risk assessment has been designed in consultation with the Faculty of Archaeology and Anthropology Safety Policy Committee.
# GM Centre Number: 908

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Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:
The GMSC consists of the CEO of Idna Genetics (20 years practical experience of GMOs), Prediction Manager of Idna Genetics (4 years practical experience GMOs), Sales and marketing Manager of Idna Genetics (4 years practical experience GMOs), Manager of the Norwich Bioincubator (10 years practical experience GMOs, serves on 2 other biological safety committees), Managing Director of Plant Biosciences Limited (20 years practical experience in plant biotechnology). The committee transacts most of its business by email, and will meet annually to review all assessments. The committee exists to assess risks to people and the environment due to Idna Genetics Limited activity with respect to genetic modification.

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial: Yes

Other (please specify)

Tick if confidential

Bacteriology
Parasitology
Transgenic Birds
Microbiology Research
The activity does not involve GMMs. The activity is restricted to non-notifiable activities involving genetically modified plant material. It is not envisaged that the company will be undertaking genetic modification itself, but will be handling genetically modified plant material produced by other parties. The majority of samples will be grain (or occasionally leaves) for destructive laboratory testing. Before disposal all material will be rendered non-viable by grinding to a fine powder, or autoclaving as appropriate. In order to monitor the efficiency of grinding or autoclaving in order to render the samples non-viable, for each new type of sample ground or autoclaved material will be tested for germination in the laboratory.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The comments received from the GMSC indicated that the risk assessment was satisfactory.
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Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GMSC is set up and includes a Biosafety Practitioner, a virologist, a pharmacist, the clinical trial principal investigator and representatives from infection prevention and control waste management and research and innovation

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste will be trial specific (verified by the GMSC) and comply with Trust Policies and procedures

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: Yes

- **Give brief details of the genetic modification safety committee**
  
  East Malling Research has an overarching GMSC (Genetic Modification Safety Committee) consisting of a BSO, a member of east malling staff, a GroDome (= Class II Greenhouse) representative and one or more representatives from Empharm. Empharm will report directly to the BSO

<table>
<thead>
<tr>
<th>Laboratory</th>
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- **Other (please specify)**
  
  Tick if confidential

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**02/03/2022**
For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC was convened to discuss this notification, and agreed recommendations have been incorporated into the sections above.

---

**Project Ref 910/05.1**

<table>
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<th>CU2 Project Title</th>
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Withdrawn N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

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Project Additional Information
### Purposes of the contained use

The purpose of the project is to use the plant viruses, Potato virus X (PVX) and Tobacco mosaic virus (TMV), as tools for the expression of foreign genes in plants, with the aim to investigate potential of the PVX and TMV vectors as tools for foreign protein production.

### Recipient or parental organism

Both PVX and TMV are endogenous viral pathogens and neither is considered to have any serious consequences on infected plants. Both viruses are transmitted in nature by mechanical means, generally plant-plant contact and neither virus is known to be transmitted by arthropod or nematode vectors. In the unlikely event of release of the modified virus to the environment a rapid selection for "wild type" virus is observed and recombinant viral progeny lacking foreign genetic sequences rapidly arise and dominate quickly dominate the viral population.

### Host/vector system

The modified PVX and TMV cDNA's with a duplicated viral coat protein subgenomic promoter is cloned in plasmid pBluescript KS (M13-) for propagation in suitable Escherichia coli K12 strains. Nucleic acid sequences of interest will be inserted into vector using standard molecular biology procedures. Gene expression will be under the control of the coat protein promoter and the expression level will not exceed that of the viral coat protein.

### Origin & function

The gene encoding the human endostatin protein will be inserted into cDNA copies of the PVX and TMV viral genomes in such a way that infection of plants with in vitro synthesized viral RNA transcripts will permit the infection of host plants and production of recombinant endostatin. Endostatin is a protein that acts as a negative regulator of angiogenesis and therefore has a potentially important role as a therapeutic agent to prevent tumour growth and metastases.

### Evaluation of foreseeable effects

Endostatin is normally produced in humans and animals (e.g. during wound healing processes) and therefore neither endostatin-coding sequences nor endostatin are considered to pose any health threat to humans and animals. It is unlikely that introduction of the endostatin gene into the PVX or TMV genome will alter the routes of viral vector transmission. The modified PVXs or TMVs are likely to be less fit than wild type. It is unlikely that expression of human endostatin, a protein that is not normally expressed in plants, will make PVX or TMV pollen or seed transmissible. It is also very unlikely that the modified PVX or TMV will become a vector transmissible (e.g. by insect, mite, fungal or nematode vectors) and that the host range of the modified PVX will be altered. Therefore, the genetically modified PVX or TMV will not be any more harmful than its parent. Inoculation of plant material may be performed in a Unigro Gro Dome The construction method means that the facility is virtually airtight. The compartments can be negatively pressurised (to approx 40 pascals) to ensure that insects or pollen do not escape and an air exhaust bag filter will be used. The method of construction also means that the steel superstructure and polycarbonate dome can withstand winds up to 120mph and resist impact from flying objects produced by such winds. Close adherence to standard operating procedures, training of staff, and rigorous implementation of containment measures should protect the environment. Furthermore, the isolated location of the growth room inside a building in the middle of a very large field of perennial fruit crops (which PVX or TMV is not able to infect), should prevent the modified viruses from escaping to infect any susceptible natural host plants. Gene expression vectors based on PVX and TMV have been used for well over a decade in the UK under containment conditions that are inferior to those of the GroDome without any evidence of escape to the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Infection of whole plants with modified PVX will be undertaken in a compartment of the Unigro Gro Dome Containment Facility. The facility is designed to meet containment level II and is therefore suitable for the proposed research. Access to the facilities (growth room) will be restricted to authorised personnel only.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Disposal of all inoculated plant material is by autoclave in laboratories located close to the GroDome House. The plant viral particles, plant tissues and their recombinant products are easily destroyed by normal autoclaving temperatures and pressures. Experimental material will be placed in sealable autoclavable bags and then into
sealable plastic bins before being transported to the labs nearby.

In the laboratory all media in contact with GM materials as well as GM cultures and plants will be placed in identified containers and autoclaved prior to disposal. Infected plants will be grown in compost in pots in waterproof trays in a containment growth room inside a building. Only a limited amount of water will be used daily for watering plants. There will be no run-off water. Accidentally contaminated water will be contained. Plant material and other solid waste will be transported from the Unigro GroDome compartment in secure closed containers, for autoclaving and disposal. Following recommendation from Defra Plant Health Division, liquid waste and condensate from the cooling coils will be collected, treated with Virkon to give a final concentration of 1% (or another suitable disinfectant at an appropriate concentration) and then autoclaved.

Is an emergency plan required according to regulation 20? 

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The GMSC was convened to discuss this notification, and agreed recommendations have been incorporated into the sections above.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units

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**Name**

POWDERMED LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

THE OXFORD SCIENCE PARK

**Road Name**

4 ROBERT ROBINSON AVENUE

**Building**

**District**

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX4 4GA

**Country**

ENGLAND

**Tel Number**

01865 501 500

**Fax Number**

01865 501 501

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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<td>ME19 6BJ</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

H&S Officer; Biological safety officer; Section Head Pre-clinical and clinical immunology; Immunologist; Research Scientist.

Quarterly meetings to discuss current practices and review safety.

<table>
<thead>
<tr>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

1. Virkon solution to kill cells.
2. Use of disposable plasticware; goes into clinical waste bags for incineration.
3. 70% Ethanol for cleaning surfaces after Virkon treatment.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

1. Biosafety cabinet work area to be cleaned immediately after administrations.
2. Task training is recorded in the operators log book.
3. SOP to be prepared.
<table>
<thead>
<tr>
<th><strong>GM Centre Number:</strong> 912</th>
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| **Date at Which Additional Info Submitted:** 02/03/2022 | **Page 11913 of 15326** |
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes](Y)

Give brief details of the genetic modification safety committee

- Committee comprises four members - MD and 3 Project Managers
- Meetings held on a monthly basis
- GM issues are raised and discussed with decisions and action points being documented in the meeting minutes.
- Both safety and GM issues are disseminated to board members (at board meetings) and all employees (via line managers and monthly company meetings)

<table>
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Tick if confidential

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- Parasitology
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- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

Culture media and cells to be disinfected/deactivated in a solution of at least 1% v/v Trigene (Medichem International) overnight before disposal down drain with plenty of water.

Pipettes, culture flasks and any other plasticware will be disinfected in a solution of at least 1% v/v Trigene overnight and then sent for incineration.

All other waste material will be autoclaved on site before being sent for incineration.

Trigene has been validated by the manufacturer and meets the following standards at the concentration used:
EN 13704, EN 14204, EN 12634, EN 13727.

Further information can be found at http://www.medichem.co.uk/mainmedical.html

Trigene has also been shown to deactivate genetic material (http://www.medichem.co.uk/dna.html; copy of personal correspondence attached)

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Other(s): Cell culture of GM human cells

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC reviewed the attached risk assessment and considered that it accurately reflected the risks of culturing the cells, and that appropriate control measures had been suggested.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

Due to the limited amount of GM worked planned for the department GM issues will be addressed by the Departmental Safety Committee which consists of the Head of Department, Departmental Biological safety Officer, Departmental Radiation Protection Supervisor, Clinical School Safety Officer, a representative from the Orthopaedic Research Unit, a laboratory representative, and the Department Administrator.

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Other (please specify): Tick if confidential: **No**
All solid waste will be double bagged and destroyed on site by incineration.
All liquid waste will be rendered safe by either treatment with 10% (v/v) hypochlorite, 1% (w/v) Virkon, or by autoclaving prior to disposal down the drain.
Any contaminated glassware etc. will be rendered safe by either treatment with 10% (v/v) hypochlorite, 1% (w/v) Virkon, or by autoclaving.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The GMSC are happy with the Risk Assessment.

Project Ref 913/21.1

Generation of human organoid cell lines expressing fluorescent proteins and luciferase

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 < 1 Litre
Non-GMM Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work
Project Additional Information

**Purposes of the contained use**

This work aims to create human organoid cell lines expressing reporter genes including GFP or RFP and firefly luciferase for monitoring our cells in in vivo and in vitro assays. We will transduce our human organoid cell lines with second generation amphotropic recombinant HIV-1 lentiviral particles generated in-house, containing our transgenes of interest—e.g., RFP and firefly luciferase.

**Recipient or parental organism**

2. Lentiviruses—HIV-1 derived, replication defective, amphotropic
3. Mammalian cells (human primary cells and cell lines)—considered as especially disabled
   - HEK293T (human kidney cells)
   - cholangiocyte organoids
   - hepatoblast organoids
   - pancreas organoids
   - pancreas and liver tumoroid lines
   - LX2 hepatic stellate cells.

**Host/vector system**

1. E.Coli (DH5 α derivative - NEB 5 α) - K12 derivative / plasmids: pLL-EF1α-rFluc-T2A-mRFP-mPGK-Puro, pWPT-GFP, psPAX2, pMD2.G
   K-12 derived strains of E.Coli are non-colonising and disabled. Having auxotrophic requirements that are unlikely to be satisfied outside the laboratory they are not considered pathogenic to humans or animals.
2. HEK293T - plasmids: pLL-EF1α-rFluc-T2A-mRFP-mPGK-Puro, pWPTGFP, psPAX2, pMD2.G
   This is a second generation lentiviral system where no structural genes are present and as a consequence no replication-competent virions can be produced. pLL-EF1α-rFluc-T2A-mRFP-mPGK-Puro co-expresses rFluc and...
mRFP from the EF1α promoter, which delivers moderate expression in most cell types (including primary and stem cells), and co-expresses the puromycin resistance gene for selection in vitro. pWPT-GFP allows for constitutive expression of eGFP driven by a EF1α promoter. HIV-1 virulence genes Vif, Vpr, Vpu and Nef have been deleted in the helper plasmid. The vector plasmid has the 3'LTR U3 region that encompasses the native viral promoter and enhancer deleted resulting in a self-inactivating vector. Packaging vector - psPAX2 - codes for Gag, Pol, Rev, and Tat but lacks both LTRs and has no viral packaging signal. Envelope (VSV-G) is expressed on a separate vector - pMD2.G

3. Human primary cells and cell lines (holangiocyte organoids, hepatoblast organoids, pancreas organoids, pancreas and liver tumoroid lines, LX2 hepatic stellate cells) / pseudotyped recombinant lentiviral particles

The lentiviral particles will be used to transduce organoid cell lines to induce stable over expression of the factor of interest (e.g. GFP and luciferase) in the transduced cell. The production of fluorescent proteins and luciferase will have no biological effect.

**Origin & function**

Inserts carried by lentivirus will express mRFP, rFLuc or eGFP

**Evaluation of foreseeable effects**

The reporter genes GFP, RFP and luciferase are not believed to harmful when expressed in mammalian cells. Lentivirus do however integrate into the host cell genome and this is associated with a risk of insertional mutagenesis. These systems are not believed to show an integration bias toward the transcriptional start site regions of DNA like other viral systems therefore the risk from this is minimal.

The vectors are all amphotropic (VSV-G pseudotyped) recombinant HIV-1 lentiviral particles that are replication defective. The viral vector system has been disabled by removal of the HIV-1 virulence genes Vif, Vpr, Vpu and Nef in the helper plasmid. The vector plasmid has the 3'LTR U3 region that encompasses the native viral promoter and enhancer deleted resulting in a self-inactivating vector. Key elements for AAV replication are deleted in the packaged viral particles which renders the vectors incapable of selfreplication. In the event of accidental contamination (sharps, aerosol) the amount of cells transduced will be much lower than that of cultured cells as the protocols are optimised for this system. The VSV-G pseudotype of this lentivirus results in complement sensitivity which increases the likelihood of an immune response against it. This means that only a low number of cells may be transduced through accidental contamination. Environmental stability tends to be increased by VSV-G pseudotyping compared to retroviruses displaying the native envelope. However, retroviral particles remain sensitive to air, temperature and pH and will have short lifespan in an open environment.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

CL2 waste is autoclaved in-house prior to disposal. Bags and Biobins containing CL2 waste should be clearly labelled and should always be sealed using autoclave tape prior to disposal. When a CL2 waste container is full the lid should be sealed using autoclave tape and taken to the autoclave for autoclaving followed by incineration. Waste bags, autoclave tape and Biobins are supplied by the department and should be collected from the distribution points.

Pipette tips can be collected in beakers containing Virkon or Distel. When full, drain the fluid and place the tips in a Biobin. When the Biobin is full put the lid on and seal for disposal.

CL2 liquid waste can be aspirated if it does not contain any chemicals that cannot be disposed of down the sink. Empty aspirator bottles should have Virkon added to them prior to use. If they are more than ¾ full when you finish please empty the contents down the sink and add fresh Virkon.

Chemicals that cannot be disposed of down the sink should be collected in bottles, treated with Virkon or other validated disinfectant compatible with the chemical being used, and put into the Chemical Fume Hood for disposal.

Sharps use must be kept to a minimum in CL2 and must be thoroughly risk assessed. Sharps should be decontaminated (by soaking in Virkon, avoid wiping) and placing in sharps bins. Sharps bins should not be filled past the lid. Once they are full pull the lid across, label as CL2 and place them in the designated collection point.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

This work was considered as part of the Department of Surgery Safety Committee Meeting held on 1st October 2020. Whilst the committee felt that the workers should be encouraged to consider third/fourth generation lentivirus systems for their work, it was acknowledged that the prior experience of the researchers and familiarity with the second generation system provided a compelling argument to use this approach. Risk Assessment Designated Class 2, Containment 2 Activity.

Project Containment
<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Name**

FISHER CLINICAL SERVICES UK LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

LANGHURSTWOOD ROAD

**District**

**Town**

Horsham

**County**

WEST SUSSEX

**Postcode**

RH14 9TE

**Country**

ENGLAND

**Tel Number**

01403 212700

**Fax Number**

01403 212797

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Fisher Clinical Services provide a packaging, storage and distribution service for clinical studies to the pharmaceutical industry and as such do not process GMOs in any way. The site is licensed by the MHRA and the licence number is MA(IMP)18693. Processes are conducted according to current GMP 2003/94/EC.

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For activities involving GMMs, describe the waste management measures which will apply to the activity.
With regard to waste management, the process has been designed at Fisher so that we are only handling 1 vial at any time. The clean up will follow the specific clean up as stated in the MSDS provided by the manufacturer.

In the unlikely event of a vial being damaged the procedure will be followed and the waste will be separately bagged and labelled as GMO waste for disposal via our licensed contractor.

I have attached the MSDS and our internal SOP for clean up and spillage.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

We have an H&S committee on site here who review H&S matters. COSHH assessments are carried out primarily by myself with an independent review and sign off by a H&S professional. Risk assessments are performed in a similar way.

The COSHH assessment for this product was handled in the same way as above using information supplied by the client.
### GM Centre Number: 915

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Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

[ ] Yes

Give brief details of the genetic modification safety committee

The STL GMSC has been set up to address Health and Safety requirements associated with the use of the ER-CALUX bioassay. This assay uses genetically modified mammalian cells. The GMSC is composed of a range of STL staff whose responsibility it is to ensure that the controls documented in the Genetically Modified Organisms (Contained Use) Regulations 2000 are followed. The GMSC committee (April 2005) is comprised of: Laboratory Manager, Biological Safety Officer, Technical Development Manager (Chair), Principle Scientist (ER-CALUX Project Manager), Site Safety Coordinator, Health, Safety and Environment Manager.

<table>
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<th>Animal Unit</th>
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<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  
Tick if confidential [ ]

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<th>Bacteriology</th>
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<th>Microbiology Research</th>
</tr>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

All material and solutions that have been in contact with genetically modified cells, both directly and indirectly will be placed in a discrete autoclave bag. The bag will then be carefully sealed, autoclaved and then stored in a secure place until disposal via a recognised waste disposal company.

Please enter comments of the GM safety committee on the risk assessment

The STL GMSC met on 06/04/05 to consider the risk assessment and safety precautions implemented at STL Bridgend to undertake oestrogenic potency testing using the ER-CALUX bioassay.

The GMSC is satisfied that all associated risk assessments have been completed and the committee agrees with the conclusions of these documents that the use of ER-CALUX meets the category, Provisional containment 1. The committee is also satisfied that the safety procedures implemented meet the requirements on risk minimisation of a Class 1 activity.
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| Comments                           |

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### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The VASTox GMSC consists of four members, the COO, and heads of biology and invertebrate biology. All are experienced scientists directly supervising any proposed GM work. All members have previous hands on experience of the GM regulations.

The committee also includes a lay person.

The committee meets every three months with the provision for additional meetings to assess new proposals in order to expedite work.

<table>
<thead>
<tr>
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<td>Yes</td>
<td>Parasitology</td>
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<td>Microbiology Research</td>
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Tick if confidential
Solid biological waste is collected in clear autoclave bags in laboratory waste bag holders e.g. Bacterial contaminated disposables. This waste is treated by Autoclaving for 30min@136°C (12mins free steaming), it is then removed to Yellow UN registered sacks and taken for incineration. All records of waste removal are kept for a minimum of 2 years.

Liquid biological waste and associated glassware is disinfected at source by the addition of 11% by volume. The mixed solution is left to stand for a minimum of 1 hour before being discarded down a laboratory sink with cold running water which is left to run for at least 1 minute. The glassware is rinsed before being placed in the designated glassware collection tray located in each laboratory prior to cleaning and autoclaving.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

No specific comments made. Authorised with recommendation that appendix of hosts and vectors is reviewed every three months.
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Data Premises Notified (Originally) 01/07/2005

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Date at Which Additional Info Submitted 02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

This function is carried out by the Biological Safety Committee which comprises representatives from each of the biology groups (currently 4), the BSO and a deputy BSO, the Quality and Operations Director and a member of the senior management team.

<table>
<thead>
<tr>
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<td></td>
</tr>
</tbody>
</table>
All liquid waste from bacteriological and cell-line work will be treated with Chemgene disinfectant (from Medimark Scientific). This has been tested for bactericidal, viricidal, fungicidal and sporicidal activity by the manufacturer and working dilutions recommended (see http://medi-mark.co.uk/products/product/chemgene-hld4h-ce-marked-high-level-medical-surface-disinfectant).

The recommended dilution for bio-hazard contamination (blood, urine, vomit etc) dilute to 1:20 (5%), for high risk disinfection of organisms in dirty conditions dilute at 1:50 and for general disinfection dilute at 1:100 (1%), (see http://medi-mark.co.uk/images/uploads/CHEMGENE_HLD4H_EN_TEST_SUMMARY.pdf).

All solid biologically contaminated waste will be collected in 30 L or 60 L sealable, leak-proof waste bins prior to incineration. All solid biologically contaminated contained waste is collected by Babraham Research Campus waste management and taken to an off-site facility at the Cambridge Pet Crematorium for incineration.

All solid waste will be autoclaved prior to incineration. An autoclave provided as a central service by the Babraham Institute will be used. This autoclave is tested on an annual basis for an insurance pressure test and serviced at six monthly intervals. The next service and pressure test is due 2/6/05. Autoclaved waste is then taken to an off-site incineration facility at the Cambridge Pet Crematorium for incineration. Information form the crematorium is attached to this form.

The risk assessments have been reviewed and deemed appropriate for the activities to be undertaken.
The cloning and mutagenesis of diptheria toxin, modified diptheria toxin with the membrane targeting domain replaced by human IL-2, and mutants thereof.

E. coli strains: XL1-blue, TG1, BL21 (DE3)

XL1-blue and TG1 are multiple auxotrophs that cannot survive outside a laboratory environment. BL21 (DE3) is disabled and has limited survivability in the environment.

The following vectors will be used with the above hosts:
- pUC derived plasmids - these are non-mobilisable.
- pET51b+, or similar. This vector is also non-mobilisable.

The genetic material will be made by gene synthesis. The diptheria toxin gene will be mutated and tested for retention of function in in vitro and cellular assays. Successful mutants will be modified by replacement of the membrane targeting domain with human IL-2. The modified protein is ultimately intended as an anti-cancer therapeutic.

Both whole toxin and modified toxin and mutants thereof will be expressed using the pET system in small cultures. It is anticipated that the toxin will be secreted to the periplasmic space in an active form. Accidental short-term exposure
To small amounts of native or mutant toxins is likely to cause minimal risk, considering the small amounts of protein being used. In addition, the modified toxin is anticipated to be less toxic than the native toxin since it will be targeted to a much narrower tissue range, i.e. those cell types expressing the IL-2 receptor. Accidental exposure is likely to be at the body surface tissues, e.g. skin and mucous membranes, whereas the most severe effects of the toxin and modified toxin are manifested upon blood exposure. Therefore the use of good microbiological practice in association with PPE will minimize exposure and consequent risk. The use of sharps should be avoided.

The cloned protein is unlikely to alter the host-range or tissue tropism of the GMM since these traits are complex and conferred by more then one protein. However, this enteric bacterium could have additional toxicity, compared to wild-type, if it were able to become established in a human host. However, this risk is negligible due to the disabled nature of the bacterial strain being used. The vectors being used are considered to be non-mobilisable, Gene transfer is thus a very remote possibility. The host organism does not express 6. cili pathogenic determinants and has been shown to be unable to persist in the guts of experimental animals. It is unable to adhere to and colonize the gut and therefore would not be able to accumulate to sufficient levels to cause harm. Contamination is most likely to occur through aerosols and low level splashes, therefore exposure can be minimized by using good microbiological practice in conjunction with PPE. In addition, to minimize the risk from aerosols, all procedures likely to generate these will be carried out in a class II microbiological safety cabinet.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The GM will be handled according to good microbiological practices at containment level 2. All liquid waste will be treated with 1% Trigene for at least 30 min prior to disposal down the sink. Contaminated glassware and plasticware will also be disinfected as above. Contaminated solid waste will be double bagged and autoclaved prior to incineration. Spillages will be contained using absorbant material that will then be treated as solid waste. The contaminated area will be wiped clean with 1% Trigene. Trigene has been certified by the manufacturer to effectively kill E.coli at concentrations of 0.5% (see http://www.medichem.co.uk/trigenelab-laboratory.html).

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

Laboratory Activities  Glass Houses  Growth Rooms

The GMSC found that this assessment identified the potential hazards associated with this work and that the containment measures described are sufficient to protect human health and the environment. The committee approves the work to be undertaken in containment 2 laboratories 2020 and 2018 by competent, authorised staff only.
## Project Ref

### 917/13.1

**Date Ackn'd**: 03/04/2013  
**CU2 Project Title**: The cloning and mutagenesis of fungal ribotoxins and the linking of these to cellular targeting domains

**Class**: Class 2  
**CultureVolClass2**: < 1 Litre

**Date Project Ceased**:  
**Non-GMM**: Consent Granted

**Withdrawn**: N  
**Tick if notifying a connected programme of work**: N

### Historical Significant Changes

#### Historical Date of Additional Info

#### Significant Change ID

#### Date of Significant Change

### Project Additional Information

**Purposes of the contained use**  
To create toxin fusion proteins with potential therapeutic use

**Recipient or parental organism**

E. coli strains XL1-blue, DH5alpha, TG1, BL21 (DE3) and derivatives, Origami2(DE3) and derivatives.  
XL1-blue, DH5alpha and TG1 are multiple auxotrophs that cannot survive outside the laboratory. BL21(DE3) and Origami2(DE3) derivatives are disabled and have limited survivability in the environment

**Host/vector system**

The following vectors will be used with the above hosts:  
pUC derived plasmids - non-mobilisable  
pET series plasmids - non-mobilisable

**Origin & function**
The ribotoxins are naturally found in various species of fungi; however for the purposes of this project, the genes will be synthetic. The genes will be cloned into bacterial expression plasmids for the production of mutant proteins for testing for their ability to kill mammalian cell lines.

<table>
<thead>
<tr>
<th>Evaluation of foreseeable effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole toxin, toxin mutants and toxin fusion proteins will be expressed in bacteria in small cultures sufficient for producing material for activity testing. It is anticipated that soluble active toxin will be either secreted to the periplasm or accumulate in the cytoplasm. Accidental short-term exposure to the small amounts of toxin being produced is likely to cause minimal risk considering the small amounts of protein being produced. Accidental exposure is likely to be at body surface tissues, e.g. skin and mucous membranes, whereas the most severe effects of the toxin are manifest upon blood exposure. Therefore the use of good microbiological practice in association with appropriate PPE will minimise exposure and consequent risk. The use of sharps should be avoided.</td>
</tr>
<tr>
<td>The cloned protein is unlikely to alter the host-range or tissue tropism of the GMM since these traits are complex and conferred by more than one protein. However, this enteric bacterium could have additional toxicity, compared to wild-type, if it were able to become established in a human host. This risk is negligible due to the disabled nature of the bacterial strains being used. Furthermore, the plasmid vectors being used are non-mobilisable so that gene transfer is a remote possibility. The host organism does not express E. coli pathogenic determinants and has been shown to be therefore not be able to accumulate to sufficient levels to cause harm. Contamination is most likely to occur through aerosols and low level splashes, therefore exposure can be minimised by using good microbiological practice in conjunction with PPE. The risk from aerosols can be further minimised by carrying out any procedures likely to generate them in a class II microbiological safety cabinet.</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
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<table>
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<tr>
<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
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</thead>
<tbody>
<tr>
<td>The GMM will be handled according to good microbiological practice at containment level 2. All liquid waste will be treated with 1% trigene prior to disposal down the sink. Contaminated glassware and plasticware will be similarly treated prior to washing. Contaminated solid waste will be disposed in a labelled, sealable waste container and incinerated. Spillages will be contained using absorbent material that will then be treated as solid waste. The contaminated area will be wiped clean with 1% Trigene.</td>
</tr>
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</table>

| Trigene has been certified to effectively kill E. coli at concentrations of 0.5% (see http://www.medichem.co.uk/trigene-laboratory.html) |

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<th>Is an emergency plan required according to regulation 20?</th>
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<th>Tick to confirm that you have attached a risk assessment to this form</th>
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<th>Tick if you are claiming exemption from disclosure for section of the risk assessment</th>
</tr>
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<tbody>
<tr>
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</table>

Please enter comments on the GM safety committee on the risk assessment

The GMSC found that this assessment was appropriate for the potential hazards associated with the proposed work and that the containment measures described are sufficient to protect human health and the environment. The committee approves this work to be undertaken in containment 2 laboratory 1010 by trained, authorised staff only.
## Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Human Clinical Applications</th>
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<td>L3 L4 L2</td>
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Name
GENESERVICE LIMITED

Name 2

Department

Campus Estate or Research Centre
2 CAMBRIDGE SCIENCE PARK

Building

Road Name
MILTON ROAD

District

Town
CAMBRIDGE

County
CAMBRIDGESHIRE

Postcode
CB4 0FE

Country
ENGLAND

Tel Number
01223 432600

Fax Number
01223 494595

E-mail

HSE Division
EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted
02/03/2022
### Premises Addresses

<table>
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<td>2 CAMBRIDGE SCIENCE PARK</td>
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<td>CB4</td>
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</tr>
</tbody>
</table>

### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Y

**Give brief details of the genetic modification safety committee**

A GMSC has been composed to reflect the balance of the workforce at Geneservice Ltd (GSL). The main duties of the committee are to ensure that the facilities are adequate for the purpose and that equipment is correct and properly maintained. Relevant training will be provided to all of the staff concerned, and local rules have been drawn for all procedures. Finally, emergency plans have also been drawn. The committee has reviewed the risk assessments.

The following members of staff constitute the GMSC:

- Chief Operations Officer
- Resource Development Manager
- Biological services
- Biological Reagents & Credit Control

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Non-microbial</td>
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<tr>
<td>Other (please specify)</td>
<td></td>
<td></td>
<td></td>
<td>Tick if confidential</td>
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</table>
Disposal of biohazard waste

Used tips, tubes, and inoculating loops are to be placed in 'sweetie jars', these are then taped shut with biohazard tape and placed in the yellow biohazard bags. Items such as disposable gloves or paper tissues contaminated with low hazard waste should be disposed of as Biohazard waste. Solid microbial waste will be placed in yellow bags (double bagged), these are then sealed and then autoclaved prior to collection via a designated waste disposal agent for incineration. The agent used is: Vetspeed Limited.

Disposal of microbial cultures

Aqueous microbial cultures are to be treated with Virkon at a final concentration of 1% w/v, left for a minimum of overnight before discarding via the drain with copious amounts of water.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessment Tomlinson doc and Clone doc

The host vector systems and constructs satisfy the requirements for Group 1. The host strains are all K12 disabled, and the vectors are non-mobilisable. The likelihood of the hazard is therefore negligible and the environmental risk effectively zero.

Risk assessment Sequencing doc

This service aims to sequence already cloned DNA templates, which are sent in by customers. The cloned DNA is effectively random, as far as the service is concerned, but will already have been cloned into the appropriate host/vector system. Once received, the service grows up small volumes of the cells, to obtain a sufficient quantity to extract the DNA and then sequence it. The host/vector systems are all standard and since the inserts are highly unlikely to enhance the pathogenicity, the work is correctly assessed as Class 1. One additional factor is that some DNAs could be potentially hazardous and it is recommended that all naked DNA should be handled appropriately to cover this hazard, ie DNA should be handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras. 8-10; ie gloves should be worn, sharps avoided and all wastes be rendered harmless before disposal.

Clearance has been given by the GMSC that work can be started, pending receipt from the HSE.
### StylaCats Limited

**Name:** StylaCats Limited

**Campus Estate or Research Centre:** THE HEATH BUSINESS & TECHNICAL PARK

**Road Name:**

**Town:** Runcom

**County:** CHESHIRE

**Postcode:** WA7 4QX

**Country:** ENGLAND

**Tel Number:** 01928 513011

**Fax Number:** 01928 513222

**E-mail:**

**HSE Division:** NORTH WEST

**Date at Which Additional Info Submitted:** 02/03/2022
Premises Addresses

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<th>Name</th>
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<td>31/12/2005</td>
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<td>The Heath Business &amp; Technical Park</td>
<td></td>
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<td>Runcom</td>
<td>CHESHIRE</td>
<td>WA7 4QX</td>
<td>ENGLAND</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Composition of committee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Operations Director</td>
</tr>
<tr>
<td>2. Head of Biotransformations</td>
</tr>
<tr>
<td>3. Senior Microbiologist</td>
</tr>
<tr>
<td>4. Health &amp; Safety Advisor</td>
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</tbody>
</table>

The committee will implement and improve policies to ensure StylaCats is compliant with or exceeds the standards for GM Activities as defined in the relevant legislation. (Health, Safety, Environment)

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
<th>Level 4 (GMMs)</th>
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<tr>
<td>Yes</td>
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</table>
The principle method of inactivation of cultures of GMMs will be by treatment in an autoclave (heat inactivation) at 120 degrees C, 15 psi, 20 minutes to achieve effective 100% kill, and following the manufacturer's recommendations for use. All cultures whether in glass or disposable containers will be autoclaved before disposal or recovery of containers. Liquid medium from which micro-organisms have been recovered and contaminated plasticware (Petri dishes, Eppendorf tubes, disposable pipettes and culture tubes etc) will also be sterilised in the autoclave prior to disposal. Day to day monitoring of the autoclave's effectiveness will be checked by inspection of records of each autoclave run. Electronic and printed records of each autoclave run will be retained. The autoclave will be regularly services and tested for effectiveness by the manufacturer.

Apills and leakages of contaminated liquids are to be treated with a suitable disinfectant (eg Virkon) according to manufacturer's recommendations and in accordance with appropriate COSHH guidelines. Disposable tissues used for mopping up disinfectant-treated spills will be autoclaved prior to disposal.

Contaminated sharps will be collected in an approved designated sharps container and disposed of by incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

For activities involving GMMs, describe the waste management measures which will apply to the activity
**GM Centre Number: 920**

<table>
<thead>
<tr>
<th>Data Premises Notified</th>
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</table>

**Name**

CENTRE FOR ENVIRONMENT, FISHERIES & AQUACULTURE SCIENCE (CEFAS)

**Name 2**

Department

**Campus Estate or Research Centre**

LOWESTOFT LABORATORY

**Road Name**

PAKEFIELD ROAD

**District**

Town

Lowestoft

**County**

SUFFOLK

**Postcode**

NR33 OHT

**Country**

ENGLAND

**Tel Number**

01502 562 244

**Fax Number**

01502 513 865

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

<table>
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<tr>
<th>Date Premises Closed</th>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Cefas AHH Division Biosecurity and Biosafety Panel
The panel comprises of chair, divisional director, biological safety officers from Lowestoft and Weymouth sites and representatives from the various science areas involved in work with pathogens and GMOs.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs)

Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
Yes

All equipment used in GM procedures including glassware for re-use, and waste materials are autoclaved on site at 121 degrees C for at least 15 minutes, with full steam penetration. The procedure ensures 100% kill and inactivation of the GMO and sterilisation of equipment.

Validation of autoclave conditions is undertaken twice yearly by service testing. Between scheduled service checks confirmation of correct functioning is carried with each load using a steam-chemical integrator device. In event of unsatisfactory operation the load is retained pending suitable repair of the autoclave.

After autoclaving waste material is disposed to landfill with consignments of general waste arising from the laboratory.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The proposal and risk assessments were distributed to members of the Burnham health and safety panel and discussed at a meeting on 14th October 2004. The risk assessments were subsequently updated to include ergonomic factors for operators of pipettes and discussed again at a meeting in January 2005. The committee were content with the assessments and endorsed the Notification of Intention to use premises for contained use activities.

Risk assessments updated, reviewed by Divisional Risk Assessment Champions and accepted as ‘HS16 AHH-EAH-ECORA RA-18 YES-YAS Assays’ in January 2013.
### MRC MITOCHONDRIAL BIOLOGY UNIT

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**Emergency Plan Required?**

**Transferred from 1992 Regs?**

**Transitional Premises Class**

**Non-GMMs**

**Withdrawn**

**Data Premises Notified (Originally)**: 02/08/2005

**Data Premises Closed**

**Transitional Premises**

**Date at Which Additional Info Submitted**: 02/03/2022
# Premises Addresses

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<td>Elsie Widdowson Laboratory</td>
<td>Fulbourn Road</td>
<td>Cambridge</td>
<td>CAMBRIDGES HIRE</td>
<td>CB1 9NL</td>
<td>ENGLAND</td>
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<td>MRC Cancer Cell Unit &amp; University of Cambridge</td>
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# Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

GM risk assessments will be considered by the already established GM safety committee of the MRC Laboratory of Molecular Biology (HSE ref. GM20).

This committee includes management, staff and user representatives, together with the Biological Safety and Medical Officers and also external representation.

| Level 1 (GMMs) | Yes | Level 2 (GMMs) | Yes | Level 3 (GMMs) | Yes | Level 4 (GMMs) | Non-microbial | Laboratory | Animal Unit | Growth Room | Glass House | Large Scale |
|----------------|-----|----------------|-----|----------------|-----|----------------|---------------|-------------|-------------|-------------|-------------|-------------|-------------|

Page 11951 of 15326
Solid waste is collected and transferred in secure containers to an incinerator, which is notified for Class 1 GM work, and then incinerated. This is considered to give 100% killing.

Liquid waste will be inactivated by autoclaving, which is considered to give 100% killing, or else will be inactivated by validated chemical means. The disinfectant used will be chosen depending upon the host cells involved, but in all cases will be validated for the purpose.

Work on human genes (including primary cell cultures) for cancer or nutritional studies.

For activities involving GMMs, describe the waste management measures which will apply to the activity

This work involves the growth and screening of a commercial yeast library in which individual genes have been knocked out. The aim is to identify genes involved in ubiquinone transport from mitochondria. The host is disabled and there is nothing to increase its hazard. The work is therefore correctly assessed as Class 1.
Project Additional Information

Purposes of the contained use
Overproduction of the V-ATPase of E. hirae into which the V-ATPase operon has been cloned. (This work is self-cloning and the GMM has already been constructed and characterised elsewhere.)
The overproduction is wanted to allow purification of the V-ATPase for structural studies.

Recipient or parental organism
Enterococcus hirae (formerly Streptococcus faecalis), ATCC9790 and 25D.

Host/vector system
E. hirae (as above) as host; pHY300LK vector.
This is a commercially available, mobilisation defective vector from Yakuruto Co. (Japan). It is one of the smallest hybrids of a series of chimeric plasmids using the parental plasmids pACYC177 of E. coli and pAM-a of Streptococcus faecalis. This shuttle vector for E.coli and B. subtilis contains an RNA primer gene for ori-177; the R-Tc gene; the R-Ap gene; Rep-alpha-1 gene, which is the plasmid replication gene; two replication origins, one for E. coli and the other for B. subtilis; and a polylinker.

Origin & function
The genetic material cloned is derived from E. hirae and is cloned back into the micro-organism as host, i.e. the work is self-cloning. The resultant construct has already been characterised elsewhere (in Japan) and the work within the UK will involve growth, cell lysis and purification of the cloned protein.

Evaluation of foreseeable effects
Enterococcus hirae is a human commensal, which occurs in the intestine of normal healthy people and only occasionally causes disease due to getting into the wrong environment (e.g. the bloodstream). Since it can cause disease, it is classified as a Hazard Group 2 pathogen - in the same way as Eschericia coli.
The genetic modification involves self-cloning of an operon from E. hirae, in order to obtain increased yields of the protein product from this operon. The protein is membrane bound and will not increase the pathogenicity of the host (which it naturally).
The only foreseeable affect is the disabling of the E. hirae, due to overproduction of one protein rather than the appropriate amount being made as in the wild-type cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Only micro-organisms involved in this work.
Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

After growth, the micro-organisms will be centrifuged and collected. The culture supernatant will be disinfected with a clear phenolic disinfectant. During the initial experiments the optimum disinfection conditions will be determined, to obtain <1 viable cell/ml, and these conditions then be applied to all supernatants before discharge down the drain into the public sewer. The cell pellet will be resuspended and the cells lysed to prepare the protein. Any substantially solid residues will be inactivated by autoclaving at 135 for 15min, which is expected to give - 100% killing.

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Containment

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<td>Class 2</td>
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### Project Additional Information

**Purposes of the contained use**

To study the pathways regulating differentiation of human cells, in particular how stem cell fate is regulated.

**Recipient or parental organism**

Human and mouse tissue culture cell lines.

**Host/vector system**

Laboratory strains of E. coli K12.  
Phoenix-ecotropic and amphotropic retroviral producer cells.

**Origin & function**

Inserts used are all derived from mouse, human and Xenopus cDNA libraries and comprise wild type and mutated forms of Notch ligands, Notch receptors, suppressor of hairless homologues, mastermind homologues, and Notch target genes including the hairy/enhancer of split family of transcription factors. In addition mutant Xenopus beta catenin and mutants of MAP kinase will be expressed to determine how the Notch pathway interacts the wnt and Map kinase signalling pathways. Cell cycle inhibitory proteins such as p21Cip1 and Geminin will also be used.

In addition reporter constructs derived from the pGL3 series of vectors will be used; these consist of Notch and wnt responsive promoter elements, driving expression of the firefly and renilla luciferase genes.  
In some experiments green fluorescent protein will be expressed as a reporter.

The intended use is to study how these genetic elements control the cell development.

**Evaluation of foreseeable effects**

The main hazard arises directly from the recombinant retroviruses produced from the packaging cells. These yield both ecotropic and amphotropic retrovirions, depending upon the envelope protein supplied by the cell.
The packaging cell lines used to generate the retrovirus have either ecotropic or amphotropic envelope proteins, and the retrovirus therefore can either infect only mouse cells, or have a wider host range including human cells. Their infectivity is unstable and infection is only obtained by co-cultivation of the packaging cell line with their recipient cells. The retrovirus is self-inactivating and there is thus no possibility of further transfer. With the ecotropic packaging cell line, there is therefore negligible risk to the worker or the environment.

With the amphotropic packaging cell line, the risk of infection of a worker during the packaging/infection step is small, but still not negligible. Although the inserted genes are not known to be oncogenic, they have this potential and it is considered that this part of the work is Class 2. In view of the, at worst, low-oncogenic nature of the inserted genes and also the self-inactivating nature of the retroviruses, there likelihood of any hazardous replication competent virus being generated is negligible and is no need to test for such RCV.

The single non-negligible hazard arises from the potentially oncogenic or cytotoxic nature of some of the inserts. This risk can be controlled if DNA grown up from such clones is handled appropriately; ie gloves should be worn, sharps avoided and all wastes be rendered harmless before disposal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 mins), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

Is an emergency plan required according to regulation 20? No

Tick to confirm that it is attached to this form No

Tick确认 that you have attached a risk assessment to this form Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment No

Please enter comments on the GM safety committee on the risk assessment

This work involved the cloning of some potentially oncogenic genes into disabled retrovirus vectors, with the use of packaging cell lines which have an amphotropic envelope protein.

From the ACGM Compendium of Guidance, Part 2B, Annexe III, particularly para. 30, this requires Class 2 (as already concluded in the risk assessment).

The Committee noted that the risk of the potentially oncogenic DNA had already been considered in the assessment.

**Project Containment**
PROJECT REF 20/02.4

FUNCTIONAL SILENCING OF GENES INVOLVED IN DNA REPAIR AND CELL CYCLE: 2

Date Ackn'd: 02/08/2005
Date Project Ceased: 03/09/2013

Class 2
Culture Volume Class 3-4

Consent Granted: Not Applicable

Tick if notifying a connected programme of work: N

Historical Significant Changes: Transferred from GM20 on 02/08/2005, Project transferred to GM3201 03/09/2013

Project Additional Information

Purposes of the contained use
To functionally silence genes whose inactivation in cancers gives rise to genetic instability, using a recently developed stable RNA interference approach, to ascertain the molecular basis for the abnormal division of cancer cells and their increased genetic instability.

Recipient or parental organism
Tissue culture cells of human origin.

Host/vector system
Laboratory strains of E. coli K12 derivatives; tissue culture cells of human origin.
pUC-based plasmid vectors with RNA polymerase III promoter (e.g., pSUPER);
Phoenix-amphotropic retroviral producer cells.

Origin & function

The plan is to use vectors based on pSUPER in which an RNA polymerase III promoter drives expression of a short (~25 mer) interfering RNA from a synthetic DNA insert. Details of the categories of interfering RNAs to be inserted are as follows:

A) RNAs which target DNA double-strand break repair pathways: The proteins encoded by BRCA1, BRCA2, RAD51 and its paralogs, Nbs1, ATM, ATR, Ku70, Ku80 and DNA-PK are nuclear molecules which participate in the sensing and repair of DNA double-strand breaks. In mice, homozygosity for null mutations in these genes leads to cell death, embryonal lethality and a DNA repair defect accompanied by genetic instability.

B) RNA which target cell cycle regulators: The proteins encoded by the mammalian cyclin/CDK genes, the mitotic kinases Pik1, nek1, Aurora-A/B/C, Bub1/BubR1 and the chromatid cohesion molecules Scc1, SMC1/3 and Eco1, are required for cell cycle progression and mitosis.

C) RNAs which target regulators of the G1, S and G2/M checkpoints: These inserts will include proteins encoded by checkpoint regulators such as p53, chk1/chk2 and chfr, or proteins involved in the downstream enforcement of these checkpoints (including cdc14, ranGAP, cyclin G, cdc25 or PP1).

Evaluation of foreseeable effects

The inserts encode short interfering RNAs which are only active within the cell, and cannot encode an exogenously expressed protein. Expression of these RNAs will not enhance the pathogenicity of the host cells. The effect of expression of the insert RNAs will be to functionally silence the target genes. In most cases, functional silencing is known to impair cellular function resulting in defective cell cycle progression or in genetic instability. In no instance is functional silencing of the target genes known to work directly to transform primary cell cultures.

A non-negligible hazard arises from the potentially oncogenic or cytotoxic nature of the cellular effects of some of the interfering RNA inserts. It is considered that DNA from such clones can be appropriately handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras 8-10, in which the potential hazard is specifically considered.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing. Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
This work involves the cloning of short DNA inserts under a eukaryotic promoter, in order to generate ~25-mer interfering RNA. The genes targeted in this work are those for sensing and repair of DNA double-stranded breaks, cell cycle progress and mitosis, and checkpoint regulators or downstream enforcement proteins. The cloned DNA is therefore potentially cytotoxic or oncogenic.

Initial cloning is into non-mobilisable vectors grown in laboratory E. coli K12 strains. The vectors are pUC-based and are disabled retroviral vectors, which require a helper t.c. line to produce virus, which in turn is only capable of a single infectious cycle. This stage is therefore Class 1.

Subsequently the retroviral vectors will be transfected into mouse emphotropic cell lines, with the packaging construct in more than one component, to produce the disabled retrovirus with an amphotropic envelope. Following consideration of the advice in the ACGM Compendium of Guidance, Part 2B, Annex III, paras 21-30, it was agreed that Class 2 is appropriate for this work. If cell lines are being maintained for a long time, consideration should be given to testing them for the presence of replication competent virus.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref** 20/03.1

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<td>STUDY OF THE REGULATION OF EPITHELIAL PROLIFERATION AND DIFFERENTIATION USING SMALL INHIBITORY RNAs (SIRNA)</td>
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**Project Additional Information**

**Purposes of the contained use**

The project aims to determine the molecular basis of the differentiation of human and mouse epithelial cells. Pathways regulating differentiation include Notch, wnt and the MAP kinase signalling cascade. In addition we wish to explore the interaction of Notch with cell cycle regulators such as p21 Cip1 and Geminin.

**Recipient or parental organism**

Tissue culture cells of human or murine origin.

**Host/vector system**

Laboratory strains of E. coli K12 derivatives; tissue culture cells of human origin.

pUC-based plasmid vectors with RNA polymerase III promoter; Phoenix-amphotropic retroviral producer cells.

**Origin & function**

The expression of inserts encoding siRNAs, consisting of sense and antisense 21-29 base sequences derived from the coding region of the target mRNA with a spacer to generate a hairpin RNA, in human primary epidermal keratinocytes, mouse primary keratinocytes and mouse and human cell lines, including squamous carcinoma cells and telomerase immortalised keratinocytes. Once expressed the siRNA oligonucleotides are expected to decrease levels of RNA encoding the target protein by over 70%.

Target genes are those of the pathways regulating epithelial stem cell fate, including Notch receptors, suppressor of hairless homologues, mastermind homologues, and the hairy/enhancer of split family of transcription factors. In addition components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins such as p21 Cip1 and Geminin will also be targeted.

Control siRNAs directed against enhanced green fluorescent protein and luciferase will be required for some experiments.

**Evaluation of foreseeable effects**

The inserts encode short interfering RNAs which are only active within the cell, and cannot encode an exogenously expressed protein. Expression of these RNAs will not
enhance the pathogenicity of the host cells. The effect of expression of the insert RNAs will be to functionally silence the target genes. In most cases, functional silencing is known to impair cellular function resulting in defective cell cycle progression or in genetic instability. In no instance is functional silencing of the target genes known to work directly to transform primary cell cultures.

A non-negligible hazard arises from the potentially oncogenic or cytotoxic nature of the cellular effects of some of the interfering RNA inserts. It is considered that DNA from such clones can be appropriately handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras. 8-10 in which the potential hazard is specifically considered.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

**Tick to confirm that you have attached a risk assessment to this form** Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N

Please enter comments on the GM safety committee on the risk assessment

This work involves the cloning of short DNA inserts under a eukaryotic promoter, in order to generate ~25-mer interfering RNA. The genes targeted in this work are those for pathways controlling epithelial stem cell fate, components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins. The cloned DNA is therefore potentially cytotoxic or oncogenic.

Initial cloning is into non-mobilisable vectors grown in laboratory E. coli K12 strains. The vectors are pUC-based and are disabled retroviral vectors, which require a helper t.c. line to produce virus, which in turn is only capable of a single infectious cycle. This stage is therefore Class 1.

Subsequently the retroviral vectors will be transfected into mouse cell lines, with the Phoenix packaging constructs, which give either ecotropic or amphotropic envelopes, to produce the disabled retrovirus with either type of envelope. Following consideration of the advice in the ACGM Compendium of Guidance, Part 2B, Annex III, paras 21-30, it was agreed that Class 1 is appropriate with the ecotropic envelope and Class 2 with the amphotropic. If cell lines from the amphotropic constructs are being maintained for a long time, consideration should be given to testing them for the presence of replication competent virus.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.
### Project Additional Information

**Purposes of the contained use**

The project aims to determine the molecular basis of the differentiation of human and mouse epithelial cells. Pathways regulating differentiation include Notch, wnt and the MAP kinase signalling cascade. In addition we wish to explore the interaction of Notch with cell cycle regulators such as p21 Cip1 and Geminin.

**Recipient or parental organism**

Tissue culture cells of human or murine origin.

**Host/vector system**
Laboratory strains of E. coli K12 derivatives; tissue culture cells of human or mouse origin, including murine cells expressing helper functions for defective retrovirus production.

pUC-based plasmid vectors with RNA polymerase III promoter; Plasmids containing lentiviral helper proteins - see diagrams attached to risk assessment.

Origin & function

The expression of inserts encoding siRNAs, consisting of sense and antisense 21-29 base sequences derived from the coding region of the target mRNA with a spacer to generate a hairpin RNA, in human primary epidermal keratinocytes, mouse primary keratinocytes and mouse and human cell lines, including squamous carcinoma cells and telomerase immortalised keratinocytes. These cells will include those infected with lentiviruses or retroviruses engineered to include a LoxP sequence flanked cDNA sequence.

Also expression of non-oncogenic genes involved in these pathways.

Target genes are those of the pathways regulating epithelial stem cell fate, including Notch receptors, suppressor or hairless homologues, mastermind homologues, and the hairy/enhancer of split family of transcription factors. In addition components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins such as p21 Cip1 and Geminin will also be targeted.

Control siRNAs directed against enhanced green fluorescent protein and luciferase will be required for some experiments.

Evaluation of foreseeable effects

The inserts encode either non-oncogenic proteins or short interfering RNAs which are only active within the cell, and cannot encode an exogenously expressed protein. Expression of these RNAs will not enhance the pathogenicity of the host cells. The effect of expression of the insert RNAs will be to functionally silence the target genes. In most cases, functional silencing is known to impair cellular function resulting in defective cell cycle progression or in genetic instability. In no instance is functional silencing of the target genes known to work directly to transform primary cell cultures.

The expression of the Cre recombinase will recombine the LoxP sites. Among other resulting deletions will be the gene for Cre itself.

Anon-negligible hazard arises from the potentially oncogenic or cytotoxic nature of the cellular effects of some of the interfering RNA inserts. It is considered that DNA from such clones can be appropriately handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras. 8-10 in which the potential hazard is specifically considered.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.
This work involves the cloning and expression of non-oncogenic proteins involved in pathways controlling epithelial cell fate. It also involves the cloning of short DNA inserts under a eukaryotic promoter, in order to generate ~25-mer interfering RNAs. These genes targeted in this work are those for pathways controlling epithelial stem cell fate, components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins. The cloned DNA is therefore potentially cytotoxic or oncogenic.

Initial cloning is into non-mobilisable vectors grown in laboratory E. coli K12 strains. The vectors are pUC-based and are disabled lentiviral or retroviral vectors, which require a helper t.c. line (or helper plasmids in a t.c. cell line) to produce virus, which in turn is only capable of a single infectious cycle. This stage is therefore Class 1.

Subsequently the retroviral vectors will be transfected into mouse cell lines, together with plasmids supplying helper functions, which give either ecotropic or amphotropic envelopes, to produce the disabled lentivirus with either type of envelope. Following consideration of the advice in the ACGM Compendium of Guidance, Part 2B, Annex III, paras. 21-30, it was agreed that Class 1 is appropriate with the ecotropic envelope and Class 2 with the amphotropic.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.

Project Containment

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Animal Units

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Large Scale Activities

| L3 | L4 | L2 |

Human Clinical Applications

| L3 | L4 |

Project Ref: 20/04.3

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</table>
Purposes of the contained use
To use somatic cell line knockout technology to generate assay reagents for combinatorial libraries of potential therapeutic compounds and for pathway analysis of potential new therapeutic targets.
This project requires gene disruption through homologous recombination in human epithelial cells in vitro; rAAV (adeno-associated virus) vectors permit such knockouts in human cells.
Inserts will encode LoxP sites, human genomic targets and positive selectable marker genes (eg neomycin or hygromycin). Target cells are human epithelial cell lines, including HCT-116, DLD1, A549, CAL51 and MT3 cell lines. These cells will be infected with rAAV (eg AAV-293).
Target genes are those of the pathways regulating DNA repair, apoptosis, histone acetylation, including BRCA2, HDCA1, SIRT1 and ADA3.

Origin & function
Target genes are those of the pathways regulating DNA repair, apoptosis, histone acetylation, including BRCA2, HDCA1, SIRT1 and ADA3.

Evaluation of foreseeable effects
The planned knockout of the target genes in human t.c. cell lines is not foreseen as increasing their pathogenicity from its negligible level for the cell lines.
The one significant hazard arises from the rAAV produced from the packaging cells. This is capable of infecting human cells and contains inserts designed to knockout target genes in the pathways regulating DNA repair, apoptosis, histone acetylation, including BRCA2, HDCA1, SIRT1 and ADA3. Their effect is therefore considered as potentially oncogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The planned knockout of the target genes in human t.c. cell lines is not foreseen as increasing their pathogenicity from its negligible level for the cell lines.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The work involves the cloning of inserts to knockout various genes, their incorporation into recombinant adeno-associated virus (rAAV) and the subsequent infection of human epithelial cell lines, in order to establish stable cell lines with the appropriate genes knocked out. The target genes are those in the pathways regulating DNA repair, apoptosis and histone acetylation, including BRCA2, HDCA1, SITR1 and ADA3. The host cells are all highly disabled and the modifications will not increase their pathogenicity. With the exception of the rAAV, the vectors are non-mobilisable and present no risk. The exception is the AAV, which can infect human cells.

Knocking out the target genes is potentially oncogenic and, while this is highly unlikely to increase the pathogenicity of the intended host cells, it does present a risk to workers who might become infected with the rAAV.

For this reason the packaging and infection steps are considered as Class 2, while the preliminary cloning steps and subsequent propagation of any modified cells are Class 1.

(Note from BSO: I have taken advice from HSE and the Inspector, to whom I was referred, agreed with this assessment.)

Project Containment
Project Ref 20/05.1

Date Ackn'd 02/08/2005

CU2 Project Title Use of lentiviral vectors in cloning and expression of genes involved in DNA repair and the cell cycle.

Date Project Ceased 03/09/2013

Class 2

CultureVolClass 2 < 1 Litre

Consent Granted Not Applicable

Non-GMM

Tick if notifying a connected programme of work N

Historical Significant Changes Project Transferred from GM20 on 02/08/2005, Project transferred to GM3

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Cancer cells exhibit abnormalities in cell cycle progression, chromosome structure and segregation and in the capacity to repair DNA damage that are central to transformation. These abnormalities are often the result of mutations in tumour suppressor genes, such as the breast cancer gene BRCA2. The overall goal of our studies is to ascertain the molecular basis for the abnormal division of cancer cells, and their increased genetic instability.

Recipient or parental organism

Murine-embryo-fibroblasts and HeLa and other cancer cell lines.

Host/vector system

E.coli laboratory K12 derivatives/pUC based vectors; Vertebrate tissue culture cells including 293T cells, murine-embryo-fibroblasts and HeLa and other cancer cell lines/Lentiviral vectors, with polytropism through viral
pseudo-typing using VSV-G protein.

Lentiviral system:  The viral system to be used employs a well-characterised lentiviral vector and packaging cell lines(Refs below). A number of safety features are incorporated to eliminate or very greatly reduce the possibility of generating productive recombinants. The vector lacks gag, pol, env, tat, rev, vpr, vpu, vif and nef functions. A chimeric LTR offsets the requirement for Tat in expressing genes transcribed from the LTR. The Rev function is provided in trans from a separate plasmid in the packaging cells, as are the Gag/Pol functions. The viral vectors are self-inactivating due to deletions in both the LTRs, and thus incapable of replication after one round of infection of target cells. The packaging cell lines are effectively helper-free, since the rev, gag/pol and env (in this case, pseudo-typed with VSV-G protein) genes are expressed from separate plasmids. Rubinson et al Nat Genetics 34:231 (2003); Dull et al., J Virol 72:8463 (1998); Myoshi et al J. Virol 72:8150 (1998).

Origin & function

Specific aims with details of the categories of inserts to be used are as follows:

A)  BRCA2-deficient cells exhibit defects in DNA repair (1). The defect is likely related to the inability of mutants BRCA2 to form functional complexes with RecA homologs in the Rad51 family (eg Rad51, xrc2 & 3, Rad51B-D). The molecular basis for this phenotype will be studied by functional and biochemical analyses in transfected cells. Wild-type or mutant forms (including point mutations, deletions and fragments) will be studied.

B)  Abnormalities of chromosome structure and number are abundant in BRCA2-deficient cells (2-4). To investigate the molecular basis for these phenotypes, the function of genes controlling chromatin structure (eg histones, histone-modifying-enzymes, HP1), the cell cycle (eg cyclins A, B, D, E; cdks 1, 2 & 4, polo-like kinases), chromosome number and structure (eg SMCs1 & 3, scc1, Ecol), cytokinesis (eg chromokinesins, INCENP, surviving) and the centrosome cycle (eg nek1, nap, g-tubulin, aurora kinases) will be investigated by functional & biochemical analyses in transfected cells. Wild-type or mutant forms (including point mutations, deletions and fragments) will be studied.

C)  We have shown that mutations inactivating mitotic checkpoint genes including Bub1 or Mad3L (BubR1), are necessary for the neoplastic transformation of cells lacking the BRCA2 tumour suppressor (5). Besides these, we will test the possibility that genes (eg chk1/chk2, cdc14A/cdc14B, PP1delta, cdc25C, m TERT or h TERT, ranGAP & cdt1) whose normal function is to regulate cell cycle progression and chromosome stability, particularly during the S phase and in mitosis, will be targets for inactivation by secondary genetic changes during tumour evolution in cells that lack genes such as BRCA2, using studies of transfected cells. Wild-type or mutant forms (including point mutations, deletions and fragments) will be studied.

D)  To identify novel genes that regulate these processes, cells will need to be transfected with pools (libraries) of vertebrate cDNAs or short-hairpin RNA molecules that interfer with the expression of vertebrate genes, for phenotypic screens. Here, the precise nature of the insert cannot be specified in advance.

In A-C above, fusions of wild-type & mutant genes to GFP or other fluorescent tags will be used to determine intracellular localisation.

3)  Yu et al., Genes Dev 14:1400 (2000)
5)  Lee et al., Molecular Cell 4:1-10 (1999)

Evaluation of foreseeable effects

Possible changes in the behaviour of cells in tissue culture, helping to elucidate the abnormal division of cancer cells, and their increased genetic instability.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work.

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before subsequent discharge doen the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

02/03/2022  Page 11968 of 15326
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

None

Project Containment

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Human Clinical Applications

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Project Ref 921/07.1

CU2 Project Title

Study of the EMSY using lentiviral vectors encoding EMSY siRNAs.

Date Ackn'd 11/01/2007

Class CultureVol

Class 2 < 1 Litre

Non-GMM Consent Granted

Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
## Project Additional Information

### Purposes of the contained use
The project aims to determine the molecular basis of the function of EMSY in mammalian cell lines. EMSY binds to the tumour suppressor protein BRCA2, inhibiting its function and is over expressed in breast and ovarian cancer.

### Recipient or parental organism
Target cells will be established mammalian cell lines, including human cells.

### Host/vector system
Target cells will be established mammalian cell lines, including human cells. Replication incompetent lentiviral supernatants from commercial sources, typically packaged with VSG or an amphotropic receptor. If it is found necessary for sufficiently stable siRNA production, a WPRE element may be incorporated in the vector.

### Origin & function
Inserts will encode RNA silencing constructs directed against the EMSY protein. Typically the inserts consist of sense and antisense 21-29 base sequences derived from the coding region of the target mRNA with a spacer to generate a hairpin RNA. Control siRNA constructs directed against housekeeping transcripts or reporter genes and non functional mutant siRNAs may also be expressed. Vectors may also encode reporters such as EGFP or selectable markers such as puromycin.

### Evaluation of foreseeable effects
EMSY is amplified in breast and ovarian cancer. Knockdown of EMSY is not predicted to have any oncogenic effects. The aim of this study is to see what effect such knockdown does have on mammalian cell lines.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Only micro-organisms involved in this work.

- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
  
  Full CL2 will be used, with no derogation.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Containment level 2 waste will be autoclaved (136°C for 10min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

### Is an emergency plan required according to regulation 20?
N

- If yes, tick to confirm that it is attached to this form
  
  N

- Tick to confirm that you have attached a risk assessment to this form
  
  Y

- Tick if you are claiming exemption from disclosure for section of the risk assessment
  
  N

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02/03/2022

Page 11970 of 15326
This work involves the use of purchased replication incompetent retroviral vectors, with amphotropic envelope proteins, which contain and express siRNAs against the mRNA for EMSY. In view of its involvement with BRCA2, it is considered that this may be potentially oncogenic. The recombinant retrovirus will be used to infect mammalian (including human) cell lines. In view of the potentially oncogenic nature of the insert into amphotropic disabled retrovirus, it was agreed that classification as Class 2 was correct. It was noted that this classification would also allow the use of vectors containing the WPRE element, should this prove desirable.

### Project Containment

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

### Project Ref 921/09.1

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<td>03/08/2009</td>
<td>Use of adenoviral and lentiviral vectors in cloning and expression in mammalian cells of prokaryotic genes kis and kid</td>
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<td>&lt; 1 Litre</td>
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- **Historical Significant Changes**: PROJECT TRANSFERRED TO GM921
- **Withdrawn**: N

### Project Additional Information

**Purposes of the contained use**

Kid protein is toxic to mammalian cells and Kis protein protects from this effect. When they are expressed together in mammalian cells, death or cell survival depends on the ratio between Kid and Kis that cells are able to sustain. This is potentially a therapeutic or experimental tool. The ratio can be controlled at the level of transcription,
mRNA stability and protein stability. We want to exploit the genetic differences between normal cells and cancer cells to regulate, differentially, the Kid-to-Kis ratio in these cells, so that cancer cells are killed and normal cells are protected from toxicity.

Recipient or parental organism

Murine and human cells (primary cells, cancer cell lines and immortalized cell lines)

Host/vector system

1) E. coli, laboratory K12 derivatives/pUC based vectors;

2) Vertebrate tissue culture cells, human and mouse, including as examples 293T cells, murine-embryo-fibroblasts and HeLa and other human cancer cell lines and immortalised normal cells; and primary cultures of human and mouse cells.

3) Adenoviral vectors and Lentiviral vectors (the latter with polytropism through viral pseudo-typing using VSV-G protein)

3.1.) Adenoviral system: The adenoviral system to be used employs a well-characterised vector and packaging cell lines (Refs below). Second generation replication defective human adenovirus serotype 5 derived adenoviral vectors will be used for expression of Kis and/or Kid proteins. Vectors are rendered replication defective by the deletion of the E1 and E3 genes. The E1 gene is essential for the assembly of infectious virus particles and is complemented in vivo by an adenovirus packaging cell. The E3 gene encodes proteins involved in evading host immunity and is dispensable (Luo et al, Nature Prot., 2 (5): 1236 (2007))

3.2.) Lentiviral systems: The two types of viral system to be used employs two well-characterised lentiviral vector and packaging cell lines (Refs below).

3.2.1.) Type A (eg. pLenti6-UbC-V5DEST from Invitrogen):

- The number of genes from HIV-1 that are used in this system has been reduced to three (i.e. gag, pol, and rev).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replicant-competent virus (Dull et al., J Virology 72 8463-8471 1998).
- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain LTR's or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced,
- The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.
- Expression of the gag and pol genes from pLP1 has been rendered Rev-dependant by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev.
- A constitutive promoter (RSV promoter) has been placed upstream of the 5 LTR in the lentiviral vector to offset the requirement for Tat in the efficient production of viral RNA. The 3LTR region has a deletion that promotes self inactivation of the viral genome upon integration in the host genome.

3.2.2.) The lentiviral packaging systems mentioned above (type A) is a third generation versions that utilize split-genes to provide the viral packaging elements on individual plasmids that physically separate the viral envelope, env (usually VSV-G), sequence from the gag-pro-por sequences. These split-gene packaging strategies reduce the risk of generating RCL because multiple recombination events are necessary to create a virus that harbors the sequences required for independent replication. Type B (Clontech’s Lenti-X HT) Packaging System also uses a split-gene packaging strategy, but adds another level of safety by further uncoupling pol (RT and IN) from gag-pro. The result is that gag, pol and env reside on three physically distinct entities, rather than the standard two. This approach further reduces the possibility of creating RCV to a level below that of standard 3rd generation packaging systems, because extra recombination events are required to create such viruses. In fact, the emergence of RCV is undetectable from systems using this approach (Wu, X., et al. (2000) Mol. Ther. 2 (1):47-55.). These improvements significantly increase the safety profile of our Lenti-X HT Packaging System.

Clontech’s Lenti-X Vectors contain less than one-third of the wild-type HIV-1 genome. These wild-type sequences mainly consist of the viral LTRs and packaging signal. All
essential replication genes have been completely removed and are instead supplied as separate DNA entities in the Lenti-X HT Packaging Mix (described above).

In brief, the number of genes from HIV-1 that are used in type B system has been reduced to four (i.e. gag, vpr-pol, rev and tat).

. Genes encoding the structural and other components required for packaging the viral genome are separated onto five plasmids. This reduces the incidence of replication competent lentivirus to levels below that of the system A above.

. Although the four packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain HIV-1 LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.

. The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.

. Expression of the gag, tat and rev genes from the packaging plasmids is directed by tetracycline dependant promoters (PrTRE). Therefore their expression requires supplying TetR- VP16 in trans, and it is only active in the absence of doxycycline in the growth medium.

. In this system, expression of tat is required in the packaging cell line, because the lentivirus vector contains an intact HIV-1 5LTR, which allows very high titers in viral preparations.

Origin & function

The overall purpose is to introduce Kis and Kid protein expression into mammalian cells, control that expression and monitor expression. Inserts will encode reporters, such as EGFP, Ds Red; transcriptional regulators such as TetR; prokaryotic genes encoding ribonuclease Kid and its counteracting protein Kis; and transcription and post-transcription-regulating sequences of prokaryotic and mammalian origin.

Genes Kis and Kid will include wildtype, inactive mutants and fusion variants (e.g. fused to unstable protein domains), and they will be expressed under the control of viral and mammalian promoters, both constitutive (e.g PrCMV, PrUbC, PrEF-Ialpha) and/or repressible (e.g regulated by TetR).

Evaluation of foreseeable effects

Some of the virus vectors will kill infected mammalian cells, so some of the vectors will be toxic to human and mammalian cells. Also because of the presence of the WPRE element in the lentivirus vectors, the lentivirus constructs might transform infected mammalian cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work.

For only GMMS - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136º C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Adenovirus vectors: (Risk assessment H2008-009)

This individual RA H2008-009 is an assessment for adeno vectors, and so the main issue is whether, and in what way, the inserts are hazardous to human cells. Oncogenic inserts would be Class 2, most other inserts Class 1. As some of the inserts are toxic, we have chosen precautionary Class 2 containment for the active toxic insert work only. The majority of the work proposed will be concerned with establishing control of expression and this can be done with inactive mutants, in Class 1.

The principal concern here is that, for some of the inserts, the virus generated and the packaging/producer cells are effectively toxins that could kill one human cell per virus particle, though the act of killing the cell would disable the virus. Handling and storing the producer cells and supernatants is therefore similar to handling cholera toxin, ricin, or phalloidin. Those involved must be made aware of this.

A subsidiary concern would be propagation of the virus, but the virus is sufficiently disabled to eliminate this. E1/E3 deleted adenovirus is generally accepted as disabled and, with wild-type adeno being HG2 this allows the mutant to be HG1. Once the virus has infected a target cell those cells pose no particular hazards, since the toxin involved is intra cellular.

Lentivirus (RA H2009-002)

The issues in this work are (i) the expression of the toxin Kid in lentiviral vectors, and (ii) the lentivirus vectors themselves, which, if they carry the complete WPRE, are potentially oncogenic and current guidance is to use them in CL2 regardless of their tropism. Both these have been considered by us before. (i): toxin-expressing human host range virus is toxic to cells in a similar way to a strongly toxic lab reagent and should be treated as such. They are borderline CL1/CL2 but we have previously chosen to recommend CL2. (ii) The lentivirus work does require CL2 when the vectors incorporate the putatively-oncogenic complete WPRE element. The E.coli work raises no particular issues as the vectors do not express in bacteria.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.

The construction work in E. coli is correctly assessed as Class 1.

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Project Ref 921/11.1

Date Ackn'd CU2 Project Title

02/03/2022
Analysis of the changes of human epithelium adenoviral transfection of genes of interest

Date Project Ceased
03/09/2013

Historical Significant Changes
Project transferred to GM3201 on 03/09/2013

Historical Date of Additional Info

Date of Significant Change

Project Additional Information

Purposes of the contained use

We aim to identify genes that could play a significant role in the transformation of human oesophageal epithelium towards cancer via the development of Barrett's oesophagus and the acquisition of a malignant phenotype. Several candidate genes have been identified in literature as potential regulators for this switch in phenotype. Most of them are transcription factors, they include HoxGenes (identified in a current project in the lab), NHF4a, p63, CDX2 and C/EBP and they are potential oncogenes.

Recipient or parental organism

Packaging cells HEKs93
Oesophageal cells:
1. Primary cells will be isolated from human squamous oesophageal resection specimens and processed to obtain single cell suspension (separate Risk Assessment RCF 041 for human tissue). For this purpose freshly isolated, cultured cells will be used.
2. Other established human oesophageal cell lines (normal and from cancer).

Host/vector system

HEK293 (human embryonic kidney) cells, that, by producing the adenovirus E1 gene in trans, allow the production of infectious virus particles when cells are transfected with E1- deleted adenovirus vectors such as the pAdEasy-1.

Vector:
Purified recombinant Ad plasmid DNA is digested with the restriction enzyme Pac I to expose its inverted terminal repeats (ITR), and then used to transfect AD-293 (or HEK293) cells which have complemented in vivo viral assembly genes are:

Oesophageal cells/Adenoviral particles
Hosts:
1. Primary cells will be isolated from human squamous oesophageal resection specimens and processed to obtain single cell suspension (Risk Assessment RCF 041). For this purpose freshly isolated, cultured cells will be used.
2. Other established human oesophageal cell lines (normal and from cancer) might be used.
Vector:
The adenoviral particles generated as above. The regions E1 and E3 of the viral genome have been deleted. E1 deletion renders the viruses defective for replication and incapable of producing infectious viral particles in target cells (provided there is no complementation by the host cell); the E3 region encodes proteins involved in evading host immunity. Therefore the host cells which do not have the E1 region cannot replicate the virus or integrate it into the genome.

Origin & function
Inserts will be from Human genomic DNA and cDNA sequences cloned in bacteria. These inserts will be genes thought to be relevant to the transformation of human oesophageal epithelium.

Evaluation of foreseeable effects
The bacterial construction system cannot generate hazardous particles from the cloned inserts and for this reason a Cat I containment level would be adequate and this part of the work is not described here.

The target cells (human oesophageal cannot replicate the virus or integrate its DNA in the genome. However, it is possible that the pathogenicity of the host cells would be enhanced by the inserts. In addition, the nature of the host cells (untested human primary cells) requires a containment level (Cat II) adequate to this risk.

Furthermore, the regions E1 and E3 of the viral genome have been deleted. E1 deletion renders the viruses defective for replication and incapable of producing infectious viral particles in target cells (provided there is no complementation by the host cell); the E3 region encodes proteins involved in evading host immunity.

HEK293 contain the E1 gene that allows gene replication and therefore also the "amplification" of the viral stock solution requires an adequate (Cat II) containment level.

The main Hazard is potential infection of persons working in the lab by the GM virus, for which a Cat II containment level is adequate.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Only micro-organisms and tissue culture cells are involved

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Full containment level 2 will be applied

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% kill

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This is a relatively typical case of adenovirus vector transduction of human cells in culture, and should be Class 2. The lab concerned already is familiar with Class 2 working, as the target cells are human primary material. The application specifically acknowledges the need to take care when storing adenovirus stocks outside the Class 2 lab, as discussed by the Hutchison-MRC safety committee.

### Project Containment

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- **Large Scale Activities**: L2 L3 L4 L2 L3 L4 L2 L3 L4
- **Human Clinical Applications**: L2 L3 L4 L2 L3 L4

### Project Ref 921/12.1

- **Date Ackn'd**: 26/01/2012
- **CU2 Project Title**: Use of lentiviral vectors in cloning and expression in mammalian cells of genes controlling cell division and differentiation
- **Class**: Class 2
- **CultureVol**: ≤ 1 Litre
- **CultureVolumeClass**: Class 3-4
- **Non-GMM**: Consent Granted

- **Date Project Ceased**: 03/09/2013
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

- **Historical Date of Additional Info**: Project transferred to GM3201 on 03/09/2013

### Project Additional Information

**Purposes of the contained use**

The project aims to investigate strategies to modulate the differentiation of human and mouse embryonic stem cells, differentiated cells and tumour cells by introduction of genes including transcription factors and cell cycle regulators using lentiviral vectors.

**Recipient or parental organism**

...
Murine and human cells (packaging cells, primary cells, cancer cell lines and immortalized cell lines).

**Host/vector system**

1) E.coli, laboratory K12 derivatives/pUC based vectors;

2) Vertebrate tissue culture cells including 293 T cells, embryonic stem cells, human cancer cell lines, and cells including fibroblasts and neuroblastoma and glioblastoma cell lines derived from patient samples.

3) Lentiviral vectors

3.2) Lentiviral systems: the two types of viral system to be used employs two well characterised lentiviral vector and packaging cell lines.

3.2.1) Type A (e.g. pFUV and tet-ON derivatives thereof), 3rd generation:

. The number of genes from HIV-1 that are used in this system has been reduced to three (i.e. gag, pol, and rev).
. Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids.
. Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain LTRs or the packaging sequence. This means that none of the HIV-1 structural and other components required for packaging the viral genome are separated onto four plasmids.
. Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
. The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.
. In some cases expression of our gene insert of interest will be drive expression off a tet-responsive CMV promoter, and regulated by a co-transduced lentivirus doxycyclin-responsive Tet regulator, giving an added level of safety for insert expression.

3.2.2) Type B (e.g. LentiX0HTX from Clonetech), 4th generation

The lentiviral packaging systems mentioned above are 3rd generation versions that utilize split-genes to provide the viral packaging elements on individual plasmids that physically separate the viral envelope, env (usually VSV-G), sequence from the gag-pro-pol sequences. These split-gene packaging strategies reduce the risk of generating RCL because multiple recombination events are necessary to create a virus that harbours the sequences required for independent replication. Type B (Clontech’s Lenti-X HT) Packaging System also uses a split gene packaging strategy, but adds another level of safety by further uncoupling pol (RT and IN) from gag-pro. The result is that gag, pol and env reside on three physically distinct entities, rather than the standard two. This approach further reduces the possibility of creating RCL to a level below that of standard 3rd generation packaging systems, because extra recombination events are required to create such viruses. In fact, the emergence of RCL is undetectable from systems using this approach (Wu, X, et al. 2000) Mol. Ther. 2(1):47-55.) These improvements significantly increase the safety profile of our Lenti-X HTX Packaging System.

Clontech’s Lenti-X Vectors contain less than one-third of the wild-type HIV-1 genome. These wild-type sequences mainly consist of the viral LTRs and packaging signal. All essential replication genes have been completely removed and are instead supplied as separate DNA entities in the Lenti-X HT Packaging Mix (described above).

In brief, the number of genes from HIV-1 that are used in type B system has been reduced to four (i.e. gag, vpr-pol, rev and tat).

. Although the four packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293 T derived producer cell lines, none of them contain HIV-1 LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.

In this system, expression of tat is required in the packaging cell line, because the lentivirus vector contains an intact HIV-1 5LTR, which allows very high titers in viral preparations.

In some cases expression of our insert gene of interest will be driven expression of a tet-responsive CMV promoter, and regulated by a co-transduced lentivirus doxycyclin-responsive Tet regulator, giving an added level of safety for insert expression.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard.

**Origin & function**

Genes and mutant derivatives thereof, usually of human, mouse or Xenopus frog origin, encoding both growth control proteins and transcription factors, along with marker genes such as GFP and antibiotic resistance genes.

**Evaluation of foreseeable effects**

Some of the virus vectors will lead to growth arrest and differentiation of mamalian cells, while there is potential to promote cell proliferation. Because of the presence of the WPRE element in the lentivirus vectors, the lentivirus constructs might transform infected mammalian cells.

The main Hazard is potential infection of persons working in the lab by the GM virus, for which a Cat II containment level is adequate.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class I level of classification.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms and tissue culture cells are involved.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class I level of classification.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
This is a fairly typical lentivirus project, designed to introduce genes into mouse and human cells in vitro. It does not seem to raise any unusual issues.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<th>Animal Units</th>
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<th>Human Clinical Applications</th>
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**Project Ref** 921/12.2

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<th>Class</th>
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<th>CultureVolumeClass3-4</th>
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<th>Significant Change ID</th>
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<td>20/06/2012</td>
<td>Use of lentiviral vectors in cloning and expression/knock-down if genes functioning as molecular regulators of lymphatic-stroma interactions</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
<td>Project transferred to GM3201 on 03/09/2013</td>
<td>II</td>
<td>03/09/2013</td>
<td>Purposes of the contained use: The project aims to determine how candidate genes may function in tumour progression or stromal cell function (e.g. gp38). We will study their phenotypic and functional changes following modification: e.g. a) in cancer cells: the acquisition of malignant features such as increased motility and invasive capacity, b) in stromal cells: changes to junctional properties and interaction with infiltrating immune cells.</td>
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- **Date Project Ceased**: 03/09/2013
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Project notified under transitional arrangements**: N

**Project Additional Information**

The project aims to determine how candidate genes may function in tumour progression or stromal cell function (e.g. gp38). We will study their phenotypic and functional changes following modification: e.g. a) in cancer cells: the acquisition of malignant features such as increased motility and invasive capacity, b) in stromal cells: changes to junctional properties and interaction with infiltrating immune cells.
Recipient or parental organism

a) Murine cells (for example, isolated tumour and stromal cells and commercially available, immortalized well-established cell lines).
b) Human cells (including packaging cells e.g. HEK293, established well-characterised cancer and stromal cell lines).

Host/vector system

1) E. coli, laboratory K12 derivatives/pUC based vectors;
2) Mammalian tissue culture cells including human cancer and mouse cell lines. Human hosts include for example, HEK293T packaging cells, well-established human cancer and stromal cell lines. Examples of mouse hosts include stromal cells such as fibroblasts (normal dermal, lymph node stromal and tumour-associated) and tumour cells either isolated or purchased as commercially available cell lines.
3) Viral vectors: The viral systems to be used employ well characterized vector systems packaging line
   3.1) Lentiviral systems:
   3.1.1) Commercially available, verified transduction ready viral transduction particles e.g. MISSION sequence verified shRNA lentiviral transduction particles (pLKO.1-puro).
   3.1.2) Third generation lentiviral particle generation using the 3-plasmid method
   ● The number of genes from HIV-1 that are used in this system has been reduced to three (i.e. gag, pol, and rev).
   ● Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids.
   ● Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
   ● The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.

Virally transduced cells will not leave Class II containment until negative results are obtained from HIV-1 p24 ELISAS. This measures viral titres and ensures no residual viral proteins are present in the supernatant of transduced cells.

3.2) Adenoviral systems expressing Cre recombinase (AdCMV-Cre) or fluorescent marker proteins
   3.2.1) Commercially sourced, pre-titred replication deficient virus constructs
   ● The Adenoviral particles purchased will be replication incompetent due to deletions in the E1 and E3 regions. The virus arrives pre-screened to ensure replication deficiency.
   3.2.2) Assembly of virus using the AdEasy system (or similar)
   ● Cre recombinase is a Type I topoisomerase from P1 bacteriophage that catalyzes site-specific recombination of DNA between loxP sites. P1 sequences are not found in the human genome, therefore cre-recombinase expression would be expected to have no impact on the cell
   ● Recombinant adenoviruses exhibit a wide tissue tropism and could potentially infect a range of mammalian cells, however, the particles being used are replication deficient due to elimination of E1 and E3 genes.
   ● The Vectors do not integrate into the genome.

Origin & function

Gene constructs and mutant derivatives thereof, usually of human or mouse origin, a) encoding candidates genes deemed important in tumour progression and stroma function e.g. gp38, b) cre recombinases to mediate specific recombination events, along with marker genes such as GFP and antibiotic resistance genes.

Evaluation of foreseeable effects

Some of the virus vectors will lead to growth arrest and differentiation of mammalian cells, while there is potential to promote cell proliferation. Because of the presence of the WPRE element in the lentivirus vectors, the lentivirus constructs might transform infected mammalian cells.
The main Hazard is potential of persons working in the lab by the GM virus, for which a Class II containment level is adequate.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Once negative viral titres have been measured, work with these genetically modified cells can be undertaken at a Class I level of classification.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Micro-organisms and tissue culture cells are involved

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class 1 level of classification.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

As per Hutchinson /MRC research Centre standard practice guidelines (unless the risk assessment demands more stringent measures)

CL2: all solid waste is autoclaved in the building and then incinerated off site by a contractor (Novus)

Liquid waste is disinfected with Virkon to final concentration of 5%; contact time of 30 minutes minimum before disposal to drain.

Bacterial Plates are autoclaved in the building.

Solid waste (including for example culture flasks, plastic pipettes) is bagged, and incinerated off site by a contractor (Novus).

It is expected that these measures will give effectively 100% killing.

---

**Is an emergency plan required according to regulation 20?**  

N

If yes, tick to confirm that it is attached to this form

N

**Tick to confirm that you have attached a risk assessment to this form**  

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  

N

---

This is a fairly standard proposal to use lentivirus (for various genes) and adenovirus (for cre) to genetically manipulate murine and human cells. The inserts proposed do not seem particularly hazardous.

**Project Containment**

02/03/2022
### Project Additional Information

**Purposes of the contained use**

We plan to use viral vectors to insert or delete candidates identified by transcriptomics or metabolomics analyses as being important in cell metabolism of both epithelia and stromal cell during tumorigenesis. We will study their phenotypic and biochemical changes following modification: e.g. a) in cancer cells: the changes of their malignant features such as increased motility and invasive capacity and changes in core metabolic pathways, b) in stromal cells: changes of core metabolic pathways and transdifferentiation into cancer associated stromal cells.

**Recipient or parental organism**

Murine fibroblasts and murine kidney epithelial cells obtained from Fumarate Hydratase deficient mice, primary and immortalised cell lines and tissues from other genetically engineered mice and well characterised human normal and cancer cell lines (including HEK293T that will be used as packaging cells for virus production).

**Host/vector system**
1) E. coli, laboratory K12 derivatives/pUC based vectors;

2) Mammalian tissue culture cells including human and mouse cell lines. Human hosts include for example, HEK 293T packaging cells, well-established human cancer and stromal cell lines. Examples of mouse hosts include epithelial cells, stromal cells such as fibroblasts and tumour cells either isolated or purchased as commercially available cell lines.

3) Viral vectors including retrovirus, lentivirus and adenovirus as follows:

**RETROVIRUS:** Modified cell lines will be created by the transduction with retroviral particles generated from appropriate packaging cell lines (see below) after their transduction with retroviral plasmids and will be based mostly on the following backbones:
1. The pBABE vector is derived from the Moloney murine leukemia virus (MMLV).
2. The pRETROSUPER vector is derived from the Murine Embryonic Stem Cell virus (MSCV).

**LENTIVIRUS:** Modified cell lines will be created by the transduction with lentiviral particles generated from appropriate packaging cell lines (see below) after their transfection with lentiviral plasmids and will be based mostly on the following backbones:
2. TRC1 and TRC2 pLKO.1-puro
3. pGIPz
4. pTripz
5. Transduction ready particles generated from the above and purchase directly from the suppliers

**ADENOVIRUS:** modified cells will be obtained by direct infection with commercially available adenoviral particles of adenoviral particles generated as follows.

1) Commercially sourced, pre-titred replication deficient virus constructs
   - The Adenoviral particle purchased will be replication incompetent due to deletions in the E1 and E3 regions. The virus arrives pre-screened to ensure replication deficiency.
2) Assembly of virus using the AdEasy system (or similar)

**Origin & function**

Gene constructs and mutant derivatives thereof, usually of human or mouse origin, a) encoding candidates genes deemed important in cell metabolism, b) cre recombinases to mediate specific recombination events, along with marker genes such as GFP and antibiotic resistance genes. c) fluorescent markers targeted to different subcellular compartments.

**Evaluation of foreseeable effects**

**ADENOVIRUS**

The adenoviral particles purchased will be replication incompetent due to deletions in the E1 and E3 regions. The virus arrives pre-screened to ensure replication deficiency.

Cre recombinase is a Type 1 topoisomerase from P1 bacteriophage that catalyzes site-specific recombination of DNA between loxP sites. P1 sequences are not found in the human genome, therefore cre-recombinase expression would be expected to have no impact on the cell.

Cells isolated from murine tissues with loxP sites will be from normal or tumour-associated tissues and are expected to be similar in nature to established fibroblast cell lines. Therefore, these cells are not anticipated to pose a hazard to human health.

Recombinant adenoviruses exhibit a wide tissue tropism and could potentially infect a range of mammalian cells, however the particles being used are replication deficient.
To elimination of E1 and E3 genes.

These Vectors do not integrate into the genome.

RETROVIRUS AND LENTIVIRUS

The main hazard arises directly from the recombinant viruses produced from the packaging cells. These yield both ecotropic and amphotropic lentivirions and ecotropic and amphotropic retrovirions, depending upon the envelope proteins supplied by the cell. Some of the virus vectors will lead to growth arrest and differentiation of mammalian cells, while there is potential to promote cell proliferation. Retroviruses and lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. To further reduce the risks of virus utilization, we will

1. Use retroviral infections targeted to murine cell or human cells designed to express ecotropic receptors

2. Use third generation lentiviral expression vectors, where genes encoding the structural and other component required for packaging the viral genome are separated onto three plasmids. All three plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus.

Some of the indicated vector might contain the WPRE (Woodchuck hepatitisB virus post transcriptional regulatory element). This element is used to increase lentiviral vector titre and gene expression. It includes the promoter for the X-protein and the part to the coding sequence for such protein, which may have oncogenic properties. Whenever possible we aim to use WPRE-less vectors such as the TRC-1-pLKO.1, thus limiting the oncogenic potential of the viral particles.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Micro-organisms and tissue culture cells are involved

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Containment level 2 will be applied only in specific circumstances such as:

1. The use of lentivirus containing the WPRE elements
2. The modulation of the expression of known or suspected oncogenes or tumour suppressors.
3. The expression of toxic or inflammatory (e.g. cytokines) genes

Other infection and virus production work will be carried out in class l.

After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class 1 level of classification.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

As per Hutchinson/MRC Research Centre standard practise guidance (unless the risk assessment demands more stringent measures).

CL2: all solid waste is autoclaved in the building and then incinerated off site by a contractor (Novus).

Liquid waste is disinfected with Virkon to final concentration of 5%; contact time of 30 minutes minimum before disposal to drain.

Bacterial Plates are autoclaved in the building.
Solid waste (including for example culture flasks, plastic pipettes) is bagged, and incinerated off site by a contractor (Novus). It is expected that these measures will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This is a fairly standard proposal to use lentivirus (for various genes) and adenovirus (for cre) to genetically manipulate murine and human cells, but the human host range virus work falls on the borderline between class I and class II: innocuous inserts may be transduced in class I, but toxic, pathogenic or oncogenic inserts or vector components require class II.

Since we discourage use of Class II where it is not necessary - to minimize the number of people exposed to more hazardous material particularly clinical material - we consider that it is helpful to identify much of this work as acceptable in class I.

Most of the inserts are not overly hazardous, being marker genes or genes involved in metabolism, so in these cases use of lentivirus that lacks the WPRE element in class I seems acceptable. However, class II is required for experiments involving vectors with the WPRE element, or with inserts that might be oncogenic (including knockdown constructs for a tumour suppressor gene) or toxic or inflammatory (such as cytokines, peptide hormones).

Project Containment

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Project Ref 921/12.4

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<td>&lt; 1 Litre</td>
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02/03/2022
The overall aim of the lab is to study genes that are altered by structural rearrangement of the cancer genome: these can be inactivated tumour suppressor genes or fusion genes analogous to those traditionally associated with leukaemias.

This project is to investigate the functions of these candidate genes by manipulating their expression using siRNA or expression of cDNAs in cultured cells. We plan to use a number of human cancer and normal cell lines, principally breast and ovarian, that can be particularly difficult to transfect by standard methods. We therefore plan to use viral vectors. The work will be carried out in vitro.

Description of procedure: cDNAs or shRNAs that target the gene of interest will be cloned into retroviral and lentiviral vectors. Recombinant retro/lentiviral vectors carrying the gene or shRNA of interest will be packaged in virus packaging cells. The viruses harvested will be used to infect human or mouse cell lines. The transformed cell line will be used in vitro for functional assays such as cell cycle, apoptosis and cell-mobility assays.

E. coli and established human and mouse cell lines in vitro. We may also use human cell lines engineered to express the ecotropic receptor (but such lines will not be constructed under the risk assessment).

Recipient or parental organism

The host/vector systems are (i) plasmids in E. coli for construction steps; (ii) transfection of retrovirus or lentivirus vector into packaging cell line systems to generate virus; and (iii) infection, with the packaged retroviral or lentiviral constructs, of human or mouse cell lines. Retrovirus/lentivirus and the packaging lines will be chosen to match the target cells and to repeat published experiments. Broadly, lentivirus will be used on less vigorous human cancer cell lines.

i) Generation of the construct to express the genes of interest.

INSERT: the insert is chosen to modulate the expression levels of genes of interest by overexpression or downregulation. We will begin for example with NRG1, which has been proposed as both a tumour suppressor gene and an oncogene. However, we will apply this technology to other known and candidate cancer genes. We will include knockdown of the known tumour suppressor gene, p53, and overexpression of the known oncogene, Ras, as controls.

a) Constructs for overexpression: these constructs are engineered using cDNAs that will encode for candidate cancer genes and rearranged forms of these genes, such as fusions of these genes, internal partially duplicated mutants and deletion mutants. These inserts will be either purchased or designed ad hoc and inserted into lentiviral or retroviral vectors.
b) Constructs for downregulation of gene expression: these constructs are shRNAs that will downregulate the expression of chosen target genes. These constructs will be purchased or designed ad hoc and inserted into lentiviral or retroviral vectors. For custom design of shRNAs, to be cloned into the MSCV-miR30 vector, we will use the Hannon lab shRNA retriever (http://katahdin.cshl.org/siRNA/RNAi.cgi?type=shRNA). We will also use microRNA-adapted shRNAs constructs already cloned into appropriate plasmids (see below) mostly, but not exclusively from Open Biosystems (thermoscientific) (http://www.openbiosystems.com/RNAi/LentiviralshRNAmir/) or Cellecta (http://www.cellecta.com/products-services/pooled-shRNA-libraries/) and Santa Cruz. We will also use shRNAs in vectors designed by others to replicate their work, notably constructs of Sheng et al Cancer Cell 2010;17, 298.

VECTOR: the vectors described here will be used to express the insert in proliferating and non-proliferating mammalian cells by means of viral transduction and could be either retrovirus- or lentivirus -based.

RETOVIRAL
The retrovirus vectors to be used are derived from Moloney murine leukaemia virus (MMLV) and its relative Murine Embryonic Stem Cell virus (pMSCV). Currently, these are pBABE, MSCV-miR30, pRETROSUPER and LPC. For all the vectors, the env genes, provided by the packaging plasmid, determines host range.
Vectors mainly for cDNAs.
1. pBABE (Morgenstern & Land) is derived from Moloney murine leukaemia virus (MMLV). The vector provides the viral package signal, transcription and processing elements, and a target gene. Transfection into a packaging cell line, such as Phoenix, produces high titer replication-incompetet viruses.
2. LPC system. The LPC vector was constructed at CSHL by S L (Cold Spring Harbor Lab) for efficient expression of cDNAs.
Vectors Specifically designed for shRNA inserts:
3. The MSCV-miR30 vector (also developed by S L’s lab) is derived from the Murine Embryonic Stem Cell virus (pMSCV), closely related to MMLV. The vector contains the mir 30-styled shRNA expression cassette expressed from the U6 promoter. The vector has a specifically designed 3’LTR that has a deletion in the LTR promoter elements. This deletion results in inactivation of the LTR mediated transcription upon retroviral integration. The phosphoglycerate kinase (PGK) promoter drives the expression of the puromycin resistance gene for selection in eukaryotic cells. The MSCV-miR30 plasmid can be propagated in E. coli under ampicillin (AMP) selection.
4. pRETROSUPER vector is also derived from the Murine Embryonic Stem Cell virus (pMSCV) and is commercially available.
Either cDNA or shRNA:
5. pOZ-FH-C is a retrovirus vector that has been used to coexpress an insert of interest with IL-2, so that infected cells can be affinity purified by IL-2 antibody (Nakatani and Ogryzko, 2003).
6. pWZL, a commercially available retroviral vector available with various selection markers, made by J M, the maker of pBABE.
7. Related established retroviral vectors to achieve coexpression with marker genes.

LENTIVIRAL
For cell lines where proliferation is limited we may need to use lentiviral vectors. We will use metabolisation-defective lentivirus (third-generation lentiviral vectors). One packaging plasmid lacks both LTRs, viral packaging signal (y) and viral genes (env, tat, rev, ypr, vpu, vif and nef). Rev is supplied in-trans on a second plasmid. Envelope is expressed on a third plasmid. The vector to be packaged has a self-inactivating LTR and expresses no viral gene products.
Lentiviral vectors used include:
1. PLKO vector is widely used and commercially available - see below.
Specifically designed for shRNA inserts:
2. Lenti-miR30 is a vector adapted by CSHL to express shRNAs as part of simulated microRNAs. Other lentiviral vectors in current use by our colleagues may also be used. They include:
1. pRSI-U6-(sh)-UbiC-RFP-2A-Puro: HIV-based lentiviral shRNA cloning vectors with H1, U6, or H1 tet-regulated promoters for expression of shRNA and a choice of a single or dual selection marker (GFP, RFP, PuroR, BleoR, NeoR, Hygro-HK, etc.) expressed from a single CMV EF1α, PGK, UbiC, or other promoters. It contains 18-nt bar code compatible with the Illumina HT Sequencing platform.
2. pLKO1-puro: The viruses generated using these plasmids were used to generate the TRC1 and TRC2 collection of commercially available shRNAs. This construct contains a 3' self-inactivating long terminal repeat. pLKO-puro is available in two forms. The TRC1-pLKO vector does not contain WPRE (Woodchuck hepatitis B viruspost-transcriptional regulatory element), while the TRC2-pLKO vector does not contain it.

3. pGIPz: this vector contains a TurboGFP expressed by an IRES in tandem with the shRNA. These constructs contain a WPRE.

4. pTripz-inducible shRNAs plasmids with TurboRFP that marks inducible shRNAmir expression

5. Transduction ready particles generated from the above and purchased directly from the suppliers.

Generation and expansion of plasmids carrying construct/gene of interest

cDNAs and shRNA plasmid constructs will be generated by subcloning cDNAs and shRNAs of interest into the empty backbones indicated above.

VECTOR: Recombinant lentiviral and retroviral plasmids will be transformed and amplified in laboratory strains of E. coli after selection with the appropriate antibiotics. Lentiviral and retroviral plasmid DNA will be purified by maxi prep.

HOST: E. coli: laboratory TOP10 chemically competent E. coli will be used for selection and amplification of plasmid DNA.

(ii) Transfection of retrovirus or lentivirus vector into packaging cell line systems to generate virus.

RETROVIRUS

Cell lines such as Phoenix cells (http://www.stanford.edu/group/nolan/retroviral_systems/phx.html) will be used as packaging cell lines. Phoenix, based on the 293T cell line, is a second-generation retrovirus producer line for the generation of helper free ecotropic and amphotropic retroviruses and has stably integrated three plasmids that provide the retrovirus proteins in trans. In order to infect human cell lines we will use the packaging cells Phoenix Ampho. Also, well characterised and commercially available PT67 cells with appropriate tropism would be used if the Phoenix system proves inadequate.

Virus will be packaged by transient transfection.

LENTIVIRUS

Lentivirus will be packaged by co-transfection of lentivirus vector and packaging plasmids into 293T cells.

(iii) Infection, with the packaged retroviral or lentiviral constructs, of human or mouse cell lines.

VECTOR: Replication deficient lentiviral or retroviral transduction particles harbouring construct/gene of interest and appropriate resistance gene or fluorescent marker. These particles can be obtained as indicated above or purchased as 'Ready-to-use lentiviral particles' from certified sources.

HOST: Well characterised human normal and cancer cell lines, mouse cell lines, or human cell lines engineered to express the ecotropic receptor. Successfully transduced cells will be selected with appropriate antibiotics and in the case of inducible of an inducible system activated by doxycycline.

Determination of viral titre in packaging cells and GM-cells supernatant

Generally, we will determine the viral titre of transducing particles suspension only if a specific MOI is required for the infection. We will determine the viral titre in the supernatant of the genetically-modified cells to assess the suitability to transfer infected cells from CL2 to CL1, when CL2 tissue culture has been used for the infection. To determine the viral titre of cell supernatant we will use well characterised methods such as the Molecular Probes' EnzChek® Reverse Transcriptase Assay, a convenient, efficient and inexpensive assay for measuring reverse transcriptase activity, a direct readout of the presence of viral particles

Genes will be genes that are mutated or rearranged in cancers, from human or mouse, and may be in normal form or mutant/rearranged variants of the genes, such as fusion genes. cDNAs-either cloned or synthesised- or shRNAs that target the gene of interest will be cloned into retroviral and lentiviral vectors
HAZARD IDENTIFICATION: Host

MODIFIED BACTERIA TO AMPLIFY THE CONSTRUCTS
Laboratory strains of modified E. coli K12 such as TOP10 are recognised as non-colonising and may be considered to be ACDP hazard group 1.

CELL LINES
Phoenix cells are widely used and they have a history of safe usage. The gag-pol and env genes are expressed from non-moloney promoters to minimise recombination potential. Different promoters for gag-pol and envelope were used to minimise their inter-recombination potential. Amphotropic Phoenix cell lines have been extensively tested for helper virus production and established as being helper-virus free. The 293T cell line, in addition to other well characterised human normal and cancer cell lines, has a history of safe usage and is not expected to represent a hazard to human health.

HAZARD IDENTIFICATION: Vector

RECOMBINANT RETRO/LENTIVIRAL VECTORS
Retroviruses and lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission.

1. The retroviral vectors used are replication defective. To produce a replication-competent retroviral genome three recombination events are required between the vector of interest, the gag-pol regions of the packaging cell line and introduction of the split genome packaging cell lines.

2. We will use third generation lentiviral expression vectors, which contain multiple safety features, as follows;

   • The majority of lentiviral genes have been eliminated (Δ vpr, vif, vpu and nef). The number of genes from HIV-1 that are used in this system has therefore been reduced to three (i.e. gag, pol, and rev).
   • Genes encoding the structural and other components required for packaging the viral genome are separated onto three plasmids. All three plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al J Virology 72 8463-8471 1998).
   • Although the packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gag,pol, rev, env) in the 293T derived producer cell lines, none of them contain 3’ LTRs. This results in elimination of the promoter-enhancer region, which avoids promoter interference issues and further negates the possibility of viral replication.
   • No single plasmid contains all the genes necessary to produce packaged lentivirus. None of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
   • The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.

Some of the indicated vector might contain the WPRE (Woodchuck hepatitis B virus post-transcriptional regulatory element). This element is used to increase lentiviral vector titre and gene expression. It includes the promoter for the X-protein and the part to the coding sequence for such protein, which may have oncogenic properties.

The main hazard arises directly from the recombinant retroviruses and lentiviruses produced from the packaging cells. These yield amphotropic lentivirions and retrovirions.

HAZARD IDENTIFICATION: Insert

Our genetic experiments will include knockdown of known (p53) and potential tumour suppressor genes (e.g. NRG1) and overexpression of known (Ras) and potential oncogenes (e.g. NRG1 again). However, at least 4 different genes should be expressed for tumorigenesis in human tissues (W.C. Hahn et al., Nature 400, 464) and we expect that a contamination of more than 4 virus supernatants would be extremely unlikely.

None of the cell lines used is capable of colonising a healthy individual and we do not expect any of our proposed manipulations to change this. The amphotropic retrovirus and lentivirus would be able to infect human but could not replicate unless the improbable event occurs that it recombines with endogenous retroviruses. Even then, it is
unlikely that expression of a single oncogene or suppression of a tumour suppressor could cause tumours, as multiple genetic alterations (between 4 and 6) are required for inducing tumours, and safeguard mechanisms exist impeding the accumulation of these alterations in the organisms.

HAZARD IDENTIFICATION: Lab Personnel
The principal hazard is gene transfer to human cells when using the human host-range packaged viruses. Attention should be paid to staff awareness of this and to storage and labelling of any stocks. Virus will be removed from the lab Cat II rooms only for storage and kept in designated -80°C freezers according to the agreed guidelines and SOPs in the Hutchinson/MRC Research Centre. Work will therefore be done in:
Class II: production and infection work with lentivirus and amphotropic retrovirus.
Cell lines produced by infection that are virus-free after infection using the methods indicated above will be transferred to Class 1
Class I for work with plasmids in E. coli.

HAZARD IDENTIFICATION ENVIRONMENT
The prokaryotic and eukaryotic cells and cell lines are highly disabled and the possibility of competent virus being generated is negligible. Therefore they should be unable to propagate in the environment. The plasmid vectors are non mobilisable, therefore neither has any possibility of further spread.
The viruses are replication-incompetent and no live virus should reach the environment.
The risk to the environment is therefore effectively zero

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Containment level 2 will be applied to work packaging and infecting with the human host-range viruses.
Infection and virus production of ecotropic retrovirus will be carried out in class 1.
After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will be transferred to class 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

CL2: all solid waste is autoclaved in the building and then incinerated off site by a contractor (Novus).
Liquid waste is disinfected with Virkon to final concentration of 5%; contact time of 30 minutes minimum before disposal to drain.
CL1: Liquid waste is disinfected with Virkon to final concentration of 1%; contact time of 30 minutes minimum before disposal to drain.
Bacterial Plates are autoclaved in the building.
Solid waste (including for example culture flasks, plastic pipettes) is bagged, and incinerated off site by a contactor (Novus).
It is expected that these measures will give effectively 100% killing

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
This is similar to many RAs for using non-replicating lentivirus and retrovirus vectors to express genes and shRNAs in human cell lines in vitro, with Class II for production and infection by human host-range virus vectors. It is quite broadly based as a variety of genes and host cells may be used, but raises no particular issues.

Since the applicant group leader is the BSO (competent person) note that the committee that has considered this risk assessment includes two others with long experience of GM risk assessment. Dr P B, formerly of MRC-LMB, and Dr P J, the previous BSO for this building.

Project Containment

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Project Ref 921/13.1

<table>
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<tr>
<th>Date Ackn'd</th>
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<th>CultureVol</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<tr>
<td>02/07/2013</td>
<td>Immortalisation of mitochondrial patient-derived primary cells and modifying expression levels of nuclear-encoded mitochondrial proteins in mammalian cells</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Tick if notifying a connected programme of work N

Project Additional Information
Purposes of the contained use

We wish to study the role of mitochondrial proteins in human disease. Respiratory chain structural subunits and assembly factors as well as proteins involved in mitochondrial gene expression and maintenance will be our primary subjects. This necessitates the use of human and mouse cells, both established continuous cultures and primary cultures from patients and/or mice. After a certain passage number, primary cells become senescent and stop dividing. To avoid this, it is possible to transform them into immortal cell lines by transfection of the SV40 T Antigen. Moreover, the cells to be analysed often need to undergo genetic modification as part of the research, in particular to allow stable knock-down expression or to overexpress a protein of interest. Lentivirus systems allow highly efficient transfection of the shRNA or cDNA for the gene of interest and its stable integration into the host cell. We will then be able to study the phenotypic and biochemical changes produced by these modifications and their relationship to mitochondrial disease. Lentiviral vectors can be exploited to produce iPSCs from both human and murine primary cultures, by using vectors expressing reprogramming proteins such as Oct-4, Sox2 etc. A further application of lentiviral vectors is the possibility to rapidly produce transgenic mice by injection of high titer virus into the embryos.

Recipient or parental organism

Mammalian cells and cell lines and bacterial cells that are disabled (non-colonising) and unable to survive or propagate outside of laboratory culture, even when transformed.

Host/vector system

The following bacterial strains will be used:
E. coli K12 derivatives such as DH5α or XL-10 gold.

The following mammalian cells will be used:
Human and murine primary fibroblast cells, murine primary cultures such as muscle cells or neurons, or cell lines expressing the SV40 T Antigen, cell lines such as L929, HEK293T (packaging), HeLa, 143B, A549 cells (stably expressing the protein of interest).

The following systems of Lentiviral transduction will be used:
1. pLOX-Ttag-iresTK+ psPAX2 + pMD2.G (Didier Trono lab). This system will be used for immortalisation of primary fibroblasts. The pLOX-Ttag-iresTK lentiviral vector (Tronolab) contains the SV40 Large T antigen and HSV1-TK as inserts. The plasmids have been derived from the HIV-1 genome. HIV lentiviral vectors however have been modified so they are safe to use in research labs. The lentiviruses are replication defective, using separate plasmids encoding various HIV genes and require multiple separate recombination events to generate replicating virions. To further prevent these recombination events, the system includes a large deletion of the 3’ LTR in the transfer plasmid (SIN system).
2. The RNAi Consortium, TRC + pMISSIONgagpol (Sigma) + pMISSIONvsrg (Sigma).
3. pWPXLd (pWPXLd-ires-Puro/Neo) + psPAX2 + pMD2.G (Didier Trono Lab).
All the above systems (including pLOX-Ttag-iresTK) are considered “Second-Generation” because they use one packaging plasmid containing: Gag, Pol, Rev and Tat and one packaging plasmid encoding VSV-G. These vectors contain the woodchuck hepatitis B virus (WHV) post-transcriptional regulatory element (WPRE) to increase viral vector titre and enhance expressions of the transgenes.
4. pLenti6.3/V5-TOPO + pLP1 + pLP2 + pLP/VSVG (Invitrogen).
The pLenti6.3/V5-TOPO+pLP1+pLP2+pLP/VSVG system is considered “Third-Generation” because the use two packaging plasmids pLP1 containing: Gag/Pol and pLP2 containing Rev and one packaging plasmid encoding VSV-G. The proteins to be expressed are not believed to have oncogenic or other pathogenic properties. It also contains the woodchuck hepatitis B virus (WHV) post-transcriptional regulatory element (WPRE).

Origin & function

The genes targeted for overexpression and knockdown are involved in aspects of mitochondrial function including, but not limited to, respiratory chain structural subunits.
and assembly factors as well as proteins involved in mitochondrial gene expression and maintenance. They are not expected to be toxic or oncogenic.

**Evaluation of foreseeable effects**

Mammalian primary cells or cell lines and bacterial cells are unable to survive or propagate outside of laboratory culture.

The genes targeted for over-expression and knockdown are involved in aspects of mitochondrial function and are not expected to be toxic or oncogenic. The SV40 large T antigen which is used to immortalise cell lines, and the reprogramming genes used to produce iPS may have oncogenic properties.

The lentiviral vectors used will be self-inactivating (SIN). A deletion inactivates transcription from the proviral LTR, so reducing the potential for transcriptional activation of cellular genes and also prevents mobilization of any RCL. However as the virus integrates into the host cell DNA there is still a potential for insertional mutagenesis to occur.

The WPRE is similar to the post-transcriptional regulatory element found in human Hepatitis B (HBV), which is closely related to WHV and may have oncogenic properties.

A plasmid encoding the envelope protein from vesicular stomatitis virus envelope protein G (VSV-G) increases the host cell range/tissue tropism and makes it able to infect a variety of human and other mammalian cells, including quiescent cells. Such particles are also physically more stable. The genes or other sequences of interest will be delivered by an integrative process leading to potential long-term expression for the life of the target cell. The results of such expression for most of the sequences will not be severe and will be limited to the cells targeted and no further. VSV-G enables the vector virus to infect a wide variety of mammalian cells. There may also be enhanced environmental stability. However, the viruses are replication disabled; they cannot produce progeny virus and so cannot spread to the wider human population or other animals. The possibility of RCL being generated and released is negligible. The lentiviruses have unstable infectivity and efficient infection generally requires forced contact with the virus and so the risk is extremely low.

None of the modifications will alter the broad properties of the vectors. They will remain replication disabled and so are biologically contained.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All disposable plastics and other solid waste will be autoclaved and incinerated. Culture medium will be treated using 1% Virkon for a minimum of 20' and disposed of via the Containment Level 2 disposal route.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

02/03/2022
Even though the lentivirus can enter human cells the overall risk is low. The SV40 large T Antigen and the reprogramming genes for iPS may have oncogenic properties and therefore the associated work is Class 2. All work with lentiviral vectors using an WPRE, such as the pWPXLd system is Class 2 regardless of the insert. Therefore the final classification is Class 2 (Containment Level 2).

Project Containment

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Project Ref 921/16.1

Date Ackn’d 30/11/2016

Date Project Ceased

CU2 Project Title Production, purification and expression of cDNA using recombinant Adenovirus vector systems

Class 2

CultureVolumeClass3-4 ≤ 1 Litre

Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

We wish to study the role of mitochondrial proteins in cellular function and human disease. The genes of interest are involved in, or thought to be involved in mitochondrial function and include respiratory chain structural subunits, assembly factors, mitochondrial dynamic regulators,
### Recipient or parental organism

Mammalian cells and cell lines and bacterial cells that are disabled (non-colonising) and unable to survive or propagate outside of laboratory culture, even when transformed

### Host/vector system

1. **Vectors:**
   (a) Recombinant Adenovirus:
      (i) Ad Easy Adenoviral vector system (Agilent Technology) Backbone: pADEasy 1, Adenoviral type S dE1/E3 including pShuttle and pShuttle-CMV
   
   Description of the recombinant Adenovirus: Both recombinant Adenoviruses are replication defective by the deletion of the E1 and E3 genes. The E1 gene is essential for the assembly of infectious virus particles and is complemented in vivo by an adenovirus packaging cell line (e.g. AD-293). The E3 gene encodes proteins involved in evading host immunity and is dispensable. Not only do these deletions render the virus incapable of replicating itself, but they also create space for up to 7.5 kb of foreign DNA.

   The plasmid pAdEasy-1, containing most of the human adenovirus serotype S (AdS) genome, is deleted for the genes E1 and E3. The removal of these two viral genes creates space for foreign DNA and eliminates self-replication capabilities. The E1 deletion renders the viruses defective for replication and incapable of producing infectious viral particles in target cells (provided there is no complementation by the host cell); the E3 region encodes proteins involved in evading host immunity and is dispensable. The deletion of both genes creates room for up to 7.5 kb of foreign DNA that can be inserted into the AdS genome. The E1 gene, which is necessary for production of viral particles, is provided in trans by AD-293 cells. pAdEasy-1 carries the ampicillin resistance gene, which is lost after recombination with a shuttle vector. The vector pShuttle-CMV contains a multiple cloning site sandwiched between the CMV promoter and the SV40 polyadenylation signal and is suitable for insertion of a large cDNA (up to 6.6 kb). pShuttle contains only a multiple cloning site. This allows for the insertion of an entire expression cassette, including specialized promoters and termination signals (up to 7.5kb). The regions indicated as arms are the stretches of sequence homology with pAdEasy-1 where the homologous recombination occurs. The R-ITR and L-ITR regions are short inverted terminal repeats (Left and Right) which have a role in replication of the viral DNA.

2. **Hosts for amplification and production**
   (a) Bacterial strains: E.coli K12 derivatives such as XL 10 Gold, BJ5183 (Addgene)
   (b) Mammalian cell lines: Established human cell lines such as HEK293 and AD-293 (Agilent)

3. **Hosts for infection and experiments**
   Classical mammalian cell lines: HeLa, Cos-7, mouse and human fibroblast

### Origin & function

Inserted cDNA coding for human proteins involved in mitochondrial function including respiratory chain structural subunits, assembly factors, mitochondrial dynamic regulators, mitochondrial and ER markers, as well as proteins involved in mitochondrial gene expression and maintenance mitochondrial dynamics and functions will be used. None of these proteins are known to be biological toxins, oncogenes or prion-type agents.

### Evaluation of foreseeable effects

The recombinant virus elements used are replication deficient due to deletions in the E1 and E3 regions. The E1 gene is essential for the assembly of infectious virus particles and is complemented in vivo (in trans) by an adenovirus packaging cell line (e.g. AD-293). The E3 gene encodes proteins involved in evading host immunity and is dispensable. These deletions render the virus incapable of replicating itself. Since the mammalian cells used possess...
integrated human AdS DNA, there is a low frequency of homologous recombination between the E1-deleted vector and the host DNA resulting in the production of some replication competent adenovirus (RCA). The frequency of occurrence is very low, but the percentage of RCA in a given virus stock goes up with each amplification of that stock. Because the recombinant adenovirus remains epichromosomal in the human host cell, there is only a remote possibility of activation or inactivation of host cell genes resulting from interruption by the transfected gene(s). Infection of bacterial cells with the recombinant Adenovirus elements presents no risk for human health and environment since at this stage the virus is still deleted of the E1 and E3 regions, and is not replicative. Following transfection, production and amplification of Adenovirus in AD-293 or HEK293 are considered as Hazard Group2/Containment Level 2: since cells possess integrated human AdS DNA, there is a low frequency of homologous recombination between the E1-deleted vector and the host DNA resulting in the production of some replication competent adenovirus (RCA). The frequency of occurrence is very low, but the percentage of RCA in a given virus stock goes up with each amplification of that stock. Therefore there is a potential risk to the worker at this stage. Adenoviridae are classified as Hazard Group 2 by the ACDP. Wild type, replication competent adenoviruses provoke cold symptoms and strong immune responses in healthy individuals and generally do not cause serious illness.

The mammalian cells infected for the experiements are established cell lines with history of safe use and could be classified as Hazard Group 1. These cells do not contain the E1 and E3 regions required for the virus replication. Infection in those cells and the corresponding experiments are classified as Class2/Containment Level 2 due to Adenovirus infection; whilst the Adenovirus within these cells is uncoated therefore non-infectious, the virus in the supernatent may be infectious for some time. However, after at least one passsage, the supernatent will be discarded and the cells washed to eliminate all the viral particules. As the uncoated Adenovirus inside the cells is not infectious the subsequent experiments will be considered as Class1/Containment Level 1. None of the modifications will alter the broad properties of the vectors. They will remain replication disabled and so are and unable to propagate in the environment. For the RCA, Adenoviruses are generally species specific; AdS infects humans and does not naturally infect other animals (but can experimentally infect cotton tail rats, which are not endogenous in the UK). Therefore there is no risk to other organisms. The host cell lines, even when transformed, are highly disabled and unable to propagate in the environment. The overall risk to the environment is therefore effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All wastes generated will be disposed of according to the MBU CL2 Code of Practice. Disposable plastics and other solid waste will be autoclaved and incinerated. Culture medium will be treated using 10f0Virkon for a minimum of 20' and disposed of via the Containment Level 2 disposal route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

02/03/2022
Class 1 for cloning stages involving recombinant viral vectors and experiments on classical mammalian cell lines after removal of the infected supernatant and cells washes. Class 2 for amplification and production of viral construct following transfection in HEK 293 and AD293 cells specifically. Class 2 for infection of mammalian cell lines, E1 and E3 defective.

Please enter comments on the GM safety committee on the risk assessment

Class 1 for cloning stages involving recombinant viral vectors and experiments on classical mammalian cell lines after removal of the infected supernatant and cells washes. Class 2 for amplification and production of viral construct following transfection in HEK 293 and AD293 cells specifically. Class 2 for infection of mammalian cell lines, E1 and E3 defective.

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GM Centre Number: 922

Data Premises Notified: 12/08/2005
(Originally)

Data Premises Closed: 30/09/2007

Transferred from 1992 Regs?: N

Emergency Plan Required?:

Transitional Premises Notified

Transitional Premises Closed

Transitional Premises Withdrawn: N

Name

REOX LIMITED

Name 2

Department

Campus Estate or Research Centre

OXFORD SCIENCE PARK

Building

THE MAGDALEN CENTRE

Road Name

District

Town

Oxford

County

OXFORDSHIRE

Postcode

OX4 4GA

Country

ENGLAND

Tel Number

01865 784422

Fax Number

01865 784425

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

Date: 07/04/2010
Name: ReOx Limited
Department: ReOx Limited
Address: OXFORD SCIENCE PARK
Building: THE MAGDALEN CENTRE
Town: OXFORD
County: OXFORDSHIRE
Postcode: OX4 4GA
Country: ENGLAND
Withdrawn: N

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GMSC shall consist of the Research Director, Director of Biochemistry and the Senior Scientist. Between them they have a lot of essential experience over a minimum of 20 years.

Laboratory
Level 1 (GMMs) Yes
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Animal Unit
Level 1 (GMMs) Yes
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Growth Room
Level 1 (GMMs) Yes
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Glass House
Level 1 (GMMs) Yes
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Large Scale
Level 1 (GMMs) Yes
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

Bacteriology Yes
Parasitology
Transgenic Birds
Microbiology Research Yes

Virology Transgenic Animals
Transgenic Fish
Gene Therapy

02/03/2022
Contaminated disposable plastics will be collected in designated biohazard waste sacks, which will be autoclaved (in a validated disposal cycle). Non-disposable items will be autoclaved as above and re-used. Waste growth media will be treated with Virkon (disinfectant) according to the manufacturer's instructions before disposal into drains. Bacterial cells will be lysed with sonication and the resulting cell debris treated with Virkon as above, or treated whole with Virkon. No viable GMMs will remain at the end of this treatment.

<table>
<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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</thead>
<tbody>
<tr>
<td>Other(s)</td>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

The risk assessment seeks to identify those strains, vectors and insertsto be used during the designated project and to identify any potential hazards. This includes hazards to both human health and safety, but also environmental consequences. In the context of this particular risk assessment there is negligible risk associated with a total breach of containment and consequently the project can be designated class 1.
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<thead>
<tr>
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<th>LUX BIOTECHNOLOGY LIMITED</th>
</tr>
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<tbody>
<tr>
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</table>
# Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

- Senior Scientist - oversees lab procedures. Responsible for health and safety. Involved in projects using GMOs.
- Director - involved in projects using GMOs.
- Lab technician - day to day running of lab, involved in projects using GMOs
- CEO - oversees LUX procedures.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Invertebrates</td>
<td>Plants</td>
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</table>

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity
Containment and control measures:
Fungal cultures shall not leave the laboratory (except as waste in dedicated sealed containers)
Lab coats used in the laboratory shall not leave the laboratory.
A record shall be kept of every culture generated and shall include information on:
Location, number, vial type, organism, strain type (including donor vectors), date of generation, expiry date and date of disposal (by autoclaving).
New and existing staff be training in necessary procedures.
Records shall be monitored by the Senior Scientist.
Waste:
Disposable plasticware will be used wherever possible. Non sharps waste (including gloves and cleaning materials) will be retained in autoclave bags in dedicated waste bins. These shall be autoclaved on site in sealed containers, in a licensed autoclave facility. Lab coats shall be autoclaved before cleaning. GMM Sharps waste and toxic waste will be disposed on through a licensed waste disposal company.
Spillages of biological nature are to be cleared with Virkon (5%). Safety cabinet and benches are to be wiped down with Virkon/70% suitable alcohol eg ethanol at the end of each day. Gloves and cleaning materials to be discarded to autoclave waste bins.
The GMM strains will not remain viable (100% kill) after autoclaving. Indicator tape shall be used to ensure the autoclave is performing as required. Autoclaved material shall then be disposed of through normal domestic channels.
The autoclaving process shall be described in a standard operating procedure to ensure it is carried out in a reproducible manner.
Autoclaving shall be carried out within 10 days of waste being generated to minimise the risk of container breakage and contamination.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Health and Safety training to be given to new and existing employees. This is to include:
1. Location of first aid equipment eg eye wash, fire-fighting equipment, Risk assessment forms, COSHH information and safety procedures (eg dealing with spillages).
2. Filling in and use of risk assessment forms.
3. Record keeping of GM strains including generation and disposal of material.
Generation of fungal biosensors. This involves expressing marker proteins eg fluorescent and luminescent proteins in a range of wild type and mutant (deleterious mutations such as those used for DNA repair or oxidative stress) …

Recipient organisms:
E. coli strain XL1 (Stratagene). (non-pathogenic). Class 1 organism.
Neurospora crassa, Aspergillus nidulans, Aspergillus niger, Magnaporthe grisea etc. wild type and mutant (deleterious mutations) strains (obtained from Fungal genetics stock Centre). Spores and mycelia to be transformed.

Vector:
Based on BlueScript (Stratagene)
Commercially sensitive.
Contain luminescent and fluorescent proteins obtained from deep sea organisms under the control of various fungal promoters.

Genetic material:
Fluorescent and luminescent proteins. Derived from marine coelenterates (non-pathogenic). No homology to known human genes.
A variety of promoters, mainly isolated from filamentous fungi. Including constitutive and inducible promoters.

Selection systems:
Bacteria: ampicillin, chloramphenicol, kanamycin
Fungi: hygromycin, and auxotroph selection

Cloning protocol:
Digestion of insert from commercially available vectors.
PCR amplification of above inserts
Insertion of PCR fragment of new vector, containing appropriate fungal selection (eg hygromycin/auxotrophic selection) and fungal promoters.
Transformation.

Note, these experiments are small scale <100ml

Foreseeable effects:
No hazards associated with insertion of gene. None of the genes are involved in pathogenesis and instead act as luminescent of fluorescent markers for assessment of promoter-driver gene expression.

No change from non-pathogenic to pathogenic state or increased virulence is expected.

The genes inserted pose no danger to health and in the unlikely event of transmission to other organism would not be expected to cause damage or health/environmental dangers.

It is HIGHLY unlikely that the GMM could cause human infection.

The fitness of GMM is likely to be comparable, or slightly reduced compared to unmodified strains.

No consequences for the environment following accidental release of modified XL1 bacteria are forseen. Previous genetic manipulations of N. crassa and other fungi tend to show some reduction in fitness compared to wild-type.

Host/vector system
Using E. coli to generate plasmids.
Transformation of fungi using an electroporation or protoplasting protocol.

Origin & function
E. coli strains: commonly used in molecular work. Obtained from commercial company eg Stratagene or Invitrogen. For use only in lab, not for general release.

Fungi are to be transformed with fluorescent and luminescent markers. These are derived from marine coelenterates (non-pathogenic). Most are commercially available from Nanolight (www.nanolight.com). They have no homology to known human genes.

Transformed fungal strains shall be used in laboratory-based assays for drug screening and environmental testing. They are NOT for general release.

Evaluation of foreseeable effects
Neurospora crassa, Aspergillus nidulans and A. niger are saprotrophic (non-pathogenic) organism. No cases of pathogenic behaviour of unmodified or modified N. crassa reported. Please see article 'Evidence for Safety of Neurospora species for Academic and Commercial Uses' (Perkins and Davis, 2000, Applied and Environmental Microbiology) for more details.

Antibiotic reporter genes used in vectors: ampicillin, kanamycin, hygromycin and chloramphenicol. Safety procedures will be followed when using such antibiotics eg gloves, mask and goggles. Used in microbiological safety cabinet level 1.

No hazards associated with insertion of gene. None of the genes are involved in pathogenesis and instead act as luminescent of fluorescent markers for assessment of promoter-driver gene expression.
No charge from non-pathogenic to pathogenic state expected.

The gene inserted pose no danger to health and in the unlikely event of transmission to other organism would not be expected to cause damage or health/environmental dangers.

It is highly unlikely that the GMM could cause human infection.

The fitness of the GMM is likely to be comparable, or slightly reduced compared to unmodified strains.

No consequences for the environment following accidental release of modified XL1 bacteria N. crassa or Aspergillus strains are foreseen. Previous genetic manipulations of fungi tend to show some reduction in fitness compared to wild-type.

Health and Safety
First aid kits, eye wash and fire-fighting equipment provided.
Risk assessment forms, for each procedure undertaken in lab are available in the lab and contain information regarding appropriate steps following spillages and for correct wast disposal.

Signs for information on coping with spillages and contact details are provided.

Labs are locked when not in use and signs forbidding unauthorised entry are clearly visible, to prevent unauthorised personnel entering labs.
All members of staff are trained in working with GMMs and general lab safety.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment and control measures:
Fungal cultures shall not leave the laboratory (except as waste in dedicated sealed containers)
Lab coats used in laboratory shall not leave the laboratory
A record shall be kept of every culture generated and shall include information on: location, number, vial type, organism, strain type (including donor vectors), date of generation, expiry date and date of disposal (by autoclaving).

Records shall be monitored by Senior Scientist.

Waste:
Disposable plasticware will be used wherever possible. Non sharps waste (including gloves and cleaning materials) will be retained in autoclave bags in dedicated waste bins. These shall be autoclaved on site (in sealed containers), 121°C> 15 min. Lab coats shall be autoclaved before cleaning. GMM sharps waste and toxic waste will be disposed of through a licensed waste disposal company.

Spillages of biological nature to be cleared with Virkon (5%). Safety cabinet and benches to be wiped down with Virkon/70% ethanol at the end of each day. Gloves and cleaning materials to be discarded to autoclave waste bins.
The GMM strains will not remain viable (effectively 100% kill) after autoclaving. The autoclave shall be serviced annually and indicator tape shall be used to ensure the autoclave is performing as required. Autoclaved material shall then be disposed of through normal domestic channels.

The autoclaving process shall be described in a standard operating procedure to ensure it is carried out in a reproducible manner. Autoclaving shall be carried out within 10 days of waste being generated to minimise the risk of container breakage and contamination.

Please enter comments on the GM safety committee on the risk assessment

Health and Safety training to be given to new and existing employees. This is to include:
1. Location of first aid equipment eg eye wash, fire-fighting equipment, Risk assessment forms, COSHH information and safety procedures (eg dealing with spillages)
2. Filling in and use of risk assessment forms.
3. Record keeping of GM strains including generation and disposal of material.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<td>Emergency Plan Required?</td>
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**Name**

INION LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

UNIT 9B CAMBRIDGE SCIENCE PARK

**Road Name**

MILTON ROAD

**Town**

Cambridge

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 OFG

**Country**

ENGLAND

**Tel Number**

01223 394 200

**Fax Number**

01223 394 210

**E-mail**

HSE Division

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Chair - Senior Scientist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Scientist</td>
</tr>
<tr>
<td>External Health and Safety Adviser</td>
</tr>
<tr>
<td>Vice president of research and development</td>
</tr>
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</table>

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Other (please specify) |

Tick if confidential

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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity
Solid waste will be placed into autoclave bags within dedicated biological waste bins in the laboratory. These bags will be closed with autoclave indicator tape before being transported to the autoclave for sterilisation.

To decontaminate equipment and liquid waste precept will be used at 1,000 pm for 20 minutes. Following decontamination liquid waste will be disposed of via a sink. Glass-ware will be cleaned via the normal washing procedure.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC reviewed the risk assessment and found it satisfactory.
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<thead>
<tr>
<th>Field</th>
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Data Premises Notified: 07/09/2005 (Originally)
Transferred from 1992 Regs?: N
Transitional Premises Class: N
Data Premises Closed: N
Non-GMMs: N
Withdrawn: N
Emergency Plan Required?: N
Transitional Premises: N

Date at Which Additional Info Submitted: 02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee (GMSC) consists of all competent personnel directly involved with the company (currently 3 people). All three members of the Helperby Therapeutics GMSC have at least 10 years experience in molecular biology/microbiology and have a track record in handling genetically modified organisms. One member of the Helperby GMSC was previously a member of the GMSC of St George’s, University of London. The Helperby Therapeutics GMSC will convene whenever a new project needs to be reviewed or changes to a current project will occur.

Helperby Therapeutics Limited is currently renting its laboratory space from St George’s, University of London. Therefore all risk assessments regarding the work with genetically modified organisms will also be presented to the GMSC of St George’s University of London for their evaluation.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

02/03/2022
Helperby Therapeutics rents its laboratories from St George's University of London within the Medical Microbiology Department. As part of this agreement Helperby therapeutics has access to the waste disposal facilities of St George's University of London.

All biological waste will be autoclaved prior to disposal.

When autoclaving is not feasible the biological material will be inactivated by soaking the material in 2% hycolin for at least 24 h (>5.29 log10 reduction after 5 min contact time according to the manufacturer validation procedure).

Laboratory equipment that cannot be autoclaved will be disinfected using Vikan sanitising agent according to the manufacturers recommendations.

All other laboratory waste will be disposed of through the clinical waste route within St. George's University of London.

---

Please enter comments of the GM safety committee on the risk assessment

The GMSC did not have any specific comments regarding the risk assessment for the project entitled "E. coli strains expressing proteins of the chaperonin regulon of M. tuberculosis and M. leprae". The GMSC was satisfied with the information provided and approved of the project and its classification as class 1.
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**Name**

ALGENTECH LTD

**Name 2**

INSTITUTE OF FOOD RESEARCH, ROOM NO.317

**Department**

IFR2

**Campus Estate or Research Centre**

NORWICH RESEARCH PARK

**Building**

**Road Name**

COLNEY LANE

**District**

**Town**

NORWICH

**County**

NORFOLK

**Postcode**

NR4 7UA

**Country**

ENGLAND

**Tel Number**

44 1603 251 426

**Fax Number**

44 1603 501 088

**HSE Division**

EAST AND SOUTH EAST

**Comments**

GM Centre found to have ceased trading in 2010

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: Y
- **Give brief details of the genetic modification safety committee**:
  
  The Genetic Modification Safety Committee (GMSC) includes Managing Director, a Biological Safety Officers and all principal Investigators. One annual general meeting is held to review and update the safety procedures. Additional meetings will be held for reviewing of risk assessments for new projects related to GMO.

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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- **Bacteriology**: Yes
- **Parasitology**: Yes
- **Transgenic Birds**: Yes
- **Microbiology Research**: Yes
- **Microbiology**: Yes
- **Transgenic Animals**: Yes
- **Transgenic Fish**: Yes
- **Gene Therapy**: Yes

02/03/2022
Waste disposal procedure at the Institute of Food Research (IFR) site

1. Introduction

This code of practice (COP) covers the disposal of biological waste at Algentech Ltd in accordance with Institute of Food Research general procedure. Biological waste is defined here as that contaminated with micro-organisms (ACDP Hazard Groups 1 and 2 and ACGM Genetically Manipulated (GM) Organism Classes I and II, mammalian cells, human body fluids/wastes or transgenic plant material. All such waste will be inactivated before final disposal, i.e. release to the environment. The inactivation procedure will be one (or more) of chemical treatment, autoclaving or incineration.

2. Inactivation by autoclaving

Waste in re-usable items such as glass bottles will be placed in the stainless steel containers available from the Media Laboratory of the Institute of Food Research. These containers will be labelled with the number of the room generating the waste, and will be collected by support staff for autoclaving.
Non-reuseable solid or semi-solid wastes (e.g. Petri dish culture) will be put in a transparent/blue autoclave bag (the recognised colour coding for biohazard waste for autoclaving), and contained within a white polypropylene bucket. Care will be taken that no sharp items that might penetrate the bags are included. Such items will be placed in a dedicated ‘sharps’ container in a separate autoclave bag. Buckets will be labelled on a piece of tape with the number of the room generating the waste, and the lid clipped on to the bucket ready for collection. Buckets will be routinely collected from the laboratory by support staff. After sterilisation, waste in autoclave bags will be further bagged by the support staff using yellow incinerator bags (see below), and dispatched for incineration.

Note that other safety requirements, such as COSHH, will also be met.

3. Chemical inactivation

Chemical inactivation will use an approved disinfectant such as alcohol at concentration 70-85% for 10-30 minutes, VIRCON at concentration 1% for 10 minutes, and chlorine compounds like bleach or hypochlorite at the concentrations 5-10% for 15-20 minutes. Although chemically-inactivated waste no longer presents a biological hazard, other requirements of COSHH will be met when considering further disposal.

4. Inactivation by incineration

Waste for incineration will be double-bagged and the outer bag will be yellow (The recognised colour coding for biohazard waste for incineration). Sharp items will be placed in a dedicated ‘sharps’ container in a separate bag. Bags will be sealed with tape, and will then be routinely collected from the laboratory by support staff. Bags of waste for incineration will be transported by a licensed contractor in secondary containment appropriate for transport on public roads.

Note that other safety requirements, such as COSHH will also be sent.

5. Transgenic plant material

As noted in 1 above, in general, waste containing genetically modified material will be inactivated prior to leaving the laboratory site. However, as an exception, genetically modified plant waste originating from IFR glasshouses, and which IFR Genetic Manipulation Safety Committees consider to be Containment Level A or B, will be sent off-site direct for incineration without prior inactivation, via an approved contractor, using yellow bags held inside rigid transport bins.

6. General Administration

All waste bins will be clearly labelled. All staff (particularly visiting workers) will be instructed on the disposal procedures for biological waste, particularly the differences between biological waste for autoclaving and ordinary non-hazardous waste e.g. office materials (black bags).

Waste Disposal Procedure at the John Innes Centre (JIC) Site.

Only genetically modified plant waste originating from JIC glasshouses, and which JIC Genetic Manipulation Safety Committees consider to be Containment Level A or B, will be disposed at JIC site. All JIC genetically modified plant waste will be autoclaved and subsequently composted on the site in accordance with JIC Safety Procedure.
More details on genes and bacterial strains will be used for research should be introduced;

Nicotiana spp have a commercial crop in the UK as ornamental varieties, and this issue should be taken in consideration.

The final version of the risk assessment was modified in accordance with comments and was accepted as adequate for the project.

---

### Project Additional Information

**Disarmed Ti plasmid derivative vectors SLJ, pBIN19 or pGreen/pSoup and strains GV3101, AGL1 and LBA4404 of Agrobacterium tumefaciens will be used for infiltration into leaves of Arabidopsis, N.tobacum, N.benthamiana, Lycopersicon spp., and Solanum spp, as a mean of transient assays for gene targeting. The transient assay will be performed to assess efficiency of gene targeting for visual marker genes like GUS and GFP, with subsequent experiments to target endogenous plant genes.**

Dipping procedure for Arabidopsis transformation will be performed to generate stable transformants with selectable markers like NPTI or NPTII for kanamycin resistance, bar for BASTA herbicide resistance, hyg gene for hygromycin resistance and aadA gene for spectinomycin resistance, and visual markers like GUS and GFP. Derivatives of plant endogenous plant genes will be used to induce gene targeting.

**Recipient or parental organism**

As plant expression vectors and constructs these vectors would not cause a human health hazard. Agrobacterium strains, Arabidopsis, Nicotiana spp., Lycopersicon spp.,...
and Solanum spp. Plants would survive in the environment but none are likely to cause damage to the environment, and neither disarmed Agrobacterium, or transgenic Arabidopsis, Nicotiana spp.; Lycoperscion spp., and Solanum spp. Would cause disease. Infiltrated plants containing high concentrations of Agrobacterium will be held in a category B greenhouse to prevent escape. The Arabidopsis plants could cross with naturally occurring plants, although the level of cross-fertilisation in this species is extremely low, especially out of controlled conditions. Therefore no harmful effects are foreseen.

**Host/vector system**

Wild type or transgenic plants of Arabidopsis, tobacco, tomato or potato with visual marker genes (GUS, GFP) will be used to assess gene targeting in transient assays or stable transformants.

Agrobacterium strains GV3101, AGL1 and LBA4404 will be used for plant transformations or infiltrations.

Disarmed vectors pBIN19, pGreen/pSoup, SLJ and pRK290 derivatives will be used for delivery of transgenes into plants.

**Origin & function**

The majority of genes used for transformation have bacterial origin. They are NPTI, NPTII for kanamycin resistance, addA gene for spectinomycin resistance, bar for BASTA resistance, hyg for hygromycin resistance and uidA (GUS) as a visual marker. GFP is an eukaryotic gene from jellyfish and is used as a visual markers. Plant endogenous genes like maize Spm transposable element and tabacco Tnt1 retrotransposon will be used to facilitate gene targeting in plants. Disarmed vectors pBIN19, pGreen/pSoup, SLJ generated at the John Innes Centre and Sainsbury Laboratory, as well as pRK290 derivatives will be used for delivery of transgenes into plants.

**Evaluation of foreseeable effects**

Arabidopsis: this is a small short-lived species of no economic importance, either as a weed or crop and of limited environmental significance (being primarily a weed of waste ground). It does not cross breed with any crop species and there are no known bridging species. Therefore, even in the event of an escape, the GMO is highly unlikely to present an environmental threat.

Nicotiana species: The Nicotiana species used (tabacum, benthamiana) are not native species in the UK. However Nicotiana spp are used as ornamental varieties in the UK. We are unaware of any such commercial activities in the local area. In the event of an escape, GM Nicotiana species are unlikely to overwinter and survive more than one growing season.

Lycoperscion species: GM Lycopersicon species are unlikely to overwinter in the UK. All fruit is bagged in the glasshouse and processed within the laboratory, so seed escape to the environment is highly unlikely.

Potato: Solanum species: a major local crop but does not generally propagate itself by seed in the UK, so escape by pollen or seed is highly unlikely. All plant material is destroyed by autoclaving, so tuber escape is also highly unlikely.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Genetically modified plants (GMP) of Arabidopsis are produced either in culture by co-cultivation with Agrobacterium or by particle bombardment or by dipping flowering plants into Agrobacterium cultures. Where Agrobacterium is used in co-cultivation experiments, as for Nicotiana spp., Lycoperscion spp. And Solanum spp., the GMM will be removed using antibiotics before planting out in Cat A greenhouses. Where dipping is used, dipped plants will be contained within Cat B glasshouses. Theses glasshouses facilities are designed to contain microbes and plant pathogens and operate under strict MAFF DEFRA approved guidelines. Briefly, access is limited by an ID card-operated double door containing foot dips, biological waste leaves by a double-doored autoclave, waste water is treated by a verified chemical treatment before release into the waste water system, the entire facility operates at positive pressure to prevent insect entry (this is supplemented by a rigorous control program) and air is filtered on release.

Transgenic plants that are free of GMMs may be grown in Cat A or B glasshouses. These have insect screens in place over vents and doors and have a systematic insect...
control program to prevent pollen dispersal. Where pollen or seed dispersal may provide an escape route for the GM plant, plants are either prevented from flowering or are bagged to contain the seed. Greenhouses are surrounded by a gravel strip that is sprayed on a regular basis with a broad spectrum herbicide.

As IFR has a limited space in greenhouse facilities transgenic or wild type plants will be grown for generation of leaf biomass only which will be subsequently used for molecular biological analyses. The Agrobacterium infiltration or dipping of plants for transformation will be performed at JIC greenhouse facilities.

We consider that our procedural and cultural precautions will minimise the risk of GM escape into the environment and that level 1 containment is sufficient to reduce the hazard to effectively zero.

Genetically modified Arabidopsis
Plants will be transformed by infiltration with Agrobacterium and grown in containment level B glasshouse. Seeds will be collected in glasshouse, double contained in the bags before transferring to the laboratory and stored in the locked up laboratory. Remaining plant material between glasshouses and laboratory will be controlled and limited as far as possible. The transgenic material will be double contained in bags and transported in rigid container. The progeny of these plants will be selected with antibiotics under sterile conditions in the laboratory before transfer to a glasshouse or growth chambers at containment level B as appropriate. Alternatively the progeny plants will be grown under containment level B and selected with a herbicide such as BASTA. After harvesting plant material will be autoclaved. In category B conditions disinfectant foot dip is provided for workers entering and leaving the glasshouse. Arabidopsis is predominately self-fertilising. This, together with the strict control of insect pests maintained in the glasshouse restricts the possibility of sexual transfer of novel genetic material. All matting and plant material is autoclaved after use.

Genetically modified Nicotiana species.
Only ornamental varieties of Nicotiana species are grown in the UK. Normal Cat. A glasshouse practice (bagging of flowering plants) is considered sufficient to contain any seed that might be produced.

Genetically modified Solanum and Lycopersicon species.
Transgenic plants of these species will be produced in tissue culture within the laboratory and mature plants grown under Cat. A containment. Plants will be grown in screened glasshouses (to prevent insect transfer of pollen). For Solanum spp. Flowers will be removed or bagged if seed set seems likely. For Lycopersicon spp. All fruit will be bagged and is processed within the laboratory. All material will be autoclaved after experiments are finished. Cat A glasshouses are considered sufficient to contain all risk.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Disposal procedure at the Institute of Food Reasearch (IFR) site.
1. Introduction
This code of practice (COP) covers the disposal of biological waste at Algentech Ltd in accordance with Institute of Food Research general procedure. Biological waste is defined here as that contaminated with micro-organisms (ACDP Hazard Groups 1 and 2 and ACGM Genetically Manipulated (GM) Organisms Classes I and II), mammalian cells, human body fluids/wastes or transgenic plant material.
All such waste will be inactivated before final disposal, i.e. release to the environment. The inactivation procedure will be one (or more) of chemical treatment, autoclaving or incineration.

2. Inactivation by autoclaving
Waste in re-usable items such as glass bottles will be placed in the stainless steel containers available from the Media Laboratory of the Institute of Food Research. These
containers will be labelled with the number of the room generating the waste, and will be collected by support staff for autoclaving.

Non-re-usable solid or semi-solid wastes (e.g. Petri dish cultures) will be put in a transparent/blue autoclave bag (the recognised colour coding for biohazard waste for autoclaving), and contained within a white polypropylene bucket. Care will be taken that no sharp items that might penetrate the bags are included. Such items will be placed in a dedicated 'sharps' container in a separate autoclave bag. Buckets will be labelled on a piece of tape with the number of the room generating the waste, and the lid clipped on to the bucket ready for collection. Buckets will be routinely collected from the laboratory by support staff. After sterilisation, waste in autoclave bags will be further bagged by the support staff using yellow incinerator bags (see below), and dispatched for incineration.

Note that other safety requirements, such as COSHH, will also be met.

3. Chemical inactivation

Chemical inactivation will use an approved disinfectant such as alcohol at concentration 70-85% for 10-30 minutes, VIRCON at concentration 1% for 10 minutes, and chlorine compounds like bleach or hypochlorite at the concentrations 5-10% for 15-20 minutes. Although chemically-inactivated waste no longer presents a biological hazard, other requirements of COSHH will be met when considering further disposal.

4. Inactivation by incineration

Waste for incineration will be double-bagged and the outer bag will be yellow (the recognised colour coding for biohazard waste for incineration). Sharp items will be placed in a dedicated 'sharps' container in a separate bag. Bags will be sealed with tape, and will then be routinely collected from the laboratory by support staff. Bags of waste for incineration will be transported by a licensed contractor in secondary containment appropriate for transport on public roads.

Note that other safety requirement, such as COSHH, will also be met.

5. Transgenic plant material

As noted in 1 above, in general, waste containing genetically modified material will be inactivated prior to leaving the laboratory site. However, as an exception, genetically modified plant waste originating only from IFR glasshouses, and which IFR Genetic Manipulation Safety Committee consider to be Containment Level A or B, will be sent off-site direct for incineration without prior inactivation, via an approved contractor, using yellow bags held inside rigid transport bins.

6. General Administration

All waste bins will be clearly labelled. All staff (particularly visiting workers) will be instructed on the disposal procedures for biological waste, particularly the differences between biological waste for autoclaving and ordinary non-hazardous waste e.g. office material (black bags).

Waste Disposal procedure at the John Innes Centre (JIC) Site.

Only genetically modified plant waste originating from JIC glasshouses, and which JIC Genetic Manipulation Safety Committee consider to be Containment Level A or B, will be disposed at JIC site. All JIC genetically modified plant waste will be autoclaved and subsequently composted on the site in accordance with JIC Safety Procedure.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
More details on genes and bacterial strains which will be used for research should be introduced;
Nicotiana spp have a commercial crop in the UK as ornamental varieties, and this issue should be taken in consideration.
The final version of the risk assessment was modified in accordance with comments and was accepted as adequate for the project.

### Project Containment

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<th>Growth Rooms</th>
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### Project Additional Information

**Purposes of the contained use**
Potex and Gemini - viruses will be used as vectors for expression of plant and bacterial genes in dicot and monocot plants.
The following geminivirus will be used: members of the Begomovirus (Tomato Golden Mosaic Virus, TGMV), Curtovirus (Beet Curly Top Virus, BCTV) and Mastrevirus (Maize Streak Virus, MSV; Wheat Dwarf Virus, WDV) genera.

The following potexvirus will be used: Potato Virus X (PVX).

Recipient or parental organism

Studies will use a range of plant hosts including Nicotiana ssp. Arabidopsis, Tomato, Maize, Barley, Rice and Wheat. E.Coli strains DH5a, DH10B and SCS110 will be used for cloning viral vector sequences into disarmed Ti plasmid derivatives (pRK290, pBIN19, Bin 400 and pGreen/pSoup derivatives).

The Agrobacterium strains GV3101, AGL1 and LBA4404 will be used to inoculate plants or cultured explants for transient and stable expression of virus derivatives.

Host/vector system

The investigation will include the construction of virus derivatives for plant inoculation and transformation:

1. Full length double stranded (dsDNA) derivatives of PVX, BCTV, TGMV, MSV, WDV and part thereof.
2. Recombinant viruses for expression of cloned genes from plants and bacteria (aadA for spectinomycin resistance; visual marker genes uidA (GUS), GFP, luciferase, amturase genes and intron sequences).

None of the genes to be used will be associated with toxic effects or genes known to affect virus virulence, and if such genes become of interest we will submit a separate or modified proposal, as appropriate.

Origin & function

Potential scenarios include the construction of virus derivatives for plant inoculation and transformation:

1. Geminiviruses:

With the exception of horticulturally desirable ornamentals that are propagated vegetatively (e.g. honeysuckle, abutilon), geminivirus diseases do not occur in the UK. All geminiviruses are transmitted by either whitefly, leaphopper or treehopper vectors. These vectors do not occur in the UK, hence the viruses could not become established in the environment even if released. Some begomoviruses are mechanically transmissable but no accidental transmission between adjacent touching plants has been observed under glasshouse conditions. In addition, the viruses do not gain access to the germ - line and hence are not seed transmissable.

-GM Geminiviruses: no harmful effects should occur because neither the wild-type virus nor the genetically modified viruses would be able to survive if released. In the worst case, it is conceivable that recombination could occur between viral DNA and plant chromosomal DNA, but as the viruses are unable to gain access to the germ line, such events would be limited to individual cells of infected plants and would be transmitted vertically. In the event of pollen release from transgenic plants, the possibility of recombination between a geminiviral transgene and an endemic virus is considered to be extremely remote (no such events have been reported).

2. Potexvirus (Potato Virus X):

PVX is transmitted mechanically. It has a narrow host range infecting potato, tomato, Brassica campestris and Nicotiana species. Experiments will be carried out with an indigenous UK isolate that is normally causing mild symptoms in infected plants. PVX is not seed transmissable. There is no known vector for transmission of PVX. We intend to use mainly sequences from the chloroplast and mitochondrial genome, these are not known to affect the virulence of PVX, and will minimise the risk of producing a more virulent form of the virus.

The most severe consequence of survival, establishment and dissemination would be the mild disease caused by PVX in potato and tomato. Given the use of containment facilities and the known fact that PVX is not transmitted by invertebrate vectors or true seed there is only low likelihood of hazard from these experiments.

3. The virus and host plants used in this project are not known as hazard to human health and nor is there any
The Agrobacterium infiltration or dipping of plants for transformation and growth of transgenic plants carrying viral sequences will be performed at the John Innes Centre (JIC) category B (level 2) greenhouse facilities where access is limited by an ID card-operated double door containing foot dips, biological waste leaves by a double-doored autoclave, waste water is treated by a verified chemical treatment before release into the waste water system, the entire facility operates at positive pressure to prevent insect entry (this is supplemented by a rigorous control program) and air is filtered on release.

The glasshouses facilities at JIC are designed to contain microbes and plant pathogens and operate under strict MAFF DEFRA approved guidelines.

The most likely routes of escape of geminivirus and potexvirus sequences into the environment is via pollen from transgenic plants. For this reason, all infectively studies in which cloned viral genome components are infiltrated into plants will be conducted under category 2 containment. Transgenic plants carrying virus sequences will be grown in category 2 containment and will be allowed to flower only when seed is to be collected, and plants will be suitably bagged to minimise pollen release.

We consider that our procedural and cultural precautions will minimise the risk of genetically modified virus escape into the environment and that plant growth level 2 containment is sufficient to reduce the hazard to effectively zero.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Transgenic plant waste:
the glasshouses facilities at JIC are designed to contain microbes and plant pathogens and operate under strict MAFF DEFRA approved guidelines: access is limited by an ID card-operated double door containing foot dips, biological waste leaves by a double-doored autoclave, waste water is treated by a verified chemical treatment before release into the waste water system, the entire facility operates at positive pressure to prevent insect entry (this is supplemented by a rigorous control program) and air is filtered on release. All JIC genetically modified plant waste will be autoclaved and subsequently composted on the site according to JIC Safety Procedure.

E.coli and Agrobacteria cultures carrying viral constructs:
Standard IFR waste disposal practises will be strictly adhered to.

All materials coming in contact with the GMM and all biological waste is activated by autoclaving, incineration or verified chemical means before leaving the building. Waste in re-usable items such as glass bottles will be first inactivated by approved chemical means (e.g 1% Virkon for 10 minutes) placed in stainless steel containers and autoclaved. Non-reuseable waste (e.g Petri dish cultures) will be autoclaved and dispatched for incineration. Bags will be transported by a licensed contractor in secondary containment appropriate for transport on public roads.

Personnel are trained in GMO/GMP and the uses of appropriate protective clothing (lab coats, gloves etc) to enable effective decontamination of personnel in the event of an accident.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
Comments of the GM Safety Committee on the risk assessment:
1) Your description of growth of transformed plants implies that there will be no soil transferred to the laboratory from the greenhouse. Is this so? If soil will be transferred, can you detail how this will be made safe and subsequently disposed of?
2) Please include details of the double containment procedures you will use when transferring plants and plant samples between greenhouse and laboratory.
3) Seeds must also be transferred under double containment: they must also be stored in a locked, designated container/refrigerator (either in the greenhouse or laboratory).
4) Please be aware of which greenhouses will be suitable for different aspects of your work (Particularly if your work progresses to a different stage). Those at JIC operate under both DEFRA and the HSE licences; those at JIC operate under both Defra and HSE licences; those at IFR only have an HSE licence, so cannot be used for e.g plant virus work.
5) Nicotiana spp do have a commercial crop in the UK, as ornamental varieties. We are unaware of any such commercial activity in the locality, but it might be useful to be aware of this possibility.

The final version of the risk assessment was modified in accordance with comments and was accepted as adequate for the project.

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Name

MEDIMMUNE UK LTD

Name 2

ASTRAZENECA/ SUPPLY BIOLOGICS

Department

Campus Estate or Research Centre

BOULEVARD INDUSTRY PARK

Building

Road Name

PLOT 6 RENAISSANCE WAY

District

SPEKE

Town

LIVERPOOL

County

MERSEYSIDE

Postcode

L24 9JW

Country

ENGLAND

Tel Number

0151 485 7817

Fax Number

0151 485 7730

E-mail

HSE Division

NORTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

**Y**

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee is lead by the Biological Safety Officer  
The committee is comprised of site functional representatives  
Senior Director - Site Operations  
Director - Quality Control  
Manager, Environmental, Health and Safety  
Manager, Manufacturing  
Oversight by Corporate GMO committee members  
Vice President Manufacturing - Vaccine  
Sr. Director, Scientific and Regulatory Affairs  
Vice President, Research & Development

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<th>Laboratory</th>
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<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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FluMist vaccine contains two cold-adapted (ca), temperature-sensitive (ts) and attenuated (att) influenza strains of type A (ie A/H1N1 and A/H3N2), and one ca, ts and att influenza strain of type B. Seeds used to produce FluMist are 6:2 reassortant viruses, which are produced by a process of genetic reassortment between a contemporary wild-type virus to produce the appropriate 6:2 vaccine strain is currently achieved using classical techniques, in which the initial steps are co-infection of cells which the MDV and a wild-type virus, followed by antibody selection and screening to identify the appropriate 6:2 vaccine strain. Recent advances in reverse genetics have made it possible to significantly improve the reassortment process using the plasmid rescue technique. Transfection of certain mammalian cells with plasmids, designed to produce each of the eight viral gene segments containing unmodified genetic sequences derived from the wild-type virus HA and NA genes and the remaining six genes derived from the MDV, results in the derivation of a specific 6:2 vaccine strain. Details regarding the two reassortment methods (classical co-infection or plasmid rescue methods) and the biological and genetic properties of 6:2 vaccine strains prepared using either of these two reassortment methods are provided in Risk Assessment. Because the viruses that are the subject of this application are attenuated vaccine viruses, Medimmune believes that it is reasonable that they should be classified below Hazard Group 2, ie as representing no hazard to human health. All waste materials containing vaccine virus are either thermally or chemically inactivated by validated procedures prior to removal from the facility. Alternatively contaminated material is sealed in clinical waste drums and incinerated at approved off-site facility.
The risk assessment has been discussed and agreed as valid and correct by all members of the safety committee and by Corporate oversight committee. The committee agree with the report conclusions that:

Rescue of infectious 6:2 reassortants by plasmid rescue methods yields MVSs that are indistinguishable from those generated by classical methods. Classically derived reassortants and plasmid rescued 6:2 reassortants have been shown by several criteria to be comparable vaccine strains. Regardless of the method used, 6:2 reassortants created by either method have highly consistent genomic sequences, express the ca, ts, and att phenotypes, and have the appropriate antigenicity.

All of the testing and characterization currently performed on the MVS of the licensed vaccine will be continued for MVS produced by plasmid rescue. Because the plasmid rescue method uses controlled reagents (use of reagents that have no animal-derived materials) and cells (complete testing for adventitious agents and tumorigenicity) no additional testing or characterization of plasmid rescued MVSs appears to be warranted. Because creation of the 6:2 by plasmid rescue is more predictable, reliable and may potentially produce vaccine with greater similarity to the wild type HA and NA antigens, it may become the preferred approach for the production of MVS. However, plasmid rescue is intended to supplement, not necessarily replace, the classical reassortment procedures. Both methods are adequate and both should be acceptable to produce vaccine in a timely manner. In addition to the above described advantages of the plasmid rescue method, rescue by plasmids may enable use of wt strains that were not originally isolated in eggs to produce reassortants (because the methodology would be expected to eliminate adventitious agents present in the original wt isolate). In summary, plasmid rescue of 6:2 reassortants should enhance the overall availability and choice of vaccine strains, should increase the genetic and antigenic authenticity of the reassortant, and should improve the predictability and reliability of production of new MVSs.

Project Ref 927/06.1

Date Ackn'd 25/08/2006

CU2 Project Title Production of a Pandemic Live attenuated Influenza vaccine virus bulk.

Class 2

Consent Granted

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Project Additional Information

Purposes of the contained use

The subject of the current application is commercial-scale manufacturing of pandemic live attenuated influenza vaccine (P-LAIV) using reassortant vaccine seeds prepared...
using either classical reassortment techniques or plasmid rescue techniques. P-LAIV is similar to the seasonal FulMist® vaccine (Influenza Virus Vaccine Live, Intranasal); however, whereas seasonal FulMist contains antigens that protect against seasonal epidemic influenza strains, P-LAIV contains antigens to protect against pandemic influenza strains.

**Recipient or parental organism**

Each live, attenuated influenza vaccine (LAIV) strain is produced by genetic reassortment of a master donor virus (MDV) and a wild-type (wt) influenza virus. The pandemic vaccine strains have a genetic composition similar to that of the seasonal vaccine strains in the licensed LAIV product, FulMist®. The Type A MDV was originally derived from wild-type A/Ann Arbor/6/60 (H2N2).

**Host/vector system**

Each pandemic vaccine strain has a 6:2 gene constellation; the six gene segments encoding internally-located viral proteins, (PB1, PB2, PA, NP, M and NS), are derived from the MDV, and the two gene segments encoding the surface glycoproteins (HA and NA) are derived from a contemporary wt strain.

**Origin & function**

All of the production of the GMO (Master Virus Seed) is performed in the United States of America. The UK site only expands the Master Virus Seed by growing the Master Virus Seed in SPF eggs to produce the Virus Harvest. The Virus Harvest is tested by Genotypic and Phenotypic methods to confirm that it is identical to the MVS. A detailed explanation of the GMO preparation process is given in the risk assessment and annex 1 of the attached document.

The Virus Harvest produced in the UK is commercial-scale manufacturing of pandemic live attenuated influenza vaccine (P-LAIV) using reassortant vaccine seeds prepared using either classical reassortment techniques or plasmid rescue techniques. P-LAIV is similar to the seasonal FulMist® vaccine (Influenza Virus Vaccine Live, Intranasal); however, whereas seasonal FulMist contains antigens that protect against seasonal epidemic influenza strains, P-LAIV contains antigens to protect against pandemic influenza strains.

**Evaluation of foreseeable effects**

The final vaccine seeds are used to produce a live attenuated influenza pandemic vaccine, which is designed to prevent influenza in humans. Therefore, the final P-LAIV vaccine seeds are not hazardous to humans. Furthermore, results of studies of seasonal vaccine in a variety of domestic and farm animals support the conclusion that the vaccine strains do not represent a hazard to the environment. We would expect the P-LAIV strains to behave in a similar way. Available shedding data for the H9N2 pandemic vaccine suggests that the median human infectious dose is greater than 10^7 TCID 50 whereas adults shed lower than 10^1 TCID 50 for less than one day; therefore in the unlikely event that the manufacturing staff would be infected by P-LAIV, it is extremely unlikely that secondary transmission outside the facility could occur. The relatively low replication of the H9N2 pandemic vaccine strain in humans and its expected inability to replicate in avian species or in farm animals (e.g. pigs) suggests that reassortment between influenza strains and the pandemic H9N2 pandemic vaccine would be unlikely to occur in either humans or in animals. The H5N1 pandemic vaccine is also highly attenuated in animals; it does not replicate in chickens and its replication is attenuated in mice and ferrets. Based on the clinical results for the H9N2 pandemic vaccine we expect the H5N1 vaccine to be highly attenuated in humans. Based on these considerations we are confident that, even in the event of a total breach of containment, the P-LAIV vaccine seeds covered by the assessment would not be hazardous to humans or the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste materials containing vaccine virus are either thermally or chemically inactivated by validated procedures prior to removal from the facility. Alternatively...
contaminated material including "sharps" are sealed in Clinical waste drums and incinerated at approved off-site facility. Liquid waste is thermally inactivated in an inactivation tank at a minimum temperature of 80°C for 15 minutes and the resultant liquid is further pH neutralised. The temperature of the cycle is monitored throughout the process. The inactivation achieves 100% influenza virus kill. Metal, glass, paper and plastic materials are inactivated by autoclaving at a minimum load temperature of 121°C for a minimum of 15 minutes. The autoclave cycle loads are validated and the temperature and pressure is monitored throughout the process. The inactivation achieves 100% influenza virus kill. Plastic materials including sharps and all laboratory biological test reagents are placed in Clinical Waste drums and incinerated. This achieves 100% influenza virus kill at an approved off-site facility.

Eggs - Eggs are macerated, and in a sealed system, the solid material is initially thermally inactivated for viruses at above 80°C for 15 minutes. The temperature is monitored throughout the process. The resultant dry material is placed in sealed drums and incinerated to ensure 100% kill of influenza virus at an approved off-site facility.

Although MedImmune believes that designation of this vaccine as Class 1 provides more than adequate assurance of safety of our employees and the environment., the company accepts the WHO classification of Class 2 for the manufacturing process.

Please enter comments on the GM safety committee on the risk assessment

Although MedImmune believes that designation of this vaccine as Class 1 provides more than adequate assurance of safety of our employees and the environment, the company accepts the WHO classification of Class 2 for the manufacturing process.

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 927/17.1

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<td>03/03/2017</td>
<td>Generation of novel live attenuated reassortant influenza virus from seasonal wild type influenza strains (H1, H3 and B strains) using reverse genetics</td>
<td>Class 2</td>
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Consent Granted
**Project Additional Information**

**Purposes of the contained use**

The aim of these studies are to generate reassortant attenuated influenza viruses (H1, H3 and B subtypes) using reverse genetics. These reassortant viruses are engineered to express the hemagglutinin (HA) and neuraminidase (NA) genes from seasonal influenza isolates on the master donor virus (MDV) backbone (A/Ann Arbor/6/60 or B/Ann Arbor/1/66).

**Recipient or parental organism**

Seasonal influenza virus of human origin - A strains (restricted to H1 and H3) and B strains which are classified as ACDP hazard group 2

E. Coli (strains NEB 5-alpha F'Iq competent E.coli, Veggie NovaBlue Singles Competent Cells or Invitrogen Top 10 Cells) - Disabled non-pathogenic, ACDP category 1

Continuous cell lines such as MDCK (CL1) or SPF eggs (CL1) (RA17722 – hazard group 1 cell lines)

Primary chicken or human cells (CL2) (RA 14917- working with primary cells)

**Host/vector system**

Reassortant viruses are engineered to express the hemagglutinin (HA) and neuraminidase (NA) genes from seasonal influenza isolates on the master donor virus (MDV) backbone (A/Ann Arbor/6/60 or B/Ann Arbor/1/66). Master donor virus is an influenza virus that was modified to confer cold-adapted (ca), temperature sensitive and attenuated phenotype and is the backbone for the FluMist/Fluenz vaccine. Attenuated viruses will be generated by reverse genetics (generally 6:2, occasionally 5:3 or 7:1 reassortants) from plasmid rescue. Novel viruses may also be engineered using single point mutagenesis of viral genome. The rescued viruses will then be characterized in vitro to confirm attenuated phenotype.

**Origin & function**

Host Organism

Seasonal influenza virus of human origin - A strains (restricted to H1 and H3) and B strains which are classified as ACDP hazard group 2

E. Coli (strains NEB 5-alpha F'Iq competent E.coli, Veggie NovaBlue Singles Competent Cells or Invitrogen Top 10 Cells) - Disabled non-pathogenic, ACDP category 1

Continuous cell lines such as MDCK (CL1) or SPF eggs (CL1) (RA17722 – hazard group 1 cell lines)

Primary chicken or human cells (CL2) (RA 14917- working with primary cells)

Source and nature of genes

Hemagglutinin (HA), neuraminidase (NA) or internal genes from seasonal influenza clinical isolates.

Source and nature of Vector

pAD 3000 plasmid - non-mobilisable
**Influenza A/Ann Arbor/6/60** - attenuated, cold adapted and temperature sensitive ACDP category 1

**Influenza A/Ann Arbor/1/66** – attenuated, cold adapted and temperature sensitive ACDP category 1

**Evaluation of foreseeable effects**

**Potential for Harm to human health**

For 6:2 and 7:1 reassortant viruses containing all Ann Arbor internal genes - The Ann Arbor master donor virus internal genes are well characterised and have been shown to confer attenuated phenotype for a large number of different 6:2 LAIVs in cells, eggs, ferrets and human safety studies. For reassortant viruses containing wildtype virus internal genes – the impact of wildtype virus internal genes on the attenuated phenotype of reassortant viruses cannot be predicted and a wildtype virus phenotype may be seen.

There is therefore the potential to develop flu-like symptoms following exposure. Personnel at risk of developing severe disease following influenza infection (e.g. pregnant women, asthmatics, immune compromised) should seek occupational health advice and undertake an individual risk assessment prior to undertaking work covered by this risk assessment.

**Potential for Harm to the Environment**

none – HA and NA genes from wild-type seasonal influenza viruses already circulating in the environment

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste must be inactivated prior to disposal as Non-hazardous Biological waste or where inactivation is not possible as Hazardous Biological waste. Inactivation should be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method). Detail: All waste from the handling of this material will be inactivated at source by chemical treatment and/or autoclave. All material is further treated by incineration.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

The Risk assessment was reviewed and approved by the organisation’s Biological Safety Committee which oversees GMO safety onsite in Nov 2016

**Project Containment**

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<td>L3 L4</td>
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The aims of these studies are to generate and work with 6:2 reassortant influenza viruses containing the hemagglutinin (HA) and neuraminidase (NA) genes from low pathogenic animal origin influenza viruses and the internal genes from the cold adapted, temperature sensitive, attenuated master donor virus (MDV) backbone A/Ann Arbor/6/60. Animal origin means isolated from an animal (for example, avian or swine) as well as isolated from a human with a zoonotic infection.

Live attenuated 6:2 reassortant viruses will be generated by reverse genetics and plasmid rescue. Novel LAIV may also be engineered using single point mutagenesis of the viral genome.

Recipient or parental organism

Wild-type animal origin low pathogenic influenza viruses do not have the multi-basic amino acid peptide insertion at the HA cleavage site (PENPKTR*GLF), are not pathogenic to chickens and are not therefore designated a SAPO agent by DEFRA. As such the wildtype viruses are handled at CL2.

Host/vector system

HOST Wild-type animal origin low pathogenic influenza viruses do not have the multi-basic amino acid peptide insertion at the HA cleavage site (PENPKTR*GLF), are not pathogenic to chickens and are not therefore designated a SAPO agent by DEFRA. As such the wildtype viruses are handled at CL2.
VECTOR E. Coli (strains NEB 5-alpha F'Iq competent E.coli, Veggie NovaBlue Singles Competent Cells or Invitrogen Top 10 Cells) - Disabled non-pathogenic, ACDP category 1
pAD 3000 plasmid - non-mobilisable
Influenza A/Ann Arbor/6/60 - attenuated, cold adapted and temperature sensitive ACDP category 1

Live attenuated 6:2 reassortant viruses will be generated by reverse genetics and plasmid rescue. Novel LAIV may also be engineered using single point mutagenesis of the viral genome.

Origin & function

Wild-type animal origin low pathogenic influenza viruses do not have the multi-basic amino acid peptide insertion at the HA cleavage site (PENPKTR*GLF), are not pathogenic to chickens.

Hemagglutinin (HA) genes from low pathogenic animal origin influenza isolates. The HA protein on the surface of the virus is responsible for virus/cell attachment and fusion. The HA protein does NOT contain the 7 amino acid multi-basic peptide insertion at the HA cleavage site (PENPKTR*GLF), characteristic of highly pathogenic influenza viruses.

Neuraminidase (NA) genes from low pathogenic animal origin influenza isolates. The NA of influenza viruses has enzymatic activity and is responsible for cleaving the HA from the cell receptor thus releasing new virus particles budding from the cell surface. There are no known regions or sequences that have been shown to be solely responsible for a highly pathogenic phenotype.

Internal genes PB2, PB1, PA, NP, M or NS from Influenza A/Ann Arbor/6/60 - these are all well characterised genes that are used in the commercial vaccine and have been shown to be safe.

E. Coli (strains NEB 5-alpha F'Iq competent E.coli, Veggie NovaBlue Singles Competent Cells or Invitrogen Top 10 Cells) - Disabled non-pathogenic, ACDP category 1
pAD 3000 plasmid - non-mobilisable
Influenza A/Ann Arbor/6/60 - attenuated, cold adapted and temperature sensitive ACDP category 1

Evaluation of foreseeable effects

The wildtype viruses are designated CL2 by HSE and DEFRA as long as they meet the following:

- presence of a HA cleavage site consistent with low pathogenic influenza viruses
- shown in assays not to cause pathogenicity in the chickens

Combination of the wildtype HA and NA genes with the internal genes of A/An Arbor/6/60 is known to result in highly attenuated cold adapted temperature sensitive viruses.

Potential for harm to human in the event of exposure - Potential for limited and mild upper respiratory tract infection

Potential to harm and disseminate to the environment - The HA and NA genes may be from low pathogenic animal origin influenza strains that are NOT currently circulating in the UK. In this case there is a low risk to the environment if the 6:2 virus was accidently released because the HA and NA genes could potentially reassort with genes from currently circulating wild type influenza to generate an influenza virus with a novel HA that is not currently in the UK environment.

This risk assessment therefore takes into consideration the differing level of risk to the environment depending whether the HA and NA genes originate from a wildtype virus that is in current circulation in the UK or not.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMO waste must be inactivated prior to disposal as Non-hazardous Biological waste or where inactivation is not possible as Hazardous Biological waste. Inactivation should be by autoclaving or by chemical treatment with 1% final Virkon for at least 30 minutes (or other validated method). Details: All waste from the handling of this

02/03/2022
material will be inactivated at source by chemical treatment and/or autoclave. All material is further treated by incineration.

**Project Containment**

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**Project Ref 927/18.1**

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<td>Generation of seasonal wild type and attenuated influenza virus using reverse genetics</td>
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<td>&lt; 1 Litre</td>
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Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
### Project Additional Information

#### Purposes of the contained use

The seasonal influenza viruses produced by reverse genetics will be used in experiments to determine the mechanism of action of vaccine viruses. These experiments will include in vitro cell culture work as well as in vivo animal model work.

#### Recipient or parental organism

The combination of the 8 gene segments will produce seasonal influenza virus which is capable of causing illness in humans.

#### Host/vector system

The viruses will be rescued in cell culture using continuous cell lines such as MDCK and 293T cell lines. The virus will be grown in specific pathogen-free (SPF) embryonated chicken eggs according to standard protocols.

#### Origin & function

The genetic material used originates from seasonal wild type influenza virus segments (HA, NA, PB2, PBi, PA, NP, M and NS). These wild type genes are restricted to currently circulating subtypes of influenza (Hi, H3 and B viruses). These segments will be used to produce wild type seasonal influenza viruses by reverse genetics. In addition to this, mutations that have been previously shown to produce a temperature sensitive phenotype will be introduced into the segments either by point mutagenesis or via replacing one or more internal gene segment (PB2, PBi, PA, NP, NS, M) with that of the cold adapted, temperature sensitive, attenuated master donor virus that is currently used for live attenuated vaccine production.

Site-directed mutagenesis will also be employed to introduce mutations into the wild type virus that have been shown to impact key characteristics of live attenuated influenza virus such as immunogenicity, antigenicity and viral fitness on human nasal epithelial cells.

#### Evaluation of foreseeable effects

As the viruses to be rescued will contain wild type influenza internal genes there is a risk of developing influenza after exposure. All procedures will be undertaken by appropriately skilled and experienced staff who have completed site biosafety training. All work will be performed at BSL2 containment. Personnel at risk of developing severe disease following influenza infection (eg pregnant women, asthmatics, immune compromised) should seek occupational health advice and undertake and individual risk assessment prior to undertaking work covered by this risk assessment.

The influenza viruses generated by reverse genetics will contain gene segments from viruses that are already circulating in the environment and so pose no additional risk to seasonal influenza strains.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All BSL2 waste will be handled as per on site procedures (listed below).
All GMO waste will be inactivated prior to disposal as non-hazardous biological waste (EWC 1801 04)
Liquid culture will be treated with a suitable disinfectant for the contact time outlined according to the manufacturer,
e.g. 10 minutes for presept, minncare or biocide x.
Eggs will be handled in a BSL2 safety cabinet with any waste being double bagged in biohazard bags containing
disinfectant powder (e.g. virkon or presept). The bags will be sealed and placed into biohazard bins. The biohazard
bins are incinerated off site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMO risk assessment (attached) was reviewed and approved at the June 2018 biosafety committee meeting at
AstraZeneca, Liverpool.

CU

Project Containment

Laboratory Activities Glass Houses Growth Rooms

L2 Yes L3 L4 L2 L3 L4 L2 L3 L4

L2 L3 L4 L2 L3 L4 L2 L3 L4

Animal Units Large Scale Activities Human Clinical Applications

L2 L3 L4 L2 L3 L4 L2 L3 L4

Project Ref 927/19.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
The seasonal influenza reassortant A/Ann Arbor/06/1960 (wtAA60) viruses produced by reverse genetics will be used as controls in experiments to determine the mechanism of action of seasonal vaccine reassortant A/Ann Arbor/06/1960 viruses with a cold adapted, temperature sensitive and attenuated phenotype (caAA60). These experiments will include in vitro cell culture work as well as in vivo animal model work.

Recipient or parental organism

The combination of the 8 gene segments will produce seasonal influenza virus which is capable of causing illness in humans.

Host/vector system

The viruses will be rescued in cell culture using continuous cell lines such as MDCK and 293T cell lines. The virus will be grown in specific pathogen-free (SPF) embryonated chicken eggs according to standard protocols.

Origin & function

The genetic material will originate from two sources:
1) HA and NA gene segments from currently circulating seasonal influenza viruses (pdm09H1N1 and H3N2)
2) PB2, PB1, PA, NP, M and NS gene segments from the wt A/Ann Arbor/06/1960 isolate (H2N2)

Reassortant viruses will be generated by reverse genetics which will contain HA and NA from seasonal circulating viruses and PB2, PB1, PA, NP, M and NS from wt A/Ann Arbor/06/1960.

Live Attenuated vaccine candidates are produced using reverse genetics with a cold adapted (ca), temperature sensitive (ts) and attenuated (att) A/Ann Arbor/06/1960 virus. Therefore the function of the genetic material is to generate reassortant viruses to be used as controls for the live attenuated vaccine candidates to determine how ca, ts, and att relate to the mechanism of action of live attenuated vaccine candidates.
As the viruses to be rescued will contain wild type influenza internal genes there is a risk of developing influenza after exposure. All procedures will be undertaken by appropriately skilled and experienced staff who have completed site biosafety training. All work will be performed at BSL2 containment.

Personnel at risk of developing severe disease following influenza infection (e.g. pregnant women, asthmatics, immune compromised) should seek occupational health advice and undertake an individual risk assessment prior to undertaking work covered by this risk assessment.

The influenza viruses generated by reverse genetics will contain HA and NA gene segments from viruses that are already circulating in the environment and so pose no additional risk to seasonal influenza strains.

PB2, PB1, PA, NP, M and NS gene segments are from a non circulating influenza subtype (H2N2) and present a risk of reassortment with other influenza viruses if there was accidental exposure. This could generate novel influenza viruses with unknown phenotypes. Although, it is unlikely that these novel viruses would have increased pathogenicity when compared to the currently circulating isolates. This is supported by the fact that risk assessments carried out on H2N2 viruses of which A/Ann Arbor/06/1960 is a part of, show that there is a low to intermediate risk of non reassorted isolates from this subtype (Risk Assessment of H2N2 Influenza Viruses from the Avian Reservoir: Jones et al, 2014. Journal of Virology).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All BSL2 waste will be handled as per on site procedures (listed below).

All GMO waste will be inactivated prior to disposal as non-hazardous biological waste (EWC 18 01 04)

Liquid culture will be treated with a suitable disinfectant for the contact time outlined according to the manufacturer, e.g. 10 minutes for presept, minncare or biocide x.

Eggs will be handled in a BSL2 safety cabinet with any waste being double bagged in biohazard bags containing disinfectant powder (e.g. virkon or presept). The bags will be sealed and placed into biohazard bins. The biohazard bins are incinerated off site.

01PDSOP0011: Cleaning and Spill Clean Up for MS&T Laboratories
01PDSOP0016: Handling of Waste from MS&T Laboratories
01PDSOP0018UK-1: MS&T Laboratory Manual

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
The GMO risk assessment (attached) was reviewed and approved at the December 2019 biosafety committee meeting at AstraZeneca, Liverpool.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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### GM Centre Number: 928

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### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

CBT has convened a GMO committee comprising experts each with a minimum of 10 years experience in the fields of molecular biology, pharmacology and drug discovery. This meets on a quarterly basis (or additionally when required) to review new applications and to review existing approved GMO procedures.

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential
Yes

Only class one organisms and bacterial waste will be produced on site. Waste will be disposed of by appropriate routes as detailed in CBT-Standard operating procedures. Solid waste will be collected in double containment waste bags and inactivated by autoclaving, prior to disposal as clinical waste destined for incineration (contractor Vetspeed). Liquid waste will be collected in suitable containers and inactivated overnight with either Presept disinfectant tablets (dichloroisocyanurate sodium salt, Johnson and Johnson) or Virkon and autoclaved. Inactivated waste will then be disposed of to the drain.

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<td>specify below)</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
All GMM will be genetically modified recombinant E. coli (these will always be attenuated strains such as JM101, DH5a, XL1 blue or similar lines). These lines will be for the purpose of producing plasmid constructs for transfection into animal cell lines to create recombinant cell lines. Examples of typical plasmid cloning vectors include: pCMVScript(Stratagene), pcDNA3 (Invitrogen), TOPO TA(Invitrogen). These constructs will have minimal anticipated detrimental effects on human health and the environment, as they will be non-oncogenic, non-viral, non-transmissible genes of human or animal origin. As such they pose virtually no risk to the individual researcher, to the environment either as naked DNA, a gene or gene product within a transfected prokaryotic or eukaryotic cell or as a translated protein. These genes also have either no or minimal anticipated combinatorial effects in the host bacterium. Examples of tenes used in such construct include GPCRs, metabolic enzymes and receptors, none of these will be for the purpose of protein production for purification. Each construct will be assessed prior to manufacture by the GM safety committee and considered class one if it meets the criteria detailed below:


II) The inserted genetic material. The inserted genetic material is already present in living cells and is unlikely to significantly change the properties of the host organisms. Overall risk: High/Medium/Low/EFFECTIVELY ZERO.


IV) Vector. The vectors are considered non-mobilisable, with negligible risk of transferring to insect, plant or human cells. Overall risk: High/Medium/LOW/Effectively Zero.

V) the resulting genetically modified micro-organisms are non-pathogenic and non-infectious. Overall Risk: High/Medium/LOW/Effectively Zero.
Project Additional Information

Purposes of the contained use

The aim of this project is to use retroviral, lentiviral, and baculoviral delivery systems to introduce various mammalian DNA sequences into mammalian cells in culture. These sequences direct expression of either (i) RNA species (siRNAs or antisense RNAs) that interfere with the expression of various mammalian proteins or (ii) the corresponding mammalian proteins themselves; for in vitro use and for the generation of stable mammalian cell lines (and subsequent use of such stable lines in vitro) with the aim of generating stable cell lines expressing fluorescent proteins, potential drug targets or therapeutic proteins, etc.

Recipient or parental organism

systems (and in the case of baculovirus - also for the transposition of inserts into Bacmid) will take place in K12 strains of E. coli (with a history of safe use).

Insect cells (e.g. sf21 - with a history of safe use) will be used for amplification of infective baculovirus.

Mammalian cell lines (e.g. immortalized lines derived from human cancer tissues) fall into two types:

a) Packaging/helper cell lines into which plasmids containing lenti- or retroviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines, i.e. a negligible risk.

Host/vector system

The gamma-RETROVIRAL vectors which will be used are derived from either Moloney Murine sarcoma Virus (MoMLV), Mouse Mammary Tumour Virus (MMTV) or Feline Leukaemia Virus (FeLV), all of which are ACDP Hazard Group 1 biological agents. On the other hand, the virus will be packaged by transfecting transfer vector into specific amphoteric ‘helper’ cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types. This means that the viruses produced for this experiment could potentially infect a number of species, including man.

However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle:

- The genes encoding structural and other components of the viral genome have been separated. These genes have been engineered to minimise the risk of recombination that could lead to production of a replicationcompetent virus.
- The packaging cell lines allow expression of proteins, required to produce progeny virus: But the transfer vector is the only genetic material transferred to the target cells, consequently these cells cannot produce the proteins which are essential for viral assembly and infectivity.
- Second, third generation or Self INactivating vectors retrovirus vectors will be used in all experiments (see SACGM compendium of guidance part 2, section 2.11 (Retroviruses) pp117

The LENTIVIRAL vectors that will be used are derived from HIV-1 , which is an ACDP Hazard Group 3 biological agent. However, second and third generation lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase biosafety and viral packaging is achieved by providing three helper
constructs in trans containing gag, pol and rev sequences.

For example, second and third generation Lentiviral Expression Systems include the following key safety features:

In the second and successive generation lentiviral vectors several lentiviral accessory genes (vif, vpr, vpu and net) are deleted from the transfer plasmid since they are not required for in vitro replication and the products they encode have cytotoxic activities.

In addition to this in the third generation lentiviral vectors:

- The Lenti expression vectors contain a deletion in the 3'LTR (IIM3) that does not affect generation of the viral genome in the producer cell line but instead results in "self-inactivation" of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a packageable viral genome.

- The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).

- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).

- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the 4' packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication competent virus can be produced.

Despite the above safety features, use of these lentiviral vectors (which include WPRE) falls within SACGM 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. Also, the virus will be packaged by transfecting transfer vector into specific amphotropic "helper" cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range of mammalian species (including humans) and cell types.

This means that the viruses produced for this experiment could potentially infect a number of species, including man. The BACULOVIRAL vector technology that we will use is based on double-stranded DNA insect viruses (baculovirus - which are ACDP Hazard Group 1 biological agents) as vehicles to efficiently deliver and express genes in mammalian cells. Bacmid/baculovirus particles are taken up by endocytosis, released for transcription and expression following migration to the nucleus. Gene expression begins within 4-6 hours of transduction and is at near maximum level within 24 hours of transduction. The baculovirus have been modified by engineering of a mammalian expression cassette for transgene expression in mammalian cells. Baculoviruses are non-replicating in mammalian cells and thus have an excellent safety profile combined with being well-tolerated by cells. Baculovirus transduction of mammalian cells is transient in nature as the foreign DNA does not integrate into the host genome.

### Origin & function

**Selectable markers - examples (but not restricted to):**

- Ampicillin resistance: E.coli derived
- Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene

**Health and Safety Executive**

Puromycin resistance (PAC): Puromycin acetyl transferase is derived from Streptomyces alboniger

Reporter proteins such as (but not restricted to):
• Fluorescent proteins as reporters:
  o GFP derived from the jellyfish Aequorea victoria and variants of this
• Luciferase - class of oxidative enzymes used in bioluminescence
  o renilla luciferase derived from the Sea pansy (Renilla reniformis)
  o firefly luciferase derived from the firefly Photinus pyralis.
Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and l or
shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding
potential drug targets or therapeutic proteins) - all human derived.
This could include the expression of potentially harmful genes e.g. encoding known proto-oncogenes or genes with
known oncogenic mutations that can contribute to cellular transformation.

Evaluation of foreseeable effects

All viral vectors employed in this protocol exhibit broad tropism and potential to infect humans. Risks conferred
fo llowing infection are identified as:

i) genetic insertion of viral sequences with potentially deleterious effects on endogenous genes: we will
employ transgene promoters and other viral sequences that may affect host gene function in a wide
range of cell types (e.g. CMV promoter). We assess retro-, lenti- or adenovirus infection might induce
permanent changes in infected cells including a risk for tumorigenesis. Risks conferred are previously
described and categorised under Class 2 risks [SACGM compendium of guidance part 2, page 121]:
..... The effects of integration upon the infected cell should be considered. For instance, promoter
sequences present in the provirus might activate genes adjacent to the integration site or,
alternatively, insertion may disrupt genes and prevent their expression.”

ii) expression of human-derived or homologous transgenes with potentially deleterious effects: various
transgenes may be employed, wherein intrinsic function of the transgene confers potentially
deleterious effects. For example, expression human oncoproteins could induce transformation of
infected cells. Beyond endogenous homeostatic mechanisms that may lessen this risk (e.g. apoptosis
and other host tumour suppression responses), we assess that standard precautions under Class 2
risk mitigation procedures are adequate to address such risks.

iii) expression of exogenous transgenes directed to host genes with potentially deleterious effects: multiple
transgene technologies (antisense, RNAi, CRISPR or related gene conversion) may be employed to
modify the function of endogenous genes with potentially deleterious effects. For example, RNAi or
antisense RNA could reduce the function of genes necessary to control cell apoptosis, potentially
resulting in tumorigenesis. As above, we assess that Class 2 risk management procedures
adequately address these risks.

iv) expression of heterologous genetic sequences with potentially deleterious effects: we will employ diverse
collections of non-human derived, exogenous transgene sequences that carry potential risks
following infection. Risks from expression of these collected transgene sequences are mitigated and
attenuated through several means. First, known pathogenic transgenes systemically removed, and
other exogenous gene products are fragmented or rearranged, such that potentially pathogenic or
other biologically deleterious genetic functions are not recapitulated in their complete endogenous
configuration. Second, potentially deleterious transgenes in any infectious viral preparation are highly
titrated, such that any single deleterious sequence comprises less than 1 part in 100,000 parts of an
inoculum with infectious potential. In total, we assess these risks are adequately managed via the
same Class 2 risk precautions employed above.

We assess environmental hazards are adequately addressed through the proposed personnel risk management
measures. We employ multiple attenuation strategies and protocols to severely limit independent virus
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)  

| Not applicable |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)  

| Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:  
1. We do not have access to an autoclave within our area of the building  
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.  
All liquid waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization is GM898) according to disposal notification GM105/4.1.  
Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from Class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.  

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)  

| All liquid waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Solid waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class 1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached. |

| Is an emergency plan required according to regulation 20? | N  |

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

02/03/2022
The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

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Please enter comments on the GM safety committee on the risk assessment

Project Containment
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes]

Give brief details of the genetic modification safety committee

Genetic Modification Safety Committee was formed on 17/10/05. The committee comprises of the health and safety committee members. The members include: Vice President Science and Technology (supervisor of GM activities). Office Manager, Quality Assurance Manager, Facilities Manager, Analytical Chemist, Senior Scientist Molecular Diagnostics (Biological Safety officer), Customer Services Manager, Operations Manager. Meetings will be held monthly in conjunction with health and safety meetings. Updates on GM activities and analysis of risk assessment forms will take place in these meetings. Risk assessments will be reviewed individually and discussed as a group.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
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Other (please specify)

Tick if confidential

![No]

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<th>Microbiology Research</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

None

**Project Ref 929/05.1**

<table>
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<tr>
<th>Date Ackn'd</th>
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<th>Class</th>
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<th>CultureVolumeClass3-4</th>
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<td>Development of geneotypic resistance assays for Hepatitis C Virus (HCV)</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

The genotypic drug resistance assays will consist of a nested RT-PCR amplification of the drug target gene followed by direct sequencing. Run off transcripts will be used as templates for the optimisation of the nested RT-PCR. A panel of constructs will also be generated to provide positive controls for the assay; their inserts will cover the viral gene of interest (e.g., NS3/4A or NS5B) under the control of a promoter such as T7 to generate in vitro transcripts. This panel of positive controls will represent different genotypes and sequences containing drug resistance mutations. If necessary, known drug resistance mutations will be introduced by site-directed mutagenesis to construct appropriate positive controls.

**Recipient or parental organism**

Disabled E. coli (JM109, XL-1 Blue, DH5alpha, TOP10 or HB101)

**Host/vector system**

Non-mobilisable plasmid vectors pUC8 and pGEM

**Origin & function**

Pre-existing HCV clones containing up to 90% of the genome and lethal mutations within the open reading frame (both making them non-infectious) will be used as PCR templates to provide positive controls for the assay. Additional positive controls representing other viral genotypes will be generated by cloning the drug target gene from clinical samples.

**Evaluation of foreseeable effects**

All experiments will be undertaken with incomplete genomic clones which are incapable of generating infectious virus. No harmful proteins will be encoded within the inserts. E. coli carrying plasmid coding for HCV proteins do not pose any significant risk to human health and safety or to the environment. Recombination and gene transfer to other microorganisms are highly unlikely.

**Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be decontaminated by treatment with 10,000 ppm chlorine overnight (100% kill) and all solid waste will be autoclaved at 134 degrees Celsius for 20 minutes.

**Is an emergency plan required according to regulation 20?**

- N

**If yes, tick to confirm that it is attached to this form**

- N

**Tick to confirm that you have attached a risk assessment to this form**

- Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

- N
Please enter comments on the GM safety committee on the risk assessment

The risk assessment was reviewed by the genetic modification safety committee; minor wording changes were agreed. Generally, the risk assessment was accepted as appropriate.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
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<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2 L3 L4 L2 L3 L4</td>
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### Project Ref 929/10.1

<table>
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<tr>
<td>05/01/2010</td>
<td>Hepatitis B Virus (HBV) phenotypic testing</td>
<td>Class 3</td>
<td>1 L</td>
<td>20 l</td>
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<table>
<thead>
<tr>
<th>Withdrew</th>
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<tbody>
<tr>
<td>N</td>
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</table>

### Project Additional Information

Purposes of the contained use

Analysis of clinical samples for in vitro susceptibility of antiviral drugs against HBV. The work will involve cloning viral gene sequences amplified from clinical samples into a plasmid containing the HBV genome from which the gene of interest was deleted. The resulting construct will contain a full-length HBV genome. Only after transfection of...
the resulting construct into host cells, such as HepG2, an infectious recombinant virus can be generated. All full-length HBV transfection experiments will be carried out in a biosafety category 3 laboratory.

**Recipient or parental organism**

Disabled E. coli hosts such as DH5α, Top10, or HB101.

**Host/vector system**

Non-mobilisable plasmid vectors based around, pBluescript, pUC or pGEM will be used as vectors in the cloning procedures in E. coli

**Origin & function**

A pre-existing construct of full length HBV lab strain is used as a backbone to clone the antiviral drug target from clinical samples. The final construct will be transfected into human hepatoma cells, such as HepG2. The transfected DNA will not be inserted into the genome of the host; instead it will result in viral replication equivalent to HBV infection of the cells. System like the one described in Tenney et al: Antimicrob Agents Chemother. 2004 September; 48(9): 3498-3507.

**Evaluation of foreseeable effects**

HBV full-length constructs used in this study could be accidentally administered. Although no evidence exists to suggest that this would cause infection, all staff handling these clones will be vaccinated against HBV and checked for protective levels of antibody prior to initiation of experiments. Moreover, handling of all HBV infectious virus stocks will be carried out in a designated Category 3 laboratory under strict operating procedures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid waste will be de-contaminated with 2500ppm chlorine, 10% TriGene ADVANCE, 3% (w/v) Virkon or an equivalent disinfectant which ensures 100% kill. All solid waste will be autoclaved at 135°C for 30 min providing 100% kill.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment

None

**Project Containment**

02/03/2022
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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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GM Centre Number: 930

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Name
OUNDLE SCHOOL

Name 2

Department
BIOLOGY DEPARTMENT

Campus Estate or Research Centre

Building
SIR PETER SCOTT BUILDING

Road Name
GLAPTHORN ROAD

District

Town
Peterborough

County
BEDFORDSHIRE

Postcode
PE8 4ES

Country
ENGLAND

Tel Number
01832 277 206

Fax Number
01832 273 564

E-mail

HSE Division
EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

- Head of Biology (Biological Safety Officer)
- SAPS representative (with access to a network of experts)
- Head of Science and Technology
- Senior Lab Technician (with 21 years experience in microbiology and medical laboratories and 4 years in school)

The Committee will be in regular contact and will be able to come and visit the lab at any time.

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<td>Other (please specify)</td>
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Tick if confidential  

No
**For activities involving GMMs, describe the waste management measures which will apply to the activity**

All biological waste will be autoclaved prior to disposal. Randomly selected autoclaved cultures will be used to inoculated fresh growth medium to check viability.

All GM plant material to be autoclaved, including the soil they were grown in.

All biologically contaminated sharps will be soaked in 10% bleach for a minimum of 20 minutes before disposal via the sharps waste system.

Any biological spills (e.g., E. coli and Agrobacterium cultures) will be bleached in freshly made 10% bleach for a minimum of 20 minutes.

**Tick to confirm that you are attaching a summary of the risk assessment**

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

**Please enter comments of the GM safety committee on the risk assessment**

All committee members were satisfied with the containment level and the measures that will be employed to minimise any risks.
## GM Centre Number: 931

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Y
The Safety Committee already established at one of the hospitals involved (St James's university Hospital) has conducted the risk assessment:

Leeds Teaching Hospitals NHS Trust Biological Safety Committee
Trust Headquarters
St James's University Hospital
Beckett Street
Leeds
LS9 7TF

Composition:
For composition, operating procedures and frequency of meetings see separate sheet.

Information on separate sheet:
Committee composition:
Associate Director of Trust (Chair), Microbiologist, Senior Research Nurse, Post Doctoral Scientist, Medical Oncologist, Pharmacist, Virologist, Infection Control Respresentative, Pharmacist, HSE Representative, Clinical Oncologist.

Operating Procedures:
A Biological Risk Assessment Form is completed for proposed study. This details background to study, Principal Investigator (PI), where the work will be carried out, details of biological agent, details of construct, details of cell line, containment level, risk to human health and environment. This form is sent to the Chair of the committee along with SOPs detailing the specifics of how the study will be conducted within the Trust (where it will be done, who will do what), protocol, Investigator Brochure, GTAC Submission if available and PI's CV. The committee ensures the study has been submitted to the necessary bodies and all the necessary approvals are in place.
The PI attends the meeting itself, presents the study and answers any questions before leaving the room to allow the committee to discuss freely. Following discussion, the PI rejoins the meeting and any issues raised are discussed. A letter is issued to the PI detailing the outcome of the meeting and giving feedback. Any necessary amendments to SOP's are made based on this feedback.

Frequency of meetings:
3 monthly but can be arranged more frequently if necessary.

<table>
<thead>
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Tick if confidential
Yes

All needles and syringes will be discarded into a designated sharps bin. This will be sealed and disposed of using normal hospital procedures.

All other materials will be treated as clinical waste and disposed of in yellow clinical waste bags under normal hospital procedures.

Unused vials will be returned to Pharmacy and stored in the refrigerator until they are collected periodically by courier.

If a spill occurs use absorbent materials to soak up spill and discard with spent vials in Biological Waste. Clean area with cleaning agent to remove residue. No specific disinfectants are required.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

All needles and syringes will be discarded into a designated sharps bin. This will be sealed and disposed of using normal hospital procedures.

All other materials will be treated as clinical waste and disposed of in yellow clinical waste bags under normal hospital procedures.

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If a spill occurs use absorbent materials to soak up spill and discard with spent vials in Biological Waste. Clean area with cleaning agent to remove residue. No specific disinfectants are required.

**Tick to confirm that you are attaching a summary of the risk assessment**

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

**Please enter comments of the GM safety committee on the risk assessment**

Date of meeting: 10th October 2005
Present: Associate Director of Trust (Chair), Microbiologist, Senior Research Nurse, Post Doctoral Scientist, Medical Oncologist, Pharmacist, Virologist, Infection Control Representative.

Apologies: Pharmacist, HSE Representative, Clinical Oncologist.

Note: The Senior Research Nurse (Debbrie Berine) and Medical Oncologist (Dr John Chester) on this committee will be working on this project. Dr Chester is the principal investigator at St James's University Hospital.

See attached:
1) Leeds Teaching Hospitals NHS Trust Biological Risk Assessment form
2) Notes of the Extra-ordinary meeting 10th October 2005 for feedback on this risk assessment.
| Data Premises Notified                         | 12/01/2006 |
| Data Premises Closed                         |            |
| Name                                          | HARPER ADAMS UNIVERSITY COLLEGE |
| Name 2                                       |            |
| Campus Estate or Research Centre             |            |
| Road Name                                     |            |
| Town                                         | Newport    |
| County                                       | SHROPSHIRE |
| Postcode                                     | TF10 8NB   |
| Country                                      | ENGLAND    |
| Tel Number                                   | 01952 820 280 |
| Fax Number                                   | 01952 814 783 |
| HSE Division                                 | WALES AND SOUTH WEST |
| Date at Which Additional Info Submitted      | 02/03/2022 |
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GM ethics and safety committee consists of internal members of staff, two of whom are not involved in GM work, together with a lay representative of the Board of Governors. The committee is required to consider applications to conduct GM work and to address ethical, safety, security and propriety concerns arising in the conduct of such work.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs) | Yes | | | |
Level 2 (GMMs) | | | | |
Level 3 (GMMs) | | | | |
Level 4 (GMMs) | | | | |
Non-microbial | | | | |
Other (please specify) | | | | |
Tick if confidential

Bacteriology | Parasitology | Transgenic | Microbiology | Yes
---|---|---|---|---
Virology | Transgenic | Transgenic | Gene Therapy
Mycology | Transgenic | Transgenic | Other (please specify below) | Yes
Other(s) | Teaching molecular biology

For activities involving GMMs, describe the waste management measures which will apply to the activity
All waste and glassware will be autoclaved at 121 degrees C for 15 minutes. Steam sterilisation will be monitored using cycle verification indicators (TST Class 6) (degree of kill 100%).

All surfaces will be cleaned after use with 1% Virkon (degree of kill greater than 99.999%).

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM ethics and safety committee meeting of the 9th December 2005 reviewed the attached risk assessment. The committee agreed to the following statement:

The genetic transformation is deemed to have no perceived hazard to humans, animals or the environment over and above an equivalent microbiology procedure using a non-modified, non-pathogenic micro-organism.

The genetic transformation can be performed in a laboratory suitable for the use of Hazard Group 1 micro-organisms. Procedures must comply with “Good Microbiological Practice” as detailed in the Harper Adams University College Code of Practice for the use of microbiology and molecular biology laboratories.
## Project Additional Information

### Purposes of the contained use

The purpose of the contained use is to safely propagate fungal spores and use such spores to inoculate detached leaves or whole plants to assess the plant pathogenic ability and virulence of various knockout mutants and wild-type Fusarium isolates, on small grain cereals. Mutants have specific genes implicated in pathogenicity knocked out. Comparison to wild-type isolates in pathogenicity assays will identify role of specific genes within pathogenicity of the Fusarium species.

### Recipient or parental organism

<table>
<thead>
<tr>
<th>GMO</th>
<th>Parental organism</th>
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<tr>
<td>GMO1</td>
<td>Fusarium graminearum</td>
</tr>
<tr>
<td>GMO2</td>
<td>Fusarium langsethiae</td>
</tr>
</tbody>
</table>

Both GMOs are natural pathogens of small grain cereals such as wheat, barley and oat and are endemic within Europe.

### Host/vector system

- **No vector systems have been identified for any of the GMO listed**

### Origin & function

**GMO1** carries a mutation in an endogenous gene encoding a hydrophobin protein. Selectable marker is the gene carrying hygromycine resistance (hph). Deletion of the hydrophobin gene is expected to reduce surface hydrophobicity of the fungus.

**GMO2** carries a mutation in an endogenous gene encoding the first enzyme (Tri5) in the biosynthetic pathway for trichothecene mycotoxins, including HT-2 and T-2. Selectable marker will be the gene carrying hygromycin resistance (hph). Deletion of Tri5 will abolish trichothecene toxin production.

### Evaluation of foreseeable effects

Both GMO1 and 2 are deletion mutants, hence reducing the risk of reversion to a minimum.

**GMO1**: Reduced surface hydrophobicity is expected to reduce pathogenicity and maybe also spore production. These are known effects from other studies. Reduced spore production will reduce the ability to disseminate if, accidentally, released into the environment.

**GMO2**: Abolished toxin production might reduce pathogenicity/aggressiveness of the GMO. The aim of the research is to test the function of HT-2/T-2 toxins as fungal virulence factors in F. langsethiae. Similar studies with Tri5 deletion in F. graminearum abolished production of deoxynivalenol (DON) and reduced pathogenicity drastically. The probable effect of deletion of Tri5, if any, is therefore a weakened pathogen. Additionally, if GMO2, accidentally, should be able to spread in the environment, the threat to human and animal health would be negligible as it would not produce toxins.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Fusarium species will be used to inoculate detached leaves and/or leaves of seedlings of small grain cereals. As such they are plant-associated GM micro-organisms.

All operations will be conducted in a Class 2 Microbiology Research Suite. The Microbiology Suite has swipe card controlled restricted access.

All manipulations of fungal cultures will be performed in a Class II Microbiological Cabinet.

Inoculated plants will be contained in a laboratory plant growth cabinet within the Class 2 Microbiology Research Suite (S25).
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All work will be conducted in a Level 2 Containment Facility, no derogation requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All work will follow the Harper Adams University College code of practice for working in the microbiology research laboratory S25 - attached.

Waste will be in the form of fungal spores and mycelia on agar plates, and remains of spore suspensions in shake flasks, tubes, filters, pipette tips, microscope slides, plant material, plant growth medium and containers.

Glass flasks with spore suspensions will be put in autoclaveable biohazard bags and sterilized by passage through a waste autoclave cycle (121 C for 1 hour) before washing (degree of kill 100%).

Glass slides will be placed in a beaker containing fresh 1% Virkon solution for a minimum of 10 minutes before disposal with general waste (landfill) (degree of kill greater than 99.999%).

All surfaces will be wiped with fresh 1% Virkon solution (degree of kill greater than 99.999%).

All other consumables and plant material will be placed in autoclaveable biohazard bags and sterilized by passage through a waste autoclave cycle (121 C for 1 hour) before disposal with general waste (landfill) (degree of kill 100%). Waste is autoclaved in a certified autoclave, load temperatures are recorded throughout the autoclave cycle and checked before waste disposal.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The College Genetic Modification Ethics and Safety Committee met on the 1st June 2009. There was a thorough review of the application and the Committee concluded there are satisfactory facilities and procedures available in college to conduct the experiments detailed within this application. A full discussion of the risk assessment was conducted and key issues of containment, disposal and decontamination were addressed. The Committee agreed that a requirement for dedicated technical staff should be clarified with the laboratory manager. On the basis of the considerations detailed above the Committee approved the submission for Class 2 GM contained use activity.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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### GM Centre Number: 933

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#### Name

NORTH WEST THAMES REGIONAL GENETICS SERVICE

#### Name 2

NORTHWICK PARK HOSPITAL

#### Campus Estate or Research Centre

CYTOGENETICS, 8V

#### Road Name

WATFORD ROAD

#### Town

Harrow

#### District

MIDDLESEX

#### County

HA1 3UJ

#### Country

ENGLAND

#### Tel Number

0208 869 2795

#### Fax Number

0208 869 3106

#### HSE Division

EAST AND SOUTH EAST

#### Comments

PREMISES CLOSED 04/03/2010

#### Date at Which Additional Info Submitted

02/03/2022
**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Committee meets annually unless new projects/procedures are to be reviewed. Consists of:
- Lecture in Human Genetics Imperial College London
- Departmental Safety Office
- Non-clinical risk manager
- Principle Clinical Scientist
- Cytogenetic Technologist

<table>
<thead>
<tr>
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<th>Glass House</th>
<th>Large Scale</th>
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**Non-microbial**

Other (please specify)  
Tick if confidential

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<td>Mycology</td>
<td>Transgenic</td>
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<tr>
<td>Other(s)</td>
<td>Propagation and purification of human genomic DNA in non-expressing vectors.</td>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity.
Liquid waste: 5% hypochlorite solution for 16 hours prior to disposal via appropriate sinks 100% kill.

Solid waste: Surfaces decontaminated with 70% ethanol, 1% Virkon used for 16 hours to decontaminate glassware. Autoclave to sterilise agar plates etc. prior to sealing in clinical waste bags for specialist disposal off site (arranged via Microbiology department). Above methods provide 100% kill.

Monitoring: Quarterly monitoring by attempting to grow up host bacteria following decontamination procedure.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

It was felt that the assessment would cover the work currently planned. However, if work with complete oncogene sequences were required a further risk assessment specifically for these sequences may be needed.

---

**Project Ref 933/06.1**

**Date Ackn’d** 26/01/2006

**CU2 Project Title** Propagation and purification of human genomic DNA in non-expressing vectors, in E. coli, for use as probes in fluorescent in situ hybridisation experiments.

**Class** Class 2

**CultureVolClass** < 1 Litre

**CultureVolumeClass** Class 3-4

**Non-GMM** Not Applicable

**Consent Granted** Non-GMM

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
### Purposes of the contained use

Propation of vectors containing fragments of human genomic DNA, in competent E. coli. Vectors are extracted, purified and labelled with fluorochromes. Labelled human genomic DNA fragments are then hybridised to human metaphase chromosomes to provide clarification of chromosome rearrangements.

### Recipient or parental organism

DH10B E Coli

### Host/vector system

- pBACe3.6
- pCYPAC2
- pPAC4

### Origin & function

Human genomic DNA
To be used as region specific fluorescently labelled probes for molecular cytogenetic analysis of human metaphase chromosomes.

### Evaluation of foreseeable effects

No ill effect foreseeable, vectors are non-expressing and inserts do not encode complete genes. Vectors are non-mobilisable.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Liquid waste: 5% hypochlorite to virkon to 1% final strength solution for 16 hours prior to disposal via appropriate sinks. 100% kill.</th>
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<td>Solid waste: Surfaces decontaminated with 70% ethanol. 1% Virkon used for 16 hours to decontaminate glassware if used. Autoclave to sterilise agar plates etc. after sealing in clinical waste bags for specialist disposal off site (arranged via Microbiology department). Above methods provide 100% kill.</td>
</tr>
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<td>Monitoring: Quarterly monitoring by attempting to grow up hosts following decontamination procedure.</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
It was felt that the assessment would cover the work currently planned. However, if work with complete oncogene sequences were required a further risk assessment specifically for these sequences may be needed.

### Project Containment

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<th>Laboratory Activities</th>
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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**
GM Centre Number: 934

Data Premises Notified (Originally) 01/02/2006

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

MARS SYMBIOSCIENCE UK

Name 2

Department

Campus Estate or Research Centre

NOTTINGHAM SCIENCE & TECHNOLOGY PAR

Road Name

UNIVERSITY BOULEVARD

Building

UNIT 2-4 FARADAY BUILDING

District

Town

NOTTINGHAM

County

NOTTINGHAMSHIRE

Postcode

NG7 2QP

Country

ENGLAND

Tel Number 0115 951 7260

Fax Number 0115 951 7261

E-mail

HSE Division MIDLANDS

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

<table>
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<tr>
<th>Date Premises Closed</th>
<th>Name</th>
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<td>NG7 2QP</td>
<td>ENGLAND</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes]

Give brief details of the genetic modification safety committee

We will use an existing Genetic Modification Safety Committee based at the Waltham Centre for Pet Nutrition (WCPN), Waltham-on-the-Wolds, Leicestershire - a different division of Masterfoods, UK. This committee consists of three WCPN associates (Head of Research, Laboratory Manager, Site Safety Manager) and an expert Molecular Biologist from Mars Symbioscience. The committee meet as necessary to discuss new work as requested whether it falls within the current containment level or if an increase in containment level is required. The committee also meets quarterly to review the progress of GMO studies, whether new work has been requested or not.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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Non-microbial

Other (please specify) Tick if confidential

Bacteriology Yes Parasitology Transgenic Birds Microbiology Research

02/03/2022
As work will be conducted with commercially available attenuated strains. All material resulting from studies with GMMs will be autoclaved or treated with a suitable antibacterial detergent (e.g., Trigene) and then transported for incineration by a registered waste carrier and disposer or disposed of to the sewage system respectively. GMM material undergoing inactivation procedures will be periodically tested for viability using standard counting methods.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The genetic modification safety committee (GMSC) considers that the work outlined in the risk assessments will be confined to specified laboratories and falls well within the existing safety protocols. As a result, the work poses minimal risk to individuals working with the genetically modified micro-organisms. As the work involves commercial attenuated strains, there will be minimal risk to the environment following accidental release. Equally, as this work is confined to the laboratory and will be used as a laboratory tool, it poses minimal risk to the employees. Therefore, the GMSC considers that this work requires level 1 containment.
### GM Centre Number: 935

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| Tel Number                          | 01235554747             |
| Fax Number                          |                         |

| E-mail                               |                         |

| HSE Division                         | EAST AND SOUTH EAST     |

| Comments                             | Name Change from Syntaxin Ltd notified on 29/07/2015, Relocation from 4-10 The Quadrant, Barton Lane, August 2016 |

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### Premises Addresses

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<td>4-10 THE QUADRANT</td>
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<td>PORTON DOWN</td>
<td>SALISBURY</td>
<td>WILTSHIRE</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Y

Give brief details of the genetic modification safety committee

Chair, Biological Safety Officer, Management, Scientist, Secretary and Lay member will meet as required.

<table>
<thead>
<tr>
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Tick if confidential

- [ ] Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Please see CU2 form for the ‘Expression of Novel Therapeutic Proteins’ notification GM36/06.1 and the submitted ‘Co-expression of novel therapeutic agents and activation protease’ GM935/06.1 with reference to the covering letter for GM935 explaining these notifications.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

None.

Project Ref 935/12.1

Date Ackn'd 23/04/2012

CU2 Project Title Lentiviral shRNA SNARE knock-down in mammalian cell lines

Class Class 2

Culture Vol Class 2 1-50 Litres

Consent Granted

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The aim of the work is to use small hairpin RNA (shRNA) Lentiviral particles to silence SNARE expression via RNA interference (RNAi) in mammalian cell lines to cause knockdown of the SNARE proteins and to generate data to investigate the relationship between the inactivation of specific SNARE proteins and inhibition of cellular secretion.

#### Recipient or parental organism


#### Host/vector system

A number of vectors used are TRV vectors, the RNAi consortium (TRC) is a collaborative effort based at the Broad Institute of MIT and Harvard, and includes six MIT and Harvard associated research institutions and five international life science organizations. These vectors contain a pUC origin of replication and U6 promoter. Each MISSION™ shRNA clone will be constructed within the Lentivirus plasmid vector pLKO.1-Puro or TRC2-pLKO-Puro. The pLKO.1-Puro and TRC2-pLKO-Puro vectors contain bacterial (Ampicillin) and mammalian (Puromycin) antibiotic resistance genes. The MISSION™ Lentivirus particles use the third generation of Lentivirus system and is a three-plasmid system consisting of three main components:

1. The packaging vector, which contains the minimal set of Lentiviral genes required to generate the virion structural proteins and packaging functions.
2. The vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector, which provides the heterologous envelope for pseudotyping.
3. The shRNA transfer vector, which contains the sequence of interest as well as the cis-acting sequences necessary for RNA production and packaging. The multi-plasmid approach results in no single plasmid containing all the genes necessary to produce packaged Lentivirus.

The pLKO.1 vector is a Lentiviral (HIV)-based plasmid. The vector is regarded as a Biosafety Level 2 material and safe to use due to its modified features (deletion of a number of accessory genes implicated in the virulence of HIV, minimal genome of the viral particles, non-replicating and self-inactivation features), making it incapable of producing virus once infected into the host cell. The only change between the pLKO.1 vector and the TRC2 vector is the addition of the WPRE (Woodchuck Hepatitis Post-Transcriptional Regulatory Element). This allows for enhanced expression of transgenes delivered by Lentivirus.

#### Origin & function

The lentiviruses will encode shRNA sequences that cause knock-down of SNARE proteins in mammalian cells

#### Evaluation of foreseeable effects

The most hazardous Lentiviruses will encode shRNA sequences that cause knock-down of SNARE proteins in mammalian cells but as the replication deficient viral particles will be used to stably transduce mammalian cell lines, after a short period of culture the GMM cell lines created from Lentiviral transduction will be free of any functional virus and will not therefore be of any harm to human health and the environment.

The lentiviruses are replication deficient so secondary infections should not occur. The cell lines that will be infected require complex media and controlled conditions for survival so escape into the environment is not expected.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

TriGene ADVANCE will be used as the general disinfectant/decontaminating agent and has been shown by the manufacturer to be effective against bacteria, viruses and cells. TriGene ADVANCE is certified Sporicidal, Mycobactericidal, Virucidal Fungicidal and Bactericidal under EN and HIRL protocols. It breaks down and inactivates DNA/RNA. TriGene ADVANCE is included on the DEFRA list of approved disinfectants.

For general disinfection on non-soiled surfaces, TriGene ADVANCE will be used at 2 parts concentrate in 100 parts water (2% v/v). 2% Trigene will be sprayed over the surface, the surface wiped with tissue, then rinsed with milliQ water and wiped followed by spraying with 70% v/v ethanol and wiped.

All spent contaminated growth media containing mammalian cells or serum within plasticware will be decontaminated by adding Trigene ADVANCE to 5% v/v and allowed to stand for 24h before disposal down the drain. All plasticware will be placed after decontamination in yellow clinical waste burn bins.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Syntaxin Ltd GMSC reviewed the risk assessment on 15/03/3012 and recommended that the proposed work had been adequately reviewed and a suitable risk assessment was in place.

Project Containment

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Animal Units

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GM Centre Number: 936

Data Premises Notified (Originally) 27/02/2006

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

GLYCOFORM LIMITED

Name 2

Department

Campus Estate or Research Centre

Building UNIT 44C

District MILTON PARK

Town ABINGDON

County OXFORDSHIRE

Postcode OX14 4RU

Country ENGLAND

Tel Number 01235 820 463

Fax Number 01235 820 362

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

GMSC approves all risk assessments in Glycoform. No work can commence unless it is authorised by GMSC. The committee is staffed by senior managers and scientists with a wide range of experience and education. Signed copies of risk assessments are filed/scanned and kept for 10 years. COSHH assessments must be carried out in addition to any GMM assessments. In case of uncertainty over risk an external body/contractor will be consulted and HSE informed. GMSC members have wide range of experience and training covering biology, microbiology, medicine, chemistry and engineering: Research Manager, Clinical Manager/Consultant, Research Scientist, Head of Protein Manufacturing.

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Non-microbial

Other (please specify) Tick if confidential

Bacteriology |

Yes

Parasitology |

Transgenic

Birds |

Microbiology

Research

Virology |

Transgenic

Animals |

Transgenic

Fish |

Gene Therapy

Mycology |

Transgenic

Invertebrates |

Transgenic

Plants |

Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity
Any equipment that come into contact with GMM will be inactivated using validated autoclave (6 log kill). All areas in which GMM work is done will have 70% aqueous solution of ethanol and 1% Virkon solution (validated for E. coli inactivation). Fresh Virkon solutions will be made daily after each use. All work surfaces on completion of work or after any spill will be wiped with a 1% Virkon and sprayed with 70% ethanol, then allowed to air dry. In case of spillage, the area will be covered with Virkon powder, left for 10 min then scraped using paper towels, then disposed into biohazard waste container. Virkon provides ~5 log kill. During all times protective clothing will be used. Care will be taken to minimise aerosol formation.

Risk assessment approved by the GM Safety Committee.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment  

Risk assessment approved by the GM Safety Committee.
**GM Centre Number: 937**

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**Name**

TRIPOS DISCOVERY RESEARCH LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

BUDE-STRATTON BUSINESS PARK

**Road Name**

**District**

**Town**

Bude

**County**

DEVON

**Postcode**

EX23 8LY

**Country**

ENGLAND

**Tel Number**

01288 359 359

**Fax Number**

01288 359 222

**E-mail**

**HSE Division**

WALES AND SOUTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

A genetic modification safety committee comprising the Safety and Environment Officer, Director of Research Operations and a Biologist will review and advise on risk assessments, good microbiological practice, containment and control measures and waste management as necessary. All other employees will be consulted on the safety issues associated with the GMO activities through the Tripos safety committee.

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Other (please specify)  
Tick if confidential

Bacteriology  
Parasitology  
Transgenic  
Microbiology  
Research  
Virology  
Transgenic Animals  
Transgenic Fish  
Gene Therapy  
Mycology  
Transgenic Invertebrates  
Transgenic Plants  
Other (please specify below)  
Yes

Other(s)  
The storage and small scale culture of established genetically modified containment level 1 cell lin

For activities involving GMMs, describe the waste management measures which will apply to the activity
Solid waste will be autoclaved before off site incineration with existing Tripos hazardous solid wastes. Liquid wastes will be treated with Virkon and then autoclaved before off site incineration with existing Tripos bulk hazardous liquid wastes.

The cells are thought highly unlikely to readily survive for significant durations outside their precise culture conditions and temperatures and autoclaving will be 100% effective. Autoclave cycle records will be filed and autoclave efficacy will be tested once a year.

Regular biological testing of the autoclave operations will be carried out at a suitable frequency.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Satisfied that the cell culture work proposed presents a suitably low risk to humans or to the environment and the containment and waste management arrangements are suitable and appropriate for the genetically modified organisms involved. The work does not involve genetic modifications and the cell lines involved are not readily viable outside their laboratory culture conditions. Employee safety should be adequately controlled by the operation of the biology laboratory at containment level 1.
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<td>BUILDING 260</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Committee comprises a member of the management team, the biological safety officer and two employee representatives. The committee will discuss the requirements for the assessment of new procedures and review existing procedures. The committee will meet on a monthly basis or as required by the biological safety officer.

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All liquid waste resulting from bacteriological procedures or activities involving the propagation of cells or non-pathogenic viruses will be treated with Virkon tablets to a final concentration of greater than 1% (weight/vol). Virkon is recommended by the manufacturer for the treatment of liquid waste.

All solid waste resulting from bacteriological procedures or activities involving the propagation of cells or non-pathogenic viruses will be placed in designated waste bags or bins. Waste will be clearly labelled as biohazard. Bags will be closed but not sealed and autoclaved prior to incineration. The autoclave is sited in building 405, Babraham Institute. The location of the autoclave in the vicinity of the laboratory complies to containment level 2 operation. The autoclave is routinely tested on a six monthly basis to confirm working temperatures and pressures are achieved. Autoclave waste is transported to an off site facility. Vetspeed, (GM Ref. GMGM898) for incineration.
Withdrawn N  

Tick if notifying a connected programme of work N  

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use

To develop safer and more immunogenic vaccines against Mycobacterium tuberculosis using cell lysate preparations obtained from the recombinant Mycobacterium bovis strain rBCG AFR-O. The lysates will be enriched in heat shock proteins and antigens cross reactive with Mycobacterium tuberculosis.

Recipient or parental organism

The parental organism is Mycobacterium bovis, strain Danish 1331 (Staten Serum Institute Lot No. #103063) ACDP Classification 2. This strain is a commercially available vaccine strain and as such is used as a live vaccine.

Host/vector system

Mycobacterium bovis BCG strain Danish 1331 has been modified by site specific recombination into the UreC site and transfection with the plasmid pAF105. Plasmid pAF105 was synthetically derived and encodes the Kanamycin gene and promoter, the OriM, which will permit plasmid replication, Mycobacterium bovis antigens Ag85a, Ag85b and TB10.4 controlled by the Ag85b promoter and does not contain E. coli derived sequences required for plasmid mobilisation.

Origin & function

Mycobacterium bovis BCG strain Danish 1331 is modified by site specific Recombination into the UreC site and transfection with the plasmid pAF105. The combined affect of the expression of the selected inserts is to enhance immunogenicity of the Mycobacterium bovis BCG strain by the over-expression of selected secreted antigens and the expression of a cytolysin which will facilitate the availability of antigens to the immune system.

The pfo A cytolysin gene from Clostridium perfringens (GenBank Accession No. CPE0163) was integrated into the genome of the host organism by site specific recombination at the UreC locus. The pfo A gene encodes perfringolysin O (Pfo), a cytolysin, cloned from Clostridium perfringens (ACDP Class 2). Cytolysin mediates escape of the organism from phagosomal vacuoles into the cytoplasm of host cells, following ingestion of the organism by the host macrophage. Phagosomal escape represents a unique mechanism to facilitate MHC class 1 antigen presentation of mycobacterium antigens. The pfo A gene, has been modified by substitution of a cystine at position 137 to a glutamine, to limit the inherent cytotoxicity of cytolysin in mammalian cells. The mutant pfoAg137q demonstrates a shortened half life in the host cytosol and is more sensitive than the unmodified protein to proteolytic degradation, these properties combined render the cytolysin less toxic than the native protein in mammalian cells. Experimental evidence suggests that cells exposed to mutant pfo Ag137q are not killed when mutant pfo Ag137q is expressed by a comparable intracellular pathogen, Listeria monocytogenes.

The 'homologous' antigens Ag85a, Ag85b and TB10.4 are encoded by the plasmid pAF105. Antigens Ag85A (Genbank Accession No. P0A4V2) and Ag85B (Genbank Accession No. P12942) from Mycobacterium bovis possess a mycolyltransferase activity required for the biogenesis of trehalose dimycolate (cord factor), a dominant structure necessary for maintaining cell wall integrity. Ag85a and Ag85b are secreted proteins and represent major antigens in the host response.
TB10.4 (Genbank Accession No. AF2122/97) of Mycobacterium bovis has been identified as a major antigen in human immune responses to Mycobacterium tuberculosis and is listed in the genbank database as an uncharacterised protein of 10kDa, and is highly conserved in bacteria, ESAT-6 like and of unknown function. This protein is not a known toxin or oncogene and has previously been over expressed in E. coli. The plasmid sequence, pAF105 is maintained in the host organism using antibiotic (Kanamycin) selection.

Evaluation of foreseeable effects

ALTERATIONS IN HOST RANGE; Hazards from the alteration of existing pathogenic traits (eg alteration of host range or tissue tropism):

* expression of the 'homologous' Ag85B and Ag85A and TB10.4 in the host organism is unlikely to modify the host range or tissue tropism of BCG Mycobacterium bovis. Although the proteins will be expressed at higher than normal levels, the majority of the protein will be secreted and the antigens will be highly enriched in the culture supernatants (in culture) or cellular cytosol. Over expression of the antigens Ag85A and Ag85B and TB10.4 is likely to increase the immunogenicity of Mycobacterium bovis as has been previously shown. No changes in tissue tropism or alterations in host range are anticipated.

* the pfo Ag137q protein is secreted, is susceptible to proteolytic degradation, and the consequences of expression are understood and have been studied extensively in other recombinant host organisms. No changes in tissue tropism or alterations in host range are anticipated.

ALTERED PATHOGENESIS: altered interactions with the human immune response:

* the overexpression of the 'homologous' antigen Ag85A has previously been shown to increase the immunogenicity of Mycobacterium bovis. Immunogenicity is commonly linked to pathogenicity, and it is unlikely that a 'more immunogenic' organism will be more pathogenic in the immune competent animal.

* The mutant pfoAg137q gene from Clostridium perfringens has previously been expressed in the intracellular pathogen Listeria monocytogenes. Both the parental strain of Listeria monocytogenes and the strain expressing the mutant cytolsin, pfoAg137q, had similar LD50 values in a virulence model in mice. No increased pathogenesis is anticipated in the selected host organism, Mycobacterium bovis.

LOSS OF DRUG SUSCEPTIBILITY. AFR-01 is resistant to Kanamycin however in the event of infection with this recombinant strain it is anticipated that the antibiotics rifampicin and isoniazid derivatives which would be the antibiotics of choice in cases of infection with Mycobacterium bovis, would remain effective.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste resulting from bacteriological procedures involving the propagation, disruption and analysis of the GMO will be treated with Virkon tablets to a final concentration of greater than 1% (weight/volume). Virkon is recommended by the manufacturer for the treatment of liquid waste. Detailed information is available at www.antechh.com. Laboratory benches and equipment will be wiped down with 1% (weight/volume) Virkon solution or where this is inappropriate a solution of 70% ethanol in water on absorbent paper and then paper will be disposed of as solid waste. Spills will be treated with Virkon as above and soaked up on absorbent paper, and treated as solid waste.

All solid waste resulting from bacteriological procedures involving the propagation, disruption and analysis of the GMO will be placed in waste bags, which will be closed but not sealed, the bags will be placed in designated bins. Waste will be clearly labeled as biohazard. Bins will be closed but not sealed and autoclaved prior to incineration. The autoclave is sited in Building 405 Babraham Institute. The location of the autoclave in the vicinity of the laboratory complies with containment level 2 operations. The autoclave is routinely tested on a six monthly basis to confirm working temperatures and pressures are achieved. Autoclaved waste is transported to an off site facility, Vetspeed, (GMRef. GMGM898) for incineration.
The risk assessment has been reviewed and containment level and activity class are deemed appropriate to the procedure.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick to confirm that it is attached to this form

Is an emergency plan required according to regulation 20?

Yes

Project Containment

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

Given the low containment level of the work to be done, the genetic modification safety committee overlaps with the biological safety committee. This committee currently consists of:
- Departmental Safety Officer
- Biological Safety Officer
- Safety Officer for the School of Biological Sciences
- Technician and Chief Electron Microscopist

The committee will initially meet every 6 months. Should substantially more biological/GM work be done, this frequency will increase. Should the work carried out on the premises change to a higher containment level, a separate genetic modification safety committee will be set up.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 1 (GMMs)</td>
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<tr>
<td>Non-microbial</td>
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</table>

Other (please specify)  

Tick if confidential  

02/03/2022
All solid waste will be autoclaved on site and subsequently disposed of with normal waste. All liquid waste will be sterilised using Virkon or another, similarly effective, reagents following the manufacturer's guidelines in terms of dosage and exposure times. Waste will subsequently be disposed of as normal, non-contaminated, non-dangerous liquid waste.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The members of the genetic modification safety committee have studies the attached risk assessment and are happy that all foreseeable risks have been identified. The preventative measures contained within the risk assessment are deemed adequate for the hazards associated with the proposed work.

Project Ref 939/17.1

Date Ackn’d 19/01/2017

Date Project Ceased

CU2 Project Title Study of GM P. falciparum parasites using optical microscopy to understand the invasion mechanism of parasites in the blood-stage of malaria disease

Consent Granted Yes

Non-GMM

Consent Granted

Project notified under transitional arrangements No
### Purposes of the contained use

To investigate by live optical imaging the molecular details of human-parasite host-pathogen interactions during the *P. falciparum* blood stages, with a particular focus on erythrocyte invasion and how that process is influenced by natural genetic variation in both host and parasite. A better understanding of critical interactions during the invasion process may lead directly to the development of new prevention and control measures such as drugs and or vaccines. This work is linked to work at the Wellcome Trust Sanger Institute which seeks to develop and apply methods for genome-scale genetic manipulation of *P. falciparum* parasites, which has been approved by HSE.

### Recipient or parental organism

**Plasmodium falciparum**

### Host/vector system

Host: standard lab-adapted *Plasmodium falciparum* parasite strains will be used, primarily 307, as that is the strain from which the *P. falciparum* reference genome sequence was generated. In certain limited circumstances other lab-adapted strains, such as 7GB, HB3, Dd2, or strains recently adapted from clinical isolates will be used in place of 307.

Vector system: vectors will be obtained from ATCC through the Malaria Research and Reference Reagent Resource Center (www.mr4.org) and then adapted by Dr Julian Rayner's group (Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA) to contain the gene or gene fragments of interest. Essentially all vectors consist of three elements: antibiotic resistance gene with associated expression elements to allow propagation in *E. coli* (usually encoding Ampicillin or Kanamycin resistance); drug resistance cassette with associated *Plasmodium* expression elements to allow positive selection in *P. falciparum* (usually anti-folate drug resistance encoded by *T. gondii* or *H. sapiens* DHFR; Blasticidin and Gentamicin resistance are also used); an expression or gene targeting cassette to either express the gene of interest in *Plasmodium falciparum* or direct insertion of the construct to a specific genomic location in order to ablate or modify the endogenous gene.

### Origin & function

We expect that the majority of modified lines tested at the Physics Department will focus on genes predicted to be involved in *P. falciparum* erythrocyte invasion, estimated to be approximately 400 genes and covering several broad classes of proteins including invasion ligands (EBL family, RBL family, AMA1), merozoite surface proteins (MSPs), invasion motor proteins (MyoA, MTIP, GAP45, GAP50), cytoskeleton components (IMCs, GAPMs) and regulatory enzymes (kinases and palmitoyl transferases). However, as larger-scale screens expand, other classes of genes may be included.
Fluorescent protein fusions and other marker proteins will also be inserted in some strains to facilitate fluorescence imaging, and these have no biological action.

**Evaluation of foreseeable effects**

The recipient organism does not contain any disabling mutations and is classified by the ACDP as a Hazard Group 3 organism. If altered from the wild-type, there is no available evidence to suggest that any of the proposed engineered mutations could increase the virulence, infectivity or stability of the pathogen. There is no available evidence that any disabling mutation in the recipient organism could be overcome e.g. due to insertion of a foreign gene or reversion. The GMOs have no altered tissue tropism and drug resistance to frontline prophylactics should not arise. The GMM would not elicit any alteration in the immune response of the host. The fluorescent and marker protein insertions are not expected to have biological activity in humans. GM *P. falciparum* parasites tend to grow more slowly, and are rapidly outgrown by wildtype parasites under experimental conditions. Therefore GM *P. falciparum* strains are expected to pose no increased hazard to human health or the environment relative to non-GM *P. falciparum* strains. The GM and wild-type *P. falciparum* will be handled in restricted access laboratories. Access will only be given to workers that have undergone induction and training to work in the derogated CL3 laboratory (CL3*). It is derogated for air handling aspects as there is no evidence for aerosol based transmission (see Section 11). All workers are instructed to seek medical attention immediately if they experience flu-like symptoms or an unexplained fever. Infection would be readily treatable with anti-malaria Is. Infection by *P. falciparum* occurs solely by inoculation into the bloodstream. Sharps will be banned in the laboratory, apart from the use of glass slides to assess culture growth. There is no evidence for aerosol based transmission, therefore the risk of infection by *P. falciparum* will be low. There are no natural non-human hosts of *P. falciparum* in the environment surrounding the laboratory, therefore *P. falciparum* would not be able to subsist outside of the laboratory environment. Therefore the risk of foreseeable effects through work with GM *P. falciparum* is judged to be low.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Does not apply here.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

The same derogations that apply to non-GM *P. falciparum* (and which follow primarily from the fact the parasite is not airborne) will apply to the culturing and experiment procedures with these GM strains. These derogations are:

- The requirement that the laboratory is sealable for fumigation
- The requirement that the laboratory is at negative pressure relative to the pressure of the immediate surroundings
- The requirement that an autoclave be sited in the laboratory suite
- The requirement for HEPA filtered extract and input air

The derogations are justified as there is no air-borne infection route and the parasites cannot survive dessication. Infection of personnel in the absense of a vector requires contact with the blood stream, which is unlikely as sharps use is restricted in the laboratory.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All solid waste that has contacted GM *P. falciparum* will be immersed in Distel disinfectant and then double bagged in unmarked autoclaved bags to be autoclaved in an autoclave validated for waste to reach 135°C for 10 minutes. Once autoclaved, provided that the autoclave is displaying a completed autoclave cycle, waste is removed from the autoclave by trained personnel and taken to the landfill waste stream.

Liquid waste containing the GM *P. falciparum* parasites will have Distel added at a 10% concentration, and will be left...
overnight, before being poured down the sink and flushed with tap water.

The GM safety committee considered this application on the 5th October 2016 and commented that 'as there is no airborne route of infection, the activity can be carried out at derogated CL3' and that 'no sharps are permitted in the laboratory, apart from the use of glass slides for the assessment of parasites that have been inactivated by the staining process'.

Please enter comments on the GM safety committee on the risk assessment

The GM safety committee considered this application on the 5th October 2016 and commented that 'as there is no airborne route of infection, the activity can be carried out at derogated CL3' and that 'no sharps are permitted in the laboratory, apart from the use of glass slides for the assessment of parasites that have been inactivated by the staining process'.

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Project Ref 939/20.1

Date Ackn'd 04/09/2020

Date Project Ceased

CU2 Project Title The Physics of Antimicrobial Resistance

Class

CultureVolClass2

CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

02/03/2022
### Project Additional Information

#### Purposes of the contained use

Bacteria are simple life forms, whose understanding of their basic processes will begin to allow us to unravel those processes that underpin all life. They are made more important by the fact that some of them can cause human disease and that existing antibiotics are becoming useless due to the spread of resistance.

**Aim:** The aim of the project is to understand how the bacterium Staphylococcus aureus is able to grow and divide.

**Objectives:**
1. To use fluorescent reporters to map cellular components during growth and division.
2. To determine the role of specific components in growth and division.
3. To elucidate the mechanisms of antibiotic action and associated resistance.

#### Recipient or parental organism

- **Staphylococcus aureus**

#### Host/vector system

We will use Staphylococcus aureus SH1000, a rbsU+ derivative of S. aureus 8325-4 (Horsburgh et al., 2002) which we will refer to as the wild-type, and its methicillin resistant mutants (MRSA), some of them carrying markers for erythromycin, kanamycin and ampicillin.

For localization experiments, derivatives of pMUTIN4 vector (Vagner et al., 1998). This vector harbours E. coli origin of replication and kanamycin and ampicillin resistance markers. Also, the derivatives of this vector that will be used in the study will carry transcriptional fusion of fluorescent proteins’ genes (mNeonGreen and eYFP) with gene of RNA polymerase β’ s.u. (thought to be involved in meticillin resistance) for localization experiments.

None of the vectors used will encode resistance to antibiotics routinely used in the treatment of infections caused by S. aureus. None of the vectors used are hazardous and they are very unlikely to confer any advantage to the host bacterium.

#### Origin & function

Genetic material modified in the bacteria will include

1. inserted fluorescent reporter fusions, mNeonGreen derived from Branchiostoma lanceolatum and eYFP a mutant of GFP originally derived from the jellyfish Aequorea victoria) to allow subcellular localisation of components
2. inactivation of existing genes involved in bacterial physiology, particularly in cell wall homeostasis, to determine biological function.

#### Evaluation of foreseeable effects

The S. aureus strains considered here are capable of causing treatable disease in humans. Symptoms vary depending on infection site. Laboratory practices that could lead to infection are those that have the potential of causing trauma of the cutaneous barrier and contact with aerosols. Good laboratory practice and the specific containment level 2 control measures including use of containment laboratories, appropriate handling of sharps and avoidance of sample treatments that may produce aerosols will minimise both the risk to the worker and escape of the organisms.

In the event of accidental exposure, the worker will be advised to seek immediate medical advice and will be provided with information on strain characterisation, including...
antibiotic susceptibility. Some of the donor strains will contain resistance markers to erythromycin and some vectors to be used encode for resistance markers to kanamycin and ampicillin.

Uncontrolled dissemination of genetic material containing antibiotic resistance markers might theoretically lead to increase of antimicrobial resistance in other bacterial species. Good laboratory microbiological practice and the specific containment level 2 control measures will provide a Contained Use of the organisms that will minimise the risk to the environment.

All alterations in the genetic material will have no impact on the level of harm that the used strains may cause to human health or to the environment. All the vectors that will be used in the experiments have no known negative impact on human health and are stably inserted to the bacteria before arrival in Cambridge. The fluorescent proteins that will be synthesized from the fusion constructs are considered as non-hazardous for human health or the environment.

It is therefore highly unlikely that the generated strains will be able to harm human health or the environment to a greater degree than the parental strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated solid waste (agar plates, disposable plasticware, etc.) will be autoclaved in an autoclave validated to reach 135 degrees celsius for 10 minutes and subsequently disposed via the standard waste route.

Liquid cultures will be chemically-inactivated (Virkon 5% for a minimum of 30 minutes) or autoclaved as for solid waste prior to disposal down the sink.

All contaminated glassware and reusable plasticware will be decontaminated as above.

In the event of the waste autoclave breaking, either a spare autoclave will be validated to deactivate waste, while the waste autoclave is fixed, or alternatively waste will be chemically decontaminated and transferred in sealed containers to an alternate autoclave at the nearby Department of Veterinary Medicine for final destruction.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The initial draft of this GM project and attendant GM assessment and related Risk Assessment for the base organism were provided to the Committee on 12th May 2020.

Requests were made to the researchers submitting the assessments to provide more details on the organisms used, details of certain equipment and their safety measures and the role of certain genes mentioned.

The researchers added these to the assessments which were returned to the Committee for re-review on the 4th June 2020.

On the 9th July 2020, after no further changes or details were requested by any member of the Committee, the assessments were passed locally to move this Notification.

02/03/2022
The project will attempt to visualise the growth patterns of microorganisms on materials.

Aims:
1. Visualise the bacterium on glass slide alone and validate microscopic techniques.
2. Use the pre-validated methodologies to visualise the growth patterns of PA on materials.

Recipient or parental organism

Pseudomonas aeruginosa and Burkholderia thailandensis
Pseudomonas aeruginosa is a Gram-negative bacterium principally found deep in tropical soils. In immunocompetent individuals, it can only cause eye infections but in immunocompromised people, it can cause skin and lung infections. It is the prototypical organism for studying bacterial biofilms.

Burkholderia thailandensis is also a Gram-negative bacterium principally found deep in soils. It rarely causes disease in animals or humans. It is being used here as a low risk model for more dangerous Burkholderia bacteria species.

We will be using laboratory adapted, highly characterised and sequenced wild-type strains both with modifications and unmodified controls.

Wild-type PA has been transduced with the pSEDQS plasmid, facilitating constitutive expression of genes for various fluorescent proteins. Wild-type BT has been transduced with a combination of pk18mocsacB (Schafer, et al., 1994) and pSLC plasmids (Eshaghi, Mehershahi, & Chen, 2016) or transposon libraries to allow expression of fluorescent reporter protein markers.

The plasmids also carry genes for carbenicillin, kanamycin and no resistance, respectively. None of the vectors used are hazardous and they are very unlikely to confer any advantage to the host bacterium.

Host/vector system

Genetic material modified in the bacteria may include
1) inserted fluorescent reporter fusions,
2) antibiotic resistance (Kanamycin and carbenicillin) markers

The bacteria were modified by transducing the vectors named above into the specified bacterial strains. The P. aeruginosa strain was modified in the lab of Professor Marvin Whiteley, then at UT Austin. The B. thailandensis was modified in the lab of Associate Professor Gan Yunn When at NUS.

These modifications are intended to allow easy detection and localisation of bacteria in experiments on their growth and survival on material surfaces.

Origin & function

The P. aeruginosa strains considered here are capable of causing disease in humans. Symptoms vary depending on infection site. Laboratory practices that could lead to infection are those that have the potential of causing trauma of the cutaneous barrier and contact with aerosols. Good laboratory practice and the specific control measures including appropriate handling of sharps and avoidance of sample treatments that may produce aerosols will minimise the risk to the worker. In the event of accidental exposure, the worker will be advised to seek immediate medical advice and will be provided with information on strain characterisation, including antibiotic susceptibility.

The B. thailandensis strains considered here are not considered capable of causing disease in immunocompetent humans.

In the event of accidental exposure, the worker will be advised to seek immediate medical advice and will be provided with information on strain characterisation, including antibiotic susceptibility. Some of the donor strains will contain resistance markers to kanamycin or carbenicillin.

Uncontrolled dissemination of genetic material containing antibiotic resistance markers might theoretically lead to increase of antimicrobial resistance in other bacterial species. Good laboratory microbiological practice and the specific containment level 2 control measures will provide a Contained Use of the organisms that will minimise the risk to the environment.

All alterations in the genetic material will have no impact on the level of harm that the used strains may cause to human health or to the environment. All the vectors that will be used in the experiments have no known negative impact on human health and are stably inserted to the bacteria before arrival in Cambridge. The fluorescent proteins that will be synthesized from the fusion constructs are considered as non-hazardous for human health or the environment.

Evaluation of foreseeable effects

The P. aeruginosa strains considered here are capable of causing disease in humans. Symptoms vary depending on infection site. Laboratory practices that could lead to infection are those that have the potential of causing trauma of the cutaneous barrier and contact with aerosols. Good laboratory practice and the specific control measures including appropriate handling of sharps and avoidance of sample treatments that may produce aerosols will minimise the risk to the worker. In the event of accidental exposure, the worker will be advised to seek immediate medical advice and will be provided with information on strain characterisation, including antibiotic susceptibility.

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It is therefore highly unlikely that the generated strains will be able to harm human health or the environment to a greater degree than the parental strains.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated solid waste (agar plates, disposable plasticware, etc.) will be autoclaved in an autoclave validated to reach 135°C for 10 minutes. Autoclaved material disposed via the standard waste disposal route. The autoclave is calibrated and validated yearly. Autoclave runs are run regularly through the year with bio-indicator test systems (e.g. 3M’s Attest, a Geobacillus spore tube system). Liquid cultures will be chemically-inactivated (Virkon 5% for minimum 30 min) or autoclaved prior to disposal down the sink. All contaminated glassware and reusable plasticware will be decontaminated as above.

In the event of the waste autoclave breaking, waste will be chemically decontaminated and sent to an approved contractor for incineration (Novus).

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units  | Large Scale Activities | Human Clinical Applications

02/03/2022
**GM Centre Number: 940**

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**Name**

THE WELLCOME TRUST

**Name 2**

THE WELLCOME TRUST ADVANCED COURSES

**Campus Estate or Research Centre**

THE WELLCOME TRUST GENOME CAMPUS

**Road Name**

**Town**

HINXTON

**County**

CAMBRIDGESHIRE

**Postcode**

CB10 1SA

**Country**

ENGLAND

**Tel Number**

01223 495 121

**Fax Number**

01223 495 130

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Premises now merged with GM552 03/09/2013

**Date at Which Additional Info Submitted**

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee consists of Advanced Courses Programme Manager, Advanced Course Laboratory Manager, Wellcome Trust Sanger Institute Biological Safety Adviser, 2 x guest instructors who teach on the course that will use containment level 1 organisms.

The lab manager and the two instructors will be responsible for ensuring that containment level 1 regulations are not breached and that all waste disposal regulations are complied with.

The committee will meet annually (the course is held annually) to discuss any changes to the protocols and to review the risk assessments.

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<td>Non-microbial</td>
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Other (please specify)  

Bacteriology  

Parasitology  

Transgenic Birds  

Transgenic Animals  

Transgenic Fish  

Transgenic Invertebrates  

Transgenic Plants  

Microbiology Research  

Gene Therapy  

Other (please specify below)  

For activities involving GMMs, describe the waste management measures which will apply to the activity
PPE
Laboratory coats must be worn in the laboratory and removed when leaving the laboratory suite.
Personal protective equipment including protective clothing must be
i) stored in a well defined place
ii) checked and cleaned at suitable intervals
iii) when discovered to be defective, repaired or replaced before further use.

Personal protective equipment which may be contaminated by biological agents must be
i) removed on leaving the work area.
ii) kept apart from uncontaminated clothing
iii) decontaminated and cleaned or if necessary, destroyed.

Waste disposal.
The chemical waste from the Advanced Course Laboratory is disposed of by a registered contractor.
Incinerated waste and culture waste (including that relating to organisms classified as containment level 1) is disposed of by the Sanger Centre core facility.

**Chemical Waste:**
Dispose of waste chemicals in the labelled waste containers and under no circumstances tip any waste chemical down the sink.
Do not mix different chemicals in the waste containers; use separate containers for each chemical as mixing may cause chemical reactions to occur.
If in doubt about disposal ask a member of the Advanced Courses team.
Chemical containers are then stored in chemical cabinets until collected by a registered chemical disposal contractor.

**Clinical waste:**
Clinical waste should be placed in the yellow clinical waste bags. This includes items such as disposable gloves and azole wipes.
The yellow bags are then collected and stored in UN approved transport containers to await regular collection and incineration by a designated waste contractor.

**Single use disposable items:**
Single use disposable items such as pipette tips, toothpicks, universal bottles, falcon tubes, appendorf tubes should be discarded into disposable plastic (sweet) jars.
When full the jars are sealed with biobazard tape, collected by the waste team and placed in UN approved containers (for collection by a designated waste contractor and final inactivation by incineration).
Biological agents are 100% inactivated using this method.

**Recyclable items**
Any items such as glassware which have been in contact with biological organisms including those subject to containment level 1 regulations should either be treated with disinfectant at the appropriate concentration and exposure time or autoclaved at an appropriate cycle prior to being sent for wash up.

**Sharps**
Sharps for example blades, scalpels, needles, small items of glassware, must be discarded into an approved sharps bin. Never try to retrieve an item placed into a sharps bin. Only fill sharps bins to that approved level (usually the three quarters line, at this point the lid must be secured and the bin put in a designated waste collection point. Bins are disposed of via incineration with residues going to landfill. This method is 100% effective at inactivating biological agents.

**Solid biological agent waste.**
Solid waste including that produced at containment level 1, ie biological organisms grown in Petri dishes, contaminated paper towels or gloves, should be placed in the grey leak proof plastic autoclave boxes lined with an autoclave bag and put in the designated waste collection point. These are collected by the Sanger waste handling team for inactivation by autoclaving. After autoclaving waste is 100% inactivated in terms of biological agents, inactivated material is then placed in yellow incineration bags for storage in UN approved transport containers (to await regular collection and incineration by a designated waste contractor).

**Liquid biological**
Liquid biological waste including that produced at containment level 1 is inactivated using disinfectant eg Virkon, Presept, Tegodyne) at a concentration and contact time appropriate to the level of contamination, which is known to be effective for sterilisation. Where this is not possible (eg chemical incompatibility from a constituent of the liquid waste) then a suitable autoclave cycle will be used. Both of these methods are 100% effective at inactivating biological agents. Where chlorine based disinfectant has been used then liquid waste must not be autoclaved.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

02/03/2022
Page 12114 of 15326
The Advanced Courses Genetic Modification Safety Committee believes that this project should be designated containment level 1. They do not consider the genetically modified organisms covered by this assessment to have any harmful properties associated with the recipient strain, the vector or the inserted material and they are confident that none of the final genetically modified organisms could be hazardous to humans or the environment.

Project Ref  940/06.1

Date Ackn’d  31/05/2006

CU2 Project Title  Inactivation of specific genes, complementation and expression of non-toxic reporter molecules in enteric bacteria

Date Project Ceased  03/09/2013

Class  Class 2

Culture Vol  1-50 Litres

Class Culture Vol Class 2 Culture Volume Class 3-4

Non-GMM  Not Applicable

Consent Granted  Not Applicable

Project notified under transitional arrangements  N

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

We will use classical gene replacement technology, based on PCR or suicide vector mediated gene conversion, to generate specific mutations in specific genes encoded within the genomes of selected enteric bacteria. Also we will introduce genes encoding non-toxic reporter molecules including Green Fluorescent Protein, Luciferase, Chloramphenicol acetyl transferase, beta-galactosidase and non-toxic vaccine antigens into different enteric bacteria. These reporter genes will be expressed from either constitutive or regulated bacterial promoters. The aim of the work is to use the reporter gene products to (1) track bacteria to different cells or sub-cellular locations within tissue culture cell lines or tissue derived from eucaryotic hosts; (2) to detect the activity of different promoters under different conditions.

Recipient or parental organism

The reporter genes will be supplied from existing plasmids as cloned DNA sequences or purchased from commercial suppliers. They will be introduced into any enteric bacteria other than those classified as Class II or above. Examples will be Escherichia coli (other than verocotoxigenic forms), Shigella spp. Salmonella enterica (other than class II agents such as S.Typhi and S.Paratyphi), Citrobacter spp etc.

Host/vector system
The host bacteria will be any enteric bacteria. The genes will either be introduced directly into the chromosome of the bacteria (via suicide vectors or PCR methodologies). Alternatively the genes will be cloned onto non-transferable plasmids such as those based on Col1E1 replicons or pAYC184 (pUC plasmids for example).

**Origin & function**

The genetic material involved is from a variety of sources such as jelly fish (Green Fluorescent Protein), bacteria (beta-galactosidase, luciferase, chloramphenicol acetyl transferase) but will not be cloned from these sources. We will use well-defined existing plasmids or genes from commercial sources. The DNA is simply to be used to report gene expression activity in enteric bacteria under different conditions.

**Evaluation of foreseeable effects**

The genes we have selected are all extremely well characterised and have been used without incident in thousands of research laboratories around the world. We will only use the named reporters associated with this application. If we decide to use any novel reporters we will amend the local risk assessment. To our knowledge there have been no foreseeable effects of these reporters other than high level expression could cause toxicity to the host bacteria (as with over expression of many proteins).

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable other than to disabled S.Typhi derivatives specifically derogated to CL2

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All the experiments will be performed in a well maintained CL2 facility. A copy of the protocol for handling enteric pathogens in these facilities is enclosed for inspection and has been approved by the local safety committee. Briefly we have detailed policies for handling contaminated liquid and solid waste that involved the contained removal of materials for autoclaving. Hence, all contaminated material is autoclaved or incinerated using chemical disinfection procedures using a variety of accepted and validated disinfectants including phenolics (hycolin etc), Virkon S or chloros. All disinfectants are made up and utilised as described in the enclosed protocol. We routinely validate inactivation using a viable count method based on the killing of control cultures of bacteria within accepted ranges. All inactivated waste material is removed using approved clinical waste disposed by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022

Page 12116 of 15326
The aim of the project is to utilise specific strains of Staphylococcus aureus derivatives in an Advanced training course designed to train Research Staff to perform research on the modular basis of infection.

Staphylococcus is an ACDP hazard group 2 pathogen and is consequently able to cause disease in humans and domestic animals. Inserted DNA normally comes from hazard group 2 bacteria with the exception of the non-toxic biological reporter genes.

Inserted genes can encode potential virulence genes but during the construction of the GMMs virulence genes may be inactivated and attenuated variants may be generated. It is unlikely that the manipulation procedures will enhance the virulence of any of the GMMs to a higher level than that of the donor, as a consequence all work will be carried out at containment level 2. Although antibiotic resistance genes such as Kanamycin will be used in the experiments the GMMs will be sensitive to appropriate therapeutic antibiotics. Such as cephalosporins and fluoroquinolones.
Although active genes may be used initially in E.Coli K12, they are unlikely to cause harm out if the context of their natural bacteria. However, it should be recognised that deliberate large scale production of some of the cloned proteins could be hazardous but this is not the aim of these experiments or within the remit of this assessment. Thus, the risk is negligible. Plasmids used in the experiments are non-conjugative and should not promote the transfer of DNA to unintended recipient bacteria.

Origin & function

The genetic material for reporter functions or antibiotic resistance is from a variety of sources such as jellyfish (Green Fluorescent Protein), bacteria (beta-galactosidase, luciferase, chloramphenicol acetyl transferase) but will not be cloned from these sources. We will use well defined existing plasmids or genes from commercial sources. The DNA is simply to be used to report gene expression activity in enteric bacteria under different conditions.

Evaluation of foreseeable effects

In most cases genetic modifications that will be carried out will result in loss of function and therefore is likely to result in attenuation of pathogenesis. Reporter genes we have selected are all extremely well characterised and have been used without incident in thousands of research laboratories around the world. If we decide to use any novel reporters we will amend the local risk assessment. To our knowledge there have been no foreseeable effects of these reporters.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not Applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All the experiments will be performed in a well maintained CL2 facility. A copy of the protocol for handling pathogens in these facilities is enclosed for inspection and has been approved by the local safety committee. Briefly we have detailed policies for handling contaminated liquid and solid waste that involved the contained removal of materials for autoclaving. Hence, all contaminated material is autoclaved or inactivated using chemical disinfection procedures using a variety of accepted and validated disinfectants including phenolics (hycolin etc). Virkon S or chloros. All disinfectants are made up and utilised as described in the enclosed protocol. We routinely validate inactivation using a viable count method based on the killing of control cultures of bacteria within accepted ranges. All inactivated waste material is removed using approved clinical waste disposal by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

There were no specific comments form the local committee other than minor typographic changes or requests for more detail.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
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**Page 12119 of 15326**

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**Name**

MTS CRYOSTORES LIMITED

**Campus Estate or Research Centre**

BLENDIEM INDUSTRIAL ESTATE

**Road Name**

BENNERLEY ROAD

**Town**

Nottingham

**District**

BULWELL

**County**

NOTTINGHAMSHIRE

**Postcode**

NG6 8UY

**Country**

ENGLAND

**Tel Number**

0115 975 3743

**Fax Number**

0115 979 7361

**HSE Division**

MIDLANDS

**Date at Which Additional Info Submitted**

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

Two members of MTS Cryostores Ltd plus one external person (with experience in Biological Safety) make up the Committee who will ensure that the facilities are fit for purpose, that all equipment is adequate and properly maintained. All local rules and procedures will be prepared and staff are correctly trained and that emergency plans are in place. The said Committee will meet on a monthly basis and minutes will be recorded, signed and archived. The Committee will meet on each consideration of a client's request.

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Other (please specify)</td>
<td>A room dedicated to the storage of material in low temperature freezers</td>
<td>tick if confidential</td>
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Bacteriology | Parasitology | Transgenic | Microbiology | Research |
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<td>Other (please specify below)</td>
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<td>GMMs will be stored in appropriate storage containers within the freezer units. There will be no ..</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity
Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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<tr>
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<td>GREATER LONDON</td>
<td>WC2A 3PX</td>
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<tr>
<td>020 7242 0200</td>
<td>020 7269 3647</td>
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Premises Addresses

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<td>Northwick Park</td>
<td>HARROW</td>
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<td>MIDDLESEX</td>
<td>HA1 3UJ</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

LGMSC meets annually consists of:
The Head of Health and Safety
General and Biological Safety Officer
2 Laboratory heads
Research Scientist
Senior Scientific Officer
Maintenance Manager
Occupational Health Physician

The Head of Health and Safety reports to Cancer Research UK’s Safety Committee which meets 4 times a year.

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  Tick if confidential
| Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research |
| Virology | Transgenic Animals | Transgenic Fish | Gene Therapy |
| Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below) |
| Other(s) | Molecular biology research |

For activities involving GMMs, describe the waste management measures which will apply to the activity:

All liquid waste will be deactivated in a Virkon trap (1%) and disposed of after 24hr, as per manufacturer's instructions; all solid waste will be autoclaved at 121°C for 30 minutes prior to incineration by validated means through a licensed contractor. Accidental spillages of liquid waste will be cleaned with 2% Virkon.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

There is nothing unusual or hazardous in the proposal.
GM Centre Number: 943

<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
<th>Transferred from 1992 Regs?</th>
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Name

PIRAMAL HEALTHCARE UK LIMITED

Name 2

Department

Campus Estate or Research Centre

LABORATORY R431

Building

THE WILTON CENTRE

Road Name

District

Town

REDCAR

County

CLEVELAND

Postcode

TS10 4RF

Country

ENGLAND

Tel Number

01484 433 513

Fax Number

E-mail

HSE Division

YORKSHIRE AND NORTH EAST

Comments

Previously NPIL Pharmeuticals (UK) Ltd

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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<td>LEEDS ROAD</td>
<td>HUDDERSFIELD</td>
<td>YORKSHIRE</td>
<td>HD1 9GA</td>
<td>ENGLAND</td>
<td>N</td>
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<td>CLEVELAND</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Composition of the Genetic Modification Safety Committee:
Technology Director, Microbiological Safety Officer, Safety, Health and Environment Committee representative, at least two laboratory based research staff.

Mode of operation:
The committee will meet monthly as part of the overall safety, health and environment review and will review ongoing projects. Ad-hoc meetings will be arranged as required due to changes in existing projects and the emergence of new projects. The outcome of the GMSC meeting will be shared with the whole research and development team through the monthly R&D review and more widely through the site Safety Committee.

<table>
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<tr>
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</table>
All laboratory liquid microbiological waste will be treated either by autoclaving in a validated autoclave, by chemical disinfection according to manufacturers instructions or by a method validated to give at least a 5-log reduction in viable count. Solid laboratory microbiological waste will be disposed of by incineration off site by a licensed contractor according to the site standard procedure. All solid waste destined for off-site incineration will be transported in labelled, sealed, unopenable plastic incineration bins. Microbiological waste derived from large scale activities will be inactivated by validated heat treatment, or under exceptional circumstances if heat treatment is not possible then treatment with acid and base will be used to inactivate prior to disposal via the site effluent treatment plant.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The GMSC have reviewed the risk assessment and confirm that the control and containment measures proposed are appropriate for the proposed work.
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**Name**
ADDENBROOKE'S HOSPITAL, CAMBRIDGE UNIVERSITY HOSPITALS NHS FOUNDATION TRUST

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**
HILLS ROAD

**District**

**Town**
CAMBRIDGE

**County**
CAMBRIDGESHIRE

**Postcode**
CB2 2QQ

**Country**
ENGLAND

**Tel Number**
01223 336946

**Fax Number**
01223 216926

**E-mail**

**HSE Division**
EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**
02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Chair - Lecturer in Neurosurgery, Principal Investigator - Consultant Neurosurgeon, Pharmacy Rep, UNISON Rep, Neurotheatres Rep, Hospital R & D Department Rep, Nursing Staff Rep, Reader in Virology, University Safety Officer, Clinical Director of the Department of Neurosciences, Domestic Services Manager.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) 
A Block: Neurotheatres & Neurosurgery Pharmacy

Tick if confidential

Bacteriology

Parasitology

Transgenic Birds

Microbiology Research

Virology

Transgenic Animals

Transgenic Fish

Gene Therapy

Yes
Waste will be generated during the preparation of Cerepro by the appropriately trained pharmacy staff and following the injection of Cerepro into the tumour bed of patients with high grade Glioma.

The waste will be disposed of in the following manner:
Disposable equipment will be placed in biohazard bags or sharps bins and autoclaved.
Any spillages in the safety cabinet or in open areas will be covered with 2% Virkon or 2% sodium hypochlorite for a minimum of 30 minutes. Any clothing, gloves, cleaning materials or any other material suspected of being contaminated with Cerepro will be autoclaved.
The theatre will be washed with 2% Virkon or 2% sodium hypochlorite after the surgery in which Cerepro was delivered.
All surgical instruments will be autoclaved as per normal hospital procedures.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Please see the attached minutes of the GMSC meetings held on 17th March 2005 and 11th January 2006.
GM Centre Number: 945

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Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Genetically Modification Safety Committee (GMSC) within Cyprotex is a sub-committee of the main safety committee and consist of four members of staff. These include, the chief scientific officer, the operations manager and other scientists who are exposed to working with the GMM as well as an office-based scientist (qualified to PhD level). The committee will oversee the applicability of the GMO regulations and advise the end users on the risk assessments. The committee will meet every six months to review the risk assessment document.

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<tr>
<td>Other (please specify)</td>
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</table>

Tick if confidential
Waste generated within Cyprotex falls into two main categories, solid and liquid waste. All waste material that has been in contact with cells or exposed to the microbiological cabinet is treated as a potential biological hazard and/or clinical waste. Solid waste covers generally, disposable plastic wares with no electrical connections (e.g. tissue culture flasks, tissue culture plates and gloves. All plastic disposable wares (including gloves) are treated as clinical waste and disposed of in yellow clinical bin bags secured with tape highlighting the types of waste. Once full, the waste is placed in a secured (assess restricted) bin and transported offsite by a contracted waste disposal company; Ideal Solutions Ltd
152 Brookland Road
Wythenshaw
Manchester
M23 9HD
Sharps are placed in the sharps bin and considered as clinical waste.
Liquid waste generated from cell culture is treated with Virkon or effective alternative disinfectant for at least 1hr prior to disposing to drain. Solvent waste is collected in Duran bottles and is secured in an access restricted area for collection by a contracted waste disposal company; P&R Waste Disposal Services Ltd
Brindley Road
St Helen's
WA9 4HY

For activities involving GMMs, describe the waste management measures which will apply to the activity

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St Helen's
WA9 4HY

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Cyprotex Discovery Ltd has a UK Environmental Agency premises code of AAK 284 which recognises us as a registered waste producer.
|-----------------------------------|-----------|-----------|-----------------------------|----------------------|-----------------------|--------------------------|----------|----------|

**Name**

REBIO TECHNOLOGIES LIMITED

**Campus Estate or Research Centre**

THE SURREY TECHNOLOGY CENTRE

**Road Name**

40 ALAN TURING ROAD

**Town**

GUILDFORD

**District**

SURREY

**County**

GU2 7YF

**Country**

ENGLAND

**Tel Number**

01483 688 290

**Fax Number**

01483 688 292

**E-mail**

HSE Division

EAST AND SOUTH EAST

**Comments**

02/03/2022
### Premises Addresses

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<td>40 ALAN TURING ROAD</td>
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<td>GU2 7YF</td>
<td>ENGLAND</td>
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<td>STOVOLDS HILL</td>
<td></td>
<td></td>
<td>CRANLEIGH</td>
<td>SURREY</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The GMSC comprises a Chairman who is on the company board (Company Secretary), a GM Safety Officer who is an experienced scientist (Associate Director), the head of process development, and a member of the admin staff (as a non-expert). Other technical staff may attend meeting as required. The GMSC meets quarterly or as required to review new GM work proposals.

The GMSC oversees all GM work and operating procedures to ensure they comply with all current legislation and reviews all GM work prior to it beginning - it forms part of the Company Health and Safety organisation and the key members also sit on the H&S committee.

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<td>Non-microbial</td>
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</table>

Other (please specify) 

Tick if confidential
There are two main methods of waste inactivation in operation at TMO’s labs.

1. Autoclaving: The bulk of waste generated is preferentially inactivated by autoclaving using a discard cycle of 121°C for 15 mins with full steam penetration to the load. This cycle is electronically monitored and controlled to ensure correct temp and pressure are maintained throughout the run. The effectiveness of the cycle is further monitored by the addition of a steam cycle verification indicator (TST strip, 121°C, 15 mins). The autoclave cycle performance is checked and validated on an annual basis during maintenance by competent contractors following relevant manufacturers guidelines.
   EFFECTIVE KILL: 100%

2. Chemical Inactivation: where waste cannot be inactivated by autoclaving, e.g. treating spills or for soaking sensitive equipment, then chemical inactivation is used. TMO use 1% (w/v) solution of Virkon (Antec International) which is used in accordance with the manufacturers instructions. Contact time is a minimum of 10 minutes. For soaking, the solution is discarded once the pink colour has faded or after 7 days (whichever is soonest).
   EFFECTIVE KILL: >1 x 10e7 reduction in viability.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment was passed without further comment - the activity class was agreed to be 1.
<table>
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<td>Downs Road</td>
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<td>University of Surrey</td>
<td>Daphne Jackson Road</td>
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<td>Guildford</td>
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<td>St James's University hospital</td>
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<td>Beckett Street</td>
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<td>NG5 1PB</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

02/03/2022
The Safety Committee already established at one of the hospitals involved (St Jame's University Hospital) has conducted the risk assessment:
Leeds Teaching Hospitals NHS Trust Biological Safety Committee
Trust Headquarters
St James University Hospital
Beckett Street
Leeds LS9 7TF
Composition: For composition, operating procedures and frequency of meetings see separate sheet.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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Tick if confidential

Bacteriology
- Parasitology
- Transgenic
  - Birds
- Microbiology
  - Research

Virology
- Transgenic
  - Animals
- Transgenic
  - Fish
- Gene Therapy
  - Yes

Mycology
- Transgenic
  - Invertebrates
- Transgenic
  - Plants
- Other (please specify below)

Other(s)
- Vaccination with 2 human prostate cancer cell lines which have been gen modified to express GM-CSF

For activities involving GMMs, describe the waste management measures which will apply to the activity
All needles and syringes will be discarded into a designated sharps bin. This will be sealed and disposed of using normal hospital procedures.

All other materials will be treated as clinical waste and disposed of in yellow clinical waste bags under normal hospital procedures.

Unused vials will be returned to pharmacy and stored in the refrigerator until full accountability has been conducted by the monitor of the study and approval to destroy has been received from sponsor.

If a spill occurs use absorbent material to soak up spill and discard with spent vials into Biological Waste. Clean area with cleaning agent to remove residue. No specific disinfectants are required.

Date of meeting: 10th October 2005
Present: Associate Director of Trust (Chair), Microbiologist, Senior Research Nurse, Post Doctoral Scientist, Medical Oncologist, Pharmacist, Virologist, Infection Control Representative

Apologies: Pharmacist, HSE Representative, Clinical Oncologist

Note: The senior Research Nurse (Debbie Berine) and Medical Oncologist (Dr John Chester) on this committee will be working on this project. Dr Chester is the principal Investigator at St James's University Hospital.

See attached:
1) Leeds Teaching Hospitals NHS Trust Biological Risk Assessment form
2) Leeds Teaching Hospitals NHS Trust SOPs.
## GM Centre Number: 948

<table>
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<th>Data Premises Notified (Originally)</th>
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### Name

**THE VETERINARY SURGERY**

### Name 2

### Department

### Campus Estate or Research Centre

**STANFORD BUSINESS COURT**

### Road Name

21-23 HIGH STREET

### District

STANFORD-IN-THE-VALE

### Town

FARINGDON

### County

OXFORDSHIRE

### Postcode

SN7 8LH

### Country

ENGLAND

<table>
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### E-mail

### HSE Division

EAST AND SOUTH EAST

### Comments

### Date at Which Additional Info Submitted

02/03/2022
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

PROFESSOR OF PAEDIATRICS, UNIVERSITY OF WASHINGTON, SEATTLE.
VETERINARY SURGEON
CLIENT
TO BE ASSISTED BY CLINICAL VETERINARY SCIENTISTS.

<table>
<thead>
<tr>
<th>Level (GMMs)</th>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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<tr>
<td>Other (please specify)</td>
<td>Assuming no higher classification than level 2, initial containment will be</td>
<td>Tick if confidential</td>
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Bacteriology
Parasitology
Transgenic
Birds

Virology
Transgenic
Animals

Transgenic
Fish

Mycology
Transgenic
Invertebrates

Transgenic
Plants

Microbiology
Research

Gene Therapy
Yes

Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity
Although advised that level 2 will apply, a full risk assessment is enclosed with this application; data on efficacy of povidone iodine is also supplied.

Controlled scientific studies at the University of Washington have demonstrated the safety of the Lentivirus vector in the transfer of c G-CSF into the target muscle tissue, using a standard protocol of operation pre-peri-and post administration of the vector by deep intra-muscular injections. Studies on the dog and rat by a team totally devoted to its research and safe development have enabled a gene therapy to be developed specifically to treat children with Cyclic Neutropaenia for a 30 year period.

The Collie dogs involved in the study have demonstrated the efficiency and safety of the gene transfer as they are a very good research tool showing classic probability-inherited cyclic neutropaenia.

Peer-reviewed papers describing virus generation & use in rats and dogs have been frequently published.

Collie and normal dogs successfully treated this way have been returned to the community as they have been considered to be of no risk to the families concerned - on a par with the use in similarly affected children. In excess of 100 rats have received lentivirus vectors and have been handled and routinely bled by investigators. These studies only required precautions normal employed in handling rodents.

Virus is generated in a biosafety level 2 laboratory and after virus administration, the staff are only required to house the animals at biosafety level 1. This also means that they can be returned to the community with no restrictions on their movements.

We are fully prepared to apply the risk assessment SOP in full as good veterinary medical practice, as well as making this request for derogation, in the earnest understanding of a fast-track result that will enable us to treat a client's puppy who is at risk from a common infection that could totally overwhelm her defences when around the Neutrophil Nadir.

The method of treatment proposed for the puppy will conform exactly to the standard operating procedure used on the dogs at the University of Washington.

Project Ref 948/06.1

<table>
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<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<td>06/06/2006</td>
<td>Multiple Deep Intra-Muscular Injections of Lentivirus Vector Encoding Canine G-CSF c DNA.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Not Applicable</td>
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</table>
**Project Additional Information**

**Purposes of the contained use**

First treatment by gene therapy of an immunologically deficient young working collie suffering from Cyclic Neutropaenia, and who carries a very grave prognosis if the situation is not treated. We do not anticipate any further Lentivirus administration.

**Recipient or parental organism**

Expression plasmid pRRL-cPPT-CMV-cGCSF-PRE-SIN was constructed by inserting the canine G-CSF cDNA into the multiple cloning site of pRRL-cPPT-CMV-X-PRE-SIN.

**Host/vector system**

A well-established Lentivirus vector is used, with non-replicating characteristics, generated by Calcium Phosphate cotransfection of the above plasmid plus 3 other "packaging" plasmids. Assays for replication-competent lentivirus continue to be negative.

**Origin & function**

Sustained delivery of cG-CSF to the target cells (muscle tissue) to permit long-term gene expression. The intended aim is to allow the dog to generate a sustained, elevated therapeutic neutrophil production, thereby allowing in turn normal growth rate to resume and no further recurrent cycles of potentially-fatal persistent infections that would be rapidly and spontaneously resolved in healthy immuno-competent dogs.

**Evaluation of foreseeable effects**

We remain confident that the use of this gene therapy presents no risk to other animals and people associated with the puppy. We have established a treatment SOP (attached with this form) to provide sensible medical safeguards to the patient, operator and client. Regular blood tests will be continued to provide simple data for on-going statistical analysis.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

This is specific canine gene therapy for a specific canine genetic immuno-deficient disease. For the sake of complete identification, the patient will be uniquely micro-chipped and will be spayed when considered well enough following achievement of a satisfactory and sustained response to the therapy. Please refer to PCR and southern blot checks in the risk assessment on tissue harvested from animals involved in the university study which demonstrates that there is no spread of any virus vector material to other body tissues apart from the intended target thigh muscle tissue.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Controlled scientific studies at the University of Washington have demonstrated the safety of the lentivirus vector in the transfer of c G-CSF into the target muscle tissue, using a standard protocol of operation PRE-,PERI and post administration of the vector by deep intra-muscular injections. Studies on the dog and rat by a team totally
devoted to its research and safe development have enabled a gene therapy to be developed specifically to treat children with cyclic neutropaenia for a 30 year period. The collie dogs involved in the study have demonstrated the efficiency and safety of the gene transfer as they are a very good research tool showing classic probably-inherited cyclic neutropaenia. Peer-reviewed papers describing virus generation and use in rats and dogs have been frequently published. Collie & normal dogs successfully treated this way have been returned to the community as they have been considered to be of no risk to the families concerned - on a par with the use in similarly affected children. In excess of 100 rats have received lentivirus vectors and have been handled and routinely bled by investigators. These studies only required precautions normally employed in handling rodents. Virus is generated in a biosafety level 2 laboratory and after virus administration, the staff are only required to house the animals at biosafety level 1. This also means that they can be returned to the community with no restrictions on their movements. We are fully prepared to apply the risk assessment SOP in full as good veterinary medical practice, as well as making this request for derogation, in the earnest understanding of a fast-track result that will enable us to treat a client's puppy who is a risk daily from a common infection that could totally overwhelm her defences when around the Neutrophil Nadir.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Procedure will leave little or no vector on the skin surface post-needling due to depth, angle and choice of min. needle gauge cosistant with safe practice.
1. Povidone Iodine will be applied to saturate the area with virucidal activity.
2. The treated, virus free area will be covered by suitable dressings for at least 24 hours.
3. Pre-treatment assay on the quality and safety of that batch of non-replicating lentivirus.
4. All materials used will be placed in 10% bleach then transferred to clinical waste bags and sharps bins for transfer to the approved incineration plant of our licensed clinical & hazardous waste disposal contractor.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The method of treatment proposed for the puppy will conform exactly to the standard operating procedure used on the dogs at the University of Washington.

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tr>
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<td>L3 L4</td>
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Animal Units

| L2 Yes | L3    | L4 |

Large Scale Activities

| L2 L3 L4 | L2 L3 L4 | L2 L3 L4 |

Human Clinical Applications

| L2 L3 L4 | L2 L3 L4 | L2 L3 L4 |

02/03/2022
## GM Centre Number: 949

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### Name

SMART HOLOGRAMS LTD

### Name 2

Department

### Campus Estate or Research Centre

Building

291 CAMBRIDGE SCIENCE PARK

### Road Name

MILTON ROAD

### Town

CAMBRIDGE

### County

CAMBRIDGESHIRE

### Postcode

CB4 0WF

### Country

ENGLAND

### Tel Number

01223 393 400

### Fax Number

01223 393 401

### E-mail

### HSE Division

EAST AND SOUTH EAST

### Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

Date Premises Closed

Name Department Name 2 Building Road Name District Town County Post-code Country Withdrawn

SMART HOLOGRAMS LTD

291 CAMBRIDGE SCIENCE PARK MILTON ROAD CAMBRIDGE CAMBRIDGES HIRE CB4 0WF ENGLAND N

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The GM safety committee consists of a founder of Smart Holograms, the BSO and the Deputy BSO of Smart Holograms. All are trained to PhD level and the BSO and deputy BSO are experienced in the safe handling and genetic modification of class 1 and class 2 micro-organisms. The GM safety committee convene on a quarterly basis. Experiments involving the genetic modification of micro-organisms are discussed on an individual basis and assessed on individual merit. The necessity of the experiment is discussed, as are the safety implications of the experiment and how GMO's will be disposed of safely. Only if all members of the GMSC agree that all safety issues have been addressed will the experiment be given the go ahead.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Yes Parasitology Transgenic Birds Microbiology Research Yes

02/03/2022
Laboratory bench top surfaces are thoroughly cleaned with a 1% Virkon solution before and after any potential exposure from GMO. Virkon solution is prepared and replaced on a weekly basis. Virkon solution has been shown to effectively kill a wide range of micro-organisms in both vegetative and spore forms. Protective items that have come into contact with GMO's e.g. gloves, are disposed of in yellow biological waste bins. Agar plates containing GMO's are also placed in yellow bin. Yellow bins are sealed when three quarters full and collected by an external licensed company for disposal by incineration. Flasks that have been used in the cultivation of GMO's are sterilised by addition of 1% Virkon solution before autoclave treatment.

Sharps that have come into contact with GMO's e.g. pipette tips, syringes, are disposed of in biological waste sharp bench bins. These are sealed when three quarters full and collected by an external licensed company for disposal by incineration.

For activities involving GMMs, describe the waste management measures which will apply to the activity

After discussion of the experiment no sources of unnecessary risk were identified. The methods of disposal of organism, lab-ware and sterilisation of equipment were deemed as suitable. No foreseeable increase in Bacillus Subtilis pathogeneisis could be identified by the attempted increase in spore sensitivity to Ca2+ DPA.
### GM Centre Number: 950

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**Name**

GENZYME THERAPEUTICS LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

310 CAMBRIDGE SCIENCE PARK

**Road Name**

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 0WG

**Country**

ENGLAND

**Tel Number**

01223 394 000

**Fax Number**

01223 394 190

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

SAFETY, HEALTH AND ENVIRONMENTAL MANAGER/BIOLOGICAL SAFETY OFFICER
HEAD OF PHAGE
SAFETY MANAGER
SCIENTISTS
MEET AT LEAST EVERY 6 MONTHS OR MORE FREQUENTLY IF NEW WORK IS TO BE CONDUCTED.

Laboratory

<table>
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<th>Level 1 (GMMs)</th>
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Animal Unit

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Growth Room

Glass House

Large Scale

Other (please specify)

Tick if confidential

Bacteriology

Parasitology

Transgenic Birds

Microbiology Research
CELL CULTURES/LINES WILL BE CULTURED IN DISPOSABLE PLASTICWARE AT A SCALE FROM 50"P1" TO 500 ML UNDER ASEPTIC CONDITIONS AND HARVESTED BY CENTRIFUGATION. ALL WASTE RESULTING FROM THESE PROCEDURES WILL BE EITHER CHEMICALLY INACTIVATED OR AUTOCLAVED PRIOR TO DISPOSAL. ALL EQUIPMENT AND MATERIALS WITHIN THE PROCEDURES WILL ALSO BE STERILISED BY AUTOCLAVING FOR 20 MINUTES AT A TEMPERATURE OF 121°C OR CHEMICALLY DISINFECTED (EG WITH VIRKON, TRIGENE OR PROCEINE 40) PRIOR TO DISPOSAL OR REUSE. ALL OPERATIONS WILL BE PERFORMED IN LABORATORY FACILITIES IN WHICH SPILLAGES CAN BE EFFECTIVELY DISINFECTED, SO THE WIDER ENVIRONMENT IS UNLIKELY TO BE CONTAMINATED.

For activities involving GMMs, describe the waste management measures which will apply to the activity

CELL CULTURES/LINES WILL BE CULTURED IN DISPOSABLE PLASTICWARE AT A SCALE FROM 50"P1" TO 500 ML UNDER ASEPTIC CONDITIONS AND HARVESTED BY CENTRIFUGATION. ALL WASTE RESULTING FROM THESE PROCEDURES WILL BE EITHER CHEMICALLY INACTIVATED OR AUTOCLAVED PRIOR TO DISPOSAL. ALL EQUIPMENT AND MATERIALS WITHIN THE PROCEDURES WILL ALSO BE STERILISED BY AUTOCLAVING FOR 20 MINUTES AT A TEMPERATURE OF 121°C OR CHEMICALLY DISINFECTED (EG WITH VIRKON, TRIGENE OR PROCEINE 40) PRIOR TO DISPOSAL OR REUSE. ALL OPERATIONS WILL BE PERFORMED IN LABORATORY FACILITIES IN WHICH SPILLAGES CAN BE EFFECTIVELY DISINFECTED, SO THE WIDER ENVIRONMENT IS UNLIKELY TO BE CONTAMINATED.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

THE RISK ASSESSMENT HAS BEEN ENDORSED BY THE GMO SAFETY COMMITTEE.

Project Ref 301/05.1

Date Ackn'd 05/07/2006

CU2 Project Title Testing of human antibodies developed from phage libraries for therapeutic use of assessing their effect on a variety of cell lines grown in the laboratory.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

To minimise the risk of infection from class II cell lines, in accordance with good microbiological practice and the protection of both work and worker. Work carried out at the premises will be limited to research activities.

#### Recipient or parental organism

The cell lines used may include adenovirally transformed lines (eg HEK-293) or cells classed as Biosafety level 1 that have been transfected with mammalian expression vectors or via adenovirus. The cell lines themselves are not infectious.

#### Host/vector system

The GM cell lines may be virally transformed, adenovirally transfected or stably transfected using expression vectors. Adenovirus has been identified by the ACDP as a Level 2 Biosafety Hazard. However, the risk of transmission to humans in this activity is considered negligible. In some cases the inserted product may be expressed as a membrane bound protein. The antigens under study are not in themselves pathogenic but should be handled according to good microbiological practice.

#### Origin & function

Adenovirus has been used to transform some commercially available cell lines, in order to render them immortal for long-term culture. Adenovirus is also as a means of introducing a gene of interest for over-expression, usually at the cell surface, for use as a selection target. The product may occasionally be released into the medium as a soluble protein, or purified from the cell, but will then be captured for use as a selection target or for in vitro characterisation and testing of the antibodies under development.

#### Evaluation of foreseeable effects

Biosafety level 2 hazard is introduced to the proposed work by use of adenovirally transformed or transfected cell lines. Other cell lines or products are currently evaluated at level 1. The risk of transmission of adenoviruses from these systems to humans is negligible and the cell and genetic material will not survive in the external environment. Standard level 2 decontamination and containment facilities are in operation. Overall, the foreseeable effects are considered negligible.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cell lines will be cultivated in disposable plasticware at a scale from 50ul to 500ml under aseptic conditions and harvested by centrifugation. All waste resulting from these procedures will be either chemically inactivated or autoclaved prior to disposal. All equipment and materials used within the procedures will also be sterilised by autoclaving for 20 minutes at a temperature of 121 degrees C or chemically disinfected (eg with Virkon, TriGene, or Proceine40) prior to disposal or re-use. All operations will be performed in laboratory facilities in which spillages can be effectively disinfected so the wider environment is unlikely to become contaminated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This project has been approved as a class 2 GM activity on the basis of the attached risk assessment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

Project Activities

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Date Ackn'd 23/08/2007

CU2 Project Title Use of bacterial expression vector producing immunotoxin.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 1-50 Litres

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N
### Project Additional Information

**Purposes of the contained use**

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>To express and purify human antibody fragments genetically fused to a truncated form of Pseudomonas Aeruginosa Exotoxin A.</td>
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**Recipient or parental organism**

<table>
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**Host/vector system**

<table>
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<td>pIG6 - based vector system</td>
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**Origin & function**

<table>
<thead>
<tr>
<th>Function</th>
<th>Details</th>
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</thead>
<tbody>
<tr>
<td>To assess mechanism of action for any given antibody candidate - specifically internalisation using a variety of cell lines.</td>
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</table>

**Evaluation of foreseeable effects**

As a disabled E. coli strain, the GMM has limited potential for survival outside laboratory culture conditions and would therefore be unlikely to become established in the environment.

The risk of exposure to a scientist would be for eg. By a splash on an open wound or through the eye and where the antibody is directed against a human antigen, the result would be hazardous if the antigen was expressed. However the risk of transmission to humans in this activity is considered unlikely.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- Not applicable as work will not involve GM animals or plants.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

- No derogation from class 2 containment will be allowed.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Equipment and materials - to be decontaminated by autoclaving at 121C for 20 minutes. This will result in a 100% kill. Following decontamination, waste will go to landfill.
Liquids - to be decontaminated by autoclaving as above. Following decontamination liquid will be disposed of down the sink. References supporting decontamination route: "Full length toxin has enzymatic activity identical to that of Diphtheria toxin" - Merck KGaA - Chemicals - Merck Biosciences (www.merckbiosciences.com).

GMSC discussed the risk assessment at a meeting on Monday 30 July 2007. Some amendments were made to the assessment, following which the committee were satisfied with the assessment and signed it off on Monday 6 August 2007.

Please enter comments on the GM safety committee on the risk assessment

GMSC discussed the risk assessment at a meeting on Monday 30 July 2007. Some amendments were made to the assessment, following which the committee were satisfied with the assessment and signed it off on Monday 6 August 2007.

Project Containment

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee
The duties of the Genetic Modification Safety Committee are fulfilled by the Gentronix Health and Safety (H&S) Committee. There is no provision within Gentronix for a separate Genetic Modification Safety Committee at this time, due to the small size of the organisation.

The H&S committee is comprised from members of staff from different tiers of the organisation:

H&S Co-ordinator & Chair
Scientific or other Director
Laboratory Manager
Member of the Technical Staff
Researcher/ Student (by invitation)

Operating Procedures & frequency of meetings
1. Chair invites members to a quarterly meeting to discuss H&S matters and to review either new, or current, risk assessments.
2. During the meeting the relevant risk assessments will be discussed and any amendments required will be made with the consensus of the committee, after liaison with the staff involved in drafting the original risk assessment.
3. The amended risk assessment will then be approved by the committee and circulated to all staff.

The Chief Scientific Officer of Gentronix is also a member of staff in the Faculty of Life Sciences at the University of Manchester. Some university research students will also be working within the Gentronix Laboratory using the same panel of organisms and procedures and the laboratory is based within the University of Manchester. Hence, the Gentronix Health and Safety Committee will solicit advice on matters concerning GMMs from the local, University of Manchester, Genetic Modification Safety Sub-Committee. This Modification & Biohazards Safety Sub-Committee and the University of Manchester Genetic Modification and Biohazards Safety Advisory Group. The Genetic Modification & Biohazards Safety Sub-Committee comprises a Chairman and representatives of the local University Genetic Modification Safety Sub-Committee associated with the various faculties and buildings within the University, as well as other University Health & Safety representatives.
H&S Co-ordinator & Chair
Scientific or other Director
Laboratory Manager
Member of the Technical Staff
Researcher/Student (by invitation)

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<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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<th>Large Scale</th>
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<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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</table>
The GMMs proposed for use in this study are as follows: (Also see accompanying Working Practices document).

- Specially-disabled strain of Saccharomyces cerevisiae (FF18984), ACDP Classification - Class 1
- Disabled strains of mammalian cell lines, principally human lymphoblastoid line (TK6) and human hepatocellular carcinoma (Hep G2), ACDP Classification - Class 1
- Disabled strains of Escherichia coli (K12 derivatives), ACDP Classification - Class 1

Standardised Working Procedures will be adopted when handling Class 1 GMMs, consistent with, and often exceeding, those required for Class 1 containment.

Inactivation and disposal of items contaminated with Class 1 GMMs

- All re-useable items contaminated with Class 1 GMMs, such as laboratory glassware and small pipetting troughs, will be disinfected by total immersion in freshly prepared 1% (w/v) Virkon solution (Antec International) for 24 hours (see note 1). Items will then be washed and autoclaved following standard laboratory procedures.
All disposable items contaminated with Class 1 GMMs, such as polypropylene pipette tips, sample vials, microplates and gloves, will be sealed in small bags containing absorbent material, labelled as “biohazard” and either inactivated via autoclave before appropriate disposal by conventional routes (see note 2), or sent for incineration by approved contractors. Materials sent for incineration will be further encapsulated in one-way sealed burn units, appropriately labelled with details of their contents and the sender's contact details.

Stock cultures of Class 1 GMMs, when no longer needed, will be sealed in small bags containing absorbent material, labelled as "biohazard", and either inactivated via autoclave before appropriate disposal (see note 2), or sent for incineration by approved contractors. Materials sent for incineration will be further encapsulated in one-way sealed burn units, appropriately labelled with details of their contents and the sender's contact details. Alternatively, larger volumes of culture can be inactivated by autoclave whilst contained within culture flasks, before appropriate disposal by conventional routes (see note 2).

Work areas will be disinfected after use using 70% ethanol/water solution (see note 3).

Liquid spills of Class 1 GMMs on the bench or floor will be contained with absorbent paper tissue, or Virkon powder. Absorbent paper and powered Virkon waste will be sealed in small bags containing absorbent material, labelled as "biohazard", and either inactivated via autoclave before appropriate disposal by conventional routes (see note 2), or sent for incineration by approved contractors. Materials sent for incineration will be further encapsulated in one-way sealed burn units, appropriately labelled with details of their contents and the sender's contact details. The affected floor/bench area will be disinfected with 70% ethanol/water solution (see note 3).

Contamination broken glass will be inactivated by total immersion in freshly prepared 1% Virkon solution for 24 hours before appropriate disposal by conventional routes (see note 1). The affected bench/floor area will be disinfected with 70% ethanol/water solution (see note 3).

Note 1: Virkon is a peroxygen compound that was found to be effective against strains of Saccharomyces cerevisiae, bacteria and viruses after 5 minutes contact time at 1% (see www.antecint.co.uk and references therein). It has minimum environmental impact and a red colour in solution indicating its activity. 99.999% degree of kill is assumed after 24 hours with 1% virkon solution.

Note 2: Items sterilised, and GM cells inactivated, by autoclave are subject to a temperature of 121°C for a duration of 20 minutes. 100% degree of kill would be achieved. A boxer autoclave is to be provided by the University of Manchester Incubator Company (The Buildin Managers) in an area adjacent to the laboratory. The autoclave is maintained regularly under a service contract with Lab3 Ltd, 1 Dragon Court, Crofts End Road, Bristol BS5 7XX.

Note 3: Alcohols give a very rapid kill of micro-organisms, but do not provide sustained microbial action. 99.999% kill would be achieved.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
All members of the current Gentronix Health and Safety Committee were involved in the drafting of risk assessments for this work. The Committee has subsequently approved the final risk assessment as an accurate account of the risks inherent to the work and a correct description of the protocols, sufficient for safe containment and use of GMMs in these projects, within the Gentronix laboratory. The risk assessments will be reviewed annually and as any significant variations in protocols arise.

In addition, the work described in this form, in the associated risk assessment and in supporting documentation has been carried out over the previous 15 years in the laboratory at the University of Manchester (formally University of Manchester Institute of Science and Technology - UMIST). Over the past 7 years this research work has been largely on behalf of Gentronix Limited. This application concerns the movement of activities to a new laboratory adjacent to the University premises. During the previous 15 years, whilst working within University premises, the work was subject to approval by the various UMIST/University of Manchester Biological Safety Committees. All work, containment procedures and risk assessments were approved by these committees.
## GM Centre Number: 952

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### Name

**PHARMARON BIOLOGICS (UK) LTD**

### Name 2

**Department**

### Campus Estate or Research Centre

### Road Name

**ESTUARY COMMERCE PARK**

### Town

**LIVERPOOL**

### District

**SPEKE ROAD**

### Building

**ESTUARY BANKS**

### County

**MERSEYSIDE**

### Postcode

**L24 8RB**

### Country

**ENGLAND**

### Tel Number

0151 728 1750

### Fax Number

0151 728 1751

### HSE Division

**NORTH WEST**

### Comments

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- (TECHNOLOGY TRANSFER MANAGER) - BIOLOGICAL SAFETY OFFICER, CHAIRMAN
- (PROCESS DEVELOPMENT MANAGER) - LABORATORY REPRESENTATIVE, SECRETARY
- (DIRECTOR OF SCIENCE) - COMPANY SAFETY OFFICER
- (GMP MANAGER) - MANUFACTURING REPRESENTATIVE
- (ANALYTICAL MANAGER) - LABORATORY REPRESENTATIVE
- (ACCOUNTANT) - LAY PERSON (OFFICE REPRESENTATIVE)

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<th>Level 1 (GMMs)</th>
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<th>Animal Unit</th>
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Other (please specify)  
Tick if confidential

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Inactivation of large scale waste (max. 200L fermenter vessel volumes) by dedicated effluent inactivation treatment plant supported by validation studies.

Inactivation of small to medium scale solid and liquid waste by autoclave decontamination cycles supported by validation studies.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

RISK ASSESSMENT REVIEWED AND DETERMINED TO BE SATISFACTORY WITH REGARDS TO CLASSIFICATION, CONTROL AND CONTAINMENT OF GMM.
**GM Centre Number: 953**

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**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Yes

Give brief details of the genetic modification safety committee

- Vice President of Research and Development - Chairman of GMM Safety Committee
- Research and Development Group Leader - Biological Safety Officer
- New Technology Group Leader - Committee member
- Safety Officer - Safety advice and information.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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<td>Level 4 (GMMs)</td>
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<td>Non-microbial</td>
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<tr>
<td>Other (please specify)</td>
<td>Tick if confidential</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All contaminated waste and cell culture waste, disposable plastics, laboratory glassware, or other contaminated materials, including waste destined for incineration, will be autoclaved at 121°C for at least 15 minutes to inactivate GMOs (100% kill) prior to disposal or cleaning and recycling. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST sterilisation control strips with colour change dot to show successful autoclaving. The dot changes from yellow through brown to purple when exposed to steam at 121°C for 15 minutes. Periodic swabs shall be taken from the culture media after autoclaving to ensure effective operation. Inactivated autoclaved waste will be removed from the premises and disposed of by incineration by a commercial contractor.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

The comments on the risk assessment were all positive and it was decided that the risk assessments met the criteria that were set down in the ACGM Compendium of Guidance.

Issues such as security, biohazard and GMO signage on the door of the room, information on the qualifications and the training of staff in standard operating procedures were also discussed:

Access to the GMO laboratory will be restricted and the laboratory area will only be accessible through the use of a swipe card.

Biohazard and signs will be displayed on the doors to the laboratory.

Staff will be trained on all standard operating procedures relating to GMO procedures and waste disposal before commencement of any work involving GMO. These standard operating procedures will be reviewed on an annual basis.
<table>
<thead>
<tr>
<th>Name</th>
<th>THE NATIONAL BLOOD SERVICE, OXFORD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name 2</td>
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<tr>
<td>Campus Estate or Research Centre</td>
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<tr>
<td>JOHN RADCLIFFE HOSPITAL</td>
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<td>Building</td>
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<td>LEVEL 2</td>
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**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

| Level 1 (GMMs) | Yes |
| Level 2 (GMMs) | Yes |
| Level 3 (GMMs) |       |
| Level 4 (GMMs) |       |
| Non-microbial |       |
| Other (please specify) |       |

Tick if confidential

---

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Composition: Chair, biological safety officer, line managers (or their delegates) representing each laboratory covered by the committee, a national blood service health and safety advisor (ex-officio capacity), representatives from each level of researcher working in the laboratories where GMM are used (eg. Post-doctoral scientist, Ph.D. student), external experts as required (eg: The Oxford Radcliffe Trust biological safety officer).

Meetings: At least every 6 months, and more often as required. Between meetings, email will be used to discuss issues that arise and can be dealt with without a formal meeting of the committee.

Procedural note: Risk assessments can only be approved following review by at least 3 members of the committee that are not directly involved in the work proposed.
| Bacteriology | Yes | Parasitology | Yes | Transgenic Birds |
| Virology | Yes | Transgenic Animals | Yes | Gene Therapy |
| Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below) |

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC is happy with the risk assessment in its current form. It was noted that the work poses low or very low risks, and that both containment and waste disposal measures are well established.

This work was initially presented as a CL1 activity. However the committee decided it should be upgraded to a CL2 due to the reported association of vectors containing WPRE and murine liver cancer.

Project Ref 954/06.1

Date Ackn’d | CU2 Project Title
07/07/2006 | To produce self-inactivating (SIN) HIV-1 based Lentiviral Vector (LV) particles as vehicles for gene transfer into mammalian cells.

Date Project Ceased

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N
### Project Additional Information

#### Purposes of the contained use

The work will involve the production of lentiviral vector particles as vehicles for gene transfer into mammalian cells. Transient and stably transfected cell lines expressing the chosen transgene will be expanded for the purposes of further study such as gene and protein analysis. Transfected cells may also be assessed in vitro assays related to cell proliferation and cell migration/adhesion.

#### Recipient or parental organism

Disabled E. coli strain used for cloning and propagation of the plasmids represent negligible risks. Mammalian cell line used to produce the lentiviral vector particles (e.g., HEK 293T cells) contain the SV40 T antigen and therefore present a medium risks under COSHH. Primary human cells represent negligible risks, since they are naturally occurring, unless they are from blood. All blood will be screened by PCR for HIV and HSV. All recipients are unlikely to survive outside the laboratory and therefore the GMOs will carry no additional hazard.

#### Host/vector system

The plasmids described in the attached risk assessment will not produce stable transfection. The lentiviral vector (LV) systems described in the same risk assessment are disabled, minimal, self-inactivating vectors that will be unable to establish an infection or propagate to other cells.

#### Origin & function

All transgenes described in the risk assessment will be of human origin.
- LacZ, Luciferase and GFP are markers used to assess transduction efficiency.
- The Nkx2.5 transcription factor is required for heart formation during development. Nkx2.5 is expressed during adult life in cardiac tissue, and it is more abundant in ventricular than atrial cardiomyocytes. Therefore its function is likely to be induction of cardiac muscle phenotype and cardiac-specific gene expression.
- The receptors CD164, PZR, JAM-A, LSP-1, CXCR4 and EPHA3 may be involved in cell adhesion and cell migration. Their overexpression may enhance cell motility and cell adhesion in human primary cells.
- Similarly, the scaffolding protein HEF-1 may be involved in cell proliferation and cell migration/motility.
- The EDG-1 (endothelial differentiation gene) is a member of the sphingosine-1-phosphate family of receptors involved in the regulation of essential cellular processes such as proliferation, migration, cytoskeletal organisation, adherens junction assembly and morphogenesis.

#### Evaluation of foreseeable effects

The expression of a standard marker gene such as LacZ, which is naturally occurring in high levels in mammalian cells due to its stability, mean that even in the unlikely event that accidental exposure occurs that no harmful effect will follow. Similarly the other transgenes are transcription factors, receptors and enzymes that naturally occur in mammalian cells, reducing the risk of harm.
Furthermore, although the potential exists that almost any gene that is involved in processes such as cell-to-cell or intracellular signalling, cell cycle control, differentiation or apoptotic control, could become an oncogene under certain circumstances e.g. Overexpression, it is rare that a single oncogene would cause cancer. The worst case scenario will occur if the overexpression of the transcription factor includes the expression of several oncogenes or inhibits the expression of tumour suppressor genes. None of the trangenes chosen are known to have harmful physiological or pharmacological properties. They are not known to be oncogenic and as they are naturally occurring they are not expected to affect host or normal human defence mechanisms. There is no evidence in literature that any of them cause an adverse effect. Gene transfer would not seem to be hazardous and the resulting GM cells are not expected to carry any additional risks to that of the unmodified recipients. The inserts code for genes which are not expected to have harmful physiological or pharmacological properties or to affect animals, plants or other organisms should they be accidently released. Classification: Class 1. There is no evidence in the literature that any of them cause an adverse effect, neither would they be likely to impact the environment should they accidently be released. It is highly unlikely that the vector system will be hazardous to animals, plants or the environment should accidental exposure occur.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable. This work will not involve the use of GM plants or animals in the premises.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The work will be carried out avoiding sharps and with the appropriate Personal Protective Equipment (PPE). All GMO and GM waste must be inactivated before disposal. Waste inactivation will either be by Virkon at 2%, for a minimum of 2 hours, or by autoclaving. All waste to be autoclaved must be placed in grey boxes, sealed and placed in the metal wheelie-bin in the Blood Research Laboratory (BRL) for inactivation in the JRH Trust’s autoclave and subsequent disposal. This is in agreement and is already well established.

Following inactivation, waste is to be placed in yellow biohazard bins, which must be sealed and dated before being passed to Grundon for removal/disposal.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The GMSC is happy with the risk assessment in its current form. It was noted that the work poses low or very low risks, and that both containment and waste disposal measures are well established. The work was initially presented as a CL1 activity. However the committee decided it should be upgraded to a CL2 due to the reported association of vectors containing WPRE and murine liver cancer.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
</table>

02/03/2022

Page 12175 of 15326
The work will involve the production of retroviral and adenoviral vector particles as vehicles for gene transfer into mammalian cells. Transient (adenovirus) and stably (retroviral) transfected cell lines expressing the chosen transgenes will be expanded for the purpose of further study such as gene and protein analysis. Transfected cells will also be assessed in in vitro assays related to stem cell phenotype, proliferation and differentiation into various lineages.

Recipient or parental organism

Disabled E. coli strain used for cloning and propagation of the plasmids represent negligible risks. Mammalian cell lines used to produce the lentiviral vector particles (eg: HEK 293T cells) contain the SV40 T antigen and therefore present a medium risk under COSHH. Primary human cells represent negligible risks, since they are naturally occurring, unless they are from blood. All blood will be screened by PCR for HIV and HSV. All recipients are unlikely to survive outside the laboratory and therefore the GMOs will carry no additional hazard.

Host/vector system

The plasmids described in the attached risk assessment will not produce stable transfection. The adenovirus vector (AdS) system described in the same risk assessment
are disabled, minimal, non-replicating vectors that will be unable to establish an infection or propagate to other cells. The retrovirus vectors used in this study are also also packaging deficient and non-replicating virus unable to establish infection or propagate to other cells.

Origin & function

All transgenes described in the attached risk assessment will be of human origin. LacZ, Luciferase and GFP are markers used to assess transduction efficiency. The pluripotency associated genes Oct4, Nanog, Sox2, and Klf4 are highly expressed in embryonic stem cells, and with varying expression profiles in other more committed multipotent stem cells. The intended function of these genes is to use them in combination, to induce a stem cell phenotype from human tissues of varying origins.

Evaluation of foreseeable effects

The expression of a standard marker gene such as LacZ, which is naturally occurring in high levels in mammalian cells, is expected to be safe due to its stability. Even in the unlikely event that accidental exposure occurs, no harmful effect will follow. Similarly, the other marker transgene GFP, has no reports of adverse reactions associated with it. Furthermore, although the potential exists that almost any gene that is involved in processes such as cell-to-cell or intracellular signalling, cell cycle control, differentiation or apoptotic control, could become an oncogene under certain circumstances e.g. overexpression, it is rare that a single oncogene would cause cancer. The worse case scenario will occur is the overexpression of the transcription factor induces the expression of several oncogenes or inhibits the expression of tumour suppressor genes.

Some of the transgenes used in this study are thought to have oncogenic characteristics, in specific cell types: Oct4 is associated exclusively with germline tumours; and Nanog can induce a lymphoproliferative disorder- However, these effects have been observed only causally, and it is not known whether they mediate oncogenesis directly, and is unlikely that exposure to these viruses will occur, given that correct PPE and Class 2 procedures will be employed. Gene transfer to target cultured cells would not seem to be hazardous and the resulting GM cells are not expected to carry any additional risks to that of the unmodified recipients. The inserts code for genes that are not expected to have harmful physiological or pharmacological properties or to affect animals, plants or other organisms should they be accidentally released. Classification: Class 2. There is no evidence in the literature that any of them cause and adverse effect, neither would they be likely to impact the environment should they accidently be released. It is highly unlikely that the vector system will be hazardous to animals, plants or the environment should accidentally exposure occur.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable. This work will not involve the use of GM plants or animals in the premises.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The work will be carried out avoiding sharps and with the appropriate Personal Protective Equipment (PPE). All GMO and GM waste must be inactivated before disposal. Waste inactivation will either be by Virkon at 2%, for a minimum of 2 hours, or by autoclaving. All waste to be autoclaved must be placed in the grey boxes, sealed and placed in the metal wheeble-bin in the Blood Research Laboratory (BRL) for inactivation in the JRH Trust’s autoclave and subsequent disposal. This is in agreement with Derrick Crook and is already well established. Following inactivation, waste is to be placed in yellow biohazard bins, which must be sealed and dated before being passed to White Rose for removal/disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The GMSC is happy with the risk assessment in its current form, and its classification as CL2.

Please enter comments on the GM safety committee on the risk assessment

The GMSC is happy with the risk assessment in its current form, and its classification as CL2.

### Project Containment

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Tick if you are claiming exemption from disclosure for section of the risk assessment

Yes

Tick to confirm that you have attached a risk assessment to this form

Yes
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Name
BIOPHARMA TECHNOLOGY LIMITED

Campus Estate or Research Centre

Road Name
WINNALL VALLEY ROAD

Town
WINCHESTER

County
HAMPshire

Postcode
SO23 0LD

Country
ENGLAND

Tel Number
01962 841092

Fax Number
01962 841147

E-mail

HSE Division
EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted
02/03/2022   

Page 12179 of 15326
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Our GM Safety Committee comprises the Director of Research & Development and a Research Scientist. This meets on an ad hoc basis in addition to the regular safety meetings. For handling specific client samples, whereupon we refer to the findings and recommendations of the GM safety committee at the client organisation and act appropriately on the information provided. We do not foresee the need for us to handle any GM materials greater than Category 1 at this stage and therefore the risk to our staff is minimal. Since there are no trade unions represented in our company (we have only 4 employees), all staff will be consulted prior to the possible handling of such materials.

<table>
<thead>
<tr>
<th>Laboratory</th>
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Tick if confidential

Bacteriology

Virology

Mycology

Other(s)

Parasitology

Transgenic

Birds

Animals

Transgenic

Fish

Transgenic

Invertebrates

Transgenic

Plants

Microbiology

Gene Therapy

Other (please specify below)

Yes

For activities involving GMMs, describe the waste management measures which will apply to the activity
We currently dispose of all laboratory waste as though it were clinical waste (EWC 18 01 03) in accordance with the recommended UK government procedure for the disposal of wastes whose collection and disposal is subject to special requirements in order to prevent infection. We have a contract with Cliniserve Ltd. Who dispose of the waste by incineration in accordance with The Environment Protection Act (1996). This includes our providing a description of the waste in accordance with European regulations (EPA 1990 SEC 34). Our premises have been registered as being "low risk" since we produce less than 200kg of hazardous waste per annum. We understand that for disposal of Class 1 GM waste, our current procedure is sufficient to cover this.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  Y

Please enter comments of the GM safety committee on the risk assessment

For the activities we wish to carry out at Biopharma Technology Ltd., we have identified the activity risks relating to the handling of the Category 1 GM material itself as being minimal, since we would be handling dilute liquid formulations, freeze-drying the material and sealing the resulting material in containers under negative pressure, thereby minimising the risk of any direct contact with our staff or the environment.

All handling operations will be carried out in a Class II microbiological safety cabinet (HEPA filtered air under laminar flow) with the operators using the following personal protective equipment: gloves, safety spectacles, laboratory coat. Additionally, a face mask will be used if it is necessary to handle powders.

Our freeze-dryers' vacuum pumps are fitted with filters to minimise the risk of organisms or oil mist being emitted into the laboratory. All accessible potential equipment contact surfaces will be disinfected using bacteriocidal solution and swabbed with 70% ethanol or isopropanol solution after use, in accordance with our standard operating procedures.
GM Centre Number: 956

|-----------------------------------|------------|-----------------------------|---|----------------------------|-----------------------------------------------|---|----------|---|-----------|---|

Name

CENTRE FOR PROCESS INNOVATION (CPI)

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

District

<table>
<thead>
<tr>
<th>Town</th>
<th>County</th>
<th>Postcode</th>
<th>Country</th>
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</thead>
<tbody>
<tr>
<td>REDCAR</td>
<td>CLEVELAND</td>
<td>TS10 4RF</td>
<td>ENGLAND</td>
</tr>
</tbody>
</table>

Tel Number 01642 455 340
Fax Number 01642 447 298

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

Date Premises Name Department Name 2 Campus Estate or Research Centre Building Road Name District Town County Post-code Country Withdrawn

CENTRE FOR PROCESS INNOVATION (CPI) WILTON CENTRE WILTON REDCAR CLEVELAND TS10 4RF N

CENTRE FOR PROCESS INNOVATION (CPI) NATIONAL FORMULATION CENTRE THE COXON BUILDING JOHN WALKER ROAD NETPARK, SEDGEFIELD COUNTY DURHAM TS21 3FE N

CENTRE FOR PROCESS INNOVATION (CPI) THE NATIONAL HEALTHCARE PHOTONICS CENTRE, THE COXON BUILDING JOHN WALKER ROAD, NETPARK SEDGEFIELD COUNTY DURHAM TS21 3FE N

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee (GMSC) consists of the biological safety officer (BSO) and up to two subject matter experts. Their remit is to review the relevant risk assessments pertaining to GMO’s

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 2 (GMMs)</td>
<td></td>
<td></td>
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<td>Yes</td>
</tr>
<tr>
<td>Level 3 (GMMs)</td>
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<tr>
<td>Level 4 (GMMs)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Non-microbial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (please specify)</td>
<td>Tick if confidential</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Laboratory waste will be treated by heat inactivation. Disinfection may also be employed. Inactivation will be by validated means where and to the extent the risk assessment shows it to be required.

For larger scale processes the cells will typically be removed by centrifugation or filtration for further processing. Disinfection or chemical treatment with caustic solution may also be employed.

Inactivation of GMM's in contaminated material and waste including those in the process effluent before discharge will be validated where and to the extent the risk assessment shows it to be required.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Laboratory waste will be treated by heat inactivation. Disinfection may also be employed. Inactivation will be by validated means where and to the extent the risk assessment shows it to be required.

For larger scale processes the cells will typically be removed by centrifugation or filtration for further processing. Disinfection or chemical treatment with caustic solution may also be employed.

Inactivation of GMM's in contaminated material and waste including those in the process effluent before discharge will be validated where and to the extent the risk assessment shows it to be required.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

The risk assessment fully considers the laboratory operations and correctly identifies the risks and the containment level required. The NIBF Development Laboratory more than meets the requirements for the containment level 1 such that the risks to humans and the environment are minimised.
<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
<th>17/08/2006</th>
<th>Transferred from 1992 Regs?</th>
<th>N</th>
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<td>Emergency Plan Required?</td>
<td></td>
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<td>N</td>
</tr>
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</table>

**Name**

FISHER CLINICAL SERVICES (T/A THERMO FISHER ELECTRON LTD)

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

1 WOODSIDE

**Town**

BISHOPS STORTFORD

**District**

**County**

HERTFORDSHIRE

**Postcode**

CM23 5RG

**Country**

ENGLAND

**Tel Number**

01279 713 320

**Fax Number**

01279 713 329

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

Name change from Thermo Electron Biorepository Services 05/06/2015

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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<th>Date Premises Closed</th>
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<td></td>
<td>1 WOODSIDE</td>
<td></td>
<td>BISHOPS STORTFORD</td>
<td>HERTFORDSHIRE</td>
<td>CM23 5RG</td>
<td>ENGLAND</td>
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<tr>
<td></td>
<td>THERMO ELECTRON LTD T/A FISHER BIOSERVICES</td>
<td>CELL GENE THERAPY CATAPULT</td>
<td></td>
<td>GLAXOSMITHK LINE CAMPUS</td>
<td>GUNNELSWOOD ROAD</td>
<td>STEVENAGE</td>
<td>HERTFORDSHIRE</td>
<td>SG12 2FX</td>
<td>N</td>
<td></td>
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</tr>
</tbody>
</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Committee comprises Head of Operations, Quality Manager, Facilities Manager and Customer Services Manager. Weekly meetings held include Health & Safety issues. Issues relating to storage of GM material will be incorporated into these meetings.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</tr>
<tr>
<td>Non-microbial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (please specify)</td>
<td>Temporary storage only. All samples will be kept frozen. No sample handling</td>
<td>Tick if confidential</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

02/03/2022
Yes

No intention to use premises for genetic modification. Frozen storage only.

Frozen storage only (-20, -80 & -150 degrees).

For activities involving GMMs, describe the waste management measures which will apply to the activity

No intention to use premises for genetic modification. Frozen storage only.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Genetic Modification Safety Committee agreed that the risk assessment had been correctly carried out and that control measures were sufficient to minimise any risks involved.

Project Ref 957/19.1

Date Ackn’d 09/05/2019

CU2 Project Title Storage and logistical distribution of biological material (Cold chain supply) on behalf of client to UK and global consignees.

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Literature

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

02/03/2022 Page 12187 of 15326
### Project Additional Information

#### Purposes of the contained use

Storage in - 80 degrees Freezer and shipping (logistical distribution) of GM material on behalf of the client. In some cases materials may need to be discarded as per client's request. No GM manipulation will be undertaken as part of this contained use.

Client notification number for contained use: GM3392/18.1.

Title: "rMV manufacture and rMV Process Development"

#### Recipient or parental organism

Non applicable as storage and cold chain logistical distribution only.

Performed by the client as per:

Client notification number for contained use: GM3392/18.1.

Title: "rMV manufacture and rMV Process Development"

#### Host/vector system

Non applicable as storage and cold chain logistical distribution only.

Performed by the client as per:

Client notification number for contained use: GM3392/18.1.

Title: "rMV manufacture and rAAV Process Development"

#### Origin & function

Non applicable as storage and cold chain logistical distribution only.

Performed by the client as per:

Client notification number for contained use: GM3392/18.1.

Title: "rMV manufacture and rAAV Process Development"

#### Evaluation of foreseeable effects

Non applicable as storage and cold chain logistical distribution only.

Performed by the client as per:

Client notification number for contained use: GM3392/18.1.
### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Non applicable as storage and cold chain logistical distribution only. Performed by the client as per:

Client notification number for contained use: GM3392/18.1.

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Non applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Class 2 GMM waste is only generated as per client's request of material disposal, as result of a faulty container or breakage of container.

The Class 2 GMM waste is autoclaved at 123°C for 30 minutes, 2 bar pressures, prior to disposal to landfill by a registered waste contractor (Veolia). Spore strips are included in each Class 2 GMM waste load for purposes of validation of destruction. The autoclaved waste is then stored in a sealed wheele bin with biohazard symbols displayed, for 48 hours, while the spore strips are incubated to validate destruction. After 48 hours and a positive destruction result with the spore strips, the inactivated waste is removed by a registered waste contractor to landfill.

The autoclave is validated annually. Class 2 sharps containers are autoclaved in accordance with an SOP for Class 2 GMM waste inactivation before being removed by a registered waste contractor (Veolia) for disposal.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

---

**Please enter comments on the GM safety committee on the risk assessment**

FBS UK bio-repository is equipped with the appropriate safe systems to store and distribute the AAV - Multiple recombinant Adeno Associated Vector material safely. These include appropriate waste and spill procedures. No laboratory manipulation will be performed for this material.

In order to undertake this contained use work, FBS UK will be required to follow notification requirement as per under The Genetically Modified Organisms (Contained Use) Regulations 2014 - GMO(CU), using CU2 form available on the Health and Safety Executives website.

This proposed contained use work is low risk. Please also refer to Product Risk Assessment FBS-P-368.

Please also refer to GMO Risk Assessment performed under:

Client notification number for contained use: GM3392/18.1.

Title: "rAAV manufacture and rAAV Process Development"
Project Ref 957/19.2

Date Ackn'd 09/08/2019

CU2 Project Title
Storage, primary labelling, secondary packaging, secondary labelling and logistical distribution of biological material
(Cold chain supply), contained in capped vial on behalf of client to UK and global consignees

Class
Class 2

CultureVolClass 2
Not Applicable

CultureVolumeClass 3-4

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
Provide storage, labelling, secondary packaging and logistical services to clients carrying out Research, Development, Clinical Trials and Manufacturing of Biotechnology and Therapy Solutions, including Cell Gene Therapy.
Storage is performed in temperature conditions ranging approximately between:
Ambient
Cold: +2 to +8 °C Fridge/walk-in fridge
Ultra Cold -20 to -30°C walk-in freezer
- 40 to -80 °C Upright freezer/Robotic freezer
-150 to -200°C vapour phase Liquid Nitrogen
Primary Labelling: Performed on closed sealed vials in ambient temperature (performed in GMP packaging suite or CL2 laboratory), cold and ultra cold (with the use of dry ice trolley or inside cold rooms).
Secondary Packaging: GM may be packaged in singular containers or multi container depending on clients request.
Secondary Labelling: External labels applied upon request of the client.
Logistical Distribution : Shipping of GM material is performed on behalf of the client using UN3245. Staff performing the task are fully trained on related shipping regulations.
Discard: GM may need to be discarded as per client's request. In these occasions material will be autoclaved prior to disposal.
No open laboratory work with GM will be undertaken as part of this contained use notification.

Recipient or parental organism
Non applicable as storage, labelling, secondary packaging and cold chain logistical distribution only.

Host/vector system
Non applicable as storage, labelling, secondary packaging and cold chain logistical distribution only.

Origin & function
Non applicable as storage, labelling, secondary packaging and cold chain logistical distribution only.

Evaluation of foreseeable effects
Non applicable as storage, labelling, secondary packaging and cold chain logistical distribution only.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Non applicable as storage, labelling, secondary packaging and cold chain logistical distribution only.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Non applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Class 2 GMM waste is only generated as per client's request of material disposal, as result of a faulty container or breakage of primary container.
The Class 2 GMM waste is autoclaved at 123,C for 30 minutes, 2 bar pressures, prior to disposal to landfill by a registered waste contractor (Veolia). Spore strips are included in each Class 2 GMM waste load for purposes of validation of destruction. The autoclaved waste is then stored in a sealed whee lie bin with biohazard symbols displayed, for 48 hours, whi le the spore strips are incubated to validate destruction. After 48 hours and a positive destruction result with the spore strips, the inactivated waste is removed by a registered waste contractor to landfill. The autoclave is validated annually. Class 2 sharps containers are autoclaved in accordance with an SOP for Class 2 GMM waste inactivation before being removed by a registered waste contractor for disposal.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
FBS UK bio-repository is equipped with the appropriate safe systems to store and distribute Class 2 GMO safely. These include appropriate waste and spill procedures. No laboratory manipulation will be performed for this material. In order to undertake this contained use work, FBS UK will be required to follow notification requirement as per under The Genetically Modified Organisms (Contained Use) Regulations 2014 - GMO(CU), using CU2 form available on the Health and Safety Executives website.

This proposed contained use work is low risk, as no laboratory work is undertaken. This contained use notification will cover:
1. All Class 2 GMO materials from clients that have already notified their Class 2 GMO activity to the HSE as far not listed on:
   a. the Counter-Terrorism and Security Act 2016
   b. the Specified Animal Pathogen Order 2008

Important notes below:

All GMO microorganisms listed on the Counter-Terrorism and Security Act 2015 will be risk assessed by the Biosafety/GMO Committee. The committee will decide if additional notification will be required on an individual basis.

All GMO microorganisms assigned Activity Class 2 or BSL2 listed on the Specified Animal Pathogen Order 2008 will be risk assessed by the Biosafety/GMO Committee. Additional CU2 notification will be required and SAPO License application will be completed and submitted to the HSE.

All GMO assigned as BSL2 or equivalent (e.g. Risk Group 2) from international non-UK clients, whom do not have a contained use notification with the HSE, will be risk assessed by the Biosafety/GMO Committee. The committee will decide if additional GMO notification will be required on an individual basis.

Any laboratory manipulation of GMO Activity Class 2 will be risk assessed by the Biosafety/GMO Committee. Additional CU2 notification will be required.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
<td>L2 L3 L3</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L2</td>
<td>L3 L4 L2</td>
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02/03/2022
Data Premises Notified (Originally) 22/08/2006

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed 22/12/2011

Transitional Premises Emergency Plan Required? N

Emergency Plan Required? N

Transferred from 1992 Regs? N

Non-GMMs N

Withdrawn N

Name

CAMBRIDGE BIOSTABILITY LTD

Name 2

Department

Campus Estate or Research Centre

UNIT 184

Building

CAMBRIDGE SCIENCE PARK

Road Name

MILTON ROAD

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB4 0GA

Country

ENGLAND

Tel Number 01223 437 400

Fax Number 01223 279 089

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<tr>
<th>Date</th>
<th>Premises Closed</th>
<th>Name</th>
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<td>CB4 OGA</td>
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Y

**Give brief details of the genetic modification safety committee**

The company currently numbers 26 personnel. The GMO safety committee comprises 3 persons:

1. Cambridge Biostability H&S appointed person.
2. Laboratory scientist (with previous experience of research project management of molecular biology projects and membership of GMO safety committees in 2 previous employments).
3. Laboratory scientist (previous experience in molecular biology).

The committee meets at 6 monthly intervals or as required.

All decisions taken in GMO safety committee meetings are also reported at general Company H&S committee meetings (Company H&S committee comprises 5 members including a senior management representative).

<table>
<thead>
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<tr>
<td>Non-microbial</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Disposable waste:
All contaminated disposable waste is steam sterilised (on site) using an autoclave. Sterilised waste is then disposed of by incineration (of site) through a contract waste disposal company. The sterilisation (autoclave) process is monitored by use of temperature indicating tape and vials included in each run.

Recycled waste;
Recycled waste e.g. glassware, is chemically disinfected using a proprietary disinfectant with quoted virucidal activity (e.g Trigene ® 2% v/v).

For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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</thead>
<tbody>
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</table>

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<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
<th>Gene Therapy</th>
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<tbody>
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<table>
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<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

Other(s) Vaccine research & development.

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Risk assessment for procedures involving GMO's (attached) were approved by the GMO safety committee.
GM Centre Number: 959

<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
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<td>N</td>
</tr>
<tr>
<td>Emergency Plan Required?</td>
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<tr>
<td>Non-GMMs</td>
<td>N</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>N</td>
</tr>
</tbody>
</table>

**Name**

OXFORD NANOPORE TECHNOLOGIES LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

23 FIVE MILE DRIVE

**District**

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX2 8HT

**Country**

ENGLAND

**Tel Number**

0870 486 1966

**Fax Number**

01865 515 657

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

NAME CHANGE FROM OXFORD NANOLABS LTD ON 19/05/2008

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

<table>
<thead>
<tr>
<th>Date Premises Closed</th>
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<th>Campus Estate or Research Centre</th>
<th>Building</th>
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<td>BEGBROKE CENTRE FOR INNOVATION &amp;…</td>
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<td>SANDY LANE</td>
<td>YARNTON</td>
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<td>OX5 1PF</td>
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<td>4 ROBERT ROBINSON AVENUE</td>
<td>OXFORD SCIENCE PARK</td>
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<td>OX4 4GA</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Chairman - (CEO)
- Health and Safety Officer - (Head of R&D)
- Secretary - (Principal Scientist)
- Biological Safety Officer - (Senior Scientist)

Committee formed 11/7/06, and will meet at least on a quarterly basis, with a brief to review all GM risk assessments and procedures.

Laboratory  | Animal Unit  | Growth Room  | Glass House  | Large Scale
---|---|---|---|---
Level 1 (GMMs) | Yes | | | |
Level 2 (GMMs) | | | | |
All liquid cultures will be autoclaved and/or treated with Virkon-S (2% w/v) prior to discarding. Contaminated areas will be treated with wiping with 2% Virkon-S (contact time > 5 minutes). All contaminated materials will be inactivated (100% kill) by autoclaving prior to disposal or recycling. The autoclave will be validated by annual thermocouple mapping and each run monitored by digital recording of the temperature/time profile. All test output will be signed off and stored for reference. A notice describing the status of the validation will be attached to the instrument. Material to be autoclaved will be placed in autoclave bags which are tied and placed in autoclave bins. Prior to autoclave taking place, the bags will be opened to allow steam to penetrate efficiently. The autoclave will be set such that the temperature in the coolest part of the chamber reaches 121°C for 30 minutes. The effectiveness of the sterilisation will be monitored by TTS tape added to each bag, and the temperature profile monitored by the use of a dedicated data printer attached to the autoclave. Once the run is complete, all bags will be identified and sealed with autoclave tape. A record of each autoclave run will be completed, signed off and filed. A Log Book of autoclave usage will require to be filled in and signed off for each run. This will be retained in the laboratory. These steps are laid out in detail in our Standard Operating Procedure ONL1.1 (Disinfection and Sterilisation of GMO material). (See attached document).

Virkon-S at 1% w/v with a contact time of 5 minutes has been shown to be effective (>5 log orders kill) against both E.coli and S. aureus (http://relyon.dupont.com/myRelyOn/activated.htm).

Work areas will be swabbed on a regular basis to check for residual bacterial contamination.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The GM Safety Committee reviewed the notification documents, risk assessments and standard operating procedures relevant to the GMO work being proposed, and have formally given approval for the application to proceed.

### Project Ref 959/14.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
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<td>Processing of Bacterial Cell Pellets Cleaning of retuned DNA sequencing flowcells</td>
<td>Class 2</td>
<td>1-50 Litres</td>
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- **Date Project Ceased**: 11/02/2014
- **Withdrawn**: Y
- **Historical Significant Changes**: Notification withdrawn

#### Project Additional Information

**Purposes of the contained use**
- Production of recombinant protein
- Cleaning returned flow cells that have been used with collaborator samples

**Recipient or parental organism**
- E. coli

**Host/vector system**
- PT7

**Origin & function**
- Growth of recombinant DNA
- Production of plasmid DNA
- Analysis of purified DNA
**Evaluation of foreseeable effects**

- Contained use will limit any pathogenic effects - PPE and mechanical procedures in place
- E. coli cell lines used are incapable of colonising the human gut
- Contained Use (MSC containment hood class 2) will limit any environmental effects

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- All items used with GMMs will be cleaned with 1% Virkon (1 hour exposure) and autoclaved.
- Disposable items will be cleaned with 1% virkon (1 hour exposure), autoclaved and disposed of in 60L yellow clinical waste bins - our current waste disposal contractor is Grundon Clinical Waste.
- Autoclaves are checked by our external maintenance engineer every 6 months to ensure kill/inactivation temperatures are reached.
- Bowie Dick tests are carried out weekly.

**Project Containment**

- The majority of the work will be Cat 1, however all work will be carried out to Cat 2 standard.
- In the case of returned flow cells, collaborator will advise us of any sample used before receipt so potential Cat 2 samples can be segregated so that staff involved will be fully aware.
- Full staff training and supervision will be carried out by the company Health & Safety Officer and the Biological Safety Officer.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
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<th>Animal Units</th>
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Name
SYSTEMS BIOLOGY LABORATORY

Name 2

Department

Campus Estate or Research Centre
UNIT 7

Road Name
WEST CENTRAL 127

District
MILTON PARK

Town
ABINGDON

County
OXFORDSHIRE

Postcode
OX14 4SA

Country
ENGLAND

Tel Number
01235 827400

Fax Number
01235 834965

E-mail

HSE Division
EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted
02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Entire organisation has 12 employees in total, so there is frequent, open dialogue particularly between those lab staff performing contained use activities, and their line management. Company director and Medical projects Manager meet on a regular basis to discuss laboratory matters, including risk assessment of contained use activities. Office and laboratory managers are both trained in health and safety issues, and ensure that staff complete risk assessments of contained use activities.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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</table>

02/03/2022
All material that has been in contact with GMM's will be disposed of in house via autoclaving of double bagged clinical waste bags. These bags are taped before disposal in such a way that successful sterilisation can be guaranteed, i.e. by use of autoclave tape and keeping the neck of the bag open for steam penetration. At the end of the autoclave process on site, sterilisation is confirmed by a paper printout of the temperature reached and the duration at this temperature during the autoclave process. These printouts are archived on site.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

All material that has been in contact with GMM's will be disposed of in house via autoclaving of double bagged clinical waste bags. These bags are taped before disposal in such a way that successful sterilisation can be guaranteed, i.e. by use of autoclave tape and keeping the neck of the bag open for steam penetration. At the end of the autoclave process on site, sterilisation is confirmed by a paper printout of the temperature reached and the duration at this temperature during the autoclave process. These printouts are archived on site.

**Tick to confirm that you are attaching a summary of the risk assessment**

Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**


**Please enter comments of the GM safety committee on the risk assessment**

The risks associated with performing the described protocols do not represent a significant hazard to those members of staff involved. The Breener scheme results in a calculation of 10 (-18) clearly placing this at containment level 1 activity.

All bacteria used are K12 derived and as such are incapable of surviving outside containment if this was breached by accident. None of the vectors used produce substances of any hazard, the vectors themselves are based on vectors considered non-hazardous by the ACGM and all methods are standard laboratory techniques.

Good laboratory practice should still be used at all times, and appropriate COSHH and risk assessments performed.
<table>
<thead>
<tr>
<th><strong>Data Premises Notified</strong></th>
<th>20/09/2006</th>
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<td><strong>Non-GMMs</strong></td>
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**Name**

STEM CELL TECHNOLOGIES UK LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

7100 BEACH DRIVE

**Road Name**

**Building**

CAMBRIDGE RESEARCH PARK

**District**

WATERBEACH

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB25 9TL

**Country**

ENGLAND

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<th><strong>Tel Number</strong></th>
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<td><strong>Fax Number</strong></td>
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**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<th>Building</th>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

Committee is composed of employees of the company - our Chief Scientific Officer, Business Unit Manager (who is our Biological Safety Officer) and 4 Scientists all of which have science or engineering qualifications. The committee will meet 6 monthly but consider new risk assessments submitted electronically and meet ad hoc to discuss if required.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity:

Waste is placed in yellow sharps bins or yellow bags labelled as clinical waste with our company name, the date and environmental agency registration number. Bags are sealed, placed in a yellow "clinical waste" wheelie bin until collected for transportation off-site for incineration.

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment:

Committee felt that there was no risk to human health or the environment from the proposed activity and therefore agreed that it should be classed as level 1.
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| Page 12208 of 15326 |
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The composition of the genetic modification safety committee is as follows:
Senior Analyst
Analyst
Technical Manager
Safety, Health and Environment Manager.

It is not thought likely that this method will be subject to change. As such the initial risk assessment is likely to remain valid. A review of the risk assessment will be conducted by the committee if new information comes to light.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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Tick if confidential
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Other(s) Use of a genetically modified cell-line to test for the presence of dioxins in our products.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Contaminated solid waste such as plastic disposables, tissue paper etc. will be collected in autoclave bags. These will be autoclaved at 121 degrees C for 22 minutes. Contaminated liquid wastes will be autoclaved under the same conditions. Any materials that cannot be autoclaved will be treated as medical waste and incinerated. Spillages of contaminated material will be treated with a full spectrum disinfectant (Vircon) and wiped up with tissue. Wipes will be autoclaved as above. The autoclave will be validated and serviced annually. Indicator disks will be used with every operation to show that appropriate conditions have been achieved. The expected level of kill for these treatments is 100 percent.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment of the organism shows that it is a class 1. Staff will be appropriately trained, follow approved procedures and use specified personal protective equipment and containment measures. Inactivation methods will give an expected 100 percent kill of the organism. The genetic modification safety committee considers that risks to humans and the environment from the use of this organism at our premises will be negligible.
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## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

St George's University of London's Genetic Modification Safety Committee (GMSC) and Biological Safety Officer for Genetic Modification (BSO-GM) provide risk assessment review and approval in an advisory capacity to St George’s NHS in accordance with regulation 8 of the Genetically Modified Organisms (Contained Use) Regulations 2014. The GMSC has representation from biological and lab safety, health and safety, microbiology/virology, infection control, clinical infectious disease and cellular biology.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>

02/03/2022
No class 1 activity at this time. First use will be at Class 2.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The St George's GMSC were all asked to review the risk assessment on the 18/07/06. This was completed by 30/08/06; there were no outstanding issues of concern from the GMSC.

Project Ref 963/06.1

A Phase 2, Randomized, Double-blind, Placebo-controlled, Multicentre, Dose-Selection Study of AD/2 Hypoxia Inducible Factor (HIF)-1alpha/VP16 in Patients with Intermittent Claudication. Protocol No: PADHIF00704.

Class 2

< 1 Litre

Consent Granted

Not Applicable

Project notified under transitional arrangements N
Project Additional Information

**Purposes of the contained use**

For 300 patients (up to 10 patients at St George's) with intermittent claudication, where they can only walk between 1 and 10 minutes before having to stop with severe leg pain. This phase 2 clinical study will examine the safety and efficacy of an experimental gene transfer agent (via a single treatment of 40 injections). And its ability to stimulate the growth of new blood vessels from existing blood vessels (angiogenesis) in an attempt to improve the blood flow in the patients' legs. To evaluate if patients have improvement in their walking ability, 6 months after receiving the study drug standardised walking treadmill tests will be performed.

Potential patients after signing informed consent and having thorough screening will be randomised to receive either 1 of 3 doses of Ad2/HIF-1a/VP16 or placebo in a 1:1:1:1 ratio. The study treatment for each patient will consist of a single dose administered intramuscularly to both legs in the Vaccine Institute at St George’s Trust a level 2 biosafety area. The drug will be stored in the locked –70C freezer in the restricted area of the Vaccine Institute S2 facility. Trial drug will be drawn up under antiseptic conditions in a BL2 biosafety cabinet in the Vaccine Institute at St George’s NHS Healthcare Trust. The syringes with prepared drug will then be placed in a closed metal container and taken to an adjacent room within the S2 facility for the patient injections. The patient will be treated with study drug in the S2 facility and will rest for at least an hour post injections to minimise any risk factors. All waste products from the preparation and treatment doses of the study drug will be contained, packaged under biohazard conditions prior to autoclaving and/or incinerating. As per risk assessment.

Recipient or parental organism

The HIF-1 sequence (1-390) amino acids within the Ad2/HIF-1 a/VP16 vector were derived from the full length HIF-1 a sequence (aa1-826) generated by polymerase chain reaction (PCR) from a HeLa cell cDNA library (available commercially from Clontech). The correct sequence was verified by DNA sequencing performed by the sponsor Genzyme Corporation.

The patient will receive the gene therapy agent as intramuscular injections to the legs. In theory growth of new blood vessels in unintended areas may occur. However the patients will be screened rigourously as per protocol. The gene is attached to an adenovirus; this may survive in the environment in the event of accidental release. However the Ad2 vector used in this study is replication deficient, so it is unlikely.

Host/vector system

Ad2/HIF-1 a/VP16 is a replication-deficient Type 2 Adenovirus (Ad2) used to express the Ad2/HIF-1 a/VP16 gene. The Ad2 DNA, used to derive the AD2/hif-1a/vp16final product, was obtained from American Type Culture Collection (ATCC). All recombinant DNA cloning and virus construction steps were completed at Genzyme Corporation (USA). The upstream manufacture of Ad2/HIF-1 a/VP16 involves cell culture. Adenovirus infection, harvest and upstream processing. The downstream
purification of Ad2/HIF-1 a/VP16 consists of several chromatography steps. After sterile filtration, the formulated bulk undergoes final dilution and is filled into glass tubing vials (3ml solution in 5ml vials). Although the wild type adenovirus type 2 naturally can infect and replicated in the respiratory tract, eye, gastrointestinal tract and bladder, the Ad2 viral vector used to express the HIF-1 a/VP16 gene is replication deficient. Therefore there is minimal risk to an individual if exposed.

## Origin & function

### Source of the HIF-1a gene.

The HIF-1 sequence (1-390) contained within the Ad2/HIF-1 a/VP16 vector was derived from the full length HIF-1 a sequence (aa1-826) generated by polymerase chain reaction (PCR) from a HeLa cell cDNA library (available commercially from Clontech). The correct sequence was verified by DNA sequencing performed by the sponsor. The study requires 300 patients (a maximum of 10 patients at St George’s Healthcare NHS Trust) male or female between the ages of 40-80 years of age who have a diagnosis of peripheral arterial disease in both lower limbs. Symptoms of severe intermittent claudication which manifests as a pain, cramping, discomfort or fatigue in various muscle groups. The patient must have had these for at least 6 months. All patients will as part of the protocol is only able to walk for no longer than 10 minutes using a standardised exercise treadmill test. Only once the patient has given informed consent and has had a vigorous cancer screening, will the patient be randomised to one of three treatment doses or placebo.

## Evaluation of foreseeable effects

### Direct Therapeutic Effect: Angiogenesis and Therapeutic Angiogenesis

Angiogenesis, the formation of new blood vessels from existing blood vessels, is a complex physiologic process involving numerous mediators including the angiogenic growth factors.

The term “therapeutic angiogenesis” has been used to describe a strategy employing gene transfer or protein formulations of angiogenic growth factors, which stimulate or augment new blood vessel development, to target vascular inefficiency. Gene transfer, when administered into ischaemic tissue, permits targeted delivery of the therapeutic transgene, and maintenance of a concentration of the angiogenic protein in that region for days to weeks following a single administration. The patients will have repeat standardised treadmill tests to assess their ability to walk further. Also repeat standardised quality of life questionnaires, to assess if their quality of life has changed since their treatment dose.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The syringes and vials containing the inoculated material will be placed in a sharps container, with 10% sodium hyperchlorite and then placed in a Denley container to be transported via trolley to be autoclaved via the SAL autoclave in an adjacent building within the hospital site, (1st floor Jenner Wing). After autoclaving, a completed form from the medical microbiology dept will be issued to verify destruction of the material.

Any used bed-linen will be cleaned using routine hospital procedures. However if there is any blood/faecal contamination then a high temperature wash will be used. The linen being placed in red bags, the contents will not then be handled again as the bags are placed directly into the laundry machines. According to the Draft copy of Guidance on the use of genetically modified micro-organisms in a clinical setting.

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Used vials and other miscellaneous materials (mat, gloves, syringes) used in the preparation of the test material will be placed in a plastic bag which is then sealed. The sealed plastic bag will be removed from the biosafety cabinet, and placed into a biohazard waste container. The contents will be autoclaved and incinerated. After each intramuscular injection any refluxed fluid must be wiped clean with sterile gauze, which will immediately be placed in biohazard bags for autoclavin and
incineration.
The used treatment syringes will be placed directly in a container with 10% sodium hypochlorite, autoclaved and disposed of in biohazard bags.
All waste from the trial patient after treatment will be treated as potentially infected. The biosafety workspace should be cleaned with 10% sodium hypochloride (bleach) solution, followed by a rinse with water and ethanol.

The autoclave is located in an adjacent building within the hospital site, (1st floor Jenner Wing ext 5738) and is a SAL autoclave. This has daily Bowie Dick checks, weekly extensive checks by hospital engineers and quarterly servicing and checks by the autoclave installers. The department of Medical Microbiology where the autoclaving occurs will also complete a confirmation form of the destruction of any waste products for research records.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The St George's GMSC were all asked to review the risk assessment on the 18/07/06. This was completed by 30/08/06; there were no outstanding issues of concern from the GMSC.

Project Containment

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Yes
**GM Centre Number: 964**

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Data Premises Closed

Transitional Premises Emergency Plan Required?  

**Name**  
ALBA BIOSCIENCE, A DIVISION OF THE SCOTTISH NATIONAL BLOOD TRANSFUSION SERVICE

Name 2

Department

Campus Estate or Research Centre

Road Name

21 ELLEN'S GLEN ROAD

District

LIBERTON

Town

EDINBURGH

County

EAST LOTHIAN

Postcode

EH17 7QT

Country

SCOTLAND

Tel Number

0131 536 5907

Fax Number

0131 536 5897

E-mail

HSE Division

SCOTLAND

Comments

**Date at Which Additional Info Submitted**

02/03/2022
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

A Genetic Modification Safety Committee (GMSC) has been established and is based at 21 Ellen's Glen Road, Edinburgh. The committee comprises six members of staff from various representative functions and scientific expertise as well as staff side representation. Meetings are currently held on a monthly basis.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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<th>Large Scale</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

a) Inactivation of liquids containing the biological agent to an appropriate disinfectant at a known concentration.
b) Disposable items of equipment/materials will be heat treated.
c) As clinical waste in a yellow bag, properly labelled and identified, to incinerator or other appropriate end process.
d) Additionally, process waste is disposed if by flushing to drain using excessive amounts of water. The drainage system of the facility is directly linked to a 100,000 US gallon (378,00 litres) reservoir before being directed to the environmental drainage system.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC have reviewed the Risk Assessment and agree that the GMO activities performed at either of Albs Bioscience's premises at both 21 Ellen's Road, Edinburgh and Pentlands Science Park, Bush Loan, Penicuik and find that the overall risk factor is low for the following reasons:
1) No genetic modification is performed within either of the premises.
2) The gene product is deemed to be non-toxic and synthesised as a fusion product and is unlikely to be hazardous.
3) The gene product will be stored, grown and expanded using standard monoclonal procedures and equipment for use in the manufacture of in vitro diagnostic reagent only (21 Ellen's Glen Road site only).

Other(s)

Cell culture expansion from small scale to large scale fermentation.
**GM Centre Number: 965**

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**Name**

| CYTOCELL LTD |

**Campus Estate or Research Centre**

| 418 CAMBRIDGE SCIENCE PARK |

**Road Name**

| MILTON ROAD |

**District**

| MILTON |

**Town**

| CAMBRIDGE |

**County**

| CAMBRIDGESHIRe |

**Postcode**

| CB4 8PZ |

**Country**

| ENGLAND |

**Tel Number**

| 01223 935080 |

**Fax Number**

| 01223 294986 |

**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Y

**Give brief details of the genetic modification safety committee**

Our GMSC is comprised of: - a) A management representative; b) chairman/BSO; c) A senior employee representative and d) An employee representative.

When we relocate to our new premises we intend to meet once per month to specifically discuss any new projects or amendments to our current risk assessments (as appropriate). There will also be a more general discussion concerning laboratory safety matters with an emphasis on the GMO work.

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**Other (please specify)**: 

Tick if confidential
Solid waste such as plastics, agar, contaminated paper etc. and liquid volumes below 50 mls are placed into open-necked autoclave bags (double thickness) and sterilised at 126°C for 20 minutes. As a means of validation a Brown "Black-dot" glass sterilisation indicator is taped to the inside of the autoclave bag (in addition to the quickly visualised autoclave tape which is taped to the outside of the bag) these two indicators are routinely checked after each autoclave run. The autoclave has its physical parameters (pressure, temperature and chamber integrity) checked 2x per year by external qualified technicians.

Solution volumes above 50 mls (bacterial culture supernatants) are disinfected chemically with commercially available "Persept" or "Klorsept" chlorine releasing tablets. Our internal validation experiments have determined that a final available chlorine concentration of 1000ppm (freshly made) for at least 3 hrs is entirely adequate. This level produces 1 100% kill rate with our supernatants.

To deduce this ppm level a ppm titration was used on bacterial supernatants, for specified periods of time, followed by spreading the disinfected solution onto agar plates with no antibiotic added; No colonies grew after an overnight incubation at 37°C.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The Cytocell GMSC has carefully considered all the risk assessments and is content that they correctly assign the procedures to containment level 1 / activity class 1.
**Name**

| HVIVO LTD |

| Name 2 | Department |

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**Comments**

Name change from Retroscreen Virology Ltd on 12/05/2015

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

02/03/2022
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<td>Invertebrates</td>
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<td>Other (please</td>
<td>specify below)</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
To use reverse genetics to create attenuated influenza virus. We will use an attenuated virus strain such as PR8 as the background strain and replace the neuraminidase and Haemagglutinin with those from current influenza A and B strains. Mutations…….

### Project Additional Information

**Purposes of the contained use**

To produce an attenuated influenza virus that could be used as a live attenuated vaccine against current strains of influenza virus. Current influenza virus vaccines are only about 70% effective, live attenuated virus vaccines like those we are trying to develop may be more effective.

**Recipient or parental organism**

The extensively studies PR8 strain of the influenza virus will be used as the recipient Influenza strain. The PR8 strain of influenza virus is highly attenuated and does not cause disease in humans. Influenza viruses are classified as ACDP category 2 pathogens.

**Host/vector system**

Plasmids containing the cloned genes of Influenza virus strain. PR8 are being generously donated to us by Professor Brownll (Sir William Dunn School of Pathology, University of Oxford). The plasmids are PPOLY-PB1-PR8, PPOLI-PA-PR8, PPOLI-PA-PR8, PPOLI-PR8, PPOLI-M-PR8, PPOLI-NS, PR8, PPOLI-Sapl-Rib, pcDNA-PBI-PR8, pcDNA-PB2-PR8, pcDNA-PA-PR8, pcDNA-NP-PR8. These plasmids are all Puc18 derivatives. These will be transfected into either Vero (African Green Monkey) cells or 293t (Human Kidney) cells. Recombinant virus will then be retrieved from these transfected cells (see Fodor E etal. Rescue of influenza A virus from recombinant DNA, J Virol 1999;73:9679-9682).
The introduced genetic material will be the influenza virus neuraminidase and Haemagglutinin genes cloned from recent strains of the influenza virus such as A/New Caledonia/11/99. Mutations will be added to these cloned genes to attenuate the virus. Alterations to the neuraminidase gene have been shown to lead a reduction of virulence (see Solorzano et al. Reduced levels of neuraminidase and Haemagglutinin which will come from recent strain of the influenza virus. The introduced gene products will be expressed on the surface of the recombinant influenza virus and are intended to act as antigens to provoke a protective immune response to any later challenge by the wild type influenza virus.

Evaluation of foreseeable effects

The influenza virus normally causes an upper respiratory infection in humans, however, the attenuated PR8 strain is non-pathogenic in humans. The mutations we add to the influenza virus genome are intended to be stable and to attenuate the virus. The haemagglutinin and the neuraminidase proteins have been shown to be non-toxic to humans and have been used extensively in vaccines. Vaccine are available to protect against Influenza virus infection and there are antiflu drugs such as Tamiflu and Relenza.

Influenza viruses pose limited environmental risk as they are degraded quickly in the environment (UV, dessication) the genetic modifications we perform will not change this.

Work with the mutated influenza virus being conducted in Microflow Biological Safety Cabinets in category 2 GLP laboratory.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste produced containing recombinant influenza virus will be treated with Basol, a quaternary ammonia compound based detergent. This detergent is known to very rapidly destroy lipid encapsulated viruses such as influenza. To ensure a one hundred percent kill the waste containing the recombinant virus will be left in the basol for 24 hours. The waste will then be disposed of down the sink. This is a standard technique for disposing of Influenza waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Both the parent virus and the neuraminidase and haemagglutinin proteins from recent strains have a history of safe use in humans.

Project Containment

<table>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
To use reverse genetics to create attenuated influenza virus.

Purposes of the contained use

To produce an attenuated influenza virus that could be used as a live attenuated vaccine against current strains of influenza virus. Current influenza virus vaccines are only about 70% effective, live attenuated virus vaccines like those we are trying to develop may be more effective.

Recipient or parental organism

The extensively studied PR8 strain of the influenza virus will be used as the recipient Influenza strain. The PR8 strain of Influenza virus is highly attenuated and does not cause disease in humans. Influenza viruses are classified as ACDP category 2 pathogens.

Host/vector system

Plasmids containing the cloned genes of Influenza virus strain, PR8 are being generously donated to us by Professor B (Sir William Dunn School of Pathology, University of Oxford). The plasmids are PPOLY-PB1-PR8, PPOLI-PA-PR8, PPOLI-HA-PR8, PPOLI-NP-PR8, PPOLI-NA-PR8, PPOLI-M, PR8, PPOLI-NS, PR8, PPOLI-Sap1-Rib, pcDNA-PB1-PR8, pcDNA-PB2-PR8, pcDNA-PA-PR8, pcDNA-NP-PR8. These plasmids are all Puc18 derivatives. These will be transfected into either Vero (african Green Monkey) cells or 293 (Human Kidney) cells. Recombinant DNA, J. Virol 1999;7.3:9679-9682).
The introduced genetic material will be the influenza virus neuraminidase and Haemagglutinin genes cloned from the recent strain of the influenza virus, A/California/7/2009(H1N1)sw1. Mutations will be added to these cloned genes to attenuate the virus. Alterations to the neuraminidase gene have been shown to lead a reduction of virulence (see Solorzano et al. Reduced levels of neuraminidase of Influenza A viruses correlate with attenuated phenotypes in mice. J. Gen Virol 2000: 81 Pt3:737-742). The recombinant virus will thus contain all the PR8 gene segments except those encoding the neuraminidase and HAEmagglutinin which will come from a recent strain of the influenza virus. The introduced gene products will be expressed on the surface of the recombinant influenza virus and are intended to act as antigens to provoke a protective immune response to any later challenge by the wild type influenza virus.

Origin & function

The influenza virus normally causes an upper respiratory infection in humans, however, the attenuated PR8 strain is non-pathogenic. The mutations we add to the influenza virus genome are intended to be stable and to attenuate the virus. The haemagglutinin and neuraminidase proteins have been shown to be non-toxic to humans and have been used extensively in vaccines. Vaccine are available to protect against Influenza virus infection and there are antiflu drugs such as Tamiflu and Relenza. Influenza viruses pose limited environmental risk as they are degraded quickly in the environment (UV, dessication) the genetic modifications we perform will not change this.

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Evaluation of foreseeable effects

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Influenza viruses pose limited environmental risk as they are degraded quickly in the environment (UV, dessication) the genetic modifications we perform will not change this.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste produced containing recombinant influenza virus will be treated with Biocleanse, a quaternary ammonia compound based detergent. This detergent is known to very rapidly destroy lipid encapsulated viruses such as influenza. To ensure a one hundred percent kill the waste containing the recombinant virus will be left in the basol for 24 hours. The waste will then be disposed of down the sink. This is a standard technique for disposing of Influenza waste.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

Both the parent virus and the neuraminidase and haemagglutinin proteins from recent strains have a history of safe use in humans.

Project Containment
Project Additional Information

Purposes of the contained use

Four Genetically Modified (GM) viruses will be used in the assay. They were produced by reverse genetics using established techniques (Neumann et al., 1999) to create and attenuated influenza virus consisting of the HA and NA gene segments from recent strains and the six internal gene segments from a cold adapted Master Donor Virus (MDV). This MDV has previously been used to generate commercially available influenza vaccines, this is described in more detail in Section 7.

The assay itself will consist of the mixing of serial dilutions of human sera with the GM virus across a 96 wells-microplate coated with the fetuin substrate. After a suitable incubation period, the enzymatic reaction will be read colorimetrically.

This assay has been very well described and is widely used to measure NA protein activity (Aymard et al., 2003).

Stocks of the GM viruses will be received at RVL ready for use and there will be no further propagation of the virus onsite.
Influenza viruses are classified as ACDP category 2 pathogens. The GM influenza viruses we will use are highly attenuated, cold adapted vaccine strains. The methodology used to generate the GM virus for use in these assays is the same as used to produce Live Attenuated Influenza Vaccine (LAIV) that is currently licensed and marketed in the USA and has recently received approval for use within the EU. Over 40 million doses of vaccine have been manufactured.

The level of attenuation of the GMO virus for use in the assay will be similar to that for the LAIV.

Host/vector system

In the assays RVL will use four different GM viruses representing different subtypes of influenza and different NA proteins. These four viruses are summarised in Table 1. The six internal gene segments of the MDVs impart the cold adapted (ca), temperature-sensitive (ts) and attenuation (att) phenotype to the vaccine strains.

Reassortant viruses such as these, carrying the six internal genes of MDV-A or MDV-B and the wild type HA and NA segments of another virus, consistently maintain three key characteristics:

1) They are cold adapted and replication is limited to the upper respiratory tract.

2) They do not replicate in the lungs of ferrets,

3) They are safe and attenuated in children and adults

(Massab et al., 1982; Monto et al., 1982; Odagiri et al., 1982; Wright et al., 1982)

Table 1. Summary of the GM influenza viruses and the origins of the respective gene segments.

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<tr>
<th>Virus name</th>
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<th>Origin of NA gene</th>
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<td>MDV-A (H6N2)</td>
<td>A/Ann Arbor/6/60 MDV-A</td>
<td>A/teal/HK/W312/97 (H6N1)</td>
<td>A/Uruguay/716/07 (H3N2)</td>
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Abbreviations PB2, PB1 and PA are the three polymerase subunits, PB for polymerase basic

HA Hemagglutinin, NA neuraminidase, NP nucleoprotein

Origin & function

Origins

A/Ann Arbor/6/60 (MDV-A) and B/Ann Arbor/1/66 strain (MDV-B)

The MDV viruses were generated by cold adaptation of an influenza type A strain (A/Ann Arbor/6/60 H2N2) and a type B strain (B/Ann Arbor/1/66) by serial passage at sequentially lower temperatures in specific pathogen-free primary chick kidney cells (Jin et al., 2003).
Influenza type A GMO viruses (Table 1)

These viruses contain 6 internal genes from the MDV-A, the HA segment of A/teal/HK/W312/97 (H6N1). The Neuraminidase (NA) genes are cloned from the influenza virus A/South Dakota/6/06 (H1N1) for the first GMO and from A/Uruguay/716/07 (H3N2) for the second.

Influenza type B GMO viruses (Table 1)

These viruses contain 7 genes from the MDV-B. The Neuraminidase (NA) genes are cloned from the influenza virus B/Florida/4/06 for the first GMO and from B/Malaysia/2506/04 for the second.

A/South Dakota/06/06 (H1N1), A/Uruguay/716/07 (H3N2), B/Florida/4/06

These are vaccines strains contained in the vaccine formulation.

B/Malaysia/2506/04

This strain is used in this project to check the cross protection of the vaccine against another type B neuraminidase.

A/teal/HK/W312/97 (H6N1)

In this project the HA of the virus (subtype H6) is used to avoid non-specific neutralisation of the NA by the antibody directed against the H1 and H3 contained in sera after vaccination.

Intended function.

The intended function of the GMO described here is to provide the NA protein, which acts enzymatically on the fetuin substrate in the assay. The assay is then used to titrate anti NA antibodies in sera from people pre and post vaccination.

Evaluation of foreseeable effects

Influenza virus normally causes an acute self-limiting upper respiratory infection in healthy humans with no underlying health conditions.

The GM viruses intended to be used in the assays are attenuated strains with limited ability to replicate in human upper respiratory tract. In general cold adapted vaccine strains, as the ones that will be used in this study, are highly restricted in there replicative ability and virus is rarely recovered by culture from the nasal wash after inoculation for volunteers (Belshe et al., 2008; Talaat et al., 2011)

The viruses have been sequenced and do not possess any known mutations that would give rise to drug resistance to the anti-neuraminidase compounds.

Influenza viruses pose limited environmental risk as they are degraded quickly in the environment due to UV radiation and desiccation.

All work with the live GM influenza viruses will be conducted in Microflow Biological Safety Cabinets in category 2 GLP laboratory.

Please see risk assessment attached to this application for more details

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No application for derogation

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste produced containing GM influenza virus will be treated with 5% (v/v) Biocleanse (final concentration), a quaternary ammonia compound based detergent. This detergent is known to very rapidly destroy lipid encapsulated viruses such as influenza. The GM virus will be left fully immersed in the 5% (v/v) Biocleanse ™ (final concentration) for 24 hours. The liquid waste will then be autoclaved and the final liquid will be disposed of via normal drainage.

Solid wastes such as 96 well microplate, pipettes tip, empty vials, etc will be treated first with biocleanse solution as mentioned above. After 24 hours being fully immersed, solid waste will be autoclave on site and then treated as clinical waste i.e. incinerated

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The RVL GMO Committee met on 12th May 2011, chaired by Prof J O, and reviewed the use of the proposed GM influenza viruses at the Company’s facility.

The following key points were considered:

**VIRUS PROVIDENCE:**
Both the MDVs, and the NA and HA proteins, are from characterised strains. The MDVs have a history of safe use in humans.

**GMO VIRUS BACKGROUND:**
These recombinant viruses are generated from cold adapted virus back-bone. For all recombinant virus evaluated here, both NA and HA segments are from well described strains and have been completely sequenced. The parental MDVs are well characterised and not known to be resistant to zanamivir and oseltamivir.

**CONDUCT OF THE ASSAY**
During the assay the 96 well plates will be washed repeatedly with PBS-Tween. Tween is a detergent and will inactivate the influenza virus.

**HANDLING OF H6 RECOMBINANT VIRUS at RVL:** There is no poultry farm in the vicinity of the RVL facility, H6 viruses tend to be of low pathogenicity.

Type B viruses are generally accepted to be less pathogenic than type A viruses and are not known to have pandemic potential.

Retroscreen’s GM committee did not believe that there was a risk associated from working with different GMO strains concurrently as there will be no culture of virus ongoing in the laboratory during the conduct of this assay and no cell culture will be conducted in the incubator dedicated to this project. All equipment will be very clearly labelled to state its dedicated use.

All virus samples will be used only within a safety cabinet. Plates will be removed from the cabinet once washed with PBS-Tween to inactivate the virus.

The committee considered the following aspect of health monitoring.

Health Monitoring (Tick relevant boxes)

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<tr>
<td>Are staff encouraged to be vaccinated?</td>
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* If required, Oseltamivir can be used prophylactically
** Vaccination of staff is encouraged, this will protect against current seasonal strains of influenza

Conclusion of the RVL GMO committee
The committee concluded that the H6N1, H6N2 and B GMO viruses could be used within the RVL labs

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**Project Containment**

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02/03/2022

Page 12234 of 15326
### Project Additional Information

**Purposes of the contained use**

Seasonal influenza strains produced by reverse genetics using established techniques (Neuman et al, 1999) will be:
1. used as challenge agents in human volunteer studies
2. investigated in standard laboratory assays.

The human volunteer studies will be used to improve understanding of influenza infection, illness and host response, in addition studies may be conducted to evaluate novel IMPs (all clinical studies subject to ethics approval).

Stocks of the GM virus will be received at hVIVO ready for use. There will be no further modification of the virus on hVIVO’s premises.

**Recipient or parental organism**

Not applicable (work to be outsourced).

**Host/vector system**

An eight plasmid system will be prepared each containing the influenza genes and transfected into a qualified cell line and recombinant virus rescued (Hoffmann E, et al., Eight-plasmid system for rapid generation of influenza virus vaccines. Vaccine 20(25-26):3165-70 2002; Hoffmann E and Webster RG, Unidirectional RNA polymerase
I-polymerase II transcription system for the generation of influenza A virus from eight plasmids” J Gen Virol. 81(12):2843-7 2000. This work will be outsourced and not take place at the applicant's premises.

Origin & function

The influenza strains from which the plasmids will be derived will be seasonal influenza strains, classified as ACDP Hazard Group 2, that naturally circulate in the human population. The seasonal influenza strains selected will be well characterised and cause mild to moderate influenza like illness. The full nucleotide sequences will be obtained from Genbank. The recombinant virus seed stocks will contain the same gene sequences as the original wildtype viruses and will be used to produce challenge virus stocks under Good Manufacturing Practice (GMP) conditions at an outsourced facility.

The recombinant virus seed stock and/or the manufactured virus will be used in laboratory assays (e.g. virus titre determinations; haemagglunination inhibition assays).

The recombinant manufactured virus may be investigated in animal models (e.g. ferret studies) to assess virus replication, clinical symptoms, pathology and biomarkers.

The recombinant GMP manufactured virus will be used in human challenge studies.

Evaluation of foreseeable effects

Influenza viruses generally cause upper respiratory tract infections in humans. Symptoms are usually resolved after 4-5 days but complete recovery can take up to 10 days or longer. The GMM will be representative of clinical isolates that naturally circulate in the human population globally. The influenza viruses to be produced by reverse genetics are classified as ACDP category 2 pathogens. The sequences will be based on recent circulating seasonal influenza strains which have been extensively studied. The use of reverse genetics for the production of influenza virus rather than the propagation of a clinical isolate will enable far greater control of the virus sequence (influenza viruses exist as a heterogeneous population of quasi-species) and hence the properties of the virus stock.

One strain of virus that may be produced by reverse genetics is H1N1. A GMM H1N1 influenza strain has previously been shown to cause mild to moderate influenza disease with no severe complications in human challenge studies (Memoli et al., Validation of the wild-type influenza A human challenge model H1N1pdmMIST: an A(H1N1)pdm09 dose-finding investigational new drug study. Clin Infect Dis. 60(5):693-702 2015).

There are no additional hazards associated with the GMM compared to that of the parental wild-type strain from which it was derived; the pathogenic traits of the GMM virus will not be increased by reverse genetics; the severity of infection is expected to be the same as for the parental wild-type. The GMM will be screened to ensure the virus sequence is consistent with the parental sequence and no known virulence associated mutations present. In addition the GMM will be checked to confirm that no mutations associated with drug resistance to neuraminidase inhibitors are present. Susceptibility testing to one or more classes of approved anti-influenza drugs may be conducted if necessary. The GMM will be no more hazardous than the parental seasonal circulating influenza strain from which it was derived.

There are two aspects to the work outlined in this application: The first involves laboratory work involving the GMM; the second is the use of the GMM in human challenge studies.

The standard procedures used for handling influenza ACDP category 2 pathogens will also be appropriate for handling the GMM. Laboratory work will take place in containment level 2 laboratories; these have restricted access and hand wash basins. Only trained personal will work with the GMM; training records will be retained. All laboratory work with the GMM will be performed in Biological Safety Cabinets (BSC) to contain aerosols. All workers will wear appropriate PPE in accordance with category 2 laboratories. Centrifugation will take place using sealed rotors.

Work on different viruses will not be performed in the same cabinet or work space to negate cross contamination. All equipment used for GMM work will be designated for work involving the particular GMM only and will be cleaned thoroughly before the equipment is used with other work. A fresh solution of an appropriate quaternary ammonia compound based detergent (5% (v/v) Biocleanse (final concentration) and 70%(v/v) Industrial methylated spirits(IMS) will be used for cleaning BSCs, benches, centrifuges and other surfaces at the end of each working session and dealing with spillages. Sealed centrifuge buckets and small items of equipment will be swabbed prior to removal from cabinets. A period of at least 30 minutes will be allowed before work with a different influenza virus commences. Once work is completed for the day, the Ultra Violet
The second aspect is the use of the GMM in human challenge studies. The hazards associated with the GMM is seen as representing an equivalent risk to those challenge studies conducted using wild-type influenza virus strains of the same ACDP Hazard Group 2. All human challenge studies will be carried out under 'contained use' conditions. Human challenge studies will be conducted within hVIVO’s specialised quarantine facility (Virus Challenge Unit, VCU). The VCU has restricted access with a mechanical air handling system and hand wash facilities. Only trained personal work within the VCU; training records will be retained. Infection control measures are in place to prevent secondary transmission and transfer of virus to outside of the VCU. As per hVIVO’s documented procedures, VCU staff will wear PPE (scrubs, apron, full face respirator or mask with eye protection and gloves). All VCU staff will change from outer clothes into PPE before entering the VCU, including a change in footwear or the use of shoe covers; and will disrobe prior to departure within the VCU’s changing area before exit; showers are available in the changing area.

Human volunteers participating in challenge studies will reside within the VCU. The GMM inoculum dose will be based on the minimum titre required to achieve infection with adequate virus shedding and symptomology. The minimal volume of GMM will be taken into the secure VCU for volunteer challenge. As such, it is expected that the GMM titres handled within the VCU to be low and the volume of virus used small (<50 mL at any one time) thereby minimising the risk of potential contamination. Volunteers are inoculated intranasally via a pipettor using a filter pipette tip to minimise the risk of aerosols.

Post volunteer inoculation, the GMM virus is expected to replicate and the new progeny virus will be representative of the season influenza parental virus from which the GMM was derived. All volunteer samples obtained post virus challenge, which may contain infectious virus (e.g. nasal washes), will be treated as clinical samples and will be handled under the parental virus ACDP category.

Volunteers will remain in the VCU for the duration of the clinical study, usually a minimum of 7 days post-inoculation (to cover the virus replication and clearance stages of influenza infection) and discharged on Day 8 or later. All volunteers must have a negative result for influenza by a rapid viral antigen test conducted on the day of discharge (or earlier) to confirm they are no longer shedding virus. Any volunteers who remain symptomatic will only be discharged from the VCU at the discretion of the study Principal Investigator. Prior to exiting the volunteers will be instructed to disinfect their rooms and personal effects with disinfectant wipes. They will then shower and change into clean clothes for departure. The clothes worn by the volunteer within the VCU are double bagged using an inner water soluble laundry bag and outer protective bag. Volunteers are instructed to wash all worn garments and are provided with disinfectant wipes and hand sanitizers to maintain infection control on departure. In accordance with the Clinical Protocol, volunteers are asked to refrain from interaction with vulnerable individuals for a fixed period post-discharge.

Floors in the VCU are steam-cleaned twice each day and following viral inoculation of volunteers. There will be at least a 24 hour period between discharge and admission of another volunteer cohort. At the end of a study the VCU is decontaminated using disinfectant (Dettol or 1% Virkon). Mops and non-disposable cloths are disinfected overnight in a mild hypochlorite (bleach) solution prior to use (2% or one part bleach to four parts warm water).

During an emergency reasonable effort is made to adhere to existing infection control measures. If external teams require access to the VCU, they will be fully briefed regarding risks and appropriate infection control measures prior to entering. Procedures are in place in the event of an emergency evacuation from the VCU during a quarantine study or in the event a volunteer requires hospitalisation. During an evacuation of the VCU, volunteers will be provided with PPE (face mask, gloves). Where possible, staff will be supplied with portable hand sanitisers for themselves and volunteers.

As a result of these precautions, release of the GMM once the volunteer has left the VCU is considered negligible. It should be acknowledged that any virus shed by an infected individual could be considered analogous to a clinical isolate and therefore pose no increased risk over those posed by seasonally circulating influenza strains.

All external transportation will be performed using approved couriers. The GMM virus will be shipped on dry ice and temperature monitored with chain of custody documentation. Large quantities of GMP virus will be shipped in accordance with Good Distribution Practice (GDP). The bulk of the GMP GMM challenge virus stocks will be retained at off-site facilities in accordance with the requirements for a ACDP category 2 pathogen and a GMM. Limited GMM stocks will be retained at hVIVO’s GMO licensed premises in secure, locked -80 oC freezers in areas with restricted access. All freezers are continually temperature monitored and linked to an alarm system. In the event of a temperature deviation that would affect the integrity of the GMM, the GMM will be transferred to another similarly secure and monitored freezer.
records will be held indicating the storage location of the GMM in accordance with hVIVO's documented procedures. These records are updated following removal of GMM stocks from the freezer enabling full traceability.

hVIVO staff are offered the annual influenza vaccination. Vaccination is not viewed as a primary control measure but as a supplementary precaution. hVIVO's infection control policy recommends staff to monitor their own health and when respiratory or influenza-like illness is recognised are advised not come into the workplace, thus minimising transmission of influenza by staff and reducing the possible risk of co-infection for those staff working with different influenza strains and possible reassortment. It is an employer’s responsibility to ensure that a worker’s health or immune status is sufficient for the activity in question. A system for the monitoring of health and immune status should therefore be implemented where the nature of the work demands it. Periodic monitoring of immune status may be required.

The GMM will be transmissible by an airborne route. The most likely routes for the release of the virus into the environment are via aerosol dissemination and contaminated waste. The survivability and stability of the GMM can be assumed to be comparable to wild-type. Influenza viruses are enveloped RNA viruses; the envelope (lipid membrane) is sensitive to desiccation, heat, UV exposure and detergents. The virus degrades quickly in the environment. The GMMs detailed here will not have been modified in any way to change this.

In common with some wild-type strains, the GMM may have the ability to infect humans and other animals (e.g. ferrets, pigs and birds). The infectivity and host-range of the GMM can be assumed to be the same as the parental wild-type. The effects of accidental exposure could result in influenza illness in humans and animals. The severity of illness with the GMM can be assumed to be the same as the parental wild-type strain. Some humans will have previously been infected with influenza and may have developed protecting antibodies. There is some evidence of cross-protection between influenza viruses of the same subtype. In general, immunity to a specific strain is thought to be life-long. In addition, some humans will have received annual vaccination against circulating influenza strains which may offer a level of protection against the GMM. Exchange of genetic material is possible between the GMM and wild-type strains in the environment. The exchange of genetic material is extremely unlikely as this would require co-infection of the GMM with another influenza virus in the same host. As detailed above, adequate control measures are in place for both the handling of the GMM in the laboratory and within the VCU, and appropriate management of study volunteers to mitigate the risk of GMM release.

Not applicable.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Request derogation for virus challenge in the VCU which do not operate under containment level 2.

Human challenge studies will be conducted in hVIVO's purpose built access controlled VCU. All staff will be appropriately attired in PPE. Infection control measures will be in place to prevent secondary transmission and transfer of virus to outside of the VCU. Surface decontamination is used to clean the VCU.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable, heat resistant plasticware and heat resistant glassware (borosilicate) will be used for the handling of GMMs in the laboratory.

A quaternary ammonium compound (Biocleanse, 5% v/v final concentration) will be used for GMM inactivation. This detergent is known to very rapidly destroy lipid encapsulated viruses such as influenza. Flasks/bottles/tubes containing GMM virus will be filled with the quaternary ammonium compound (5%(v/v) final concentration) within a BSC and sealed overnight (10 hours). All liquids, solid waste and other items that may have been contaminated with the GMM (e.g. pipette tips and gloves) will be discarded into the quaternary ammonium compound (5% (v/v) final concentration) within a BSC and soaked overnight (10 hours). After overnight inactivation, the liquid will be separated from the solid waste by decanting the liquid waste into glass Duran bottles. The solid waste, along with clinical waste and sharps (disinfected in alcohol within Cinbins) from the VCU will be placed in sharps bins and sealed. GMM waste will be stored separately from all other waste. All GMM associated waste and recyclable materials (e.g. glass bottles and waste pots) will be autoclaved for sterilisation. Post autoclaving (and confirmation of a successful autoclave run), liquid waste will be disposed via normal drainage and glassware cleaned. Solid waste will be transferred to clinical waste bags, labelled appropriately, and incinerated. All waste will be handled in accordance with hVIVO’s documented procedures.
Biocleanse (5% v/v), Dettol Antibacterial Surface Wipes, industrial methylated spirit (70% v/v) and/or Virkon will be used to disinfect medical equipment and surfaces. Gowns, scrubs and linen used within the VCU will be contained within soluble laundry bags and placed in Cinbins, sealed and transferred to external laundry facilities.

Has been reviewed and no Comments

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment Y
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**Name**

| Name                  | INGENZA LIMITED        |

**Campus Estate or Research Centre**

| Name 2 Department    |                       |

**Building**

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| Road Name | EASTER BUSH CAMPUS |

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**E-mail**

| HSE Division | SCOTLAND |

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee contains 3 members, consisting of Operations Director, Laboratory Manager and a Senior Scientist - Molecular Biology. A general meeting takes place once a year and covers the maintenance of previous assessments. Meetings are also convened when assessment of new projects are required.

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Tick if confidential  

Yes

Bacteriology  

Yes  

Parasitology  

Transgenic Birds  

Microbiology Research  

Yes
All waste will be disposed of through the Roslin Institute (BBSRC Institute) Waste Management Programme.

Solid waste will be placed in an appropriate bag provided by the waste management programme, and autoclaved before disposing of through the waste management biological waste system.

Liquid waste will be treated with approved biological waste disinfectant (e.g. Virkon) following manufacturers guidelines and again disposed of as outlined by the waste management programme.

For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste will be disposed of through the Roslin Institute (BBSRC Institute) Waste Management Programme.

Solid waste will be placed in an appropriate bag provided by the waste management programme, and autoclaved before disposing of through the waste management biological waste system.

Liquid waste will be treated with approved biological waste disinfectant (e.g. Virkon) following manufacturers guidelines and again disposed of as outlined by the waste management programme.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The safety committee, after a review of the risk assessment, recommends that all work will be class 1 and that the assessment describes all work using GMM's to be undertaken on the premises.
The purpose of the contained use application is to produce an intermediate compound in a chemical process, which will instead be produced in a biological system, where the finite nature of the chemical industry is removed. It is believed that the constructed strain will have no greater affect on humans and the environment than is seen already in the parental wild type strain.

The parental organism is a Burkholderia strain, and is available for purchase from DSMZ.

The vector system being used are pBBR1 derivative plasmids harbour a mobilization region required to mediate the transfer of the plasmids only when the IncP group transfer functions are supplied in trans from the vector pRK2013. This work was described previously by Antoine et al: Isolation and molecular characterization of a novel broad-host-range plasmid from Bordetella bronchiseptica with sequence similarities to plasmids from Gram-positive organisms Antoine et al. Mol. Microbiol 1992, 6, 1785-1799.

The target gene will be produced synthetically by a third party company.

The affects of the alterations to the parental strain are expected to result in the production of the compound of interest. The projected production levels are low with no more than 1 g/L of the compound being produced.

The engineering steps to be performed on the Burkholderia cepacia strain described above will result in diversion of carbon flux from a well characterized endogenous compound into the compound of interest which is not naturally produced by the strain. Previous deletion of the gene encoding the key enzyme involved in the synthesis of the endogenous compound did not result in any other phenotype changes than the alteration of the compound synthesis. The gene to be inserted in the deletion strain is present in a wide range of organisms from bacteria to single cell eukaryotic organisms, mammals and plants. The natural product of the reaction is a known intermediate in an ubiquitous synthesis pathway and therefore do not represent any hazards or potential risk for increased pathogenicity.
Previous work on the generation of a compound similar to the target compound using the gene of interest in yeast did not result in any detectable toxic effect for the cells and the environment.

Therefore we predict that Burkholderia cepacia pathogenicity will not likely be affected by either the deletion of the endogenous gene or the introduction of the heterologous target gene.

| Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants) |
| Not applicable |

| For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification) |
| Not applicable |

| Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate) |
| The main method of inactivation is through sterilisation using an autoclave where the water is heated under pressure to a temperature of 122 oC for 1 hour. This system has previously been validated for the Class I work that is currently performed in the laboratory. Upon receipt of a Class II licence tests will be performed to determine whether the current inactivation method completely kill the Class II organism that will be grown before any genetic modification takes place. Following validation of the inactivation process liquid waste will be disposed of into the local drainage system while a national waste disposal company will remove solid waste. The integrity of the microbiological safety cabinet, in which the organisms will be manipulated, will be maintained through thorough cleaning using a biocide spray, a further bactericidal, fungicidal, virucidal, mycobactericidal, sporocidal spray which also inactivates and breakdown DNA/RNA. Finally surfaces are wiped with 70 % IPA solution. |

| Is an emergency plan required according to regulation 20? |
| N |

| If yes, tick to confirm that it is attached to this form |
| N |

| Tick to confirm that you have attached a risk assessment to this form |
| Y |

| Tick if you are claiming exemption from disclosure for section of the risk assessment |
| Y |

Please enter comments on the GM safety committee on the risk assessment
The safety committee wanted a statement inserted into the risk assessment to confirm that Class II work will be performed with laboratory operators wearing Blue lab coats, while Class I work will continue to wear white lab coats.

The committee also requested that the risk assessment clarifies that Class II work is performed in a dedicated safety cabinet, incubator and fermentor separate from all Class I work.

All operators must risk assess the tasks that are required to be performed and should understand that immunocompromised personnel should refrain from performing work which involves Class II organisms. No laboratory work can be performed until the risk assessment has been reviewed and issued.

An additional point that was raised by the Biological Safety Committee, following their review of the risk assessment is that one way in which it can be attempted to show that mobilization has been reduced is to ensure that IncP stays in the donor host (a Class I organism) and is not transferred to Burkholderia cepacia as the presence of IncP would increase the ability to mobilise foreign DNA. To ensure that IncP has been removed from Burkholderia cepacia the committee has requested IncP is tested for removal either by a marker selection test or a PCR test. The absence of IncP should confirm that mobilization to other strains is reduced.

It was agreed by all members of the Biological Safety Committee that the work be assigned to Class II.

**Project Containment**

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#### Name

**PROMETIC BIOSCIENCES**

#### Name 2

#### Department

#### Campus Estate or Research Centre

**211 SCIENCE PARK**

#### Road Name

**MILTON ROAD**

#### Building

#### District

#### Town

**CAMBRIDGE**

#### County

**CAMBRIDGESHIRE**

#### Postcode

**CB4 0ZA**

#### Country

**ENGLAND**

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#### E-mail

#### HSE Division

**EAST AND SOUTH EAST**

#### Comments

#### Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The committee consists of 4 individuals who meet on a quarterly basis. The individuals in question hold the following positions. R & D Director, Chief Chemist and Health & Safety Officer, Project Manager Bioassay Development.

### Laboratory
- Level 1 (GMMs): Yes

### Animal Unit
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)

### Growth Room
- Non-microbial

### Glass House
- Other (please specify)
  - Tick if confidential

### Large Scale
- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

- Any samples containing the original cell culture material or its processed products should be disposed of into 1% Virkon. This includes unstopped assay plates, eg "BCA".
- Virkon-treated paper waste etc can be disposed of in the normal waste.
- Dry contaminated items should be autoclaved unlidded, and then disposed of in the yellow bin, from which an approved agent then takes them for incineration. Our autoclave process is not accredited for complete pathogen removal but decreases it substantially so that the waste may be stored before collection.
- Contaminated sharps, broken glass etc should go into the sharps bin.
- Polyacrylamide must not be autoclaved; this is hazardous as acrylamide monomer can be generated. Gels and disposable items contaminated with polyacrylamide which have been used for plasma fractions, whether or not treated with SDS, should be incinerated by an approved agent with an incinerator which works at a temperature suitable for the safe degradation of polycrylamide, eg that at Addenbrookes. Place in the bin provided, when full this is transferred to the yellow skip.
- ELISA plates which have been "stopped" using 2M Sulphuric acid may be considered non-infective. The wall contents are flushed down the sink with plenty of water and the rinsed plates disposed of in normal waste. NB these must not be autoclaved because acid may damage the autoclave.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

The committee has reviewed the proposed work and does not consider the elements of this activity to be of significant risk.
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Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The iQur GM Safety Committee comprises:

- The Head of Scientific Operations who is a member of the company Management
- The Health and Safety Coordinator (the company does not have a Biological Safety Officer)
- The Laboratory Manager, to represent the technical staff
- The Immunotherapeutics team leader from iQur (Southampton) (Chair)
- A Senior Research Scientist from 1Qur (Southampton) to represent the Immunotherapeutics team.
- A Research Scientist from 1Qur (Leeds) to represent the Vaccine Research and Development team.

The first meeting of the iQur GMSC was held on 19th December 2006 at which the chairperson was elected. It is anticipated that a meeting will be held annually to review existing risk assessments and containment measures. Additional meetings will be scheduled as and when risk assessments for new activities are submitted. Since the company is small, the GMSC will also take responsibility for ensuring that staff are appropriately trained and that full SOPs are prepared for activities involving GMMs.
| Level 1 (GMMs) | Yes |
| Level 2 (GMMs) | Yes  | Yes |
| Level 3 (GMMs) |  |
| Level 4 (GMMs) |  |
| Non-microbial |  |
| Other (please specify) |  |

| Bacteriology | Parasitology | Transgenic Birds | Microbiology Research |
| Virology | Yes | Transgenic Animals | Transgenic Fish | Gene Therapy |
| Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below) | Yes |

Other(s) | in vitro immunological assays of virus-specific immune function serological assays. |

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 969/07.1
The use of recombinant influenza virus to test if immunisation with virus-like particles carrying a haemagglutinin insert, induces specific and protective immunity.

Purposes of the contained use

The GMM in question is an existing recombinant influenza virus, which will be obtained through a Material Transfer Agreement with Reading University. It will be used to test if immunization with non-replicating virus-like particles (VLPs) carrying a protein domain from influenza haemoglobin (HA), induces specific immunity and protects against infection with the recombinant influenza virus.

Recombinant virus will be propagated by culture in Madin Darby canine kidney (MDCK) cells, the TCID50 will be determined and the virus will be concentrated and stored frozen. The virus will be used as follows:

• inactivated virus will be used in in vitro assays e.g. haemagglutination assays and to coat plates for the detection of HA-specific serum antibodies by ELISA.
• Viable virus will be used in in vitro micro-neutralisation assays.
• Viable virus will be used to determine if immunisation with HA-VLPs induces protective immunity against influenza infection.

Recipient or parental organism

The recombinant virus to be used comprises the 6 gene segments that encode for the internal proteins of influenza A/PR/8/34 recombined through reserve genetics with the haemagglutinin (HA) and neuraminidase (NA) genes from A/Panama/2007/99. For the purpose of the following discussion, A/PR/8/34 is regarded as the recipient microorganism and A/Panama/2007/99 as the donor of the insert.

Influenza A/PR/8/34 is a laboratory strain of influenza A, favoured for its high growth in embryonated chicken eggs and in mammalian cells. It is, however, completely attenuated for man and although antigenic, is not infectious. Because of these properties it is the backbone strain of choice for the manufacture of human influenza vaccines. A/PR/8/34 is, however, virulent in some animal models, including mice.

Host/vector system
The PR8 x Panama recombinant virus already exists. All steps requiring plasmids and vectors for the construction of this recombinant virus by reverse genetics have already been undertaken at the University of Reading.

**Origin & function**

The PR8 x Panama recombinant was produced at the University of Reading using reverse genetics. DNA corresponding to the haemagglutinin (HA) and neuraminidase (NA) genes of wild-type (wt) circulating influenza A virus H3N2 A/Panama/2007/99 was recovered by RT-PCR and cloned into appropriate mammalian transcription vectors. Plasmids containing these two genes were then pooled with six other plasmids that carried the remaining internal influenza virus genes (PA, PB1, PB2, NP, M and NS) from strain A/PR/8/34, which is attenuated for infectivity in humans. Each of these eight plasmids contained the relevant influenza gene in a negative sense between an RNA polymerase I promoter and RNA polymerase I terminator sequence, yielding eight vRNA-like negative sense RNA segments in the transfected cell. All eight RNA and pol I plasmids were co-transfected with an additional four expression plasmids, each containing one of the influenza genes PA, PB2 and NP from strain A/PR/8/34 in a positive sense under the control of RNA polymerase II promoters. These four genes encode the proteins that form the viral ribonucleoprotein complex of influenza viruses and are minimally sufficient to promote transcription, replication and packaging of the viral genome. Together, all 12 plasmids yielded infectious recombinant influenza virus that displays the HA and NA surface proteins of A/Panama/2007/99 and is attenuated for man by the A/PR/8/34 backbone. The virus produced by this reverse genetics approach is genetically similar to reassortant viruses produced in eggs during the manufacture of influenza vaccines.

Recombinant influenza PR8 x Panama virus infects and replicates in Madin Darby canine kidney (MDCK) cells and is shed into the culture supernatant. This will be the source of recombinant virus for the proposed experiments.

Recombinant influenza PR8 x Panama expresses Panama H2N2 at its surface. It thus mimics the cell binding and entry properties of native A/Panama/2007/99, without the associated virulence and risk to human health, making it more suitable for laboratory investigations.

For the purpose of the current study, the PR8 x Panama virus has been chosen because it expresses the H3 haemagglutinin subtype, as does the insert in the HA-VLPs that will be used for immunisations. Immunisation with the HA-VLPs is expected to induce an immune response directed against the H3 insert, which should afford protection against infection with an H3 influenza A virus. The PR8 x Panama recombinant has been chosen for this purpose because it offers a safer alternative for handling in the laboratory than wild-type A/Panama/2007/99.

**Evaluation of foreseeable effects**

Influenza reassortants, comprising the 6 genes encoding the internal influenza proteins from the PR8 strain with only the HA and NA derived from another influenza strain, have been used routinely for production of influenza vaccines for over 25 years. According to the WHO, there have been no reported infections among production workers exposed to an environment that harbours substantial aerosols of reassortant virus, arguing in favour of the very low risk of PR8 reassortant viruses to human health.

The recombinant PR8 x Panama virus to be used in this study was produced by reverse genetics rather than reassortment, but this is not expected to alter the fact that the resulting GM virus will be of low virulence to man. It has been chosen for these studies in preference to the wild-type A/Panama/2007/99 strain, because of its predicted attenuation for man compared to the wild-type. It is not considered to pose a significant risk to human health. At worst it might cause a mild respiratory infection with local symptoms such as nasal congestion.

The wild-type A/Panama/2007/99 strain is already circulating within the human population and there can be expected to be some level of immunity through:

- the presence of residual protective immunity to A/Panama/2207/99 as a result of prior infection
  A/Panama/2007/99 was prevalent between 1999 and 2003, or
- the presence of residual protective immunity to A/Panama/2007/99 as a result of prior vaccination.

Influenza viruses readily exchange genes by the process of reassortment. Therefore, there is a theoretical possibility that the recombinant PR8 x Panama virus could form secondary reassortments if replicating in the presence of a naturally occurring influenza virus. Depending on the nature of the reassortment this could theoretically give rise
To a more virulent virus. However, given the laboratory containment measures that will be taken and the low virulence of the PR8 x Panama in man, the risk of a secondary reassortant event occurring is minimal.

Effects on the Environment

For the in vitro laboratory work the containment measures that will be taken to safeguard human health should also serve to reduce any attendant environmental risk to negligible levels.

Despite being attenuated for man, influenza A/PR/8/34 is virulent in some animals, including mice. The recombinant PR8 x Panama virus is considered to be of similar virulence to the PR8 parent and thus poses a risk of adventitious infection of mice or possibly other small mammals in the environment. During the protection experiments the possibility of inadvertent transmission of the PR8 x Panama recombinant virus will be minimised by adherence to strict class 2 containment measures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Influenza A viruses are inactivated by heat (>71°C) and by chemical treatments including formalin, oxidising agents, dilute acids and ammonium ions. Virkon® is a virucidal disinfectant containing an inorganic peroxygen compound that oxidises proteins within the influenza virus, thus destroying its physical structure and activity. Virkon® retains its activity in the presence of organic matter and is recommended for the inactivation of micro-organisms in tissue culture supernatants.

Waste will be treated as follows:

Spent cell culture supernatants that have been in contact with the recombinant virus, will first be chemically inactivated by soaking for 15 minutes in a 1% solution of Virkon® (final concentration). Spent liquid will then be flushed down the drain with copious amounts of water.

Contaminated tissue culture plasticware will be soaked in a 1% solution of Virkon® for 15 minutes. It will be drained and thoroughly flushed with water terminal inactivation by autoclaving.

All waste generated during the protection experiments will be loaded into an autoclave integral to the class 2 suite. It will be inactivated by autoclaving at 121°C for 20 minutes. Biological waste will then be removed for terminal inactivation by incineration. The empty containers will be autoclaved a second time, ready for re-use.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
The risk assessment was reviewed by the iQur GMSC on 19/12/2006.

The committee discussed the designation of this contained use as a class 2 activity. Bearing in mind that both parental organisms are hazard group 2 pathogens and that the predicted activities of the recombinant virus will remain similar to those of the A/PR/8/34 parent, it was agreed that the work required containment level 2 was justified as a class 2 activity. Evidence was given where in similar situations, recombinant viruses which retain the same virulence as the parental strain, also retained the same GM classification.

The possibility for secondary reassortment events to take place was discussed. Given that the recombinant virus is expected to be of low virulence in man, and the containment measures that will be taken, the chances of a laboratory worker harbouring a wild-type influenza infection becoming dually infected with the recombinant virus were slim. Some members of the committee felt this risk was so small that special measures were unnecessary, others suggested that measures should be taken to prevent workers with influenza-like symptoms from handling the recombinant virus. The risk assessment has been modified to include this latter measure.

Other minor comments were made about sections of the risk assessment as follows. These have all been amended in the attached version (version 2).

- Factual errors in the overview, about how the recombinant virus was generated required correction
- Further clarification was required about how the virus would be inactivated for some in vitro assays
- Clarification was required about whether viable or inactivated virus would be used in the in vitro assays.

### Project Containment

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Name

LIPOXEN PLC

Name 2

Department

Campus Estate or Research Centre

LONDON BIOSCIENCE INNOVATION CENTRE

Road Name

2 ROYAL COLLEGE STREET

Building

District

Town

LONDON

County

GREATER LONDON

Postcode

NW1 0NH

Country

ENGLAND

Tel Number

020 7691 3583

Fax Number

020 7419 4653

E-mail

HSE Division

LONDON

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes](Y)

Give brief details of the genetic modification safety committee

- Principal Scientist, Biological Safety Officer
- Laboratory Manager, Health & Safety Officer, GM Committee Secretary
- Senior Scientist, GM Safety Officer, Chairperson of GM Committee

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<thead>
<tr>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

All solid waste to be autoclaved prior to incineration. Thermolog strip used in autoclave to validate each run, as well as digital print out of each run. Autoclave serviced annually.

Liquid waste to be inactivated by the use of Virkon to manufacturers standards (1% solution, see manufacturers killing data).

Decontamination of work benches with Virkon, tissue culture hoods with Proline Biocontrol (see manufacturer for killing data), followed by 70% IMS. All tissue culture waste to be autoclaved.

Any contaminated equipment or spills to be treated with either Proline (for metal equipment) or Virkon (particularly for spills) followed by 70% IMS. Contamination of murine and human cells to be kept at a minimum by the use of hoods, aseptic technique and spraying with IMS everything prior to the entry into hood.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All mammalian cell lines to be used to be Biosafety class 1 characterised authenticated continuous cell lines as determined by the ATCC.

Risk assessment for the work detailed approved by all concerned.
GM Centre Number: 971

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Name

BAKHU PHARMA LTD

Name 2

Department

Campus Estate or Research Centre

CROFT BUSINESS PARK

Road Name

UNIT 34 THURSBY ROAD

Building

District

BROMBOROUGH

Town

MERSEYSIDE

County

Postcode

CH62 3PW

Country

ENGLAND

Tel Number

0151 334 9044

Fax Number

0151 334 9045

E-mail

HSE Division

NORTH WEST

Comments

Phoenix Chemicals acquired by Bakhu Pharma Ltd in June 2011

Date at Which Additional Info Submitted

02/03/2022

Page 12259 of 15326
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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<th>Members:</th>
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<tbody>
<tr>
<td>Compliance Manager</td>
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<td>EHS Manager</td>
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<td>Technical Manager</td>
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Committee first met on 23 November.

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Other (please specify)  

Tick if confidential

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From information supplied by the supplier, it is expected that the GMM will be completely inactivated under the reaction conditions employed for its use. The reaction medium is both corrosive and highly toxic to the organism.

After reaction, the biomass will be filtered off, drummed up and sent for off-site disposal using a licensed waste contractor. The liquid filtrate will undergo further chemical processing including distillation at around 160°C.

The biomass will be tested using an approved and validated method by a contract microbiology lab to confirm that no living GMM remains after reaction.

Tick to confirm that you are attaching a summary of the risk assessment: Yes

Tick if you are claiming exemption from disclosure for sections of the risk assessment: 

Please enter comments of the GM safety committee on the risk assessment:

The risk assessment is comprehensive and covers all foreseeable situations whereby personnel or the environment may be exposed to the GMM. Appropriate decontamination procedures have been proposed for each situation.
GM Centre Number: 972

Data Premises Notified: 09/01/2007
Transferred from 1992 Regs?: N
Transitional Premises Class: N

Data Premises Closed: N
Transitional Premises Emergency Plan Required?: N
Non-GMMs: N
Withdrawn: N

Name: DIAMOND LIGHT SOURCE LTD

Name 2:

Department:

Campus Estate or Research Centre: HARWELL SCIENCE & INNOVATION CAMPUS

Road Name: DIAMOND HOUSE

District: CHILTON

Town: DIDCOT

County: OXFORDSHIRE

Postcode: OX11 0DE

Country: ENGLAND

Tel Number: 01235 778 000
Fax Number: 01235 778 499

E-mail:

HSE Division: EAST AND SOUTH EAST

Comments:

Date at Which Additional Info Submitted: 02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

See the attached terms of reference for details of the GM safety committee.

Committee is made up of:

- Senior Scientists
- DLS Biological Safety Officer
- DLS Laboratory Manager
- DLS employee representative
- Representatives of other scientists working with biological material
- An external consultant (Dr MRC Cambridge)

<table>
<thead>
<tr>
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<td>Level 4 (GMMs)</td>
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</table>
Live GM waste will be disposed of in a rigid plastic (meeting UN 3921 transport requirements) clinical waste bins (sometimes called cliniwaste bins).

Full bins will be collected by an Environment Agency approved clinical waste contractor.

The Clinical waste contractor has HSE approval to collect, transport, store and dispose (via incineration) of GM waste.

Controlled incineration at high temperature has been well validated to achieve a 100% kill rate of viable organisms (viable organisms not detected in plume or ash).

Validation is undertaken by the waste contractor and results will reported to Diamond as per the "duty of care".

See the attached Waste Disposal Policy.

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**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Comments from the Biological Safety Committee (14/12/2006)

Review of Risk Assessment

Brief discussion of TC work. Question regarding other types of CL1 work to be assessed; other CL1 work will be assessed as/when required. Emailed comments from external consultant relayed to committee. Committee agreed the project had correctly been assessed as Class 1.

Review of DLS Biological Safety Handbook

Transport of biological material, how are DLS employees protected when material sent by non DLS staff, in the process of completing DLS biological material transport policy - this will be communicated to individuals intending to send material to DLS.

Why waste covered in two separate documents; brief description of waste disposal in handbook to allow personnel to correctly follow procedures, in-depth description in supporting document detailing DLS policy toward biological waste.

Laboratory coat colour - suggested need to specify colour to distinguish different jobs (also allows separation of laundry) (bio-workers/chemists/workshop/cleaners etc.).

ACTION - to investigate lab coat colours and report back to committee. First aiders not currently trained to deal with injuries arising from work with biological material. CL 1 organisms will not cause harm, two new first aiders to be trained (both with a history of working in a standard biological laboratory). Own is validation of disinfectants to be done, and does assessment of incorrect use of disinfectant need to be carried out. For hazard group 1 organisms the manufacturers instructions sufficient, plus, a complete breach of containment for HG1 organisms would not present risk to humans/environment.

Committee agreed to handbook.

Comment (13/12/2006)

On the risk assessment, I have no qualms that things are under-assessed - my major feeling is that in several places the most remotely possible risks are being discussed, where I think that it would be fully justified to simplify things. As an example, it is normal to consider standard t.c. cell lines as HG 1, without needing any further justification - they are simply too disabled to present any hazard, so the discussion can move directly on to possible hazards from the vector (s) and inserts.

If the vectors are non-mobilisable and do not increase the pathogenicity of the host cells (in fact, pretty unlikely), then the only consideration becomes whether or not the transgene could increase the pathogenicity of the host or, if highly expressed, the coded protein could present any hazard. My feeling is that the vectors do not present any risk, nor do the transgenes increase the pathogenicity. The question of expressed proteins is more than adequately discussed - in typical practice if there is no mammalian promoter being used one can usually take it that there will be little expression and so negligible hazard.

I hope these comments are helpful.

Project Ref 972/19.1

Date Ackn'd 18/07/2019

CU2 Project Title Generation, usage and storage of material, including genetically modified material, from Hazard Group 2 agents

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted

Date Project Ceased 02/03/2022
Purposes of the contained use

To work with Hazard Group 2 (HG2) organisms which include those that may be genetically modified. The work would involve the generation, growth, imaging and storage of material. Expression of the material (i.e. protein) will be in either prokaryotic or eukaryotic organisms which will be no greater than HG2 and maybe GM material. The expressed material is prepared for usage, primarily imaging. Imaging will be completed at DLS or RCaH. Storage of the material will also take place in DLS and RCaH.

There are 2 routes for these samples, SS1 and SS2. The samples could be any of the following:
- Non-hazardous proteins genetically modified with a tag for structural and bio-chemical characterisation
- Non hazardous proteins originating in higher than Hazard Group 2 agents classification
- Prokaryotic material from no greater than HG2 classification
- Eukaryotic material from no greater than HG2 classification
- Virus no greater than HG2 classification

For SS1: Diamond Light Source (DLS) is a user facility where external people use the facilities at DLS. The Research Complex at Harwell (RCaH) is also a user facility used by external scientists. Samples which may include GM samples are generated and prepared before arrival to DLS or RCaH. This work will be assessed by their home institution. The home institution will be responsible for the transport to DLS and RCaH. Sample and experimental details must be submitted via the User Administration System (UAS) prior to bringing anything on site. Diamond Safety, Health and Environment (SHE) will assess the risk of the proposed work and can accept or deny the application to complete the work at DLS. The samples arriving at DLS or RCaH will be handled on DLS beamlines and/or RCaH facility. Only short term storage of up to a week will be permitted, followed by either inactivation of the sample or transport of the sample back to the home institution.

For SS2, samples will be generated, stored and transported on site between DLS and RCaH. Assessment will be completed at a local level for every sample. This assessment will detail the experiment to be completed, including details of hosts, vectors, inserts and mutations to be used. This completed assessment be submitted to the GMS management committee for review and approval/rejection of the proposed work.

Work will be rejected if:
- Any biological agent to be used is classified as HG3 or HG4
- Any biological agent to be used is a SAPO agent
- Any biological agent is listed on ATCSA Schedule 5

In the case of an experiment using multiple live bio-material, this should not have more than one biological agent from the same domain carrying a genetic modification (i.e. The experiment will be rejected where a GM virus is used in co-infection with a non-GM/GM virus but an experiment will be accepted using a GM virus infecting a GM.
mammalian cell line.
If the genetic modification confers potentially harmful biological activity (e.g. virulence factor, toxin, determinant of immune evasion)
If the genetic modification increases the pathogenicity and/or fitness of the sample.

Recipient or parental organism
Any prokaryotic or eukaryotic organism.

Host/vector system
Any prokaryotic or eukaryotic organism.
Vectors used will be commercially available non-mobilisable vectors

Origin & function
The genetic material will originate from either a prokaryotic or eukaryotic organism.

Health and Safety
Executive
The inserted genetic material will be expressed in either a prokaryotic and eukaryotic system and will not
• Confer potentially harmful biological activity (e.g. a virulence factor, a toxin, an allergen or determinant of immune evasion)
• Increase its pathogenicity and/or fitness.
• Alter the host range
• Alter the susceptibility to prophylaxis
• Confer potentially harmful activity to the environment
• Increase its survival fitness.

Any work to be completed must not increase the virulence, pathogenicity or robustness of the recipient host (classification up to HG2 prokaryotic or eukaryotic organism)
Manipulation using sharps will be avoided at all times, however the use of sharps is unavoidable in some cases (e.g. some imaging tools) These must be handled only by members of staff with experience in using these tools
Any manipulation of an organism that can cause harm by the airborne route must be able to be handled in a microbiological safety cabinet or the proposed work will be rejected

Evaluation of foreseeable effects
No hazards are specified due to no existing pathogenic traits. Any mutation must not confer any pathogenic traits or alter the host range. If any alteration of the host range is likely further assessment must be completed.

No hazards are envisaged as the inserted material must not code any hazardous products.
Additionally, to avoid transfer of genetic material, in the case of an experiment using multiple live bio-material, there will not be more than one biological agent from the same domain of life carrying a genetic modification.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
No use of larger GMOs will take place

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No derogation measures required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The work will produce both solid waste and liquid waste.

**Health and Safety**

**Executive**

Solid waste: Will be treated by disposal into autoclave bags or bins and autoclaved at 121°C for 15 mins prior to either off site incineration or disposal to landfill. In the case of lab coats, they will be treated by autoclaving at 135°C for 25 mins. Autoclaves are available for use at both DLS and RCaH. Autoclaves are serviced and validated annually.

Liquid waste: Will be treated in one of the following ways depending on the concentration and organism in use.

- Immersion in 70% ethanol for 5 mins
- Immersion in 1% virkon for 15 mins
- Immersion in Sodium hypochlorite for at least 30 mins. Concentration dependent on organism used and work being completed
- Immersion in Distel for 15 mins. Concentration dependent on organism used and work being completed.

Autoclaving at 121°C for 15 minutes for disposal

Surfaces and tools will be decontaminated with one of the following depending on the concentration and organism in use, along with the surface and tools to be treated:

- 70% ethanol
- 1% virkon
- Sodium hypochlorite. Concentration dependent on organism used and work being completed.
- Distel. Concentration dependent on organism used and work being completed.

The use of antibiotics that are used as a treatment for that infection must be avoided for work involving that organism.

**Is an emergency plan required according to regulation 20?**  
[ ] Yes  [X] No

Tick to confirm that it is attached to this form

[X] Yes

Tick to confirm that you have attached a risk assessment to this form

[X] Yes

Tick if you are claiming exemption from disclosure for section of the risk assessment

[ ] Yes

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tr>
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<td>L3 L4 L2</td>
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**Name**

CANCER RESEARCH UK, CAMBRIDGE INSTITUTE

**Name 2**

**Department**

SCHOOL OF CLINICAL MEDICINE UNIVERSITY OF CAMBRIDGE

**Campus Estate or Research Centre**

LI KA SHING CENTRE

**Road Name**

ROBINSON WAY

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 0RE

**Country**

ENGLAND

**Tel Number**

020 7242 0200

**Fax Number**

020 7269 3647

**E-mail**

**HSE Division**

LONDON

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Significant Change
973/09.1a
Date of Additional Information (significant change only)
17/06/2014

Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Chairperson - Senior Scientist
Scientific Advisors
Biological Safety Officer
Occupational Health Physician
Members of: Management Team, Scientific Technical Staff, Staff Representative.
The committee will meet as required but at least annually to review current projects.

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<tr>
<td>Level 2 (GMMs)</td>
<td>Yes</td>
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Yes

All waste will be deactivated in a Virkon trap (1%) and disposed of after 24 hours as per manufacturers instructions. All GMM solid waste will be incinerated by licenced contractor. All waste from retrovirus work will be handled seperately from other tissue culture waste. Accidental spillages of liquid waste will be cleaned up with 2% Virkon solution. We do not expect the scale of low viral work to exceed more than 200ml for amphoteric viruses and 1 litre for ecotropic viruses.

For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste will be deivated in a Virkon trap (1%) and disposed of after 24 hours as per manufacturers instructions. All GMM solid waste will be incinerated by licenced contractor. All waste from retrovirus work will be handled seperately from other tissue culture waste. Accidental spillages of liquid waste will be cleaned up with 2% Virkon solution. We do not expect the scale of low viral work to exceed more than 200ml for amphoteric viruses and 1 litre for ecotropic viruses.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

It is the view of the CRI committee that, all GMM's handled in these facilities are regarded as relatively safe. There is a very low risk to humans as the host vector systems have been specifically designed to be of a non-transmmisable form and are replication incompetent. These organisms are mostly unstable and cannot survive outside of the environment in which they grown.
The GM work undertaken in these facilities is regarded as safe and without threat to the users or the environment.

Project Ref 2/01.10

Date Ackn'd 02/02/2007

CU2 Project Title MODULATION OF RAS FUNCTION IN HUMAN EPIDERMAL KERATINOCYTES

Class 2

CultureVol

Class

CultureVolume

Class3-4
Date Project Ceased

Withdrawn          Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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<th>Project notified under transitional arrangements</th>
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Withdrawn  

Tick if notifying a connected programme of work

Historical Significant Changes

TRANSFERRED FROM GM CENTRE 2 (2/2/07)

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 2/01.12

Date Ackn'd 02/02/2007

Date Project Ceased

CU2 Project Title INTEGRIN SIGNALLING IN MOUSE KERATINOCYTES

Class 2

Culture Vol Class 2

Consent Granted Not Applicable

Non-GMM

Historical Significant Changes TRANSFERRED FROM GM CENTRE 2 (2/2/07)

Project notified under transitional arrangements Y

Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
**Project Containment**

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<td>L3 L4 L2 L3</td>
<td>L3 L4 L2 L3</td>
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<tr>
<td><strong>Animal Units</strong></td>
<td><strong>Large Scale Activities</strong></td>
<td><strong>Human Clinical Applications</strong></td>
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**Project Ref 2/01.6**

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**Withdrawn**  N

**Historical Significant Changes**  
TRANSFERRED FROM GM CENTRE 2 (2/2/07)
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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02/03/2022
**Project Ref** 2/01.7

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**Historical Significant Changes**

TRANSFERRED FROM GM CENTRE 2 (2/2/07)

**Project Additional Information**

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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Project Ref  2/01.8

Date Ackn’d  02/02/2007  CU2 Project Title  MANIPULATION OF EPIDERMAL STEM CELL FATE BY VERTEBRATE  Class  Class 2  CultureVolClass2  CultureVolumeClass3-4
### Project Additional Information

- **Purposes of the contained use**
- **Recipient or parental organism**
- **Host/vector system**
- **Origin & function**
- **Evaluation of foreseeable effects**

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Please enter comments on the GM safety committee on the risk assessment

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**Project Ref 2/01.9**

**Date Ackn’d**
02/02/2007

**Date Project Ceased**

**CU2 Project Title**
EXPRESSION OF SIGNALLING MOLECULES IN HUMAN AND MOUSE KERATINOCYTES

**Class**
Class 2

**CultureVolClass2**

**CultureVolumeClass3-4**

**Non-GMM**
Not Applicable

**Consent Granted**

**Tick if notifying a connected programme of work**
N

**Historical Significant Changes**
TRANSFERRED FROM GM CENTRE 2 (2/2/07)

02/03/2022
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**
Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

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Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 20/04.3

Date Ackn’d 06/06/2007
CU2 Project Title Using Somatic Knockouts for novel compound screening and target/pathway
Class 2
Culture Volume Class 2 Culture Volume Class 3-4

Class 2 < 1 Litre

02/03/2022
Page 12287 of 15326
Date Project Ceased: validation

Non-GMM

Consent Granted: Not Applicable

Project notified under transitional arrangements: N

Withdrawn: N

Tick if notifying a connected programme of work: N


Historical Date of Additional Info: 

Significant Change ID: 

Date of Significant Change: 

## Project Additional Information

### Purposes of the contained use

To use somatic cell line knockout technology to generate assay reagents for combinatorial libraries of potential therapeutic compounds and for pathway analysis of potential new therapeutic targets.

This project requires gene disruption through homologous recombination in human epithelial cells in vitro; rAAV (adeno-associated virus) vectors permit such knockouts in human cells.

Inserts will encode LoxP sites, human genomic targets and positive selectable marker genes (e.g., neomycin or hygromycin). Target cells are human epithelial cell lines, including HCT-116, DLD1, A549, CAL51 and MT3 cell lines. These cells will be infected with rAAV (e.g., AAV-293).

Target genes are those of the pathways regulating DNA repair, apoptosis, histone acetylation, including BRCA2, HDCA1, SIRT1 and ADA3.

### Recipient or parental organism

Tissue culture cells of human origin.

### Host/vector system

Laboratory strains of E. coli K12 derivatives;

Tissue culture cells of human origin.

Human t.c. lines both for recombinant virus production (e.g., AAV-293 cells derived from HEK 293 cells will be used as producer cells) and for subsequent infection to produce recombinant t.c. cell lines.

pUC-based plasmid vectors; Moloney Leukaemia virus based vectors.

AAV Helper-Free system (Stratagene) (pCMV-MCS for initial cloning, pAAV-LacZ to produce the ssDNA for encapsidation, pAAV-RC and pHelper to provide the adenoviral proteins required for encapsidation); the resulting recombinant adeno-associated viral vectors will be used for transduction of human cells.

### Origin & function

Target genes are those of the pathways regulating DNA repair, apoptosis, histone acetylation, including BRCA2, HDCA1, SIRT1 and ADA3.

### Evaluation of foreseeable effects

The planned knockout of the target genes in human t.c. cell lines is not foreseen as increasing their pathogenicity from its negligible level for the cell lines.
The one significant hazard arises from the rAAV produced from the packaging cells. This is capable of infecting human cells and contains inserts designed to knockout target genes in the pathways regulating DNA repair, apoptosis, histone acetylation, including BRCA2, HDCA1, SIRT1 and ADA3. Their effect is therefore considered as potentially oncogenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The planned knockout of the target genes in human t.c. cell lines is not foreseen as increasing their pathogenicity from its negligible level for the cell lines.

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For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The planned knockout of the target genes in human t.c. cell lines is not foreseen as increasing their pathogenicity from its negligible level for the cell lines.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The work involves the cloning of inserts to knockout various genes, their incorporation into recombinant adeno-associated virus (rAAV) and the subsequent infection of human epithelial cell lines, in order to establish stable cell lines with the appropriate genes knocked out. The target genes are those in the pathways regulating DNA repair, apoptosis and histone acetylation, including BRCA2, HDCA1, SITR1 and ADA3. The host cells are all highly disabled and the modifications will not increase their pathogenicity. With the exception of the rAAV, the vectors are non-mobilisable and present no risk. The exception is the AAV, which can infect human cells.

Knocking out the target genes is potentially oncogenic and, while this is highly unlikely to increase the pathogenicity of the intended host cells, it does present a risk to workers who might become infected with the rAAV.

For this reason the packaging and infection steps are considered as Class 2, while the preliminary cloning steps and subsequent propagation of any modified cells are Class 1.

(Note from BSO: I have taken advice from HSE and the Inspector, to whom I was referred, agreed with this assessment.)

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**Animal Units**

**Large Scale Activities**

**Human Clinical Applications**

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**Project Ref** 678/03.2

**Date Ackn'd**

23/05/2007

**CU2 Project Title**

ADENOVIRAL EXPRESSION OF NOVEL TIMP MUTANTS AND CHIMERAS IN MAMMALIAN CELLS

**Class**

Class 2

**CultureVol**

1-50 Litres

**Non-GMM Consent Granted**

Not Applicable

**Project notified under transitional arrangements**

N

**Withdrawn**

N

**Tick if notifying a connected programme of work**

N

**Historical Significant Changes**

TRANSFERRED FROM GM 678 - 23/5/07.

**Recipient or parental organism**

**Characterisation of novel Tissue inhibitor of metalloproteinase (TIMP) 1, 2, 3 and 4 mutants and chimeras and their comparison with wild-type TIMPs in a variety of mammalian cell models to assess their affects on cellular processes dependent on metalloproteinase activity.**

**Host/vector system**

Plasmid DNA will be produced in E. coli K12 strains which are class 1 pathogens and are assessed to be containment level II microorganisms.
TIMP-Ad5 adenovirus are to be made by Flp dependent recombination by co-transfection of plasmid vectors pDC515 or pDC516 with pBHGfrt(triangle)E1E3, 3flp in HEK293T cells. Vectors pDC515/6 are non mobilisable and contain the TIMP gene and CMV promotor. pBHGfrt(triangle)E1E3,3flp encodes the Ad5 genome and can only produce adenovirus in mammalian cells which express adenoviral proteins E1 and E3, such as HEK293T after transfection. Further information can be obtained from www.Microbix.com who are the manufacturers of the vector system. TIMP-Ad5 will be used to infect a variety of mammalian cell lines and primary cells. These are not expected to contain the adenoviral genes E1 and E3 and should not produce viral particles after infection.

**Origin & function**

TIMPs 1, 2, 3 and 4 are from human cDNA and are inhibitors of metalloproteinases. Chimeras are made from the cDNAs encoding parts of human Furin, murine TACE and GFP, or the polypeptides KDEL, [Leu]17, V5 epitope and [His]6. These are to target TIMPs to particular cellular organelles (Fur, TACE, KDEL, (Leu)17, or to aid detection (GFP, V5, [His]6).

Plasmid vectors pDC515/6 and pBHGfrt(triangle)E1E3,3flp are derived from vector pUC18 and recombinant adenovirus Ad5.

**Evaluation of foreseeable effects**

Replication deficient Ad5 are unlikely to cause disease for purpose of group II classification. However the nature of the inserted gene should be considered in the context of the recombinant virus. Work with adenovirus carries risk of allergy after repeated exposure. This effect is idiosyncratic and may manifest as conjunctivitis and rhino-tracheo-bronchitis. Ad5-CMV based virus are not known to cause these symptoms. However although the virions lack E3 protein and are compromised in their ability to establish and maintain an infection in human cells, should infection occur, the effect of elevated TIMP expression is difficult to predict due to the varied functions of these proteases they inhibit. However, decreases in ECM turnover through MMP inhibition, or inhibition of shedding of growth factors, receptors and cytokines, on balance, is likely to decrease cell survival. All TIMPs have been shown to decrease tumour survival in murine models when expressed by adenoviral infection (variety of transformed cells including epithelial derived cells). The Ad5 adenovirus is non-oncogenic and TIMPs, GFP, ADAM17 and Furin are not recognised as oncogenes. The oncogenic potential of short peptide sequences consisting of the V5 epitope, KDEL and the 17 amino acid Leucine rich peptide is not known.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated for 24hrs with 2% Virkon or Presept tablets before disposal into the laboratory drainage system. Alternatively disinfected waste will be gelled with Vernagel in sealed containers and removed for incineration. Solid waste such as plastics will be double bagged before transfer to a waste autoclave within the building, and then disposed of by incineration on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The project has been reviewed by the CIMR Biological and Genetic Modification Safety Committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.

### Project Containment

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### Project Ref 678/04.1

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<td>CO-CULTURE OF CONDITIONALLY IMMORTALISED HUMAN MAMMARY FIBROBLASTS AND ENDOTHELIAL CELLS WITH HUMAN CANCER CELL LINES</td>
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Project notified under transitional arrangements

### Purposes of the contained use

The use of co-culture of tumour epithelial cells with tumour stroma (fibroblasts and endothelial cells) to mimic aspects of tumour cell-cell interactions in vitro.

### Recipient or parental organism

Human Mammary Fibroblasts (HMF clones A to D) and Human Mammary Microvascular Endothelial Cells (HMME clones 2 and 7) were obtained from Ludwig Institute for...
Cancer Research, London. The characteristics of the cells are described in the publication by O'Hare et al, PNAS, 2001, 98 p646-51. In brief, primary cells derived from breast tissue were immortalised by separate transduction with helper-free amphotropic murine-lukaemia-virus (MLV) encoding the catalytic subunit of human telomerase (hTERT) and a heat labile mutant of the simian virus 40 large tumour antigen (U19tsA58). The resulting cells are immortal, yet retain most genetic and phenotypic characteristics of the primary parental cells. The cells only retain immortality when cultured at the permissive temperature of 34 degrees C, and senesce after 15 passages at 37 degrees C. These cells have the hTERT and U19tsA58 genes stably integrated into the host cell genome by MLV retroviral transduction. They have been tested negative for the presence of LMV and are unlikely to produce LMV containing hTERT and U19tsA58 in the future. However as hTERT and U19tsA58 genes are potentially oncogenic, it is advised that the cells are cultured under containment level II conditions and the cell lines are classified as Class II organisms.

Host/vector system

Cells are derived from primary fibroblasts and microvascular endothelial cells obtained from breast tissue explants.

The vector system used originally used to transform these cells is described in the publication by O'Hare et al, PNAS, 2001, 98p646-51. In brief the vectors used were helper-free amphotropic murine-lukaemia-retrovirus (MLV) containing resistance genes for antibiotics hygromycin and G418 to aid selection of clones which had integrated MLV stably into their genome.

NB. Only the cells derived from this vector system are to be used by the applicants. NOT the vector system itself.

Origin & function

The human telomerase catalytic domain (hTERT) is derived by removal of the regulatory domains of human telomerase. Human telomerase maintains telomere length which is a means of regulating cell life-span. HTERT prevents telomere shortening and can lead to immortalisation of cells which express it.

The simian virus 40 large tumour antigen is a known oncogene and induces DNA synthesis by by-passing key cell cycle check points through interacting with regulatory proteins (c-myc, Ras). It also blocks p53 dependent apotosis U19tsA58 is a temperature sensitive mutant of SV40LTA which is inactive at 37 degrees C yet is active at the permissive temperature of 34 degrees C.

Expression of these constructs individually does not always lead to cell immortalisation, although expression of both will usually lead to complete transformation and immortalisation.

Evaluation of foreseeable effects

Due to the nature of the hTERT and U19tsA58 genes stably inserted into the genome by MLV transduction there exists a very small but finite risk of a recombination event which could lead to production of amphotropic replication competent MLV containing either the hTERT or U19tsA58 genes should the cells come into contact with MLV or a derivative thereof. To further reduce the risk of recombination, the following will be adhered to.

1. Culture in Containment Category II facility following CIMR Containment II Code of Practice.
2. The cells are not to be cultured in the presence of murine cells.
3. All human cell lines which will be co-cultured with the immortalised fibroblasts or endothelial cells will be tested for the presence of MLV by PCR.
4. The fibroblast and endothelial cells will be tested monthly for the presence of MLV by PCR.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Liquid waste will be treated for 24 hrs with 2% Virkon or Presept tablets before disposal into the laboratory drainage system. Alternatively disinfected waste will be gelled with Vernagel in sealed containers and removed for incineration. Solid waste such as plastics will be double bagged before transfer to a waste autoclave within the building, and then disposed of by incineration on site.

The project has been reviewed by the CIMR Biological and Genetic Modification Safety Committee. We are satisfied that the risks have been properly assessed and that the work will be carried out under the appropriate conditions and controls.

Please enter comments on the GM safety committee on the risk assessment

The project has been reviewed by the CIMR Biological and Genetic Modification Safety Committee. We are satisfied that the risks have been properly assessed and that the work will be carried out under the appropriate conditions and controls.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4 L2</td>
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<tr>
<td>Human Clinical Applications</td>
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Project Ref 678/05.2

Date Ackn'd 23/05/2007

CU2 Project Title Adenoviral over-expression and siRNA knock-down of metalloproteinases of the MMP and ADAM families of proteinases

Class 2 1-50 Litres

Consent Granted Not Applicable

Historical Significant Changes TRANSFERRED FROM GM 678. (23/5/07)
Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

**Purposes of the contained use**

We will produce viral particles using the FLP recombinase kit from Microbix. The gene of interest is cloned into pDC515 or pDC516, which contains a CMV promotor or pDC511 and 512 containing the SV40 promotor, allowing expression to be checked under Containment Level 1 conditions prior to generation of virus. Virus is made by co-transfection of the pDC vector with pBHGfrDE1E3, 3FLP in NautCell 293T cells, where FLP recombination occurs producing viral particles. This will be performed under Containment Level 2 conditions in compliance with the institute code of practice.

**Recipient or parental organism**

E.coli: All strains are K12 derived thus disabled and non-colonising. Vectors are mobilisation defective. Mammalian cells: pDC515/6. Vector is non-mobilisable.

Adenovirus: Microbix adenovirus and all commercially prepared adenoviral genomes are based on Ad5 which is replication deficient but can enter a variety of human cells.

**Host/vector system**

E. coli, K12 pBr322 based
Mammalian Cell pBR322 based
Mammalian cell Ad5 based virion post infection (E1 deficient).
NautCell293T Ad5 based virion.

**Origin & function**

Human origin
SiRNA is synthetically derived- used to inhibit expression
cDNAs - used for over expression

**Evaluation of foreseeable effects**

Replication deficient Ad5 are unlikely to cause disease for purpose of group II classification. However the nature of the inserted gene should be considered in the context of the recombinant virus. Work with adenovirus carries risk of allergy after repeated exposure. However although the virions lack E1/E3 proteins and are compromised in their ability to establish and maintain and infection inhuman cells. Over expression of MMPs through inadvertent infection by RAd5 virus expressing the enzymes via a CMV or SV40 promotor may result in increases in ECM degradation and hence increases in cellular proliferation and migration. High levels of over expression of most MMPs in tumour cell lines in mouse models of subcutaneous xenotransplantation results in increased tumour growth. It is possible therefore that increased rates of cell growth may occur on adenoviral expression. Although increased MMP expression is increased on neoplastic transformation in most tumour types,increased MMP expression itself is not thought to cause cellular transformation at the genetic level, hence MMPs are not considered oncogenes. Effects of ADAM knock down are even harder to predict in vivo through inadvertent infection of sirRNA expressing adenovirus. With the exception of ADAM10 (embryonic lethal due to lack of Notch/Delta processing) or TACE (EGF like knockout phenotype due to poor shedding of EGF ligands) all other mouse knockouts made so far as ADAMs are viable and have no severe phenotypic differences from their wild type litter mates which have been defined as yet.
The virus is physically relatively stable, so need good practice to ensure it isn't spilled and left to present a hazard at some future point; the host range of Ad5 is naturally limited to man and therefore the virus will not infect animals or plants nor, due to its disablement, spread to the wider human community. Bacteria and mammalian cells are unable to survive outside of laboratory culture conditions.

See attached protocol for further details.

In summary, there is little evidence that either knock down of MPs or their over expression would have long term effects, should any pathology result from inadvertent infection by adenovirus. Adherence to the code of practice for containment 2 facilities should be adequate for preventing inadvertent infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be treated with either 2% virkon overnight in labelled containers. Liquid post disinfection will be gelled with Vernagel and placed in sealed containers and taken to room 1.23 for incineration. Solid waste will be placed in double autoclave bags, secured with autoclave tape labelled with room no and user name, followed by autoclaving and incineration. Liquid spills will be treated with 2% Virkon (non metallic surfaces) or 10% Trigene (metallic surfaces), absorbed with paper towels which will then be autoclaved. Surfaces will be routinely cleaned with 10% Trigene.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

This project has been reviewed by the Institutes Biological and Genetic Safety Committee. This is satisfied that the risks have been properly addressed and the work will be carried out under the appropriate controls and conditions.

Project Containment

<table>
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<tr>
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<td>L2 Yes</td>
<td>L3 L4 L2 L3 L4</td>
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</table>

Animal Units | Large Scale Activities | Human Clinical Applications
### Project Additional Information

**Purposes of the contained use**

To study the activities and mechanisms of suppressor gene function by recombinant means in human and mouse cell lines.

**Recipient or parental organism**

Established human (IMR90, W138 & BJ), and 293T human embryonic kidney cells transformed with E1a and carrying a temperature sensitive T antigen, also mouse embryonic fibroblasts cell lines.

**Host/vector system**

Retroviral vectors based on Moloney Murine Leukaemia Virus, ecotropic (for mouse cells) or amphotropic (for human cells) packaging system. Mobilisation-defective Lentiviral vectors will be used for non-dividing cell lines.

See attached project proposal.

**Origin & function**

cDNAs and shRNAs, tumour suppressors (p53, Rb, p16) and over expression of oncogenes (HMGA, ras, mdm2, etc) involved in tumourigenesis.

See attached project proposal.

**Evaluation of foreseeable effects**

None of the cell lines used are capable of colonising a healthy individual and we do not expect any of our proposed manipulations to change this. The amphotropic
retrovirus and lentivirus would be able to infect human but could not replicate unless the improbable event occurs that it recombines with endogenous retroviruses. Even then it is unlikely the expression of a single oncogene or suppression of a tumour suppressor causes tumours, as multiple genetic alterations (between 4 and 6) are required for inducing tumours and safeguard mechanisms exist impeding the accumulation of these alterations in the organisms (i.e. single events like ras or myc expression instead of unrestrained proliferation induce growth arrest or apoptosis).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| All work, where directed by the Biological Safety Committee, will be carried out in the higher containment level 2 facility. Other work will be conducted with good laboratory practice and in accordance with the biosafety local rules. All virus production will be undertaken in the CL2 facility. Liquid waste will be rendered harmless by treatment with Virkon (1%) over 16 hours and in accordance with recommended manufacturers instruction. Spills will be treated with an excess of 2% Virkon or other approved validated disinfecting agent. All solid waste loads from CL2 will be autoclaved at 121C for the requisite time, validated and bagged for disposal by incineration through an approved, validated, licensed contractor (White Rose). Solid waste arising from Class 1 work will be bagged and collected in containers for incineration by the above named contractor. Staff undertaking collection of waste will be trained to deal with the clean-up of accidental spills. Autoclaves are subject to regular routine maintenance, insurance inspection and annual validation. |

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project was submitted for comment was considered at the meeting of the Biological Committee on Friday 15th December 2007. The committee agreed that the project assessment was correct and parts of this proposal would need to be carried out at Containment Level 2. All virus production work ecotropic or amphotropic would be done in the CL2 facilities. Staff engaged in work at CL2 would need to be made familiar with the requirements and local rules and sign acknowledgement that training had been provided, evidence of past experience would also be required.

Should treated cells need to be removed from the CL2 laboratory, evidence would be needed to demonstrate absence of virus in the cells and supernatant.

The committee agreed that this proposal should be submitted under the requirements of the Genetically Modified (Contained Use) Regulations 2000 activity notification.

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**Project Ref** 973/09.1

**Date Ackn'd** 21/07/2009

**CU2 Project Title** Genetic modifications of human and mouse cells using four different types of viral vector

**Class** Class 2

**CultureVol** 1-50 Litres

**Consent Granted** Non-GMM Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID** 973/09.1a

**Date of Significant Change** 17/06/2014

**Project Additional Information**

**Purposes of the contained use**

Translational research on cancer pathology and treatment

**Recipient or parental organism**

Human or mouse cell lines, including both primary and established cultures

**Host/vector system**

- 'Third generation' lentiviral vector systems, and also second generation lentiviral vector systems only if derived from non-human viruses. *Second generation* retroviral (other than lentiviral) vector systems. Adenoviral vector systems. Herpes simplex 1 'amplicon' vector systems.

02/03/2022
A wide variety of inserts will be used, according to the different areas of research across the institute, many of them essentially harmless, but will sometimes include inserts that are inherently oncogenic, that activate oncogenes, that repress tumour suppressor genes, that promote the expression of growth factors, or are otherwise potentially harmful.

**Evaluation of foreseeable effects**

All vectors are well characterised, with a history of safe use, and are inherently safe. The lentiviral vectors are self-inactivating, replication incompetent, with the genes for assembly of live vector viral particles on three different plasmids, with viral accessory genes deleted, and with expression of vector contingent on upstream elements and trans complementation. However, the lentiviral vectors may also carry enhancements to host range & stability and/or to vector titre & transgene expression. The retroviral vectors are self-inactivating, replication incompetent, with the genes for assembly of live vector viral particles on two different plasmids, with packaging signals and 5'LTR typically deleted, and with expression of vector contingent on trans complementation. However, the retroviral vectors may also carry enhancements to host range & stability and/or to vector titre & transgene expression. The adenoviral vectors non-replicative and non-mobilisable with the E1 gene cassette deleted, in most cases the E3 cassette deleted, and in many cases the E2 cassette deleted, and the insertion site is at the site of disablement (E1). The HSV-1 amplicons are generated using a bacterial artificial chromosome but with the packaging signal, origin of replication and the ICP27 gene (essential for replication) all deleted and with 'stuffer' sequence inserted to render the HSV sequence too large to be packaged into viral particles, and with the missing elements supplied from separate BAC's/plasmids, and then the final amplicon containing largely only insertion sequence with little or no room for viral sequence and thus non-infectious. The risk to human health will be very low in most cases, but in some cases, with potentially harmful inserts, and/or enhanced vectors, and/or human primary material, the risk to human health may become significant. The risk to the environment is very low in all cases because of the highly attenuated and disabled nature of all the vectors and recipient cell lines used.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste is inactivated with the peroxygen disinfectant DuPont RelyOn Virkon at a freshly prepared overall concentration of 1% for at least 20 minutes prior to disposal to drain. Based on the manufacturer's efficacy data, this will inactivate all cell lines and viral vectors used with effectively 100% kill. Solid waste is autoclaved prior to removal from site by a suitably approved carrier to a suitably approved disposal site for final incineration. The autoclave is regularly serviced and tested. This will inactivate all cell lines and viral vectors used with 100% kill.

Any spills are treated with Virkon as above. All work is carried out in a class II microbiological safety cabinet conforming to BS EN 12469:2000, and regularly serviced and validated.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N
Four risk assessments are attached, one for each vector type. All were approved by the GM safety committee as GM Class 2 and/or 'COSHH' Hazard Group 2 (although certain aspects may be at GM Class 1 and/or 'COSHH' Hazard Group 1 as indicated in the assessments. These risk assessments represent generic templates, and the committee require that a detailed specific risk assessment be submitted to them for approval for every new/separate project. These specific assessments may be based on these generic assessments, but must clearly show that the proposed work (ie, the specific cell-lines, vectors, inserts and procedures to be used) fall within these generic risk assessments and give a level of risk consistent with the generic assessments, or else a further notification under regulation 10 will be made.

## Project Containment

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<table>
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<tr>
<th>Animal Units</th>
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### Project Ref 973/17.1

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<th>Date Project Ceased</th>
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<td>05/04/2017</td>
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</table>

### Project Additional Information

**Purposes of the contained use**

Mice will be infected with influenza Strain A virus isolates including some previously GM virus (altered to express peptide epitope marker) and their ability to raise effective
Immune responses will be monitored. The model uses well characterised and commonly used laboratory mouse-adapted strains of Influenza A virus (H1N1 isolates A/WS/N/33 (WSN) and A/PR/8/34 (PR8) and H3N2 isolate A/HK/X31) to study immune response to virus infection and rechallenge.

Recipient or parental organism

Parental organisms: Influenza virus strain A/WSN/33 (WSN;H1N1) and A/PR8/34 (PR8;H1N1).

Host/vector system

Orthomyxoviridae, Influenza virus type A, Subtype H1 N1, strains A/WSN/33 (WSN;H1N1) and A/PR8/34 (PR8;H1N1) containing the OVA 257-264 peptide was generated by reverse genetics.


The host organism will comprise of either:
(a) characterized mammalian cell lines (such as canine cell line, MDCK) maintained in tissue culture for viral plaque assays.
(b) C57BL/6 and transgenic mice for virus immunity studies.

Origin & function

Ovalbumin sequence 257-264 (SIINFEKL) as a peptide epitope marker. To allow virus-specific immune cell responses to be monitored using MHC-tetramer technology and transgenic T cell receptor (TCR) expressing mice (such as OT1 mice).

Evaluation of foreseeable effects

We classify the work with these H1 N1 influenza virus strains as GM Containment Level 2 for the following reasons:
Strain A/WSN/33 is classified as BSL-2 by the ATCC
Strain A/PR8/34 is classified as BSL-2 by the ATCC

The OVA 257-264 is a MHC I Kb restricted epitope and is presented on the surface by antigen presenting cells to activate a CD8+ T lymphocyte response specific for the OVA peptide (Walker et al., J. Immunol. 1997, 159:2563-2566; Topham et al., J. Immunol. 2001, 167:6983-6990). The insertion of OVA is not predicted to increase the virulence or pathogenicity of the virus.

The viral influenza model involves the exposure of mice to live virus to give a subclinical infection. The parental influenza virus strains WSN and PR8 are well characterised and have a low or no pathogenicity in human. They are highly passaged isolates used routinely in laboratories are considered non pathogenic. The virus required direct intranasal administration of viral particles to infect cells of the respiratory tract. There is no evidence of transmission between mice or from mouse to human (Lowen, A.C., Mubareka, S., Tumpey T.M., Garcia-Sastre, A. & Palese P. (2006). The guinea pig as a transmission model for human influenza virus. Proc Natl Acad Sci USA 103, 9988-9992). For mouse infections all work will be carried out in a containment 2 area within the biological services unit. Mice will be handled within MSC II cabinets and housed in individually ventilated (HEPA filtered) sealed cages. As such, the risk of spread to the wild murine population is very unlikely.

The viral particles cannot survive outside of the mouse, and are rapidly inactivated by dehydration or other environmental insult, and so scope for spread via the environment is severely limited. The virus is fragile and is quickly inactivated by detergents, lipid solvents, phenolic disinfectants or chlorinating agents. The virus is to be stored in sealed labelled vials within an -80:°C freezer, as such the environmental risk is correspondingly extremely low. No production of viral stocks will be undertaken, existing GM virus will be used..

The Advisory committee on dangerous pathogens document - ‘Advice on Experimental working with Influenza
Viruses of Pandemic Potential" states that, 'Viruses with pandemic potential should be handled at higher containment (at CL3 or above as determined by risk assessment). These include subtypes H2, H1 and H3 if, in the case of the latter two, the viruses being handled are sufficiently different from currently circulating human viruses to warrant an assumption of little or no cross-reactive immunity in the human population.' The viruses to be used in this case are predicted to be antigenically similar to components of the currently available seasonal human influenza vaccine and this indicates containment level 2 will be adequate.

The GM insert is known to be harmless.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated for 24 hrs with 2% Virkon or Presept tablets before disposal into the laboratory drainage system. Alternatively disinfected waste will be gelled with Vernagel in sealed containers and removed for incineration.

Solid waste such as plastics or cage waste will be double bagged before transfer to a waste autoclave within the building, and then disposed of by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Risk Assessment was approved by the GM safety committee on 2nd February 2017 as GM Class 2 and/or 'COSHH' Hazard Group 2.

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| Comments |

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

We have 5 members in our newly established committee with between 12 and 30 years practical experience in the following disciplines; Microbiology, molecular biology, production of recombinant proteins, cell culture and diagnostics development. 3 members have PhD's, 1 a MSc and another BSc Hons. The group aims to meet 2-3 times a year or as required to look at risk assessments.

One of the members has several years training and experience as a safety advisor whilst working in a class 2 facility at Unilever Research, and is responsible for organising meetings.

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<thead>
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Laboratory waste is placed into autoclave bags which sit in autoclavable boxes. At the end of the day a lid is placed on the box and it is transported to the autoclave. The lid is removed and autoclaved along with the box containing the waste, an automatic discard cycle is used. Each run is monitored by thermocouples that are placed in the waste load and chamber to record temperatures reached. This is recorded and registered. The bags and boxes also carry autoclave tape that is thermosensitive.

Any spill that occur in the lab during work are treated with 70% ethanol and cleaned up with absorbent paper. This waste is also autoclaved. The degree of kill has been determined by testing the viability of microorganisms in heat. Class 1 heat sensitive organism show a 1 log kill per second at 70°C.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM safety committee agree that the activities under assessment fall into the Class 1 category.
### GM Centre Number: 975

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**Name**

UK RESEARCH & INNOVATION

**Name 2**

**Department**

**Campus Estate or Research Centre**

ISIS

**Building**

RUTHERFORD APPLETON LABORATORY

**District**

CHILTON

**Town**

DIDCOT

**County**

OXFORDSHIRE

**Postcode**

OX11 0QX

**Country**

ENGLAND

**Tel Number**

01235 44 5610

**Fax Number**

01235 44 5808

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Please see the attached terms of reference for the ISIS Biological Safety Committee.

The committee is currently made up of;

- Senior Scientist, Biomolecular Sciences
- ISIS Safety Officer
- ISIS Biological Safety Officer
- ISIS Laboratory Manager
- Representatives of other scientists working with biological material (2 persons)
- Representatives of senior management (Head ISIS Diffraction and Muons division, Head ISIS Operations division)
- An expert external consultant
- ISIS Safety Officer
- ISIS Biological Safety Officer
- ISIS Laboratory Manager
- Representatives of other scientists working with biological material (2 persons)
- Representatives of senior management (Head ISIS Diffraction and Muons division, Head ISIS Operations division)
- An expert external consultant

### Laboratory

- Level 1 (GMMs)
  - Yes

### Animal Unit

- Level 2 (GMMs)

### Growth Room

- Level 3 (GMMs)

### Glass House

### Large Scale
Level 4 (GMMs)
Non-microbial

Other (please specify)

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Tick if confidential

For activities involving GMMs, describe the waste management measures which will apply to the activity

Please see attached waste disposal policy.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Comment 30/11/2006
On the specific documents you sent, I am happy with both risk assessments. One minor point on both is that I am a "former member of ACGM", not being on its successor SACGM.

Review by the BSC 3/12/2006
The risk assessments were discussed and it was suggested that a list of workers should be included in the risk assessment. However, the procedure for access to the Bio Lab is addressed in the Biological Safety Handbook. Access will only be given to trained and competent persons.

There was a discussion about record keeping for expressed proteins which will be appropriately catalogued.

Asked that in section 3 1 litre was changed to 2 litres.

Stated that the risk assessment will be available in the Bio Lab.

Concern was expressed that the risk assessments did not clearly state that the work was class 1 and that a qualifying table should be included. Explained that this format is only used for hazard group 1 organisms but that he would add a table into the risk assessment. Action.

There was a discussion about generic risk assessments for the Bio Lab.
GM Centre Number: 976

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Name

CELLAURA TECHNOLOGIES LTD

Name 2

Department

Campus Estate or Research Centre

BIOCITY

Road Name

PENNYFOOT STREET

Town

NOTTINGHAM

County

NOTTINGHAMSHIRE

Postcode

NG1 1GF

Country

ENGLAND

Tel Number

0115 912 4415

Fax Number

0115 912 4289

E-mail

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>NG1 1GF</td>
<td>ENGLAND</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The genetic modification safety committee consists of five members who meet annually and on a Ad Hoc basis if a new project assessment is required. The members include two company directors, the Operations Manager, the research pharmacologist who will be conducting the work on site and an external biological safety advisor from the University of Nottingham. Meetings will involve a review of all risk assessments for our operating procedures and a discussion of any further safety aspects.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs) | | Yes | | |
Level 2 (GMMs) | Yes | | | |
Level 3 (GMMs) | | | | |
Level 4 (GMMs) | | | | |
Non-microbial | | | | |
Other (please specify) | Tick if confidential | |
Bacteriology | Parasitology | Transgenic Birds | Microbiology Research | Yes |
All work will be conducted using the appropriate safety equipment and the aseptic technique to prevent contamination. All bacterial plates (used and unused) will be rinsed in 70% IMS before temporary storage in a sealed metal container. All waste will then be autoclaved on a discard cycle to achieve complete disinfection prior to transport for incineration in sealed biohazard bags by a licensed contractor. There is an autoclave present in the same building, which can be used for this purpose. This incinerator is validated for this purpose on an annual basis.

Any liquid bacterial cultures or tissue culture medium will be treated overnight with 2% Trigene to kill off any living micro-organisms (as per manufacturers instructions and data sheet), after which they will be discarded down the sink. Tissue culture flasks and plates where cells may be living will be treated with 2% Trigene for 24 hours to kill any remaining culture after which they will be discarded into sealed biohazard bags and sent for incineration. All gloves will be discarded into the incineration waste bags. All pipette tips, which may have come into contact with GMO's, will be treated with 2% Trigene and then discarded into a sealed sharps bin, which will be incinerated when full.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Two risk assessments (CA-07-1 and CA-07-2) were considered by the genetic modification safety committee at its meeting on the 22nd February 2007 (copies attached). These were both approved as Class 1 activities.

Immediately prior to the meeting the Chairman and external Biological Safety advisor from the University of Nottingham, undertook an inspection of the laboratories and confirmed that the physical condition, safety procedures and administration of the laboratories complied with level 2 containment.
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**Name**

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

**Name 2**

**Department**

Campus Estate or Research Centre

THE LABORATORY

Road Name

CITADEL HILL

**Building**

**District**

**Town**

PLYMOUTH

**County**

DEVON

**Postcode**

PL1 2PB

**Country**

ENGLAND

**Tel Number**

01752 633 207

**Fax Number**

01752 633 102

**E-mail**

HSE Division

WALES AND SOUTH WEST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

A joint GM Safety committee is held between the Marine Biological Association and Plymouth Marine Laboratory. Both sites have their own Biological Safety Officer and GMSO who are on the committee, a union representative, postdoctoral researcher and a student representative. There is also an external advisor from the University of Plymouth. All members of the committee are required to look at the risk assessments of new and reviewed projects. The committee will meet 4 times a year and will feedback to the Joint Health and Safety Management Committee.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste is bagged within the lab using suitable autoclave bags, these are transported in an aluminium box to the autoclave (this is situated along the corridor and down one floor in the lift) this is done using a trolley. The waste is autoclaved at 121°C for 40 minutes. The autoclave is validated on a monthly basis using bioindicators within the load, which are incubated for 72 hrs to make sure they have been killed. This is carried out in house. Annually the autoclave is pressure tested by an engineer as part of planned maintenance, as part of this a 12 point test will be carried out on a test load.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

"With the addition of the method of deactivation of any waste as well as making clear the assessment of risk to the environment, as recommended during the HSE visit, the committee are happy with the risk assessment for the project and agree that it is a class one activity.

The risk assessment should be reviewed in a year, or sooner if there are any significant changes"
### Emergency Plan Required?

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### Comments

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The NovaBiotics Genetic Modification Safety Committee consists of the Health & Safety Officer, Deputy Health & Safety Officer, Fire Officer, Biological Safety Officer, First Aid Representative, Laboratory Staff Representative and Chief Scientific/Chief Executive Officer. At present, NovaBiotics Ltd Genetic Modification Safety Committee meets on an ad hoc basis, but once GM work begins it is anticipated that the committee will meet monthly, at least initially, with meeting frequency requirements under constant review.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale |
------------|-------------|-------------|-------------|-------------|
Level 1 (GMMs) | Yes       |            |             |             |
Level 2 (GMMs) |           |            |             |             |
Level 3 (GMMs) |           |            |             |             |
Level 4 (GMMs) |           |            |             |             |
Non-microbial |            |             |             |             |
Other (please specify) | Tick if confidential |  |
Bacteriology | Yes       | Parasitology | Transgenic Birds | Microbiology Research |
Virology | Transgenic Animals | Transgenic Fish | Gene Therapy | Yes |
For activities involving GMMs, describe the waste management measures which will apply to the activity

All GM waste will be inactivated by autoclaving. GM activity in Novabiotics' Molecular Biology laboratory will be limited to routine cloning and expression work using attenuated Escherichia coli strains. All work will be carried out in small volumes (<50ml) using disposable plastic-ware at all times. All GM-contaminated plastic-ware will be inactivated by autoclaving, and none will be re-used following autoclaving. No unautoclaved GM waste of any description shall be kept in the Molecular Biology Laboratory for more than 48 hours. All GM waste must be autoclaved prior to disposal. Any waste in the GM Biohazard waste bins on Friday afternoon must be autoclaved rather than being left over the weekend, where practicable. If this is not possible, for example due to breakdown or malfunction of the autoclave, then the bags should be clearly labelled, sealed with autoclave tape and left in water-tight container in the Molecular Biology Laboratory to wait later treatment. An agreement is in place with Sterile Technologies Ltd to dispose of all BioHazard waste (including GM waste) on an ad hoc basis in the event of a breakdown of the Varioklav 135S Autoclave.

All GM waste must be autoclaved in the Varioklav 135S Autoclave in the "Wet" Laboratory. The correct cycle, "destruction EV" applies a vacuum to the load before heating it to 134°C for 15 min. This is cycle P2 on the front panel of the autoclave. A separate Standard Operating Procedure is available for operation of this autoclave, as is a risk assessment for the use of autoclaves in general.

Waste bags (marked "Biohazard") will, when full, be placed inside larger clear plastic autoclaveable bags and these bags will be sealed with autoclave tape, leaving a small opening to permit the ingress of steam. The top of the bag will be folded over and taped down with a small piece of tape for transport to the autoclave. Bags will be placed in securely lidded plastic boxes and taken to the "Wet" Laboratory for autoclaving. Once the bag is placed in the autoclave, this small piece of tape will be removed and operators should ensure that the unsealed hole left in the top of the bags is left open to allow air to be evacuated and for the ingress of steam during the autoclave cycle. After autoclaving, the waste bag and contents will be placed inside the orange clinical waste bag provided and loaded into the waste storage bin prior to collection and disposal by Sterile Technologies Ltd.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The members of the Genetic Modification Safety Committee agreed unanimously that the Risk Assessment was entirely adequate for the purposes for which it was designed, and will be implemented.
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**Name**

ERBA MANNHEIM MOLECULAR LTD

**Campus Estate or Research Centre**

4 BARTHOLEMEW'S WALK

**Road Name**

4 BARTHOLEMEW'S WALK

**Town**

ELY

**County**

CAMBRIDGESHIRE

**Postcode**

CB7 4EA

**Country**

ENGLAND

**Tel Number**

01353 667 880

**Fax Number**

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Advisor: Cambridge University. The committee will meet quarterly to review all GM activity or when a new GM activity is planned. Full details on request.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|-------------|-------------|-------------|-------------|-------------|-------------|-------------|

**Level 1 (GMMs)**

Yes

**Level 2 (GMMs)**

**Level 3 (GMMs)**

**Level 4 (GMMs)**

**Non-microbial**

**Other (please specify)**

Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research
---|--------------|------------------|---------------------|---------------------|-------------|-------------|-------------|

Yes |           |                  | Yes

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Class 1 GMM waste is disposed of by autoclaving. Autoclave cycles are validated to ensure waste reaches the required criteria. The criteria to pass a cycle are that the contents of the waste must undergo a sterilisation cycle greater than or equivalent to sterilisation at 121°C for at least 15 minutes.

Autoclaved waste is then disposed of via the clinical waste route, using an authorised contractor to dispose of it by incineration.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

The risk assessment has been studied by the GM committee who agree that the GM work to be carried out by Lumora is designated as class 1 GM activity.
<table>
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<th>GM Centre Number: 980</th>
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Date at Which Additional Info Submitted: 02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Y]

Give brief details of the genetic modification safety committee

Composition: GM Biological Safety Officer (1), Management representative (1), Staff/Technical representative (1). Operating procedures: The Committee will meet as frequently as required (at least once per quarter). Full agendas and full minutes of meetings will be available from the GM Biological Safety Officer.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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Non-microbial

Other (please specify)

Tick if confidential

Bacteriology Yes

Parasitology

Transgenic Birds

Microbiology Research Yes

Virology

Transgenic Animals

Transgenic Fish

Gene Therapy
Although not required for class 1 activities, two autoclaves are available in the laboratory where the work will be carried out for inactivation of waste material as well as a class 11 safety cabinet.

All waste material containing viable GMMs, including spent culture fluid and other media, will be inactivated by heat inactivation (autoclave) or chemical means (where appropriate).

Contaminated waste and contaminated items will be autoclaved using a validated cycle, e.g. at 121 degrees C for 20 minutes before disposal and may include a holding time.

The autoclaves used for waste disposal will be validated regularly for performance.

Contaminated laboratory glassware and other materials awaiting disinfection will be stored in a safe manner and pipettes will be totally immersed in disinfectant, where necessary.

Spillage is treated by chemical methods such as spraying the contaminated area with 70% ethanol and Virkon if required.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

This will be a continuation of class 1 activities previously approved by Manchester Interdisciplinary Biocentre. No additional risks to human health or the environment are indicated. The laboratory where the work is proposed to be carried out, has both autoclaves and a class 11 safety cabinet available in the same room.
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**Name**

SYNAIRGEN RESEARCH LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

MAILPOINT 810, LEVEL F

**Road Name**

TREMONA ROAD

**Building**

SOUTH BLOCK, SOUTHAMPTON GENERAL HOSPITAL

**District**

**Town**

SOUTHAMPTON

**County**

HAMPSHIRE

**Postcode**

SO16 6YD

**Country**

ENGLAND

**Tel Number**

023 8051 2800

**Fax Number**

023 8051 2800

**E-mail**

**HSE Division**

WALES AND SOUTH WEST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
**Premises Addresses**

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<td>TREMONA ROAD</td>
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<td>SO16 6YD</td>
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</table>

**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Members of the committee are: Founding member; Head of Bioscience Discovery; Head of Bioscience Development; Discovery Team Leaders; Development Team leader.

The committee has quarterly meetings to review GM and Health and Safety related matters or earlier intervals as required.

The GMO work currently under consideration has been reviewed by members of the committee and their comments incorporated into safety considerations + protocols.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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<td>Tick if confidential</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

The GM safety committee were satisfied that the correct protocols were in place for handling class 2 GMO. The disposal of contaminated material was discussed and it was found to be acceptable to use the disinfection methods described as approved by the manufacturer for retrovirus culture. Additional comments relating to the format and content of the risk assessment have been integrated into the supporting document enclosed.

Project Ref 981/07.1

Date Ackn’d 23/05/2007

CU2 Project Title Conditional Immortalisation of Primary Human Bronchial Epithelial Cells using Retroviral delivery of / and integration of Thermolabile SV40 Tantigen, or C-MYC.

Class 2

Culture Volume Class 2 < 1 Litre

Consent Granted Not Applicable

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info
### Project Additional Information

#### Purposes of the contained use

Generation of conditionally immortalised cell lines derived from Bronchoscopic brushings taken from well characterised individuals for research purposes.

#### Recipient or parental organism

Retrovirus - MoMLV based retrovirus vector (pZIP-Neo or NIT-Tag) (pBABE or pLNCX-2). Recipient cells are human primary bronchial epithelial cells and carry a risk of T.B. infection, the risk is considered low in choice of donors.

#### Host/vector system

**Packaging cell lines**

1) BING/CAK 8 A 293T derived (human kidney epithelial origin) cell line expressing amphotropic envelope protein.
2) TEFLY A Human sarcoma derived cell line expressing amphotropic envelope protein, gag and pot genes.

**Origin & function**

The pZIP-Neo vector and TEFLY A cell line originated from the Ludwig Research Institute and will be supplied by Lonza Nottingham Ltd.

The NIT-TAG vector originated from research at the University of Michigan and Laboratory of Genetics, Salk Institute, La Jolla, California and will be supplied by the latter.

The BING/CAK 8 cell line will be purchased from ATCC Cell biology cell cultures collection (Teddington, TW11 0LY, UK).

The intended function is to generate retroviral packaging, delivery and integration of thermolabile SV40 T antigen, into primary human bronchial epithelial cells. The generation of stable conditionally immortalised cell lines from well characterised donors.

To the same purpose C-MYC will be introduced in the commercially available vector pLNCX-2 (Clontech) (a format licenced from Reneuron Ltd).

#### Evaluation of foreseeable effects

The GMO produced will be amphotropic retrovirus capable of delivering and integration of heat sensitive T antigen. The GMO is replication deficient.

Infected cells will be resistant to the amphotropic neomycin and have extended proliferative potential at permissive temperatures below 32C.

Accidental exposure to retrovirus carries a potential risk of infection. Inoculation with significant viral titre may cause infection with tumorigenic potential. The risk is deemed low as proviral integration would be required a site deleterious and oncogenic to the cell or there would need to be conversion of the T antigen to wild type (now thermolabile) and co-expression of oncogenic RAS (Thomas M 2002 Neoplasia 4: 493-500). All work will be carried out in class 2 microbiological safety hoods and designated incubators and equipment, routinely disinfected will be used to minimise exposure risk.

Transformed cells will not be derived from the operator or other laboratory personnel. If inoculation due to sharp injury occurred it would be likely to invoke a strong allogenic immune response resulting in rejection of injected transformed cells.

The retrovirus is replication deficient and susceptible to degradation on dehydration. Host cells are unable to survive outside cell culture conditions these characteristics mean accidental release into the environment carries negligible risk of hazard, particularly as likely exposure would be from small quantities.

The GMO produced will be amphotropic retrovirus capable of delivering the oncogene C-MYC. The retrovirus is replication deficient itself.

The activity of CMYC is regulated by cellular localisation and the fusion gene introduced will produce a chimer of C-MYC and a modified estradiel receptor. The chimeric protein must dimerise to enable nuclear translocation and subsequent C-MYC activity. Dimerisation is controlled by ligand binding where the ligand is 4-Hydroxy Tamoxifen (4-OHT).

Accidental exposure to the retrovirus carries a potential risk of infection, however the tumorigenic risk is negligible without the coincidental use of 4-OHT.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

---

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection of biohazardous tissue culture waste in liquid form or contaminated plastics will be performed by treatment with chemical disinfectant Virkon. Virkon is DEFRA approved disinfectant active against a broad range of viruses including retrovirus.

UV irradiation and disinfection of class 2 microbiological safety cabinets where biohazardous cultures are opened and where aerosols can potentially form will be routine procedure.

In addition to disinfection, solid waste will be double bagged and sealed autoclaved and incinerated - by clinical waste contractors appropriately trained.

It is noted that retroviruses are susceptible to dehydration, transformed cells only survive in controlled tissue culture conditions, the inserted gene product is thermolabile.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Details of the proposed GM project, Risk assessment and code of practice for handling retrovirus and related class 2 material was circulated to members of the GM safety committee for consideration on 23/4/07. The comments of the committee have been incorporated into the supporting documents to this application regarding risk assessment and code of practice for handling GMO.

The committee reviewed the disposal of contaminated waste and were satisfied with the manufacturers stipendation that Virkon was suitable for disinfection of retrovirus contaminated material. Additional steps ensuring containment during disinfection, use of UV and autoclaving to sterilise materials and work space were approved.

Project Containment

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02/03/2022
GM Centre Number: 982

Data Premises Notified: 07/06/2007

Transferred from 1992 Regs?: N

Transitional Premises Class: N

Data Premises Closed: N

Transitional Premises Emergency Plan Required?: N

Non-GMMs: N

Withdrawn: N

Name

PUBLIC HEALTH ENGLAND

Name 2

Department

Campus Estate or Research Centre

Building

Road Name: 2 NEWARK STREET

District

Town: LONDON

County: GREATER LONDON

Postcode: E1 2AT

Country: ENGLAND

Tel Number: 020 7377 5895

Fax Number: 020 7539 3459

E-mail

HSE Division: LONDON

Comments

Date at Which Additional Info Submitted: 02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The HPA is located at the Institute of Cell and Molecular Sciences (ICMS), Barts and the London School of Medicine, Queen Mary College. A GMO safety committee has been constituted within the ICMS and this proposal has been reviewed by it. We now have created a joint ICMS-HPA GMO committee with representatives of the HPA MRU to provide a single site-wide oversight. The GMO committee has a chairman (a senior virologist at this time) and a secretary (a bacterial molecular biologist) plus bacteriologists and virologists with scientific, medical training and direct experience of the handling and containment of pathogenic microorganisms at cat. 1, 2 and 3 including GMOs.

Level 1 (GMMs)

- Yes

Level 2 (GMMs)

- Yes

Level 3 (GMMs)

- Yes

Level 4 (GMMs)

- Yes

Non-microbial

- Yes

Other (please specify)

Tick if confidential

Bacteriology

- Yes

Parasitology

Transgenic Birds

Microbiology Research

Yes
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For activities involving GMMs, describe the waste management measures which will apply to the activity

The HPA MRU conducts all its activities at class 2 or class 3 (ie. activities appropriate to class 1 would be conducted in class 2 laboratories)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Not applicable.
## GM Centre Number: 983

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### Name

**OXOID (ELY) LTD**

**Department**

### Campus Estate or Research Centre

**Road Name**

ANGEL DROME

**District**

ELY

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB7 4ET

**Country**

ENGLAND

**Tel Number** 01353 646 200

**Fax Number** 01353 646 246

**E-mail**

**HSE Division** EAST AND SOUTH EAST

### Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Y

Give brief details of the genetic modification safety committee

- R & D Manager
- Regulatory Compliance Manager
- H&S advisor

The GM safety committee operates as a sub committee of the Oxoid (Ely) Ltd Health & Safety Committee. The committee meets monthly to discuss all H&S related matters, and the GM committee meets as required to review new procedures or specific GM related issues.

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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  

Tick if confidential [ ]

- Bacteriology: Yes
- Parasitology
- Transgenic Birds
- Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

All disposable waste, disposable labware, gloves and materials used for treatment of spillages are treated by autoclaving on a validated sterilisation cycle of 121°C for a minimum of 15 minutes. The degree of kill is expected to be 100%. Standard autoclave indicators are used to verify that the cycle has completed successfully.

Prior to autoclaving all re-usable laboratory glassware is also treated with commercially available sodium dichlorosocyanurate based liquid disinfectant.

Surfaces are routinely disinfected with commercially available propan-1-ol based surface disinfectant. Laboratory environmental monitoring is carried out monthly, surfaces are sampled and any contaminants isolated. Environmental monitoring records indicate routine sanitation procedures are sufficient to eliminate any bacterial contaminants that might occur.

Following autoclaving all materials are removed from the premises by a licensed contractor for disposal via incineration.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The proposed procedures are considered to be low-risk with regard to the health and safety of personnel involved in the work, those working within the same laboratory space, and to the environment. There is no known pathogenicity attached to the organism to be used in the GMM work, and the gene fragments to be inserted will not produce functional proteins, therefore the modified organism is considered to pose a very low risk.
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Name

**NEUTEC PHARMA LTD**

Name 2

Department

Campus Estate or Research Centre

**MANCHESTER SCIENCE PARK**

Road Name

**LLOYD STREET NORTH**

Building

**WILLIAMS HOUSE**

District

Town

**MANCHESTER**

County

**GREATER MANCHESTER**

Postcode

**M15 6SE**

Country

**ENGLAND**

Tel Number

**07967 778 717**

Fax Number

**0161 276 8826**

E-mail

HSE Division

**NORTH WEST**

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Biohazzards & Genetic Modification Committee, NeuTec Pharma Ltd., Williams House

- Committee Members: Chair & Biological Safety Officer, External/Independent Expert, Safety Officer, Laboratory Manager, Facilities Designer, Senior Microbiologist, Committee Secretary & Preclinical Expert, Medical Adviser, Occupational Health Representative, Building Facilities Representative.
  - The committee performs review/approves GM and Biological hazards risk assessments, promotes best practice, staff consultation.
  - The Chair & Biological Safety Officer has over 10 years experience chairing health & safety and GM committee meetings.
  - Company staff are consulted and provide input (by and including the various experts).
  - Minutes of meetings are freely circulated within the company through the H&S portal (server based).
  - Meetings as needed (minimum bi-annually)

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02/03/2022
Two GMOs will be used at the NeuTec facility, both class I: Escherichia coli K-12 and Saccharomyces cerevisiae.

Waste Management: All waste material containing viable GMMs, including contaminated disposable plastics, spent culture fluid and other media, are inactivated by chemical treatment (Trigene Advance Laboratory 2.5% final concentration, exposure overnight) prior to incineration. Chemically inactivated waste is stored in leak-proof, sealed containers prior to collection by Astec Waste Services. Contaminated laboratory glassware and pipettes are totally immersed in Trigene Advance Laboratory (2.5% final concentration) and incubated overnight. Decontaminated glassware is washed in water and reused. Our waste disposal policy document detailing processing of different types of waste is attached. Attachment 1.

Validation of Inactivation: This has been performed by the manufacturer of Trigene Advance Laboratory, and certificates of testing and microbial inactivation for different microorganisms are available. Attachment 2.

Degree of Kill: No Test certificate is available for Saccahromyces. However data for Candida albicans (a similar non-spore forming yeast) is available. Here Trigene (1% final) added to a fungal suspension containing albumin (3 mg/mL) and erythrocytes 3 mL/L) resulted in a >106 reduction in viable yeast cells after 15 min at 20 deg C. The test method used was BS EN 13624 “Candida Quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants used in medical areas”.

For E.coli, a >106 reduction in viable E.coli cells was observed under similar conditions. Test method used was EN1276. “Bactericidal activity of Trigene Advance using phase 2 step 1 suspension test”

Monitoring Measures: No monitoring is planned. However, by September 2007 an autoclave will be available in our facility and contaminated waste will be subject to sterilization using standard autoclave conditions. This autoclave will be subject to “under load” measurements of temperature and pressure, autoclave tape indicators and in some instances spore strip tests.
The assessment was considered by our GM safety committee on the 7th June 2007. There were no specific comments from our GM safety committee. There were minor changes in the wording of the assessment.
GM Centre Number: 985

Data Premises Notified (Originally) 05/07/2007

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed Transitional Premises

Emergency Plan Required? N Non-GMMs N Withdrawn N

Name

CLATTERBRIDGE CENTRE FOR ONCOLOGY NHS FOUNDATION TRUST

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

CLATTERBRIDGE ROAD

District

BEBINGTON

Town

WIRRAL

County

CHESHIRE

Postcode

CH63 4JY

Country

ENGLAND

Tel Number 0151 334 1155

Fax Number 0151 482 7673

E-mail

HSE Division NORTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

- 6 monthly meetings are held.
- Representation of Medical Oncology, Nursing, Occupational Health, Risk Management, Infection Control, University of Liverpool BSO, Clinical Trials, Health & Safety.
- Terms of reference and code of conduct with organisational reporting mechanism to trust board.

#### Laboratory

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial
Other (please specify) | Level 1 clinical trial on day therapy unit at Clatterbridge Centre for Oncology | Tick if confidential |  

| Bacteriology | Parasitology | Transgenic | Microbiology |  
| Virology | Transgenic | Transgenic | Gene Therapy | Yes |  
| Mycology | Transgenic | Transgenic | Other (please specify below) |  

Other(s)  

For activities involving GMMs, describe the waste management measures which will apply to the activity  

Clinical waste will be treated as "GMO waste" according to the Trust Code of Practice.  

Waste will be double bagged, sealed with a red identification tag and transported to an incineration site approved for the disposal of GM waste.  

All waste will be labelled as GM waste and disposed of via contractor who is registered for handling disposal of GM waste.  

Tick to confirm that you are attaching a summary of the risk assessment | Y |  

Tick if you are claiming exemption from disclosure for sections of the risk assessment |  

Please enter comments of the GM safety committee on the risk assessment  

The risk is considered low with standard good microbiological practice and within existing standard operating procedures.  

Administration of the agent will take place in a defined room and named healthcare staff wearing apron, gloves, mask and goggles. Existing standard operating procedures within Code of Practice is appropriate to cover pharmacy, laboratory and waste disposal.  

Project Ref | 985/18.1 |  

| Date Ackn'd | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4 |  
| 04/10/2018 | Gene therapy product RP1 (Common name rHSV_1hGM-CSF/GALV-GP) for the | Class 2 | < 1 Litre |  

02/03/2022
RP1 will be administered to subjects as an anti-tumour therapy, as part of a Phase I clinical trial to treat a wide range of solid tumour types. RP1 has a particular utility in combination with immune co-inhibitory pathway blockade. Intended indications to study include soft tissue sarcoma, breast cancer including triple negative breast cancer (TNBC), lung cancer including non-small cell lung cancer (NSCLC), melanoma, non-melanoma skin cancers, head and neck cancer, primary liver and kidney cancer and colorectal cancer. The initial clinical trial protocol intends to test RP1 in several indications as a monotherapy and in combination with anti-PO-1 therapy.

Recipient or parental organism

RP1 (rHSV_1hGM-CSF/GALV-GP) is a selectively replication competent Herpes Simplex Virus-1 (HSV-1). The virus contains a codon-optimised sequence for human granulocyte macrophage colony stimulating factor (hGM-CSF) and a codon optimised sequence for the gibbon ape leukemia virus surface glycoprotein (GALV-GP) with the R- sequence deleted (R-)[GALV-GP-R-]. GALV-GP-R- expression leads to cell to cell fusion (syncytial) formation in infected tumour cells through binding to the constitutively expressed PIT-1 receptor for GALV. This results in the death of the cells by membrane fusion and is also intended to enhance the spread of the virus through the tumour. Since the RP1 selectively replicates in tumour cells, the expression of the GALV-GP-R- is minimised in normal tissues. The oncolytic destruction of tumour cells leads to the release of tumour associated antigens that are intended to engender an antitumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further enhanced through GALV-GP-R- mediated killing, fusion associated cell death which also results in the production of the highly immunogenic exosomes, which is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, uninjected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

Host/vector system

RP1 (rHSV-1 hGM-CSF GALV-GP) is derived from the RH018A strain of Herpes Simplex Virus-1. RP1 is produced in the Vero cell and released into the culture media during cell lysis, prior to purification.
RP1 was constructed using a new strain of HSV-1 (strain RH018A). Replimune obtained and compared 30 clinical strains of HSV-1 on a panel of human tumour cell lines and selected the most promising of these (strain RH018A) for further development.

RP1 expresses the immune stimulatory protein GM-CSF, which augments therapeutic activity. GALV-GP-R- binds to the Pit1 receptor, which is widely expressed on mammalian cells including human tumour cells. Pit1 is also critical for cell proliferation, and its expression is therefore unlikely to be lost or down-regulated in response to cancer treatment. The truncated R- version of the protein provides constitutive fusion activity without GALV (i.e. the virus) itself. Expressing GALV-GP-R- together with GM-CSF is expected to increase clinical activity as compared to only expressing GM-CSF. As well as causing direct tumour cell death by cell to cell fusion, cell to cell fusion followed by death is highly immunogenic and includes the release of immunogenic tumour antigen-containing exosomes. Expression of GALV-GP-R- from an oncolytic virus is therefore expected to improve systemic, immune mediated, effects, as well as effects in the directly treated tumour thereby increasing synergy with other immunemediated approaches to cancer therapy such as immune co-inhibitory pathway blockade.

**Origin & function**

As described above (under Recipient or Parental Organism), the oncolytic destruction of tumour cells (upon transduction with RP1) leads to the release of tumour associated antigens that are intended to engender an antitumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further amplified through GALV-GP-R- mediated killing, fusion associated cell death which results in the production of the highly immunogenic exosomes and is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, uninjected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

**Evaluation of foreseeable effects**

As described above (under Recipient or Parental Organism), the oncolytic destruction of tumour cells (upon transduction with RP1) leads to the release of tumour associated antigens that are intended to engender an antitumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further amplified through GALV-GP-R- mediated killing, fusion associated cell death which results in the production of the highly immunogenic exosomes and is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, uninjected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation is requested

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All used and unused RP1 and diluent vials used in preparation and syringes will be destroyed per institutional policy. As per the wild-type HSV-1 virus, the recombinant HSV-1 vector particles that represent RP1 are highly susceptible to dehyration, rapidly inactivated outside the host and easily inactivated (for example with 1 % Virkon solution). As part of phase I of the clinical trial, biodistribution and shedding will be monitored. RP1 DNA levels in blood and urine will be determined at time-points outlined in the Schedule of Assessments of the clinical protocol (day 1, day 3/7/10, day 14, day 21, day 28, day 35, day 42, day 49, day 56 and as part of the follow up, 30 days after the last dose). Blood will be collected after the first, second and third RP1 injections at the following time-points: 1 (±15min), 2 (±15min), and 4 (±15min) hours and also immediately prior to dosing at the second and third dose. RP1 DNA levels and virus from saliva/oral mucosa, injection sites, injection site dressings, and lesions that appear to be herpetic will be determined on the same days.
The feedback was around notification of the laboratory staff for routine bloods for participants on study. These are marked with a red identifier and the lab staff are notified of a participant on study. The risk assessment designated a class I for this GMO in line with a similar study. The Chair has sent a letter on behalf of the Committee for the study to proceed.

Project Containment

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Project Ref 985/19.1

Date Ackn'd: 24/10/2019

CU2 Project Title: RP2 is a selectively replication competent herpes simplex virus 1 (HSV-1) which expresses exogenous genes, and is administered by intratumoural injection

Class: Class 2

CultureVolClass2: ≤ 1 Litre

Non-GMM Consent Granted

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Historical Date of Additional Info

02/03/2022
### Project Additional Information

#### Purposes of the contained use

RP2 is an interlesionally delivered oncolytic immunotherapy comprised of a genetically engineered HSV-1 (herpes simplex virus type 1) that selectively replicates in tumour cells as part of the Replimune Clinical Trial: An Open-Label, Multicenter, Phase 1 Study of RP2 as a Single Agent and in Combination with PD1 Blockade in Patients with Solid Tumours.

#### Recipient or parental organism

RP2 is a selectively replication competent herpes simplex virus 1 (HSV-1) which expresses exogenous genes, and is administered by intratumoral injection. RP2 was constructed using a new strain of HSV-1 (strain RH018). The neurovirulence factor (ICP34.5) encoding genes and the ICP47 encoding gene are deleted from the virus. The ICP34.5 deletion allows the virus to replicate selectively in tumours. The virus contains a codon-optimised sequence for human granulocyte macrophage colony stimulating factor (hGM-CSF), a cytokine involved in the stimulation of immune responses. In addition, the virus contains a codon-optimised sequence for the gibbon ape leukemia virus surface glycoprotein (GALV-GP) with the R- sequence deleted (R-). GALV-GP-R- causes cell to cell fusion resulting in cell death. The truncated R- version provides constitutive fusion activity, which is intended to be beneficial for tumour treatment. RP2 has been engineered to express anti-human CTLA-4 antibody-like molecule. Anti-human CTLA-4 interferes with the interaction of CTLA-4 (expressed as a subset of activated T cells) with B7 (CD80/CD86) molecules on professional antigen presenting cells. The design and mode(s) of action is similar to that of the previous RP1 trial.

#### Host/vector system

RP2 was constructed using a new isolate of HSV-1 (strain RH018; ECACC Accession Number 1612904). RP2, rHSV-1 hGM-CSF/GALV-GP-R-/ahCTLA-4 RP2 consists of (a) a lipid bilayer envelope derived from host cell membranes, including polyamines, lipids and glycoproteins; (b) a tegument of amorphous material; (c) a capsid made of capsomers arranged in icosapentahedral symmetry; (d) an internal core containing double-stranded DNA of ~160 kilobase pairs.

#### Origin & function

RP2 is a selectively replication competent HSV-1 intended for direct injection into suitable solid tumors. All herpes viruses are comprised of a large double-stranded linear DNA genome that is packaged into an icosahedral nucleocapsid which is approximately 100 nm in diameter. The herpes virus family is classified into different groups based on biological properties such as host cell range, length of replication cycle, and the cell type in which they become latent. Wild type HSV-1 is an alpha herpes virus with a wide host cell range. RP2 is a modified HSV-1 containing the genes coding for hGM-CSF, GALV-GP-R- and anti-hCTLA-4. RP2 was constructed using a new isolate of t1SV-1 (strain RH018; ECACC Accession Number 1612904). The neurovirulence factor ICP34.5 genes and the ICP47 gene are deleted from the virus. The ICP34.5 deletion renders the virus non-pathogenic and allows the virus to replicate selectively in tumor tissue. The role of ICP47 is to block antigen presentation to MHC class I and II molecules by blocking the transporters associated with antigen processing (TAP). This deletion also allows the increased and earlier expression of US11, a gene that promotes growth in tumor
cells without decreasing tumor selectivity. The virus contains the coding sequence for human GM-CSF, a cytokine involved in the stimulation of immune responses. GM-CSF expression is under the control of the hCMV IE promoter. The virus also contains the coding sequence GALV-GP-R-. The truncated R- version provides constitutive fusion activity, which is intended to enhance the spread of virus through the tumor. GALV-GP-R-expression is under the control of the RSV L TR promoter. RP2 also expresses anti-hCTLA-4 with the intent to enhance anti-tumor activity. As stated above, anti-hCTLA4 interferes with the interaction of CTLA-4 (expressed on a subset of activated T cells) with B7 (CD80/CD86) molecules on professional antigen presenting cells. This is intended to result in enhanced T-cell activation due to blockade of the inhibition otherwise mediated by the CTLA-4/B7 interaction. The resulting enhanced local anti-tumor T-cell activation, proliferation, and lymphocyte infiltration into tumors, is intended to lead to improved local and systemic anti-tumor effects both alone and in combination with antibodies targeting PD-1 or PD-L1. Intratumoral expression of the anti-hCTLA4 antibody-like molecule would also be expected to reduce toxicity as compared to the systemic administration of an anti-hCTLA4 antibody, including in combination with antibodies targeting PD-1 or PD-L1.

Evaluation of foreseeable effects

No studies have yet been conducted with RP2 and therefore no data is available. However, as previously described RP2 is an oncolytic immunotherapy product intended for the treatment of solid tumors and is identical to RP1 other than the expression of anti-CTLA-4 from RP2 which RP1 does not express. Whilst no studies have yet begun with RP2, approximately 26 patients have been treated in a phase 1 trial of RP1 in the UK. Patients have been treated with up to 10 mL of 108 PFU/mL in superficial and deep (visceral) cohorts at dose levels of between 104 and 108 PFU/mL. One patient in the low dose deep cohort had a dose limiting toxicity (DLT) of a clinically asymptomatic lipase increase after the third virus dose was administered (106 PFU/mL). Although this resolved without intervention, an additional three subjects were enrolled at the same dose and no further DLTs were observed. Side effects otherwise have been as expected for an oncolytic immunotherapy with low-grade febrile and constitutional symptoms with mild injection site reactions having been seen. The RP2D for use by superficial injection was determined to be a first dose of 106 PFU/mL followed by subsequent doses of 107 PFU/mL.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We would need a derogation as we do not have an autoclave on site.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Ali used and unused vials will be disposed of as per eee policies and procedures and in line with the IB for RP2. Spills will be treated with a virucidal agent such as 1% sodium hypochlorite or Virkon. All materials contaminated with RP2 will be disposed of in compliance with local institutional eee policies and SOPs. Incineration is appropriate. In the event of exposure to broken skin or needle stick, the site will be cleaned thoroughly with soap and water or a skin disinfectant. A physician for monitoring for signs of infection, occupational health will be contacted for support. Acyclovir or other anti-viral drugs may be administered prophylactically. Our patients will be given full instructions and support from our designated Principal and Co-Investigators and Research Practitioner Team which will include care of any -lesions and advice on any shedding. Our patients will be provided with spare dressings, instruction for use and safe disposal equipment which they can return to eee, they will be given out of hours contact numbers and advice will be on hand by the eee Team if needed and all advice will be in line with Sponsor instruction and the study protocol.
The committee felt that this is an important follow on study to RP1 supported at CCC. The committee felt that the risk assessment for class 1 was appropriate for RP2 as we will only be handling small quantities of virus and not any large mixing as per Sponsor IB; this in our assessment substantially reduces risk and given the structure and attenuation of the IMP, we feel becomes class I. The Chair has sent a letter to the effect that the committee supports the study proceeding.

Please enter comments on the GM safety committee on the risk assessment

The committee felt that this is an important follow on study to RP1 supported at CCC. The committee felt that the risk assessment for class 1 was appropriate for RP2 as we will only be handling small quantities of virus and not any large mixing as per Sponsor IB; this in our assessment substantially reduces risk and given the structure and attenuation of the IMP, we feel becomes class I. The Chair has sent a letter to the effect that the committee supports the study proceeding.

Project Containment

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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
| Data Premises Notified (Originally) | 11/07/2007 |
| Data Premises Closed |  |
| Transferred from 1992 Regs? | N |
| Transitional Premises Class |  |
| Transitional Premises | N |
| Emergency Plan Required? |  |
| Non-GMMs | N |
| Withdrawn | N |

Name

ALPHA BIOLOGICS

Name 2

Department

Campus Estate or Research Centre

ALPHA BIOLOGICS EUROPE

Building

Daly Labs 201/204, Babraham Research Campus

Road Name

BABRAHAM

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB22 3AT

Country

ENGLAND

Tel Number

01223 496 070

Fax Number

01223 496 071

E-mail

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee comprises one member of the senior management team and two members of the laboratory scientific team. The committee meets every two months to discuss the new arising GMO assessments or more frequently should this be required by the Biologics Safety Officer and the initiation of work programmes.

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<th>Laboratory</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

All spent liquid waste that has come into contact with the cell lines or the cell product will be treated with Virkon to a final concentration of a minimum of 1%. The Virkon treatment will last a minimum of overnight approx. 10 hours prior to being disposed of through the normal Babraham drainage system. Virkon is the EPA approved disinfectant. Liquid spillages will be dealt with by the use of an approved spillage kit. Solid waste including plasticware from procedures and laboratory work with the GMO will be placed in a plastic autoclave bag which will be sealed but not completely closed. The waste will be autoclaved and labelled clearly as a biohazard. The autoclave used is sited in the same building as the laboratory. The autoclave is routinely tested every six months ensure the reading of temperature and pressure are correct. The autoclaved waste is transported off site for incineration by Vet Speed (GM ref 98).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The host and vector systems being used are all standard and as the inserts are unlikely to cause or enhance any pathogenicity the work is assessed as a Class 1 activity.

The GMSC has cleared the work to start on receipt from the HSE that this is applicable.
GM Centre Number: 987

Data Premises Notified (Originally) 12/07/2007

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required?

Non-GMMs N

Withdrawn N

Name

UNIVERSITY OF LINCOLN

Name 2

Department

COLLEGE OF SCIENCE

Campus Estate or Research Centre

Building

Road Name

BRAYFORD POOL

District

Town

LINCOLN

County

LINCOLNSHIRE

Postcode

LN6 7TS

Country

ENGLAND

Tel Number 01522 882 000

Fax Number 01522 886 041

E-mail

HSE Division MIDLANDS

Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

School of Life Sciences – Technical Managers (2 – also act as Biological Safety Officers),
Senior Lecturer (biochemistry),
Associate Professor in Bioveterinary Science (Avian and comparative immunology) (Chair)
Senior Technician (Avian Immunology)
Commercial Manager (proteomics/protein structure determination)
Senior Lecturer (microbial and molecular genetics).

Health & Safety Department – Head of Department.

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Non-microbial

Other (please specify) Tick if confidential

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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

All plastic and glassware and microbiological plates and cultures will be autoclaved at 134°C for 30 mins (waste decont cycle) prior to disposal. This autoclave cycle is designed to give 100% kill of all bacteria and GMMs. All work surfaces and spills will be disinfected with 1% Virkon solution in accordance with the manufacturers recommendations.

Autoclaves are validated annually for insurance purposes and will be serviced annually. NB the autoclaves were purchased in Jan 2006 therefore the first service will be Aug/Sept. 2007 after the end of the warranty period.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Genetic Modification Safety Committee (GMSC) met on 20-06-07 to evaluate the risk assessments for the proposed GMO activities (see attached documentation). The committee reviewed and approved both risk assessments following minor modifications.

1. Include details about disinfecting surfaces and spill where GMM activities are performed (section 4 applications GMO001 & GMO002)
2. Provide further details of associated hazards relating to work with bacteriophages (section 2 application GMO002)

All GMO activities were approved as class1.

NB - the project supervisors for these applications are members of the GMSC. However neither supervisor was present when the committee discussed or approved their risk assessment. Likewise, for GMO001, relinquished the role of Biological Safety Officer so as not to be involved in the approval of there own risk assessment. For this application only a suitably qualified member of the GMSC acted as Biological Safety Officer.
Purposes of the contained use

To enable the generation of self-inactivating third generation recombinant lentiviral particles that encode:
- Specific open reading frames (ORFs)
- encoding for wild type proteins involved in DNA replication and repair
- mutated / truncated forms of above proteins
- tagged forms of wild-type and mutated / truncated forms of above proteins
- Short hairpin (shRNA) sequences for the knockdown of expression of ORFs by RNA interference
- The generation and in vitro use of stable mammalian cell lines for the purpose of
- studying the cellular effect of knockdown of gene expression of above proteins
- studying the cellular effect of overexpressing ORFs
- establishing cellular / in vitro disease models
- identifying druggable targets in DNA replication and repair pathways
- verifying such druggable targets

Recipient or parental organism

i) E. coli K12 strains for facilitating cloning and propagation of plasmid DNA:
- DH5alpha, SURE for generic use
- STBL3 for cloning and propagating shRNA sequences
E. coli K12 strains are attenuated, non-colonising, non-pathogenic strains, unlikely to survive outside the laboratory and have a long history of safe use, and are regarded as non-hazardous
ii) the HEK293T packaging cell line for the assembly and packaging of lenticiral particles. Infectious viral particles are generated by the packaging cell line and secreted into the medium at high viral titre. Risk is associated with the high titer of infectious viral particles.

iii) recipient cell lines. These cells are exposed to the high titre virus suspension generated in the packaging cells. The viral genome is integrated into the genome of the recipient cell line but because of the design of the system (third generation lentiviral vectors) the virus is incapable of further replication. The transduced cell lines, therefore, pose no further risk compared to the recipient cell lines.

Host/vector system

Third generation lentiviral vectors will be used throughout the work, which are replication incompetent and self inactivating (SIN). The systems separate the packaging signals and viral LTRs on the expression plasmid from the viral structural and expression genes (gag, pol and rev from FIV or HIV and viral envelope glycoproteins such as the VSV-G gene from Vesicular Stomatitis Virus, in place of HIV or FIV env). The viral structural and expression genes and the envelope glycoprotein genes are separated on at least two additional plasmids. Deletion of accessory genes vpr, vpu, vif and nef mean that the vector is unable to replicate once it has transduced the target cell. In addition third generation lentivirus are deleted for the tat gene and carry a SIN deletion of the 3' LTR (ΔU3) which results in "selfinactivation" of the lentivirus following transduction of the target cell, precluding adventitious activation of the vector by endogenous retroviruses and minimising the risk of recombination with ERVs. In particular, the pLKO.1, pLenti6, and pLenti-DEST vectors will be used.

The packaging cell line HEK293T (and its derivatives) is generated from the well-characterised 293 human embryonic kidney cell line (Pear et al., PNAS 1993; 80; 8392; ATCC CRL-11268). This cell line stably and constitutively expresses a temperature-sensitive version of the SV40 large T antigen.

The recipient cell lines are well established laboratory cell lines (for example, but not exclusively, HeLa, U2OS); normal / transformed / immortalised fibroblasts (for example, but not exclusively, MRC5, GM00637, WI38, HS68tert); normal and immortalised mesenchymal stem cells

Origin & function

i) The cDNA fragments of ORFs will be produced from RNA prepared from laboratory cell lines, reverse transcribed with reverse transcriptase (e.g. AccuScript, Agilent) and PCR amplified with purpose designed primers (for truncation, mutagenesis or Gateway cloning). PCR amplified cDNA fragments will be inserted into suitable primary cloning vectors (Gateway or TA cloning vectors). Insert from the primary constructs will be transferred into suitable expression vectors with or without tagging.

ii) shRNA -encoding DNA fragments will be chemically synthesised and inserted into pLKO.1 vector. Transduction with shRNA-bearing vectors will result in siRNA mediated knockdown of target genes. Physiological effect of knockdown will be studied in vitro using microscopic and molecular biology techniques. The effect of overexpression of wild type and mutated / truncated forms of proteins will be studied in similar techniques. Expressing of wild type and mutated / truncated forms in the background of knocked-down expression will permit ‘rescue’ of the knock-down phenotype, and will provide a functional map of the re-expressed protein.

Evaluation of foreseeable effects

i) Characteristics of the host, virus or viral vector and any hazards associated with it

The transfer vector systems are derived from FIV or HIV (feline or human immunodeficiency virus, respectively) and have been specifically engineered for biosafety by separating the packaging signals and viral LTR's on the expression plasmid from the viral structural and expression genes (gag, pol and rev from FIV or HIV and the VSV-G gene from Vesicular Stomatitis Virus, or similar alternatives, in place of FIV or HIV env) encoded on three or four separate
plasmids, which remain in the packaging cell line, effectively precluding the production of replication competent virus in the target cell or should the viral vector escape containment. FIV vectors are included as they may be more efficient for some cell types. It does not imply any different containment or risk considerations than HIV based vectors. The plasmids expressing these gene products carry no packaging signals or LTRs and so cannot themselves be mobilised with the vector and have been engineered not to contain any regions of homology to each other or to the viral vector, to prevent undesirable recombination events that might result in replication competent virus being produced. Reversion to wild type is extremely unlikely given that several recombination events would be needed to reconstitute an active viral genome, the viral genes are present on three different plasmids, which have minimal sequence homology and the viruses are self-inactivating following insertion (i.e. the LTR’s are destroyed upon genome insertion).

The viral vector would be able to transduce many tissues should it come in contact with them. The major hazard is therefore represented by the packaged virus prior to infection of the target cells and residual virus in the medium of infected cells. The two potential transmission routes are by external exposure (either skin lesions or mucous membranes; only in the case of very high titres and aerosol production) and by accidental injection/inoculation using sharps. The following safety measures will be employed to minimise these risks:
- The work will be carried out in a restricted area in a designated Class II laminar flow safety cabinet.
- The usage of sharps of any kind is strictly forbidden.
- Researchers working with active viral particles will be wearing double-gloves
- Additional PPE: safety goggles, labcoat, disposable plastic sleeve covers will provide additional barrier for the protection of experimenters.
- Liquid waste will be disinfected with 1% Virkon solution, which is a widely used disinfection reagent capable of inactivating all known viruses.
- Culture vessels and other plasticware that has been in contact with live virus particles are collected in Biohazard bags inside the cabinet. Closed bags are decontaminated with 70% ethanol on the outer surface and autoclaved.
- All centrifugation step is conducted in an aerosol tight container and rotor; contaminated liquids and solids are treated as above.

Severity of harm is MINOR given that infection of human tissue if achieved would be with non-replicable virus and low-efficiency. Likelihood of harm is deemed to be LOW. The Standard Operating Procedures document set out rules for safe working with lentiviral vectors, in particular for avoiding contamination. The usage of sharps is strictly forbidden. Viral titre is expected to be between 0.5-5x10^6 IU/ml. The virus is generally weak and does not survive long outside of buffered solutions. Infection requires optimal conditions and the use of additives in cell culture. Immune competent mice show sterilising immunity with wild-type virus infection; humans would also show such sterilising immunity. Risk is generally speaking no greater than that of handling human tissue or bodily fluids i.e. relatively safe given that proper precautions are taken to avoid infection.

ii) Characteristics of the inserted sequences, and any hazards associated with it

Ectopic expression / overexpression of ORFs will be driven by strong constitutive promoters. Lentiviral transfer of such constructs is associated with hazards of modifying cells, including those of the researchers should accidents happen. However, due to the very low infecting efficiency of the virus, and its inability to replicate, the risk is low unless the viral vectors carry oncogenes / proto-oncogenes. ORFs with known oncogenic potential will be excluded from the work.

There is further risk associated with lentivirus vectors carrying shRNA cassettes. Here the major risk is knockdown of expression of tumour suppressors. ORFs with known tumour suppressor activity will be excluded from the work. In addition, target sequences that may affect virulence genes or associated regulatory pathways will also be excluded. Off-target effect of shRNA cassettes are minimised by careful design of the target sequence that includes database searches to exclude sequences with effective homology to other targets.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

There is not intention to use larger GMOs.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be disinfected using Virkon (oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers) which is expected to give 100% kill of any organisms. Virkon is widely accepted as the correct agent to disinfect bacteria, viruses, fungi and cultured cells, and approved for use against HIV. Solid waste is collected in Biohazard bags inside the biological safety cabinet. Closed bags are sprayed with 70% ethanol on the outside surface, then transferred for autoclaving. Autoclaving is performed at 134 deg C at 15 psi for 45 minutes to give 100% kill of organisms.

This project has been considered by the full GMSC and following revisions recommended by the committee, has been approved at containment level 2.

Project Containment

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## Premises Addresses

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<td>CLEVELAND</td>
<td>TS4 3BW</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
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02/03/2022
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

ILLUMINA CAMBRIDGE LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

19 GRANTA PARK

**District**

GREAT ABINGDON

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB21 6DF

**Country**

ENGLAND

**Tel Number**

01223 408000

**Fax Number**

01223 408001

**E-mail**

**HSE Division**

MIDLANDS

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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<td>CB2 5LD</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee (GMSC) consists of the Chief Executive Officer, the Biological Safety Officer and VP Engineering.

The GMSC meets on a quarterly basis with additional meetings as and when necessary. The committee seeks to understand the risks both to human health and the environment. The GMSC advise, discuss and judge on all GMO risk assessments, which supersedes other laboratory risk assessments. The committee will seek and appoint extra sources of advice, where necessary. The committee is also responsible for consulting all staff on health and safety issues. The committee will discuss and act upon any recommendations by the Biological Safety Officer.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|-------------|-------------|-------------|-------------|
Level 1 (GMMs) | Yes         |             |             |             |
Level 2 (GMMs) |             |             |             |             |
Level 3 (GMMs) |             |             |             |             |
Level 4 (GMMs) |             |             |             |             |
Non-microbial |             |             |             |             |
Work surfaces are wiped down with chemical disinfectants (Virkon or equivalent) at the end of each procedure. Spillages are decontaminated with powder chemical disinfectants (Virkon or equivalent). As much as practically possible, the work will be done in a class 2 biological safety cabinet (serviced annually) to reduce contamination to samples and reduce the aerosol exposure to workers and the environment. The solid and liquid waste generated from the GMO work is deactivated by autoclaving at 121°C for 22 mins. The autoclaving process is validated via use of commercial autoclave tape which gives a visual indication that it has been exposed to the correct sterilisation conditions. The autoclave equipment is serviced annually to ensure that performance of the sterilisation equipment. The degree of kill via a correctly-operating autoclave is 100%. Treated waste are then collected and disposed by licenced laboratory hazardous waste companies.

Tick if you are claiming exemption from disclosure for sections of the risk assessment □

Tick to confirm that you are attaching a summary of the risk assessment Y

Please enter comments of the GM safety committee on the risk assessment

The risk assessment is appropriate and has considered all potential hazards to humans and the environment. The committee agrees with the classification of the activity as class 1.
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Date at Which Additional Info Submitted: 02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

A Genetic Modification Safety Committee (GMSC) has been established within the Trust, reporting through the Risk Management Committee of the Renal Unit within the South West Thames Institute for Renal Research (SWITRR) co-locates, from here to the Clinical Networks Division Risk Management Committee and finally into Epsom and St. Helier University Hospitals Trust Health and Safety Committee (HSC) who are responsible for Health & Safety within the Trust, reporting to the Trust Executive Board. The GMSC comprised Scientific Director (SWITRR) as Chair and Biological Safety Advisor, Deputy Scientific Director (SWITRR) as Biological Safety Officer, Trust Risk Management Advisor and HSC liason, Microbiology Medical Scientist, Consultant Immunologist, R&D Manager, post-doctoral research scientist and PhD student working in this area, and two external scientific advisors available as necessary. The committee meets 3x annually to assess proposals, risk assessments, incident reports, assign containment levels and to monitor GM work in the Trust to ensure safe and best practice.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
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<td>Tick if confidential</td>
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Yes

All procedures will follow the standards of Good Microbiological Practice and Good Occupational Safety & Hygiene. All work will be conducted to the requirements for containment level 1. The bench surfaces in the laboratory are easily cleaned, impervious to water, resistant to acids, alkalis, solvents, disinfectants and other decontamination agents in use. A spill tray with disposable covers will be used to provide environmental and personal protection and ease of decontamination should such a need be identified. Routine disinfection will use 1% hypochlorite solution on all surfaces except metal and equipment where 70% aqueous alcohol will be used in accordance with the Trust Infection Control Guidelines. At least a 5log kill in the treated areas is expected. Hand washing facilities and a supply of soap are provided in the laboratory. The laboratory is equipped with a dedicated safety cabinet and all associated equipment for experimental procedures involving GMM. An autoclave and dishwasher are also located in the laboratory for safe decontamination and disposal of GMM waste at source. The autoclave is serviced routinely under contract to ensure calibration and proper working order. All contaminated material, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Each run will be monitored using TST (Time, Steam and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min). All inactivated waste will then be disposed of through Trust clinical waste management procedures. Work surface and equipment contamination will be tested quarterly by Microbiology excepting in the event of a spill where affected areas will be tested by Microbiology for the presence of relevant organisms after appropriate decontamination. The laboratory will be inspected on a quarterly basis. Remedial action will be identified and enforced as necessary.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The committee accepted that the organism and vectors under discussion fell under the category of a recognised disabled micro-organism and that the vectors were not mobilisable. The modifications proposed were considered unlikely to alter these characteristics. The appropriate and due diligence had been paid to the potential risk of harm to personnel and the environment and that appropriate control measures had been described. The committee accepted the proposal for and assigned an activity class 1 and containment level 1 status for this proposal. The facilities provided were demonstrated to exceed the requirements for such a containment level and approval was given to the project subject to the Chair notifying HSE that this Institution was wanting to start work under the Genetically Modified Organisms (Contained Use) Regulations 2000 and acknowledgement by the HSE of receipt of that notification.
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Name

BEATSON WEST OF SCOTLAND CANCER CENTRE

Name 2

Department

Campus Estate or Research Centre

GARTNAVEL GENERAL HOSPITAL

Road Name

1053 GREAT WESTERN ROAD

Town

GLASGOW

Building

County

LANARKSHIRE

Postcode

G12 0YN

Country

SCOTLAND

Tel Number

0141 301 7000

Fax Number

E-mail

HSE Division

SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Newly established committee - as part of Glasgow Biomedicine (joint university and nhs).

Contact - Clinical Research & Development, NHS Greater Glasgow & Clyde, Ward 11, Dykebar Hospital, Grahamston Road, Paisley PA2 7DE

<table>
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Other (please specify) Department of Pharmacy Aseptic unit for assembly of Level 1&2 products

Tick if confidential

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Bacteriology | Parasitology | Transgenic Birds | Microbiology Research
Yes

Gene Therapy

Virology

Transgenic Animals

Transgenic Fish

Mycology

Transgenic Invertebrates

Transgenic Plants

Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Viricide to be used for Class 1

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

In our view there is no problem with this proposal. The vaccinia strain is highly attenuated and the data on its safety are impressive. That other sites have approved its use without significant modification and that it has already been given to my patients with renal and other cancers convinces us that we are happy for the study to go ahead.
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
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<td>Department</td>
</tr>
<tr>
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<tr>
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<tr>
<td>Tel Number</td>
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Date at Which Additional Info Submitted: 02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Comprises 5 individuals: (i) Operations Director, serving as senior management representative; (ii) Director of Virology, serving as acting Health & Safety Manager/BSO, and chair of committee; (iii) Molecular Biology Manager and (iv) Virology Manager serving as representatives from the two technical divisions; and (v) QA Manager, serving as representative for the non-technical, support staff who may be present in laboratory areas where GMO work conducted, and/or provide equipment maintenance etc. Individuals also provide relevant experience in the areas of virology, molecular biology, and microbiology. Meetings will be held every 2 months, and more often if required. Minutes will be reported to general safety committee, and made available to all staff, either electronically or by posting on H&S noticeboard.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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</table>
Yes

Yes

See attached RA for further details.

Briefly: Liquid waste will be disinfected using a minimum of 1% Virkon, or by autoclaving (with/without prior chemical inactivation). Solid waste contaminated with GMMs will be autoclaved on site, prior to uplift for secondary inactivation (incineration). At least 5 log kill would be envisaged, based on manufacturers information, for Virkon. Surfaces potentially in contact with GMMs will be cleaned routinely using 2% virkon. Validation studies to confirm the effectiveness of procedures used for inactivation of model agents will be performed, once assays are in place. SOPs govern all working practices, and all equipment is maintained and checked / validated at regular intervals, including the autoclave.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment
The risk assessment is intended to cover the performance of all envisaged class 1 and 2 activities (form CU2 for class 2 activities also enclosed).

The RA was reviewed by the GMSC members, and comments incorporated. Comments were minor, and principles covered were agreed/approved by the committee. Committee noted for internal purposes that storage boxes for the transport of samples between laboratory areas should be sourced, and training in biological safety, including GM regulations, should be arranged.

### Project Ref 992/07.1

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
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<th>Project notified under transitional arrangements</th>
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<td>05/09/2007</td>
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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID** 992/07.1a

**Date of Significant Change** 28/04/2014

**Project Additional Information**

**Purposes of the contained use**

To perform biological safety testing of GMMs used in the production of biopharmaceuticals, or intended for use as vaccines, gene therapy vectors, or anti-cancer therapies. The majority of tests are designed to allow the detection of adventitious agents present within the client's materials, through the use of cell culture or PCR-based assays. These assays will form part of regulatory studies to support the safety of products at various stages of development (pre-clinical, clinical, licenced).

**Recipient or parental organism**

May include prokaryotic or eukaryotic cells, and plasmid or virus vectors (see also below). See The risk assessment for further details.

**Host/vector system**
Prokaryotic cells, such as E.coli, containing plasmid vectors (typically disabled or non-colonising host, with non-mobilisable / mobilisation defective vectors); mammalian cell lines containing stably inserted gene constructs:

- virus vectors (e.g., baculovirus, avipoxviruses, sendai virus, adenovirus, herpesvirus, retrovirus (usually based on simple oncoviruses), vaccinia, MVA)

See attached risk assessment for further details

**Origin & function**

The majority of samples submitted for testing will include: prokaryotic and eukaryotic cells expressing therapeutic proteins, including monoclonal antibodies; mammalian cells expressing helper functions to support the growth of replication defective virus vectors (helper and/or packaging cell lines); and virus vectors and attenuated viruses typically intended for use as vaccines, or for use in gene therapy or the treatment of neoplasia.

The inserted genes will therefore typically encode: therapeutic proteins (such as hormones (e.g., erythropoietin, growth factor, insulin), cytokines, coagulation factors) intended to replace normal human proteins deficient or absent in certain individuals or conditions; monoclonal antibodies; vaccine antigens, derived from various pathogens, often representing surface proteins; and detectable I selectable markers. Immune-modulatory genes may also be incorporated in some constructs, to enhance the immune response to the expressed immunogen.

See attached risk assessment for further details, and additional examples.

**Evaluation of foreseeable effects**

The majority of the GMMs submitted for testing will be derived from hazard group 1 or 2 agents, and contain inserts considered unlikely to significantly increase the risk of the construct, or containment level required, with respect to human health and the environment.

Most recombinant cells expressing therapeutic products would be expected to fall into GM activity class 1, although as many of the products may represent hormones or cytokines or novel constructs where the range of functions may not be known, additional precautions or higher containment may be required in some cases (e.g., pregnant women may be advised not to handle GMM5 expressing growth hormones where high level exposure could theoretically occur).

Vectors intended for use as live vaccines or for cancer! gene therapy are likely to be capable of entering, if not productively replicating, in human cells, and thus the potential for integration into the host cell genome, and/or long term expression, will be considered in deriving the final classification. For example, retrovirus vectors based on simple oncoviruses such as MLV, pseudotyped with an Env protein with tropism for human cells, will be classed as GM activity class 2, due to the potential for integration, and insertional mutagenesis. As replication competent virus (RCV) can be generated during the construction of most vectors, replication incompetent vectors will be classified at a similar level to the parent virus, as a minimum, unless evidence to show freedom from RCV is provided. Although in most instances it is unlikely that inserted genes would significantly increase the risk of a construct, there may be uncertainties with respect to the impact on pathogenicity (eg, inclusion of immune modulatory genes, or virus envelope proteins that could in theory alter the host range or tropism of the parent organism), and there may be some situations where additional precautions and/or higher containment is required.

Most processes are not envisaged to significantly impact on the containment requirements for most constructs, but some situations, e.g., tissue culture work where there may be a risk of recombination / rescue due to superinfection or the presence of endogenous virus sequences within a cell, may require additional precautions / higher containment.

Further discussion is contained within the attached risk assessment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| n/a |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| n/a |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Liquid waste will be inactivated by incubating overnight in a minimum of 1% virkon, or by autoclaving (with/without prior chemical inactivation). 5 log kill would be anticipated from the manufacturers’ information for virkon.
Solid waste contaminated with GMMs will be autoclaved on site, prior to uplift for incineration. Surfaces potentially in contact with GMMs will be cleaned routinely using 2% virkon. Spills will be disinfected using virkon (solid powder or 2% solution), and if necessary, biosafety cabinets may be disinfected by fumigation, using either formaldehyde or vapourised hydrogen peroxide (VHP).

Validation studies to determine the effectiveness of virkon, and fumigation, in inactivating a range of model agents will be performed once the relevant assays are in place. Initially, formaldehyde fumigation will be used for cabinets, as this is known to be effective for a broad range of organisms. However, it is intended to replace this with VHP, if this is shown to be effective in our hands.

Standard Operating Procedures govern all working practices, including the preparation of disinfectants, cleaning and spills policies, and disposal of waste. All equipment is validated and monitored on a regular basis, including the autoclave, air handling and biosafety cabinets. See attached risk assessment for further detail.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was reviewed by the GMSC members, and their comments incorporated. There were no major comments, and the committee was satisfied with the containment measures proposed. The principles I working practices described in the assessment were agreed / approved by the committee.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2</td>
<td>L3 L4 L2</td>
<td>L3 L4 L2</td>
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<td>L4</td>
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<td><strong>Emergency Plan Required?</strong></td>
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**Name**

EUROFINS PHARMA BIOANALYSIS SERVICES UK LTD

**Campus Estate or Research Centre**

Name 2

**Road Name**

90 PARK DRIVE, MILTON PARK

**District**

ABINGDON

**Town**

OXON

**County**

OXFORDSHIRE

**Postcode**

OX14 4RY

**Country**

ENGLAND

**Tel Number**

01235 444100

**Fax Number**

01235 444199

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Name change 09/12/2013 formerly Bioanalab Ltd. Name change 01/04/2014 formerly Millipore UK Ltd

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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</table>

## Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Yes

**Give brief details of the genetic modification safety committee**

The members of the Company Health and Safety Committee are ex officio members of the Genetic Modification Committee. Current membership of the Health and Safety Committee include the CEO, the Health and Safety Officer, the Radiation Protection Officer and a first Aider. The committee includes employee representatives. It meets as required to consider and approve applications for projects involving genetic modification.

<table>
<thead>
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<tr>
<td>Other (please specify)</td>
<td>Tick if confidential</td>
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</table>

02/03/2022
All waste containing GMM's or exposed to GMM's will be treated according to the Company's standard operating procedure for biohazardous waste. It will be decontaminated by treatment with a suitable disinfectant prior to being securely bagged, sealed and sent for incineration by an approved contractor. Liquid waste will be decontaminated with disinfectant prior to disposal by a suitable route depending on the nature of the liquid. If these methods are deemed not to be suitable for particular items, they will be made safe by autoclaving in the company's on-site autoclave prior to disposal by incineration. The estimated degree of kill by these combined methods is extremely close to 100%.

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
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<td>Mycology</td>
<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
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<td>Other(s)</td>
<td>Research and testing using genetically modified cells in vitro.</td>
<td></td>
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</tbody>
</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

none. The application was approved by all members of the committee.
**GM Centre Number: 994**

<table>
<thead>
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<th>Data Premises Notified (Originally)</th>
<th>08/11/2007</th>
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<td>Non-GMMs</td>
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</table>

**Name**

SOURCE BIOSCIENCE

**Name 2**

**Department**

**Campus Estate or Research Centre**

UNITS 24 AND 25

**WILLIAM HAMES HOUSE**

**Road Name**

COWLEY ROAD

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 0WU

**Country**

ENGLAND

**Tel Number**

01223 432600

**Fax Number**

0122 343 2601

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

The requirement for this notification has come about because of Medical Solutions acquisition of Geneservices Ltd and that some of the Geneservice business is being relocated to Nottingham. A GMSC had already been established at Geneservices and this will remain, incorporating those individuals from the previous GMSC still present within the new company (Medical Solutions) and all the present members of the Medical Solutions Safety Review Committee. The GMSC will meet concurrently with the Medical Solutions Safety Committee. The following members of staff constitute the GMSC: (Quality Assurance Manager), (Head of Operations), (Resource Development Manager, BSO), (Safety Officer), (Deputy Safety Officer), (Chief Laboratory Scientist),

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<tr>
<td>Level 3 (GMMs)</td>
<td></td>
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</tbody>
</table>
Disposal of biohazard waste

Used tips, tubes and inoculating loops are to be placed in ‘sweetie jars’, these are then taped shut with biohazard tape and placed in the yellow biohazard bags. Items such as disposable gloves or paper tissues contaminated with low hazard waste should be disposed of as Biohazard waste. Solid microbial waste will be placed in yellow bags and disposed in the same way as biological waste. All biohazard waste will be placed in yellow bags (double bagged), these are then sealed and autoclaved prior to collection via a designated waste disposal agent for incineration.

The agent used is:
Polkacrest

Disposal of microbial cultures

Aqueous microbial cultures are to be treated with Virkon at a final concentration of 1% w/v, left for a minimum of overnight before discarding via the drain with copious amounts of water.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Risk assessment

The host vector systems and constructs satisfy the requirements for Group 1. The host strains are all K12 disabled, and the vectors are non-mobilisable. The likelihood of the hazard is therefore negligible and the environmental risk effectively zero.

Risk assessment Sequencing doc

This service aims to sequence already cloned DNA templates, which are sent in by customers. The cloned DNA is effectively random, as far as the service is concerned, but will already have been cloned into the appropriate host/vector system. Once received, the service grows up small volumes of the cells, to obtain a sufficient quantity to extract the DNA and then sequence it. The host/vector systems are all standard and since the inserts are highly unlikely to enhance the pathogenicity, the work is correctly assessed as Class 1.

One additional factor is that some DNAs could be potentially hazardous and it is recommended that all naked DNA should be handled appropriately to cover this hazard, ie DNA should be handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex I, paras. 8-10; ie gloves should be worn, sharps avoided and all wastes be rendered harmless before disposal.

Clearance has been given by the GMSC that work can be started, pending receipt from the HSE.
GM Centre Number: 995

Data Premises Notified
(Originally) 08/11/2007

Transferred from
1992 Regs? N

Transitional Premises
Class

Data Premises Closed
Transitional Premises
Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name
NOVA LABORATORIES LTD

Name 2

Department

Campus Estate or Research Centre

Building MARTIN HOUSE

Road Name GLOUCESTER CRESCENT

District WIGSTON

Town LEICESTER

County LEICESTERSHIRE

Postcode LE18 4YL

Country ENGLAND

Tel Number 0116 223 0100

Fax Number 0116 223 0101

E-mail

HSE Division MIDLANDS

Comments

Date at Which Additional Info Submitted
02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Technical Director
- Quality Assurance Director (responsibility for site Health & Safety)
- Manufacturing Director
- External Project Manager(s) — Expert advice from clients responsible for producing GMM’s

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<td>Other (please specify)</td>
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Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research

02/03/2022
Yes

Prior to unloading an Isolator the contents will be decontaminated by gassing with a mixture of Hydrogen Peroxide and Peracetic Acid vapours.

Solid re-usable items of equipment will be subjected to further chemical decontamination (hypochlorite treatment) and dry heat sterilization.

Solid disposable items will be subjected to further chemical decontamination (hypochlorite treatment) and/or disposal as clinical waste (off site Incineration).

Liquid waste will be subjected to further chemical decontamination (hypochlorite treatment) prior to discharge from the facility. No active residues will be discharged into the facilities drainage system.

The facilities drainage system is "stand alone" there being no connection with the local mains drainage. Waste will be discharged into a bunded holding tank for disposal by a licensed contractor.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk Assessment agreed by Nova members of safety committee.

Project Ref 995/07.1

Date Ackn'd 09/11/2007

CU2 Project Title Aseptic filling of a human vaccine containing a modified HSV 1.

Class 2

Culture Volume Class 2

Culture Volume Class 3-4

Non-GMM Consent Granted

Not Applicable
**Project Additional Information**

**Purposes of the contained use**

To prevent filling operators being infected by live virus and to prevent product cross-contamination within the Nova “live biologics” facility (Building C).

**Recipient or parental organism**

Confidential customer information. Nova receives pre-modified bulk virus for filling into final containers that are used for human vaccination. No fermentation or modification of micro-organisms is performed by Nova.

**Host/vector system**

Modified
HSV 1. Nature of modification is commercially confidential.
Tests have
confirmed that modification does not increase the pathogenicity of the virus beyond Level 2.

**Origin & function**

For human vaccination.

**Evaluation of foreseeable effects**

The primary route of infection is oral.
Since aerosols are not believed capable of transmitting infective virus, normal cGMP handling including the use of isolator technology to maintain sterility of the product will provide protection for the operators.
For infection to occur the virus would need to enter cells that are capable of sustained rapid division, consequently "sharps injuries" are unlikely to lead to infection. Even so the process is designed to minimise the use of sharps during the filling procedures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Heat treatment and/or disinfectant treatments to achieve 100% kill. Prior to unloading an isolator the contents will be decontaminated by gassing with a mixture of hydrogen peroxide and Per-Acetic Acid vapours. Solid re-useable items of equipment will be subjected to further chemical decontamination (hypochlorite treatment) and dry heat sterilization. Solid disposable items will be subjected to further chemical decontamination (hypochlorite treatment) and/or disposal as clinical waste (off site incineration). Liquid waste will be subjected to further chemical decontamination (hypochlorite treatment) prior to discharge from the facility. No active residues will be discharged into the facilities drainage system. The facilities drainage system is “stand alone” there being no connection with the local mains drainage. Waste will be discharged into a bunded holding tank for disposal by a licensed contractor.

Is an emergency plan required according to regulation 20?  
N
If yes, tick to confirm that it is attached to this form
N
Tick to confirm that you have attached a risk assessment to this form
Y
Tick if you are claiming exemption from disclosure for section of the risk assessment
N

Please enter comments on the GM safety committee on the risk assessment
Risk assessment agreed by Nova members of safety committee.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
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Project Ref 995/12.1

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<tr>
<td>29/03/2012</td>
<td>Aseptic processing of a batch of drug substance into sealed vials in accordance with good manufacturing practice (GMP)</td>
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Date Project Ceased

29/03/2012

Class  
Class 2

CultureVolClass2  
1-50 Litres

CultureVolumeClass3-4

Non-GMM

Consent Granted

Project notified under transitional arrangements
N
Project Additional Information

Purposes of the contained use
Filling of an investigational, genetically modified adenovirus into vials for therapeutic use.
Full containment in a flexible film isolator to assure maintenance of mono-culture status (i.e. ensure purity) of the product during aseptic filling (i.e. to prevent ingress of contaminating organisms derived from personnel and the environment).

Recipient or parental organism
The therapeutic product is a genetically modified replication deficient, human adenvirus serotype 5.
It is ACDP level 2 requiring Containment level 2.

Host/vector system
The GMM was produced in HEK293 packaging cell lines and only infects CAR expressing cells.

Origin & function
The GMM was produced, purified and released in Switzerland in accordance with good manufacturing practice (GMP) regulations, and is fully characterised and safety tested. Nova Laboratories has the facility and capability to fill the virus into sealed vials, which are then intended for further human clinical trials in North America.
The material has been developed to activate immune cells to enhance the recognition of infection and viral resistance. Data from nonclinical and human clinical studies have shown acceptable safety profiles.

Evaluation of foreseeable effects
The therapeutic product has demonstrated a good safety profile with the effects of exposure to this and similar organisms well documented. Other than potential host organisms, it is considered that there are no environmental niches or habitats that would be affected, either directly or indirectly, by exposure to this product. The GMM is replication deficient, and is deemed a weaker pathogen than the native adenovirus. It is also susceptible to common disinfectants and cleaning agents (e.g. 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulfate, and 70% ethanol) and is thermally unstable above 56°C, pH sensitive, and will be contained within an isolator during aseptic processing to protect the product and ensure its purity. The bulk drug substance and finished product will be frozen during storage and transportation. No laboratory manipulation or clinical administration will occur at Nova’s facility. There are systems in place to prevent the vaccine product or any waste associated with the manufacture from affecting the surrounding ecosystem.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
The containment for process requirements & good manufacturing practice (GMP) are more stringent than those required for protection of human health and the environment. In this case Class 2 activities requiring Containment Level 2 are performed at a significantly higher level in order to ensure product purity and prevent contamination from operators or the environment, hence the use of gassed isolators which are commonly used for such purposes at Nova Laboratories.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The isolator is pre- & post-sterilised by gassing using hydrogen peroxide/peracetic acid vapour that will be validated with respect to lethal efficacy against the virus dried on to various test substrates. All liquid waste is chemically inactivated prior to disposal through a contained drain into a holding tank. The virus is also sensitive to pH, common disinfectants (e.g. sodium hypochlorite, glutaldehyde, sodium dodecyl sulfate) and 1N sodium hydroxide (NaOH).

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The safety committee has taken note of the full risk assessment and the procedure to be deployed at Nova. The processes required to ensure aseptic manufacture in a gassed isolator are deemed more than adequate for handling a class 2 adenovirus in small batch quantities.

Project Containment

<table>
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Project Ref 995/15.1

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<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>05/08/2015</td>
<td>Aseptic formulation and filling of bulk GMM provided by a Client Company, into injection vials or pre-filled syringes to develop a therapeutic medicinal product with view to the manufacture and launch of a commercially licensed drug product following marketing authorisation.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
</tr>
<tr>
<td>Date Project Ceased</td>
<td>Consent Granted</td>
<td></td>
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</tr>
</tbody>
</table>
**Project Additional Information**

**Purposes of the contained use**

This is an aseptic fill and finish activity for manufacturing studies with view to developing patient-ready doses of an Investigational Medicinal Product, and later licensed drug product from a live attenuated viral vaccine in the future.

Full containment in a flexible film isolator is primarily to assure maintenance of mono-culture status of the product during aseptic filling (i.e. prevent ingress of contamination from personnel and/or the environment).

**Recipient or parental organism**

AAV2-hRPE65v2 employs the adeno-associated virus (AAV) as a delivery vehicle for the normal human RPE65 gene; the recombinant vector is a non-enveloped icosahedral virion of approximately 65nm in diameter. Recombinant AAV will be used to express human retinal pigment epithelial 65kDa protein (hRPE65).

**Host/vector system**

The AAV2-hRPE65v2 gene transfer vector is based on Adeno-Associated Virus (AAV), a non-pathogenic, helper virus-dependent member of the parvovirus family of small, non-enveloped viruses. AAV’s single-stranded DNA genome only contains two genes, the Rep gene that codes for proteins involved in DNA replication, and the Cap gene, which through a differential splicing mechanism encodes three amino-terminal variant virus proteins, VP1, VP2 and VP3, that make up the coat of the virus.

**Origin & function**

The drug substance is to be supplied from our U.S. based client. It is intended for manufacturing process development with view to launch of a medicinal drug product. As an Investigation Medicinal Product it has demonstrated an excellent safety profile in human clinical studies.

**Evaluation of foreseeable effects**

AAV is not known to cause any diseases in humans or animals. AAV viruses are not associated with any human disease; however, there is evidence of AAV infection in the human embryo and an association of AAV with male infertility.

Biosafety level 2 contaminant is required, however all handling of this material will be in fully contained isolators to prevent contamination of the product from organisms derived from personnel or the environment. Normal cGMP handling using isolator technology to maintain the monoculture status of the drug product will provide more than sufficient protection for operators.

Methods of inactivation have been identified and approved including chemical treatment with sodium hypochlorite for 20 minutes.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

02/03/2022
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None - Biosafety level 2 containment will be utilised throughout the process. In addition, product filling activities will be undertaken in an isolator in order to prevent contamination of the product from organisms derived from personnel or the environment. In addition, this will provide more than sufficient protection for operators.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The isolator is pre- & post-sterilised by gassing using hydrogen peroxide/peracetic acid vapour that will be validated with respect to lethal efficacy against the virus dried on to various test substrates. All solid waste is chemically inactivated prior to disposal by a specialist licensed contractor. All liquid waste is chemically inactivated prior to disposal through a contained, dedicated drain to a dedicated holding tank, which is emptied by a specialist licensed contractor.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

The viral vaccine can be handled in accordance with standard operating procedures for handling live biologics in Nova Laboratories dedicated facilities. The GMM has demonstrated an excellent safety profile during use in Human Clinical Trials and can be readily deactivated via the use of Sodium hypochlorite (1-10% dilution)

The safety committee has taken note of the full risk assessment and the procedure to be deployed at Nova. The processes required to ensure aseptic manufacture in a gassed isolator are deemed more than adequate for handling a class 2 virus in small batch quantity

Project Containment

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<td>L2</td>
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GM Centre Number: 996

Data Premises Notified (Originally) 13/11/2007

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

PEACEFUL PETS LIMITED

Name 2

Department

Campus Estate or Research Centre

Building THE GRANGE

Road Name

District WEST RUDHAM

Town KINGS LYNN

County NORFOLK

Postcode PE31 8SY

Country ENGLAND

Tel Number 01485 528 141

Fax Number 01485 528 444

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ]

Give brief details of the genetic modification safety committee

Peaceful Pets Ltd is a small company. We would submit our risk assessment to seek guidance and advice from the genetic modification safety committee of the Royal Veterinary College, North Mymms. the Chair of the RVC GM committee

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
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<td>Laboratory</td>
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<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Genetically Modified Material (GMM), double-bagged and labelled by the waste producer at the point of collection will be transported directly to our site in 660 litre double skinned galvanised lockable trucks. The material will be destroyed by incineration in a HS Thermal 500 incinerator at temperatures in excess of 550 degrees centigrade under Section 5.1 Part B of the Pollution Prevention Control Regulations 2000. The incinerator of a hot hearth design, is strictly operated under the Secretary of State’s Guidance for Animal Carcase Incineration. Temperatures and emissions are monitored and regularly inspected by Environmental Protection & the Local Authority- The inert ash remains will be transported by a Registered Waste Carrier to an approved landfill site.

Waste material will be transported under Environment Agency Waste Carriers Registration number AEA/792563. Biosecurity for the transport of the waste from the producer to the Peaceful Pets site is of the utmost importance. To minimise the risk of contamination whilst the waste is in transit the Biosecurity Guidance for the National Fallen Stock Scheme will be implemented. The Guidance covers specific vehicle design, safe handling of waste, use of disinfectants and vehicle signage. DEFRA through the State Veterinary Service visit the site on a regular basis to inspect incinerators, vehicles and relevant paperwork.

The double-skinned galvanised 660 litre bins containing the labelled, double-bagged GMM will be tail-lifted onto a specifically designed vehicle for transport. The driver will be competent and familiar with the biosecurity guidance. Bins will be mechanically tipped and loaded into the incinerator. Throughout transportation and loading into the incinerator a complete spillage containment kit will be close by. The galvanised bins are thoroughly washed and disinfected using a DEFRA approved chemical at the recommended concentration. Throughout the transport and disposal procedure appropriate protective clothing and footwear will be worn. Relevant records of collection, incineration and disposal of the inert ash will be maintained for traceability and Duty of Care.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The producer of the material’s genetic modification safety committee will consider the risk assessment to the environment appropriate for such a Class 1 activity. The Chair of the GM safety committee advises the inactivation or containment of the GMM’s in the infected animals is the responsibility of the waste producer who deposits the carcasses into hermetically sealed bags before transportation.
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<td>Name</td>
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<tr>
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<td>EAST AND SOUTH EAST</td>
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<td>CB22 3AT</td>
<td>ENGLAND</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Vice President Biochemistry
Vice President Medicinal Chemistry
Head of Applied Biology
Head of Chemistry
External advisor from Babraham Research Institute

<table>
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<tr>
<th>Laboratory</th>
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</table>

Other (please specify) Tick if confidential

Bacteriology Yes
Parasitology
Transgenic Birds
Microbiology Research Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity

Amura will dispose of its materials using services provided by the Babraham Bioscience Technologies ("BBT"). In summary- Category 1 waste will be disposed of in hermetically sealed and labelled 30L or 60L yellow boxes with yellow lids. Boxes will be labelled and logged (on clipboards) before collection and disposal by BBT. The labels must show: The BRC registration number (NFK 619), the Company (Amura), Building number and Date. The box must be logged on the clipboard provided before placing in the external yellow bins located outside the above BBT buildings. Manual handling guidelines should be observed by all staff moving the bins. BBT has contracted VetSpeed (Royston) to remove materials off-site, treat materials to inactivate material (autoclaving) and subsequent disposal of materials.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Amura has adequately and appropriately addressed the health and safety assessment in relation to:
i. operators and non-operators (e.g. visitors and non-scientific staff)
ii. environmental issues (e.g. site access, storage, waste management, etc.) required to fulfil its objectives.
Amura is advised to continually and actively review its procedures to ensure that the processes and associated risk! assessment remains relevant for the use intended.
### GM Centre Number: 998

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**Name**

LECTUS THERAPEUTICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

BABRAHAM RESEARCH CAMPUS

**Building**

BUILDING 260

**Road Name**

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB22 3AT

**Country**

ENGLAND

**Tel Number**

01223 499 050

**Fax Number**

01226 499 055

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

PREMISES CLOSED 08/03/2010

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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<th>Campus Estate or Research Centre</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee:

- Director of Biology (Management Representative)
- Head of Assay Development & Screening
- Senior Research Scientist Biology
- Biological Safety Officer
- Safety Manager

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential  


Yes

Only small scale liquid waste will be generated (cs litres at any one time). Liquid waste will be collected and inactivated using chlorine based disinfectant (eg. Chloros) prior to sink disposal.
Solid waste will either be collected as sharps solid waste and treated as described below, or will be collected in buckets lined with autoclave bags, which will be autoclaved in a cycle of 121 degrees Celsius for 15 mins, and will then be collected as hazardous waste by an external contractor. A fully calibrated autoclave will be used for the inactivation. Calibration will be carried out annually.
Sharp solid waste will be collected in sharpsafe bins and then sealed in a 60 litre burn-bins, which will be collected for off-site incineration by a hazardous waste disposal company.
Chlorination and autoclaving are accepted procedures which will result in complete killing.
The waste disposal company used would be either Grundon or Vetspeed.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment Y

Please enter comments of the GM safety committee on the risk assessment
attached risk assessment has been approved by the committee.
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Data at Which Additional Info Submitted

Date at Which Additional Info Submitted: 02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

We are using Imperial College’s GM safety committee. All GM risk assessments have been and will be examined by this committee

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<tr>
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<th>Parasitology</th>
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<th>Microbiology</th>
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**LIQUID WASTE**
Detail of type of waste — Liquid waste from cell culture
Inactivation — Chemical inactivation using Virkon. Freshly made Virkon will be made up with to give a final concentration & 1% (1000 parts per million of available chlorine) for at least 30 minutes.
Validation of treatment - Virkon has been independently validated to kill virus and a 5 minute exposure is enough to kill 5 to 6 logs of virus (Antiviral Research Volume 64, Issue 1, October 2004, Pages 27-33).

**SOLID WASTE**
Detail of type of waste - glass vials, rubber stoppers, plastic pipettes, cell culture flasks, 96 well cell culture plates
(other than - reusable glassware is soaked in 1% Virkon overnight, rinsed thoroughly, sent for washing and autoclaving)
Autoclave cycle — temp 131°C for 30 minutes.
Monitoring — monitored by chart recorder attached to the autoclave
Validation — no microbes can survive effective autoclaving. There will be an annual 12 point thermocouple testing of autoclave
Route of final disposal — incineration

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Below are the comments of the Imperial College GM safety committee. Our alterations are in bold
Following the discussion of your proposal entitled “Freeze d.ylnq of Adenovirus with GEP and detection in HEK 293 cell culture” (GM1C -01675.1), the committee request that the following amendments are made to the proposal prior to approval:

Reviewer I
Please clartI the classification, is the pmject a containment level I or 2?

containment level 1 - see Part I - 1.4

Reviewer 2
Part I
I agree that this is a classi GMO and therefore part 2 does not need to be submitted to the committee with the assessment. The part 2 assessment is still a valuable exercise for the research team.

Part 2
Hopefully there is another separate access to the office from a non lab area.
Unfortunately access to the lab and office share the same entrance

Part 3
a i. la Please state that the final concentration of virkon including dilution with waste liquid will be no less than 1%.

It is advised that yukon is not allowed to come into contact with culture media for longer than necessary as chlorine gas has been observed to be evolved after prolonged exposure. The manufacturers recommended minimum contact time is 1% under ideal conditions. Therefore I would recommend 30 minutes as a generally effective, but not overly long, contact time.

This has now been changed to include final concentration. Virkon exposure has been reduced to 30 minutes ff11 is easily available autoclaving using a properly validated waste autoclave is the preferred mute lbr solid waste as it gives more reliable complete sterilisation. Solid waste can form air pockets especially in pipettes where the liquid agent may not reach.

We have access to an autoclave and disposal of solid waste has been changed to using this route-- see 3.I.lb

3.2a It is common practice in many other labs to use closed lunch box style containers for transport of the flat culture vessels as they have been known to split without warning (suspect that they are damaged during shipping). The bottom of the lunch box also provides a handy spill fray during incubation.

Agreed - amended

3.5a Virken should always be used such that the final concentration after dilution with any waste is no less than 1%.

Amended - final concentration should be at least 1% 

3.5b Mien and to whom will you report the incident?

Amended - safety officer

3.8 please tick the box if what you are saying is true.

Box ticked

Reviewer 3
No requirement for part 2 of the form.

Part 3:

Please confirm whether chemical disinfection only will be used to inactivate solki GM waste, if autoclaving is going to be used, please complete 3.1. lb.

Autoclaving is now included in part 3.1.lb

Reviewer 4

part 3 section 3.2
description of transport of any viable GMM to autoclave

Amended - Plastics containing GMMs will be double contained in plastic bag (not sealed to allow steam penetration) which will be placed Inside a plastic box for transport to the autoclave.
### Project Additional Information

**Purposes of the contained use**
To improve stability of the vaccine components at elevated temperature in either a liquid or lyophilised presentation

**Recipient or parental organism**
Vero cells

**Host/vector system**
DEN-2 PDK-53 virus (highly attenuated Dengue type 2 backbone) which has demonstrated safety and efficacy in man and grown in Vero cells

**Origin & function**
Origins of the genetic material is from the remaining three Dengue serotypes (1, 3 and 4)

**Evaluation of foreseeable effects**
Immunofocus assay in Vero cells
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Please see attached Risk Assessment for full details of the waste management procedures.
Basically all liquid waste will be decontaminated by contact with freshly made up virkon - final concentration will be 1% (1000 parts per million of available chlorine) – for a minimum of 30 minutes contact time. Solid waste soaked in freshly made up virkon prior to autoclaving (Holding temperature of 121°C for 15 mins autoclave serviced regularly (arranged landlord) a copy of service record held by Stabilitech

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
3. RISK EVALUATION OF THE DENVAX VACCINE

The DENVax dengue vaccine represents an important potential tool in the fight to address an infectious disease that threatens over half of the world's population. Inviragen is addressing this challenge with a novel tetravalent genetically modified chimeric vaccine. The design of this vaccine is based on careful consideration of needing to provide immunogenicity against all four serotypes while ensuring safety to patients and the environment.

As outlined in Sections 1 and 2 of this document, significant effort has gone into developing and manufacturing an attenuated chimeric tetravalent vaccine that provides a strong neutralizing antibody response in several animal models. Data have also been generated showing the parent attenuated virus (DEN-2 PDK-53) is safe while generating a strong immune response in humans in Phase 1 clinical trials. Based on this proof-of-concept of potential safety and effectiveness, the scientific effort was expanded to include the other serotypes and the DENVax tetravalent vaccine is now approaching clinical trials for evaluation of safety and immunogenicity in human volunteers.

The modifications to the viruses were designed to address the balance required between effectiveness and safety to patients and the environment. These effects of these modifications can be summarized as follows:

- **Reduced replication:** The modified viruses do not replicate as efficiently as wild type dengue viruses in mammalian cells (e.g., Vero, LLC-MK 2), mosquito cells or in mosquitoes. In addition, the replicative potential for the viruses is reduced by >90% at temperatures characteristic of a mild fever (i.e., 39°C).

- **Reduced pathogenicity:** As demonstrated in the neonatal mouse neurovirulence data and the plaque size test, the pathogenicity of the modified viruses is significantly attenuated compared to wild type dengue viruses.

- **Retained immunogenicity:** The data demonstrating immunoprotein in animal models (e.g., mice and monkeys) and neutralization of viral infectivity in the Plaque Reduction Neutralization Test clearly show that attenuation of the viruses for safety was achieved without compromising the ability to neutralize the viruses in the vaccine with an effective immune response.

- **Since all four DENVax components share the identical attenuating mutations, recombination between vaccine strains cannot generate more pathogenic viruses.**

Extensive controls have been implemented to ensure the vaccine meets the above characteristics of reduced replicative potential, attenuated pathogenicity and retained immunogenicity. As outlined in Section 1, an exhaustive effort went into thoroughly characterizing the genotype and phenotype of the candidate DEN-2 PDK-53 vaccine virus. This same rigor was applied in development of the subsequent research grade ChiDEN-1-V, ChiDEN-2-V, and ChiDEN-3-V viruses. Thereafter, Pre-Master, Master and Working Virus Seeds and the ultimate tetravalent vaccine, which are produced under cGMP conditions by a highly reputable vaccine manufacturer, are thoroughly tested and characterized to ensure fidelity to the genotypic and phenotypic requirements of this attenuated vaccine.

The DENVax vaccine has been designed to meet the challenge of balancing safety and effectiveness. The temperature sensitivity and reduced replicative phenotype of the vaccine viruses in mammals and mosquitoes significantly reduces the potential for spread between vaccinated humans by mosquitoes. Furthermore, the reduced pathogenicity of the vaccine viruses and strong immunogenicity they stimulate ensure that any viral replication subsequent to vaccine administration will be very short-lived, non-pathogenic and minor in terms of magnitude.

In summary, this combination of features makes this candidate dengue vaccine well suited for clinical testing in humans as the safety risk for both humans and the environment has been considered and the vaccine has been designed such that there is virtually no environmental risk associated with use of this vaccine in humans.

### Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications
Data Premises Notified: 09/07/2018
(Originally)

Transferred from 1992 Regs?: No

Transitional Premises Class: NA

Data Premises Closed

Transitional Premises

Emergency Plan Required?: No

Non-GMMs: No

Withdrawn: No

Name

NHS GRAMPIAN

Name 2

Department

Campus Estate or Research Centre

Building

ABERDEEN ROYAL INFIRMARY

District

FORESTERHILL

Town

ABERDEEN

County

ABERDEENSHIRE

Postcode

AB25 2ZB

Country

SCOTLAND

Tel Number

01224 551121

Fax Number

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E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

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Give brief details of the genetic modification safety committee

University of Aberdeen Foresterhill Genetic Modification Safety Committee.

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<thead>
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**Other (please specify)**

Tick if confidential  

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Yes
Consumables shall be inactivated by autoclaving at 134°C for 10-15 minutes, which achieves a 100% kill, and then disposed of through normal hospital clinical waste routes. The autoclave is located within an accredited laboratory in the hospital and is calibrated annually by Health Facilities Scotland. A record is maintained of all autoclave cycles. This waste is collected by a specialist waste disposal operative (Healthcare Environmental Group) for appropriate disposal.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

None
## Data Premises Notified

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### Data Premises Closed
- **N**

### Transitional Premises Emergency Plan Required?
- **N**

### Non-GMMs Withdrawn
- **N**

### Withdrawn
- **N**

### Name

**GREAT ORMOND STREET HOSPITAL FOR CHILDREN NHS FOUNDATION TRUST**

### Name 2

### Department

### Campus Estate or Research Centre

### Building

### Road Name

**GREAT ORMOND STREET**

### District

### Town

**LONDON**

### County

**GREATER LONDON**

### Postcode

**WC1N 3JH**

### Country

**ENGLAND**

### Tel Number

**020 7405 9200**

### Fax Number

**0**

### E-mail

### HSE Division

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### Comments

### Date at Which Additional Info Submitted

**02/03/2022**
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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Give brief details of the genetic modification safety committee:

Joint UCUUCLH/RFH Genetic Modification Safety Committee (clinical trials). The GMSC comprises a number of academic clinicians and clinical trials people from the 3 named centres with experience in the clinical application of GMOs. On such academic clinician from GOSH is already on this committee and the responsible person named below will also attend. The committee is a virtual one such that RAs are dealt with by email with face to face meetings as required.
**For activities involving GMMs, describe the waste management measures which will apply to the activity**

For routine cleaning all surfaces are wiped down with 70% ethanol after use. Liquid GMM waste is first disinfected using Sanichlor (effervescent chlorine tablets) to give a final concentration of 2500ppm chlorine overnight before being disposed of down a laboratory sluice. This is a standard procedure and viability is below detectable levels. 100% No specific testing for vector contamination. Environmental monitoring as routine for this laboratory. Waste will be taken by the dedicated personnel to be autoclaved at Camelia Botnar Laboratories or the Institute of Child Health (back-up) and this process logged in the batch manufacturing record. Solid materials are double-wrapped then autoclaved. The autoclaving cycle is carried out at 134 degrees celsius for 3 minutes. All sharps are placed in puncture-proof containers and disposed of through the central collection service for contaminated waste. All sharps are placed in puncture-proof containers and disposed of through the central collection service for contaminated waste. Waste is incinerated via a clinical waste process stream.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

**Please enter comments of the GM safety committee on the risk assessment**

There has been a GMSC at UCL for many years with a long history of reviewing GM risk assessments and interaction with the HSE for approvals. The new committee has continuity of personnel and has separated the risk assessments with a clinical impact. Professor Wasim Qasim is on the committee and has a more than 10 years experience of submitting GM Risk Assessments for review.
### GM Centre Number: 1065

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### Name

BRIGHTON & SUSSEX UNIVERSITY HOSPITALS NHS TRUST

### Name 2

**Department**

### Campus Estate or Research Centre

ROYAL SUSSEX COUNTY HOSPITAL

### Road Name

EASTERN ROAD

### Town

BRIGHTON

### District

SUSSEX

### County

BN2 5BE

### Country

ENGLAND

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**HSE Division**

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**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Sussex University Biological Safety Committee

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| Other (please specify) | | | | | Tick if confidential
Contaminated needles, syringes, used/unused vials and swabs will be discarded in a designated labelled sharps box in the treatment area. The sealed box will be stored in the designated treatment area or approved clinical waste area until collection for incineration. Drug destruction certificates will be completed. The local Labs will analyse the routine bloods and dispose of waste according to local procedures. The TRIOC protocol requires all staff who handle trial drug to wear gloves, apron, goggles and mask. Disposable PPE is recommended which will be available in pharmacy, in the treatment area and in spillage kits. Waste will be disposed of by incineration or other such locally approved method for GM waste. All waste potentially contaminated with GM culture material must be rendered non-viable (inactivated by a validated means) prior to leaving the site for final disposal. This includes GM material disposed of at the preparation of the gene therapy product stage, administration to patient stage, waste generated when removing and analysing samples.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Contaminated needles, syringes, used/unused vials and swabs will be discarded in a designated labelled sharps box in the treatment area. The sealed box will be stored in the designated treatment area or approved clinical waste area until collection for incineration. Drug destruction certificates will be completed. The local Labs will analyse the routine bloods and dispose of waste according to local procedures. The TRIOC protocol requires all staff who handle trial drug to wear gloves, apron, goggles and mask. Disposable PPE is recommended which will be available in pharmacy, in the treatment area and in spillage kits. Waste will be disposed of by incineration or other such locally approved method for GM waste. All waste potentially contaminated with GM culture material must be rendered non-viable (inactivated by a validated means) prior to leaving the site for final disposal. This includes GM material disposed of at the preparation of the gene therapy product stage, administration to patient stage, waste generated when removing and analysing samples.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

GMM defined as hazard group 1. Risks to human health from vector or gene insert are low. Risk of transfer to other organisms, animals or the environment are low. Drug tracking, storage and handling measures acceptable. Waste disposal measures appropriate. PPE (gloves, apron, mask and goggles) required for pharmacy and administering staff. Pregnant staff should not handle the drug. An appropriate emergency plan is in place.
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Salford Royal NHS Foundation Trust genetic modification safety committee - The committee consists comprises the following members: Chair - Principal Clinical Research Lead, Director of R & I operations, Biological Safety Officer, Pharmacist, R&I Nurse Delivery lead, carious other clinical/research staff, other staff as appropriate.

The remit of the committee is to advise on the adequacy of any risk assessments undertaken relating to GM activities at this site.

The committee will meet at appropriate intervals based on the level of activity of relevant work.

In order to fulfil this requirement the committee will:

- Receive and review each proposal for genetic modification work and judge the adequacy of the risk assessment and the identification of appropriate containment and other risk control measures.
- Periodically review risk assessments depending on the level of activity and level of risk.
- Act as a source of advice on the preparation of risk assessments.
- Consider any accidents or incidents relevant to genetic modification work.
- Consider and recommend arrangements for health monitoring or surveillance where appropriate.
- Act as a source of information relating to GM activity.
- Assist and advise the Authorised Person (in the first instance biological safety officer) to discharge the responsibilities for statutory notifications and record keeping.
- Provide an annual report to the relevant assurance committees and risk management team.
- Liaise with the University of Manchester where work at site is being conducted by University of Manchester employees at this site.

The committee has now met for the first time and will meet at least annually or as needed depending on level of activity on the site.

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<th>Glass House</th>
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Level 1 (GMMs)  
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Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  
Clinical and pharmacy areas

Tick if confidential

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<td>Plants</td>
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Other(s)

The activity to be undertaken is a clinical trial of an advanced therapeutic agent as defined by the

For activities involving GMMs, describe the waste management measures which will apply to the activity

Any disposable materials used will be immediately disposed of by incineration as clinical waste.
Syringes, cannulas and all contaminated procedure related material used in implantation will be placed in a single patient use, procedure specific biohazard sharps box for disposal by incineration. This will be a size 15 litre or 25 litre capacity to accommodate all related waste. All contaminated equipment will be cleaned as per local guidelines.
Waste management will be in line with local disposal procedures as used for clinical waste.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The genetic modification committee met and thoroughly discussed the risk assessment. Various suggestions were made and it was agreed that with the inclusion of these suggestions, the risk assessment was an appropriate reflection of the level of risk involved.
The Genetic Modification Committee will review future risk assessments relating to proposed studies.
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**Name 2**

**Department**

**Campus Estate or Research Centre**

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**Building**

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**District**

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**Comments**

**Date at Which Additional Info Submitted**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

4 members from York Teaching Hospital NHS Foundation Trust (Clinical lead for Research, Consultant Microbiologist, Chief Pharmacist, Research Adviser) and 1 external member (Biological Safety Officer with GMO experience from University)

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Other (please specify)  

Clinical Research Trial(s) involving a Class 1 GM, presenting no/negligible risk to human health ...

Tick if confidential  

Bacteriology  

Parasitology  

Transgenic Birds  

Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

Unused product will be returned to the study sponsor who will provide instructions, packaging and labels for safely returning vials for inactivation and disposal. Used vials will be securely held in quarantine until reconciliation has taken place by the CRA then disposed of via a documented local procedure. Contaminated equipment or packaging will be placed into a clearly labelled sharps bin or clinical waste disposal container, sealed and incinerated.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The purpose of the assessment (from a GMSC perspective) is to consider risks to staff and other workers who could be exposed to the GM vaccine. The conclusion of the assessment is acceptable (a Class 1 GM trial activity, presenting no / negligible risk to human health and the environment, requiring the application of Containment Level 1 measures to limit contact with humans / environment) on the basis that the trial involves use of a replication deficient recombinant lymphocytic choriomeningitis vaccine vector (rILCMV) with a safe history of use, and use of vaccine antigens with no inherently harmful properties.
### GM Centre Number: 1140

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#### Name
- **BOURNEMOUTH UNIVERSITY**

#### Name 2

**Department**

#### Campus Estate or Research Centre

- **Campus Estate or Research Centre**

#### Road Name
- **FERN BARROW**

#### District
- **WALLISDOWN**

#### Town
- **POOLE**

#### County
- **DORSET**

#### Postcode
- **BH12 5BB**

#### Country
- **ENGLAND**

#### Tel Number
- **01202 965001**

#### Fax Number
- **01202 965255**

#### E-mail

#### HSE Division
- **WALES AND SOUTH WEST**

#### Comments
- **coin case 4299740 site 1959959**

#### Date at Which Additional Info Submitted

- **02/03/2022**
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Y
Membership
The membership of the School of Applied Sciences GMSC depends on the nature of the work to be undertaken. But the committee consists of the following core members or their delegated representatives. Additional members may attend meetings if their expertise are required, e.g., medical doctor, veterinary surgeon, or University Health & Safety Advisor.

1. Deputy Dean of School - management representative
2. Director of Operations - management representative
3. Academic representative and health & safety specialist
4. Technical staff representative
5. UCU representative
6. Unison representative
7. School Health & Safety Co-ordinator and Biological Safety Officer
8. Principal investigator

Responsibilities of the committee
a) To review new risk assessments relating to work involving the genetic modification of organisms (GMOs) in order to judge the adequacy of the risk assessment to protect the health of humans and the environment.

b) Consider whether the identified containment and other control measures are appropriate and available.

c) To advise on the requirements for training, equipment, facilities, and other resources necessary to undertake work involving the use of GMOs.

d) To review existing risk assessments if there is cause to do so.

e) Consider and approve reports from any inspections made of facilities or any compliance audits.

f) To keep under review the health and safety measures, such as local rules and standard operating procedures, relating to work involving the use of GMOs.

g) To scrutinise accidents or incidents related to work involving GMOs to ensure that control measures are appropriate to protect individuals and the environment.

h) To keep accurate records of the Committee's meetings. These must be consisting of:
   - Date of Meeting
   - Attendees
   - Apologies/absences
   - Title of the project(s) submitted to the committee, principal investigator, department, division, and location of work to be carried out in.
   - Reference number assigned to the project
   - Agreed classification of the project and whether notification to the HSE is required.
   - Containment level required
   - Any further control measures to be taken by the personnel involved with the project.
   - Amendments to be added to the risk assessment form.
   - Comments and concerns raised by the GMSC members regarding the project.
   - Dissenting opinion and agreed outcome.
   - Actions to be taken and the named responsible person.

h) The Biological Safety officer is responsible for keeping all records of the committee.

Frequency of meetings
Little work involving GMOs occurs in the school. Consequently, the Committee does not meet on a regular basis. The Biological Safety Officer will convene the committee when they have received GMO risk assessments or there is another reason for the committee to convene. However, the committee will meet at least once a year to review the work involving GMOs/GMMs within the School, including points B through G in section 3 above.

| Laboratory | Animal Unit | Growth Room | Glass House | Large Scale |
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste will be managed by autoclaving the GMMs at 126 for 15 minutes, before disposal of the material as clinical waste via a commercial contractor. Autoclave indicator tape will be included in each batch to the required conditions have been achieved.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Review risk assessment for genetically modified microorganisms ref. GMM/01

Risk assessment GMM/01

The committee was satisfied that the strains to be used were derived from E.coli strain K12 and that the strains themselves were not considered pathogenic (i.e. conforming to class 1) The committee accepted that the proposed modifications should not cause increased pathogenicity though the production of toxic gene products, altered metabolism and that the proposed modifications should not alter the allergenicity of the strains. The committee also accepted that the proposed modifications should not alter the allergenicity of the strains. The committee also accepted that the modified strains would still come under class 1 and that class 1 containment was adequate. The committee was satisfied that the risk to the health of humans or plants and animals in the environment was negligible. The facilities, staff training and containment and disposal measures in place were also considered acceptable. The risk assessment was therefore accepted without change.

Risk assessment GMO/01 & GMO/02

In the case of both risk assessments, the committee discussed the techniques, genes involved, the containment measures and possibility of the modifications causing increased harm to human health though the production of toxic gene products, altered metabolism and increased allergenicity. The committee discussed the potential for modified organisms to cause harm to the environment and concluded that the proposed modification should pose no threat to native species. The committee also accepted the facilities, expertise of staff, safety and containment measures were adequate, But asked that a second laboratory be added to the risk assessment as it was foreseen that the controlled environment facilities in that lab (room DG43B) would be required.

The committee agreed that the work involved class 1 GMMa and non-notifiable GM animals amd, therefore, new premises notification must be made to the HSE before the work outlined in all three risk assessments could take place.
<table>
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| LANCASHIRE  
TEACHING  
HOSPITALS  
NHS  
FOUNDATION  
TRUST | ROYAL  
PRESTON  
HOSPITAL | AVONDALE  
UNIT | SHAROE  
GREEN LANE | PRESTON | PR2 9HT | N |

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Tick if confidential

Give brief details of the genetic modification safety committee

Advice and support received from the Biological Safety Officer at The Clatterbridge Cancer Centre NHS Foundation Trust.

<table>
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<td>Other (please specify)</td>
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Bacteriology  
Parasitology  
Transgenic  
Birds  
Microbiology  
Research
All sharps that have been in contact with the vaccine or vaccination site will be disposed of into sharps bins provided by Stericycle and all materials used will be disposed as clinical waste. All waste will be immediately placed into a suitable receptacle (e.g. sharps into a sharps box, swabs and dressings into a clinical waste bag) which will be located within the area where the GMO is being prepared. The clinical waste will be double bagged and tied before leaving the clinical area where the vaccine is being delivered. Sharps boxes will be locked before leaving the clinical area.

Sharps boxes and waste bags will be placed into a dedicated lockable GMO clinical waste bin and stored within a locked room in the NIHR Lancashire Clinical Research Facility (LCRF) until collected by the licensed waste contractor (Stericycle). Only trained staff will have access to the key for this lockable bin. This include any waste or sharps created to manage an accidental spillage in pharmacy or on transport from pharmacy to the LCRF. No GMO waste will enter the Lancashire Teaching Hospitals waste streams.

All GMO waste will be collected and removed from the sites by contractors (Stericycle) who are registered for handling GMO material. The waste management company contracted will be ultimately responsible for the disposal of the material as clinical waste (via incineration or any other validated means, according to current guidelines and regulations).

NOTE: Waste which has been fully chemically deactivated does not require further deactivation prior to disposal, e.g. swabs and paper towels used to mop up GMO spills using chlorine based disinfectants should still be treated as GM waste, items fully immersed in disinfectant do not need to be further deactivated prior to disposal.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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| **Data Premises Notified (Originally):** | 15/01/2020 |
| **Transferred from 1992 Regs?:** | N |
| **Transitional Premises Class:** |  |
| **Data Premises Closed:** |  |
| **Transitional Premises:** | N |
| **Emergency Plan Required?:** |  |
| **Non-GMMs:** | N |
| **Withdrawn:** | N |

**Name**
UNIVERSITY HOSPITALS OF LEICESTER NHS TRUST

**Name 2**

**Campus Estate or Research Centre**
LEICESTER ROYAL INFIRMARY

**Building**
LEVEL 3, BALMORAL BUILDING

**Road Name**
INFIRMARY SQUARE

**District**

**Town**
LEICESTER

**County**
LEICESTERSHIRE

**Postcode**
LE1 5WW

**Country**
ENGLAND

**Tel Number**
0300 303 1573

**Fax Number**
0

**E-mail**
blank

**HSE Division**

**Comments**

**Date at Which Additional InfoSubmitted**
02/03/2022
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- **Y**

**Give brief details of the genetic modification safety committee**

The Genetic Materials Committee meets once a month to discuss feasibility of any advanced therapy, genetic, cell therapy studies as well as early phase clinical trials. The representatives include:

Director of Safety and Risk, Health and Safety Services Manager, Health, Safety And Local Security Specialist, Pharmacists, Deputy Chief Pharmacist, Assistant Director, Research & Development, Head of Research Operations, Director Of Research & Development, Lead Nurse for Research, HOPE Senior Finance & Contracts Manager, Hope Unit, Manager, Lead Biomedical Scientist, Biomedical Safety Officer. Research team are invited to discuss and present potential studies.

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Non-microbial

Other (please specify)  

Tick if confidential  

Bacteriology  

Parasitology  

Transgenic  

Microbiology  

Research  

Virology  

Transgenic  

Animals  

Transgenic  

Fish  

Gene Therapy  

Mycology  

Transgenic  

Invertebrates  

Transgenic  

Plants  

Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Empty vials and used vials with needles and syringes, components used for collecting body fluid sample will be put in to a purple lid bin and sealed (as per local Trust policy). PPE and gauze will be put in to orange clinical waste bins and sealed for incineration following participant visit. Not inactivated at site prior to disposal is necessary. Sealed purple lid bins and orange clinical waste bags are incinerated. Standard disposal of samples by standard SOPs by local laboratories. Central laboratories will perform the safety analysis of complete blood count with differential, liver function test, renal function test and efficacy analysis (CMV PCR). Central laboratories will perform immunogenicity analysis. Central laboratories will dispose residual biosamples per their SOPs for disposing of biohazardous material.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

The information available regarding the GMM supports the proposed Class 1 risk categorisation. On this basis containment level 1 with no additional measures is the appropriate containment necessary to control the risk. The arrangements described are entirely in line with containment level 1.

It is difficult to envisage any process by which this replication incompetent vaccine virus could prove any infective risk to the individual to whom it is administered to or to staff or other patients or to non-human animals. The very low risk that the replication incompetent vaccine virus could in theory reassort with wild type virus in a wild rodent reservoir and regain the ability to produce infectious virus is not in itself of significant consequence as there is no basis for the resulting virus to be of higher pathogenicity than wild type virus and the UK is already an endemic area for LCMV.

No special precautions are required for transport or disposal over and above what is normally done in clinical areas. Waste can be processed as clinical waste. No special precautions are required for dealing with spillage or accidents.
**GM Centre Number: 1230**

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**Name**

SHEFFIELD TEACHING HOSPITALS NHS FOUNDATION TRUST

**Name 2**

**Department**

**Campus Estate or Research Centre**

TRUST HEADQUARTERS

**Road Name**

8 BEECH HILL ROAD

**Building**

**District**

**Town**

SHEFFIELD

**County**

**Postcode**

S10 2SB

**Country**

ENGLAND

**Tel Number**

0114 2269696

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y
- Give brief details of the genetic modification safety committee
Purpose:
1. To ensure that the structures and processes are in place for the safe conduct of gene therapy research on human subjects at Sheffield Teaching Hospitals NHS Foundation Trust.
2. Interface with the Gene Therapy Advisory Committee and comply with the recommendations of other agencies with statutory responsibilities including the Medicines and Healthcare Products Regulatory Agency and the Health & Safety Executive.
3. This Group will not consider the ethical or the scientific merits of the research projects which would have been assessed by the Research Ethics Committees and the Gene Therapy Advisory Committee.
4. This Group will not consider the Clinical Trials Application assessments by the competent authority which would have been assessed by the MHRA in the UK.

Accountability:
1. To report to the Director of R&D of STH
2. Informing the UoS Faculty MDH Research Committee
3. See reporting structure below

Frequency of meetings:
• Virtual meeting on an as needed basis for approval of studies
• Physical Meeting on an annual basis to review procedures

Areas of Expertise:
Pharmacy, including:
• Storage
• Disposal
• Staff Training
• SOPs
Laboratories, including:
• Virology
• Transport of specimens
• Personal Protection Equipment
• Staff Training
• SOPs
Waste Management, including:
• Spillages
• Disposal
• Personal Protective Equipment
• Staff Training
• SOPs
Patient pathway and staff involvement, including:
• Patient facilities
• Transport of specimens
• Infection Control
• Personal Protection Equipment
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For activities involving GMMs, describe the waste management measures which will apply to the activity:

Storage of the modified herpes simplex virus HSV1716 at -70°C
Inactivation Methods:
The vials will be injected with 2% VIRKON solution and left for 1 hour to deactivate the virus. 2% VIRKON will be aspirated into the three-way valve and Rocket IPC drainage line. All the above equipment will be left in contact with the VIRKON for at least 1 hour to inactivate the virus.

After deactivation of residual virus the vials, needle, syringe three-way valve and Rocket IPC drainage line will be disposed of in a sharps box and local policy for disposal of this type of waste will be followed. All other solid decontaminated waste (e.g paper towels and gloves) will be disposed of in clinical waste bags.

Degree of kill:
When used as per the manufacturer's instructions, Virkon will provide complete inactivation of HSV1716.

Proposed process testing/monitoring measures:
Not required as validated by manufacturer.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

1. Waste Manager - In reference to page 9 of "The HSV1716 Handling and Administration Instructions Booklet", comments to point 2) are:
a) Disposal is to be carried out in accordance with the STH Waste Strategy and Policy
b) Vials, needles and syringes are to be disposed of in an approved sharps container with a yellow body and lid.
c) The 3 way Rocket IPC will contain a large volume of loose liquid and should be disposed of in a hermetically sealed container.  
d) The Herpes simplex virus not in a cultured form is classified as a category B infectious substance and should be disposed of as infectious Clinical waste for Alternative Treatment (orange lidded container).
e) Soiled PPE used in process should be disposed of as Infectious Clinical Waste In Orange bags.

2. Patient Safety Manager - potential risk to staff from splashes etc, what staff should do if they did get a splash to their eye/mouth etc - it shouldn't happen but if it does what are the contingencies.

Other reviewers provided confirmation of their review only. The above comments were followed up until resolution
Data Premises Notified (Originally) 01/04/2019

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name
ROYAL CORNWALL HOSPITALS NHS TRUST

Name 2

Department

Campus Estate or Research Centre
RESEARCH MANAGERS OFFICE (F50)

Building
KNOWLEDGE SPA

Road Name

District
TRELISKE

Town TRURO

County

Postcode TR1 3LG

Country ENGLAND

Tel Number 01872 256422

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>TRURO</td>
<td>TR1 3LG</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Microbiology Lead Scientist, Research Lead Pharmacist, Chief Biomedical Officer, Research Governance Lead, Research Manager, Research Lead Nurse (Matron)

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Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

Virology Transgenic Animals Transgenic Fish Gene Therapy

02/03/2022
The medicinal product is a mouthwash containing the modified bacterium which is non-infectious, non-pathogenic, non-replicating and has minimal survival capabilities outside of an artificial culture environment. After rinsing their mouths patients are advised to discard into the sink or toilet.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

| Other(s) | The storage and dispensing of a genetically modified medicinal product |

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
GM Centre Number: 3001

Data Premises Notified: 15/02/2008
(Originally)

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

VEOLIA ENVIRONMENTAL SERVICES

Name 2

Department

Campus Estate or Research Centre

Road Name

BRIDGES ROAD

District

Town

ELLESMERE PORT

County

CHESIRE

Postcode

CH65 4EQ

Country

ENGLAND

Tel Number

0151 348 5000

Fax Number

0151 348 5206

E-mail

HSE Division

NORTH WEST

Comments

Date at Which Additional Info Submitted

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities — **Y**

Give brief details of the genetic modification safety committee

- Comprises Plant Materials Manager, Control Operator, Safety representative, Health and Safety Advisor, Technical Control Manager.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 1 (GMMs)</td>
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</table>

Bacteriology  | Parasitology | Transgenic Birds | Microbiology Research |
|-------------|--------------|------------------|-----------------------|
### For activities involving GMMs, describe the waste management measures which will apply to the activity

Disposal via High Temperature Incineration

**Tick to confirm that you are attaching a summary of the risk assessment**

Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**


---

### Project Ref 3001/08.1

**Date Ackn'd:** 15/02/2008  
**Date Project Ceased:** 04/03/2009  
**Withdrawn:** Y

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**Non-GMM Consent Granted**  
Not Applicable

**Project notified under transitional arrangements**  
N
Project Additional Information

Purposes of the contained use

Destruction of GMO via high temperature incineration

Recipient or parental organism

Information to be provided by waste producer and new risk assessment conducted for any new materials. Current requirement is for material contaminated with attenuated vaccine viruses (influenza). As attenuated vaccines they should not represent a hazard to human health so should be classified as below hazard group 2 in the ACDP Approved list of biological agents. In any event, in a complete breach of containment the virus would not survive long in the environment which greatly reduces the risk of infection. (evaporation rate of influenza-containing droplets under ambient conditions ranges from 3 to 30 seconds and Flumist undergoes a 100-fold loss in infectious titre following desiccation).

Host/vector system

Information to be provided by waste producer and new risk assessment conducted for any new materials. Current requirement is for Flumist vaccines which do not cause disease symptoms associated with the wild-type influenza virus (they actually prevent infection). If the virus did manage to survive in the environment, a simultaneous infection of the same cell with vaccine virus and wild-type virus would be required to transfer vaccine gene sequences to the wild-type strain. This event would not be expected to have adverse consequences, studies have shown the attenuated reassortants would be less virulent than the wild-type virus.

Bacterial host or viral vector studies have shown no environmental hazards — the plasmid rescue seed preparation method encodes the same human influenza viral proteins that are contained in seeds prepared using classical non GMO techniques.

Origin & function

Information to be provided by waste producer and new risk assessment conducted for any new materials. Current requirement results in the production of only unmodified human influenza genes — no GM material will be present in the material to be destroyed. It is the harvesting method — plasmid rescue technique — that brings this material under the umbrella of these regulations Material is intended for vaccine production

Evaluation of foreseeable effects

Information to be provided by waste producer and new risk assessment conducted for any new materials. Current requirement - Flumist vaccine seeds made either by non-GMM and GMM (the classical reassortment or by plasmid rescue) are genetically, biologically, and phenotypically comparable and do not contain detectable levels of plasmid cell culture DNA. Therefore there should be no adverse effects from the material resulting from this process.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Material will be presented in sealed containers which will be incinerated without being opened.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All materials will be presented to the facility in sealed containers and will not be opened. We therefore wish to be exempted from the following schedule 8 measures:

Schedule 8 part II table 2:
- General — Paragraphs 1 — 9
- Equipment — Paragraphs 10 — 14
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containers will be incinerated reaching a temperature of 1300°C using our High Temperature Incinerator, achieving a 100% degree of kill. Ultimate form will be vitrified slag and ash.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The risk assessments have been carried out by the plant materials manager and safety advisor and cross referenced to relevant safety instructions and risk assessments.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L3 L4</td>
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**Name**

INSPIRALIS LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

LABORATORY 253

**Road Name**

NORWICH RESEARCH PARK

**District**

COLNEY

**Town**

NORWICH

**County**

NORFOLK

**Postcode**

NR4 7UH

**Country**

ENGLAND

**Tel Number**

01603 450924

**Fax Number**

01603 450923

**E-mail**

contact@inspiralis.co.uk

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022

Page 12452 of 15326
### Premises Addresses

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<th>Building</th>
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<th>District</th>
<th>Town</th>
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<td>253 NORWICH BIONCUBATOR</td>
<td>NORWICH RESEARCH</td>
<td>COLNEY NRWICH</td>
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<td>NR4 7UH</td>
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- [Y]

**Give brief details of the genetic modification safety committee**

- Composition
- Director of Inspiralis
- Director of Inspiralis
- Non exec director of Inspiralis and scientific consultant. Head of Dept. Biological Chemistry, John Innes Centre, Norwich.
- Head Support Services, John Innes Centre, Norwich

<table>
<thead>
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<tr>
<td>Yes</td>
<td></td>
<td></td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid waste will be placed in unsealed bags placed in lidded but unsealed plastic boxes and removed from the laboratory to the John Innes Centre central autoclave service where it will be sterilized. The autoclave cycle will include heating the material to 120°C, for 15 minutes to ensure 100% kill for all material. Liquid waste and contaminated glassware will be treated with hycolin disinfectant, diluted according to the manufacturers instructions, overnight at room temperature. An initial check will be carried out the first time the process is carried out to check that no culturable cells remain before disposal. For low level risk this check will be repeated on an annual basis to ensure that the procedure ensures a 100% kill. Higher risk work may require more frequent monitoring.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

Assessment approved by committee. No other comments.
**GM Centre Number: 3003**

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**Name**

EUROFINS INTEGRATED DISCOVERY UK LTD

**Campus Estate or Research Centre**

FYFIELD BUSINESS & RESEARCH PARK

**Building**

BUILDING 025

**Road Name**

FYFIELD ROAD

**District**

ongar

**Town**

ESSEX

**County**

CM5 0GS

**Postcode**

ENGLAND

**Country**

**Tel Number**

01277 367000

**Fax Number**

01277 367 099

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee (GMSC), is a subcommittee of the Selcia Health and Safety Consultative Committee, and consists of:

1. GM Biological Safety Officer (GMBSO) (Chair).
2. Selcia Health and Safety Officer (Secretary).
3. A General Staff Representative - to represent all persons who have access to genetic modification facilities e.g. technical staff, research workers, ancillary workers, students etc.
4. A member of the Selcia Management Team (General Manager and Head of Bioanalytics and Discovery Technologies to alternate).
5. Where necessary, a Technical Expert(s) - to provide expertise on particular work areas, e.g. on viral vectors, medical or environmental aspects.

The GMSC will meet six times a year, just prior to the main Selcia Health and Safety Consultative Committee. The GMBSO will circulate and archive written minutes of each meeting to the GMSC, the Selcia Health and Safety Consultative Committee, affected employees and the Management Team. A copy of the minutes will be posted on the Selcia Health and Safety Consultative Committee notice board in the tearoom.

The company GMBSO will chair the GMSC to ensure consistency in approach and outcome of risk assessments and will present a summary of key issues to the Selcia Health and Safety Consultative Committee at each meeting. The company’s notification for genetic modification work will be submitted to the HSE on this basis.

The GMBSO may refer matters to the Company Doctor and Occupational Health Adviser where appropriate.

The GMSC will advise on risk assessments and ensure that:

1. Proper and valid assessments have been made of the risks to human health and safety and to the environment
2. Satisfactory decisions about the appropriate containment and control measures have been made
3. The approach to risk assessment is in accordance with the guidance provided by ACGM taking into account the parameters detailed in the Regulations.
Live GM organisms must be inactivated by validated means. Usually, solid VirkonTNII (available from Antec International Ltd) will be added, to aqueous biological waste, to obtain a percentage of VirkonfM (usually 1%) that will render the OMO inactive. Alternatively media waste will be aspirated into Duran bottles containing TriGene (Medichem International). For each new cell line, the degree of kill will be determined at the first use of a cell line, either with a haemocytometer, after Trypan Blue staining, or with a NucleocounterTM (Chemometec). Waste will be treated overnight with the chemical agent before disposing of via a designated sink with copious amounts of water. Note: Detergent treated waste will not be autoclaved. Untreated aqueous biological waste, such as used culture media, may also be aspirated into 5L glass bottles and autoclaved2 on a regular basis to minimise build up of adventitious contaminants. Autoclaved aqueous waste will be disposed of down a designated sink with copious amounts of water. For each new cell line, the degree of kill will be determined after autoclaving, by counting the number of viable cells remaining. This will either be with a haemocytometer, after Trypan Blue staining, or with a NucleocounterTM (Chemometec). The degree of kill is expected to be 100%, if this is not the case, the autoclave cycle conditions will be modified to obtain 100% kill.

Plastic waste
Plastic waste such as disposable culture flasks, pipettes, tips and centrifuge tubes will be placed into appropriate autoclave bags (transparent with blue writing) and autoclaved.
autoclaved2 The autoclaved waste will then be treated as clinical waste: autoclaved bags will be put into yellow bags and, when full, the bag will be sealed with a numbered tag. The tag number will be written in the waste management book and on the print outs corresponding to the autoclaved waste. Details of the waste type will also be put in the waste management book. The yellow bags will be placed in a designated wheelie bin and taken to the waste store to be picked up by the designated waste contractor.

All empty reusable plasticware or glassware will be immersed in 1% VirkonTM overnight, after discarding down the designated sink with copious amounts of water; the plasticware will be rinsed and washed as normal.

Sharps

The use of sharps will be minimised, where possible. Hypodermic needles, glass pipettes and scalpels etc must be placed in square yellow sharpsafe bins, autoclaved1 then treated as clinical sharps waste. The bins will be numbered and details of the contents recorded in the waste management book. The sharps bins will be placed in a designated wheelie bin and taken to the waste store to be picked up by the designated waste contractor.

1. VirkonTM: “The Ultimate Broad Spectrum Virucidal Disinfectant” - independently proven effective against all seventeen virus families and bacteria and fungi including Mycoplasmae, Salmonallae, E. coli, Candida and Aspergillus.
2. Waste will be autoclaved at 121°C for at least 30mm. A printout generated for each run, will be recorded and filed. The autoclave has an internal load sensor to ensure the actual load reaches the required temperature. The autoclave will be serviced every 6 months and calibrated annually.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment GMRAOO 1 was approved unanimously by the Genetic modification Safety Committee on 28.02.08, There were no further comments.

Project Ref 3003/08.1

Date Ackn'd
07/04/2008

CU2 Project Title
The establishment, culture and use of GM and non-GM cell lines in cell-based assays to characterise drug activity.

Class
Class 2

CultureVol
Consent Granted
1-50 Litres

Not Applicable

 Withdraw
N

Tick if notifying a connected programme of work
N

Historical Significant Changes

Historical Date of Additional Info
02/03/2022
### Project Additional Information

#### Purposes of the contained use

- To maintain a small-scale (<8 litres) cell culture of GM cell lines for routine culture.
- To maintain small-scale (<100ml) cryopreserved samples of GM cell lines.
- To perform studies to determine the effect of compounds/reagents/drugs on GM cell lines using cell-based assays, such as cell viability, ELISA assays, adhesion and migration assays.

#### Recipient or parental organism

HCE-2 [50,Bl1 is a commercially available GM cell line, which can be purchased from the American Type Culture Collection (Number CRL-1135). It is a human corneal epithelial cell line generated by immortalisation of a primary culture of normal corneal epithelium with adenovirus I2-SV4O hybrid virus. This commercially available, GM cell line is epithelial in origin but contains Adeno-I2/SV-40 viral sequences. See attached risk assessment.

#### Host/vector system

See above and attached risk assessment

#### Origin & function

The aim is to perform studies to determine the effect of compounds/reagents/drugs on this cell line using cell-based assays, such as cell viability, ELISA assays, adhesion and migration assays. See attached risk assessment.

#### Evaluation of foreseeable effects

It is possible that accidental injection of the cells may be tumorigenic or cause an allergic or autoimmune response in humans although the risk of this happening is quite low. It is not considered to be pathogenic in humans, and would be expected to have limited ability to survive outside of the culture medium. The likelihood of the GMO getting into the environment is very low.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

#### For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Treatment of Aqueous Waste**

Live GM organisms must be inactivated by validated means. Usually, solid Virkon®MI (available from Antec International Ltd) will be added, to aqueous biological waste, to obtain a percentage of VirkonTM (usually 1%) that will render the GMO inactive. Alternatively media waste will be aspirated into Duran bottles containing TriGene (Medicem International). For each new cell line the degree of kill will be determined at the first use of a cell line either with a haemocytometer, after Trypan Blue staining, or with a NucleocounterTM (Chemometec). Waste will be treated overnight with the chemical agent before disposing of via a designated sink with copious amounts of water. Note: Detergent treated waste will not be autoclaved.

Untreated aqueous biological waste, such as used culture media, may also be aspirated into 5L glass bottles and autoclaved2 on a regular basis to minimise build up of...
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Plastic waste
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All empty reusable plasticware or glassware will be immersed in 1% VirkonM overnight, after discarding down the designated sink with copious amounts of water; the plasticware will be rinsed and washed as normal.

Sharps
The use of sharps will be minimised, where possible. Hypodermic needles, glass pipettes and scalpels etc must be placed in square yellow sharpsafe bins, autoclave& then treated as clinical sharps waste. The bins will be numbered and details of the contents recorded in the waste management book. The sharps bins will be placed in a designated wheelie bin and taken to the waste store to be picked up by the designated waste contractor.

1. Virko11: "The Ultimate Broad Spectrum Virucidal Disinfectant" - independently proven effective against all seventeen virus families and bacteria and fungi including Mycoplasmae, Salmonallae, F. coli, Candida and Aspergillus.

2. Waste will be autoclaved at 121°C for at least 30mm. A printout generated for each run, will be recorded and filed. The autoclave has an internal load sensor to ensure the actual load reaches the required temperature. The autoclave will be serviced every 6 months and calibrated annually.

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The risk assessment GMRA002 was approved unanimously by the Genetic modification Safety Committee on 28.02.08. Th1 were no further comments.

Project Containment

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**Name**

F-STAR BIOTECHNOLOGY LTD

**Campus Estate or Research Centre**

BABRAHAM RESEARCH CAMPUS

**Building**

EDDEVA B920

**Road Name**

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB22 3AT

**Country**

ENGLAND

**Tel Number**

0044 676 393 2194

**Fax Number**

0043 1 865 5556

**HSE Division**

EAST AND SOUTH EAST

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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<th>Department</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The committee is made up of the H&S Adviser/Biological Safety Officer and their deputy, as well as 2 volunteer scientists. The H&S adviser chairs the committee. The committee will meet formally once a year to review the procedures and inspect the laboratories, and meet on an ad hoc basis each time a new GM project is proposed. All meeting will be documented and minutes released to the staff.

<table>
<thead>
<tr>
<th>Laboratory</th>
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<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Containment level 1 GM waste is disposed of following the Babraham Research Site waste policy (application GM1 05):

Solid waste is disposed of in hermetically sealable bins (UN approved Type 3H21Y30/S/2003). The bins are sealed and labeled (The BRC registration number: NFK 619; F-Star Biotechnology Ltd; Building 280 & date of disposal). The bins are then collected outside of building 280 in a central collection point where they will be placed into 210 liter yellow labeled “Eurobins”. Collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities are maintained on site, if required.

Liquid Waste is inactivated by the addition of “Virkon” prior to disposal into the drainage system. Babraham Research site has its own sewage treatment plant which is closely monitored.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Bacteria used are well characterized laboratory strains routinely used in molecular biology laboratories,
The bacteriophage M13 derivatives (KMIS, M13-K07) are only able to infect bacteria and not associated with human disease.
All work is classified as category Level I
Testing of human antibodies isolated from yeast, phage and ribosome display libraries. Antibodies are ultimately intended for therapeutic use and will initially be assessed for their affect on primary cells and a variety of cell lines grown in the laboratory.

Project Additional Information

Purposes of the contained use

To minimise the risk of infection from class II cell lines and blood borne infections, in accordance with good microbiological practice and the protection of both work and worker. Work carried out at the premises will be limited to research activities.

Recipient or parental organism

The cell lines used may include adenovirally transformed lines (e.g. HEK-293) or cells classed as Biosafety Level 1 that have been transfected with mammalian expression vectors or via adenovirus-retrovirus. The cell lines themselves are not infectious.

Host/vector system

The GM cell lines may be virally transformed, adenovirally transfected or stably transfected using expression vectors. Cell lines will be stably or transiently transfected with a gene encoding the antigen of interest. Transfection will be carried out using generic mammalian expression vectors, eg. pCEP4. Adenovirus has been identified by the ACDP as a Level 2 Biosafety Hazard. However, the risk of transmission to humans in this activity is considered negligible. The inserted products may be expressed as a soluble or membrane bound proteins. The antigens under study are not in themselves pathogenic but should be handled according to good microbiological practice.

Origin & function

Adenovirus has been used to transform some commercially available cell lines, in order to render them immortal for long-term culture. Transfection of cells is a means of introducing a gene of interest for over-expression, usually at the cell surface, for use as a selection target. The product may also be released into the medium as a soluble protein, or purified from the cell, but will then be captured for use as a selection target or for in vitro characterisation and testing.

Evaluation of foreseeable effects

Biosafety level 2 hazard is introduced to the proposed work by use of adenovirally transformed or transfected cell lines. Other cell lines or products are currently evaluated at level 1. The risk of transmission of adenoviruses from these systems to humans is negligible and the cell and genetic material will not survive in the external environment. Standard level 2 decontamination and containment facilities are being set up and will be in operation prior to commencing this work. Overall, the foreseeable effects are considered negligible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Disposable waste material will not be autoclaved prior to incineration - this derogation follows the allowance specific for the Babraham Research Site. Waste material will be placed in sealed 30 or 60l eurobins and sent offsite for incineration by Vetspeed (Their GM authorization is GM898) according to Babraham Institute disposal notification GM105/4.1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cell lines will be cultured in disposable plasticware at a scale from 500ul to 5l under aseptic conditions and harvested by centrifugation. All liquid waste will be chemically inactivated (e.g. with Virkon, TriGene, or Proceine40) prior to disposal. All operations will be performed in laboratory facilities in which spillages can be effectively disinfected so the wider environment is unlikely to become contaminated. Disposable waste material will be placed in sealed 30 or 60l eurobins and sent offsite for incineration by Vetspeed (GM authorization ref. GM898) according to Babraham Institute disposal notification GM105/4.1.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project has been approved as a class 2 GM activity on the basis of the attached risk assessment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
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<td>Animal Units</td>
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Project Ref 3004/13.1

Date Ackn'd 08/08/2013

CU2 Project Title Retroviral Transduction of Mammalian Cells

Class 2 CultureVolClass 1-50 Litres

Project Title Class CultureVolume Class 3-4

Class 2 CultureVolClass 1-50 Litres

02/03/2022  Page 12466 of 15326
**Project Additional Information**

**Purposes of the contained use**
To create stable cell lines containing genes of interest

**Recipient or parental organism**
Mammalian cells classed as HG1 by the ACDP.

**Host/vector system**
2nd Generation lentiviral vector system. Replication deficient, but can still infect and integrate genome into a broad range of human cells.

**Origin & function**
Human oncogenes, with the potential for tumorigenesis.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
No animals or plants will be used.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
We would like to use the same derogation as the Babraham Institute, who are responsible for waste disposal for all companies on the research Campus, to not use an autoclave to deactivate waste but send it for incineration. The Babraham Institute use Vetspeed (GM authorisation GM898 and their derogation number is GM105/4.1)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
All culture work will be carried out in disposable plasticware. All liquid waste will be inactivated with Virkon (validated to fully inactive lentivirus) overnight before being disposed of to the drains. All solid waste will be sealed in 60L eurobins and sent for incineration by approved contractor, Vetspeed. Surfaces (Benchtop, safety cabinet interior etc) can be disinfected with Biocleanse or Trigene, both of which are validated to fully inactivate lentivirus.
The GMSC has reviewed the risk assessment and is happy that all preventative measures are in place, and have given their approval for the work to take place.

Please enter comments on the GM safety committee on the risk assessment

The GMSC has reviewed the risk assessment and is happy that all preventative measures are in place, and have given their approval for the work to take place.

**Project Containment**

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Pharmaceutical Services Director
- Director of Quality
- HS&E Manager
- Project Manager
- Compliance officer (Microbiologist)
- Microbiologist

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

The activity (secondary packaging and storage) is not expected to generate any waste. In case of an accidental release of damage to the primary containment, materials are collected and disinfected by treatment with a standard house-hold disinfectant or collected in a double sealed bag and disposed of as medical waste.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The material containing GMMO will be delivered to Pen in the primary packaged form, but sealed inside clear plastic bags. This will allow for safer opening of transit packaging without the risk of damaged primary packaging creating a spillage situation. All surfaces will be wiped clean using 70% IPA.

The handling of GMO material will be similar to that for handling any other primary packaged clinical drug at Penn. All staff are familiar in the handling requirements of primary packaged drug material, and receive initial and ongoing training Penn has well established spillage control procedures and these will be supplemented by the use of 70% IPA in the event of the release of the GMO material.

Following the review of the risk assessment Penn is confident that the procedures and training already in place are suitable for the safe handling of this material.
GM Centre Number: 3006

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**Name**

BABRAHAM RESEARCH CAMPUS LTD

**Campus Estate or Research Centre**

THE BABRAHAM RESEARCH CAMPUS

**Road Name**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB22 3AT

**Country**

ENGLAND

**Tel Number**

01223 696 004

**Fax Number**

01223 696 020

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

02/03/2022
BBT Chief Business Officer
BBT Head of Biology
BBT Bioincubator Manager
BBT Development Scientist/BSO
Research scientist (protein biochemistry), Babraham Institute BSO and GMSC chairman
Health and safety consultant
Risk assessments will be circulated amongst the committee for scrutiny and commented on electronically & revisions will be made as necessary. The GMSC will meet twice per year or when necessary to discuss RA5 & GM safety

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Babraham Institute biosafety policy and guidance will be followed. In particular, GMM cell cultures will be inactivated overnight with 1% Virkon and working surfaces disinfected with 2% Trigene (see attached documents: SOP “Disinfection (Inactivation) in the Containment Level I Lab” and COP “Dealing with Biological Spills”). Waste will be disposed of in 30 or 60 litre eurobins in accordance with SI biosafety policy (see attached document: SOP “Clinical Waste Management: Level 1”)

02/03/2022
GMSC discussed all RAs associated with this CU1 form on 14 Feb 2008. Final approval given 14 May 2008.
GM Centre Number: 3007

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**Name**

BIO OUTSOURCE LTD

**Campus Estate or Research Centre**

TODD CAMPUS

**Road Name**

WEST OF SCOTLAND SCIENCE PARK

**Town**

GLASGOW

**District**

LANARKSHIRE

**County**

SCOTLAND

**Postcode**

G20 0XA

**Tel Number**

0141 946 4222

**Fax Number**

0141 946 2221

**HSE Division**

SCOTLAND

**Comments**

GM3139 Mablyte Ltd closed and class 1 work transferred to BioOutsource

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes](https://example.com/yes.png)

Give brief details of the genetic modification safety committee

- Managing Director.
- Non Executive Director (ex GMBSO).
- Head of In Vitro Services (Deputy BSO).
- Head of Quality.
- Genetic Modification Biological Safety Officer (Chair). Biological Safety Officer.
- Employee representative.
- The GMSC meets monthly on a regular basis and more frequently if required.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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<tr>
<td>Level 2 (GMMs)</td>
<td>Yes</td>
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</table>
Liquid waste will be inactivated by either autoclaving at 121°C for 15 mm (with or without prior chemical inactivation), or by incubation overnight in the presence of Virkon at a final concentration of at least 1% (w/v), prior to discard. Virkon has been demonstrated to be effective against a wide range of viruses and bacteria (Dupont Global Website) with published data demonstrating greater than 5 logs of inactivation of virus. Autoclaving of material will result in approximately 100% kill or inactivation of the agents.

The company is a cGMP compliant organisation for the outsourcing of vaccine and biological product.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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<td>Parasitology</td>
<td>Transgenic</td>
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<tr>
<td>Transgenic</td>
<td>Plants</td>
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Other(s) | The company is a cGMP compliant organisation for the outsourcing of vaccine and biological product |

Tick to confirm that you are attaching a summary of the risk assessment | Y |

Tick if you are claiming exemption from disclosure for sections of the risk assessment | |

Please enter comments of the GM safety committee on the risk assessment

The risk assessment has been approved by the GMSC.
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<thead>
<tr>
<th>Field</th>
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## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Sr Scientist, Dep. Site Manager, Cellartis AR
- Sr Scientist, Cellartis AB
- Head of Safety Services, University of Dundee
- Res. Assistant, Cellartis AB
- Res. Assistant, Cellartis AR
- Glasgow University Glasgow University

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify) Tick if confidential

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
All solid waste that has been in the microbiological safety cabinets (MSC) will be disposed of in dedicated waste containers in autoclavable biohazard bags. These bags will be autoclaved in order to kill off any infected cells. All cells in suspension will be killed by exposure to Perform (www.uk.schulke-mayr.com) according to the manufacturer’s instructions (2 % solution, over night), suspension will subsequently be poured out in the drain. All gloves used for work with infected cells will be autoclaved together with other solid waste. The MSC will be wiped with distilled water and 70 % ethanol before and after use. Autoclave will be validated for typical loads. Expected degree of kill: 100%.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick if notifying a connected programme of work

It is concluded that the work proposed offers a negligible risk to the environment as well as to the staff involved in the work.

Project Ref 3008/08.1

Ticker: 3008/08.1

Date Ackn’d 17/07/2008

Date Project Ceased 24/05/2012

Project notified under transitional arrangements

Historical Significant Changes

02/03/2022

Page 12481 of 15326
Project Additional Information

Purposes of the contained use

Research and development of stem cell function and differentiation.

Recipient or parental organism

Well characterised human stem cell lines.

Host/vector system

Well characterised vector systems with a history of safe use, well characterised human stem cells. This notification does not include research to develop new viral vector systems or to investigate virus life cycles. Most commonly, adenoviral, retroviral and lentiviral vector systems will be used.

Origin & function

Tissue specific promoters, germ layer specific promoters, transcription factors, reporter genes, siRNA, shRNA, microRNA.

Evaluation of foreseeable effects

Significant risks to human health arise during the generation of cell lines: once established the cell lines are unlikely to cause harm to human health or the environment.

1. Risks arising from recombination/complementation events

The worst case scenario is that a replication competent virus expressing a harmful gene (e.g., oncogene) is generated either during manufacture of the infectious viral vector particle (i.e., between transfer vector and packaging cell line components) or through co-infection of a person with wild-type virus and viral vector. This scenario is unlikely given the number of recombination events required.

It is also possible that the transfer vector reverts to a replication competent virus. The level of risk depends upon the pathogenicity of the wild-type virus, and for high-risk viruses this possibility is unlikely given the number of recombination events required when packaging the transfer vector.

2. Risks arising from integration of viral vector into persons genome

It is possible that random insertion could lead to activation of cellular genes, disruption of important genes (e.g., tumor suppressor) or acquisition of a harmful gene (e.g., oncogene). The worst case is that permanent changes are induced in an infected cell resulting in tumorigenesis.

3. Risks arising from inserted genetic material

Wild-type and mutant forms of genes and genetic material may have harmful effects (as detailed in Section 2.2 SACGM compendium of Guidance) including known and putative oncogenes, growth factors, cytokines, non-coding regulatory elements, anti-sense constructs, and siRNA.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be autoclaved (expected degree of kill 100%), and then collected for further incineration. Liquid waste will be treated with 2% Perform over night before being autoclaved (expected degree of kill 100%) and then put to drain. Trace contaminated reusable labware and equipment with will be autoclaved if possible- if this is not possible then it will be disinfected with 2% Perform overnight before being rinsed to drain.

<table>
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<th>N</th>
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<tr>
<td>If yes, tick to confirm that it is attached to this form</td>
<td>N</td>
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<tr>
<td>Tick to confirm that you have attached a risk assessment to this form</td>
<td>Y</td>
</tr>
<tr>
<td>Tick if you are claiming exemption from disclosure for section of the risk assessment</td>
<td>N</td>
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</table>

Please enter comments on the GM safety committee on the risk assessment

The foreseeable risks of this work using well characterised viral vectors with a history of safe use are controlled by dedicating a specific laboratory to this work. Access to these laboratories is restricted to staff who are directly involved in the work, and who have been instructed in Local Rules. Infectious viral vector particles (eq waste, samples for storage at low temperature) will only be removed from these laboratories when a safe method of transport has been agreed with the Biological Safety Adviser.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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GM Centre Number: 3009

| Data Premises Notified (Originally) | 18/07/2008 | Transferred from 1992 Regs? | N |
| Data Premises Closed | Transitional Premises Class | N |
| Emergency Plan Required? | Non-GMMs | N |
| Withdrawn | N |

Name

DOMAINEX LTD

Name 2

Department

Campus Estate or Research Centre

CHESTERFORD RESEARCH PARK

Road Name

District

LITTLE CHESTERFORD

Town

SAFFRON WALDEN

County

ESSEX

Postcode

CB10 1XL

Country

ENGLAND

Tel Number

01223 433187

Fax Number

E-mail

HSE Division

WALES AND SOUTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

---

Give brief details of the genetic modification safety committee

Chair BSc PhD over 25 years experience in microbiology and molecular biology ex chairmna of Glaxo-whichcome GMSC
BSO over 10 years experience in molecular biology
Scientist over 5 years experience in molecular biology
Chemist scientist over 10 years experience in research non-expert contribution
The committee will meet at least twice a year and deal with applications electronically. Ad hoc meetings to discuss issues or proposals may be held.

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<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<tr>
<td>Level 2 (GMMs)</td>
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</tr>
</tbody>
</table>
8. WASTE MATERIAL - DISINFECTION, AUTOCLAVING, DISPOSAL

8.1. Liquid cultures and liquid medium from which micro-organisms have been recovered should be sterilised by treatment overnight with Virkon powder (1% w/v) before being discarded via the sink.

8.2. GM Waste material should not be allowed to accumulate and placed promptly in the yellow clinical waste incineration bins. Used laboratory glassware and other materials awaiting disinfection must be stored in a safe manner in plastic boxes provided.

8.3. Re-usable contaminated glass and plastic vessels should be disinfected as described earlier, than rinsed and put in the washer. Eppendorf tubes, petri-dishes etc., should be placed directly into yellow clinical waste incineration bins.

8.4. Non GM waste, but chemically contaminated waste, except needles, scalpel blades and sharps, shall be disposed of in yellow waste incineration bags or bins. Sharp items should be placed in an appropriate container. Containers and bags for incineration must NOT be overfilled. Needles discarded into them must NEVER be re-sheathed.

5.4. Virkon powder - peroxygen compound proven to have a wide range of microbial, antiviral and antifungal activity. 'Used as a 1% solution, which is stable for 7 days. Must be discarded if pink colour is lost. Active agent is potassium monopersulphate. Contains anionic surfactant.
At the first meeting the committee approved the proposal as recommended. As this risk assessment is used for CU1 application and to further benchmark future proposals it was sent to the company’s Health and Safety Advisor, for comment. raised no significant comments
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**Name**

ABCAM PLC

**Name 2**

**Department**

**Campus Estate or Research Centre**

CAMBRIDGE BIOMEDICAL CAMPUS

**Building**

**Road Name**

DISCOVERY DRIVE

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 0AX

**Country**

ENGLAND

**Tel Number**

01223 696 000

**Fax Number**

01223 696 001

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional InfoSubmitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

| Level 1 (GMMs) | Yes |
| Level 2 (GMMs) |      |
| Level 3 (GMMs) |      |
| Level 4 (GMMs) |      |
| Non-microbial  |      |

Give brief details of the genetic modification safety committee

The GMSC is a sub-committee of the Laboratory Health and Safety Committee. The GMSC is composed of six employees with significant experience and knowledge of molecular biology (Biological Safety Officer, Senior Product Development Manager, Senior Characterisation Scientist, Protein Expression Scientist, Cell Culture Scientist and two Development Scientists). All are qualified to degree level and 4 of the 6 hold PhDs in relevant subjects. The BSO/Senior Product Development Manager is on the management team of the facility.

The Laboratory Health and Safety Committee meets at least quarterly, with the GMSC meeting as required (e.g. as new risk assessments are submitted or when specific GM issues are raised by the main committee).
All laboratory solid waste and contaminated consumables (flasks! pipettes, etc) will be inactivated by autoclaving. This will be monitored for each run by a print-out and logs retained.

All liquid waste will be inactivated with a validated disinfectant according to manufacturers instructions (e.g. 1% final concentration virkon for at least 30 minutes).

Waste inactivated via the autoclave or by a validated disinfectant will be placed in sealable waste bins, distinctly from other waste, and processed by our waste contractor (whom are licensed to handle Class 1 GM waste)

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment Y

Please enter comments of the GM safety committee on the risk assessment

The GMSC reviewed the risk assessment and no further comments were suggested.

### Project Ref 3010/12.1

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<td>20/11/2012</td>
<td>The production of recombinant proteins in mammalian cell lines</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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**Project Additional Information**

**Purposes of the contained use**

The proposed objective of this project is to generate recombinant proteins, of mammalian origin, primarily for use in research as reagents. This will involve the batch production of protein (full length or fragment) using established cell lines in combination with CMV based vectors to produce milligram quantities of active proteins.

**Recipient or parental organism**

CHO cells and their derivates. CHO cells are classified as a Hazard Group 1 cell line, are well characterised and have a long history of safe use.

HEK293 cells and their derivates. HEK293 cells have been immortalised by transfection with adenovirus DNA and contain a partial virus genome, on this basis HEK293 cells are normally classified as Hazard Group 2. The cell line itself is well characterised and has a long history of safe use. Furthermore HEK cells do not shed active virus and will not be used for viral production in this project.

In addition, mammalian cell lines have very poor chances of survival outside of the nutrient rich and sterile cell culture environment, therefore they pose minimal environmental risk.

Disabled (auxotrophic for nutrients and non-colonising) lab strains of E. coli (e.g. DH5α, Novablue) will be used for cloning and vector generation. All strains have a long history of safe use, are unlikely to survive in the environment and will be sourced from reputable suppliers.

**Host/vector system**

All plasmids will be non-mobilising in bacteria, and will carry CMV and/or HTLV viral promoters to enable mammalian gene expression (e.g. pCMV6-AC)

**Origin & function**

All target proteins will be derived from mammalian sources, including humans, rabbits and mice. The cloned genes will include enzymes, structural proteins but, in the interest of safety, exclude more hazardous products such as oncogenes. The source material will be purchased as cDNA from a supplier or derived from tissues produced by a third party animal house.

End uses will include the production of lysates for screening by western blot and detection reagents/enzymes for assays such as ELISA.

**Evaluation of foreseeable effects**

The project scope allows for the cloning and expression of a wide range of functionally distinct genes. The most hazardous gene products will be those known to code for potentially harmful proteins; cytokines, growth factors. The production of cytokines and growth factors will, where possible, be restricted to non-functional fragments, with each protein requiring its own risk assessment. Furthermore, the use of sharps, in conjunction with any GMO work will further reduce operator risk.
The accidental transfer of cloned genes to humans is unlikely to present a high level of risk as the plasmids used will be naked DNA and, unaided, unlikely to cross a cell membrane to establish protein production within the cell.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste is to be autoclaved at 121°C for 15 minutes to sterilise prior to disposal via Abcam's established clinical waste disposal route. Solid waste is double-bagged in clear autoclave bags, prior to sterilisation and, post-sterilisation, placed in clearly marked-yellow clinical waste bags for disposal off-site. All clinical waste is kept in sealable yellow-bins, distinct from all other waste and is removed from the site for incineration by a licensed contractor. Our designated GM autoclave, keeps electronic records of each run, will display a warning alarm if a cycle fails, and is serviced by the manufacturer on a yearly basis.

Disinfection:
70% ethanol/industrial methylated spirits and blue roll is used in combination with Trigene to sterilise work surfaces by spraying and wiping down with blue roll both before and after Cell culture work.
Where necessary, liquid cultures are disinfected by mixing 1:1 with 2% Virkon and left overnight to ensure maximum killing time (in excess of manufacturer’s recommendations). The same Virkon solution is used to sterilise any spills that occur, and serological pipettes are soaked in 1% Virkon prior to disposal via the clinical waste route.
Both Virkon and Trigene have been shown to be broad spectrum and fast acting disinfectants by their manufacturers.

Is an emergency plan required according to regulation 20? Y
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This project is broadly GM class 1 with the exception of a Hazard Group 2 host organism (HEKs). All work to be treated as Hazard group/GM class 2 and carried out in Class 2 laminar flow hood. A cell culture room within Abcam's existing facilities will be set aside for the GM work.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
<td>Yes</td>
<td>L3</td>
<td>L4</td>
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02/03/2022
### Project Additional Information

**Purposes of the contained use**

To transform E. coli with genes derived from Hazard group 2 organisms to help antibody production or as an aid to protein production (e.g. receptors and chaperones) primarily for use in research as reagents.

**Recipient or parental organism**

The recipient strain will be a recognised disabled non-colonising E. coli, classified as an ACDP Hazard Group 1 organism. These strains of E. coli have a long history of safe use, are unlikely to survive in the environment and will be sourced from reputable suppliers.

- Mach1™ T1R E. coli (Invitrogen)
  - (recA1398 endA1 tonA 80 lacM15 lacX74 hsdR(rk-mk+))
- BL21 Star™ (DE3) (Invitrogen)
  - (F- ompT hsdSB (rB-mB-) gal dcm me131 (DE3))
- BL21 Star™ (DE3)pLysS (Invitrogen)
One Shot® MAX Efficiency® DH5α™ T1R (Invitrogen)
(F- 80lacZ M15 (lacZA-argF) U169 recA1 endA1 hsdR17(rk-, mk+) phoA supE44 thi-1 gyrA96 relA1 tonA)

One Shot® ccdB Survival™ T1R TOP10 (Invitrogen)
(F- mcrA (mrr-hsdRMS-mcrBC) 80lacZ M15 lacX74 recA1 ara 139 D(arai-leu)7697 galU galK rpsL (StrR ) endA1 nupG tonA::Ptrc –ccdA)

BL21-A1 (Invitrogen)
F- ompT hsdSB(rB- mB-) gal dcm araB::T7RNAP-tetA

BL21 CodonPlus(DE3)-RPIL (Stratagene)
(F- ompT hsdS(rB- mB-) Tetr gal λ(DE3) endA Hte [argU proL Camr] [argU ileY leuW Strep/Specr])

BL21-Gold (DE3)LysS (Stratagene)
(F- ompT hsdS(rB- mB-) dcm+ gal λ(DE3) endA Hte [pLysS Camr])

BL21 CodonPlus-RPIL (Stratagene)
(F- ompT hsdS(rB- mB-) Tetr gal endA Hte [argU proL Camr] [argU ileY leuW Strep/Specr])

BL21-Gold LysS (Stratagene)
(F- ompT hsdS(rB- mB-) dcm+ gal endA Hte [pLysS Camr])

NovaBlueTM Gigasingles (Novagen)
endA1 hsdR17(rK12– mK12+) supE44 thi-1 recA1 gyrA96 relA1 lac F'[proA+B+ lacIqZ ΔM15::Tn10 (TcR)]

BL21 (Novagen)
F- ompT hsdSB(rB- mB-) gal dcm

Subcloning Efficiency DH5α (Invitrogen)
F- φ80lacZΔM15 Δ(lacZA-argF)U169 recA1 endA1 hsdR17(rk-, mk+) phoA supE44 thi-1 gyrA96 relA1 λ-

SHuffle® Express (New England BioLabs)
ofu2A [lon] ompT ahpC gal λatt::pNEB3-r1-cDsbC (SpecR, lacIq) ΔtxB sulA11 R(mcr-73::miniTn10--TetS)2 [dcm] R(zgb-210::Tn10 --TetS) endA Δgor Δ(mcrC-mrr)114::IS10

WK6
Δ(lac-proAB)galEstrA [F' lacIq lacZΔM15 proAB+]

Host/vector system

All plasmids will be non-mobilising in bacteria, and will not carry mammalian promoters. Example plasmids include:

pJ421 (DNA2.0) (pACYC-ori, KanR, T5 promoter)
pENTR™ 221 (Invitrogen) (pUC ori, KanR, attL, attL2)
pDONR™ 221 (Invitrogen) (pUC ori, KanR, attL, attL2)
pET300/NT-DEST (Invitrogen) (pBR322 ori, AmpR, CmpR, ccdB, lacO, T7 promoter, attR, attR2, ROP)
pQE-80L (and derivatives) (Qiagen) (pBR322 ori, AmpR, lacO, T5 promoter)
pET-19, pET-22 (and derivatives) (Novagen) (pBR322 ori, AmpR, lacI, lacO, T7 promoter)
pET-28 (Novagen) (pBR322 ori, KanR, lacI, lacO, T7 promoter)
pET200/D-TOPO (Invitrogen) (pBR322 ori, KanR, lacI, lacO, T7 promoter, ROP)

Origin & function

The majority of proteins will be derived from mammalian sources or non-verotoxic strains of E. coli. Hazardous mammalian products such as oncogenes and cytokines may be expressed and some sequences will be derived from pathogenic (but not verotoxic) E.coli. Where possible target selection will be restricted to proteins known to be non-hazardous when expressed in isolation. The source material will be purchased as cDNA from a supplier or derived from tissues produced by a third party animal house.

End uses will include the production of protein for use as antigens, lysates for screening by western blot and detection reagents/enzymes for assays such as ELISA.

Evaluation of foreseeable effects

The project scope allows for the cloning and expression of a wide range of functionally distinct genes. The most hazardous of which are potentially harmful proteins (such as cytokines, growth factors, oncogenes). The production of these proteins will, where possible, be restricted to non-functional fragments, with each protein requiring its own risk assessment. Furthermore, prohibiting the use of sharps, in conjunction with any GMO work will further reduce operator risk.

The accidental transfer of sequences to other hosts is highly unlikely due to the disabled nature of the host organism and non-mobilisable vectors used. The vector will not carry genetic factors for mobilisation and expression vectors will not contain eukaryotic promoter sequences (further reducing the risk to humans).

All genetically modified micro-organisms (GMMs) will originate from recognised, disabled, non-colonising E. coli strains, classified as ACDP Hazard Group 1 organisms. Therefore the GMM is highly unlikely to survive in the ‘outside environment’ (outside the media). The risk posed by expressed proteins will be minimised, as they will be expressed in small quantities (mg), and the likelihood of delivery to the site of action is minimal (no sharps).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Precautions for safe disposal:
All waste is to be autoclaved at 121°C for 15 minutes to sterilise prior to disposal via Abcam's established clinical waste disposal route. Solid waste is double-bagged in...
clear autoclave bags, prior to sterilisation and, post-sterilisation, placed in clearly marked-yellow clinical waste bags for disposal off-site. All clinical waste is kept in sealable yellow-bins, distinct from all other waste and is removed from the site for incineration by a licensed contractor. Our designated GM autoclave, keeps electronic records of each run, will display a warning alarm if a cycle fails, and is serviced by the manufacturer on a yearly basis. NB Containment: prior to inactivation all class 2 materials are to be handled in a class 2 biosafety cabinet (these are are maintained, and serviced on an annual basis, by an approved contractor).

Disinfection:
70% ethanol/industrial methylated spirits and blue roll is used in combination with Trigene to sterilise work surfaces by spraying and wiping down with blue roll both before and after Cell culture work.
Where necessary, liquid cultures are disinfected by mixing 1:1 with 2% Virkon and left overnight to ensure maximum killing time (in excess of manufacturer’s recommendations). The same Virkon solution is used to sterilise any spills that occur, and serological pipettes are soaked in 1% Virkon prior to disposal via the clinical waste route.
Both Virkon and Trigene have been shown to be broad spectrum and fast acting disinfectants by their manufacturers.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
All members are satisfied with the assessment and note: All work to be treated as Hazard group/GM class 2 and carried out in Class 2 laminar flow hood. This work is to take place in a designated room within Abcam’s existing Hazard group 2/Class 2 facilities.

Project Containment

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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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| Project Ref | 3010/15.1 |

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The proposed objective of this project is to generate recombinant antibodies, of mammalian origin, to small molecules, proteins and viruses in mammalian cells. These antibodies will be used primarily in research as reagents. This will involve the batch production of antibodies using established cell lines, in combination with CMV based vectors to produces milligram quantities antibody.

HEK293 cells and their derivates. HEK293 cells have been immortalised by transfection with adenovirus DNA and contain a partial virus genome. on this basis HEK293 cells are normally classified as Hazard Group 2. The cell line itself is well characterised and has a long history of safe use. Furthermore HEK cells do not shed active virus and will not be used for viral production in this project.

CHO cells and their derivates. CHO cells are classified as a Hazard Group 1 cell line, are well characterised and have a long history of safe use.

Mammalian cell lines, such as CHO and HEK293 cells, have very poor chances of survival outside of the nutrient rich and sterile cell culture environment. therefore they pose minimal environmental risk.

Disabled (auxotrophic for nutrients and non-colonising) lab strains of E. coli (e.g. DH5a, Novablue) will be used for cloning and vector generation. All strains have a long history of safe use, are unlikely to survive in the environment and will be sourced from reputable suppliers.

The adenoviruses which will be used in aspects of this work have been rendered replication deficient by deletion of the E1 region of the genomes. Purified adenovirus will be provided for us by a reputable supplier. and will not be propagated on-site. The adenoviruses which will be used in this work will not contain any genetic Insertion.

All plasmids will be non-mobilising in bacteria, and will carry CMV and/or HTLV viral promoters to enable mammalian gene expression (e.g. pCMV6-AC)

The source material will be plasmids or synthetic DNA obtained from a reputable supplier (e.g. Origene) or, in the
case of antibody production, B cells obtained from the PBMCs or spleens of immunized rabbits produced by a third-party animal house. In the case of antibody production B cells will be fused to create hybridomas and the antibody sequences from these cells will be subsequently cloned into antibody expression vectors. These will be transfected into HEK293 (and/or CHO cells, or similar mammalian cells) to allow expression and the secretion of the antibody. End uses will include the harvesting cell supernatant for screening by ELISA and, in some cases, Western blot, immunohistochemistry, immunocytochemistry and flow cytometry.

In the case of anti-viral antibody production, purified replication-incompetent virus adenovirus, provided by a reputable supplier will be used at the antigen in an ELISA assay to identify specific antibodies. Purified virus will either be adsorbed directly onto the surface of polystyrene microtitre plates or will be added to plates previously coated with anti-viral polyclonal antibody, in a standard sandwich ELISA method.

**Evaluation of foreseeable effects**

This project will involve cloning of a variety of functionally distinct genes and expressing them in mammalian cells. The most hazardous gene products will be those known to code for potentially harmful proteins; cytokines, growth factors. The production of cytokines and growth factors will, where possible, be restricted to non-functional fragments, with each protein requiring its own risk assessment. Furthermore, the use of sharps, in conjunction with any GMO work will be prohibited to further reduce operator risk.

In the case of recombinant antibody production, these antibodies will have been produced in rabbits, in response to immunisation, and therefore no potential hazards from the encoding plasmids is expected.

The accidental transfer of cloned genes to humans is unlikely to present a high level of risk as the plasmids used will be naked DNA and, unaided, unlikely to cross a cell membrane to establish protein production within the cell. In the case of anti-viral antibody production, purified replication-incompetent virus adenovirus, provided by a reputable supplier will be used at the antigen in an ELISA assay to identify specific antibodies. Purified virus will either be adsorbed directly onto the surface of polystyrene microtitre plates or will be added to plates previously coated with anti-viral polyclonal antibody, in a standard sandwich ELISA method. The adenoviruses which will be employed in this work are replication deficient and will not contain any genetic insertion. No replication competent viruses have been found following standard Replication Competent Adenovirus assays, so there is very minimal risk of any live virus being present in the final preparation. Purified virus will be provided by a reputable supplier and virus will not be propagated on site at any stage.

In the very unlikely event of exposure to replication competent adenoviruses, it should be noted that adenoviruses are ubiquitous pathogens of both mammals and birds, usually causing only mild self-limiting infection in human which does not require any specific treatment. No allergic or toxic effects are associated with these viruses.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste is to be autoclaved at 121 °C for 15 minutes to sterilise prior to disposal via Abcam's established clinical waste disposal route. Solid waste is double-bagged in clear autoclave bags, prior to sterilisation and, poststerilisation, placed in clearly marked-yellow clinical waste bags for disposal off-site. All clinical waste is kept in sealable yellow-bins, distinct from all other waste and is removed from the site for incineration by a licensed contractor. Our deSignated GM autoclave, keeps electronic records of each run, will display a warning alarm if a
cycle fails, and is serviced by the manufacturer on a yearly basis.

Disinfection:
70% ethanol/industrial methylated spirits and blue roll is used in combination with Distel to sterilise work surfaces
by spraying and wiping down with blue roll both before and after Cell culture work.
Where necessary, liquid cultures are disinfected by mixing 1:1 with 2% Virkon and left overnight to ensure
maximum killing time (in excess of manufacturer's recommendations). The same Virkon solution is used to sterilise
any spills that occur, and serological pipettes are soaked in 1% V"kon prior to disposal via the clinical waste route.
Both Virkon and Distel have been shown to be broad spectrum and fast acting disinfectants by their manufacturers.

Is an emergency plan required according to regulation 20?  N
If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y
Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

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**Name**

CANCER RESEARCH UK THERAPEUTIC DISCOVERY LABORATORIES

**Name 2**

**Department**

**Campus Estate or Research Centre**

WIBR, UNIVERSITY COLLEGE LONDON

**Building**

THE CRUCIFORM BUILDING

**Road Name**

GOWER STREET

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

WC1E 6BT

**Country**

ENGLAND

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<tr>
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**E-mail**

**HSE Division**

LONDON

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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<td>CANCER RESEARCH UK THERAPEUTIC DISCOVERY LABS LTD</td>
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<td>THE MILNER THERAPEUTIC S INSTITUTE</td>
<td>JEFFREY CHEAH BIOMEDICAL CENTRE, UNIVERSITY OF CAMBRIDE</td>
<td>Puddicombe Way</td>
<td>CAMBRIDGE</td>
<td>CB2 0AW</td>
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</tbody>
</table>
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  Y

Give brief details of the genetic modification safety committee

Chair: Director of Discovery
Secretary: Health and Safety Manager
Members:
- Head of Biology (biological safety officer)
- Molecular Biology Group Leader
- Lay Member
- Outside, independent member
- Occupational Health Physician
- Two Employee representatives
- Chief Scientific Officer Please see attached sheet for operating procedures

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Bacteriology</td>
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<td>Parasitology</td>
<td>Transgenic Birds</td>
<td>Microbiology Research</td>
</tr>
</tbody>
</table>

Tick if confidential
Yes

All waste will be autoclaved at a minimum of 121 deg C for 20 minutes before removal from the premises for incineration. This procedure is expected to kill 99.999% of all GMO’s. A print out of each cycle will be kept on file for a minimum of two years. Virkon in a 1% solution for laboratory discard jars left for at least 12 hours in a 2% solution for laboratory spills.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

It was decided that the procedures should be amended to the effect that all Activity Class 1 projects should be sent by e-mail to all members of the committee for interim approval, without the need for a formal meeting. This interim approval to be ratified by a formal meeting of the committee that should be held annually in the first instance. There was some discussion about how our risk assessments should be arranged. Originally each gene was given a different risk assessment for each cell type. After some discussion it was decided that putting a series of genes from a gene family into a series of cell types could go under one risk assessment provided each situation was considered on its merits and they all had the same classification. This would cut down on the paperwork considerably. It was emphasised that the committee should be aware of the potential for higher risks within these assessments. Risk assessments will be reviewed approximately annually, and those we are not actively working on will be designated as parked. Each completed risk assessment was examined. It was decided that a single assessment would suffice for all kinases in all expression systems. It was also agreed that all expression systems could be grouped together provided they all represented the same level of risk.

Date Ackn’d: 18/06/2010
CU2 Project Title: Genomic modification of human cells using lentiviral vectors
Project Ref: 3011/10.1
Purposes of the contained use

CRT undertakes focussed translational research on cancer pathology & treatment. This necessitates extensive use of human cells (established cell lines). These cells very often need to undergo genomic modification as part of the research. This GM includes use of shRNA or shRNAmir (microRNA-adapted shRNA) to perform gene knockouts, insertion of reporter genes to label and detect cellular processes, insertion of genes.

Lentiviruses are a type of retrovirus with more complex genomes than what are usually classified as 'retroviruses' for the purpose of GM work, and include the human immunodeficiency virus (HIV). The genome comprises the gag, pol, env genes (as do 'retroviruses') plus tat & rev genes that regulate viral gene expression and genes such as vif/vpr/vpu/nef needed for infectivity & pathogenesis. Lentiviruses, as per all retroviruses, are enveloped single-stranded RNA viruses. Lentiviruses infect both dividing and non-dividing cells. Lentiviruses are stably integrated into the genome of infected cells.

The lentiviral vectors that will be used for the work do not use the env gene but rather are pseudotyped with vesicular stomatitis virus membrane glycoprotein (VSV-G) to increase host range and stability. They will also include the woodchuck hepatitis B virus (WHV) post-transcriptional regulatory element (WPRE) to increase viral vector titre and genomic integration into, dividing and non-dividing human cells of a wide variety of tissue types with relatively high efficiency. Lentiviral vectors thus provide an excellent vehicle of great utility for achieving the genomic modifications needed for the research objectives of CRT. High titres of viral particles may be required. Examples of lentiviral vector systems used include TRIPZ and GIPZ with Trans-Lentiviral packaging system PTLA1-Pak, pTLA1-Enz, pTLA1-Env, pTLA1-Rev and pTLA1-TOFF (Open Biosystems), or pLENTI4 and pLENTI6 with ViraPower packaging system pLP1, pLP2, and pLP/VSVG (Invitrogen).

Given the nature & objectives of the research at CRT, the genetic targets are commonly oncogenes and tumour suppressor genes.

Recipient or parental organism

The bacterial strains used to produce the plasmid vectors are attenuated non-colonising strains that are harmless to Humans and cannot survive outside culture conditions, and are therefore harmless to the environment.

The producer cells that will package the virus after being transfected are harmless to humans. They are also unable to survive outside culture conditions, and are therefore harmless to the environment.

The recipient cells that will eventually receive and integrate the DNA are harmless to humans. They are also unable to survive outside culture conditions, and are therefore harmless to the environment.

Host/vector system

All the plasmids used are non-mobilizable
The lentiviral vectors used will all be well characterised, with a history of safe use, and inherently safe. These vectors will all be self-inactivating & replication-incompetent, that is, each lentiviral vector particle infects and gives genomic modification of one cell, and that cell will not (and indeed cannot) subsequently produce and release further viral particles. This is due to a deletion in the 3’ LTR (TATA deletion) which prevents excision after genomic integration.

These vectors will all be at least third generation, that is, the genes needed for packaging of the construct into live viral particles are present on at least three different vectors that cannot themselves be packaged into viral particles, and cannot recombine with each other or with the lentiviral vector. This means that cells can be infected with viral particles made in the packaging cell lines, but infectious viral particles cannot be made in the target cells. This then means that the probability of recombination events (especially with wild type lentivirus) generating a 'reverted' viral vector capable of inducing live viral production & release by infected cells is very unlikely - it would require three recombination events, each of which is very unlikely. Furthermore, the accessory genes (vif, vpr, vpu, nef) and the tat gene are deleted, and expression of the vector construct is contingent on upstream elements and trans complementation packaging acts as an inherent safeguard against generation of productive recombinants.

The lentiviral particles cannot survive outside of closed controlled cell culture conditions and are rapidly inactivated by dehydration or other environmental insults. They require close contact with body fluids or percutaneous inoculation for transmission. The virus could, in principle, in the unlikely event of it being introduced to the cells of a worker, give genomic modification of cells of the worker. The inserted sequences could be oncogenic, or could activate oncogenes, or could repress tumour suppressor genes, or could lead to other deleterious effects and dangerous changes to gene expression.

Origin & function

The bacterial strains used to produce the plasmid vectors are attenuated non-colonising strains that are harmless to Humans and cannot survive outside culture conditions, and are therefore harmless to the environment.

The recipient cell lines cannot survive outside the laboratory incubators and as such pose no threat to health or environment.

None of the plasmids, which are non-mobilizable, are harmful to health or to the environment. The packaging components are from the HIV-1 virus (harmful to Humans) but many components that are critical for HIV-1 infection have been removed. Only those essential for viral packaging remain in the vectors.

The most dangerous GMM is the lentiviral particles and the most hazardous steps are their collection, concentration and infection of recipient cells with them. However, the viral particles require close contact with body fluids or percutaneous inoculation for transmission. The virus could, in principle, in the unlikely event of it being introduced to the cells of a worker, give genomic modification cells of the worker. The inserted sequences could be oncogenic, or dangerous changes to gene expression.

The lentiviral particles cannot survive outside of closed controlled cell culture conditions and are rapidly inactivated by dehydration or other environmental insults. In order to minimize risks, all work involving viruses will be performed in Class 2 cabinets in containment level 2 laboratories specified particularly for viral GMM work. All DNA plasmids containing viral proteins will be grown separately to avoid opportunity for recombination. To avoid any contamination of workers by viral particles, no glass or other sharps will be used in the rooms where the virus is prepared and used, and workers will be protected by a lab coat and disposable gloves. Skin lesions will be covered by a bandage in addition to the protective wear described above. Specific guidelines will be in place to ensure all viral preparations are handled, labelled, stored transported and cleaned-up correctly. All those involved in the work will be made aware of the guidelines and the associated risks, as well as anyone else who may use the laboratory.

Evaluation of foreseeable effects

The genetic material included use of shRNA or shRNAmir (microRNA-adapted shRNA) to perform gene knockouts, insertion of reporter genes to label and detect cellular processes, insertion of genes (generated from IMAGE clones coming from human cDNA Libraries). Given the nature & objectives of the research at CRT, the genetic targets are commonly oncogenes and tumour suppressor genes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1-5% Virkon (v/v) will be used to treat liquid waste (18 hours) and to decontaminate any spills and for disinfection of any reusable bottles or other equipment and laboratory materials used. Liquid waste will be poured down the sink after treatment with 105% Virkon. Work surfaces will be wiped down with 5% TriGene Advanced and 70% ethanol treatment, solid waste will be double bagged in biological waste bags, sealed and autoclaved at 132°C with a hold time of 15 min by trained staff, then bagged according to UCL waste services procedure. Autoclave runs are regularly validated by waste management staff via thermocouple print out. Autoclave runs are regularly validated by waste management staff.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

University College London Genetic Modification Safety Committee:
This committee commented that the fact that sharps/glass etc was not used was mentioned on form CU2 it should be mentioned on the accompanying risk assessment.

Cancer Research Technology’s Biological and Genetic Modification Safety Committee.
This committee commented that we explain the abbreviation and include a reference to shRNA.
They ask for more explanation of a “third generation vector” this was added to section 2.06
In section 1.10 they requested we give some idea of the size, purpose and reference for the shRNA.
In section 1.15 they asked that we add “No likelihood of further cell to cell transfer”
In section 1.36 there was some discussion about the additional training required. It was decided that this was not needed at this level of risk.
A typographical error was corrected in section 2.07

Project Containment

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The use of 2nd generation lentiviral packaging vectors in combination with 3rd generation lentiviral transfer vectors to produce lentivirus for modification of mammalian cell lines

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Non-GMM Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

The aim is to use 2nd generation or later lentiviral packaging systems in combination with 3rd generation Lentiviral transfer vectors to achieve single or multiple genetic mutations by utilising shRNA, CRISPR/Cas9 (and derivatives thereof) in genome wide screening efforts and in a range of mammalian cell lines in order to facilitate drug discovery projects. The extent of the planned work is limited to in vitro studies. Approaches include both transfection of plasmid vectors into eukaryotic packaging cell lines such as HEK-293T to generate replication-defective viral particles and, use of such replication-defective viral particles to transduce mammalian cell lines. The use of 2nd generation packaging systems will enable the generation of virus with appropriate virus titers required for whole genome wide pooled CRISPR screening in a wide range of mammalian cell lines. Virus titers from wholly 3rd generation lentiviral systems may not be suitable for a wide range of difficult to transduce mammalian cell lines. Restricting the use of of lentiviral systems to 3rd generation would therefore prevent successful genome wide screening in certain cell lines. 2nd generation lentiviral packaging systems will only be used if 3rd or 4th generation systems will not be suitable as described above. Additionally, close scrutiny of the transgene in the context of 2nd generation systems will be ensured in order to prevent the use of vectors containing packaged Tat and to minimize the likelihood of generating replication-competent viruses. Oncogenes and cytokines are not used as transgenes.

**Recipient or parental organism**

Lentiviruses are a type of retrovirus with more complex genomes than what are usually classified as ‘retroviruses’ for the purposes of GM work and include the human immunodeficiency virus (HIV). The genome comprises the gag, pol, env genes (as do ‘retroviruses’) plus tat & rev genes that regulate viral gene expression and genes such as vif / vpr / vpu / nef needed for infectivity and pathogenesis. Lentiviruses, as per all retroviruses, are enveloped single-stranded RNA viruses. Lentiviruses infect both dividing and non-dividing cells and stably integrate into the genome of infected cells. All vector systems will be split to minimise possibility of replication and where packaging cell lines are to be used these will be consistent with 2nd generation or later systems. Hence these viral vectors are extremely unlikely to be able to replicate in human cells or in the wider environment and are unable to present the pathogenesis associated with the originating pathogen. The lentiviral vectors that will be used for the work do not use the env gene but rather are pseudotyped with vesicular stomatitis
virus membrane glycoprotein (VSV-G) to increase host range and stability. They will also include the woodchuck hepatitis B virus (WHV) post-transcriptional regulatory element (WPRE) to increase viral vector titre and enhance expression of the transgenes (WPRE is similar to the post-transcriptional regulatory element found in human hepatitis B virus (HBV), which is closely related to WHV). Lentiviral vectors thus allow transfection of, and stable genomic integration into, dividing and non-dividing human cells of a wide variety of tissue types with relatively high efficiency. Lentiviral vectors thus provide an excellent vehicle of great utility for achieving the genomic modifications needed for the research objectives of CRT. High titres of viral particles may be required. Examples of lentiviral vector systems used include TRIPZ and GIPZ with Trans-Lentiviral packaging system pTLA1-Pak, pTLA1-Enz, pTLA1-Env, pTLA1-Rev and pTLA1-TOFF (Open Biosystems), or pLENTI4 and pLENTI6 with ViraPower packaging system pLP1, pLP2, and pLP/VSVG (Invitrogen), pMD2.G (https://www.addgene.org/12259/) and psPAX2 (https://www.addgene.org/12260/).

### Host/vector system

For lentiviral systems, viral particles will be generated by introducing plasmids containing elements of the virus genome into established packaging cell lines, e.g. human embryonic kidney (HEK293T) cells. There may be instances where the essential viral packaging genes will be expressed stably by the packaging cell, with a single plasmid carrying the vector backbone and transgene being introduced by transfection, and in other cases the viral functions and the vector backbone/transgene will be encoded on separate plasmids which will be transfected together.

Such viral vectors will then be used to transduce a range of human and animal cell lines. Target cell lines for transduction may comprise established and primary mammalian cell lines not known or suspected to contain pathogens at Hazard Group 3 or above. This could include human, rodent and primate sources.

### Origin & function

Given the nature and objectives of the research at CRT - TDL, the genetic targets are commonly oncogenes and tumour suppressor genes. Short hairpin RNA (shRNA) molecules intended to inhibit the expression of a range of genes including those known or suspected to be involved in pathways of oncogenesis, and genes intended to induce immortalisation and the extension of the proliferative lifespan of cells. CRISPR/Cas9 systems may be expressed which are intended to cause double-stranded breaks in DNA, leading to mutation by non-homologous end-joining or insertion of specific mutations or tags with homology-directed repair constructs. Variants of the CRISPR/Cas9 system include, S. pyogenes (sp) Cas9, spCas9-HF-1, saCas9, catalytically dead mutants of Cas9 fused to effectors such as cytidine deaminase or transcriptional/epigenetic regulators (CRISPRi/CRISPRa) and non Cas9 modifiers such as Cpf1. This work does not include the use of viral vectors expressing toxin genes or major pathogenesis factors (e.g. oncogenes as transgenes).

### Evaluation of foreseeable effects

For lentiviral vectors, use of second generation (or later) viral vector systems in which all accessory genes have been deleted, and where gag, pol, env and rev genes are provided in trans (either on additional accessory plasmids or integrated into the genome of packaging cell lines), means that the risk of production of replication-competent or infectious virus is extremely low. In addition the use of self-inactivating (SIN) viral vector systems in which promoter and enhancer elements have been removed from the long terminal repeats (e.g U3 deleted 3'LTR for lentiviral transfer vectors), reduces the risk of transactivation of genes proximal to the insertion site, but there remains the possibility gene activation by constitutive promoter/enhancer sequences within the inserted genetic material. Insertion of the viral genome could also result in disruption of genes involved in cellular regulation (e.g. tumour-suppressor genes). WPRE sequences used to increase the levels of expressed mRNA will be present in many vector systems. Although some versions of these elements also express a promoter fragment of the Woodchuck Hepatitis Virus X protein for which there is published evidence for its association with oncogenic activity (Hohne et al., EMBO 9, 1137-1145.1990; Schuster et al., Oncogene 19, 1173-1180. 2000), it is anticipated that this would not result in a need for a higher containment level (>CL2). Nevertheless to improve safety considerations, the preference will be for vectors devoid of the WPRE fragment or containing the mutated/deleted non X protein fragment containing form of WPRE.

In the instance of any transduction event, even by a viral vector that is non-infectious and replication-defective, the resultant integration of the inserted gene into the genome of the host cell means that expression will be permanent. Hence whilst the vector itself will not proliferate, cell division may result in a population of cells expressing the transgene. Specific hazards associated with inserted genes include:-

1. Expression/over-expression of known or potential oncogenes: while oncogenesis is known to be a multi-factorial process, the risk of insertional activation of other oncogenes by the vector, or of insertional inactivation of tumour suppressor genes is unpredictable, and could act in concert to contribute to oncogenic pathways.
2. Expression of short hairpin RNAs designed to knock down known or potential tumour suppressor genes
3. Intentional CRISPR/Cas9 modifications leading to inactivation of tumour suppressor genes or increased activity of endogenous oncogenes.
4) Expression of cytokines, growth factors and other bioactive molecules: these proteins have the potential to cause inappropriate growth, differentiation or apoptosis of cells, which are associated with oncogenesis. Growth factors and cytokines may also be teratogenic, and have other effects on the immune response. Such hazards may be associated with the viral vector or the final GMO (ie. cell line).

The highest level of risk associated with the viral vector or insert hazards is exposure by direct contact, self inoculation and aerosol generation. Controls measures required at Containment Level 2 include a no sharps policy, gloves and use of microbiological biosafety class II cabinet to reduce likelihood of exposure. The risks to workers associated with the plasmids used to generate the viral vectors are considerably less than the virus itself, as the passive uptake of circular DNA into human cells is a very inefficient process and chromosomal integration is much less likely.

All risk assessments for GM activities with retroviral vectors will continue to be subject to scrutiny and approval by the CRUK Research & Innovation (R&I) GM committee. The following activities will NOT be permitted within the work:-
1) Preparation of high-titre retroviral or lentiviral stocks by methods that require the use of needles to harvest the virus (e.g. density gradient centrifugation).
2) The use of sharps, including hypodermic needles and scalpels,
3) Expression of a major pathogenesis factor or a toxin gene (eg oncogenes and cytokines).
4) The use of lentiviral vectors for which there is evidence or potential for the presence of replication-competent virus.
5) Aerosol generation by ultracentrifugation of supernatants.
6) Any other activity which the R&I GM committee decides which constitutes one or more risks that might consign it to a higher Class.

According to the SACGM Compendium of Guidance, the risks are negligible 'where the cells do not incorporate any helper function and where residual virus titres have been reduced by replacing the potentially infectious cell supernatant medium'. Where validated procedures are not available or number of passages/dilution factors are unknown, screening (eg p24 ELISA for lentivirus) for presence of viral contamination in supernatant will be performed before any derogation of containment measures. Cells or samples shown to be free from viral vectors used after that may therefore be re-assigned to Class 1 GMMs for subsequent use, storage and transport, so long as the parental strain is Hazard Group 1.

No animals or plants to be used in this work

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No animals or plants to be used in this work

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be inactivated with a final concentration of 2% Virkon for a minimum of 20 minutes. (Dupont specification says it has been independently proven that 1% solution kills bacteria within 5 minutes and parvovirus within 10 minutes.) Surfaces are cleaned with a solution of 1% Virkon or Chemgene 1:10 spray. Chlorasept tablets are also used on some occasions at a minimum of one 2.5gm tablet per litre of solution, again left for a minimum of 20 minutes.

All solid Biohazard and GM waste is double contained in autoclave bags within autoclave bins, autoclaved on site at a minimum of 131deg C with a hold time of 20 minutes followed by offsite disposal. The autoclave runs are monitored for temperature and hold time automatically.

Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with waste protocols correctly as specified above

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
Comments from CRUK R&I's GM committee. The proposed notification is an extension of TDL's existing class 2 GM notification to allow the use of 2nd generation lentiviral packaging systems with 3rd generation transfer vectors. Points discussed included:

1. General view was that risk assessment and notification were all within class 2.
2. There was discussion over the types of KO/KI being generated – the aim is to screen genome wide by creating pools of targeted insertions.
3. A question regarding whether additional signage was required whilst viruses are ‘actively in use’ – the existing signage and access control meets regulatory requirements and are sufficient.
4. A comment was made that re-assignment of cells or samples to Class 1 GMMs for subsequent use, storage and transport can only occur if the parental strain is Hazard Group 1.
5. A comment was made on ‘plates being lidded and sealed’ and if there would be secondary containment in the incubators? All the incubators are in CL2, the lidding and sealing of plates is to allow plates to be taken out of CL2 in secondary containers for reading on equipment in CL1 – out of scope risk assessments are in place to allow this.
6. A question was also raised around spillages and whether a procedure was in place to cover a spill on a person. This is not covered in the risk assessment but is included as part of the local Milner institute CL2 induction and standard procedures.

Project Containment

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Animal Units

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Large Scale Activities

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</tbody>
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### Name

PROZOMIX LIMITED

### Name 2

#### Campus Estate or Research Centre

#### Road Name

WEST END INDUSTRIAL ESTATE

#### Town

HALTWHISTLE

#### County

NORTHUMBERLAND

#### Postcode

NE49 9HA

#### Country

ENGLAND

#### Tel Number

01434 400 455

#### Fax Number

01434 322 822

#### E-mail

#### HSE Division

YORKSHIRE AND NORTH EAST

### Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

Date Premises Closed | Name | Department | Name 2 | Building | Road Name | District | Town | County | Post-code | Country | Withdrawn
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
06/11/2019 | PROZOMIX LIMITED | UNIT 7G | HALTWHISTLE | 3 STATION COURT | HALTWHISTLE | NORTHERN LND | NE49 9HN | ENGLAND | N

06/11/2019 | PROZOMIX LIMITED | BUILDING 4 | HALTWHISTLE | WEST END INDUSTRIAL ESTATE | HALTWHISTLE | NORTHERN LND | NE49 9HA | ENGLAND | N

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The GMSC is chaired by an experienced senior research scientist (PhD, University of Newcastle upon Tyne, 3 yrs Molecular Biology and Enzymology), Postdoc, University of York! 3.5 yrs [Molecular Biology and Structural Enzymology, Senior Lecturer, Northumbria University, 1.5 yrs [Course Leader BSc Biotechnology, GMSC], Industrial Experience, biotechnology company (20 employees), 4.5 yrs [Head of Molecular Biology Division, Safety Officer, Biological Safety Officer, Chair of GMSC1]. Another Prozomix senior researcher also sits on the GMSC, along with a non-scientific Director of the company. Formal meetings are held every 6 months. Formal emails containing risk assessments or each propose new enzyme are prepared by the chair for immediate circulation.

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Spent liquid growth media to be sterilised by addition of 10% (v/v) £0.5 L Virkon for >60 mm. Treated waste to be discarded down designated sink (in containment level I laboratory where all work will take place) with copious water. All other waste to be sterilised by autoclaving following “waste procedure” with monitoring by way of TST Control Integrator Test Strips (Browne), to confirm effectiveness of each run. Inactivation effectiveness of the “waste procedure” for small volume cultures will also be confirmed by frequent (monthly) viability testing performed by plating 0.2 mL of a healthy 16 h culture of the strains used after inactivation by way of the “waste procedure”. During every small scale production run (1 L scale), the production of aerosols will be monitored using open agar plates containing (1) no antibiotic and (2) the selection antibiotic(s). The results of this monitoring will be recorded along with results from the “waste procedure” viability spread plate tests. Toxic materials will be disposed of by special contractor arrangement, as will sharps (collected in sharps bin for eventual incineration). However, toxic waste will be limited to very small quantities of ethidium bromide bound to a flow through cartridge, as per any small molecular biology laboratory. Sharps waste will be emptied annually, and this will only be to ensure the inside of the bin is free from any growth in the bottom.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

With reference to the attached class 1 activity risk assessment, it is reiterated that individual risk assessments will be performed in advance of the development / production of new recombinant enzyme / protein. It is also reiterated that a consensus is required before any practical work can begin with respect to the development! production of a new recombinant enzyme! protein. The GMSC is wholly satisfied that the restricted access purpose designed research and development! production laboratory at Prozomix is perfectly suitable for the small scale recombinant enzyme! protein development / production and other proposed operations as proposed. Notable features with respect to protecting human health and that of the environment and that all work falls into Class 1. Inactivation, monitoring and aerosol production are performed routinely and records kept, all autoclave runs (regardless of reason) are monitored with TST test strips and results logged in a record book, a clear and simple bacterial spill protocol is employed, and full training is provided even to previously experienced members of staff regarding safe performance of the microbiological, molecular biological and GMM aspects of the proposed activities (and records kept).
| Data Premises Notified (Originally) | 01/09/2008 |  |  |  |
| Data Premises Closed | 06/06/2013 |  |  |  |
| Transferred from 1992 Regs? |  | N |  |  |
| Transitional Premises |  |  |  | N |
| Emergency Plan Required? |  | N |  |  |
| Transitional Premises |  |  |  |  |
| Non-GMMs |  |  | N |  |
| Withdrawn |  |  |  | N |

**Name**

SHELL RESEARCH LIMITED

**Campus Estate or Research Centre**

**Road Name**

POOL LANE

**Town**

INCE

**District**

**County**

CHESHIRE

**Postcode**

CH2 4NU

**Country**

ENGLAND

**Tel Number**

01513735984

**Fax Number**

01513735182

**E-mail**

**HSE Division**

blank

**Comments**

TRADING AS SHELL GLOBAL SOLUTIONS UK

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

They tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Tick if confidential

Give brief details of the genetic modification safety committee

The GMSC will meet at least once per quarter to discuss GM risk assessments, and other issues associated with GM and microbiological safety. The GMSC consist of people with training (to PhD level) and experience (many years academic and/or industrial) in molecular and microbiology, biochemistry and genetic manipulation, as well as HSE experts. The GMSC members have also been chosen from the managerial, scientist, and technical levels, and there will also be a representative from the site safety group. This combination of members has been chosen to ensure that all possible issues are raised, discussed, correctly assessed and documented at the GMSC meetings.

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<th>Glass House</th>
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Other (please specify) Tick if confidential

Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research Yes |
All GM work will be carried out in a class 2 laboratory, with autoclave facilities in a room within the class 2 laboratory suite. Liquid waste containing GMMs will usually be autoclaved for 1 hour at 121°C (15psi), except where significant amounts of solvent are present in the growth medium, in which case the GMMs will be inactivated with Virkon treatment for at least 1 hour at room temperature. Autoclave efficiency will be monitored during every autoclave run by checking the autoclave run data (from the internal thermocouple). In addition, inactivation efficiency will be screened on a regular basis by placing biological indicators in the samples being autoclaved, and by attempting to reculturing the autoclaved (or Virkon treated) material after inactivation. After inactivation, non-solvent waste will be disposed of down drains with copious amounts of water, whereas waste containing significant amounts of solvent will follow normal solvent disposal routes.

Solid waste will be put into autoclave bags in the laboratory (themselves contained in distinctive plastic bins to distinguish them from standard laboratory waste bins), and then autoclaved by trained staff. After inactivation, the solid waste will be treated as standard laboratory waste, and then taken for incineration by laboratory stewards.

Spills will be cleaned using standard microbiological spill kits, and if appropriate, surfaces will also be sterilized with 70% ethanol.

The GMSC was happy with this risk assessment, although a few minor changes were suggested, and these have been incorporated into the modified version (attached). These minor changes were around the use of ‘commercial’ vectors; it was felt that work on creating novel plasmid backbones was beyond the scope of this risk assessment, and should be risk assessed separately.

It was agreed that the GMMs would not pose an increased risk to human health. However, there was a discussion about the hazard that these organisms might pose to the environment. It was pointed out that GMMs expressing a whole suite of carbohydrolases might be able to degrade plant biomass, and thus thrive in the natural environment. Counter to this, it was pointed out that these organisms would endure a high metabolic load - due to the amount of protein that they are synthesizing - and would therefore likely be less fit in the natural environment than the wild-type organisms. To degrade natural, untreated plant biomass, these organisms would have to produce, and export cellulases and ligninases (5 or more enzymes in total), and it was agreed that with this metabolic load, they are unlikely to have increased fitness in the natural environment. Furthermore, the use of ligninases is beyond the scope of this work. In light of this, it was decided that treating the GMMs as HG2 organisms provided more than adequate containment measures.
**GM Centre Number: 3017**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The genetic modification safety committee is part of Lab901 Ltd's health and safety committee. All risk assessments and working procedures will be reviewed prior to start of work.

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Tick if confidential

Bacteriology

Parasitology

Transgenic Birds

Microbiology Research

Virology

Transgenic Animals

Transgenic Fish

Gene Therapy
Post analysis, the disposable microfluidic device will be treated with 5% Virkon solution and autoclaved prior to disposal. For bench work, all samples will be treated with 5% Virkon and then autoclaved prior to appropriate disposal to biological waste.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Other(s) Application of GMM as an ideal model target for a development diagnostics instrument. HCV and MLV...

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment was authored by ** and reviewed by **. The health and safety committee will fully review the risk assessment at it's next meeting on 14th August 2008.
GM Centre Number: 3018

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Name
NATURAL ENVIRONMENTAL RESEARCH COUNCIL

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

District

Town
WALLINGFORD, OXON

County
OXFORDSHIRE

Postcode
OX10 8BB

Country
ENGLAND

Tel Number
01491 838800

Fax Number
01491 692424

E-mail

HSE Division
blank

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>OX19 8BB</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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Give brief details of the genetic modification safety committee

Professor Robert Possee, Chair

BSO, Union Side representative, Exotoxicologist, Ecological entomologist, Hydrologist

Tick if confidential

Bacteriology, Parasitology, Transgenic Birds, Transgenic Animals, Transgenic Fish, Microbiology Research, Gene Therapy

02/03/2022
Autoclaving will be used to inactivate waste material giving a degree of kill of 100%. Commercial spore strips will be used to test the efficiency of the process.

Tick to confirm that you are attaching a summary of the risk assessment Y
Tick if you are claiming exemption from disclosure for sections of the risk assessment 

Please enter comments of the GM safety committee on the risk assessment

WLGM-01 and WLGM-02 were reviewed by the GM safety Committee on 10th July 2008. There were some minor changes suggested to WLGM-02, to remove some of the abbreviations. The committee were in agreement that both risk assessments should be sent to the HSE as part of the notification of intention to use the premise for contained use activities.
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**Name**

SPD DEVELOPMENT COMPANY LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

PRIORY BUSINESS PARK

**Road Name**

**District**

**Town**

Bedford

**County**

BEDFORDSHIRE

**Postcode**

MK44 3UP

**Country**

ENGLAND

**Tel Number**

01234 835495

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01234 835006

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**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

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| Comments                          |                   |

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Environmental Health & Safety Manager
Process Science Manager
Scientist Process Science Group

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Tick if confidential

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<th>Microbiology Research</th>
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<th>Transgenic Fish</th>
<th>Gene Therapy</th>
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## For activities involving GMMs, describe the waste management measures which will apply to the activity

All consumables will be single use items disposed of in line with current legislation i.e. Fully licensed hazardous waste contractor. All associated consumables and viosle cells will be incinerated (including lysed cell extract).

<table>
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<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: ___

**Please enter comments of the GM safety committee on the risk assessment**

risk assessment sufficient and satisfactory for control of work being undertaken.
### GM Centre Number: 3021

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**Name**

THE UNIVERSITY OF CAMBRIDGE

**Name 2**

**Department**

**Campus Estate or Research Centre**

UNIVERSITY FORVIE SITE

**Building**

WEST FORVIE BUILDING

**Road Name**

ROBINSON WAY

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 0SZ

**Country**

ENGLAND

**Tel Number**

01223 748405

**Fax Number**

01223 748404

**E-mail**

**HSE Division**

Blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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<th>Building</th>
<th>Road Name</th>
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<td>West Forvie Building</td>
<td>Robinson Way</td>
<td>CAMBRIDGE</td>
<td>CB2 0SZ</td>
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<td>UNIVER</td>
<td>THE WEST FORVIE BUILDING</td>
<td>ROBINSON WAY</td>
<td>CAMBRIDGE</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

ADDED 21/07/2010 & amended 26/05/2016
BSO For the Stem Cell Institute Ann McLaren Laboratory (formerly the Ann McLaren Laboratory for Regenerative medicine/LRM
BSO for Central Biomedical Resources (WFB)
Clinical School Safety Officer
BSO for Cardiovascular Medicine
WFB Biofacility Manager

Biofacility Manager
Yes

Yes

Yes

Yes

All liquid waste will be disposed of in designated containers containing 10% sodium hypochlorite or Virkon and left overnight to ensure inactivation of all potential pathogens and then discarded. All solid waste will be disposed of in designated biological waste bags, autoclaved and then sent for incineration.

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

To be advised.
Purposes of the contained use

Originating from early developing cells after fertilization, embryonic stem cells (ESC) are unique in their capacity to generate all differentiated cell types composing their original organism. The ability to cultivate ESC in vitro offers a tremendous opportunity to the scientific community to study fundamental mechanisms acting through embryonic development as well as opening exciting clinical perspectives by offering wide possibilities for in vitro pharmacological models and cell sources for regenerative medicine. Our global objective is thus to gain better knowledge in molecular factors and mechanisms controlling early ESC fate specification towards the three primitive embryonic germ layers (endoderm, mesoderm and ectoderm) and further differentiation towards mature functional cells. By bringing important insights in fundamental development processes, our project should allow us to gain in parallel a better control in ex vivo differentiation protocols from ESC and improve the production of therapeutically relevant cell types like cardiomyocytes, neurons, pancreatic cells or haematopoietic stem cells for instance.

This general perspective will develop into three leading projects implying genetic modification of various cell types:
- Project1. Factors controlling cell fate specification of pluripotent stem cells
- Project2. Transcriptional factor mediated reprogramming of somatic and embryonic stem cells
- Project3. Gene targeting in human embryonic stem cell

Since they are highly efficient vectors to genetically modify numerous cell types (notably ESC), we plan to use recombinant viral vectors derived from gamma-retroviruses, lentiviruses and adenoviruses to achieve our goal.

We will use recombinant retroviral particles (MuLV derived, from the company Vectalys, France) and recombinant lentiviral particles (HIV-1 derived, production in situ) which have amphotropic ranges of tropism (VSV-G pseudotyped). For some experiments, recombinant adenoviral particles (Ad5 derived, from the company Hybrid Systems, UK) will be also used. All recombinant viral vectors will be replication defective and each production batch
properly tested for absence of infectious particles. Inserts (cDNA, shRNA) will be cloned into these vectors, alone or in combination (through the use of independent promoters, IRES or 2A sequences) with GFP, antibiotic resistance gene or another insert of interest. In situ production of lentiviral particles will be achieved by transient co-transfection of 3 plasmids (vector, helper and envelope) into 293T/17 cells. All lentiviral particles will be produced from SIN (Self Inactivating) backbones using the 2nd generation production system (Vif, Vpr, Nef and Vpu deleted helper plasmid). Work involving recombinant virus handling (production, transduction) and human primary cell culture will be performed under containment level 2 conditions in compliance with institution practice.

Recipient or parental organism
1. Escherichia coli - K12 derived strains, disabled, non colonising
2. Gamma oncoretroviruses – MuLV derived, replication defective, amphotrope
3. Lentiviruses – HIV-1 derived, replication defective, amphotrope
4. Adenoviruses – Ad5 derived, replication defective, ecotrope
5. Mammalian cells (primary cells and cell lines) – considered as especially disabled
   - Human, murine embryonic stem cell lines (H9, H1, HSF6, ...)
   - Human, murine induced pluripotent stem cell lines (produced in situ)
   - Human, murine primary cells (embryonic and adult skin fibroblasts, keratinocytes, peripheral blood cells)
   - Human, murine somatic cell lines (HEK-293T, HCT-116, ...)

Host/vector system
1. E.coli K12 / pUC derived plasmids - no insert expression, plasmid non mobilisable.
2. HEK-293T / pUC derived plasmids – transient co-transfection, viral gene and insert expression, production of amphotrope replication deficient lentiviral particles (each batch to be tested).
5. Mammalian cells / Ad5 vectors – replication defective adenoviruses, transient genetic modification.

Origin & function
1. Human, murine, xenopus cDNA (+/- TAG sequences) coding for transcription factors, signal transducers, growth factors and surface receptors. These inserts are expected to play a role in pluripotency, cell fate decision and differentiation from pluripotent stem cells (PSCs). Their expression may enhance/block commitment and differentiation of PSCs towards one of the three primitive germ layers and further differentiated progeny.
2. shRNA and miRNA sequences against cell endogenous coding sequences. These inserts are non coding RNA sequences targeting cell endogenous mRNA from above mentioned factor families (in 1) in order to inhibit protein expression by RNA interference (RNAi). Their expression may enhance/block commitment and differentiation of PSCs towards one of the three primitive germ layers and further differentiated progeny.
3. Reporter genes (GFP, LacZ, Luciferase, possibly fused to cDNA (type1 inserts)). These inserts will code for proteins with fluorescent or enzymatic properties allowing easy identification of genetically modified (GM) cells. They are thought to have no deleterious biological effect on expressing cells.
4. Selection genes (NPT, PAC). These inserts will produce enzymatic proteins able to inactivate specific antibiotic families allowing selection of GM cells. They are thought to have no deleterious biological effect on expressing cells.
5. Functional non coding sequences. These inserts will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES). They will be used (independently or in combination) into viral expression cassettes to achieve best
control of transgene expression depending on targeted cells.
6. Neutral non coding sequences (introns, isolated exons). These inserts will be used in particular for gene targeting projects to promote homologous recombination at defined genomic loci (intronic/exonic genomic sequences). They should not have biological effect by themselves.

**Evaluation of foreseeable effects**

**Human Health considerations -**

The most obvious risk for human health is linked to accidental infection of worker by recombinant viral particles. Indeed, because only replication defective viral particles (non infectious) will be used for experimental work through this project and inserts will not produce compounds with acute toxicity (like bacteria toxins), no additional risk will be associated with the final GMO itself (mammalian cells) compared to initial organism. Importantly, primary cells coming from human samples are uncharacterised and may contain adventitious agents: they will be handled at containment Level 2 (COSHH regs).

First, risks associated with the nature and design of recombinant viral particles has to be assessed. Retroviral recombinant particles (MuLV and HIV-1 derived) will be VSV-G pseudo-typed (protein G from the Vesicular Stomatitis Virus will replace the native envelope protein). VSV-G packaging alters the tropism of the retroviral particles, requiring interaction only with phospholipids present in the plasma membrane of all cells rather than interaction with a cognate receptor as occurs with ecotropic packaging. Thus, VSV-G pseudo-typed retroviruses acquire the ability to efficiently infect virtually all human cell types. Moreover, VSV-G pseudo-typing improves stability of the viral particle and may represent an aerosol means of transmission in addition to the expected percutaneous risk. Retroviruses stably integrate the host cell genome. This property is associated with a risk of insertional mutagenesis, i.e. ectopic activation/inhibition of host gene expression after colocorized provirus integration. Subsequent deregulation of endogene expression could theoretically lead to transduction associated detrimental effects like initiation of oncogenic processes. Moreover, the addition of the WPRE (Woodchuck Hepatitis Post-transcriptional Regulatory Element) to the expression cassette in several retroviral backbones could also enhance the oncogenic potential of such vectors (SACGM, annual report 2004). Importantly, among retroviral vectors, the use of lentiviral derived backbone is thought to be safer. First, genomic integration profile of lentiviral derived vectors do not show an integration bias toward the transcriptional start site region of host cell genes like MuLV ones. Moreover, we will use exclusively SIN (Self Inactivating) vectors devoid of viral enhancer activity which reduces further the likelihood of endogene activation. In contrast, recombinant adenoviral vectors are considered safer. Indeed, adenoviral genome is rarely seen to integrate into the host cell genome and transgene expression remains transient in host cells. Moreover, the disease caused by wild type adenovirus is usually just a mild and self limited common cold.

Risks associated with inserted coding sequences should also be assessed. cDNA (or shRNA/miRNA) inserts carried by viral vectors will direct ectopic expression (or inhibition) of transcription factors, signal transducers, growth factors and receptors which are expected to play a role in pluripotency, cell fate decision and differentiation from pluripotent stem cells. It is difficult to predict in vivo consequences of ectopic expression for all these genes on human health. However, since they are key regulator of cell identity, their overexpression could lead to pathological modifications of cell phenotype/function. Moreover, several of these genes have been involved in oncogenic processes and uncontrolled expression could subsequently initiate tumoral transformation. However, malignancy is a complex multistep process which involves multiple genomic alterations and a single “hit” is unlikely to trigger alone the oncogenic process. Experiments involving co-transduction or even co-expression by the same vector of several inserts should consequently be considered at higher risk for the worker. Notably, reprogramming experiments of somatic cells toward pluripotency (co-transduction with the 4 factors OCT4, SOX2, MYC, KLF4) could imply a specific risk since accidental genetic modification of worker cell could theoretically lead to teratoma formation.

Above mentioned risks will be tempered by the fact we will use strictly replication defective viral vectors unable to propagate further after transduction. For all viral vector type, in the likelihood of an accidental contamination...
(percutaneous, aerial), amount of viral vectors able to effectively contaminate worker cells will be smaller and transduction much less efficient compared to improved in vitro transduction protocols used on cultivated cells. Of note, MuLV derived vectors are only efficient in transducing proliferating cells that limits the number of potential accidental targets. Importantly, VSV-G pseudotyping of retroviral particles results also in complement sensitivity increasing the likelihood of immunological neutralisation in human hosts. Together, these considerations suggest that only low number of cells may be effectively transduced through accidental contamination. Moreover, malignancy is a complex multistep process which involves multiple genomic alterations and it is unlikely that any single “hit” corresponding to an accidental infection would initiate transformation. Notably, cells exposed to non percutaneous accidental infection (stratum corneum, respiratory epithelium) are terminally differentiated cells with limited lifespan and high turnover, which greatly limits the risk of effective tumoral transformation. Nevertheless, in the context of experiments using polycistronic viral vectors for combined expression of several cDNA, tumoral transformation risk may be higher: appropriate safety measures will be undertaken to limit accidental contamination and all workers will be informed of additional risk associated with the use of such specific viral constructions.

- Environment considerations -
VSV-G pseudotyping of recombinant retroviral particles (MuLV and HIV-1 derived) would enable vectors to infect a variety of animal cell types including those of different animal species. Moreover, environmental stability tends to be increased by VSV-G pseudotyping compared to retroviruses displaying the native envelope. However, retroviral particles remain sensitive to air, temperature and pH and will have short lifespan in open environment. Importantly, recombinant retroviral vectors will be replication defective and could not produce progeny viruses able to spread to the wider human or other animal populations.

Adenoviruses are generally species specific. Indeed, serotype 5 Adenoviruses infect humans and does not naturally infect other animals. Therefore, the risk of accidental infection of other organisms is very low. Because recombinant adenoviruses will be replication incompetent, they could not spread to the wider human population from any accidentally infected individual.

*Transgene mobilization issue for retroviruses.* Recombinant viral vectors which will be used to genetically modify mammalian cells will be replication deficient and thus unable to propagate to the environment. However, a risk of recombinant vector mobilization through concomitant wild type retrovirus contamination of the targeted cell still exists. Indeed, recombinant retroviruses encompass a native encapsidation sequence which could allow production of transgene carrying viral particles if complementing viral proteins (enzymes, capsid and envelope) are provided by a compatible wild type retrovirus. The likelihood of such mobilization is still very low, even less with SIN Lentiviruses which are devoid of provirus LTR activity. Importantly, mobilized recombinant retroviruses would still be unable to replicate in other cells than those carrying the complementing wild type retrovirus. Moreover, amphotropism associated with VSV-G pseudotyping would no longer exist for viral particles encompassing mobilized recombinant retroviral genomes.

Since generation of replication competent viral particles (RCV) is associated with the highest risk factor for human health and environment, production steps will be given the highest risk factor in the whole process (i.e. production, transduction, GM cell analysis). All lentiviral productions will be carried out in strict Class2 conditions by trained and experienced workers. To decrease the likelihood of infection standard measures to decrease percutaneous as well as aerosolised transmission of viral particles will be adopted, such as no use of sharps and all work to be performed within a class II microbiological safety cabinet. Moreover, configuration of the laboratory premises will allow direct
autoclaving of all equipment in the Class 2 related lobby. All lentivirus batches will be tested for absence of RCV before use for cell transduction. In the very unlikely event of RCV generation, decontamination procedures will be carefully carried out and the event notified to the local BSO. Notably, no RCV generation involving 2nd generation SIN lentiviral vectors has ever been reported as today in the literature. MuLV derived retroviral particles and adenoviral particles will be produced by external manufacturers (Vectaly and Hybrid Systems respectively) and production batches demonstrated RCV free by internal validated methods.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For some experiments (teratoma formation, hematopoietic engraftment), genetically modified mammalian cells (human, murine) could then be transplanted by tissular or systemic injection into immunodeficient recipient mice to generate chimaeras. Importantly, protocols involved will not allow germ line transmission of the genetic modification. Since all viruses utilised to genetically manipulate the cells before transplant will have been tested for absence of RCV, there will be no risk of further infection and there will also be a minimal risk that the animals could escape from the animal facility.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- All disposable culture/lab ware from the CL2 laboratory will be double-bagged, properly labelled as biohazard and autoclaved before leaving the building for incineration (autoclaving in adjacent lobby). [100% kill]
- All highly contaminated material, i.e. all stuff in contact with viruses (pipettes, tips, culture plates, liquid waste bottles), will be separately decontaminated (Virkon 2%/Trigene Advanced 10% for 16 hours) and liquids solidified (Vernagel) before autoclaving. [100% kill]
- Small spills will be removed after one minute surface soaking with Trigene Advanced 1% and further decontamination by 70% Ethanol. [effectively 100% kill]
- Large accidental spills will be sprinkled with Virkon powder before cleaning. [effectively 100% kill]
- Bench and cabinet surfaces will be wiped down with Trigene Advanced 1% followed by 70% Ethanol. [effectively 100% kill]
- All GM modified cell cultures will be destroyed (soaking with Trigene Advance 1% and autoclaving/incineration) [100% kill] or chemically fixed/lysed (PFA 1-4%, Guanidin, RIPA) before leaving containment [effectively 100% kill].

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
This project has been reviewed on 8th April 2009 by the West Forvie Building biological and genetic safety committee. This is satisfied that the risks have been properly addressed and that the work will be carried out under the appropriate controls and conditions. Nevertheless, the committee stressed on the need for additional risk assessment and specific information for workers undertaking experimental work with multiple insert (polycistronic) viral vector constructs which are associated with a higher risk for human health through accidental infection. Moreover, the committee emphasized that work with replication defective adenoviral vectors could be actually done at containment level 1 using MSC II.

**Project Containment**

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- **Animal Units**: L2 L3 L4
- **Large Scale Activities**: L2 L3 L4
- **Human Clinical Applications**: L2 L3 L4

**Project Ref**: 3021/10.1

- **Date Ackn'd**: 10/09/2010
- **CU2 Project Title**: Pre-clinical study of the therapeutic plasticity of somatic neural stem cells: secreted membrane particles, transcription factors and cell transplants in rodents with experimental autoimmune encephalomyelitis

- **Class**: Non-GMM
- **Culture Vol**: Consent Granted
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

Project: "Secreted membranes vesicles: role in the therapeutic plasticity of neural stem cells.
We use a bidirectional lentiviral vector (Bd.LV), as a sensitive reporter system. This vector coordinately expresses two transgenes, green fluorescent protein (GFP) and low-affinity nerve growth factor receptor (ΔNGFR), as distinct transcripts. By inserting perfectly complementary miRNA target (miT) sites into the 3' untranslated region (3' UTR) of the GFP cDNA, GFP expression is subject to miRNA-mediated regulation; ΔNGFR expression is unaffected by miRNA activity and serves as a control.
We have two kinds of LVs carrying respectively: the wild type GFP as a reporter gene or a rapid turnover or destabilized GFP as a reporter gene.

2) Project 2: Programming the oligodendroglial cell fate in neural stem/percursor cells.
We will take advantage of a lentiviral vector-based system to induce the expression (both inducible and constitutive) of either of three main transcription factors (TFs) involved in oligodendrogenesis: Olig2, Mash 1 and Sox10. In particular, we will use a first set of bidirectional lentiviral vectors (Bd.LV) that allow the coordinate constitutive expression of one of the given TFs as a reporter gene (either GFP or ΔNGFR) with the final aim of investigating the effect of the constitutive expression of each given TF on NPCs biology and differentiation potential. Subsequently, a second set of Bd.LVs (allowing the expression of a TF under the control of a Tetracycline inducible promoter (TetON) and the reverse transactivator (rTA) under the control of a constitutive promoter) will be used to achieve the temporal control of the transgene expression.

3) Project 3: transplantation of pluripotent stem cell-derived NOCs in experimental autoimmune encephalomyelitis. The transition from the neural crest to the SCP stage is marked by the appearance between others of myelin protein zero (PO) mRNA and cadherin 19. Cadherin 19 is down-regulated again as SCPs generate Schwann cells and is therefore the only well characterised marker that is restricted to the SCP stage (in vivo). Immature Schwann cells express S100β. Myelination is accompanied by equally extensive down-regulation of a set of molecules that characterise the immature Schwann cell stage such as the p75 neurotrophin receptor.
These constructs are intended to create a battery of hES cell lines expressing a fluorescent marker (GFP) under the control of promoters concerning the expression of stage-specific genes along the Schwann cell differentiation lineage. These markers will be used to selectively follow out hES cells after their differentiation towards a peripheral glial lineage.
Instead, a lentiviral vector carrying a double fusion reporter gene that stably expresses enhanced green fluorescene protein (eGFP) and firefly luciferase (fluc) reporter genes will be used to carry out an in vivo monitoring (through bioluminescence and magnetic resonance) of the presence, proliferation, migration and fate of the infected cells once transplanted into the host.

Recipient or parental organism
One Shot TOP10 Chemically Competent E. coli - disabled
Recombinant lentiviruses - HIV-1 derived, 3rd generation, replication defective, amphotrope
Mammalian cells (primary cells and cell lines) - considered as especially disabled.
- Human, murine somatic neural stem/precursor cell (NPCs);
- Human, murine somatic cell lines (HEK-293T, NIH-3T);
- Human embryonic stem cells;
- Human induced pluripotent stem cells.

Host/vector system
- TOP10 E. coli/pUC derived plasmids - no insert expression, non mobilisable
- HEK-293T/pUC derived plasmids - transient co-transfection, viral gene and insert expression (3rd generation system/4 plasmids) production of amphotrope replication deficient HIV1 derived lentiviral particles (each production batch to be tested). All transfer vectors were built from plasmid pCCL.sin.cPPT.PGK.GFP.WPRE or pRRL plasmid (see Follenzi et al., Nat Genet, 25: 217, 2000)
- Mammalian cells/ HIV-1 vectors - replication defective lentiviruses, stable genomic integration

Origin & function
Type 1 (cDNA) inserts will code for transcription factors, which are expected to play a role in cell fate decision and differentiation of neural stem/precursor cell (NPCs) or...
hES cells after their differentiation towards a central vs peripheral glial lineage, and are not expected to affect either proliferation or differentiation of targeted cells. It is difficult to predict in vivo consequences of ectopic expression of all these genes on human health. Since they are key regulator of cell identity, their overexpression could lead to pathological modifications of cell phenotype/function. However, all these transcription factors are mammalian derived, naturally occurring and does not affect cell proliferation thus suggesting the absence of major risks for human health and environment.

Type 2 (miRNA/miRNA target sequences) inserts will produce non-coding miRNA sequences targeting cell endogenous mRNA or exogenous miRNA target sequences on miRNA-regulated vectors, possibly inhibiting protein expression. As for Type 1, it is difficult to predict in vivo consequences of ectopic expression of these sequences on human health. However, since they target key regulators of cell identity, their expression could lead to pathological modifications of cell phenotype/function. Notably, several miRNA have been implied in oncogenic processes.

Type 3 (reporter genes) inserts will code for proteins with fluorescent or enzymatic properties allowing easy identification of genetically modified (GM) cells. They are thought to have no deleterious biological effect on expressing cells expected acute toxicity at very high concentration.

Type 4 (selection genes) inserts will code for enzymatic proteins able to inactivate specific antibiotic families allowing selection of GM cells. They are thought to have no pathological effect on expressing cells excepted acute toxicity at very high concentration.

Type 5 (functional non-coding sequences) inserts will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES). They will be used (independently or in combination) into the expression cassette to achieve best control of insert expression depending on the targeted cell. No direct pathological effect is expected for these sequences. However, indirect deleterious effects should be considered for promoter and enhancer sequences through retrovirus mediated genomic integration in the host cell and potential insertional mutagenesis. Indeed, strong promoter/enhancer could lead to ectopic activation of neighbour endogene expression. Moreover, the WPRE sequence has been shown to have oncogenic properties by itself.

**Evaluation of foreseeable effects**

- **Human Health considerations**

The most obvious risk for human health is linked to accidental infection of worker by recombinant viral particles. Indeed, because only replication defective viral particles (non infectious) will be used for experimental work through this project and inserts will not produce compounds with acute toxicity (like bacteria toxins), no additional risk will be associated with the final GMO itself (mammalian cells) compared to initial organism. Importantly, primary cells coming from human samples are uncharacterised and may contain adventitious agents: they will be handled at containment Level 2 (COSHH regis).

First, risks associated with the nature and design of recombinant viral particles has to be assessed. Lentiviral recombinant particles (HIV-1 derived) will be VSV-G pseudo-typed (protein G from the Vesicular Stomatitis Virus will replace the native envelope protein). VSV-G packaging alters the tropism of the retroviral particles, requiring interaction only with phospholipids present in the plasma membrane of all cells rather than interaction with a cognate receptor as occurs with ecotropic packaging.

Thus, VSV-G pseudo-typed retroviruses acquire the ability to efficiently infect virtually all human cell types. Moreover, VSV-G pseudo-typing improves stability of the viral particle and may represent an aerosol means of transmission in addition to the expected percutaneous risk. Retroviruses stably integrate the host cell genome. This property is associated with a risk of insertional mutagenesis, i.e. ectopic activation/inhibition of host gene expression after colocalized provirus integration. Subsequent deregulation of endogene expression could theoretically lead to transduction associated detrimental effects like initiation of oncogenic processes. Moreover, the addition of the WPRE (Woodchuck Hepatitis Post transcriptional Regulatory Element) to the expression cassette in several retroviral backbones could also enhance the oncogenic potential of such vectors (SACGM, annual report 2004). Importantly, among retroviral vectors, the use of lentiviral derived backbone is thought to be safer. First, genomic integration profile of lentiviral derived vectors do not show an integration bias toward the transcriptional start site region of host cell genes like MuLV ones. Moreover, we will use exclusively SIN (Self Inactivating) vectors devoid of viral enhancer activity which reduces further the likelihood of endogen activation.

Risks associated with inserted coding sequences should also be assessed. cDNA or (shRNA/miRNA) inserts carried by viral vectors will direct ectopic expression (or inhibition) of transcription factors, signal transducers, growth factors and receptors which are expected to play a role in pluripotency, cell fate decision and differentiation from pluripotent stem cells. It is difficult to predict in vivo consequences of ectopic expression for all these genes on human health. However, since they are key regulator of cell identity, their overexpression could lead to pathological modifications of cell phenotype/function. However, malignancy is a complex multistep process which involves multiple genomic alterations and a single "hit" is unlikely to trigger alone the oncogenic process. Experiments involving co-transduction or even co-expression by the same vector of several inserts should consequently be considered at higher risk for the worker.

Above mentioned risks will be tempered by the fact we will use strictly replication defective viral vectors unable to propagate further after transduction. For all viral vector type, in the likelihood of an accidental contamination (percutaneous, aerial), amount of viral vectors able to effectively contaminate worker cells will be smaller and transduction much less efficient compared to improved in vitro transduction protocols used on cultivated cells. Importantly, VSV-G pseudotyping of retroviral particles results in complement sensitivity increasing the likelihood of immunological neutralisation in human hosts. Together, these considerations suggest that only low number of
cells may be effectively transduced through accidental contamination. Moreover, malignancy is a complex multistep process which involves multiple genomic alterations and it is unlikely that any single "hit" corresponding to an accidental infection would initiate transformation. Notably, cells exposed to non percutaneous accidental infection (stratum corneum, respiratory epithelium) are terminally differentiated cells with limited lifespan and high turnover, which greatly limits the risk of effective tumoral transformation. Nevertheless, in the context of experiments using polycistronic viral vectors for combined expression of several cDNA, tumoral transformation risk may be higher: appropriate safety measures will be undertaken to limit accidental contamination and all workers will be informed of additional risk associated with the use of such specific viral constructions.

Environmental considerations -

VSV-G pseudotyping of recombinant lentiviral particles would enable vectors to infect a variety of animal cell types including those of different animal species. Moreover, environmental stability tends to be increased by VSV-G pseudotyping compared to retroviruses displaying the native envelope. However, retroviral particles remain sensitive to air, temperature and pH and will have short lifespan in open environment. Importantly, recombinant retroviral vectors will be replication defective and could not produce progeny viruses able to spread to the wider human or other animal populations.

*Transgene mobilization issue for retroviruses.

Recombinant viral vectors which will be used to genetically modify mammalian cells will be replication deficient and thus unable to propagate to the environment. However, a risk of recombinanat vector mobilization through concomitant wild type retrovirus contamination of the targeted cell still exists. Indeed, recombainant retroviruses encompass a native encapsidation sequence which could allow production of transgene carrying viral particles if complementing viral proteins (enzymes, capsid and envelope) are provided by a compatible wild type retrovirus. The likelihood of such mobilization is still very low, even less with SIN Lentiviruses which are devoid of provirus LTR activity. Importantly, mobilized recombinant retroviruses would still be unable to replicate in other cells than those carrying the complementing wild type retrovirus. Moreover, amphotropism associated with VSV-G pseudotyping would no longer exist for viral particles encompassing mobilized recombinant retroviral genomes.

Since generation of replication competent viral particles (RCV) is associated with the highest risk factor for human helath and environment, production steps will be given the highest risk factor in the whole process (i.e. production, transduction, GM cell analysis). All lentiviral productions will be carried out in strict Class 2 conditions by trained and experienced workers. To decrease the likelihood of infection standard measures to decrease percutaneous as well as aerosolised transmission of viral particles will be adopted, such as no use of sharps and all work to be performed within a class II microbiological safety cabinet. Moreover, configuration of the laboratory premises will allow direct autoclaving of all equipment in the Class 2 related lobby. All lentivirus batches will be tested for absence of RCV before use for cell transduction. In the very unlikely event of RCV generation, decontamination procedures will be carefully carried out and the event notified to the local BSO. Notably, no RCV generation involving 3rd generation SIN lentiviral vectors has ever been reported as today in the literature.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

In the context of project 3 "transplantation of pluripotent stem cell-derived NPCs in experimental autoimmune encephalomyelitis" genetically modified mammalian cells will be transplanted by tissular or systemic injection into mice. Importantly, protocols involved will not allow germ line transmission of the genetic modification. Since all viruses used to genetically manipulate the cells before transplant are replication deficient, there will be no risk of further infection and risk of animal escape from the animal facility is minimal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All disposable culture/lab ware from the CL2 laboratory will be double bagged, properly labelled as biohazard and autoclaved before leaving the building for incineration. (Autoclave located in lobby 218, outside CL2 room)

All highly contaminated material, ie. in contact with viruses will be decontaminated separately using 2% Virkon or 10% Trigene Advanced for 16 hours prior to autoclaving.

Small surface spills will be removed after soaking with 1% Trigene Advanced followed by wiping with 70% Ethanol

Larger surface spills will be sprinkled with Vrkon powder before cleaning as above.
Bench and safety cabinet surfaces will be wiped down with 1% Trigene Advanced followed by 1% Ethanol before and after working.

All GM modified cell cultures will be destroyed by soaking in 1% Trigene Advanced followed by autoclaving as 134 degrees C for 3 minutes and then incineration on the Addenbrooke's Hospital site or will be chemically fixed using 1-4% Paraformaldehyde solution, Guanidin, RIPA) before leaving the laboratory.

Autoclaving process is validated by the use of Browne Steriliser Tubes, type 2 for steam cycles at 134C for 3 mins. Each autoclaving cycle is monitored using an internal temperature probe properly fit into waste bags.

This project has been reviewed on 6th June 2010 by the West Forvie Biolding biological and genetic safety committee. This is satisfied that the risks have been properly addressed and that the work will be carried out under the appropriate controls and conditions.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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GM Centre Number: 3028

Data Premises Notified (Originally) 14/10/2008

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed 14/10/2008

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name
LIVERPOOL JOHN MOORES UNIVERSITY

Name 2

Department
LIFE SCIENCE SUPPORT UNIT

Campus Estate or Research Centre
FACULTY OF SCIENCE

Building

Road Name
BYROM STREET

District

Town
LIVERPOOL

County MERSEYSIDE

Postcode L3 3AF

Country ENGLAND

Tel Number 0151 231 2218

Fax Number

E-mail

HSE Division blank

Comments
Merged with GM257 (this premises notified in error)

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<th>Date Premises Closed</th>
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<td>Life Sciences Support Unit</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The Life Sciences Support Unit Management Committee meets quarterly and issues of genetic modification safety will be addressed at these meetings and a safety officer present to offer advice.

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Non-microbial

Other (please specify)  

Tick if confidential

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<th>Microbiology Research</th>
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<td>Gene Therapy</td>
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<tr>
<td>Virology</td>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste generated will be treated as clinical waste and disposed of by a designated commercial contractor off-site.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

At the Committee's previous meeting, this research was discussed and no safety issues were raised.
<table>
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**Name**

HEPTARES THERAPEUTICS LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

STEINMETZ BUILDING

**District**

GREAT ABINGDON

**Town**

CAMBRIDGE

**County**

**Postcode**

CB21 6DG

**Country**

ENGLAND

**Tel Number**

020 8906 7100

**Fax Number**

020 8816 2044

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

**Y**

Give brief details of the genetic modification safety committee

Composition - Biological Safety Officer (Secretary), Chief Scientific Officer, Cell Culture Scientist, Molecular Biologist, Chair person, Safety Advisor

The Committee will consider all applications for work involving genetic modification and will advise management on their suitability. The Committee will call on outside expertise if necessary.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

All liquid waste will be chemically inactivated using 2500 ppm available chlorine before discharge to sewers.

Solid waste will be autoclaved at 121 degrees for 15 mins using a vacumed pulse autoclave.

All these are standard approved methods of disposal. See attached Code of Practice.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Biological Safety Committee at the National Institute for Medical Research, Mill Hill, London NW7 1AA has approved this work at Class 1 to be carried out at Containment Level 1.

**Project Ref 3031/22.1**

**Date Ackn'd** 02/03/2022  **CU2 Project Title** Expression and functional characterisation of GPCRs and associated biomarkers in HG2 cells of mammalian origin  **Date Project Ceased** 02/03/2022  **Class** Class 2  **CultureVolClass2** < 1 Litre  **CultureVolumeClass3-4**  **Non-GMM Consent Granted**  **Project notified under transitional arrangements** N
**Project Additional Information**

**Purposes of the contained use**

This assessment describes the expression of a protein of interest in cell lines classified as Hazard Group 2 to facilitate relevant biological assays to demonstrate target engagement and proximal pharmacology as well as pertinent downstream effects for the purposes of drug discovery.

Work that may be carried out on these cell lines includes, but is not limited to:
- Transient transfection or baculovirus infection of primary cells or HG2 cell lines.
- Generation of stable cell lines expressing express GPCRs or their associated proteins.
- Assessing the expression of GPCRs and their associated proteins by RNA analysis or labelling by means of antibodies, fluorescent ligands nanobodies or label free methods.
- Functional characterisation of these receptors by signalling assays or other downstream assays such as cytokine or mediator release.

Future work along these lines is likely to include the use of lentiviruses to express these proteins, subject to suitable risk assessment and at the appropriate containment level.

**Recipient or parental organism**

A cell line or primary cell is classified hazard group 2 (HG2) if it is possible that it contains a biological agent that can cause human disease and may be a hazard to employees but is unlikely to spread to the community and there is usually effective prophylaxis or treatment available. These cell lines have often been immortalised using a retrovirus that may express oncogenes such as v-myc or v-raf. They are often well characterised and will be obtained from reliable sources, yet cannot be guaranteed to be virus-free, posing a small risk of infection to users.

This work may include, but is not limited to:
- Well characterised cell lines from the National Institute of Health or the American Type Culture Collection for which work is recommended to be carried out at containment level 2, eg hTERT immortalized cells, HeLa cells etc.
- Cell lines transformed/immortalised with viral agents (e.g. SV40) and/or oncogenes eg microglial cell lines such as IMG or induced pluripotent stem cells (IPSCs).
- Primary cells or cells without a well-defined history.

**Host/vector system**

Work with these cells may involve utilization of endogenously expressed receptors or signalling proteins, or these may be introduced into the cell by transient transfection, or by means of a viral vector system such as, but not limited to, baculovirus- based technologies such as BacMam.

Transfection of mammalian cell lines is achieved with plasmids, into which the cDNA encoding a wild-type or mutant G protein coupled receptor (GPCR) or accessory protein, such as a co-receptor or signalling molecule, has been ligated. In addition to the cDNA of the protein being analysed, additional coding sequence may be present. This may encode Enhanced Green Fluorescent Protein, a biotin-acceptor peptide or affinity tags such as His10, myc tag, Flag tag, hemagglutinin tag, Strep-tag or Avi-tag. A sequence corresponding to a protease cleavage site may also be present. In addition, the GPCR coding sequence may contain an insertion coding for a protein or protein fragment such as T4 lysozyme or apocytochrome b(562)RIL. The plasmid will also contain gene(s) encoding antibiotic resistance. Expression of genes may be driven by a strong promoter such as pCMV or a weaker one such as the pTK. These systems may also be used to generate a cell line stably expressing the insert protein.

The gene of interest may also be delivered into the cells using baculovirus- based technologies such as BacMam (derived from pFastBac from Life Technologies) and
derivatives thereof including pHepBacMam with a variety of tags and signal sequences, and may additionally encode genes for antibiotic resistance. While baculovirus are capable of infecting mammalian cells and the mammalian promoter they contain permit expression of the gene of interest, they are incapable of replication either within these cells or in insects; these systems in themselves are safe to use at containment level 1.

Origin & function

The inserted genes encode GPCRs or accessory proteins such as GPCR co-receptors and downstream signalling molecules. The function of GPCRs is to initiate different signalling cascades upon ligand binding. The signalling cascades result in variety of cellular responses such as transcription, proliferation, differentiation, development, inflammation, or apoptosis.

In addition to the cDNA of the protein being analysed, additional coding sequence may be present. This may encode Enhanced Green Fluorescent Protein, a biotin-acceptor peptide or affinity tags such as His10, myc tag, Flag tag, hemagglutinin tag, Strep-tag or Avi-tag. A sequence corresponding to a protease cleavage site may also be present. In addition, the GPCR coding sequence may contain an insertion, coding for a protein or protein fragment designed to aid crystallography, such as T4 lysozyme, apocytochrome b(562)RIL, flavodoxin, rubredoxin, glycogen synthase or similar fragments. These are not known to be toxic, oncogenic, or otherwise harmful.

Evaluation of foreseeable effects

Risk from host cell lines:
Primary human cells may contain adventitious agents that can cause human disease. Cell lines that have been immortalised using oncogenes or a retrovirus that may express oncogenes such as v-myc or v-raf, are often well characterised and will be obtained from reliable sources, yet cannot be guaranteed to be completely virus-free, posing a small risk of infection to users. IPSCs are also known to pose a potential risk of in vivo teratoma formation. Manipulation of these cells such as centrifugation, sonication etc may give rise to potentially infectious aerosols. Another possible route would be direct inoculation of cell material. Large volumes of culture carry greater risk of exposure and should be handled with care.

These cell lines are all adapted to growth under laboratory conditions and would not survive without specific media and atmospheric requirements, whereas primary cells are incapable of survival beyond a few passages. Therefore, they would be unable to survive outside of the laboratory and the risk of any transfer is negligible. Subsequent addition of a retroviral packaging mix to cell lines that have already been manipulated through retroviral transduction, which has the potential to mobilise the integrated genes, is also a risk to be considered.

Risk from vector/insert genes:
The genes to be expressed exogenously are not known to be toxic or oncogenic and are not expected to alter the pathogenicity of the cell lines. The vectors used do contain genes for antibiotic resistance.

The wearing of lab coat, gloves and safety goggles is required when working with HG2 material. The routes of entry for the gene product or cell derived material into the user would be via direct inoculation, or inhalation of aerosols. For this reason, work must take place in a Class II MSC and the use of sharps is prohibited. Reagents should be risk assessed prior to use on these cells to ensure absence of retroviral packaging elements. Staff will never use their own cells for work. Culture volumes may vary with the experiment being performed, but staff will strive to keep the volumes to the minimum necessary.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste generated from this contained use include cell culture supernatants as well as solid waste in the form of cell waste, plasticware etc. Liquid cultures from HG2 cell lines or GM Class 2 material will be inactivated with 1% Chemgene or similar disinfectant for at least 30 minutes prior to disposal to drain with plenty of water. The efficacy of Chemgene has been documented by the manufacturer (https://www.starlabgroup.com/Documents/eng/1167785.pdf) and is safe and effective for use as a disinfectant of CL2 waste.

All solid waste, including cell waste and plasticware will be disinfected with 1% Chemgene for 30 minutes prior to disposal in double bagged clinical waste sacks. These are collected by an external contractor for incineration.
A defined spill control procedure that takes into account the potential for class 2 GMs is in place—training has been provided to all users and the protocol has been practiced.

The GM committee was presented an overview of the planned work, including a summary of the existing facilities and working practices already in place (adequate containment facilities with access provided to trained personnel and appropriate waste disposal measures). The risk assessments for this work were then presented to the committee (also circulated before the meeting). A query was raised about the risks of teratoma to pregnant women. In reply it was pointed out that the risk was not known to be higher than for the general population, which is already significantly reduced as the cells will not be maintained in culture onsite. A member of the committee with experience of working with iPSCs confirmed all potential risks have been included in the assessment and the control measures would adequately cover these. The nature of the planned GM work was reviewed. The insert genetic material will not alter the category of risk associated with the HG2 cell line. The plasmid construction is class 1 and can be undertaken at containment level 1, but work with HG2 cell lines must be undertaken at containment level 2. The proposed modifications will not alter the tropism, pathogenicity or otherwise of the cell lines and would not increase the category of risk associated with them.

The committee also discussed safety and decontamination of instruments used in the analysis of HG2 material and reviewed cleaning protocols and user training requirements and recommended the use of secondary containment for transport, clear labelling and warning stickers. Existing cleaning protocols were found to be adequate for the level of risk posed by HG2 material. Provision of training with updated safety guidelines for all users was agreed. The disposal of GM class 2 waste was also discussed. The protocols recommended in the GM risk assessment were deemed satisfactory for the intended use. The committee also discussed storage requirements for HG2 material, and recommended that this could be safely stored in clearly labelled cryoboxes (which would constitute secondary containment) within a -150°C freezer.

The committee agreed this was a GM Class 2 project and the potential risks to users and the environment were sufficiently addressed by the risk assessments; by following the control measures indicated, these risks were considered to be effectively minimised.

Please enter comments on the GM safety committee on the risk assessment

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The committee agreed this was a GM Class 2 project and the potential risks to users and the environment were sufficiently addressed by the risk assessments; by following the control measures indicated, these risks were considered to be effectively minimised.

Please enter comments on the GM safety committee on the risk assessment

The GM committee was presented an overview of the planned work, including a summary of the existing facilities and working practices already in place (adequate containment facilities with access provided to trained personnel and appropriate waste disposal measures). The risk assessments for this work were then presented to the committee (also circulated before the meeting). A query was raised about the risks of teratoma to pregnant women. In reply it was pointed out that the risk was not known to be higher than for the general population, which is already significantly reduced as the cells will not be maintained in culture onsite. A member of the committee with experience of working with iPSCs confirmed all potential risks have been included in the assessment and the control measures would adequately cover these. The nature of the planned GM work was reviewed. The insert genetic material will not alter the category of risk associated with the HG2 cell line. The plasmid construction is class 1 and can be undertaken at containment level 1, but work with HG2 cell lines must be undertaken at containment level 2. The proposed modifications will not alter the tropism, pathogenicity or otherwise of the cell lines and would not increase the category of risk associated with them.

The committee also discussed safety and decontamination of instruments used in the analysis of HG2 material and reviewed cleaning protocols and user training requirements and recommended the use of secondary containment for transport, clear labelling and warning stickers. Existing cleaning protocols were found to be adequate for the level of risk posed by HG2 material. Provision of training with updated safety guidelines for all users was agreed. The disposal of GM class 2 waste was also discussed. The protocols recommended in the GM risk assessment were deemed satisfactory for the intended use. The committee also discussed storage requirements for HG2 material, and recommended that this could be safely stored in clearly labelled cryoboxes (which would constitute secondary containment) within a -150°C freezer.

The committee agreed this was a GM Class 2 project and the potential risks to users and the environment were sufficiently addressed by the risk assessments; by following the control measures indicated, these risks were considered to be effectively minimised.
**GM Centre Number: 3032**

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**SERVIER RESEARCH AND DEVELOPMENT LTD**

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

**District**

**Town**

**County**

**Postcode**

**Country**

**Tel Number**

**Fax Number**

**E-mail**

**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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<td>Centre for Biopharmacy Research</td>
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<td>Framewood Road</td>
<td>Rowley</td>
<td>Wexham Springs, Slough</td>
<td>SL3 6PJ</td>
<td>ENGLAND</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

Scientific staff from Servier Research and Development Ltd (technical and managerial) and an External Consultant from the University of Reading.

<table>
<thead>
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<th>Level 1 (GMMs)</th>
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<th>Level 3 (GMMs)</th>
<th>Level 4 (GMMs)</th>
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<td>Animal Unit</td>
<td>Growth Room</td>
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<td>Large Scale</td>
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</tbody>
</table>
Yes

For activities involving GMMs, describe the waste management measures which will apply to the activity

No waste material will be removed from the isolated culture suite prior to heat inactivation. - Liquid effluent (eg waste culture media buffers) will be transferred to 1 litre plastic bottles and autoclaved (123°C for 15 minutes). Autoclaved liquid waste will be placed in hermetically sealed bins supplied by an approved contractor, who disposes and incinerates the contents. No liquid effluent will be disposed of down the sink.
- Solid waste (eg plastic culture plates/flasks, plastic pipettes) will be placed in plastic biohazard bags and autoclaved (121°C for 15 minutes). Autoclaved waste will be placed in hermetically sealed bins supplied by an approved contractor, who disposes and incinerates the contents.

Testing/Monitoring: autoclave service and performance was validated by a company with UKAS certification; autoclave will be serviced regularly; computerised systems are in place to monitor temperatures during each autoclave cycle.

All activities involving modified HEK293 will be conducted under Level 2 Containment measures.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Following detailed assessment of the recipient, vectors, donor organism, genetic inserts and resulting genetically modified cell line, the Genetic Modification Safety Committee has assigned the work as a Class 2 activity, requiring Level 2 Containment measures.

---

Project Ref 3032/08.1

Date Ackn'd 18/12/2008

Date Project Ceased 03/12/2009

Withdrawn Y

Tick if notifying a connected programme of work N

CU2 Project Title Pharmacokinetic, metabolism and drug-drug molecular interactions, using HEK293 cell lineages genetically modified to express transporters from the Solute Carriers (SLC) family …..

CultureVol

Class 2 < 1 Litre

Consent Granted Not Applicable

Project notified under transitional arrangements Y

Class 2

Consent Granted

Project notified under transitional arrangements N

CultureVolumeClass2

Consent Granted

Project notified under transitional arrangements N

CultureVolumeClass3-4

Consent Granted

Project notified under transitional arrangements N

---

Other(s) Use of cell cultures (HEK293 Human Embryonic Kidney)

---
### Project Additional Information

#### Purposes of the contained use

Genetically modified HEK293 cells will be produced by Vectalys, France. Modified HEK293 cells will be used in diverse study protocols to determine if our compounds are substrates and/or inhibitors of the over-expressed transporters, to anticipate eventual in vivo studies in human at the clinical development stage. Studies will be carried out using cell suspensions and/or plated cells in various incubation buffers.

#### Recipient or parental organism

(Please see attached risk assessment for a more detailed explanation)

HEK293 Human Embryonic Kidney cell line immortalised with Adenovirus 5 DNA.

Due to the presence of adenoviral sequences, cells have been categorised as Class 2.

Level 2 Containment measures are in place to reduce hazards to human health and the environment.

#### Host/vector system

(Please see attached risk assessment for a more detailed explanation)

Third generation lentiviral vector (pLV) produced from a packaging line in which helper genes are provided in trans by helper constructs pENV (contains VSVG glycoprotein sequence) and pHIV-gag-pol (does not encode env, Vif, Vpr, Vpr, Vpu and Nef).

Vectors pLV and pENV are capable of infecting human cells and have been classified as Class 2. Level 2 Containment measures are in place to reduce hazards to human health and the environment.

#### Origin & function

(Please see attached risk assessment for a more detailed explanation)

Donor Organism: Immortalised epithelial colorectal adenocarcinoma cells (Caco-2) (Class 1)

Genetic inserts encode proteins of the Solute Carrier (SLC) family of transmembrane transport proteins and have been categorised as Class 1: SLC01A2 (protein OATP1A2); SLC02B1 (protein OATP2B1); SLC04C1 (protein OATP4C1); SLC10A1 (protein NTCP); SLC16A1 (protein MCT1); SLC22A1 (protein OCT1)

The SLCs contain a number of hydrophobic transmembranous alpha helixes connected via intra- and extra-cellular hydrophilic loops. Depending on the SLCs transporters are functional either monomerically or oligomerically (homo or hetero-oligomers), and are genetically localised on the external side of the plasma membrane.

The expressed sequences to do not alter the survivability/fitness of the HEK293 cell line, nor do they confer pathogenicity to the cells.
Evaluation of foreseeable effects

No genetic manipulation will be conducted at Servier Research & Development Ltd UK and the cell line will be propagated for experimental purposes only.

All work will be conducted under Level 2 Containment measures, and suitable inactivate modified cells prior to removal from the isolated cell culture suite.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

No waste material will be removed from the isolated culture suite prior to heat inactivation. - Liquid effluent (eg waste culture media buffers) will be transferred to 1 litre plastic bottles and autoclaved (123°C for 15 minutes). Autoclaved liquid waste will be placed in hermetically sealed bins supplied by an approved contractor, who disposes and incinerates the contents. No liquid effluent will be disposed of down the sink.

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Testing/Monitoring: autoclave service and performance was validated by a company with UKAS certification; autoclave will be serviced regularly; computerised systems are in place to monitor temperatures during each autoclave cycle.

All activities involving modified HEK293 will be conducted under Level 2 Containment measures.

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Please enter comments on the GM safety committee on the risk assessment

Following detailed assessment of the recipient, vectors, donor organism, genetic inserts and resulting genetically modified cell line, the Genetic Modification Safety Committee has assigned the work as Class 2 activity, requiring Level 2 Containment measures.

Project Containment

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02/03/2022
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GM Centre Number: 3033

Data Premises Notified
(Originally) 08/12/2008

Transferred from 1992 Regs? N

Transitional Premises
Class

Data Premises Closed

Transitional Premises
Emergency Plan Required?

Non-GMMs N Withdrawn N

Name
UNIVERSITY OF NORTHAMPTON

Name 2
Department

Campus Estate or Research Centre
NW107, AVENUE CAMPUS

Road Name
STGEORGE'S AVENUE

Building

District

Town
NORTHAMPTON

County
BLANK

Postcode
NN2 6JD

Country
ENGLAND

Tel Number
01604 735500

Fax Number
01604 791954

E-mail

HSE Division
blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Associate Dean of Research, School of Health (Chair of GMSC)
Occupational Health and Safety Manager, University
School Manager, School of Health
Laboratory Manager, School of Health, Union Representative, University Staff Member, School Health Postgraduate Student, School of Health
The University GMSC meets annually to ensure the risk assessment is current and compliant with the HSE regulations.

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Other (please specify) Tick if confidential

Yes

Bacteriology Yes
Parasitology
Transgenic Birds
Microbiology Research Yes

02/03/2022
The favoured method of disposal of GMO waste is through heat inactivation by autoclaving at 121°C for 15 minutes, reducing viability 100%. Plastics, agar and small volumes (30 ml universals) will be autoclaved in double lined autoclave bags. Larger volumes of liquids should be autoclaved in double lined autoclave bags. Larger volumes of liquids should be autoclaved in Pyrex containers before disposal. Autoclaved disposal bags should be clearly labelled, stating GMO waste, identifying the laboratory name and building. Autoclaved waste will be disposed of via yellow biohazard bins provided (Wastecare (GB)), these are subsequently collected and disposed of by Wastecare (GB), Laydon Road, Stevenage, Herts SG1 2BW.

Inactivation by chemical means, either a phenolic, halogenated tertiary amine or hyperchlorite solution, maybe used to the manufacturers validated specification. Plastic ware, glassware and media may be disinfected using this method. Plastic ware should then be disposed of via Wastecare (GB) in yellow biohazard bins provided, liquid waste should be disposed of via the drains whilst glassware should be washed and dried using standard good laboratory practice.

Spillages will be disinfected using aforementioned solutions and dealt with using relevant COSHH guidelines and good laboratory practice methods as required. Autoclaved waste will be disposed of via yellow biohazard bins provided (Wastecare (GB)), these are subsequently collected and disposed of by Wastecare (GB), Laydon Road, Stevenage, Herts, SG1 2BW.

The risk assessment is suitable and adequate against all foreseeable risks in accordance with Part 2 and 3 of the SACGM compendium of Guidance. As a result of the risk assessment and in conjunction with Schedule 8 Table 1a, the committee have reached a decision that this work falls under containment level 1 for laboratories working with GMMs.
Cloning and sub-cloning of Campylobacter sp. To identify the role of virulence genes in pathogenesis

Purposes of the contained use

Various cloning and sub-cloning techniques, such as directed mutagenesis or random mutagenesis, will be used to assess the role of virulence factors in the pathogenesis of Campylobacter infection.

Recipient or parental organism

The most hazardous Campylobacter strains, including Campylobacter jejuni are ACDP hazard category 2. Strains produced in this study are not likely to be more virulent than any reference strain used.

Campylobacter sp. are ubiquitous in the environment and the infection they cause is usually self-limiting.

Host/vector system

The disabled E.coli strains DH5α, TOP10, JM109 and CA434 will be used as the plasmid host and are not pathogenic. Due to the differences in codon usage between E.coli and Campylobacter, it is very unlikely that expression of Campylobacter genes will occur in E.coli. Cloning plasmids to be used, include pUC19 which does not replicate in Campylobacter and is not transmissible. These plasmids will also contain antibiotic resistance markers which are regularly used in the laboratory environment and E.coli. The plasmids are not self-transmissible.

Origin & function

Genomic DNA will be extracted from Campylobacter sp. and amplified by PCR. This DNA will then be cloned into pUC19 vectors for propagation in E. coli and transformation into Campylobacter.

The approach of creating mutants for testing, either by deletion of a gene or insertion of a transposon will make the GMM less virulent.

The majority of the genes to be expressed are innocuous bacterial enzymes used for basic metabolic functions and they are unlikely to function in isolation or in E.coli.

Evaluation of foreseeable effects

Campylobacter sp.

The duplication of metabolic genes in these studies are highly unlikely to increase virulence or survival in the environment.
The effect of gene dosage on the only known virulence determinants of Campylobacter is unknown. Expression levels of these genes may vary widely in clinical strains and the experiments here are unlikely to exceed clinical levels even in the worst case scenario. Random mutagenesis or directed gene deletion may increase or decrease virulence factor expression. None of the antibiotic markers used correspond to antibiotics that are routinely used to treat Campylobacter infection.

E.coli: 
The strains used are disabled, non-pathogenic laboratory strains which are unlikely cause disease even with the addition of fragmented DNA or genes. As such infection with recombinant E.coli or survival in the environment is deemed highly unlikely.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Waste will be autoclaved to achieve 100% kill and will then be sent to landfill after processing by approved waste disposal contractors.

**Is an emergency plan required according to regulation 20?**  N

**If yes, tick to confirm that it is attached to this form**  N

**Tick to confirm that you have attached a risk assessment to this form**  Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  N

**Please enter comments on the GM safety committee on the risk assessment**

The GMSC has considered the proposal for work with Campylobacter, and after initial discussion to clarify the text of the risk assessment we have no concerns about the genetic modification as planned, and note that it is highly unlikely that a GMO more hazardous than the host will be generated. The main concerns expressed were regarding the safe transport, handling and disposal of the pathogenic host organism. Acceptable safe working practices have been cited in the risk assessment, and reference has been included to indicate the expertise and experience of the key post-doc who has safely and successfully worked with Campylobacter, and with other Level 2 and 3 pathogens for 13 years. One committee member has extensive experience of pathogen work including MRSA and has no problem with this straightforward application using category 2 bacteria. The GMSC has approved this proposal.

**Project Containment**

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<thead>
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02/03/2022
### Project Ref 3033/11.2

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<td>Cloning and sub-cloning of Clostridium sp. To identify the role of virulence genes in pathogenesis</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements N

### Historical Significant Changes
- N

### Project Additional Information

#### Purposes of the contained use

Various cloning and sub-cloning techniques, such as directed mutagenesis or random mutagenesis, will be used to assess the role of virulence factors in the pathogenesis of Clostridium sp. Infection. This includes but is not limited to the species Clostridium difficile (work will not include the use of Clostridium botulinum).

#### Recipient or parental organism

- The most hazardous Clostridium sp. Strains are ACDP hazard category 2 and this includes Clostridium difficile strains. Clostridium difficile R20291 is regarded as the PCR ribotype 027 reference strain. Strains produced in this study are not likely to be more virulent than this. Clostridium difficile 630 and 630 delta erm will also be used in this study but are known to be less virulent than strain R20291.

- C. difficile only presents a hazard when the normal bacterial flora is compromised and is carried by 25% of the population with no effect to health

#### Host/vector system

- The disabled E. coli strains DH5α, TOP10, JM109 and CA434 will be used as the plasmid host and are not pathogenic. Due to the differences in codon usage between E. coli and C. difficile, it is very unlikely that expression of C. difficile genes will occur in E. coli. Also due to the random fragmentation of the genomic DNA the required elements for expression may not be cloned with the genomic DNA.
- The pMTL vectors based on the CoIE1 replicon will be used as shuttle vectors in E.coli and C.difficile. These plasmids are nic mob minus and are derived from Gram
positive and negative components, many of which (except the Gram positive replicon) do not function in clostridia. These plasmids will also contain antibiotic resistance markers which are regularly used in the laboratory environment and E.coli. The plasmids are not self-transmissable.

### Origin & function

Genomic DNA will be extracted from the Clostridium difficile sequence strains CD630 and R20291 and amplified by PCR. This DNA will then be cloned into pMTL80000 or pMTL007 series vectors for propagation in E. coli and conjugation into Clostridium difficile.

The approach of creating mutants for testing, either by deletion of a gene or insertion of a transposon will make the GMM less virulent.

The majority of the genes to be expressed are innocuous bacterial enzymes for use for basic metabolic functions and they are unlikely to function in isolation or in E coli.

### Evaluation of foreseeable effects

**C. difficile:**
- The duplication of metabolic genes in these studies are highly unlikely to increase virulence or survival in the environment.
- The effect of gene dosage on the only known virulence determinants of C. difficile. Toxins A and B is unknown. Expression levels of the genes encoding toxin A and B vary widely in clinical strains and the experiments here are unlikely to exceed clinical levels even in the worst case scenario.
- Random mutagenesis or directed gene deletion may increase or decrease virulence factor expression. None of the antibiotic markers used correspond to antibiotics that are routinely used to treat C. difficile infection.

**E. coli:**
- The strains used are disabled, non-pathogenic laboratory strains which are unlikely to cause disease even with the addition of fragmented clostridial DNA or genes. As such infection with recombinant E. coli or survival in the environment is deemed highly unlikely

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**Not Applicable**

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Not Applicable**

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be autoclaved to achieve 100% kill and will then be sent to landfill after processing by approved waste disposal contactors

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Please enter comments on the GM safety committee on the risk assessment

---

02/03/2022
The GMSC has considered the proposal for work with C. difficile, and after initial discussion to clarify the text of the risk assessment we have no concerns about the genetic modification as planned, and note that it is highly unlikely that a GMO more hazardous than the host will be generated. The main concerns expressed were regarding the safe transport, handling and disposal of the pathogenic host organism. Acceptable safe working practices have been cited in the risk assessment, and reference has been included to indicate the expertise and experience of the key post-doc who has safely and successfully worked with C. difficile, and other Level 2 and 3 pathogens for 13 years. One committee member has extensive experience of pathogen work including MRSA and has no problem with this straightforward application using category 2 bacteria. The GMSC has approved this proposal.

Project Containment

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Project Ref 3033/12.1

Date Ackn'd 12/12/2012

Date Project Ceased

CU2 Project Title Site directed mutagenesis, in Listeria spp.

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Site directed mutagenesis using plasmids such as pORI19 and pVE6007 will be used to inactivate target genes in order to assess their roles in stress response and virulence mechanisms following exposure to various antimicrobials in Listeria spp. Gene up-regulation using pIMK2 will be used to alter expression levels of target genes to assess contribution in resistance mechanisms to antimicrobials and virulence characteristics of subsequent isolates. This includes but is not limited to the species Listeria monocytogenes. No known antibiotic resistance genes will be targeted for mutagenesis.

Recipient or parental organism

The most hazardous Listeria strains, including Listeria monocytogenes are ACDP category 2. Listeria spp. are ubiquitous in the environment and the infection they cause is usually self-limiting.

Host/vector system

The disabled E. coli strains DH5α, EC101, TOP10, JM109 and CA434 will be used as the plasmid host and are not pathogenic. Due to the differences in codon usage between E. coli and Listeria spp., it is very unlikely that expression of Listeria spp. genes will occur in E. coli.

Vector systems will include, but not be limited to, the pORI19-pVE6007 gene inactivation system and the pIMK2 overexpression system. pORI19 and pIMK2 vectors will be used as shuttle vector in E. coli and Listeria spp. The pORI10 lacks RepA and therefore requires a co-plasmid pVE6007 to replicate. These plasmids contain antibiotic resistance markers which are regularly used in the laboratory environment and E. coli. pVE6007 is a temperature sensitive plasmid with a chloramphenicol resistance marker. The plasmids are not self-transmissible.

Origin & function

Genomic DNA will be extracted from the Listeria monocytogenes sequence strains EDGe and FSL R2-449 and genes of interest will be amplified by PCR/ Amplicons will then be cloned into pIMK2 or pORI19 vectors for propagation in E. coli and subsequent transformation of Listeria monocytogenes. Mutant generation through the use of pORI19-pVE6007 is likely to inactivate key resistance genes; as such it is unlikely such mutants will be more pathogenic than the wild-type strain. The pIMK2 system used to increase target gene expression. This may increase resistance to antimicrobials (non-antibiotic) but it is not envisaged that will render the organisms more pathogenic. The majority of the genes to be targeted code for innocuous bacterial enzymes used for basic metabolic functions. These proteins are unlikely to function in isolation or in E. coli.

Evaluation of foreseeable effects

Listeria spp.: The duplication of metabolic genes in these studies are highly unlikely to increase virulence or survival in the environment.

E. coli: The strains used are disabled, non-pathogenic laboratory strains which are unlikely to cause disease even with the addition of fragmented Listeria spp. DNA or genes. As such infection with recombinant E. coli or survival in the environment is deemed highly unlikely.

L. lactis: The strain used has a sole purpose in harbouring/maintaining the pVE6007 plasmid. As such no manipulations will be undertaken in L. lactis. Owing to plasmid possession strain MG1363 possesses chloramphenicol resistance.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be autoclaved to achieve 100% kill and liquid waste will either be autoclaved to achieve 100% kill or will be disinfected to achieve a 5 log reduction (99.999%). Solid waste will either be sent to landfill or incinerated by approved waste disposal contractors.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 3033/19.1

Date Ackn’d 03/05/2019

CU2 Project Title Tolerance of oxidative and metal stresses in Streptococcus pneumoniae: Transcriptional responses and novel mechanisms

Class 2

Consent Granted

Date Project Ceased 02/03/2022
### Project Additional Information

#### Purposes of the contained use

The functions of genes involved in oxidative stress responses and metal homeostasis in *Streptococcus pneumoniae* (including those of transcriptional regulators) will be investigated by constructing specific deletions of the genes of interest and by integrating luciferase or ~-galactosidase genes into the genome for use as transcriptional reporters.

#### Recipient or parental organism

*S. pneumoniae* 039 is a pathogenic bacterium from a clinical isolate. It is a well-characterised and commonly-used laboratory strain of *S. pneumoniae*, in part due to its genetic tractability, and has been used in research for almost 100 years. Other *S. pneumoniae* strains that will be used as recipients include TIGR4 and the non-pathogenic 039-derived strain R6.

#### Host/vector system

Escherichia coli K12-derived, non-pathogenic strains ego DH5a, JM199, TOP10 will be used as host for vectors.

#### Origin & function

- **E. coli and click beetle/firefly** - derived genes will be obtained from plasmid DNA, Streptococcal derived-genes will be amplified by PCR from genomic DNA.
- For transcriptional reporters:
  - Firefly/ click beetle luc genes - bioluminescence, partial protein coding sequence without introns
  - E. coli LacZ' - partial protein coding sequence for ~-galactosidase enzyme
- For antibiotic resistance genes:
  - E. coli bla - ~-lactamase conferring ampicillin resistance
  - *S. pneumoniae* rpsL* - mutant form of the S12 ribosomal protein gene conferring streptomycin resistance
  - *S. pneumoniae* tetM - GTPase conferring tetracycline resistance
  - *S. pneumoniae* ada9 - adenylytransferase conferring spectinomycin resistance
  - *S. pneumoniae* emrB - methylase conferring resistance to erythromycin
  - *S. faecalis* aphlll - aminoglycoside phosphotransferase conferring kanamycin resistance

#### Evaluation of foreseeable effects

GM *S. pneumoniae* are unlikely to pose an increased risk to human health compared with the parental strain as most genetic modifications will remove or mutate genes that could contribute to pathogenicity. Erythromycin resistant
organisms are unlikely to have any increased risk to health as erythromycin is not a first-line antibiotic for treatment of S. pneumoniae infections and the ermB gene confers specific resistance to erythromycin and will not affect resistance to other clinically relevant antibiotics.

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For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A                                                                                             |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Autoclaving at 121 DC for 15 minutes with moist heat should give 100 % kill. Validation of the autoclave to effectively sterilise waste is done at least annually; where the nature or composition of the load changes further validation must be carried out. During validation, a worst case load is simulated and temperature probes are inserted - this is known as the 12 point thermocouple test. The thermocouples are connected to a recording device and readings are then taken during the autoclave cycle. The test is carried out by a competent engineer using calibrated equipment in accordance with the British Standard (BS 2646: 1993). Calibration of the autoclave is also performed to check that the control panel of the autoclave is functioning correctly and that it is regulating the decontamination process and displaying the operating parameters accurately. The Astell autoclave for autoclaving of waste creates a digital log of each run, this data is stored in the 'Technician's Folder' on the HEA drive to indicate successful decontamination of each load. Unusual loads should be monitored on a load-by-load basis. All records of performance testing are kept for a minimum of 5 years. Disinfection with Presept should give 100 % kill. Presept tablets to be used at 1,000 ppm for general laboratory disinfection. Used as per manufacturer's instructions, Presept is bactericidal for all Gram positive bacteria |

| Is an emergency plan required according to regulation 20? N |

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
GMO safety committee met on 10/10/2018 to consider the risk assessment. Suggested changes were:
- Project Activity end date can be left open as not tied to a funded or time-limited project
- Review of any changes to risks after the move to new Waterside campus August 18
- Confirmation that no materials contain pathogens or toxins covered by the Anti-Terrorism Crime and Security Act
- Clarification of what is meant by sharps (scalpel blades, needles but not pipette tips)
- Method of verifying waste treatment to be added
- Confirmation that Hepatitis B immunisation is not required
- Addition of JS as a project worker
- Risk assessments were reviewed on 08/05/18 and the work approved by the committee.

**Project Containment**

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<tr>
<td>Building</td>
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| Town                             | STEWARTBY       |
| County                          | BEDFORDSHIRE    |
| Postcode                        | MK34 9ND        |
| Country                         | ENGLAND         |

| Tel Number                      | 01234 765773    |
| Fax Number                      | 01234 765778    |
| HSE Division                    | blank           |

| Comments                        |                 |

Date at Which Additional Info Submitted

02/03/2022
**Premises Addresses**

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</table>

**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The genetic modification Safety Committee (GMSC) is comprised of four individuals who have various roles and levels of expertise within the company.

The GMSC will meet once every two months to review ongoing experimental progress and monitoring of containment. Minutes of the GMSC meetings will be maintained and copies circulated to all members. The chair will be responsible for making the committee members aware of any intended changes to experimental plans so that updated risk assessments can be performed and reviewed in a timely manner. The chair has an extensive background in GM activities.

<table>
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</table>

| Level 2 (GMMs) | |

| Level 3 (GMMs) | |

| Level 4 (GMMs) | |

| Non-microbial | |

Other (please specify) Tick if confidential

Bacteriology Yes

Parasitology

Transgenic Birds

Microbiology Research Yes

02/03/2022
All experiments will be performed within a clean room environment. All work surfaces are easy to clean, impervious to water and resistant to acids, alkalis, solvents, disinfectants and decontamination agents. Personal protective equipment will be issued to all laboratory staff and will include disposable gloves, clean room suits and eye-protection when necessary. Personal protective clothing will not be worn out of the laboratory. All generated wastes will be treated on-site through either chemical or physical (autoclave) inactivation.

Work surfaces and small equipment items will be wiped down after use at the end of each day using a solution of 60% isopropanol. Ovens, incubators and other large pieces of equipment will be wiped down weekly with a solution of 60% isopropanol. Liquid wastes (cultures containing live cells) will be chemically inactivated by addition of bleach to a final working dilution of 2% and allowing to stand for a minimum of 15 minutes after which inactivated liquid wastes will be disposed of down the drain with plenty of water.

Solid wastes including agar culture plates containing live Ecoli or algae colonies, disposable pipettes, pipette tips, culture tubes and microcentrifuge tubes will be segregated at the point of creation and inactivated by autoclave treatment for 15-20 minutes at 121°C. Operating conditions during autoclave runs will be monitored and recorded as well as the nature of the load being treated. Chemical indicator tape will be used to indicate the effectiveness of the treatment. Degrees of kill are estimated to be in the 6 log range using these inactivation methods. Surface swabs will be taken at a minimum frequency or fortnightly from autoclaved solid waste streams and from disinfected work surfaces, and liquid samples taken from chemically-inactivated cultures. Serial plating will be performed on the appropriate growth media to assess survival of bacterial or algal GMMs. Results will be recorded and reviewed by the GMSC at each meeting.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Could an alternate selectable marker, such as GFP (green fluorescent protein) be used in place of an antibiotic resistance gene to identify algal cells that have integrated the incoming DNA?

Yes, we could consider alternates, especially for longer-term developments including scale-ups, but antibiotic resistance markers will be used for initial proof of concept.

As the workspace (cleanroom) will also be used for other activities (electronics) can there be a separate set of distinguishable cleanroom suits for those staff using GMMs?

Yes this will be done. Cleanroom suits will also be laundered offsite on a twice monthly or as needed schedule.

Will there be a log of experiments with instructions in the event of employee absence due to sickness? This would include handling and/or shutdown instructions for pausing or terminating an experiment?

Yes, this will be implemented.
GM Centre Number: 3035

Data Premises Notified (Originally) 12/12/2008

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

ROYAL BOTANIC GARDENS

Name 2

Department

MOLECULAR SYSTEMATICS

Campus Estate or Research Centre

JODRELL LABORATORY

Building

Road Name

District

Town

RICHMOND

Country

ENGLAND

County

SURREY

Postcode

TW9 3AB

Tel Number 0208 332 5312

Fax Number 0208 332 5310

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Committee formed by:
Keeper of the Jodrell
Head of Molecular Systematics Section
Jodrell laboratory Manager
Molecular Systematics laboratory manager
Mycology laboratory manager

Committee meets every 6 months. Extraordinary meetings are held if needed.

<table>
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<th>Glass House</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All GMM work to be carried out in controlled areas of the lab-laminar flow cabinet and adjacent bench containing water bath and growth cabinet. GMMs are stored in a labelled and controlled area of the cold room.

Waste containers should be properly labelled.

A log book is placed in the lab to record name of user, date, bacterial line used for the experiment, method and date of killing of all material.

All material in contact with GMMs (vials, test tubes, pipettes, petri dishes, gloves, bottles) to be autoclaved at 145 degrees for 45 minutes.

A monitor strip must be inserted in the centre of the load to monitor sterilization and be kept as a record in the autoclave log book.

Autoclaved waste is transferred to a yellow plastic bag and placed in yellow bin outside for collection and incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All members of the committee have seen the risk assessment and all comments were included.
**GM Centre Number: 3036**

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**Campus Estate or Research Centre**

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**Comments**

This address is for LIMAGRAIN LTD who are part of the same company.

**Date at Which Additional Info Submitted**

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Premises Addresses

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<td>SUFFOLK</td>
<td>IP30 9UP</td>
<td>ENGLAND</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Research Manager (Chairman) Limagrain UK Ltd
- Biological Safety Officer, Limagrain UK Ltd
- Health and Safety Representative, Limagrain UK Ltd
- Wheat Project Leader, Biogemma
- Transgenesis platform leader, Biogemma

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 2 (GMMs)</td>
<td>Level 3 (GMMs)</td>
<td>Level 4 (GMMs)</td>
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Other (please specify) Tick if confidential

02/03/2022
No activities involving GMM will be performed in these premises. Only GM plants (first generation or further, no primary transformant) will be grown in these premises. GM plant seeds will be imported in double layered and sealed boxes. Upon reception, the seeds will be sown and boxes autoclaved. GM plants will be grown in closed growth chambers until their seeds are ready to harvest. The collected seeds will be shipped back to France in the same double layered and sealed boxes. The remaining non fertile biological material (stalk and leaves essentially) will be destroyed by heat treatment (autoclave or incineration). Depending on the waste volume, this material will be either autoclaved (low quantity) or collected in sealed container by a service provider specialised in hazardous waste management to be incinerated. The waste management procedures will destroy in any case the germination capacity of left over seeds if any.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

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<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
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Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: 

Please enter comments of the GM safety committee on the risk assessment:

The risk assessment has been reviewed and approved by the safety committee.
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Name

NHS GREATER GLASGOW AND CLYDE

Name 2

Department

Campus Estate or Research Centre

Road Name

Grahamston Road

Town

Paisley

Building

WARD 11, DYKEBAR HOSPITAL

District

Tel Number 0141 314 0232

Fax Number

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>Southern General Hospital</td>
<td>1345 Govan Road</td>
<td>Glasgow</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee within NHS Greater Glasgow & Clyde consists of Dr W C (Chairman) and D C P (Deputy Director of the MRC Virus Unit). The Committee enlist the services of any suitably qualified personnel when required. The committee generally meet on a monthly basis but do convene as required.

Committee operating procedures - The committee closely review protocols and risk assessment for each relevant trial. The committee then liaise closely with the study sponsor to resolve any outstanding queries. Once the committee are satisfied with all arrangements they will issue either a favourable or non-favourable opinion for the trial.
The investigational medicinal product (IMP) used in this study is a suspension of cells contained within a plastic screw cap vial and will only be handled openly within the operating theatre. IMP is drawn into a grafting syringe via a loading cannula, thus minimising the risk of stick injury. There is, however, a risk of aerosol generation should the grafting syringes be emptied in air. This risk will be controlled by emptying grafting syringes into a gauze as described in local rules.

Grafting syringes and canulas used in the procedure will be disposed directly into biohazard sharp bins. In addition, all other materials coming into contact with the IMP will be disposed directly into biohazard bins for incineration as clinical waste. Waste disposal contractors involved in the disposal of waste from this study hold appropriate and valid HSE and SEPA licences for the disposal of genetically modified materials, details of which can be provided on request.

Safety spill kits will be made available in all locations where IMP will be handled. Any spillage will be wiped clean with a biocidal spray and swabs disposed of as clinical waste. Within the theatre environment, all staff will wear gloves and gowns as per normal practice. All contaminated equipment will be thoroughly cleaned and all other contaminated materials will be incinerated as clinical waste.
The genetic modification safety committee were satisfied with the risk assessment.

### Project Ref 3037/19.1

**Date Ackn’d**
30/10/2019

**Date Project Ceased**

**CU2 Project Title**
Gene therapy product RP1 (Common name rHSV-1 hGM-CSF/GALV-GP) for the treatment of solid tumours.

**Class**
Class 2

**CultureVolClass2**
< 1 Litre

**CultureVolumeClass3-4**

**Non-GMM Consent Granted**

**Project notified under transitional arrangements**
N

**Withdrawn**
N

**Tick if notifying a connected programme of work**
N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

### Project Additional Information

**Purposes of the contained use**

RP1 will be administered to subjects as an anti-tumour therapy, as part of a Phase I clinical trial to treat a wide range of solid tumour types. RP1 has a particular utility in combination with immune co-inhibitory pathway blockade. Intended indications to study include soft tissue sarcoma, breast cancer including triple negative breast cancer (TNBC), lung cancer including non-small cell lung cancer (NSCLC), melanoma, non-melanoma skin cancers, head and neck cancer, primary liver and kidney cancer and colorectal cancer. The initial clinical trial protocol intends to test RP1 in several indications as a monotherapy and in combination with anti-PD-1 therapy.

**Recipient or parental organism**

RP1 (rHSV-1 hGM-CSF/GALV-GP) is a selectively replication competent Herpes Simplex Virus-1 (HSV-1). The virus contains a codon-optimised sequence for human granulocyte macrophage colony stimulating factor (hGM-CSF) and a codon optimised sequence for the gibbon ape leukemia virus surface glycoprotein (GALV-GP) with the R- sequence deleted (R-) [GALV-GP-R-]. GALV-GP-R- expression leads to cell to cell fusion (syncytial) formation in infected tumour cells through binding to the constitutively expressed PIT-1 receptor for GALV. This results in the death of the cells by...
membrane fusion and is also intended to enhance the spread of the virus through the tumour. Since the RP1 selectively replicates in tumour cells, the expression of the GAL V-GP-R- is minimised in normal tissues. The oncolytic destruction of tumour cells leads to the release of tumour associated antigens that are intended to engender an antitumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further enhanced through GALV-GP-R- mediated killing, fusion associated cell death which also results in the production of the highly immunogenic exosomes, which is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, un injected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

Host/vector system

RP1 (rHSV-1hGM-CSF/GAIV-GP) is derived from the RH018A strain of Herpes Simplex Virus-1. RP1 is produced in the Vero cell and released into the culture media during cell lysis, prior to purification.

Origin & function

RP1 was constructed using a new strain of HSV-1 (strains RH018A). Replimune obtained and compared 30 clinical strains of HSV-1 on a panel of human tumour cell lines and selected the most promising of these (strain RH018A) for further development. RP1 expresses the immune stimulatory protein GM-CSF, which augments therapeutic activity. GALV-GP-R- binds to the PiT1 receptor, which is widely expressed on mammalian cells including human tumour cells. PiT1 is also critical for cell proliferation, and its expression is therefore unlikely to be lost or down-regulated in response to cancer treatment. The truncated R- version of the protein provides constitutive fusion activity without GAL V (i.e. the virus) itself. Expressing GALV-GP-R- together with GM-CSF is expected to increase clinical activity as compared to only expressing GM-CSF. As well as causing direct tumour cell death by cell to cell fusion, cell to cell fusion followed by death is highly immunogenic and includes the release of immunogenic tumour antigen-containing exosomes. Expression of GAL V-GP-R- from an oncolytic virus is therefore expected to improve systemic, immune mediated, effects, as well as effects in the directly treated tumour thereby increasing synergy with other immunemediated approaches to cancer therapy such as immune co-inhibitory pathway blockade.

Evaluation of foreseeable effects

As described above (under Recipient or Parental Organism), the oncolytic destruction of tumour cells (upon transduction with RP 1) leads to the release of tumour associated antigens that are intended to engender an antitumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further amplified through GALV-GP-R- mediated killing, fusion associated cell death which results in the production of the highly immunogenic exosomes and is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, un injected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All used and unused RP1 and diluent vials used in preparation and syringes will be destroyed per institutional policy. As per the wild-type HSV-1 virus, the recombinant HSV-1 vector particles that represent RP1 are highly susceptible to dehydration, rapidly inactivated outside the host and easily inactivated (for example with 1 % Virkon solution).

Phase 1
As part of phase I of the clinical trial, biodistribution and shedding will be monitored. RP1 DNA levels in blood and urine will be determined at time-points outlined in the Schedule of Assessments of the clinical protocol, (i.e. day 1, day 2, day 3, day 15, day 16, day 17, day 29, day 30, day 31, day 43, day 57, day 71, day 85, day 99 and as part of the follow up, 30 days and 60 days after the last dose of RP1).
• Blood, saliva/oral mucosa, urine samples and injection site dressing swabs will be collected at the first, second and third RP1 injections at the following time points: pre-close, 6 (+/-2hr) hours, 21 hours (+/-3hr) and 48 hours (+/-6hr) and also immediately prior to dosing at fourth and fifth dose.
• Specimens (swabs) will be collected at any time there is a suspicion of RP1-related viral infection occur such as vesicular eruptions or other signs of herpes viral infection. Samples should be obtained as soon as possible after symptoms arise to maximize the possibility of detection of virus, optimally within 24 hours, but samples may be collected later if collection within 24hrs is not possible. Subjects will be asked to take swabs at home for the subsequent 7 days after the initial test.

Phase 2
As part of phase 2 portion of the clinical trial, biodistribution and shedding will be also monitored.
• RP1 DNA levels in Blood, Saliva/oral mucosa, urine samples and injection site dressing swabs will be collected pre-dose at the first through last RP1 injections (cycles 1 to 8). Additionally, for each of the tumour types enrolled, the first six patients the following additional time points will be required: Doses 1, 2 and 3: 6 eighth injections. Samples on injection days will be collected prior to any injections and handling of RP1. Samples will also be collected at the 30 and 60 day follow-up visits.
• Specimens (swabs) will also be collected at any time there is a suspicion of RP1-related viral infection occur such as vesicular eruptions or other signs of herpes viral infection

In phases 1 and 2 of the study, samples on injection days will be done prior to any injections and handling of RP1.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GMS committee reviewed the risk assessment on two occasions
Committee review on 2nd May 2018
Reviewer 1
I agree with the risk assessment, in general it is very detailed and covers all the main issues- a few minor points
below. An HSV oncolytic equivalent to this one was recently approved by the FDA for melanoma treatment so there is
a long history of assessment in humans of related HSV vectors. The novel GMO in this trial has been developed by
the same team (At Replimmue) so they have all the necessary expertise. The GMO is attenuated for tumour-selective
replication and is injected directly into the tumour.
Comments below
Section 2.2. This does not cover all the inserted genes as GMCSF is not described. In general more details are
needed in this section for all inserted genes and function.
Section 4.1 . It would be good to know the expected routes of shedding and timeframe for the GMO in comparison to
wt HSV. Appreciate that assessing shedding is part of the protocol but some indication based on previous knowledge
of Imglytic inserted here would be helpful, or any of the pre-clinical data briefly mentioned in 5.2.4k.
Section 5.1. Needs to be confirmed that the NHS contractors are licensed to transport GMO waste since it will not be
inactivated (unless there is a plan to use disinfectant before storage of waste). This isn't clear? How long before
removal and inactivation of GMO waste?
with residual virus in are not mentioned? Will sharps boxes be removed and inactivated immediately after
administration etc? Is autoclaving being used? There are no details on disinfectants etc.
Reviewer 2
With reference to Risk assessment Page 2 Notification to HSE- the sponsor has received notification to use CLASS 2
under contained Use. Does this notification include notification of GGC to Use Class 2 under contained Use or are
you using a previous notification. Each site needs to be notified The Beatson Oncology site is likely to have a
notification number. Please submit.
Section 5.1 - When the patient returns home are certain groups of people still restricted?
Who will collect the orange bags containing dressings from the homes of participants
Page 11 section 5.2.4 d and e- how will information in relation to the trial be shared with the local NHS GG&C
laboratories? e.g. information on cleaning if samples are spilled or there is a needle stick injury
Page 15 Section 6d Emergency procedures - Are there any specific safety instructions for those handling a dead
body? How will information be relayed if post mortem required?
Page 15 6e - Please discuss the trial with HAU and HDU so they are aware when the first patient is being treated and
what information will be available to be transferred with the patient in the event of an emergency e.g. Resuscitation.
Are pregnant women or immune compromised staff restricted or do they need to be made aware of any additional
risk? Please confirm this will take place and that the meeting will be documented within the local site file held by the
Page 16 section 8.- please complete
- employer details and
- other trial team members who will have contact with the patient and
- Possible interaction with HAU and HDU personnel- names may not be known but just write HAU and HDU
personnel and please discuss with leads for HAU and HDU
- Will NHS GG&C provide occupational Health support for non-NHS GGC staff? (e.g. Glasgow University staff)

When the trial is running
- Please forward any SUSARs to NHS Governance that occur with patients under the care of NHS GG&C

Page 17, Section 10- please submit the names of two reviewers for the appropriateness of this trial. In order to safe
time we would be happy for the PI to contact the reviewers and for their reviews to be sent to the GM committee.

Reviewer 3
Clarification needed on the following:
Has BWOSCC been notified to HSE as a site - the letter supplied may only cover the Sponsor.
GGCRA
- 5.2.7 doesn't cover disposal of sharps
- 5.3.1 - other than pharmacy no detail on actual routes of disposals. Need detail on any of the items
used in clinical area - giving sets etc. Looks like no specific waste handling being applied other than
for routine biological waste. Need to confirm/document this. Section 3.3.3 of Sponsor RA permits
this but suspect in pharmacy will use the rapid inactivation in the gene therapy isolator - need the
detail.
- 8.1.1 not completed - should be for domestic staff etc

Reviewer 4
I agree that more details are required as others have indicated with respect to prior data on shedding and on disposal
of dressings, sharps etc, especially once out of the hospital setting. The section on action in the event of patient death
has not been completed (it only has a "7" in the box) but I would expect this to be a potential event in this type of study
and some plan needs to be in place for this eventuality - notification to mortuary and pathology staff about any
required precautions etc.

Reviewer S
I had no fundamental safety concerns regarding the processes - there are some procedural issues around sharps
boxes etc which have been picked up by others and as long as these are addressed then that will be fine. I can't
comment on the specific GMO risks but support expert advice of reviewer.
The local investigator updated the risk assessment to take into consideration the comments of the committee above
and on the 20th February 2019 the committee requested minor updates before approving pending HSE notification
and approval.
Under local risk assessment section relating to training further confirmation was requested relating to code of practice.
- If a code of practice has not been prepared confirm in this section that appropriate training is in place to cover
risk of exposure and precautions required to hospital staff
- Please ensure that each of the local labs involved in primary, secondary or exploratory endpoints have lab
manuals according to GGC policy.
- Under emergency procedures rather than referring to a Health board policy for accidental exposure describe
how staff will be trained to deal with this event.
The study will be audited by the NHS GG&C Governance auditor to ensure patient safety after the first patient
has been recruited. The study was approved by the GMS committee pending HSE approval and local R&D
approval.
## Project Containment

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GM Centre Number: 3039

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Name
TOUCHLIGHT GENETICS LTD

Name 2

Department

Campus Estate or Research Centre

Road Name
LOWER SUNBURY ROAD

Town
HAMPTON

Country
ENGLAND

Tel Number
020 8481 9200

Fax Number
01372 825 148

E-mail

HSE Division
blank

Comments

Date at Which Additional Info Submitted
02/03/2022
Premises Addresses

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<th>Name</th>
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<th>Building</th>
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<td>LEATHERHEAD FOOD INTERNATIONAL</td>
<td>R189</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The GM Safety Committee consists of the Director of Research, Senior Research Officer (Safety Officer) and a Research Assistant. Meetings will be held monthly. As the unit is small (5 people) any immediate issues can be addressed quickly; it is intended that (1) The Safety Officer will chair the meetings. (2) The Research Assistant will bring any concerns regarding GM and Microbiological activities to the attention of the committee. (3) The Director of Research will determine the direction of future research both in the immediate and long term. (4) As a direct consequence of which the Safety Officer will implement such measures to facilitate any modification of GM safety procedure as maybe required.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
--- | --- | --- | --- | ---
Level 1 (GMMs) | Yes | | | |
Level 2 (GMMs) | | | | |
Level 3 (GMMs) | | | | |
Level 4 (GMMs) | | | | |
Non-microbial | | | | |
Other (please specify) | | | Tick if confidential | |
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<th>Parasitology</th>
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<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
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</table>

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Sterilization of all microbiological waste, GM contaminated disposable plastics (Eppendorf tubes, pipette tips and associated glassware at 121-124 C for 30 mins. By means of an autoclave within the premises (R189). Disposal of sterile waste using normal methods provided by the Leatherhead Food Institute within which the Touchlight Genetics is situated. The efficacy of sterilization procedures is to be monitored by Black Spot Brownes tubes and standard autoclave tape.

Tick to confirm that you are attaching a summary of the risk assessment **Y**

Tick if you are claiming exemption from disclosure for sections of the risk assessment ****

**Please enter comments of the GM safety committee on the risk assessment**

It is the GM committees view that the bacterial strains and vectors which Touchlight Genetics Ltd intended to use pose minimal, if any risk to laboratory personnel or the environment. It is also considered that the proposed safety measures and decontamination procedures are adequate for Class 1 organisms; a laminator flow cabinet and an autoclave are present within the laboratory. The committee recognises that all current members of the laboratory have extensive experience within the fields of microbiology and molecular biology.

Director of Research Ph.D (20 years experience at Universities of Warwick, Queen Mary College, Royal Dental School, Imperial College & the Royal Holloway.)

Senior Research Officer Ph.D (25 years at the Universities of Plymouth, Kent, Warwick and Imperial College). Research Assistant Ph.D (3 years Royal Holloway)

Research Assistant MSc (5 years at the University if Nicklaus Copernicus, 4 years as Microbiologist within Leatherhead Food International.

As senior research personnel the Director of Research and the Senior Research Officer have attended safety courses within the institutions listed above. The second research assistant has completed safety courses within the Leatherhead Food Institute.
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
<th>Level 4 (GMMs)</th>
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<tr>
<td>Yes</td>
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Gmms will be inactivated by exposure by exposure to disinfectants (1% Virkon solution) for the appropriate time (overnight). This is enough to kill/inactivate >99.99% of all micro-organisms and viruses. Waste material will then be placed in bins, autoclaved and then incinerated by an off-site incineration company (Vetspeed - Their GM authorisation in GM898) according to disposal notification GM104/4.1, specific for the Brabham Research Campus.

**Other (please specify)**

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<th>Bacteriology</th>
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<td>Invertebrates</td>
<td>Plants</td>
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**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

Gmms will be inactivated by exposure by exposure to disinfectants (1% Virkon solution) for the appropriate time (overnight). This is enough to kill/inactivate >99.99% of all micro-organisms and viruses. Waste material will then be placed in bins, autoclaved and then incinerated by an off-site incineration company (Vetspeed - Their GM authorisation in GM898) according to disposal notification GM104/4.1, specific for the Brabham Research Campus.

**Tick to confirm that you are attaching a summary of the risk assessment**

Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

Non-GMM

Please enter comments of the GM safety committee on the risk assessment

In our opinion the risk assessment for the project 'Use of genetically modified human 'isogenic' cell-lines' outlines the nature of the work clearly and the characteristics of the GM organisms to be used. The hazards to human health are clearly defined and, in our opinion, we agree to the level 1 containment to be appropriate.

**Project Ref** 3042/09.1

<table>
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<th>Date Ackn'd</th>
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<td>07/08/2009</td>
<td>Adeno-Associated Virus (AAV) mediated introduction of human DNA sequences into human cells in culture</td>
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<td>Class 2</td>
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</table>
**Purposes of the contained use**

The purpose is to study the effect of modulating gene function in human cells in culture either by protein modification or reduction in protein levels.

**Recipient or parental organism**

The recipient organisms will comprise various characterized human cell lines maintained in tissue culture.

The human cell lines fall into 2 types:

- a) A packaging/helper cell line into which AAV DNA will be introduced and from which infectious virus will be secreted into the medium to high titre. The media from these cell lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.
- b) Recipient cell lines in culture. These cell will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines i.e. a negligible risk.

**Host/vector system**

The viral system which will be used is derived from the Adeno-Associated Virus (AAV) which is not categorised by ACDP.

On the other hand the virus will be packaged by transfecting transfer vector and helper vectors into specific packaging cell lines.

- This means that the viruses produced for these experiments could infect man.

However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle.

- The genes encoding structural and other components of the viral genome have been separated.
- The packaging cell lines allow expression of proteins required to produce progeny virus. The transfer vector is the only genetic material transferred to the target cells; consequently these cells cannot produce the proteins which are essential for viral assembly and infectivity.

**Origin & function**

The genetic material involved will be of 3 types:

1) For exon loss to knock-out gene function

- human genomic DNA surrounding the region to be targeted and a selection cassette to knock-out gene function.
2) For introduction of a SNP/small deletion/small insertion into the endogenous gene
   o human genomic DNA encompassing the region to introduce the SNP/small deletion/small insertion of interest and a selection cassette

3) For a tagged version of the endogenous gene using a reporter gene e.g. luciferase
   o human genomic DNA encompassing the region to be targeted, a reporter gene and a selection cassette.

Evaluation of foreseeable effects

Although the AAV's are not by categorized ADCP, a number of factors could increase the containment that will be required to work with them. We therefore need to consider the worst case scenarios:

The genetic material inserted into between the viral ITR's will be of 3 types:

1) For exon loss to knock-out gene function
   o genomic DNA surrounding the region to be targeted and a selection cassette to knock-out gene function

2) For introduction of a SNP/small deletion/small insertion into the endogenous gene
   o genomic DNA encompassing the region to introduce the SNP/small deletion/small insertion of interest and a selection cassette

3) For a tagged version of the endogenous gene using a reporter gene e.g. luciferase
   o genomic DNA encompassing the region to be targeted, a reporter gene and a selection cassette.

In every case, we will not be inserted a complete gene from the endogenous locus. Our technology specifically targets the endogenous gene locus and therefore we only need to target the region of interest. For example, if we want to create a point mutation in exon 1 of a gene we do not need to insert a complete cDNA with the exon 1 mutation; we just need to insert the mutated version of exon 1 with surrounding genomic DNA.

For each of the 3 types of inserted genetic material the hazards are as follows:

1) genomic DNA surrounding the region to be targeted and a selection cassette to knock-out gene function
   o This will have very few hazards associated with it as we are typically removing gene function. If the gene to be targeted is a tumour suppressor (e.g PTEN, p53) there may be an effect on cell characteristics depending on the particular gene. For example, PTEN has been shown to regulate levels of PI3K signalling and thus cell proliferation.
   o Worst case scenario: If an end-user were to infect themselves with this type of recombinant AAV vector, it is possible that the targeting construct could remove the function of the endogenous gene in the infected cells.
   o However, it would only target one allele of the gene rendering the infected cell heterozygous. For the genes which we would target (tumour suppressors) the tumorigenic effect is only seen when both alleles have been removed.
   o Also, as cancer is a multistep process removal of gene function is very unlikely to lead to complete cancer. At very worst it would make the few targeted cells one step along the path to tumorigenesis

2) genomic DNA encompassing the region to introduce the SNP/small deletion/small insertion of interest and a selection cassette
   o This is the insertion that has the most risk associated with it. For example, we may want to introduce a mutation which has been shown to be pro-oncogenic i.e. in K-Ras changing Glycine 12 to Valine. Transformation of cells has been shown to be a multi-step process requiring mutations in more than one gene. Therefore the introduction of a single mutation in the inserted genetic material provides a low risk of transforming any cell line. There is a potential for it to be oncogenic when recombined into the gene’s endogenous locus. However the expression levels of this altered version of the gene will generally be lower as it is expressed under the control of its endogenous promoter. This is in comparison to the effects on cells of the same mutation under the control of a high level exogenous (e.g. viral) promoter which has been shown to be transforming to cell lines.
   o Worst case scenario: If an end-user were to infect themselves with this type of recombinant AAV vector, it is possible that the targeting construct could change the function of the endogenous gene in the infected cells.
   o It would only target one allele of the gene rendering the infected cell heterozygous but has been shown to be associated with progression along the tumorigenesis process.
   o However, as cancer is a multistep process this gain of gene function is very unlikely to lead to complete cancer. At very worst it would make the few targeted cells one
step along the path to tumorigenesis.

- The degree of infection is likely to be small in the end-user as the targeting process is not very efficient so even if cells are infected with the recombinant AAV virus, very few of these would be correctly targeted.
- Overall, the highest level of risk does come from introducing SNP/small deletion/small insertion of interest but even if an end-user were to be infected, there is a very low risk of the infected cells being correctly targeted. If the cells are correctly targeted, these cells are very unlikely to be cancerous – at worst they will be one step along the path to cancer.

3) genomic DNA encompassing the region to be targeted, a reporter gene and a selection cassette.
- This will have very low hazards associated with it as we are not changing the endogenous expression or characteristics of the targeted gene. Reporter genes such as luciferase have no hazards associated with them.
- Worst case scenario: If an end-user were to infect themselves with this type of recombinant AAV vector, it is possible that the targeting construct could make a fusion protein with the reporter gene. However, as the reporter gene has no hazards associated with it, the only result would be that the infected cells would express the reporter gene.

There is no evidence that when the inserted genetic material recombines with the host cell lines genomic DNA that any sequences of the AAV viral genome are incorporated. In the recombinant AAV viral particles, the only AAV genomic sequences present are the ITR’s and there is no evidence that these are incorporated into the host genome during homologous recombination.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material will be inactivated by treatment with 1% w/v Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - their GM authorization is GMGM898) according to disposal notification GM105/4.1.

The disposal method is expected to achieve 100% inactivation of the GMM.

The data sheets describing inactivation by Virkon are attached

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The local genetic modification safety committee approved the risk assessment on Tuesday 4th August 2009.
### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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#### Project Ref 3042/12.1

**Date Ackn'd**: 29/06/2012  
**CU2 Project Title**: Lentiviral introduction of short hairpin (sh) RNA sequences into cells  
**Class**:  
**CultureVolClass2**: Class 2  
**CultureVolumeClass3-4**: < 1 Litre  
**Non-GMM Consent Granted**: Consent Granted  
**Withdrawn**: N

Tick if notifying a connected programme of work: N

#### Project notified under transitional arrangements

#### Historical Significant Changes

- **Historical Date of Additional Info**: 
- **Significant Change ID**: 
- **Date of Significant Change**: 

#### Project Additional Information

**Purposes of the contained use**

To study the effect on cellular function of decreasing protein levels in human cells in culture

**Recipient or parental organism**

The recipient organisms will comprise various characterised human cell lines in tissue culture. The human cell lines fall into 2 types:

- a) A packaging/helper cell line into which lentiviral transfer, packaging and envelope vectors will be introduced and from which infectious virus will be secreted into the medium at high titre. The media from these cell lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the
media.

b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines (a negligible risk).

Host/vector system

The viral system is a third-generation lentiviral vector system, which is classified as Hazard Group Level 2.

The virus will be packaged by transflecting transfer vectors and helper vectors into specific packaging cell lines.
- This means the viruses produced could infect man.

However, the viruses being used have been modified in a number of ways to make them safe to handle.
- The genes encoding structural and other components of the viral genome have been separated so that no single vector contains the components necessary to produce replication-competent virus.
- Transfection of the three vectors into a packaging cell line allows expression of proteins required to produce progeny virus, the transfer vector is the only genetic material transferred to the target cells; consequently these cells cannot produce the proteins essential for viral assembly and infectivity.

The genetic material involved will code for small hairpin RNA (shRNA) sequences. The shRNAs will be directed against proteins that are involved in promotin cancer; therefore knockdown of the targets should no be associated with increased risks, over and above original cell lines.

Origin & function

Evaluation of foreseeable effects

In a very rare case, a user handling human cancer cell lines could be histocompatible with the original donor of the cancer cells. This poses a colonization risk if exposed to broken skin or needle stick injury. No glass or other sharps will be used and workers will be protected by a lab coat, nitrile gloves and eye protection. Skin lesions will be covered with a bandage in addition to the protective wear described above.

Possible risk of infection with virus particles. It is a possibility that the end-user could infect themselves with the virus particles. This may result in introduction of the shRNA sequence into the user's cells. We are investigating the effect of knocking down protein levels in tumour cells with the aim of impairing tumour cell growth. Knockdown of the individual target is very unlikely to have an untoward effect. No single plasmid contains all the genes necessary to produce packaged lentiviruses - resistant particles are replication-incompetent. Should the unlikely scenario of end-user infection occur, further virus production could not occur in infected cells.

Control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the laboratory (either by autoclaving or use of Virkon). Work will be carried out in containment 2 laboratories in class II microbiological safety cabinets with routine disinfection of work surfaces. All staff will be suitably trained in working at containment level 2 laboratories and be aware of the necessary risk control measures.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid waste is expected to be generated.
All liquid waste (including tissue culture fluids) will be inactivated by treatment with 1%w/v Virkon (or 1% Trigene) for 12 hours prior to autoclaving. Waste material will then be incinerated by an off-site incineration company (Grundon - their GM authorization is attached) according to disposal notification.

The disposal method is expected to achieve 100% inactivation of the GMM.

Datasheet information from Relyon.dupont_virkon has validated the use of this disinfectant for use with a broad range of infectious agents (including Herpes simplex virus, and retroviridae).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC opinion is that the use of lentiviral vectors should be conducted at containment level 2. The individuals reviewing these documents are aware of the space implications for increasing the numbers of projects requiring access to category II containment facilities. For all new projects, an agreed system of work will need to be put in place, so not to compromise any other activities within the facility. This will need to be agreed prior to commencement of activities

**Project Containment**

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**Project Ref** 3042/15.1

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Purposes of the contained use
To study the effect on cellular function of expression of a protein in human, and or mammalian cells in culture.

Recipient or parental organism
The recipient organisms will comprise various characterised human, and or mammalian cell lines maintained in tissue culture.
The cell lines fall into 2 types:
a) A packaging/helper cell line into which lentiviral transfer, packaging and envelope vectors will be introduced and from which infectious virus will be secreted into the medium at high titre. The media from these cell lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.
b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines (a negligible risk).

Host/vector system
Packaging cells are transfected with the (replication-defective) retroviral vector and associated packaging vectors; mRNAs that are transiently transcribed from the vector are encapsidated and bud off into the cell supernatant. These supernatants, containing the retroviral particles are collected, and used to infect target cells. Upon infection of the target cell, the viral RNA molecule is reverse transcribed by RT (which is present in the virus particle), and the cDNA of the gene of interest, flanked by the LTRs, is integrated into the host DNA. Because the vector itself carries none of the viral proteins, once a target cell is infected the LTR expression cassette is incapable of proceeding through another round of virus production.

Origin & function

Evaluation of foreseeable effects
In a very rare case, a user handling human cancer cell-lines could be histocompatible with the original donor of the cancer cells. This poses a colonization risk if exposed to broken skin or needle stick injury. No glass or other sharps will be used and workers will be protected by a lab coat, nitrile gloves and eye protection. Skin lesions will be covered with a bandage in addition to the protective wear described above.
Possible risk of infection with virus particles. It is possible that the end-user could infect themselves with the virus particles. This may result in introduction of the DNA
sequence into the user’s cells. The cDNA may encode an oncogene, in which case it is possible that infection may result in a proportion of cells with tumourigenic potential. Retroviruses (distinct from lentiviruses) are only capable of infecting dividing cells; therefore exposure of any non-dividing cells is unlikely to result in infection with viral RNA. No single plasmid contains all the genes necessary to produce packaged retrovirus - therefore resultant particles are replication-incompetent. Should the unlikely scenario of end-user infection occur, further virus production could not occur in infected cells.

Control measures employed will minimise risks to the environment. These control measures include rendering all solid or liquid waste inactive within the laboratory (either by autoclaving or use of an appropriate disinfectant for example Virkon). Work will carried out in Class 2 containment laboratories in class II microbiological safety cabinets with routine disinfection of work surfaces. All staff will be suitably trained to work in Class 2 containment laboratories, in the experimental procedures undertaken for the study, and be made aware of the risk assessment and control measures being applied.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid waste is expected to be generated.

All liquid waste (including tissue culture fluids) will be inactivated by treatment with 1%w/v Virkon (or 1% Chemigene) for 12 hours prior to disposal to drains.

All solid waste material will be inactivated by treatment with 1% w/v Virkon (or 1% Chemigene) solution for 12 hours prior to autoclaving. Waste material will then be incinerated by an off-site incineration company (Grundon - their GM authorization is attached) according to disposal notification.

The disposal method is expected to achieve 100% inactivation of the GMM.

Datasheet information from Relyon.dupont _virkon has validated the use of this disinfectant for use with a broad range of infectious agents (including Herpes simplex virus, and retroviridae).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

1. The risk assessment HD_GMO_294 covers the use of the Agilent retroviral pCFB system (cat#217568 and cat#240028). Any deviation from this vector backbone will require review to insure the necessary control measures have been incorporated in the vector to limit risk of exposure and release.

2. The use of retroviral version of Agilent's Complete Control Inducible Mammalian Expression System will allow high transduction efficiencies in difficult to transfect cell lines and proviral copy number can be easily controlled to aid the application of the inducible expression system.

3. Initial an optimised protocol using 10cm dishes will be used. Once this process has been setup within Horizon it is recommended that the virus production be tested in containers with closures (T flasks) to reduce risk of spills.

Project Containment
Project Ref 3042/17.1

Date Ackn'd 11/08/2017

CU2 Project Title Delivery of recombinant oncolytic adenoviral vectors into cell lines of mammalian origin

Date Project Ceased

Class 2 Culture Vol Class 2

Consent Granted

Non-GMM

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To study the ability of recombinant oncolytic adenoviruses to kill tumour cells growing in culture.

Recipient or parental organism

The recipient organisms will comprise various characterised human cancer cell lines maintained in tissue culture. These cell lines have a long history of safe use across the research community and represent minimal risks to health or the environment.

Host/vector system

- Proprietary Oncolytic Adenovirus Species C Type 5 oncolytic viral vector. This vector is attenuated to ensure it selectively replicates in human tumour cells and not in normal cells via deletions in the regulator sequences of key genes.
genes involved in replication of the virus. In addition, several regions of the adenoviral genome have been deleted to enable insertion and expression of an exogenous transgene.

- Hazards associated with risk of infection: Adenoviruses cause generally mild respiratory tract infections which are self-limiting and generally asymptomatic. As the recombinant oncolytic adenovirus selectively replicates in tumour cells, in the event of accidental infection of a worker's normal cells, further virus production would not occur, which minimizes risks to health.
- Potential hazards to the environment: Although human Ad5 adenovirus vectors have been shown to be capable of entering some animal cells, they do not replicate efficiently. Furthermore, the virus is selective for tumour cells and not normal cells. Therefore it is unlikely that work with this vector will represent a significant risk to the environment.
- Control measures employed will minimize risks to workers and the environment. These control measures include rendering all solid or liquid waste inactive within the laboratory (either by autoclaving, incineration or use of disinfectant). Work will be carried out in containment 2 laboratories in a class II microbiological safety cabinets with routine disinfection of work surfaces. All staff will be suitably trained in working at containment 2 laboratories and be aware of the necessary risk control measures.
- Note that this type of human adenovirus serotype 5 (Ad5) vectors have been extensively used across the research community and have been approved for use in solid tumours and Ad5 has been tested in multiple forms in clinical trials.

Origin & Function

Evaluation of foreseeable effects

- After infection of human cancer cell lines in vitro, the oncolytic adenovirus replicates, which then results in lysis of the tumour cells.
- Therefore the main potential hazards to health or the environment associated with the genetically modified tumour cell lines is the release of infective adenovirus particles when the tumour cell is lysed by the virus.
- Hazards associated with risk of infection: Adenoviruses cause generally mild respiratory tract infections which are self-limiting and generally asymptomatic. As the recombinant oncolytic adenovirus selectively replicates in tumour cells, in the event of accidental infection of a worker's normal cells, further virus production would not occur, which should minimize risks to health.
- Potential hazards to the environment: Although human Ad5 adenovirus vectors have been shown to be capable of entering some animal cells, they do not replicate efficiently. Furthermore, the virus is selective for tumour cells and not normal cells. Therefore it is unlikely that work with this vector will represent a significant risk to the environment.
- Control measures employed will minimize risks to workers and the environment. These control measures include rendering all solid or liquid waste inactive within the laboratory (either by autoclaving, incineration or use of disinfectant). Work will be carried out in containment 2 laboratories in class II microbiological safety cabinets with routine disinfection of work surfaces. All staff will be suitably trained in working at containment 2 laboratories and be aware of the necessary risk control measures.
- Note that these types of adenoviruses have been used for decades in both gene therapy and oncology applications in the laboratory and the clinic. For example human adenoviruses serotype 5 (Ad5) have been approved for human use in solid tumors and Ad5 have been tested in multiple forms in clinical trials. For example Ph I - III clinical trials have been conducted with replicating Ads having deletions or specific promoter use conferring tumor replication selectivity (e.g. ONYX-015).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Liquid and solid waste is expected to be generated.
- All liquid waste (including tissue culture fluids) will be inactivated by treatment with 1 %w/v Virkon (or 1 % Trigene) for 12 hours prior to disposal to drains.
- All solid waste material will be inactivated by treatment with 1% w/v Virkon (or 1% Trigene) solution for 12 hours prior to autoclaving. Waste material will then be incinerated by an off-site incineration company (Grundon - their GM authorization is attached) according to disposal notification.
- The disposal method is expected to achieve 100% inactivation of the GMM.
- Datasheet information from Relyon.dupont_virkon has validated the use of this disinfectant for use with a broad range of infectious agents (including adenovirus).

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The GMSC opinion is that the use of adenovirus is appropriate at containment level 2. All equipment in the process has been assessed and will be suitable for the process with the risk of aerosol production minimal. All waste routes are also effective and approved

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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<tr>
<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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</table>

Project Ref 3042/19.1
To study the ability of recombinant oncolytic herpes simplex virus vectors to kill tumour cells growing in culture.

The recipient organisms will comprise various characterised human cancer cell lines maintained in tissue culture. These cell lines have a long history of safe use across the research community and represent minimal risks to health or the environment.

Host/vector system

- Proprietary oncolytic HSV-1 Herpes simplex virus vectors. These vectors contain deletions (e.g. deletion of the gamma34.5 neuropathogenesis gene) that effectively attenuates the virulence of HSV-1 to ensure it selectively replicates in tumour cells but not normal neuronal cells (i.e. neurovirulence has been eliminated).
- Typically the temporal expression of one or more viral proteins are modified so that they are expressed earlier in the viral replication cycle. This is sufficient to overcome the partial innate antiviral response that exists in tumor cells, as during tumorigenesis mutations occur in pathways that confer growth advantages to the cancer cell that render the cell incapable of mounting a complete antiviral response. However, this mutation is insufficient to overcome the full antiviral response that occurs in normal cells.
- In some cases the vector will be modified via the introduction of immunomodulatory transgenes, to help the virus evade anti-viral T-cells while promoting anti-tumour immune cell recruitment and/or stimulation of anti-tumour immune cell activity.
- These types of attenuations/modifications are the basis of Talimogene laherparepvec (T-Vec/Imlygic), the first FDA-approved oncolytic virus (Conry et al 2018. Human Vaccines and Immunotherapeutics, 14, 839-846). Therefore, the safety profile of Herpes simple viruses modified in this way are excellent.

Origin & function

- The genetic material is a proprietary modified herpes simplex virus type 1 (HSV-1) that has been modified as described above to attenuate the virulence of HSV-1 to ensure it selectively replicates in tumour cells but not normal neuronal cells (i.e. neurovirulence has been eliminated).
- In some cases the vectors will contain human immunomodulatory transgenes to help the virus evade anti-viral T-cells while promoting anti-tumour immune cell recruitment and/or stimulation of anti-tumour immune cell activity.
- After Infection of human cancer cell lines in vitro, oncolytic herpes simplex virus vectors replicate, which then results in lysis of the tumour cells.
- Therefore the main potential hazards to health or the environment associated with the genetically modified tumour cell lines is the release of infective herpes virus particles when the tumour cell is lysed by the virus.
- For recombinant oncolytic HSV1. As the recombinant oncolytic herpes virus selectively infects tumour cells, in the event of accidental infection of a workers normal cells, further virus production would not occur, which should minimizes risk to health. Furthermore, antiviral drugs are available for the treatment of infections like acyclovir, foscarnet valacyclovir, famciclovir, and penciclovir that can inhibit viral replication. However, to our knowledge there have been no reports of laboratory acquired infections resulting from work with these types of viral vector.
-For completeness the following applies to Parental or Wild-type HSV-1 the following infection risks have been identified. Hazards associated with risk of infection: Parental HSV-1 is associated mainly with "above the waist" infections involving the mouth, pharynx, face, eye, and central nervous system (CNS), but can be associated with "below the waist" infections of the genital region (although these symptoms are mainly associated with HSV-2 infection). Herpes labialis/cold sores: Caused mainly by HSV-1. Primary infections with HSV-1 are acquired usually in childhood and may be asymptomatic or subclinical. Symptomatic primary infections present mainly as gingivostomatitis, with fever, sore throat, fetor oris, anorexia, cervical adenopathy, and mucosal edema and vesicular and ulcerative painful lesions involving the buccal mucosa, tongue, gums, and pharynx. Ulcers heal without scarring within 2-3 weeks. Recurrent infections have generally milder symptoms and clinical course. Recurrent lesions due to HSV-1 occur mainly on a specific area of the lip (vermilion border of the lip), and are called "cold sores" or "fever blisters". The lesions heal in approximately 8-10 days. Herpetic whitlow: Characterized by formation of painful vesicular lesions on the nail or finger area. Infections of the eye: Characteristic dendritic ulceration occurs on conjunctiva, and cornea. HSV infection may cause other ocular diseases, including blepharitis/dermatitis, conjunctivitis, dendritic epithelial keratitis, and corneal ulceration. Encephalitis: Serious infections of the CNS, affecting both children and adolescents. It may occur due to primary or latent infection with HSV-1 virus. HSV encephalitis affects one temporal lobe, leading to focal neurologic signs and edema. Genital herpes: It is a sexually transmitted disease. Genital herpes is caused mainly by HSV-2. Primary genital herpes is characterized by formation of multiple, bilateral, painful, and extensive genital ulcers, which heal without scarring within 12 days. Patients also present with tender enlarged lymph nodes, fever, malaise, and myalgia. Rarely, the disease may also cause aseptic meningitis with neck rigidity and severe headache. Recurrent genital herpes disease is of shorter duration, is milder and does not have systemic symptoms. The main manifestation of the disease is prodromal paresthesias in the perineum, genitalia or buttocks, followed by formation of grouped lesions on the external genital area. The lesions heal without scarring in 2-5 days. Neonatal Herpes: Neonatal herpes is an extremely severe disease with a very high mortality rate. Neurological complications may occur in infants who survive the infection.
- Potential hazards to the environment: Although, non-human primates in captivity can be accidentally infected and rabbits and rodents have been infected experimentally the host range of human herpes simplex virus 1 is generally restricted to humans in the wild. Furthermore, this recombinant version of the virus has been engineered to be attenuated and selective for tumour cells and not normal cells. Therefore it is unlikely that work with this vector will represent a significant risk to the environment.
- Control measures employed will minimise risks to workers and the environment. These control measures include rendering all solid or liquid waste inactive within the laboratory (either by autoclaving, incineration or use of ). Work will carried out in containment 2 laboratories in class II microbiological safety cabinets with routine disinfection of work surfaces. All staff will be suitable trained in working at containment 2 laboratories and be aware of the necessary risk control measures.
- Note that a number of herpes simplex virus 1-based viral vectors have been evaluated and approved for human therapy e.g. Talimogene laherparepvec (T-Vec/Imlygic), which is the first FDA-approved oncolytic virus (Conny et al 2018. Human Vaccines and Immunotherapeutics, 14, 839-846). Therefore, the safety profile of Herpes simplex virus 1-based vectors are excellent.

Evaluation of foreseeable effects

- For recombinant oncolytic HSV1. As the recombinant oncolytic herpes virus selectively infects tumour cells, in the event of accidental infection of a workers normal cells, further virus production would not occur, which should minimizes risk to health. Furthermore, antiviral drugs are available for the treatment of infections like acyclovir, foscarnet valacyclovir, famciclovir, and penciclovir that can inhibit viral replication. However, to our knowledge there have been no reports of laboratory acquired infections resulting from work with these types of viral vector.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid and solid waste is expected to be generated.
All liquid waste (including tissue culture fluids) will be inactivated by treatment with 1%w/v Virkon (or 2% Chemgene) for 12 hours prior to disposal to drains.
All solid waste material will be inactivated by treatment with 1% w/v Virkon (or 2% Chemgene) solution for 12 hours prior to autoclaving. Waste material will then be
Incinerated by an off-site incineration company (Grundon - their GM authorization is attached) according to disposal notification. The disposal method is expected to achieve 100% inactivation of the GMM. Datasheet information from Relyon.dupont _virkon has validated the use of this disinfectant for use with a broad range of infectious agents.

Datasheet information from Relyon.dupont _virkon has validated the use of this disinfectant for use with a broad range of infectious agents.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC opinion is that the use of herpes simplex virus 1-based viral vectors is appropriate at containment level 2. All equipment in the process has been assessed and will be suitable for the process with the risk of aerosol production minimal. All waste routes are also effective and approved.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>Animal Units</td>
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### GM Centre Number: 3043

| Data Premises Notified (Originally) | 09/04/2009 |
| Data Premises Closed | 02/12/2016 |
| Transferred from 1992 Regs? | N |
| Transitional Premises Class | |
| Emergency Plan Required? | |
| Transferred from Transitional Premises | |
| Non-GMMs | N |
| Withdrawn | N |

#### Name

TAKEDA CAMBRIDGE LTD

#### Campus Estate or Research Centre

**Road Name:** 418 CAMBRIDGE SCIENCE PARK

**Town:** CAMBRIDGE

**County:** CAMBRIDGESHIRE

**Postcode:** CB4 0PZ

**Country:** ENGLAND

**Tel Number:** 01223 477910

**Fax Number:** 01223 477911

**E-mail**

**HSE Division:** EAST AND SOUTH EAST

#### Comments

Takeda cambridge merged with Paradigm Therapeutics 03/12/2015

#### Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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<th>County</th>
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<td>UNIT 60</td>
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<td>TAKEDA CAMBRIDGE LTD</td>
<td>Takeda Cambridge Ltd</td>
<td>418 CAMBRIDGE SCIENCE PARK</td>
<td>430 Cambridge Science Park</td>
<td>Milton Road Cambridge</td>
<td>CB4 0QA</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The committee is a sub-committee of the Biological Safety Committee and consists of a representative from all the sub sections of the company involved in GM Organism work.

The committee will meet not less than four times per year to review the use of GMOs. However, it is not anticipated that there will be any major changes in the types and varieties of GMOs in use.

4.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Non-microbial</td>
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</table>
The GMMs that are currently generated by Takeda Cambridge Ltd are completely destroyed by standard autoclaving. All laboratory waste (for example gloves, used plasticware, pipettes, and anything else contaminated) will be subject to autoclaving before leaving the premises. In the case of liquids, a strong disinfectant (for example Chloros) will be used to treat samples and regular testing will take place to gauge the level of killing. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment. For minor spillages it is anticipated that a solution of 70% Ethanol or 60% Isopropanol will be used to decontaminate – any solid waste from the decontamination process will be autoclaved before leaving the building. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are deemed inappropriate. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. In accordance with Good Laboratory Practice, Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching “clean” surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Hands are washed following removal of gloves.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Takeda Cambridge Ltd (formerly Paradigm Therapeutics Ltd.) has been working under licence #GM 860 (to Paradigm Therapeutics Ltd, 418 Cambridge Science Park) with the GMMs outlined in Appendix 1. In accordance with this, the GMSC has recommended that Takeda Cambridge continue to work only with fully verified commercial sources of Host strains and vectors to maintain all work at Hazard Level 1 and Containment Level 1.

### Project Ref 3043/10.1

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<td>16/12/2010</td>
<td>Use of SH-SY5Y-HTT-LC3 cells that express the Huntingtin and LC3 proteins to investigate cellular mechanisms of protein degradation</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Date Project Ceased: 02/12/2016

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Project notified under transitional arrangements: N

### Project Additional Information

**Purposes of the contained use**

SH-SY5Y-HTT-LC3 cells expressing fluorescently tagged huntingtin protein (HTT-GFP (green fluorescent protein)) and fluorescently tagged microtubule-associated protein 1-light chain 3 (LC3-mCherry) will be used to investigate how the cellular process of protein degradation is regulated.

All humans have the Huntingtin gene (HTT) which codes for the huntingtin protein (Htt). This gene contains a repeated section called a trinucleotide repeat. When the length of this repeated section reaches a certain threshold, it produces an altered form of the protein, mutant Htt (mHtt). The differing functions of mHtt from Htt are the cause of the pathological changes which cause the disease symptoms of Huntington's disease. The Htt protein interacts with over 100 other proteins and appears to have multiple biological functions. In this system a recombinant Htt protein that is tagged with the well-characterized fluorescent protein, GFP, will be over expressed in order to generate fluorescently-tagged protein aggregates which can be visualized. The clearance of these protein aggregates by the cellular process of autophagy can then be monitored.

Autophagy is a catabolic process involving degradation of a cells own components through the lysosomal machinery. Autophagy involves the formation of a membrane around a targeted region of the cell (generating an autophagosome) which separates the contents from the rest of the cytoplasm. This autophagosome then fuses with a
lysosome (which contains the enzymes required for degradation) and the contents are degraded. The LC3 protein is the only known mammalian protein that specifically associates with the autophagosome membrane. By tagging LC3 with mCherry, another well-characterized fluorescent protein, it can be used in the cell line detailed here as a marker to identify autophagosomes.

SH-SY5Y-HTT-LC3 cell monolayers will be cultured according to containment level II (CL2) regulations. Microscopic visualization of the fluorescent tagged Htt and LC3 proteins under a variety of conditions (e.g. alterations in nutrient supply) will enable a better understanding of the protein degradation process.

The parental cell line SH-SY5Y, is a human neuroblastoma which was derived as a subclone of the SK-N-SH cell population originating from a human bone marrow sample (ATCC #CRL-2266). The SH-SY5Y cell line displays neuronal-like properties and continuous cultures were established in vitro (Biedler et al., 1973. Cancer Res. 33, 2643-2652; Biedler et al., 1978, Cancer Res. 38, 3751-3757) without the need for immortalization using retrovirus. SH-SY5Y cells can be maintained in a containment level 1 facility (as per instructions from ATCC). SH-SY5H-HTT-LC3 cells can not survive outside the laboratory and this cell line can not produce viral particles (Section 7). The SH-SY5Y-HTT-LC3 cell line will be treated as a CL2 cell line and all material will be totally inactivated (by exposure to Trigene advance (Medichem)) at the end of each experiment. No foreseeable effects on human health and safety are expected.

The human neuroblastoma SH-SY5Y cell line is a subclone of the SK-N-SH cell population which originates from a human bone marrow sample (ATCC #CRL-2266). This cell line has neuronal-like properties and continuous cultures were established in vitro by selection for growth (Biedler et al., 1973. Cancer Res. 33, 2643-2652; Biedler et al., 1978, Cancer Res. 38, 3751-3757) without the need for immortalization. SH-SYSY-HTT-LC3 cells can not survive outside the laboratory and this cell line can not produce viral particles (detailed below). The SH-SYSY-HTT-LC3 cell line will be treated as a CL2 cell line and all material will be totally inactivated (by exposure to Trigene advance *) at the end of each experiment. All solid material that has been in contact with this cell line, following inactivation, will be autoclaved on site. Inactivated liquid waste and inactivated, autoclaved solid waste material will be collected and taken off site by Grundons and either disposed of according to liquid waste regulations or in the case of solid waste, incinerated. No foreseeable effects on human health and safety are expected.

*As per manufacturer's information (Medichem) Trigene advance formula is a nanoemulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms. Rapid penetration of cell membranes by Trigene advance disinfectant constituents ensures cell death is equally rapid. Trigene advance works quicker than conventional high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products.

The SH-SYSY-HTT-LC3 cell line was generated in the laboratory of a collaborator at the University of British Columbia. An overview of the process taken to generate this cell line is detailed below and full details of the vectors and delivery systems employed can be found on the Clontech website.

Proteins to be overexpressed in SH-SYSY cells:
1) HTT: All humans have the Huntingtin gene (HTT) which codes for the huntingtin protein (Htt). Part of this gene is a repeated section called a trinucleotide repeat. When the length of this repeated section reaches a certain threshold, it produces an altered form of the protein, mutant Htt (mHtt). The differing functions of mHtt are the cause of the pathological changes which cause the disease symptoms of Huntington's disease. The Htt protein interacts with over 100 other proteins and appears to have multiple biological functions. This protein will be expressed as a fusion with green fluorescent protein to allow microscopic visualisation (HTT-GFP).

2) LC3: The LC3 (microtubule-associated protein 1-light chain 3) protein is the only known mammalian protein that specifically associates with the autophagosome membrane and thus can be used as a specific marker of autophagosomes. This protein will be expressed as a fusion with the fluorescent protein mCherry to allow microscopic visualisation (LC3-mCherry).

Expression System and Delivery System
Expression of both LC3-mCherry and HTT-GFP was achieved by adaptation of the Lenti-X Tet-On Advanced system (Clontech). Detailed information about this system can
be found: http://www.clontech.com/products/detail.asp?product_id=172013&product_group_id=171916&product_family_id=1419&tabno=2 Briefly, this system is based on the generation of lentiviral expression vectors, the expression from which can be modulated by the presence of doxycycline (see below). These vectors are packaged into replicon-incompetent lentivirus using lentivirus packaging systems and the lentiviral packaging 293T cell line (Clontech). As detailed below, the system deploys a number of specific measures to ensure that viral replication can only occur in the packaging 293T cell line and can not occur in the cell line which is transduced. The replicon-incompetent lentivirus were then used to transduce the SH-SY5Y cells. The resultant SH-SY5Y-HTT-LC3 cells can not generate replicon-competent lentivirus (RCL; discussed below).

The gene expression system consists of a vector construct that stably expresses the tet-On advanced transcriptional activator (rtTA), and response elements (pLVX-Tight-Puro) into which the genes of interest are cloned (in this case, HTT-GFP and LC3-mCherry). The pLVX vector (Clontech) is an HIV-1-based lentiviral expression vector. This vector contains the viral processing elements necessary for the production of replicon-competent lentivirus. This vector does not contain the total complement of all viral packaging proteins and thus by itself lacks all of the necessary components to produce replicon-competent lentivirus. The other required components are expressed in the packaging cell line. The rtTA and IRES m-cherry fused to LC3 are driven by the CMV promoter. The HTT-GFP is driven by the pTight promoter (Tet On). The LC3-mCherry is constitutively expressed where as HTT-GFP is under rtTA control and is only expressed in the presence of doxycycline.

As indicated above, the lentiviral expression vectors and the lentivirus packaging systems and 293T cells lines that were used to generate the SH-SY5Y-HTT-LC3 cell line were obtained from Clontech. As detailed on the Clontech website: there are several measures that have been taken to ensure that viral replication is specifically restricted to specific packaging cells to minimize the possibility of generating a RCL. Clontech state that: 'the emergence of RCL is undetectable from systems using this approach'. Clontech’s lenti-X vectors contain less than one third of the wild-type HIV-1 genome. All essential replication genes have been completely removed and are instead supplied as separate DNA entities in the Lentiviral HTX packaging mix. The Lenti-X HTX packaging mix is only utilized with 293T cells. Clontech's lentiviral packaging systems utilize split-genes to provide the viral packaging elements on individual plasmids that physically separate the viral envelope, env (usually VSV-G), sequence from the gag-pro-pol sequences. As an additional safety measure, pol is also separated from gag-pro. The result is that gag, pol and env reside on three physically distinct entities. This split-gene packaging strategy reduces the risk of generating RCL because multiple recombination events are necessary to create a virus that harbours the sequences required for independent replication. Use of the system generates replicon-competent virus particles. Such virus particles were used to generate the SH-SY5Y-HTT-LC3 cell line.

Measures are in place to ensure that all matter (solid and liquid) that has been in contact with the SH-SY5Y-HTT-LC3 cell line will be disposed of so that it has no impact on human health and safety. SH-SY5H-HTT-LC3 cells can not survive outside the laboratory environment and this cell line has been created in such a manner to prevent the production of viral particles (detailed above). Due to the fact that the SH-SY5Y-HTT-LC3 cell line was generated using a lentivirus system it will be treated as a CL2 cell line and all material will be totally inactivated (by exposure to Trigene advance*) at the end of each experiment. All solid material that has been in contact with this cell line, following inactivation, will be autoclaved on site. Inactivated liquid waste and inactivated, autoclaved solid waste material will be collected and taken off site by Grundons and either disposed of according to liquid waste regulations or in the case of solid waste, incinerated. No foreseeable effects on human health and safety are expected.

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Origin & function

The vector construction, preparation of lentivirus and the generation of stable cell lines (detailed above) was carried out in the laboratory of a collaborator at the University of British Columbia.

SH-SY5Y-HTT-LC3 cells that can express fluorescently tagged huntingtin protein (HTT-GFP (green fluorescent protein)) and fluorescently tagged LC3 protein (LC3-mCherry) will be used to investigate how the cellular process of protein degradation is regulated. All humans have the Huntingtin gene (HTT) which codes for the protein huntingtin (Htt). Part of this gene is a repeated section called a trinucleotide repeat. When the length of this repeated section reaches a certain threshold, it produces an altered form of the protein, mutant Htt (mHtt). The differing functions of mHtt to Htt are the cause of the pathological changes which cause the disease symptoms of Huntington's disease. The Htt protein interacts with over 100 other proteins and appears to have multiple biological functions. In this system the Htt protein...
cell walls of micro-organisms. Rapid penetration of cell membranes by Trigene advance disinfectant constituents ensures cell death is equally rapid. Trigene advance works

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regulations or in the case of solid waste, incinerated. No foreseeable effects on human health and safety are expected.

Inactivated liquid waste and inactivated, autoclaved solid waste material will be collected and taken off site by Grundons and either disposed of according to liquid waste

exposure to Trigene Advance*) at the end of each experiment. All solid material that has been in contact with this cell line, following inactivation, will be autoclaved on site.

machinery to produce viral particles (detailed above). The SH-SY5Y-HTT-LC3 cell line will be treated as a CL2 cell line and all material will be totally inactivated (by exposure to Trigene advance* (Medichem)) at the end of each experiment. No foreseeable effects on human health and safety are expected.

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cell walls of micro-organisms. Rapid penetration of cell membranes by Trigene advance disinfectant constituents ensures cell death is equally rapid. Trigene advance works

quicker than conventional high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products.

**Evaluation of foreseeable effects**

The SH-SY5Y-HTT-LC3 cell line will be maintained in a CL2 facility and will be treated as CL2 material (see 8). The vector construction, preparation of lentivirus and the generation of the SH-SY5Y-HTT-LC3 stable cell line (detailed above) was carried out in the laboratory of a collaborator at the University of British Columbia. The parental

SH-SY5Y cell line can be maintained in CL1 facilities (in accordance with ATCC guidelines). In addition, this cell line was transformed with replicon-incompetent lentivirus (according to Clontech literature; detailed above) and does not contain the necessary virus machinery to shed replicon-competent particles. However, to ensure maximal safe practice, the SH-SY5Y-HTT-LC3 cell line will be maintained in a CL2 facility and will be treated as CL2 material.

All humans have the Huntingtin gene (HTT) which codes for the protein huntingtin (Htt). Part of this gene is a repeated section called a trinucleotide repeat. When the length of this repeated section reaches a certain threshold, it produces an altered form of the protein, mutant Htt (mHtt). The differing functions of mHtt to Htt are the cause of the pathological changes which cause the disease symptoms of Huntington's disease. The Htt protein interacts with over 100 other proteins and appears to have multiple biological functions. The LC3 protein is involved in the formation of the autophagosome during autophagy. We do not anticipate any foreseeable effects on human health and safety during and after the use of this cell line.

As previously indicated, measures are in place to ensure that all matter (solid and liquid) that has been in contact with the SH-SY5Y-HTT-LC3 cell line is inactivated and disposed of so that it has no impact on human health and safety. SH-SYSH-HTT-LC3 cells can not survive outside the laboratory and this cell line lacks the cellular machinery to produce viral particles (detailed above). The SH-SY5Y-HTT-LC3 cell line will be treated as a CL2 cell line and all material will be totally inactivated (by exposure to Trigene Advance*) at the end of each experiment. All solid material that has been in contact with this cell line, following inactivation, will be autoclaved on site. Inactivated liquid waste and inactivated, autoclaved solid waste material will be collected and taken off site by Grundons and either disposed of according to liquid waste regulations or in the case of solid waste, incinerated. No foreseeable effects on human health and safety are expected.

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cell walls of micro-organisms. Rapid penetration of cell membranes by Trigene advance disinfectant constituents ensures cell death is equally rapid. Trigene advance works

quicker than conventional high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Assessment of the SH-SY5Y-HTT-LC3 line risk assessment (please see attached risk assessment number XXXX) by the internal GMO committee dictated that this cell line should be maintained as a CL2 cell line because it was generated using replicon incompetent lentivirus. The tissue culture laboratories have been set up according to the relevant regulations. The tissue culture laboratory where this work will take place has the following features:

1. Benches are impervious to water and resistant to acids, alkalis, solvents, decontamination agents and are easy to clean
2. Labs are under negative pressure
3. The maintenance of the cell line will be carried out in class II biosafety hoods which have double hepa filters and have been certified as functional to perform such work
4. All solid waste (pipettes; plastic ware etc) will be treated with a solution of Trigene to inactivate the material. The waste is then autoclaved on site prior to collection and disposal by Grundons
5. All liquid waste, any contaminated solid waste and cellular material is totally inactivated by exposure to Trigene advance (1:10 dilution; Medichem*) prior to collection and disposal by Grundons
6. All personnel using the TC facilities are made aware (by verbal means and the use of appropriate hazard stickers) as to the nature of the work being undertaken in the lab
7. All personnel using the TC facilities receive appropriate training for which there are written records
8. All personnel will wear the appropriate personal protective equipment. By adhering to the CL2 guidelines together with knowledge regarding the SH-SY5Y-HTT-LC3 cell line, no foreseeable effects on human health and safety are expected.

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**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

The SH-SY5Y-HTT-LC3 cell line can not survive outside the laboratory. This cell line does not contain the cellular machinery to shed replicon-competent viral particles (discussed above). SH-SY5Y-HTT-LC3 cultures will be treated as CL2 material and the following details our waste management measures:

1. Culture medium will be aspirated with plastic pipettes and will be collected in a suitable vessel which is appropriately labelled.
2. Media will be decontaminated and 100% inactivated by exposure to Trigene advance* (1:10 dilution; Medichem).
3. Decontaminated/inactivated media will be collected and disposed of by Grundons.
4. Plastic ware that has been in contact with the cell line (e.g. pipettes, flasks etc) will be exposed to trigene to inactivate any remaining material.
5. De-contaminated plastic ware will be stored in autoclave boxes and autoclaved on site.
6. Any solid tissue culture material (e.g. gloves, paper towels etc) will be placed in autoclave bags and autoclaved.
7. All autoclaved material will be collected and disposed of by Grundons.

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The Genetic Modification Safety Committee of Takeda Cambridge Ltd (Jonathan Ellery PhD, Alan Hendrick PhD, 2010) upon review of the risk assessment for the SH-SY5Y-HTT-LC3 cell line determined that:

The parental cell line SH-SY5Y requires class 1 containment and has not been immortalised deliberately with retrovirus. This cell line represents no hazard to human health.

The lentivirus systems used to introduce the HTT-GFP and LC3-mCherry genes into the SH-SY5Y cell line are designed to minimise the risk of generating a cell line that is capable of producing replicon competent virus particles.

It has been noted that there is no supporting data to show this to be the case for SH-SY5Y-HTT-LC3.

Of the two proteins to be expressed only one of these (a mutant form not being expressed) has been shown to be involved with a human pathology.

The risk from exposure to a tagged version of the protein is negligible.

It was decided to categorise this work as class 2 because the cell line was generated using a lentiviral system and that a mutant version of the Htt protein has been implicated in a human pathology.

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Project Ref 3043/11.1

Date Ackn'd: 28/03/2011

CU2 Project Title: Use of BV-2 cells to investigate cellular mechanisms of neurodegeneration

Class: Class 2

CultureVolClass2: ≤ 1 Litre

Consent Granted

Non-GMM

Date Project: 02/03/2022
The BV2 cell line is an immortalized microglia cell line, which will be used to analyse mechanisms of neurodegeneration. Immortalized cell lines proliferate and therefore represent a valuable source for in vitro cell assays. This is in contrast with primary rodent microglia, which do not appear to proliferate in vitro. Furthermore, the protocol to isolate primary microglia is lengthy and requires confluent mixed glial preparations to be obtained (2 weeks in vitro) before the microglia can be isolated. Following isolation, the yield of microglia is generally very low and thus the number of experiments/glial preparation is limited.

Microglia activation has been reported in Alzheimer's disease. This results in release of cytokines and reactive oxygen species (ROS) that have neurotoxic effects. It is therefore critical to analyse microglia function and potentially the interplay between microglia and neurons in order to validate potential drug targets. BV-2 cells will be stimulated with beta-amyloid or other stimuli eg lipopolysaccharide to induce cell activation. Cytokine release and ROS production will be measured. Target expression analysis by immocytochemistry and Western blotting will be performed in microglia cell lysates and the effect of blocking the target on interest on ROS and cytokine release will be analysed. Compound treatment to inhibit the target of interest as well as RNAi experiments will be performed. For the latter, transient target knockdown studies will be performed using si-RNA transfection via lipid or defined polymers.

BV-2 cells have been generated by infecting mouse primary microglial cells with a v-raf/v-myc oncogene carrying retrovirus (J2). Since BV-2 cells retain most of the morphological, phenotypical and functional properties described for freshly isolated microglial cells, it can be concluded that J2 virus infection resulted in the immortalization of active microglial cells (Blasi E., et al J Neuroimmunology 1990).

In more detail, microglial cells were purified by shaking and subsequent selective plastic adherence of 1-week old mixed brain cultures obtained from C57BL/6 mice, as detailed in Giulian and Baker, 1986; Bocchini et al., 1988. The cultures were incubated overnight with control supernatants or J2 virus containing supernatants, fed biweekly with RPMI 1640, supplemented with 10% heat-inactivated fetal bovine serum, gentamicin and L-glutamine (4mM) (complete medium). Foci of proliferating cells (BV-2 cells) were observed in the infected cultures, whereas the uninfected cells gradually lost adherence properties and eventually died. BV-2 cell cultures appeared as a population composed of adherent, variably shaped cells. When assessed for histochemical markers, BV-2 cells exhibited a high degree of homogeneity, being >90% positive for nonspecific esterase activity. All cells lacked peroxidase activity. Upon continuous in vitro culture (>18 months), morphological, phenotypical and functional properties of BV-2 cells remained unchanged and, as assessed by Northern blot analysis, such cells constitutively expressed high levels of J2 virus mRNA. Most of the cells were similar to primary newborn microglia (Giulian and Baker, 1986), therefore indicating that J2 virus infection resulted in the immortalization of active microglial cells. Phagocytosis studies using C. Albicans microorganisms strengthen this conclusion. In fact, it is well-known that astrocytes and oligodendrocytes have phagocytic ability to some extent (Noske et al., 1982; Saito et al., 1986; Bjerkness et al., 1987); however, only microglial cells are able to ingest large-size particles such as Candida (Bocchini et al., 1988). Thus, also in this respect, BV-2 cells and primary microglial cells were indistinguishable. Upon opsonization the percentage of phagocytic cells as well as the phagocytosis index were enhanced, indicating that a functionally active Fc receptor system is expressed on BV-2 cells. Similarly to primary microglia (Giulian et al., 1986; Frei et al., 1986), BV-2 cells retain constitutive as well as inducible secretory functions. From these literature data, we have therefore decided to use this cell line for our in vitro studies involving microglia in neurodegeneration.
References:
Blasi E. et al., J Neuroimmunol. 1990
Bocchini V. et al., Dev. Neurosci. 1988
Frei K. et al., J Immunol. 1986

Recipient or parental organism

The BV-2 cell line was generated using v-raf/v-myc oncogene carrying retrovirus expressing the nuclear v-myc and the cytoplasmic v-raf oncogene products as well as the env gp70 antigen at the surface level. V-myc is the viral homolog of c-myc. The v-myc oncogene can transform several lineages of cells either alone or in co-operation with other oncogenes. V-raf is a murine leukemia viral oncogene homolog 1, also capable of transforming cells. When mutated, oncogenes have the potential to cause normal cells to become cancerous.

Importantly, the BV-2 cell line produces an enveloped recombinant ecotropic retrovirus capable of infecting murine cells only (http://bioinformatics.istge.it/cldb/cl7130.html); such virus is known for its in vitro transforming ability and in vivo tumorigenic potential. Although BV-2 cells can not survive outside the laboratory, all BV-2 biological material will be totally inactivated (by exposure to Trigene advance*) at the end of each experiment. No foreseeable effects on human health and safety are expected.

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Host/vector system

There will be no stable introduction of genetic material

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Evaluation of foreseeable effects

Health considerations
The use of the cell line for research purposes should not represent a risk to humans as BV-2 cells are capable of infecting murine cells only (http://bioinformatics.istge.it/cldb/cl7130.html). Although BV-2 cells can not survive outside the laboratory, all BV-2 biological material will be totally inactivated (by exposure to Trigene advance*) at the end of each experiment. No foreseeable effects on human health and safety are expected

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Care should be taken in order not to infect other murine cells present in the laboratory. Care should be taken in order not to spread the cell line in the environment as there is potential to infect murine cells. All BV-2 biological material will be totally inactivated (by exposure to Trigene advance*) at the end of each experiment. It is therefore highly unlikely that the virus could survive any unintentional discharge into a sanitary sewage system. Solid waste will be autoclaved prior to incineration offsite.

The BV-2 cell line is recognised as an important tool for the study of neurodegenerative processes of its phenotype and that it has been immortalised. The GMO committee is satisfied that because of the ectropic nature of the retrovirus generated by the cell line and that no further genetic modification has been proposed that this material can be adequately controlled within a class 2 environment. The procedures detailed in this assessment clearly demonstrate that contaminated material will be contained and inactivated before leaving the laboratory environment protecting both staff and the environment.

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**Project Ref** 3043/13.1

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<td>Lentiviral vector mediated RNA interference (RNAi) in rodent primary neurons following virus addition to knockdown and study the role of agmatinase in vitro, etc</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Project notified under transitional arrangements N
Agmatinase is an enzyme (EC3.5.3.11) that belongs to the arginase family. It hydrolyses agmatine to form putrescine and urea. Agmatine is a putative neurotransmitter acting on imidazoline, alpha-2 adrenergic and NMDA receptors. Agmatine shows neuroprotection in several in vitro models. Furthermore, agmatine improves memory in aged rats as well as after scopolamine challenge. It is of interest to determine whether downregulating agmatinase similar effects can be measured. These experiments will therefore be of importance for potential novel treatments to improve cognitive function.

Recipient or parental organism
Rodent primary neurones and in vivo Mouse/Rat

Host/vector system
The lentiviral vector expression system used as well as the transgene used have a very low risk for Human Health and safety. These 2 points are discussed below Lentiviral Expression System

Lentiviruses belong to the family Retroviridae, which forms a diverse and extensive family affecting both human and animal species. Retroviruses are characterised by a unique replication mechanism involving reverse transcription of the viral RNA genome, giving rise to a DNA provirus that contain two positive sense copies of the RNA genome, encased within a capsid that is surrounded by a host-cell derived envelope glycoprotein and cell-surface determinants. These interactions are generally specific and are believed to be the principal factor affecting cell type and species specificity. Receptor binding triggers membrane fusion mediated by the transmembrane subunit of the virion envelope glycoprotein, resulting in delivery of the virus capsid to the target cell.

The first risk to human health from the use of this expression system comes from the fact that it is based on HIV-1. However retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and thus lentiviral vectors are not infectious via the airborne route. The main risk of infection is consequently the risk of injection but it is highly unlikely to happen as all measure to avoid have been considered (see part C for detailed measures).

Origin & function
The transgene has a very low risk because it would be highly unlikely that expression in a few cells would be sufficient to lead to a disease state. The inability of the lentiviral vector to propagate in mammalin cells also reduces the risk.

Proteins that are commonly used as reporter genes and genes used for the identification and selection of translated cells (EGFP) will be used in this system and are not known to have toxic or oncogenic effects. Genes that will be excluded from this expression system are genes encoding a) proteins of retroviral origin b) toxins c) oncogenes

Evaluation of foreseeable effects
Human health hazard
Oral ingestion will be prevented by standard laboratory working practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosa' cells with modified viruses. However, these modified virus particles carry an extremely low risk for humans. Firstly, they are replication-incompetent. Secondly, these particles carry an extremely low infection risk, and transduction of
a laboratory worker's skin or mucosa is virtually impossible. In addition no genes encoding toxins, oncogenes, or pathogen-derived genes are used in our studies. In the unlikely event of accidental transduction of a laboratory worker's skin and mucosal cell layers. The most likely route of accidental infection with a lentivirus will be via inadvertent percutaneous inoculation however the control of sharps to minimise inadvertent exposure decrease considerably the chances of such accident (see Part C for procedure and control measure with sharp). Moreover use of personal protective equipment designed to prevent a mucosal exposure/splash to the face and exposure of hands (especially in persons with broken skin or open cuts). Infection of the community at large with lentiviruses particles is impossible for the above described reasons of replication incompetence.

HAZARDS TO THE ENVIRONMENT

As the viral systems used are self-inactivating and non-replicative, it is extremely unlikely that accidental release into the environment will cause any environmental damage or risk to others. The potential risks for lentiviral expression are released into a human population already infected with HIV, allowing for potential recombination events that would incorporate the inserted sequence into a viable RCL. This scenario is extremely unlikely, and would in any case be unlikely to cause any additional problem.

This lentiviral vector is designed for gene expression in mammalian cells and transduces a wide range of host human and non-human cell lines which can be dividing or non-dividing. Indeed the vector is pseudotyped with VSV-G envelope and therefore has a greater host range however it cannot survive even after infection (which is highly unlikely). Although VSVG-pseudotyped virus particles on transduce in theory all mammalian cell, transduction is also highly dependent on the concentration of the virus particles in a given medium. Once produced they are aliquoted and stored in small amounts (each tube contains at maximum 10µl) at -80C, considering infectivity of the vector drops markedly at room temperature it is hard to envisage an accidental mechanism for release that would allow survival.

Moreover lentiviral vectors are enveloped RNA viruses that are relatively unstable and highly susceptible to dehydration. Lentivirus particles have thus a short half-life and are unstable at room temperature. However, they can survive for long periods in high protein media. Retroviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. Furthermore, many retroviruses require high titre inoculations to establish an infection. Therefore, the survivability of retroviruses is not thought to pose a risk to the environment.

Rodents such as wild-type mice and rats, cannot support replication of infectious HIV-1 (Morrow 1987, Lewis 1995, Bieniasz 2000, Goffinet 2007). As a result, the potential for shedding of replication-competent lentiviruses (RCL) from such animals is very low (even if RCL were present in the original vector inoculum).

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and are mostly designed to be protective. EGFP will be primarily expressed in the CNS (as it uses neuron specific promoters). Similarly, shRNA and miRNA sequences will not survive as they are highly unstable and thus would disappear very quickly if accidentally spilled into the environment. Sequences within the viruses cannot be transferred to other micro-organisms without a rare recombination event as described above. Thus we see no conceivable hazard associated with transfer of the expressed sequences.

Summary

Environmental risk is very low because accidental release is very unlikely to result in production of RCL. Although the virus is capable of transducing mammalian cells it lacks the genes for replication and expression of proteins required for packaging into capsids.

The viral materials have been classified as CL2 and to minimise the risk of release to the environment from the laboratory. However, if accidental release into the environment does occur the vectors/plasmids could not survive and the risk is minimal.

References
The work outlined above has been assessed as a Class 2 activity even though the risk of pathogenicity to the end worker is very low as the virus is severely replication defective and encodes no toxic proteins. Biosafety 2 conditions will be followed as prescribed by the HSE for handing and usage of the stock vials of lentivirus and when working with primary neuronal cells. However, it is permissible to reduce the containment level at some point following vector delivery into animal brain as there is no expectation of infection, the site of inoculation has been thoroughly cleansed, and the bedding changed. Indeed for experimental animals that have been inoculated with non-replicating virus, the risk of operator exposure from the infected animals is minimal as is the potential for virus shedding. It is therefore appropriate to house these animals in a containment level 1 facility after recovery (Karlen 2007).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

As the viral systems used are self-inactivating and non-replicative, it is extremely unlikely that accidental release into the environment will cause any environmental damage or risk to others. The potential risks for lentiviral expression are release into a human population already infected with HIV, allowing for potential recombination events that would incorporate the inserted sequence into a viable, replication competent virus. This scenario is extremely unlikely, and would in any case be unlikely to cause any additional problem.

This virus is designed for gene expression in mammalian cells and transduces a wide range of host human and non-human cell lines which can be dividing or non-dividing. Indeed the vector is pseudotyped with VSV-G envelope and therefore has a greater host range however it cannot survive even after infection (which is highly unlikely). Although VSVG-pseudotyped virus particles can transduce, in theory all mammalian cells, transduction is also highly dependent on the concentration of the virus particles in a given medium. Once produced they are aliquoted and stored in small amounts (each tube contains maximum 100µl) at -80C, considering infectivity of the vector drops.
markedly at room temperature it is hard to envisage an accidental mechanism for release that would allow survival. Moreover lentiviral vectors are enveloped RNA viruses that are relatively unstable and highly susceptible to dehydration. Lentivirus particles have thus a short half-life and are unstable at room temperature. However, they can survive for long periods in high protein media. Retroviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required to transmission. Furthermore, many retroviruses require high titre to the environment.

Some animals, such as wild-type mice, cannot support replication of infectious HIV-1. As a result, the potential for shedding of replication-competent lentiviruses (RCL) from such animals is very low (even if RCL were present in the original vector inoculum).

There are no conceivable hazards associated directly with the plasmids (containing the inserts) as they will not replicate/survive outside the laboratory, are mostly designed to be protective, and are primarily active in the CNS (as they use neuron specific promoters). Similarly, shRNA and miRNA sequences will not survive as they are highly unstable and this would disappear very quickly if accidentally spilled into the environment. Sequences within the viruses cannot be transferred to other micro-organisms without a rare recombination event as described above. Thus we see no conceivable hazard associated with transfer of the expressed sequences.

Waste handling

- Solid waste will then be neutralised by soaking in a validated chemical reagent prior to being bagged up in double autoclave bags, then autoclaved prior to incineration offsite.
- Liquid volumes will be disposed of into sealed biohazard containers filled with Virkon* for incineration. It is routine that all liquid waste material is neutralised before disposal to allow transportation in leak-proof containers to prevent spillage

In vivo
- Needles should be used with extreme care, only used when absolutely necessary and should never be resheathed, but disposed of directly into a suitable waste container (sharp bin). Sharps will be discarded in a yellow glass container (sharp bin) to be stored in a safe manner prior to incineration.
- Animal waste is considered non-infectious and disposed of along with the waste from non-infected animals so animal carcasses will be incinerated offsite.

It is worth highlighting that there is no aqueous disposal to sewers on site and that all waste is sent off site for incineration. If necessary any material can get autoclaved prior to being taken off site for incineration.

Following class 2 procedures for the stereotaxic injection, good laboratory practice and extreme care with sharps will be sufficient to insure that all contaminated material will be contained and inactivated before leaving the laboratory environment protecting both staff and the environment.

All workers using animals or primary cells are required to attend an in-house training prior to starting work and are then closely supervised by experienced research scientist until competent in all procedures. No worker will be precluded from the work proposed here and no vaccinations are required. We do not feel that specific health monitoring is required, although all workers will be made aware of the nature of the viruses that are being used.

Workers performing the surgical and behavioural tests are required to follow agreed procedures for the handling of animals to reduce the risk from allergens and bites… etc including the use of appropriate protective clothing and face masks, but there are no extra risks associated with the fact that the animal has undergone the procedures detailed. All in vivo work is carried out with the appropriate licenses and permissions from UK regulatory authorities.

Summary

All work with viral stocks and primary cultures will be carried out in class 2 safety cabinets. The lentivirus is unable to propagate in the primary cells so RCL will not be generated. In addition all plasticware and solutions that are used will be chemically inactivated with validated reagents before autoclaving prior to disposal for incineration. This will effectively prevent release of lentivirus to the environment and protect workers.

The maximum injection into an animal is 8µl. the Hamilton syringe is the only sharp tool in contact with the virus, it will be filled in a class 2 safety cabinet and it will be either a closed box inside a closed ice bucket or securely attached to a rig. Thus there is minimal risk of accidental puncturing of the skin with a contaminated syringe.
Once injected the viral particles cannot re-infect any other tissues as: (i) the coat protein is lost following attachment and the DNA is incorporated into the host cell replication deficient; (ii) they are replication deficient. Hence, lentiviral vectors cannot re-infect once injected into the brain.

Biological material will be totally inactivated (by exposure to Virkon* and/or DISTEL and/or Trigene advance*) at the end of each experiment. It is therefore highly unlikely that the virus could survive any unintentional discharge into a sanitary sewage system. No foreseeable effects on human health and safety are expected.

Project Containment

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Animal Units

| L2 | L3 | L4 | L2 |

Large Scale Activities

| L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |

Project Ref 3043/13.2

- Date Ackn'd: 19/03/2013
- CU2 Project Title: Lentiviral vector mediated RNA interference (RNAi) in rodent primary cells (neuron or glia) following virus addition to knockdown and study the role of histone deacetylase 2 (HDAC2) in vitro etc
- Date Project Ceased: 02/12/2016
- Class: Class 2
- CultureVolClass2: ≤ 1 L
- Consent Granted
- Project notified under transitional arrangements: No

Historical Significant Changes

Tick if notifying a connected programme of work: No
Project Additional Information

Purposes of the contained use

Aim:
HDAC2 is a histone deacetylase belonging to class I HDACs. HDACs regulate histone acetylation resulting in modulation of gene transcription. In particular, HDCA2 ablation has been shown to improve cognitive function possibly by increasing brain derived neurotrophic factor (BDNF) levels. Reduction of HDAC2 via adeno-associated viral vectors carrying shRNAs directed against HDAC2 injected into the CA1 region alleviated memory deficits in CK-p25 mice. Conversely, HDAC2 over expression in mice resulted in decreased dendritic spines and impaired memory. HDAC2 inhibition has therefore the potential of improving cognition and representing an innovative treatment for neurodegenerative diseases such as Alzheimer's.

A lentivirus delivering a shRNA and mediating gene knockdown of HDAC2 in brain cells (glial and neurons) would be a very useful tool to confirm the role of HDAC2 in regulation of BDNF levels and on cell viability/morphology. Moreover, testing the effect on HDAC2 knockdown in vivo in cognition tests would be useful to confirm literature findings. These experiments are important given the current interest in HDAC2 in the pathophysiology and therapy of neurodegenerative diseases.

Recipient or parental organism

Primary rodent neurones and Mouse/Rat

Host/vector system

The lentiviral vector expression system used as well as the transgene used have a very low risk for Human Health and safety. Theses 2 points are discussed below.

Lentiviral vector Expression System

Lentiviruses belong to the family Retroviridae, which forms a diverse and extensive family affecting both human and animal species. Retroviruses are characterised by a unique replication mechanism involving reverse transcription of the viral RNA genome, giving rise to a DNA provirus that contain two positive sense copies of the RNA genome, encased within a capsid that is surrounded by a host-cell derived envelope. Cellular entry involves interaction between the surface subunit of the virion envelope glycoprotein and cell-surface determinants. These interactions are generally specific and are believed to be the principal facotr affecting cell type and species specificity. Receptor binding triggers membrane fusion mediated by the transmembrane subunit of the virion envelope glycoprotein, resulting in delivery of the virus capsid to the target cell.

The first risk to human health from the use of this expression system comes from the fact that it is based on HIV-1. However retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and thus lentiviral vectors are not infectious via the airborne route. The main risk of infection is consequently the risk of injection but it is highly unlikely to happen as all measure to avoid have been considered (see part C for detailed measures).
The transgene has a very low risk because it would be highly unlikely that expression in a few cells would be sufficient to lead to a disease state. The inability of the lentiviral vector to propagate in mammalian cells also reduces the risk.

Proteins that are commonly used as reporter genes and genes used for the identification and selection of transfected cells (EGFP) will be used in this system and are not known to have toxic or oncogenic effects. Genes that will be excluded from this expression system are genes encoding a) proteins of retroviral origin b) toxins c) oncogenes.

Evaluation of foreseeable effects

Human health hazard

Oral ingestion will be prevented by standard laboratory working practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosa' cells with modified viruses. However, these modified virus particles carry an extremely low risk for humans. Firstly, they are replication-incompetent. Secondly, these particles carry an extremely low infection risk, and transduction of a laboratory worker's skin or mucosa is virtually impossible. In addition no genes encoding toxins, oncogenes, or pathogen-derived genes are used in our studies. In the unlikely event of accidental transduction of a laboratory worker's skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus will be via inadvertent percutaneous inoculation however the control of sharps to minimise inadvertent exposure decrease considerably the chances of such accident (see Part C for procedure and control measure with sharp). Moreover use of personal protective equipment designed to prevent a mucosal exposure/splash to the face and exposure of hands (especially in persons with broken skin or open cuts). Infection of the community at large with lentivirus particles is impossible for the above described reasons of replication incompetence.

HAZARDS TO THE ENVIRONMENT

As the viral systems used are self-inactivating and non-replicative, it is extremely unlikely that accidental release into the environment will cause any environmental damage or risk to others. The potential risks for lentiviral expression are released into a human population already infected with HIV, allowing for potential recombination events that would incorporate the inserted sequence into a viable RCL. This scenario is extremely unlikely, and would in any case be unlikely to cause any additional problem.

This lentiviral vector is designed for gene expression in mammalian cells and transduces a wide range of host human and non-human cell lines which can be dividing or non-dividing. Inded the vector is pseudotyped with VSV-G envelope and therefore has a greater host range however it cannot survive even after infection (which is highly unlikely). Although VSVG-pseudotyped virus particles can transduce in theory all mammalian cell, transduction is also highly dependent on the concentration of the virus particles in a given medium. Once produced they are aliquoted and stored in small amounts (each tube contains at maximum 10µl) at -80C, considering infectivity of the vector drops markedly at room temperature it is hard to envisage an accidental mechanism for release that would allow survival.

Moreover lentiviral vectors are enveloped RNA viruses that are relatively unstable and highly susceptible to dehydration. Lentivirus particles have thus a short half-life and are unstable at room temperature. However, they can survive for long periods in high protein media. Retroviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. Furthermore, many retroviruses require high titre inoculations to establish an infection. Therefore, the survivability of retroviruses is not thought to pose a risk to the environment.

Rodents such as wild-type mice and rats, cannot support replication of infectious HIV-1 (Morrow 1987, Lewis 1995, Bieniasz 2000, Goffinet 2007). As a result, the potential for shedding of replication-competent lentiviruses (RCL) from such animals is very low (even if RCL were present in the original vector inoculum).

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and are mostly designed to be protective. EGFP will be primarily expressed in the CNS (as it uses neuron specific promoters). Similarly, shRNA and miRNA sequences will not survive as they are highly unstable and thus would disappear very quickly if accidentally spilled into the environment. Sequences within the viruses cannot be transferred to other micro-organisms without a rare recombination event as described above. Thus we see no conceivable hazard associated with transfer of the expressed sequences.
Environmental risk is very low because accidental release is very unlikely to result in production of RCL. Although the virus is capable of transducing mammalian cells it lacks the genes for replication and expression of proteins required for packaging into capsids. The viral materials have been classified as CL2 and to minimise the risk of release to the environment from the laboratory. However, if accidental release into the environment does occur the vectors/plasmids could not survive and the risk is minimal.

References

For further information, see also the safety guidance "Biosafety Considerations for Research with Lentiviral Vectors" published by the US NIH as well as the following articles:

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

The work outlined above has been assessed as a Class 2 activity even though the risk of pathogenicity to the end worker is very low as the virus is severely replication defective and encodes no toxic proteins. Biosafety 2 conditions will be followed as prescribed by the HSE for handling and usage of the stock vials of lentivirus and when working with primary neuronal cells. However, it is permissible to reduce the containment level at some point following vector delivery into animal brain as there is no expectation of infection, the site of inoculation has been thoroughly cleaned, and the bedding changed. Indeed for experimental animals that have been inoculated with non-replicating virus, the risk of operator exposure from the infected animals is minimal as is the potential for virus shedding. It is therefore appropriate to house these animals in a containment level 1 facility after recovery (Karlen 2007).

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
As the viral systems are self-inactivating and non-replicative, it is extremely unlikely that accidental release into the environment will cause any environmental damage or risk to others. The potential risks for lentiviral expression are release into a human population already infected with HIV, allowing for potential recombination events that would incorporate the inserted sequences into a viable, replication competent virus. This scenario is extremely unlikely, and would in any case be unlikely to cause any additional problem.

This virus is designed for gene expression in mammalian cells and transduces a wide range of host human and non-human cell lines which can be dividing or non-dividing. Indeed the vector is pseudotyped with VSV-G envelope and therefore has a greater host range however it cannot survive even after infection (which is highly unlikely). Although VSVG-pseudotyped virus particles can transduce, in theory all mammalian cells, transduction is also highly dependent on the concentration of the virus particles in a given medium. Once produced they are aliquoted and stored in small amounts (each tube contains at maximum 10µl) at -80C, considering infectivity of the vector drops markedly at room temperature it is hard to envisage an accidental mechanism for release that would allow survival.

Moreover lentiviral vectors are enveloped RNA viruses that are relatively unstable and highly susceptible to dehydration. Lentivirus particles have thus a short half-life and are unstable at room temperature. However, they can survive for long periods in high protein media. Retroviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. Furthermore, many retroviruses require high titre inoculations to establish an infection. Therefore, the survivability of retroviruses is not thought to pose a risk to the environment.

Some animals, such as wild-type mice, cannot support replication of infectious HIV-1. As a result, the potential for shedding of replication-competent lentiviruses (RCL) from such animals is very low (even if RCL were present in the original vector inoculum).

There are no conceivable hazards associated directly with the plasmids (containing the inserts) as they will not replicate/survive outside the laboratory, are mostly designed to be protective, and are primarily active in the CNS (as they use neuron specific promoters). Similarly, shRNA and miRNA sequences will not survive as they are highly unstable and thus would disappear very quickly if accidentally spilled into the environment.

Sequences within the viruses cannot be transferred to other micro-organisms without a rare recombination event as described above. Thus we see no conceivable hazard associated with transfer of the expressed sequences.

Waste handling

In vitro and in vivo
- Solid waste will be neutralised by soaking in a validated chemical reagent prior to being bagged up in double autoclave bags, then autoclaved prior to incineration offsite.
- Liquid volumes will be disposed of into sealed biohazard containers filled with Virkon* for incineration. It is routine that all liquid waste material is neutralised before disposal to allow transportation in leak-proof containers to prevent spillage

In vivo
- Needles should be used with extreme care, only used when absolutely necessary and should never be resheathed , but disposed of directly into a suitable waste container (sharp bin). Sharps will be discard in a yellow glass container (sharp bin) to be stored in a safe manner prior to incineration.
- Animal waste is considered non-infectious and disposed of along with the waste from non-infected animals so animal carcasses will be incinerated offsite. It is worth highlighting that there is no aqueous disposal to sewers on site and that all waste is sent off site for incineration. If necessary any material can get autoclaved prior to being taken off site for incineration.

Following class 2 procedures for the stereotaxic injection, good laboratory practice and extreme care with sharps will be sufficient to insure that all contaminated material will be contained and inactivated before leaving the laboratory environment protecting both staff and the environment.

All workers using animals or primary cells are required to attend an in-house training prior to starting work and are then closely supervised by experienced research scientist until competent in all procedures. No workers will be precluded from the work proposed here and no vaccinations are required. We do not feel that specific health monitoring is required, although all workers will be made of the nature of the viruses that are being used.
Workers performing the surgical and behavioural test are required to follow agreed procedures for the handling of animals to reduce risk from allergens and bites... etc including the use of appropriate protective clothing and face masks, but there are no extra risks associated with the fact that the animal has undergone the procedures detailed. All in vivo work is carried out with the appropriate licences and permissions from UK regulatory authorities.

Summary
All work with viral stocks and primary cultures will be carried out in class 2 safety cabinets. The lentivirus is unable to propagate in the primary cells so RCL will not be generated. In addition all plastic ware and solutions that are used will be chemically inactivated with validated reagents before autoclaving prior to disposal for incineration. This will effectively prevent release of lentivirus to the environment and protect workers.

The maximum injection into an animal is 8µl. The Hamilton syringe is the only sharp tool in contact with the virus, it will be filled in a class 2 safety cabinet and it will be either a closed box inside a closed ice bucket or securely attached to a rig. Thus there is minimal risk of accidental puncturing of the skin with a contaminated syringe. Once injected the viral particles cannot re-infect any other tissues as: (i) the coat protein is lost following attachment and the DNA is incorporated into the host cell replication deficient; (ii) they are replication deficient. Hence, lentiviral vectors cannot re-infect once injected into the brain.

Biological material will be totally inactivated (by exposure of Virkon* and/or DISTEL and/or Trigene advance*) at the end of each experiment. It is therefore highly unlikely that the virus could survive any unintentional discharge into a sanitary sewage system. No foreseeable effects on human health and safety are expected.

Is an emergency plan required according to regulation 20?  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref    3043/13.3

Date Ackn'd 26/03/2013  CU2 Project Title Lentiviral vector mediated RNA interference (RNAi) in rodent brain (mouse or rat)  Class  Class 2  CultureVolClass2 < 1 Litre  ClassVolumeClass3-4

02/03/2022  Page 12631 of 15326
following stereotaxic injection to knockdown and study the role of D-amino-acid oxidase (DAAO)

Aim:
The flavo enzyme D-amino-acid oxidase (DAAO) metabolizes D-serine, which is the endogenous N-methyl D-aspartate receptor (NMDAR) co-agonist. As such, it has the potential to modulate the function of NMDAR and to contribute to the widely hypothesized involvement of NMDAR signalling in schizophrenia.

A lentivirus delivering a shRNA and mediating gene knockdown of the glial enzyme DAAO in specific brain structures would be a very useful tool to confirm the role of DAAO in D-serine metabolism. These issues are important given the current interest in DAAO in the pathophysiology and therapy of neuropsychiatric disorders.

Recipient or parental organism
Mouse/Rat

Host/vector system
Lentiviral Vector Expression System
Lentiviruses belong to the family Retroviridae, which forms a diverse and extensive family affecting both human and animal species. Retroviruses are characterised by a unique replication mechanism involving reverse transcription of the viral RNA genome, giving rise to a DNA provirus that contain two positive sense copies of the RNA genome, encased within a capsid that is surrounded by a host-cell derived envelope. Cellular entry involves interaction between the surface subunit of the virion envelope glycoprotein and cell-surface determinants. These interactions are generally specific and are believed to be the principal factor affecting cell type and species specificity. Receptor binding triggers membrane fusion mediated by the transmembrane subunit of the virion envelope glycoprotein, resulting in delivery of the virus capsid to the target cell.

The first risk to human health from the use of this expression system comes from the fact that it is based on HIV-1. However retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and thus lentiviral vectors are not infectious via the airborne route. The main risk of infection is consequently the risk of injection but it is highly unlikely to happen as all measure to avoid have been considered (see part 8 and 11 for detailed measures).

Origin & function
Summary
The transgene has a very low risk because it would be highly unlikely that expression in a few cells would be sufficient to lead to a disease state. The inability of the lentiviral
vector to propagate in mammalian cells also reduces the risk. Proteins that are commonly used as reporter genes and genes used for the identification and selection of transfected cells (EGFP) will be used in this system and are not known to be toxic or oncogenic. Genes that will be excluded from this expression system are genes encoding a) proteins of retroviral origin b) toxins c) oncogenes

**Evaluation of foreseeable effects**

**HUMAN HEALTH HAZARD**
The lentiviral vector expression system used as well as the transgene used have a very low risk for human health and safety as explained in the GMO characteristics description above.

Oral ingestion will be prevented by standard laboratory working practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory workers' skin or mucosa' cells with modified viruses. However, these modified virus particles carry an extremely low risk for humans. Firstly, they are replication-incompetent and self inactivating (see part 7 - Host/Vector system). Secondly, these particles carry an extremely low infection risk, and transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus will be via inadvertent cutaneous inoculation however the control of sharps to minimise inadvertent exposure decrease considerable the chances of such accident (see 11 for procedure and control measure with sharp). Moreover use of personal protective equipment designed to prevent a mucosal exposure/spit to the face and exposure of hands (especially in persons with broken skin or open cuts). Infection of the community at large with lentivirus particles is more than unlikely for the above described reasons of replication incompetence.

**HAZARDS TO THE ENVIRONMENT**
As the viral systems used are self-inactivating and non-replicative, it is extremely unlikely that accidental release into the environment will cause any environmental damage or risk to others. The potential risks for lentiviral expression are release into a human population already infected with HIV, allowing for potential recombination events that would incorporate the inserted sequence into a viable RCL. This scenario is extremely unlikely, and would in any case be unlikely to cause any additional problem.

This lentiviral vector is designed for gene expression in mammalian cells and transduces a wide range of host human and non-human cell lines which can be dividing or non-dividing. Indeed the vector is pseudotyped with VSV-G envelope and therefore has a greater host range however it cannot survive even after infection (which is highly unlikely). Although VSVG-pseudotyped virus particles can transduce in theory all mammalian cells, transduction is also highly dependent on the concentration of the virus particles in a given medium. Once produced they are aliquoted and stored in small amounts (each tube contains at maximum 10µl) at -80C, considering infectivity of the vector drops markedly at room temperature it is hard to envisage an accidental mechanism for release that would allow survival.

Moreover lentiviral vectors are enveloped RNA viruses that are relatively unstable and highly susceptible to dehydration. Lentivirus particles have thus a short half-life and are unstable at room temperature. However, they can survive for long periods in high protein media. Retroviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. Furthermore, many retroviruses require high titre inoculations to establish an infection. Therefore, the survivability of retroviruses is not thought to pose a risk to the environment.

Rodents such as wild-type mice and rats, cannot support replication of infectious HIV-1 (Morrow 1987, Lewis 1995, Bieniasz 2000, Goffnet 2007). As a result, the potential for shedding of replication-competent lentiviruses (RCL) from such animals is very low (even if RCL were present in the original vector innoculum).

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/ survive outside the laboratory and are mostly designed to be protective. EGFP will be primarily expressed in the CNS (as it used neuron specific promoters). Similarly, shRNA and miRNA sequences will not survive as they are highly unstable and thus would disappear very quickly if accidentally spilled into the environment. Sequences within the viruses cannot be transferred to other micro-organisms without a rare recombination event as described above. Thus we see no conceivable hazard associated with transfer of the expressed sequences.

Summary
Environmental risk is very low because accidental release is very unlikely to result in production of RCL. Although the virus is capable of transducing into mammalian cells it
lacks genes for replication and coat proteins. The viral materials have been classified as CL2 to minimise the risk of release to the environment from the laboratory. However, if accidental release into the environment does occur the vectors/plasmids could not survive and the risk is minimal. For further information, see also:
- The safety guidance "Biosafety Considerations for Research with Lentiviral Vectors" published by the US NIH.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All surgical procedures are carried out in a procedural room attached to the animal facility that was built within the last five years to a high standard. The surgery room is mechanically ventilated and an inward airflow is maintained extracting room air to atmosphere. The workplace is maintained at air pressure negative to atmosphere. In put air and extract air to the workplace is filtered using high efficiency particulate absorption (HEPA). However since these agents are not airborne pathogens, the use of a safety cabinet, negative air pressure and HEPA filtration of exhaust air would not be mandatory. Moreover the access to the laboratory is restricted to authorised persons (by using badge)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The work outlined above has been assessed as a Class 2 activity even though the risk of pathogenicity to the end worker is very low as the virus is severely replication defective and encodes no toxic proteins. Biosafety 2 conditions should be followed as prescribed by the HSE for handling and usage. However, it is permissible to reduce the containment level at some point following vector delivery into animal brain as there is no expectation of infection, the site of inoculation has been thoroughly cleansed, and the bedding changed. Indeed for experimental animals that have been inoculated with non-replicating virus, the risk of operator exposure from the infected animals is minimal as is the potential for virus shedding. It is therefore appropriate to house these animals in a containment level 1 facility.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Solid waste will be neutralised by soaking into Virkon* prior to being bagged up in double autoclave bags, then autoclaved prior to incineration offsite.
- Needles should be used with extreme care, only used when absolutely necessary and should never be resheathed, but disposed of directly into a suitable waste container (sharp bin). Sharps will be discarded in a yellow glass container (sharp bin). Sharps will be discarded in a yellow glass container to be stored in a safe manner prior to incineration.
- Liquid volumes will be disposed of into sealed biohazard containers filled with Virkon* for incineration. It is routine that all liquid waste material is neutralised before disposal to allow transportation in leak-proof containers to prevent spillage.
- Animal waste is considered non-infectious and disposed of along with the waste from non-infected animals so animal carcasses will be incinerated offsite. It is worth highlighting that there is no aqueous disposal to sewers on site and that all waste is sent off site for incineration. If necessary any material can get autoclaved prior to being taken off site for incineration.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
The GMO committee has deemed that the handling of the virus stock supplied by the Uney laboratory in Bristol University is a Class 2 activity. This decision has been reached that, although the virus will not be generated at Takeda Cambridge Ltd, and that the lentivirus particles have been generated using a third generation 4 plasmid expression system, the possibility of a recombination event leading to replication competent virus can never be discounted. It is also noted that the Woodchuck Post-transcriptional Regulatory Element (WPRE) has been associated with potential oncogenic effects with some viral gene delivery systems. The EGFP and the shRNA encoded by the lentivirus are not known to possess any inherent toxic or pathogenic hazard to either individuals or to the environment. The committee is satisfied that the necessary procedures have been put in place to allow the safe injection of virus into animals within the animal facility and that there is no risk to the environment due to the inherent instability of the lentiviral particles, non-replicative nature and self inactivation of the virus after insertion into the host genome.

### Project Containment

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**Animal Units**

- L2
- L3
- L4

**Large Scale Activities**

- L2
- L3
- L4

**Human Clinical Applications**

- L2
- L3
- L4

### Project Ref 3043/13.4

**Date Ackn’d**

- 06/11/2013

**CU2 Project Title**

- Rodent (rat/mouse) lentiviral vector mediated RNA interference (RNAi) knockdown studies to investigate the role of several proteins of interest in the central nervous system (CNS)

**Class**

- Class 2

**Culture Vol Class 2**

- < 1 Litre

**Culture Volume Class 3-4**

- Consent Granted

**Non-GMM**

- Yes

**Date Project Ceased**

- 02/12/2016

**Withdrawn**

- No

**Tick if notifying a connected programme of work**

- No

**Historical Significant Changes**

- Withdrawn

**Historical Date of Additional Info**

- 02/03/2022

**Significant Change ID**

- 02/03/2022

**Date of Significant Change**

- 02/03/2022
Purposes of the contained use

Aim
The objective of the proposed studies is to investigate the role of a number of proteins of interest in the CNS, using lentiviral vectors expressing small hairpin RNAs (shRNAs), which act to downregulate the translation of specific proteins by binding and promoting the breakdown of their coding mRNA transcripts. The studies will focus on discovering the role of the proteins in both physiological (cognition and neurotransmission for instance) and pathological (in CNS disease states) processes that occur in the brain, with the overarching aim of identifying potential new drug targets for CNS diseases. The genes/proteins of interest that will be targeted by the shRNAs do not include any growth factors, confirmed oncogenes, cytotoxins, or immunomodulators, and none of them are of retroviral origin.

Methods
Target material will be transduced with replication incompetent lentiviral vectors expressing shRNA transcripts either targeting specific mRNAs/proteins of interest, or with vectors expressing scrambled shRNAs, in the case of the negative control treatment groups. The lentiviral vectors will also express enhanced green fluorescent protein (EGFP) to aid in the identification, visualisation and isolation of transduced cells. The extent of mRNA and protein knockdown that is achieved will then be assessed at different time points (e.g. 24h-48h-72h), post-transduction, using suitable in vitro assays, including, RT-PCR, Western Blotting, and immunocytochemistry. Once the conditions that lead to the optimal knockdown of the mRNAs/proteins have been determined, what effects the knockdown of the proteins have on various physiological parameters will be evaluated using standard experimetal techniques.

Description of the lentiviral vectors used for the experiment
The lentiviral vectors will be produced and supplied by James Uney’s Lab, which is based at the University of Bristol. Each aliquot of lentiviral reagent comprises of a small volume (5 to 10 μl) that contains no more than 1x10e08 viral particles which decreases the risk to the operator. The lentiviral vectors are generated with the following safety features to minimise the chance of a random recombination event occurring that leads to the generation of replication competent lentivirus (RCL).

Self inactivating (SIN) non replication-competent HIV based lentiviral vectors
The virus integrates into the target cell's genome via long terminal repeats (LTRs). These have had the U3 region of the 3' LTR deleted such that when they integrate in the genome of the target cell the same deletion is transferred to the 5' LTR and is sufficient to prevent transcription of full length vector RNA.

Segregation of the viral genes required for production of lentiviral particles
The lentiviral vectors are generated using four standard plasmid vectors that must be co-transfected into a packaging cell line for the production of lentivirus particles in trans. Three plasmids carry one of the essential genes required for viral capsid production whilst the fourth carries the transgene genetic material to be packaged into the resultant viral vector (target specific shRNA or scrambled shRNA and EGFP). By segregating each of the essential viral genes required for viral generation the chance of a multiple recombination event that generates a viral genome capable of generating RCL is minimised. Together with the SIN this makes the likelihood of generating RCL very small.

Recipient or parental organism
Mammalian Cells

Host/vector system
Lentiviruses belong to the Retroviridae virus family, a diverse and extensive family of viruses, which are capable of infecting both human and animal species. Retroviruses are characterised by a unique replication mechanism involving reverse transcription of the viral RNA genome, which gives rise to a DNA provirus that contains two positive sense copies of the viral RNA genome; and in a functional assembled virus, the viral genome is encased within a capsid that is surrounded by a host-cell derived envelope. Cellular entry of the virus involves interactions between glycoproteins contained in the virion’s envelope and extracellular plasma membrane proteins on target cells. These interactions are generally specific and are believed to be the principal factor determining which species and type of cells a specific virus is capable of infecting. The binding of a virus’s envelope glycoproteins to an appropriate receptor site on a target cell triggers the fusion of the virion’s envelope membrane with the plasma membrane of the target cell, which results in the delivery of the virus capsid to the intracellular space of the target cell.

In this proposal cells targeted for transduction by lentiviral vectors are biosafety 2 or lower and are not infected with wildtype lentivirus. The lentiviral vector system proposed for use in these studies is based on HIV-1 and therefore a theoretical risk to human health exists. Retroviruses are, however, generally transmitted via exposure to contaminated body fluids or percutaneous inoculation, and they are generally not transmitted via the airborne route. Accidental
piercing of the skin or other surface tissues with virus containing objects, therefore, represents the main potential route by which accidental infection could occur. The consequence of accidental infection can be minimised by using lentiviral vectors that are unable to replicate and hence unable to transduce further cells and to contain genetic material that is highly unlikely to have a detrimental effect on human health or the environment. It is also possible that the random integration of the viral genome into cells can lead to the trans-activation of genes downstream of the integration site.

Production of these proposed lentiviral vectors will take place in the lab of James Uney, at the University of Bristol. The lentiviral vector stock will be received from James Uney’s lab in aliquots of 5 to 10 µl containing between 1x10^8 to 1x10^10 transducing units (TU)/µl of vector in TSSM buffer (20mM Tromethamine, 100mM NaCl, 10mg/mL sucrose, 10mg/mL mannitol). The material should be essentially free of the HEK-293T helper cell line used to produce the viral titre. Each aliquot of lentiviral vector solution should contain not more than 1x10^8 viral particles.

The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to, inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats. The material is normally shipped frozen within manufacturer designed racks and will be stored in a designated -80°C freezer.

The Lentiviral Expression System that will be used include the following key safety features:

Self Inactivation (SIN)

All of the transgene/EGFP containing plasmid vectors that will be used contain a deletion in the 3' LTR (ΔU3) that does not affect generation and packaging of the viral genome in the HEK-293T producer cell line. After transduction of target cells the process of integration into the genome of the target cell copies the deletion to the 5' LTR preventing transcription of the integrated lentiviral genome and subsequently is no longer capable of producing packagable viral genomic material. This reduces the risk of mobilisation of the genetic material even following a superinfection with wild-type HIV. Such activity has not been observed even under permissive in vitro conditions (Bukovsky et al 1999). Moreover, deletion of enhancer and promoter elements from the 3' U3 region in the vector construct will result in a provirus that is entirely devoid of the U3 enhancer sequences, which will result in a reduction in the potential for transactivation of cellular genes due to an insertion event. Insertion can also cause loss of gene expression with harmful effects and such as event can not be ruled out with any system that integrates into the genome of a cell.

Segregation of genes required for generation of lentiviral particles

The retroviral genes required to generate viral particles has been reduced to the essential gag, pol, rev and env genes. Each gene is delivered to the packaging cell line on a separate plasmid vector. The genetic material to be packaged into the viral particle is supplied on an additional fourth bacterial plasmid. The four plasmids do not contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a RCL.

The HIV-1 Env gene has been replaced by the VSV-G gene from Vesicular Stomatitis Virus in the pMD2-VSVG.env plasmid. Pseudotyping HIV-1 vectors obviates safety concerns associated with the use of HIV-1 gp120, which has known pathogenic consequences. However, pseudotyping has a big impact on the biodistribution and tropism of viral vectors. In particular, HIV-1 gp120 restricts transduction of HIV-1 vectors to CD4+ cells, which limits its use to CD4+ cells, like T cells and macrophages. In contrast, heterologous envelopes, like VSV-G, typically broaden the tropism and allow gene transfer into a broad variety of cells.

The transgene/EGFP containing plasmid vectors (pRRL plasmid vectors) that will be used, only contain essential viral cis-acting sequences, including the LTRs and will be the only plasmids to include the packaging signal Ψ. Although the packaging plasmids used in these systems allows for the expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK-293T producer cell lines, none of them contain LTRs or the Ψ packaging sequence. The lentiviral accessory genes (vif, vpr, vpu and nef) that are dispensable for lentiviral vector production/transduction have been deleted from the packaging construct as they are not required for in vitro replication but the products they encode have cytotoxic activities.

Expression of the gag and pol genes from pMDLgpl-RRE has been rendered Rev-dependent by virtue of the presence of the HIV-1 Rev Responsive Element (RRE) in the gag/pol mRNA transcript. All the transgene/EGFP containing plasmid/vectors that will be used also include RRE. Addition of the RRE in these plasmids prevents gag and pol expression in the absence of Rev, which is supplied by the pRSV-REV plasmid. The viral rev protein is provided in trans to ensure efficient nuclear export of the full-length viral RNA genome through binding to the RRE by enhancing and stabilising the export of viral mRNAs from the nucleus. The HIV-1 Rev protein mediates nuclear export of both unspliced full-length and partially spliced viral RNAs by bridging an interaction between the Rev response element (RRE) in the genomic DNA and the host cell CRM1 nuclear export pathway. The Rev/RRE system is highly conserved among lentiviruses, and removal of the RRE sequence and associated splice donor/acceptor
sequences results in a loss of transduction efficiency.

The LTR used to drive expression from the pRRL plasmids has been modified so as to increase lentiviral vector production but to also allow lentiviral vector production to be independent of tat expression, which has been removed. A constitutive promoter (RSV promoter) has been placed upstream of the 5' LTR in the pRRL plasmids to offset the requirement for Tat. Tat is essential for the replication of wild-type HIV-1, as it is required for the efficient production of viral RNA. It is known that Tat-deleted mutants of wild-type HIV are not replication competent (Vendel and Lumb 2003; O'Brien et al. 1990). The deletion of Tat further decreases the risk of generating a putative RCL.

Lentiviral vectors have a very low potential to cause immunogenicity.

The pRRL vector includes the Woodchuck Post-transcriptional Regulatory Element (WPRE) to promote mRNA export and stabilise transgene mRNA levels, which should give rise to increased transgene expression. There have been concerns surrounding the potential oncogenic activity of the native WPRE, because of the expression of an oncogenic WHV-X protein from an open reading frame found within the element. The WHV-X ORF translation start site has been mutated in the WPRE used in these lentiviruses to prevent expression of WHV-X protein removing the oncogenic risk (Zanta-Boussif et al. 2009).

Transcription of the transgene/EGFP will either be under the control of the U6 and CMV promoters respectively or the Polymerase II human synapsin I (SynI) promoter. It is noted that although the LTRs have been mutated to remove U3 enhancer sequences this does not prevent trans-activation by readthrough from strong promoters such as CMV. The U6 promoter is well suited to drive the expression of shRNAs due to it possessing the following favourable characteristics: (i) it initiates from position +1 of the transcripts, and (ii) it yields transcripts that do not terminate with a poly-A tail but with a series of four to five thymidine residues, which results in a series of 3' U residues leading to a fully functional shRNA sequence. Indeed, the structure of the transcribed product closely resembles synthetic double-stranded siRNAs, except for the fact that the two strands are linked by a spacer sequence. This system has been used to successfully inhibit gene expression in mammalian cells lines, with efficiencies comparable with that of synthetic siRNA.

The hSynI promoter directs high expression specifically in neuronal cell types which negates the chance of trans-activation of genes in non-neuronal cells.

It is not considered that the use of these lentiviral vectors will result in a significantly increased risk of oncogenic activation compared to the risk possessed by any other viral delivery system. Moreover, the risk of transduction leading to harmful consequences following exposure is related in part to the titre of the viral vectors; and exposure of workers to quantities of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of lentiviral vectors.

REFERENCES

All the references below have been cited in the review attached to the present document: Boro Dropulic (2011). Lentiviral vectors: their molecular design, safety, and use in laboratory and preclinical research. Hum. Gene Ther. 22:649-657.

For further information, see also the safety guidance “Biosafety Considerations for Research with Lentiviral Vectors” published by the US NIH.


Origin & function

Transgene: proteins to be expressed:

EGFP

EGFP is a modified form of green fluorescent protein (GFP) that has enhanced spectral properties making it a superior tool as a reporter of gene expression in biological research. GFP is a 238 amino acid residue (26.9kDa) protein that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range and was first isolated from jellyfish Aequorea victoria. EGFP is not known to have any disease association and is regarded as non-toxic to living cells. The expression of EGFP will be used to identify cells that have been transduced with lentiviral vectors.

shRNAs targeting a number of different mRNAs

As shRNA tends to lead to a loss of activity it is inherently regarded as being safer than gain of function experiments provided processes that are known to be involved in

02/03/2022
cell cycle regulation, tumour suppressors and are growth factors are not affected. None of the targets proposed in this study are known to be cytotoxic, tumour suppressors, growth factors or to be involved in the control of the cell cycle. None of the sequences are of retroviral origin.

RNA interference (RNAi) is an antisense technology that exploits a normal cellular antiviral response that acts to inhibit viral protein synthesis through the production of short hairpin RNAs that bind and target viral double-stranded RNA (dsRNA) molecules for degradation. The shRNA molecules are processed by cellular enzymatic pathways to produce small inhibitory RNA (siRNA) species, and it is the latter RNA molecules that bind and target complementary RNA sequences for degradation by the RNA-induced silencing complex (RISC). With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be ‘knocked-down’, in order to study their functions.

The effects of the siRNA species that are generated in these experimental systems may have broader effects on the cell than just modulating the expression of a particular gene/protein. There may be sequences within a specific shRNA molecule that are homologous to other coding sequences within the mammalian genome that are not necessarily linked or closely related to the intended target. It is, therefore, theoretically possible that a specific siRNA may knock down the expression of genes other than the intended target one, and for this reason, the RNAi systems have been designed carefully to minimise the likelihood that there will be unwanted or potentially adverse effects arising from a non-target gene being inadvertently targeted. This is done by screening designed shRNA sequences against databases of known mammalian gene/mRNA sequences. Additionally, with the use of this approach, the shRNAs are also designed so as to bind complementary sequences that are present in rodents, but not in humans, which reduces potential risks to humans. The likelihood that a specific shRNA will bind to a human form of the target mRNA is extremely low, but it cannot be completely excluded.

Morever, RNAi interference may, additionally, also have deleterious effects upon cellular metabolism due to the triggering of antiviral responses. It has been shown that siRNA molecules (even if less than 30nt in length) can trigger dsRNA antiviral responses. Such responses not only lead to the degradation of dsRNA molecules, but also results in interferon production, which, in turn, leads to inflammation and the non-specific inhibition of protein synthesis.

MicroRNAs (miRNAs) are endogenously encoded 22-nt-long RNAs that are generally expressed in a highly tissue and/or developmental-stage specific fashion, and they function to post-transcriptionally regulate the expression of target genes. In certain experimental RNA interference approaches, this miRNA system can be harnessed/manipulated to study the functions of specific genes/proteins. For example, regulatable RNA polymerase II promoters can be experimentally activated so as to over-express endogenous microRNAs in cell culture systems. Alternatively, artificial microRNAs can also be engineered to match the features of existing microRNA genes, such as the gene encoding the human miR-30 microRNA. Some of the vectors that will be used in the proposed studies, contain a miR-30 cassette (see section 6). In these vectors, the expression of shRNA will be under the control of the PolymeraseII SynI promoter, and these vectors will yield single miR-30-EGFP transcripts, from which the miRNA will be excised by endogenous pathways before translation of EGFP.

Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. These vectors will be employed as negative controls to demonstrate that any observed effects are due to the knockdown of a specific mRNA/protein of interest, rather than being due to any non-specific effects that the delivery of the viral vectors might cause. This sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of mouse, rat or human origin. It will only serve as the negative control for the vector producing shRNA. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health.

Evaluation of foreseeable effects

Human health hazards

There is a very low risk of human infection due to the small quantities of virus to be handled. The method by which the lentivirus vector is produced means that it is highly unlikely that RCL can be generated even if an infection occurs in a person who is HIV-1 positive. Infection can lead to a permanent transduction of cells and the possibility of a harmful event either by transactivation or disruption of gene expression can not be ruled out. For this reason the work with stock viral aliquots will be conducted as a class II activity. Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker’s skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the genes/proteins of interest that will be targeted by shRNAs do not include any growth factors, confirmed oncogenes, cytotoxins, or immunomodulators, and none of them are of retroviral origin. In the unlikely event of accidental transduction of a laboratory worker’s skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being
replication incompetent and being extremely sensitive to desiccation.

B) HAZARDS TO THE ENVIRONMENT

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable RCL. This scenario is, however, extremely unlikely, and even if it was to occur, it is unlikely that it would lead to any untoward effects.

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and they are, in theory, capable of transducing all dividing and non-dividing mammalian cell types. As emphasised before, the vectors, however, cannot self-propagate after infection, and successful transduction is also critically dependent on the presence of high enough concentrations of virus particles; and viral vector stock solutions are aliquoted and stored in small volumes (each tube contains at maximum 10μl) at -80°C so the potential for a high titre dose is reduced. The infectivity of the vectors rapidly decreases at room temperature so it is considered highly unlikely that the vectors could survive long enough in the environment to pose a risk if accidentally released. Furthermore, lentiviral vectors have a short half-life at room temperature due to their structural characteristics making them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, and this is illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

Rodents, such as wild-type mice and rats, cannot support replication of infectious HIV-1 (Morrow 1987, Lewis 1995, Bieniasz 2000, Goffinet 2007). As a result, the potential for shedding of replication-competent lentiviruses (RCL) from such animals is very low (even if RCL were present in the original vector inoculum).

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA and miRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

Summary

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will undertaken have been classified as CL2, and effective containment procedures will be adhered to. However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems (see above discussions).

References

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General waste handling procedures
Solid waste will be neutralised by soaking it in a validated disinfectant solution (Virkon or Distel), after which the material will be ‘double bagged’, autoclaved and, ultimately, incinerated offsite by a licensed contractor. Liquid waste will be disposed of into sealed biohazard containers, neutralised with Virkon* and then sent for incineration by a licensed contractor. It will be routine practice that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages.

* Virkon
Rely+On™ Virkon® is a multi-purpose disinfectant. It contains oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds, such as proteins, leading to widespread, irreversible damage and subsequent deactivation/destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance against over 500 strains of viruses, bacteria and fungi. Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control. Importantly, Virkon has specifically been validated for lentiviral inactivation (Antec-Biosentry). Virkon is sold as a powder which dissolves readily in water. It is intended to be mixed with water to form a 1% solution (i.e. 10g per litre) for hard surface and equipment disinfection. 1:100 is also the dilution rate advised for virucidal efficacy against HIV-1. The product has a pink colour, which is useful in that it helps to gauge the concentration of a prepared solution, and importantly, as Virkon ages, it discolours, making it obvious when it needs to be replaced. The solution is generally stable for five to seven days. Moreover, there is no evidence to suggest that bacterial disease-causing organisms develop resistance towards Virkon, as opposed to some other disinfectant products. Moreover, Virkon is not classified as R53, it is classified as readily biodegradable, and it does not persist in the environment.

* Distel (formally Trigene advance)
Distel is formulated as a nanoemulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms, ensuring the rapid induction of cell death in treated micro-organisms. Distel works quicker than conventional high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products. Distel has been reported to be bactericidal, fungicidal as well as virucidal and sporicidal. It is recommended to be used at a 1:200 dilution for general purposes, 1:100d for high risk areas, and 1:50 for disinfection of blood and bio-hazard spillages.

Summary
As any active viral particles on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.
The GMSC has deemed that the handling of the virus stock supplied by the Uney laboratory in Bristol University is a Class 2 activity. This decision has been reached that, although the virus will not be generated at Takeda Cambridge Ltd, and that the lentivirus particles have been generated using a third generation 4 plasmid expression system, the possibility of a recombination event leading to replication competent virus can never be discounted. It is also noted that the Woodchuck Post-transcriptional Regulatory Element (WPRE) has been associated with potential oncogenic effects with some viral gene delivery systems. The EGFP and shRNA encoded by the lentivirus are not known to posses any inherent toxic or pathogenic hazard to either individuals or to the environment. The GMSC is also satisfied that the knock down in expression of the target genes in this study do not pose any known hazard. The necessary procedures have been put in place to allow the safe use of the virus on primary neuronal cells and for the injection of virus into animals within the animal facility and that there is no risk to the environment due to the inherent instability of the lentiviral particles, non-replicative nature and self inactivation of the virus after insertion into the host genome.

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**Project Ref** 3043/14.1

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Tick if notifying a connected programme of work N

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

**Aim**
The objective of the proposed studies is to investigate the role of a protein of interest in the CNS, using adenoviral vectors expressing small hairpin RNAs (shRNAs), which act to downregulate the translation of the protein of interest by binding and promoting the breakdown of its mRNA transcripts. The studies will be focused on discovering the role of the protein of interest in both physiological and pathological processes that occur in the brain, with the overarching aim of identifying potential new drug targets for CNS diseases.

The gene/protein of interest that will be targeted by the shRNA is not of adenoviral origin, and it has not been reported to have any oncogenic, tumour suppressor, immunomodulatory, or cytotoxic activities.

**Methods**
Target material will be transduced with replication incompetent adenoviral vectors expressing shRNA transcripts either targeting the mRNA/protein of interest. Or with vectors expressing scrambled shRNAs. In the case of the negative control treatment groups. The adenoviral vectors will also express enhanced green fluorescent protein (EGFP) to aid in the identificiation, visualisation and isolation of transduced cells. The extent of mRNA and protein knockdown that is achieved will then be assessed at different time points (e.g. 24h-48h-72h), post-transduction, using suitable in vitro assays, including, RT-PCR, Western Blotting, and immunocytochemistry. Once the conditions that lead to the optimal knockdown of the mRNAd/proteins have been determined, what effects the knockdown of the proteins have on various physiological parameters will be evaluated using standard experimented techniques.

**Recipient or parental organism**

Mouse/Rat

**Host/vector system**

Wild Type Adenoviruses
Adenoviruses are ubiquitous pathogens of both mammals and birds. Over 100 serotypes are known, 51 of which infect humans. The severity of adenoviral infections varies from acute respiratory diseases (ARD) in adults (Ad2, Ad7) to mild respiratory symptoms in children (Ad2; Ad5), gastroenteritis (Ad40; Ad41), conjunctivitis (Ad8; Ad19; Ad37), cystitis or subclinical infection (Ad12). Certain serotypes have also been shown to be tumorigenic in neonatal rats (Ad12; Ad7), although this has never been observed in humans.

Primary infection generally occurs in childhood via the airborne or faecal-oral routes and can be persistent with viral shedding continuing for month. Latent infection of
lymphoid tissue can also occur and reactivation in the immunocompromised can lead to serious complications. However, the precise mechanism of latency remains unknown. Immunity is thought to be lifelong and over 90% of individuals are seropositive for Ad2 and Ad5.

The adenovirus virion comprises a non-enveloped icosahedral capsid containing a 36 kb double stranded DNA genome (the general structure of adenoviruses is illustrated in figure 1 below). Adenoviruses can infect a broad variety of cell types including non-dividing cells) via interaction between the viral fibre protein and the cellular Coxsackie B Adenovirus Receptor (CAR) - a widely expressed, 48 kDa member of the immunoglobulin superfamily. Following virus absorption, RGD motifs on the penton base interact with cell surface αv integrins, stimulating an intracellular signalling cascade and clathrin-mediated endocytosis. Not all serotypes share the same affinity for CAR and some utilise alternate receptors and cell-surface integrins.

Adenoviruses replicate in the nucleus, where the viral genome is maintained epichromosomally, and viral gene expression is divided into two distinct phases - Early and Late transcription. Early transcription occurs 6 to 8 hours after infection, generating early proteins from four major regions, E1, E2, E3 and E4. The E1 promoter directs expression of the E1 proteins, E1A and E1B that subvert the cellular environment and control transcription of the other early genes. E1A disrupts cell-cycle regulation by binding to key regulators of transcription and mitosis. This results in the expression of the pro-apoptotic factors, including p53, which is bound and inactivated by an E1B protein. E2 proteins are required for genome replication and packaging. E3 proteins aid the evasion of the immune system by disrupting the processing of class 1 Major Histocompatibility Complexes and inhibition of Fas- and TNF-mediated apoptosis. One E3 protein, the so-called Adenovirus Death Protein (ADP), promotes cytolysis and 79 release of progeny virions. E4 proteins further subvert the cellular environment and modulate the activities of E1 proteins. Late transcription, directed by the Major late Promoter, occurs 4 to 6 hours after the onset of Early transcription and results in the expression of the structural proteins L1, L2, L3, L4 and L5. The lytic cycle lasts for 24-48 hours (depending on subtype and target cell) generating up to 1x105 viral particles per infected cell.

Disabled Recombinant Adenovirus Vector Systems.

To date, most genetic modification work involving adenoviruses has involved the development of transduction vectors derived from human Ad2, Ad5 and Ad12. `First Generation' vectors comprise the majority of Adenovirus vectors used to date and harbour a genomic deletion that removes the E1 expression cassette. E1A and E1B are usually supplied in trans using a complementing cell line that contains the E1 expression cassette (such as HEK293 or PerC6). Packaging sequences are retained in order to generate viable progeny. Since adenoviruses have a strict packaging limit (105% of the wild-type genome size), the E3 cassette is also commonly deleted since it is dispensable for growth in vitro. `Second Generation' vectors also have much of the E2 cassette deleted, increasing its packaging capacity and further disabling the virus by removing its capability to replicate and process viral DNA. This deletion also virtually eliminates the possibility of a recombination event that might result in Replication Competent Viruses (RCV). `Third Generation', or 'Gutless' vectors generally retain only packaging sequences and therefore have the largest capacity for inserted genetic material. The latter vectors, however, require extensive complementation in trans from a helper virus.

Adenoviruses can infect a wide variety of cell types, although individual serotypes have more restricted tropisms, and in most transduced tissues, expression form Ad vectors is transient due to clearance of the virus by the immune system, and expression lasts only one to two weeks. In some 'immune privileged' tissue expression may be longer persisting for a year or more. Integration into the host genome represents the only significant mechanism by which long-term expression can be maintained by disabled Ad vectors. This is relatively rare, occurring at a frequency of approximately 1 in 105 pfu in human primary cell cultures. Deletions in the viral vector or the genetic insert may alter the immunogenic or pathogenic nature of the virus. For example, proteins derived from the E3 cassette (which is often deleted in adenoviral vectors) are involved in immune evasion strategies in vivo. Their deletion, while facilitating the clearance of virus by the host immune system, might result in an increased inflammatory response and increased pathogenicity.

There is a possibility that recombination events could result in harmful sequences being transferred between related viruses. This could take place between a vector and a wild-type adenovirus or viral sequences present in a cell; for example. It has been shown that 20% of normal healthy adults have E1A sequences present in their respiratory epithelium. When engineering recombinant viruses, it is, therefore, common practice to locate an insert in place of the E1 cassette. This, any homologous recombination that restores E1 sequences to the vector will also delete the insert and vice-versa. Inserts cloned into other areas of the viral genome could be maintained in the event that E1 sequences are restored, resulting in a GM RCV. The probability of acquisition of sequences form a complementing cell line or helper virus can be minimised if there
are no overlapping sequences. For example, HEK293 cells carry cell line or helper virus can be minimised if there are no overlapping sequences. For example, HEK293 cells carry 11% of the adenovirus genome containing the E1 cassette; This includes at least 800 bp of sequence present within most E1-deleted adenovirus vectors, providing the potential for recombination that restores the E1 region in the virus.

In contrast, PerC6 and similar cell lines have been engineered to express the minimal E1A and E1B genes from heterologous promoters, and thus have no sequence overlap with most newer E1-deleted vectors, greatly reducing the frequency of generating replication-competent virus.

Most adenovirus vectors have been derived from human viruses, which are not thought to be able to replicate efficiently in animal cells. Therefore, it is unlikely that activities with these vectors will represent any significant risk to the environment. However, human Ad5 vectors have been shown to enter some animal cells and there may be environmental risk associated with inserted genetic material. Human Ad5 vectors have been shown to enter (but not replicate efficiently in) cells of mouse, rat and canine origin, which raises the question of whether or not recombination between human and animal adenoviruses might occur. There is, however, no evidence to suggest that this is possible.

Description of the recombinant pacAd5 adenoviral vector system to be used

pacAd5 Vectors produced using the RAPAd Production System

The adenoviral vectors that will be used are all recombinant disabled shRNA expressing pacAd5 vectors, which will be produced using the RAPAd4 Adenoviral Expression System (Anderson et al, 2000). In this system, replication-deficient adenoviral vectors are produced in HEK293 packaging cells using pacAd5 9.2-100 backbone and pacAd5 RSVK-NpA transgene-containing shuttle vectors, which undergo homologous recombination after co-transfection.

The recombinant pacAd5 vectors that are produced with the RAPAd system are rendered replication-deficient as a consequence of the pacAD5 9.2-100 backbone vector being devoid of the left-hand ITR, the packaging signal and E1 sequences (complete E1a and partial E1b deletions); and virus replication can, therefore, only take place in HEK293 packaging cells, in which the deleted E1 sequences that are essential for replication are provided in trans. The recombinant viruses do, however, still contain all of the other wild-type adenovirus sequences, including those that code for immune evasion proteins (E3), viral structural/assembly proteins (L1-L5), and proteins that are responsible for replication levels of E1a copy number by quantitative PCR.

Characteristics of the Recombinant Adenoviral Vecotr supplied by J U Lab.

The adenoviral vector will be produced and supplied by JU's Lab, which is based at the University of Bristol, and virus production will not take place at Takeda Cambridge's site. Each aliquot of adenoviral reagent will comprise of a small volume (5-20μl) containing up to ~8 x 10e12 viral particles/ml (2 x 10e10 IU/ml) dissolved in TSSM buffer (20mM Tromethamine, 100mM NaCl, 10mg/mL sucrose, 10mg/mL mannitol). The viral stock solution will consist of viral particles containing in the vector genome (full capsids) and also of a variable number of empty viral capsids, and it should be essentially free of the HEK293 helper cell line used to produce the vectors. Importantly, all virus preps are tested for replication competent adenovirus (RCA contamination by immune-staining procedures. Other trace components that might be present include, but are not limited to, inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids and fats. The material is normally shipped and stored frozen within manufacturer designed racks in a -80° freezer.

Post-transfection of target cells, the vectors will produce both a small hairpin RNA (shRNA) that targets the mRNA of the protein of interest (or a scrambled shRNA), and the enhanced green fluorescent protein (EGFP) reporter protein to facilitate the identification of transduced cells.

Transgenes of Interest Contained in the Vectors.

The transgene of interest that will be inserted into the vector will contain sequences that code for a shRNA molecule targeting the mRNA of the protein of interest (or a scrambled shRNA in the case of the negative control) and/or the transduction marker protein, EGFP. Control viral vectors that code for and express only EGFP and no shRNA will also be used in some experiments. The shRNA (or scrambled shRNA) sequences will be under the control of the U6 promoter, while the EGFP gene will be under the control of the Cytomegalovirus (CMV) promoter.
**U6 shRNA Promoter**

The U6 promoter used in the U6.shRNA-CMV.GFP constructs is well suited to drive the expression of shRNAs due to it possessing the following favourable characteristics:

(i) it initiates from position +1 of the transcripts, and (ii) it yields transcripts that do not terminate with a poly-A tail but with a series of four to five thymidine residues that results in a series of 3’ U residues which gives rise to a fully functional shRNA sequence. Indeed, the structure of the transcribed product closely resembles synthetic double-stranded siRNAs, except for the fact that the two strands are linked by a spacer sequence. This system has been used to successfully inhibit gene expression in mammalian cell lines, with efficiencies comparable with that of synthetic siRNA.

**EGFP CMV Promoter**

EGFP will be produced by all of the vectors, and it will be controlled by the CMV immediate-early enhancer, which directs high-level expression in most cell types, although expression levels vary between cell and tissue types.

**Origin & function**

**Transgene: proteins to be expressed:**

**EGFP**

EGFP is a modified form of green fluorescent protein (GFP) that has enhanced spectral properties making it a superior tool as a reporter of gene expression in biological research. GFP is a s38 amino acid residue (26.9kDa) protein that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range and was first isolated from jellyfish Aequorea victoris. EGFP is not known to have any disease association and is regarded as non-toxic to living cells. The expression of EGFP will be used to identify cells that have been transduced with adenoviral vectors.

**shRNAs targeting the protein of interest**

As shRNA tends to lead to a loss of activity it is inherently regarded as being safer than gain of function experiments provided that tumour suppressors, growth factors, and processes that are known to be involved in cell cycle regulation are not affected. The gene/protein of interest that will be targeted by the shRNA is not of adenoviral origin, and it has not been reported to have any cytotoxic tumour suppressor, growth stimulating, or cell cycle disrupting activities.

**RNA interference (RNAi)** is an antisense technology that exploits a normal cellular antiviral response that acts to inhibit viral protein synthesis through the production of short hairpin RNAs that bind and target viral double-stranded RNA (dsRNA) molecules for degradation. The shRNA molecules are processed by cellular enzymatic pathways to produce small inhibitory RNA (siRNA) species, and it is the latter RNA molecules that bind and target complementary RNA sequences for degradation by the RNA-induced silencing complex (RISC). With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be ‘knocked-down’, in order to study their functions.

The effects of the siRNA species that are generated in these experimental systems may have broader effects on the cell than just modulating the expression of a particular gene/protein. There may be sequences within a specific shRNA molecule that are homologous to other coding sequences within the mammalian genome that are not necessarily linked or closely related to the intended target. It is, therefore, theoretically possible that a specific siRNA may knock down the expression of genes other than the intended target, and for this reason, the RNAi systems have been designed carefully to minimise the likelihood that there will be unwanted or potentially adverse effects arising from a non-target gene being inadvertently targeted. This is done by screening designed shRNA sequences against databases of known mammalian gene/mRNA sequences. Additionally, with the use of this approach, the shRNAs are also designed so as to bind complementary sequences that are present in rodents, but not in humans, which reduces potential risks to humans. The likelihood that a specific shRNA will bind to a human form of the target mRNA is extremely low, but it cannot be completely excluded.

Moreover, RNAi interference may, additionally, also have deleterious effects upon cellular metabolism due to the triggering of antiviral responses. It has been shown that siRNA molecules (even if less than 30 nt in length) can trigger dsRNA antiviral responses. Such responses not only lead to the degradation of dsRNA molecules, but also results in interferon production, which, in turn, leads to the inflammation and the non-specific inhibition of protein synthesis.
Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complimentary to any known mammalian sequences. These vectors will be employed as negative controls to demonstrate that any observed effects are due to the knockdown of a specific mRNA/protein of interest, rather than being due to any non-specific effects that the delivery of the viral vectors might cause. This sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of mouse, rat or human origin. It will only serve as the negative control for the vector producing shRNA., Consequently, these sequences are unlikely to pose any safety risks for the environment or human health.

**Evaluation of foreseeable effects**

As the adenovirus vector system that will be used is based on wild-type adenovirus serotype that is capable of infecting humans (Ad5), a theoretical risk to human health cannot be excluded even though recombinant replication-deficient viral preparations will be used, which have been validated to be virtually free of any wild-type virus. Moreover, the recombinant viruses do still contain the functional wild-type virus sequences that code for viral DNA replication proteins (E2 Polymerase/DNA binding proteins), which means that copies of the viral genome are likely to be generated in transfected cells. There is, therefore, a theoretical risk that replication-competent transgenic viruses could be formed from a rare recombination event if the vectors infect cells that are also infected with wild-type adenovirus. The probability of such an event occurring is, however, considered to be extremely low.

Adenoviruses are generally transmitted primarily via the airborne and/or faecal-oral routes, and inhalation of viral aerosols and/or oral ingestion of viral stock solution, therefore, represent the main potential routes by which accidental infection is likely to occur. The probability of a worker becoming accidentally infected is, however, considered to be low, as appropriate risk reduction measures will be implemented, Notably, micro-syringes will be filled with viral vectors only in a Class 2 MSC to avoid the production of aerosols, and the risk of infection occurring due to needle stick injuries will be negated by using blunt needles, in most cases, and by using suitable containment conditions. Oral ingestion will be prevented by standard laboratory safety practices.

Accidents resulting in spillage of viral solutions or the production of viral-containing aerosols outside of a safety cabinet could potentially lead to transduction of a limited number of a laboratory worker's skin or mucosal cells with recombinant virus. Such an event is, however, highly unlikely to have any negative consequences on the health of the infected worker, as the recombinant viral particles are replication-deficient, and modulation of the shRNA-targeted protein of interest has not been reported to cause any oncogenic, immunomodulatory, or cytotoxic activities. The vectors also contain no modifications that are known to cause pathogenicity to plants, vertebrate animals, or humans. Natural shedding of superficial epithelial skin and mucosal cell layers and rapid phagocytosis of virus by epithelial immune cells will also act to self-limit any such infections. As adenoviral vectors are generally maintained epichromosomally after transfection, there is a minimal risk of the vectors causing undesirable effects as a result of insertion events disrupting host genome functioning.

Moreover, the risk of infection leading to tumourigenesis or other untoward effects is related in part to the titre of the viral vectors; and exposure of workers to quantitites of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of adenoviral vectors (viruses will be handled as aliquots no larger than 20ul). Importantly, as mentioned earlier, the production of the adenoviral vectors will only take place in the lab of J U which is based at the University of Bristol, and it will, in no instance, take place at Takeda Cambridge's site. This means that large volumes of viruses will not be generated on-site, which consequently decreases the risk of infection.

Infection of the community at large is highly unlikely due to the recombinant vectors being replication deficient.

**B) HAZARDS TO THE ENVIRONMENT**

Adenoviruses infect most mammalian and bird cell types (both replicative and non-replicant), and they are able to survive for prolonged periods of time outside of their host systems due to them being highly stable even in favourable conditions. The recombinant viral vectors could, therefore, potentially infect susceptible organisms if accidentally released into the environment. The chance that active viral particles will be accidentally released into the environment is, however, considered to be highly unlikely, as effective containment procedures will be strictly adhered to. Furthermore, in the unlikely event that active viral particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be negligible, due to the vectors being replication-deficient. Moreover, effective infection of organisms is critically dependent on the presence of high enough concentrations of viral particles; and viral vector stock solutions will be manages so as to prevent contaminations with relatively high viral titres (after production, the vectors are stored/handled in small aliquots not bigger than 20ul).
As mentioned above, the recombinant viruses do still contain the functional wild-type virus sequences that code for viral DNA replication proteins (E2 Polymerase/DNA binding proteins), which means that copies of the viral genome are likely to be generated in transfected cells. There is, therefore, a theoretical risk that replication-competent transgenic viruses could be formed from a rare recombination event if the vectors infect cells of an organism that are also infected with wild-type adenovirus. The probability of such an event occurring is, however, considered to be extremely low.

Accidental environmental contamination with any shRNA molecules is also considered to pose negligible environmental safety risks, as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if released into the environment. There is also a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General waste handling procedures
Solid waste will be neutralised by soaking it in a validated disinfectant solution (Virkon or Distel) for a minimum period of 24 hours, after which the material will be 'double bagged', autoclaved and, ultimately, incinerated offsite by a licensed contractor. Liquid waste will be disposed of into sealed biohazard containers, neutralised with Virkon* for a minimum of 24 hours, and then sent for incineration by a licensed contractor. It will be routine practice that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages.

*Virkon
Rely+On™ Virkon® is a multi-purpose disinfectant. It contains oxone(potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds, such as proteins, leading to widespread, irreversible damage and subsequent deactivation/ destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance agains over 500 strains of viruses, bacteria and fungi. Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control. Importantly Virkon is sold as a powder which dissolves readily in water to form a 1% solution (i.e. 10g per litre) for hard surfaces and equipment disinfection. 1:100 is also the dilution rate advised for virucidal The product has a pink colour, which is useful in that it helps to gauge the concentration of a prepared solution, and importantly, as Virkon ages, it discoulors, making it obvious when it needs to be replaced. The solution is generally stable for five to seven days. Moreover, there is no evidence to suggest that bacterial disease-causing organisms develop resistance towards Virkon, as opposed to some other disinfectant products. Moreover, Virkon is not classified as R53, it is classified as readily biodegradable, and it does not persist in the environment.

Distel (formerly Trigene advance)
Distel is formulated as a nanoemulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms, ensuring the rapid induction of cell death in treated micro-organisms. Distel works quicker than conventional high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products. Distel has been reported to be bactericidal, fungicidal as well as virucidal and sporicial. It is recommended to be used at a 1:2000 dilution for general purposes, 1:100d for high risk areas, and 1:50 for disinfection of blood and bio-hazard spillages.

Summary
As any active viral particles on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.
The GMSC has deemed that the handling of the adenovirus virus stock supplied by the Uney laboratory in Bristol University is a Class 2 activity. This decision has been reached that although the virus will not be generated at Takeda Cambridge Ltd, and that the adenovirus particles have been generated using an expression system that should render the virus non-replicative, the possibility of a recombination event or infection into cells containing wild-type adenovirus leading to replication competent virus can never be discounted. The EGFP and shRNA encoded by the adenovirus are not known to possess any inherent toxic or pathogenic hazard to either individuals or to the environment. The GMSC is also satisfied that the knock down in expression of the target gene in this study does not pose any known hazard. The necessary procedures have been put in place to allow the safe use of the virus.

Please enter comments on the GM safety committee on the risk assessment

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Project Containment

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Project Ref 3043/14.2

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Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes transferred to GM3344 02/12/2016
The purpose of this project is to validate positive and negative modulators of cellular processes that might be useful drug targets that work to delay or halt Neurodegeneration, the process that underlines a number of diseases including dementia. These positive and negative modulators will be identified through a druggable RNAi high content screen in a human non-neuronal cell line that is currently being performed by an outside academic collaborator. Selected targets will be further assessed by looking at their effects in neuronal and non-neuronal cells. To ensure that the targets affect specific cellular processes each of the selected RNAi targets will also be coexpressed with a fluorescent protein (FP) reporter that is directed to a specific organelle. It is expected that depletion will not necessarily cause any major alterations in unstressed neurons. Target dependent depletion through RNAi will have to be performed in a disease relevant model. For this, a lentiviral construct containing a FP tagged or untagged human Neuro degeneration relevant disease mutation or Neurodegeneration disease relevant RNAi will be combined with the RNAi dependent depletion of the relevant positive/negative regulator. Through this strategy, it is hoped that the identity and validity of one or more targets that may be useful targets for the treatment of Neurodegeneration can be determined. This work will involve the use of particles of lentiviral origin and so require containment to minimise the risk of exposure to both humans and the environment. It should be noted that we will be using third generation lentiviral self-inactivating replication defective (SIN-RD) lentiviral particles. The fact that four different plasmids are required to generate these SIN-RD lentiviral particles reduces the risk even further. Nevertheless according to current statutes and as a precautionary measure, all generation and application of virus will be done under Level 2 containment.
Origin & function

The SIN-RD lentiviral particles will contain the following sequences:

1. shRNA/miRNA directed towards the knock down of the expression of a particular endogenous mammalian target (Mouse, Rat, Human). These de novo derived sequences are not derived from an organism and will be designed to only target an individual species. In addition the sequences chosen will not target known oncogenes, tumour suppressors, cell cycle regulators, Immunomodulators and will not be of retroviral origin. Resources that will be used to assess these criteria will include the interrogation of genome wide RNA screens to assess Mitosis and cell death (www.mitochek.org).

The particles will deliver shRNA/miRNA constructs that will either target one or two genes. For shRNA constructs transcription will be driven by a constitutively active Polymerase III U6 promoter. For some targets shRNA may not represent an effective methodology for knock-down of expression so miRNA constructs prove more effective. This methodology utilises an endogenous cellular mechanism for post-transcriptionnally regulation of target genes. Artificial miRNA sequences are generated using the human miR-30 microRNA cassette under the control of a polymerase II miRNA sequences or an additional protein. The miRNA sequences will be excised by the endogenous cellular machinery before expression of the protein.

2. Fluorescent protein (FP markers which have been identified from a range of different organism; are known to be benign in terms of their effects when expressed in mammalian cells but are essential for visualisation of these cells. When these in addition are fused to a subcellular marker gene, that is expressed only in a particular sub-cellular structure within a cell, will enable the visualisation of a particular process and any changes that occur in response to the knockdown of the expression of a particular gene. Expression of the FP will be either under the control of a CMV promoter which is constitutively active and commonly used in biological research or a promoter that only allows expression in neuronal cells.

3. Human disease relevant mutated gene expression which is intended to recreate a certain aspect of disease pathology. It is expected that its expression will be cytotoxic but will not be a growth factor (which does not include trophic factors), confirmed oncogene, tumour suppressor gene (TSG), cell cycle regulator or immunomodulator, and none of them will be of retroviral orgin. Expression of the mutant proteins will be either under the control of a polymerase II promoter that is either constitutively active or only active in neuronal cells.

Lentiviral production - on site B60CRP or 430CSP

Where lentivirus is not available from the collaborator; lentiviral production will be carried out on-site. Lentiviral production will only be carried on HEK293T cells and will be carried out in a class 2 microbiological safety cabinet within a Biosafety level 2 laboratory. The HEK293T cells used for lentiviral production will be seeded in filter containing screw cap culture flasks in order to minimize the risk of spills and/or aerosol mediated viral spread. The modified PRL plasmid containing the necessary shRNA/miRNA & protein encoding sequences together with the chemical or lipid based transfection reagent (e.g. Polyethylenimine (PEI), Lipopectamine) into the HEK293T packaging cell line. After 6-12 hours, the medium is replaced with fresh serum-free medium. (e.g. Neurobasal medium with 2% B-27 supplement and 1X Pen/Strep) and grown for an additional 72-96 hours. After 24-48 hours, so as to ensure the viability of the transfected HEK293T cells, medium from the cells is collected into screw cap tube and stored at 4°C and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the first harvest tube. All plastic ware and solutions will be treated as if contaminated.

Still within a Biosafety level 2 microbiological safety cabinet in a Biosafety level 2 laboratory, the harvested supernatants will be filtered using a 0.45 um filter before either being dispensed as aliquots or further purified by chromatography and/or concentrated by centrifugation. For certain application the lentiviral particles will need to be a concentrated. The lentiviral particles may need to be purified to be either chromatography (e.g. ViraBind™) and eluted in a high salt solution (50mM Tris, pH 7.5,5 mM MgC12, 2 M NaCl) and/or concentrated by ultrafiltration (e.g. LentiSelect™). For certain application where a highly concentrated, highly purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimise the possible risk of accidental viral contamination and spread due to the need for an ultracentrifuge, all loading and unloading will be done in the Biosafety level 2 cabinet. Once the sucrose cushions have been added to the centrifuge tubes, they will be transferred into the Biosafety level 2 Safety Cabinet. The unpurified or chromatography purified lentiviral particles will then be added to the sucrose cushion. The tubes will then be transferred to their respective bucket holders and the bucket sealed using their respective screw caps and marked as containing virus. The bucket will be transferred to a weighing scale and a counterpart bucket and centrifuge with water (and without lentivirus) will be prepared that is weight matched. The balanced buckets will then be transferred to the rotor before being placed within the centrifuge chamber. The ultracentrifuge will be run at 70000g for 2 hours at 20°C. Upon return of the bucket to the Biosafety level 2 cabinet, the supernatant will be removed from the tubes before transfer of the resuspended virus pellet into individual screw cap croyvials. As
the greatest risk for contamination occurs within the centrifugation buckets themselves during transport to and from the weigh scales and ultracentrifuge, not only will the centrifuge tubes also be decontaminated with DISTEL 10% and 70% isopropanol before removal from the Biosafety level 2 laboratory. In any case, at no point will concentrated virus stock be exposed to outside of the Biosafety level 2 hood environment.

The lentiviral titre will be determined in terms of the number of viral particles (VP) or Transduction units (TU) per ml. Assessing the number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based enrichment assay (e.g. QuickTitre™ Quantification Kit) or a p24 ELISA assay (e.g.QuickTitre™ Lentivirus Titre Kit).will be chosen. The VP quantification methods tends to overestimate the TU by 10-1000 fold. Thus, the TU/ml will in certain instances also be determined. For this, a titration range of lentiviruses expressing a fluorescent tag, serial dilution will be prepared and added to a 24 well cluster plate of T25 TC-flasks containing HEK293T for 48 hours. The percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using FACS analyser. The biological titre (TU/ml) according to the following formula: 

$$\text{TU}/\mu l = \frac{(P \times N/100 \times V) \times 1/DF}{V}$$

where P= % GFP+ cells, N= number of cells at time of transduction = 105, V= volume of dilution added to each well and DF=dilution factor.

Aliquots with not more than 1x10^8 viral per viral are subsequently stored at -80°C in individual screw capped cryotubes. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats.

Assessment of effects

Assessing whether the knock down has worked in neurons can be challenging. Consequently, initially the RNAi depletion of the target gene (using the FP tagged version followed by the RNAi sequence) will be assessed in immortalised cell lines (mouse NIH/3T3 and human HEK293T and human HEK293T). This work will be done initially at Biosafety level 2. In order to determine whether the knock-down has worked; the levels of mRNA and protein will be assessed post-transduction, using suitable in vitro assays, including for example, RT-PCR, Western Blotting, and immunocytochemistry. These results will subsequently be confirmed on neurons such as those derived from human induced Pluripotent stem cells (iPSCs) using immunocytochemistry.

The correct distribution and perturbed cellular functions of the relevant human Neurodegeneration relevant disease mutations will also be assessed by subsequent immunocytochemistry and Bioenergetic analysis (using Seahorse™). Other physiological parameters, including, for example, the release of specific neurotransmitters from neurons or the expression of related proteins will be measured using suitable in vitro assays, including, for example, ELISA, PCR, neurite outgrowth, electrophysiology, and neuroprotection assays. In some instances, the vectors might need to be studied in rodent neuron-glia co-cultures in order to investigate the interaction between these two cell types.

Evaluation of foreseeable effects

Human health hazards

The method by which the lentivirus vector is produced means that it is highly unlikely that RCL can be generated even if an infection occurs in a person who is HIV-2 positive. Infection can lead to a permanent transduction of cells and the possibility of a harmful event either by transactivation or disruption of gene expression cannot be ruled out. For this reason the work with stock viral aliquots will be conducted as a class ll activity. Oral ingestion will be prevented by involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication -incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the genes/proteins of interest that will be targeted by shRNAs do not include any growth factors, confirmed oncogenes, cytotoxins, or immunomodulators, and none of them are of retroviral origin. The mutant genes express proteins known to be cytotoxic to neurons but it is highly unlikely such cells would be transduced via the mucosal or skin routes. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus, this will be self-limited due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent and being extremely sensitive to desiccation.

Hazard to the Environment
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable RCL. This scenario is, however, extremely unlikely, and even if it was to occur, it is unlikely that the vectors could survive long enough in the environment to pose a risk if accidentally released.

Furthermore, lentiviral vectors have a short half-life at room temperature due to their structural characteristics making them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, and this is illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA and miRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

Summary
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as Biosafety level 2, and effective containment procedures will be adhered to (see section C for details). However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems (see above discussions).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Following transduction of cell lines that are designated as requiring Biosafety Containment Level 1 with lentivirus we wish to be able to use these cells at BioSafety Containment Level 1. This can be justified by the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24 hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as only requiring Biosafety Containment Level 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General waste handling procedures:
Solid waste will be neutralised by soaking it in a validated disinfectant solution (Virkon* or Distel) for at least 24 hours, after which the material will be 'double bagged', autoclaved and, ultimately, incinerated offsite.
At B60CRP, liquid waste will be disposed of into sealed biohazard containers filled with Virkon* for incineration. It will be routine practice that all liquid waste material be neutralised for at least 24 hours before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. At 430CSP, liquid waste will be inactivated by Virkon* for 24 hours prior to drain disposal via a designated sink.

*Virkon
Rely+On™Virkon® is a multi-purpose disinfectant. It contains oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds such as proteins, leading to widespread, irreversible damage and subsequent deactivation/destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance against over 500...
strains of viruses, bacteria and fungi. Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control. Importantly, Virkon is sold as a powder which dissolves readily in water. It is intended to be mixed with water to form a 1% solution (i.e. 10g per litre) for hard surface and equipment disinfection. 1:100 is also the dilution rate advised for virucidal efficacy against HIV-1.

The product has a pink colour, which is useful in that it helps to gauge the concentration of a prepared solution, and importantly, as Virkon ages, it discours, making it obvious when it needs to be replaced. The solution is generally stable for five to seven days. Moreover, there is no evidence to suggest that bacterial disease-causing organisms develop resistance towards Virkon, as opposed to some other disinfectant products. Moreover, Virkon is not classified as R53, it is classified as readily biodegradable, and it does not persist in the environment.

*Distel (formally Trigene advance)*
Distel is formulated as a nanoemulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms, ensuring the rapid induction of cell death in treated micro-organisms. Distel works quicker than concentrated high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products. Distel has been reported to be bactericidal, fungicidal as well as virucidal and sporicidal. It is recommended to be used at a 1:200 dilution for general purposes, 1:100d for high risk areas, and 1:50 for disinfection of blood and bio-hazard spillages.

Summary
As any active viral particles on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

The GMSC has agreed that the production and handling of virus stocks is a Class 2 activity. This decision has been reached because although the lentivirus will be generated using a third generation, self-inactivating, 4 plasmid expression system, the possibility of a recombination event occurring that leads to replication competent virus can never be discounted with this type of virus. The potential oncogenic effects of the Woodchuck Post-transcriptional Regulatory Element have been negated by the use of the mutant form that prevents expression of the X protein. The production of large amounts of virus represents the most hazardous aspect of the proposal and the GMSC is satisfied that the necessary precautions have been put in place to ensure containment is maintained. Some of the proposed nervous system. The procedures put in place to ensure containment is maintained. Some of the proposed lentivirus will encode for proteins that are known to have neurotoxic effects and cause diseases of the human central nervous system. The procedures put in place will effectively prevent any chance of virus being delivered to the necessary tissues within a human in sufficient quantities to represent a hazard to human health. The FP and shRNA/miRNA encoded by the other lentivirus are not known to pose any inherent risk to human health or the environment. The proposed targets to be knocked down in this study also pose no known risk. The request to derogate the Biosafety Containment Level from 2 to 1 post transduction has been deemed acceptable given that the transduced cells will be washed extensively to remove any remaining viral particles and will be tested for the absence of transducing virus. It has been made clear that human iPSC cell lines that have been generated using lenti- or retrovirus methods tested negative for the absence of virus at least. These cells will always be tested for the absence of infectious particles before they can be used as if they only required Biosafety Containment Level 1.
Project Containment

Laboratory Activities

L2 Yes L3 L4 L2 L3 L4 L2 L3 L4

Animal Units

L2 L3 L4 L2 L3 L4 L2 L3 L4

Glass Houses

L3 L4 L2 L3 L4 L2 L3 L4

Large Scale Activities

L2 L3 L4 L2 L3 L4 L2 L3 L4

Growth Rooms

L2 L3 L4 L2 L3 L4 L2 L3 L4

Human Clinical Applications

Laboratory Activities Glass Houses Growth Rooms

Project Ref 3043/14.3

Date Ackn'd 03/12/2014

CU2 Project Title Lentiviral siRNA knockdown studies to identify and validate drug targets for psychiatric and neurodegenerative diseases

Date Project Ceased 02/12/2016

Class 2 CultureVolClass2 < 1 Litre

Consent Granted

Historical Significant Changes

transferred to GM3344 02/12/2016

Project notified under transitional arrangements N

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Aim

The lentiviral siRNA knockdown studies that will be carried out as part of this body of work will be focused on discovering the role of specific proteins in both physiological and pathological eNS processes, with the overarching aim of identifying and validating potential new drug targets for psychiatric and/or neurodegenerative diseases. The proteins that will be selectively knocked down by lentiviralUy expressed siRNA will include those that have been either directly or indirectly linked to relevant eNS disease states; and, in most cases, the targeted proteins will play a role in well characterised relevant disease-related biologicaVpathological processes. Relevant neurodegeneration

02/03/2022

Page 12655 of 15326
related processes, for example, are likely to include, bioenergetics, oxidative stress, neutre-inflammation, protein
misfolding, excitotoxicity, and autophagy. Psychiatric disease related protein targets, on the other hand, are likely to
play a role in neurogenesis, neural plasticity, or the functioning of one or more disease-linked neurotransmitter
systems.

Importantly, the genes/proteins of interest that will be targeted by siRNAs do not include any growth factors,
confirmed oncogenes, tumour suppressors, cell cycle regulators, cytotoxins, or immunomodulators, and none of
them are of retroviral origin. All selected targets will be presented to the GMSC for approval.

Methods

The approach to be taken will be the application of lentiviral vectors to cell lines that are deemed biosafety level 2
or lower, including well characterised immortalised cell lines that are known not to contain lentiviral genetic
material, and human induced Pluripotent stem cells (iPSCs). The lentiviral vectors will either express siRNA
transcripts targeting specific mRNAs/proteins of interest, or with vectors expressing scrambled siRNAs, in the case
of the negative control treatment groups. The extent of mRNA and protein knockdown that is achieved will then be
assessed at different time points (e.g. 24h-48h-72h), post-transduction, using suitable in vitro assays, including for
example, RT-PCR, Western Blotting, and immunocytochemistry. Once the conditions that lead to the optimal
knockdown of the mRNAs/proteins have been determined, it will be evaluated what effects the knockdown of the
proteins have on various physiological parameters, including, for example, the release of specific neurotransmitters
from neurons or the expression of related proteins. These siRNA-induced alterations will be measured using
suitable in vitro assays, including, for example, ELISA, PCR, neurite outgrowth, electrophysiology, and
neuroprotection assays. In some instances, the vectors might need to be studied in neuron-glia co-cultures in order
to investigate the interaction between these two cell types.

Description of the lentiviral vectors used for the experiment

Third generation HIV based shRNNmiRNA expressing lentiviral vectors will be produced either internally or
sourced from external suppliers, which will include reputable commercial suppliers, and an academic group at the
University of Bristol headed by Prof. James Uney. Each aliquot of lentiviral reagent will comprise of a small volume
(5 to 30μl) that contains no more than 1x10^8 viral particles. Post-transduction of target cells, the vectors will
produce both a shRNNmiRNA targeting the mRNAs of a protein of interest, and a reporter protein to facilitate the
identification of transduced cells.

Recipient or parental organism

Mammalian Cells

Host/vector system

Vector system = self inactivating (SIN) non replication-competent HIV based lentiviral vectors

Health and Safety

Executive

The viral vectors that will be used in the proposed studies are third generation self-inactivating (SIN) non
replication-competent HIV based lentiviral vector systems, which have been designed to produce stable gene
expression in mammalian cells; and they are generated by co-transfecting a suitable immortalized packaging cell
line (e.g. HEK 293T cells) with four separate plasmids. Each of the plasmids used (described below) expresses a
different set of genes, and all of the genes from the different vectors, when combined (following co-transfection),
provide the smallest possible set of essential viral genes that is still compatible with virus production. Moreover, the
resultant vectors are all vesicular stomatitis virus (VSV-G) pseudotyped lentiviral vectors, which are self-inactivating
and highly unlikely to undergo recombination. Importantly, the viral vector system is inherently incapable of
replication in mammalian cells. Only in a patient with HIV could any form of recombination occur, i.e. the probability
of the production of replication competent lentiviral vectors (RCL) is very small.

The plasmids

The sequences that are required to generate the viral vectors are sub-cloned into 4 standard bacterial plasmid vectors. The transgene expression plasmid will contain the transgene that codes for a relevant siRNA sequence and/or a transduction marker protein (e.g. GFP), while the other 3 packaging plasmids will contain 3 different sets of sequences that code for different viral packaging proteins. A brief overview of the 4 different plasmids is provided below:

Plasmids containing the transgene of interest

The transgene expression plasmid will contain the sequences that code for the siRNA transcript that will target a specific mRNA protein of interest (or a scrambled siRNA in the case of the negative control), and also for a transduction marker protein. Control viral vectors that express only a marker protein and no siRNA will also be used in some experiments, and the corresponding plasmids for these vectors will only contain the sequence coding for the marker protein. Depending on the knockdown strategy taken, either shRNA or miRNA based constructs will be used to express the artificial siRNA sequences. In some of the constructs used, siRNA and marker protein sequences will be expressed as two separate transcripts under the control of two separate promoter elements. In other instances, siRNA and marker protein expression will be under the control of a single promoter system. The promoter elements used to control transgene expression will vary depending on the specific plasmid that is employed. Only well characterized promoter systems will, however, be used to drive transgene expression. These will include the H1, U6 small nuclear, cytomegalovirus (CMV), elongation factor 1 alpha (EF1α), phosphoglycerate kinase (PGK), ubiquitin C (UbC), and murine stem cell virus (MSCV) promoters; or in instances where siRNA expression in a specific cell subtype is desired, polymerase III promoters such as the synapsin I (SynI), neuron specific enolase (NSE), tyrosine hydroxylase (TH), calcium/calmodulin-dependent protein kinase II (CamKII), or glial fibrillary acidic protein (GFAP) promoters.

Additionally, all of the transgene constructs will contain the WPRE, cPPT, RRE, and 41 sequences, as well as the modified LTR, and these sequences are the only other coding sequences (apart from the siRNA and transduction marker proteins) that will be present in the viral vectors. The latter elements are important for the expression of the shRNA in targeted cells, and they will all be stably expressed in infected cells.

Plasmids containing the packaging related sequences

The three other plasmids that make up the 4 plasmid system will each contain a different set of sequences that code for proteins that are required for production and packaging of the viral vectors. Gag-pol will be expressed by one of the plasmids, rev by a second, and VSV-G by a third, and the latter proteins will only be provided in trans during the production phase. The specific packaging plasmids that will be used will vary depending on the packaging system that is employed. Only well characterized packaging plasmids will, however, be used in all cases. For example, in one system, gag-pol, rev and VSV-G will be expressed separately by pMDLgp-RRE, pRSVREV, and pMD2-VSVG.env packaging plasmids, respectively, whereas, in a second, they will be expressed by pPACKH1-GAG, pPACKH1-REV, and pVSV-G, respectively. Importantly, all of the above genes, that are essential for production of full length viral particles, have been removed from the expression plasmids containing the transgenes of interest. The 4 plasmids used in the system have also been engineered so as to have no common sequences, which greatly reduces the risk of a recombination event that would result in the insertion of production/packaging genes into the transgene expression plasmid.

The expression systems and the transgenes that comprise the lentiviral vectors that will be used have a very low risk for human health and safety, and the reasons for this are discussed below.

Lentiviral Vector Expression System
Lentiviruses belong to the Retroviridae virus family, a diverse and extensive family of viruses, which are capable of infecting both human and animal species. Retroviruses are characterised by a unique replication mechanism involving reverse transcription of the viral RNA genome, which gives rise to a DNA provirus that contains two positive sense copies of the viral RNA genome; and in a functional assembled virus, the viral genome is encased within a capsid that is surrounded by a host-cell derived envelope. Cellular entry of the virus involves interactions between glycoproteins contained in the virion's envelope and extracellular plasma membrane proteins on target cells. These interactions are generally specific and are believed to be the principal factor determining which species and type of cells a specific virus is capable of infecting. The binding of a virus's envelope glycoproteins to an appropriate receptor site on a target cell triggers the fusion of the virion's envelope membrane with the plasma membrane of the target cell, which results in the delivery of the virus capsid to the intracellular space of the target cell.

As the lentiviral vector system that will be used in these studies is based on HIV-1, a theoretical risk to human health exists. Retroviruses are, however, generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and, they are, generally, not transmitted via the airborne route. Accidental piercing of the skin or other surface tissues with virus containing objects, therefore, represent the main potential route by which accidental infection could occur. Appropriate risk reduction measures will, however, be implemented to reduce the likelihood of this occurring.

In all of the transgene expression plasmids, the genes of interest are flanked by non-coding retroviral TRs, and no retroviral genes are encoded on the transgene plasmids. Therefore, no retroviral genes will be transferred into generated viral particles. The transgene construct is packaged into particles using an immortalised cell based packaging system, which requires the co-transfection of packaging cells (e.g. HEK293 cells) with three additional separate packaging plasmids (pMDLgp-RRE, pRSV-REV and PMD2-VSVG.env, for example). The latter 3 plasmids express the envelope protein from VSVg and the non-structural proteins of the virion, and, importantly, none of these genes will be transferred into the assembled viral vectors, since they lack the packaging signal (+), which is only present on the transgene containing plasmids.

The lentiviral Expression System that will be used include the following key safety features:

All of the lentiviruses will be generated using a 4 plasmid/3 retroviral gene (gag, pol, rev) system, which yields replication-incompetent vectors that are devoid of all viral sequences, apart from essential cis-acting sequences, including the L TRs and the packaging signal '+', and only the transgene plasmids will contain +1. This system allows for the expression in trans of proteins required to produce viral progeny (e.g. gal, pOl, rev, env) in producer cell lines, which do not contain L TRs or the'+ packaging sequence. None of the retrovirus structural genes will, therefore, actually be present in the packaged viral genome, which means that no new replication-competent lentivirus (RCL) can be produced.

Importantly, following infection of a target cell, lentiviral vectors are self-inactivating on integration into the host genome. This is achieved because the process of reverse transcription uses the 3' long terminal repeat (3' TR) as a template to produce the 5'TR. In the transgene expression plasmids that will be used, the U3 region of the 3'TR has been deleted, resulting in deletions in both 3' and 5' L TRs on integration into the host genome. The deletion in the 3'TR (uU3) does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" (SIN) of the vector after transduction of target cells, which ensures that the viral genome cannot be released from the host genome.

Moreover, deletion of enhancer and promoter elements from the 3' U3 region in the vector constructs will result in a provirus that is entirely devoid of the U3 enhancer sequences, which will result in a reduction in the potential for transactivation of cellular genes due to an insertion event. Self-inactivating vectors are also less likely to be
mobilized following a superinfection with wild-type virus (HIV), and the VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes, obviating safety concerns associated with the use of HIV-1 gp120, which has known pathogenic consequences. Importantly, all of the 4 plasmids used in the system have been engineered not to contain any regions of homology with each other so as to prevent undesirable recombination events that could lead to the generation of a (RCI).

Lentiviral production - on site B60CRP or 430CSP

Lentiviral production will also be carried out on-site. Lentiviral production will only be carried on HEK293T cells and will be carried out in a class 2 microbiological safety cabinet (MSC) within a dedicated Level 2 laboratory. The HEK293T cells used for lentiviral production will be seeded in filter containing screw cap culture flasks in order to minimize the risk of spills and/or aerosol mediated viral spread. For this, a modified pRRL transgene expression plasmid together with three additional separate packaging plasmids (pMDLgp-RRE, pRSV-REV and PMD2-VSVG.env) will be transfected using a transfection reagent (e.g. Polyethylenimine (PEI), Lipofectamine) into HEK293T cells. After 6-12 hours, the medium is replaced with fresh serum-free medium (Neurobasal medium with 2% B-27 supplement and 1X Pen/Strep) and grown for an additional 72-96 hours. After 24-48 hours, so as to ensure the viability of the transfected HEK293T cells, medium from the cells is collected into screw cap tube and stored at 4°C and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the first harvest tube. After lentivirus collection has been completed, the cells in the flask will be treated with a validated anti-microbial agent (e.g. DISTEL 10% or another equivalent product) for at least 24 hours prior to autoclaving on site. Autoclaved material will then subsequently be incinerated. Still within a Class 2 MSC in a Level 2 Biosafety laboratory, the harvested supernatants will be filtered using a 0.45 micron filter before either aliquoted or further purified by chromatography and/or concentrated by centrifugation. For certain applications, the lentiviral particles will need to be concentrated. The lentiviral particles may need to be purified by either chromatography (e.g. ViraBindTM) and eluted in a high salt solution (50mM Tris, pH 7.5, 5mM MgCl2, 2M NaCl) and/or concentrated by ultrafiltration (e.g. LentiSelectTM). For certain applications where highly concentrated, highly purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimise the possible risk of accidental viral contamination and spread due to the need for an ultracentrifuge, all loading and unloading will be done in a class 2 MSC. Once the sucrose cushions have been added to the centrifuge tubes, they will be transferred into a class 2 MSC. The unpurified or chromatography purified lentiviral particles will then be added to the sucrose cushion. The tubes will then be transferred to their respective bucket holders and the bucket sealed using their respective screw caps and marked as containing virus. The bucket will be transferred to a weighing scale and a counterpart bucket and centrifuge with water (and without lentivirus) will be prepared that is weight matched. The balanced buckets will then be transferred to the rotor before being placed within the centrifuge chamber. The ultracentrifuge will be run at 70000g for 2 hours at 20°C. Upon return of the buckets to the class 2 MSC, the supernatant will be removed from the tubes before transfer of the resuspended virus pellet into screw cap cryovials. As the greatest risk for contamination occurs within the centrifugation buckets themselves during transport to and from the weigh scales and ultracentrifuge, not only will the centrifuge tubes but also the buckets decontaminated with DISTEL 10% and 70% isopropanol before removal from the level 2 laboratory. In any case, at no point will concentrated virus stock be exposed to outside of the class 2 MSC environment.

The lentiviral titre will be determined in terms of the number of viral particles (VP) or Transduction units (TU) per mL. Assessing the number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based enrichment assay (e.g. QuickTitre™ Quantification Kit) or a p24 ELISA assay (e.g. QuickTitre™ Lentivirus Titre kit) will be chosen. The VP quantification methods tend to overestimate the TU by 10-1000 fold.
Thus, the TU I ml will in certain instances also be determined. For this, a titration range of lentiviruses expressing a fluorescent tag, serial dilution will be prepared and added to a 24 well cluster plate or T25 TC-flasks containing HEK293T for 48 hours. The percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using FACs analyser.

Aliquots with not more than 1x10^8 virus per vial are subsequently stored at -80°C in screw capped cryotubes. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to, inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats.

**Origin & function**

Transgene: proteins to be expressed

Health and Safety

Executive Additional hazards that also arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.

A. Transduction Marker Proteins

Only well characterised transduction marker proteins that are not associated with any cytotoxic, immunogenic or oncogenic responses will be employed, including, for example, Fluorescent proteins such as GFP, GFPem, YFP, OsRed, mCherry, CFP.

B. shRNNmiRNA: Endogenous proteins to be depleted

The proteins that will be selectively knocked down by lentivirally expressed siRNA will include proteins that have been either directly or indirectly linked to psychiatric and/or neurodegenerative disease states; and, in most cases, the targeted proteins will play a role in well characterised relevant disease-related biological/pathological processes. Relevant neurodegeneration related processes, for example, are likely to include, bioenergetics, oxidative stress, neuro-inflammation, protein misfolding, excitotoxicity, and autophagy. Psychiatric disease related targets, on the other, are likely to play a role in neurogenesis, neural plasticity, or the functioning of one or more disease-linked neurotransmitter systems.

Importantly, the genes/proteins of interest that will be targeted by siRNAs do not include any growth factors, confirmed oncogenes, tumour suppressors, cell cycle regulators, cytokotoxins, or immunomodulators, and none of them are of retroviral origin. Depending on project objectives and human vs. rodent mRNA sequence homology, siRNA sequences will be designed either to specifically target only the human or mouse/rat mRNA sequence of a target protein, or both the human and mouse/rat sequences.

RNA interference (RNAi)

RNA interference (RNAi) is an antisense technology that exploits a normal cellular antiviral response that acts to inhibit viral protein synthesis through the production of short hairpin RNAs (shRNAs) that bind and target viral double-stranded RNA (dsRNA) molecules for degradation. The shRNA molecules are processed by cellular enzymatic pathways to produce small inhibitory RNA (siRNA) species, and it is the latter RNA molecules that bind and target complementary RNA sequences for degradation by the RNA-induced silencing complex (RISC). With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be 'knocked-down', in order to study their functions.

Potential deleterious effects such off-target and immunomodulatory responses can be minimised through screening designed siRNA sequences against databases of known mammalian gene/mRNA sequences during the design stage.

MicroRNAs (miRNAs) are endogenously encoded 22-nl-long RNAs that are generally expressed in a highly tissue and/or developmental-stage specific fashion, and they function to post-transcriptionally regulate the expression of
target genes. In certain experimental RNA interference approaches, this miRNA system can be
harnessed/manipulated to study the functions of specific genes/proteins. The additional benefit is that one can
place these miRNA sequences under the control of a Polymerase II promoter in which the miRNA or multiples
thereof are co-transcribed with a coding sequence. For example, RNA polymerase II promoters can be
experimentally activated so as to over-express endogenous microRNAs in cell culture systems. Alternatively,
artificial microRNAs can also be engineered to match the features of existing microRNA genes, such as the gene
encoding the human miR-30 microRNA. In an analogous method, the BLOCK-iTTM Pol II miR RNAi expression
vectors from life Technologies allow for selected miRNA sequences that are flanked on both ends with flanking
sequences that allows for their successful transcription under a Polymerase II promoter. Additional coding
sequences containing a fluorescent protein tag are placed 5’ to the miRNA.

Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled
shRNAlmiRNA), and which is not complementary to any known mammalian sequences. These vectors will be
employed as negative controls to demonstrate that any observed effects are due to the knockdown of a specific
mRNA/protein of interest, rather than being due to any non-specific effects that the delivery of the viral vectors
might cause. This sequence will adopt a hairpin structure as with any shRNA/miRNA, but it should not target any
mRNA of mouse, rat or human origin. It will only serve as the negative control for the vector producing
shRNA/miRNA. Consequently, these sequences are unlikely to pose any safety risks for the environment or human
health.

Summary
The transgenes that will be expressed by the viral vectors have a very low safety risk to human health. This is
because accidental contamination with a vector would lead to only a small number of cells becoming infected, and
it would be highly unlikely that expression in only a few cells would be sufficient to lead to a disease state. The
inability of the lentiviral vectors to propagate in mammalian cells also reduces the risk.
The transduction marker proteins that will be employed are not known to cause any relevant toxicities, and the the
genes/proteins of interest that will be targeted by siRNAs do not include any growth factors, confirmed oncogenes,
cytotoxins, tumour suppressors or immunomodulalors, and none of them are of retroviral origin.

Evaluation of foreseeable effects

Human health hazards
Although the lentiviral vectors that will be used are replication incompetent and contain only -20% of the original
HIV-1 genome, there is a small risk that subsequent infection of cells already infected with the lentiviral genome by
HIV-, could lead to a rare recombination event in which the transgene is transferred to a replication-competent
virus. Thus, the sequences in the vector that will be expressed could potentially be transferred to surrounding cells.
This event is, however, extremely unlikely to occur, and it has been shown that, even under permissive in vitro
conditions, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host
genome (Bukovsky et al 1999).
Additionally, there is a theoretical risk that an infection event with the vectors could lead to the infected cells
becoming cancerous, as lentiviral integration into a host genome could potentially lead to the activation of an
endogenous oncogene. All transcriptionally active long-terminal repeats (LTRs) have, however, been removed from
the viruses as well as all promoter-like elements other than that required to drive expression of the transgene.
which should prevent unforeseen activation of such genes. The deletion of the retroviral enhancer in self-inactivating
systems reduces the risk of activation but not of disruption and therefore, retroviral infection might still
have permanent effects upon a cell (including oncogenic effects).
Importantly, the likelihood of a worker becoming accidently infected is, however, considered to be low, as
appropriate risk reduction measures will be implemented. Notably, micro-syringes will be filled with viral vectors
only in a class 2 MSC to avoid the production of aerosols. The most likely route of accidental infection with a
fentivirus will be via inadvertent percutaneous inoculation. The likelyhood of this occurring will be minimised by following the correct procedures. Oral ingestion will be prevented by standard laboratory safety practices. Moreover, the genes/proteins of interest that will be targeted by shRNAs do not include any growth factors, confirmed oncogenes, tumour suppressors, cytotoxins, or immunomodulators, and none of them are of retroviral origin. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

HAZARDS TO THE ENVIRONMENT
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active viral vector particles do accidently get released into the environment, the safety risks posed by such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable RCL. This scenario is, however, extremely unlikely, and even if it was to occur, it is unlikely that it would lead to any untoward effects.

The vectors have been pseudolyped with the VSV-G envelope in order to increase tropism, and they are, in theory, capable of transducing all dividing and non-dividing mammalian cell types. As emphasised before, the vectors, however, cannot self-propagate after infection, and successful transduction is also critically dependent on the presence of high enough concentrations of virus particles; and viral vector stock solutions will be managed in a way that will prevent contaminations with relatively high viral titres. After production, the vectors are aliquoted and stored in small volumes (each tube contains at maximum 30μl) at -80°C, and when taking into account that the infectivity of the vectors rapidly decrease at room temperature, it is considered highly unlikely that the vectors could survive in the long term after being accidentally released into the environment.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA and miRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

Summary
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will undertaken have been classified as CL2, and effective containment procedures will be adhered to. However, in the unlikely event that active viral vector particles do accidently get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems (see above discussions).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Following transduction of eel lines that are designated as requiring Biosafety Containment level1 with lentivirus, we wish to be able to subsequently use these cells at Biosafety Containment level1. This can be justified by the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24
hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as only requiring Biosafety Containment Level 1. Sources of iPSC cells that have been shown to lack viral integration and test negative for infectious virus can be regarded as requiring biosafety level 1 containment. Such iPSC cells can also be derogated to biosafety level 1 containment once tested for the absence of infectious virus.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General waste handling procedures:
SoUt waste will be neutralised by soaking it in a validated disinfectant solution for 24 hours, after which the material will be 'double bagged', autoclaved and, ultimately, incinerated offsite. At B60CRP, liquid waste will be disposed of into sealed biohazard containers filled with Virkon® for incineration. It will be routine practice that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. At 430CSP, liquid waste will be inactivated by Virkon® for 24 hours prior to drain disposal.

• Virkon

Virkon® is a multi-purpose disinfectant. It contains oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds, such as proteins, leading to widespread, irreversible damage and subsequent deactivation/destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance against over 500 strains of viruses, bacteria and fungi, Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control. Importantly, Virkon has specifically been validated for lentiviral inactivation (Antec-Biosentry); Virkon is sold as a powder which dissolves readily in water. It is intended to be mixed with water to form a 1% solution (i.e. 10.9 per litre) for hard surface and equipment disinfection. 1:100 is also the dilution rate advised for virucidal efficacy against HIV-1.

The product has a pink colour, which is useful in that it helps 10 gauge the concentration of a prepared solution, and importantly, as Virkon ages, it discoulers, making it obvious when it needs to be replaced. The solution is generally stable for five to seven days. Moreover, there is no evidence to suggest that bacterial disease-causing organisms develop resistance towards Virkon, as opposed to some other disinfectant products. Moreover, Virkon is not classified as R53, it is classified as readily biodegradable, and it does not persist in the environment.

• Distel

Distel is formulated as a nanoemulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms, ensuring the rapid induction of cell death in treated micro-organisms. Distel works quicker than conventional high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products. Distel has been reported to be bactericidal, fungicidal as well as virucidal and sporicidal. It is recommended to be used at a 1:200 dilution for general purposes, 1:100 for high risk areas, and 1:50 for disinfection of blood and bio-hazard spillages.

Summary

As any active viral particles on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The GMSC has agreed that the production, handling and use of lentivirat siRNA stocks is a Class 2 activity. This decision has been reached because although the lentivirus will be generated using a third generation, self-inactivating, 4 plasmid expression system, the possibility of a recombination event occurring that leads to replication competent virus can never be discounted with this type of virus. The potential oncogenic effects of the Woodchuck Post-translational Regulatory Element have been negated by the use of the mutant form that prevents expression of the X protein. The proposal includes the potential to produce large amounts of virus. This represents the most hazardous aspect of the proposal and the GMSC is satisfied that the necessary precautions have been put in place to ensure containment is maintained. Some of the proposed lentivirus may encode for proteins that are known to have neurotoxic effects and are associated with diseases of the human central nervous system. The procedures put in place will effectively prevent any chance of virus being delivered to the necessary tissues within a human in sufficient quantities to represent a hazard to human health. The FP and shRNmiRNA encoded by the other lentivirus are not known to pose any inherent risk to human health or the environment. The proposed targets to be knocked down in this study also pose no known risk. The request to derogate the Biosafety Containment level from 2 to 1 post transduction has been deemed acceptable given that the transduced cells will be washed extensively to remove any remaining viral particles and will be tested for the absence of transducing virus. It has been made clear that human iPSC cell lines that have been generated using lenti- or retrovirus methods tested negative for the absence of virus at least. These cells will always be tested for the absence of infectious particles before being can be used as if they only required Biosafety Containment level 1.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 3043/15.1

Date Ackn'd | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4 |
------------|-------------------|-------|------------------|-----------------------|
26/02/2015  | Generation, production and utilisation of lentiviral particles for delivery of | Class 2 | < 1 Litre |
The purpose of this project is to use lentivirus to deliver programmable nucleases, specifically zinc finger nucleases and Cas9/CRISPR, to disrupt gene function in mammalian cultured cells. The primary aim is to use gene targeting to validate novel targets for drug discovery and to set up disease relevant model systems in vitro.

Recipient or parental organism

Rodent Origin: Immortalised cell lines.
Human Origin: HEK293T, immortalised cell lines and induced pluripotent stem cells

Host/vector system

Lentivirus vectors: The four plasmids that will be used will be a modified version of pRRL, pMDLgp-RRE, pRSV-REV and PMD2-VSVG.env. The latter three are required for the production of the SIN replication defective lentiviral particles in HEK293T cells. The target shRNA/miRNA or gene will be inserted into the modified pRRL vector. This modified vector will have the following safety features: a deletion in the 3’LTR making it SIN in the infected cells, the packaging sequence is only found in the pRRL with the packaging constructs being provided by the remaining non-LTR containing plasmids. Lentivirus vectors: pMDLgp-RRE, pRSV-REV and PMD2-VSVG.env. The Tat promoter has been replaced by Polymerase Type 2 promoter, making them replication incompetent. The inclusion of these features ensures that the production of these SIN replication defective lentiviral particles only occurs in the transfected HEK293T. Outside of this setting, viral replication cannot take place and so pose a minimal risk to both humans and the environment.

Origin & function

The proposal involves the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site. These constructs will contain a combination of programmable nuclease plus accessory sequences and a fluorescent protein as lineage marker. In some cases constructs will contain a combination of programmable nuclease plus accessory sequences and a selectable marker giving rise to a selectable trait in transduced cell lines. Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the cleavage site or by recombination with user-supplied synthetic vector containing sequences complementary to the target gene. Insertion of the sequences into the third generation lentiviral vector on site will be achieved by either conventional cloning or Recombineering. Lentiviral particles will either be purchased ready for use or produced in-house using the third generation system described below. Nuclease encoding lentivirus may be used in isolation or co-transduced with lentivirus encoding a targeting construct designed to recombine and insert defined sequences into the target gene.
The SIN-RD (Self-Inactivating-Replication Deficient) lentiviral particles will contain a combination of the following sequences:

1. Programmable nucleases (Zinc fingers and Cas9/CRISPR) designed to cause double stranded breaks in particular endogenous mammalian targets (Mouse, Rat, Human).

2. Fluorescent protein markers that are known to be benign in terms of their effects when expressed in mammalian cells but are essential for visualisation of these cells. When these in addition are fused to a subcellular marker gene, subcellular visualisation of a particular process can be visualised.

3. Selectable marker for conferring a selectable trait to mammalian cells in vitro. Exposure to the relevant compound will result in survival of cells containing the viral vector (positive selection) or elimination of cells containing the viral vector (negative selection).

A. Programmable nucleases: zinc finger nucleases and Cas9/CRISPR

Zinc finger nucleases and Cas9/CRISPR are both programmable nucleases systems that are designed to cause cleavage of a pre-determined DNA sequence. Zinc fingers are proteins of mammalian origin comprising a DNA binding domain which confers sequence specificity and a nuclease domain which cleaves the target sequence subsequent to precise binding. Cas9/CRISPR is a bacterial protein/RNA complex that contains a nuclease domain and an RNA-binding domain; the sequence specificity of Cas9 is determined by the sequence of the RNA (referred to as the guide RNA) with which it is complexed. To effect cleavage the guide RNA has to be supplied by co-expression with Cas9.

Zinc fingers or Cas9 protein will be directed against genes of therapeutic interest identified by literature reports or by in-house screens. Since the purpose of programmable nucleases is to cause mutations in defined DNA sequences the primary hazard is that such a mutation would lead to deleterious cellular effects. The genes/proteins of interest that will be targeted do not and will not include any growth factors (which does not include trophic factors), confirmed oncogenes, tumour suppressors or immunomodulators. In isolation these sequences are unlikely to pose any safety risks for the environment or human health.

B. Fluorescent protein tag proteins:

Genetically encoded Fluorescent proteins, such as green fluorescent protein (GFP), have been widely used as a reporter for biological research over the past decades. Their exogenous expression is not associated with any cytotoxic, immunogenic or oncogenic response. A large selection of different FP constructs have been and continue to be generated, which differ in their optical and stability properties. A current non-exhaustive list of such FPs that will be used include, GFP, GFPem, YFP, DsRed, mCherry, CFP. Further variants that may be used can be found here (http://www.einstein.yu.edu/research/facilities/fluorescent/)

C. Selectable markers

Genes that confer resistance or sensitivity to antibiotic compounds are routinely used in mammalian cell culture. Examples include Neo (G418 resistance), Puro (puromycin resistance), Bsd (blasticidin resistance), Hyg (hygromycin resistance), Zeo (zeocin resistance), and HSVTK (gancyclovir sensitivity). All of these genes have been used extensively in mammalian cell genetics with no reported adverse effects in humans or animals.

Evaluation of foreseeable effects

Procedures

During all procedures that involve the handling/use of the viral vectors, all workers (incl. those that are not working directly with the vectors) that are present within a lab where the work is undertaken are required to wear personal protective equipment (incl. both appropriate clothing and gloves) at all times. The use of two pairs of gloves is advised so the external pair can be disposed in an autoclave bag and replaced whenever necessary (e.g. in the case of contact with the viral reagent). Face masks are also available for use, where appropriate. In addition, all workers are made aware of the nature of the viral work that is going on within the laboratory, and they have to follow appropriate procedures to ensure that there is no cross-contamination into non-viral working areas. All the workers using the viral delivery systems are experienced research scientists, and junior scientists will be closely supervised until they are competent in the handling of the viruses.

In vitro assessment

Assessing whether the genome manipulation has worked will be carried out by a combination of PCR and sequencing, and will normally take place initially in biosafety level 1 immortalised cell lines that are known not to contain lentiviral genetic material. Application of lentiviral particles will be done at Biosafety level 2. After at least 24 hour incubation and extensive washing, the cells will be treated as Biosafety level 1 (see below the justification for derogation of cells post-infection to Biosafety level 1). Once validated the same procedure will be carried out in the target cell line which will be immortalised biosafety level 1 cell lines or human induced Pluripotent stem cells (iPSCs).
In the latter case these will subsequently be differentiated into defined lineages for phenotypic analysis by manipulating the culture conditions. Where viral transduction is undertaken on a class 2 cell line the resultant material will remain under biosafety level 2 conditions. In all cases phenotypic analysis will be performed using suitable in vitro assays, including for example, RT-PCR, Western Blotting, immunocytochemistry and imaging.

Human health hazards
The methods by which the lentivirus vector is produced means that it is highly unlikely that replication competent lentivirus (RCL) can be generated even if an infection occurs in a person who is HIV-1 positive. Infection can lead to a permanent transduction of cells and the possibility of a harmful event either by transactivation or disruption of gene expression cannot be ruled out. For this reason the work with stock viral aliquots will be conducted as a class II activity. Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosal cells with modified viruses, due to the inherent properties of the viral vectors. Moreover, the genes/proteins of interest that will be targeted by shRNAs do not include any growth factors, confirmed oncogenes, cytotoxins, or immunomodulators, and none of them are of retroviral origin. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adherence to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent and being extremely sensitive to dessication.

Hazard to the Environment
The probability that active viral particles will be accidentally released into the environment is considered to be extremely low, as effective containment procedures will be adhered to. However, in the unlikely event that active vector particles do accidentally get released into the environment, the safety risk posed by such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable RCL. This scenario is, however, extremely unlikely, and even if it was to occur, it is unlikely that it would lead to any untoward effects.

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and they are, in theory, capable of transducing all dividing and non-dividing mammalian cell types. As emphasised before, the vectors cannot self-propagate after infection, and successful transduction is also critically dependent on the presence of high enough concentrations of virus particles; also viral vector stock solutions are aliquoted and stored in small volume (each tube contains at maximum 10µl at 80° so the potential for a high titre dose is reduced. The infectivity of the vectors rapidly decreases at room temperature so it is considered highly unlikely that the vectors could survive long enough in the environment to pose a risk if accidentally released.

Furthermore, lentiviral vectors have a short half-life at room temperature as their structural characteristics make them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, and this is illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA and miRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

Summary
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as Biosafety level 2, and effective containment procedures will be adhered to (see section C for details). However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems (see above discussions).
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Following transduction of cell lines that are designated as requiring Biosafety Containment Level 1 with lentivirus, we wish to be able to subsequently use these cells at Biosafety Containment Level 1. This can be justified by the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24 hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as only requiring Biosafety Containment Level 1. Sources of IPSC cells that have been shown to lack viral integration and test negative for infectious virus can be regarded as requiring biosafety level 1 containment. Such IPSC cells can also be derogate to biosafety level 1 containment once tested for the absence of infectious virus.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General cleaning procedures
Surfaces will be thoroughly cleaned with Virkon* Or Distel 10% (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all of the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Virkon* or Distel 10% before being autoclaved/incinerated.

Waste handling procedures
Solid waste will be inactivated by soaking it in a validated disinfectant solution for 24 hours, after which the material will be ‘double bagged’, autoclaved and, ultimately, incinerated offsite.

Liquid waste will be disposed of into sealed biohazard containers filled with Virkon* for incineration. It will be routine practice that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Neutralised liquid waste will be securely sealed for collection and off-site disposal by an approved agent (B60CRP) or discarded down an approved sink (430CSP).

Summary
As any active viral particles on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered very unlikely that active virus particles would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.

*Virkon
Rely+On™ ® is a multi-purpose disinfectant. It contains oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds, such as proteins, leading to widespread, irreversible damage and subsequent deactivation/destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance against over 500 strains of viruses, bacteria and fungi. Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control. Importantly, Virkon has specifically been validated for lentiviral inactivation (Antec-Biosentry).

Virkon is sold as a powder which dissolves readily in water. It is intended to be mixed with water to form a 1% solution (i.e. 10g per litre) for hard surface and equipment disinfection. 1:100 is also dilution rate advised for virucidal efficacy against HIV-1.

*DISTEL (formerly known as Trigene advance)
DISTEL is formulated as a nonmulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms, ensuring the rapid induction of cell death in treated micro-organisms. DISTEL works quicker than conventional disinfectants and achieves apoptosis (cell death) rather than merely
suspended activity as with conventional disinfectant products. DISTEL has been reported to be bactericidal, fungicidal as well as virucidal and sporicidal
(http://tristel.com/products/healthcare/laboratories/distel-high-level-laboratory-disinfectant/). It is recommended to be used at a 1:100 dilution for general purposes, 1:10
dilution for high risk disinfection

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMSC has agreed that the production, handling and use of the lentiviral siRNA stocks is a Class 2 activity. This decision has been reached because although the
lentivirus will be generated using a third generation, self-inactivating, 4 plasmid expression system, the possibility of a recombination event occurring that leads to
replication competent virus can never be discounted with this type of virus. The potential oncogenic effects of the Woodchuck Post-translational Regulatory Element have
been negated by the use of the mutant form that prevents expression of the X protein. The proposal includes the potential to produce large amounts of virus. This
represents the most hazardous aspect of the proposal and the GMSC is satisfied that the necessary precautions have been put in place to ensure containment is
maintained. The encoded Cas9 protein and guide RNA to be delivered by lentivirus in this proposal is now an established and widely used technique. Cas9 itself is not
known to have any detrimental effects on human health or the wider environment. The proteins targeted by the guide RNAs will be assessed on a case by case basis to
ensure their potential risk is minimal. The request to derogate the Biosafety Containment Level 2 from 2 to 1 post transduction has been deemed acceptable given that the
transduced cells will be washed extensively to remove any remaining viral particles and will be tested for the absence of transducing virus. It has been made clear that
human iPSC cell lines that have been generated using lentiv- or retrovirus methods must be negative for the absence of virus at least. These cells will always be tested for
the absence of infectious particles before they can be used as if they only require Biosafety Containment Level 1

Project Containment

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</tr>
</thead>
<tbody>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 3043/15.2

Date Ackn'd: 26/02/2015
CU2 Project Title: Generation and production of lentiviral particles for delivery of modifiers and gene
Class: 2
CultureVolClass2: < 1 Litre
This work will involve the use of particles of lentiviral origin to deliver modifiers of gene expression to mammalian cells. The aim is to dysregulate key disease-related pathways and then assess the effect of such manipulations on gene expression with a view to identifying novel disease-modifying targets.

Recipient or parental organism

Rodent Origin: Mouse cell lines
Human Origin: immortalised cell lines and induced pluripotent stem cells

Host/vector system

lentivirus vectors: The four plasmids that will be used will be modified versions of pRRI, pMDLgp-RRE, pRSV-REV and PMD2-VSVG.env. The latter three are required for the production of the SIN replication defective lentiviral particles in HEK293T cells. The target shRNNmiRNA or gene will be inserted into the modified pRRL vector. This modified vector will have the following safety features: a deletion in the 3'LTR making it SIN in the infected cells, the packaging sequence is only found in the pRRL vector. This modified vector will have the following safety features: a deletion in the 3'LTR making it SIN in the infected cells, the packaging sequence is only found in the pRRL vector. The Tat promoter has been replaced by Polymerase Type 2 promoter, making them replication incompetent. The inclusion of these features ensures that that the production of these SIN replication defective lentiviral particles only occurs in the transfected HEK293T. Outside of this setting, viral replication cannot take place and so pose a minimal risk to both humans and the environment.

Origin & function

The SIN-RD lentiviral particles will contain the following sequences:

Health and Safety Executive
1. shRNNmiRNA directed towards the knock down of the expression of a particular endogenous mammalian target (Mouse, Rat, Human). These de novo derived sequences are not derived from an organism.
2. Fluorescent protein marker which are known to be benign in terms of their effects when expressed in mammalian cells but are essential for visualization of these cells. When these in addition are fused to a subcellular marker gene, subcellular visualization of a particular process can be visualized.

3. Cre recombinase which catalyzes excision of sequences flanked by its recognition site, loxP.

4. Fluorescent marker protein fused to a ribosomal subunit to enable visualization of cells where vitally delivered gene expression is taking place and immunoprecipitation of ribosomes from such cells.

Methods

This proposal involves the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site. These constructs will contain a combination of shRNAmiRNA and a fluorescent protein as lineage marker. In some cases constructs will contain a combination of shRNAmiRNA and Cre recombinase giving rise to a pre-determined deletion event in transduced cell lines. Insertion of the sequences into the third generation lentiviral vector on site will be achieved by either conventional cloning or Recombineering. Lentiviral particles will either be purchased ready for use or produced in-house using the third generation system described below.

Lentiviral production - Uney laboratory

Some of the lentiviral vectors will be produced on site whilst others will be obtained from the Uney laboratory, which is based at the University of Bristol. The lentiviral vectors provided by the Uney laboratory will be stock solution containing up to 1x10^10 transducing units (TU) l ml of vector in TSSM buffer (20 mM TRIS, 100 mM NaCl, 10 mg/mL sucrose, 10 mg/ml mannitol), and will be essentially free of the HEK293T helper cell line used to expand the viral titre. Each purified and concentrated aliquot of lentiviral vector solution will comprise of small volumes (up to 10 1-11) containing not more than 1x10^8 viral particles. The material is normally shipped frozen and stored within manufacturer designed racks in a _80°C freezer.

Lentiviral production - on site 860CRP or 430CSP

Lentiviral production will also be carried out on-site using HEK293T cells and will be carried out in a class 2 microbiology safety cabinet within a dedicated Biosafety level 2 laboratory. The HEK293T cells used for lentiviral production will be seeded in filter containing screw cap culture flasks in order to minimize the risk of spills and aerosol mediated viral spread. For this the modified pRRI plasmids together with three additional separate plasmids (pMDLgp-RRE, pRSV-REV and PMD2-VSVG.env) will be transfected using a transfection reagent (e.g. Polyethylenimine (PEI), lipofectamine) into HEK293T packaging line. After 6-12 hours, the medium is replaced with fresh serum-free medium (e.g. Neurobasal medium with 2% B-27 supplement and 1X PeniStrep) and grown for an additional 72-96 hours. After 24-48 hours, so as 10 ensure the viability of the transfected HEK293T cells, medium from the cells is collected into screw cap tubes and stored at 40C and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the first harvest tube. After lentivirus collection has been completed, the cells in the flasks will be treated with a validated anti-microbial agent (e.g. DISTEL 10% or another equivalent product) for at least 24 hours. liquid waste will be disposed off-site via an approved waste disposal agent (B60CRP) or via a designated sink (430CSP) after at least 24 hours neutralisation with 10% DISTEL or equivalent. Solid material will be autoclaved on-site and then collected for incineration by an approved waste disposal agent.

Still within a Class 2 microbiology safety cabinet in a Biosafety level 2 laboratory, the harvested supernatants will be filtered using a 0.45um filter before being either aliquoted or further purified by chromatography and or concentrated by centrifugation. The lentiviral particles may need to be purified by either chromatography (e.g. ViraBindTlo1) and eluted in a high salt solution (50 mM Tris, pH 7.5, 5 mM MgCl2, 2 M NaCl) and or concentrated by ultrafiltration (e.g. lentiSelect™). For certain application where highly concentrated, highly purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimise the possible risk of accidental viral
contamination and spread due to the need for an ultracentrifuge, all loading and unloading will be done in the Class 2 microbiology safety cabinet. Once the sucrose cushions have been added to the centrifuge tubes, they will be transferred into the Class 2 microbiology safety cabinet. The unpurified or chromatography purified lentiviral particles will then be added to the sucrose cushion. The individually capped tubes will then be transferred to their respective bucket holders and the bucket sealed using their respective screw caps and marked as containing virus. The bucket will be transferred to a weighing scale and a counterpart bucket and tubes with water (and without lentivirus) will be prepared that is weight matched. The balanced buckets will then be transferred to the rotor before being placed within the centrifuge chamber. The ultracentrifuge will be run at 70000g for 2 hours at 20°C. Upon return of the buckets to the Class 2 microbiology safety cabinet, the supernatant will be removed from the tubes before transfer of the resuspended virus pellet into individual screw cap cryovials. As the greatest risk for contamination occurs within the centrifugation buckets themselves during transport to and from the weigh scales and during ultracentrifuge, not only will the centrifuge tubes but also the rotor be decontaminated with DISTEL 10% before removal from the Level 2 laboratory. Optionally the centrifuge rotor can be autoclaved at 121°C. In any case, at no point will concentrated virus stock be exposed to outside of the class 2 hood environment. All supernatants will be treated as above. The lentiviral titre will be determined in terms of the number of viral particles (VP) or Transduction units (TU) per ml. The number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based enrichment assay (e.g. QuickTitre™ Quantification Kit) or a p24 ELISA assay (e.g. QuickTitreTN Lentivirus Titre kit) will be chosen. The VP quantification methods tend to overestimate the TU by 10-1000 fold. Thus, the TU 1 ml will in certain instances also be determined. For this, a titration range of lentiviruses expressing a fluorescent tag, serial dilution will be prepared and added to a 24 well cluster plate or T25 Te-flasks containing HEK293T for 48 hours. The percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using FACS analyser. The biological titre (TU/ml) according to the following formula: TUIIJI = (P x N x 1100 x V) x 1/DF, where? = % GFP+ cells, N = number of cells at time of transduction = 10[5], V = volume of dilution added to each well and DF = dilution factor = 1 (undiluted), 10-1 (diluted 1/10), 10-2 (diluted 1/100), and so on. Aliquots with not more than 1x10B viral per vial are subsequently stored at -80°C in individual screw capped cryovials. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to, inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats.

Transgene: proteins to be overexpressed

Additional hazards could also arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.

A. Fluorescent protein tag proteins:

The green fluorescent protein (GFP) is a protein composed of 238 amino acid residues (26.9 kDa) that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. GFP traditionally refers to the protein first isolated from the jellyfish Aequorea victoria, which has a major excitation peak at a wavelength of 395 nm and a minor one at 480 nm. Its emission peak is at 509 nm, which is in the lower green portion of the visible spectrum. EGFP is a variant of GFP containing two point mutations; S65T and F64L. These mutations dramatically improve the spectral characteristics of GFP, resulting in increased fluorescence, photostability, and a shift of the major excitation peak to 488 nm, with the peak emission kept at 509 nm. This matches the spectral characteristics of commonly available FITC filter sets and also improves folding efficiency at 37 °C thereby allowing practical use in mammalian cells. Genetically encoded fluorescent proteins such as green fluorescent protein have been widely used as a reporter for biological research over the past decades. Their exogenous expression is not associated with any cytotoxic, immunogenic or oncogenic response.
B. ere recombinase

Gre Recombinase is a tyrosine recombinase enzyme derived from the P1 Bacteriophage. The enzyme uses a topoisomerase I like mechanism to carry out site specific recombination events. The enzyme (3SkOa) is a member of the Integrase family of site specific recombinase and it is known to catalyse the site specific recombination event between two DNA recognition sites (loxP sites). This 34 base pair (bp) 10xP recognition site consists of two 13bp palindromic sequences which flank an 8bp spacer region. Two separate DNA species both containing lox? sites can undergo fusion as the result of Gre mediated recombination. DNA sequences found between two 10xP sites are said to be "10xed". The enzyme plays important roles in the life cycle of the P1 Bacteriophage such as cyclization of the linear genome and resolution of ~imeric chromosomes that form after DNA replication.

ere recombinase is a widely used tool in the field of molecular biology. The enzyme's unique and specific recombination system is exploited to manipulate genes and chromosomes in a huge range of research, such as gene knock out or knock in studies. The enzyme's ability to operate efficiently in a wide range of cellular environments (including mammals, plants, bacteria, and yeast) enables the ere-Lox recombination system to be used in a vast number of organisms, making it a particularly useful tool in scientific research.

The simplicity and robustness of the Gre-loxP systems has enabled scientists to exploit the Gre enzyme in order to manipulate DNA both in vivo and ex vitro. As the enzyme has a specific 34bp DNA substrate the genome of the organism would have to be 1018bp in length for there to be a likely occurrence of a loxP site. As mammalian genomes are on average in the region of 3x10e9 bp there is a very low chance of finding an endogenous 10xP site. For ere to be functional in a foreign host, exogenous loxP sites must be engineered. This allows precise control over the activity of the ere enzyme in test organisms.

ere recombinase is not known to cause any diseases in humans or animals.

C. shRNA: endogenous proteins to be depleted

RNA interference (siRNA)

RNA interference (siRNA) is an antisense technology that exploits a normal cellular antiviral response that acts to inhibit viral protein synthesis through the production of short hairpin RNAs that bind and target viral double-stranded RNA (dsRNA) molecules for degradation. The shRNA molecules are processed by cellular enzymatic pathways to produce small inhibitory RNA (siRNA) species, and it is the latter RNA molecules that bind and target complementary RNA sequences for degradation by the RNA-induced silencing complex (RISC). With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be 'knocked-down', in order to study their functions.

The effects of the siRNA species that are generated in these experimental systems may have broader effects on the cell than just modulating the expression of a particular gene/protein. There may be sequences within a specific siRNA molecule that are homologous to other coding sequences within the mammalian genome that are not necessarily linked or closely related to the intended target it is, therefore, theoretically possible that a specific siRNA may knock down the expression of genes other than the intended target one, and for this reason, the siRNA systems have be designed carefully to minimise the likelihood that there will be unwanted or potentially adverse effects arising from a non-target gene being inadvertently targeted. This is done by screening designed siRNA sequences against databases of known mammalian gene/JmRNA sequences.

RNA interference may also have deleterious effects upon cellular metabolism due to the triggering of antiviral responses. It has been shown that siRNA molecules (even if less than 30nJ in length) can trigger dsRNA antiviral responses. Such responses not only lead to the degradation of dsRNA molecules, but also results in interferon production, which, in turn, leads to inflammation and the non-specific inhibition of protein synthesis.

In some of the lentiviral vectors that will be used in the proposed studies the eukaryotic U6 Polymerase III promoter
will be used to drive the expression of specific shRNAs of interest. The U6 promoter is well suited to drive the expression of shRNAs as it possesses the following favourable characteristics: (i) it initiates from position +1 of the transcripts, and (ii) it yields transcripts that do not terminate with a poly-A tail but with a series of four to five thymidine residues, which results in a series of 3' U residues leading to a fully functional shRNA sequence. Indeed, the structure of the transcribed product closely resembles synthetic double-stranded siRNAs, except for the fact that the two strands are linked by a spacer sequence. This system has been used to successfully inhibit gene expression in mammalian cell lines, with efficiencies comparable with that of synthetic siRNA.

Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. These vectors will be employed as negative controls to demonstrate that any observed effects are due to the knockdown of a specific mRNA protein of interest, rather than being due to any non-specific effects that the delivery of the viral vectors might cause. This sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of mouse, rat or human origin. It will only serve as the negative control for the vector producing shRNA. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health.

**Evaluation of foreseeable effects**

**Procedures**

During all procedures that involve the handling/use of the viral vectors, all workers (including those that are not working directly with the vectors) that are present within a lab where the work is undertaken are required to wear personal protective equipment (including both appropriate clothing and gloves) at all times. The use of two pairs of gloves is advised so the external pair may be disposed in an autoclave bag and replaced whenever necessary (e.g. in the case of contact with the viral reagent). Face masks are also available for use, where appropriate. In addition, all workers are made aware of the nature of the viral work that is going on within the laboratory, and they have to follow appropriate procedures to ensure that there is no cross-contamination into non-viral working areas. All the workers using the viral delivery systems are experienced research scientists, and junior scientists will be closely supervised until they are competent in the handling of the viruses.

**In vitro assessment**

Assessing whether the gene modulation has worked will be carried out by a combination of PCR and fluorescence microscopy, and will normally take place initially in biosafety level 1 immortalised cell lines that are known not to contain lentiviral genetic material. Application of lentiviral particles will be done at Biosafety level 2. After at least 24 hour incubation and extensive washing, the cells will be treated as Biosafety level 1 (see below the justification for removal of cells post-infection to Biosafety Level 1). Once validated the same procedure will be carried out in the target cells. Where viral transduction is undertaken on a class 2 cell line the resultant material will remain under biosafety level 2 conditions. In all cases phenotypic analysis will be performed using suitable in vitro assays, including for example, RT-PCR, Western Blotting, immunocytochemistry and imaging.

**In vitro studies**

Transduction of cells (e.g. human derived iPSC neurons, mouse and human immortalised cell lines) with the lentiviral vectors will be carried out in a Class 2 microbiology safety cabinet within a dedicated Biosafety level 2 laboratory. In the in vitro studies, the TUs, and consequently volumes added, will be dependent on the number of cells in the tissue culture dish/flask to be infected. However, as the maximum number of cells to be handled per experiment is around 1 x 10^8, not more than 1 x 10^8 viral particles per experiment will be used. None of these cells will be cultivated in the filter screw cap flasks used for HEK293T mediated lentiviral production. This is to ensure that, in particular, the HEK293T cells used for lentiviral production are not inadvertently used for lentiviral assessment! Any plastic ware or solutions that are used to handle the transduced cells will be chemically inactivated in the Biosafety level 2 laboratory with a validated anti-microbial agent (e.g. DISTEL 10% or another equivalent product) for at least 24 hours. Liquid waste will be disposed of off-site via an approved waste disposal agent (B60CRP) or via a designated sink (430eSP).
Solid material will be autoclaved on-site and then collected for incineration by an approved waste disposal agent. Once the medium containing the lentiviral particles has been removed and the cells (e.g. mouse NIH/3T3, human HEK293T) have been extensively washed, which all is done in a Class 2 microbiology safety cabinet, the cells can be used following Biosafety level 1 guidelines, as no virus will be present and the latent virus in the transduced cells is replication incompetent, unless the cell line was Originally classified as biosafety level 2 in which case the material will continue to be treated as such. A viral titre determination assay may on occasion be used to verify the absence of virus in the medium from transduced cell lines. This will be performed by PCR using oligonucleotides specific to the conserved regions within the viral genome.

An additional level of safety and screening procedure for the handling human iPSC cells will be applied for the following reason. The procedure for the generation of iPSC neurons was originally developed in 2006 by Yamanaka allows for the reprogramming of adult differentiated cells into stem cells through the introduction of four transcription factors using replication incompetent retrovirus. Since then the biosafety level of the retroviral constructs has been improved, such that both retroviral and non-viral methods of reprogramming have been reported. Even assuming that the source of our human iPSC cells resulted from work using first or second generation replication incompetent lentiviral particles, the risk of producing active lentivirus from the additional exposure to a third generation replication incompetent virus is considered negligible. Nevertheless, a number of precautionary measures will be implemented for use of these cells. Any new human iPSC line that is brought onto our site and was generated using SIN-RD lentiviruses or retroviruses (preference will be given to those lines for which there is documentation from the provider that no viral particles are being made and released) will be initially cultured at Biosafety Level 2 and tested by titre assessment to confirm that no virus is being generated. Further, after the removal of our SIN-RD lentivirus and extensive washing, the cells will be incubated in fresh medium and will be maintained at Biosafety Level 2 safety for at least 24 hours. These cells can then only be treated as Biosafely Level 1, once a virus titre assay has confirmed that no virus is being released into the medium.

Human health hazards

The method by which the lentivirus vector is produced means that it is highly unlikely that replication competent lentivirus (RCL) can be generated even if an infection occurs in a person who is HIV-1 positive. Infection can lead to a permanent transduction of cells and the possibility of a harmful event either by transactivation or disruption of gene expression cannot be ruled out. For this reason the work with stock viral aliquots will be conducted as a class II activity. Oral ingestion will be prevented by standard laboratory safely practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the genes/proteins of interest that will be targeted by shRNAs do not include any growth factors, confirmed oncogenes, cytotoxins, or immunomodulators, and none of them are of retroviral origin. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent and being extremely sensitive to desiccation.

Hazard to the Environment

The probability that active viral particles will be accidentally released into the environment is considered to be extremely low, as effective containment procedures will be adhered to. However, in the unlikely event that active viral
vector particles do accidentally get released into the environment. The safety risk posed by such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable RCL. This scenario is, however, extremely unlikely, and even if it were to occur, it is unlikely that it would lead to any untoward effects. The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and they are, in theory, capable of transducing all dividing and non-dividing mammalian cell types. As emphasised before, the vectors cannot self-propagate after infection, and successful transduction is also critically dependent on the presence of high enough concentrations of virus particles; also viral vector stock solutions are aliquoted and stored in small volumes (each tube contains at maximum 10^11) at -80°C so the potential for a high titre dose is reduced. The infectivity of the vectors rapidly decreases at room temperature so it is considered highly unlikely that the vectors could survive long enough in the environment to pose a risk if accidentally released. Furthermore, lentiviral vectors have a short half-life at room temperature as their structural characteristics make them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, and this is illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media. There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. siRNA and miRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen. Summary

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to (see section C for details). However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems (see above discussions).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Following transduction of cell lines that are designated as requiring Biosafety Containment level 1 with lentivirus, we wish to be able to subsequently use these cells at Biosafety Containment level 1. This can be justified by the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24 hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as only requiring Biosafety Containment Level 1. Sources of iPSC cells that have been shown to lack viral integration and test negative for infectious virus can be regarded as requiring biosafety level 1 containment. Such iPSC cells can also be derogated to biosafety level 1 containment once tested for the absence of infectious virus.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
General cleaning procedures
Surfaces will be thoroughly cleaned with Virkon® or Distel 10%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all of the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Virkon® or Distel 10% before being autoclaved/incinerated.

Waste handling procedures
Solid waste will be neutralised by soaking it in a validated disinfectant solution, after which the material will be 'double bagged', autoclaved and, ultimately, incinerated. Liquid waste will be disposed of into sealed biohazard containers filled with Virkon® for incineration. It will be routine practice that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Neutralised liquid waste will be securely sealed for collection and off-site disposal by an approved agent (B60CRP) or discarded down an approved sink (430eSP).

Summary
As any active viral particles on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered very unlikely that active virus particles would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.

*Virkon
Rely+OnTM Virkon® is a multi-purpose disinfectant. It contains oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds, such as proteins, leading to widespread, irreversible damage and subsequent deactivation/destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance against over 500 strains of viruses, bacteria and fungi, Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control. Importantly, Virkon has specifically been validated for lentiviral inactivation (Antec-Biosentry).

Virkon is sold as a powder which dissolves readily in water. It is intended to be mixed with water to form a 1% solution (i.e. 10 g per litre) for hard surface and equipment disinfection. 1:100 is also the dilution rate advised for virucidal efficacy against HIV-1.

DISTEL (formerly known as Trigene advance)
DISTEL is formulated as a nanoemulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms, ensuring the rapid induction of cell death in treated micro-organisms. DISTEL works quicker than conventional high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products. DISTEL has been reported to be bactericidal, fungicidal as well as virucidal and sporicidal (http://www.tristel.com/products/healthcarelaboratories/Distel-high-levellaboratory-disinfectantU). It is recommended to be used at a 1:100 dilution for general purposes, 1:10 dilution for high risk disinfection.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment Y
The GMSC has agreed that the production, handling and use of the lentiviral siRNA slacks is a Class 2 activity. This decision has been reached because although the lentivirus will be generated using a third generation, self-inactivating, 4 plasmid expression system, the possibility of a recombination event occurring that leads to replication competent virus can never be discounted with this type of virus. The potential oncogenic effects of the Woodchuck Post-translational Regulatory Element have been negated by the use of the mutant form that prevents expression of the X protein. The proposal includes the potential to produce large amounts of virus. This represents the most hazardous aspect of the proposal and the GMSC is satisfied that the necessary precautions have been put in place to ensure containment is maintained. The encoded fusion-protein to be delivered by lentivirus in this proposal has been used extensively in the literature and is not known to have any detrimental effects on human health or the wider environment. The request to derogate the Biosafety Containment Level from 2 to 1 post transduction has been deemed acceptable given that the transduced cells will be washed extensively to remove any remaining viral particles and will be tested for the absence of transducing virus. It has been made clear that human iPSe cell lines that have been generated using lentiviral methods must be negative for the absence of virus at least. These cells will always be tested for the absence of infectious particles before being can be used as if they only required Biosafety Containment Level 1.

Project Containment

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Project Ref 3043/15.3

Date Ackn'd 29/07/2015

Date Project Ceased 02/12/2016

Withdrawn N

Tick if notifying a connected programme of work N

Class CultureVol Class 2 CultureVolume Class 3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N
Dysregulation of podocyte function and loss of kidney podocytes contributes to the progression of human kidney disease. An immortalised human podocyte cell line generated at Bristol University will be used to investigate the cellular pathways involved in the development of human renal disease. To better understand these cellular pathways, the expression or the activity of the gene of interest will be modulated by several methods:

1. The utilisation of tool compounds
2. The knock-down of the gene of interest by shRNA
3. The generation of knockout somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
4. The generation of reporter gene cell lines expressing a reporter gene (e.g.: fluorescence protein or enzyme) under the promoter of a gene of interest

The experiments involving the transfection of vectors such as shRNA, programmable nucleases or reporter genes will be performed by the lentivirus.

The effect of modulating the expression or the activity of a target gene of interest will be determined by the expression level of proteins, mRNA or mediators involved in the target signalling pathway.

The generation of immortalised human podocyte cell line has been performed in Doctor Richard Coward's laboratory at Bristol University. Primary human podocytes that tested negative for HIV and Hepatitis B have been immortalised by the insertion of SV40 large tumour antigen and the hTERT into the genome. These immortalised human podocytes have been genetically modified to allow the cells to proliferate at the permissive temperature of 33°C and to enter growth arrest and express markers of differentiated in vivo podocytes at the temperature of 37°C. While SV40 T antigen can transform cells and is oncogenic, the U19tsA58 variant used here is unstable at 37°C minimising the risk involved with its use.

The immortal podocyte cell line has been generated by retroviral delivery of the pZip-Neo SV(X)I shuttle vector containing SV-40 large tumour antigen and the hTERT sequences. The generation of the immortal podocytes is briefly described below:

a. Generation of the pZip-Neo SV(X)I shuttle vector
   As described by Cepko et al (1), the pZip-Neo SV(X)I shuttle vector is composed of:
   - Moloney murine leukaemia virus (M-MuLV) transcriptional unit including the long terminal repeats (L TRs) necessary
for the initiation of viral transcription and the polyadenylation of viral transcripts, as well as for integration; sequences necessary for the reverse transcription of the viral genome; sequences for the encapsidation of viral RNA; and 5' and 3' splicing signals involved in the generation of the subgenomic viral env RNA.

- DNA sequences derived from the transposon Tn5 for G418 and kanamycin resistance
- Sequence coding for simian virus 40 large tumour antigen, strain U19tsA58; The tsA58 SV40 mutation (A438V) encodes a thermolabile large tumour (T) antigen (TAG) capable of immortalization only at the permissive temperature of 33°C, becoming unstable and inactive at 37°C
- Sequence coding for hTERT, the essential catalytic subunit of the human telomerase gene. Overexpression of hTERT into the cells stabilises telomere length and therefore prevents cellular senescence.

b. Generation of retroviral particle containing the pZIP-Neo SV(X)I vector
To generate the retroviral particle containing the pZIP-Neo SV(X)I vector, the pZIP-Neo SV(X)I vector has been transfected into the packaging cell line, TEFL Y-A cell line. This cell line stably expresses M-MuLV gag-pol and env genes. The TEFLY-A packaging cell line does not produce replication-competent retroviruses (1). This cell line allows the production of retrovirus containing the pZIP-Neo SV(X)I vector but the retroviruses produced are replication-defective (replication-incompetent). The structure of the SVX genome that is transferred to recipient cells via virus infection was characterized in a random G418 resistant cell line. Southern blot analysis confirmed that these retroviruses transfer a single proviral genome of the expected structure into the infected cells genome. Supernatants from the TEFL Y-A cells containing replication-defective retroviruses were used to infect the primary human glomerular epithelial cultures.

c. Generation of immortal human podocyte cell line
The donor of the human kidney nephrectomy sample has been tested negative for HIV and Hepatitis B. Primary human kidney glomeruli were isolated by differential sieving of the tissue and cultured until epithelial outgrowths (podocytes) reached confluence. At this stage the cells were passaged and supernatants from the TEFL Y-A cells containing recombinant retroviruses were used to transduce the primary human glomerular epithelial cultures. The transduced podocyte cells contain the sequence of SV40 Large tumour antigen and the sequence of hTERT. The tsA58 SV40 mutation (A438V) encodes a thermolabile large tumour (T) antigen (TAG) capable of immortalization only at the permissive temperatures. Cells are proliferative at the permissive temperature of 33°C. Transfer to the nonpermissive temperature of 37°C results in the inactivation and degradation of large T antigen. The transgene will inactivate fully at 39.5°C but normally complete quiescence occurs at 37°C after 1-3 days. The retrovirus also conferred G418 resistance. Once these cultures reached confluence, genomic integration of the transgene was selected using G418 and a switch to 33°C culture, a permissive temperature for SV40T expression. Subcloning of these cultures produced the clonal cell population used here (the host cells). Following establishment of quiescence at 3rC, the cells then enter growth arrest and express markers of differentiated in vivo podocytes.

Bibliography

Host/vector system
To study the function of podocytes in human disease, the expression of a target of interest may be decreased in the immortalised human podocyte cell line by the transduction with shRNA or programmable nuclease sequences (CRISPR or Zinc-finger nucleases) by lentivirus. The modulation of a particular cellular pathway may be studied with the use of a reporter gene assay. In this case, the vector containing the reporter gene under the regulating sequence from the promoter of a gene of interest will be introduced by lentivirus into the immortalised human podocytes. This proposal involves both the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site. Lentiviral particles will either be purchased ready for use or produced in-house using the third generation system described below. Insertion of the shRNA, programmable nuclease sequences or the luciferase
genes under the promoter of the gene of interest into the third generation lentiviral vector system will be achieved on site by either conventional cloning or recombination-mediated genetic engineering. Both commercially acquired and in-house lentivirus will use the same vector backbones containing identical promoters. Target genes will be selected based on the needs of drug discovery projects; programmable nucleases and shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. The production of lentivirus containing the vectors of interest are described below:

1. Generation of lentivirus

Lentiviral vectors will be purchased commercially or produced in-house using the third generation system described below. Each purified and concentrated aliquot of lentiviral reagent comprises of a small volume (5 to 101FL) that contains no more than 1x10^8 viral particles.

Vector System = self-inactivating (SIN) non replication-competent HIV based lentiviral vectors

The viral vectors that will be used in the proposed studies are self-inactivating (SIN) non replication-competent HIV based lentiviral vector systems, which have been designated to produce stable gene expression in mammalian cells; and they are generated by co-transfecting HEK 293T cells with four separate plasmids. It is worth noting that HEK293T express the SV40 T antigen but the genetic material encoding for this protein is non-mobile and will therefore not become incorporated into lentivirus. Each of the plasmids used expresses a different set of genes which, when combined following co-transfection, provide the smallest possible set of essential viral genes that is still compatible with virus production. Moreover, the vectors are all vesicular stomatitis virus (VSV-G) pseudotyped lentiviral vectors, which are self-inactivating and highly unlikely to undergo recombination. Importantly, the viral vector system is inherently incapable of replication in mammalian cells. Only in a patient with HIV could any form of recombination occur, i.e. the probability of the production of replication competent lentiviral vectors (RCL) is very small.

The plasmids

Health and Safety

The sequences that will be included in the viral vectors are sub-cloned into 4 standard bacterial plasmid vectors. One of the plasmids, pRRL, will contain the transgene that codes for a programmable nuclease and accessory, or shRNA, or the reporter protein under the promoter of the gene of interest.

Plasmids containing the packaging related sequences

The three other plasmids (pMDLgp-RRE, pRSV-REV and pMD2-VSVG.env), that make up the 4 plasmid system, each contain a different set of sequences that code for proteins that are responsible for packaging the viral vectors. All the genes (gag-pol, rev and env) required for production and packaging of the full length viable viral RNA particles have been removed from the pRRL lenti-plasmids containing the transgenes of interest. The absence of the majority of the viral structural genes renders the lentiviral vectors replication-defective. The replication and capsid genes are provided in trans during the production phase: gag-pol and rev are expressed separately (pMDLgp-RRE and pRSVREV respectively), as is the VSV-G coat protein (pMD2-VSVG.env), and it is, therefore, theoretically impossible for a purified recombinant lentiviral vector to replicate. The plasmids have been engineered so as to have no common sequences that would promote recombination, and, hence, plasmid genes do not become inserted into the pRRL plasmid. There are no known cases of recombination occurring with third generation lentiviral systems. This allows for a viral particle to be produced that can enter any cell, but, once inside, it does not have the relevant genes necessary to either re-package itself or to re-generate full length viral RNA following genomic integration.

Following infection of a target cell, lentiviral vectors are self-inactivating on integration into the host genome. This is achieved because the process of reverse transcription uses the 3' long terminal repeat (3'L TR) as a template to
produce the 5’ L TR. In the pRRL lenti-plasmids, the U3 region of the 3’L TR has been deleted, resulting in deletions in both 3’ and 5’ L TRs on integration into the host genome. These deletions are designed to ensure that the viral genome cannot be released from the host genome. This system, therefore, allows for the long term expression of the gene of interest with little risk of the production of further viral particles.

Plasmids containing the transgene of interest

One of the 4 plasmids, pRRL, will contain the sequences that code for shRNA, or programmable nuclease sequence, or reporter gene sequence under the promoter of the gene of interest.

a. Vector containing shRNA

As mentioned above, pRRL plasmid will contain the sequences that code for one shRNA transcript that will target one mRNAs/protein of interest (or a scrambled shRNA in the case of negative control). The shRNA or scrambled shRNA sequences will be under the control of the Polymerase III U6 promoter. The shRNA sequences will be chosen to target a single human gene. Target genes of the shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. Additionally, the above transgene constructs contain the WPRE, cPPT, RRE, and 4’ sequences, as well as the modified L TR (see below for a description of each of the elements), and these sequences are the only other sequences with biological activity apart from the shRNA that will be present in the viral vectors. These elements are important for the expression of the inserts in the targeted cells, and they will all be stably expressed in a majority of the infected cells.

b. Vector containing CRISPR or Zinc-finger nucleases vector

Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the cleavage site or by recombination with a user-supplied synthetic vector containing sequences complementary to the target gene.

For Cas9/CRISPR, nuclease target specificity is determined by an accessory sequence, the guide RNA, which consists of a short (18 - 20 nucleotide) sequence homologous to the target gene and an additional short sequence that forms a complex with the Cas9 enzyme. In that case, the plasmid pRRL will contain the sequences that code for a Cas9/CRISPR plus accessory sequences comprising a short guide RNA that determines target specificity.

In the case of zinc finger nucleases target specificity is dependent upon the zinc finger protein sequence. The pRRL will contain in that case the sequence of zinc finger nucleases without accessory sequences.

In addition, a fluorescent protein or enzyme under the control of the Cytomegalovirus (CMV) promoter, EF1A promoter or the promoter of a gene of interest may be included. Control viral vectors that express only the fluorescent protein/enzyme and no programmable nuclease will be used in some experiments. Programmable nuclease genes will be under the control of either the CMV promoter or the EF1A promoter. For Cas9/CRISPR based constructs, the guide RNA sequences will be under the control of the Polymerase III U6 promoter.

c. Reporter gene vector: vector containing a reporter protein under the promoter of the gene of interest

Genetic reporter systems are widely used to study eukaryotic gene expression and cellular physiology. Applications include the study of receptor activity, transcription factors, intracellular signalling, mRNA processing and protein folding. For example when a particular cellular pathway is activated, it will induce the translocation of transcription factor into the nucleus. The transcription factors will then bind the regulating elements in the promoter of a gene of interest and it will induce the transcription of this gene. The reporter gene system uses this process to measure the activation of the pathway of interest.

The pRRL vector will contain the sequence coding for a reporter protein under the control of the regulating elements of the promoter of the gene of interest or the CMV or EF1A promoter. For example this reporter protein could be a fluorescent protein, an enzyme such as firefly luciferase or β-galactosidase, or a FRET-based reporter designed to detect endogenous enzymatic activity or molecular interactions. An appropriate selection marker Le.; neomycin could also be included to allow the selection of stable immortal human podocytes containing the luciferase sequence under the control of the promoter of the gene of interest.
a. Assessing efficacy of the lentivirus into a BSL 1 cell line
Assessing whether the genome manipulation or the knock-down has worked will be carried out by a combination of PCR and sequencing, and will normally take place initially in Biosafety level 1 immortalised cell lines (human HEK293T) that are known not to contain lentiviral genetic material. Application of lentiviral particles will be done at Biosafety level 2. After at least 24 hour incubation and extensive washing, the cells will treated as Biosafety level 1 (see below the justification for removal of cells post-infection to Biosafety Level 1). In order to determine whether the knock-down or the genome manipulation has worked, the levels of mRNA and protein will be assessed posttransduction, using suitable in vitro assays, including for example, RT-PCR, Western Blotting or immunocytochemistry.

b. Transduction of the lentivirus and the generation of stable expression into the immortalised human podocytes
Once validated, the same procedure will be carried out in the immortalised human podocyte cell line. As the immortalised human podocytes are maintained in a Biosafety level 2 containment, the inoculation of lentiviral particles, the transformed cells and the phenotypic analysis will also be performed in a Biosafety level 2 containment. When a lentivirus containing a programmable nuclease or the reporter gene vector will be used, a stable cell line could be established by selecting the cells in which the genome has been modified by the programmable nucleases or the integration of the reporter gene. In all cases phenotypic analysis will be performed using suitable in vitro assays, including for example, RT-PCR, Western Blotting, immunocytochemistry and imaging, or cellular bioenergy (i.e. mitochondria respiration function or glycolysis function).

Origin & function

Evaluation of foreseeable effects

* Human Health and Safety
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Executive
The immortal human cell that will be used has a low risk for human health. The expression systems and the transgenes that comprise the lentiviral vectors that will be used have also a very low risk for human health and safety, and the reasons for this are discussed below.
A) Lentivirus containing shRNA, or programmable nucleases sequence or reporter gene vectors
The expression systems and the transgenes that comprise the lentiviral vectors that will be used have a very low risk for human health and safety, and the reasons for this are discussed below.
Lentiviral Vector Expression System
Lentiviruses belong to the Retroviridae virus family, a diverse and extensive family of viruses, which are capable of infecting both human and animal species. Cellular entry of the virus involves interactions between glycoproteins contained in the virion's envelope and extracellular plasma membrane proteins on target cells. These interactions are generally specific and are believed to be the principal factor determining which species and type of cells a specific virus is capable of infecting. The binding of a viruses' envelope glycoproteins to an appropriate receptor site on a target cell triggers the fusion of the virion's envelope membrane with the plasma membrane of the target cell, which results in the delivery of the virus capsid to the intracellular space of the target cell.
As the lentiviral vector system that will be used in these studies is based on HIV-1, a theoretical risk to human health exists. However, retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airborne route. Therefore, accidental piercing of the skin or other surface tissues with virus containing objects represents the main potential route by which accidental infection could occur. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring (see below for detailed measures).
The lentiviral vector production process is thoroughly described in the section below to highlight the key safety
features of the lentiviral vectors. In all of the modified pRRL plasmids (containing shRNA, or programmable nucleases or reporter gene sequences), the sequences inserted are flanked by non-coding retroviral LTRs, and no retroviral genes are encoded on the modified pRRL plasmids. Therefore, no retroviral genes will be transferred into generated viral particles. This construct is packaged into particles using a HEK293T cell based packaging system, which requires the cotransfection of HEK293T cells with three additional separate plasmids (pMDLgp-RRE, pRSV-REV and pMD2-G). The latter 3 plasmids express the envelope protein from VSVg and the non-structural proteins of the virion, and, importantly, none of these genes will be transferred into the assembled viral vectors, since they lack the packaging signal (4'-), which is only present on the modified pRRL containing plasmids.

The Lentiviral Expression System that will be used include the following key safety features:

• All of the pRRL containing plasmids/vectors that will be used contain a deletion in the 3' LTR (LlU3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" (SIN) of the vector after transduction of target cells. Once integrated into a transected target cell, the lentiviral genome is no longer capable of producing viral genomic material than can be packaged. Moreover, deletion of enhancer and promoter elements from the 3' U3 region in the vector construct will result in a provirus that is entirely devoid of the U3 enhancer sequences, which will result in a reduction in the potential for transactivation of cellular genes due to an insertion event. Furthermore, the development of self-inactivating vectors improves the biosafety of vectors, as they are less likely to be mobilized following a superinfection with Wild-type virus (HIV).

• The number of retroviral genes that are used in the four plasmid system has been reduced to three (i.e. gag, pol, and rev).

• The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes. Pseudotyping HIV-1 vectors obviate safety concerns associated with the use of HIV-1 gp120, which has known pathogenic consequences. However, pseudotyping has a big impact on the biodistribution and tropism of viral vectors. In particular, HIV-1 gp120 restricts transduction of HIV-1 vectors to CD4+ cells, which limits its use to CD4+ cells, like T cells and macrophages. In contrast, heterologous envelopes, like VSV-G, typically broaden the tropism and allow gene transfer into a broad variety of cells: This risk will mitigated by the use of self-inactivating virus and limiting the number of viral particles that will be handled at anyone time.

• Sequences encoding the proteins required for packaging of the viral genome are separated onto three plasmids, and all of the 4 plasmids used in the system have been engineered not to contain any regions of homology with each other so as to prevent undesirable recombination events that could lead to the generation of a replication-competent lentivirus (RCL), which could potentially be harmful to humans. It is important to note that no such RCL has ever been observed despite large-scale production and testing of lentiviral vectors.

• All of the pRRL containing plasmids/vectors that will be used (see above) are devoid of all viral sequences apart from essential cis-acting sequences, including the LTRs and the packaging signal 4'. Only the pRRL plasmids will contain the packaging sequence 4'. Although the packaging plasmids used in these systems allows for the expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK293T producer cell lines, none of them contain LTRs or the 4' packaging sequence. Several of the lentiviral accessory genes (vif, vpr, vpu and net) that are dispensable for lentiviral vector production/transduction have been deleted from the packaging construct. Therefore, none of the retrovirus structural genes will actually be present in the packaged viral genome, as they will never be expressed in the transduced target cells, which means that no new RCL can be produced.

• The lentiviral particles produced in this system are replication-incompetent, only carry the sequences of interest, and no other viral species are produced.

• Expression of the gag and pol genes from pMDLgp-RRE has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. All the modified plasmid/vectors that will be used
also include RREs. Addition of the RRE in these plasmids prevents gag and pol expression in the absence of Rev, which is contained in the pRSV-REV plasmid only. The viral rev protein is provided in trans to ensure efficient nuclear export of the full-length viral RNA genomes through binding to the RRE. The Rev/RRE system is highly conserved among lentiviruses, and removal of the RRE sequence and associated splice donor/acceptor sequences results in a loss of transduction efficiency.

- Initially, the endogenous LTR was used to drive the pRRLs plasmid expression via transactivation by the tat protein, but LTR has been modified so as to increase lentiviral vector production, and also to allow lentiviral vector production to be independent of tat expression. A constitutive promoter (RSV promoter) has been placed upstream of the 5’ LTR in the pRRL plasmids to offset the requirement for Tat, which is essential for replication of wild-type HIV-1, as it is required for the efficient production of viral RNA. It is known that Tat-deleted mutants of wild-type HIV are not replication competent. Therefore, the deletion of Tat should decrease the risk of generating a putative RCL.

- Lentiviral vectors have a very low potential to cause immunogenicity.

To be noted:
The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction. In the lentiviral vector system that will be used, the WPRE (Woodchuck Post-transcriptional Regulatory Element) is used to promote mRNA export and also to stabilise transgene mRNA levels, which should give rise to increased transgene expression. However, it is important that such elements and their associated functions are carefully scrutinised as, in the case of WPRE, there have been unforeseen effects. There have been concerns surrounding the potential oncogenic activity of the native WPRE, because of the expression of an oncogenic WHV-X protein from an open reading frame found within the element. However, in the only study in which increased tumourigenesis has been demonstrated, EIAV and FIV vectors were injected in utero at very high titres. HIV vectors have been shown not to mediate similar effects. The expression of the protein X was abrogated simply by mutating the WHV-X ORF translation start site, which did not affect WPRE function, thereby, removing the oncogenic safety risks.

While the lentiviral vectors that are produced using this system contain only about 20% of the original genome of HIV-1, there is a very small risk that subsequent HIV-1 infection of cells already infected with the lentiviral genome could lead to a rare recombination event in which the transgene is transferred to a replication-competent virus. Thus, the sequences in the vector that will be expressed could potentially be transferred to surrounding cells. This event is, however, extremely unlikely to occur for a number of reasons:

1. The lentiviral vector is replication-incompetent and self-inactivating. A four plasmid HIV lentiviral system minimises the amount of viral genes expressed from one plasmid even further. Consequently, the lentivirus genome lacks critical components required for packaging and proliferation. In the case of a subsequent HIV-1 infection, following lentiviral integration, it has been previously shown that, even under permissive in vitro conditions, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host genome, although non-self-inactivating vectors can be. In the case of a worker already infected with HIV-1, the HIV genome will have already integrated into the host genome of infected cells. While it is conceivable that the lentiviral genome will infect the same cells and integrate into a location that will allow for homologous recombination with the native HIV-1 genome, the probability of that occurring is extremely small.

2. In order for the spread of the gene of interest to occur following accidental infection (assuming that this has led to viral integration), a series of unlikely events would have to occur:
   a. The worker would have to become infected with HIV-1 or to be already infected with the virus.
   b. The viral and lentiviral genomes would have to integrate into the host worker's genome in the same cells and in a position where they could interact to effect homologous recombination (point 1 above)
   c. Recombination would have to occur in just the right regions to allow for transfer of the gene of interest from the
lentivirus to the HIV-1 genome, which would also involve the transfer of the HIV-1 genes to the lentiviral genome. In this case, it is conceivable that a non-self-inactivating HIV could be generated that contained the gene of interest but not the rest of the genome it requires. The other gene products could be provided in trans from the lentiviral genome that may now contain the HIV-1 genes or from other HIV-1 integrants.

d. The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest, but again no genes necessary for subsequent replication. In order for another round of infection to occur, the process would have to begin again.

In these circumstances, the effects of lentiviral infection are likely to be minor in comparison to the effects of the HIV-1 infection, which would be required to affect the spread of the gene of interest. In addition, the scenario described is essentially equivalent to the rescue of the lentiviral genome from the host, which has already been shown not to occur.

3. It is extremely unlikely that any worker would infect themselves with a significant dose of lentivirus as the volumes that are used in transfection experiments are small (aliquots contain a maximum of 10 IU of vector solution).

4. Moreover, insertional mutagenesis into the host genome may be considered as an oncogenic risk. We cannot rule out the possibility that, when the lentiviral genome integrates into the host genome, it will not lead to the activation of an endogenous oncogene. However, all transcriptionally active long-terminal repeats (LTRs) have been removed as well as all promoter-like elements other than that required to drive expression of the transgene. This should prevent unforeseen activation of such genes. It is noted that deletion of the retroviral enhancer in self-inactivating systems reduces the risk of activation but not of disruption, and, therefore, retroviral infection might still have permanent effects upon a cell (including oncogenic effects).

Importantly, we do not consider that the use of these lentiviral vectors will result in a significantly increased risk of oncogenic activation compared to the risk possessed by any other viral delivery system. Moreover, the risk of transduction leading to tumourigenesis or other untoward harm following exposure is related in part to the titre of the viral vectors; and exposure of workers to quantities of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of lentiviral vectors.

Use of lentivirus with immortalised human podocyte cells

As outlined previously, the immortalised human podocytes contain a partial viral genome from Moloney murine leukemia virus (M-MuLV). Subsequent infection of these podocytes with other viruses could permit mobilisation of the viral genome and SV40T/hTERT transgenes contained within. However, this would require a very rare recombination event following a spontaneous double strand break at the site of M-MuLV integration. Sequence alignment between M-MuLV genomes and the lentiviral vectors pMDLgp-RRE, pRSV-REV, pMD2-VSVG-env and pRRL plamids shows no significant overlap or sequence homology that could facilitate recombination following a double strand break. Therefore the probability of recombination between these viral sequences is extremely low and the risk to human health is minimal.

Transgenes:

Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.

ShRNA

RNA interference (RNAi) is an antisense technology that exploits a normal cellular antiviral response that acts to inhibit viral protein synthesis through the production of short hairpin RNAs that bind and target viral double-stranded RNA (dsRNA) molecules for degradation. The shRNA molecules are processed by cellular enzymatic pathways to produce small inhibitory RNA (siRNA) species, and it is the latter RNA molecules that bind and target complementary RNA sequences for degradation by the RNA-induced silencing complex (RISC). With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be 'knocked-down', in order to study
their functions. Potential deleterious effects such as off-target and immunomodulatory responses can be minimised through screening designed shRNA sequences against databases of known mammalian gene/mRNA sequences during the design stage. The genes/proteins of interest that will be targeted by shRNA do not and will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. These vectors will be employed as negative controls to demonstrate that any observed effects are due to the knockdown of a specific mRNA/protein of interest, rather than being due to any non-specific effects that the delivery of the viral vectors might cause. This sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of human origin. It will only serve as the negative control for the vector producing shRNA. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health.

Programmable nucleases: zinc fingers and Cas9/CRISPR
Zinc fingers or Cas9 protein will be directed against genes of therapeutic interest identified by literature reports or by in-house screens. Since the purpose of programmable nucleases is to cause mutations in defined DNA sequences the primary hazard is that such a mutation would lead to deleterious cellular effects. The genes/proteins of interest that will be targeted do not and will not include any growth, tropic factors, confirmed oncogenes, tumour suppressors or immunomodulators. In isolation these sequences are unlikely to pose any safety risks for the environment or human health.

Green and red fluorescent proteins
The green fluorescent protein (GFP) is a protein composed of 238 amino acid residues (26.9 kDa) that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. GFP traditionally refers to the protein first isolated from the jellyfish Aequorea Victoria.
Red fluorescent protein (DsRed) and its derivatives are structurally very similar to the green fluorescent proteins but are derived from Discosoma sp. mushroom anemone.
In biological research, fluorescent proteins are frequently used as a reporter of gene expression. While most small fluorescent molecules such as FITC (fluorescein isothiocyanate) are strongly phototoxic when used in live cells, fluorescent proteins are usually much less harmful when illuminated in living cells. Fluorescent proteins are not known to cause any diseases in humans or animals and have no direct effect on cellular processes.

Enzymes
Firefly luciferase is a 61 kDa monomeric protein first isolated from Photinus pyralis. The enzyme allows the photon emission through the oxidation of the beetle luciferin in a reaction that requires ATP, Mg+ and 02. The "flash" of light from the photon emission can be measured. In biological research, the luciferase protein is frequently used as reporter of gene expression. Luciferase is not known to cause any diseases in humans or animals and has no direct effect on cellular processes. The expression of luciferase under the control of a promoter of a gene of interest is unlikely to pose any safety risks for the environment or human health.
Beta-galactosidase is a 464kDa homotetrameric protein that catalyses the hydrolysis of beta-galactosides into monosaccharides and is present in the lysosomes in mammalian cells. In biological research, this activity can be utilized as a reporter of gene expression by catalysing substrates that result in luminescent, fluorescent or coloured products. Overexpression of beta-galactosidase is associated with cellular senescence but the activity of betagalactosidase is not required for senescence and cannot initiate it. Beta-galactosidase has been safely expressed as part of a gene reporter system in transgenic rodents and is not expected to pose any safety risks for the environment or human health.

FRET-based reporters of enzymatic activity or molecular interactions
Fluorescence resonance energy transfer (FRET) functions through a proximity-based transfer of energy from one fluorophore to a closely-located second fluorophore, such that the wavelength of the emitted signal is altered. In biological research this phenomenon can be utilised to assess direct interactions of molecules such as proteins. A FRET-based system can be conceivably designed to examine activity of enzymes such as proteases or kinases based on a change in proximity of two tethered fluorophores, or protein-protein interactions using fusion proteins with fluorophores like GFP. Only benign molecules will be used in these systems to ensure that these approaches have no adverse impact on human safety or the environment.

Summary on lentivirus

The transgenes that will be expressed by the viral vectors have a low safety risk to human health. This is because accidental contamination with a vector would lead to only a small number of cells becoming infected, and it would be highly unlikely that expression in only a few cells would be sufficient to lead to a disease state. The inability of the lentiviral vectors to propagate on mammalian cells also reduces the risk.

The programmable nucleases (CRISPR or Zinc Finger) that will be employed in this viral vector system are not known to cause any relevant toxicity that might represent a safety risk to human health. The Fluorescence Proteins and the enzymes that will be employed in this viral vector system are not known to cause any relevant toxicities that might represent a safety risk to human health. For both programmable nucleases and shRNA, the genes/proteins of interest that will be targeted will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators, and none of them are or will be of retroviral origin. Therefore the risk of use will be low.

B) Immortalised human podocytes and transduced immortalised human podocytes

The immortalised human podocytes cell line described in this proposal have been immortalised by the insertion of U19tsA58-SV40T and hTERT sequences into the genome of primary human podocytes isolated from a donor that tested negative for Hepatitis Band HIV. The generation of this immortalised human podocyte cell line will not take place at Takeda Cambridge. This cell line has been generated in the laboratory of Doctor Richard Coward at Bristol University, UK. Due to the presence in the genome of the sequence coding for the SV40T antigen and the hTERT, this cell line will be maintained at the Biosafety level 2 containment.

As described above, this immortalised human podocyte cell line contains retroviral-integrated U19tsA58-SV40T and hTERT along with a partial viral genome. The tsA58 SV40 mutation (A438V) encodes a thermolabile large tumour (T) antigen (Tag) capable of immortalization only at the permissive temperatures. The U19 mutation (S152N and R154K) prevents binding to the SV40 origin of replication, imparting greater transformation efficiency and additionally preventing Tag-mediated replication of viral DNA, improving safety as the partial viral genome cannot be recovered from the integration site by U19tsA58-SV40 Tag. hTERT or telomerase reverse transcriptase, is the catalytic subunit of telomerase. Its overexpression stabilises telomere length preventing replicative senescence. These 2 elements induce immortalisation of the human podocytes. However, these cells do not contain the viral genes gag-pol and env required for virus production and therefore cannot produce viruses. We will confirm lack of gag-pol and env integration by PCR and lack of virus production in the podocytes by ensuring that supernatants from podocyte cultures do not transduce HEK293T cells.

Further modification of the immortalised human podocyte cell line may enhance the risk of a recombination of a replication-competent viral genome in the cells. Application to these cells of additional retrovirus or lentivirus particles could result in liberation of replication-competent retroviruses by genetic recombination events. This risk will be minimised by confirming that the viral genes gag-pol and env are not present in the genome of the immortalised human podocytes before the application of the lentivirus on the cells. Furthermore, this risk can be minimised by ensuring that any lentivirus (subclasses of retrovirus) used in experiments with the host cells does not contain gagpol, env or any other significant retroviral genes involved in packaging and replication.
The major risk factor associated with the utilisation of this cell line or the genetically modified podocyte cells would be accidental percutaneous inoculation of a worker. As described above, the immortalised podocyte cells will have to be in an environment at 33 degree Celsius to be able to proliferate. In the case of percutaneous inoculation of a worker it is unlikely that the cells can proliferate.

The SV40 large T antigen is capable of transforcting mammalian cells. However the U19tsA58 variant used here has two important safety features - (i) the U19 mutation prevents binding to SV40 origin of replication, preventing release of the integrated viral genome in the absence of further modifications or viral infections; (ii) the tsA58 mutation renders the TAg unstable at 37OC and thus would be incapable of transforming cells following infection of a human or animal host. Due to the integration of M-MuLV long terminal repeats (LTRs) and the presence of SV40 origin of replication in these podocytes, expression of wild-type SV40 TAg would result in liberation of the integrated MuLV sequences including U19tsA58-SV40T and hTERT. This risk will be avoided by ensuring that no SV40 sequence is introduced into the immortalised podocytes.

Another risk factor associated to the accidental percutaneous inoculation of a worker with immortalised human podocytes or transduced podocytes will be the potential recombination of the retroviral genome present in the podocytes and retroviral genome present in the accidentally inoculated worker. Even in that case, a series of unlikely events would have to occur to:

a. The worker would have to become infected with HIV-1 or to be already infected with the virus.

b. The viral and retroviral genomes would have to integrate into the immortal human podocyte's genome in a position where they could interact to effect homologous recombination.

c. Recombination would have to occur in precise regions to allow for transfer of the gene of interest (hTERT, SV40T antigen, shRNA, programmable nucleases, or reporter gene sequence) from the lentivirus to the HIV-1 genome, which would also involve the transfer of the HIV-1 genes to the lentiviral genome. In this case, it is conceivable that a non-self-inactivating HIV could be generated that contained the gene of interest but not the rest of the genome it requires. The other gene products could be provided in trans from the lentiviral genome that may now contain the HIV-1 genes or from other HIV-1 integrants.

d. The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest, but again no genes necessary for subsequent replication. In order for another round of infection to occur, the process would have to begin again.

Although the risk for human health is low, all measures will be in place to minimize the risk of human health to avoid any accidental inoculation of a worker (see measures below).

Even if the events are unlikely, the immortalised human podocyte cell line and the transduced podocytes will be maintained at Biosafety level 2 containment. Therefore all measures will be in place to minimize the risk to human health.

Human health hazards

Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the programmable nucleases and shRNA that will be expressed or targeted will not be designed to disrupt any growth factors, trophic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.
The most likely route of accidental infection with a lentivirus or the podocyte cell line will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures (for the detailed measures see Measures used in the Biosafety level 2 containment). Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent. Infection of the community at large by the immortalised podocyte cells is highly unlikely due to the impossibility of the cells to survive outside of a laboratory environment.

* Environment and Activity Considerations
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to (see Measures used in the Biosafety level 2 containment). However, in the unlikely event that active lentiviral vector particles do accidently get released into the environment, the safety risk posed by such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable replication-competent lentivirus. However, this scenario is extremely unlikely and even if it was to occur, it is unlikely that it would lead to any untoward effects. As the HIV virus is a human pathogen infection of another species would not be expected to allow any form of recombination event leading to a viable transmissible entity and so the risk from this scenario is considered to be negligible. Rodents, such as Wild-type mice and rats, cannot support replication of infectious HIV-1 (2). As a result, the potential for shedding of replication-competent lentiviruses from such animals is very low (even if they were present in the original vector inoculum).

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and in theory they are capable of transducing all dividing and non-dividing mammalian cell types. As emphasised before, the vectors cannot self-propagate after infection, and successful transduction is critically dependent on the presence of high enough concentrations of virus particles. Viral vector stock solutions will be managed in a way that will prevent contaminations with relatively high viral titres. After production, the vectors are aliquoted and stored in screw capped cryovials at ao° c. Taking into account that the infectivity of the vectors rapidly decreases at room temperature, it is considered highly unlikely that the vectors could survive in the long term after being accidentally released into the environment. Lentiviral vectors have a short half-life at room temperature due to their structural characteristics making them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, as illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

The risk to the environment following accidental release of the immortalised human podocytes would be negligible since the cells are incapable of surviving outside of laboratory conditions. If live podocyte cells were to directly inoculate animals, they could theoretically survive but would be quickly cleared by the immune system, as previously mentioned would be unproliferative at 37°C body temperatures and do not produce active viruses (this will be confirmed). An additional risk is associated to the accidental inoculation of rodents already infected by the Moloney murine leukaemia virus. In that case, the recombination between the genome of the Moloney murine leukaemia virus and the genome of the immortalised human podocytes could occur leading to the production of M-MuLV viruses containing the sequences of SV40 large tumour antigen and hTERT. Even if the SV40 variant present in the
Podocytes were liberated into replication-competent M-MuLV following recombination in an already-infected rodent, the tsA58 variant is incapable of transforming cells in an infected organism due to instability at 37°C body temperatures. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures and ensuring that all potentially contaminated material is totally inactivated before disposal.

* Containment Measures

The work outlined above has been classified as a Biosafety level 2 activity, even though the risk of pathogenicity to the end worker is considered to be very low. In all cases, HSE prescribed Biosafety 2 conditions should be adhered to when handling the immortalised human podocyte cells, generating the viral vectors in HEK293T cells, as well as their subsequent storage at -80°C. For subsequent in vitro transduction of the cells types mentioned in this proposal (e.g. human HEK293T, immortalised human podocyte cells) this will be performed under Biosafety level 2 conditions. The human transduced HEK293T used to assess the efficacy of the vectors will only be converted to Biosafety level 1 once the medium containing the virus has been removed and the infected cells have been extensively washed. The immortalised human podocytes transduced by lentivirus will be maintained under Biosafety level 2.

Procedures

During all procedures that involve the handling/use of the viral vectors or cells, all workers (incl. those that are not working directly with the vectors) that are present within a lab where the work is undertaken are required to wear personal protective equipment (at all times. The use of two pairs of gloves is advised so the external pair can be disposed in an autoclave bag and replaced whenever necessary (e.g. in the case of contact with the viral reagent). Face masks are also available for use, where appropriate. In addition, all workers are made aware of the nature of the viral work that is going on within the laboratory, and they have to follow appropriate procedures to ensure that there is no cross-contamination into non-viral working areas. All the workers using the viral delivery systems are experienced research scientists, and junior scientists will be closely supervised until they are competent in the handling of the viruses.

Lentiviral production

Production of lentiviruses will also be carried out on-site using a modified procedure based on Bukovsky et al (3). Lentiviral production will only be carried out in HEK293T cells in a Biosafety level 2 cabinet within a dedicated Biosafety level 2 laboratory. The HEK293T cells used for lentiviral production will be seeded in filter containing screw cap culture flasks in order to minimize the risk of spills and/or aerosol mediated viral spread. For this the modified pRRL plasmids together with three additional separate plasmids (pMDLgp-RRE, pRSV-REV and pMD2-VSVG.env) will be transfected using a transfection reagent (e.g. Polyethylenimine (PEI), Lipofectamine) into HEK293T packaging line. After 6-12 hours, the medium is replaced with fresh serum-free medium and grown for an additional 72-96 hours. After 24-48 hours, so as to ensure the viability of the transfected HEK293T cells, medium from the cells is collected into screw cap tube and stored at 4°C and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the first harvest tube. After lentivirus collection has been completed, the cells and the flasks will be decontaminated and disposed of as detailed later (see Waste Handling).

Still within a Biosafety level 2 Safety Cabinet in a Biosafety level 2 Biosafety laboratory, the harvested supernatants will be filtered using a 0.45 μm filter before either aliquotted or further purified by chromatography and/or concentrated by centrifugation. The lentiviral particles may need to be purified by either chromatography (e.g. ViraBind™) and eluted in a high salt solution (50 mM Tris, pH 7.5, 5 mM MgCl2, 2 M NaCl) and/or concentrated by ultrafiltration (e.g. LentiSelectTM). For certain application where highly concentrated, highly purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimise the possible risk of accidental viral contamination and spread due to the need for an ultracentrifuge, all loading and unloading will be done in the Biosafety level 2 Safety Cabinet. Once the sucrose cushions have been added to the centrifuge tubes, they will be
transferred into the Biosafety level 2 cabinet. The unpurified or chromatography purified lentiviral particles will then be
added to the sucrose cushion. The individually capped tubes will then be transferred to their respective bucket holders
and the bucket sealed using their respective screw caps and marked as containing virus. The bucket will be
transferred to a weighing scale and a counterpart bucket and tubes with water (and without lentivirus) will be prepared
that is weight matched. The balanced buckets will then be transferred to the rotor before being placed within the
centrifuge chamber. The ultracentrifuge will be run at 70000g for 2 hours at 20°C. Upon return of the buckets to the
Biosafety level 2 cabinet, the supernatant will be removed from the tubes before transfer of the resuspended virus
pellet into individual screw cap cryovials. As the greatest risk for contamination occurs within the centrifugation
buckets themselves during transport to and from the weigh scales and during ultracentrifuge, the centrifuge tubes and
also the buckets will be decontaminated with Chemgene HLD4L 5% for 24 hours before removal from the Level 2
laboratory. In any case, at no point will concentrated virus stock be exposed to the environment outside of the class 2
hood. All supernatants will be treated as above.

The lentiviral titre will be determined in terms of the number of viral particles (VP) or Transduction units (TU) per ml.
The number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based
enrichment assay (e.g. QuickTitre™ Quantification Kit) or a p24 ELISA assay (e.g. QuickTitre™ Lentivirus Titre kit)
will be chosen. The VP quantification methods tend to overestimate the TU by 10-1000 fold. Thus, the TU 1 ml will in
certain instances also be determined. For this, a titration range of lentiviruses expressing a fluorescent tag, serial
dilution will be prepared and added to a 24 well cluster plate or T25 TC-flasks containing HEK293T for 48 hours. The
percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using
FACS analyser. The biological titre (TU/ml) according to the following formula: $TU/ml = \left( \frac{P}{N} \times 10^10 \times V \times 10DF \right)$,
where $P =$ % GFP+ cells, $N =$ number of cells at time of transduction = 105, $V =$ volume of dilution added to each well
and $DF =$ dilution factor = 1 (undiluted), 10-1 (diluted 1/10), 10-2 (diluted 1/100), and so on.

Aliquots with not more than 1x10^18 viral per vial are subsequently stored at -80°C in individual screw capped
cryotubes. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a
variable number of empty viral capsids. Other trace components that might be present include, but are not limited to,
inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats.

In vitro studies

Transduction of cells (e.g. human HEK293T or immortalised human podocyte cells) with the lentiviral vectors will be
carried out in a Biosafety level 2 cabinet within a dedicated Biosafety level 2 laboratory. In the in vitro studies, the
TUs, and consequently volumes added, will be dependent on the number of cells in the tissue culture dish/flask to be
infected, up to the maximum number of viral particles to be handled per experiment (around 1 x 10^18). None of these
cells will be cultivated in the filter screw cap flasks used for HEK293T mediated lentiviral production. This is to ensure
that, in particular, the HEK293T cells used for lentiviral production are not inadvertently used for lentiviral assessment.
Any plastic ware or solutions that are used to handle the transduced cells will be decontaminated and disposed of as
detailed later (see Waste Handling). Once the medium containing the lentiviral particles has been removed and the
cells (e.g. human HEK293T or immortalised human podocyte cells) have been extensively washed, which all is done
in a Biosafety level 2 cabinet, the human HEK293T cells can be used following Biosafety level 1 guidelines, as no
virus will be present and the latent virus in the transduced cells is replication-incompetent. The immortalised human
podocytes will be used following Biosafety level 2 guidelines. A viral titre determination assay may on occasion be
used to verify the absence of virus in the medium from human HEK293T cells and immortalised human podocyte
cells.

Cleaning SOPs

General cleaning procedures

Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each
experiment or after the occurance of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all of the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being autoclaved/incineration.

* Chemgene HLD4L

Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls of micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. The Synergetic blend of technology and active ingredients enables Chemgene HLD4L to work quicker than conventional high level disinfectants and ensure that apoptosis (cell death) is achieved rather than merely suspending activity as with conventional products. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, Hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1 %) for general purposes, 1 :20d for high risk areas, and 1 :20 (5%) for disinfection of blood and bio-hazard spillages.

References

For further information, see also the safety guidance "Biosafety Considerations for Research with Lentiviral Vectors" published by the US NIH

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Cleaning SOPs
General cleaning procedures
Health and Safety
Executive
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all of the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being autoclaved/incinerated.

Waste Handling
Solid waste will be neutralised by soaking it in a validated disinfectant solution, after which the material will be ‘double bagged’, autoclaved and, ultimately, incinerated offsite. It will be routine practice that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. At 860°C, liquid waste will be inactivated by Chemgene HLD4L* for 24 hours prior to drain disposal as per 430CSP and 418CSP permit requirements.

Following all work requiring biosafety level 2 containment including work with lentiviruses and immortalised human podocytes, any solutions, plastic ware and consumables that have been used will be decontaminated with Chemgene HLD4L.
HLD4L for at least 24 hours prior to removal from the biosafety level 2 laboratory and disposal as outlined above. As any active viral particles or immortalised human podocytes cells on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles or immortalised human podocytes would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected. Chemgene HLD4L

Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls of micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. The Synergetic blend of technology and active ingredients enables Chemgene HLD4L to work quicker than conventional high level disinfectants and ensure that apoptosis (cell death) is achieved rather than merely suspending activity as with conventional products. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, Hepatitis B, Herpes Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1 %) for general purposes, 1 :20d for high risk areas, and 1 :20 (5%) for disinfection of blood and bio-hazard spillages.

The GMSC has agreed that the work outlined above can be safely carried out under biosafety class 2 containment. The immortalised podocyte cell line has been generated (from an individual shown to be negative for HIV or Hep8) using a retroviral system that delivers a thermolabile SV40T antigen, a human telomerase but retains its L TR. The cell line has been shown to not generate infectious particles. Although there is a chance that the retrovirus can mobilise out of the genome the thermolability of the SV40T antigen mitigates the potential transformative and oncogenic effects of subsequent accidental infection. As the planned work calls for the use of lentivirus to deliver shRNA, targeted nucleases or reporter constructs there is an increased risk of mobilisation through recombination events. Sequence analysis has shown that there is no shared common sequence between the lentiviral vectors that will be used to deliver additional genetic material and the Moloney murine leukaemia virus system used to immortalise the human podocytes essentially minimising this risk. The lentivirus themselves are generated using a third generation system whereby the essential elements required for virus production have been separated out onto different vectors and are only delivered in a transient manner to minimise the chance of a recombination event leading to replication competent virus. Even when the immortalised podocytes are transduced with a reporter gene and either shRNA or programmable nucleases in combination the likely hood of recombination is minimal. This virus system is also self-inactivating so that once integrated into the target cell's genome it cannot mobilise. As the virus integrates into the genome of cells no tumour suppressors, oncogenes, genes involved in quiescence or immune-modulators will be targeted. As recombination and mutation events cannot be ruled out this work will be carried out under class 2 containment. Transduced cells will be tested for absence of infectious particles.
**Project Additional Information**

**Purposes of the contained use**

The human immortalised proximal tubules cells will be used to investigate the cellular pathway involved in the development of human renal disease, in particular acute kidney injury.

To better understand these cellular pathways, the expression or the activity of the gene of interest will be modulated by several methods:

1. The utilisation of tool compounds
2. The knock-down of the gene of interest by shRNA
3. The generation of knockout somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
4. The generation of reporter gene cell line (i.e. fluorescence protein or enzyme) under the promoter of the gene of interest.
The experiments involving the transfection of vectors such as shRNA or programmable nucleases will be performed by lentivirus. The effect of modulating the expression or the activity of the target of interest will be determined by the expression level of the proteins, mRNA or mediators involved in the target signalling pathway.

Recipient or parental organism

TH1 cells are derived from primary human renal proximal tubule epithelial cells (RPETCs) immortalised by two lentiviral vectors carrying the human telomerase (hTERT) and the SV40 large and small T antigen (Tag). The generation of the TH1 cells have been described by Kowolik and al. (Kowolik et al, 2004, Oncogene). Primary human RPTECs from third and fourth passage were transduced with both HIV-7/CPNO-Tag and HIV-7/CPNO-hTERT vectors.

Description of the vectors used to immortalize primary cells: This vector pHIV7/CNPO is the “backbone” of the pHIV7/CNPO-hTERT and pHIV7/CNPO-Tag. To generate the pHIV7/CNPO, the neomycin resistance (neoR) gene and an internal ribosome entry site (IRES) flanked by two LoxP sites controlled by the immediate early (IE) gene promoter of cytomegalovirus (CMV) were inserted into the pHIV7 (Kowolik and Yee, 2002). The pHIV7/CNPO-hTERT is composed of the pHIV7/CNPO in which the cDNA for the catalytic subunit of the human telomerase (hTERT) has been inserted. The pHIV7/CNPO-Tag is composed of the pHIV7/CNPO in which the cDNA for SV40 large and small T antigen has been inserted.

The resultant cell line, TH1, contains the sequence coding for SV40 Large and Small T antigen and hTERT under the CVM IE enhancer and the promoter of the HIV 5’LTR. The cell line also contains 190bp of central polypurine tract sequence (cPPT) from HIV-1, 800bp post-transcriptional regulatory element from Woodchuck hepatitis virus (WPRE) and the U3 region of the HIV 3’LTR deleted of 400bp that completely removed the enhancer and promoter sequences in this U3 region.

The immortalised cells failed to form anchorage-independent colonies in soft agar. They also failed to form tumours in nude mice. Furthermore transient Cre expression in TH1 cells leads to efficient proviral deletion and upregulation of some renal specific activities. Removal of the genes for cell immortalisation also significantly decreases the rate of cell proliferation and ultimately leads to cell senescence, suggesting that the cell cycle control remains intact in TH1 cells despite continuous passage of these cells in culture. These results strongly suggests that the immortalised cells were not transformed.

Host/vector system

To study the function of proximal tubules cells in human disease, the expression of a target of interest may be decreased in the TH1 cell line by the transduction of shRNA or programmable nuclease by lentivirus. The modulation of a particular cellular pathway may be studied with the use of a reporter gene assay. In this case, the vector contained the reporter gene under regulating sequence from the promoter of the gene of interest will be introduced by lentivirus into these TH1 cells.

This proposal involved both the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site using the third generation system. Both commercially acquired and in-house lentivirus will use the same vector backbones containing identical promoters. Target genes will be selected based on the needs of drug discovery projects; programmable nucleases and shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators.

The production of lentivirus containing the vectors of interest are described below:

1- Generation of lentivirus
Lentiviral vectors will be purchased commercially or produced in-house using the third generation system described below. Each purified and concentrated aliquot of lentiviral reagent comprised of a small volume (5 to 10ul) that contains no more than 1X10e8 viral particles.

Vector System = self-inactivating (SIN) non replication-competent HIV based lentiviral vectors
The viral vectors that will be used in the proposed studies are self-inactivating non replication-competent HIV based lentiviral vector systems, which have been designated to produce stable gene expression in mammalian cells; and they are generated by co-transfecting HEK 293T cells with four separate plasmids. Each of the plasmids used expressed a different set of genes which, when combined following co-transfection, provide the smallest possible set of essential viral genes that is still compatible with virus production. Moreover, the vectors are all vesicular stomatitis virus (VSV-G) pseudotyped lentiviral vectors, which are self-inactivating and highly unlikely to undergo recombination. Importantly, the viral vector system is inherently incapable of replication in mammalian cells.

The plasmids
The sequences that will be included in the viral vectors are sub-cloned into 4 standard bacterial plasmid vectors.

- Plasmids containing the packaging related sequences
Each of the three other plasmids, pMDLgp-RRE, pRSV-REV and pMD2-VSVG.env contain a different set of sequences that code for proteins that are responsible for packaging the viral vectors. All the genes (gag-pol, rev and env) required for production and packaging of the full length viable viral RNA particles have been removed from the pRRL lenti-plasmids containing the transgenes of interest.

- Plasmids containing the transgene of interest
  - Vector containing shRNA
    As mentioned above, pRRL plasmid will contain the sequences that code for one shRNA transcript that will target one mRNA/protein of interest (or a scrambled shRNA in the case of negative control). The shRNA or scrambled shRNA sequences will be under the control of polymerase III U6 promoter. The shRNA sequences will be chosen to target a single mouse or rat gene. Target genes of the shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators.
  - Vector containing CRISPR or Zinc-finger nucleases or Cre recombinase
    Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the cleavage site or by recombination with a user-supplied synthetic vector containing sequences complementary to the target gene.
    For Cas9/CRISPR, nuclease target specificity is determined by an accessory sequence, the guide RNA, which consists of a short (18-20 nucleotide) sequence homologous to the target gene and an additional short sequence that forms a complex with the Cas9 enzyme. In the case, the plasmid pRRL, will contain the sequences that code for a Cas9/CRISPR plus accessory sequences comprising a short guide RNA that determines target specificity. The guide RNA sequences will be under the control of the Polymerase III U6 promoter.
    In the case of Zinc finger nucleases target specificity is dependent upon the zinc finger protein sequence. The pRRL will contain in that case the sequence of zinc finger nucleases under the control of either the CMV promoter or the EF1A promoter. In addition, a fluorescent protein or enzyme under the control of cytomegalovirus (CMV) promoter, EFIA promoter or the promoter of a gene of interest may be included. Control viral vectors that express only the fluorescent protein/enzyme and no programmable nuclease will be used in some experiments.
    The pRRL vector will contain the sequence coding for the Cre recombinase under the the control of the CMV promoter to allow the removal of the SV40 T antigen sequence and the hTERT sequence from the TH1 genome.
  - Reporter gene vector
    The pRRL vector will contain the sequence coding for a reporter protein (enzyme or fluorescent protein) under the control of the regulating elements of the promoter of the gene of interest or the CMV or EF1A promoter. An appropriate selection marker i.e.; neomycin could also be included to allow the selection of stable human tubule cells containing the reporter gene sequence under the control of the promoter of the gene of interest.
    The pRRL vector will contain the sequence coding for the Cre recombinase under the the control of the CMV promoter to allow the removal of the SV40 T antigen sequence and the hTERT sequence from the TH1 genome.

**Origin & function**

- Assessing efficacy of the lentivirus into a BSL1 cell line
  Assessing whether the genome manipulation or the knock-down has worked will normally take place initially in biosafety level 1 immortalised cell line (human HEK293T) that are known not to contain lentiviral genetic material. Application of lentiviral particles will be done at biosafety level 2 confinement. After at least 24 hour incubation and extensive washing, the cells will treated as biosafety level 1 (see below the justification for removal of cells post-infection to biosafety level 1). In order to determine whether the knock-down or the genome manipulation has worked, the levels of mRNA and protein will be assessed post-transduction, using suitable in vitro assays.

- Transduction of the lentivirus and the generation of stable expression into TH1 cells
  Once validated the same procedure will be carried out in the human TH1 cells. As the human proximal tubule cells are maintained in a biosafety level 2 confinement, the inoculation of lentiviral particles, the transformed cells and the phenotypic analysis will also be performed in a biosafety level 2 containment. When a lentivirus containing a programmable nuclease or the reporter gene will be used, a stable cell line could be established by selecting the cells in which the genome has been modified. In all cases phenotypic analysis will be performed using suitable in vitro assays, including for example, RT-PCR, Western Blotting, immunocytochemistry and imaging, or cellular bioenergy (i.e.: mitochondria respiration function or glycolysis function).
Evaluation of foreseeable effects

* Human Health

The immortal human proximal tubule cell that will be used has a low risk for human health. The expression systems and the transgenes that comprise the lentiviral vectors that will be used have also a very low risk for human health and safety, and the reasons for this are discussed below.

A- Lentivirus containing shRNA, or programmable nuclease sequence or reporter gene vectors

Lentiviral Vector Expression System

Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. As the lentiviral vector system that will be used in these studies is based on HIV-1, a theoretical risk to human health exists. However, retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airborne route. Therefore, accidental piercing of the skin or other surface tissues with virus containing objects represents the main potential route by which accidental infection could occur. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

In all of the modified pRRL plasmids (containing shRNA, or programmable nuclease or reporter gene sequences), the sequences inserted are flanked by non-coding retroviral LTRs, and no retroviral genes are encoded on the modified pRRL plasmids. Therefore, no retroviral genes will be transferred into generated viral particles. This construct is package into particles using a HEK293T cell based packaging system, which requires the co-transfection of cells with three additional separate plasmids (pMDLgp-RRE, pRSV-REV and pMD2-VSVG.env). The latter 3 plasmids express the envelope protein from VSVg and the non-structural proteins of the virion, and, importantly, none of these genes will be transferred into the assembled viral vectors, since they lack the packaging signal, which is only present on the modified pRRL containing plasmids.

The lentiviral Expression System what will be used include the following key safety features:

* All of the pRRL contain a deletion in the 3'LTR (DeltaU3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" (SIN) of the vector after transduction of target cells. Once integrated into a transfected target cell, the lentiviral genome is no longer capable of producing viral genomic material that can be packaged. Moreover, deletion of enhancer and promoter elements from the 3' U3 region in the vector construct will result in a provirus that is entirely devoid of the U3 enhancer sequences, which will result in a reduction in the potential for transactivation of cellular genes due to an insertion event. Furthermore, the development of self-inactivating vectors improves the biosafety of vectors, as they are less likely to be mobilised following a superinfection with wild-type virus (HIV).

* The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes. Heterologous envelopes, like VSV-G, typically broaden the tropism and allow gene transfer into a broad variety of cells. The risk will mitigated by the use of self-inactivating virus and limiting the number of viral particles that will be handled at any one time.

* Sequences encoding the proteins required for packaging of the viral genome are separated onto three plasmids, and all of the 4 plasmids used in the system have been engineered not to contain any regions of homology with each other so as to prevent undesirable recombination events that could lead to the generation of a replication-competent lentivirus (RCL), which could potentially be harmful to humans. It is important to note that no such RCL has ever been observed despite large-scale production and testing of lentiviral vectors.

* All of the pRRL containing plasmids/vectors will be used are devoid of all viral sequences apart from essential cis-acting sequences, including the LTRs and the packaging signal. Although the packaging plasmids used in these systems allows for the expression in trans of protein required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK293T producer cell lines, none of them contain LTRs or the packaging sequence. Several of the lentiviral accessory genes (vif, vpr, vpu and nef) that are dispensable for lentiviral vector production/transduction have been deleted from the packaged construct. Therefore, none of the retrovirus structural genes will actually be present in the packaged viral genome, as they will never be expressed in the transduced target cells, which means that no new RCL can be produced.

* The lentiviral particles produced in this system are replication-incompetent, only carry the sequences of interest, and no other viral species are produced.

* Expression of the gag and pol genes from pMDLgp-RRE has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Addition of the RRE in these plasmids prevents gag and pol expression in the absence of rev, which is contained in the pRSV-REV plasmid only. The Rev/RRE system is highly conserved among lentiviruses, and removal of the RRE sequence and associated splice donor/acceptor sequences result in a loss of transduction efficiency.

* LTR has been modified so as to increase lentiviral vector production, and also to allow lentiviral vector production to be independent of tat expression. It is known that Tat-deleted mutants of wild-type HIV-1 are not replication competent. Therefore, the deletion of Tat should decrease the risk of generating a putative RCL.

* Lentiviral vectors have a very low potential to cause immunogenicity.
In the lentiviral vector system that will be used, the WPRE (woodchuck post-transcriptional regulatory element) has been mutated to remove the oncogenic WHV-X protein from an open reading frame found in WPRE sequence. The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction.

While the lentiviral vectors that are produced using this system contain only about 20% of the original genome of HIV-1, there is a very small risk that subsequent infection of cells already infected with the lentiviral genome of HIV-1 could lead to a rare recombination event in which the transgene is transferred to a replication-competent virus. Thus, the sequences in the vector that will be expressed could potentially be transferred to surrounding cells. This event is, however, extremely unlikely to occur for a number of reasons:

1- The lentiviral vector is replication-incompetent and self-inactivating. In the case of a subsequent HIV-contamination, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host genome, although non-self-inactivating vectors can be.

2- In order for the spread of the gene of interest to occur following accidental infection (assuming that this has lead to viral integration), a series of unlikely events have to occur:
   a- The worker would have to become infected with HIV-1 or to be already infected with the virus.
   b- The viral and lentiviral genomes would have to integrate into the host worker's genome in the same cells and in a position where they could interact to effect homologous recombination (point 1 above)
   c- Recombination would have to occur in just the right regions to allow for transfer of the gene of interest from the lentivirus to the HIV-1 genome, which could also involve the transfer of the HIV-1 genes to the lentiviral genome. In that case, it is conceivable that a non-self-inactivating HIV could be generated that contained the gene of interest but not the rest of the genome it requires. The other gene products could be provided in trans from the lentiviral genome that may now contain the HIV-1 genes or from other HIV-1 integrants.
   d- The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest, but again no genes neccessary for subsequent replication. In order for another round of infection to occur, the process would have to begin again.

These circumstances, the effects of lentiviral infection are likely to be minor in comparison to the effects of the HIV-1 infection, which would be required to affect the spread of the gene of interest. In addition, the scenario described is essentially equivalent to the rescue of the lentiviral genome from the host, which has already been shown not to occur.

3- It is extremely unlikely that any worker would infect themselves with a significant dose of lentivirus as the volumes that are used in transfection experiments are small (aliquots contain a maximum of 10μl of vector solution).

4- Moreover, insertional mutagenesis into the host genome may be considered as an oncogenic risk. We cannot rule out the possibility that, when the lentiviral genome integrates into the host genome, it will not lead to the activation of an endogeneous oncogene. However, all transcriptionally active long-terminal repeats (LTRs) have been removed as well as all promoter-like elements that required to drive expression of the transgene. This should prevent unforeseen activation of such genes. It is noted that deletion of retroviral enhancer in self-inactivating systems reduces the risk of activation but not of disruption, therefore, retroviral infection might still have permanent effects upon a cell (including oncogenic effects).

Importantly, we do not consider that the use of these lentiviral vectors will result in a significant increased risk of oncogenic activation compared to the risk possessed by any other viral delivery system. Moreover, the risk of transduction leading to tumourigenesis or other untoward harm following exposure is related in part to the titre of the viral vectors; exposure of workers to quantities of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of lentiviral vectors.

Transgenes:
Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.
• shRNA
With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be knocked-down, in order to study their functions. Potential deleterious effects such as off-target and immunomodulatory responses can be minimised through screening designed shRNA sequences against databases of known mammalian/mRNA sequences during the design stage. The genes/proteins of interest that will be targeted by shRNA do not and will not include any growth factors.
tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors and immunomodulators. Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. This sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of human, mouse or rat origin. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health.

- Programmable nucleases: zinc fingers or Cas9/Crispr or Cre recombinase
  
  Zinc fingers or Cas9 protein will be directed against genes of therapeutic interest. Since the purpose of programmable nucleases is to cause mutations in defined DNA sequences the primary hazard is that such a mutation would lead to deleterious cellular effects. The genes/proteins that will be targeted do not and will not include any growth, trophic factors, confirmed oncogenes, tumor suppressors or immunomodulators. In isolation, these sequences are unlikely to pose any safety risks for the environment or human health.

  The Cre recombinase enzyme is not known to cause any diseases in human and animals and have direct effect on cellular processes. The application of Cre recombinase in TH1 will induce the deletion of the sequences between the 2 sequences LoxP such as the sequences for coding for neomycin, hTERT and Tag and the IRES, leaving the sequence of CMV/LTR, cPPT, CMV, WPRE and LTR in the TH1 genome. In that case, the resultant cells have been shown to down-regulate proliferation and becoming senescent after few passages (Kowolik et al. 2004).

- Reporter genes: Fluorescent protein or enzymes

  The reporter gene could be a fluorescent protein or an enzyme. Fluorescent proteins, for example GFP or dsRed, are not known to cause any diseases in human or animals and have no direct effect on cellular processes. Enzymes used frequently in reporter gene assay ie luciferase of b-galactosidase are not known to cause any diseases in humans or animals and as no direct effect on cellular processes. The expression of fluorescent protein or enzymes under the control of a promoter of a gene of interest is unlikely to pose any safety risks for the environment or human health.

**Summary on lentivirus**

The transgenes have a low safety risk to human health because accidental contamination with a vector would lead to only a small number of cells becoming infected, and it would be highly unlikely that expression in only a few cells could be sufficient to lead to a disease state. The inability of the lentiviral vectors to propagate on mammalian cells also reduces the risk. The programmable nucleases (CRISPR or Zinc finger or Cre recombinase) that will be employed in this viral vector system are not known to cause any relevant toxicity that might represent a safety risk to human health. The fluorescent proteins or the enzymes are not known to cause any relevant toxicities that might represent a safety risk to human health. For both programmable nucleases and shRNA, the genes/proteins of interest that will be targeted will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators, and none of them are or will be of retroviral origin. Therefore the risk of use will be low.

**B- Immortalised human proximal tubules cells TH1 and transduced immortalised TH1 cells**

The immortalised TH1 cell line have been immortalised by the presence of the coding region for both large and small T-antigen and hTERT. This cell line will be purchased from Kerafast.

Large and Small T antigen have been described to be sufficient to transform human cells. hTERT or telomerase reverse transcriptase is the catalytic subunit of telomerase. Its overexpression stabilises telomere length preventing replicative senescence. These 2 elements induce immortalisation of the human proximal tubules cells. These cells also contain the sequences for WPRE, cPPT and deltaU3 of HIV-1 virus. However these cells do not contain the viral genes gag-pol and env required for virus production and therefore cannot produce viruses. We will confirm lack of gag-pol and env integration by PCR and lack of virus production in the TH1 by ensuring that supernatants from TH1 culture do not transduce HEK293T cells.

As mentioned above, the TH1 cells contain the sequence coding for the WPRE (woodchuck post-transcriptional regulatory element). According to Kowolik and Yee 2002, the WPRE sequence hasn’t been mutated to remove the oncogenic WHV-X protein from an open reading frame found in WPRE sequence. The WPRE sequence will be sequenced to confirm the presence of the unmutated form in TH1. One of the major risk factor associated with the utilisation of this cell line would be in that case accidental percutaneous inoculation of a worker. As described by Kowolik et al, TH1 cells failed to form anchorage-independent colonies in soft agar. They also failed to form tumours in nude mice. Furthermore, this TH1 cell line could theoretically not survive into the inoculated worker but would be quickly cleared by the human immune system.

One of the major risks would be if these constructions coding for the small T-antigen and/or Large T antigen are mobilised. This could happen if the TH1 cells are accidentally inoculated to a worker already infected by a retroviral genome. Even in that case, as series of unlikely events would have to occur to:
a. The worker would have to be already infected with the HIV-1 virus.

b. The retroviral genomes would have to integrate into the immortal mouse or rat mesangial cells genome in a position where they could interact to effect homologous recombination.

c. Recombination would have to occur in precise regions to allow for transfer of the SV40 T antigen sequences from the TH1 genome to the HIV-1 genome.

d. The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest.

Although the risk for human health is low, all measures will be in place to minimize the risk of human health to avoid any accidental inoculation of a worker (see measures below).

Further modification of the TH1 cell line may enhance the risk of the mobilisation of the sequence coding for small and/or large T antigen sequences, or WPRE and the sequence of DeltaU3. Application to these cells of additional lentivirus particules could result to the mobilisation of these sequences and the liberation of replication-competent retroviruses by genetic recombination events. This risk will be minimised by confirming that the viral genes gag-pol and env are not present in the genome of the immortalised TH1 cells before the application of the lentivirus used on the cells. Furthermore, the risk can be minimised by ensuring that any lentivirus used in experiments with the host cells does not contain gag-pol, env or any other significant retroviral genes involved in packaging and replication. To ensure that any replication-competent retroviruses by genetic recombination events had occurred, the supernatant of the TH1 cells will be tested for the production of retroviruses by application on HEK293T cells. In the case of DeltaU3 in the TH1, the Delta U3 contained in the TH1 will be sequenced to ensure that the sequence contains in the TH1 can not be recombined to the sequence DeltaU3 contained in the lentivirus to generate a full 3’ LTR that can affect the production of the viral genome.

Even if the events are unlikely, the immortalised human proximal tubule cell line and the transduced TH1 cells will be maintained at biosafety level 2 containment. Therefore all measures will be in place to minimize to risk to human health.

Human health hazards

Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker’s skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the programmable nucleases and shRNA that will be expressed or targeted will not be designed to disrupt any growth factors, trophic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. In the unlikely event of accidental transduction of a laboratory worker’s skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus or with the TH1 cell line will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent. Infection of the community at large by TH1 cells is highly unlikely due to the impossibility of the cells to survive outside of a laboratory environment.

* Environement considerations

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active lentiviral vector particles do accidentally get released into the environment, the safety risk posed such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable replication-competent lentivirus. However, this scenario is extremely unlikely and even if it was to occur, it is unlikely that it would lead to any untoward effects. As the HIV virus is a human pathogen infection of another species would not be expected to allow any form of recombination event leading to a viable transmissible entity and so the risk from this scenario is considered to be negligible. Rodents, such as wild-type mice and rats, cannot support replication of infectious HIV-1 (Goffinet et al, 2007 Retrovirology). As a result, the potential for shedding of replication-competent lentiviruses from such animals is very low (even if they were present in the original vector inoculum).

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and in theory they are capable of transducing all dividing and non-dividing
mammalian cell types. As emphasized before, the vectors cannot self-propagate after infection, and successful transduction is critically dependent on the presence of high enough concentrations of virus particles. Viral vector stock solutions will be managed in a way that will prevent contaminations with relatively high viral titres. After production, the vectors are aliquoted and stored in screw capped cryovials at -80°C. Taking into account that the infectivity of the vectors rapidly decreases at room temperature, it is considered highly unlikely that the vectors could survive in the long term after being accidentally released into the environment. Lentiviral vectors have a short half-life, at room temperature due to their structural characteristics making them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, as illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media. There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen. The risk to the environment following accidental release of the immortalised human proximal tubule cells would be negligible since the cells are incapable of surviving outside of laboratory conditions. If TH1 cells were to directly inoculate animals, they would be quickly cleared by the immune system and would not produce active viruses. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures and ensuring that all potentially contaminated material is totally inactivated before disposal.

Summary
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to. In the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems. It is also unlikely that the immortalised human proximal tubule cells, TH1 cells, and the transduced TH1 cells will be accidentally released into the environment, as the cells handling and studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
The work outlined above has been classified as a Biosafety level 2 activity, even though the risk of pathogenicity to the end worker is considered to be very low. In all cases, biosafety level 2 conditions must be adhered to when handling the immortalised human proximal tubule cells, generating the viral vectors in HEK293T cells, as well as their subsequent storage at -80°C. For subsequent in vitro transduction of the cells types mentioned in this proposal (e.g. human HEK293T, TH1 cells) will be performed under biosafety level 2 conditions. The human transduced HEK293T used to assay the efficacy of the vectors will be converted to biosafety level 1 once the medium containing the virus has been removed and the infected cells have been extensively washed. The TH1 cells transduced by lentivirus will be maintained under biosafety level 2.

Procedures:
During all procedures that involve the handling/use of the viral vectors or cells, all workers (incl. those that are not working directly with the vectors) that are present within a lab where the work is undertaken are required to wear personal protective equipment at all times. The use of two pairs of gloves is advised so the external pair can be disposed in an autoclave bag and replaced whenever necessary (e.g. in the case of contact with the viral reagent). Face masks are also available for use, where appropriate. In addition, all workers are made aware of the nature of the viral work that is going on within the laboratory, and they have to follow appropriate procedures to ensure that there is no cross-contamination into non-viral working area. All the workers using the viral delivery systems are experienced research scientist, and junior scientists will be closely supervised until they are competent in the handling of the viruses.

Lentiviral production
Lentiviral production will also be carried out on-site using a modified procedure based on Bukovsky et al. (Bukovsky et al, 1999, J. of virology). Lentiviral production will only be carried out in HEK293T cells in a biosafety level 2 cabinet within a culture flask with screw cap filter in order to minimize the risk of spills and/or aerosol mediated viral spread. For this the modified PRRL plasmids together with three additional separate plasmids (pMDLgp-RRE, PRSV-REV and pMD2-VSVG.env) will be transfected using a transfection reagent (e.g. Polyethylenimine (PEI), Lipofectamine) into HEK293T packaging line. After 6-12 hours, the medium is replaced with fresh serum-free medium and grown for an additional 72-96 hours. After 24-48 hours, so as to ensure the viability of the transfected HEK293T cells, medium form the cells is collected into
screw cap tube and stored at 4oC and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the first harvest tube. After lentivirus collection has been completed, the cells and the flasks will be decontaminated and disposed of a detailed later. Still within a biosafety level 2 safety cabinet in a biosafety level 2 laboratory, the harvested supernatants will be filtered using a 0.45um filter before either aliquoted or further purified by chromatography and/or concentrated by centrifugation. The lentiviral particles may need to be purified by either chromatography and eluted in a high salt solution and/or concentrated by ultrafiltration. For certain application where highly concentrated and purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimize the possible risk of accidental viral contamination and spread due to the need for an ultracentrifuge, all loading and unloading will be done in the biosafety level 2 safety cabinet. Once the sucrose cushions have been added to the centrifuge tubes, the unpurified or chromatography purified lentiviral particles will be added to the sucrose cushion. The individually capped tubes will then be transferred to their respective bucket holders and the bucket sealed using their respective screw caps and marked as containing virus. The buckets containing viruses will be balanced with a buckets containing a tube with water and without lentivirus. The balanced buckets will then be transferred to the rotor before being placed within the centrifuge chamber. The ultracentrifuge will be run at 70000g for 2 hours at 20oC. Upon return of the buckets to the biosafety level 2 cabinet, the supernatant will be removed from the tubes before transfer of the resuspended virus pellet into individual screw cap cryovials. As the greatest risk for contamination occurs within the centrifugation buckets themselves during transport to and from the weight scales and during ultracentrifuge, the centrifuge tubes, the buckets and the centrifuge rotor will be decontaminated by spraying Chemgene HLD4L 5%. Optionally, the centrifuge rotors could be autoclaved at 121oC. In any case, concentrated virus stock will not be exposed to the environment outside of the biosafety level 2 cabinet. All supernatants will be treated as above.

The lentiviral titre will be determined in terms of number of viral particles (VP) or transduction units (TU) per mL. The number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based enrichment assay or a p24 ELISA assay will be chosen. The VP quantification methods tend to overestimate the TU by 10-1000 fold. Thus, the TU/mL will in certain instances also determined. For this, a titration range of lentiviruses expressing a fluorescent tag will be prepared and added to a 24 well cluster plate or T25 TC-flasks containing HEK293T for 48 hours. The percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using FACS analyser.

Aliquots with not more than 1x10⁸ viruses per vial are stored at -80oC in individual screw capped cryotubes. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to, inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats.

Cleaning SOPs
General cleaning procedures
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being autoclaved/incinerated.

* Chemgene HLD4L
Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1%) for general purposes, 1:20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General cleaning procedures
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being autoclaved/incinerated.
being autoclaved/incinerated.

Waste Handling
Following all work requiring biosafety level 2 containment including work with lentiviruses and immortalised mouse or rat mesangial cells, solid waste will be neutralised by soaking in a validated disinfectant solution, after which the material will be “double bagged”, autoclaved and, ultimately, incinerated offsite. It will be routine practise that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Liquid waste will be inactivated by Chemgene HLD4L* for 24 hours prior to drain disposal as per 430CSP and 418CSP permit requirements. As any active viral particles or immortalised mouse or rat mesangial cells on surface or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles or immortalised mouse or rat mesangial cells would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.

* Chemgene HLD4L
Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1%) for general purposes, 1:20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages.

The TH1 cell line has been immortalised by the use of hTERT and SV40 large & small T antigen delivered by a Self-inactivating retrovirus (SIN). Retrovirus delivery includes integration of a WPRE element, 5' LTR and a 3'LTR. There is limited information about the actual deletion in the 3'LTR or if the WPRE has been mutated to remove the WHV-X oncogene. This region of the TH1 genome will be sequenced to test for deletion in the potential risk. Knowing the sequence will allow us to confirm that the retroviral vectors that will be used to deliver genetic material will not have homologous sequences that might enable recombination and increase the risk of mobilisation (especially if Cre-recombinase is used as it releases hTERT and SV40 T-antigen genetic sequence). Lentivirus will be generated using a third generation methodology which requires 4 different plasmids, that do not share sequence homology, to be transfected into the same packaging cell. The isolation of the genes required for virus production minimises the chance of accidentally generating replication competent virus (RCV). The desired packaged genetic material also undergoes self-inactivation upon integration into a host genome after infection of target cells. As this is a permanent change it is noted that no tumour suppressors, oncogenes or immune modulators will be targeted. The host cells contain the transformative SV40 T antigens so it is important to prevent mobilisation of this genetic material. There will be no homologous sequence present between the lentivirus and the SV40 T antigen coding region. If Cre-recombinase is used the hTERT and SV40 T antigens are removed leading to senescence of the cells and a removal of potential risk. In this situation as the genetic code is released a lack of homologous sequence will ensure recombination does not occur. Combined with the minimal chance of RCV being present will effectively prevent the generation of virus containing SV40 T antigen. The lentivirus being produced, although pseudotyped to have broad tropism, poses a minimal risk to the environment as it is highly unlikely to be able to infect an animal outside of the laboratory environment in the case of accidental release and the material will also rapidly deactivate at room temperature. The GMSC has agreed that this work will be conducted at biosafety level 2 because retroviral vectors are being used to deliver genetic material to host cells that have been immortalised with SV40 T antigen and hTERT.
### Project Additional Information

**Purposes of the contained use**

The mesangial cells, SV40 MES 13 and RMC cell line, will be used to investigate the cellular pathway involved in the development of human renal disease, in particular glomerulosclerosis disease.

To better understand these cellular pathways, the expression or the activity of the gene of interest will be modulated by several methods:

1. The utilisation of tool compounds
2. The knock-down of the gene of interest by shRNA
3. The generation of knockout somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
4. The generation of reporter gene cell line (i.e.: fluorescence protein or enzyme) under the promoter of the gene of interest.
The experiments involving the transfection of vectors such as shRNA or programmable nucleases will be performed by lentivirus. The effect of modulating the expression or the activity of the target of interest will be determined by the expression level of the proteins, mRNA or mediators involved in the target signalling pathway.

**Recipient or parental organism**

- **SV40 MES 13 cell line**
  The immortalised SV40 MES 13 cell line will be purchased from ATCC. The generation of this cell line has been described by MacKay et al (MacKay et al. Kidney Int. 1988). The transgenic mice used contain the construct SV Tag 188 composed of the coding region for both large and small T-antigen along with its 72 and 21 base-pair repeats. These mice have been described to develop glomerulosclerosis and the large T antigen has been detected by immunoprecipitation in the kidney. The mesangial cells have therefore been isolated from these mice. These cells have been described to form colonies in soft agar and are positive for SV40 large T antigen.

- **RMC cell line**
  The immortalised RMC cell line will be purchased from ATCC. Primary mesangial cells have been isolated from a 3 month old rat. The cells are maintained in culture until passage 8, then they are immortalised by the pSV3-Neo vectors and maintained in the presence of G418. The cells contain the sequences of SV40 large T-antigen and neomycin genes.

**Host/vector system**

Description of the lentivirus containing shRNA, programmable nucleases sequences or gene reporter vectors

To study the function of mesangial cells in human disease, the expression of a target of interest may be decreased in the mouse or rat mesangial cell line by the transduction of shRNA or programmable nuclease by lentivirus. The modulation of a particular cellular pathway may be studied with the use of a reporter gene assay. In this case, the vector contained the reporter gene under regulating sequence from the promoter of the gene of interest will be introduced by lentivirus into these immortalised mesangial cells.

This proposal involved both the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site using the third generation system. Both commercially acquired and in-house lentivirus will use the same vector backbones containing identical promoters. Target genes will be selected based on the needs of drug discovery projects; programmable nucleases and shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators.

The production of lentivirus containing the vectors of interest are described below:

Lentiviral vectors will be purchased commercially or produced in-house using the third generation system described below. Each purified and concentrated aliquot of lentiviral reagent comprised of a small volume (5 to 10ul) that contains no more than 1X10e8 viral particles.

**Vector System = self-inactivating (SIN) non replication-competent HIV based lentiviral vectors:**

The viral vectors that will be used in the proposed studies are self-inactivating non replication-competent HIV based lentiviral vector systems, which have been designated to produce stable gene expression in mammalian cells; and they are generated by co-transfecting HEK 293T cells with four separate plasmids. Each of the plasmids used expressed a different set of genes which, when combined following co-transfection, provide the smallest possible set of essential viral genes that is still compatible with virus production. Moreover, the vectors are all vesicular stomatitis virus (VSV-G) pseudotyped lentiviral vectors, which are self-inactivating and highly unlikely to undergo recombination. Importantly, the viral vector system is inherently incapable of replication in mammalian cells.

The plasmids:

- The sequences that will be included in the viral vectors are sub-cloned into 4 standard bacterial plasmid vectors.
  - Plasmids containing the packaging related sequences
    Each of the three other plasmids, pMDLgp-RRE, pRSV-REV and pMD2-VSVG.env contain a different set of sequences that code for proteins that are responsible for packaging the viral vectors. All the genes (gag-pol, rev and env) required for production and packaging of the full length viable viral RNA particles have been removed from the pRRL lent-plasmids containing the transgenes of interest.
  - Plasmids containing the transgene of interest
    The fourth plasmid, pRRL, will contain the sequences that code for shRNA, or programmable nuclease sequence, or reporter gene sequence under the promoter of the gene of interest.
- **a- Vector containing shRNA**
As mentioned above, pRRL plasmid will contain the sequences that code for one shRNA transcript that will target one mRNA/protein of interest (or a scrambled shRNA in the case of negative control). The shRNA or scrambled shRNA sequences will be under the control of polymerase III U6 promoter. The shRNA sequences will be chosen to target a single mouse or rat gene. Target genes of the shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators.

b- Vector containing CRISPR or Zinc-finger nucleases vector
Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the cleavage site or by recombination with a user-supplied synthetic vector containing sequences complementary to the target gene.

For Cas9/CRISPR, nuclease target specificity is determined by an accessory sequence, the guide RNA, which consists of a short (18-20 nucleotide) sequence homologous to the target gene and an additional short sequence that forms a complex with the Cas9 enzyme. In the case, the plasmid pRRL, will contain the sequences that code for a Cas9/CRISPR plus accessory sequences comprising a short guide RNA that determines target specificity. The guide RNA sequences will be under the control of the Polymerase III U6 promoter.

In the case of Zinc finger nucleases target specificity is dependent upon the zinc finger protein sequence. The pRRL will contain in that case the sequence of zinc finger nucleases under the control of either the CMV promoter or the EF1A promoter. In addition, a fluorescent protein or enzyme under the control of cytomegalovirus (CMV) promoter, EFIA promoter or the promoter of a gene of interest may be included. Control viral vectors that express only the fluorescent protein/enzyme and no programmable nuclease will be used in some experiments.

c- Reporter gene vector
The pRRL vector will contain the sequence coding for a reporter protein (enzyme or fluorescent protein) under the control of the regulating elements of the promoter of the gene of interest or the CMV or EF1A promoter. An appropriate selection marker i.e.; neomycin could also be included to allow the selection of stable immortal mesangial cells containing the reporter gene sequence under the control of the promoter of the gene of interest.

Origin & function

a- Assessing efficacy of the lentivirus into a BSL1 cell line
Assessing whether the genome manipulation or the knock-down has been achieved will normally take place initially in biosafety level 1 immortalised cell line (human HEK293T) that are known not to contain lentiviral genetic material. Application of lentiviral particles will be done at biosafety level 2 confinement. After at least 24 hour incubation and extensive washing, the cells will treated as biosafety level 1 (see below the justification for removal of cells post-infection to biosafety level 1). In order to determine whether the knock-down or the genome manipulation has worked, the levels of mRNA and protein will be assessed post-transduction, using suitable in vitro assays.

b- Transduction of the lentivirus and the generation of stable expression into mesangial cells (mouse or rat)
Once validated the same procedure will be carried out in the mouse or rat mesangial cells. As the mouse/rat mesangial cells are maintained in a biosafety level 2 confinement, the inoculation of lentiviral particles, the transformed cells and the phenotypic analysis will also be performed in a biosafety level 2 containment. When a lentivirus containing a programmable nuclease or the reporter gene will be used, a stable cell line could be established by selecting the cells in which the genome has been modified. In all cases phenotypic analysis will be performed using suitable in vitro assays, including for example, RT-PCR, Western Blotting, immunocytochemistry and imaging, or cellular bioenergy (i.e.: mitochondria respiration function or glycolysis function).

Evaluation of foreseeable effects

* Human health considerations
The immortal mouse/rat mesangial cell that will be used has a low risk for human health. The expression systems and the transgenes that comprise the lentiviral vectors that will be used have also a very low risk for human health and safety, and the reasons for this are discussed below.

A- Lentivirus containing shRNA, or programmable nucleases sequence or reporter gene vectors
Lentiviral Vector Expression System
Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. As the lentiviral vector system that will be used in these studies is based on HIV-1, a theoretical risk to human health exists. However, retroviruses are generally transmitted via exposure to contaminated body fluids or
percutaneous inoculation and generally not transmitted via the aribone route. Therefore, accidental piercing of the skin or other surface tissues with virus containing objects represents the main potential route by which accidental infection could occur. Appropriate measures will be implemented to reduce the likelihood of this occurring.

In all of the modified pRRL plasmids (containing shRNA, or programmable nucleases or reporter gene sequences), the sequences inserted are flanked by non-coding retroviral LTRs, and no retroviral genes are encoded on the modified pRRL plasmids. Therefore, no retroviral genes will be transferred into generated viral particles. This is constructed is packaged into particles using a HEK293T cell based packaging system, which requires the co-transfection of HEK293T cells with three additional separate plasmids (pMDLgp-RRE, pRSV-REV and pMD2-VSVG.env). The latter 3 plasmids express the envelope protein from VSVG and the non-structural proteins of the virion, and, importantly, none of these genes will be transferred into the assembled viral vectors, since they lack the packaging signal, which is only present on the modified pRRL containing plasmids.

The lentiviral Expression System will be used include the following key safety features:
* All of the pRRL contain a deletion in the 3’LTR (deltaU3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" (SIN) of the vector after transduction of target cells. Once integrated into a transfected target cell, the lentiviral genome is no longer capable of producing viral genomic material that can be packaged. Moreover, deletion of enhancer and promoter elements from the 3’ U3 region in the vector construct will result in a provirus that is entirely devoid of the U3 enhancer sequences, which will result in a reduction in the potential for transactivation of cellular genes due to an insertion event. Furthermore, the development of self-inactivating vectors improves the biosafety of vectors, as they are less likely to be mobilised following a superinfection with wild-type virus (HIV).
* The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes. Heterologous envelopes, like VSV-G, typically broaden the tropism and allow gene transfer into a broad variety of cells. The risk will mitigated by the use of self-inactivating virus and limiting the number of viral particles that will be handled at any one time.

* Sequences encoding the proteins required for packaging of the viral genome are separated onto three plasmids, and all of the 4 plasmids used in the system have been engineered not to contain any regions of homology with each other so as to prevent undesirable recombination events that could lead to the generation of a replication-competent lentivirus (RCL), which could potentially be harmful to humans. It is important to note that no such RCL has ever been observed despite large-scale production and testing of lentiviral vectors.

* All of the pRRL containing plasmids/vectors will be devoid of all viral sequences apart from essential cis-acting sequences, including the LTRs and the packaging signal psi. Although the packaging plasmids used in these systems allows for the expression in trans of protein required to produce viral progeny (e.g. gag, pol, rev, env) in the HEK293T producer cell lines, none of them contain LTRs or the psi packaging sequence. Several of the lentiviral accessory genes (vif, vpr, vpu and nef) that are dispensable for lentivector production/transduction have been deleted from the packaged construct. Therefore, none of the retrovirus structural genes will actually be present in the packaged viral genome, as they will never be expressed in the transduced target cells, which means that no new RCL can be produced.

* The lentiviral particles produced in this system are replication-competent, only carry the sequences of interest, and no other viral species are produced.
* Expression of the gag and pol genes from pMDLgp-RRE has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Addition of the RRE in these plasmids prevents gag and pol expression in the absence of rev, which is contained in the pRSV-REV plasmid only. The Rev/RRE system is highly conserved among lentiviruses, and removal of the RRE sequence and associated splice donor/acceptor sequences result in a loss of transduction efficiency.

* LTR has been modified so as to increase lentiviral vector production, and also to allow lentiviral vector production to be independent of tat expression. It is known that Tat-deleted mutants of wild-type HIV-1 are not replication competent. Therefore, the deletion of Tat should decrease the risk of generating a putative RCL.

* Lentiviral vectors have a very low potential to cause immunogenicity.
* In the lentiviral vector that will be used, the WPRE (woodchuck post-transcriptional regulatory element) has been mutated to remove the oncogenic WHV-X protein from an open reading frame found in WPRE sequence.

While the lentiviral vectors that are produced using this system contain only about 20% of the original genome of HIV-1, there is a very small risk that subsequent infection of cells already infected with the lentiviral genome of HIV-1 could lead to a rare recombination event in which the transgene is transferred to a replication-competent virus. Thus, the sequences in the vector that will be expressed could potentially be transferred to surrounding cells. This event is, however, extremely unlikely to occur for a number of reasons:
1. The lentiviral vector is replication-competent and self-inactivating. In the case of a subsequent HIV-contamination, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host genome, although non-self-inactivating vectors can be.
2- In order for the spread of the gene of interest to occur following accidental infection (assuming that this has lead to viral integration), a series of unlikely events have to occur:

a- The worker would have to become infected with HIV-1 or to be already infected with the virus.
b- The viral and lentiviral genomes would have to integrate into the host worker's genome in the same cells and in a position where they could interact to effect homologous recombination (point 1 above).
c- Recombination would have to occur in just the right regions to allow for transfer of the gene of interest from the lentivirus to the HIV-1 genome, which could also involve the transfer of the HIV-1 genes to the lentiviral genome. In that case, it is conceivable that a non-self-inactivating HIV could be generated that contained the gene of interest but not the rest of the genome it requires. The other gene products could be provided in trans from the lentiviral genome that may now contain the HIV-1 genes or from other HIV-1 integrants.
d- The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest, but again no genes neccessary for subsequent replication. In order for another round of infection to occur, the process would have to begin again.

In these circumstances, the effects of lentiviral infection are likely to be minor in comparison to the effects of the HIV-1 infection, which would be required to affect the spread of the gene of interest. In addition, the scenario described is essentially equivalent to the rescue of the lentiviral genome from the host, which has already been shown not to occur.

3- It is extremely unlikely that any worker would infect themselves with a significant dose of lentivirus as the volumes that are used in transfection experiments are small (aliquots contain a maximum of 10μl of vector solution).

4- Moreover, insertional mutagenesis into the host genome may be considered as an oncogenic risk. We cannot rule out the possibility that, when the lentiviral genome integrates into the host genome, it will not lead to the activation of an endogeneous oncogene. However, all transcriptionally active long-terminal repeats (LTRs) have been removed as well as all promoter-like elements that required to drive expression of the transgene. This should prevent unforeseen activation of such genes. It is noted that deletion of retroviral enhancer in self-inactivating systems reduces the risk of activation but not of disruption, therefore, retroviral infection might still have permanent effects upon a cell (including oncogenic effects).

Importantly, we do not consider that the use of these lentiviral vectors will result in a significant increased risk of oncogenic activation compared to the risk possessed by any other viral delivery system. Moreover, the risk of transduction leading to tumourigenesis or other untoward harm following exposure is related in part to the titre of the viral vectors; exposure of workers to quantities of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of lentiviral vectors.

Transgenes:
Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.
• shRNA
With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be knocked-down, in order to study their functions. Potential deleterious effects such as off-target and immunomodulatory responses can be minimised through screening designed shRNA sequences against databases of known mammalian/mRNA sequences during the design stage. The genes/proteins of interest that will be targeted by shRNA do not and will not include any growth factors, trophic factors, quiescence factors, confirmed oncogenes, tumor suppressors and immunomodulators. Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. This sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of human, mouse or rat origin. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health.
• Programmable nucleases: zinc fingers and Cas9/CRISPR
Zinc fingers or Cas9 protein will be directed against genes of therapeutic interest. Since the purpose of programmable nucleases is to cause mutations in defined DNA sequences the primary hazard is that such a mutation would lead to deleterious cellular effects. The genes/proteins that will be targeted do not and will not include any growth, trophic factors, confirmed oncogenes, tumor suppressors or immunomodulators. In isolation, these sequences are unlikely to pose any safety risks for the environment or human health.
• Reporter genes: Fluorescent protein or enzymes
The reporter gene could be a fluorescent protein or an enzyme. Fluorescent proteins, for example GFP or dsRed, are not known to cause any diseases in human or
animals and have no direct effect on cellular processes. Enzymes used frequently in reporter gene assay ie luciferase of b-galactosidase are not known to cause any diseases in humans or animals and as no direct effect on cellular processes. The expression of fluorescense protein or enzymes under the control of a promoter of a gene of interest is unlikely to pose any safety risks for the environment or human health.

Summary on lentivirus
The transgenes have a low safety risk to human health because accidental contamination with a vector would lead to only a small number of cells becoming infected, and it would be highly unlikely that expression in only a few cells could be sufficient to lead to a disease state. The inability of the lentiviral vectors to propagate on mammalian cells also reduces the risk. The programmable nucleases (CRISPR or Zinc finger) that will be employed in this viral vector system are not known to cause any relevant toxicity that might represent a safety risk to human health. The fluorescent proteins or the enzymes are not known to cause any relevant toxicities that might represent a safety risk to human health. For both programmable nucleases and shRNA, the genes/proteins of interest that will be targeted will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators, and none of them are or will be of retroviral origin. Therefore the risk of use will be low.

B- Immortalised mouse or rat mesangial cells and transduced immortalised mesangial cells

The SV40 MES 13 cell line have been immortalised by the presence of the coding region for both large and small T-antigen along with its 72 and 21 base-pair repeats in the genome.

The immortalised RMC cell line contains the sequences coding for SV40 large T-antigen and neomycin. SV40 virus is a polyomavirus that is found in both monkeys and humans. As described, both cell lines, SV40 MES 13 and RMC, are from rodent origin and contain the sequences coding for SV40 small and or large T-antigen. Large and Small T antigen have been described to be sufficient to transform human cells. One of the major risk factor associated with the utilisation of this cell line would be accidental percutaneous inoculation of a worker. These mouse and rat cell lines could theoretically not survive but would be quickly cleared by the human immune system. One of the major risks would be if these constructions coding for the small T-antigen and/or Large T antigen are mobilised. This could happens if the mesangial cells are accidentally inoculated to a worker already infected by a retroviral genome. Even in that case, as series of unlikely events would have to occur to:

a. The worker would have to be already infected with the HIV-1 virus
b. The retroviral genomes would have to integrate into the immortal mouse or rat mesangial cells genome in a position where they could interact to effect homologous recombination.
c. Recombination would have to occur in precise regions to allow for transfer of the SV40 T antigen sequences from the mesangial genome to the HIV-1 genome.
d. The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest.

Although the risk for human health is low, all measures will be in place to minimize the risk of human health to avoid any accidental inoculation of a worker (see measures below).

Further modification of the immortalised mouse or rat mesangial cell lines may enhance the risk of the mobilisation of the sequence coding for small and/or large T antigen sequences.

Application to these cells of additional lentivirus particules could result to the mobilisation of the small and or large T antigen sequence and the liberation of replication-competent retroviruses by genetic recombination events. This risk will be minimised by confirming that the viral genes gag-pol and env are not present in the genome of the immortalised mouse and rat mesangial cells before the application of the lentivirus used on the cells. Furthermore, the risk can be minimised by ensuring that any lentivirus used in experiments with the host cells does not contain gag-pol, env or any other significant retroviral genes involved in packaging and replication. To ensure that any replication-competent retroviruses by genetic recombination events had occurred, the supernatant of the mesangial cells will be tested for the production of retroviruses by application on HEK293 cells. Even if the events are unlikely, the immortalised mouse and rat mesangial cell line and the transduced mesangial cells will be maintained at biosafety level 2 containment. Therefore all measures will be in place to minimize to risk to human health.

Human health hazards
Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosal cells with modified viruses. However, these modified virus
particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the programmable nucleases and shRNA that will be expressed or targeted will not be designed to disrupt any growth factors, trophic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. In the unlikely event of accidental transduction of a laboratory worker’s skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus or the mesangial cell line will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent. Infection of the community at large by immortalised mouse or rat mesangial cells is highly unlikely due to the impossibility of the cells to survive outside of a laboratory environment.

* Environment considerations

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active lentiviral vector particles do accidently get released into the environment, the safety risk posed such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable replication-competent lentivirus. However, this scenario is extremely unlikely and even if it was to occur, it is unlikely that it would lead to any untoward effects. As the HIV virus is a human pathogen infection of another species would not be expected to allow any form of recombination event leading to a viable transmissible entity and so the risk from this scenario is considered to be negligible. Rodents, such as wild-type mice and rats, cannot support replication of infectious HIV-1 (Goffinet et al, 2007 Retrovirology). As a result, the potential for shedding of replication-competent lentiviruses from such animals is very low (even if they were present in the original vector inoculum).

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and in theory they are capable of transducing all dividing and non-dividing mammalian cell types. As emphasized before, the vectors cannot self-propagate after infection, and successful transduction is critically dependent on the presence of high enough concentrations of virus particles. Viral vector stock solutions will be managed in a way that will prevent contaminations with relatively high viral titres. After production, the vectors are aliquoted and stored in screw capped cryovials at -80°C. Taking into account that the infectivity of the vectors rapidly decreases at room temperature, it is considered highly unlikely that the vectors could survive in the long term after being accidentally released into the environment. Lentiviral vectors have a short half-life, at room temperature due to their structural characteristics making them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, as illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

The risk to the environment following accidental release of the immortalised rat or mouse mesangial cells would be negligible since the cells are incapable of surviving outside of laboratory conditions. If live mouse or rat mesangial cells were to directly inoculate animals, they could theoretically survive but would be quickly cleared by the immune system and would not produce active viruses. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures and ensuring that all potentially contaminated material is totally inactivated before disposal.

Summary

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to. In the unlikely event that active viral vector particles do accidently get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems. It is also unlikely that the immortalised mouse and rat mesangial cells and the transduced mesangial cells will be accidentally released into the environment, as the cells handling and studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to.
The work outlined above has been classified as a Biosafety level 2 activity, even though the risk of pathogenicity to the end worker is considered to be very low. In all cases, HSE prescribed biosafety level 2 conditions should be adhered to when handling the immortalised mouse or rat mesangial cells, generating the viral vectors in HEK293T cells, as well as their subsequent storage at -80°C. For subsequent in vitro transduction of the cells types mentioned in this proposal (e.g. human HEK293T, immortalised mouse or rat mesangial cells) will be performed under biosafety level 2 conditions. The human transduced HEK293T used to assess the efficacy of the vectors will be converted to biosafety level 1 once the medium containing the virus has been removed and the infected cells have been extensively washed. The immortalised mouse or rat mesangial cells transduced by lentivirus will be maintained under biosafety level 2.

Procedures:
During all procedures that involve the handling/use of the viral vectors or cells, all workers (incl. those that are not working directly with the vectors) that are present within a lab where the work is undertaken are required to wear personal protective equipment at all times. The use of two pairs of gloves is advised so the external pair can be disposed in an autoclave bag and replaced whenever necessary (e.g. in the case of contact with the viral reagent). Face masks are also available for use, where appropriate. In addition, all workers are made aware of the nature of the viral work that is going on within the laboratory, and they have to follow appropriate procedures to ensure that there is no cross-contamination into non-viral working area. All the workers using the viral delivery systems are experienced research scientist, and junior scientists will be closely supervised until they are competent in the handling of the viruses.

Lentiviral production
Lentiviral production will also be carried out on-site using a modified procedure based on Bukovsky et al. (Bukovsky et al, 1999, J. of virology). Lentiviral production will only be carried out in HEK293T cells in a biosafety level 2 cabinet within a culture flask with screw cap filter in order to minimize the risk of spills and/or aerosol mediated viral spread. For this the modified PRRL plasmids together with three additional separate plasmids (pMDLgp-RRE, pRSV-REV and pMD2-VSVG.env) will be transfected using a transfection reagent (e.g. Polyethylenimine (PEI), Lipofectamine) into HEK293T packaging line. After 6-12 hours, the medium is replaced with fresh serum-free medium and grown for an additional 72-96 hours. After 24-48 hours, so as to ensure the viability of the transducted HEK293T cells, medium form the cells is collected into screw cap tube and stored at 4°C and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the harvest tube. After lentivirus collection has been completed, the cells and the flask will be decontaminated and disposed of a detailed later. Still within a biosafety level 2 safety cabinet, the harvested supernatants will be filtered using a 0.45um filter before either aliquotted or further purified by chromatography and/or concentrated by centrifugation. The lentiviral particles may need to be purified by either chromatography and eluted in a high salt solution and/or concentrated by ultrafiltration. For certain application where highly concentrated and purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimize the possible risk of accidental viral contamination and spread due to the need for an ultracentrifuge, all loading and unloading will be done in the biosafety level 2 safety cabinet. Once the sucrose cushions have been added to the centrifuge tubes, the unpurified or chromatography purified lentiviral particles will be added to the sucrose cushion. The individually capped tubes will then be transferred to their respective bucket holders and the bucket sealed using their respective screw cap marks and marked as containing virus. The buckets containing viruses will be balanced with a buckets containing a tube with water and without lentivirus. The balanced buckets will then be transferred to the rotor before being placed within the centrifuge chamber. The ultracentrifuge will be run at 70000g for 2 hours at 20°C. Upon return of the buckets to the biosafety level 2 cabinet, the supernatant will be removed from the tubes before transfer of the resuspended virus pellet into individual screw cap cryovials. As the greatest risk for contamination occurs within the centrifugation buckets themselves during transport to and from the weight scales and during ultracentrifuge, the centrifuge tubes and the buckets and the rotor will be decontaminated with spraying Chengene HLD4L 5%. Optionally, the centrifuge rotor can be autoclaved at 121°C. In any case, concentrated virus stock will not be exposed to the environment outside of the biosafety level 2 cabinet. All supernatants will be treated as above.

The lentiviral titre will be determined in terms of number of viral particles (VP) or transduction units (TU) per mL. The number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based enrichment assay or a p24 ELISA assay will be chosen. The VP quantification methods tend to overestimate the TU by 10-1000 fold. Thus, the TU/mL will in certain instances also determined. For this, a titration range of lentiviruses expressing a fluorescent tag will be prepared and added to a 24 well cluster plate or T25 TC-flasks containing HEK293T for 48 hours. The percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using FACS analyser.

Aliquots with not more than 1x10^6 viruses per vial are stored at -80°C in individual screw capped cryotubes. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to, inorganic
In vitro studies
Transduction of cells (e.g., human HEK293T, mouse or rat mesangial cells) with the lentiviral vectors will be carried out in a Biosafety level 2 cabinet within a dedicated Biosafety level 2 laboratory. In the in vitro studies, the TUs, and consequently volumes added, will be dependent on the number of cells in the tissue culture dish/flask to be infected, up to the maximum number of viral particles to be handled per experiment (around 1 x 10^8). None of these cells will be cultivated in the filter screw cap flasks used for HEK293T mediated lentiviral production. This is to ensure that, in particular, the HEK293T cells used for lentiviral production are not inadvertently used for lentiviral assessment. Any plastic ware or solutions that are used to handle the transduced cells will be decontaminated and disposed of as detailed later (see Waste Handling). Once the medium containing the lentiviral particles has been removed and the cells (e.g., human HEK293T, mouse or rat mesangial cells) have been extensively washed, which all is done in a Biosafety level 2 cabinet, the human HEK293T cells can be used following Biosafety level 1 guidelines, as no virus will be present and the latent virus in the transduced cells is replication-incompetent. The immortalised human podocytes will be used following Biosafety level 2 guidelines. A viral titre determination assay may on occasion be used to verify the absence of virus in the medium from human HEK293T cells and immortalised human podocyte cells.

Cleaning SOPs

General cleaning procedures
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all of the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being autoclaved/incinerated.

* Chemgene HLD4L
Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls of micro-organisms. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, Hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1%) for general purposes, 1:20d for high risk areas, and 1:20 (5%) for disinfection of blood and bio-hazard spillages.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General cleaning procedures
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being autoclaved/incinerated.

Waste Handling
Following all work requiring biosafety level 2 containment including work with lentiviruses and immortalised mouse or rat mesangial cells, solid waste will be neutralised by soaking in a validated disinfectant solution, after which the material will be “double bagged”, autoclaved and, ultimately, incinerated offsite. It will be routine practise that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Liquid waste will be inactivated by Chemgene HLD4L* for 24 hours prior to drain disposal as per 430CSP and 418CSP permit requirements.

As any active viral particles or immortalised mouse or rat mesangial cells on surface or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles or immortalised mouse or rat mesangial cells would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.
Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1%) for general purposes, 1:20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The host cell lines have been immortalised using genetic material encoding SV40 T antigens which are capable of immortalising human cells. This coding sequence cannot be mobilised. In the event of accidental injection into a healthy worker the immune system would rapidly destroy any cells so negating any risk. The proposed work involves the use of lentivirus to deliver various constructs into the aforementioned cells. The lentivirus will be generated using a third generation methodology which requires 4 different plasmids, that do not share sequence homology, to be transfected into the same packaging cell. The isolation of the genes required for virus production minimises the chance of accidentally generating replication competent virus (RCV). The desired packaged genetic material also undergoes self-inactivation upon integration into a host genome after infection of target cells. As this is a permanent change it is noted that no tumour suppressors, oncogenes or immune modulators will be targeted. The host cells contain the transformative SV40 T antigens so it is important to prevent mobilisation of this genetic material. There will be no homologous sequence present between the lentivirus and the SV40 T antigen coding region. Combined with the minimal chance of RCV being present will effectively prevent the generation of virus containing SV40 T antigen. The lentivirus being produced, although pseudotyped to have broad tropism, poses a minimal risk to the environment as it is highly unlikely to be able to infect an animal outside of the laboratory environment in the case of accidental release and the material will also rapidly deactivate at room temperature.

Although the risks presented here are minimal the GMSC agrees that this work will be conducted at biosafety level 2 to ensure containment is maintained because the material is being delivered into cells using lentiviruses and those cells contain genetic material encoding SV40 T antigens.

Project Containment

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02/03/2022
Use of the Expi293 MembranePro Expression technology system (Life Technologies) for the expression and display of mammalian cell surface membrane proteins including G-protein coupled receptors (GPCR), ion channels transporter and other membrane bound molecules in an aqueous-compatible format.

Expi293F cells are based on the HEK293 (primary human embryonic kidney cells) immortalised cell line. The Expi293F will be used to generate the VLPs, these cells have been adapted from HEK293 cells to grow in high density suspension culture. The HEK293 cell line is a permanent line established from primary embryonic human kidney cells transformed with sheared human adenovirus type 5 DNA. The E1A adenovirus gene expressed in HEK293 cells participates in the transactivation of some viral promoters, allowing these cells to produce very high levels of protein. This enables the efficient generation of viral capsids when these genes are introduced transiently into the the cell. The adenovirus genetic sequence present in HEK293 cells has been found to be non mobilisable and as such is not packaged into viral particles. This means that the cells themselves are essentially non-hazardous. These properties have meant that many virus packaging cell lines have been derived from the HEK293 cell line.

Recipient or parental organism
Expi293F cells are based on the HEK293 (primary human embryonic kidney cells) immortalised cell line. The Expi293F will be used to generate the VLPs, these cells have been adapted from HEK293 cells to grow in high density suspension culture. The HEK293 cell line is a permanent line established from primary embryonic human kidney cells transformed with sheared human adenovirus type 5 DNA. The E1A adenovirus gene expressed in HEK293 cells participates in the transactivation of some viral promoters, allowing these cells to produce very high levels of protein. This enables the efficient generation of viral capsids when these genes are introduced transiently into the the cell. The adenovirus genetic sequence present in HEK293 cells has been found to be non mobilisable and as such is not packaged into viral particles. This means that the cells themselves are essentially non-hazardous. These properties have meant that many virus packaging cell lines have been derived from the HEK293 cell line.

Host/vector system
The system uses pEF-V5-His TOPO to express the gene of interest. This plasmid does not contain any lentiviral
genes and therefore poses no risk. The lentiviral gag gene or protein (packaging mix) is provided in enhancer solutions provided with the Expi293 Membrane Pro Expression Technology system kit which will be used in the production of the VLPs. The exact nature of this enhancer material is propriety to Life Technologies but as it contains the ability to supply the gag gene or protein it will be managed as needing to be treated as a class 2 material. This system lacks any other lentiviral genes and as such does not produce or encapsulate viral DNA. Once the VLPs have been generated and purified the enhancer solution will have been removed.

Origin & function

The system relies upon the presence of the lentiviral gag core structural protein to drive the self assembly of sub viral particles that should not encapsulate DNA. These VLPs bud off from the cell surface taking membrane proteins with them. As such they will contain the proteins of either GPCR, ion channel, enzymes, transporters or other cell surface membrane proteins that are expressed in the cell.

Targets of interest that will be over-expressed for incorporation into VLPs will not be known to have cytotoxic, oncogenic or immunomodulatory effects. The purified VLPs can then be used in a variety of vitro assays to assess small molecule, peptide and protein interactions with the protein of interest present in the VLP membrane.

Evaluation of foreseeable effects

Human Health Hazard
The production method for VLPs means that it is highly unlikely that infectious virus can ever be generated. The only lentiviral gene present in the production of the VLPs is the gag gene. As the system lacks the env and pol genes infectious virus particles can not be generated and there is no viral genome to be packaged. The vectors used to express the genes of interest do not contain packaging sequences and will not therefore be packaged into the VLPs. These vectors will essentially be absent once the VLPs have been purified. There is a small chance that the gag gene and/or the vector encoding the gene of interest introduced in trans may become trapped with a VLP as it forms. As such there is a theoretical risk that if fusogenic particlles do come in to contact with cells it may introduce DNA and lead to a permanent genetic change in the cell. This risk is minimal because the number of VLPs containing any DNA will be minimal, they will be dilute and as they lack the env protein they are highly unlikely to be able to infect cells. To ensure that the other lentiviral genes are not introduced into the system VLPs will not be used with other cell cultures and production will be kept separate from cells being used for the production of lentiviral vectors. As the system does utilise a lentiviral gene during the generation of the VLPs this work will be carried out at biosafety level 2. The generation of VLPs will therefore be treated as a class II activity. Standard health and safety procedures of working will be followed throughout to eliminate any risk of infection.

Hazard to the environment
The chance that infectious lentivirus is released into the environment is considered to be negligible. The VLPs that are generated are designed to only contain the gag protein of the lentiviruses and not the gag gene. Infectious VLP generation would have to involve the gag gene encountering the remaining pol or env genes and infecting cells in the environment, the risk of this is negligible given that the VLPs lack the product of the env gene and are highly unlikely to be able to infect cells. In the event of release of the VLPs into the environment, they are labile outside of the laboratory environment being sensitive to temperature and will rapidly degrade at room temperature.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
The production of the VLPs is the point at which large volumes of concentrated material will be generated in a system.
that has had the gene encoding gag present. The purification process will essentially remove genetic material. Once aliquots of this final material has been dispensed there should be essentially no genetic material present. Subsequent experiments will not involve the use of cells and limited amounts of material will be diluted and it is thought appropriate to handle this material with good laboratory practice.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste, including pipette tips and tissue culture flasks will be neutralised by soaking in a validated disinfection solution (5% HLD4L solution, Chemgene) for 24 hours, after which the material will be double bagged, autoclaved and taken off site for incineration. Liquid waste will be chemically deactivated with 5% HLD4L solution, Chemgene for a minimum of 24 hours prior to drain disposal via a designated sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Project Containment

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Project Ref 3043/16.1
The human immortalised neural progenitor cells will be used as a tool cell line in Neurodegeneration to investigate cellular pathways involved in the development of neurodegenerative disorders, in particular Huntington's.

To better understand these cellular pathways, the expression or the activity of the gene of interest will be modulated by several methods:

1. The utilisation of tool compounds
2. The knock-down of the gene of interest by shRNA
3. The generation of modified somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
4. The generation of reporter gene cell line (e.g., fluorescence protein or enzyme) under the promoter of the gene of interest.

The experiments involving the transfection of vectors such as shRNA or programmable nucleases may be performed by lentivirus. The effect of modulating the expression or the activity of the target of interest will be determined by the expression level of the proteins, mRNA or mediators involved in the target signalling pathway.

Recipient or parental organism

1. The human immortalised neural progenitor cells, ReNcell 197VM
   - ReNcell VM is an immortalized human neural progenitor cell line with the ability to readily differentiate into neurons and glial cells.
   - ReNcell 197VM was derived from the ventral mesencephalon region of a human fetal brain tissue. Immortalized by retroviral transduction, using replication incompetent retrovirus, with the avian v-myc myelocytomatosis viral oncogene.
   - These immortalised human neural progenitor cells, ReNcell 197VM, will be purchased from Millipore. Millipore have tested the cell line and shown that it does not produce infectious virus particles.
2. Human immortalised neural progenitor cells, ReNcell mHTT Exon1 197VM
• ReNcell 197VM cells were transduced with lentiviral virus particles containing varying length HTT exon 1, eGFP, cPPT, WPRE, LTR U3 del (pHRsincpptUCOE+htt exon1 IRES eGFP 29CAG/71CAG/129 CAG WPRE).
These immortalised human neural progenitor cells, ReNcell mHTT Exon1 197VM, will be provided by UCL. This cell line contains the v-myc oncogene, a WPRE element that may contain the WHV-X oncogene ORF and therefore will be maintained in a biosafety level 2 confinement.

Description of the lentivirus containing shRNA, programmable nucleases sequences or gene reporter vectors
To utilise the neural progenitor cells to study neurodegeneration, the expression of a target of interest may be decreased in the ReNcell line by the transduction of shRNA or programmable nuclease by lentivirus.

The modulation of a particular cellular pathway may be studied with the use of a reporter gene assay. In this case, the vector containing the reporter gene under the regulation of the promoter of the gene of interest may be introduced by lentivirus into the ReNcells. In addition other exogenous genes may also be introduced using lentiviral vectors.

This proposal may involve both the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site using the third or fourth generation system. Both commercially acquired and in-house lentivirus will use the same transfer vector plasmid. Target genes will be selected based on the needs of drug discovery projects; programmable nucleases and shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators (these targets will be presented to the GMSC for approval prior to any work taking place).

The production of lentivirus containing the vectors of interest are described below:
1- Generation of lentivirus

Executive

Lentiviral vectors will be purchased commercially or produced in-house using the third or fourth generation system described below. Each purified and concentrated aliquot of lentiviral reagent comprised of a small volume (5 to 10ul) that contains no more than 1 X 10^8 viral particles.

Vector System = self-inactivating (SIN) non replication-competent HIV based lentiviral vectors

The viral vectors that will be used in the proposed studies are self-inactivating non replication-competent HIV based lentiviral vector systems, which have been deSignated to produce stable gene expression in mammalian cells; and they are generated by co-transfecting HEK 293T cells with four to six separate plasm ids. Each of the plasm ids used expresses a different set of genes which, when combined following co-transfection, provide the smallest possible set of essential viral genes that is still compatible with virus production. Moreover, the vectors are all vesicular stomatitis virus (VSV-G) pseudotyped lentiviral vectors, which are self-inactivating and highly unlikely to undergo recombination. Importantly, the viral vector is inherently incapable of replication in mammalian cells.

The plasm ids

Third Generation

The sequences that will be included in the viral vectors are sub-cloned into 4 standard bacterial plasmid vectors.

• Plasm ids containing the packaging related sequences

Each of the three other plasm ids, pCgpV, pRSV-Rev and pCMV-VSVG contain a different set of sequences that code for proteins that are responsible for packaging the viral vectors. All the genes (gag-pol, rev and env) required for production and packaging of the full length viable viral RNA particles have been removed from the pSMPUW lentiplasm ids containing the transgenes of interest.

Fourth Generation

The sequences that will be included in the viral vectors are sub-cloned into 6 standard bacterial plasmid vectors

• Plasm ids containing the packaging related sequences
Each of the other plasmids, pTRE-gag-pro, LTRHIV2-vpr-pol, penvVSV-G, pTet-Off and ptat-IRES-rev contain a different set of sequences that code for proteins that are responsible for packaging the viral vectors. All the genes (gag-pol, rev and env) required for production and packaging of the full length viable viral RNA particles have been removed from the pSMPUW lenti-plasmids containing the transgenes of interest. An integrase deficient version of the fourth generation packaging mix is available which contains a mutation in the sequence encoding the viral integrase. The resulting integrase-deficient lentivirus (IDLV) generates circular vector episomes in transduced target cells that are gradually lost by dilution in dividing cells (transient expression), but are stable in quiescent cells.

### Plasmids containing the transgene of interest

The fourth plasmid, pSMPUW, will contain the sequences that code for shRNA, or programmable nuclease sequence, or reporter gene sequence under the promoter of the gene of interest.

**a- Vector containing shRNA**

As mentioned above, pSMPUW plasmid will contain the sequences that code for one shRNA transcript that will target one mRNA/protein of interest (or a scrambled shRNA in the case of negative control). The shRNA or scrambled shRNA sequences will be under the control of polymerase II or III promoter. The shRNA sequences will be chosen to target a single gene. The shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators.

**b- Vector containing CRISPR or Zinc-finger nucleases**

Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the cleavage site or by recombination with a user-supplied synthetic vector containing sequences complementary to the target gene allowing insertion of exogenous genetic sequence. For Cas9/CRISPR, nuclease target specificity is determined by an accessory sequence, the guide RNA, which consists of a short (18-20 nucleotide) sequence homologous to the target gene and an additional short sequence that forms a complex with the Cas9 enzyme. In this case, the plasmid pSMPUW, will contain the sequences that code for a Cas9/CRISPR plus accessory sequences comprising a short guide RNA that determines target specificity. The guide RNA sequences will be under the control of a Polymerase III promoter.

### Health and Safety Executive

In the case of Zinc finger nucleases target specificity is dependent upon the zinc finger protein sequence. The pSMPUW will contain in that case the sequence of zinc finger nucleases under the control of either a polymerase II promoter. In addition, a fluorescent protein or enzyme under the control of a polymerase II promoter or the promoter of a gene of interest may be included. Control viral vectors that express only the fluorescent protein/enzyme and no programmable nucleases will be used in some experiments.

The Cas9/CRISPR, if introduced into any cell line by lentivirus, will use the integrase deficient version of fourth generation packaging mix.

**c- Reporter gene vector**

The pSMPUW vector will contain the sequence coding for a reporter protein (enzyme or fluorescent protein) under the control of the regulating elements of the promoter of the gene of interest or the a polymerase II promoter. An appropriate selection marker i.e.; neomycin could also be included to allow the selection of stable human neural progenitor cells containing the reporter gene sequence under the control of the promoter of the gene of interest. Target genes will be selected based on the needs of drug discovery projects; programmable nucleases, shRNA or exogenous genetic material will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators (proposed targets will require approval internally by
Procedures:

During all procedures that involve the handling/use of the viral vectors or cells, all workers (incl. those that are not working directly with the vectors) that are present within a lab where the work is undertaken are required to wear personal protective equipment at all times. The use of two pairs of gloves is advised so the external pair can be disposed in an autoclave bag and replaced whenever necessary (e.g. in the case of contact with the viral reagent). In addition, all workers are made aware of the nature of the viral work that is going on within the laboratory, and they have to follow appropriate procedures to ensure that there is no cross-contamination into non-viral working area. All the workers using the viral delivery systems are experienced research scientist, and junior scientists will be closely supervised until they are competent in the handling of the viruses.

Lentiviral production

Lentiviral production will also be carried out on-site using a modified procedure based on Bukosvsky et al. (Bukosvsky et al, 1999, J. virology). Lentiviral production will only be carried out in HEK293T cells in a biosafety level 2 cabinet within a culture flask with screw cap filter in order to minimize the risk of spills and/or aerosol mediated viral spread. For this the modified pSMPUW plasmids together with three to five additional separate plasmids (pCgpV, pRSV-Rev and pCMV-VSVG or pTRE-gag-pro, L TRHIV2-vpr-pol, penv(VSV-G), pTet-Off and p tat-IRES-rev) will be transfected, using a standard chemical transfection method, into HEK293T packaging line. After 6-12 hours, the medium is replaced with fresh serum-free medium and grown for an additional 72-96 hours. After 24-48 hours, so as to ensure the viability of the transfected HEK293T cells, medium from the cells is collected into screw cap tube and stored at 40°C and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the first harvest tube. After lentivirus collection has been completed, the cells and the flasks will be decontaminated and disposed of (detailed later). Still within a biosafety level 2 safety cabinet in a biosafety level 2 laboratory, the harvested supernatants will be filtered using a 0.45um filter before either aliquoted or further purified by chromatography and/or concentrated by centrifugation. The lentiviral particles may need to be purified by either chromatography and eluted in a high salt solution and/or concentrated by ultrafiltration. For certain applications, where highly concentrated and purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimize the possible risk of accidental viral contamination and spread due to the need for an ultracentrifuge, all loading and unloading will be done in the biosafety level 2 safety cabinet. Once the sucrose cushions have been added to the centrifuge tubes, the unpurified or chromatography purified lentiviral particles will be added to the sucrose cushion. The individually capped tubes will then be transferred to their respective bucket holders and the bucket sealed using their respective screw caps and marked as containing virus. The buckets containing viruses will be balanced with buckets containing a tube with water and without lentivirus. The balanced buckets will then be transferred to the rotor before being placed within the centrifuge chamber. The ultracentrifuge will run at 70000g for 2 hours at 200°C. Upon return of the buckets to the biosafety level 2 cabinet, the supernatant will be removed from the tubes before transfer of the resuspended virus pellet into individual screw cap cryovials. As the greatest risk for contamination occurs within the centrifugation buckets themselves during transport to and from the weight scales and during ultracentrifuge, the centrifuge tubes, the buckets and the centrifuge rotor will be decontaminated by spraying Chemgene HLD4L 5%. Optionally, the centrifuge rotors could be autoclaved at 1210°C. In any case, concentrated virus stock will not be exposed to the environment outside of the biosafety level 2 cabinet. All supernatants will be treated as above.

The lentiviral titre will be determined in terms of number of viral particles (VP) or transduction units (TU) per mL. The number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based enrichment assay or a p24 ELISA assay will be chosen. The VP quantification methods tend to overestimate the TU
by 10-1000 fold. Thus, the TU/mL will in certain instances also be determined. For this, a titration range of lentiviruses expressing a fluorescent tag will be prepared and added to a 24 well cluster plate or T25 TC-flasks containing HEK293T for 48 hours. The percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using FACS analyser/Bioanalyser. The biological titre (TU/mL) according to the following formula: 

\[
\text{TU/ul} = (P \times N \times 100 \times V) \times 1/10D, \quad \text{where } P = \% \text{ GFP+ cells}, \quad N = \text{number of cells at time of transduction} = 10^5, \\
V = \text{volume of dilution added to each well and } OF = \text{dilution factor} = 1 \text{ (undiluted), 10-1 (diluted 1/10), 10-2 (diluted 1/100), and so on.}
\]

Aliquots with not more than 1x10^8 viruses per vial are stored at -80°C in individual screw capped cryotubes. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to, inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats.

a- Assessing efficacy of the lentivirus into a BSL 1 cell line

Assessing whether the genome manipulation or the knock-down has worked will normally take place initially in biosafety level 1 immortalised cell line that are known not to contain lentiviral genetic material. Application of lentiviral particles will be done at biosafety level 2 confinement. After at least 24 hour incubation and extensive washing, the cells will treated as biosafety level 1 (see below the justification for removal of cells post-infection to biosafety level 1).

In order to determine whether the knock-down or the genome manipulation has worked, the levels of mRNA and protein of the gene of interest will be assessed post-transduction.

b- Transduction of the lentivirus and the generation of stable expression into ReNcell VM neural progenitor cells

Once validated the same procedure will be carried out in the ReNcell VM neural progenitor cells. As these cells are maintained in a biosafety level 2 confinement, the inoculation of lentiviral particles, the transformed cells and the phenotypic analysis will also be performed in a biosafety level 2 containment. When a lentivirus containing a programmable nuclease or the reporter gene will be used, a stable cell line could be established by selecting the cells in which the genome has been modified. In all cases phenotypic analysis will be performed using suitable in vitro assays, including for example, RT-PCR, Western Blotting, immunocytochemistry and imaging, or cellular bioenergy (Le.: mitochondria respiration function or glycolysis function).

**Evaluation of foreseeable effects**

* Human Health

The immortal human ReNcell VM neural progenitor cell that will be used has a low risk to human health. The expression systems and the transgenes that comprise the lentiviral vectors that will be used have also a very low risk for human health and safety, and the reasons for this are discussed below.

A- Lentivirus containing shRNA, or programmable nucleases sequence or reporter gene vectors

**Lentiviral Vector Expression System**

Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. As the lentiviral vector system that will be used in these studies is based on HIV-1, a theoretical risk to human health exists. However, retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airbone route. Therefore, accidental piercing of the skin or other surface tissues with virus containing objects represents the main potential route by which accidental infection could occur. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

In all of the modified pSMPUW plasmids (containing shRNA, or programmable nucleases or reporter gene sequences), the sequences inserted are flanked by non-coding retroviral L TRs, and no retroviral genes are encoded on the modified pSMPUW plasmid ids. Therefore, no retroviral genes will be transferred into generated viral particles. This construct is packaged into particles using a HEK293T cell based packaging system, which requires the cotransfection of these cells with three to five additional separate plasmid ids (as detailed above). The additional plasmid ids express the envelope protein from VSVg and the non-structural proteins of the virion, and, importantly, none of these
genes will be transferred into the assembled viral vectors, since they lack the packaging signal (psi), which is only present on the modified pSMPUW plasmids. The lentiviral Expression System what will be used include the following key safety features:
* All of the pSMPUW contain a hybrid 3'LTR that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" (SIN) of the vector after transduction of target cells. Once integrated into a transfected target cell, the lentiviral genome is no longer capable of producing viral genomic material that can be packaged. Moreover, presence of an SV40 polyA after the hybrid 3'L TR in the vector construct will result in a provirus which should reduce the potential for transactivation of cellular genes due to an insertion event. Furthermore, the development of self-inactivating vectors improves the biosafety of vectors, as they are less likely to be mobilised following a superinfection with wild-type virus (HIV).
* The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes. Heterologous envelopes, like VSV-G, typically broaden the tropism and allow gene transfer into a broad variety of cells. The risk will mitigated by the use of self-inactivating virus and limiting the number of viral particles that will be handled at anyone time.
* Sequences encoding the proteins required for packaging of the viral genome are separated onto three to five plasmids, and all of the 4-6 plasmids used in the system have been engineered not to contain any regions of homology with each other so as to prevent undesirable recombination events that could lead to the generation of a replication-competent lentivirus (RCL), which could potentially be harmful to humans. It is important to note that no such RCL has ever been observed despite large-scale production and testing of lentiviral vectors.
* All of the pSMPUW containing plasmids/vectors will be used are devoid of all viral sequences apart from essential cis-acting sequences, including the L TRs and the packaging signal psi. Although the packaging plasmids used in these systems allows for the expression in trans of protein required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK293T producer cell lines, none of them contain L TRs or the psi packaging sequence. Several of the lentiviral accessory genes (vif, vpr, vpu and nef) that are dispensable for lentiviral vector production/transduction have been deleted from the packaged construct. Therefore, none of the retrovirus structural genes will actually be present in the packaged viral genome, and they will never be expressed in the transduced target cells, which means that no new RCL can be produced.
* The lentiviral particles produced in this system are replication-incompetent, only carry the sequences of interest, and no other viral species are produced.
* Expression of the gag and pol genes from pgag-pol-RRE has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Addition of the RRE in these plasmids prevents gag and pol expression in the absence of rev, which is contained in the pRSV-REV plasmid only. The Rev/RRE system is highly conserved among lentiviruses, and removal of the RRE sequence and associated splice donor/acceptor sequences result in a loss of transduction efficiency.
* L TR has been modified so as to increase lentiviral vector production, and also to allow lentiviral vector production to be independent of tat expression. It is known that Tat-deleted mutants of wild-type HIV-1 are not replication competent. Therefore, the deletion of Tat should decrease the risk of generating a putative RCL.
* Lentiviral vectors have a very low potential to cause immunogenicity.
* The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction.

Health and Safety

Executive

While the lentiviral vectors that are produced using this system contain only about 20% of the original genome of HIV-1, there is a very small risk that subsequent infection of cells already infected with the lentiviral genome of HIV-1 could
lead to a rare recombination event in which the transgene is transferred to a replication-competent virus. Thus, the sequences in the vector that will be expressed could potentially be transferred to surrounding cells. This event is, however, extremely unlikely to occur for a number of reasons:

1- The lentiviral vector is replication-incompetent and self-inactivating. In the case of a subsequent HIV-contamination, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host genome, although non-self-inactivating vectors can be.

2- In order for the spread of the gene of interest to occur following accidental infection (assuming that this has lead to viral integration), a series of unlikely events have to occur:
   a- The worker would have to become infected with HIV-1 or to be already infected with the virus.
   b- The viral and lentiviral genomes would have to integrate into the host worker's genome in the same cells and in a position where they could interact to effect homologous recombination (point 1 above)
   c- Recombination would have to occur in just the right regions to allow for transfer of the gene of interest from the lentivirus to the HIV-1 genome, which could also involve the transfer of the HIV-1 genes to the lentiviral genome. In that case, it is conceivable that a non-self-inactivating HIV could be generated that contained the gene of interest but not the rest of the genome it requires. The other gene products could be provided in trans from the lentiviral genome that may now contain the HIV-1 genes or from other HIV-1 integrants.
   d- The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest, but again no genes neccessary for subsequent replication. In order for another round of infection to occur, the process would have to begin again.

In these circumstances, the effects of lentiviral infection are likely to be minor in comparison to the effects of the HIV-1 infection, which would be required to affect the spread of the gene of interest. In addition, the scenario described is essentially equivalent to the rescue of the lentiviral genome from the host, which has already been shown not to occur.

3- It is extremely unlikely that any worker would infect themselves with a significant dose of lentivirus as the volumes that are used in transfection experiments are small (aliquots contain a maximum of 10IJL of vector solution).

4- Moreover, insertional mutagenesis into the host genome may be considered as an oncogenic risk. We cannot rule out the possibility that, when the lentiviral genome integrates into the host genome, it will not lead to the activation of an endogeneous oncogene. However, all transcriptionally active long-terminal repeats (LTRs) have been removed as well as all promoter-like elements that required to drive expression of the transgene. This should prevent unforeseen activation of such genes. It is noted that deletion of retroviral enhancer in self-inactivating systems reduces the risk of activation but not of disruption, therefore, retroviral infection might still have permanent effects upon a cell (including oncogenic effects).

Importantly, we do not consider that the use of these lentiviral vectors will result in a significant increased risk of oncogenic activation compared to the risk possessed by any other viral delivery system. Moreover, the risk of transduction leading to tumourigenesis or other untoward harm following exposure is related in part to the titre of the viral vectors; exposure of workers to quantities of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of lentiviral vectors.

Transgenes:
Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.

shRNA
With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be knocked-down, in order to study their functions. Potential deleterious effects such as off-target and immunomodulatory responses can be minimised through screening designed shRNA sequences against databases of
known mammalian/mRNA sequences during the design stage. The genes/proteins of interest that will be targeted by
shRNA do not and will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor
suppressors and immunomodulators. Some of the viral vectors that will be used contain a sequence of interest that
has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. This
sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of human, mouse or rat
origin. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health (this
will be approved by the GMSC).

Programmable nucleases: zinc fingers or Cas9/CRISPR
Zinc fingers or Cas9 protein will be directed against genes of therapeutic interest. Since the purpose of
programmable nucleases is to cause mutations in defined DNA sequences the primary hazard is that such a mutation
would lead to deleterious cellular effects. The genes/proteins that will be targeted do not and will not include any
growth, tropic factors, confirmed oncogenes, tumor suppressors or immunomodulators. In isolation, these sequences
are unlikely to pose any safety risks for the environment or human health.

Reporter genes: Fluorescent protein or enzymes
The reporter gene could be a fluorescent protein or an enzyme. Fluorescent proteins, for example GFP or dsRed,
are not known to cause any diseases in human or animals and have no direct effect on cellular processes. Enzymes
used frequently in reporter gene assay ie luciferase or b-galactosidase are not known to cause any diseases in
humans or animals and as no direct effect on cellular processes. The expression of fluorescent protein or enzymes
under the control of a promoter of a gene of interest is unlikely to pose any safety risks for the environment or human
health.

Summary on lentivirus
The transgenes have a low safety risk to human health because accidental contamination with a vector would lead to
only a small number of cells becoming infected, and it would be highly unlikely that expression in only a few cells
could be sufficient to lead to a disease state. The inability of the lentiviral vectors to propagate on mammalian cells
also reduces the risk. The programmable nucleases (CRISPR or Zinc finger or Cre recombinase) that will be
employed in this viral vector system are not known to cause any relevant toxicity that might represent a safety risk to
human health. The fluorescent proteins or the enzymes are not known to cause any relevant toxicities that might
represent a safety risk to human health. For both programmable nucleases and shRNA, the genes/proteins of interest
that will be targeted will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes,
tumor suppressors or immunomodulators, and none of them are or will be of retroviral origin. Therefore the risk of use
will be low.

8- Immortalised human neural progenitor cells ReNcell 197VM & mHTT Exon1 197VM and transduced immortalised
ReNcell197VM & mHTT Exon1 197VM cells
Immortalised ReNcell197VM & mHTT Exon1 197VM neural progenitor cells
The immortalised ReNcell 197VM cell line has been immortalized by retroviral transduction, using replication
incompetent retrovirus, with the avian v-myc myelocytomatosis viral oncogene.
One of the major risks would be if the sequences coding for the v-myc oncogene were mobilised. This could happen if
the ReNcell 197VM or mHTT Exon1 197VM cells are accidentally inoculated to a worker already infected by a
retroviral genome. Even in that case, a series of unlikely events would have to occur to:
a. The worker would have to be already infected with the HIV-1 virus
b. The retroviral genomes would have to integrate into the immortal human neural progenitor cells genome in a
position where they could interact to effect homologous recombination.
c. Recombination would have to occur in precise regions to allow for transfer of the v-myc oncogene sequences from
the ReNcell197VM or mHTT Exon1 197VM genome to the HIV-1 genome.
d. The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest. Although the risk for human health is low, all measures will be in place to minimize the risk of human health to avoid any accidental inoculation of a worker (see measures below). The ReNcell mHTT Exon1197VM cells also contain the sequences for WPRE, cPPT and deltaU3 of HIV-1 virus. However these cells should not contain the viral genes gag-pol and env required for virus production and therefore cannot produce viruses. It is not known if the WPRE sequence has been mutated to remove the oncogenic WHV-X protein from an open reading frame found in WPRE sequence. One of the major risk factors associated with the utilisation of this cell line would be in that case accidental percutaneous inoculation of a worker. This ReNcell mHTT Exon1 197VM cell line could theoretically not survive in the inoculated worker but would be quickly cleared by the human immune system. Further modification of the ReNcell197VM or mHTT Exon1197VM cell line may enhance the risk of mobilisation of the sequence coding for v-myc oncogene, or WPRE and the sequence of OU3 in the case of ReNcell mHTT Exon1 197VM. Application to these cells of additional lentivirus particles could result in the mobilisation of these sequences but should not result in the liberation of replication-competent retroviruses as the viral genes gag-pol and env should not be present in the genome of the immortalised ReNcell mHTT Exon1 197VM neural progenitor cells. The deltaU3 region contained in the ReNcell197VM mHTT Exon1 cells contains a deletion within the 3’L TR region rendering it self-inactivating and the 3’L TR contained in any virus generated in house, using pSMPUW, contains a hybrid enhanced polyA in the U3 region. If further modification is performed using lentivirus in the ReNcell 197VM mHTT Exon1 cells, the L TR regions will be sequenced to check for lack of homology. The ReNcell 197VM cells are likely to contain retroviral L TR sequences, with the v-myc oncogene. These L TR sequences would be derived from MMLV and L TRs present in any lentivirus generated in house using pSMPUW lentivirus backbone would derive from HIV-1. There should not be sequence homology between these regions. If further modifications are performed using lentivirus, these regions will be sequenced to check. Furthermore, the risk can be minimised by ensuring that any lentivirus used in experiments with the host cells does not contain gag-pol, env or any other significant retroviral genes involved in packaging and replication. The ReNcell mHTT Exon1 197VM cells contain a mutant form of Huntingtin Exon 1. Data indicate that exon 1 proteins are highly neurotoxic (Landles et al., 2010; Barbaro et al., 2015), and models that express either mutant full length HTT or mutant exon 1 of HTT can be used effectively to study HD. Exon-1 expressing models demonstrate disease phenotypes such as aggregate formation (Landles et al., 2010). One of the major risks would be if the sequence coding for the mHTT Exon1 were mobilised. This could happen if the mHTT Exon1 197VM cells are accidentally inoculated to a worker already infected by a retroviral genome. Even in that case, a series of unlikely events would have to occur to (as described above for v-myc mobilisation). Even if the events are unlikely, the immortalised human neural progenitor cell line and the transduced ReNcell 197VM & mHTT Exon1 197VM cells will be maintained at biosafety level 2 containment. Therefore all measures will be in place to minimize the risk to human health.

Human health hazards

Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker’s skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the programmable nucleases and shRNA that will be expressed or targeted will not be designed to disrupt any growth factors, trophic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. In the unlikely event of accidental transduction of a laboratory worker’s skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell
layers. The most likely route of accidental infection with a lentivirus or with the ReNcell 197VM & mHTT Exon1 197VM cell line will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent. Infection of the community at large by ReNcell 197VM or mHTT Exon1 197VM cells is highly unlikely due to the impossibility of the cells to survive outside of a laboratory environment.

* Environment Considerations

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active lentiviral vector particles do accidently get released into the environment, the safety risk posed in such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable replication-competent lentivirus. However, this scenario is extremely unlikely and even if it was to occur, it is unlikely that it would lead to any untoward effects. As the HIV virus is a human pathogen, infection of another species would not be expected to allow any form of recombination event leading to a viable transmissible entity and so the risk from this scenario is considered to be negligible. Rodents, such as wild-type mice and rats, cannot support replication of infectious HIV-1 (Goffinet et ai, 2007 Retrovirology). As a result, the potential for shedding of replication-competent lentiviruses from such animals is very low (even if they were present in the original vector inoculum).

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and in theory they are capable of transducing all dividing and non-dividing mammalian cell types. As emphasized before, the vectors cannot self-propagate after infection, and successful transduction is critically dependent on the presence of high enough concentrations of virus particles. Viral vector stock solutions will be managed in a way that will prevent contaminations with relatively high viral titres. After production, the vectors are aliquoted and stored in screw capped cryovials at -80°C. Taking into account that the infectivity of the vectors rapidly decreases at room temperature, it is considered highly unlikely that the vectors could survive in the long term after being accidentally released into the environment. Lentiviral vectors have a short half-life, at room temperature due to their structural characteristics making them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, as illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

The risk to the environment following accidental release of the immortalised human neural progenitor cells would be negligible since the cells are incapable of surviving outside of laboratory conditions. If ReNcell 197VM cells or mHTT Exon1 197VM cells were to directly inoculate animals, they would be quickly cleared by the immune system and would not produce active viruses. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures and ensuring that all potentially contaminated material is totally inactivated before disposal.

Summary
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to. In the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems. It is also unlikely that the immortalised human neural progenitor cells, ReNcell 197VM/mHTT Exon 197VM cells, and the transduced ReNcell 197VM/mHTT Exon 1 197VM cells will be accidentally released into the environment, as the cell handling and studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Following transduction of cell lines that are designated as requiring Biosafety Containment Level 1 with lentivirus, we wish to be able to subsequently use these cells at Biosafety Containment Level 1. This can be justified by the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24 hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as only requiring Biosafety Containment Level 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General cleaning procedures
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these validated chemical inactivators will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being autoclaved/incinerated.

Waste Handling
Following all work requiring biosafety level 2 containment including work with lentiviruses and ReNcell VM cells, solid waste will be neutralised by soaking in a validated disinfectant solution for 24 hours, after which the material will be "double bagged", autoclaved and, ultimately, incinerated onsite. It will be routine practise that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Liquid waste will be inactivated by Chemgene HLD4L * for 24 hours prior to drain disposal.
As any active viral particles or ReNcell VM cells on surface or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles or ReNcell VM cells would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.

* Chemgene HLD4L
Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1 : 100 dilution (1 %) for general purposes, 1 : 20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages.
The proposal outlined above uses ReNcelis that have been immortalised by self-inactivating retroviral integration of vMyc. A derivative of this cell line has been generated by the random integration of genetic sequence predominantly to express mHTT Exon 1 to generate a useful tool cell line for the study of Huntington's disease. It is not known if the WHV-X protein ORF has been deleted from the WPRE element in the lentivirus used for the expression of the mHTT Exon 1. The proposal then covers the knock down of gene targets via either shRNA, CRISPR/Cas9 or ZFN that could be delivered using lentivirus. In addition certain genes or variants of genes involved in disease may be integrated into the cell genome again by lentiviral vectors. Although it is noted that no viral particles are reported to be shed from the ReNcelis due to the presence of oncogenes delivered via retroviral & lentiviral integration into the host genome the GMSC confirms that the cell lines must be maintained under biosafety containment level 2. The GMSC is satisfied that because of the pre-cautions put in place around the lentiviral vectors to be used and genetic sequences to be delivered that this level of containment is suitable for the subsequent use of the cell lines.

Please enter comments on the GM safety committee on the risk assessment

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Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Project Ref 3043/16.2

Date Ackn'd 18/05/2016
CU2 Project Title Use of human induced pluripotent stem cells generated from primary human cells and integrating retroviral vectors
Class Class 2
CultureVolClass2 < 1 Litre
Consent Granted
To utilise human induced pluripotent stem cells (hiPSC) generated using retroviral reprogramming by third party organisations for target identification, target validation and compound/biologics testing to facilitate drug discovery. HiPSC generated from healthy control donors and patients with disease-relevant mutations, will be used to investigate genes and cellular mechanisms involved in the initiation and development of human disease, for example neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, ALS, Huntington's disease. The hiPSC will be differentiated into a variety of both central nervous system (CNS) and non-CNS like mature cell types using non-genetic methodologies. These differentiated cells will be used in experiments including, but not limited to, cell-based assays, ELISA, western blotting, electrophysiology, immunocytochemistry/microscopy.

To expand understanding of the cellular mechanisms of a disease, or to use the cells for drug discovery-related work, the expression or the activity of genes of interest may be further modulated in the iPSC by several methods:

1. The utilisation of tool compounds
2. The over-expression of a protein of interest
3. The knock-down of the gene of interest by shRNA
4. The generation of modified somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
5. The generation of reporter gene cell line (i.e. fluorescence protein or enzyme) under the promoter of the gene of interest.

HiPSC derived from primary human cells, most commonly fibroblasts. The hiPSC that will be used will have been generated using 3rd and or 4th generation self-inactivating (SIN) nonmobilisable integrating retroviral systems based on, but not limited to, mouse Maloney murine leukaemia virus (MMLV), Maloney murine sarcoma virus (MMSV), myeloproliferative sarcoma virus (MPSV), or human HIV. Some of the original donor material will not have been screened for human pathogens but donors will have a known medical history and will be classified as being low risk for retroviral pathogens. As such the hiPSC are highly unlikely to be producing replication competent lentivirus particles (RCLs).

Reprogramming of the hiPSC is achieved using a variety of factors, that are integrated into the cell genome by the retroviral vectors, that induce pluripotency in the donor cells. The following factors may be present in the supplied hiPSCs: SOX2: is a transcription factor that is essential for maintaining the pluripotency of undifferentiated embryonic stem cells.
KLF4: is a transcription factor that regulates proliferation, differentiation, apoptosis and somatic cell reprogramming. KLF4 may also act as a tumour suppressor gene.

OCT4: is a homeodomain transcription factor of the POU family. This protein is critically involved in the self-renewal of undifferentiated embryonic stem cells and is used as a marker for undifferentiated stem cells. It is indispensable for generating iPSC.

CMYC: is a transcription factor that plays a role in cell cycle progression, apoptosis and cellular transformation. Mutated c-Myc is found in many cancers, where it is constitutively expressed, leading to the unregulated expression of many genes, some of which are involved in cell proliferation leading to oncogenesis. This factor poses a risk if mobilised into RCL.

• NANOG: is a transcription factor critically involved with self-renewal of undifferentiated embryonic stem cells.
• LIN28: encodes a microRNA-binding protein; overexpression of which in mice can cause gigantism and a delay in puberty onset. Human GWAS studies indicates the LIN28B gene to be associated with human height and puberty timing. The biosafety risk is low for adults.
• GLI1: is a highly promiscuous transcription factor, positively or negatively regulating the expression of a number of genes.

Host/vector system

The vectors and factors used to generate any hiPSC that is supplied will be fully described prior to receipt and be reviewed by the Genetic Modification Safety Committee (GMSC) at Takeda Cambridge Ltd (TCB). Secondary transduction of the hiPSC with lentiviral vectors is planned. The hiPSC already contain integrated lentiviral sequences so subsequent lentiviral vectors will be non-homologous to mitigate the small risk of a recombination event leading to mobilisation of genetic material, such as the myc oncogene or immunomodulatory genes, that might pose a risk to human health. The integrated murine based lentivirus pose no additional risk to the environment. Secondary transduction would modify the hiPSCs to explore the underlying disease process or to generate tools for drug discovery. To this end the following could be used based upon the needs of drug discovery programs:

• Integration of shRNA constructs to 'knock down' expression of a gene of interest
• The over-expression of particular genes
• Specific modification of the host genome to using programmable nucleases:
  • Cas9/CRISPR
  • Zinc finger
• Reporter genes under the control of selected transcriptional regulatory sequences to study effects on cellular pathways

The lentivirus used will either be obtained commercially or generated de novo internally using third of fourth generation vector systems. The vector backbone and promoters used to drive vector generation in these systems will be consistent. Genes selected for study will not include growth factors, tropic factors, quiescence factors, confirmed oncogenes or tumour suppressors.

Vectors will be generated using a non replication-competent HIV based lentiviral system that upon integration into the host genome looses part of its long terminal repeat (LTR) preventing excision at a later time point. Such self-inactivating (SIN) vectors are designated to produce stable gene expression in target mammalian cells. The viral particles can only be generated upon co-transfecting a packaging cell line (HEK 293T) with at least four separate plasmids. Three of which provide the minimal set of genes required for viral production (gag-pol, rev and env). The env gene used expresses the vesicular stomatitis virus (VSV-G) to increase tropism. The final plasmid supplies the genetic material to be supplied to the target cell and as such contains packaging sequences directing it to be incorporated into nascent viral particles. Only the minimum amount of lentiviral genome is used in the system and all of the plasmids lack homologous sequences to minimise any chance of recombination. The resultant lentiviral
particles are VSV-G pseudotyped, SIN and replication incompetent in mammalian cells. Small aliquots of no more than 1 x 1 De8 viral particles in 5 to 10 microlitres will be used further reducing the risk.

Third Generation Systems use 4 standard bacterial plasmid vectors. Three plasmids encode for proteins required for production and packaging of full length viral RNA (pCgpV, pRSV-Rev and pCMV-VSVG). The gene of interest is contained in the pSMPUW plasmid. This is the only plasmid that contains the packaging sequence for incorporation into the virus particle.

Fourth Generation Systems use 6 standard bacterial plasmid vectors. The system uses tetracycline to control when viral particles are produced adding yet another level of control to production. pTRE-gag-pro, L TRHIV2-vpr-pol, penv(VSV-G), pTet-Off and plat-IRE-s-rev contain the sequences that code for proteins that are responsible for the tetracycline control and packaging the viral vectors. As above pSMPUW only contains the genetic material to be packaged into the vector and no other lentiviral gene sequence. An integrase deficient version of the fourth generation is available which contains a mutation in the sequence encoding the viral integrase.

Origin & function

The plasmid, pSMPUW, will contain the genetic sequence to be introduced. These will not include growth factors, tropic factors, quiescence factors, confirmed oncogenes or tumour suppressors. All proposed sequences to be used will require approval by the GMSC.

a-Vector containing shRNA - each vector will code for one shRNA transcript that will be designed to target one mRNA/protein of interest (or a scrambled shRNA in the case of negative control). This will be under the control of a polymerase II or III promoter.

b-Vector containing CRISPR or Zinc-finger nuclease - Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This instigates a robust DNA repair response which can either be used to disrupt the target sequence by deletion at the cleavage site or introduce user-supplied genetic material via recombination with a vector containing complementary sequences to the target gene.

For Cas9/CRISPR, nuclease target specificity is determined by a guide RNA, which consists of a short (18-20 nucleotide) sequence homologous to the target gene and an additional short sequence that forms a complex with the Cas9 enzyme. Vector will encode Cas9/CRISPR plus a guide RNA under the control of a Polymerase III promoter.

Zinc finger nuclease target specificity is dependent upon the zinc finger protein sequence which will be under the control of a polymerase II promoter.

A fluorescent protein or enzyme under the control of a polymerase II promoter or the promoter of a gene of interest may be included. Control vectors only expressing fluorescent protein/enzyme will also be used.

c-Reporter or overexpression gene vector - The vector will contain either sequence coding for a reporter protein, a protein to be overexpressed under the control of regulatory elements of a promoter of a gene of interest or a polymerase II promoter. An appropriate selection marker i.e.; neomycin could also be included to allow the selection of cells stably containing either the introduced genetic material.

The efficacy of lentiviral vectors will first be assessed using cell lines that routinely require biosafety level 1 containment (BSL 1). Application of lentiviral particles will be done at biosafety level 2 containment. At least 24 hours post transduction the cells will be washed to remove any residual viral particles and then treated as BSL 1 as the potential risk has been minimised. The efficacy of the genome manipulation will be assessed using in vitro assays.

hiPSC will be transduced with validated lentivirus. All procedures will be conducted at BSL2. Stable cell lines will be documented. Resultant cells will be studied to further understand disease processes or used for drug discovery efforts. This will be done by phenotypic analysis using a range of common in vitro assays, including but not limited to RT-PCR, Western Blotting, immunocytochemistry and imaging, cellular bioenergy or electrophysiology.
hiPSC are pluripotent and are capable of uncontrolled proliferation. Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. The lentiviral vector system that will be used is based on HIV-1. Retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airborne route. Piercing of the skin represents the main potential route by which material could be accidentally introduced into an individual. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

Accidental introduction of hiPSC into healthy individuals should not lead to the formation of teratomas because the immune system will rapidly destroy these cells. As mentioned above the pathogen status of some cells is not known. However the material is not derived from high-risk clinical patients and procedures are in place to deal with needle stick incidents. Individuals with compromised immune systems are not permitted to work with this material.

The genetic material to be incorporate into the viral particles is flanked by non-coding retroviral L TRs in the pSMPUW plasmid. No retroviral genes are encoded on this plasmid minimising the chance any will be packaged into viral particles. As the system requires the co-transfection of three to five additional separate plasmids into a permissive cell line the chance of recombination occurring that leads to the incorporation of any or all of the retroviral genes necessary for the production of a replication competent lentivirus (RCL) is very low and the risk is therefore is low. The lentiviral Expression System includes the following key safety features:

* Only pSMPUW includes L TR and packaging signal sequence required for incorporation into viral particles and integration into the genome of a transduced cell. A hybrid 3’LTR is used that does not affect generation of the viral genome in the producer cell line, but upon integration into the genome of a target cell, the 3’LTR SIN and prevents production of viral genomic material that can be packaged and reduces the chance of mobilisation due to secondary infection with lentivirus. No transcriptionally active L TRs are present in the system and an SV40 polyA is included after the hybrid 3’LTR to reduce the potential for transactivation of cellular genes due to an insertion event that might promote inappropriate gene expression leading to oncogenic effects.

* The LTR has been modified to enable lentiviral production independent of Tat expression. HIV-1 devoid of Tat is known to be replication incompetent. This decreases the risk of RCL occurring.

* The essential genes encoding the proteins required for packaging of the viral genome are separated onto three to five plasmids. These express the proteins (gag, pol, rev, env) required to generate viral particles transiently in the HEK-293T packaging cell line. Other retroviral structural genes are not present in the system. None of the plasmids have regions of homology which will prevent undesirable recombination. As multiple recombination events would be required to generate a RCL the risk of this happening is very low and has so far not been observed in large-scale production and testing of lentiviral vectors.

* Expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Expression requires the presence of rev, which is supplied by the pRSV-REV plasmid. The Rev/RRE system is highly conserved among lentiviruses and loss of the RRE sequence and associated splice donor/acceptor sequences results in a loss of transduction efficiency.

* Lentiviral vectors have a very low potential to cause immunogenicity.

* The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction.

Whilst pseudotyped VSV-G is used as the envelop protein to increase the cell types that can be infected this increase in risk is mitigated by the SIN non-replication nature of the viral vectors generated. Insertional events that disrupt gene function can occur and in some instances might lead to undesirable effects and could be oncogenic. The resultant lentiviral vectors contain about 20% of the original HIV-1 genome. Recombination with wild-type HIV-1 is still potentially possible and would result in mobilisation of the transgene. This is highly unlikely because:

1. Wild-type HIV-1 is not used with in the laboratory environment.
2- hiPSC are not derived from donors either known to be HIV positive or from a high-risk population; so HIV contamination is highly unlikely.

3- Wild type HIV-1 cannot rescue a SIN HIV-1 based lentivirus once integrated into the host genome.

4- It is likely that any recombination would actually decrease the ability of HIV-1 to infect other cells or to replicate.

5- Even if a worker was already or became HIV-1 positive and was accidentally infected with lentiviral vector any recombination would require infection of the same cells. Generation of RCL would require homologous recombination to be in the right regions to enable mobilisation and incorporation to the HIV-1 genome without loss of replication ability. If such a rare event were to occur it is likely to be self limiting and the HIV-1 infection itself is of greater concern to the worker.

The use of lentiviral vectors is an efficient manner with which to deliver genetic material to numerous cell types. The design of the vectors minimises the chance for subsequent mobilisation of transgenes and inappropriate activation of endogenous oncogenes. Insertional events can lead to harmful side effects but are still very unlikely. As such the greatest risk from these vectors comes with their production where much larger volumes and numbers of vectors are being generated. The risk here is mitigated by the correct use of containment measures and the absence of sharps.

Lentiviral vectors are susceptible to dehydration and loss of viability if they are not stored in high protein conditions which reduces the risk to workers and the wider environment.

Transgenes to be delivered to target cells:
Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected hiPSC cells.

Gene products to be overexpressed will not include any growth factors, confirmed oncogenes, or tumor suppressors. The focus of the research is on neurodegenerative diseases so with justification to and with GMSC approval immunomodulators or proteins known to be involved in neurodegeneration may be delivered by lentivirus. The 'knock-down' of specific gene expression using shRNA can lead to off-target and immunomodulatory responses in vivo. shRNA sequences wil be screened against databases of known mammalian/mRNA sequences during the design stage to avoid such complications. Genes encoding any growth factors, tropic factors, quiescence factors, confirmed oncogenes, or tumor suppressors will not be targeted. Control vectors containing a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to and therefore do not target any known mammalian sequences. Such sequences are unlikely to pose any safety risks for the environment or human health.

Programmable nucleases: zinc fingers or Cas9/CRISPR will be directed against genes of therapeutic interest. Again genes that will be targeted will not include any growth, trophic factors, confirmed oncogenes, tumor suppressors or immunomodulators. In isolation, these sequences are unlikely to pose any safety risks for the environment or human health. Programmable nucleases may be used to generate isogenic controls lines by correcting disease causing mutations back to wild-type sequence. This is low risk because the sequence is aimed to revert back to non-disease forms of the gene.

Reporter genes: Commonly used fluorescent proteins or enzymes under the regulatory control of promoters of interest may be used to monitor effects on cellular pathways. Such proteins are not known to cause any human or animal disease and pose no-risk.

In all cases justification for any target must be submitted to the GMSC for approval prior to any work commencing.

Summary
hiPSC will be derived from low risk patient populations but may not be screened for human pathogens. The lentiviral systems are relocation incompetent and SIN minimising the potential for transgene mobilisation and propagation. The transgenes that can be used will on their own have a low risk to human health. Infection of mucosal cells may occur via aerosols but is highly unlikely due to the use of microbiological safety cabinets and secondary containment. Such
infection is self limiting due to the natural shedding of epithelial cells coupled with the replication incompetent nature of the vectors. The most likely route of accidental infection with a lentivirus or with the hiPSC line will be via inadvertent percutaneous inoculation via stick injury or open wound. The likelihood of this occurring will be minimised by following standard BSL2 containment practices. Infection of the community and environment with lentivirus particles is highly unlikely due to small quantities used, their intrinsic instability and rapid loss of viability with time. hiPSC can not survive outside of the laboratory environment so pose no wider risk. Therefore the risks towards workers, co-workers, the public and the environment associated with the use of these lentiviral vectors with hiPSC will be low and BSL2 containment is sufficient.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Handling
Solid waste will be neutralised by soaking in a validated disinfectant solution (5% Chemgene HLD4L), after which the material will be autoclaved and then sent for incineration via a registered waste disposal company. All liquid waste will be inactivated for 24hrs prior to disposal to drains via a designated sink. Chemgene HLD4L: is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1: 100 dilution (1 %) for general purposes, 1 :20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages. General cleaning procedures Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being autoclaved/incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
It is noted that the primary human cell lines used for the generation of hiPSes will be taken from patient populations with a known medical history meaning that there is a low risk of material containing harmful human pathogens. This does not rule out the presence of latent viral infection but the use of third or fourth generation integrating lentiviral systems that are self-inactivating and essentially non-mobilisable makes it highly unlikely that infective replication competent virus will be generated. Important due to the presence of the potentially oncogenic Mye sequence. Additional rounds of lentiviral transduction should not be able to mobilise any genetic elements because self-inactivating systems will be used that lack the necessary genes for viral replication and packaging. As an additional measure the GMSe will have a clear understanding of how each cell line was generated and steps will be taken to ensure overlapping and therefore potential sites for recombination are not present in lentiviral vectors. These vectors are for the introduction of further modifications. Those aimed at correcting disease mutations do not pose a risk to health. The introduction of non-disease causing genetic material for exogenous expression is not seen as a risk either. In the instance where immune-modulators may be targeted the risk although increased is well contained by the avoidance of sharps during transduction procedures. Once cells are transduced and virus removed the risk posed here is very low. Due to the use of iPSe derived from primary human cells and integrating lentiviral vectors this dictates propagation, differentiation and further transduction of cell lines at BSL2. Subsequent testing of the cells will also continue at BSL2 although the genetic modification of the cells is of very low risk.

**Project Containment**

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**Project Ref** 3043/16.3

**Date Ackn'd** 18/05/2016

**CU2 Project Title** Use of human induced pluripotent stem cells generated from primary human cells by episomal methodologies

**Date Project Ceased** 02/12/2016

**Class** Class 2

**Culture Vol** < 1 Litre

**Non-GMM Consent** Consent Granted

**Historical Significant Changes** transferred to GM3344 02/12/2016
**Project Additional Information**

**Purposes of the contained use**

To utilise human induced pluripotent stem cells (hiPSC) generated using episomal reprogramming by third party organisations for target identification, target validation and compound/biologics testing to facilitate drug discovery. HiPSC generated from healthy control donors and patients with disease-relevant mutations, will be used to investigate genes and cellular mechanisms involved in the initiation and development of human disease, for example neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, ALS, Huntington's disease.

The hiPSC will be differentiated into a variety of both central nervous system (CNS) and non-CNS like mature cell types using non-genetic methodologies. These differentiated cells will be used in experiments including, but not limited to, cell-based assays, ELISA, western blotting, electrophysiology, immunocytochemistry/microscopy.

To expand understanding of the cellular mechanisms of a disease, or to use the cells for drug discovery-related work, the expression or the activity of genes of interest may be further modulated in the iPSC by several methods:

1. The utilisation of tool compounds
2. The over-expression of a protein of interest
3. The knock-down of the gene of interest by shRNA
4. The generation of modified somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
5. The generation of reporter gene cell line (i.e. fluorescence protein or enzyme) under the promoter of the gene of interest.

**Recipient or parental organism**

HiPSC derived from primary human cells, most commonly fibroblasts.

**Host/vector system**

The hiPSC will have been generated using an Epstein Barr Virus episomal system by a third party. By the time of transfer to Takeda Cambridge Ltd these vectors will have been lost and the cells deemed 'foot-print' free. The cells are also checked for absence of active expression of the genes present on the episomes. Although the gene expression profile has been altered they are essentially not genetically modified and the only risk is associated with any human pathogens present in culture. This risk is mitigated by donors having a known medical history and
belonging to low risk populations. Secondary transduction would modify the hiPSCs to explore the underlying disease process or to generate tools for drug discovery. To this end the following could be used based upon the needs of drug discovery programs:

- Integration of shRNA constructs to ‘knock down’ expression of a gene of interest
- The over-expression of particular genes
- Specific modification of the host genome to using programmable nucleases:
  - Cas9/CRISPR
  - Zinc finger
- Reporter genes under the control of selected transcriptional regulatory sequences to study effects on cellular pathways

The lentivirus used will either be obtained commercially or generated de novo internally using third of fourth generation vector systems. The vector backbone and promoters used to drive vector generation in these systems will be consistent. Genes selected for study will not include growth factors, tropic factors, quiescence factors, confirmed oncogenes or tumour suppressors.

Vectors will be generated using a non replication-competent HIV based lentiviral system that upon integration into the host genome looses part of its long terminal repeat (LTR) preventing excision at a later time point. Such selfinactivating (SIN) vectors are designated to produce stable gene expression in target mammalian cells. The viral particles can only be generated upon co-transfecting a packaging cell line (HEK 293T) with at least four separate plasmids. Three of which provide the minimal set of genes required for viral production (gag-pol, rev and env). The env gene used expresses the vesicular stamatitis virus (VSV-G) to increase tropism. The final plasmid supplies the genetic material to be supplied to the target cell and as such contains packaging sequences directing it to be incorporated into nascent viral particles. Only the minimum amount of lentiviral genome is used in the system and all of the plasmids lack homologous sequences to minimise any chance of recombination. The resultant lentiviral particles are VSV-G pseudotyped, SIN and replication incompetent in mammalian cells. Small aliquots of no more than 1x10e8 viral particles in 5 to 10 microlitres will be used further reducing the risk.

Third Generation Systems use 4 standard bacterial plasmid vectors. Three plasmids encode for proteins required for production and packaging of full length viral RNA (pCgpV, pRSV-Rev and pCMV-VSVG). The gene of interest is contained in the pSMPUW plasmid. This is the only plasmid that contains the packaging sequence for incorporation into the virus particle.

Fourth Generation Systems use 6 standard bacterial plasmid vectors. The system uses tetracycline to control when viral particles are produced adding yet another level of control to production. pTRE-gag-pro, LTRHIV2-vpr-pol, penV(VSV-G), pTet-Off and ptat-IRES-rev contain the sequences that code for proteins that are responsible for the tetracycline control and packaging the viral vectors. As above pSMPUW only contains the genetic material to be packaged into the vector and no other lentiviral gene sequence. An integrase deficient version of the fourth generation is available which contains a mutation in the sequence encoding the viral integrase.

### Origin & function

The plasmid, pSMPUW, will contain the genetic sequence to be introduced. These will not include growth factors, tropic factors, quiescence factors, confirmed oncogenes or tumour suppressors. All proposed sequences to be used will require approval by the GMSC.

a-Vector containing shRNA - each vector will code for one shRNA transcript that will be designed to target one mRNA of interest (or a scrambled shRNA in the case of negative control). This will be under the control of a polymerase II or III promoter.

b-Vector containing CRISPR or Zinc-finger nucleases - Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This instigates a robust DNA repair response which can either be used to disrupt
the target sequence by deletion at the cleavage site or to introduce user-supplied genetic material via recombination with a vector containing complementary sequences to the target gene.

For Cas9/CRISPR, nuclease target specificity is determined by a guide RNA, which consists of a short (18-20 nucleotide) sequence homologous to the target gene and an additional short sequence that forms a complex with the Cas9 enzyme. Vector will encode Cas9/CRISPR plus a guide RNA under the control of a Polymerase III promoter. Zinc finger nuclease target specificity is dependent upon the zinc finger protein sequence which will be under the control of a polymerase II promoter.

A fluorescent protein or enzyme under the control of a polymerase II promoter or the promoter of a gene of interest may be included. Control vectors only expressing fluorescent protein/enzyme will also be used.

c-Reporter or overexpression gene vector - The vector will contain either sequence coding for a reporter protein, a protein to be overexpressed under the control of regulatory elements of a promoter of a gene of interest or a polymerase II promoter. An appropriate selection marker Le.; neomycin could also be included to allow the selection of cells stably containing either the introduced genetic material.

The efficacy of lentiviral vectors will first be assessed using cell lines that routinely require biosafety level 1 containment (BSL 1). Application of lentiviral particles will be done at biosafety level 2 containment. At least 24 hours post transduction the cells will be washed to remove any residual viral particles and then treated as BSL 1 as the potential risk has been minimised. The efficacy of the genome manipulation will be assessed using in vitro assays.

hiPSC will be transduced with validated lentivirus. All procedures will be conducted at BSL2. Resultant cell lines will be documented. Resultant cells will be studied to further understand disease processes or used for drug discovery efforts. This will be done by phenotypic analysis using a range of common in vitro assays, including but not limited to RT-PCR, Western Blotting, immunocytochemistry and imaging, cellular bioenergy or electrophysiology. This activity is low risk but without data on the pathogen status of the hiPSC cell line all work will continue at BSL2.

**Evaluation of foreseeable effects**

hiPSC are pluripotent and are capable of uncontrolled proliferation. Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. The lentiviral vector system that will be used is based on HIV-1. Retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airborne route. Piercing of the skin represents the main potential route by which material could be accidentally introduced into an individual. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

Accidental introduction of hiPSC into healthy individuals should not lead to the formation of teratomas because the immune system will rapidly destroy these cells. As mentioned above the pathogen status of some cells is not known. However the material is not derived from high-risk clinical patients and procedures are in place to deal with needle stick incidents. Individuals with compromised immune systems are not permitted to work with this material.

The genetic material to be incorporated into the viral particles is flanked by non-coding retroviral L TRs in the pSMPUW plasmid. No retroviral genes are encoded on this plasmid minimising the chance any will be packaged into viral particles. As the system requires the co-transfection of three to five additional separate plasmids into a permissive cell line the chance of recombination occurring that leads to the incorporation of any or all of the retroviral genes necessary for the production of a replication competent lentivirus (RCL) is very low and the risk is therefore is low.

The lentiviral Expression System includes the following key safety features:

* Only pSMPUW includes L TR and packaging signal sequence required for incorporation into viral particles and integration into the genome of a transduced cell. A hybrid 3'L TR is used that does not affect generation of the viral genome in the producer cell line, but upon integration into the genome of a target cell, the 3'L TR SIN and prevents production of viral genomic material that can be packaged and reduces the chance of mobilisation due to secondary infection with lentivirus. No transcriptionally active L TRs are present in the system and an SV40 polyA is included after the hybrid 3'L TR to reduce the potential for transactivation of cellular genes due to an insertion event that might
promote inappropriate gene expression leading to oncogenic effects.

- The LTR has been modified to enable lentiviral production independent of Tat expression. HIV-1 devoid of Tat is known to be replication incompetent. This decreases the risk of RCL occurring.
- The essential genes encoding the proteins required for packaging of the viral genome are separated onto three to five plasmids. These express the proteins (gag, pol, rev, env) required to generate viral particles transiently in the HEK-293T packaging cell line. Other retroviral structural genes are not present in the system. None of the plasmid have regions of homology which will prevent undesirable recombination. As multiple recombination events would be required to generate a RCL the risk of this happening is very low and has so far not been observed in large-scale production and testing of lentiviral vectors.
- Expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Expression requires the presence of rev, which is supplied by the pRSV-REV plasmid. The Rev/RRE system is highly conserved among lentiviruses and loss of the RRE sequence and associated splice donor/acceptor sequences results in a loss of transduction efficiency.
- Lentiviral vectors have a very low potential to cause immunogenicity.
- The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction.

Whilst pseudotyped VSV-G is used as the envelop protein to increase the cell types that can be infected this increase in risk is mitigated by the SIN non-replication nature of the viral vectors generated. Insertional events that disrupt gene function can occur and in some instances might lead to undesirable effects and could be oncogenic.

The resultant lentiviral vectors contain about 20% of the original HIV-1 genome. Recombination with wild-type HIV-1 is still potentially possible and would result in mobilisation of the transgene. This is highly unlikely because:

1- Wild-type HIV-1 is not used within the laboratory environment.
2- hiPSC are not derived from donors either known to be HIV positive or from a high-risk population; so HIV contamination is highly unlikely.
3- Wild type HIV-1 cannot rescue a SIN HIV-1 based lentivirus once integrated into the host genome.
4- It is likely that any recombination would actually decrease the ability of HIV-1 to infect other cells or to replicate.
5- Even if a worker was already or became HIV-1 positive and was accidentally infected with lentiviral vector any recombination would require infection of the same cells. Generation of RCL would require homologous recombination to be in the right regions to enable mobilisation and incorporation to the HIV-1 genome without loss of replication ability. If such a rare event were to occur it is likely to be self-limiting and the HIV-1 infection itself is of greater concern to the worker.

The use of lentiviral vectors is an efficient manner with which to deliver genetic material to numerous cell types. The design of the vectors minimises the chance for subsequent mobilisation of transgenes and inappropriate activation of endogenous oncogenes. Insertional events can lead to harmful side effects but are still very unlikely. As such the greatest risk from these vectors comes with their production where much larger volumes and numbers of vectors are being generated. The risk here is mitigated by the correct use of containment measures and the absence of sharps.

Lentiviral vectors themselves rapidly lose ability to transduce cells if not stored in high protein buffer and are susceptible to dehydration. This reduces the risk of accidental exposure to workers and the environment.

Transgenes to be delivered to target cells:

Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transduced hiPSC cells.

Gene products to be overexpressed will not include any growth factors, confirmed oncogenes, or tumor suppressors. The focus of the research is on neurodegenerative diseases so with justification to and with GMSC approval immunomodulators or proteins known to be involved in neurodegeneration may be delivered by lentivirus.
The 'knock-down' of specific gene expression using shRNA can lead to off-target and immunomodulatory responses in vivo. shRNA sequences will be screened against databases of known mammalian/mRNA sequences during the design stage to avoid such complications. Genes encoding any growth factors, trophic factors, quiescence factors, confirmed oncogenes, or tumor suppressors will not be targeted. Control vectors containing a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to and therefore do not target any known mammalian sequences. Such sequences are unlikely to pose any safety risks for the environment or human health.

Programmable nucleases: zinc fingers or Cas9/CasPR will be directed against genes of therapeutic interest. Again genes that will be targeted will not include any growth, trophic factors, confirmed oncogenes, tumor suppressors or immunomodulators. In isolation, these sequences are unlikely to pose any safety risks for the environment or human health. Programmable nucleases may be used to generate isogenic controls lines by correcting disease causing mutations back to wild-type sequence. This is low risk because the sequence is aimed to revert back to non-disease forms of the gene.

Reporter genes: Commonly used fluorescent proteins or enzymes under the regulatory control of promoters of interest may be used to monitor effects on cellular pathways. Such proteins are not known to cause any human or animal disease and pose no-risk.

In all cases justification for any target must be submitted to the GMSC for approval prior to any work commencing.

Summary
hiPSC will be derived from low risk patient populations but may not be screened for human pathogens. The cell supplied to Takeda Cambridge Ltd will be ‘foot-print’ free and can be regarded as not being genetically modified. Subsequent lentiviral vector systems are relitigation incompetent and SIN essentially preventing transgene mobilisation and propagation. The transgenes that can be used will on their own have a low risk to human health. Infection of mucosal cells may occur via aerosols but is highly unlikely due to the use of microbiological safety cabinets and secondary containment. Such infection is self limiting due to the natural shedding of epithelial cells coupled with the replication incompetent nature of the vectors. The most likely route of accidental infection with a lentivirus or with the hiPSC line will be via inadvertent percutaneous inoculation via stick injury or open wound. The likelihood of this occurring will be minimised by following standard BSL2 containment practices. Infection of the community and environment with lentivirus particles is highly unlikely due to small quantities used, their intrinsic instability and rapid loss of viability with time. hiPSC can not survive outside of the laboratory environment so pose no wider risk. Therefore the risks towards workers, co-workers, the public and the environment associated with the use of these lentiviral vectors with hiPSC will be low and BSL2 containment is sufficient.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Handling
Solid waste will be neutralised by soaking in a validated disinfectant solution (5% Chemgene HLD4L), after which the material will be autoclaved and then sent for incineration via a registered waste disposal company. All liquid waste will be inactivated for 24hrs prior to disposal to drains via a designated sink.
Chemgene HLD4L: is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell...
death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1%) for general purposes, 1:20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages.

General cleaning procedures
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L * before being autoclaved/incinerated.

It is noted that the primary human cell lines used for the generation of hiPSCs will be taken from patient populations with a known medical history meaning that there is a low risk of material containing harmful human pathogens. This does not rule out the presence of latent viral infection but the use of third or fourth generation integrating lentiviral systems that are self-inactivating and essentially non-mobilisable makes it highly unlikely that infective replication competent virus will be generated. None of the facets used to generate the hiPSC will still be present in the cells. The lentiviral vectors described here pose a low risk because they non-mobilisable, self-inactivating and replication incompetent. In addition they lack the genetic elements necessary for viral replication and packaging. As an additional measure the GMSC will have a clear understanding of the proposed modifications. Vectors aimed at correcting disease mutations do not pose an additional risk to health. The introduction of non-disease causing genetic material for exogenous expression is not seen as a risk either. In the instance where immune-modulators may be targeted the risk although increased is well contained by the avoidance of sharps during transduction procedures. Once cells are transduced and virus removed the risk posed by the lentivirus is essentially removed. The main continuing risk is from the hiPSC and the potential presence of human pathogens. For this reason any propagation, differentiation, transduction and testing of cell lines will be carried out at BSL2.

Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications
Human induced pluripotent stem cells (hiPSC), generated from healthy control and patients with disease-relevant mutations (specifically one healthy parental control & three offspring samples with varying CAG expansions within Huntington exon 1 coding sequence), will be used to investigate genes and cellular mechanisms involved in the initiation and development of neurodegenerative disorders, for example, Alzheimer's disease, Parkinson's disease, ALS, Huntington's disease etc.

To better understand these genes and cellular mechanisms, the expression or the activity of the gene of interest will be modulated by several methods:

1- The utilisation of tool compounds
2- The knock-down of the gene of interest by shRNA
3- The generation of modified somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
4- The generation of reporter gene (Le. fluorescence protein or enzyme) under the control of a promoter of the gene of interest or an overexpressing cell line under the control of a promoter of the gene of interest or a ubiquitously expressing promoter

The experiments involving the transfection of vectors such as shRNA or programmable nucleases may be performed by lentivirus. The effect of modulating the expression or the activity of the target of interest will be determined by the expression level of the proteins, mRNA or mediators involved in the target signalling pathway.
Description of the human induced pluripotent stem (iPS) cells

The iPS cells are established by reprogramming human fibroblasts. These fibroblast cells have been derived from healthy and patient skin biopsies following informed consent.

Reprogramming of human fibroblast cells to iPS cells was performed at the Rockefeller University using a commercially available kit sold by Life Technologies, Cytotune - iPS 2.0 Sendai Reprogramming Kit. The reprogramming vectors include the four Yamanaka factors, Oct, Sox2, Klf4 & c-Myc, shown to be sufficient for efficient reprogramming (Takahashi et al, 2007). These are expressed from the following vectors, CytoTune 2.0 KOS, CytoTune 2.0 hc-Myc & CytoTune 2.0 hKlf4.

iPS cells have been tested with Applied Biosystems TaqMan iPSC Sendai Detection kit. This kit is used to detect the presence of and determine the levels of Sendai virus and exogenous transcription factors (cMyc, Oct3/4, Klf4, Sox2) delivered by the Sendai virus. No expression was detected in RNA from the reprogrammed iPS cells. This would indicate that there is no virus present in these iPS cells. Sendai virus is a negative sense single stranded RNA virus which must be converted to positive RNA (mRNA) before translation. The virus remains in the cytoplasm, does not enter the nucleus and does not go through a DNA intermediary, therefore the reprogramming should not result in a permanent genetic modification of these cells.

Fibroblast lines from donors have been provided as a basic research tool to investigate human disease. Consent to screen for major human pathogens is not sought at the time of donation. The donors are not known to be positive for any human pathogens. However, screening the cells for major human pathogens would have potentially significant clinical health implications for patients (e.g. HIV), for which explicit consent has not been given at the time of donation. As such, the cells and subsequent iPSC lines generated, have not and cannot be screened for human pathogens and must therefore be handled at Biological Safety Level 2.

B- Description of the lentivirus containing shRNA, programmable nucleases sequences or gene reporter vectors

To utilise the iPS cells to study neurodegeneration, the expression of a target of interest may be decreased in the iPSC line by the transduction of shRNA or programmable nuclease by lentivirus.

The modulation of a particular cellular pathway may be studied with the use of a reporter gene assay. In this case, the vector containing the reporter gene under the regulation of the promoter of the gene of interest may be introduced by standard transfection methods or lentivirus into the iPS cells.

This proposal may involve both the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site using either a third or fourth generation system. Both commercially acquired and in-house lentivirus will use the same transfer vector plasmid. Target genes will be selected based on the needs of drug discovery projects; programmable nucleases and shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators (these targets will be approved internally by the GMSC).

The production of lentivirus containing the vectors of interest are described below:

1- Generation of lentivirus

Lentiviral vectors will be purchased commercially or produced in-house using either a third or fourth generation system described below. Each purified and concentrated aliquot of lentiviral reagent comprised of a small volume (5 to 100 l) that contains no more than 1x10^8 viral particles.

Vector System = self-inactivating (SIN) non replication-competent HIV based lentiviral vectors

The viral vectors that will be used in the proposed studies are self-inactivating non replication-competent HIV based lentiviral vector systems, which have been deSignated to produce stable gene expression in mammalian cells. They are generated by co-transfecting HEK 293T cells with four to six separate plasmids. Each of the plasmids used expresses a different set of genes which, when combined following co-transfection, provide the smallest possible set
of essential viral genes that is still compatible with virus production. Moreover, the vectors are all vesicular stomatitis
toxin virus (VSV-G) pseudotyped lentiviral vectors, which are self-inactivating and highly unlikely to undergo recombination.
Importantly, the viral vector is inherently incapable of replication in mammalian cells.

The plasmids

Third Generation

The sequences that will be included in the viral vectors are sub-cloned into 4 standard bacterial plasmid vectors.
Plasmids containing the packaging related sequences
Each of the three other plasmids, pCgpV, pRSV-Rev and pCMV-VSVG contain a different set of sequences that code
for proteins that are responsible for packaging the viral vectors. All the genes (gag-pol, rev and env) required for
production and packaging of the full length viable viral RNA particles have been removed from the pSMPUW lentiplasmids containing the transgenes of interest.

Fourth Generation

The sequences that will be included in the viral vectors are sub-cloned into 6 standard bacterial plasmid vectors
Plasmids containing the packaging related sequences
Each of the other plasmids, pTRE-gag-pro, LTRHIV2-vpr-pol, peny(VSV-G), pTet-Off and ptat-RES-rev contain a
different set of sequences that code for proteins that are responsible for packaging the viral vectors. All the genes
(gag-pol, rev and env) required for production and packaging of the full length viable viral RNA particles have been
removed from the pSMPUW lenti-plasmids containing the transgenes of interest. An integrase deficient version of the
fourth generation packaging mix is available which contains a mutation in the sequence encoding the viral integrase.
The resulting integrase-deficient lentivirus (IDLV) generates circular vector episomes in transduced target cells that
are gradually lost by dilution in dividing cells (transient expression), but are stable in quiescent cells.

Plasmids containing the transgene of interest
The plasmid, pSMPUW, will contain the genetic sequence that codes for either shRNA, programmable nuclease, a
reporter gene under the promoter of the gene of interest or protein of interest under the control of the regulating
elements of the promoter of the gene of interest or a ubiquitously expressing promoter.

a- Vector containing shRNA
As mentioned above, pSMPUW plasmid will contain the sequence that code for a selected shRNA transcript that will
target one mRNA/protein of interest (or a scrambled shRNA in the case of negative control). The shRNA or
scrambled shRNA sequences will be under the control of polymerase II or III promoter. The shRNA sequences will be
chosen to target a single gene. The shRNA will not be produced against any growth factors, tropic factors, quiescence
factors, confirmed oncogenes, tumor suppressors or immunomodulators.

b- Vector containing CRISPR or Zinc-finger nucleases
Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn
instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the
cleavage site or by recombination with a user-supplied synthetic vector containing sequences complementary to the
target gene.

For Cas9/CRISPR, nuclease target specificity is determined by an accessory sequence, the guide RNA, which
consists of a short (18-20 nucleotide) sequence homologous to the target gene and an additional short sequence that
forms a complex with the Cas9 enzyme. In this case, the plasmid pSMPUW, will contain the sequences that code for
a Cas9/CRISPR plus accessory sequences comprisIng a short guide RNA that determines target specificity. The
guide RNA sequences will be under the control of a Polymerase III promoter.
In the case of Zinc finger nucleases target specificity is dependent upon the zinc finger protein sequence. The
pSMPUW will contain in that case the sequence of zinc finger nucleases under the control of a polymerase II
Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the cleavage site or by recombination with a user-supplied synthetic vector containing sequences complementary to the target gene.

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In the case of Zinc finger nucleases target specificity is dependent upon the zinc finger protein sequence. The pSMPUW will contain in that case the sequence of zinc finger nucleases under the control of a polymerase II promoter. In addition, a fluorescent protein or enzyme under the control of a polymerase II promoter or the promoter of a gene of interest may be included. Control viral vectors that express only the fluorescent protein/enzyme and no programmable nuclease will be used in some experiments.

The Cas9/CRISPR, if introduced into any cell line by lentivirus, will use the integrase deficient version of fourth generation packaging mix.

c- Reporter gene/Overexpression vector

The pSMPUW vector will contain the sequence coding for a reporter protein (enzyme or fluorescent protein) or protein of interest under the control of the regulating elements of the promoter of the gene of interest or a ubiquitously expressing promoter. An appropriate selection marker i.e.; neomycin could also be included to allow the selection of stable human iPS cells containing the reporter gene sequence or gene of interest under the control of the promoter of the gene of interest or a polymerase II promoter. Any overexpression will not be of growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators. Any protein to be overexpressed will be approved by GMSC beforehand.

**Origin & function**

Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the cleavage site or by recombination with a user-supplied synthetic vector containing sequences complementary to the target gene.

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In the case of Zinc finger nucleases target specificity is dependent upon the zinc finger protein sequence. The pSMPUW will contain in that case the sequence of zinc finger nucleases under the control of a polymerase II promoter. In addition, a fluorescent protein or enzyme under the control of a polymerase II promoter or the promoter of a gene of interest may be included. Control viral vectors that express only the fluorescent protein/enzyme and no programmable nuclease will be used in some experiments.

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**Evaluation of foreseeable effects**

- **Human Health**

  The human iPS cells that will be used have a low risk to human health. Samples are derived from patients that are not known to be infected but the disease status cannot be confirmed. Hence treated with caution at BSL2. The expression systems and the transgenes that comprise the lentiviral vectors that will be used have also a very low risk.
for human health and safety, and the reasons for this are discussed below.

A- Reprogrammed human iPS cells

The reprogrammed human iPS cells have been generated by reprogramming human skin fibroblasts using Sendai virus expressing a number of reprogramming factors. This Sendai reprogramming kit includes a number of safety features, as described below:

The host species for the Sendai virus (SeV) reported so far are mouse, rat, hamster and guinea pigs, all of which have been described to be serologically positive so should be non-pathogenic to humans. SeV is transmitted by aerosol and contact with respiratory secretions. The virus is highly contagious but the infection does not persist in immunocompetent animals.

Cytotune Sendai reprogramming vectors are based on a modified, non-transmissible form of SeV, which as a Fusion protein (F) deleted, rendering the virus incapable of producing infectious particles from infected cells. The presence of functional mutations such as temperature sensitivity in the amino acid sequence of several SeV proteins (SeVITS~F, SeVITS12~F, and SeVITS15~F) renders the vectors easily removable from transduced cells. As mentioned previously in the description of the human iPS cells, the RNA from one of the cell lines has been tested with Applied Biosystems TaqMan iPSC Sendai Detection kit. This kit is used to detect presence of and determine levels of Sendai virus and exogenous transcription factors (cMyc, Oct3/4, Klf4, Sox2) delivered by the Sendai virus. No expression was detected in RNA from the reprogrammed iPS cells. This would indicate that there is no virus present in these iPS cells.

The time needed to derive vector-free iPSCs may vary depending on culture and passage conditions. In the case of human neonatal foreskin fibroblast cells (strain BJ), it takes about 1-2 months after gene transduction to obtain vector free iPSCs. The iPS cells were cultured at the Rockefeller post transduction for at least 2 months prior to being received at UCL. All cells have been treated in the same manner and cultured for the same length of time post transduction so should be free of virus but RNA from the other 3 cell lines will be tested.

The major risks of this line is the unknown human pathogen status. Fibroblast lines from donors have been provided as a basic research tool to investigate human disease. Consent to screen for major human pathogens is not sought at the time of donation. Screening the cells for major human pathogens would have potentially significant clinical health implications for patients (e.g. HIV), for which explicit consent has not been given at the time of donation. As such, the cells and subsequent iPS lines generated, have not and cannot be screened for human pathogens and must therefore be handled at Biological Safety Level 2.

Due to the unknown pathogen status of the iPS cells, using lentivirus on these cells could pose a risk regarding potential recombination of lentivirus with retrovirus that could already be present within the cells. As consent has not been given to screen for human pathogens the supernatant from transduced cells cannot be tested for the presence of viral particles. Therefore iPS cells treated with lentivirus must be handled at Biological Safety Level 2. Therefore all measures will be in place to minimize the risk to human health.

B- Lentivirus containing shRNA, or programmable nucleases sequence or reporter gene/overexpression vectors

Lentiviral Vector Expression System

Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. As the lentiviral vector system that will be used in these studies is based on HIV-1, a theoretical risk to human health exists. However, retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airborne route. Therefore, accidental piercing of the skin or other surface tissues with virus containing objects represents the main potential route by which accidental infection could occur. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

In all of the modified pSMPUW plasmids (containing shRNA, programmable nucleases or reporter gene sequences),
the sequences inserted are flanked by non-coding retroviral L TRs, and no retroviral genes are encoded on the modified pSMPUW plasmids. Therefore, no retroviral genes will be transferred into generated viral particles. This construct is packaged into particles using a HEK293T cell based packaging system, which requires the cotransfection of these cells with three to five additional separate plasmids (as detailed above). The additional plasmids express the envelope protein from VSVg and the non-structural proteins of the virion, and, importantly, none of these genes will be transferred into the assembled viral vectors, since they lack the packaging signal (0), which is only present on the modified pSMPUW plasmids.

The lentiviral Expression System what will be used includes the following key safety features:
* All of the pSMPUW contain a hybrid 3’L TR that does not affect generation of the viral genome in the production cell line, but results in “self-inactivation” (SIN) of the vector after transduction of target cells. Once integrated into a transfected target cell, the lentiviral genome is no longer capable of producing viral genomic material that can be packaged. Moreover, presence of an SV40 polyA after the hybrid 3’L TR in the vector construct will result in a provirus which should reduce the potential for transactivation of cellular genes due to an insertion event. Furthermore, the development of self-inactivating vectors improves the biosafety of vectors, as they are less likely to be mobilised following a superinfection with wild-type virus (HIV).
* The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes. Heterologous envelopes, like VSV-G, typically broaden the tropism and allow gene transfer into a broad variety of cells. The risk will mitigated by the use of self-inactivating virus and limiting the number of viral particles that will be handled at anyone time.
* Sequences encoding the proteins required for packaging of the viral genome are separated onto three to five plasmids, and all of the 4-6 plasmids used in the system have been engineered not to contain any regions of homology with each other so as to prevent undesirable recombination events that could lead to the generation of a replication-competent lentivirus (RCL), which could potentially be harmful to humans. It is important to note that no such RCL has ever been observed despite large-scale production and testing of lentiviral vectors.
* All of the pSMPUW containing plasmids/vectors will be used are devoid of all viral sequences apart from essential cis-acting sequences, including the LTRs and the packaging signal o. Although the packaging plasmids used in these systems allows for the expression in trans of protein required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK293T producer cell lines, none of them contain L TRs or the 0 packaging sequence. Several of the lentiviral accessory genes (vif, vpr, vpu and nef) that are dispensable for lentiviral vector production/transduction have been deleted from the packaged construct. Therefore, none of the retrovirus structural genes will actually be present in the packaged viral genome, and they will never be expressed in the transduced target cells, which means that no new RCL can be produced.
* The lentiviral particles produced in this system are replication-incompetent, only carry the sequences of interest, and no other viral species are produced.
* Expression of the gag and pol genes from pCpgV has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Addition of the RRE in these plasmids prevents gag and pol expression in the absence of rev, which is contained in the pRSV-REV plasmid only. The Rev/RRE system is highly conserved among lentiviruses, and removal of the RRE sequence and associated splice donor/acceptor sequences result in a loss of transduction efficiency.
* L TR has been modified so as to increase lentiviral vector production, and also to allow lentiviral vector production to be independent of tat expression. It is known that Tat-deleted mutants of wild-type HIV-1 are not replication competent. Therefore, the deletion of Tat should decrease the risk of generating a putative RCL.
* Lentiviral vectors have a very low potential to cause immunogenicity.
* The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to
improve the nuclear import of the proviral DNA and hence accelerate transduction. While the lentiviral vectors that are produced using this system contain only about 20% of the original genome of HIV-1, there is a very small risk that subsequent infection of cells already infected with the lentiviral genome of HIV-1 could lead to a rare recombination event in which the transgene is transferred to a replication-competent virus. Thus, the sequences in the vector that will be expressed could potentially be transferred to surrounding cells. This event is, however, extremely unlikely to occur for a number of reasons:

1- The lentiviral vector is replication-incompetent and self-inactivating. In the case of a subsequent HIV-contamination, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host genome, although non-self-inactivating vectors can be.

2- In order for the spread of the gene of interest to occur following accidental infection (assuming that this has lead to viral integration), a series of unlikely events have to occur:
   a- The worker would have to become infected with HIV-1 or to be already infected with the virus.
   b- The viral and lentiviral genomes would have to integrate into the host worker's genome in the same cells and in a position where they could interact to effect homologous recombination (point 1 above)
   c- Recombination would have to occur in just the right regions to allow for transfer of the gene of interest from the lentivirus to the HIV-1 genome, which could also involve the transfer of the HIV-1 genes to the lentiviral genome. In that case, it is conceivable that a non-self-inactivating HIV could be generated that contained the gene of interest but not the rest of the genome it requires. The other gene products could be provided in trans from the lentiviral genome that may now contain the HIV-1 genes or from other HIV-1 integrants.
   d- The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest, but again no genes necessary for subsequent replication. In order for another round of infection to occur, the process would have to begin again.
   e- It is conceivable the above could also happen if the iPS cells are already retrovirus positive.

In these circumstances, the effects of lentiviral infection are likely to be minor in comparison to the effects of the HIV-1 infection, which would be required to affect the spread of the gene of interest. In addition, the scenario described is essentially equivalent to the rescue of the lentiviral genome from the host, which has already been shown not to occur.

3- It is extremely unlikely that any worker would infect themselves with a significant dose of lentivirus as the volumes that are used in transfection experiments are small (aliquots contain a maximum of 10J.l1 of vector solution).

4- Moreover, insertional mutagenesis into the host genome may be considered as an oncogenic risk. We cannot rule out the possibility that, when the lentiviral genome integrates into the host genome, it will not lead to the activation of an endogeneous oncogene. However, all transcriptionally active long-terminal repeats (LTRs) have been removed as well as all promoter-like elements that required to drive expression of the transgene. This should prevent unforeseen activation of such genes. It is noted that deletion of retroviral enhancer in self-inactivating systems reduces the risk of activation but not of disruption, therefore, retroviral infection might still have permanent effects upon a cell (including oncogenic effects).

Importantly, we do not consider that the use of these lentiviral vectors will result in a significant increased risk of oncogenic activation compared to the risk possessed by any other viral delivery system. Moreover, the risk of transduction leading to tumourigenesis or other untoward harm following exposure is related in part to the titre of the viral vectors; exposure of workers to quantities of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of lentiviral vectors.

Transgenes:
Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.
shRNA
With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be
knocked-down, in order to study their functions. Potential deleterious effects such as off-target and
immunomodulatory responses can be minimised through screening designed shRNA sequences against databases of
known mammalian/mRNA sequences during the design stage. The genes/proteins of interest that will be targeted by
shRNA do not and will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor
suppressors and immunomodulators. Some of the viral vectors that will be used contain a sequence of interest that
has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. This
sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of human, mouse or rat
origin. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health (this
will be approved by the GMSC).

Programmable nucleases: zinc fingers or Cas9/CRISPR
Zinc fingers or Cas9 protein will be directed against genes of therapeutic interest. Since the purpose of
programmable nucleases is to cause mutations in defined DNA sequences the primary hazard is that such a mutation
would lead to deleterious cellular effects. The genes/proteins that will be targeted do not and will not include any
growth, trophic factors, confirmed oncogenes, tumor suppressors or immunomodulators. In isolation, these sequences
are unlikely to pose any safety risks for the environment or human health.

Reporter genes: Fluorescent protein or enzymes/ Overexpression of proteins
The reporter gene could be a fluorescent protein or an enzyme, this reporter gene may be fused to a ribosomal
subunit (L 1 Oa). Fluorescent proteins, for example GFP or dsRed, are not known to cause any diseases in human or
animals and have no direct effect on cellular processes. Enzymes used frequently in reporter gene assay ie luciferase
or b-galactosidase are not known to cause any diseases in humans or animals and as no direct effect on cellular
processes. The expression of fluorescecent protein or enzymes under the control of a promoter of a gene of interest is
unlikely to pose any safety risks for the environment or human health.

Proteins of therapeutic interest will be targeted for potential overexpression. Genes/proteins that will be
overexpressed do not and will not include any growth factors, tropic factors, quiescence factors, confirmed
oncogenes, tumor suppressors or immunomodulators. Any protein to be overexpressed will be approved by GMSC
beforehand.

Summary on lentivirus

Health and Safety
Executive
The transgenes have a low safety risk to human health because accidental contamination with a vector would lead to
only a small number of cells becoming infected, and it would be highly unlikely that expression in only a few cells
could be sufficient to lead to a disease state. The inability of the lentiviral vectors to propagate on mammalian cells
also reduces the risk. The programmable nucleases (CRISPR or Zinc finger or Cre recombinase) that will be
employed in this viral vector system are not known to cause any relevant toxicity that might represent a safety risk to
human health. The fluorescent proteins or the enzymes are not known to cause any relevant toxicities that might
represent a safety risk to human health. For both programmable nucleases and shRNA, the genes/proteins of interest
that will be targeted will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes,
tumor suppressors or immunomodulators, and none of them are or will be of retroviral origin. Therefore the risk of use
will be low.

Human health hazards
Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident
outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited
number of the laboratory worker's skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the programmable nucleases and shRNA that will be expressed or targeted will not be designed to disrupt any growth factors, trophic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus or with the human iPS cell line will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent. Infection of the community at large by human iPS cells is highly unlikely due to the impossibility of the cells to survive outside of a laboratory environment.

* Environmental Considerations

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active lentiviral vector particles do accidently get released into the environment, the safety risk posed in such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable replication-competent lentivirus. However, this scenario is extremely unlikely and even if it was to occur, it is unlikely that it would lead to any untoward effects. As the HIV virus is a human pathogen, infection of another species would not be expected to allow any form of recombination event leading to a viable transmissible entity and so the risk from this scenario is considered to be negligible. Rodents, such as wild-type mice and rats, cannot support replication of infectious HIV-1 (Goffinet et al, 2007 Retrovirology). As a result, the potential for shedding of replication-competent lentiviruses from such animals is very low (even if they were present in the original vector inoculum).

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and in theory they are capable of transducing all dividing and non-dividing mammalian cell types. As emphasized before, the vectors cannot self-propagate after infection, and successful transduction is critically dependent on the presence of high enough concentrations of virus particles. Viral vector stock solutions will be managed in a way that will prevent contaminations with relatively high viral titres. After production, the vectors are aliquoted and stored in screw capped cryovials at -aOOC. Taking into account that the infectivity of the vectors rapidly decreases at room temperature, it is considered highly unlikely that the vectors could survive in the long term after being accidentally released into the environment. Lentiviral vectors have a short half-life, at room temperature due to their structural characteristics making them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, as illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

The risk to the environment following accidental release of the human iPS cells would be negligible since the cells are
incapable of surviving outside of laboratory conditions. Despite the cells being reprogrammed using Sendai virus they
do not produce virus and therefore would not be able to infect any rodent population. If human iPS cells were to
directly inoculate animals, they would be quickly cleared by the immune system and would not produce active viruses.
The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures
and ensuring that all potentially contaminated material is totally inactivated before disposal.

Summary
The chance that active viral particles will be accidentally released into the environment is considered to be highly
unlikely, as the viral vector studies that will be undertaken have been classified as biosafety level 2, and effective
containment procedures will be adhered to. In the unlikely event that active viral vector particles do accidentally get
released into the environment, the safety risks posed by such an event is considered to be low due to the vectors
being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their
host systems. It is also unlikely that the human iPS cells will be accidentally released into the environment, as the cell
handling and studies that will be undertaken have been classified as biosafety level 2, and effective containment
procedures will be adhered to.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General cleaning procedures
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each
experiment or after the occurrence of an accidental spillage of infectious material. Application of these validated
chemical inactivators will totally inactivate any viral vectors that might be present. According to the biosafety literature,
all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact
with the virus will be treated with Chemgene HLD4L * before being autoclaved/incinerated.

Waste Handling
Following all work requiring biosafety level 2 containment including work with lentiviruses and ReNcell VM cells, solid
waste will be neutralised by soaking in a validated disinfectant solution for 24 hours, after which the material will be
"double bagged", autoclaved and, ultimately, incinerated offsite. It will be routine practise that all liquid waste material
be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent
spillages. Liquid waste will be inactivated by Chemgene HLD4L * for 24 hours prior to drain disposal as per 430CSP
and 418CSP permit requirements.

As any active viral particles or ReNcell VM cells on surface or in waste material will be completely inactivated at the
end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly
unlikely that active virus particles or ReNcell VM cells would unintentionally become discharged into a sanitary
sewage system. No foreseeable adverse effects on human health and safety are expected.

* Chemgene HLD4L
Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to
be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is
equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B,
Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1: 1 00 dilution (1 %) for
general purposes, 1 :20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages.
The GMSC has reviewed the current risk assessment and agrees with the class 2 categorisation for this work. It is noted that although the hiPSC were generated using Sendai virus that needs to be handled at BSL2 the actual cells that will be provided to TCB will be free of Sendai virus and will have lost all detectable levels of plasmid. The main risk from the hiPSCs is their unknown pathogen status but this is mitigated because all donors are of low risk clinical populations. The lentiviral systems that will be subsequently used are inherently designed to prevent inappropriate recombination and subsequent mobilisation of transgenes into replication competent viral particles. Third and fourth generation systems deliver replication incompetent, self-inactivating viral particles that lack even the minimum set of genes required for virus production. The cells themselves cannot survive outside of a lab environment and the lentiviral particles loss viability rapidly if not stored properly being susceptible to desiccation. Risk associated with these activities to humans and the environment is deemed low. The use of biosafety level 2 containment is appropriate and sufficient because of the use of lentiviral elements and the unknown pathogen status of the cells.

Please enter comments on the GM safety committee on the risk assessment

The GMSC has reviewed the current risk assessment and agrees with the class 2 categorisation for this work. It is noted that although the hiPSC were generated using Sendai virus that needs to be handled at BSL2 the actual cells that will be provided to TCB will be free of Sendai virus and will have lost all detectable levels of plasmid. The main risk from the hiPSCs is their unknown pathogen status but this is mitigated because all donors are of low risk clinical populations. The lentiviral systems that will be subsequently used are inherently designed to prevent inappropriate recombination and subsequent mobilisation of transgenes into replication competent viral particles. Third and fourth generation systems deliver replication incompetent, self-inactivating viral particles that lack even the minimum set of genes required for virus production. The cells themselves cannot survive outside of a lab environment and the lentiviral particles loss viability rapidly if not stored properly being susceptible to desiccation. Risk associated with these activities to humans and the environment is deemed low. The use of biosafety level 2 containment is appropriate and sufficient because of the use of lentiviral elements and the unknown pathogen status of the cells.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Human Clinical Applications

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Project Ref 860/03.1

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<td>Commercial Elisa Kits containing human sourced materials, total RNA extraction from human tissue samples and use of attenuated adenovirus in the tag on demand system for protein expression studies</td>
<td>Class 2</td>
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Date Project Ceased 02/03/2022

Consent Granted Not Applicable
Project Additional Information

Purposes of the contained use

1. Measure the concentration of specific materials in samples.
2. Use extracted RNA for cDNA synthesis for expression profiling of human genes.
3. To transiently generate proteins incorporating an antibody detectable tag to confirm protein expression.

Recipient or parental organism

Adenovirus - recipient - mammalian cells grown in culture.

Host/vector system

Adenovirus
- attenuated (non-replicative) adenovirus particles supplied as a suspension
- no production of virus to be carried out

Origin & function

Adenovirus
- Originator: Invitrogen Corporation
- the virus suspension is supplied frozen by the above company
- an aliquot of supernatant is added to cultured mammalian cells and allowed to infect them
- the virus encodes a mutated tRNA that permits read through of the TAG stop codon in the gene sequence
- mRNA encoding a TAG stop codon is read through and the subsequent nucleotide sequence used to add additional amino acids to the protein being synthesised
- in recombinant cells the cDNA encoding the gene of interest contains a TAG stop codon followed by gene sequence encoding a number of amino acids that when synthesised generate an epitope that can be recognised by an antibody
- the addition of the epitope will allow confirmation of protein expression of the gene of interest in the recombinant cell line

Evaluation of foreseeable effects

The adenovirus particles can infect human cells. In the unlikely event of a user being infected the following effects are likely to happen:
1. Viral particles will infect cells
2. The tRNA will be expressed
3. There will be read through of any mRNA which has a TAG stop codon
4. It is estimated that this will be about 33% of all transcribed genes in each cell infected
5. The adenovirus does not replicate in the human cells so any effect will be transient, a maximum of 8 days
6. The resultant change in the proteins is most likely to be toxic to the cells
7. This may result in cell death of those cells infected
8. As a result of cell death in the local area of administration and an inflammatory immune response will be elicited
9. It is not anticipated that there will be any long term effects due to the attenuated nature of the adenovirus

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

ELISA and human tissue
- human sample material and plastics contaminated with material to be treated with strong disinfectant
- human samples processed for RNA will be effectively neutralised in the extraction process
- all disinfected material will then be incinerated
- for spills, solid materials will be collected and put in a container with disinfectant, liquid spills will be treated with disinfectant and then absorbed with paper towels, the area will then be further cleaned with 1% virkon solution. All collected materials will be incinerated.

Adenovirus
- all contaminated materials and plastics to be treated with strong disinfectant
- all disinfected material will then be incinerated
- for spills, solid materials will be collected and put in a container with disinfectant, liquid spills will be treated with disinfectant and then absorbed with paper towels, the area will then be further cleaned with 1% virkon solution. All collected materials will be incinerated

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Paradigm Therapeutics Ltd has been working under license GM 860 with fully verified commercial sources of host strains and vectors at hazard level 1 and containment 1. Due to forseen expansion of experimentation, the GMSC has identified that Paradigm Therapeutics Ltd applies that the license be modified to include hazard level 2 and containment 1. All ELISA materials will be sourced commercially and have undergone routine screenings for infectious agents. The adenovirus materials will be sourced commercially from a reputable company and human tissue samples if used will be stored in appropriate containers at low temperature prior to extraction in chaotropic salts and then phenol/chloroform, which will effectively neutralise any biological hazard. The GMSC feels that these measures detailed above reduce the risk significantly.
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### Name

**PLASTICELL LTD**

### Campus Estate or Research Centre

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| HSE Division               | LONDON              |

### Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

Our GM committee comprises of the company's CEO, one of the senior scientists and an external consultant. The committee meets twice a year to discuss safety measures in place in the laboratory and approve Genetic Modification Risk Assessments. When scientists plan to use a new genetically modified organism, the consultant advises on safety procedures.

The CEO and senior scientist make sure the suggested safety measures are incorporated into SOPs and are being followed.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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<tr>
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</table>
Contaminated sharps are collected in closed sharp bins, tagged and are removed by clinical waste contractors for incineration.

Culture flasks, tubes and other lab consumables used in experiments are collected in a closed bin and once this is filled, are autoclaved at 134 deg C for 15 minutes for de-contamination. Then it is disposed with clinical waste for incineration.

Liquid waste (culture media etc.) in disinfected and de-contaminated using 1% solution of Virkon following manufacturers instructions. Then it is poured down the sink and flushed with copious amounts of water.

The autoclave used for de-contamination is serviced twice a year.
Comments form the GM Committee meeting on 18th March 2009.
GM Committee considered the risk assessment and approved category 1 work involving GM organisms on Plasticell premises. The following changes were suggested and carried out for the GM risk assessment forms.
HSE notification Form should include broader description of work conducted in the lab.
GM Form Part 1:
1.21. It should be prefaced with "The following cell lines will be obtained and grown".
Because GM cell lines are already constructed elsewhere, there is nothing in this RA about hosts/vectors for their construction, and so a possible comment would be as follows:
"This work involves the maintenance and growth of existing, stable GM cell lines. These are not infected with any adventitious agent, nor are they secreting the vector used for their construction. As they pose negligible risk to either workers or the environment, they are correctly classified as Class 1."
GM Part 3:
3.1.1 Disinfectants are not considered to give "100% kill rate" (only autoclaving or incineration are considered to do this). This phrase should be modified to ">10e5 kill rate".

Project Ref 3044/14.1

Date Ackn'd  24/09/2014
CU2 Project Title  1. Knock-down with lentiviral vectors of targets discovered in our small molecule screening campaigns. 2. Generation of PAX7 reporter cell lines via lentiviral transduction

Date Project Ceased

Class  Class 2
CultureVol Class 2 < 1 Litre
CultureVolume Class 3-4

Non-GMM Consent Granted

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

1. We want to validate selected targets from our small molecule screening campaigns through shRNA delivered by lentiviral particles. 2. We want to generate reporter cell lines to screen for small molecules activating the expression of this gene.
Recipient or parental organism

1. Recipient cells will be human embrionic stem cells or other types of stem cells and progenitor cells. After lentiviral transduction, the expression of one of their genes will be downregulated. Recipients cells will be characterised by qPCR and Western Blotting to assess the function of the lentiviral constructs. Cells transduced with lentiviral particles will be kept in a dedicated incubator. After processing the samples they will be conveniently inactivated and discarded. 2. Same as in 1 but upon differentiation of the stem cell in muscle they will express GFP.

Host/vector system

1. Self-inactivating replication incompetent lentivirus based on pLKO backbone and purchased from established vendors like Sigma-Aldrich. These vectors carry an shRNA used to knock-down the expression of a particular gene. 2. Self-inactivating replication incompetent lentivirus based purchased from Applied Biological Materials

Origin & function

1. shRNAs inserted in Lentiviral vector (PLKO1 backbone) with the intended function of downregulating specifically one gene. 2. Human PAX7 promoter sequence followed by GFP sDNA

Evaluation of foreseeable effects

1. The cell lines transduced with the lentiviral particles will show downregulation of a specific gene. 2. The cell lines transduced with the Pax7 reporter gene will not show any new phenotype unless PAX7 expression is stimulated by induced differentiation into muscle; in that case they will express GFP

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable material exposed to lentiviral particles will be inactivated with virkon, disposed in appropriate plastic bags and autoclaved. All surfaces of the cell culture hood will be cleaned and sterilized with 70% ethanol

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment
<table>
<thead>
<tr>
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**Animal Units**

**Large Scale Activities**

**Human Clinical Applications**
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Name

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Name 2

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HSE Division

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Comments

Date at Which Additional Info Submitted

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Valneva SE is a European company registered in France and with operations in France, Austria, Sweden and Scotland with a corporate biological safety committee which is composed of competent members of staff with experience in aspects of biological safety from each operational site. The committee meets to review ongoing and new aspects of the companies biological safety interests in research, development and manufacturing operations. The Biological Safety committee chairperson is the EOHS Manager from the Biological production site in Livingston Scotland (Japanese Encephalitis Virus vaccine IXIARO manufacturing site). A broad range of experience of aspects of biological safety and contained use is held on site and across the organisation where commercially manufactured and CTM development products are manufactured (CAT 21 BSL2) including Japanese Encephalitis virus, Polio virus, Zika virus, recombinant Newcastle disease virus. Valneva has experience of determination and assessment of biological safety of other recombinant virus constructs and submissions to regulatory authorities. Biological safety assessment and completion of BioCOSHH and Biological Risk Assessments for viral inactive and active viral vaccine products is carried out at Valneva Scotland Ltd.

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02/03/2022
Waste Control measures are in place as part of VSL procedures and as described in controlling documentation;
- LlV-SOP-0333 Waste Disposal and Spills Procedure
- Prevention of Breach of Containment of Pathogens
A range of validated disinfection agents are used to clean laboratory surfaces and equipment.
Liquid Waste from all clean rooms is disposed of via a secure drainage system. All drains feed the contained waste holding tank within the VSL facility and independent drain pipework with non-return valves are in place from the CTM facility to prevent cross contamination.
Waste is held in a 2000 L holding tank. When the level in this tank reaches 900 L, the system automatically transfers 700 L of waste to a 1000 L killing tank. Using a heated steam supply, the system heats the kill tanks to 121.50°C for 20 minutes. Using chilled water the system then cools the kill tank to 40°C. All contents are then automatically put to drain.
Solid biological waste is held on site in Yellow lidded and locked hazardous clinical waste bins provided by the licenced waste contractor. Waste bins are uplifted by the licenced waste contractor and transferred to the licenced waste contractor disposal site for incineration.
Autoclave for viral kill is available on site
In general terms measures in place to prevent a breach of containment are described specifically in all controlling documentation and SOP’s. Training in all aspects of the SOP’s is carried out and documented ensuring all requirements for control and containment are understood. Further control and prevention measures for the facility and equipment are described in the following controlling documentation;
- LlV-SOP-0333 Waste Control and Spills Procedure
- LlV-SOP-0008 Planned Preventative Maintenance
- LlV-SOP-1024 Calibration of Equipment
- LlV-SOP-0018 Plant and Equipment Monitoring Checks and Routine Inspection
- LlV-SOP-0010 Maintenance Policy
- LlV-SOP-0116 and 0060 Operation of Decontamination Autoclave
[ ] LlV-SOP-0022 Parameter Configuration of the FMS and Critical Process Parameter Recording Systems
[ ] LlV-SOP-0585 Receipt and handling of CAT 2 material in OB1 QC suite

For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste Control measures are in place as part of VSL procedures and as described in controlling documentation;
- LlV-SOP-0333 Waste Disposal and Spills Procedure
Prevention of Breach of Containment of Pathogens
A range of validated disinfection agents are used to clean laboratory surfaces and equipment.
Liquid Waste from all clean rooms is disposed of via a secure drainage system. All drains feed the contained waste holding tank within the VSL facility and independent drain pipework with non-return valves are in place from the CTM facility to prevent cross contamination.
Waste is held in a 2000 L holding tank. When the level in this tank reaches 900 L, the system automatically transfer 700 L of waste to a 1000 L killing tank. Using a heated steam supply, the system heats the kill tanks to 121.50°C for 20 minutes. Using chilled water the system then cools the kill tank to 40°C. All contents are then automatically put to drain.
Solid biological waste is held on site in Yellow lidded and locked hazardous clinical waste bins provided by the licenced waste contractor. Waste bins are uplifted by the licenced waste contractor and transferred to the licenced waste contractor disposal site for incineration.
Autoclave for viral kill is available on site
In general terms measures in place to prevent a breach of containment are described specifically in all controlling documentation and SOP’s. Training in all aspects of the SOP’s is carried out and documented ensuring all requirements for control and containment are understood. Further control and prevention measures for the facility and equipment are described in the following controlling documentation;
- LlV-SOP-0333 Waste Control and Spills Procedure
- LlV-SOP-0008 Planned Preventative Maintenance
- LlV-SOP-1024 Calibration of Equipment
- LlV-SOP-0018 Plant and Equipment Monitoring Checks and Routine Inspection
- LlV-SOP-0010 Maintenance Policy
- LlV-SOP-0116 and 0060 Operation of Decontamination Autoclave
[ ] LlV-SOP-0022 Parameter Configuration of the FMS and Critical Process Parameter Recording Systems
[ ] LlV-SOP-0585 Receipt and handling of CAT 2 material in OB1 QC suite

Tick to confirm that you are attaching a summary of the risk assessment

02/03/2022 Page 12765 of 15326
## Project Ref 3045/18.1

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- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

### Project notified under transitional arrangements
- N

### Historical Significant Changes
- **Historical Date of Additional Info**: 
- **Significant Change ID**: 
- **Date of Significant Change**: 

### Project Additional Information

**Purposes of the contained use**

Preparation of Clinical Trial Manufacturing lots in a CTM licensed facility for GMP manufacture at a process scale of 66 x 850cm² roller bottles producing approximately 11.5L viral harvest volume with associated downstream viral purification processing producing a Bulk Drug product of -1.1 L and required microbiological and analytical method Quality Control lot release testing.

**Recipient or parental organism**

Chickungunya wild type strain LR2006-0PY1 CHIKV

**Host/vector system**

02/03/2022
ChikV ~5nsP3 attenuated live ChikV GMO was derived from wild type strain LR2006-0PY1 CHIKV infectious clone attenuated by deleting amino acid (aa) in the hypervariable region of the nsP3 protein, residues 1656 to 1717 of the P1234 polyprotein which were substituted with a linker (aa sequence AYRAAAG).

Full detail of the attenuation process is described in the attached ChikV BioCOSHH assessment and summarised below;

**Origin & function**

Attenuated Chikungunya virus strain "~5nsP3" was obtained on Licence from the group of Peter Liljestrom at the Karolinska Institutet in Stockholm, Sweden.

Based on the virus sequence a full-length synthetic genome was produced by GeneArtTM Gene Synthesis. The virus was adapted to Vero cells by serial passaging.

A full description of the cloning strategy and the plasmid construct used to generate virus seed banks is given in the extract from the IND submission; section 3.2.S.2.3.2.2 (Attachment 1)

Successful assembly of the ChikV ~5nsP3 genome was verified by sequencing and is described in the following document;

Investigational Medicinal Product Dossier (IMPD) Chikungunya vaccine VLA1553 Verification of ChikV ~5nsP3 Sequence (Attachment 1)

Using attenuated Chikungunya virus strain "~5nsP3 " to generate ChikV live vaccine candidate for clinical evaluation. Preparation of Clinical Trial Manufacturing lots in a CTM licensed facility for GMP manufacture at a process scale of 66 x 850cm2 roller bottles producing approximately 11.5L viral harvest volume with associated downstream viral purification processing producing a Bulk Drug product of ~1.1 L and required microbiological and analytical method Quality Control lot release testing.

**Evaluation of foreseeable effects**

A full assessment of risk was carried out and documented as a BioCOSHH assessment, Attachment 2 and are summarised below;

Attenuated ChikV ~5nsP3 GMO Virus will be held at VSL as a Working Viral Seed Bank (WVSB) and used to produce GMP CTM manufacturing and development batches of vaccine. All manipulations of ChikV virus are completed within a BSL2 laboratory facility. All work is completed and controlled using standard operating procedures (SOP's) with training completed and documented for all staff. An EOHS risk assessment has been completed and protective measures and equipment have been identified, implemented with training to be carried out and documented prior to the ChikV virus being brought on site. There is no hazard or risk of infection identified following exposure to the ChikV ~5nsP3 GMO virus. However transmission by direct blood contact could act as a mode of transmission of the attenuated non-infectious strain.

The hazard and risk to the individual as an outcome of the potential mode of transmission of blood transfer is considered to be effectively zero with control measures applied offering full protection to all personnel.

This is further documented in the Environmental I occupational health and safety risk assessment to be prepared prior to the attenuated ChikV ~5nsP3 GMO being brought on site. The likelihood of any residual risk being realised is unlikely and the risk rating is identified as Effectively Zero.

The manufacturing process for Attenuated ChikV ~5nsP3 GMO viral vaccine operates within a GMP manufacturing clean room rated at BSL2 and is operated as a sterile process in closed vessels e.g. Roller Bottle cell culture and viral infection. All manual operations where live virus is manipulated in open vessels are carried out in Class II microbiological safety cabinets within the ISO 7 Classified Manufacturing Clean Rooms. Small volumes (~3mL) of live virus are sampled and used for analysis in a range of assays including; SOS PAGE analysis and safety testing.
aerosols are generated with the possible exception of a single manufacturing process step (Virus concentration using Tangential Flow Filtration) which is completed in a fully closed disposable system made of Plastics with no aerosol release into the atmosphere.

Valneva Scotland Ltd has a GMP licenced (MHRA) manufacturing facility for CTM batch manufacture and implements full control of all aspects of the manufacturing process with the live ChikV virus, implementing strict procedural, access and management control, as per the requirement of BSL2 operations including; storage and release of the virus WVSB, handling and transport of live virus samples, inactivation and control of biological waste removed from site. All testing of viral product will be outsourced to Valneva Austria or an external Contract Services Organisation (CSO).

All liquid biological waste containing attenuated ChikV ~5nsP3 GMO are treated in a steam pressurised Kill tank prior to disposal as per SEPA licence I permit.

All operational activities are controlled with Standard Operating Procedures.

Training in all aspects of the manufacturing process requirements is detailed in the controlling documentation (SOP's) with training carried out and documented in individuals training record.

All virus disinfection agents will be assessed to ensure the effectiveness of the viral kill.

VSL holds a Scottish Environment Protection Agency (SEPA) permit which documents and controls all aspects of waste disposal. SEPA conducts an annual inspection of the VSL facility and an annual report documenting waste generated including both liquid and solid biological waste is submitted.

Procedures are in place to prevent potential contact of staff operating in the ChikV manufacturing clean rooms with other members of staff during emergency procedures and evacuation of the CTM manufacturing facility. Further details of control measures are included into an Occupational Risk Assessment for ChikV operations; OHSE/001/18-01

Live WVSB is stored as frozen aliquots in a locked freezer with controlled access and requiring documented approval to release material for manufacturing and process development use.

All areas of the manufacturing and testing facility have controlled restricted access with documented training requirements and approval for access to areas required.

Based on the evaluation of risk presented in the BioCOSHH assessment the risk to the individual and the environment with all control measures in place has been determined as effectively zero.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Schedule 8 Part 2, Table 1 a, 17;

'Inactivation of GMM's in contaminated material and waste'.

Kill tank as described in section 6,

Liquid Waste from all clean rooms is disposed of via a secure drainage system. All drains feed the contained waste holding tank within the VSL facility and independent drain pipework with non-return valves are in place from the CTM facility to prevent cross contamination.

Waste is held in a 2000 L holding tank. When the level in this tank reaches 900 L, the system automatically transfer 700 L of waste to a 1000 L killing tank. Using a heated steam supply, the system heats the kill tanks to 121.50 C for 20 minutes. Using chilled water the system then cools the kill tank to 40°C. All contents are then automatically put to drain.

This is a generic method for waste disposal and not a validated method for biological pathogen 'Kill'. However for each individual pathogen the method is assessed in a Biological COSHH Risk Assessment and a justification for use is
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Control measures are in place as part of VSL procedures and as described in controlling documentation;
LIV-SOP-0333 Waste Disposal and Spills Procedure
Prevention of Breach of Containment of Pathogens
A range of disinfection agents are used to clean laboratory surfaces and equipment including 70% Iso Propyl Alcohol (IPA) and sodium hypochlorite which have been shown to inactivate Chikungunya virus (American Association of Blood Banks FEB 2014 update to Transfusion 2009;49 (Suppl):59-61 S and Infectious Disease and Microbial Agents: Chikungunya Virus; Chandrakant Lahariya MD).

Liquid Waste from all clean rooms is disposed of via a secure drainage system. All drains feed the contained waste holding tank within the VSL facility and independent drain pipework with non-return valves are in place from the CTM facility to prevent cross contamination.

Waste is held in a 2000 L holding tank. When the level in this tank reaches 900 L, the system automatically transfer 700 L of waste to a 1000 L killing tank. Using a heated steam supply, the system heats the kill tanks to 121.50°C for 20 minutes. Using chilled water the system then cools the kill tank to 40°C. All contents are then automatically put to drain.

Complete inactivation of ChikV on incubation at a temperature of 60 degrees C for 90 minutes (2015 Science Writers Symposium Developing Tools to Keep Dengue and Chikungunya Out of Our Blood Supply; Maria Rios, Ph.D. Principal Investigator FDA Center for Biologics Evaluation and Research September 18, 2015)

Solid biological waste is held on site in Yellow lidded and locked hazardous clinical waste bins provided by the licenced waste contractor. Waste bins are uplifted by the licenced waste contractor and transferred to the licenced waste contractor disposal site for incineration. . .

Autoclave for viral kill is available on site

In general terms measures in place to prevent a breach of containment are described specifically in all controlling documentation and SOP's. Training in all aspects of the SOP's is carried out and documented ensuring all requirements for control and containment are understood. Further control and prevension measures for the facility and equipment are described in the following controlling documentation;
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- LIV-SOP-0010 Maintenance Policy
- LIV-SOP-0116 and 0060 Operation of Decontamination Autoclave
- LIV-SOP-0022 Parameter Configuration of the FMS and Critical Process Parameter Recording Systems

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

Approved with no additional comments received

## Project Containment

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**Name 2**

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**Campus Estate or Research Centre**

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**Building**

| DUNDEE UNIVERSITY INCUBATOR |

**District**

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**E-mail**

**HSE Division**

| SCOTLAND |

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Genetic Modification safety committee of the company is composed of the Scientific Director, one employee and the Managing Director of the company. The committee meets quarterly to advise on risk assessments of contained activities.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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- Bacteriology: Yes
- Parasitology: 
- Transgenic Birds: 
- Microbiology Research: 
- Virology: Yes
- Transgenic Animals: 
- Transgenic Fish: 
- Gene Therapy: 

02/03/2022
Autoclaving of solid and liquid waste to >121°C for 60 minutes. Temperature probe is used to monitor temperature of waste material to ensure that all areas of the material being autoclaved reach 121°C.

Used bacteria media and mammalian tissue culture media is treated with 1% Virkon overnight or Sodium hypochlorite (Chloros) at:
- 1,000 ppm for general wiping of equipment and benches
- 2,500 ppm for discard containers (if required)
- 10,000 ppm for spillages
- 20,000 ppm for work surfaces, including microbiological safety cabinets

Please enter comments of the GM safety committee on the risk assessment

The Genetic Modification Safety Committee agrees that the assessment for notifiable activity is accurate, the containment facilities are appropriate and recommends the committee and HSE be notified appropriately if Class 2 or Class 3 activity is to be carried out subsequently.

This risk assessment should be kept under review and the Biological and Genetic Modification Safety Committee should be informed if there are any significant changes in procedures, personnel or location that might affect risk of harm to humans or the environment.
This application was not progressed and the fee was refunded
Premises Addresses

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify)  Tick if confidential

Bacteriology  Parasitology  Transgenic Birds  Microbiology Research
Virology  Transgenic Animals  Transgenic Fish  Gene Therapy
Mycology  Transgenic Invertebrates  Transgenic Plants  Other (please specify below)
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Y
A GM Safety Committee has been established and held its first meeting in January 2009. Its composition includes the University Biological Safety Officer, 2 senior scientists and 3 academic clinicians with a research or clinical interest in gene therapy, the chair UCL/UCLH research safety subcommittee, the Divisional Manager Joint UCLH/UCL biomedical research unit, a microbiologist/infectious disease clinician with experience on GM safety committees, the infection control nurse consultant and relevant pharmacy representatives will be co-opted where appropriate. The GMSC has direct accountability and reports to the joint UCL, UCLH, RFH research governance committee (cross representation by GMSC chair and 2 other GMSC members). There is also cross representation from Research Safety Sub-Committee (Chair of RSSC on GMSC). The joint unit research governance committee (cross representation by GMSC Chair and 2 other GMSC members) There is also cross representation from research safety sub-committee (Chair of RSSC on GMSC). The joint unit research governance committee will report to UCL Faculty of Faculty of Biomedical Sciences and UCLH and RFH Trust Boards via the appropriate routes within those trusts.

Remit of the Committee:
Risk Assessment (RA) of planned clinical trials involving GMO/GMMs involving UCLH, RFH and QS.
RA of planned clinical trials involving GMOs where UCL is acting as sponsor.
Provision of safety advice and recommendations specific for individual clinical trails to UCL (UCL & UCLH research governance and research safety sub-committees) and involved trusts.
Notification of high-risk trails to the HSE where indicated.
More general provision of advice.
To support/provide education to clinical staff participating in running clinical trails involving GMOs.
Generation of SOP templates.
The GMSC will meet on an ad-hoc basis and will provide a Risk Assessment for a Clinical trial involving GMMs within 28 days of its submission for consideration.

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Other (please specify) Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy Yes
- Mycology
- Transgenic Invertebrates
- Transgenic Plants
- Other (please specify below)
For activities involving GMMs, describe the waste management measures which will apply to the activity:

For clinical trials involving the administration of replication incompetent viral and non-viral vectors (i.e., Class 1 activities) usual existing hospital waste management procedures will be used. Contaminated material such as swabs and dressings will be placed in the yellow bag waste stream. Contaminated sharps such as needles, syringes and glass vials should be placed into sharps bins and disposed of as normal.

In the event of spillage or contamination of clinical equipment/clothes, soaking in an effective disinfectant such as virkon 1% and/or autoclaving before washing is sufficient.

Clinical staff taking blood or tissue samples from treated patients are trained to avoid exposing themselves or others to blood-borne pathogens and in the prevention and in the prevention of needle stick injuries. In the event of a needlestick injury, normal procedures should be undertaken as stipulated by the UCLH Department of Occupational Health.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

WT1 TCR Gene Therapy for Leukaemia: A Phase I/II Safety and Toxicity Study (WT1 TCR-001):
[Trial ID: EudraCT 2006-004950-25; MHRA CTA 20363/0253/001-0001; GTAC 128; UKCRN 5099; UCL Sponsor ID 06/154]

Summary of GMSC comments on the Risk Assessment:
The clinical trial is correctly classified as a Class 1 project and falls under the Contained Use regulations. The precautions stated in the risk assessment are commensurate with the (low) risk of the work. Transportation and spillage issues are well covered as is the risk to trial workers.
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

| Yes

**Give brief details of the genetic modification safety committee**

- Committee Comprises Chief Scientist (25+ Yrs Experience infectious disease research e.g Common Cold, FMDV, HIV)
- Laboratory Manager (Chemist)
- Head Technician (Prions, blood diagnostic lab)

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</thead>
<tbody>
<tr>
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**Tick if confidential**

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
### For activities involving GMMs, describe the waste management measures which will apply to the activity

All Biological materials and contact materials will be put in clinical waste bags and incinerated by a commercial service.

<table>
<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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**Other(s)**

Tick to confirm that you are attaching a summary of the risk assessment  
Tick if you are claiming exemption from disclosure for sections of the risk assessment

**Please enter comments of the GM safety committee on the risk assessment**

The Baculovirus samples to be analysed in the detaDOT laboratories are not pathogenic. All baculovirus work is carried out under best practice conditions. The accompanying risk assessment (External version) forms will be sent to all customers.
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| Comments                         |             |

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

<table>
<thead>
<tr>
<th>Chairperson</th>
<th>Project lead</th>
<th>Research Nurse Specialist</th>
<th>Director of research &amp; development</th>
<th>Quality Assurance manager - pharmacy</th>
<th>Acting Helath &amp; Safety Adviser</th>
<th>Consultant Micro-biologist</th>
<th>Biological Safety Officer, University of Bristol with honorary contract</th>
<th>Divisional Clinical Risk Lead</th>
<th>Administration Assistant</th>
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</table>

Give brief details of the genetic modification safety committee

### Laboratory

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

### Animal Unit

### Growth Room

### Glass House

### Large Scale

02/03/2022
### For activities involving GMMs, describe the waste management measures which will apply to the activity

University Hospitals Bristol NHS Foundation Trust Waste Management Policy enclosed

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Trust Gene Therapy Committee are satisfied with the proposed study. No comments of concern.
**GM Centre Number: 3051**

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### Name

**KINGSTON RESEARCH LIMITED**

### Name 2

**BANKS COOPER ASSOCIATES**

### Campus Estate or Research Centre

### Building

### Road Name

**21 MARINA COURT**

### District

### Town

**HULL**

### County

### Postcode

**HU1 1TJ**

### Country

**ENGLAND**

### Tel Number

**01482 892304**

### Fax Number

**01482 892392**

### E-mail

### HSE Division

**LONDON**

### Comments

### Date at Which Additional Info Submitted

**02/03/2022**
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Y
The KRL General Manager is ultimately accountable for the safety performance of KRL. The Operations Manager is responsible for ensuring safety of operations on a daily basis. The Genetic Modification Safety Committee (GMSC) provides advice to the Operations Manager for practical implementation of GM related safety, as outlined below:

- Advise on and review in detail new and amended activities involving the use of GMM
- Advise on the risk assessment and any updates to the risk assessment have been completed in accordance with the Genetically Modified Organism (Contained Use) Regulations 2000 and Advisory Committee on Genetic Modification Compendium of Guidance (ACGM)
- Advise on the bio-safety training requirements needed for personnel expected to carry out work with genetically modified micro-organisms

Composition
During the project phase, the GMSC comprises the following members:

- Biobutanol OSBL Project Manager (Chairperson)
- BP Hull Site Occupational (Health & Hygiene) Advisor
- Biobutanol Demonstration Plant Biological Safety Officer
- Biobutanol Demonstration Plant Operations Representative
- HRTC Safety Manager
- Representative of DuPont SHE Department
- BP Biofuels Technology Representative
- DuPont Technology Representative

In order for a meeting to be considered quorate, 5 of the 8 members must be present. It is the responsibility of the Chair to ensure that the attendees provide adequate expertise to make justifiable decisions and recommendations. This team will be supported by a number of consultants who can be called on to give advice as required.

Upon moving into the operations of the Biobutanol Demonstration unit and introducing GMM to the site, the GMSC composition will be revised as follows:

- KRL Operations Manager (Chairperson)
- KRL Biological Safety Officer
- BPCL HRTC HSSE Manager
- DuPont Technology Representative
- KRL Operations Team Representative
- BP Biofuels Technology Representative
- BPCL Hull Site Occupational (Health & Hygiene) Advisor

In order for a meeting to be considered quorate, 4 of the 7 members must be present. It is the responsibility of the Chair to ensure that the attendees provide adequate expertise to make justifiable decisions and recommendations. This team will be supported by a number of consultants who can be called on to give advice as required.

Meetings of the GMSC
The committee will meet on an ad hoc basis to discuss proposed modifications to the genetically modified micro-organism or the Standard operating procedures. However, scheduled meetings will be planned at least twice a year in order to review operations, near miss reports and incident reports. Dates of these meetings will be fixed in advance.

Notice of the meetings will normally be circulated one week in advance and accompanied by a meeting agenda, minutes of the last meeting and papers for discussion. The Minutes of the Committee must be issued within two weeks of the date of the meeting and will be distributed to all committee members and invited attendees. A copy will be placed in reading file stored in the facility safety corner.
Depending on the type of waste (disposables, liquids, residual GMMs on equipment, off-gas), one of the following management measures will be used for inactivation and/or removal of viable GMMs: chemical inactivation, physical inactivation by heat (off-site incineration, autoclave, steam, fermentor heaters or the process GMM inactivation system) or filtration. Any off-site disposal will be carried out using licensed waste handling contractors and registered waste disposal sites appropriate for the waste type.

The largest volumes of liquids containing GMMs will be handled via the process GMM inactivation system that consists of two heat exchangers and residence time piping to ensure GMM inactivation. Viable GMM-containing process streams from the fermenters will be pumped through an exchanger where the temperature of the process fluid is raised. Both flow rate (residence time) and temperature can be adjusted based on proven inactivation time/temperature profiles.

Each inactivation method is expected to result in a minimum log 5 reduction of GMM viability. For each inactivation method, control of the relevant parameters (such as temperature, concentration, expiry date, treatment duration) is foreseen.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
The GMSC reviewed the risk assessment for the activity entitled “Bio-butanol Demonstration Plant” during its meeting of 9th June 2009.

Given the description of the GMMs and the activity, the GMSC agreed that the proposed containment measures are adequate. The GMSC acknowledges that in some cases the measures that will be implemented exceed the safety requirements as they are inspired by other aspects such as quality of product.

Any modifications to the process will be subject to Management of Change (MoC) which will highlight the requirement to consider whether the GMM risk assessment should be reviewed as part of any process-related modification.

Finally, the GMSC stressed that the reviewed risk assessment is accepted in connection with the specific organisms specified therein. If a new GMM is to be introduced to the process or the existing GMM further modified, then the MoC process will also highlight the requirement to review the risk assessment and amend as required to suit the specifics of the new/modified organism.
### GM Centre Number: 3054

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**Name**

SCOTTISH ENVIRONMENT PROTECTION AGENCY

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

5 REDWOOD CRESCENT

**District**

PEEL PARK

**Town**

EAST KILBRIDE

**County**

LANARKSHIRE

**Postcode**

G74 5PP

**Country**

SCOTLAND

**Tel Number**

01355 574200

**Fax Number**

01355 574688

**E-mail**

**HSE Division**

SCOTLAND

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The project has been considered by GM Safety Committee has agreed with the classification of GM1 and risk assessment for notification to the HSE.
The committee comprises the Microbiology Unit Manager, Marine Scientist, Senior Marine Chemist, Ecotoxicologist, Health and Safety Unit, Emergency Planning Advisor, Head of Marine Science and the Head of Chemistry.

OPERATING PROCEEDURES AND FREQUENCY OF MEETINGS:-
1. Chair invites members to a 6 monthly meeting to discuss H&S matters and to review either new, or current risk assessments.
2. Risk Assessments are circulated prior to the meeting.
3. During the meetings the relevant risk assessments will be discussed and any amendments required will be made by the assessment originator.
4. The amended risk assessment will then be circulated to all staff in the GMSC for further comments/suggestions.
5. Finalised risk assessments are passed to the Head of Health and Safety for approval.

<table>
<thead>
<tr>
<th>Laboratory</th>
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<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>
The GMM proposed for use in this study is a modified strain of Saccharomyces cerevisiae (FF18984). ACDP Classification - Class 1: Gentronix Greenscreen EM™

The Ecotoxicology laboratory is a UKAS accredited facility, where the Class 1 GMM will be handled in accordance to the Standardised Working Procedure and GLP in Class 1 + containment level.

* All Solid and liquid waste is sealed in biohazard bags containing an absorbent material and inactivated in an autoclave at 134º C for 34 minutes. Inactivated waste is disposed of accordingly to local Microbiological rules with final disposal as either general laboratory waste or hazardous waste as identified appropriate.

* Contaminated glassware will be decontaminated in a final volume of 1% (w/v) Virkon solution for 24 hours prior to going through an automated glasswash cycle, followed by autoclaving as required.

* Work areas/hard surfaces will be disinfected after use using 70% Ethanol solution/Aquet 1% Solution.

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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
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02/03/2022
* Hands and wrists of laboratory staff will always be washed prior to leaving the GMM area (CTR1). Furthermore, following contamination with viable yeast cells, hands and wrists will be washed immediately.

Spillage Containment -

* Liquid spills of class 1 GMMs on the bench or floor will be contained with absorbent paper tissue. The area would then be decontaminated with 1% (w/v) Virkon. Contaminated absorbent material will be put into specified biohazard waste bag/container which will be sent for autoclaving at a cycle of 134°C/34min prior to final disposal. Liquid spills will be recorded and reported to the line manager and Health and Safety.

Laboratory clothing which is contaminated/potentially contaminated with Class 1 GMMs must be sealed in large 'biohazard' bags and will be decontaminated through heat inactivation in the autoclave prior to commercial laundering.

* Contaminated broken glass will be inactivated by autoclave before appropriate disposal. The affected bench/floor area will be disinfected with 70% ethanol/water solution. Final disposal of inactivated broken glassware will be as either general laboratory waste, or hazardous waste - depending on any contamination of the glassware by hazardous chemicals.

Autoclave -
Items inactivated via autoclave are subject to a temperature of 134°C for 34 minutes. The temperature probe used in the autoclave, the autoclave cycle chart recorder and the temperature variation within the autoclave chamber are assessed and calibrated annually by a UKAS certified calibration agent. 100% degree of kill would be achieved.

Chemical agents -
Alcohols give a very rapid kill of micro-organisms, but do not provide sustained microbial action. 99.999% kill would be achieved.
Virkon is a peroxygen system that is widely used for laboratory disinfection, and is effective against bacteria, viruses and yeast. In addition, Virkon was found to be effective against 3 strains of Saccharomyces cerevisiae (the Host organism proposed for use in this study) after 5 minutes contact time at 1% using a suspension test (Alegente et al). 99.999% degree of kill is assumed after 24 hours contact with 1% Virkon solution.
And anti-fungal agent that is effective in destroying a broad spectrum of organisms including Saccharomyces spp. (Day-Impex, 2004) 99.999% kill would be achieved.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

SEPA's GM Safety Committee has approved the risk assessment and believes that the activities described present no risk to the environment or to human health.
The committee certifies that the laboratories in which this work will be done fully satisfy the requirements for the Advisory Committee on Genetic Modification containment level 1, with good practice being observed to containment level 2.
**GM Centre Number: 3058**

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**Comments**

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**  
[ ] Y

**Give brief details of the genetic modification safety committee**

The safety officer with overall responsibility for Hypoxium is the CEO. The safety committee consists of the safety officer and the laboratory safety officer (senior scientist). All safety procedures are overseen by an external qualified safety advisor (CFIOSH, MRSC, MISTR) with twenty years of experience. The committee meets quarterly where use is reviewed. Risk assessments are reviewed and updated annually.

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Waste is inactivated using virkon, and waste removed from site by a professional specialised waste management company (Grundon) who perform high temperature incineration and dispose of the waste.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste is inactivated using virkon, and waste removed from site by a professional specialised waste management company (Grundon) who perform high temperature incineration and dispose of the waste.

Please enter comments of the GM safety committee on the risk assessment

This risk assessment covers the use of genetically modified cell lines supplied to Hypoxium Ltd by Horizon Discovery. All host cell lines are established lines of human origin, which are unable to survive in the outside environment. Modifications carried out do not increase the risks associated with the host cell lines, therefore for safety purposes these cells can be considered to be ‘as parental’. The committee therefore approves this risk assessment and the classification of the cell lines for use at containment level 1, and corresponding GMM Class 1.
GM Centre Number: 3059

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Name

HENDERSON MORLEY PLC

Name 2

Department

Campus Estate or Research Centre

Road Name

2 SALISBURY ROAD

District

MOSELEY

Town

BIRMINGHAM

County

MIDLANDS

Postcode

B13 8JS

Country

ENGLAND

Tel Number

01214424600

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01214424611

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HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022

Page 12800 of 15326
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Composition: Chair
- Biological Safety Officer
- Senior Scientific Project Manager

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<thead>
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Other (please specify) Tick if confidential

- Bacteriology: Yes
- Parasitology
- Transgenic Birds
- Microbiology Research: Yes
- Virology: Yes
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
The proposed GMMs will be GM Herpes Simplex Virus type 1 (GM-HSV1); ACDP2. Modified to incorporate polyepitopes from other human pathogens; namely, human cytomegalovirus, Epstein Barr virus or influenza virus. Others to incorporate polyepitopes derived from certain human tumours; namely, CD20 or MART1. None of these GMMs are expected to pose an increased hazard. There is no increased risk to humans nor risk to the environment (nil).

The risk assessment concludes, therefore, that precautions and procedures already in place for the production and disposal of HSV1 (ACDP2) are sufficient. All associated Standard Operating Procedures, Chemical and Biological Hazard Risk assessments pertaining to the cultivation and purification of GM-HSV1 (appended) will be complied with at all times.

Hazard Risk assessments specific to the Genetic Modification of HSV will be considered within the application for 'individual contained use activities'.

---

**Project Ref**  3059/09.1

**Date Ackn'd**  25/09/2009

**CU2 Project Title**  The creation of recombinant human Herpesvirus 1 (HHV-1) in which novel antigenic sequence is present as a fusion protein with either the major tegument protein (vp11/12), the envelope and glycoprotein D (gD) or both 11/12 and gD

**Date Project Ceased**  

**Class**  Class 2

**CultureVol**  1-50 Litres

**Class Culture Volume**

**Culture Volume Class**

**Non-GMM**

**Consent Granted**

**Project notified under transitional arrangements**  N

**Withdrawn**  N

**Tick if notifying a connected programme of work**  N

**Historical Significant Changes**
# Project Additional Information

## Purposes of the contained use

Infection of cell culture with HHV-1 results in the formation of non-infectious virus particles which lack the viral capsid and DNA. These Light (L) particles have a similar structure to infectious HHV-1 particles and have potential as vaccine candidates. The aim of this work is to create recombinant HHV-1 in which novel antigenic sequence is displayed as a fusion protein with the viral gD and vp11/12 proteins. These recombinant viruses will then be used to make L particles with the novel antigen present in the viral structural proteins.

## Recipient or parental organism

Human herpesvirus 1 strain 17. Class 2 pathogen.

## Host/vector system

- E. coli strains DH5, DH5-T1R, TOP10 dam-/dcm-

## Origin & function

Genomic DNA from HHV-1 strain 17 will be used as a template for the amplification of the viral US6 gene (gD), UL46 gene (vp11/12) and UL23.

## Evaluation of foreseeable effects

The modifications will be carried out by the insertion of novel sequence into the viral US6 gene (gD) or UL46 gene (vp11/12) as an in-fram fusion. Sequences to be inserted into these genes include (but may not necessarily be limited to) antigenic determinants for Epstein-Barr virus, Human Cytomegalovirus, Human and Canine CD20, Human Melan-A, Gallid ovalbumin and influenza A. Based on published data, insertion of sequence of sequence into these sites is not predicted to alter the pathogenicity of the virus. Recombinant viruses may be constructed using a thymidine kinase (TK) selection method. These would not be expected to have increased virulence or pathogenicity but would have resistance to 5’ bromo-deoxyctydine (B CdR) treatment.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All infectious waste to be sterilised:
I. Liquid waste; hypochlorite tabs. Manufacturer's instructions followed explicitly.
II. Solid waste; Autoclave then incineration at 34 Redfern Road, Tysley, Birmingham, B11 2BH. Autoclave cycle parameters monitored and recorded electronically and verified by additional thermal indicators (tapes and tubes).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**Name**

SIMON LANGTON GRAMMAR SCHOOL FOR BOYS

**Campus Estate or Research Centre**

**Road Name**

LANGTON LANE, NACKINGTON ROAD

**Town**

CANTEBURY

**County**

KENT

**Postcode**

CT4 7AS

**Country**

ENGLAND

**Tel Number**

01227 463567

**Fax Number**

01227 456486

**E-mail**

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**HSE Division**

**Comments**

Date at Which Additional Info Submitted

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GMM/GMO committee at the Biosciences Department, School and the Biosciences department.
The Committee consists of the chair, a staff representative and postgraduate student representative. They meet on an adhoc basis to consider individual applications.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential
Contaminated plasticware (tips, eppendorfs etc.) will be collected in beakers containing 1% Virkon solution. Waste materials will be collected in autoclavable Biohazard bags. These will be autoclaved at 121 degrees C and all bags and materials will be tagged with autoclave tape to ensure that the correct operating temperature has been reached. We have two manual autoclaves and one automatic autoclave.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The project was classified as Class 1
### GM Centre Number: 3062

| Data Premises Closed | | Transitional Premises | N | Non-GMMs | N | Withdrawn | N |

**Name**

BIOVITAL RESEARCH

**Name 2**

**Department**

**Campus Estate or Research Centre**

SOMERTON BUSINESS PARK

**Road Name**

UNIT 7, CAMELOT COURT

**Building**

**District**

**Town**

SOMERTON

**County**

SOMERSET

**Postcode**

TA11 6SB

**Country**

ENGLAND

**Tel Number**

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**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Biological Safety Officer (Chair), Research scientist (Employee representative), Management representative, External Expert advisor.

The committee will consider any project involving GMMs and will meet on a 6 monthly basis.

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<thead>
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<th>Transgenic Animals</th>
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02/03/2022
The maximum amount of waste generated at one time will be 150ml of culture media/liquid waste and small quantities of solid non-sharp standard laboratory waste (for example wipes, plates).

All liquid and solid waste will be inactivated by autoclaving at 124 centigrade for 30 minutes in a dedicated autoclave giving a 100% kill effectiveness. Disposal will then be as standard laboratory waste via a commercial laboratory waste disposal company. The autoclave facility is tested for compliance by use of temperature monitoring and by use of colour change tape to confirm sterilization confirmation tests using a commercial test strip system are undertaken.

All surfaces will be regularly cleaned with a commercial bacteriocidal surface cleaner during the work and all operatives will wear PPE’s (including laboratory coats and gloves) that are decontaminated before leaving the facility.

The waste management and decontamination procedures fully comply with the guidelines contained in the SACGM compendium of guidance for handling waste.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Given the use of attenuated host strains, vectors incapable of expression in mammalian cells and the low toxicity of the inserted sequences, the GMM's are appropriately classified at level 1. An accidental release would be low impact. The proposal includes working at level 2 containment despite only Level 1 GMM usage, as such all sensible precautions will be taken and the risk of release is also low. Overall the risk of the project is therefore low to negligible and the risk, management procedures are adequate.
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Name

**PHARMINOX LTD**

Name 2

Department

Campus Estate or Research Centre

**BIOCITY**

Road Name

**PENNYFOOT STREET**

Town

**NOTTINGHAM**

District

County

**NOTTINGHAMSHIRE**

Postcode

**NG1 1GF**

Country

**ENGLAND**

Tel Number

01159124524

Fax Number

01159124521

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee (GMSC) will consist of the following members:

Chief Scientific Officer (company director), Head of Chemistry, two Pharmacologists and an external academic biological research consultant.

The GMSC will meet once yearly to review existing GMM risk assessments. Monthly meetings are held where the above gather so an agenda item to review a new GMM risk assessment can be added on an ad hoc basis.

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<th>Laboratory</th>
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Tick if confidential

Bacteriology  Parasitology  Transgenic Birds  Microbiology Research
GMM cell lines, cell culture media and labware used in the culture of GMM cell lines will be disposed of through the use of commercially available certified detergents (e.g. Trigene) using dilutions and disinfection times recommended by the manufacturer. Decontaminated liquid cell culture waste will be disposed of down the sink, decontaminated solid waste will be sealed in biohazard bags (or sharps containers where appropriate) and disposed of through a commercial waste disposal company.

Some GMM cell lines will be used in an assay whereby the final step requires the direct application of DMSO to the cells thus killing them. Once the cells are removed from their correct culture medium they rapidly become non-viable. Cell viability assays will be carried out to monitor the effectiveness of this method of cell kill.

Once finished with the plates are bagged, sealed in an appropriate container supplied by a commercial waste disposal company who will then dispose of them appropriately.

Screening and research into the effects of novel small molecules on established mammalian cell lines

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment PHX-GM-001 (attached) was considered by the genetic modification safety committee at its meeting on 14th September 2009. The activities included in the risk assessment were approved as Class 1.
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Date at Which Additional Info Submitted: 02/03/2022
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee has the same make-up as the Biological Safety Committee is made up of Biological Safety Officer (Chair), Director of the Research School of Health and Life Sciences or his nominee, Professor of Pharmaceutical Engineering (representing the Faculty of Engineering), Head of School Sport and Exercise Science (representing the Faculty of Social Sciences, Nominees from Chemistry and Human Science (representing the Faculty of Science) and upto 4 co-opted members. The full University Biological Policy can be found at, http://www.lboro.ac.uk/admin/hse/policies/download/biological%20safety%20policy.pdf

There are two levels of Risk Assessment. If work is to be carried out with non-GM material then a Biological Risk assessment must be completed. If the work is with GM material then in addition a GM risk assessment must also be carried out with non-GM material then a Biological Risk assessment must be completed. If the work is with GM material then in addition a GM risk assessment must also be carried out (see exemplar attached). Once a GM risk assessment has been completed it is submitted to the GM safety for approval. Here it is peer reviewed by at least two members of the committee comments fed-back to the applicant(s) where necessary and revised before formal approval is given. Work cannot commence before formal approval is obtained from the GM safety committee. The GM safety committee meets formally at least once annually however the assessment and approval of GM risk assessments can be dealt with by circulation. The committee also inspects and approves new laboratories for GM and biological safety compliance before any work can be carried out. Laboratories with existing approval for GM/biological work are inspected and re-approved annually.

Level 1 (GMMs) Yes
All work will be carried out in a Biological Hazard Group II Containment laboratory.

All solid and liquid waste will be autoclaved i.e. 121 degrees C for 20 minutes prior to disposal by incineration. The autoclaves have been professionally validated against the specific types of waste we intend to use and they will be re-validated and serviced every 12 months unless a problem is identified sooner. Validation at point of use via the visual inspection of applied ‘autoclave tape’.

Liquid spills are dealt with by flushing with 1-2% v/v Virkon solution (industrial standard disinfectant for such purposes validated against a range of common cell types), the spill will then be mopped up, autoclaved (121 degrees C for 20 minutes) prior to incineration.

In all cases a 100% kill is expected prior to disposal.

Tick to confirm that you are attaching a summary of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Committee member 1.

1) Page 2: Are the same sequences being inserted into both the 51R and foetal liver cells? This is somewhat unclear. Would it be appropriate to maybe replicate the table, once for each cell line, to make this clearer?
2) Page 4: Quantity of organism - does this refer to each cell line or in total? (I would assume each based on selection B2.4.2 in risk assessment)
3) Page 5: Work likely to produce aerosols - although it is detailed in the biological risk assessment it may be worth reiterating the fact that the work is also carried out in the self-contained Class II environment of the CompacT SelecT.
4) Page 10: Room numbers are missing from the top table. For training - mention the "records of training" which are mentioned in C4.2 of the biological risk assessment.

Committee member 2

The risk assessment appears to be good so I hope this allows the project to progress.

The risk assessment appended was accepted after these had been addressed and the Risk Assessment attached is that which was accepted after modification.
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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Chair
- BS officer
- 3 principal investigators
- Representative of other employers conducting research in this establishment
- Secretary

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<tr>
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Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

02/03/2022 Page 12819 of 15326
Virology  Transgenic Animals  Transgenic Fish Gene Therapy

Mycology  Transgenic Invertebrates  Transgenic Plants Other (please specify below) Yes

Other(s)  work with established cell lines

For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid waste - autoclave on the premises. Autoclaves validated and tested regularly (annual test), each load will be monitored and recorded.
Standard autoclaving temperature and pressure will be used to achieve greater than 5 log kill.

Liquid waste - hypochlorite solution at strength of 10,000 ppm active chlorine

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The committee has approved this work to be conducted in the Research Complex at Harwell as a transfer from GM Centre 553 (Oxford University). Formal transfer of this project, along with other Class 1 activities, will be made by GM Centre number 553 to the Research Complex at Harwell, once the Complex has been assigned a centre number.
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| Comments                             |              |

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

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<tr>
<td>1. Director</td>
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<td>2. Project Manager (Microbiology)</td>
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<td>4. Health &amp; Safety Advisor</td>
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<td>5. Engineering Services Manager</td>
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The committee meets regularly (quarterly), and also as required, to review and make recommendation on proposed work to be carried out at the company's premises.

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Other (please specify) Tick if confidential

02/03/2022
The principle method of inactivation of cultures of GMM's will be by treatment in an autoclave (heat inactivation) at 15 p.s.i. for 20 minutes following the manufacturer's recommendations for use.

All cultures, whether in glass or disposable containers, will be autoclaved before disposal or recovery of containers. Liquid medium from which micro-organisms have been recovered by centrifugation or filtration, and contaminated plasticware (Petri dishes, Eppendorf tubes, disposable pipettes and culture tubes etc), will also be sterilised in the autoclave prior to disposal. The autoclave will be regularly serviced and tested for effectiveness according to the manufacturer's recommendations.

Spills and leakages of contaminated liquids are to be treated with a suitable disinfectant (eg Virkon™) according to manufacturer's recommendations and in accordance with appropriate COSHH guidelines. Disposable tissues used for mopping up disinfectant-treated spills will be autoclaved prior to disposal.

Contaminated sharps will be collected in an approved designated sharps container and disposed of by incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM Safety Committee reviewed the risk assessment and requested that COSHH assessments relating to host microorganisms were filed together (locally) with the risk assessment.
**GM Centre Number: 3069**

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**Name**

TANGENT REPROFILING LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

CHERWELL INNOVATION CENTRE

**Road Name**

77 HEYFORD PARK

**District**

UPPER HEYFORD

**Town**

BICESTER

**County**

OXFORDSHIRE

**Postcode**

OX25 5HD

**Country**

ENGLAND

**Tel Number**

07870 110654

**Fax Number**

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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<td>77 HEYFORD PARK</td>
<td>UPPER HEYFORD</td>
<td>BICESTER</td>
<td>OXFORDSHIRE</td>
<td>OX25 5HD</td>
<td>ENGLAND</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- CEO of Tangent Reprofiling Limited
- CSO of PepeTcell Limited
- Laboratory Manager of DiagnOx
- Senior Consultant for Oxford Innovations
- Professor of Biology at the University of Warwick (Academic Expert)

<table>
<thead>
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Tick if confidential

Bacteriology: Yes

Parasitology: 

Transgenic Birds: 

Microbiology Research: Yes
Inactivation of GMMs is by autoclaving and/or by chemical disinfection. Chemical disinfection is achieved by treatment with a final concentration of Virkon of a minimum of 5% for a minimum of 30 minutes. Autoclaving will be monitored daily by use of indicator tap, and the kill sufficiency measured monthly by the attempted growth of an appropriate GMM containing solution after autoclaving.

All disposable consumables that have been in contact with GMMs are autoclaved before removal from the laboratory for disposal. Autoclaving is carried out in the same laboratory as the work has been carried out. Material for autoclaving is contained using a double-bagging protocol where multiple small autoclave bags are collected in a larger autoclave bag within the microbiology safety cabinet, and then transferred to the autoclave. After autoclaving the materials are disposed of as clinical waste.

Disposable lab coats and nitrile disposable gloves are worn at all times and are not removed from the laboratory except after autoclaving for disposal.

Surfaces are cleaned daily by liberal application of 5% Virkon. Spills will be contained with disposable absorbent materials which will be treated with Virkon before autoclaving for disposal as clinical waste. The area of the spill will be treated by liberal application of 5% Virkon solution.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

The GMSC has read and understood the risk assessment and classifies the work as Group 1. The Academic Expert has recommended that filter tips are used on all pipetting actions involving GMOs to minimise the risk of contamination of the pipettes. This has been implemented, and added to the risk assessment accordingly.
### GM Centre Number: 3070

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**Name**

CAMBRIDGE BIOFREEZE LTD

**Name 2**

**Department**

Campus Estate or Research Centre

**Road Name**

UNIT 12, CARLTON PLACE

**District**

SHIRE HILL INDUSTRIAL ESTATE

**Town**

SAFFRON WALDEN

**County**

ESSEX

**Postcode**

CB11 3AQ

**Country**

ENGLAND

**Tel Number**

0844 335 2819

**Fax Number**

01799 528430

**E-mail**

Blank

**HSE Division**

Blank

**Comments**

### Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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<td>CB11 3AQ</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

1st Member - Entrepreneur with 40 years experience working with and for the biomedical field, transporting and storing low temperature GM samples.

2nd Member - Retired laboratory manager with 40 years experience working in a leading government backed molecular biology research institute. He has a vast knowledge base on handling and storing GM samples.

3rd Member - Chemistry graduate (University of Bristol) with several years laboratory experience, registered Dangerous Goods Safety Advisor and ADR trained driver with experience handling, transporting and storing low temperature GM samples.

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Other (please specify) Storage/Warehousing

Tick if confidential
Yes

In the highly unlikely event of a vial breakage/spillage, staff would wear protective gloves and eye wear, then undertake disinfectant procedures using Hypochlorite solution, place broken vial/spilt materials in to autoclave bags which will then be sealed and collected by an approved GM waste disposal contractor for incineration.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessment compiled with all three members involved. After speaking to our customer, considering all risks and consulting with the HSE, we are satisfied that we are an exceptionally low risk class one activity and have all procedures and operating measured in place to minimise risk and all eventualities are covered.
GM Centre Number: 3071

Data Premises Notified (Originally) 01/12/2009
Transferred from 1992 Regs? N
Transitional Premises Class

Data Premises Closed
Transitional Premises Emergency Plan Required? N
Non-GMMs N
Withdrawn N

Name
WELLCOME -MRC STEM CELL INSTITUTE

Name 2
UNIVERSITY OF CAMBRIDGE

Campus Estate or Research Centre
CAMBRIDGE BIOMEDICAL CAMPUS

Building
JEFFREY CHEAH BIOMEDICAL CENTRE

Road Name
PUDDICOMBE WAY

District

Town
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County
CAMBRIDGESHIRE

Postcode
CB2 0AW

Country
ENGLAND

Tel Number 01223 760240
Fax Number 01223 760241

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted
02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The CSCR GM committee reports to the Biological Safety Committee, which in turn reports to the main Centre Safety Committee. It oversees all current and potential projects within the research centre. The committee reviews current and proposed projects as a "virtual committee" and will sit formally as required e.g. to discuss a complex project proposal or significant change to an existing project. It is composed of the BSO and Deputy, Centre Safety Officer and other experienced GM users.

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02/03/2022 | Page 12831 of 15326
All Class 1 solid waste is inactivated on site using a steam autoclave. Validation of waste inactivation by autoclaving is in place. All liquid waste is inactivated by treatment with Virkon or Trigene according to the manufacturer’s instructions.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The seven members of the Biological Safety Committee did not raise concerns about the project and unanimously approved the project as containment level 1 work.
Project Additional Information

Purposes of the contained use

Originating from early developing cells after fertilization, embryonic stem cells (ESC) are unique in their capacity to generate all differentiated cell types composing their original organism. The ability to cultivate ESC in vitro offers a tremendous opportunity to the scientific community to study fundamental mechanisms acting through embryonic development as well as opening exciting clinical perspectives by offering wide possibilities for in vitro pharmacological models and cell sources for regenerative medicine. Our global objective is thus to gain better knowledge in molecular factors and mechanisms controlling early ESC fate specification towards the three primitive embryonic germ layers (endoderm, mesoderm and ectoderm) and further differentiation towards mature functional cells. By bringing important insights in fundamental development processes, our project should allow us to gain in parallel a better control in ex vivo differentiation protocols from ESC and improve the production of therapeutically relevant cell types like cardiomyocytes, neurons, pancreatic cells or haematopoietic stem cells for instance.

This general perspective will develop into three leading projects implying genetic modification of various cell types:
- Project1. Factors controlling cell fate specification of pluripotent stem cells
- Project2. Transcriptional factor mediated reprogramming of somatic and embryonic stem cells
- Project3. Gene targeting in human embryonic stem cell

Since they are highly efficient vectors to genetically modify numerous cell types (notably ESC), we plan to use recombinant viral vectors derived from gamma-retroviruses, lentiviruses and adenoviruses to achieve our goal. We will use recombinant retroviral particles (MuLV derived, from the company Vectalsys, France) and recombinant lentiviral particles (HIV-1 derived, production in situ) which have amphotropic ranges of tropism (VSV-G pseudotyped). For some experiments, recombinant adenoviral particles (Ad5 derived, from the company Hybrid Systems, UK) will be also used. All recombinant viral vectors will be replication defective and each production batch properly tested for absence of infectious particles. Inserts (cDNA, shRNA) will be cloned into these vectors, alone or in combination (through the use of independent promoters, IRES or 2A sequences) with GFP, antibiotic resistance gene or another insert of interest. In situ production of lentiviral particles will be achieved by transient co-transfection of 3 plasmids (vector, helper and envelope) into 293T/17 cells. All lentiviral particles will be produced from SIN (Self Inactivating) backbones using the 2nd generation production system (Vif, Vpr, Nef and Vpu deleted helper plasmid). Work involving recombinant virus handling (production, transduction) and human primary cell culture will be performed under containment level 2 conditions in compliance with institution practice.

Recipient or parental organism

1. Escherichia coli - K12 derived strains, disabled, non colonising
2. Gamma oncoretroviruses – MuLV derived, replication defective, amphotrope
3. Lentiviruses – HIV-1 derived, replication defective, amphotrope
4. Adenoviruses – Ad5 derived, replication defective, ecotrope
5. Mammalian cells (primary cells and cell lines) – considered as especially disabled
   - Human, murine embryonic stem cell lines (H9, H1, HSF6,…)
   - Human, murine induced pluripotent stem cell lines (produced in situ)
   - Human, murine primary cells (embryonic and adult skin fibroblasts, keratinocytes, peripheral blood cells)
   - Human, murine somatic cell lines (HEK-293T, HCT-116,…)

1. E.coli K12 / pUC derived plasmids - no insert expression, plasmid non mobilisable.
2. HEK-293T / pUC derived plasmids – transient co-transfection, viral gene and insert expression, production of amphotrope replication deficient lentiviral particles (each batch to be tested).
5. Mammalian cells / Ad5 vectors – replication defective adenoviruses, transient genetic modification.

Host/vector system

1. Human, murine, xenopus cDNA (+/- TAG sequences) coding for transcription factors, signal transducers, growth factors and surface receptors. These inserts are expected to play a role in pluripotency, cell fate decision and differentiation from pluripotent stem cells (PSCs). Their expression may enhance/block commitment and differentiation of PSCs towards one of the three primitive germ layers and further differentiated progeny.
2. shRNA and miRNA sequences against cell endogenous coding sequences. These inserts are non coding RNA sequences targeting cell endogenous mRNA from above mentioned factor families (in 1) in order to inhibit protein expression by RNA interference (RNAi). Their expression may enhance/block commitment and differentiation of PSCs towards one of the three primitive germ layers and further differentiated progeny.
3. Reporter genes (GFP, LacZ, Luciferase, possibly fused to cDNA (type1 inserts)). These inserts will code for proteins with fluorescent or enzymatic properties allowing easy identification of genetically modified (GM) cells. They are thought to have no deleterious biological effect on expressing cells.
4. Selection genes (NPT, PAC). These inserts will produce enzymatic proteins able to inactivate specific antibiotic families allowing selection of GM cells. They are thought to have no deleterious biological effect on expressing cells.
5. Functional non coding sequences. These inserts will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES). They will be used (independently or in combination) into viral expression cassettes to achieve best control of transgene expression depending on targeted cells.
6. Neutral non coding sequences (introns, isolated exons). These inserts will be used in particular for gene targeting projects to promote homologous recombination at defined genomic loci (intronic/exonic genomic sequences). They should not have biological effect by themselves.

Origin & function

Evaluation of foreseeable effects

Human Health considerations -

The most obvious risk for human health is linked to accidental infection of worker by recombinant viral particles. Indeed, because only replication defective viral particles (non infectious) will be used for experimental work through this project and inserts will not produce compounds with acute toxicity (like bacteria toxins), no additional risk will be associated with the final GMO itself (mammalian cells) compared to initial organism. Importantly, primary cells coming...
from human samples are uncharacterised and may contain adventitious agents: they will be handled at containment Level 2 (COSHH regs).

First, risks associated with the nature and design of recombinant viral particles has to be assessed. Retroviral recombinant particles (MuLV and HIV-1 derived) will be VSV-G pseudo-typed (protein G from the Vesicular Stomatitis Virus will replace the native envelope protein). VSV-G packaging alters the tropism of the retroviral particles, requiring interaction only with phospholipids present in the plasma membrane of all cells rather than interaction with a cognate receptor as occurs with ecotropic packaging. Thus, VSV-G pseudo-typed retroviruses acquire the ability to efficiently infect virtually all human cell types. Moreover, VSV-G pseudo-typing improves stability of the viral particle and may represent an aerosol means of transmission in addition to the expected percutaneous risk. Retroviruses stably integrate the host cell genome. This property is associated with a risk of insertional mutagenesis, i.e. ectopic activation/inhibition of host gene expression after colocalized provirus integration. Subsequent deregulation of endogene expression could theoretically lead to transduction associated detrimental effects like initiation of oncogenic processes. Moreover, the addition of the WPRE (Woodchuck Hepatitis Post-transcriptional Regulatory Element) to the expression cassette in several retroviral backbones could also enhance the oncogenic potential of such vectors (SACGM, annual report 2004). Importantly, among retroviral vectors, the use of lentiviral derived backbone is thought to be safer. First, genomic integration profile of lentiviral derived vectors do not show an integration bias toward the transcriptional start site region of host cell genes like MuLV ones. Moreover, we will use exclusively SIN (Self Inactivating) vectors devoid of viral enhancer activity which reduces further the likelihood of endogene activation. In contrast, recombinant adenoviral vectors are considered safer. Indeed, adenoviral genome is rarely seen to integrate into the host cell genome and transgene expression remains transient in host cells. Moreover, the disease caused by wild type adenovirus is usually just a mild and self limited common cold.

Risks associated with inserted coding sequences should also be assessed. cDNA (or shRNA/miRNA) inserts carried by viral vectors will direct ectopic expression (or inhibition) of transcription factors, signal transducers, growth factors and receptors which are expected to play a role in pluripotency, cell fate decision and differentiation from pluripotent stem cells. It is difficult to predict in vivo consequences of ectopic expression for all these genes on human health. However, since they are key regulator of cell identity, their overexpression could lead to pathological modifications of cell phenotype/function. Moreover, several of these genes have been involved in oncogenic processes and uncontrolled expression could subsequently initiate tumoral transformation. However, malignancy is a complex multistep process which involves multiple genomic alterations and a single “hit” is unlikely to trigger alone the oncogenic process. Experiments involving co-transduction or even co-expression by the same vector of several inserts should consequently be considered at higher risk for the worker. Notably, reprogramming experiments of somatic cells toward pluripotency (co-transduction with the 4 factors OCT4, SOX2, MYC, KLF4) could imply a specific risk since accidental genetic modification of worker cell could theoretically lead to teratoma formation. Above mentioned risks will be tempered by the fact we will use strictly replication defective viral vectors unable to propagate further after transduction. For all viral vector type, in the likelihood of an accidental contamination (percutaneous, aerial), amount of viral vectors able to effectively contaminate worker cells will be smaller and transduction much less efficient compared to improved in vitro transduction protocols used on cultivated cells. Of note, MuLV derived vectors are only efficient in transducing proliferating cells that limits the number of potential accidental targets. Importantly, VSV-G pseudotyping of retroviral particles results also in complement sensitivity increasing the likelihood of immunological neutralisation in human hosts. Together, these considerations suggest that only low number of cells may be effectively transduced through accidental contamination. Moreover, malignancy is a complex multistep process which involves multiple genomic alterations and it is unlikely that any single “hit” corresponding to an accidental infection would initiate transformation. Notably, cells exposed to non percutaneous accidental infection (stratum corneum, respiratory epithelium) are terminally differentiated cells with limited lifespan and high turnover,
which greatly limits the risk of effective tumoral transformation. Nevertheless, in the context of experiments using polycistronic viral vectors for combined expression of several cDNA, tumoral transformation risk may be higher: appropriate safety measures will be undertaken to limit accidental contamination and all workers will be informed of additional risk associated with the use of such specific viral constructions.

- Environment considerations -
VSV-G pseudotyping of recombinant retroviral particles (MuLV and HIV-1 derived) would enable vectors to infect a variety of animal cell types including those of different animal species. Moreover, environmental stability tends to be increased by VSV-G pseudotyping compared to retroviruses displaying the native envelope. However, retroviral particles remain sensitive to air, temperature and pH and will have short lifespan in open environment. Importantly, recombinant retroviral vectors will be replication defective and could not produce progeny viruses able to spread to the wider human or other animal populations.

Pubic Register
Health and Safety
Executive
CU 2 2000 (rev 11/08) Page 5 of 11
Adenoviruses are generally species specific. Indeed, serotype 5 Adenoviruses infect humans and does not naturally infect other animals. Therefore, the risk of accidental infection of other organisms is very low. Because recombinant adenoviruses will be replication incompetent, they could not spread to the wider human population from any accidentally infected individual.

*Transgene mobilization issue for retroviruses.
Recombinant viral vectors which will be used to genetically modify mammalian cells will be replication deficient and thus unable to propagate to the environment. However, a risk of recombinant vector mobilization through concomitant wild type retrovirus contamination of the targeted cell still exists. Indeed, recombinant retroviruses encompass a native encapsidation sequence which could allow production of transgene carrying viral particles if complementing viral proteins (enzymes, capsid and envelope) are provided by a compatible wild type retrovirus. The likelihood of such mobilization is still very low, even less with SIN Lentiviruses which are devoid of provirus LTR activity. Importantly, mobilized recombinant retroviruses would still be unable to replicate in other cells than those carrying the complementing wild type retrovirus. Moreover, amphotropism associated with VSV-G pseudotyping would no longer exist for viral particles encompassing mobilized recombinant retroviral genomes.

Since generation of replication competent viral particles (RCV) is associated with the highest risk factor for human health and environment, production steps will be given the highest risk factor in the whole process (i.e. production, transduction, GM cell analysis). All lentiviral productions will be carried out in strict Class2 conditions by trained and experienced workers. To decrease the likelihood of infection standard measures to decrease percutaneous as well as aerosolised transmission of viral particles will be adopted, such as no use of sharps and all work to be performed within a class II microbiological safety cabinet. Moreover, configuration of the laboratory premises will allow direct autoclaving of all equipment in the Class2 related lobby. All lentivirus batches will be tested for absence of RCV before use for cell transduction. In the very unlikely event of RCV generation, decontamination procedures will be carefully carried out and the event notified to the local BSO. Notably, no RCV generation involving 2nd generation SIN lentiviral vectors has ever been reported as today in the literature.

MuLV derived retroviral particles and adenoviral particles will be produced by external manufacturers (Vectalys and Hybrid Systems respectively) and production batches demonstrated RCV free by internal validated methods.

For some experiments (teratoma formation, haematopoietic engraftment), genetically modified mammalian cells (human, murine) could then be transplanted by tissular or systemic injection into immunodeficient recipient mice to
generate chimaeras. Importantly, protocols involved will not allow germ line transmission of the genetic modification. Since all viruses utilised to genetically manipulate the cells before transplant will have been tested for absence of RCV, there will be no risk of further infection and there will also be a minimal risk that the animals could escape from the animal facility.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- All disposable culture/lab ware from the CL2 laboratory will be double-bagged, properly labelled as biohazard and autoclaved before leaving the building for incineration (autoclaving in adjacent lobby). [100% kill]
- All highly contaminated material, i.e. all stuff in contact with viruses (pipettes, tips, culture plates, liquid waste bottles), will be separately decontaminated (Virkon 2%/Trigene Advanced 10% for 16 hours) and liquids solidified (Vernagel) before autoclaving. [100% kill]
- Small spills will be removed after one minute surface soaking with Trigene Advanced 1% and further decontamination by 70% Ethanol. [effectively 100% kill]
- Large accidental spills will be sprinkled with Virkon powder before cleaning. [effectively 100% kill]
- Bench and cabinet surfaces will be wiped down with Trigene Advanced 1% followed by 70% Ethanol. [effectively 100% kill]
- All GM modified cell cultures will be destroyed (soaking with Trigene Advance 1% and autoclaving/incineration) [100% kill] or chemically fixed/lysed (PFA 1-4%, Guanidin, RIPA) before leaving containment [effectively 100% kill].

Is an emergency plan required according to regulation 20?  
- Yes, tick to confirm that it is attached to this form  
- No

Tick to confirm that you have attached a risk assessment to this form
- Yes, tick to confirm that you are claiming exemption from disclosure for section of the risk assessment
- No

Please enter comments on the GM safety committee on the risk assessment

This project has been reviewed on 8th April 2009 by the West Forvie Building biological and genetic safety committee. This is satisfied that the risks have been properly addressed and that the work will be carried out under the appropriate controls and conditions. Nevertheless, the committee stressed on the need for additional risk assessment and specific information for workers undertaking experimental work with multiple insert (polycistronic) viral vector constructs which are associated with a higher risk for human health through accidental infection. Moreover, the committee emphasized that work with replication defective adenoviral vectors could be actually done at containment level 1 using MSC II.

Project Containment
The project is concerned with introducing genes to human somatic cell lines, embryonic stem (ES) cells or induced pluripotent stem (iPS) cells.

Recipient or parental organism

Human somatic cell lines, embryonic stem (ES) cell lines and induced pluripotent stem (iPS) cells will be co-infected with a combination of genes that have or might have oncogenic activity. The expressed genes will provide the host cell with a proliferation advantage and they will induce an undifferentiated, pluripotent state. The Sendai Virus infects human cells with high efficiency. Thus, exposure of an individual to the Sendai Virus, by possibly a break in the skin, could result in a localised transfer of the pathogenic properties of the particular gene.

Host/vector system

The target genes will be cloned into the non-transmissible sendai viral vector, SeV/deltaF, which does not encode F protein, so that this defective vector can amplify specifically in an F-expressing packaging cell line but cannot spread to F protein-nonexpressing cells. SeV vector is the cytoplasmic RNA vector that replicate in the form of negative-sense single stranded RNA in the cytoplasm of infected cells, which do not go through a DNA phase nor integrate into the host genome (Li et al. J Virol. 2000)
All DNA will be propagated in E coli (e.g. DH10B or derivatives).

Pathogenicity and transmissibility of SeV are not changed by any inserted sequences. SeV vector is the Cytoplasmic RNA vector that replicates in the form of negative-sense single stranded RNA in the cytoplasm of infected cells, which do not go through a DNA phase nor integrate into the host genome.

293T cells will be transfected with F-defective SeV vector carrying a transgene and pCAGGS-plasmids carrying the T7 RNA polymerase, NP, P, F5R, and L gene. They are cultured for 1 to 3 days to generate the seed SeV/deltaF vector. Then, these are propagated using the LLC-MK2 cells that are a monkey kidney cell line and carrying the F gene (LLC-MK2/F7). And the recovered F-defective SeV vectors are harvested.

SeV is able to infect cells from a broad host range, including humans but as this SeV is a murine parainfluenza virus, pathogenicity to humans has not been reported. As this SeV vector is a non-transmissible vector lack of F gene, transmissible virus is never produced, so that they have no possibility of horizontal transmission. This SeV vector is excluded from in vivo host within a few days to a few weeks after infection because of this vector’s immunogenicity.

None of the cell lines would be expected to remain viable outside the controlled culture conditions and infection of virus and expression of gene will be transient. We do not anticipate that any of the genetic manipulations we are proposing would alter this.

Recipient cells expressing the individual genes under study will be created which are likely to have some growth advantage or induce increased proliferation in an autocrine or paracrine manner.

Evaluation of foreseeable effects

Bacterial strain of E coli such as DH10B and its derivatives are not capable of colonizing a healthy individual.

Exposure of an individual to SeV, by possibly a break in the skin, could result in a localized transfer of the pathogenic properties of the particular gene. But no localized transfer caused by SeV has been reported.

The inserted genes are likely to have an autocrine or paracrine proliferative effect when expressed. Infection normally lasts only a few days to a few weeks. During this term, it is predicted that over-expression of pluripotent genes, oncogenes and signalling molecules could lead to an increased or decreased rate of cell division, although tumour progression and disease in the whole organism is unlikely.

The major possible source of hazard to the environment would be accidental escape of high titre virus solutions expressing cDNAs, which could then infect rodents.

SeV requires close contact for their transmission and their survival in the general environment is poor due to their fragile envelope. The vector is non-mobilisable and the successful infection of a cell would be transient without stable integration of the viral genes into host DNA.

The SeV would be able to infect a wide host range but could not be spread. There is no possibility of recombination because of RNA virus. A mutated form that is seen in flu is not produced because of a single-strand genome instead of segmented genome.

The use of sharps will be strictly controlled and avoided whenever possible. Needles and scalpels will not be permitted. Plastic Pasteur pipettes will be used for aspirating culture medium.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No animals or plants will be used.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be sealed in double autoclave bags and transported to the departmental autoclave where it will be exposed to steam at 127°C for 25 minutes. Effectively 100% kill. Run information of autoclaves is stored and can be accessed or printed off at any time to ensure temperatures within the autoclave were maintained during the cycle.

All liquid waste will be inactivated by treatment with Virkon or Trigen at a final concentration of 1% for 12 hours before disposal via the drains. Effectively 100% kill as determined by manufacturer.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The overall assessment for the vector itself (the Sendai Virus; HG1) would be CL1 because it is a non-integrating virus. Although the infection efficiency is very high and applies to humans and rodents, the infection would not persist and our local rules would still cover the risks under CL1.

The factor that puts this assessment on CL2 is the actual genes that are expressed. At least one of it is a known oncogene and all of them are likely to have a positive effect on cell proliferation. Furthermore, it is the aim of the project to increase proliferation and pluripotency and all genes are expressed at the same time. Taken together the hazard identification for the expressed genes and especially the fact that they are co-expressed is CL2.

The assessment was generally well written and the committee has only two comments on the form itself:
1. Define human somatic cell lines by including ‘commercially available’ otherwise refer to the Centre’s risk assessments regarding use of human cell lines.
2. Run information of autoclaves is stored and can be accessed or printed off at any time to ensure temperatures within the autoclave were maintained during the cycle.

In summary, we have to assign CL2 to the project.

Project Containment

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Animal Units  
Large Scale Activities  
Human Clinical Applications
The purpose of this project is to study the biology of haematopoietic stem cells using the experimental approach of transgene delivery by means of retroviral vector systems. The biological questions we aim to address include the molecular controls regulating stem cell formation and stem cell plasticity as well as the molecular defects in stem cells that lead to leukaemia. The proposed work has therefore the potential to open up new areas for both fundamental and applied biomedical research with potential clinical relevance for the treatment of leukaemia.

Recipient or parental organism

E. coli transformed with pUC-based retroviral vectors will be handled at containment level 1. The retroviral work will be performed at containment level 2.

The proposed retroviral systems incorporate the following safety modifications to prevent viral replication:

1. The packaging genes are separated into two plasmids, which lack both LTRs and have no viral packaging signal.
2. The following viral genes have been deleted from the packaging vector: env, vpr, vpu, vif and nef.
3. The vectors expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion) and expresses no viral gene products.
4. Envelope is expressed on a separate vector.

Host/vector system

The following host/vector system will be used:

E. coli; K12/pUC derivatives carrying the ampicillin gene driven by a bacterial promoter together with viral genes and cDNAs driven by eukaryotic promoters that do not express in bacteria.
The purpose of the project is studying both normal and malignant haemopoietic stem cell biology. All the genes we want to deliver to cells are of known function. We will use disabled retro and lenti vectors and virus pseudotypes that only infect mouse cells whenever the inserts are potential oncogenes.

Insert DNA categories:
1. Reporter genes (lacZ, GFP + variants, luciferase, PLAP) together with regulatory regions enhancers, promoters, silencers
2. Cre recombinase gene, self-exising with regulatory regions
3. SV40 large T antigen, HoxB4, Hox11, Notch1: potential oncogenic function (only to be used with ecotropic envelopes)
4. SCL, Myod, myf5, pax3, pax7, pdx1, ngn3: transcription factors (only to be used with ecotropic envelopes)

Evaluation of foreseeable effects

The risk of infection with retroviruses is through direct inoculation, which is unlikely as no needles are used for any experimental procedures involving retroviruses. Moreover, we will use virus pseudotypes that only infect mouse cells whenever the inserts are potential oncogens. The viruses used in this project are replication defective and thus not pathogenic when tested in mice.

The proposed retroviral systems incorporate the following safety modifications to prevent viral replication:
1. The packaging genes are separated into two plasmids, which lack both LTRs and have no viral packaging signal.
2. The following viral genes have been deleted from the packaging vector: env, vpr, vpu, vif and nef.
3. The vectors expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion) and expresses no viral gene products.
4. Envelope is expressed on a separate vector.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

We propose to generate transgenic mice using the lentiviruses. These mice will be housed in CBS (Addenbrooke's site Cambridge) together with all our other transgenic mice. Transgenic mice will only be produced for the inserts of category 1 (ie. inert reporter genes). Due to the design of the proposed experiments (see above) and the containment procedures of CBS, the environmental risk after introduction of the transgene is negligible.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is treated using 5% chloros for 24 hours prior to disposal.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The project has been reviewed by the CIMR Biological & Genetic Modification Safety Committee, and we are satisfied that the risk assessment is accurate and that the work will be carried out under the appropriate conditions.
### Project Containment

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Tick if notifying a connected programme of work: N

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### Project Additional Information

**Purposes of the contained use**

We will produce viral particles which have both ecotropic and amphotropic ranges of tropism, using replication defective MSCV v2.1 based retroviral vectors. The gene of interest is cloned into these vectors which concomitantly either express GFP or an antibiotic resistance marker. Viral particles are produced following transient co-transfection of 293T cells with the retro/lentiviral vector and one (ecotropic) or 2 (amphotropic) packaging constructs. This will be performed under containment level 2 conditions in compliance with institute practice.

**Recipient or parental organism**

E Coli - All strains are K12 derived thus disabilingn and non-colonising. Vectors are non-mobilisable. Mammalian cells - MSCV vector is non-mobilisable.
Retrovirus - MSCV vectors are based on replication defective retroviral genomes and FUGW vectors on replication defective lentiviral genomes.

**Host/vector system**

- **Host** - 293T cells/vector - MSCV retrovirus or FUGW lentivirus
- **Host** - Mammalian cell, post infection/vector - MSCV retrovirus or FUGW lentivirus (replication incompetent).

**Origin & function**

- **Human origin**
- **cDNA** - for overexpression.
- **siRNA** - synthetically derived to be complimentary to human sequence, used to inhibit protein expression

**Evaluation of foreseeable effects**

**Risk Assessment for human health:**

The most obvious potential risk to human health is the expression of oncogenes within accidentally infected human cells. This would be impossible for exotropically packaged retrovirus or lentivirus, but is theoretically possible for amphotropically packaged retrovirus or lentivirus. Theoretically these viruses can infect human cells and lead to integration and expression of the insert in dividing cells. In turn, expression of oncogenes or potential oncogenes or of endogenous genes through insertional mutagenesis could potentially be harmful to human health. There is also the theoretical potential for propagation of the virus through recombination and the generation of replication competent retrovirus (RCR). The MSCV vector is replication defective and the production of RCR will be minimised by the use of a 3 plasmid transfection system to prevent recombination. However, testing for RCR will be performed for each batch of amphotropic virus using a marker recovery assay (each virus also expresses either a GFP marker or an antibiotic resistance marker) and only batches negative for RCR will be utilised further.

For lentiviruses, theoretically these viruses can also infect human cells and lead to integration and expression of the insert in dividing and non-dividing cells. In turn, expression of oncogenes or potential oncogenes or of endogenous genes through insertional mutagenesis could potentially be harmful to human health. There is also the theoretical potential for propagation of the virus through recombination and the generation of replication competent retrovirus (RCR). The FUGW vector is replication defective and the production of RCR will be minimised by the use of a 3 plasmid transfection system to prevent recombination. However, testing for RCR will be performed for each batch of amphotropic virus using a marker recovery assay (each virus also expresses either a GFP marker or an antibiotic resistance marker), and only batches negative for RCR will be utilised further. In addition, although the lentivirus has modifications to increase transcription and nuclear import (the WRE and HIV-1 flap element respectively) which might theoretically increase the risk of expression in infected human cells, it is replication defective and the SIN modification of the 3' LTR is duplicated in the 5'LTR upon reverse transcription.

This results in the transcriptional inactivation of the provirus and decreases the likelihood of insertional mutagenesis. These vectors also have a 5' LTR modification (U3 region replaced with CMV promoter) which along with the use of a 3 plasmid transfection system reduces the possibility of recombination and minimises the risk of production of RCR. Recently concern has been raised with regard to the WRE element, as mice transplanted with control vectors containing this element have developed tumors. Therefore, in keeping with GTAC and SACGM recommendations these vectors will be handled at containment level 2.

To decrease the likelihood of infection standard measures to decrease percutaneous as well as aerosolised transmission of the virus, such as no use of sharps ans work to be performed within a class II microbiological safety cabinet will be adopted. Aerosolised virus is theoretical risk to infect the respiratory epithelium, but its production would be minimised as above. In addition the respiratory epithelium is terminally differentiated, has a high turn-over and it is unlikely that the haematopoietic specific oncogenes involved in this proposal would have any effects on this tissue in the unlikely event of infection. Moreover, malignancy is a multistep process and it is unlikely that any single "hit" would initiate transformation and in occasional experiments where oncogenes were coexpressed this would be by cotransduction making dual infection of the same human cell highly unlikely. Also VSVg pseudotyping results in complement sensitivity increasing the likelihood of immunological neutralisation in human hosts. However, during the production and concentration of retrovirus, the transduction of cells and the subsequent analysis of these cells, CL2 conditions will be strictly adhered to.

Both viral particles are relatively unstable and standard practices will ensure adequate decontamination of facilities.
Bacterial and mammalian cells are unable to survive outside of laboratory culture conditions. See attached protocol for further details.

In summary, there is little evidence for the likelihood of hazardous long-term effects from accidental infection and overexpression of an oncogene. Adherence to the code of practice for containment 2 facilities should be adequate for prevention of inadvertent infection.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be treated with 2% Virkon overnight in labelled containers. Liquid post disinfection will be gelled with vernagel, placed in sealed containers and taken to room 1.23 for incineration. Solid waste will be placed in double autoclave bags, secured with autoclave tape and labelled with the room number and user name, followed by autoclaving and incineration. Liquid spills will be treated with 2% Virkon (non-metallic surface) or 10% trigene (metallic surfaces) and absorbed with paper towels which will then be autoclaved. Surfaces will be routinely cleaned with 10% trigene.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

This project has been reviewed by the institute's biological and genetic safety committee. This is satisfied that the risks have been properly addressed and that the work will be carried out under the appropriate controls and conditions.

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02/03/2022

Page 12845 of 15326
The project aims to investigate strategies to modulate the differentiation of human and mouse embryonic stem cells, differentiated cells and tumour cells by introduction of genes including transcription factors and cell cycle regulators using lentiviral vectors.

Recipient or parental organism
Murine and human cells (packaging cells, primary cells, cancer cell lines and immortalized cell lines).

Host/vector system
1) E.coli, laboratory K12 derivatives/pUC based vectors;

2) Vertebrate tissue culture cells including 293 T cells, embryonic stem cells, human cancer cell lines, and cells including fibroblasts and neuroblastoma and glioblastoma cell lines derived from patient samples.

3) Lentiviral vectors

3.2) Lentiviral systems: the two types of viral system to be used employs two well characterised lentiviral vector and packaging cell lines.

3.2.1) Typa A (eg. pFUV and tet-ON derivatives thereof), 3rd generation:
. The number of genes from HIV-1 that are used in this system has been reduced to three (i.e. gag, pol, and rev).
. Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids.
. Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell
lines, none of them contain LTRs or the packaging sequence. This means that none of the HIV-1 structural and other components required for packaging the viral genome are separated onto four plasmids.

Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.

The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.

In some cases expression of our gene insert of interest will be driven expression off a tet-responsive CMV promoter, and regulated by a co-transduced lentivirus doxycyclin-responsive Tet regulator, giving an added level of safety for insert expression.

### 3.2.2. Type B (eg. Lentix0HTX from Clontech), 4th generation

The lentiviral packaging systems mentioned above are 3rd generation versions that utilize split-genes to provide the viral packaging elements on individual plasmids that physically separate the viral envelope, env (usually VSV-G), sequence from the gag-pro-pol sequences. These split-gene packaging strategies reduce the risk of generating RCL because multiple recombination events are necessary to create a virus that harbours the sequences required for independent replication. Type B (Clontech's Lenti-X HT) Packaging System also uses a split gene packaging strategy, but adds another level of safety by further uncoupling pol (RT and IN) from gag-pro. The result is that gag, pol and env reside on three physically distinct entities, rather than the standard two. This approach further reduces the possibility of creating RCL to a level below that of standard 3rd generation packaging systems, because extra recombination events are required to create such viruses. In fact, the emergence of RCL is undetectable from systems using this approach (Wu, X, et al. 2000) Mol. Ther. 2(1):47-55.) These improvements significantly increase the safety profile of our Lenti-X HTX Packaging System.

Clontech’s Lenti-X Vectors contain less than one-third of the wild-type HIV-1 genome. These wild-type sequences mainly consist of the viral LTRs and packaging signal. All essential replication genes have been completely removed and are instead supplied as separate DNA entities in the Lenti-X HT Packaging Mix (described above).

In brief, the number of genes from HIV-1 that are used in type B system has been reduced to four (i.e. gag, vpr-pol, rev and tat).

Although the four packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293 T derived producer cell lines, none of them contain HIV-1 LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.

The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.

In this system, expression of tat is required in the packaging cell line, because the lentivirus vector contains an intact HIV-1 5LTR, which allows very high titers in viral preparations.

In some cases expression of our insert gene of insert of interest will be driven expression off a tet-responsive CMV promoter, and regulated by a co-transduced lentivirus doxycyclin-responsive Tet regulator, giving an added level of safety for insert expression.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard.

### Origin & function

Genes and mutant derivatives thereof, usually of human, mouse or Xenopus frog origin, encoding both growth control proteins and transcription factors, along with marker genes such as GFP and antibiotic resistance genes.

### Evaluation of foreseeable effects

Some of the virus vectors will lead to growth arrest and differentiation of mammalian cells, while there is potential to promote cell proliferation. Because of the presence of the
WPRE element in the lentivirus vectors, the lentivirus constructs might transform infected mammalian cells.

The main Hazard is potential infection of persons working in the lab by the GM virus, for which a Cat II containment level is adequate.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class I level of classification.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms and tissue culture cells are involved.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class I level of classification.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

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The project is concerned with understanding the mechanisms involved in lineage selection upon stem cell differentiation in the epidermis.

The work requires the use of human keratinocytes which are difficult to transfect by standard methods. Recombinant retroviral infection is therefore needed as a means of delivering the appropriate cDNA constructs into the cells. Human keratinocyte cell lines will be derived from a reliable source of normal infant skin which has been surgically removed from screened healthy individuals having a negligible risk of contamination with adventitious human pathogens.

Host/vector system

Constructs will be cloned into the non-mobilisable viral plasmid vector pBabe (puro) and derivatives based on Moloney Murine Leukaemia Virus (Morgenstern and Land 1990, Nucleic Acid Research 18 3587-3596).

RNAi constructs will be cloned into pRETRO SUPER and its commercially available derivatives.

Origin & function

All DNA will be propagated in E Coli (e.g DH10B or derivatives).

The plasmid will first be transfected into an ecotropic packaging cell line, Phoenix-eco, and the retrovirus produced will be harvested over 1-2 days.

The helper-free retrovirus producer cell line Phoenix-eco, was developed by Dr. G. Nolan at Standfors University, (http://www.standfors.edu/group/nolan/index.html) and has
been used by over 2500 labs. Phoenix-eco cells express the gag-pol and envelope genes using different non-Moloney promoters to minimize both recombination and inter-typic recombination potential. This cell line has a history of safe usage.

This ectropic retrovirus will then be used to stably infect a second packaging cell line, GP + envAm12, which produces disabled amphotropic retrovirus. The mouse cell line GP +enAm12 has been widely used for the production of amphotropic virus. Tests for the safety of the GP + envAm12 packaging line showed no evidence for the generation of wild type virus and it has a history of safe usage. Markowitz, Goff and Bank. Virology 167, 400-406 (1998). Defective amphotropic virus would then be used for the infection of human keratinocytes. Transduced cell would be selected and maintained in medium containing an appropriate antibiotic before further use in experiments.

All genes will be inserted downstream of the viral LTR with the intention of achieving a moderate level of expression in recipient cells. Infection of keratinocytes will be by transfer of supernatant culture fluids with any concentration of the virus particles. Recipient human keratinocytes expressing the individual genes under study will be created which are likely to have some growth advantage or induce proliferation in an autocrine or paracrine manner.

Evaluation of foreseeable effects

Bacterial strains of Ecoli such as DH10B and its derivatives are not capable of colonising a healthy individual.

The amphotropic retrovirus is able to infect cells from a broad host range, including humans but can not replicate unless it recombines with endogenous retrovirus. Exposure of an individual to amphotropic retrovirus, by possibly a break in the skin, could result in a localised transfer of the pathogenic properties of the particular gene. None of the cell lines would be expected to remain viable outside the controlled culture conditions and we do not anticipate that any of the genetic manipulations we are proposing would alter this.

It is predicted that over-expression of oncogenes and signalling molecules could lead to an increased rate of cell division although tumour progression and disease in the whole organism is unlikely. The major possible source of hazard to the environment would be accidental escape of high titre virus solutions expressing cDNAs, which could then infect rodents. Retroviruses require close contact for their transmission and their survival in the general environment is poor due to their fragile envelope. The vector is non-mobilisable and the successful infection of a cell would include stable integration of the viral genes into host DNA. Although the amphotropic virus would be able to infect humans it could not replicate unless it recombined with endogenous retrovirus. Even then it is unlikely that expression of a single oncogene or inactivation of a tumours, as multiple genetic alterations (between 4 and 6) are required for inducing tumours, and a safeguard mechanisms exist impeding the accumulation of these alterations in the organisms (I.e.single events like Ras or myc expression instead of unrestrained proliferation, induce growth arrest or apoptosis) (Hanahan & Weinberg, Cell 100, 57-70;2000).

The use of sharps will be strictly controlled and avoided wherever possible. Needles and scalpels will not be permitted. Plastic Pasteur pipettes will be used for aspirating culture medium.

No animals or plants will be used.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will be sealed in double autoclave bags, and autoclaved with steam at 127°C for 25 minutes. Effectively 100% kill. Autoclaves are rountinely monitored and records are kept by the department.

All liquid waste will be inactivated by treatment with Virkon at a final concentration of 1 % for 12 hours before disposal via the drains. Effectively 100% kill as determined by
The committee noted that the consequences are unknown of infection of cells of the worker by the modified virus, and have the potential to lead to cell proliferation. However, the committee felt that the risk is extremely low, because of the method of handling the virus including the avoidance of sharps. Secondly, the rate of infection is likely to be very low as the virus has a low infectivity. This is clear since in vitro infection is increased by optimisation of conditions using eg lipophilic substances. Thirdly, the virus genome will only integrate in proliferating cells, which are relatively rare in the skin.

Please enter comments on the GM safety committee on the risk assessment

The committee noted that the consequences are unknown of infection of cells of the worker by the modified virus, and have the potential to lead to cell proliferation. However, the committee felt that the risk is extremely low, because of the method of handling the virus including the avoidance of sharps. Secondly, the rate of infection is likely to be very low as the virus has a low infectivity. This is clear since in vitro infection is increased by optimisation of conditions using eg lipophilic substances. Thirdly, the virus genome will only integrate in proliferating cells, which are relatively rare in the skin.

**Project Containment**

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**Name**

VIVOMEDICA (UK) LTD

**Name 2 Department**

**Campus Estate or Research Centre**

KENT SCIENCE PARK

**Road Name**

BUILDING 130 ABBOTT DRIVE

**Town**

SITTINGBOURNE

**County**

KENT

**Postcode**

ME9 8AZ

**Country**

ENGLAND

**Tel Number**

01795 414460

**Fax Number**

01795 414461

**E-mail**

blank

**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Consists of CEO, Financial Director, Group Leader, Senior Scientist and Biological Safety Officer.
Meeting every 6 months, or more frequently if new technology/procedures are to be introduced, or an accident occurs

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<thead>
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Tick if confidential

Bacteriology | Parasitology | Transgenic | Transgenic | Microbiology | Research |
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02/03/2022 Page 12853 of 15326
Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below) | Yes
---|---|---|---
Other(s) | Use of commercially available human induced pluripotent stem (IPS) cell derived cardiomyocytes

For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste disposal will be carried out according to an SOP. All solid waste is to be autoclaved before being placed in plastic theatre bins and taken to incineration by a licensed outside contractor. All liquid waste is disinfected using a Virkon solution and following the manufacturers instructions for maximum kill. Non-disposable labware is disinfected prior to re-use. All spills to be cleaned using propriatory reagents e.g. spill kits. No further, monitoring is deemed necessary due to the low/zero hazard presented by the cells involved.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The committee pointed out that correct signage on the laboratories was required and that the control of visitors would be reviewed.

A member of the committee asked if this notification covered the use of embryonic stem cells. The committee was informed that the notification covered the genetic manipulation of induced pluripotent stem cells only, and that the ethical requirements for using embryonic stem cells fell under a different set of regulations.

A member of the committee asked if we needed to name our waste provider, and was told that the waste disposal must be carried out by a contractor licensed by the Environment Agency.
GM Centre Number: 3075

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Name
NATIONAL HEALTH SERVICE BLOOD AND TRANSPLANT

Name 2
NATIONAL BLOOD SERVICE

Campus Estate or Research Centre

Road Name
14 ESTUARY BANKS

District
SPEKE

Town
LIVERPOOL

County
MERSEYSIDE

Postcode
L24 8RB

Country
ENGLAND

Tel Number
0151 268 7200

Fax Number
0151 268 7124

E-mail

HSE Division
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Comments

Date at Which Additional Info Submitted
02/03/2022

Page 12855 of 15326
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

NBS Liverpool Management Representative (Chair) Senior Scientist/Biological Safety Officer NBS Liverpool
Health and Safety Advisor NBS Liverpool Safety Representative NBS Liverpool
Technology Centre Manager Liverpool Tissue Services Manager Liverpool
Senior Scientist University Manchester External Independent GM Advisor

The committee meets on an annual basis due to the low level of activity. All new submissions or accepted submissions that require amendment for consideration are made electronically to the Biological Safety officer for distribution to the committee for review. Any comments must be returned within two weeks and any required amendments resubmitted within 2 weeks. After acceptance the risk assessment is signed by the Biological Safety Officer or the GM Safety Committee Chair.

<table>
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Other (please specify) Tick if confidential
All liquid and dry waste will be sent for incineration by SRCL Ltd. All material for disposal will be placed in 30 litre yellow containers which are sealed prior to removal from the room where the class one material is being used. The boxes are then labelled to clearly identify them as GM waste and placed into wheelie bins for collection. The wheelie bin containing the GM waste is clearly marked to identify from other wheelie bins for transport to the incineration depot.

Any minor liquid spillages (<20ml) will be removed by soaking up with an absorbent wipe or for larger liquid spills by the addition of absorbent granules and transfer in to a yellow container. The area will then be disinfected with either Klericide A or B (a rotational disinfectant with demonstrated virucidal activity) according to the manufacturers instructions. After inactivation, the area will be wiped and the wipe placed into the yellow container. The area is then sprayed with 70% isopropyl alcohol and the wipe placed into the yellow container. The container will be sealed and sent for incineration as above.

Overall, I think the proposal is fine aside from concerns about the way the range of transgenes are assessed in Part 2. Numerous cDNA’s have been listed and covered with the comment that they are wild type and therefore not pathogenic. I don’t think this can be assumed. A number of these cell surface proteins have essentially an unknown function and so it would ne nwise to just use this coverall phrase for all of them particularly with respect to expression of these genes in cells which wouldn't normally express the particular protein. There should be som consideration of each of the genes with respect to safety.
### GM Centre Number: 3076

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**Campus Estate or Research Centre**

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**Town**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee consists of the CEO, Chief Operations Officer, Technical Manager (Cell Culture) and Veterinary Manager. The committee meets quarterly when the premises are being used for contained use activities.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

All work is carried out in a Category 2 laboratory and laboratory waste is autoclaved prior to incineration

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The genetic modification safety committee considered this application and determined that there should be nor harmful properties associated with the strain, vector or inserted material and that these have a negligible associated risk.
GM Centre Number: 3078

Data Premises Notified (Originally) | 11/01/2010

Transferred from 1992 Regs? | Y

Transitional Premises Class | N

Data Premises Closed

Transitional Premises Emergency Plan Required? | N

Non-GMMs | Y

Withdrawn | Y

Name

ORLA PROTEIN TECHNOLOGIES LTD

Name 2

Department

Campus Estate or Research Centre

BIOSCIENCE INCUBATOR

Building

INTERNATIONAL CENTRE FOR LIFE

Road Name

TIMES SQUARE

District

Town

NEWCASTLE UPON TYNE

County

TYNE AND WEAR

Postcode

NE1 4EP

Country

ENGLAND

Tel Number | 0191 231 3127

Fax Number

E-mail

HSE Division | blank

Comments

Date at Which Additional Info Submitted | 02/03/2022
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### Premises Conditions

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<td>Transgenic Fish</td>
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<tr>
<td>Gene Therapy</td>
<td></td>
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</table>

Newcastle University Safety Office (Head of Safety) provide health and safety assistance as a consultancy service contract to Orla Proteins Ltd. As part of this contract University GMSC will provide advice to Orla on its GM risk assessments.
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd. TST class 6 emulating indicator 121°C for 20 min).

<table>
<thead>
<tr>
<th>Mycology</th>
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<th>Transgenic</th>
<th>Other (please specify below)</th>
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<tbody>
<tr>
<td>Invertebrates</td>
<td>Plants</td>
<td></td>
<td></td>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

- All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd. TST class 6 emulating indicator 121°C for 20 min).

- **Tick to confirm that you are attaching a summary of the risk assessment**

- **Tick if you are claiming exemption from disclosure for sections of the risk assessment**

Please enter comments of the GM safety committee on the risk assessment

- The University GM Safety Committee has approved the following GM risk assessments which were previously carried out under the University premises notification.

- Principal Investigator: (Orla Proteins Ltd).

- Local GM reference GM03/24.
  Expression of truncated insoluble E. coli OmpA protein in E. coli.

  Heterologous expression of isolated proteins of human respiratory viruses in E. coli.
GM Centre Number: 3079

Data Premises Notified (Originally) 11/02/2010

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

GRANOX LTD

Name 2

Department

Campus Estate or Research Centre

Building

3MG DEVELOPMENT

Road Name

DESO TO ROAD

District

Town

WIDNES

County

CHESHIRE

Postcode

WA8 0PB

Country

ENGLAND

Tel Number 0151 424 6731

Fax Number 0151 495 1895

E-mail

HSE Division NORTH WEST

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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<thead>
<tr>
<th>Date</th>
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<th>Building</th>
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<tr>
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<td>DESOTO ROAD</td>
<td>WIDNES</td>
<td>CHESHIRE</td>
<td>WA8 0PB</td>
<td>ENGLAND</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The committee is represented by the Site Operations Manager, the Energy Division Operations Manager and the Compliance Officer.

A full risk assessment has been carried out which covers the containment measures for the loading procedures at Novartis through to off-loading and processing at the Granox site. The committee are responsible for ensuring that all individuals with responsibility for this procedure have been fully trained to minimise the risks to both humans and the environment. The committee will carry out routine inspections and audits to ensure that the contained use procedure is adhered to and reviewed where necessary. Records will be kept of these audits.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Level 2 (GMMs)</td>
<td>Level 3 (GMMs)</td>
<td>Level 4 (GMMs)</td>
<td>Non-microbial</td>
</tr>
</tbody>
</table>

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

02/03/2022
The waste will be chemically pretreated at the suppliers premises. The treatment will involve the use of a chemical agent which will reduce the risk associated with handling the GM virus strains.

The material is produced at the Novartis plant and is poultry derived liquid egg waste containing…

For activities involving GMMs, describe the waste management measures which will apply to the activity

The waste will be chemically pretreated at the suppliers premises. The treatment will involve the use of a chemical agent which will reduce the risk associated with handling the GM virus strains.

Please enter comments of the GM safety committee on the risk assessment

The risk assessment has been carried out by the members of the GM Safety Committee and includes all the appropriate measures to ensure that the risks to humans and the environment are minimised by robust contained used procedures. Advice was sought from Specialist Inspectors from the HSE Biological Agents Unit and Novartis to ensure that all the appropriate measures are included within the risk assessment.

Project Ref 3079/10.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Date Project Ceased</th>
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<tr>
<td>12/02/2010</td>
<td>Disposal of Level 2 GMMs produced from the manufacture of influenza vaccines by incineration and energy recovery.</td>
<td>Class 2</td>
<td>Not Applicable</td>
<td>Non-GMM</td>
<td></td>
<td>Consent Granted</td>
</tr>
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</table>

Historical Significant Changes
Project Additional Information

Purposes of the contained use

The purpose of the contained use is to provide a secure auditable disposal route for Level 2 GMMs produced from the manufacture of influenza vaccines at the Novartis plant. There is approximately 120 tonnes of the Level 2 GMM material produced per week which is predominantly liquid. The material will be off-loaded in the Blood plant tanks which have a capacity of 300 tonnes. From here it will be pumped to tanks 1 & 2 which have a capacity of 140 tonnes each. From here is is pumped to the fluidised bed combustion plant. The material can also be off-loaded in the Empire building and pumped directly to tanks 1 & 2 prior to incineration in the fluidised bed combustion plant.

Recipient or parental organism

Various attenuated strains of influenza virus prepared by reverse genetics and used in the manufacture of influenza vaccine e.g. AH1N1 NIBRG X 121. The materials are of relatively low pathogenicity and pose a very low risk of contracting influenza to humans and animals as the strains have been attenuated to thrive in fertilised hen eggs as host organism. There is a very remote risk of reassortment with a circulating wild virus strain.

Host/vector system

Fertilised hen eggs. Liquid egg waste. Vector could conceivably be mammalin species, human, pig, rodents and birds.

Origin & function

Lab prepared attenuated influenza virus strains used solely for the manufacture of influenza vaccines.

Evaluation of foreseeable effects

Very low probability of viral proliferation in a human/animal host, generating influenza.

Production of conventional Fluvirin (Northern Hemisphere) vaccine is considered to be safe to human health due to attenuation of human pathogenicity by egg adaptation. This is achieved through the growth of the virus through a series of passages in eggs. The new strains of A California/7/2009 x PR8 e.g. NIBRG - 121xp A/California/07/09 will also have limited pathogenicity similar to conventional Fluvirin however slightly enhanced transmissibility as a new strain, this accounts for the higher level of control applied.

There is a remote possibility of secondary reassortent with normal human/animal viruses giving rise to new strains which are replication-competent in man/animal. Note: It is generally considered to be technically difficult to produce reassortants in vitro.

When such difficulties are considered, together with the contained handling and treatment used for the waste, then the chances of an A(H1N1) attenuated virus strain...
Infecting man and producing a secondary reassortant is EXTREMELY LOW. Containment procedures from off-loading through to destruction by incineration ensure that the risk to humans and the environment is minimised.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

see attached file *Granox Ltd Novartis GMO Draft Procedures Jan 10th*

The waste is pretreated at the suppliers facility with a chemical agent that will reduce the risks associated with the virus. It will be transported in a contained manner by road tanker. On receipt at Granox it will be directed to the Blood plant connecting hose will be flushed through with Virkon prior to disconnection and the contents pumped to the storage tanks. In the unlikely event that there is insufficient storage capacity in the Blood Plant tanks, the material will be off-loaded in the Empire Building and the contents will be pumped in an enclosed system to tanks 1 & 2 prior to incineration. Both tanker offloading areas are served by sumps. In the event of a leak or spillage the split material can be recovered from the sump and pumped directly back to the Blood plant tanks or tanks 1 & 2. Spill management procedures are in place and there is a high degree of physical containment of storage tanks and associated equipment. Working losses from tanks 1 & 2 breath directly via extraction to the secondary air system of the fluidised bed combustors. The liquid material is pumped from storage to the fuel holding bin mixed with solids and pumped into the fluidised bed reactors and combusted at 850 degrees celcius, yielding complete destruction of the material. Vermin controls in place. Availability of prophylaxis in form of vaccination.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

The risk assessment has been carried out by members of the GM Safety Committee and includes all the appropriate measures to ensure that the risks to humans and the environment are minimised by robust contained used procedures. Advice was sought from Specialist Inspectors from HSE Biological Agents Unit and Novartis to ensure that all the appropriate measures are included within the risk assessment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L2</td>
<td>L3</td>
<td>L4</td>
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02/03/2022
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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### GM Centre Number: 3080

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#### Name

EUROFINS AGROSCIENCE SERVICES

#### Name 2

Department

#### Campus Estate or Research Centre

Building

#### Road Name

SLADE LANE

#### District

WILSON

#### Town

MELBOURNE

#### County

DERBYSHIRE

#### Postcode

DE73 8AG

#### Country

ENGLAND

#### Tel Number

01332 864800

#### Fax Number

01332 864763

#### E-mail

blank

#### HSE Division

blank

#### Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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<td>DERBYSHIRE</td>
<td>DE73 8AG</td>
<td>ENGLAND</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Compliance Accreditation Manager
- Elisa Scientist
- Senior Quality Auditor
- Trials Co-ordinator
- Group Operations Director

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Yes

Yes

Other (please specify)

Tick if confidential

Bacteriology

Parasitology

Transgenic Birds

Microbiology Research

02/03/2022
<table>
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<tr>
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<tr>
<td>Mycology</td>
<td>Transgenic Invertebrates</td>
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<td>Other (please specify below)</td>
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</tbody>
</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

GMSC met on 25th Jan 10 to comment on risk assessment.
All containment measures and hazard prevention were agreed.
<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
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</table>

**Name**

CRYO-STORE LTD

**Campus Estate or Research Centre**

2 GREENWICH CENTRE BUSINESS PARK

**Building**

2 GREENWICH CENTRE BUSINESS PARK

**Road Name**

NORMAN ROAD

**District**


**Town**

GREATER LONDON

**County**

SE10 9QF

**Postcode**

ENGLAND

**Country**

**Tel Number** 020 8858 4854

**Fax Number** 020 8858 4853

**E-mail**

**HSE Division** blank

**Date at Which Additional Info Submitted**

02/03/2022
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The material is owned by our client (Domantis Ltd) and their genetic safety committee provide advice to Cryo-Store in the form of risk assessments etc. Cryo-store itself, is a ultra low temperature storage facility that will simply store the supernatant material in sealed containers in a dedicated -80 freezer unit.
All materials stored at Cryo-Store remains the research property of the client and will be returned to their premises at some time.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
<table>
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<th><strong>GM Centre Number:</strong> 3082</th>
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<td>Withdrawn</td>
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**Name:**

THERMOFISHER SCIENTIFIC LTD

**Campus Estate or Research Centre:**

**Building:**

REME HOUSE

**Road Name:**

CLIPPER BOULEVARD WEST

**District:**

CROSSWAYS

**Town:**

DARTFORD

**County:**

KENT

**Postcode:**

DA2 6PT

**Country:**

ENGLAND

**Tel Number:** 01322 295600

**Fax Number:** 01322225413

**E-mail:**

**HSE Division:** blank

**Comments:**

Date at Which Additional Info Submitted: 02/03/2022
Premises Addresses

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<td>DA2 6PT</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Technical Manager
- Technical process Officer
- Manufacturing Manager
- EIA manager
- Team leader
- UK Health, Safety & Environment Manager

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Tick if confidential</td>
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<table>
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<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

All liquid waste, disposable labware, gloves and materials used for the treatment of spillages are treated by autoclaving on a validated sterilisation cycle of 121° C for minimum of 15 minutes. The degree of kill is expected to be 100%. Standard autoclave indicators are used to check the cycle has completed successfully.

Prior to autoclaving all re-usable laboratory glassware is also treated with 2% Hycolin solution for 24 hours.

Surfaces are routinely disinfected with commercially available surface disinfectants.

Following autoclaving all materials are removed from the premises by a licensed contractor for final disposal via incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The GM Safety Committee have reviewed the risk assessments and are satisfied that all the hazards have been identified and the associated risks reduced to their lowest level.

**Project Ref** 3082/10.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<th>CultureVolClass2</th>
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<tbody>
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<td>23/07/2010</td>
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<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</tr>
<tr>
<td>Date Project Ceased</td>
<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
<td></td>
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</tbody>
</table>
**Project Additional Information**

**Purposes of the contained use**
The LPS antigen is used as a positive control in a commercial ELISA kit.

**Recipient or parental organism**
Salmonella minnesota

**Host/vector system**
Plasmid pFEN207


**Origin & function**
The origin of the recombinant is unknown. The strain was inherited from a previous company involved in the culture and production of ELISA kits.
The plasmid inserted into S. minnesota results in expression of a Chlamydial epitope on the LPS of the recombinant.
The LPS antigen is used as a positive control antigen in a commercial ELISA kit.

**Evaluation of foreseeable effects**
The recombinant S. minnesota has no increased pathogenicity as a result of the transformation.
S. minnesota mutants have been frequently reported in the scientific literature.
S. minnesota can be currently found in the environment in the form of human and animal carriers and is transferred via the fecal-oral route.
Salmonellosis is a self-limiting form of gastroenteritis.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All culture work involving the live organism will be carried out in a Class II Biosafety cabinet. All disposable equipment will be initially decontaminated in 2% hycolin* (non-phenolic based) for 24 hours, then autoclaved on a validated sterilisation cycle of 121°C for 15 minutes. The degree of kill is 100%. Standard autoclave indicators are used to check the cycle has been completed successfully.

Following autoclaving all materials are removed from the premises by a licensed contractor for final disposal via incineration.

*Hycolin composition: Alkyl dimethyl benzyl ammonium chloride, fatty alcohol ethoxylate, tetrasodium EDTA)

Surfaces are routinely disinfected with commercially available surface disinfectants.

The Salmonella culture is heat killed by boiling for 15 minutes. A viability test is then performed to confirm inactivation. The viability test consists of testing a sample of the heat killed culture in both broth and agar plate mediums. The viability is checked at 24 and 48 hours after inoculation for growth.

If there is any growth present, the culture must be re-boiled for 15 minutes then retested. The process is repeated until 100% kill is confirmed.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

the risk assessment has been reviewed and the Genetic Modification Safety Committee is satisfied that all hazards have been identified and the associated risks reduced to their lowest level”.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4 L2</td>
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<tr>
<td>Animal Units</td>
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<td>Large Scale Activities</td>
<td>L2 L3 L4 L2</td>
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**Name**

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**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

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<table>
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**Comments**

**Date at Which Additional Info Submitted**

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Page 12881 of 15326
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

Chair of the committee: Head of Department

Other members of the committee include the teacher supervising the project, another member of teaching staff and two biology department technicians.

Note on the qualification of committee members: The head of department has previously acted as chair of a different genetic modification safety committee when working in a government research laboratory and two other members of staff hold PhDs in aspects of molecular biology.

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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Tick if confidential

<table>
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<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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</table>

02/03/2022
Agar plates and all incidental waste including gloves will be autoclaved in a benchtop autoclave used at 121°C for 20 min (the autoclave will be serviced by the manufacturer every six months). Micropipette tips will be disposed of into disinfectant (0.25% sodium hypochlorite) and left overnight before autoclaving. Following autoclave treatment the waste will be sent for incineration as clinical waste.

PCR based site directed mutagenesis of well understood genes such as those encoding lysozyme.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

There were no concerns raised
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**Date at Which Additional Info Submitted:**

02/03/2022
### Premises Addresses

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<td>D41 5LR</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GM Safety Committee is a sub-committee of the main safety committee. Membership is augmented by co-option of scientific personnel from within the company who are conversant with GM methodology and requirements

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
<th>Large Scale</th>
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- **Bacteriology**: Yes
- **Parasitology**: Yes
- **Transgenic**: Yes
  - Birds
  - Fish
- **Microbiology Research**: Yes
- **Gene Therapy**: Yes

02/03/2022
GMO's are harvested by centrifugation or cross-flow membranes and then used to recover recombinant protein. All waste is sterilized prior to disposal by a combination of chemical inactivation or autoclaving. Use of standard nutritionally stringent strains limits survivability in the environment.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

- GMO's are harvested by centrifugation or cross-flow membranes and then used to recover recombinant protein.
- All waste is sterilized prior to disposal by a combination of chemical inactivation or autoclaving.
- Use of standard nutritionally stringent strains limits survivability in the environment.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

E. coli and Baculovirus risk assessments routinely reviewed in January 2010.
Both assessed as still current
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### Name

LYOSOLUTIONS LTD

### Campus Estate or Research Centre

227 TETRICUS SCIENCE PARK

### Road Name

227 TETRICUS SCIENCE PARK

### District

PORTON DOWN

### Town

SALISBURY

### County

WILTSHIRE

### Postcode

SP4 0JQ

### Country

ENGLAND

### Tel Number

01980 556490

### Fax Number

01980 556491

### E-mail

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### HSE Division

blank

### Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Committee consists of two members of staff

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Large Scale</th>
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Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity

- All waste material will be incinerated as hazardous material
- All utensils will be treated with Zirkon
- The laboratory does have restricted access to named personnel only

Tick to confirm that you are attaching a summary of the risk assessment ✗

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The committee will compromise of two Company Employees and Premises Director

02/03/2022
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Name

ROBERT GORDON UNIVERSITY

Name 2

SCHOOL OF PHARMACY AND LIFE SCIENCES,

Campus Estate or Research Centre

GARTHDEE ROAD

District

RIVRSIDE EAST

Town

ABERDEEN

County

ABERDEENSHIRE

Postcode

AB10 7GJ

Country

SCOTLAND

Tel Number

01224 262500

Fax Number

01224 262555

E-mail

HSE Division

SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>Faculty of Health and Social Care</td>
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<td>ABERDEENSHPRE</td>
<td>AB10 1FR</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Membership of GMO Safety Committee- Chairperson, Biological Safety Officer, Health and Safety co-ordinator, Current users and two lay members.

Meetings will be conducted quarterly.

Further details such as job titles to be added

<table>
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</table>
Microbiological waste is bagged and autoclaved at a minimum of 121 degrees centigrade, with a pressure of at least 2 bar for a minimum of 15 minutes. Thereafter liquid waste is disposed of via the drains and solid waste is bagged and sent with the standard waste disposal system.

Tissue culture waste is deactivated using 'Haz Tabs' (a biological bleaching agent) for 24 hours and then disposed of via the drains.

Cell transformations using microorganisms or eukaryotic cell lines

For activities involving GMMs, describe the waste management measures which will apply to the activity

Microbiological waste is bagged and autoclaved at a minimum of 121 degrees centigrade, with a pressure of at least 2 bar for a minimum of 15 minutes. Thereafter liquid waste is disposed of via the drains and solid waste is bagged and sent with the standard waste disposal system.

Tissue culture waste is deactivated using 'Haz Tabs' (a biological bleaching agent) for 24 hours and then disposed of via the drains.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Forms GMA1 and GMR1 with regards to the study of the role of GPR55 in cardiovascular disease were considered and approved subject to minor modifications. Coveners action was taken with regards to the changes requested.
**GM Centre Number: 3090**

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**Name**

BIGDNA LTD

**Name 2**

**Department**

ROSLIN BIOCENTRE

**Building**

WALLACE BUILDING

**Campus Estate or Research Centre**

**Road Name**

**Town**

ROSLIN

**County**

**Postcode**

EH25 9PP

**Country**

SCOTLAND

**Tel Number** 0131 200 6342

**Fax Number** 0131 440 9521

**E-mail**

**HSE Division** blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- R & D Manager, Biological Safety Officer
- Commercial Director, responsible for general health and safety
- Senior R & D Scientist
- R & D Scientist

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Non-microbial

Other (please specify)

Tick if confidential

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02/03/2022
Equipment, plasticware etc will be decontaminated by soaking in Virkon®, which will be prepared as directed by the manufacturer (10g/1L). Studies have already shown that Virkon® is effective at killing the phage and at the working concentration a 10^9 fold reduction in phage titre occurs immediately. This was the limit of detection of our assay and the actual reduction is probably significantly more. This work has not been published. Decontaminated waste will either be disposed of via the sink or stored in autoclave bags and then autoclaved within the Roslin Biocentre (RBC). Published studies have shown that it takes 6 min to inactivate phage at 100°C (dry heat, Jepson and March, 2004, Vaccine 22: 2413-2419) and we anticipate it would be quicker at the higher temperature of 121°C (used in autoclaving material in the RBC) with a wet environment. The autoclave is based in the RBC is used routinely to dispose of contaminated material. After autoclaving all waste is sent for incineration.

Several disinfectants have been tested with lambda phage vaccines and under normal laboratory conditions, when used at the recommended concentrations all standard disinfectants (e.g. Virkon® http://ww2.dupont.com/Products/en_TR/Virkon.html;) will inactivate the phage.

For general work volumes are low <500ml. All procedures are carried out in dedicated laboratories. Any spillages would be mopped up with blue roll and the contaminated towel disposed of into an autoclave bag. After each experiment the area would then be treated with Virkon® prepared as directed by the manufacturer, and then mopped up again. This process would be repeated twice to ensure that any spillage is decontaminated. Waste from any spillages will be autoclaved as soon as possible in the RBC. Where larger volumes of material are present, e.g. fermentor volume 3.5L, spillages would be cleaned up initially using the spill kit provided. The area would then be treated with Virkon®, prepared as directed by the manufacturer, and mopped up again.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Please enter comments of the GM safety committee on the risk assessment

The Committee reviewed both the Risk Assessment and Notification of Intention to Use form and made some general comments, which Dr *** will incorporate. It was agreed that the five Hepatitis B variants will be included in the same Risk Assessment form. Each of the variants are very similar.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Hypha Discovery has formed a 5-person GMSC to consider and address the risks of working with genetically-modified microorganisms (GMMs) in the company. The committee is chaired by a member of the company's management team, who has previously managed research involving GMMs. The other members of the committee are the company's H&S and Biological Safety Officer, who has over 30 years' experience of microbiological R&D including working with GMMs, the key research scientist responsible for the work involving GMMs and representatives of the departments where the work involving GMMs and their products will be handled. The committee meets as needed, and GMMs are an agenda item in the company's bimonthly H&S meetings. The GMMs to be used in the current project at Hypha have been, and will be, constructed in a laboratory at the University of London and advice on the risk assessment for the GMMs has been received from the leader of the group responsible for constructing the GMMs, a highly experienced researcher in this field.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
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Liquid waste, unwanted cultures and contaminated non-glass items are either autoclaved (chamber temperature at 128 degrees C and probe temperature at 121 degrees C for 30 minutes, with simulated load of fresh cold water [0.8l); expected degree of kill effectively 100%) using an autoclave that is validated annually with the simulated load, pressure and temperature probes, or inactivated by adding virkon to 1% and leaving for at least 4h (expected degree of kill effectively 100%, will be validated with the GMMs).

Contaminated non-disposable glassware is immersed in 1% virkon solution and left to soak for at least 4h. The inactivated washings are disposed of via the sewer drainage system.

Degree of kill achieved by autoclaving or 1% virkon exposure will be validated for these GMMs by streaking appropriate materials onto nutrient agar plates, incubating and examining for evidence of viability.

Inactivated, post-autoclave solid waste is double-bagged for removal via the general waste collection.
The GMM risk assessment has been presented to the GMSC with the following summary findings: the environmental and health risks are low and acceptable; the GMM host strains and their engineered products are of low risk and they will be handled by trained staff using procedural and containment controls and personal protective equipment. The GMMs are constructed in Escherichia coli and Streptomyces lividans host strains to express three-gene operons encoding cytochrome P450 (CYP), ferredoxin (Fd) and ferredoxin reductase (Fdr) genes from wild-type actinomycete bacteria. These will be stored and cultured for biocatalytic purposes by Hypha Discovery using whole-cell biotransformations or the via the preparation of cell-free enzyme extracts. This risk assessment is based on the first examples of GMMs expressing 3 different CYP/Fd/Fdr systems but is intended to cover subsequent GMMs expressing similar enzyme systems sourced from environmental organisms. The donor and recipient microorganisms belong to ACDP hazard group 1 and both of the recipient microorganisms have long histories of safe laboratory use. The GMMs resulting from the insertion of the three-gene CYP-Fd-Fdr operons are considered to have a low risk for environmental harm as they are unlikely to persist in the environment and the products of the inserted genes are enzymes that already exist in the environment. These enzymes are non-hazardous and their expression does not pose a risk to human health or increase the pathogenicity of the host organisms. The containment level required for handling these GMMs is level 1.
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**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: [Y]

Give brief details of the genetic modification safety committee:

GMSC is constituted as follows:

- chair by Pharmaceutic Specialist supported by;
- associate director for Specialist Operations;
- advisory function provided by the EHS Manager who will also initially act as the Biological Safety Officer with expertise in GMO work and a background in Biochemistry/Genetics;
- managers from within the user department who will oversee the work;
- biological specialists from the user department;
- Employee Safety Representative from the user department;
- Other experts seconded as required e.g. Quality

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Non-microbial

Other (please specify) Tick if confidential [ ]

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**Other(s)**

Growth of well established class 1 eukaryotic GM lines expressing human and other genes

For activities involving GMMs, describe the waste management measures which will apply to the activity

All biological waste will initially be inactivated using a disinfectant, autoclaved on site and sealed in UN approved leakproof containers for off-site incineration by Patheon's Waste Management Contractor. Cell kill will be confirmed through validated processes.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC reviewed the information available on 13 July and classified this work as class 1. No additional measures over and above that required by risk assessment are required. Staff carrying out GMO work will have the details noted on their medical records and any required changes in health surveillance will be undertaken.

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02/03/2022

Page 12902 of 15326
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**Name**

DEPT OF APPLIED MATHEMATICS & THEORETICAL PHYSICS, UNIVERSITY OF CAMBRIDGE

**Name 2**

**Department**

**Campus Estate or Research Centre**

CENTRE FOR MATHEMATICAL SCIENCES

**Road Name**

WILBERFORCE ROAD

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB3 0WA

**Country**

ENGLAND

**Tel Number**

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**Fax Number**

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**E-mail**

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**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The genetic modification safety committee includes three members. One of these is a principal investigator in the Department of Plant Sciences whose expertise is in the areas of GMOs such as arabidopsis and green algae. The others are principal investigators in the Department of Applied Mathematics and Theoretical Physics (DAMTP) who have overall responsibility for the laboratories which carry out biophysical research. One of these is both the Safety Officer and the Biological Safety Officer in DAMTP.

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Tick if confidential

Yes
1) For liquid cultures:
Treat with 0.1% sodium hypochlorite for 24h. This kills any microorganisms and thus can be safely dumped down the drain. This widely-used protocol also degrades any recombinant DNA. Occasional random plating of deactivated solution will be used to verify protocol's efficacy.

2) For contaminated solid waste:
2a) For solid agar plates and serological pipettes:
Place a heavy-duty biohazard bag in designated pedal bin. When bag is full it will be collected for incineration by Vetspeed, Ltd., a University-approved commercial waste disposal company.
2b) For contaminated pipette tips, polypropylene tubes, paper towels and gloves:
Place in a yellow designated tub which is lined with clear autoclavable bag. When bag is full it will be autoclaved at 135 C on site and disposed with regular trash in black bags.
2c) For glass chambers used in microscopy and other sharps such as needles (including syringe barrels): Place in designated sharps container. When the container is full it will be collected for incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

In its first meeting, the safety committee suggested several simplifications and extensions of the risk assessment which have now been incorporated. These include disposing of GM Arabidopsis prior to bolting (seed production), thus eliminating concerns about seed dispersal. This also allows all earlier growth to be conducted in dedicated growth chambers in the laboratory. All experiments in this laboratory will be done on recently-germinated plants grown in Petri dishes. When necessary, seeds from mature plants will be obtained from the Plant Sciences Department, which has state of the art facilities for contained growth of mature plants.

In addition, the committee recommended that the risk assessment include procedures for decontamination of microscopes as a matter of general usage, and also in the event of spillage (reported to the Safety Committee)
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**Name**

MIDDLESEX UNIVERSITY

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

THE BURROWS

**District**

**Town**

HENDON

**County**

MIDDLESEX

**Postcode**

NW4 4BT

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ENGLAND

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020 8411 5000

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<th>Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Give brief details of the genetic modification safety committee</td>
<td></td>
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</tr>
</tbody>
</table>

02/03/2022
Genetic Modification Safety Committee
(Sub-Committee of School of Health and Social Sciences Health and Safety Committee)

Role of the Genetic Modification Safety Committee.
To monitor and keep under review arrangements for the control of all activities involving genetic modification (GM). To meet the statutory purpose to advise on risk assessments made in compliance with the regulations as described in the Genetically Modified Organisms (Contained Use) Regulations 2000 To provide reports, give advice and make recommendations to the HSSC Health and Safety Committee about any matter relating to the control of experimental genetic modification. To advise on safety training requirements.

Membership
The committee shall be composed of:

a. Head of Department of Natural Sciences (Chair)
b. The University Biological Safety Officer
c. Associate Dean Research, School of Health and Social Sciences
d. Professor of Cancer Biology/Person responsible for supervision and safety of GM activities on the premises
e. 2 nominees from academic staff in the department of Natural Sciences
f. Chair of the Natural Sciences Ethics Sub-committee
g. Technical Manager
h. Representative from Estates and Facilities Management Services (EFMS)
i. Elected Health and Safety Officers, UCU/UNISON

Co-opted members:
Additional members may be co-opted by the sub-committee as felt appropriate by the committee.

Quorum
A minimum of 5 members to include the Chair (or deputy), the Biological Safety Officer and the person responsible for supervision and safety of GM activities on the premises.

Frequency of meetings:
The full sub-committee shall normally meet twice per year (normally June and September). Extra meetings may be scheduled if required.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
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Other (please specify)  

Tick if confidential

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<tr>
<th>Bacteriology</th>
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<th>Transgenic</th>
<th>Microbiology</th>
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<tr>
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<td>Birds</td>
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<td>Gene Therapy</td>
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<tr>
<td></td>
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<td>Plants</td>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

- **Solid Waste** - All GM solid waste will be treated on site typically by autoclaving. Subsequent disposal by normal laboratory routes. Clinical waste and sharps bins are removed for disposal by authorised contractor and official waste disposal notes generated. There may be instances where chemical disinfection by Virkon™ (following manufacturer's defined usage) is the method of preference.

- **Sharps boxes** - All sharps boxes containing GM materials will be autoclaved on site and then disposed as per solid waste (above).

- **Liquid waste** - All GM liquid waste will be treated on site, either by autoclaving or chemical disinfection using Virkon™. Larger volumes will subsequently be discarded via drains; smaller volumes may be discarded within primary containers via solid waste route.

- **Surface disinfection** - All lab surfaces where GM activities have taken place will be disinfected after use, using Virkon™.

- **Spillages** - Any spillages involving GM materials will be disinfected using Virkon™.

- **Autoclaves** - All autoclaves are subject to annual service and calibration by competent engineer (Priorclave contract: ISO9000 certification and UKAS approval as a calibration laboratory). Each run cycle on the waste autoclave produces a printout for validation.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
**Project Ref** 3096/15.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
<th>CultureVolume</th>
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<tbody>
<tr>
<td>26/02/2015</td>
<td>Ecology and genetics of Salmonella-plant interactions</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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</table>

**Purposes of the contained use**

This project is focused on the identification of metabolic, regulatory and structural genes in Salmonella enterica serovar typhimurium that are involved in successful colonization of plants, in particularly tomatoes and lettuce. Proliferation of Salmonella within the tissue of these plants is responsible for the number and severity of produce associated gastroenteritis outbreaks increasing, so we are coming to recognize that we know little about the ecology of Salmonella and enterovirulent Escherichia coli outside of their animal hosts. It is clear that these human pathogens can contaminate roots, stems, vegetables, and/or post-harvest production stages. The goal is to define the ecology of Salmonella during colonization within fresh produce by the identification and characterization of Salmonella "plant-specific" genes and host responses. This information will help to establish which
mechanisms Salmonella needs in order to colonize fresh produce and overcome physical barriers and host restrictions on colonization. Any vegetable and fruit may be susceptible to the proliferation of Salmonella. We expect to work with tomato, lettuce, spinach, cantaloupe, onions, peppers, pears, apple and other vegetables and fruit eaten raw.

The aim of this project is to determine the role of Salmonella transcriptional regulators, capsule formation genes and other genes important during Salmonella proliferation in fresh produce. Our targets are the -4000 genes and proteins of Salmonella. In addition, we expect to modify proteins to screen possible catalytic sites or binding regions involved in Salmonella proliferation in plants.

Strong evidence exists in the literature, supported by the PI's preliminary results, which suggests that there is a key role of different regulatory and structural genes during plant infection. The basis for the proliferation in vegetables is divisible into roughly three general categories: direct acting genes, regulatory genes, and recognition genes.

Salmonella mutants will be produced and their expression will be studied during proliferation in plants. Mutants will be generated by routine mutagenesis protocols, such as the Datsenko and Wanner "One step" protocol. According with the PI's preliminary results, initial experiments will be carried out by generating mutants with deletions in rcsA and rcsB genes, two transcriptional regulator involved in capsule and biofilm formation of Salmonella in tomato. We expect to expand this research to other genes.

Gene expression will be analyzed in vivo by the recombinase-based in vivo expression technology (RIVET). The fitness of the mutants compared to the wild type will be tested in different varieties of vegetables. If we learn that a mutation shows a modified fitness, further complementation of the deleted genes will be produced to restore the phenotype and confirm the contribution of each gene during plant infection.

Recipient or parental organism

The following strains represent what we intend initially, but we may wish to take this further to other parental strains of Escherichia coli and Salmonella enterica as research proceeds.

| E. coli | DHSa F- (lacZYA-argFjU169 recA1 endA 1 hsdR17 supE44 thi-1 gyrA96 relA1. This strain is used to host plasmids for the genetic modifications. |

Example of mutants that will be generated according with the recipient strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mutations</th>
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<tbody>
<tr>
<td>14028 rcsA1::cm rcsB1::kan</td>
<td></td>
</tr>
<tr>
<td>14028 rcsA::cm</td>
<td></td>
</tr>
<tr>
<td>14028 resA1::kan</td>
<td></td>
</tr>
<tr>
<td>14028 resB2::frt</td>
<td></td>
</tr>
<tr>
<td>14028 rcsA1::frt-kan-frt</td>
<td></td>
</tr>
<tr>
<td>pBAD33-rcsA or (csB</td>
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</table>

Host/vector system

The following plasmids represent what we need initially, but we may wish to take this further to other vectors as research proceeds.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCP20 FIP+; &quot;A cl857+ ; &quot;A pR Repts, amp, cm Noel et al. 2010</td>
<td></td>
</tr>
<tr>
<td>pBAD33 general cloning vector, low copy Wang RF, Kushner SR (1991)</td>
<td></td>
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</tbody>
</table>

Construction of versatile low- copy-number vectors for cloning, sequencing and gene expression
All the strains and plasmids that will be used in this project are tools routinely used for genetic studies in Salmonella enterica sv. Typhimurium 14028 and Escherichia coli. They are widely used by the scientific community and currently published in peer-reviewed scientific literature. The following references are included to provide detailed information on the origins and intended use of the genetic material involved.

**Bacterial mutagenesis. Plasmids pKD3, 4, 46. Gene deletion.**


Plasmid pCP20:


Salmonella enterica sv. Typhimurium 14028 and Escherichia coli DH50:


RIVET (recombinase-based in vivo expression technology) construction:

In RIVET, the promoter of interest is cloned upstream of a promoterless bicistronic InpR-lacZ operon in a genetic background that contains res-tet-res sites cloned in a neutral site of the genome. When the promoter of interest is activated, this drives expression of tnpR. TnpR acts upon "res" sites and excises the tetracycline marker cloned within them. The loss of the tetracycline resistance then creates a heritable marker of gene expression.


**Evaluation of foreseeable effects**

The bacterial strains will become kanamycin, ampicillin or chloramphenicol resistant.

**Health and Safety**

Executive

The plant tissue(s) infected with GMO Salmonella or E. coli will NOT be used to generate any GMO plants.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste containing GMO materials will be autoclaved before final disposal as clinical waste (including sharps waste). Liquid waste will either be autoclaved as above or will be chemically disinfected with 1% Virkon (final concentration). Please see Risk Assessment for more details.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GM Safety Committee made a number of procedural recommendations, all of which have been incorporated into the final risk assessment (attached). After consideration, it was agreed was that this work fits comfortably within the definition of a class 2 activity.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<td>L3</td>
<td>L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L4</td>
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02/03/2022
**GM Centre Number: 3098**

<table>
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<th>Transitional Premises Class</th>
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<th>Non-GMMs</th>
<th>Withdrawn</th>
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<tbody>
<tr>
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<td>N</td>
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</tbody>
</table>

**Name**

BICYCLE THERAPEUTICS LTD

**Name 2**

Department

**Campus Estate or Research Centre**

BABRAHAM RESEARCH CAMPUS

**Building**

BUILDING 900

**Road Name**

District

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB22 3AT

**Country**

ENGLAND

**Tel Number**

01223 497395

**Fax Number**

E-mail

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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<td>BABRAHAM RESEARCH CAMPUS</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- GMO Adviser (external Consultant) (Technical expert)
- CEO - Bicycle Therapeutics (Management representative)
- Senior Scientist - Bicycle Therapeutics (responsible for GMO work)
- Staff representative - Scientist at Bicycle Therapeutics (BSO)

Genetic Modification Safety Committee will advise on risk assessments and these risk assessments will be reviewed and approved by the Committee in advance of any work commencing. The GMSC will review established risk assessments, at minimum, annually

<table>
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<tr>
<td>Other (please specify)</td>
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</tbody>
</table>

Tick if confidential

02/03/2022
| Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research |
| Virology | Transgenic Animals | Transgenic Fish | Gene Therapy |
| Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below) |
| Other(s) | Bacteriological work including the use of bacteriophage |

For activities involving GMMs, describe the waste management measures which will apply to the activity

All GMM contaminated materials will be decontaminated in Trigene, Vircon or hypochlorite solution

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM Safety Committee has reviewed the risk assessment and have approved it
GM Centre Number: 3099

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<td>N</td>
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<tr>
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</table>

Name

FOUNDATION FOR LIVER RESEARCH

Name 2

Department

Campus Estate or Research Centre

INSTITUTE OF HEPATOLOGY

Road Name

111 COLDHARBOUR LANE

Town

LONDON

County

GREATER LONDON

Postcode

SE5 9NT

Country

ENGLAND

Tel Number

020 7255 9847

Fax Number

020 7380 0405

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<th>Name 2</th>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes](Yes)

Give brief details of the genetic modification safety committee

The Committee will be made up of the Institute Manager, Deputy Manager, Administration Manager, Safety Consultant/Biological Safety Officer

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<th>Transgenic Birds</th>
<th>Microbiology Research</th>
</tr>
</thead>
</table>
Various research on liver failure and associated liver disease

For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Assessment was reviewed and approved

Project Ref 14/03.1

Date Ack'n'd 09/02/2011

CU2 Project Title THE IN VITRO STUDIES OF HEPATITIS B VIRUS REPLICATION, INVESTIGATING THE EFFECTS OF ANTIVIRAL DRUGS ON VIRAL REPLICATION AND ANTIGEN EXPRESSION

Date Project Ceased

Class CultureVol Class2 CultureVolume Class3-4

Class 2 250 ml max

Non-GMM Consent Granted Yes

Project notified under transitional arrangements N

Withdrawn

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID...

Date of Significant Change
Project Additional Information

Purposes of the contained use
To study the replication and immunobiology of Hepatitis B virus in vitro by:

1. Transfecting plasmids containing HBV-DNA in to hepatoma cell lines with transient viral expression.
2. Culturing cell lines that have HBV integrated into their genome and produce full infectious virus in culture.

Recipient or parental organism
Recipient organism: The plasmid containing HBV-DNA will be transfected into 2 human hepatoma cell lines - HepG2 and HUH-7. These cells pose no risk to workers.

Parental organism: A cell line which has the HBV genome integrated into their genomic DNA - HepG2215 cells. These cells produce full infectious Hepatitis B virions in culture. The foreseeable risk in handling these cells is the innoculation of the worker with the cells or the culture supernatant and thereby infecting the worker with HBV.

The most hazardous risk to workers would be of infection by the virus therefore -

1. All workers will be vaccinated against HBV and have proven protective antibody titres prior to commencing the work.
2. All work will be carried out in our containment level 3 (CL3) facility
3. As the major risk of infection is through needle stick injury there is a NO SHARPS policy in the CL3 laboratory

If control measure failed the most severe risk would be inoculation of the worker with the virus.

Host/vector system
Hosts: Bacterial - E. coli: Disabled host
Vector: PUC 19 plasmid: non-mobilisable, BOM (-)/NIC (-), MOB (-), TRA (-) and with ampicillin resistance

There is no foreseeable effects with this host/vector system.

Origin & function
The gene sequence to be inserted into the plasmid is the full HBV-DNA extracted from human patients infected with HBV.

This sequence will be inserted into the PUC19 plasmid vector and transformed into E. coli (DH5-alpha). This DNA will then be extracted and transfected into the HepG2 and HUH-7 cell lines. These cells will be cultured and experiments to monitor viral replication will be carried out.

Evaluation of foreseeable effects
The foreseeable effect of this project, if control measures failed is the infection of the person handling the cell line with HBV.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste generated in the containment level 3 facility will be autoclaved in the double-ended autoclave.

1. Solid waste:
This includes laboratory plastics, laboratory overalls, gloves, paper wastes etc will be collected in double autoclave bags within a sealed container and when 3/4 full loaded into the ‘dirty’end of the autoclave which is within the CL3 laboratory. Once the cycle is complete the worker the waste is collected from the ‘clean’ end of the autoclave situated outside of the CL3 facility. The receipt from the autoclave is checked to ensure the run has passed (100% kill) and the waste is collected into yellow bags for incineration.

2. Liquid Waste
The liquid waste is decontaminated with Virkon powder - using 1g of powder per 100 ml of culture medium. This has been tested and proven to be 100% effective against Hepatitis B virus.

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
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<td>L2</td>
<td>L3 L4</td>
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</tr>
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Project Ref 3099/10.1

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<th>CultureVolumeClass3-4</th>
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<td>The role of the immune response in the control of Hepatitis C virus infection</td>
<td>Class 3</td>
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Project Additional Information

Purposes of the contained use

To study the interactions in-vitro between human cells of the immune response and HCV by:

1. Transforming bacteria with pJFH-1 plasmid containing full HCV sequence of a patient with fulminant hepatitis.
2. Transscribing HCV RNA from this plasmid, pJFH-1
3. Transfecting this HCV RNA, by electroporation, into a human hepatoma cell line HUH-7 to produce infectious HCV viral particles
4. Co-culturing the lymphocytes isolated (from control subjects and patients with HCV infection) with the JFH-1 transfected hepatoma cell line
5. Assessing viral kinetics and virus-specific immune responses

Recipient or parental organism

Recipient Organism 1: The plasmid pJFH-1 will be transformed into competent E.coli Bacteria DH5a which will be grown in LB medium. The amplified plasmid will be extracted from the E. coli.

Recipient organism 2: RNA transcribed from the plasmid pJFH-1 containing full length HCV cDNA will be transfected into Huh-7 human hepatoma cell line. These cells will produce full infectious HCV virions in culture. The foreseeable risk in handling these cells is the inoculation of the worker with the cells or the culture supernatant and thereby infecting the worker with HCV.

The most hazardous risk to workers would be of infection by the virus therefore:
1- All the work will be carried out in our containment level 3 (CL3) facility
2- As the major risk of infection is through needle stick injury, there is a NO SHARPS policy in the CL3 laboratory.

If control measure failed the most severe risk would be inoculation of the worker with HCV.

Host/vector system

Host 1: E. coli DH5a strain
Host 2: HUH-7 human hepatoma cell line
Vector: Full length HCV RNA
There are no foreseeable effects with this host/vector system.

**Origin & function**

HCV RNA will be transcribed and purified from the JFH-1 plasmid and used to transfect Huh-7 cells. The transfected cells will be cultured in presence or absence immune cells and experiments to monitor viral replication and host immune responses will be carried out.

**Evaluation of foreseeable effects**

The foreseeable effect if control measures failed is the infection of the worker with HCV.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the class of activity. (Measures & Justification)

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All the waste generated in the CL3 will be autoclaved in the double-ended autoclave.

**Solid waste:**

Include, gloves, suits, plasticware, paper waste will be decontaminated by autoclaving. Solid waste will be collected in double autoclavable bags within a sealed container. When 3/4 full, the bag will be loaded into the 'dirty' end of the autoclave which is situated within the CL3 and a 'discard' cycle (134°C for 25 min.) will be run. Once the cycle is complete, the worker will collect the autoclaved bag from the 'clean' end of the autoclave situated outside the CL3. The receipt form of the run will be checked to ensure the run has passed and the waste will be collected into yellow bags for incineration.

**Liquid waste:**

The liquid waste will be decontaminated by autoclaving. Empty containers will be half filled with the contaminated liquid waste. The containers will be disposed in 3/4 full double autoclavable bags which will be autoclaved using the 'discard' cycle (25 min at 134°C).

2. HCV is sensitive to heat treatment and temperatures from 56°C or higher can be used for complete loss of infectivity therefore the treatment of the waste load at 134°C for 25 min will completely inactivate HCV.

3. The receipt form of the run will be checked to ensure the run has passed.

4. The waste will be collected into yellow bags, closed and removed for incineration.

Contaminated laboratory surfaces and equipments are disinfected with a 40% 1-propanol solution. The surfaces will be treated with the solution for 5 min. then wiped clean.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

02/03/2022
This project has been reviewed by the GM safety committee and approved, protocols for disinfection and waste disposal have been amended to show a consistent approach throughout all experimental procedures.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L2</td>
<td>L2</td>
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<tr>
<td>L3 Yes</td>
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<thead>
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<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
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**Project Ref 3099/12.1**

- **Date Ackn’d**: 16/04/2012
- **CU2 Project Title**: Understanding the role of MAPK cascade in cancer and inflammation
- **Class**: Class 2
- **CultureVolClass2**: ≤ 1 Litre
- **CultureVolumeClass3-4**: Non-GMM
- **Consent Granted**: Consent Granted

- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**
- **Historical Date of Additional Info**: Non-GMM
- **Significant Change ID**: Class 2
- **Date of Significant Change**: Consented

**Project Additional Information**

**Purposes of the contained use**

MAPK and NF-κB pathways control cell survival, and this control is crucial for different biological function including apoptosis, proliferation and survival. Studies aimed at understanding the molecular mechanisms of these pathways of intracellular signalings might unveil crucial target for anti-inflammatory and anti-cancer therapies.
To perform our studies, we will generate several DNA plasmids containing Flag- or HA-tagged genes and transformed them into the E. coli bacteria in order to amplify the DNA. The purified DNA allows us to conduct transfection assay in cells. We prepared glycerol stocks of the transformed bacteria. These GMMs are essential tools to perform studies aimed at gaining better understanding of the basis for the MAPK-mediated control of cell survival.

We are planning to perform the following procedures involving GMMs:

1) Transforming the E. coli strain DH5a and other K12 E. coli strains with vectors pcDNA3.1, pSRa, pMIGR1, pSUPER, pVSV, pLentiLox 3.7, pENV, pMDL, pREV, pWPT, R8.91, MD2G, pMT2T, pRSV, pBluescript, pET, pWPI. With the aim of amplifying the DNA vectors;

2) Transfecting standard mammalian cell lines (including 293T, HeLa, NIH-3T3, HuH-7, HepG2, Hep3B, PLC5, Huh-28, Mz-ChA-1, TFK1, HF60, HuCCT-1, H69, SG231, RPMI8266, JNJ3, MM1S, ) with mammalian expression vectors (in which cells would you use the bacterial vectors?);

3 Transducing standard mammalian cell lines (such as 293T, HeLa, NIH-3T3, fibroblasts, etc) with non-replicative variants of lenti-viruses (such as pLentiLox, pWPT, pWPI, etc.) or retro-viruses (such as pMIGR1, pSUPER, etc.) The lenti-viral vectors pLentiLox and pWPT contain the woodchuck post- transcriptional regulatory element (WPRE), which has been demonstrated to have some oncogenic properties, whilst the retro-viral vectors pMIGT1 and pSUPER do not contain this element. Both lentiviruses and retro-viruses are completely unable to replicate into the transduced cells, because the packaged viruses lack Gag, Pol, and Env, which are the genes necessary for the viral replication, and the transduced mammalian cell lines do not express Gag, Pol and Env proteins.

These tools and procedures will give us the possibility to perform cellular studies aimed at gaining a better understanding of the basis for the MAPK- and NF-kB mediated control of cancer cell survival.

**Recipient or parental organism**

- E. coli strain DH5a and other K12 that present high efficiency strain for cloning and subcloning.
- Mammalian cell lines: 293T, HeLa, NIH-3T3, HuH-7, fibroblasts, and other standard mammalian cell lines.
- Fibroblasts and other primary cells (such as lymphocytes, hepatocytes, etc.) from mice, including KO and Tg mice.

**Host/vector system**


LentiLox 3.7 (PLL3.7) or other lentiviral vectors are designed for transducing cDNA or short-hairpin RNA (shRNA), which includes RNA interference in a wide range of cell types, tissues and organisms. We will use these vectors to infect and efficiently silence proteins in mammalian cells and their progeny, as described in (Rubinson and Dillon et al, Nature Genetics, 2003). These lentiviruses are pseudo-coated with VSV-G and are capable of infecting human cells, and thus present important biosafety issues. CL2 conditions should be used at all times when handling lentiviral preparations, transected cells or the combined transfection reagent.

For the purpose of using vectors in a BL2 laboratory and eliminate potential harm, the odifications described in section 1.24 have been made to prevent viral replication.

More information regarding these widely used techniques for transducing mammalian cells can be found at [http://www.sciencegateway.org/protocols/lentivirus/index.htm](http://www.sciencegateway.org/protocols/lentivirus/index.htm)

**Origin & function**

The purpose of the use of these plasmids in the host organism is to propagate and obtain recombinant proteins produced directly into the host organism.
The mammalian cells transfected and transduced with the different vectors will be expressing as well as silencing MAOK and NF-κB proteins. The lenti-viral vectors pLentiLox and pWPT used for transduction have oncogenic properties.


The following modifications have been made to prevent viral replication:

1. Packaging vector lacks both LTRs and has no viral packaging signal.
2. The following viral genes have been deleted from the packaging vector: env, tat, rev, vpr, vpu, vif and nef.
3. Rev is supplied in trans on a different vector (RSV-Rev)
4. The vector expressing the packaged viral genome has a self-inactivating LTR (TATA box deletion and expresses no viral gene products.
5. Envelope, in this case VSVG, is expressed on a separate vector.

LentiLox 3.7 (pLL3.7) or other lentiviral vectors are designed for transducing cDNA or short-hairpin RNA (shRNA), which induces RNA interference in a wide range of cell types, tissues and organisms. We will use these vectors to infect and efficiently silence proteins in mammalian cells and their progeny, as described in (Rubinson and Dillon et al, Nature Genetics, 2003). These lentiviruses are pseudo-coated with VSV-G and are capable of infecting human cells, and thus present important biosafety issues. CL2 conditions should be used at all times when handling the virus and/or the transduced cells. All decontamination steps should be performed using 2% virkon and 70% ethanol 1% SDS Gloves, lab coat should be worn at all times when handling lentiviral preparations, transfected cells or the combined transfection reagent.

For the purpose of using these vectors in a BL2 laboratory and eliminate potential harm, the modifications described in section 1.24 have been made to prevent viral replication.

More information regarding these widely used techniques for transducing mammalian cells can be found at http://www.sciencegateway.org/protocols/lentivirus/index.htm

Evaluation of foreseeable effects

N/A

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste:
Includes gloves, suits, plastic ware, paper waste will be disinfected by autoclaving. Solid waste will be collected in double autoclavable bags within a sealed container. When 3/4 full, the bag will be loaded into the autoclave and a 'discard' cycle (134° for 25 min.) will be run. Once the cycle is complete, the receipt form of the run will be checked to ensure the run has passed and the waste will be collected into yellow bags for incineration.

Liquid waste:
The liquid waste will be decontaminated by virkon (2.5g tablet/500ml infectious liquid waste) for at least 30 minutes, followed by disposal in the sink. Virkon has been tested.
by independent laboratories and been proven to be effective against a total of 20 virus families (including HIV/AIDS), 43 bacterial genera and 27 fungal genera.

Surfaces
Contaminated laboratory surfaces and equipments are disinfected with a 1% virkon solution for at least 10 min before being wiped dry. The surfaces will be further cleaned with a 70% Ethanol solution.

The Institute of Hepatology genetic modification safety committee (GMSC) has received and approved project GMOO2. The GMSC is happy for project GMOO2 to start at the Institute of Hepatology once authorized by the HSE.

Please enter comments on the GM safety committee on the risk assessment

The Institute of Hepatology genetic modification safety committee (GMSC) has received and approved project GMOO2. The GMSC is happy for project GMOO2 to start at the Institute of Hepatology once authorized by the HSE.

Project Containment

<table>
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<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>Animal Units</td>
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<td>Human Clinical Applications</td>
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<td>L4 L3</td>
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</table>

Is an emergency plan required according to regulation 20? [ ] N

If yes, tick to confirm that it is attached to this form [ ] N

Tick to confirm that you have attached a risk assessment to this form [ ] Y

Tick if you are claiming exemption from disclosure for section of the risk assessment [ ] N
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<th><strong>Transitional Premises Class</strong></th>
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<td>01223 839 133</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:
Our GM safety committee is comprised of company employees. Currently the committee has four members; three hold PhDs in the life sciences and the fourth is a senior-level technical staff member.

The committee will hold its first meeting in early September 2010 and reconvene monthly thereafter. The committee will consider all of the GM work undertaken at the company as described in submitted Risk Assessment Forms. It will consider all of the GM work undertaken at the company as described in submitted Risk Assessment Forms. It will also be tasked with auditing compliance to all risk mitigation strategies.

Added 28/01/2022 - The GMSC now meets (at least) quarterly and has expanded. It now comprises of; Chair, Nominated Deputy Chair, Biological Safety Officer, external expert, lay person and Departmental Representatives (11 people in total).

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>

Non-microbial

Other (please specify)  
Tick if confidential

Bacteriology  
Parasitology  
Transgenic Birds  
Microbiology Research

Virology  
Transgenic Animals  
Transgenic Fish  
Gene Therapy

Mycology  
Transgenic Invertebrates  
Transgenic Plants  
Other (please specify below)  
Yes

Other(s)  
Basic DNA cloning using bacterial and tissue culture procedures with mammalian cells

For activities involving GMMs, describe the waste management measures which will apply to the activity.
All GMMs used in these Class 1 activities will be effectively 100% inactivated as follows.

For solid waste containing GMMs, the materials will be autoclaved at the appropriate temperature and pressure for an adequate length of time. The autoclaved waste will then be sent for incineration. For liquid waste containing GMMs, a decontamination liquid such as Virkon (peroxygen-based and the referred method) or hypochlorites (bleach) will be used. For example, a working concentration of Virkon will be prepared fresh and then used to dilute the contaminated liquid to 1-2% final. The activity of the Virkon stock solution will be verified by colour indicators. DMM-containing liquids will be held in Virkon treatment for a minimum of 20- minutes. Treated liquids will be disposed down normal drains with running water provided no non-biological hazards are present. Empty containers will be autoclaved.

Both solid and liquid waste will be appropriately labeled. Solid waste will be placed in bio-hazard plastic bags for autoclaving and incineration.

A meeting of the Safety Committee was conducted on September 9th, 2010 to consider the Risk Assessment of certain work conducted at the company using GMMs.

The committee considered the nature of the organisms as well as the details for handling, inactivating and disposing of them and came to the consensus that these activities should be considered Level 1 risks.

The committee approved the procedures outlined for the handling of GMMs. A copy of the Risk Assessment along with more detailed committee comments is attached to this form.
This work is aiming to develop monoclonal antibodies as a drug to treat Ebola. The lenti pseudo-type viruses are classified by the Advisory Committee on Dangerous Pathogens (ACOP) as hazard group two organisms. The viruses will be produced and provided by our collaborator in the University of Westminster and are genetically modified to contain Ebola glycoprotein (GP), which is crucial for the infection of Ebola virus, and non-toxic marker genes, such as luciferase. By looking into the monoclonal antibodies binding to the GP, the Ebola infection will be blocked at this stage.

In vivo work:
These pseudo-type viruses will be used for immunization of Kymice (hybrid of C57/B6 and 129 mouse strains). The mice are housed in the Research Support Facility (RSF) building at the Wellcome Trust Sanger Institute (WTSI) in a purpose built CL2 facility. Serial bleed, terminal bleed and samples (spleen, legs) collection will happen on the same premises. The WTSI has a large scale programme for generating mice. All details of the immunization procedures are approved by the Home Office Licence held by Professor Gordon Dougan/Or Simon Clare and immunizations will be performed in accordance with this approved licence and appropriate project licence holder.

In vitro work:
The immuno-response of mice will be checked by ELISA with the polyclonal serum against the pseudo-type viruses. The leniviruses used for vaccination also serve as reporter viruses in neutralisation assays. Tissue samples (spleens and bone marrows) will be dispensed into single cells, stained for cell sorting. These will be all conducted under BSL II conditions.

Recipient or parental organism
The parentallenti-viruses are CI 2 organism. The recipient organisms (mouse and assay cell lines) are CI1.

In vivo work:
The lenti pseudo-type viruses to be used will include viruses harbouring EboJa glycoprotein and non-toxic reporter gene (GFP or Luciferase). The mice for immunization is a genetically modified outbred strain (C57B6 with 129). These mice won't pose a higher risk to human health or the environment than the parental strains (wild type C57B6 or 129). We anticipate that mice will be infected by the viruses used for immunization and some will go on to develop clinical symptoms. Such mice will be humanly sacrificed according to the methods outlines in Sanger's Home Office License if their clinical symptoms reach a certain well documented level of severity. We foresee no unusual outcomes since the viruses we will use is defected, so can’t replicate. After inoculation, the viruses will be cleared out very quickly by the mouse natural immuno system.

In vitro work:
The lentiv pseudotype viruses we will be using are produced by our collaborator in the University of Westminster who will be responsible for the health and safety assessment and control for the production process. There is good evidence that such replication defective viruses are not pathogenic when tested in mice and a minimum of 3 recombination events are necessary to produce replication competent, recombinant virus. In addition such virus vectors are single cycle infection agents not able to form productive infections or to recombine with endogenous retroviruses and become productively infectious.

There is a very low likelihood that exposure to any components of this project will cause harm. The main risk is accidental infection and transfer of the lentivirus to the laboratory personnel directly handling the lentivirus. This risk is extremely low since all the procedure will be carried out following the requirement for protection of working in CL2. Also the lentivirus is carrying a non-toxic, non-human reporter gene, so the potential harm by insertional mutagenesis/overexpression of cellular genes by random integration is extremely low. The likelihood that this will cause harm is low as the replicative defective nature of the lenti vectors ensures that even in the case of a breach of containment and accidental injection, no virus can spread within or between individuals.

Several serial bleed will be taken after each boost to monitor the titre by ELISA. The ELISA will be performed in a class II hood to provide a control measure to prevent exposure of the operative to any aerosols produced within the hood. Plates will be read on a plate reader outside of the class II lab but prior to removal plates will be sealed with a self adhesive polypropylene seal and surface of plates which are accessible will be decontaminated with distel wipes. Functional activity of immunised mouse serum samples and antibodies will be determined using an E Bola lentiviral infection assay. The assay uses lentivirus expressing the E bala glycoproteins on the surface to infect HEK 293 or Vero E6 cell lines. All work will be carried out in class II hood to minimise exposure to aerosols in accordance with cat II procedures. Plates will be processed on a plate machine outside of the class II lab but prior to removal will be sealed with a self adhesive polypropylene seal and surface of plates which are accessible will be decontaminated with distel wipes. Any plasticware to be removed from the lab will be decontaminated with distel wipes. Concentrated stocks of the lentivirus will be stored in a tocked -80 freezer within the category II lab which has entry control to restrict access to stocks. Liquid waste from in vitro work containing lentivirus will be decontaminated by adding virkon (final 1% v/v) in a class II hood to minimise aerosol exposure when carrying out decontamination process.

Samples of mouse cens and tissue or lentiviral supernatant will be transferred between the Sanger Centre and Kymab but all samples will be packaged well with absorbent materia l, secondary contained in a sealed container specifically for the purpose of CL2 sample transportation and placed in designated packaging with correct safety labelling. All packages will be transported with the relevant safety documentation and drivers are insured to do so.

Host/vector system

Host cell lines
Host cest lines will include: 293T, Vero-E6, murine embryonic fibroblasts.
No cell lines or primary cells used will be from the workers.

The foreseeable effects of the infection of these cells with recombinant retroviruses would be the production of a cell line with altered properties able to establish a tumour in the laboratory workers, but the likelihood of this hazardous event to happen is estimated to be LOW under the strict BSL II procedure.

Mice

All immunization and sample collection work: will be carried out in purpose built CL2 facilities in Sanger center and animal cadavers win be autoclaved before disposal.

Origin & function

Origins of lenti pseudotype virus - These are supplied to us from our collaborator Dr Edward Wright, University of Westminster.
Evaluation of foreseeable effects

 Lentiviral Gene expression
The main risk is accidental exposure of laboratory workers to recombinant lentiviruses. As these lentiviral vectors are self-inactivating, the risk of onwards transmission to another person is impossible. The main risk is therefore alteration of the cellular function of the exposed person through insertional inactivation/activation of tumour suppressor genes/oncogenes respectively, or the alteration of cellular function by gene overexpression or ablation from the transferred genetic material within the vector. THESE ARE EXTREMELY UNLIKELY EVENTS THAT HAVE ONLY OCCURRED IN A CLINICAL SETTING FOLLOWING PROLONGED INFECTION WITH HIGH TITRE RETROVIRAL VECTORS AND SELECTION IN VIVO FOR CELLS WITH ALTERED GROWTH POTENTIAL. THEREFORE THE OVERALL RISKS ARE EXTREMELY LOW.

Mice
All immunization work will be carried out in purpose built CL2 facilities in Sanger center and animal cadavers will be autoclaved within this facility before disposal. Immunocompromised or pregnant workers will not be allowed to work in these facilities. OVERALL THE RISKS OF ADVERSE EVENTS FROM HANDLING GM VIRUSES FOR MURINE WORK ARE LOW.

Together the risks identified within this integrated programme of work can be minimised to acceptable low levels by working to existing laboratory standards and practices in designated Containment Level 2 laboratories. This is supported by the accompanying risk assessments.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
All mice are housed in a CL2 laboratory suite built for purpose.
Animal cadavers will be autoclaved within this facility before disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
We operate a number of comprehensive and proven waste management measures which are conducted according to our focal protocol for handling e12 pathogens in both sites (Sanger Center animal house and Kymab CL2 lab). The protocols for the two sites are as follows:

Sanger Centre:
The GMM will be used within a containment level 2 laboratory in which all cultures/liquid waste is decontaminated prior to disposal using a final concentration of 1% Virkon solution for at least 16 hours (concentration validated by manufacturers) before disposal to the drains. Thus drains, sinks etc do not pose a mode of transmission to the environment. Air movement is also strictly regulated in the laboratory environment. Solid biological waste (including cadavers) is autoclaved using temperature cycles and conditions appropriate for the inactivation of biological material. Autoclaving is performed by departmental staff using approved conditions (134 degrees C for 3 minutes per cycle). Plasticware etc is decontaminated with 1 % Virkon solution prior to being autoclaved as described above.

Kymab Ltd:
At Kymab all cultures/liquid waste is decontaminated prior to disposal using a final concentration of 1% Virkon solution for at least 0.5 hours (concentration validated by manufacturers) before disposal to the drains. The virkon will be
added to liquid in a class Is hood to minimise aerosol exposure during the process. Thus drains, sinks etc do not pose a mode of transmission to the environment. waste should be secondary contained in a sealed container if left outside the safety cabinet during decontamination periods. Plastic pipettes should be secondary contained by re-sheathing in wrapper before placing in bins. All solid waste from Kymab is placed in hermetically sealable yellow bins and sent for incineration off site. Air movement is also strictly regulated in the laboratory environment. All the work will be carried out in Cat Is hood to minimize aerosol exposure.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Non-GMM Consent Granted

Consent Granted: Not Applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Withdrawn

02/03/2022
Project Additional Information

Purposes of the contained use

This connected programme of work aims to discover and develop monoclonal antibodies and vaccines against medically-important human targets or pathogens using the Kymouse™ (hybrid of mouse strains C57B6 and 129) platform. The pathogens we will investigate are all classified by the Advisory Committee on Dangerous Pathogens (ACDP) as hazard group two (HG2) organisms and will include:
1) human orthomyxoviruses, including influenza viruses
2) human herpesviruses, including human cytomegalovirus, Epstein-Barr virus and herpes simplex virus 1
3) human pneumoviruses, including respiratory syncytial virus
4) Lentiviral-vectors and lentiviruses
5) Gram-negative bacteria, including Escherichia coli and Acinetobacter baumannii.

Human targets or pathogen-associated antigens will be used for the immunisation of the Kymouse™, which has a human B cell repertoire. No live GM pathogens will be used for immunisations. Once an immune response is observed, tissue samples (spleens, lymph nodes and bone marrow) will be taken and antigen specific B-cells or plasmablasts sorted by flow cytometry. The heavy and light chains of each B-cell receptor will be sequenced and the antibodies synthesised and expressed.

This connected programme will use a number of standard molecular biology/microbiology methods to test the efficacy of these monoclonal antibodies in in vitro systems. In some cases, viruses produced will be derived from defined genotype recombinant sources, such as herpesvirus BACs or reverse-genetics influenza virus and pathogens engineered to express non-toxic marker genes.

Lentivirus vectors containing human or pathogen genes may be used for gene delivery and expression in eukaryotic cells. Genes will be cloned from human and pathogen DNA or cDNA into bacterial vectors that contain bacterial and/or eukaryotic selectable markers and bacterial or eukaryotic expression signals. A 3rd generation lentivirus vector system will be used for this work. To generate lentiviral vectors the gene of interest will be transferred into a modified HIV vector, with viral genes vif, vpu and nef deleted, containing a strong promoter (e.g. CMV or SFFV promoter) driving the expression of a marker, such as GFP, and the single gene of interest from the bacterial plasmids. This will be transfected into eukaryotic cells together with two HIV packaging plasmids (one encoding gag/pol, one encoding rev) and an envelope expressing plasmid (VSV-G) to produce recombinant lentiviruses. These will be used to transduce cell lines and primary cells, producing cells that express the gene of interest and after selection or sorting produce stable cell lines. The resulting stable cell lines will be used to assess antibodies in binding or effector function assays.
The recipient or parental pathogens are all hazard group 2 organisms. The recipient organisms (cell lines or primary human cells) are hazard group 1 or 2. Work with hazard group 3 or 4 pathogens is excluded from this coordinated programme of work.

Identifying and testing monoclonal antibodies against pathogens or pathogen-associated proteins:
Genetic modifications for all pathogens within this body of work will involve insertion of marker genes such as GFP or luciferase or deletion/ modification of pathogen genes to cause attenuation. The foreseeable effects from the production of such recombinant pathogens will be to produce a pathogen that is equivalent to or attenuated relative to wild type. Insertion of oncogenes is excluded from this work.

All pathogens used are ADCP HG2 and will be worked with in CL2 containment facilities.

Pathogens to be worked with include:
HERPESVIRUSES (wild type and recombinant, which may be generated from bacterial artificial chromosomes (BACs));
Herpes Simplex Virus Type 1 (HSV-1)
Human Cytomegalovirus (HCMV)
Epstein-Barr virus (EBV)

These are transmitted primarily as cell-free virus in saliva and through close physical contact. The potential for accidental exposure comes from virus containing medium that is ingested, exposing the oral cavity or via needle stick injuries. With HSV-1 there is also an infection risk to the eye, which may lead to herpes keratitis and ulceration of the cornea. In most people, infection with any of the herpesviruses causes a self-limited primary infection with the potential for the virus to reactivate sporadically.

Herpes Simplex Virus type 1 (HSV-1)
HSV-1 causes cold sores and genital herpes. More than 70% of the population is infected and retains the virus for life in latent form. Herpes infections are most contagious when symptoms are present but can still be transmitted to others in the absence of symptoms. Most infected people do not suffer from recurrent cold sores, but nevertheless occasionally secrete virus in their saliva. Symptoms of herpes include painful blisters or ulcers at the site of infection.

In immunocompromised people, such as those with advanced HIV infection, HSV-1 can have more severe symptoms and more frequent recurrences. Rarely, HSV-1 infection can also lead to more severe complications such as encephalitis or keratitis (eye infection).

Neonatal herpes can occur when an infant is exposed to HSV in the genital tract during delivery. This is a rare condition, occurring in an estimated 10 out of every 100,000 births globally, but can lead to lasting neurologic disability or death. The risk for neonatal herpes is greatest when a mother acquires HSV infection for the first time in late pregnancy.

Human Cytomegalovirus (HCMV)

Human cytomegalovirus (HCMV) causes lifelong infection and has a prevalence of 55-100% within the human population. Primary HCMV infection is generally asymptomatic in healthy hosts but can cause severe and sometimes fatal disease in immunocompromised individuals and neonates. HCMV is the leading infectious cause of congenital abnormalities in the developed world, affecting 1-2.5% of all live births. HCMV intrauterine infection and can cause significant morbidity, including low birth weight, hearing loss, visual impairment, microcephaly, hepatosplenomegaly, and varying degrees of mental retardation. HCMV also causes serious disease in organ transplant recipients and AIDS patients, either after primary infection or reactivation of latent infection. Epstein Barr virus (EBV)

EBV is known to have tropisms for B cells and epithelial cells and in some circumstances, may infect T cells,
EBV can cause infectious mononucleosis (glandular fever). Symptoms include fatigue, fever, inflamed throat, swollen lymph nodes in the neck, enlarged spleen, swollen liver, rash. People who get symptoms from EBV infection, usually teenagers or adults, get better in two to four weeks. However, some people may feel fatigued for several weeks or even months.

After an EBV infection, the virus becomes latent (inactive) within the body. In some cases, the virus may reactivate. This does not always cause symptoms, but people with weakened immune systems are more likely to develop symptoms if EBV reactivates.

There are three major types of B cell malignancy linked to EBV - Burkitt, Hodgkin and diffuse large B cell lymphomas, as well as rarer EBV-associated tumours of B cell origin (PBL- plasmablastic lymphoma and PEL primary effusion lymphoma), which are seen in late stage AIDS patients and heavily immunocompromised individuals. ORTHOMYXOVIRUSES (wild type, produced from reverse genetics systems and recombinant)

Influenza A
Influenza B
Health and Safety
Executive

No influenza viruses classified above hazard group 2 will be used. Some strains of influenza virus that are highly infectious to agriculturally important species (e.g. H5 or H7 'avian' influenza) are controlled under the Specified Animal Pathogens Order 2008, but these will NOT be used in this project. Influenza viruses classified above Hazard group 2 are excluded from this programme of work.

Influenza viruses are airborne pathogens transmitted primarily as foamites. Therefore the primary risk to personnel from GM influenza viruses is exposure to tissue culture medium containing viruses, either as an aerosol or as a large volume exposure to the nose, mouth and eyes (mucous membranes).

Influenza is a contagious respiratory illness caused by influenza viruses that infect the nose, throat, and sometimes the lungs. Influenza A & B can cause the same spectrum of disease, ranging from mild to severe illness, and at times can lead to death. People who have influenza often feel some or all of these signs and symptoms that usually start suddenly, not gradually:
- Fever or feeling feverish/chills, cough, sore throat, runny or stuffy nose, muscle or body aches, headaches, fatigue.
- Some people may have vomiting and diarrhoea, though this is more common in young children than in adults.
- People at high risk from developing serious influenza-related complications include those over age 65, pregnant women, people with chronic medical conditions (such as asthma, diabetes, weakened immune system or heart disease) and young children.

All personnel that work in the laboratories which use influenza will be offered annual influenza vaccinations. Only low pathogenicity laboratory strains of influenza will be used and these may be GM. Different strains of influenza will not be worked with at the same time to avoid the possibility of reassortment between viruses.

PNEUMOVIRUSES
Respiratory syncytial virus

Human respiratory syncytial virus (RSV) causes respiratory tract infections. It spreads easily by direct contact. It is a major cause of lower respiratory tract infections and hospital visits during infancy and childhood. For adults, RSV produces mainly mild symptoms, often indistinguishable from common colds and minor illnesses. For some
children, RSV can cause bronchiolitis, leading to severe respiratory illness requiring hospitalization and, rarely, causing death. Severe disease is most likely to occur in premature infants or the immunocompromised. **GRAM-NEGATIVE BACTERIA**

**Escherichia coli**
Pathogenic strains are classified as hazard group 2. The exceptions are E.coli, verocytotoxigenic strains (eg 0157:H7 or 0103), which are classed as hazard group 3. We will NOT be working with any verocytotoxigenic strains of E. coli. E.coli classified above Hazard group 2 are EXCLUDED from this programme of work.

E. coli bacteria are transmitted primarily by faecal-oral route and normally live in the intestines of people and animals. Most E. coli are harmless and actually are an important part of a healthy human intestinal tract. However, some E. coli are pathogenic, meaning they can cause illness, either diarrhoea or illness outside of the intestinal tract. Extra-intestinal pathogenic E. coli (or ExPEC), is the most common cause of urinary tract infections and Gram-negative bacteraemia. Before the 2000s, ExPEC was mostly susceptible to first-line antibiotics (e.g. cephalosporins and fluoroquinolones) that are often used to treat infections. However, resistance to fluoroquinolones among E. coli is very widespread and they are now ineffective in more than half of patients. Of special concern is that FQ-R is often accompanied by resistance to the cephalosporins.

**Acinetobacter baumannii**
A. baumannii is a Gram-negative bacteria, which is usually considered to be a low-virulent pathogen. It is an opportunistic pathogen in humans, infecting critically ill patients or immunocompromised individuals and can cause serious therapeutic problems in the clinical setting, due to its multidrug resistance to clinically available antimicrobial agents. Acinetobacter causes a variety of diseases, ranging from pneumonia to serious blood or wound infections, and the symptoms vary depending on the disease. Acinetobacter may also "colonize" or live in a patient without causing infection or symptoms, especially in tracheostomy sites or open wounds. Acinetobacter can be spread to susceptible persons by person-to-person contact or contact with contaminated surfaces.

**LENTIVIRAL VECTORS**
Lentiviruses based on Human immunodeficiency virus type 1 and 2 (HIV-1/-2) are classified as Hazard Group 3. However, we will NOT be working with HG3 lentiviruses but modified lentiviral vectors which are suitable for use at Containment Level 2.

The lenti pseudo-type viral particles to be used will be replication-deficient viruses harbouring the genes of interest. There is good evidence that such replication defective viruses are not pathogenic and a minimum of 3 rare recombination events are necessary to produce replication competent, recombinant virus. In addition, such virus vectors are single cycle infection agents not able to form productive infections or to recombine with endogenous retroviruses and become productively infectious.

The viral genes vif, vpu, nef and tat are deleted in this system and for 3’ generation vectors the necessary viral genes are split across four plasmids therefore making each vector attenuated by lack of intact full length genome. Extensive recombination events (a minimum of three) would be needed to produce a replication-competent virus, and there is good evidence that replication-defective viruses are not pathogenic when tested in mice. In addition such virus vectors are single cycle infection agents not able to form productive infections or to recombine with endogenous retroviruses and become productively infectious. If exposure to such viral vectors occurred this would be a very self-limiting infection.

CL2 containment for the molecular biology, bacterial, viral and tissue culture work is sufficient for both the production and use of the GM pathogens mentioned above and for production of lentivirus plasmids and replication defective viral vectors. This level of containment is also sufficient for the transduced cell lines that result from exposure to the viral vector preparations. The primary risk of working with any of the pathogens listed above results from immunodeficiency or infection during pregnancy. Therefore, immunosuppressed workers or pregnant women will be excluded from working with these organisms.
Host/vector system

Plasmids
Cloning plasmids will be pGEM derivatives, pUC series, pBLUESCRIPT series, pcDNA, pCMV/Zeo, and pEGFP-1. We will be using a commercial 3rd-generation system: ViraPower™ Lentiviral expression system (Thermofisher). The components of both these systems are as follows: A pLenti-based expression vector into which the gene of interest will be cloned. The vector also contains the elements required to allow packaging of the expression construct into virions (e.g., 5' and 3' LTRs, 4J packaging signal). The ViraPower™ Packaging Mix contains an optimized mixture of the three packaging plasmids, pLP1, pLP2, and pLPNSVG. These plasmids supply the helper functions as well as structural and replication proteins in trans required to produce the lentivirus. These have been extensively engineered to be self-inactivating and are only capable of a single round of infection.

For construction of GM bacteria expressing GFP/RFP and luciferase, random insertion into the bacterial chromosome with the Tn5 transposon, for example containing kanamycin resistance genes and genes for GFP/RFP and luciferase will be used. Once inserted into the bacterial genome the inserted sequence is nonmobilisable. Bacteria may be transformed with plasmid vectors to allow deletion of specific genes resulting in over production of OMVs. In this example, TolR will be replaced with a kanamycin cassette using the plasmid pAJD433. The plasmid will be removed from the mutant construct resulting in a chromosomally-inserted sequence that is non-mobilisable.

GM viruses will be generated externally by collaborators and will be assessed for effect on activity, and confirmed safe to handle at CL2, prior to transfer to Kymab. They are generated using reverse genetics plasmid systems (e.g. influenza, RSV) or bacterial artificial chromosomes (herpes viruses). Vectors for modification will NOT be handled at Kymab, and residual plasmids become insignificant upon first passage of the virus, which is also performed externally, so there are no risks associated with vectors.

Origin & function

Cell lines/Primary cells
Cell lines, such as (but not limited to) A549, MRC-5, WI-38, Hep-2, RMK, MDCK, 293T, will be obtained from commercial sources (e.g. ATCC) they may require handling at either CL 1 or CL2. Primary human cells (e.g. leukocyte cones) may also be used. These must be handled at CL2. Primary cells from the workers will NOT be used.

Embryonated chicken eggs.
Where high titre influenza virus is required, such that growth in tissue culture would not be practical, embryonated chicken eggs may be used to produce stocks of influenza A or B. The embryonated chicken eggs to be used will be obtained from a commercial source, the eggs being suitable for medical research. The supplier is registered under DEFRA's Poultry Health scheme, which means all disease control programmes are up to the latest UK and EU standards. Prior to infection, embryonated eggs can be handled at CL 1.

Viruses and bacterial strains may be purchased from Public Health England's (PHE) culture collections, for instance the National Collection of Pathogenic Viruses (NCPV) or the National Collection of Type Cultures for bacteria (NCTC). Primary isolates from hospital laboratories and diagnostic service laboratories will be obtained under appropriate agreements and licenses. GM pathogens or those not commercially available may be supplied by collaborating parties, under appropriate licensing agreements.

Evaluation of foreseeable effects

Bacterial hosts
The bacterial host for all plasmid propagations within this programme of work is E.coli K12, which is disabled in key
bacterial functions (i.e. recombination defective) and unable to survive in the environment or pass genetic material onto host bacterial flora following a breach of containment. For the production of lentiviral vectors the lentiviral genome is split over four separate plasmids such that virus production is only initiated when all four plasmids are present in a eukaryotic cell. Therefore there is no risk of lentiviral particles being produced from bacterial culture. Overall the risk of adverse events from the handling of plasmids or bacteria is low.

Lentiviral Gene expression

The main risk is accidental exposure of laboratory workers to recombinant lentiviruses. This risk is reduced significantly by working in microbiological safety cabinets (MSC). As these lentiviral vectors are self-inactivating the risk of onwards transmission to another person is extremely unlikely. The primary risk is therefore alteration of the cellular function of exposed personnel through insertional inactivation/activation of tumour suppressor genes/oncogenes respectively, or the alteration of cellular function by gene overexpression or ablation from the transferred genetic material within the vector. These are extremely unlikely events that would only occur following prolonged infection with high titre retroviral vectors and selection in vivo for cells with altered growth potential. Therefore the overall risks are low.

GM viruses

For all viruses within this body of work genetic modifications will involve insertion of marker genes such as GFP, luciferase or deletion/modification of viral genes to cause attenuation. The foreseeable effects from the production of such recombinant viruses will be to produce a virus that is equivalent to, or attenuated relative to, wild type virus. All viruses used in this programme of work are ADCP hazard group 2 pathogens and both wild type and GM viruses will be handled in CL2 containment facilities. If available, laboratory workers will also receive vaccinations against parental virus strains. Immunocompromised or pregnant workers will not be allowed to work in the laboratory. Overall the risks of adverse events from handling GM viruses within this project are low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Kymab Ltd operates a number of comprehensive and proven waste management measures which are conducted according to our Code of Practice for handling CL2 pathogens:

Liquid Waste:
All cultures/liquid waste must be decontaminated prior to disposal using a final concentration of 1 % Virkon solution for at least 1 hour (concentration validated by manufacturers) before disposal to the drains.
2 % Virkon must be provided in the MSC in a sealable container. Liquid waste is added to this, so minimising aerosol production, to achieve a final 1 % solution. The container must be sealed shut and the external surfaces decontaminated with IPASEPT before removal from the MSC. Inactivating liquid waste must be retained for at least 1 hour before disposal in the sink.
Drains, sinks etc. do not pose a mode of transmission to the environment, as liquid waste has been inactivated.

Solid Waste:
Solid waste (e.g. plates, flasks, tubes) will be sealed (lids tightened or plates taped shut) before being disposed of in yellow biohazard bins. Other solid waste e.g. used gloves, tissue, packaging is also disposed of in yellow bins.

Stripettes:
A final concentration of 1% Virkon must be aspirated up and down in contaminated plastic stripettes prior to soaking in 1% virkon for at least 1 hour. Stripettes are then placed into hermetically sealable yellow bins.

Small plastic ware:
Small plastic ware e.g. tips/eppendors are disposed of in sweetie jars which are retained within the MSC. Once full sweetie jars are sealed and the external surfaces sprayed with IPASEPT before removal from the MSC and disposal in yellow bins.

When yellow bins are full they are hermetically sealed, wiped down with IPASEPT, marked as CL2 waste and removed to the secure collection point where they are collected by the local registered contractor (arranged by Babraham Bioscience Technologies) and sent for incineration off site.

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Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

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Please enter comments on the GM safety committee on the risk assessment

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Relevant Comments from the Minutes of the Meeting of the Biological Safety Committee, 29th March 2018

... assessments coming before the committee for approval must have already undergone consultation within the originators own group and will have been seen by JP (the Biological Safety Officer).

For approval by April 6th:

RAG 12 (formerly RAF 088), RAG 013 and RAG 014

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Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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<th>Name</th>
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<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
<th>Withdrawn</th>
</tr>
</thead>
</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Chair of Genetic Modifications Safety Committee: Biological Safety Officer and Chair of GMSC, a senior manager with an appropriate scientific background. Responsible for ensuring the committee reviews all risk assessments, co-ordinating comments from the committee and communicating with scientists and maintaining the record of GMSC activity. Liaison with HSE, preparation and submission of notifications/applications. Maintenence of statutory records responsible for biological safety and genetic manipulations.

Laboratory supervisor: Responsible for operations, working practice in the laboratory and training, also the primary person responsible for the origin and integrity of recombinant cell lines.

Members: Include a range of individuals with sufficient depth and scope of knowledge in their particular discipline to enable them to understand the risks arising from the normal range of activities undertaken and be able to judge the adequacy of GM risk assessments submitted to the committee. Responsible for highlighting changes in the work to the committee.

The Biological safety Committee will meet monthly and the Genetic Modifications Safty Committee will meet quarterly or ad hoc in case of a request at intervening Biological Safety Committee meetings. The Convergence Biological Safety Committee will interface with Babraham Research Institute BSO to ensure flow of communication and sharing of best practice.

<table>
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<th>Large Scale</th>
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</table>
Solid waste from laboratories carrying out GM work at Class 1 will be placed into a suitably labelled hermetically sealable 60L UN approved Type 3H2/Y30/S/2002. They will be sealed by the producer of the waste and removed to a central collection point where they will be placed into a 210L yellow labelled "Eurobins" for collection by the local registered clinical/GM waste incinerator contractor (Vetspeed) in accordance with Babraham Institute procedures (derogation notification to HSE GM105/04.1).

Liquid waste is inactivated chemically. Where possible buffers not containing interfering substances, such as phenol red, will be decontaminated using "Microsol", according to the manufacturer's instructions on correct concentration and duration to ensure complete decontamination, then diluted to drain. Cell culture supernatants will be decontaminated using "Virkon", according to the manufacturer's instructions on concentration and duration of exposure to ensure complete decontamination, then diluted and disposed to drain. Drainage is processed through the Babraham Institute sewage treatment plant which is closely monitored. Volume to drain <10L per week.

Please enter comments of the GM safety committee on the risk assessment

The committee has considered the risk to human health and to the environment associated with the organisms and consider the proposal to be Activity Class 1

Agreed ACGM containment level 1
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Name

EPISTEM LTD

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

48 GRAFTON STREET

District

Town

MANCHESTER

County

GREATER MANCHESTER

Postcode

M13 9XX

Country

ENGLAND

Tel Number

0161 606 7258

Fax Number

0161 606 7348

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Person responsible for supervision and safety of GM activities at the premises
Epistem management representative
Staff representative
Health and Safety representative
Internal GM advisor

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

All the GMOs will be eukaryotic cell lines that cannot survive outside of an incubator. Disinfectant protocols will be employed using inactivation with 1-3% Virkon disinfectant for 24 hours followed by autoclaving of all contaminated waste prior to disposal as clinical waste. The process to ensure that autoclaving has occurred will be retention of the autoclave read-out and the use of autoclave tape.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Since no bacterial host is being used, the overall risk is very low
GM Centre Number: 3105

Data Premises Notified (Originally) 25/02/2011

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed Transitional Premises Non-GMMs N Withdrawn N

Emergency Plan Required?

Name

THE NATIVE ANTIGEN COMPANY LTD

Name 2

Department

Campus Estate or Research Centre

Building

BUILDING B

District

KIDLINGTON

Road Name

LANGFORD LOCKS

Town

OXFORDSHIRE

County

Postcode

OX5 1LH

Country

ENGLAND

Tel Number 01865 595230 Fax Number

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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<td>Cherwell</td>
<td>Unit 77 Heyford Park, Upper Heyford</td>
<td>Oxford</td>
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<td>OXFORDSHIRE</td>
<td>OX25</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The Native Antigen Company GMO Biological Safety Committee has been established to assess risk assessments and give guidance on the effect of any project on the safety to the members of NAC, the safety to co-workers and other members of staff at the premises, the safety to the general public, the risk of cross contamination or recombination, the risk of release into the community of GMOs and any ethical considerations that may be relevant. Its members are drawn from a variety of backgrounds and bring a diverse range of expertise both within and outside of the company itself, from University lecturers to MDs. The Committee meets on an ad hoc basis whenever new GMO projects are proposed.

<table>
<thead>
<tr>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<th>Bacteriology</th>
<th>Parasitology</th>
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From our Code of Practice:

Any Solid GMM Biological Waste (including flasks, pipettes, tips etc) is to be fully soaked in 1% Virkon for a minimum of 24 hours, after which it should be bagged in the yellow clinical waste bags no more than 3/4 full and closed with a black cable tie. Full clinical waste bags are put in the locked right hand green cabinet for collection by the waste disposal service where they will be incinerated. Liquid GMM Biological Waste is to have sufficient Virkon powder added to it to make it at least 1% overall and left for a minimum of 24 hours to inactivate any viable pathogenic material. Thereafter it is to be disposed of down the sink with plenty of water. Contaminated sharps such as needles used in banding should be soaked in 1% Virkon for 24 hours then disposed of in the sharps bins provided. Full sharps bins are collected by the waste disposal contractors and incinerated off site. The process of disinfecting with Virkon has been demonstrated to completely destroy any infectivity or viability of every micro organism by independent labs and by the manufacturer of the powder.

Please enter comments of the GM safety committee on the risk assessment

The GMO Biological Safety Committee reported that they were happy for this project to go ahead and agree with the classification and proposed control measures. It was noted that a Risk Assessment number should be used to help track Risk Assessments (this has now been added) and the committee wanted to remind the company to keep all manufacturers literature and product information for any vectors, organisms or cells that are being purchased to add to the background safety information. The Committee also noted that every risk assessment should be reviewed on an annual basis to ensure they are still accurate and applicable.
<table>
<thead>
<tr>
<th>Name</th>
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<tbody>
<tr>
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Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

GM safety committee meets at least every six month and comprises:
- Chief Scientific Officer/Chair of H&S committee
- Director of Assay Development and Screening
- Director of Transgenic Platforms and Cell Engineering
- Director of Molecular Biology
- Senior Scientist/Biological Safety Officer

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<tr>
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<td>Tick if confidential</td>
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</table>
Pellets and spent growth medium containing viable E.coli cells will be inactivated with the appropriate disinfectant and then discharged. The disinfection processes are in accordance with the manufacturers instructions and expected to achieve a 99.9999% kill (greater than five log reduction in viability). Glassware will be similarly disinfected before reuse.

Solid media and plasticware (pipettes, tips etc) will be disposed of into suitably labelled hermetically sealable bins. The bins are 60 litre UN approved (to Class 3) Type 3H2/Y30/S/2003. They will be sealed by the producer of the waste and removed to a secure central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Under an approved derogation, collections by the local registered clinical /GM waste incinerator contractor will be made three times a week to prevent a build up. The site has 24/7 Security.

The control measures described above are sufficient for the containment of Class 1 organisms and therefore these activities are approved by the Crescendo Biologics GMSC.
### Project Additional Information

#### Purposes of the contained use
- To produce proteins for use in the discovery and development of antibody-based therapeutics.
- To knock-down or knock-out expression of constitutive proteins in human or animal cells or cell lines.
- To test candidate antibody-based therapeutics using the human and/or animal material.

#### Recipient or parental organism
- E. coli K-12 derivatives such as XL1-BLUE, XL10-GOLD, BL21, AVB101, JM109, Rosetta blue, NovaBlue and TG1 are recognised as non-colonising and disabled, and may be considered equivalent to ACDP hazard group 1. These will be used for the production of vectors to be used in the transfection of eukaryotic cells.
- A variety of human and animal cells and cell lines will be used as recipients for the vectors (e.g., HEK293 cells, CHO cells, Dendritic cells, T cells and T cell lines). Cells may be hazard group 1. However, some cells may carry adventitious agents and will therefore be hazard group 2. Human or simian donors of primary blood cells or lymphoid cells will be assessed and only material from Low Risk donors will be used and material will be handled at containment level 2.

#### Host/vector system
- cDNA coding for human or animal genes will be obtained from tissue or isolated from cells or obtained from synthetic sources.
- K12 E coli host cells will be used for the construction and production of vectors. Vectors including pcDNA, pUC, pET, pCR2.1 and pJET based vectors will be used. These are considered to be non-mobilisable. Gene transfer is thus a remote possibility.
- Eukaryotic cells appropriate for the given experiment will be used. In most cases, the introduced genes will not increase the hazards associated with the parent eukaryotic cell lines. However, some genes, such as those encoding for growth factors, may have the potential to increase the rate of tumour growth or metastatic potential of well characterised continuous cell lines that are usually handled as containment level 1. In these circumstances, the cells will be handled at containment level 2. Similarly, some siRNA targets may also increase the potential hazard of the parent cell line and containment level 2 will be used where appropriate. The eukaryotic host cells are likely to retain stringent growth requirements and remain susceptible to dehydration reducing the risk to humans and the environment.

#### Origin & function
- cDNA coding for human or animal genes will be obtained from tissue or isolated from cells or obtained from synthetic sources.
- The genetic material will be used to produce reagents for use in therapeutic antibody discovery and development.

#### Evaluation of foreseeable effects
- It is unlikely that the GMM would survive and become established in the environment. The inserted (or silenced) genes may alter existing traits of the eukaryotic cell lines. In extreme cases, there may be potential for some tumour cells to escape normal immune surveillance and survive and replicate following accidental inoculation. However, the eukaryotic hosts are likely to retain stringent growth requirements and the use of good microbial practice in a containment level 2 facility will be sufficient to limit contact.

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02/03/2022
with humans and the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Pellets and spent growth medium containing viable E.coli or eukaryotic cells will be inactivated with the appropriate disinfectant and then discharged. The disinfection processes are in accordance with the manufacturers instructions and expected to achieve a 99.9999% kill (greater than five log reduction in viability). Glassware will be similarly disinfected before reuse.

Solid media and plasticware (pipettes, tips etc) will be disposed of into suitably labelled hermetically sealable bins. The bins are 30 litre UN approved (to Class 3) Type 3H2/Y30/S/2003. They will be sealed by the producer of the waste, sprayed with disinfectant and removed to a secure central collection point. Under an approved derogation, collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build up. The site has 24/7 Security.

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The Risk Assessment should be used in conjunction with COSHH forms detailing further information on the particular genetic manipulation. All COSHH procedures relating to the particular experimental process should be used in conjunction with the risk assessment.

Project Containment

<table>
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Project Ref  3106/16.1
Production and in vitro activity testing of 'armed' Enadenotucirev (EnAd) virus for delivery of therapeutic proteins to tumour cells

To genetically modify enadenotucirev (EnAd), an attenuated adenovirus that selectively replicates in tumour cells, to express therapeutic genes (antibody or antibody fragments) that have the potential for treatment of human cancer.

To test activity of the genetically modified virus in non-purified (cell supernatants) or purified form in in vitro assays such as infectivity assays, potency assays, selectivity assays, transgene expression and binding assays using established cell lines or primary immune cells.

Testing of infectivity, potency, selectivity, transgene expression and transgene product binding to target protein will use established well characterised human and murine cell lines with a long history of safe use. These cells will be primarily hazard group 1 however some may carry adventitious agents and will be hazard group 2. Primary peripheral blood cells may be used in some functional experiments to assess activity of expressed transgenes and will be handled under containment level 2. Cells will be cultured in closed culture vessels within an incubator creating the specialised environment required for the propagation of the cells (e.g. an humidified incubator at 37°C with an atmosphere of 5% CO2 and 95% air). Only authorized personnel will conduct the work and it will be take place within a designated laboratory with controlled access via pin entry lock system or card access. Samples will be stored in fridges, incubators or freezers located inside the containment facility.

The host cell range for enadenocutirev virus replication is limited. All animals apart from higher primates do not express the primary receptor for viral entry (CD46). Even when the receptor is present it is insufficient for virus replication to occur due to attenuation of the virus to selectively replicate in human tumour cells (Kuhn et al 2008, PLoS One 3. e2409). Virus replication in infected tumour cells will eventually result in cell lysis. The use of good microbiological practice in a containment level 2 facility will be sufficient to limit contact of any released virus or infected cells with humans and the environment.

Enadenotucirev (EnAd) - an Ad11p/Ad3 chimeric group B attenuated adenovirus that demonstrates selective replication in tumour cells. It has a non-enveloped, icosahedral structure and a linear, double stranded DNA genome replicated by a high fidelity polymerase. The enadenotucirev virus was selected from a library of chimeric adenoviruses for the ability to replicate in colorectal cells at short time points after infection. The virus selected has a minimal genome to permit replication with a wild type
Ad11p backbone, a chimeric E2 region and deletions in E3/4.

Background to safety of Wild type Ad11 virus
In healthy adults symptomatic infections of wild type adenoviruses are rare even though the viruses are ubiquitous in the environment (Schmittzz et al 1983 American Journal of Epidemiology 117, 455-466). Adenovirus infection is usually associated with epithelial surface of the lungs and GI tract, although wild type Ad11 is associated with conjunctivitis and urinary tract infections. Once exposed to a given serotype of adenovirus individuals usually develop lifelong immunity. Immune compromised individuals can be at higher risk. For example adenovirus infections have been reported in transplant patients receiving immunosuppressive drugs and HIV patients (Kojaoghlanian et al 2003, Reviews in Medical Virology 13, 155-171) resulting in viraemia and on rare occasions death if untreated. In such patients withdrawal of immunosuppressive drugs can result in recovery indicating that the wild type virus can be effectively controlled by the human immune system. A specific risk of working with adenoviruses is the the possibility of conjunctivitis with splashes of high titre virus into the eye and the resulting local inflammatory response. Eye protection will be worn and aerosol production minimised when working with high virus titres in the laboratory. Adenoviruses have an ADCP classification of 2.

Safety of enadenotucirev virus
In comparison to the wild type Ad11 enadenotucirev is attenuated in normal cells through loss of genomic sequences and thus presents a reduced risk to human health. It has no gain of function genetic insertions that may increase virulence or pathology from the wild type Ad11. It is also further vulnerable to the immune system due to the absence of the E3 genes which are responsible for hiding virus infected cells from destruction by CTL and NK cells. Harm is unlikely to be caused by accidental release of the virus. The receptor required for virus entry into a cell, CD46, is only present in higher primates and even when the receptor is present virus replication is unlikely to occur due to the selectivity of the virus for malignant cells. Enadenotucirev is sensitive to the antiviral agent cidofovir (Bauzon et al. 2003, Molecular Therapy 7, 526-534). The risk from release of the virus into the environment is minimal due to the limited host cell range for virus replication. Furthermore clinical experience has now been gained in over 50 patients receiving repeated, high doses of EnAd intravenously over periods of up to 12 weeks. At high doses (above 1e12 virus particles) intravenous EnAd causes a transient inflammatory response due to the number of particles interacting with macrophages that resolves over 1-3 days and is consistent with previous clinical trials with other viruses and vaccines. There have been no signs or symptoms of EnAd causing any active infection in any of the subjects. Overall, the clinical experience following multiple systemic injections (at levels more than 1000-fold in excess of natural or accidental exposure) suggests that attenuated EnAd has limited potential of causing human disease.

The enadenotucirev virus will be genetically modified to express transgenes that encode antibodies or antibody fragments specific for tumour associated proteins, proteins that modulate the immune system and for proteins that are not expressed in humans (controls). All cDNA's encoding the transgenes will be generated by DNA synthesis and inserted into the transgene cassettes using PCR and standard cloning vectors.

Transgene expression will be under the control of an inserted mammalian promoter such as CMV or under the control of the endogenous enadenotucirev major late promoter (MLP). Protein expression may also be controlled by other regulator elements including internal ribosome entry sequences (IRES) or P2A peptides. Exogenous promoters such as CMV could potentially drive expression in any mammalian cell types however transgene expression is restricted by the cell types permissive to enadenotucirev virus infection. Expression of the transgene should not alter the enadenotucirev virus host cell range (i.e. cell types it can infect) or potentiate the virus activity.

The genetic material will be used to produce reagents for use in therapeutic antibody discovery and development. Therapeutic biological agents such as antibodies are typically dosed at level of 1-10mg (or higher)/kg of body weight to be efficacious and have higher relatively good safety profiles due to target specificity. Typical culture in the laboratory setting produces antibody/antibody fragments in the ng/ml range from the modified enadenotucirev virus infected cells and thus the quantities produced are unlikely to be of significant risk to human health.

Expression of antibody/antibody fragments targeting tumour specific antigens can be considered relatively harmless to humans. Tumour associated antigens are a class of proteins that are expressed on many tumour types and are sufficiently different from normal cell proteins through their high expression levels on the tumour cells or different modification (e.g. glycosylation).
Antibody/antibody fragments targeting immune modulating proteins may have potential deleterious activities in humans if their expression is not tightly regulated. The use of the tumour specific promoter or virus specific MLP will restrict their expression to cells susceptible to virus infection (human tumour cells) and allow expression transgene expression late in the virus life cycle when the virus is ready to leave the cell. If the inserted product is considered to be less hazardous following tightly controlled expression a less selective promoter may be subsequently used to enhance gene expression and efficacy. Examples of antibodies targeting immune modulating proteins include the clinically approved CTLA4 antagonist Ipilimumab (Starz 2012 Expert Opin Biol Ther), PD1 and PDL1 antagonists (Topalian et al 2012, N Engl J Med., Brahmenr et al 2012, New Englan Journal Med.). Systemic administration of such antibodies can have off target effects thus restriction of the expression of similar agents to the tumour environment through infection with using genetically modified enadenotucirev virus may reduce these effects.

Evaluation of foreseeable effects

Addition of the transgene into the enadenotucirev virus is not expected to impact viral infectivity or the types of cells that enadenotucirev virus can infect or replicate. It is therefore it is unlikely that the GMO would survive and become established in the environment as the enadenotucirev virus is only permissive in restricted cell types i.e. tumour cells. and is highly species specific (higher primates, no reporter replication in rodent cell lines or mouse/rat primary cells). Cells infected with the virus will lyse following the viral replication cycle. The potential harm to human health due to accidental inoculation of the modified virus is minimal due to the cell type specific replication which limits the expression of the transgene to low levels unless in the tumour microenvironment.

In healthy adults symptomatic infections of wild type adenoviruses are rare however for laboratory work the GMO will be handled under containment level 2 to minimise risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be treated as category 2 waste

All areas will be decontaminated after used using 2% Virkon for a 5 minute contact time (6 log kill McCormick et al 2004, AntiViral Research 64 (1): 27-33 or 10% bleach (>4 log kill Rutala et al. 2006 Antimicrob Agents Chemother. 50 (4) 1419-1424.

Liquid waste will be disinfected with 2% virkon for at least 1 hour then discharged to drains.

Solid waste (pipettes, tips, culture flasks etc) will be treated with 2% virkon then disposed of into suitably labelled hermetically sealable bins. The bins are 30 litre UN approved (to Class 3) Type 3H2/Y30/S/2003. They will be sealed by the producer of the waste, sprayed with disinfectant, labelled with a Category 2 hazard sticker and removed to a secure central collection point for incineration under an approved derogation.

All waste disposed of according to the Babraham Research Campus -Hazard Group 2 Waste disposal S.O.P (waste sealed in 30L Euro bins and sent offsite for incineration by Vetspeed (GM authorisation GM898, notification GM105/4.1).

Sharps will be disposed on into sharps bind and sent for incineration as described above for solid waste, however the use of sharps is unlikely to be required.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y
The Risk Assessment should be used in conjunction with COSSH forms detailing further information on the particular genetic manipulation. All COSSH procedures relating to the particular experimental process should be used in conjunction with the risk assessment.

Please enter comments on the GM safety committee on the risk assessment

The Risk Assessment should be used in conjunction with COSSH forms detailing further information on the particular genetic manipulation. All COSSH procedures relating to the particular experimental process should be used in conjunction with the risk assessment.

**Project Containment**

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**Name**

MIDAS MEDISCIENCE LIMITED

**Name 2**

Department

**Campus Estate or Research Centre**

Building

**Road Name**

BUILDING 110 ABBOTT DRIVE

**District**

KENT SCIENCE PARK

**Town**

SITTINGBOURNE

**County**

KENT

**Postcode**

ME9 8NP

**Country**

ENGLAND

**Tel Number**

020 8133 5177

**Fax Number**

01795 500299

**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities Y

Give brief details of the genetic modification safety committee

Consists of CEO, Group Leader and Biological Safety Officer
Meeting every 6 months, or more frequently if new technology/procedures are to be introduced, or an accident occurs

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02/03/2022
Waste disposal will be carried out according to an SOP. All solid waste is to be autoclaved before being placed in plastic theatre bins and taken to incineration by a licensed outside contractor. All liquid waste is disinfected using a Virkon solution and following the manufacturers instructions for maximum kill. Non-disposable labware is disinfected prior to re-use. All spills to be cleaned using proprietary reagents e.g. spill kits. No further monitoring is deemed necessary due to the low/zero hazard presented by the cells involved.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste disposal will be carried out according to an SOP. All solid waste is to be autoclaved before being placed in plastic theatre bins and taken to incineration by a licensed outside contractor. All liquid waste is disinfected using a Virkon solution and following the manufacturers instructions for maximum kill. Non-disposable labware is disinfected prior to re-use. All spills to be cleaned using proprietary reagents e.g. spill kits. No further monitoring is deemed necessary due to the low/zero hazard presented by the cells involved.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

(The committee asked how the hazardous waste would be disposed of, and wether double-bagging of waste is necessary. The committee asked how the regulations controlling GMOs would affect other laboratory activities. The committee asked whether the first use notification was transferable to new premises , if required.)
**GM Centre Number: 3109**

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**Name**

BBI SOLUTIONS OEM LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

BROAD OAK ROAD

**District**

**Town**

SITTINGBOURNE

**County**

KENT

**Postcode**

ME9 8AQ

**Country**

ENGLAND

**Tel Number**

01795 423077

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01622 331296

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**Comments**

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

A GMO safety committee has been established on site and has been made up of three individuals from varying disciplines (QA, R&D and Technical/Production). This committee meets up periodically to review all GMO activities within the company.

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Yes

All GMO material is documented on arrival onto the site and stored in dedicated areas. Culture is handled and disposed of under the guidance of SOPs and the company's ISO9001 Quality system. Before disposal, all culture is inactivated using disinfectants and, or autoclaving. Dedicated equipment is used for the culturing of microbes and processing of culture. Equipment is cleaned following strict SOPs. No live culture is transported from this site.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMO committee believes that all GMO activities at SCIPAC fall under containment level 1 (Class 1). We do not envisage exceeding this class in any future work, but will continue to assess projects on a case by case basis before they commence.
**GM Centre Number: 3110**

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**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  
Y

Give brief details of the genetic modification safety committee

As our company is currently small (2 members of staff). The GMSC will consist of these 2 individuals both highly experienced in working within GMO legislation and with risk assessment for GMSC. The make up of the GMSC thus contains both management and staff and because of the size of the company is fully representative of the organisation. The GMSC meets at the start of each new project (or monthly) to examine the adequacy of risk assessment and to test conclusions of those assessments through discussion. Even though those creating GMSC forms are the members of the GMSC, the GMSC is not taken as a rubber stamp body but is an essential discipline within the GMO approval process. The GMSC has created a risk assessment proformer as follows:-

The risk assessment process is divided into 4 sections. Section one gathers information about the GMM and assigns a provisional level of containment for the protection of human health. Section 2 considers the work activity and the suitability of control measures identified in section one. Section three considers environmental risks and section 4 determines the Classification and Containment Level.

A completed form is provided as required.

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Tick if confidential  

For activities involving GMMs, describe the waste management measures which will apply to the activity

All reagents, plasticware and glassware which have come into contact with GMMs or indeed any intermediate in the creation of GMM, will be inactivated by either, incineration, autoclaving or chemical sterilisation. Incineration will be undertaken for all disposable plasticware (pipette tips, plastic pipettes, loops, spreaders, petri dishes etc).

Autoclaving will be used to sterilise most glassware which is to be reused (on an autoclave "kill" cycle") used in the creation of or use of GMMs. Liquid and solid wastes (bacterial supernatants, pellets and waste cultures etc) will also be inactivated by autoclaving. Chemical disinfection will be used for glassware for which this is more convenient (e.g glass pipettes) and for reusable plasticware that cannot be sterilised (e.g. some centrifuge tubes). The disinfectant to be used will be Virkon, used according to the manufacturers instructions for the disinfection of bacteria (contact time and concentration).

Monitoring of waste steam in ativation will be performed periodically. In the case of autoclaves, autoclave kill cycle efficiency will be determined by validation and calibration annually by thermometric monitoring equipment, by a competent trained individual and the certification displayed.

In the case of chemical disinfection (with Virkon) - efficiency of killing and log fold reduction of the agent is widely available from the manufacturer and the literature. Except where it is necessary to depart from the manufacturers conditions no in house testing of Virkon efficiency will be performed.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

We believe that the constructs proposed in the submission clearly fall within the CL1 assessment.

Specifically the plasmids are well established to be non mobilisable, the host E. coli strains are widely used and known to provide no human or environmental harm or impact. The inserts (which have been previously expressed in the literature) are bacterial genes with no direct human equivalent and we can forsee no direct (or indirect) impact of their expression on either human health or of environmental impact. The disposal methods outlined are more than adequate.

We agree with the conclusion of the assessment that the risk to human health and the environment is effectively zero.
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**Name**

**UK RESEARCH & INNOVATION (UKRI) MRC HARWELL INSTITUTE**

**Campus Estate or Research Centre**

2ND FLOOR DAVID PHILLIPS BUILDING

**Road Name**

NORTH STAR AVENUE

**Building**

POLARIS HOUSE

**District**

**Town**

SWINDON

**County**

WILTSHIRE

**Postcode**

SN2 1FL

**Country**

ENGLAND

**Tel Number**

01793 416200

**Fax Number**

**E-mail**

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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Project Ref 3111/11.1

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<td>Development of a novel Otitis Media model for translational research using non-typeable Haemophilus influenzae as a bacterial challenge</td>
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Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

The purpose of this work is to further the genetics of Otitis Media (OM) by development of a well characterized intranasal challenge model with defined strains of non-typable Haemophilus influenzae (NTHi) at known CFU doses. This will allow a thorough understanding of the dynamics of nasal carriage, ascending Eustachian tube infection and colonization of the middle ear using in vivo imaging, conventional bacteriology and host inflammatory and immune response. We want to explore the effects of
vaccination as a prophylactic measure, treat the infection using antibiotics and to explore the role of genes in OM pathways using conditional knock out models

Recipient or parental organism

Host/vector system

The vector system is non-typeable (non-capsular) Haemophilus influenzae (NTHi) and genetically modified versions of these strains bearing mutations that reduce virulence

Origin & function

The Haemophilus influenzae strains originate from a collaborators laboratory and/or are available commercially. The intended function of these organisms is to provide a bacterial challenge to an Otitis media model and to develop a full understanding of the dynamics of infection via the nasal passages to the middle ear.

Evaluation of foreseeable effects

NTHi is a highly adapted commensal whose only niche is the upper respiratory tract of its human host. Ordinarily there is passive carriage (in 60-70%) of normal individuals.
NTHi only invades the middle ear secondarily to upper respiratory tract infection with a virus (e.g. common cold or influenza). It does not survive in the environment outside its human host. Transmission between hosts is via aerosol droplets.
NTHi is not a natural pathogen of the mouse and inbred mouse strains develop only transient self-resolving otitis media which lasts 7-10 days

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid culture waste will be rendered non viable by overnight decontamination with Virkon at 3% working volume before disposal to drain.
Tips and loops used during culture procedures will be soaked in 3% Virkon for 24 hours prior to autoclave sterilisation.
All solid culture waste, including sharps in sealed containers, will be collected in Sterilin autoclave bags within lidded metal bins. Solid waste materils (e.g. gloves, plasticware etc) are rendered inactive by autoclaving (100% kill) in a route and goes for high temperature incineraton.
The autoclave is performance validated annually by the manufacturer (Gettinge). Records are kept on site.
Virkon is routinely used as per the manufacturers recommendations:-
Solid surfaces are disinfected with 1% Virkon solution.
Plasticware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
A query was made about the likelihood of worker infection with the Haemophilus influenzae. A discussion about needle stick injury and aerosol eye injury followed and the committee were reassured by the safety measures put in place via the risk assessment and the availability of broad-spectrum antibiotic treatment if required. A question was raised about increased risk to vulnerable staff (such as pregnant women) and information was given detailing that no additional risk was identified for pregnancy and that work will be carried out in an access controlled laboratory with access granted to only those working on the project.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tr>
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<td>L3 L4</td>
<td>L2 L3 L4</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L2 Yes</td>
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Project Ref 3111/12.1

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<tr>
<td>20/04/2012</td>
<td>Use of viral vectors to alter the expression of a variety of genes linked to human disease both in vitro in cell culture and in vivo in mouse models</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<td>N</td>
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Withdrawn Y Tick if notifying a connected programme of work Y

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The purpose of this work is to determine the specific role of a variety of genes thought or known to be involved in the pathogenesis of a number of different human diseases.
conditions including but not limited to neurological disorders, developmental disorders, deafness and diabetes.

Recipient or parental organism

primary and established cell cultures derived from mutant and wild type mice and cell lines derived from other mammalian species including but not limited to rat, monkey and human.

Tissue slices ex vivo and maintained in culture

other recipient organisms detailed in section 17

Host/vector system

Lentivirus: established 3rd generation lentiviral vectors originally derived from HIV, defective for replication and contransfected with 3rd generation core packaging plasmids into 293T cells to produce virus containing the gene sequence of interest.

Adenovirus: commercially available 3rd generation replication deficient adenoviral vectors

Retrovirus: commercially available 3rd generation replication deficient retroviral vectors including but not limited to pBABE-puro derived from MoMTV

Origin & function

commercially available SiRNA
commercially available transgenes
cDNA

Evaluation of foreseeable effects

effects on cell biology due to knock-down, altered expression or over-expression of the genes of interest in cell cultures. See section 17 for foreseeable effects on other recipient organisms.

The foreseeable effects on human health are minimal as the vectors being used are 3rd generation and are replication deficient, therefore unable to replicate in the event of an accidental exposure by a laboratory worker. Any inoculation of a laboratory worker with these virus vectors will have a localised effect only and all projects are designed to minimise or eliminate the use of sharps to prevent accidental inoculation.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Detailed in section 17

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid culture waste will be rendered non viable by overnight decontamination with Virkon at 3% working volume before disposal to drain.
Tips and loops used during culture procedures will be soaked in 3% Virkon for 24 hours prior to autoclave sterilisation.
All solid culture waste, including sharps in sealed containers, will be collected in Sterilin autoclave bags within lidded metal bins. Solid waste materials (e.g gloves, plasticware etc) are rendered inactive by autoclaving (100%kill) in a validated machine located within the same building. The solid waste then enters the yellow bag clinical waste route and goes for high temperature incineration.
The autoclave is performance validated annually by the manufacturer (Getinge or BMM Weston). Records are kept on site.
Virkon is routinely used as per the manufacturers recommendations :-
Solid surfaces are disinfected with 1% Virkon solution.

02/03/2022
Plasticware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

The committee had no comment to pass forward on this assessment and are happy for the work to go ahead as planned following receipt of notification acceptance.

Please enter comments on the GM safety committee on the risk assessment

The committee had no comment to pass forward on this assessment and are happy for the work to go ahead as planned following receipt of notification acceptance.

Project Containment

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Animal Units

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**Name**

CENSO BIOTECHNOLOGIES GROUP LTD/ROSLIN CELL SCIENCES

**Name 2**

**Department**

**Campus Estate or Research Centre**

ROSLIN BIOCENTRE

**Building**

WALLACE BUILDING

**Road Name**

**District**

**Town**

ROSLIN

**County**

**Postcode**

EH25 9PP

**Country**

SCOTLAND

**Tel Number**

0131 200 6407

**Fax Number**

0131 200 6401

**E-mail**

**HSE Division**

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**Comments**

Name change from Roslin Cellab 30/03/2017

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

<table>
<thead>
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Give brief details of the genetic modification safety committee

Senior Project Manager & Biological Safety Officer
Business Development Manager
R&D Scientist

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<tr>
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Tick if confidential

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Solids (e.g. plastic ware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or disinfect with freshly prepared Precept (2x 2.5g tablets per 1L water) for 1hr (minimum), discharge any excess liquids to drains, dispose of solids via clinical waste stream for microwave treatment or via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), or disinfect with freshly prepared Precept (2x 2.5g tablets per 1L water) for 1hr (minimum), discharge any excess liquids to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes) dispose via clinical waste stream for microwave treatment.

Degree of kill

Autoclaving - effectively 100% kill (annual validation using 12 point thermocouple of worst case loads)

Disinfection - a 1% solution of Virkon disinfectant with a contact time of at least 10 minutes gives a >4.25 log reduction of HIV-1 (data supplied by manufacturer).

Heat treatment or incineration - not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Please enter comments of the GM safety committee on the risk assessment

Topics discussed:
2. Potential hazards associated with transgene cassettes: None.
3. Waste disposal procedures: As outlined in section 6 of this form.
4. Discussed proposal to establish a book to record the receipt, use and disposal of lentivirus samples. Will adopt.
5. On day of viral transduction, will ensure that no one other than the operator is present in the tissue culture lab.
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Name

ARECOR LTD

Name 2

Department

Campus Estate or Research Centre

Road Name

2 CAMBRIDGE SCIENCE PARK

District

2 CAMBRIDGE SCIENCE PARK

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB4 0FE

Country

ENGLAND

Tel Number

01223426060

Fax Number

01223423111

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>Virology</td>
<td>Department</td>
<td>2 Cambridge Science Park</td>
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<td>CAMBRIDGE</td>
<td>CB4 0FE</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: [Y]

Give brief details of the genetic modification safety committee:

The committee consists of QA Manager, Project Manager- Virology and QC Lab technician. The committee have monthly meetings to review risk assessments, COShh assessments and training records. All staff are trained prior to performing procedures, SOPs are in places for procedures. The committee also review any incoming material for client projects and ensure that adequate information is gathered. This information includes MSDS, COA’s, safety data on the product. No material is allowed on the Arecor Ltd site without the correct paperwork.

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</table>
| Other (please specify) |                 |             |             |             | Tick if confidential [ ]
| Bacteriology        | Parasitology    | Transgenic | Microbiology | Research    |
| Virology            | Yes             | Birds       |Transgenic  | Gene Therapy |
|                     | Transgenic      | Fish        | Birds       |             |
|                     | Animals         |             | Transgenic  |             |

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

No genetic modification will be undertaken at Arecor Ltd. We will be stabilising attenuated human and animal vaccines. All work will carried out in a class II safety cabinet in a class II lab. Only in vitro work will be performed with these vaccines. The lab is segregated and only trained staff will be working in there. All solid waste will be autoclaved at 130C for 30 minutes prior to disposal. All liquid waste will be auto claved at 130C for 60 minutes prior to disposal down the drain. The autoclave is regulary serviced. If an autoclave run does not complete properly the screen will say failed at the end of the run. In the event of this happening the run will be started again. Autoclaving effectively has a 100% kill of GMMs. Surfaces will be cleaned with 1% Virkon if necessary.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Genetic modification committee are satisfied by the preventative and precautionary control measures that have been put in place to work with these attenuated vaccines. At the monthly meetings risk assessments, safety date and coshh assessments will be reviewed.
**Project Additional Information**

**Purposes of the contained use**

To stabilise attenuated human and animal vaccines. The vaccines are class I and II organisms and will be used in a class II safety cabinet in a class II laboratory. All personnel will be trained and wear appropriate PPE.

**Recipient or parental organism**

Attenuated vaccines are a weakened form of the parent virus. They have all been modified to be less pathogenic than the parent virus because they will be used to vaccinate humans or animals. There are no foreseeable detrimental effects to human health or to the environment because these are vaccines and there are procedures in place, such as working in a class II BSC in a class II lab and effective kill waste disposal methods.

**Host/vector system**

No genetic modification will be performed at Arecor Ltd. All testing will solely be performed in vitro. There is no extended host range. The risk of working with these vaccines is negligible because all work will be carried out by trained personnel in a class II laboratory.

**Origin & function**

The genetically modified organisms will be supplied by clients. The GMM's are attenuated vaccines that will be used in humans and animals to vaccinate them against diseases. At Arecor we are formulating solutions to stabilise the vaccines for greater world-wide distribution. All work will be performed in vitro in a contained class II environment. Prior to importation from outside the EU (if applicable) Defra pathogens will be notified and an import license will be obtained. The origin is the parent virus that causes the disease that the vaccines is being developed against and the function of the genetically modified organism is to vaccinate individuals or animals against diseases.

**Evaluation of foreseeable effects**

The foreseeable effects would not be more severe than infection with the wild-type virus and most likely less so because all GMM's being used at Arecor Ltd have been specifically designed for vaccination purposes. There is no foreseeable effect to the environment because after reconstitution the vaccines shelf-life is approximately 30 minutes at room temperature. If any of these vaccines escaped into the environment they would not be able to survive or disseminate. The likelihood that the GMM will be released into the environment under the requirements of the containment level to protect human health is negligible. The vaccines will only be handled in class II laboratory conditions by trained personnel, there are effective waste disposal procedures in place to ensure a 100% kill of the GMM's. PPE will be worn, there are SOPs in place and risk assessments in place. The lab is limited to authorised personnel only and all manipulations of the vaccines will be carried out in a class II biological safety cabinet.

Arecor believe there is minimal risk to humans, animals and the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All liquid waste will be treated by autoclaving at 130C for 60 minutes, before disposal to drains. Disposable solid waste which is or may be contaminated with GMMs is also...
inactivated by autoclaving at 130°C for 30 minutes before removal as clinical waste by specialist contactors. The exceptions to the above are:
"Sharps", including scalpel blades, needles (with or without attached syringes), and disposable plastic tips for micropipettors, may be decontaminated by drawing up Virkon disinfectant, and SOAKING IN Virkon for a minimum of 15 minutes. Disposable plastic pipettes may also be decontaminated by immersion in Virkon as above.

Expected degree of kill:

Autoclaving achieves effectively 100% kill of all GMMs.

The manufacturer's information indicates efficacy of Virkon against 65 strains of virus over 19 viral families including class III and IV organisms, such as Newcastle disease and foot and mouth. Virkon is recognised by industry and government worldwide as a disinfectant of choice for livestock disease prevention and control. The mode of action of virkon is to achieve deactivation and/or destruction of target organism through general oxidative disruption of key structures and compounds vital to normal activity (e.g. proteins and lipids). It has been demonstrated that 15 minutes exposure to 1% Virkon> 4-6 log10 kill of seed stocks

Please enter comments on the GM safety committee on the risk assessment

Genetic modification committee are satisfied by the preventative and precautionary control measures that have been put in place to work with these attenuated vaccines. At the monthly meetings risk assessments, safety data and coshh assessments will be reviewed.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
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Animal Units

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Name

LONDON METROPOLITAN UNIVERSITY

Name 2

Department

Campus Estate or Research Centre

FACULTY OF LIFE SCIENCES

Building

TOWER BUILDING

Road Name

166-220 HOLLOWAY ROAD

District


Town

LONDON

County

GREATER LONDON

Postcode

N7 8DB

Country

ENGLAND

Tel Number

020 7133 4545

Fax Number

020 7133 4149

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>N7 7DD</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

| Yes

Give brief details of the genetic modification safety committee

- Chair (Faculty academic staff member - GMM user)
- Faculty Biological Safety Officer
- University Health & Safety Office representative
- Faculty management representative
- Faculty academic staff member - GMM user
- Faculty technical staff representative/Faculty Safety Officer
- Faculty research student representative - GMM user

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<th>Laboratory</th>
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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste to be autoclaved in dedicated GMM autoclave and disposed via drains.

Solid waste (larger plasticware, agar plates) to be autoclaved in dedicated GMM autoclave and disposed via private contractor who handles clinical waste (Dynamiq Cleaning Ltd., Chester House, 1-3 Brixton Road, London SW9 6DE).

Smaller solid waste (pipette tips, microfuge tubes, other small disposable tubes) collected in dedicated disposal bottles, autoclaved, disposal by private contractor (Dynamiq Cleaning Ltd.).

All waste is processed (autoclaved and deposited in dedicated bins for collection) in a dedicated disposal room connected to the laboratories via corridor or service lift (i.e. lift connecting laboratory preparation room with teaching laboratory used solely for transport of materials and associated laboratory personnel between the two rooms; it is separate from public access lifts or staircases).

GMM autoclave to operate at a cycle of 136 degrees C for 10 minutes, achieving a 100% kill for class 1 GMMs, and is serviced annually by Priorclave.

Other(s) Molecular cell biology in cultured eukaryotic cells

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Faculty Management Representative: "To my eyes these are very well-considered documents. Just one small point- with double containment would it be expected that the containers are normally unbreakable or does that not need to be the case or not need to be stated ."

University H & S representative: "It looks fine to me. Does the word 'safe' fulfill the requirement to explain the 'extent to which it is disabled' in Section 2? In Section 5 should 'the most hazardous GM' read the most hazardous GMM'? Should there be a copy of Part 3?"

These comments have been used to modify the form
**GM Centre Number: 3115**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Essen BioScience Ltd genetic modification safety committee will be chaired by the UK lab manager. Meetings will be held quarterly (or upon notification of new GMO activities) and will consist of the Director of Essen BioScience Europe, alongside relevant knowledgable individuals from within the UK organisation or when required the safety officer from the US parent company (Essen BioScience Inc)

- **Level 1 (GMMs)**
  - Yes
- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- **Non-microbial**
- **Other (please specify)**
  - Tick if confidential

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
Within the cell culture suite there are three routes for waste disposal: yellow biohazard bags, yellow burn bins and sharp bins.

Yellow Biohazard bags are for disposal of lightly contaminated & non-puncturing waste e.g. plastic wrappers, gloves, waste paper, wipes, tissues.

Yellow burn bins are for disposal of all other (including biologically contaminated plastic) waste - pipette tips, stripettes, flasks, etc.

Sharps bins are for disposal of biologically contaminated needles, glass and other sharp objects.

Solid waste (biologically contaminated plasticware) will be either autoclaved (121°C for 30 mins, using indicator tape to verify sterilisation) or chemically inactivated with disinfectant (troclosene sodium or bleach) prior to placing in yellow burn bins.

Once full yellow biohazard bags are closed with a cable tie and sharp and burn bins sealed with locking lids. These are sent for incineration on a monthly schedule.

Biologically contaminated liquid waste is collected and appropriately decontaminated with disinfectant (Presept tablets; troclosene sodium), incubated for 24hrs and discarded down the sink.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The use of well-characterised mammalian cells, such as Chinese hamster ovary (CHO) and human embryonic kidney (HEK293) cells for recombinant expression of human proteins is appropriate under hazard group 1 conditions.

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<td>Predominantly the work will involve the use of mammalian cell lines recombinantly expressing protein</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Project Ref 3115/12.1

Date Ackn’d 07/03/2012
CU2 Project Title Expression of fluorescent probes, such as green fluorescent protein (GFP), in

Class 2
Culture Vol Class 2 ≤ 1 Litre
Culture Volume Class 3-4

02/03/2022 Page 12991 of 15326
The labelling of mammalian cells as a method to selectively track biological processes such as proliferation, apoptosis and migration for identifying/understanding modulators of these complex pathways. To this end lentiviral gene transfer systems to express fluorescent probes operating in the green and red wavelengths have been developed. Generation of the lentiviral vectors and the production of virus will not take place in the Essen BioScience Ltd lab, but the use of the viral particles forms the basis of this notification.

Well characterised mammalian cells (e.g. HEK293, HT1080 and HUVEC cells) will be used as the recipient for the fluorescent probes delivered using the lentivirus gene transfer system. These cell lines will be obtained from reputable sources, such as ATCC/ECACC, with appropriate certification regarding the absence of adventitious agents. In all cases the biological safety level of the host cell lines will be 1.

Lentivirus system based on the pLentiLox lentiviral vector map; incorporating either the TagGFP2 or mKate 2 fluorescent markers, with or without the nuclear localisation signal (3xNLS), an internal ribosome entry site (IRES) and either a puromycin or bleomycin (zeocin) selection marker. The human elongation factor-1 alpha (EF1a) promoter is used to drive the expression of the gene(s). Vector maps are included as part of the risk assessment document.

The vectors used are supplied by the University of Michigan and encode the fluorescent probes GFP (green fluorescent protein) or mKate (far-red fluorescent protein). Viral particle production occurs at Essen BioScience Inc. (A A, Michigan) with transfection of HEK293 cells with the vector, alongside the 3 packaging plasmids. The supernatant is harvested containing replication deficient lentivirus.

Both GFP and mKate2 have been widely used as fluorescent probes and are well characterised and are not known to possess any oncogenic potential.

The constructs are third generation lentiviral SIN (self-inactivating) vectors. The vector is packaged using third generation packaging plasmids (3 packaging plasmids). The design of all four vectors used in viral production incorporate safety measures against the generation of replication competent virus. The vectors’ LTRs are self-inactivating.
(SIN), thus restricting mRNA production from integrating vectors to the internal promoter, severely reducing full-length vector transcripts. The lentiviral vector is pseudotyped with the VSV-G envelope serotype providing a greater margin of personal and public safety and reducing the risk of recombination. According to the literature, viruses packaged under these conditions were found to contain no detectable level of replication competent virus.

The vector uses a WPRE element. The post-transcriptional regulatory element (PRE) of the woodchuck hepatitis virus (WHV) improves 3’ RNA processing and thus increases transgene expression efficiency. However, some versions of this element are capable of expressing part of the x protein from WHV which may have oncogenic properties. The WPRE used in the plasmids used in the current study have been modified, mutating the x protein promoter(1488-1492, GCTGA.ATCAT), thus greatly reducing the expression of X protein( see Schambach et al, 2006, Gene Therapy 13, 641-45). Therefore the risk of oncogenic properties has been decreased.

Neither GFP (green fluorescent protein) nor mKate 2 (far-red fluorescent protein) possess oncogenic properties, thus presenting a low risk to human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Within the cell culture suite there are four routes for waste disposal: autoclave bags, yellow biohazard bags, yellow burn bins and sharps bins.

Autoclave bags are for the disposal of biologically contaminated solid waste and are autoclaved at 131C for > 30 mins, using indicator tape to verify sterilisation. All solid waste potentially class 2 contaminated will be autoclaved. The autoclave is situated within the building where all work will be conducted.

Yellow Biohazard bags are for disposal of lightly contaminated & non puncturing waste e.g. plastic wrappers, gloves, waste paper, wiped, tissues.

Yellow burn bins are for disposal of lightly contaminated waste which may pose a risk or puncturing the biohazard bags e.g. stripettes.

Sharps bins are for disposal of biologically contaminated needles, glass and other sharp objects (the use of sharps whilst undertaking class 2 work will not be conducted).

Autoclaved waste is placed in yellow biohazard bags, sealed with cable ties and sent for incineration under code 19 01 03.

Once full, yellow biohazard bags will be closed with cable ties and sharps and burn bins sealed with locking lids. These are sent for incineration on a monthly schedule. Storage of waste bags/bins prior to disposal is in locked 770 litre container located within the BioPark security perimeter.

Biologically contaminated liquid waste is collected and appropriately decontaminated with disinfectant (Presept tablets; troclosene soium, 2,500ppm) incubated for 24 hrs and discarded down the sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The safety committee approved the risk assessment. It was confirmed that once washed sufficiently, infected mammalian cells could be used at the risk level of the cells prior to infection. The implementation of appropriate training was stated as essential prior to any personnel working with lentiviral reagents. Finally the use of sharps during any aspect of handling the lentiviral reagents is not appropriate and if required a further risk assessment would be needed.

### Project Containment

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### Project Ref 3115/15.1

- **Date Ackn'd**: 30/09/2015
- **Expression of fluorescent probes, such as green fluorescent protein (GFP) using a lentivirus gene transfer system, into mammalian cell lines potentially contaminated by Hepatitis B virus (HBV)**
- **Class**: 2
- **Culture Volume**: ≤ 1 Litre
- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

### Purposes of the contained use

The labelling of mammalian cells as a method to selectively track biological processes such as proliferation, apoptosis and migration for identifying/understanding modulators of these complex pathways. To this end lentiviral gene transfer systems to express fluorescent probes operating in the...
green and red wavelengths will be used, but not developed within the Essen BioScience Ltd lab. The use of hepatocyte cell lines for the study of potential anti-cancer therapies has been well established. Within the current project we wish to combine our existing approaches of using the lentiviral expression system with relevant cell types potentially contaminated by HPV.

Recipient or parental organism

The parental cell types to be used within the project include Hep3b (HB-8064) and SNU-449 (CRL-2234) cells, to be supplied by a client, originally obtained from ATCC. Both have evidence for HPV contamination and as such additional precautions need to be applied to reduce the risk of potential infection.

Host/vector system

Lentivirus system based on the pLentiLox lentiviral vector map; incorporating either the TagGFP2 or mKate2 fluorescent markers, with or without the nuclear localisation signal (3xNLS), an internal ribosome entry site (IRES) and either a puromycin or bleomycin (zeocin) selection marker. The human elongation factor-1 alpha (EF1a) promotor is used to drive the expression of the gene(s). Vector maps are included as part of the risk assessment document.

Origin & function

The vectors used are supplied by the University of Michigan and encode the fluorescent probes GFP (green fluorescent protein) or mKate (far-red fluorescent protein). Viral particle production occurs at Essen BioScience Inc. (Ann Arbor, Michigan) with transfection of HEK293 cells with the vector, alongside the 3 packaging plasmids. The supernatant is harvested containing replication deficient lentivirus. Both GFP and mKate2 have been widely used as fluorescent probes and are well characterised and are not known to possess any oncogenic potential.

Evaluation of foreseeable effects

Host cell lines: HepG3 and SNU-449 cells contains HBV genome integrated into the eukaryotic chromosomes (see Su et al. 2010 for HepG3 and Ku et al 2005 for SNU-449). However there is currently no evidence that these cell lines produce infectious Hepatitis B virus. Lentiviral infection: The constructs are third generation lentiviral SIN (self-inactivating) vectors. The vector is packaged using third generation packaging plasmids (3 packaging plasmids). The design of all four vectors used in viral production incorporate safety measures against the generation of replication competent virus. The vectors’ LTRs are self-inactivating (SIN), thus restricting mRNA production from integrating vectors to the internal promoter, severely reducing full-length vector transcripts. The lentiviral vector is pseudotyped with the VSV-G envelope serotype, providing a greater margin of personal and public safety and reducing the risk of recombination.
According to the literature, viruses packaged under these conditions were found to contain no detectable level of replication competent virus. The vector uses a WPRE element. The post-transcriptional regulatory element (PRE) of the woodchuck hepatitis virus (WHV) improves 3’ RNA processing and thus increases transgene expression efficiency. However, some versions of this element are capable of expressing part of the X protein from WHV which may have oncogenic properties. The WPRE used in the plasmids used in the current study have been modified, mutating the X protein promoter (1488-1492, GCTGA>ATCAT), thus greatly reducing the expression of X protein (see Schambach et al, 2006, Gene Therapy 13, 641-45). Therefore the risk of oncogenic properties has been decreased. Neither GFP (green fluorescent protein) nor mKate2 (far-red fluorescent protein) possess oncogenic properties, thus presenting a low risk to human health.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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Sharps bins are for disposal of biologically contaminated needles, glass and other sharp objects (the use of sharps whilst undertaking class 2 work will not be conducted).

Autoclaved waste is placed in yellow biohazard bags, sealed with cable ties and sent for incineration under code 18 01 03.

Once full, yellow biohazard bags will be closed with cable ties and sharps and burn bins sealed with locking lids. These are sent for incineration on a monthly schedule. Storage of waste bags/bins prior to disposal is in locked 770 litre container located within the BioPark security perimeter.

Biologically contaminated liquid waste is collected and appropriately decontaminated with disinfectant (Presept tablets; troclosene sodium, 2,500ppm), incubated for 24hrs and discarded down the sink.
The safety committee approved the risk assessment. It was confirmed that once washed sufficiently, infected mammalian cells could be used at the risk level of the cells prior to infection. The implementation of appropriate training was stated as essential prior to any personnel working with either the host cells or lentiviral reagents. Although the risk of HBV infection is negligible, as vaccination is available all employees who are to work with these host cells should be vaccinated. Finally, the use of sharps during any aspect of handling the lentiviral reagents is not appropriate and if required a further risk assessment would be needed.

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Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form Y

Tick to confirm that it is attached to this form N
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**Premises Addresses**

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</table>

**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Composition. - consists of three individuals
- UK EH&S Manager - EH&S representative
- Dept Manager/Supervisor - Management representative
- staff representative

Frequency of meeting
Initially to approve the risk assessment covering the Class 1 GM (CU) activity to be carried out, namely storage and despatch of cell lines, thereafter in the event of any change in the activity (eg scale) or regulations.

<table>
<thead>
<tr>
<th>Laboratory</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

In the event of a spillage, material would be deactivated with an appropriate method (eg bleach/disinfectant solution) and disposed of through an approved licenced waste contractor.
Solid (or frozen) waste including time expired product would be placed in clinical waste bag for incineration through licenced waste contractor

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The committee note that the activities consist solely of receipt, storage and despatch of sealed containers. Any risks to human helath or the environment arising from a leaking or damaged container would be readily dealt with by the routine spillage procedures.
Overall the risk to human health and the envrionment from this activity is very small.
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Name

SAINSBURY LABORATORY UNIVERSITY OF CAMBRIDGE

Name 2 Department

Campus Estate or Research Centre Building

Road Name

47 BATEMAN STREET

District

Town

CAMBRIDGE

County Postcode Country

CAMBRIDGESHIRE CB2 1LR ENGLAND

Tel Number Fax Number

01223 761100 01223 76100

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Members of the GM safety committee will be:
Departmental safety officer, Biological Safety Officer, Glasshouse and Plant Growth Manager, Two Principal Investigators and a representative from the junior Scientific staff

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<tr>
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</table>
### Laboratories:

Solid biological waste that is not contaminated by any hazardous chemicals which would be released into the atmosphere, or by sharps (including yellow tips etc) which would puncture the bags, is autoclaved.

Sharps (including yellow and blue tips) contaminated with biological waste will be thoroughly decontaminated in Hycollin or autoclaved in an appropriate container (see department safety officer for advice) before disposal with the other sharps.

Liquid Biological waste in plastic containers will be decontaminated with Hycollin or bleach.

Liquid biological waste in glassware will be decontaminated with Hycollin or by autoclaving via the Kill Boxes.

Glassware (flasks, bottles etc.) contaminated with biological waste should have their caps removed and placed in the blue plastic boxes for autoclaving.

Any spills should be cleaned with either bleach or Hycollin.

---

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<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<th>Transgenic Plants</th>
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### Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity.
Autoclaves are routinely serviced and regular checks to ensure autoclave are reaching the required temperatures using thermo logs.

This applies to all glasshouses and CERs marked as 'transgenic' areas.

Disposal of unwanted plants and soil
NB Users should see the Glasshouse Manager for glasshouse and CERs for waste disposal procedures.
If transgenic plants are grown within a glasshouse then by default all plants in the glasshouse are treated as transgenic and these procedures must be followed.

Exceptions to this practice must be made clear and justified in the GMNRA and practical arrangements agreed with the Glasshouse Manager.

Plants and soil shall be put into the black bins marked "Transgenic" and not into any other bins. No soil or tops will be accepted in autoclave bags.

Pots, labels and trays shall be put in the transparent autoclave bags. Clearly separate the items for disposal will be autoclaved.

No bags of mixed items such as soil and plastic pots will be accepted. These will be left for science staff to sort correctly. (v) The soil and tops in the black bins shall be tipped into a steam cart. The cart must not be filled higher than level with top of cart. The cart shall be taken to the autoclave area and attached to the steam line. The contents will be steam sterilized at 100°C for 1 hour.

Care must be taken to avoid spilling plant material onto roadways and paths. Any spills must be cleaned up using the dust pan and brush provided and split material returned to the steam cart.

After sterilizing is complete the contents from the steam cart should be deposited in the compost bays.
After sterilization all autoclave bags containing disposable items shall be put into rubbish skips.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment Y

Please enter comments of the GM safety committee on the risk assessment
The risk is low. The risk assessment is appropriate

Project Ref 3117/13.1

<table>
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<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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The oomycetes Phytophthora palmivora, Phytophthora infestans and Phytophthora capsici cause disease on vegetable crops. On the other hand, plants engage in beneficial symbiosis with Mycorrhiza fungi. This research will focus on the host interface with the pathogen and symbiont to address how Phytophthora and mycorrhizal fungi are similarly or differentially perceived and the extent to which they employ the same development plant program to establish interactions. We will identify and characterize genetic elements with common functions in Phytophthora and mycorrhizal colonization. Furthermore, we will elucidate the role of microbial effector proteins in the intracellular accommodation process. More specifically, gene sequences will be generated for genes from host plants and Phytophthora spp. and used in laboratory experiments to determine their subcellular distribution and their biochemical activities. We will also employ a range of other microbial plant pathogens (such as Xanthomonas campestris, Agrobacterium rhizogenes and tobacco rattle virus) to enable delivery of the genes under study or the encoded proteins into host cells. Furthermore, we will explore similarities of fungal and oomycete interfaces by comparative studies between fungal pathogens and oomycete pathogens.

Ultimately, this work will reveal the boundaries between symbiosis and pathogenesis and will provide novel mechanisms for pathogen control.

Recipient or parental organism

Recipients will be strains of prokaryotic (A. rhizogenes, X. campestris pv. Vesicatoria, TRV) and eukaryotic plant pathogens (oomycetes and fungi) some of which are native to the UK environment, some of which will have been imported and held under Plant Health Licence from FERA/DEFRA.

Recipients/hosts will be: Agrobacterium rhizogenes, Xanthomonas campestris pv. Vesicatoria, Phytophthora infestans, Phytophthora palmivora, Phytophthora capsici and Tobacco rattle virus (TRV) without Nematode transmission.

There research could generate transgenic plant pathogens with altered host ranges and pathogenicity

Host/vector system

Vector systems will be:
- Binary vectors and bacterial expression vectors
- Phytophthora expression vectors
- Phytophthora expression vectors (pTOR backbone) (can only be replicated in E. coli)
- The TRV genome, split and embedded in the T-DNA of a binary vector in separate disabled Agrobacterium tumefaciens strains

Origin & function

Vector inserts will be genes from either plants or plant associated microorganisms that are supposedly involved in plant microbe interactions such as microbial effectors;
Candidate eukaryotic plant pathogen effector molecules will be identified from beneficial mycorrhiza fungi and oomycete pathogens including Phytophthora infestans, Phytophthora capsici, Phytophthora palmivora, and the ascomycete fungi such as Blumeria spp., Mycosphaerella sp., Leptosphaeria maculans, Botrytis cinerea, Sclerotinia sclerotiorum, Fusarium spp., Pyrenophora spp. Using bioinformatic approaches.

Genes will be cloned from P. palmivora, P. capsici and P. infestans strains included in this notification. Genes of interest form other plant pathogens will not be cloned via cultivation of these microorganisms but by de-novo gene synthesis or by obtaining DNA fragments through collaboration.

Many of these genes will be transferred back into recipient strains which may be eukaryotic plant pathogens Phytophthora palmivora. Phytophthora capsici and Phytophthora infestans as well as prokaryotic plant pathogens tobacco rattle virus (TRV), Agrobacterium rhizogenes and Xanthomonas campestris pv. vesicatoria.

Stably transformed genetically modified eukaryotic plant pathogens will be inoculated onto host plants and tested for pathogenicity. Host plants include tomato, potato, and Nicotiana spp., sweet potato (Ipomoea batatas), Mirabilis jalapa, cereals (wheat, barley, rice), Brassicus including Arabidopsis thaliana and Legumes (Medicago sp., Lotus sp.). Other species plants may be tested for their ability to support establishment of a plant-microbe interaction.

**Evaluation of foreseeable effects**

**Human Health;**

None of the recipient organisms used in these projects is a human pathogen and does not, therefore, constitute a hazard to human health. The proposed genetic modifications of plant pathogens will not cause the modified strains to become human or animal pathogens and are therefore of no risk to human or animal health.

**Environmental Safety;**

The plant pathogens Tobacco rattle virus (TRV), Phytophthora infestans and Agrobacterium rhizogenes are all present in the local environment, even if the unmodified recipient strains here used may be imported from outside the UK and held under licence from DEFRA. Therefore any risk posed by their potential accidental escape is not related to the introduction of a new plant disease, but rather to any risks relating to their genetic modification. Because these organisms can survive in the UK, GM strains could also survive in the local environment. Some of the genetic modifications could result in pathogens that are more virulent than the recipient strain. However, the recipient strains have relatively low virulence and hence none of the GM strains are likely to be significantly more virulent than naturally occurring isolates of the same species.

The plant pathogens; Xanthomonas campestris pv. Vescatoria, Phytophthora capsici and Phytophthora palmivora are unlikely to be present in the local environment since they are pathogens of subtropical/tropical plants and are unable to survive to be present in the local environment since they are pathogens of subtropical/tropical plants and are unable to survive in the local environment, due both to the absence of suitable hosts and environmental adversity. P. palmivora will be used as a model organism, receiving candidate effector genes from different donor plant pathogens and beneficial microorganisms. The hazard identified is that P. palmivora may have an altered host range and be able to infect plants that may grow in the local environment. However, it is extremely unlikely that the genetically modified P. palmivora will differ from wild type strains in its ability to survive in the UK environment. Moreover P. palmivora is not likely to be present in the UK and therefore not able to reproduce sexually with the GM strain.

The silencing of candidate plant pathogen effector genes could lead to the growth of the GM-pathogen on an otherwise resistant plant host, especially if the silencing resulted in a loss of avirulence determinant(s). However, potato varieties harbouring genes conferring P. infestans resistance remain susceptible to other diverse naturally occurring virulent races of the pathogen.

The silencing of effector genes is not likely to alter the GM-pathogen's host range, but is likely to have a negative effect on virulence.
For genetically modified plant pathogens that could survive in the UK environment. It is envisaged that none of the modifications will lead to races of pathogen that are more virulent than naturally occurring races; the recipient strains are not the most virulent and the most virulent naturally occurring strains will not be modified. Moreover since an important objective of these investigations is to monitor the infection mechanisms of eukaryotic plant pathogens, any significant increases in virulence will be identified.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For plant material infected with GMMs Phytophthora sp., Xanthomonas sp. And TRV, containment level 2 is appropriate.

Xanthomonas or TRV infected plants will be maintained in containment level 2 growth facilities at the Sainsbury Laboratory (SLCU). Standard operating procedures for the use of plant growth facilities and for the disposal of waste will be strictly adhered to.

GM-Phytophthora sp. Will be used only in the laboratory under in vivo conditions in containment level 2.

GM-A rhizogenes containing plant material will be maintained solely under in vitro conditions in containment level 1.

Plant pathogen infected plant material and compost will be killed by autoclaving prior to disposal.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMM material will be handled using good microbiological practice (GMP). All GMM material will be autoclaved prior to disposal.

The class II safety cabinet and growth chamber surfaces will be disinfected by virkon/trigene spray and wiping with appropriate disinfectants. All contaminated glassware/equipment will be disinfected after use and before cleaning by autoclaving where possible, otherwise by immersion in disinfectant solution or spray wiped with ethanol.

Residual plant material and compost is placed in lidded stainless steel containers and stored within a latched lidded blue container clearly marked as containment 2 material. The containers are then separately autoclaved. The stainless steel container is autoclaved using the Compost Waste cycle (see below) and the blue container is autoclaved using the Plastic Discard cycle.

Plastic waste is placed within an autoclave bag and stored in a blue container (as above). This is then disposed of using the Plastic Discard cycle.

Oomycete and bacterial cultures an culture plates in the lab will be autoclaved prior to disposal.

Any condensate from CER's is collected into a steel bucket. This is regularly checked and the bucket and water waste then autoclaved using our Fluid Discard Programme (See below). All drains within the growth chamber (CER) are sealed. Any water residue is then limited within the chamber, but should run off occur then this is vacuumed with a clearly marked designated wet vac and waste autoclaved as above.

Autoclave procedures are as follows;

* Soil and plant waste generated in growth rooms are autoclaved in stainless steel containers using the Compost Waste cycle. This has a temperature 134°C for 30 minutes. The whole process also includes 5 negative and positive pushes.

* Plastic waste is autoclaved using the Plastic Discard cycle. This has a temperature of 121°C for 30 minutes. The whole process also included 5 negative and positive pulses.

* All water is treated using the Fluid Discard cycle. This has a temperature of 121°C for 30 minutes. The whole process also includes 6 negative pulses.

Additional information: All autoclaves are validated once a year using maximum loads for each cycle. We have a contract with the autoclave company for 3 additional onsite
service visits. Regular validation using chemical indicators for sterilisation are also carried out.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<th>Glass Houses</th>
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GM Centre Number: 3118

Data Premises Notified (Originally) 20/04/2011

Transferred from 1992 Regs? N

Transitional Premises

Class

Data Premises Closed

Transitional Premises

Emergency Plan Required?

N

Non-GMMs N

Withdrawn N

Name

UNIVERSITY OF WORCESTER

Name 2

Department

Campus Estate or Research Centre

INSTITUTE OF SCIENCE AND THE ENVIRO

Building

ST JOHNS CAMPUS

Road Name

HENWICK GROVE

District

Town

WORCESTER

County

WORCESTERSHIRE

Postcode

WR2 6AJ

Country

ENGLAND

Tel Number 01905 542242

Fax Number 01905 855132

E-mail

HSE Division blank

Comments

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee (GMSC) will be chaired by the University Biological Safety Officer (BSO); this is a person who has experience of working with GM in another UK University and is thoroughly familiar with the administrative procedures at the University of Worcester at UW. There will be at least four other members of the GMSC, all of whom have familiarity with GM technologies and with the related risk assessments and safety procedures. All of theses (except the trade union representative) have been gained experience in GM at other UK Universities. All scientific members of the GMSC will be involved in research that involves GM technology (although none of this has been allowed to commence at UW until the appropriate permission has been granted).

All GM work will be carried out within the Institute of Science and the Environment (ISE) and its associated Research Centre (the National Pollen and Aerobiology Research Unit or NPARU) on the St John's campus at UW. The GMSC members are drawn from the ISE and NPARU and will report directly to the joint ISE/NPARU Safety Committee which in turn reports to the University's Health, Welfare and Safety Committee.

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02/03/2022
| Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research | Yes |
| Virology | Transgenic Animals | Transgenic Fish | Gene Therapy |
| Mycology | Transgenic Invertebrates | Transgenic Plants | Yes | Other (please specify below) |

**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

After use, bacteria and plant material will be autoclaved to achieve 100% kill. The material will be autoclaved at 127°C for 35 minutes. The autoclave indicates whether the load has reached temperature for the required time and shows a 'failed load' in this case. We also use temperature strips that provide an additional method of identifying any failure in a run. The autoclave will be routinely serviced and receive an annual Certificate of Calibration.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Reviewer 1

GM Micro-organism risk assessment
I agree with your assessment that there is not a significant risk to human health or the environment associated with the work described.

I have a number of points that I wish to be considered before the assessment is accepted.

1. I have marked on the form a few minor clerical points (mis-spellings etc). Please place all species names in italics.
2. Major point: The GMO (plant) risk assessment (commented on below) refers to work in which alleles of Arabidopsis resistance genes are transferred to other genotypes of Arabidopsis. This will require preliminary work in E. coli and Agrobacterium. However, this has not been described here. You MUST include in this risk assessment a clear description of the work relating to the work in micro-organisms with Arabidopsis R genes.
3. Major point 1: The GM Micro-organism risk assessment (commented on above) refers to experiments in which genes encoding ‘effectors’ are taken from Hyaloperonospora arabidopsidis and, after work in E. coli and Agrobacterium, are expressed in a plant (Arabidopsis). However, the activities described in this GMO (plant) risk assessment describes work in which alleles of Arabidopsis resistance genes are transferred to other genotypes of Arabidopsis. You MUST include in this risk assessment a clear description of the work relating to the expression in plants of the H. arabidopsidis effector genes.
4. Major point 2: We have organised the risk assessment forms on the basis that one deals with potential risks associated with work on micro-organisms and the other on plants. This division is fundamental to the risk assessment process even where, as quite often, the actual experiments being performed require work with the same sequences in both micro-organisms and plants. I am concerned that the inclusion of information on bacteria in the ‘plant’ document sets a precedent that will confuse future compilers of risk assessments. I have therefore marked several short sections that I suggest you remove from the plant risk assessment document. Actually, the information included on p. 5 (marked) could be transferred to the GMO risk assessment document since it contains more detail than is currently included.
5. Missing information: no submission date has been included (p.1). You have not filled in Tables 1 at the end of the document; see the marked sentence on p.13 for guidance. This is very simple, requiring only crosses in appropriate boxes and a final statement of the containment level, but must be completed.

GM Organism (plant) risk assessment
I agree with your assessment that there is not a significant risk to human health or the environment associated with the work described.

I have a number of points that I wish to be considered before the assessment is accepted.

2. Again, I have marked on the form a few minor clerical points (mis-spellings etc). Please place all species names in italics.
3. Major point 1: The GM Micro-organism risk assessment (commented on above) refers to experiments in which genes encoding ‘effectors’ are taken from Hyaloperonospora arabidopsidis and, after work in E. coli and Agrobacterium, are expressed in a plant (Arabidopsis). However, the activities described in this GMO (plant) risk assessment describes work in which alleles of Arabidopsis resistance genes are transferred to other genotypes of Arabidopsis. You MUST include in this risk assessment a clear description of the work relating to the expression in plants of the H. arabidopsidis effector genes.
4. Major point 2: We have organised the risk assessment forms on the basis that one deals with potential risks associated with work on micro-organisms and the other on plants. This division is fundamental to the risk assessment process even where, as quite often, the actual experiments being performed require work with the same sequences in both micro-organisms and plants. I am concerned that the inclusion of information on bacteria in the ‘plant’ document sets a precedent that will confuse future compilers of risk assessments. I have therefore marked several short sections that I suggest you remove from the plant risk assessment document. Actually, the information included on p. 5 (marked) could be transferred to the GMO risk assessment document since it contains more detail than is currently included.
5. Missing information: no submission date has been included (p.1). You have not filled in Tables 1 at the end of the document; see the marked sentence on p.13 for guidance. This is very simple, requiring only crosses in appropriate boxes and a final statement of the containment level, but must be completed.
Reviewer 2

Report on risk assessment of genetically modified organisms, Dr M T – M W

The research contained in this proposal appears to be of high scientific value. There is little to be identified in terms of either risk to the environment by contamination or risk to human health. Containment procedures appear to be adequate and monitored effectively by regular servicing and monitoring of autoclave equipment. As long as the following points are rectified on the proposal then there are no reasons to stop the proposal from proceeding.

1. Page 1 - Institute/research centre. This should read “National Pollen and Aerobiological Research Unit”
2. Page 1 – ‘Pichia pastori’ should read ‘Pichia pastoris’
3. Page 1 several typographical errors in the box at the bottom of the page need to be corrected. What does ‘shuffled’ mean?
5. Page 4 – what are the host ranges of these plasmid vectors?
6. Page 4 - the large box. It would be useful to state that these host organisms have a long history of safe use in many laboratories worldwide.
7. Page 4 – the large box. Previously it is stated that some of the genes may be expressed in planta. Should the plant host be added here?
8. Page 5 – upper box – a comment about the non-GM component of this proposal – why are you planning to use anti-His antibodies rather than Ni-agarose columns which is the standard way of purifying His-tagged proteins?
9. Page 5 – upper box – please state the autoclave conditions being used.
10. Page 6 – upper box – in terms of protein expression and isolation you are likely to need much more than 5-15ml of culture. Dependent on level of expression the amount of culture you will need is more likely to be in the range of 100ml – 1litre, possibly even more if proteins are expressed at a very low level.
11. Page 6 – third box – add Agrobacterium to the potential for aerosol contamination.
12. Page 8 – box 3 – you state that H. arabidopsidis is ‘not-toxic or allergenic’. Do you have evidence for this? Otherwise change the wording to ‘not known to be toxic or allergenic’.
13. Page 9 box 2 – change ‘none identified’ to ‘effectively zero’ as the form states.

Report on risk assessment of genetically modified organisms (plants) Dr Mahmut Tor – Mike Wheeler

The research outlined in this proposal is of high scientific value and as long as the conditions outlined below are addressed in full by the researcher I see no problem with proceeding with this research. There appears to be negligible risk in terms of the environment and human and plant health with proceeding with this plan of work.

1. Page 1 - Institute/research centre. This should read “National Pollen and Aerobiological Research Unit”
2. Throughout – ensure all species and gene names are italicised correctly please.
3. Page 3 – box, section 2 – please add antibiotic resistance genes that are being used for selection in E.coli (e.g. kanamycin phosphotransferase). These are noted in the box at the bottom of page 4.
4. Page 5 – change ‘heterologues’ to ‘heterologous’
5. Page 6 - top box. The last sentence suggests that expression of the gen under its native promoter ‘is not expected to cause any significant alterations in phenotype’. In that case why is this experiment being done? I think that it is quite possible that there will be a change in resistance phenotype in Arabidopsis thaliana Col-0 if the mutant version of the gene that it currently contains is supplemented with an active gene. The researcher needs to make this much clearer here.
6. Page 8 – second box. It states that inoculated plants are to be ‘transferred by hand between facilities in the building’ (my underlining). Would it be more appropriate to lessen transfer between parts of the building as this is likely to increase the risk of contamination of the environment either by Agrobacterium or more especially by pollen as no doubt the plants are flowering if they are being used for a ‘floral dip’ method (see box page 1). If transfer between parts of the building must
take place then plants should be contained in a box that is able to be autoclaved to minimize pollen dispersal.
7. Page 8 – box 4 – autoclave conditions need to be made explicit here. In terms of validation please refer to the method of validation outlined in box 6 on this page.
8. Page 9 – box 2 – This box should be filled in in more detail than is there at present.
9. Page 9 – box 3 – There are a couple of issues here. First, release of plants would mean release of the RHAC1 gene under control of 35S which is not something that currently occurs in the wild population of Arabidopsis at present. This needs to be made explicit. There is quite possibly no reason why this would be any particular issue compared to expression under control of the native promoter but this needs to be made explicit. Secondly, insertion of transgenes using Agrobacterium T-DNA transformation inevitable means that the sites of insertions are not under control of the researcher. Therefore it is not possible to say whether there would be an effect or not on wild plants. I agree that this is a negligible hazard but it is one that should be discussed in this section to at least illustrate that the researcher has considered this.

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Project Ref 3118/16.1

Retroviral transduction of immortalised mammalian cell lines

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Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The genetic modification of several commercially available mammalian cell lines to constitutively overexpress or downregulate wild-type / mutant BCAT1 protein for the intracellular functional characterisation of the redox active CXXC motif.

Recipient or parental organism

Immortalised mammalian cell lines.

Host/vector system
Human BCAT1 cDNA pCMV6-Entry Vector construct (OriGene) transformed into E. coli alpha select competent cell and HEK293T hosts.

Host characteristics:
E.coli alpha-select competent cells. Genotype: F- deoR endA1 recA1 relA1 gyrA96 hsdR17(rk-, mk+) supE44 thi-1 phoA ΔlacZYA-argF)U169 Φ80lacZΔM15 λ – cell we be used to expand BCAT1-pCMV6 and BCAT1-pLENTI constructs.

HEK293T immortalised cell line, is a highly transfectable derivative of human embryonic kidney 293 cells, and contains the SV40 T antigen. This cell line is competent to replicate vectors carrying the SV40 region of replication. It gives high titers when used to produce retroviruses. It has been widely used for retroviral production, gene expression and protein production. DNA profile: CSF1PO: 11,12; D13S317: 12,14; D16S539: 9,13; DSS818: 8,9; D7S820: 11; TH01: 7, 9.3; TPOX: 11; vWA: 16,19; Amelogenin: X

These host organisms have a long history of safe use in many laboratories worldwide and pose negligible risk to human health for the usage described in this document.

Vector system details:
pCMV6-Entry Vector (OriGene) characteristics include: Myc/DDK tag, f1 ori (origin of repication for f1 phage host e.g. M13), SV40 ori (for replication inside mammalian cell systems carrying the Simian Virus large T antigen e.g. HEK293T immortalised human kidney cells), ColE1 (bacteria host origen of replication; low copy number ~15-20), Neor (neomycin resistance in mammalian cells), Kanr (kanamycin resistance for plasmid recovery in E. coli), CMV promotor (for constitutive expression in mammalian cell host e.g. HEK293T), Kozak (eukaryotic mRNA translatio initiation sequence).

pLENTI-C-Myc-DDK-IRES-Neo Vector (OriGene) characteristics include: Myc/DDK tag, Ori, Neor, CAT (CAMr – cloramphenicol resistance for selection in E.coli host), 5’ LTR (contains upstream RSV promoter in place of TAT), 3’ LTR (contains SIN preventing viral genome self replication). **Note that the vector does not contain gag, pol, rev and VSV-G (essential for viral replication and packaging), thus replication defective**.

OriGene Lenti-Vpak packaging kit characteristics:
The lentiviral replication and packaging genes gag, pol, rev and VSV-G have been separated onto three different plasmids pCMV6-Entry vectors, which have been optimised for transfection use in the Vpak kit.

Description of the system:
Combination of construct with Vpak plasmids will transfect and stably produce pseudo-lentiviral particles containing BCAT1 mRNA via the SV40 promotor in mammalian packaging cell lines that carry the Simian Virus large T antigen e.g. HEK293T cells. These are pseudo-lentiviral particles since VSV-G (Vesicular Stomatitis Virus pseudo-envelope) is used in place of the lentiviral envelope as a safety feature. The pseudo-lentiviral particles packaged by HEK293T cells will subsequently be used to, immortalised mammalian to express or silence wild-type of CXXC motif mutant BCAT1 protein under control of the CMV promotor (Naldini et al, 1996; Burns et al, 1993).

Unlike other retroviruses, pseudo-lentiviral particles can enter non-dividing cells (Lewis et al, 1992). Lentiviral expression is replication defective in target cells, since the only mRNA packaged are those which contain 3’ and 5’ LTR (ψ sequence packaging signal). Only the pLENTI plasmid contains 3’ and 5’ LTR’s, thus only BCAT1 mRNA is transfected and not the mRNA required for lentiviral replication and packaging as they are contained on a pCMV6-Entry Vector (which does not contain 3’ and 5’ LTR’s).

This system is a 3rd generation lentiviral system where the replication and viral packaging genes; *gag*, *pol*, *rev* and *VSV-G are separated onto three separate constructs absent of 3’ and 5’ LTR’s (Dull et al, 1998) i.e. the Lenti-Vpak packaging kit. Thus the packaging of pseudo-lentiviral particles containing mRNA of the gene of interest by HEK293T cells requires co-transfection of four separate constructs. Since neomycin selection is under control of the SV40 promoter, thus only cells carrying the Simian Virus large T antigen will produce pseudo-lentiviral particles in the presence of neomycin – which is a further safety feature. Furthermore, many nucleotide changes have been made to sequences used in these vectors to render sequence similarity to wild strains of the parent viruses even lower. This makes the 3rd generation system extremely safe, which is exemplified through its global usage (Sinn et al, 2005). The generation of replication competent pseudo-lentiviral particles (RCL) requires four
separate recombination events to occur in the HEK293T cells. This can be monitored via RT-PCR (viral replication genes) or ELISA (Sastry et al., 2005). At the time of writing this, no reported cases of RCL generation are evident from the literature. Pathogenicity is negligible.

*gag = group specific antigen (matrix, nuclear capsid and major capsid), pol = reverse transcriptase, protease and integrase, rev = regulator auxiliary gene and VSV-G = replaced rev (envelope glycoprotein)

References:


Origin & function
Human BCAT1 cDNA pCMV6-Entry Vector construct is commercially available as naked DNA in lyophilised form obtained from OriGene.

Functions:
BCAT1 encodes the human cytosolic branched-chain aminotransferase isoenzyme (hBCATc). hBCATc belongs to the fold type-V PLP dependent protein family. The canonical role for hBCATc is to transfer the α-amino group from the branched-chain amino acids, Leu, Ile, & Val to α-ketoglutarate generating glutamate and the respective α-keto acid. hBCATc has a defined role in glutamate signalling in the brain and is the drug target for Gabapentin (an anti-convulsant). There are 5 transcript variations. This project involves transcript variant 5 (the closest match in terms of sequence alignment to Goto et al. J Biol Chem; 280(44): 37246-56). Recent studies indicate that the over-expression of BCAT1 in ovarian (Wang et al, 2015) and nasopharyngeal carcinoma (Zhou et al, 2013) was linked to increased cell proliferation. Whilst the oncogenic role for BCAT1 is undefined, overexpression in certain cancer cells may relate to tumour progression.

Our research has highlighted a potential and novel 'anti-oxidant' role for BCATc which centres on the CXXC motif (Coles et al, 2012). To date these studies have been performed using biochemical analysis on purified wild-type and CXXC motif mutant BCATc protein. The next logical step is to evaluate this process intracellularly. If our hypothesis is correct, we would expect to observe a reduction in the cellular redox potential where wild-type BCATc is over-expressed. No difference will be observed for CXXC motif mutants.

References:


Evaluation of foreseeable effects

The expression of hBCATc protein will be evident in the HEK293T cell line. Expression of hBCATc is under control of a CMV promotor. Expression is constitutive and high. Lentiviral and pseudo-lentiviral genes will also be expressed in the HEK293T cell line, which are under control of the SV40 promotor. These HEK293T cells will constitutively express and package pseudo-lentiviral particle that contain hBCATc mRNA. The pseudo-lentiviral particles will subsequently be used to transduce several immortalised human leukaemia target cell lines, including; THP-1, MV-411, K562, NB-4 and U937. These cells once transduced will stability and constitutively express hBCATc protein under control of the CMV promotor. No other lentiviral or pseudo-lentiviral genes should be expressed in these target cells. hBCATc is a cytosolic protein. Low expression of hBCATc may be observed in the alpha-select cells.

Summary of the key biosafety features of 3rd generation lentiviral systems:

a) 5'LTR is wildtype for 2nd generation and inactivated for 3rd generation.
b) 2nd generation contains 4 lentiviral genes (gag, pol, rev and tat), whereas 3rd generation only contain 3 (gag, pol and rev).
c) 2nd generation uses 3 plasmids in total with gag, pol, rev and tat being expressed on the same construct. Whereas 3rd generation split packing and replication onto separate plasmids i.e. rev is express on a plasmid separate from gag. Thus for 3rd generation systems a total of 4 plasmids are used.
d) For 2nd generation a single-step crossover could cause the wildtype 5' LTR and packaging ψ sequence to be placed in front of the lentiviral genes, thus generating RCL. Whereas for 3rd generation the 3' LTR is truncated, reducing the likelihood of producing RCL, plus the number of recombination to produce RCL events is greatly increased, thus improving the biosafety.

Effects on human health:

Having considered the nature of the modified organisms, the nature of the transgenes added and the nature of the operations in culturing the cells, (to be cultured in class-2 biological safety cabinets), we cannot identify any direct risks to human health. Although no oncogenic role for BCAT1 is defined, over-expression of this gene may promote cell proliferation in certain cancer types. The HEK293T cells will synthesise and package pseudo-lentiviruses (which can transduce primary human cells) and in light of the cancer studies we are not 100% sure how the over-expression of hBCATc will effect the growth and survival of healthy cells. Thus all mammalian cell culture working involving HEK293T cells and the afore mentioned immortalised leukaemia cell lines will be carried out at biosafety containment level 2. This recommendation is in accordance with OrgiGene (suppliers of the 3rd generation lentiviral system we are using) and ATCC (suppliers of the HEK293T cells).

Effects on human health, high risk individuals e.g. immunocompromised, pregnant or breastfeeding women:

Immunocompromised, pregnant and breast feeding women are at risk of pseudo-lentiviral exposure, however, biosafety containment level 2 is sufficient to ameliorate the risk of exposure. Whilst there are no reports of hBCATc directly causing human disease, we are not 100% sure how the over-expression of hBCATc will effect cell growth (no known oncogenic role). To this end, we will adhere to biosafety containment level 2 during these procedures.

Immunocompromised, pregnant and breast feeding women are at risk of pseudo-lentiviral exposure, however, biosafety containment level 2 is sufficient to ameliorate the risk of exposure. Whilst there are no reports of hBCATc causing human disease, we are not 100% sure how the over-expression of hBCATc will effect cell growth (no known oncogenic role). To this end, we will adhere to biosafety containment level 2 during these procedures.
In spite of the potential for BCAT1 to increase proliferation in certain cancer types, it is unlikely that accidental infection with BCAT1 pseudo-lentivirus contracts itself causes cancer.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This project does not involve larger GMOs.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection / inactivation of GMMs in waste and subsequent disposal

Disposal of waste generated inside the biological safety cabinet (liquid, cellular & solid waste e.g. pipette tips etc) will be in 10% hypochlorite solution prepared fresh prior to the tissue culture session (important since hypochlorite loses its activity after 24 hours in solution) and leave for 48 hours (kills enveloped viruses at 1000ppm in <10 minutes). All waste is handled inside the biological safety cabinet (recommend 500 ml Duran bottles containing 100 ml 10 % hypochlorite solution and labelled, in which waste supernatant, cells and pipette tips are ejected). Following 48 hours the waste solution can be disposed of. The tips can be recovered and autoclaved (15 psi; 121 oC / 103.4 kPa, for 15 minutes), along with all other solid waste e.g. flasks, gloves etc. Surfaces inside the class-2 biological safety cabinet must be swabbed with 70% ethanol before and after any cell culture session. 1% Virkon will be used to decontaminate any spills on the surface inside the biological safety cabinet (spill volume should not exceed 10 ml at any one time); decontamination of metal surfaces and parts using 1% Virkon is achieved in <10 minutes. This will be followed by the UV decontamination cycle built into the biological safety cabinet. During this period, the biological safety cabinet will be decommissioned. 10% hypochlorite, 70% ethanol and 1% Virkon are all effective at destroying enveloped viruses, such as pseudo-lentiviral particles as generated here (http://www.cleaning-for-health.org/disinfectant-chart/).

GMO waste supernatants (liquid and cellular) from tissue culture procedures carried out inside the biological safety cabinet will be inactivated using 10 % hypochlorite solution (inactivation occurs at 1000ppm in <10 minutes). Solid waste and any GMO remaining waste will be autoclaved (15 psi; 121 oC / 103.4 kPa, for 15 minutes) in EG071. Autoclave conditions will kill all organisms in 15 minutes.

Monitoring at point of use:

In general, all cell culture work will be carried out using tissue culture flasks (T75 or T25) with screw cap lids, which will contain cells and viral supernatant. Flasks will be labelled clearly labelled ‘Genetically Modified Organisms’ and ‘Lentivirus’. Where 6 well culture plates are used (volume <6 ml), transport between equipment will be carried inside a separately sealed container labelled ‘Genetically Modified Organisms’ and ‘Lentivirus’. No tissue culture flask (or plate) containing packaging cells (HEK293T) or pseudo-lentiviral particles will leave the EG071 laboratory (all of the equipment for cell culture; class 2 biological safety cabinet and 5% CO2 incubator are in EG071). GMO containing tissue culture flasks will be placed on the designated bottom shelf of the incubator. Where 6 well plates are used (volume <6 ml), incubations will be carried out inside a separately sealed container labelled ‘Genetically Modified Organisms’ and ‘Lentivirus’.

Monitoring of waste inactivation methods:

The liquid waste in 10 % hypochlorite solution will be tested for viability following 48 hours by inoculating 5 ml RPMI tissue culture medium and subsequent incubation. If inactivation method is efficient, no growth should be evident after 24 hours.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

02/03/2022  Page 13018 of 15326
The committee requested further clarification relating to the leukaemia cell lines that will be used in the final transductions. These have been added to the document.

The committee requested more detail relating to the respective vector characteristics. This has been completed in full and added to the document.

The committee wanted further clarification relating to the containment of viral producing HEK293T cells, including the volume of liquid culture. This information was completed and added to the document.

The committee was happy to approve the work subject to HSE approval. The committee chair recommended that CU2 was to consulted before work begins.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Data Premises Notified: 10/03/2011
(Originally)

Transferred from 1992 Regs?: N

Transitional Premises Class:

Data Premises Closed

Transitional Premises

Emergency Plan Required?: N

Non-GMMs: N

Withdrawn: N

Name

PROTEIN TECHNOLOGIES LTD

Name 2

Department

Campus Estate or Research Centre

MANCHESTER SCIENCE PARK

Building

GREENHEYS BUILDING (UNIT 51)

Road Name

District

Town

MANCHESTER

County

GREATER MANCHESTER

Postcode

M15 6JJ

Country

ENGLAND

Tel Number

07950847647

Fax Number

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Y

**Give brief details of the genetic modification safety committee**

The Genetic Modification Safety Committee (GMSC) comprises of the two qualified individuals: The Biological Safety Officer (BSO) who holds a Chief Scientific Officer position at the company and the Biological Training Officer (BTO) who is an experienced post-doctoral scientist. The specific roles of the BSO will be the management and implementation of health and safety legislation (Management of Health & Safety at Work Regulations) by inspecting the laboratory, ensuring sufficient stocks of disinfectants etc and observing workers and will write regular reports to ensure a safe working environment. The role of the BTO will be the daily implementation of GM safety induction check list and making sure they understand and implement GM safety procedures. The BSO will be immediately informed if there are GM incidences and containment measures will be taken and a report written and filed for HSE inspection. In the context of the GM safety and Contained Use Regulations, meetings between the BSO and BTO will be held six months to review safety measures, disposal issues and implementation of further safety measures will be implemented and a written report will be filed.

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The company will use Class 1 organisms that will include bacteria (E. coli) and other microbes such as yeast and pichia. Note that all of the strains used are non-pathogenic for humans. For example the E. coli BL21 strain will be used which is a normal laboratory strain which is considered harmless. The E. coli are BL21 strains are found to be poor gut colonizers even in an animal model highly susceptible to the establishment of a persistent infection (J Applied Micro 89 (2000) 1048-1058). To safeguard human health measures for inactivation of microbes will be done with an autoclave on site, disinfectants and cleaning materials will be available, laboratory coats and gloves will be worn, no drinking/eating will be done at the laboratories.

Waste management: Microbial samples will be placed within a biohazard bag which will be removed by the BTO or technical staff for autoclaving and incineration (ii), in case of spillage, a solution of disinfectant (Virkon 1% w/v in water-stable for 7 days). Spills of infected material will be attended to immediately by laboratory personnel (wearing gloves and lab coats) followed by treatment of the affected area with diluted Virkon disinfectant.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The company's GM safety committee seeks to maintain good microbiological practice for handling of E. coli and yeast cultures, i.e. containment measures for level 1 organisms. Neither the K12 or BL21 commercial E. coli or yeast strains used are considered pathogenic to humans or animals. The E. coli strains used are very unlikely to survive or thrive in the environment as they have auxotrophic requirements or other debilitating mutations. The E. coli strains used would not be hazardous to the environment even if they did survive. The GMOs are unlikely to be any more harmful or better able to survive than the original host. There are no special measures necessary to protect the environment & details of waste disposal and emergency clean-up procedures have been described in Note 6. Agar plates, batch cultures and all other contaminated materials will be autoclaved before disposal. E. coli K12 strains are recognised as non-colonising to humans and are unlikely to persist in the gut, lung, or survive outside of the special culture medium which provides their auxotrophic requirements. The BL21 strains are E. coli B derivatives sold commercially for use with T7 promoter-driven vectors, principally the pET series, and are also found to be poor gut colonizers even in animal model highly susceptible to the establishment of a persistent infection. (J Applied Micro 89 (2000) 1048-1058)
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**Name**

MORVUS TECHNOLOGY LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

TY MYDDFAI

**District**

LLANARTHNE

**Town**

CARMARTHEN

**County**

**Postcode**

SA32 8HZ

**Country**

WALES

**Tel Number**

01558 667182

**Fax Number**

01558 67199

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

We have established a genetic modification safety committee to advise on risk assessment to human health and environment.

The membership consists of the following persons.

- Biological Safety Officer
- Deputy Biological Safety Officer
- Staff Representative
- Safety Officer
- Management Representative

The Biological safety officer will act as a Chairperson and the Deputy Biological Safety Officer will act as a Secretary.

Membership will be renewed annually at the first meeting each year.

The committee may co-opt or invite other persons to be in attendance as it requires, including those are personally involved in the work under consideration.

Responsibilities of Officers

The Biological Safety Officer will be the Chairperson and responsible for receiving proposals on behalf of the committee and ensuring that they are prepared correctly. He/She will monitor compliance with the relevant statutory Regulations and call meetings through the committee Secretary.

Duties of the Members

All the members shall report relevant matters to the Committee and promote safety awareness among all the members of staff. Members shall acquaint themselves with safety as applied to genetic modifications and biological agents.

Meetings

To be held twice annually at not more than six-monthly intervals. A record of the Biological Safety Committee meetings in the form of Minutes to be kept and circulated to Committee members, Safety Committee and Senior Management.
Liquid biological waste: The liquid waste will be collected in sealed glass containers, decontaminated with Virkon or Tri Gene and then discharged.

Disinfectant to be used, exposure time and working concentration:

- **Virkon**: Concentration 1-3%                  Exposure Time > 20min
- **Tri Gene**: Concentration 1:50% dilution    Exposure Time > 20min

Source of validation data: (e.g. manufacturer's data or own studies)

Manufacturer's Data

Solid biological waste: The solid waste (disposable plates, pipette tips, etc) will be collected in autoclavable bags, decontaminated by two cycles of autoclaving and then disposed.
Decontamination by autoclaving cycles.

Each waste bag will be autoclaved at 136 degrees celcius for 60 minutes

Number of autoclaving cycles per bag: 2

Source of validation data: (e.g manufacturers data or own studies)

Manufacturers data

Autoclave maintenance and validation method

Autoclave will be annually serviced by the certified engineer

During the autocalve runs, heat-sensitive autoclave tape will be placed on flasks and test tube racks as a rapid autoclave validation method.

Tubes of Bacillus subtilis spores (3 per autoclave) will be used as biological indicators. They will be labeled and placed at the front, middle and back of the chamber.

After the cycle, the B. subtilis tubes will be allowed to cool down to room temperature. 0.1 ml from each tube will be plated onto separate labeled TSA (tryptic soya agar) plates and will be incubated for 48 hr at 37°C.

Source of validation data: (e.g. manufacturer's data or own studies)

Manufacturers Data

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Tubes of Bacillus subtilis spores (3 per autoclave) will be used as biological indicators. They will be labeled and placed at the front, middle and back of the chamber.

The micro-organisms which will be used in all three projects, MTL-101, MTL-103, and MTL-201 belongs to Hazard Group 1, as designated by the UK Advisory Committee on Dangerous Pathogens (ACDP), that is a ‘biological agent unlikely to cause human disease’. The routine cloning and expression work in attenuated E. coli strains have a long history of safe use and most can be handled safely at Containment Level 1. The low risk host-vector systems will be used in these projects. The general risk assessment as well as risk assessment to human health and risk assessment to the environment is found to be satisfactory. The activity is assigned as class 1 and will be handled at containment level 1 laboratories.
GM Centre Number: 3121

Data Premises Notified (Originally) 01/07/2011
Transferred from 1992 Regs? N
Transitional Premises Class
Data Premises Closed
Transitional Premises
Emergency Plan Required? N
Non-GMMs N
Withdrawn N

Name
LIFE TECHNOLOGIES LTD

Name 2
Department

Campus Estate or Research Centre

Road Name
7 KINGSLAND GRANGE

District
WOOLSTON

Town
WARRINGTON

County
CHESHIRE

Postcode
WA1 4SR

Country
ENGLAND

Tel Number 01925 282715
Fax Number 01925 282702

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**
  - Y

- **Give brief details of the genetic modification safety committee**
SOP 4384445 EHS Committee. Meetings take place on a monthly basis which include safety representatives from all departments on site including management. Site Director chairs the meeting.
Committee members will be volunteers from any department and are elected in line with the Health and Safety (Consultation with Employees) Regulations.
Membership shall be for a minimum of twelve months.
When their term of office is near to an end, Committee members will be responsible, along with the departmental manager, in finding a replacement volunteer member if they no longer wish to carry on this activity.
EHS Committee meetings will be held every month at Kingsland Grange and quarterly at Lingley House.
The meeting will require that at least 90% of the committee members are present.
Members who will be absent from any meeting, for whatever reason, should notify the EHS Advisor as soon as possible before the meeting and nominate upwards for another employee to attend.
EHS Committee meetings shall be conducted to an Agenda e.g
Minutes of last meeting/action items review.
Accidents / Incidents / Near Misses / Property Damage / Occupational Health
Inspection findings
Policies/Regulatory Compliance
Personal Protective Equipment (PPE)
Biosafety / GMM
Review of metrics
Environmental Matters
Any Other Business
Minutes will be taken at each EHS committee meeting and shall be reviewed by the EHS Advisor and the Senior Management committee representative who signs and dates the minutes as being a true record.
The minutes will then be communicated to the site no more than 7 working days after the meeting and a copy posted on the EHS notice board and site Shared Public Drive

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Bacteriology | Parasitology | Transgenic | Microbiology |
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Any Solid waste will be autoclaved and incinerated
Glassware will be treated with disinfectant at the recommended concentration for the stipulated length of time before being washed and autoclaved.
I. SOP for disinfection of CL1 waste for drain disposal
1. Effectiveness:
All liquid waste will be treated with disinfectant and autoclaved (e.g. Trigene at the recommended concentration).
2. Recommended Personal Protective Equipment:
   • Lab coat
   • Latex or nitrile gloves
   • Safety glasses
3. Concentration:
The appropriate concentration of Trigene at the recommended concentration shall be used dependant on volume of waste.
4. Contact time:
An appropriate contact time of Trigene with liquid waste is >180 minutes before drain disposal. After >180 minutes of contact, disinfected liquid waste is poured down the sink and the drain is flushed with copious amounts of water.
II. SOP for autoclaving CL1 liquid waste for drain disposal
1. Effectiveness:
   Autoclaving is an effective means of sterilizing CL1 liquid waste. Sterilization refers to the complete killing of all living organisms, including spores. The autoclave is validated for effectiveness at each use by using Indicator tape autoclave which conforms to BS7893, 1997 and BS EN 867-2, 1997
2. Recommended Personal Protective Equipment:
   • Lab coat
   • Latex or nitrile gloves
   • Heat resistant gloves
   • Safety glasses
3. Procedure:
a. Collect BL1 liquid waste in autoclavable, leak proof containers that are never more than ¾ full.
b. Place containers in an autoclavable tray in the autoclave. LOOSEN each container top and place indicator tape on each top.
c. Adequate cycle time varies depending on load, type of autoclave, and secondary containment.

Tick to confirm that you are attaching a summary of the risk assessment ✗
Project Ref 3121/11.1

Date Ackn'd 01/07/2011

CU2 Project Title
Work with recombinant bovine viral diarrhea virus (BDV)

Class
Class 2

Culture Vol
1-50 Litres

Class Culture Vol
Class 2 Culture Volume 1-50 Litres

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purpose of the contained use
The aim is to produce viral vectors that are not infectious to humans which can be used to calibrate or control molecular biological extraction, amplification and detection processes and systems (e.g. diagnostic assay for RNA viruses) by utilising the pathogen target sequence in the recombinant BVDV.

Recipient or parental organism
The recipient organism is a non cytopathic BVDV (NCP7) which contains a deletion of the CP7 specific 27 nucleotide insert. The cDNA of this construct has been further modified by the insertion of four unique cloning sites in the 3’ UTR (Baroth et al., JGV, 2010, 91, 1213-7). BVDV is a member of the Pestiviruses genus, in the family Flaviviridae and is an animal pathogen. Bovine viral diarrhea is a disease of cattle, it causes a mild infection in adult animals, however if
a fetus is infected in the first third of gravidity a persistent infection occurs, if infection with another BVDV occurs it
can be fatal. Symptoms of the virus infection include fever, diarrhea, respiratory disease and a discharge from the
eyes and nose. Most BVDV infections in adult animals are self limiting and there is no effective treatment. Cattle in
the UK can be immunised against BVDV using an inactivated vaccine.

Host/vector system

The Vector
The vector is BVDV NCP7 which harbors four unique restriction sites in the 3' UTR (NCP7 MCS) (Baroth et al., JGV,
2010, 91, 1213-7). Construction of the recombinant BVDV will be via a subclone which contains a fragment of
approximately 1200 base pairs of the BVDV 3' UTR in a pblue script vector, once the target sequence has been
inserted in to the subclone it will be transferred back into the NCP7 MCS vector.

The Host
The host cell line is Madin-Darby Bovine Kidney (MDBK) cells, it was derived from kidney cancer of an apparently
normal Bos Taurus and is BVDV free. MDBK cells would not be able to survive outside of the laboratory culture
conditions.

Origin & function

Quantitation standard and internal controls for molecular diagnostic kit / products based on quantitative PC2
technology, origin as host vector system as described above. The aim is to produce viral vectors that are not
infectious to humans which can be used to calibrate or control molecular biological extraction, amplification and
detection processes and systems (e.g. diagnostic assay for RNA viruses) by utilising the pathogen target sequence in
the recombinant BVDV.

Activity

Evaluation of foreseeable effects

The Insert
Recombinant BVDV will contain RNA inserts. This insert will be located in the 3' UTR of BVDV and will therefore not be
translated, only replicated. The inserts will pose no risk of infection to the natural host of the insert, the environment and
no further risk of infection to cattle. E.g. an insert is genetic material from HCV, a member of the genus hepacvirus of the
family Flaviviridae this is a human pathogen.

Human health considerations
The parental BVDV is non infectious into humans and poses no threat to human health. The inserts however, can be
derived from human pathogens, but are situated in the 3' UTR of the BVDV and thus not translated, posing no threat to
human health. This insert is a small portion of a human pathogen genome which is not infectious when isolated from the
parental virus and is thus not a threat to human health.

Environmental considerations
The recombinant virus is able to replicate and release infectious virus to similar levels observed for the parental wild type
(WT) virus. If this was released into the environment it would pose a risk to animal health, this risk would be very low
due to the instability of the virus when exposed to the environment and when it is outside of its normal transmission
route. The main transmission route is generally by direct contact with that are infected with BVDV.

Level II containment and following good microbiological practice will be implemented in the laboratory. Access to the
laboratory will be restricted to personnel trained to work with category II contained microorganisms when work with
infectious agents is in progress. A sign on the door will notify people of the microorganism being used, the personal
protective equipment required and entry and exit procedure if required. Work will be performed whilst wearing personal
protective equipment and biosafety procedures for handling the virus will be outlined in the standard operating
The virus will normally be completely inactivated with \( \beta \)-propiolactone, an agent used in inactivate BVDV for vaccines, before it leaves the facility.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Determination of final classification and containment measures

WT BVDV is classified as Class II, it is a common virus and whilst most BVDV infections are self limiting except if the fetus is infected during early pregnancy the virus can cause a persistent infection. There is no effective treatment against BVDV, however, cattle in the UK can be immunised against BVDV using an inactivated vaccine.

The genetic inserts are from human viral pathogens and pose no risk to human health or the environment.

The recombinant BVDV will replicate less efficiently or to a similar level to WT parental NCP7 BVDV, it is therefore allocated to Class II, GMM activities of low risk for which level II containment is appropriate to protect human and animal health as well as the environment.

The GMM activity is therefore classified as Class II.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Once the RNA has been introduced into the MDBK cell line the RNA can replicate and produce infectious virus.

- Gloves will be worn when handling infectious material
- Once RNA has been transfected into MDBK cells pipette tips will be washed with disinfectant and discarded into a sharps bin or bag located in the BSC II tissue culture hood.
- Pipettes (disposable stripettes) will be washed out with disinfectant and then discarded into pipette disinfection chambers that are filled with disinfectant. After being soaked overnight the pipettes will be autoclaved and disposed of by incineration by an authorised third party.
- Plastic wear that has come into contact with MDBK cells that are infected with BVDV will be soaked in disinfectant for at least the minimum time indicated on the chosen disinfectant or overnight.
- All waste once treated with disinfectant will be autoclaved and incinerated.

To bring virus out of the hood and opened on the bench in the laboratory to do downstream work cells will either be fixed or lysed in the BSC level II tissue culture hood.

No floor drains are present in the lab and potential of spillage is low.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
SOP 4384445 EHS Committee. Meetings take place on a monthly basis which include safety representatives from all departments on site including management. Site Director chairs the meeting. Committee members will be volunteers from any department and are elected in line with the Health and Safety (Consultation with Employees) Regulations. Membership shall be for a minimum of twelve months. When their term of office is near to an end, Committee members will be responsible, along with the departmental manager, in finding a replacement volunteer member if they no longer wish to carry on this activity. EHS Committee meetings will be held every month at Kingsland Grange and quarterly at Lingley House. The meeting will require that at least 90% of the committee members are present. Members who will be absent from any meeting, for whatever reason, should notify the EHS Advisor as soon as possible before the meeting and nominate upwards for another employee to attend. EHS Committee meetings shall be conducted to an Agenda e.g Minutes of last meeting/action items review. Accidents / Incidents / Near Misses / Property Damage / Occupational Health Inspection findings Policies/Regulatory Compliance Personal Protective Equipment (PPE) Biosafety / GMM Review of metrics Environmental Matters Any Other Business Minutes will be taken at each EHS committee meeting and shall be reviewed by the EHS Advisor and the Senior Management committee representative who signs and dates the minutes as being a true record. The minutes will then be communicated to the site no more than 7 working days after the meeting and a copy posted on the EHS notice board and site Shared Public Drive

Project Containment

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02/03/2022 Page 13035 of 15326
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Name

VAXIN UK LTD

Name 2

Department

Campus Estate or Research Centre

LONDON BIOSCIENCE INNOVATION CENTRE

Road Name

2 ROYAL COLLEGE STREET

Town

LONDON

County

GREATER LONDON

Postcode

NW1 0NH

Country

ENGLAND

Tel Number

0207 691 4908

Fax Number

0207 681 9129

E-mail

HSE Division

LONDON

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Immune Targeting Systems (ITS) has established a GM safety committee chaired by the Research and development Director and members include the responsible person for the health and safety and at least one member of the scientific staff. ITS will employ an external health and safety advisor to provide additional advice if and when required. The GM safety committee will follow regulations and guidelines in accordance with the Genetically Modified Organisms (Contained Use) Regulations 2000.

ITS’s projects involving the use of genetically modified cells will require a risk assessment and review by IT’s GM Safety Committee before work will commence.

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Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential
Decontamination and disposal procedures will be followed according to guidance described in IT’s operational handbook. Decontamination procedures will use Virkon solution which is a reliable broad spectrum high level disinfectant. Virkon is effective against Hepatitis A, B, and C, HIV, MRSA, VRE, Norovirus, Influenza A Virus. Virkon is rapidly effective killing 99.999% of micro-organisms in less than 10 minutes, even in the presence of organic matter. The manufacturer recommends using Virkon at 1%.

All work surfaces including equipment, the MSC enclosure or other surfaces will be decontaminated with 1% Virkon solution (Hard surfaces) or 3% Virkon solution (for absorbent surfaces). All disposable items such as plasticware and pipette tips will be decontaminated with a 1% Virkon solution overnight before disposal to the clinical waste bag prior to autoclaving. GMM material will be inactivated by exposure to excess volumes of 1% Virkon solution for 24 hours. Prior to disposal in the autoclaved clinical waste. Monitoring of the 121°C, 1.5 hour autoclave cycle is achieved using a chart recorder and records of individual autoclave cycles are tracked. Autoclaved waste is disposed of in the general clinical waste.

For animal carcasses' will be incinerated.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

ITS's GMSC reviewed the risk assessment for the project 'Assessment of antigen-specific T cells responses induced by vaccination with fluoropeptides to induce effective anti-tumour responses on 26.05.2011. The risk assessment was considered to be appropriate for the GMM used. The GMSC also agreed that the classification as a class 1 activity was appropriate.
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<td>01530 223351</td>
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<tr>
<td>Fax Number</td>
<td>0870 166 6233</td>
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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
# Premises Addresses

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# Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

- Associate Professor at University of Nottingham with 10+ years of experience on GMSC, including chairing GMSC. Biological Safety Officer. 24 years experience with GMOs and BSO at large industrial site in Loughboough for several years.
- Deputy BSO: Post doc with 8 years working with GMOs
- Technical director: 12+ years experience working with GMOs
- Chief Operations Officer: 14 years experience working with GMOs
- Site health and safety officer. Professional with considerable experience of health and safety issues
- Administrator: Company administrator and non expert rep on committee

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  

Tick if confidential  

02/03/2022
For all risk class 1 and 2 activities involving GMMs, Trigene Advance will be used as the general disinfectant/decontaminating agent and has been shown by the manufacturer to be effective against bacteria, viruses and cells.

For general disinfection on non-soiled surfaces, Trigene Advance will be used at 2 part concentrate in 100 parts water (2% v/v) which is greater than the manufacturer's recommended concentration. 2% Trigene will be sprayed over the surface, the surface wiped with blue towel, then rinsed with milliQ water and wiped followed by spraying with ethanol and wiped.

For disinfection and cleaning of surfaces in the presence of split media containing 10% serum, the media would be mopped up with a paper towel (which would be placed in a yellow clinical waste bag) and 2% Trigene used to decontaminate the area as above as recommended by the manufacturer.

All spent contaminated growth media containing E. coli, mammalian cells or serum within plasticware will be decontaminated by adding Trigene advance to 5% v/v and allowed to stand for 24h before disposal down the drain.

All contaminated plastic pipettes will be immediately sluiced with 5% Trigene before disposal into plastic clinical waste e.g. plastic gloves and paper waste.

Validation of inactivation of waste disinfectants. The concentrations of Trigene disinfectant to be used are based on the manufacturer's recommendations for these organisms, taking into account the protein concentration.

Solid waste containing GMOs that has not been decontaminated e.g. plastic petri dishes containing agar growth media and live bacterial colonies (risk class 1) will be placed in sealed leakproof clinical waste bins which will then be placed in autoclave bags and autoclaved to inactivate the GMOs. These will then be bagged and treated as normal clinical waste. The autoclave is routinely inspected and maintained according to the manufacturer's instructions.

All inactivated clinical waste material is double bagged and sealed and kept in leak-proof containers. All clinical waste (bagged and in incineration bins) will be taken off site by the accredited company SRCL (formerly White Rose) for disposal by incineration.
The generation of replication deficient lentiviral particles will be carried out in a designated class 2 safety cabinet and transfected and transduced cells likely to have "live" lentiviral particles will be incubated in a designated incubator.

An up to date record of all the genes used in this programme of work as well as the workers involved in generating the replication deficient lentiviruses is to be kept.

**Project Ref** 3125/11.1

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<th>CultureVolumeClass3-4</th>
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<td>15/07/2011</td>
<td>Replication deficient lentiviral transduction cell-lines and primary cells for evaluation of growth and tumorigenicity in vitro and in vivo</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The intention is to generate cell lines which can be used with cancer research for the evaluation of tumour cell growth and proliferation in both in vitro and in vivo settings. Mammalian cells, including epithelial cells, mesenchymal stem cells (MSCs), fibroblasts, and endothelial cells will be transduced using lentiviral particles with fluorescent markers (Green, Red, Yellow, etc., Clontech) or luciferase markers (Firefly, Renilla, Promega), driven by a constitutive or inducible mammalian promoters (CMV, EF1α, s100a4, etc) in order to label the cells with these markers, so that cell numbers/proliferation and biological processes can be monitored. The cells will also be transduced with genes or shRNAs (small hairpin RNAs that cause gene knockdown) against genes involved in tumorigenic processes, including for example growth factors and their receptors, proteolytic enzymes or oncogenes.

The transduced cell-lines will be co-cultured in various 2 dimensional and 3 dimensional in vitro models to examine the role of the target genes on cell viability, effects within cells and cell-cell interactions. In addition, the transduced or transfected cells will be used to establish xenografts in rodent models, to study the effects of anti-cancer research agents and the effect of target gene up-regulation or knock down on tumours within that in vivo system.
Recipient or parental organism

During routine cloning non colonising or disabled strains of E. coli K12 will be used e.g. DH5α and XL1-blue. These have a long history of safe use and are non-pathogenic.

The recipient cell lines will be human MSCs, primary tumor- and normal tissue-derived fibroblasts, and a variety of colorectal, pancreatic, prostatic, mammary, gastro-oesophageal, ovarian, lung, melanoma, leukemic, glioblastoma, renal, and bladder epithelial tumour cell lines, including those in the NCI-60 panel, as well as primary human tumour derived cells, and cells of endothelial origin. All work will be performed at containment level II as some of the cell-lines to be used require this level of containment due to the potential of endogenous pathogens (e.g. primary tumour samples).

Host/vector system

Non-viral vectors

All vectors to be used are standard non-mobilisable or mobilisation defective plasmids in E. coli.

Replication-deficient lentiviral vector systems

Examples of the lentiviral vectors to be used are from the Lenti-X system (Clontech) for constitutive or inducible gene expression, and the TripZ system (OpenBiosystems) for inducible shRNA-mediated knockdown. These systems produce lentivirus particles which are third- or fourth-generation viruses, according to SACGM guidelines because the viral components required to make infectious particles are enclosed on multiple plasmids separate from the expression vector. These viral components gag (nucleocapsid), pol (reverse transcriptase) env (envelope protein, determines cells which can be infected) and rev (involved in translation of gag and env) must be transiently transfected into the virus-producing cells (the standard HEK293T cells will be used for this purpose) along with the expression vector, in order to produce viral particles. Typically 4 packaging plasmids containing these essential lentiviral genes are required. None of the packaging plasmids contain effective LTRs or Ψ packaging sequence, and none of the HIV-1 structural genes are present in the packaged viral genome, and therefore never expressed in transduced cells, so no new replication-competent virus can be produced following transfection of the HEK293T cells, the genes are not transferred into the viral particles created, or the cells transduced by these particles, unless multiple recombination events occur which bring all of the viral components into the expression vector. This specific series of recombination events is considered extremely unlikely. Although the replication deficient viral particles produced by the HEK293Ts are able to transduce a broad range of human cell-types, including primary cells, no further viral particles are reproduced by the transduced cells.

Origin & function

The inserted genes encode for either
a) Coloured fluorescent proteins (GFP or RFP from bacterial and marine sources) or luciferase protein (from firefly or Renilla) which will be used to monitor cell numbers/proliferation/promoter activity. No harmful effects on cell biology are known to be associated with these gene products.

b) DNAs encoding potential human oncogenes and tumour suppressor genes from commercial sources, including growth factors (e.g. gastrin, TGF-beta, hepatocyte growth factor) and their receptors (e.g. CCK-2R, c-met), transcription factors (e.g. Snail, Slug, Zeb, Twist), proteolytic enzymes (e.g. matrix metalloproteinases), genes involved in cell cycle (e.g.PLK-1) genes involved in cell metabolism, genes encoding for kinases, involved in protein synthesis and degradation.

c) shRNA (small hairpin RNAs that cause gene knockdown) against specific target genes that may be involved in tumorigenic processes, including for example growth factors and their receptor, proteolytic enzymes, kinases and potential oncogenes. These are typically obtained from commercial sources as cloned DNAs.

Evaluation of foreseeable effects

The most hazardous GMM used will be the lentiviral particles enabling expression of potential oncogenes and tumour suppressor genes.

Rationale for this statement:

With regard to the unmodified vector, these are third generation lentiviruses which are replication deficient and thus can be handled at containment level 1. SACGM guidance states: “Replication defective vectors that cannot infect human cells can generally be considered class 1. For replication defective retroviruses and letiviruses capable of infecting human cells, if the risk assessment demonstrates they are adequately attenuated, it is possible to designate the activity as class 1:- Factors supporting this classification will include: low risk of generation of Replication Competent Viruses (e.g. a third generation packaging system), self inactivating (SIN) LTR and non-harmful insert. However, contaminated sharps represent a significant hazard (see paragraph 31), and their use should be excluded for vectors that can infect human
cells, if the activity is to be designated class 1.

The lentiviral system to be used is a third generation system producing virus particles which are replication deficient, thereby minimising the possibility of the resulting stable cell-lines producing viral particles. However the genes encoded in the lentiviral particles are potential oncogenes and tumour suppressors; upregulation of oncogenes and downregulation of tumour suppressor genes is potentially carcinogenic. The greatest risk would be associated with accidental infection of someone during handling of the viral particles with the viral particles themselves which may be oncogenic. To reduce the likelihood of this occurring, the viral particles will be handled at containment level 2 in a microbiological class 2 safety cabinet, gloves will be worn by the worker at all times and no sharps will be used. However, even in this worst case scenario, no further infectious virus would be produced by the individual's infected cells and only single genes can possibly be modulated. NB modulation of multiple genes is generally thought to be involved in transformation of cells, thus reducing the risks involved with accidental infection. Other GMOs to be generated:

The replication deficient viral particles will be used to stably transduce primary tumour cells and cell lines. After a short period of culture the GMM cell lines created from lentiviral transduction will be free of any functional virus and will not therefore be of any harm to human health and the environment. The cell lines are unlikely to be harmful even if accidentally injected into a worker as the worker's immune system would naturally clear foreign cells. No workers will be allowed to work on their own or a colleagues endogenous cells.

The original replication deficient viral stock (applied at up to 2.5 million per ml to achieve a multiplicity of infection (MOI) of 50, the upper end of range recommended) will have been diluted more than 10xlog10-fold following 5 washes (10ml reduced to 100ul at each wash), and in addition, these viruses can survive in culture conditions for only a few days. Transduction efficiencies vary for different cell-lines and for all cell-lines, transduction efficiency is correlated with MOI (Zhang et al, 2004). The significance of controlled conditions in lentiviral vector titration and in the use of multiplicity of infection MOI (from 32 to 2) resulted in a reduction of the transduction efficiency from 80 to 20% in the cell-line most susceptible to transduction, and to below 10% in the other cell-lines. A 10xlog-fold reduction in the viral titres is regarded as more than adequate to ensure the absence of any functional virus in the culture supernatant, and the transduced cells can then be handled at containment level 1. There will be no free or functional lentivirus particles present when lentivirus-transduced cell lines are implanted into recipient mice, so the establishment of xenografts can also be performed at containment level 1.

However, the animals to be used are kept at containment level II and thus, this work will also be carried out at this level.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Generally mutant mice e.g. MF1 nude or NOS/SCID mice will be used. However occasionally transgenic mice may be used for the Xenograft models. There is a lower risk of infection in workers derived from bites (than from animals naturally existing in the wild) due to high health status of animals. The consequences of all environmental hazards from these animals are of either an equal or lower risk to that which is posed by animals naturally occurring in the wild. The containment and control measures in place are:

1. All transgenic rodents are housed in isolators with independent supplies of filtered air and water or quarantine approved rooms.
2. All waste is bagged up and disposed through an approved waste disposal contractor.
3. Rederivation is carried out by trained staff. All equipment is sterilised before and after each use.
4. All carcasses are disposed through an approved controlled waste disposal contractor.
5. Site pest control policies are in place using a combination of rodent barriers and the services of pest control companies.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For all risk class 1 and 2 activities associated with this programme of work, Trigene Advance will be used as the general disinfectant/decontaminating agent and has been shown by the manufacturer to be effective against bacteria, viruses and cells.

For general disinfection on non-soiled surfaces, Trigene Advance will be used at 2 part concentrate in 100 parts water (2% v/v) which is greater than the manufacturer's recommended concentration. 2% Trigene will be sprayed over the surface, the surface wiped with blue towel, then rinsed with milliQ water and wiped followed by spraying with 70% ethanol and wiped.
For disinfection and cleaning of surfaces in the presence of spilt media containing 10% serum, the media would be mopped up with a paper towel (which would be placed in yellow clinical waste bag) and 2% Trigene used to decontaminate the area as above as recommended by the manufacturer.

All spent contaminated growth media containing E. coli, mammalian cells or serum within plasticware will be decontaminated by adding Trigene advance to 5% v/v and allowed to stand for 24h before disposal down the drain.

All contaminated plastic pipettes will be immediately sluiced with 5% Trigene before disposal into plastic clinical waste bins. All plastic ware will be placed after decontamination in yellow clinical waste bags together with other solid waste e.g. plastic gloves and paper waste.

Validation of inactivation of waste disinfectants. The concentrations of Trigene disinfectant to be used are based on the manufacturer's recommendation for these organisms, taking into account the protein concentration.

For generation of replication deficient lentiviruses, the use of sharps is forbidden. Specific separate class 2 microbiological safety cabinets and CO2 incubator will be designated for use with the repication deficient lentivirus.

Solid waste containing GMOs that has not been decontaminated e.g. plastic petri dishes containing agar growth media and live bacterial colonies (risk class 1) will be placed in sealed leakproof clinical waste bins which will then be placed in autoclave bags and autoclaved to inactivate the GMOs. These will then be treated as normal clinical waste. The autoclave is routinely inspected and maintained according to the manufacturer's instructions.

All inactivated clinical waste material is double bagged and sealed and kept in leak-proof containers. All clinical waste (bagged and in incineration bins) will be taken off site by the accredited company SRCL (formally White Rose) for disposal by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Handling of transduced cells following 5 passages is assigned to class 1. However, it will be carried out at containment level II since many of the mammalian cell lines to be used are assigned to class 2.

Generation and handling of the lentiviral particles are assigned to class 2.

The generation of replication deficient lentiviral particles will be carried out in a designated class 2 safety cabinet and transfected and transduced cells likely to have "live" lentiviral particles will be incubated in a designated incubator.

An up to date record of all the genes used in this programme of work as well as the workers involved in generating the replication deficient lentiviruses is to be kept as an appendix at the end of this risk assessment.

Risk assessment PRA1 approved, subject to minor amendments.
**Project Containment**

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<th>Growth Rooms</th>
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**Laboratory Activities**
- Animal Units
- Large Scale Activities
- Human Clinical Applications

**Project Ref** 3125/20.1

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<td>The use of recombinant strain of Herpes Simplex Virus (RBT Herpes Simplex Virus ?3) with deletion of ICP34.5 and US12 genes for in vivo administration</td>
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**Project notified under transitional arrangements** N

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**Historical Significant Changes**
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**
The targeting of cancers using viruses has significant therapeutic potential. The virus used in this work has been modified to increase specific targeting of tumour cells. This project will assess the antitumoural activity of this modified virus.

**Recipient or parental organism**
- Mice bearing tumour xenograft or allograft of human or mouse origin.
Host/vector system

HSV-1 with alteration of the genome in two regions resulting in the inactivation of 2 genes (ICP34.5 and US12).

Origin & function

Inactivation of two specific genes will result in reduced viral replication in normal tissue, while maintaining replication in tumour tissue.

Evaluation of foreseeable effects

The genetic modifications to HSV will make it safer than the parental strain, and reduce viral replication in healthy tissue. HSV with deleted ICP34.5 has been used in phase I clinical trials (PMID 10845724), with confirmation of safety and tumour selectivity reported. Viral strains with mutation of ICP34.5 and US12 have previously been characterised and used for in vivo studies (PMID 11333900).

Modified HSV can be infectious to humans with primary infection being usually mild (results in fever blisters or cold sores, usually on the face and lips which crust and heal within a few days). It is not related to livestock or avian species. Modified HSV contains modifications reducing its virulence and replication in normal tissue.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For this programme of work, Distel will be used as the general disinfectant/decontaminating agent. This is a halogenated tertiary amine compound with a blend of surface active disinfectants and detergents that has been shown by the manufacturer to be effective against bacteria, viruses and cells.

For general disinfection of surfaces, Distel will be used at 5% v/v, which is higher than the manufacturer's recommended concentration. 5% Distel will be sprayed over the surface, the surface wiped with blue towel, followed by spraying with 70% isopropanol and wiped.

In the event of a spillage, the area will be disinfected with 10% Distel.

All spent contaminated liquid and consumables will be placed into a sealed container and decontaminated with 5% Distel overnight.

All decontaminated materials will be placed in clinical waste bins for disposal by incineration.

Following completion of the work, the Class II biosafety cabinet will be disinfected and fumigated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

02/03/2022
It was noted that the material will be handled as class 2. It was noted that the modifications to the virus make increased the safety of the material.

**Project Containment**

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**Project Ref** 3125/22.1

Date Ack'n'd 06/01/2022

CU2 Project Title

The aim of this study is to investigate anti-tumour efficacy of an attenuated vaccinia virus. It will investigate the tolerability of the virus in mouse oncology models, and the effects of virus administration on tumour growth

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Non-GMM Consent Granted

Tick if notifying a connected programme of work N

Withdrawn N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Purposes of the contained use

To assess anti-tumour efficacy of an attenuated vaccinia virus in mouse models of cancer.

### Recipient or parental organism

Attenuated virus will be used to treat mice, which will either be non-tumour bearing (to confirm tolerability at doses tested) or tumour bearing (to assess anti-tumour efficacy).

### Host/vector system

The viral vector has been attenuated with mutations in the RR1(J2R) and TK1(I4L) genes. Green Fluorescent protein (GFP) gene has been inserted by homologous recombination in TK1 locus. The genetic modifications will reduce viral replication and virulence.

### Origin & function

RR1 mutation reduces viral replication, while TK1 mutation will reduce the virulence of the virus. Green Fluorescent Protein, which is a fluorescent protein exhibiting green fluorescence when exposed to light in the blue or ultraviolet range.

### Evaluation of foreseeable effects

Attenuated vaccinia virus CTx_oVV1-GFP with RR1 and TK1 mutations (western reserve strain). Exposure can potentially occur from mouse urine, droppings, saliva or nesting material.

With appropriate containment and PPE, risks will be very small. If exposure does occur, symptoms are mild, and asymptomatic infection is common in healthy individuals. When symptoms do develop, they consist of a mild rash and low grade fever. In rare cases (usually immunocompromised individuals), a more serious rash can develop, along with fever, tiredness, headache and backache.

If exposure does occur, the following actions will be taken:

a. Sharps injury (needle stick and subcutaneous biological exposure)
   • Wash exposed area thoroughly for 15 minutes using warm water and sudsing soap.

b. Skin Exposure (non-intact skin)
   • Immediately go to the sink and thoroughly wash the skin wound with soap and water.

b. Skin Exposure (non-intact skin)
   • Immediately go to the sink and thoroughly wash the skin wound with soap and water.

c. Splash to Eye(s), Nose or Mouth
   • Immediately flush the area with running water for at least 15 minutes.
   • For eyes, use the eye wash for 15 minutes while holding eyelids open.

d. Inhalation
   • Move out of contaminated area. Get medical help.

e. Splash Affecting Garments
   • Remove garments that may have become soiled or contaminated and place them in a double biowaste bag.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Distel will be used as the general disinfectant/decontaminating agent. This is a halogenated tertiary amine compound with a blend of surface active disinfectants and detergents. It has been shown by the manufacturer to be effective against viruses.

For general disinfection on non-soiled surfaces, Distel will be used at 2 part concentrate in 100 parts water (2% v/v). 2% Distel will be sprayed over the surface, the surface wiped with blue towel, then rinsed with milliQ water and wiped followed by spraying with 70% ethanol and wiped.

For disinfection and cleaning of surfaces in the presence of liquid, the liquid would be treated with 5% Distel, mopped up with a paper towel (which would be placed in yellow clinical waste bag) and 5% Distel used to decontaminate the area.

All spent contaminated liquid will be decontaminated by adding Distel to 5% v/v and allowed to stand for 24 h before disposal. All contaminated plastic pipettes will be immediately sluiced with 5% Trigene before disposal into plastic clinical waste bins. All plastic ware will be placed after decontamination in yellow clinical waste bags together with other solid waste.

All inactivated clinical waste material is doubled bagged and sealed and kept in leak-proof containers. All clinical waste (bagged and in incineration bins) will be taken off site by an accredited company for disposal by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was prepared by a PhD level scientist and was approved by the committee

**Project Containment**

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Name
SYGNATURE DISCOVERY LTD

Name 2

Department

Campus Estate or Research Centre
BIOCITY

Road Name
PENNYFOOT STREET

Town
NOTTINGHAM

County
NOTTINGHAMSHIRE

Postcode
NG1 1GF

Country
ENGLAND

Tel Number
0115 941 5401

Fax Number
0115 924 2788

E-mail

HSE Division
blank

Comments
Name change on 05/09/2011 from Signature Chemical Services Ltd

Date at Which Additional Info Submitted
02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

### Level 1 (GMMs)
- Laboratory: Yes
- Animal Unit: Yes
- Growth Room: 
- Glass House: 
- Large Scale: 

### Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial
- Other (please specify): Tick if confidential

Give brief details of the genetic modification safety committee

The genetic modification safety committee will compromise of at least 3 biologists (2 senior research scientists and 1 biology group leader), one of whom will be an assigned Biological Safety Officer or Deputy Biological Safety Officer. This committee is responsible for safety issues concerning genetically modified organisms and will meet on a monthly basis to assess all proposals to work with GMOs. The meetings will be chaired by the Biological Safety Officer, or a Deputy Biological Safety Officer, and minutes of the committee meetings will be captured and stored electronically.
Disinfection Procedure (All Sites) - GMOs will be fully inactivated using Chemgene or Virkon disinfectant used according to the manufacturer's recommendations. An autoclave is also available which will allow heat inactivation of GMOs in the event of an emergency spill.

Waste Disposal (BioCity) - Solids: Solid waste, once inactivated by Chemgene (2 % (v:v) for general cleaning, 20 % (v:v) for higher risk disinfection) or Virkon (1-2 %) disinfection, will be double bagged in yellow biohazard bags, sealed and will go into the lab biohazard waste stream. All waste awaiting incineration will be stored in leak proof containers which will allow easy disinfection if required. Laboratory waste will be collected by an external waste management contractor (Stericycle) for processing off-site. Liquids: Liquid waste, following Chemgene or Virkon inactivation (2 % or 1-2 % respectively, overnight) will be disposed of via a suitable aqueous waste route.

In Vivo Procedures (Nottingham) - Spillages are likely to be small volumes only – the area will be disinfected with 1 % Virkon (contact time 10 minutes); absorbed onto absorbent pad and disposed in clinical waste bin. Stock AAV will be disinfected with 1% Virkon for at least 10 minutes before being absorbed onto absorbent material and disposed in the clinical waste stream. Sharps and other consumables in direct contact with the AAV will be bagged/sealed and autoclaved in the licensed establishments autoclave at 135 oC for 30-45 minutes in line with validated cycle. Bedding/enrichment materials will also be autoclaved in this way before cages are washed under normal washing procedures. Carcasses will be disposed by incineration via the licensed establishment's GMO clinical waste stream.

In vivo Procedures (Alderley Park) - For in vivo studies, following IMPACT testing GM cell lines will be grown under standard tissue culture conditions using validated inactivation/disinfection procedures with approved waste streaming. Culture volumes will be <10 litres. All liquid waste (e.g. spent tissue culture media) will be inactivated using 2 % Virkon for a period of at least 1 hour, before discharging to drain flushed with copious amounts of water. Cage waste will be single bagged in yellow bags with black ties and sent for incineration offsite. Animal carcasses and tissues will be double bagged with red ties and sent offsite for incineration. Sharps will be placed in Sharpsafes, sealed, then autoclaved and sent for incineration.

Waste Disposal (Alderley Park) - All waste will be streamed according to the Alderley Park Site waste guidelines. Contaminated solid waste will be inactivated in a centralised autoclave that operates under an environmental permit. All solid waste will be transported to the autoclave in drop-sided wheele bin to the autoclave facility by trained waste operators. The autoclave has a sterilising cycle of 125 oC for 30 minutes and once completed the waste will be sent for incineration. Liquid waste will be inactivated using 2 % Virkon for a period of at least 1 hour, before discharging to drain flushed with copious amounts of water.

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Please enter comments of the GM safety committee on the risk assessment

The GMO risk assessment has been presented to the genetic modification safety committee. Summary findings: The health and environment risks with the handling of GMOs has been reduced to low, acceptable levels by the use of trained staff, procedural and containment controls and personal protective equipment.

Project Ref 3126/11.1

Date Ackn'd 18/07/2011

CU2 Project Title Using in vitro biological cellular assays to assess structure/activity properties of research compounds

Class 2

Culture Volume Class 2 1-50 Litres

Non-GMM Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To identify and optimise research compounds for progression to candidate drugs. Working as a contract research organisation and also on internal research programmes.

Recipient or parental organism

Mammalian cells including HEK, CHO, HeLa, and THP-1. All cells will be handled under Class II containment.

Host/vector system

Standard mammalian expression vectors including Pcdna3.1 and derivatives. Plasmids/vectors will be non-mobilisable or mobilisation defective.

Origin & function

Mammalian genes encoding for major classes of cellular proteins e.g. receptors, ion channels, and enzymes. The sequence of the coding regions will be known and
Evaluation of foreseeable effects

There is expected to be a very low risk of potentially harmful effects associated with the genetically modified organisms in terms of changes to host cell pathogenicity or toxicity to humans. The GMOs will have negligible ability to survive independently in the environment if accidentally released from containment. The likelihood of potentially harmful effects being realised as a consequence of exposure to the operator or environment is negligible. The intended protein classes (see above) would represent gene sequences where any hazardous biological effects are considered unlikely because of the known properties of the protein. It is highly unlikely that these proteins could be delivered to a site in the body in an active form. Mammalian cell lines are especially disabled hosts and vectors are non-transmissible. No foreseeable adverse effects to the environment are expected due to the containment and control measures put in place.

Working Practices - The named laboratory for GMO work will comply with HSE Containment Level 2 facilities and working practices. Stock GMO material will be stored in a fully maintained freezer with access restricted to named personnel only. All handling of GMOs will be performed in a Class II biological safety cabinet. All operators will wear the appropriate PPE i.e. lab coats, gloves and safety glasses and will be fully trained in the safe use of GMOs by an experienced user. The use of sharps will be kept to a minimum and all contaminated sharps will be disposed of in a dedicated sharps bin. Aerosol generation will also be minimised e.g. by using sealed centrifuge tubes that will only be opened in a Class II cabinet. In addition, the laboratory will be maintained under negative pressure. Scientists not directly involved in the GMO work but who share the laboratory facilities will be made fully aware of the working procedure. GMOs will not be transported from the designated laboratory to other areas/buildings.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The risk assessment for working with GMOs requires all control measures to be necessary and in place

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Type/form of waste - The majority of waste will be in liquid form (e.g. spent tissue culture medium, media in microtitre plates), but some solid waste will also be generated (e.g. stripette tips, reagent vials)

Disinfection Procedure - GMOs will be fully inactivated by Trigene Advance disinfectant used according to the manufacturers recommendations (MediChem International Ltd) Trigene is an industry leading disinfectant composed of halogenated tertiary amines that have broad spectrum activity against bacteria, fungi, virus spores, mycobacteria and protozoa. This reagent is DEFRA approved and uses nanotechnology to carry the active ingredients through cell walls for rapid and complete cell death (apoptosis), rather than inhibiting cell activity as with conventional disinfectant products. It is non-hazardous (non-toxic, non-corrosive) and is fully biodegradable. Please see attached document for manufacturers microbiological report describing effectiveness of inactivation. An autolave is also available which will allow heat inactivation of GMOs in the event of an emergency spill. For specific decontamination methodology, see attached risk assessment.

Waste stream - Solids, Solid waste, once inactivated by Trigene disinfection overnight (2% (v/v) for general cleaning, 20% (v/v) for higher risk disinfection), will be double bagged in yellow biohazard bags, sealed and will go into the lab biohazard waste stream. All waste will be collected by an external waste management contractor (PHS Waste Management) for processing off-site. Liquids: Liquid waste, following Trigene inactivation (overnight) will be disposed of down the laboratory drains and flushed with copious amounts of water.

Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y
The GMO risk assessment has been presented to the genetic modification safety committee. Summary findings: The health and environment risks with the handling of GMOs has been reduced to low, acceptable levels by the use of trained staff, procedural and containment controls and personal protective equipment.

**Project Containment**

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<th>Laboratory Activities</th>
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**Project Ref** 3126/15.1

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<td>03/02/2015</td>
<td>Generation of stable immortalised human cell lines in which shRNA knockdown of human genes such as EP300 or CTNS, inducible via IPGAL and delivered using a lentiviral system</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</tr>
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</table>

Withdrawn

Tick if notifying a connected programme of work

**Project Additional Information**

**Purposes of the contained use**

Knockdown of the indicated genes will be used to confirm phenotypic changes observed via treatment with drug candidate molecules are through molecule engagement.
with the intended protein target.

### Recipient or parental organism

The cell lines to be transformed with the lentiviral vectors will be immortalized human cancer cell lines. These cell lines are able to propagate in mammalian culture conditions but are unable to survive outside of a laboratory setting. The cell lines selected for transformation do not harbour any other viruses and are checked regularly for mycoplasma infection to reduce the risk of any events which may generate replication competent virus.

### Host/vector system

The cell lines will be transformed with a lentiviral vector based on HIV. This vector will be purchased from the reagent supplier Sigma-Aldrich as a product from their mission shRNA line. These virus are prepared in such as way as to be replication incompetent, including biosafety features such as splitting the viral genome over three plasmids during production to minimise the risk of recombination events. Further to this Sigma-Aldrich regulatory QC production for any evidence of recombination/replication competence.

### Origin & function

The genetic material will have originated from Sigma-Aldrich LLC. The genetic material will consist of a lentiviral genome which will be incorporated into a mammalian genome without replication of the lentiviral system. Once incorporated the DNA of lentiviral origin will encode an antibiotic selection marker (such as puromycin or neomycin) to select clones of the transformed cells. The DNA will also encode a shRNA transcript which when expressed will cause knockdown of a targeted gene such as EP300 or CTNS. The shRNA transcript will be under the control of a 3* lac operon, thus in the presence of lactose the shRNA is not expressed, however upon treatment with IPGAL repression of the shRNA is removed and the transcript is freely expressed.

### Evaluation of foreseeable effects

Although the lentiviral vector has the ability to infect humans, and random integration of lentiviral DNA to the human genome may cause cancer, the viral titre to be used in the experiments (10^6 viral particles per vector) is too small to pose a significant risk to the operator.

HIV is a significant human health concern, the viral vectors used in the above project are replication incompetent and have biosafety features built in (see 7: Host/ vector system) and thus would not cause a significant risk to human health.

Lentivirus is easily decontaminated by the use of either alcohol (destroys viral envelope) or Trigene (Destroys viral envelope and inactivates RNA) which are the routine decontamination procedures our labs, thus the risk of residual virus surviving on laboratory surfaces is low.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Larger GMOs will not be employed

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The majority of waste will be in liquid form (e.g. spent tissue culture medium, media in microtitre plates), but some solid waste will also be generated (e.g. stripette tips, reagent vials).

**Disinfection Procedure – liquid waste will be fully inactivated by 2% (v:v) Trigene Advance disinfectant used according to the manufacturers recommendations (MediChem International Ltd).**

**Solids:** Solid waste, once inactivated by 2% (v:v) Trigene disinfection overnight will be double bagged in yellow biohazard bags, sealed and will go into the lab solid waste stream. All contaminated plasticware will be immersed in 20% (v:v) Trigene overnight before disposal. Laboratory waste will be collected by an external waste management contractor (PHS Waste Management) for processing off-site. **Liquids:** Liquid waste, following 2% (v:v) Trigene/Chemgene inactivation (overnight), liquid
waste is decanted into 10 litre plastic bottles (labelled ‘Bioscience aqueous Trigene waste’)

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
no comments

Project Containment

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Animal Units

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Project Ref  3126/18.1

Date Ackn'd 14/12/2018

CU2 Project Title Development of cytopathic effect assays in DF-1 cells for the screening of MVA vaccines.

Class 2
CultureVol Class 2 < 1 Litre
Non-GMM Consent Granted

Withdrawn N
Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Sygnature Discovery intends to perform this work in order to determine the infectivity of vaccine formulations post manufacture and to determine the effect of long term storage on vaccine infectivity. Formulations are expected to have improved infectivity/stability over time compared to current formulations of the vaccine.

The parental organism/GMOs are Modified Vaccinia virus Ankara (MVA) and Sementis Copenhagen Vector (SCV). The recipient will be the DF-1 cell line (chicken embryonic fibroblasts) for the MVA vaccine and ST01 (1438 osteocarcinoma cell line. expressing recombinant D13 from vaccinia virus) for the SCV virus.

The vector system are MVA and SCV, with the host systems as DF-1 (MVA) and ST01 (SCV) cells.

Regarding MVA:
MVA was originally derived from the Chorioallantois Vaccinia virus Ankara (CVA) through serial passage (>570 passages) in chicken embryonic fibroblast (CEF) cells. Due to the high number of passages MVA lost approximately 30 kbp of genetic material, and largely lost its capacity to propagate in mammalian cells. This was done initially to produce a safer alternative to the smallpox vaccine. In 1977 MVA was licensed in Germany, and was administered to over 120000 persons with nominal side effects. Although attenuated, MVA remains able to stimulate the immune system by expressing viral genes. This enables a recombinant MVA expressing genes from a different virus to be used as an effective and safe vaccine. This has been attempted with other diseases such as HIV/AIDS, tuberculosis, malaria, influenza and hepatitis 8.

The MVA to be used here encodes antigens from Makona Ebolavirus. The inserts were created in vitro by gene synthesis and cloned into an expression plasmid before amplification in E. coli. Plasmid DNA was purified and used for recombination with the MVA genome.
MVA will undergo abortive infection in host DF-1 cells, allowing the replication of MVA DNA and expression of the Makona Ebolavirus antigens. Infection in DF-1 cells will enable the quantification of MVA infectivity, enabling the determination of vaccine stability.

Regarding SCV:
SCV was generated by deleting the D13L gene from the Copenhagen strain of vaccinia virus by Sementis. The D13L gene encodes an essential viral assembly protein (D13). Deletion of the D13L gene therefore renders SCV incapable of generating viral progeny in vaccine recipients. As such, SCV undergoes abortive infection when infecting host cells. This gene deletion still enables SCV to infect host cells and preserves genome amplification, allowing expression of antigens to modulate a host immune response. SCV has been previously used to produce a vaccine against chikungunya virus, and was able to immunize mice against chikungunya virus infection. As a modified strain of vaccinia virus, SCV can also act as a vaccine for smallpox. The vaccinia virus has previously been used as a
smallpox vaccine in Denmark and the Netherlands. The SCV to be used here encodes the red fluorescent protein from Discosoma nummiforme (dsRed). The purpose of dsRed expression is to be a marker of virus infection. The inserts were created in vitro by gene synthesis and cloned into an expression plasmid before amplification in E.coli. Plasmid DNA was purified and used for recombination with the SCV genome.

ST01 cells express the D13 protein deleted in SCV. Therefore, infection of ST01 cells with SCV will rescue the replicative capacity of SCV in ST01 cells alone, allowing the assessment and quantification of SCV titer.

Evaluation of foreseeable effects

Regarding MVA:
MVA is considered stable within the environment and has the capacity to infect a broad range of host cells, including human cells. However, in the unlikely event of the genetically modified MVA being unintentionally released there is a low likelihood of infection, as this would be dependent on exposure to mucosal membrane or open wounds. In the event of infection, symptoms of MVA in healthy adults are mild, potentially including a rash or fever. Additionally, the capacity of MVA to replicate is attenuated and any infection would be expected to be rapidly cleared by the host immune response. Expression of the Makonavirus Ebolavirus genes is not anticipated to be harmful, and is intended to elicit a protective immune response. However, the true effects of these antigens are untested in humans.
Evaluation of foreseeable effects includes the intended effect of generating data on the infectivity of the MVA vaccine in DF-1 cells the unintended effect of generating small quantities of biohazardous waste, which will be disposed of appropriately as determined by in-house risk assessments (See section 12).

Regarding SCV:
Vaccinia virus, similarly to MVA, is considered stable within the environment and has a capacity to infect a broad host range, including human cells. However, in the unlikely event of SCV being unintentionally released into the environment, the likelihood of infection is low as SCV requires direct contact with a mucosal membrane or an open wound. If this were to happen, infection of healthy individuals with vaccinia virus may produce mild symptoms including rash or fever. The capacity of SCV to replicate is attenuated due to the deletion of the D13L gene. As a consequence it would be expected that SCV would be quickly removed by the host immune response. However, infection of ST01 cell culture will enable a local replication of SCV, which may increase the likelihood of infection. Expression of dsRed by SCV is not expected to cause harm, as its intention is to act as a marker only. However, the true effect of SCV coupled with dsRed are untested in humans.
Evaluation of foreseeable effects includes the intended effect of generating date on the infectivity of SCV in ST01 cells, and the unintended effect of generating small quantities of biohazardous waste, which will be disposed of appropriately as determined by in-house risk assessments (See section 12).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All operations will be perform by trained personnel within a containment level 2 laboratory. Appropriate signage warning of the biohazard risk will be placed on all doors to the laboratory. All activities involving the vaccine will take place in a Class II microbiological safety cabinet (MSC). All aqueous waste material potentially contaminated with vaccine will be decontaminated with 1 % (v:v) Virkon overnight inside a Class II
MSC before removal into designated Bioscience aqueous waste. Potentially contaminated solid waste (powder, wipes, gloves etc.) is to be deconaminated with 1% (v:v) Virkon and sealed in a clinical waste bin for disposal by an approved waste-handling agent. Potentially contaminated surfaces will be treated 1% (v:v) Virkon, 20% (v:v) Chemgene and 70% (v:v) ethanol before being left to try. Equipment used will similarly be treated with 1% (v:v) Virkon, 20% (v:v) Chemgene and 70% (v:v) ethanol. Before leaving the laboratory all users are to thoroughly wash and dry hands.

The GMO risk assessment has been presented to the genetic modification safety committee. Summary findings: The health and environmental risks associated with the handling of GMOs has been reduced to low, acceptable levels by the use of trained staff, procedural and containment controls and personal protective equipment and as such the committee have endorsed the assessment.

Please enter comments on the GM safety committee on the risk assessment

The GMO risk assessment has been presented to the genetic modification safety committee. Summary findings: The health and environmental risks associated with the handling of GMOs has been reduced to low, acceptable levels by the use of trained staff, procedural and containment controls and personal protective equipment and as such the committee have endorsed the assessment.

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Project Ref 3126/19.1

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<td>01/05/2019</td>
<td>Respiratory Syncytial Virus with Red Fluorescent Protein (RSV-RFP1) from ViraTree (Cat. no. R131) for anti-virals therapeutics</td>
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<td>Non-GMM Consent Granted</td>
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Project Additional Information

Purposes of the contained use

Sygnature Discovery intends to perform this work in order to identify novel molecules that block RSV infectivity. Human respiratory syncytial virus (RSV) is an enveloped, nonsegmented, negative-sense, single-stranded RNA virus belonging to the Pneumovirus genus of the subfamily Pneumovirinae, the family Paramyxoviridae. RSV is the most common virus responsible for acute and severe lower airway disease in infants and young children worldwide. Despite the enormous burden of RSV disease, there is no efficacious vaccine or antiviral drug therapy yet available.

Recipient or parental organism

The parental organism/GMOs is the RSV A2 strain. The RSV genome (15.2 kb) contains 10 mRNAs encoding 11 proteins. The nucleocapsid (N) protein binds the negative-strand RNA genome and associates with the phosphoprotein (P), the large (L) polymerase protein, and the M2-1 protein to form the nucleocapsid. The matrix (M) protein is present between the nucleocapsid and the outer envelope and plays a structural role in virion assembly and budding. There are three envelope glycoproteins: the attachment glycoprotein (G), the fusion (F) protein, and the small hydrophobic (SH) protein. The genome also encodes two nonstructural proteins (NS1, NS2) which suppress the interferon response and M2-2 protein (the second product of the M2 gene) which governs the transition from transcription to replication of genomic RNA.

Host/vector system

The Red Fluorescent Protein (RFP) gene was inserted in front of the NS1 gene (as the first gene). Recombinant, RFP-expressing RSV (RSV-RFP1) was generated from the full-length RSV-GFP1 (ViraTree Product# R121) plasmid, by replacing its first GFP gene with the wild-type Discosoma RFP gene from pDsRed plasmid (Clontech cat. no. 632412). To accomplish this, the BstXI restriction site within the RFP gene was disrupted by PCR mutagenesis, and this modified RFP gene was amplified by PCR with primers that added the gene start and NS1 untranslated region preceding the RFP gene and the L gene end following the RFP gene.

Origin & function

The DsRed fluorophore was isolated from the wild-type Discosoma RFP gene. The RFP protein is expressed by the host cell (HEp-2 cells, ATCC® CCL-23™ or A549 cells, ATCC® CCL-185™) upon successful infection by the virus. The fluorescence readout can be used as a measure of RSV infectivity. RSV-RFP will be be propagated in HEp-2 cells and upon release into the supernatant, frozen down into 1 ml aliquots for anti-viral screening assays. The anti-viral assays will be performed in different formats in HEp-2 cells or A549 cells:
- Cytopathic effect assay: readout of RSV-induced cell death as a measure of infectivity using CellTiter-Glo® Luminescent Cell Viability Assay (Promega). Cells and virus are lysed at the end-point.
- RSV RFP expression from the host cell as a measure of infectivity: infected cells are fixed using 4% paraformaldehyde.
- Plaque assay to determine RSV titer: quantifying RSV-induced plaque formation in cells at different dilutions. Cells and virus are fixed using 10% formaldehyde.
- Plaque assay for anti-viral screening: quantifying RSV-induced plaque formation in cells after 10% formaldehyde fixation.
The insertion of the RFP gene has little or no effect on RSV-A2 host infectivity, and according to the manufacturer, the recovered virus replicates to near-parental RSV-A2 titers.

RSV PATHOGENICITY/TOXICITY: RSV primarily infects human epithelial cells within the nasopharynx; however, it can also infect other types of cells, including cell lines, but with much lower efficacy. Infection may lead to the formation of syncytia within the infected cell. Primary infection with RSV is generally exhibited as lower respiratory tract disease, pneumonia, bronchiolitis, tracheobronchitis, or upper respiratory tract illness. Common clinical symptoms include rhinorrhea, sneezing, cough, pharyngitis, bronchitis, headache, fatigue, and fever. In some cases, otitis media may occur. RSV infections usually begin with upper respiratory tract disease, which has the tendency to progress to lower respiratory tract disease (in ~50% cases). Severe infection (involving pneumonia) may develop among elderly patients with underlying respiratory conditions. Children and immunocompromised individuals are more susceptible to developing severe disease.

RSV EPIDEMIOLOGY: RSV occurs worldwide and is the most common cause of bronchiolitis and pneumonia among infants and young children. Within USA, 100,000 hospitalizations and 4,500 deaths annually are attributed to RSV infections. RSV is also a major cause of nosocomial infections. Morbidity and mortality is highest among children with underlying illness and individuals with immunodeficiency or immunosuppression. Virtually all children are infected by age 2 to 3. Repeated infections are common, particularly in young children with up to 5 or 6 infections per year. Although all individuals can be infected with RSV, those at high risk include premature infants, young children, elderly, immunocompromised, and children under age 2 with chronic lung conditions. Other factors that may predispose to RSV infection include: crowding (schools and day care centers), exposure to tobacco and smoke, low socioeconomic status, and family history of atopy and asthma. Infection among healthy and immunocompetent individuals tends to be less severe. RSV follows a seasonal pattern. Annual outbreaks occur during fall, winter, and early spring among urban centers. In the Northern hemisphere, epidemics peak in February and March, and may last up to 5 months. In tropical and subtropical regions, most outbreaks occur during the rainy season. RSV outbreaks involving lower respiratory illness have been reported in nursing homes and institutions.

HOST RANGE: Humans; however, various animal species can be experimentally infected with RSV including cotton rats, mice, ferrets, guinea pigs, hamsters, marmosets, lambs, and nonhuman primates.

INFECTIOUS DOSE: The infectious dose for RSV is > 160 - 640 viral units, administered through intranasal spray, as listed by the National Institutes of Health.

MODE OF TRANSMISSION: RSV is most likely transmitted through direct contact with infectious secretions (via fomites) and/or large-particle aerosols; however, close contact with infected individuals, or significant exposure of nasal or conjunctival mucosa with contaminated hands is required for transmission. Transmission via small-particle aerosols is less likely.

INCUBATION PERIOD: Incubation period for RSV infection ranges from 2 to 8 days.

COMMUNICABILITY: Communicable during the period of active disease. The disease is likely not readily transmitted from person-to-person, since significant and prolonged contact is required with infected individuals. Children are known to shed virus for long periods (up to weeks) even after clinical recovery.

DRUG SUSCEPTIBILITY: RSV has been shown to be susceptible to ribavirin, which has been used to treat severe RSV infections; however, recent studies suggest that its use produces no significant benefit.

SUSCEPTIBILITY TO DISINFECTANTS: RSV has been shown to be susceptible to ether, chloroform, and a variety of detergents, including 0.1% sodium deoxycholate, sodium dodecyl sulphate, and Triton X100. It may also be sensitive to hypochlorites (1% sodium hypochlorite), formaldehyde (18.5 g/L; 5% formalin in water), 2% glutaraldehyde, and iodophores (1% iodine). The broad spectrum disinfectant Rely+On Virkon is reported by the manufacturer to be effective against RSV.

PHYSICAL INACTIVATION: RSV is sensitive to heating above 55°C for 5 minutes (up to 90% decrease in infectivity). It is also sensitive to freezing and thawing (~90% loss in infectivity following each freeze-thaw cycle). It is also sensitive to acidic media (pH<7).
SURVIVAL OUTSIDE HOST: RSV is generally very vulnerable to environmental changes, particularly temperature and humidity. It is sensitive to high and low temperature, and to drying; i.e., low humidity levels. It loses up to 90% infectivity at room temperature after 48 hours and up to 99% at 1°C after 7 days. The optimal pH is 7.5. It may survive for about 3 to 30 hours on nonporous surfaces at room temperature.

SURVEILLANCE: Monitor for symptoms. Other than monitoring for symptoms, there are four main techniques for diagnosing RSV, including virus cultures, serology, immunofluorescence and/or antigen detection, and nucleic acid based tests. Being labor-intensive and time consuming, the first two techniques are rarely employed for diagnostic purposes in epidemiological studies. Rapid diagnostic techniques for viral antigen detection, including immunofluorescent-antibody assay, optical immunoassay, enzyme immunoassay, and chromatographic immunoassay are preferred. Most are commercially available, easy to perform and produce rapid results. Nucleic acid tests (such as RT-PCR) are generally more sensitive.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All operations will be performed by trained personnel within a containment level 2 laboratory. Appropriate signage warning of the biohazard risk will be placed on all doors to the laboratory.

All activities involving RSV will take place in a Class II microbiological safety cabinet (MSC).

All aqueous waste material potentially contaminated with RSV will be decontaminated with 1% (v:v) Rely+On Virkon overnight inside a Class II MSC before disposal into a liquid waste container within the containment level 2 suite. Before final disposal, the liquid waste container will be placed inside a 30 litre clinical sealed unit waste bin.

Potentially contaminated solid waste (wipes, gloves etc.) will be decontaminated with 1% (v:v) Rely+On Virkon and sealed in a 50 litre clinical sealed unit waste bin.

Both liquid and solid waste will be packaged as clinical waste in their respective sealed unit waste bins, collected and incinerated by an approved waste contractor.

Potentially contaminated surfaces will be treated 1% (v:v) Rely+On Virkon, 20% (v:v) Chemgene and 70% (v:v) ethanol before being left to try. Equipment used will similarly be treated with 1% (v:v) Virkon, 20% (v:v) Chemgene and 70% (v:v) ethanol. Before leaving the laboratory all users will thoroughly wash and dry hands.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All operations will be performed by trained personnel within a containment level 2 laboratory. Appropriate signage warning of the biohazard risk will be placed on all doors to the laboratory.

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The GMO risk assessment has been presented to the genetic modification safety committee.
Summary findings: The health and environmental risks associated with the handling of GMOs has been reduced to low, acceptable levels by the use of trained staff, procedural and containment controls and personal protective equipment and as such the committee have endorsed the assessment.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Project Ref** 3126/21.1

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Tick if notifying a connected programme of work N

Historical Significant Changes
Selected reporter genes will be introduced in the human NK cell line NK92 and the resulting cell lines will be cultured for use in various in vitro cell signalling assays to research candidate molecules’ activity against selected cellular targets. Reporter genes can include CD16-EGFP (Enhanced Green Fluorescent protein), destabilised luciferase gene and other fluorescent reporter genes under the control of CARE.

The parental NK-like cell line NK-92 was discovered in the blood of a Male 50YO subject suffering from a non-Hodgkins lymphoma. NK-92 is a NK-like cell line that was initially isolated from the blood of a Subject suffering from a large granular lymphoma and subsequently propagated in cell culture. The NK-92 cell line has been described (Gong et al., 1994; Klingemann, 2002). NK-92 cells have a CD3-/CD56+ phenotype that is characteristic of NK cells. They express all of the known NK cell-activating receptors except CD16, but lack all of the known NK cell inhibitory receptors except NKG2A1/CD94 and IL T2/L1R1, which are expressed at low levels. Furthermore, NK-92 is a clonal cell line that, unlike the polyclonal NK cells isolated from blood, expresses these receptors in a consistent manner with respect to both type and cell surface concentration. Similarly, NK-92 cells are not immunogenic and do not elicit an immune rejection response.

CD16 was introduced into NK-92 cells by means of retroviral transduction in the following manner. Complementary DNA encoding the gene for either the low or high affinity form of CD16 was sub-cloned into a bi-cistronic retroviral expression vector, pBMN-IRES-EGFP (obtained from G. Nolan, Stanford University, Stanford, Calif.) using the BamHI and Not restriction sites in accordance with standard methods (e.g., (Ausubel, 2002; Sambrook and Russell, 2001)). This expression vector was then transfected into the Phoenix-Amphotropic retroviral packaging cell line and the resulting virus-containing supernate was used to transduce NK-92 cell ls. Transduced NK-92 cells expressing CD16 on their surface (NK-92-CD16, also known as CD16/FcεRIy-NK-92) were separated from the residual non-transduced NK-92 cells using a fluorescence activated cell sorter (FACS). When appropriate to the intended use, the NK-92-CD16 cells were further sub-sorted on the basis of CD16 expression level using a FACS, based upon coordinate expression of Enhanced Green Fluorescent Protein (EGFP). The resulting NK-92 CD16 cells stably express CD16 in cell culture without the need for periodic antibiotic selection.

Characterization of the parental NK-92 cell line (Gong et al., 1994; Van et al., 1998) revealed that NK-92 cells are cytotoxic to a significantly broader spectrum of tumour and infected cell types than NK cells, and further that they often exhibit higher levels of cytotoxicity toward these targets. NK-92 cells do not, however, attack normal cells nor do they elicit an immune rejection response.
Since the NK-92 cell line was isolated from a large granular lymphoma subject, the cells have the potential to establish tumors in recipient Subjects. However, no evidence of tumorigenicity has been reported so far. Hazards include: percutaneous injury, ingestion, mucous membrane exposure.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All operations will be performed by trained personnel within a containment level 2 laboratory. Appropriate signage warning of the biohazard risk will be placed on all doors to the laboratory.

All activities will take place in a Class II microbiological safety cabinet (MSC).

Cells will be manipulated in a Class II cabinet - manipulations outside of the cabinet should be avoided.

Users will change gloves regularly to minimise risks of any breach of containment Any contaminated gloves must be disposed of via the clinical waste route

Centrifugation will be performed with the cells contained in screw capped tubes or sealed plates to prevent spillages and aerosol formation. Centrifuge caps will be employed for all centrifugations to provide double containment Pipette boys & multichannel pipettes will be decontaminated with 2% (v:v) Chemgene, followed by 70% (v:v) ethanol before removal from the cabinet.

All aqueous waste material potentially contaminated with RSV will be decontaminated with 2% (v:v) Chemgene overnight inside a Class II MSC before disposal into a liquid waste container within the containment level 2 suite. Before final disposal, the liquid waste container will be placed inside a 30 litre clinical sealed unit waste bin.

Potentially contaminated solid waste (wipes, gloves etc.) will be decontaminated with 2% (v:v) Chemgene and sealed in a 50 litre clinical sealed unit waste bin.

Both liquid and solid waste will be packaged as clinical waste in their respective sealed unit waste bins, collected and incinerated by an approved waste contractor.

Potentially contaminated surfaces will be treated with 2% (v:v) Chemgene and 70% (v:v) ethanol before being left to dry. Equipment used will similarly be treated with 2% (v:v) Chemgene and 70% (v:v) ethanol. Before leaving the laboratory all users will thoroughly wash and dry hands.

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
The GMO risk assessment has been presented to the genetic modification safety committee. Summary findings: The health and environmental risks associated with the handling of GMOs has been reduced to low, acceptable levels by the use of trained staff, procedural and containment controls and personal protective equipment and as such the committee have endorsed the assessment.

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<td>L2</td>
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#### Project Ref 3126/21.2

- **Date Ackn’d**: 25/08/2021
- **CU2 Project Title**: Class 2 genetic modification of eukaryotic cells using viral vectors
- **Class**: Class 2
- **CultureVolClass2**: < 1 Litre
- **Project notified under transitional arrangements**: N

#### Purposes of the contained use

This contained use notification covers a range of activities utilising lentiviral, retroviral and adenoviral vectors to genetically modify cells of human or animal origin. The genetic modification in question may include, but is not limited to: expression of known and putative oncogenes, growth factors and other proteins, with or without tags to
facilitate identification and/or purification. Modification of expression levels of endogenous proteins using methods such as shRNA or CRISPR-Cas9. Transformation and/or immortalisation of non-cancerous cells. Induction of pluripotency in cells. Use of packaging cells to generate replication-incompetent viral particles for research.

Recipient or parental organism

Lentiviral, retroviral and adenoviral vectors based on a range of human and animal viruses, such as HIV, EIAV, MMLV, AAV, MMTV and MSCV. To ensure safety of users and public, all viral vectors will be generated using a split-vector system of second generation design or later. Resulting viral particles will be demonstrably incapable of replication outside of a compatible packaging cell line and will be incapable of dissemination in the environment. Vectors may be modified to have wider tropisms through expression of ecotropic and amphotropic envelope proteins, including VSV-G, and may include either wild-type or mutated forms of the Woodchuck Hepatitis Virus Post-transcriptional Response Element (WPRE).

Host/vector system

All viral vectors will be produced using a split-vector system, where components of the viral genome are separated on different plasmids to preclude viral replication outside of their packaging cell lines. Packing cell lines such as HEK293 (or other validated packing cell lines) will be transfected with the relevant packaging plasmids and the vector carrying the genetic cassette of interest. Viral particles will be harvested from the culture medium of the packaging cell line and used to transduce human or animal cells not exceeding Hazard Group 2. Example cell lines include cancer cell lines derived from human tissue, primary human cells, mouse embryonic fibroblasts, among others.

Origin & function

Genetic material in question will be from a wide variety of origins and have a range of functions, some unknown. Example genes include hTERT for immortalisation of primary cells. p53DD for transformation of primary cells. Activated oncogenes for study of oncogenesis. Introduction of genes involved in metabolism, cell cycle regulation, or other functions, to elucidate disease mechanisms. These genes may be fused with tags and proteins such as GFP or luciferase to aid in detection and analysis. CRISPR-Cas9 may be introduced into cells to facilitate manipulation of gene expression, or insertion of identifying tags into the genome. Similarly shRNA constructs may be introduced to modulate expression of endogenous genes. Genetic material known to encode toxins or pathogenic factors which could enable infection or dissemination, is explicitly excluded from this notification.

Evaluation of foreseeable effects

All vector systems in this contained use will use split-packaging to eliminate the possibility of self-replication or dissemination into the environment. Gag, pol, env and rev genes are provided in trans (either on separate plasmids or expressed in the packaging cell lines), meaning that viral particles generated using these systems are not capable of producing new virus themselves. No credible evidence exists of replication competent virus arising from third-generation systems. From this information the risk of these viruses creating systemic disease in an individual, or disseminating amongst a population, are considered negligible.

Insertional mutagenesis resulting in cell transformation is a highly unlikely, but theoretically possible outcome of self-inoculation with these viruses. Contemporary viral vectors incorporate self-inactivating motifs to prevent activation of genes proximal to the insertion site, by removing enhancer and promoter sequences from long terminal repeats (LTRs). However, inactivation of tumor suppressor genes by insertion is a remote possibility. Special consideration will be given to viruses carrying known oncogenes, or cassettes (shRNA, CRISPR-Cas9 etc) which disrupt endogenous expression of tumour suppressor genes.

Viral titre during transduction is unlikely to exceed \(10^{E+7}\) particles / ml. Cells will be transduced sequentially with each plasmid. These factors together mean that even in the event of self-inoculation, it is highly improbable that the correct combination of genetic events would occur to induce a cancerous cell.

All work described in this notification will be subject to local risk assessment and appropriate remediations put in place. These will include, but are not limited to; elimination of sharps in cell culture experiments, adherence to Containment Level 2 procedures, provision of sleeve-covers, isolation of experiments using virus from non-virus activities in the lab, visual indicators that viral activities are being carried out on relevant communal equipment and lab areas.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

This notification only applies to cells. Whole organisms are not covered.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The majority of waste will be in liquid form. A small amount of solid waste will also be generated (e.g. stripette tips, reagent vials). Our procedures for both sites is detailed below.

Nottingham BioCity procedures:
Liquid waste: All liquid waste will be decontaminated with 20% chemgene. Chemgene inactivates a broad spectrum of viruses, including those listed here. Following this, waste will be decanted into 20 litre bottles (labelled bioscience aqueous waste, with biohazard symbol) and when full this can be decanted into the chemistry non-halogenated (non-chlorinated) waste stream for disposal by an approved contractor (Stericycle).
Solid waste: Solid waste will be soaked in 20% v/v Chemgene overnight. After soaking, liquid waste will be transferred to the liquid waste stream (see above). Solid waste will be disposed of in 24L or 60L sharps bins labelled biohazard and disposed of by an approved contractor (Stericycle).

Alderley Park Procedures:
Liquid waste: All liquid waste will be decontaminated with 1% w/v Virkon. Virkon inactivates a broad spectrum of viruses, including those listed here. Following decontamination, liquid waste will be disposed of down the sink with copious amounts of water.
Solid waste: Solid waste will be soaked in 1% w/v Virkon overnight. After soaking, liquid waste will be disposed of down the sink with copious amounts of water. Solid waste will be disposed of in 24L or 60L sharps bins labelled biohazard and disposed of by an approved contractor (Stericycle).

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Extensive discussion was conducted with the Bioscience Safety Health and Environment (SHE) team, which has responsibility for reviewing risk assessments in the department. This includes work involving genetic modification, with the SHE team constituting our Genetic Modification Safety Committee (GMSC). Our team has considerable expertise in the biology and handling of viral vectors and transduced cell lines. It was decided unanimously that these activities should be assigned contained use Class 2.

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02/03/2022
**GM Centre Number: 3127**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
# Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Yes**

Give brief details of the genetic modification safety committee

- Biological safety officer, based in the UK. Has completed "Biological Safety Officers Level 1" course and registration with ITSR. More than 20 years general safety experience gained from sitting on the laboratory safety committee, being a COSHH assessor and managing an electronics cleanroom facility.
- Biological safety officer, based in the Netherlands with responsibility for a large and varied biological research facility. Activities at this facility include bacterial genetic modifications and also use of mammalian cells, general chemistry, radio chemistry, molecular biology.
- Research Scientist with PhD in molecular microbiology, experience in the construction and use of genetically modified microorganisms

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Other (please specify) Tick if confidential

- **Yes**

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

02/03/2022
All waste generated to GMMs is to be disposed of by incineration by a licenced contactor. Before leaving the laboratory itself, the waste will be disinfected using a Rodwell Monach 50 autoclave. Samples will be heated for 15 minutes at 121°C under pressure with steam. The temperature profile within the autoclave will be monitored by the integral data logging system, which takes readings of the vessel temperature and load temperature by means of a probe. If larger volumes of liquid are to be sterilised, a matched volume of water in an identical container will be used for monitoring the temperature of the load. Confirmation that the correct temperature has been reached will be obtained firstly using the confirmation display of the autoclave. To provide a backup to this message, temperature sensitive indicator strips (Browne TST or similar), and temperature sensitive autoclave tape (3M or similar), will be used. Only when positive confirmation has been supplied by both the autoclave and by temperature sensitive indicators will the load be considered safe to leave the laboratory. Periodically the activity of temperature indicators will be tested using a biological indicator (Attest, 3M, or similar).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Overall the risk assessment covers the necessary procedures well. Include the use of a biological indicator periodically to ensure that the effectiveness of the multiple overlapping sterilisation measures is confirmed.

Project Ref 3127/11.1

Date Ackn'd 20/12/2011

Date Project Ceased

CU2 Project Title Growing Streptococcus mutans strains which contain plasmids for producing fluorescent proteins

Class Class 2

CultureVolClass2 < 1 litre

Consent Granted

Non-GMM

Project notified under transitional arrangements
### Project Additional Information

**Purposes of the contained use**

To prevent release of genetic material coding for antibiotic resistance genes and fluorescent proteins into the environment

**Recipient or parental organism**

Streptococcus mutans UA159

**Host/vector system**

pDM15

**Origin & function**

The plasmid pDM15 (Deng et al. 2009), is to be used to constitutively produce protein fluorophores such as GFP to allow visualisation of bacteria by microscopy. pDM15 is a derivative of pVA838, a non-mobilisable Escherichia coli-Streptococcus shuttle vector. The genetic material carried by this plasmid is the gene coding for green fluorescent protein from Aequoria victoria. In addition, the vector is to be used for carrying derivatives of the fluorescent protein dsRed from Discosoma sp.

**Evaluation of foreseeable effects**

The encoded genes within the pDM15 vector encode non-toxic fluorescent proteins which would have no selective benefit in human, animal or plant pathogens. It is not foreseeable that these carry any risk to the wider environment. The vectors are non-mobilisable, reducing the likelihood of the spread of genetic material from the plasmid. The vectors encode antibiotic resistance genes for erythromycin and cloramphenicol, which have both been in use for a significant amount of time, meaning resistance is likely to be widespread and easily acquired. Release of these sequences should however be minimised at all times so as to reduce the prevalence of these determinants in the environment. The plasmids have previously been found to be lost after a short time without antibiotic selection (Macrina et al. 1982), and as such would be expected to be poorly maintained in the environment if accidentally released. Streptococcus mutans UA159 is a category 2 microorganism, and causes disease in the mouth only through production of organic acids from sugar provided in the diet. It is not known to have specialist virulence determinants such as secreted toxins.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste generated related to GMMs is to be disposed of by incineration by a licensed contractor. Before leaving the laboratory itself, the waste will be disinfected using a Rodwell Monarch 50 autoclave. Samples will be heated for 15 minutes at 121°C under pressure with steam. The temperature profile within the autoclave will be monitored.
by the integral data logging system, which takes readings of the vessel temperature and load temperature by means of a probe. If larger volumes of liquid are to be sterilised, a matched volume of water in an identical container will be used for monitoring the temperature of the load. Confirmation that the correct temperature has been reached will be obtained firstly using the confirmation display of the autoclave. To provide a backup to this message, temperature sensitive indicator strips (Browne TST or similar), or temperature sensitive autoclave tape (3M or similar), will be used. Only when positive confirmation has been supplied by both the autoclave and by temperature sensitive indicators will the load be considered safe to leave the laboratory. Periodically the ativity of temperature indicators will be tested using a biological indicator (Attest, 3M, or similar).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Overall the risk assessment covers the necessary procedures well. Include the use of biological indicator periodically to ensure that the effectiveness of the multiple overlapping sterilisation measures is confirmed.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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### GM Centre Number: 3128

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**Name**

INTEGRATED MAGNETIC SYSTEMS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

DUNDEE TECHNOLOGY PARK

**Road Name**

GEMINI CRESCENT

**Building**

PROSPECT BUSINESS CENTRE

**District**

**Town**

DUNDEE

**County**

**Postcode**

DD2 ITY

**Country**

SCOTLAND

**Tel Number**

01382 561087

**Fax Number**

**E-mail**

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

IMSL's Genetic Modification Safety has three (total number of employees is currently four) and comprises the laboratory Manager (convenor), Chief Operations Officer (member) and chief Scientific Officer (member). The committee meets quarterly, or more frequently as needed. The committee members also communicate regularly by email and face to face discussions.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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Tick if confidential

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</table>

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

No GMM production will be performed in the laboratory. Only small back-up samples will be stored, to a maximum of 10ml in the first twelve months. Any spillage or unwanted GMMs will be inactivated by validated disinfection using Virkon disinfectant before being disposed of to the drain.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Each member of the Genetic Modification Safety Committee has read and approved the attached Risk Assessment summary for Class 1 activities
### GM Centre Number: 3129

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**Name**

| IPSEN BIOPHARM LTD |

**Name 2**

| Department |

**Campus Estate or Research Centre**

| WREXHAM INDUSTRIAL ESTATE |

**Road Name**

| UNIT 9 ASH ROAD |

**Town**

| WREXHAM |

**County**

| CLWYD |

**Postcode**

| LL13 9UF |

**Country**

| WALES |

**Tel Number**

| 01978 661181 |

**Fax Number**

| 01978 664223 |

**E-mail**

| blank |

**HSE Division**

| blank |

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

| Membership: |
| Management representative (Site Leadership Team member) |
| Environment, Health and Safety representative |
| BSO |
| Scientist (1) |
| Scientist (2) |
| Quality/Lay member |

The Genetic Modification Safety Committee (GMSC) will consist of at least four members to be quorate and must include an EHS representative and the BSO. Where it is not possible for all designated persons to attend a committee meeting, comments should be provided in advance of the meeting on any documents under review or an appropriate deputy appointed. A deputy will be selected on the basis of their experience; however the proposers of the activity under discussion will be excluded from consideration. Invites will also be extended to risk assessment authors and/or area Responsible Persons as appropriate. The frequency of meeting will be biannual at minimum, with committee meetings arranged as and when is necessary to ensure that risk assessment are reviewed within a timely manner. The role of Chairperson and Secretary will be assigned to the aforementioned members as appropriate and meeting minutes will be kept. The remit of the committee is to advise management on the adequacy of GMO risk assessments, to provide a beneficial influence on ensuring good practice on safety, training and laboratory discipline for the GMOs, to help with the formation of local GMO rules, to review risk assessments at least every two years and to review any accidents and near misses involving GMOs.

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02/03/2022
Level 4 (GMMs)

Non-microbial

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<th>Use of GMMs for the production of Biologics</th>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Not Applicable

Tick to confirm that you are attaching a summary of the risk assessment

Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Not Applicable
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Name
CHARLES RIVER RESEARCH SERVICES UK LTD

Name 2

Campus Estate or Research Centre
LAB E4

Building
GROUND FLOOR BUILDING 500

Road Name
DISCOVERY PARK

District

Town

County
KENT

Postcode
CT13 7ND

Country
ENGLAND

Tel Number
01799533561

Fax Number
0

E-mail
blank

HSE Division

Comments
Previously Cangenix Ltd changed to Argenta Discovery Ltd 29/04/2013. Name change from Argenta Discovery 06/07/2018

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

- Level 1 (GMMs)
  - Laboratory: Yes
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)

Give brief details of the genetic modification safety committee

Cangenix will utilise the existing biology safety committee in place in the Biosciences department at the University of Kent at Canterbury, currently consisting of the BSO, a representative of the academic staff and a post-doctoral researcher. Meetings of the committee are held on an ad hoc basis several times during the year. It is envisaged that a Cangenix representative will be present at all appropriate meetings of the committee.
To minimise the risk of escape of GMOs to the environment, all contaminated waste streams generated during the course of the work will be decontaminated within the laboratory within the laboratory buildings. Cultures will be grown within leak proof vessels. Fermenters be set up in such a way that accidental spillage will be contained. Solid and liquid waste will be autoclaved within the laboratory block prior to incineration. Autoclaves are maintained and tested as part of the wider university safety policies. All laboratory work surfaces will be regularly cleaned using Virkon disinfectant. Spillages will be treated with Virkon and any associated waste (paper towels, etc) will be treated as per solid waste streams. No live GMOs will be present at the end of the decontamination procedures.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

There were no comments made by the Biology Safety Officer with respect to the assessments. All such assessments had previously been approved by the full GM Safety Committee at Pfizer in Sandwich where the work was previously undertaken.

Project Ref 3130/19.1

Date Ackn'd CU2 Project Title
09/01/2019 Purification of recombinant human myosin protein from adenovirus-infected murine

Class CultureVolClass2 CultureVolumeClass3-4
Class 2 1-50 Litres

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Generation of human recombinant myosin protein for structural biological studies. Cell pellet derived from this process will be supplied to Charles River Discovery Services at Sandwich from an external client. No expression work will be undertaken at the Sandwich location, only the recombinant myosin protein purification which is the purpose of this notification.

**Recipient or parental organism**

The GMO in question will be the adenovirus vector used for gene delivery into mouse myoblasts for the purposes of over-expression of myosin protein.

**Host/vector system**

Commercial adenovirus gene delivery system (pAdEasy) from Qbiogene.

**Origin & function**

Gene encoding human myosin IIa protein. This is a cytoskeletal protein widely expressed in human muscles.

**Evaluation of foreseeable effects**

There is the potential for residual infectious/non-replicative adenoviruses to be present in the cells that will be provided. Whilst these viruses are nonreplicative, they can technically still be infectious to human cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Any liquid waste derived from lysis of the cell pellets will be treated with Virkon disinfectant (2%) or bleach (10%) for a minimum of 2 hours before disposal to drainage. Glassware will similarly be treated with Virkon disinfectant (2%) before washing. Any solid waste will be kept to a minimum and autoclaved within the laboratory where the work is being conducted before disposal into site waste streams. 1% Virkon has been determined to be 100% effective against similar viruses in external manufacturer's testing.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

The local committee indicated that the work needed to be notified as CU2 due to the potential for residual adenoviral particles and this was confirmed by the organisation BSO.

Project Containment

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02/03/2022
GM Centre Number: 3131

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Name

BIOCONVERSION TECHNOLOGIES LTD

Name 2

Department

Campus Estate or Research Centre

LONDON BIOSCIENCE INNOVATION CENTRE

Road Name

2 ROYAL COLLEGE STREET

Town

LONDON

County

GREATER LONDON

Postcode

NW1 0NH

Country

ENGLAND

Tel Number

020 7691 4912

Fax Number

020 7681 9129

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022

Page 13088 of 15326
### Premises Addresses

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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: Y

- **Give brief details of the genetic modification safety committee**

  > We have formed a Genetic Modification Safety Committee to advise on risk assessments in advance of the work starting. This committee is comprised of the company's Chief Technical Officer (as Biological Safety Officer), Chief Science Officer, Molecular Biologist, Fermentation Scientist and a Senior Geneticist with experience from several food companies and ICI.

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<th>Glass House</th>
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- **Tick if confidential**: 

- **Bacteriology**

- **Parasitology**

- **Transgenic Birds**

- **Microbiology Research**: Yes
Liquid microbial cultures in flasks and tubes and fermentation spent cultures are disinfected by strong industrial disinfectant 'Virkon' and left for at least 2 hours before being disposed in normal drains. This way all the live microbial cells in our cultures are killed as checked and verified by plate viable cell count afterwards.

Solid microbial cultures on various types of medium agar plates are put into special sealed autoclavable buckets and autoclaved at 121 degrees Celcius for 20 minutes before being disposed of in normal municipal waste skips.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid microbial cultures in flasks and tubes and fermentation spent cultures are disinfected by strong industrial disinfectant 'Virkon' and left for at least 2 hours before being disposed in normal drains. This way all the live microbial cells in our cultures are killed as checked and verified by plate viable cell count afterwards.

Solid microbial cultures on various types of medium agar plates are put into special sealed autoclavable buckets and autoclaved at 121 degrees Celcius for 20 minutes before being disposed of in normal municipal waste skips.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Genetic Modification Safety Committee of the company believes that the risk assessment done for Class 1 activities (see attached) is carried out correctly and comply with HSE regulations on contained use of genetically modified organisms
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

A four member GM safety committee led by a chair has been established to advise on risk assessments of contained use of genetic modification research activities.

Members of the committee are experienced researchers who have previously led active research programmes and supervised and trained research students and staff and have widely published in biological and biomedical areas using microbiological and mammalian cell culture systems. Members of the committee have experience in research at Class I and Class II levels and also in compliance of HSE and Defra regulations.

The committee meets periodically at one to three month intervals as per the requirements of the departmental activities. GM safety committee members also sit on the Departmental H & S committee and the Chair of the GM committee also sits on the University H & S committee. These arrangements ensure coordination of the general H & S and COSHH/GM safety requirements.

Minutes of the committee proceedings approved by members are available on BREO, the University electronic resources system accessed by staff and students, based on appropriate levels of access set.

GM safety committee approved form for Risk Assessment of Work with Genetically Modified Organisms with relevant guidelines as well as appropriate HSE guidelines documents are available to academics and members of the research groups via BREO.

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02/03/2022
Liquid waste will be decontaminated by disinfecting with 1% Virkon overnight. Waste will then be disposed of down the sink with running water.

Glassware will be decontaminated by disinfecting with 1% Virkon overnight. Glassware will then be rinsed thoroughly with water, washed in the glass washer and autoclaved.

Solid waste will be placed into clear waste bags which will be tied and autoclaved on site, prior to disposal in yellow waste bags for incineration.

Virkon treatment and autoclaving are both routine methods of inactivating biological material with very high efficacy, and these methods have been standardly used in most laboratories for many years and are in accordance with the waste management policy document on the Division's BREO site.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The GM safety committee reviewed the GM-Risk assessment forms completed by the academics for each of the project areas described in the attached document.

For each project, forms including the description of 1) research and methodology; 2) donor, vector and host and their characteristics; 3) characteristics of any GMO produced including any protein expression, effects and risk to human health, quantity of organism used; 4) containment conditions and level including use of PPE, disinfection, inactivation, transport and storage; 5) monitoring of containment and control methods and 6) Table of control measures and containment levels were scrutinised by the members individually ahead of the committee meetings and collectively during the committee meetings.

Any clarifications and amendments required were communicated to individual academics and the revised forms were scrutinised by the committee, as above.

Following the amendments, it was confirmed by the UoB-DoS-GM safety committee that all approved submissions included in the current CU1 are deemed appropriate for Class 1/CU1 classification.

Project Ref 3132/13.1

To characterise the biological roles of PI3K enzymes

Date Ackn'd 02/05/2013

CU2 Project Title

Tick if notifying a connected programme of work N

Class 2

Consent Granted

Class Culture Vol Class 2 Culture Volume Class 3-4

< 1 Litre

Non-GMM

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

E.coli strains will be used to amplify cDNA vectors and plasmid constructs. This will be achieved by heat shock and overnight culture, selection of colonies and their subsequent amplification. Glycerol stocks will be kept at -80oC. cDNA vectors and constructs will be transfected and amplified in this way. This includes all cDNA described below. Escherichia coli will also be transformed with cDNA plasmids to express recombinant fusion proteins. Typically these will be glutathione S-transferase (GST) fusions and a commercial pGex2T vector used (GE Healthcare). Finally, Escherichia coli will be used to propagate lentiviral plasmids, each plasmid will be generated separately and no single plasmid will carry sufficient genetic information to produce a complete lentiviral particle.
Mammalian cell lines will be produced that express recombinant protein or have specific gene expression attenuated using silencing shRNA. Again a wide variety of mammalian cell lines (typically) will be used. This will be achieved in several ways that allow direct introduction of cDNA plasmids or oligos. For this purpose inorganic (calcium phosphate), physical (electroporation) and lipid based approaches (Lipofectamine Invitrogen) will be used. cDNA of interest will be sub-cloned into mammalian expression vectors that include pcDNA3.1 and pEGFP. Mammalian cells will be used to generate lentiviral particles that will be used to introduce shRNA for the purpose of gene silencing. The use of such packaging cells confers additional biosafety by reducing the possibility of a recombination event that may generate lentiviral particles capable of self replication.

Recipient or parental organism

| Disabled E.coli strains | XL1- Blue, DH5a, DH5aT1R, STBL-3, BL21, JM109 |
| Mammalian cells including Murine: Swiss 3T3, NIH3T3, WT-M8+13 & Human: A wide variety of cell lines that include A431, Human embryonic kidney (HEK293), HMT3522, HeLa, ABB/13 |

Host/vector system

| pBluescript family to allow amplification and sub-cloning of cDNA sequences |
| pGex family and others to express recombinant proteins in bacteria |
| pcDNA, TOPo, pMT2, pEGFP and others to express recombinant proteins in mammalian cells |
| Lentivirus plasmid vector, pLKO.1-puro5 or TRC2- pLKO-puro for transduction of shRNA sequences into mammalian cells. |

Origin & function

Gene sequences used are primarily mammalian – human or mouse but possibly zebrafish.
Platelet derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), hepatocyte growth factor receptor, (MET), GM-CSF receptor (FMS), Insulin receptor, Adaptors and Scaffold proteins: p85, nck, grb-2 , Linker of activated T-cells (LAT), intersectin
Phospholipases and kinases: Phosphoinositide 3-kinase enzymes p110a, p110b, p110d, p110g, PI3K-C2a, PI3K-C2b, PI3K-C2g and vps34, Phospholipase A2; Phospholipase C; Phospholipase D
Non-receptor tyrosine kinases: Src, Fyn, ZAP70
It is important to stress that although oncogenic forms of some proteins above exist (ie. src, PDGFR, EGFR), the cDNAs we will use do not encode oncogenes and the products are non-toxic.
Protein expression in E.coli is typically at a 1- 20 mg / l level under a T7 / lac promotor. All cloned inserts are intron-free to allow expression in a prokaryotic system and have ampicillin resistance to allow for selection. Mammalian expression vectors primarily employ the CMV promoter for the expression of recombinant protein. Puromycin, kanamycin, or hygromycin resistance genes are the selectable markers typically used in mammalian expression vectors.
Lentiviral expression vectors primarily employ the U6 or Human phosphoglycerate kinase eukaryotic promoter (TRC2 only) and have puromycin and ampicillin resistance genes to allow selection. Lentiviral work will be used for downregulation of gene expression in host cells.

Evaluation of foreseeable effects

The highest containment level required for this risk assessment is level 2, which corresponds to class 2 GMO to cover the lentiviral work. The host systems used are either non-pathogenic or disabled, and host systems have been routinely used in containment 1 / 2 laboratories safely for many years. The bacterial strains to be used are mobilisation-deficient (Mob-) and recombination deficient. Protein sequences are non-toxic. The generation of separate lentiviral DNA plasmids at different times (envelope vector, shRNA transfer vector and the packaging vector) in E.coli minimises the risk of recombination events. Modification of mammalian host systems with shRNA does have the potential to increase the hazard level. However, recombinant lentiviruses produced with the MISSION
third generation lentiviral system have been shown to produce replication incompetent viral particles because of the designed safety features:
The packaging vector, which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions. The vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector, which provides the heterologous envelope for pseudotyping. The shRNA transfer vector, which contains the sequence of interest as well as the cis acting sequences necessary for RNA production and packaging. The multi-plasmid approach results in no single plasmid containing all the genes necessary to produce packaged lentivirus. The resulting particles are replication-incompetent and deletion in the U3 portion of the 3’ LTR eliminates the promoter-enhancer region, further negating the possibility of viral replication. The system has also removed virulence genes which are not necessary for shRNA packaging. These features combined have improved biosafety and handling. There are no known incidents of third generation systems producing infectious, replication competent virus, but replication competency will be monitored (see below). NIH guidelines recommend replication-incompetent lentiviral particles be handled as Risk Group-Level 2 (RGL2). A packaging cell (HEK293) will be used to generate the lentiviral particles, the cells will be destroyed (see disinfection and inactivation) within 72 hours as an additional containment measure. All work will be carried out in a restricted access area using a designated class II laminar flow hood. Routine monitoring will be conducted to ensure that the viral vector remains replication incompetent. This will be achieved using PCR to detect the presence of deleted sequences in transduced target cells.

Work with lentivirus will not be permitted without prior training by the Principal Investigator who will supervise work (or their designated technical expert). The worker should demonstrate good microbiological and tissue culture technique and an understanding of this risk assessment prior to being permitted to work with lentivirus. Risk assessments will be provided for all areas of work.

The only procedure involved in these experiments that might produce aerosols is the centrifugation of cells, where a spillage may result in aerosols. Sealed tubes/containers holding up to 50ml of resuspended transfected mammalian cell lines or 250ml E.coli cultures will be centrifuged in sealed buckets to contain aerosols. If a spillage should occur in the centrifuge, we will allow time for aerosols to settle, and then buckets will be opened in a class II hood (transfected mammalian cells) or a microbiological class II hood (E.coli cultures). Spillage will be absorbed with tissues and placed in clear waste bags for autoclaving prior to incineration. Rotor and buckets will be removed and washed with 3% Virkon/ 10% bleach (in the appropriate Class II hood), and then rinsed clean with distilled water and allowed to dry. Inside of the centrifuge will be wiped with tissues soaked in 3% Virkon or 10% bleach, and then sprayed with 70% ethanol and wiped dry. Laboratory coats and nitrile gloves will be worn for all work. The following PPE will be worn when working with lentiviral vectors: gloves; lab coat. A surgical mask and eye protection (goggles) or face shield will be worn any time that there is a risk of a ‘splash’ of lentiviral particles to the face (such as clearing up a spillage).

E. coli has the potential to be a gastrointestinal pathogen, and although the strains we are using are non pathogenic, good microbiological practice and good occupational hygiene will be observed at all times. A single Class 2 microbiological hood will be designated for GMO work to minimise the risk of creating pathogenic strains by cross contamination. Additionally, propagation of separate lentiviral DNA plasmids (envelope vector, shRNA transfer vector and the packaging vector) in E.coli minimises the risk of a recombinant event.

Transfected mammalian cells may be viable if ingested or taken through broken skin. shRNA has the potential to inhibit expression of a tumourogenic regulator, however this risk is deemed to be very low. To minimise risks, eating or drinking is strictly prohibited in all laboratory spaces. PPE; wearing gloves and a labcoat, while working with GMOs should prevent any direct contact of the GMO with the workers skin, greatly reducing the risk of GMOs entering the worker via broken skin. The use of a surgical mask and eye protection (goggles) or face shield (if there is a risk of a ‘splash’ to the face, such as clearing up a spillage), will reduce the risk of inhalation, or contact with mucosal membranes. All work will be carried out in a restricted access area using a designated class II cabinet. Sharps will not be used.

The lentival system is an HIV based plasmid and is used because of its efficacy at infecting mammalian cells, giving it the potential to infect the worker through broken skin, aerosols or ingestion. To minimise this risk, we will use a third generation lentiviral system which has been developed with enhanced safety features: The packaging vector, which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions. The vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector, which provides the heterologous envelope for pseudotyping. The shRNA transfer vector, which contains the sequence of interest as well as the cis acting sequences necessary for RNA production and packaging. The multi-plasmid approach results in no single plasmid containing all the genes necessary to produce packaged lentivirus. The resulting particles are replication-incompetent and a deletion in the U3 portion of the 3’ LTR eliminates the promoter-enhancer region, further negating the possibility of viral replication. The system has also removed virulence genes which are not necessary for shRNA packaging. These features combined have improved biosafety and handling. There are no known incidents of third generation systems producing infectious, replication competent virus, but, replication
**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

<table>
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**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

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**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
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<tr>
<th>Liquid waste will be decontaminated by disinfecting with 3% Virkon or 10% bleach overnight. Waste will then be disposed of down the sink with running water. Glassware will be decontaminated by disinfecting with 3% Virkon overnight. Glassware will then be rinsed thoroughly with water, washed in the glass washer and autoclaved. Solid waste will be placed into clear waste bags which will be tied and autoclaved on site, prior to disposal in yellow waste bags for incineration. Virkon treatment and autoclaving are both routine methods of inactivating biological material with very high efficacy, and these methods have been standardly used in most laboratories for many years and are in accordance with the waste management policy document on the Division's BREO site. Any GMOs associated with lentivirus will be decontaminated in 10% bleach for at least 20 minutes before autoclaving and disposal as per manufacturer's instructions. Disinfection procedures will be as in accordance with the Laboratory Disinfection DoS Policy document on the Division BREO site. Surfaces will be cleaned with 70% alcohol solution (ethanol or IMS). For spillages, aerosols will be allowed to settle and will then be absorbed with tissues and placed in clear waste bags for autoclaving. Contaminated areas will be washed with 3% Virkon and rinsed with either water, or 70% alcohol. For spillages associated with lentivirus particles, paper towel soaked in 10% bleach will be placed on top of the spill for 20 minutes, inactivating the virus. The paper towels will then be placed in clear, labelled bags for autoclaving. The surface will then be wiped down with 3% virkon, followed by 70% ethanol. GMOs will be disinfected using 3% Virkon. This disinfectant has been validated by the manufacturer and is used in accordance with manufacturer’s specifications. All equipment used in conjunction with lentiviral work will be decontaminated with 3% Virkon or 10% bleach for at least 20 minutes before disposal, as per manufacturer's instructions.</th>
</tr>
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**Is an emergency plan required according to regulation 20?**

Y

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

02/03/2022
The GM safety committee reviewed the GM-Risk assessment form completed by the academic responsible for the project area described.

Description of 1) research and methodology; 2) donor, vector and host and their characteristics; 3) characteristics of any GMO produced including any protein expression, effects and risk to human health, quantity of organism used; 4) containment conditions and level including use of PPE, disinfection, inactivation, transport and storage; 5) monitoring of containment and control methods and 6) Table of control measures and containment levels were scrutinised by the members individually ahead of the committee meetings and collectively during the committee meetings.

Any clarifications and amendments required were communicated to the concerned academic and the revised form was scrutinised by the committee, as above.

Following the amendments, it was confirmed by the UoB-DLS-GM safety committee that the approved submission included in the current CU2 is deemed appropriate for Class 2/CU2 classification.

### Project Containment

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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<th>Country</th>
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<tr>
<td></td>
<td>PCI GROUP</td>
<td>BIOTEC SERVICES INTERNATIONAL</td>
<td></td>
<td>BRIDGEND INDUSTRIAL ESTATE</td>
<td>BIOTEC HOUSE</td>
<td>CENTRAL PARK, WESTERN AVENUE</td>
<td>BRIDGEND</td>
<td>CARDIFF</td>
<td>CF31 3RT</td>
<td>WALES</td>
<td>N</td>
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<td>BIOTEC SERVICES INTERNATIONAL</td>
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<td>UNIT 2100 CENTRAL PARK</td>
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<td>CF31 3TY</td>
<td>WALES</td>
<td>N</td>
<td></td>
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</tbody>
</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

- Biological Safety Officer (Company Director)
- General Safety Officer
- Head of Technical Support
- Operations Manager
- Quality Assurance Manager

The committee will be responsible for Biotec's procedures associated with handling of GMOs, conducting and reviewing individual GMO product's risk assessments, auditing for compliance to GMO handling procedures, reviewing and reporting (where required) any incidents associated with Biotec's handling of GMOs

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
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<td>Level 1 (GMMs)</td>
<td>Yes</td>
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<td></td>
<td></td>
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<td>Level 2 (GMMs)</td>
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<td>Level 3 (GMMs)</td>
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<td>Level 4 (GMMs)</td>
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<tr>
<td>Non-microbial</td>
<td></td>
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</tbody>
</table>
Before taking receipt of any GMO product, the Genetically Modified Safety Committee at Biotec will identify a suitable chemical disinfectant for inactivating the GMO and the exposure time to the disinfectant required for inactivation. Biotec will be handling primary packed GMOs and the only time in which waste management methods will be employed is if a GMO's primary packaging is damaged. At this point, Biotec's Standard Operating Spill Procedure will be activated. Spilled material will be covered and contained with absorbent pads from a SPC Spillage Kit; the selected disinfectant will be applied to the spillage area. The spillage area will be in contact with the disinfectant for a predetermined time, and then all items associated with the spill will be placed into a biohazard bag. The biohazard bag will be labelled with the date of spill, GMO, number of damaged items, cause of breakage, and names of persons involved in cleaning the spill. The biohazard bag will be placed in a designated location ready for destruction and the spillage clearance record will be completed and handed to the Biological Safety Officer for review. The Biohazard bag will be sent for incineration; incineration will be undertaken by a company licensed to handle GMOs.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

The only risk of exposure to material would be if there is an accidental spill because no primary manufacturing takes place on Biotec's premises. The type of illness caused by exposure will be described in each GMO's risk assessment. Generally, Containment Level 2 GMOs could cause human disease but this is unlikely and treatment is available.

### Other (please specify)

- **Bacteriology**
- **Parasitology**
- **Transgenic**
- **Birds**
- **Microbiology**
- **Research**
- **Virology**
- **Transgenic**
- **Animals**
- **Transgenic**
- **Fish**
- **Gene Therapy**
- **Mycology**
- **Transgenic**
- **Invertebrates**
- **Transgenic**
- **Plants**
- **Other (please specify below)**

- **Other(s)**: Storage and labelling of primary packed material only

---

**Project Ref** 3136/13.1

**Date Ackn'd** 02/03/2022  **CU2 Project Title**

---

**Class**  **CultureVolClass2**  **CultureVolumeClass3-4**
Receipt and storage of modified adenovirus CRAd (serotype 5)

<table>
<thead>
<tr>
<th>Date Project</th>
<th>Class 2</th>
<th>&lt; 1 Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceased</td>
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</tbody>
</table>

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

Long term storage of virus at -80 degrees.

**Recipient or parental organism**

ORCA-1010 is a CRAd (serotype 5) vector containing a distinct mutation in the adenoviral E1A proteins (called "Delta" or dl922-947") enabling selective virus replication in pRb-(Retinoblastoma protein)-deregulated tumour cells and the incorporation of an Arg-Gly-Asp (RGD) sequence, known to interact with αV integrins, into the adenovirus fiber to enhance tumour infection. Further, a mutation within the adenoviral E3/19K protein allows for more efficient virus release and spread within permissive tumour tissue.

**Host/vector system**

Not applicable, only virus stored on site.

**Origin & function**

The ORCA-010 virus is based on human Adenovirus serotype 5 (Ad5).

ORCA-010 comprises the following genetic modifications:

1. Deletion of bp 922-947 of the adenoviral genome. This causes an eight amino-acid deletion in the conserved region-2 (CR2) of adenoviral E1A proteins. This mutation provides tumour-selective virus replication in pRB-deregulated tumour tissue /cells.
2. Insertion of one adenine nucleotide at position bp 29174 equivalent to bp 445 of the adenoviral E3/19K protein. This insertion causes a shift in the open reading frame (ORF) of E3/19K that changes the amino acid sequence from position 149 onwards and generates a stop-codon at position 154 resulting in a truncated version of E3/19K. Whereby wild-type E3/19K is retained in the endoplasmatic reticulum the mutated E3/19K as expressed from ORCA-010 is re-localised to the plasma membrane (Gros et al., Cancer Research 2008, 68: 8928-8937).
3. The Fiber protein of ORCA-010 contains an RGD sequence in the HI loop of the knob domain. This Arg-Gly-Asp amino acid sequence provides the virus with the capacity to infect cells using αV integrins as a receptor, therby increasing tumour cell infectivity (Suzuki K et al., Clin Cancer Res 2001, 7: 120-126).
Ad5 is transmissible via the mouth, nasopharynx or ocular conjunctiva and has been uniquely recorded as being transmissible via the airborne route in a military setting. Ad5 is most commonly spread in children by the faecal/oral route although other strains may largely spread via contaminated water (Fields, Virology (1996): page 2153). Whilst adenoviruses can transform rodent cells numerous screens have failed to correlate the presence of adenoviruses with oncogenesis in humans. Wild-type Ad5 is classified as ACDP class 2 biologic according to UK HSE standards. The infection with Ad5 is very common within populations and within the Western world over 90% of individuals are sero-positive for Ad5 resulting in lifelong immunity.

The virus may be transmitted by inhalation, ingestion, skin absorption or via needle stick injury. The determination of toxicity of replicating adenoviruses in animal models is difficult as human Ad5 does not replicate in mice. Semi-permissive animal models for replication of human Ads are the Syrian hamster and the cotton rat.

In case of accidental inoculation of an operator with ORCA-010 it is expected that the infection is cleared efficiently in sero-positive individuals. Additionally, the capacity to replicate in normal, non-dividing tissue should be decreased compared to wild-type Ad5. However, it is expected to replicate in normal, non-dividing tissue should be decreased compared to wild-type Ad5. However, it is expected that the level of cancer-specific virus replication is not complete and some leaky, residual replication in normal cells might be present (see also Sauthoff et al., Mol Ther 2004, 10: 749-757). The level of residual replication in normal cells seems to be dependent on the cell type used. For example Fueyo et al. reported resistance of normal fibroblasts to the dl922-947 virus replication.

Virus replication/propagation could happen if ORCA-010 does infect cells that are actively dividing. However, again this event should be controlled by the operator's immunity against Ad5.

Further, at least two clinical studies (US-624 and US-625) have been initiated with the Ad5 dl922-947 virus (also called Delta24 deletion). (http://www.wiley.co.uk/genetherapy/clinical). One clinical trial has been performed with Ad5 dl922-947 RGD in ovarian cancer and one clinical study with the same virus is ongoing in glioma. The Phase III data in ovarian cancer indicated that the virus was safe and well tolerated up to dosages of 1x10^12 virus particles per day over three consecutive days (KJ Kimball et al., Clin Cancer Res 2010, 16(21): 5277-5827).

Expected effects of mutations on the virus phenotype/properties:

1. The Retinoblastoma protein (pRb) is a key cell-cycle regulator by controlling the transcriptional activity of the transcription factor family E2F. The dissociation of pRB from promoter-bound E2F protein allows the entry of the cell into the S-phase of the cell cycle. The interaction of pRB with E2F is regulated by phosphorylation within the cell. This pathway is deregulated in many tumour types and as a consequence pRB can not interact with E2F anymore leading to uncontrolled entry into S-phase and therefore cell proliferation. Viral replication within the host cell also requires the entry of the host cell into S-phase and this is enabled by the binding of viral E1A proteins to the cellular pRb protein thereby releasing it from promoter-bound E2F protein. Therefore, the dl922-947 deletion in E1A enables selective virus replication in pRB-deregulated tumour cells, which are constitutively deregulated for the E2F pathway. On the other hand, if ORCA-010 infects a non-tumour host cell, it can not dissociate pRB from E2F and therefore not drive the cell into the S-phase of the cell cycle. In this situation the cell will go into apoptosis or cell growth arrest dependent on the cellular background of the infected cell.

2. The re-localisation of the truncated E3/19K protein, lacking the intra-cellular domain, causes an increase in influx of Ca2+ and this leads to an increase in cell permeability leading to an earlier release of viral progeny during the replication cycle. Therefore ORCA-010 does spread more efficiently through permissive tumour tissue compared to wild-type Ad5 virus.

3. The endogenous receptor for Ad5 viruses, the CAR receptor, is often downregulated or not present at all at the surface of cancer cells. Integrins however, are widely present on many different types of cancer cells and thus the integration of the RGD sequence into the receptor binding domain of the Fiber protein increases the infectivity of ORCA-010 significantly. It should be noted however, that the TGD modification applied to ORCA-010 does not preclude the binding of the fiber to CAR, and the modified virus can enter the cells through αv integrins and CAR.

In summary, it is expected that ORCA-010 is less cytotoxic after accidental inoculation than wild-type Ad5. The presence of the RGD insertion and of the E3/19k mutation are not expected to increase the pathogenicity of the virus in both sero-positive and sero-negative operators. The potential increase in viral spread caused by the E3/19K mutation and the RGD insertion should be counter-balanced by the reduced replication potential caused by the E1A mutation.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No autoclave in the building: Only storage of material is performed at the site. Therefore contamination will only occur if there is the breakage of a bottle. Spill procedures are in place for spills, all material in contact with the spill is collected, treated with 10% sodium hypochlorite and sent off site for incineration with a registered waste management company. There is no equipment used during storage that would come into contact with the virus and require autoclaving.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virus

Spills of the GMO will be inactivated by treatment with 10% sodium hypochlorite solution (1.2% available chloride) for a minimum of 5 minutes, the inactivated spill will then be absorbed with spill kits (kept in the storage area) and all material disposed of as class II solid waste or placed in a biohazard waste bin and treated with 10% sodium hypochlorite for 1hr to inactivate and then dispose of via incineration by an approved contractor.

Is an emergency plan required according to regulation 20? [N]  
If yes, tick to confirm that it is attached to this form [N]  
Tick to confirm that you have attached a risk assessment to this form [Y]  
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
</tbody>
</table>

Animal Units

<table>
<thead>
<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
</tr>
</tbody>
</table>

Project Ref 3136/13.2

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
**Storage and labelling of vials containing live influenza virus reagent**

**Date Project Ceased**: 26/11/2013

**Class 2**: < 1 Litre

**Non-GMM Consent Granted**: Yes

**Project notified under transitional arrangements**: No

**Withdrawn**: No

**Tick if notifying a connected programme of work**: No

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

<table>
<thead>
<tr>
<th>Project Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purposes of the contained use</strong></td>
</tr>
<tr>
<td>Long term GMP storage of virus reagent on behalf of Retroscreen virology</td>
</tr>
<tr>
<td>storage below -70</td>
</tr>
<tr>
<td><strong>Recipient or parental organism</strong></td>
</tr>
<tr>
<td>The influenza reference virus NIBRG-14 is a reassortant prepared by reverse genetics from A/Vietnam/1194/2004 (H5N1) virus (in which the polybasic HA cleavage site has been excised and A/PR/8/34 (H1N1) virus</td>
</tr>
<tr>
<td><strong>Host/vector system</strong></td>
</tr>
<tr>
<td>Material is freeze dried in allantoic fluid from embryonated hens eggs</td>
</tr>
<tr>
<td><strong>Origin &amp; function</strong></td>
</tr>
<tr>
<td>Virus reference standard</td>
</tr>
<tr>
<td><strong>Evaluation of foreseeable effects</strong></td>
</tr>
<tr>
<td>The material can cause influenza in humans. Any one exposed to the material (only in the event of a spill) would be offered the seasonal flu vaccine and advised not to come into contact with avian or porcine species for 14 days. Any staff handling the spill will wear respiratory protection</td>
</tr>
<tr>
<td><strong>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</strong></td>
</tr>
<tr>
<td>not applicable</td>
</tr>
<tr>
<td><strong>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</strong></td>
</tr>
<tr>
<td>No autoclave in the building; Only storage of material is performed at the site. Therefore contamination will only occur if there is the breakage of a vial. Spill procedures are in place for spills, all material in contact with the spill is collected treated with virkon and sent off site for incineration with a registered waste management company. There is...</td>
</tr>
</tbody>
</table>
no equipment used during storage that would come into contact with the virus and require autoclaving.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste management following a spill is described in section 11.

Destruction of the material being stored is not planned to take place at our premises, but if required it would be sent for incineration as a GMO with our licensed waste handling company. The vials would not be opened on site.

Is an emergency plan required according to regulation 20? [N]

Tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The material is commercially manufactured within the UK. Less information is available on the organism compared to other materials stored, only the product insert. However as only storage is required this will be acceptable

Project Containment

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<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units L2 L3 L4</td>
<td>Large Scale Activities L2 L3 L4</td>
<td>Human Clinical Applications L2 L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 3136/14.1

Date Ackn'd 12/11/2014

CU2 Project Title Storage, labelling and distribution of TM1050 for Clinical Trial

Class 2

Culture Vol Class 2 Not Applicable

Consent Granted

02/03/2022
Project Additional Information

Purposes of the contained use
Supply of clinical sites with the investigational medicinal product (GMO base product) and the placebo (salt buffer) in the context of the clinical study TG1050.02. The clinical study is to study a HBV therapeutic vaccine candidate able to induce strong and multispecific T cell responses in order to prime or recall T cells in HBV chronically infected patients.

Recipient or parental organism
The parental organism is the wild type Adenovirus type 5 (GENBANK access of hAd5 sequence: AY339865). Adenovirus is a non enveloped, double-stranded DNA virus.

Host/vector system
The transfer system used is a viral transfer system with a human adenovirus vector of group C and serotype 5 (Ad5) deleted of several regions of its genome (E1 and E3). Adenoviral vectors of this type are commonly used in humans (Vorburger S.A. and Hunt K.K., 2002). There is no use of transposons for the construction of the GMM. The genome of the vector Ad5 E1° E3° is assembled in a plasmid with gene of interest and sequences for functional expression. The plasmid genome, used to generate the viral vector, was obtained by homologous recombination (Chartier C. et al., 1996) (Degryse E., 1996) between two plasmids, one carrying the backbone of the Ad5 viral vector body and the other the expression cassette with the sequence coding for HBV. The transfer system used is a viral transfer system with a human adenovirus vector of group C and serotype 5 (Ad5) deleted of several regions of its genome (E1 and E3). Adenoviral vectors of this type are commonly used in humans (Vorburger S.A. and Hunt K.K., 2002). There is no use of transposons for the construction of the GMM. The genome of the vector Ad5 E1° E3° is assembled in a plasmid with gene of interest and sequences for functional expression. The plasmid genome, used to generate the viral vector, was obtained by homologous recombination (Chartier C. et al., 1996) (Degryse E., 1996) between two plasmids, one carrying the backbone of the Ad5 viral vector body and the other the expression cassette with the sequence coding for HBV Pol, Env and Core antigens and the regulation genetic elements.
Hepatitis B virus (HBV)
Orthohepadnavirus
Hepadnaviridae family.
Accession number Y07587 described by Stoll-Becker (Stoll-Becker S. et al., 1997)
Genotype D, serotype ayw
The three following genes are encoded in the Adenovirus vector:
Core protein: has been demonstrated as being the target of T cell responses in patients resolving their HBV infection spontaneously or under treatment (Ferrari C. et al., 1990) (Penna A. et al., 1997), with 2 epitopes described to be associated with resolution (Rossol S. et al., 1997; Tsai S.L. et al., 1992) (Bertoni R. et al., 1997; Webster G.J. et al., 2004). The Core protein was considered as the key antigen of the vaccine.
Polymerase protein: has been demonstrated as being rich in T cell epitopes, including one epitope described to be associated with resolution (Webster G.J. et al., 2004) even if less studied than the Core protein.
HBsAg protein or Envelope protein: has also been demonstrated as being rich in T cell epitopes, including 2 epitopes described to be associated with resolution (Webster G.J. et al., 2004).
The original HBV design encoded by the vaccine candidate is a fusion protein Core-Pol-Env being composed of a truncated Core protein (aa 1-148) fused to a polymerase protein deleted of the amino acid residues involved in pol-associated polymerase and RNase H enzymatic activities and mutated at 4 amino acid positions to further abolish RNaseH activity with 2 HBsAg domain inserted in Pol deletion sites, Env1 corresponding to amino acids 14 to 51 of the small HBsAg inserted in the polymerase-abolishing deletion site and Env2 corresponding to amino acids 165 to 194 of the small HBsAg inserted in the RNaseH-abolishing deletion site.

Origin & function
The strain Ad5 E1° and E3° is non replicative on mammalian normal cells. It need for its propagation, a complementation cell line which bring it up missing functions, that are, at minimum, proteins coded by E1A and E1B genes.
The absence of sequence homology between the vector and the complementation cell line genomes should eliminate the occurrence of Replication Competent Adenovirus (RCA), during the production process.
The genome of the GMM contains no gene for antibiotic resistance. Ampicillin resistance gene used as a selection marker in the plasmid is completely deleted during the transfection to produce the GMM (plasmid digested by Pac I prior to the transfection).
One cannot exclude that the missing E1 function, necessary for viral replication, could be complemented during coinfection of the vector with a wild type (Ad5) adenovirus. However, our assessment of this risk concludes that this event is not likely to cause any significant harm in anyone, for the following reasons:
- This complementation will not extend beyond the viremia of Ad5, which is known to be limited. The vector will be complemented only as long as the parental virus propagates.
- This complementation will be limited to areas where both types of viral particles are numerous enough to simultaneously infect the same cell. Consequently, only a fraction of the recombinant vector will be amplified, and this amplification will be less efficient than the amplification of the wild type parental virus (Ad5).

Evaluation of foreseeable effects
The strain Ad5 E1° and E3° is non replicative on mammalian normal cells. It need for its propagation, a complementation cell line which bring it up missing functions, that are, at minimum, proteins coded by E1A and E1B genes.
The absence of sequence homology between the vector and the complementation cell line genomes should eliminate the occurrence of Replication Competent Adenovirus (RCA), during the production process.
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- This complementation will not extend beyond the viremia of Ad5, which is known to be limited. The vector will be complemented only as long as the parental virus propagates.
- This complementation will be limited to areas where both types of viral particles are numerous enough to simultaneously infect the same cell. Consequently, only a fraction of the recombinant vector will be amplified, and this amplification will be less efficient than the amplification of the wild type parental virus (Ad5).
• The most likely recombination event which could have occurred is the homologous recombination, that could lead to the generation of either a recombinant vector AdE1+ E3+ competent for replication but having lost the transgene or the recombinant vector TG1050 E1+E3+, deficient for the replication. Thus, these recombinants do not present higher risk than the recombinant vector TG1050. Much more unlikely, illegitimate recombination events (TG1050 E1+E3+) only remain theoretical and thus undetectable since there are so rare.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All material will remain frozen whilst at Biotec. Therefore the main risk is to staff handling the material and exposed to spillage of a vial. The material is non-replicating, therefore the risk to staff is minimal. All staff handling the material are trained in the required spillage procedures for class 2 organisms. There is minimal risk to the environment as the material will remain within controlled storage areas.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any unopened material will be sent to a waste company licensed for GMO incineration. Any waste from spills (including all materials used to clean the spill) will be treated with 2% virkon for 1 hr prior to being sent for incineration. Any contaminated surfaces will have 2% virkon in contact for 30 minutes prior to cleaning.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The client has provided all the information for the completion of the form and MSDS. A risk assessment from the client has not been supplied. The risk assessment for handling the material at Biotec has been supplied.

Project Containment

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</tbody>
</table>

Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
Project Ref 3136/15.1

Date Ackn'd 04/03/2015

Date Project Ceased

CU2 Project Title: Storage, packaging, labelling and distribution of Thymidine-kinase deactivated vaccinia virus containing GM-CSF; Pexastimogene Devacirepvec; Pexa-Vec; JX-594

Class 2

Consent Granted

Non-GMM

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Oncolytic viral vaccine for human use in Phase 1-3 Clinical Trials

Recipient or parental organism

Human

Host/vector system

Vaccinia Virus (Wyeth strain, New York Board of Health)

Origin & function

1. Thymidine kinase (TK) gene deactivation
   Origin: see #3 below
   Function: to enhance vaccinia virus selection to cancer cells

2. human GM-CSF insert (human granulocyte-macrophage colony stimulating factor)
   Origin: human
   Function: stimulate the immune system against tumor cells

8. Containment and control measures for larger GMOs (e.g. GM animals and plants) - Public
Register
9. Maximum culture volumes per experiment - for GMMs only - Public Register
10. For GMMs only - the level of containment that will be applied - Public Register
Level 2 - Laboratory contained use
11. For GMMs only - Application for any derogation from full containment for the class of contained use (measures and justification) - Public Register
I would like to request derogation from full containment measures
12. Description of waste management measures - Public Register
3. Beta-galactosidase insert
Origin: E. coli
Function: to disrupt and deactivate the TK gene and to provide a marker.

Evaluation of foreseeable effects

General vaccinia virus information:
Based on use of the vaccine (wild type vaccinia): As a vaccine, wild-type vaccinia has been intentionally given to hundreds of millions of people, many of whom were young children; Serious toxicities occurred rare; At risk populations are now well defined; No new, dangerous virus arose
Transmission very rare and only with direct inoculation and touching of infectious material – open pustule, contaminated dressings (i.e. NOT airborne).
Complications are very rare with wild-type vaccinia: Vaccine overall effective and safe in people with healthy immune systems
Per CDC:
~1,000 of 1 million 1st time vaccinees → serious, non-life threatening side effects
14-52 of 1 million 1st time vaccinees → potentially life-threatening reactions
1-2 of 1 million: life-threatening reactions result in death
Rare side effects include: Eczema vaccinatum, progressive vaccinia, postvaccinal encephalitis myopericarditis
“At Risk” populations who should not receive the vaccine have been identified
Pexa-Vec (GMO):
is an attenuated vaccinia vaccine being investigeted for treatment of multiple types of solid cancers. To date, over 300 adult and pediatric patients with advanced, refractory cancer have been treated with over 1100 doses of Pexa-Vec. Treatment with Pexa-Vec has been well-tolerated, and no transmission to patient contacts for health care workers has been reported.
The GM-CSF and beta-galactosidase are well characterized that do not induce any negative reactions in humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
NA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
I would like to request derogation from full containment measures.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Only closed vials of material will be handled therefore contamination will only occur if there is a spill. Self contained biological spill kits are held in the areas where the material is stored. These contain all PPE required and absorbent materials. Spills are cleaned and all contaminated material and PPE is treated with 1% sodium hypochloride for 1 hour prior to sending for incineration by our licensed provider.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The material is in line with our current storage capability and no additional activities are required prior to receipt.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
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<td>L3 L4</td>
<td>L2 L3 L4</td>
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Project Ref 3136/16.1

<table>
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<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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</thead>
<tbody>
<tr>
<td>20/04/2016</td>
<td>Storage and distribution of retroviral vector for use in producing drug product for clinical trials</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Date Project Ceased

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Storage below -70 and distribution in dry ice of SFG.iCasp9.2A.dCD19 retroviral supernatent.

**Recipient or parental organism**

Murine origin, PG13 cells ATCC #CRL-10686 base on NIH 3T3

**Host/vector system**

The infectious agent is attenuated (see vector map in attached risk assessment)

The infectious agent is replication incompetent

The producer cell system used to generate the vector supernatant is safety optimised by deletion of packaging signals from viral genes expressed in the packaging cell line. There is no RCR formation reported so far for this host.

**Origin & function**

The BPZ-1001 (SFG.iCasp9.2A.dCD19) retroviral vector was constructed at the Baylor College of Medicine, Cell and Gene Therapy (CAGT) Translational Research Laboratory in a GLP environment. The SFG backbone uses a Moloney murine leukemia virus (MoMLV)–based retroviral vector. All of env and most of gag/pol except the segment constituting the packaging sequence (psi) have been removed from the SFG backbone. As a result, the vector is replication incompetent.

The intended function is the expression of CD19 after transduction in T cells

**Evaluation of foreseeable effects**

Replication deficient retroviral vectors pseudotyped with GALV env are able to infect a broad range of mammalian cells including human cells during their cell division. But they require MLV gag/pol as well as the envelope protein, both provided by the helper cell line, for replication. For this reason uncontrolled spread is not expected.
Material is stored on site and distributed frozen

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No autoclave in the building: Only storage of material is performed at the site. Therefore contamination will only occur if there is the breakage of a bottle. Spill procedures are in place for spills, all material in contact with the spill is collected, treated with 2% Virkon and sent off site for incineration with a registered waste management company. There is no equipment used during storage that would come into contact with the virus and require autoclaving.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Secondary storage only on site. All materials would be sent off site to a licensed facility for incineration. In the event of a spill all materials/surfaces would be treated with 2% virkon for 1 hour. All contaminated materials would be sent for incineration following decontamination in virkon.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

no further comments

Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2 L3 L4 L2 L3 L4 L2</td>
<td>L3 L4 L2 L3</td>
<td>L4</td>
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</tbody>
</table>

Project Ref 3136/16.2
**Project Additional Information**

**Purposes of the contained use**

Storage and distribution of material for clinical trials.

**Recipient or parental organism**

The recipient organism is the 293 HEK cell line which was derived from human embryonic kidney (HEK) cells transfected with fragments of mechanically sheared adenovirus S DNA (Ad5) by selection for cells that exhibited many of the characteristics of Ad transformation.

**Host/vector system**

Recombinant AAV vector serotype 2 containing the human mitochondrial ND4 gene (rAAV2-ND4 vector)

**Origin & function**

Human derived:

Insert 1.1:
- Name: NADH deshydrogenase type 4 gene.
- Characterization/nature of the sequence: cDNA encoding for the mitochondrial enzyme ND4 gene. This sequence has been codon optimized for an optimal gene expression in humans.
- Activity: the ND4 protein is involved in the mitochondrial respiratory chain, in particularly in the ATP synthesis used as a source of chemical energy for cell.

Insert 1.2:
- Name: HBB2, human beta globin intron
- Characterization/nature of the sequence: intronic sequence derived from the human beat globin human gene.
The material will be used for an in vivo gene therapy approach based on a recombinant AAV2 (rAAV2) viral vector expressing the human mitochondrial NADH deshydrogenase type 4 gene. The final GMO is the recombinant vector rAAV2/2-ND4 produced by tri-transfection of plasmids into adherent HEK293 cells. The final GMO will be administered by single intravitreal (IVT) injection into LHON patients bearing a mutation into their ND4 gene. Activity this sequence is used to enhance gene expression by facilitating the transport of mRNA from nucleus to cytoplasm.

Insert 1.3:
- **Name**: COX10 MTS
- **Characterization/nature of the sequence**: mitochondrial targeting sequence (MTS) derived from the COX10 mitochondrial gene (cytochrome c oxidase assembly homolog 10), gene which belongs to the mitochondrial respiratory chain complex assembly factors
- **Activity**: this sequence is used to translocate the mRNA into the mitochondria.

Insert 1.4:
- **Name**: COX10 UTR
- **Characterization/nature of the sequence**: Untranslated region (UTR) of the COX10 mitochondrial gene (cytochrome c oxidase assembly homolog 10), gene which belongs to the mitochondrial respiratory chain complex assembly factors
- **Activity**: this sequence is used to stabilize ND4 mRNA.

Cytomegalovirus derived:

Insert 2.1:
- **Name**: CMV promoter
- **Characterization/nature of the sequence**: Non coding sequence, promoter sequence.
- **Activity**: Used as binding DNA sequence for transcriptional factors. Allow the transcription of the cDNA ND4.

**DONOR 3: VIRUS**

1. **Scientific Name**: Adeno-associated virus
2. **Taxonomy**:
   (i) **order and/or higher taxon**: Parvoviriidae
   (ii) **genus**: Dependovirus
   (iii) **species**: Adeno-associated virus
   (v) **strain**: serotype 2
3. **Other names (usual name, strain name, etc.)**: The common name is AAV2
4. **Phenotypic and genetic markers**: NA
5. **Pathogenicity**: No

**Evaluation of foreseeable effects**

No pathological, ecological and physiological traits are present. In natural conditions, wild type AAV2 in the presence of a helper virus (adenovirus) is found to transmit to humans or non human primates only and is not known to colonize other species.

Wt AAV does not appear to cause any diseases in humans, and none of its close zootropic relatives cause any known diseases in animals.

Wt AAV2 as well as GS010 viral vector are non pathogenic.

When documented, the pathogenicity of parvoviruses is related to the Rep proteins. rAAV vectors contain no viral coding sequence, more especially they do not express Rep proteins which play a key role not only for DNA replication but also for site-specific integration and cellular growth inhibitory effects. Recently accumulated data enlighten the specific biological properties of rAAV such as integration specificity and efficiency.

Because they lack all viral encoded proteins (especially the Rep proteins), rAAV are doubly defective in comparison to
their wild type counterpart.
Most of the in vivo studies of rAAV mediated gene transfer have led to the conclusion that the vector is maintained into the transduced cells essentially as extrachromosomal episomes. However depending on the cell type, maintenance of the AAV genome occurs under multiple forms. In muscle, episomal forms appear to be almost the exclusive mode of persistence whereas, in liver cells, integrated DNA forms up to 5% have been reported. Accidental insertional mutagenesis and subsequently adverse effects such as gene disruption or oncogene activation associated with the use of AAV vectors is seen currently as a very infrequent possibility. It should be emphasize that no real and unambiguous adverse effect attributable to rAAV as been reported in the literature so far, notably in animals that have been followed over long period. [7]. Clinical trials performed up to now with AAV vectors has not raised this issue.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
NA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No Autoclave in the building. Only labelling, storage and distribution of primary packaged material being performed. Any contaminated material on site will be sent for incineration by licenced waste company. The only exposure on site will be if there is a spill, procedures are inplace to ensure all spilled material is decontaminated with 2% virkon prior to sending for incineration. No equipment is used on site that would require autoclaving.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
The only exposure on site will be if there is a spill, procedures are inplace to ensure all spilled material is decontaminated with 2% virkon prior to sending for incineration. All contaminated material is sent for incineration by a licenced waste company. Any material at the end of the trial is sent for incineration by a licenced waste company.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
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<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
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</table>

02/03/2022
### Project Additional Information

**Purposes of the contained use**

Storage and distribution of 5 retroviral supernatants material.

**Recipient or parental organism**

Human cells

**Host/vector system**

*murine gamma Retroviral SIN vector*

**Origin & function**

*synthetic therapeutic gene of Human origin*

The intended function is chimeric antigen receptor (CAR) like protein.

The 4 retroviral vectors and inserts are named as follows:

- CG_CAR_001.1 (HE-DN35)
- NCARGD2MP10413
the full vector map for each insert is shown on each risk assessment

**Evaluation of foreseeable effects**

The supernatent contains replication deficient, recombinant gamma-retroviral SIN vector particles pseudotyped with Gibbon Ape Leukimia virus (GALV) envelope, thus are infectious for human but not mouse (xenotropic). Infection requires cell division. Accidental uptake (wounding) of vectors will result in rapid degradation by the host immune system.

The vectors do not encode any toxin and are unable to pread in the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

NA

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No Autoclave in the building. Only storage and distribution of primary packaged material being performed.

Any contaminated material on site will be sent for incineration by licenced waste company. The only exposure on site will be if there is a spill, procedures are inplace to ensure all spilled material is decontaminated with 2% virkon prior to sending for incineration. No equipment is used on site that would require autoclaving.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The only exposure on site will be if there is a spill, procedures are inplace to ensure all spilled material is decontaminated with 2% virkon prior to sending for incineration. All contaminated material is sent for incineration by a licenced waste company. Any material at the end of the project is sent for incineration by a licenced waste company. Material is kept frozen whilst on site and during distribution so there is minimal risk of aerosols from vial breakage.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

**Project Containment**

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02/03/2022
**Project Ref ** 3136/16.4

<table>
<thead>
<tr>
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<th>CU2 Project Title</th>
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</thead>
<tbody>
<tr>
<td>18/08/2016</td>
<td>Storage and distribution of numerous virus vectors on behalf of clients, which have been altered to make them replication deficient within the environment</td>
</tr>
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<table>
<thead>
<tr>
<th>Date Project Ceased</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<th>Consent Granted</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Project notified under transitional arrangements N

- **Historical Significant Changes**
- **Historical Date of Additional Info**
- **Significant Change ID**
- **Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The material may be for storage only, or for labelling and distribution to clinical trial sites. PCI Bridgend is a clinical trial cold chain storage and distribution specialist.

**Recipient or parental organism**

The majority of materials are for use in Humans as various therapies in clinical trial. Influenza is for use in testing of flu vaccines.

**Host/vector system**

- murine, human or avian

**Origin & function**

Various genetic inserts are inserted into the recombinant vectors. These are all identified on individual risk assessments held by PCI. Consideration of the effect of the vector insert and position of the insert is made when assessing the class of the
GMO. Only class 1 and 2 materials are handled at the site. These remain fully closed at all times and held with appropriate containment measures (frozen, doubly contained with absorbant materials within each box).

**Evaluation of foreseeable effects**

Materials held at site are generally replication deficient or altered to ensure they are unable to infect and spread between hosts. An assessment of the effect of each individual GMO held at PCI Bridgend is completed on the individual risk assessments.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

NA

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No Autoclave is present in the building. Only storage, distribution and occasional labelling of primary packaged material being performed. Any contaminated material on site will be sent for incineration by a licenced waste company. The only exposure on site will be if there is a spill, procedures are in place to ensure all spilled material is decontaminated with 2% virkon prior to sending for incineration. No equipment is used on site that would require autoclaving. All staff handling influenza material are vaccinated with the annual flu vaccine.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

The only exposure on site will be if there is a spill, procedures are in place to ensure all spilled material is decontaminated with 2% virkon prior to sending for incineration. All contaminated material is sent for incineration by a licenced waste company. Any material at the end of the project is sent for incineration by a licenced waste company. Material is kept frozen whilst on site and during distribution so there is minimal risk of aerosols from vial breakage.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This is a generic application to cover the contained use performed at the site for the range of materials we currently handle. All the material types listed have already been accepted by the HSE for handling at PCI Bridgend. This would allow us to bring in new genetic inserts for the current organism types and activities without completing individual CU2’s. However every GMO brought into the PCI Bridgend facility would undergo full risk assessment and if it did not meet the criteria covered within this notification for organism type or activity a separate notification would be made. No new influenza strains would be brought onto the site without discussing with the HSE to determine whether the current notification and precautions are sufficient.
## Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
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</tr>
</thead>
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<td>Emergency Plan Required?</td>
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**Name**

QUOTIENT SCIENCES LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

RUDDINGTON FIELDS BUSINESS PARK

**Road Name**

MERE WAY

**Town**

NOTTINGHAMSHIRE

**County**

NG11 6JS

**Postcode**

ENGLAND

**Country**

**Tel Number**

0115 974 9000

**Fax Number**

0115 974 8000

**E-mail**

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

<table>
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<tr>
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<td>MERE WAY</td>
<td>NOTTINGHAM</td>
<td>NOTTINGHAM SHIRE</td>
<td>NG11 6JS</td>
<td>ENGLAND</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Committee is comprised of the following - VP Pharmaceutical Sciences (senior management), Health & Safety Manager, Clinical Research Physician, Scientists with relevant microbiology background and experience.

As we do not routinely perform GMM work, the committee will meet on an as required basis, prior to each program of work. The committee will be responsible for ensuring a suitable and sufficient risk assessment of all work is performed as required by the Regulations. The H & S Manager will be responsible for ensuring employees handling GMMs are trained appropriately and are made aware of the outcome of risk assessments.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Other (please specify) Tick if confidential

02/03/2022
An autoclave is available on the premises and will be used to inactivate GMM waste prior to disposal, where the risk assessment dictates this is required. The inactivated waste will then be disposed of as clinical or chemical waste via an appropriately licensed waste contractor.

For activities involving GMMs, describe the waste management measures which will apply to the activity

An autoclave is available on the premises and will be used to inactivate GMM waste prior to disposal, where the risk assessment dictates this is required. The inactivated waste will then be disposed of as clinical or chemical waste via an appropriately licensed waste contractor.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC have reviewed the risk assessment and are satisfied that all hazards associated with handling the GM material have been adequately assessed. The Committee is satisfied that the program of work will be of negligible risk to both human and environmental safety. This was confirmed at a meeting held on 10th October 2011
GM Centre Number: 3139

Data Premises Notified (Originally) 20/01/2012
Transferred from 1992 Regs? N
Transitional Premises Class

Data Premises Closed 27/04/2015
Transitional Premises Emergency Plan Required? N
Non-GMMs N
Withdrawn N

Name
MABLYTE LTD

Name 2

Department
SCIENCE AND TECHNOLOGY CENTRE

Campus Estate or Research Centre
UNIVERSITY OF READING

Building
EARLEY GATE

Road Name
WHITEKNIGHTS ROAD

District

Town
READING

County
BERKSHIRE

Postcode
RG6 6BX

Country
ENGLAND

Tel Number 01189 357368 Fax Number 0

Date at Which Additional Info Submitted
02/03/2022

Comments
GM Centre closed class 1 work transferred to GM3007 other project (class2) closed

HSE Division blank

E-mail

02/03/2022
### Premises Addresses

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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The members of the safety committee are ex officio members of the GM committee. They comprise the owner and the Principal Scientist. Both of them have extensive prior experience of working with the type of GMMs to be used. Both members are based at the laboratory where the work will be carried out. Meetings are held whenever a new project is to be initiated and at least once a year to review all projects.

#### Laboratory
- Level 1 (GMMs): Yes
- Level 2 (GMMs): Yes
- Level 3 (GMMs):
- Level 4 (GMMs):
- Non-microbial

#### Animal Unit
- Tick if confidential

#### Growth Room
- Transgenic Animals
- Transgenic Fish

#### Glass House
- Transgenic Birds

#### Large Scale
- Bacteriology
- Parasitology
- Microbiology Research: Yes
- Virology
- Transgenic Animals
- Gene Therapy

02/03/2022
All waste material containing GMMs or exposed to GMMs will be treated according to the standard operating procedure for biohazardous waste. It will be decontaminated by treatment with a suitable disinfectant prior to being securely bagged, sealed and sent for incineration by an approved contractor. Liquid waste will be decontaminated with disinfectant prior to disposal by a suitable route depending on the nature of the liquid. If these methods are deemed not to be suitable for particular items, they will be made safe by autoclaving in the on site autoclave prior to disposal by incineration. The estimated degree of kill by these combined methods is extremely close to 100%.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Mammalian cell lines will be transfected with gene vectors for research-scale production of recombinant proteins.

Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: N

Please enter comments of the GM safety committee on the risk assessment:
none. The risk assessment was approved by all members of the committee

Project Ref: 3139/13.1

Date Ackn'd: 28/11/2013
CU2 Project Title: Expression of Fc receptors in human lymphocytes
Date Project Ceased: 27/04/2015
Class: Class 2
Culture Vol: 1-50 Litres
Non-GMM: Consent Granted

Project notified under transitional arrangements: N

Tick if notifying a connected programme of work: N

Historical Significant Changes: CM3139 closed. Class work transferred to GM3007 class 2 project closed

Historical Date of Additional Info: 02/03/2022
### Purposes of the contained use

The purpose of the project is to develop human lymphocyte cell lines which express immunoglobulin Fc receptors and are able to kill antibody-coated target cells. The cell lines will be used in research and quality control of antibodies.

### Recipient or parental organism

The recipient cells will be human lymphocyte cell lines with characteristics of natural killer cells, such as NK-92 and/or KHYG-1. NK-92 was established in 1992 from the blood of a 50-year-old man with non-Hodgkin lymphoma. The cell line has been routinely used by others for the planned purpose. The cell line is EBV positive, with a latent infection, and is classified Level 1.

KHYG-1 was established in 1970 from the pleural effusion of a 53-year-old woman with chronic myeloid leukemia. The cell line is commonly used in laboratories for cytotoxicity assays. The cell line is classified Level 1.

The intermediate host cell line for packaging of the retroviral vector will be HEK-293T. This is a very widely used human embryonic kidney cell line which has been transformed with adenovirus E1a and carries a temperature sensitive T antigen co-selected with neomycin. The cell line is classified Level 1.

### Host/vector system

The Gryphon expression vector pCHAC is a standard vector based on murine moloney leukaemia virus (MMLV). Such viruses can potentially cause cell transformation and tumour formation. The vector has been rendered replication-incompetent by deletion of its envelope (env) genes and therefore needs to be produced in a packaging cell which can complement the env gene in order to produce infectious virus. The host range depends on the specificity of the viral envelope. We will be using an amphitropic env gene to allow infection of murine and non-murine, including human cells.

Following transduction, the virus integrates into the host genome and is present in the infected cells as a DNA provirus. The main safety concern is the possibility of recombination between the provirus and an endogenous helper virus which might theoretically result in the production of replication competent virus.

Gryphon is an upgraded version of the Phoenix retroviral transduction system which has an excellent safety record over many years of use with a wide variety of host cells, including NK cells such as those proposed in this project. Gryphon includes a gene encoding a green fluorescent protein (mWasabi) for easy visualisation of transfectants and to prevent potential recombination, different non-Moloney promoters for the gag-pol and env genes are used. Nevertheless, due to the theoretical possibility of producing replication competent viruses, the genetically modified cell lines resulting from this project will be classified Class 2 unless and until the absence of replication-competent virus is proved.

### Origin & function

There are several types of immunoglobulin Fc receptors (FcR) which are naturally found on the surface of various human white blood cells. They include CD16, CD32 and CD64. We propose to use CD16 which is a low affinity receptor found on the surface of natural killer cells. It is involved in antibody-dependent cell-mediated cytotoxicity (ADCC). Natural killer cell lines such as NK-92 and KHYG-1 do not express CD16. By transfecting them with a vector which contains the CD16 gene, we expect that the CD16 Fc receptor will be expressed on their surface and they will be enabled to carry out ADCC in a similar fashion to normal natural killer cells.

The CD16 gene to be used in these experiments will be designed to code for the published protein sequence of human CD16 protein. The natural protein consists of two subunits. It has been shown that the corresponding genes may be fused together to code for a single protein chain. This may increase the chance of success of the transfection and improve the expression levels. The codon usage of the synthetic CD16 gene will be optimised for expression in human cells.

### Evaluation of foreseeable effects

This project includes in-frame insertion of expressible DNA downstream of a promoter with the intention of expressing Fc receptors (e.g. CD16) on the surface of mammalian cells. Therefore, it is possible that biologically active proteins will be produced. However, the quantities will be extremely small (micrograms) and it is not...
suspected or known that these molecules are toxic, oncogenic or would be detrimental if administered to a target tissue. No adverse effects are expected to be associated with the genetic insert.

The vectors used in this project will be well-characterised replication-incompetent retrovirus. The vectors will contain only selective markers (ampicillin resistance) that are already in routine use in standard cloning vectors and will contain no recognisable harmful sequences. This specifically excludes cloning any sequences that are known or suspected to be oncogenic, encode a toxin or allergen or encode a product that could be detrimental if delivered to a target tissue. No adverse effects are expected to be associated with the vector per se.

The final engineered cells will contain the stably integrated vector as a DNA provirus. A theoretical safety concern is the possibility of recombination between the provirus and an endogenous helper virus (if such exists in the host cells) which might theoretically result in the production of replication competent virus. We are not aware of any reports of such recombination events associated with the cell lines or vector system to be used. Nevertheless, as a result of this theoretical concern, the resulting GMM will be treated as Class 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

none

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste material which might be contaminated with the GMM will be disposed as follows:

Cell culture ware, plastic ware etc will be immersed in 10% bleach or 1% Virkon for at least 1 h. Aqueous liquid will be discarded to the laboratory drain, solid waste will be securely bagged and taken for incineration by an approved contractor (Select Environmental Services Limited, Prosper Park • Bennet Road • Reading • Berkshire RG2 0QX)

Culture medium, other liquids will be treated with bleach to final concentration of 10% or Virkon to a final concentration of 1%, for at least 1 h and discarded to the laboratory drain.

Sharps, pipettor tips, broken glass etc will be stored in sealed sharps container and taken for incineration by the approved contractor.

Our risk assessment indicates that these proposed waste management measures are at least as effective, and overall less inherently hazardous than inactivation by autoclaving, and therefore the availability of an autoclave within the facility is not considered necessary.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was approved by the genetic modification safety committee without any objections

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
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GM Centre Number: 3140

Data Premises Notified (Originally) 26/01/2012

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

DISCUVA LTD

Name 2

Department

Campus Estate or Research Centre

THE MERRIFIELD CENTRE

Building

Road Name

ROSEMARY LANE

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB1 3LQ

Country

ENGLAND

Tel Number 0

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Committee composed of Biological Safety Officer, Deputy Biological Safety Officer, Associate Director, Anti-Infectives Research. This structure has inputs from three biologists and one chemist. All have extensive experience working in research and biotech drug discovery environments and previous involvement with health and safety, COSHH assessments and/or GMO safety committees. Any new planned GM work requires preparation of a GM risk assessment, which is reviewed by the GMO safety committee. GMO Safety Committee will meet ahead of new planned GMO activities to ensure procedures, safeguards and compliance to regulations are in place in advance of initiation of those projects. These meetings will be minuted and records of these meeting retained.

<table>
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</table>
| Other (please specify) |             |             |             | Tick if confidential

02/03/2022
Solid waste that is contaminated with bacteria (including the parent strain and the GMO) will be autoclaved (heating with steam under pressure at 121°C for 30 minutes) to achieve 100% kill before removal from the containment facility. This will be monitored by taking samples from the autoclaved waste (e.g., from small liquid cultures) onto solid growth medium with incubation to allow any viable bacteria to grow into visible colonies, and reviewed by the GM safety committee at quarterly intervals.

Liquid waste that is contaminated will be treated with "Virkon" disinfectant according to the manufacturer's instructions to achieve 100% kill. This will be monitored by taking samples from treated waste onto solid growth medium with incubation to allow any viable bacteria to grow into visible colonies, and reviewed by the GM safety committee at quarterly intervals.

The safety committee discussed the potential for modulation of pathogenicity determinants by transposon insertion, but agreed that the number of endogenous genes so modulated would be limited and would therefore not confer a pathogenic advantage. Moreover, the experimental process does not specifically select for any pathogenic derivatives.
**Project Additional Information**

**Purposes of the contained use**
- To identify essential genes of bacteria that may indicate targets for the development of new antibiotics.
- To identify as yet unknown mechanisms of bacterial resistance to antibiotics.
- To assist in the identification of the bacterial targets for newly identified antibiotics.
- These results will be obtained for clinically relevant bacteria, as indicated above and below.

**Recipient or parental organism**
- Pseudomonas aeruginosa, Acinetobacter baumanii and Klebsiella pneumoniae that do not normally cause disease in healthy individuals but are associated with infection of patients in hospitals.
- Uropathogenic Escherichia coli that causes infection of the urogenital tract, both in hospitals and in the community, and like Klebsiella pneumoniae is transmitted in contaminated water and food. Neisseria gonorrhoeae which causes gonorrhoea and is transmitted by sexual contact.

**Host/vector system**
- The transposon mutagenesis procedure involves the introduction of a short DNA fragment of 1 to 2 kb (the transposon) directly into the bacterial cell without the need for an additional bacterial or DNA host or vector.

**Origin & function**
- The transposon used in the mutagenesis procedure consists of components of a naturally occurring transposon (Tn5) originally identified in an antibiotic resistance strain of a bacterium called Klebsiella. However, the transposon is modified to include only the minimal components required for the experiment. The functional components of the modified transposon consist only of a chosen antibiotic resistance gene and the short 19bp transposon ends which are recognised by the transposase enzyme during the transposition process. The antibiotic resistance gene of the transposon allows the selection of the GMO from the parental strain. The modified transposon does not include the gene coding for transposase which is present in the naturally occurring transposon. Instead, the transposase enzyme is bought commercially and mixed with the minimal transposon to generate the active transposition bodies. Thus, once the mutagenesis procedure is finished the minimal transposon is fixed in the bacterial genome, no longer able to insert anywhere else, as it does not possess the necessary transposase gene. The locations in the genome of the sites of transposon insertion can then be determined and used to identify targets for new antibiotics.

**Evaluation of foreseeable effects**
- The GMOs will become resistant to the antibiotic against which the resistance gene encoded by the transposon is active. These resistance genes will be chosen against...
antibiotics that would not normally be used in therapy, so that should any of the GMOs escape into the environment, or be transmitted inadvertantly to susceptible individuals, they will possess no selective advantage over non-GM bacteria and so would be unlikely to spread. The inserted DNA (transposon) does NOT possess any additional traits that would be expected to increase the GMM's ability to cause disease or survive in it's natural habitat. The transposon mutagenesis procedure will result in the modulation of genes in the genome as a result of the insertion of the transposon, most likely resulting in attenuation of the bacteria or reduced ability to survive in the environments normally inhabited.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation of containment level is requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste that is contaminated with bacteria (including the parent strain and the GMO) will be autoclaved (heating with steam under pressure at 121C for 30 minutes) to achieve 100% kill before removal from the containment facility. This will be monitored by taking samples from the autoclaved waste (eg. From small liquid cultures) onto solid growth medium with incubation to allow any viable bacteria to grow into visible colonies, and reviewed by the GM safety committee or quarterly intervals.

Liquid waste that is contaminated will be treated with "Virkon" disinfectant according to the manufacturers instructions to achieve 100% kill. This will be monitored by taking samples from treated waste onto solid growth medium with incubated to allow any viable bacteria to grow into visible colonies, and reviewed by the GM safety committee at quarterly intervals.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The safety committee discussed the potential for modulation of pathogenicity determinants by transposon insertion, but agreed that the number if endogenous genes so modulated would be limited and would therefore not confer a pathogenic advantage. Moreover, the experimental process does no specifically select for any pathogenic derivatives

Project Containment

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</table>

02/03/2022
Manipulation of HG2 pathogen genomes to aid antibiotic target identification and reveal resistance mechanisms by overexpression (complementation) or gene product downregulation of endogenous proteins

Purposes of the contained use
To elucidate mechanisms of resistance and sensitivity to known and new antibiotic compounds with the aim of revealing their modes of action and potential intracellular targets, and so aid in the development of new compounds as therapeutic antibiotics

Recipient or parental organism
Discova programs involve research using the following HG2 microorganisms:
Pseudomonas aeruginosa, Acinetobacter baumanii and Klebsiella pneumoniae that do not normally cause disease in healthy individuals, but are associated with infection of patients in hospitals.
Uropathogenic escherichia coli that causes infection of the urogenital tract, both in hospitals and in the community, and like Klebsiella pneumoniae is transmitted in contaminated water and food. Neisseria gonorrhoeae which causes gonorrhoea and is transmitted by sexual contact. Non-pathogenic E. coli K12.

Host/vector system
Laboratory strains of E. coli will be the host for the DNA vectors.
Bacterial plasmid DNA vectors will be used, and will include standard cloning vector plasmids, which are often commercially available. Suicide vectors will also be utilised which do not normally replicate in bacteria other than specific laboratory strains of E. coli.

Origin & function
Plasmid vectors to be used will be constructed from components that occur naturally in the bacteria being used in the studies. These components include a replication origin to maintain the plasmid through replication cycles and an antibiotic resistance determinant to allow for selection of bacteria harbouring the plasmid vector. Some of the plasmid vectors may also include bacterial gene transcriptional promoters, such as the araBAD promoter from E. coli, which function to promote expression of genes inserted into the plasmid, and genes such as sacB from Bacillus subtilis that codes for levansucrase and which allows counter-selection against the plasmid in the presence of sucrose. None of the plasmid vectors to be used are self-transmissible, unlike the many naturally occurring plasmids from which the plasmid vector components are derived.

The chromosomally-inserted DNA will normally code for a gene, or genes, of interest from the HG2 micro-organism, and may include the gene's native promoter, or potentially a heterologous inducible promoter (such as araBAD). The inserted DNA functions to complement existing gene function, or to mediate homologous recombination with the existing gene in the HG2 recipient in order to specifically replace, mutate, or knockout the gene or promoter of interest. Such mutations may serve to specifically inactivate the gene, or in some cases to specifically regulate expression of the gene.

**Evaluation of foreseeable effects**

The modern DNA manipulation techniques to be employed allow the precise construction of DNA molecules, thus unforeseeable effects due to the presence of DNA not necessary for the experiment can be avoided.

Some GMOs will become resistant to the antibiotics to which the resistance gene encoded by the plasmid vector is active. However, only one or two antibiotics to which the resistance gene encoded by the plasmid vector is active. However, only one or two antibiotic resistance determinants are used at a time, so the generation of multiple resistance GMOs will normally be avoided. In the case of chromosomally-inserted sequences, the ultimate GMO will not possess a selectable resistance gene.

Any resistance genes are chosen against antibiotics that would not normally be used in therapy, so they will possess no selective advantage over non-GM bacteria, would be expected to respond to treatment, and would be unlikely to spread.

The inserted DNA does NOT possess any additional traits that would be expected to increase the GMO's ability to cause disease or survive in its natural habitat, or any other environment. The inserted DNA would normally code for genes that have been identified as modulators of sensitivity to antibacterial agents (novel antibiotics). As such, some GMOs will possess reduced susceptibility to these agents, while others will demonstrate increased susceptibility. This reduced susceptibility is unlikely to be sufficient to cause the GMO to show clinical (high levels of) resistance to those agents, and is unlikely to persist in the absence of continued selection pressure.

The work does not involve specifically the modulation or investigation of genes coding for pathogenicity determinants; rather, it involves those that may be involved in the modulation action of resistance to known and new antimicrobial compounds, which would be expected to be genes important for growth under the laboratory conditions which are used to study antibiotic resistance.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- The work does not involve any non-micro-organisms.

- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
  - No derogation of containment level is requested

- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
  - Solid waste that is contaminated with bacteria (including the parent strain and the GMO) will be autoclaved (heating with steam under pressure at 125°C for 30 minutes) to achieve 100% kill before removal from the containment facility. This will be monitored by taking samples from the autoclaved waste (e.g. From small liquid cultures) onto solid growth medium with incubation to allow any viable bacteria to grow into visible colonies, and reviewed by the GM safety committee at quarterly intervals. The autoclave used for processing is validated annually. All processed solid waste will be transferred to an authorised Waste Management provider where waste will be processed via incineration.
Liquid wasr that is contaminated will be treated with "Virkon" disinfectant according to the manufacturer's instructions to achieve 100% kill. This will be monitored by taking samples from treated waste onto solid growth medium with incubated to allow any viable bacteria to grow into visible colonies, and reviewed by the GM safety committee at quarterly intervals.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The safety committee discussed the potential for modulation of pathogenicity by genetic manipulation procedures, but agreed that such modulation would most likely result in no change or reduced pathogenicity, although scientists will be vigilant for changes to pathogen characteristics. In addition, current working practices are sufficient to prevent exposure. The safety committee concluded that the work involved is no more risk than a microbiology lab handling different strains of HG2 pathogens which themselves will vary in their levels of virulence.

Moreover, the experimental process does not specifically select for any derivatives with increased pathogenicity.

Please enter comments on the GM safety committee on the risk assessment

The safety committee discussed the potential for modulation of pathogenicity by genetic manipulation procedures, but agreed that such modulation would most likely result in no change or reduced pathogenicity, although scientists will be vigilant for changes to pathogen characteristics. In addition, current working practices are sufficient to prevent exposure. The safety committee concluded that the work involved is no more risk than a microbiology lab handling different strains of HG2 pathogens which themselves will vary in their levels of virulence.

Moreover, the experimental process does not specifically select for any derivatives with increased pathogenicity.

**Project Containment**

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### Name

NEUSENTIS (PFIZER)

### Name 2

### Department

### Campus Estate or Research Centre

### Building

PORTWAY BUILDING (UCB)

### Road Name

GRANTA PARK

### Town

CAMBRIDGE

### District

### County

CAMBRIDGESHIRE

### Postcode

CB21 6GS

### Country

ENGLAND

### Tel Number

01304 643708

### Fax Number

0

### E-mail

### HSE Division

Blank

### Comments

### Date at Which Additional Info Submitted

02/03/2022

Page 13140 of 15326
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

Biological Safety Officer attends monthly safety meetings to raise any biological safety issues. All lab stewards attend and disseminate information to lab users. Risk assessments are created in an electronic registration system and are circulated to registered Gmusers with appropriate expertise and experience, before final approval by BSO and discussion at safety meeting.

<table>
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Tick if confidential: 
| Bacteriology | Yes | Parasitology | Yes | Transgenic Birds | Yes |
| Virology | Yes | Transgenic Animals | Yes | Transgenic Fish | Gene Therapy |
| Mycology | Transgenic Invertebrates | Transgenic Plants | Other (please specify below) |

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Solid biological waste is autoclaved and liquid waste is inactivated in 2% Virkon, before disposal down the sink. See attached document 'Virkon efficacy data'.

**Tick to confirm that you are attaching a summary of the risk assessment** Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

**Please enter comments of the GM safety committee on the risk assessment**

Please see the attached documents 'Lentivirus notification' and Significant Change to risk GM331/08.1 - GMSC approved document'

Neusentis was originally covered under GM centre GM331 and the attached assessment was originally notified at Sandwich. The significant change was submitted to include Neusentis work. This activity will be transferring entirely to Neusentis and to the new GM centre reference ie Neusentis will carry out class 1 activities exactly as previously notified at Sandwich, but now independently of Sandwich and class 2 activity only in terms of in vivo use of lentivirus, where the use of sharps increases risk to human health.

---

**Project Ref 331/08.1**

**Date Ackn’d** 06/03/2012

**CU2 Project Title** Construction and use of Lentivirus vectors for the expression of heterologous proteins and inhibitory RNA sequences in mammalian cells.

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<tbody>
<tr>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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**Date Project Ceased**
**Project Additional Information**

**Purposes of the contained use**

The lentivirus vector system will be used for the following experimental purposes:

1. To evaluate gene function in normal body cell biology and disease by overexpression or RNAi-mediated knockdown of the relevant gene function in primary cultured cells from normal and diseased tissues.

2. To generate stable cell lines expressing genes of interest to be used in the development of cell-based screening assays and disease-relevant in vitro cell models. These assays will be used to explore gene function and to screen compounds for activity at drug targets.

**Recipient or parental organism**

Recipient organisms will be cultured mammalian cells.

**Host/vector system**

The lentiviral vector systems used for these studies will be third generation replication-defective lentiviral vector systems. Only gag, pol and rev genes from HIV will be employed and these will be supplied in trans to facilitate virus packaging. All other HIV genes have been deleted. Examples of commercially available vectors with these properties include invitrogen’s ViraPower (pLenti vectors), and Stigma’s pLKO.1 vector systems. High litre virus stocks will be generated by cotransfecting a 293FT packaging cell line with transfer and packaging vectors.

**Origin & function**

Genes to be overexpressed will be derived from human or other relevant mammalian species (e.g. rodent, dog, non-human primate). Genes will be those considered to be of potential relevance in disease processes and will be overexpressed in cultured cells to determine their functional activity and to enable discovery of novel drugs targeting the gene product. Genes will include those encoding cell receptors, ion channels, enzymes and regulatory proteins from cellular signalling pathways. Genes will be derived from in-house cloning efforts or from academic or commercial sources.

Inhibitory RNA sequences will be targetted to genes considered to be of potential relevance in disease processes. Targetted genes will include those encoding cell receptors, ion channels, enzymes and regulatory proteins from cellular signalling pathways. Inhibitory RNA sequences will be obtained from commercial sources.

**Evaluation of foreseeable effects**

The risk associated with overexpression or knockdown of individual genes will be considered on a case by case basis and reviewed locally by the GM safety committee prior to work being started. Particular consideration will be given to genes that may be oncogenic or immunomodulatory.

VSV-G pseudotyped lentivirus strains will be used and these strains will have the ability to infect human cells and cells from other mammalian species. However, because these strains are replication defective, the virus can only carry out a single round of infection. Structural genes and other components required for packaging the viral
genome are separated onto three plasmids and have been engineered so as not to contain any homologous sequences to prevent undesirable recombination events that could lead to the generation of a replication-competent virus. Cells that may contain virus sequences able to mobilise the lentivirus vectors by providing structural genes in trans will be excluded from these experiments. Provirus insertion into the host genome will occur with high frequency and while this is unlikely to generate a harmful phenotype in the cultured mammalian cell (host cells from workers will not be used), there is a low probability of infection of a worker e.g. through accidental inoculation with packaged virus. Integration and long term expression of the recombinant gene or cDNA encoding inhibitory RNA could occur. There is a theoretical risk of transformation of cells by insertion of oncogenes or by knock-down of tumour suppressor genes. Because of the replication-defective nature of these vectors, this could only occur at low frequency and therefore unlikely to have a detrimental effect as a single event is unlikely to result in cellular transformation. Lentiviruses can also act as insertional mutagens. The likelihood of this occurring is reduced by the use of a self-inactivating vector which disables the LTR regions in the integrated vector and thus reduces the risk of oncogene activation at the site of insertion. Additionally, because the vector is replication-defective, the level of exposure that might occur following accidental introduction of the virus will be low and thus reduces the probability of virus insertion at a site likely to promote tumourigenesis. However, because these vectors incorporate strong heterologous promoters and because provirus insertion is a feature of retrovirus biology, work with these vectors is assessed as a class 2 activity and the use of appropriate containment is used to minimise exposure.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

To minimise the risk of escape of GMO's to the environment, all work will be performed in containment level 2 biology laboratory. Cultures will be grown within leakproof vessels. All contaminated waste streams generated during the course of this work will be decontaminated on site. Virkon disinfectant will be added to liquid waste and left for a defined contact period prior to disposal. Solid waste will be autoclaved and subsequently incinerated prior to disposal. Additional information on disinfection procedures is included in departmental safety manuals. The only foreseeable release of viable waste from our facility is in the form of aqueous waste that has been inadequately treated, possibly in the event of an accidental spill to a sink. Such waste is emitted via our Waste Water Treatment Facility (WWTF) before final discharge to the River Stour. Processing via the WWTF is such that the virus concentration will be massively diluted and will not reach the river Stour for approximately 5 days after leaving the laboratory. The treatment regimen in the WWTF includes a 24hr disinfection step. Thus the risk of viable organisms entering the local environment is effectively zero.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
This project was discussed with the HSE during a routine inspection by the Biological Agents Unit of the Pfizer facilities in April 2007. Under the new guidance from SACGM the Lentivirus work could be assigned as a class 2 activity. The HSE recommended that a generic notification and risk assessment should be submitted as a broad connected programme of work covering all Lentiviral vector work when the next Lentivirus project was proposed. Individual assessments would then be written at Pfizer for each proposed project but there would be no need to notify HSE of any future projects unless they fell outside the connected programme.
A copy of the risk assessment was circulated to the full GMSC membership who gave their approval.

### Project Containment

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<tr>
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GM Centre Number: 3143

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Name

ARGONAUT THERAPEUTICS LIMITED

Name 2

Department

Campus Estate or Research Centre

OXFORD SCIENCE PARK

Road Name

ROBERT ROBINSON AVENUE

Building

MAGDALEN CENTRE NORTH

District

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX4 4GA

Country

ENGLAND

Tel Number

01505 874184

Fax Number

0

E-mail

HSE Division

blank

Comments

Formerly Oxford Cancer Biomarkers -Informed 5/9/16 of name change

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Genetic Modification Safety Committee is comprised of Laboratory Safety Officer and Deputy Laboratory Safety Officer with input from other Company employees as appropriate. The Committee meets bi-monthly to discuss safety concerns and review risk assessments as required. More frequent meetings will be arranged as laboratory activities dictate.

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<thead>
<tr>
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<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

Yes

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<tr>
<th>Bacteriology</th>
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<td>Yes</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid waste: all solid waste will be collected and autoclaved before removal by waste contractors (Grundon). All liquid waste will be disinfected (Virkon solution, final concentration 2% before disposal down sink with plenty of water (in compliance with Thomas Water Trade effluent consent). Appropriate disposal will be monitored by the Safety Officer.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Safety committee review of risk assessment: no significant risks were identified and it was agreed that all procedures described were correctly classified as Class 1 risk and Containment Level 1 measures were appropriate.
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**DNA ELECTRONICS LTD**

**Name**

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

10 JOHN STREET

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

WC1N 2EB

**Country**

ENGLAND

**Tel Number**

020 7594 0822

**Fax Number**

020 7681 2821

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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<td>W12</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The committee consists of the following roles:
- Biosafety Officer
- Health and Safety Officer
- Deputy Health and Safety Officer
- Senior Molecular Biologist

<table>
<thead>
<tr>
<th>Laboratory</th>
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</table>

Other (please specify)

Tick if confidential

Yes

Bacteriology

Yes

Parasitology

Transgenic

Birds

Microbiology

Research
Waste management is controlled by SOP.

Contaminated microbiology consumables will be immediately discarded into liquid disinfectant (10% Trigene) and left for a minimum of 12 hours. Preparation of liquid disinfectant will be controlled by training and SOP.

Contaminated liquid, solid media and glassware will be sterilised by autoclave (121C for 15 mins). Autoclave usage will be controlled by training and SOP. Autoclave waste cycles will be validated and users will record confirmation that cycling has reached correct temperature and time before disposing of autoclaved waste via the clinical waste (yellow bag) route.

Contaminated surfaces will be wiped with liquid disinfectant before and after each use.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Standard class 1 GMM project. Happy to proceed with experiments outlined in risk assessment. Local rules must be followed at all times.
GM Centre Number: 3145

<table>
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Data Premises Closed | Transitional Premises | Emergency Plan Required? | N | N |

Name
AURELIA BIOSCIENCE LTD

Name 2

Campus Estate or Research Centre
BIOCITY

Road Name
PENNYFOOT STREET

Town
NOTTINGHAM

County
NOTTINGHAMSHIRE

Postcode
NG1 1GF

Country
ENGLAND

Tel Number
0115 8370503

Fax Number
0

E-mail
blank

HSE Division
blank

Comments

Date at Which Additional Info Submitted
02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee (GMSC) is comprised of the Chief Scientific Officer, Science Director and the Managing Director of the company. These three individuals have greater than 70 years combined experience of working within the research environment of the pharmaceutical industry. The CSO has previously held the role of Safety Officer for a group of 30 individuals. The Science Director has trained and supervised other members of staff in working with GMO's. The Managing Director has a background in Molecular Biology has served on GMO committees in the past and was chair of a cross-functional safety committee in previous employment.

All members of the GMSC have formal qualifications at postgraduate level in the form of a PhD or an MSc.

The committee meets as required due to the size of the company but at least once every 3 months to review risk assessments and to discuss proposals for new GMO work.

<table>
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The spent culture material from plastic culture ware will be inactivated using the disinfectant Trigene as per the manufacturer's recommendations. This will involve inactivation overnight using a 2% solution (final concentration) of the disinfectant Trigene. Following overnight treatment the inactivated liquid waste will be flushed down the sink. The disinfected plastic ware will then be bagged as biohazard waste and disposed of by a contractor (e.g. SRCL) via incineration. Contaminated sharps including pipette tips are disposed of in “sharps bins” that once full are sealed and sent for incineration by waste contractor. Monitoring and checks will be made on a weekly basis to ensure that liquid waste is being inactivated correctly and that the plastic waste is correctly bagged. Standard Operating Procedures will be used to ensure compliance.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The spent culture material from plastic culture ware will be inactivated using the disinfectant Trigene as per the manufacturers recommendations. This will involve inactivation overnight using a 2% solution (final concentration) of the disinfectant Trigene. Following overnight treatment the inactivated liquid waste will be flushed down the sink. The disinfected plastic ware will then be bagged as biohazard waste and disposed of by a contractor (e.g. SRCL) via incineration. Contaminated sharps including pipette tips are disposed of in "sharps bins" that once full are sealed and sent for incineration by waste contractor. Monitoring and checks will be made on a weekly basis to ensure that liquid waste is being inactivated correctly and that the plastic waste is correctly bagged. Standard Operating Procedures will be used to ensure compliance.

Other(s)

Use of immortal cell lines (e.g. HEK293 or CHO-K1) that have been transfected with human or animal D

For activities involving GMMs, describe the waste management measures which will apply to the activity

The GMSC discussed the operational plans and the remit of Aurelia Biosciences service offerings with respect to GMO's. The summary risk assessment represents the current scope of Aurelia Bioscience services. A decision was reached that the work to be conducted would be restricted to Class I GMO's at this time. The GMSC acknowledged that this policy would be reviewed in the future as the business need required.
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**Name**

CAMBIVAC LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

ROOM 2031, BUILDING 280 MONETA

**Road Name**

BABRAHAM RESEARCH CAMPUS

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGEShire

**Postcode**

CB22 3AT

**Country**

ENGLAND

**Tel Number**

01223 497481

**Fax Number**

0

**E-mail**

blank

**HSE Division**

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The committee is composed of four members including the biosafety officer, a technician (providing staff representation) and an executive director of the company along with a University Academic, with extensive experience in human and environmental safety, acting as an expert advisor. The Safety Committee fulfils the responsibilities under GMO Contained Use Regulations to approve risk assessments and provides guidance and direction on good bio-safety at the laboratory. The Safety Committee is representative of all people having access to the laboratory and will encourage discussion to ensure the views of all members are taken into account. Risk assessments will be circulated amongst the committee for scrutiny and commented on electronically & revisions will be made as necessary. The committee will meet at minimum twice a year with the ability to additionally meet promptly as and when needed.

<table>
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Other (please specify)

Tick if confidential

Yes

Bacteriology

Parasitology

Transgenic

Birds

Microbiology Research

Yes
Babraham Institute biosafety and guidance policy and guidance will be followed. In particular cell cultures will be inactivated by adding 1% Virkon and being left to stand for min 1 hour.

Waste material will be disposed of in 30 or 60l eurobins in accordance with BI biosafety policy "Clinical Waste Management Level 1"
**GM Centre Number:** 3148

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**Name**

GENZYME LIMITED

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

12 ROOKWOOD WAY

**District**

**Town**

HAVERHILL

**County**

SUFFOLK

**Postcode**

CB9 8PB

**Country**

ENGLAND

<table>
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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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<td>12 ROCKWOOD WAY</td>
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<td>SUFFOLK</td>
<td>CB9 8PB</td>
<td>ENGLAND</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The committee is made up of; (i) Biological safety officer, (ii) head of safety at Genzyme (Haverhill), (iii) environment representative, (iv) project coordinator and (v) site director.

Meetings will be held every 6 months

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<thead>
<tr>
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<th>Growth Room</th>
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<tr>
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</table>

Level 1 (GMMs)

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential

Bacteriology: Yes
Parasitology
Transgenic Birds
Microbiology Research: Yes

02/03/2022
All contaminated materials, including waste destined for incineration, will be inactivated by chemical sterilisation using Trigene or Virkon followed by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Based off the risks associated with the procedure and the genetic modifications also not posing any significant risks, the committee found that the activity was assigned as a class 1 activity.
<table>
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<tr>
<th>Data Premises Notified (Originally)</th>
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Name
IKSUDA THERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre
THE BIOSPHERE

Road Name
DRAYMANS WAY

District
NEWCASTLE HELIX

Town
NEWCASTLE UPON TYNE

County
TYNE AND WEAR

Postcode
NE4 5BX

Country
ENGLAND

Tel Number
0191 6031680

Fax Number
0

E-mail

HSE Division
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Comments

Date at Which Additional Info Submitted
02/03/2022
Premises Addresses

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Glythera's microbiological safety committee will compromise two (2) members of Glythera (COO and Principal Scientist), both of whom have been previously responsible as BSO for safety at Eden Biodesign, Spek, UK. In addition, the inex facility and it's tenant are represented by two (2) further persons. Meetings will be monthly whilst the company sets up its laboratory presence then revert to quarterly. All activities will be underpinned by risk assessment according to GMO policy.

<table>
<thead>
<tr>
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<tr>
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Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Inactivation of biological waste will be in accordance with local rules and will include chemical (liquid waste) and/or decontamination (solid waste) by autoclaving. Decontaminated waste will be removed from site by a registered waste disposal contractor.

<table>
<thead>
<tr>
<th>Virology</th>
<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
<th>Gene Therapy</th>
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<tr>
<td>Mycology</td>
<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
</tr>
</tbody>
</table>

Other(s)

Selected recombinant products will be expressed at between 2.5L and 5L scale and purified in order t

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessment will be carried out on a case by case basis based on strategic decisions. Risk assessments will be available upon request.
<table>
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| Comments | |

| Date at Which Additional Info Submitted | 02/03/2022 |
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

- General Manager (Chair)
- Senior Packaging Representative
- Warehouse Representative
- Environmental Health & Safety Representative
- Quality Representative

GMSC meetings held for individual projects as and when required as projects dictate

<table>
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<td>Other (please specify)</td>
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Tick if confidential
Waste disposal

The only waste that should be produced will be that arising from any breakages or rejected stock. As the material is supplied in vials and will be maintained frozen at all times while on site. It is not anticipated that significant quantities of waste will be produced. Any waste that is generated will be consigned as genetically modified clinical waste for incineration at a site notified to the HSE under the GMO (Contained Use) Regulations. Such waste will be transported in UN approved sealed waste units.

Spillage

Any spillage of HSV1716 will be inactivated by immersion in Virkon or Chloros overnight. Non-disposable items will also be immersed in Virkon or Chloros overnight prior to normal cleaning. Manufacturer's data on the disinfectant and its methods of use will be kept in the area spillage kits.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste disposal

The only waste that should be produced will be that arising from any breakages or rejected stock. As the material is supplied in vials and will be maintained frozen at all times while on site. It is not anticipated that significant quantities of waste will be produced. Any waste that is generated will be consigned as genetically modified clinical waste for incineration at a site notified to the HSE under the GMO (Contained Use) Regulations. Such waste will be transported in UN approved sealed waste units.

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Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The work covers the receipt, storage, labelling and distribution of pre-packaged vials (within a clinical supplies facility). A full assessment has been carried out and through the GMSC has been assigned Containment Level 1 and Activity Class 1.
### Project Additional Information

**Purposes of the contained use**

Storage and Distribution

**Recipient or parental organism**

The recipient organism is Adenovirus type 5; the taxonomy of which is detailed below:
- **Scientific Name:** Adenovirus Type 5 (Ad5)
- **Group:** Group III, Subgroup C
- **Family:** Adenoviridae
- **Genus:** Mastadenovirus
- **Species:** Human adenovirus
- **Strain name:** Serotype 5

**Host/vector system**

The gene transfer vector is a recombinant type 5 adenovirus vector containing the human interferon α2b (IFNα2b) gene. The vector was generated by recombination between an IFN-α2b-containing plasmid and a derivative of adenovirus type 5.

**Origin & function**

Replication deficiency: the final vector is replication deficient, as a consequence of removing the adenovirus E1a and E1b regions.
- Transfer of human IFN-α2b complementary DNA (cDNA): when administered locally to the pleural space, the vector transfects both normal mesothelial and malignant mesothelioma cells, resulting in the production of high and sustained local concentrations of IFN-α2b protein within the pleural space and tumor.

**Evaluation of foreseeable effects**

Expression is measured for each batch of rAd-IFN clinical material.
- Expression of IFNα2b is determined using a limit assay which is reported qualitatively (expresses/does not express IFN). The assay is complementary to the infectivity and potency assays.
- rAd-IFN has been engineered to be replication-incompetent and is naturally integration deficient. Therefore infection leading to replication of the GMO (and therefore potential for dispersal) is not possible under normal circumstances.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A - Bathate facility is Storage and Distribution only

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

If a spill occurs on site the waste will be processed as follows:
Disposable items should be placed in biohazard bags (or other appropriate designated containers) and incinerated (rAd-IFN is sensitive to heat; 1 hour at 56°C is used to inactivate the virus, or autoclaving at 121°C for a minimum of 15 minutes). Autoclaving will be carried out via an approved contractor. Once the GMO has been autoclaved it will be sent for incineration via an approved contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Storage and Distribution Only, recipient sites will access the risk to there operations/ people/ environment and consult with their own site GMO Safety Committee.

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 3150/21.2

Date Ackn'd: 16/06/2021
CU2 Project Title: Secondary Packaging, Storage and Distribution at the Catalent Bathgate facility
Class: Class 2
CultureVolClass2: Not Applicable
CultureVolumeClass3-4: Not Applicable
### Project Additional Information

#### Purposes of the contained use
Secondary Packaging (labelling vials), Storage and Distribution of Clinical Trial material.

#### Recipient or parental organism
No hazard identified from 293 or 293TR cells

#### Host/vector system
293 cells (human cells with part of the adenovirus genome integrated, so that adeno protein E1 is expressed). 293TR cells (293 cells expressing TetR repressor protein)

The recombinant adenoviral vector is replication incompetent, and can only form infectious particles in 293 cells, not in other human or animal cells, and cannot therefore cause a disseminated infection or disease.

#### Origin & function
Antigen to be used are targets of immune response. MAGEA3-NYESO is not allergenic or toxic but could potentially be oncogenic or unknown function.

Transfection of 293 cells with the recombinant adeno genome and production of adeno particles for animal immunisations and pre-Master Virus Seed intended for clinical manufacture. Max volume 10 x 500 ml

#### Evaluation of foreseeable effects
The resulting genetically modified virus will be infectious but will be replication defective. Expression of the inserted MAGEA3-NYESO may increase the hazard of the virus.

The vector is therefore assigned to hazard group 2.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A - Bathgate facility is Secondary Packaging (labelling of vials), Storage and Distribution only

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation from autoclave on site. As per client information the use of Virkon solution is sufficient to deactivate the material.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
If a spill occurs on site the waste will be processed as follows:
Area will be evacuated and a Risk Assessment completed. Disposable items should be placed in biohazard bags (or other appropriate designated containers) and incinerated. The material will be sent for incineration via an approved contractor.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

Please enter comments on the GM safety committee on the risk assessment
Secondary packaging (labelling of vials), Storage and Distribution, recipient sites will access the risk to there operations/people/environment and consult with their own site GMO Safety Committee.

Project Containment

<table>
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Animal Units

<table>
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Project Ref 3150/21.3

Date Ackn'd 16/06/2021

CU2 Project Title Secondary Packaging, Storage and Distribution at the Catalent Bathgate facility (MVA-NYESO)

Class 2

CultureVolClass2 Not Applicable

CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements [N]

Tick if notifying a connected programme of work [N]

Historical Significant Changes [N]

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

Secondary Packaging (labelling vials), Storage and Distribution of Clinical Trial material.

**Recipient or parental organism**

NONE: Primary chicken embryo fibroblasts from an SPF flock may be handled at containment level 1. Hazard Group 1 for all mammalian cell lines. Cell lines have been screened for the presence of contaminating agents.

**Host/vector system**

NONE: The recombinant MVA vector can only form infectious particles in the relevant permissive cells, not in other human or animal cells, and cannot therefore cause a disseminated infection or disease.

**Origin & function**

NONE: The resulting genetically modified virus will be infectious but will be replication defective. The inserted NYESO gene may increase the overall hazard group, as the function of the antigen is not fully known.

The NYESO gene is synthetically produced and cloned and will be derived from plasmids for this work.

NYESO is not allergenic or toxic and not known to be oncogenic. However, the function is not fully known.

Infection of CEF cells with the recombinant MVA and production of recombinant MVA particles. Maximum cell culture volume 2 x 1.3 litres ml in Vaccitech laboratories.

**Evaluation of foreseeable effects**

The recombinant MVA-NYESO particles generated in the permissive cells will transduce animal cells and express the NYESO transgene. The proteins will then be processed by the normal cellular machinery. If injected into animals, an immune response will be generated against the recombinant protein. However no further infectious particles can be formed.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A - Bathgate facility is Secondary Packaging (labelling of vials), Storage and Distribution only.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Derogation from autoclave on site. As per client information the use of Virkon solution is sufficient to deactivate the material.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

a spill occurs on site the waste will be processed as follows:

Area will be evacuated and a Risk Assessment completed. Disposable items should be placed in biohazard bags (or other appropriate designated containers) and incinerated. The material will be sent for incineration via an approved contractor.
Please enter comments on the GM safety committee on the risk assessment

Secondary packaging (labelling of vials), Storage and Distribution, recipient sites will access the risk to their operations/ people/ environment and consult with their own site GMO Safety Committee.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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#### Animal Units

<table>
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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2</td>
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### Project Ref 3150/21.4

- **Date Ackn’d**: 16/06/2021
- **CU2 Project Title**: Secondary Packaging, Storage and Distribution at the Catalent Bathgate facility (MVA-MAGE-A3)
- **Class**: Class 2
- **Culture Volume Class 3-4**: Not Applicable
- **Non-GMM**: Consent Granted

#### Historical Significant Changes

- **Historical Date of Additional Info**: 02/03/2022
- **Significant Change ID**: Not applicable

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## Project Additional Information

### Purposes of the contained use

Secondary Packaging (labelling vials), Storage and Distribution of Clinical Trial material.

### Recipient or parental organism

Primary chicken embryo fibroblasts from an SPF flock may be handled at containment level 1. Hazard group 1 for all mammalian cell lines. Cell lines have been screened for the presence of contaminating agents. CEF can be considered as especially disabled hosts and are unable to survive outside of the laboratory environment. CEF are primary cell cultures with a limited passage capability and no tumorigenic potential.

### Host/vector system

The recombinant MVA vector can only form infectious particles in the relevant permissive cells, not in other human or animal cells, and cannot therefore cause a disseminated infection or disease.

### Origin & function

MAGE-A3 is one of the melanoma antigen gene (MAGE-A3)-type antigens. This protein is expressed in various tumour cells but not expressed in normal tissues except in male-germline cells that lack MHC class I molecules. Since the MAGE-A3 protein is restricted to germ cell of testis and trophoblast of placenta which are immune privileged tissues, the protein is highly immunogenic and recognized by CTLs when expressed elsewhere. MAGE-A3 is the most frequently expressed MAGE-A3-type antigen in human cancers.

Infection of CEF cells with the recombinant MVA and production of recombinant MVA particles. Maximum cell culture volume 2 x 1.3 litres ml in Vaccitech laboratories.

### Evaluation of foreseeable effects

Negligible. MVA-MAGE-A3 this poses no environmental impact as the recombinant vector cannot disseminate. In additional, all waste is inactivated, and any transported materials are suitably packaged according to UN3373. The resulting genetically modified virus (MVA-MAGEA3) will be infectious but will be replication defective. There potential tumorigenic nature of the MAGE-A3 inserted gene increases the potentially hazardous nature of the recombinant MVA. MVA-MAGEA3 is therefore classified as Hazard Group 2.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A - Bathgate facility is Secondary Packaging (labelling of vials), Storage and Distribution only

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation from autoclave on site. As per client information the use of Virkon solution is sufficient to deactivate the material.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

If a spill occurs on site the waste will be processed as follows:

Area will be evacuated and a Risk Assessment completed. Disposable items should be placed in biohazard bags (or other appropriate designated containers) and incinerated. The material will be sent for incineration via an approved contractor.
Secondary packaging (labelling of vials), Storage and Distribution, recipient sites will assess the risk to their operations/ people/ environment and consult with their own site GMO Safety Committee.

Please enter comments on the GM safety committee on the risk assessment

Secondary packaging (labelling of vials), Storage and Distribution, recipient sites will assess the risk to their operations/ people/ environment and consult with their own site GMO Safety Committee.

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Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Is an emergency plan required according to regulation 20? N
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**Name**

<table>
<thead>
<tr>
<th>Name</th>
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**Name 2**

<table>
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**Campus Estate or Research Centre**

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**Building**

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**Town**

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**County**

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**Postcode**

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**Country**

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**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
**Premises Addresses**

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<td>OX4 4GE</td>
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  
Y

Give brief details of the genetic modification safety committee

Oxford Genetics GM Risk Assessments are reviewed and approved by the Biological Safety Committee that we have established within the company. This committee provides guidance on all safety aspects of projects that are proposed within the company. It attempts to identify possible hazards associated with the proposed work that could compromise the safety to the general public, the risk of cross contamination or recombination, the risk of release into the community of GMOs, and any ethical considerations that may be relevant. Its members are drawn from a variety of scientific backgrounds and bring a diverse range of expertise, both within and outside, of the company itself, from University lecturers to MDs. The Committee conducts its business both electronically and in meetings on an ad hoc basis whenever new GMO projects are proposed.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
</table>

02/03/2022
Any Solid GMM Biological Waste (including flasks, pipettes, tips etc) is to be sterilised in an autoclave that has been validated to ensure the sterility of autoclaved waste. After autoclaving, bags will be transferred to yellow clinical waste bags no more than 3/4 full and closed with a black cable tie. Full clinical waste bags are put in a locked cabinet for collection by the waste disposal service where they will be incinerated.

Liquid GMM Biological Waste is to have sufficient Virkon powder added to it to make it at least 1% overall and left for a minimum of 24 hours to inactivate any viable pathogenic material. Thereafter it is to be disposed of down the sink with plenty of water.

Contaminated sharps such as needles used in banding should be soaked in 1% Virkon for 24 hours then disposed of in the sharps bins provided. Full sharps bins are collected by the waste disposal contractors and incinerated off site. The process of disinfecting with Virkon has been demonstrated to completely destroy any infectivity or viability of every microorganism used by our own staff by the manufacturere of the powder. Susceptibility of any new GMO to killing in this way will be validated prior to use.

**Please enter comments of the GM safety committee on the risk assessment**
The GMO Biological Safety Committee reported that the genetic engineering conducted at Oxford Genetics poses little to no risk to any of the companies' members, the environment, or the general public. It is of a non-infectious, non-hazardous nature and the genes that are being encoded within their genetic material have no inherent biological toxicity. It was noted that a Risk Assessment number should be used to help track Risk Assessments (this has now been added), and the committee wanted to remind the company to keep all manufacturer's literature and product information for any vectors, organisms or cells that are being purchased, to add to the background safety information. The committee also noted that every risk assessment should be reviewed on a regular basis to ensure they are still accurate and applicable.

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**Project Ref** 3151/17.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
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<tbody>
<tr>
<td>10/02/2017</td>
<td>Engineering and generation of retroviral and lentiviral vectors (small scale)</td>
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<table>
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<tr>
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**Historical Significant Changes**

<table>
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<tr>
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<tr>
<td>Significant Change ID</td>
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<td>Date of Significant Change</td>
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**Project Additional Information**

**Purposes of the contained use**

To improve the production processes for replication incompetent retrovirus and lentivirus particles in terms of viral titres, safety and infectivity in a range of different cell types.

**Recipient or parental organism**

The parental organism of retroviral vectors are Moloney Murine Leukemia Virus (MoMLV) and/or Murine Stem Cell Virus (MSCV) belonging to the gammaretroviral genus of the Retroviridae family. The parental organism of lentiviral vectors is Human immunodeficiency virus (HIV) belonging to the lentiviral genus of the same family of viruses. Following infection with retroviruses/lentiviruses, the viral genome stably integrates into the genome.
of the host cell whereby it uses the host cell replication machinery to replicate, giving rise to a persistent infection. Particularly the gammaretroviruses have a bias for integration near proliferation-associated genes or transcriptional start sites endowing them with a high potential for oncogenesis through insertional mutagenesis. Lentiviruses on the other hand show a stronger preference for integrating within active transcription units with no such bias and so are generally considered nononcogenic. Gammaretroviruses can infect only actively dividing cells whereas lentiviruses can also target nondividing cells.

Wild-type MoMLV can infect only mice and is oncogenic as chronic productive retroviral infection allows insertional mutagenesis leading to cell transformation and tumor formation. However, amphotrophic envelope MoMLV or recombinant viral particles pseudotyped with non-native envelope can have a wider host range which might include humans and other vertebrates. Therefore, infection of humans can occur only with such amphotrophic or pseudotyped viruses following direct injection or exposure of mucosal membranes. However, as highlighted, MoMLV derived vectors can infect only actively dividing cells, though, following cell entry they are unstable and lose their ability to integrate with a half-life in the range of 5.5 to 7.5 h.

MSCV is a recombinant virus, derived from PCC4-cell-passaged myeloproliferative sarcoma virus (PCMV) with further mutations in the 5’ untranslated region, with the ability to infect mouse embryonal carcinoma and embryonal stem cells. Once again, only viral vectors derived from this virus pseudotyped with a glycoprotein targeting human cells can pose a potential risk to humans following direct exposure.

Wild-type HIV infects vital cells in the human immune system such as helper T cells (specifically CD4+ T cells), macrophages, and dendritic cells. Transmission occurs via blood, semen, vaginal fluid, pre-ejaculate, or breast milk. It causes life-long infection through integration into the host genome as well as host immunity evasion. Within 2-4 weeks of infection follows the acute infection stage which most often manifests with flu-like symptoms which include fever, swollen glands, sore throat, rash, muscle and joint aches and pains, and headache. During this time the virus replicates in large amounts, destroying CD4+ T cells in the process. This is followed by a response from the immune system which lowers the level of virus in the body, bringing up the number of CD4+ T cells again. The disease is then in a mostly asymptomatic clinical latency stage whereby the virus replicates at very low levels. Eventually, the disease progresses to AIDS whereby the number of CD4+ T cells falls below a defined threshold, whereby the infected person becomes vulnerable to dangerous opportunistic infections.

**Host/vector system**

The vector system is lentiviral or retroviral delivery of transgenes into mammalian cells. The most common cell type used for production will be
either adherent HEK 293/HEK 293T cells or suspension 293 cells. These cells are derived from human embryonic kidney cells and are widely used as they are easily maintained in culture and are very amenable to transfection. They differ from normal embryonic kidney cells as they have been transformed with adenovirus 5 DNA. Specifically, a 4.5 kb insert from the left arm of the viral genome is found in human chromosome 19 which includes the E1 genes, which normally function in replication of the wild-type adenovirus. The E1 proteins, originally described as immortalising oncproteins, can induce mitogenic activity and allow the HEK293 to proliferate indefinitely. Suspension 293 cells are HEK293 cells that have been adapted to growth in high-density suspensions in serum-free chemically defined medium. HEK 293T cells have an additional insertion of the SV40 Large T-antigen, another viral oncogene which acts by suppressing the tumour suppressor Retinoblastoma protein, enabling the by-pass of the G1/S checkpoint and entry into the S-phase of the cell cycle. Additionally, it allows episomal DNA replication from SV40 ORIs providing extra plasmid amplification within the cells.

The viral transfer and packaging plasmids will be transfected into HEK 293/293T or 293 cells either for transient expression or transfected linearised to allow stable integration into the cell genome to generate stable virus producer cell lines. This should not alter the characteristics of the cell line beyond the ability to produce recombinant retroviral or lentiviral particles. Target cells that will be subsequently transduced with such generated retroviral/lentiviral particles will stably integrate the delivered DNA into their genome. It will be ensured that the transgenes used will not be oncogenic (or potentially oncogenic) or any known cytotoxins. The intended effect of the vector in the host is that the transgene is permanently expressed. This will change the characteristics of the transduced cell at the level of gene and protein expression. In a therapeutic system, such change would constitute for example restoring the function of a defective gene or adding an additional functionality to the cell such as for example expression of a chimeric antigen receptor in a T-cell enabling such altered T-cell to target cancer cells for destruction, and as such this change will be desirable.

The plasmid backbones used will be designed at Oxford Genetics, whereas the viral sequences will be taken from NCBI records and synthesized externally as required. The work to be undertaken will involve engineering or modification of pre-existing retroviral and lentiviral packaging and transfer plasmids to improve the production of replication incompetent retrovirus and lentivirus particles in terms of viral titres, safety and infectivity in a range of different cell types. Genetic inserts for the transgenes will be predominantly human genes, either wild-type or various fusion/truncated genes, reporter genes and tags. Inserts will not include known protein encoded toxins, superantigens or oncogenes and so should not confer potentially harmful
biological activity. Transgenes delivered by the recombinant virus are intended to be stably integrated and so permanently expressed in transduced cells. In some cases, the transgenes may be intended for therapeutic use to supply defective function or provide an additional functionality of therapeutic benefit. In the case of reporter genes, the intended function of the insert is likely to be assessment of the viral titre and as a measure of infectivity and level of gene expression during optimisation of the transient and stable cell production methods.

Evaluation of foreseeable effects

The generated recombinant retroviral/lentiviral particles will only contain the gene of interest flanked by viral packaging signals in terms of genetic information and so will lack the ability to self-propagate. Yet such particles will still be able to infect target cells, effectively delivering the gene of interest which subsequently becomes stably integrated into the genome. The major risks to be considered are potential for generation of replication-competent viral particles (RCP) via homologous recombination and the potential for oncogenesis or other deleterious effect through insertional mutagenesis. The risk of generation of RCP during virus manufacture is very low due to employing a plasmid system where essential viral genes will be separated onto different plasmids with very low homology. Reconstitution of a replication-competent retrovirus or lentivirus particles would require multiple specific recombination events to take place in the presence of a wild-type virus and is thus, highly unlikely. Similarly, this holds true for transduction of target cells where no prior viral sequences should be present precluding any recombination events.

As the vectors will stably integrate into the genome of transduced cells, if the site of integration is near protooncogenes or tumour-suppressor genes, their expression pattern could be altered such that cell transformation will follow. This risk is higher with retroviral rather than lentiviral vectors given the bias of the parental organisms towards such sites. Additionally, there is a risk of insertion within endogenous gene sequences, potentially disrupting their function.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Derogation from containment level 2 measure (schedule 8 table 1a) are not needed.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid biological hazardous waste and contaminated consumables shall be generated. Liquid waste is to be treated with 1% Virkon for at least 30 mins before being flushed down the sink with plenty of water.
Any consumables that come into contact with viral material are to be packaged appropriately and sent for incineration by a licenced third party contractor as biological hazardous waste. Virkon is a disinfectant shown to be active against all families of virus as well as large numbers of bacteria and fungi. The comprehensive range of biocides within virkon target the virus in varying ways, this means that resistance against disinfection can not occur and the virus is at minimum deactivated but most likely destroyed. The efficacy of virkon has been sufficiently validated through testing by the manufacturer and its users. Using incineration by a third party is the only way to ensure a 100% kill of all biological materials on contaminated consumables.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been reviewed to have been carried out correctly and sufficiently with control measures in place that are appropriate to minimise personnel and environmental risks associated as well as being in line with the regulatory requirements. The genetic modification safety committee acknowledges that this contained use excludes the use of working with any sharps or harmful genetic inserts. If the stated project needs change with regards to these exclusions then it is acknowledged that a full review of the contained use be carried out. The information outlined in this notification is in-line with the risk assessment carried out for the generation and production of retroviral and lentiviral vectors and particles.

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Project Ref 3151/19.1

02/03/2022
Production of human Adenovirus particles (Adenovirus type 5)

To improve and carry out the production, purification and concentration processes for type 5 Adenovirus and to assist in the titration of Adeno-associated virus. Additional ly, further modifications to the Ad5 GMO may include encoding reporter (fluorescent and luminescent) transgenes to enable tracking of viral infection in vitro and in vivo.

The Ad5 GMO in question is a replication-competent form that resembles wildtype-adenovirus infection with some modification causing a reduction in pathogenicity. Wild type adenovirus infection varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, tonsillitis, cough and conjunctivitis; common cause of nonstreptococcal exudative pharyngitis among children under 3 years; more severe diseases include laryngitis, croup, bronchiolitis, or severe pneumonia; a syndrome of pharyngitis and conjunctivitis (pharyngoconjunctival fever). Most of the general population harbour neutralising antibodies to Ad5 and infections are generally self-resolved. Incubation period: 1-10 days. The virus is stable at temperatures of 4°C or less and cannot infect organisms other than humans and some primates.

The vector system in question is a replication-competent Ad5 vector transgene delivery into mammalian cells. The genome of the vector is encoded in a plasmid DNA vector to allow propagation of the DNA in bacterial cells and the viral vector is produced by transfection into mammalian cell lines. The most common cell type used for production of the viral vector will be either adherent HEK 293/HEK 293T cells or suspension 293 cells. These cells are derived from human embryonic kidney cells and are widely used as they are easily maintained in culture and are very amenable to transfection. They differ from normal embryonic kidney cells as they have been transformed with adenovirus 5 DNA. Specifically, a 4.5 kb insert from the left arm of the viral genome is found in human chromosome 19 which includes the E1 genes, which normally function in replication of the wild-type adenovirus. The E1 proteins, originally described...
as immortalising oncoproteins, can induce mitogenic activity and allow the HEK293 to proliferate indefinitely. Suspension 293 cells are HEK293 cells that have been adapted to growth in high-density suspensions in serum-free chemically defined medium. HEK 293T cells have an additional insertion of the SV40 Large T-antigen, another viral oncogene which acts by suppressing the tumour suppressor Retinoblastoma protein, enabling the by-pass of the G1/S checkpoint and entry into the S-phase of the cell cycle. Additionally, it allows episomal DNA replication from SV40 ORIs providing extra plasmid amplification within the cells.

**Origin & function**

The Ad5 GMO will be constructed by de novo DNA synthesis encoding the full (or partial) genome of human adenoviruses. Viral particles will be produced by transfection into producer cells (HEK293, HELA) for production of viral particles. Viral particles recovered from plasmid DNA constructs will be further amplified in producer cells at midscale (HYPERflask) and purified using caesium chloride density centrifugation. Adenoviruses will be used as a helper virus to induce replication of recombinant adenov-associated viruses (rAAV) in producer cell lines. rAAV are replication deficient AAV that need factors provided in trans for replication. Adenovirus will be used to infect the cells to provide factors necessary for rAAV replication. Another potential use is to produce recombinant proteins - native, bacterial and viral antigens, structural proteins, reporter proteins, antibodies and single-chain antibody molecules. Proteins associated with tumorigenesis or toxins will not be produced and are not in the remit of this notification.

**Evaluation of foreseeable effects**

The Ad5 GMO in question is a replication-competent form that resembles wildtype-adenovirus type 5 infection that can cause a self-limiting infection of the upper respiratory tract and the common cold in humans. This form of Ad5 is only able to infect humans and some primates so poses little risk to the environment and most of the general population harbour neutralising antibodies to Ad5 and infections so would generally be self-resolved with little effect to human health if infection were to occur. The specific modifications to this vector will include the addition of encoding reporter (fluorescent and luminescent) transgenes to enable tracking of viral infection in vitro and in vivo, which will pose no additional effects to human health or the environment compared to the wild-type virus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Derogation from containment level 2 measure (schedule 8 table 1a) are not needed.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid biological hazardous waste and contaminated consumables shall be generated. Liquid waste is to be treated with 5% Chemgene for at least 30 minutes before being flushed down the sink with plenty of water. Any consumables that come into contact with viral material are to be packaged appropriately and sent for incineration by a licenced third party contractor as biological hazardous waste or autoclaved using a validated method before disposal. Chemgene is a disinfectant shown to be active against all families of virus as well as large numbers of bacteria and fungi. The comprehensive range of biocides within Chemgene target the virus in varying ways, this means that
resistance against disinfection can not occur and the virus is at minimum deactivated but most likely destroyed. The efficiency of Chemgene has been sufficiently validated through testing by the manufacturer and its users.

The risk assessment has been reviewed to have been carried out correctly and sufficiently with control measures in place that are appropriate to minimise personal and environmental risks associated as well as being in line with the regulatory requirements. The genetic modification safety committee acknowledges that this contained use excludes the use of any harmful genetic inserts. If the stated project needs change with regards to these exclusions then it is acknowledged that a full review of the contained use be carried out. The information outlined in this notification is in-line with the risk assessment carried out for the production of human adenovirus type 5.

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been reviewed to have been carried out correctly and sufficiently with control measures in place that are appropriate to minimise personal and environmental risks associated as well as being in line with the regulatory requirements. The genetic modification safety committee acknowledges that this contained use excludes the use of any harmful genetic inserts. If the stated project needs change with regards to these exclusions then it is acknowledged that a full review of the contained use be carried out. The information outlined in this notification is in-line with the risk assessment carried out for the production of human adenovirus type 5.

Project Containment

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**Data Premises Notified**

*(Originally)*

**29/01/2021**

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**Emergency Plan Required?**

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### Name

**AGENDA RESOURCE MANAGEMENT**

**Name 2**

**Department**

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### Tel Number

**03456 445566**

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### HSE Division

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### Comments

**Date at Which Additional Info Submitted**

**02/03/2022**
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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Give brief details of the genetic modification safety committee

| Level 1 (GMMs) | 
| Level 2 (GMMs) | 
| Level 3 (GMMs) | 
| Level 4 (GMMs) | 
| Non-microbial | 

- Other (please specify)  

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<th>Laboratory</th>
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<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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Tick if confidential

02/03/2022
As a standard, waste from these laboratories is disposed of by heat treatment to give a 100% kill, such as by high temperature incineration or autoclave.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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 Tick to confirm that you are attaching a summary of the risk assessment: Y

 Tick if you are claiming exemption from disclosure for sections of the risk assessment: Y

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 3154

Data Premises Notified (Originally) 26/07/2019

Transferred from 1992 Regs? N

Transitional Premises

Data Premises Closed

Transitional Premises Withdrawn

Emergency Plan Required?

Non-GMMs

N

Name

BIRMINGHAM CITY UNIVERSITY

Name 2

Department

Campus Estate or Research Centre

FACULTY OF HEALTH, EDUCATION & LIFE

Building

SCIENCES, CITY SOUTH CAMPUS

Road Name

WESTBOURNE ROAD

District

EDGBASTON

Town

BIRMINGHAM

County

MIDLANDS

Postcode

B15 3TN

Country

ENGLAND

Tel Number

0121 331 5000

Fax Number

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E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The genetic modification safety committee is a sub-group of the Biological Safety Committee, which has members from the Life Sciences Department, the Life Sciences Technician Team and from the central University Health and Safety and the Environmental Sustainability Departments.

The genetic modification safety committee has received advice and guidance from a biological safety officer from the neighboring, University of Birmingham

<table>
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Other (please specify)  

Tick if confidential

Yes

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Liquid cultures and contaminated laboratory glassware will be disinfected by submersion and soaked in 1% Virkon solution for at least 4 hours. Samples will be regularly tested by plating on agar plates to assess killing efficiency.

Larger culture volumes may be sterilised by autoclave, at 126°C for 15 minutes, as an alternative.

GMMs contained on solid agar media will be sterilised by autoclave, at 126°C for 15 minutes.

Contaminated tissues, gloves, wipes etc will be sterilised by autoclave, at 126°C for 15 minutes.

All autoclaved waste will be disposed of to landfill in black bags.

Pipette tips, cuvettes, centrifuge tubes and other laboratory plastics contaminated with GMMs will be sterilised by autoclave, at 126°C for 15 minutes.

Sterilisation by autoclave will be done in the two large capacity autoclaves that are present in the technical preparation room adjacent to the research laboratories. All autoclaved waste will be disposed of to landfill in black bag waste.

The waste stream described above is supplemented through a contract for sharps collection through Cannon Hygene. This contract is currently being extended to include yellow bag waste - which will alleviate the requirement to autoclave tissues, gloves, wipes etc.

Contaminated spills will be sterilised locally by wiping with 1% Virkon solution.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM safety committee is a sub-group of the Biological Safety Committee. Membership of the Biological Safety Committee includes representatives of the University Health and Safety committees who oversee the implementation of risk assessments.
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Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification committee at MISSION Therapeutics is composed of the chief operating officer of the company acting as head of health and safety, the biological safety officer (head of biology) and a health and safety laboratory manager (senior scientist).

The health and safety committee meets approximately every month and all aspects of the company health and safety are reviewed, including matters related to genetic modifications.

Risk assessments related to genetic modifications are established by scientists planning on performing the procedures and reviewed by the biological safety officer as well as the head of health and safety.

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At MISSION Therapeutics, we have implemented the Babraham Research Campus (BBT) waste management policies. Regarding genetically modified microorganisms, all solid waste will be disposed of in clinical waste bins that will be incinerated. All liquid culture waste will be combined with an equal volume of Virkon 1% and left at room temperature for at least 1 hour, after which it will be disposed of down the sink. All scientist performing procedures involving GMM will sign risk assessments and will be monitored by the biological safety officer as well as the health and safety lab manager.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

At MISSION Therapeutics, we have implemented the Babraham Research Campus (BBT) waste management policies. Regarding genetically modified microorganisms, all solid waste will be disposed of in clinical waste bins that will be incinerated. All liquid culture waste will be combined with an equal volume of Virkon 1% and left at room temperature for at least 1 hour, after which it will be disposed of down the sink. All scientist performing procedures involving GMM will sign risk assessments and will be monitored by the biological safety officer as well as the health and safety lab manager.

**Tick to confirm that you are attaching a summary of the risk assessment**

Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**


Please enter comments of the GM safety committee on the risk assessment

The MISSION Therapeutics genetic safety committee has evaluated the risk assessment for the proposed GMM procedures and has agreed that the proposed activities will fall into level 1 and the committee supports the CU1 application.
To enable the generation and use of recombinant self-inactivating second or third generation lentiviral particles (by commercial research organisations or in house using commercially available 3rd generation lentivirus systems) encoding:

1) Open Reading Frames (ORFs), cDNAs, peptides
2) Specific gene sequences
3) Short hairpin RNAs (shRNAs) for the knockdown of Open Reading Frames (ORFs), cDNAs or specific gene sequences by RNA Interference (RNAi)

for in vitro use and for the generation of stable mammalian cell lines (and subsequent use of such stable lines in vitro)

Recipient experimental systems are cells derived from mammalian organisms, predominantly in the form of immortalized cell lines derived from human cancer tissues.

The mammalian cell lines fall into two types:

a) A packaging/helper cell line into which plasmids containing lentiviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines i.e. a negligible risk.

The lentiviral vectors which will be used are derived from HIV-1, which is an ACDP Hazard Group 3 biological agent.

However, second and third generation lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase biosafety and viral packaging is achieved by providing three helper constructs in trans containing gag, pol and rev sequences.

For example, second and third generation Lentiviral Expression Systems include the following key safety features:

In the second and successive generation lentiviral vectors several lentiviral accessory genes (vif, vpr, vpu and nef) are deleted from the transfer plasmid since they are not required for in vitro replication and the products they encode have cytotoxic activities.

In addition to this in the third generation lentiviral vectors:
• The Lenti expression vectors contain a deletion in the 3' LTR (boU3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell (Vee et al., 1987; Vu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
• The number of genes from HIV-1 that are used in the system has been reduced to three (Le. gag, pol, and rev).
• Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).
• Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them containLTRs or the 4J packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication competent virus can be produced. Despite the above safety features, use of these lentiviral vectors (which include WPRE) falls within SACGM 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. Also, the virus will be packaged by transfecting transfer vector into specific amphoteric 'helper' cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types. This means that the viruses produced for this experiment could potentially infect a number of species, including man.

Origin & function

Selectable markers - examples (but not restricted to);
D Ampicillin resistance: E.coli derived
D Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
D Puromycin resistance (PAC) : Puromycin acetyl transferase is derived from Streptomyces alboniger

Reporter proteins such as (but not restricted to);
D Fluorescent proteins as reporters;
• GFP derived from the jellyfish Aequorea victoria and variants of this
D Luciferase - class of oxidative enzymes used in bioluminescence
• renilla luciferase derived from the Sea pansy (Renilla reniformis)
• firefly luciferase derived from the firefly Photinus pyralis.

Open reading frames, cDNAs and gene sequences encoding GFP or Luciferase proteins and I or shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding GFP or Luciferase proteins).

Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and I or shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins) - all human derived.
This could include the expression of potentially harmful genes e.g. encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation. Full length cDNA encoding wild type and disease relevant mutants of these types of genes will be expressed in third generation SIN lentiviral vectors. Any use of lentiviral particles encoding oncogenic inserts will require appropriate controls and operator training.
The lentiviruses are, at worst, amphotropic or pseudo typed with VSV G protein, either of which confers a broad host tropism including human cells. However, the lentivirus is self inactivating and there is thus no possibility of it multiplying further.

Since some of the inserted DNA could code for potentially hazardous RNA or protein, the work is assessed as Class 2. This accords with HSE SACGM Compendium of Guidance (Part 2, section 2.11 "Retroviruses", para. 18-20).

Even for the non oncogenic inserted DNA, there is a slight but non negligible risk due to the presence in the lentiviral vector of the Woodchuck Post-transcriptional Regulatory Element. The WPRE containing vector DNA will be treated as potentially oncogenic and is assigned to class 2 (see HSE SACGM Compendium of Guidance (Part 2, section 2.11 "Retroviruses", para.13)).

However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle:

- The genes encoding structural and other components of the viral genome have been separated. These genes have been engineered to minimise the risk of recombination that could lead to production of a replication-competent virus.
- The packaging cell lines allow expression of proteins, required to produce progeny virus. But the transfer vector is the only genetic material transferred to the target cells, consequently these cells cannot produce the proteins which are essential for viral assembly and infectivity.

Second or third generation lentivirus vectors will be used in all experiments.

Procedures and controls measures will therefore follow HSE SACGM Compendium of Guidance (Part 2, section 2.11 "Retroviruses", para.30-36) i.e. using multiple plasmids with minimum sequence homology (e.g. our 2nd or 3rd generation lentivirus vector systems), gloves should be worn, use of class II safety cabinets, sharps avoided and all wastes be rendered harmless before disposal etc.

It is not thought that the modified virus would pose a serious risk to animals or plants in the environment. Although the VSV coat protein permits invasion of other mammalian cells, as in the case of humans, infection would be restricted to primary cells and productive virus would not be produced. In addition the control measures to protect human health will minimise release of virus to the environment. Therefore the environmental risk is low.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

- described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:
  1. We do not have access to an autoclave within our area of the building
  2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

  All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization is GM898) according to disposal notification GM1 05/4.1.
  
  Waste from our GM work at Class2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from class 1 waste) 60 litre UN approved (to Class 3) Type 3H21 Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled “Eurobins”. Contents of the bin are recorded and collections by the local
registered clinical/GM waste incinerator contractor will be made three times a week to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor’s facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major road traffic accident, however, the containers are designed to withstand this.

This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM1 05/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:

1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization is GM898) according to disposal notification GM1 05/4.1.

Waste from our GM work at Class2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from class 1 waste) 60 litre UN approved (to Class 3) Type 3H21 Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled “Eurobins”. Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor’s facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major road traffic accident, however, the containers are designed to withstand this.

This disposal method is expected to achieve 100% inactivation of the GMM.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<td>L3</td>
<td>L4</td>
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<tr>
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- **Animal Units**
  - L2

- **Large Scale Activities**
  - L2

- **Human Clinical Applications**
  - L2
## OSSIANIX UK LTD

### General Information

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<tr>
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### Contact Information

- **Name**: OSSIANIX UK LTD
- **Department**: 
- **Campus Estate or Research Centre**: 
- **Road Name**: GUNNELS WOOD ROAD
- **District**: STEVENAGE
- **Town**: HERTFORDSHIRE
- **County**: ENGLAND
- **Postcode**: SG1 2FX
- **Tel Number**: 01438 906821
- **Fax Number**: 01224437822
- **E-mail**: blank
- **HSE Division**: blank

### Additional Information

- **Date at Which Additional Info Submitted**: 02/03/2022

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### Premises Addresses

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<td>SG1 2FX</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Company CEO
- Site Director & Operational Manager
- Senior Scientist Director
- Senior Scientist Group Leader

To meet at a minimum 6 monthly or ad hoc to assess any new activities and risk assessments

<table>
<thead>
<tr>
<th>Laboratory</th>
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<tr>
<td>Non-microbial</td>
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Other (please specify)

Tick if confidential

Bacteriology  Y  Parasitology  Transgenic Birds  Microbiology Research

02/03/2022
<table>
<thead>
<tr>
<th>Virology</th>
<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
<th>Gene Therapy</th>
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<tr>
<td>Mycology</td>
<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
</tr>
</tbody>
</table>

Other(s)

Bacteriophage library screening and expression; Modified gene expression in mammalian cells in tissue

For activities involving GMMs, describe the waste management measures which will apply to the activity

All GM classified laboratory waste will be completely inactivated by autoclaving in the building for subsequent collection and disposal by incineration. All potentially contaminated work surfaces routinely disinfected with Virkon and 70% ethanol.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

GMSC comment. The risk assessments for protocols and GMMs calculate all potential activity appropriately as Class 1 activities to be carried out at the Life Science Innovations Building (address as above) designed at containment level 2.
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**Name**

REDX ONCOLOGY LTD

**Campus Estate or Research Centre**

BLOCK 33F

**Road Name**

MERESIDE

**Building**

ALDERLEY PARK

**District**

ALDERLEY

**Town**

ALDERLEY

**County**

CHESHIRE

**Postcode**

SK10 4TG

**Country**

ENGLAND

**Tel Number**

0151 7064812

**Fax Number**

0

**E-mail**

blank

**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
**Premises Addresses**

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<td>DAULBY STREET</td>
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The committee consists of: Biological Safety Officer (BSO), Genetic Modifications Safety Officer (GMSO), SHE Officer, Head of Medicinal Chemistry (Senior Management Representative), Scientist from Pharmacology lab (representative of technical staff), a representative from the University of Liverpool (UoL Biological Safety officer), and a project manager (Management Representative). The Committee meets every two months, or more often if required. Risk Assessments and background reading is circulated to committee members by the GMSO a minimum of two weeks in advance of a committee meeting. The meeting is chaired by the GMSO. Risk Assessments are discussed, and either passed with comments or further information may be requested and risk assessment is put back for discussion at the next committee meeting. The Minutes are circulated after the meeting and should be agreed upon, sent to senior management and stored.

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</table>
Oncology research will be carried out.

Solid Waste will be bagged and autoclaved at 126°C for approximately 1hr, it will then go through a drying process in the autoclave for approximately 1hr, for 100% kill. The autoclave will give a print out which shows the temperature it reaches and the duration it is at temperature for, printouts will be checked to ensure temperature is reached and to ensure temperature is maintained for required length of time, printouts will be retained & logged. Autoclaved waste will then be sent for incineration with SRCL waste disposal company. Waste will be incinerated at one of three incineration sites (at Speke, Crewe or Bolton), used by SRCL. Liquid waste will be handled in the same way. If the autoclave is unavailable or breaks down, Redx Oncology has access to an autoclave on the 5th floor of the Duncan Building. If both autoclaves are broken/inaccessible liquid waste will be mixed with Virkon (final concentration of Virkon 1.25%), for 24 hrs to inactivate (99.99% kill).

Bacteriology
Parasitology
Transgenic
Birds
Microbiology
Research

Virology
Transgenic
Animals
Transgenic
Fish
Gene Therapy

Mycology
Transgenic
Invertebrates
Transgenic
Plants
Other (please specify below)

Other (please specify) 

For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid Waste will be bagged and autoclaved at 126°C for approximately 1hr, it will then go through a drying process in the autoclave for approximately 1hr, for 100% kill. The autoclave will give a print out which shows the temperature it reaches and the duration it is at temperature for, printouts will be checked to ensure temperature is reached and to ensure temperature is maintained for required length of time, printouts will be retained & logged. Autoclaved waste will then be sent for incineration with SRCL waste disposal company. Waste will be incinerated at one of three incineration sites (at Speke, Crewe or Bolton), used by SRCL. Liquid waste will be handled in the same way. If the autoclave is unavailable or breaks down, Redx Oncology has access to an autoclave on the 5th floor of the Duncan Building. If both autoclaves are broken/inaccessible liquid waste will be mixed with Virkon (final concentration of Virkon 1.25%), for 24 hrs to inactivate (99.99% kill).

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment Y

Please enter comments of the GM safety committee on the risk assessment

The Genetic Modifications Safety Committee (GMSC), agreed with the classification of the Risk Assessment as Class I. The genetic modification of the cells does not make the original parental cell line any more dangerous/infective. The technical representative on the GMSC indicated that they had worked with this commercially available Gli-bla 22 Rv1 cell line before and had no concerns relating to the safety classification.

Project Ref 3158/12.1

Date Ackn'd 20/06/2012 CU2 Project Title Smoothened HEK293/D473H Smoothened HEK293 Class 2 CultureVolClass2 1-50 Litres
Project Additional Information

Purposes of the contained use

Please See Section 17
These stably transfected cell lines will be used to assess the efficacy of novel compounds ability to inhibit signalling in the Hedgehog signaling pathway, via inhibition of the Smoothened receptor. We will use these stably transfected cell lines to assess the efficacy of novel Smo Inhibitors at inhibiting both WT Smo and D473H Smo receptors, in functional assays and in binding assays.
Aberrant activation of the Hedgehog signalling pathway (of which smoothened is an integral part), is present in 25% of all cancers.

Recipient or parental organism

Please See Section 17
HEK 293 (human embryonic kidney) cells will be purchased from ATCC and contain Adenovirus and are classified as biosafety level 2.

Host/vector system

Please See Section 17
MMLV-derived retroviral vector, will be used to transfect HEK293 cells with our gene of interest (human wild type smoothened of human D473H smoothened). There are no known hazards associated with the use of the MMLV-derived retroviral vector, a marker rescue assay has been done on this vector virus showing there is no replication competent virus left.

Origin & function

Please See Section 17
Smo (Smoothened) is a protein involved in signalling in the Hedgehog pathway. Hedgehog pathway activation is initiated when Hedgehog ligand binds Patched receptor and relieves its inhibition of Smoothened. This allows Smoothened to move into the cilia of cells and interact with complex of proteins resulting in activation of the Glioma associated family of zinc finger transcription factors which translocate to the nucleus, and result in transcription of Hedgehog target genes. Tumours in about 25% of all cancer deaths are estimated to involve aberrant Hh pathway activation. The Hh pathway is also thought to be important for driving self-renewal of cancer stem cells.

Evaluation of foreseeable effects

Please See Section 17
The inserted gene does not encode a pathogenicity determinant, such as an adhesin, a penetration factor or a surface component providing resistance to host defence.
mechanisms. The inserted gene does not encode a surface component, envelope protein or capsid protein that might bind to a different receptor to that used by the recipient microorganism. Thus it is unlikely to have any indirect effects. Gene transfer or recombination of the GMM with a wild-type microorganism, is unlikely to be a matter of concern. In the event of a breach of containment the GMM is unlikely to survive in the environment for long enough for any such gene transfer to take place. If gene transfer did take place it could result in expression of WT Smo or D473H Smo in cell. Overexpression of Smo could result in aberrant signalling in the Hh pathway, giving rise to an oncogenic phenotype. D473H Smo, gives rise to Smo resistant to inhibition by several Smo antagonists.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste will be bagged and autoclaved at 126°C for approximately 1hr, it will then go through a drying process in the autoclave for approximately 1hr, for 100% kill. The autoclave will give a print out which shows the temperature it reaches and the duration it is at temperature for, printouts will be checked to ensure temperature is reached and to ensure temperature is maintained for required length of time, printouts will be retained & logged. Autoclaved waste will then be sent for incineration with SRCL waste disposal company. Waste will be incinerated at one of three incineration sites (at Speke, Crewe or Bolton), used by SRCL. Liquid waste will be handled in the same way. If the autoclave is unavailable or breaks down, Redx Oncology has access to an autoclave on the 5th floor of the Duncan Building. If both autoclaves are broken/inaccessible liquid waste will be mixed with Virkon (final concentration of Virkon 1.25%), for 24 hrs to inactivate (99.99% kill).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Solid Waste will be bagged and autoclaved at 126°C for approximately 1hr, it will then go through a drying process in the autoclave for approximately 1hr, for 100% kill. The autoclave will give a print out which shows the temperature it reaches and the duration it is at temperature for, printouts will be checked to ensure temperature is reached and to ensure temperature is maintained for required length of time, printouts will be retained & logged. Autoclaved waste will then be sent for incineration with SRCL waste disposal company. Waste will be incinerated at one of three incineration sites (at Speke, Crewe or Bolton), used by SRCL. Liquid waste will be handled in the same way. If the autoclave is unavailable or breaks down, Redx Oncology has access to an autoclave on the 5th floor of the Duncan Building. If both autoclaves are broken/in accessible liquid waste will be mixed with Virkon (final concentration of Virkon 1.25%), for 24 hrs to inactivate (99.99% kill).

Project Containment

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<td>L2</td>
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<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

02/03/2022
pcDNA3.1-ABCB1 will be stably transfected into He La cells to generate the cell line HeLa-ABCB1.

The generated stably transfected cell line will be used to assess a variety of in-house generated compounds if they are substrates for ABCB1. To be more specific, a clone will be chosen with ABCB1 expression levels similar to that of human tumours. This will then be used in comparison to the parental cells to determine the susceptibility of compounds towards ABCB1 using a proliferation assay.

He La cells will be purchased from ECACC and are classified as BioSafety level 2.

A pcDNA3.1-derived vector will be used to transfect HeLa cells with ABCB1. As this vector has a non-viral backbone and gene expression is driven by a CMV promoter there is no interaction between the vector and HPV-18 (which is present in HeLa cells) possible.

In cancer, multiple mechanisms can contribute to tumour resistance, and one implicated in causing multidrug resistance (MDR), whereby resistance is acquired against mechanistically and structurally distinct drugs, is the P-glycoprotein pump (Pgp); also known as multidrug resistance protein 1 (MDR1) or ATP-binding cassette (ABC).
sub-family B member 1 (ABCB1)]. This is a 170 kDa membrane integrated transporter encoded by the ABCB1 gene which is responsible for the efflux of xenobiotics from cells and controlling their absorption across the intestinal and blood brain barriers. Known substrates of Pgp include chemotherapy drugs from the taxane, vinca alkaloid and anthracycline families, which are rapidly effluxed in vitro by certain tumour cell lines over-expressing Pgp. Although the physiological relevance of Pgp to MDR in patients remains to be fully discerned, clear correlations between levels of Pgp expression and the overall efficacy of chemotherapy treatments have been found in breast cancer and certain leukaemias.

**Evaluation of foreseeable effects**

The inserted gene does not encode a pathogenecity determinant, such as an adhesin, a penetration factor or a surface component providing resistance to host defence mechanisms. The inserted gene does not encode a surface component, envelope protein or capsid protein that might bind to a different receptor to that used by the recipient microorganism. Gene transfer or recombination of the GMM with a wild-type microorganism, is unlikely to be a matter of concern. In the event of a breach of containment the GMM is unlikely to be a matter of concern. In the event of a breach of containment the GMM is unlikely to survive in the environment for long enough for any such gene transfer to take place. If Gene transfer did take place it could result in expression of ABCB1 in a cell. Overexpression of ABCB1 results in a reduced sensitivity to a large number of approved drugs. Still, there are a large number of compounds not affected by this transporter protein.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste will be bagged and autoclaved at 126°C for approximately 1hr, it will then go through a drying process in the autoclave for approximately 1hr, for 100% kill. The autoclave will give a print out which shows the temperature it reaches and the duration it is at temperature for, printouts will be checked to ensure temperature is reached and to ensure temperature is maintained for required length of time, printouts will be retained & logged. Autoclaved waste will then be sent for incineration with Initial Medical Services waste disposal company. Liquid waste will be handled in the same way. If the autoclave is unavailable or breaks down, Redx Oncology has access to an autoclave on the 5th floor of the Duncan Building. If both autoclaves are broken/inaccessible liquid waste will be mixed with Virkon (final concentration of Virkon 1.25%), for 24 hrs to inactivate (99.99% kill).

Is an emergency plan required according to regulation 20?  
If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

The Genomic Modification Safety Committee (GMSG), agreed with the classification of the cell line as Class II. This is due to the fact that insertion of ABCB1 into HeLa cells (the parental cell line is already biosafety level 2) makes them less susceptible to a number of drugs, but a large number of drugs are still efficacious on them.
The assay represents an invaluable system for testing the inhibitory activities of small molecules against a specific kinase of interest in the context of human cells. Cell based compound potency measurements are important components of the drug discovery process, since biochemical potency values often do not translate to cellular activity for a number of reasons, including compound membrane permeability, cellular ATP concentration, compound localisation etc.

Recipient or parental organism

HEK 293 (human embryonic kidney) cells transiently expressing either full length Human BTK WT or Mutant contain Adenovirus and are classified as biosafety level 2.
pCMV vector will be used. pCMV is a eukaryotic vector in which gene of interest is expressed under the control of CMV promoter. Vector pCMV, there is no known hazards associated with use of this vector; the vector has been commonly used to express gene of interest in mammalian cells. pCMV is a eukaryotic vector in which gene of interest is expressed under the control of CMV promoter. There is no evidence that the vector could potentially mobilize.

Origin & function

BTK is a member of the Tee family of protein tyrosine kinases and plays an important role in regulation of B-Cell receptor development & signaling. Targeted therapies that suppress B-cell receptor signalling have emerged as promising agents in the treatment of several B-cell malignancies and autoimmune disease. We have developed a number of small molecules capable of inhibiting BTK, as potential treatments for B-cell malignancies.

The activity of BTK is regulated by a variety of mechanisms, including membrane translocation & phosphorylation. LYN is usually associated with CD79B in unstimulated B cells. Upon antigen activation CD79A and CD79B are phosphorylated on their ITAMs by Tyrosine Kinases Lyn & Fyn. This results in recruitment of Syk & activation of Syk. Multiple proteins including PI3K, BLNK and BTK are also recruited to the PM. PI3K phosphorylates PIP2 to PIP3 which accumulates on inner surface of the PM and acts as a docking site for BTK recruited to the PM upon antigen activation. In unstimulated cells a very small proportion of BTK is localised to the PM. During BCR ligiation- 30% of BTK is transported to PM, through interactions of its PH domain with PIP3, generated by PI3K. Here BTK comes into close proximity of an array of proteins including cell surface receptors, Src family protein tyrosine kinases & PI3-K. Membrane targeting of BTK is a prerequisite for its phosphorylation and activation. BTK is first phosphorylated by SYK or LYN at tyrosine 551 (in its catalytic domain) and later is autophosphorylated at tyrosine 223 in SH3 domain. There are two possibilities about how BTK autophosphorylates through an intramolecular interaction involving catalytic & SH2 domains, BTK could autophosphorylate itself at tyrosine 223 autophosphorylation at tyr 223 could take place via dimerization.

Evaluation of foreseeable effects

Direct Effects: The sequence inserted does not encode an insect or animal toxin or a product which can cause silencing of a gene encoding a crucial metabolic enzyme in susceptible hosts. Overexpression of BTK could result in aberrant signalling in the BCR pathway, giving rise to an oncogenic phenotype. Mutant BTK, gives rise toibrutinib acquired resistance.

Indirect Effects: The inserted sequence does not encode a pathogenicity determinant, such as an adhesin, a penetration factor or a surface component providing resistance to host defence mechanisms. The inserted gene does not encode a surface component, envelope protein or capsid protein that might bind to a different receptor to that used by recipient microorganism.

Gene transfer or recombination of the GMM with a wild-type micro-organism, is unlikely to be a matter of concern. In the event of a breach of containment the GMM is unlikely to survive in the environment for long enough for any such gene transfer to take place. If Gene transfer did take place it could result in expression of WT BTK or mutated BTK in a cell.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste will be bagged and autoclaved at 126°C for approximately 1hr, it will then go through a drying process in the autoclave for approximately 1hr, for 100% kill. The autoclave will give a print out which shows the temperature it reaches and the duration it is at temperature for, printouts will be checked to ensure temperature is reached and to ensure temperature is maintained for required length of time, printouts will be retained & logged. Autoclaved waste will then be sent for incineration with SRCL waste disposal company. Waste will be incinerated at one of three incineration sites (at Speke, Crewe or Bolton), used by SRCL. Liquid waste will be handeled in the same way. If the autoclave is unavailable or breaks down, Redx Oncology has access to an autoclave on the 5th floor of the Duncan Building. If both autoclaves are broken/inaccessible liquid waste will be mixed with Virkon (final concentration of Virkon 1.25%), for 24 hrs to inactivate (99.99% kill).
The Genetic Modification Safety Committee (GMSC), agreed with the classification of the cell lines as Class II. The GMSC felt that transient expression of BTK WT and Mutant could result in an oncogenic phenotype and as such warranted a Class II Classification.

Project Containment

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<th>Growth Rooms</th>
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<tr>
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Project Ref 3158/14.1

Date Ackn’d 10/04/2014  
Date Project Ceased  
CU2 Project Title Expression of genetically encoded fluorescently tagged proteins in order to visualize the cellular behaviour of primary human pancreatic stellate cells in a 3d co-culture model with the immortalized epithelial carcinoma cell line Panc-1

Class CultureVolClass2 CultureVolumeClass3-4  
Class 2 < 1 Litre  
Non-GMM Consent Granted  
Project notified under transitional arrangements N

Historical Significant Changes  
Historical Date of Additional Info  
Significant Change ID
## Project Additional Information

### Purposes of the contained use

In order to study pancreatic adenocarcinoma we aim to create 3D co-culture system to study the interaction of cancer cells with stellate cells and eventually to test the effect of compounds on this interaction. The transfection of fluorescent proteins will allow us to distinguish the two cell types from each other. This model would be a useful tool to test compounds taking into account the effect of the tumour microenvironment and an extracellular matric on the efficacy of the compounds.

### Recipient or parental organism

- **Panc-1 cells** are available from the ATCC and are classified as biosafety level 1.
- Human primary stellate cells isolated from samples collected at the Royal Liverpool University hospital. (Primary Cells: Biosafety level 2)

### Host/vector system

Pre-made lentiviral particles (from pPACKH1) will be used to transfect cells with our genes of interest. Replication-defective lentiviral vectors, such as the 3rd Generation vector provided in this product, are not known to cause any diseases in humans or animals. However, lentiviruses can integrate into the host cell genome and thus pose some risk of insertional mutagenesis. Material is a Risk Group 2 and should be handled under BSL2 controls.

### Origin & function

Both β-actin and Vimentin are housekeeping genes and have no oncogenic role. Their insertion in the proposed procedures is to allow the monitoring of different cell types within the same co-culture. Links to the products are provided below:

2. [https://www.millipore.com/catalogue/item/17-10203](https://www.millipore.com/catalogue/item/17-10203)

### Evaluation of foreseeable effects

The inserted gene does not encode a pathogenicity determinant, such as an adhesion, a penetration factor or a surface component providing resistance to host defence mechanisms. The inserted gene does not encode a surface component, envelope protein or capsid protein that might bind to a different receptor to that used by the recipient microorganism.

Though highly unlikely, recombination could occur between vector coding sequences and the helper packaging sequences of any virus contained in the primary cells which will not have been screened for viruses, however its not expected to result in the formation of functional and replication competent recombinant viruses. Gene transfer or recombination of the GMM with a wild-type microorganism, is unlikely to be a matter of concern. In the event of a breach of containment the GMM is unlikely to survive in the environment for long enough for any such gene transfer to take place. If Gene transfer did take place it could result in expression of full-length human b-actin-RFP or Human Vimentin-GFP

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid Waste will be autoclaved using an autoclave cycle for solids (>120C for 1 hour followed by a drying process for 1hr to give 100% kill). This inactivated waste will then be sent for incineration by Initial Medical Services. Liquid Waste will be autoclaved using an autoclave cycle for decontamination before being sent for incineration by Avanti.
parent company SRCL. If not possible to autoclave, liquid waste will be mixed with Vircon (final conc 10%), for 2 hrs (99.99% kill) before being sent for incineration by Initial Medical Services

The Genetic Modification Safety Committee (GMSC), agreed with the classification of the proposed GMO work as Class II. The GMSC felt that due to the proposed use of primary cells that this work warranted a Class II Classification. The GMSC recommended that secondary containment should be used when plates and cultures are in the incubator, to transport plates and flasks, and to store viruses in the freezer. The GMSC also suggested that waste should be sealed within the tissue culture hood.

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Safety Committee (GMSC), agreed with the classification of the proposed GMO work as Class II. The GMSC felt that due to the proposed use of primary cells that this work warranted a Class II Classification. The GMSC recommended that secondary containment should be used when plates and cultures are in the incubator, to transport plates and flasks, and to store viruses in the freezer. The GMSC also suggested that waste should be sealed within the tissue culture hood.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 3158/14.2

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<td>Immortalization of primary pancreatic stellate cells and creation of an immortal stable fluorescent pancreatic stellate</td>
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Historical Significant Changes

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N
### Project Additional Information

**Purposes of the contained use**

Reproducible 3D systems for the exploration of the interactions between the stroma and pancreatic cancer cells are not readily available. Immortalized stellate cell lines in combination with our 3D co-culture system will allow us to study the tumour-stoma interaction of pancreatic adenocarcinoma for an extended period of time.

**Recipient or parental organism**

Human primary stellate cells isolated from samples collected at the Royal Liverpool University hospital (Primary Cells: Biosafety level 2)

**Host/vector system**

The lentiviral particles used will be the following:
- Vector Name: pLenti-hTERT, pLenti-EF1α-hTERT-RFP
- Vector Type: Lentiviral Vector
- Antibiotic Information: Puromycin

**Origin & function**

Reproducible 3D systems for the investigation stroma-pancreatic cancer cell interaction are not readily available. We therefore aim to create immortalized stellate cell lines to use in 3D co-culture system. We will create immortalized stellate cell lines isolated from a normal, chronic pancreatitis and cancer by inserting human telomerase reverse transcriptase protein (hTERT) when this protein is exogenously expressed the cells are able to maintain sufficient telomere lengths to avoid replicative senescence.

**Evaluation of foreseeable effects**

The direct effect is that the human telomerase reverse transcriptase protein is an oncogene and has the capacity to immortalize any human cell which is inoculated with the virus.

With regards to indirect effects, they inserted gene does not encode a pathogenicity determinant, (e.g. and adhesin). The inserted gene does not encode a surface component, envelope protein or capsid protein that might bind to a different receptor to that used by the recipient microorganism. An enhancer deletion in the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral vector following transduction and integration into genomic DNA of the target cells. The number of lentiviral genes necessary for packaging, replication and transduction is limited to three (gag, pol, rev), and their expression is derived from different plasmids, which all lack packaging signals. These plasmids share no significant homology to the expression vector, preventing the generation of replication-competent virus. None of the gag, pol, or rev genes will be present in the packaged viral genome, thus making the mature virus replication-incompetent.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid Waste will be autoclaved using an autoclave cycle for solids (>120°C for 1 hour followed by a drying process for 1 hr to give 100% kill). This inactivated waste will then be sent for incineration by Initial Medical Services. Liquid Waste will be autoclaved using an autoclave cycle for decontamination before being sent for incineration by Avanti parent company SRCL. If not possible to autoclave, liquid waste will be mixed with Vircon (final conc 10%), for 2 hours (99.995 Kill) before being sent for incineration by Initial Medical Services.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Safety Committee (GMSC, agreed with the classification of the proposed GMO work as Class II. The GMSC felt that due to the proposed use of primary cells that this work warranted a Class II classification. Committee also felt that due to the human gene insert's (hTERT) ability to immortalise cells, this work warranted a Class II classification.

The GMSC recommended that secondary containment should be used when plates and cultures are in the incubator, to transport plates and flasks, and to store viruses in the freezer. The GMSC also suggested that waste should be sealed within the tissue culture hood.

Project Containment

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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Project Ref 3158/14.3

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<td>08/07/2014</td>
<td>The use of reporter cell lines for receptor specificity assays</td>
<td>Class 2</td>
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Date Project Ceased
### Project Additional Information

**Purposes of the contained use**

Stably transfected cell lines will be used to assess the efficacy of novel compounds ability to stimulate signalling of target receptors

**Recipient or parental organism**

Commercially available HEK293 cells. Classified as Biosafety Level 2

**Host/vector system**

Prior to purchase of the HEK293 cells they have been co-transfected with the human target receptor genes of interest and an inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. The SEAP reporter gene is placed under the control of a transcription factor. Stimulation with receptor ligands activated the transcription factor which induces the production of SEAP

**Origin & function**

The inserted sequence does not encode a pathogenicity determinant, such as an adhesin, a penetration factor or a surface component providing resistance to host defence mechanisms. The inserted gene does not encode a surface component, envelope protein or capsid protein that might bind to a different receptor to that used by recipient microorganism. Gene transfer or recombination of the GMM with a wild-type microorganism, is not likely to be a matter of concern. In the event of a breach of containment the GMM is unlikely to survive in the environment for long enough for any such gene transfer to take place. Overexpression of target receptor could result in over-stimulation of pro-inflammatory signalling pathways. HEK293 cells are tumorigenic

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be disinfected with a suitable detergent (e.g. 10% Distel or Chemgene) then autoclaved using an autoclave cycle (held at a minimum of 125 degrees C for at least 15 minutes). The inactivated waste will then be sent for incineration by a waste management contractor.
Liquid Waste will be disinfected by incubating with equal volume of suitable detergent (e.g. Distel or Chemgene) for a minimum of 2 hours before disposing to drain.

Autoclave monitoring and validation:
1. Daily Efficacy Monitoring is achieved by Chemical Indicator strips used in each cycle every day and each container of waste is sealed with colour reactive autoclave tape.
2. Alongside this are printout report checks which are used to determine if a successful cycle has been achieved in terms of temperatures and times. The printer report will also be checked to see if any alarms have been recorded thereafter.
4. Servicing is to be completed twice a year and Validation Testing annually by an authorised, competent recognised 3rd party contractor.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

The Risk assessment was circulated on the 19th May 2014.

The risk assessment was discussed by the GMSC on 22nd May 2014. All committee members agreed that Class 2 is the appropriate classification for this work and that GMM_RA_A1001 is approved subject to the following actions, which have been undertaken.

Actions from meeting:

- Safety procedure documents "REDXAI PSP_002 Tissue Culture" and "REDX AI PSP_003 Waste Disposal" have been referenced in the document.
- Autoclave operating and validation procedures have been sourced from third party contractor and relevant information included in risk assessment.
- Clarification sought on Biological Safety Officer and Team Leader being one and same - agreed this is ok since on other signatory still required for approval.
- Reference made to all disinfectants currently or previously used in laboratory.
- Wording in Section 4.5.2 revised to ensure all staff working on this project have had or will be offered HBV immunisation
- Question raised as to whether cells to be segregated in cell culture incubator and liquid nitrogen store. Incubators in cell culture laboratory can be designated according to cell type/risk. Cells can be segregated into separate racks within liquid nitrogen store.
- Added comment to risk assessment confirming that no genetic modifications will be performed on Redx premises, the cell lines are commercially available with modifications in place.
- Volumetric estimates of cell usage added to risk assessment
- Wording in Section 4.6.1 amended to ensure that employees known to be immunocompromised refrain from working with these cells

Project Containment

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02/03/2022
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**Name**

OXFORD BIOThERAPEUTICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

94 MILTON PARK

**District**

ABINGDON

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX14 4RY

**Country**

ENGLAND

**Tel Number**

01235 861770

**Fax Number**

01235 861771

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  Y

Give brief details of the genetic modification safety committee

The committee contains the following members:

- Oxford Therapeutics safety officer
- Scientific staff involved in GMM (Junior and Senior Scientist)
- Manager involved in initiating and supervising the GMM work (Director of Proteomic Discovery)

The committee will meet quarterly. An approved form detailing all the work in a project will be generated. The decisions of the committee with regard to a project and its risk assessment will be minuted.

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Tick if confidential  

02/03/2022  
Page 13221 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

All solid waste must be soaked in a final concentration of 1% Virkon overnight and disposed of in the grey autoclave box. Autoclave all waste by the end of the week.

All liquid waste must be made up to at least a final concentration of 1% Virkon overnight. Discard down the sink with excess water the following day.

All glassware and plastics must be soaked in at least a final concentration of 1% Virkon for at least 1 hour. Rinse thoroughly and place into the washing-up bowls or yellow bins as appropriate.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The committee reviewed the risk assessment and were satisfied that all the safety issues associated with the work had been fully addressed
### Data Premises Notified

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| N |

### Transitional Premises

| Class | N |

### Data Premises Closed

| Emergency Plan Required? | N |

### Non-GMMs

| N |

### Withdrawn

| N |

### Name

| TRANSFARMATION LIMITED |

### Name 2

| BIOLOGICAL SERVICES UNIT |

### Campus Estate or Research Centre

| Building |

### Road Name

| ROYAL COLLEGE STREET |

### District

| LONDON |

### Town

| LONDON |

### County

| GREATER LONDON |

### Postcode

| NW1 0TU |

### Country

| ENGLAND |

### Tel Number

| 01707642162 |

### Fax Number

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### E-mail

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### HSE Division

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### Comments

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### Date at Which Additional Info Submitted

| 02/03/2022 |
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Transpharmation is comprised of several highly experienced (both academic and pharmaceutical) scientists with a wealth of knowledge about genetically modified animals coupled with molecular biology. We act as our own genetic modification safety committee

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Bacteriology Parasitology Transgenic Birds Microbiology Research
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<td>Other(s)</td>
<td>Behavioural pharmacology</td>
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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

N/A We are using rodents only

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment

**Please enter comments of the GM safety committee on the risk assessment**

We aren't using a GMM (genetically modified microorganism) we use only mice
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Name

VIVA BIOSCIENCE LTD

Name 2

Department

Campus Estate or Research Centre

UNIT 64, BASEPOINT BUSINESS CENTRE

Road Name

YEOFORD WAY

Building

Town

EXETER

District

County

DEVON

Postcode

EX2 8LB

Country

ENGLAND

Tel Number

01392953020

Fax Number

0

E-mail

HSE Division

blank

Comments

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02/03/2022
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Scientific Director
- Managing Director
- Laboratory Manager

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- Tick if confidential

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste material containing viable GMMs, including spent culture fluid and media inactivated by autoclave before final disposal. Glassware disinfected/autoclaved before cleaning/re-use. Autoclave performance monitored by autoclave indicator strips, details of each run (conditions, materials, outcome) recorded in log book.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The written Risk Assessment has been thoroughly reviewed and is considered to be appropriate for the work planned. This work is wholly in line with the company's strategic plans. The detailed nature of the products to be produced is regarded as being commercially confidential and should not be disclosed.
### UNIVERSITY HOSPITAL PLYMOUTH NHS TRUST

#### Name

**UNIVERSITY HOSPITAL PLYMOUTH NHS TRUST**

#### Campus Estate or Research Centre

**DERRIFORD HOSPITAL**

#### Road Name

**DERRIFORD ROAD**

#### Town

**PLYMOUTH**

#### County

**DEVON**

#### Postcode

**PL6 8DH**

#### Country

**ENGLAND**

#### Tel Number

**01752 431045**

#### Fax Number

**01752 315110**

#### Date at Which Additional Info Submitted

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Y
Role of the Genetic Modification Safety Committee
● To advise on and review new and amended GM research activities within the Trust
● Ensure the risk assessment has been completed in accordance with the GMO (Contained Use) Regulations and Advisory Committee on Genetic Modification Compendium of Guidance (ACGM).
● Advise on the genetic modification safety training requirements needed to carry out the work of the Constitution of the Committee
The Committee should be composed of:
● Chairperson
● Secretary
● Representative from the Pharmacy Clinical Trials Research Management
● The Principal Investigator or a representative from the project group intending to carry out a particular GM activity can be invited.
● The Committee also has the right to co-opt members to serve for a period specified by the Chair of the Committee
Role of the GM Chair
The GM Chairperson should be a member of the Trusts staff. They must be nominated by the R & D Committee and formally appointed by the Trust's Associate Medical Director of Research. Their appointment must be ratified by the Medical Director on behalf of the Trust.
The GM chair must:
● Be familiar with the GMO (Contained Use) Regulations & GMO (deliberate release) Regulations
● Ensure that each application is dealt with in an unbiased manner.
● Provide advice regarding proposed GM projects.
● Give Trust approval for the project if they meet all regulatroy and local requirements.
● If approval is conditional, ensure that the risk assessment form has been amended as requested.
Role of the GM Secretary
● Be familiar with the GMO (Contained Use) Regulation & GMO (deliberate release) Regulations.
● Issue an acknowledgement to the Principal Investigator following the successful submission of a GM risk assessment form to the GMSC.
● Issue formal approval or rejection to the Principal Investigator following a GMSC meeting
● Ensure minutes of meetings are recorded and distributed as required
Duty/Role of GMSC Members
The GMSC members must be familiar with the following:
● GMO (Contained Use) Regulation & GMO (deliberate release) Regulations
● ACGM Compendium of Guidance.
● Advisory Committee on Dangerous Pathogens (ACDP) "The management, design and operation of microbiological containment laboratories" ISBN 0 71762034 4.
● Control of Substances Hazardous to Health Regulations (COSHH).
● Other relevant publications.
The chair members should:
● The Committee can seek confidential technical advice from sources outside the committee as required.
● Have an understanding of the risks to both human health and the environment that may arise from the proposed GM activity.
● Review the accuracy and detail of GM risk assessments presented to the Committee and advise accordingly.
● Ensure the risk assessment has been completed in accordance with the GMO (Contained Use) Regulations and ACGM guidance.
● Advise on the genetic modification safety training requirements needed to carry out the work.
● Consider wether the appropriate containment facilities, as indicated by the risk assessment, are available.
Meetings of the Genetic Modification Safety Committee:
The committee will only meet as required. Dates of the meetings will be fixed in advance; notice of the meetings will be circulated one week in advance and
accompanied by a meeting agenda, minutes of the last meeting and papers for discussion. The Minutes of the Committee must be issued within the two weeks of the
date of the meeting and should be reported to the R & D Committee.

An accurate record of the meeting must be kept of:
- Date of meeting.
- Attendees.
- Apologies/absences.
- Title of the projects submitted to the Committee, principal Investigator, directorate and location of work to be carried out in.
- R & D number assigned to the project.
- Agreed classification of the project and whether the notification to the HSE is required.
- Containment level required.
- Any further control measures to be taken by the personnel involved with the project.
- Amendments to be added to the risk assessment form.
- Comments and concerns raised by the GMSC members regarding the project.
- Dissenting opinion and agreed outcome.
- Actions to be taken and the named responsible person
- Minutes must be distributed to the GMSC and R & D Committee members

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Tick if confidential: [ ]

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
- Mycology
- Transgenic Invertebrates
- Transgenic Plants
- Other (please specify below) Yes

Other(s): Gene therapy study
For activities involving GMMs, describe the waste management measures which will apply to the activity

All wastes resulting from the IMP handling are treated according to regular hospital procedure for infectious wastes. Work area must be disinfected according to the standard hospital procedure. Standard methods of waste treatment will be used, such as off the shelf disinfectants, standard heat treatment and incineration.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
TG4010 is a viral suspension of the recombinant vector MVATG9931. The recombinant vector MVATG9931 is a recombinant vaccinia virus carrying sequences coding for human mucin 1 (MUC1) antigen and interleukin-2 (IL2). MVATG9931 was generated by homologous recombination between a transfer plasmid (pTG9931) which carries a gene coding for MUC1 flanked by sequences surrounding the deletion II of Modified Vaccinia Virus Ankara (MVA) and a subclone of MVA, named N33.1, isolated in Transgene laboratory.

Recipient strain: Modified Vaccinia virus Ankara (MVA)
MVA is a substrain of the vaccinia virus, belonging to the Poxviridae family and attenuated after several passages on primary chicken embryo fibroblasts (CEF). It has been developed by Mayr and Stickl during the course of the smallpox eradication campaign. The MVA strain was obtained after 570 passages of the Chorioallantois vaccinia virus Ankara (CVA) on primary CEF. Since the 516th passage in CEF, due to the stability of its new properties described below, the attenuated virus was re-named MVA.

Human mucin-1 (MUC1) antigen
The main function of mucin, normally found at the apical surface of mucin-secreting epithelial cells in many types of tissue, is to lubricate and protect epithelial cells from the harsh environment of the lumen. The MUC1 protein is over-expressed by tumor cells and less glycosylated than the normal form of the MUC1, revealing new peptide and carbohydrate antigenic epitopes. These immunological differences between MUC1 in normal cells and in tumors make it a target for immunotherapy.

Human interleukin-2 (IL2)
IL2 cytokine is included to act as an adjuvant in the immune response as IL2 is naturally secreted by antigen-stimulated T lymphocytes and is capable of stimulating the proliferation and differentiation of lymphoid cells by binding to specific surface cell receptors. The presence of IL2 is essential for the manifestation of cell-mediated and humoral immunity as well as for primary and secondary immune responses.

The MVA strain is not found in natural ecosystems and is a highly attenuated vaccinia virus. The clinical drug product TG4010 is provided in single dose 4-mL glass vials. Each vial contains the GMO in suspension in the formulation buffer named S 08. Active chlorine at 0.6% and some chemical disinfectants like aldehydes, alcohols (e.g., isopropyl alcohol 30% or ethyl alcohol 40%), hydrogen peroxide, phenols and quaternary ammonium compounds have been established to be active on the GMO.

GMO preparation is performed in advance by the sponsor which supplies all participating clinical sites with clinical drug product individual glass vials. Then, the most hazardous procedure is the preparation of a clinical drug product dose to be administered to the patient.

Wild-type vaccinia virus may cause local reactions including erythema, edema and systemic reactions such as fever and malaise, as has been observed with conventional vaccination to smallpox.

There is no established pre-existing medical condition that increase the risk associated with this agent. In addition, to be on the safe side in any case:
Healthcare personnel: Individuals who are pregnant, who are breast feeding, who have eczema or other exfoliative skin conditions or are immunocompromised should not handle the clinical drug product or come in contact with contaminated dressings.
Patients: Individuals who are pregnant, who are breast feeding, who are under chronic treatment with any immunosuppressive drug are not allowed to take part to the
The MVA strain is not directly classified by the French biosafety commission (Haut Comite de Biotechnologies). On the other hand, the resulting recombinant MVA (MVATG9931) as well as other recombinant MVA vectors developed by the company Transgene SA have been classified as class 1 Laboratory and other health-care personnel who work with highly attenuated strains of vaccinia virus (e.g., MVA) do not require routine vaccinia vaccination. Furthermore, no reports of transmission to health-care personnel from vaccine recipients have been published. Although no formal surveillance system has been established to monitor laboratory workers, no laboratory-acquired infections resulting from exposure to this highly attenuated strain or recombinant vaccines derived from this strain have been reported in the scientific literature or to Centers for Disease Control and Prevention (CDC). Vaccinia (Smallpox) Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP), June 22, 2001/50(RR10):1-25 (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5010a1.htm) US National Institutes of Health (NIH): Biosafety Level 1 recommended Swiss classification: Risk Group 1, Biosafety Level 1 recommended German classification: Risk Group 1, Biosafety Level 1 recommended Containment Level 1; Activity Class 1. Notification of HSE required as first GM study at this site.
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**Name**

| STRATOPHASE LTD |

**Name 2**

**Department**

**Campus Estate or Research Centre**

| UNIT 10A THE QUADRANGLE |

**Road Name**

| PREMIER WAY |

**District**

**Town**

| ROMSEY |

**County**

| HAMPSHIRE |

**Postcode**

| SO51 9DL |

**Country**

| ENGLAND |

**Tel Number**

| 01794 511226 |

**Fax Number**

| 08704 580754 |

**E-mail**

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**HSE Division**

**Comments**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

Stratophase is a company with 15 employees. The committee is comprised of 5 members of staff representing a cross section of the stake holders in the company.

The chairman holds a PhD in Physics and has worked within and been responsible for Health and Safety in multidisciplinary (physics, chemistry and biology) laboratories for >13 years including safety policies, risk assessments and COSHH (including Containment Level II facilities).

The Biological Safety Officer and Genetic Modification Safety Officer roles are held by the same employee with >14 years of commercial experience using GM cell lines at containment levels I, II and III and >5 years experience on the Health and Safety Committee (including GM H&S) of a major biopharmaceutical company. Qualified to BSc (Hons) Microbiology.

The Committee is advised by an international expert with >20 years experience in containment level I, II and III environments for Biopharmaceutical production.

The company Health and Safety Officer sits on the committee with >15 years facility management experience and is responsible for the company's integrated pest management activity (IPM).

The committee is completed by a member of technical staff that works daily within the lab.

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02/03/2022
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The scientific goal is the identification and characterisation of the life cycle of a mammalian cell.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

- Liquid waste will be autoclaved.
- All components will be autoclaved.
- The autoclave will be verified through annual thermocouple mapping (and service). Validation of the autoclave cycle will be carried out by the operator using the system controls to verify that the autoclave program was successfully carried out, and in addition Class 6 Time, steam and Temperature test strips will be used every run to verify a successful autoclave run.
- If necessary, whilst awaiting autoclave sterilisation all components will be soaked in 1% Virkon disinfectant.

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

The risks have been fully considered and addressed for the proposal "Proposal and Risk Assessment for Work with Genetically Modified Microorganisms (Class 1)"
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### Name

SGS M-SCAN

### Campus Estate or Research Centre

2-3 MILLARS BUSINESS CENTRE

### Road Name

FISHPONDS CLOSE

### Town

WOKINGHAM

### County

BERKSHIRE

### Postcode

RG41 2TZ

### Country

ENGLAND

### Tel Number

01189 896940

### Fax Number

01189 896941

### E-mail

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### Comments

Date at Which Additional Info Submitted

02/03/2022

Page 13239 of 15326
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee comprises the safety officer, a member of the sales team, project manager and/or study director. Where further information concerning a particular GMM is required, an external biological safety officer may be consulted.

GMM members will be received from external organisations (the "client") for the purpose of analysis at SGS M-Scan Ltd. All relevant risk assessments, storage requirements and waste procedures pertaining to the GMM will be obtained from the client. On submission of the relevant information, the GMSC will meet to discuss the proposed submission and decide whether to accept the GMM for analysis. Once a submission has been authorised, an internal risk assessment will be completed and circulated to company staff prior to receipt of the GMM on the premises.

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Material will be received from various organisations for the purpose of analysis at SGS M-Scan. This may include proteins, viral vectors and/or toxins, all of which may pose a risk of harm to the operator and/or the environment. All material will be submitted from the client to SGS M-Scan Ltd with the relevant risk assessments containing waste management procedures. Internal risk assessments will be produced in relation to the work to be completed as SGS M-Scan Ltd. Such procedures will be adhered to during all work completed with the material at SGS M-Scan Ltd.

Solid waste: Solid waste (e.g. tips, tubes, etc.) where possible, will be decontaminated with 1% [final concentration] virkon for 60 minutes. Virkon will then be drained and washed down the sink with plenty of fresh water. The solid waste will then be placed into orange biobins. Once filtered, orange biobins will be sealed, bagged in an autoclave bag and placed into a rigid waste container (yellow) designated GM waste. Once filtered, rigid waste containers will be sealed and placed ready for collection for incineration by a licensed waste contractor (e.g. Grundon).

Liquid waste: All liquid waste will be decontaminated with 1% [final concentration] virkon for 60 minutes. Inactivated liquid waste will then be washed down the sink with plenty of fresh water.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Material will be received from various organisations for the purpose of analysis at SGS M-Scan. This may include proteins, viral vectors and/or toxins, all of which may pose a risk of harm to the operator and/or the environment. All material will be submitted from the client to SGS M-Scan Ltd with the relevant risk assessments containing waste management procedures. Internal risk assessments will be produced in relation to the work to be completed as SGS M-Scan Ltd. Such procedures will be adhered to during all work completed with the material at SGS M-Scan Ltd.

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Liquid waste: All liquid waste will be decontaminated with 1% [final concentration] virkon for 60 minutes. Inactivated liquid waste will then be washed down the sink with plenty of fresh water.

Please enter comments of the GM safety committee on the risk assessment

Genetic modification safety committee (GMSC) of SGSM-Scan Ltd are prepared to accept samples given that the risk of replication competent lentivirus being produced being extremely low. All in house procedures will be modified for handling a biological hazard and waste kept separate from all other waste. The GMSC recommends that all standard protective laboratory protective clothing must be worn including laboratory coats, safety glasses and disposable gloves. All work should be completed in a way that minimises the production of aerosols.
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: 

Y

Give brief details of the genetic modification safety committee:

The Genetically Modified Organisms (Contained Use) Regulations (2000), places a statutory duty on Avacta Group businesses, and specifically on Aptuscan Ltd as an employer intending to carry out genetic modification activities, to establish a Genetic Modification Safety Committee (GMSC) prior to the start of these activities. The role of this Committee is to review all risk assessments relating to the creation, use and disposal of genetically modified organisms. Given the scope of operations in Avacta Animal Health (AAH) and Avacta Analytical (AA), we have extended the role of the Genetic Modification Safety Committee (GMSC) to take account of general biological, chemical and physical safety. As a consequence of the widened roles, the GMSC will be renamed the Laboratory Safety Committee (LSC). The proposed membership of the Committee is as follows:

- Chair: Director of Aptuscan, Head of Discovery Technology, also responsible for Laboratory Safety in Unit 651
- Biological Safety Officer: several years of post-doctoral experience in lab work, quality systems and operations management, mainly in industry; also management representative for AAH
- Staff representative for Aptuscan and lab technician, responsible for safe operations in labs E and F
- Management representative for AAH with responsibility for labs E and B
- Staff representative for AAH and lab B
- Management representative for AA and lab C
- Staff representative for AA and lab C

The combined laboratory experience of this committee exceeds 50 person years in both academic and commercial settings.

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02/03/2022
Waste in labs B, C, E and F falls into 3 categories: non-hazardous eg packing; clinical waste eg from diagnostic assays performed with animal samples in Avacta Animal Health (AAH); and waste potentially contaminated with Class I GMM, such as pipette tips and other disposable plastics; solid microbiological growth media and liquid waste such as biological growth media and waste from the cleaning of glassware.

Liquid waste will be inactivated by incubation and cleansing with a proprietary cleaner such as Virkon, and disposed of via the drain.

Solid waste, including microbiological growth media and plastics, will be inactivated by autoclaving before disposal via incineration by an approved contractor (SRCL). The autoclave will be onsite (to be installed in laboratory E on August 14th) as SRCL are not approved for the transport or storage of GMMs.

E. coli strains and M13 bacteriophage will be used for the creation and screening of libraries of en [Level 4 (GMMs)]

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Review of Risk Assessment received
There was a brief discussion of molecular biological (PKF) and veterinary diagnostic (HS, RH, GS) work (ALL). It was agreed that there are potential hazards when handling biological material from animals that will need to be monitored by this committee. PKF noted that as he is also responsible for overall lab safety in 651, he will also be reviewing COSHH assessments, and making sure that we are as "joined up" as possible.

The risk assessment from Avacta Technology entitled "Affimer Arrays" had been circulated on July 12th, and had been read by all. It was agreed that technical and assessment aspects were entirely satisfactory, although the title should be changed to "Affimer Arrays and Assay Development", so as to reflect that work on assay development will also require cat I work. The committee unanimously agreed that the project had correctly been assessed as Class 1, and that the risk assessment should be passed on to AS with a recommendation that he sign off on our notification to the HSE. We remain on course to start work on Monday, September 3rd, as long as the CU1 notification form is received by HSE in the week beginning July 23rd.

[Note - this internal deadline has been missed and we will not be able to start work until September 17th 2012]
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Shyden Biotechnology Ltd is a small start-up R&D company who are tenants of the newly established Stevenage Bioscience Catalyst (SBC) within the campus site of GlaxoSmithKline (GSK) PLC. The Genetic Modification Safety Committee is made up of 2 representatives from Shyden Biotechnology Ltd, 1 representative from another tenant company, the building manager of the SBC and 1 representative from GSK. All members are experienced in GMM work and are able to assess risk assessments and how to manage projects in the SBC.

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Bacteriology Yes Parasitology Transgenic Birds Microbiology Research
All liquid waste containing Class 1 GMMs will be chemically inactivated using 1% Virkon for at least 30 minutes and washed down designated laboratory sinks with copius amounts of cold water. Virkon kills 99.9995% of GMMs in 10 minutes. This will be routinely tested using agar plate colony assays.

All solid waste containing Class I GMMs will be chemically treated as above (if it contains any liquids) and after liquid disposal, the solid material will be autoclaved (124°C for 15 minutes). This effectively kills 100% if the GMMs and will be tested annually as above. The autoclaved material will then be disposed of as laboratory waste by Grundon contractors.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

This risk assessment was reviewed by the committee and after minor modifications and suggestions, it was approved. This risk assessment was also approved by Imperial College when the project was carried out there.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GMSC will be comprised of the Chief Scientific Officer, Biological Safety Officer, two Senior Scientists and the staff health and safety representative. Note that we are a small company, and the Chief Scientific Officer is currently also the Biological Safety Officer. A quorum for the meeting is a minimum of three individuals, to include the CSO, Biological Safety Officer (or deputy), and staff health and safety representative.

The Committee will meet a minimum of once per year to discuss routine matters, review all existing risk assessments and audit the existing procedures. The GMSC will meet more frequently to discuss risk assessments etc. that require more urgent attention

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)

Use of genetically-modified animal cell lines. Use of tissue samples from

Tick if confidential

Tick if confidential

Bacteriology

Parasitology

Transgenic Birds

Microbiology Research

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste, including cell cultures, will be inactivated by aspirating into a concentrated virkon solution, such that the final concentration remains above 2% and disinfected for at least 1 hour. It will then be disposed of down the sink with copious quantities of water.

Contaminated solid waste such as plastic disposables, tissue paper and culture plates, etc will be disposed of into suitable clinical waste disposal bins. Clinical waste disposal bins are sealed by the user and disposed of together with other such material generated on the Babraham Research Campus by incineration offsite.

Sharps materials will be disposed of in yellow sharps containers, which are disposed of by incineration offsite, together with other such material generated on the Babraham Research Campus.

All waste management measures will follow as a minimum the Babraham Research Campus biosafety and guidance policy.

Tick to confirm that you are attaching a summary of the risk assessment  
Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC found that the risk assessment adequately stated the risks of carrying out the activities described, and confirmed the activity class of the work to be 1. The control measures in place were found to be suitable and indeed largely already in place due to the fact that the laboratory in which the work is due to be carried out is already categorised as category 2 for carrying out work on human tissue samples.
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Date at Which Additional Info Submitted: 02/03/2022
Premises Addresses

Date Premises Closed | Name | Department | Name 2 | Campus Estate or Research Centre | Building | Road Name | District | Town | County | Post-code | Country | Withdrawn
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
ROYAL SURREY COUNTY HOSPITAL NHS FOUNDATION TRUST | | | | EGERTON ROAD | GUILDFORD | | SURREY | GU2 7XX | N

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The Trust Biosafety Committee is chaired by the Trust Medical Director. It has representatives from medical and nursing staff, pharmacy (including Chief Pharmacist and pharmacist responsible for the Trust Pharmacy Aseptic Unit Gene Therapy Facility) microbiology/virology, the Trust Infection Control team, the Trust Infection Control Officer, Trust Research Governance & Development Manager, department of oncology, Health and Safety Representative, Occupational Health, Trust Biological Safety Officer, University of Surrey. Other specialists are seconded ad hoc as required. The committee operates under the Trust's "Guidelines for the use of clinical biological agents (including genetically modified organisms)". It meets as required to assess risk assessments and consider biosafety issues.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
--- | --- | --- | --- | ---
Level 1 (GMMs) | | | | Yes
Level 2 (GMMs) | | | | 
Level 3 (GMMs) | | | | 
Level 4 (GMMs) | | | | 
Non-microbial | | | | 
Other (please specify) | | | | 
Tick if confidential

02/03/2022
2% Virkon used according to manufacturer's guidelines. In validation studies two representatives examples from the poxviridae have been tested, these being vaccinia (the parent of TroVax®) and bovine pseudocowpox virus. For vaccinia, a 10 min exposure to both 0.5% and 1% solutions of Virkon caused 1000-fold reduction in infectious titre, with complete inactivation being demonstrated after 30 minutes. Staff will be trained in the preparation and use of Virkon and monitoring the deactivating efficacy of the solution in use. After inactivation waste will enter the hospital contaminated waste streams for incineration appropriate for sharps or non-sharps.

Tick to confirm that you are attaching a summary of the risk assessment 

Tick if you are claiming exemption from disclosure for sections of the risk assessment 

Please enter comments of the GM safety committee on the risk assessment

None

**Project Ref** 3170/18.1

**Date Ackn'd** 06/09/2018

**CU2 Project Title** Gene therapy product RP1 (Common name rHSV-1 hGM-1-CSF/GALV-GP) for the treatment of solid tumours

**Class** Class 2

**CultureVol Class 2** < 1 Litre

**CultureVolume Class 3-4**

Non-GMM Consent Granted

Project notified under transitional arrangements N

02/03/2022
RP1 will be administered to subjects as an anti-tumour therapy, as part of a Phase I clinical trial to treat a wide range of solid tumour types. RP1 has a particular utility in combination with immune co-inhibitory pathway blockade. Intended indications to study include soft tissue sarcoma, breast cancer including triple negative breast cancer (TNBC), lung cancer including non-small cell lung cancer (NSCLC), melanoma, non-melanoma skin cancers, head and neck cancer, primary liver and kidney cancer and colorectal cancer. The initial clinical trial protocol intends to test RP1 in several indications as a monotherapy and in combination with anti-PD-1 therapy.

Recipient or parental organism

RP1 (rHSV:1hGM-CWALV-GP) is a selectively replication competent Herpes Simplex Virus-1 (HSV-1). The virus contains a codon-optimised sequence for human granulocyte macrophage colony stimulating factor (hGM-CSF) and a codon optimised sequence for the gibbon ape leukemia virus surface glycoprotein (GALV-GP) with the R- sequence deleted (R-) [GALV-GP-R-]. GALV-GP-R- expression leads to cell to cell fusion (syncytial) formation in infected tumour cells through binding to the constitutively expressed Pit-1 receptor for GALV. This results in the death of the cells by membrane fusion and is also intended to enhance the spread of the virus through the tumour. Since the RP1 selectively replicates in tumour cells, the expression of the GALV-GP-R- is minimised in normal tissues. The oncolytic destruction of tumour cells leads to the release of tumour associated antigens that are intended to engender an antitumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further enhanced through GALV-GP-R- mediated killing, fusion associated cell death which also results in the production of the highly immunogenic exosomes, which is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, un.injected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

Host/vector system

RP1 (rHSV_1 hGM-CSF/GALV-GP) is derived from the RH018A strain of Herpes Simplex Virus-1. RP1 is produced in the Vero cell and released into the culture media during cell lysis, prior to purification.

Origin & function

RP1 was constructed using a new strain of HSV-1 (strain RH018A). Replimune obtained and compared 30 clinical strains of HSV-1 on a panel of human tumour cell lines and selected the most promising of these (strain RH018A) for further development. RP1 expresses the immune stimulatory protein GM-CSF, which augments therapeutic activity. GALV-GP-R- binds to the Pit1 receptor, which is widely expressed on mammalian cells including human tumour.
cells. PI1 is also critical for cell proliferation, and its expression is therefore unlikely to be lost or down-regulated in response to cancer treatment. The truncated R- version of the protein provides constitutive fusion activity without GALV (i.e. the virus) itself. Expressing GALV-GP-R- together with GM-CSF is expected to increase clinical activity as compared to only expressing GM-CSF. As well as causing direct tumour cell death by cell to cell fusion, cell to cell fusion followed by death is highly immunogenic and includes the release of immunogenic tumour antigen-containing exosomes. Expression of GALV-GP-R- from an oncolytic virus is therefore expected to improve systemic, immune mediated, effects, as well as effects in the directly treated tumour thereby increasing synergy with other immunemediated approaches to cancer therapy such as immune co-inhibitory pathway blockade.

Evaluation of foreseeable effects

As described above (under Recipient or Parental Organism), the oncolytic destruction of tumour cells (upon transduction with RP1) leads to the release of tumour associated antigens that are intended to engender an antitumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further amplified through GALV-GP-R- mediated killing, fusion associated cell death which results in the production of the highly immunogenic exosomes and is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, un.injected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Spills should be treated with 70% isopropanol. All materials contaminated with RP1 must be disposed of in compliance with institutional procedures for the disposal of GMO (genetically modified organism) waste. Used and unused RP1 and diluent vials used in preparation and syringes will be destroyed per institutional policy. As per the wild-type HSV-1 virus, the recombinant HSV-1 vector particles that represent RP1 are highly susceptible to dehydration, rapidly inactivated outside the host and easily inactivated (for example with 1 % Virkon solution). As part of phase I of the clinical trial, biodistribution and shedding will be monitored. RP1 DNA levels in blood and urine will be determined at time-points outlined in the Schedule of Assessments of the clinical protocol (day 1, day 2/3, 015, Day 16/17, Day 29, Day 30/31, Day 43, Day 57, and as part of follow up at 30 day post last dose and 60 day post last dose. 3/7/10, day 14, day 21, day 28, day 35, day 42, day 49, day 56 and as part of the follow up, 30 days after the last dose).

Blood and urine will be collected at the first, second and third RP1 injections at the following timepoints: pre-dose, 6 (+/-2hr) hours, 21 hours (+/-3hr) and 48 hours (+/-6hr) and also immediately prior to dosing at fourth and fifth dose. Samples on injection days will be done prior to any injections and handling of RP1. Samples will also be collected at the 30 day and 60 day follow-up visit.

Saliva/oral mucosa samples will be collected at the first, second and third RP1 injections at the following timepoints: pre-dose, 6 (+/-2hr) hours, 21 hours (+/-3hr) and 48 hours (+/-6hr) and also immediately prior to dosing at fourth and fifth dose. Samples on injection days will be done prior to any injections and handling of RP1. Samples will also be collected at the 30 day and 60 day follow-up visit.

Injection site and exterior of dressing swabs samples will be collected at the first, second and third RP1 injections at the following timepoints: pre-dose (note - no exterior of dressing swab at this time), 6 (+/-2hr) hours, 21 hours (+/-3hr)
and 48 hours (+/-6hr) and also immediately prior to dosing at the fourth and fifth dose. Samples will also be collected at the 30 day and 60 day follow-up visit.

Specimens (swabs) will be collected at any time there is a suspicion of RP1-related viral infection occur such as vesicular eruptions or other signs of herpes viral infection. Samples should be obtained as soon as possible after symptoms arise to maximize the possibility of detection of virus, optimally within 24 hours, but samples may be collected later if collection within 24hrs is not possible. Subjects will be asked to take swabs at home for the subsequent 7 days after the initial test.

As part of Phase 2 Schedule of Assessments (TNBC, NMSC, NSCLC, melanoma), blood and urine will be collected at the following time points: Day 1, day 2/3, day 22, day 23/34, day 43, day 44/45, day 64, day 85, and as part of follow up, day 60 post last dose. Blood and urine will be collected, pre-dose at the first, second, third and fifth RP1 injections. Additionally, for each of the tumor types enrolled, the first six patients the following additional timepoints will be required: Doses 1, 2 and 3: 6 (+/-2hr) hours, 21 (+/-3hr) and 48 (+/-6hr) hours and also immediately prior to dosing at the fourth and fifth dose. Samples on injection days will be collected prior to any injections and handling of RP1. Samples will also be collected at the 60 day follow-up visit.

Saliva/oral mucosa samples will be collected pre-dose at the first, second, third and fifth RP1 injections. Additionally, for each of the tumor types enrolled, the first six subjects the following additional timepoints will be required: Doses 1, 2 and 3: 6 (+/-2hr) hours, 21 (+/-3hr) and 48 (+/-6hr) hours and also immediately prior to dosing at the fourth and fifth dose. Samples on injection days will be collected prior to any injections and handling of RP1. Samples will also be collected at the 60 day follow-up visit.

Injection site/dressing swabs samples will be collected pre-dose at the first (no exterior of dressing), second, third and fifth RP1 injections. Additionally, for each of the tumor types enrolled, in the first six subjects the following additional timepoints will be required: Doses 1, 2 and 3: 6 (+/-2hr) hours, 21 (+/-3hr) and 48 (+/-6hr) hours and also immediately prior to dosing at the fourth and fifth dose. Samples on injection days will be collected prior to any injections and handling of RP1. Samples will also be collected at the 60 day follow-up visit.

Specimens (swabs) will be collected at any time there is a suspicion of RP1-related viral infection occur such as vesicular eruptions or other signs of herpes viral infection. Samples should be obtained as soon as possible after symptoms arise to maximize the possibility of detection of virus, optimally within 24 hours, but samples may be collected later if collection within 24hrs is not possible. Subjects will be asked to take swabs at home for the subsequent 7 days after the initial test.

The timepoints for samples have been update for phase I. Risk assessment to include sample information for phase II as this is relevant. Section 6.3 is ticked no autoclaving therefore "NEEDS COMPLETION" in the other boxes can be removed. No further comments made, proceed to submission.
**Project Contention**

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<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref** 3170/20.1

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<td>18/11/2020</td>
<td>An Open-label Phase 1 Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics and Preliminary Efficacy of MEDI5395 in Combination with Durvalumab in Subjects with Select Advanced Solid Tumors (D645C00001)</td>
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**Project notified under transitional arrangements** N

Withdrawn N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Clinical trial in patients with selected advanced solid tumours.

**Recipient or parental organism**

MEDI5395 is a novel genetically modified recombinant Newcastle Disease Virus (NDV) expressing human granulocyte macrophage colony-stimulating factor (huGM-CSF). NDV has been classified as an avian paramyxovirus that has demonstrated strong oncolytic activity against human tumor cells (Reichard et al, 1992; Amarasinghe et al, 2017). NDV is classified as velogenic, mesogenic, or lentogenic, depending on its virulence in birds. NDV is not a
human pathogen and there is little or no pre-existing immunity against NDV.

**Host/vector system**

MEDI5395 OV is a non-pathogenic (lentogenic) variant of the WT NDV 73T strain. Inserted a huGM CSF transcriptional unit at the phosphoprotein (P) and matrix protein (M) gene junction. The huGMCSF transgene functions to enhance presentation of tumour antigens generated following MEDI5395-induced tumour lysis. MEDI5395 is a rNDV that has been modified to reduce avian virulence (via F-protein cleavage site modification and incorporation of 198 nt intergenic stretch sequence) and to incorporate a human GM-CSF transgene.

**Origin & function**

MEDI5395 is produced in HeLa-S3 cells. MEDI5395 is a systematically administered Oncolytic Virus that selectively replicates, produces GM-CSF, and exerts cytolytic activity in tumour cells. NDV enters cells via cell surface sialic acid, which is ubiquitous; it is selectively cytolytic for tumour cells vs non-tumour cells as a result of defects in the interferon signalling pathway that are common among diverse tumour types. This study will treat subjects with selected advanced solid tumours with Intravenous MEDI5395 in combination with durvalumab. The synergistic activity of this combination is anticipated to provide greater benefit to subjects than either treatment alone with MEDI5395 enhancing durvalumab activity through upregulating PD-L1 on tumour cells and immune cells within the tumour, releasing tumour specific antigens through tumour lysis, triggering both the innate and adaptive immune systems, and in vivo delivery of GM-CSF to the TME enhancing immunologic responsiveness to checkpoint inhibition.

**Evaluation of foreseeable effects**

As described above, MEDI5395 has the potential to enhance durvalumab activity through upregulating PD-L1 on tumour cells and immune cells within the tumour, releasing tumour specific antigens through tumour lysis, triggering both the innate and adaptive immune systems, and in vivo delivery of GM-CSF to the TME enhancing immunologic responsiveness to checkpoint inhibition.

The combination of MEDI5395 (which may induce tumor lysis and prime an antitumor immune response) and durvalumab (which may sustain an effective antitumor immune response) has the potential to provide improved clinical activity versus either monotherapy.

The potential risks for MEDI5395 to the trial subjects are based on the mechanism of action of MEDI5395. Based on nonclinical data and shedding data from human clinical studies with other NDVs it is anticipated that some transient, low-level shedding of MEDI5395 into subject secretions or excretions may occur, however this level of shedding should not constitute a hazard to human health or to birds. Due to its viral attenuation, the Sponsor believes MEDI5395 could be considered biologically contained even if released in the environment and the HSE agreed with the sponsor appraisal of the very low risk of MEDI5395 to human health and the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The Trust received confirmation from the HSE on the 18th October 2018 that the competent authority agreed to the omission of autoclaving at Royal Surrey NHS Foundation Trust due to the delegation of derogation to the GM
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Spills should be treated with Virkon 1% or 10% hypochlorite solution. All materials contaminated with MEDI5395 must be disposed of in compliance with institutional procedures for the disposal of GMO (genetically modified organism) waste. Used and unused vials used in preparation will be destroyed as per institutional policy and procedures. The disposal and moving of the waste will be organised by a delegated member of the contracted estates team. The waste will be tagged ready for SRCL Ltd. to collect, the delegated member of the contracted estates team will inform SRCL Ltd. that the site has GM waste to collect for disposal under the GM waste account. Shedding of infectious viral particles into saliva and urine have been observed following IV administration of nonrecombinant NDVs to mice, non-human primates, and humans. Virus shedding in subjects will be assessed using a quantitative real time-reverse transcription PCR method. The following clinical lab tests will be performed: Serum chemistry, Hematology, Coagulation, Thyroid function tests, Cardiac labs. The genome copies of MEDI5395 will be measured in saliva and urine pre and post dose administration of MED15395, at the end of treatment (within 28 days of the decision) and 90 days (+1- 7 days) post last dose follow-up. Viral infectivity will be determined for the shedding samples which have MEDI5395 genome copies greater than the genome copy cut-point that will be established based on the correlation of genome copies and virus infectivity by an in vitro study.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
No comments made. Risk assessment complete and approved by GMSC, proceed to submission.

Project Containment

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Fahy Gurteen is a startup company. The newly formed genetic modification safety committee comprises:
- Senior Cell Biologist
- Cell Biologist
- Operation Executive
- External Consultant

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Non-microbial

Other (please specify) Tick if confidential

Tick if confidential

Bacteriology Yes Parasitology Transgenic Birds Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

GMMs will be inactivated by overnight exposure to 1% Virkon disinfectant solution, guaranteeing killing of greater than 99.99% of microorganisms and viruses. Liquid waste material will be treated with disinfectant overnight before autoclaving and sink disposal. Solid waste in the form of consumable plasticware exposed to GMMs will be autoclaved and disposed by an external contractor (Grundon).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The project "Expression of proteins involved in oncogenic signalling pathways" and the associated risks are adequately described in the risk assessment. The work poses little risk to health or the environment and we agree on working under level 1 containment.
### Velindre Cancer Centre

**Address:**

**Velindre Road**

**Whitchurch**

**Cardiff, CF14 2TL, Wales**

**Tel Number:** 029 20314909

**Fax Number:** 0

**E-mail:** blank

**HSE Division:**

**Comments:**

**Date at Which Additional Info Submitted:**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes]

Give brief details of the genetic modification safety committee

Velindre Trust GMSC committee. This committee meets monthly, Composition - biological safety officer, health & safety officer, infection control officer, pharmacy rep, specialist nurse, responsible person for waste, Consulatant Oncologist, Clinical Trial Unit Manager, union rep, patient representative, virologist.

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Tick if confidential

![No]

Bacteriology

Parasitology

Transgenic Birds

Microbiology Research

Virology

Transgenic Animals

Transgenic Fish

Gene Therapy

![Yes]
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For activities involving GMMs, describe the waste management measures which will apply to the activity:

Safe discard cycle by autoclave. Autoclave will be maintained within Welsh Health Estates (Shared Services) approved parameters. Advice sought from Senior Decontamination Engineer for Wales. In the event of an autoclave failure lasting longer than 12 hours, the live GM product will be transported in a sealed rigid container by an approved clinical waste contractor, directly to an incineration facility.

Tick to confirm that you are attaching a summary of the risk assessment  

**Y**

Tick if you are claiming exemption from disclosure for sections of the risk assessment  


Please enter comments of the GM safety committee on the risk assessment:

The GMSC were satisfied that potential risks related to the conduct of this study had been identified by the research team and mitigated within local SOPs for implementation within Velindre Cancer Centre.
| Data Premises Notified (Originally) | 23/01/2013 | Transferred from 1992 Regs? | N | Transitional Premises Class |  |
| Data Premises Closed |  | Transitional Premises |  | Non-GMMs | N | Withdrawn | N |

| Name | CANTAB ANTI-INFECTIVES |
| Name 2 | Department |

| Campus Estate or Research Centre | BIOPARK |
| Road Name | BROADWATER ROAD |
| Town | WELWYN GARDEN CITY |
| County | HERTFORDSHIRE |
| Postcode | AL7 3AX |
| Country | ENGLAND |

| Tel Number | 01707 356130 |
| Fax Number | 01707 356131 |

| E-mail |  |
| HSE Division | blank |

| Comments |  |

| Date at Which Additional Info Submitted | 02/03/2022 |
Premises Addresses

Date Premises Closed Name Department Name 2
Premises Addresses

Campus Estate or Research Centre Building Road Name District Town County Post-code Country Withdrawn
CANTAB BIOPARK BROADWATER ROAD WELWYN GARDEN CITY HERTFORDSHIRE AL7 3AX ENGLAND N

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Research Director, Associate Director of Microbiology (Biological Safety Officer), Associate Director of natural Product Chemistry (H&S Chair).

The committee will meet annually to review all risk assessments and HSE notifications. Additional meetings will be held to consider new risk assessments and notification requirements as required. All assessments and notifications will be circulated by email and comments from the committee taken into account prior to any new procedures being adopted. Any assessments which fall outside the expertise of the committee will be sent to external advisors drawn from experienced scientists working in the relevant area.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Yes Parasitology Transgenic Birds Microbiology Research Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid biological waste will be placed in biological waste bags in plastic bins. When full the waste will be transferred to autoclave and sterilized using a 'killing-off' cycle. This autoclave cycle will include heating the material to at least 121°C, for 30 minutes to ensure 100% kill for all material.

Liquid waste and contaminated glassware will be treated in one of two ways:
1) Autoclaving: The autoclave cycle will include heating the material to at least 121°C, for 30 minutes to ensure 100% kill for all material
2) Presept disinfectant: Presept disinfectant, diluted according to the manufacturer's instructions, overnight at room temperature.

In either case an initial check will be carried out the first time the process is used to check that no culturable cells remain before disposal. This check will be repeated periodically depending on classification of risk and whenever waste from different cell types or quantities of culture are produced to ensure that the procedure results in a 100% kill.

Members of the Genetic Modification Safety Committee have read the attached risk assessment and have agreed with its conclusions.
GM Centre Number: 3181

Data Premises Notified (Originally) 10/04/2013

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

OIL PLUS LTD

Name 2

Department

Campus Estate or Research Centre

Building

DOMINION HOUSE

Road Name

KENNET SIDE

District

Town

NEWBURY

County

BERKSHIRE

Postcode

RG14 5PX

Country

ENGLAND

Tel Number 01635 30226

Fax Number 01635 49618

E-mail

HSE Division Blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Technical Manager, Laboratory Supervisor, Principal Technologist (Microbiology), 2 x Technologists (both Microbiologists) comprise the committee. Meetings to be held every 6 months to review working practices, advise on the health and safety measures used regarding GMMs, to advise on technical matters.

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<th>Gene Therapy</th>
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Virology | Microbiology Research | Gene Therapy

Yes |
All solid waste contaminated with GM microorganisms (JM109 competent escherichia coli) will be placed in clear autoclave bags labelled ‘Autoclave Waste Only’. The solid waste (and liquid cultures) will be autoclaved at 121°C for 60 min. Solid waste can then be disposed of in the biohazard waste (although regular waste is acceptable). Liquid cultures will be disposed of down the sink after autoclaving agar will be autoclaved, then bagged and disposed of in regular waste.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Other(s)

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Another risk control measure is to dispose of culture by incineration

02/03/2022

Page 13273 of 15326
<table>
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Name

CYCLOFLUIDIC LTD

Name 2

Department

Campus Estate or Research Centre

OXFORD BUSINESS PARK

Road Name

9400 GARSINGTON ROAD

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX4 2HN

Country

ENGLAND

Tel Number

01707358676

Fax Number

01707358676

HSE Division

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

| Head Of Biology: BSc (Hons), Dphil, Basic Health & Safety Certificate (Charted Institute Of Health) : Sixteen Years Pharmaceutical R&D Experience.  |
|Chief Technology Officer:BSC Twenty-Seven years pharmaceutical R&D experience.  |
|The genetic modification safety committee will meet on a monthly basis. The meeting will be minuted and such minutes filed appropriailey. The genetic modification safety committee will also give a verbal report at the monthly Health and Safety meeting.  |

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</table>
Laboratory use of non-commercial and commercially available transfected cell lines. Conduct cell-based assays using genetically modified organisms to evaluate the biological activity of test articles on such cell lines. Genetic modifications may include over-expression of target proteins and/or immortalisation of cell lines using induced (e.g. Viral infection) or un-induced (e.g. Cancerous) Genetic modification will not be carried out.

Solid and liquid waste will be disposed of in biohazard weaver bins (e.g. GRIFF BIN300, Griffiths & Neilsen; ThermoFisher; Rexam, Etc) These are fully lockable and sealable plastic containers. Once locked, the lid has a fluid-tight adhesive seal to prevent any residual fluid leakage after final closure. These bins will be disposed of by full containment followed by incineration. Based on the risk assessment and control measures, on-site autoclaving will not be required.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

For activities involving GMMs, describe the waste management measures which will apply to the activity

A risk assessment and COSHH assessment by the users (Laboratory Scientists) have identified the hazards and who might be harmed and how. Users have evaluated the risks and decided on the precautions. The assessments have been submitted to the appropriate individuals for review and the findings have been recorded ready for implementation pending approval. There is also a review and update procedure should the process require amendment or on a chronological basis.
GM Centre Number: 3183

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Name

MERCK CHEMICALS LTD

Name 2

Department

Campus Estate or Research Centre

BOULEVARD INDUSTRIAL PARK

Road Name

PADGE ROAD

District

BEESTON

Town

NOTTINGHAM

County

NOTTINGHAMSHIRE

Postcode

NG9 2JR

Country

ENGLAND

Tel Number

01159430840

Fax Number

01159430951

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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Page 13279 of 15326
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee comprises three staff members of BioServ UK Ltd. The committee members have worked with genetically modified organisms for many years and are familiar with the SACGM guidance for the containment and control of activities involving genetically modified microorganisms including the risk assessment of such activities. One of the committee members has served on the Genetic Modifications Advisory Group within the University of Sheffield to advise academic researchers on safety considerations for work involving the genetic modification of microorganisms.

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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All the work will be carried out in designated laboratories suitable for the procedures. Bulk liquid waste will be neutralised by addition of 2% Virkon or autoclaved in designated and validated discard autoclaves. Glassware will be decontaminated by autoclaving or by filling with 2% Virkon solution or 5% hypochlorite. Solid waste will be disposed of by incineration, using the appropriate waste stream as defined by the Biological Safety Committee.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Autoclaving will be performed using validated equipment. Each of these processes shall be tested for effectiveness by time-course sampling followed by determination of degree of kill.
**GM Centre Number: 3186**

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**Name**

**CENSO BIOTECHNOLOGIES GROUP LTD/ROSLIN CELL SCIENCES**

**Name 2**

**Department**

**Campus Estate or Research Centre**

**ROSLIN BIOCENTRE**

**Building**

**WALLACE BUILDING**

**Road Name**

**District**

**ROSLIN**

**Town**

**County**

**Postcode**

**EH25 9PP**

**Country**

**SCOTLAND**

**Tel Number** 0131 440 6541

**Fax Number** n/a

**E-mail**

**HSE Division** blank

**Comments**

Name change from Roslin Cells 01/12/2015, Name change from Roslin Cell Sciences 30/03/2017

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Y
<table>
<thead>
<tr>
<th>Laboratory</th>
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Other(s):

- Transfection of mammalian cell lines with episomal and RNA vectors. Transduction of mammalian cell li

Tick if confidential

Bacteriology  Parasitology  Transgenic  Microbiology
- Birds

Virology  Transgenic
- Animals
- Fish

Mycology  Transgenic
- Invertebrates
- Plants

Other (please specify below) Yes

For activities involving GMMs, describe the waste management measures which will apply to the activity.
Solids (e.g. plasticware: pipettes, flasks, tubes, culture plates, etc.) Autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 mins, or 126-130°C for at least 10 mins, or 134-138°C for at least 3 mins), or disinfect with freshly prepared Haz-Tabs (2x2.5g tablets/litre water) for 1hr minimum, discharge excess liquids to drains, dispose of solids via clinical waste stream for microwave treatment or via the industrial (landfill) waste stream.

Liquids (e.g. samples, cell culture supernatants, culture media) - Autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 mins, or 126-130°C for at least 10 mins, or 134-138°C for at least 3 mins), or disinfect with freshly prepared 2% Virkon or Haz-Tab (2x2.5g tablets/litre) solution overnight (min 1hr) discharge to drains.

One procedure requires the use of sharps (manipulation of cell colonies with Swemed Stem Cell cutting Tool or 25 gauge needles) Procedures require that sharps are never re-sheathed and placed directly in a sharps bin located within the culture hood after use. Sharps are autoclaved using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 mins, or 126-130°C for at least 10 mins, or 134-138°C for at least 3 mins), dispose via clinical waste stream for microwave treatment.

Degree of kill:
Autoclaving: Effectively 100% kill (annual qualification using 12 point thermocouple of worst case loads)
Disinfection: A 1% solution of Virkon disinfectant with a contact time of at least 10 minutes gives a >4.25 log reduction of HIV-1 (data from manufacturer).
Heat treatment or incineration: Not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
BACKGROUND FOR COMMITTEE DISCUSSION

The potential methodologies to be used include delivery of gene encoding and non-encoding nucleic acids in vectors that cannot integrate into the Deoxynbonucleic acid (DNA) genome of a cell in its nuclear compartment or replicate. This includes a modified variant Sendai viruses composed of ribonucleic acid (RNA) which does not permit its replication (which would normally only occur in the cytoplasmic compartment of a cell). Replicating sendai have been used safely in the context of gene therapy. We will also use DNA or RNA sequences which cannot replicate and may be unmodified or modified chemically to facilitate their ability to enter into the cytoplasmic or nuclear compartment of stability within the cell. There are eventually destroyed by the cell or diluted out with successive cell divisions.

The Sendai Virus, constitutes as defective mutant clone of the normal cytoplasmic RNA vector which normally resides in a murine host. This mutant clone is non-pathogenic and replication incompetent due to the removal of the M,F and HN genes. The vector includes functional mutations such as temperature sensitivity to aid their safe removal.

Non-integrating, non-replicating gene encoding DNA vectors are composed of "episomal" circular plasmid DNA which contains genes that are expressed by the cell phenotype which is the objective of the reprogramming process.

For purposes of reprogramming to a pluripotent stem cell state, such as manifest by embryonic stem cells, gene encoding vectors may contain genes which promote cell proliferation such as C-myc, that are known tumour promoting oncogenes. As the vectors are replication incompetent, the effect of this gene is transient and the risk of tumour formation is unlikely.

Non-gene encoding nucleic acids may constitute micro RNA or short interfering RNA from 10 to a few hundred nucleotides in length whose effects are to destabilise specific endogenous gene encoding mRNAs, thereby inhibit their expression to facilitate reprogramming of cellular phenotype/identity. They may also include similarly sized anti-sense oligonucleotide DNA which can transiently destabilise endogenous mRNAs or control which variant of a mRNA are expressed from the genome.

The purpose of the research is to reprogram human cells to alternative phenotypes/identities. This may include embryonic or adult derived somatic cells such as for example fibroblasts or blood derived mononuclear cells) to create induced pluripotent (IPS) cell lines, lineage committed stem or progenitor cells or alternative differentiated cells.

CONCLUDING REMARKS

In summary, the committee agreed that the risk posed by the reprogramming vectors should be considered containment level 1, however as the work also involves the culture of human cells and tissues we will be conducting all activities within containment level 2 for COSHH reasons.

The committee also felt it was important to stress that all cellular reprogramming activities will involve the use of vectors which are non-integrating and are gradually removed from the culture over a number of passages. The reprogrammed cells are as such because they will have undergone changes to their gene expression and not due to any actual modification of their genome.

The reprogrammed cell lines are only capable of survival in strictly controlled cell culture conditions within a controlled laboratory. Removal, or even significant variance, from these conditions would cause the cells to perish in a very short time. The reprogramming process will not confer any irreversible survival benefits.

The cells will be non-pathogenic and are expected to pose no additional risk compared to the parental cell line/culture.

Cell culture volumes are expected to be multiples of 4-10mL individual culture volumes. Maximum of 250-500mL at any one time.
The purpose of the research is to reprogram human cells to alternative phenotypes/identities. This may include embryonic or adult derived somatic cells (such as for example fibroblasts or blood derived mononuclear cells) to create induced pluripotent (iPS) cell lines, lineage committed stem or progenitor cells or alternative differentiated cells.

Unmodified or non-genome integrating genetically modified human cells do not survive easily outwith controlled laboratory culture conditions. The genetic modification of the cell is transient and experienced during reprogramming of cellular phenotype/identity, with genetic vectors either being destroyed by the cell or diluted out following successive cell divisions.

The Sendai Virus, constitutes a defective mutant clone of the normal cytoplasmic RNA vector which normally resides in a murine host. This mutant clone is non-pathogenic and replication incompetent due to the removal of the M, F and HN genes. The vector includes functional mutations such as temperature sensitivity to aid their safe removal.
Non-integrating, non-replicating gene encoding DNA vectors are composed of "episomal" circular plasmid DNA which contains genes that are expressed by the cell phenotype which is the objective of the reprogramming process.

For purposes of reprogramming to a pluripotent stem cell state, such as manifest by embryonic stem cells, gene encoding vectors may contain genes which promote cell proliferation such as C-myc, that are known tumour promoting oncogenes. As the vectors are replication incompetent, the effect of this gene is transient and the risk of tumour formation is unlikely.

Non-gene encoding nucleic acids may constitute micro RNA or short interfering RNA form 10 to a few hundred nucleotides in length whose effects are to destabilise specific endogenous gene encoding mRNAs, thereby inhibit their expression to facilitate reprogramming of cellular phenotype/identity. They may also include similarly sized anti-sense oligonucleotide DNA which can transiently destabilise endogenous mRNAs or control which variant of a mRNA are expressed from the genome.

Origin & function
The genetic material in the Sendai and all nucleic acid vectors originate from synthetic manufacture.

Evaluation of foreseeable effects
The reprogrammed cell lines are only capable of survival in strictly controlled cell culture conditions within a controlled laboratory. Removal, or even significant variance, from these conditions would cause the cells to perish in a very short time. The reprogramming process will not confer any irreversible survival benefits.

The cells will be non-pathogenic and are expected to pose no additional risk acompanied to the parental cell line/culture.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g. plasticware: pipettes, flasks, tubes, culture plates, etc): Autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125C for at least 15 mins, or 126-130C for at least 10 mins, or 134-138C for at least 3 mins), or disinfect with freshly prepared Haz- Tabs (2x2.5g tablets/litre of water) for 1 hr minimum, discharge excess liquids to drains, dispose of solids via clinical waste stream for microwave treatment or via the industrial (landfill) waste stream.

Liquids (e.g. samples, cell culture supernatants, culture media) - Autoclave using a make safe cycle as specified in BS2646, Part 3, 1993 (either 121-125C for at least 15 mins, or 126-130C for at least 10 mins, or 134-138C for at least 3 mins), or disinfect with freshly prepared Haz- Tabs (2x2.5g tablets/litre of water) for 1 hr minimum, discharge to drains.

Sharps may be used but there are procedures in place to minimise risk of injury including no re-sheathing of needles and placing of sharps directly into sharps bins within the MSC class II cabinets after use.

Degree of kill:
Autoclaving: Effectively 100% kill (annual qualification using 12 point thermocouple of worst case loads)
Disinfection; A 1% solution of Virkon disinfectant with a contact time of at least 10 mins gives a >4.25 log reduction of HIV-1 (data from manufacturer).
Heat treatment or incineration: Not applicable, all waste is autoclaved prior to disposal by heat treatment or incineration.
The vectors are considered low risk as they are non-pathogenic and are replication incompetent. The committee agreed that the vectors would normally be classified as Class 1, however, since some vectors may contain the oncogene C-myc the containment level should be class 2. Therefore any risks considered to be greater than Class 1 requirements are limited to those presented by the presence of this oncogene.

There is no determinable risk to the environment.

Any risk to human health relates to individual operators and is negligible. There is no risk of transmission or dissemination.

**Project Containment**

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**Project Ref** 3186/15.1

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Withdrawn N

Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**

The purpose of the work on the premises is to expand and bank human induced pluripotent (iPS) cell lines. These have not been derived on site, but may still contain reprogramming material on receipt. The potential methodologies that will have been used to generate the iPS cell lines include delivery of gene encoding and non-encoding nucleic acids in different vectors. These include integrating vectors like retro- or lenti-viral or non-integrating vectors such as a modified variant Sendai viruses or nonintegrating, non-replicating gene encoding DNA vectors composed of "episomal" circular plasmid DNA.

**Recipient or parental organism**

Unmodified or non-genome integrating genetically modified human cells perish in a very short time outwith controlled laboratory culture conditions. The genetic modification of the cell is transient and experienced during reprogramming of cellular phenotype/identity, with genetic vectors either being destroyed by the cell or diluted out following successive cell divisions.

**Host/vector system**

The Sendai and episomal vectors are replication incompetent and are not integrated into the genome, rather their genes are expressed in the cytoplasm and resultant transcription factors influence changes in the genome using normal cellular mechanisms. Integrating virus used to generate iPS cells is disabled and unable to replicate in any host and the virions produced are extremely labile and do not persist in the environment. By the time iPS cells generated using retro- or lentiviral stragegies are received, virions will no longer be present.

**Origin & function**

The inserted material includes genes leading to the expression of factors related to cell pluripotency. When expressed, these transcription factors can
induce reprogramming in the host cell genome. By the time iPS cells are received on site, the DNA sequences will no longer be in a gene delivery system and unlikely to cause harm to operators. The genetic material in the viral and all nucleic acid vectors are expected to have originated from synthetic manufacture.

**Evaluation of foreseeable effects**

The reprogrammed cell lines are only capable of survival in strictly controlled cell culture conditions within a controlled laboratory. Removal, or even significant variance, from these conditions would cause the cells to perish in a very short time. The reprogramming process will not confer any irreversible survival benefits. The cells will be non-pathogenic and are expected to pose no additional risk compared to the parental cell line/culture.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

<table>
<thead>
<tr>
<th>Solids (e.g. plasticware: pipettes, flasks, tubes, culture plates, etc.): disposed of according to the Babraham Research Campus - Hazard Group 2 Waste disposal S.O.P (waste sealed in 30L Euro bins and sent offsite for incineration by Vetspeed (GM authorisation GM898, notification GM105/4.1).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquids (e.g. samples, cell culture supernatants, culture media): Disinfect with freshly prepared 2% Virkon or Haz-Tab (2x2.5g tables/litre) solution overnight (min 1hr) discharge to drains. Spills will be cleared up using absorbent tissue and discarded as solids. Surfaces are decontaminated using Klercide A or B (Shield Medicare) according to Roslin Cells cleaning S.O.P (15min contact time).</td>
</tr>
<tr>
<td>Degree of kill:</td>
</tr>
<tr>
<td>Disinfection: A 1% solution of Virkon disinfectant with a contact time of at least 10 mins gives a &gt;4.25 log reduction of HIV-1 (data from manufacturer). 15min contact time using Klercide B gives minimum 2-fold log reduction of bacterial, yeast and spore load (data from manufacturer).</td>
</tr>
<tr>
<td>Incineration: Effectively 100% kill.</td>
</tr>
<tr>
<td>The procedure does not require the use of sharps.</td>
</tr>
</tbody>
</table>

**Is an emergency plan required according to regulation 20?**

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
The committee agreed that the risk posed by the reprogramming vectors should be considered containment level 2 due to the potential presence of c-myc.
The committee also felt it was important to stress that no use of reprogramming vectors will take place on site, which greatly reduced the risks for staff.
The reprogrammed cell lines are only capable of survival in strictly controlled cell culture conditions within a controlled laboratory. Removal, or even significant variance, from these conditions would cause the cells to perish in a very short time. The reprogramming process will not confer any irreversible survival benefits.
The cells will be non-pathogenic and are expected to pose no additional risk compared to the parental cell line/culture.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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**Project Ref**: 3186/18.1

- **Date Ackn’d**: 12/07/2018
- **CU2 Project Title**: Transduction of differentiated iPSC lines with recombinant lentivirus
- **Class**: Class 2
- **Culture Volume Class 2**: < 1 Litre
- **Consent Granted**: Not Applicable
- **Non-GMM**: N
- **Project notified under transitional arrangements**: N

Tick if notifying a connected programme of work: N
## Project Additional Information

### Purposes of the contained use

The objective of this work is transduce differentiated human induced pluripotent stem cell (iPSC) lines with lentivirus from a variety of primary tissue sources and from a variety of reprogramming methodologies and gene editing methodologies to enable disease relevant cellular assay to be developed.

### Recipient or parental organism

Human differentiated induced pluripotent stem cells (iPSCs)

### Host/vector system

Lentivirus. Only virus generated from 3rd generation systems or later systems will be used (Self Inactivation versions.)

### Origin & function

The inserted material includes promoter and reporter genes (fluorescent proteins and luminescent proteins) which lead to expression in mammalian cells. The reporter protein will be used to selectively track biological processes such as proliferation, apoptosis and migration.

### Evaluation of foreseeable effects

Commercially available, self in-activating /replication deficient recombinant HIV based lentiviral will be used. These have been generated from vectors with all viral genes removed and replaced with an expression cassette encoding mammalian promoters such CMV or EF1alpha promoter linked to reporter gene protein (e.g. GFP).

The GMOs generated do not have any growth or selection advantage and should not present any biological advantage or confer any survival benefits. The resulting genetically modified cells require strict culture conditions and skilled operator interventions to ensure their survival. Outside of these carefully controlled laboratory conditions the cells would perish very quickly.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solids (e.g. plasticware: pipettes, flasks, tubes, culture plates, etc.) will be collected in 30 litre Euro bins plastic bins and disposed of according to the Babraham Research Campus -Hazard Group 2 Waste.
disposal stream.
In summary each bin has a unique identifier and the bin labeled with biohazard sticker and the company name an (waste sealed in 30L Euro bins) and sent offsite for incineration by Vetspeed (GM authorisation GM898, notification GM105/4.1) or double bagged in clinical waste bags, placed in locked container and sent offsite for incineration by Healthcare Environmental (GM authorisation GM777).
Liquids (e.g. samples, cell culture supernatants, culture media): Disinfect with freshly prepared 2% Virkon or Haz-Tab (2x2.5g tables/litre) or equivalent chlorine based solution overnight (min 1hr)
Spills will be cleared up using absorbent tissue and discarded as solids. Surfaces are decontaminated using Distel, Microsol 4+ or Chemgene HDL4L, 1% virkon or equivalent.
Degree of kill:
Disinfection: A 1% solution of Virkon disinfectant with a contact time of at least 10 min gives a >4.25 log reduction of HIV-1 (data from manufacturer).
15 min contact time using Distel gives minimum 4-fold log reduction of bacterial, fungi and virus load (data from manufacturer).
15 min contact time using Microsol 4+ gives minimum 3.67-fold log reduction of bacterial, yeast, fungi and virus load. 60min contact time gives a minimum of 3.17-fold log reduction of mycobacterium and spore load (data from manufacturer).
15 min contact time using Chemgene HLD4L gives minimum 3.12-fold log reduction of bacterial, yeast and spore load (data from manufacturer).
Incineration: Effectively 100% kill.
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<thead>
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<tr>
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**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The RB&HFT GM Safety Committee (RB&HFT GMSC) is responsible for ensuring that work is conducted in accordance with the study risk assessment and is compliant with Genetically Modified Organism (Contained Use) Regulations.

Represented on the Committee are RB&HFT clinicians, Pharmacy, Infection Control, Quality and Safety Department, R&D (including the Research Governance and Regulatory Compliance Manager and the Associate Director of Research), appropriate Clinical and Research Facility managers, Clinical Trial Manager Representatives and the RB&HFT BSO. Representatives from the Project Team also attend meetings.

RB&HFT GMSC is a subcommittee of the RB&HFT Clinical Trials Oversight Committee (CTOC) which reviews and oversees all clinical trials activities in the organisation.

RB&HFT GMSC receives technical advice from Imperial College GMSC 309 if requested. Responsibility for approval of GM studies rests with RB&HFT CTOC and will be based on the recommendation from RB&HFT GMSC.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes
Level 2 (GMMs)
Level 3 (GMMs)
There are two locations where GM waste is expected to arise: The Royal Marsden Clinical Trials Pharmacy and the Biomedical Research Unit (BRU) Cardiac catheter laboratory.

Waste, pertaining to the Contained Release Regulations, will be generated as a result of preparation of the GM product (Royal Marsden Clinical Trials Pharmacy) and during the cathetization procedure (BRU).

Inactivation of GM product: The GM product for the proposed clinical trial will be inactivated according to the Study Sponsor's guidance, which has provided validation of inactivation by the dilution and mixture of 0.5% sodium hypochlorite solution, with a minimum contact period of 20 minutes.

Royal Marsden Hospital GM waste management system:

Liquid waste: During the preparation of the GM product in the Royal Marsden Hospital Pharmacy, there will be approximately 2ml of undiluted GM product at 3 x 10^{12} DRP/mL as waste and 10mL of 1:15 diluted GM product. The GM product waste will be inactivated by 0.5% sodium hypochlorite as above and then placed in

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For activities involving GMMs, describe the waste management measures which will apply to the activity:

- **Bacteriology**
  - Parasitology
- **Virology**
  - Transgenic Animals
- **Mycology**
  - Transgenic Invertebrates
- **Transgenic Birds**
- **Transgenic Fish**
- **Transgenic Plants**
- **Microbiology Research**
- **Gene Therapy** Yes
- **Other (please specify below)**

---

**Other(s)**
designated bins sealed and marked biohazard materials, for incineration off site.

Solid waste: During preparation of the GM product, one needle and three syringes will come into direct contact with the GM product. These sharps will be placed in designated sharps bins, sealed and marked biohazard materials and destroyed by incineration via the Royal Marsden gene therapy waste stream.

Royal Brompton Hospital Biomedical Research Unit (BRU) Cardiac catheterisation laboratory:

Liquid waste: After administration of the GM product to the patient approximately 10 mls of diluted GM product will remain in the syringe. This will be inactivated (as above) then placed in designated waste bins, sealed and marked biohazard. It will then be sent off site for incineration via the RB&HFT Biohazard Waste stream.

Solid waste: During the catheterisation procedure a high pressure tubing line and a catheter will contact the GM product. This will be inactivated (as above) and then disposed of in designated waste bins, sealed and marked biohazard and which will then be sent off site for incineration via the RB&HFT Biohazard Waste stream.

As patients may shed GM AAV in urine for up to 4 weeks after catheterization and perfusion of the virus, an application for deliberate release has been submitted to DEFRA. Any waste (GM or not) arising from patients post-operatively will be handled in the same way as other clinical waste at RB&HFT.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

A risk assessment has been reviewed by RB&HFT GMSC and Imperial College GMSC 309 in relation to the study:

"CUPID 2 (A Phase 2b, double-blind, placebo-controlled, multinational, multi-centre, randomised study evaluating the safety and efficacy of intracoronary administration of MYDICAR® (AAV1/SERCA2a) in subjects with heart failure.

The risk assessment was undertaken following MHRA (Medicines and Healthcare Products Regulatory Agency) and GTAC (Gene Therapy Advisory Committee) approval of the study.

Imperial College GMSC 309 recommended that the study Sponsor submit to DEFRA for deliberate release approval which it has done. THE Sponsor and Chief Investigator are anticipating a positive response from DEFRA. RB&HFT is fully aware that some aspects of the study would still be regulated by the Contained Use of GMO's Regulation and is therefore submitting notification of premises for its hospital sites.

Following an initial review by Imperial College GMSC 309 and RB&HFT GMSC, the risk assessment was revised and updated to cover:

i) Details of potential needlestick injuries and risk mitigation

ii) Clarification of issues related to viral shedding after leaving the hospital premises (and subsequent DEFRA submission).

iii) Further details on inactivation of the GMO (detailed in section 6) in particular of the use of validated disinfectant.

iv) Clarification on deep clean procedure

v) Development pf plan to mitigate risks of spillsafe either in pharmacy or the catheter laboratories and in the transport of the GM product between facilities.

The revised risk assessment, which was reviewed in conjunction with the Study Manual (detailing local study procedures and the management of the study on-site) has been approved by both Imperial College GMSC 309 and RB&HFT GMSC. Both confirmed this as a Level 1 study.
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Date at Which Additional Info Submitted: 02/03/2022
### Premises Addresses

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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Y

**Give brief details of the genetic modification safety committee**

Health and Safety Manager

10 years post-doctoral level experience in cloning and expression work with E.coli and yeast

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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- Bacteriology: Yes
- Parasitology: Transgenic Animals
- Transgenic Birds: Microbiology Research
- Transgenic Fish: Gene Therapy
- Virology: Transgenic Birds
Due to the relatively small volumes of liquid, waste will not be treated prior to disposal but will be sealed in the original sampling tubes or screw cap tubes to which material has been transferred during the testing processes. The tubes will be placed in clinical waste bin which will be irreversibly sealed before transport by a licensed contractor and incineration. If larger volumes are accumulated then the potentially infected liquid waste will be added to a large volume of disinfectant solution according to the suppliers recommended dosage rates of disinfectant e.g. Virkon 1% w/v solution for 30 mins minimum, then washed away to the public sewer. Accidental spillages will be dealt with by swabbing with disinfectant. Solid waste e.g. culture plates will be placed in a clinical waste bin as above or autoclaved prior to disposal as clinical waste by a licensed contractor.

<table>
<thead>
<tr>
<th>Mycology</th>
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<th>Transgenic Plants</th>
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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment

**Please enter comments of the GM safety committee on the risk assessment**

Commonly used lab strain of E.coli are recognised as non-colonising and disabled, and may be considered to be equivalent ACDP hazard group 1 because they have an established record of safety in the laboratory with no adverse effects on human, animal or plant health or the environment. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture.
### GM Centre Number: 3189

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**Comments**

**Date at Which Additional Info Submitted**

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## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

- Chair
- Biological Safety Officer
- Secretary
- Staff Representative(s)
- External Safety Advisor

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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Other (please specify): Tick if confidential: **No**

- Bacteriology: **Yes**
- Parasitology: **Yes**
- Transgenic Birds: **Yes**
- Microbiology Research: **Yes**
For activities involving GMMs, describe the waste management measures which will apply to the activity

E. coli liquid waste
1 tablet of HazTab per 400ml gives a 2,500ppm of chlorine suitable for killing E. coli with a contact time of at least 1 hour. Alternatively 1 hour contact with a freshly made solution of 2% Virkon diluted 50:50 with liquid waste to give a final w/v of 1% Virkon can also be used. The standard procedure with contact time at least 12 hours effectively kills E. coli 100%. We have not been able to retrieve live E. coli from this solution.

E. coli solid waste
These items (like bacterial agar plates, plasticware etc,) will be placed in autoclave bags which are filled three quarters and tied with a cable tie. Tied bags are placed in a dedicated bin situated in the laboratory and then autoclaved for 30 minutes at 121 degrees centigrade in the service room. Autoclaved waste will be yellow bagged and stored in a dedicated secure area before removal for incineration by contract waste services.

The degree of kill may be monitored by plating aliquots of treated samples on appropriate bacterial growth medium and incubating overnight under appropriate growth conditions for the relevant organism.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Spirogen GMSC was constituted and met on 26 April 2013 to consider the risk assessment. The GMSC classified the GM activity as Class 1.
### GM Centre Number: 3190

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#### Name

**UNIVERSITY OF CAMBRIDGE**

#### Name 2

**OCCUPATIONAL HEALTH & SAFETY SERVICE**

#### Campus Estate or Research Centre

**TENNIS COURT ROAD**

#### Road Name

**TENNIS COURT ROAD**

#### Town

**CAMBRIDGE**

#### District

**CAMBRIDGESHIRE**

#### County

**CB2 1PD**

#### Country

**ENGLAND**

#### Tel Number

**01223334031**

#### Fax Number

**01223334100**

#### E-mail

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#### HSE Division

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#### Comments

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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**Other(s)**

For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid waste inactivated by autoclaving in on-site autoclave followed by off-site incineration by approved contractor. Liquid waste inactivation by 1% Virkon solution and discharge to drains

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

No comment, routine practice
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee (GMSC) is a subpanel of the University of Gloucestershire Research Ethics Committee (REC). The GMSC consists of representatives from across the University, including each school, the Biological Safety officer and an external expert advisor on genetic modification.

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All waste (aside from disinfected fluids) will be autoclaved at 121 degrees C for 50 minutes - Kill rate effectively 100% Disinfection of fluids will be achieved through treatment with 1% Virkon for at least 30 min before disposal to drain. Manufacturer's information indicates efficacy of Virkon against bacteria including E.coli and Salmonella sp. at dilutions ranging from 1% to 0.125%.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Following initial review by the GMSC, it was highlighted that the risk assessment needed greater clarity in several areas. In particular, because two classes of GMM are produced in this work, the GMSC expressed concern at having a single overarching risk assessment and recommended that two separate risk assessments be formed to cover both GMM classes. All points have now been met and the GMSC has approved this assessment.

Project Ref 3192/20.1

Date Ackn’d

CU2 Project Title

Investigation of bacterial colonization and infection

Date Project Ceased

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes
Project Additional Information

Purposes of the contained use

The objective of this research is to study commensal and pathogenic bacterial interactions with eukaryotic cells, tissues and whole organisms. Our approach focuses primarily around the study of bacterial genes that are required for bacterial attachment, type three secretion systems and virulence. Type three secretion systems are required for attachment and injection of type three effector proteins into mammalian cells. Genetic modifications in bacteria will include loss-of-function mutation (deletion/insertion/point mutation) as well as introduction of plasmids encoding for fluorescent reporter proteins, wild type alleles (for functional restoration or recombinant protein production), or wild type alleles fused to fluorescent or enzymatic reporters (to generate transcriptional, translational reporters). All strains used will be a maximum of ACDP Cat. 2. Genetically manipulated strains of Escherichia coli (commensal and pathogenic strains, but excluding Shiga-like toxin producing strains), Salmonella typhimurium, Yersinia pseudotuberculosis, Shigella sonnei, Pseudomonas aeruginosa and Klebsellia pneumonia, will either be obtained from ATCC, from other research institutions or will be generated at the University of Gloucestershire by electroporation or conjugation. The project will also include genetic manipulation of Human cell lines, which will include transient transfection of fluorescent or enzymatic reporters (to generate transcriptional, translational and effector delivery reporters). To allow characterization of interaction with human cells, genetically manipulated cell lines (Hela, Caco-2, SW480, RKO) will either be obtained from ATCC, from other research institutions or will be generated at the University of Gloucestershire by transient transfection using electroporation or liposome mediated delivery. In brief, candidate genes in bacteria will be deleted by cloning the open reading frame into a pUC19 or pDM4 suicide vector (or similar), which will then be disrupted by the insertion of a Tetracycline or Gentamycin resistance marker from pSS262 or pSS87, respectively. To effectively introduce the plasmid into the bacterium, morIT will be inserted into the plasmid from pLS214, pLS217 or pSS125 (or similar) and the plasmid mobilised into the bacterium. Wild type alleles will be reintroduced back into mutant strains by cloning the ORF together with the minimal stabilisation fragment (mSF) and morIT cassettes into a plasmid. To generate reporter strains the promoter or ORF of the candidate genes will be cloned into a transcriptional (pSS223) or translational (pSS231) reporter plasmids (or similar) with Lacz, GFP or mCherry as a reporter. Where plasmids are not available, the Lacz reporter will be replaced with GFP or mCherry by standard cloning techniques. For generating human cell lines, fluorescent or enzymatic reporter proteins (CFP, YFP, GFP, mCherry, Luciferase) will be cloned into pDNA3.1 or similar.

Recipient or parental organism

E.coli used as a cloning vector for DNA manipulation, recombinant protein production and to aid for conjugation experiments (Routine laboratory strains DH5alpha, BL21, MM294) that would not survive outside the laboratory and non pathogenic Pathogenic E.coli (Shiga toxin negative or deletion strains) are Gram negative pathogens and can cause GI tract infection. Infections with EPEC or Shiga-toxin negative EHEC cause cramping, vomiting, and diarrhea but are self-limiting in otherwise healthy patients. Salmonella typhimurium is a Gram negative pathogen and can cause GI tract infection or wound infections. Infections are self-limiting in otherwise healthy patients. Yersinia pseudotuberculosis is a Gram negative pathogen and can cause Far East scarlet-like fever in the human host. Symptoms include fever, abdominal pain and, rarely, diarrhoea. Disease can last 1-3 weeks but is self-limiting in otherwise healthy patients. Shigella sonnei is a Gram negative pathogen and can cause GI tract infection. Symptoms include cramping, nausea, and watery or bloody diarrhoea. Infections are self-limiting in otherwise healthy patients but supportive treatment with beta-lactams or quinolones is common and effective. Pseudomonas aeruginosa is a Gram negative, opportunistic, aerobic coccobacillus. P. aeruginosa is commonly found as a skin
commensal, and in the environment on moist surfaces. P. aeruginosa causes infections in burns patients and can cause fatal lung infections in Cystic Fibrosis individuals. Klebsiella pneumoniae is a Gram negative organism commonly isolated from water, soil, the gastrointestinal tract, and skin, and causes nosocomial infections including UTI, pneumonia, sepsis and tissue infections, mainly in immune compromised patients. Human Cell Lines. Are commercially available and are not derived from workers in the department. Human Cell lines will be unable to colonise workers as any inoculation event would provoke a strong immune response by the individual against the cell line due to differences in MHC class I and II types.

Host/vector system

Host species: E.coli (DH5α, Class I) will be used for cloning and propagation of plasmids, E.coli (BL21, Class I) will be used for the production of recombinant protein, E. coli MM294 (Class I) will be used to aid conjugation; Escherichia coli; Only Shiga toxin negative or stx deletion strains will be used (Class II) Salmonella typhimurium (Class II) Yersinia pseudotuberculosis (Class II) Shigella sonnei (Class II) Pseudomonas aeruginosa (Class II) Klebsiella pneumoniae (Class II) Human Cell Lines: To include inclusions such as: Hela, Caco-2, SW480, RKO, HT29, MCF7, PLB985, HLO (Class I) Vector Systems mobilizable and broad host-range vectors, such as: pLS214 contains morT as a HindIII fragment in pUC1918 from E.coli plasmid R4 pLS217 contains morT as an EcoRI fragment in pUC1918 from E.coli plasmid R4 pPS124 contains mSf fragment in pUC1918 from pBR322 E.coli. P. aeruginosa shuttle vector pSS223 conjugative and suicidal lacZ transcriptional fusion plasmid containing morT from E.coli plasmid R4 pPS231 conjugative and suicidal lacZ translational fusion plasmid containing morT from E.coli plasmid R4 pPS262 Tcr cassette in pUC1918 from pBR322 pSS213 contains T7 (A1/04/03) for tight regulation of gene expression pUC19 contains origin of replication, lacZ, ApR and multiple cloning site pm4 contains origin of replication, chiR and multiple cloning site Non mobilisable vectors, to include for example: pGEX4T3, the pET and pCAL vectors, used to express protein, either from T7 promoters or from the gene's native promoter, either in its native form or as part of a his-tagged, MBP or similar fusion to an innocuous protein. Mammalian expression vectors, pCDNA3.1, pSFFV or TCF reporter plasmid or similar. Transient, constitutive expression from CMV, SV40 or native promoter in human cell lines. GentR, NeoR and AmpR. pUC ori for replication in E.coli.

Origin & function

Sequences of candidate genes will be PCR amplified from bacterial genomic DNA isolated from either Escherichia coli (commensal and pathogenic strains, but excluding Shiga-like toxin producing strain), Salmonella typhimurium, Yersinia pseudotuberculosis, Shigella sonnei, Klebsiella pneumoniae, Pseudomonas aeruginosa and cloned into the relevant vector. All donor species are ACDP Cat. 2, with the exception of commensal E. coli, which is ACDP 1. All ACDP II donor species have the potential to cause disease, and are transmitted via the faecal-oral route or via direct contact with infected people, animals or equipment and subsequent ingestion or exposure of open wounds to an infectious dose of the bacterium. Pseudomonas aeruginosa can also be transmitted via aerosols. Sequences will include open reading frames of candidate genes, or promoter regions of candidate genes for expression of reporter genes. Sequences of particular interest include those of virulence factors (e.g., adhesins, effectors, structural components of secretion systems, and secreted lytic enzymes). Reporter genes (i.e., GFP, mCherry, YFP, CFP, Luciferase, lacZ, Tev Protease) will be PCR amplified from E. coli plasmids and cloned into appropriate host plasmids for conjugation, transformation or transfection into the appropriate host species. Antibiotic resistance markers (e.g., ampR, chiR, tetR, strepR, kanR) will be PCR amplified from standard E. coli plasmids and inserted into the open reading frames of candidate genes in pUC19 or pDM4. Mammalian gene ORFs and promoter regions will be PCR amplified either from mammalian cell cultures or from commercially available E.coli plasmids and cloned into appropriate host plasmids for transient transfection into the appropriate cell line.

Evaluation of foreseeable effects

Having considered the nature of the modified organisms, the nature of the transgenes added and the nature of culturing and testing the organisms, we cannot identify any enhanced risk to human health above that normally associated with the culture of these ACDP II organisms. Our approach focuses primarily around deleting genes that are required for type three secretion systems and virulence. Type three secretion systems are required for attachment and injection of type three effector proteins into mammalian cells. Therefore, deletion of the effector proteins, or the secretion machinery itself, will inhibit this mechanism of killing mammalian cells and hence reduce the pathogenicity of the organism. Similarly, deletion of adhesins will decrease the organism's propensity to colonize and cause disease in the human host. Other methods will include tagging functional proteins with fluorescent or enzymatic reporters to enable protein localisation studies, and the reintroduction of genes to complement mutant phenotypes. Where possible and when using ACDP II organisms as hosts, genes will be expressed from native promoters to reduce the chances of over- and ectopic expression. It is possible that in some cases, the nature of the experiment (e.g., expression of constructs from a high copy number plasmid or deletion of repressor genes) may give rise to GMOs with enhanced virulence potential relative to the original strain. However, it is highly unlikely that this enhancement would confer fundamentally new abilities on the strain (such as new host range) or increase virulence to a level that would change the ACDP classification of the organism. Where overexpression is required (to enable chemical/biochemical characterisation of the protein), expression will be performed in a disabled laboratory strain of E.coli (e.g. BL-21 E.coli).
overexpression of bacterial effector proteins, it is worth noting that the catalytic subunit of any toxin cannot exert any effect unless delivered in soluble active from to the appropriate compartment of the appropriate cells within the human body. This cannot happen here as the necessary delivery mechanisms (e.g., a secretion system for getting out of the cell, a binding domain for attaching to cells and mediating entry) will be absent from the cloning host (E. coli BL21). We will never express the binding and toxic domains in the same cell. The selection genes are widely used antibiotic resistance markers. Although our GMO bacteria will be resistant to these antibiotics, we anticipate that the additional risks to human or animal health from these selectable markers will be minimal, as the GMO strains are likely to be attenuated in virulence and have reduced fitness compared to clinical isolates. Additionally, wherever possible, we will use resistance markers that do not give rise to resistance against antibiotics used to commonly treat infections arising from the host species. Reporter genes such as GFP and Lacz, which are used routinely, have not been reported to increase risk to human health.

With regards to the genetic manipulation of human cell lines. These are commercially available and are not derived from workers in the department. The cell will be unable to colonise workers as they do not share the same MHC class I and class II loci and will therefore provoke a host-versus-graft immune response. In addition, the likelihood of colonisation of an operator is extremely low as no sharps (i.e., needles) will be used during handling of the cell lines. The risk of spread to the environment is low as tissue culture cells are unable to survive outside the laboratory environment. The introduction of florescence or enzymatic reporter genes with constructs such as pCDN3.1 is common and will not alter any of these characteristics.

Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

After use, solid media and plastic/glassware that has come into contact with organisms will be autoclaved (50 mins, 121 degrees) and disposed of through the University’s disposal route. Effectiveness of waste autoclave is regularly monitored and all runs are validated with internal temperature monitoring and reporter tape. Liquid waste containing organisms will be inactivated with Virkon (exposed to a final concentration of 1% Virkon for a minimum of 30 minutes). This will result in 100% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Following initial review by the GMSC, it was highlighted that the risk assessment needed greater clarity in several areas. In particular, because two classes of GMM are produced in this work, the GMSC expressed concern at having a single overarching risk assessment and recommended that two separate risk assessments be formed to cover both GMM classes. All points have now been met and the GMSC has approved this assessment.

Project Containment

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02/03/2022 Page 13315 of 15326
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**Name**

IMANOVA LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

IMPERIAL COLLEGE LONDON

**Building**

HAMMERSMITH HOSPITAL

**Road Name**

DU CANE ROAD

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

W12 0NN

**Country**

ENGLAND

**Tel Number**

02080086000

**Fax Number**

02080086491

**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Under the terms of the agreement between Imperial College London and Imanova the ICL GM safety committee for the Hammersmith Hospital (ICL) site GM31, will review and make comments on risk assessments and related documents received from Imanova. The ICL GMSC will not approve or, in any way, condone the work. The GMSC accepts no responsibility for ensuring that the work is conducted in accordance with the risk assessment or in compliance with the relevant regulations. The GMSC (nor Imperial College Safety Department) accept no responsibility in ensuring that any of the committee's recommendations are complied with. The responsibility for all these matters rest entirely with Imanova.

The Safety Department will only forward the GMSC comments once they have been collated. The ICL Safety Department will not issue any approval forms, no will it administer any notifications required to the HSE. The responsibility for all these matters rest entirely with Imanova.

The committee has representation from Imperial College Academics, Campus Safety Managers, the Hammersmith NHS Trust Safety Manager. The College Biological Safety Officer, post graduate students, and Technical staff. There is currently no formal Union representative but they have been approached.

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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A

Tick to confirm that you are attaching a summary of the risk assessment

Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment


Please enter comments of the GM safety committee on the risk assessment


02/03/2022
Reviewer 1
Section 1.1 Typo subsequent and clinical. Please briefly describe which aspects of the work occur in ICL premises and in Imanova.
Section 2.2 Please describe briefly how the Ldtr−/− mouse line created?
Section 7.2 Is the Imanova lab a Home Office designated lab? How is escape prevented in the Imanova facility?

Reviewer
The form looks fine to me apart from 3.3 where they should say that the mice would be incapable of surviving in the wild since they are unused to external pathogens outside the CBS facility.

Reviewer 3
Section 6.3: Please add information relating to injection of PET radioligands into animals for imaging experiments.
Section 9.1: Please confirm decay storage of carcasses injected with PET radioligands before autoclaving. Please confirm what solid waste will be disposed of via the drain. Please describe disposal procedure between autoclaving and incineration.
Section 10.1: Use of holdall bag not required for transport within a laboratory. Please confirm presence of rodent barriers on the lab entrance.

Reviewer 4.
Section 9.1: This section refers to chemical inactivation of waste; although helpful to describe treatment of residues on instruments (this reference can be left in), there is no need to indicate that any solid waste is treated chemically to decontaminate, so the section on chemical treatment of solid waste can be left blank.

Reviewer 5
The main issue I believe is the possible use of radiation (which need to be confirmed). If radiation is used then consideration for its use included in the assessment e.g. listed under section 6.3 (other hazards) and in section 9.2 (autoclaving waste).
Also, section 9.1 under 'solid waste' I am not sure if they need to mention '30 min autoclave'. This is considered under section 9.2
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Date at Which Additional Info Submitted

02/03/2022
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<td>CM7 2YW</td>
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### Premises Conditions

- Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities
  - Y

- Give brief details of the genetic modification safety committee
The Catapult GMSC will meet as required to discuss and review any new GM projects or revisions to existing GM projects; minutes will be kept.

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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td><strong>Other (please specify)</strong></td>
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- **Bacteriology**: Yes
- **Parasitology**: Yes
- **Transgenic**: Yes
- **Birds**: Yes
- **Microbiology Research**: Yes
- **Virology**: Yes
- **Transgenic Animals**: Yes
- **Transgenic Fish**: Yes
- **Gene Therapy**: Yes
- **Mycology**: Yes
- **Transgenic Invertebrates**: Yes
- **Transgenic Plants**: Yes
- **Other (please specify below)**: Yes
- **Other(s)**: Assay and process development for human cell therapy product

For activities involving GMMs, describe the waste management measures which will apply to the activity.
Liquid waste is treated with freshly made Distel (formerly sold under the name of Trigene Advance) (tristel) at q 1:10 dilution for >12 hours and then sink disposal.

Solid waste is double-bagged in yellow clinical waste bags, placed in a shared large yellow bin and removed by King's College waste-handling services for off-site incineration.

Distel is guaranteed to 100% kill by the manufacturer for a wide range of viruses, bacteria and mycobacteria. Distel has been tested in accordance with BS EN 14347, 14348, 13727, 13624, 13623, 1276, 1675 protocols.

The active constituent of Distel are approved under the European Biocidal Products Directive (ref 98/8/EC) and offer REACH compliance. Distel is manufactured in the United Kingdom to the highest standards of BS EN ISO 9001:2008 and BS EN ISO 13485:2003, for the manufacturer of disinfectant and sterilising solutions for medical, dental and veterinary use.

Spillages of GMMs will be inactivated using DISTEL at a 1:10 dilution for 10 min.

The project titled 'Assay and Process development for CTX0E03 cortex cell therapy product' has been reviewed by the Cell Therapy Catapult GMSC.

The GMSC consider this to be a class 1 because:

1. No virus or other GMO will be constructed.
2. Though the inserted gene is a potential oncogene, the risk to human health is minimised to the lowest level as no sharps will be used.
3. All waste will be handled correctly; there is no risk to the environment.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 3195

<table>
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<tr>
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Name

ISOMERASE THERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre

BUILDING 60

Road Name

CHESTERFORD RESEARCH PARK

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB10 1XL

Country

ENGLAND

Tel Number

01223 911856

Fax Number

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E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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<th>Building</th>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

At least two members, at least one with at least 5 years of experience with bacterial genetic modification. Meetings will occur at least once every quarter. Any new projects involving genetic modification will need prior approval by the GMSC.

<table>
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<td>Other (please specify)</td>
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02/03/2022
All liquid and solid waste (maximum total volume 20 litres) will be deactivated by autoclaving at 125°C for 20 minutes. Deactivation will be monitored by using 3M comply indicator tape for steam. In addition, small amounts of liquid waste may be activated using RBS (final concentration of 5% v/v). There is therefore no possibility that bacteria will escape from the laboratory.

Where possible the use of sharps is avoided and plastic pipettes are used. However, any sharps used are always placed in a Cin-Bin and disposed of through licensed contractors according to the Company's Waste Disposal Procedure.

A kill curve disinfection test versus time is performed on parental organisms.

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

The GMSC has discussed the risk assessments and agree that the hazards and risks have been addressed by the use of appropriate containment.

---

**Project Ref** 3195/20.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<th>CultureVolumeClass3-4</th>
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<td>05/08/2020</td>
<td>Generation of new genetically modified microbial strains to produce novel natural products, analysis of strain and isolation of compounds of strains and the storage and maintenance of microbial cultures and vectors</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Non-GMM</td>
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Withdrawn: N

Tick if notifying a connected programme of work: N

Project notified under transitional arrangements: N

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Generation of new genetically modified microbial strains to produce novel natural products for research

Recipient or parental organism
Recipient strains are ACDP HG 1 or 2 bacterial or fungal strains.

Host/vector system
Host organisms are ACDP HG 1 or 2 bacterial or fungal strains. Vector systems are based on E.coli and are defective in one or more transfer functions and can only be mobilised by other elements which supply the missing transfer functions

Origin & function
Genetic material is derived from ACDP HG 1 or 2 bacterial or fungal strains, usually from genes coding for natural products of enzymes of medical or commercial benefit

Evaluation of foreseeable effects
Bacterial and fungal strains used are typically naturally occurring bacteria and fungus, frequently isolated from soil and are essentially wild-type. The bacteria are not normally aggressive colonisers and therefore pose minimal risk to humans or the environment

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid and solid waste (maximum total volume 20 litres) will be deactivated by autoclaving at 123°C for 20 minutes. Deactivation will be monitored by using 3M comply indicator tape for steam. In addition, small amounts of liquid waste may be deactivated using Chemgene (1% solution) or sodium hydroxide. There is therefore no possibility that bacteria will escape from the laboratory.

Where possible the use of sharps is avoided and plastic pipettes are used. However, any sharps used are always placed in a Cin-Bin and disposed of through licensed contractors.
Please enter comments on the GM safety committee on the risk assessment
This work has been discussed by and approved by the isomerase GM safety committee

**Project Containment**

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

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**Name**

NATIONAL WAITING TIMES CENTRE BOARD

**Name 2**

**Department**

**Campus Estate or Research Centre**

GOLDEN JUBILEE NATIONAL HOSPITAL

**Road Name**

AGAMEMNON STREET

**Town**

CLYDEBANK

**County**

**Postcode**

G81 4DY

**Country**

SCOTLAND

**Tel Number**

0141 951 5000

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The National Waiting Time Centre Board has established a Gene Therapy Safety Committee. The remit of the committee is to:

Review and process risk assessments and Standard Operating Procedures which result from the Board's participation in Gene Therapy trial. This covers all clinical research studies carried out under the Genetically Modified Organisms (contained use) Regulations enforced by HSE and the Genetically Modified Organism (deliberate release) regulations enforced by the Scottish Government's Rural and Environment Directorate GM Policy Unit.

The committee is co-chaired by two consultant cardiologists and includes the Board's Heads of Facilities, Infection Control and Clinical Governance. Also included are the Board's health and safety lead, clinical trials pharmacist, research manager, three external advisors (including scientific expertise) and senior research nurses.

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</table>
Pharmacy Department: The GT product (IMP) will arrive by courier to the GJNH Pharmacy in a vial on dry ice. It will be transported in sealed plastic bags in a designated sealed container to the storage location (2-8°C refrigerator) in the GJNH Pharmacy department. The IMP will be held in the Clinical Trial fridge in the Pharmacy Department, GJNH. The Pharmacy Department is alarmed and locked with entry by swipe ID card. When required, each vial will be logged out and the number recorded on the fridge door (and in the Pharmacy File). Each vial is delivered in single units from the GJNH Pharmacy to the Cardiac Catheterisation laboratories, GJNH for assembly into a single syringe. The remaining contents of each vial will be disposed of by autoclaving.

Aerosols and splashes and spillages may be generated during the transfer of material from the vial to the syringe. All personnel will take normal precautions - see Appendix 11 of the National Infection Control Manual - Part 1 Standard Infection Control Precautions: Management of blood and body fluid spillages. Note that, in common with all NHS Boards in Scotland, the disinfectant of choice is Actichlor which is effective against bacteria, spores, yeasts, moulds and viruses including Hepatitis B and HIV.

Cardiac Catheterisation Lab: The GJNH research team will prepare the vector solution in the Cardiac Catheterisation Labs. Staff will wear the PPE normally used for...
Cath Lab procedures. At the end of the session all PPE items will be placed into Clinical Waste and autoclaved prior to disposal as per GJNH procedures for disposal of all Clinicac Waste. All operators will wear gloves as per Appendix 5 of the National Infection Control Manual - Part 1 Standard Infection Control Precautions: Glove use and selection.

Transport: Route 1: Pharmacy to Cardiac Catheterisation labs. This will apply to the vial of GM material as it is received by the pharmacy department. No manipulations of the vial will take place in the pharmacy department. Route 2: Within the Cardiac Catheterisation labs. This will apply to the transfer of GM material from the transport vial to the syringe, the transport of the syringe to patient and the disposal of all GT contaminated material, including PPE, Route 3: From the Cardiac Catheterisation labs to labs (site of autoclave). This will describe the transport of waste material - sharps bins and waste bags - from the Cardiac Catheterisation labs to the autoclave. One SOP will cover all of these routes and will list the responsible personnel, the routes taken and the containment measures in place.

Waste: vials and syringes containing residual GM material, delivery devices, PPE, material used to clean spillages etc - will be stored in a locked room prior to autoclaving using a validated mixed cycle. Waste will then be disposed of through the usual hospital disposal system.

Autoclave: The maintenance/testing regime for the laboratory autoclave (labs department, Level 2, GJNH) is as follows: daily checks (user), weekly tests (CSPD Engineers), quarterly tests (CSPD Engineers), and annual validation tests (Health Facilities Scotland engineers). Maintenance is done on a reactive basis throughout the year based on weekly and quarterly test results, with an annual full service. Twice yearly pressure vessel insurance surveys are carried out using written scheme (CPSD Engineers/Sun Alliance Inspector). Daily and weekly tests are checks to ensure that the machine is performing correctly and that the independent monitoring agrees within the limits set in SHTM2010 with the control devices on the autoclave. Quarterly and annual validation test are carried out with calibrated thermocouples and pressure transducers connected in the same places as control ad independent monitoring devices as well as spread through the various load types to ensure that as well as the autoclave itself, all parts of loads to be processed attain sterilisation temperatures and pressures for at least the stated times required in SHTM2010. If there are any anomalies found calibration can be changed at this time.

The Gene Therapy Safety Committee met on the 29th May 2013 to review the risk assessment for the CUPID 2 trial. Each section was considered and members contributed suggestions and changes as required. The document was redrafted and circulated to the group on the 30th May 2013. One of the Scientific Advisors update a couple of sections - no further updates were requested.

The Risk Assessment was sent to the Gene Therapy Safety Committee Chair on the 5th June for final approval. Note that the GJNH Head of Infection Control has also signed the Risk Assessment.

Please enter comments of the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

02/03/2022
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<thead>
<tr>
<th>Name</th>
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## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

**Y**

Give brief details of the genetic modification safety committee

- Chairman + molecular biologist (at senior level)
- Secretary + biological safety officer
- Staff representative(s)
- External safety advisor

Meetings will be held annually to review the project outline and risk assessments, or sooner if significant changes to the project are proposed

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

### Liquid E. coli waste

1 x 2.5g preSept tablet (containing sodium dichloroisocyanurate, NaDCC) in 560 ml water gives a solution of 2500 ppm available chlorine, suitable for killing E. coli with a contact time of at least 1 hour (99.9% kill with a contact time of 12 hours). All solutions will be freshly prepared to ensure efficacy. Once inactivated, liquid waste will be diluted by flushing down the drain with plenty of water in accordance with MSDS disposal of small quantities of NaDCC waste.

### Solid E. coli waste

Solid waste including agar plates and plastic ware will be collected in autoclave waste bags, closed with a tie, and destroyed by autoclaving at 121° C for 15 minutes in the service room. A service contract is also in place with PHS Wastetech who are authorised to carry and dispose of GM waste.

Efficacy of inactivation methods can be tested by plating samples of the inactivated material onto appropriate bacterial growth media and culturing under suitable conditions. No growth would indicate complete inactivation had been successful.

### Surface spills

Surface spills will be contained with paper towel soaked with 2% Trigene. The area will be thoroughly cleaned with 2% trigene, with paper towels discarded into clinical waste.

---

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The BioMot genetic modification safety committee (GMSC) met on the 30th May 2013 to discuss the project application and risk assessment. Terms of reference for the committee were agreed.

The use of GMOs in the project was agreed to be an essential part of the proposed work. The project was deemed to be a Class 1 category for GM work, with overall low or negligible risk to both human health and the environment. The risk assessment was deemed suitable for the project, with minor amendments incorporated and circulated to committee members following discussion at the meeting.
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Name

ORIGIN SCIENCES LTD

Name 2

Department

Campus Estate or Research Centre

GRANTA PARK

Road Name

1 RIVERSIDE

District

GREAT ABINGDON

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB21 6AD

Country

ENGLAND

Tel Number 01223 750490

Fax Number N/A

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

![Yes](Yes.png)

Give brief details of the genetic modification safety committee

The GM Safety committee is formed of the Scientific Director and Special Projects Lead. All GM risk assessments will be reviewed and approved by the committee before any work takes place to ensure HSE guideline are met. The role of the committee is to ensure that HSE guidelines regarding the use of GMMs is adhered to and will meet monthly.

<table>
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Tick if confidential  

![Yes](Yes.png)

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
All GMMs will be inactivated by immersion in 1% Virkon solution for 30 minutes before disposal. Any material that comes into contact with GMM’s will be disposed of in Biohazard labelled bags or immersion in 1% Virkon solution for >30 minutes and collected for autoclave and incineration by initial Medical Services. Areas where work is conducted with GMMs will be designated according and kept clean before and after work with a 70% ethanol solution.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
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<td>Other(s)</td>
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</table>

Please enter comments of the GM safety committee on the risk assessment

This risk assessment has been reviewed by the committee and it is in agreement that the procedures outlined pose negligible or no risk to human and environmental health. As such, a containment level 1 designation for this work is appropriate.
### GM Centre Number: 3199

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**Name**

| SITA UK LTD |

**Campus Estate or Research Centre**

| WREXHAM HEALTHCARE |

**Road Name**

| MARLBOROUGH ROAD |

**District**

| WREXHAM INDUSTRIAL ESTATE |

**Town**

| WREXHAM |

**County**

| LL13 9RJ |

**Country**

| ENGLAND |

**Tel Number**

| 01978 729930 |

**Fax Number**

| 01978 664241 |

**Date at Which Additional Info Submitted**

| 02/03/2022 |

**Date**

| 23/08/2013 |
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Genetic Modification Committee will compromise of the Senior Site Manager, Plant Manager and the sites Health & Safety Representative

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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<td>Tick if confidential</td>
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Bacteriology Parasitology Transgenic Birds Microbiology Research
The notified premises is a dedicated clinical waste incinerator. It is a Part A process regulated by the Environment Agency and have authorisations in place via a permit issued under the Waste Incineration Directive, permit number is WP3836ZF.

The plant is of a Rotary Kiln design and has three main components: A primary combustion chamber, a secondary combustion chamber and a flue gas cooling and cleaning system. The waste is mechanically loaded into the primary combustion chamber where a minimum temperature of 1000 degrees centigrade is maintained for a minimum of two seconds in an oxygen rich atmosphere to completely oxidise the combustable gases. The flue gas is then cooled to around 130 degrees centigrade by means of a steam boiler before being treated with powdered activated carbon and hydrated lime in a baghouse to remove any remaining impurities.

The plant has a designated storage area with separate facilities for the storage of special or hazardous waste. There is a well developed bin bar coding system in place paperwork and written procedures for the handling of the different types of waste and it is intended to extend these systems to include GMO waste. The plant and site is operated around the clock by teams of Shift Leaders and Plant Operators, who are under the management of a Plant Manager and a Senior Site Manager.

To ensure compliance with the CDG regulations the waste will be delivered into site in UN approved packaging such as hermatically sealed containers. These containers are specifically made for the containment of higher risk materials such as human tissue and body parts, microbiological cultures and out of date medicines. They come in a variety of sizes ranging from 30 Litre containers to 120 Litre containers. They are fitted with a lid which when sealed, forms a very strong hermetic seal when pushed into place. The whole container is designed to be incinerated with no manual handling the contents at all.

The site specialises in the collection, transport and disposal of clinical waste in UN Approved 770 Litre containers and it is envisaged that the containers described above will be placed inside these. 770 Litre containers for mechanical loading into the incinerator. In conjunction with the smaller UN Approved containers, this will provide a UN Approved combination package ideal for the transport, storage and disposal of infectious waste.

Each bin has a unique bar code attached which is used to track bin movements from waste producer to final disposal on a computerised system which satisfies the Duty of Care requirements. There are spillage kits containing all the equipment needed to clean up spillage of waste materials and staff are well trained in the spillage procedures. Fortunately because of the mechanical handing systems employed, spillages are extremely rare and the kits and training are more of precautionary measure. In addition, the site has a Safety Representative elected by the workforce who has agreed to be a member of the GMO safety Committee.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The Risk Assessment has been carried out by the Senior Site Manager and Plant Manager and has been reviewed by the Health & Safety Representative.
# Project Additional Information

**Purposes of the contained use**

Disposal of GMO Class 1 and Class 2 waste via High Temperature Incineration

**Recipient or parental organism**

The characteristics and evaluation of foreseeable effects will be determined by the individual waste producer

**Host/vector system**

Determined by the individual waste producers

**Origin & function**

Determined by the individual waste producers

**Evaluation of foreseeable effects**

Determined by the individual waste producers

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Waste must be inactivated, or the waste producer must be able to demonstrate that the waste will pose no threat to human health or the environment.

For only GMOs - application for any derogation from full containment for the Class of activity.  (Measures & Justification)

The GMO waste will be packaged in UN Approved sealed packages and mechanically loaded into the incinerator via lockable 770 Litre containers which are also UN Approved. The waste will be incinerated at a minimum temperature of 1000 degrees centigrade and this will kill 100% of the GMO’s. The final product is a sterile ash which then goes onto landfill.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The GMO waste will be packaged in UN Approved sealed packages and mechanically loaded into the incinerator via lockable 770 Litre containers which are also UN Approved. The waste will be incinerated at a minimum temperature of 1000 degrees centigrade and this will kill 100% of the GMO’s. The final product is a sterile ash which then goes onto landfill.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Risk Assessment has been carried out by the Senior Site Manager and Plant Manager and has been reviewed by the Health & Safety Representative

**Project Containment**

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**Name**

HUTCHISON/MRC RESEARCH CENTRE, UNIVERSITY OF CAMBRIDGE

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**Campus Estate or Research Centre**

BOX 197 CAMBRIGE BIOMEDICAL CAMPUS

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The GM Safety committee was set up in 2005 when the centre formed part of the MRC Centre Cambridge (GM921). The centre is transferring employment to the University of Cambridge from the MRC; hence requires a new premises centre number.

The current committee, which is intended to remain, consists of a Chair (senior academic), the Biological Safety Officer, two other senior academics and the Lab Manager, health and safety. The committee meets at least annually and communicates face to face and by email at other times; the committee report to the Health and Safety committee and the centre's director.

<table>
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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

**Liquid Waste:** (Class 1)
Inactivation will either be autoclaving (considered to give 100% kill) or by chemical means; Virkon gives 99.999% kill in 10 mins and is the default disinfectant, used unless a risk assessment indicates otherwise. Deactivated liquid will then be poured down the sink with at least equal volumes of water.

**Solid Waste:** (Class 1)
All bacterial plates are autoclaved (considered to give 100% kill) on site by support staff. They are finally disposed by incineration offsite. We use a reputable disposal company for all our laboratory waste.

Other solid waste is collected in good quality plastic sacks which line rigid plastic bins. When two thirds full they are sealed and taken to internal wheelie bins. At least once per day the wheelie bins are collected by support staff; the sealed sacks are then put into another sack (obtained from our reputable disposal company) these large bins are kept in a secure yard. The large bins are collected by the disposal company and a fresh bin is replaced, therefore all sacks are secure at all times.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The significant GM work in this project was the construction elsewhere of the cell lines to be used and their screening for virus. The work currently planned to be done at the Hutchison is routine transfection of mammalian cells using plasmids that do not lead to expression in the host bacteria.
**Project Additional Information**

**Purposes of the contained use**

To study the pathways regulating differentiation of human cells, in particular how stem cell fate is regulated.

**Recipient or parental organism**

Human and mouse tissue culture cell lines.

**Host/vector system**

Laboratory strains of E. coli K12.

Phoenix-ecotropic and amphotropic retroviral producer cells.

**Origin & function**

Inserts used are all derived from mouse, human and Xenopus cDNA libraries and comprise wild type and mutated forms of Notch ligands, Notch receptors, suppressor of hairless homologues, mastermind homologues, and Notch target genes including the hairy/enhancer of split family of transcription factors. In addition mutant Xenopus beta catenin and mutants of MAP kinase will be expressed to determine how the Notch pathway interacts the wnt and Map kinase signalling pathways. Cell cycle inhibitory proteins such as p21Cip1 and Geminin will also be used.

In addition reporter constructs derived from the pGL3 series of vectors will be used; these consist of Notch and wnt responsive promoter elements, driving expression of the firefly and renilla luciferase genes.

In some experiments green fluorescent protein will be expressed as a reporter.

The intended use is to study how these genetic elements control the cell development.

**Evaluation of foreseeable effects**

The main hazard arises directly from the recombinant retroviruses produced from the packaging cells. These yield both ecotropic and amphotropic retrovirions, depending upon the envelope protein supplied by the cell.
The packaging cell lines used to generate the retrovirus have either ecotropic or amphotropic envelope proteins, and the retrovirus therefore can either infect only mouse cells, or have a wider host range including human cells. Their infectivity is unstable and infection is only obtained by co-cultivation of the packaging cell line with their recipient cells. The retrovirus is self-inactivating and there is thus no possibility of further transfer. With the ecotropic packaging cell line, there is therefore negligible risk to the worker or the environment.

With the amphotropic packaging cell line, the risk of infection of a worker during the packaging/infection step is small, but still not negligible. Although the inserted genes are not known to be oncogenic, they have this potential and it is considered that this part of the work is Class 2. In view of the, at worst, low-oncogenic nature of the inserted genes and also the self-inactivating nature of the retroviruses, there likelihood of any hazardous replication competent virus being generated is negligible and is no need to test for such RCV.

The single non-negligible hazard arises from the potentially oncogenic or cytotoxic nature of some of the inserts. This risk can be controlled if DNA grown up from such clones is handled appropriately; ie gloves should be worn, sharps avoided and allwastes be rendered harmless before disposal.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 mins), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

**Project Containment**

This work involved the cloning of some potentially oncogenic genes into disabled retrovirus vectors, with the use of packaging cell lines which have an amphotropic envelope protein.

From the ACGM Compendium of Guidance, Part 2B, Annexe III, particularly para. 30, this requires Class 2 (as already concluded in the risk assessment).

The Committee noted that the risk of the potentially oncogenic DNA had already been considered in the assessment.
Project Additional Information

**Purposes of the contained use**

To functionally silence genes whose inactivation in cancers gives rise to genetic instability, using a recently developed stable RNA interference approach, to ascertain the molecular basis for the abnormal division of cancer cells and their increased genetic instability.

**Recipient or parental organism**

Tissue culture cells of human origin.

**Host/vector system**

Laboratory strains of E. coli K12 derivatives; tissue culture cells of human origin. pUC-based plasmid vectors with RNA polymerase III promoter (eg pSUPER);
Phoenix-amphotropic retroviral producer cells.

Origin & function

The plan is to use vectors based on pSUPER in which an RNA polymerase III promoter drives expression of a short (~25 mer) interfering RNA from a synthetic DNA insert. Details of the categories of interfering RNAs to be inserted are as follows:

A) RNAs which target DNA double-strand break repair pathways: The proteins encoded by BRCA1, BRCA2, RAD51 and its paralogs, Nbs1, ATM, ATR, Ku70, Ku80 and DNA-PK are nuclear molecules which participate in the sensing and repair of DNA double-strand breaks. In mice, homozygosity for null mutations in these genes leads to cell death, embryonal lethality and a DNA repair defect accompanied by genetic instability.

B) RNA which target cell cycle regulators: The proteins encoded by the mammalian cyclin/CDK genes, the mitotic kinases Pik1, nek1, Aurora-A/B/C, Bub1/BubR1 and the chromatid cohesion molecules Scc1, SMC1/3 and Eco1, are required for cell cycle progression and mitosis.

C) RNAs which target regulators of the G1, S and G2/M checkpoints: These inserts will include proteins encoded by checkpoint regulators such as p53, chk1/chk2 and chfr, or proteins involved in the downstream enforcement of these checkpoints (including cdc14, ranGAP, cyclin G, cdc25 or PP1).

Evaluation of foreseeable effects

The inserts encode short interfering RNAs which are only active within the cell, and cannot encode an exogenously expressed protein. Expression of these RNAs will not enhance the pathogenicity of the host cells. The effect of expression of the insert RNAs will be to functionally silence the target genes. In most cases, functional silencing is known to impair cellular function resulting in defective cell cycle progression or in genetic instability. In no instance is functional silencing of the target genes known to work directly to transform primary cell cultures.

A non-negligible hazard arises from the potentially oncogenic or cytotoxic nature of the cellular effects of some of the interfering RNA inserts. It is considered that DNA from such clones can be appropriately handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras 8-10, in which the potential hazard is specifically considered.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing. Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
This work involves the cloning of short DNA inserts under a eukaryotic promoter, in order to generate ~25-mer interfering RNA. The genes targeted in this work are those for sensing and repair of DNA double-stranded breaks, cell cycle progress and mitosis, and checkpoint regulators or downstream enforcement proteins. The cloned DNA is therefore potentially cytotoxic or oncogenic.

Initial cloning is into non-mobilisable vectors grown in laboratory E. coli K12 strains. The vectors are pUC-based and are disabled retroviral vectors, which require a helper t.c. line to produce virus, which in turn is only capable of a single infectious cycle. This stage is therefore Class 1.

Subsequently the retroviral vectors will be transfected into mouse emphotropic cell lines, with the packaging construct in more than one component, to produce the disabled retrovirus with an amphotropic envelope. Following consideration of the advice in the ACGM Compendium of Guidance, Part 2B, Annex III, paras 21-30, it was agreed that Class 2 is appropriate for this work. If cell lines are being maintained for a long time, consideration should be given to testing them for the presence of replication competent virus.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
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Tick if notifying a connected programme of work N

Historical Significant Changes

- Project transferred from GM921 on 03/09/2013
# Project Additional Information

## Purposes of the contained use

The project aims to determine the molecular basis of the differentiation of human and mouse epithelial cells. Pathways regulating differentiation include Notch, wnt and the MAP kinase signalling cascade. In addition we wish to explore the interaction of Notch with cell cycle regulators such as p21 Cip1 and Geminin.

## Recipient or parental organism

Tissue culture cells of human or murine origin.

## Host/vector system

- Laboratory strains of E. coli K12 derivatives;
- Tissue culture cells of human origin.
- pUC-based plasmid vectors with RNA polymerase III promoter;
- Phoenix-amphotropic retroviral producer cells.

## Origin & function

The expression of inserts encoding siRNAs, consisting of sense and antisense 21-29 base sequences derived from the coding region of the target mRNA with a spacer to generate a hairpin RNA, in human primary epidermal keratinocytes, mouse primary keratinocytes and mouse and human cell lines, including squamous carcinoma cells and telomerase immortalised keratinocytes. Once expressed the siRNA oligonucleotides are expected to decrease levels of RNA encoding the target protein by over 70%.

Target genes are those of the pathways regulating epithelial stem cell fate, including Notch receptors, suppressor of hairless homologues, mastermind homologues, and the hairy/enhancer of split family of transcription factors. In addition components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins such as p21 Cip1 and Geminin will also be targeted.

Control siRNAs directed against enhanced green fluorescent protein and luciferase will be required for some experiments.

## Evaluation of foreseeable effects

The inserts encode short interfering RNAs which are only active within the cell, and cannot encode an exogenously expressed protein. Expression of these RNAs will not
enhance the pathogenicity of the host cells. The effect of expression of the insert RNAs will be to functionally silence the target genes. In most cases, functional silencing is known to impair cellular function resulting in defective cell cycle progression or in genetic instability. In no instance is functional silencing of the target genes known to work directly to transform primary cell cultures.

A non-negligible hazard arises from the potentially oncogenic or cytotoxic nature of the cellular effects of some of the interfering RNA inserts. It is considered that DNA from such clones can be appropriately handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras. 8-10 in which the potential hazard is specifically considered.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before either subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

This work involves the cloning of short DNA inserts under a eukaryotic promoter, in order to generate ~25-mer interfering RNA. The genes targeted in this work are those for pathways controlling epithelial stem cell fate, components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins. The cloned DNA is therefore potentially cytotoxic or oncogenic.

Initial cloning is into non-mobilisable vectors grown in laboratory E. coli K12 strains. The vectors are pUC-based and are disabled retroviral vectors, which require a helper t.c. line to produce virus, which in turn is only capable of a single infectious cycle. This stage is therefore Class 1.

Subsequently the retroviral vectors will be transfected into mouse cell lines, with the Phoenix packaging constructs, which give either ecotropic or amphotropic envelopes, to produce the disabled retrovirus with either type of envelope. Following consideration of the advice in the ACGM Compendium of Guidance, Part 2B, Annex III, paras 21-30, it was agreed that Class 1 is appropriate with the ecotropic envelope and Class 2 with the amphotropic. If cell lines from the amphotropic constructs are being maintained for a long time, consideration should be given to testing them for the presence of replication competent virus.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.
Project Containment

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<th>Laboratory Activities</th>
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<td>STUDY OF THE REGULATION OF EPITHELIAL PROLIFERATION USING REPLICATION INCOMPETENT LENTIVIRAL VECTORS.</td>
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Non-GMM Consent Granted: Not Applicable

Tick if notifying a connected programme of work: N

Historical Significant Changes: Project transferred from GM921 on 03/09/2013

Project Additional Information

**Purposes of the contained use**

The project aims to determine the molecular basis of the differentiation of human and mouse epithelial cells. Pathways regulating differentiation include Notch, wnt and the MAP kinase signalling cascade. In addition we wish to explore the interaction of Notch with cell cycle regulators such as p21 Cip1 and Geminin.

**Recipient or parental organism**

Tissue culture cells of human or murine origin.

**Host/vector system**
Laboratory strains of E. coli K12 derivatives; tissue culture cells of human or mouse origin, including murine cells expressing helper functions for defective retrovirus production.

pUC-based plasmid vectors with RNA polymerase III promoter; Plasmids containing lentiviral helper proteins - see diagrams attached to risk assessment.

### Origin & function

The expression of inserts encoding siRNAs, consisting of sense and antisense 21-29 base sequences derived from the coding region of the target mRNA with a spacer to generate a hairpin RNA, in human primary epidermal keratinocytes, mouse primary keratinocytes and mouse and human cell lines, including squamous carcinoma cells and telomerase immortalised keratinocytes. These cells will include those infected with lentiviruses or retroviruses engineered to include a LoxP sequence flanked cDNA sequence.

Also expression of non-oncogenic genes involved in these pathways.

Target genes are those of the pathways regulating epithelial stem cell fate, including Notch receptors, suppressor or hairless homologues, mastermind homologues, and the hairy/enhancer of split family of transcription factors. In addition components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins such as p21 Cip1 and Geminin will also be targeted.

Control siRNAs directed against enhanced green fluorescent protein and luciferase will be required for some experiments.

### Evaluation of foreseeable effects

The inserts encode either non-oncogenic proteins or short interfering RNAs which are only active within the cell, and cannot encode an exogenously expressed protein. Expression of these RNAs will not enhance the pathogenicity of the host cells. The effect of expression of the insert RNAs will be to functionally silence the target genes.

In most cases, functional silencing is known to impair cellular function resulting in defective cell cycle progression or in genetic instability. In no instance is functional silencing of the target genes known to work directly to transform primary cell cultures.

The expression of the Cre recombinase will recombine the LoxP sites. Among other resulting deletions will be the gene for Cre itself.

Anon-negligible hazard arises from the potentially oncogenic or cytotoxic nature of the cellular effects of some of the interfering RNA inserts. It is considered that DNA from such clones can be appropriately handled as set out in the ACGM Compendium of Guidance, Part 3A, Annex 1, paras. 8-10 in which the potential hazard is specifically considered.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 1 waste, generated during the preparative steps for the CL2 work, will be inactivated by either autoclaving, before discharge down the drain into the public sewer, or by incineration (in a registered facility). It is expected that these will give effectively 100% killing.

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before eithe subsequent discharge down the drain into the public sewer or incineration. It is expected that these will give effectively 100% killing.

02/03/2022
This work involves the cloning and expression of non-oncogenic proteins involved in pathways controlling epithelial cell fate. It also involves the cloning of short DNA inserts under a eukaryotic promoter, in order to generate ~25-mer interfering RNAs. These genes targeted in this work are those for pathways controlling epithelial stem cell fate, components of the MAP kinase and wnt kinase signalling pathways, and cell cycle regulatory proteins. The cloned DNA is therefore potentially cytotoxic or oncogenic.

Initial cloning is into non-mobilisable vectors grown in laboratory E. coli K12 strains. The vectors are pUC-based and are disabled lentiviral or retroviral vectors, which require a helper t.c. line (or helper plasmids in a t.c. cell line) to produce virus, which in turn is only capable of a single infectious cycle. This stage is therefore Class 1.

Subsequently the retroviral vectors will be transfected into mouse cell lines, together with plasmids supplying helper functions, which give either ecotropic or amphotropic envelopes, to produce the disabled lentivirus with either type of envelope. Following consideration of the advice in the ACGM Compendium of Guidance, Part 2B, Annex III, paras. 21-30, it was agreed that Class 1 is appropriate with the ecotropic envelope and Class 2 with the amphotropic.

It was noted that appropriate precautions are already given for the naked DNA, to allow for its potentially cytotoxic or oncogenic nature.

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**Project Ref** 20/05.1

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<td>03/09/2013</td>
<td>Use of lentiviral vectors in cloning and expression of genes involved in DNA repair and</td>
<td>Class 2</td>
<td>≤ 1 Litre</td>
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</table>
Cancer cells exhibit abnormalities in cell cycle progression, chromosome structure and segregation and in the capacity to repair DNA damage that are central to transformation. These abnormalities are often the result of mutations in tumour suppressor genes, such as the breast cancer gene BRCA2. The overall goal of our studies is to ascertain the molecular basis for the abnormal division of cancer cells, and their increased genetic instability.

Recipient or parental organism

Murine-embryo-fibroblasts and HeLa and other cancer cell lines.

Host/vector system

E.coli laboratory K12 derivatives/pUC based vectors; Vertebrate tissue culture cells including 293T cells, murine-embryo-fibroblasts and HeLa and other cancer cell lines/Lentiviral vectors, with polytropism through viral pseudo-typing using VSV-G protein.

Lentiviral system: The viral system to be used employs a well-characterised lentiviral vector and packaging cell lines(Refs below). A number of safety features are incorporated to eliminate or very greatly reduce the possibility of generating productive recombinants. The vector lacks gag, pol, env, tat, rev, vpr, vpu, vif and nef functions. A chimeric LTR offsets the requirement for Tat in expressing genes transcribed from the LTR. The Rev function is provided in trans from a separate plasmid in the packaging cells, as are the Gag/Pol functions. The viral vectors are self-inactivating due to deletions in both the LTRs, and thus incapable of replication after one round of infection of target cells. The packaging cell lines are effectively helper-free, since the rev, gag/pol and env (in this case, pseudo-typed with VSV-G protein) genes are expressed from separate plasmids. Rubinson et al Nat Genetics 34:231 (2003); Dull et al., J Virol 72:8463 (1998); Myoshi et al J. Virol 72:8150 (1998).

Origin & function

Specific aims with details of the categories of inserts to be used are as follows:

A) BRCA2-deficient cells exhibit defects in DNA repair (1). The defect is likely related to the inability of mutants BRCA2 to form functional complexes with RecA homologs in the Rad51 family (eg Rad51, xrc2 & 3, Rad51B-D). The molecular basis for this phenotype will be studied by functional and biochemical analyses in transfected cells. Wild-type or mutant forms (including point mutations, deletions and fragments) will be studied.

B) Abnormalities of chromosome structure and number are abundant in BRCA2-deficient cells (2-4). To investigate the molecular basis for these phenotypes, the function of tenes controlling chromatin structure (eg histones, histone-modifying-enzymes, HP1), the cell cycle (eg cylins A, B, D, E; cdk1, 2 & 4, polo-like kinases), chromosome number and structure (eg SMCs1 & 3, scc1, Ecol), cytokinesis (eg chromokinesins, INCENP, Surviving) and the centrosome cycle (eg nek1, nap, g-tubulin, aurora kinases)
will be investigated by functional & biochemical analyses in transfected cells. Wild-type or mutant forms (including point mutations, deletions and fragments) will be studied. C) We have shown that mutations inactivating mitotic checkpoint genes including Bub1 or Mad3L (BubR1), are necessary for the neoplastic transformation of cells lacking the BRCA2 tumour suppressor (5). Besides these, we will test the possibility that genes (eg chk1/chk2, cdc14A/cdc14B, PP1delta, cdc25C, m TERT or h TERT, ranGAP & cdh1) whose normal function is to regulate cell cycle progression and chromosome stability, particularly during the S phase and in mitosis, will be targets for inactivation by secondary genetic changes during tumour evolution in cells that lack genes such as BRCA2, using studies of transfected cells. Wild-type or mutant forms (including point mutations, deletions and fragments) will be studied.

D) To identify novel genes that regulate these processes, cells will need to be transfected with pools (libraries) of vertebrate cDNAs or short-hairpin RNA molecules that interfer with the expression of vertebrate genes, for phenotypic screens. Here, the precise nature of the insert cannot be specified in advance.

In A-C above, fusions of wild-type & mutant genes to GFP or other fluorescent tags will be used to determine intracellular localisation.

3) Yu et al., Genes Dev 14:1400 (2000)
5) Lee et al., Molecular Cell 4:1-10 (1999)

**Evaluation of foreseeable effects**

Possible changes in the behaviour of cells in tissue culture, helping to elucidate the abnormal division of cancer cells, and their increased genetic instability.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Full containment level 2 will be applied.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Containment level 2 waste will be autoclaved (136 degrees C for 10 min), before subsequent discharge doen the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

N

**Project Containment**
The aim of this study is to identify genetic dependencies in metastatic cancer cells by utilizing retro and lentiviral vectors. Murine and human cells (packaging cells, primary cells, cancer cell lines and immortalized cell lines) are used as recipients. K12-derived E.coli strains (e.g., DH5alpha) are used for plasmid transformations and amplifications.
Stable gene transduction will be achieved by Moloney murine leukemia virus (MMLV) or Murine stem cell virus (MSCV) - derived retroviral (e.g. pBabe, pRetroX) or HIV1-derived lentiviral vectors (e.g. pGIPZ, pTRIPZ, pLVX). 293T human embryonic kidney cells and derivatives will be used as packaging/producer cells.

We will use commercially available retroviral and lentiviral packaging systems of the utilize split genes to provide viral packaging elements on individual plasmids that physically separate the viral envelope, env (VSV-G), sequence from the gag-pro-pol sequences. These split-gene packaging strategies reduce the risk of generating replication-competent lentivirus (RCL) because multiple recombination events are necessary to create a virus that harbors the sequences required for independent replication. Also, these systems contain a large deletion in the 3’ LTR making packaging cells will be used. Retroviruses can only infect both dividing and non-dividing cells and no stable packaging cells will be used. Retroviruses can only infect dividing cells and are therefore less hazardous. They will be produced using packaging cells that stably express the packaging constructs. In both cases, the packaged viruses are not able to replicate independently.

The 2nd generation lentiviral vetors contain several safety mechanisms:

- Reduced number of genes from HIV-1 (i.e. gag, pol, tat and rev).
- Separation of genes encoding the structural and other components required for packaging the virus to substantially reduce the risk of undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., J Virology 72 8463-8471 1998).
- None of the HIV-1 structural genes are present in the packaged viral genome and are therefore never expressed in the transduced targe cell.
- The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral particles are produced.
- In some cases expression of the gene of insert of interest will be dependent on a tet-responsive CMV promoter, and regulated by a co-transduced lentivirus doxycyclin-responsive Tet regulator, giving an added level of safety for insert expression.

These vectors contain the WPRE (Woodchuck hepatitis B virus post-transcriptional regulatory element). This element is used to increase lentiviral vector titre and gene expression. It includes the promoter for the X-protein and the part to the coding sequence for such protein, which may have oncogenic properties.

The viral vectors will not increase the pathogenicity of the host cells. The main hazard arises directly from the recombinant retroviruses produced from the packaging cells. These yield both ecotropic and amphotropic lentivirions and ecotropic and amphotropic retrovirions, depending upon the envelope proteins supplied by the cell.

Target cells:
Tumour cell lines, both established and those derived from patient samples. After infection is complete, cells wil be propagated as cell lines. Target cells include human and mouse cancer cells. Inserts include transcription factors, epigenetic regulators, metabolic enzymes, RNAi-constructs, the Cas9 nuclease, and guide RNA constructs. Some of them may be expected to confer some growth advantage on the recipient cells. In some instances we wil be transducing cells with known or putative oncogenes or constructs that target known or putative tumour suppressor genes.

Infected cells
After infection and selection, the manipulated cells will no longer be harbouring propagating virus, so they will no longer represent an infection hazard.

Origin & function
Genes and mutant derivatives thereof, usually of human or mouse origin, encoding transcription factors, metabolic regulators, epigenetic regulators and other cancer-related gene products identified through bioinformatic analysis, also potential or known oncogenes or tumour suppressor genes. Marker genes (GFP, luciferase etc.), CRE recombinase, RNAi constructs and genetic tags (HA, FLAG), antibiotic resistance genes, targeted nucleases (e.g. Cas9 and derivatives), nuclease guide RNAs. Some of the virus vectors may lead to growth arrest of mammalian cells, while others may promote cell proliferation. Because of the presence of the WPRE element in the lentivirus vectors, the lentivirus constructs might transform infected mammalian cells.

The main Hazard is potential infection of persons working in the lab by the GM virus, for which a Class II containment level is adequate.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these
**Evaluation of foreseeable effects**

Some of the virus vectors may lead to growth arrest of mammalian cells, while others may promote cell proliferation. Because of the presence of the WPRE element in the lentivirus vectors, the lentivirus constructs might transform infected mammalian cells.

The main Hazard is potential infection of persons working in the lab by the GM virus, for which a Class II containment level is adequate.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class I level of classification.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Only micro-organisms and tissue culture cells are involved.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class I level of classification.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved on site (136°C for 10 min) before disposal (rotoclave) by a specialist company. Liquid waste may be disinfected (final concentration 5% Virkon for 30 min) before disposal to drain. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Project Containment**

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<th>Growth Rooms</th>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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This is typical lentivirus project, designed to introduce genes into mouse and human cells in vitro. It does not raise any unusual issues.
### Purposes of the contained use

The object of this study is to modulate the expression levels of Ras proteins in a number of well-established human and normal and cancerous cell lines, in order to investigate whether different levels of expression of mutant Ras proteins result in differing outcomes for the cell.

### Recipient or parental organism

Human cells (packaging cella, cancer cell lines and immortalized cell lines).

### Host/vector system

**Plasmid production:**
K12-derived E.coli strains (e.g. DH5alpha) of bacteria are used for transformations and amplification of plasmids.

**Retro/lentiviral production:**
Stable gene transduction will be achieved by Moloney murine leukemia virus (MMLV)-derived retroviral vectors (e.g. pBabe, pLPCX) or HIV1-derived lentiviral vectors (e.g. pLKO.1). 293T human embryonic kidney cells and derivatives will be used as packaging/producer cells.

We will use commercially available retroviral and lentiviral packaging systems that use split genes to provide the viral packaging elements on individual plasmids that physically separate the viral envelope sequence, env (VSV-G), from the gag-pro-pol sequences. These split-gene packaging strategies reduce the risk of generating
replication-competent lentivirus because multiple recombination events are necessary to create a virus that harbors the sequences required for independent replication. Also, these systems contain a large deletion in the 3’ LTR marking these viruses replication-incompetent. Lentiviruses can infect both dividing and non-dividing cells and no stable packaging cells will be used. Retroviruses can only infect both dividing and non-dividing cells and no stable packaging cells will be used. Retroviruses can only infect dividing cells and therefore less hazardous. They will be produced using packaging cells that stably express the packaging constructs. In both cases, the packaged viruses are not able to replicate independently.

The 2nd generation lentiviral vectors contain several safety mechanisms:

. Reduced number of genes from HIV-1 (i.e. gag, pol, tat and rev).

. Separation of genes encoding the structural and other components required for packaging the virus to substantially reduce the risk of undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., J Virology 72 8463-8471 1998).

. None of the HIV-1 structural genes are present in the packaged viral genome and are therefore never expressed in the transduced target cell.

. The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.

The viral vectors will not increase pathogenicity of the host cells. The main hazard arose directly from the recombinant retroviruses produced from the packaging cells, which yield amphotropic lentivirons and retrovirions.

Target cells:
Established tumour cell lines. After infection is complete, cells will be propagated as cell lines. Inserts include known oncogenes or constructs that target known oncogens.

Infected cells:
After infection and selection, the manipulated cells will no longer be harbouring propagating virus, so they will no longer represent an infection hazard.

Origin & function

Known oncogenes of human origin and silencing constructs (shRNA) targeting the same oncogenes, as well as marker genes (GFP) and antibiotic resistance genes (puromycin).

Evaluation of foreseeable effects

The main hazard is potential infection of persons working in the lab by the GM virus, for which a Class II containment level is adequate.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class I level of classification.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms and tissue culture cells are involved

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All class II waste will be treated as per Hutchison/MRC Research Centre guidelines. Currently, liquid waste will be treated with an equal volume of 10% Virkon solution, with a contact time of 30 minutes before disposal. This will be flushed down the sink with copious amounts of water.
Plastic waste, tissues, gloves etc. will be placed in biohazard. All class II waste will be autoclaved before leaving the building for incineration.

Class I waste will be disposed according to Hutchison/MRC Research Centre practice, currently: liquid waste will be inactivated with Virkon; bacterial plates will be autoclaved on site; remaining waste will be incinerated off site.

It is expected that this will give effectively 100% killing.

This is similar to many Ras for using mon-replicating lentivirus and retrovirus vectors to express genes and shRNAs in human cell lines in vitro, with Class II for production of, and infection by human host-range virus vectors. It raises no particular issues.

Project Containment

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Project Ref 3201/14.3

- **Date Ackn'd**: 30/10/2014
- **CU2 Project Title**: Identifying the molecular regulators of lung tumour development and progression by modulation of genes of interest using lentiviral vectors
- **Class**: Class 2
- **CultureVolumeClass2**: < 1 Litre
- **Non-GMM**: Consent Granted

Date Project Ceased: 03/01/2019

Project notified under transitional arrangements N

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This is similar to many Ras for using mon-replicating lentivirus and retrovirus vectors to express genes and shRNAs in human cell lines in vitro, with Class II for production of, and infection by human host-range virus vectors. It raises no particular issues.
The aim of this study is to identify genes involved in regulating lung tumour development and progression.

Murine and human cells (packaging cells, primary cells, cancer cell lines and immortalised cell lines).

Laboratory K12 or B derived E. coli strains will be used for transformation, selection and amplification of plasmid DNA.

Lentiviral production:
Stable gene transduction will be achieved HIV1-derived lentiviral vectors (eg. pLKO.1, pBOBI, pGIPZ, pRRL etc). 293T Human embryonic kidney cells will be used as packaging/producer cells.

We will use commercially available lentiviral packaging systems that utilize split genes to provide the viral packaging elements on individual plasmids that physically separate the viral envelope, env (VSV-G), sequence from the gag-pro-pol sequences. These split-gene packaging strategies reduce the risk of generating replication competent lentivirus (RCL) because multiple recombination events are necessary to create a virus that harbours the sequences required for independent replication. Also, these systems contain a large deletion in the 3′ LTR making these viruses replication incompetent. Lentiviruses can infect both dividing and non dividing cells and no stable packaging cells will be used. The packaged virus will not be able to replicate independently.

The generation of lentivirus has been significantly modified for biosafety:
- The viral genes (env,tat, rev, vpr, vpu, vif and nef) have been deleted from the vector, so that it expresses no viral gene products.
- The vector has a self-inactivating LTR TATA box deletion) and
- Genes encoding the structural and other components required for packaging the vector, and the envelope glycoprotein, are provided on two or three separate plasmids.
All plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al, 1998)
  - No single plasmid contains all the genes necessary to produce packaged lentivirus. None of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell so no new replication-competent virus can be produced.
  - The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.
  - In some cases expression of the gene insert of interest will be dependent on a tet-responsive promoter, and regulated by a co-transduced lentivirus doxycycline-responsive Tet regulator, giving an added level of safety for insert expression.

These vectors contain the WPRE (Woodchuck hepatitits B virus post-transcriptional regulatory element) as a means to increase virus titre and gene expression. As it included the promoter and partial coding sequence for the X-protein, it may have oncogenic properties.
**Target cells:**
Tumour cell lines, both human and murine will be used. After infection is complete, cells will be propagated as cell lines. Inserts include transcription factors, oncogenes, tumour suppressors, metabolic enzymes, and RNAi constructs. Some of them may be expected to confer some growth advantage on the recipient cells.

**Infected cells:**
After infection and selection, the manipulated cells will no longer be harbouring propagating virus, so they will no longer represent an infection hazard.

**Origin & function**
Genes and mutant derivatives thereof, usually of mouse or human origin, encoding putative oncogenes or tumour suppressor, transcription factors, metabolic enzymes. Marker genes (GFP, RFP etc), RNAi constructs and genetic tags (HA, FLAG) and antibiotic resistance genes.

**Evaluation of foreseeable effects**
Some of the virus vectors may lead to proliferation or alternatively growth arrest of mammalian cells. Because of the presence of the WPRE element in the vectors, the lentivirus constructs might transform infected mammalian cells.

The main hazard is potential infection of persons working in the lab by the GM virus, for which a Class 2 containment level is adequate.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer be an infection hazard. Work with these genetically modified cells can be undertaken at Class 1.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Only micro-organisms and tissue culture cells are involved

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
Full containment at level 2 will be applied.

After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at class 1.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Containment level 2 waste will be disposed of according to Hutchison-MRC SOPs. Currently solid waste is autoclaved (136°C for 10 min), before disposal offsite by specialist contractors; the waste is then rotoclaved. Liquid waste may be mixed with Virkon to a final concentration of 5% and stood for 30 minutes minimum before drain disposal. It is expected that his will give 100% killing.

**Is an emergency plan required according to regulation 20?**
N

**If yes, tick to confirm that it is attached to this form**
N

**Tick to confirm that you have attached a risk assessment to this form**
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**
N
This is a fairly typical lentivirus/cultured cell GM proposal requiring CL2 for production and infection with virus, and does not appear to raise any particular issues.

**Project Containment**

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**Project Ref** 3201/16.1

- **Date Ackn'd**: 12/05/2016
- **CU2 Project Title**: Functional analysis of oesophageal adenocarcinoma genomic variants
- **Class**: Class 2
- **Culture Volume Class 2**: ≤ 1 Litre
- **Non-GMM Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N

**Project Additional Information**

**Purposes of the contained use**

This project aims to better understand the functional effects of genomic aberrations in oesophageal adenocarcinoma by permanently or transiently altering the genomic and transcriptomic state of in vitro models including oesophageal cell lines and organoids derived from primary tumour tissue. This requires the use of lentiviral RNAi and cDNA over-expression vectors as well as expression of targeted nucleases that will permanently alter cancer cell genomes.

**Recipient or parental organism**
1. Established human tumour cell lines and 2. Oesophageal organoid cultures derived directly from patient samples. These Organoids can be passaged >30 times in many cases, without transformation. The patient samples are not in general screened for blood-borne viruses, and we are not permitted to screen them, for ethical/consent reasons. There is a separate risk assessment for these cultures.

**Host/vector system**

K12-derived E. coli strains (e.g. DH5alpha) of bacteria are used for transformations and amplification of plasmids.

Stable gene transduction into human cells will be achieved using HIV1-derived 2nd generation lentiviral vectors such as pCW57.1 and pLX304. For CRISPR experiments, vectors such as pCW57.1, containing dox inducible Cas9, and pKLV-U6gRNA-EF-pGKpuro2ABFP, encoding guide RNAs, will be used. 293T human embryonic kidney cells and derivatives will be used as packaging/producer cells, using packaging plasmids such as psPAX2 and psMD2.G. Later generation systems with further safety mechanisms and higher efficacy may be adopted as they become established.

**Origin & function**

Inserts will encode reporters (e.g. EGFP, DsRed, luciferase); transcriptional regulators (e.g. Tet-On); normal and modified transcription factors or epigenetic regulators and other cancer-associated transcripts, including known oncogenes; shRNAi constructs, or targeted nucleases and their associated RNA molecules (CRISPR/Cas9), including constructs that functionally ablate known tumour suppressor genes or activate known oncogenes. Many of these Constructs are intended to oncogenically transform human cells. Genes will be expressed under the control of viral and mammalian promoters, both constitutive (e.g. CMV or E2F) and/or inducible (e.g. regulated by Tet-On trans-activator protein).

**Evaluation of foreseeable effects**

DNA grown up from clones should also be handled with care as it is potentially oncogenic because of the WRPE element and oncogenic inserts; i.e. gloves should be worn, sharps avoided and all wastes be rendered harmless before disposal.

K12-derived E. coli strains (e.g. DH5alpha) of bacteria (containment level 1) are used for transformations and amplification of plasmids. No constructs will contain mammalian extra-cellular protein encoding genes driven by bacterial promoters as these could potentially enhance E.coli pathogenicity.

Viral particles will be filtered to remove any possible contamination by 293T cells, which could allow continued viral production. We will use 2nd generation lentiviral vectors containing several safety mechanisms:

- Reduced number of genes from HIV-1 in lentiviral construct (i.e. gag, pol, tat and rev are absent).
- Separation of genes encoding the structural and other components required for packaging the virus to substantially reduce the risk of undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., J Virology 72 8463-8471 1998).
- None of the HIV-1 structural genes are present in the packaged viral genome and are therefore never expressed in the transduced target cell.
- The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.
- In some cases expression of the gene insert of interest will be dependent on a tet-responsive promoter, and regulated by a co-transduced lentivirus doxycyclin-responsive Tet regulator, giving an added level of safety for insert expression.

Given the non-negligible hazards associated with the use of replication incompetent lentivirus in this case, particular attention will be paid to staff awareness when working with genes with a potential growth-promoting function (virus production, labelling, storage). No sharps will be used when working with lentiviruses. Also, appropriate personal protective equipment will be used (gloves, lab coats).

For some experiments, virus will be stored outside the Class 2 lab. For this, it will be in a designated section of a clearly designated freezer, in double containers, according to the Hutchison-MRC Research Centre Class 2 Code of Practice.

Lentiviral vectors contain the WPRE (Woodchuck hepatitis B virus post-transcriptional regulatory element), which may have oncogenic properties, so, whether or not the
inserts are oncogenic. All these lentiviruses are potentially oncogenic. The risk of Lentiviral infection of a worker during the packaging/infection step is small, but still not negligible. As the vector contains the complete WPRE element as well as tumorigenic inserts this part of the work is Class 2. However any theoretical hazard is only during initial contact since such viruses could not propagate. The prokaryotic cells are highly disabled and the plasmid vectors are non-mobilisable, therefore neither has any possibility of further spread.

In principle, there is a small risk that organoid cultures might carry HIV, which, by acting as a helper virus, would make the lentivirus inserts replication-competent. However, to our knowledge HIV does not replicate in cells such as oesophageal epithelium, so, provided the organoids have been passaged by disaggregation so that they do not also carry macrophages or lymphocytes, the risk of HIV is low. After infected organoids have been passaged further, the risk of active virus will be very low.

To cryopreserve cells in CL2, storage will be at -80 outside the CL2 lab as specified above for virus storage. When it is really necessary for long-term preservation to store cells in a vapour phase liquid nitrogen refrigerator this will be in a clearly designated location, in a double container, according to an agreed Hutchison-MRC Research Centre Class 2 Code of Practice and with explicit permission from the Hutchison Lab Manager responsible.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

After infection, selection and passaging at least once, for cell line cultures only, NOT organoids, cells that are virus negative may be transferred to CL1. To show that cells are negative for replicating lentivirus, we will use well characterised methods such as the Molecular Probes’ EnzChek® Reverse Transcriptase Assay.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be disposed of according to Hutchison-MRC SOPs.

Containment level 2: Solid waste e.g. plastic waste, tissues, gloves etc. will be placed in clear biohazard autoclave bags provided. When full, bags will be sealed with tape and placed in the appropriate biohazard bin, it is then autoclaved (136°C for 10min). Final disposal is offsite by specialist contractors; the waste is then rotoclaved.

Liquid waste should be mixed with Virkon to a final concentration of 5% and stood for 30 minutes minimum before drain disposal. It is expected that this will give 100% killing.

Containment Level I: Solid waste, e.g. plastic waste, tissues, gloves etc. will be placed in yellow bags. When full, bags will be sealed with tape and placed in the appropriate biohazard bin. Bacterial plates are collected in clear biohazard autoclave bags and are autoclaved (136°C for 10min). Final disposal of all CL1 solid waste is offsite, by specialist contractors; the waste is then rotoclaved.

Liquid waste should be mixed with Virkon to a final concentration of 1% and stood for 30 minutes minimum before drain disposal. It is expected that this will give 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
This is principally a project to use lentivirus to manipulate cancer-relevant genes in human cells. The non-standard element of the proposal is that organoid cultures of human epithelium from unscreened patients are among the target cells to be used. Screening is not permissible for ethical reasons. Since such cultures might rarely be contaminated with HIV, the general principle that modern lentivirus constructs cannot propagate could break down, because the HIV could package the lentivirus vector, in effect creating a defective virus-helper virus system. However, the HIV itself is at least as great an ‘in principle’ risk, which we accept. Self-inactivating constructs would be preferable in this case, since they are less likely to transcribe packageable RNAs. An SOP for cryopreserving CL2 cells needs to be developed. The CRISPR work does not raise ‘gene drive’ issues and so, according to current views, raises no additional concerns.

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Project Ref 3201/16.2

Date Ackn’d: 25/05/2016

CU2 Project Title: Lentiviral particles containing human open-reading frame (hORF) cDNA libraries to find novel druggable targets in human reporter cell lines

Class: 2

Culture Vol: < 1 Litre

Project notified under transitional arrangements N

Consent Granted

Date Projects Ceased

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

02/03/2022
The main purpose of the work is to create reporter cell lines in which human open-reading frame (hORF) eDNA libraries are expressed. Our starting material will consist of short coding sequences of human eDNA, encompassing much of the human transcriptome, encoding short peptides which may (or may not) represent in-frame, ordered human peptide sequences, but will not usually code for complete, functional human proteins. The above library will be subcloned, along with a selection marker into the pl VX-TetOne (Clontech), or similar vector and subsequently viral particles will be synthesised and titrated by Vectalys ltd, a contract research organisation (CRO). This virus will be used to infect human reporter cell lines in order to generate recombinant cell lines. In these cell lines, the hORF libraries will be randomly inserted into the host genome. Following selection, should tetracycline dependant vectors be used, these cell lines will be activated by Doxycycline treatment prior to assessment for reporter activity.

- Due to source material of cDNA hORF library, in-frame peptides cannot be guaranteed, therefore 66% of the library will consist of peptides coded by out-of-frame cDNA; 33% will consist of human ORF peptides.

Human cell lines including but not limited to: U20S, HCT1 16, D1 D-1, SW48, Panc-1, Hel a.

Laboratory strains of E.coli K12, pl VX-TetOne (Clontech ltd.) vectors and 3rd generation, self-inactivating lentiviral particles containing pl VX-TetOne with human open reading frame libraries. In E.coli and in tissue culture cells, the host cells are disabled and hazard group 1. Further to this, no expression of human protein should occur in bacterial cells, therefore no increase in pathogenicity of the bacteria should occur following transformation. The vectors are non-mobilisable; 3rd generation self-inactivating vectors exhibit negligible risk of replication-competent virus production. The transgenes have a limited potential to disrupt endogenous genes either by coding sequence insertion-mediated silencing, by promoter insertion-mediated dysregulation or by aberrant production of all or part of a hORF. The disruptive potential is limited by the autosomal nature of the genome together with the random nature of lentiviral insertion and secondly, in some cases by the inducible, chemically-dependant nature of the transgenes. The risk to environment is limited by the self-inactivating nature of the lentiviral particles and low probability of creating replication-competent lentivirus A small risk to user however remains, the provisional classification is therefore Class 2.

Our starting material will consist of short coding sequences of human eDNA, encompassing much of the human transcriptome, encoding short peptides which may (or may not) represent in-frame, ordered human peptide sequences, but will not usually code for complete, functional human proteins. These open-reading frames will be permanently inserted into the genomes of the target cell populations and will subsequently express protein fragments in either a constitutive or chemically regulated manner. In the case of pLVX-TetOne, the genetic material inserted between the viral L TRs will include both fragments of and intact wild-type human open-reading frame sequences (hORFs) from throughout the human transcriptome; sequences encoding a transactivation gene (TetOn3G); a tetracycline-responsive promoter (TRE3G) and a selection cassette to identify gene targeted clones.

The worst case scenario is that accidental infection of a user with lentivirus results in genetic modification of his/her cells and/or production of replication-competent lentiviral particles. However, there are multiple strong reasons why
such an event is unlikely to be hazardous in respect of human health or environmental safety. Firstly, deregulation of tumour suppressor genes by coding sequence insertion is unlikely, as the genes in question are largely encoded on autosomes, the lentiviral vectors do not encode regions of homology for specific insertion loci, and so the rate of random insertion into a specific and detrimental loci using lentiviral vectors makes it extremely unlikely that both somatic copies will be altered in a single cell. Secondly, in the case of an inducible system, the presence of an activating compound, doxycycline is required for effective transcription of the inserted transgene. As cells other than experimental cell lines are unlikely to have doxycycline at effective concentrations, accidental infection of a user is very unlikely to result in gain-of-function conditions by hORF insertion as the hORF should not be transcribed. Thirdly, when using lentiviral particles produced by 3rd generation packaging systems that utilise splitgenes to provide the viral packaging elements on individual plasmids that physically separate the viral envelope, env (usually VSV-G), sequence from the gag-pro-pol sequences. These split-gene packaging strategies reduce the risk of generating RCL because multiple recombination events are necessary to create a virus that harbours the sequences required for independent replication (see diagram, p7). There is no evidence that when the inserted genetic material recombines with the host cells genomic DNA that any rep/cap sequences of the lentiviral genome are incorporated. In the recombinant lentiviral particles, lentiviral genomic sequences present are: WPRE, RRE, cPPT/CTS and*, (see p.5-6). Also, the Long Terminal Repeats (LTRs) are included, of which the U3 region of the 3'LTR is deleted, allowing genomic integration in host cells, but preventing subsequent first strand synthesis of viral RNA and therefore replication of integrated viral DNA. Finally (as detailed below), the vector/host systems are non-mobilisable and disabled, and the lentiviral particles are replication-deficient, rendering the potential to affect environmental safety very minimal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All plasticware to be double-bagged, sealed, autoclaved and then incinerated on-site.
All solid wastes to be double-bagged, sealed, autoclaved and then incinerated on-site.
All liquid wastes to be disinfected with 1% Virkon for 1h prior to disposal by sewage with plenty of water.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N
A straightforward RA for lentivirus vector infection of human cell lines. The only unusual feature is that the virus will be prepared off site by a company, but arrangements for shipping and storage are described and conform to our building’s SOP.

**Project Containment**

<table>
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**Project Ref** 3201/18.1

- **Date Ackn’d**: 18/01/2018
- **CU2 Project Title**: Use of lentiviral vectors in cloning and expression of genes involved in breast cancer development

<table>
<thead>
<tr>
<th>Class</th>
<th>CultureVol</th>
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<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>

- **Non-GMM Consent Granted**: Not Applicable
- **Project notified under transitional arrangements**: N

**Historical Significant Changes**
- **Historical Date of Additional Info**: 02/03/2022
- **Significant Change ID**: 18/01/2018

**Project Additional Information**

**Purposes of the contained use**

To study the impact selected cancer driver genes have on the normal cellular functioning, clonal fate and proliferative dynamics in vitro using conditional expression of selected mutated genes in normal human breast cells. Some of the most commonly mutated genes in breast cancer include p53, PIK3CA, CCND1, PTEN, ERBB2, GATA3, and BRCA1/2.
genes (The Cancer Genome Atlas Network et al. Nature 2012). Cancer driver genes often contain mutations (such as frame shift, missense or truncation mutations) that lead to aberrant expression and activation of downstream growth factor Signalling cascades and activate proliferative pathways as shown in mouse transgenic models and breast cancer cell lines. It is not known how the mutations confer selection advantage in normal breast cells or whether there is cooperation between mutated and normal cells to drive tumorigenesis. The objective of this study is to induce expression of these mutations by cDNA expression of 3D organoid structures in selected mammary epithelial cells. Inserts will incluclude WT and mutated versions of the above genes of interest. Further, inserts will encode an inducible system (such as Tet-On/Off or Cre-ERT2) to induce expression at specific times and inserts will be fused to reporters such as GFP to determine cellular localisation. This work will involve difficult-to-transfect human breast cells, requiring the use of lentiviral systems for effective gene transfer. This assessment refers to work that will be carried out in vitro.

**Recipient or parental organism**

Infection of human breast cells (both primary and early passage cells) with packaged viral constructs. After infection, the cells will be cultured in media containing puromycin to select for transformants. The infected cells will then be cultured in 3D organoid systems and expression of mutations induced after 3D spheroid formation. These organoids will then be used for microscopy analysis and cells recovered for protein/gene expression analyses.

**Host/vector system**

*HOST:* Lentivirus will be packaged by co-transfection of lentiviral transfer plasmid, packaging plasmids and envelope plasmid into HEK293T cells.

*VECTOR:* Lentiviruses are produced using a second-generation packaging system. Three plasmids will be used to generate viral particles: (i) cDNA-containing vector (such as pLV), (ii) packaging plasmid (i.e. psPAX2) and (iii) envelope plasmid (e.g. pMD2.G or pCMV-VSVG). The lentiviral transfer plasmid, packaging plasmid and envelope plasmid are transfected into 293T packaging cells. Genes for replication and structural proteins are absent in the packaged viral genome since these genes are supplied by other plasmids in the packaging cells. The lentiviral vector contains a self-inactivating 3' lTR that renders it unable to produce infectious virus once it integrates into the host genome. Thus, the viral particles that form are self-inactivating and replication incompetent.

**Origin & function**

Inducible expression lentiviral vectors (such as Tet- or Cre-ERT2 systems) used for cDNA-mediated expression of genes of interest (WT and mutated versions) and commercially available via Addgene.

**Evaluation of foreseeable effects**

The prokaryotic and eukaryotic cells and cell lines, even if the latter are transformed with an oncogene, are highly disabled and the possibility of competent virus being generated is negligible. Therefore, they should be unable to propagate in the environment. The plasmid vectors are non-mobilisable, so neither has any possibility of further spread. The viruses are replication-incompetent and no live virus should reach the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All class 2 waste will be treated as per HutchisonMRC Research Centre guidelines. Currently, liquid waste will be treated with an equal volume of 10% Virkon solution, with a contact time of 30 minutes before disposal. This will achieve 100% kill. This will be flushed down the sink with copious amounts of water. Plastic waste, tissues, gloves etc. will be placed in biohazard bins provided. Double bags will be used. When full, bags will be sealed with tape and placed in the appropriate biohazard. All Class 2 waste will be autoclaved before leaving the building for incineration.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form  
N

Tick to confirm that you have attached a risk assessment to this form  
Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  
N

Please enter comments on the GM safety committee on the risk assessment

A standard CL2 lentivirus project, but the cells to be infected are going to be primary human breast epithelial cells. The most significant risk related to these cells is that they come straight from patients and hence they possibly contain also blood-derived cells. As per the associated risk assessment on human-derived cells, the primary human cell cultures will be kept in CL2 facilities until they have been confirmed to be negative for blood cells, after this they can be used also in CL1 facilities.

Project Containment

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Project Ref  3201/18.2

Date Ackn’d  20/04/2018

CU2 Project Title  Transfection and infection of mammalian cell lines or cancer cell lines

Class  Class 2
CultureVolClass2  < 1 Litre
CultureVolumeClass3-4

Non-GMM  Consent Granted

Date Project  02/03/2022
The overall aim of the project is to gain insight into the mechanisms and processes that promote cancer initiation and progression. More specifically we seek to study the interplay between cellular plasticity, stemness, senescence and tumorigenesis during lung cancer initiation and progression as well as the autocrine and paracrine roles of cellular senescence.

Human/murine primary cells (primarily with a lung origin, but not excluding other organ/tissues) and commercially available human cell lines, human tumor cell lines, mouse cell lines and mouse cancer cell lines shall be used.

Retrovirus
Retroviral plasmids will be transfected into an ecotropic packaging cell line, Phoenix-eco or amphotropic/VSV-G pseudotyped recombinant retrovirus. The ecotropic env gene produces particles that only infect rodent cells. The amphotropic env gene allows infection of both murine and non murine cells.

Lentivirus
We shall be using second third and subsequent generation lentivirus such as FUW-M2rtTA, pLKO, pWPI, and others. Both the viral vector and recombinant virus have a broad host range when pseudotyped. 293T cell line shall be used to produce lentiviral particles.

On occasion adenoiviral (Ad) vectors shall be used. These include type 5 infection-ready, replication incompetent adenovirus expressing Cre recombinase (AdCMV-Cre), FLP recombinase (AdCMV-Flp) or fluorescent marker proteins and appropriate adenoviral controls. Commerially available Adeno associated virus (AAV) shall be used on occasions. AAV vectors consist of recombinant transgene sequences flanked by AAV inverted terminal repeats (ITR) and produced by triple transfection system: the ITR-containing plasmid, the plasmid encoding AAV the capsid (VP1, VP2 and VP3 proteins) and replicate genes and the adenoviral helper plasmid.

The classes of genes to be manipulated are genes involved in the oncogenic or tumor suppression activity, inflammation, pluripotency, development and differentiation, cellular signalling, cellular senescence, transcription and chromatin regulation, cell adhesion and polarity.

Human or mouse genes to be used:
1. Genes with cell autonomous effect including wild type and mutant forms of Ras (such as K-RasG12D or K-RasG12V), Raf, Mek, Erk, Beta catenin, Tcf/Lef, Rac, Rho,

02/03/2022
cdc42, TNF alpha, PI3K, Kif4, Oct4, Sox2, Myc or histone methyltransferases.

2. Genes with autocrine and paracrine effects. These include wild type and mutant constructs of alpha and beta integrin subunits, Ephrins and E-cadherin, components of the Stat3 family, nuclear factor Kb, signalling partway, Hedgehog signalling pathway, PI3K signalling pathway, Wnt signalling pathway, Notch signalling pathway, TGF-Beta signalling pathway and EGF signalling pathways.

3. Reporter genes with no growth-promoting capacity, such as GFP, luciferase, H2B-EGFP.

4. Other poorly characterised genes

### Evaluation of foreseeable effects

**Retrovirus**

The helper-free retroviral producer cell line Phoenix-eco, express gag-pol and envelope genes using different non-Moloney promoters to minimize both recombination and inter-typic recombination potential. The mouse cell line GP+envAm12 packaging line showed no evidence for a generation of wild type virus and both systems have a history of safe usage.

**Lentivirus**

Deletion of vpr, vpu, vif and nef accessory genes renders the lentiviral vectors used unable to replicate once they have transduced target cells. The third generation lentivirus production system are deleted for the tat gene and carry a SIN deletion. This results in selfinactivation of the lentivirus following the transduction of the target cells. Amphotropic retroviruses and the lentiviruses used (VSV-G-pseudotyped) are able to infect cells from a broad host range, including humans, but cannot replicate unless they recombine with endogenous virus.

**Adenovirus**

The commercially available viral particles will be replication incompetent due to deletion of E1 and E3 regions. The viruses received are prescreened to ensure replication deficiency.

**Adenoasssociated virus (AAV)**

These viruses are infectious to humans but they are not known to cause human disease. AAV vector genomes remain primarily episomal in target cells and have a low (if any) frequency of integration in target cells.

Although the viral vectors listed above will be able to infect human cells, they will not replicate unless recombined with an endogenous virus. In this event, it is unlikely that expression of a single oncogene or inactivation of a tumor supressor would cause tumors, as multiple genetic alterations (4-6) are required for tumor induction. Safeguard mechanisms exist impeding the accumulation of these alterations in the organisms. Single events such as ras or myc expression instead of leading to unrestrained proliferation, induce growth arrest and apoptosis (Hanahan and Weinberg, 2000).

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All containment level 2 waste will be treated as per Hutchison/MRC Research Centre guidelines. All liquid waste will be inactivated by treatment with 3% Virkon for at least 30 minutes (overnight for large cultures) before disposal via the drains with copious amounts of water. This will achieve 100% kill. Any spillages will be treated with Distel or Chemgene at a final concentration of 5%. Plastic waste shall be soaked in Virkon solution overnight before disposal in the appropriate bins provided. Tissue and gloves used shall be disposed in the appropriate biohazard labeled bins. All solid waste shall be doublebagged. Bags shall be sealed with tape and placed in the biohazard containers. All solid waste shall be autoclaved before leaving the building for incineration.
To determine the viral titre of cells, well characterized methods that involves measuring reverse transcriptase activity shall be used. This is a direct readout of the presence of viral particles.

Routine disinfection of surfaces shall be achieved with 1% Distel or Chemgene solution. MSC are routinely fumigated and filters replaced, servicing every 6 months as per Hutchison/MRC Research Centre guidelines.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This is a standard virus-based gene delivery protocol. A wide range of viral vectors is to be used but they should all be replication incompetent. Primary human cells are to be risk assessed separately. Clearance given to start work following HSE notification.

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Project Ref 921/11.1

Date Ackn'd 03/09/2013

CU2 Project Title

Analysis of the changes of human epithelium adenoviral transfection of genes of interest

Date Project Ceased

Class 2

Culture Volume Class 3-4

< 1 Litre

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

02/03/2022
### Project Additional Information

**Purposes of the contained use**

We aim to identify genes that could play a significant role in the transformation of human oesophageal epithelium towards cancer via the development of Barrett's oesophagus and the acquisition of a malignant phenotype. Several candidate genes have been identified in literature as potential regulators for this switch in phenotype. Most of them are transcription factors, they include HoxGenes (identified in a current project in the lab), NHF4a, p63, CDX2 and C/EBP and they are potential oncogenes.

#### Recipient or parental organism

Packaging cells HEKs93  
Oesophageal cells:  
1. Primary cells will be isolated from human squamous oesophageal resection specimens and processed to obtain single cell suspension (separate Risk Assessment RCF 041 for human tissue). For this purpose freshly isolated, cultured cells will be used.  
2. Other established human oesophageal cell lines (normal and from cancer).

#### Host/vector system

**HEK293** (human embryonic kidney) cells, that, by producing the adenovirus E1 gene in trans, allow the production of infectious virus particles when cells are transfected with E1- deleted adenovirus vectors such as the pAdEasy-1.  
**Vector:**  
Purified recombinant Ad plasmid DNA is digested with the restriction enzyme Pac I to expose its inverted terminal repeats (ITR), and then used to transfect AD-293 (or HEK293) cells which have complemented in vivo viral assembly genes are:  
**Oesophageal cells/Adenoviral particles**  
**Hosts:**  
1. Primary cells will be isolated from human squamous oesophageal resection specimens and processed to obtain single cell suspension (Risk Assessment RCF 041). For this purpose freshly isolated, cultured cells will be used.  
2. Other established human oesophageal cell lines (normal and from cancer) might be used.  
**Vector:**  
The adenoviral particles generated as above. The regions E1 and E3 of the viral genome have been deleted. E1 deletion renders the viruses defective for replication and incapable of producing infectious viral particles in target cells (provided there is no complementation by the host cell); the E3 region encodes proteins involved in evading host immunity. Therefore the host cells which do not have the E1 region cannot replicate the virus or integrate it into the genome.
Inserts will be from Human genomic DNA and cDNA sequences cloned in bacteria. These inserts will be genes thought to be relevant to the transformation of human oesophageal epithelium.

**Evaluation of foreseeable effects**

The bacterial construction system cannot generate hazardous particles from the cloned inserts and for this reason a Cat I containment level would be adequate and this part of the work is not described here.

The target cells (human oesophageal cannot replicate the virus or integrate its DNA in the genome. However, it is possible that the pathogenicity of the host cells would be enhanced by the inserts. In addition, the nature of the host cells (untested human primary cells) requires a containment level (Cat II) adequate to this risk. Furthermore, the regions E1 and E3 of the viral genome have been deleted. E1 deletion renders the viruses defective for replication and incapable of producing infectious viral particles in target cells (provided there is no complementation by the host cell); the E3 region encodes proteins involved in evading host immunity. 

HEK293 contain the E1 gene that allows gene replication and therefore also the "amplification" of the viral stock solution requires an adequate (Cat II) containment level.

The main Hazard is potential infection of persons working in the lab by the GM virus, for which a Cat II containment level is adequate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

- Only micro-organisms and tissue culture cells are involved
- For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
  - Full containment level 2 will be applied
- Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
  - Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% kill

- Is an emergency plan required according to regulation 20? N
- If yes, tick to confirm that it is attached to this form N
- Tick to confirm that you have attached a risk assessment to this form Y
- Tick if you are claiming exemption from disclosure for section of the risk assessment N

**Please enter comments on the GM safety committee on the risk assessment**

This is a relatively typical case of adenovirus vector transduction of human cells in culture, and should be Class 2. The lab concerned already is familiar with Class 2 working, as the target cells are human primary material. The application specifically acknowledges the need to take care when storing adenovirus stocks outside the Class 2 lab, as discussed by the Hutchison-MRC safety committee.

**Project Containment**

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02/03/2022
Project Ref 921/12.1

Date Ackn'd 03/09/2013

Date Project Ceased 19/09/2019

Expression of proteins in human and mouse cells by lentiviral gene transfer

Class 2

< 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Historical Significant Changes Project transferred from GM921 on 03/09/2013, project transferred to 3071

Project Additional Information

Purposes of the contained use

The project aims to investigate strategies to modulate the differentiation of human and mouse embryonic stem cells, differentiated cells and tumour cells by introduction of genes including transcription factors and cell cycle regulators using lentiviral vectors.

Recipient or parental organism

Murine and human cells (packaging cells, primary cells, cancer cell lines and immortalized cell lines).

Host/vector system

1) E.coli, laboratory K12 derivatives/pUC based vectors;
2) Vertebrate tissue culture cells including 293 T cells, embryonic stem cells, human cancer cell lines, and cells including fibroblasts and neuroblastoma and glioblastoma cell lines derived from patient samples.
3) Lentiviral vectors

3.2) Lentiviral systems: the two types of viral system to be used employs two well characterised lentiviral vector and packaging cell lines.

3.2.1) Type A (eg. pFUV and tet-ON derivatives thereof), 3rd generation:
. The number of genes from HIV-1 that are used in this system has been reduced to three (i.e. gag, pol, and rev).
. Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids.
. Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain LTRs or the packaging sequence. This means that none of the HIV-1 structural and other components required for packaging the viral genome are separated onto four plasmids.
. Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
. The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.
. In some cases expression of our gene insert of interest will be drive expression off a tet-responsive CMV promoter, and regulated by a co-transduced lentivirus doxycyclin-responsive Tet regulator, giving an added level of safety for insert expression.

3.2.2) Type B (eg. LentiX0HTX from Clonetech), 4th generation
The lentiviral packaging systems mentioned above are 3rd generation versions that utilize split-genes to provide the viral packaging elements on individual plasmids that physically separate the viral envelope, env (usually VSV-G), sequence from the gag-pro-pol sequences. These split-gene packaging strategies reduce the risk of generating RCL because multiple recombination events are necessary to create a virus that harbours the sequences required for independent replication. Type B (Clontech’s Lenti-X HT) Packaging System also uses a split gene packaging strategy, but adds another level of safety by further uncoupling pol (RT and IN) from gag-pro. The result is that gag, pol and env reside on three physically distinct entities, rather than the standard two. This approach further reduces the possibility of creating RCL to a level below that of standard 3rd generation packaging systems, because extra recombination events are required to create such viruses. In fact, the emergence of RCL is undetectable from systems using this approach (Wu, X, et al. 2000) Mol. Ther. 2(1):47-55.) These improvements significantly increase the safety profile of our Lenti-X HTX Packaging System.

Clontech’s Lenti-X Vectors contain less than one-third of the wild-type HIV-1 genome. These wild-type sequences mainly consist of the viral LTRs and packaging signal. All essential replication genes have been completely removed and are instead supplied as separate DNA entities in the Lenti-X HT Packaging Mix (described above).

In brief, the number of genes from HIV-1 that are used in type B system has been reduced to four (i.e. gag, vpr-pol, rev and tat).
. Although the four packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293 T derived producer cell lines, none of them contain HIV-1 LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
. The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.
. In this system, expression of tat is required in the packaging cell line, because the lentivirus vector contains an intact HIV-1 5LTR, which allows very high titers in viral preparations.
In some cases expression of our insert gene of insert of interest will be driven expression off a tet-responsive CMV promoter, and regulated by a co-transduced lentivirus doxycyclin-responsive Tet regulator, giving an added level of safety for insert expression.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard.

Origin & function

Genes and mutant derivatives thereof, usually of human, mouse or Xenopus frog origin, encoding both growth control proteins and transcription factors, along with marker genes such as GFP and antibiotic resistance genes.

Evaluation of foreseeable effects

Some of the virus vectors will lead to growth arrest and differentiation of mammalian cells, while there is potential to promote cell proliferation. Because of the presence of the WPRE element in the lentivirus vectors, the lentivirus constructs might transform infected mammalian cells.

The main Hazard is potential infection of persons working in the lab by the GM virus, for which a Cat II containment level is adequate.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class I level of classification.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Only micro-organisms and tissue culture cells are involved.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class I level of classification.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Containment level 2 waste will be autoclaved (136°C for 10 min), before subsequent discharge down the drain into the public sewer or incineration. It is expected that this will give effectively 100% killing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This is a fairly typical lentivirus project, designed to introduce genes into mouse and human cells in vitro. It does not seem to raise any unusual issues
Project Ref 921/12.2

Identifying the molecular regulators of lymphatic-stroma interactions by modulation of genes of interest using viral vectors

Date Ackn'd 03/09/2013

Date Project Ceased

Class 2

Class Culture Vol Class 2 Culture Volume Class 3-4

≤ 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Project transferred from GM921 on 03/09/2013

Project Additional Information

Purposes of the contained use

The project aims to determine how candidate genes may function in tumour progression or stromal cell function (e.g. gp38). We will study their phenotypic and functional changes following modification: e.g. a) in cancer cells: the acquisition of malignant features such as increased motility and invasive capacity, b) in stromal cells: changes to junctional properties and interaction with infiltrating immune cells.

Recipient or parental organism

a) Murine cells (for example, isolated tumour and stromal cells and commercially available, immortalized well-established cell lines).

b) Human cells (including packaging cells e.g. HEK293, established well-characterised cancer and stromal cell lines).

Host/vector system

02/03/2022
1) E. coli, laboratory K12 derivatives/pUC based vectors;
2) Mammalian tissue culture cells including human cancer and mouse cell lines. Human hosts include for example, HEK293T packaging cells, well-established human cancer and stromal cell lines. Examples of mouse hosts include stromal cells such as fibroblasts (normal dermal, lymph node stromal and tumour-associated) and tumour cells either isolated or purchased as commercially available cell lines.

3) Viral vectors: The viral systems to be used employ well characterized vector systems packaging line
3.1) Lentiviral systems:
3.1.1) Commercially available, verified transduction ready viral transduction particles e.g. MISSION sequence verified shRNA lentiviral transduction particles (pLKO.1-puro).
3.1.2) Third generation lentiviral particle generation using the 3-plasmid method
   ● The number of genes from HIV-1 that are used in this system has been reduced to three (i.e. gag, pol, and rev).
   ● Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids.
   ● Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293T derived producer cell lines, none of them contain LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
   ● The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.

Virally transduced cells will not leave Class II containment until negative results are obtained from HIV-1 p24 ELISAS. This measures viral titres and ensures no residual viral proteins are present in the supernatant of transduced cells.

3.2) Adenoviral systems expressing Cre recombinase (AdCMV-Cre) or fluorescent marker proteins
3.2.1) Commercially sourced, pre-titred replication deficient virus constructs
   ● The adenoviral particles purchased will be replication incompetent due to deletions in the E1 and E3 regions. The virus arrives pre-screened to ensure replication deficiency.

3.2.2) Assembly of virus using the AdEasy system (or similar)
   ● Cre recombinase is a Type I topoisomerase from P1 bacteriophage that catalyzes site-specific recombination of DNA between loxP sites. P1 sequences are not found in the human genome, therefore cre-recombinase expression would be expected to have no impact on the cell
   ● Recombinant adenoviruses exhibit a wide tissue tropism and could potentially infect a range of mammalian cells, however, the particles being used are replication deficient due to elimination of E1 and E3 genes.
   ● The vectors do not integrate into the genome.

Origin & function
Gene constructs and mutant derivatives thereof, usually of human or mouse origin, a) encoding candidates genes deemed important in tumour progression and stroma function e.g. gp38, b) cre recombinases to mediate specific recombination events, along with marker genes such as GFP and antibiotic resistance genes.

Evaluation of foreseeable effects
Some of the virus vectors will lead to growth arrest and differentiation of mammalian cells, while there is potential to promote cell proliferation. Because of the presence of the WPRE element in the lentivirus vectors, the lentivirus constructs might transform infected mammalian cells.

The main hazard is potential of persons working in the lab by the GM virus, for which a Class II containment level is adequate.

After infection and selection, the manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Once negative viral titres have been measured, work with these genetically modified cells can be undertaken at a Class I level of classification.
Micro-organisms and tissue culture cells are involved

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Full containment level 2 will be applied.

After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class 1 level of classification.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

As per Hutchinson/MRC research Centre standard practice guidelines (unless the risk assessment demands more stringent measures)

CL2: all solid waste is autoclaved in the building and then incinerated off site by a contractor (Novus)

Liquid waste is disinfected with Virkon to final concentration of 5%; contact time of 30 minutes minimum before disposal to drain.

Bacterial Plates are autoclaved in the building.

Solid waste (including for example culture flasks, plastic pipettes) is bagged, and incinerated off site by a contractor (Novus).

It is expected that these measures will give effectively 100% killing.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

This is a fairly standard proposal to use lentivirus (for various genes) and adenovirus (for cre) to genetically manipulate murine and human cells. The inserts proposed do not seem particularly hazardous.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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Animal Units | Large Scale Activities | Human Clinical Applications
Project Additional Information

Purposes of the contained use

We plan to use viral vectors to insert or delete candidates identified by transcriptomics or metabolomics analyses as being important in cell metabolism of both epithelia and stromal cell during tumorigenesis. We will study their phenotypic and biochemical changes following modification: e.g. a) in cancer cells: the changes of their malignant features such as increased motility and invasive capacity and changes in core metabolic pathways, b) in stromal cells: changes of core metabolic pathways and transdifferentiation into cancer associated stromal cells.

Recipient or parental organism

Murine fibroblasts and murine kidney epithelial cells obtained from Fumarate Hydratase deficient mice, primary and immortalised cell lines and tissues from other genetically engineered mice and well characterised human normal and cancer cell lines (including HEK293T that will be used as packaging cells for virus production).

Host/vector system

1) E. coli, laboratory K12 derivatives/pUC based vectors;

2) Mammalian tissue culture cells including human and mouse cell lines. Human hosts include for example, HEK 293T packaging cells, well-established human cancer and stromal cell lines. Examples of mouse hosts include epithelial cells, stromal cells such as fibroblasts and tumour cells either isolated or purchased as commercially available cell lines.

3) viral vectors including retrovirus, lentivirus and adenovirus as follows:
RETROVIRUS: Modified cell lines will be created by the transduction with retroviral particles generated from appropriate packaging cell lines (see below) after their transduction with retroviral plasmids and will be based mostly on the following backbones:
1. The pBABE vector is derived from the Moloney murine leukemia virus (MMLV).
2. The pRETROSUPER vector is derived from the Murine Embryonic Stem Cell virus (MSCV).

LENTIVIRUS: Modified cell lines will be created by the transduction with lentiviral particles generated from appropriate packaging cell lines (see below) after their transfection with lentiviral plasmids and will be based mostly on the following backbones:
2. TRC1 and TRC2 pLKO.1-puro
3. pGIPz
4. pTripz
5. Transduction ready particles generated from the above and purchase directly from the suppliers

ADENOVIRUS: modified cells will be obtained by direct infection with commercially available adenoviral particles of adenoviral particles generated as follows.

1) Commercially sourced, pre-titred replication deficient virus constructs
   • The Adenoviral particle purchased will be replication incompetent due to deletions in the E1 and E3 regions. The virus arrives pre-screened to ensure replication deficiency.
2) Assembly of virus using the AdEasy system (or similar)

Origin & function
Gene constructs and mutant derivatives thereof, usually of human or mouse origin, a) encoding candidates genes deemed important in cell metabolism, b) cre recombinases to mediate specific recombination events, along with marker genes such as GFP and antibiotic resistance genes. c) fluorescent markers targeted to different subcellular compartments.

Evaluation of foreseeable effects
ADENOVIRUS
The adenoviral particles purchased will be replication incompetent due to deletions in the E1 and E3 regions. The virus arrives pre-screened to ensure replication deficiency.
Cre recombinase is a Type 1 topoisomerase from P1 bacteriophage that catalyzes site-specific recombination of DNA between loxP sites. P1 sequences are not found in the human genome, therefore cre-recombinase expression would be expected to have no impact on the cell.

Cells isolated from murine tissues with loxP sites will be from normal or tumour-associated tissues and are expected to be similar in nature to established fibroblast cell lines. Therefore, these calls are not anticipated to pose a hazard to human health.

Recombinant adenoviruses exhibit a wide tissue tropism and could potentially infect a range of mammalian cells, however the particles being used are replication deficient to elimination of E1 and E3 genes.
These Vectors do not integrate into the genome.

RETROVIRUS AND LENTIVIRUS

The main hazard arises directly from the recombinant viruses produced from the packaging cells. These yield both ecotropic and amphotropic lentivirions and ecotropic and
amphotropic retrovirions, depending upon the envelope proteins supplied by the cell. Some of the virus vectors will lead to growth arrest and differentiation of mammalian cells, while there is potential to promote cell proliferation. Retroviruses and lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. To further reduce the risks of virus utilization, we will

1. Use retroviral infections targeted to murine cell or human cells designed to express ecotropic receptors

2. Use third generation lentiviral expression vectors, where genes encoding the structural and other component required for packaging the viral genome are separated onto three plasmids. All three plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus.

Some of the indicated vector might contain the WPRE (Woodchuck hepatitisB virus post transcriptional regulatory element). This element is used to increase lentiviral vector titre and gene expression. It includes the promoter for the X-protein and the part to the coding sequence for such protein, which may have oncogenic properties. Whenever possible we aim to use WPRE-less vectors such as the TRC-1-pLKO.1, thus limiting the oncogenic potential of the viral particles.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**Micro-organisms and tissue culture cells are involved**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Containment level 2 will be applied only in specific circumstances such as:**

1. The use of lentivirus containing the WPRE elements
2. The modulation of the expression of known or suspected oncogenes or tumour suppressors.
3. The expression of toxic or inflammatory (e.g. cytokines) genes

Other infection and virus production work will be carried out in class I.

After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will no longer represent an infection hazard. Work with these genetically modified cells can be undertaken at a Class 1 level of classification.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

As per Hutchinson/MRC Research Centre standard practise guidance (unless the risk assessment demands more stringent measures).

CL2: all solid waste is autoclaved in the building and then incinerated off site by a contractor (Novus).

Liquid waste is disinfected with Virkon to final concentration of 5%; contact time of 30 minutes minimum before disposal to drain.

Bacterial Plates are autoclaved in the building.

Solid waste (including for example culture flasks, plastic pipettes) is bagged, and incinerated off site by a contractor (Novus).

It is expected that these measures will give effectively 100% killing.
This is a fairly standard proposal to use lentivirus (for various genes) and adenovirus (for cre) to genetically manipulate murine and human cells, but the human host range virus work falls on the borderline between class I and class II: innocuous inserts may be transduced in class I, but toxic, pathogenic or oncogenic inserts or vector components require class II.

Since we discourage use of Class II where it is not necessary - to minimize the number of people exposed to more hazardous material particularly clinical material - we consider that it is helpful to identify much of this work as acceptable in class I.

Most of the inserts are not overly hazardous, being marker genes or genes involved in metabolism, so in these cases use of lentivirus that lacks the WPRE element in class I seems acceptable. However, class II is required for experiments involving vectors with the WPRE element, or with inserts that might be oncogenic (including knockdown constructs for a tumour suppressor gene) or toxic or inflammatory (such as cytokines, peptide hormones).

**Project Containment**

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**Project Ref** 921/12.4

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<td>Roles of cancer genes studied by lentivirus and retrovirus-mediated gene manipulation in vitro</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

The overall aim of the lab is to study genes that are altered by structural rearrangement of the cancer genome: these can be inactivated tumour suppressor genes or fusion genes analogous to those traditionally associated with leukaemias.

This project is to investigate the functions of these candidate genes by manipulating their expression using siRNA or expression of cDNAs in cultured cells. We plan to use a number of human cancer and normal cell lines, principally breast and ovarian, that can be particularly difficult to transfect by standard methods. We therefore plan to use viral vectors. The work will be carried out in vitro.

Description of procedure: cDNAs or shRNAs that target the gene of interest will be cloned into retroviral and lentiviral vectors. Recombinant retro/lentiviral vectors carrying the gene or shRNA of interest will be packaged in virus packaging cells. The viruses harvested will be used to infect human or mouse cell lines. The transformed cell line will be used in vitro for functional assays such as cell cycle, apoptosis and cell-mobility assays.

Recipient or parental organism

E. coli and established human and mouse cell lines in vitro. We may also use human cell lines engineered to express the ecotropic receptor (but such lines will not be constructed under the risk assessment).

Host/vector system

The host/vector systems are (i) plasmids in E. coli for construction steps; (ii) transfection of retrovirus or lentivirus vector into packaging cell line systems to generate virus; and (iii) infection, with the packaged retroviral or lentiviral constructs, of human or mouse cell lines. Retrovirus/lentivirus and the packaging lines will be chosen to match the target cells and to repeat published experiments. Broadly, lentivirus will be used on less vigorous human cancer cell lines.

i) Generation of the construct to express the genes of interest.

INSERT: the insert is chosen to modulate the expression levels of genes of interest by overexpression or downregulation. We will begin for example with NRG1, which has been proposed as both a tumour suppressor gene and an oncogene. However, we will apply this technology to other known and candidate cancer genes. We will include knockdown of the known tumour suppressor gene, p53, and overexpression of the known oncogene, Ras, as controls.

a) Constructs for overexpression: these constructs are engineered using cDNAs that will encode for candidate cancer genes and rearranged forms of these genes, such as fusions of these genes, internal partially duplicated mutants and deletion mutants. These inserts will be either purchased or designed ad hoc and inserted into lentiviral or retroviral vectors.

b) Constructs for downregulation of gene expression: these constructs are shRNAs that will downregulate the expression of chosen target genes. These constructs will be purchased or designed ad hoc and inserted into lentiviral or retroviral vectors.

For custom design of shRNAs, to be cloned into the MSCV-miR30 vector, we will use the Hannon lab shRNA retriever (http://katahdin.cshl.org/siRNA/RNAi.cgi?type=shRNA)

We will also use microRNA-adapted shRNAs constructs already cloned into appropriate plasmids (see below) mostly, but not exclusively from Open Biosystems (thermoscientific)
VECTOR: the vectors described here will be used to express the insert in proliferating and non-proliferating mammalian cells by means of viral transduction and could be either retrovirus- or lentivirus-based.

RETROVIRAL
The retrovirus vectors to be used are derived from Moloney murine leukaemia virus (MMLV) and its relative Murine Embryonic Stem Cell virus (pMSCV). Currently, these are pBABE, MSCV-miR30, pRETROSUPER and LPC. For all the vectors, the env genes, provided by the packaging plasmid, determines host range.

Vectors mainly for cDNAs.
1. pBABE (Morgenstern & Land) is derived from Moloney murine leukaemia virus (MMLV). The vector provides the viral package signal, transcription and processing elements, and a target gene. Transfection into a package cell line, such as Phoenix, produces high titre replication-incompetent viruses.
2. LPC system. The LPC vector was constructed at CSHL by S L (Cold Spring Harbor Lab) for efficient expression of cDNAs.

Vectors Specifically designed for shRNA inserts:
3. The MSCV-miR30 vector (also developed by S L's lab) is derived from the Murine Embryonic Stem Cell virus (pMSCV), closely related to MMLV. The vector contains the mir 30-styled shRNA expression cassette expressed from the U6 promoter. The vector has a specifically designed 3'LTR that has a deletion in the LTR promoter elements. This deletion results in inactivation of the LTR mediated transcription upon retroviral integration. The phosphoglycerate kinase (PGK) promoter drives the expression of the puromycin resistance gene for selection in eukaryotic cells. The MSCV-miR30 plasmid can be propagated in E. coli under ampicillin (AMP) selection.
4. pRETROSUPER vector is also derived from the Murine Embryonic Stem Cell virus (pMSCV) and is commercially available.

Either cDNA or shRNA:
5. pOZ-FH-C is a retrovirus vector that has been used to coexpress an insert of interest with IL-2, so that infected cells can be affinity purified by IL-2 antibody (Nakatani and Ogryzko, 2003).
6. pWZL, a commercially available retroviral vector available with various selection markers, made by J M, the maker of pBABE.
7. Related established retroviral vectors to achieve coexpression with marker genes.

LENTIVIRAL
For cell lines where proliferation is limited we may need to use lentiviral vectors. We will use metabolisation-defective lentivirus (third-generation lentiviral vectors). One packaging plasmid lacks both LTRs, viral packaging signal (y) and viral genes (env, tat, rev, vpr, vpu, vif and nef). Rev is supplied in-trans on a second plasmid. Envelope is expressed on a third plasmid. The vector to be packaged has a self-inactivating LTR and expresses no viral gene products.

Lentiviral vectors used include:
1. PLKO vector is widely used and commercially available - see below.
2. Lenti-miR30 is a vector adapted by CSHL to express shRNAs as part of simulated microRNAs. Other lentiviral vectors in current use by our colleagues may also be used. They include:
1. pRSI-U6-(sh)-UbiC-RFP-2A-Puro: HIV-based lentiviral shRNA cloning vectors with H1, U6, or H1 tet-regulated promoters for expression of shRNA and a choice of a single or dual selection marker (GFP, RFP, Pyr, BleoR, NeoR, Hygro-HK, etc.) expressed from a single CMV EF1α, PGK, UbiC, or other promoters. It contains 18-nt bar code compatible with the Illumina HT Sequencing platform.
2. pLKO1-puro: The viruses generated using these plasmids were used to generate the TRC1 and TRC2 collection of commercially available shRNAs. This construct contains a 3' self-inactivating long terminal repeat. pLKO-puro is available in two forms. The TRC1-pLKO vector does not contain WPRE (Woodchuck hepatitis B viruspost-transcriptional regulatory element), while the TRC2-pLKO vector does not contain it.
3. pGIPz: this vector contains a TurboGFP expressed by an IRES in tandem with the shRNA. These constructs contain a WPRE.
4. pTripz-inducible shRNAs plasmids with TurboRFP that marks inducible shRNAmir expression
5. Transduction ready particles generated from the above and purchased directly from the suppliers.
Generation and expansion of plasmids carrying construct/gene of interest

cDNAs and shRNA plasmid constructs will be generated by subcloning cDNAs and shRNAs of interest into the empty backbones indicated above.

VECTOR: Recombinant lentiviral and retroviral plasmids will be transformed and amplified in laboratory strains of E. coli after selection with the appropriate antibiotics. Lentiviral and retroviral plasmid DNA will be purified by maxi prep.

HOST: E. coli: laboratory TOP10 chemically competent E. coli will be used for selection and amplification of plasmid DNA.

(ii) Transfection of retrovirus or lentivirus vector into packaging cell line systems to generate virus.

RETOVIRUS

Cell lines such as Phoenix cells (http://www.stanford.edu/group/nolan/retroviral_systems/phx.html) will be used as packaging cell lines. Phoenix, based on the 293T cell line, is a second-generation retrovirus producer line for the generation of helper free ecotropic and amphotropic retroviruses and has stably integrated three plasmids that provide the retrovirus proteins in trans. In order to infect human cell lines we will use the packaging cells Phoenix Ampho. Also, well characterised and commercially available PT67 cells with appropriate tropism would be used if the Phoenix system proves inadequate.

Virus will be packaged by transient transfection.

LENTIVIRUS

Lentivirus will be packaged by co-transfection of lentivirus vector and packaging plasmids into 293T cells.

(iii) Infection, with the packaged retroviral or lentiviral constructs, of human or mouse cell lines.

VECTOR: Replication deficient lentiviral or retroviral transduction particles harbouring construct/gene of interest and appropriate resistance gene or fluorescent marker. These particles can be obtained as indicated above or purchased as 'Ready-to-use lentiviral particles' from certified sources.

HOST: Well characterised human normal and cancer cell lines, mouse cell lines, or human cell lines engineered to express the ecotropic receptor. Successfully transduced cells will be selected with appropriate antibiotics and in the case of inducible of an inducible system activated by doxycycline.

Determination of viral titre in packaging cells and GM-cells supernatant

Generally, we will determine the viral titre of transducing particles suspension only if a specific MOI is required for the infection. We will determine the viral titre in the supernatant of the genetically-modified cells to assess the suitability to transfer infected cells from CL2 to CL1, when CL2 tissue culture has been used for the infection. To determine the viral titre of cell supernatant we will use well characterised methods such as the Molecular Probes' EnzChek® Reverse Transcriptase Assay, a convenient, efficient and inexpensive assay for measuring reverse transcriptase activity, a direct readout of the presence of viral particles.

Genes will be genes that are mutated or rearranged in cancers, from human or mouse, and may be in normal form or mutant/rearranged variants of the genes, such as fusion genes. cDNAs-either cloned or synthesised- or shRNAs that target the gene of interest will be cloned into retroviral and lentiviral vectors.

Evaluation of foreseeable effects

HAZARD IDENTIFICATION: Host

MODIFIED BACTERIA TO AMPLIFY THE CONSTRUCTS

Laboratory strains of modified E. coli K12 such as TOP10 are recognised as non-colonising and may be considered to be ACDP hazard group 1.

CELL LINES

02/03/2022 Page 13395 of 15326
Phoenix cells are widely used and they have a history of safe usage. The gag-pol and env genes are expressed from non-moloney promoters to minimise recombination potential. Different promoters for gag-pol and envelope were used to minimise their inter-recombination potential. Amphotropic Phoenix cell lines have been extensively tested for helper virus production and established as being helper-virus free. The 293T cell line, in addition to other well characterised human normal and cancer cell lines, has a history of safe usage and is not expected to represent a hazard to human health.

HAZARD IDENTIFICATION: Vector
RECOMBINANT RETRO/LENTIVIRAL VECTORS
Retroviruses and lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission.

1. The retroviral vectors used are replication defective. To produce a replication-competent retroviral genome three recombination events are required between the vector of interest, the gag-pol regions of the packaging cell line and introduction of the split genome packaging cell lines.

2. We will use third generation lentiviral expression vectors, which contain multiple safety features, as follows;

   • The majority of lentiviral genes have been eliminated (Δ vpr, vif, vpu and nef). The number of genes from HIV-1 that are used in this system has therefore been reduced to three (i.e. gag, pol, and rev).
   • Genes encoding the structural and other components required for packaging the viral genome are separated onto three plasmids. All three plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al J Virology 72 8463-8471 1998).
   • Although the packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gag, pol, rev, env) in the 293T derived producer cell lines, none of them contain 3’ LTRs. This results in elimination of the promoter-enhancer region, which avoids promoter interference issues and further negates the possibility of viral replication.
   • No single plasmid contains all the genes necessary to produce packaged lentivirus. None of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
   • The lentiviral particles produced are replication-incompetent and only carry the gene of interest. No other viral species are produced.

Some of the indicated vector might contain the WPRE (Woodchuck hepatitis B virus post-transcriptional regulatory element). This element is used to increase lentiviral vector titre and gene expression. It includes the promoter for the X-protein and the part to the coding sequence for such protein, which may have oncogenic properties.

The main hazard arises directly from the recombinant retroviruses and lentiviruses produced from the packaging cells. These yield amphotropic lentivirions and retrovirions.

HAZARD IDENTIFICATION: Insert
Our genetic experiments will include knockdown of known (p53) and potential tumour suppressor genes (e.g. NRG1) and overexpression of known (Ras) and potential oncogenes (e.g. NRG1 again). However, at least 4 different genes should be expressed for tumorigenesis in human tissues (W.C. Hahn et al., Nature 400, 464) and we expect that a contamination of more than 4 virus supernatants would be extremely unlikely.

None of the cell lines used is capable of colonising a healthy individual and we do not expect any of our proposed manipulations to change this. The amphotropic retrovirus and lentivirus would be able to infect human but could not replicate unless the improbable event occurs that it recombines with endogenous retroviruses. Even then, it is unlikely that expression of a single oncogene or suppression of a tumour suppressor could cause tumours, as multiple genetic alterations (between 4 and 6) are required for inducing tumours, and safeguard mechanisms exist impeding the accumulation of these alterations in the organisms.

HAZARD IDENTIFICATION: Lab Personnel
The principal hazard is gene transfer to human cells when using the human host-range packaged viruses. Attention should be paid to staff awareness of this and to storage and labelling of any stocks. Virus will be removed from the lab Catll rooms only for storage and kept in designated -80°C freezers according to the agreed guidelines and
SOPs in the Hutchinson/MRC Research Centre. Work will therefore be done in:
Class II: production and infection work with lentivirus and amphotropic retrovirus.
Cell lines produced by infection that are virus-free after infection using the methods indicated above will be transferred to Class I for work with plasmids in E. coli.

HAZARD IDENTIFICATION ENVIRONMENT
The prokaryotic and eukaryotic cells and cell lines are highly disabled and the possibility of competent virus being generated is negligible. Therefore they should be unable to propagate in the environment. The plasmid vectors are non mobilisable, therefore neither has any possibility of further spread.
The viruses are replication-incompetent and no live virus should reach the environment.
The risk to the environment is therefore effectively zero

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Containment level 2 will be applied to work packaging and infecting with the human host-range viruses.
Infection and virus production of ecotropic retrovirus will be carried out in class 1.
After infection and selection, the stably manipulated cells will no longer be harbouring propagatable virus, so they will be transferred to class 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
CL2: all solid waste is autoclaved in the building and then incinerated off site by a contractor (Novus).
Liquid waste is disinfected with Virkon to final concentration of 5%; contact time of 30 minutes minimum before disposal to drain.
CL1: Liquid waste is disinfected with Virkon to final concentration of 1%; contact time of 30 minutes minimum before disposal to drain.
Bacterial Plates are autoclaved in the building.
Solid waste (including for example culture flasks, plastic pipettes) is bagged, and incinerated off site by a contactor (Novus).
It is expected that these measures will give effectively 100% killing

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
This is similar to many Ras for using non-replicating lentivirus and retrovirus vectors to express genes and shRNAs in human cell lines in vitro, with Class II for production and infection by human host-range virus vectors. It is quite broadly based as a variety of genes and host cells may be used, but raises no particular issues.

Since the applicant group leader is the BSO (competent person) note that the committee that has considered this risk assessment includes two others with long experience of GM risk assessment. Dr P B, formerly of MRC-LMB, and Dr P J, the previous BSO for this building.

**Project Containment**

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**Name**

CHRONOS THERAPEUTICS LTD

**Campus Estate or Research Centre**

FRIEZE FARM

**Road Name**

WOODSTOCK ROAD

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX2 8JX

**Country**

ENGLAND

**Tel Number**

01865 518910

**Fax Number**

01865 511418

**E-mail**

blank

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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<td>WOODSTOCK ROAD</td>
<td>OXFORD</td>
<td>OXFORDSHIRE</td>
<td>OX2 8JX</td>
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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee comprises the Scientific Director, the Laboratory Manager, the Office Manager and the Chronos Research Fellow

<table>
<thead>
<tr>
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Other (please specify)  
Tick if confidential

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<th>Microbiology Research</th>
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<th>Transgenic Fish</th>
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<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</table>

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Contaminated waste will be placed into sealed containers which will then be removed from the site by an independent contractor. The contractor will be a specialist waste management company who will dispose of the waste in accordance with all relevant legislation and guidelines applicable to clinical waste. The contractor will be required to provide documentation confirming that the method of disposal complies with the relevant legislation.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

To the best of our knowledge the activities described here present negligible risk to both the environment and to workers.
<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
<th>28/08/2013</th>
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**Name**

INVISTA TEXTILES (UK) LTD

**Campus Estate or Research Centre**

THE WILTON CENTRE

**Road Name**

THE WILTON CENTRE

**District**

WILTON

**Town**

REDCAR

**County**

NORTH YORKSHIRE

**Postcode**

TS10 4RF

**Country**

ENGLAND

**Tel Number**

01642 431281

**Fax Number**

01642 431291

**E-mail**

blank

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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<td>THE WILTON CENTRE</td>
<td>LABORATORIES D210, D212 &amp;D228, D220, 218 &amp;206</td>
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<td>REDCAR</td>
<td>NORTH</td>
<td>YORKSHIRE</td>
<td>TS10 4RF</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Biological Safety Committee (BSC) is comprised of five members. The members have been chosen from senior management, bioscience and biochemical engineering functions. All members have PhD. Degrees in their respective fields, i.e. biochemistry, molecular genetics and chemical engineering. GMM risk assessments are reviewed and approved via e-mail as per procedure S01E01P01 - Genetically Modified Microorganism Risk Assessment Procedure for Laboratory Scale Activities. Briefly, the Principal Investigator submits the completed GMM risk assessment form to the Biological Safety Officer (BSO), who forwards the form for review and comment by the BSC. Once any review comments have been addressed satisfactorily, the BSO approves the GMM risk assessment on behalf of the BSC. The BSC meets once per month in person to discuss the maintenance of standards and continuous improvement with regard to contained use of GMMs.

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</table>

Tick if confidential

02/03/2022
Depending upon chemical composition, aqueous biological waste shall be classified as either conventional or special waste. All aqueous biological waste shall be sterilised using a validated autoclave cycle prior to disposal. The temperature profile for the autoclave cycle is logged by the autoclave and the trend archived. In addition to specialist quarterly and annual servicing, the waste autoclave cycle shall be revalidated once per annum using e.g. Attest indicators. Autoclaved conventional waste shall be disposed of via laboratory drains and autoclaved special waste shall be collected for disposal by authorised waste disposal specialists.

All solid waste shall be double bagged in biohazard bags ensuring steam access to the solid waste and autoclaved using autoclave tape as an indicator of sterility. Depending upon chemical composition, the solid waste shall be classified as either conventional or special waste.

Spills shall be contained using absorbant materials and spill kits (as appropriate) and disposed of with solid waste. Affected areas (e.g. bench-top) shall be sanitised with e.g. isopropanol or similar biocide (Virkon), contacting the areas with liquid disinfectant for a minimum of 15 [min]. Gass laboratory fermenters shall be bunded as appropriate.

Glass ware and other re-usable equipment contacting GMMs shall be either autoclaved prior to washing or sprayed with e.g. isopropanol or similar suitable biocide (Virkon), contacting the affected surfaces with liquid disinfectant for a minimum of 15 [min] prior to washing.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The below revisions to the original GMM risk assessment were added to the assessment as per advice from the Biological Safety Committee members:

"This is a proprietary strain to be provided by Life Technologies, Grand Island, New York, and is designed specifically as a laboratory strain to host genetically modified plasmids. The strain will be used in accordance with the manufacturer's instructions."

"A DNA sequence will be generated from this amino acid sequence that is optimised for E. coli expression and this will be synthesised by a commercial company."

"This vector is a proprietary expression vector to be supplied by Life Technologies, Grand Island, New York, and is designed specifically as a laboratory expression vector. This will not be modified beyond insertion of the synthesised gene sequence in accordance with the manufacturer's instructions."

"The expression vector is designed to over produces the enzyme of interest; in this case we would expect the quantity of MDD to be in excess of 100 fold its normal cellular concentration. However, there is no evidence to suggest this will increase to potential for hazard or increase the survivability of the modified organism."

"Clear up will involve disinfecting the area using the proprietary anti viral and anti bacterial agent Virkon."

"Loss of selective pressure is likely to result in loss of the ampicillin resistance plasmid as replication of the plasmid represents an energetic disadvantage."
**Data Premises Notified (Originally)**
- 26/09/2013

**Transferred from 1992 Regs?**
- N

**Emergency Plan Required?**
- N

**Transitional Premises**
- N

**Non-GMMs**
- N

**Withdrawn**
- N

**Name**
- TAPTON SCHOOL

**Name 2**
- 

**Department**
- 

**Campus Estate or Research Centre**
- 

**Road Name**
- DARWIN LANE

**District**
- 

**Town**
- SHEFFIELD

**County**
- YORKSHIRE

**Postcode**
- S10 5RG

**Country**
- ENGLAND

**Tel Number**
- 0114 2671414

**Fax Number**
- 0

**E-mail**
- 

**HSE Division**
- blank

**Comments**
- 

**Date at Which Additional Info Submitted**
- 02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Biosafety Committee
University of Sheffield

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Tick if confidential

Bacteriology Yes  Parasitology  Transgenic Birds  Microbiology Research Yes
Yes

All material which is known to degrade on autoclaving will be autoclaved and the remainder will be disinfected with Virkon in line with HSE recommendations. We will confirm at the start of the procedure and on a yearly basis that the disinfectant is effective by plating out bacteria on LB plates and checking that no colonies are formed.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
### Data Premises Notified
- **Date:** 12/11/2013

### Data Premises Closed
- **Date:**
- **Emergency Plan Required?** No

### Name
- **SPHERE FLUIDICS LIMITED**

### Campus Estate or Research Centre
- **BABRAHAM RESEARCH CAMPUS**

### Road Name
- **BABRAHAM**

### Town
- **CAMBRIDGE**

### District
- **BABRAHAM**

### County
- **CAMBRIDGESHIRE**

### Postcode
- **CB22 3AT**

### Country
- **ENGLAND**

### Tel Number
- **01223804202**

### Fax Number
- **01223804210**

### Date at Which Additional Info Submitted
- **02/03/2022**
### Premises Addresses

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<td>CAMBRIDGE</td>
<td>CB22 3AT</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The safety committee is composed of the CEO of Sphere Fluidics, the principal scientist in the Molecular and Cell Biology department within Sphere Fluidics, a senior scientist in the Molecular and cell Biology department within Sphere Fluidics and the Biological safety officer of Babraham research Campus.

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<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
<td>Yes</td>
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02/03/2022
Every plastic (pipettes microcentrifuge tubes) and glass wear will be inactivated with 1% virkon and disposed in a 10L yellow eurobin or autoclaved for recycling.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 3206/16.1**

Date Ackn'd 18/02/2016

CU2 Project Title The generation and use of lentiviral particles for mediating gene modulation in mammalian cells

Class 2

Culture Vol Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
**Purposes of the contained use**

To enable the generation and use of recombinant self-inactivating third generation lentiviral particles (by commercial research organisations or in house using commercially available 3rd generation lentivirus systems) encoding:

1. Open Reading Frames (ORFs), cDNAs, peptides
2. Specific gene sequences
3. Short hairpin RNAs (shRNAs) for the knockdown of Open Reading Frames (ORFs), cDNAs or specific gene sequences by RNA Interference (RNAi)

for in vitro use and for the generation of stable mammalian cell lines (and subsequent use of such stable lines in vitro)

**Recipient or parental organism**

Recipient experimental systems are cells derived from mammalian organisms, predominantly in the form of immortalized cell lines derived from human cancer tissues.

The mammalian cell lines fall into two types:

a) A packaging/helper cell line into which plasmids containing lentiviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines i.e. a negligible risk.

**Host/vector system**

The lentiviral vectors which will be used are derived from HIV-1, which is an ACDP Hazard Group 3 biological agent. However, second and third generation lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase biosafety and viral packaging is achieved by providing three helper constructs in trans containing gag, pol and rev sequences.

For example, second and third generation Lentiviral Expression Systems include the following key safety features:

- In the second and successive generation lentiviral vectors several lentiviral accessory genes (vif, vpr, vpu and nef) are deleted from the transfer plasmid since they are not required for in vitro replication and the products they encode have cytotoxic activities.
- In addition to this in the third generation lentiviral vectors:
  - The Lentiv expression vectors contain a deletion in the 3’ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
  - The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).
  - Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).
  - Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication competent virus can be produced.

Despite the above safety features, use of these lentiviral vectors (which include WPRE) falls within SACGM 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. Also, the virus will be packaged by transfecting transfer vector into specific amphoteric ‘helper’ cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types.

This means that the viruses produced for this experiment could potentially infect a number of species, including man.

**Origin & function**

Selectable markers – examples (but not restricted to);

- Ampicillin resistance: E. coli derived
Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
Puromycin resistance (PAC): Puromycin acetyl transferase is derived from Streptomyces alboniger

Reporter proteins such as (but not restricted to);
- Fluorescent proteins as reporters:
  - GFP derived from the jellyfish Aequorea victoria and variants of this
  - Luciferase – class of oxidative enzymes used in bioluminescence
  - Renilla luciferase derived from the Sea pansy (Renilla reniformis)
  - Firefly luciferase derived from the firefly Photinus pyralis.

Open reading frames, cDNAs and gene sequences encoding GFP or Luciferase proteins and/or shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding GFP or Luciferase proteins).

Evaluation of foreseeable effects

The lentiviruses are, at worst, amphotropic or pseudo typed with VSV G protein, either of which confers a broad host tropism including human cells. However, the retrovirus is self inactivating and there if thus no possibility of it multiplying further.

Since some of the inserted DNA could code for potentially hazardous RNA or protein, the work is assessed as Class 2. This accords with HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.18-20).

Even for the non oncogenic inserted DNA, there is a slight but non negligible risk due to the presence in the lentiviral vector of the Woodchuck Post-transcriptional Regulatory Element. The WPRE containing vector DNA will be treated as potentially oncogenic and is assigned to class 2 (see HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.13)).

However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle:
- The genes encoding structural and other components of the viral genome have been separated. These genes have been engineered to minimise the risk of recombination that could lead to production of a replication-competent virus.
- The packaging cell lines allow expression of proteins, required to produce progeny virus: But the transfer vector is the only genetic material transferred to the target cells, consequently these cells cannot produce the proteins which are essential for viral assembly and infectivity.

Second or third generation retrovirus vectors will be used in all experiments

Procedures and controls measures will therefore follow HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.30-36) i.e. using multiple plasmids with minimum sequence homology (e.g. our 2nd or 3rd generation lentivirus vector systems), gloves should be worn, use of class II safety cabinets, sharps avoided and all wastes be rendered harmless before disposal etc.

It is not thought that the modified virus would pose a serious risk to animals or plants in the environment. Although the VSV coat protein permits invasion of other mammalian cells, as in the case of humans, infection would be restricted to primary cells and productive virus would not be produced. In addition the control measures to protect human health will minimise release of virus to the environment. Therefore the environmental risk is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:
1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed – Their GM authorization is GM898) according to disposal notification GM105/4.1.
Waste from our GM work at Class2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Eurobin container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.

This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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<td>L3 L3 L3 L4 L3 L4</td>
<td>L3 L3 L3 L3 L4</td>
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</table>
The aim of this project is to use retroviral delivery systems to introduce various mammalian DNA sequences into mammalian cells in culture. These sequences direct expression of either (i) RNA species (siRNAs or antisense RNAs) that interfere with the expression of various mammalian proteins or (ii) the corresponding mammalian proteins themselves; for in vitro use and for the generation of stable mammalian cell lines (and subsequent use of such stable lines in vitro) with the aim of generating stable cell lines expression fluorescent proteins, potential drug targets or therapeutic proteins, etc.

Recipient experimental systems are cells derived from mammalian organisms, predominantly in the form of immortalized cell lines derived from human cancer tissues. The mammalian cell lines fall into two types:

a) A packaging/helper cell line into which plasmids containing retroviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines i.e. a negligible risk.

Host/vector system

The retroviral vectors which will be used are derived from either Moloney Murine sarcoma Virus (MoMLV), Mouse
Mammary Tumour Virus (MMTV) or Feline Leukaemia Virus (FeLV), all of which are ACDP Hazard Group 1 biological agents.

- On the other hand, the virus will be packaged by transfecting transfer vector into specific amphoteric ‘helper’ cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types.
- This means that the viruses produced for this experiment could potentially infect a number of species, including man.

However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle:

- The genes encoding structural and other components of the viral genome have been separated. These genes have been engineered to minimise the risk of recombination that could lead to production of a replication competent virus.
- The packaging cell lines allow expression of proteins, required to produce progeny virus: But the transfer vector is the only genetic material transferred to the target cells, consequently these cells cannot produce the proteins which are essential for viral assembly and infectivity.
- Second, third generation or Self INactivating vectors retrovirus vectors will be used in all experiments (see SACGM compendium of guidance part2, section 2.11 (Retroviruses) pp117

Selectable markers – examples (but not restricted to);
- Ampicillin resistance: E.coli derived
- Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene

Puromycin resistance (PAC) : Puromycin acetyl transferase is derived from Streptomyces alboniger

Reporter proteins such as (but not restricted to);
- Fluorescent proteins as reporters;
- GFP derived from the jellyfish Aequorea victoria and variants of this
- Luciferase – class of oxidative enzymes used in bioluminescence
  - renilla luciferase derived from the Sea pansy (Renilla reniformris)
  - firefly luciferase derived from the firefly Photinus pyralis.

Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and / or shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins) – all human derived.

This could include the expression of potentially harmful genes e.g. encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation.

Evaluation of foreseeable effects

Although these retroviruses are categorized at ACDP 1, a number of factors could increase the containment that will be required to work with them. We therefore need to consider worst case scenarios:

The virus is packaged using an amphoteric system to allow transfection of human cells and this may have knock on effects when considering

i) Biological properties of the gene product

ii) The expression characteristics and

iii) Provirus insertion (see below)

i) In the case of the introduction of retroviruses carrying DNA sequences encoding either antisense or RNA species into cells it is possible to envisage a scenario in which the knockdown of the mRNA targeted by the siRNA or antisense RNA, and hence the reduction in level of the encoded protein will have an effect on cell metabolism.
Perhaps the most extreme example of this would be the reduction in the levels of a protein that normally facilitates cell apoptosis i.e. a pro-apoptotic protein. In this circumstance, cells that would normally die could survive. Even in this case the risk of a serious effect is unlikely to be high as firstly, before cells could begin to proliferate in an uncontrolled manner, accumulation of additional mutations would need to occur, and secondly a large body of previous work on siRNAs suggests that the overall level of "knockdown" achieved does not generally exceed 80% and hence complete growth control is unlikely to be lost. More caution is required with respect to retroviruses encoding mammalian proteins. The worst case scenario is likely to be expression of a potential oncoprotein. Clearly this could result in transformation of infected cells.

However, over-expression of proto-oncogenes in primary mammalian cells tends to result not in transformation; instead the result is either the induction of cellular senescence or the activation of apoptotic pathways resulting in cell death.

ii) The promoter could be highly active in a wide range of cell types (e.g. the CMV promoter).

iii) (From the SACGM compendium of guidance part 2, page 121) 'The effects of integration upon the infected cell should be considered. For instance, promoter sequences present in the provirus might activate genes adjacent to the integration site or, alternatively, insertion may disrupt genes and prevent their expression. Therefore, retrovirus infection might induce permanent changes in a cell, resulting in tumourigenesis. It is recognized that, in humans, this appears so far only to have occurred in the context of deliberate transduction of large numbers of stem cells with a retrovirus vector. Furthermore, the transferred gene enabled the cells to proliferate in response to cytokines, and the many ensuing cycles of cell replication may have allowed additional, co-operating events to occur. High-titre inoculations are required to establish a clinically significant level of infection or gene transfer, and accidental infections of this magnitude are unlikely during standard laboratory-based manipulations of retroviruses. Nevertheless, retrovirus vectors have been shown to have transforming properties in vivo and a cautious approach to handling them is advised.'

Whilst it is possible that production of individual siRNAs or antisense RNAs could disrupt normal function in a single infected cell, it is unlikely that this effect will propagate further. The worse case scenario might be the reduction in the levels of a protein that normally facilitates cell apoptosis i.e. a pro-apoptotic protein. In this circumstance, cells that would normally die could survive. Even in this case the risk of a serious effect is unlikely to be high as firstly, before cells could begin to proliferate in an uncontrolled manner, accumulation of additional mutations would need to occur, and secondly a large body of previous work on siRNAs suggests that the overall level of "knockdown" achieved does not generally exceed 80% and hence complete growth control is unlikely to be lost.

More caution is required with respect to the retroviral vectors directing expression of mammalian proteins. The worst case scenario is likely to be expression of a potential oncoprotein. Clearly this could result in transformation of infected cells.

The control measures in place to protect human health should result in minimal release of the GMM and therefore little associated environmental effects. Although the retroviral vector is capable of infecting a wide range of mammalian cells the virus will be incapable of replicating in those cells and therefore no spread of the GMM is visualized.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

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<thead>
<tr>
<th>Section</th>
<th>Description</th>
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### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

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This disposal method is expected to achieve 100% inactivation of the GMM.
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The data sheets describing inactivation by Virkon are attached.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

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02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes  

Give brief details of the genetic modification safety committee

This project is approved by Pulmocide management team (including Health and Safety Officer), and this class 1 GMM use in previous premise (before relocation within Imperial College) was approved by Imperial College GM Committee. This application was also consulted by Imperial College Safety Officer.

<table>
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Tick if confidential

Bacteriology  Parasitology  Transgenic Birds  Microbiology Research
**For activities involving GMMs, describe the waste management measures which will apply to the activity**

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<tbody>
<tr>
<td>Transfection of plasmid encoding human cell line or human primary cells</td>
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Liquid waste: Disinfected by adding TriGene/Distel or Virkon to a minimum concentration of 1 % then leaving for at least 10 minutes before disposal. A log 5 reduction in viable cell numbers is described by the manufacturers.

Solid waste: decontaminated by 1 % Trigene/Distel or Virkon before disposal, and then dispose via clinical waste routine.

A log 5 reduction in viable cell numbers is described by the manufacturers and suppliers.


https://www.fishersci.co.uk/webfiles/uklweb-docs/SLSGD05.PDF

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

I made a few minor changes to the waste section and added some OH notes re the cell lines and sources of material. Agreed GM class 1, HG1.
## GM Centre Number: 3209

| Data Premises Notified (Originally) | 04/12/2013 |
| Data Premises Closed | 19/10/2015 |
| Transferred from 1992 Regs? | N |
| Transitional Premises Class | N |
| Non-GMMs | N |
| Withdrawn | N |

| Name | PROFACTOR PHARMA LTD |
| Name 2 | |
| Department | |
| Campus Estate or Research Centre | BIOCITY SCOTLAND |
| Road Name | BO’NESS ROAD |
| Building | |
| District | |
| Town | |
| County | LANARKSHIRE |
| Postcode | ML1 5UH |
| Country | SCOTLAND |
| Tel Number | 07974892562 |
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| HSE Division | blank |
| Comments | |

Date at Which Additional Info Submitted

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

We intend to begin work in our laboratory in November 2013. Work will be confined to culturing CHO DG44 cells expressing human proteins in disposable systems supplied by GE Healthcare. The Company is at present small and the Biological Safety Committee will comprise the CSO (with 35 years experience in the field) and three other senior members of staff with GM and protein purification experience. These staff begin work on 4th November. We also propose Prof J M of the University of Surrey as an Independent Chair. J is founder of the company but has no responsibility in its day to day running. He has been chair of other ACGM committees for many years.

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Bacteriology | Parasitology | Transgenic Birds | Microbiology Research
| Yes |
Solid waste will be autoclaved prior to disposal through existing routes at BioCity Scotland. Liquid waste will be treated with Trigene according to manufacturer's instructions. More details on this are provided in the attached risk assessment.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Solid waste will be autoclaved prior to disposal through existing routes at BioCity Scotland. Liquid waste will be treated with Trigene according to manufacturer's instructions. More detail on this is provided in the attached risk assessment.

Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: 

Please enter comments of the GM safety committee on the risk assessment:

The risk assessment is appropriate for the level of work proposed.
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**Name**

PROCARITA BIOSYSTEMS LTD

**Campus Estate or Research Centre**

NORWICH RESEARCH PARK

**Building**

INNOVATION CENTRE

**Road Name**

COLNEY LANE

**Town**

NORWICH

**County**

NR4 7GJ

**Country**

ENGLAND

**Tel Number**

01603 274501

**Fax Number**

0

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Yes

Give brief details of the genetic modification safety committee

Members of the GM Safety Committee will be:
- Departmental safety officer,
- Biological safety officer,
- One Principle Investigator and a representative from the junior Scientific staff

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  
Tick if confidential

- Bacteriology [Yes]  
- Parasitology             
- Transgenic Birds  
- Microbiology Research [Yes]  
- Virology    
- Transgenic Animals  
- Transgenic Fish  
- Gene Therapy  

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Laboratories:
Solid biological waste that is not contaminated by any hazardous chemicals which would be released into the atmosphere, or by sharps (including plastic pipette tips etc) which would puncture the bags is placed in appropriate plastic bags within disposable plastic containers and collected regularly.

(i) Sharps (including plastic pipette tips) contaminated with biological waste must be thoroughly decontaminated in bleach in an appropriate container before disposal with the other sharps.

(ii) Liquid biological waste in plastic containers and glassware (flasks bottles etc) would be decontaminated with bleach and then autoclaved.

(iii) Glassware (flasks, bottles etc) contaminated with biological waste should have their caps removed and placed in the blue plastic boxes for autoclaving.

(iv) Any spills should be clean with either bleach or an appropriate disinfectant (i.e. Bioguard).

(v) Labcoats used when culturing any class 1 microorganisms are placed in an appropriate plastic bag, autoclaved and washed weekly.

(vi) Autoclaves are routinely serviced and regular checks to ensure autoclave are reaching the required temperatures using thermo logs

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

This document has been read and approved by all the members of the safety committee and there are no comments at this time

**Project Ref** 3210/14.1

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<td>Design of novel treatment against drug resistant microorganisms (MRSA, Pseudomonas aeruginosa)</td>
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Non-GMM  Consent Granted
Methicillin-resistant Staphylococcus aureus (MRSA) is any strain of Staphylococcus aureus that has developed through the process of natural selection, resistance to beta-lactam antibiotics. The evolution of such resistance does not cause the organism to be more intrinsically virulent than strains of S. aureus that have no antibiotic resistance, but resistance does make MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous. People are very commonly colonized with Community associated -MRSA and are completely asymptomatic.

Pseudomonas infection is caused by strains of bacteria found widely in the environment; the most common type causing infections in humans is called Pseudomonas aeruginosa. Pseudomonas infections usually occur in people in the hospital and/or with weakened immune systems and are a common cause of ear infections and rash. In both cases, careful attention to routine infection control practices, especially hand hygiene and environmental cleaning, can substantially lower the risk of infection.

None of the organisms above mentioned neither are nor will be genetically modified in these premises.

Parental organisms: Mycobacterium smegmatis, Pseudomonas aeruginosa and methycilin resistant Staphylococcus aureus.

Non-pathogenic E. coli strains (DH5 alpha, BL21, Rosetta)

The only GMM used for the experiments will be three common E. coli strains (DH5 alpha, BL21 and Rosetta). In this instance the modifications will be mutations that will overexpress the corresponding non-toxic recombinant protein.

E. coli DH5 alpha strain and other related strains (BL21 and Rosetta) used in our premises are not known as hazards to human health and nor is there any reason to suppose that it could be unless specifically modified to express toxic proteins or proteins that produce toxic products. There is no reason known to us why these E. coli strains should be a human health hazard under the conditions used in our experimentation. These modified bacteria will be equally non-pathogenic and, therefore, will not pose an environmental hazard.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Laboratories:
Solid biological waste that is not contaminated by any hazardous chemicals which would be released into the atmosphere, or by sharps (including plastic pipette tips etc) which would puncture the bags is placed in disposable plastic containers and collected daily.

(i) Sharps (including plastic pipette tips) contaminated with biological waste must be thoroughly decontaminated in bleach in an appropriate container before disposal with the other sharps.

(ii) Liquid biological waste in plastic containers and glassware (flasks, bottles etc) should be decontaminated with bleach and then autoclaved.

(iii) Glassware (flasks, bottles etc.) contaminated with biological waste should have their caps removed and placed in the blue plastic boxes for autoclaving

(iv) Any spills should be clean with either bleach or an appropriate disinfectant (i.e. Bioguard)

(v) Labcoats used when culturing any class 2 microorganisms are placed in an appropriate plastic bag, autoclaved and washed.

(vi) Autoclaves are routinely serviced and regular checks to ensure autoclave are reaching the required temperatures using thermo logs

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This document has been read and approved by all the members of the safety committee and there are no comments at this time but to confirm that the Class 2 organisms described above neither are nor will be genetically modified in these premises.

Project Containment

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Animal Units       Large Scale Activities     Human Clinical Applications

02/03/2022
GM Centre Number: 3211

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Name

THE ROYAL INSTITUTION OF GREAT BRITAIN

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

21 ABERMARLE STREET

District

Town

LONDON

County

GREATER LONDON

Postcode

W1S 4BS

Country

ENGLAND

Tel Number

02076702969

Fax Number

0

E-mail


HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The Royal Institution has established a GMSC for the demonstration of genetically modified organisms for teaching purposes. These demonstrations will always be a class 1 activity. The GMSC of the Royal Institution has been made in conjunction with Oxford University. If in the future a GMO is to be shown as part of a lecture from another University, then that University's GMSC will be consulted by the Royal Institution GMSC in the same way.

Laboratory
Animal Unit
Growth Room
Glass House
Large Scale

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Yes

Other (please specify) Tick if confidential

Bacteriology
Parasitology
Transgenic Birds
Microbiology Research

02/03/2022
The worms will be inactivated by GM regulated autoclave at University College London.

Fluorescent reporter (GFP, RFP, YFP) tagged nematode worms will be displayed in public lectures.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The worms will be inactivated by GM regulated autoclave at University College London.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Oxford GMSC and the Royal Institution GMSC consider these activities as class 1 and are satisfied that appropriate containment measures are in place. Their comments are as follows:

"The use of gfp- or rfp-tagged nematodes on culture plates and microscopic slides for teaching purposes represents no hazard to either human health or the environment. The transgenic worms will be completely contained within their culturing plates and cannot escape nor would they survive should they escape. All genetically modified material will be destroyed by chemical disinfection and/or autoclaving off-site. Assignment of the activity to non-notifiable transgenic work at Containment level 1 is appropriate."

The GMO will be handled by trained personnel only, and will not be handled by the public at any time. The disposal of GMOs will be made in conjunction with the GM-regulated areas at University College London. A risk assessment for the demonstration has been made and is kept at the Royal Institution to be referred to by the Royal Institution GMSC.
### GM Centre Number: 3214

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**CRODA EUROPE LTD**

**Name**

**CRODA EUROPE LTD**

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

**COWICK HALL**

**District**

**SNAITH**

**Town**

**GOOLE**

**County**

**YORKSHIRE**

**Postcode**

**DN14 9AA**

**Country**

**ENGLAND**

**Tel Number**

01405 860531

**Fax Number**

01405 860205

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: [Y]

Give brief details of the genetic modification safety committee:

The Genetic Modification Safety Committee comprises:
- Chair (currently Research Manager, Microbiology)
- Secretary (appointed by Committee)
- Members: Site Safety and Health Executive (SHE) representative
  - Laboratory Safety Representative
  - 3 research scientists with expertise in Microbiology and Use of GMO's

The committee currently meets as required but will move to regular meetings once HSE approval is received to work with GMO's. The GMSC oversees experimental work involving GMO's, including facility and procedures, considering potential impact on human health and environment. The GMSC operates under the aegis of the group SHE and in accordance with Croda corporate social responsibility (CSR) goals and policies.

<table>
<thead>
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02/03/2022
**Non-microbial**

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**Other(s)**

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

All waste potentially containing viable GM material is deactivated by autoclaving (using regularly maintained and calibrated autoclaves) before disposal to drain (for liquid waste) or incineration (solid waste). The laboratory drains discharge through a dedicated effluent treatment plant, operated under EA permit. Regular monitoring of the water treatment plant will be supplemented with specific tests for any GM material used onsite. Spills of material containing GM components are deactivated by treatment with suitable disinfectant (e.g., Vircon) which is validated against each GMO used in the facility. Samples derived from manipulation of GMO's are sterile filtered and routinely monitored for content of GM material by validated PCR test before further processing in other laboratories.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

**Please enter comments of the GM safety committee on the risk assessment**

None
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**Name**

SCOTIA BIOLOGICS LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

5 JAMAICA STREET

**District**

**Town**

ABERDEEN

**County**

**Postcode**

AB25 3UX

**Country**

SCOTLAND

**Tel Number**

01224438570

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The local GM Safety Committee at the University of Aberdeen has agreed to advise on the risk assessments and contained use activities

<table>
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Tick if confidential

Yes

- Bacteriology: Yes
- Parasitology: Yes
- Transgenic Birds: Yes
- Microbiology Research: Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid wastes (e.g. culture medium): 1% Virkon according to manufacturer's guidelines.
Plasticware: autoclaving (126°C, 11 min). Plastics are soaked in 1% Virkon and then double-bagged before being taken in a large plastic box with lid to autoclave room, the performance of which are inspected every 6 months.
Autoclaved wastes are stored in orange bags in designated plastic boxes which are kept in a metal cage outside the laboratory building. These orange bags are collected by the NHS waste disposal services regularly.
Sharps: We anticipate that the level of sharp waste generated will be zero, and any contaminated broken glass will be soaked in 1% Virkon, placed in Cin Bins, bagged and incinerated.
Laboratory benches are wiped down daily with 1% Virkon.

Autoclaved waste from the autoclaves is regularly monitored (6 monthly checks) to verify the effectiveness of the inactivation procedure. The procedures are expected to kill as close to 100% of bacteria and bacteriophage as can be expected, although an absolute guarantee of 100% kill is not possible. According to its manufacturers (Antec) Virkon is effective against gram negative bacteria such as E. coli, and bacteriophage such as M13K07.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Section 7: Confirmation is required that all of those researchers named will have a supervisory role - only these people need to be listed at this point.
Section 8: Rather than summing the experience with GM of all the supervisors give the individual experience of key persons.
General: Where possible define abbreviations to ensure the clarity of the text and ease of understanding for the non-specialist
Additional information section: Question 7 - There are two chemical kill methods where possible we normally recommend avoiding multiple chemical disinfectants unless there are good reasons. The concentration of the Presept should be stated.

N C sent the following comments
Section 2: the applications should be from the person who is an employee or officer of Scotia Biologics and who is leading on the work. The forms then need to be signed by the same person.
Section 3: it would be better if section 3 said just "Scotia Biologics Ltd"
### GM Centre Number: 3217

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**Name**

ABSYNTH BIOLOGICS LTD

**Name 2**

Department

**Campus Estate or Research Centre**

THE SHEFFIELD BIOINCUBATOR

**Road Name**

40 LEAVYGREAVE ROAD

**Town**

SHEFFIELD

**County**

SOUTH YORKSHIRE

**Postcode**

S3 7RD

**Country**

ENGLAND

**Tel Number**

01189882183

**Fax Number**

0

**E-mail**

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**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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<td>BLOCK 3</td>
<td>ALDERLEY EDGE</td>
<td>CHESHIRE</td>
<td>SK10 4TF</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee (GMSC) in the company comprises of four people from different roles within the company including workers and management (expertise in many areas including microbiology and working with GMM). Any work involving GMM will be reviewed and approved by the GMSC. Before any new work is to be carried out a risk assessment will be submitted to the GMSC who will review the risk assessment and discuss the work and assessment in a meeting with all committee members present (any discussions are documented by meeting minutes and actions circulated after the meeting). The committee will then ask for further information if needed by submitting individual, approve or reject the risk assessment. If any work is required or deemed as level 2 GMM then the HSE will be notified.

### Laboratory

- Level 1 (GMMs): Yes
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial

### Animal Unit

- Other (please specify) Tick if confidential
**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Disinfection and autoclaving will be carried out where required. All waste materials will be disposed of into autoclave bags, yellow clinical waste bags/bins or sharps bins as required. All contaminated solid waste (agar plates, disposable plasticware etc) will be placed in autoclave bags and will be autoclaved and disposed of via designated route. Liquid cultures will be chemically-inactivated (Virkon 1% or PeraSafe 0.16% for 30 mins) or autoclaved prior to disposal. All contaminated glassware and reusable plasticware will be decontaminated as above.

- **a) Disinfection.**
  1% Virkon will be used for disinfection. The surfaces of the microbiological safety cabinet will be disinfected with 1% Virkon after use. Laboratory benches will be swabbed with 1% Virkon after any activity.

- **b) Autoclaving.**
  Autoclaves are not present in the laboratory for waste treatment. However, a large central autoclave facility is located on site at Alderley Park (please see next section for the further details of the Waste management process and derogation request)
  The autoclave is subject to twice yearly servicing and annual validation using a 12-point thermocouple procedure which includes a probe placed in a representative load.

**Tick to confirm that you are attaching a summary of the risk assessment**

- [ ]

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

- [ ]

---

**Please enter comments of the GM safety committee on the risk assessment**

The GMSC held a meeting on 24th February to discuss the Risk assessment for Class 1 activities reference number 13AB004. The committee went through each section of the risk assessment and discussed each section in turn.

Where unclear the text from the original document was clarified e.g. the use of sharps was asked to be removed from the risk assessment as no activities would be conducted with these organisms with the use of any sharps. It was agreed by all parties of the committee that the risk assessment was fit for purpose and that the activities outlined by the risk assessment were deemed satisfactory with no significant risk to human health or the environment. In particular the expression of the proteins/genes of interest do not give the host organism any increase in selective advantage or virulence. The organisms themselves cannot, even with these genetic modifications survive outside the confines of a laboratory environment.
GM Centre Number: 3219

Data Premises Notified (Originally) 26/04/2019

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

PHARMIDEX PHARMACEUTICAL SERVICES LTD

Name 2

Department

Campus Estate or Research Centre

EMEA KNOWLEDGE CENTRE

Building

Road Name

MOSQUITO WAY

District

Town

HATFIELD

County

HERTFORDSHIRE

Postcode

AL10 9SN

Country

ENGLAND

Tel Number 07377725605

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Genetic modification safety committee established within the company

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<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research |
| Transgenic Animals | Transgenic Fish | Gene Therapy | |
For activities involving GMMs, describe the waste management measures which will apply to the activity

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Other(s) | Oncology drug discovery

Detailed risk assessment and protocol prepared, which will involve all waste being incubated with appropriate reagent, e.g. microsol 4+ for a minimum of 1 hr. All contaminated class 2 surfaces also to be cleaned with the same agent, followed by 70% ethanol and followed by use of UV light overnight.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Genetic modification safety committee established at Pharmidex, with appropriate expertise gained within the pharmaceutical industry.
### GM Centre Number: 3220

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**Date at Which Additional Info Submitted:**

02/03/2022

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Page 13447 of 15326
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Genetic Modification Safety Committee formed to discuss requirements for handling GMM material on site at Braintree
- Senior member from each department included on committee - plan to meet quarterly unless change in plans require more frequent meetings.
- Will be included on agenda a monthly management team meetings

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Contaminated equipment and unwanted material to be decontaminated via autoclave on a validated autoclave cycle

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessment for the E. coli B21 strain discussed - material to be handled in a Biosafety level 2 containment facility. Risk is deemed risk low.
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Name

DEPARTMENT OF MEDICAL GENETICS, UNIVERSITY OF CAMBRIDGE

Name 2

Department

Campus Estate or Research Centre

ACADEMIC LABORATORU OF MEDICAL GEN

Road Name

ADDENBROOKES TREATMENT CENTRE

District

CAMBRIDGE BIOMEDICAL CAMPUS

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB2 0QQ

Country

ENGLAND

Tel Number

01223 746714

Fax Number

01223 746777

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>DEPARTMENT OF MEDICAL GENETICS, UNIVERSITY OF CAMB</td>
<td>ACADEMIC LABORATORY OF MEDICAL GENETICS</td>
<td></td>
<td>BOX 238, LEVEL 6</td>
<td>ADDENBROOK ES TREATMENT CENTRE</td>
<td>CAMBRIDGE BIOMEDICAL CAMPUS</td>
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<td>CAMBRIDGE HIRE</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The Departmental Genetic Modification Safety Committee is incorporated into the Departmental Biological Safety Committee (DBSC). The DBSC includes management representatives, e.g. the Head and Administrator of the Department, the Departmental Biological Safety Officer, the Departmental Safety Officer, two Principal Investigators belonging to the Department, one representative of the general staff who have access to the genetic modification facilities, as well as the Safety Officer for the School of Clinical Medicine.

The members of the DBSC meet formally face to face on a termly basis. The agenda is sent prior to the DBSC meeting, the points are raised/ discussed/ actions to be taken agreed during the meeting and the minutes are sent back to each member by email.

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Non-microbial

Other (please specify)  

Tick if confidential  

02/03/2022
Fundamental and translational research involving disabled hosts and vectors

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref 3221/14.1**

**Date Ackn’d** 24/04/2014

**CU2 Project Title** Genetic Studies in inherited diseases: renal cysts, renal cell carcinoma (RCC), phaeochromocytoma and paraganglioma

**Date Project Ceased**

**Class** Class 2

**CultureVolClass2** < 1 Litre

**CultureVolumeClass3-4**

**Non-GMM Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

02/03/2022
**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

This project aims to functionally investigate the mechanisms of tumourigenesis in renal cell carcinoma, phaeochromocytoma and paraganglioma, as there are currently no curative therapies and better treatments are urgently required. We believe that the identification of the genetic basis of the inherited forms of those diseases will highlight those genes and pathways that are critical for tumourigenesis and will provide insights into the pathogenesis of sporadic diseases.

The objectives are therefore to generate and characterise human cell line models for renal cell carcinoma, phaeochromocytoma and paraganglioma. We will compare paired isogenic cell lines transiently or stably transfected with wild-type/mutated/knocked down versions of established genes involved in those kinds of tumours and we will particularly monitor the effects on cell viability, proliferation, apoptosis and cell metabolism. We will also study in the same way newly identified genes. In addition, gene editing (specific gene knockout with complete loss of function) will be engineered using the RNA-guided CRISPR (clustered regularly interspaced short palindromic repeats) – associated nuclease Caspase 9 technology. In parallel, we will test therapeutic agents that selectively target cells deficient in those genes, hoping that these new drug screening studies will enable advances in the treatment of those diseases.

**Recipient or parental organism**

We will use disabled or especially disabled E. coli bacterial strains, non-pathogenic to humans such as DH5α. They are unable to survive or propagate outside of laboratory culture.

We will use a panel of human cell lines from kidney origin (derived from normal or cancerous tissues), commercially available, well characterised and considered ACDP Hazard Group (HG) 1 (such as HK2, UOK262, SKRC39, KTCK26, 786-0 cells). All of these hosts have a safe history of use and can be classified as especially disabled or non-colonising as defined in ACGM Compendium of Guidance. We may use in the future primary human cells and finite lines which, while of similar low risk to established cell lines themselves, are not fully characterised and authenticated; they may contain adventitious infectious agents, therefore we will handle these at CL2.

The packaging cells (Phoenix) will be of second-generation, amphotropic, based on the human embryonic kidney cell line 29.

**Host/vector system**

For bacteria: commercially available cloning (e.g. pBluescript) vectors and mammalian expression vectors (see below).

For mammalian cell lines: commercially available cloning and expression vectors (e.g. pcDNA3.1, pIRESneo, pEGFP-V-RS shRNA and derivatives).

For viruses: replication defective retroviruses based on the Murine Moloney Leukemia Virus (such as pBabe) will be used to infect cell lines, as well as replication incompetent versions of lentiviruses based on the human immunodeficiency virus HIV1 backbone. For gene editing, we will use the plentiCRISPR plasmid, which is based on the lentiviral backbone; it expresses the human codon-optimized Cas9 protein and puromycin resistance from EFS promoter and CRISPR chimeric RNA element with customizable single guided RNA (sgRNA) from the U6 promoter. Packaging vectors have been modified to make development of pathogenic recombination-competent virus extremely unlikely. All plasmid vectors used are non mobilisable and free from harmful sequences, except the plentiCRISPR plasmid which contains the WPRE motif (that may contribute to oncogenic potential of the viral vector) and will not survive when removed from tissue culture conditions.

**Origin & function**

In most cases the inserted genetic material will be cDNAs encoding recognised wild-type tumour suppressor genes of human origin (e.g. von Hippel-Lindau (VHL), Fumarate Hydratase (FH), Folliculin (FLCN), succinate dehydrogenase subunit B (SDHB)), as well as the corresponding mutated variants. These genes will also be knocked down via shRNA, siRNA and CRISPR-Cas9 technology. The same approach will be used to transfect cell lines with newly identified genes involved in hereditary RCC, phaeochromocytoma and paraganglioma diseases. In parallel, we will selectively target cells deficient in all those inherited gene functions by screening phosphatase and kinase siRNA libraries and comparing the effects with their wild-type counterparts. Apart from their role in causing RCC, phaeochromocytoma and paraganglioma, none of the proteins that we are dealing with has any known toxicity (e.g. production of toxins, cytokines, allergens, hormones) or oncogenicity, is of no selective advantage to other organisms and none of the inserts will be secreted proteins. It is anticipated however that the products of the mutated versions, as well as the knock-downs will
cause reduction/ loss of tumour suppressor ability and have some effects on cellular processes, such as growth, differentiation, apoptosis and metabolism, coupled with the resulting effects on downstream genes and pathways.

The following is a summary of what is known of the function of the main proteins we are studying:

VHL tumour suppressor gene product has multiple functions, the best characterised function is the ability to regulate the HIF-1 and HIF-2 transcription factors (as part of an E3-ligase complex that targets HIF-alpha subunits for proteasomal degradation in normoxic conditions). Inactivation of VHL protein with consequent stabilisation of HIF-1/HIF-2 causes activation of an extensive repertoire of downstream target genes such as VEGF, PDGF, TGFα and CCND1. Recent work from a number of groups has indicated that HIF2 is the major driver of renal oncogenesis while HIF-1 may be anti-oncogenic.

In contrast to VHL, most other inherited RCC genes have not been reported to be frequently mutated in sporadic forms of RCC. Nevertheless, though the function of these genes is less well characterised than that of the VHL gene product, the pathways linked to inherited RCC genes have been implicated in the pathogenesis of sporadic RCC. Thus inactivation of SDHB and FH is associated both in vivo and in vitro with activation of HIF-1/HIF-2 related pathways (mimicking that observed in VHL deficient cells).

The FLCN gene product has been linked to regulation of the AMPK/mTOR pathways though more recent studies have suggested that the effects of folliculin on mTOR signalling are complex and context dependent. In unpublished studies we have identified novel folliculin interacting proteins that link folliculin function to Wnt, cadherin and Rho signalling pathways. A recent report has identified deregulation of the KEAP/NRF2 pathway in FH-associated and sporadic Type 2 papillary cancer.

Evaluation of foreseeable effects

The genetic modifications are not expected to overcome disablement of the host cells, nor affect host specificity, tissue tropism or susceptibility to host defence mechanisms. The organisms will remain enfeebled and unable to infect or cause pathology in wider animal or human populations. The environmental fitness of the modified hosts will remain severely limited and the modified organisms will be unable to survive.

The retroviral vector system used is based on the Murine Moloney Leukemia Virus. Replication defective retroviruses will be made by transient transfection of retroviral transfer plasmids (such as pBMN, pLXIN and pQXCI series) into packaging cells lines such as Phoenix. These viral vectors could deliver oncogenic genes or down-regulate tumour suppressor functions. The vectors can integrate into the host cell DNA so transgene expression is long term possibly leading to an oncogenic phenotype. However, the risk of human infection using the above viral system is extremely low as the virus can only infect via direct inoculation. Thus, if no sharps are used in combination with good laboratory practice the risk of infection is extremely low. In addition, these vectors only infect dividing cells.

The lentiviral vectors we will use are based on the HIV backbone. Although this is a HG 3 pathogen, vectors are all third generation replication defective and considered class 1; virus replicative, packaging and envelope functions and the transgene vector are split over four or more plasmids, requiring multiple recombination events to generate replication competent retrovirus (RCR). Any RCR generated would lose the transgene during recombination events. Many accessory functions/pathogenicity determinants have been deleted and the vectors used will be self-inactivating (SIN). A deletion inactivates transcription from the proviral LTR so reducing the potential for transcriptional activation of cellular genes and also prevents mobilization of any RCR. However these vectors can infect non-dividing cells and can integrate into the cell genome so could deliver oncogene or depress suppressors on a long term basis leading possibly to an oncogenic phenotype.

The resultant GM cell lines will not be viable outside the provided growth conditions, therefore pose little or no risk to human health and environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste containing GMMs will be inactivated via chemical disinfection with 1% Virkon (final concentration) for 16 hours, then disposed to drains. Expected degree of kill – 100%. All solid, disposable culture/lab ware will be directly incinerated on site at Addenbrooke’s Hospital. Recyclable lab-ware will be soaked in a 1% Virkon solution (stocks replaced every 3 days) for 16 hours. Bench/cabinet surfaces will be wiped down with 70% ethanol. Any spills of media/cell lines will be covered by Virkon powder for 3 minutes, mopped up, rinsed with 1% Virkon and wipes incinerated. Validation of GMM inactivation methods will be performed and recorded termly: aliquots of GMMs will undergo the same treatment as GMM waste and will be plated back in normal growth media for 24 hours before being tested for viability. Access to an autoclave is also granted in case of emergencies on level 4 of the Addenbrooke’s Treatment Centre/ Institute of Metabolic Science building.
Following review of the enclosed risk assessment, all GMSC members were satisfied that the genetic modifications involved will not overcome disablement of the recipient cells, nor affect host specificity, tissue tropism or susceptibility to host defence mechanisms. Therefore, the panel agreed that the resulting genetically modified micro-organisms will pose little risk to human health and no risk to the environment and confirmed that the safety control measures put in place will be adequate and sufficient to fully protect the workers and the environment of any harm.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<td>L3 L4</td>
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Project Ref 3221/18.1

Date Ackn'd | 30/08/2018
Date Project Ceased | 

CU2 Project Title | Exploring the mechanisms of double strand break repair and rearrangement signatures in human cancers

Class | CultureVolClass2 | CultureVolumeClass3-4
Class 2 | ≤ 1 Litre
Non-GMM | Consent Granted

Withdrawn | N
Tick if notifying a connected programme of work | N

Historical Significant Changes

Historical Date of Additional Info
Mutational processes leave characteristic imprints in the genome, which we term mutational signatures. To date, work from our group has identified six rearrangement signatures from studying 560 breast cancer genomes. Out of the six rearrangement signatures (RS), three are associated with defective homologous recombination (HR) DNA repair: RS-3 with defective BRCA1 function, RS-5 with defective BRCA1 or BRCA2 function, and the mechanism of RS-1 is currently unknown.

To identify the mechanistic underpinnings of these signatures, we attempt to generate genome-wide targeted double strand breaks (OSBs) in human cells using (a) a well-characterized restriction enzyme system (AsiSI-ER); and (b) an engineered multiplex CRISPR-Cas9 system. The inducible nature of both of these systems will allow us to modulate and control the DSB induction inside host cells. Following induction, we intend to map the induced DSBs using a targeted DSB sequencing technique and to study their repair profiles and rearrangement patterns using whole-genome sequencing (WGS), generating mutational signatures as readouts. By contrasting the DSB damage maps and rearrangement signatures in repair-deficient cells against their repair-proficient wild type counterparts, we aim at systematically determine the qualitative and quantitative features of how cells accrue different somatic rearrangements, and how the rearrangement patterns change given the annulment of critical components of compensatory DNA repair pathways.

AIM 1
First, to derive direct causative evidence for the mutagenic effect of BRCA 1/2 loss-of-function mutations, we plan to knock out BRCA1 and BRCA2 in a near-haploid cell line, HAP-1 as well as some other human cell lines (such as MCF10A, MDA-MB-231 and H1 299) using CRISPR-Cas9 either in the form of (a) ribonucleoprotein complex (RNP) nucleofaction or (b) plasmid based transfection with lipid-based transfection reagents. In cases where knock-out of a gene induces cell lar toxicity (i.e. essential genes), we would use inducible shRNA’s (e.g. Tet-pLKO, SMARTvector inducible lentiviral shRNA) for conditional knock-down. In addition to normal cell culture condition, we also plan to induce genome-wide targeted breaks in these BReA1 or BRCA2 deficient cell lines using a well-characterised restriction enzyme system, AsiSI-ER. In this system, restriction enzyme AsiSI is fused to an oestrogen receptor binding domain and is only actively translocated into the nucleus to cut the DNA in the presence of 4-hydroxytamoxifen (4-OHT). Under this stress condition, we expect to see an increased mutation accumulation rate and more rearrangements. Retroviral plasmids pBABE-AsiSI-ER and pAID-AsiSI-ER will be used for this purpose. For pAID-AsiSI-ER, the engineered AsiSI-ER is further fused to an auxin-induced degron (AID) such that the enzyme would be depleted upon auxin addition.

AIM 2
To allow for a more flexible and refined system where we could control both the numbers and the sites of damage (in this case DSBs) in cells, we plan to engineer an all-in-one multiplex CRISPR-Cas9 system. In th is system, individual single guide RNAs (sgRNAs) are flanked by tRNA sequences such that one could clone in multiple sgRNAs for targeting breaks to different parts of the genome. A Tet-On inducible promoter drives the expression of a fusion Cas9 protein (DD-Cas9) in which Cas9 is fused to a destabilizing domain (~O) such that it is only stabilized and active in the presence of the ligand Shield-1. With this system, we envision that Cas9 expression would be tightly controlled with doxycycline; and Cas9 protein activity could be induced and tuned with Shield-1 post-translationally. The vector will be constructed in a PiggyBac vector backbone because of its large cargo size limit for lentivirus particle packaging.
and used in conjunction with a transposase plasmid (HyPbase) for establishing stable cell lines capable of being induced for DNA breaks. Note that should the final transfer plasmid size be small enough to be packaged into lentivirus particles, we may use the lenti CRISPRv2 plasmid backbone (Addgene 52961), and pTRE3G vectors from Takara (Clontech) to establish these stable cell lines.

AIM 3 & OVERARCHING GOALS

Health and Safety

Executive

To systematically investigate how cells with different DNA repair defects modulate their repair outcomes post damage induction, we plan to use the genome-wide OSB induction system developed in AIM2 across a panel of CRISPRCas9-edited DNA repair mutant cells (RNP or plasmid-based gene editing, non-virus method). Here, we aim to primarily target key components of the DNA repair pathways, including but not restricted to common tumour suppressor genes such as BRCA1, BRCA2, TP53, etc. For a more comprehensive list of examples of the genes we plan to study in the project, please refer to supplementary Table S1 attached at the end of this form. The data generated from this study could potentially offer mechanistic insights into how certain rearrangement patterns arise in human cancers and provide an avenue for leveraging them for therapeutic purposes.

Recipient or parental organism

Molecular cloning and transformation will be done in common competent E.coli cells for cloning such as NEB Stable, DH5a, NEB10 beta and their derivatives. All are commercially available from NEB. XL 10-Gold ultra-competent cells are available from Agilent. E. coli IOG SUPREME electro competent cells from Cambridge Bioscience.

For lentivirus or retrovirus work, packaging of viral particles will be achieved in eukaryotic helper packaging cell lines, such as the well characterised and commercially available HEK-293T, Plat-A (Cell Biolabs), Phoenix-AMPHO (ATCC).

Most of the CRISPR editing work will be done in HAP-lor eHAP cell lines (Horizon Discovery). To validate or reproduce some of the findings in other human cell lines, we might use a panel of lines from multiple tissue lineages (can be normal and cancerous) such as MCF10A, MDA-MB-231, MDA-MB-436, EUFA423F, hTERT-HME1, HCC1937, DLD-1, etc. All cell lines are publicly or commercially available.

All bacterial cells and mammalian cell lines are considered to be disabled and pose no risk to human health.

Host/vector system

All of the plasmid constructs will be made through Gibson assembly-based methods. Molecular cloning and transformation will be done in common competent E.coli cells for cloning such as NEB Stable, DH5a, NEB10 beta and their derivatives (all are commercially available from NEB). XL 10-Gold ultra-competent cells are available from Agilent. E. coli IOG SUPREME electro competent cells from Cambridge Bioscience.

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For CRISPR-Cas9 knockout of DNA repair genes

CRISPR-Cas9 cloning backbones for sgRNA such as pX458 (#48138), pX459 (#62988), pX330 (#98750), lentiCas9-Blast (#52962), lentiGuide-puro (#52963), pCFD5/6 (#73914. 73915) - all vectors are available from Addgene.
Alternatively, RNP nucleofaction with synthetic guide RNAs and Gas9 protein will be used. Non-virus based.

For knockdown of essential genes (i.e. BRGA1/BRGA2)
Inducible shRNA vector Tet-pLKO-puro (Addgene #21915) for inducible expression of shRNA or SMARTvector
Inducible lentiviral shRNA (Dharmacon). Both vectors differ in the selection markers in host cells. Lentiviral-based.
Lentiviral Gasg vectors for targeted DSBs
Lentiviral vectors are HIV1/2 based 3rd generation virus, e.g. DO-Cas9 with filler sequence and Venus (#90085),
inducible pTRE3G vectors, pGMV-Tet3G and pEFa-Tet3G regulator plasmids with pTRE3G, pTRE3G-mCherry,
pTRE3G-Bi-mCherry, pTRE3G-Bi-ZsGreen1 response plasmids - all from Takara (Clontech ). These vectors are all based on Tet-inducible promoters in combination with different fluorescence markers.

Retroviral vectors for targeted DSBs (restriction enzyme)

Health and Safety

Executive

Retrovectors based on MMLV, MSCV, (e.g. pBABE-puro plasmid from Addgene, pRetroQ and pRetroX from Takara Clontech). AsiSI restriction enzyme plasmids were modified from pBASE backbone, in which the enzyme is fused to an oestrogen receptor binding domain as detailed in AIM1 (EMBO J. 2010 Apr 21 ; 29(8): 1446-1457). AsiSI-ER restriction enzyme was cloned into pAID1.1-N vector (BioROIS) to produce pAIO-AsiSI-ER in which the enzyme can be depleted with the addition of auxin. Retroviral-based.

For stable integration if plasmid cargo size> 10.5 kb
PiggyBac plasmids pCAG/pCMV-hyPBase (Yusa et al., 2011) were obtained through Sanger Institute clone requests service (https://www.sanger.ac.uk/form/-CXJaC-oRKGLQziN7jxNw).

All viral vectors to be used in the study are either the originals or in some instances, modified from the above without changing its tropism or its inability to replicate. E.g. to change the reporter from an antibiotic resistance gene to a fluorescence protein without altering the backbone sequences. Note that though all of our lentil retrovirus associated work will be amphotropic in nature, we are ONLY working with human cell lines.

Origin & function

Lentiviruses based on Human Immunodeficiency Virus type 1 and 2 (HIV-1/-2) however vectors to be used will be disabled 3rd generation and so risk level is reduced.
Retroviruses: used in vector systems are murine in origin but are amphotropic targeting only dividing cells. Mammalian cell lines from a mix of cancerous and normal human derived - will be transfected with plasmids encoding bacterial-derived Cas9 endonuclease together with single guide RNAs (sgRNAs) for generating stable cell lines with the desired targeted gene edits or with the ability to generate genome wide targeted double strand break (DSB). In addition to Cas9, we will also use restriction enzyme AsiSI-ER (EMBO J. 2010 Apr 21 ; 29(8): 1446-1457) to generate DSBs in these cells as a means to induce DNA damage.
Wherever possible we will use a similar Cas9 targeted system which can be tightly controlled and induced with the addition of Doxycycline (Tet-On 3G vectors from Takara Clontech) in cell culture media (e.g. Tetracycline inducible promoter, and DD-Cas9). Such a system would allow the transcript expression under Tet-promoter to be mediated by controlling the presence and/or concentration of doxycycline. The Tet-On system used in the studies will only be activated in the presence of Dox. With OD-Cas9 system (Addgene Plasmid# 90085), Cas9 will only be active in the presence of the ligand Shield-1. In Tet-pLKO-puro system, shRNA will only be expressed in the presence of Oox.

Evaluation of foreseeable effects

The genetic modifications are not expected to overcome disablement of the host cells or susceptibility to host defence mechanisms. The organisms will remain enfeebled; the environmental fitness of the modified hosts will remain severely limited and the modified organisms will be unable to survive.
The consequence of inducing or stabilising Cas9 expression, and hence DSBs would result in activation of DNA repair pathways to mediate the damage and initiate repair predominantly through cellular pathways: non-homologous end joining (NHEJ), homologous recombination (HR) or alternative NHEJ (alt NHEJ)). Mutations (e.g. indels, frameshift mutations, premature stop codons) at the DSB sites may also potentially result in target genes being knocked out as a result of misrepair. In the instance of Tet-plKO-puro/SMARTvector for shRNA, targeted transcripts will be knocked down.

The viral vectors we use could potentially down-regulate tumour suppressor functions. The vectors can integrate into the host cell DNA so transgene expression is long term possibly leading to an oncogenic phenotype. The resultant GM cell lines will not be viable outside the provided growth conditions, therefore pose little or no risk to human health.

Lentiviral vectors are generated with an amphotropic envelope (VSV-G) which may broaden the tropism of the vectors, rendering them capable of infecting human cells of many tissue types. Lentiviral vectors are also capable of transducing non-dividing cells. As a result they may pose a potential increased risk of infectivity to humans.

This connected programme of work will change the expression of a variety of genes which may be hazardous. It should be noted that a transduction event (even by a viral vector that is replication-defective) will result in integration of the gene edit into the genome of the host cell. If the expression of the gene is driven by a constitutive promoter, that expression will be permanent. While the vector itself will not proliferate, cell division may result in a population of cells expressing the transgene.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste containing GMMs will be inactivated via chemical disinfection with 1 % Virkon (final concentration) overnight (expected degree of kill 100%): or autoclave inactivated prior to disposal to drains (expected kill 100%). All disposable culture/lab ware will be chemically or autoclave inactivated prior to incineration on site at Addenbrooks Hospital.

Bench cabinet surfaces will be wiped down with disinfectant solution followed by 70% ethanol. Any spills of media/cell lines will be treated with 1% Virkon solution or covered by Virkon powder for 5 minutes and mopped up; rinsed with 1% Virkon followed with 70% Ethanol.

All general laboratory waste will be incinerated on site.

An autoclave is based in the laboratory where work is performed. Access is also granted in case of emergencies on level 4 of the Addenbrooke's Treatment Centre Institute of Metabolic Science building.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

Following initial review of the enclosed risk assessment and given the genome-wide scope of the project, the GMSC members requested to clarify the vectors used by detailing and grouping the plasmids, retroviral vectors and lentiviral vectors separately, as well as depending on the aim of the study. In order to be more specific, the GMSC also demanded to insert a table (in appendix) listing the main DNA damage response genes targeted by the CRISP-Cas9 system. Finally, the GMSC noted that the viral particles of VSV G pseudotype may have the potential to alter the mode of transmission and tropism, however the members were satisfied that the genetic modifications involved will not overcome disablement of the recipient cells, nor should decrease the susceptibility to host defence mechanisms. Therefore, the panel agreed that the resulting genetically modified micro-organisms will pose little risk to human health and no risk to the environment and confirmed that the safety control measures put in place will be adequate and sufficient to fully protect the workers and the environment of any harm.

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Transferred from 1992 Regs? N
Transitional Premises Class
Data Premises Closed Transitional Premises Non-GMMs N Withdrawn N
Emergency Plan Required?

Name
BIOSCEPTRE UK LTD

Name 2
Department

Campus Estate or Research Centre Building
BABRAHAM RESEARCH CAMPUS THE JONAS WEBB BUILDING (910)

Road Name
District

Town
County
Postcode
Country
CAMBRIDGE CAMBRIDGESHIRE CB22 3AT ENGLAND

Tel Number 01223 496095 Fax Number 01223 496069

E-mail
HSE Division blank

Comments

Date at Which Additional Info Submitted
02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The primary contact and person involved in the GM safety committee will be Senior Research Scientist and Biological Safety Officer, previously trained to PhD level in biology.

Supporting members of the committee will include the Chief Scientific Officer, Office Manager and a member from the Babraham Research Institute Health and Safety Team including BBT H&S Officer. A supporting role will be played by a contracted external Health & Safety Advisor

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Other (please specify) Tick if confidential

Tick if confidential

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02/03/2022
Virology  | Yes  | Transgenic Animals  | Transgenic Fish  | Gene Therapy  
Mycology  | Yes  | Transgenic Invertebrates  | Transgenic Plants  | Other (please specify below)  
Other(s)  | In the first instance only Microbiology & Bacteriology will be carried out with the cell lines  

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tissue culture will be treated with 1% Virkon for a minimum of 12 hours before disposal down sinks or in yellow biological safety bins. Any contamination of work surfaces will be wiped with a 1% Virkon solution. Periodically we will assess the degree of killing by trypan blue counts or fluorescent viability dyes.

All other solid waste disposed of via autoclaving at 121 C for 15 minutes.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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**Project Ref**  
3222/14.1

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### Project Additional Information

#### Purposes of the contained use

The study objectives are to:
- Characterise the surface expression of markers on cell lines including P2X7 -transfected hosts
- To assess the biology of P2X7 ion channels on transfected cells
- To utilise a variety of in vitro assays including FACS, ELISA, Western blot, cell imaging, cell death, antibody binding, gene expression on cells transfected HEK293 cells
- Analyse in vitro cell killing by antibody-dependent cell-mediated cytotoxicity using the stably transfected Jurkat cells.

#### Recipient or parental organism

**Cell Lines**

Commonly used tissue culture human cell lines characterised as Biohazard Group 1 and 2: Examples include
- HEK293 cells (Cl2), K562 (Cl1), Molt4 (Cl1), KG-1a (Cl1), THP-1 (Cl1), KG-1 (Cl1), Hel (el1), Ramos (Cl1), Rec-1 (el1), MV4-11 (el1), U266B1 (Cl1), RPMI8226 (el1), SP2I0 (CL1), SK-A-NS, Kelly (CL1), Colo205 (Cl1).
- HCT -116 (C1), HT -29-(C1), MiaPaCa2 (el1), PC3 (elL 1), DU145 (el1), InCap (C1), LU2 (C1), NCI-H460 (CL 1), MCF-10a (Cl1), NC’-H520 (C1), MCF-7 (Cl1), A375 (CL 1), HT-1080 (Cl1), SCC-9 (C1), SK-MEL-5 (Cl1), SK-MEL-28 (Cl1) and Jurkat cells stably expressing the FcyRllla (As part of ADCC Reporter Bioassay (Promega).

#### Host/vector system

Mammalian cells will be transfected with non-viral expression vector using commercially available pcDNA3.1 plasmid as previously described in Adinolfi et al 2005. pCMV6-AN-mRFP and pEZ-M02 p/asmids will be used as well. Where necessary plasmids will be propagated using commercially available Escherichia coli k-12 derivatives (TOP 10 series and DH5-alpha, genotype F- mcrA A(mrr-hsdRMS-mcrBC) lp801ac.ltl.M1511.JacX74 nupG recA1 araD139 A(araleu) 7697 galE15 9alK16 rpsl(StrR) endA 1, - ) as hosts. TOP10 and DH5-alpha bacteria have been reported to be auxotrophic for various essential amino acids.

The source of these vectors will be Prof. Francesco di Virgillio. University of Ferrara and/or D C, Biosceptre International Ud, Sydney.

#### Origin & function

**Insert ONAs**

**Health and Safety**

**Executive**

- Forms of the P2X7 gene, including full length, splice variants, and SNPs, from various mammalian species including but not limited to humans, sheep, mouse, rat, rabbit and dog.
- Fluorescent proteins i.e. GFP and RFP to monitor transgene transfection and expression.
- One commercially available cell line is transfected with human FcgRllla. This cell line is composed of Jurkat cells transfected as part of the ADCC Reporter Bioassay Kit sold by Promega.
We intend to transfect various cell lines with siRNA targeting P2X7 mRNA. Using this we can elucidate the biology of the P2X7 gene and identify the target of our therapeutic.

**Evaluation of foreseeable effects**

**BACTERIAL SPECIES:**
The hazardous potential of the GMMs created is considered no greater than that of the parental line. The E. coli strains used are K-12 derivatives and these have been recognised as non-colonising and disabled. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and are auxotrophic for various essential amino acids that are unlikely to be satisfied outside the laboratory environment. It is unlikely that the expression of human P2X7 gene will have an effect on the pathogenicity of bacterial species, furthermore the bacteria are auxotrophic for essential amino acids and this will impair their accidental release into the environment.

**siRNA OF MAMMALIAN CELLS:**
Introduction of siRNA constructs is designed to remove intracellular mRNA for the target genes. The predicted effect is the down regulation of the corresponding protein expression from the target cells. This treatment is expected to have little impact on the nature of the target cell.

**TRANSFECTION OF MAMMALIAN CELLS WITH P2X7:**
The major risk with the transfection of mammalian cell lines is the introduction of viral promotores, i.e. CMV. Waste management measures to avoid accidental exposure and release of potentially hazardous material is described in section 12. Furthermore, overexpression of the P2X7 gene in human cell lines, HEK293 cells, has been shown to increase replication in vitro and in vivo (Adinolfi et al.2012). Therefore, great care will be taken to prevent exposure of the operator to the bacterial vector containing human P2X7 or the transfected GM cell lines.

Introduction of constructs expressing siRNA or fluorescent proteins is expected to have little impact on the nature of the target cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not applicable, only microorganisms (including tissue culture cells) are involved in this work.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
All waste disposed of according to the Babraham Research Campus -Hazard Group 2 Waste disposal S.O.P (waste sealed in 30L Euro bins and sent offsite for incineration by Velspeed (GM authorisation GM898, notification GM105/14.1).

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Tissue culture waste will be treated with 1% Virkon for a minimum of 12 hours before disposal down sinks or in yellow biological safety bins. Any contamination of work surfaces will be wiped with a 1% Virkon solution. Periodically we will assess the degree of killing by trypan blue counts or fluorescent viability dyes. The primary risk is the generation of viral particles and the Virkon S by Du Pont records 99.9999% killing of 58 viral, 31 bacterial and 6 fungal species. All other solid waste (e.g. gloves, plastic ware, cell pellets) will be rendered inactive by autoclaving at 121c for 15 minutes.

Glass ware will be treated with 1% Virkon for a minimum of 1 hour before cleaning.
The primary contact and person involved in the GM safety committee will be a Senior Research Scientist and Biological Safety Officer, previously trained to PhD level in biology. The bso has attended an IOSH accredited Biological Safety course by One Nucleus entitled Biological Safety: Management and Practise. Supporting members of the committee will include the Chief Scientific Officer, a research scientist and a member from the Babraham Research Institute Health and Safety Team including BBT H&S Officer. A supporting role will be played by a contracted external Health and Safety Advisor.

Comments from GMO Safety Committee:
- Change group leader name and number of research staff (as mentioned in section 1.2.7) to accommodate the restructuring of the company.
- Remove 'Examples of from 'Examples of cell lines being used in the lab include: HEK293 Cells' from section 1.2.6.
- Add two additional plasmid vectors and their details to section 2.3.1. These include pCMV-AN-mRFP and Pezm02

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes L3 L4 L2 L3 L4 L2 L4 L2 L3 L4</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
<td>L2 L3 L4</td>
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</table>

Project Ref 3222/15.1

<table>
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<tr>
<th>Date Ackn'd</th>
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<th>Class</th>
<th>CultureVol</th>
<th>CultureVol</th>
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</thead>
<tbody>
<tr>
<td>01/04/2015</td>
<td>Viral transduction of cell lines with vectors for expression or knockdown of P2X receptors</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Non-GMM Consent Granted
The study objectives are to:
- Modify expression of P2X receptors by cancer and 1*100 cancer cell lines to gain insights into its role in cancer biology.
- Create cell lines stably knocked down for P2X.
- Create cell lines for overexpressing variants of P2X.

Recipient or parental organism

Human derived cells such as Neonatal Fibroblasts (C12), Adult Fibroblasts (C11), Keratinocytes (C11) purchased from ATCC.

Host/vector system

Cells will be transduced with commercial available viral shRNA vectors such as PIK01 (sigma) or pLVX-shRNA2 vector (Clontech) containing shRNA targeted to P2X receptors or scrambled controls and a selection marker such as antibiotic resistance (Puromycin, Blasticidin or G418) or Fluorescent protein expression (RFP or GFP).

Mammalian cells will be transduced with viral expression vectors such as lenti X expression system (Clontech) containing P2X receptor expression constructs and a selection marker such as antibiotic resistance (Puromycin, Blasticidin or G418) or fluorescent protein expression (RFP or GFP).

Lentiviral particles for transduction will be made in HEK293T packaging cells with 3rt1 generation packaging systems. lentiviral partides may also be bought ready made from Sigma (Mission shRNA lentiviral particles) or Santa Cruz (sc-42S75-Vor sc:108080).

All lentiviral transduction systems used will involve replication incompetent lentivirus, made with at least 3 separate
vectors, which do not contain the necessary 'liral elements for viral replication. Where necessary plasmids will be propagated using commercially available Escherichia coli K-12 derivatives (TOP 10 series and DH5-alpha, genotype F- mcrA (mrr-hsdRMS-mcrBC) <p80lacZIil.M1 5 il.IacX74 nupG recAI araD139 6(ara- leu)7697 galE15 galK16 rpsL(StrR) endA 1 ) as hosts.

<table>
<thead>
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<th>Insert DNAs</th>
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</tr>
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<td>Executive</td>
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<td>- Forms of the P2X receptor genes, including full length, splice variants, and SNPs, from various mammalian species including but not limited to humans, sheep, mouse, rat, rabbit and dog.</td>
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<tr>
<td>- Fluorescent proteins i.e. GFP and RFP to monitor transgene transfection and expression.</td>
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<tr>
<td>- Antibiotic resistance selection markers including puromycin, blasticidin and G418</td>
</tr>
<tr>
<td>- shRNA sequences targeted within the coding sequences and UTR of P2X receptors.</td>
</tr>
</tbody>
</table>

**Evaluation of foreseeable effects**

**BACTERIAL SPECIES:**
The hazardous potential of the GMMs created is considered no greater than that of the parental line. The E. coli strains used are K-12 derivatives and these have been recognised as non-colonising and disabled. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and are auxotrophic for various essential amino acids that are unlikely to be satisfied outside the laboratory environment. It is unlikely that the expression of human P2X7 gene will have an effect on the pathogenicity of bacterial species, furthermore the bacteria are auxotrophic for essential amino acids which will impair their accidental release into the environment.

**shRNA OF MAMMALIAN CELLS:**
Introduction of shRNA constructs is designed to remove intracellular mRNA for the target genes. The predicted effect is the down regulation of the corresponding protein expression from the target cells. This treatment is expected to have little impact on the nature of the target cell.

**TRANSDUCTION OF MAMMALIAN CELLS WITH vectors expressing P2X receptors:**
The major risk with the transfection of mammalian cell lines is the introduction of viral promoters, i.e. CMV. Waste management measures to avoid accidental exposure and release of potentially hazardous material is described in section 12. Furthermore, over expression of the P2X7 gene in human cell lines, HEK293 cells, has been shown to increase replication in vitro and in vivo (Adinolfi et al 2012). Therefore, great care will be taken to prevent exposure of the operator to the vector containing human P2X7 or the GM cell lines.

**TRANSDUCTION OF CELLS WITH LENTIVIRAL CONSTRUCTS**
Lentiviral transduction system are capable of infecting human cells and must be handled with care. However, all lentiviral transduction systems used will involve replication incompetent lentivirus, made with at least 3 separate vectors, which do not contain the necessary viral elements for viral replication. This makes production of whole replicating virus essentially impossible.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not applicable, only microorganisms (including tissue culture cells) are involved in this work.
All waste disposed of according to the Babraham Research Campus -Hazard Group 2 Waste disposal S.O.P (waste sealed in 30L or 60L Euro bins and sent offsite for incineration by Vetspeed (GM authorisation GM898, notification GM105/4.1 ).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Tissue culture waste will be treated with 1% Virkon for a minimum of 12 hours before disposal down sinks or in yellow biological safely bins. Any contamination of work surfaces will be wiped with a 1% Virkon solution. Periodically we will assess the degree of killing by Irypan blue counts or fluorescent viabiliy dyes. The primary risk is the generation of viral particles and the Virkon S by Du Pont records 99.999910 killing of 58 viral, 31 bacterial and 6 fungal species.

Disposable waste material will be placed in sealed 30 or 601 eurobins and sent offsite for incineration by Vetspeed (GM authorization ref. GM898) according to Babraham Institute disposal notification GM1054.1. Prior to disposal clip a RED LID onto the eurobin and ensure it is firmly attached. Then close the lid and push down firmly around the edges of the bin to seal. Place a category 2 sticker, which contains the UN 3373 diamond sign, onto the eurobin. Spray the bin all over with 2% Trigene.

Glass ware will be treated with 1% Virkon for a minimum of 1 hour before cleaning.

Liquid waste from lenti viral work will be collected and solidified with absorbeZe powder in plastic containers which will be disposed as solid waste in sealed 30l or 60l Euro bins and sent offsite for incineration by Vetspeed (GM authorisation GM898, notification GM105/4.1).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The primary contact and person involved in the GM safety committee will be a Senior Research Scientist and Biological Safety Officer, previously trained to PhD level in biology. The BSO has attended an IOSH accredited Biological Safety course by One Nucleus entitled Biological Safety: Management and Practise.

Supporting members of the committee will include the Chief Scientific Officer, a research scientist and a member from the Babraham Research Institute Health and Safety Team including BBT H&S Officer. A supporting role will be played by a contracted external Health and Safety Advisor.

Commnts from GMO Safety Committee:
The GMO safety committee has reviewed and agreed the risk assessment attached.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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02/03/2022
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Animal Units  Large Scale Activities  Human Clinical Applications

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**Name**

BRITISH GEOLOGICAL SURVEY

**Campus Estate or Research Centre**

ENVIRONMENTAL SCIENCE CENTRE

**Road Name**

NICKER HILL

**Town**

NOTTINGHAM

**District**

KEYWORTH

**County**

NOTTINGHAMSHIRE

**Postcode**

NG12 5GG

**Country**

ENGLAND

**Tel Number**

0115 936 3100

**Fax Number**

0115 936 3200

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

**Give brief details of the genetic modification safety committee**

Composition: Geomicrobiology Laboratory Manager, Health & Safety Officer, 2 x Union reps. Centre for Ecology and Hydrology (CEH) Health and Safety Officer will be occasional member of the committee in an advisory capacity

Frequency of meeting: Annually, or when new activities are proposed.

Operation procedures: Agenda distributed in advance, meetings minuted and the minutes made freely available to all staff to consider issues of safety around the GM work.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Level 2 (GMMs)</td>
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<td>Other (please specify)</td>
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Tick if confidential

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02/03/2022
Microorganisms will be deactivated either by autoclaving for 20 minutes at 121°C (e.g., solid cultures).

Autoclave is serviced every six months.

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<thead>
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<th>Bacteriology</th>
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<td></td>
<td>Invertebrates</td>
<td>Plants</td>
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</table>

Other(s) | Cloning of PCR products from a variety of organisms into E. coli

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Microorganisms will be deactivated either by autoclaving for 20 minutes at 121°C (e.g., solid cultures).

Autoclave is serviced every six months.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

Non-committee members are happy to proceed with this notification.
## Data Premises Notified
- **Date:** 23/07/2014
- **(Originally) Data Premises Closed**

## 1992 Regs?
- **Transferred from 1992 Regs:** N

## Transitional Premises
- **Transitional Premises Class:**
- **Emergency Plan Required:** N
- **Non-GMMs:** N
- **Withdrawn:** N

### Name
- **CANTERBURY CHRIST CHURCH UNIVERSITY**

### Campus Estate or Research Centre

### Road Name
- **NORTH HOLMES ROAD**

### Town
- **CANTERBURY**

### Building
- **BUILDING 2 - STEHM**

### District
- **KENT**

### County
- **CT1 1QU**

### Country
- **ENGLAND**

### Tel Number
- **01227 767700**

### Fax Number
- **0**

### E-mail
- **blank**

### HSE Division
- **blank**

### Comments

### Date at Which Additional Info Submitted
- **02/03/2022**
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Y

02/03/2022
Role of the Genetic Modification Safety Committee

- To advise on risk assessment
- To monitor and review GM activities carried out within the Department
- To develop Local Rules for work involving genetic modification
- To consider accidents or incidents involving genetic modification
- To consider the findings from safety inspections where these relate to activities involving genetic modification.

Constitution of the Committee

The Committee should be composed of:

- Chairperson (elected by the Committee)
- Secretary
- Head of Department or representative (may be Chair of Committee).
- Biological Safety Officer
- Departmental Health and Safety Officer
- Technical Staff representative
- Representatives from University Safety Office.

The BSO should no be the chair of the Committee.

There should be a mechanism for consulting and informing organisations affected by the GM activities conducted within the department (e.g. cleaning and maintenance etc.).

The occupational helath representative should be invited to attend in connection with issues relating to the management of health risks.

The committee should report to the Health and Safety Group.

Role of the GM Chair

The GM Chairperson should be an academic member of Canterbury Christ Church University staff. They must be nominated by the GMSC and formally appointed by the Head of Dean of Faculty.

The GM Chair must:

- Ensure that local rules are drawn up and followed for the safety of all.
- Advise on the training of personnel in appropriate microbiological practice.
- Investigate accidents or spillage etc in the laboratories and taking any action they consider necessary.
- Appropriate records and reports should be made.
- Ensure that accurate records are kept.
• Ensure that appropriate disinfection procedures for the laboratories are in place and are followed.
• Participate in locally organised inspections.
• Ensure that control measures and equipment are tested and appropriately maintained.
• Ensure appropriate waste disposal procedures are used and records kept.
• Provide technical support to the GMMSC on risk assessment and classification.
• Ensure all statutory notifications are made to the HSE with copies to University's RPG.
• Ensure the adequacy of arrangements for the physical security of the laboratories.

Duty/Role of GMSC Members

The GMSC members must be familiar with the following:

• GMO (Contained Use) Regulations.
• ACGM Compendium of Guidance.
• Advisory Committee on Dangerous Pathogens (ACDP) "The management, design and operation of microbiological containment laboratories" ISBN 0 71762034 4.
• Control of Substances Hazardous to Health (COSHH).
• All other relevant publications.

Awareness of these documents will be raised to the committee members by the BSO in the first instances and any other persons who may receive relevant documentation.

The Chair and members should:

• Seek advice from the BSO, when necessary. The BSO will seek confidential technical advice from sources outside the committee as and when appropriate.
• Have an understanding of the risks to both human and animal health, and to the environment that may arise from any proposed GM activity.
• Check the accuracy of the risk assessment presented to the committee.
• Review in detail GM risk assessments presented to the committee.
• Review in detail GM risk assessments presented to the Committee and advise accordingly. Ensure the risk assessment has been completed in accordance with the GMO (Contained Use) regulations and other relevant ACGM guidance.
• Advise on the genetic modification safety training requirements needed to carry out the work.
• Consider whether the appropriate containment facilities, as indicated by the risk assessment, are suitable and by what means such work may otherwise be carried out.
• Keep projects discussed within the Committee confidential.

Meetings of the Genetic Modification Safety Committee (GMSC)

The Committee should meet regularly, at least termly. Dates of the meetings should be fixed in advance. Notice of the meetings should be circulated one week in advance and accompanied by an agenda, minutes of the last meeting and papers for discussion. The Minutes of the Committees should be reported to the University RPG.

An accurate record of the meeting must be kept of:
- Date of meeting.
- Attendees.
- Apologies/absences.
- Title of projects submitted to the Committee, principal investigator, department, division and location of work to be carried out in.
- Risk assessment and other forms submitted for the Committee’s consideration.
- Agreed classification of projects and whether notification to the HSE is required.
- Containment level required.
- Any further control measures to be taken by the personnel involved with the project.
- Amendments to be made to the risk assessment form.
- Comments and concerns raised by the GMSC members regarding the project.
- Dissenting opinion and agreed outcome.
- Actions to be taken and the named responsible person.

Minutes must be distributed to Head of Department and to the University Safety Manager.

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Tick if confidential

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<th>Parasitology</th>
<th>Transgenic</th>
<th>Microbiology</th>
<th>Yes</th>
</tr>
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<td>Virology</td>
<td>Transgenic</td>
<td>Transgenic</td>
<td>Gene Therapy</td>
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</tr>
<tr>
<td>Mycology</td>
<td>Transgenic</td>
<td>Transgenic</td>
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<tr>
<td>Other(s)</td>
<td>Invertebrates</td>
<td>Plants</td>
<td></td>
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</table>

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Prior to final discharge/disposal of waste from contained use activities, any risks to humans and the environment associated with any GMO must be removed by use of validated inactivation methods. Inactivation refers to the complete or partial destruction of GMMs so as to ensure that any contact between the GM plant material and humans or the environment is limited to provide a high level of protection to both humans and the environment.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental materials, including soil, should be clearly marked and be treated by a validated means before disposal to kill any residual organisms, such as seeds, tubers etc.</td>
</tr>
<tr>
<td>Plastic consumables should be made safe by autoclaving (126-132°C for 15 mins) and solids disposed of via the industrial waste stream for landfill or incineration. Liquids (eg samples, culture supernatants, tissue culture media) should be made safe by autoclaving (126-132°C for 15 mins) and discharged to drains. Agar plates - should be autoclaved (126-132°C for 15 mins) and disposed of via the industrial waste stream for landfill or incineration. 1% (w/v) Virkon disinfectant solution should be used to decontaminate surfaces (10 min) contact time). Large spills will be treated with Virkon powder and mopped up with paper towel. This will be autoclaved as for solid waste.</td>
</tr>
<tr>
<td>Autoclaving, effectively 100% kill (annual validation)</td>
</tr>
<tr>
<td>Chemical disinfection with Virkon, used according to manufacturers instructions under standard conditions, manufacturers validation (e.g. 99.998% kill)</td>
</tr>
</tbody>
</table>

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

| GMSC members reviewed the proposed risk assessment and deemed the questions asked would thoroughly and comprehensively cover any potential GM activities proposed to be undertaken within the department.  
It was noted that no GM activities of a class 2 nature or above would be carried out in the department within the foreseeable future |
<table>
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<tr>
<th>Data Premises Notified (Originally)</th>
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<tbody>
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<td>Transferred from 1992 Regs?</td>
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<td>Transitional Premises</td>
<td></td>
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<tr>
<td>Emergency Plan Required?</td>
<td></td>
</tr>
<tr>
<td>Non-GMMs</td>
<td>N</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>N</td>
</tr>
</tbody>
</table>

Name
SYNPROMICS LTD

Name 2

Department

Campus Estate or Research Centre
THE ROSLIN INNOVATION CENTRE

Road Name
EASTER BUSH CAMPUS

District

Town
MIDLOTHIAN

County

Postcode
EH25 9RG

Country
SCOTLAND

Tel Number
0131 658 5301

Fax Number
0131 658 5302

E-mail

HSE Division
blank

Comments
address change from 28/08/2017

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
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<tr>
<td>28/08/2017 SYNPROMICS LTD</td>
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<td></td>
<td></td>
<td>9 LITTLE FRANCE ROAD</td>
<td>EDINBURGH</td>
<td>EH16 4UX</td>
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<tr>
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<td>EASTERN BUSH CAMPUS</td>
<td>MIDLOTHIAN</td>
<td>EH25 9RG</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

02/03/2022

Page 13481 of 15326
Genetic Modification Safety Committee

a) Terms of Reference
1. To receive and review in detail and in a systematic manner every proposal for genetic modification work and to judge the adequacy of the risk assessment and the identification of appropriate containment and other risk control measures.
2. To periodically review risk assessments.
3. To advise on the preparation and review of local rules to cover work involving genetic modification.
4. To advise on the needs for training, equipment and resources for genetic modification work.
5. To consider any accidents or incidents relevant to genetic modification work.
6. To consider and recommend arrangements for health monitoring or surveillance where appropriate.
7. To disseminate legislation and approved guidance relevant to work with genetic modification.
8. To promote a knowledge and understanding of genetic modification within the University.
9. To assist and advise the Authorised Person to discharge the responsibilities for statutory notifications and record keeping.
10. To annually report on its activities to the Health and Safety Manager

b) Frequency
The Committee will meet 6 monthly, or more frequently should the need arise.

c) Constitution
There are no hard-and-fast rules governing the make-up of a GMSC. Ideally it should have balanced representation of both management and employees with its members also being representative of all persons having access to the genetic modification facilities or who might otherwise be exposed to such work.

It should have enough members, with sufficient depth and range of knowledge and experience to:-
• Understand the risks to both human health and the environment arising from the normal range of activities undertaken, and the extent to which risks are uncertain.
• Judge the adequacy of the risk assessment.
• Test its emerging conclusions by discussion so that the advice given is genuinely that of a committee and not an individual.

d) Composition
• A Chairperson.
• Representatives of management with responsibility for GM work.
• Representatives of those leading work with GM.
• Representatives of Technical Staff supporting work with GM.
• Representatives of persons having access to the GM facilities or who might otherwise be exposed to GM work, e.g. ancillary staff, administrative support staff, contractors.
• The Biological Safety Officer (the person appointed to comply with the legally required notifications and record keeping).
• Co-opted persons to supplement expertise where necessary.
• Note: Medical surveillance is not a specific requirement and a supervisory medical officer is not required to be appointed.

The composition (but not names) of the GMSC must be notified to the HSE as part of notification of first use of premises for GM activities. Any changes to the composition should also be notified.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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<td></td>
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</table>
Non-microbial

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<th>Other (please specify)</th>
<th>Tick if confidential</th>
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<td>Tick if confidential</td>
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<td>Mycology</td>
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<td>Transgenic</td>
<td>Other (please</td>
</tr>
<tr>
<td></td>
<td>Invertebrates</td>
<td>Plants</td>
<td>specify below)</td>
</tr>
</tbody>
</table>

Other(s) Mammalian cell lines derived from human, mouse or any other group depending on the project requirements

For activities involving GMMs, describe the waste management measures which will apply to the activity

- **Solids** (e.g. plastic-ware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

- **Liquids** (e.g. samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

- **Sharps** (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste stream for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All Risk Assessment were approved with no further action required.

The committee agreed classification of the projects/products and that the HSE should be notified that Synpromics Ltd is ready to commence work.
**Project Ref** 3226/15.1

**Date Ackn'd** 30/04/2015

**CU2 Project Title** Culture of non-human and human primary and cell recombinant viral vectors

**Date Project Ceased**

**Class** Class 2

**CultureVol** 1-50 Litres

**CulturesVolume**

**Class** Class 2

**Non-GMM Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Mammalian cell line cultures derived from human, mouse or any other origin depending on the project requirements.

Primary cell cultures of animal and human origin.

Transient and stable transfection of promoter activity reporter vectors.

Screening of promoter libraries using the following recombinant viral vectors: AAV (adeno-associated virus), adenoviral, baculoviral, retroviral and lentiviral vectors.

**Recipient or parental organism**

Human, mouse, rat or any other mammalian organism depending on the needs of each project.

**Host/vector system**

**Host**

Mammalian cell line cultures derived from human, mouse or any other origin depending on the project requirements.

Primary cell cultures of animal and human origin.

**Vector system:**

Commericially available recombinant viral vectors including: AAV (adeno-associated virus), adenoviral, baculoviral, retroviral and lentiviral vectors.

Libraries of promoter elements/parts will be cloned in recombinant viral vectors. GFP, Luc or any other reporter gene will be used to detect transcriptional activity depending on the particular needs of each project.

**Origin & function**
The intended use of the GMOs is to screen libraries of premoter elements/parts cloned upstream of a reporter gene (GFP, Luc or any other gene of interest depending on the detection method used for the screen) in mammalian cells to identify premoter elements/parts active in the cells and/or conditions of interest depending on each individual project.

For biosafety reasons, the viral genes for packaging in the recombinant viral vectors have been split in different plasmids and these plasmids do not share any sequence homology between them and the lentiviral screening vector. This strategy prevents the generation of replication-competent virus and the resultant viral particles cannot replicate autonomously in target cells.

### Evaluation of foreseeable effects

<table>
<thead>
<tr>
<th>Event</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
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</tbody>
</table>

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

<table>
<thead>
<tr>
<th>Type of Waste</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids</td>
<td>Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.</td>
</tr>
<tr>
<td>Liquids</td>
<td>Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes) discharge to drains.</td>
</tr>
</tbody>
</table>

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors), 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol Presept may be used as an alternative to Virkon: 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Waste Type</th>
<th>Treatment Method</th>
<th>Disposal Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids</td>
<td>Autoclave</td>
<td>Clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.</td>
</tr>
<tr>
<td>Liquids</td>
<td>Autoclave</td>
<td>Discharge to drains.</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
All Risk Assessments were approved with no further action required.

The committee agreed classification of the projects/products and that the HSE should be notified that Synpromics Ltd is ready to commence work.

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 Yes</td>
<td>L3 L4 L2 L3</td>
<td>L3 L4 L2 L3</td>
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<td>L3 L4</td>
<td>L3 L4</td>
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### GM Centre Number: 3227

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#### Name

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#### Name 2

#### Department

#### Campus Estate or Research Centre

<table>
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<th>WHITE HORSE BUSINESS PARK</th>
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#### HSE Division

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#### Comments

#### Date at Which Additional Info Submitted

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Page 13487 of 15326
### Premises Addresses

<table>
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<tr>
<th>Date Premises Closed</th>
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<th>Building</th>
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<td>ATLAS GENETICS</td>
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<td>WILTSHIRE</td>
<td>BA14 OXG</td>
<td>N</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

There are three members of the genetic modification committee and they also hold positions in the health and safety committee; the head of both committees is the CEO of the company. The Biological Safety Officer sits on both committees and the third member comes from the scientific staff and has experience of working with GMOs. The committee meets every three months and any issues that require discussion are communicated beforehand. The status of any impending actions is followed up at meetings.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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</tr>
<tr>
<td>Level 2 (GMMs)</td>
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<td>Level 3 (GMMs)</td>
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<td>Level 4 (GMMs)</td>
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<td>Bacteriology</td>
<td>Yes</td>
<td>Parasitology</td>
<td>Transgenic Birds</td>
<td>Microbiology Research</td>
</tr>
</tbody>
</table>
All waste will be autoclaved at 134°C for 20mins to ensure sterilisation. The sterile waste will then be disposed of as clinical waste for incineration. In addition to using autoclave tape, the autoclave prints out the conditions achieved each run and indicates if the program passed or failed and this information is logged and stored. The autoclave is serviced 6 monthly and calibrated annually.

DNA diagnostics; bacteria are to be transformed with truncated target genes

For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste will be autoclaved at 134°C for 20mins to ensure sterilisation. The sterile waste will then be disposed of as clinical waste for incineration. In addition to using autoclave tape, the autoclave prints out the conditions achieved each run and indicates if the program passed or failed and this information is logged and stored. The autoclave is serviced 6 monthly and calibrated annually.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The work is of low risk as the bacterium is derived from a non-pathogenic strain and none of the genetic material to be transformed into the bacteria are functioning genes. Nonetheless, the work will be carried out under containment level two conditions to further minimise risk.
### AVACTA LIFE SCIENCE

#### Name 2

<table>
<thead>
<tr>
<th>Name 2</th>
<th>Department</th>
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#### Road Name

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<table>
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<tbody>
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#### Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee
Promexus Ltd is a fully owned subsidiary of the Avacta Group plc (Unit 706, Thorp Arch Estate, Wetherby, LS23 7FZ).
The Avacta Group consists of the following companies which are all located on the Thorp Arch Estate, Avacta Life Sciences, Avacta Animal Health and Avacta Analytical

As both Promexus and Avacta Life Sciences use the same core protein technology platform, we plan to use the Genetic Modification Safety Committee (GMSC) that has already been established at Avacta Life Sciences to review all risk assessments relating to the creation, use and disposal of genetically modified organisms.

Given the scope of operations within the Avacta Group, they have extended the role of the Genetic Modification Safety Committee (GMSC) to take account of general biological, chemical and physical safety. As a consequence of the widened role, the GMSC has been renamed the Laboratory Safety Committee (LSC).

The membership of the Committee is as follows:

Chair:
PhD (University of Nottingham) – Received training in molecular biology techniques at Queen’s Medical Centre, Nottingham and transferred systems to research lab. Gained extensive molecular biology experience including cloning, PCR, DNA purification and mutational analysis. Optimised protein expression in E. coli and devised protein purification protocols.

2003-2010 PDRA (University of Leeds) – Used standard molecular biology methods regularly and frequently cultured E. coli in various media.
2010-2012 Application Scientist (Farfield Group, Manchester) – Responsibilities included oversight of lab health and safety.
2012-present Applications Scientist (Avacta Analytical, Wetherby) – Member of lab and biological health and safety committee.

Biological Safety Officer (at Avacta Life Sciences):
University of Sheffield (2000-04), PhD: Cat 2 work with Bacillus cereus.
Abcam (2005-2013): Leaving Position - Head of R&D. Corporate BSO, management of Cat 2 work and Class 1 GMO work.
Avacta Life Sciences (2013-present), Head of R&D.

Other committee members: Lab based staff and management representatives from Avacta Life Sciences, Avacta Animal Health, Avacta Analytical and Promexus Ltd.
The combined laboratory experience of this committee exceeds 50 person years in both academic and commercial setting

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 1 (GMMs)</td>
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<tr>
<td>Non-microbial</td>
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Other (please specify) Tick if confidential
E. coli K12 strains (and their associated DNA plasmids) will be used to manipulate the gene for the creation of engineered protein variants of human Stefin A. The engineered proteins will be expressed and purified from a range of hosts to produce enough material for pre-clinical animal studies and stability testing (100's mgs). The expression hosts will include E. coli, mammalian cells e.g. CHO cells (a well established hamster cell line that cannot survive outside the lab environment) and yeast (pichia pastoris). There will also be a need to occasionally express proteins of human or animal origin to support pre-clinical research. Again these will be expressed either using E. coli, CHO or yeast.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

E. coli K12 strains (and their associated DNA plasmids) will be used to manipulate the gene for the creation of engineered protein variants of human Stefin A. The engineered proteins will be expressed and purified from a range of hosts to produce enough material for pre-clinical animal studies and stability testing (100's mgs). The expression hosts will include E. coli, mammalian cells e.g. CHO cells (a well established hamster cell line that cannot survive outside the lab environment) and yeast (pichia pastoris). There will also be a need to occasionally express proteins of human or animal origin to support pre-clinical research. Again these will be expressed either using E. coli, CHO or yeast.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Waste potentially contaminated with Cat I GMM, such as pipette tips and other disposable plastics; solid microbiological growth media and liquid waste such as biological growth media and waste water from the cleaning of glassware.

Liquid waste will be inactivated by incubation and cleansing with a proprietary cleaner such as Virkon, and disposed of via the drain.

Solid waste, including microbiological growth media, cell pellets and plastics, will be inactivated by autoclaving onsite before disposal via incineration by an approved contractor.
**Project Additional Information**

**Purposes of the contained use**

Phage display selection, production of proteins using mammalian cell line for transient or stable expression and use of transient cell lines to assess the potency effect of the selected proteins.

**Recipient or parental organism**

E.coli, HEK293 (Human embryonic Kidney cells) for transient expression, CHO (Chinese Hamster Ovarian) cell line for stable expression. Fully characterised cell lines, including but not limited to, Jurkat and RAW cell lines or transient cell lines commercially available. Those can be used to assess potency.

**Host/vector system**

Mammalian expression vector

**Origin & function**

1-For example HEK 293 is a virally transformed, stable well established human epithelial kidney cell line that is routinely used in expressing plasmid derived proteins. This cell line has been established for laboratory use only and would not survive long without specific culturing conditions. The HEK293 cells contain DNA from Adenovirus 5. The cell line will be cultured for production of therapeutic proteins.

Regarding other expression cell line as CHO, it is established that 2-In order to assess the potency of the therapeutic proteins reporter genes such as GFP, RFP and luciferase have been used in various transgenic animal systems and have been shown to have no harmful effects on the organisms.
HEK293, CHO or the other cell lines are unable to survive outside their laboratory environments and require cell maintenance therefore they are unlikely to cause any harm to other organisms. Also, the work involving these cells will be undertaken in a Containment Level 2 facility. The cells used are to the best of our knowledge, free of adventitious agents and will be non-self cell lines that will not be able to colonise those involved with the work. Indirect hazards to human health associated with possible adventitious agents have been considered separately in a general Risk Assessment relating to work with human derived materials. All human derived samples are treated as though capable of causing disease.

**Evaluation of foreseeable effects**

HEK293, CHO or the other cell lines are unable to survive outside their laboratory environments and require cell maintenance therefore they are unlikely to cause any harm to other organisms. Also, the work involving these cells will be undertaken in a Containment Level 2 facility. The cells used are to the best of our knowledge, free of adventitious agents and will be non-self cell lines that will not be able to colonise those involved with the work. Indirect hazards to human health associated with possible adventitious agents have been considered separately in a general Risk Assessment relating to work with human derived materials. All human derived samples are treated as though capable of causing disease.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A - full containment of the live cells will be used

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

GMM liquid waste will be disposed off into appropriate disinfectant (2% sodium hypochlorite, 1 % Virkon or a 1:10 dilution of Distel or Chemgene) within a Class II Microbiological Safety Cabinet (MSC) where all work using these organisms will be carried out. After 24 hours, this waste will be discarded into the drains. Bacterial culture plates will be sealed, double-bagged inside the cabinet before being autoclaved on site (see attached Risk Assessment for details of cycles). Contaminated tips will be soaked in appropriate disinfectant (see above) and disposed of in a sharps container. Any contaminated glassware (kept to minimum) will be soaked in appropriate disinfectant for 24 hours, then rinsed prior to normal cleaning. Other solid waste (plasticware) will be autoclaved on site prior to disposal via the clinical waste route. Autoclave logs will be monitored and waste will only be disposed of if the run is successful. Fresh dilutions of each disinfectant will be made weekly. Virkon is a bactericidal agent, which is used at a concentration of 1 %. It has been shown to cause a 5-log reduction in bacterial counts (Hernandez, A. 2000. Journal of Hospital Infection. 46: 203-209). Low concentrations of sodium hypochlorite (bleach) have a biocidal effect on mycoplasma and vegetative bacteria.
in seconds in the absence of high amounts of organic material (Dychdala GR Disinfection, sterilization, and preservation. 2001 :135-157). S. aureus, Salmonella choleraesuis, and P. aeruginosa have been shown to be inactivated in <10 minutes by sodium hypochlorite (Rutala WA et al. Infect Control Hosp. Epidemiol. 1998;19:323-7). Distel I Chemgene are bactericidal and effective in destroying type strain cultures of P. aeruginosa, S.aureus, MRSA and VRE with contact time of 1-5 minutes. Solid waste are incinerated by a laboratory waste management company.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment will be reviewed as the company is moving in a new site.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L2 L3 L4</td>
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Project Ref 3228/18.1

<table>
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<td>04/07/2018</td>
<td>Protein engineering and expression of Affimer therapeutics using human cell lines</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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such as HEK-293 and derivatives

E.coli, HEK293 (Human embryonic Kidney cells) for transient expression, CHO (Chinese Hamster Ovarian) cell line for stable expression. Fully characterised cell lines, including but not limited to, Jurkat and RAW cell lines or transient cell lines commercially available. Those can be used to assess potency.

Mammalian expression vector

HEK293, CHO or other cell lines to be used are unable to survive outside
their laboratory environments and require cell maintenance therefore they are unlikely to cause any harm to other organisms. Also, the work involving these cells will be undertaken in a Containment Level 2 facility. The cells used are to the best of our knowledge, free of adventitious agents and will be non-self cell lines that will not be able to colonise those involved with the work. Indirect hazards to human health associated with possible adventitious agents have been considered separately in a general Risk Assessment relating to work with human derived materials. All human derived samples are treated as though capable of causing disease.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A - full containment of the live cells will be used

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The disinfectants and concentrations (unless otherwise stated) we use are: Virkon (at a final concentration of 1 % [w/v]; hereafter referred to as “Virkon”), 1 % (v/v) Distel and 70 % (v/v) ethanol. Virkon is a high-level laboratory disinfectant that is used as a bactericidal, fungicidal, and virucidal agent. It is effective in the presence of blood-borne diseases (HIV/Hepatitis B).

Cleaning and recycling of reusable laboratory equipment, such as glassware, that has been in contact with biological agents of HG 2 will require a minimal 10-30 minute incubation with Virkon (as per SOP ALS-WE000002) prior to dishwashing (as per SOP ALS-WE000144; wash at 80 °C, drying at maximum temperature), or manual dishwashing (as per SOP ALS-WE000114) and autoclaving (as per SOP ALS-WE000006) at 121°C for 15 minutes. Autoclaves will be validated by twice yearly servicing and autoclave tape with each run.

Liquid waste will be inactivated by an incubation of minimum 30 minutes to overnight with Virkon prior to disposal via the sink.

Aspirators are used whenever possible when liquid is being removed from flasks, bottles, reservoirs etc. to waste. Aspirators provide a closed system whereby aerosol production is kept to an absolute minimum. Aspirators must always be used with the correct filter, cleaned with Virkon and water after each use the waste is disinfected with Virkon as per SOP ALS-WE000115. Where possible the contents of the reservoir should be left overnight to ensure decontamination before discarding to waste down the sink. Minimum contact time is 30 minutes.

Surfaces are to be cleaned with 1 % Distel followed by 70% ethanol.

All disposable plasticware that has been in contact with cells of HG 2 (including pipette tips, stripettes, plates, falcon tubes, reservoirs and flasks) must, after removal of liquid waste, be placed into a fully-sealable clinical waste burn-bin, UN3291 labelled, and stored in a secure bin shed on site. Bins must be sealed before leaving the laboratory to prevent risk of release into the environment during transport. All solid biological wastes are incinerated off-site by waste management company LabWaste, permit no. EPR/SP3895VG, Waste Carriers License: CBDU66492.
The risk assessment will be reviewed frequently during H&S meetings.

Please enter comments on the GM safety committee on the risk assessment

The risk assessment will be reviewed frequently during H&S meetings.

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Animal Units

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Project Ref 3228/19.1

Date Ackn'd 08/02/2019

CU2 Project Title

This is a connected Programme of work: Class 2 activities involving the use of lentiviral and retroviral vectors to overexpress proteins and affimers intracellularly or on the surface of mammalian cells (primary cells and cell lines).

Date Project Ceased

Class 2

< 1 Litre

Non-GMM Consent Granted

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
**Project Additional Information**

### Purposes of the contained use

Overexpression of human, primate and rodent genes in various cells and cell lines using lentiviral/retroviral vectors in order to assess the activity/potency of Affimer therapeutic in immuno-oncology in vitro.

### Recipient or parental organism

- Primary Cells (human T cell)
- Primary cells (Mouse T cell)
- LK35.2 (mouse hybridoma B cell line)
- DO1110 (mouse hybridoma T cell line)
- HEK293 (Human Embryonic Kidney cell line)

### Host/vector system

**Lentivirus:**

The major risks to be considered for work with HIV-1 based lentivirus vectors are the potential for generation of replication-competent lentivirus (RCL), and the potential for oncogenesis via random chromosomal integration. The viral regulatory genes gag, pol, and rev are encoded in a separate plasmid and the gene of interest is encoded by the transfer vector which lacks any transacting structural genes. These constructs have been altered to contain non-overlapping sequences, hence minimizing the possibility of recombination. In addition non-essential genes have been eliminated from the virus, thus any replication-competent virus generated would lack essential factors for replication and virulence in vivo. Thus, even though the virus particles could infect human cells at the virus packaging stage, the virus is replication deficient.

The nature of the transgene must also be considered in assessing risk. These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector (e.g., expression of a known oncogene with a constitutive strong promoter may require heightened safety precautions). The genes used in this work are not oncogenes with constitutive promoters, thus the risk is minimal.

**Evaluation of foreseeable effects.**

The potential for generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters, the most important of which are the number of recombination events necessary to reassemble a replication competent virus genome and the number of essential genes that have been deleted from the vector/packaging system. On this basis, later generation lentivirus vector systems (3rd and 4th generation) that are used in this project are likely to provide a greater margin of personal and public safety than earlier vectors, because they use a heterologous coat protein (e.g., VSV-G) in place of the native HIV-1 envelope protein, thus reducing the risk of RCL generation. Later generation vector systems also separate vector and packaging functions onto three or four plasmids and they include additional safety features such as the deletion of Tat, which is essential for replication of wild-type HIV-1, and altered 3' LTR that renders the vector “self-inactivating” (SIN).

### Origin & function

The genes of interest that are transferred to host cells using the lentiviral vector system are genes of human, rodent or primate origin and normally involved the regulation the immune system during inflammatory activation and/or in immuno-oncology. The intended use of these genes is exploiting their natural function to evaluate the anti-tumor potency of affimer therapeutics in vitro. The genes will be expressed on host cells and their role in immune
Evaluation of foreseeable effects

There are three different GMO's being produced during this process:

1) Bacteria with the individual components: Bacteria will not contain more than one viral component and therefore are unable to produce viral particles. Bacterial strain used is standard K-12 derivative DH5α E.coli, which is the most popular and widely available laboratory strain, and the presence of the vectors is not expected to alter pathogenicity of the bacteria, nor alter their survivability in the environment.

2) Lentivirus/retrovirus: Individual components for the virus are transfected into HEK293 cells where they are packaged and released. The lentivirus particles obtained from the packaging cells is infectious and will be handeled accordingly. As lenti viral particles are capable of infecting non-dividing cells, the most probable route of exposure for this work would be dermal via sharps (needle-sticks), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure of the eyes, nose, and mouth. Another route would be inhalation via aerosols depending on the use of equipment such as centrifuges or vortex mixers. Care will be taken when pipeting in order to avoid splashing or generation of aerosols. Immunocompromised individuals will not work with lentivirus. Work involving the virus particles will be in a microbiological safety cabinet level 2. Gloves and laboratory coats and other PPE will be worn and no sharps will be used. Furthermore, it should be borne in mind that the viral particles produced are non-replicative and self inactivating. The presence of the inserts (gene of interest) will not alter this and therefore unlikely to alter survivability in the environment.

3) Infected mammalian cell lines: As the viral particles are non-replicative and self inactivating, once the target cells are infected, the virus will integrate and express the desired proteins, but will not cause any further viral production. Thus, after about 5 days of transduction, the infected mammalian cells will be free of infective viruses and safe to handle. In the mean time during the 5 days transduction period the cells will be considered infective and handeled accordingly. As the genes used to generate the GMOs are normally found in mammalian cells, the trasduced cell lines are not expected to have altered survivability and impact on the envionment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No large GMO are involved as this is an in vitro work using cultured cells.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid Waste: All solid materials that come in contact with virus-containing solutions or vessels will be decontaminated by spraying with 1% sodium hypochlorite (10% bleach) or 1% Virkon and exposed for 20-30 min, contained before exiting the biosafety cabinet. Solid waste will be collected in a double layer biohazard bag inside the Biosafety Cabinet. Pipet tips are considered to be sharps and will be collected in a disposable plastic box (such as an empty P-1000 box), and the box closed and deposited into the biohazard bag (in the Biosafety Cabinet) at the end of the work session. At the end of the work session, the biohazard bag will be closed, sprayed with 70% EtOH, and deposited into a biohazardous waste container for incineration. The biohazard waste container will be closed all the time.

Liquid Waste: Liquid waste including media and solutions will be pipetted into a 500 ml bottle containing bleach with 1% final concentration of sodium hypochlorite or 1% Virkon. For example, 50 ml of bleach that contains 10% sodium hypochlorite will be added to the 500 ml bottle before starting the lentivirus work and placed in the BSC.
After finishing work with the lentivirus, the liquid waste will be left in the bleach for 20-30 minutes for effective
decontamination. The decontaminated liquid waste will then be poured down the sink and cold water will be run for
minimum 5 minutes to flush the corrosive disinfectant from the sink. The bottle can be rinsed and reused. Liquids
disinfected with bleach will not be autoclaved to minimize generation of chlorinie gas.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

The overall risk is low as all the organisms to be used are classified at hazard group 2 and the activity is classified
2

Project Containment

<table>
<thead>
<tr>
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<td>L4</td>
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Animal Units  
Large Scale Activities  
Human Clinical Applications

Project Ref  3228/19.2

Date Ackn’d  08/03/2019  
Date Project Ceased  
Withdrawn  
Tick if notifying a connected programme of work  

CU2 Project Title  Raising Affimers Against Adenovirus 5

Class  Class 2  
CultureVolClass2  Not Applicable  
CultureVolumeClass3-4  
Non-GMM  Consent Granted  

Project notified under transitional arrangements  

Project Additional Information

Purposes of the contained use

The project is to raise Affimers against attenuated Adenovirus 5. This Risk Assessment specifically covers the work involving attenuated Adenovirus 5. Risk Assessments for the screening and growing of Affimers are covered elsewhere.

Recipient or parental organism

The wild type Adenovirus serotype 5 causes sub-clinical infections and is categorised by the ACDP as Hazard Group 2.

Host/vector system

Attenuated Adenoviral Vector System

The adenoviral vectors to be used are attenuated through deletion of the E1 region of the genome (containing E1A and E1B) and through insertions and deletions within the E3 gene that inactivate it. This has several effects on the virus. Firstly, deletion of the E1A gene eliminates the potential for viral transformation of cells, since the E1A gene product is absolutely required for this process. It is also required for activation of all other early genes (E1B, E2, E3 and E4). The E1B gene product (also deleted) co-operates with E1A in transformation. The E2 region (E2A and E2B) contains DNA binding (E2A) and DNA polymerase (E2B) activities that are absolutely essential for viral replication. The E3 gene product helps in viral avoidance of the immune system by binding to the major histocompatibility complex MHC polypeptides in the endoplasmic reticulum. However, this gene is also inactivated in this strain of adenovirus, resulting in the virus being highly susceptible to immune surveillance. The E4 gene product is required for formation of an active complex between the E1B gene product and the E4 gene product.

The attenuated virus cannot recombine with wild-type virus in any way that produces viable virus (Beck T. C et al Methods Cell. Biol. (1994) 43, 161-89). Recombination with a wild-type virus would, by the nature of the
recombination event, remove the E1 region from the wild-type virus thereby inactivating its ability to replicate. Taken together, the above means that the recombinant virus is essentially unable to propagate in the external environment.

**Origin & function**

The adenoviral vectors to be used are attenuated through deletion of the E1 region of the genome (containing E1A and E1B) and through insertions and deletions within the E3 gene that inactivate it. This has several effects on the virus. Firstly, deletion of the E1A gene eliminates the potential for viral transformation of cells, since the E1A gene product is absolutely required for this process. It is also required for activation of all other early genes (E1B, E2, E3 and E4). The E1B gene product (also deleted) co-operates with E1A in transformation. The E2 region (E2A and E2B) contains DNA binding (E2A) and DNA polymerase (E2B) activities that are absolutely essential for viral replication. The E3 gene product helps in viral avoidance of the immune system by binding to the major histocompatibility complex MHC polypeptides in the endoplasmic reticulum. However, this gene is also inactivated in this strain of adenovirus, resulting in the virus being highly susceptible to immune surveillance. The E4 gene product is required for formation of an active complex between the E1B gene product and the E4 gene product. The attenuated virus cannot recombine with wild-type virus in any way that produces viable virus (Beck T, C et al Methods Cell Biol. (1994) 43, 161-89). Recombination with a wild-type virus would, by the nature of the recombination event, remove the E1 region from the wild-type virus thereby inactivating its ability to replicate. Taken together, the above means that the recombinant virus is essentially unable to propagate in the external environment.

**Evaluation of foreseeable effects**

Under conditions where an organism (human or non-human mammalian) already was suffering from an adenoviral infection, it is possible that the recombinant virus could be replicated and packaged. Since this is likely to be a relatively rare event, and not one that could sustain a population of recombinant virus, the risks are extremely low. Additionally, any wild-type virus would be at a significant growth advantage with respect to the recombinant virus (for the reasons mentioned above concerning attenuation of the virus), meaning that the wild-type virus would outgrow the recombinant one very quickly.

There will a GFP gene inserted in the adenovirus. The GFP gene product is non-hazardous and non-oncogenic. There are no additional hazards of the GFP sequence being transferred to related Adenovirus. The adenovirus is attenuated by deletion of the E1 region of the genome and unable to propagate in the environment. They are replication incompetent, so do not have any possibility of further spread. They will be easily outcompeted.
by any wild-type Adenovirus.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Laboratories where GMM will be used have chemically impervious flooring (linoleum) that is easy to clean. Laboratory benches and sinks are made of impervious materials (Trespa or steel) that are easy to clean. Any vessels containing GMMs that are being shaken or centrifuged must be closed. Aerosol formation must be avoided.

Pipettes must be cleaned after they have been used with adenovirus with 1% (v/v) Distel solution followed by 70% (v/v) ethanol.

GMMs are stored in safe areas as (incubators, fridges or freezers) within the laboratories. Entry into the building is via key fob only. The building and all internal doors are locked overnight, and the building is protected by a security system.

All work involving open virus shall be undertaken in a Class 2 BioSafety Cabinet, or if not practical in a fume cabinet. This includes phage selection up to the stage the bacteriophages are eluted; as the phages are eluted in 0.2M Glycine, pH2.2, this will permanently inactivate the adenovirus. As an extra precaution, the eluted phage will be heated at 60°C for 15 minutes to ensure complete inactivation of any residual adenovirus. Care will be taken with the eluted bacteriophage, but amplification of phage will take place on the bench in the R&D laboratory. Any ELISA involving viable adenovirus will be carried out wholly in the Class 2 BioSafety Cabinet, until reading the ELISA plate on the plate reader, where the plate will be sealed with an adhesive plate seal and read on the plate reader in the main lab.

**Personal Protection**

Staff and visitors are required to wear a lab coat and protective eyewear when in the lab, whether at the bench or not, and to wash hands in a dedicated sink when leaving the lab. Gloves are provided and are recommended when working with GMMs.

Mouth pipetting is explicitly forbidden, as is eating or drinking or the application of cosmetics in the lab.

**Waste and Spills**

All waste potentially contaminated with GMM is to be treated as detailed in section 4.3 prior to disposal.

Spill kits are available under the sink in both labs. Liquid spills may be treated with powdered Virkon, and mopped up from outside the spill. All solid waste resulting from cleaning the spill should be autoclaved prior to disposal.

All solid contaminated materials, including waste destined for incineration, will be placed into a yellow clinical waste bin. These bins will be collected by a specialist lab waste disposal company (LabWaste Ltd., Hinckley, UK or an equivalent waste contractor). Cleaning and recycling of reusable laboratory equipment, such as glassware, will require a minimal 30 minutes incubation with 1% Virkon (or an equivalent bactericidal detergent) (10 min for metal items due to corrosive nature of Virkon) prior to dishwashing (wash at 80°C, drying 65°C) and autoclaving. Glassware that has been contacted with bacteriophage will be treated with 2% Virkon.

Liquid waste will be inactivated for at least 30 min or overnight with 2% Virkon prior to disposal via the sink.
Surfaces are cleaned with 1% Virkon or 70% ethanol (if no open flame is present). Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The genetic safety committee was consulted and comments were incorporated in the risk assessment.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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#### Name

| AGALIMMUNE LIMITED |

#### Campus Estate or Research Centre

| DISCOVERY PARK HOUSE |

#### Road Name

| RAMSGATE ROAD |

#### Town

| SANDWICH |

#### District

| KENT |

#### County

| CT13 9ND |

#### Postcode

| ENGLAND |

#### Tel Number

| 01304728757 |

#### Fax Number

| 0 |

#### E-mail

| blank |

#### HSE Division

| blank |

#### Date at Which Additional Info Submitted

| 02/03/2022 |

#### Comments

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## Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**  
Y

**Give brief details of the genetic modification safety committee**

The genetic modification committee will comprise the Laboratory Manager and the Head of Translation Research, both of whom are senior scientists with in depth experience of working with GMOs under containment level 2 conditions.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

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<th>Transgenic Birds</th>
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<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All solid waste (e.g. plasticware) will be discarded in designated GMM waste bins containing Biohazard marked waste bags. This waste will be collected and removed for incineration at Discovery Park by Park Serve, a contractor that specialises in the handling and disposal of hazardous biological waste. All liquid waste will be inactivated by adding to Virkon to make a 1% Virkon solution and incubated for >15 minutes, likewise pipettes and glassware will be soaked in 1% Virkon solution and incubated for >15 minutes before disposal down a sink. Areas where GMM work has taken place will be disinfected with a 1% Virkon solution once completed.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Genetic Safety Committee deems that the use of cell lines with biosafety level 1 can be used in the laboratory under containment level 1 conditions with the correct waste management and handling as laid out in the risk assessment.
## Project Additional Information

**Purposes of the contained use**

| Use of biosafety level 2 designated mammalian cell lines and primary human cells in experiments to determine the efficacy and mode of action of novel therapeutics |

**Recipient or parental organism**

| Not applicable |

**Host/vector system**

| Mammalian cell lines and primary human cells |

**Origin & function**

The origins of the biosafety level 2 designated cell lines are mammalian and are all available from commercial sources such as Public Health England. In most cases these cells have been transformed with a viral vector to immortalise them or they may have been demonstrated to be infected with a pathogen. This requires them to be handled and disposed of under containment level 2 conditions. Human primary cells such as peripheral blood mononuclear and natural killer cells will be obtained from commercial sources where they have been tested for the presence of pathogens. However, the cells still need to be treated as potentially infectious and will therefore also be handled under containment level 2 conditions. The cell lines may be transiently or stably transfected with human proteins using non-viral methods in order to express that protein on the cell surface for functional studies. These proteins will be non-infectious.

**Evaluation of foreseeable effects**

The cell lines are potentially infectious, although with organisms that do not cause serious human disease and have been designated BSL-2 by ATCC and ECACC. The pathogens and their risks are well documented and all cell lines will be purchased from commerical sources. The risks to human and environmental health are thus low. The primary human cells to be purchased from commercial sources are potentially infectious, although they will have been screened for the serious human pathogens HIV, Hep B & Hep C. The risk to human and environmental health is therefore moderate, but warrants use under containment level 2 conditions. The proteins that may be transfected to the cell lines will be naturally occurring human wild-type or mutant variants of human proteins and are not infectious.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| Not applicable |

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

| All solid genetically modified waste (e.g. plasticware) will be removed by contractors (Park Serve) and incinerated on site according to validated methods |

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

| All solid waste (e.g. plasticware) will be discarded in designated GMM waste bins containing Biohazard marked waste bags. This waste will be collected and removed for incineration at Discovery Park by Park Serve, a contractor that specialises in the handling and disposal of hazardous biological waste. All liquid waste will be completely inactivated by adding to Virkon to make a 1% solution for >15 minutes before disposal down a sink. Areas where GMM work has taken place will be disinfected with a 1% Virkon solution once completed. Staff will receive full training on the handling and disposal of GMM waste |
The committee deems that containment level 2 conditions will be suitable for the use of both BSL-2 designated cell lines and potentially infectious human cells, provided that they are procured from a source that has prescreened the primary cells for the presence of HIV, HCV and HBV. Staff must be fully trained in the requirements for working under containment level 2 conditions as laid out in the risk assessment and local and HSE guidelines. Written records of this training must be kept on record.

Please enter comments on the GM safety committee on the risk assessment

The committee deems that containment level 2 conditions will be suitable for the use of both BSL-2 designated cell lines and potentially infectious human cells, provided that they are procured from a source that has prescreened the primary cells for the presence of HIV, HCV and HBV. Staff must be fully trained in the requirements for working under containment level 2 conditions as laid out in the risk assessment and local and HSE guidelines. Written records of this training must be kept on record.

Project Containment

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Project Ref 3230/16.1

Assessment of infectivity and recombinant gene expression by conditionally replication competent adenoviruses encoding non-pathogenic animal proteins

1-50 Litres

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Purposes of the contained use
To determine the ability of tumour specific adenoviruses to infect and lyse human cancer cell lines and to express reporter proteins or other non-pathogenic animal proteins encoded by the engineered virus.

### Recipient or parental organism
Human cancer cell lines e.g. A549

### Host/vector system
Adenovirus, e.g. type 5 background

### Origin & function
Genetic manipulation of the starting adenovirus background will be performed by an external contractor, who will then produce and supply purified virus particles for use in in vitro assays. Genetic manipulations will be performed in shuttle vectors before the full length viral plasmid is created. Manipulations will involve 1) deletion/disruption of one or more viral genes to confer conditional replicative ability of the virus in human cancer cells and 2) insertion of non-pathogenic animal proteins including common reporter proteins such as green fluorescent proteins.

### Evaluation of foreseeable effects
Naturally occurring adenovirus is a hazard group 2 human pathogen. Adenovirus infection is often asymptomatic. Symptomatic infection is most commonly associated with mild respiratory illness but can also cause, particularly in children or immune-compromised individuals, gastroenteritis, cystitis, conjunctivitis, bronchitis or pneumonia. Most infections are mild and require no therapy. Where required, symptomatic therapy is provided. The engineered viruses to be used carry mutation(s) to reduce replication in normal human cells whilst maintaining replicative ability in human cancer cell lines. However, it is likely that the strains will still be able to replicate to a degree in normal human cells. Inserted genes encoding non-pathogenic animal proteins will not alter the infectivity or pathogenesis of the virus. The animal proteins encoded by the virus are not endogenous human proteins but the risk to human health from expression in an individual through accidental exposure is low. Human adenovirus may be poorly replicative/infectious in certain mammalian species. The animal proteins encoded by the virus are either endogenous to all mammalian species (except humans and Old World Monkeys) or are completely harmless reporter proteins that do not affect cellular processes.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

An autoclave is not available in the laboratory or building for heat-inactivation of solid waste contaminated with GMMs, therefore the following arrangements for disposal of GMM solid waste have been made. All solid genetically modified waste (e.g. plasticware) will be removed from the laboratory in sealed bags by contractors (ParkServe) and autoclaved then incinerated at an on site waste disposal facility run by a specialist hazardous waste management contractor (Augean).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid genetically modified waste (e.g. plasticware) will be removed from the laboratory in sealed bags by contractors (ParkServe) and autoclaved then incinerated at an on site waste disposal facility run by a specialist hazardous waste management contractor (Augean). All liquid waste will be inactivated in a minimum 1% Virkon solution for at least 30 minutes before disposal via the drains. Laboratory areas where GMM work has taken place, i.e. class II microbiological safety cabinets, will be disinfected with a 1% Virkon solution at the end of each working sessions. Staff will receive full training on the handling and disposal of GMM waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The committee have reviewed and approved the associated risk assessment, which recommends all work be performed under containment level 2. Staff undertaking the work must be sufficiently competent in working under containment level 2 conditions and must be trained in the hazards and risks associated with this work as laid out in the associated risk assessment. This training must be recorded.

Project Containment

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02/03/2022
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#### Name

XCELLR8 LTD

#### Name 2

Department

#### Campus Estate or Research Centre

TECHSPACE ONE

#### Road Name

KECKWICK LANE

#### Building

SCI-TECH DARESBURY

#### Town

DARESBURY

#### County

CHESHIRE

#### Postcode

WA4 4AB

#### Country

ENGLAND

#### Tel Number

01925607134

#### Fax Number

0

#### E-mail

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#### Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities Y

Give brief details of the genetic modification safety committee

The Duties of the Genetic Modification Safety Committee are fulfilled by the XCellR8 Health and Safety (H&S) Committee. There is no provision within XCellR8 for a separate Genetic Modification Safety Committee at this time, due to the small size of the organisation.

The H&S Committee is comprised from members of staff from different tiers of the organisation:

- H&S Co-ordinator & Chair
- Scientific or other Director
- Laboratory Manager
- Member of the Technical Staff
- Researcher / Student (by invitation)

Operating Procedures & frequency of meetings

1. Chair invites members to a quarterly meeting to discuss H&S matters and to review either new, or current, risk assessments.
2. During the meeting the relevant risk assessments will be discussed and any amendments required will be made with the consensus of the committee, after liaison with the staff involved in drafting the original risk assessment.
3. The amended risk assessment will then be approved by the committee and circulated to all staff.

<table>
<thead>
<tr>
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<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>
The GMMs proposed for use in this study are as follows: (Also see accompanying Working Practices document).

• Disabled strains of mammalian cell lines, principally human lymphoblastoid line (TK6) and human keratinocyte cell line (KeratinoSens), ACDP Classification - Class 1

Standardised Working Procedures will be adopted when handling Class 1 GMMs, consistent with, and often exceeding, those required for Class 1 containment.

Inactivation and disposal of items contaminated with Class 1 GMMs

• All re-usable items contaminated with Class 1 GMMs, such as laboratory glassware and small pipetting troughs, will be disinfected by total immersion in freshly prepared 1% (w/v) Virkon solution (Antec International) for 24 hours (see note 1). Items will then be washed and autoclaved following standard laboratory procedures, prior to re-use (See note 2).

• All disposable items contaminated with Class 1 GMMs, such as polypropylene pipette tips, sample vials, microplates and gloves, will be sealed in small bags containing absorbent material, labelled as
'biohazard', and either inactivated via autoclave before appropriate disposal by conventional routes (see note 2), or sent for incineration by approved contractors. Materials sent for incineration will be further encapsulated in one-way sealed burn units, appropriately labelled with details of their contents and the sender’s contact details.

- Stock cultures of Class 1 GMMs, when no longer needed, will be sealed in small bags containing absorbent material, labelled as 'biohazard', and either inactivated via autoclave before appropriate disposal (see note 2), or sent for incineration by approved contractors. Materials sent for incineration will be further encapsulated in one-way sealed burn units, appropriately labelled with details of their contents and the sender’s contact details. Alternatively, larger volumes of culture can be inactivated by autoclave whilst contained within culture flasks, before appropriate disposal by conventional routes (see note 2).

Work areas will be disinfected after use using 70% ethanol / water solution (see note 3).

Spillage Containment

- Liquid spills of Class 1 GMMs on the bench or floor will be contained with absorbent paper tissue, or Virkon powder. Absorbent paper and powdered Virkon waste will be sealed in small bags containing absorbent material, labelled as 'biohazard', and either inactivated via autoclave before appropriate disposal by conventional routes (see note 2), or sent for incineration by approved contractors. Materials sent for incineration will be further encapsulated in one-way sealed burn units, appropriately labelled with details of their contents and the sender’s contact details. The affected floor/bench area will be disinfected with 70% ethanol/water solution (see note 3).

- Contaminated broken glass will be inactivated by total immersion in freshly prepared 1% Virkon solution for 24 hours before appropriate disposal by conventional routes (See note 1). The affected bench/floor area will be disinfected with 70% ethanol/water solution (see note 3).

Note 1: Virkon is a peroxygen compound that was found to be effective against strains of Saccharomyces cerevisiae, bacteria and viruses after 5 minutes contact time at 1% (see www.antecint.co.uk and references therein). It has minimum environmental impact and a red colour in solution indicating its activity. 99.999% degree of kill is assumed after 24 hours contact with 1% Virkon solution.

Note 2: Items sterilised, and GM cells inactivated, by autoclave are subject to a temperature of 121°C for a duration of 20 minutes. 100% degree of kill would be achieved.

Note 3: Alcohols give a very rapid kill of micro-organisms, but do not provide sustained microbial action. 99.999% kill would be achieved.

Tick to confirm that you are attaching a summary of the risk assessment [Y]  
Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

All members of the current XCellR8 Health and Safety Committee were involved in the drafting of risk assessments for this work. The Committee has subsequently approved the final risk assessment as an accurate account of the risks inherent to the work and a correct description of the protocols, sufficient for safe containment and use of GMMs in these projects, within the XCellR8 laboratory. The risk assessments will be reviewed annually and as any significant variations in protocols arise.

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  Y

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee (GMSC) is a subcommittee of the main Biological Safety Committee (BSC). It consists of the Biological Safety Officer (BSO) and three subject matter experts. Their remit is to review the relevant risk assessments pertaining to GMMs and recommend appropriate action.

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Any liquid solutions in contact with GMMs will be hard piped from laboratories or production suites to hold tanks and subjected to sterilization by heat treatment prior to discharge. The effectiveness of the procedure will be fully validated and tested periodically. Plate counts will be used to monitor the presence of GMM in the waste. There will also be the option to remove waste by tanker should the heat treatment be incapacitated.

Disinfection will also be employed when necessary.

Any plasticware, laboratory consumables and personal protective equipment (PPE) in contact with GMMs will be autoclaved prior to disposal. Where this is not possible, they will be discarded in biohazard waste containers for incineration.

The development of bioprocesses, up to 200L in scale, using cultured mammalian cells

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The attached risk assessment was circulated to the BSO and GMSC. There were no further comments as the proposed work is not disimilar to that being undertaken at the existing CPI facility at Wilton. Safeguards and risk assessments already in place at the Wilto facility will be employed at the NBMC.
Adeno-associated viral vector (AAV) mediated therapy has some potentially interesting applications in clinical research. As the wild type virus is not associated with any diseases in humans or any other organism, its use as a vehicle to deliver functional genes into cells is very compelling. For its potential to be realised we need to develop robust and scaleable manufacturing process for the production of AAV. To do this, we need to develop analytical techniques to measure and quantify the AAV. We need to develop ways of producing the viral vector in host cells and methods of purifying the viral vector.

The work undertaken at CPI will allow these promising therapies to be further realised.

**Recipient or parental organism**

Parental organism is adeno associated virus (AAV). Parental organism will be used to infect HEK293 cells (the recipient)

**Host/vector system**

HEK293 host cells.

**Origin & function**

The genetic material of the viral vector is derived from adeno associated virus genome (145 nucleotides from the virus termini). The function is to allow the transfer of genes into host cells for therapeutic purposes. Other external genetic elements (adeno associated virus genes Rep and Cap and adenoviral genes E1, E2, E4 and Va) will allow the replication of the vector in the host cells. These genes will not be present in the replication deficient, non-pathogenic viral vector generated.

**Evaluation of foreseeable effects**

AAV is not known to cause any disease in humans. Research carried out by using wild type AAV and AAV derived vectors (rAAV) has been designated
as BSL1 by the NIH [http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html, Appendix B-I. Risk Group 1 (RG1) Agents]. The viral vector genome is significantly deleted and will be replication deficient outside specific controlled systems. Once used to transduce a cell, it is highly improbable for these viral vectors to form more transducing particles since we are using replication-deficient vectors which lack the essential gene products (supplied by plasmids/producer cells during the initial production of the particle) required to produce a novel viral vector particle. Since the viral vector plasmid shares no DNA sequence homology with the helper plasmids or the genome in the producer cells, therefore there is no likelihood of homologous recombination events resulting in reconstitution of a replication competent virus. The proposed host cell line HEK-293 is of human origin, however it is widely used in the field since 1973 without any incidents. Since the integrated DNA in the cells is of adenoviral (and not adeno associated viral) origin, any GMM resulting from this work would not contain the AAV viral elements making it more pathogenic than the wild type. All waste produced will be bagged and autoclaved prior to disposal. All viral vector and viral particles in the liquid waste will be destroyed by the addition of virkon to a final concentration of 1%. Waste will be transported in sealed and bunded containers from the lab. All research carried out will be documented. In addition, the generation of any GMOs (new viral vectors, transduced cells, etc), their storage and their destruction, will be documented.

The only likely methods of these replication deficient, non-pathogenic, viral vectors ever escaping containment are through the escape of treated animals, untreated waste or through a needle stick injury or aerosol inhalation by the laboratory workers. The laboratory personnel would be trained in the correct treatment of the waste and in the use of the viral vectors, to prevent any contamination to them. Animal models are not used at CPI. Since there is a very small likelihood that the viral vectors might, potentially, transduce laboratory workers through needle stick injuries or open wounds or as aerosols, we will limit the use to needles to procedures where they are absolutely essential [with safeguards such as double gloving and proper training], prohibit any personnel with open wounds and scratches from working with them and ensure that the personnel use masks when the vectors are taken outside the microbiology safety cabinets. We will also carry out routine analysis for the detection of potential AAV contamination of any escape routes (doors, shoes, wash basins, switches, equipment, etc) using qPCR for the viral termini.

Thus, all measures have been taken to train the personnel in the correct use of these non-pathogenic viral vectors and in their destruction. However, since they require two additional adeno-associated virus genes along with four adenovirus genes, should these replication-deficient, non-pathogenic viral vectors ever escape the laboratory, it is impossible for them to form a self-sustaining population. Even in the highly unlikely event of a needle stick injury, these vectors would be cleared by the host immune system.
## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| n/a |

## For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| n/a |

## Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| All the details about the generation, storage and/or destruction of GMOs generated in the facility will be recorded and will be available to internal and external auditors. Following the use of GMOs in a safety cabinet, clean the surface of the cabinet with 1% Chemgene, and switch on the UV (wherever available) for a minimum of an hour. Disinfection: Decontaminate work areas with 1% Virkon (for non-metallic surfaces) or 1% Chemgene (Trigene) solutions for 30 minutes. Follow with water to rinse of residue. Spill and Accident Procedures. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure. Cover the spill with absorbent material. Starting at the edges and work towards the centre. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes: viscous spills require 30 minutes. Use paper towels to wipe up the spill, working from the edge to centre. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves. Discard absorbent material in Chemical waste bags. Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, allow to disinfect for 15-20 minutes longer, and wipe with towels. Discard all cleanup materials (soaked with disinfectant) in Chemical bag, and any contaminated PPE in a biohazard bag. Close and secure the bags. Place bag in a second biohazard bag, secure and remove for incineration. Destruction of GMOs: Cells transduced with GMOs will be exposed to 1% Virkon for an hour (alternatively, the flasks/dishes can be bagged and autoclaved) before disposed. Fluids/containers in contact with GMOs (cell culture medium, PBS, etc) will be made up to 1% Virkon and left for an hour prior to disposal. Gloves that come in contact with GMOs will be sprayed with 1% Chemgene and binned. Sealed samples of GMOs (vials) will be sterilised by autoclaving. Bacteria and flasks (used for transformation of GMO plasmids) would be destroyed either by the process (plasmid preparation) or with a final concentration of 1% Virkon. The facility is provided the capability for Vaporised Hydrogen Peroxide decontamination of the room. |

## Is an emergency plan required according to regulation 20?

| N |

## If yes, tick to confirm that it is attached to this form

| N |

## Tick to confirm that you have attached a risk assessment to this form

| Y |

## Tick if you are claiming exemption from disclosure for section of the risk assessment

| N |
## Project Containment

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**Name**

NANOTHERICS LTD

**Campus Estate or Research Centre**

**Road Name**

SCIENCE AND BUSINESS PARK

**Town**

STOKE ON TRENT

**Building**

IC4 KEELE UNIVERSITY

**District**

**County**

STAFFORDSHIRE

**Postcode**

ST5 5NL

**Country**

ENGLAND

**Tel Number**

01782 622112

**Fax Number**

01782 621919

**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
# Premises Addresses

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# Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

- Composed of CEO, Laboratory Staff, Scientific Advisor.
- Meet once per quarter or more frequently if experimental plans change.
- Discuss experiment plans/SOP's/General H&S and Risk analysis.

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste is autoclaved, yellow bagged and passed on to Labswaste Limited for disposal.

| Other(s) | Transfection (primarily GFP) of immortalised cell lines and occasionally commercially supplied prima |

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessment is completed and in place.
Low risk procedure and all appropriate measures are in place.
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**Name**

BIO-RAD ABD SEROTEC LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

ENDEAVOUR HOUSE

**District**

KIDLINGTON

**Road Name**

LONGFORD LANE

**Town**

OXON

**County**

OXFORDSHIRE

**Postcode**

OX5 1GE

**Country**

ENGLAND

**Tel Number**

01865 852700

**Fax Number**

01865 373899

**E-mail**

**HSE Division**

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**Comments**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

Two levels of assessment, review and monitoring:

i) GMM Safety Team comprising Lab Manager, QC Manager and H & S Advisor/Consultant. Meets quarterly or whenever a new procedure, involving use of new GMO's proposed for introduction

ii) .Quarterly H & S Committee chaired by Ops Director with Lab Manager, QA Manager, H & S Consultant and HR Director plus 10 employee representatives

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Other (please specify) Tick if confidential

Bacteriology   Parasitology   Transgenic Birds   Microbiology Research
Virology       Transgenic Animals Transgenic Fish Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

6.1 - Waste Management measures?
Consequences of a spillage is assessed as 'Nil' GMM media has no effect on humans nor general environment. See attached 'Risk assessment'
6.2 Inactivation measures? Degree of kill? - A 1% solution of 'Virkon' is used to kill GMM cell media solutions after conclusion of QC tests. See attached 'Risk Assessment'
6.3 Process testing and monitoring measures?
- to prevent contamination of water drainage systems? Class 1 media only. Nil risk to drainage in event of spillage
- to prevent transfer by air or clothing. Class 1 V limited life

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

GMO Safety Team reviewed 'Risk Assessment' attached at Endeavour House and found it "suitable and sufficient"
"Risk assessment" and explanation of GMO Regs 2014 to be presented to Co. H & S Committee 15 October 2014, for discussion plus Q's & A's and acceptance
### GM Centre Number: 3238

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

The safety committee is composed of three senior scientists with a background in DNA manipulation and cell culture

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
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Tick if confidential

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<th>Bacteriology</th>
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<th>Microbiology Research</th>
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<th>Virology</th>
<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
<th>Gene Therapy</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste will be disposed of via the Babraham waste disposal derogation GM1 05/04.1 which states that "Liquid Waste is inactivated by the addition of "Virkon" according to the manufacturer's instructions prior to disposal to the drainage system. Waste from laboratories carrying out GM work at Class 1 and Class 2 will be placed into suitably labelled hermetically sealable bins. The bins are 60 litre UN approved to Class 3 Type 3H21Y30/S/2003. They will be sealed by the producer of the waste and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections by the local registered clinical /GM waste incinerator contractor (VetSpeed) will be made three times a week to prevent a build-up. The site has 24/7 security.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Agree with the GM class but work needs to be carried out at ACDP containment 2 to over safety of working with oncogenes and cells with adventitious agents.
<table>
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Name

READING SCIENTIFIC SERVICES LTD

Name 2

Department

Campus Estate or Research Centre

WHITEKNIGHTS CAMPUS

Road Name

PEPPER LANE

Building

READING SCIENCE CENTRE

District

Town

READING

County

BERKSHIRE

Postcode

RG6 6LA

Country

ENGLAND

Tel Number

0118 818 4000

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [Y]

Give brief details of the genetic modification safety committee

GMSC includes the Facilities and EHS Manager, Biological Safety Officer and Microbiological and Molecular Biologists and Cell Culture Scientists, all with experience of working at activity class 1 and 2. Collectively many decades worth of experience in similar organisations undertaking preclinical research and development. Where additional support is required the staff needed will be added/adapted to allow for the most relevant expertise and input. External advice from H&S Consultant/Biosafety Practitioner will be contracted on an ad hoc basis where necessary to supplement internal expertise if required.

The committee will meet every 4 months to review biosafety and whenever a new risk assessment is required. The first meeting will review the initial risk assessments.

<table>
<thead>
<tr>
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Non-microbial
**Other (please specify)**  

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<tr>
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<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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<td>Other(s)</td>
<td>No genetic manipulation is carried out on the premises</td>
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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Briefly, Liquid waste will be disinfected using a minimum of 1% Virkon or other suitable alternative for the specified contact time, or by autoclaving (with/without prior chemical inactivation). Solid waste contaminated with GMMs will be autoclaved on site, prior to uplift for secondary inactivation (incineration). Surfaces potentially in contact with GMMs will be cleaned routinely using 1% virkon, 70% Ethanol or other commercial disinfectant. SOPs govern all working practices and emergency procedures. All equipment is maintained and checked / validated at regular intervals, including autoclaves. SOPs govern all working practices, and all equipment is maintained and checked / validated at regular intervals, including autoclaves.

**Tick to confirm that you are attaching a summary of the risk assessment**

 Tick if you are claiming exemption from disclosure for sections of the risk assessment  

**Please enter comments of the GM safety committee on the risk assessment**

The GMSC has reviewed all of the Health & Safety Implications of the proposed work. We have not identified any risks that are greater than working with non-GM versions of the species.
Each cell line will be risk assessed prior to acceptance on site.
We unanimously believe that the research should be classified as Containment Level 1.
## GM Centre Number: 3240

<table>
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### Name

DRAYTON ANIMAL HEALTH LTD

### Name 2


### Campus Estate or Research Centre

PENDEFORD BUSINESS PARK

### Building

PENDEFORD HOUSE

### Road Name

WOBASTON ROAD

### District


### Town

WOLVERHAMPTON

### County


### Postcode

WV9 5AP

### Country

ENGLAND

### Tel Number

01902 271300

### Fax Number

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### E-mail

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### HSE Division


### Date at Which Additional Info Submitted

02/03/2022
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GM safety committee is made up of the Resource Manager at ADAS Drayton who will chair the committee, the head of ADAS's biotechnology group as the Biological Safety Officer, and the ADAS Drayton's trained risk assessor. They will report directly to the overall Health & Safety group established at ADAS Drayton

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Tick if confidential

Y

- Bacteriology
  - Yes
- Parasitology
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- Transgenic Animals
- Transgenic Fish
- Gene Therapy
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Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

### Project Ref 3240/14.1

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<th>Project notified under transitional arrangements</th>
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<td>18/12/2014</td>
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<td>Evaluation of the safety and efficacy of double recombinants of Herpesvirus for turkeys (HVT) expressing foreign genes from Newcastle disease virus and infectious bronchitis virus as vaccines in broiler chickens</td>
<td>Class 2</td>
<td></td>
<td></td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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Withdrawn  

Tick if notifying a connected programme of work  

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
**Purposes of the contained use**

Evaluation of the safety and efficacy of a recombinant HVT vaccine in broiler chickens under semi-commercial conditions required as part of the registration package.

**Recipient or parental organism**

The HVT parent strain is FC-126

**Host/vector system**

None

**Origin & function**

Herpesvirus of turkeys (HVT) expressing the following genes:
1. The F gene of Newcastle disease virus and
2. The VP2 gene of infectious bursal disease virus (IBVO)

These genes have the following functions within the native viral particle:

- **NOV F** protein is an envelope glycoprotein required for virus cell fusion and is important for vaccine induced immunity.
- **VP2** protein is a major structural protein and the host protective immunogen of IBOV.

The expression of these gene products from the recombinant HVT will serve as vaccines that should induce protective immunity in vaccinated poultry against the original virus whose gene is expressed.

**Evaluation of foreseeable effects**

<table>
<thead>
<tr>
<th>Hazards to human health:</th>
</tr>
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<tbody>
<tr>
<td>(i) Hazards associated with the recipient microorganisms (e.g. bacterial host or viral vector)</td>
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</table>

HVT is currently classified in the subfamily of alphaherpesvirinae, and is also known as MeJeagrid herpesvirus 1, turkey herpesvirus, or Marek's disease virus serotype 3. The virus was first described around 1970 as a herpesvirus infecting turkeys and sharing antigenic features in common with Marek's disease virus (MDV). Whilst MDV is highly pathogenic for chickens, HVT is non-pathogenic and could therefore be used for effective vaccination against infection and disease caused by MDV (Okazaki et al., 1970, Avian Diseases, vol. 14, p. 413-429). Since then, vaccination of chickens against MDV by using HVT has become part of the standard vaccination program of billions of chickens produced worldwide every year.

The HVT virion has all the features of a typical herpesvirus, and is about 160nm in size in its enveloped form. It possesses a large genome (approximately 159 kb) comprising linear double stranded DNA. The HVT genome has a long history of manipulation; in particular its non-pathogenic properties have led to research into the use of HVT as a viral vector for expression and delivery of various proteins to the chicken. Examples are the expression of genes coding not only for antigens from other poultry pathogens such as: infectious bursal disease virus (IBDV) (Darteil et al., 1995, Virolology, vol 21, p. 481-490), Newcastle disease virus (NOV) (Sondermeijer et al., 1993, Vaccine, vol. 11, p. 349-358) and avian influenza (U et al., 2001, Vaccine, vol. 29, p. 8257-8266). But also the expression has been described of a parasite antigen (Cronenberg et al., 1999, Acta Virol., vol. 43, p. 192-197), or of a cytokine, to manipulate the chicken's immune response (NO 2009/156, 367; Tarpey et al., 2007, Vaccine, vol. 25, p. 8529-8535).

Such work has led to the development of commercial vaccines using such technology, including the Vaxxitech product range (Meria), the Vectormune HVT product range (Ceva) and the Innovax product range (Merck Animal Health), thus providing convincing data in a large number of birds of the safety of HVT vectored IBO and ND vaccines. Therefore there are no perceived hazards associated with the recipient microorganism.

(ii) Hazards arising directly from the inserted gene product (e.g. cloning of a toxin or oncogene)
The fusion (F) protein in NOV is involved in attachment and entry into host cells and is the major antigen responsible for immunity against NOV infection. It is also a determinant for virulence based on the presence of a basic Cleavage site which is typically present in high virulence field strains such as Herts 33/56. The gene encoding the fusion protein of NOV was however originally cloned from a lentogenic NOV vaccine strain (Clone 30) which does not contain this basic cleavage signal.

VP2 protein is a major structural protein and the host protective immunogen of IBDV. The VP2 gene is derived from a virulent classic type of IBOV field strain. Overall the risk of hazards arising directly from the inserted gene products is considered low.

(iii) Hazards arising from the alteration of existing traits (e.g. alteration of pathogenicity, host range, tissue tropism, mode of transmission or host immune response)

HVT is a non-pathogenic alpha herpesvirus with a host range restricted to chickens and turkeys. The F protein of NOV is a typical membrane anchored glycoprotein containing a secretion signal, it is likely to be expressed only partially at the surface of HVT/F infected cells. NOV has been associated with mild conjunctivitis and influenza-like symptoms in man and it is therefore possible that the presence of the F protein in the virus envelope could result in transmission to man. HVT however is typically cell associated and does not generate enveloped viral particles in the supernatant of infected cultures such as is seen with most other herpesviruses, although infectious cell-free virus has been detected in the feather dust of chickens vaccinated with HVT. The VP2 protein of IBDV does not have features like a signal sequence or transmembrane anchor region as is found with the F protein from NOV or most of the glycoproteins from herpesviruses and is therefore not expected to be exposed on the cell surface.

Therefore overall the risk of hazards arising from alteration of the existing traits of the HVT vector is considered low.

(iv) The potential hazards of sequences within the GMM being transferred to related microorganisms

The HVT backbone of the HVT-ND-IBD construct has been modified by the insertion of fragments containing the NOV F and IBDV VP2 genes and their associated promoter sequences. There have been no gene deletions. The phenotype of the recombinant strain is therefore the same as that of the HVT backbone. If the inserted fragments were to be lost, this would result in the wild type HVT, which is also avirulent. There have never been reports of the recombination of HVT with other related herpesviruses of poultry, e.g. virulent (serotype 1) MDV or serotype 2, and the possibility of recombination with a virus capable of infecting humans is therefore considered extremely small. Genetic transfer to other organisms in the environment has never been described for herpesviruses, and is therefore considered to be unlikely.

Hazards to the environment:

(i) Hazards associated with the recipient microorganisms (e.g. bacterial host or viral vector)

The host range for HVT is limited to avian species and replication does not occur in mammals. Furthermore HVT is non-pathogenic in all avian species tested and in consequence would be classified as ACDP hazard group 1. In laboratory studies the parent HVT strain has been shown to replicate in chickens and turkeys but not in ducks or pigeons after direct inoculation. The natural route of infection is via inhalation of feather follicle dust. The shed and spread capability of the HVT ND-IBD construct is expected to be similar to its parent HVT. Shedding of HVT from inoculated chickens is of low level and sporadic in nature (Zygraich and Huygelen 1972, Avian Diseases, 16: 793-798; Cho, 197 1, Poultry Science, 50: 881-887; Cho, 1974, Avian Diseases, 19: 136-141; Cho, 1976, Poultry Science, 55, 1830-1833). Further, the inserted genes provide no competitive advantage for the GMO and it is anticipated, based on similar constructs, that replication efficiency will be reduced, with no significant spread observed between chickens. HVT is endemic and ubiquitous in domestic turkeys so the likelihood of spread in turkeys is also potentially constrained by prior immunity. There are therefore no perceived environmental hazards arising from recipient organism.
(ii) Hazards arising directly from the inserted gene product (e.g. cloning of a toxin or oncogene)
The fusion (F) protein in NOV is involved in attachment and entry into host cells and is a major determinant for
virulence based on the presence of a basic cleavage site which is typically present in high virulence field strains such as
Herts 33/56. It is also the major antigen responsible for immunity against NOV infection. The gene encoding the
fusion protein was however originally cloned from a lentogenic NOV vaccine strain (Clone 30) which does not contain
this basic cleavage signal.
VP2 protein is a major structural protein and the host protective immunogen of IBDV. The VP2 gene is derived from a
virulent classic type of IBDV field strain. Overall the risk of environmental hazards arising directly from the inserted
gene products is considered low.
(iii) Hazards arising from the alteration of existing traits (e.g. alteration of pathogenicity, host range, tissue tropism)
HVT is a non-pathogenic alpha herpesvirus with a host range restricted to chickens and turkeys. The HVT virion has
all the features of a typical herpesvirus, it is about 160 nm in size in its enveloped form and possesses a large
genome (approximately 159 kb) comprising linear double stranded DNA. Key proteins for interaction with host cells
are glycoproteins inserted in the viral envelope. In cell culture HVT does not produce enveloped viral particles
however infectious cell-free virus has been detected in the feather dust of chickens vaccinated with HVT. The gene
encoding the fusion protein was originally cloned from a lentogenic NOV vaccine strain (Clone 30) which does not
contain the basic cleavage signal. Since F is a typical membrane anchored glycoprotein containing a secretion signal,
it is likely to be expressed at least partially at the surface of HVT/F infected cells although this has not been confirmed
by immunofluorescence staining using F-specific antibodies. Any effect on virulence of an HVT recombinant is very
unlikely since the vector is proven to be non-pathogenic and the inserted F gene originates from a non-virulent NOV
strain. This conclusion has meanwhile been confirmed in many laboratory studies as well as years of experience with
the commercial product Innovax-NO that contains the HVT/F vector strain. The VP2 protein does not have features
like a signal sequence or transmembrane anchor region such as F from NOV or most of the glycoproteins from
herpesvirus and is therefore not expected to be exposed on the cell surface. Commercial vaccines using recombinant
HVT technology, including the Vaxxitech product range (Merial), the Vectormune HVT product range (Ceva) and the
Innovax product range (Merck Animal Health), have been available for many years with no adverse effects reported,
thus providing convincing data in a large number of birds of the safety of HVT vectored IBO and NO vaccines. Overall
the risk of environmental hazards arising directly from the alteration of existing traits is considered low.
(iv) The potential hazards of sequences within the GMM being transferred to related microorganisms
The HVT backbone of the HVT-NO-IBD construct has been modified by the insertion of fragments containing the NDVF
and IBDV VP2 genes and their associated promoter sequences. There have been no gene deletions. The
phenotype of the recombinant strains is therefore the same as that of the HVT backbone. If the inserted fragments
would be lost, this would result in the wild type HVT, which is also avirulent.
The potential for recombination of the HVT recombinants with virulent Marek's Disease virus would be no greater than
can occur with current vaccines containing HVT. HVT is commonly present in vaccinated chickens that become
"superinfected" with virulent MDV. Furthermore, serotype 3 (HVT) is often given with serotype 2 and/or serotype 1
strains as a polyvalent vaccine. As there have never been reports on the recombination of HVT with either the
virulent (serotype 1) MDV or serotype 2, this possiblity can be considered extremely small. Genetic transfer to other
organisms in the environment has never been described for herpes viruses, and is therefore considered to be unlikely.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

(i) Animal carcasses, bedding and waste from animal accommodation will be bagged and sealed. The surface of these bags will then be disinfected with Virkon S as per manufacturers instructions for Mareks disease (1:200) before bags are stored in a sealed clinical waste skip and/or burn bins, prior to being sent off site for incineration by a licensed contractor.
(ii) The room and all equipment will be sprayed with Virkon S as per manufacturers instructions for Mareks disease. Following the appropriate contact lime, equipment will be cleaned, allowed to dry and placed in storage. The room will be steam cleaned, disinfected again and left to dry as per the local SOP for biosecurity.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

This study and its associated risk assessments have been reviewed by the GMSC and has been categorised as class 2.

Project Containment

<table>
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<th>Laboratory Activities</th>
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<td>Human Clinical Applications</td>
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</table>

Project Ref 3240/16.1

Date Ackn’d 28/04/2016  
CU2 Project Title To evaluate the efficacy and safety of a live Salmonella vaccine for use in chickens to aid in preventing infection and colonisation by Salmonella enterica  
Date Project Ceased  
Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Liter
Non-GMM Consent Granted
### Project Additional Information

#### Purposes of the contained use

To generate data suitable for use in licensing a live Salmonella vaccine for use in chickens.

#### Recipient or parental organism

- Salmonella enterica of serogroups B, C, 0 and E typically
- S. Enteritidis
- S. Typhimurium
- S. Anatum
- S. Hadar
- Other serogroup representative serovars may be used.

#### Host/vector system

None

#### Origin & function

- Salmonella enterica of serogroups B, C, 0 and E typically
- S. Enteritidis
- S. Typhimurium
- S. Anatum
- S. Hadar
- Other serogroup representative serovars may be used.

#### Evaluation of foreseeable effects

Hazards to human health:

1. Hazards associated with the recipient microorganisms (e.g. bacterial host or viral vector)
   - Salmonella infection may cause acute gastroenteritis with sudden onset of abdominal pain, diarrhoea, nausea and vomiting.
   - The routes of infection may be ingestion or parenteral inoculation; the importance of aerosol exposure is not known.
   - The infectious dose varies with the susceptibility of the host, the strain of the organism and the form of ingestion. The infectious dose was thought to be > 10^5 in healthy individuals, but outbreaks caused by chocolate and egg borne organisms suggests that the dose may be as low as 1 - 100 organisms.
Salmonella infections usually resolve in 5-7 days and often do not require treatment unless the patient becomes severely dehydrated or the infection spreads from the intestines.

(ii) Hazards arising directly from the inserted gene product (e.g. cloning of a toxin or oncogene)

Not applicable

(iii) Hazards arising from the alteration of existing traits (e.g. alteration of pathogenicity, host range, tissue tropism, mode of transmission or host immune response)

None foreseeable.

None foreseeable.

(iv) The potential hazards of sequences within the GMM being transferred to related microorganisms

The genes are only mobilisable by transducing phage or recombination. In the unlikely event of this happening the receiving strain would lose its ability to produce flagellin and or survive in vivo. This would not be considered as having the potential to increase its virulence.

Hazards to the environment:

(i) Hazards associated with the recipient microorganisms (e.g. bacterial host or viral vector)

The recipient organisms were originally isolated from poultry although Salmonella enterica strains of these serovars are not normally a pathogen for poultry. However, they may be capable of infecting other, more susceptible species and must be considered as being still capable of surviving in the environment.

(ii) Hazards arising directly from the inserted gene product (e.g. cloning of a toxin or oncogene)

Not applicable.

(iii) Hazards arising from the alteration of existing traits (e.g. alteration of pathogenicity, host range, tissue tropism)

None foreseeable.

(iv) The potential hazards of sequences within the GMM being transferred to related microorganisms

The genes are only mobilisable by transducing phage or recombination. In the unlikely event of this happening the receiving strain would lose its ability to produce flagellin and or survive in vivo. This would not be considered as having the potential to increase its virulence.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

In a facility where class 2 work is undertaken it is a legal requirement to have an autoclave in the building. There is an autoclave on site in the laboratory, however there is not an autoclave in each specific animal accommodation building. For this project no requirement for an autoclave in the animal accommodation is forseen and we therefore request a derogation from this requirement.

Waste on this project from the animal accommodation will be managed as follows:

There will be dedicated PPE (gloves, overalls, hairnets, wellies) in rooms containing vaccinated birds, when not being used these will be kept in lockers with in those rooms to prevent contamination with dust. PPE except wellies will be disposable. Wellies will be washed and disinfected with Virkon S 1 % prior to being removed from the room. Waste will only leave the room(s) in sealed, disinfected bags (with Virkon S) or sharps boxes and be placed in burn bins.

Animals will be euthanased with in the rooms and carcasses double bagged, bags will then be disinfected and removed from the rooms and placed in burn bins.

At study end, waste material will be double bagged, bags will then be disinfected and removed from the rooms.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

(i) Solid and liquid waste including animal carcasses, bedding and laboratory waste will be placed in suitable containers as applicable and then bagged in clinical waste bags. The surface of these bags will then be disinfected with a Virkon S at 1% before bags are stored in a sealed clinical waste skip and/or burn bins, prior to being sent off site for incineration by a licensed contractor.

(ii) The room and all equipment will be sprayed with Virkon S at 1% which will be allowed contact time of at least 12 hours. Following the appropriate contact time, equipment will be cleaned, allowed to dry and placed in storage. The room will be steam cleaned, disinfected again and left to dry as per the local SOP for biosecurity.

(iii) Re-usable glassware will be decontaminated by autoclaving using a validated cycle before cleaning.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

This study and its associated risk assessments have been reviewed by the GMSC and it has been categorised as Class 2.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
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### Campus Estate or Research Centre

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### Date at Which Additional Info Submitted

<table>
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<th>Date</th>
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Premises Addresses

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<th>Department</th>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Person has many years of experience working with GM cell lines from previous employment in the pharmaceutical industry. All their previous GMO activities were covered by risk assessments.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste that is potentially contaminated with the GMOs will be de-contaminated over night in disteland disposed of as clinical waste by SRCL for incineration. This procedure is well established at XenoGesis for non-GMO clinical waste. Due to the fragile nature of animal cells outside the body and outside controlled environment (37C, humidified atmosphere, in specific culture media etc) and because distel and its predecessor, Trigene are well established for this purpose, assessment of the disinfection efficiency is considered unnecessary.

The person suggested the following:

- Treatment of GMO waste with Distel which was the approved procedure in previous employment.
- Use of a biological safety class II cabinet for all cell culture manipulations.
- It was discussed that the planned work using cell lines commercially available from reputable sources is class 1
- Waste from the lab should be removed frequently.
- It was discussed that the negative air pressure in premises used for GMO's class 1 is not necessary.
# GM Centre Number: 3242

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## Name

ADAPTIMMUNE LTD

## Name 2

Department

## Campus Estate or Research Centre

Road Name: 60 JUBILEE DRIVE

## Building

District: MILTON PARK

Town: ABINGDON

County: OXFORDSHIRE

Postcode: OX14 4RX

Country: ENGLAND

## Tel Number

01235 430000

## Fax Number

0

## E-mail

HSE Division: blank

## Comments

Change of address from 25/04/2017

## Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Y

**Give brief details of the genetic modification safety committee**

Originally registered jointly with Immunocore Ltd as GM centre no. 742, Adapimmune Ltd established its own independent Biological and Genetic Modification Safety Committee (GM centre no. 3242) in 2014 when both companies separated. The Committee conducts its business both electronically and in regular and ad hoc meetings. The Committee's main remit is to provide guidance on safe and compliant working practices with biological materials proposed within the company and to review and approve biological and genetic modification risk assessments.

The Committee currently consists of 7 Adapimmune Ltd members:

Chairman of the Committee (Head of Platform Research Group)
Biological Safety Officer
Health and Safety Officer
Four additional representatives from a selection of main laboratory groups
First activity will be class II, therefore see activity notification form CU2

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

n/a
The primary aim of this work is to generate T cells expressing wildtype or engineered T cell receptors with specificities to the following cell types:

a) Cells expressing cancer antigens, including but limited to, NY-ESO, gp100 and MAGE A3.
b) Cells expressing viral epitopes from viruses, including but not limited to, human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein Barr Virus (EMV) & human papilloma virus (HPV).
c) Cells expressing putative-immune disease antigens or in graft rejection antigens. The auto-immune diseases to be studied include but are not limited to diabetes mellitus type 1, rheumatoid arthritis, autoimmune hepatitis, & multiple sclerosis.

The T cells will also express reporter genes that allow for the selection of transformants and subsequent detection in immunological assays. These include but are not limited to: Green Fluorescent Protein (GFP), Red Fluorescent Protein (RFP), dsRED and/or luciferase genes.

The secondary aim of this work is to generate antigen presenting cells (APCs) carrying the relevant HLA to allow specific antigen presentation for testing the potency and safety of the T cells engineered to express the TCRs above.

The lentivirus system would allow us to derive T cell clones stably expressing the TCR of interest, or T cells transiently expressing these genes. T cell clones expressing ectopic TCR would be used in cellular assays to test antigen specific response, including using the APCs produced above.

Recipient or parental organism

The recipient cells will be:

a) Human T cells not derived from the blood of the user.
b) Fully characterised cell lines, including but not limited to HEK293T, Jurkat and SupT1 cell lines.

The work involving these cells will be undertaken in a Containment Level 2 facility. The cells used are to the best of our knowledge, free of adventitious agents and will be non-self cell lines that will not be able to colonise those involved with the work. Indirect hazards to human health associated with possible adventitious agents have been considered separately in a general Risk Assessment relating to work with human derived materials. All human derived samples are treated as though capable of causing disease.

Host/vector system

Two types of vectors will be used:

Type A:
Commercially acquired vectors, typically third (or sometimes fourth) generation Lentiviral expression systems including but not limited to:
ViraPower lentiviral Expression systems sold by Invitrogen Ltd. The vectors are: pLenti6iVs-Directional TOPO, pLenti6.2-GW/EniGFP, pLP1, pLP", and pLP/VSVG plasmids.
Lenti-X lentiviral Expression systems sold by Clontech Laboratories, Inc. The vectors include: pLVX-Puro, pLVXDsRed-Monomer-CI, Lenti-X FIT packaging system.

Type B
Vectors constructed by our academic collaborators e.g. University of Pennsylvania (Upenn).
These will typically be third generation type systems including but not limited to:
Vectors obtained from 'University of Pennsylvania; Upenn': Lentiviral vector derived from the 'Dull' vector (Dull et al. J Viral 1998), and 3 packaging plasmids (gag/pal, rev, VSVG envelope) similar to that used by Zufferey (Zufferey et al, Nat Biotech 1997)

These Lentiviral Expression Systems are third or fourth generation lentivirus systems. The lentivirus can infect mammalian cells but cannot replicate in these cells. These systems include some or all of the following safety features:
1. Expression vectors containing a deletion of the 3' LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell, Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a viral particle.
2. The number of HIV-1 genes that are used in the system has been reduced to three or four only: gag, pol, tat and sometimes rev.
3. The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope gene (env)
4. Genes encoding the structural and other components required for packaging the viral genome are separated onto multiple plasmids. These plasmids have been engineered not to contain any homology regions to prevent recombination events.
5. Although the plasmids allow expression in trans of proteins to produce lentivirus progeny in producer cell lines, none of them contain LTRs or the packaging sequence. This means that none of the HIV-1 structural genes are present in the packaged viral genome and are thus never transduced in the target cell. No new replication-competent virus can be produced.
6. The lentiviral particles produced in these systems are replication-incompetent and only carry the genes of interest.
7. Expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript.

Any T cells transduced using these vectors would only present a potential hazard to the original T cell donor. Transduced T cells and derived T cell clones will not be derived from the blood of any personnel working in Adaptimmune's Cell Biology laboratories thus removing this potential hazard.

Origin & function

Reporter genes such as GFP, RFP and luciferase have been used in various transgenic animal systems and have been shown to have no harmful effects on the organisms.
The TCR gene inserts encode for proteins known to be functionally expressed by T cells only, as they require the presence of cellular CD3 proteins only found in T cells. Expression of TCRs by cells other than T cells renders the TCR non-functional. In the case of cancer and viral epitope-specific TCR genes, their expression in T cells
should only induce a response to cells expressing tumour antigens or viral antigens (including virally infected cells).

TCR genes specific for putative auto-antigens or self antigens do present a hazard to the worker performing the work but only if the worker's own T cells were transfected to express the genes. This could result in T cells that would target normal tissue and would not be recognised by the workers immune system as non-self. For this reason, the use of self cells is strictly prohibited.

HLA is expressed by the majority of human cells and confers no harmful properties.

**Evaluation of foreseeable effects**

The hazards to workers and the environment posed by the vector with insert are considered negligible due to the containment level II measures employed. All work will be carried out in purpose-built, access-controlled laboratories. Skin contact with the organisms or the transfected cells will be avoided by minimising aerosols (by working in a Class II biosafety cabinet), and by use of gloves and safety glasses. If a worker does accidentally infect themselves with the virus, in all likelihood it will be rapidly destroyed by the workers' immune system. The routine procedures employed (e.g., autoclaving or chemical inactivation of all cellular material prior to disposal) will ensure an adequate level of safety.

In the extremely unlikely event of transfected T cell clones gaining entry to the worker, the cells in all likelihood would be rapidly destroyed by the workers' own immune system by virtue of being "non-self" HLA mis-matched cells (i.e. similar to the immune rejection of non-matched transplant tissue).

Transfected mammalian cells are considered especially disabled and are unlikely to survive in the environment in the absence of strict osmotic, buffering, temperature, and nutrient conditions.

Lentiviruses are highly susceptible to dehydration. However, they can survive for long periods in high protein-buffered media. Lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. The lentivirus cannot replicate and so the consequences of escape are considered negligible, therefore risk is effectively zero. Infected mammalian cells are not able to establish themselves and spread in nature and the vectors selected are either disabled or attenuated.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

This solid waste and the majority of the liquid waste generated during this procedure will be collected in specialised containers and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Registration Number; NAAo5o. The autoclaved solid waste will be disposed of via incineration through an appointed specialist waste management company; Grundon Waste Management Limited. Autoclaved liquid waste will be disposed of through a sink to public sewer. Any remaining liquid waste will be treated with an over-kill concentration Virkon (2% final wt/vol) or 10% ChemGene according to the manufacturers instructions (DuPont/MediMark Scientific for which independent validated efficacy against retrovirus exists.

**Is an emergency plan required according to regulation 20?**

Y

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment
The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tbody>
<tr>
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### Animal Units

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<tr>
<th>Large Scale Activities</th>
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<td>L2 L3 L4 L2 L3 L4 L2 L3 L4</td>
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### Project Ref 3242/16.1

- **Date Ackn’d:** 21/04/2016
- **CU2 Project Title:** Expression of TCRs and reporter genes in pluripotent human Embryonic Stem (ES) cells and in ES-derived differentiated T cells
- **Class:** Class 2
- **CultureVol:** 1-50 Litres

### Project Additional Information

**Purposes of the contained use**

The primary aim of this work is to generate human T cells derived from ES cells that express native or engineered T cell receptors with recognition specificities to the following cell types:

1. Cells expressing cancer antigens, including but not limited to, NY-ESO and other cancer antigens expressed in cancer cells.
b) Cells expressing viral epitopes from viruses, including but not limited to, human immunodeficiency virus (HIV),
cytomegalovirus (CMV), Epstein Barr Virus (EMV) & human papilloma virus (HPV).
c) Cells expressing putative-immune disease antigens or in graft rejection antigens. The auto-immune diseases to be
studied include but are not limited to diabetes mellitus type 1, rheumatoid arthritis, autoimmune hepatitis, & multiple
sclerosis.
(Similar work with non-ES cells has previously been submitted and approved under reference: GM3242/14.1 entitled
“Lentivirus carrying genes encoding for T Cell Receptors, HLA and marker molecules”).
The modified ES cells will also express non-pathogenic genes that allow for the detection and/or selection of
transformants and/or subsequent analysis in immunological assays. These include but are not limited to: Green
Fluorescent Protein (GFP), Red Fluorescent Protein (RFP), tdTomato Red and/or luciferase and/or puromycin
resistance genes.

Recipient or parental organism

The recipient cells will be:
Human ES cells and several derived cell lineages resulting from biological treatment regimes designed to differentiate
these ES cells into what are ultimately stable T cells.
The ES progenitor cells will have been genetically-modified prior to receipt and use by Adaptimmune (details of these
modifications are included in Section 17). These stable genetic modifications are designed to reduce allogeneic
rejection by the host immune system. Where appropriate, these GM ES cells will have also been gene modified prior
to Adaptimmune's use by the inclusion of cell fate "suicide genes" which provides an additional measure of safety
against immune tolerance in vivo. These genetically-modified ES cells do not produce any viruses (GM or otherwise).
The work involving these cells will be undertaken in a Containment Level 2 facility. The cells used are to the best of
our knowledge, free of adventitious agents.

Host/vector system

Two types of lentivectors will be used to transduce the previously-modified ES cells at Adaptimmune (as previously
described in GM3242/14.1):

Type A:
Commercially acquired third (and fourth) generation lentiviral systems including but not limited to:
ViraPower Lentiviral Expression systems sold by Invitrogen Ltd. The vectors are: pLenti6N5-Directional TOPO,
pLenti6.2-GW/EmGFP, pLP1, pLP2, and pLPNSVG plasmids. Lenti-X Lentiviral Expression systems sold by Clontech
Laboratories, Inc. The vectors include: pLVX-Puro, pLVX-DsRed-Monomer-C1 , Lenti-X HT packaging system.

Type B.
These are third generation type systems, including but not limited to: lentiviral vectors derived from the 'Dull' lentiviral
vector transgene plasmid (Dull et al. J Viral 1998), and its three packaging plasmids, e.g. pMDLgpRRE (gag/pol),
 pRSV-rev (rev) and pMDG.1 (VSVG envelope) or Adaptimmune pCI-VSVG (VSVG envelope), and other systems
similar to that used by Zufferey et al., (Zufferey et al, Nat Biotech 1997).
The WPRE region (which contains the potentially oncogenicX-protein gene sequence) of the recombinant lentiviral
vector genome has been removed.
The above Lentiviral Expression Systems are third or fourth generation replication-defective lentiviral vector systems.
The lentiviral vector particles can infect mammalian cells but cannot replicate in these cells. These systems include
the following safety features:
1. The number of HIV-1 genes that are used in the system has been reduced to three or four only: gag, pol and rev
with or without tat.
2. The VSV-G envelope gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope protein (env) thus
reducing the chance of creating an HIV-1 virus.
3. Genes encoding the structural and enzymes required for packaging the viral genome are separated into multiple plasmids and does not contain L TR or packaging signal sequences. All three/four plasmids have been engineered to contain minimal homology regions. This reduces the chance for homologous recombination events to occur which may lead to the formation of an HIV-1 virus.
4. The lentiviral particles produced in these systems are replication-incompetent and only express the transgene. The integrated lentiviral genome is no longer capable of producing a viral particle simply because it only contains a small portion of the HIV gag structural gene and the transgene. It requires the presence of at least the whole HIV gag-pol polygene and the HIV env gene within the two L TRs in order to produce a viral particle.
5. The transgene cassette contains a deletion of the 3' L TR (U3) to remove the promoter/enhancer element that does not affect generation of the viral genome in the producer host cell line, but results in a "self-inactivation" of the lentiviral vector after transduction of the target cell. Once integrated into the transduced target cell, there is no promoter/enhancer element present in both L TRs to transcribe any gene downstream.

Origin & function

The pre-modified ES cells will contain several knockout mutations and the ectopic expression of non-classical HLA genes that separately and together will cause a stepwise increased reduction in the potential for recognition and rejection by the immune system. ES cells will also be modified to include selection genes (e.g. puromycin selection) All genes expressed are synthetic genes codon-optimised for expression in mammalian cells. Where relevant, the risks to the workers associated with these host immunity reduction modifications will be removed by the prior inclusion of cell fate "suicide" genes so that these cells can be selectively killed by the administration of approved small molecule drugs that selectively activate intracellular death pathways. The TCR genes to be used encode for proteins known to be functionally expressed by T cells only, as they require the presence of cellular CD3 proteins which are only found in T cells. Expression of TCRs by cells other than T cells renders the TCR non-functional. In the case of cancer and viral epitope-specific TCR genes, their expression in T cells should only induce a response to cells expressing tumour antigens or viral antigens (including virally-infected cells).

Reporter genes such as GFP, RFP and luciferase have been used in various cellular and transgenic animal systems and have been shown to have no harmful effects on the organisms.

Evaluation of foreseeable effects

The hazards to workers and the environment posed by the vector with insert are considered negligible due to the containment level II measures employed. All work will be carried out in purpose-built, access-controlled laboratories. Skin contact with the organisms or the transfected cells will be avoided by minimizing aerosols (by working in a Class II biosafety cabinet), and by the use of gloves and safety glasses. The use of sharps during procedures is prohibited. The routine procedures employed (e.g., autoclaving or chemical inactivation of all cellular material prior to disposal) will ensure an adequate level of safety. Transfected mammalian cells are considered especially disabled and are unlikely to survive in the environment in the absence of strict osmotic, buffering, temperature, and nutrient conditions. Lentiviruses are highly susceptible to dehydration. However, they can survive for long periods in high protein-buffered media. Lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. The lentivirus cannot replicate and so the consequences of escape are considered negligible, therefore risk is effectively zero. Infected mammalian cells are not able to establish themselves and spread in nature and the vectors selected are either disabled or attenuated.

Although the aims of the project are to confer immunological avoidance properties to the ES cells, modifications will
be added in a logical step-wise manner, with the addition of safety systems such as suicide genes prior to the knockout of molecules that would normally allow the hosts immune system to recognise and destroy the ES cells.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The solid waste and the majority of the liquid waste generated during this procedure will be collected in specialised containers and sterilised by autoclaving at 121 °C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste under Adaptimmune Limited's Environment Agency Waste Registration Number; OL T130. The autoclaved solid waste will be disposed of via incineration through an appointed specialist waste management company; Grundon Waste Management Limited. Autoclaved liquid waste will be disposed of through a sink to public sewer. Any remaining liquid waste will be treated with an over-kill concentration of Virkon (2% final w/vol) or 10% ChemGene according to the manufacturers instructions (DuPonUMediMark Scientific) for which independent validated efficacy against retrovirus exists.

Is an emergency plan required according to regulation 20? 

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity.

**Project Containment**

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<td>Large Scale Activities</td>
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</tr>
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02/03/2022
The primary aim of this work is to generate human T cells derived from iPS cells that express native or engineered T cell receptors with recognition specificities to the following cell types:

a) Cells expressing cancer antigens, including but not limited to, NY-ESO, gp100 and MAGE A3.
b) Cells expressing viral epitopes from viruses, including but not limited to, human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein Barr Virus (EMV) & human papilloma virus (HPV).
c) Cells expressing putative-immune disease antigens or in graft rejection antigens. The auto-immune diseases to be studied include but are not limited to diabetes mellitus type 1, rheumatoid arthritis, autoimmune hepatitis, & multiple sclerosis.

The modified iPS cells will also express non-pathogenic reporter genes that allow for the selection of transformants and subsequent detection in immunological assays. These include but are not limited to: Green Fluorescent Protein (GFP), Red Fluorescent Protein (RFP), dsRED and/or luciferase genes.

(This work expands on embryonic stem cell work previously submitted and approved under reference GM3242/ entitled "The use and genetic modification of human embryonic stem cells")

The recipient cells will be:
Human iPS cells and several derived cell lineages resulting from biological treatment regimes designed to differentiate these iPS cells into what are ultimately stable T cells.

The iPS progenitor cells will be generated by an external company using somatic cells derived from healthy donor tissue (e.g. peripheral blood/cord blood). Somatic cells will be reprogrammed using a combination of transcription factors (e.g. OCT3/4, SOX2, c-MYC, KLF4, NANOG and LIN28). Gene delivery systems to induce reprogramming of somatic cells could include: Adenovirus mediated transduction, sendai virus mediated transduction, mRNA transfection or episomal transfection. Vectors used in the reprogramming phase should not persist beyond a few cell passages. For added safety, vector clearance will be demonstrated and confirmed prior to release of iPS cells to Adaptimmune or external collaborators.

Genetic modification of iPS cell lines will be conducted by our collaborator Universal Cells prior to use by Adaptimmune. A recombinant adeno-associated virus delivery system will be used to knock-out (RFXANK, RAG and beta-2 microglobulin) and knock-in (HLA-E and TCR) genes. These stable genetic modifications are designed to reduce allogeneic rejection by the host immune system. Where appropriate, these GM iPS cells will have also been gene modified prior to Adaptimmune's use by the inclusion of a cell fate "suicide gene" which provides an additional measure of safety against immune tolerance in vivo. These genetically-modified iPS cells do not produce any viruses (GM or otherwise).

The work involving these cells will be undertaken in a Containment Level 2 facility. The cells used are to the best of our knowledge, free of adventitious agents.

### Host/vector system

Two types of lentivectors will be used to transduce the modified iPS cells:

**Type A:**
- Commercially acquired vectors, typically third (or sometimes fourth) generation Lentiviral expression systems including but not limited to:
  - ViraPower Lentiviral Expression systems sold by Invitrogen Ltd. The vectors are: pLenti6/Vs-Direction TOPO, pLenti6.2-GW/EnIgFP, pLP1, pLP2, and pLP/VSVG plasmids.
  - Lenti-X Lentiviral Expression systems sold by Clontech Laboratories, Inc. The vectors include: pLVX-Puro, pLVXDsRed-Monomer-Cl, Lenti-X FIT packaging system.

**Type B:**
- Vectors constructed by our academic collaborators e.g. University of Pennsylvania (UPenn).
  - These will typically be third generation type systems including but not limited to:
    - Vectors obtained from 'University of Pennsylvania; UPenn': Lentiviral vector derived from the ‘Dull’ vector (Dull et al. J Viral 1998), and 3 packaging plasmids (gag/pal, rev, VSVG envelope) similar to that used by Zufferey (Zufferey et al, Nat Biotech 1997)

These Lentiviral Expression Systems are third or fourth generation lentivirus systems. The lentiviruses can infect mammalian cells but cannot replicate in these cells. These systems include some or all of the following safety features:

1. Expression vectors containing a deletion of the 3’ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a viral particle.
2. The number of HIV-I genes that are used in the system has been reduced to three or four only: gag, pol, tat and sometimes rev.
3. The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-I envelope gene (env).
4. Genes encoding the structural and other components required for packaging the viral genome are separated onto multiple plasmids. These plasmids have been engineered not to contain any homology regions to prevent recombination events.
5. Although the plasmids allow expression in trans of proteins required to produce lentivirus progeny in producer cell lines, none of them contain LTRs or the packaging sequence. This means that none of the HIV-I structural genes are present in the packaged viral genome and are thus never transduced in the target cell. No new replication-competent virus can be produced.
6. The lentiviral particles produced in these systems are replication-incompetent and only carry the genes of interest.
7. Expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-I RRE in the gag/pol mRNA transcript.
### Origin & function

The pre-modified IPS cells will contain several knockout mutations and the ectopic expression of non-classical HLA genes that separately and together will lead to a stepwise reduction in the potential for recognition and rejection by the immune system. IPS cells will also be modified to include selection genes (e.g., puromycin selection). All genes expressed are synthetic genes codon-optimised for expression in mammalian cells.

Where relevant, the risks to the workers associated with these host immunity reduction modifications will be removed by the prior inclusion of cell fate "suicide" genes so that these cells can be selectively killed by the administration of small molecules that selectively activate intracellular death pathways.

The TCR gene to be used encode for proteins known to be functionally expressed by T cells only, as they require the presence of cellular CD3 proteins which are only found in T cells. Expression of TCRs by cells other than T cells renders the TCR non-functional. In the case of cancer and viral epitope-specific TCR genes, their expression in T cells should only induce a response to cells expressing tumour antigens or viral antigens (including virally-infected cells).

Reporter genes such as GFP, RFP, and luciferase have been used in various cellular and transgenic animal systems and have been shown to have no harmful effects on the organisms.

### Evaluation of foreseeable effects

The hazards to workers and the environment posed by the vector with insert are considered negligible due to the containment level II measures employed. All work will be carried out in purpose-built, access-controlled laboratories. Skin contact with the organisms or the transfected cells will be avoided by minimizing aerosols (by working in a Class II biosafety cabinet), and by the use of gloves and safety glasses. The routine procedures employed (e.g., autoclaving or chemical inactivation of all cellular material prior to disposal) will ensure an adequate level of safety.

Transfected mammalian cells are considered especially disabled and are unlikely to survive in the environment in the absence of strict osmotic, buffering, temperature, and nutrient conditions. Lentiviruses are highly susceptible to dehydration. However, they can survive for long periods in high protein-buffered media. Lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. The lentivirus cannot replicate and so the consequences of escape are considered negligible, therefore risk is effectively zero. Infected mammalian cells are not able to establish themselves and spread in nature and the vectors selected are either disabled or attenuated.

### Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]
The solid waste and the majority of the liquid waste generated during this procedure will be collected in specialised containers and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste under Adaptimmune Limited’s Environment Agency Waste Registration Number; OLT130. The autoclaved solid waste will be disposed of via incineration through an appointed specialist waste management company; Grundon Waste Management Limited. Autoclaved liquid waste will be disposed of through a sink to public sewer. Any remaining liquid waste will be treated with an over-kill concentration of Virkon (2% final wt/vol) or 10% ChemGene according to the manufacturers instructions (DuPont/MediMark Scientific) for which independent validated efficacy against retrovirus exists.

**Project Containment**

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**Project Ref** 3242/17.2

- **Date Ackn’d**: 03/05/2017
- **CU2 Project Title**: Lentiviral vectors co-expressing TCR genes and additional genes to enhance the effectiveness of T cells in specific disease environments.
- **Class**: Class 2
- **CultureVolClass2**: 1-50 Litres
- **Consent Granted**

**Project notified under transitional arrangements**

**Historical Significant Changes**

- **Historical Date of Additional Info**: 14/08/2018

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<th>Significant Change ID</th>
<th>Date of Significant Change</th>
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<td>3242/17.2a</td>
<td>14/08/2018</td>
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**Project Additional Information**

- **Date Ackn’d**: 03/05/2017
- **Date Project Ceased**: 
- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: Y
- **Non-GMM Consent Granted**
Adaptimmune genetically modifies human T cell receptors (TCRs) and then uses lentiviral vectors to transduce these TCRs into human T cells so that these transduced T cells are better able to recognise and kill cancer cells or cells infected with viruses. The transduction of T cells with modified TCRs can also be adapted to create regulatory T cells that can potentially block the natural T cells that cause autoimmune disease.

The primary aim of this connected programme of work is to transduce human T-cells with a range of non-replicating self-inactivating transgene-integrating lentiviral vectors. Each vector encodes a disease-associated α/β heterodimeric TCR together with one of several additional genes designed to enhance the effectiveness of T cells in specific situations.

The gene combinations assessed here comprise α/β TCR genes alongside either genes with co-stimulatory properties; genes with anti-inhibitor properties; genes to enhance T cell killing, “Suicide” genes (and their activating pro-drugs) and genes that help create antigen-dependent immunosuppression. These properties may affect not only the recipient T cells but may also act on other immune and non-immune cell types as bystanders to potentiate the anti-disease immune response.

The resulting genetically-modified human primary T cells and T cell lines will be used for in vitro cell-based assays of T cell function. Additional assays will assess if the T cells still retain their target antigen-specific functionality.

### Recipient or parental organism

**Recipients:**
- Human HEK293T cell line and its derivatives - Lentiviral vector packaging cell line
- Human primary T cell lymphocytes
- Human T cell lines - not virally infected (e.g. SupT1, Jurkat and T cell hybridomas e.g., 58α- β-) - Blood-derived CD4+ and CD8+ T cells.

### Host/vector system

Replication-defective, self-inactivating lentiviral particles of one or more alternative types of 3rd and 4th generation lentiviral vector systems (commercially-acquired or made in-house):

All lentiviral systems are based on lentiviral transgene plasmids that carry a “self-inactivation (SIN)” mutation that inactivates the promoter and enhancer functions in both of the LTR sequences that flank the integrated transgene region Zufferey et al., (J.Virol 72, 9873-9880 1998).

Characteristics of the vectors:
- The number of HIV-1 open reading frame genes that are used in the system has been reduced to a minimum of gag, pol and rev.
- Genes encoding the above structural proteins and enzymes which required for packaging the viral genome are separated onto multiple plasmids and do not contain LTR or packaging signal sequences. All plasmids have been engineered to contain minimal homology regions. This reduces the chance for homologous recombination events to occur which may lead to the formation of a viable retrovirus.
- The VSVg envelope gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope protein (gp160 env) gene thus further reducing the chance of creating a viable retrovirus by homologous recombination events. NB the host range of the VSVg is very broad allowing the lentiviral particles to potentially transduce any dividing mammalian cell type.
- The lentiviral particles produced in these systems are replication-incompetent and only express the transgene in the transduced cells. The integrated lentiviral genome is no longer capable of producing a viral particle
- The transgene vector plasmid contains a deletion in the 3’ LTR (U3) to remove the promoter and enhancer elements. This does not affect the generation and packaging of the viral RNA genome in the lentiviral transgene particle producer host cell line, but results in “self-inactivation (SIN)” of the lentiviral transgene vector after its reverse transcription in the target cell. Prior to integration into the DNA of the transduced target cell, the 3’ SIN LTR is copied and in the process replaces the wt 5’ LTR.
• None of our lentiviral transgene constructs contains a WPRE sequence (and hence none contain the open reading frame for the potentially-oncogenic Protein X that this sequence encodes).

Replication-defective lentiviral particles will be produced by co-transfection of human HEK293T cells with both a lentiviral TCR-based transgene vector plasmid together with a complimentary set of lentiviral packaging plasmids (All of these recombinant DNA transfection plasmids had previously been generated in E.coli).

The resulting lentiviral vector particles will be used to transduce human T cells and T cell lines. The transduced T cells are designed to express both the affinity-enhanced TCR and a companion gene that enhances the functionality of subsequent T cell immunotherapy. Functionality will be assessed first in vitro using culture human cells that naturally or ectopically express and display cognate or non-cognate TCR peptide/MHC antigens.

Origin & function

Parental organism of inserts:
Human cDNA genes or synthetic equivalents of these genes without introns and based on the amino acid sequences of WT/GM human proteins - Full length, truncated and/or mutated forms of human genes. Genes not associated with oncogenicity, viral virulence or any pathogenicity.

WT or mutant alpha / beta TCR chain pairs (These TCR chains are sequence-modified to enhance specific binding to peptide/HLA antigens relevant to cancer). The transduced T cells are designed to express both the affinity-enhanced TCR and a companion gene that enhances the functionality of subsequent T cell immunotherapy. Functionality enhancement will be achieved by one or more of the following:
Gene(s) with co-stimulatory functions including various genes enhancing / complimenting antigen-dependent TCR signalling in T cells;
Gene(s) to block inhibitory signals including various genes enhancing the activity of T-cells by blocking anti-T-cell inhibitory signals;
Stimulatory cytokines / cytokine receptor gene(s) includes various cytokine and/or cytokine receptor genes that stimulate T cells and/or act on surrounding cells types to enhance immunotherapy;
Genes to modify T cell metabolism includes various genes enhancing the metabolism of T cells especially in the tumour microenvironment;
Gene(s) to enhance T cell killing includes various genes that potentiate antigen-specific target cell killing;
Genes (and their activating pro-drugs) confer conditional cell fate "suicide" mechanisms;
Gene(s) to enable an immunosuppressive environment by helping to create antigen-dependent immunosuppression.

Refer to Box 17 for confidential details of inserts.

Evaluation of foreseeable effects

The most hazardous GM vector/insert combinations in this assessment are the packaged lentiviral TCR transgene vector particles. These particles are pseudo-typed with the VSV-G envelope protein which allows them to transduce virtually any mammalian cell line. These particles are not in themselves viable viruses, they cannot replicate in their transduced host cells. They can however functionally express the genes they carry in other cells in the body they transduce.

The lentiviral transgene vectors express TCR genes plus accessory genes designed to further enhance T cell function. The TCR alpha and beta chains that are expressed can only function in T cells as they require the presence of CD3 signalling proteins which are themselves only found in T cell lineages (except for TCR-CD3z fusion proteins). Should the TCR molecules find themselves in a permissive cell type they are unlikely to cause harm as the TCR will only signal in response to cognate peptide/HLA antigens.

The conceivably most hazardous GM lentiviral vectors would be:
1. Those with inserts that, when expressed by any cell transduced by the lentiviral vector, produce:
   a. a transient local inflammatory response (transduced body cells are not immortal). This response can however be managed by generalised immunosuppressive drugs.
   b. a transient local bystander cell death response. This may be additionally limited by self-triggering of the death response in the insert-expressing cells themselves;
   c. a transient local immunosuppressive environment, temporarily potentiating an existing infectious or other chronic or acute disease that is normally controlled by
immune surveillance and/or temporarily inhibiting "non-self" protection. Some such inserts may act as growth factors for established tumours and as a factor that assists the differentiation of immunosuppressive Treg T cells. However, as above, these effects would only be local and limited.

Most Hazardous GMMs:
HEK293T cells packaging lentiviral TCR transgene vector particles present no more risk than the particles themselves. This cell-based vector packaging system is only able to produce vector particles under in vitro tissue culture conditions. Production of particles in these cells is transient (maximum ~72 hours). Packaging requires a genomic vector RNA transcript and a set of packaging proteins; both are transiently expressed from non-integrated non-replicating plasmids transiently introduced into the HEK293T packaging host cell line by transfection with these DNA plasmids. Primary T cells and T cell lines transduced by these lentiviral particles present negligible risk to the laboratory worker as they cannot produce functional virus. Transactivation of host genes by inserted LTR-flanked TCR transgene cassettes is blocked because each LTR carries a large deletion that inactivates both the promoter and enhancer activities of the LTRs. To avoid the issue of transduced T cell tolerance the use of self T cells by laboratory workers is expressly forbidden. This self T cell barrier is effectively managed by written protocols and training.

Packaged lentiviral particles can infect human primary cells of multiple lineages. The genes co-expressed with the TCR genes might suppress bystander cell proliferation but this will be self-limiting. All transgene-expressed genes other than very short animal picornavirus 2A skipping sequences all genes are of non-oncogenic human amino-acid sequence origin and are unlikely to elicit any deleterious immunological or allergic responses even if the individual had previously received an anti-human picornaviral virus vaccination.

The research concerned combines enhanced affinity TCRs which have not previously been tested for safety in a clinical setting with additional genes enhancing T cell function. If lentiviral particles containing both were to be inadvertently injected into the bloodstream of the scientist and enter their own T cells, and subsequently these T cells were to encounter cognate antigen to switch them on (which is unlikely), there would be a potential risk of an adverse immune reaction in the worker. For this reason the use of needles and sharps and working with "self" T cells is strictly prohibited during lentivirus work.

Inadvertently injected transduced/transfected GMO T-cells may attack normal tissue but these T-cells are normally recognised by the individual’s immune system as non-self and are efficiently eliminated by the host-immune system. Theoretically however an allergenic reaction and/or an allergic response could be generated that might ultimately prove fatal.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We do not require the measure "Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs" as there are no disease vectors which could disseminate GM human T-cells.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Most liquid waste is inactivated in freshly prepared 1% Virkon (final concentration) for at least one hour contact time before being poured down the foul drain. Non-human tissue solid waste and some liquid waste generated during this procedure will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste under Adaptimmune Limited’s Environment Agency Waste Registration Number; OLT130. The autoclaved solid waste will be disposed of via incineration through an appointed specialist waste management company; Grundon Waste Management Limited. Autoclaved liquid waste will be disposed of through a sink to public sewer.

HTA-defined human tissue solid and contained liquid waste is disposed of directly (without prior disinfection) into dedicated bins which are wiped on the outside with 70% ethanol before disposal as Human Tissue Waste for special incineration through Grundon Waste Management Limited as above.
Any remaining liquid waste will be treated with Virkon (1% final wt/vol) or 10% ChemGene according to the manufacturers instructions (DuPont/MediMark Scientific) for which independent validated efficacy against retrovirus exists.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity

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Large Scale Activities

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Human Clinical Applications

- L2 L3 L4 L2 L3 L4 L2

Project Ref 3242/17.3

Date Ackn'd 03/05/2017

CU2 Project Title Overexpression of gene(s) with oncogenic potential in mammalian cell lines

Date Project Ceased

Class 2

Culture Vol Class 2 1-50 Litres

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
# Project Additional Information

## Purposes of the contained use

The primary aim of this project is to overexpress notch receptor 1 in a mammalian cell line. This genetically modified cell line will then be used as a tool to select ("pan") from Antibody Phage Displayed Libraries for agonistic antibodies against notch receptor 1. The GM cells may also be used as part of a signalling reporter system aimed at assessing the agonistic properties of notch specific antibodies in in vitro assays.

## Recipient or parental organism

The recipient cells will be human (HG2) and mouse (HG1) cell lines. For further confidential details of recipients please refer to Section 17.

## Host/vector system

Two types of lentivectors could be used to transduce the mammalian cell lines:

**Type A:**
Commercially acquired vectors, typically third (or sometimes fourth) generation Lentiviral expression systems including but not limited to:

- ViraPower Lentiviral Expression systems sold by Invitrogen Ltd. The vectors are: pLenti6iVs-Dfrecional TOPO, pLenti6.2-GW/EniGFP, pLP1, pLP2, and pLPNSVG plasm ids.
- Lenti-X Lentiviral Expression systems sold by Clontech Laboratories, Inc. The vectors include: pLVX-Puro, pLVXDsRed-Monomer-CI, Lenti-X FIT packaging system

**Type B:**
Vectors constructed by our academic collaborators e.g. University of Pennsylvania (UPenn). These will typically be third generation type systems including but not limited to:

- Vectors obtained from 'University of Pennsylvania; UPenn): Lentiviral vector derived from the 'Dull' vector (Dull et al. J Viral 1998), and 3 packaging plasm ids (gag/pal, rev, VSVG envelope) similar to that used by Zufferey (Zufferey et al, Nat Biotech 1997)

These Lentiviral Expression Systems are third or fourth generation lentivirus systems. The lentiviruses can infect mammalian cells but cannot replicate in these cells. These systems include some or all of the following safety features:

1. Expression vectors containing a deletion of the 3’ L TR (L1U3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell. Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a viral particle.
2. The number of HIV-I genes that are used in the system has been reduced to three or four only: gag, pol, tat and sometimes rev.
3. The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-I envelope gene (env).
4. Genes encoding the structural and other components required for packaging the viral genome are separated onto multiple plasmids. These plasm ids have been engineered not to contain any homology regions to prevent...
**Origin & function**

Human cDNA genes or synthetic equivalents of these genes without introns and based on the amino acid sequences of WT/GM human proteins: Full length synthetic forms of human genes codon-optimised for expression in human and mouse cells, e.g. Notch Receptor 1 (a potential oncogene); constitutive promoters; selection genes (e.g., puromycin resistance) and reporter genes (such as GFP, RFP and luciferase).

Notch receptor 1 over-expression will be used to select agonistic antibodies against Notch1 for further study. For further confidential details of inserts please refer to Section 17.

**Evaluation of foreseeable effects**

The most hazardous GM vector/insert combinations in this assessment are the packaged lentiviral transgene vector particles. These particles are pseudo-typed with the VSV-G envelope protein which allows them to transduce virtually any mammalian cell line. These particles are not in themselves viable viruses, they cannot replicate in their transduced host cells. They can however functionally express the genes they carry in other cells in the body they transduce.

The lentiviral transgene vectors express the Notch 1 gene plus an accessory constitutive promoter gene designed to induce overexpression of notch receptor 1 on the surface of human or mouse cell lines. Inhibition of Notch1 has been shown to have an anti-proliferative effect in certain cancers. Notch1 is therefore considered to be a potential oncogene and co-expression of Notch1 stimulatory genes is likely to enhance this effect. If the lentiviral vector inadvertently enters the body, it is able to transduce any body cell due to the broad host range of the VSV-G enveloping gene and such cells may then induce malignant tumours or cancer in the recipient.

Packaging cell lines transduced with above vectors present no more risk than the particles themselves and are only able to transiently produce vector particles under in vitro tissue culture conditions. Human primary cells in general are considered as especially disabled hosts, provided they are unable to colonise the worker (i.e. non-self), and to the best of our knowledge they contain no adventitious agents which are potentially harmful. Should infected cells gain entry to the worker, the cells would in all likelihood be rapidly destroyed by the workers' immune system by virtue of being antigenically "non-self".

Packaged lentiviral particles can infect human primary cells of multiple lineages. There is therefore a risk of transducing your own cells if the lentivirus should enter your body. Although mutations that constitutively activate Notch 1 have been associated with a number of leukemias (e.g., T-All and Cll) there is no direct evidence that NOTCH1 directly induces cellular transformation in mature mammalian cells. In such cases it is an additional driving force but not the initial cause of the cancer.

Inadvertently injected transduced/transfected GMO cells will be picked up by the individual's immune system as nonself and should be efficiently eliminated by the host-immune system. Severely immunocompromised individuals may be at risk as they may have issues detecting and clearing genetic modified cells should they enter the body. However, such severely immunocompromised people would be ill and would not have access to the lab.

**Containment and control measures for GMOs that are not microorganisms (e.g., GM animals & plants)**

N/A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We do not require the measure "Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs" as there are no disease vectors which could disseminate GM human T-cells.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste is inactivated in freshly prepared 1% Virkon (final concentration) or 10% ChemGene for at least one hour contact time before being poured down the foul drain.

All CL2 waste is autoclaved before disposal (121°C for 25 minutes for 100% kill) - the autoclave is efficacy-tested using Thermalog strips every 3 months and a complete validation programme is performed annually during maintenance.

Upon autoclaving, solid waste is disposed of as 'hazardous waste' for off-site incineration by an approved and licenced hazardous waste contractor.

Human Tissue waste is disposed of directly (without prior disinfection) into dedicated bins and collected by an approved and licenced hazardous waste contractor for specialist off-site incineration.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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<td>L2</td>
<td>L3</td>
<td>L4</td>
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Project Ref 3242/17.4

Date Ackn'd 03/05/2017  
CU2 Project Title Expression of the parent proteins for peptide MHC/HLA antigens  
Class 2  
CultureVolClass2 1-50 Litres  
CultureVolumeClass3-4
**Project Additional Information**

**Purposes of the contained use**

This document relates to studies aimed at producing human cell lines expressing the genes for the parent proteins of T cell antigens. These proteins are processed into peptides that are presented by MHC/HLA molecules. These peptide-MHC antigens are then recognised by antigen-specific T cell receptors (TCRs). We wish to study the peptide/MHC binding properties of engineered TCRs so that we can evaluate their suitability as components to be used in T cell-mediated adoptive cell therapy.

**Recipient or parental organism**

Recipients:
- Human primary cells and established cell lines (HG2 - potential but not confirmed pathogen presence) to be transduced with lentiviral antigen-presenting cells
- Human HEK293T cell line and its derivatives (for lentiviral vector packaging)

**Host/vector system**

Replication-defective, self-inactivating lentiviral particles. One or more alternative types of 3rd and 4th generation lentiviral vector systems.

**Type A:**
Commercially-acquired
Third and fourth generation lentiviral vector packaging systems including but not limited to:
- "ViraPower" Lentiviral vector expression systems (Invitrogen Ltd). The plasmids include pLenti6N5-Directional TOPO, pLenti6.2-GW/EmGFP, pLP1, pLP2, and pLPNSVG plasmids.
- "Lenti-X" Lentiviral expression systems (Clontech Laboratories, Inc). The vectors include: pLVX-Puro, pLVXDsRed-Monomer-C1, Lenti-X HT packaging system.

**Type B:**
Made in-house:
These are third generation type systems, including but not limited to:
Lentiviral vectors derived from the ‘Dull’ lentiviral vector transgene plasmid (Dull et al, J Virol 72, 8463-8471 1998),
and its three packaging plasmids i.e., pMDLgpRRE (Gag/Pol), pRSV-rev (Rev) and pMDG.1 (VSVg envelope) or an alternative Adaptimmune pCI-VSVG (VSVg envelope) plasmid. Allentiviral systems are based on lentiviral transgene plasmids that carry a "self-inactivation (SIN)" mutation that inactivates the promoter and enhancer functions in both of the LTR sequences that flank the integrated transgene region Zufferey et al. (J. virol. 72, 9873-9880, 1998)).

Characteristics:

- The number of HIV-1 open reading frame genes that are used in the system has been reduced to a minimum of gag, pol and rev.
- Genes encoding the above structural proteins and enzymes which required for packaging the viral genome are separated onto multiple plasmids and do not contain LTR or packaging signal sequences. All plasmids have been engineered to contain minimal homology regions. This reduces the chance for homologous recombination events to occur which may lead to the formation of a viable retrovirus.

• The VSVg envelope gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope protein (gp160 env) gene thus further reducing the chance of creating a viable retrovirus by homologous recombination events. NB the host range of the VSVg is very broad allowing the lentiviral particles to potentially transduce any dividing mammalian cell type.

• The lentiviral particles produced in these systems are replication-incompetent and only express the transgene in the transduced cells. The integrated lentiviral genome is no longer capable of producing a viral particle.

• The transgene vector plasmid contains a deletion in the 3' LTR (U3) to remove the promoter and enhancer elements. This does not affect the generation and packaging of the viral RNA genome in the lentiviral transgene particle producer host cell line, but results in "self-inactivation (SIN)" of the lentiviral transgene vector after its reverse transcription in the target cell. Prior to integration into the DNA of the transduced target cell, the 3' SIN LTR is copied and in the process replaces the wt 5' LTR.

• None of our in-house lentiviral transgene constructs contains a WPRE sequence (and hence none contain the open reading frame for the potentially-oncogenic Protein X that this sequence encodes).

Origin & function

Origins: Human (synthetic or semi-synthetic functional and non-functional gene sequences, some of which are associated with oncogenicity or tumourigenesis); Jellyfish (reporter genes) and Bacterial HG1 (drug resistance genes)

All inserts will consist of wholly or partially synthetic human genes based on the amino acid sequences of wildtype or modified human proteins. Sometimes they will be co-expressed with reporter genes (e.g., eGFP) and/or drug selection genes (e.g., Puromycin resistance). In other circumstances the parent gene will be co-expressed with a Class I or Class II MHC/HLA antigen-presenting gene (this can be with or without the additional expression of a beta 2m gene). On occasion the presentation of the peptide antigen will be assisted by genetic fusion of the gene to a ubiquitination signal or by genetic fusion to ubiquitin itself to promote efficient peptide antigen processing.

The parent protein can be a cancer-specific protein e.g., from a mutated K-ras gene. Alternatively, the gene product can be a Cancer-Testes antigen (e.g., from a MAGE family or NY-ESO gene). If the parent protein gene is a known or potential oncogene (e.g., K-ras) it will only be expressed in a non-functional form e.g., as a truncated gene fragment. These genes for TCR antigen presentation are not restricted to cancer. We also wish to express and study other antigenic human proteins as targets for TCR-based autoimmune disease therapy.

In addition to these intended TCR target antigen genes we wish to express control parent antigen genes. These can be non-mutated wild-type versions of the antigen genes. Alternatively, the control genes can be other human genes that are potentially recognised by a TCR but not associated with tumourigenesis or autoimmunity.
The most hazardous GM vector/insert combinations in this assessment are the packaged lentiviral transgene vector particles. These particles are pseudo-typed with the VSVg envelope protein which allows them to transduce virtually any mammalian cell line. These particles are not in themselves viable viruses, they cannot replicate in their transduced host cells. They can however functionally express the genes they carry in other cells in the body they transduce.

The lentiviral transgene vectors express proteins associated with cancer or autoimmunity. However, the cloning and expression of functional known or putative oncogene proteins is not permitted.

Mammalian cells (including the HEK293T lentiviral vector packaging cells) that are transduced with antigen parent protein gene lentiviral transgene vector particles should present no more risk than the particles themselves. Furthermore, the transduced human cells produced will have a HLA tissue type different from the laboratory staff and hence will be detected and efficiently eliminated by the immune systems of any inadvertently contaminated worker. Working with 'self' materials is strictly prohibited.

Severely immunocompromised people, who conceivably would be most at risk, do not have access to the lab.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We do not require the measure "Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs" as there are no disease vectors which could disseminate GM human T-cells.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Most liquid waste is inactivated in freshly prepared 1% Virkon (final concentration) for at least one hour contact time before being poured down the foul drain.

Non-human tissue solid waste and some liquid waste generated during this procedure will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste under Adaptimmune Limited's Environment Agency Waste Registration Number: OL T130. The autoclaved solid waste will be disposed of through an appointed specialist waste management company. Autoclaved liquid waste will be disposed of through a sink to public sewer.

HTA-defined human tissue solid and contained liquid waste is disposed of directly (without prior disinfection) into dedicated bins which are wiped on the outside with 70% ethanol before disposal as Human Tissue Waste for special incineration through Grundon Waste Management Limited as above.

Any remaining liquid waste will be treated with Virkon (1% final w/vol) or 10% ChemGene according to the manufacturers instructions (DuPonUMediMark Scientific) for which independent validated efficacy against retrovirus exists.

### Is an emergency plan required according to regulation 20?  

N

### If yes, tick to confirm that it is attached to this form 

N

### Tick to confirm that you have attached a risk assessment to this form 

Y
The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
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#### Project Ref 3242/17.5

- **Date Ackn'd**: 04/05/2017
- **CU2 Project Title**: Disruption of genes in human primary stem cells and cell lines
- **Class**: Class 2
- **Culture Vol Class**: 1-50 Litres
- **Consent Granted**: Non-GMM

#### Project Additional Information

- **Project notified under transitional arrangements**: N
- **Tick if notifying a connected programme of work**: N
### Purposes of the contained use

The primary aim of this project is to disrupt and thereby inactivate genes in human cells. The gene disruptions(s) are designed to stably change the genotype and phenotype of the cells. Cells with disrupted genes will be used for various purposes such as:

1. Evaluating if a host cell protein or an MHC-presented peptide derived from this protein is immunogenic. e.g. to attempt to confirm if the peptide/antigen target for a T cell receptor (TCR) is derived from the product of a known host gene.
2. Determining if gene disruption(s) will alter the pathways of differentiation of a stem cell line
3. Determining if disruption of a host gene(s) can boost the viral vector packaging capability of a cell line.

### Recipient or parental organism

**Recipients:**
- Human primary cells, established cell-lines and stem cells (HG2 - potential but not confirmed pathogen presence):
  - Human primary cells
  - Established human cell lines
  - Human Haematopoietic Stem Cells (Adult bone marrow-derived Haematopoietic Stem Cells)
  - Human Embryonic Stem (ES) Cells (Embryo-derived Stem Cells)
  - Human Induced Pluripotent Stem Cells (Stem cells induced by Yamanaka factors)
  - Human HEK293T cell line and its derivatives (Lentiviral vector packaging cell line (human neuro-adrenallineage))

Refer to Box 17 for confidential details of recipients.

### Host/vector system

Gene editing technology (Plasmid for expression of the functional components needed for CRISPR/Cas9 genome editing in mammalian cells with an additional orange fluorescent protein (OFP) transfection reporter gene (OFP not knocked in))

All-in-one lenti-Cas9 (System Biosciences) (3rd generation lentiviral vector packaging system:

- Lentiviral vector transgene plasmid to be packaged into VSVg pseudotyped replication incompetent lentivirus.
- Contains 5’LTR, 3’SIN LTR, RRE and WPRE sequences and expresses Cas9 and CRISPR gRNA sequence in the target cell.

All plasmids non-mobilisable assembled and propagated in E.coli laboratory strains.
- The VSVg envelope gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope protein (gp160 env) gene thus further reducing the chance of creating a viable retrovirus by homologous recombination events. NB the host range of the VSVg is very broad allowing the lentiviral particles to potentially transduce any dividing mammalian cell type.
- The lentiviral particles produced in these systems are replication-incompetent and only express the transgene in the transduced cells. The integrated lentiviral genome is no longer capable of producing a viral particle.

### Origin & function

Synthetic gene disruption sequences with partial homology to the gene targeted for disruption are chemically synthesised DNA insert genes assembled by standard Molecular Biology techniques. They function as transcribed guide RNAs to direct Cas9 to cleave DNA at specific sequences within human genes.

Knock in gene sequences are synthetic or semi-synthetic DNA sequences bearing partial homology to the gene targeted for disruption. They also contain either additional synthetic gene sequences that allow for genotypic screening or semi-synthetic sequences that additionally allow for the expression of either reporter genes (e.g. GFP)
for the phenotypic screening of gene disruption or drug resistance genes (e.g. puromycin resistance) that are expressed to provide a mechanism for selecting cells carrying disrupted genes.

NB All gene knock-in sequences are designed for gene disruption screening or selection. The knocking-in of any other type of functional gene will require specific risk assessment.

The genes to be disrupted are:-
1. Genes in human tissue culture cell lines that are associated with the adaptive immuno-surveillance of cancer or autoimmunity.
2. Genes in human stem cells (Haematopoietic Stem Cells, Embryonic Stem Cells, Induced Pluripotent Stem Cells) especially those that are associated with cell lineage differentiation.
3. Genes in human cell lines whose disruption may boost the production of packaged lentiviral vector particles.

It must be stressed that the inserts in the vectors that are used for gene knock-in contain only non-functional truncated versions of parts of the targeted gene. Furthermore, no known tumour suppressor genes will be disrupted.

Refer to Box 17 for confidential details of inserts.

### Evaluation of foreseeable effects

The most hazardous GM vector/insert combinations in this assessment are the packaged lentiviral vector particles. These particles are pseudo-typed with the VSVg envelope protein which allows them to transduce virtually any human cell type. These particles are not in themselves viable viruses, they cannot replicate in their transduced host cells. They can in theory unintentionally express the targeted CRISPR/Cas9 genes they carry in other cells transduced with these lentiviral vectors.

CRISPR/Cas9 constructs disrupting anti-apoptotic genes could, if inadvertently entered into the body, cause local cell death. To minimise the likelihood of this occurring such constructs will not be used in any lentiviral vectors. Instead these gene disruptions will be limited to non-viral vectors that require additional cell transfection reagents and protocols.

The human pluripotent stem cells are potentially tumorigenic. However, stem cells modified to be able to evade the immune system will not be used in this project and would quickly be destroyed by a person's own immune system. Cells transduced with non-viral or lentiviral CRISPR/Cas9 gene disruption constructs should be no more hazardous than the equivalent non-transduced cells.

Other potentially hazardous GM lentiviral vectors would be those with expressable inserts that, when expressed by any cell transduced by the lentiviral vector, produce a potentially antigenic Cas9, screening and/or drug selection protein resulting in a transient local inflammatory response (transduced body cells are not immortal). This response can however be managed by generalised immunosuppressive drugs.

Packaged lentiviral particles can infect human primary cells of multiple lineages. Introducing potentially antigenic inserts into the body may result in a transient local inflammatory response. CRISPR/Cas9 constructs disrupting anti-apoptotic genes could, if inadvertently entered into the body, cause local cell death. Severely immunocompromised people, who conceivably would be most at risk, do not have access to the lab.

Refer to Box 17 for confidential details of vectors.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We do not require the measure “Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs” as there are no disease vectors which could disseminate GM human T-cells.
Most liquid waste is inactivated in freshly prepared 1% Virkon (final concentration) for at least one hour contact time before being poured down the foul drain. Non-human tissue solid waste and some liquid waste generated during this procedure will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste under Adaptimmune Limited’s Environment Agency Waste Registration Number; OL T130. The autoclaved solid waste will be disposed of via incineration through an appointed specialist waste management company. Autoclaved liquid waste will be disposed of through a sink to public sewer. HTA-defined human tissue solid and contained liquid waste is disposed of directly (without prior disinfection) into dedicated bins which are wiped on the outside with 70% ethanol before disposal as Human Tissue Waste for special incineration through Grundon Waste Management Limited as above. Any remaining liquid waste will be treated with Virkon (1% final w/v) or 10% ChemGene according to the manufacturers instructions (DuPont/MediMark Scientific) for which independent validated efficacy against retrovirus exists.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity.

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity.

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<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Project Ref 3242/17.6
The primary aim of this project is to produce HEK293T cell lines with increased capability to produce packaged lentiviral particles. This will be achieved by transducing and/or transfecting them with vectors carrying genes that change the packaging cell's phenotype to enable it to produce higher amounts of packaged lentiviral vectors for longer. GMO RA 248 describes the work of packaging lentiviral vectors into HEK293T cells. These packaged lentiviral vectors are subsequently transduced back into HEK293T cells, where this time they integrate into the genome and coexpress the inserts of interest together with the reporter or selection gene.

Recipient or parental organism

Recipients:
- Human HEK293T cell line and its derivatives - Lentiviral vector packaging cell line

Host/vector system

Recipients:
- Human HEK293T cell line and its derivatives - Lentiviral vector packaging cell line
  - None of our lentiviral transgene constructs contains a WPRE sequence (and hence none contain the open reading frame for the potentially-oncogenic Protein X that this sequence encodes).

As alternative to inserting genes into lentiviral vectors to integrate them stably into the packaging cell genome, some inserts will be cloned into non-viral plasmid vectors or EBV-origin based plasmid vectors. These are subsequently transfected into HEK293T cells. The EBV-origin plasmid vectors will remain in the cytoplasm of the HEK293T cells and will enhance the production of packaged particles without integrating into the genome of these cells. In the absence of continued drug selection these plasmid vectors will then gradually be lost as the cells divide. Plasmid vectors include pCI, pClneo,
pPur plasm ids; pREP4, pCEP4 plasmids (All non-viral and EBV virus oriP origin/EBNA-1 recombinant DNA mammalian gene expression plasmids that co-express a detection/selection gene. Non-mobilisable).

**Origin & function**

**Parental organism of inserts:**
Human cDNA genes or synthetic codon-optimised equivalents of these genes based on the amino acid sequences of these proteins - Full length, truncated and/or mutated forms of human genes. Genes not associated with oncogenicity, viral virulence or any pathogenicity.

Synthetic sequence-modified genes derived from the sequences of mammalian viral genes; Synthetic gene constructs that express shRNA molecules for the specific knockdown of host cell mRNAs (Gene based on viral sequence are substantially altered to prevent hypothetical homologous recombination).

**Overview of Inserts:**
1. Genes expressing human proteins to enhance lentiviral vector production
2. Synthetic genes expressing viral protein sequences (but not homologous to viral gene sequences) to enhance lentiviral vector production
3. Synthetic genes to express shRNA sequences that target host mRNA molecules to reduce expression of host genes that impede lentiviral vector production
4. Reporter genes to detect transfected/transduced mammalian cells
5. Drug resistance genes to allow selection of transfected/transduced mammalian cells
6. tRNA genes

Refer to Box 17 for confidential details of inserts.

**Evaluation of foreseeable effects**

The most hazardous GM vector/insert combinations in this assessment are the packaged lentiviral transgene vector particles. These particles are pseudo-typed with the VSVg envelope protein which allows them to transduce virtually any mammalian cell line. These particles are not in themselves viable viruses, they cannot replicate in their transduced host cells. They can however functionally express the genes they carry in other cells in the body they transduce.

The lentiviral transgene vectors express pro-viral genes or genes that antagonise anti-viral genes. These genes are not expected to cause significant changes to any host cells that are transduced.

HEK293T cells transduced with pro-viral lentiviral transgene vector particles present no more risk than the particles themselves. Furthermore, these novel cell-based vector packaging system are only able to produce vector particles under in vitro tissue culture conditions.

Packaged lentiviral particles can infect human primary cells of multiple lineages.

The conceivably most hazardous GM lentiviral vectors would be those with inserts that, when expressed by any cell transduced by the lentiviral vector, produce an antigenic protein resulting in a transient local inflammatory response (transduced body cells are not immortal). This response can however be managed by generalised immunosuppressive drugs. Severely immunocompromised people, who might conceivably be most at risk, do not have access to the lab.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We do not require the measure "Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs" as there are no disease vectors which...
could disseminate GM human T-cells.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Most liquid waste is inactivated in freshly prepared 1% Virkon (final concentration) for at least one hour contact time before being poured down the foul drain.

Non-human tissue solid waste and some liquid waste generated during this procedure will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste under Adaptimmune Limited's Environment Agency Waste Registration Number; OL T130. The autoclaved solid waste will be disposed of via incineration through an appointed specialist waste management company. Autoclaved liquid waste will be disposed of through a sink to public sewer.

HTA-defined human tissue solid and contained liquid waste is disposed of directly (without prior disinfection) into dedicated bins which are wiped on the outside with 70% ethanol before disposal as Human Tissue Waste for special incineration through Grundon Waste Management Limited as above.

Any remaining liquid waste will be treated with Virkon (1% final w/v) or 10% ChemGene according to the manufacturers instructions (DuPonUMediMark Scientific) for which independent validated efficacy against retrovirus exists.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity

Project Containment

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02/03/2022
The primary aim of this project is express human or viral proteins in human cells that are known to be or are suspected to be oncogenic. The purpose of this work is to produce positive control cell lines to be used when analysing the levels of mRNA for oncogenes/potential oncogenes that are found in human tumour samples and in human cell lines.

A second use of these cell lines is to study the production of antigenic peptides that are derived from oncogenic proteins which are then presented on the surface of cells by human leukocyte antigen proteins (HLAs). Peptide-HLA antigens (pHLAs) are recognised by T Cell Receptors (TCRs) on T cells. We will use these oncogene-derived pHLA antigen cell lines as targets for human T cell clones and for human primary T cell lines stably transduced with lentiviral vectors that express recombinant TCR genes.

Recipient or parental organism
Recipient cells: Human primary cells and human cell lines.

Host/vector system
Bacterial non-mobilisable mammalian expression shuttle plasmids that do not contain a viral origin of replication (e.g., pCl, pClneo and pPur plasm ids). These plasm ids cannot be used to produce packaged virus-like transduction particles

Origin & function
Parental organisms for inserts: Oncogenes or potential oncogenes in the form of human and viral cDNA or fully synthetic DNA.
Full length, truncated and/or mutated forms of human or viral genes whose proteins have oncogenic or potentially oncogenic properties:
Human cDNA genes encoding proteins or synthetic codon-optimised equivalents of these genes based on their amino acid sequences.
cDNA or synthetic sequence-modified oncogenes derived from the sequences of mammalian viral genes.
NB: No actual virus will be handled at any stage, only DNA based on viral DNA sequences.
Further detail is provided in Section 17

**Evaluation of foreseeable effects**

The most hazardous GM vector/insert combinations in this assessment are bacterial plasmids that express known oncogenic proteins.
Plasmids carrying oncogenes do not present a hazard in themselves as they lack the mechanism to enter cells without special techniques.
The most hazardous GMOs in this assessment are human primary cells that have been immortalised by the expression of oncogenic proteins.
Oncogenes on plasmids will only very infrequently be integrated into the DNA of the transfected cells. Cells transformed with plasmids carrying an oncogene could in principle cause cancer:
If the oncogenes are integrated into DNA of primary cells such cells could potentially cause cancer on accidental introduction into the bloodstream if not recognised by a worker's own immune system (e.g. own cells; defective immune system; cells manipulated to evade the immune system)
As long as workers only work with non-self cells, these transformed cell lines will be efficiently recognised and destroyed by the worker's immune system if their immune system is healthy. It is for this reason that no one is allowed to transfet their own cells or to use cells that are engineered to evade immune surveillance / destruction.
If plasmids carrying oncogenes are integrated into the DNA of established cell lines they would not present more risk than the immortalised cell line in itself for workers with a healthy immune system.
It is recognised that workers with a (temporarily) ineffective immune system will be at increased risk of developing cancer if the cell lines carrying them were inadvertently introduced into their bloodstream.
Such people include but are not limited to HIV carriers; diabetics; those on steroids; those with latent other infections etc.
Since it is impossible to know at any moment whether a worker's immune system is functioning adequately, we will ensure we build in the controls of strict prohibition of handling sharps when working with these oncogenic GMs / GMOs and any skin abrasions are adequately covered.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We do not require the measure "Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs" as there are no disease vectors which could disseminate these GMOs.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Non-human tissue solid waste and some liquid waste generated during this procedure will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of...
chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste. The autoclaved solid waste will be disposed of via incineration through an appropriately licenced hazardous waste company. Autoclaved liquid waste will be disposed of through a sink to public sewer.

HTA-defined human tissue solid and contained liquid waste is disposed of directly (without prior disinfection) into dedicated bins which are sealed once full and wiped on the outside with 70% ethanol before disposal as Human Tissue Waste for special incineration through an appropriately licenced waste company as above. Any remaining liquid waste will be treated with 1% Virkon (final wt/vol) or 5% ChemGene (final wt/vol) for both of which independent validated efficacy against human tissue exists. The waste cycle on the autoclave is fully validated and temperature-tested every 12 months and the efficacy of steam penetration is tested using Thermalog strips every 3 months.

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Animal Units

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Project Ref 3242/17.8

Date Ackn'd | CU2 Project Title | Class | CultureVolClass2 | CultureVolumeClass3-4 |
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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

The Genetic Modification Committee discussed and commented on the proposed work, and after consultation with the Scientists that will be undertaking the labortory work, the project was approved on 7 September 2017 and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent Class 2 activity.
### Project Additional Information

#### Purposes of the contained use

Commercially available KILR retroviral vector particles (DiscoverX) expressing a beta-galactosidase/human "housekeeping" gene reporter fusion protein will be transduced into human primary cells and human cancer cell lines so that these cells can be used as targets in improved T cell cytotoxicity assays.

#### Recipient or parental organism

- Human primary cell lines - ACDP Hazard Group 2
- Human immortalised cell lines - ACDP Hazard Groups 1-2

#### Host/vector system

- Moloney Murine leukemia virus (MoMLV) - with wildtype L TRs (not Self Inactivating SIN L TRs)
- pMoMLV backbone with wildtype 5' and 3' L TRs, G418 antibiotic resistance (expression driven by 5' retroviral L TR promoter), KILR reporter gene insert expression driven by CMV promoter.

#### Origin & function

- Non-pathogenic laboratory E.coli strain (beta galactosidase gene) - Non-hazardous
- Normal human amino acid sequence for a "housekeeping" gene - Non-hazardous

(NB: two returns were entered in the website link to fit it onto the page).
Evaluation of foreseeable effects

The most hazardous GM constructs are the retroviral vector particles. Although replication-defective these expression vectors stably integrate into the DNA of human cells in a near-random fashion and hence this might possibly cause oncogenic insertional transformation of primary cells.

A more serious concern is that these retroviral particles contain wildtype 5' and 3' LTR as direct repeat sequences which function both as promoters and as enhancers (indeed the vector G418 drug resistance selection gene is expressed from the functional 5' LTR). These promoters/enhancers could increase the expression of host genes that are adjacent to the sites of random insertion of the vector; again with potential oncogenic effect.

The retroviral vector particles are pseudo-typed with the VSV-G envelope protein significantly broadening the host range of cell lines that can be transduced to include any human cell or cell lines. However, once inside the cell these particles cannot replicate or generate progeny that can further transduce cells.

The most hazardous vector/insert/host combinations are cell lines transduced with KILR-retroviral vector particles but these present significantly less risk than the viral vector particles themselves. These cells are not able to produce more vector particles.

Effects on human health:

The 'gene expressed in human cells is a non-hazardous beta-galactosidase/ host protein fusion gene reporter, which is unlikely to elicit any deleterious immunological or allergic responses.

Since the LTRs are not attenuated or disabled they may act as a promoter and/or enhancers to express genes adjacent to the insertion, which may cause cell immortalisation. Therefore, using these KILR retroviral vector particles carries a slightly higher risk of initiating tumours than corresponding LTR self-inactivating (SIN) viral vector particles if they were to be accidentally introduced into a worker. This increased risk is mitigated with enhanced controls.

If transduced "self" cells were inadvertently reintroduced into the worker, the immune system would be unable to recognise and eliminate the cells. However, the recipient cell lines are always from another individual or are commercially available cancer cell lines both of which will present non-self HLA allotypes.

These non-self transduced GM cells, if accidentally injected into the worker, would at most cause a temporary and limited local immune reaction in those with a healthy immune system.

Neither the expressed reporter gene nor the cancer cells themselves are expected to adversely affect human health. However, in the unlikely event that insertional mutagenesis or insertion adjacent to an oncogene upregulated by the retroviral LTRs creates a tumour cell, those whose immune response is less acute (e.g. those with latent infections, diabetics, people on steroid medication or pregnant-breastfeeding women) which does not recognise non-self cells sufficiently, could potentially contract cancer in a worst-case scenario.

Since we cannot effectively exclude such people from coming in contact with the GMOs we are putting robust controls in place to minimise the likelihood of this happening.

8.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We do not require the measure "Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs" as there are no disease vectors which could disseminate these GMOs.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Non-human tissue solid waste and some liquid waste generated during this procedure will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. The waste cycle on the autoclave is fully validated and temperature-tested every 12 months and the efficacy of steam penetration is tested using Thermalog strips every 3 months.

Autoclaved solid waste will be treated as Hazardous Waste. The autoclaved solid waste will be disposed of via incineration through an appropriately licenced hazardous waste company. HTA-defined human tissue solid and contained liquid waste is disposed of directly (without prior disinfection) into dedicated bins which are sealed once full and wiped on the outside with 70% ethanol before disposal as Human Tissue Waste for special incineration through an appropriately licenced waste company as above.

Any remaining liquid waste will be treated for a minimum contact time of 1 hour (preferably overnight) with 1% Virkon (final wt/vol) or 5% ChemGene (final wt/vol). For both chemicals proven independent validated efficacy exists against human tissue.

Autoclaved or chemically treated liquid waste will be disposed of through a sink to public sewer.

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Committee discussed and commented on the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved on 7 September 2017 and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent Class 2 activity.

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Project Ref 3242/17.9
GMO RA 256 Gene disruption in human cells using packaged Adeno-Associated Viral (AAV) vectors

Date Project Ceased: 14/12/2017

Consent Granted: Non-GMM

Class 2: < 1 Litre

Project notified under transitional arrangements: N

Historical Significant Changes:

Historical Date of Additional Info:

Withdrawn: N

Tick if notifying a connected programme of work: N

Project Additional Information

Purposes of the contained use:

Individual genes will be permanently disrupted by transducing human cells with packaged Adeno-Associated Viral (AAV) vectors. These single-stranded DNA vectors will be constructed as shuttle plasmids in E. coli then packaged as infectious particles in modified HEK293 cells. The resultant non-replicating and integration-deficient viral vector particles will then be used to transduce human cell lines and stem cells to cause gene disruption. The gene-disruption AAV vectors covered by this GMO RA differ from the wildtype AAV virus and some MV-derived gene expression vectors as they do not use the AAV-encoded viral integration pathway. Instead these MV-derived vectors carry regions of DNA that are homologous to the gene chosen for disruption. They also contain a promoter and drug selection gene combination that is inserted between the two regions of DNA homology. Drug selection can then be used on vector-transduced cells to select for rare DNA copying events where the AAV vector DNA replaces the matching host DNA. Disabling modifications in the vector DNA homology regions can be used to permanently disrupt the target host gene. If required, the promoter/drug selection gene that is used can be precisely removed at a later date. The removal of the drug selection combination is achieved by the transient introduction of a Cre recombinase gene expression plasmid. Expressed Cre recombinase protein precisely deletes the promoter/section DNA by catalysing an exact homologous recombination event between two sequence-defined short tandem DNA repeat LoxP motifs that are included in the design of the AAV gene disruption vector.

Recipient or parental organism:

Escherichia coli - Disabled laboratory K12 strains of E. coli for standard gene cloning and shuttle plasmid vector preparation. ACGM Class 1.

AAV-293 cells - Human HEK293-derived cell line that stably expresses the Adenoviral E1 gene. ACGM Class 2. This will be used to package AAV vectors.

AAV-HT1080 cells - Human cell line which stably expresses the Adenoviral E1 gene. ACGM Class 2. This will be used for titration of AAV vectors.
Our gene editing vectors are based on the Agilent AAV Helper-Free System which eliminates the requirement for viral co-infection during both the AAV vector production and AAV stock titration steps, thereby making the system safer. Nevertheless, the Agilent System still requires co-transfection of the vector plasmid with a helper plasmid that expresses a limited number of the viral genes that are essential for viral vector packaging. The AAV Helper-Free System (Agilent Technologies, https://www.genomics.agilent.com/enNiral-MediatedDelivery/AAV-Helper-Free-System?cid=AG-PT-183&tabid=AG-PR-1006) will be adapted for use in our studies. It allows the production of packaged infectious recombinant human Adeno-associated virus-2 (AAV-2) vector virions without the use of a helper virus.

The AAV Helper-Free System takes advantage of the known Adenovirus gene products that mediate the replication and assembly of the AAV virus and the demonstration that these gene products can be introduced into the host packaging cell line by a combination of the stable transfection of the packaging cell line and co-transfection with a helper plasmid. In the AAV Helper-Free System, most of the Adenovirus gene products required for the packaging of infective (but non-replicating) AA V vector particles are supplied on the plasmid pH-helper (which expresses the Adenoviral E2A and E4 proteins, and the Adenoviral VA RNA gene). This is co-transfected into cells alongside the plasmid DNA that encodes the human AAV-2 based viral vector. The remaining essential Adenoviral gene product is supplied by the AAV-293 host cells, a modified HEK293 human cell line that stably expresses the Adenoviral E1 gene.

The wild-type AAV-2 viral genome contains just two coding sequences rep and cap (encoding the replication and capsid genes, respectively). These are flanked by inverted terminal repeats (ITRs) that contain all the cis-acting elements necessary for viral replication, packaging and chromosomal integration. In the Agilent AAV Helper-Free System, the rep and cap genes have been removed from the viral vector and are supplied in trans on the plasmid pAAV-RC. The removal of the AAV rep and cap genes allows for insertion of up to 4.5Kb of DNA between the ITR sequences.

In our gene disruption protocols (see Khan et al., Nature Protocols 16, 482-501 2011) we will use 2 DNA regions of ~1.5Kb which are homologous to one or two exons of the gene targeted for disruption. These homology regions are separated by a promoter-drug selection gene together with a termination/polyadenylation Signal sequence. After drug selection for the relatively rare stable disruption of the target gene the drug selection moiety can be removed by the transient delivery of Cre recombinase (via plasmid-mediated transient transfection). Cre causes site-specific recombination between the LoxP sequences that flank the selection moiety. Recombination leaves just the disrupted target gene behind containing just a single copy of the LoxP sequence. In traditional viral delivery systems, regeneration of wild-type virus by recombination is a major concern. In our system, as with the parental Agilent AAV Helper-Free system, the AAV-2 ITR-containing gene disruption plasmids do not share any regions of homology with the rep and cap-gene expression plasmid (pAAV-RC), thereby preventing the production during packaging of viable AAV virus through homologous recombination.

Conventional AA V vector titration methods involve co-infection by wild-type Adenovirus and the AA V vector stock. The Agilent AAV Helper-Free system uses a virus-free AAV vector titration method, which removes the requirement for Adenoviral co-infection.

AAV-2-derived vectors have proven to be especially valuable for gene-editing due to the high vector titre achievable (enabling a very high multiplicity of infection for efficient gene disruption). The gene-disruption AAV vectors covered by this GMO RA differ from the wildtype AAV virus and some MV-derived gene expression vectors as they do not use the AAV-encoded viral integration pathway. Instead these MV-derived vectors carry regions of DNA that are homologous to the gene chosen for disruption. They also contain a promoter and drug selection gene combination that is inserted between the two regions of DNA homology. Drug selection can then be used on vector-transduced cells to select for rare DNA copying events where the AAV vector DNA replaces
the matching host DNA. Disabling modifications in the vector DNA homology regions can be used to permanently disrupt the target host gene.

Vectors used:
1. Modified pAAV-MCS plasmids (based on the pAAV-MCS plasmid supplied by Agilent); 2. pCMV-MCS plasmid (containing the same expression cassette as pAAV-MCS but without ITRs); 3. pAAV-LacZ plasmid (contains the AAV backbone -including ITRs but excluding rep and cap- with a gene expression cassette for lacZ); 4. pAAV-hrGFP plasmid (as 3 but with cassette for hrGFP); 5. pAAV-RC plasmid (encoding AAV genes rep and cap); 6. pH helper plasmid (containing the Adenoviral helper genes E2A, E4 and VA); 7. pCMV-Cre (System BioSciences).

Plasmids 5 and 6 will be co-transfected into AAV-293 cells with the engineered AAV vector gene homology plasmid (modified Plasmid 1) or with Plasmid 3 or 4 for the production of AAV homology vectors alongside their respective LacZ and/or hrGFP AAV vector controls.

All AAV vector, helper plasmid, vector control and the Cre recombinase plasmid are shuttle plasmids that are nonmobilisable in E.coli (all derived from pUC plasmids).

These plasmids all carry a bacterially-expressed beta-lactamase penicillin-resistance genes (as is standard in many laboratory E.coli plasmids).

Gene inserts are promoter/drug resistance gene combinations flanked by LoxP recombination sites and then flanked on either side by approximately 1.5kb of human genomic DNA. Each -1 .5kb sequence is matched with an exon (or two exons) of the human gene that is targeted for disruption.

For safety reasons tumour suppressor genes will not be targeted for disruption using this technology.

Origin & function

GM constructs:
The most hazardous GM constructs are the packaged AAV viral vector particles. This is because they could potentially infect both non-dividing and proliferating human cells including those of laboratory workers (e.g., through needlestick injury).

However, these AAV vector constructs are not replicating viruses. They do not express viral proteins and cannot easily integrate into host data via their ITR sequences. Recombination by homology-driven targeting of human genes is very inefficient. Non-homologous targeting is even less efficient. Hence, although the transient extrachromosomal expression of the AAV vector and its drug resistance gene is likely, stable exogenous gene expression is unlikely. The packaged AAV virion envelope proteins are encoded by the packaging system and not by the AAV vector so cannot be expressed and, in any case, they are poorly immunogenic in man (this is supported by the safe use of AAV gene expression vectors in human gene-modified cell therapy).

There are three types of GMOs created:
*1. E. coli containing the AAV transgene plasmid - these GMOs are not more hazardous than the disabled E. coli itself.
*2. HEK293T containing AAV transgene and packaging plasmids. This cell creates packaged viral vector (see above). The hazard of these GMOs is equal to that of the packaged virions they produce.
03. Human cell lines / stem cells / lab worker's own cells containing packaged AAV viral vector.

If tumour suppressor genes would be knocked out in lab workers' own cells this could theoretically result in cancer. For this reason we will strictly prohibit the use of AAV virions to knock out tumour suppressor genes. Otherwise, the cells will not gain additional hazards to either humans or the environment from being transduced with these virions.

It is theoretically possible to generate a fully immuno-privileged cell line by a combination of several gene
disruptions and Class1 b MHC gene expression. The generation of an immuno-privileged cell line is therefore prohibited unless the cell line already contains a functional and validated cell fate "suicide" gene. The AAV virus does not cause disease or a significant immune response in humans. The AAV-based gene disruption vectors used in this study are expected to be further disabled. We will not disrupt any tumour suppressor genes. No categories of human individuals are thought to be at increased risk. This is also true of humans latently infected with either Adenovirus or Herpes virus. This is because our AAV-based vectors do not carry the rep and cap genes that required for both AAV viral replication and viral packaging and for ITR-driven chromosomal insertion. None of the drug resistance genes used provide resistance to antibiotics that are currently clinically-relevant and none of the proteins expressed from these drug resistance genes are known to be especially toxic or abnormally allergenic.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

1) Containment Level 2 is not required for the GM Class 1 work producing non-expression shuttle plasmids in E. coli.
2) We do not require the measure "Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs" as there are no such disease vectors which could disseminate these GMOs.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Non-human tissue solid waste and some liquid waste generated during this procedure will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Chemical Hazardous Waste. The autoclaved solid waste will be disposed of via incineration through an appropriately licenced hazardous waste company. Autoclaved liquid waste will be disposed of through a sink to public sewer.

HTA-defined human tissue solid and contained liquid waste is disposed of directly (without prior disinfection) into dedicated bins which are sealed once full and wiped on the outside with 70% ethanol before disposal as Human Tissue Waste for special incideration through an appropriately licenced waste company as above. Any remaining liquid waste will be treated with 1 % Virkon (final w/w) or 5% ChemGene (final w/w) for both of which independent validated efficacy against human tissue exists.

The waste cycle on the autoclave is fully validated and temperature-tested every 12 months and the efficacy of steam penetration is tested using Thermalog strips every 3 months.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y
The Genetic Modification Committee discussed and commented on the proposed work, and after consultation with the Scientists who will be undertaking the laboratory work, the project was approved on 29 November 2017 and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent Class 2 activity.

### Project Containment

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  - L3
  - L4

- **Large Scale Activities**
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- **Human Clinical Applications**
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  - L3
  - L4

### Project Ref 3242/18.1

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- **Non-GMM**
  - Not Applicable

- **Consent Granted**
  - Not Applicable

- **Project notified under transitional arrangements**
  - N

### Project Additional Information

**Purposes of the contained use**

- The objective of these projects is to use gene editing techniques to edit the host cell genome.

- The genome editing can be used to correct or introduce specific mutations in target genes or the targeted “knock-in” of desired transgene sequences and associated
regulatory elements. In addition, this approach can also be adopted to knock out specific gene expression by the introduction of stop codons into the reading frame of the target gene and the expression of the transgene from the endogenous promoter.

Furthermore, the approach can be used to completely knock out the function of a target gene by the incorporation of a selectable marker into the reading frame of the targeted sequence.

Cells which undergo the genome editing will be used for various purposes such as:
1. The incorporation of fluorescent proteins into the coding sequence of host cell genes that are only expressed in certain cell lineages. This will allow the real-time imaging and monitoring of cell differentiation from pluripotent stem cells.
2. The expression of a single exogenous human T cell receptor (TCR) α and/or β-chain sequence from a specific locus.
3. The knock-in of specific genes e.g. HLA alleles or genes encoding host proteins that we wish to confirm are immunogenic in cells that normally lack the expression of these proteins.
4. The addition of epitope tags to the coding sequence of host cell proteins e.g., to enable antibody-mediated purification or detection by FACS.
5. The mutation of host cell proteins in order to modify function e.g., mutation of an amino acid that is post translationally modified.

**Recipient or parental organism**

Recipient organisms:
- Escherichia coli - Disabled laboratory K12 strains of E.coli for standard gene cloning and shuttle plasmid vector preparation. ACDP Hazard Group 1
- AAV-293 cells - Human HEK293-derived cell line that stably expresses the Adenoviral E1 gene. ACGM Class 2. This will be used to package AAV vector
- AAV-HT1080 cells - Human cell line which stably expresses the Adenoviral E1 gene. ACGM Class 2. This will be used for titration of AAV vectors
- Human primary cells ((ACDP HG2 - potential but not confirmed pathogen presence); established human cell lines; human HEK293T cell line and its derivatives - Human HEK293-derived cell line that stably expresses the Adenoviral E1 gene. ACGM Class 2. This will be used to package AAV vector.

**Host/vector system**

Adeno-associated viruses (AAVs) are naturally replication-deficient small ssDNA genome human parvoviruses (aka Dependoparvoviruses, ACDP HG2). AAV viruses infect the cells of humans and some other primate species. Natural AAV replication requires co-infection with a helper Adenovirus or Herpes virus. AAV by itself does not cause any disease in humans and only elicits a very mild immune response. Recombinant AAV vectors can infect both dividing and quiescent cells and persist for many cell divisions in an extrachromosomal state without integrating into the genome of the host cell. The native virus and some AAV-derived viral vectors can undergo ITR-dependent integration into a single genetic on the long arm of chromosome 19 (19q13-qter), termed the AAVS. The AAV vectors that we will use are unable to perform ITR-dependent integration and are further disabled by the removal of both the replication (rep) and structural capsid (cap).

Our AAV genome editing vectors are designed to contain regions of DNA that are homologous to the sequence of the target locus to be edited with the transgene insertion. During cell division and DNA replication this homology permits the site-specific incorporation of extrachromosomal recombinant AAV vector ssDNA at a low frequency. These relatively rare homology-driven gene editing events require drug selection for the identification and isolation of resultant gene disrupted cell lines. If required, the drug selection gene can be removed afterwards using a plasmid that expresses a site-specific recombinase such as Cre or FLP.

Our gene editing vectors are based on the AAV Helper-Free System which eliminates the requirement for viral co-infection during both the AAV vector production and AAV stock titration steps, thereby making the system safer. The AAV Helper-Free System requires co-transfection of the vector plasmid with a helper plasmid that expresses a limited number of the viral genes that are essential for viral vector packaging.

rAAV vectors for transgene expression incorporating the AAV Internal Tandem Repeat sequences (ITRs) at the extreme 5’ and 3’ ends.
The packaging plasmids encode REP CAP proteins and adenoviral accessory proteins. These plasmids may incorporate:

- fluorescent proteins and reporter genes;
- T-cell receptor proteins;
- HLA proteins;
- Suicide genes;
- Adenoviral accessory proteins;
- REP CAP proteins

### Origin & function

**Originating organisms of inserts:**

- **Human** - (Synthetic codon-optimised sequences from gDNA and mRNA) - ACDP HG1
- Various non-pathogenic species (e.g. jellyfish Aequorea Victoria, tomato, plum, Discosoma sp.) - Reporter sequences - ACDP HG1
- Picornavirus-derived sequences – e.g. Poliovirus; Rhinovirus; Encephalomyocarditis virus (IRES), e.g. Foot and Mouth virus, Porcine Teschovirus; Equine Rhinitis A virus; Thosea asigna virus (2A-like skip sequence peptides). - ACDP HG3 / SAPO Group 3-4 (but all sequences used are non-pathogenic synthetic sequences)
- Simian virus 40; Cytomegalovirus (gene expression promoter for drug resistance; polyA sequences) - ACDP HG2 (synthetic non-pathogenic sequences)
- Streptomyces alboniger; Escherichia coli; Aspergillus terreus); Streptocolloteichus hindustanus; Klebsiella pneumoniae (synthetic antibiotic resistance genes) - ACDP HG1-2 (synthetic non-pathogenic sequences)
- Herpes simplex virus Type 1– (synthetic thymidine kinase) - ACDP HG2 (synthetic non-pathogenic sequences)
- Cow, rabbit – (synthetic polyA sequences) - ACDP HG1 (synthetic non-pathogenic sequences)
- E. coli bacteriophage P – (synthetic Cre recombinase gene) - ACDP HG1 (synthetic non-pathogenic sequences)
- Saccharomyces cerevisiae – (synthetic FLP recombinase) - ACDP HG1 (synthetic non-pathogenic sequences)

The AAV vectors will target transgene expression to a defined loci. The AAV vector will contain the AAV ITR sequence, homology arms transgene and selectable marker. Several loci may be targeted for transgene expression. For example:

1. Loci that express genes with high constitutive expression e.g genes with house-keeping function
2. Knock in of the transgene with exogenous promoters into genomic safe harbour sites e.g AAVS1
3. Insertion of transgenes into loci associated with a defined cell lineage so that lineage differentiation from stem cells can be followed
4. Insertion of transgene sequences into human cell lines which may be used for the production and packaging of lentiviral vector particles.
5. Knock in of a specific HLA allele or another gene encoding a human protein/variant

Intentional insertion of oncogenes or targeting of known tumour-suppressors will not be permitted.
The most hazardous GM constructs are the packaged AAV viral vector particles. This is because they could potentially infect both non-dividing and proliferating human cells including those of laboratory workers (e.g., through needle stick injury).

However, these AAV vectors are not replicating viruses. They do not express viral proteins and cannot easily integrate into host data via their ITR sequences. Recombination by homology-driven targeting of human genes is very inefficient. Non-homologous targeting is even less efficient. Hence, although the transient extrachromosomal expression of the AAV vector and its drug resistance gene is likely, stable exogenous gene expression is unlikely. The packaged AAV virion envelope proteins are encoded by the packaging system and not by the AAV vector so cannot be expressed and, in any case, they are poorly immunogenic in man (this is supported by the safe use of AAV gene expression vectors in human gene-modified cell therapy).

Other gene constructs disrupting anti-apoptotic genes could, if inadvertently entered into the body, cause local cell death. To minimise the likelihood of this occurring such constructs will not be used in any AAV vectors. Instead these gene disruptions will be limited to non-viral vectors that require additional cell transfection reagents and protocols e.g. electroporation of ribonucleoprotein complexes.

There are four types of GMOs created:

- E. coli containing the AAV transgene plasmids or lentiviral vector expressing sequence specific nuclease – these GMOs are not more hazardous than the disabled E. coli itself.
- HEK293T containing AAV transgene and packaging plasmids. This cell creates packaged viral vector (see above). The hazard of these GMOs is equal to that of the packaged virions they produce.
- HEK293T containing the lentiviral vector and associated packaging plasmids. This cell creates packaged viral vector (see above). The hazard of these GMOs is equal to that of the packaged virions they produce.
- Human cell lines / stem cells / lab worker’s own cells containing packaged AAV viral vector.

- If tumour suppressor genes would be knocked out in lab workers’ own cells this could theoretically result in cancer. For this reason we will strictly prohibit the use of AAV virions to promote the integration of the transgene at loci with known tumour suppressor function, nor will we permit the expression of functional known human oncogenes (e.g. KrasV12) from the transgene. Otherwise, the cells will not gain additional hazards to either humans or the environment from being transduced with these virions. In addition, we strictly prohibit the use of this technology to promote the expression of pathogenic proteins (e.g. bacterial toxins) that may be harmful to human health.
- It is theoretically possible to generate a fully immuno-privileged cell line by a combination of several gene disruptions and lack of Class1b MHC gene expression. The generation of an immuno-privileged cell line is therefore prohibited unless the cell line already contains a functional and validated cell fate “suicide” gene.
- Cells whose genome has been edited should be no more hazardous than the equivalent non-transduced cells.

The AAV virus does not cause disease or a significant immune response in humans. The AAV-based gene disruption vectors used in this study are expected to be further disabled. We will not target any tumour suppressor genes for transgene insertion nor will we express oncogenic proteins in the transgene. In addition, we strictly prohibit the use of this technology to promote the expression of pathogenic proteins (e.g. bacterial toxins) that may be harmful to human health.

Constructs disrupting anti-apoptotic genes could, if inadvertently entered into the body, cause local cell death. Given the nature of the project the risk of any disruption is very low. In addition suicide systems will be incorporated.

No categories of human individuals are thought to be at increased risk. This is also true of humans latently infected with either Adenovirus or Herpes virus. This is because our AAV-based vectors do not carry the rep and cap genes that required for both AAV viral replication and viral packaging and for ITR-driven chromosomal insertion. None of the drug resistance genes used provide resistance to antibiotics that are currently clinically-relevant and none of the proteins expressed from these drug resistance genes are known to be especially toxic or abnormally allergenic.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

1) Containment Level 2 is not required for the GM Class 1 work producing non-expression shuttle plasmids in E. coli.

2) We do not require the measure “Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs” as there are no disease vectors which could disseminate these GMOs.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Most liquid waste is inactivated in freshly prepared 1% Virkon or 5% Chemgene (both final concentration wt/vol) for at least one hour contact time before being poured down the foul drain.

At CL1, solid waste is disinfected where practicable and disposed of as hazardous waste for off-site incineration.

At CL2, non-human tissue solid waste will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. The autoclaved solid waste will be disposed of as Hazardous Waste via incineration through an appointed specialist waste management company.

HTA-defined human tissue solid and contained liquid waste is disposed of directly (with or without prior disinfection) into dedicated bins which are wiped on the outside with 70% ethanol before disposal as Human Tissue Waste for special incineration through an appointed specialist waste management company.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Committee discussed the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the project was approved and the Biological Safety Officer was actioned to complete the HSE Notification for a subsequent class 2 activity.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
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Animal Units  
Large Scale Activities  
Human Clinical Applications
# Project Additional Information

## Purposes of the contained use

The purpose of this project is to produce virus-based gene expression vectors that will be used for transducing primary human cells or cell lines of immune effector cell types such as lymphocytes, macrophages, dendritic cells or natural killer cells for research and development purposes. The vectors will encode Immuno-modulatory proteins/complex or pools/libraries of Immuno-modulatory proteins/complexes. Immuno-modulatory proteins/complexes are members of the immunoglobulin superfamily (IgSF) which control innate and adaptive immunity, and are possible candidates for use in cell-based treatment of malignancies, autoimmune diseases and infectious diseases.

## Recipient or parental organism

- **Bacteria, ACDP Hazard Group 1:** Laboratory strains of diasabled Escherichia coli K12. For shuttle plasmid preparation, not for gene expression.
- **Human cell lines, ACDP Hazard Group 2, ACGM Class 2:** e.g., HEK293T host cell line for viral vector packaging, Jurkat cell line as vector target cells to evaluate Immuno-modulatory proteins/complexes.
- **Viral vector, ACGM Class 2:** Packaged viral vector particles.
- **Human primary cells, ACDP Hazard Group 2:** Immune effector cell types as vector target cells to evaluate Immunomodulatory proteins/complexes.
Packaged replication-defective, self-inactivating lentiviral vector particles:
Third Generation lentiviral vector packaging systems (without the HIV-1 Tat gene) made in-house or commercially acquired: Consisting of 3 or more shuttle plasmids that express viral packaging proteins plus a transgene vector expression plasmid. The transgene vector is transcribed into RNA in the packaging host cell line. Packaged vector RNA is converted to DNA in the transduced target cell, then integrated into the target cell genome to permit stable transgene expression.

Origin & function

Origins:
Human immuno-modulatory genes (cDNA and synthetic)
Human primary T cell lymphocytes and cell lines (ACDP HG2)
Human primary Natural Killer cells and cell lines (ACDP HG2)
Human primary Macrophages and cell lines (ACDP HG2)
Human primary Dendritic Cells and cell lines (ACDP HG2)
Human HEK293 and HEK293T cell lines and their derivatives (ACDP HG2)
Lentiviral vector host cell lines, containing adenoviral E1 genes. HEK293T cell line also contains the SV40 Large T-antigen.

Intended functions:
The above cell lines will be transduced with lentiviral vectors so that they then stably express immuno-modulatory proteins as possible candidates for use in cell-based treatment of malignancies, autoimmune diseases and infectious diseases.
The GMM produced (vectors and cells) will be used for in vitro studies performed in Containment level 2 laboratories and as such have been assessed as ACGM Class 2

Evaluation of foreseeable effects

The most hazardous foreseeable effect is transduction of the laboratory workers own "self" cells with lentiviral vector particles.
The consequences of this are potentially significant but are limited to the cells transduced as the vector is not capable of producing viable progeny vector.
The cell-based consequences of the transduction of self cells are limited to the lifespan of the primary cells transduced. None of the immuno-modulatory genes are associated with onogenic transformation. In addition, the immunomodulatory genes to be studied are only functional in a limited number of cell types.
In respect of both primary cells and cell lines there are a number of immune compatibility barriers such as MHC. For this reason workers are not allowed to use self cells.
Furthermore the use of sharps and needles is prohibited.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

1. Containment Level 2 is not required for the E.coli GM Class 1 work where shuttle plasmids are produced which cannot express the immuno-modulatory genes being studied in human cells.
2. We do not require the measure "Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs" as no disease vectors capable of dissemination will be in use.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste and some liquid waste generated during this procedure will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste. The autoclaved solid waste will be disposed of via incineration through an appropriately licenced hazardous waste company. Autoclaved liquid waste will be disposed of through a sink to public sewer.

The waste cycle on the autoclave is fully validated and temperature-tested every 12 months and the efficacy of steam penetration is tested using Thermalog strips every 3 months

Any remaining liquid waste will be treated with 1% Virkon (final w/vol) or 5% ChemGene (final w/vol) for both of which independent validated efficacy against human tissue exists.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Please enter comments on the GM safety committee on the risk assessment

The Genetic Modification Committee discussed and commented on the proposed work, and after consultation with the Scientists that will be undertaking the laboratory work, the risk assessment was approved by the Committee on 7 September 2017

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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Project Ref 3242/19.2

Date Ackn’d 02/03/2022  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4
The purpose of this project is to produce or purchase non-viral gene expression vectors that will be used for transfecting either primary or immortalised mammalian cell lines with oncogenic, potentially-oncogenic and/or mitogenic mammalian and viral genes. In some circumstances gene expression will be controlled not by the direct expression of ORF sequences but by other means including siRNA. Potentially-oncogenic and/or mitogenic proteins and the inhibition of the function of tumour suppressor genes will be restricted to non-viral vector systems that, for safety reasons, rely on nucleic acid-based mammalian cell transfection. These cells / cell lines will be used, amongst other purposes, as systems to elicit and/or measure responses from immunologically-active cells (e.g., T cell responses to target cell-produced cancer antigens).

Recipient or parental organism

Bacteria, ACDP Hazard Group 1: Laboratory strains of disabled Escherichia coli K12. For shuttle plasmid preparation, not for bacterial gene expression.

Mammalian cell lines, ACDP Hazard Group 2, ACGM Class 2 (e.g., human lines including HEK293T, Jurkat, A375, SupT1, HT1080 and animal cell lines such as COS-7, CHO-K1 and BHK21 and their derivatives).

Transfected mammalian primary cells, ACDP Hazard Group 2, ACGM Class 2 (e.g., Human primary T cells, NK cells, NKT cells, follicular dendritic cells, macrophages, hepatocytes, cardiac myocytes).

Host/vector system

All gene expression plasmids will be non-viral, non-mobilising and non-mobilisable in E.coli. The only exception being mobilisable plasmids based on the pBR322 origin of replication which will be restricted to E.coli strains that lack the mobilising function of F factor (as either free or integrated F plasmid).

Gene expression shuttle plasmids requiring transfection include transient and/or stable gene expression plasmid vectors based on the SV40 virus T antigen coupled with the SV40 origin or, alternatively, vectors based on the EBV viral EBNA-1 gene coupled with the EBV OriP origin of replication.
The non-viral vector transfection of mammalian cells with potential oncogenes can be transient or stable. Stable transfection can be via random integration or by specific gene targeting. Targeting can be assisted by co-transfection of a gene editing gene knock-in system. The knock-in template oncogene must not be supplied via a viral vector. Transposon-based gene expression systems.

**Origin & function**

**Origins:**
- Health and Safety
- Executive

**Potentially-oncogenic and/or mitogenic genes including known or suspected mammalian or viral oncogenes and natural or synthetic genes that decrease or inactivate tumour suppressor genes or proteins (cDNA or synthetic origin). These genes can be expressed as proteins with antigenic epitope and/or purification tags (e.g., Biotin, His-tag, HA-tag, Myc-tag, FLAG, GST, MAP, CAT, V5) or co-expressed with reporter genes (e.g., GFP and other fluorescent protein genes from marine invertebrates, luciferase from insect species and beta-galactosidase genes from bacteria), gene expression control protein genes (e.g., Tet-On or Tet-Off repressor proteins from bacteria), RNA-silencing suppressor genes (plant virus and mammalian viral origin) and/or antibiotic resistance genes (e.g., G418/Kanamycin, Puromycin, Zeocin, Blasticidin and other antibiotic resistance genes of bacterial origin).

**Replication-defective, self-inactivating retroviral vectors derived from murine retroviruses (ACDP HG2)**

**Plasmid vectors based on the SV40 T antigen with the SV40 origin (ACDP HG2) or alternatively vectors based on the EBV viral EBNA-1 gene with the EBV OriP origin of replication (ACDP HG2).**

**Transposon-based gene expression systems adapted from eukaryotic cell transposons (ACDP HG2).**

**Mammalian primary cells and cell lines (ACDP HG2)**

**Intended functions:**

The above cells and cell lines will be transfected with non-viral vectors so that they then express potentially oncogenic/mitogenic proteins. These cells / cell lines will be used, amongst other purposes, as systems to elicit and/or measure responses from immunologically-active cells (e.g., T cell responses to target cell-produced cancer antigens). The GMM produced (plasmids, packaged viral vectors, transposon systems and mammalian cells) will be created using shuttle plasmids assembled and prepared in E.coli (ACGM Class 1). Plasmid DNA and transposon expression vector transfections of mammalian cells (ACGM Class 2) will performed in Containment level 2 laboratories as will all experiments using the resulting transfected mammalian cells.

**Evaluation of foreseeable effects**

The most hazardous foreseeable effect is the inadvertent (albeit extremely unlikely) transfection of the laboratory workers own primary "self" cells which might then result in their oncogenic transformation. It is for this reason that viral vectors are expressly disallowed.

The likelihood of serious consequences of such a transfection of "self" cells is limited as there are a number of immune compatibility "non-self" barriers to cell growth including MHC alloreactivity. To retain these barriers workers are not allowed to deliberately transfected their own "self" cells. Furthermore, the use of sharps and needles is prohibited.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

1. Containment Level 2 is not required for the E.coli GM Class 1 work on the assembly and production of DNA shuttle
plasmids as the transgenes are not expressed in bacteria.

2. We do not require the measure "Efficient control of disease vectors (e.g., for rodents and insects) which could disseminate GMMs" as no such disease vectors capable of dissemination will be in use.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste and some liquid waste generated during this procedure will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste. The autoclaved solid waste will be disposed of via incineration through an appropriately licenced hazardous waste company. Autoclaved liquid waste will be disposed of through a sink to public sewer.

The waste cycle on the autoclave is fully validated and temperature-tested every 12 months and the efficacy of steam penetration is tested using Thermalog strips every 3 months.

Any remaining liquid waste will be treated with 1 % Virkon (final w/vol) or 5% ChemGene (final w/vol) for both of which independent validated relevant decontamination efficacy exists.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The Biological Genetic Modification Safety Committee discussed and commented on the proposed work, and after consultation with the scientists that will be undertaking the laboratory work, the attached representative risk assessment GMO RA 270: Expressing surface target antigens (oncogenes and potential oncogenes) was approved by the Committee on 20th March 2019.

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<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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</tr>
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Project Ref 3242/19.3
The purpose of this project is to produce or purchase both non-viral and virus-based gene expression vectors that will be used for transfecting or transducing either primary or immortalised mammalian cell lines with various mammalian and viral genes. In some circumstances gene expression will be controlled not by the direct expression of ORF sequences but by other means including siRNA. Most importantly, the expression of potentially-oncogenic and/or mitogenic proteins and the inhibition of the function of tumour suppressor genes will be avoided.

Recipient or parental organism

Bacteria, ACDP Hazard Group 1: Laboratory strains of disabled Escherichia coli K12. For shuttle plasmid preparation, not for bacterial gene expression.

Mammalian cell lines, ACDP Hazard Group 2, ACGM Class 2: i.e., Host cell lines for viral vector packaging (e.g. human HEK293T) as well as vector-transfected/transduced recipient cell lines (e.g., human lines including HEK293T, Jurkat, A375, SupT1, HT1080 and animal cell lines such as COS-7, CHO-K1 and BHK21 and their derivatives)

Packaged viral vector particles ACDP Hazard Group 2, ACGM Class 2 (e.g., packaged AAV or lentiviralretroviral vectors)

Vector transfected/transduced mammalian primary cells, ACDP Hazard Group 2, ACGM Class 2 (e.g., Human primary T cells, NK cells, NKT cells, follicular dendritic cells, macrophages, hepatocytes, cardiac myocytes).

Host/vector system

All virus-based and non-viral gene expression plasmids will be non-mobilising and non-mobilisable in E.coli. The only exception being non-mobilisable plasmids based on the pBR332 origin of replication which will be restricted to E.coli strains that lack the mobilising function of F factor (as either free or integrated F plasmid).

Packaged replication-defective, self-inactivating retroviral and lentiviral transduction vector particles:
Murine retroviral and third generation lentiviral vector packaging systems (without the HIV-1 Tat gene, without WPRE sequences) made in-house or commercially acquired: Packaging of retroviral and lentiviral vectors with or without shuttle plasmids that express viral packaging proteins (including various alternative viral pseudotyping proteins) plus a transgene vector expression plasmid. The transgene vector is transcribed into genomic RNA in the packaging host cell line. Packaged vector gRNA is converted to DNA in the transduced target cell, then usually integrated (some vectors will be intentionally integration-deficient) into the target host cell genome to permit stable transgene expression. These virus-based vectors are incapable of self-replication, unable to produce infectious progeny and will use LTR insertion sequences that contain "self-inactivating" deletions that disable their LTR promoters. None of these vectors will use potentially-oncogenic WPRE sequences to boost gene expression.

Non self-replicating AAV gene expression transduction vectors (both episomal and stably-integrated).

Transfection of mammalian cells with non-oncogenic genes can also be transient or stable. Stable transfection can be via random integration or by specific gene targeting. Targeting can be assisted by co-transfection of a gene editing gene knock-in system. The non-oncogenic gene to be knocked in could be supplied on a viral (e.g., AAV) or non-viral (e.g., plasmid or transposon) template.

Non-packaged expression shuttle plasmids requiring transfection including vectors based on the SV40 virus T antigen with the SV40 origin or, alternatively, vectors based on the EBV viral EBNA-1 gene with the EBV OriP origin of replication.

Transposon-based gene expression systems.

**Origin & Function**

**Origins:**
- Non-oncogenic, non-mitogenic genes (cDNA and synthetic) including mammalian HLA-MHC class I (with or without beta 2m), HLA-MHC class II, reporter genes (e.g. GFP and other fluorescent protein genes from marine invertebrates, luciferase from insect species and beta-galactosidase genes from bacteria), gene expression control protein genes (e.g., Tet-On or Tet-Off repressor proteins from bacteria), RNA-silencing suppressor genes (plant virus and mammalian viral origin), antibiotic resistance genes (e.g., G418/Kanamycin, Puromycin, Zeocin, Blasticidin and other antibiotic resistance genes of bacterial origin), genes for the parent proteins of immunological epitopes, viral protein genes used for viral vector packaging (lentiviral vector packaging plasmids: Gag-Pol and Rev from HIV-1 plus pseudotyping envelope protein genes from VSV and other viruses. AAV vectors: Adenoviral and AAV genes) (ACDP HG1 and HG2).
- Expressed ORFs to optionally include antigenic epitope and/or purification tags (e.g., Biotin, His-tag, HA-tag, Myc-tag, FLAG, GST, MAP, CAT, V5).
- Plasmid vectors based on the SV40 T antigen with the SV40 origin (ACDP HG2) or alternatively vectors based on the EBV viral EBNA-1 gene with the EBV OriP origin of replication (ACDP HG2).
- Transposon-based gene expression systems adapted from eukaryotic cell transposons (ACDP HG2).
- Replication-defective, self-inactivating retroviral vectors derived from murine retroviruses (ACDP HG2).
- Replication-defective Adeno-associated viral (AAV) expression vectors (ACDP HG2).
- Mammalian primary cells and cell lines (ACDP HG2).
- Human HEK293 and HEK293T lentiviral and AAV viral vector packaging cell lines and their derivatives containing integrated adenoviral E1 genes. The HEK293T cell line also contains the SV40 Large T-antigen (ACDP HG2).

**Intended functions:**
- The above cells and cell lines will be transfected with non-viral vectors or transduced with viral vectors so that they then stably express non-oncogenic non-mitogenic proteins or so they express genes that suppress host genes not including host tumour suppressor genes.
The GMM produced (plasmids, packaged viral vectors, transposon systems and mammalian cells) will be created using shuttle plasmids assembled and prepared in E.coli (ACGM Class 1). Plasmid DNA and transposon expression vector transfections and the viral vector transduction of mammalian cells (ACGM Class 2) will performed in Containment level 2 laboratories as well all experiments using the resulting transfected/transduced mammalian cells.

**Evaluation of foreseeable effects**

The most hazardous foreseeable effect is the inadvertent transduction of the laboratory workers own primary "self" cells with viral vector particles that have broad cellular tropisms. The consequences are limited to the properties of and host reaction to these transduced "self" cells. GM viral vectors used are not capable of producing viable infectious self-replicating progeny and are designed not to recombine with endogenous viral sequence to produce viable progeny. Furthermore the consequences of the transduction of "self" cells are limited to the lifespan of the primary cells transduced as no oncogenic or mitogenic transformation will occur. Lastly, for both primary cells and cell lines there are a number of immune compatibility "non-self" barriers to cell growth including MHC alloreactivity. To retain these barriers workers are not allowed to transfet or transduce their own "self" cells. Furthermore, the use of sharps and needles is prohibited.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

1. Containment Level 2 is not required for the E.coli GM Class 1 work on the assembly and production of DNA shuttle plasmids as the transgenes are not expressed in bacteria.
2. We do not require the measure "Efficient control of disease vectors (e.g. for rodents and insects) which could disseminate GMMs" as no such disease vectors capable of dissemination will be in use.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste and some liquid waste generated during this procedure will be collected and sterilised by autoclaving at 121°C for 25 minutes for 100% kill. Thermal mapping of the autoclave cycles and the use of chemical indicator strips will be used as methods of validating the autoclave sterilisation cycles. Autoclaved solid waste will be treated as Hazardous Waste. The autoclaved solid waste will be disposed of via incineration through an appropriately licenced hazardous waste company. Autoclaved liquid waste will be disposed of through a sink to public sewer. The waste cycle on the autoclave is fully validated and temperature-tested every 12 months and the efficacy of steam penetration is tested using Thermalog strips every 3 months. Any remaining liquid waste will be treated with 1% Virkon (final wt/vol) or 5% ChemGene (final wt/vol) for both of which independent validated relevant decontamination efficacy exists.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

Y
The Biological Genetic Modification Safety Committee discussed and commented on the proposed work, and after consultation with the scientists that will be undertaking the laboratory work, the attached representative risk assessments GMO RA 269 - "Expressing non-oncogenic proteins under inducible promoters" and GMO RA 271 "Overexpression of recombinant proteins, protein domains and peptides in mammalian cells" were approved by the Committee on 20th March 2019.

### Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Name

PEPTCELL LIMITED

Name 2

Department

Campus Estate or Research Centre

(C/O SEEK GROUP)

Road Name

45 BEECH STREET

Building

CENTRAL POINT

District

Town

LONDON

County

GREATER LONDON

Postcode

EC2Y 8AD

Country

ENGLAND

Tel Number

0207 153 6570

Fax Number

0

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

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<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<td>Gene Therapy</td>
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02/03/2022
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [  ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [  ]

Please enter comments of the GM safety committee on the risk assessment
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**Name**

ANTIKOR BIOPHARMA LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

LABS46, STEVENAGE BIOSCIENCES CAT

**Road Name**

GUNNELS WOOD ROAD

**Building**

**District**

**Town**

STEVENAGE

**County**

HERTFORDSHIRE

**Postcode**

SG1 2FX

**Country**

ENGLAND

**Tel Number**

01438 906906

**Fax Number**

0

**E-mail**

**HSE Division**

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**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>SG1 2FX</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The director of research has over 20 years experience in GM research and has been a GM safety officer at Imperial College and a principle investigator carrying out GM activities before moving to commercial R&D

<table>
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<th>Animal Unit</th>
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- Bacteriology: Yes
- Parasitology: |
- Transgenic Birds: |
- Microbiology Research: Yes
- Virology: |
- Transgenic Animals: |
- Transgenic Fish: |
- Gene Therapy: |
All liquid waste containing Class I GMMs will be chemically inactivated using 1% Virkon for at least 30 minutes and washed down designated laboratory sinks with copious amounts of cold water. Virkon kills 99.9995% of GMMs in 10 minutes. This will be routinely tested using agar plate colony assays.

All solid waste containing Class I GMMs will be chemically treated as above (if it contains any liquids) and after liquid disposal, the solid material will be autoclaved (124°C for 15 minutes). This effectively kills 100% of the GMMs and will be tested annually as above. The autoclaved material will then be disposed of as laboratory waste by Grundon contractors.

For activities involving GMMs, describe the waste management measures which will apply to the activity

This risk assessment was reviewed by the committee and after minor modifications and suggestions, it was approved. This risk assessment was also approved by Imperial College when the project was carried out there, prior to being spun out into a commercial enterprise.
### Demuris Limited

**GM Centre Number:** 3245

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**Name:**

- DEMURIS LIMITED

**Name 2**

**Department**

- blank

**Campus Estate or Research Centre**

- THE BIOSPHERE

**Road Name**

- DRAYMANS WAY

**District**

- NEWCASTLE HELIX

**Town**

- NEWCASTLE UPON TYNE

**County**

- TYNE AND WEAR

**Postcode**

- NE4 5BX

**Country**

- ENGLAND

**Tel Number**

- 0191 580 6177

**Fax Number**

**E-mail**

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**HSE Division**

- blank

**Comments**

**Date at Which Additional Info Submitted**

- 02/03/2022
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<td>FACULTY OF MEDICAL SCIENCES</td>
<td>FRAMLINGTON PLACE</td>
<td>NEWCASTLE UPON TYNE</td>
<td>NE2 4HH</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The Committee includes the Chairman and Director of Drug Development, the CEO, Company Advisor - Microbiology, the Principal Scientist, and the Senior Bioscientists. Meeting of the committee will take place in December of each year.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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Bacteriology | Yes | Parasitology | | Transgenic | Birds | Microbiology | Research | Yes |
Yes

All bacterial and bio-contaminated waste will be managed and disposed of in accordance with Newcastle University procedures. This involves autoclaving (134°C for 30 minutes) in appropriate containers prior to incineration by the University - approved contractor.

(For information - Demuris Limited is located within Newcastle University)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment has been reviewed and approved by the Demuris GMSC
GM Centre Number: 3246

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**Name**

CAMBRIDGE EPIGENETIX LTD

**Campus Estate or Research Centre**

CHESTERFORD RESEARCH PARK

**Building**

TRINITY BUILDING (B400)

**Road Name**

LITTLE CHESTERFORD

**Town**

SAFFRON WALDEN

**County**

ESSEX

**Postcode**

CB10 1XL

**Country**

ENGLAND

**Tel Number**

01223804260

**Fax Number**

0

**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

R&D Manager is responsible for H&S and will act as the Biological Safety Officer. Long term user of GMO in previous role, have taken advice on Biological Safety, GMO Risk Assessments are in place.

<table>
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<tr>
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Inactivation with bleach or autoclave. All GMO waste will be disposed of in yellow biohazard bins or bags. Biohazard collection bins are provided by BBT (authority who run the Babraham Research Campus) for disposal.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Inactivation with bleach or autoclave. All GMO waste will be disposed of in yellow biohazard bins or bags. Biohazard collection bins are provided by BBT (authority who run the Babraham Research Campus) for disposal.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

None applicable
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Symbiosis GM safety committee has been established and has met.

The committee comprises of experienced Technical staff from core staff representatives, project management, Quality, manufacturing and Engineering. Further expert specialist technical advice will be available as required from external sources or the QP.

The GM safety committee meet to review each new potential GM project. Routine process's will be monitored and feedback collated via the routine 'SAFE' meetings with the minutes circulated to all of the GM safety committee.

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<thead>
<tr>
<th>Laboratory</th>
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<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

02/03/2022
The Symbiosis process utilises single use disposable items for all product contact materials throughout the MHRA licensed formulation and vial filling process. Further to this, Symbiosis utilise a specialist licensed contract waste management company with a pathway for clinical waste incineration. It is intended that all waste materials used in the manufacturing of the vector will be decontaminated with an appropriate agent and contained post treatment for disposal by incineration.

Agents for Decontamination:
A technical evaluation of disinfectant antiviral activity for the proposed project has been completed. Based on this study, it is proposed to utilise a suitable concentration of NaOH. After sodium hydroxide treatment items are placed in sealable containers in preparation for collection by a licensed contractor for incineration.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The GM safety committee noted, that the product has been previously assessed and approved by the client at the primary/upstream manufacturing facility as requiring containment level 1. It was further noted that the purified viral vector is not considered as 'live' at the formulation and filling stages to be completed at Symbiosis. The project involves the use of a minimal HIV-1 vector, in that most of the viral genes have been removed from the vector genome. Furthermore, these vectors are unable to replicate and are self-inactivating and would not survive beyond the facility to cause infection.

It was noted that modifications to the waste stream are required to incorporate exposure to NaOH. It is also proposed to install a Cat II Microbiological safety cabinet for the bulk pooling step which currently presents the greatest risk of operator exposure to the largest volume of product.

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fill/Finish processing of Oxford Biomedica's (OBX) proprietary lentiviral vector platform for the de

Tick to confirm that you are attaching a summary of the risk assessment
Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM safety committee noted, that the product has been previously assessed and approved by the client at the primary/upstream manufacturing facility as requiring containment level 1. It was further noted that the purified viral vector is not considered as 'live' at the formulation and filling stages to be completed at Symbiosis. The project involves the use of a minimal HIV-1 vector, in that most of the viral genes have been removed from the vector genome. Furthermore, these vectors are unable to replicate and are self-inactivating and would not survive beyond the facility to cause infection.

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Name

IONTAS LTD

Name 2

Department

Campus Estate or Research Centre

SUITE 2, THE WORKS

Road Name

LONDON ROAD

District

PAMPISFORD

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB22 3FT

Country

ENGLAND

Tel Number

01223 750801

Fax Number

0

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The IONTAS Ltd genetic modification committee will consist of:

1. The biosafety officer who has extensive experience of genetic modification issues, being currently in charge of biosafety for a large research institute. The biosafety officer will chair all genetic modification committee meetings.
2. The Chief Executive Officer has 26 years experience of genetic modification and previously ran research groups both in industry and academia. His function on the committee is to advise and provide the necessary resources for the actions of the committee to be implemented.
3. Group Leader who has 25 years experience of genetic modification in E. coli and mammalian cells. He will provide advice and ensure the actions arising from the committee meetings are implemented.
4. Research Scientist who is a specialist in mammalian cell culture has 8 years experience. She will provide advice and write the minutes of the meetings.

The committee will meet every 6 months to review biosafety and whenever a new risk assessment is required. The first meeting will review the existing risk assessments.

Level 1 (GMMs)

Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)
We intend to adopt the waste disposal procedure currently used by the Babraham Institute (GM105) and other companies on the Babraham Research Campus (BRC) under derogation (GM105/04.1): Waste from laboratories carrying out GM work at Class1 will be placed into suitably labelled hermetically sealable bins. The bins are 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003. They will be sealed by the producer of the waste and removed to a central collection point where they will be placed into 210 litre yellow labelled “Eurobins”. Collections by the local registered clinical/GM waste incinerator contractor (Vetspeed – GM 898) will be made three times a week to prevent a build-up. The BRC has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be maintained on site, as required by the Regulations, in case of loss of the contractor's facilities.

Liquid waste is inactivated by the addition of "Virkon" according to the manufacturer's instructions prior to disposal. Validation experiments will be carried out in-house using microbiological testing. (Smaller volumes (i.e. <10ml) will then be disposed of in sealed tubes with the solid waste. Larger volumes will be disposed of via the drainage system. This is then processed through our own sewage treatment plant which is closely monitored. This results in approximately 5 litres of diluted inactivated waste from each

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The risk assessments were assessed at the Department of Biochemistry, University of Cambridge as class 1 in November 2012. We have recently reviewed these after our relocation to the Babraham Research Campus.
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**Name**

QUAY PHARMACEUTICALS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

DEESIDE INDUSTRIAL PARK

**Road Name**

28 PARKWAY

**Building**

**District**

**Town**

DEESIDE

**County**

CLWYD

**Postcode**

CH5 2NS

**Country**

WALES

**Tel Number**

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**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Check box: Yes

Give brief details of the genetic modification safety committee

Microbiology Technical Expert for the site has led the risk assessment for contained usage of genetically modified microorganisms (GMMs) in consultation with the manufacturer of the genetically modified microorganism drug substance whom have extensive experience in safe handling of GMMs.

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<th>Glass House</th>
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<td>Microbiology Research</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Prior to handling of GMM on site a verified liquid decontamination method is to be transferred in from the manufacturer of the GMM drug substance. For decontamination of waste from GMP manufacture if waste is present from operations within an isolator a clear bag is to be used and sealed with a cable tie prior to removal from the isolator as captured under “safe bag” procedure and placed within a UN3291 yellow biohazard waste bin. If waste is generated in GMP manufacture outside of an isolator then waste is to be added into a yellow UN3291 biohazard waste bag and prior to placing in a yellow biohazard waste bin sealed with a cable tie. All surfaces of the yellow biohazard waste bin are decontaminated with 1% Virkon and given a 20 minute contact time prior to removal from the GMP suite. Subsequently the yellow biohazard waste bins are removed from the GMP suite and stored in the dedicated biohazard storage area ready for collection by the licenced waste collection service for incineration.

Should GMMs be used for analytical development activities samples and consumables where feasible will be submerged in 1% Virkon with a minimum contact time of 30 minutes prior to disposal in a yellow biohazard waste bin. Where submerging of consumables is not feasible they are to be directly placed into a yellow biohazard waste bag within a yellow biohazard waste bin.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment Y

Please enter comments of the GM safety committee on the risk assessment

The laboratory and manufacturing facilities at Quay Pharmaceuticals are prepared for working with human hazard class 2 microorganisms and would leverage these biosafety controls for safe handling of class 1 GMMs. Due to the nature of Quay's operations in handling a diverse range of microorganisms effective cleaning and disinfection procedures are at the forefront of Quay’s practices and will continue to be applied for handling and manufacture with class 1 GMMs.
To assess the impact to viability when lyophilising or spray drying M. pneumoniae liquid cultures to establish a low moisture, stable drug substance material. Should success be seen then further development work would be performed to create a stable drug product formulation that could proceed to GMP manufacture and usage in a human clinical trial.
Client procedure and literature have been consulted to determine a suitable decontamination procedure and this has been verified using the wildtype strain.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

A chemical disinfection procedure is to be performed to dispose of waste generated. Waste to be generated can include the liquid culture samples received from the client that are no longer required, liquid samples generated in analytical testing and dehydrated samples generated from freeze drying and spray drying studies not consumed in analytical testing.

Samples are to be submerged in 1% Virkon for 30 minutes and subsequently disposed in UN3291 yellow biohazard. The UN3291 yellow bins are stored in the dedicated biohazard storage area ready for collection by the licenced waste collection service for incineration.

The Virkon solution is to be made fresh on the day of usage and where a liquid dilution is taking place from the sample it will not exceed 5% of the 1% Virkon volume, if so a Virkon solution should be created to account for dilution factor to maintain a 1% concentration. Following the contact time in 1% Virkon the Virkon solution itself is to be held for a minimum of 24 hours before being disposed of.

Should the project being performed be successful and a GMP manufacture desired by the client a review of decontamination procedure will be performed to assess practices to be employed for waste management and decontamination of GMP facilities.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

Although gene mutations have been made to the microorganism to attenuate its virulence and gene inserts are not anticipated to influence virulence there is not sufficient evidence to assign its hazard group assessment lower than that of the wildtype strain.

Project Containment

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Name

UNIVERSITY OF ROEHAMPTON

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

ROEHAMPTON LANE

District

Town

LONDON

County

GREATER LONDON

Postcode

SW15 5PJ

Country

ENGLAND

Tel Number

020 8392 3000

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
**Premises Addresses**

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</tr>
</tbody>
</table>

**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The Committee comprises the following members:

Five full-time University of Roehampton academic staff members (2 Lecturers, 2 Senior Lecturers, 1 Reader) with research experience in mammalian cell biology and microbiology (including the designated co-chairs below).

One full-time University of Roehampton technical laboratory manager with extensive H&S experience.

Two full-time staff members from the University's Health and Safety Office with extensive H&S regulatory experience;

One external consultant (staff at Medical Research Council Head Office) with H&S and GMO regulatory experience.

All academic staff members have PhDs in relevant disciplines and several years of expertise in CL1 and CL2 work and work with GMOs.

The Committee will meet quarterly to discuss shared issues (external consultant may participate by videoconference), and will be chaired by one of the co-chairs in rotation; the other will take minutes. Laboratory inspections will be performed at least twice a year to review compliance, in conjunction with these meetings. Risk assessments will be drafted by the staff member responsible for each project, reviewed internally by a member of staff with relevant expertise (mammalian cell biology or microbiology, as appropriate) and, in case of any doubt or unresolved issues, referred to the external consultant for input. The final risk assessments will be circulated to the Committee with a recommendation to approve/revise/reject, and generally approved by email.

<table>
<thead>
<tr>
<th>Laboratory</th>
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</tr>
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<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
<td></td>
<td></td>
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<tr>
<td>Level 4 (GMMs)</td>
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</tbody>
</table>

02/03/2022
### Microbiology

Bacterial cultures will be grown in liquid or on solid media in sealed containers/flasks in microbiological incubators. According to established laboratory procedures, solid waste will be autoclaved (121°C for 15 min), liquid waste will be de-activated using 10% bleach overnight. Small spillages will be treated with 70% ethanol, while large spillage will be trapped with congealing powder (which will then be autoclaved) and the area further deactivated with 70% ethanol. Bacterial isolates will be cryopreserved in glycerol stocks at -80°C. Work will take place in CL1 laboratory facilities. Laboratory workers will wear personal protective equipment at all times. All stored samples will be labelled in compliance with regulations.

### Mammalian cell culture

Live cell cultures will be handled in a Class II Biosafety cabinet, and cryopreserved in a liquid nitrogen Dewar container. All work will take place in a dedicated tissue facility suitable for CL1 work. All stored samples will be labelled in compliance with regulations.

Inactivation will proceed as follows. Liquid waste will be treated with 10% bleach overnight before sink disposal. Solid waste will be collected in double autoclave bags, and autoclaved on site.

Cell preparations for further analysis will be chemically fixed (2% paraformaldehyde or alcohols) or subjected to detergent extraction following frozen storage, and following use will be further inactivated as described above for liquid waste.

---

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Microbiology: Bacterial cultures will be grown in liquid or on solid media in sealed containers/flasks in microbiological incubators. According to established laboratory procedures, solid waste will be autoclaved (121°C for 15 min), liquid waste will be de-activated using 10% bleach overnight. Small spillages will be treated with 70% ethanol, while large spillage will be trapped with congealing powder (which will then be autoclaved) and the area further deactivated with 70% ethanol. Bacterial isolates will be cryopreserved in glycerol stocks at -80°C. Work will take place in CL1 laboratory facilities. Laboratory workers will wear personal protective equipment at all times. All stored samples will be labelled in compliance with regulations.

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---

**Tick to confirm that you are attaching a summary of the risk assessment**

---

**Please enter comments of the GM safety committee on the risk assessment**

CU 1 2000 (rev 11/08) Page 4 of 9

The GM safety committee has approved the risk assessment.
This is a class 2 connected programme of multidisciplinary work with the broad aim of elucidating how genetic and environmental variables and cell interactions conspire to determine the outcomes of infectious, immune-mediated, neurological, and malignant diseases.

The microbiological arm of this project is based on the use of unbiased genome-wide screens for the identification of molecular factors involved in S. aureus and P. aeruginosa infection. The objective is to dissect the interaction of S. aureus with mammalian host cells during intracellular survival and proliferation, to gain biologically relevant insight into the host factors that are co-opted by this pathogen or used against it. For P. aeruginosa, the aim is to determine how genes control and affect virulence in this extracellular pathogen and how this is linked to the pathogen’s antibiotic resistance. This work has the potential to lead to the identification of novel chemotherapeutic targets and a better understanding of the determinants of susceptibility to S. aureus and of virulence and susceptibility in P. aeruginosa, respectively.

A complementary arm of this project involves the genetic manipulation of the mammalian host to study the role of host determinants of resistance or susceptibility to infection. One focus will be on antigen presentation to T cells as a critical step in activating an adaptive immune response. These studies will have broader implications, as well, for antigen presentation in transplantation, vaccine design, autoimmunity, and antitumour immunity. Related pathways contributing to inflammation and immunity (e.g., phagocytosis, activation of respiratory burst, autophagy, pathogen sensing) will also be investigated.
- Escherichia coli DH5 alpha and other K12-derived host strains commonly used in molecular biology
- Staphylococcus aureus NCTC13626
- Pseudomonas aeruginosa PA14

Eukaryotic
- Human immortalised (transformed or malignant) cell lines, such as HeLa, 293T, U937, THP-1, KG-1, and existing mutants and transfectants of these cell lines
- Murine immortalised cell lines, such as A2O-II, and existing mutants and transfectants of these cell lines
- Dedicated packaging cell lines for retro- and lentiviral transduction, such as Phoenix-A, derived from immortalised cell lines
- Human Epstein Barr virus (EBV)-transformed B-cell lines, and derived somatic cell mutants and transfectants
- Short-term cultures derived from human leukocytes, such as monocyte-derived dendritic cells or peripheral blood mononuclear cells.

**Host/vector system**

E. coli and S. aureus / pALC1743 and pIMAY
Pseudomonas aeruginosa PA14 / Mariner based MAR2xT7 transposon

Eukaryotic cells:
- These will be transformed with plasmid, retroviral, or lentiviral vectors, for example:
  - HeLa, U937, or KG-1 transduced with Mission LentIPlex Human Pooled shRNA Library based on pLKO.1-puro vector (Sigma-Aldrich Co.), or similar specific shRNA constructs
  - 293T cells transfected with P12-MMP, pMD-MLV-ogp, pMD2.G
  - EBV-transformed B cell lines transduced with retroviral particles produced in Phoenix-A cells transfected with pBMN-IRES-<marker> vectors, where <marker> refers to genes any screenable or selectable marker, such as GFP, LacZ, or resistance to antibiotics such as neo(R), hygro(R), puromycin(R).

**Origin & function**

Origins:
- The genetic material to be inserted will be obtained from commercial sources (e.g., Sigma-Aldrich), from collaborating academic or industry laboratories, or amplified from appropriate cells of origin (which include the cells identified above as recipient cells).

The intended functions of the genetic material include:
- Expression or repression of genes that mediate or regulate microbial antibiotic resistance or virulence
- Expression or repression of genes that mediate or regulate host cell responses to bacterial infection
- Expression or repression of genes that mediate or regulate antigen presentation to T cells of the immune system

**Evaluation of foreseeable effects**

a) Foreseeable effects on human health.
- from vectors carrying selectable markers

Selectable markers used commonly in plasmid vectors include genes that encode bacterial antibiotic resistance. The possibility exists of spreading antibiotic resistance to lab workers' commensal flora on accidental exposure. This risk is normally managed at CL1 unless other factors necessitate use of CL2.
- from viral vectors

This project will rely heavily on the use of lentiviral or retroviral particles. Lentiviral and retroviral vector constructs are highly efficient vehicles for in vivo gene delivery into mammalian cells. Use of lentiviral vector systems is particularly desirable because of their ability to integrate transgenes into dividing, as well as non-dividing cells.

However, there are risks associated with working with lentiviral or retroviral vectors, and these must be carefully controlled. The major hazards to exposed lab workers are the potential generation of replication competent virus, the potential of viral particles to trigger an antiviral immune response with possibility of immune pathology, and the potential for oncogenesis through insertional mutagenesis. These hazards necessitate the use of Containment Level (CL) 2 for all work with these vectors.

To minimise the risk of re-creating replication-competent virus, we will use vector systems and producer cell lines with inherent safety features, which greatly reduce this
possibility. This is accomplished by splitting the vector system into several plasmids, which produce different proteins required for viral assembly (at least two helper plasmids and one vector containing the vector genome plus transgene). Thus, a number of recombination events would be required to form a complete replication-competent virus increases, which is extremely unlikely.

In order to minimise the risk of insertional mutagenesis or virally-induced inflammation following accidental infection of lab workers with viral vectors, lentiviral/retroviral samples will be handled at CL-2 (Containment Level 2). The cell lines producing viral particles and the particles will be handled in a CL-2 microbiological safety cabinet; double gloves pulled over the cuffs of the laboratory coats will be used at all times. All work will be conducted in a lab with access restricted to authorised and trained personnel. Further, Pasteur pipettes will be substituted with plastic disposable tips. Contaminated labware and equipment will be treated with appropriate decontaminants, e.g., bleach, immediately after use.

-- from inserted genes of interest

Many of the inserts are expected to cause gene expression changes which, in themselves, carry minimal risk to human health (requiring no containment above CL1), but will be performed at CL2 if the host cell requires this level of containment (e.g., use of EBV-transformed B-cell lines as hosts).

The expression of potential oncogenes or the knocking down of tumour suppressor genes may lead to cellular tumorigenic transformation. The silencing of genes by using the RNAi technology will rarely generate a complete lack of gene expression. The heterogeneous expression of the targeted genes may alter the regulatory networks and the stoichiometry of different proteins involved in a variety of cellular processes. RNAi may also trigger a cellular antiviral response, leading to inflammation and the non-specific inhibition of protein synthesis.

-- from recipient cells

One of the sub-projects involves work with Methicillin-resistant Staphylococcus aureus strains. S. aureus is an important cause of skin and soft tissue infections, abscesses, pneumonia, endocarditis, osteomyelitis, foreign-body infections, toxic shock syndrome and sepsis. Because of this, S. aureus is classified as a class II human pathogen. Vancomycin and daptomycin are the first-line antibiotic choices for MRSA bacteremia. The genetic modification of S. aureus NCTC13626 will not involve the generation of either vancomycin nor daptomycin resistant strains. We will generate transiently S. aureus mutants resistant to chloramphenicol. Nevertheless, the deletion mutants will have no added antibiotic resistances.

In the agr deletion mutants, we will inactivate the master regulator of the virulence of this S. aureus. This could make an unpredicted increase of virulence and/or transmissibility in the strain. However, based on previous literature (Infect. Immun. May 2011 vol. 79 no. 5 1927-1935), agr affected mutants are fully-attenuated strains of MRSA. The deletion mutants will be made in-frame to avoid the polar effects on the expression of adjacent genes. The complementation of the deletion mutants will most likely not be fully achieved due to the cost of harboring extra-chromosomal material to the fitness of the mutant strains.

For work related to P. aeruginosa: All the bacteria are loss-of-function mutants, making it very unlikely that they will outcompete the wild-type. PA14NR mutants do carry a gentamicin-resistance cassette for selection purposes, rendering this antibiotic ineffective in treating hypothetical infections.

EBV-transformed B cells generally are latently infected with low levels of infectious virus, or none, being produced in culture. Moreover, adult lab workers will generally have been infected with EBV already and carry immunity to the virus. Nonetheless, there is the possibility of de novo EBV infection of previously unexposed lab workers, which might trigger infectious mononucleosis and increase the long-term risk of malignancy. All work with EBV-transformed B-cell lines will therefore be carried out at CL2, even if the genetic modification itself does not carry hazards to human health.

b) Foreseeable effects on the environment.

Work on host responses to S. aureus

The retroviral vectors will be pseudotyped with the surface glycoprotein of vesicular stomatitis virus (VSV-G), which will increase their range of host tropism becoming pan tropic. In addition, their resistance to dehydration and their ability to survive in the environment may be higher. However, we will generate replication-incompetent viral particles, thus diminishing their chances of spreading.

The pIMAY vector used for this work has an E. coli origin of transfer on its sequence, which could potentially make it a mobilizable plasmid. However, this vector has been adapted to produce deletion mutants on MRSA strains efficiently. Circumventing a strong restriction barrier, combined with an improved deletion and transformation protocol, has allowed the genetic manipulation of previously untransformable MRSA strains. In addition, the set of genes that code for the bacterial conjugation systems are species specific (Microbiol. Mol. Biol. Rev. June 2003 vol. 67 no. 2 277-301) and therefore the chances of transmission of this plasmid from the MRSA target strain to other bacteria are very low. To propagate the pIMAY vector and its derivatives, we will only use E. coli strains that lack the specific set of genes to allow the transfer of this vector via conjugation, minimizing the possibility of its spread.

S. aureus is not considered a specified animal pathogen by the DEFRA. In addition, MRSA strains have lower fitness in comparison with commensal staphylococci part of the natural flora of different animals. However, the transmission of MRSA strains to domestic animals has been increasingly reported by veterinarians (ILAR J.
2010;51(3):233-44).
P. aeruginosa
As all the bacteria are loss-of-function mutants of a lab adapted strain (PA14) and it is very unlikely that they will out-compete environmental wild-types. As it is the nature of the project to investigate determinants of virulence using a near-whole genome library, it is not impossible that some mutants will have altered and heightened patterns of virulence factor production, leading to potentially more severe disease phenotype in the bacterium’s plant, invertebrate or vertebrate hosts.
Eukaryotic host cells
These cells are not viable in the natural environment, so no risk to the environment (other than that to human health, see above) is expected to arise from accidental release.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Waste Management Measures</th>
<th>Details</th>
</tr>
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<tbody>
<tr>
<td>E. coli will be grown in liquid or on solid media in sealed containers/flasks in microbiological incubators. According to established laboratory procedures, solid waste will be autoclaved (121°C for 15 min), liquid waste will be de-activated using 10% bleach overnight. Small spillages will be treated with 70% ethanol, while large spillage will be trapped with congealing powder (which will then be autoclaved) and the area further deactivated with 70% ethanol. Bacterial isolates will be cryopreserved in glycerol stocks at -80°C. Work will take place in CL1 laboratory facilities. Laboratory workers will wear personal protective equipment at all times. All stored samples will be labelled in compliance with regulations.</td>
<td></td>
</tr>
<tr>
<td>Work involving MRSA and P. aeruginosa will be carried out following the rules of class 2 containment level supplemented by the principles of good microbiological practice. Lab coat and gloves are used as a matter of routine in the lab. Class II Safety cabinets are inspected and validated annually. Bacterial cultures will be grown in liquid or on solid media in sealed containers/flasks in microbiological incubators. According to established laboratory procedures, solid waste will be autoclaved (121°C for 15 min), liquid waste will be de-activated using 10% bleach overnight. Small spillages will be treated with 70% ethanol, while large spillage will be trapped with congealing powder (which will then be autoclaved) and the area further deactivated with 70% ethanol. Bacterial isolates will be cryopreserved in glycerol stocks at -80°C. Work will take place in CL2 laboratory facilities with restricted access. Laboratory workers will wear personal protective equipment at all times. All stored samples will be labelled in compliance with regulations.</td>
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All students/staff receive general safety information. Students receive advice and guidance on safety procedures within the laboratory, including culture and disposal of pathogenic micro-organisms. Specialist guidance or training in handling of microorganisms can be provided by the laboratory technicians. Risks of infection are not large providing good microbial practice is maintenance. If skin is breached whereupon individuals should seek medical attention at the nearest available hospital. Such individuals should declare if the organism being handled carried any antibiotic resistance markers. Trained First Aiders would normally be contacted in the event of emergency and incidents would be recorded in the accident book.

Mammalian cell culture: Live cell cultures will be handled in a class 2 safety cabinet, and cryopreserved in a liquid nitrogen Dewar container. All work will take place in a dedicated tissue facility suitable for CL2 work. All stored samples will be labelled in compliance with regulations. Inactivation will proceed as follows. Liquid waste will be treated with 10% v/v household bleach overnight before sink disposal. Solid waste will be collected in double autoclave bags, and autoclaved on site. Cell preparations for further analysis will be chemically fixed (2% paraformaldehyde or alcohols) or subjected to detergent extraction following frozen storage, and following use will be further inactivated as described above for liquid waste.

For the work carried out with viral particles, the following measures will be applied:
- Good laboratory techniques will be strongly emphasised. Designated workers will be trained in good laboratory techniques before commencing work with potentially oncogenic DNA sequences. They will be made fully aware of the potential hazards of such work.
- Access to the laboratory will be limited to authorised personnel and designated workers.
- All experimental procedures will be performed so as to minimise aerosol production. Procedures which are likely to generate aerosols such as the use of sonicators, vigorous shaking and mixing etc. will be avoided, or where necessary, will be carried out in closed containers or a class II microbiological safety cabinet.
- Procedures using lentiviral particles, packaging cell lines and exposed cells will only be carried out in the tissue culture suite.
- Safety cabinets will be routinely disinfected prior to and following use.
- Double gloves pulled over the cuffs of the laboratory coats will be used at all times.
- Designated microbiological safety cabinets to be used.
- Only plastic pipettes will be used in the tissue culture facilities which will be treated with bleach immediately after use. Furthermore, all contaminated materials and media including waste destined for subsequent incineration will be autoclaved. The use of sharps will be prohibited and all precautions will be taken to identify procedures or devices that might be used in these projects which could cause an injury to the researchers.

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile. Appropriate disinfectants for inactivating lentiviral particles on surfaces include 70% ethanol and 1% Virkon solution. Liquid waste should be treated with 10% bleach overnight before sink disposal.

The Committee comprises the following members:

- Five full-time University of Roehampton academic staff members (2 Lecturers, 2 Senior Lecturers, 1 Reader) with research experience in mammalian cell biology and microbiology (including the designated co-chairs below).
- One full-time University of Roehampton technical laboratory manager with extensive H&S experience.
- Two full-time staff members from the University's Health and Safety Office with extensive H&S regulatory experience;
- One external consultant (staff at Medical Research Council Head Office) with H&S and GMO regulatory experience.

All academic staff members have PhDs in relevant disciplines and several years of expertise in CL1 and CL2 work and work with GMOs.

The Committee will meet quarterly to discuss shared issues (external consultant may participate by videoconference), and will be chaired by one of the co-chairs in rotation; the other will take minutes. Laboratory inspections will be performed at least twice a year to review compliance, in conjunction with these meetings. Risk assessments will be drafted by the staff member responsible for each project, reviewed internally by a member of staff with relevant expertise (mammalian cell biology or microbiology, as appropriate) and, in case of any doubt or unresolved issues, referred to the external consultant for input. The final risk assessments will be circulated to the Committee with a recommendation to approve/revise/reject, and generally approved by email.

**Project Containment**

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<th>Growth Rooms</th>
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<th>Human Clinical Applications</th>
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**Name**

INSTIL BIO UK

**Name 2**

**Department**

**Campus Estate or Research Centre**

UMIC BIO-INCUBATOR

**Road Name**

GRAFTON STREET

**District**

**Town**

MANCHESTER

**County**

GREATER MANCHESTER

**Postcode**

M13 9XX

**Country**

ENGLAND

**Tel Number**

0161 603 7732

**Fax Number**

0161 606 7284

**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GM safety committee will comprise four individuals from within, and external, to the establishment and all familiar with the activity performed within the organisation. All four members have 10 or more years of experience of working with the same or similar GMOs at multiple research institutes and have experience of generating risk assessments for GMO activity. Members roles are: Director of Cell Therapy Research (Formerly Research Fellow, Cardiff Medical School), Director of Cell Production (Formerly Senior Research Scientist, University of Manchester), Professor of Immunology (University of Manchester) & Senior Research Fellow, Institute of Cancer Sciences, University Of Manchester who has also been a member on the GMO committee within the host academic institution for >5 years.

The committee will meet annually to discuss any necessary issues related to the work, but can also meet at intervening periods to discuss any pressing issues that may need to be addressed. New and amended applications will be circulated and reviewed by the committee members and where appropriate additional expertise will be sought on an ad hoc basis. Once there is a unanimous agreement between the committee members for a new project risk assessment which meets the contained use licence for the organisation the project will be approved to start.

Any incidents or changes to the use or risk of GMO activity will be reported to the committee for review to identify the best course of action if required.

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Decontamination
Contaminated plasticware will be decontaminated using hyperchlorite solution, or where minimal residual GMM material remains will be disposed of in yellow container for incineration. Contaminated liquids will be decontaminated in hyperchlorite solution for >1 hour. Decontamination in hyperchlorite solution such as Actichlor provides 100% decontamination of all bacteria, fungi and viruses. The decontaminated liquid can then be disposed of down the sink with excess water.

Spillages
Any minor liquid spillages (<20ml) will be removed by soaking up with an absorbent wipe or for larger liquid spillages by the addition of absorbent matts and transfer into a yellow container prior to autoclave. The area will then be sprayed with an anti-microbial agent (with demonstrated virucidal activity i.e. Klercide A, S, or C (GMP use) or hyperchlorite solution (non-GMP use)). After inactivation, the area will be wiped and the wipe placed into a yellow container. The area will then be sprayed and wiped with 70% isopropyl alcohol and the wipe placed into the yellow container. The container will be sealed and sent for incineration as above.

Centrifuge spillage
If breakage occurs during a run, the centrifuge will be stopped, and left for 30 minutes to allow the aerosol to disperse. On opening all liquid will be absorbed using absorbent material and placed into a yellow box. The rotor and/or buckets will be removed and all surfaces sprayed and wiped with an anti-microbial agent (with demonstrated virucidal activity Klercide C (GMP use) or hyperchlorite solution (non-GMP use)). After inactivation, the area will be wiped and the wipe placed into a sharp proof container. The area will then be sprayed and wiped with 70% alcohol and the wipe placed into the sharp proof container. The container will be sealed and sent for incineration as above.

All clinical lab waste is sent for incineration and will leave the building within locked large yellow thick plastic transport containers.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Decontamination
Contaminated plasticware will be decontaminated using hyperchlorite solution, or where minimal residual GMM material remains will be disposed of in yellow container for incineration. Contaminated liquids will be decontaminated in hyperchlorite solution for >1 hour. Decontamination in hyperchlorite solution such as Actichlor provides 100% decontamination of all bacteria, fungi and viruses. The decontaminated liquid can then be disposed of down the sink with excess water.

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If breakage occurs during a run, the centrifuge will be stopped, and left for 30 minutes to allow the aerosol to disperse. On opening all liquid will be absorbed using absorbent material and placed into a yellow box. The rotor and/or buckets will be removed and all surfaces sprayed and wiped with an anti-microbial agent (with demonstrated virucidal activity Klercide C (GMP use) or hyperchlorite solution (non-GMP use)). After inactivation, the area will be wiped and the wipe placed into a sharp proof container. The area will then be sprayed and wiped with 70% alcohol and the wipe placed into the sharp proof container. The container will be sealed and sent for incineration as above.

All clinical lab waste is sent for incineration and will leave the building within locked large yellow thick plastic transport containers.
One member suggested changes which further highlight the safety of the GMO. The wording was altered slightly to clarify the safety of these class 1 modified GMO.

Three members had no comments to make and were happy with the risk assessment.

**Project Ref** 3252/15.1

**Date Ack'n'd** 08/04/2015  **CU2 Project Title** Pre-clinical and clinical research into re-targeting the immune system against cancer

**Class** Class 2  **CultureVol** 1-50 Litres  **CultureVolume** Class 2-4

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N  **Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Purpose:

1. Generation of plasmids using Biosafety Class I disabled prokaryotic strains
2. Generation of retro/lentiviral vectors using packaging lines or Transient transfections using Biosafety Class I lines such as PG13 or 293T.
3. Genetic modification using plasmid or retro/lentiviral transfection of Biosafety Class I/II cell lines such as Jurkat, RAJI, K852, Daudi, HT-1080 as models and targets to assess and enhance T cell function in the anticancer cell.
therapy context.

[4] Pre-clinical and clinical trial development for Human cell therapies using Plasmid, Retro/Lentiviral transfection of primary human cells to produce anti-cancer specificities

Recipient or parental organism

[1] Preliminary plasmid vector creation will be performed using Biosafety Class I bacterial strains such as the disabled E.coli strains including Xl1 Blue, Xl-10 Gold or Top10

[2] Transfection of Biosafety Class III cell lines such as PG13, 293T, Jurkat, RAJI, K562, Daudi, or HT-1080 with TCR, CAR, cancer-antigens or stimulatory or co-stimulatory molecules using plasmids, retro- or lenti-viral vectors

[3] Transfection of Primary human cells such as peripheral blood mononuclear cell derived T-cells, Monocytes or NK cells, Tumour derived cells with TCR, CAR, cancer-antigens or stimulatory of co-stimulatory molecules using plasmids, retro- or lenti-viral vectors

Host/vector system

Non-viral systems
Plasmid vectors such as pcDNA3.1, pBluescript (Strategene) and TOPO (Invitrogen) based plasmid vectors.

Viral systems
Second, third or fourth generation lentiviral and retroviral vectors deleted of any viral expressed sequences and packaged using either: transient multi-plasmid transfection in cell lines such as 293T or pre-transfected stable packaging cell lines such as PG13.

Origin & function

The genetic material which will be transferred within the vector system will be derived from human cells and synthesised by an external company to generate genetic material which is codon optimised for mammalian expression. The material will then be transferred to mammalian cells in vitro to generate cells with enhanced antitumour activity, with the anti-tumour activity monitored by one or more standard immunological assays.

The genes will form several groups:

1) Cancer specific T-cell receptors (TeR): TeR specific for antigens, including but not limited to, NY-ESO, gp100, MAGE A3, WT1, p53 and surr ...

2) Virus specific TeR: T-cell receptors specific for viruses implicated in oncogenic transformation, including but not limited to, cytomegalovirus, Epstein-Barr virus and human papilloma virus

3) Chimeric antigen receptors (CAR): CARs specific for tumour associated antigens, including but not limited to, carcinoembryonic antigen (CEA), 5T4 and melanoma-associated chondroitin sulphate proteoglycan (MCSP).

4) Pro-survival genes: Genes which enhance the pro-survival potential of modified cells

5) Synthetic genes: Synthetic genes which can be used to modulate the growth or presence of gene-modified T-cells in vitro or in vivo. For example suicide gene which can be used to selectively deplete gene modified T-cells after infusion into patients.

Evaluation of foreseeable effects

Introduction of genetic material into cells in vitro will generate genetically modified cells with the capacity for enhanced anti-tumour activity; as models to assess function; and as replication disabled co-culture targets to enhance cell expansion. The genetically modified cells will be monitored in vitro using standard assays including basic microscopy, flow cytometry and ELISA.

Potential hazards to human health and control methods:

1) Contamination of user with retro/lentiviral supernatant: The risk to the user of the lentiviral supernatant is low, The
main risk to health would occur in an instance where long-lived dividing somatic cells come into contact with retro/lentiviral particles for a period long enough for infection to occur. This risk is reduced further by the operator using standard biosafety PPE (lab coat, nitrile gloves etc) and manipulating the contained use within a biosafety class 2 cabinet, Class IIIIE isolator or negative pressure cabinet.

2) Contamination of user/operator with GMO: The main risk to the operator would come with the unintentional administration of GMO to themselves. This only poses a risk when the GMO is derived from self tissue as there will be no immune rejection. To prevent this the operator will be strictly forbidden from performing manipulations or cell culture on cells derived from themselves.

3) The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines are replication incompetent Thus whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal (Details of the origins and deletions within the specific retro/lentiviral constructs are provided within the specific risk assessments).

4) The primary dangers in working practice are expected to be the formation of aerosols and use of sharps. We propose to carry out all manipulations in a Class II microbiological safety cabinet following GLP and any additional local safety guidelines appropriate to this work and minimise the use of sharps where possible and where sharps are necessary operators must work within the constraints of the sharps usage and safety policy.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Decontamination**

Contaminated plasticware will be decontaminated using hyperchlorite solution, or where minimal residual GMM material remains will be disposed of in yellow container for incineration. Contaminated liquids will be decontaminated in hyperchlorite solution for >1 hour. Decontamination in hyperchlorite solution such as Actichlor provides 100% decontamination of all bacteria, fungi and viruses. The decontaminated liquid can then be disposed of down the sink with excess water.

**Spillages**

Any minor liquid spillages <20ml) will be removed by soaking up with an absorbent wipe which will be submerged in hyperchlorite solution for > 1 hour prior to transfer into a yellow container for autoclave. The area will then be sprayed with an anti-microbial agent (with demonstrated virucidal activity Le. Klercide A, a, or C (GMP use) or hyperchlorite solut ion (non-GMP use)). After inactivation, the area will be wiped and the wipe placed into a yellow container. The area will then be sprayed and wiped with 70% isopropyl alcohol and the wipe placed into the yellow container. The container will be sealed and sent for incineration as above.

**Centrifuge spillage**

If breakage occurs during a run, the centrifuge will be stopped, and left for 30 minutes to allow the aerosol to disperse, On opening all liquid will be absorbed using absorbent material and decontaminated in hyperchlorite solution for > 1 hour. The rotor and/or buckets will be removed and all surfaces sprayed and wiped with an anti-microbial agent (with demonstrated virucidal activity Klercide C (GMP use) or hyperchlorite solution (Non-GMP use)). After inactivation, the area will be wiped and the wipe placed into a sharp proof container. The area will then be sprayed and wiped with
70% alcohol and the wipe placed into the sharp proof container. The container will be sealed and sent for incineration as above. All clinical lab waste is sent for incineration and will leave the building within locked large yellow thick plastic transport containers.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

One member suggested the risk assessment was modified to include extra vectors that may be used in production of medicinal products and/or research and development. One member suggested the policies regarding the use of sharps was modified. The use of sharps in the production of medicinal products cannot be avoided due to the nature of the containers that the virus is supplied in. This is indicated in the risk assessment. This policy does not cover the R&D lab and as such the use of sharps remains prohibited in this situation. One member had no comments and was happy with the risk assessment. One member raised some queries regarding viral stability. This was clarified by rewording the section to highlight differences in stability of viruses at 37°C and at room temperature. Queries were also raised over provision of vaccinations for staff and the wording of the risks associated with tumour targeting of GMOs. Reworking was again sufficient to clarify these points.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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GM Centre Number: 3253

Data Premises Notified (Originally) 09/12/2014

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

PROGENITOR LABS

Name 2

Department

Campus Estate or Research Centre

STEVENAGE BIOSCIENCE CATALYST

Building

Road Name

GUNNELS WOOD ROAD

District

Town

STEVENAGE

County

Postcode

SG1 2FX

Country

ENGLAND

Tel Number 0203 384 0809

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Our GM committee comprises of the company's CEO, one of the senior scientist and an external consultant. The committee meets twice a year to discuss safety measures in place in the laboratory and approve Genetic Modification Risk Assessments. When scientist plan to use a new genetically modified organism, the consultant advises on safety procedures.

The CEO and senior scientist make sure the suggested safety measures are incorporated into SOPs and are being followed

<table>
<thead>
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<th>Laboratory (GMMs)</th>
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<td>Other (please specify)</td>
<td>Tick if confidential</td>
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Bacteriology Parasitology Transgenic Birds Microbiology Research

02/03/2022
Contaminated sharps are collected in closed sharp bins, tagged and are removed by clinical waste contractors for incineration.

Culture flasks, tubes and other lab consumables used in experiments are collected in a closed bin and once this is filled, are autoclaved at 134 deg C for 15 minutes for de-contamination. The it is disposed with clinical waste for incineration.

Liquid waste (culture media etc.) is disinfected and de-contaminated using 1% solution of Virkon following manufacturer's instructions. Then it is poured down the sink and flushed with copious amounts of water.

The autoclave used for de-contamination is serviced twice a year.

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Please enter comments of the GM safety committee on the risk assessment:

Comments from the GM Committee meeting on 19 November 2014:

GM Committee consider the risk assessment and approved category 1 work involving GM organisms on Progenitor Labs premises.

Project Ref 3253/14.1

1. Knockdown with lentiviral particles discovered in our small molecule screening campaigns. 2. Generation of PAX7 (and other reporter cell lines via lentiviral transduction)

Class 2  Consent Granted

Non-GMM
Project Additional Information

Purposes of the contained use

1. We want to validate selected targets from our small molecule screening campaigns through shRNA delivery by lentiviral particles.
2. We want 10 generic reporter cell lines to screen small molecules activating the expression of a particular gene.

Recipient or parental organism

1. Recipient cells will be human embryonic stem cells or other types of stem cells and progenitor cells. After lentiviral transduction, the expression of one of their genes will be downregulated. Recipient cells will be characterised by qPCR and Western Blotting to assess the function of the lentiviral constructs. Cells transduced with lentiviral particles will be kept in a dedicated incubator. After processing the samples, waste will be conveniently inactivated and discarded.
2. Same as in 1. but upon differentiation of the stem cell, they will express GFP or another colour marker.

Host/vector system

1. Self-incactivating replication incompetent lentivirus based on pKO backbone and purchased from established vendors like Sigma-Aldrich. These vectors carry an shRNA used to knock-down the expression of a target gene.
2. Self-incactivating replication incompetent lentivirus purchased from Applied Biological Materials or other vendors.
Vector system are lentiviral vectors (PlK01 backbone)

Origin & function

Evaluation of foreseeable effects

1. The cell lines transduced with lentiviral particles will show downregulation of a specific gene.
2. The cell lines transduced with the reporter construct will not show any phenotype unless the target gene is stimulated, in that case they will express GFP or another colour marker. Cells lines will be characterised by qPCR and Western Blotting.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disposable material exposed to lentiviral particles will be inactivated with Virkon, disposed in appropriate plastic bags and autoclaved. All surfaces of the cell culture hood will be cleaned and sterilized with 70% ethanol.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Comments from the GM Committee meeting on 19 November 2014:
GM Committee consider the risk assessment and approved category 1 work involving GM organisms on Progenitor labs premises

Project Containment

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GM Centre Number: 3255

Data Premises Notified (Originally) 09/12/2014

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required?

Non-GMMs N

Withdrawn N

Name

CAMBIMUNE LTD

Name 2

Department

Campus Estate or Research Centre

BABRAHAM RESEARCH CAMPUS

Building

MONETA BUILDING

Road Name

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB22 3AT

Country

ENGLAND

Tel Number 01223 497101

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The committee is composed of four members including the biosafety officer, a research scientist (providing staff representation and an executive director of the company along with a University Academic, with extensive experience in human and environmental safety, acting as an expert advisor, The Safety Committee fulfills the responsibilities under GMO Contained Use Regulations to approve risk assessments and provides guidance and direction on good bio-safety practice at the laboratory. The Safety committee is representative of all people having access to the laboratory and will encourage discussion to ensure the views of all members are taken into account. Risk assessments will be circulated amongst the committee for scrutiny and commented on electronically & revisions will be made as necessary. The committee will meet at minimum twice a year with the ability to additionally meet promptly as and when needed.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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Tick if confidential

Yes

Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research |
|------------|------|--------------|------------------|----------------------|

02/03/2022
Babraham Institute biosafety guidance and policy will be followed. In particular cell cultures will be inactivated by adding 1% Virkon and being left to stand for min 1 hour. Waste material will be disposed of in 30 or 60l eurobins in accordance with Babraham Institute biosafety policy "Clinical Waste Management: Level 1"

For activities involving GMMs, describe the waste management measures which will apply to the activity

Babraham Institute biosafety guidance and policy will be followed. In particular cell cultures will be inactivated by adding 1% Virkon and being left to stand for min 1 hour. Waste material will be disposed of in 30 or 60l eurobins in accordance with Babraham Institute biosafety policy "Clinical Waste Management: Level 1"

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC reviewed and approved the Risk Assessment on the 25/11/2014 and it was agreed that the risk assessments for the activity to be undertaken was entirely appropriate. The SOPs for the laboratory were also considered in conjunction with the risk assessment and deemed to be appropriate for the work to be carried out.
GM Centre Number: 3256

Data Premises Notified (Originally) 14/04/2015

Transferred from 1992 Regs? N

Transitional Premises

Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

CN BIO INNOVATIONS LIMITED

Name 2

Department

Campus Estate or Research Centre

Building

BUILDING 332

Road Name

CAMBRIDGE SCIENCE PARK ROAD

District

MILTON

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB4 0WN

Country

ENGLAND

Tel Number 01707 358719

Fax Number 0

E-mail

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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities:** Yes

- **Give brief details of the genetic modification safety committee:**

  The Genetic Modification/Biosafety Committee meets quarterly and is composed of CTO, laboratory manager (15 yrs experience with GMO), Senior Scientist and external biosafety consultant (former head of biosafety at large pharmaceutical company, member of the UK Scientific Advisory Committee on Genetic Modification)

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For activities involving GMMs, describe the waste management measures which will apply to the activity

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<td>Plants</td>
<td>Other (please specify below)</td>
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</table>

Solid waste and liquid waste - chemical disinfection using disinfectants with literature precedent for the viruses in the study.
Excess virus stock - validated autoclave cycle.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
1. The material safety data sheets supplied identify Biosafety Level 2 practices and containment facilities as required for use of their vectors. You should make sure that you are satisfied that these are not required for the proposed work.

The SACGM Compendium of guidance Part 2: Risk assessment of genetically modified microorganisms (other than those associated with plants) Page 13 states that:

‘Wild-type adenoviruses are ACDP hazard group 2 pathogens and should be handled at Containment Level 2. Many adenoviral vector strains have been constructed that are deleted for E1, encoding key genes required for viral growth. These strains are disabled and incapable of establishing a productive, transmissible infection in humans. These vector strains can be considered to be avirulent and may be handled safely at Containment Level 1.’

To ensure replication deficiency of the virus particles the vendors have deleted the E1 and E3 regions allowing the particles to safely be used as a gene delivery tool. In addition, the gene delivered by the viruses in this project encodes enhanced Green Fluorescent Protein (eGFP) that has no direct effect on cellular processes. For these reasons, although the viruses will be used under containment levels 2 conditions due to the use of primary human cells, the GMO activity is class I.

2. I note from SignaGen's website that some of their vectors include WPRE sequences. As described in the SACGM Compendium (Part 2 section 2.2 para 45, some versions of this element "are capable of expressing part of the X protein from WHV which may have oncogenic properties, and risk assessments should take into account the possible harmful effects of this sequence. Vectors containing these forms of WPRE should be assigned to class 2 or higher." If the vectors you are using do incorporate WPRE sequences you should check whether or not they include sequences encoding part or app of the X-protein and modify the assessment accordingly.

Communication with the vendors indicated the plasmids used to generate the viral particle to be sent do not contain WPRE and do not present oncogenic risk. The comprehensive risk assessment has been amended to reflect this confirmation.

3. Is there any potential for insertional activation (e.g. of potentially oncogenic genes) by the vectors?

Delivery of any nucleic acid could theoretically cause insertional mutagenesis if it integrates into the genome. There has been much debate around Viral gene delivery but studies suggest the risk is low. Integration is usually at the site of active genes.

References


4. Is there supporting data regarding the methods used for disinfection

AD-eGFP and AAV-eGFP vendor documentation (attached) states both viruses are susceptible to 1% sodium hypochlorite and recommends 30 minute contact times.
It is also reported that 0.9% virkon for more than 5 minutes is an effective disinfectant for adenoviruses reducing potency by greater than six logs upon a five minute exposure (McCormick L, Maheshwari G 2004, Sakudo A & Shintani H, 2011).

References
1) Mammalian Expression Plasmids (pcDNA3.1, pcDNA6, pCRII-TOPO) for transfection of HepG2 cells with HBV genome (either full or over length) or human sodium taurocholate co-transporting peptide (hNTCP), the entry receptor for HBV.

2) HepG2.2.15 and HepAD38 cell lines which are transfected HepG2 cells which stably express infectious HBV, in the case of HepAD38 under tetracycline control.

3) Cell culture derived virus from the above cell lines used to infect primary human hepatocytes.

Origin & function

The Hepatitis B genome used to stably transflect HepG2.2.15 and HepAD38 cells was isolated from patient serum of HBsAg sub type ayw. Full or overlength HBV genonomes used to transiently tranfect HepG2 cells will be chemically synthesised from publically available sequences for HBV of different genotypes. The function of the inserted HBV genetic material is to enable the cells to replicate HBV virus and produce infectious virions. The virus produced is itself a GMO and it will be used to infect cultures of primary human hepatocytes and optionally non-parenchymal cells, resulting in these cells exhibiting all steps of the viral life cycle and producing infectious virus.

The sequence for hNTCP will be chemically synthesised from publically available sequences. Transfection of hNTCP into HepG2 causes overexpression of NTCP which renders the cells susceptible to infection by HBV.

Evaluation of foreseeable effects

The purpose of the genetic modification is in all cases to cause cells to replicate HBV, except in the case of hNTCP overexpression where the result is a cell which is more susceptible to infection.

Hazard to human health

The transfected and infected cells will produce HBV at titres up to 10 billion IU/ml which has been shown to be infectious in in vitro cell cultures, humanised liver chimeric mice and chimpanzees. It is highly likely this virus would be infectious to humans.

Hazard to the environment

Minimal as the cell cultures are only viable in specialised cell culture conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No larger GMO will be used in this work.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogations

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be autoclaved at 121oC for 20 min before being disposed of by incineration as clinical waste. Prior to autoclaving the solid waste contaminated with GM material, e.g. flasks or plates containing GM cells, will be contacted with 2% Virkon disinfectant for at least 1 hour.

The cycles used on the autoclave for make safe of waste will be validated using 12 point thermocouple tests which will be repeated every 6 months. Only loads similar to those validated will be run. A thermal indicator will be included in each load and records for each load will be kept.

Liquid waste, to include cell culture media containing virus will be contacted with 2% Virkon for at least 1 hour before disposal to drain. Virkon contains an indicator to show it remains active, fresh Virkon to be made up at least every 7 days. Literature shows 1% Virkon for 10 minutes is sufficient to reduce HBsAg to undetectable levels.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
CN Bio Innovations competent person reviewed the risk assessments and found 2 areas to be addressed: i) The description of the mammalian expression plasmids to be used was too generic, ii) Risks posed by the presence of long tail repeats from Moloney murine leukemia virus (MoMLV) needed to be assessed. Following addition of descriptions of extra mammalian expression plasmids and a risk assessment of MoMLV the competent person considered the risk assessment satisfactory.

**Project Containment**

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Data Premises Notified (Originally) 27/01/2015

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

CAMBRIDGE ACADEMY FOR SCIENCE AND TECHNOLOGY

Name 2

Department

Campus Estate or Research Centre

Road Name

ROBINSON WAY

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB2 0SZ

Country

ENGLAND

Tel Number 01223 724300

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Biological Safety Officer ~ 20 years experience in working with GMOs, formerly Biological Safety Officer for Dept, Surgery, Cambridge University, 2003 completed (and passed with and past MRC/University of Sussex Biological Safety traininf course. Risk assessments will also be scrutinised by a Safety Officer from Cambridge University

Laboratory Animal Unit Growth Room Glass House Large Scale

- Level 1 (GMMs)
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)
- Non-microbial: Yes
- Other (please specify): Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research: Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste will be inactivated by autoclaving or by disinfection with Virkon (according to the manufacturers instructions). Treated samples will periodically be tested for viable organisms and records kept.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Biological Safety Officer ~ 20 years experience in working with GMOs, formerly Biological Safety Officer for Dept. Surgery Cambridge University, 2003 completed (and passed with and past MRC/University of Sussex Biological Safety training Course. Risk assessments will also be scrutinised by a Safety Officer from Cambridge University
### Data Premises Notified (Originally)
- **GM Centre Number:** 3258
- **Date:** 26/01/2015

### Transferred from 1992 Regs?
- **Transferred from Transitional Premises:** N

### Data Premises Closed
- **Transitional Premises Emergency Plan Required:** N

### Non-GMMs Withdrawn
- **Non-GMMs:** N

### Withdrawn
- **Withdrawn:** N

---

### Name
- **BLINK THERAPEUTICS LTD**

### Name 2

### Department

### Campus Estate or Research Centre
- **STEFENAGE BIOSCIENCE CATALYST**

### Building
- **GUNNELS WOOD ROAD**

### Road Name
- **STEFENAGE**

### District
- **HERTFORDSHIRE**

### County
- **SG1 2FX**

### Postcode
- **ENGLAND**

### Tel Number
- **01438906945**

### Fax Number
- **0**

### E-mail
- **blank**

### HSE Division

### Comments

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### Date at Which Additional Info Submitted
- **02/03/2022**
# Premises Addresses

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# Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

- Company CEO
- Principal Scientist
- Senior scientist
- To meet at a minimum 6 monthly or ad hoc to assess any new activities and risk assessments

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Other (please specify)  

Tick if confidential

Bacteriology  

Yes  

Parasitology  

Transgenic Birds  

Microbiology Research
All GMM's classified laboratory waste will be completely inactivated by autoclaving in the building premises for subsequent collection and disposal by incineration. All potentially contaminated work surfaces routinely disinfected with Virkon and 70% ethanol. Liquid waste potenitally contaminated will be inactivated using Virkon.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

GMSC comment: the risk assessment for working with GMMs calculate all potential activity appropriately as Class 1 activities to be carried at the Stevenage Bioscience Catalyst Building (address as above) designed at containment level 2.
**GM Centre Number: 3260**

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**Name**

BLUEBERRY THERAPEUTICS LTD

**Campus Estate or Research Centre**

THE BIOHUB

**Road Name**

ALDERLEY PARK

**Town**

MACCLESFIELD

**Building**

3F60

**District**

**County**

CHESHIRE

**Postcode**

SK10 4TG

**Country**

ENGLAND

**Tel Number**

01625 238776

**Fax Number**

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**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee (GMSC) consists of new people both of whom have more than 20 years experience handling GMMs. One is a member of the management who additionally has 4 years experience of being on the GMSC of a global pharmaceutical company which included reviewing over 50 GMM proposals every year. Any new pieces of work will be assessed by a written proposal which will then be reviewed.

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<td>Non-microbial</td>
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Other (please specify)  
Tick if confidential

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<tr>
<td>Yes</td>
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</table>
Disinfection and autoclaving will be carried out where required. All waste materials will be disposed of into autoclave bags, yellow clinical waste bags/bins or sharps bins as required. All contaminated solid waste (agar plates, disposable plasticware etc) will be placed in autoclave bags and will be autoclaved and disposed of via designated route. Liquid cultures will be chemically-inactivated (Virkon 1% or PeraSafe 0.16% for 30 mins) or autoclaved prior to disposal. All contaminated glassware and reusable plasticware will be decontaminated as above.

a) Disinfection.
1% Virkon will be used for disinfection. The surfaces of the microbiological safety cabinet will be disinfected with 1% Virkon after use. Laboratory benches will be swabbed with 1% Virkon after any activity.

b) Autoclaving.
Autoclaves are not present in the laboratory for waste treatment. However, a large central autoclave facility is located on site at Alderley Park (please see next section for the further details of the Waste management process and derogation request).
The autoclave is subject to twice yearly servicing and annual validation using a 12-point thermocouple procedure which includes a probe placed in a representative load.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC met on 29th April 2015 to review Risk Assessment 004 which had been given a provisional class 1 risk assessment. It was agreed that this was appropriate as there was no significant risk to either the environment or operator.
GM Centre Number: 3262

Data Premises Notified (Originally) 18/02/2015
Data Premises Closed

Transferred from 1992 Regs? N
Transitional Premises
Emergency Plan Required? N

Transitional Premises
Non-GMMs N
Withdrawn N

Name
UNIVERSITY OF SOUTH WALES

Name 2

Department
MICROBIOLOGY RESEARCH

Campus Estate or Research Centre

Building

Road Name

District
PONTYPRIDD

Town
CARDIFF

County
SOUTH WALES

Postcode
CF37 1DL

Country
WALES

Tel Number 03455760101
Fax Number BLANK

E-mail

HSE Division WALES AND SOUTH WEST

Comments

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<td>Alfred Russel Wallace Building</td>
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<td>CARDIFF</td>
<td>CF37 4AT</td>
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<td>N</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

A risk assessment has been carried out by a competent person. This assessment is subject to the university's Sign-off and review procedures. The main findings of the risk assessment, along with the necessary control measures, will be brought to the attention of all the relevant staff and students and discussed as appropriate by the quartely school and University Health and Safety Committees. Where necessary, specific representation will be made at these Committees from Biologically qualified staff, with expertise in GM work, to supplement existing School/University Management and Occupational Health and Safety staff.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

QUOTIENT BIORESEARCH (RADIOCHEMICALS) LTD

**Campus Estate or Research Centre**

**Road Name**

NETTLEFOLD ROAD

**Town**

CARDIFF

**County**

WALES

**Postcode**

CF24 5JQ

**Tel Number**

02920 474 900

**Fax Number**

02920 474 974

**E-mail**

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**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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<td>WALES</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

An independent competent person has reviewed the risk assessment for the proposed work with GMMs and has confirmed that it has been classified correctly as class 1. This person has an Honours Degree and a PhD in microbiology and over 10 years of experience in GMO risk assessment and biorisk management

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Tick if confidential

Bacteriology: Yes

Parasitology

Transgenic Birds

Transgenic Animals

Microbiology Research

Transgenic Fish

Gene Therapy

02/03/2022
The GMMs to be used will be class 1 and the risk assessment for the work does not identify risks to either human health or to the environment. It is, however, Quotient's policy to inactivate all biological waste and 100% kill is anticipated. All solid waste containing class 1 GMMs will be autoclaved in the laboratory. Autoclave cycles (121°C for 30 minutes) will be validated using biological indicator strips. The autoclave is subject to statutory test and examinations. All liquid waste will be incinerated or treated with 1% (final concentration) Virkon solution overnight prior to disposing to drain. Surfaces will be cleaned with azowipes (79% isopropanol aq.) or 70% ethanol aq.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The competent individual has reviewed the risk assessment for the proposed work and concluded that the activities to be performed are class 1: the modified progenitor bacteria are Hazard Group 1, as classified under Part I of Schedule 3 of the Control of Substances Hazardous to Health Regulations 2002 (as amended) and: the planned genetic modification will alter metabolic pathways and are considered very unlikely to alter pathogenicity traits or present a hazard to the environment.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The proposed work will involve culturing class 1 GMMs (e.g. actinomycetes)
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Name
EDGE HILL UNIVERSITY

Name 2

Department

Campus Estate or Research Centre
BIOSCIENCES

Road Name
ST HELENS ROAD

Building

District

Town
ORMSKIRK

County
LANCASHIRE

Postcode
L39 4QP

Country
ENGLAND

Tel Number
01695 575171

Fax Number
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E-mail

HSE Division
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Comments

Date at Which Additional Info Submitted
02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The Edge Hill University Genetically Modified Organisms Committee has been formed as a reporting sub-group to the Bioscience Building Health and Safety Committee. The first meeting of the group was held on the 8th May 2014, followed by a secondary meeting on the 3rd June 2014. During these meetings the remit and terms of reference for the committee was agreed. The group will be in charge of reviewing all risk assessments for new practical’s and research that involve genetic modification of organisms. Responsibility to ensure that facility containment is appropriate will also rest with this committee. The group is chaired by the Senior Technician in charge of research facilities and is attended by the Head of Department, Senior Research Academics, Chair of the Biosciences Health Safety Committee, with invitations to Student Union representatives from each academic year and PhD students with relevant experience. Any individual who has a working interest within the institution will also be allowed to attend.

Level 1 (GMMs)

Yes

Level 2 (GMMs)
Yes

Any material contaminated by GMO’s such as tips, petri dishes, syringes, swabs, and cleaning materials will be fully autoclaved to kill contaminants. Autoclaved waste will then be double bagged, or placed into specified sharps bins and will be destroyed via incineration. All surfaces will be sanitised using ethanol or Vercon based cleaning products.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Any material contaminated by GMO’s such as tips, petri dishes, syringes, swabs, and cleaning materials will be fully autoclaved to kill contaminants. Autoclaved waste will then be double bagged, or placed into specified sharps bins and will be destroyed via incineration. All surfaces will be sanitised using ethanol or Vercon based cleaning products.

**Tick to confirm that you are attaching a summary of the risk assessment**

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

**Please enter comments of the GM safety committee on the risk assessment**

The committee agreed with the suitability of the current risk assessment format. No future proposals were made for work that required containment levels above that of level 1. It also agreed that the facilities and disposal methods are appropriate for this level of containment and that it would meet and review this status on at least an annual basis or as new GMO related projects arise.
### Project Additional Information

#### Purposes of the contained use

The purpose of the contained use is to enable the development and/or use of viral and plasmid vectors to manipulate the expression of genes associated with development, disease pathogenesis and treatment. Gene expression changes, such as overexpression or knock down, are anticipated to result in changes to cellular structure, signalling, interactions, metabolism and ultimately function. These changes can lead to cellular death, cellular reprogramming and directed differentiation into specific cell types.

All vectors used are disabled either by being devoid of the genes required for replication and/or being split between separate plasmids which must be combined to produce a viral particle. In addition to this, packaging plasmids are required in order to aid entry into the cell. These vectors will have no impact on the environment as they will be created, used, contained and disposed of under strict class 2 laboratory conditions.

#### Recipient or parental organism

Recipient organisms include primary cell cultures obtained from human and mammalian tissue, induced pluripotent stem cells (iPSCs), embryonic stem cell (ESC) lines, adult stem cells, established mammalian and invertebrate cell lines and bacterial and fungal species.

Example tissues include placenta, lung, skin, liver, brain, spleen, heart, intestines, kidneys, foetal and embryonic material. Additional primary cell cultures include keratinocytes, fibroblasts, epithelial, CFBE cells.

IPSCs will be derived from human and mouse cell cultures.

Human and mouse ESCs and adult stem cells will be obtained commercially or from collaborators or repositories.

Established mammalian cell line examples include HeLa, HaCaT, KBM-7, HAT1, A549, ISHIKAWA, BeWo, HEK 293, 293T, H441, NHBE, CFBE, A549, KELLY, CHO, 1HAEO, A549, HBE, SK-N-BE(2).

Insect cell line examples include Sf21.

Example bacterial cells include Agrobacterium tumefaciens and LBA4404.
Plasmids for direct gene expression of foreign genetic material include pcDNA-DEST53, 47, 40
Plasmids for lentivirus production include pLenti6/UbC/V5-DEST, pLenti6.2/C-Lumio/V5-DEST, pBABE-puro, STEMCCA, MISSION® pLKO.1-puro
Plasmids for adenovirus production include pShuttle-Cre-HA
Binary Plasmids include Pgreeni
Helper plasmids and entry vectors include pENTR
Transcribed lentiviruses, adenoviruses
Nanovesicles

Cell hosts and viral packaging cells include HEK 293, AdenoX 293 and Agrobacterium tumefaciens cells.
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) CAS9

The foreign genetic material inserted includes human and mammalian cDNAs that are involved in aspects of cellular transformation, self-renewal, proliferation, signalling, structure, metabolism, ion transport, differentiation and apoptosis. These genes would include, but are not limited to transcription factors, enzymes, growth factors, signalling molecules, ion channels and angiogenic factors and cytokines, some of which can be seen in the list below:

- **Transformation** - hTert, Bmi1, cMYC
- **Self renewal** - cMyc, Klf4, Oct4, Sox2
- **Proliferation** - NUDT2, TP53, MYCN
- **Signalling** - IGF
- **Structural** - JUP, NF1, DNAH5,
- **Metabolism** - ALOX12B, ALOXE3, PNPL2, ApoE, LCAT
- **Ion transport** - ENaC, CFTR,
- **Differentiation** - TGM1, CERS3, ABCA12, LIPN, NIPAL4, IL6, MRTF-B,
- **Apoptosis** - MRTF
- **Joint lubrication** - PRG4

Viral DNA to be inserted into insect cells - BaculoDirect™ C-term Linear DNA Gateway® Cassette contains the genetic information that enables cutting and ligation of insect DNA in one reaction.

In addition to the above, reporter genes would be expressed constitutively or conditionally as cellular markers. Reporter genes include fluorescent markers (GFP, YFP, CFP, mCherry, PicoGreen, DY677, Cy3), luminescent markers (firefly, gaussia and vargula luciferases) and biochemical markers (AFP, CAT).

The expression of genes, such as those above, would be manipulated by overexpressing genes (introduction of trangege, gene correction), causing exon skipping, or by expressing specific complementary nucleotide sequences in the form of siRNA, shRNA and GAPMERS to reduce or prevent gene expression. The CRISPR CAS9 system will also be used to produce mutations in target genes and reduce or prevent their expression.

### Evaluation of foreseeable effects

Primary human tissues and cells have the potential to carry pathogenic organisms such as viruses and bacteria. Established cell lines and embryonic and adult stem cells are often pre-screened for such pathogenic organisms. Under no circumstances will researchers be permitted to culture their own cells.
To reduce the risk of generating replication competent lentivirus (RCL), the parts of the genome critical for viral replication are deleted or severely modified enabling the transduction of target cells but preventing the production of new virions. After transduction and integration into the target cell's DNA, self-inactivating mutations prevent further transcription. Lentiviral genes necessary for packaging are carried in separate plasmids reducing the risk of generating RCL by increasing the number of recombination events required for their generation.

Adeno
Adenoviruses are DNA viruses capable of causing respiratory tract and intestinal infections and can be spread via mucous membrane contact including the inhalation of droplets. Shuttle and adenoviral genes are carried on separate plasmids. To minimise the potential for adenoviral vectors to replicate, portions of the Early Regions 1 (E1) and 3 (E3) of wild-type adenovirus have been deleted from the genome. The elimination of E1 elements means that it requires a cell line that expresses E1 genes for viral replication and transcription of the viral DNA. Exposure of somatic cells that do not express E1 genes leads to a transient non-cytopathic infection that is not replicated or actively transcribed. Packaging of this viral vector after the transfection of E1 gene containing cells, is capable of infecting human cells. Therefore, adenoviruses capable of expressing oncogenes will not be created so as to avoid any chance of these genes being expressed in vivo.

Adeno Associated Viruses (AAV)
AAVs are replication-limited and typically not known to cause disease in humans. AAVs have a small packaging capacity and can transduce both dividing and non-dividing cells with a low immune response and low toxicity.

The majority of the gene products expressed are expected to affect cellular properties such as proliferation, apoptosis, migration, adhesion and fate. Also, some have known or suspected oncogenic or tumour suppressor properties.

Gene products in plasmid form exist in non-mobilisable constructs and are therefore of minimal environmental risk. Shuttle and adenoviral genes are carried on separate plasmids. Viral vector expressing potential oncogenes are a potential environmental risk as host infection could elicit a transformation event. All work is carried out in a class II cabinet and all liquid waste inactivated by virkon treatment and solid waste autoclaved.

Primary cells infected with viral vector containing potential oncogene would pose minimal threat as expression would most likely be restricted to the infected cell, which in itself would be rejected by the host immune system.

The recombinant viral vectors are all highly disabled vectors, the design of which has greatly reduced the risks associated with them. In addition, to minimise contact with viral particles, all research involving viral vector production and use will take place under strict containment level 2 conditions in class II biological safety cabinets. This will minimise exposure to liquid aerosols. The use of sharps will be minimal and restricted to pipette tips. Single entry biohazard labelled sharps containers for tips will be present in the work space to prevent handling.

Bacterial and fungal species
Group 2 bacterial and fungal species have the potential to cause human disease via contact with mucous membranes including inhalation and sharps injuries. Agrobacterium tumefaciens causes crown gall disease of a wide range of plants.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All surfaces will be disinfected pre and post carrying out GMO work use using 70% ethanol, 1% virkon or an anti-viral disinfectant such as Bacillolcid. Spillages will be...
cleaned using 70% ethanol, 1% virkon or Baccilocid. Solid waste (mainly plastic ware such as flasks and tubes) will be collected in autoclave bags and autoclaved using a make safe cycle (121-125oC for a minimum of 15 minutes). Excess liquids will be discharged into drains and solids disposed of solids via clinical waste stream for incineration. Solid waste can also be collected in biohazard labelled clinical waste bags and taped up when 80% full, using biohazard tape. These bags will be transferred, by trained laboratory staff, to large, plastic clinical waste eco-lock bins that will be collected by a registered waste contractor for off site incineration. Sharps, including pipettes and tips, will be disposed of in plastic biohazard labelled sharps containers. Sharps boxes will be autoclaved at 134 oC for a minimum of 15 minutes and removed and incinerated by waste contractors. Liquids (eg samples, culture supernatants, tissue culture media) will be autoclaved using a make safe cycle (121-125oC for a minimum of 15 minutes) and discharged to drains. Agar plates will be autoclaved using a make safe cycle (121-125oC for a minimum of 15 minutes). Excess liquid will be discharged into drains and solid waste will be disposed of via the clinical waste stream for incineration.

Autoclaves used to inactivate waste must will be subject to regular validation, calibration and maintenance. Validation of inactivation of waste will take place using a biological indicator such as Geobacillus stearothermophilus. Autoclave tape will be used to ensure that sterilisation temperatures have been reached. Calibration of the autoclaves will be carried out annually or as required. An autoclave will be located in the same building as the GMO work is being carried out. Records of GMM inactivation will be kept by the University for a period of 12 months and available on request.

The Genetic Modification Health and Safety (GMOHS) Committee at Edge Hill University reviewed the CU2 application and risk assessment at a meeting on 26.01.18. Minor revisions were suggested and made including changes to inactivation and waste disposal procedures, the addition of validation measures for autoclave inactivation and it was decided that pregnant personnel would be advised against carrying out work with class II GMOs. The revised document was subsequently approved for submission by the committee.

Please enter comments on the GM safety committee on the risk assessment

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Project Containment

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02/03/2022
## GM Centre Number: 3266

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Directors of London Biological Laboratories have appointed a Safety Committee (SC) which is responsible for reviewing and providing advice on contained use risk assessments to the management. The SC consists of the Biological Safety Officer (BSO), two directors of London Biological Laboratories, and any additionally appointed advisers with relevant expertise as necessary. Principal investigators are required to submit risk assessments to the SC, who will review and provide advice on adequacy of the risk assessment. The BSO will integrate this advice into the risk assessment and give final advice, with respect to approval for the contained use to take place, to the person responsible. The members of the SC represent a balance of experienced scientists, management, and lab users. The committee members include individuals with extensive postgraduate biological research experience. The BSO, particularly, has an extensive background working in class 2 contained use settings. Through partnerships with local universities and other scientific institutions, the SC has access to a wide range of experts from who are willing to provide advice on risk assessments for contained use.

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All lab users receive an induction into the procedures for the handling and containment of GMO, and this includes a section on waste management and monitoring measures. These procedures are regularly reviewed and updated by our internal safety committee, and also by external review by competent assessors and are detailed in our Standard Operating Procedures documentation.

Ensuring that lab users follow the practices in compliance with the published procedures is enforced by the BSO with the full support of the management.

According to established practice all waste (solid and liquid) and equipment that have come into contact with GMO are autoclaved at 121°C at 15 psi for at least 15 minutes. This is reported to reduce the number of viable organisms by six logs (99.9999% degree of kill) and therefore this is our preferred method for GMO inactivation.

Autoclaving is done on site in the biolab, and all users are trained to operate the autoclave and to run a daily test. Monitoring of the level of inactivation is performed monthly by the use of a plate growth assay.

For activities involving GMMs, describe the waste management measures which will apply to the activity
The autoclave is tested to ensure that it is functioning to the manufacturer’s specification, and this is done in two ways, firstly using a programmed pressure/vacuum test is run and secondly autoclave test strips are used to ensure that the necessary temperature has been obtained. The results of these tests are recorded in a log book.

These requirements are codified in our SOPs and all lab users are required to follow them strictly. Further, to ensure surfaces are free of contamination, worktops are cleaned with 1% virkon solution before and after a protocol is performed.

In the case of unanticipated spillages and similar accidents in the lab, a dedicated GMO spill kit is provided. The Spill Kit is inspected regularly by the BSO or another competent lab user, and its use is described during the safety induction and is detailed further in its own SOP. The Spill Kit contains at least two separate disinfectants that are suitable for inactivation and disinfection of a spillage, the spill kit also contains plastic disposable aprons, gloves, shoe covers, absorbent materials, and autoclave bags, to enable efficient and complete clean up. Once the spill kit has been used to clear up a spillage, the materials that have been used are autoclaved prior to disposal.

This risk assessment was submitted to the SC and after minor modifications it was approved. The risk assessment was also reviewed by an external adviser who provided advice to the committee.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

This risk assessment was submitted to the SC and after minor modifications it was approved. The risk assessment was also reviewed by an external adviser who provided advice to the committee.
**GM Centre Number: 3267**

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**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Vice President Preclinical and Clinical Development - Chairman of GMM Safety Committee
- HSE Manager (Safety Officer) - Secretary of GMM safety committee
- Senior Scientist (Biological Safety Officer, Scotland)
- Senior Scientist (Biological Safety Officer, Northern Ireland)
- Laboratory Support Team Leader (Biological Safety Officer)

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify) Tick if confidential

Bacteriology Yes Parasitology Transgenic Birds Microbiology Research

02/03/2022
All contaminated waste and cell culture waste, including cultured cells and vessels, growth media, disposable plastics, laboratory glassware and other contaminated materials will be autoclaved at 121°C for at least 15 minutes to inactivate GMOs (100% kill) prior to disposal or cleaning and recycling. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored with autoclave indicator tape to show the target temperature of 121°C is reached. Periodic swabs will be taken from the culture media after autoclaving to ensure effective operation. Inactivated, autoclaved waste will be uplifted and disposed of by incineration by a commercial contractor.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
## GM Centre Number: 3268

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Quarterly health and safety meetings for the whole of the laboratory
Monthly health and safety meetings for the cell culture team

<table>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

02/03/2022
Inactivation of all plasticware and media that has come into contact with the GMM with 1% virkon solution. The liquid and/or plasticware will then be autoclaved at 121°C for 15 minutes (internal probe in the autoclave and TST test strips included in the load). The autoclave waste is then put into 60L incineration bins off site by SRCL. SRCL provide us with a quarterly notification of destruction.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

HEK293 eukaryotic cells are unable to survive outside their laboratory environment. All waste that is generated from the use of these GMMs will be disinfected or autoclaved. The risk level of using this cell line is low.

Project Ref 3268/15.1

Date Ackn’d 12/03/2015

CU2 Project Title Handling of GMO cell lines (class 1 and class II)

Class 2

CultureVol

< 1 Litre

Consent Granted

Class CultureVol

Class 2

< 1 Litre

Non-GMM

Consent Granted

Project notified under transitional arrangements

Historical Date of Additional Info

Significant Change ID

02/03/2022
### Project Additional Information

#### Purposes of the contained use

Biological cell potency assays

#### Recipient or parental organism

HEK293 cells - human embryonic kidney cells

#### Host/vector system

Adenovirus 5

#### Origin & function

HEK 293 is a virally transformed, stable well established human epithelial kidney cell line that is routinely used in expressing plasmid derived proteins. This cell line has been established for laboratory use only and would not survive long without specific culturing conditions. The HEK293 cells contain DNA from Adenovirus 5. The cell line will be cultured for a cell-based potency assay for a chemotactic drug product.

#### Evaluation of foreseeable effects

HEK293 cells are unable to survive outside their laboratory environments and are therefore unlikely to cause harm to other organisms

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A - full containment of the live cells will be used

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Inactivation of all plasticware and media that has come into contact with the GMM with 1% virkon solution. The liquid and/or plasticware will then be autoclaved at 121°C for 45 minutes (internal probe in the autoclave and TST test strips included in the load). The autoclave waste is then put into 60L incineration bins for incineration off site by SRCL. SRCL provide us with a quarterly notification of destruction.

#### Is an emergency plan required according to regulation 20?

No

#### If yes, tick to confirm that it is attached to this form

No

#### Tick to confirm that you have attached a risk assessment to this form

Yes

#### Tick if you are claiming exemption from disclosure for section of the risk assessment

No

---

02/03/2022
HEK293 eukaryotic cells are unable to survive outside their laboratory environment. All waste that is generated from the use of these GMMs will be disinfected or autoclaved. The risk level of using this cell line is low.

### Project Containment

<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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#### Project Ref 3268/18.1

- **Date Ackn’d**: 25/01/2018
- **CU2 Project Title**: Analysis of modified adenovirus ONCOS-102 by Dynamic Light Scattering
- **Class**: Class 2
- **Culture Volume**: ≤ 1 Litre
- **Non-GMM Consent Granted**: Consent Granted
- **Project notified under transitional arrangements**: N

#### Purposes of the contained use

The organism is used as a cancer therapy, samples of the virus will be submitted to our site for analysis. The virus will be analysed by Dynamic Light Scattering (DLS), this is a non-destructive test used to determine the degree of aggregation of the virus particles. This information will be used to characterise the product to provide data to support stability and process development.
### Recipient or parental organism

Adenovirus type 5

### Host/vector system

None

### Origin & function

The virus contains three modifications these are:

i. A deletion in the E1A gene preventing the virus from replicating in none dividing cells

ii. Introduction of the gene for human Granulocyte Macrophage Colony Stimulating Factor (GMCSF), a chemokine added to enhance the immune response

iii. Replacement of the Adenovirus type 5 fibre knob sequence with than of the Adenovirus type 3 sequence.

### Evaluation of foreseeable effects

Adenovirus type 5 is an ACDP hazard group 2 pathogen which causes mild respiratory symptoms and is infectious via the aerosol and the faecal-oral routes. The modified virus contains a deletion in the E1A gene, therefore preventing transformation of infected cells. This will limit replication to cells which are already undergoing cell division, as this process will no longer be triggered by the virus.

Expression of GMCSF is expected to cause an upregulation of the immune response at the site of infection due to activation of T-cells, this would not be expected to cause a hazard. Replacement of the Ad5 fibre knob with the Ad3 fibre knob will change the cell surface receptor used for viral entry. The receptor for Ad5 is down regulated in many tumour cells, both rectors are widely expressed in normal tissues. This modification would therefore not be expected to cause a change in the hazards associated to the virus (both Ad5 and Ad3 are hazard group 2 organisms, which exhibit the same clinical symptoms).

The pathogenic traits of the virus will be reduced compared to the parent as viral replication will be limited to dividing cells, due to the E1A deletion. The construct does not contain a known mobilisable genetic element (mob elements), it is unlikely that the virus would transfer genetic material to another organism.

The virus could cause an infection, however due to the E1A deletion this would be expected to be less productive than the wild type virus. Wild type adenovirus causes mild flu like or gastrointestinal symptoms, the modified virus is highly unlikely to be more pathogenic than the wild type.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Liquid waste will be treated over night with Virkon resulting in a least a 6 log reduction in virus titre. Contaminated plastics (pipette tips, tubes and cuvettes) will be autoclaved resulting in a 100% kill, prior to disposal as clinical waste for incineration.
Further detail requested on clean down for SSC and spill procedure, added to assessment prior to approval.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Is an emergency plan required according to regulation 20?

If yes, tick to confirm that it is attached to this form

Tick to confirm that it has been attached to this form

Is an emergency plan required according to regulation 20?

Project Containment

Laboratory Activities

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Glass Houses

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Growth Rooms

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Large Scale Activities

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Human Clinical Applications

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Project Ref 3268/19.1

Date Ackn’d 28/11/2019

Date Project Ceased

CU2 Project Title

Biophysical Characterisation of Vaccine Viruses

Class

Class 2

Consent Granted

Culture Volume

< 1 Litre

Project notified under transitional arrangements

Historical Significant Changes

Withdrawn

Tick if notifying a connected programme of work
## Project Additional Information

### Purposes of the contained use
- Analytical testing to support product development

### Recipient or parental organism
- Arenaviruses, Lymphocytic Choriomeningitis Virus Armstrong strain and Pichinde Virus

### Host/vector system
- None

### Origin & function
- non-ocogenic fusion protein of Human Papilloma Virus 16 early proteins E6 and E7

### Evaluation of foreseeable effects

The parent Arenaviruses LCMV Armstrong strain and PIC are both hazard group 2 organisms. It should be noted that per the approved list of biologics the majority of LCMV strains are class 3, however the Armstrong strain is specifically named as a class 2 (this is a lab adapted strain). These organisms cause flu like symptoms with a typical incubation period of 5-21 days. The strains have been attenuated and are expected to be less pathogenic than the wild type parent organisms, however at the present time it can not be confirmed that the attenuation is sufficient to support moving to class 1. Expression of the wild type versions of the E6 and E7 proteins leads to oncogenic transformation, the constructs contain a fusion protein of E6 and E7 which has been in engineered to be non-oncogenic. This non-functional protein is expected to generate an immune response against E6 and E7 proteins.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated overnight with Virkon resulting in at least a 6 log reduction in virus titre. Contaminated plastics will be treated as clinical waste and removed from site in sealed containers for incineration.
The committee requested that a clarification was added that the majority of LCMV strains are class 3, while the Armstrong strain used is specifically named as a class 2.

**Project Containment**

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**Project Ref** 3268/19.2

**Date Ackn’d** 25/12/2019

**CU2 Project Title** Analysis of MVrhS-HEXA Recombinant Adeno-Associated Virus (MV) by DLS

**Class** Class 2

**Culture Vol** < 1 Litre

**Culture Volume Class** Class 3-4

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**
**Project Additional Information**

**Purposes of the contained use**
The GMO is under evaluation as a gene therapy product. Intertek will perform biophysical analysis to provide data to support manufacturing of this product.

**Recipient or parental organism**
Adeno Associated Virus

**Host/vector system**
None

**Origin & function**
The gene encoding the human Beta-hexosaminidase subunit alpha has been introduced. The construct is designed for use in gene therapy to treat individuals with mutations in the Beta-hexosaminidase subunit alpha gene which results in Tay-Sachs disease.

**Evaluation of foreseeable effects**
A previous study using a similar construct demonstrated that intracranial injections of AAVrh8 encoding monkey i3-NAcetylhexosaminidase, resulting in overexpression of the HEXA gene caused neurotoxicity in the non-human primate brain (Golebiowski et al., 2017). However, this transgene was under control of a CSA (chicken beta actin) promoter/WPRE which may induce expression at different levels versus the promoter used in the constructs here. Of note, AAV8 does not cross the blood-brain-barrier reducing the risk of neurotoxicity for handlers of the GMO. In addition to neuronal tissue, AAV8 is capable of transducing muscle, liver, retina and pancreas the effect of expression of the transgene in these tissues is not known.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
NA

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Liquid waste will be treated with Virkon resulting in at least a 6 log reduction in virus titre. Contaminated plastics (pipette tips, tubes and cuvettes) will be autoclaved resulting in a 100% kill, prior to disposal as clinical waste for incineration.

**Is an emergency plan required according to regulation 20?**
N

**If yes, tick to confirm that it is attached to this form**
N
**Project Containment**

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<th>Laboratory Activities</th>
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- Animal Units
- Large Scale Activities
- Human Clinical Applications

**Project Ref** 3268/20.1

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**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
### Purposes of the contained use

Analytical testing GMO to support product development

### Recipient or parental organism

Adenovirus serotype 5

### Host/vector system

None

### Origin & function

The inserted sequence encodes two ligands which activate the human immune system

### Evaluation of foreseeable effects

Adenovirus serotype 5 cause acute respiratory disease and occasionally pneumonia in both children and adults. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection that can cause more systemic diseases. Unmodified Ad5 is classified in hazard group 2 under the COSHH Regulations.

While the precise identity of the transgene is unknown, the information provided states that it encodes for two ligands that activate the immune system. This may result in several undesirable effects including cytokine storm, direct activation of cytotoxic lymphocytes or other non-specific proinflammatory responses, which if untreated may be detrimental to health

The supplied vector is a first-generation recombinant adenovirus with deletions in the E1NE3A genes rendering it replication incompetent. The coding sequence of the proteins has been situated in the E3A cassette.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NA

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated overnight with Virkon resulting in at least a 6 log reduction in virus titre. Contaminated plastics will be autoclaved on site prior to being treated as clinical waste and removed from site in sealed containers for incineration.

---

**Is an emergency plan required according to regulation 20?**  

- **N**  

**If yes, tick to confirm that it is attached to this form**  

- **N**  

**Tick to confirm that you have attached a risk assessment to this form**  

- **Y**  

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  

- **N**
Please enter comments on the GM safety committee on the risk assessment

None

Project Containment

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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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Animal Units
Large Scale Activities
Human Clinical Applications

L2 L3 L4 L2 L3 L4 L2 L3 L4

Project Ref 3268/21.1

Date Ackn’d 14/01/2021
CU2 Project Title Analysis of Equine Infectious Anaemia Virus (EIAV) gene therapy vector

Date Project Ceased

Class 2 CultureVolClass2 < 1 Litre CultureVolumeClass3-4
Non-GMM Consent Granted

 Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
Historical Date of Additional Info

Significant Change ID
Date of Significant Change

Project Additional Information

Purposes of the contained use
Analytical testing GMO to support product development

Recipient or parental organism
Equine Infectious Anaemia Virus (EIAV)

Host/vector system

none

Origin & function

The inserted sequence encodes three human genes required for the biosynthesis of neurotransmitters under the control of the CMV promoter

Evaluation of foreseeable effects

EIAV can cause mild flu like symptoms in humans, however in this case the construct is a 3rd generation self inactivating (SIN) gene therapy vector which lacks all accessory and replication genes required for viral propagation. During production these functions were provided in trans by simultaneous transfection with multiple separate plasmids, which are not carried forward to the final construct. The vector is pseudotyped with the vesicular stomatitis virus (VSV) capsid to allow infection of multiple cell types. While the virus is expected to be infectious the infected cells would not generate any new progeny virus particles. Expression of the transgenes is not expected to cause toxicity.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

NA

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste will be autoclaved on site prior to disposal as clinical waste for incineration

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

None

Project Containment
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Name change from - KWS BioTest Ltd 02/04/2019

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Biological and Genetic Modification Safety Committee meets at least quarterly but extraordinary meetings may also be held. The committee is chaired by the company's Biological Safety Officer and is composed of the Senior Health and Safety Scientist and scientists from the infectious disease, cell biology and immunology teams. All members have at least 3 years experience working in bioscience and have received formal training in biosafety. The precise composition at each meeting may vary depending on the agenda items. This is to ensure that the committee has the right level of expertise to review submissions/issues and any conflicts of interest are removed. Other specialists are invited to attend as and when required.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs) | Yes | | | |
Level 2 (GMMs) | Yes | | | |
Level 3 (GMMs) | Yes | | | |
Level 4 (GMMs) | Yes | | | |
Non-microbial

Other (please specify)  

Tick if confidential  

Bacteriology  Yes  Parasitology  Transgenic  Transgenic  Transgenic  Transgenic  Transgenic  Transgenic  Transgenic

Bacteriology  Yes  Parasitology  Transgenic  Transgenic  Transgenic  Transgenic  Transgenic  Transgenic  Transgenic

Virology  Yes  Transgenic  Transgenic  Transgenic  Transgenic  Transgenic

Virology  Yes  Transgenic  Transgenic  Transgenic  Transgenic  Transgenic

Mycology  Transgenic  Transgenic  Transgenic  Transgenic  Transgenic

Mycology  Transgenic  Transgenic  Transgenic  Transgenic  Transgenic

Other(s)  

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

Project Ref  3269/15.1

Date Ackn'd  09/03/2015

Date Project Ceased

CU2 Project Title  Use of bioluminescence to quantify bacteria in infection

Class  Class 2  CultureVolClass2  < 1 Litre

Consent Granted

Non-GMM  Consent Granted
**Purposes of the contained use**

Our laboratory performs a number bacterial infection models, which are used in the investigation of novel antimicrobial agents. The ability of the novel compounds to inhibit growth of bacteria is tested. The test will be a determination of the numbers of bacteria present and the hope is that treatment will reduce these numbers.

For some of our studies, we aim to quantify the numbers of bacteria at serial timepoints through the course of infection, by using imaging methods. In order to do this it is necessary to use bacteria which have been genetically modified to produce light (bioluminescence). These light-producing bacterial strains are commercially available and the light production is due to the introduction of a gene such as luciferase, exploiting the same principle as the production of light in many species in nature, for example the firefly. By quantifying the amount of light, the number of bacteria can be determined at several different timepoints during the course of infection.

**Recipient or parental organism**

This work encompasses the following bacterial strains: *Staphylococcus aureus*, *Streptococci pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.

These recipient (host) micro-organisms are bacteria which are ACDP hazard group 2 and are genetically modified to render them bioluminescent (in the presence of luciferin substrate).

Acinetobacter is readily found throughout the environment including drinking and surface waters, soil, sewage and various types of foods.

*Escherichia coli* is commonly found in the intestines & faeces of humans and animals.

*Pseudomonas aeruginosa* is commonly in soil and water.

*Staphylococcus aureus* is a common coloniser of human skin and can be found on environmental surfaces around infected individuals.

*Streptococcus pneumoniae* is part of the normal human upper respiratory tract flora & has been isolated from aquatic environments such as seawater.

These bacteria do not tend to cause disease in immunocompetent individuals, but can be problematic in the immunosuppressed, neonates or the elderly. The risk assessment outlines primary containment and PPE measures which have been assessed for the handling and containment of these organisms.

**Host/vector system**

The host organisms covered by this risk assessment are bacteria which are ACDP hazard group 2 and are genetically modified to render them bioluminescent (in the presence of luciferin substrate).
The genetically modified bacteria will be obtained from a commercial source or from a scientific collaborator. This risk assessment covers the use of these genetically modified bacteria and their wild type equivalents, not the generation of genetically modified bacteria.

The vector for bioluminescent transformation was the gram-positive lux transposon cassette, Tn4001 luxABCDE Kmr. Examples of the transformation strategy are further detailed in K.P. Francis, et al, infection & Immunity 2001 (69(5)3350-3358) and J.L. Kadurugamuwa et al Infection & Immunity, 2003 (71(2) 882-890).

Origin & function

Insertion of genetic material from the luciferase gene (Photorhabdus luminescens lux operon) renders the bacteria bioluminescent (in the presence of oxygen and the enzymatic substrate, luciferin), hence allow their detection using non-invasive in vivo imaging using a Caliper-Xenogen IVIS system.

The modified bacteria can also be selectively grown on kanamycin, by virtue of a resistance gene. GMO are bioluminescent bacteria which can be used to quantify a bacterial infection by monitoring the level of light produced in the presence of the luciferin substrate.

Evaluation of foreseeable effects

The main risks of these products used relate to the risk to human health by these ACDP hazard group 2 organisms. The bacteria used are each commonly found either in the environment or in human hosts. Furthermore, the genetically modified bioluminescent strains are no more virulent than the parental strains. This project should not contribute any additional risk to the environment even if there were to be an accidental release.

The containment and disposal measures detailed will minimise any chance of release of the viable bacteria into the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon is a bactericidal agent, which is used at a concentration of 1%. It has been shown to cause a 5-log reduction in bacterial counts (Hernandez, A. 2000. Journal of Hospital Infection. 46: 203-209).

In vitro GMM liquid waste will be disposed off into 1% Virkon. After 24 hours, this will be discarded to drains. Bacterial plates will be sealed and autoclaved. Contaminated tips will be soaked in 1% Virkon prior to autoclaving. Any contaminated glassware (kept to minimum) will be soaked in Virkon for 24 hours, then rinsed prior to collection for cleaning.

Other solid waste (plasticware eg homogenizing tubes, pipettes, pipette tips, flasks, tubes) will be disposed of as biohazard waste and autoclaved on site. Disposable plastic waste will be disposed of via the biological waste route. Biohazard waste will be removed from the laboratory to autoclaves for on site destruction.

Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains,
dispose of solids via the industrial (black bag) waste stream for landfill.

13. * Is an emergency plan required according to regulation 21?
   Yes No

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**Project Ref**  3269/19.1

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### Project Additional Information

**Purposes of the contained use**

medical research

**Recipient or parental organism**

Embryonal human kidney cells HEK-293FT. The following information is published by Thermo Fisher Scientific in the user guide for the 293FT cell line:

The 293 Cell Line is a permanent line established from primary embryonal human kidney transformed with sheared human adenovirus type 5 DNA (Graham et al., 1977; Harrison et al., 1977). The E1A adenovirus gene is expressed in these cells and participates in transactivation of some viral promoters, allowing these cells to produce very high levels of protein. The 293-FT Cell Line is a fast-growing variant of the HEK 293 cell line, and was originally obtained from Robert Horlick at Pharmacopeia.

293FT cells stably express the neomycin resistance gene from pCMVSPORT6TAg.neo. The pCMVSPORT6TAg.neo plasmid is derived from pCMVSPORT6, which has been modified to include the following features:

- The neomycin resistance gene for stable selection in mammalian cells (Southern & Berg, 1982). Expression of the neomycin resistance gene is controlled by the SV40 early enhancer/promoter from which the SV40 origin of replication has been removed.
- The gene encoding the SV40 large T antigen to facilitate optimal virus production (e.g., Thermo Fisher Scientific’s ViraPower™ Lentiviral Expression System) and to permit episomal replication of plasmids containing the SV40 early promoter and origin. Expression of the SV40 large T antigen is controlled by the human cytomegalovirus (CMV) promoter.

**References:**


Harrison, T., Graham, F., and Williams, J. (1977) Host-range Mutants of Adenovirus Type 5 Defective for Growth in HeLa Cells. Virology 77, 319-329

Studies have demonstrated maximal virus production in human 293 cells expressing SV40 large T antigen (Naldini et al., 1996), making the 293FT Cell Line a particularly suitable host for generating lentiviral constructs using the ViraPower™ Lentiviral Expression System available from Thermo Fisher Scientific.
References:


Host/vector system

A commercially available kit from Invitrogen. The ViraPower lentiviral expression system uses a replicon-incompetent lentivirus. Based on the lentikat system developed by Cell Genesys (Dull et al, 1998).

The ViraPower system is a third-generation lentiviral system and includes the following key safety features:

- The pLenti expression vector contains a deletion in the 3′ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
- The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).
- The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns et al., 1993; Emi et al., 1991; Yee et al., 1994).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).
- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
- The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.
- Expression of the gag and pol genes from pLP1 has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull et al., 1998).
- A constitutive promoter (RSV promoter) has been placed upstream of the 5’ LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA (Dull et al., 1998).

Various non-harmful constructs will be tested within the system.

Origin & function

The production of a reporter cell line using a previously genetically modified commercial cell line. The 293 FT cell line is a commercially available cell line that has been previously transformed using SV40 large T antigen. When a ViraPower expression vector and the ViraPower lentiviral packaging mix are co-transfected into 293FT cells, high levels of the viral RNA and the gag/pol and rev proteins required for packaging are produced.

The presence of the large T antigen within the cell line allows very high levels of protein to be expressed from vectors containing the SV40 origin.

Evaluation of foreseeable effects

The information below is published by Washington State University regarding hazards associated with HEK293 cells (the parental cell line of the cells in this study; with the exception of neomycin resistance, it is expected that 293FT cells will be highly similar). For more information, see: https://biosafety.wsu.edu/hek-293-cell-lines/
Family Adenoviridae—Genus Mastadenovirus— Human adenovirus Group C—Human adenovirus 5 (HadV-5). Non-enveloped double stranded DNA virus (1). Adenovirus has been completely sequenced and it includes a total of 35,937 nts (15 pp2359). The 1-4344 nts of HadV-5 represents approximately 12% of the full genome. This agent is unusually stable to chemical, physical and adverse pH conditions (2). Proteins transcribed from this left portion of the Adenovirus include proteins that are involved in oncogenic transformation and in positive regulation of transcription of the early genes of host infection (3), as well as cell cycle genes (4). However, Group C viruses are not known to be tumorigenic (15 pp.2379). It is well established that E1A proteins have mitogenic activity (4). In mammalian cells, the human adenovirus type 5 early region 1A (E1A) oncoprotein functions as a thyroid hormone (TH) dependent activator of the thyroid hormone receptor (TR) (5). Transcription of viral early genes leads to synthesis of some 17 early proteins, many of which perturb host cell or host physiology (4). The Adenovirus DNA genome provides it with the advantage that its DNA can persist in host cells as either a circular extra chromosome (plasmid) or by integration into the host DNA after the complete viral replication has stopped. It has been recently noted that adenoviral DNA from the E1A gene is correlated with COPD (6). The DNA segment which induces transformation is located between 1 and 6% from the left end of the HadV-5 DNA molecule (7). A 2002 study indicates that HEK-293 cells exhibit a pattern of intermediate filament expression similar to that seen in early differentiating neurons as opposed to cells derived from typical kidney epithelial or mesenchymal cells. This fact could have significant implications for experiments that use these cells as kidney cell controls or as non-neuronal control cells (8).

Primary hazards
Ingestion via the fecal oral route (9) Droplet exposure of the mucous membranes (9) Inhalation (through respiratory droplets) (10) Contact/hand-to-eye transfer (10)

Special hazard notes
Generalized infections can occur in immunocompromised individuals. Exposure to this agent can cause serious infection in the congenitally immunocompromised, in patients undergoing immunosuppressive treatment for organ and tissue transplants and for cancers, and in human immunodeficiency virus-infected patients. Adenovirus infections in these patients tend to become disseminated and severe. In all immunocompromised patients, generalized illness involving the central nervous system, respiratory system, hepatitis, and gastroenteritis usually have a fulminate course and result in death. Treatments for adenovirus infections are of little proven value (11). People who smoke may be at higher risk from exposure to HadV-5 (6). Using Adenoviral vectors in conjunction with these cell lines will result in replication-competent viruses as the portion of HadV-5 incorporated into chromosome 19 contains both the E1 and E3 regions that are generally deleted in commonly used Adenoviral vectors (12). Lab workers exposed to HEK-Cells may present an in vivo recombination opportunity between the 12% Adenovirus and latent adenoviruses in the human lab worker (host). Alternatively, poor work technique can cause propagation of adenovirus in the HEK cell lines by introduction of adenovirus from the lab worker (13)."

References
1. Boundless.com, Double-Stranded DNA Viruses: Adenoviruses
3. Harvard Catalyst, Harvard Catalyst Profiles, Adenovirus E1A Proteins
4. “Adenovirus type 5 exerts genome-wide control over cellular programs governing proliferation, quiescence, and survival,” by Daniel L. Miller, Chad L. Myers, Brenden Rickards, Hilary A. Coller, and S. Jane Flint, BioMed Central, Genome Biology, April 12, 2007
5. “Cellular Context of Coregulator and Adaptor Proteins Regulates Human Adenovirus 5 Early Region 1A-Dependent Gene Activation by the Thyroid Hormone Receptor,” Xianwang Meng, Yong-Fan Yang, Xiemin Cao, Manjapra V. Govindan, Michael Shuen, Anthony N. Hollenberg, Joe S. Mymryk, Paul G. Walfish; Molecular Enocrinology, June 1, 2003
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In vitro GMO waste will be disposed of in 1% virkon and after soaking overnight, autoclaved. Tissue culture flasks and pipettes will be soaked over night in 1% virkon and then autoclaved. Contaminated tips will be soaked in 1% virkon prior to autoclaving. Any contaminated glassware (kept to a minimum) will be soaked in 1% virkon for 24hours, then rinsed prior to collection for cleaning.

Other solid waste (plastic ware, eg tissue culture flasks, pipettes, tips, tubes) will be disposed of as biohazard waste and autoclaved on site. Disposable plastic waste will be disposed of via the biological waste route.

Autoclave programme is a make safe programme as specified in BS 2646 part 3 1993 (either 121-125 degrees C for at least 15 minutes, 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) any excess liquids after autoclaving are discarded to drains and solid waste disposed of via the clinical waste route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The committee members were in agreement that this work should go ahead at containment level 2 pending approval from the HSE. The experience and competency of our scientists performing the experimentation was noted to be very competent.

### Project Containment

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#### Project Ref 3269/20.1

**Date Ackn’d** 18/06/2020  
**CU2 Project Title** GM Influenza virus for use in vaccination studies or Influenza infection models  
**Class** Class 2  
**CultureVol/Class 2** < 1 Litre  
**CultureVolumeClass 3-4**  
**Non-GMM** Consent Granted  
**Project notified under transitional arrangements** N

**Withdrawn** N  
**Tick if notifying a connected programme of work** N

### Project Additional Information

**Purposes of the contained use** Medical research.

**Recipient or parental organism**
<table>
<thead>
<tr>
<th>Host/vector system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permissive cell lines (obtained from a reputable supplier), or infection models.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>All viral strains are purchased from NIBSC or ATCC or another reputable supplier. We do not intend to Genetically Modify or alter any of the strains in-house.</td>
</tr>
</tbody>
</table>

| The influenza reference virus NIBRG-23 is a re-assortant prepared by reverse genetics from A/turkey/Turkey/1/2005 (H5N1) virus (in which the polybasic HA cleavage site has been excised) and A/PR/8/34(H1N1) virus. (NIBSC) |
| The influenza reference virus NIBRG-301 is a re-assortant prepared by reverse genetics from A/duck/Vietnam/NCVD-1584/2012 (H5N1) virus (in which the polybasic HA cleavage site has been excised) and A/PR/8/34 (H1N1) virus. The known passage history of 18/134 is attached. |
| The influenza reference virus NIBRG-14 is a re-assortant prepared by reverse genetics from A/Vietnam/1194/2004 (H5N1) virus (in which the polybasic HA cleavage site has been excised) and A/PR/8/34(H1N1) virus. |
| Other re-assorted commercially available Influenza strains BSL2 level only. |

<table>
<thead>
<tr>
<th>Evaluation of foreseeable effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>The wild type strains of these reassortants (all H5N1 strains) are classed as highly pathogenic avian influenza viruses (HPAIV) and as such possess a polybasic HA cleavage site. The possession of this cleavage site has been shown to be one of the main virulence determinants in HPAIV, by allowing the HA to be cleaved intracellularly by ubiquitously occurring proteases and therefore have the capacity to infect various cell types and cause systemic infections.</td>
</tr>
<tr>
<td>The HA of all low-pathogenic avian viruses (LPAIV) and human influenza viruses carry a mono- or dibasic motif. These are cleaved extracellularly, which limits their spread in hosts to tissues where the appropriate proteases are encountered.</td>
</tr>
<tr>
<td>Excision of the polybasic HA cleavage site renders the strains a low pathogenic phenotype and limits the infection to the digestive and respiratory tract.</td>
</tr>
<tr>
<td>All staff directly working in the project must be vaccinated with the seasonal influenza vaccine.</td>
</tr>
<tr>
<td>Any member of staff displaying flu-like symptoms must not work with influenza strains.</td>
</tr>
<tr>
<td>Only one strain of influenza will be used at a time in both in studies to reduce the risk of reassortment.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n/a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>For only GMMs - application for any derogation from full containment for the Class of activity. (Measures &amp; Justification)</th>
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<tbody>
<tr>
<td>N/A</td>
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<table>
<thead>
<tr>
<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro GMO waste will be disposed of in 1% virkon and after soaking overnight and then autoclaved. Contaminated tips will be soaked in 1% virkon prior to autoclaving. Any contaminated glassware (kept to a minimum) will be soaked in 1% virkon for 24 hours, then rinsed prior to collection for cleaning. Other solid waste (plastic ware, eg tissue culture flasks, pipettes, tips, tubes) will be disposed of as biohazard waste and autoclaved on site. Autoclave programme is a make safe programme as specified in BS 2646 part 3 1993 (either 121-125 degrees C for at least 15 minutes, 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) any excess liquids after autoclaving are discarded to drains and solid waste disposed of via the clinical waste route. All waste is collected by a registered clinical waste company for incineration.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Is an emergency plan required according to regulation 20?</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
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<table>
<thead>
<tr>
<th>If yes, tick to confirm that it is attached to this form</th>
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<tbody>
<tr>
<td>N</td>
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</table>
The committee members were in agreement that this work should go ahead at containment level 2 pending approval from the HSE. The experience and competency of our scientists performing the experimentation was noted to be very competent.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L2</td>
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**Animal Units**

<table>
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<tr>
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<th>L4</th>
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**Large Scale Activities**

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**Human Clinical Applications**

<table>
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<tr>
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**Project Ref** 3269/22.1

**Date Ackn’d** 04/03/2022

**CU2 Project Title**

In vivo and in vitro immunogenicity study of Vaccitech Viral Vector Baboon Cytomegalovirus (BaCMV) Expressing GFP Antigen

**Class** 2

**Culture**< 1 Litre

**Culture Vol**

**Consent Granted**

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**
Purposes of the contained use

A baboon cytomegalovirus (BaCMV) strain originally acquired from ATCC was cloned into a bacterial artificial chromosome (BAC) to generate BaCMVBAC. BaCMVBAC harbours the entire BAC cassette that encodes several genes, including GFP. To rescue BaCMVBAC, DNA will be delivered by nucleofection into human fibroblasts (MRC-5 cells) and cells will be cultured until high titres of virus are produced. Viral stocks will be used in a variety of assays within a Bioscafet (Class II) cabinet in our BSL2 facilities.

Recipient or parental organism

Upon nucleofection into MRC-5 cells, BaCMVBAC DNA undergoes a full viral life cycle, i.e. the DNA is capable of replicating and producing virion components that assemble into infectious virus particles that are released to productively infect new MRC-5 cells. Many of the genes present in the BAC cassette serve no function in MRC-5 cells (since they are involved in bacterial cell regulation). However, GFP permits the visualisation of BaCMV-infected cells, allowing viral spread to be monitored over time.

Host/vector system

BaCMVBAC DNA will delivered into MRC-5 cells by nucleofection. Once virus is rescued, subsequent MRC-5 infection can be performed simply by culturing cells with virus-containing supernatant. BaCMVBAC carries no additional risks over BaCMV i.e. the presence of the BAC cassette does not alter the risks posed by working with BaCMV. Therefore it should still be handled as a BSL-2 agent.

Origin & function

The BAC cassette harbours genes important for maintenance and replication of the DNA in bacterial cells. These proteins provide no function when expressed in MRC-5 cells and therefore their risk to human health is essentially zero. The BAC will also express GFP, which has been widely used in thousands of studies and has never caused harmful effect in humans.

Evaluation of foreseeable effects

MRC-5 cells pose no risk to human health and have been classified as a BSL-1 agent. BaCMV is a cell culture adapted baboon strain of cytomegaloviruses. The fact that cytomegaloviruses are highly species-tropic combined with the adaptation to cell culture means the risk of BaCMV producing pathogenesis in humans is minimal. However, since other BaCMV strains have been shown to be transmitted following baboon-to-human liver xenotransplantation (Michaels et al, 2001), our risk assessment of BaCMV has been modelled on human CMV (HCMV) which is a BSL-2 agent. As a precaution, immunocompromised or vulnerable individuals should avoid working with BaCMV.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

n/a
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

In vitro GMO waste will be disposed of in 1% virkon and after soaking overnight and then autoclaved. Contaminated tips will be soaked in 1% virkon prior to autoclaving. Any contaminated glassware (kept to a minimum) will be soaked in 1% virkon for 24 hours, then rinsed prior to collection for cleaning. Other solid waste (plastic ware, eg tissue culture flasks, pipettes, tips, tubes) will be disposed of as biohazard waste and autoclaved on site. Disposable plastic waste will be disposed of via the biological waste route. Chicken eggs will be processed and taken directly to the autoclave.

Autoclave programme is a make safe programme as specified in BS 2646 part 3 1993 (either 121-125 degrees C for at least 15 minutes, 126-130 degrees C for at least 10 minutes or 134-138 degrees C for at least 3 minutes) any excess liquids after autoclaving are discarded to drains and solid waste disposed of via the clinical waste route.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The committee members were in agreement that this work should go ahead at containment level 2 pending approval from the HSE. The experience and competency of our scientists performing the experimentation was noted to be very competent.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2  Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>L2  L3 L4</td>
<td>L2</td>
<td>L3</td>
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<tr>
<td>L2  L3 L4</td>
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Animal Units

<table>
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<tr>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
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<td>L2  L3 L4</td>
<td>L2  L3 L4</td>
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<tr>
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<td>Non-GMMs</td>
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**Name**

JOHNSON MATTHEY CCT

**Campus Estate or Research Centre**

28 CAMBRIDGE SCIENCE PARK

**Road Name**

MILTON ROAD

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 0FP

**Country**

ENGLAND

<table>
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**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The role of BSO is taken on by the Biology Teamleader of the Biocatalysis Team. The BSO is a molecular microbiologist with over 15 years of lab experience in microbial molecular biology, recombinant protein production in E. coli, strain engineering. The BSO successfully registered as an ISTR Biological Practitioner Level 1 (Foundation) in 2014/15 and was appointed BSO by the technical director of Johnson Matthey CCT in February 2015.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Level 2 (GMMs)</td>
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<tr>
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<tr>
<td>Level 4 (GMMs)</td>
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<tr>
<td>Non-microbial</td>
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</tr>
<tr>
<td>Other (please specify)</td>
<td>Tick if confidential</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Sterilisation: All liquid and solid waste will be deactivated by autoclaving at 123°C for at least 20 minutes in a Systec
autoclave. Deactivation will be monitored by using 3M Thermalog Indicator Strips for steam. An annual autoclave
servicing and UKAS validation of two waste cycles is performed, using a recognised method with temperature and
pressure sensors on a full cycle. Labcoats are sterilised in a special autoclave cycle before leaving the building for
dry-cleaning.

Decontamination: Work surfaces are decontaminated using 70% ethanol at the end of each work period. Any spills
are decontaminated with freshly prepared Virkon for 10 minutes prior to cleanup. Virkon is inexpensive, highly
effective against a broad range of microbes, and does not have the associated problems of metal corrosion or odour
compared to bleach. Both methods of inactivation are validated by standard means. Both methods give 100% kill and
there is therefore no possibility that bacteria will escape from the laboratory. Lab benches are regularly swabbed and
checked for antibiotic resistant recombinant E. coli.

Waste removal: Solid autoclaved waste of waste code EWG18-02-03 and contaminated sharps (placed in an
approved plastic biohazardous sharp container) are disposed of by a special disposal service. Liquid autoclaved
waste is treated as household waste water (a trade effluent form was approved by Anglian Water).

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  Y

Please enter comments of the GM safety committee on the risk assessment

Two risk assessments for work in the field of biocatalysis are attached. One RA covers the storage and maintenance
of risk assessed strain and plasmid collections from X-Zyme GmbH in Germany (which belongs to JM) and other
external sources, the other one covers genetic engineering and small-scale production of biocatalysts undertaken at
the new premises. Both risk assessments focus exclusively on recombinant E. coli lab strains and E. coli plasmids
and standard techniques for manipulation and expression of genes of biocatalytic value. Transferred collections have
been previously assessed as classI or equivalent. In both assessments the level of risk is considered negligible and
the highest containment level to protect human health and environment is 1. The work is classified as GM activity
class 1 and requires therefore no contained use notification.
<table>
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<tbody>
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Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

 Tick if confidential  

Give brief details of the genetic modification safety committee

Porton Biopharma will form as a result of a corporatisation of the Development & Production group of Public Health England. It is intended to use Public Health England's GMSC for any proposals and risk assessments associated with Porton Biopharma. Porton Biopharma will form on 1st April 2015

<table>
<thead>
<tr>
<th>Laboratory</th>
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Non-microbial

Other (please specify)  

Tick if confidential  

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</table>
All waste streams will be inactivated by heat or chemical methods. 100% kill is achieved by autoclaving (121°C min residence 20 minutes). Chemical methods would be used for spills.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

Risk assessment currently undergoing renewal. This work has previously been carried out within Public Health England.

Project Ref 3273/18.1

Date Ackn'd 04/04/2018

CU2 Project Title Assessing the Dickeya dadantii recombinant microorganisms previously made at PHE for potential viability of increased asparaginase yield as a new product stream for Erwinase® manufacture

Class 2

Culture Vol

Class 2

1-50 Litres

Consent Granted

Project notified under transitional arrangements N
**Project Additional Information**

### Purposes of the contained use

The GMM is assigned Class 2 ACDP containment level. Although the host microorganism is classified as ACDP 1, the containment level is increased as the host is an APHA regulated plant pathogen.

### Recipient or parental organism

Genetically modified strains of Dickeya dadantii, formally known as Erwinia chrysanthemi will encode for Lasparaginase gene for overexpression of the enzyme, The inserted L-asparaginase enzyme is native to Dickeya dadantii thus it is unlikely to modify the host in any way. There are no harmful properties associated with the inserted gene product. Dickeya is Animal and Plant Health Agency (APHA) licensed as it is a plant pathogen, The GMOs will only be used in APHA licensed laboratories as specified below under strict guidance of such regulations.

### Host/vector system

**Host:** Dickeya dadantii (Erwinia chrysanthemi) NCPPB 1066 and SCI 193  
**Non-mobilisable vectors:** pASN32 (7.4kbp), pASN230 (13.5kbp) and pj401 :129213 (4983bp)

### Origin & function

The genetic material inserted is a gene encoding for over-expression of asparaginase enzyme that is naturally produced by the host Dickeya dadantii (Erwinia chrysanthemi). The encoded gene sequence, including signal peptides, will be synthesised and inserted into a vector incorporating a promoter sequence, antibiotic resistance and lac system for IPTG induction, L-asparagine is deaminated to aspartate in an enzymatic reaction catalysed by Lasparaginase, The enzyme also has a lesser activity towards L-glutamine for conversion to L-glutamate, The GMOs will be assessed for increased yield of asparaginase enzyme in the fermentation.

### Evaluation of foreseeable effects

The host organism is not a human pathogen and is classed as ACDP1.  
The host organism is a plant pathogen and can cause soft rot in succulent fleshy plant organs such as roots, tubers, stem cuttings and thick leaves and is controlled by APHA for handling and waste management. All the activities relating to the keeping and use of the GMO will be carried out in accordance with APHA regulation, confined within secure APHA registered containment labs.  
The inserted L-asparaginase enzyme is native to Dickeya dadantii thus it is unlikely to modify the host in any way. There are no harmful properties associated with the inserted gene product.  
The vectors to be used are non-mobilisable plasmid vector, therefore, negating the possibility of horizontal gene transfer to other microorganisms. Transfer of the sequences within the GMM to other microorganisms is extremely unlikely.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

N/A
All activities concerned with handling of the GMM will be carried out by APHA registered staff within APHA registered
labs. All samples and any associated waste will be incinerated via on-site incineration or by autoclaving at 121 °C for a
minimum of 15 minutes. All equipment and containment facilities will be disinfected using sodium hypochlorite under
validated conditions of use.

<table>
<thead>
<tr>
<th>Project Containment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Laboratory Activities</strong></td>
</tr>
<tr>
<td>L2: Yes</td>
</tr>
<tr>
<td><strong>Animal Units</strong></td>
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**Project Ref** 3273/20.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
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<tbody>
<tr>
<td>15/01/2020</td>
<td>The aim of this work is to produce recombinant protein in Escherichia coli</td>
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<table>
<thead>
<tr>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>Class 2</td>
<td>1-50 Litres</td>
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</tr>
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</table>

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Tick if notifying a connected programme of work

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

NIA
### Project Additional Information

#### Purposes of the contained use

The GMM is assigned Class 2 ACDP containment level. Although the host microorganism is classified as ACDP1, the containment level is increased as the vector is mobilisation defective with a strong promoter and the protein is a biologically active substance which is very unlikely to have a deleterious effect.

#### Host/vector system

#### Origin & function

#### Evaluation of foreseeable effects

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All activities concerned with handling of the GMM will be handled in containment level II laboratories by staff trained in the associated risk assessments. All samples and waste will be killed with available chlorine solution and be incinerated via on-site incineration or by autoclaving at 121 °C for a minimum of 15 minutes. All equipment will be disinfected using chlorine or >70% isopropyl alcohol solution under validated conditions of use.
Is an emergency plan required according to regulation 20? Y

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment N/A

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
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<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<td>L3</td>
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<th><strong>District</strong></th>
<th><strong>Town</strong></th>
<th><strong>County</strong></th>
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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- VP Drug Discovery - responsible for GMO work
- CTO - Expertise in natural peptides and experienced molecular biologist
- CEO - Management representative

<table>
<thead>
<tr>
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<tr>
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<tr>
<td>Other (please specify)</td>
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</table>

Tick if confidential

Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research |
Virology | Transgenic Animals | Transgenic Fish | Gene Therapy |
All GMM contaminated materials will be decontaminated in Distel, Vircon or hypochloride solution. Solid level 1 waste will be disposed of in 30 or 60 litre eurobins in accordance with Babraham Institute rules (derogation notification to the HSE GM 105/04).

<table>
<thead>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

- All GMM contaminated materials will be decontaminated in Distel, Vircon or hypochloride solution. Solid level 1 waste will be disposed of in 30 or 60 litre eurobins in accordance with Babraham Institute rules (derogation notification to the HSE GM 105/04).

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [   ]

Please enter comments of the GM safety committee on the risk assessment

The GM Safety Committee has reviewed the risk assessment and have approved it.
### GM Centre Number: 3275

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**Name**

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF ENGINEERING

**Department**

Name 2

**Campus Estate or Research Centre**

**Road Name**

TRUMPINGTON STREET

**District**

Town

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 1PZ

**Country**

ENGLAND

**Tel Number**

01223 332600

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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<td>OF ENGINEERING</td>
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<td>CAMBRIDGE</td>
<td>CB2 1PZ</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The department's biological safety committee serves as the genetic modification safety committee, and is composed of:
- The departmental biological safety officer (chairman)
- The local safety coordinator
- The departmental safety officer
- The bioengineering laboratory technician
- A university biological safety adviser

The committee meets three times per year. It reviews risk assessments and provides advice and training for about 20 researchers using biological agents in the department.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
<td></td>
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<td></td>
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<tr>
<td>Level 2 (GMMs)</td>
<td></td>
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<td>Non-microbial</td>
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<td>Other (please specify)</td>
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<td>Bacteriology</td>
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<td>Parasitology</td>
<td>Transgenic Birds</td>
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<td>Virology</td>
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<td>Transgenic Fish</td>
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<tr>
<td>Mycology</td>
<td></td>
<td>Transgenic Invertebrates</td>
<td>Yes</td>
<td>Transgenic Plants</td>
</tr>
</tbody>
</table>

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Maximum culture volume that could be released at any one time is less than one litre.

Disinfectant: Virkon® & Distel (formerly Trigene), following the manufacturer's guidelines for dosage and exposure time for maximum kill, pipettes will be totally immersed in disinfectant before disposal.

Solid contaminated waste is disposed in robust and leakproof containers, which are taken monthly by an approved external incineration contractor for certified disposal.

On site autoclave can be used for prior inactivation of solid waste in puncture proof containers, cycles at 121°C for 15 to 30 minutes, provides 100% kill, monitored with Browne's tube or steam tape, serviced, calibrated and validated for waste inactivation annually. Contaminated laboratory glassware and other materials awaiting disinfection will be stored in a safe manner.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The members of the genetic modification safety committee have studied the attached risk assessment and agree that all foreseeable risks have been identified. The preventative measures contained within the risk assessment are deemed adequate for the hazards associated with the proposed work.
**GM Centre Number: 3277**

<table>
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**Name**

PEAK PROTEINS LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Building**

BIRCHWOOD HOUSE

**District**

TYTHERINGTON BUSINESS PARK

**Town**

MACCLESFIELD

**Road Name**

LARKWOOD WAY

**County**

CHESHIRE

**Postcode**

SK10 2XR

**Country**

ENGLAND

**Tel Number**

07968 848917

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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<th>Date Premises Closed</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Tick if confidential

The genetic modification safety committee (GMSC) consists of new people both of whom have more than 20 years experience handling GMMs. One is a member of the management who additionally has 5 years experience of being on the GMSC of a global pharmaceutical company which included reviewing over 50 GMM proposals every year. Any new pieces of work will be assessed by a written proposal which will then be reviewed and classed as either class 1 or class 2.

<table>
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</table>
Disinfection and autoclaving will be carried out where required. All waste materials will be disposed of into autoclave bags, yellow clinical waste bags/bins or sharps bins as required. All contaminated solid waste (agar plates, disposable plasticware etc) will be placed in autoclave bags and will be autoclaved and disposed of via designated route. Liquid cultures will be chemically-inactivated (Virkon 1% or PeraSafe 0.16% for 30 mins) or autoclaved prior to disposal. All contaminated glassware and reusable plasticware will be decontaminated as above.

a) Disinfection.
1% Virkon will be used for disinfection. The surfaces of the microbiological safety cabinet will be disinfected with 1% Virkon after use. Laboratory benches will be swabbed with 1% Virkon after any activity.

b) Autoclaving.
Autoclaves are not present in the laboratory for waste treatment. However, a large central autoclave facility is located on site at Alderley Park (please see next section for the further details of the Waste management process and derogation request). The autoclave is subject to twice yearly servicing and annual validation using a 12-point thermocouple procedure which includes a probe placed in a representative load.

The GMSC met on 20th April to review PP - 2015-1 which had been given a provisional class 1 risk assessment. It was agreed that this was appropriate as there was no significant risk to either the environment or operator.
### Data Premises Notified (Originally)

- Date: 10/06/2015

### Transferred from 1992 Regs?

- Yes / No: No

### Transitional Premises Class

- Non-GMMs: Yes / No: No

### Data Premises Closed

- Emergency Plan Required?: Yes / No: No

### Name

- OXSONICS THERAPEUTICS LIMITED

### Name 2

- Department

### Campus Estate or Research Centre

- 2ND FLR EAST WING MAGDALEN CENTRE N

### Building

- MAGDALEN CENTRE

### Road Name

- ROBERT ROBINSON AVENUE

### Town

- OXFORD

### County

- OXFORDSHIRE

### Postcode

- OX4 4GA

### Country

- ENGLAND

### Tel Number

- 01865784772

### Fax Number

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### E-mail

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### HSE Division

- blank

### Comments

### Date at Which Additional Info Submitted

- 02/03/2022
## Premises Addresses

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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The committee consists of three scientists with extensive combined experience working with genetically modified organisms. The committee hold monthly biosafety meetings.
- An senior academic with research interests in the development of viral gene delivery systems. Experienced in designing studies and supervising postgraduate students undertaking research projects with class II genetically modified viruses.
- A research scientist with 10+ years postdoctoral experience in containment level II laboratories working with genetically modified bacteria and viruses and acting as Biosafety Manager in a biotechnology company for 2 years.
- A research scientist who's recent PhD project involved regular work with class II genetically modified viruses.

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
<th>Level 4 (GMMs)</th>
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02/03/2022
Other (please specify)  Tick if confidential  

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| Other(s)     |              |            |                             |

For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

N/A

Project Ref  3278/15.1

Date Ackn'd  11/06/2015

Date Project Ceased

CU2 Project Title  Assisted delivery of vaccinia virus in the treatment of cancer

Class  2

CultureVol  ≤ 1 Litre

Class2 CultureVolume  3-4

Consent Granted

Project notified under transitional arrangements  N

Tick if notifying a connected programme of work  N

Withdrawn  N
Project Additional Information

Purposes of the contained use

A conditionally replicating vaccinia virus capable of lytic replication within cancer cells is planned to be used to treat cancer by either:
- improving the delivery of the virus into tumours by 'pumping' the virus into tumours using ultrasound induced cavitation;
or
- developing polymer modified vaccinia virus capable of extending the circulation time of the virus and further targetting the virus to tumours.

Recipient or parental organism

In vitro and in vivo experiments will be performed with established cell lines and primary cells of human and mouse origin. Established cell lines may include, but not limited to SKOV, HepG2, SNU-5, and HT116 and with primary cells may include human erythrocytes, human peripheral blood lymphocytes (PBL), mouse erythrocytes and mouse splenocytes.

Host/vector system

Attenuated vaccinia virus - Copenhagen and possibly, Western Reserve, Wyeth, and MVA strains. The inserted foreign genes luciferase or FCU1 (at the JR2 locus) replace the Thymidine kinase gene resulting in disruption of the thymidine kinase reducing the virulence. Further deletion of the ribonucleotide reductase gene (from the I4L locus) reduces virulence further.

Origin & function

Host cells are acquired commercially while the vaccinia virus is a gift from collaborators.

The luciferase gene from firefly is a widely used reporter gene not considered to be harmful. Luciferase is a natural oxidative enzyme, which catalyses the ATP dependent conversion of luciferin to oxyluciferin a process which results in the emission of a photon of light. Luciferase from firefly is widely used as reporter genes and not considered to have harmful properties.

FCU1 is a cytosine deaminase-phosphoribosyl transferase fusion enzyme for the conversion of the prodrug 5-fluorocytosine to the active metabolite 5-fluorouracil monophosphate F-dUMP. E. coli cytosine deaminase activates 5-fluorocytosine to 5-fluorouracil (5-FU), a well established anticancer drug. Phosphoribosyl transferase enhances the efficacy of the system, by improving the conversion of 5-FU to F-dUMP in the cell.

Cytotoxicity of these prodrug-activating systems is conditional upon the presence of prodrug.

The intended function of the genetic material is to enable the luminescent detection and quantification of virus infection (Luciferase) or the conversion of prodrug to
chemotherapeutic drug (FCU1) specifically in vaccinia-virus infected tumour cells.

**Evaluation of foreseeable effects**

Humans are not a natural host of vaccinia. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous infection, but it contains antigens that stimulate an immune response that is cross-reactive with smallpox antigens and thereby confers protection from the human disease. Serious complications occurred in less than 1 in 4,000 people, mainly in immunosuppressed and extremely young individuals. Rare complications include eczema vaccinatum, disseminated vaccinia rash, progressive vaccinia (in T-cell-deficient individuals) and encephalitis (1-2 per million vaccinated) [Fields, 1996]. Although Vaccinia viruses are replicating DNA viruses, they do not insert their DNA into the host genome and there has been no known transfer of viral genetic material into the host genome.

Vaccinia virus shows an inherent selectivity for cancerous tissues relative to normal tissues. This appears to be influenced by at least three underlying biological characteristics of tumours: their “leaky” blood vessels, for example due to Vascular Endothelial Growth Factor (VEGF) effects; their proliferative state and the activity of the Epidermal Growth Factor (EGF) receptor pathway. In addition to the inherent tumour tropism exhibited by vaccinia, several vaccinia strains (VVTK-RR-/FCU1) have been designed to be additionally attenuated in normal cells and tissues through a deletion of the TK gene. TK gene-deleted vaccinia viruses, are significantly attenuated in normal tissues. Virulence can be further decreased by removal of the ribonucleotide reductase (RR) gene, the product of which catalyzes the formation of deoxyribonucleotides from ribonucleotides. Deoxyribonucleotides in turn are used in the synthesis of the vaccinia DNA genome. RR gene-deleted vaccinia viruses, are significantly attenuated in normal tissues in vivo and are, therefore, less toxic whilst maintaining anti-tumoural activity. Work using polymers to modify the virus will further attenuate its ability to infect human cells as the regions which provide it with tropism for its target cell surface receptors will be covered. Selective tropism for cancer cells may then be provided by the addition of ligands targeting cancer specific receptors.

Some vaccinia strains have had genes inserted to produce FCU1. It is a cytosine deaminase-phosphoribosyl transferase fusion enzyme for the conversion of the prodrug 5-fluorocytosine to the active metabolite 5-fluorouracil monophosphate F-dUMP. E. coli cytosine deaminase activates 5-fluorocytosine to 5-fluorouracil (5-FU), a well established anticancer drug. Phosphoribosyl transferase enhances the efficacy of the CD system, by improving the conversion of 5-FU to F-dUMP in the cell. Cytotoxicity of these prodrug-activating systems is conditional upon the presence of prodrug.

Animals inoculated with conditionally-replicating vaccinia are unlikely to support a productive infection except within tumour cells and possibly in proliferating skin epithelium. There is a small risk from transfer of infected animal fluids to open wounds, and operators will be careful to protect their hands from such risks. The procedures put in place for handling and disposal of virus make escape into the environment highly improbable. The use of attenuated laboratory strains results in a modified virus that is less pathogenic than wild type strains and the inserted genes pose no foreseeable risk to human or environment in the unlikely event of loss of containment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Animals injected with vaccinia will be housed in isolators in a Containment Level 2 wing of the animal unit. They will be handled in class II safety cabinets until/unless it is shown there is no risk from shedding of the virus. Cages and bedding will be treated according to standard Containment Level 2 procedures (inclucding autoclaving of animal bedding and chemical disinfection of liquid wastes) as detailed in the appended detailed risk assessment. All handling of live animals will be performed by people trained to do so during a prescribed Home Office training course. The greatest risk to humans will be from any percutaneous injury sustained during the inoculation procedure. This risk will also be minimised by following the procedures prescribed in the risk assessment.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

2% Virkon is used for soaking pipettes and tissue culture plates/flasks for at least 1 hour followed by transfer of solids into biological hazard bags for autoclaving. Virkon treated liquid waste will be washed down a drain. Virkon contact achieves a 100% degree of kill within 10 minutes (see www.antechh.com/virkonapps.html).

Virkon disinfection will be tested. Virus will be exposed to 2% Virkon for 15 minutes, a dialysis step will be performed to remove virkon and the sample diluted in culture media. This is then put on to cells and transgene expression, which indicates viability of the virus, is assayed after 24 hours.
The genetic modification safety committee concluded that the risk assessment is comprehensive and the equipment and processes in place to deal with the handling and disposal of this agent are appropriate.

Project Containment

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
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Project Ref 3278/16.1

Date Ackn'd 07/04/2016

CU2 Project Title Conditionally replicating herpes virus HSV-1 (JS1) for use in cancer research

Class 2 Culture Vol Class 2 < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
### Project Additional Information

#### Purposes of the contained use

A conditionally replicating Herpes simplex virus -1 capable of lytic replication within cancer cells is planned to be used to treat cancer by either:

- improving the delivery of the virus into tumours by ‘pumping’ the virus into tumours using ultrasound induced cavitation;
- or
- developing polymer modified virus capable of extending the circulation time of the virus and further targetting the virus to tumours.

#### Recipient or parental organism

In vitro and in vivo experiments will be performed with established cell lines and primary cells of mouse origin. Established cell lines may include, but not limited to, A20 cells, CT-26 cells, and with primary cells may include mouse erythrocytes and mouse splenocytes.

#### Host/vector system


#### Origin & function

Host cells are acquired commerically while the modified HSV-1 is provided by collaborators (Amgen).

The Granulocyte-macrophage colony-stimulating factor (GM-CSF) is of mouse origin. GM-CSF is a glycoprotein that functions as a cytokine. It stimulates the production of immune cells, granulocytes and monocytes. GMSCF is part of the immune/inflammatory cascade.

The intended function of the genetic material is to elicit an immune/inflammatory response to HSV-1 infected tumour cells. This will be achieved by stimulation of an immune response through virus mediated GMC-SF production and a direct response to HSV-1 antigens. Further tumour destruction will be achieved through the oncolytic properties of the virus.

#### Evaluation of foreseeable effects

Wild type herpes simplex virus type 1 (HSV-1) infection causes blisters on mucosal membranes or epithelial cells of the mouth lips or genitals. Virus infection occurs through contact with wounds or infection of the mucosal epithelia. After initial infection the virus spreads to neuronal cells, it then travels along the neurone (by a process called retrograde transport) to the ganglion. In the case of herpes infections of the oral mucosa, the virus goes to the trigeminal ganglia whereas infections of the genital mucosa lead the virus entering the sacral ganglia. The virus can also travel in the opposite direction to arrive at the mucosa that was initially infected; hence the site of primary infection determines the site of blisters which can re-occur after periods of latency. Vertical transmission of HSV-1 to neonates is asymptomatic in 98.3% of cases, however, when symptoms do manifest they can be fatal. However, in this project the virus used is the JS1 strain with the neurovirulence gene ICP34.5 gene is removed from the virus in order to attenuate neurovirulence and restrict tropism to transformed or malignant cells. Furthermore the deletion of ICP47 enables antigen presentation and limits the virus’ immune evasion mechaism, and insertion of GM-CSF actively stimulates an immune response. As the HSV-1 thymidine kinase gene has not been deleted in this strategy, antiviral agents such as ganciclovir and acyclovir will still be effective against genetically modified virus variants.

Animals inoculated with this HSV-1 are unlikely to support a productive infection except within tumour cells and possibly in proliferating skin epithelium. There is a small risk from transfer of infected animal fluids to open wounds, and operators will be careful to protect their hands from such risks.

02/03/2022
The procedures put in place for handling and disposal of virus make escape into the environment highly improbable. The use of attenuated laboratory strains results in a modified virus that is less pathogenic than wild type strains and the inserted genes pose no foreseeable risk to human or environment in the unlikely event of loss of containment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Animals injected with modified herpes simplex 1 virus will be housed in isolators in a Containment Level 2 wing of the animal unit. They will be handled in class II safety cabinets until/unless it is shown there is no risk from shedding of the virus. Cages and bedding will be treated according to standard Containment Level 2 procedures (including autoclaving of animal bedding and chemical disinfection of liquid wastes) as detailed in the appended detailed risk assessment. All handling of live animals will be performed by people trained to do so during a prescribed Home Office training course. The greatest risk to humans will be from any percutaneous injury sustained during the inoculation procedure. This risk will also be minimised by following the procedures prescribed in the risk assessment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

2% Virkon is used for soaking pipettes and tissue culture plates/flasks for at least 1 hour followed by transfer of solids into biological hazard bags for autoclaving. Virkon treated liquid waste will be washed down a drain. Virkon contact achieves a 100% degree of kill within 10 minutes (see www.antechh.com/virkonapps.html).

Virkon disinfection will be tested. Virus will be exposed to 2% Virkon for 15 minutes, a dialysis step will be performed to remove virkon and the sample diluted in culture media. This is then put on to cells and transgene expression, which indicates viability of the virus, is assayed after 24 hours.

**Project Containment**

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</tr>
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</table>

The genetic modification safety committee concluded that the risk assessment for working with this modified HSV-1 is comprehensive and the equipment and processes in place to deal with the handling and disposal of this agent are appropriate for safe containment.
### Project Additional Information

**Purposes of the contained use**

We plan to show improved effectiveness of a conditionally replicating vaccinia virus carrying reporter genes, or immuno-stimulatory genes for tumour cells expression. The virus is also capable of lytic replication within cancer cells only. We will improve the delivery of the virus into tumours by ‘pumping’ the virus into tumours using ultrasound induced cavitation.

We also plan to study the effect of ultrasound on the shedding of viral antigens and its immunostimulatory effects. A transgenic cell line expressing ovalbumin will be used for this purpose.

**Recipient or parental organism**

In vitro and in vivo experiments will be performed with established human cell lines and mouse cell lines. In vivo experiments will be performed in mice with subcutaneously implanted/grown tumours composed of human or mouse cell lines. Human cells will include, but not be limited to, UM-UC-3 cells. Mouse cell lines may be transgenic and include (but not limited to), A20, A20 - OVA, A20 - OVA +AsRed, B16-F10, B16-F10 - OVA and B16-F10 - OVA +AsRed.

Mouse strain may be spontaneous immunosuppressive strains to enable human cell line engraftment such as (CD-1 nude) but not transgenic. Mice will be commercially sourced.

GM cell lines will be a gift from a collaborator. Human cell line will be commercially sourced.

**Host/vector system**

Both SKV-Luc and TBio-6517 are attenuated derivatives of the well established Copenhagen vaccinia smallpox vaccine genetically engineered to selectively replicate in...
Origin & function

The genetic modifications of SKV-Luc are designed to attenuate the virus and make it more selective to tumour cells and will have the effect of reduced pathogenicity potential in non-transformed cells. Insertion of the luciferase gene into this virus enables this reporter to be used to track infection and replication in tumours. The modification made to produce TBio-6517 are designed to induce an immunological response to the tumour cells.

A20-ova expressing ckOVA (clone C2), A20-ova expressing ckOVA and AsRed (clone D6), B16F10-ova expressing ckOVA (clone D7), B16F10-ova expressing ckOVA and AsRed (clone C9) will be used for this section of the project. Both genes are expressed under the EF1alpha promoter. The genes have been integrated at Rosa26 safe harbour locus using CRISPR/Cas9 system.

Evaluation of foreseeable effects

Humans are not a natural host of vaccinia. Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous infection, but it contains antigens that stimulate an immune response that is cross-reactive with smallpox antigens and thereby confers protection from the human disease. Serious complications occurred in less than 1 in 4,000 people, mainly in immunosuppressed and extremely young individuals. Rare complications include eczema vaccinatum, disseminated vaccinia rash, progressive vaccinia (in T-cell-deficient individuals) and encephalitis (1-2 per million vaccinated) [Fields, 1996]. Although Vaccinia viruses are replicating DNA viruses, they do not insert their DNA into the host genome and there has been no known transfer of viral genetic material into the host genome.

Vaccinia virus shows an inherent selectivity for cancerous tissues relative to normal tissues. This appears to be influenced by at least three underlying biological characteristics of tumours: their "leaky" blood vessels, for example due to Vascular Endothelial Growth Factor (VEGF) effects; their proliferative state and the activity of the Epidermal Growth Factor (EGF) receptor pathway. In addition to the inherent tumour tropism exhibited by vaccinia, these vaccinia strains (SKV-Luc and TBio-6517) are additionally attenuated in normal cells and tissues through selection of strain with deletions including 16 native viral coding segments removed through a 13,767 bp deletion in the 3 prime region, and the deletion of 16 native coding segments removed through a 11,899 bp deletion in the 5 prime region. These deletions disable the ability of the virus to suppress the normal cellular anti-viral response (e.g. interferon signalling via the cGAS/STING pathway, other viral defence effector pathways, and apoptosis) that functions to suppress late viral replication in normal cells, but is absent in many types of transformed tumour cells. Two sets of expression cassettes were added to this SKV derivative strain. One of these was inserted into the native B8R coding segment, and expresses cDNAs for the human cytokine FLT3L and the membrane-bound p35 subunit of the human cytokine IL-12, using native vaccinia virus promoters (pB19R and pLate respectively). The other was inserted into the site of the 11,899 bp CpMDSp deletion and expresses cDNAs for the light and heavy chain genes of an anti-CTLA-4 antibody. This cassette also makes use of a native vaccine virus promoter (pH5R), and includes a bi-cistronic cassette where the two cDNAs are separated by a T2A ribosome skip sequence. The FLT3L protein is a hematopoietic growth factor that drives dendritic cell survival and proliferation. The IL-12 protein has pleiotropic effects on multiple components of the tumour microenvironment and promotes the T cell response. The anti-CTLA-4 antibody is included to inactivate any native CTLA-4 protein which normally serves to attenuate T cell activation. This transgene is modelled after the anti-CTLA-4 monoclonal antibody Yervoy, an agent approved by the FDA for the treatment of advanced metastatic melanoma. The viruses will be obtained from a collaborator and no further genetic manipulation will be performed.

The genetically modified cell lines are of mouse origin and pose no risk to humans. A20-ova expressing ckOVA (clone C2), A20-ova expressing ckOVA and AsRed (clone D6), B16F10-ova expressing ckOVA (clone D7), B16F10-ova expressing ckOVA and AsRed (clone C9) will be used for this section of the project. Both genes are expressed under the EF1alpha promoter. The genes have been integrated at Rosa26 safe harbour locus using CRISPR/Cas9 system.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Animals injected with vaccinia will be housed in isolators in a Containment Level 2 wing of the animal unit. They will be handled in class II safety cabinets until/unless it is shown there is no risk from shedding of the virus. Cages and bedding will be treated according to standard Containment Level 2 procedures (including autoclaving of animal bedding and chemical disinfection of liquid wastes) as detailed in the appended detailed risk assessment. All handling of live animals will be performed by people trained to do so during a prescribed Home Office training course. The greatest risk to humans will be from any percutaneous injury sustained during the inoculation procedure. This risk will also be minimised by following the procedures prescribed in the risk assessment.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
2% Virkon is used for soaking pipettes and tissue culture plates/flasks for at least 1 hour followed by transfer of solids into biological hazard bags for incineration. Virkon treated liquid waste will be washed down a drain. Virkon contact achieves a 100% degree of kill within 10 minutes (see www.antechh.com/virkonapps.html).

Regular inspections and health and safety checks will be made and records and logs of use and disinfection of waste will be maintained.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The genetic modification safety committee concluded that the risk assessment is comprehensive and the equipment and processes in place to deal with the handling and disposal of this agent are appropriate.

### Project Containment

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GM Centre Number: 3279

Data Premises Notified (Originally) 15/06/2015

Transferred from 1992 Regs? N

Transitional Premises

Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

SIBELIUS LIMITED

Name 2

Department

Campus Estate or Research Centre

20 EAST CENTRAL

Road Name

127 OLYMPIC AVENUE

District

MILTON PARK

Town

ABINGDON

County

OXFORDSHIRE

Postcode

OX14 4SA

Country

ENGLAND

Tel Number 01865 518910

Fax Number 01865 511418

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

### Give brief details of the genetic modification safety committee

- Chief Scientific Officer, Sibelius Limited
- Research Director, Sibelius Limited
- Screening Operations manager, Sibelius Limited

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Glass House</th>
<th>Large Scale</th>
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</table>

Tick if confidential

- Bacteriology: Yes
- Parasitology: Yes
- Transgenic Birds: Yes
- Microbiology Research: Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity

Contaminated Waste will be placed into sealed containers which will then be removed from the site by an independent contractor. The contractor will be a specialist waste management company who will dispose of the waste in accordance with all relevant legislation and guidelines applicable to clinical waste. The contractor will be required to provide communication confirming that the method of disposal complies with the relevant legislation.

<table>
<thead>
<tr>
<th>Virology</th>
<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
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<tr>
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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

To the best of our knowledge the activities described here present negligible risk to both the environment and to workers.
**GM Centre Number: 3280**

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**Name**

ARQUER DIAGNOSTICS LIMITED

**Department**

**Campus Estate or Research Centre**

NORTH EAST BUSINESS & INNOVATION CE

**Road Name**

**District**

WEARFIELD

**Town**

SUNDERLAND

**County**

TYNE AND WEAR

**Postcode**

SR5 2TA

**Country**

ENGLAND

**Tel Number**

0191 516 6768

**Fax Number**

0

**E-mail**

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**HSE Division**

**Comments**

Company name changeFrom Urosens Ltd 06/07/2015

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The risk assessment has been reviewed and deemed to cover all relevant risks adequately, users were happy that the hazards of the project have been correctly identified, and that appropriate safe systems of work will be used to control those risks identified.

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Tick if confidential

Bacteriology             Parasitology  Transgenic Birds  Microbiology Research
Yes                        Yes                      Yes                        Yes                              Yes
Virology
Mycology
Other(s)
Mammalian Cell line work and Human primary culture

For activities involving GMMs, describe the waste management measures which will apply to the activity
n/a

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment Y

Please enter comments of the GM safety committee on the risk assessment
n/a

Project Ref  3280/15.1

Date Ackn'd CU2 Project Title
09/07/2015 Gene transfer into mammalian cells for the study of cancer biomarkers

Date Project Ceased

Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
# Project Additional Information

## Purposes of the contained use

The aim of the project is to validate a variety of cancer biomarker genes including oncogenes, tumour suppressor genes and genes encoding components of cellular signalling pathways in cancer in order to develop diagnostic immunoassays.

## Recipient or parental organism

**E. coli**

E. coli K12 strains such as:

- DH5α (F− Φ80lacZ∆M15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK−, mK+) phoA supE44 λ− thi-1 gyrA96 relA1)

will be used for propagation of plasmid DNA.

E. coli K12 strains are attenuated, non-colonising, non-pathogenic strains, unlikely to survive outside the laboratory and have a long history of safe use, and are regarded as non-hazardous.

**Cell lines**

Primary and established human cell lines and mammalian cell lines.

The cell lines used are not capable of survival outside the laboratory and are non-transplantable due to immuno-incompatibility. They are therefore considered to be highly disabled hosts. Established cell lines with a history of safe use may be considered hazard group 1, however primary cell lines may contain endogenous pathogens and are treated as hazard Group 2.

## Host/vector system

The following will be delivered to target mammalian (including human) cell lines:

1. cDNAs encoding wild-type or mutant known candidate biomarkers including oncogenes, tumour suppressor genes and other genes involved in cell signalling, apoptosis and/or development of cancer. Expression is under the control of cloned human promoter sequences, constitutive viral promoters (such as CMV or SV40 early promoters)).

   cDNAs may also be expressed as fusions with GFP and derivatives to allow fluorescent detection or with epitope tags such as haemagglutinin (HA) or FLAG or with poly-histidine tags to facilitate protein detection and purification.

2. shRNA or siRNA (interfering RNA) molecules under the control of human or viral promoters targeted to genes thought to be involved in cancer.

**DNA**

Human/mammalian DNA isolated from tissues, primary or established cell lines; cloned cDNA or shRNA/siRNA from collaborators or commercial sources.

## Origin & function

**Evaluation of foreseeable effects**

This work involves the use of vectors to allow targeted over-expression or knock-down of genes of interest in human and mammalian cell lines and the growth of mammalian cell lines which have been transduced with viral vectors containing expression cassettes that harbour:

- cDNA
- short hairpin RNAs

(Viral transductions will be carried out by collaborators carried out under local GM rules and risk assessments and only transferred to our facility upon testing to confirm lack of viral activity).
GMOs created would consist of:

Modified cell lines – The inserted genes/interference RNAs include those that code for/target known or potential oncogenes or tumour suppressor genes and could potentially lead to cellular transformation if expressed in an operator’s cells although several further genetic changes would be required for progression to malignancy.

E. coli K12 strains - These strains are attenuated, non-colonising, non-pathogenic strains, unlikely to survive outside the laboratory and have a long history of safe use, and are regarded as non-hazardous therefore an genetic modification should not have any effects.

In addition work to be carried out is small-scale thus there is no chance of major release of any GMO. and therefore the work is non-hazardous to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No Larger GMOs to be used

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None of legal controls will be derogated.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste will be treated with Virkon granules or an equal volume of 10% Virusolve+ (shown to be effective in killing all known pathogens in independent tests) and left for at least 12 hours before disposal down the drain with copious amounts of water.

Solid waste (plastic ware or cells) will be treated with appropriate concentrations of Virkon or Virosolve+ for at least 12 hours before being destroyed as clinical waste.

Liquid spills

Wearing appropriate gloves, liquid spills will be treated with Virkon granules until deep pink, or with an equivalent volume of 10% Virusolve+. The treated area will be left for at least 30 minutes and the area will then be mopped with plenty of water.

Solid spills

Wearing appropriate gloves, solid spills will be gathered into an autoclave bag and the area affected will be wiped liberally with 2% Virkon. The treated area will be left for 30 minutes and the area will then be mopped with plenty of water. Immediately after it has been collected and the affected area has been treated with Virkon, the solid waste will be taken for autoclaving.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been reviewed and deemed to cover all relevant risks adequately, users were happy that the hazards of the project have been correctly identified, and that appropriate safe systems of work will be used to control those risks identified.
<table>
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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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<td>Human Clinical Applications</td>
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### Name

| NEEM BIOTECH LTD |

### Campus Estate or Research Centre

### Road Name

| ROSEHEYWORTH BUSINESS PARK NORTH |

### Town

| ABERTILLERY |

### County

| GWENT |

### Postcode

| NP13 1SX |

### Country

| WALES |

### Tel Number

| 01495 292 700 |

### Fax Number

| 0 |

### E-mail

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### HSE Division

### Comments

### Date at Which Additional Info Submitted

| 02/03/2022 |
### Premises Addresses

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<td>GWENT</td>
<td>NP13 1SX</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Neem Biotech Ltd's Quality, Health & Safety Manager is qualified with the NEBOSH H&S National General Certificate (Sept 2009) and ISTR-accredited Biosafety Professional Certificates (Jul 2009 and May 2010). The SHE Manager is also registered as a Biosafety Professional (BSP) with the ISTR in the UK and is a Registered Biosafety Professional (RBP) with ABSA in the USA. The SHE Manager previously served as the Biological Safety Officer for the University of Southampton from 2009 - 2011 where he also served on the University's GM & Biosafety Committee.

<table>
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<tr>
<th>Laboratory</th>
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Tick if confidential

Y

Bacteriology Yes

Parasitology

Transgenic Birds

Microbiology Research
Yes

All biological waste is either pre-treated with a 10% bleach solution (final concentration) overnight prior to disposal down the drain (if liquid), or autoclaved at 121°C for 15 minutes where impractical to pre-treat with bleach. Alternatively, all solid biological waste is disposed of in Clinical Waste bags destined for incineration using the Company's contracted waste treatment agent (SRCL).

Virology  Transgenic Animals  Transgenic Fish  Gene Therapy
Mycology  Yes  Transgenic Invertebrates  Transgenic Plants  Other (please specify below)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Risk Assessment performed by degree-qualified Company Microbiologists and reviewed by the Company's SHE Manager.

Tick to confirm that you are attaching a summary of the risk assessment  Y
Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref  3282/16.1

Date Ackn'd  14/07/2016  CU2 Project Title  Antimicrobial compounds for treatment of biofilm and other infections
Class  CultureVolClass2  CultureVolumeClass3-4
Class 2  < 1 Litre

Non-GMM  Consent Granted

Project notified under transitional arrangements  N

Withdrawn  N  Tick if notifying a connected programme of work  N

Historical Significant Changes
Historical Date of Additional Info
### Project Additional Information

**Purposes of the contained use**

Experiments involving biofilm reporter strains with various genetic modifications will be used to demonstrate the efficacy of various antimicrobial compounds. These strains may involve plasmid or chromosomal genetic modification. Quorum sensing inhibition is a key target in novel antimicrobials for the disruption of biofilm infections and may be explored using GMMs. Another key area of target is urease inhibition with may be studied with various GMMs.

**Recipient or parental organism**

- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Salmonella enterica*
- *Ureaplasma*

**Host/vector system**

- lacZ, lux, gfp, phoA, gus
- Fluorescent proteins
- ure

**Origin & function**

All genetic material will be standard as published in peer reviewed journals or available commercially from reputable sources.

**Evaluation of foreseeable effects**

Plasmids used may carry antibiotic resistance genes for selection purposes. Genes being used are primarily used as reporters to indicate a specific metabolic activity or ability. Occasionally mutations may be introduced to knock out the function of a specific gene.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All GMM liquid waste is sterilised prior to disposal (down drain) using a standard benchtop autoclave (121°C for 15 minutes) or with 10% sodium hypochlorite (final concentration). Disposable plastic or
glass is autoclaved where possible or soaked in 10% sodium hypochlorite solution prior to disposal by
registered third party waste handler.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No comments

**Project Containment**

<table>
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### CRUK–ASTRAZENECA ANTIBODY ALLIANCE LABORATORY

#### The Cori Building

**GM Centre Number:** 3286

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**Emergency Plan Required?**

- [ ] Yes
- [x] No

**Non-GMMs**

- [ ] Yes
- [x] No

**Withdrawn**

- [ ] Yes
- [x] No

### Name

**Name:** CRUK–ASTRAZENECA ANTIBODY ALLIANCE LABORATORY

### Department

**Department:**

### Campus Estate or Research Centre

**Road Name:** GRANTA PARK

**Town:** CAMBRIDGE

**County:** CAMBRIDGESHIRE

**Postcode:** CB21 6GS

**Country:** ENGLAND

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**E-mail:**

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**HSE Division:**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

Advice has been sought from GMO/risk assessment experts at MedImmune (Granta Park, Cambridge, UK, CB21 6GS).

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid Class 1 GMO waste can be disposed of directly into tiger-stripe bags which are sent off site for incineration as Non-Infectious Healthcare Waste (EWC 18 01 04). Liquid waste will be treated with 1-5% Virkon for at least 30 minutes.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All risk assessments attached to this application have been discussed and amended under the guidance of those involved with the GMO process at MedImmune.

**Project Ref** 3286/18.1

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Withdrawn N

Tick if notifying a connected programme of work N
**Project Additional Information**

**Purposes of the contained use**

We undertake drug discovery research to generate therapeutic antibodies against rare cancers so as 1) to provide diagnostic tools, 2) to generate potential cancer therapeutics, and 3) to provide tools to the academic collaborators to deepen our understanding of the disease. This necessitates extensive use of mammalian cells in our research. To generate disease relevant models, these cell lines often need to undergo genomic modification (GM) as part of the process. This GM includes over-expression of target proteins, knock-down or knock-out of certain genes, intentional activation or interference of certain genes, insertion of marker proteins, and mutagenesis to include cancer relevant mutations.

The process involves introduction of nucleic acid materials (DNAs or RNAs) into chosen mammalian cell lines. This can be achieved by using a range of techniques, from traditional chemical or lipid based transfection, electroporation, to lentiviral transduction (using 3rd or later generation of lentiviral vectors). Following the above process, the genes are inserted randomly into the genome, and by selecting for a marker (often antibiotic resistance) and/or desired traits, we could obtain the pools and/or clonal cell lines. More controlled insertion and interference can be achieved by CRISPR-Cas9 technology which uses a single-stranded, hairpin-forming guide RNA (sgRNA) to introduce a modification at a specific site on DNA. Due to the nature and objectives of our research, the genetic targets are often genes linked to cancer development, including oncogenes and tumour suppressor genes.

**Recipient or parental organism**

Mammalian cells (recipient cell lines):

Parental mammalian cell lines are usually of commercial origin, e.g. ATCC or ECACC, and are from hazard group 1 or 2. The cell lines of interest would not knowingly contain (or being contaminated by) infectious human pathogens such as HIV or HepB. However, they might have integrated fragments of viral genome, e.g. HPV (which is often the case of HG2 cancer cell lines). Primary cells obtained from reputable sources should have been pre-screened for the presence of some of the blood-borne viruses, bacteria, yeast and fungi. Hazard Group 1 and Hazard Group 2 mammalian cell lines in continuous culture have been found unable to establish colonies in healthy/immuno-competent human adults following intradermal inoculation, ingestion or inhalation. Many of the cell lines have long history (some 40-50 years) of safe use. These cells are unlikely to infect or harm any living things in the environment, as they are unlikely to survive outside of laboratory culture conditions for any duration of time without required nutrients and temperature.
Lentivirus and producer cells:
Lentivirus particles are generated in a host mammalian cell line. The producer cells (e.g. HEK293T) have a long history of safe use and unlikely to be pathogenic to humans or environment. Lentivirus, generated from 3rd generation lentivirus vectors, is, while infectious, self-inactivating and replication incompetent. While an accidental infection of another cultured cells in a laboratory is possible, it is unlikely for virus to survive the harsh environment outside the laboratory to infect any living things.

Bacterial strains:
Health and Safety
Executive
The DNA plasmid vectors will be propagated during the molecular cloning stages. This process is done usually by transforming K-12 derivative strains of E. coli (Hazard Group 1 E. coli, e.g. DHSalpha and Top1 0) and purifying DNA from them. These strains are disabled, and have been demonstrated to be non-pathogenic and have well-understood, stable genetic lesions in the bacterial chromosome. These lesions often render the microorganism auxotrophic and dependent upon nutrients that must be supplied in the culture media. Furthermore, these strains are often rendered incapable of colonising mammalian hosts, either due to introduced biological restrictions or sensitivity to common agents.

Host/vector system

DNA plasmids:
All the plasmids used are non-mobilizable.

Lentiviral particles:
The plasmid containing the gene of interest is transfected along with plasmids containing viral packaging genes into the host producer cells. For 3rd or later generation of lentiviral systems, there should be at least 3 vectors required to generate packaged viral particles. The viruses are self-inactivating and replication incompetent, and enhanced safety feature of later generation of LVs ensures that the potential for multiple recombination events to revert to replication competent viruses are extremely rare.

Viral particles themselves are easily inactivated using a common laboratory disinfectant (e.g. Virkon, TriGene, Distel), and are susceptible to inactivation by dehydration or other environmental insults outside of controlled cell culture conditions. They require close contact with body fluids or percutaneous inoculation for transmission. The virus could, in principle, in the unlikely event of it being introduced to the cells of a worker, give genomic modification of cells of the worker.

Origin & function

Genes of interest:
The sequences of the genes of interest could be the full length or fragments of a gene from open reading frames (ORFs), cDNAs, genomic DNA or designed synthetic DNA sequences. The sequences can contain intended mutations. RNA interference and CRISPR-Cas9 sgRNA materials are single stranded RNAs (in case of CRISPR, it could be encoded in DNA plasmid), which has specific target sequences within the genes or in the regulatory elements.

The types of genes include 1) Non-hazardous genes encoding proteins which are soluble, membrane bound or secreted, including exogenous proteins to add functions e.g. reporter genes, 2) 'Target genes' known to have close link to cancer, or 3) known 'hazardous' genes, e.g. oncogenes, tumour suppressor genes, DNA repair genes and their regulatory elements. However, we do not intend to use any immediately toxic genes, e.g. toxin and proteins which cause neurodegenerative disorders of humans (Transmissible Spongiform Encephalopathies or TSEs)

CRISPR-Cas9 systems:
There are two major components, Cas9 (CRISPR associated protein 9) and single guide RNA (sgRNA), involved in this technology. The machinery is used in an RNA-guided manner for genome editing, e.g., knock-in, knock-out, artificial transcriptional activation and inhibition.

Genes from the viral vectors:
Five viral genes are present in the host and/or virus particles from lentiviral packaging:
Gag, pol, tat, rev, and an envelope protein (most likely VSV-G, Vesticular Stomatitis Virus glycoprotein G, replacing HIV's own envelope proteins).
These genes are encoded over 3 separate vectors in 3rd or later generation of lentiviral systems. The accessory genes from original HIV genome (v if, vpr, vpu, nef) are deleted from the packaging vectors.

Evaluation of foreseeable effects

**Mammalian cells:**
The final products are the mammalian cell lines (parental cell lines from Hazard groups 1 and 2) which have altered levels of expression of certain gene(s) of interest, or mutation(s):
A cell line might overexpress one or more gene(s) of interest
A cell line might have lowered (or no) expression of one or more genes of interest
A cell line might have one or more mutations in the gene of interest or in the regulatory element(s).
In most cases, resulting cell lines should not be any more hazardous than the parental cell lines. Even in the event of directly injecting them into a human subject or contaminating open cuts and wounds with the cells, a person's immune system should quickly mount a defensive immune response. Cell lines themselves are usually not known to be allergenic or toxic. There is no evidence to suggest that the host or genetically modified cell line will be able to propagate outside of a controlled laboratory environment without defined growth media and temperature requirements.
However, genetically modified cells, especially when manipulation was performed on known oncogenes, tumour suppressor genes, DNA repair genes, or signalling molecules i.e. cytokines, could have potential to impair normal biological pathways and systems if they are introduced directly into a human subjects. Again, in most cases, the immune system would quickly clear those cells by recognising them as foreign. However, in some rare cases, the risk might be increased due to the characteristics of the parental cell line, or the expressed gene(s):
Some parental and resulting cell lines are tumorigenic, and they might pose higher risk to immune-compromised individuals (note: experiments showing tumorigenicity are often done in nude mice, which have compromised immune system).
Some Hazard Group 2 cells contain genetic material from human papillomavirus (HR-HPV). In the normal 2-D culture condition, cells cannot produce HPV. However, in 3-D cultures, co-culturing or genetic modification could potentially allow production of HPV particles.

**CRISPR-Cas9:**
There is a slight risk if all the components of CRISPR machinery are injected into the operator, especially in conjunction with a lentiviral vector delivery system:
DeSigned CRISPR effects: The changes originally designed for in vitro tissue culture could occur within the operator’s cells.
Potential for insertional mutagenesis: The original viral particles generated in producer cells can infect human cells and integrate the genome in the host human cells from the accidental introduction (e.g. needle sticks). This could potentially lead to 1) integration and expression of Cas9 and sgRNA genes, 2) insertional mutagenesis depending on the site of integration of Cas9 gene and subsequently oncogenesis. The risk rating becomes higher if 1) high viral titre, 2) large volume and 3) directly injected/contaminating via open cuts/wounds.
To minimise risk, avoid route of direct infection, e.g. needle sticks and/or spills onto open wounds. Take caution when dealing with CRISPR-Cas9 machinery, especially if lentiviral vector delivery system is used. Resulting cell lines should not pose an increased risk to the operator over their parental cell lines.
Lentiviral particles:
The highest risk of infection is when virus particles are generated and handled: The resulting virus particles are infectious, although self-inactivating and replication incompetent (i.e., cannot increase the viral load after being taken out of virus producing cells). Because of engineered pseudotyping, the virus will have a broad array of cell types and species it can infect. The viral particles require close contact with body fluids or percutaneous inoculation for transmission. If the virus is introduced directly (e.g. Injection or spill over broken skin), chances of infection could be conceivable. Exposure to other mucus membrane (e.g. Splash to the eyes, nose, mouth, or inhalation of airborne particles) might have some risk of infection, although at much reduced rate. An accidental contact of the cultured cells or viral particles to intact skin should not have hazardous consequences given the affected skin is washed immediately with copious amount of water.

There are several potential risks if accidental exposure of the virus particles to human occurs:
Potential for insertional mutagenesis: The original viral particles generated in the producer cells (e.g. HEK-293) can infect human cells and integrate their genome in the host human cells from the accidental introduction (e.g. needlesticks). This could potentially lead to 1) integration and expression of insert gene (which could be an oncogene), 2) insertional mutagenesis depending on the site of integration (which could potentially change the gene or regulatory elements) and subsequently oncogenesis. The risk rating becomes higher if 1) oncogenes are inserted, 2) high viral titre, 3) large volume and 4) directly injected/contaminating via open cuts/wounds.

Potential for generating replication competent virus: the later generation of lentiviral vectors are self-inactivating and replication incompetent. The complex safety features, e.g. deleted accessory genes and ‘split-genes’ into separate vectors, requires at least 3 recombination events to generate reverted, replication competent viruses, which is extremely rare and highly unlikely.

The lentiviral particles cannot survive outside of closed controlled cell culture conditions and are rapidly inactivated by dehydration or other environmental insults. Once the virus particles are internalised, remaining virus particles washed away, and the viral genome integrated into host's DNA, the resulting established cell lines should not be any more harmful than the parental mammalian cell lines.

Bacteria cells:
The bacterial strains used to produce the plasmid vectors are attenuated non-colonising strains that are harmless to Humans and cannot survive outside culture conditions, and are therefore harmless to the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon and Distel are broad spectrum virucidal disinfectants effective in against blood borne viruses, including GM lentivirus - which are the main microbial hazards in this project. They also destroy DNA and RNA, making them suitable reagents for destroying GMOs. 2% Virkon or Distel (v/v) will be used to treat liquid waste (30 minutes) and to decontaminate any spills and for disinfection of any reusable bottles or other equipment and laboratory materials used. Liquid waste will be poured down the sink after treatment with 2% Virkon or Distel. Work surfaces will be wiped down with Distel and 70% ethanol at the end of work and when contamination is suspected. After treatment, solid waste (e.g. flasks) will be bagged in biological waste bags. This waste will then be destroyed by high temperature incineration using a reputable waste contractor, such as Grundon. High temperature incineration is a validated means used to provide assurance of complete GMO kill.
CMAL has an agreement with waste management CRO, Grundon, whose operational site is located within 6-miles of the laboratory. Building management team assists us with transportation of laboratory waste to the designated bins. The team also operates the autoclave instruments in the building.

Joint Cancer Research UK Therapeutic Discovery Laboratory and Cancer Research UK Biological and Genetic Modification Safety Committee:
This committee agreed that the proposed project falls within the activity assigned (activity class 2) and approved the project with a minor comment:
- Incubation of cultures involved in lentiviral work should be incubated in a separate incubator from other cultures.

The recommendation from the committee was reflected in the final version of the risk assessment, typographical errors were corrected, and approved by CMAL, Shurene Bishop Simon (Health and Safety Manager and secretary of the safety committee) and Lorna Stewart (chair of the safety committee).

Please enter comments on the GM safety committee on the risk assessment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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<tbody>
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Animal Units

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Project Containment

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
**Data Premises Notified**
24/08/2015

**Transferred from 1992 Regs?**
N

**Transitional Premises**
Class

**Data Premises Closed**

**Transitional Premises**
Emergency Plan Required?
N

**Non-GMMs**
N

**Withdrawn**
N

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**Campus Estate or Research Centre**
STEVENAGE BIOSCIENCE CATALYST

**Road Name**
GUNNELS WOOD ROAD

**Building**

**District**

**Town**
STEVENAGE

**County**
HERTFORDSHIRE

**Postcode**
SG1 2FX

**Country**
ENGLAND

**Tel Number**
01438 906755

**Fax Number**
0

**E-mail**

**HSE Division**
blank

**Comments**

**Date at Which Additional Info Submitted**
02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Auspherix’s GM committee comprises of the following members:

1. CEO
2. CSO who has extensive knowledge with working with GMO
3. Senior scientist
4. Biological Safety Advisor - external consultant

The committee meets at least every 6 months or ad hoc to assess any new activities and risk assessments

<table>
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Tick if confidential

Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research | Yes |

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

GMM liquid waste will be disposed off into appropriate disinfectant (2% sodium hypochlorite, 1% Virkon or a 1:10 dilution of Distel or Chemgene) within a Class II Microbiological Safety Cabinet (MSC) where all work using these organisms will be carried out. After 24 hours, this waste will be discarded into the drains. Bacterial culture plates will be sealed, double-bagged inside the cabinet before being autoclaved on site (see attached Risk Assessment for details of cycles). Contaminated tips will be soaked in appropriate disinfectant (see above) and disposed of in a sharps container. Any contaminated glassware (kept to minimum) will be soaked in appropriate disinfectant for 24 hours, then rinsed prior to normal cleaning. Other solid waste (plasticware) will be autoclaved on site prior to disposal via the clinical waste route. Autoclave logs will be monitored and waste will only be disposed of if the run is successful. Fresh dilutions of each disinfectant will be made weekly.

Virkon is a bactericidal agent, which is used at a concentration of 1%. It has been shown to cause a 5-log reduction in bacterial counts (Hernandez, A 2000. Journal of Hospital Infection. 46: 203-209). Low concentrations of sodium hypochlorite (bleach) have a biocidal effect on mycoplasma and vegetative bacteria in seconds in the absence of high amounts of organic material (Dychdala GR Disinfection, sterilization, and preservation. 2001 :135-157). S. aureus, Salmonella choleraesuis, and P. aeruginosa have been shown to be inactivated in <10 minutes by sodium hypochlorite (Rutala WA et al Infect Control Hosp. Epidemiol. 1998;19:323-7). Distel I Chemgene are bactericidal and effective in destroying type strain cultures of P. aeruginosa, S.aureus, MRSA and VRE with contact time of 1-5 minutes.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Committee has approved the project at Class 2 for work at Containment 2
**Project Ref** 3288/15.1

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<td>The use of bacterial mutants, knockouts and modified cell line to assess the antimicrobial activity of small molecules</td>
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<td></td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements

**Historical Significant Changes**

Tick if notifying a connected programme of work

**Project Additional Information**

**Purposes of the contained use**

Bacterial infection models will be used to characterise the activity of novel antimicrobial agents. Specifically, the ability of the novel compounds to inhibit bacterial growth will be tested. These experiments aim to quantify and monitor infection at serial timepoints by using bacteria that have the green fluorescent protein (gfp) gene inserted into their genome. Monitoring GFP production will allow the visual detection of infection as well as quantification of the amount of bacteria present. Mutant bacterial libraries with deletions in non-essential genes, will also be used to characterise pathways that are targeted by the antimicrobial agents being assessed.

**Recipient or parental organism**

The following bacteria will be used in the proposed work:

- *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus subtilis* and *Escherichia coli*.
- These micro-organisms are classified as ACDP hazard group 2 (or 1 in the case of B. subtilis) and are found in the environment or human host.
- *E.coli* is commonly found in the intestine and faeces of humans and animals as a commensal organism.
- *S.aureus* commonly colonises human skin and nasal passages and is found on environmental surfaces in hospitals and in community settings.
- *Acinetobacter* is found in the environment in water (including drinking and surface waters) as well as in sewage and soil.
- *Pseudomonas aeruginosa* is commonly found in soil and water.
S. typhimurium is predominately found in the intestinal lumen of humans and animals. Bacillus subtilis is classed as non-pathogenic. These bacteria do not tend to cause disease in immunocompetent individuals or cause a self-limiting disease, but can be problematic in the immunosuppressed, neonates or the elderly. Risk assessments are in place outlining the safe handling of these organisms. J-Iat cells originate from the Jurkat cell line, which are designated as biosafety level 1.

Host/vector system

The host organisms are ACDP hazard group 2 or below and are described above. The genetically modified bacteria and cells will be obtained from commercial sources or from a scientific collaborator.

Origin & function

Non essential bacterial genes are disrupted by the insertion of an antibiotic-resistance cassette or a transposon cassette. Fluorescent bacteria have a gfp gene inserted. The modified bacteria can be selectively grown, by virtue of a resistance gene. The genetic material is stably inserted into the bacterial genome and is not known to result in more virulent bacteria.

Evaluation of foreseeable effects

To human health and environment:
Any risk using these GMOs relates to them being ACDP hazard group 2 organisms. The modified strains have not been shown to be more virulent than their wildtype equivalent. Control measures will be in place when using these GMOs, as outlined in the attached risk assessment. Therefore there are no additional risks to humans or the environment as a result of any of the identified modifications. The containment and disposal measures outlined in the risk assessment below will minimise the chance of infection to the worker or release of these organisms into the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMM liquid waste will be disposed of into appropriate disinfectant (2% sodium hypochlorite, 1% Virkon or a 1:10 dilution of Distel or Chemgene) within a Class II Microbiological Safety Cabinet (MSC) where all work using these organisms will be carried out. After 24 hours, this waste will be discarded into the drains. Bacterial culture plates will be sealed, double-bagged inside the cabinet before being autoclaved on site (see attached Risk Assessment for details of cycles). Contaminated tips will be soaked in appropriate disinfectant (see above) and disposed of in a sharps container. Any contaminated glassware (kept to minimum) will be soaked in appropriate disinfectant for 24 hours, then rinsed prior to normal cleaning.
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Distel I Chemgene are bactericidal and effective in destroying type strain cultures of P. aeruginosa, S.aureus, MRSA and VRE with contact time of 1-5 minutes.

**Project Containment**

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Please enter comments on the GM safety committee on the risk assessment

The Committee has approved the project at Class 2 for work at Containment 2

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| **Name:** | PFIZER LTD |
|-----------|

| **Campus Estate or Research Centre:** | DISCOVERY PARK |
|--------------------------------------|
| **Road Name:** | SANDWICH |
| **Town:** | KENT |
| **County:** | CT13 9NJ |
| **Postcode:** | ENGLAND |
| **Country:** |

| **Tel Number:** | 01304 616161 | **Fax Number:** | 0 |
|-----------------|-------------|----------------|

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| **Date at Which Additional Info Submitted:** | 02/03/2022 |
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<td>PFIZER'S GENETIC MEDICINE INSTITUTE</td>
<td>THE LONDON BIOSCIENCE INNOVATION CENTRE</td>
<td>2 ROYAL COLLEGE STREET</td>
<td>LONDON</td>
<td>NW1 0NH</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The site BSO attends the bi-annual GM committee at pfizer Cambridge site for peer review of GM risk assessment.

Biological Safety Officer attends the local bi-weekly Leadership Team meeting including a section for raising any safety and biological safety issues.

All lab representatives attend a 3-monthly health and safety local meeting to raise issues and disseminate information to lab users including Bio safety issues and information.

Risk assessments are created in an electronic registration system and are circulated to registered GM users with appropriate expertise and experience, before final approval by BSO and discussion at safety meeting.

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02/03/2022
Solid Waste:
Any biological or potentially infectious waste must be autoclaved to sterilise prior to disposal. Waste for autoclaving should be collected in clear, autoclave bags placed inside autoclavable bins or in autoclavable Bio-bins. Once full, the bins should be sealed and labelled for disposal. All autoclave waste must be left in the appropriate collection point for collection by LBIC staff. The waste will be collected on a daily basis. Laboratory waste which does not require autoclave sterilisation should be bagged in a yellow, clinical waste bag or an appropriate Bio-bin. Once full, the bag or bin should be sealed using cable ties and labelled and left in the appropriate collection point.

Liquid Waste: Liquid waste for disposal to foul drain should be disinfected with approved method of disinfection e.g. Virkon, Chern Gene or precept. All waste material and residues will be disposed of according to the LBIC hazardous waste disposal guidelines.

Biohazardous waste is emptied daily.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Attached biohazard risk assessment has been reviewed and approved.

Project Ref 3289/16.1

Date Ackn'd CU2 Project Title Class CultureVol
08/01/2016 GMO work activities at Pfizer's Genetic Medicine Institute, based at the London Class 2 1-50 Litres

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

To engineer, design and develop rAAV vectors as well as develop rAAV manufacturing process and analytical methods to support the research and development of gene therapy medicinal products. The work involves culture of laboratory strains of non-pathogenic transmission immobilized bacteria, rAAV, produced using tissue culture methods in established mammalian or insect cell lines (supplied from external sources) in conjunction with plasmid DNA or recombinant viruses. Plasmid and viral lots will be analysed using biochemical, biological, molecular and physical characterisation methods. The biological methods include the use of HeLa RC32 (RG2ICl2) cell line (HeLa cells containing Rep/Cap Genes from AAV2) for infectious titer assay of rAAV lots and use of Adenovirus type 5 (Ad5) reference standard and HEK293T (both RG2ICl2).

**Recipient or parental organism**

Non-pathogenic transmission immobilised Bacteria (E.Coli DH5Alpha and SURE bacteria) are used to generate and engineer plasmid DNA through molecular biology techniques (cloning) and amplify stock of plasmids for further use. rAAV are derived from a non-pathogenic and replicative defective human virus (AA V) and generated using human embryonic kidney cells (HEK293T, HEK293), Chinese Hamster Ovary (CHO) or insect (SF9) cell lines in conjunction with either plasmid DNA, recombinant baculoviruses (AcMNPV) generated through cloning and transfection through SF9 cells, or recombinant baculoviruses (from external sources).

**Host/vector system**

rAAV are generated within HEK cells (HEK293, HEK293T), CHO cells or SF9 cells. AAVs are non Pathogenic, replication deficient human viruses. Characteristic of the AAV Systems to be used:

- **Virus Free System:** The rAAVs will be manufactured using a helper-virus free system by transient transfection of 2 or 3 plasmids in HEK293 or HEK293T cells (RG2ICl2):

  - The 2 plasmids System is composed of a Helper/Packaging Plasmid coding for the 5 minimum required adenoviral helper Genes/proteins, the Rep proteins of the AA V2 and the Cap proteins (VP1, VP2, VP3) of different rAAV serotypes and engineered variants (depending on the specific of each project the different serotypes use will be...
AAV-1, 2, 3, 4, 5, 6, 7, B, 9, rh10 and Variants derived/engineered from AAV 1, 2, 3, 4, 5, 6, 7, 8, 9, rh10). The second plasmid contains the expression cassette flanked by AAV2 ITR sequences. The third plasmid system is composed of a Helper plasmid containing the 5 minimum required adenoviral helper genes/proteins, the packaging plasmid containing the Rep gene from AAV V2 and the Cap gene of dille rent AAV serotypes and engineered variants (as above) and the third plasmid contains the expression cassettes flanked by AAV2ITRs.

**Origin & function**

The expression cassettes intended to be used are either reporter genes (YFP, GFP, luciferase etc...) or a therapeutic transgene (for gene therapy strategy evaluation, usually based on human genes) under the control of tissue specific promoter or constitutive ones (CMV, CAG etc...). Each Therapeutic transgene will be risk assessed separately on a project to project basis.

Nature and origin of inserts including possibility of inserted material, altering the characteristics of the recombinant AAV: rAAV genomes produced contain the 2 ITRs from AAV 2 (inverted terminal repeats) flanking an expression cassette composed of either reporter genes such as YFP, GFP or luciferase or therapeutic transgene (risk assessed separately on a project by project basis) under the control of tissue specific human promoters or constitutive promoters such as CMV or CAG promoters.

The helper functions coding for the 5 minimum required adenoviral helper Genes/proteins E1A-E1B, E2, E4, and VA RNA will be brough by the helper plasmids in the transfection system.

rAAV variant engineering, by modification of the AAV Cap sequences, generates rAAV with modified capsid resulting in increased transduction efficency, increased or modified biodistribution, modified tissue tropism and/or reduced immunogenicity.

**Evaluation of foreseeable effects**

Due to the production systems using separated entity for the expression of Rep/Cap genes and the ITRs and Genome, recombination events leading to a replication competent AAV (wtAAV) are considered very low. In the event of this recombination occurring the resulting species would be as replication deficient as the wild type AAV and nonpathogenic.

rAAV genome integration in human genome is considered extremely low risk due to the deletion of the Rep gene (involved in the site specific integration mechanism) from the rAAV genome. Rare events of random integration of rAAV genome have been reported but the probability of such an event is considered very low.

HEK293, HEK293T, Hela, Hela RC32 all carry biohazard associated with the partial viral genome they contains and potential adventitious agent, however the risk is considered low considering the controls in place.

Baculovirus does not infect humans or animal but causes hazard to environment (as is insect pathogene) however the Baculovirus expression system is based on a deleted strains for the polyhedrin gene rendering the virus sensitive to environmental factors and insect gut condition resulting in a low risk for the environment and human health.

Accidental exposure routes are determined to be:

Ingestion, Inhalation, Inoculation/Injection, Contact e.g. eyes, dermal
The likely sources of accidental exposure are due to aerosol inhalation, exposure to contaminated liquids or via sharps.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

**Solid Waste**

Any biological or potentially infectious wastes are handled through the LBIC waste management route via autoclaving. Biohazardous wastes are collected in clear biohazard bags or in autoclavable Bio-bins. Once full, the bins are sealed and labelled for disposal.

All autoclave waste are left in the appropriate collection point for collection by LBIC staff. The waste are collected on a daily basis.

Laboratory waste which does not require autoclave sterilisation are bagged in a yellow or orange waste bag or an appropriate Bio-bin. Once full, the bag or bin is sealed using cable ties and labelled and left in the appropriate collection point for collection by LBIC.

**Liquid Waste**

- Liquid waste for disposal to foul drain are disinfected with approved method of disinfection e.g. Virkon 1% (w/v) or Chern Gene (1:20 v/v) for a minimum period of 2 hours.

All waste material and residues will be disposed of according to the LBIC hazardous waste disposal guidelines.

Biohazardous waste is emptied daily.

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**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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**Project Ref** 3289/16.2

<table>
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<tr>
<th>Date Ackn'd</th>
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<tbody>
<tr>
<td>15/09/2016</td>
<td>Design, production and analysis of HBoV/AAV(human Boca Virus/Adeno-Associated-Virus) hybrid vectors for Gene Therapy</td>
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<tr>
<td>31/07/2017</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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**Non-GMM Consent Granted**

**Project notified under transitional arrangements** N

### Purposes of the contained use

The project involves the design, production and analysis of HBoV/AAV vector composed of a HBoV1 capsid and a rAAV genome encoding for either reporter genes (Luciferase, mCherry, GFP, YFP) or the human or ferret CFTR gene, under the control of CMV, CAG or F5 promoters. Design will involve molecular biology cloning techniques and DNA amplification in E.Coli lab strains, production will involve amplification of cell line (HEK293 or HEK293T), transfection with an Helper, packaging, transgene and replication plasmids. Clarification through microfluidisation, centrifugation, ultracentrifugation, filtration; purification by chromatography. Analysis of the vector will involve nucleic acid amplification techniques (PCR, qPCR, dPCR), protein analysis, (HPLC, SDS PAGE, WesternBlot, ELISA), Genome analysis (alkaline AGE, AGE, HPLC) and other methods for potency and purity determination.

### Recipient or parental organism

Non-pathogenic transmission immobilised Bacteria (E.Coli DH5Alpha and SURE bacteria) are used to generate and engineer plasmid DNA through
molecular biology techniques (cloning) and amplify stock of plasmids for further use. rAAV genomes are derived from a non-pathogenic and replicative defective human virus (AAV), Packaged into the caps of HBoV1 (human boca virus 1), a member of the Parvoviridae virus family has been suggested to cause human disease (has been associated with lower respiratory tract and gastrointestinal infections, predominantly in children) generated using human embryonic kidney cells (HEK293T, HEK293), or insect (SF9) cell lines in conjunction with either plasmid DNA, recombinant baculoviruses (AcMNPV) generated through cloning and transfection through SF9 cells.

**Host/vector system**

rAAV/HBoV vector are generated within HEK cells (HEK293, HEK293T) or SF9 cells. Characteristic of the rAAV/HBoV1 Systems to be used: Virus Free System: The rAAV/HBoV1 will be manufactured using a helpervirus free system by transient transfection of 4 plasmids in HEK293 or HEK293T cells (RG2/CL2): o The 4 plasmids system is composed of a Helper plasmid containing the 5 minimum required adenoviral helper genes/proteins, the packaging plasmid containing the early terminated non structural gene (NS) and Structural (VP1, VP2 and VP3) gene of HBoV1 under the control of CMV promoter, the Replication plasmid containing the Rep gene from AAV2 and a 1.3Kb deleted version of the Cap gene of AAV2 (disrupted capisid gene) flanked by ITRs fro Adenovirus 5 and the fourth plasmid contains the expression cassettes flanked by AAV2 ITRs. Baculovirus (AcMNPV) /Insect Cells System (RG1/CL1) for rAAV/HBoV1 production: rAAV/HBoV1 production is allowed by infecting SF9 Cell culture by 2 Baculovirus expressing Rep from AAV2, VP gene of HBoV1 gene and The expression cassettes flanked by AAV2 ITRs. HEK293 are transformed with Adenovirus 5 DNA and HEK 293T express the SV40 large from the Simian Virus 40 in addition, both cell line are handled at CL2..

**Origin & function**

The expression cassettes intended to be used are either reporter genes (YFP,GFP, Luciferase, mCherry etc..) or a fCFTR (ferret CFTR) or hCFTR (human CFTR) therapeutic transgenes under the control of tissue specific promoter or constitutive ones (CMV, CAG or F5). Nature and origin of inserts including possibility of inserted material, altering the characteristics of the recombinant AAV/HBoV1: rAAV genomes produced contain the 2 ITRs from AAV 2 (inverted terminal repeats) flanking an expression cassette composed of either reporter genes such as YFP, GFP, Luciferase or mCherry or therapeutic transgene (fCFTR or hCFTR) under the control of tissue specific human promoters or constitutive promoters such as...
CMV, CAG or F5 promoters.
The helper functions coding for the 5 minimum required adenoviral helper
Genes/proteins E1A-E1B, E2, E4, and VA RNA will be brought by the helper
plasmids in the transfection system.

rAAV/HBoV1 vector are regenerated using a HBoV1 capsid and a rAAV
genome increasing tissue tropism to the Lung.
The resultant rAAV/HBoV1 vector would be, as all rAAV (since the genome
is based on AAV), replication incompetent, and once introduced into human
cells result in CFTR expression. There is none expected harmful properties
to human as rAAV is non-pathogenic, additionally CFTR is normally expressed
in normal individuals and is not expected to be harmful in the population, at
least overexpression of CFTR in healthy animal is not known to be harmful or
toxic. rAAV are also safe for the environment (infect only mammalian) and in
the unlikely accidental infection of environmental species, AAV is not known to
cause any animal diseases and the expression of human CFTR gene in

Evaluation of foreseeable effects

animal is not know to be harmful or toxic. has the only component from
HBoV1 is the proteic capsid surrounding the rAAV genome it is not expected
to display any of the pathological effect of the wild type HBoV.

Due to the production systems using separated entity for the expression of
Rep genes, the ITRs and Genome, and the HBoV1 capsid, recombination
events leading to a replication competent AAV (wtAAV) are considered not
possible since the rAAV capsid gene in the system is disrupted.

Recombination event leading to a replication competent HBoV1 is considered
very low since most of the NS proteins, essential to HBoV1 replication, have
been removed from the system. recombination event leading to a replication
competent rAAV/HBoV1 would only be possible if AAV Rep protein could
support the replication, assembly and packaging of rAAV/HBoV1, and this
multiple recombination is considered of low risk, and it is unknown whether
AAV Rep could support all these function in a cross species system. In the
event of any of this recombination occurring the resulting species would be as
replication deficient as the wild type AAV and non-pathogenic. rAAV genome
integration in human genome is considered extremely low risk due to the
deletion of the Rep gene (involved in the site specific integration mechanism)
from the rAAV genome. Rare events of random integration of rAAV genome
have been reported but the probability of such an event is considered very
low.

HEK293, HEK293T, HeLa, HeLa RC32 all carry biohazard associated with
the partial viral genome they contains and potential adventitious agent,
however the risk is considered low considering the controls in place.
Baculovirus does not infect humans or animal but causes hazard to
environment (as is insect pathogen) however the Baculovirus expression
system is based on a deleted strains for the polyhedrin gene rendering the
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low risk for the environment and human health.
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Ingestion, Inhalation, Inoculation/Injection, Contact e.g. eyes, dermal
The likely sources of accidental exposure are due to aerosol inhalation,
exposure to contaminated liquids or via sharps.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>Solid Waste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any biological or potentially infectious wastes are handled through the LBIC waste management route via autoclaving. Biohazardous wastes are collected in clear biohazard bags or in autoclavable Bio-bins. Once full, the bins are sealed and labelled for disposal. All autoclave waste are left in the appropriate collection point for collection by LBIC staff. The waste are collected on a daily basis. Laboratory waste which does not require autoclave sterilisation are bagged in a yellow or orange waste bag or an appropriate Bio-bin. Once full, the bag or bin is sealed using cable ties and labelled and left in the appropriate collection point for collection by LBIC.</td>
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</tbody>
</table>

<table>
<thead>
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<th>Liquid Waste</th>
</tr>
</thead>
<tbody>
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<td>Liquid waste for disposal to foul drain are disinfected with approved method of disinfection e.g. Virkon 1% (w/v) or ChemGene (1:20 v/v) for a minimum period of 2 hours. All waste material and residues will be disposed of according to the LBIC hazardous waste disposal guidelines. Biohazardous waste is emptied daily.</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
No comment provided, the risk assessment was reviewed and approved by the members of the committee

Project Containment

02/03/2022
<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
<tr>
<td>L2</td>
<td>L3 L4 L2 L3</td>
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<tr>
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Animal Units

Large Scale Activities

Human Clinical Applications
### GM Centre Number: 3290

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#### Name

ATDBio Ltd

#### Name 2

Department

#### Campus Estate or Research Centre

MAGDALEN CENTRE NORTH

#### Road Name

ROBERT ROBINSON AVENUE

#### Building

OXFORD SCIENCE PARK

#### Town

Oxford

#### County

OXFORDSHIRE

#### Postcode

OX4 4GA

#### Country

ENGLAND

#### Tel Number

01865784616

#### Fax Number

none

#### E-mail

support@atdbio.com

#### HSE Division

EAST AND SOUTH EAST

#### Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

<table>
<thead>
<tr>
<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
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<th>Withdrawn</th>
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<td>Magdalen Centre North</td>
<td>Oxford Science Park</td>
<td>Robert Robinson Avenue</td>
<td>OXFORD</td>
<td>Oxford</td>
<td>OXFORDSHIRE</td>
<td>OX4 4GA</td>
<td>ENGLAND</td>
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</tr>
</tbody>
</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

In accordance with Regulation 8, which requires the person responsible to ensure that expert advice on risk assessment is obtained, due to class 1 risk assessment such advice can be provided by a competent individual. The competent individual providing the advice is the Safety Officer who is also a scientist supervising and carrying out the work.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Level 2 (GMMs)</td>
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<td>Birds</td>
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Tick if confidential

02/03/2022
Off-site treatment by High Temperature Incineration will be used. Solid and liquid waste will be separated and stored for collection by Grundon (waste contractor) and sent for High Temperature Incineration. Provisions for inactivation on-site will also be available; an autoclave on-site (serviced annually and each run will be monitored with print out of temperature/time profile) using standard methods of inactivation with a 100% degree of kill expectation will be used to inactivate solid waste.

Virkon powder to disinfect liquid waste with 1% Virkon solution. Inactivated liquid waste will be disposed of to drain once this has been approved and included in ATDBio’s Thames Water Trade Effluent Notice.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The competent individual has reviewed the risk assessment for the proposed work and concluded that the activities are Class 1. The bacteria cell lines are classified as ACDP Hazard Group 1, the vectors are existing or commercially available vectors and the DNA inserts code non-hazardous, non-toxic proteins.
### GM Centre Number: 3291

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#### Name

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#### Name 2

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#### Campus Estate or Research Centre

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#### Comments

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02/03/2022
Premises Addresses

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

All senior members of staff have been members of a GMO safety committee at their previous place of employment (BioVex Ltd). One of these staff members was also the GMO officer at their previous place of employment for 6 years. This business BioVex Ltd) also handled genetically modified viruses. This employee has received training in GMO risk assessment processes. The employee chaired the GMO committee and was responsible for employee project registration and staff training.

Replimune is also in the process of contracting an outside Health and Safety consultant with extensive experience in conducting GMO risk assessments to give further advice and training as required.

Level 1 (GMMs)
Yes

Level 2 (GMMs)
Yes

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial
For activities involving GMMs, describe the waste management measures which will apply to the activity

Not applicable

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Not applicable

Project Ref 3291/15.1

Date Ackn'd 20/08/2015

CU2 Project Title Development of oncolytic viruses for cancer therapy

Class Non-GMM

CultureVolClass2 Class 2

Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N
### Project Additional Information

#### Purposes of the contained use

To test a number of oncolytic viruses for efficacy in pre-clinical models alone and in combination with other cancer therapies.

#### Recipient or parental organism

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>Description</th>
<th>Relevant Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes Simplex Type 1 (HSV-1) or Type 2 (HSV-2)</td>
<td>The project will use strains disabled by deletion of the ICP34.5 or ICP34.5 and ICP47 genes. Each of the deletions in ICP34.5 and ICP47 have been shown individually to generate a non-pathogenic, though still replication competent virus.</td>
<td>Burgos et al. (2006) J Neurovirol 12: 420-27; Goldsmith et al. (1998) J Exp Med 187:341-348; Perng et al. (1995) J. Virol. 69:3033-41; Perng et al. (1996) J Virol 70:2883-93. This virus is designed to replicate selectively in tumors as compared to surrounding tissue.</td>
</tr>
<tr>
<td>Vesticular stomatitis virus (VSV)</td>
<td>This virus is a member of the Rhabdoviridae family. It is inherently tumor specific as replication is strongly suppressed in Interferon gamma (IFNy) response-normal tissues, but activated in IFNy pathway-defective tumor cells. Strains which have mutations in the matrix (M) protein have increased IFNy sensitivity which has been shown to increase tumor specificity.</td>
<td>Huang et al. (2003) Mol. Ther. 8: 434-440; Balachandran and Barber (2000) rUBMB Ufe 50: 135-138; Stojdl et al (2000) Nat. Med. 6, 821-825. 80th wild type and strains strains containing mutations in the matrix (M) proteins will be used in this project. Clinical trials are underway with VSV as an oncolytic agent (Clinicaltrials.gov identifier: NCT01628640)</td>
</tr>
<tr>
<td>Maraba virus</td>
<td>This virus is also a member of the Rhabdoviridae family and is also inherently tumor specific due to suppression of replication by IFNy. Like VSV, mutant strains with deletions in the M protein sequence which show increased IFNy sensitivity have been identified.</td>
<td>Brun et al. (2010) Mol Ther 18: 1440-1449. 80th wild type and strains containing mutations in the matrix (M) protein and G protein will be used in this project. Clinical trials are underway with Maraba virus as an oncolytic agent (Clinicaltrials.gov identifier: NCT02285816). Both Maraba virus and VSV may be pseudotypes with the envelope glycoprotein of lymphocytic choriomeningitis virus (ICMV), which has been shown to reduce neutralizing antibody induction in vivo (Muik et al. (2014) Cancer Research 74: 3567-3578).</td>
</tr>
</tbody>
</table>

#### Host/vector system

See above. The Green fluorescent protein (GFP; jelly fish) and/or bacteriallacZ marker genes will be inserted into each of the viruses under CMV, RSV or endogenous viral promoter control. Therapeutic genes may then be inserted which will be the subject of separate submission(s).
Origin & function

Green fluorescent protein (GFP: jelly fish) and lacZ marker genes. CMV and RSV viral promoter sequences.

Evaluation of foreseeable effects

HSV-1 is universally prevalent virus (approximately 50-80% of the human population are seropositive for HSV-1). HSV-1 rarely causes severe symptoms. The final vectors should only be capable of replicating in rapidly dividing eukaryotic cells. The viruses to be generated will be disabled by the deletion of ICP34.5 and ICP47 which has been shown to significantly reduce the pathogenicity of recombinant HSV-1 viruses compared to wild type virus and has been shown to be safe in a number of clinical trials (Andtbacka et al. (2015). J Clin Oncol. 2015 May 26. pii: JCO. 2014.58.3377; Harrington et al. (2010). Clin Cancer Res 16: 4005-15; Hu et al. (2006), Clin Cancer Res 12: 6737-47; MacKie et al. (2001). Lancet 357:525-6; Markert et al. (2000). Gen Ther 7:867-7). HSV-2 is closely related to HSV-1 and is also a universally prevalent virus although infections levels are lower than those for HSV-1 (approximately 20% of the human population are seropositive for HSV-2). The final vectors will have the same characteristics as those shown displayed by ICP34.5 and ICP47 deleted HSV-1 viruses.

VSV mainly infects horses, cattle, pigs, sand flies (Fang et al. (2012). Vaccine 30: 1313-21) and is non-lethal. The virus is passed on by insect bite or direct contact with an infected animal. Zoonotic infection of humans can occur either by directly exposed 10 infected livestock, or following an insect bite. Most human infections appear to be mild or subclinical (Letchworth et al. (1999). Vet J 157: 239-260; Lichty et al. (2004). Trends in Mol Med 10: 210-216). A clinical trial with a modified form of the virus containing IFN-13 (VSV-IFN-13) for the treatment of liver cancer is currently ongoing in the US. Oncolytic VSV has also been shown to be well tolerated in dogs (leBlanc et al. (2013) Hum. Gen. Ther. Clin. Dev. 24: 174-181) by intravenous dosing at up to 10 x 10^{10} TCID50. Maraba virus is related to VSV but is only found in insects and does not naturally infect mammals (Pauszek et al. (2011). Arch Virol 85: 9346-9358). A clinical trial with a modified form of the virus containing transgenic MAGE-A3 (MarabaMAGE-A3) is currently ongoing in Canada.

No expected effects of human exposure to any of these viruses are expected due to the attenuating deletions included (HSV1/2) or as the viruses do not naturally cause human disease (VSV, Maraba virus). Where oncolytic viruses have been tested in clinical trials, by direct injection into tumors, side effects are generally limited to mild fevers and local inflammation. However, this is at far higher doses than could occur following accidental exposure during this project.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

A 1% Virkon solution will be used for the disinfection of HSV vectors. 70% IMS and DistelTM will be used for general cleaning purposes.

All liquids that come into contact with virus will be treated with 1% Virkon solution for a minimum of 20 minutes prior to disposal.

All tissue culture plastics, tips and pipettes that come into contact with virus will be treated with 1% Virkon solution for a minimum of 20 minutes prior to disposal. After disinfecting, plastics will be drained of any excess liquid and then
placed in clinical waste bags. Once full, the bags will be sealed with biohazard tape before being removed by a regulated contractor.

Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L2</td>
<td>L2</td>
</tr>
<tr>
<td>L3</td>
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<table>
<thead>
<tr>
<th>Animal Units</th>
<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3</td>
<td>L2</td>
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<tr>
<td>L4</td>
<td>L4</td>
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</tbody>
</table>

**Project Ref** 3291/16.1

Date Ackn'd 03/03/2016

CU2 Project Title Production of oncolytic viruses expressing therapeutic molecules

Date Project Ceased

Class 2

Culture Class < 1 Litre

Non-GMM

Consent Granted

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info
The aim of this project is to develop oncolytic virus based on herpes simplex virus type 1 (HSV-1) or herpes simplex virus type 2 (HSV-2) which express proteins which may be beneficial for cancer treatment.

Herpes Simplex Virus Type 1 (HSV-1) and Type 2 (HSV-2): the project will use strains disabled by deletion of the ICP34.5 or ICP34.5 and ICP47 genes. Each of the deletions in ICP34.5 and ICP47 have been shown individually to generate a non-pathogenic, though still replication competent virus (Burgos et al. (2006) J Neurovirol 12: 420-27; Goldsmith et al. (1998) J Exp Med 187:341-348; Perng et al. (1995) J. Virol. 69:3033-41; Perng et al. (1996) J Virol 70:2883-93). This virus is designed to replicate selectively in tumours as compared to surrounding tissue.

The following genes (in various combinations) will be used to construct the viruses. These proteins are being inserted to aid in the promotion of an antitumor immune response and/or spread of the virus through tumours. Immune stimulatory proteins to be expressed will include ICOS-L, GITR-L, CD40-L, OX40-L, 4-1BB-L, FLT3-L, IL12, IL2, IL18, IL5, IL21, IL-28, IL-29, GM-CSF and TNFa. Anti-sense and/or RNAi to be expressed will include RAAi for TGFb2, IFNa and IFNg. Single chain antibodies to be expressed will include antibodies to CTLA-4, PD1, LAG-3, TIM-3 and VISTA PATENT. Molecules to aid viral spread will include viral membrane fusogenic glycoproteins from VSV-G, measles virus H or F, GALV-R-1, metalloproteinases and collagenases chondroitinases, and secreted DNAse.

One to four of these proteins will be expressed from a single virus to identify optimal combinations. Viruses expressing of marker genes (GFP, LacZ) in addition to the genes listed above. In all cases mouse or human versions of the genes may be used. These genes will be under the control of the human cytomegalovirus immediate early promoter (HCMV IE), the Rous sarcoma
virus promoter (RSV), the Simian Virus 40 (SV40) promoter, the Moloney murine leukemia virus (MMLV) LTR promoter, promoters from HSV itself or mammalian promoters such as the EF-1α/eIF4g hybrid promoter.

**Evaluation of foreseeable effects**

HSV-1 is a universally prevalent virus (approximately 50-80% of the human population are seropositive for HSV-1). HSV-1 rarely causes severe symptoms. The final vectors will selectively replicate in tumors as compared to normal tissue through the deletion of ICP34.5 or ICP34.5 and ICP47. These deletions have been shown to significantly reduce pathogenicity compared to wild type virus and have been shown to be safe in a number of clinical trials, including with the additional expression of GM-CSF (Andtbacka et al. (2015). J Clin Oncol. 2015 May 26. pii: JCO. 2014.58.3377; Harrington et al. (2010). Clin Cancer Res 16: 4005-15; Hu et al. (2006). Clin Cancer Res 12: 6737-47; Mackie et al. (2001). Lancet 357:525-6; Markert et al. (2000). Gen Ther 7:867-7).

HSV-2 is closely related to HSV-1 and is also a universally prevalent virus although infections levels are lower than those for HSV-1 (approximately 20% of the human population are seropositive for HSV-2). The final vectors will have the same characteristics as those shown displayed by ICP34.5 and ICP34.5/ICP47 deleted HSV-1 viruses.

No expected effects of human exposure are expected due to the attenuating deletions made in the viruses. Where oncolytic viruses based on HSV-1 have been tested in clinical trials by direct injection into tumors, side effects are generally limited to mild fevers and local inflammation following direct injection of up to 1e8 pfu/ml virus stocks into tumors. These are far higher doses than would be expected to occur following any accidental exposure to the viruses to be constructed in this project. In all cases, even following accidental exposure at high dose, only very localised expression of the encoded gene(s) would be expected as viruses to be constructed in the project will not replicate productively in normal tissue and would also be expected to be rapidly inactivated following any systemic exposure by immune responses to HSV.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not applicable

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

A 1% Virkon solution will be used for the disinfection of HSV vectors. 70% IMS and Distel will be used for general cleaning purposes. All liquids that come into contact with virus will be treated with 1% Virkon solution for a minimum of 20 minutes prior to disposal.
All tissue culture plastics, tips and pipettes that come into contact with virus will be treated with 1% Virkon solution for a minimum of 20 minutes prior to disposal. After disinfecting, plastics will be drained of any excess liquid and then placed in clinical waste bags. Once full, the bags will be sealed with biohazard tape before being removed by a regulated contractor.

The members of the GMO committee have been directly involved in the production of this risk assessment and has therefore agreed with its conclusions.

Project Containment

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<td>L2</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
</tr>
</tbody>
</table>

Project Ref 3291/17.1

Date Ackn’d 18/05/2017

Date Project Ceased

CU2 Project Title Gene therapy product RP1 (Common name rHSV-1hGM-CSF/ GALV-GP) for the treatment of solid tumours.

Class 2

 Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Tick to confirm that you have attached a risk assessment to this form Y

Tick to confirm that it is attached to this form

Is an emergency plan required according to regulation 20? N
RP1 will be administered to subjects as an anti-tumour therapy, as part of a Phase I clinical trial to treat a wide range of solid tumour types. RP1 has a particular utility in combination with immune co-inhibitory pathway blockade. Intended indications to study include soft tissue sarcoma, breast cancer including triple negative breast cancer (TNBC), lung cancer including non-small cell lung cancer (NSCLC), melanoma, non-melanoma skin cancers, head and neck cancer, primary liver and kidney cancer and colorectal cancer. The initial clinical trial protocol intends to test RP1 in several indications as a monotherapy and in combination with anti-PD-1 therapy.

RP1 (rHSV-1hGM-CSF/ GALV-GP) is a selectively replication competent Herpes Simplex Virus-1 (HSV-1). The virus contains a codon-optimised sequence for human granulocyte macrophage colony stimulating factor (hGM-CSF) and a codon optimised sequence for the gibbon ape leukemia virus surface glycoprotein (GALV-GP) with the Rsequence deleted (R-) [GALV-GP-R-]. GALV-GP-R- expression leads to cell to cell fusion (syncytial) formation in infected tumour cells through binding to the constitutively expressed PiT-1 receptor for GALV. This results in the death of the cells by membrane fusion and is also intended to enhance the spread of the virus through the tumour. Since the RP1 selectively replicates in tumour cells, the expression of the GALV-GP-R- is minimised in normal tissues. The oncolytic destruction of tumour cells leads to the release of tumour associated antigens that are intended to engender an anti-tumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further enhanced through GALV-GP-R- mediated killing, fusion associated cell death which also results in the production of the highly immunogenic exosomes, which is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, uninjected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

RP1 (rHSV-1hGM-CSF/ GALV-GP) is derived from the RH018 strain of Herpes Simplex Virus-1. RP1 is produced in the Vero cell and released into the culture media during cell lysis, prior to purification.
RP1 was constructed using a new strain of HSV-1 (strain RH018). Replimune obtained and compared 30 clinical strains of HSV-1 on a panel of human tumour cell lines and selected the most promising of these (strain RH018) for further development. RP1 expresses the immune stimulatory protein GM-CSF, which augments therapeutic activity. GALV-GP-R- binds to the PIIT1 receptor, which is widely expressed on mammalian cells including human tumour cells. PIIT1 is also critical for cell proliferation, and its expression is therefore unlikely to be lost or down-regulated in response to cancer treatment. The truncated R- version of the protein provides constitutive fusion activity without GALV (i.e. the virus) itself. Expressing GALV-GP-R- together with GM-CSF is expected to increase clinical activity as compared to only expressing GM-CSF. As well as causing direct tumour cell death by cell to cell fusion, cell to cell fusion followed by death is highly immunogenic and includes the release of immunogenic tumour antigen-containing exosomes. Expression of GALV-GP-R- from an oncolytic virus is therefore expected to improve systemic, immune mediated, effects, as well as effects in the directly treated tumour thereby increasing synergy with other immune-mediated approaches to cancer therapy such as immune co-inhibitory pathway blockade.

Evaluation of foreseeable effects

As described above (under Recipient or Parental Organism), the oncolytic destruction of tumour cells (upon transduction with RP1) leads to the release of tumour associated antigens that are intended to engender an anti-tumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further amplified through GALV-GP-R- mediated killing, fusion associated cell death which results in the production of the highly immunogenic exosomes and is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, uninjected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All used and unused RP1 and diluent vials used in preparation and syringes will be destroyed per institutional policy. As per the wild-type HSV-1 virus, the recombinant HSV-1 vector particles that represent RP1 are highly susceptible to dehydration, rapidly inactivated outside the host and easily inactivated (for example with 1% Virkon solution). As part of phase I of the clinical trial, biodistribution and shedding will be monitored. RP1 DNA levels in blood and urine will be determined at time-points outlined in the Schedule of Assessments of the clinical protocol (day 1, day 3/7/10, day 14, day 21, day 28, day 35, day 42, day 49, day 56 and as part of the follow up, 30 days after the last dose). Blood will be collected after the first, second and third RP1 injections at the following time-points: 1 (±15min), 2 (±15min), and 4 (±15min) hours and also immediately prior to dosing at the second and third dose. RP1 DNA levels and virus from saliva/oral mucosa, injection sites, injection site dressings, and lesions that appear to be herpetic will be determined on the same days.

Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N
Project Containment

Laboratory Activities

Glass Houses

Growth Rooms

L2 L3 L4 L2 L3 L4 L2 L3 L4

Animal Units

Large Scale Activities

Human Clinical Applications

L2 L3 L4 L2 L3 L4

Project Ref 3291/20.1

Date Ackn'd 23/04/2020

CU2 Project Title Gene therapy product RP1 (Common name rHSV-1hGM-CSF/ GALV-GP) in combination with Cemiplimab for the treatment of solid tumours

Class 2 CultureVolClass2 < 1 Litre CultureVolumeClass3-4

Consent Granted

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

RP1 will be administered to subjects as an anti-tumour therapy, as part of a phase II clinical trial to treat patients with advanced cutaneous squamous cell carcinoma. RP1 has a particular utility in combination with immune coinhibitory pathway blockade. The clinical trial protocol intends to estimate the clinical benefit of cemiplimab monotherapy versus cemiplimab in combination with RP1 for patients with locally advanced or metastatic (nodal or distant) cutaneous squamous cell carcinoma (CSCC), as assessed by overall response rate (ORR) according to central review.

Recipient or parental organism
RP1 (rHSV-1hGM-CSF/GALV-GP) is a selectively replication competent Herpes Simplex Virus-1 (HSV-1). The virus contains a codon-optimised sequence for human granulocyte macrophage colony stimulating factor (hGM-CSF) and a codon optimised sequence for the gibbon ape leukemia virus surface glycoprotein (GALV-GP) with the Rsequence deleted (R-) [GALV-GP-R-]. GALV-GP-R- expression leads to cell to cell fusion (syncytial) formation in infected tumour cells through binding to the constitutively expressed PiT-1 receptor for GALV. This results in the death of the cells by membrane fusion and is also intended to enhance the spread of the virus through the tumour. Since the RP1 selectively replicates in tumour cells, the expression of the GALV-GP-R- is minimised in normal tissues. The oncolytic destruction of tumour cells leads to the release of tumour associated antigens that are intended to engender an anti-tumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further enhanced through GALV-GP-R- mediated killing, fusion associated cell death which also results in the production of the highly immunogenic exosomes, which is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, uninjected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

Host/vector system

RP1 (rHSV-1hGM-CSF/GALV-GP) is derived from the RH018 strain of Herpes Simplex Virus-1. RP1 is produced in the Vero cell and released into the culture media during cell lysis, prior to purification.

Origin & function

RP1 was constructed using a new strain of HSV-1 (strain RH018). Replimune obtained and compared 30 clinical strains of HSV-1 on a panel of human tumour cell lines and selected the most promising of these (strain RH018) for further development.

RP1 expresses the immune stimulatory protein GM-CSF, which augments therapeutic activity.

GALV-GP-R- binds to the PiT1 receptor, which is widely expressed on mammalian cells including human tumour cells. PiT1 is also critical for cell proliferation, and its expression is therefore unlikely to be lost or down-regulated in response to cancer treatment. The truncated R- version of the protein provides constitutive fusion activity without GALV (i.e. the virus) itself. Expressing GALV-GP-R- together with GM-CSF is expected to increase clinical activity as compared to only expressing GM-CSF. As well as causing direct tumour cell death by cell to cell fusion, cell to cell fusion followed by death is highly immunogenic and includes the release of immunogenic tumour antigencontaining exosomes. Expression of GALV-GP-R- from an oncolytic virus is therefore expected to improve systemic, immune mediated, effects, as well as effects in the directly treated tumour thereby increasing synergy with other immune-mediated approaches to cancer therapy such as immune co-inhibitory pathway blockade.

Evaluation of foreseeable effects

As described above (under Recipient or Parental Organism), the oncolytic destruction of tumour cells (upon transduction with RP1) leads to the release of tumour associated antigens that are intended to engender an antitumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further amplified through GALV-GP-R- mediated killing, fusion associated cell death which results in the production of the highly immunogenic exosomes and is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, uninjected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
No derogation is requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All used and unused RP1 and diluent vials used in preparation and syringes will be destroyed per institutional policy.

As per the wild-type HSV-1 virus, the recombinant HSV-1 vector particles that represent RP1 are highly susceptible to dehydration, rapidly inactivated outside the host and easily inactivated (for example with 1% Virkon solution).

As part of phase II of the clinical trial, biodistribution and shedding will be monitored. RP1 DNA levels in blood and urine will be determined at time-points outlined in the Schedule of Assessments of the clinical protocol. Blood, urine, oral swabs, injection site and exterior of dressing samples will be collected at the first, second and third RP1 injection at the following timepoints: pre-dose (excluding dressing), and also immediately prior to dosing at the fourth through to the eighth dose (Cycle 3 Day 1). Samples on injection days will be done prior to any injections and handling of RP1. Samples will also be collected at Cycle 3 Day 43 and Cycle 4 Day 43 (note exterior of dressings will not be obtained at these timepoints). For patients who withdraw from treatment or due to PD, prior to Cycle 4 Day 43; samples will be collected at the EOT visit. NOTE: Samples will also be taken at 6 hours (+/-2hr), 21 hours (+/-3hr) and 48 hours (+/-6hr) post dose in 24 patients following the first, second and third RP1 doses.

Is an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

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02/03/2022
### Data Premises Notified

| Data Premises Notified (Originally) | 13/01/2016 | Transferred from 1992 Regs? | N | Transitional Premises Class | N | Non-GMMs | N | Withdrawn | N |

### Data Premises Closed

| Transitional Premises Emergency Plan Required? | N |

### Name

| Name | REDX IMMUNOLOGY LTD |

### Name 2

### Department

### Campus Estate or Research Centre

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### Road Name

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### Town

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### HSE Division

### Comments

Notified no work in block 19- 2/9/16

### Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Redx Anti-Infectives which is a subsidiary of the Redx Pharma business discussed the proposed work and risk assessment.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)

Tick if confidential

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<th>Transgenic Birds</th>
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<tbody>
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<td>Yes</td>
</tr>
</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All glassware, plasticware and tips that have been contaminated with GMO material will be de-activated in 2% Chemgene before disposal. Solid waste agar plates will be placed into clear Biohazard bags for onsite autoclaving followed by incineration. Liquid waste will be deactivated with 4% Chemgene solution (minimum of 2% final concentration) for at least 2 hours prior to disposal down the sink. Further information about the onsite autoclave

Validation testing on a regular monitoring schedule has been designed to comply with Environment Agency guidance: 'How to comply with your environmental permit. Additional Guidance for: Clinical Waste (EPR 5.07)' Annex 2 and 'standard rules SR2008 No 25 clinical waste and healthcare waste treatment and transfer station'. This includes daily chemical indicator tests and temperature reports, quarterly biological indicator tests and annual validation.

Further information on Chemgene

According to the manufacturer, ChemGENE has been proven to denature DNA and RNA. "Dr Martin Moncrieffe of the Department of Biochemistry, Cambridge University has certified that "Formulation HLD4 at dilutions of 1:200 or lower is very effective at precipitating/denaturing DNA and RNA". (0.5% final). In addition ChemGENE has proven virucidal activity including the ability to inactivate "Adenovirus, Hepatitis B, Herpes Simplex, Human Immunodeficiency Virus (HIV) and Norovirus". For inactivation of HIV ChemGENE is effective at a 1:50 dilution (2% final)

ChemGENE works by inducing apoptosis in the target cells at concentrations less than 1:100 (1% final).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment was reviewed in detail, and the below changes were requested to be made:
- Removal of HG2 cell lines
- Potential for and risk from any aerosols generated.
- Addition of further information on the onsite autoclave facility.
This risk assessment will cover genetic manipulation of cell lines and bacterial strains to investigate drug targets for immunology disease areas. The work is to primarily to investigate cell signalling pathways and drug interactions with the cell lines. This is to validate and screen various drug targets in these disease areas.

This specifically will include:
- The use of commercially available Hazard Group 1 & 2 and Activity Class 1 & 2 GMO cell lines.
- Transient transfection of Activity Class 1 & 2 GMO’s in Hazard Group 1 & 2 cell lines.
- Generation and propagation of plasmid expression vectors including lentiviral expression plasmids.
- Plasmid transfection of Hazard Group 1 & 2 and Activity Class 1 & 2 cell lines with expression vectors to modulate gene expression by either overexpressing open reading frames (ORFs), cDNAs or specific gene sequences and/or knocking down gene expression by RNAi using siRNAs.
- Generation of stable cell lines using plasmid transfection
- Generation of third generation, recombinant, self-inactivating (SIN) lentiviral particles using HEK293T packaging cell line, using the commercially available third generation packaging system from Systems Biosciences.
- Transduction of Hazard Group 1 & 2 and Activity Class 1 & 2 cell lines with lentiviral particles to
modulate gene expression by either overexpressing open reading frames (ORFs), cDNAs or specific gene sequences and/or knocking down gene expression by RNAi using shRNAs.
- Protein production in bacterial strains.
- Protein production in mammalian cell lines.

Recipient or parental organism

Mammalian cell lines including Hazard Group 1 and 2 and Activity Class 1 and 2 genetically modified cells all of which are covered by appropriate risk assessments.
Bacterial hosts will be for plasmid propagation or for protein expression.
Expression hosts contain a chromosomal copy of the T7 RNA polymerase gene under lacUV5 control to allow inducible expression of the gene of interest. All strains will be covered by appropriate risk assessments.

Host/vector system

This assessment will cover various vector systems:
Lentiviral vectors:
The vector system used will be the commercially available third generation packaging system for example from Systems Biosciences.
The aim is to use lentiviral transduction to produce cell lines that express cDNAs, ORFs and gene sequences encoding potential drug targets and/or shRNAs (designed to knockdown the expression of these targets). The genes of interest will be expressed by 3rd generation self-inactivating (SIN) lentivirus.
Lentiviral vectors (LV) are viral-based gene delivery systems that can stably deliver genes or shRNA into cell lines with up to 100% efficiency. LV particles bind to target cells using an envelope protein that allows for release of the LV RNA containing the gene or gene silencing sequence into the cell. The LVs RNA is then converted into DNA within the target cell line and the DNA preintegration complex enters the nucleus and integrates into the target cell’s chromosomal DNA. Gene delivery is stable because the target gene is integrated in the chromosome and is copied along with the DNA of the cell every time the cell divides. The experimental work proposed here involves the generation of lentiviral particles and the addition of lentivirus particles encoding gene sequences to mammalian cell lines in vitro. Following the transduction of the mammalian cell lines with gene specific lentivirus, RNA and protein will be extracted from the cells to determine expression levels of target gene or gene product and functional assays will be performed.
Bacterial vectors:
For bacterial protein expression the E. coli strains to be used for this work include, but not exclusively: BL21, BL21(DE3), BL21(DE3) pLysS, Lemo21 (DE3), BL21 Star pLysS, Tuner, Tuner pLysS, Origami, Origami B, Origami pLysS, Rosetta, Rosetta pLysS, Rosetta-gami-pLysS. Work with any additional strains will be reviewed internally by the GMSC.
Expression hosts contain a chromosomal copy of the T7 RNA polymerase gene under lacUV5 control to allow inducible expression of the gene of interest.
gene under lacUV5 control to allow inducible expression of the gene of interest.

**Origin & function**

These vectors will be used to generate disease model cell lines for assessment of target biology for use in the immunology therapeutic areas and to identify and/or validate open reading frames (ORFs) / cDNAs or specific gene sequences as potential drug targets and for screening assays. Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and/or shRNAs (designed to knockdown the expression of ORFs, cDNAs and gene sequences (encoding potential drug targets or therapeutic proteins) will be expressed in self-inactivating (SIN) lentiviral expression vectors.

The genetic material cloned into the viral expression vectors before being used to generate lentivirus for transduction of various cell lines.

Of particular note:

**Overexpression of Oncogenes** (ref: SACGM compendium of guidance part 2-2 p38 sections 6-9).

Genes encoding known proto-oncogenes or genes with known oncogenic mutations can contribute to cellular transformation. Any use of lentiviral particles encoding oncogenic inserts will require appropriate controls and operator training. The expression of the majority of genes is predicted to generally have little or no adverse effect. Cells expressing these gene products pose no greater risk than Hazard Group 2 cancer cell lines, many of which harbour mutations within these genes.

**Knockdown of tumour suppressors** (ref: SACGM compendium of guidance part 2-2, p42, paragraph 24).

Knockdown of genes encoding known tumour suppressors can contribute to cellular transformation. However, shRNA expression systems rarely completely silence the targeted gene. shRNAs targeting these types of genes or gene sequences with tumour suppressive functions will only be expressed in third-generation SIN lentiviral vectors. Any use of lentiviral particles capable of reducing tumour suppressor expression will require appropriate controls and operator training.

In addition to the genes/shRNAs the lentiviral vectors will encode selectable antibiotic resistance markers for example (but not limited to): ampicillin resistance, blasticidin resistance, puromycin resistance, neomycin resistance and hygromycin resistance.

**Evaluation of foreseeable effects**

These will vary depending on the cell type and can be known or unknown. Potential common pathogens might
be human pathogens such as Epstein Barr virus or rhinovirus. Primary human samples are typically screen for the presence of the high risk human pathogens hepatitis virus and HIV by the supplier. Samples containing such pathogens should not be used.

Note: Hazard Group 2 cells and Activity class 2 cells present a greater risk than Hazard Group 1 cells, usually due to their potential to harbour human pathogens.

Regarding lentiviral particles, the only potential for harm to human health could be through direct exposure to or injection of the recombinant lentiviral particles. A no sharps policy will be in place to mitigate the risk of inoculation. However even with injection of lentiviral particles, the immune system would most likely recognise this as foreign and mount an immune response. Though lentiviral particles can infect almost all human cells, the recombinant lentiviral vectors used to generate viral particles are self-inactivating and replication deficient, so they are unable to replicate and do not express any of their endogenous genes. Once within the mammalian cell no viral particles would be produced. There is therefore no risk of viral gene transfer to another host. Precautions should be taken to avoid working on one’s own cells or those of colleagues to mitigate any transmission risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste will be disinfected with a suitable detergent (e.g. 10% Distel or 5% Chemgene) then autoclaved using an autoclave cycle (held at a minimum of 125 degrees C for at least 15 minutes). The inactivated waste will then be sent for incineration by a waste management contractor. Liquid Waste will be disinfected by incubating with equal volume of suitable detergent (e.g. 10% Distel or 5% Chemgene) for a minimum of 2 hours before disposing to drain.

Autoclave monitoring and validation:
1. Daily Efficacy Monitoring is achieved by Chemical Indicator strips used in each cycle every day and each container of waste is sealed with colour reactive autoclave tape.
2. Alongside this are printout report checks which are used to determine if a successful cycle has been achieved in terms of temperatures and times. The printer report will also be checked to see if any alarms have been recorded.
3. Biological indicator testing is completed monthly for the first 6 months of autoclave operation and quarterly thereafter.
4. Servicing is to be completed twice a year and Validation Testing annually by an authorised, competent recognised 3rd party contractor.
The risk assessment was discussed by the GMSC on 5th February 2016. All committee members agreed that Class 2 is the appropriate classification for this work and that GMM_RA_IM002 is approved.

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was discussed by the GMSC on 5th February 2016. All committee members agreed that Class 2 is the appropriate classification for this work and that GMM_RA_IM002 is approved.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Is an emergency plan required according to regulation 20? Y
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
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Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The Phoremost Ltd genetic modification committee will consist of:
1. The Biosafety Officer (J. Dillon), who has extensive experience of genetic modification issues. The biosafety officer will chair all genetic modification committee meetings.
2. The Manager & Chief Operating Officer (J. Roix), who has 15 years experience of genetic modification and has supervised research groups both in industry and academia. His function on the committee is to advise and provide the necessary resources for the actions of the committee to be implemented.
3. Investigator & Lab Head (J. Dillon), who has 20 years experience of genetic modification in E. coli and mammalian cells. She will provide advice and ensure the actions arising from the committee meetings are implemented.

The committee will meet every 6 months to review biosafety and whenever a new risk assessment is required. The first meeting reviewed the existing risk assessments.

<table>
<thead>
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02/03/2022
All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below). Waste from our GM work at Class 1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins".

Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below). Waste from our GM work at Class 1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins".

Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

The safety committee was convened on 3 August 2015. The scope and particular aspects of safety risks described in this risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols for enablement of further work, pending notification and acknowledgement by relevant authorities.
The generation and use of lentiviral particles for mediating gene modulation in mammalian cells and their subsequent use in vitro for screening and target identification and validation.

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

To enable the generation and use of recombinant self-inactivating third generation lentiviral particles (by commercial research organisations or in house using commercially available 3rd generation lentivirus systems) encoding:

- Open Reading Frames (ORFs), cDNAs, peptides
- Specific gene sequences
- Short hairpin RNAs (shRNAs) for the knockdown of Open Reading Frames (ORFs), cDNAs or specific gene sequences by RNA Interference (RNAi)

for in vitro use and for the generation of stable mammalian cell lines (and subsequent use of such stable lines in vitro) with the aim of:

- Determining peptide activity in disease relevant screening platforms
- Efficiently generating cell and disease relevant models lines for assessment of target biology and target validation
- Validating data from other experiments
- Identifying and / or validating ORFs / cDNAs / or specific gene sequences as potential drug targets.

Recipient or parental organism

Recipient experimental systems are cells derived from mammalian organisms, predominantly in the form of immortalized cell lines derived from human cancer tissues.
The mammalian cell lines fall into two types: a packaging/helper cell line into which plasmids containing lentiviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines i.e. a negligible risk.

The lentiviral vectors which will be used are derived from HiV-1, which is an ACDP Hazard Group 3 biological agent. However, "Third Generation" lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase biosafety. These include the vif, vpr, vpu and nef accessory genes which are not required for in vitro replication. The tat gene is also deleted and the Tat responsive promoter present in the 5’ LTR is replaced with heterologous promoters, for example with the Rous sarcoma virus U3 region. An additional biosafety feature is achieved by deletion of the rev gene from the viral transfer vector. Viral packaging is achieved by providing three helper constructs in trans containing gag, pol and rev sequences (figure 1).

An additional biosafety feature is that these vectors are self-inactivating (SIN), whereby the U3 region of the 3’ LTR (which contains the major viral promoters and enhancers) is copied to the 5’ end of the provirus during reverse transcription. Deletion of enhancer and promoter elements from the 3’ U3 region in the vector construct will result in a provirus that is entirely devoid of U3 enhancer sequences, therefore reducing the potential for transactivation of cellular genes as a result of insertion. Furthermore, such vectors are not easily mobilisable as a result of a superinfection with wildtype virus.

Third generation Lentiviral Expression Systems include the following key safety features:

The Lenti expression vectors contain a deletion in the 3′ LTR (−U3) that does not affect generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.

The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).

Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have...
been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998). Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication competent virus can be produced. Despite the above safety features, use of these lentiviral vectors (which include WPRE) falls within SACGM 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. Also, the virus will be packaged by transfecting transfer vector into specific amphoteric ‘helper’ cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types. This means that the viruses produced for this experiment could potentially infect a number of species, including man.

**Origin & function**

Selectable markers – examples (but not restricted to);
- Ampicillin resistance: E.coli derived
- Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
- Puromycin resistance (PAC) : Puromycin acetyl transferase is derived from Streptomyces alboniger
- Reporter proteins such as (but not restricted to);
  - Fluorescent proteins as reporters;
  - GFP derived from the jellyfish Aequorea victoria and variants of this
  - Luciferase – class of oxidative enzymes used in bioluminescence renilla luciferase derived from the Sea pansy (Renilla renifomris) firefly luciferase derived from the firefly Photinus pyralis.
- Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins
  - and / or shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins) – all human derived.

**Evaluation of foreseeable effects**

The lentiviruses are, at worst, amphotropic or pseudotyped with VSVG protein, either of which confers a broad host tropism including human cells. However, infectivity is unstable and infection is only obtained by cocultivation
of the packaged viruses with the recipient cells. The retrovirus is self-inactivating and therefore exhibits a low possibility of further transfer.

Since some of the inserted DNA codes for potentially hazardous RNA or protein, the work is assessed as Class 2. This accords with HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.18-20).

Even for the nononcogenic inserted DNA, there is a slight but nonnegligible risk due to the presence in the lentiviral vector of the Woodchuck Posttranscriptional Regulatory Element. The WPRE-containing vector DNA will be treated as potentially oncogenic and is assigned to class 2 (see HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.13)).

However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle:

The genes encoding structural and other components of the viral genome have been separated. These genes have been engineered to minimise the risk of recombination that could lead to production of a replication-competent virus.

The packaging cell lines allow expression of proteins, required to produce progeny virus: But the transfer vector is the only genetic material transferred to the target cells, consequently these cells cannot produce the proteins which are essential for viral assembly and infectivity.

Third generation or Self-inactivating vectors retrovirus vectors will be used in all experiments.

Procedures and controls measures will therefore follow HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.30-36) i.e. using multiple plasmids with minimum sequence homology (e.g. our 3rd generation SIN lentivirus vector system), gloves should be worn, use of class II safety cabinets, sharps avoided and all wastes be rendered harmless before disposal etc.

It is not thought that the modified virus would pose a serious risk to animals or plants in the environment. Although the VSV coat protein permits invasion of other mammalian cells, as in the case of humans, infection would be restricted to primary cells and productive virus would not be produced. In addition the control measures to protect human health will minimise release of virus to the environment. Therefore the environmental risk is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:

1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All waste material will be inactivated by treatment with 1% (w/v) Virkon
solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed – Their GM authorization is GM898) according to disposal notification GM105/4.1. Waste from our GM work at Class2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this.

This disposal method is expected to achieve 100% inactivation of the GMM.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:

1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed – Their GM authorization is GM898) according to disposal notification GM105/4.1. Waste from our GM work at Class2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the
The risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this.

This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM.

The data sheets describing inactivation by Virkon are attached.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The safety committee was convened on 11 August 2015. The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactorily consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities, the risk assessment and related activities were deemed suitable for work.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L2</td>
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<td>L4</td>
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</tbody>
</table>

Animal Units

| L2 | L3 | L4 |

Large Scale Activities

| L2 | L3 | L4 |

Human Clinical Applications

| L2 | L3 | L4 |
The generation and use of lentiviral, retroviral and adenoviral particles for mediating gene modulation in mammalian cells and their subsequent use in vitro for screening and target identification and validation.

Project Additional Information

Purposes of the contained use

Following from our previously approved clearance of a Class 2 activity (GM3294 / 15.1), we plan to expand the repertoire of genetically engineered viral vectors used in our research: we aim to employ additional virus types, and expand the collection of GMO transgene inserts:

Specifically, these protocols will enable the generation and use of lentiviruses, retroviruses and adenoviruses encoding:

1) Open Reading Frames (ORFs), cDNAs, peptides
2) Specific gene sequences
3) Short hairpin RNAs (shRNAs) for the knockdown of Open Reading Frames (ORFs), cDNAs or specific gene sequences by RNA Interference (RNAi)

These genetically modified agents are used in vitro to generate and use stable mammalian cell lines to:

1) Determine peptide activity in disease relevant screening platforms
2) Efficiently generate cell and disease relevant models lines for assessment of target biology and target validation
3) Validate data from other experiments
4) Identify and / or validate ORFs / cDNAs / or specific gene sequences as potential drug targets.

Recipient experimental systems are cells derived from mammalian organisms, predominantly in the form of immortalized cell lines derived from human cancer tissues. The mammalian cell lines fall into two types:

a) A packaging/helper cell line into which plasmids containing lenti-, retro- and adenoviral DNA will be introduced,
and from which infectious virus will be secreted into the medium to high titre. Media extracted from these cell-lines present a risk of infection to personnel, as they typically contain high titres of infectious viral particles.

b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines are proven to not actively produce infectious virus particles, and therefore are unlikely to present greater risk than uninfected, parental cells.

**Host/vector system**

Transgene function or mutagenesis, our overall risk assessment category and risk mitigation plan are not significantly changed from protocols we previously approved for using genetically modified lentiviruses (GM3294 / 15.1). All new virus types employed here each have proven history of safe use by other parties, following from multiple viral attenuation and risk mitigation strategies employed in their construction; the methods we will employ do not differ from those established protocols.

**Lentiviral**

As described in our previously accepted protocols, we will employ second generation or later versions of self-inactivating (SIN) lentiviral vectors. Similar to our intended retroviral use, these systems confer risks of potential human infection, but the viruses employed have also been multiply attenuated to strictly limit viral propagation and infectious risk. Our anticipated protocols use established reagents, and our methods do not vary significantly from those employed with a historical record of safe use by third parties [see: SACGM containment level 2 (ref: SACGM compendium of guidance part 2-11 P119 sections 8-30)].

**Retroviral**

We will employ several retroviral vectors, including those derived from Moloney Murine sarcoma Virus (MoMLV), Mouse Mammary Tumour Virus (MMTV), Feline Leukaemia Virus (FeLV), or Murine Embryonic Stem Cell virus (pMSCV). While all vectors are ACDP Hazard Group 1 biological agents, we will utilise virus preparations that have expanded tropism and potential infectious risk in humans. All vectors are multiply attenuated to strictly limit viral propagation and infectious risk for the intended use (see SACGM compendium of guidance part 2, section 2.11 (Retroviruses) pp117).

**Adenoviral**

We will use several adenoviral vector systems, of which the commercially available AdEasy vector system is an example. As with our retroviral and lentiviral intended uses, we will employ standard reagents and protocols similar to those proven through historical use by third parties. The multiply attenuated variants of adenovirus vectors we will employ all fall within SACGM 2 criteria due to infection risks from self-replicating viruses formed via recombination or risk of insertional mutagenesis. [see SACGM containment level 2 (ref: SACGM compendium of guidance part 2 pg. 78, Section 2.7)]

**Origin & function**

Selectable markers – examples (but not restricted to);
Ø Ampicillin resistance: E.coli derived
Ø Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
Ø Puromycin resistance (PAC) : Puromycin acetyl transferase is derived from Streptomyces alboniger

Reporter proteins such as (but not restricted to);
Ø Fluorescent proteins as reporters;
• GFP derived from the jellyfish Aequorea victoria and variants of this
Ø Luciferase – class of oxidative enzymes used in bioluminescence
• renilla luciferase derived from the Sea pansy (Renilla reniformis)
• firefly luciferase derived from the firefly Photinus pyralis.
This work involves the use of DNA fragments that, when delivered into mammalian cells and integrated into the cell genome, will abrogate the ability of one or more genes to generate a complete and functional protein product that would otherwise normally be produced by the cell.

**Evaluation of foreseeable effects**

All viral vectors employed in this protocol exhibit broad tropism and potential to infect human. Risks conferred following infection are identified as:

i) genetic insertion of viral sequences with potentially deleterious effects on endogenous genes: we will employ transgene promoters and other viral sequences that may affect host gene function in a wide range of cell types (e.g. CMV promoter). We assess retro-, lenti- or adenovirus infection might induce permanent changes in infected cells including a risk for tumorigenesis. Risks conferred are previously described and categorised under Class 2 risks [SACGM compendium of guidance part 2, page 121]: “…The effects of integration upon the infected cell should be considered. For instance, promoter sequences present in the provirus might activate genes adjacent to the integration site or, alternatively, insertion may disrupt genes and prevent their expression.”

ii) expression of human-derived or homologous transgenes with potentially deleterious effects: various transgenes may be employed, wherein intrinsic function of the transgene confers potentially deleterious effects. For example, expression human oncoproteins could induce transformation of infected cells. Beyond endogenous homeostatic mechanisms that may lessen this risk (e.g. apoptosis and other host tumor suppression responses), we assess that standard precautions under Class 2 risk mitigation procedures are adequate to address such risks.

iii) expression of exogenous transgenes directed to host genes with potentially deleterious effects: multiple transgene technologies (antisense, RNAi, CRISPR or related gene conversion) may be employed to modify the function of endogenous genes with potentially deleterious effects. For example, RNAi or antisense RNA could reduce the function of genes necessary to control cell apoptosis, potentially resulting in tumorigenesis. As above, we assess that Class 2 risk management procedures adequately address these risks.

iv) expression of heterologous genetic sequences with potentially deleterious effects: we will employ diverse collections of non-human derived, exogenous transgene sequences that carry potential risks following infection. Risks from expression of these collected transgene sequences are mitigated and attenuated through several means. First, known pathogenic transgenes systemically removed, and other exogenous gene products are fragmented or rearranged, such that potentially pathogenic or other biologically deleterious genetic functions are not recapitulated in their complete endogenous configuration. Second, potentially deleterious transgenes in any infectious viral preparation are highly titrated, such that any single deleterious sequence comprises less than 1 part in 100,000 parts of an inoculum with infectious potential. In total, we assess these risks are adequately managed via the same Class 2 risk precautions employed above.

We assess environmental hazards are adequately addressed through the proposed personnel risk management measures. We employ multiple attenuation strategies and protocols to severely limit independent virus propagation beyond the intended use, and therefore anticipate minimal risks of GMM release.
Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:
1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed – Their GM authorization is GM898) according to disposal notification GM105/4.1.

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This disposal method is expected to achieve 100% inactivation of the GMM.

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All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins".

Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.

This disposal method is expected to achieve 100% inactivation of the GMM.

The data sheets describing inactivation by Virkon are attached.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.
## Project Containment

<table>
<thead>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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### Animal Units
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### Large Scale Activities
- L2 L3 L4 L2

### Human Clinical Applications
- L2 L3 L4
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Premises Addresses

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</tbody>
</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The members of the GM safety committee include the Managing Director and the Research Director of the Company. Both of them have appropriate scientific qualifications and extensive prior experience of working with the type of GMMs to be used. In addition, the committee includes a scientifically-qualified representative from Oxford Innovation (the landlord and facility manager). The committee members are based at the laboratory where the work will be carried out. Meetings are held when a new project is to be initiated and at least once a year to review all projects.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 1 (GMMs)</td>
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<td>Other (please specify)</td>
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</table>
Mammalian cell lines will be transfected with gene vectors for virology research

For activities involving GMMs, describe the waste management measures which will apply to the activity

NA CU2 attached

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

NA CU2 attached

Project Ref 3295/15.1

Date Ackn’d 18/09/2015
Date Project Ceased

Production of cells expressing shRNA and/or tetracycline repressor protein (TetR) using recombinant lentivirus

Class 2 CultureVolClass2 < 1 Litre
Class CultureVolumeClass3-4

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info
The aim of the project is to develop stable mammalian cell lines that can be infected by virus but do not allow replication of the virus.

1) HEK-293T. The stable cell line HEK-293T is a very widely used human embryonic kidney cell line which has been transformed with adenovirus E1a and carries a temperature sensitive T antigen co-selected with neomycin. The cell line was first described in 1977 and is Class 2 because of the incorporation of adenovirus genes.
2) BSC1. The stable cell line BSC1 is a very widely used Cercopithecus aethiops (African green monkey) kidney cell line and was first characterised in 1963. The cell line is Class 1.
3) HELA. The stable cell line HELA is a very widely used human cervical adenocarcinoma cell line. In 1955 HeLa cells were the first human cells successfully cloned and were used to propagate poliovirus. HELA carries genes from human papillomavirus 18 and therefore is Class 2.
4) A549. The stable cell line A549 is a very widely used human lung carcinoma cell line and was first described in 1972. The cell line is Class 1.
5) SK-Mel-28. The stable cell line SK-Mel-28 is a very widely used human melanoma cell line. It was created with a large number of other melanoma cell lines by passaging primary tumour cells in nude mice and was first described in 1972. The cell line is Class 1.

1) pRS. The commercially available pRS vector is to be used for transient transfection of recipient cells with shRNA. The plasmid contains standard elements including a bacterial origin of replication, ampicillin resistance gene, human U6 promoter and puromycin antibiotic resistance gene under the control of an SV40 early promoter.
2) pGFP-C-shLenti and pLenti-C-mGFP. These are commercially available third-generation lentivirus vectors for stable integration of genes coding for shRNA and TetR in recipient cells. The vector is multiply disabled and incapable of autonomous replication.
3) lentivirus packaging and envelope plasmids. These three commercially available trans-active plasmids code for rev and the rev response element and an envelope protein from vesicular somatitits virus, all of which are essential for expression and packaging of the lentivirus in HEK-296T cells.

1) shRNA. The shRNA genes do not code for protein, but for RNAs which will putatively specifically inhibit the replication of virus if it were infecting the recipient cell. They are not expected to have any pathological effect either in humans or animals.
2) TetR. TetR codes for tetracycline repressor protein, a component of the tetracycline-controlled transcriptional activation system, widely used since 1992. TetR is capable of binding to a bacterial tetracycline operator (TetO) which may be placed upstream of a gene which it is desired to control. Binding of TetR to TetO reduces transcription of the associated gene. TetR is not expected to have any pathological effect either in humans or animals.

The GMOs resulting from this project will be stable mammalian cell lines which have been modified to express shRNA specific for silencing genes of a virus and TetR, the
tetracycline repressor protein which is able to repress the transcription of genes which are preceded by the tetracycline operator TetO. TetO is not expected to be found in the recipient cells. The foreseeable effects of the genetic modification are that if the modified cells were to be infected with the virus, then the replication of the virus could be inhibited, and if the modified cells were to be infected with a virus that had genes controlled by TetO, then the expression of those genes could be inhibited. There is no reason to expect that these foreseeable effects are likely to have an adverse impact on human health or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste material which might be contaminated with the GMM will be disposed as follows:
Cell culture ware, plastic ware etc will be immersed in 10% bleach or 1% Virkon for at least 1 h. Aqueous liquid will be discarded to the laboratory drain, solid waste will be securely bagged and taken for incineration by an approved contractor (Select Environmental Services Limited, Prosper Park • Bennet Road • Reading • Berkshire RG2 0QX)
Culture medium, other liquids will be treated with bleach to final concentration of 10% or Virkon to a final concentration of 1%, for at least 1 h and discarded to the laboratory drain.
Sharps, pipettor tips, broken glass etc will be stored in sealed sharps container and taken for incineration by the approved contractor.
Our risk assessment indicates that these proposed waste management measures are at least as effective, and overall less inherently hazardous than inactivation by autoclaving, and therefore the availability of an autoclave within the facility is not considered necessary.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was approved by the genetic modification safety committee without any comments.

Project Containment

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</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
</tbody>
</table>
The aim of the project is to develop a modified vaccinia virus. Vaccinia virus, strain Lister. ACDP hazard group 2. This is the vaccine strain which was developed in the UK and widely used in the eradication of smallpox. Vaccinia infection is mild and typically asymptomatic in healthy individuals, but it may cause a rash and fever. Certain complications and/or vaccine adverse effects occasionally arise. The chance of this happening is significantly increased in people who are immunocompromised. Approximately one in a million individuals developed a fatal response to vaccination.

(1) Host cells will be selected from following the widely used mammalian cell lines: HEK-293T. (human embryonic kidney transformed with adenovirus E1a, class 2), BSC1 (African green monkey kidney, class 1), HELA (human cervical adenocarcinoma cell line, class 2), A549 (human lung carcinoma cell line, class1), SK-Mel-28 (human melanoma, class 1), Vero (African green monkey kidney, class 1). No risks to human health are expected to be associated with the use of these cells.

(2) The vector will be derived from pUC13-EcoGPTEGFP, a shuttle vector widely used for insertion of genes into vaccinia by homologous recombination. No additional risks to human health are expected to be associated with the
**Origin & function**
The genetic material to be inserted is not expected to be expressed and does not have any intended or expected functions in the final GMO as used in this project.

**Evaluation of foreseeable effects**
The GMOs resulting from this project will be modified vaccinia virus which have been attenuated by disruption of the thymidine kinase (TK) gene and therefore likely to be significantly less virulent than the original virus. They will incorporate inserted genetic material which is not expected to be expressed in the host cells. There is no reason to expect that these foreseeable effects are likely to have an adverse impact on human health or the environment.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
none

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
Waste material which might be contaminated with the GMM will be disposed as follows:

Cell culture ware, plastic ware etc will be immersed in 10% bleach or 1% Virkon for at least 1 h. Aqueous liquid will be discarded to the laboratory drain, solid waste will be securely bagged and taken for incineration by an approved contractor (Select Environmental Services Limited, Prosper Park • Bennet Road • Reading • Berkshire RG2 0QX).

Culture medium, other liquids will be treated with bleach to final concentration of 10% or Virkon to a final concentration of 1%, for at least 1 h and discarded to the laboratory drain.

Sharps, pipettor tips, broken glass etc will be stored in sealed sharps container and taken for incineration by the approved contractor.

Our risk assessment indicates that these proposed waste management measures are at least as effective, and overall less inherently hazardous than inactivation by autoclaving, and therefore the availability of an autoclave within the facility is not considered necessary.

---

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

---

The risk assessment was approved by the genetic modification safety committee without any comments.

**Activity**

---

**Project Containment**

Laboratory Activities | Glass Houses | Growth Rooms

02/03/2022
The aim of the project is to develop a modified vaccinia virus that expresses immunoglobulins from infected cells.

Recipient or parental organism

1) Vaccinia virus, strain Lister. ACDP hazard group 2. This is the vaccine strain which was developed in the UK and widely used in the eradication of smallpox. Vaccinia infection is mild and typically asymptomatic in healthy individuals, but it may cause a rash and fever. Certain complications and/or vaccine adverse effects occasionally arise. The chance of this happening is significantly increased in people who are immunocompromised. Approximately one in a million individuals developed a fatal response to vaccination.

Host/vector system

(1) Host cells will be selected from widely used mammalian cell lines which will include: CV1 (african green monkey kidney, class 1), BSC1 (African green monkey, class 1), A549 (human lung carcinoma cell line, class 1), HEK-293 (human embryonic kidney transformed with adenovirus E1a, class 2) and HELA (human cervical adenocarcinoma cell line, class 2). Other mammalian cell lines of class 1 or 2 biosafety may also be used. No risks to human health are expected to be associated with use.
of these cells. (2) One vector will be derived from pUC13-EcoGPTEGFP, a shuttle vector widely used for insertion of genes into vaccinia virus by homologous recombination. Another vector will be pUC57, a widely used general purpose cloning plasmid, that will have DNA inserted into it homologous recombination with vaccinia virus. No additional risks to human health are expected to be associated with the vector.

Origin & function

The resulting modified Vaccinia viruses will be attenuated as the inserted transgenes will disrupt genes in the vaccinia virus genome including Thymidine Kinase (TK) and Vaccinia Growth Factor (VGF) and may include disruption of other vaccinia virus genes. The transgenes themselves will be immunoglobulins specific for human or analogues of human proteins in animals. The immunoglobulins will not target proteins present in the GMO and are not expected to affect the host cells described above.

Evaluation of foreseeable effects

The GMOs resulting from this project will be modified vaccinia virus which have been attenuated by disruption of the thymidine kinase (TK) and/or the Vaccinia Growth Factor (VGF) gene and therefore likely to be significantly less virulent than the original virus. They will incorporate inserted DNA code for immunoglobulins which will be expressed in the host cells, though there is no reason to expect that these immunoglobulins are likely to have an adverse impact on human health or the environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste material which might be contaminated with the GMM will be disposed as follows:
Cell culture ware, plastic ware etc will be immersed in 10% bleach or 1% Virkon for at least 1 h. Aqueous liquid will be discarded to the laboratory drain, solid waste will be securely bagged and taken for incineration by an approved contractor (Select Environmental Services Limited, Prosper Park • Bennet Road • Reading • Berkshire RG2 0QX)
Culture medium, other liquids will be treated with bleach to final concentration of 10% or Virkon to a final concentration of 1%, for at least 1 h and discarded to the laboratory drain.
Sharps, pipettor tips, broken glass etc will be stored in sealed sharps container and taken for incineration by the approved contractor.
Our risk assessment indicates that these proposed waste management measures are at least as effective, and overall less inherently hazardous than inactivation by autoclaving, and therefore the availability of an autoclave within the facility is not considered necessary.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was approved by the genetic modification safety committee without any comments.
## Project Containment

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**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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- **Level 1 (GMMs)**
  - Yes

- **Level 2 (GMMs)**

- **Level 3 (GMMs)**

- **Level 4 (GMMs)**

- **Non-microbial**

- **Other (please specify)**

- **Tick if confidential**

Consultant advisor, 25 years experience in biological safety, Chartered Member of the Institute of Occupational Safety & Health

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Transgenic Animals**
- **Gene Therapy**
- **Transgenic Fish**

02/03/2022
The lentiviral vectors are not capable of surviving outside of a tissue culture environment and therefore will require no further waste treatment.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

I have reviewed the attached risk assessment and find it accurately reflects the work to be undertaken and the Class 1 assigned.
### GM Centre Number: 3297

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**Name**

AUTOLUS LIMITED

**Campus Estate or Research Centre**

**Road Name**

85 TOTTENHAM COURT ROAD

**Town**

London

**County**

GREATER LONDON

**Postcode**

W1T 4TQ

**Country**

ENGLAND

**HSE Division**

LONDON

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Guidance has been taken from the GMO Safety Committee from a major London academic institution specifically experienced in the use of GMOs planned for use by Autolus. Also from previous experience working with GMOs in a large pharmaceutical company (GSK).

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Non-microbial

Other (please specify)  

Tick if confidential

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Please refer to attached risk assessments - briefly:
Gloves, laboratory coats and safety glasses are routinely used in the labs. No needles/glass sharps will be used.
Solid GMM waste will be placed in autoclave bags and sent for incineration.
No sharps (such as glass pipettes or needles) will be used - pipette tips will be placed in a sharps container prior to
disposal in autoclave bags.
It is not possible to estimate a degree of kill of virus. However, autoclaving or incineration is sufficient for 100%
inactivation of viruses.
Liquid waste will first be disinfected with 1 % Vircon for a minimum of 30 minutes (or Presept overnight - 1x2.Sg
tabletsSOOml waste to give 2S00ppm available chlorine) before being disposed down the sink. This is a standard
procedure and enveloped viruses are known to be fully inactivated this way.
Surfaces such as hoods or benches will be disinfected with 70% ethanol - again a procedure known to inactivate
enveloped viruses.
In the case of spillages - virkon will be added at at least 1 % for 30 minutes, the area will then be wiped, wipes placed
in autoclave bags, and then surfaces treated with 70% ethanol.
* Note: For GMM work using human blood samples as starting material work will be CL2 (see specific RA for
additional measures and disposal procedures and completed CU2 form).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Dr Ekaterini (Nina) Kotsopoulou holds a DPhii in Gene Therapy using lentiviral vectors (obtained 2000) and has 19
years experience working in laboratories using GMOs. She has authored a number of risk assessments, has reviewed
and approved a number of risk assessments while at her previous employment (Director in R&D, at GSK), received
extensive training while at GSK (employed there 2005-2015) and was part of the biological safety committee for a
year.
Generation of replication-deficient retroviral vectors and use to transduce mammalian cell lines and human and rodent primary cells.

**Purposes of the contained use**

Research and Development towards the generation of novel ex vivo gene therapy based treatments for cancer

**Recipient or parental organism**

Disabled bacterial strains (E. Coli derivatives), continuous mammalian cell lines (such as HEK293) - host lines and cell lines expressing retroviral vectors, primary cells of human and rodent origin - host cells and primary cells expressing retroviral vectors

**Origin & function**

Replication deficient retroviral vectors (mainly MoMLV based), expressing CAR genes.

*See risk assessments for full details.*

**Evaluation of foreseeable effects**

The retroviral vector could theoretically transduce a broad range of cell types of multiple species and theoretically express the CAR and/or suicide gene within those cells. Expression of these proteins is unlikely to cause harm if accidentally transferred to humans. Due to the inherent instability of the virus outside of the cell the risk of this is extremely low.

The viral vector only contains the minimal elements required for genome packaging, integration and transgene expression and is replication deficient, hence in the unlikely event of accidental transfer to human, it will not propagate. Accidental insertion of the vector genome in human cells though could theoretically lead to insertional mutagenesis in the transduced cells, although the likelihood of the latter is extremely low.

The retrovirally transduced cells are not expected to be of any greater hazard than the original unmodified cells.
Human primary blood cell samples carry the risk of bearing human pathogens/viruses such as HIV-1 and/or Hepatitis S. It is possible this could lead to infection of personnel working with these samples. The risk is low as (a) samples will come from pre-screened donors where possible, (b) all personnel will be vaccinated against Hepatitis S, (c) appropriate procedures and training will be in place for work involving human blood samples and (d) the use of sharps will be avoided where possible.

Please see attached risk assessments for further details.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For any work excluding the use of human blood material (classified as CL 1):
- Solid GMM waste will be placed in autoclave bag, and sent for incineration.
- No sharps (such as glass pipettes or needles) will be used - pipette tips will be placed in a sharps container prior to disposal in autoclave bags.
- It is not possible to estimate a degree of kill of virus. However, autoclaving is sufficient for 100% inactivation of viruses.
- Liquid waste will first be disinfected with 1% Virkon for a minimum of 30 minutes (or Presept overnight -1x2.5g tableU500ml waste to give 2500ppm available chlorine) before being disposed down the sink. This is a standard procedure and enveloped viruses are known to be fully inactivated this way.
- Surfaces such as hoods or benches will be disinfected with 70% ethanol- again a procedure known to inactivate enveloped viruses.
- In the case of spillages - virkon will be added at at least 1% for 30 minutes, the area will then be wiped, wipes placed in autoclave bags, and then surfaces treated with 70% ethanol.

For any work where human blood material is used (classified as CL2) additional measures are:
- All solid waste will be soaked in a final concentration of 1% Virkon overnight before being disposed of by incineration.
- All liquid waste will be made up to at least a final concentration of 1% Virkon overnight before being disposed down the sink.
- All glassware and plastics will be soaked in at least a final concentration of 1% Virkon for at least 1 hour, rinsed thoroughly and placed into the washing-up bowls or yellow bins as appropriate.
- Stripettes will be rinsed in Virkon 5% prior to disposal by incineration. Vacutainers with minimal blood residues will be disposed of by incineration.
- Tubes with blood residues from Ficoll separation will be disposed of by incineration (or autoclaving if known infected) or liquid waste is inactivated by 1% (v/v) Virkon for a minimum period of 1 hour and then discarded down the drain.

In case of spillages the following instructions will be followed (disinfectant = 1% Virkon):

ACCIDENTAL SPILLAGE: Do not bend down to inspect the damage, as this will immediately expose you to the concentrated aerosol. If the spillage is not too big, cover promptly with paper towels or an inert binding material and gently pour disinfectant over the paper, working from the outside towards the centre of the spill. After 30 mins, the towels can be removed to solid waste. The area can then be cleaned with disinfectant.
SPILLAGE IN CABINET: Cabinets must be left switched on, and minor spillages (<10ml) absorbed onto paper towels, and the sprayed with disinfectant and left for 20-30 minutes to inactivate the spillage, then mopped up with absorbent paper, which will then be disposed of as solid waste. The grill will be checked to determine if the spillage has contaminated underneath the MSC work surface. If so, it must be decontaminated as described above. For larger spills (>10mls) the work will be made safe and advice sought from laboratory manager or departmental safety adviser to assess if fumigation is required.

SPILLAGE IN CENTRIFUGE: Failure of the centrifuge run or if a spill or leak is suspected. The centrifuge will be switched off or disconnected from the power supply, and left for 30 minutes before opening to allow any aerosol generated to settle. During this time a note will be placed on the centrifuge to inform other users of the situation and to prevent anyone else using it. Any sign of a leak/spill inside the centrifuge will be cleared up with disinfectant and paper towels.

13. Is an emergency plan required according to regulation

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No comments - approved

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Project Ref 3297/17.1

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<td>Generation of replication-deficient retroviral vectors and use to transduce mammalian</td>
<td>Class 2</td>
<td>1-50 Litres</td>
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cell lines and human and rodent primary cells in both research and clinical environments

Disabled bacterial strains (E. Coli derivatives), continuous mammalian cell lines (such as HEK293) – host lines and cell lines expressing retroviral vectors, primary cells of human and rodent origin – host cells and primary cells expressing retroviral vectors

Replication deficient retroviral vectors (mainly MoMLV based), expressing CAR genes. See risk assessments for full details.

The retroviral vector could theoretically transduce a broad range of cell types of multiple species and theoretically express the CAR and/or suicide gene within those cells. Expression of these proteins is unlikely to cause harm if accidentally transferred to humans. Due to the inherent instability of the virus outside of the cell the risk of this is extremely low.

The viral vector only contains the minimal elements required for genome packaging, integration and transgene expression and is replication deficient, hence in the unlikely event of accidental transfer to human, it will not propagate. Accidental insertion of the vector genome in human cells though could theoretically lead to insertional mutagenesis in the transduced cells, although the likelihood of the latter is extremely low.

The retrovirally transduced cells are not expected to be of any greater hazard than the original unmodified cells.

Human primary blood cell samples carry the risk of bearing human pathogens/viruses such as HIV-1 and/or Hepatitis B. It is possible this could lead to infection of personnel working with these samples. The risk is very low as (a) samples will come from pre-screened donors where possible, (b) all personnel will be vaccinated against Hepatitis B, (c) appropriate procedures and training will be in place for work involving human blood samples and (d) the use of sharps will be avoided where possible.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None - standard CL2 measures applicable (and none additional identified from risk assessments)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid GMM waste will be placed in autoclave bag, and sent for autoclaving.

Sharps will be placed in a sharps container prior to disposal in autoclave bags/incineration.

It is not possible to estimate a degree of kill of virus. However, autoclaving is sufficient for 100% inactivation of viruses.

Liquid waste will first be disinfected with 1% (minimum) Vircon (or equivalent) for a minimum of 30 minutes (or Presept overnight – 1x2.5g tablet/500ml waste to give 2500ppm available chlorine) before being disposed down the sink. This is a standard procedure and enveloped viruses are known to be fully inactivated this way.

Surfaces such as hoods or benches will be disinfected with 70% ethanol – again a procedure known to inactivate enveloped viruses.

In the case of spillages – virkon will be added at at least 1% for 30 minutes, the area will then be wiped, wipes placed in autoclave bags and sent for incineration/autoclaving, and then surfaces treated with 70% ethanol.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No comments - approved

Project Containment

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Name

NEWCELLS BIOTECH LIMITED

Name 2

Department

Campus Estate or Research Centre
THE BIOSPHERE

Road Name
DRAYMANS WAY

Building
NEWCASTLE HELIX

District
N/A

Town
NEWCASTLE-UPON-TYNE

County
TYNE AND WEAR

Postcode
NE4 5BX

Country
ENGLAND

Tel Number
01912418695

Fax Number
N/A

E-mail

HSE Division
YORKSHIRE AND NORTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

**Give brief details of the genetic modification safety committee**

The competent person that has reviewed the risk assessment has had 17 years academic experience working in laboratories involved in biochemistry and stem cell research. The individual has attended the Newcastle University GMO training course. The individual runs a large laboratory research group and is responsible for all SH&E aspects of the group.

<table>
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**Non-microbial**

**Other (please specify)**

Tick if confidential
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

For activities involving GMMs, describe the waste management measures which will apply to the activity

I have assessed the activities that will be carried out within in the premises that are the subject of this notification and am satisfied that the appropriate protocols and controls are in place to satisfy the requirements of the Class I activities.
<table>
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## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Yes
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
All waste is soaked in 10% bleach solution overnight and discarded in clinical waste bags for uplift and incineration by an authorised waste contractor (LabWaste). In addition, any spillages of CL2 materials are cleaned with Microsol/IPA and all waste materials autoclaved and discarded as described above.

Storage of CL2 GMO materials

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

Biological Safety Officer at Renishaw Diagnostics Limited
GM Centre Number: 3301

Data Premises Notified (Originally) 04/11/2015
Transferred from 1992 Regs? N
Transitional Premises Class
Data Premises Closed Transitional Premises
Emergency Plan Required? N Non-GMMs N Withdrawn N

Name
SAL SCIENTIFIC LTD

Name 2 Department

Campus Estate or Research Centre Building
UNITS 1 & 2 GLASSHOUSE STUDIOS

Road Name District
FRYERN COURT ROAD BURGATE

Town County Postcode Country
FORDINGBRIDGE HAMPSHIRE SP6 1QX ENGLAND

Tel Number 02381 290272 Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022 02/03/2022 Page 13865 of 15326
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

Committee comprising 2 directors and a senior scientist. All experienced cell biologists (2x PhDs, 1x Degree). All have multi-year experience in managing containment level 2 cell culture facilities.

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<td>Microbiology Research</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Disposal of any waste which has come into contact with the cells is disposed of in biohazard bins. Waste is double bagged before removing from the lab for off-site incineration. This method gives 100% kill.
Liquid waste is treated with Virkon and left for 24 hours. This could be monitored by removing a sample of liquid, spinning it down and culturing to look for viable material. This method is sufficient to kill any living material left in contact with it (100% kill)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

This is an initial risk assessment to cover propagation of class 1 cells. We ultimately expect to be undertaking work requiring class 2 containment. Therefore, containment level 2 containment measures will be adopted from the outset.

Project Ref 3301/15.1

Date Ackn'd 18/12/2015
Date Project Ceased

CU2 Project Title

Use of plasmid-based mammalian expression vectors and lentiviral vectors for the generation of stably-modified mammalian cell line for pre-clinical drug discovery applications (e.g. target identification/validation, cell-based screening, etc).

Class CultureVol Class 2 CultureVolumeClass3-4

Class 2 1-50 Litres
Non-GMM Consent Granted

Project notified under transitional arrangements
**Project Additional Information**

**Purposes of the contained use**

The development of cell lines (primarily as a contract research service, but also for in-house research):

1. To evaluate gene function under normal and disease conditions by overexpression or RNAi-mediated knockdown of the relevant gene function in established mammalian cell lines.
2. To generate stable cell lines (derived from established cell lines) expressing wild-type and mutant genes of interest to be used in the development of cell-based screening assays and disease-relevant in vitro cell models. These assays will be used to explore gene function and to screen compounds for activity at drug targets. In many cases, the gene products will be expressed as fusion proteins encompassing an epitope tag or fluorescent/luminescent reporter.

**Recipient or parental organism**

The recipient cells will be established, well-characterised mammalian cell lines (both immortalised cell lines and induced pluripotent stem cell lines) that will be, to the best of our knowledge, free of adventitious agents.

**Host/vector system**

Two types of vector system will be used (with gene expression typically being controlled from the immediate early Cytomegalovirus (CMV) promoter or, where required, via an inducible Tet-based system):

1) Standard, non-mobilisable mammalian expression vectors including Pcdna3.1 and derivatives. Vectors frequently contain the SV40 origin which enables episomal replication in mammalian cells that contain the SV40 large T antigen. Where this is the case, the recipient cells to be used in these procedures will be negative for the SV40 large T antigen.

2) Third generation (or later), replication-defective lentiviral vector systems based on HIV. Only gag, pol and rev genes from HIV will be employed and these will be supplied in trans to facilitate virus packaging. All other HIV genes have been deleted. Examples of commercially available vectors with these properties include Invitrogen’s ViraPower (pLenti vectors), and Sigma’s pLKO.1 vector systems. High titre virus stocks will be generated by co-transfecting a 293T packaging cell line with transfer and packaging vectors.

**Origin & function**

The genes of interest will typically be mammalian genes encoding for major classes of cellular proteins e.g. receptors, ion channels, kinases and other enzymes. The sequence of the coding regions will be known and provided by the client and gene expression will typically be under the control of a constitutive promoter (e.g. CMV). These genes will have putative or established roles in regulating cellular signalling and will often play a role in modulating cell proliferation and/or apoptosis. As such, these genes will almost always be regarded as being potentially oncogenic. Genes will either be provided by the client or obtained from commercial sources.

Inhibitory RNA sequences will be targetted to genes considered to be of potential relevance in disease processes. Targeted genes will include those encoding cell receptors, ion channels, kinases and other enzymes. Inhibitory RNA sequences will be obtained from commercial sources.
Evaluation of foreseeable effects

The risk associated with overexpression or knockdown of individual genes will be considered on a case by case basis and reviewed locally by the GM safety committee prior to work commencing. Particular consideration will be given to genes that may be oncogenic or immunomodulatory.

When developing cell lines using plasmid-based mammalian expression vectors, a range of commercially-available, non-mobilisable eukaryotic expression vectors will be used including pEGFP-derived and pcDNA3-derived vectors. Many of the genes of interest that will be carried by these vectors will be regarded as being potentially oncogenic. Expression of such sequences in mammalian cells may, in some instances, enhance the malignant properties of the cells. However, neither the DNA, encoded protein, or mammalian transfectants are likely to have any deleterious effect on the worker or the environment. No primary cultures will be used and all recipient cells will be "non-self". Therefore, in the unlikely event that a worker is infected with a transfected cell (e.g. via inadvertent percutaneous inoculation), the cells will be recognised as non-self and cleared by the workers immune system. Nevertheless, in order to minimise the potential for exposure, and given the potentially oncogenic nature of the genes of interest, the generation of cell lines using this approach is assessed as requiring level 2 containment (see below). The GM microorganisms are incapable of surviving outside of the laboratory environment, particularly in the context of the level 2 containment procedures being adhered to, effectively reducing the environmental risk to zero (see below).

When developing cell lines using lentiviral transduction, VSV-G pseudotyped lentivirus strains will be used and these strains will have the ability to infect human cells and cells from other mammalian species. However, because these strains are replication defective, the virus can only carry out a single round of infection. Structural genes and other components required for packaging the viral genome are separated onto three plasmids and have been engineered so as not to contain any homologous sequences to prevent undesirable recombination events that could lead to the generation of a replication-competent virus. When prepared lentiviral particles are not available from the client, lentiviral production will be carried out on-site using HEK293T cells with harvested supernatants being filtered using a 0.45 um filter before either being dispensed as aliquots or further purified by chromatography and/or concentrated by ultrafiltration.

Cells that may contain virus sequences able to mobilise the lentivirus vectors by providing structural genes in trans will be excluded from these experiments. Provirus integration into the host genome will occur with high frequency and while this is unlikely to generate a harmful phenotype in the cultured mammalian cell (and host cells from workers will not be used), there is a low probability of infection of a worker e.g. through accidental inoculation with packaged virus via inadvertent percutaneous inoculation. Integration and long term expression of the recombinant gene or cDNA encoding inhibitory RNA could occur. There is a theoretical risk of transformation of cells by insertion of oncogenes or by knock-down of tumour suppressor genes. Because of the replication-defective nature of these vectors, this could only occur at low frequency and therefore unlikely to have a detrimental effect as a single event is unlikely to result in cellular transformation. Lentiviruses can also act as insertional mutagens. The likelihood of this occurring is reduced by the use of a self-inactivating vector which disables the LTR regions in the integrated vector and thus reduces the risk of oncogene activation at the site of insertion. Additionally, because the vector is replication-defective, the level of exposure that might occur following accidental introduction of the virus will be low and thus reduces the probability of virus insertion at a site likely to promote tumourigenesis. However, because these vectors incorporate strong heterologous promoters and because provirus insertion is a feature of lentivirus biology, work with these vectors is assessed as a class 2 activity and the use of appropriate containment is used to minimise exposure.

To summarise, whilst many of these genes will be regarded as being potentially oncogenic, the hazards to workers and the environment posed by the vector, insert and GMO are considered negligible due to the containment level 2 measures employed. All work will be carried out in access-controlled laboratories. Skin contact with the vectors or the transfected cells will be avoided by minimising aerosols (by keeping cells in sealed vessels that are only opened in a Class II biosafety cabinet), and by use of gloves and Howie-style lab coats. Two pairs of gloves will be worn when preparing and handling viral particles, to allow disposal of possibly contaminated outer gloves whilst retaining a protective barrier. Sharps (e.g. needles or glass pipettes) will not be used. If a worker does accidentally infect themselves with the virus, it is likely that it will be rapidly destroyed by the workers’ immune system. In the extremely unlikely event of transfected cells gaining entry to the worker, the cells in all likelihood would be rapidly destroyed by the workers’ own immune system by virtue of being "non-self".

Transfected mammalian cells are highly unlikely to survive in the environment in the absence of strict osmotic, buffering, temperature, and nutrient conditions. Lentiviruses are highly susceptible to dehydration. However, they can survive for long periods in high protein-buffered media. Lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. The lentivirus cannot replicate and so the consequences of escape are considered negligible, therefore
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

GMMs derived using non-mobilisable, non-viral expression vectors from cell lines that are normally designated as requiring Biosafety Containment Level 1 will be considered as being Class 1 once stable pools have been selected (provided the cells are unable to support episomal replication - e.g. cells negative for the SV40 large T antigen).

Similarly, following transduction of cell lines that are designated as requiring Biosafety Containment Level 1 with lentivirus we propose to be able to use these cells at Biosafety Containment Level 1 based on the application of the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24 hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as requiring Biosafety Containment Level 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The majority of waste will be in liquid form (e.g. spent tissue culture medium, media in microtitre plates), but some solid waste will also be generated (e.g. stripette tips, reagent vials).

All solid waste will be sealed in double autoclave bags, removed by a specialist waste-management company (Medisort) for autoclaving with steam. Effectively 100% kill.

All liquid waste will be inactivated by treatment with Virkon at a final concentration of 1% for 12 hours before disposal via the drains. Effectively 100% kill as determined by the manufacturer (DuPont).

*Virkon
Rely+On : Virkon® is a multi-purpose disinfectant. It contains oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds such as proteins, leading to widespread, irreversible damage and subsequent deactivation/destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance against over 500 strains of viruses, bacteria and fungi. Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control. Importantly, Virkon is sold as a powder which dissolves readily in water. It is intended to be mixed with water to form a 1% solution (i.e. 10g per litre) for hard surface and equipment disinfection. 1:100 is also the dilution rate advised for virucidal efficacy against HIV-1. The solution is generally stable for five to seven days and there is no evidence to suggest that bacterial disease-causing organisms develop resistance towards Virkon, as opposed to some other disinfectant products. Moreover, Virkon is not classified as R53, it is classified as readily biodegradable, and it does not persist in the environment.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment
The GMO risk assessment has been presented to the genetic modification safety committee. Summary findings: The health and environment risks with the handling of GMOs has been reduced to low, acceptable levels by the use of trained staff, procedural and containment controls and personal protective equipment.

The GMSC has agreed the production, handling and use of the lentiviral siRNA stocks is a Class 2 activity. This decision has been reached because although the lentivirus will be generated using third generation (or later), self-inactivating, 3 or 4 plasmid expression systems, and cells that may contain virus sequences able to mobilise the lentivirus vectors by providing structural genes in trans will be excluded from these experiments, the possibility of a recombination event occurring that leads to replication competent virus can never be discounted with this type of virus. The proposal includes the potential to produce large amounts of virus. This represents the most hazardous aspect of the proposal and the GMSC is satisfied that the necessary precautions have been put in place to ensure containment is maintained. The proteins targeted for expression or knock-down will be assessed by the GMSC (meeting as and when required, but at least every three months) on a case by case basis to ensure their potential risk is minimal. The request to derogate the Biosafety Containment Level 2 from 2 to 1 post-transduction has been deemed acceptable given that the transduced cells will be washed extensively to remove any remaining viral particles and will be tested for the absence of transducing virus.

**Project Containment**

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<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Name**

SYNTHACE LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Building**

WEST WORKS BUILDING

**Road Name**

195 WOOD LANE

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

W12 7FQ

**Country**

ENGLAND

**Tel Number**

02075545877

**Fax Number**

0

**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Yes**

Give brief details of the genetic modification safety committee

- **Head of Bioprocessing**
  - 40 years of experience in industrial bioprocessing using production strains including GMMs. Extensive experience in safety assessment and containment in role as Head of Fermentation Sciences at SmithKline Beecham and as a global consultant since.

- **Chief Scientific Officer**
  - 13 years of lab experience working with diverse GMMs at containment level 1.

- **Research Scientist**
  - >5 years lab experience with diverse GMMs

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
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The majority of labware that will come into contact with GMMs will be disposable. All such material will be deposited in autoclave bags, and will be autoclaved at a minimum of 126 degrees centigrade for a minimum of 15 minutes, rebagged and tagged before disposal by London Bioscience Innovation Centre (LBIC) central autoclaving services. GMM waste in non-disposable containers will be autoclaved in the same way, before being disposed of as aqueous waste. LBIC is part of the Royal Veterinary College London, which is a registered hazardous waste producer, and all waste is disposed of in accordance with the Waste Regulations 2005.

Autoclaves at LBIC are serviced on an annual basis, and a temperature probe is placed at the centre of the waste to ensure all material reaches the required temperature for the sterilization period. All autoclaving will be verified with the use of autoclave indicator strips.
The formation of the Synthace Genetic Modification Safety Committee (GMSC) has necessitated a review of the procedure for risk assessments and seeking GMSC approval for genetic design and assembly work.

The GMRA workflow will follow a sequence based on:

i. Design of genetic constructs,
ii. Formal Risk Assessment (RA),
iii. Identification of genetic technical details and requirements,
iv. Preparation of GMSC submission,
v. Order of DNA ‘parts’ from external sources,
vi. Construct assembly and testing.

The GMSC will convene and review submitted proposals during phases iv / v above, and before:

- The Synthace GMSC will meet at least monthly.
- The use of the term ‘origin’ is taken to mean origin of sequence as well as potentially the origin of DNA, the full name and of an originating species together with the assessment of the associated biological class of risk will be determined and recorded.
- To facilitate future gene assembly studies gene sequences relating to standardised ‘parts’ will designated by an internal reference number and part name.
- Managerially, Synthace will establish a Safety and Quality Compliance Committee (SQCC) and it has been determined that the GMSC will constitute a subcommittee of this key function. Dr Markus Gershater is designated as company Safety Officer (SO) and Biological Safety Officer (BSO) until such time permits the appointment of a full-time employee to oversee these roles.

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Project Ref 3302/21.1

<table>
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<tr>
<td>13/10/2021</td>
<td>Optimization of transfection protocols and AAV infectivity assays using HEK293 and 293T cells</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
### Project Additional Information

#### Purposes of the contained use

To perform cell culturing, handling and transfection of HEK293 and 293T cells with 4 plasmids for transient overexpression of fluorescent proteins (TagBFP, mKO2, neonGreen, mKate2) and synthetic transcriptional regulators (L7Ae, Tet-3-G) acting on the promoters for the fluorescent proteins, with the aim of optimizing transfection procedures using design of experiment (DOE) protocols.

To perform cell culturing, handling and infection of HEK293 cells with AAV particles containing a GFP transgene, with the aim of quantifying percentage of GFP-positive cells by flow cytometry.

#### Recipient or parental organism

HEK-293 (ATCC CRL-1573™) and 293T (ATCC CRL-3216™) cell lines. These are considered BSL-2.

#### Host/vector system

The plasmids are all pCMV-containing Tetracycline, Ampicillin and Kanamycin for cloning and transfection selection in E. coli. No vectors to be transfected confer any selection cassette for cell line selection (antibiotics are only expressed under bacterial promoters.)

AAV8 particles carrying GFP transgene (obtained from commercial sources).

#### Origin & function

Genetic inserts for the plasmids will either be fluorescent proteins or synthetic transcriptional regulators, and do not carry harmful biological activity. Full list and origins for inserts and DNA parts can be found below:

- **TREG** (Inducible promoter (by Tet3G)) Ref sequence: pLVX-TRE3G [https://www.takarabio.com/products/genefunction/tet-inducible-expression-systems/tet-on-3g-systems/tet-on-3g-lentiviral](https://www.takarabio.com/products/genefunction/tet-inducible-expression-systems/tet-on-3g-systems/tet-on-3g-lentiviral)
- **Tet3G** (Transcriptional activator (of pTREG)) Ref sequence: pLVX-Tet3G [https://www.takarabio.com/products/genefunction/tet-inducible-expression-systems/tet-on-3g-systems/tet-on-3g-lentiviral](https://www.takarabio.com/products/genefunction/tet-inducible-expression-systems/tet-on-3g-systems/tet-on-3g-lentiviral)

Plasmid transfection will be performed through lipid-based transfection systems, cells will be discarded following assessment of gene expression (flow cytometry).

In the case of infection with AAV8-GFP particles, cells will be used for the assessment of AAV titer and as a measure of infectivity (flow cytometry) and not cultured further.
The sequences being overexpressed are highly unlikely to alter the pathogenicity or properties of the mammalian host cells. Neither the DNA, encoded protein, bacterial or mammalian transfectants are likely to have any deleterious effect on the environment.

The GM microorganisms are incapable of surviving outside of the laboratory environment. All laboratory procedures will follow good microbiological practice. GM microorganisms will be manipulated in a Class II Microbiological safety cabinet. Written records of staff training will be obtained and checked before commencement of work by individuals. All handling and manipulation of mammalian cell cultures will be carried in a Class 2 cabinet within a containment level 2 facility. Personal protective equipment (PPE) such as disposable gloves, laboratory coat and lab safety spectacles will be worn for all applications.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For preparation of plasmic DNA/protein, bacteria will be collected by centrifugation and lysed. Liquid waste material will be rendered non-viable by decontamination with Chemgene for at least 30 minutes prior to sink disposal and autoclaving.

Any contaminated solid waste (e.g. contaminated disposable plasticware, agar plates, DNA) will be bagged and autoclaved prior to disposal as normal solid waste in accordance with laboratory practice in the laboratory. Disposal and decontamination of materials used in mammalian cell culture will be done essentially as described above for bacteriological work.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been reviewed to have been carried out correctly and sufficiently with control measures in place that are appropriate to minimise personnel and environmental risks associated as well as being in line with the regulatory requirements.

The internal Health & Safety committee acknowledges that this contained use excludes the use of working with any sharps or harmful genetic inserts. If the stated project needs change with regards to these exclusions then it is acknowledged that a full review of the contained use be carried out.
# Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**Animal Units**

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**Large Scale Activities**

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**Human Clinical Applications**

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**Name**

CENTAURI THERAPEUTICS LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Building**

DISCOVERY PARK HOUSE

**Road Name**

RAMSGATE ROAD

**District**

**Town**

SANDWICH

**County**

KENT

**Postcode**

CT13 9ND

**Country**

ENGLAND

**Tel Number**

01304 728610

**Fax Number**

0

**E-mail**

**HSE Division**

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**Comments**

Name change from Altermune Ltd 18/01/2016

**Date at Which Additional Info Submitted**

02/03/2022
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<td>RAMSGATE ROAD</td>
<td>SANDWICH</td>
<td>KENT</td>
<td>CT13 9ND</td>
<td>N</td>
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<td></td>
</tr>
</tbody>
</table>

### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Y

**Give brief details of the genetic modification safety committee**

Advice has been obtained from the Safety Officer of our sister company Agalimmune prior to submitting this application and writing applicable risk assessment for Altermune. The Agalimmune safety officer is an experienced GMO(CU) scientist who has worked with class 2 GMOs for >10 years. A GMSC has now been set up for Altermune comprising of 3 individuals with extensive GMO experience who will meet on a monthly basis. This include the following:

- Head of the Translational Research Group and past manager of the containment level 3 facility at Pfizer, Sandwich.
- The laboratory steward of the Altermune laboratory with >10 years experience working with class 2 GMOs and cat 3 pathogens.
- Experienced Scientist within the laboratory with >10 years experience working with class 2 GMOs.

**Laboratory**

Level 1 (GMMs)

Yes

Level 2 (GMMs)

Yes

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

**Animal Unit**

Level 1 (GMMs)

Yes

Level 2 (GMMs)

Yes

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

**Growth Room**

Yes

**Glass House**

Yes

**Large Scale**

Tick if confidential

**Bacteriology**

Yes

**Parasitology**

**Transgenic Birds**

**Microbiology Research**

Yes

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 3303/16.1

**Date Ackn'd** 20/01/2016

**CU2 Project Title** Use of biosafety level 2 designated mammalian cell lines in experiments to determine the efficacy and mode of action of novel therapeutics

**Class** Class 2

**Culture Vol Class 2** 1-50 Litres

**Non-GMM Consent Granted**

**Project notified under transitional arrangements** N

**Withdrawn** N

**Tick if notifying a connected programme of work** N
### Project Additional Information

#### Purposes of the contained use

Use of biosafety level 2 designated mammalian cell lines in experiments to determine the efficacy and mode of action of novel therapeutics.

#### Recipient or parental organism

Not Applicable

#### Host/vector system

Mammalian cell line and primary human cells.

#### Origin & function

The origins of the biosafety level 2 designated cell lines are mammalian and are all available from commercial sources such as Public Health England. In most cases these cells have been transformed with a viral vector to immortalise them or they may have been demonstrated to be infected with a pathogen. This requires them to be handled and disposed of under containment level 2 conditions. Human primary cells such as peripheral blood mononuclear and natural killer cells will be obtaining from commercial sources where they have been tested from the presence of pathogens. However the cells still need to be treated as potentially infectious and will therefore be handled under containment level 2 conditions. The cell lines may be transiently or stably transfected with human proteins using non viral methods in order to express the protein on the cell surface for functional studies. These protein will be non infectious.

#### Evaluation of foreseeable effects

The cell lines are potentially infectious, although with organisms that do not cause serious human disease and have been designated BSL-2 by ATCC and ECACC. The pathogens and their risks are well documented and all cell lines will be purchased from commercial sources. The risk to human and environmental health are therefore low. The primary human cells to be purchased from commerical sources are potenitally infectious, although they will have been screened for the serious human pathogens HIV, Hep B and Hep C. The risk to human and environmental health is therefore moderate, but warrants use under containment level 2 conditions. The protein that may be transfected to the cell lines will be naturally occurring human wild-type or mutant variants of human protein and are not infectious.

#### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

#### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All solid genetically modified waste (e.g. plasticware) will be removed by contractors and incinerated on site according to validated methods.

#### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste (e.g. plasticware) will be discarded in designated GMM waste bins containing Biohazard waste bags. The waste will be collected and removed for incineration at Discovery Park by Augean, an onsite contractor that specialises in the handling and disposal of hazardous biologcal waste. All liquid waste will be completely inactivated by adding Virkon to make a 1% solution and incubating for >15 minutes before disposal down a sink, likewise pipettes and glassware will be soaked in 1% Virkon solution for >15 minutes before disposal. Areas where GMM work has taken place will be disinfected with a 1% Virkon solutions once completed. Staff will receive full traning on the handling and disposal of GMM waste.
The committee deems that containment level 2 conditions will be suitable for the use of both BSL-2 designated cell lines and potentially infectious human cells, providing that they are procured from a source that has been prescreened the primary cells for the presence of HIV, HCV and HBV. Staff must be fully trained in the requirements for working under containment level 2 conditions as laid out if the risk assessment and local and HSE guidelines. Written records of this training must be kept on record.

Please enter comments on the GM safety committee on the risk assessment

The committee deems that containment level 2 conditions will be suitable for the use of both BSL-2 designated cell lines and potentially infectious human cells, providing that they are procured from a source that has been prescreened the primary cells for the presence of HIV, HCV and HBV. Staff must be fully trained in the requirements for working under containment level 2 conditions as laid out if the risk assessment and local and HSE guidelines. Written records of this training must be kept on record.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
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<tr>
<td>L2</td>
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</table>

Project Ref 3303/18.1

Date Ackn'd 31/01/2018

CU2 Project Title

Use of biosafety level 2 designated mammalian cell lines in experiments to determine the efficacy and mode of action of novel therapeutics.

Class 2

CultureVol

1-50 Litres

Non-GMM

Consent Granted

Not Applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

02/03/2022

Page 13883 of 15326
Date of Significant Change

Project Additional Information

Purposes of the contained use

Use of biosafety level 2 designated mammalian cell lines in experiments to determine the efficacy and mode of action of novel therapeutics.

Recipient or parental organism

Not Applicable

Host/vector system

Mammalian cell line and primary human cells.

Origin & function

The origins of the biosafety level 2 designated cell lines are mammalian and are all available from commercial sources such as Public Health England. In most cases these cells have been transformed with a viral vector to immortalise them or they may have been demonstrated to be infected with a pathogen. This requires them to be handled and disposed of under containment level 2 conditions. Human primary cells such as peripheral blood mononuclear and natural killer cells will be obtained from commercial sources where they have been tested for the presence of pathogens. However the cells still need to be treated as potentially infectious and will therefore be handled under containment level 2 conditions. The cell lines may be transiently or stably transfected with human proteins using non viral methods in order to express the protein on the cell surface for functional studies. These proteins will be non infectious.

Evaluation of foreseeable effects

The cell lines are potentially infectious, although with organisms that do not cause serious human disease and have been designated BSL-2 by ATCC and ECACC. The pathogens and their risks are well documented and all cell lines will be purchased from commercial sources. The risk to human and environmental health are therefore low. The primary human cells to be purchased from commercial sources are potentially infectious, although they will have been screened for the serious human pathogens HIV, Hep B and Hep C. The risk to human and environmental health is therefore moderate, but warrants use under containment level 2 conditions. The protein that may be transfected to the cell lines will be naturally occurring human wild-type or mutant variants of human protein and are not infectious.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste (e.g. plasticware) will be neutralised by autoclaving. The autoclaved waste will then be discarded in designated biohazardous waste bins containing biohazard waste bags. The waste will be collected and removed for incineration at Discovery Park by Augean, an onsite contractor that specialises in the handling and disposal of hazardous biological waste.

All liquid waste will be completely inactivated by adding Virkon to make a 1% solution and incubating for >15 minutes before disposal down a sink, likewise pipettes and glassware will be soaked in 1% Virkon solution for >15 minutes before disposal. Areas where GMM work has taken place will be disinfected with a 1% Virkon solutions once completed. Staff will receive full training on the handling and disposal of GMM waste.
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Please enter comments on the GM safety committee on the risk assessment

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### Project Containment

<table>
<thead>
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<th>Growth Rooms</th>
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**Name**

<table>
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<td>YORK ST JOHN UNIVERSITY</td>
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**Name 2**

<table>
<thead>
<tr>
<th>Department</th>
</tr>
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<tbody>
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**Campus Estate or Research Centre**

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</tr>
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**Road Name**

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**Town**

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**E-mail**

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**Comments**

**Date at Which Additional Info Submitted**

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<tbody>
<tr>
<td>02/03/2022</td>
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</tbody>
</table>
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

The Genetic Modification Safety Committee (GMSC) at York St John University was formed in July 2015 as a sub-committee of the Faculty Health, Safety and Well Being committee. The Head of Programme for Biomedical Science, University Health and Safety Advisor and the Biological Safety Officer form the GMSC. The GMSC at York St John University will meet regularly (each semester) and may also include the Dean of the Faculty of Health and Life Sciences and Deputy Vice Chancellor who also have experience of working with GMO and HSE guidelines and legislation.

Both the University Health and Safety Advisor and Biological Safety Officer have undertaken the Biological Safety training course from Edinburgh University (June 2015). The Biomedical Science academic staff, Head of Programme and Dean of the Faculty of Health and Life Sciences have experience from previous institutions of working with GMO and complying with appropriate legislation. The Head of Programme has liaised with colleagues from their previous institution who were members of their GMO committee and have extensive relevant experience in this area. Additionally, advice has also been sought from HM Specialist Inspector at the Health and Safety Executive, Biological Agents Unit, by email.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tr>
<td>Other (please specify)</td>
<td></td>
<td></td>
<td>Tick if confidential</td>
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</tbody>
</table>
The work will be carried out in compliance with good laboratory practice and local safety rules for GM work in a containment level 2 facility. The category 2 laboratory will be regularly monitored by academic members of staff and the Biological Safety Officer to ensure good housekeeping and hygiene is regularly maintained with particular emphasis on treatment and disposal of infectious biological waste.

Protective clothing will be worn as necessary and laboratory coats will remain in the laboratories when in use and will be laundered regularly. GMM contaminated material and liquid waste will be inactivated in 1-3% Virkon and disposed after 24 hr, as per manufacturer's validated instructions; or autoclaved at 121 degrees C for 30 minutes 1 BAR (100% Kill) or 130 degrees C for 15 mins 2 BAR (100% Kill) prior to incineration by validated means through licensed contractor.

Autoclave:
- Liquid sterilisation 120 degrees C for 30 minutes
- Equipment/Glassware sterilisation 121 degrees C for 15 mins
- Solid waste 130 degrees for 15 mins

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
</tr>
</thead>
<tbody>
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</table>

<table>
<thead>
<tr>
<th>Virology</th>
<th>Transgenic Animals</th>
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<tbody>
<tr>
<td>Transgenic Fish</td>
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</table>

<table>
<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
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<tr>
<td>Transgenic Plants</td>
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<tr>
<th>Other(s)</th>
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</thead>
</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity
Probe in position to determine that the correct temperature has been reached internally. If required the autoclave performance can be validated by a 12-point thermocouple test on an annual basis.

Records will be kept on site.

All autoclaved solid waste will enter the yellow bag clinical waste route and goes for incineration. This will be autoclaved prior to leaving the building to be incinerated as per clinical waste.

It is unlikely that any viable GMMs would remain after this treatment, and in any case would require specialist conditions for growth.

The licensed contractor for all Biohazard waste and sharps is currently Phs Wastemanagement Head office located at PHS Group Caerphilly, Wales.

Spillages:

Virkon is routinely used as per the manufacturers recommendations:

- Solid surfaces are disinfected with 1% Virkon solution.
- Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% for 1hr prior to cleaning.
- Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation).
- Liquid waste will be treated, for at least 12 hours with Virkon to a final concentration of at least 2%. This will then be discarded to drain.
- Contaminated clothing where autoclaving is not possible/appropriate soak in 1% then rinse well in water and wash.

The licensed contractor for all Biohazard waste and sharps is currently Phs Wastemanagement Head office located at PHS Group Caerphilly, Wales.

The licensed contractor for Chemical Waste Disposal will be SLS Chemical Waste Disposal Service.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment.
The Biological Safety Officer and Head of Programme have worked together to compose the risk assessment document, based on an initial risk assessment and Health and Safety considerations outlined in an internal document for the Faculty Health, Safety and Well Being committee required by the meeting in July 2015. The internal document has been considered by the University Health, Safety and Well Being committee in October 2015.

Risks associated with the general use of the undergraduate Biomedical Science teaching laboratories are outlined in the attached risk assessment form and the persons responsible for taking action are identified.

The risks from using mainly category 1 micro organisms and well characterised cell lines are low and the normal health and safety considerations outlined in forms CU1 and CU2 should reduce these risks further. Any GMO will be used only under the supervision of the Biological Safety Officer and the trained academic staff and all students will receive bespoke training before they commence any activities using category 1 or category 2 micro organisms, GMO or cell lines: including safe use; sterile technique; handling of any spillages and correct disposal routes.

Disinfection of the equipment and laboratory benches, inactivation of all cells in virkon and autoclaving of liquid and solid media before incineration will be overseen by the Biological Safety Officer to minimise the risk of any contamination. The Biomedical Science laboratory facilities are locked and have card access. Only authorised and trained staff and students will be allowed access to the facilities and a record of laboratory users will be maintained.

Based upon our review of the techniques and procedures to be carried out in the Biomedical Science laboratories, we consider the facilities and procedures are adequate to control the level of risk presented to humans and the environment by these genetically modified Hazard Group 2 bacteria.

---

**Project Ref 3304/15.1**

**Date Ackn'd**: 11/11/2015

**CU2 Project Title**: Generation of recombinant antibody binders using a human synthetic scFv (Tomlinson I+J library) or peptide (PhD 12) phage display library etc

**Class**: Class 2

**Culture Vol**: < 1 Litre

**Project notified under transitional arrangements**: N

**Historical Significant Changes**: NOTIFICATION WITHDRAWN 13/04/2016

**Project Additional Information**

**Purposes of the contained use**

- Project 1 - For the production of in vitro recombinant binders as replacement for animal-derived antibodies.
- Project 2 - Production of recombinant proteins in prokaryotic or eukaryotic expression systems
<table>
<thead>
<tr>
<th>Project 3</th>
<th>Production of alternative splice variants using microarray/RT-PCR in a number of well characterised (immortalised) cell lines of human origin (see Appendix 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project 4</td>
<td>Incorporate aptamers for allergens into Saccharomyces species such as cerevisiae/boulardii (GRAS) conditional cell wall mutants</td>
</tr>
<tr>
<td>Project 5</td>
<td>Production of specific point mutations or deletion mutants in the beta-2-microglobulin gene</td>
</tr>
</tbody>
</table>

Recipient or parental organism

- Standard laboratory E. coli strains including K12 derivatives TG1, HB2151 BL21, BL21 (DE3), DH5alpha, Stbl2, dam-/dcm-and XL10-GOLD (see Appendix 1).
- Saccharomyces species such as cerevisiae/boulardii.
- A number of well characterised (immortalised) cell lines of human origin (see Appendix 2).

Host/vector system

Project 1 - Well characterised systems with a history of safe use will be used, for example, non-mobilisable plasmid and bacteriophage.

- A bacteriophage, or phage, is a virus that solely infects bacteria (e.g. E. Coli). Infection is mediated by binding of a phage surface protein (pII) to the bacterial F pilus. It is possible to genetically insert genes encoding antibody fragments (Tomlinson I+J) or peptide binder sequences (New England Biolabs) into the phage gene III which encodes pIIl capsid protein. The Tomlinson I+J system uses a plasmid (phagemid) to produce these virus recombinants on site whereas the peptide library supplies phage previously genetically modified at commercial source.

Project 2 - The bacterial hosts will be E coli K12 derivatives such as BL21, BL21 (DE3), DH5alpha, Stbl2, dam-/dcm-& XL10-GOLD. Also DH10Bac™, which contains a baculovirus shuttle vector (bacmid bMON14272) and a helper plasmid (pMON7124). All of these strains are disabled and unable to survive in the environment outside the lab.

- No hazard to human health or the environment.
- Bacterial plasmids are pUC-based (e.g. pUC19 DNA) pCR-Blunt, pCR-TOPO, pDONR.
- Recombinant baculovirus will be received from collaborators (Baylor College of Medicine, Houston, Texas) ready modified using the plasmid backbones pFastBac™ Dual, pBAC4x, pDEST8, pDEST10, and pDEST20, pGEX-2T. pFastBac and similar baculovirus vectors containing bacterial promoters driving expression of antibiotic resistance and a baculovirus polyhedron promoter driving expression of the inserted sequence, which is only active in the presence of insect baculovirus in insect cells.

Project 3 - The mammalian cell lines to be used in this project will include a number of well characterised (immortalised) cell lines of human origin (see Appendix 2).

Project 4 - Conditional cell wall mutants will be generated by replacing key cell wall genes with an MX cassette using long flanking homology-PCR. Several mutants will be generated in order to achieve the optimum lysis under alkaline conditions. Aptamers will be identified to specific allergens. These aptamers once optimised will be introduced into the Yeast cell wall mutants produced above. The aptamers will be placed under the control of promoters key to the stress response, again using an MX cassette for selection and long flanking homology-PCR to replace the genes. Several mutants will be produced to optimise production of aptamer before yeast cell lysis under alkaline conditions.

Project 5 - Bacterial strains used will be E. coli JM109 for DNA analysis and BL21DE3 for protein analysis, both are standard lab cloning strains. The plasmid (pET23a) will be used to insert the beta-2-microglobulin gene and express the point mutations and it contains no mobile elements.

Origin & function

Project 1 - (Tomlinson I+J) Phagemid genes encode human antibody fragments (scFv) which are used to produce recombinant antibodies.


Project 2 - Recombinant baculovirus will be received from collaborators ready modified (Baylor College of Medicine, Houston, Texas, USA). Bacterial strains will be purchased from commercial sources such as Novagen, New England Biolabs and Invitrogen technologies.
Project 3 - Immortalised cell lines will be received from collaborators from the University of Huddersfield (see Appendix 2.

Project 4 - The Saccharomyces species will be received from collaborators at Manchester University or purchased from the Saccharomyces gene deletion project. Aptamers will be purchased from a commercial source, for example Aptamer Solutions Ltd, York

Project 5 - The pET23a plasmid containing the wild type and mutated beta-2-microglobulin genes will be received from storage from the University of Bradford. Bacterial strains will be purchased from commercial sources such as Novagen, New England Biolabs and Invitrogen technologies

Evaluation of foreseeable effects

Projects 1 - 5 - None of the vectors are harmful to the environment and do not supply a survival advantage to any microorganisms. The cloning and expression vectors are also not a hazard to human health. The bacterial strains to be used are non-colonising and incapable of surviving in the environment or causing human infection. They pose minimal risk to users or the environment.

The genetically modified micro-organisms are likely to cause similar effects on human health and the environment as the wild type parent. Therefore, the containment measures for the parental micro-organisms reflect this risk and come in 2 forms:

1. Containment Level 2 conditions including use of negative pressure class 2 fume hood (sealability for fumigation, HEPA filters on extract air).

2. If working at the bench then this should take place within 18 inches of a blue flame-Bunsen burner. All reusable instrumentation in contact with GMOs (e.g. metal inoculating loops) or the mouths of open bottles containing GMO will be flame sterilized before and after use to prevent contamination of the worker or environment with GMO.

Methods 1 and 2 will be required to reduce the risks to research and support staff and to the broader environment to an acceptable level. It is extremely unlikely that the genetic modification will alter significantly the fundamental properties of the parental micro-organism such as pathogenicity, infectivity, virulence, survivability, host range and/or response to prophylaxis/treatment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The work will be carried out in compliance with good laboratory practice and local safety rules for GMO work in a containment level 2 facility. The category 2 laboratory will be regularly monitored by members of staff and the Biological Safety Officer to ensure good housekeeping and hygiene is regularly maintained with particular emphasis on treatment and disposal of infectious biological waste.

For preparation of plasmid DNA/protein, bacteria and / or cell culture will be collected by centrifugation and lysed. Liquid waste material will be decontaminated overnight with Virkon (1%) and autoclaved prior to sink disposal. Any contaminated solid waste (e.g. contaminated gloves, paper towels, disposable plastic ware, agar plates) will be bagged and autoclaved before disposal as normal solid waste. All materials in contact with bacteriophage will be soaked overnight in 2% trigene disinfectant. 2% Trigene (Distel) will be used to treat spills and wipe down surfaces and equipment after work. Trigene is used in strict accordance with the manufacturer's guidelines and efficacy.
data. 2% Trigene and 70% alcohol sprays will be kept to hand at all times for cleaning purposes and for decontamination of spillages.

The efficacy of autoclaving procedure will be validated as follows:
From the electronic schedule of autoclave cycle parameters to ensure successfully completed autoclave cycle on each occasion. If any cycle is unsatisfactory, it will be repeated. Following autoclaving, liquid waste will be disposed of as standard laboratory waste. Solid waste such as agar plates will be autoclaved inside plastic bags within a metal container at 121 degrees C for at least 15 minutes. Autoclave run records will be checked following each run, and if unsatisfactory the cycle will be repeated. During the first four years after installation an annual 12-point validation test, employing independent thermocouples, will be used to demonstrate that the autoclave holds the specified temperature and pressure for the required period of time. Thereafter, autoclaves will be serviced every 6 months by an engineer from the provider and calibrated annually to ensure the validation criteria are met.

Spillages:
Virkon is routinely used as per the manufacturers recommendations:
Solid surfaces are disinfected with 1% Virkon solution.
Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Any contaminated glassware is treated with 1% for 1hr prior to cleaning.
Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 1% final working concentration. 2% or 3% solutions of virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation).
Liquid waste will be treated, for at least 12 hours with Virkon to a final concentration of at least 2%. This will then be discarded to drain.
 Contaminated clothing where autoclaving is not possible/appropriate soak in 1% then rinse well in water and wash.

The licensed contractor for all Biohazard waste and sharps is currently Phs Wastemanagement Head office located at PHS Group Caerphilly, Wales.

The licensed contractor for Chemical Waste Disposal will be SLS Chemical Waste Disposal Service

Inspections: Safety inspections are carried out regularly to ensure local health and safety rules are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Health and Safety Advisor and Biological Safety Officer.
The Biological Safety Officer and Head of Programme have worked together to compose the risk assessment document, based on an initial risk assessment and Health and Safety considerations outlined in an internal document for the Faculty Health, Safety and Well Being committee required by the meeting in July 2015. The internal document has been considered by the University Health, Safety and Well Being committee in October 2015.

Risks associated with the general use of the undergraduate Biomedical Science teaching laboratories are outlined in the attached risk assessment form and the persons responsible for taking action are identified.

The risks from using mainly category 1 microorganisms and well characterised cell lines are low and the normal health and safety considerations outlined in forms CU1 and CU2 should reduce these risks further. Any GMO will be used only under the supervision of the Biological Safety Officer and the trained academic staff and all students will receive bespoke training before they commence any activities using category 1 or category 2 microorganisms, GMO or cell lines: including safe use; sterile technique; handling of any spillages and correct disposal routes.

Disinfection of the equipment and laboratory benches, inactivation of all cells in virkon and autoclaving of liquid and solid media before incineration will be overseen by the Biological Safety Officer to minimise the risk of any contamination. The Biomedical Science laboratory facilities are locked and have card access. Only authorised and trained staff and students will be allowed access to the facilities and a record of laboratory users will be maintained.

Based upon our review of the techniques and procedures to be carried out in the Biomedical Science laboratories, we consider the facilities and procedures are adequate to control the level of risk presented to humans and the environment by these genetically modified Hazard Group 2 bacteria.

### Project Containment

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Safety Officer at University of Oxford
A number of University of Oxford Professors actively engaged in research involving the use of GMM
Three appropriately trained scientists at Oxford Biotrans

<table>
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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
  - Yes
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Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees Celsius for at least 15 minutes or 126-130 degrees Celsius for at least 10 minutes or 134-138 degrees Celsius for at least 3 minutes), discharge to drains, or chemical disinfection with Virkon using manufacturer’s instructions under standard conditions, discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees Celsius for at least 15 minutes or 126-130 degrees Celsius for at least 10 minutes or 134-138 degrees Celsius for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) and glassware (disposable or broken) - dispose via clinical waste stream for incineration.

Degree of kill
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical disinfection with Virkon, used according to manufacturer’s instructions under standard conditions, manufacturers validation [eg4.79] log reduction ([eg99.998]% kill).

The project involves the preparation of mutants of enzymes of cytochrome P450BM3

For activities involving GMMs, describe the waste management measures which will apply to the activity

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 degrees Celsius for at least 15 minutes or 126-130 degrees Celsius for at least 10 minutes or 134-138 degrees Celsius for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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Give brief details of the genetic modification safety committee

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Mycology
Transgenic Invertebrates
Transgenic Plants
Other (please specify below)

Other(s)

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Please enter comments of the GM safety committee on the risk assessment

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Project Ref 3306/15.1

Date Ackn'd 02/12/2015

Purification and expression of viral vectors and analysis

Date Project Ceased

CU2 Project Title

Tick if notifying a connected programme of work

Class 2 Culture Volume Class 2, 3-4

< 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
### Purposes of the contained use

Improving the purification of viral vectors

### Recipient or parental organism

HEK-293 cells and HEK-293T cells

### Host/vector system

Lentivirus, Adeno Associated Virus and Adenovirus. The proprietary versions of these vectors have been modified from the wild type to make them safe for use in human therapeutics. They have been altered to be replication incompetent and contain a benign reporter transgene (green florescent protein or beta galactosidase) - minimising concerns for both operator and environment.

### Origin & function

Proprietary Lentivirus, Adeno Associated Virus and Adenovirus material will be obtained from 3rd parties. This material will be purified using Puridify’s material at the premises outlined. Samples of purified viral material will be used to infect HEK 293 or HEK 293T cells and the reporter transgene quantified (green florescent protein or beta galactosidase). Other samples of purified viral material will be used to analyse the purification e.g. protein gels.

### Evaluation of foreseeable effects

All of the vectors used in this project are being developed for human therapies and thus have been significantly engineered to alter parts of the genetic code to create proprietary vectors that have a better safety profile than wild type.

The key alteration is that all vectors have been altered to be replication incompetent. This means that they pose a minimal concern to the environment should they be released as they cannot replicate in any cell or organisms they are able to infect. The vectors used also pose minimal to no chance to infecting plant or livestock as they are developed/chosen to transfect human cells.

These vectors have the ability to infect humans via a number of methods e.g. direct contact with skin, mucous membrane of the eye, nose and mouth, ingestion, or injection. Infection could lead to the expression of the transgene; insertional mutagenesis or an immune response.

However the transgenes of the vectors used express do not encode for oncogenes, growth factor receptors or toxin molecules. The reporter transgenes used in this work will be green florescent protein and beta gal to minimise the chance of harm to either the environment or personnel. If an individual is infected the vectors are replication incompetent and so will be unable to propagate throughout an individual.

There is the possibility that if an organism is also infected with a replication competent wild type vector that that transgene vector will also gain this ability. However control measures implemented as outlined below make this a highly unlikely event.

**HEK-293 cells and HEK-293T cells**

HEK 293 cells were generated in 1973 by transformation of cultures of normal human embryonic kidney cells with sheared adenovirus 5 DNA. As this cell line includes adenovirus genes it allow the propagation of the viral vectors used which have been engineered to become replication incompetent. The HEK-293 line has been further engineered to contain the SV40 Large T-antigen for the use of retroviral propagation.

The HEK cell lines pose minimal potential damage to the environment as they have been adapted to an artificial cell culture environment under laboratory conditions and so are unlikely to thrive in the external environment if released. It is not the aim of this project to propagate virus for experimentation but rather use these cell lines to test the
infectivity of the purified vectors via quantification of successfully transfected reporter transgene. Therefore the adherent cultures will be grown in multiwall plate formats of well columns less than 5mL (<500,000 cells per well). And not more than 1L of cell culture volume total. These wells will be infected with low viral concentrations (anticipated to be less than 10E6 per well). This makes handling and destruction of the viral infected cells easier to control, therefore minimising risk to operator or environment.

Rational of Class 2 classification for all vectors

• After a review of the information sources and discussions with experts in the field there was a mix of opinion as to which of the proprietary vectors should be considered Class 1 and which should be considered Class 2 – these opinions differed between the sources consulted for the same vector variants. The case for Class 1 classification often being argued under the rational that the vectors are replication incompetent and express what were considered to be biologically benign transgenes.
• However, due to the disparities in classification of the vectors from the information sources it was decided that all vectors should be treated as Class 2 materials and appropriate control measures for Class 2 materials applied to the entire project.

Control measures to be adopted:

Records and tracking of materials used are kept as part of Puridify’s good lab practice which involves both hard copy and electronic copies of lab books. All materials are clearly labelled and stored in specific safe storage areas within the facilities.

• PPE: lab coats, gloves and goggles to be worn at all times. This is to reduce the possibility of any physical contact with the vector.
• To avoid exposure to aerosol/droplets viral manipulations should be conducted in biosafety Level 2 (BSL2) cabinets to prevent release and possible inhalation.
• No sharps to be used – removes the possibility of introduction into the blood stream
• Centrifugation must be conducted in closed containers and using sealed rotors. Rotors must be opened in a Biosafety cabinet.
• Once the vector has been disrupted during preparation for nucleic acid or protein analysis it is safe to be used outside of the cabinet (PPE as outlined above should still be used).
• All wastes treated to destroy GMO material (see disposal section below)
• Volumes of dilute virus material to be kept under <1L of 1E13 virus particles to make waste handling and disposal easier and to reduce the severity of any adverse events
• Volumes of concentrated virus material to be kept under 20mL of 1E15 virus particles. This allows easy handling in the biosafety hood.
• HEK 293, HEK 293T cell lines (volumes less than 1L). Once cells have been transfected they should only be handled in closed containers or in the BSL2 cabinet
• All vector material when stored in fridge/freezer will be clearly labelled as to its contents.
• All experiments take place in the Stevenage Bioscience Catalyst which is on a secure site as part of the Glaxo Smith Kline Campus. All areas where the virus is stored are only accessible by employees (via keycard or lock and key). Visitors are supervised and wear badges to identify them.
• Training for use of the vector material will be conducted by users who have prior experience of handling the vector material. The completion of training will involve the adding of the individuals name to this risk assessment. The signature will serve as a record of the training and be kept on file as part of the Puridify training records.
• Where vector material is transported this will only be done in sealed containers which are labelled and placed inside sealed plastic packaging. These in turn will be put into larger boxes, sealed and the box labelled with Puridify’s name, address and contact details with the instruction not to open unless authorised.
• There will be no lone, out of hours, work for this project.
• The Purifidy Health and Safety Policy covering lab practice and use of COSHH is to be followed at all times.

Protein Purification in the AKTA Avant system
• The AKTA Avant system has an enclosed fraction collector meaning the virus is not in contact with the external environment
• Viral material loaded to the loop inside the BSL2 cabinet;
• Parafilm should be wrapped around the connections;
• Delta pressure alarm should be less than 2 MPa;
• To avoid the formation of aerosols, 10 minutes should be the minimum of time between the last fraction collected and before opening the drawer.

Protein Purification in the AKTA Pure system
• The AKTA pure system does not have an enclosed fraction collector therefore all work must be completed inside a BSL2 cabinet

Together the implementation of these control measures make this project “LOW” risk

First Aid:

EYE EXPOSURE FROM SPLASH OR AEROSOLS: Rinse a minimum of 15 minutes in eye wash available at all sink hand wash areas or flush area with water. Seek medical attention.
CONTACT WITH SKIN: wash thoroughly with disinfectant. Hand sinks available in all labs for washing.
INHALATION EXPOSURE FROM AEROSOLS: Seek medical attention.
NEEDLESTICK AND/OR SHARPS EXPOSURE: No sharps are to be used

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Disposal
• Vikon is used as it is a validated cleaning method for viral vector material and transfected cells if used in the following way under manufacturers recommendations
• Hard surfaces: A solution containing 1% Virkon for 1 hour contact time, 10mins for metal parts (longer can cause corrosion)
• Safety cabinets: 1% Virkon (10mins)
• Discard jars, plastic tissue culture flasks, glassware: A solution containing 1% Virkon.
• Ensure all surfaces are in contact with the disinfectant (10mins). Added to sealed bags for autoclaving at a minimum of 1210C, 1.15bar, 15 mins hold.
• Supernatants/liquid waste: 3% Virkon diluted 2:1 in culture medium
• All autoclaved solid waste is to be placed into hazardous waste bags. There are 2x autoclaves available and the equipment is regularly serviced to ensure it maintains its ability to be a validated cleaning method. Chart recorder or display screen is attached to autoclave to monitor treatment.
• Full bags are to be transported to Purifidy’s hazardous waste bins in the SBC storage area
• Bins are collected for off site incineration by Grundons waste management

Accidental Release:
In a contained area Virkon powder should be applied all over the split area. This solid waste is then to be autoclaved
as above.
In an uncontained area, all persons should evacuate the area to avoid contact with any potential aerosols formed.
Only after 1 hour the room might be entered (to allow any aerosols to settle) and the procedures should be the same as in a contained area.
The floor is sealed and the lab is built to CL2 level

Interest an emergency plan required according to regulation 20?  

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The Stevenage Bioscience Catalyst (SBC) is an incubator hub for small and emerging biotechnology start-up and spin-out companies. It is a UK Government backed (Innovate UK) enterprise in collaboration with the Wellcome Trust and GSK. The SBC provides technical, business, organisational and commercial support to its tenant companies and also monitors Health and Safety compliance. It has set up a GMO committee to ensure that tenants are assessing the risks associated with their work appropriately and to disseminate best practice among researchers. Dr Deonarain is the chairman of this committee and has 25 years experience working with GMO in academic and commercial settings and was Chairman of Imperial College Life Sciences GMO committee for 4 years. The SBC committee has reviewed and advised Puridify on its GMO risk assessment and are satisfied that they have considered the risks in terms of GM organisms and scale of work. They have appropriate control measures to contain the work and appropriate procedures for the class of GMO activity for waste disposal.

Project Containment

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**Name**
RXCELERATE LTD

**Name 2**

**Campus Estate or Research Centre**
BABRAHAM RESEARCH CAMPUS

**Road Name**

**Building**

**District**
BABRAHAM

**Town**
CAMBRIDGE

**County**
CAMBRIDGESHIRE

**Postcode**
CB22 3AT

**Country**
ENGLAND

**Tel Number**
01223 839557

**Fax Number**
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**E-mail**

**HSE Division**
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**Comments**

**Date at Which Additional Info Submitted**
02/03/2022
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Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities [Y]

Give brief details of the genetic modification safety committee

The GMSC will be comprised of the Chief Scientific Officer/Safety Officer, two Scientific Officers and an administrator. Note that we are a small company, and the Chief Executive Officer is also the Safety Officer. A quorum for the meeting is a minimum of three individuals. The Committee will meet a minimum of once per year to discuss routine matters, review all existing risk assessments and audit the existing procedures. The GMSC will meet more frequently to discuss risk assessments etc. that require more urgent attention.

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Tick if confidential [ ]

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Other(s) | Use of genetically-modified human cell lines for protein expression |

For activities involving GMMs, describe the waste management measures which will apply to the activity
5. Nature of work to be undertaken at the premises (check all boxes which apply) (note 5)

- Bacteriology
- Virology
- Mycology
- Parasitology
- Transgenic Animals
- Transgenic Invertebrates
- Transgenic Birds
- Transgenic Fish
- Transgenic Plants
- Microbiology Research
- Gene Therapy
- Other (please specify below)

Use of a genetically-modified human cell line for protein expression

6. For class 1 contained use involving GMMs, describe the waste management measures (include the inactivation method(s), the degree(s) of kill and proposed process testing / monitoring measures), which you will apply to the contained use (note 6)

The GMSC found that the risk assessment adequately stated the risks of carrying out the activities as described (assessing the secretion of protein by ELISA using a GM cell line). The committee confirmed the activity class of the work to be 1. The control measures in place for this type of work were deemed suitable and were already in place in RxCelerate's cell laboratory as this is already categorised as Category 2 for carrying out work on human cell lines.
### GM Centre Number: 3308

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#### Name

DEPARTMENT OF CLINICAL NEUROSCIENCES, UNIVERSITY OF CAMBRIDGE

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CAMBRIDGE

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#### Comments

Date at Which Additional Info Submitted

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Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities   Y

Give brief details of the genetic modification safety committee

Clinical School Safety Officer, Intermediate Clinical Fellow, three divisional SSO’s, one 050, one PhD student, two Post Doctorates and one departmental administrator.

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Level 1 (GMMs)  Yes
Level 2 (GMMs)  Yes  Yes
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial  Yes

Other (please specify)  Tick if confidential  

Bacteriology  Yes  Parasitology  Transgenic Birds  Microbiology Research
Virology  Yes  Transgenic Animals  Yes  Transgenic Fish  Gene Therapy
Mycology  Transgenic Invertebrates  Transgenic Plants  Other (please specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Decontamination is achieved by treating with Virkon (effectively 100% kill) on site, followed by rotoclaving by the waste contractor of the building (i.e. Novus Environmental).

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

02/03/2022
Q1: Please give a fuller description of the CRISPR/Cas 9 system and explain why these bacterial systems would not result in a more harmful E. coli host when they are used.

A1: The CRISPR Cas9 system is a gene editing tool which makes use of the Streptococcus pyogenes adaptive immune system. In short, a guide RNA oligomer is designed complementary to a target DNA sequence. The guide RNA directs Cas9 nuclease to cut the DNA at the target site. The DNA break is repaired by recombination with a template containing the desired point mutation.

The CRISPR/Cas9 components are in two groups: (i) the Cas9 plasmids encode the Cas9 nuclease and a sequence targeting the nuclease to tau exon 10; (ii) the pcDNA3 plasmids encode a repair template, either normal or mutant tau exon 10.

As proteins, the Cas9 nucleases encoded have the potential to cut the human tau gene. However, only when the repair template and the Cas9 nuclease are mixed could mutant tau be inserted. To minimise the risks of this happening:

- Health and Safety Executive
- The two groups of plasmids were amplified separately using Stbl3 E. coli, and stored separately.
- Small volumes (always less than 300ul) of each plasmid were made to minimise the risk of spillage.
- Cas9 nuclease and repair template plasmids were only mixed in a volume of 100ul or less. in a Class II cell culture cabinet, 5 minutes prior to transfection into iPS cells.

Q2: No other questions brought up by the committee.
**Purposes of the contained use**

Our research focuses on understanding how diseases damage the nervous system, and developing methods to repairing this damage. Research spans basic biology through to clinical studies. Areas of research include the biology of neurons and glia, the process of myelination, the use of stem cells to repair the brain, axon regeneration, plasticity in the brain, mechanisms of neurodegeneration and inflammation. The techniques are multi-disciplinary, and include molecular and cell biology, electrophysiology, both tissue culture and in vivo work, behavioural studies, clinical studies. Specifically for this notification, human and rodent primary and cell line cultures will be transduced with different viral vectors (see below), in order to introduce coding genes or non-coding RNAs associated with pathogenicity and/or repair processes and to understand their role in the context of neurodegenerative diseases.

**Recipient or parental organism**

- Well characterised commercial bacteria (e.g. One Shot® TOP10 and Stbl3™ Chemically Competent E. coli) disabled, non colonising, non pathogenic.
- Commercial packaging cell lines, e.g. HEK-293T human cell line. This cells are highly transfecable derivative of human embryonic kidney 293 cells, containing the SV40 T-antigen. Thus this cell line is competent to replicate vectors carrying the SV40 origin of replication, giving high titers when used to produce viral particles. It has been widely used for lentiviral production, gene expression and protein production.
- Human and rodent primary neural cultures, somatic neural stem/precursor cells, embryonic stem cells, fibroblasts and induced pluripotent stem cells. Characteristic of cell cultures are never totally understood, so primary human cultures will be handled at containment level 2, considering the possibility of adventitious viruses. Rodent cultures will normally be handled at containment level 1, unless otherwise specified in the risk assessment.

**Host/vector system**

Only well characterised, replication deficient viral vector system with a history of safe use will be used:
- HIV-1 derived lentiviral 3rd and 4th generation, replication defective (e.g. eGFP pRRtsin.PPT-hCMV, pCDH-EF-1-
MCS-T2A-GFP).
- Adena virus vectors, replication defective (e.g. Adeasy and Adtrack, deleted E1 and E3 genes).
- Adena-associated virus vectors, replication defective (e.g. AAV6).

**Origin & function**

Inserts are arbitrarily grouped as follows:
Type 1. Human, murine eDNA (+/M TAG sequences) coding for proteins, for example:
Health and Safety
Executive
transcription factors (Wnt3a, Ngn2 etc.) are expected to influence cell fate decision and differentiation, but not expected to have an effect on cell proliferation. Other genes may code for disease associated proteins (alpha synuclein, tau etc).
Type 2. ncRNA sequences for example:
shRNAs and miRNAs (mmu-miR-1S5-5p, etc) will produce ncRNA sequences targeting cell endogenous mRNA (Wnt3a, p73). These sequences are not expected to affect cell proliferation. Notably, mmu-miR-1S5-5p is not implicated
in oncogenic processes,

Type 3. Reporter genes for example:
enhanced GFP (eGFP), mCherry, firefly luciferase etc will code for proteins with fluorescent properties allowing easy
identification of genetically modified (GM) cells. All of these labels are thought to have no deleterious biological effect
on expressing cells.

Type 4. Selection genes for example:
bacterial resistance (ampicillin) or mammalian resistance (puromycin) will express proteins with enzymatic activities to
allow selection of bacterial or GM cell cultures.

Type 5. Functional non-coding sequences for example:
promoter, enhancer, insulators, IRES, WPRE, of mammalian or viral orig in. Functional non-coding sequences will act
as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and
insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES). They will be used (independently or in
combination) into viral expression cassettes to achieve best control of transgene expression depending on targeted
cells.

Evaluation of foreseeable effects

- Human health considerations -
Only replication defective viral particles (non-infectious, i.e. unable to multiply and propagate after initial cell
transduction) will be used, and inserts will not produce compounds with acute toxicity (like bacterial toxins), no
additional risk will arise from the final genetically modified cells, compared to the initial cell source. A possible risk to
human health derives from the recombinant viral particles, via accidental infection of the researcher

The recombinant lentiviral particles (HIV-1 derived) will be packaged via the vesicular stomatitis virus (VSV-G). This
step will alter the tropism of the retroviral particles, as it only requires interaction with the plasma membrane of cells
rather than a specific receptor, thus allowing increased stability of the viral particles associated with the VSV-G
envelope. This may add the aerosol risk in addition to the percutaneous risk. Retroviruses stably integrate into the
host cell genome, and there may be a risk of insertional mutagenesis, with subsequent ectopic inhibition/activation of
host gene expression. Subsequent de-regulation of endogene expression could theoretically lead to transduction
associated detrimental effects such as initiation of oncogenic processes. Moreover the addition of the Woodchuck
hepatitis Post transcriptional Regulatory Element (WPRE) to the expression cassette in several retroviral backbones
could also enhance the oncogenic potential of such vectors (SACGM 2004). However, the use of a lentiviral
backbones is thought to be safer, as the genomic integration profile of lentiviral derived vectors do not show an
integration bias towards the transcriptional start site region of host cell genes.

Risk is also associated with inserted coding sequences. cDNA or shRNAmiRNA inserts carried by viral vectors will
direct ectopic expression or inhibition of transcription factors, and strong promoter/enhancer effects from non-coding
sequences could lead to ectopic activation of neighbour endogene expression. The risks described above will be
moderated by the fact that we will use the strictly replication defective viral vectors, 3rd or 4th generation system. The
third-generation HIV-1-derived lentiviral vector system consists of four plasmids. The transfer vector contains the
transgene or Silencing cassette to be delivered in a lentiviral backbone containing all the cis-acting sequences
required for genomic RNA production and packaging. Lentiviral transfer vectors can be designed to express
(constitutively or conditionally) both transgenes and shRNAs in single units or multiple combinations. Three additional
plasmids (pMDL, pRev and pVSVG) provide the trans-acting factors required for packaging. In addition, an important
safety feature is provided by a deletion of the promoter-enhancer region in the 3'L TR (SIN; self inactivating vectors).
During reverse transcription the proviral S'L TR is copied from the 3'L TR, thus transferring the deletion to the S'L TR;
the deleted S'L TR of the integrated provirus is therefore transcriptionally inactive, preventing subsequent viral
replication or mobilization in the transduced cell. In the 4th generation systems the gag is separated from the gag-propol
sequences normally found on 3rd generation vectors, making the production of recombinant lentivirus even more
unlikely, as extra recombination events would be needed. AAVs have no known link to human illness and are commonly used as vectors for the introduction of genes. In most cases Containment Level 1 will be sufficient, unless there are any additional risks due to the biological activity of the insert, identified by risk assessment.

For all viral vector types, in the event of an accidental contamination, (percutaneous or inhalation) the amount of viral vector able to effectively contaminate a workers cell will be minimal, the transduction for cells in vitro needing a specialized protocol for efficient uptake. The VSV-G pseudotyping results in complement sensitivity, increasing the likelihood of immunological neutralization in human hosts. Cells of the cornea, and lung, are terminally differentiated cells with a limited lifespan and high turnover, which greatly diminishes the chance of effective transformation.

- Environmental considerations-
VSV-G pseudotyping makes the recombinant viral particles amphotropic, able to infect a larger range of animal and cell types, however the viral particles are still sensitive to air, temperature and pH, and will have a short lifespan in the open environment. Importantly, they will be replication defective and so will not produce progeny able to spread from an infected host. Transgene mobilization is theoretically possible if infection occurs in a host cell, which is also infected by a wild-type retrovirus - if the wild-type virus is compatible with the recombinant lentivirus, it may provide encapsidation to allow production of a replication competent viral particle carrying the transgene. The likelihood of this mobilization is very low, and replacement of the VSV-G envelope would remove the amphotropism of the resulting viral particle. Viral particle production steps will be carried out under Containment level 2 conditions. This involves packaging of the separate constructs, concentration of the resultant viral supernatant, transduction of the target cells, and culture analysis of the resultant GM cell populations. All researchers will be trained in the use of hazards involved in working with recombinant viruses. To decrease the likelihood of infection, standard measures to decrease percutaneous as well as aerosolised transmission of viral particles will be adopted, such as no use of sharps and all work to be carried out within a Class II microbiological safety cabinet. All waste will be sterilised by validated means via immediate immersion disinfection and local autoclaving (see point 12 below).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Work involving GM animals will be carried out using facilities outside the Clifford Allbutt Building, namely centres GM 572, GM 793, GM 407, GM 486, GM 3021.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Decontamination for class 1 waste is achieved by treating with Virkon (effectively 100% kill) on site, followed by rotoclaving by the waste contralor of the building (i.e. Novus Environmental).

Decontamination for class 2 waste: all highly contaminated waste solid material, such as pipette tips, petri dishes. will be immersed in 2% Virkon solution for 24 hours, within the microbiological safety cabinet, effectively 100% kill.

All solid waste will be discarded to autoclave bags and autoclaved locally, at 121 degrees C for at least 45 minutes, before discard in clinical waste bag for incineration, 1 00% kill.

Small surface spills will be immediately soaked with Distel, followed by wiping with 70% Ethanol, effectively 100% kill.

Large spills will be sprinkled with Virkon powder before cleaning as above, effectively 100% kill.

Safety cabinet surfaces will be wiped down with Distel before and after working, effectively 100% kill.

All discarded GM modified cell cultures will be destroyed by soaking in 2% Virkon solution or 24 hours followed by autoclaving at 121 degrees C for at least 45 minutes and subsequent discard in clinical waste bag for incineration.
100% kill. Autoclave is temperature validated annually. All mammalian tissue which has been infected with virus will either be fixed with 4% paraformaldehyde or 2% glutaraldehyde, or if fresh will be subject to risk assessment of the possibility of infectious virus still being present and handled accordingly.

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
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<tbody>
<tr>
<td>Q1: Reference is made to adena-associated virus vectors but the examples are actually adenovirus systems (E1 and E3 are adenovirus genes). Are both MV and adenovirus vector systems to be used or is this just a mix up?</td>
<td>A1: This was corrected now. More in detail, both systems will be used separately and independently. AAVs or AdVs are packaged using HEK 293 cells (whose DNA contains crucial elements required for virus propagation that are absent from the viral vectors, thus eliminating the possibility that the viruses produced will propagate outside these cells). To propagate AdVs, HEK 293 cells contain all the components required for viral propagation. The gene of interest is cloned into a replication-defective backbone under promoters such as PGK, CAMKII or synapsin, or tyrosine hydroxylase to target dopaminergic neurons (to target neurons). For AAV production HEK 293 cells are transfected with AAV vectors and with a helper vector that permits AAV packaging but does not produce virus independently.</td>
</tr>
<tr>
<td>Q2: No other questions have been brought up by the committee</td>
<td></td>
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Project Containment

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<td>L3 L4 L2 L3 L4 L2 L3 L4</td>
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Project Ref 3308/19.1

Date Ackn'd 02/03/2022  CU2 Project Title

Class CultureVolClass2 CultureVolumeClass3-4
Transcriptional factor mediated reprogramming of somatic and human pluripotent stem cells

The generation of induced pluripotent stem cells (hiPSC, for induced pluripotent stem cells) from adult terminally differentiated somatic cells by ectopic expression of a small set of transcription factors represents a breakthrough in the field of hPSC research. This approach has eliminated the ethical issues associated with traditional ESCs and has the potential to revolutionise the manufacture of human cell types. The proposed use involves generation of new cellular identities and the development of novel reprogramming paradigms.

Recipient or parental organism

Recipient/Host(s):
- Escherichia coli, K12 derived strains – disabled
- Mammalian cells (primary cells and cell lines) – considered as especially disabled
  - Human embryonic stem cell lines (H9)
  - Human induced pluripotent stem cell lines (produced in situ)
  - Human somatic cell lines (HEK-293T)
  - Mammalian primary cells including human, rodent, bovine (embryonic and adult skin fibroblasts, keratinocytes, peripheral blood cells)

Host/vector system

Only well characterised, replication deficient viral vector system with a history of safe use will be used:
- Lentiviruses – HIV-1 derived, replication defective
- Adenoviruses – Ad5 derived, replication defective
- Sendai viruses (SeV) – Paramyxoviridae. Single chain RNA disabled virus, non-transmissible due to deletion of F protein (F), with temperature sensitive hemagglutinin-neuraminidase (HN) and M genes inhibiting the formation of non transmissible virus-like particles (NTVLP).
- Episomal vectors: vectors with an oriP/EBNA-1 (Epstein-Barr nuclear antigen-1) backbone for delivering the reprogramming genes. Episomal vectors are non-integrative. In addition, silencing of the viral promoter driving EBNA-1 expression and the loss of the episomes due to defects in vector synthesis and partitioning allows the removal of episomal vectors from the iPSCs without any additional manipulation.
Origin & function

Inserts
1. Human, murine, xenopus cDNA (+/- TAG sequences) coding for transcription factors, signal transducers, growth factors and surface receptors
2. shRNA and miRNA sequences against cell endogenous coding sequences
3. Reporter genes (GFP, LacZ, Luciferase, possibly fused to cDNA (type1 inserts))
4. Selection genes (NPT, PAC)
5. Functional non coding sequences (promoter, enhancer, insulators, IRES, WPRE, of mammalian or viral origin)
6. Neutral non coding sequences (introns, isolated exons)

Evaluation of foreseeable effects

• cDNA (Type1 inserts) will code for transcription factors, signal transducers, growth factors and receptors which are expected to play a role in pluripotency, cell fate decision and differentiation from pluripotent stem cells (PSCs).
• shRNA (Type2 inserts) will produce non-coding RNA sequences targeting cell endogenous mRNA from above mentioned families (Type1 list). Expression of both insert types may enhance/block commitment and differentiation of PSCs towards one of the three primitive germ layers and further differentiated progeny.
• Reporter genes (Type3 inserts) will code for proteins with fluorescent or enzymatic properties allowing easy identification of genetically modified (GM) cells. They are thought to have no deleterious biological effect on expressing cells.
• Selection genes (Type4 inserts) will produce enzymatic proteins able to inactivate specific antibiotic families allowing selection of GM cells. They are thought to have no deleterious biological effect on expressing cells.
• Functional non-coding sequences (Type5 inserts) will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES). They will be used (independently or in combination) into viral expression cassettes to achieve best control of transgene expression depending on targeted cells.
• Neutral non-coding sequences (Type6 inserts) will be used in particular for gene targeting projects to promote homologous recombination at defined genomic loci (intrinsic/exonic genomic sequences). They should not have biological effect by themselves.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All highly contaminated waste solid material, such as pipette tips, petri dishes, will be immersed in 2% Virkon solution for 24 hours, within the microbiological safety cabinet. All solid waste will be discarded to autoclave bags and autoclaved locally, at 134 degrees C for at least 3 minutes, with printout verification of sterilisation cycle, before discard in clinical waste bag for incineration. Small surface spills will be immediately soaked with Chemgene or equivalent followed by wiping with 70% Ethanol. Large spills will be sprinkled with Virkon powder before cleaning as above. Safety cabinet surfaces will be wiped down with Chemgene or equivalent before and after working. All discarded GM modified cell cultures will be destroyed by soaking in 2% Virkon solution for 24 hours followed by autoclaving at 134 degrees C for at least 3 minutes with printout verification of sterilisation cycle, and subsequent discard in clinical waste bag for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
1. Assume no needles are involved in the purification of the lentivirus vectors.

2. Under app D 1st para it is stated that transduced cells may be removed from CL2 – this may not be possible for all cells i.e. human primary derived cells. Addressed in revised risk assessment

3. Will the genomic loci targeting constructs present any more risk than others? The genomic loci will not present any more risk than other genomic targeting vectors. It is a PUC plasmid-based vector and the risk assessment is well characterised.

4. P9 app C bovine and mouse primary cells are given as hazard 1. How is this established? There are quite a few bovine zoonotic agents so the tissue type, source (veterinary?) and processing will determine whether these remain a risk. I think all these are bacterial so will be eliminated as part of the culturing process. For mice, this is less of an issue and one assumes these are SPF animals as source material. This is mainly a CoSHH consideration.

   The above risk has been addressed by a secondary biological risk assessment (for COSHH regs), where the hazard group has been identified. In brief, Rodent tissue is from a scientific animal facility where sentinel screening is carried out. With the bovine cell lines, the material will come from regulated farms and abattoirs, where health records are kept, and the source material has been checked by a meat inspector. Culturing process will eliminate most risk, and cells will move from CL2 to CL1 on sterility testing.

   Contained

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**Project Ref** 3308/20.1

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<tr>
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<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
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<td>04/12/2020</td>
<td>Mechanisms of pathogenicity and strategies of brain repair in neurodegenerative disorders</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
</tr>
</tbody>
</table>

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Date Project Ceased 02/03/2022

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Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

---

Please enter comments on the GM safety committee on the risk assessment

- Contained

---

CU2 Project Title

Mechanisms of pathogenicity and strategies of brain repair in neurodegenerative disorders

---

Class

Consent Granted

---

Project notified under transitional arrangements N
Purpose of the contained use

Our research focuses on understanding how diseases damage the nervous system, and developing methods to repairing this damage. Research spans basic biology through to clinical studies. Areas of research include the biology of neurons and glia, the process of myelination, the use of stem cells to repair the brain, axon regeneration, plasticity in the brain, mechanisms of neurodegeneration and inflammation. The techniques are multi-disciplinary, and include molecular and cell biology, electrophysiology, both tissue culture and in vivo work, behavioural studies, clinical studies. Specifically for this notification, human and rodent primary and cell line cultures will be transduced with different viral vectors (see below), in order to introduce coding genes or non-coding RNAs associated with pathogenicity and/or repair processes and to understand their role in the context of neurodegenerative diseases.

Recipient or parental organism

- Well characterised commercial bacteria (e.g. One Shot® TOP10 and Stbl3™ Chemically Competent E. coli) disabled, non colonising, non pathogenic.
- Commercial packaging cell lines, e.g. HEK-293T human cell line. This cell is highly transfectable derivative of human embryonic kidney 293 cells, containing the SV40 T-antigen. Thus this cell line is competent to replicate vectors carrying the SV40 origin of replication, giving high titers when used to produce viral particles. It has been widely used for lentiviral production, gene expression and protein production.
- Human and rodent primary neural cultures, somatic neural stem/precursor cells, embryonic stem cells, fibroblasts and induced pluripotent stem cells. Characteristic of cell cultures are never totally understood, so primary human cultures will be handled at containment level 2, considering the possibility of adventitious viruses. Rodent cultures will normally be handled at containment level 1, unless otherwise specified in the risk assessment.

Host/vector system

Only well characterised, replication deficient viral vector system with a history of safe use will be used:
- HIV-1 derived lentiviral 3rd and 4th generation, replication defective (e.g. eGFP pRRLSsin.PPT-hCMV, pCDH-EF-1-MCS-T2A-GFP).
- Adena virus vectors, replication defective (e.g. Adeasy and Adtrack, deleted E1 and E3 genes).
- Sendai virus vectors (hOct 3/4, hSox2, hKlf4, hc-Myc).
- Adeno-associated virus vectors, replication defective (e.g. AAV6).

Origin & function

Inserts are arbitrarily grouped as follows:
Type 1. Human, murine eDNA (+/- TAG sequences) coding for proteins, for example:
Hntth and Safety
Executive
transcription factors (Wnt3a, Ngn2 etc.) are expected to influence cell fate decision and differentiation, but not expected to have an effect on cell proliferation. Other genes may code for disease associated proteins (alpha synuclein, tau etc).

Type 2. ncRNA sequences for example:
shRNAs and miRNAs (mmu-miR-155-5p, etc) will produce ncRNA sequences targeting cell endogenous mRNA (Wnt3a, p73). This sequences are not expected to affect cell proliferation. Notably, mmu-miR-155-5p is not implicated in oncogenic processes.

Type 3. Reporter genes for example:
enhanced GFP (eGFP), mCherry, firefly luciferase etc will code for proteins with fluorescent properties allowing easy identification of genetically modified (GM) cells. All of these labels are thought to have no deleterious biological effect on expressing cells.

Type 4. Selection genes for example:
bacterial resistance (ampicillin) or mammalian resistance (puromycin) will express proteins with enzymatic activities to allow selection of bacterial or GM cell cultures.

Type 5. Functional non-coding sequences for example:
promoter, enhancer, insulators, IRES, WPRE, of mammalian or viral origin. Functional non-coding sequences will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES). They will be used (independently or in combination) into viral expression cassettes to achieve best control of transgene expression depending on targeted cells.

Evaluation of foreseeable effects

Human health considerations -
H •• ~h and Safety
Executive
A possible risk to human health derives from the recombinant viral particles, via accidental infection of the researcher.
Because only replication defective viral particles (non-infectious, Le. unable to multiply and propagate after initial cell transduction) will be used, and inserts will not produce compounds with acute toxicity (like bacterial toxins), no additional risk will arise from the final genetically modified cells, compared to the initial cell source.
The recombinant lentiviral particles (H1V-1 derived) will be packaged via the vesicular stomatitis virus (VSV-G). This step will alter the tropism of the retroviral particles, as it only requires interaction with the plasma membrane of cells rather than a specific receptor, thus allowing increased stability of the viral particles associated with the VSV-G envelope. This may add the aerosol risk in addition to the percutaneous risk. Retroviruses stably integrate into the host cell genome, and there may be a risk of insertional mutagenesis, with subsequent ectopic inhibition/activation of host gene expression. Subsequent de-regulation of endogene expression could theoretically lead to transduction associated detrimental effects such as initiation of oncogenic processes. Moreover the addition of the Woodchuck hepatitis Post transcriptional Regulatory Element (WPRE) to the expression cassette in several retroviral backbones could also enhance the oncogenic potential of such vectors (SACGM 2004). However, the use of a lentiviral backbones is thought to be safer, as the genomic integration profile of lentiviral derived vectors do not show an integration bias towards the transcriptional start site region of host cell genes.
Risk is also associated with inserted coding sequences. cDNA or shRNA/miRNA inserts canied by viral vectors will direct ectopic expression or inhibition of transcription factors, and strong promoter/enhancer effects from noncoding
sequences could lead to ectopic activation of neighbour endogene expression.
The risks described above will be moderated by the fact that we will use the strictly replication defective viral vectors, 3rd or 4th generation system. The third-generation HIV-1-derived lentiviral vector system consists of four plasmids. The transfer vector contains the transgene or silencing cassette to be delivered in a lentiviral backbone containing all the cis-acting sequences required for genomic RNA production and packaging. Lentiviral transfer vectors can be designed to express (constitutively or conditionally) both transgenes and shRNAs in single units or multiple combinations. Three additional plasmids (pMDL, pRev and pVSVG) provide the trans-acting factors required for packaging. In addition, an important safety feature is provided by a deletion of the promoter-enhancer region in the 3’LTR (SIN; self inactivating vectors). During reverse transcription the proviral 5’LTR is copied from the 3’LTR, thus transferring the deletion to the 5’LTR; the deleted 5’LTR of the integrated provirus is therefore transcriptionally inactive, preventing subsequent viral replication or mobilization in the transduced cell. In the 4th generation systems the gag is separated from the gag-pro-pol sequences normally found on 3rd generation vectors, making the production of recombinant lentivirus even more unlikely, as extra recombination events would be needed.

AAVs have no known link to human illness and are commonly used as vectors for the introduction of genes. For all viral vector types, in the event of an accidental contamination, (percutaneous or inhalation) the amount of viral vector able to effectively contaminate a worker's cell will be minimal, the transduction for cells in vitro needing a specialized protocol for efficient uptake. The VSV-G pseudotyping results in complement sensitivity, increasing the likelihood of immunological neutralization in human hosts. Cells of the cornea, and lung, are terminally differentiated cells with a limited lifespan and high turnover, which greatly diminishes the chance of effective transformation. All manipulations using lentiviral vectors will be at containment level 2.

- Senda; Virus vectors consist of the viral proteins Nucleocapsid protein (NP), Phosphoprotein (P), Matrix protein (P), Fusion protein (F), Hemagglutinin-Neuraminidase (HN) and Large protein (L). Because Sendai virus infects cells by attaching itself to surface receptor sialic acid present on the surface of many different cells, it can infect a wide range of cell types. Activation of F protein by a protease is required for the virus-cell fusion process to take place. Following infection, the virus goes through genome replication, protein synthesis and then daughter virus particles are assembled and released. However, they are no longer capable of producing infectious particles from infected cells, because the viral genome lacks the F-gene. All activities involving senda; virus manipulations will be performed at containment level 2.

Expression of the vector genes leads to reprogramming of target cells and allows them to be come pluripotent. The Sendai virus replicates independently of the cell cycle in the cytoplasm and does not intergrate into the host cells genome. In addition the presence of functional mutations such as temperature sensitivity in the amino-acid sequence of several Sendai virus proteins (SeVITS.6.F and SeVITS15.6.F) allows for easy removal of the vectors from transduced cells.

- Adenovirus vectors are replication defective due to removal of E1 /3/4 regions. Because they can infect humans, containment Level 2 practices and facilities will be used for activities involving adenoviral vectoF5.

- Microbiological safety cabinets wi ll be used for all manipulations that can generate aerosols (e.g. pipetting, harvesting glinfected cells, opening sealed centrifuge containers). Aerosol containment devices will be used when centrifuging and V3ccum lines used for aspiration will be protected with liquid disinfectant traps and a micron filter.

Environmental considerations

VSV-G pseudotyping makes the recombinant viral particles amphotropic, able to infect a larger range of animal and cell types, however the viral particles are still sensitive to air, temperature and pH, and will have a short lifespan in the open environment. Importantly, they will be replication defective and so will not produce progeny able to spread from an infected host. Transgene mobilization is theoretically possible if infection occurs in a host cell, which is also
infected by a wild-type retrovirus - if the wild-type virus is compatible with the recombinant lentivirus, it may provide encapsidation to allow production of a replication competent viral particle carrying the transgene. The likelihood of this mobilization is very low, and replacement of the VSV-G envelope would remove the amphotropism of the resulting viral particle. Viral particle production steps will be carried out under Containment level 2 conditions. This involves packaging of the separate constructs, concentration of the resultant viral supernatant, transduction of the target cells, and culture/analysis of the resultant GM cell populations. All researchers will be trained in the use of hazards involved in working with recombinant viruses. To decrease the likelihood of infection, standard measures to decrease percutaneous as well as aerolised transmission of viral particles will be adopted, such as no use of sharps and all work to be carried out within a Class 11 microbiological safety cabinet. All waste will be sterilised by validated means via immediate immersion disinfection and local autoclaving (see point 12 below).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Work involving GM animals will be carried out using facilities outside the Clifford Allbutt Building, namely centres GM 572, GM 793, GM 407, GM 486, GM 3021.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

NJA

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Decontamination for class 1 waste is achieved by treating with Virkon (effectively 100% kill) on site, followed by rotoclaving by the waste contractor of the building (i.e. Novus Environmental). Decontamination for class 2 waste: all highly contaminated waste solid material, such as pipette tips, petri dishes, will be immersed in 2% Virkon solution for 24 hours, within the microbiological safety cabinet, effectively 100% kill. All solid waste will be discarded to autoclave bags and autoclaved locally, at 121 degrees C for at least 45 minutes, before discard in clinical waste bag for incineration, 100% kill. Small surface spills will be immediately soaked with Chemgene, followed by wiping with 70% Ethanol, effectively 100% kill. Large spills will be sprinkled with Virkon powder before cleaning as above, effectively 100% kill. Safety cabinet surfaces will be wiped down with Chemgene before and after working, effectively 100% kill. All discarded GM modified cell cultures will be destroyed by soaking in 2% Virkon solution or 24 hours followed by autoclaving at 121 degrees C for at least 45 minutes and subsequent discard in clinical waste bag for incineration 100% kill. Autoclave is temperature validated annually. All mammalian tissue which has been infected with virus will either be fixed with 4% paraformaldehyde or 2% glutaraldehyde, or if fresh will be subject to risk assessment of the possibility of infectious virus still being present, and handled accordingly.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Please enter comments on the GM safety committee on the risk assessment

On the CU2:
Could there be some indication of the CLs used for the lentiviral vectors and the Sendai vectors?
Second to last paragraph, line 2 - Containment level instead of biQsafety level.
Nextflast para - Microbiological safety cabinets ...
On the risk assessment:
P6 - can the overall risk (of the recipient microorganisms) be low if one of the sub sections is medium?
Can there be some information of the potential modes of transmission of each of the viruses?
P9 - potential for transmission of naked NA is checked yes - is that correct?
P10 - will the host range be different to parental virus is checked no. It is in the case of lentiviral vectors bearing
VSV G. P12 ditto.
P10 - next question - above lentiviral VSVG are more susceptible to human complement, so yes.

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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Transitional Premises

Emergency Plan Required?

Non-GMMs

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Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

---

Give brief details of the genetic modification safety committee

Operations and Site Director - 34 years laboratory experience and currently Operations and Site Director at TC BioPharm with responsibilities for Process Development, GMP Manufacture, Clinical operations, Engineering and Quality. Previous experience includes responsibility as GMO officer at contract manufacturing organisations; Excell Biotechnology and Angel Biotechnology. Conducted clinical trials to include ReNeurn - first in man treatment using genetically modified stem cells (primary human neuronal cells) with C-myc gene regulated by 4-OHT.

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A - proceed to section 90

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

N/A - proceed to section 9

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**Project Ref 3310/15.1**

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Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022   Page 13928 of 15326
### Purposes of the contained use

The aim of this work will be the use of lentivirus particles to deliver transgenes to mammalian cells. Human cells (e.g. PBMCs, T cells, cell lines) will be co-cultured with the appropriate titre of lentiviral particles in a suitable cell culture medium to facilitate viral-mediated transfer of the genetic information transgene into the target cell line. The transduced cells will be cultured and used in immune or other assays - all assays will be performed as in vitro studies only. A genetically modified, replication defective lentivirus will be used to transduce the cells.

### Recipient or parental organism

Human cells; peripheral blood mononuclear cells (PBMCs), T cells and cell lines.

### Host/vector system

Recombinant, replication-defective lentivirus. The viruses are classified as Risk Group 2 by the World Health Organization (WHO).

### Origin & function

Please see section 17 for full details.

### Evaluation of foreseeable effects

The major risks associated with lentiviral vectors are 1) the potential generation of replication-competent lentivirus (RCL) and 2) the potential for oncogenesis via random chromosomal integration. These risks can be mitigated by the nature of the vector system and the nature of the transgene inserted. The potential generation of replication competent lentivirus is reduced due to the modification the recombinant virus has undergone to render it replication incompetent. This means that outside of a specific, complimentary packaging cell line, the viral DNA construct does not contain sufficient genetic material to produce infectious virions or the ability to self-replicate. Please see section 17 for a detailed description of the lentiviral vectors intended for use at TC BioPharm.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

None intended for use.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste will be decontaminated by incubation with an appropriate volume and concentration of virkon solution for at least 16 hours. Virkon is a virucidal disinfectant known to be effective at killing at 47 strains from 35 different viruses. After this time, the decontaminated waste will be dipsoed of down the drain with copious amounts of running water.

All contaminated solid waste will be double bagged and uplifted by SRCL - a specialist clinical waste uplift service.

All contaminated sharps will be disposed into a sharps containers. The sharps container will be sealed and uplifted by SRCL - a specialist clinical waste uplift service.
SRCL's clinical waste disposal processes are fully compliant with all regulations, including those defined in the Controlled Waste Regulations act of 2012.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

The genetic modification safety committee was consulted regarding the proposed scheme of work detailed within this contained use notification. The committee was comprised of the Health & Safety Officer, the Biological Safety Officer, the CEO and three experienced laboratory scientists. The proposed scheme of work was considered and discussed in detail, as was the BioCOSHH assessment associated with this work. The committee were satisfied that the BioCOSHH assessment adequately identified and assessed the risks of this work and details the measures by which to minimise the risks to both the operators and the environment.

Project Containment

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: 

- [ ] Yes
- [ ] No

Give brief details of the genetic modification safety committee:

Hologic Ltd. GM and Environmental Health and Safety committee has been established and has regular monthly meetings. The committee comprises of experienced Technical staff and Scientists representative of core areas of expertise in Microbiology and Pharmaceutical Chemistry. Further expert specialist technical advice is available if required from Hologic Inc. EH&S department and specialist scientists.

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Other (please specify): 

Tick if confidential: 

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- [ ] No

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Following sterility analysis, the closed sterility unit (BioFlex® B90/S Flexible Film Sterility Test Isolator System or TPC Flexible Film Sterility Test Isolator System; each are equivalent) will be washed down with 70% IPA and sterilised with 35% Hydrogen Peroxide. It is intended that all waste product and non-reusable consumables used in the conduct of the sterility test will be de-contaminated with an appropriate agent (Hydrogen Peroxide 35%) and post analysis double bagged in sealed sterile plastic bags within the closed Isolator system, removed and placed in waste disposal bins prior to collection and incineration by a specialist contract waste management company licenced for clinical waste incineration. Reusable scissors and forceps are placed in sterile bags and autoclaved. Re-usable bottles following sterility analysis are placed in the autoclave using a 100% killing cycle. The facility has three Astell autoclaves available in the facility for this purpose.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Following sterility analysis, the closed sterility unit (BioFlex® B90/S Flexible Film Sterility Test Isolator System or TPC Flexible Film Sterility Test Isolator System; each are equivalent) will be washed down with 70% IPA and sterilised with 35% Hydrogen Peroxide. It is intended that all waste product and non-reusable consumables used in the conduct of the sterility test will be de-contaminated with an appropriate agent (Hydrogen Peroxide 35%) and post analysis double bagged in sealed sterile plastic bags within the closed Isolator system, removed and placed in waste disposal bins prior to collection and incineration by a specialist contract waste management company licenced for clinical waste incineration. Reusable scissors and forceps are placed in sterile bags and autoclaved. Re-usable bottles following sterility analysis are placed in the autoclave using a 100% killing cycle. The facility has three Astell autoclaves available in the facility for this purpose.

The Hologic GM and EH&S safety committee noted that the product has been previously assessed and approved by the manufacturer and by our client as requiring containment level 1. It was further noted that the purified viral vector is not considered ‘live’ at the formulation and filling stages to be completed at our clients facility nor at the sterility testing stage to be completed at Hologic Ltd. The project conducted at Hologic Ltd. involves the sterility testing of the finished product HIV-1 lentiviral vector.
GM Centre Number: 3313

Data Premises Notified: 22/02/2016
(Originally)

Transferred from 1992 Regs?: N

Transitional Premises

Class:

Data Premises Closed: N

Transitional Premises

Emergency Plan Required?

Non-GMMs: N

Withdrawn: N

Name

LEEDS BECKETT UNIVERSITY

Name 2

Department

Campus Estate or Research Centre

CITY CAMPUS

Building

PORTLAND BUILDING

Road Name

District

Town

LEEDS

County

WEST YORKS

Postcode

LS1 3HE

Country

ENGLAND

Tel Number

0113 812 3612

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

A Health and Safety Consultant has advised the school on setting up premises notification and risk assessment for proposed work with class 1 and 2 GMMS. They have also advised on the setting up of a GMSC to consist of the GMBSO, Faculty H&S Advisor, and appropriate expertise from the faculty. Additional advice will be sought for any proposed works outwith the experience or expertise of the committee members.

The consultant is a chartered Fellow of IOSH with a BSc (hons) Applied Biology and Masters degree in a Biological subject, and has worked in molecular biology laboratories. They have acted as a GMSO and chaired and administered GMSC's for over 10 years, dealing with assessments for work up to class 3 for a range of hosts (including plants, animals, insects) and a range of GMM's, inserts and vectors. They have organised BSO network meetings as well as teaching on a professional biosafety practitioner course.

<table>
<thead>
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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Non-microbial</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

Maximum culture volume (multiples of up to): 1 litre.
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving by autoclaving at 15 lbs/sq inch pressure and 121 degrees C for at least 20 minutes (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. The autoclave is tested by the manufacturers (LTE Scientific Ltd) twice yearly.
Runs may also be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121ºC for 20 min).

Consultant comments - The attached risk assessment is a broad assessment to cover class 1 work in undergraduate teaching laboratories using low risk, commercially available vectors and hosts. Research work at class 1 will also be undertaken (subject to review of the risk assessments already in place at the current host institutions) and if outside the scope of this assessment will be assessed separately. Class 2 work will be notified separately and appropriate acknowledgements and permissions received before the work is transferred to the facility.
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**Name**

EARLHAM INSTITUTE

**Name 2**

**Department**

**Campus Estate or Research Centre**

NORWICH RESEARCH PARK

**Road Name**

**District**

COLNEY

**Town**

NORWICH

**County**

NORFOLK

**Postcode**

NR4 7UZ

**Country**

ENGLAND

**Tel Number**

01603 450001

**Fax Number**

01603450021

**E-mail**

**HSE Division**

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**Comments**

name change 19/07/2016 from The Genome Analysis Centre

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

Three members of the site Biological Safety Committee (Genetic Modification Safety Committee) who possessed appropriate knowledge, experience and training in the scientific area in question, reviewed the risk assessment and provided comments on aspects of the risk assessment that required additional supporting information. Following provision of this additional information the risk assessment was agreed to be appropriate and provided a sufficient assessment of the risks posed by the activities.

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Material containing Class 1 GMMs will be disposed of by placing in a designated BLUE plastic box lined with a clear autoclavable bag. The petri dishes and bottles placed in these boxes will not be sealed.

Tips, Eppendorf tubes and sharps contaminated with the above materials will be placed in open plastic bottles to allow penetration of steam and then placed in a designated BLUE plastic box lined with a clear autoclavable bag.

The lid of the blue box must close properly, not rest on the contents and must not be sealed down with tape to allow penetration of steam.

All solid materials coming in contact with the GMM and all biological waste is inactivated by autoclaving at 121 C at 15psi for 15 minutes or by validated chemical means.

Small volumes of liquid GMM contaminated waste will be disinfected by validated means followed by sink disposal with copious amounts of water.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Reviewer 1: "If anyone is going to be involved in generating GM Agrobacterium tumefaciens strains, then they should also assess the risks of that activity in the same way they have for E.coli. It depends on where the service ends and the user requesting synthesis takes over."

[Note: this work will not involve generating GM Agrobacterium tumefaciens strains]

Reviewer 2: No comments

Reviewer 3: Provided minor edits to the draft risk assessment with particular emphasis on hazard identification with respect to environmental safety, assessment of class and containment level and double checking that all hazards are properly considered by the proposal.
The research projects in the Korcsmaros group aim at deciphering host-microbe interactions that take place particularly in the digestive tract. Evidences in the literature demonstrate that pathogens (in particular facultative intracellular pathogens like Salmonella enterica) highjack the host cell machinery for their benefit. Multiple microbial proteins have been shown to interfere with host cellular processes but the full regulatory mechanisms that modulate this effect remain unelucidated. We are interested in understanding how microbial proteins affect homeostatic host processes and functions (e.g. Autophagy, Antimicrobial protein production) during host-microbe interactions in health and disease. Investigating this crosstalk requires to modify the model system we use so that we can visualise and measure the specific impact microbial proteins have on specific signalling cascade of host cells.

Two approaches will be employed as alternative or complement of each other:
• either remove the gene from the bacteria and observe how it changes the phenotype in host cells; in some cases, this will also involve tagging the bacteria with a fluorescent report fusion (e.g. GFP, mCherry)
• or express only the bacterial protein inside mammalian cells from a genetic construct to exclude additional/synergistic effect of other bacterial factors and only assess the effect of the candidate bacterial protein on the mammalian cell function.
Salmonella enterica
Salmonella enterica serovar Typhimurium is a zoonotic food-borne pathogen affecting animals and humans, through consumption of contaminated food or water. In healthy individuals it causes a self-limiting gastroenteritis. Symptoms include headache and nausea, vomiting, diarrhoea, abdominal pain or cramps, fever.

Staphylococci spp.
S. aureus for example is a Gram-positive spherical bacteria, member of the Firmicutes of important clinical and biotechnological relevance. It is frequently found in the nose, respiratory tract and on the skin. It has been estimated that 20% to 30% of the human population are long-term carriers of S. aureus. Pathogenic S. aureus strains have been isolated from around the world. Symptoms of Staphylococcal infection caused by food intoxication are nausea, abdominal pain, vomiting, cramps, diarrhoea. Skin conditions caused by the bacterial exfoliative toxins include blisters, skin loss, pimples, furuncles, impetigo, folliculitis, abscesses, poor temperature control, fluid loss, and secondary infection. In immunocompromised individuals S. aureus can cause (very rarely) necrotizing fasciitis which is life-threatening and causes severe morbidity. Certain strains of S. aureus produce the superantigen TSST-1, which is responsible for 75% of toxic shock syndrome (TSS) cases.

Campylobacter spp.
Campylobacter jejuni and Campylobacter coli are the most common causes of campylobacteriosis. The typical symptoms are severe diarrhoea and abdominal pain.

Escherichia coli
Among the many strains of E. coli, only a few trigger diarrhoea. Other symptoms are severe stomach cramps and vomiting. The symptoms usually last up to seven days if there are no complications, but some infections can be severe and may be life threatening.

Listeria monocytogenes
Listeriosis affects primarily pregnant women, older adults and persons with weakened immune systems due to a disease (e.g., diabetes, organ transplant, cancer, age) or due to medications (e.g., steroids). Symptoms of listeriosis are fever, muscle aches, and sometimes gastrointestinal symptoms, such as nausea or diarrhoea. If infection spreads to the nervous system, symptoms can include headache, stiff neck, confusion, loss of balance, or convulsions.

Clostridium difficile
Clostridium difficile is commonly found in the gut without causing any symptoms. The symptoms associated with toxin-producing C. difficile infection (CDI) can include diarrhoea (mild to severe), fever, loss of appetite, nausea, abdominal pain or tenderness. In the most severe cases the pseudomembranous colitis may require emergency bowel surgery and can be fatal. C. difficile is able to form spores that are able to survive for long periods in the environment and contribute to its ability to spread between hosts.

While preparing the bacterial infectious inoculum staff will wear appropriate PPE and will be working in hazard group 2 containment laboratory and safety cabinet. New and expectant mothers are regarded as being at particular risk.

Additional COSHH risk assessments will be performed for new and expectant mothers as needed.

Adverse effects from establishment or dissemination of GMMs in the environment:
The generated mutants and plasmids used contain antibiotic resistance genes; selective antibiotic resistance markers will be chosen different from the drugs of choice for the treatment of infections by these infectious agents and thus would present no selective advantage in the event of an accidental release. For example, for culturing Salmonella antibiotics such as kanamycin, ampicillin, chloramphenicol will be used. Accidental release of the organisms should not occur as spillages are immediately disinfected and all contaminated liquid and solid waste is autoclaved to sterilise prior to discard.
Adverse effects from transfer of genetic material to or from other micro-organisms:
mCherry and GFP reporter/tag proteins are harmless proteins, and do not increase the risk associated with the strains or transfected cells.

Adverse effects from the interaction of GMMs with other organisms in the containment facility:
GMMs should not be able to accidentally interact directly with other GMMs within the facility. Nonetheless, coculturing of different bacterial strains with or without potentially GM mammalian cells is planned. This process will not raise the Hazard Group of either of the organisms to a higher Hazard Group, even in cases where genetic material might be transferred from one organism to another.

Host/vector system

We will use Hazard group 2 human and zoonotic foodborne pathogens (such as Salmonella enterica serovars, Staphylococci spp., Campylobacter spp., Escherichia coli, Listeria monocytogenes, Clostridium difficile) as well as commensal bacterial strains (such as Bifidobacteria spp., Lactobacillus spp., Bacteroides thetaiotaomicron, Ruminococcus spp.) usually not causing human disease. All strains that will be used are classified maximum as hazard group 2 organisms as per the HSE Approved List of biological agents (http://www.hse.gov.uk/pubns/misc208.pdf).

- We will construct transient or stable plasmid-borne expression systems in mammalian cells (e.g. intestinal epithelial cells like HT-29 colon cancer cell line, or organoid culture) enabling them to produce a bacterial protein of interest. For transient expression system, vectors such as CoIE1 replicon derivatives will be used. For stable expression system, integrated system such as the Flp-In Complete System (ThermoFisher Scientific, https://www.thermofisher.com/order/catalog/product/K601001). This will involve using weak to medium strength promoter such as that of the human Ubiquitin C gene, to reduce the toxicity risk associated with high level of expression of the microbial protein within the host cells. When strong expression of a protein is required, the expression systems will be based on commonly used viral promoters such as those from polyomavirus simian virus 40 (SV40) or Cytomegalovirus (CMV).

- The plasmids we use to replace a gene by a resistance cassette and remove that cassette post-gene deletion if necessary (such as pKD46 and pCP20 plasmids), express the RepA101ts thermosensitive DNA binding and polymerase activity. This is necessary for the plasmid replication (pSC101 ori), permissive at 30°C but no longer at 37°C to 42°C. Losing these plasmids will ensure no additional untraceable genome modification can occur in the recipient strains.

- The plasmids (such as pWKS.30 or pBR322) to be used for complementation do not harbour the genes necessary for self-mobilisation, however it may contain a bom site (such as pBR322) which can make the plasmid mobilisable and transferrable to other strains in the presence of a helper plasmid. Because of that, the risk is minimal for antibiotic resistances encoded by the plasmid to be transferred to other strains should infection with this modified organism happen in a context where gut microbiota strains harbour appropriate helper plasmids. The overall risk is therefore considered very low.

- Constructing GM bacterial strains will involve prokaryotic plasmid vectors (e.g. CoIE1 or pSC101 derivatives). These plasmids contain antibiotic resistance cassette(s) such as Chloramphenicol, Kanamycin, Ampicillin, etc used as selective markers. Some of these plasmids possess a thermosensitive replication system which allow the loss of the plasmid upon bacterial growth at increased non-permissive temperature for the plasmid replication (for gene replacement, fusion insertion or resistance cassette deletion).

- Reporter proteins such as GFP or mCherry will be used to monitor gene expression or to tag protein or bacterial cells and localise them within a biological sample.

During that process the organisms will be at their highest infectious state.
- The modified bacteria will be used in parallel to wildtype parental strains in in vitro (mammalian cell culture models) or in vivo (animal models) infection assays. At the end of the assays, the infected mammalian cells or the cells expressing candidate bacterial proteins will be analysed by techniques such as bioimaging, qPCR, RNAseq or Western blot analysis. Part of the sample collecting will involve physical separation of cells of interest from other cells. This will be performed using a flow cytometer.

### Origin & function

Strains we are going to use are a) commonly used in expert groups of the research field, and b) likely to be isolated from infection cases or human or animal gut.

For example S. enterica strain SL1344 is a hisG auxotroph derivative from the 4/74 strain, isolated from a calf with salmonellosis in the United Kingdom (Hoiseth and Stocker, 1981, Nature, 291: 238–239). S. aureus strains will derive from the parental strain NCTC8325-4 (RN450), a modified S. aureus NCTC8325 strain originally isolated in 1960 from a sepsis patient and primarily utilized in the laboratory for the study of S. aureus genetics.

All genes manipulated in these experiments will either be derived from the species under observation, or will be well-characterised genes that will not affect the pathogenicity or virulence of the species under investigation e.g. GFP, mCherry, antibiotic cassettes used in cloning.

Other related genes may be used, these would present no greater risk to the environment, humans or animals.

### Evaluation of foreseeable effects

All handling of the microorganisms will be performed in designated containment level 2 laboratories. The flow cytometer (such as a BD FACSMelody sorter instrument) is also in a containment level 2 laboratory and placed inside a containment level 2 safety cabinet to minimise the risk of exposure to generated aerosol.

Transmission and infection of laboratory personnel is most likely via the oral route for Salmonella, Campylobacter, Listeria, Staphylococci, Clostridium or by skin exposure (e.g. Staphylococci). Good laboratory practice and implementation of the recommendations outlined in Schedule 8 in The Genetically Modified Organisms (Contained Use) Regulations 2014 Guidance L29 will minimise risks. This includes personal protective equipment (PPE) appropriate for all pathogens used, and will include wearing lab coats, gloves, and safety glasses when necessary. All in vitro work will involve standard laboratory procedures where every procedural risk will be assessed. Given the level 2 containment and control measures, it is unlikely that the GMMs will reach the environment and cause harm to humans or animals outside the laboratory. Standard laboratory procedures will include restricting access to authorised workers, ensuring that all areas that have been in contact with potentially hazardous micro-organisms will be routinely swabbed with a recommended disinfectant, no laboratory clothing will be allowed outside the laboratory and all outdoor clothing will be stored in offices. All contaminated waste will be autoclaved and/or incinerated.

While transporting the GM Hazard Group 2 material to the Containment Level 2 lab across Containment Level 1 working area, samples will be fully contained. When transporting materials outside the containment laboratories they will be triple contained according to the SOP QA-SJB-009.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All GMM material will be handled using good microbiological practice (GMP). All GMM material will be inactivated by

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All GMM material will be handled using good microbiological practice (GMP). All GMM material will be inactivated by
autoclaving prior to disposal. All residual microbiological material will be stored in sealed containers and disposed of by autoclaving. Autoclave procedures are as follows: Microbiological solid waste is sterilised by autoclaving at 121 degrees C for a minimum of 30 minutes at 15p.s.i. Routine monitoring of autoclave efficiency is completed using Thermalog S strips. Annual 12 point validation of autoclaves is performed.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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Project Ref 3315/20.1

Date Ackn'd 28/02/2020

 CU2 Project Title Use of Lentiviral vectors for expression of cellular proteins and non-coding RNAs in eukaryotic cells

Class CultureVol

Class CultureVolume

Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

02/03/2022
Research activities in Macaulay group focus on studying the effect of cellular proteins involved in signalling pathways and metabolic processes in eukaryotic cells of mesodermal and endodermal origin and in haematopoietic system. Furthermore, we want to utilize non-coding DNA and RNA for divisional history studies and lineage tracing purposes. We intend to modify eukaryotic cells using CRISPR-Cas9 system to evaluate in vitro effect of proteins and non-coding RNAs of interest.

Transduced cells will be used for in vitro experiments including imaging and biochemical analysis (e.g., qPCR, Western blotting, pull down or mass spectrometry).

Defective lentiviruses will be generated using a lentivirus vector plasmid which contains the LTR from HIV or MLV with SFFV, CMV, CAG, EF1alpha orland U6 promoters. This plasmid will be co-transfected with packaging plasmids: pVSV-G (encoding the envelope proteins under the control of the CMV promoter) and pCMVDR8.91 (encoding replication proteins). The resulting virus secreted from HEK293T/FT producer cells is defective in packaging signal and will be used for infection of recipient mammalian cells listed above. No productive virus that is infectious will be created.

Components of the TPO-cMpl, JAK-STAT signalling pathways, metabolic pathways including glycolysis, OXPHOS, Krebs cycle and gain/loss of function mutants, components of CRISPR-Cas9 system (Cas9 protein or/and its mutants). Short non-coding DNA sequence encoding the cellular barcode (semi-random sequence of 50-80 nucleotides long) or/and non-coding sequence consisting of un methylated DNA 200-500 bp. Specific shRNA and miRNA sequences for various proteins of metabolic pathways and key haematopoietic signalling pathways (e.g., TPO, JAK-STAT etc).

Fluorescent proteins GFP, eGFP, emGFP, DsRed, mCherry, tdTomato, BFP, Venus, luciferase
The mammalian cell hosts are non-pathogenic and unlikely to survive outside cell culture. It is recognised that lentivirus vectors can in principle cause insertional mutagenesis in the cells they enter, but the likelihood of a worker infecting themselves and causing a hazard in this manner is extremely low. Primary human lymphocytes, monocytes, stromal cells, adipocytes and leukaemia cells will be screened for HIV at the Norfolk and Norwich Hospital before being brought into the laboratory.

The replication incompetent lentivirus is not a risk as it will not infect further cells. That the lentivirus has a genome split into 3 plasmids means that recombination between packaging helper and vector is very low and the potential for generation of replication competent lentivirus is extremely reduced. The virus envelope protein is from VSV and there is no HIV envelope protein in the system and the minimal required HIV functions are from the helper plasmid that contains no packaging or L TR sequences. There is no chance that it will recombine with HIV from a HIV infected worker.

Fluorescent proteins are non-toxic and have been used in many laboratories over years with no adverse effects. Noncoding DNA fragments to be inserted are not oncogenes. Some of the proteins act as tumour suppressors and their knock-down could therefore pose a potential risk if a worker infected himself/herself. However, cancer formation is a multistep process in humans requiring at least six genetic changes, so it is highly unlikely that an infection would contribute to tumorigenesis. Also, as the lentiviral vectors are replication-defective they would not propagate in an infected worker.

EI tissue culture laboratory is CL2 lab and procedures in place will be followed to dispose of contaminated material. Plasticware will be autoclaved 1210C for 20 minutes, or 30 minutes for dense loads prior to disposal to landfill. Liquids will be autoclaved 1210C for 20 minutes and accidental spills disinfected using minimum 2% Chemgene.

All procedures will be performed in Class II Microbiological Safety Cabinets in a designated CL2 laboratory. Workers are required to wear lab coats and gloves at all times. Needles will not be used. Cultures will be grown in tissue culture flasks (T75 cm2) or dishes (P1 OOs) in DMEM or RPMI tissue culture media containing 10% FBS. The cultures will be grown in designated incubators.

Viral stocks will be routinely tested for the extremely rare event that the 'helper' virus (replication competent) has been generated. This is done by infecting 3T3 cells, collecting supernatants and testing if this supernatant can infect fresh 3T3 cultures.

All stocks will be destroyed by autoclaving should we find helper viruses.

Nitrile gloves will be worn during all experimental procedures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No genetically modified plants and animals will be created.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogation is requested.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid biological waste will be sterilised by autoclaving at 121 °C for 20 minutes, or 30 minutes for dense loads. Liquid biological waste will be sterilised by autoclaving at 121 °C for 20 minutes. Any chemically contaminated biological waste will be sent for incineration by a licenced waste contractor.
Please enter comments on the GM safety committee on the risk assessment

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<tbody>
<tr>
<td>L2 Yes</td>
<td>L3 L4</td>
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Project Ref 3315/20.2

Date Ackn’d 28/02/2020
CU2 Project Title Molecular genetics of plant responses to the environment

Class 2
Culture Vol Class 2 < 1 Litre
Non-GMM Consent Granted

Dates

- Date Project Ceased

- Withdrawn

- Historical Significant Changes

- Significant Date of Additional Info

- Significant Change ID

- Date of Significant Change

Project notified under transitional arrangements N

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The purpose of these experiments is to study the role of plant genes in coordinating plant growth in response to changes in the environment e.g. changes in the availability of nutrients or water.

#### Recipient or parental organism

DNA sequences will be cloned into plasmid vectors in E. coli for experimental purposes. These will be introduced into non-disarmed Agrobacterium spp. and used for gene delivery to plant tissues. The GMO Agrobacteria are not expected to have any new characteristics except resistance to the selective agent encoded on the plasmid vector. The plant GMOs are expected to have resistance to the selective agent, to express a reporter gene such as green fluorescent protein and/or to have altered patterns of gene expression.

#### Host/vector system

Plasmid vectors: disarmed Ti plasmid derivative vectors used to modulate gene expression include pRK290 derivatives, Bin19 derivatives, Bin 400, pMSP1, PTA7002, PZP200, pCAMBIA, pICH, pAGM and pGreen/pSoup derivatives, pAgricola, pHellsgate, and pJawohl series.

E.coli: Strains include disabled strains of K-12 such as DH5a, HB101, JM109, XL-1Blue and commercial derivatives that are unable to reproduce in the natural environment and can only survive in laboratory conditions.

Agrobacterium: non-disarmed Agrobacterium spp. strains including A. rhizogenes LBA9402, 8196, ATCC15834, AR 1193, K599)

Plant species: Solanaceae; Solanum lycopersicon (tomato), S. tuberosum (potato) and Nicotiana spp., Gramineae; Hordeum vulgare (barley), Triticum aestivum (wheat); Brassicaceae; Arabidopsis thaliana (thale cress) and B. oleracea (cabbage); Asteraceae (Calendula spp., Inula Spp, Helianthus spp.).

#### Origin & function

Genes and/or other DNA sequences that express programmable nucleases. For example, constructs based on the CRISPR/Cas9 or Cas12a system will be used to generate mutations in plant genes.

Genes and/or other DNA sequences that express non-coding RNAs that are used to silence the expression of plant genes.

Genes and/or DNA sequences that express proteins involved in plant metabolic processes e.g. assimilation of nutrients, production of metabolites in response to stress.

Promoters: the expression of the transgene(s) can be driven either by its own promoter or by that from another plant species. Chemically inducible promoters (e.g. dexamethasone, glucocorticoid and estradiol) and/or viral promoters (e.g. CaMV, TMV and PVX) and/or bacterial promoters such as pNos and pOcs will also be used. Synthetic promoters comprised of motifs that bind eukaryotic transcriptional factors and/or transcriptional enhancers and repressors.

Reporter genes: fluorescent marker genes such as GFP or luciferase.

Marker genes: antibiotic or herbicide resistance genes such as those for resistance to kanamycin, hygromycin, streptomycin/streptovomycin and/or phosphinothricin.

#### Evaluation of foreseeable effects

GMO Agrobacteria are not expected to have any characteristics except resistance to the antibiotic conferred by the selectable marker gene in the plasmid backbone. All other foreign genetic material will have regulatory elements.
designed to confer expression in plant cells are therefore unlike to confer any new characteristics to the bacterial cells. GMO plants produced using A. rhizogenes are expected to be composite plants with wild-type shoots and transgenic roots. They must be cultured on solid media in the laboratory and clonally propagated. The root tissues are expected to have resistance to the antibiotic or herbicide conferred by the selectable marker gene. The root tissues is also expected to either express the reporter gene (e.g. GFP or luciferase) or to have an altered gene expression or metabolic profile depending on the plant gene that has been expressed or disrupted.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Composite plants with transgenic roots produced using Agrobacterium-mediated delivery will be maintained in the laboratory on solid selective media. They will be clonally propagated and will not be transferred to soil. All plant material and all laboratory consumables (petri dishes, tips), culture vessels and residual culture material will be sterilised and disposed of by autoclaving (blue kill boxes). All work will be performed following the Standard Operating Procedure (Laboratory work for work with Plant Health Licensed Pathogens) associated with the DEFRA licence held for the use non-disarmed Agrobacteria. Metal utensils such as forceps, scalpels and blades are sterilised using a dry bead sterilizer at 250°C or above. Any spills are treated using sodium hypochlorite with 2,500 ppm active chlorine before being cleaned up using paper towels which can then be discarded in biological waste (blue box) for autoclaving. The benches on which manipulations are carried out will be cleaned using Bioguard spray after each lab session. Laboratory coats and gloves are worn during the manipulation of the licensed material. Gloves are disposed of immediately after use or after any accidental contamination. These are autoclaved at 121°C, 15 psi for 20 minutes.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is requested.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid biological waste will be sterilised by autoclaving at 121 °C for 20 minutes, or 30 minutes for dense loads. Liquid biological waste will be sterilised by autoclaving at 121 °C for 20 minutes. Any chemically contaminated biological waste will be sent for incineration by a licenced waste contractor.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

02/03/2022
<table>
<thead>
<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

Large Scale Activities

Human Clinical Applications
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| **(Originally)** | |
| **Transferred from 1992 Regs?** | N |
| **Transitional Premises Class** | |
| **Data Premises Closed** | |
| **Transitional Premises** | N |
| **Emergency Plan Required?** | |
| **Non-GMMs** | N |
| **Withdrawn** | N |

| **Name** | CATALENT UK PACKAGING LIMITED |
| **Name 2** | |
| **Department** | |

| **Campus Estate or Research Centre** | WINGATES INDUSTRIAL ESTATE |
| **Road Name** | LANCASTER WAY |
| **District** | WESTHOUGHTON |
| **Town** | BOLTON |
| **County** | LANCASHIRE |
| **Postcode** | BL5 3XX |
| **Country** | ENGLAND |

| **Tel Number** | 01942 790000 |
| **Fax Number** | 0942 799799 |

| **E-mail** | |
| **HSE Division** | blank |

| **Comments** | |

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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</tbody>
</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Two other Catalent sites are registered, both sites EHS lead head up the genetic modification safety committee

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Transgenic</td>
<td>Microbiology</td>
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<td>Virology</td>
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<td>Gene Therapy</td>
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</tr>
</tbody>
</table>

02/03/2022
Via a registered waste provider, Veolia, limited to returns of finished product from investigator sites

Secondary packaging activities on finished products only

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

It is considered that the activities are of low risk and that the emergency procedures are in place should a spillage occur

Project Ref  3316/19.1

Storage and Distribution at the Catalent Bolton facility

Date Ackn'd  15/03/2019

CU2 Project Title  Storage and Distribution at the Catalent Bolton facility

Date Project Ceased

Class  Class 2

CultureVolClass2  Not Applicable

Consent Granted

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
Purposes of the contained use

Storage and Distribution Only.

Recipient or parental organism

The recipient organism is Adenovirus type 5; the taxonomy of which is detailed below:

- Scientific Name: Adenovirus Type 5 (Ad5)
- Group: Group III, Subgroup C
- Family: Adenoviridae
- Genus: Mastadenovirus
- Species: Human adenovirus
- Strain name: Serotype 5

Host/vector system

The gene transfer vector is a recombinant type 5 adenovirus vector containing the human interferon α2b (IFNα2b) gene.
The vector was generated by recombination between an IFN-α2b-containing plasmid and a derivative of adenovirus type 5.

Origin & function

- Replication deficiency: the final vector is replication deficient, as a consequence of removing the adenovirus E1a and E1b regions.
- Transfer of human IFN-α2b complementary DNA (cDNA): when administered locally to the pleural space, the vector transfects both normal mesothelial and malignant mesothelioma cells, resulting in the production of high and sustained local concentrations of IFN-α2b protein within the pleural space and tumor.

Evaluation of foreseeable effects

Expression is measured for each batch of rAd-IFN clinical material. Expression of IFNα2b is determined using a limit assay which is reported qualitatively (expresses/does not express IFN). The assay is complementary to the infectivity and potency assays.
rAd-IFN has been engineered to be replication-incompetent and is naturally integration deficient. Therefore infection leading to replication of the GMO (and therefore potential for dispersal) is not possible under normal circumstances.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A - Bolton facility is Storage and Distribution only.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A
If a spill occurs on site, the waste will be processed as follows:
Disposable items should be placed in biohazards bags (or appropriated designated containers) and
incinerated (rAd-IFN is sensitive to heat: 1 hours at 56 degrees is used to inactivate the virus, or
autoclaving at 121 degrees for A MINIMUM OF 15 MINUTES). Autoclaving will be carried out by an
approved contractor. Once the GMO has been autoclaved, it will be sent for incineration via an
approved contractor.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  N

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

Storage and Distribution Only.
Recipient sites will assess the risk for their operations/ people/ environment and consult with their own
site GMO Safety Committee.

Project Containment

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<td>26/01/2017</td>
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### Name

KESIOS THERAPEUTICS LIMITED

### Name 2

### Department

### Campus Estate or Research Centre

IMPERIAL COLLEGE INCUBATOR

### Building

BESSEMER BUILDING LEVELS 1&2

### Road Name

IMPERIAL COLLEGE LONDON

### District

SOUTH KENSINGTON CAMPUS

### Town

LONDON

### County

GREATER LONDON

### Postcode

SW7 2AZ

### Country

ENGLAND

### Tel Number

0203 818 9280

### Fax Number

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### E-mail

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### HSE Division

### Comments

Date at Which Additional Info Submitted

02/03/2022
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</tbody>
</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

We have a Memorandum of Understanding that gives us access to advice from the Imperial College Biological Safety Officer who has 16 years relevant experience. We also have access to the ICL South Kensington GM committee (GMB) should we need to submit a proposal for Class 2 GMO's. This is a diverse committee with academic expertise from microbiologists, virologists and plant biologists as well as the ICL BSO.

4.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
Level 1 (GMMs) | Yes
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial
Other (please specify)
Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research
Yes
Liquid wastes will be inactivated using Distel at a minimum of 1% final concentration after dilution with the liquid waste, and will be incubated for at least 30 minutes, however will be left for 24 hours. Distel is known to inactivate mammalian cell lines under these conditions. Additionally the cell lines are unable to survive outside of the laboratory environment. Degree of kill is anticipated to be at least a Log 5 reduction in viable material.

Solid wastes are autoclaved. Solid wastes are then disposed of via Imperial Incubator hazardous waste dumpsters. The autoclave equipment is validated annually using certified, calibrated and traceable equipment to national standards through a UKAS accredited laboratory. The Degree of kill will be at least a log 5 reduction in viable material.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

14th Jan 16 - Hi Sl- My comments for your consideration. Please send the completed and adjusted Bi01 back to Biosafety@imperial.ac.uk-thanks I
Title - The Title should be the title of the project in which the GMO's are being used. The Bi01 is a project registration and is specific to the PI and project.
1.1 A little more detail here. What are the aims and objectives of the project?
2.3 Just a little more information here. Explain why there are fragments of AD5 genome present.
2.13 to 2.18 I thought this was a GM project? Under the GM contained use regs cell lines form all eukaryotic species are considered as being microorganisms. Please complete the GM section. I assume you are transfecting or transducing the cell line with a vector, perhaps a viral vector containing a DNA insert? The recipient cell will be the HEK 293 cells (perhaps also the viral vector depending on how attenuated it is, a wild type viral vector would also be considered as a viable host organism), the vector (includes highly attenuated and replication deficient viral vectors)
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Biological Safety Officer at the Clinical Biomanufacturing Facility, Jenner Institute, Churchill Hospital, Oxford

Tick if confidential

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</table>

Other (please specify)
Virkon is used in accordance with the manufacturer's instructions (i.e. 1% solution). It has been validated as being effective within 10 minutes at this concentration. See www.antechh.com/virkonapps.html.

We routinely decontaminate laboratory consumables by full immersion in 1% Virkon and then leaving to soak for at least 1 hour.

Virkon powder is added directly to liquid waste to a final concentration of 1%.

Decontaminated waste is double bagged into clinical waste bags, these are then transferred into large locked clinical wastebins which are collected from the site by a specialist commercial company (Grundon) for incineration.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 3318/16.1

Date Ackn'd 31/03/2016

Date Project Ceased

CU2 Project Title The use of genetically modified cells and gene delivery vectors

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 L

Non-GMM Consent Granted

Project notified under transitional arrangements N

Tick if notifying a connected programme of work N

Historical Significant Changes
### Purposes of the contained use

For drug development purposes.

### Recipient or parental organism

The cell lines to be used are commercially available genetically modified cell lines e.g. encoding SV40 temperature sensitive elements or those created in house by introduction of genes encoded by viral vectors or in plasmid based systems.

Viral vectors (lentiviral and retroviral vectors) encoding genes of interest will be propagated.

The viral vectors are all attenuated and replication deficient in the absence of accessory genes. The genes encoded in the viral vectors are likely to be reporter genes such as GFP. Other genes which may be encoded have known biological functions which are unlikely to present any human risk.

The inserted gene products are expressed at very low levels, however it is possible that they may cause an immune response in healthy subjects. Immunocompromised individuals may be less likely than normal healthy individuals to mount an immune response to incoming viruses, but as the modified viruses are replication defective, this should not lead to replicative spread of the virus. With respect to the environment, the viruses require a host to replicate; it is very unlikely that the GM strains will be able to form a successful infection.

### Host/vector system

Well characterised, commercially available cell lines and viral vectors e.g. lentiviral vectors such as PKO-1.

### Origin & function

Commercial sources, such as Sigma-aldrich, Thermoscientific.

### Evaluation of foreseeable effects

The risk of infection with plasmids is extremely low. The use of biological safety cabinets and appropriate PPE, negate the risk of this possibility.

In viral vectors packaged via the third generation (3 plasmid) system, the requirement for lentiviral Tat is removed via modification of the LTR, and Rev is supplied in trans in another plasmid. As a result, the third generation system
distributes the HIV genome elements over four rather than three plasmids, further improving safety profiles. Plasmids supplied by commercial companies (e.g. Thermo Scientific, Sigma-Aldrich, etc.) are of a similar format and have been already risk assessed and quality controlled by the companies, further reducing their risk. Cells, plasmids and material used are treated 2% virkon or disposed by incineration through approved disposal routes. The LV particles are self-inactivating (SIN) vectors since they have deletions in the parental U3 region of their long terminal repeat (L TR) which results in the deletion of the 5' and 3' L TR promoters on integration. As such, these vectors will only integrate once. The vector is pseudotyped with an alternative non-native HIV envelope, normally from Vesicular Stomatitis Virus G protein (VSV-G). This expands the host range of the vector but limits infection of CD4+ T cells which are the natural target of HIV-1.

All cells are screened by PCR for HIV and HSV, so the possibility of transfer of any of the sequences to a related virus through homologous recombination is very remote.

Replication competent lentivirus (RCL) is a very small but potential risk - this is tested for by serial infections of HeLa cells with viral stocks, and two rounds of removal of supernatant and transfer to uninfected stocks after 48 hours. The presence of RCL can easily be detected by transgene expression or Q-PCR.

The maximum volume of cultures or culture supernatants to be processed is up to 0.5 litre for medium scale vector production. In this dilute form, viral vector titres are relatively low and hence risk is mostly conferred by the volume of material to which there is a risk of exposure. Unconcentrated virus should be considered to carry mild risk in small quantities, as compared to the ultra-centrifuged high titre virus, which should be considered moderate risk. All work involving the use of viral vectors is only performed by trained personnel who use PPE which includes as a minimum of gloves, lab coat and eye protection and performed in biological safety cabinet in designated category 2 facilities. All staff are shown how to prevent and deal with spillages that may arise e.g. spill granules and virkon solutions and or powder.

Viral vectors are aliquoted and stored in minimal volumes in double sealed tubes to prevent inadvertent spillage. All centrifugation steps are performed in sealed rotors containing sealed tubes in order to prevent the risk of viral vector or transduced cell spillages. All directly-contaminated material is Virkon-treated prior to disposal, and transduced cellular material disposed of by normal GMO routes. The route of infection of HIV is via blood/mucosal exposure, which our SOPs are explicitly designed to prevent (e.g. no sharps during virus production and concentration).

Downstream assays using washed transduced cells should pose no extra risk beyond that inherent in the assays themselves, since the virus is internalised within 24 hours of transduction and no detectable residual virus remains. During virus collection risk is minimised by the availability of liquid Virkon for dousing spills of any size prior to their absorption and safe disposal. Smaller high titre viral spills, e.g. from dropped tips, are dealt with in the same manner, commensurate with risk being affected by both viral titre and volume.

The above measures ensure that the process and procedures are adequately controlled

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Virkon is used in accordance with the manufacturer's instructions (i.e. 1% solution). It has been validated as being effective within 10 minutes at this concentration. See www.antechh.com/virkonapps.html

We routinely decontaminate laboratory consumables by full immersion in 1% Virkon and then leaving to soak for at
least 1 hour. Virkon powder is added directly to liquid waste to a final concentration of 1%. Decontaminated waste is double bagged into clinical waste bags, these are then transferred into large locked clinical wastebins which are collected from the site by a specialist commercial company (Grundon) for incineration.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

Risks are adequately controlled.

Project Containment

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**Name**

MOORFIELD EYE HOSPITAL NHS FOUNDATION TRUST

**Name 2**

**Department**

**Campus Estate or Research Centre**

R&D DEPARTMENT

**Road Name**

162 CITY ROAD

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

EC1V 2PD

**Country**

ENGLAND

**Tel Number**

0207 253 3411

**Fax Number**

0207 608 6925

**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

University College London has an established Genetic Modification Safety Committee (GMSC) which will advice Moorfields Eye Hospital NHSFT on the risk assessments of the use of genetically modified organisms prepared by the Trust.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

Contaminated materials including syringes, vials, swabs and materials used for cleaning will be placed in sharps bins or clinical waste bins designated to be disposed of by incineration. Such waste materials generated by the Trust are sent to external waste contractors for incineration.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

As the adenoassociated viral vectors proposed to be used here do not carry trans gene sequences that are expected to aggravate the risk (oncogenes, toxins etc) these vectors can be handled at biosafety level 1 (BSL-1). Similar vectors have been used in animal studies at UCL (intraocularly and systemically) for a considerable time without raising safety concerns. Previous clinical trials of adenoviral mediated gene therapy in the eye have shown that shedding of vector through the tear fluid does occur rarely and in very small amounts. As most tear fluid is ingested, the likelihood of release of vector into the environment is minimal.

Waste disposal as 'contaminated clinical waste' will be sufficiently rigorous to guarantee inactivation of the GMM and integration of the waste disposal route with an existing system will minimise the chances of breaches occurring.
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Page 13968 of 15326
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Genetic Modification Safety Committee is a subgroup of the Health and Safety Committee which is composed of the two founders (Managing Director (qualified to MSc) and Operations Director (qualified to PhD) of the company. These individuals have over 15 years prior experience working with genetically modified mammalian cell lines in the laboratory with GMP-compliant facilities and have been required to prepare and review a range of related COSHH assessments and GMO risk assessments.

The Genetic Modification Safety Committee is a subgroup of the Health and Safety Committee which is composed of the two founders (Managing Director (qualified to MSc) and Operations Director (qualified to PhD) of the company. These individuals have over 15 years prior experience working with genetically modified mammalian cell lines in the laboratory with GMP-compliant facilities and have been required to prepare and review a range of related COSHH assessments and GMO risk assessments.

The company’s procedures require the HSC to meet on a quarterly basis, along with the HSMC. I meetings can be arranged as and when required. The company has established Health and Safety procedures D... including HSOP004 (Use of Genetically Modified Organisms) which details the company’s approach to managing the risk associated with the contained use of GMOs.
Liquid waste will be inactivated by incubation overnight with 1% Virkon overnight; documented procedures are in place for preparation of the disinfectant with a defined expiry dates. Virkon has been demonstrated to be effective against a wide range of viruses and bacteria and will kill 100% of the agents. Solid waste, including sealed microtiter plates, will be collected by a third party and inactivated using a validated rotoclave/autoclave procedure that will result in 100% kill or inactivation of the agents.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The risk assessment has been approved by the GMSC.
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Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Advice was received from the Chair of a well established GMSC from an independent academic institution. This person has >15 years experience in conducting research involving GM of mammalian cells with apoptosis regulatory genes, similar to the work proposed here.

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Tick if confidential

Yes

Bacteriology | Yes |
Parasitology |     |
Transgenic Birds |    |
Microbiology Research | Yes |
For activities involving GMMs, describe the waste management measures which will apply to the activity:

All materials will be routinely inactivated on site by autoclaving at 121 °C for fifteen minutes, which will be sufficient to obtain 100% kill of micro-organisms. The effectiveness of the procedure will be monitored at monthly intervals by inclusion of steriliser control tubes (Browne tubes; control indicator type 1: 121 °C 15 min) and the results recorded in a log book. The autoclave will be serviced and undergo validation testing by the service engineer on a yearly basis.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

'The procedures described are entirely appropriate for work at Biosafety Level 1 with disabled hosts E.coli K12 derivatives and the mammalian cell lines you mention which all have a history of safe use.'
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**Name**

BIOTANGENTS LIMITED

**Name**

**Department**

**Campus Estate or Research Centre**

INTERNATIONAL RESEARCH CENTRE

**Road Name**

PENTLANDS SCIENCE PARK

**District**

BUSH LOAN

**Town**

PENICUIK

**County**

**Postcode**

EH26 0PZ

**Country**

SCOTLAND

**Tel Number**

0131 514 0871

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**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Advice was received from a Professor of Microbial Biotechnology at the University of Edinburgh, who has considerable experience planning and supervising research, with numerous high-impact publications on bacterial manipulation, and who is a local Biological Safety Officer at the University of Edinburgh. Advice was also received from the Genetic Modification Safety Advisor at the Moredun Research Institute.

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Other (please specify) Tick if confidential

Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research

Yes
Solids (e.g. plastic-ware such as pipettes, flasks, tubes, etc., and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste stream for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

Particular care will be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that involves broken glass) unless they are aware of the potential risks and trained in safe working practices.

If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste stream.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

considerable experience planning and supervising research, with numerous high-impact publications on bacterial manipulation, and who is a local Biological Safety Officer at the University of Edinburgh.
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Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Stevenage Bioscience Catalyst (SBC) is an incubator hub for small and emerging biotechnology start-up and spin-out companies. It is a UK Government backed (Innovate UK) enterprise in collaboration with the Wellcome Trust and GSK. The SBC provides technical, business, organisational and commercial support to its tenant companies and also monitors Health and Safety compliance. It has set up a GMO committee to ensure that tenants are assessing the risks associated with their work appropriately and to disseminate best practice among researchers.

The SBC committee's chairman has reviewed and advised us on our GMO risk assessments.

<table>
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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Bacteriology | Parasitology | Transgenic Birds | Microbiology Research |
Waste Disposal

- Vikon is used as it is a validated disinfectant for transfected cells if used in the following way under manufacturers recommendations for 100% kill rate.
- Hard surfaces: A solution containing 1% Virkon for 1 hour contact time, 10mins for metal parts (longer can cause corrosion)
- Safety cabinets: 1% Virkon (10mins)
- Discard jars, plastic tissue culture flasks, glassware: A solution containing 1% Virkon.
- Ensure all surfaces are in contact with the disinfectant (10mins).
- Supernatants/liquid waste: 3% Virkon diluted 2:1 in culture medium
- All solid waste is going to be incinerated.
- Full bags (properly labelled and closed) are to be transported to the hazardous waste bins in the SBC storage area. Howie type Lab coats, safety glasses and nitrile gloves will be used during the waste handling.
- Bins are collected for off-site incineration by Grundon waste management

Accidental Release:
In our laboratories for class 1 contain use the floor is sealed and the labs are built to CL2 level, all the MSC are Class 2.
In an uncontained area, all persons should evacuate the area to avoid contact with any potential aerosols formed.
Only after 1 hour the room might be entered (to allow any aerosols to settle) and the procedures should be the same as in a contained area.
In a contained area Virkon powder should be applied all over the spill area. The spillage to be mopped up with damp cloths, it must not be brushed. This solid waste is then to be incinerated as described before.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Dr Mahendra Deonarain is the chairman of a 10-member SBC GM committee. Dr Deonarain has 25 years’ experience working with GMOs and has previously served as GM Officer in the Dept of Life Sciences at Imperial College. The GM committee has a wide range of experiences in GMO R&D.
Dr Deonarain and the committee have advised Tusk Therapeutics on evaluating GMO handling and waste disposal risks and are satisfied that they are acting appropriately, responsibly and with the best practice of the SBC. The R&D does not contain anything exceptional that requires special consideration. The SBC GM Committee have approved the associated risk assessment and support this notification.
Purposes of the contained use

Black Belt TX Limited (formerly known as Black Belt Therapeutics Ltd) is developing monoclonal antibody and small molecule inhibitor therapeutics aimed at stimulating the immune system for the treatment of various cancers. A key part of developing such therapeutics is to evaluate their biological activity in cell based bioassays. Non-GMO cell lines can often serve this purpose but sometimes because high levels of the target protein are required or because there is no suitable assay readout, cells must be transformed and genetically modified by means of viral infection or other methods, including CRISPR/Cas9 genome editing, as described below.

When non-viral transfection is suitable for bioassay development, the process will be preferred and carried out at Black Belt TX, utilising Karpas299, SUDHL-1, SR786, Jurkat, MDA-MB-231, SK-MEL, THP-1 and other cell lines, as well as pre-screened (for common blood borne pathogens) primary blood cells, including T cells and myeloid cells, such as monocytes or in vitro-differentiated macrophages and antigen presenting cells.

When viral transduction (infection of a cell with viral particles) is necessary, this can be either carried out at commercial providers, providing virally-infected cells to Black Belt TX (BBTX), or performed in house at BBTX utilising replication-incompetent and non-mobilizable lentiviral vectors, which are sourced from commercial providers as ready-to-transduce particles (e.g. from Horizon Discovery and Cellecta).

A series of different procedures involving GMOs will be carried out. Activities listed below under points 1) and 2) are BSL-1 level, while activities under 3) and 4) involve BSL-2 level.
1) The transfection reagent Lipofectamine 3000 (Life Technologies) is utilized to express the firefly luciferase enzyme under the control of Stat5 activation. This generates a Stat5 reporter assay. The DNA vector/plasmid utilized for that purpose is pGL4.52[luc2P/STAT5 RE/Hygro] from Promega. This vector does not contain oncogenes. After the transfection, a specific antibiotic (hygromycin) is utilized to ensure selection of cells that have been transfected and so express the Stat5 reporter gene (luciferase). The process might occasionally and eventually result in stable incorporation of the plasmid DNA, i.e. The cell line might become stably-transfected with the plasmid and kept in culture for indefinite time without losing the Stat5-reporter functionality. This stably incorporated Stat5-reporter plasmid DNA is non-mobilizable.

2) For some of the virally-transformed cells, the lentivirus vector construction and infection of HEK293 and CHO cells is performed at Vectalys (Toulouse, France). The cell-lines transferred to Black Belt TX will have the vector sequence verified, will not contain oncogenes in the vector and the cell-line multiplicity of infection will be reported. All vectors are non-mobilizable.

3) Lentiviral transduction at BBTX premises will be performed utilizing ready-to-transduce viral preparations sourced from commercial providers (e.g. Horizon Discovery, Cellecta and Flash Therapeutics). The lentiviral vectors are sequence verified by the providers, do not contain known oncogenes, are 2nd or 3rd generation, replication incompetent and non-mobilizable once integrated in the DNA of the infected cells. All work involving lentiviral particles/vectors is dealt with inside a BSL-2 laboratory following the appropriate risk assessments and SOPs, which are in place at BBTX.

4) CRISPR/Cas9-mediated genome editing will be carried out to knock-out specific genes of interest, including GCN2, PERK and other stress-related proteins, which can play some role in cellular energetics & metabolism. Knock-out of these proteins is not associated with cancer development: i.e. they are not known onco-suppressors. However, in some cases screening approaches with pooled gRNA, derived from lentiviral transduction will be carried out. For such screening, it is not possible to predict the biological effect of the knock down of each individual knock down or combination of them, where more than one gRNA can be expressed in a given cell. All screening work is performed in the BSL-2 laboratory. The Cas9 protein is transfected into the cells by either electroporation, liposome-mediated delivery (e.g. lipofectamine reagents from ThermoFisher), utilizing recombinant Cas9 protein or mRNA encoding the protein, or by employing genome non-incorporating LentiFlash Technology (from Flash Therapeutics). Moreover, the cell delivery of gRNA and Cas9 expression is temporally separated by at least 24h, with cell culture replacement in between of the two steps. Hence, there is no risk for recombination between different vectors and there is low and contained risk to human health and the environment.

Recipient or parental organism

- Chinese hamster ovary cells (CHO)
- Human embryonic kidney cells (HEK293)
- Human lymphoma cell lines (KARPAS 299, SU-DHL-1 and SR-786)
- Human breast cancer cell line (MDA-MB-231)
- Human melanoma cell line (SK-MEL)
- T-cell leukemia cell line (Jurkat)
- Acute monocytic leukemia cell line (THP-1)
- Primary immune cells derived from healthy donor blood (T cells, monocytes)
- In vitro differentiated macrophages and antigen presenting cells (derived from blood immune cells)

Host/vector system

1) Helper plasmid#1: pHIVgagpol: human Retrovirus derived from HIV.
   This plasmid carries the structure genes (gag) and functional genes (pol), but not the envelope gene.
   This plasmid carries the gag and pol genes under the control of CMV promoter/enhancer, allowing their constitutive expression in cells transfected by this plasmid. It is non-coding for the accessory proteins Vif, Vpr, Vpu and Nef.

2) Helper plasmid#2: pEnv:
   This plasmid carries the sequence allowing the expression of the VSV (Vesicular Stomatitis Virus) envelope G-glycoprotein, VSV-G. Only the sequence of the G protein of serotype Indiana (NCBI accession number: AM_690336.1) is manipulated in the laboratory.

02/03/2022
This plasmid carries the VSV-G gene under the control of CMV promoter/enhancer, allowing its constitutive and strong expression in cells transfected by this plasmid.

3) Expression plasmid pLV-EF1-hCD137
This plasmid is a Self Inactivating (SIN) HIV derived lentiviral vector, containing a Multi-Cloning Site to insert cDNA
Δ [U3]: SIN (self inactivating) vector: Self-inactivating (SIN) vectors lacks viral enhancers/promoters in their 3’ long terminal repeat (LTR). The deletion of the U3 region in the 3’ LTR allows:
- decrease the number of HIV-1 internal viral sequences
- LTR inactivation
- Prevent the CIS activation of genes closed to the integration site

4) PLASMID pGL4.52[luc2P/STAT5RE/Hygro] Vector
The pGL4.52[ luc2P /STAT5 RE/Hygro] Vector contains five copies of a STAT5 response element (STAT5 RE) that drives transcription of the luciferase reporter gene luc2P (Photinus pyralis). luc2P is a synthetically derived luciferase sequence with humanized codon optimization that is designed for high expression and reduced anomalous transcription. The luc2P gene contains hPEST, a protein destabilization sequence, which allows luc2P protein levels to respond more quickly than those of luc2 to induction of transcription. The vector backbone contains an ampicillin resistance gene to allow selection in E. coli and a gene for hygromycin resistance to allow selection of stably transfected mammalian cell lines.

5) Vector Edit-R lentiviral sgRNA from Horizon Discovery.
The vector backbone contains a gRNA sequence under the control of the U6 promoter and a Puromycin resistance gene under the control of the mCMV promoter to allow for selection of stably transduced mammalian cell lines.

6) Vector pRSGUP-U6-(sg)-UbiC-Puro from Cellecta
https://www.cellecta.com/resources/vector-information/#sgRNA2
The vector backbone contains gRNA sequence under the control of the U6 promoter and a Puromycin resistance gene under the control of the UbiC promoter to allow selection of stably transduced mammalian cell lines.

7) Vector pRSEGP-U6-(sg)-EF1-Puro from Cellecta
https://www.cellecta.com/resources/vector-information/#sgRNA2
The vector backbone contains a gRNA sequence under the control of the U6 promoter and a Puromycin resistance gene under the control of the EF1 promoter to allow selection of stably transduced mammalian cell lines. In other similar version of this vector, reporter genes such as GFP or mCherry are integrated and co-expressed with the antibiotic resistance gene.

8) pRSG21-U6-(sg)-CMV-TagGFP2-2A-Puro from Cellecta.
https://www.cellecta.com/resources/vector-information/#sgRNA2
The vector backbone contains a gRNA sequence under the control of the U6 promoter and a Puromycin resistance gene under the control of the CMV promoter jointly with the GFP2 reporter gene, allowing selection of stably transduced mammalian cell lines, while also monitoring efficiency of transduction. In other similar version of this vector, a different reporter gene is used, such as RFP.

9) The CRISPR/Cas9- dependent genome editing of cells in the absence of viral vectors
In some instances, genome editing is performed without contribution from DNA plasmids or viral vectors. Transfection can be carried out with the recombinant Cas9 protein and synthetic guide RNA directly incorporated into the Lipofectamine CRISPRMAX Cas9 reagent from ThermoFisher, which is subsequently fused with cell membranes, releasing protein and gRNA inside the cells. (https://www.thermofisher.com/order/catalog/product/CMAX00015). Alternatively, the NEON electroporation system is used.

02/03/2022
10) The CRISPR/Cas9-dependent genome editing of cells using genome non-incorporating lentiFlash particles from Flash Therapeutics (Vectalys)

In this case, genome editing is performed utilizing a transducing lentiviral particle, where all viral genes have been removed, rendering the vector incapable of genome integration. The particles are rather designed for direct delivery of mRNA into the target cell, allowing exogenous and transient over-expression of proteins, such as the Cas9 for the purpose of genome editing, when combined with appropriate gRNA.

https://www.vectalys.com/our-lentiviral-platform/for-research-project/premade-lentiflash-particles/

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**GENE INSERTION:**

**CD38:** also known as cyclic ADP ribose hydrolase is a glycoprotein found on the surface of many immune cells (white blood cells), including CD4+, CD8+, B lymphocytes and natural killer cells.

**CD137:** is a member of the tumor necrosis factor (TNF) receptor family. Its alternative names are tumor necrosis factor receptor superfamily member 9 (TNFRSF9), 4-1BB and cell surface expression is induced by lymphocyte activation.

**CR-TAM:** CRTAM, (class-I MHC-restricted T-cell associated molecule), is homodimer protein belonging to Ig superfamily. It is expressed transiently on the surface of activated NK cells, NKT cells and a subset of CD8+ T cells. Nectin-like molecule-2 (Nect2, TSCL1) is the ligand of CRTAM. CRTAM plays an important role in cell adhesion and migration.

**Firefly Luciferase:** Firefly luciferase is a euglobulin protein that catalyses the oxygenation of luciferin using ATP and molecular oxygen to yield oxyluciferin, a highly unstable, singlet-excited compound that emits light upon relaxation to its ground state.

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**GENE KNOCK-OUT:**

**GCN2:** Metabolic-stress sensing protein kinase that phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (eIF-2-alpha/EIF2S1) on 'Ser-52' in response to low amino acid availability (PubMed:25329545). Plays a role as an activator of the integrated stress response (ISR) required for adaptation to amino acid starvation. Converts phosphorylated eIF-2-alpha/EIF2S1 either to a competitive inhibitor of the translation initiation factor eIF-2B, leading to a global protein synthesis repression, and thus to a reduced overall utilization of amino acids, or to a translational initiation activation of specific mRNAs, such as the transcriptional activator ATF4, and hence allowing ATF4-mediated reprogramming of amino acid biosynthetic gene expression to alleviate nutrient depletion.

**PERK:** Metabolic-stress sensing protein kinase that phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (eIF-2-alpha/EIF2S1) on 'Ser-52' during the unfolded protein response (UPR) and in response to low amino acid availability. PERK converts phosphorylated eIF-2-alpha/EIF2S1 either in a global protein synthesis inhibitor, leading to a reduced overall utilization of amino acids, or to a translation initiation activator of specific mRNAs, such as the transcriptional activator ATF4, and hence allowing ATF4-mediated reprogramming of amino acid biosynthetic gene expression to alleviate nutrient depletion. Serves as a critical effector of unfolded protein response (UPR)-induced G1 growth arrest due to the loss of cyclin-D1 (CCND1). Involved in control of mitochondrial morphology and function.

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**POOLED SCREENING and TARGET ID:** For the purpose of new target identification and validation, screening with pooled lentiviral vectors will be carried out in primary human cells, including T cells isolated from PBMCs or established cancer cells, such as Jurkat cells. Targets might be unknown in function and one given cell can be infected with one or more vectors, carrying different gRNAs. In such screening approach, it is not possible to predict the specific biological effect of the knock down for every single target or for a combination of them, when more than one infection per cell happens. All work involving lentiviral vectors is carried out in the BSL-2 laboratory. All vectors are 2nd or 3rd generation, sequence verified by the commercial provider (e.g. Horizon, Cellecta and Flash Therapeutics), engineered to be replication-incompetent and non-mobilizable once integrated in the DNA of the host cell, minimizing risk for human health and the environment.
The most hazardous procedure is the lentiviral transduction of human cells, utilising ready-to-transduce viral particles (from commercial providers). These engineered lentiviruses are classified as Risk Group 2 by the World Health Organization (WHO) and Biosafety Level 2 (BL2) or Enhanced BL2 (depending on the circumstances) by the U.S. Centers for Disease Control (CDC) Office of Health and Safety. Vector hazard includes tropism towards human cells, possibility of transducing his/her cells in the event of being in contact with wounds from the researcher. All vectors are 2nd or 3rd generation, sequence-verified, engineered as replication-incompetent and non-mobilizable once integrated in the DNA of the host cell. All work is performed in BSL-2 laboratory. Hence, the risk for human health and the environment is contained and low. Apart from the above, the rest of the work is Class 1 thus posing little or no risk.

BSL-1 GMO will comprise of:
A) Karpas299, SUDHL-1, SR786 and Jurkat cells transfected with pGL4.52[ luc2P /STAT5 RE/Hygro] Vector from Promega. The genes encoded in the plasmid are luciferase and hygromycin resistance gene, which are not oncogenes. The reporter gene is Luciferase. The plasmid is non-mobilizable.
B) CHO or HEK293 cells transfected with three plasmids: helper plasmid 1 comprising pHIV-gagpol, helper plasmid 2 comprising pVSVG and expression plasmid comprising pLV-EF1-Black Belt selected gene. The current list of selected genes is CD38, CD137 and CR-TAM. These are not oncogenes. All plasmids are non-mobilizable.
C) MDA-MB-231, SK-MEL, Jurkat, THP-1, Primary blood cells, including T cells, myeloid cells (monocytes, in-vitro differentiated macrophages and antigen presenting cells) transiently transfected with Cas9 recombinant protein and synthetic guide RNA (gRNA) to selectively knock-out genes of interests, which include PERK and GCN2. Knock-out of these genes is not known to cause cancer. Notably, no plasmid or viral vectors are utilised for this procedure. Either Lipofectamine CRISPRMAX Cas9 or NEON electroporation system from ThermoFisher is utilized to incorporate gRNA and Cas9 into the cells.

BSL-2 GMO will comprise of:
D) Primary cells, including Pan T cells isolated from blood PBMCs of healthy donors, which are pre-screened/ free of common blood borne pathogens (blood sourced via NHSBT or commercial providers), and established, commercially available cancer cells, such as Jurkat cells are transduced with vectors expressing gRNA. These viral preparations are sourced from commercial providers as ready-to-transduce particles. The work includes vector backbones such as the edit R Lentiviral sgRNA from Horizon Discovery (https://dharmacon.horizondiscovery.com/gene-editing/crispr-cas9/crispr-controls/edit-r-lentiviral-sgma-pooled-screening-library/) and vectors pRSGU6-6g-Ubic-Puro and Vector pRSGEP-U6-(sg)-EF1-Puro from Cellecta (https://www.cellecta.com/resources/vector-information/#sgRNA2). Alternative, similar vectors from these or other validated providers might be utilized. The vectors might further include reporter genes, such as GFP, mCherry or turboRFP, which are all established, non-oncogenic reporter genes utilized to monitor the efficiency of cell transduction. In all cases, the vectors are either 2nd or 3rd generation lentiviral, sequence-verified, replication-incompetent and non-mobilizable once integrated in the DNA. All work is carried out inside the BSL-2 laboratory at BBTX, following appropriate risk assessment and SOPs.

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Disposal:
- Presept tablets (from Johnson & Johnson) are used as it is a validated desinfectant for virally-transduced cells if used under manufacturers recommendations for 100% kill rate.
- Safety cabinets: cleaned with tissue soaked in Chemgene laboratory disinfectant, disposed of in bio-hazard waste bins.
- Plastic jars are used to disinfect both solid and liquid waste overnight.
The tissue culture plasticware are soaked in Presept solution overnight, ensuring that all surfaces are in contact with the disinfectant. Solid waste is then double bagged.
into bio-hazard yellow bins or lid-secured plastic yellow bins. All GMO LvL-2 solid waste is autoclaved before being transported to the hazardous waste bins of the SBC area. Autoclave cycle: minimum of 121°C, 1.15bar, 15 mins hold. 100% kill rate validation of BSL LvL2 waste in the autoclave is validated by Annual 12 point thermocouple testing of autoclave + routine/each run confirmation of 100% kill rate. Routine confirmation of kill rate either by built-in thermocouple recorder or by use of commercially available autoclave indicator (e.g., Browne TST) in each load, jointly with log book records. Full bags (properly labelled and closed) are transported to the hazardous waste bins in the SBC storage area. Howie type Lab coats, safety glasses and nitrile gloves will be used during all phases of the waste handling.

- Bins are collected for off-site incineration by Grundon waste management.

Accidental Release:
All our laboratories for contained use have sealed floor and working surfaces. The labs are built to CL2 level and all Biosafety cabinets are Class 2. In an uncontained area, all persons should evacuate the area to avoid contact with any potential aerosols formed. Only after 1 hour the room might be entered (after aerosols have settled) and the procedures should be the same as in a contained area.

In a contained area, Specific Biological spill kits are applied all over the spill area. The spillage is to be mopped up with damp cloths, it must not be brushed. This solid waste is then pre-soaked overnight in Presept solution and disposed as per above procedure (under waste Disposal measures).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

D is the chairman of a 10-member SBC GMO committee. D has 25 years’ experience working with GMOs and has previously served as GM Officer in the Dept of Life Sciences at Imperial College. The GMO committee has a wide range of experiences in GMO R&D. D and the committee have advised Black Belt TX on evaluating GMO handling and waste disposal risks and are satisfied that they are acting appropriately, responsibly and with the best practice of the SBC. The R&D does not contain anything exceptional that requires special consideration. The SBC GMO Committee have approved the associated and updated risk assessment on 15th July, 2019 and support this notification.

**Project Containment**

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**Name**
CAMBRIDGE CONSULTANTS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**
SCIENCE PARK

**Road Name**
MILTON ROAD

**District**

**Town**
CAMBRIDGE

**County**
CAMBRIDGESHIRE

**Postcode**
CB4 0DW

**Country**
ENGLAND

**Tel Number**
01223420024

**Fax Number**
n/a

**E-mail**

**HSE Division**
EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Biology SHE oversight committee
Role is to ensure Cambridge Consultants meets all necessary safety and legal requirements to commission and operate the microbiology lab, to provide expert advice to the commissioning team where necessary, to perform training for new staff where necessary; responsibilities include creating and supporting the commissioning and maintenance team, specifying tasks to be performed and documents to be created to commission and run the lab, review and sign-off of documents (particularly Risk assessments (PUWER, CoSHH, GMO), protocols and SOPs, equipment IQ/OQ/PQ), ensuring staff working in the lab are trained and competent to work there, and on-going oversight of lab use and safety

Members of the SHE oversight committee
1. CHAIR - Head of Quality Assurance (QA)
2. SHE Officer (20+ years experience in Health & Safety, including 8 years in biotechnology laboratory setting)
3. Analytical Lab Manager
4. Biological Safety Officer (PhD level and postdoctoral work, in total 9 years experience in microbiology / molecular biology laboratory); undertaking ISTR-accredited course (Biosafety Practitioner Level 1) starting 20 April, and
5. Technology Director

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Yes

To minimise the risk of escape of GMMs to the environment, all contaminated waste streams generated through the course of this work will be disinfected within the laboratory premises covered by this notification.

All waste suitable for the local authority refuse collection, except recyclable paper and glass, will be placed in clearly marked waste bins ('Domestic-type waste') and these will be made available for collection by the cleaners. No cleaner staff will be given access to the laboratory. Recyclable (non-contaminated) paper and cardboard will be collected separately. Broken glass bins will be available in the laboratory (NB. distinct from ‘Sharps’ bins).

Items which are not allowed to be put in the domestic-type waste bins is regarded as ‘controlled waste’ and must be disposed of as follows:

- Glassware that has been in contact with cells must be subjected to disinfection by exposure to suitable disinfectant (e.g. Virkon® S or similar products) according to manufacturer’s instructions (e.g. 1% solution for at least 2 hours); glassware must be completely filled and swabbed on the outside, then thoroughly rinsed in water before washing in washing machine.
- Contaminated cultures and used media must be disinfected by exposure to suitable disinfectant (e.g. Virkon® or similar products) according to manufacturer’s instructions (e.g. 1% solution for at least 2 hours); once disinfected the liquid will be disposed of in accordance with Cambridge Consultants internal policies (either down the drain or via waste management providers); disposable solids must be disposed of in an appropriate manner (see below).
- Broken glassware and sharps must be disinfected before being discarded in the requisite Broken Glass or Sharps bins as appropriate.

The aim of the project is to design and construct new synthetic biology parts.

For activities involving GMMs, describe the waste management measures which will apply to the activity.
Plasticware that has been in contact with cells must be disinfected before placed in double autoclavable bags.

- Waste for autoclaving must be put in double autoclavable bags, sealed with autoclave tape, autoclaved as per manufacturer’s instructions and validated for full decontamination (autoclave tape turned black) before placing in black bag for disposal as domestic waste.
- Sharps (including all needles and glass Pasteur pipettes) must be placed in a regulation standard Sharps bin (orange-lidded yellow sharps box for incineration).
- All waste removal will be conducted by waste management providers as appointed by Cambridge Consultants Building Services.


- Any small or moderate (< 100 ml) spills on surfaces outside the safety cabinet must be dealt with immediately, and other workers in the laboratory must be made aware of the spill. Disinfectant must be kept at hand for these types of event. If contaminated, any PPE (Personal Protective Equipment) such as lab coats, gloves or safety spectacles must be removed, disinfected and autoclaved. The spill must be wiped with paper towels after disinfection for at least 30 minutes, paper towels are then disposed of in biohazard bin. After disinfection, sharps or broken glass must be picked up with forceps/tweezers and disposed of in sharps or broken glass bins.
- Any reusable item such as dustpan and brush that may be exposed to biological materials must be autoclaved after use; floor surfaces must be mopped after disinfection if exposed to a spill.
- Small spills (< 100 ml) inside the safety cabinet are dealt with by leaving the biosafety cabinet running and wiping equipment and the interior surfaces with an appropriate disinfectant. If the spill has gone into the interior of the cabinet (e.g. through grilles or similar), the cabinet must be disassembled as much as possible and decontaminated. The cabinet must be left running for at least 10 minutes after clean-up.
- Major spills (> 100 ml) inside or outside the safety cabinet must be dealt with by closing laboratory doors and evacuating the room, leaving the room empty for at least 30 minutes to let aerosols settle. The laboratory must then undergo clean-up procedure as for the small or moderate spills.
- All major spills must be recorded in the Laboratory Accident Log Book.

In the event of a spill leading to an employee being exposed to a hazard (e.g. face or eye splash, cut or puncture with sharps, exposure of non-intact skin), the following emergency protocol will be followed, depending on the nature of the accident:
- Remove gloves, encourage needle sticks and cuts to bleed, wash with soap and water for 15 minutes (no scrubbing)
- Flush eyes at eyewash station with clean water for 15 minutes; remove contact lenses if present
- Flush nose, mouth or skin with water for 15 minutes
- For all injuries and biological exposures, seek medical attention if employee has been exposed to synthetic nucleic acid molecules

Incident report forms must be completed following adverse events and kept with the Laboratory Accident Log Book. All biohazard exposures must be reported to the SHE Officer.

The aim of the project is to design and construct new synthetic biology parts and systems and to re-design existing genetic parts and systems for specific purposes. This will be done by assembling synthetic DNA for insertion into bacterial cells. The constructs will be grown at small scale (less than 10 litres). Taken together, the potential hazards of the GMM, the inserted genetic material and the vector will yield a system which is highly unlikely to cause harm to human health or the environment.
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**Comments**

**Date at Which Additional Info Submitted**

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**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The company internal safety committee encompasses the genetic modification safety committee and has reviewed the projects and risk assessments. The committee was set up and held its first meeting on 29th April 2016. The risk assessments were discussed and the planned genetic modification work was assessed to be class 1 by all members of the committee.

The members of the Safety committee includes the company chief scientific officer and 2 individuals who have over 10 years experience each in working with and reviewing genetic modification projects.

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste will be inactivated with Distel disinfectant for at least 8 hours prior to sink disposal. Solid waste (plasticware) will be rinsed with Distel prior to collection by a specified waste disposal contractor for incineration. The contractor is HSE licensed as a waste disposal contractor for GM waste.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The safety committee requested further information on Distel disinfectant to be added to the risk assessments, as well as staff training logs. Both these items were added to the risk assessments and the safety committee approved the work as GM class 1.

Project Ref 3326/18.1

Date Ackn'd 01/08/2018

CU2 Project Title Generation of a subgenomic Respiratory Syncytial Virus (RSV) reporter system

Class 2

Culture Volume < 1 Litre

Consent Granted Not Applicable

Project notified under transitional arrangements N
**Project Additional Information**

**Purposes of the contained use**

To establish a subgenomic reporter system for RSV in which small molecule inhibitors of RSV can be tested and the effect of amino acids variations in the RSV genes in this system can be assessed.

**Recipient or parental organism**

Hep2 cells or BHK cells. Both are laboratory-adapted cell lines that have demonstrated safe use in GM projects.

**Host/vector system**

pcDNA3 or pBR322 based plasmid vectors which are considered nonmobilisable containing single RSV genes or a luciferase reporter gene flanked by RSV leader and trailer regulatory elements. Vaccinia virus modified to express bacteriophage T7 RNA polymerase, strain vTF-7 purchased from ATCC (VR-2153)

**Origin & function**

RSV genes P protein, L polymerase, N-protein, and M2-1 form the RSV ribonucleoprotein replication complex. Infectious RSV will not be produced. Firefly luciferase is a benign reporter gene that will be transcribed by the RSV replication complex. T7 RNA polymerase from bacteriophage provided by vaccina T7 drives the expression of the RSV genes to form the replication complex

**Evaluation of foreseeable effects**

The vectors used in this project individually express 4 proteins from RSV that form the RSV replication complex. No other RSV proteins, nor a replication competent RSV genome is expressed and therefore infectious RSV will not be produced. Mutations made in the individual RSV proteins are not expected to change the properties. The luciferase reporter will only be expressed if T7 RNA polymerase is present and the RSV replication complex is formed and therefore poses a minimal concern to the environment, this reporter construct contains RSV regulatory elements but is not replication competent and infectious RSV will
Vaccinia stably accepts large foreign sequences into its genome so it is a very popular tool in immunology and vaccine research and is a well-established and studied vector.

Vaccinia virus produced from a Vaccinia vector with the T7 polymerase gene insert is not likely to be any more or less infectious than a wild type Vaccinia virus.

Vaccinia virus is listed in the ACDP classification as a group 2 pathogen. Vaccinia virus was historically used as a live-virus vaccination strain against smallpox in humans and does not demonstrate any major health effects. Accidental inoculation with vaccinia can occur if the virus interacts with broken skin, or penetrates the dermis via a needlestick injury. Typical inoculation with vaccinia virus may result in skin lesions and scabbing, though serious complications such as ocular vaccinia, myopericarditis, and eczema vaccinatum have been reported in extremely rare cases.

Infectious Vaccina T7 will be handled at containment level 2 at all times. All manipulation of infectious material will be performed within a class II microbiological safety cabinet (MSC) to reduce the risk of exposure. Centrifuge bucket lids will be used, with lids only removed inside the MSC following centrifugation.

Volumes of vaccinia virus stock (titre not more than 10E7) will be kept under 30ml with dilute (titre <10E3) culture volumes kept below 200mL.

The Laboratory has controlled entry access to authorised individuals only. Any transport of virus stocks between laboratories will be in leak-proof locklid boxes. Anyone with known immunodeficiency or other predisposing medical conditions, including exfoliative skin conditions and pregnant staff will not be permitted to work with pathogenic viruses. The use of sharps is prohibited within the laboratory. Lab coats and disposable nitrile gloves will be worn at all times and hands will be washed after the work activity is completed and prior to leaving the lab. Safety spectacles will also be worn when handling vaccinia virus to reduce the risk of ocular exposure.

All work will be undertaken in a Class II MSC to minimise the risk of release to the environment. Some isolates of Vaccinia are stable on surfaces when dried, but are susceptible to disinfection by detergents and are heat-labile at 60°C. All surfaces will be cleaned with 1% Distel before and after use to minimise the risk of vaccinia survival on fomite surfaces, liquid waste will be disinfected with 1% Distel and solid waste will be disinfected with 1% Distel prior to autoclaving at 121°C, 1.15 bar with 15 mins hold time prior to disposal by incineration as described in waste management section below.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
1% Distel will be used for disinfection of liquid waste, following the manufacturer’s instructions and left to decontaminate overnight for a minimum period of 16 hours prior to laboratory sink disposal with large quantities of water. Distel (formerly TriGene) – is a high-level surface disinfectant that destroys RNA and DNA, and is tested and approved as bactericidal, fungicidal, virucidal and tuberculocidal. Hard surfaces such as the Microbiological Safety Cabinet will be disinfected with 1% Distel. Solid waste/plasticware including pipette tips will be submerged in 1% Distel and left to decontaminate overnight for a minimum period of 16 hours. Subsequently, solid waste will be autoclaved on a minimum of 121°C, 1.15 bar 15 minutes hold cycle which is expected to be sufficient for 100% kill. An indicator strip will be included in every autoclave run to ensure temperature and hold time is achieved. The autoclave is serviced annually and there is a back-up autoclave available if required. All autoclaved waste is transferred to hazardous waste bags and placed in hazardous waste bins in the SBC storage area. Bins are collected for off site incineration by an authorised waste management contractor (Grundons waste management).

Any spillages in MSC are decontaminated with 1% Distel, overnight if required. Trolleys and sealed packaging are used to minimise any spillage during any transportation e.g. of waste to the autoclave. Solid waste will be decontaminated by soaking in 1% Distel overnight prior to autoclaving and disposal by incineration, while liquid waste is decontaminated with Distel solution overnight, prior to disposal in the appropriate lab sink with large quantities of water. If a spillage occurs in a sealed centrifuge bucket, sealed buckets will be left to stand for 30 mins to allow aerosols to settle, and then opened in the MSC. All the contaminated components will be decontaminated with 1% Distel-soaked paper towel to remove worst of contamination, followed by a 30-minute soak in 70% ethanol. All solid waste, tissues, gloves etc., from the clean up will be autoclaved prior to waste disposal by incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The Stevenage Bioscience Catalyst (SBC) is an incubator hub for small and emerging biotechnology start-up and spin-out companies. SBC provides technical, business, organisational and commercial support to its tenant companies and also monitors Health and Safety compliance. It has set up a >10-member GMO committee from tenant companies to ensure that tenants are assessing the risks associated with their work appropriately and to disseminate best practice among researchers. The chairman of this committee and has 25 years experience working with GMO in academic and commercial settings and was Chairman of Imperial College Life Sciences GMO committee for 4 years. The SBC committee has reviewed and advised ReViral on its GMO risk assessment and are satisfied that they have considered the risks in terms of GM organisms and scale of work. They have appropriate control measures to contain the work and appropriate procedures for the class of GMO activity for waste disposal.

In addition, the ReViral internal safety committee encompasses the genetic modification safety committee and has reviewed this projects and risk assessment. The members of the Safety committee includes the company chief scientific officer and 2 individuals who each have over 10 years experience in working with and reviewing genetic modification projects. The ReViral safety committee agreed that the control measures and waste disposal are appropriate for the class and scale of the planned GMO activity.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
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**Project Ref** 3326/19.1

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Project notified under transitional arrangements N
### Project Additional Information

#### Purposes of the contained use

To establish a reverse system for RSV in which small molecule inhibitors of RSV can be tested and the effect of amino acids variations in the RSV genes in this system can be assessed.

#### Recipient or parental organism

| Lab adapted cell lines: Hep-2 and BSR-T7/5 cell line (BHK-21 cells that stably expressing phage T7 RNA polymerase under control of the cytomegalovirus promoter, pSC6-T7-NEO (Buchholz et al J Virol 1999. 73:251-259). | Differentiated airway epithelial cultures derived from healthy primary human cells. |

#### Host/vector system

| pcDNA3 based plasmid vectors which are considered non-mobilisable containing single RSV genes. | The RSV antigenome construct, pSynk-RSV119F is a BAC Vector which are F-factor-based plasmids which are conjugated-deficient. |

#### Origin & function

| RSV genome consisting of genes encoding the following RSV proteins: P protein, L polymerase, N-protein, M2, NS1, NS2, M, SH, G and F protein. mKate2 a benign reporter gene. T7 RNA polymerase from bacteriophage within BSR-T7 cells drives the expression of the RSV genes |  |

#### Evaluation of foreseeable effects

| When co-transfected into BSR-T7 cells along with the helper plasmids, the RSV antigenomic construct will produce infectious, replication competent RSV. |  |
RSV is listed in the ACDP classification as a group 2 pathogen. RSV is normally a mild and self-limiting infection without long term sequelae. Infectious RSV will be handled at containment level 2 at all times. All manipulation of infectious material will be performed within a class II microbiological safety cabinet (MSC) to reduce the risk of exposure. Centrifuge bucket lids will be used, with lids only removed inside the MSC following centrifugation. Volumes of RSV stock (titre not more than 10E7) will be kept under 60ml with dilute (titre <10E3) culture volumes kept below 200mL. The Laboratory has controlled entry access to authorised individuals only. Any transport of virus stocks between laboratories will be in leak-proof lock lid boxes. Anyone with known immunodeficiency or other possible predisposing medical conditions will not be permitted to work with RSV. The use of sharps is prohibited within the laboratory. Lab coats and disposable nitrile gloves will be worn at all times and hands will be washed after the work activity is completed and prior to leaving the lab. All work will be undertaken in a Class II MSC to minimise the risk of release to the environment. RSV is susceptible to disinfection by detergents and is heat-labile at 60°C. All surfaces will be cleaned with 1% Distel before and after use to minimise the risk of RSV survival on fomite surfaces, liquid waste will be disinfected with 1% Distel and solid waste will be disinfected with 1% Distel prior to autoclaving at 121°C, 1.15 bar with 15 mins hold time prior to disposal by incineration.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1% Distel will be used for disinfection of liquid waste, following the manufacturer’s instructions and left to decontaminate overnight for a minimum period of 16 hours prior to laboratory sink disposal with large quantities of water. Distel (formerly TriGene) – is a high-level surface disinfectant that destroys RNA and DNA, and is tested and approved as bactericidal, fungicidal, virucidal and tuberculocidal. Hard surfaces such as the Microbiological Safety Cabinet will be disinfected with 1% Distel. Solid waste/plasticware including pipette tips will be submerged in 1% Distel and left to decontaminate overnight for a minimum period of 16 hours. Subsequently, solid waste will be autoclaved on a minimum of 121°C, 1.15 bar 15 minutes hold cycle which is expected to be sufficient for 100% kill. An indicator strip will be included in every autoclave run to ensure temperature and hold time is achieved. The autoclave is serviced annually and there is a back-up autoclave available if required. All autoclaved waste is transferred to hazardous waste bags and placed in hazardous waste bins in the SBC storage area. Bins are collected for off site incineration by an authorised waste management contractor (Grundons waste management). Any spillages in MSC are decontaminated with 1% Distel, overnight if required. Trolleys and sealed
packaging are used to minimise any spillage during any transportation e.g of waste to the autoclave. Solid waste will be decontaminated by soaking in 1% Distel overnight prior to autoclaving and disposal by incineration, while liquid waste is decontaminated with Distel solution overnight, prior to disposal in the appropriate lab sink with large quantities of water. If a spillage occurs in a sealed centrifuge bucket, sealed buckets will be left to stand for 30 mins to allow aerosols to settle, and then opened in the MSC. All the contaminated components will be decontaminated with 1% Distel-soaked paper towel to remove worst of contamination, followed by a 30-minute soak in 70% ethanol. All solid waste, tissues, gloves etc., from the clean up will be autoclaved prior to waste disposal by incineration.

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

### Please enter comments on the GM safety committee on the risk assessment

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The responsible person has worked in and headed groups that worked with similar types of cell lines in industry and academia previously.

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**Other(s)**

transfected mammalian cell lines for the production of antibodies and antigens for use in research

For activities involving GMMs, describe the waste management measures which will apply to the activity

NA

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All waste items (pipette tips, containers, flasks etc.) produced in the use of the GMM materials will be treated with Virkon to a final concentration of 10% and left overnight before final disposal. Solid materials will be placed in clinical waste bins post treatment with Virkon.
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Name

CITY OF LONDON SCHOOL

Campus Estate or Research Centre

Road Name

QUEEN VICTORIA STREET

Town

LONDON

County

GREATER LONDON

Postcode

EC4V 3AL

Country

ENGLAND

Tel Number

0207 4890291

Fax Number

0207 3296887

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Yes

Give brief details of the genetic modification safety committee:
Senior Lecturer - Faculty of Natural Sciences - Department of Life Sciences - Imperial College, London
Works in the Centre for Synthetic Biology and Innovation and is a Principal Investigator for the Imperial College's iGEM team; Course leader for Biotechnology Applications of Proteins Course; Extensive research and teaching experience in the field of Synthetic Biology and Genetic Modifications

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</table>
Both liquid and solid waste will be autoclaved with 100% expected degree of kill. Our autoclaves are examined and validated annually. Virkon disinfectant will be used to deal with any spills in the laboratory.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The organisms employed are all ACDP Hazard Group 1 and not harmful to humans. The E coli strains for plasmid construction and maintenance are non-pathogenic laboratory strains. Synechocystis is not an infectious agent. The proposed genetic modifications will not change the infectivity of the microorganisms or allow them to persist in the environment. Although the plasmids used in the cloning procedures contain an antibiotic resistance marker that can be used for selection, the antibiotics used are not the same ones used in frontline patient treatment.

The proposed waste disposal by autoclaving should be sufficient to decontaminate the waste produced during the experiments.
**GM Centre Number: 3329**

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**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Y**

Give brief details of the genetic modification safety committee

- Person is chief scientist in the company with 20yrs experience in GM techniques and risk assessment

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Transient and stable expression of ion channel proteins in mammalian cell culture

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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

All cell cultures will be killed with Virkon and autoclaved prior to disposal.

---

**Tick to confirm that you are attaching a summary of the risk assessment**

Y

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**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

---

**Please enter comments of the GM safety committee on the risk assessment**

Growth constraints on mammalian cell cultures mean that the cell lines pose negligible risk to human health and the environment. The inserts being expressed are from non-viral vectors. Whilst the protein they encode is a functional ion channel expressed at the cell membrane, the protein is non-oncogenic, not cytotoxic, not immunomodulatory, non-pathogenic and non-allergenic.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Dublin City University Biological Safety Committee
This committee is comprised of academic and technical staff from the Faculty of Science and Health,
Dublin City University, Glasnevin, Dublin 9, Ireland.

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Bacteriology        Parasitology   Transgenic Birds   Microbiology Research
All GMM waste is deactivated and disposed of via the James Cook University Hospital Pathology Decontamination Facilities (26.A.102a) Autoclaves. Deactivation is carried out using Getinge (The Sterilisation Company) GE6913-AR1 Lab autoclaves. This autoclave is a pre-vacuum sterilizer which operates with saturated steam as the sterilizing agent, and has a temperature range of up to 137°C (279ºF) and pressure up to 2.3 bar (34 psi). Deactivation is load dependant. Materials loaded will not exceed the “Validated Load” stated in the configuration manual.

Solid GMM waste is placed in a biohazard autoclave bag, filled to no more than 2/3 in the hinged lid autoclave tins provided (including plastic pipettes). All GMM waste will be transferred to the Pathology Decontamination Facility (26.A.102a) for deactivation. Solid waste is deactivated by autoclaving at 121ºC, 15 p.s.i. for 30 minutes. Deactivated material is then disposed of within the general waste stream.

Light clinical waste bags will be provided for non GMM waste.

Only clinical waste should be disposed of in these bags.

The bags are changed daily or as soon as they are full.

When 2/3 full the neck of the bag twisted and tied in a ‘swan neck’ using the green/white tape provided.

The bags should then be placed in the waste disposal hold in LRI centre for the Porters to collect.

Liquid GMM waste will be placed in 1L autoclavable containers no more than 2/3 full. Prior to use 2/3 sachets of safety gel will be placed in the container. When 2/3 full a further 2/3 sachets of safety gel will be added and the contents left overnight to solidify. These containers are then placed within the hinged lid autoclave tins and the hinges secured prior to transportation. The use safety gel minimizes the risk of spillage due to fluid leakage during transfer to Pathology decontamination facility. Liquid waste is deactivated by autoclaving at 121ºC, 15 p.s.i. for 30 minutes. Deactivated liquid waste is then disposed of down the drain.

6.4.1. Transfer of GMM Waste for Deactivation: GMM Waste for deactivation will be transferred from GlycoSeLect’s operation facility within the Second Floor, Learning and Resource Centre’s Research Laboratory (Room 12.C.008) to the Pathology Decontamination Area Room (26.A.102a) of the Trusts Pathology building. The transfer of the material (date, type, volume & deactivation cycle/run number) will be recorded by completion of a “GlycoSeLect: GMM Waste Transfer Log” form.

All GMM waste deactivation procedures executed by the Pathology Decontamination Facility (26.A.102a) are documented using established Pathology SOPs and logging systems. All deactivation runs are recorded by completion of an “Autoclave documentation monitoring system” form.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

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<tr>
<th>Virology</th>
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<th>Transgenic Fish</th>
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Other(s) - Molecular Biology: production of recombinant proteins using non-pathogenic E.coli cloning and expression.
Project Title:
To clone, mutagenize, express, purify and characterise carbohydrate-binding proteins (CBP’s –
including lectins & adhesins), carbohydrate-binding domains (CBD’s) and carbohydrate active enzymes
including lectins & adhesins), carbohydrate-binding domains (CBD’s) and carbohydrate active enzymes
(CAE’s) from a number of class 1 and class 2 microorganisms.
Decision
On behalf of the Biological Safety Committee, I hereby confirm approval for the project detailed above.
The committee have reviewed the application and associated risk assessment with no comment for
amendments.
MB
Chair of the Biological Safety Committee
Date of Approval: 23rd October 2014
Submission on behalf of: Dr. PC
Affiliated School/ Centre: Glycoselect Ltd
**GM Centre Number: 3332**

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**Name 2**

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**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022 |
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Risk assessments were written by the senior biologist in conjunction with the Head of Biology. Health and safety documentation including GMO risk assessment and Local Rules are reviewed and approved by independent health and safety advisors.

Experience:
- Head of Biology - PhD in Cellular Biology, 28 years experience in laboratory setting including hands on experience of mammalian cell culture of GMOs plus 20 years in Pharmaceutical Industry as line manager of scientific staff.
- Senior Biologist - PhD in Integrated Biology. 10 years practical experience with the contained use of GMO microbiological organisms (Mammalian and bacterial cells) under Class I and II biosafety classification.

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Other (please specify) Tick if confidential

02/03/2022
Yes

All genetic modification work within Mironid biology will be conducted on microorganisms which are incapable of surviving in runoff water. However, all waste from bacterial (e.coli and K12 derivatives) cell and mammalian culture growth will be inactivated prior to disposal.

Liquid waste from Mammalian cell culture media and buffers will be chemically inactivated with a final concentration of 10% (v/v) Chloros bleach (minimum 2,500ppm chloros (hypochlorite)), and incubated for a minimum of 1 hour prior to sink disposal (100% kill).

Solid waste from mammalian cell culture (plastic flasks, plastic pipettes, gloves, plastic culture plates) will be autoclaved for 30 minutes at 126 C at -15 psi (100% kill) using an onsite autoclave which is annually validated using thermocoupled testing by independent inspectors.

Liquid waste from Bacterial (k12 derived e.coli) cells culture media will be inactivated using 2% (v/v) virkon disinfectant which has been validated for 100% kill by the manufacturer and in independent study.

Solid waste (pipettes, 15ml and 50 ml tubes) will be disinfected with 2% virkon prior to autoclaving for 30 mins at 1260C at - 15psi (100% kill) using an onsite autoclave which is annually validated using thermocoupled testing by independent inspectors.

LB agar plates used in the culture of debilitated e.coli strains will be autoclaved (as above, 100% kill) prior to disposal. Autoclave waste is disposed (incinerated) by outside contractor at a site distant to Mironid Biology Labs.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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LB agar plates used in the culture of debilitated e.coli strains will be autoclaved (as above, 100% kill) prior to disposal. Autoclave waste is disposed (incinerated) by outside contractor at a site distant to Mironid Biology Labs.

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Genetic modification of established cell lines and debilitated e.coli bacteria proposed by Mironid Ltd poses no risk of adverse health effect to humans or the environment. All organisms are incapable of survival in runoff water, the delivery systems pose no additional risk and inserts pose low risk to human health and pose no threat to environmental safety. All GMMs created and grown as part of this work will be inactivated prior to disposal (100% kill).

Although all GM work proposed by mironid is considered Class 1, containment level 2 systems of work will be applied.
**GM Centre Number:** 3335

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**Name**

KARUS THERAPEUTICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

UNIT1, GENESIS BUILDING

**Road Name**

LIBRARY AVENUE

**District**

HARWELL CAMPUS

**Town**

DIDCOT

**County**

OXFORDSHIRE

**Postcode**

OX11 0SG

**Country**

ENGLAND

**Tel Number**

01235 829140

**Fax Number**

01235 420517

**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

GMSC: Chief Scientific Officer (management representative, 20 years life sciences management experience), Chair and Secretary: Group Leader, Biology (PhD molecular biology, 14 years experience in public sector, academia and industry), Senior Scientist, Biology (14 years industry life science experience).

Advice on the risk assessment was provided by an external Health and Safety consultant

GMSC meets on a regular bimonthly basis to review project specific risk assessment and safety procedures involving GMO. Minutes of the GMSC meetings and approved risk assessments are maintained

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Other (please specify) Tick if confidential
GMM contaminated waste (disposable plasticware and spent culture liquid) is treated by chemical disinfection overnight followed by autoclaving in a benchtop autoclave (121°C for 22 minutes) to inactivate the GM, with each run monitored using TST (time, steam and temperature) test strips. Autoclaved waste is disposed of in a clinical waste stream by incineration by a contractor to achieve 100% kill.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

No additional comments from GMSC
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

QuantumDX’s Biological Safety Committee will also stand as the Genetic Modification Safety Committee and has been consulted throughout approval of risk assessments. The committee comprises a range of representatives from various safety and technical disciplines of the Company, representing both management and employees, health and safety advisors and, if necessary, input from clinicians. Between them they have extensive experience in biological safety and containment laboratory management and practice with specific experience handling elevated biological agent hazard groups and GMM. The committee meets frequently (minimum of 4 times a year) with additional meetings where required to discuss new procedures, use of new organisms, development of new assay targets or facilities requirements.

QuantumDX are also in the process of training two Biological Safety Officers (undertaking training APR-JUN 2018 with the Biosafety Training Institute at Edinburgh University).

The committee has reviewed and approved the risk assessment (QHS-342 appended) relating to the level 1 GMM work proposed to be undertaken.

An external Health and Safety consultant was also contacted for specific risk assessment review.

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

GMMs will be handled under containment level (CL) 2 conditions due to additional work undertaken on the premises (HSE notified)

Waste Disposal
All waste (solid and liquid) is subject to autoclave (autoclave located in laboratory suite) before disposal as biological waste by an external contractor (Stericycle). Waste disposal procedures are fully detailed in internal procedure QLAB-65. Autoclaving: Autoclave housed within the laboratory suite serviced and calibrated twice a year, by a reputable service provider, to ensure the validation criteria are met. During normal, daily operation each run is recorded to ensure the required conditions are achieved (121C degrees for 47 minutes).

Disinfection
Virkon, as a 1% solution, or Acitchlor, as a 0.1% solution, is used to treat spills and wipe down surfaces and equipment after work. Both disinfectants are used in strict accordance with the manufacturer’s guidelines.

Monitoring
Safety Inspections are carried out annually to ensure internal policies and procedures are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Compliance Officer.

Please enter comments of the GM safety committee on the risk assessment

The committee has reviewed and approved the risk assessment (QHS-342 appended) relating to the level 1 GMM work proposed to be undertaken.
The H37Ra attenuated strain of M. tuberculosis is to be used as a model for the pathogenic strain of M. tuberculosis to allow development of a novel diagnostic system for pulmonary Tuberculosis infection. Bacteria expressing a coloured tag are required for visual analysis of bacterial extraction from a medium, concentration and lysis. M. tuberculosis is a Hazard Group 3 organism and as such a well-defined, attenuated substitute will be used to allow safer handling during early development.

Recipient or parental organism

Mycobacterium tuberculosis H37Ra is a fully sequenced (ATCC- 25177) attenuated laboratory strain of TB classified as a Hazard Group 2 pathogen.

Host/vector system

The plasmid the strains contain is based on pFPV2 first published/created in 1996 (Valdivia, Hromockyj, Monack, Ramakrishnan, & Falkow, 1996) and widely used in the mycobacterium community since. The plasmid encodes a fluorescent tag (mCherry) fused to a constitutive promoter in addition to a hygromycin resistance cassette to maintain selection. The plasmid does not integrate into the host genome and has no impact upon pathogenicity. The resistance cassette does not convey resistance to any front line TB treatment.

Origin & function
The mCherry protein, initially isolated from Discosoma sea anemones is widely used as a fluorescent reporter. When grown under selection (100ug/ml Hygromycin), the GMM H37Ra strain will express the mCherry protein and can be hence used for fluorescence imaging with 587/610 nm Excitation and Emission properties. No mutations or alterations will be made to the host genome.

**Evaluation of foreseeable effects**

No alterations or mutation to the host genome are made and no genetic material is inserted or excised from the genome.

The plasmid will only be maintained in the strain under selective pressures, namely the addition of hygromycin to the growth media at a concentration of 100ug/ml.

The addition of a fluorescent protein will not affect the viability or infectious capabilities of the host organism so poses no threat to humans.

The plasmid cannot propagate outside of the host cell and will not be maintained in the host without the addition of antibiotics to the growth media. As such, this modification poses no threat to the environment.

The antibiotics used to maintain selection are not frontline drugs used for the treatment of TB and the introduction of the hygromycin resistance cassette will not impact the efficacy of drugs used to treat active TB.

All GM material or potentially contaminated waste is subject to autoclave before waste disposal, further reducing the risk of environmental exposure.

The parental organism is an attenuated strain of TB classified as a HG2 pathogen

Plasmid is well characterised and has been previously used by QMDx in M. smegmatis stains (HSE notified in GM 3336 premises notification).

The GMM requires additional growth supplements to maintain GM status and as such pose no threat to the environment

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All culture and analysis of GMM is completed under CL2 conditions by fully trained staff.

**Waste Disposal**

All waste (solid and liquid) is subject to autoclave (autoclave located in laboratory suite) before disposal as biological waste by an external contractor (Stericycle). Waste disposal procedures are fully detailed in internal procedure QLAB-65.

Autoclaving: Autoclave housed within the laboratory suite serviced and calibrated twice a year, by a reputable service provider, to ensure the validation criteria are met. During normal, daily operation each run is recorded to ensure the required conditions are achieved (121C degrees for 47 minutes).

**Disinfection**

Disinfection of surfaces and equipment not compatible with autoclave is completed using Acichlor, as a 0.1% solution or Tristel as provided at working concentration. Tristel is used to treat spills. Both disinfectants are used in strict accordance with the manufacturer's guidelines and have been demonstrated to achieve a suitable degree of kill.
Monitoring
Safety Inspections are carried out annually to ensure internal policies and procedures are adhered to and that risk assessments and training records are in order. Inspection reports are kept by the Compliance Officer.

Spill response
Procedures are documented for dealing with accidental spillage of liquid culture.

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Animal Units

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment was approved by the committee 19 SEP 2018. Committee agrees class 2 notification to be made to the HSE.

Project Containment
**GM Centre Number: 3337**

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Risk assessment was prepared by a molecular biologist with a PhD and more than a decade of experience handling GMMs and larger GMOs. It was reviewed both by internal health and safety personnel and by a University safety officer who has served on University GM safety committees.

Tick if confidential

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Yes

Yes

All cultures and materials in contact with cultures will be autoclaved at times and temperatures validated for 100% killing. Spills will be treated with Virkon according to the manufacturers instructions.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessment was prepared by a molecular biologist with a PhD and more than a decade of experience handling GMMs and larger GMOs. It was reviewed both by internal health and safety personnel and by a University safety officer who has served on University GM safety committees.

Public Register
Public Register
Public Register
Public Register
Public

Project Ref 3337/21.1

Designing gene regulatory logic for lentiviral-vector-based cell and gene therapy using a toolkit of regulatory elements

Class 2   < 1 L

Non-GMM

Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info
A system that enables a precise control over target gene expression with inducer-molecules is highly sought after. The design of such inducible systems that meets required performance metrics poses a great challenge in mammalian cell synthetic biology. The aim of our work will be to assemble a toolkit from well-characterised, highly tunable, synthetic genetic regulatory components that will enable precise control of gene expression for the production and targeting of lentiviral vectors used in cell and gene therapy applications.

Candidate gene regulatory elements will be assessed in the context of HEK293, Jurkat and primary T cells using transient transfection, CRISPR and 3rd generation lentiviral systems. The knowledge gained will also be used to address the problem of balancing viral yield optimisation with cellular metabolic burden in HEK293 cells.

Common, well-characterised fluorescent proteins will be used to assess the performance of chosen gene regulatory elements and as genetic payloads to assess lentiviral production and titres.

Cells: HEK293, Jurkat and primary T cells from commercial sources providing screened material.
Other than introducing metabolic burden associated with exogenous protein expression, genetic manipulation of these cells is not expected to alter their characteristics or safety profile.

Microorganisms: standard laboratory strains of non-pathogenic E. coli will be used for plasmid propagation and cloning.

Third generation lentiviral plasmids will be cloned and propagated in E. Coli and chemically transfected into HEK293 cells. Resulting lentiviral vectors will be used to transduce HEK293, Jurkat and primary T cells.

In order to optimise viral vector yields in HEK293 cells to a target level, we will fine-tune promoter strengths of the constituent viral plasmids using validated site-specific DNA modifications. Plasmids will be fully sequenced to ensure no off-site sequence alterations prior to their use for viral production. Promoter strength modification will not impact the safety profile of the lentiviral system.

Lentiviral vector will be used for delivery of inducible synthetic genetic circuits consisting of fluorescent proteins under the control of regulatory elements from well-characterised, published libraries of synthetic transcription factors, promoters and inhibitors developed for programmable control of gene expression in mammalian cells such as COSMOS (Donahue et. al. Nat Commun., 2020) and CRISPR-based toolbox for synthetic biology such as dCAS (Xu et. al. J of Mol Bio, 2019).

Gene regulatory elements are designed to target specific sequences located within the synthetic circuit and not to have interactions with the host cell's endogenous gene regulatory networks. The output of our synthetic circuits will be a precisely tunable intracellular expression of fluorescent reporter proteins controlled by exogenously added inducer-molecules.
Synthetic gene regulatory components we aim to utilise have been previously developed and tested in mammalian cell lines (HEK293 and Jurkat) and are designed to target specific DNA sequences not present in the host genome. Other than imposing additional metabolic burden onto the host cell, we don't expect further changes in cells' characteristics or safety profile following our genetic modifications.

In case of an accidental operator exposure to GM cells, we don't foresee additional hazards beyond ones posed by unmodified cells.

In case of an accidental operator exposure and infection with our lentiviral vector carrying synthetic circuits, we do not expect any active gene expression to occur from the circuit itself due to its dependency on the presence of inducer-molecules and its inability to interact with the host cell's endogenous gene regulatory networks.

However, there is a potential risk of insertional mutagenesis due to chromosomal integration of lentivirally delivered transgene that may disrupt a tumour-suppressor gene or activate an oncogene.

In addition, a very small risk is a self-reversion to replication competent lentivirus during viral vector production. This is highly unlikely with the 3rd generation of self-inactivating lentiviral system that will be used in our work. Moreover, to exclude the risk of vector recombination with a wild-type HIV-1 virus, we will be using fully screened primary T cells obtained from a commercial bloodbank.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Small sharp plastic wear such as pipette tips will be collected into a hard-plastic, sealable container such as a sweetie jar or an empty 500 ml medium bottle. Plastic wear such as tissue culture plates and flasks will be sprayed with Virkon solution, placed inside a double ziploc bag. At the completion of work, tip-containing bottles and double Ziploc bags will be sealed, surface disinfected with Virkon spray or 70% ethanol and collected in a double autoclave bag in a dedicated waste bin. When the bag is full, it will be sealed with autoclave tape and decontaminated in our bench-top autoclave to achieve 100% kill of GM organisms. Virkon solution will be made according to manufacturer's instructions (10 g Virkon in 1L of water) and has been validated by the manufacturer to be highly effective against a broad spectrum of microorganisms.

Liquid waste will be decontaminated in the BSC using 500 ml plastic container such as a used medium bottle containing 1x5g Virkon tablet (which is enough to disinfect 500 ml liquid waste). Waste volume will not be allowed to exceed 500ml per bottle. The waste will be left to decontaminate for at least 20 min. After which, 2 sachets of VernaGel will be added to solidify the liquid. Once solid has formed (about 20 min), the lid will be closed, the bottle will be surface decontaminated and placed in a dedicated laboratory waste bin.

All autoclaved and chemically decontaminated waste will be stored in a dedicated laboratory waste bin for collection by a commercial disposal service.

Use of sharp materials such as glass pipettes, scalpels, needles, etc in conjunction with lentiviral work will not be permitted.

Small spillages will be immediately covered with a Virkon-soaked absorbent tissues, allowed to decontaminate for 20 min and wiped with additional tissues soaked in 70% ethanol. Absorbent tissues will be disposed of together with solid waste in a dedicated waste bin for subsequent autoclaving.
GM Safety Committee had the following comments:

- avoid use of sharps and large viral culture volumes or high viral titres (above 5x10e9 pfu/ml). In addition, aerosol generating procedures such as centrifugation or vortexing should be undertaken in sealed vessels. Flow cytometric analysis of virally transduced cells or other aerosol-generating analysis techniques should be performed on PFA-fixed, inactivated samples.

- restrict access to the class 2 activity area to authorised and trained personnel only

- keep written training records

- avoid the use of transgenes with known biological activity in host cells such as oncogenes, toxins, cytokines, growth factors and immunomodulatory proteins.

We have taken measures to address these points as outlined in the risk assessment and local rules for CL2 work. In particular, only 3rd generation lentiviral system will be used and the choice of transgenes for our work will include only common fluorescent proteins under the control of well-characterised inducible synthetic regulatory elements not expected to interact with host cells' endogenous gene regulatory pathways.

Project Containment

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<tr>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Tick if you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Page 14035 of 15326

02/03/2022
### GM Centre Number: 3338

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### Name

**DNAWRITE LTD**

### Name 2

**Department**

### Campus Estate or Research Centre

**THE SCIENCE VILLAGE**

### Road Name

**CHESTERFORD RESEARCH PARK**

### Building

### District

**CAMBRIDGE**

### Town

**Campus Estate or Research Centre**

### County

**CAMBRIDGESHIRE**

### Postcode

**CB10 1XL**

### Country

**ENGLAND**

### Tel Number

**01223789161**

### Fax Number

**N/A**

### E-mail

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### HSE Division

### Comments

### Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Yes

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**Give brief details of the genetic modification safety committee**

The health and safety officer has over 13 years experience of using GMMs in research and has PhD and BSc (Hons) qualifications. Risk assessments are performed by the lead research scientist in collaboration with the H&S officer. All risk assessments are signed off by the H&S officer with annual reviews.

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**Tick if confidential**

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**Bacteriology** | Yes

**Parasitology**

**Transgenic**

**Birds**

**Microbiology**

**Research**
Work will be performed within the designated laboratory and all staff will be compliant with DNAwrite health and safety policy, which can be provide upon request. This requires risk assessment, the use of PPE (gloves, protective eyewear, laboratory coats) when in the designated area and also compliance with our waste disposable and management procedures.

Waste management:
Any contaminated solid waste (such as LB plates, pipette tips or gloves) will be inactivated by autoclaving and then disposed in Low-risk Hazardous Waste containers. These containers are collected and disposed of by Anglian Environmental Services Ltd and records of disposal will be kept.
Liquid waste will be decontaminated by exposure to 1% Virkon for >30mins. Liquid will then be disposed down the sink and rinsed with water. We anticipate no more than 500ml of liquid waste per month.
GMM work will be performed in a designated zone within the lab and the surfaces of the lab benches, will be de-contaminated by wiping with 1% Virkon and azowipes.

Monitoring:
The efficiency of our waste management will be routinely monitored using common microbiology techniques, such as dip slides, swabs and LB plates containing antibiotics.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
GM Centre Number: 3339

Data Premises Notified (Originally) 11/10/2016

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

NANNA THERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre

THE MERRIFIELD CENTRE

Building

Road Name

12 ROSEMARY LANE

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB1 3LQ

Country

ENGLAND

Tel Number 01223 394 200

Fax Number 0

E-mail

HSE Division blank

Comments

Change of company name from Bactevo Ltd notified 19/06/2019

Date at Which Additional Info Submitted

02/03/2022
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Bactevo currently shares the same site as parent company Discuva. The GMO safety committee is made up of members from both companies including the Director of Biology, and Principal scientist and Senior research scientists. All have extensive experience working in research, biotech and drug discovery environments and previous involvement with health and safety, CoSHH assessments and/or GMO safety committees.

Any new planned GM work requires preparation of a GM Risk assessment, which is reviewed by the GMO safety committee prior to commencement of work.

The GMO Safety Committee will ensure procedures, safeguards and compliance to regulations are in place in advance of initiation of those projects. These meetings will be minuted and records of these meeting retained.

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Tick if confidential

02/03/2022
Solid waste that is biologically contaminated (including the parent strain and the GMO) will be autoclaved (heating with steam under pressure at >121°C for >15 minutes) to achieve 100% kill before removal from the containment facility. This will be monitored by taking samples from the autoclaved waste (e.g., from small liquid cultures) onto solid growth medium with incubation to allow any viable bacteria to grow into visible colonies, and reviewed by the GM safety committee at quarterly intervals. The autoclave used for processing is validated annually. All processed solid waste will be transferred to an authorised Waste Management provider where waste will be processed via incineration.

Liquid waste that is contaminated will be treated with "Virkon" disinfectant according to the manufacturer’s instructions to achieve 100% kill. This will be monitored by taking samples from treated waste onto solid growth medium with incubation to allow any viable bacteria to grow into visible colonies, and reviewed by the GM safety committee at quarterly intervals.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

The safety committee discussed the potential for modulation of pathogenicity by genetic manipulation procedures, but agreed that such modulation would most likely result in no change or reduced pathogenicity, although scientists will be vigilant for changes to pathogen characteristics. In addition, current working practices are sufficient to prevent exposure as all work is carried out within a class II microbiological safety cabinet.

Our screening platform allows the identification of individual 'producer clones' in very small volumes (<1nl) and are screened for desired phenotypes such as antimicrobial activity or specific killing of cancer cells. The small volume and nature of the phenotypic screen makes it extremely unlikely that we would isolate increased pathogenic strains or those producing toxins. Scale up only occurs once the clones have been genetically characterised and Mass-spec identification of the compounds produced by the clone has occurred making any risk to staffs minimal.
Manipulation of organisms isolated from various sources principally environmental such as soil but also other biomes so as to allow them to continuously produce the full chemical diversity they are naturally capable of making.

Purposes of the contained use

To allow easy identification, isolation and production of chemicals naturally made by organisms for use as pharmaceuticals such as anti-cancer and antibiotics avoiding chemical synthesis. This will allow the development of new drugs and chemicals to improve therapeutics across a broad range of diseases.

Recipient or parental organism

Bactevo are working with microorganisms from the natural environments and engineering them to constitutively produce compounds that are rarely or only made in specific conditions to allow identification of those that have value to society such as a therapeutic agent. All bacteria isolated are identified by sequencing the 16S rRNA prior to creation of transposon libraries. Libraries will only be made in bacteria that are not known to cause significant human or plant diseases. Initially we are targeting groups such as Pseudomonas and streptomycyes. None of the organisms will belong to ACDP hazard group 1 or 2 and will generally be opportunistic human pathogens at worst and unlikely to infect healthy individuals even with the modifications made.

Host/vector system

Well characterised laboratory strains of E. coli, Streptomycetes and Bacilli will be used as hosts for DNA vectors. It may be that we also utilise naturally occurring phage and plasmids from the species of interest such as Streptomycyes as vectors. These will already be present in the environment and so in the unlikely release from Class II laboratory are already prevalent in the natural populations including any resistance or other genes they carry so represent no additional hazard to the environment or people than that already present.
Bacterial plasmid DNA vectors will be used, and will include standard cloning vector plasmids, which are often commercially available. Suicide vectors will also be utilised which do not normally replicate in bacteria other than specific laboratory strains of E. coli, Bacillus or streptomycetes. Generally the plasmid vectors are not self-transmissible, significantly reducing the likelihood of their unwanted spread. In all cases and especially with ‘native’ vectors where there is a possibility of self-transmission. No resistance cassettes will be used that are not already found commonly in the environment and/or used to treat humans so as to further reduce the risk of spread or transmission of markers in the environment and prevent potential resistance to clinically relevant antibiotics. All assessment will have to specify cassettes to be used and no deviation from this is allowed without additional assessment by the GMM committee.

Origin & function

Plasmid vectors to be used will be constructed from components that occur naturally in the bacteria being used in the studies. These components include a replication origin to maintain the plasmid through replication cycles and an antibiotic resistance determinant to allow for selection of bacteria harbouring the plasmid vector. Some of the plasmid vectors may also include bacterial gene transcriptional promoters, such as the E. coli or Pseudomonas mB promoter, which function to promote expression of genes inserted into the plasmid, and genes such as sacB from Bacillus subtilis that codes for levansucrase and which allows counter-selection against the plasmid in the presence of sucrose.

The chromosomally-inserted DNA will normally code for a gene, or genes, of interest from the micro-organism, and may include the gene's native promoter, or potentially a heterologous inducible promoter (such as araBAD). The inserted DNA functions to complement existing gene function, or to mediate homologous recombination with the existing gene in the recipient in order to specifically replace, mutate, or knockout the gene or promoter of interest. Such mutations may serve to specifically inactivate the gene, or in some cases to specifically regulate expression of the gene.

Alternatively the chromosomal insertion may comprise an antibiotic resistance determinant (such as those found commonly in the environment, e.g. kanamycin resistance) and a functional promoter to increase expression of genes downstream of the insertion.

Evaluation of foreseeable effects

The DNA manipulation techniques to be employed allow the precise construction of DNA molecules, thus unforeseeable effects due to the presence of DNA not necessary for the experiment can be avoided. Some GMOs will become resistant to the specific antibiotics as a result of the resistance gene encoded by the plasmid vector or if inserted into the chromosome. However, only one or two antibiotic resistance determinants are used at a time, so the generation of multiple resistance GMOs will be avoided. Antibiotic resistance genes are chosen against antibiotics that would not normally be used in therapy, so they will possess no selective advantage over non-GM bacteria, would be expected to respond to treatment, and would be unlikely to spread outside the laboratory as no selective pressure. We also ensure that the cassettes used are already common in the environment and have in most cases been isolated from environmental strains meaning the spread and dissemination into the environment has already occurred and we are unlikely to enhance this propagation.

The inserted DNA does NOT possess any additional traits that would be expected to increase the GMO's ability to cause disease or survive in it's natural habitat, or any other environment. The inserted DNA would normally code for genes that have been identified to produce a defined product or enhance its production further. As such, some GMOs will possess increased production of a compound of interest, however this will be characterised by this stage including any toxicology and human health risk.

The work does not involve specifically the modulation or investigation of genes coding for pathogenicity determinants;
rather, it involves genes involved in producing valuable compounds such as human pharmacological compounds.
Scale up of production of the compound may be undertaken, but only once the compound has been identified and
evaluated for toxicity at a very small scale.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste that is contaminated with bacteria (including the parent strain and the GMO) will be autoclaved (heating with steam under pressure at >121°C for 15 minutes) to achieve 100% kill before removal from the containment facility. This will be monitored by taking samples from the autoclaved waste (e.g. from small liquid cultures) onto solid growth medium with incubation to allow any viable bacteria to grow into visible colonies, and reviewed by the GM safety committee at quarterly intervals. The autoclave used for processing is validated annually. All processed solid waste will be transferred to an authorised Waste Management provider where waste will be processed via incineration.

Liquid waste that is contaminated will be treated with "Virkon" disinfectant according to the manufacturer's instructions to achieve 100% kill. This will be monitored by taking samples from treated waste onto solid growth medium with incubation to allow any viable bacteria to grow into visible colonies, and reviewed by the GM safety committee at quarterly intervals.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The safety committee discussed the potential for modulation of pathogenicity by genetic manipulation procedures, but agreed that such modulation would most likely result in no change or reduced pathogenicity, although scientists will be vigilant for changes to pathogen characteristics. In addition, current working practices are sufficient to prevent exposure.

Our screening platform allows the phenotypic identification of producer clones by co-culture with indicator or reporter cells in very small volumes (<1nl) and are screened for desired phenotypes such as antimicrobial activity or specific killing of a cancer cells. The small volume and nature of the phenotypic screen makes it extremely unlikely we would isolate increased pathogenic strains or those producing toxins. Scale up only occurs once the clones have been characterised genetically by sequencing and metabolically by Mass-spectrometry of the compounds produced. This massively reduces the risk of toxin production or strains that show enhanced pathogenic traits being isolated and cultured. Strains used are isolated from environments that individuals are commonly exposed to, such as soil or skin surface swab and are thus unlikely to infect healthy individuals.
## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>L3 L4</td>
<td>L2 L3</td>
</tr>
<tr>
<td>Yes</td>
<td>L3 L4</td>
<td>L2 L3 L4</td>
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- **Laboratory Activities**
  - L2
  - Yes

- **Glass Houses**
  - L3
  - L4

- **Growth Rooms**
  - L3
  - L4

### Laboratory Units
- Animal Units
- Large Scale Activities
- Human Clinical Applications

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Data Premises Closed

Emergency Plan Required?

Transitional Premises

Non-GMMs

Withdrawn

Name

AXOL BIOSCIENCE LTD

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

District

Town

County

Postcode

Country

CAMBRIDGE

CAMBRIDGESHIRE

CB10 1XL

ENGLAND

Tel Number

Fax Number

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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<tr>
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<td>AXOL BIOSCIENCE LTD</td>
<td></td>
<td></td>
<td>SUITE 3, THE SCIENCE VILLAGE</td>
<td>CHESTERFOR D RESEARCH PARK</td>
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<td>CAMBRIDGE</td>
<td>CB10</td>
<td>1XL</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The Axol Bioscience Ltd genetic modification committee will consist of:
1. The Biosafety Officer who has extensive experience of genetic modification issues, being currently in charge of biosafety for a large research institute. The biosafety officer will chair all genetic modification committee meetings.
2. The Director of Operations has over 8 years experience of genetic modification in E.coli and mammalian cells, and previously ran research groups both in industry and academia. His function on the committee is to advise, provide the necessary resources for the actions of the committee and ensure that the actions are implemented.
3. Research & Scientist who is a specialist in mammalian cell culture has over 4 years experience. She will provide advice and write the minutes of the meetings.

The committee will meet every 6 months to review biosafety and whenever a new risk assessment is required. The first meeting will review the existing risk assessments.

<table>
<thead>
<tr>
<th>Laboratory (GMMs)</th>
<th>Animal Unit (GMMs)</th>
<th>Growth Room (GMMs)</th>
<th>Glass House (GMMs)</th>
<th>Large Scale (GMMs)</th>
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<tr>
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<td>Non-microbial</td>
<td>Tick if confidential</td>
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<tr>
<td>Other (please specify)</td>
<td></td>
<td>Tick if confidential</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>
**For activities involving GMMs, describe the waste management measures which will apply to the activity**

### GENERAL

All biological waste including reagents (cell culture media, washings, etc.) and materials (consumables such as pipettes, culture vessels, etc.) that have had contact with the organisms, GMM or otherwise, are contained within yellow sacks or in labelled yellow clinical bins and exit from the facility by 'controlled waste transfer' via a validated, contracted waste disposal party (Grundon Waste Management LTD). All contained organisms are rendered waste by HI041 pre-treatment and inactivation with a minimum of 24hrs to ensure 100% kill as per manufacturer's instructions and advice.

**SOLIDWASTE**

We intend to adopt similar waste disposal procedures used by the Babraham Institute (GM105) and other companies on the Babraham Research Campus (BRC) under derogation (GM105.1): Waste from laboratories carrying out GM work at Class1 will be placed into either yellow sacks (18kg, EWC Code 180103) or 60 litre yellow Clinical Poly Bin (EWC Code 020202) as appropriate. They will be sealed by the producer of the waste and removed to a central, lock secured collection point where they will be placed into 770 litre yellow bin (EWC Code 180103). Collections by the local registered clinical GM waste incinerator contractor (Grundon Waste Management LTD) will be made once a week to prevent a build-up. The Chesterford Research Park has 24/7 Security & Surveillance. The contractor uses solid bodied transport for waste transfer to the Colnbrook Clinical Incinerator (Berkshire SL3 0EG), and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be maintained on site, as required by the Regulations, in case of IOSs of the contractor's facilities.

**LIQUIDWASTE**

Liquid waste is inactivated by the addition of HI041 (CHEMGENE, tested under EN protocols, biodegradable under OECD conditions) according to the manufacturer's instructions prior to disposal. Validation experiments will be carried out in-house using microbiological testing. Smaller volumes (i.e., <10ml) will then be disposed of in sealed tubes with the solid waste. Larger volumes will be disposed of via the common drainage system. This results in approximately 5 l of diluted, inactivated waste from each laboratory involved in GM activities. This treated liquid waste is then processed through our own sewage treatment plant which is closely monitored. The total eHluent volume from the Chesterford Research Park site is approximately 110,000 cubic metres per day.

Tick to confirm that you are attaching a summary of the risk assessment

---

02/03/2022
**Project Ref** 3341/17.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<td>09/03/2017</td>
<td>Sendai virus-based Reprogramming of human Peripheral Blood Mononuclear Cell (PBMNC), fibroblasts or other</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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<tr>
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<td>somatic cell types for induced Pluripotent Stem Cell (iPSC) generation</td>
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</table>

- **Project notified under transitional arrangements**: N
- **Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

**Project Additional Information**

**Purposes of the contained use**

Induced pluripotent stem cells (iPSCs) are genetically reprogrammed somatic cells which exhibit a pluripotent stem cell-like state similar to embryonic stem cells. iPSCs can be derived by inducing selected gene expression via various methods including retrovirus-mediated gene transduction and chemical induction.

**Recipient or parental organism**

Recipient experimental systems are human peripheral blood mononuclear cell (PBMNC), fibroblasts or other somatic cell types. SeV vectors used in this kit consist of viral proteins NP, P, M, F (activated), HN, and L, and the SeV genome RNA, from which the F gene is deleted. Because SeV infects cells by attaching itself to cell surface receptor sialic acid, present on the surface of many cell types of different species, the vectors are able to transduce a wide range of cell s. However, they are no longer capable of producing infectious particles from infected cells, because the
viral genome lacks the F-gene. In addition, the presence of functional mutations such as temperature sensitivity in the amino acid sequence of several SeV proteins (SeV(TS F, SeV(TS12 F, and SeV(TS15 F) renders the vectors easily removable from transduced cells.

SACGM containment classification: Level 2

Host/vector system

The CytoTune TM_iPS 2.0 Sendai Reprogramming Kit is a non-integrating system that uses Sendai virus (wild type ACDP 1) vectors to reprogram somatic cells into induced pluripotent stem cells (iPSCs). The CytoTune TM_iPS 2.0 Sendai Reprogramming Kit contains three CytoTune ™ 2.0 reprogramming vectors that are used for delivering and expressing key genetic factors necessary for reprogramming somatic cells into iPSCs (see below).

CytoTune® Sendai hOct3/4 (Human Oct3/4 ; NM_002701.4) Pluripotency
CytoTune® Sendai hSox2 (Human Sox2; NM_003106.2) Pluripotency
CytoTune® Sendai hKlf4 (Human Klf4; BC029923.1) Proliferation
CytoTune® Sendai hc-Myc (Human c-Myc; K02276.1) Proto-oncogene, Proliferation

Origin & function

Human transcription factor genes used to reprogram human fibroblasts and PBMCs to produce iPSCs including:
- Oct4
- Sox2
- Klf4
- c-Myc (a known oncogene linked with several forms of cancer)

Sendai virus vector is a Cytoplasmic RNA vector. Sendai virus vector replicates its RNA genome and produces proteins exclusively in the cytoplasm. It does not enter cell nucleus. In principle, the virus should not alter the chromosomes in the cell nucleus.

Evaluation of foreseeable effects

SeV vectors used in this kit consist of viral proteins NP, P, M, F (activated), HN, and L, and the SeV genome RNA, from which the F gene is deleted. Because SeV infects cells by attaching itself to cell surface receptor sialic acid, present on the surface of many cell types of different species, the vectors are able to transduce a wide range of cells. However, they are no longer capable of producing infectious particles from infected cells, because the viral genome lacks the F-gene. In addition, the presence of functional mutations such as temperature sensitivity in the amino acid sequence of several SeV proteins (SeV(TS F, SeV(TS12 F, and SeV(TS15 F) renders the vectors easily removable from transduced cells.

Regarding the sequences being carried by this vector, some transcription factors are oncogenic e.g. c-Myc is a known oncogene linked with several forms of cancer, therefore to minimise any potential risk of this oncogene getting into those working with this system use SACGM containment Level 2

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building, we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM1 05/4. 1 (see also above section 6 above for description). Reasons for

02/03/2022
adopting this method of waste disposal are:
1. We the company were prior tenants at the Babraham Institute & Research Campus and adherents to their waste management & disposal procedures and policies.
2. We do not have access to a waste autoclave within our facility or in the building area.
All liquid waste material will be inactivated by treatment with between 1-5% (w/v) "HL04L" solution prior to disposal.
Solid waste material will be placed in sealed plastic bags or bins and incinerated by an off-site incineration company (Grundon Waste Management LTD).
Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable 18kg yellow plastic bags or 60 litre bins. Sealed bags and bins are placed into a secondary 770 litre yellow bin in a secured and under surveillance enclosure. Contents and weight of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made one times a week to prevent a buildup. The Chesterford site has 24/7 Security & Surveillance. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

See above 11.

Furthermore;
GENERAL
ALL biological waste including reagents (cell culture media, washings, etc.), materials (consumables such as pipettes, culture vessels, etc.) that have had contact with the organisms, GMM or otherwise, are contained within yellow sacks or in labelled yellow clinical bins and exit from the facility by 'controlled waste transfer' via a validated, contracted waste disposal party (Grundon Waste Management LTD). ALL contained organisms are rendered waste by HL04L pre-treatment and inactivation with for a minimum of 24hrs to ensure 100% kill as per manufacturer's instructions and advice.

SOLID WASTE
We intend to adopt similar waste disposal procedures used by the Babraham Institute (GM105) and other companies on the Babraham Research Campus (BRC) under derogation (GM105/04.1): Waste from laboratories carrying out GM work at Class1 and 2 will be placed into either yellow sacks (18kg, EWC Code 180103) or 60 litre yellow Clinical Poly Bin (EWC Code 020202) as appropriate. They will be sealed by the producer of the waste and removed to a central, lock secured collection point where they will be placed into 770 litre yellow bin (EWC Code 180103). Collections by the local registered clinical/GM waste incinerator contractor (Grundon Waste Management LTD) will be made once a week to prevent a build-up. The Chesterford Research Park has 24/7 Security & Surveillance. The contractor uses solid bodied transport for waste transfer to the Colnbrook Clinical Incinerator (Berkshire SL3 OEG), and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be maintained on site, as required by the Regulations, in case of loss of the contractor's facilities.

LIQUID WASTE
Liquid waste is inactivated by the addition of "HL04L" (CHEMGENE, tested under EN protocols, biodegradable under OECD conditions) according to the manufacturer's instructions prior to disposal. Validation experiments will be carried out in-house using microbiological testing. Smaller volumes (i.e. <10ml) will then be disposed of in sealed tubes with the solid waste. Larger volumes will be disposed of via the common drainage system. This results in approximately 5
litres of diluted, inactivated waste from each laboratory involved in GM activities. This treated liquid waste is then processed through our own sewage treatment plant which is closely monitored. The total effluent volume from the Chesterford Research Park site is approximately 110,000 cubic metres per day.

The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2</td>
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<td>L2 L3 L4 L2</td>
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<td>L2</td>
<td>L3 L4 L2 L3 L4</td>
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</table>

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**
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Name
CEREVANCE LTD

Name 2
Department

Campus Estate or Research Centre
418 CAMBRIDGE SCIENCE PARK

Road Name
MILTON ROAD

District

Town
CAMBRIDGE

County
CAMBRIDGESHIRE

Postcode
CB4 0PZ

Country
ENGLAND

Tel Number
01223 477910

Fax Number
01223 477911

E-mail
blank

HSE Division

Comments

Date at Which Additional Info Submitted
02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee:
The committee is a sub-committee of the Health & Safety Committee and consists of key representatives from sub-sections of the company involved in GM Organism work. The committee is chaired by a senior member of the scientific staff.
The committee will meet not less than four times per year to review the use of GMOs.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>

Other (please specify) Tick if confidential

Bacteriology Yes Parasitology Transgenic Birds Microbiology Research Yes
The class 1 GMMs that will be generated by Cerevance Ltd will be destroyed by the following regime. All laboratory waste (for example gloves, used plastic ware, pipettes, and anything other potentially contaminated material) will be subject to autoclaving before leaving the premises. Autoclaves are validated yearly by an independent contractor. Solid waste is then sent on site for incineration. In the case of liquid waste, a certified laboratory disinfectant (for example HDL4) will be used to inactivate material overnight before disposal via a designated sink. Bench tops are impervious to water and are resistant to moderate heat and the chemicals used to decontaminate work surfaces and equipment. For minor spillages it is anticipated that a solution of 70% industrial methylated spirit will be used to decontamination - any resultant solid waste will be autoclaved before leaving the building. The laboratory is designed so that it can be easily cleaned. Floors are sealed and impervious to liquids. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Protective laboratory coats, designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the company. Gloves are worn when working with any material or equipment. Gloves are changed frequently or when known to be contaminated and disposed of as solid waste. Gloves are not used for touching "clean" surfaces (keyboards, telephones, etc.), and they are not worn outside of the laboratory. Hands are washed following removal of gloves and before leaving the laboratory environment.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The proposed class 1 activities fall into two categories. The first is the use of attenuated commonly used fully verified commercial non-pathogenic laboratory strains of bacteria for generating plasmid DNA or the expression of non-toxic proteins. The bacteria represent a negligible risk to laboratory staff, the public and the environment. The second is the use of commonly used fully verified commercial laboratory level 1 mammalian cell lines that may be used to exogenously express proteins that are not known to be harmful to humans or the environment. These cell lines can not survive outside of the laboratory environment and represent a negligible risk to laboratory workers, the public and the environment. The methods for inactivating/killing the material will ensure no viable material leaves the laboratory.

**Project Ref 3043/14.2**

**Date Ackn'd**
02/12/2016

**CU2 Project Title**
Generation and production of lentiviral particles for RNA interference (RNAi) mediated knockdown of selected target genes to investigate their role in pathological conditions of the central nervous system

**Class CultureVolClass2 CultureVolumeClass3-4**
Class 2 < 1 Litre

**Non-GMM Consent Granted**

**Project notified under transitional arrangements**
N

**Withdrawn**
N

Tick if notifying a connected programme of work N

**Historical Significant Changes**
transferred from GM3043 02/12/2016

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The purpose of this project is to validate positive and negative modulators of cellular processes that might be useful drug targets that work to delay or halt Neuroregeneration, the process that underlines a number of diseases including dementia. These positive and negative modulators will be identified through a druggable RNAi high content screen in a human non-neuronal cell line that is currently being performed by an outside academic collaborator. Selected targets will be further assessed by looking at their effects in neuronal and non-neuronal cells. To ensure that the targets affect specific cellular processes each of the selected RNAi targets will also be coexpressed with a fluorescent protein (FP) reporter that is directed to a specific organelle. It is expected that depletion will not necessarily cause any major alterations in unstressed neurons. Target dependent depletion through RNAi will have to be performed in a disease relevant model. For this, a lentiviral construct containing a FP tagged or untagged human Neuro degeneration relevant disease mutation or Neurodegeneration disease relevant RNAi will be combined with the RNAi dependent
depletion of the relevant positive/negative regulator. Through this strategy, it is hoped that the identity and validity of one or more targets that may be useful targets for the treatment of Neurodegeneration can be determined. This work will involve the use of particles of lentiviral origin and so require containment to minimise the risk of exposure to both humans and the environment. It should be noted that we will be using third generation lentiviral self inactivating replication defective (SIN-RD) lentiviral particles. The fact that four different plasmids are required to generate these SIN-RD lentiviral particles reduces the risk even further. Nevertheless according to current statutes and as a precautionary measure, all generation and application of virus will be done under Level 2 containment.

Recipient or parental organism
Mammalian cells

Host/vector system

Lentivirus vectors: These are generated using a third generation production system. This uses four plasmids that will include modified versions of pRRL, pMDLgp-RRE, pRSV-REV and PMD2-VSVG. Env. The latter three are the minimum structural genes required for the production of the Self In-Activating (SIN) replication defective lentiviral particles in HEK293T cells that are commonly used for the packaging of lentiviral particles in laboratories. The target shRNA/miRNA or protein encoding gene will be inserted into the modified pRRL vector. This modified vector will have the following safety features: a deletion in the 3’ Long Terminal Repeat (LTR) making it SIN in the infected cells, the LTR containing plasmids Lentivirus vectors: pMDLgp-RRE, pRSV-REV and PMD2-VSVG. Env. The tat promoter has been replaced by a Polymerase Type 2 promoter, making them replication incompetent. The inclusion of these transfected HEK293T. Outside of this setting, viral replication cannot take place and so pose a minimal risk to both humans and the environment. Lentiviral particles are also unstable outside of cell environments or solutions with high protein content at room temperature. Particles are also sensitive to dessication reducing their ability to disseminate. The selected target mammalian cells do not express retroviral elements and due to the replication defective and SIN nature of the lentiviral vectors will not generate viral particles.

Origin & function

The SIN-RD lentiviral particles will contain the following sequences:
1. shRNA/miRNA directed towards the knock down of the expression of a particular endogenous mammalian target (Mouse, Rat, Human). These de novo derived sequences are not derived from an organism and will be designed to only target an individual species. In addition the sequences chosen will not target known oncogenes, tumour suppressors, cell cycle regulators, Immunomodulators and will not be of retroviral origin. Resources that will be used to assess these criteria will include the interrogation of genome wide RNA screens to assess Mitosis and cell death (www.mitocheck.org).

The particles will deliver shRNA/miRNA constructs that will either target one or two genes. For shRNA constructs transcription will be driven by a constitutively active Polymerase III U6 promoter. For some targets shRNA may not represent an effective methodology for knock-down of expression so miRNA constructs prove more effective. This methodology utilises an endogenous cellular mechanism for post-transcriptionlly regulation of target genes. Artificial miRNA sequences are generated using the human miR-30 microRNA cassette under the control of a polymerase II miRNA sequences or an additional protein. The miRNA sequences will be excised by the endogenous cellular machinery before expression of the protein.

2. Fluorescent protein (FP markers which have been identified from a range of different organism; are known to be benign in terms of their effects when expressed in mammalian cells but are essential for visualisation of these cells. When these in addition are fused to a subcellular marker gene, that is expressed only in a particular sub-cellular structure within a cell, will enable the visualisation of a particular process and any changes that occur in response to the knockdown of the expression of a particular gene. Expression of the FP will be either under the control of a CMV promoter which is constitutively active and commonly used in biological research or a promoter that only allows expression in neuronal cells.

3. Human disease relevant mutated gene expression which is intended to recreate a certain aspect of disease pathology. It is expected that its expression will be cytotoxic but will not be a growth factor (which does not include trophic factors), confirmed oncogene, tumour suppressor gene (TSG), cell cycle regulator or immunomodulator, and none of them will be of retrovirial orgin. Expression of these mutant proteins will be either under the control of a polymerase II promoter that is either constitutively active or only active in neuronal cells.

Lentiviral production - on site B60CRP or 430CSP
Where lentivirus is not available from the collaborator, lentiviral production will be carried out on-site. Lentiviral production will only be carried on HEK293T cells and will be carried out in a class 2 microbiological safety cabinet within a Biosafety level 2 laboratory. The HEK293T cells used for lentiviral production will be seeded in filter containing screw cap culture flasks in order to minimize the risk of spills and/or aerosol mediated viral spread. The modified pRL plasmid containing the necessary shRNA/miRNA & protein encoding sequences together with the chemical or lipid based transfection reagent (e.g. Polyethyleneimine (PEI), Lipofectamine) into the HEK293T packaging cell line. After 6-12 hours, the medium is replaced with fresh serum-free medium. (e.g. Neurobasal medium with 2% B-27 supplement and 1X Pen/Strep) and grown for an additional 72-96 hours. After 24-48 hours, sufficient to ensure the viability of the transfected HEK293T cells, medium from the cells is collected into screw cap tube and stored at 4°C and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the first harvest tube. All plastic ware and solutions will be treated as if contaminated.

Still within a Biosafety level 2 microbiological safety cabinet in a Biosafety level 2 laboratory, the harvested supernatants will be filtered using a 0.45 um filter before either being dispensed as aliquots or further purified by chromatography and/or concentrated by centrifugation. For certain application the lentiviral particles will need to be concentrated. The lentiviral particles may need to be purified be either chromatography (e.g. ViraBind™) and eluted in a high salt solution (50mM Tris, pH 7.5, 5 mM MgCl2, 2 M NaCl) and/or concentrated by ultrafiltration (e.g. LentiSelect™). For certain application where a highly concentrated, highly purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimise the possible risk of accidental viral contamination and spread due to the need for an ultracentrifuge, all loading and unloading will be done in the Biosafety level 2 cabinet. Once the sucrose cushions have been added to the centrifuge tubes, they will be transferred into the Biosafety level 2 Safety Cabinet. The unpurified or chromatography purified lentiviral particles will then be added to the sucrose cushion. The tubes will then be transferred to their respective bucket holders and the bucket sealed using their respective screw caps and marked as containing virus. The bucket will then be transferred to a weighing scale and a counterpart bucket and centrifuge with water (and without lentivirus) will be prepared that is weight matched. The balanced buckets will be transferred to the rotor before being placed within the centrifuge chamber. The ultracentrifuge will be run at 70000g for 2 hours at 20°C. Upon return of the bucket to the Biosafety level 2 cabinet, the supernatant will be removed from the tubes before transfer of the resuspended virus pellet into individual screw cap cryovials. As the greatest risk for contamination occurs within the centrifugation bucket itselfs then during transport to and from the weigh scales and ultracentrifuge, not only will the centrifuge tubes but also the buckets decontaminated with DISTEL 10% and 70% isopropanol before removal from the Biosafety level 2. In any case, at no point will concentrated virus stock be exposed to outside of the Biosafety level 2 hood environment.

The lentiviral titre will be determined in terms of the number of viral particles (VP) or Transduction units (TU) per ml. Assessing the number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based enrichment assay (e.g. QuickTitre™ Quantification Kit) or a p24 ELISA assay (e.g.QuickTitre™ Lentivirus Titre Kit).will be chosen. The VP quantification methods tends to overestimate the TU by 10-100 fold. Thus, the TU/ml will in certain instances also be determined. For this, a titration range of lentiviruses expressing a fluorescent tag, serial dilution will be prepared and added to a 24 well cluster plate of T25 TC-flasks containing HEK293T for 48 hours. The percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using FACS analyser. The biological titre (TU/ml) according to the following formula: TU/ml = (P x N/100 x V) x 1/DF, where P=% GFP+ cells, N=number of cells at time of transduction =105, V=volume of dilution added to each well and DF=dilution factor.

Aliquots with not more than 1x108 viral per viral are subsequently stored at -80°C in individual screw capped cryotubes. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats.

Assessment of effects
Assessing whether the knock down has worked in neurons can be challenging. Consequently, initially the RNAi depletion of the target gene (using the FP tagged verison followed by the RNAi sequence) will be assessed in immortalised cell lines (mouse NIH/3T3 and human HEK293T and human HEK293T). This work will be done initially at Biosafety level 2. In order to determine whether the knock-down has worked; the levels of mRNA and protein will be assessed post-transduction, using suitable in vitro assays, including for example, RT-PCR, Western Blotting, and immunocytochemistry. These results will subsequently be confirmed on neurons such as those derived from human induced Pluripotent stem cells (iPCs) using immunocytochemistry.

The correct distribution and perturbed cellular functions of the relevant human Neurodegenerative disease mutations will also be assessed by subsequent
Immunocytochemistry and Bioenergetic analysis (using Seahorse™). Other physiological parameters, including, for example, the release of specific neurotransmitters from neurons or the expression of related proteins will be measured using suitable in vitro assays, including, for example, ELISA, PCR, neurite outgrowth, electrophysiology, and neuroprotection assays. In some instances, the vectors might need to be studied in rodent neuron-glial co-cultures in order to investigate the interaction between these two cell types.

Evaluation of foreseeable effects

Human health hazards

The method by which the lentivirus vector is produced means that it is highly unlikely that RCL can be generated even if an infection occurs in a person who is HIV-2 positive. Infection can lead to a permanent transduction of cells and the possibility of a harmful event either by transactivation or disruption of gene expression can not be ruled out. For this reason the work with stock viral aliquots will be conducted as a class II activity. Oral ingestion will be prevented by involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the genes/proteins of interest that will be targeted by shRNAs do not include any growth factors, confirmed oncogenes, cytotoxins, or immunomodulators, and none of them are of retroviral origin. The mutant genes express proteins known to be cytotoxic to neurons but it is highly unlikely such cells would be transduced via the mucosal or skin routes. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus, this will be self-limited due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent and being extremely sensitive to desiccation.

Hazard to the Environment

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable RCL. This scenario is, however, extremely unlikely, and even if it was to occur, it is unlikely that the vectors could survive long enough in the environment to pose a risk if accidentally released.

Furthermore, lentiviral vectors have a short half-life at room temperature due to their structural characteristics making them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, and this is illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA and miRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

Summary

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as Biosafety level 2, and effective containment procedures will be adhered to (see section C for details). However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems (see above discussions).
### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Following transduction of cell lines that are designated as requiring Biosafety Containment Level 1 with lentivirus we wish to be able to use these cells at Biosafety Containment Level 1. This can be justified by the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24 hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as only requiring Biosafety Containment Level 1.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- **General waste handling procedures:**
  - Solid waste will be neutralised by soaking it in a validated disinfectant solution (Virkon* or Distel) for at least 24 hours, after which the material will be 'double bagged', autoclaved and, ultimately, incinerated offsite.
  - At B60CRP, liquid waste will be disposed of into sealed biohazard containers filled with Virkon* for incineration. It will be routine practice that all liquid waste material be neutralised for at least 24 hours before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. At 430CSP, liquid waste will be inactivated by Virkon* for 24 hours prior to drain disposal via a designated sink.

  *Virkon*
  - Rely+On™Virkon® is a multi-purpose disinfectant. It contains oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds such as proteins, leading to widespread, irreversible damage and subsequent deactivation/destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance against over 500 strains of viruses, bacteria and fungi. Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control. Importantly, Virkon is sold as a powder which dissolves readily in water. It is intended to be mixed with water to form a 1% solution (i.e. 10g per litre) for hard surface and equipment disinfection. 1:100 is also the dilution rate advised for virucidal efficacy against HIV-1.
  - The product has a pink colour, which is useful in that it helps to guage the concentration of a prepared solution, and importantly, as Virkon ages, it discolours, making it obvious when it needs to be replaced. The solution is generally stable for five to seven days. Moreover, there is no evidence to suggest that bacterial disease-causing organisms develop resistance towards Virkon, as opposed to some other disinfectant products. Moreover, Virkon is not classified as R53, it is classified as readily biodegradable, and it does not persist in the environment.

  *Distel (formally Trigene advance)*
  - Distel is formulated as a nanoemulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms, ensuring the rapid induction of cell death in treated micro-organisms. Distel works quicker than concentrated high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products. Distel has been reported to be bactericidal, fungicidal as well as virucidal and sporicidal. It is recommended to be used at a 1:200 dilution for general purposes, 1:100d for high risk areas, and 1:50 for disinfection of blood and bio-hazard spillages.

**Summary**

As any active viral particles on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles would unintentionally become discharged into a sanitary sewage system. No forseeable adverse effects on human health and safety are expected.

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**Is an emergency plan required according to regulation 20?**  N

**If yes, tick to confirm that it is attached to this form**  N
The GMSC has agreed that the production and handling of virus stocks is a Class 2 activity. This decision has been reached because although the lentivirus will be generated using a third generation, self-inactivating, 4 plasmid expression system, the possibility of a recombination event occurring that leads to replication competent virus can never be discounted with this type of virus. The potential oncogenic effects of the Woodchuck Post-transcriptional Regulatory Element have been negated by the use of the mutant form that prevents expression of the X protein. The production of large amounts of virus represents the most hazardous aspect of the proposal and the GMSC is satisfied that the necessary precautions have been put in place to ensure containment is maintained. Some of the proposed nervous system. The procedures put in place to ensure containment is maintained. Some of the proposed lentivirus will encode for proteins that are known to have neurotoxic effects and cause diseases of the human central nervous system. The procedures put in place will effectively prevent any chance of virus being delivered to the necessary tissues within a human in sufficient quantities to represent a hazard to human health. The FP and shRNA/miRNA encoded by the other lentivirus are not known to pose any inherent risk to human health or the environment. The proposed targets to be knocked down in this study also pose no known risk. The request to derogate the Biosafety Containment Level from 2 to 1 post transduction has been deemed acceptable given that the transduced cells will be washed extensively to remove any remaining viral particles and will be tested for the absence of transducing virus. It has been made clear that human iPSC cell lines that have been generated using lenti- or retrovirus methods tested negative for the absence of virus at least. These cells will always be tested for the absence of infectious particles before they can be used as if they only required Biosafety Containment Level 1.

Project Containment

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<th>Growth Rooms</th>
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<td>L3 L4</td>
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<td>Human Clinical Applications</td>
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Project Ref  3043/14.3

Lentiviral siRNA knockdown studies to identify and validate drug targets for psychiatric and neurodegenerative diseases

Date Ackn'd  02/12/2016
Date Project Ceased

Class 2
Consent Granted

Project notified under transitional arrangements  N
The lentiviral siRNA knockdown studies that will be carried out as part of this body of work will be focused on discovering the role of specific proteins in both physiological and pathological eNS processes, with the overarching aim of identifying and validating potential new drug targets for psychiatric and/or neurodegenerative diseases. The proteins that will be selectively knocked down by lentivirally expressed siRNA will include those that have been either directly or indirectly linked to relevant eNS disease states; and, in most cases, the targeted proteins will play a role in well characterised relevant disease-related biologicaVpathological processes. Relevant neurodegeneration related processes, for example, are likely 10 include, bioenergetics, oxidative stress, neure-inflammation, protein misfolding, excitotoxicity, and autophagy. Psychiatric disease related protein targets, on the other hand, are likely to play a role in neurogenesis, neural plasticity, or the functioning of one or more disease-linked neurotransmitter systems.

Importantly, the genes/proteins of interest that will be targeted by siRNAs do not include any growth factors, confirmed oncogenes, tumour suppressors, cell cycle regulators, cytotoxins, or immunomodulators, and none of them are of retroviral origin. All selected targets will be presented to the GMSC for approval.

Methods
The approach to be taken will be the application of lentiviral vectors to cell lines that are deemed biosafety level 2 or lower, including well characterised immortalised cell lines that are known not to contain lentiviral genetic material, and human induced Pluripotent stem cells (iPSCs). The lentiviral vectors will either express siRNA transcripts targeting specific mRNAs/proteins of interest, or with vectors expressing scrambled siRNAs, in the case of the negative control treatment groups. The extent of mRNA and protein knockdown that is achieved will then be assessed at different time points (e.g. 24h-48h-72h), post-transduction, using suitable in vitro assays, including for example, RT-PCR, Western Blotting, and immunocytochemistry. Once the conditions that lead to the optimal knockdown of the mRNAs/proteins have been determined, it will be evaluated what effects the knockdown of the proteins have on various physiological parameters, including, for example, the release of specific neurotransmitters from neurons or the expression of related proteins. These siRNA-induced alterations will be measured using suitable in vitro assays, including, for example, ELISA, PCR, neurite outgrowth, electrophysiology, and neuroprotection assays. In some instances, the vectors might need to be studied in neuron-glia co-cultures in order to investigate the interaction between these two cell types.

Description of the lentiviral vectors used for the experiment
Third generation HIV based shRNAmiRNA expressing lentiviral vectors will be produced either internally or sourced from external suppliers, which will include reputable commercial suppliers, and an academic group at the University of Bristol headed by Prof. James Uney. Each aliquot of lentiviral reagent will comprise of a small volume (5 to 301-Jl) that contains no more than 1x10e08 viral particles. Post-transduction of target cells, the vectors will
produce both a shRNmiRNA targeting the mRNAs of a protein of interest, and a reporter protein to facilitate the identification of transduced cells.

Recipient or parental organism
Mammalian Cells

Host/vector system

Vector system = self inactivating (SIN) non replication-competent HIV based lentiviral vectors

Health and Safety

Executive

The viral vectors that will be used in the proposed studies are third generation self-inactivating (SIN) non replication-competent HIV based lentiviral vector systems, which have been designed to produce stable gene expression in mammalian cells; and they are generated by co-transfecting a suitable immortalized packaging cell line (e.g. HEK 293T cells) with four separate plasmids. Each of the plasmids used (described below) expresses a different set of genes, and all of the genes from the different vectors, when combined (following co-transfection), provide the smallest possible set of essential viral genes that is SIN compatible with virus production. Moreover, the resultant vectors are all vesicular stomatitis virus (VSV-G) pseudotyped lentiviral vectors, which are self-inactivating and highly unlikely to undergo recombination. Importantly, the viral vector system is inherently incapable of replication in mammalian cells. Only in a patient with HIV could any form of recombination occur, i.e. the probability of the production of replication competent lentiviral vectors (RCL) is very small.

The plasmids

The sequences that are required to generate the viral vectors are sub-cloned into 4 standard bacterial plasmid vectors. The transgene expression plasmid will contain the transgene that codes for a relevant siRNA sequence and/or a transduction marker protein (e.g. GFP), while the other 3 packaging plasmids will contain 3 different sets of sequences that code for different viral packaging proteins. A brief overview of the 4 different plasmids is provided below:

Plasmids containing the transgene of interest

The transgene expression plasmid will contain the sequences that code for the siRNA transcript that will target a specific mRNA/protein of interest (or a scrambled siRNA in the case of the negative control), and also for a transduction marker protein. Control viral vectors that express only a marker protein and no siRNA will also be used in some experiments, and the corresponding plasmids for these vectors will only contain the sequence coding for the marker protein. Depending on the knockdown strategy taken, either shRNA or miRNA based constructs will be used to express the artificial siRNA sequences. In some of the constructs used, siRNA and marker protein sequences will be expressed as two separate transcripts under the control of Ivo separate promoter elements. In other instances, siRNA and marker protein expression will be under the control of a single promoter system. The promoter elements used to control transgene expression will vary depending on the specific plasmid that is employed. Only well characterized promoter systems will, however, be used to drive transgene expression. These will include the H1, U6 small nuclear, cytomegalovirus (CMV), elongation factor 1 alpha (EF10), phosphoglycerate kinase (PGK), ubiquitin C (UbC), and murine stem cell virus (MSCV) promoters; or in instances where siRNA expression in a specific cell subtype is desired, polymerase III promoters such as the synapsin I (SynI), neuron specific enolase (NSE), tyrosine hydroxylase (TH), calcium/calcmodulin-dependent protein kinase II (CamKII), or glial fibrillary acidic protein (GFAP) promoters.

Additionally, all of the transgene constructs will contain the WPRE, cPPT, RRE, and 41 sequences, as well as the modified LTR, and these sequences are the only other coding sequences (apart from the siRNA and transduction marker proteins) that will be present in the viral vectors. The latter elements are important for the expression of the
shRNA in targeted cells, and they will all be stably expressed in infected cells.

Plasmids containing the packaging related sequences
The three other plasmids that make up the 4 plasmid system will each contain a different set of sequences that code for proteins that are required for production and packaging of the viral vectors. Gag-pol will be expressed by one of the plasmids, rev by a second, and VSV-G by a third, and the latter proteins will only be provided in trans during the production phase. The specific packaging plasmids that will be used will vary depending on the packaging system that is employed. Only well characterized packaging plasmids will, however, be used in all cases. For example, in one system, gag-pol, rev and VSV-G will be expressed separately by pMDLgp-RRE, pRSVREV, and pMD2-VSVG.env packaging plasmids, respectively, whereas, in a second, they will be expressed by pPACKH1-GAG, pPACKH1-REV, and pVSV-G, respectively. Importantly, all of the above genes, that are essential for production of fulllength viral particles, have been removed from the expression plasmids containing the transgenes of interest. The 4 plasmids used in the system have also been engineered so as to have no common sequences, which greatly reduces the risk of a recombination event that would result in the insertion of production/packaging genes into the transgene expression plasmid.

The expression systems and the transgenes that comprise the lentiviral vectors that will be used have a very low risk for human health and safety, and the reasons for this are discussed below.

Lentiviral Vector Expression System
Lentiviruses belong to the Retroviridae virus family, a diverse and extensive family of viruses, which are capable of infecting both human and animal species. Retroviruses are characterised by a unique replication mechanism involving reverse transcription of the viral RNA genome, which gives rise to a DNA provirus that contains two positive sense copies of the viral RNA genome; and in a functional assembled virus, the viral genome is encased within a capsid that is surrounded by a host-cell derived envelope. Cellular entry of the virus involves interactions between glycoproteins contained in the virion's envelope and extracellular plasma membrane proteins on target cells. These interactions are generally specific and are believed to be the principal factor determining which species and type of cells a specific virus is capable of infecting. The binding of a virus's envelope glycoproteins to an appropriate receptor site on a target cell triggers the fusion of the virion's envelope membrane with the plasma membrane of the target cell, which results in the delivery of the virus capsid to the intracellular space of the target cell.

As the lentiviral vector system that will be used in these studies is based on HIV-1, a theoretical risk to human health exists. Retroviruses are, however, generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and, they are, generally, not transmitted via the airborne route. Accidental piercing of the skin or other surface tissues with virus containing objects, therefore, represent the main potential route by which accidental infection could occur. Appropriate risk reduction measures will, however, be implemented to reduce the likelihood of this occurring.

In all of the transgene expression plasmids, the genes of interest are flanked by non-coding retroviral TRs, and no retroviral genes are encoded on the transgene plasmids. Therefore, no retroviral genes will be transferred into generated viral particles. The transgene construct is packaged into particles using an immortalised cell based packaging system, which requires the co-transfection of packaging cells (e.g. HEK293 cells) with three additional separate packaging plasmids (pMDLgp-RRE, pRSV-REV and pMD2-VSVG.env, for example). The latter 3 pJasmids express the envelope protein from VSVG and the non-structural proteins of the virion, and, importantly, none of these genes will be transferred into the assembled viral vectors, since they lack the packaging signal (+), which is only present on the transgene containing plasmids.

The lentiviral Expression System that will be used include the following key safety features:
All of the lentiviruses will be generated using a 4 plasmid/3 retroviral gene (gag, pol, rev) system, which yields replication-incompetent vectors that are devoid of all viral sequences, apart from essential cis-acting sequences, including the L TRs and the packaging signal '+', and only the transgene plasmids will contain l['1. This system allows for the expression in trans of proteins required to produce viral progeny (e.g. gal, pOl, rev, env) in producer cell lines, which do not contain L TRs or the '+' packaging sequence. None of the retrovirus structural genes will, therefore, actually be present in the packaged viral genome, which means that no new replication-competent lentivirus (RCL) can be produced.

Importantly, following infection of a target cell, lentiviral vectors are self-inactivating on integration into the host genome. This is achieved because the process of reverse transcription uses the 3' long terminal repeat (3' l TR) as a template to produce the S' l TR. In the transgene expression plasmids that will be used, the U3 region of the 3' l TR has been deleted, resulting in deletions in both 3' and S' L TRs on integration into the host genome. The deletion in the 3' l TR (aU3) does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" (SIN) of the vector after transduction of target cells, which ensures that the viral genome cannot be released from the host genome.

Moreover, deletion of enhancer and promoter elements from the 3' U3 region in the vector constructs will result in a provirus that is entirely devoid of the U3 enhancer sequences, which will result in a reduction in the potential for transactivation of cellular genes due to an insertion event. Self-inactivating vectors are also less likely to be mobilized following a superinfection with wild-type virus (HIV), and the VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes, obviating safety concerns associated with the use of HIV-1 gp120, which has known pathogenic consequences. Importantly, all of the 4 plasmids used in the system have been engineered not to contain any regions of homology with each other so as to prevent undesirable recombination events that could lead to the generation of a (RCI).

Lentiviral production - on site B60CRP or 430CSP

Lentiviral production will also be carried out on-site. Lentiviral production will only be carried on HEK293T cells and will be carried out in a class 2 microbiological safety cabinet (MSC) within a dedicated Level 2 laboratory. The HEK293T cells used for lentiviral production will be seeded in filter containing screw cap culture flasks in order to minimize the risk of spills and/or aerosol mediated viral spread. For this, a modified pRRL transgene expression plasmid together with three additional separate packaging plasmids (pMDLgp-RRE, pRSV-REV and PMD2-VSVG/env) will be transfected using a transfection reagent (e.g. Polyethylenimine (PEI), Lipofectamine) into HEK293T cells. After 6-12 hours, the medium is replaced with fresh serum-free medium (Neurobasal medium with 2% B-27 supplement and 1X Pen/Strep) and grown for an additional 72-96 hours. After 24-48 hours, so as to ensure the viability of the transfected HEK293T cells, medium from the cells is collected into screw cap tube and stored at 4°C and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the first harvest tube. After lentivirus collection has been completed, the cells in the flasks will be treated with a validated anti-microbial agent (e.g. DISTEL 10% or another equivalent product) for at least 24 hours prior to autoclaving on site. Autoclaved material will then subsequently be incinerated. Still within a Class 2 MSC in a Level 2 Biosafety laboratory, the harvested supernatants will be filtered using a 0.45 micron filter before either aliquotted or further purified by chromatography and/or concentrated by centrifugation. For certain applications, the lentiviral particles will need to be concentrated. The lentiviral particles may need to be purified by either chromatography (e.g. ViraBindTM) and eluted in a high salt solution (50mM Tris, pH 7.5, 5mM MgCl2, 2M NaCl) and/or concentrated by ultrafiltration (e.g. LentiSelectTM). For certain applications where highly concentrated, highly purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimise the possible risk of accidental viral contamination and spread due to the need for
an ultracentrifuge, all loading and unloading will be done in a class 2 MSC. Once the sucrose cushions have been added to the centrifuge tubes, they will be transferred into a class 2 MSC. The unpurified or chromatography purified lentiviral particles will then be added to the sucrose cushion. The tubes will then be transferred to their respective bucket holders and the bucket sealed using their respective screw caps and marked as containing virus. The bucket will be transferred to a weighing scale and a counterpart bucket and centrifuge with water (and without lentivirus) will be prepared that is weight matched. The balanced buckets will then be transferred to the rotor before being placed within the centrifuge chamber. The ultracentrifuge will be run at 70000g for 2 hours at 20°C. Upon return of the buckets to the class 2 MSC, the supernatant will be removed from the tubes before transfer of the resuspended virus pellet into screw cap cryovials. As the greatest risk for contamination occurs within the centrifugation buckets themselves during transport to and from the weighing scales and ultracentrifuge, not only will the centrifuge tubes but also the buckets decontaminated with DISTEL 10% and 70% isopropanol before removal from the level 2 laboratory. In any case, at no point will concentrated virus stock be exposed to outside of the class 2 MSC environment.

The lentiviral titre will be determined in terms of the number of viral particles (VP) or Transduction units (TU) per ml. Assessing the number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based enrichment assay (e.g. QuickTitre™ Quantification Kit) or a p24 ELISA assay (e.g. QuickTitre™ Lentivirus Titre Kit) will be chosen. The VP quantification methods tend to overestimate the TU by 10-1000 fold. Thus, the TU/ml will in certain instances also be determined. For this, a titration range of lentiviruses expressing a fluorescent tag, serial dilution will be prepared and added to a 24 well cluster plate or T25 TC-flasks containing HEK293T for 48 hours. The percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using FACS analyser. Aliquots with not more than 1x10⁸ virus per vial are subsequently stored at -80°C in screw capped cryotubes. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to, inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats.

Transgene: proteins to be expressed

Health and Safety

Executive

Additional hazards that arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.

A. Transduction Marker Proteins

Only well characterised transduction marker proteins that are not associated with any cytotoxic, immunogenic or oncogenic responses will be employed, including, for example, fluorescent proteins such as GFP, GFPem, YFP, OsRed, mCherry, CFP.

B. shRNmiRNA: Endogenous proteins to be depleted

The proteins that will be selectively knocked down by lentivirally expressed siRNA will include proteins that have been either directly or indirectly linked to psychiatric and/or neurodegenerative disease states; and, in most cases, the targeted proteins will play a role in well-characterised relevant disease-related biological/pathological processes. Relevant neurodegeneration-related processes, for example, are likely to include, bioenergetics, oxidative stress, neuro-inflammation, protein misfolding, excitotoxicity, and autophagy. Psychiatric disease-related targets, on the other, are likely to play a role in neurogenesis, neural plasticity, or the functioning of one or more disease-linked neurotransmitter systems. Importantly, the genes/proteins of interest that will be targeted by siRNAs do not include any growth factors.
confirmed oncogenes, tumour suppressors, cell cycle regulators, cytotoxins, or immunomodulators, and none of them are of retroviral origin. Depending on project objectives and human vs. rodent mRNA sequence homology, siRNA sequences will be designed either to specifically target only the human or mouse/rat mRNA sequence of a target protein, or both the human and mouse/rat sequences.

RNA interference (RNAi)

RNA interference (RNAi) is an antisense technology that exploits a normal cellular antiviral response that acts to inhibit viral protein synthesis through the production of short hairpin RNAs (shRNAs) that bind and target viral double-stranded RNA (dsRNA) molecules for degradation. The shRNA molecules are processed by cellular enzymatic pathways to produce small inhibitory RNA (siRNA) species, and it is the latter RNA molecules that bind and target complementary RNA sequences for degradation by the RNA-induced silencing complex (RISC). With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be 'knocked-down', in order to study their functions. Potential deleterious effects such as off-target and immunomodulatory responses can be minimised through screening designed siRNA sequences against databases of known mammalian gene/mRNA sequences during the design stage.

MicroRNAs (miRNAs) are endogenously encoded 22-nl-long RNAs that are generally expressed in a highly tissue and/or developmental-stage specific fashion, and they function to post-transcriptionally regulate the expression of target genes. In certain experimental RNA interference approaches, this miRNA system can be harnessed/manipulated to study the functions of specific genes/proteins. The additional benefit is that one can place these miRNA sequences under the control of a Polymerase II promoter in which the miRNA or multiples thereof are co-transcribed with a coding sequence. For example, RNA polymerase II promoters can be experimentally activated so as to over-express endogenous microRNAs in cell culture systems. Alternatively, artificial microRNAs can also be engineered to match the features of existing microRNA genes, such as the gene encoding the human miR-30 microRNA. In an analogous method, the BLOCK-iTTM Pol II miR RNAi expression vectors from life Technologies allow for selected miRNA sequences that are flanked on both ends with flanking sequences that allows for their successful transcription under a Polymerase II promoter. Additional coding sequences containing a fluorescent protein tag are placed 5' to the miRNA. Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA/miRNA), and which is not complementary to any known mammalian sequences. These vectors will be employed as negative controls to demonstrate that any observed effects are due to the knockdown of a specific mRNA/protein of interest, rather than being due to any non-specific effects that the delivery of the viral vectors might cause. This sequence will adopt a hairpin structure as with any shRNA/miRNA, but it should not target any mRNA of mouse, rat or human origin. It will only serve as the negative control for the vector producing shRNA/miRNA. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health.

Summary

The transgenes that will be expressed by the viral vectors have a very low safety risk to human health. This is because accidental contamination with a vector would lead to only a small number of cells becoming infected, and it would be highly unlikely that expression in only a few cells would be sufficient to lead to a disease state. The inability of the lentiviral vectors to propagate in mammalian cells also reduces the risk. The transduction marker proteins that will be employed are not known to cause any relevant toxicities, and the genes/proteins of interest that will be targeted by siRNAs do not include any growth factors, confirmed oncogenes, cytotoxins, tumour suppressors or immunomodulators, and none of them are of retroviral origin.

Evaluation of foreseeable effects

Human health hazards
Although the lentiviral vectors that will be used are replication incompetent and contain only \(-20\%\) of the original HIV-1 genome, there is a small risk that subsequent infection of cells already infected with the lentiviral genome by HIV-, could lead to a rare recombination event in which the transgene is transferred to a replication-competent virus. Thus, the sequences in the vector that will be expressed could potentially be transferred to surrounding cells. This event is, however, extremely unlikely to occur, and it has been shown that, even under permissive in vitro conditions, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host genome (Bukovsky et al. 1999).

Additionally, there is a theoretical risk that an infection event with the vectors could lead to the infected cells becoming cancerous, as lentiviral integration into a host genome could potentially lead to the activation of an endogenous oncogene. All transcriptionally active long-terminal repeats (LTRs) have, however, been removed from the viruses as well as all promoter-like elements other than that required to drive expression of the transgene, which should prevent unforeseen activation of such genes. The deletion of the retroviral enhancer in self-inactivating systems reduces the risk of activation but not of disruption, and, therefore, retroviral infection might still have permanent effects upon a cell (including oncogenic effects).

Importantly, the likelihood of a worker becoming accidently infected is, however, considered to be low, as appropriate risk reduction measures will be implemented. Notably, micro-syringes will be filled with viral vectors only in a class 2 BSL to avoid the production of aerosols. The most likely route of accidental infection with a lentivirus will be via inadvertent percutaneous inoculation. The likelihood of this occurring will be minimised by following the correct procedures. Oral ingestion will be prevented by standard laboratory safety practices. Moreover, the genes/proteins of interest that will be targeted by shRNAs do not include any growth factors, confirmed oncogenes, tumour suppressors, cytotoxins, or immunomodulators, and none of them are of retroviral origin. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

HAZARDS TO THE ENVIRONMENT

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active viral vector particles do accidently get released into the environment, the safety risks posed by such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable RCL. This scenario is, however, extremely unlikely, and even if it was to occur, it is unlikely that it would lead to any untoward effects.

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and they are, in theory, capable of transducing all dividing and non-dividing mammalian cell types. As emphasised before, the vectors, however, cannot self-propagate after infection, and successful transduction is also critically dependent on the presence of high enough concentrations of virus particles; and viral vector stock solutions will be managed in a way that will prevent contaminations with relatively high viral titres. After production, the vectors are aliquoted and stored in small volumes (each tube contains at maximum 30μl) at -SO·C, and when taking into account that the infectivity of the vectors rapidly decrease at room temperature, it is considered highly unlikely that the vectors could survive in the long term after being accidentally released into the environment.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA and miRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed
above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

Summary
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will undertaken have been classified as CL2, and effective containment procedures will be adhered to. However, in the unlikely event that active viral vector particles do accidently get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems (see above discussions).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Following transduction of eel! lines that are designated as requiring Biosafety Containment level 1 with lentivirus, we wish to be able to subsequently use these cells at Biosafety Containment level 1. This can be justified by the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24 hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as only requiring Biosafety Containment Level 1. Sources of iPSC cells that have been shown to lack viral integration and test negative for infectious virus can be regarded as requiring biosafety level 1 containment. Such iPSC cells can also be derogated to biosafety level 1 containment once tested for the absence of infectious virus.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General waste handling procedures:
Solid waste will be neutralised by soaking it in a validated disinfectant solution for 24 hours, after which the material will be 'double bagged', autoclaved and, ultimately, incinerated offsite. At B60CRP, liquid waste will be disposed of into sealed biohazard containers filled with Virkon* for incineration. It will be routine practice that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. At 430CSP, liquid waste will be inactivated by Vir<on* for 24 hours prior to drain disposal.

• Virkon
Rely+OnT~ Virkon® is a multi-purpose disinfectant. It contains oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds, such as proteins, leading to widespread, irreversible damage and subsequent deactivation/destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance against over 500 strains of viruses, bacteria and fungi, Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control. Importantly, Virkon has specifically been validated for lentiviral inactivation (Antec-Biosentry).

Virkon is sold as a powder which dissolves readily in water. It is intended to be mixed with water to form a 1% solution (i.e. 10 g per litre) for hard surface and equipment disinfection. 1:100 is also the dilution rate advised for virucidal efficacy against HIV-1.

The product has a pink colour, which is useful in that it helps to gauge the concentration of a prepared solution, and importantly, as Virkon ages, it discolors, making it obvious when it needs to be replaced. The solution is generally
stable for five to seven days. Moreover, there is no evidence to suggest that bacterial disease-causing organisms develop resistance towards Virkon, as opposed to some other disinfectant products. Moreover, Virkon is not classified as R53, it is classified as readily biodegradable, and it does not persist in the environment.

• Distel
Distel is formulated as a nanoemulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms, ensuring the rapid induction of cell death in treated micro-organisms. Distel works quicker than conventional high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products. Distel has been reported to be bactericidal, fungicidal as well as virucidal and sporicidal. It is recommended to be used at a 1:200 dilution for general purposes, 1:100 for high risk areas, and 1:50 for disinfection of blood and bio-hazard spillages.

Summary
As any active viral particles on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.

Please enter comments on the GM safety committee on the risk assessment

The GMSC has agreed that the production, handling and use of lentivirat siRNA stocks is a Class 2 activity. This decision has been reached because although the lentivirus will be generated using a third generation. self-inactivating 4 plasmid expression system, the possibility of a recombination event occurring that leads to replication competent virus can never be discounted with this type of virus. The potential oncogenic effects of the Woodchuck Post-translational Regulatory Element have been negated by the use of the mutant form that prevents expression of the X protein. The proposal includes the potential to produce large amounts of virus. This represents the most hazardous aspect of the proposal and the GMSC is satisfied that the necessary precautions have been put in place to ensure containment is maintained. Some of the proposed lentivirus may encode for proteins that are known to have neurotoxic effects and are associated with diseases of the human central nervous system. The procedures put in place will effectively prevent any chance of virus being delivered to the necessary tissues within a human in sufficient quantities to represent a hazard to human health. The FP and shRNmiRNA encoded by the other lentivirus are not known to pose any inherent risk to human health or the environment. The proposed targets to be knocked down in this study also pose no known risk. The request to derogate the Biosafety Containment level from 2 to 1 post transduction has been deemed acceptable given that the transduced cells will be washed extensively to remove any remaining viral particles and will be tested for the absence of transducing virus. It has been made clear that human iPSC cell lines that have been generated using lenti- or retrovirus methods tested negative for the absence of virus at least. These cells will always be tested for the absence of infectious particles before being they can be used as if they only required Biosafety Containment level 1.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
# Project Containment

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**Project Ref** 3043/15.1

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<td>Generation, production and utilisation of lentiviral particles for delivery of programmable nucleases to disrupt selected target genes in vitro</td>
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Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

**Historical Significant Changes**

Transferred from GM3043 02/12/2016

**Project Additional Information**

**Purposes of the contained use**

**Aim**
The purpose of this project is to use lentivirus to deliver programmable nucleases, specifically zinc finger nucleases and Cas9/CRISPR, to disrupt gene function in mammalian cultured cells. The primary aim is to use gene targeting to validate novel targets for drug discovery and to set up disease relevant model systems in vitro.

**Recipient or parental organism**

Rodent Origin: Immortalised cell lines.
Human Origin: HEK293T, immortalised cell lines and induced pluripotent stem cells
Host/vector system

Lentivirus vectors: The four plasmids that will be used will be a modified version of pRRL, pMDLgp-RRE, pRSV-REV and PMD2-VSVG.env. The latter three are required for the production of the SIN replication defective lentiviral particles in HEK293T cells. The target shRNA/miRNA or gene will be inserted into the modified pRRL vector. This modified vector will have the following safety features: a deletion in the 3'LTR making it SIN in the infected cells, the packaging sequence is only found in the pRRL with the packaging constructs being provided by the remaining non-LTR containing plasmids Lentivirus vectors: pMDLgp-RRE, pRSV-REV and PMD2-VSVG.env. The Tat promoter has been replaced by Polymerase Type 2 promoter, making them replication incompetent. The inclusion of these features ensures that the production of these SIN replication defective lentiviral particles only occurs in the transfected HEK293T. Outside of this setting, viral replication cannot take place and so pose a minimal risk to both humans and the environment.

Origin & function

The proposal involves the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site. These constructs will contain a combination of programmable nuclease plus accessory sequences and a fluorescent protein as lineage marker. In some cases constructs will contain a combination of programmable nuclease plus accessory sequences and a selectable marker giving rise to a selectable trait in transduced cell lines. Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the cleavage site or by recombination with user-supplied synthetic vector containing sequences complementary to the target gene. Insertion of the sequences into the third generation lentiviral vector on site will be achieved by either conventional cloning or Recombineering. Lentiviral particles will either be purchased ready for use or produced in-house using the third generation system described below. Nuclease encoding lentivirus may be used in isolation or co-transduced with lentivirus encoding a targeting construct designed to recombine and insert defined sequences into the target gene.

The SIN-RD (Self-Inactivating-Replication Deficient) lentiviral particles will contain a combination of the following sequences:
1. Programmable nucleases (Zinc fingers and Cas9/CRISPR) designed to cause double stranded breaks in particular endogenous mammalian targets (Mouse, Rat, Human).
2. Fluorescent protein markers that are known to be benign in terms of their effects when expressed in mammalian cells but are essential for visualisation of these cells. When these in addition are fused to a subcellular marker gene, subcellular visualisation of a particular process can be visualised.
3. Selectable marker for conferring a selectable trait to mammalian cells in vitro. Exposure to the relevant compound will result in survival of cells containing the viral vector (positive selection) or elimination of cells containing the viral vector (negative selection).

A. Programmable nucleases: zinc finger nucleases and Cas9/CRISPR
Zinc finger nucleases and Cas9/CRISPR are both programmable nucleases systems that are designed to cause cleavage of a pre-determined DNA sequence. Zinc fingers are proteins of mammalian origin comprising a DNA binding domain which confers sequence specificity and a nuclease domain which cleaves the target sequence subsequent to precise binding. Cas9/CRISPR is a bacterial protein/RNA complex that contains a nuclease domain and an RNA-binding domain; the sequence specificity of Cas9 is determined by the sequence of the RNA (referred to as the guide RNA) with which it is complexed. To effect cleavage the guide RNA has to be supplied by co-expression with Cas9.
Zinc fingers or Cas9 protein will be directed against genes of therapeutic interest identified by literature reports or by in-house screens. Since the purpose of programmable nucleases is to cause mutations in defined DNA sequences the primary hazard is that such a mutation would lead to deleterious cellular effects. The genes/proteins of interest that will be targeted do not and will not include any growth factors (which does not include trophic factors), confirmed oncogenes, tumour suppressors or immunomodulators. In isolation these sequences are unlikely to pose any safety risks for the environment or human health.

B. Fluorescent protein tag proteins:
Genetically encoded Fluorescent proteins, such as green fluorescent protein (GFP), have been widely used as a reporter for biological research over the past decades. Their exogenous expression is not associated with any cytotoxic, immunogenic or oncogenic response. A large selection of different FP constructs have been and continue to be generated, which differ in their optical and stability properties. A current non-exhaustive list of such FPs that will be used include, GFP, GFPem, YFP, DsRed, mCherry, CFP. Further variants that may be used can be found here (http://www.einstein.yu.edu/research/facilities/fluorescent/)
Genes that confer resistance or sensitivity to antibiotic compounds are routinely used in mammalian cell culture. Examples include Neo (G418 resistance), Puro (puromycin resistance), Bsd (blasticidin resistance), Hyg (hygromycin resistance), Zeo (zeocin resistance), and HSVTK (gancyclovir sensitivity). All of these genes have been used extensively in mammalian cell genetics with no reported adverse effects in humans or animals.

Evaluation of foreseeable effects

Procedures
During all procedures that involve the handling/use of the viral vectors, all workers (incl. those that are not working directly with the vectors) that are present within a lab where the work is undertaken are required to wear personal protective equipment (incl. both appropriate clothing and gloves) at all times. The use of two pairs of gloves is advised so the external pair can be disposed in an autoclave bag and replaced whenever necessary (e.g. in the case of contact with the viral reagent). Face masks are also available for use, where appropriate. In addition, all workers are made aware of the nature of the viral work that is going on within the laboratory, and they have to follow appropriate procedures to ensure that there is no cross-contamination into non-viral working areas. All the workers using the viral delivery systems are experienced research scientists, and junior scientists will be closely supervised until they are competent in the handling of the viruses.

In vitro assessment
Assessing whether the genome manipulation has worked will be carried out by a combination of PCR and sequencing, and will normally take place initially in biosafety level 1 immortalised cell lines that are known not to contain lentiviral genetic material. Application of lentiviral particles will be done at Biosafety level 2. After at least 24 hour incubation and extensive washing, the cells will be treated as Biosafety level 1 (see below the justification for derogation of cells post-infection to Biosafety level 1). Once validated the same procedure will be carried out in the target cell line which will be immortalised biosafety level 1 cell lines or human induced Pluripotent stem cells (iPSCs). In the latter case these will subsequently be differentiated into defined lineages for phenotypic analysis by manipulating the culture conditions. Where viral transduction is undertaken on a class 2 cell line the resultant material will remain under biosafety level 2 conditions. In all cases phenotypic analysis will be performed using suitable in vitro assays, including for example, RT-PCR, Western Blotting, immunocytochemistry and imaging.

Human health hazards
The methods by which the lentivirus vector is produced means that it is highly unlikely that replication competent lentivirus (RCL) can be generated even if an infection occurs in a person who is HIV-1 positive. Infection can lead to a permanent transduction of cells and the possibility of a harmful event either by transactivation or disruption of gene expression cannot be ruled out. For this reason the work with stock viral aliquots will be conducted as a class II activity. Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosal cells with modified viruses, due to the inherent properties of the viral vectors. Moreover, the genes/ proteins of interest that will be targeted by shRNAs do not include any growth factors, confirmed oncogenes, cytotoxins, or immunomodulators, and none of them are of retroviral origin. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent and being extremely sensitive to dessication.

Hazard to the Environment
The probability that active viral particles will be accidentally released into the environment is considered to be extremely low, as effective containment procedures will be adhered to. However, in the unlikely event that active vector particles do accidentally get released into the environment, the safety risk posed by such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable RCL. This scenario is however, extremely unlikely, and even if it was to occur, it is unlikely that it would lead to any untoward effects.

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and they are, in theory, capable of transducing all dividing and non-dividing mammalian cell types. As emphasised before, the vectors cannot self-propagate after infection, and successful transduction is also critically dependent on the presence of
high enough concentrations of virus particles; also viral vector stock solutions are aliquoted and stored in small volume (each tube contains at maximum 10µ at 80\(^\circ\) so the potential for a high titre dose is reduced. The infectivity of the vectors rapidly decreases at room temperature so it is considered highly unlikely that the vectors could survive long enough in the environment to pose a risk if accidentally released.

Furthermore, lentiviral vectors have a short half-life at room temperature as their structural characteristics make them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, and this is illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA and miRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

Summary
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as Biosafety level 2, and effective containment procedures will be adhered to (see section C for details). However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems (see above discussions).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Following transduction of cell lines that are designated as requiring Biosafety Containment Level 1 with lentivirus, we wish to be able to subsequently use these cells at Biosafety Containment Level 1. This can be justified by the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24 hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as only requiring Biosafety Containment Level 1. Sources of IPSC cells that have been shown to lack viral integration and test negative for infectious virus can be regarded as requiring biosafety level 1 containment. Such IPSC cells can also be derogate to biosafety level 1 containment once tested for the absence of infectious virus.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General cleaning procedures
Surfaces will be thoroughly cleaned with Virkon* Or Distel 10% (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all of the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Virkon* or Distel 10% before being autoclaved/incinerated.

Waste handling procedures
Solid waste will be inactivated by soaking it in a validated disinfectant solution for 24 hours, after which the material will be 'double bagged', autoclaved and, ultimately, incinerated offsite.

Liquid waste will be disposed of into sealed biohazard containers filled with Virkon* for incineration. It will be routine practice that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Neutralised liquid waste will be securely sealed for collection and off-site disposal by an approved agent (B60CRP) or discarded down an approved sink (430CSP).
Summary

As any active viral particles on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered very unlikely that active virus particles would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.

*Virkon

Rely+On™ ® is a multi-purpose disinfectant. It contains oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, sulphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds, such as proteins, leading to widespread, irreversible damage and subsequent deactivation/destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance against over 500 strains of viruses, bacteria and fungi. Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control. Importantly, Virkon has specifically been validated for lentiviral inactivation (Antec-Biosentry).

Virkon is sold as a powder which dissolves readily in water. It is intended to be mixed with water to form a 1% solution (i.e.10g per litre) for hard surface and equipment disinfection. 1:100 is also dilution rate advised for virucidal efficacy against HIV-1.

*DISTEL (formerly known as Trigene advance)

DISTEL is formulated as a nonmulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms, ensuring the rapid induction of cell death in treated micro-organisms. DISTEL works quicker than conventional disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products. DISTEL has been reported to be bactericidal, fungicidal as well as virucidal and sporicidal (http://tristel.com/products/healthcare/laboratories/distel-high-level-laboratory-disinfectant/). It is recommended to be used at a 1:100 dilution for general purposes, 1:10 dilution for high risk disinfection.

Please enter comments on the GM safety committee on the risk assessment

The GMSC has agreed that the production, handling and use of the lentiviral siRNA stocks is a Class 2 activity. This decision has been reached because although the lentivirus will be generated using a third generation, self-inactivating, 4 plasmid expression system, the possibility of a recombination event occurring that leads to replication competent virus can never be discounted with this type of virus. The potential oncogenic effects of the Woodchuck Post-translational Regulatory Element have been negated by the use of the mutant form that prevents expression of the X protein. The proposal includes the potential to produce large amounts of virus. This represents the most hazardous aspect of the proposal and the GMSC is satisfied that the necessary precautions have been put in place to ensure containment is maintained. The encoded Cas9 protein and guide RNA to be delivered by lentivirus in this proposal is now an established and widely used technique. Cas9 itself is not known to have any detrimental effects on human health or the wider environment. The proteins targeted by the guide RNAs will be assessed on a case by case basis to ensure their potential risk is minimal. The request to derogate the Biosafety Containment Level 2 from 2 to 1 post transduction has been deemed acceptable given that the transduced cells will be washed extensively to remove any remaining viral particles and will be tested for the absence of transducing virus. It has been made clear that human iPSC cell lines that have been generated using lentiviral or retroviral methods must be negative for the absence of virus at least. These cells will always be tested for the absence of infectious particles before they can be used as if they only require Biosafety Containment Level 1.
**Project Additional Information**

**Purposes of the contained use**

This work will involve the use of particles of lentiviral origin to deliver modifiers of gene expression to mammalian cells. The aim is to dysregulate key disease-related pathways and then assess the effect of such manipulations on gene expression with a view to identifying novel disease-modifying targets.

**Recipient or parental organism**

- **Rodent Origin:** Mouse cell lines
- **Human Origin:** Immortalised cell lines and induced pluripotent stem cells
lentivirus vectors: The four plasmids that will be used will be a modified versions of pRRI, pMDlgp-RRE, pRSV-REV and PMD2-VSVG.env. The latter three are required for the production of the SIN replication defective lentiviral particles in HEK293T cells. The target shRNmiRNA or gene will be inserted in to the modified pRRl vector. This modified vector will have the following safety features: a deletion in the 3'L TR making it SIN in the infected cells, the packaging sequence is only found in the pRRl with the packaging constructs being provided by the remaining non-L TR containing plasmids Lentivirus vectors: pMDLgp-RRE, pRSV-REV and PMD2NSVG.env. The Tat promoter has been replaced by Polymerase Type 2 promoter, making them replication incompetent. The inclusion of these features ensures that that the production of these SIN replication defective lentiviral particles only occurs in the transfected HEK293T. Outside of this setting, viral replication cannot take place and so pose a minimal risk to both humans and the environment.

### Host/vector system

The SIN-RD lentiviral particles will contain the following sequences:

#### Health and Safety

1. shRNmiRNA directed towards the knock down of the expression of a particular endogenous mammalian target (Mouse, Rat, Human). These de novo derived sequences are not derived from an organism.
2. Fluorescent protein marker which are known to be benign in terms of their effects when expressed in mammalian cells but are essential for visualisation of these cells. When these in addition are fused to a subcellular marker gene, subcellular visualisation of a particular process can be visualised.
3. Cre recombinase which catalyses excision of sequences flanked by its recognition site, loxP.
4. Fluorescent marker protein fused to a ribosomal subunit to enable visualisation of cells where vitally delivered gene expression is taking place and immunoprecipitation of ribosomes from such cells.

#### Methods

This proposal involves the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site. These constructs will contain a combination of shRNmiRNA and a fluorescent protein as lineage marker. In some cases constructs will contain a combination of shRNmiRNA and Cre recombinase giving rise to a pre-determined deletion event in transduced cell lines. Insertion of the sequences into the third generation lentiviral vector on site will be achieved by either conventional cloning or Recombineering. Lentiviral particles will either be purchased ready for use or produced in-house using the third generation system described below.

#### Lentiviral production - Uney laboratory

Some of the lentiviral vectors will be produced on site whilst others will be obtained from the Uney laboratory, which is based at the University of Bristol. The lentiviral vectors provided by the Uney laboratory will be stock solution containing up to 1x10^10 transducing units (TU) l ml of vector in TSSM buffer (20 mM TRIS, 100 mM NaCl, 10 mg/mL sucrose, 10 mg/ml mannitol), and will be essentially free of the HEK293T helper cell line used to expand the viral titre. Each purified and concentrated aliquot of lentiviral vector solution will comprise of small volumes (up to 10 1-11) containing no more than 1x10^8 viral particles. The material is normally shipped frozen and stored within manufacturer designed racks in a _80°C freezer.

#### Lentiviral production - on site 860CRP or 430CSP

Lentiviral production will also be carried out on-site using HEK293T cells and will be carried out in a class 2 microbiology safety cabinet within a dedicated Biosafety level 2 laboratory. The HEK293T cells used for lentiviral production will be seeded in filter containing screw cap culture flasks in order to minimize the risk of spills and aerosol mediated viral spread. For this the modified pRRI plasmids together with three additional separate plasmids (pMDLgp-RRE, pRSV-REV and PMD2-VSVG.env) will be transfected using a transfection reagent (e.g.
Polyethylenimine (PEI), lipofectamine) into HEK293T packaging line. After 6-12 hours, the medium is replaced with fresh serum-free medium (e.g. Neurobasal medium with 2% B-27 supplement and 1X PeniStrep) and grown for an additional 72-96 hours. After 24-48 hours, so as to ensure the viability of the transfected HEK293T cells, medium from the cells is collected into screw cap tubes and stored at 40C and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the first harvest tube.

After lentivirus collection has been completed, the cells in the flasks will be treated with a validated anti-microbial agent (e.g. DISTEL 10% or another equivalent product) for at least 24 hours. Liquid waste will be disposed off-site via an approved waste disposal agent (B60CRP) or via a designated sink (430CSP) after at least 24 hours neutralisation with 10% DISTEL or equivalent. Solid material will be autoclaved on-site and then collected for incineration by an approved waste disposal agent.

Still within a Class 2 microbiology safety cabinet in a Biosafety level 2 laboratory, the harvested supernatants will be filtered using a 0.45um filter before being either aliquoted or further purified by chromatography and/or concentrated by centrifugation. The lentiviral particles may need to be purified by either chromatography (e.g. ViraBindTlo1) and eluted in a high salt solution (50 mM Tris, pH 7.5, 5 mM MgCl2, 2 M NaCl) and/or concentrated by ultrafiltration (e.g. lentiSelect™). For certain applications where highly concentrated, highly purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimise the possible risk of accidental viral contamination and spread due to the need for an ultracentrifuge, all loading and unloading will be done in the Class 2 microbiology safety cabinet. Once the sucrose cushions have been added to the centrifuge tubes, they will be transferred into the Class 2 microbiology safety cabinet. The unpurified or chromatography purified lentiviral particles will then be added to the sucrose cushion. The individually capped tubes will then be transferred to their respective bucket holders and the bucket sealed using their respective screw caps and marked as containing virus. The bucket will be transferred to a weighing scale and a counterpart bucket and tubes with water (and without lentivirus) will be prepared that is weight matched. The balanced buckets will then be transferred to the rotor before being placed within the centrifuge chamber. The ultracentrifuge will be run at 70000g for 2 hours at 20oC. Upon return of the buckets to the Class 2 microbiology safety cabinet, the supernatant will be removed from the tubes before transfer of the resuspended virus pellet into individual screw cap cryovials. As the greatest risk for contamination occurs within the centrifugation buckets themselves during transport to and from the weigh scales and during ultracentrifugation, not only will the centrifuge tubes but also the rotor be decontaminated with DISTEL 10% before removal from the Level 2 laboratory. Optionally the centrifuge rotor can be autoclaved at 121oC. In any case, at no point will concentrated virus stock be exposed to outside of the Class 2 hood environment. All supernatants will be treated as above.

The lentiviral titre will be determined in terms of the number of viral particles (VP) or Transduction units (TU) per ml. The number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based enrichment assay (e.g. QuickTitre™ Quantification Kit) or a p24 ELISA assay (e.g. QuickTitre™ Lentivirus Titre kit) will be chosen. The VP quantification methods tend to overestimate the TU by 10-100 fold. Thus, the TU 1 ml will in certain instances also be determined. For this, a titration range of lentiviruses expressing a fluorescent tag, serial dilution will be prepared and added to a 24 well cluster plate or T25 Te-flasks containing HEK293T for 48 hours. The percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using FACS analyser. The biological titre (TU/ml) according to the following formula: 

\[
TU/ml = \left( \frac{P \times N \times 1000 \times V}{DF} \right),
\]

where? = % GFP+ cells, N = number of cells at time of transduction = 10^5, V = volume of dilution added to each well and DF = dilution factor = 1 (undiluted), 10-1 (diluted 1/10), 10-2 (diluted 1/100), and so on.

Aliquots with not more than 1x10^8 viral per vial are subsequently stored at -800C in individual screw capped cryotubes. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to,
inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats.

Transgene: proteins to be overexpressed

Additional hazards could also arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.

A. Fluorescent protein tag proteins:

The green fluorescent protein (GFP) is a protein composed of 238 amino acid residues (26.9 kDa) that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. GFP traditionally refers to the protein first isolated from the jellyfish Aequorea victoria, which has a major excitation peak at a wavelength of 395 nm and a minor one at 480 nm. Its emission peak is at 509 nm, which is in the lower green portion of the visible spectrum.

EGFP is a variant of GFP containing two point mutations; S65T and F64L. These mutations dramatically improve the spectral characteristics of GFP, resulting in increased fluorescence, photostability, and a shift of the major excitation peak to 488 nm, with the peak emission kept at 509 nm. This matches the spectral characteristics of commonly available FITC filter sets and also improves folding efficiency at 37 °C thereby allowing practical use in mammalian cells. Genetically encoded Fluorescent proteins such as green fluorescent protein have been widely used as a reporter for biological research over the past decades. Their exogenous expression is not associated with any cytotoxic, immunogenic or oncogenic response.

B. ere recombinase

Gre Recombinase is a tyrosine recombinase enzyme derived from the P1 Bacteriophage. The enzyme uses a topoisomerase I like mechanism to carry out site specific recombination events. The enzyme (3SkOa) is a member of the Integrase family of site specific recombinase and it is known to catalyse the site specific recombination event between two DNA recognition sites (loxP sites). This 34 base pair (bp) loxP recognition site consists of two 13bp palindromic sequences which flank an 8bp spacer region. Two separate DNA species both containing loxP sites can undergo fusion as the result of Gfe mediated recombination. DNA sequences found between two loxP sites are said to be "flxed". The enzyme plays important roles in the life cycle of the P1 Bacteriophage such as cyclization of the linear genome and resolution of 1meric chromosomes that form after DNA replication.

ere recombinase is a widely used tool in the field of molecular biology. The enzyme's unique and specific recombination system is exploited to manipulate genes and chromosomes in a huge range of research, such as gene knock out or knock in studies. The enzyme's ability to operate efficiently in a wide range of cellular environments (including mammals, plants, bacteria, and yeast) enables the ere-Lox recombination system to be used in a vast number of organisms, making it a particularly useful tool in scientific research.

The simplicity and robustness of the Gre-loxP systems has enabled scientists to exploit the Gre enzyme in order to manipulate DNA both in vivo and ex vitro. As the enzyme has a specific 34bp DNA substrate the genome of the organism would have to be 1018bp in length for there to be a likely occurrence of a loxP site. As mammalian genomes are on average in the region of 3x10^9 bp there is a very low chance of finding an endogenous 10xP site. For ere to be functional in a foreign host, exogenous loxP sites must be engineered. This allows precise control over the activity of the ere enzyme in test organisms.

ere recombinase is not known to cause any diseases in humans or animals.

C. shRNA: endogenous proteins to be depleted

RNA interference (siRNA)

RNA interference (siRNA) is an antisense technology that exploits a normal cellular antiviral response that acts to inhibit viral protein synthesis through the production of short hairpin RNAs that bind and target viral double-stranded RNA (dsRNA) molecules for degradation. The shRNA molecules are processed by cellular enzymatic pathways to produce small inhibitory RNA (siRNA) species, and it is the latter RNA molecules that bind and target complementary
RNA sequences for degradation by the RNA-induced silencing complex (RISC). With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be 'knocked-down', in order to study their functions.

The effects of the siRNA species that are generated in these experimental systems may have broader effects on the cell than just modulating the expression of a particular gene/protein. There may be sequences within a specific siRNA molecule that are homologous to other coding sequences within the mammalian genome that are not necessarily linked or closely related to the intended target. It is, therefore, theoretically possible that a specific siRNA may knock down the expression of genes other than the intended target one, and for this reason, the siRNA systems have been designed carefully to minimise the likelihood that there will be unwanted or potentially adverse effects arising from a non-target gene being inadvertently targeted. This is done by screening designed siRNA sequences against databases of known mammalian gene/mRNA sequences.

RNA interference may also have deleterious effects upon cellular metabolism due to the triggering of antiviral responses. It has been shown that siRNA molecules (even if less than 30nt in length) can trigger dsRNA antiviral responses. Such responses not only lead to the degradation of dsRNA molecules, but also results in interferon production, which, in turn, leads to inflammation and the non-specific inhibition of protein synthesis.

In some of the lentiviral vectors that will be used in the proposed studies the eukaryotic U6 Polymerase III promoter will be used to drive the expression of specific shRNAs of interest. The U6 promoter is well suited to drive the expression of shRNAs as it possesses the following favourable characteristics: (i) it initiates from position +1 of the transcripts, and (ii) it yields transcripts that do not terminate with a poly-A tail but with a series of four to five thymidine residues, which results in a series of 3' U residues leading to a fully functional shRNA sequence. Indeed, the structure of the transcribed product closely resembles synthetic double-stranded siRNAs, except for the fact that the two strands are linked by a spacer sequence. This system has been used to successfully inhibit gene expression in mammalian cell lines, with efficiencies comparable with that of synthetic siRNA.

Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. These vectors will be employed as negative controls to demonstrate that any observed effects are due to the knockdown of a specific mRNA/protein of interest, rather than being due to any non-specific effects that the delivery of the viral vectors might cause. This sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of mouse, rat or human origin. It will only serve as the negative control for the vector producing shRNA. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health.

### Evaluation of foreseeable effects

#### Procedures

During all procedures that involve the handling/use of the viral vectors, all workers (incl. those that are not working directly with the vectors) that are present within a lab where the work is undertaken are required to wear personal protective equipment (incl. both appropriate clothing and gloves) at all times. The use of two pairs of gloves is advised so the external pair may be disposed in an autoclave bag and replaced whenever necessary (e.g. in the case of contact with the viral reagent). Face masks are also available for use, where appropriate. In addition, all workers are made aware of the nature of the viral work that is going on within the laboratory, and they have to follow appropriate procedures to ensure that there is no cross-contamination into non-viral working areas. All the workers using the viral delivery systems are experienced research scientists, and junior scientists will be closely supervised until they are competent in the handling of the viruses.

#### In vitro assessment

Assessing whether the gene modulation has worked will be carried out by a combination of PCR and fluorescence microscopy, and will normally take place initially in biosafety level 1 immortalised cell lines that are known not to contain lentiviral genetic material. Application of lentiviral particles will be done at Biosafety level 2. After at least 24
hour incubation and extensive washing, the cells will be treated as Biosafety level 1 (see below the justification for removal of cells post-infection to Biosafety Level 1). Once validated the same procedure will be carried out in the target cells. Where viral transduction is undertaken on a class 2 cell line the resultant material will remain under biosafety level 2 conditions. In all cases phenotypic analysis will be performed using suitable in vitro assays, including for example, RT-PCR, Western Blotting, immunocytochemistry and imaging.

In vitro studies

Transduction of cells (e.g. human derived iPSC neurons, mouse and human immortalised cell lines) with the lentiviral vectors will be carried out in a Class 2 microbiology safety cabinet within a dedicated Biosafety level 2 laboratory. In the in vitro studies, the TUs, and consequently volumes added, will be dependent on the number of cells in the tissue culture dish/flask to be infected. However, as the maximum number of cells to be handled per experiment is around 1 x 10^8, not more than 1 x 10^8 viral particles per experiment will be used. None of these cells win be cultivated in the filter screw cap flasks used for HEK293T mediated lentiviral production. This is to ensure that, in particular, the HEK293T cells used for lentiviral production are not inadvertently used for lentiviral assessment! Any plastic ware or solutions that are used to handle the transduced cells will be chemically inactivated in the Biosafety level 2 laboratory with a validated anti-microbial agent (e.g. DISTEL 10% or another equivalent product) for at least 24 hours. Liquid waste will be disposed of on-site via an approved waste disposal agent (B60CRP) or via a designated sink (430eSP). Solid material will be autoclaved on-site and then collected for incineration by an approved waste disposal agent.

Once the medium containing the lentiviral particles has been removed and the cells (e.g. mouse NIH/3T3, human HEK293T) have been extensively washed, which all is done in a Class 2 microbiology safety cabinet, the cells can be used following Biosafety level 1 guidelines, as no virus will be present and the latent virus in the transduced cells is replication incompetent, unless the cell line was originally classified as biosafety level 2 in which case the material will continue to be treated as such. A viral titre determination assay may on occasion be used to verify the absence of virus in the medium from transduced cell lines. This will be performed by PCR using oligonucleotides specific to the conserved regions within the viral genome.

An additional level of safety and screening procedure for the handling human iPSC cells will be applied for the following reason. The procedure for the generation of iPSC neurons was originally developed in 2006 by Yamanaka, which allows for the reprogramming of adult differentiated cells into stem cells through the introduction of four transcription factors using replication incompetent retrovirus. Since then the biosafety level of the retroviral constructs has been improved, such that both retroviral and non-viral methods of reprogramming have been reported. Even assuming that the source of our human iPSC cells resulted from work using first or second generation replication incompetent lentiviral particles, the risk of producing active lentivirus from the additional exposure to a third generation replication incompetent virus is considered negligible. Nevertheless, a number of precautionary measures will be implemented for use of these cells. Any new human iPSC line that is brought onto our site and was generated using SIN-RD lentiviruses or retroviruses (preference will be given to those lines for which there is documentation from the provider that no viral particles are being made and released) will be initially cultured at Biosafety Level 2 and tested by titre assessment to confirm that no virus is being generated. Further, and after the removal of our SIN-RD lentivirus and extensive washing, the cells will be incubated in fresh medium and will be maintained at Biosafety Level 2 safety for at least 24 hours. These cells can then only be treated as Biosafety Level 1, once a virus titre assay has confirmed that no virus is being released into the medium.

Human health hazards

The method by which the lentivirus vector is produced means that it is highly unlikely that replication competent lentivirus (RCL) can be generated even if an infection occurs in a person who is HIV-1 positive. Infection can lead to a permanent transduction of cells and the possibility of a harmful event either by transactivation or disruption of gene expression cannot be ruled out. For this reason the work with stock viral aliquots will be conducted as a class II.
activity. Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the genes/proteins of interest that will be targeted by shRNAs do not include any growth factors, confirmed oncogenes, cytotoxins, or immunomodulators, and none of them are of retroviral origin. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent and being extremely sensitive to desiccation.

Hazard to the Environment

The probability that active viral particles will be accidentally released into the environment is considered to be extremely low, as effective containment procedures will be adhered to. However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risk posed by such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable RCL. This scenario is, however, extremely unlikely, and even if it was to occur, it is unlikely that it would lead to any untoward effects.

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and they are, in theory, capable of transducing all dividing and non-dividing mammalian cell types. As emphasised before, the vectors cannot self-propagate after infection, and successful transduction is also critically dependent on the presence of high enough concentrations of virus particles; also viral vector stock solutions are aliquoted and stored in small volumes (each tube contains at maximum 101J1) at -80°C so the potential for a high titre dose is reduced. The infectivity of the vectors rapidly decreases at room temperature so it is considered highly unlikely that the vectors could survive long enough in the environment to pose a risk if accidentally released.

Furthermore, lentiviral vectors have a short half-life at room temperature as their structural characteristics make them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, and this is illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. siRNA and miRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

Summary

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to (see section C for details). However, in the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems (see above discussions).
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Following transduction of cell lines that are designated as requiring Biosafety Containment level 1 with lentivirus, we wish to be able to subsequently use these cells at Biosafety Containment level 1. This can be justified by the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24 hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as only requiring Biosafety Containment Level 1.

Sources of iPSC cells that have been shown to lack viral integration and test negative for infectious virus can be regarded as requiring biosafety level 1 containment. Such iPSC cells can also be derogated to biosafety level 1 containment once tested for the absence of infectious virus.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General cleaning procedures
Surfaces will be thoroughly cleaned with Virkon* or Distel 10%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all of the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Virkon- or Distel 10% before being autoclaved/incinerated.

Waste handling procedures
Solid waste will be neutralised by soaking it in a validated disinfectant solution, after which the material will be ‘double bagged’, autoclaved and, ultimately, incinerated offsite.
liquid waste will be disposed of into sealed biohazard containers filled with Virkon- for incineration. It will be routine practice that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Neutralised liquid waste will be securely sealed for collection and off-site disposal by an approved agent (B60CRP) or discarded down an approved sink (430eSP).

Summary
As any active viral particles on surfaces or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered very unlikely that active virus particles would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.

*Virkon
Rely+OnTM Virkon® is a multi-purpose disinfectant. It contains oxone (potassium peroxymonosulphate), sodium dodecylbenzenesulfonate, suiphamic acid; and inorganic buffers. Virkon oxidizes key structures and compounds, such as proteins, leading to widespread, irreversible damage and subsequent deactivation/destruction of the microorganism. It is typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. With powerful, proven performance against over 500 strains of viruses, bacteria and fungi. Virkon offers a broad spectrum efficacious anti-microbial activity, and it is used by governments worldwide for Emergency Disease Control.

Importantly, Virkon has specifically been validated for lentiviral inactivation (Antec-Biosentry).
Virkon is sold as a powder which dissolves readily in water. It is intended to be mixed with water to form a 1% solution (i.e. 10 9 per litre) for hard surface and equipment disinfection. 1:100 is also the dilution rate advised for virucidal efficacy against HIV-1.
DISTEL (formerly known as Trigene advance)
DISTEL is formulated as a nanoemulsion that enables the active molecules in the product to be carried rapidly through cell walls of micro-organisms, ensuring the rapid induction of cell death in treated micro-organisms. DISTEL works quicker than conventional high level disinfectants and achieves apoptosis (cell death) rather than merely suspending activity as with conventional disinfectant products. DISTEL has been reported to be bactericidal, fungicidal as well as virucidal and sporidical (http://www.tristel.com/products/healthcarelaboratories/distel-high-levellaboratory-disinfectant). It is recommended to be used at a 1:100 dilution for general purposes, 1:10 dilution for high risk disinfection.

The GMSC has agreed that the production, handling and use of the lentiviral siRNA slacks is a Class 2 activity. This decision has been reached because although the lentivirus will be generated using a third generation, self-inactivating, 4 plasmid expression system, the possibility of a recombination event occurring that leads to replication competent virus can never be discounted with this type of virus. The potential oncogenic effects of the Woodchuck Post-translational Regulatory Element have been negated by the use of the mutant form that prevents expression of the X protein. The proposal includes the potential to produce large amounts of virus. This represents the most hazardous aspect of the proposal and the GMSC is satisfied that the necessary precautions have been put in place to ensure containment is maintained. The encoded fusion-protein to be delivered by lentivirus in this proposal has been used extensively in the literature and is not known to have any detrimental effects on human health or the wider environment. The request to derogate the Biosafety Containment Level from 2 to 1 post transduction has been deemed acceptable given that the transduced cells will be washed extensively to remove any remaining viral particles and will be tested for the absence of transducing virus. It has been made clear that human iPSe cell lines that have been generated using lentiv- or retrovirus methods must be negative for the absence of virus at least. These cells will always be tested for the absence of infectious particles before being they can be used as if they only required Biosafety Containment Level 1.

Please enter comments on the GM safety committee on the risk assessment

The GMSC has agreed that the production, handling and use of the lentiviral siRNA slacks is a Class 2 activity. This decision has been reached because although the lentivirus will be generated using a third generation, self-inactivating, 4 plasmid expression system, the possibility of a recombination event occurring that leads to replication competent virus can never be discounted with this type of virus. The potential oncogenic effects of the Woodchuck Post-translational Regulatory Element have been negated by the use of the mutant form that prevents expression of the X protein. The proposal includes the potential to produce large amounts of virus. This represents the most hazardous aspect of the proposal and the GMSC is satisfied that the necessary precautions have been put in place to ensure containment is maintained. The encoded fusion-protein to be delivered by lentivirus in this proposal has been used extensively in the literature and is not known to have any detrimental effects on human health or the wider environment. The request to derogate the Biosafety Containment Level from 2 to 1 post transduction has been deemed acceptable given that the transduced cells will be washed extensively to remove any remaining viral particles and will be tested for the absence of transducing virus. It has been made clear that human iPSe cell lines that have been generated using lentiv- or retrovirus methods must be negative for the absence of virus at least. These cells will always be tested for the absence of infectious particles before being they can be used as if they only required Biosafety Containment Level 1.

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications
Use of the Expli293 MembranePro Expression technology system (Life Technologies) for the expression and display of mammalian cell surface membrane proteins including G-protein coupled receptors (GPCR), Ion channels transporter and other membrane bound molecules in an aqueous-compatible format.

Expi293F cells are based on the HEK293 (primary human embryonic kidney cells) immortalised cell line. The Expi293F will be used to generate the VLPs, these cells have been adapted from HEK293 cells to grow in high density suspension culture. The HEK293 cell line is a permanent line established from primary embryonic human kidney cells transformed with sheared human adenovirus type 5 DNA. The E1A adenovirus gene expressed in HEK293 cells participates in the transactivation of some viral promoters, allowing these cells to produce very high levels of protein. This enables the efficient generation of viral capsids when these genes are introduced transiently into the the cell. The adenovirus genetic sequence present in HEK293 cells has been found to be non mobilisable and as such is not packaged into viral particles. This means that the cells themselves are essentially non-hazardous. These properties have meant that many virus packaging cell lines have been derived from the HEK293 cell line.
Host/vector system

The system uses pEF-V5-His_ TOPO to express the gene of interest. This plasmid does not contain any lentiviral genes and therefore poses no risk. The lentiviral gag gene or protein (packaging mix) is provided in enhancer solutions provided with the Expi293 Membrane Pro Expression Technology system kit which will be used in the production of the VLPs. The exact nature of this enhancer material is proprietary to Life Technologies but as it contains the ability to supply the gag gene or protein it will be managed as needing to be treated as a class 2 material. This system lacks any other lentiviral genes and as such does not produce or encapsulate viral DNA. Once the VLPs have been generated and purified the enhancer solution will have been removed.

Origin & function

The system relies upon the presence of the lentiviral gag core structural protein to drive the self assembly of sub viral particles that should not encapsulate DNA. These VLPs bud off from the cell surface taking membrane proteins with them. As such they will contain the proteins of either GPCR, ion channel, enzymes, transporters or other cell surface membrane proteins that are expressed in the cell.

Targets of interest that will be over-expressed for incorporation into VLPs will not be known to have cytotoxic, oncogenic or immunomodulatory effects. The purified VLPs can then be used in a variety of vitro assays to assess small molecule, peptide and protein interactions with the protein of interest present in the VLP membrane.

Evaluation of foreseeable effects

Human Health Hazard

The production method for VLPs means that it is highly unlikely that infectious virus can ever be generated. The only lentiviral gene present in the production of the VLPs is the gag gene. As the system lacks the env and pol genes infectious virus particles can not be generated and there is no viral genome to be packaged. The vectors used to express the genes of interest do not contain packaging sequences and will not therefore be packaged into the VLPs. These vectors will essentially be absent once the VLPs have been purified. There is a small chance that the gag gene and/or the vector encoding the gene of interest introduced in trans may become trapped with a VLP as it forms. As such there is a theoretical risk that if fusogenic particles do come in to contact with cells it may introduce DNA and lead to a permanent genetic change in the cell. This risk is minimal because the number of VLPs containing any DNA will be minimal, they will be dilute and as they lack the env protein they are highly unlikely to be able to infect cells. To ensure that the other lentiviral genes are not introduced into the system VLPs will not be used with other cell cultures and production will be kept separate from cells being used for the production of lentiviral vectors. As the system does utilise a lentiviral gene during the generation of the VLPs this work will be carried out at biosafety level 2. The generation of VLPs will therefore be treated as a class II activity. Standard health and safety procedures of working will be followed throughout to eliminate any risk of infection.

Hazard to the environment

The chance that infectious lenti virus is released into the environment is considered to be negligible. The VLPs that are generated are designed to only contain the gag protein of the lentiv virus and not the gag gene. Infectious VLP generation would have to involve the gag gene encountering the remaining pol or env genes and infecting cells in the environment, the risk of this is negligible given that the VLPs lack the product of the env gene and are highly unlikely to be able to infect cells. In the event of release of the VLPs into the environment, they are labile outside of the laboratory environment being sensitive to temperature and will rapidly degrade at room temperature.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
The production of the VLPs is the point at which large volumes of concentrated material will be generated in a system that has had the gene encoding gag present. The purification process will essentially remove genetic material. Once aliquots of this final material has been dispensed there should be essentially no genetic material present. Subsequent experiments will not involve the use of cells and limited amounts of material will be diluted and it is thought appropriate to handle this material with good laboratory practice.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste, including pipette tips and tissue culture flasks will be neutralised by soaking in a validated disinfection solution (5% HLD4L solution, Chemgene) for 24 hours, after which the material will be double bagged, autoclaved and taken off site for incineration. Liquid waste will be chemically deactivated with 5% HLD4L solution, Chemgene for a minimum of 24 hours prior to drain disposal via a designated sink.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The system described above for the production of VLP for the enrichment of membrane proteins for subsequent analysis of molecular interactions with other molecules poses a very low risk to human health and the environment. The system utilises genetic material from HIV, which is known to mutate rapidly, and there is the small possibility that genetic material may be enclosed in the VLP. With this in mind the Genetic Modification Safety Committee (GMSC) agrees that the production of VLPs should be carried out at biosafety level 2 when concentrated VLP suspensions will be generated. Once generated the resultant VLP material should contain no viral genetic material and will be diluted for use in further experiments. This reduces the risk and the GMSC views that it is appropriate that subsequent downstream work can be carried out using good laboratory practices.

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Project Ref 3043/16.1

Use and genetic modification of an immortalised human neural progenitor cells, ReNcell 197VM & ReNcell mHTT Exon1 197VM

Date Ackn'd 02/12/2016
CU2 Project Title

Date Project Ceased

Class Consent Granted
CultureVolClass2 < 1 Litre
CultureVolumeClass3-4

Non-GMM

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes
transferred from GM3043 02/12/2016

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

The human immortalised neural progenitor cells will be used as a tool cell line in Neurodegeneration to investigate cellular pathways involved in the development of neurodegenerative disorders, in particular Huntingtons. To better understand these cellular pathways, the expression or the activity of the gene of interest will be modulated by several methods:

1- The utilisation of tool compounds
2- The knock-down of the gene of interest by shRNA
3- The generation of modified somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
4- The generation of reporter gene cell line (Le. fluorescence protein or enzyme) under the promoter of the gene of interest.

The experiments involving the transfection of vectors such as shRNA or programmable nucleases may be performed by lentivirus. The effect of modulating the expression or the activity of the target of interest will be determined by the expression level of the proteins, mRNA or mediators involved in the target signalling pathway.

Recipient or parental organism

1- The human immortalised neural progenitor cells, ReNcell 197VM
• ReNcell VM is an immortalized human neural progenitor cell line with the ability to readily differentiate into neurons and glial cells.
• ReNcell 197VM was derived from the ventral mesencephalon region of a human fetal brain tissue. Immortalized by retroviral transduction, using replication incompetent retrovirus, with the avian v-myc myelocytomatosis viral oncogene.
These immortalised human neural progenitor cells, ReNcell197VM, will be purchased from Millipore. Millipore have tested the cell line and shown that it does not produce infectious virus particles.

2- Human immortalised neural progenitor cells, ReNcell mHTT Exon1 197VM

• ReNcell 197VM cells were transduced with lentiviral virus particles containing varying length HTT exon 1, eGFP, cPPT, WPRE, LTR U3 del (pHRsincpptUOCE+htt exon1 IRES eGFP 29CAG/71CAG/129 CAG WPRE).

These immortalised human neural progenitor cells, ReNcell mHTT Exon1 197VM, will be provided by UCL. This cell line contains the v-myc oncogene, a WPRE element that may contain the WHV-X oncogene ORF and therefore will be maintained in a biosafety level 2 confinement.

Host/vector system

Description of the lentivirus containing shRNA, programmable nucleases sequences or gene reporter vectors

To utilise the neural progenitor cells to study neurodegeneration, the expression of a target of interest may be decreased in the ReNcell line by the transduction of shRNA or programmable nuclease by lentivirus.

The modulation of a particular cellular pathway may be studied with the use of a reporter gene assay. In this case, the vector containing the reporter gene under the regulation of the promoter of the gene of interest may be introduced by lentivirus into the ReNcells. In addition other exogenous genes may also be introduced using lentiviral vectors.

This proposal may involve both the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site using the third or fourth generation system. Both commercially acquired and in-house lentivirus will use the same transfer vector plasmid. Target genes will be selected based on the needs of drug discovery projects; programmable nucleases and shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators (these targets will be presented to the GMSC for approval prior to any work taking place).

The production of lentivirus containing the vectors of interest are described below:

1- Generation of lentivirus

Health and Safety

Executive

Lentiviral vectors will be purchased commercially or produced in-house using the third or fourth generation system described below. Each purified and concentrated aliquot of lentiviral reagent comprised of a small volume (5 to 10ul) that contains no more than 1 X 1 Oe8 viral particles.

Vector System = self-inactivating (SIN) non replication-competent HIV based lentiviral vectors

The viral vectors that will be used in the proposed studies are self-inactivating non replication-competent HIV based lentiviral vector systems, which have been designated to produce stable gene expression in mammalian cells; and they are generated by co-transfecting HEK 293T cells with four to six separate plasmids. Each of the plasmids used expresses a different set of genes which, when combined following co-transfection, provide the smallest possible set of essential viral genes that is still compatible with virus production. Moreover, the vectors are all vesicular stomatitis virus (VSV-G) pseudotyped lentiviral vectors, which are self-inactivating and highly unlikely to undergo recombination. Importantly, the viral vector is inherently incapable of replication in mammalian cells.

The plasmids

Third Generation

The sequences that will be included in the viral vectors are sub-cloned into 4 standard bacterial plasmid vectors.

• Plasmids containing the packaging related sequences

Each of the three other plasmids, pCgpV, pRSV-Rev and pCMV-VSVG contain a different set of sequences that code for proteins that are responsible for packaging the viral vectors. All the genes (gag-pol, rev and env) required for production and packaging of the full length viable viral RNA particles have been removed from the pSMPUW lentiplasmids containing the transgenes of interest.
Fourth Generation
The sequences that will be included in the viral vectors are sub-cloned into 6 standard bacterial plasmid vectors
• Plasmids containing the packaging related sequences
Each of the other plasmids, pTRE-gag-pro, LTRHIV2-vpr-pol, penv(VSV-G), pTet-Off and ptat-RES-rev contain a
different set of sequences that code for proteins that are responsible for packaging the viral vectors. All the genes
(gag-pol, rev and env) required for production and packaging of the full length viable viral RNA particles have been
removed from the pSMPUW lenti-plasmids containing the transgenes of interest. An integrase deficient version of the
fourth generation packaging mix is available which contains a mutation in the sequence encoding the viral integrase.
The resulting integrase-deficient lentivirus (IDLV) generates circular vector episomes in transduced target cells that
are gradually lost by dilution in dividing cells (transient expression), but are stable in quiescent cells.

Origin & function

Plasmids containing the transgene of interest
The fourth plasmid, pSMPUW, will contain the sequences that code for shRNA, or programmable nuclease sequence,
or reporter gene sequence under the promoter of the gene of interest.

a- Vector containing shRNA
As mentioned above, pSMPUW plasmid will contain the sequences that code for one shRNA transcript that will target
one mRNA/protein of interest (or a scrambled shRNA in the case of negative control). The shRNA or scrambled
shRNA sequences will be under the control of polymerase II or III promoter. The shRNA sequences will be chosen to
target a single gene. The shRNA will not be produced against any growth factors, tropic factors, quiescence factors,
confirmed oncogenes, tumor suppressors or immunomodulators.

b- Vector containing CRISPR or Zinc-finger nucleases
Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn
instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the
cleavage site or by recombination with a user-supplied synthetic vector containing sequences complementary to the
target gene allowing insertion of exogenous genetic sequence.
For Cas9/CRISPR, nuclease target specificity is determined by an accessory sequence, the guide RNA, which
consists of a short (18-20 nucleotide) sequence homologous to the target gene and an additional short sequence that
forms a complex with the Cas9 enzyme. In this case, the plasmid pSMPUW, will contain the sequences that code for
a Cas9/CRISPR plus accessory sequences comprising a short guide RNA that determines target specificity. The
guide RNA sequences will be under the control of a Polymerase III promoter.

Health and Safety

Executive
In the case of Zinc finger nucleases target specificity is dependent upon the zinc finger protein sequence. The
pSMPUW will contain in that case the sequence of zinc finger nucleases under the control of either a polymerase II
promoter. In addition, a fluorescent protein or enzyme under the control of a polymerase II promoter or the promoter
of a gene of interest may be included. Control viral vectors that express only the fluorescent protein/enzyme and no
programmable nuclease will be used in some experiments.
The Cas9/CRISPR, if introduced into any cell line by lentivirus, will use the integrase deficient version of fourth
generation packaging mix.

Health and Safety

Executive
In the case of Zinc finger nucleases target specificity is dependent upon the zinc finger protein sequence. The
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programmable nuclease will be used in some experiments.
The Cas9/CRISPR, if introduced into any cell line by lentivirus, will use the integrase deficient version of fourth
generation packaging mix.

a- Reporter gene vector
The pSMPUW vector will contain the sequence coding for a reporter protein (enzyme or fluorescent protein) under the
control of the regulating elements of the promoter of the gene of interest or the a polymerase II promoter. An
appropriate selection marker i.e.; neomycin could also be included to allow the selection of stable human neural
progenitor cells containing the reporter gene sequence under the control of the promoter of the gene of interest.
Target genes will be selected based on the needs of drug discovery projects; programmable nucleases, shRNA or exogenous genetic material will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators (proposed targets will require approval internally by the company GMSC before work can start)

Procedures:
During all procedures that involve the handling/use of the viral vectors or cells, all workers (incl. those that are not working directly with the vectors) that are present within a lab where the work is undertaken are required to wear personal protective equipment at all times. The use of two pairs of gloves is advised so the external pair can be disposed in an autoclave bag and replaced whenever necessary (e.g. in the case of contact with the viral reagent). In addition, all workers are made aware of the nature of the viral work that is going on within the laboratory, and they have to follow appropriate procedures to ensure that there is no cross-contamination into non-viral working area. All the workers using the viral delivery systems are experienced research scientist, and junior scientists will be closely supervised until they are competent in the handling of the viruses.

Lentiviral production
Lentiviral production will also be carried out on-site using a modified procedure based on Bukovsky et al. (Bukovsky et al, 1999, J. of virology). Lentiviral production will only be carried out in HEK293T cells in a biosafety level 2 cabinet within a culture flask with screw cap filter in order to minimize the risk of spills and/or aerosol mediated viral spread. For this the modified pSMPUW plasmids together with three to five additional separate plasmids (pCgpV, pRSV-Rev and pCMV-VSVG or pTRE-gag-pro, L TRHIV2-vpr-pol, penV(VSV-G), pTet-Off and plat-IREs-rev) will be transfected, using a standard chemical transfection method, into HEK293T packaging line. After 6-12 hours, the medium is replaced with fresh serum-free medium and grown for an additional 72-96 hours. After 24-48 hours, so as to ensure the viability of the transfected HEK293T cells, medium from the cells is collected into screw cap tube and stored at 40°C and fresh serum-free medium is added to the cells. The cells are grown for an additional 24-48 hours before the second medium solution is added to the first harvest tube. After lentivirus collection has been completed, the cells and the flasks will be decontaminated and disposed of (detailed later). Still within a biosafety level 2 safety cabinet in a biosafety level 2 laboratory, the harvested supernatants will be filtered using a 0.45μm filter before either aliquoted or further purified by chromatography and/or concentrated by centrifugation. The lentiviral particles may need to be purified by either chromatography and eluted in a high salt solution and/or concentrated by ultrafiltration. For certain applications, where highly concentrated and purified virus may be required, the traditional method of sucrose cushion based centrifugation may be necessary. To minimize the possible risk of accidental viral contamination and spread due to the need for an ultracentrifuge, all loading and unloading will be done in the biosafety level 2 safety cabinet. Once the sucrose cushions have been added to the centrifuge tubes, the unpurified or chromatography purified lentiviral particles will be added to the sucrose cushion. The individually capped tubes will then be transferred to their respective bucket holders and the bucket sealed using their respective screw caps and marked as containing virus. The buckets containing viruses will be balanced with buckets containing a tube with water and without lentivirus. The balanced buckets will then be transferred to the rotor before being placed within the centrifuge chamber. The ultracentrifugation will be run at 70000g for 2 hours at 200°C. Upon return of the buckets to the biosafety level 2 cabinet, the supernatant will be removed from the tubes before transfer of the resuspended virus pellet into individual screw cap cryovials. As the greatest risk for contamination occurs within the centrifugation buckets themselves during transport to and from the weight scales and during ultracentrifugation, the centrifuge tubes, the buckets and the centrifuge rotor will be decontaminated by spraying Chemgene HLD4L 5%. Optionally, the centrifuge rotors could be autoclaved at 1210°C. In any case, concentrated virus stock will not be exposed to the environment outside of the biosafety level 2 cabinet. All supernatants will be treated as above.
The lentiviral titre will be determined in terms of number of viral particles (VP) or transduction units (TU) per mL. The number of VP can be assessed relatively quickly and does not involve cell infection. In this case, a bead based enrichment assay or a p24 ELISA assay will be chosen. The VP quantification methods tend to overestimate the TU by 10-1000 fold. Thus, the TU/mL will in certain instances also be determined. For this, a titration range of lentiviruses expressing a fluorescent tag will be prepared and added to a 24 well cluster plate or T25 TC-flasks containing HEK293T for 48 hours. The percentage of fluorescent positive cells will be quantified manually under a fluorescent microscope or ideally using FACS analyser/Bioanalyser. The biological titre (TU/mL) according to the following formula: TU/uL = (P x N 100 x V) x 1DF, where P = % GFP+ cells, N= number of cells at time of transduction = 105, V = volume of dilution added to each well and OF = dilution factor = 1 (undiluted), 10-1 (diluted 1/10), 10-2 (diluted 1/100), and so on.

Aliquots with not more than 1x10e8 viruses per vial are stored at -80°C in individual screw capped cryotubes. The viral stock will consist of viral particles containing the vector genome (full capsids) and also of a variable number of empty viral capsids. Other trace components that might be present include, but are not limited to, inorganic salts, vitamins, other nutrients, human cellular proteins, carbohydrates, amino acids, and fats.

a- Assessing efficacy of the lentivirus into a BSL 1 cell line
Assessing whether the genome manipulation or the knock-down has worked will normally take place initially in biosafety level 1 immortalised cell line that are known not to contain lentiviral genetic material. Application of lentiviral particles will be done at biosafety level 2 confinement. After at least 24 hour incubation and extensive washing, the cells will treated as biosafety level 1 (see below the justification for removal of cells post-infection to biosafety level 1). In order to determine whether the knock-down or the genome manipulation has worked, the levels of mRNA and protein of the gene of interest will be assessed post-transduction.

b- Transduction of the lentivirus and the generation of stable expression into ReNcell VM neural progenitor cells
Once validated the same procedure will be carried out in the ReNcell VM neural progenitor cells. As these cells are maintained in a biosafety level 2 confinement, the inoculation of lentiviral particles, the transformed cells and the phenotypic analysis will also be performed in a biosafety level 2 containment. When a lentivirus containing a programmable nuclease or the reporter gene will be used, a stable cell line could be established by selecting the cells in which the genome has been modified. In all cases phenotypic analysis will be performed using suitable in vitro assays, including for example, RT-PCR, Western Blotting, immunocytochemistry and imaging, or cellular bioenergy (Le.: mitochondria respiration function or glycolysis function).

Evaluation of foreseeable effects

* Human Health
The immortal human ReNcell VM neural progenitor cell that will be used has a low risk to human health. The expression systems and the transgenes that comprise the lentiviral vectors that will be used have also a very low risk for human health and safety, and the reasons for this are discussed below.

A- Lentivirus containing shRNA, or programmable nucleases sequence or reporter gene vectors
Lentiviral Vector Expression System
Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. As the lentiviral vector system that will be used in these studies is based on HIV-1, a theoretical risk to human health exists. However, retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airbone route. Therefore, accidental piercing of the skin or other surface tissues with virus containing objects represents the main potential route by which accidental infection could occur. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.
In all of the modified pSMPUW plasmids (containing shRNA, or programmable nucleases or reporter gene sequences), the sequences inserted are flanked by non-coding retroviral L TRs, and no retroviral genes are encoded on the modified pSMPUW plasm ids. Therefore, no retroviral genes will be transferred into generated viral particles.
This construct is packaged into particles using a HEK293T cell based packaging system, which requires the cotransfection of these cells with three to five additional separate plasmids (as detailed above). The additional plasmids express the envelope protein from VSVg and the non-structural proteins of the virion, and, importantly, none of these genes will be transferred into the assembled viral vectors, since they lack the packaging signal (psi), which is only present on the modified pSMPUW plasmids.

The lentiviral Expression System what will be used include the following key safety features:

* All of the pSMPUW contain a hybrid 3'LTR that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" (SIN) of the vector after transduction of target cells. Once integrated into a transfected target cell, the lentiviral genome is no longer capable of producing viral genomic material that can be packaged. Moreover, presence of an SV40 polyA after the hybrid 3'L TR in the vector construct will result in a provirus which should reduce the potential for transactivation of cellular genes due to an insertion event. Furthermore, the development of self-inactivating vectors improves the biosafety of vectors, as they are less likely to be mobilised following a superinfection with wild-type virus (HIV).

* The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes. Heterologous envelopes, like VSV-G, typically broaden the tropism and allow gene transfer into a broad variety of cells. The risk will mitigated by the use of self-inactivating virus and limiting the number of viral particles that will be handled at anytime time.

* Sequences encoding the proteins required for packaging of the viral genome are separated onto three to five plasmids, and all of the 4-6 plasmids used in the system have been engineered not to contain any regions of homology with each other so as to prevent undesirable recombination events that could lead to the generation of a replication-competent lentivirus (RCL), which could potentially be harmful to humans. It is important to note that no such RCL has ever been observed despite large-scale production and testing of lentiviral vectors.

* All of the pSMPUW containing plasmids/vectors will be used are devoid of all viral sequences apart from essential cis-acting sequences, including the L TRs and the packaging signal psi. Although the packaging plasmids used in these systems allows for the expression in trans of protein required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK293T producer cell lines, none of them contain L TRs or the psi packaging sequence. Several of the lentiviral accessory genes (vif, vpr, vpu and nef) that are dispensable for lentiviral vector production/transduction have been deleted from the packaged construct. Therefore, none of the retrovirus structural genes will actually be present in the packaged viral genome, and they will never be expressed in the transduced target cells, which means that no new RCL can be produced.

* The lentiviral particles produced in this system are replication-incompetent, only carry the sequences of interest, and no other viral species are produced.

* Expression of the gag and pol genes from pgag-pol-RRE has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Addition of the RRE in these plasmids prevents gag and pol expression in the absence of rev, which is contained in the pRSV-REV plasmid only. The Rev/RRE system is highly conserved among lentiviruses, and removal of the RRE sequence and associated splice donor/acceptor sequences result in a loss of transduction efficiency.

* L TR has been modified so as to increase lentiviral vector production, and also to allow lentiviral vector production to be independent of tat expression. It is known that Tat-deleted mutants of wild-type HIV-1 are not replication competent. Therefore, the deletion of Tat should decrease the risk of generating a putative RCL.

* Lentiviral vectors have a very low potential to cause immunogenicity.

* The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction.

Health and Safety
Executive

While the lentiviral vectors that are produced using this system contain only about 20% of the original genome of HIV-1, there is a very small risk that subsequent infection of cells already infected with the lentiviral genome of HIV-1 could lead to a rare recombination event in which the transgene is transferred to a replication-competent virus. Thus, the sequences in the vector that will be expressed could potentially be transferred to surrounding cells. This event is, however, extremely unlikely to occur for a number of reasons:

1- The lentiviral vector is replication-competent and self-inactivating. In the case of a subsequent HIV-contamination, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host genome, although non-self-inactivating vectors can be.

2- In order for the spread of the gene of interest to occur following accidental infection (assuming that this has lead to viral integration), a series of unlikely events have to occur:
   a- The worker would have to become infected with HIV-1 or to be already infected with the virus.
   b- The viral and lentiviral genomes would have to integrate into the host worker's genome in the same cells and in a position where they could interact to effect homologous recombination (point 1 above)
   c- Recombination would have to occur in just the right regions to allow for transfer of the gene of interest from the lentivirus to the HIV-1 genome, which could also involve the transfer of the HIV-1 genes to the lentiviral genome. In that case, it is conceivable that a non-self-inactivating HIV could be generated that contained the gene of interest but not the rest of the genome it requires. The other gene products could be provided in trans from the lentiviral genome that may now contain the HIV-1 genes or from other HIV-1 integrants.
   d- The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest, but again no genes necessary for subsequent replication. In order for another round of infection to occur, the process would have to begin again.

In these circumstances, the effects of lentiviral infection are likely to be minor in comparison to the effects of the HIV-1 infection, which would be required to affect the spread of the gene of interest. In addition, the scenario described is essentially equivalent to the rescue of the lentiviral genome from the host, which has already been shown not to occur.

3- It is extremely unlikely that any worker would infect themselves with a significant dose of lentivirus as the volumes that are used in transfection experiments are small (aliquots contain a maximum of 10μl of vector solution).

4- Moreover, insertional mutagenesis into the host genome may be considered as an oncogenic risk. We cannot rule out the possibility that, when the lentiviral genome integrates into the host genome, it will not lead to the activation of an endogeneous oncogene. However, all transcriptionally active long-terminal repeats (LTRs) have been removed as well as all promoter-like elements that required to drive expression of the transgene. This should prevent unforeseen activation of such genes. It is noted that deletion of retroviral enhancer in self-inactivating systems reduces the risk of activation but not of disruption, therefore, retroviral infection might still have permanent effects upon a cell (including oncogenic effects).

Importantly, we do not consider that the use of these lentiviral vectors will result in a significant increased risk of oncogenic activation compared to the risk possessed by any other viral delivery system. Moreover, the risk of transduction leading to tumourigenesis or other untoward harm following exposure is related in part to the titre of the viral vectors; exposure of workers to quantities of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of lentiviral vectors.

Transgenes:

Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.
With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be knocked-down, in order to study their functions. Potential deleterious effects such as off-target and immunomodulatory responses can be minimised through screening designed shRNA sequences against databases of known mammalian/mRNA sequences during the design stage. The genes/proteins of interest that will be targeted by shRNA do not and will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors and immunomodulators. Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. This sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of human, mouse or rat origin. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health (this will be approved by the GMSC).

Programmable nucleases: zinc fingers or Cas9/CRISPR

Zinc fingers or Cas9 protein will be directed against genes of therapeutic interest. Since the purpose of programmable nucleases is to cause mutations in defined DNA sequences the primary hazard is that such a mutation would lead to deleterious cellular effects. The genes/proteins that will be targeted do not and will not include any growth, trophic factors, confirmed oncogenes, tumor suppressors or immunomodulators. In isolation, these sequences are unlikely to pose any safety risks for the environment or human health.

Reporter genes: Fluorescent protein or enzymes

The reporter gene could be a fluorescent protein or an enzyme. Fluorescent proteins, for example GFP or dsRed, are not known to cause any diseases in human or animals and have no direct effect on cellular processes. Enzymes used frequently in reporter gene assay ie luciferase or b-galactosidase are not known to cause any diseases in humans or animals and as no direct effect on cellular processes. The expression of fluorescent protein or enzymes under the control of a promoter of a gene of interest is unlikely to pose any safety risks for the environment or human health.

Summary on lentivirus

The transgenes have a low safety risk to human health because accidental contamination with a vector would lead to only a small number of cells becoming infected, and it would be highly unlikely that expression in only a few cells could be sufficient to lead to a disease state. The inability of the lentiviral vectors to propagate on mammalian cells also reduces the risk. The programmable nucleases (CRISPR or Zinc finger or Cre recombinase) that will be employed in this viral vector system are not known to cause any relevant toxicity that might represent a safety risk to human health. The fluorescent proteins or the enzymes are not known to cause any relevant toxicities that might represent a safety risk to human health. For both programmable nucleases and shRNA, the genes/proteins of interest that will be targeted will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators, and none of them are or will be of retroviral origin. Therefore the risk of use will be low.

8- Immortalised human neural progenitor cells ReNcell 197VM & mHTT Exon1 197VM and transduced immortalised immortalised ReNcell197VM & mHTT Exon1 197VM cells

Immortalised ReNcell197VM & mHTT Exon1 197VM neural progenitor cells

The immortalised ReNcell 197VM cell line has been immortalized by retroviral transduction, using replication incompetent retrovirus, with the avian v-myc myelocytomatosis viral oncogene. One of the major risks would be if the sequences coding for the v-myc oncogene were mobilised. This could happen if the ReNcell 197VM or mHTT Exon1 197VM cells are accidentally inoculated to a worker already infected by a retroviral genome. Even in that case, a series of unlikely events would have to occur to:

a. The worker would have to be already infected with the HIV-1 virus

b. The retroviral genomes would have to integrate into the immortal human neural progenitor cells genome in a
position where they could interact to effect homologous recombination.
c. Recombination would have to occur in precise regions to allow for transfer of the v-myc oncogene sequences from
the ReNcell197VM or mHTT Exon1 197VM genome to the HIV-1 genome.
d. The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest.
Although the risk for human health is low, all measures will be in place to minimize the risk of human health to avoid
any accidental inoculation of a worker (see measures below).
The ReNcell mHTT Exon1197VM cells also contain the sequences forWPRE, cPPT and deltaU3 of HIV-1 virus.
However these cells should not contain the viral genes gag-pol and env required for virus production and therefore
cannot produce viruses. It is not known if the WPRE sequence has been mutated to remove the oncogenic WHV-X
protein from an open reading frame found in WPRE sequence. One of the major risk factors associated with the
utilisation of this cell line would be in that case accidental percutaneous inoculation of a worker. This ReNcell mHTT
Exon1 197VM cell line could theoretically not survive in the inoculated worker but would be quickly cleared by
the human immune system. Further modification of the ReNcell197VM or mHTT Exon1197VM cell line may enhance the
risk of mobilisation of the sequence coding for v-myc oncogene, or WPRE and the sequence of OU3 in the case of
ReNcell mHTT Exon1 197VM. Application to these cells of additional lentivirus particles could result in the
mobilisation of these sequences but should not result in the liberation of replication-competent retroviruses as the viral
genes gag-pol and env should not be present in the genome of the immortalised ReNcell mHTT Exon1 197VM neural
progenitor cells. The deltaU3 region contained in the ReNcell197VM mHTT Exon1 cells contains a deletion within
the 3'L TR region rendering it self-inactivating and the 3'L TR contained in any virus generated in house, using
pSMPUW, contains a hybrid enhanced polyA in the U3 region. If further modification is performed using lentivirus in
the ReNcell 197VM mHTT Exon1 cells, the L TR regions will be sequenced to check for lack of homology.
The ReNcell 197VM cells are likely to contain retroviral L TR sequences, with the v-myc oncogene. These L TR
sequences would be derived from MMLV and L TRs present in any lentivirus generated in house using pSMPUW
lentivirus backbone would derive from HIV-1 . There should not be sequence homology between these regions. If
further modifications are performed using lentivirus, these regions will be sequenced to check.
Furthermore, the risk can be minimised by ensuring that any lentivirus used in experiments with the host cells does
not contain gag-pol, env or any other significant retroviral genes involved in packaging and replication.
The ReNcell mHTT Exon1 197VM cells contain a mutant form of Huntingtin Exon 1. Data indicate that exon 1 proteins
are highly neurotoxic (Landles et al., 2010; Barbaro et al., 2015), and models that express either mutant full length
HTT or mutant exon 1 of HTT can be used effectively to study HD. Exon-1 expressing models demonstrate disease
phenotypes such as aggregate formation (Landles et al., 2010). One of the major risks would be if the sequence
coding for the mHTT Exon1 were mobilised. This could happen if the mHTT Exon1 197VM cells are accidentally
inoculated to a worker already infected by a retroviral genome. Even in that case, a series of unlikely events would
have to occur to (as described above for v-myc mobilisation).
Even if the events are unlikely, the immortalised human neural progenitor cell line and the transduced ReNcell 197VM
& mHTT Exon1 197VM cells will be maintained at biosafety level 2 containment. Therefore all measures will be in
place to minimize the risk to human health.
Human health hazards
Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident
outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited
number of the laboratory worker's skin or mucosal cells with modified viruses. However, these modified virus particles
carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the
particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the
viral vectors. Moreover, the programmable nucleases and shRNA that will be expressed or targeted will not be
designed to disrupt any growth factors, trophic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus or with the ReNcell 197VM & mHTT Exon1 197VM cell line will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent. Infection of the community at large by ReNcell197VM or mHTT Exon1 197VM cells is highly unlikely due to the impossibility of the cells to survive outside of a laboratory environment.

* Environment Considerations

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active lentiviral vector particles do accidently get released into the environment, the safety risk posed in such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable replication-competent lentivirus. However, this scenario is extremely unlikely and even if it was to occur, it is unlikely that it would lead to any untoward effects. As the HIV virus is a human pathogen, infection of another species would not be expected to allow any form of recombination event leading to a viable transmissible entity and so the risk from this scenario is considered to be negligible. Rodents, such as wild-type mice and rats, cannot support replication of infectious HIV-1 (Goffinet et al, 2007 Retrovirology). As a result, the potential for shedding of replication-competent lentiviruses from such animals is very low (even if they were present in the original vector inoculum).

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and in theory they are capable of transducing all dividing and non-dividing mammalian cell types. As emphasized before, the vectors cannot self-propagate after infection, and successful transduction is critically dependent on the presence of high enough concentrations of virus particles. Viral vector stock solutions will be managed in a way that will prevent contaminations with relatively high viral titres. After production, the vectors are aliquoted and stored in screw capped cryovials at -80°C. Taking into account that the infectivity of the vectors rapidly decreases at room temperature, it is considered highly unlikely that the vectors could survive in the long term after being accidentally released into the environment. Lentiviral vectors have a short half-life, at room temperature due to their structural characteristics making them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, as illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.

There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA sequences will also not survive as they are highly unstable, and they would, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

The risk to the environment following accidental release of the immortalised human neural progenitor cells would be negligible since the cells are incapable of surviving outside of laboratory conditions. If ReNcell 197VM cells or mHTT Exon1 197VM cells were to directly inoculate animals, they would be quickly cleared by the immune system and would not produce active viruses. The likelihood of this occurring will, however, be minimised by adhering to
appropriate risk management measures and ensuring that all potentially contaminated material is totally inactivated before disposal.

Summary
The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to. In the unlikely event that active viral vector particles do accidentally get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems. It is also unlikely that the immortalised human neural progenitor cells, ReNcell 197VM/mHTT Exon 197VM cells, and the transduced ReNcell197VM/mHTT Exon1 197VM cells will be accidentally released into the environment, as the cell handling and studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Following transduction of cell lines that are designated as requiring Biosafety Containment Level 1 with lentivirus, we wish to be able to subsequently use these cells at Biosafety Containment Level 1. This can be justified by the following procedure: under Biosafety Containment Level 2 media containing virus will be removed, the transduced cells will be extensively washed to remove any remaining virus. This liquid waste will be neutralised for at least 24 hours before disposal. The washed cells will then be cultured for a further 24 hours before being tested for the absence of infectious virus before being allowed to be treated as only requiring Biosafety Containment Level 1.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General cleaning procedures
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these validated chemical inactivators will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being autoclaved/incinerated.

Waste Handling
Following all work requiring biosafety level 2 containment including work with lentiviruses and ReNcell VM cells, solid waste will be neutralised by soaking in a validated disinfectant solution for 24 hours, after which the material will be "double bagged", autoclaved and, ultimately, incinerated offsite. It will be routine practise that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Liquid waste will be inactivated by Chemgene HLD4L * for 24 hours prior to drain disposal.
As any active viral particles or ReNcell VM cells on surface or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles or ReNcell VM cells would unintentionally become discharged into a sanitary sewage system. No foreseeable adverse effects on human health and safety are expected.

* Chemgene HLD4L
Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is
equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpes Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1%) for general purposes, 1:20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages.

Please enter comments on the GM safety committee on the risk assessment

The proposal outlined above uses ReNcelis that have been immortalised by self-inactivating retroviral integration of vMyc. A derivative of this cell line has been generated by the random integration of genetic sequence predominantly to express mHTT Exon 1 to generate a useful tool cell line for the study of Huntington's disease. It is not known if the WHV-X protein ORF has been deleted from the WPRE element in the lentivirus used for the expression of the mHTT Exon 1. The proposal then covers the knock down of gene targets via either shRNA, CRISPR/Cas9 or ZFN that could be delivered using lentivirus. In addition certain genes or variants of genes involved in disease may be integrated into the cell genome again by lentiviral vectors. Although it is noted that no viral particles are reported to be shed from the ReNcelis due to the presence of oncogenes delivered via retroviral & lentiviral integration into the host genome the GMSC confirms that the cell lines must be maintained under biosafety containment level 2. The GMSC is satisfied that because of the pre-cautions put in place around the lentiviral vectors to be used and genetic sequences to be delivered that this level of containment is suitable for the subsequent use of the cell lines.

Project Containment

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Project Ref 3043/16.2

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Project Additional Information

**Purposes of the contained use**

To utilise human induced pluripotent stem cells (hiPSC) generated using retroviral reprogramming by third party organisations for target identification, target validation and compound/biologics testing to facilitate drug discovery. HiPSC generated from healthy control donors and patients with disease-relevant mutations, will be used to investigate genes and cellular mechanisms involved in the initiation and development of human disease, for example neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, ALS, Huntington's disease. The hiPSC will be differentiated into a variety of both central nervous system (CNS) and non-CNS like mature cell types using non-genetic methodologies. These differentiated cells will be used in experiments including, but not limited to, cell-based assays, ELISA, western blotting, electrophysiology, immunocytochemistry/microscopy.

To expand understanding of the cellular mechanisms of a disease, or to use the cells for drug discovery-related work, the expression or the activity of genes of interest may be further modulated in the iPSC by several methods:

1. The utilisation of tool compounds
2. The over-expression of a protein of interest
3. The knock-down of the gene of interest by shRNA
4. The generation of modified somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
5. The generation of reporter gene cell line (i.e. fluorescence protein or enzyme) under the promoter of the gene of interest.

**Recipient or parental organism**

HiPSC derived from primary human cells, most commonly fibroblasts.

The hiPSC that will be used will have been generated using 3rd and or 4th generation self-inactivating (SIN) nonmobilisable integrating retroviral systems based on, but not limited to, mouse Maloney murine leukaemia virus (MMLV), Maloney murine sarcoma virus (MMSV), myeloproliferative sarcoma virus (MPSV), or human HIV. Some of the original donor material will not have been screened for human pathogens but donors will have a known medical history and will be classified as being low risk for retroviral pathogens. As such the hiPSC are highly unlikely to be producing replication competent lentivirus particles (RCLs).

Reprogramming of the hiPSC is achieved using a variety of factors, that are integrated into the cell genome by the retroviral vectors, that induce pluripotency in the donor cells. The following factors may be present in the supplied
hiPSCs:
SOX2: is a transcription factor that is essential for maintaining the pluripotency of undifferentiated embryonic stem cells.
KLF4: is a transcription factor that regulates proliferation, differentiation, apoptosis and somatic cell reprogramming. KLF4 may also act as a tumour suppressor gene.
OCT4: is a homeodomain transcription factor of the POU family. This protein is critically involved in the self-renewal of undifferentiated embryonic stem cells and is used as a marker for undifferentiated stem cells. It is indispensable for generating iPSC.
CMYC: is a transcription factor that plays a role in cell cycle progression, apoptosis and cellular transformation. Mutated c-Myc is found in many cancers, where it is constitutively expressed, leading to the unregulated expression of many genes, some of which are involved in cell proliferation leading to oncogenesis. This factor poses a risk if mobilised into RCL.
NANOG: is a transcription factor critically involved with self-renewal of undifferentiated embryonic stem cells.
LIN28: encodes a microRNA-binding protein; overexpression of which in mice can cause gigantism and a delay in puberty onset. Human GWAS studies indicates the LIN28B gene to be associated with human height and puberty timing. The biosafety risk is low for adults.
GLIS1: is a highly promiscuous transcription factor, positively or negatively regulating the expression of a number of genes.

Host/vector system

The vectors and factors used to generate any hiPSC that is supplied will be fully described prior to receipt and be reviewed by the Genetic Modification Safety Committee (GMSC) at Takeda Cambridge Ltd (TCB). Secondary transduction of the hiPSC with lentiviral vectors is planned. The hiPSC already contain integrated lentiviral sequences so subsequent lentiviral vectors will be non-homologous to mitigate the small risk of a recombination event leading to mobilisation of genetic material, such as the myc oncogene or immunomodulatory genes, that might pose a risk to human health. The integrated murine based lentivirus pose no additional risk to the environment. Secondary transduction would modify the hiPSCs to explore the underlying disease process or to generate tools for drug discovery. To this end the following could be used based upon the needs of drug discovery programs:
• Integration of shRNA constructs to ‘knock down’ expression of a gene of interest
• The over-expression of particular genes
• Specific modification of the host genome to using programmable nucleases:
  • Cas9/CRISPR
  • Zinc finger
• Reporter genes under the control of selected transcriptional regulatory sequences to study effects on cellular pathways.

The lentivirus used will either be obtained commercially or generated de novo internally using third of fourth generation vector systems. The vector backbone and promoters used to drive vector generation in these systems will be consistent. Genes selected for study will not include growth factors, tropic factors, quiescence factors, confirmed oncogenes or tumour suppressors.

Vectors will be generated using a non replication-competent HIV based lentiviral system that upon integration into the host genome loses part of its long terminal repeat (LTR) preventing excision at a later time point. Such selfinactivating (SIN) vectors are designated to produce stable gene expression in target mammalian cells. The viral particles can only be generated upon co-transfecting a packaging cell line (HEK 293T) with at least four separate plasmids. Three of which provide the minimal set of genes required for viral production (gag-pol, rev and env). The env gene used expresses the vesicular stomatitis virus (VSV-G) to increase tropism. The final plasmid supplies the...
genetic material to be supplied to the target cell and as such contains packaging sequences directing it to be incorporated into nascent viral particles. Only the minimum amount of lentiviral genome is used in the system and all of the plasmids lack homologous sequences to minimise any chance of recombination. The resultant lentiviral particles are VSV-G pseudotyped, SIN and replication incompetent in mammalian cells. Small aliquots of no more than 1 x 10^8 viral particles in 5 to 10 microlitres will be used further reducing the risk.

Third Generation Systems use 4 standard bacterial plasmid vectors. Three plasmids encode for proteins required for production and packaging of full length viral RNA (pCagV, pRSV-Rev and pCMV-VSVG). The gene of interest is contained in the pSMPUW plasmid. This is the only plasmid that contains the packaging sequence for incorporation into the virus particle.

Fourth Generation Systems use 6 standard bacterial plasmid vectors. The system uses tetracycline to control when viral particles are produced adding yet another level of control to production. pTRE-gag-pro, LTR HIV2-vpr-pol, penv(VSV-G), pTet-Off and ptat-IRES-rev contain the sequences that code for proteins that are responsible for the tetracycline control and packaging the viral vectors. As above pSMPUW only contains the genetic material to be packaged into the vector and no other lentiviral gene sequence. An integrase deficient version of the fourth generation is available which contains a mutation in the sequence encoding the viral integrase.

The plasmid, pSMPUW, will contain the genetic sequence to be introduced. These will not include growth factors, tropic factors, quiescence factors, confirmed oncogenes or tumour suppressors. All proposed sequences to be used will require approval by the GMSC.

- Vector containing shRNA - each vector will code for one shRNA transcript that will be designed to target one mRNA/protein of interest (or a scrambled shRNA in the case of negative control). This will be under the control of a polymerase II or III promoter.
- Vector containing CRISPR or Zinc-finger nucleases - Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This instigates a robust DNA repair response which can either be used to disrupt the target sequence by deletion at the cleavage site or to introduce user-supplied genetic material via recombination with a vector containing complementary sequences to the target gene.
  - For Cas9/CRISPR, nuclease target specificity is determined by a guide RNA, which consists of a short (18-20 nucleotide) sequence homologous to the target gene and an additional short sequence that forms a complex with the Cas9 enzyme. Vector will encode Cas9/CRISPR plus a guide RNA under the control of a Polymerase III promoter.
  - Zinc finger nuclease target specificity is dependent upon the zinc finger protein sequence which will be under the control of a polymerase II promoter.

A fluorescent protein or enzyme under the control of a polymerase II promoter or the promoter of a gene of interest may be included. Control vectors only expressing fluorescent protein/enzyme will also be used.

- Reporter or overexpression gene vector - The vector will contain either sequence coding for a reporter protein, a protein to be overexpressed under the control of regulatory elements of a promoter of a gene of interest or a polymerase II promoter. An appropriate selection marker i.e.; neomycin could also be included to allow the selection of cells stably containing either the introduced genetic material.

The efficacy of lentiviral vectors will first be assessed using cell lines that routinely require biosafety level 1 containment (BSL 1). Application of lentiviral particles will be done at biosafety level 2 containment. At least 24 hours post transduction the cells will be washed to remove any residual viral particles and then treated as BSL 1 as the potential risk has been minimised. The efficacy of the genome manipulation will be assessed using in vitro assays. hiPSC will be transduced with validated lentivirus. All procedures will be conducted at BSL2. Stable cell lines will be documented. Resultant cells will be studied to further understand disease processes or used for drug discovery efforts. This will be done by phenotypic analysis using a range of common in vitro assays, including but not limited to
Evaluation of foreseeable effects

hiPSC are pluripotent and are capable of uncontrolled proliferation. Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. The lentiviral vector system that will be used is based on HIV-1. Retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airborne route. Piercing of the skin represents the main potential route by which material could be accidentally introduced into an individual. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

Accidental introduction of hiPSC into healthy individuals should not lead to the formation of teratomas because the immune system will rapidly destroy these cells. As mentioned above the pathogen status of some cells is not known. However the material is not derived from high-risk clinical patients and procedures are in place to deal with needle stick incidents. Individuals with compromised immune systems are not permitted to work with this material.

The genetic material to be incorporated into the viral particles is flanked by non-coding retroviral LTRs in the pSMPUW plasmid. No retroviral genes are encoded on this plasmid minimising the chance any will be packaged into viral particles. As the system requires the co-transfection of three to five additional separate plasmids into a permissive cell line the chance of recombination occurring that leads to the incorporation of any or all of the retroviral genes necessary for the production of a replication competent lentivirus (RCL) is very low and the risk is therefore low.

The lentiviral Expression System includes the following key safety features:

* Only pSMPUW includes LTR and packaging signal sequence required for incorporation into viral particles and integration into the genome of a transduced cell. A hybrid 3’LTR is used that does not affect generation of the viral genome in the producer cell line, but upon integration into the genome of a target cell, the 3’LTR SIN and prevents production of viral genomic material that can be packaged and reduces the chance of mobilisation due to secondary infection with lentivirus. No transcriptionally active LTRs are present in the system and an SV40 polyA is included after the hybrid 3’LTR to reduce the potential for transactivation of cellular genes due to an insertion event that might promote inappropriate gene expression leading to oncogenic effects.

* The LTR has been modified to enable lentiviral production independent of Tat expression. HIV-1 devoid of Tat is known to be replication incompetent. This decreases the risk of RCL occurring.

* The essential genes encoding the proteins required for packaging of the viral genome are separated onto three to five plasmids. These express the proteins (gag, pol, rev, env) required to generate viral particles transiently in the HEK-293T packaging cell line. Other retroviral structural genes are not present in the system. None of the plasmids have regions of homology which will prevent undesirable recombination. As multiple recombination events would be required to generate a RCL the risk of this happening is very low and has so far not been observed in large-scale production and testing of lentiviral vectors.

* Expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Expression requires the presence of rev, which is supplied by the pRSV-REV plasmid. The Rev/RRE system is highly conserved among lentiviruses and loss of the RRE sequence and associated splice donor/acceptor sequences results in a loss of transduction efficiency.

* Lentiviral vectors have a very low potential to cause immunogenicity.

* The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction.

Whilst pseudotyped VSV-G is used as the envelop protein to increase the cell types that can be infected this increase in risk is mitigated by the SIN non-replication nature of the viral vectors generated. Insertional events that disrupt gene function can occur and in some instances might lead to undesirable effects and could be oncogenic. The resultant lentiviral vectors contain about 20% of the original HIV-1 genome. Recombination with wild-type HIV-1 is still potentially possible and would result in mobilisation of the transgene. This is highly unlikely because:
1. Wild-type HIV-1 is not used with in the laboratory environment.
2. hiPSC are not derived from donors either known to be HIV positive or from a high-risk population; so HIV contamination is highly unlikely.
3. Wild type HIV-1 cannot rescue a SIN HIV-1 based lentivirus once integrated into the host genome.
4. It is likely that any recombination would actually decrease the ability of HIV-1 to infect other cells or to replicate.
5. Even if a worker was already or became HIV-1 positive and was accidentally infected with lentiviral vector any recombination would require infection of the same cells. Generation of RCL would require homologous recombination to be in the right regions to enable mobilisation and incorporation to the HIV-1 genome without loss of replication ability. If such a rare event were to occur it is likely to be self limiting and the HIV-1 infection itself is of greater concern to the worker.

The use of lentiviral vectors is an efficient manner with which to deliver genetic material to numerous cell types. The design of the vectors minimises the chance for subsequent mobilisation of transgenes and inappropriate activation of endogenous oncogenes. Insertional events can lead to harmful side effects but are still very unlikely. As such the greatest risk from these vectors comes with their production where much larger volumes and numbers of vectors are being generated. The risk here is mitigated by the correct use of containment measures and the absence of sharps. Lentiviral vectors are susceptible to dehydration and loss of viability if they are not stored in high protein conditions which reduces the risk to workers and the wider environment.

Transgenes to be delivered to target cells:
Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected hiPSC cells.
Gene products to be overexpressed will not include any growth factors, confirmed oncogenes, or tumor suppressors. The focus of the research is on neurodegenerative diseases so with justification to and with GMSC approval immunomodulators or proteins known to be involved in neurodegeneration may be delivered by lentivirus. The 'knock-down' of specific gene expression using shRNA can lead to off-target and immunomodulatory responses in vivo. shRNA sequences will be screened against databases of known mammalian/mRNA sequences during the design stage to avoid such complications. Genes encoding any growth factors, tropic factors, quiescence factors, confirmed oncogenes, or tumor suppressors will not be targeted. Control vectors containing a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to and therefore do not target any known mammalian sequences. Such sequences are unlikely to pose any safety risks for the environment or human health.
Programmable nucleases: zinc fingers or Cas9/CRISPR will be directed against genes of therapeutic interest. Again genes that will be targeted will not include any growth, tropic factors, confirmed oncogenes, tumor suppressors or immunomodulators. In isolation, these sequences are unlikely to pose any safety risks for the environment or human health. Programmable nucleases may be used to generate isogenic controls lines by correcting disease causing mutations back to wild-type sequence. This is low risk because the sequence is aimed to revert back to non-disease forms of the gene.
Reporter genes: Commonly used fluorescent proteins or enzymes under the regulatory control of promoters of interest may be used to monitor effects on cellular pathways. Such proteins are not known to cause any human or animal disease and pose no-risk.
In all cases justification for any target must be submitted to the GMSC for approval prior to any work commencing.

Summary
hiPSC will be derived from low risk patient populations but may not be screened for human pathogens. The lentiviral systems are replication incompetent and SIN minimising the potential for transgene mobilisation and propagation. The transgenes that can be used will on their own have a low risk to human health. Infection of mucosal cells may occur...
via aerosols but is highly unlikely due to the use of microbiological safety cabinets and secondary containment. Such infection is self limiting due to the natural shedding of epithelial cells coupled with the replication incompetent nature of the vectors. The most likely route of accidental infection with a lentivirus or with the hiPSC line will be via inadvertent percutaneous inoculation via stick injury or open wound. The likelihood of this occurring will be minimised by following standard BSL2 containment practices. Infection of the community and environment with lentivirus particles is highly unlikely due to small quantities used, their intrinsic instability and rapid loss of viability with time. hiPSC can not survive outside of the laboratory environment so pose no wider risk. Therefore the risks towards workers, co-workers, the public and the environment associated with the use of these lentiviral vectors with hiPSC will be low and BSL2 containment is sufficient.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Handling
Solid waste will be neutralised by soaking in a validated disinfectant solution (5% Chemgene HLD4L), after which the material will be autoclaved and then sent for incineration via a registered waste disposal company. All liquid waste will be inactivated for 24hrs prior to disposal to drains via a designated sink.
Chemgene HLD4L: is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpes Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1: 1 00 dilution (1 %) for general purposes, 1 :20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages.
General cleaning procedures
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being autoclaved/incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
It is noted that the primary human cell lines used for the generation of hiPSES will be taken from patient populations with a known medical history meaning that there is a low risk of material containing harmful human pathogens. This does not rule out the presence of latent viral infection but the use of third or fourth generation integrating lentiviral systems that are self-inactivating and essentially non-mobilisable makes it highly unlikely that infective replication competent virus will be generated. Important due to the presence of the potentially oncogenic Mye sequence. Additional rounds of lentiviral transduction should not be able to mobilise any genetic elements because self-inactivating systems will be used that lack the necessary genes for viral replication and packaging. As an additional measure the GMSe will have a clear understanding of how each cell line was generated and steps will be taken to ensure overlapping and therefore potential sites for recombination are not present in lentiviral vectors. These vectors are for the introduction of further modifications. Those aimed at correcting disease mutations do not pose a risk to health. The introduction of non-disease causing genetic material for exogenous expression is not seen as a risk either. In the instance where immune-modulators may be targeted the risk although increased is well contained by the avoidance of sharps during transduction procedures. Once cells are transduced and virus removed the risk posed here is very low. Due to the use of iPSe derived from primary human cells and integrating lentiviral vectors this dictates propagation, differentiation and further transduction of cell lines at BSL2. Subsequent testing of the cells will also continue at BSL2 although the genetic modification of the cells is of very low risk.

Project Containment

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<th>Large Scale Activities</th>
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**Project Ref** 3043/16.3

**Date Ackn'd** 02/12/2016

**CU2 Project Title** Use of human induced pluripotent stem cells generated from primary human cells by episomal methodologies

**Class** Class 2

**Culture Vol** < 1 Litre

**Consent Granted** Non-GMM Consent Granted

**Tick if notifying a connected programme of work** N

**Historical Significant Changes** transferred from GM3043 02/12/2016
Project Additional Information

Purposes of the contained use

To utilise human induced pluripotent stem cells (hiPSC) generated using episomal reprogramming by third party organisations for target identification, target validation and compound/biologics testing to facilitate drug discovery. HiPSC generated from healthy control donors and patients with disease-relevant mutations, will be used to investigate genes and cellular mechanisms involved in the initiation and development of human disease, for example neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, ALS, Huntington's disease. The hiPSC will be differentiated into a variety of both central nervous system (CNS) and non-CNS like mature cell types using non-genetic methodologies. These differentiated cells will be used in experiments including, but not limited to, cell-based assays, ELISA, western blotting, electrophysiology, immunocytochemistry/microscopy. To expand understanding of the cellular mechanisms of a disease, or to use the cells for drug discovery-related work, the expression or the activity of genes of interest may be further modulated in the iPSC by several methods:

1. The utilisation of tool compounds
2. The over-expression of a protein of interest
3. The knock-down of the gene of interest by shRNA
4. The generation of modified somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
5. The generation of reporter gene cell line (i.e. fluorescence protein or enzyme) under the promoter of the gene of interest.

Recipient or parental organism

HiPSC derived from primary human cells, most commonly fibroblasts.

Host/vector system

The hiPSC will have been generated using an Epstein Barr Virus episomal system by a third party. By the time of transfer to Takeda Cambridge Ltd these vectors will have been lost and the cells deemed 'foot-print' free. The cells are also checked for absence of active expression of the genes present on the episomes. Although the gene expression profile has been altered they are essentially not genetically modified and the only risk is associated with any human pathogens present in culture. This risk is mitigated by donors having a known medical history and
Secondary transduction would modify the hiPSCs to explore the underlying disease process or to generate tools for drug discovery. To this end the following could be used based upon the needs of drug discovery programs:

- Integration of shRNA constructs to 'knock down' expression of a gene of interest
- The over-expression of particular genes
- Specific modification of the host genome to using programmable nucleases:
  - Cas9/CRISPR
  - Zinc finger
- Reporter genes under the control of selected transcriptional regulatory sequences to study effects on cellular pathways

The lentivirus used will either be obtained commercially or generated de novo internally using third of fourth generation vector systems. The vector backbone and promoters used to drive vector generation in these systems will be consistent. Genes selected for study will not include growth factors, tropic factors, quiescence factors, confirmed oncogenes or tumour suppressors.

Vectors will be generated using a non replication-competent HIV based lentiviral system that upon integration into the host genome loses part of its long terminal repeat (LTR) preventing excision at a later time point. Such self-inactivating (SIN) vectors are designated to produce stable gene expression in target mammalian cells. The viral particles can only be generated upon co-transfecting a packaging cell line (HEK 293T) with at least four separate plasmids. Three of which provide the minimal set of genes required for viral production (gag-pol, rev and env). The env gene used expresses the vesicular stamatis virus (VSV-G) to increase tropism. The final plasmid supplies the genetic material to be supplied to the target cell and as such contains packaging sequences directing it to be incorporated into nascent viral particles. Only the minimum amount of lentiviral genome is used in the system and all of the plasmids lack homologous sequences to minimise any chance of recombination. The resultant lentiviral particles are VSV-G pseudotyped, SIN and replication incompetent in mammalian cells. Small aliquots of no more than 1x10⁸ viral particles in 5 to 10 microlitres will be used further reducing the risk.

Third Generation Systems use 4 standard bacterial plasmid vectors. Three plasmids encode for proteins required for production and packaging of full length viral RNA (pCgpV, pRSV-Rev and pCMV-VSVG). The gene of interest is contained in the pSMPUW plasmid. This is the only plasmid that contains the packaging sequence for incorporation into the virus particle.

Fourth Generation Systems use 6 standard bacterial plasmid vectors. The system uses tetracycline to control when viral particles are produced adding yet another level of control to production. pTRE-gag-pro, LTRHIV2-vpr-pol, penv(VSV-G), pTet-Off and ptat-RES-rev contain the sequences that code for proteins that are responsible for the tetracycline control and packaging the viral vectors. As above pSMPUW only contains the genetic material to be packaged into the vector and no other lentiviral gene sequence. An integrase deficient version of the fourth generation is available which contains a mutation in the sequence encoding the integrase domain.

The hiPSC will have been generated using an Epstein Barr Virus episomal system by a third party. By the time of transfer to Takeda Cambridge Ltd these vectors will have been lost and the cells deemed ‘foot-print’ free. The cells are also checked for absence of active expression of the genes present on the episomes. Although the gene expression profile has been altered they are essentially not genetically modified and the only risk is associated with any human pathogens present in culture. This risk is mitigated by donors having a known medical history and belonging to low risk populations. Secondary transduction would modify the hiPSCs to explore the underlying disease process or to generate tools for drug discovery. To this end the following could be used based upon the needs of drug discovery programs:
Integration of shRNA constructs to 'knock down' expression of a gene of interest
• The over-expression of particular genes
• Specific modification of the host genome to using programmable nucleases:
  • Cas9/CRISPR
  • Zinc finger
• Reporter genes under the control of selected transcriptional regulatory sequences to study effects on cellular pathways

The lentivirus used will either be obtained commercially or generated de novo internally using third of fourth generation vector systems. The vector backbone and promoters used to drive vector generation in these systems will be consistent. Genes selected for study will not include growth factors, tropic factors, quiescence factors, confirmed oncogenes or tumour suppressors.

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Third Generation Systems use 4 standard bacterial plasmid vectors. Three plasmids encode for proteins required for production and packaging of full length viral RNA (pCgpV, pRSV-Rev and pCMV-VSVG). The gene of interest is contained in the pSMPUW plasmid. This is the only plasmid that contains the packaging sequence for incorporation into the virus particle.

Fourth Generation Systems use 6 standard bacterial plasmid vectors. The system uses tetracycline to control when viral particles are produced adding yet another level of control to production. pTRE-gag-pro, LTRHIV2-vpr-pol, penv(VSV-G), pTet-Off and plat-IRES-rev contain the sequences that code for proteins that are responsible for the tetracycline control and packaging the viral vectors. As above pSMPUW only contains the genetic material to be packaged into the vector and no other lentiviral gene sequence. An integrase deficient version of the fourth generation is available which contains a mutation in the sequence encoding the viral integrase.

Evaluation of foreseeable effects

hiPSC are pluripotent and are capable of uncontrolled proliferation. Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. The lentiviral vector system that will be used is based on HIV-1. Retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airborne route. Piercing of the skin represents the main potential route by which material could be accidentally introduced into an individual. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

Accidental introduction of hiPSC into healthy individuals should not lead to the formation of teratomas because the immune system will rapidly destroy these cells. As mentioned above the pathogen status of some cells is not known. However the material is not derived from high-risk clinical patients and procedures are in place to deal with needle stick incidents. Individuals with compromised immune systems are not permitted to work with this material.

The genetic material to be incorporate into the viral particles is flanked by non-coding retroviral LTRs in the pSMPUW plasmid. No retroviral genes are encoded on this plasmid minimising the chance any will be packaged into viral
particles. As the system requires the co-transfection of three to five additional separate plasmids into a permissive cell line the chance of recombination occurring that leads to the incorporation of any or all of the retroviral genes necessary for the production of a replication competent lentivirus (RCL) is very low and the risk is therefore is low. The lentiviral Expression System includes the following key safety features:

* Only pSMPUW includes LTR and packaging signal sequence required for incorporation into viral particles and integration into the genome of a transduced cell. A hybrid 3’LTR is used that does not affect generation of the viral genome in the producer cell line, but upon integration into the genome of a target cell, the 3’LTR SIN and prevents production of viral genomic material that can be packaged and reduces the chance of mobilisation due to secondary infection with lentivirus. No transcriptionally active LTRs are present in the system and an SV40 polyA is included after the hybrid 3’LTR to reduce the potential for transactivation of cellular genes due to an insertion event that might promote inappropriate gene expression leading to oncogenic effects.

* The LTR has been modified to enable lentiviral production independent of Tat expression. HIV-1 devoid of Tat is known to be replication incompetent. This decreases the risk of RCL occurring.

* The essential genes encoding the proteins required for packaging of the viral genome are separated onto three to five plasmids. These express the proteins (gag, pol, rev, env) required to generate viral particles transiently in the HEK-293T packaging cell line. Other retroviral structural genes are not present in the system. None of the plasmids have regions of homology which will prevent undesirable recombination. As multiple recombination events would be required to generate a RCL the risk of this happening is very low and has so far not been observed in large-scale production and testing of lentiviral vectors.

* Expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Expression requires the presence of rev, which is supplied by the pRSV-REV plasmid. The Rev/RRE system is highly conserved among lentiviruses and loss of the RRE sequence and associated splice donor/acceptor sequences results in a loss of transduction efficiency.

* Lentiviral vectors have a very low potential to cause immunogenicity.

* The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction.

Whilst pseudotyped VSV-G is used as the envelop protein to increase the cell types that can be infected this increase in risk is mitigated by the SIN non-replication nature of the viral vectors generated. Insertional events that disrupt gene function can occur and in some instances might lead to undesirable effects and could be oncogenic. The resultant lentiviral vectors contain about 20% of the original HIV-1 genome. Recombination with wild-type HIV-1 is still potentially possible and would result in mobilisation of the transgene. This is highly unlikely because:

1. Wild-type HIV-1 is not used with in the laboratory environment.
2. hiPSC are not derived from donors either known to be HIV positive or from a high-risk population; so HIV contamination is highly unlikely.
3. Wild type HIV-1 cannot rescue a SIN HIV-1 based lentivirus once integrated into the host genome.
4. It is likely that any recombination would actually decrease the ability of HIV-1 to infect other cells or to replicate.
5. Even if a worker was already or became HIV-1 positive and was accidentally infected with lentiviral vector any recombination would require infection of the same cells. Generation of RCL would require homologous recombination to be in the right regions to enable mobilisation and incorporation to the HIV-1 genome without loss of replication ability. If such a rare event were to occur it is likely to be self limiting and the HIV-1 infection itself is of greater concern to the worker.

The use of lentiviral vectors is an efficient manner with which to deliver genetic material to numerous cell types. The design of the vectors minimises the chance for subsequent mobilisation of transgenes and inappropriate activation of endogenous oncogenes. Insertional events can lead to harmful side effects but are still very unlikely. As such the
greatest risk from these vectors comes with their production where much larger volumes and numbers of vectors are
being generated. The risk here is mitigated by the correct use of containment measures and the absence of sharps.
Lentiviral vectors themselves rapidly lose ability to transduce cells if not stored in high protein buffer and are
susceptible to dehydration. This reduces the risk of accidental exposure to workers and the environment.
Transgenes to be delivered to target cells:
Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and
expressed in transduced hiPSC cells.
Gene products to be overexpressed will not include any growth factors, confirmed oncogenes, or tumor
suppressors. The focus of the research is on neurodegenerative diseases so with justification to and with GMSC
approval immunomodulators or proteins known to be involved in neurodegeneration may be delivered by lentivirus.
The 'knock-down' of specific gene expression using shRNA can lead to off-target and immunomodulatory
responses in vivo. shRNA sequences will be screened against databases of known mammalian/mRNA sequences
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Programmable nucleases: zinc fingers or Cas9/CRISPR will be directed against genes of therapeutic interest.
Again genes that will be targeted will not include any growth, trophic factors, confirmed oncogenes, tumor
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disease causing mutations back to wild-type sequence. This is low risk because the sequence is aimed to revert back
to non-disease forms of the gene.
 Reporter genes: Commonly used fluorescent proteins or enzymes under the regulatory control of promoters of
interest may be used to monitor effects on cellular pathways. Such proteins are not known to cause any human or
animal disease and pose no-risk.
In all cases justification for any target must be submitted to the GMSC for approval prior to any work commencing.
Summary
hiPSC will be derived from low risk patient populations but may not be screened for human pathogens. The cell
supplied to Takeda Cambridge Ltd will be 'foot-print' free and can be regarded as not being genetically modified.
Subsequent lentiviral vector systems are replication incompetent and SIN essentially preventing transgene mobilisation
and propagation. The transgenes that can be used will on their own have a low risk to human health. Infection of
mucosal cells may occur via aerosols but is highly unlikely due to the use of microbiological safety cabinets and
secondary containment. Such infection is self limiting due to the natural shedding of epithelial cells coupled with the
replication incompetent nature of the vectors. The most likely route of accidental infection with a lentivirus or with the
hiPSC line will be via inadvertent percutaneous inoculation via stick injury or open wound. The likelihood of this
occurring will be minimised by following standard BSL2 containment practices. Infection of the community and
environment with lentivirus particles is highly unlikely due to small quantities used, their intrinsic instability and rapid
loss of viability with time. hiPSC can not survive outside of the laboratory environment so pose no wider risk.
Therefore the risks towards workers, co-workers, the public and the environment associated with the use of these
lentiviral vectors with hiPSC will be low and BSL2 containment is sufficient.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Handling

Solid waste will be neutralised by soaking in a validated disinfectant solution (5% Chemgene HLD4L), after which the material will be autoclaved and then sent for incineration via a registered waste disposal company. All liquid waste will be inactivated for 24hrs prior to disposal to drains via a designated sink.

Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1 %) for general purposes, 1 :20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages.

General cleaning procedures
Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L * before being autoclaved/incinerated.

Please enter comments on the GM safety committee on the risk assessment

It is noted that the primary human cell lines used for the generation of hiPSCs will be taken from patient populations with a known medical history meaning that there is a low risk of material containing harmful human pathogens. This does not rule out the presence of latent viral infection but the use of third or fourth generation integrating lentiviral systems that are self-inactivating and essentially non-mobilisable makes it highly unlikely that infective replication competent virus will be generated. None of the factors used to generate the hiPSC will still be present in the cells. The lentiviral vectors described here pose a low risk because they non-mobilisable, self-inactivating and replication incompetent. In addition they lack the genetic elements necessary for viral replication and packaging. As an additional measure the GMSC will have a clear understanding of the proposed modifications. Vectors aimed at correcting disease mutations do not pose an additional risk to health. The introduction of non-disease causing genetic material for exogenous expression is not seen as a risk either. In the instance where immune-modulators may be targeted the risk although increased is well contained by the avoidance of sharps during transduction procedures. Once cells are transduced and virus removed the risk posed by the lentivirus is essentially removed. The main continuing risk is from the hiPSC and the potential presence of human pathogens. For this reason any propagation, differentiation, transduction and testing of cell lines will be carried out at BSL2.

Project Containment
**Laboratory Activities** | **Glass Houses** | **Growth Rooms**
---|---|---
L2 Yes | L3 | L4
L2 | L3 | L4
L2 | L3 | L4

**Animal Units** | **Large Scale Activities** | **Human Clinical Applications**
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L2 | L3 | L4
L2 | L3 | L4
L2 | L3 | L4

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**Project Ref** 3043/16.4

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<td>Use of genetic modification of human induced pluripotent stem cells generated using primary human fibroblasts and Sendai virus</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM Consent Granted</td>
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Tick if notifying a connected programme of work: Y

Historical Significant Changes: transferred from GM3043 02/12/2016

**Project Additional Information**

**Purposes of the contained use**

Human induced pluripotent stem cells (hiPSC), generated from healthy control and patients with disease-relevant mutations (specifically one healthy parental control & three offspring samples with varying CAG expansions within Huntingtin exon 1 coding sequence), will be used to investigate genes and cellular mechanisms involved in the initiation and development of neurodegenerative disorders, for example, Alzheimer's disease, Parkinson's disease, ALS, Huntington's disease etc.

To better understand these genes and cellular mechanisms, the expression or the activity of the gene of interest will be modulated by several methods:

1. The utilisation of tool compounds
2. The knock-down of the gene of interest by shRNA
3. The generation of modified somatic cells using programmable nucleases (CRISPR or Zinc finger nucleases)
4. The generation of reporter gene (e.g., fluorescence protein or enzyme) under the control of a promoter of the gene of interest or an overexpressing cell line under the control of a promoter of the gene of interest or a ubiquitously expressing promoter

The experiments involving the transfection of vectors such as shRNA or programmable nucleases may be performed by lentivirus. The effect of modulating the expression or the activity of the target of interest will be determined by the expression level of the proteins, mRNA or mediators involved in the target signalling pathway.

Recipient or parental organism

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| The iPS cells are established by reprogramming human fibroblasts. These fibroblast cells have been derived from healthy and patient skin biopsies following informed consent. Reprogramming of human fibroblast cells to iPS cells was performed at the Rockefeller University using a commercially available kit sold by Life Technologies, Cytotune - iPS 2.0 Sendai Reprogramming Kit. The reprogramming vectors include the four Yamanaka factors, Oct, Sox2, Klf4 & c-Myc, shown to be sufficient for efficient reprogramming (Takahashi et al, 2007). These are expressed from the following vectors, CytoTune 2.0 KOS, CytoTune 2.0 h-c-Myc & CytoTune 2.0 hKlf4. iPS cells have been tested with Applied Biosystems TaqMan iPSC Sendai Detection kit. This kit is used to detect the presence of and determine the levels of Sendai virus and exogenous transcription factors (cMyc, Oct3/4, Klf4, Sox2) delivered by the Sendai virus. No expression was detected in RNA from the reprogrammed iPS cells. This would indicate that there is no virus present in these iPS cells. Sendai virus is a negative sense single stranded RNA virus which must be converted to positive RNA (mRNA) before translation. The virus remains in the cytoplasm, does not enter the nucleus and does not go through a DNA intermediary, therefore the reprogramming should not result in a permanent genetic modification of these cells. Fibroblast lines from donors have been provided as a basic research tool to investigate human disease. Consent to screen for major human pathogens is not sought at the time of donation. The donors are not known to be positive for any human pathogens. However, screening the cells for major human pathogens would have potentially significant clinical health implications for patients (e.g., HIV), for which explicit consent has not been given at the time of donation. As such, the cells and subsequent iPSC lines generated, have not and cannot be screened for human pathogens and must therefore be handled at Biological Safety Level 2.

Host/vector system

B- Description of the lentivirus containing shRNA, programmable nucleases sequences or gene reporter vectors

To utilise the iPS cells to study neurodegeneration, the expression of a target of interest may be decreased in the iPSC line by the transduction of shRNA or programmable nuclease by lentivirus. The modulation of a particular cellular pathway may be studied with the use of a reporter gene assay. In this case, the vector containing the reporter gene under the regulation of the promoter of the gene of interest may be introduced by standard transfection methods or lentivirus into the iPS cells This proposal may involve both the use of existing lentiviral constructs purchased commercially and the generation of new constructs on site using either a third or fourth generation system. Both commercially acquired and in-house lentivirus will use the same transfer vector plasmid. Target genes will be selected based on the needs of drug discovery projects; programmable nucleases and shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators (these targets will be approved internally by the GMSC). The production of lentivirus containing the vectors of interest are described below:
1- Generation of lentivirus
Lentiviral vectors will be purchased commercially or produced in-house using either a third or fourth generation system described below. Each purified and concentrated aliquot of lentiviral reagent comprised of a small volume (5 to 100 μl) that contains no more than 1x10^8 viral particles.

Vector System = self-inactivating (SIN) non replication-competent HIV based lentiviral vectors

The viral vectors that will be used in the proposed studies are self-inactivating non replication-competent HIV based lentiviral vector systems, which have been desiganted to produce stable gene expression in mammalian cells. They are generated by co-transfecting HEK 293T cells with four to six separate plasmids. Each of the plasmids used expresses a different set of genes which, when combined following co-transfection, provide the smallest possible set of essential viral genes that is still compatible with virus production. Moreover, the vectors are all vesicular stomatitis virus (VSV-G) pseudotyped lentiviral vectors, which are self-inactivating and highly unlikely to undergo recombination. Importantly, the viral vector is inherently incapable of replication in mammalian cells.

The plasmids

Third Generation
The sequences that will be included in the viral vectors are sub-cloned into 4 standard bacterial plasmid vectors. Plasmids containing the packaging related sequences

Each of the three other plasmids, pCgpV, pRSV-Rev and pCMV-VSVG contain a different set of sequences that code for proteins that are responsible for packaging the viral vectors. All the genes (gag-pol, rev and env) required for production and packaging of the full length viable viral RNA particles have been removed from the pSMPUW lentiplasmids containing the transgenes of interest.

Fourth Generation
The sequences that will be included in the viral vectors are sub-cloned into 6 standard bacterial plasmid vectors
Plasmids containing the packaging related sequences

Each of the other plasmids, pTRE-gag-pro, L TRHIV2-vpr-pol, penv(VSV-G), pTet-Off and p tat-IRES-rev contain a different set of sequences that code for proteins that are responsible for packaging the viral vectors. All the genes (gag-pol, rev and env) required for production and packaging of the full length viable viral RNA particles have been removed from the pSMPUW lentiplasmids containing the transgenes of interest. An integrase deficient version of the fourth generation packaging mix is available which contains a mutation in the sequence encoding the viral integrase. The resulting integrase-deficient lentivirus (IDLV) generates circular vector episomes in transduced target cells that are gradually lost by dilution in dividing cells (transient expression), but are stable in quiescent cells.

Plasmids containing the transgene of interest

The plasmid, pSMPUW, will contain the genetic sequence that codes for either shRNA, programmable nuclease, a reporter gene under the promoter of the gene of interest or protein of interest under the control of the regulating elements of the promoter of the gene of interest or a ubiquitously expressing promoter.

a- Vector containing shRNA
As mentioned above, pSMPUW plasmid will contain the sequence that code for a selected shRNA transcript that will target one mRNAs/protein of interest (or a scrambled shRNA in the case of negative control). The shRNA or scrambled shRNA sequences will be under the control of polymerase II or III promoter. The shRNA sequences will be chosen to target a single gene. The shRNA will not be produced against any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators.

b- Vector containing CRISPR or Zinc-finger nucleases
Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the
Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This in turn instigates a robust DNA repair response which ultimately leads to disruption of the target sequence by deletion at the cleavage site or by recombination with a user-supplied synthetic vector containing sequences complementary to the target gene.

For Cas9/CRISPR, nuclease target specificity is determined by an accessory sequence, the guide RNA, which consists of a short (18-20 nucleotide) sequence homologous to the target gene and an additional short sequence that forms a complex with the Cas9 enzyme. In this case, the plasmid pSMPUW, will contain the sequences that code for a Cas9/CRISPR plus accessory sequences comprising a short guide RNA that determines target specificity. The guide RNA sequences will be under the control of a Polymerase III promoter.

In the case of Zinc finger nucleases target specificity is dependent upon the zinc finger protein sequence. The pSMPUW will contain in that case the sequence of zinc finger nucleases under the control of a polymerase II promoter. In addition, a fluorescent protein or enzyme under the control of the polymerase II promoter or the promoter of a gene of interest may be included. Control viral vectors that express only the fluorescent protein/enzyme and no programmable nuclease will be used in some experiments.

The Cas9/CRISPR, if introduced into any cell line by lentivirus, will use the integrase deficient version of fourth generation packaging mix.

c- Reporter gene/Overexpression vector

The pSMPUW vector will contain the sequence coding for a reporter protein (enzyme or fluorescent protein) or protein of interest under the control of the regulating elements of the promoter of the gene of interest or a ubiquitously expressing promoter. An appropriate selection marker i.e.; neomycin could also be included to allow the selection of stable human iPS cells containing the reporter gene sequence or gene of interest under the control of the promoter of the gene of interest or a polymerase II promoter. Any overexpression will not be of growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators. Any protein to be overexpressed will be approved by GMSC beforehand.
the gene of interest or a polymerase II promoter. Any overexpression will not be of growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators. Any protein to be overexpressed will be approved by GMSC beforehand.

**Evaluation of foreseeable effects**

* Human Health

The human iPS cells that will be used have a low risk to human health. Samples are derived from patients that are not known to be infected but the disease status cannot be confirmed. Hence treated with caution at BSL2. The expression systems and the transgenes that comprise the lentiviral vectors that will be used have also a very low risk for human health and safety, and the reasons for this are discussed below.

A- Reprogrammed human iPS cells

The reprogrammed human iPS cells have been generated by reprogramming human skin fibroblasts using Sendai virus expressing a number of reprogramming factors. This Sendai reprogramming kit includes a number of safety features, as described below:

- The host species for the Sendai virus (SeV) reported so far are mouse, rat, hamster and guinea pigs, all of which have been described to be serologically positive so should be non-pathogenic to humans.
- SeV is transmitted by aerosol and contact with respiratory secretions. The virus is highly contagious but the infection does not persist in immunocompetent animals.

Cytotune Sendai reprogramming vectors are based on a modified, non-transmissible form of SeV, which as a Fusion protein (F) deleted, rendering the virus incapable of producing infectious particles from infected cells. The presence of functional mutations such as temperature sensitivity in the amino acid sequence of several SeV proteins (SeVITS~F, SeVITS12~F, and SeVITS15~F) renders the vectors easily removable from transduced cells. As mentioned previously in the description of the human iPS cells, the RNA from one of the cell lines has been tested with Applied Biosystems TaqMan iPSC Sendai Detection kit. This kit is used to detect presence of and determine levels of Sendai virus and exogenous transcription factors (cMyc, Oct3/4, Klf4, Sox2) delivered by the Sendai virus. No expression was detected in RNA from the reprogrammed iPS cells. This would indicate that there is no virus present in these iPS cells.

The time needed to derive vector-free iPSCs may vary depending on culture and passage conditions. In the case of human neonatal foreskin fibroblast cells (strain BJ), it takes about 1-2 months after gene transduction to obtain vector free iPSCs. The iPS cells were cultured at the Rockefeller post transduction for at least 2 months prior to being received at UCL.

All cells have been treated in the same manner and cultured for the same length of time post transduction so should be free of virus but RNA from the other 3 cell lines will be tested.

The major risks of this line is the unknown human pathogen status. Fibroblast lines from donors have been provided as a basic research tool to investigate human disease. Consent to screen for major human pathogens is not sought at the time of donation. Screening the cells for major human pathogens would have potentially significant clinical health implications for patients (e.g. HIV), for which explicit consent has not been given at the time of donation. As such, the cells and subsequent iPSC lines generated, have not and cannot be screened for human pathogens and must therefore be handled at Biological Safety Level 2.

Due to the unknown pathogen status of the iPS cells, using lentivirus on these cells could pose a risk regarding potential recombination of lentivirus with retrovirus that could already be present within the cells. As consent has not been given to screen for human pathogens the supernatant from transduced cells cannot be tested for the presence of viral particles. Therefore iPS cells treated with lentivirus must be handled at Biological Safety Level 2.

Therefore all measures will be in place to minimize the risk to human health.

B- Lentivirus containing shRNA, or programmable nucleases sequence or reporter gene/overexpression vectors

**Lentiviral Vector Expression System**
Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. As the lentiviral vector system that will be used in these studies is based on HIV-1, a theoretical risk to human health exists. However, retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airbone route. Therefore, accidental piercing of the skin or other surface tissues with virus containing objects represents the main potential route by which accidental infection could occur. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

In all of the modified pSMPUW plasmids (containing shRNA, programmable nucleases or reporter gene sequences), the sequences inserted are flanked by non-coding retroviral LTRs, and no retroviral genes are encoded on the modified pSMPUW plasmids. Therefore, no retroviral genes will be transferred into generated viral particles. This construct is packaged into particles using a HEK293T cell based packaging system, which requires the cotransfection of these cells with three to five additional separate plasmids (as detailed above). The additional plasmids express the envelope protein from VSVg and the non-structural proteins of the virion, and, importantly, none of these genes will be transferred into the assembled viral vectors, since they lack the packaging signal (O), which is only present on the modified pSMPUW plasmids.

The lentiviral Expression System what will be used includes the following key safety features:
* All of the pSMPUW contain a hybrid 3'LTR that does not affect generation of the viral genome in the production cell line, but results in "self-inactivation" (SIN) of the vector after transduction of target cells. Once integrated into a transfected target cell, the lentiviral genome is no longer capable of producing viral genomic material that can be packaged. Moreover, presence of an SV40 polyA after the hybrid 3'LTR in the vector construct will result in a provirus which should reduce the potential for transactivation of cellular genes due to an insertion event. Furthermore, the development of self-inactivating vectors improves the biosafety of vectors, as they are less likely to be mobilised following a superinfection with wild-type virus (HIV).
* The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes. Heterologous envelopes, like VSV-G, typically broaden the tropism and allow gene transfer into a broad variety of cells. The risk will mitigated by the use of self-inactivating virus and limiting the number of viral particles that will be handled at anyone time.
* Sequences encoding the proteins required for packaging of the viral genome are separated onto three to five plasmids, and all of the 4-6 plasmids used in the system have been engineered not to contain any regions of homology with each other so as to prevent undesirable recombination events that could lead to the generation of a replication-competent lentivirus (RCL), which could potentially be harmful to humans. It is important to note that no such RCL has ever been observed despite large-scale production and testing of lentiviral vectors.
* All of the pSMPUW containing plasmids/vectors will be used are devoid of all viral sequences apart from essential cis-acting sequences, including the LTRs and the packaging signal O. Although the packaging plasmids used in these systems allows for the expression in trans of protein required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK293T producer cell lines, none of them contain LTRs or the 0 packaging sequence. Several of the lentiviral accessory genes (vif, vpr, vpu and nef) that are dispensable for lentiviral vector production/transduction have been deleted from the packaged construct. Therefore, none of the retrovirus structural genes will actually be present in the packaged viral genome, and they will never be expressed in the transduced target cells, which means that no new RCL can be produced.
* The lentiviral particles produced in this system are replication-incompetent, only carry the sequences of interest, and no other viral species are produced.
* Expression of the gag and pol genes from pCpgV has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Addition of the RRE in these plasmids prevents gag and pol expression in the absence of rev, which is contained in the pRSV-REV plasmid only. The Rev/RRE system is
highly conserved among lentiviruses, and removal of the RRE sequence and associated splice donor/acceptor sequences result in a loss of transduction efficiency.

* LTR has been modified so as to increase lentiviral vector production, and also to allow lentiviral vector production to be independent of tat expression. It is known that Tat-deleted mutants of wild-type HIV-1 are not replication competent. Therefore, the deletion of Tat should decrease the risk of generating a putative RCL.
* Lentiviral vectors have a very low potential to cause immunogenicity.
* The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction.

While the lentiviral vectors that are produced using this system contain only about 20% of the original genome of HIV-1, there is a very small risk that subsequent infection of cells already infected with the lentiviral genome of HIV-1 could lead to a rare recombination event in which the transgene is transferred to a replication-competent virus. Thus, the sequences in the vector that will be expressed could potentially be transferred to surrounding cells. This event is, however, extremely unlikely to occur for a number of reasons:

1- The lentiviral vector is replication-incompetent and self-inactivating. In the case of a subsequent HIV-contamination, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host genome, although non-self-inactivating vectors can be.

2- In order for the spread of the gene of interest to occur following accidental infection (assuming that this has lead to viral integration), a series of unlikely events have to occur:
   a- The worker would have to become infected with HIV-1 or to be already infected with the virus.
   b- The viral and lentiviral genomes would have to integrate into the host worker's genome in the same cells and in a position where they could interact to effect homologous recombination (point 1 above)
   c- Recombination would have to occur in just the right regions to allow for transfer of the gene of interest from the lentivirus to the HIV-1 genome, which could also involve the transfer of the HIV-1 genes to the lentiviral genome. In that case, it is conceivable that a non-self-inactivating HIV could be generated that contained the gene of interest but not the rest of the genome it requires. The other gene products could be provided in trans from the lentiviral genome that may now contain the HIV-1 genes or from other HIV-1 integrants.
   d- The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest, but again no genes necessary for subsequent replication. In order for another round of infection to occur, the process would have to begin again.
   e- It is conceivable the above could also happen if the iPS cells are already retrovirus positive.

In these circumstances, the effects of lentiviral infection are likely to be minor in comparison to the effects of the HIV-1 infection, which would be required to affect the spread of the gene of interest. In addition, the scenario described is essentially equivalent to the rescue of the lentiviral genome from the host, which has already been shown not to occur.

3- It is extremely unlikely that any worker would infect themselves with a significant dose of lentivirus as the volumes that are used in transfection experiments are small (aliquots contain a maximum of 10J.l1 of vector solution).

4- Moreover, insertional mutagenesis into the host genome may be considered as an oncogenic risk. We cannot rule out the possibility that, when the lentiviral genome integrates into the host genome, it will not lead to the activation of an endogeneous oncogene. However, all transcriptionally active long-terminal repeats (LTRs) have been removed as well as all promoter-like elements that required to drive expression of the transgene. This should prevent unforeseen activation of such genes. It is noted that deletion of retroviral enhancer in self-inactivating systems reduces the risk of activation but not of disruption, therefore, retroviral infection might still have permanent effects upon a cell (including oncogenic effects).

Importantly, we do not consider that the use of these lentiviral vectors will result in a significant increased risk of
oncogenic activation compared to the risk possessed by any other viral delivery system. Moreover, the risk of transduction leading to tumourigenesis or other untoward harm following exposure is related in part to the titre of the viral vectors; exposure of workers to quantities of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of lentiviral vectors.

Transgenes:
Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cells.

shRNA
With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be knocked-down, in order to study their functions. Potential deleterious effects such as off-target and immunomodulatory responses can be minimised through screening designed shRNA sequences against databases of known mammalian/mRNA sequences during the design stage. The genes/proteins of interest that will be targeted by shRNA do not and will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors and immunomodulators. Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. This sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of human, mouse or rat origin. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health (this will be approved by the GMSC).

Programmable nucleases: zinc fingers or Cas9/CRISPR
Zinc fingers or Cas9 protein will be directed against genes of therapeutic interest. Since the purpose of programmable nucleases is to cause mutations in defined DNA sequences the primary hazard is that such a mutation would lead to deleterious cellular effects. The genes/proteins that will be targeted do not and will not include any growth, trophic factors, confirmed oncogenes, tumor suppressors or immunomodulators. In isolation, these sequences are unlikely to pose any safety risks for the environment or human health.

Reporter genes: Fluorescent protein or enzymes/ Overexpression of proteins
The reporter gene could be a fluorescent protein or an enzyme, this reporter gene may be fused to a ribosomal subunit (L 1 Oa). Fluorescent proteins, for example GFP or dsRed, are not known to cause any diseases in human or animals and have no direct effect on cellular processes. Enzymes used frequently in reporter gene assay ie luciferase or b-galactosidase are not known to cause any diseases in humans or animals and as no direct effect on cellular processes. The expression of fluorescent protein or enzymes under the control of a promoter of a gene of interest is unlikely to pose any safety risks for the environment or human health.

Proteins of therapeutic interest will be targeted for potential overexpression. Genes/proteins that will be overexpressed do not and will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators. Any protein to be overexpressed will be approved by GMSC beforehand.

Summary on lentivirus
Health and Safety
Executive
The transgenes have a low safety risk to human health because accidental contamination with a vector would lead to only a small number of cells becoming infected, and it would be highly unlikely that expression in only a few cells could be sufficient to lead to a disease state. The inability of the lentiviral vectors to propagate on mammalian cells also reduces the risk. The programmable nucleases (CRISPR or Zinc finger or Cre recombinase) that will be employed in this viral vector system are not known to cause any relevant toxicity that might represent a safety risk to human health. The fluorescent proteins or the enzymes are not known to cause any relevant toxicities that might

02/03/2022 Page 14120 of 15326
represent a safety risk to human health. For both programmable nucleases and shRNA, the genes/proteins of interest that will be targeted will not include any growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumor suppressors or immunomodulators, and none of them are or will be of retroviral origin. Therefore the risk of use will be low.

Human health hazards

Oral ingestion will be prevented by standard laboratory safety practices. Aerosol formation resulting from an accident outside of the safety cabinet and involving lentivirus particle-containing liquid may lead to transduction of a limited number of the laboratory worker's skin or mucosal cells with modified viruses. However, these modified virus particles carry an extremely low safety risk for human health, as they are replication-incompetent, and also because the particles carry an extremely low infection potential via the mucosal/skin routes, due to the inherent properties of the viral vectors. Moreover, the programmable nucleases and shRNA that will be expressed or targeted will not be designed to disrupt any growth factors, trophic factors, quiescence factors, confirmed oncogenes, tumour suppressors or immunomodulators. In the unlikely event of accidental transduction of a laboratory worker's skin or mucosa by lentivirus particles, this will be self-limited, due to natural shedding of superficial epithelial skin and mucosal cell layers.

The most likely route of accidental infection with a lentivirus or with the human iPS cell line will be via inadvertent percutaneous inoculation. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures. Infection of the community at large with lentivirus particles is highly unlikely due to the viral vector particles being replication incompetent. Infection of the community at large by human iPS cells is highly unlikely due to the impossibility of the cells to survive outside of a laboratory environment.

* Environmental Considerations

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as effective containment procedures will be adhered to. However, in the unlikely event that active lentiviral vector particles do accidentally get released into the environment, the safety risk posed in such an event is considered to be negligible, due to the vectors being self-inactivating and non-replicative. The main potential risk in such an event would be the subsequent contamination of a human population already infected with HIV, which would potentially allow for recombination events that could incorporate the inserted sequences into a viable replication-competent lentivirus. However, this scenario is extremely unlikely and even if it was to occur, it is unlikely that it would lead to any untoward effects. As the HIV virus is a human pathogen, infection of another species would not be expected to allow any form of recombination event leading to a viable transmissible entity and so the risk from this scenario is considered to be negligible. Rodents, such as wild-type mice and rats, cannot support replication of infectious HIV-1 (Goffinet et al, 2007 Retrovirology). As a result, the potential for shedding of replication-competent lentiviruses from such animals is very low (even if they were present in the original vector inoculum).

The vectors have been pseudotyped with the VSV-G envelope in order to increase tropism, and in theory they are capable of transducing all dividing and non-dividing mammalian cell types. As emphasized before, the vectors cannot self-propagate after infection, and successful transduction is critically dependent on the presence of high enough concentrations of virus particles. Viral vector stock solutions will be managed in a way that will prevent contaminations with relatively high viral titres. After production, the vectors are aliquoted and stored in screw capped cryovials at -80°C. Taking into account that the infectivity of the vectors rapidly decreases at room temperature, it is considered highly unlikely that the vectors could survive in the long term after being accidentally released into the environment. Lentiviral vectors have a short half-life, at room temperature due to their structural characteristics making them highly susceptible to dehydration, and relatively unstable. For this reason, retroviruses become rapidly inactivated outside host systems, as illustrated by the fact that close contact is required for transmission. They can, however, survive for long periods in high protein media.
There are no conceivable hazards associated directly with the lentiviral vectors (containing the inserts) as they will not replicate/survive outside the laboratory and they have been designed to include many protective safety features. shRNA sequences will also not survive as they are highly unstable, and they WOUId, consequently, be rapidly inactivated/degraded if accidentally spilled into the environment. Moreover, as discussed above, there is a very low risk that the sequences contained within the viral vectors could be transferred to other organisms, as a rare recombination event would have to occur to allow this to happen.

The risk to the environment following accidental release of the human iPS cells would be negligible since the cells are incapable of surviving outside of laboratory conditions. Despite the cells being reprogrammed using Sendai virus they do not produce virus and therefore would not be able to infect any rodent population. If human iPS cells were to directly inoculate animals, they would be quickly cleared by the immune system and would not produce active viruses. The likelihood of this occurring will, however, be minimised by adhering to appropriate risk management measures and ensuring that all potentially contaminated material is totally inactivated before disposal.

Summary

The chance that active viral particles will be accidentally released into the environment is considered to be highly unlikely, as the viral vector studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to. In the unlikely event that active viral vector particles do accidently get released into the environment, the safety risks posed by such an event is considered to be low due to the vectors being self-inactivating and non-replicative, and also due to the poor ability of the vectors to survive outside of their host systems. It is also unlikely that the human iPS cells will be accidentally released into the environment, as the cell handling and studies that will be undertaken have been classified as biosafety level 2, and effective containment procedures will be adhered to.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

General cleaning procedures

Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these validated chemical inactivators will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L * before being autoclaved/incinerated.

Waste Handling

Following all work requiring biosafety level 2 containment including work with lentiviruses and ReNcell VM cells, solid waste will be neutralised by soaking in a validated disinfectant solution for 24 hours, after which the material will be "double bagged", autoclaved and, ultimately, incinerated offsite. It will be routine practise that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Liquid waste will be inactivated by Chemgene HLD4L * for 24 hours prior to drain disposal as per 430CSP and 418CSP permit requirements.

As any active viral particles or ReNcell VM cells on surface or in waste material will be completely inactivated at the end of each experiment by the highly effective cleaning/disinfection protocols outlined above, it is considered highly unlikely that active virus particles or ReNcell VM cells would unintentionally become discharged into a sanitary
sewage system. No foreseeable adverse effects on human health and safety are expected.

* Chemgene HLD4L
Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1%) for general purposes, 1:20 dilution (5%) for high risk areas, and for disinfection of blood and bio-hazard spillages.

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

Please enter comments on the GM safety committee on the risk assessment

The GMSC has reviewed the current risk assessment and agrees with the class 2 categorisation for this work. It is noted that although the hiPSC were generated using Sendai virus that needs to be handles at BSL2 the actual cells that will be provided to TCB will be free of Sendai virus and will have lost all detectable levels of plasmid. The main risk from the hiPSCs is their unknown pathogen status but this is mitigated because all donors are of low risk clinical populations. The lentiviral systems that will be subsequently used are inherently designed to prevent inappropriate recombination and subsequent mobilisation of transgenes into replication competent viral particles. Third and fourth generation systems deliver replication incompetent, self-inactivating viral particles that lack even the minimum set of genes required for virus production. The cells themselves cannot survive outside of a lab environment and the lentiviral particles lose viability rapidly if not stored properly being susceptible to desiccation. Risk associated with these activities to humans and the environment is deemed low. The use of biosafety level 2 containment is appropriate and sufficient because of the use of lentiviral elements and the unknown pathogen status of the cells.

Project Containment

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**GM Centre Number: 3345**

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TRADEBE HEALTHCARE NATIONAL LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

ATLAS HOUSE

**Road Name**

THIRD AVENUE

**District**

GLOBE BUSINESS PARK

**Town**

MARLOW

**County**

BUCKINGHAMSHIRE

**Postcode**

SL7 1EY

**Country**

ENGLAND

**Tel Number**

07776990667

**Fax Number**

0

**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Yes

Give brief details of the genetic modification safety committee

Person: Cell & Molecular Sciences Genetic Toxicologist with 21 years experience and BSc & Post Grad Diploma Applied Toxicology

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<th>Level 1 (GMMs)</th>
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Tradebe Healthcare will collect and dispose of Level 1 GMMs from a customer's laboratory at a Tradebe Healthcare high temperature incinerator that is permitted and regulated by either the Environment Agency or Natural Resources Wales. Other than the current testing and monitoring of the incinerators as required by the Environmental Permits, no further testing will be carried out as the waste will be completely destroyed and there will be nothing to test.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tradebe Healthcare will collect and dispose of Level 1 GMMs from a customer's laboratory at a Tradebe Healthcare high temperature incinerator that is permitted and regulated by either the Environment Agency or Natural Resources Wales. Other than the current testing and monitoring of the incinerators as required by the Environmental Permits, no further testing will be carried out as the waste will be completely destroyed and there will be nothing to test.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

None of the measures in containment level 2 of Table 1 (of Schedule 8) are required for safety reasons, however some of these may be employed to prevent contamination of the GMM. The activity is therefore Class 1.
### General Information

- **GM Centre Number:** 3346
- **Data Premises Notified (Originally):** 19/12/2016
- **Transferred from 1992 Regs?:** N
- **Transitional Premises Class:** N
- **Data Premises Closed:**
- **Transitional Premises Emergency Plan Required?:** N
- **Non-GMMs:** N
- **Withdrawn:** N

### Company Information

- **Name:** ENTOMICS BIOSYSTEMS LTD
- **Legal Name:**
- **Name 2:**
- **Department:**

### Address Information

- **Campus Estate or Research Centre:** FUTURE BUSINESS CENTRE
- **Road Name:** KINGS HEDGES ROAD
- **Town:** CAMBRIDGE
- **County:** CAMBRIDGESHIRE
- **Postcode:** CB4 2HY
- **Country:** ENGLAND
- **Tel Number:** 07449 557134
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### Comments

**Date at Which Additional Info Submitted:**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Counsel was sought within an extended network of academic and private researchers, whom have all worked with GMM in the past, and are acquainted with the implications of GMM class 1 contained use.
- Work will be limited to Class 1 GMM only, suggesting a single, qualified individual is sufficient to provide advice on the risks involved with GMM work. The skilled individual in question has held a post for over 8 years as Biological Safety Officer at a large, Cambridge-based research organisation dealing with GMM and GM plants on a regular basis.

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Tick if confidential

Bacteriology | Yes |
Parasitology |      |
Transgenic Birds |      |
Microbiology Research | Yes |
For activities involving GMMs, describe the waste management measures which will apply to the activity

To prevent release of viable Class 1 GMMs, the following inactivation protocols are to be implemented:

- **Inactivation of liquid GM matter** - including liquid cultures, their derivatives, containers and accidental spills, is to be performed using a 1% solution of DuPont RelyOn Virkon and left for at least 10 minutes. After this, liquid waste can be safely washed down the drain with generous amounts of water, or absorbed with lab tissues and disposed of in the correct bin.

- **Inactivation of solid GM matter** - including GM biomass, tools and agar plates, is to be performed via autoclaving. GM waste should be placed in an autoclavable bag, and put through a 121 degrees Celsius 115 min inactivation programme. Following inactivation, the waste should be disposed of in the correct bin.

- **Microbiological Safety Cabinet (MSC) Inactivation** - Following GMM work in the Level 2 MSe, a germicidal UV light (UVe, 254-280 nm) will be turned on and left for a minimum of 2 hours. This will ensure any viable leftover GMM are destroyed. The risks of such GMM remaining in the MSe in the first place will be mitigated by disinfection of the MSC with 70% isopropanol solution before and after each session.

- **Inactivated waste disposal** will be performed by a licenced subcontractor. If inactivation is not possible, disposal will be performed by inceneration (subcontractor).

- **Inactivation quality controls** will be performed when commencing work with new GMM organisms, and routinely every 6 months to ensure inactivation protocols work appropriately.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The skilled individual's suggestions were incorporated into the above sections and relevant risk assessments. After outlining the final containment and inactivation strategies for GMM, no further concerns were brought forward by the skilled individual mentioned in Section 3.
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Page 14130 of 15326
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

An external biosafety consultant has reviewed the risk assessment.

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Tick if confidential

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All genetically-modified human cells will be fully inactivated by cell fixation (using formalin). Glass flasks containing residual transformed bacterial cells (in LB broth) will be inactivated by treatment with the bacterial agent Virkon (freshly made) at the manufacturers' specified concentration of 10g per litre for at least 2 hours. All solid waste, including LB/agar plates covered with bacterial colonies, pipettes/stripettes and plastic centrifuge tubes, will also be inactivated by submerging in Virkon (as above) for at least 2 hours. All inactivated solid waste will be discarded using strong, leakproof yellow biological waste bags (or plastic incineration bins), which will be sealed and finally incinerated off-site by a reputable commercial biological waste service provider. All liquid waste will be inactivated by chemical treatment with Virkon (as above) for 2 hours before discarding down the sink. As a matter of course, a fresh solution of formalin cell fixative will be prepared for every experiment. A strict procedure has been developed to ensure fresh Virkon solutions are made on a routine basis. Both methods are well-established to fully inactivate the GMOs (i.e. the transfected human cells and the transformed bacteria).

For activities involving GMMs, describe the waste management measures which will apply to the activity

The host organisms meet the criteria for work to be safely carried out under CL 1 containment and the genetic modifications involved are not foreseen to alter this. The work is therefore appropriate for categorisation as a risk class 1 activity.
**Project Additional Information**

**Purposes of the contained use**

To determine if x2 BSL-2 viruses interact with selected protein targets included in CR protein library with interactions being detected using cell microarray technology (on fixed slides only).

**Recipient or parental organism**

The study will use human HEK293 cells which are especially disabled recipients of the virus, and not expected to survive in the environment, nor able to colonise the operator (non-self). HEK293 is a human embryonic epithelial cell line stably expressing the transforming gene of the human Type 5 adenovirus. However, HEK293 cells fall into the category of Low Hazard under the SACGM guidance for determining containment level for cell cultures.

**Host/vector system**

The viruses supplied by the client are a Adenovirus type 5- based virus. These are non-enveloped double-stranded DNA virus with a linear DNA genome.

The Adenovirus provided by the client is based on human adenoviral type 5 backbone and is replication-competent, permissive in cell lines such as HEK293 cells and A549 cells. However, it is important to note that viral particles will only be added to fixed HEK293 cells in this study.

**Origin & function**

The targets being screened against in this study are part of the CR extensive library of plasma membrane and secreted proteins. The CR DNA library is expressed in a custom vector which is based upon Clontech's non-mobilisable pIRES2-ZsGreen vector, which has been adapted to receive human ORF cDNAs using Invitrogen's Gateway™-mediated recombination technology. In this vector, expression of the human cDNA insert is driven by the powerful and constitutive CMV IE promoter. This is followed by an IRES-ZsGreen element, such that the fluorescent protein ZsGreen will be co-expressed in transfected HEK293 cells.

The client will provide CR with 3 x 10^11 particles for each test Adenovirus type virus. CR will screen each test virus at 0, 5,000 and 10,000 virus particles.

**Evaluation of foreseeable effects**

RC Ads are being studied intensely for cancer therapeutics. Their mechanism of action is to induce the lysis of cancer cells with the released viral particles then targeting neighboring tumor cells. In cancer cells, the pattern of gene expression makes the cells more permissive for Ad replication whereas in non-cancerous (healthy) cells multiple Ad proteins must be expressed to force cells into a permissive state. The Ad virus supplied by the client have been further engineered to permit replication only in cancer cells and that limit replication in non-cancerous cells [1].
HEK293 cells transiently transfected with CRL High Peak custom vectors+ gene inserts where any risk to human health has previously been assessed to be very low. Under routine cell microarray tests these transfected cells are handled under Containment level 1. The recombinant test Ad particles will be only added to fixed cells and so the likelihood of generating replicative competent Adenovirus (Ad) is effectively zero.

References

All work will be carried out in a single designated Class II Safety cabinet and the particles will be added to cell microarray slides (after cell fixation). The cells will then be washed to remove excess viral particles, and the bound particles will be further fixed in place at this point. Excess viral particles removed in the wash step will be Disinfectant treated and disposed of in hazardous waste. Any viral stocks will be autoclaved prior to disposal (see below for details) In the unlikely scenario that the operator was exposed to the adenoviral particles it is unlikely further adenoviral particles would be created since the virus is engineered to not replicate in healthy cells. However, the existing particles could trigger a strong immune response in the operator. To mitigate the risk of exposure by aerosols all work will be done within a Class II Safety cabinet

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All genetically-modified HEK293 cells will be fully destroyed by cell fixation (using formalin).

Any viral stock will be disposed of by autoclaving at 121°C for 15 minutes.

All inactivated solid waste will be discarded using yellow biological waste bags (or plastic incineration bins), which will be sealed and finally incinerated off-site by a reputable commercial biological waste service provider.

All liquid waste will be inactivated by chemical treatment with the bactericidal agent Virkon (freshly-made) at the manufacturer’s specified concentration of 10 g per litre for at least 2 hours before discarding down the sink.

As a matter of course, a fresh solution of formalin cell fixative will be prepared for every experiment. A strict procedure has been developed to ensure fresh disinfectant solutions are made on a routine basis. Both methods are well-established to inactivate the GMOs (i.e. the transfected human cells).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Collective agreement of BSL2, requires a CU2 application to the HSE

**Project Containment**

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Name

| EVOTEC UK LTD                       |            |

Name 2

Department

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Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

This person is the Biological Safety Officer at our main site in Abingdon and is responsible for overseeing all GMO work carried out there.

<table>
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<th>Laboratory</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

n/a

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The genetic modification safety committee has reviewed related risk assessments and confirms that the recombinant organisms are not expected to be more hazardous than the parent strain (both considered to be BSL category 2). The inserted vector provides no advantage to the host bacterium in terms of virulence, a very modest increase in resistance to antimicrobial agents (as selectable markers), and low risk of escape into the environment.
Project Additional Information

Purposes of the contained use
To visualise microorganism during growth phases

Recipient or parental organism
Staphylococcus aureus NR-46158

Host/vector system
Escherichia coli - staphylococcal shuttle vector pCN5? Vector pCN5? contains the E. coli ColE1 replication origin, the S. ·aureus pT181 cop-wt-repC replicon, the Pbla promoter and a promoterless B-lactamase reporter gene, gfpmut2 .

Origin & function
Vector pCNS? is a member of a series of novel shuttle vectors that were developed using PCRdesigned cassettes to allow for easy exchange of vector components. The function of the vector is to insert a DNA sequence that will allow the production of green fluorescent protein (GFP) such that the bacterium will generate GFP.

Evaluation of foreseeable effects
Vector pCNS? is a member of a series of novel shuttle vectors that were developed using PCRdesigned cassettes to allow for easy exchange of vector components. The function of the vector is to insert a DNA sequence that will allow the production of green fluorescent protein (GFP) such that the bacterium will generate GFP.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
The recombinant organisms are not expected to be more hazardous than the parent strain.
Risk 1) Infection of staff by pathogenic organisms.
Risks are low as Personal Protective Equipment, including laboratory coat, disposable gloves and safety goggles will be used to avoid direct contact with these samples. Organism is not spread by the aerosol route and is of low pathogenicity with an infectious challenge of >10119 cfu required to establish an infection.
Risk 2) Release into the environment.
The risk is low as all procedures will be performed in a BSL Containment level 2 laboratory. The laboratory benefits from...
from design and equipment suitable for working with BSL 2 organisms. All contaminated waste is autoclaved within
the facility. Autoclaved waste is transported in sealed bins for final disposal by incineration by a registered company.
Risk 3) Accidental transfer of plasmid into an unrelated microbe ..
The risk is low as isolates will be handled as BSL 2 pathogens and will not be knowingly mixed with other cultures.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

GM cultures (cell culture media, agar or broth) will be handled according to standard practice (BSL class 2 microbes)
for the prevention of contamination. All spent organism / waste media will be decontaminated by autoclaving at 134C
for 20 minutes followed by incineration; spillages will be treated with 1 % Virkon left in place for 15 minutes with wipe
up materials disposed in a sealed bin for incineration. No additional precautions are required compared to the wild
type isolates.

Project Containment

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GM Centre Number: 3352

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Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Health & Safety Manager, The Royal (Dick) School of Veterinary Studies and The Roslin Institute, The University of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG

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Non-microbial

Other (please specify)  
Tick if confidential  

Bacteriology  
Parasitology  
Transgenic Birds  
Microbiology Research  
Virology  
Transgenic Animals  
Transgenic Fish  
Gene Therapy
The proposed GMO is a vaccine. Disposal of the vaccine vial, the needle and syringe used to draw up the vaccine and the plastic dart and needle used to deliver it to the animal will be via the same techniques used to dispose of contaminated medical sharps in a lock-topped sharps container followed by heat rendering by a registered medical waste contractor. Health Care Environmental (Shotts, Lanarkshire) are our contracted medical and animal by-product disposal contractors and are licensed to handle waste disposal of Genetically Modified materials.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Other(s)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

As a vaccine is a sealed unit and the product will not be exposed to the environment directly, containment is likely from vial to animal. Disposal of vials, needles and syringes should be by standard medical grade waste disposal (heat sterilisation) to ensure product inactivation.
**GM Centre Number: 3354**

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**Comments**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Chief Scientific Officer PhD qualified with 20+ years working in microbiology and specifically with long history of working with GMOs.

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Tick if confidential

Bacteriology Yes

Parasitology

Transgenic Birds

Microbiology Research Yes

Virology

Transgenic Animals

Transgenic Fish

Gene Therapy

Gene Therapy

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid cultures inactivated using known, effective biocides (1% Virkon); solid cultures disinfected through autoclaving and waste sent for incineration using on site (Dstl, Porton Down) facilities.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The work being carried out at these premises involves disabled E.coli hosts and non-mobilisable vectors containing non-harmful gene fragments. Work with these strains will be infrequent.
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**Name**

LEAF EXPRESSION SYSTEMS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

NORWICH RESEARCH PARK

**Building**

BUILDING7, ZONE 2

**Road Name**

COLNEY LANE

**District**

NORWICH

**Town**

COLNEY

**County**

NORFOLK

**Postcode**

NR4 7UJ

**Country**

ENGLAND

**Tel Number**

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**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Discussions have taken place with a Biological Safety Officer (Level 1 Biosafety Practitioner and M-ISTR) from the Norwich Research Park. Discussions included classification of GM work and appropriate containment measures that should be employed and risk assessment of work activities.

Two members of the Biological Safety Committee (Genetic Modification Safety Committee) from the JI Site, who possessed appropriate knowledge, experience and training in the scientific area in question, reviewed the risk assessment for the proposed work and provided comments on aspects of the risk assessment that required additional supporting information. Following provision of this additional information the risk assessment was agreed to be appropriate and provided a sufficient assessment of the risks posed by the activities.

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</table>
**Waste liquid treatment** - All process solutions and laboratory sink waste feed into a waste liquid effluent treatment facility located outside the facility. The treatment facility inactivates any residual microbiological material that is contained in the solutions by heat treatment at a minimum of 121 degrees C at 15psi for 20 minutes. This will be validated upon commissioning of the system. Controls (procedural and system) exist on the effluent treatment plant so that loads of waste effluent cannot be discharged to the foul sewer without the required parameters (temperature and time) being achieved. The system works in a batch mode by filling a tank and processing accordingly. If the required parameters are not met an alarm is activated and the system will stop with no risk of discharge before appropriate rework or assessment is made.

**Autoclave** - Any solid waste (plant debris, soil, pots etc.) will be autoclaved on a validated cycle to ensure complete inactivation of any microbiological material. Media containing bacterial cultures will be inactivated using the same equipment. All autoclave loads will be treated at 1210C for a minimum of 20 minutes, but the length of the cycle will be dependent on the validation that is performed. For denser loads the length of time is likely to be increased to 30 minutes. The cycle will be validated to ensure that the kill requirements have been achieved before operation. Controls (procedural and system) exist to ensure loads are processed to the required settings (time, temperature and pressure). Disinfection of accidental spillages will be performed using validated processes followed by sink disposal with copious amounts of water.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

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Other(s) Using plants as bioreactors for scaling up of processes. The plants are not genetically modified and

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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
<table>
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<tr>
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Name

ASCUS ART & SCIENCE LTD

Name 2

Campus Estate or Research Centre

SUMMERHALL

Road Name

SUMMERHALL PLACE

Town

EDINBURGH

Building


District


County

Postcode

EH9 1PL

Country

SCOTLAND

Tel Number

079 74300 465

Fax Number

0

E-mail


HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

Details of the person with expertise in risk assessments relating to contained use:
ASCUS Lab Genetic Modification Safety Officer:
Job title: ASCUS Lab Technician
Relevant credentials:
Bsc(Hons) Biotechnology at the University of Barcelona
MSc Synthetic Biology and Biotechnology at the University of Edinburgh
Experience with GMO class 1 and 2.

<table>
<thead>
<tr>
<th>Laboratory</th>
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Tick if confidential: 

02/03/2022
Validation tests of autoclave will be documented and performed placing a biohazard bag into a secondary autoclavable container with an autoclave indicator tape strip inside the bag. The program will run for 20 min at 125°C and 1 Ba. After completing the cycle the state of the chemical integrator strip will be checked. If the validation is not successful, the autoclaving cycle will be run again extending 10 min the program. For bench-top collection of contaminated goods small plastic containers will be used. This will be transferred to a larger leak-proof container with the biohazard symbol and an autoclave bag until is autoclaved. The bags containing the waste must be clearly identified as biohazardous at any point of the treatment. GMM inactivation and removal of biological components in dry contaminated solid (plastic disposables, tissue paper, gloves, culture plates…) material will be performed through sterilisation via autoclaving at 125°C this will be achieved by maintaining 1 bar pressure for 20 minutes prior to disposal. The autoclave is located in the laboratory and is composed of a pressure cooker and an electrical induction hob. The autoclaved waste will be placed in a non-see-through bag or container and finally disposed as regular trash. Additional chemical inactivation methods are also available to dispose liquids or disinfect glass containing genetically modified organisms. A 10% concentration of Virkon or bleach can be used to decontaminate liquids when allowing to sit overnight. Volumes larger than 1 litre of waste will be autoclaved. After sterilisation the liquids can be discarded on the sink with a copious amount of water. Glass will be immersed in the disinfectant solution. Surface and equipment disinfection will be performed with 70% denatured alcohol. If sharps contaminated with GMM are generated they should be disposed of in a designated sharps collection bin marked with the biohazard symbol. Sharps container must be permanently closed, puncture-resistant and leak-proof container. Sharps disposal should be via the organisation's authorised waste contractor only. ASCUS Lab has contacted two waste disposal agencies that have agreed to one-off uplifts from Summerhall.

For activities involving GMMs, describe the waste management measures which will apply to the activity
The ASCUS Lab premises are equipped and prepared to work with class 1 GMM. The projects taking place in the ASCUS Lab will be focused on the genetic modification of model or well-characterised microorganisms. These modifications will mostly involve performing routine transformations in disabled E. coli strains therefore containment level 1 is adequate for the nature of the premises and the projects undertaken. Please find the risk assessment summary attached to this form.

The ASCUS lab premises are also capacitated to work with larger non-notifiable GMO’s, which do not pose a greater risk to humans than its unmodified organism as described in Part 2 Schedule 12 paragraph 5, The Genetically Modified Organisms (Contained Use) Regulations 2014. This will include active ties involving C. elegans for which risk assessment for human health and safety is attached. Although this form indicates that work that involves plants can be undertaken within these premises, work is not expected to start and there are no currently projects planned to be started in the near future and for this reason risk assessment is not included. The ASCUS Lab will perform a risk assessment for these potential projects and determine whether they can be undertaken in the premises and comply with the regulations and containment measures of a BSL class 1 laboratory. Activities with plants would potentially involve modifications of Rhizobium or Agrobacterium after additional risk assessments have been undertaken. These projects would include non-toxic plants for human or animal health that cannot disseminate in the environment. The modifications would not be heritable and the plants will be grown in small numbers. There should not be evidence that the resulting plants could survive and spread if accidentally released into the environment and therefore would not represent an environmental risk. Risk assessments for plant use will be undertaken with the advice of GM plant experts.
### GM Centre Number: 3357

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### Name
AZOTIC TECHNOLOGIES LTD

### Name 2

### Department

### Campus Estate or Research Centre
CHORLEY BUSINES & TECHNOLOGY CENTRE

### Building

### Road Name
EUXTON LANE

### District
EUXTON

### Town
CHORLEY

### County
LANCASHIRE

### Postcode
PR7 6TE

### Country
ENGLAND

### Tel Number
01252 668631

### Fax Number
0

### E-mail

### HSE Division
blank

### Comments

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

GMO Committee with biannual meetings, comprised of Laboratory Manager, Scientist (Microbiology) and Scientist (Molecular Biology). The scope of the Committee is to:
Review risk assessments covering the acquisition, storage, use, disposal and transport of these materials and approve such activities before commencement;
Approve containment facilities for undertaking work with these materials;
Monitor activities involving these materials by receiving inspection/audit reports, training information and reports of accidents/incidents.

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02/03/2022
* All GMO work is conducted inside a Class II Biosafety cabinet that is thrice sterilised with Virkon or Distel before and after use.  
* Solid waste and materials are collected into Biohazard bags and autoclaved at 121 degrees Celsius for 80 minutes.  
* Liquid waste is mixed with an equal volume of double-concentrated sterilising agent such as Virkon or Distel for 1 hour before being poured down the sink.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment  
Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment
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**STORM THERAPEUTICS LTD**

**Name**

STORM THERAPEUTICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

BABRAHAM RESEARCH CAMPUS

**Building**

MONETA BUILDING

**Road Name**

**District**

BABRAHAM

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB22 3AT

**Country**

ENGLAND

**Tel Number**

01223 804174

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Y**

Give brief details of the genetic modification safety committee

The Storm Therapeutics genetic modification safety committee consists of VP R&D, Head of Biology and a health and safety laboratory manager.

The health and safety committee meets monthly to address all aspects of health and safety including all matter relating to genetic modifications.

All risk assessments of experiments involving genetic modifications and genetically modified organisms are reviewed by the biological safety officer and the head of health and safety.

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In accordance with the Babraham Research Campus Biosafety policy for containment level 1, all solid waste including agar plates and contaminated consumables will be disposed in sealable yellow clinical waste Eurobins that will be incinerated. All liquid cultures contaminated with GMMs and genetically modified cells will be inactivated by adding 1% Virkon and being left to stand for at least 1 hour before diluting and disposing via the sink flushing with plenty of water.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The Storm Therapeutics safety committee contains VP R&D and Head of Biology who are competent in risk assessment. The Head of Biology is the company Biological Safety Officer and has recently updated his training.

Please enter comments of the GM safety committee on the risk assessment

The generation and use of Adeno Associated Virus (AAV), Y-retrovirus and lentivirus vectors for investigating the biological function of RNA modifying enzymes in disease

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Project notified under transitional arrangements

 Withdrawn

Tick if notifying a connected programme of work
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to use AAV, \(\gamma\)-retroviral and lentiviral delivery systems to introduce various mammalian DNA sequences into mammalian cells in culture. These sequences direct expression of either (i) RNA species (aptamers, siRNAs, gRNAs, miRNAs or shRNAs) that modulate the expression of various mammalian genes or (ii) the corresponding mammalian mRNAs/proteins themselves. The generation of AAV, \(\gamma\)-retroviral and lentiviral vectors is intended as in vitro tool for the generation of transient and stable mammalian cell lines and the subsequent use of such modified cell lines in vitro for the validation of novel oncogenes and putative drug targets.

**Recipient or parental organism**

Cloning, propagation and amplification of component plasmids for AAV, \(\gamma\)-retroviral and lentiviral vector systems will take place in K12 strains of E. coli (with a history of safe use).

Mammalian cell lines (e.g. immortalized lines derived from human cancer tissues) fall into two types:

a) Packaging/helper cell lines into which plasmids containing lentiviral or retroviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

b) Recipient mammalian cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection. Viral particles will either be derived from packaging cell cultures (as above in a) or will be purchased commercially from approved suppliers. However, the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines, i.e. once infected they pose a negligible risk to staff.

**Host/vector system**

The AAV vector technology that we will use is based on a non-enveloped single-stranded DNA virus (Adeno Associated Virus - which are ACDP Hazard Group 1 biological agents) as vehicles to efficiently deliver and express genes in mammalian cells. AAV viruses are taken up by endocytosis, released for transcription and expression following migration to the nucleus. Given the low pathogenicity of the recipient virus, the major hazards that will be posed by recombinant AAV vectors will depend upon the properties of the inserted genetic material and any products that it may encode.

The \(\gamma\)-RETOVIRAL vectors which will be used are derived from either Moloney Murine sarcoma Virus (MoMLV), Mouse Mammary Tumour Virus (MMTV) or Feline Leukaemia Virus (FeLV), all of which are ACDP Hazard Group 1 biological agents. On the other hand, the virus will be packaged by transfecting transfer vector into specific amphoteric 'helper' cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types. This means that the viruses produced for this experiment could potentially infect a number of species, including man.
However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle:

- The genes encoding structural and other components of the viral genome have been separated. These genes have been engineered to minimise the risk of recombination that could lead to production of a replication-competent virus.

- The packaging cell lines allow expression of proteins, required to produce progeny virus: But the transfer vector is the only genetic material transferred to the target cells, consequently these cells cannot produce the proteins which are essential for viral assembly and infectivity.

- Second, third generation or Self inactivating vectors retrovirus vectors will be used in all experiments (see SACGM compendium of guidance part 2, section 2.11 (Retroviruses) pp117)

The LENTIVIRAL vectors that will be used are derived from HIV-1, which is an ACDP Hazard Group 3 biological agent. However, second and third generation lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase biosafety and viral packaging is achieved by providing three helper constructs in trans containing gag, pol and rev sequences.

For example, second and third generation Lentiviral Expression Systems include the following key safety features:

In the second and successive generation lentiviral vectors several lentiviral accessory genes (vif, vpr, vpu and nef) are deleted from the transfer plasmid since they are not required for in vitro replication and the products they encode have cytotoxic activities.

In addition to this in the third generation lentiviral vectors:

- The Lenti expression vectors contain a deletion in the 3’ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line but instead results in “self-inactivation” of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a packageable viral genome.

- The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).

- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).

- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication competent virus can be produced.

Despite the above safety features, use of these lentiviral vectors (which include WPRE) falls within SACGM 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. In addition, the virus will be packaged by transfecting transfer vector into specific amphoteric ‘helper’ cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types.

This means that the viruses produced for this experiment could potentially infect a number of species, including man.

### Origin & function

1) **Selection markers** – examples such as (but not restricted to):
   - Ampicillin resistance gene Beta lactamase derived from E. coli.
   - Neomycin resistance gene Aminoglycoside phosphotransferase derived from Streptomyces.
   - Puromycin resistance gene Puromycin acetyl transferase derived from Streptomyces alboniger.

2) **Reporter proteins** such as (but not restricted to):
   - Fluorescent proteins as reporters - e.g. GFP derived from the jellyfish Aequorea victoria and its derivatives.
   - Luciferase – class of oxidative enzymes used in bioluminescence such as Renilla luciferase derived from the Sea pansy (Renilla reniformris) or Firefly luciferase derived from the firefly Photinus pyralis and its derivatives.

3) **Open reading frames (ORFs), cDNAs and gene sequences encoding potential drug targets or therapeutic proteins** derived from human or other mammalian genomes.
This could include the expression of potentially harmful genes, for example encoding known proto-oncogenes, or genes with known oncogenic mutations that can contribute to cellular transformation.

4) shRNAs, siRNAs, miRNAs and gRNAs derived from human or other mammals designed to modulate the expression of gene sequences, open reading frames and mRNAs encoding potential drug targets or therapeutic proteins. Modulating the expression of either oncogenes (e.g. upregulation) or tumour suppressor genes (e.g. downregulation) could lead to cellular transformation.

**Evaluation of foreseeable effects**

All viral vectors employed in this protocol exhibit broad tropism and potential to infect human. Risks conferred following infection are identified as:

1) Genetic insertion of viral sequences with potentially deleterious effects on endogenous genes: we will employ transgene promoters and other viral sequences that may affect host gene function in a wide range of cell types (e.g. CMV promoter). We assess retro-, lenti- or adenovirus infection might induce permanent changes in infected cells including a risk for tumorigenesis. Risks conferred are previously described and categorised under Class 2 risks [SACGM compendium of guidance part 2, page 121]: “…The effects of integration upon the infected cell should be considered. For instance, promoter sequences present in the provirus might activate genes adjacent to the integration site or, alternatively, insertion may disrupt genes and prevent their expression.”

2) Expression of human-derived or homologous transgenes with potentially deleterious effects: various transgenes may be employed, wherein intrinsic function of the transgene confers potentially harmful effects. For example, expression of human oncoproteins could induce transformation of infected cells. Beyond endogenous homeostatic mechanisms that may lessen this risk (e.g. apoptosis and other host tumour suppression responses), we assess that standard precautions under Class 2 risk mitigation procedures are adequate to address such risks.

3) Expression of exogenous transgenes directed to modulate host genes with potentially deleterious effects: multiple transgene technologies (antisense, RNAi, CRISPR or related gene conversion) may be employed to modify the function of endogenous genes with potentially deleterious effects. For example, RNAi or gene editing by CRISPR could reduce the function of genes necessary to control cell apoptosis, potentially resulting in tumorigenesis. As above, we assess that Class 2 risk management procedures adequately address these risks.

4) Expression of heterologous genetic sequences with potentially deleterious effects: we will employ diverse collections of non-human derived, exogenous transgene sequences that carry potential risks following infection. Risks from expression of these collected transgene sequences are mitigated and attenuated through several means. First, known pathogenic transgenes were systemically removed and other exogenous gene products are fragmented or rearranged, such that potentially pathogenic or other biologically deleterious genetic functions are not recapitulated in their complete endogenous configuration. Second, potentially deleterious transgenes in any infectious viral preparation are highly titrated, such that any single deleterious sequence comprises less than 1 part in 100,000 parts of an inoculum with infectious potential. In total, we assess these risks are adequately managed via the same Class 2 risk precautions employed above.

We assess environmental hazards are adequately addressed through the proposed personnel risk management measures. We employ multiple attenuation strategies and protocols to severely limit independent virus propagation beyond the intended use, and therefore anticipate minimal risks of GMM release.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:

1. We do not have access to an autoclave within our area of the building;
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by
Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from Class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this.

This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.

This disposal method is expected to achieve 100% inactivation of the GMM.

Is an emergency plan required according to regulation 20? N

Tick to confirm that it is attached to this form N

Tick if you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

The scope of work and the particular aspects of safety risks described in the risk assessment were discussed and satisfactory consensus was achieved regarding the adequacy of Safe Operating Procedures (SOPs) and Code of Practice (COP) as well as risk management measures and protocols.

**Project Containment**

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Name
THE TECHNOLOGY PARTNERSHIP PLC

Name 2

Department

Campus Estate or Research Centre
MELBOURN SCIENCE PARK

Road Name

District

Town
ROYSTON

County
HERTFORDSHIRE

Postcode
SG8 6EE

Country
ENGLAND

Tel Number
01763 262626

Fax Number
0

E-mail

HSE Division
blank

Comments

Date at Which Additional Info Submitted
02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

TTP encourages a healthy and safe working environment, and believes this will be achieved through cooperation at all levels within the organisation. This includes the development of its staff, monitoring and improvement to working practices and facilities, and implementing procedures and practices to ensure that any hazards or risks involving GM research are assessed and controlled.

A biological safety advisor with significant experience will review all research projects involving genetically modified organisms. This biological safety advisor will provide advice where required on GM biological safety issues, as well as maintain a record of all GM projects, competent persons performing this work, and relevant project risk assessments (completed by the project leader of each GM project). This person will ensure that all GM work at TTP complies with the Genetically Modified Organisms (Contained Use) Regulations (2014), at a level appropriate for the type of research undertaken. This person will additionally periodically inspect laboratory and workshop areas to ensure safety precautions are adequate. Any deficiencies will be reported to the appropriate Division Manager, Safety Officer, and Project leader. Where necessary the biological safety adviser will report concerns and other information to the TTP Health and Safety committee, who are responsible for overall monitoring and review of TTP Health and Safety.

The biological safety advisor is a senior cell biologist with a PhD in Cell biology and Molecular Physiology. This person has eighteen years research experience in academia including 7 years leading a research group, as well as extensive experience in the safe handling, storage, and transportation of GMOs. Experience includes cloning, RNA and DNA handling, stable and transient transfections, lentivirus and retrovirus preparation and transduction, and genetic modification of bacterial and mammalian cells (including established and primary human cell lines, organoid cultures, tissue slice cultures, and human primary stem cells). From 2011-2016 this person acted as a University Genetic Modification Safety Officer, responsible for risk assessment and review of numerous GMO applications. They also held a GMO license for lentiviral transduction and modification of mammalian cells during this time.
Mammalian cell culture

Liquid waste from GMM use is to be aspirated into a DuPont Rely+On-containing bottle and left for at least 2 hours before disposal with waste water (as per manufacturer's instructions, attached). Monitoring of decontamination and disinfectant replenishing schemes will be according to the manufacturer's instructions (DuPont, optically). Solid waste, including wipes, gloves, test tubes and culture vessels, are to be disposed in biohazard waste containers [e.g., DisposaFlatPak 50], collected by Biffa [carrier licence, insurance permit and PPC permit in appendix] and destroyed by incineration [Veolia Tyseley - site permit in appendix]. Mammalian cells will be cultivated in a CO2 incubator which has a dry heat cycle to disinfect the interior. At the end of each experiment, the incubator disinfection cycle will be executed to disinfect all interior surfaces. GMO laboratory bench surfaces and biological safety cabinets (including laminar flow hoods) will be cleaned after every use with 70% ethanol which will be sprayed and allowed to remain for at least 1 minute. This ethanol concentration and contact time kills most bacteria, e.g. Pseudomonas aeruginosa, Serratia marcescens, E. coli and Salmonella typosa in 10 seconds. It also deactivates lipophilic viruses like Herpes, Influenza or Vaccinia and many hydrophilic viruses (eg Adenovirus, Enterovirus, Rhinovirus and Rotavirus. While
effective against most bacteria and viruses, some organisms can survive this disinfection process. Therefore, once every three months, all surfaces will be treated with 10% sodium hypochlorite. Sodium hypochlorite treatment for >5min will decontaminate any residual bacteria or other organisms.

Bacterial cell culture
Liquid waste from non-pathogenic bacterial cultures (e.g., laboratory strain E. coli such as TOP10, DH5alpha) will be treated with Rely+On Virkon for >2h and then disposed of with waste water (as per the manufacturer's instructions). Monitoring will be achieved optically as outlined above. Solid waste, including bacterial plates, tips, tubes and wipes will be disposed of in biohazard waste containers as detailed above (disposed in biohazard waste containers [e.g. DisposaFlatPak 50], collected by e.g. Biffa [carrier licence, insurance permit and PPC permit in appendix] and destroyed by incineration [e.g. Veolia Tyseley - site permit in appendix]).

All solid waste material from mammalian and bacterial cultures will be stored in a room with electronic (key fob) access control. GMO facilities will be regularly inspected (at least 2x/month) to ensure appropriate cleaning procedures are followed, and all laminar and microbiological safety cabinet surfaces cleaned at least every 3 months using appropriate agents (e.g., 10% sodium hypochlorite). Biohazard containers once full will be sealed, labelled with a unique project identifier and date of storage, and held until collection (collection schedule every 2 weeks). The number, content and origin of biohazard waste bins will be recorded.

Please enter comments of the GM safety committee on the risk assessment
GMO work at TTP will be at Level 1 and risk assessment procedures and documentation have been put in place to cover all work. The requirements for Level 1 GMO handling, storage and waste management have been analysed and provisions put in place to meet these requirements. Additional provisions for record keeping, staff training and GMO material tracking, including procedures of waste storage and incineration, have been put in place. A quarterly, and on demand, review schedule of provisions and processes is in place and is designed to ensure provisions match current requirements and to keep recording processes up to date.
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Name

STEMNOVATE LTD

Name 2

Department

Campus Estate or Research Centre

IDEA SPACE

Road Name

3 CHARLES BABBAGE ROAD

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB3 0GT

Country

ENGLAND

Tel Number

07865928070

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: [Y]

Give brief details of the genetic modification safety committee:

The GM safety committee person at the Babraham research campus where Stemnovate's laboratory work will be conducted is MSc, PhD, Tech IOSH, MISTR, and works as Health, Safety and Quality Assurance of Research Manager & Biosafety Officer, Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT. The risk assessment documentation for the Babraham research campus have been duly submitted by the company following a meet up and discussion with facilities management.

At company, CEO is B.V.Sc, M.V.Sc, M.Res (Stem Cell Research) and PhD. She has over 10 years experience in induced pluripotent stem cell (iPSC) research including reprogramming with several vector systems including retroviruses, lentiviruses, sendai viral systems. She did postdoc at University of Cambridge in a MRC (UK-RMP) project for generation of iPSCs for cellular therapies followed by working as operation manager in a company dealing in cellular products for in vitro R&D and was responsible for all risk assessments and implementation of biosafety.

<table>
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<th>Glass House</th>
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There is dedicated waste management system at Babraham Research Campus. The work is conducted in Cat-2 Lab with lock and batch identification system where trained personnel are only allowed to conduct work who are also trained on proper waste disposal. Work is conducted in a safety hood, all tissue culture plastic is treated in Virkon as disinfectant solution before disposal in yellow bin with lid for Cat-2 waste properly lined with autoclave bags for Cat-2 waste. All microtips, pipettes are disposed off in disinfectant solution and disposed after 24 hrs in Cat-2 waste bags. The safety hoods are wiped with 70% ethanol and disinfectant wipes and UV irradiated before and after use. All cryovials and microcentrifuge tubes are properly sealed. The spillage is treated with disinfectant and proper cleaning and reporting is mandatory. All liquid culture waste is directly disposed off in a container with virkon solution through proper aspirator tubing and a vacuum pump. Disinfectants: Virkon as virucidal disinfectant; HAZ tabs and 70% ethanol. All cat-2 waste bins are disposed off at designated location on campus and thereafter are managed by on campus facilities management team and waste disposal service.
The comments from GM safety committee personnel for the human iPS project using Sendai vector system: the ACDP do not classify the Sendai virus as a class 2 (containment 2), but using it as a vector of a proto-oncogene (e.g. c-myc) would require containment 2.

The comments from GM safety committee personnel for the animal iPS project using retroviral vector system: Though MMLV retroviral system is considered Cat1 work but as the pCl-10A1 is used for packaging, the envelope protein confers a wide host range (including humans) and, together with the use of proto-oncogenes e.g. c-myc, would move it into ACGM2 = containment 2 (see SACGM compendium of guidance part 2 (attached), section 2.11 (Retroviruses) pp 121).

---

**Project Ref**  3361/17.1

**Date Ackn'd**  04/05/2017
**Project Title**
The use of retroviral vector systems and Sendai viral vector system for generation of induced pluripotent stem cells from human and animal primary cells.

**Class**  Class 2  **Culture Vol**  < 1 Litre

**Date Project Ceased**

**Consent Granted**

**Non-GMM**

**Project notified under transitional arrangements**  N

**Tick if notifying a connected programme of work**  N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**
Generation of induced pluripotent stem cells (human and animal) for cellular differentiation and develop organ on chip technology for in vitro toxicology studies

**Recipient or parental organism**
Human primary cells and animal (canine, bovine, porcine, equine, mouse) primary cells

**Host/vector system**
The retrovirus vector systems have been based on the oncoviruses of the murine (MuLV) groups of retroviruses. These can be assigned to ACGM containment level 1 on the basis of their inherent properties. These are disabled vectors.

CytoTuneTM 2.0 Sendai reprogramming vectors in this kit are based on a modified, non-transmissible form of SeV, which has the Fusion protein (F) deleted, rendering the virus incapable of producing infectious particles from infected cells. (see attached risk assessment for details)

Origin & function

Human OCT4, SOX2, MYC, KLF4, Pluripotency genes and cell proliferation
Mouse OCT4, SOX2, MYC, KLF4

Evaluation of foreseeable effects

SeV vectors used in this kit consist of viral proteins NP, P, M, F (activated), HN, and L, and the SeV genome RNA, from which the F gene is deleted. Because SeV infects cells by attaching itself to cell surface receptor sialic acid, present on the surface of many cell types of different species, the vectors are able to transduce a wide range of cells. However, they are no longer capable of producing infectious particles from infected cells, because the viral genome lacks the F-gene. In addition, the presence of functional mutations such as temperature sensitivity in the amino acid sequence of several SeV proteins (SeV/TSΔF, SeV/TS12ΔF, and SeV/TS15ΔF) renders the vectors easily removable from transduced cells.

For Retroviral based vectors The packaging cell line is HEK, The inserted genes (OCT4, SOX2, KLF4 and C-MYC).

The final virus is replication defective. Retroviruses require close contact for their transmission and their survival in the general environment is poor. Members of the MuLV oncovirus group frequently require high titres of virus to establish persistent infections in immunologically competent animals so that the risk of harm to the environment associated with accidental release of vectors is generally low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

There is dedicated waste management system at Babraham Research Campus. The work is conducted in Cat-2 Lab with lock and batch identification system where trained personnel are only allowed to conduct work who are also trained on proper waste disposal. Work is conducted in a safety hood, all tissue culture plastic in treated in Virkon as disinfectant solution before disposal in yellow bin with lid for Cat-2 waste properly lined with autoclave bags for Cat-2 waste. All microtops, pipettes are disposed off in disinfectant solution and disposed after 24 hrs in Cat-2 waste bags. The safety hoods are wiped with 70% ethanol and disinfectant wipes and UV irradiated before and after use. All cryovials and microcentrifuge tubes are properly sealed. The spillage is treated with disinfectant and proper cleaning and reporting is mandatory. All liquid culture waste is directly disposed off in a container with virkon solution through proper aspirator tubing and a vaccum pump. Disinfectants: Virkon as virucidal disinfectant; HAZ tabs and 70% ethanol. All cat-2 waste bins are disposed off at designated location on campus and thereafter are managed by on campus facilities management team and waste disposal service.
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### Project Containment

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**Name**

GAMMADELTA THERAPEUTICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

38 JERMYN STREET

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

SW1Y 6DN

**Country**

ENGLAND

**Tel Number**

0207 388 9771

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Company Chief Operating Officer responsible for Business Operations at GammaDelta Therapeutics who has a degree and DPhil in Pharmacology followed by over 15 years of experience working in operational roles supporting R&D activities within biotechnology companies. The Company Chief Operating Officer is the Company nominated Safety Champion.
- Company Vice President of Research is leading Research and Gene Engineering activities at GammaDelta Therapeutics since January 2019. She holds a PhD in Biochemistry and has 14 years’ experience working with genetically modified cells and viral vectors. 9 years experience were gained in an industry setting, where she has also held positions as chair of a GMSC and been a member of H&S committees.
- Company Gene Engineering Team Lead is leading the gene engineering activities at GammaDelta Therapeutics since 2017 October. He holds a medical (MD, University of Debrecen, Hungary) and Phd degree (UCL, London) and has over 10 years of experience working with gene modified cells, lentiviral vector production gained both in academic and biotechnology environment.
- Company Laboratory Manager & Safety Officer who manages the facilities at GammaDelta Therapeutics and who has received considerable formal Health & Safety training. She holds an Honours Bachelor of Science in Biology and has over 15 years’ experience working in the research field in both academic and biotechnology environments.
- An external Safety Advisor who is laboratory based and a chartered member of the Institution of Occupational Safety and Health (12 years). They are a member of a University GMSC.

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<td>Level 1 (GMMs)</td>
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</table>
- Any surface/equipment which is known or thought to be contaminated with GMOs will be disinfected using 1:10 Distel or 1:10 Chemgene solution. A contact time of at least 5 minutes is required. After contact time, any residue is wiped away using absorbent tissue, which is then disposed of via autoclave bags.
- Liquid wastes are to be inactivated by adding Virkon solution to a concentration of 2% (contact time in solution of 24 hours). After contact time, liquid wastes are disposed of via a designated laboratory sink, flushing with plenty of water.
- At a concentration of 1:10 Distel or 1:10 Chemgene solution there is a greater than 5 log degree of kill on a range of bacterial, fungal and viral strains. Please see link for Distel [https://www.starlabgroup.com/Documents/eng/186645.pdf](https://www.starlabgroup.com/Documents/eng/186645.pdf) and link for Chemgene [https://www.starlabgroup.com/Documents/eng/339784.pdf](https://www.starlabgroup.com/Documents/eng/339784.pdf).
- At 1% Virkon solution with contact time of 10 minutes kills a wider range of bacterial, fungal, yeast and viruses. Please see link for Virkon [https://relyondisinfection.com/fileadmin/user_upload/RelyOn_Virkon_UK.pdf](https://relyondisinfection.com/fileadmin/user_upload/RelyOn_Virkon_UK.pdf).
- Consumables or re-usable equipment contaminated with GMO waste will be autoclaved with a holding time of 15 minutes at 121 degree C. GammaDelta Therapeutics Limited (GDT) facilities will be used to autoclave waste. Designated staff at GDT are fully trained to treat and handle waste effectively and safely. Autoclaves are 12-point thermocouple and load tested annually. Bagged waste for autoclaving will be transported from GDT laboratories inside sealed containers, containers will remain closed when being transported from laboratories to Lab 4.01 where autoclaves are located. There is more than one autoclave present in GDT facilities, meaning that there is a back-up autoclave if required.
- Untreated waste is stored securely inside GDT laboratories.
- After waste is autoclaved by GDT staff it is sent for incineration at a site certified to deal with clinical waste, contracted by GDT. The company handling the waste is Stericycle.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment □
The Company's GMSC reviewed the draft risk assessment on 30 March 2017 and the external Safety Advisor additionally reviewed the updated risk assessment on 13 April 2017.

Comments from the Company's GMSC (30 March 2017):
- The draft GMO risk assessment (annotated by the external Safety Advisor) for proposed lentiviral work at GammaDelta Therapeutics Limited ("GDT") was reviewed and discussed by the GMSC.
- It was agreed that the risk assessment provided a good overview of the proposed activity and described the risks, hazards and control measures. Minor suggestions were made concerning the wording and presentation of information. It was noted that the viral titre should be added and agreed that, subject to confirmation of viral titre, the described work would likely be classified as a GM class 1 activity.
- The Safety Officer was actioned to update the assessment for final review by the external Safety Advisor.

Comments from the final review by external Safety Advisor (13 April 2017):
- Following confirmation of the viral titre (less than 6e6 transducing units per ml) using a third generation lentiviral vector and bearing in mind that the project does not use sharps or glass and that staff are protected by a number of control measures such as MSC2 cabinets and PPE with negligible dissemination of GMO products into the environment, the project was assigned to GM class 1 activity.
- Final minor changes were made to the risk assessment, including adding that GDT labs have been designed to CL2 standard and that the holding time for autoclave is 15 mins.

The final risk assessment was circulated again to the GMSC on 20th April 2017 and there were no further comments.
GM Centre Number: 3364

Data Premises Notified (Originally) 10/09/2021

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

BETATEC HOP PRODUCTS LTD

Name 2

Department

Campus Estate or Research Centre
MALVERN HILLS SCIENCE PARK

Road Name
GERALDINE ROAD

Building

District

Town
GREAT MALVERN

County
WORCESTERSHIRE

Postcode
WR14 3SZ

Country
ENGLAND

Tel Number
01684217351

Fax Number
n/a

E-mail

HSE Division
EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted
02/03/2022
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

The person advising holds an MSc, eMIOSH, BSP and has over 20 years experience in reviewing GM and Wild type risk assessments.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
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</table>

Other(s)

- Fermentation experiments as per RA.

- All cultures to be clearly labelled “YKO”.
- Maximum volume of concentrated cultures will be 5 ml, 10A8 cfu/ml yeast strain) in screw-top tubes.
- Maximum volume of fermentation will be 300 ml with 10A7 cfu/ml yeast strain using airlocks.
- Storage of YKO strain cultures will be at -80°C in 96-well plates (2001-li/well) or in screw top-vials (1.5 ml).
- Cultures on solid agar plates may be temporarily stored in the fridge sealed with parafilm.
- Material containing Class-1 GMO YKO Saccharomyces cerevisiae strains will be discarded through the landfill waste route.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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### Name
EXMOOR PHARMA CONCEPTS LTD

### Name 2

### Department

### Campus Estate or Research Centre

### Building
PROSPECT HOUSE

### Road Name
58 QUEENS ROAD

### Town
READING

### District

### County
BERKSHIRE

### Postcode
RG1 4RP

### Country
ENGLAND

### Tel Number
0117 48 5739

### Fax Number
0

### E-mail

### HSE Division
blank

### Comments

### Date at Which Additional Info Submitted
02/03/2022
## Premises Addresses

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<td>FILTON ROAD</td>
<td>BRISTOL</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

No

Give brief details of the genetic modification safety committee

The GOI and vector derivative material are already in use by our contract process development client. As such a competent member of the client team (the Process & Technology Transfer Lead) has provided relevant safety information for use in our own risk assessment, has reviewed the completed risk assessment and visited the lab itself.

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Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research
Two autoclaves are available for relevant waste. Solid/contaminated waste will be disposed into biohazard bins/boxes, sealed and placed into lockable clinical waste containers and disposed of by GMO registered waste management company (http://www.labwaste.co.uk). Decontamination of surfaces will be conducted using elevated levels of the disinfectant ChemGene HLD4L and 70%(v/v) Kleercide spray. Inactivation of liquids will be performed using Virkon tablets as per manufacturer instructions.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The GOI and vectors have been evaluated and are deemed safe for use in the CL 1 lab at Exmoor Pharma. Concepts based on a thorough risk assessment exercise conducted both on the use of this GOI and vector/host cell type at an academic centre operating a cell and gene therapy MHRA approved GMP facility which was witnessed and conducted by their GM safety committee and a repeat risk assessment based on the prior but localised for eXmoor Pharma's lab, which is attached in this premises 1° use of a CL 1 GMO. Alan is content that all minimum requirements are met according to best practice. Furthermore, the natural invaribility of the material in question along with defunct replication competent ability and the inability of rAAV to integrate into its preferred site of genomic integration on human chromosome 19 as seen in wt AAV, also adds a layer of risk mitigation when using this GOI and GMO in a controlled environment. Due diligence has been followed against the most contentious questions for handling of this material and there is no substantial proof that any mishandling of this GMO and derivatives will impact the safety of personnel or the environment it is contained within.

Project Ref 3365/19.1

Date Ackn'd 02/03/2022
### Purposes of the contained use

This work is a part of a process development program to help a client develop and optimise an efficient lentiviral vector manufacturing process at a smaller laboratory scale, which will enable them to take products through the clinic to commercial manufacture. The work in the lab involves cell culture in shake flasks and bioreactors (both in a closed system) between 1L and 40L working volume, using a common bioreactor operating and control platform. Cell culture is followed by induction of the cells to produce viral vector particles, and various steps of downstream purification and analysis of the material. Closed processing is maximised with all open processing operations conducted within a Class II Biological Safety Cabinet.

### Recipient or parental organism

Human embryonic kidney cells (HEK293T).

### Host/vector system

Lentiviral beta-globin (GLOBE) vector system induced from stable HEK293T cell line using doxycycline, that is replication-defective and intended for ex vivo use for modification of autologous CD34+ stem cells.

### Origin & function

Replication-defective lentivirus encoding beta-globin (GLOBE) transgene. Genetic sequence of interest: transgene genetic material is of human origin and intended for ex vivo use for therapeutic modification of autologous CD34+ stem cells. Lentiviral genes: genes for packaging and encapsulation. Sequences are available from client 87363.

### Evaluation of foreseeable effects

HEK293T cells containing genetic sequences of stable lentivirus engineered to be replication-defective. The host HEK293T cell line is a stable lentiviral vector (LVV) producer where LVV production is induced thus transfection is not required. Genetic sequences with stable lentivirus are intended in therapeutic use to increase the expression of beta-globin, which in itself would not have a detrimental effect on human host cells.
Replication-defective lentiviral vectors are non-infectious according to the client's Material Safety Data Sheet. Stable suspension HEK293T cells pose no significant hazard for members of the laboratory or environment.

Closed processing and single use disposable equipment and components are used wherever possible to minimise personnel exposure in the lab and to contain process materials. A biological safety cabinet class II is used for all open processing steps.

The work will use standard laboratory and bioreactor methods and will not involve any processes that generate significant quantities of aerosols.

Post cell growth in the bioreactor, cells are immediately induced to release the viral vector of interest which is subsequently clarified free of cells and cellular debris for further purification. Spent cell culture media and process fluids generated during downstream purification will be inactivated, along with single use materials, as previously described.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

A derogation request is sought for the secure storage, transport and incineration off-site of Class 2 solid waste. It is proposed that a licensed contractor will remove disinfected solid waste from the laboratory location where it is generated for off-site inactivation and disposal (also see section 12. below).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

There is no autoclave of sufficient size in the building, so inactivation in the laboratory is by initial treatment with a disinfectant. Class 2 liquid waste will be chemically inactivated by the addition of Virkon (1% final working concentration) and overnight treatment at room temperature prior to being disposed of down the laboratory drain with copious amounts of water. As per manufacturer's data these conditions result in complete inactivation of GMOs. Class 2 solid waste including plastic ware will be inactivated within a sealed container by immersion in Virkon (1% final working concentration) overnight at room temperature, prior to collection for off-site incineration by a licensed 3rd party waste management contractor (www.srl.com). Inactivated solid waste will be placed in double yellow clinical waste bags within contractor-supplied bins fitted with a sealed lid, located in each laboratory room. When filled, waste bags are securely closed and taken to a larger contractor-supplied 770 litre lockable wheeled bin which will be tagged with a unique barcode. The waste management contractor will remove the filled bin and replace with an empty bin on a weekly collection schedule. A consignment note is generated for each bin of waste material removed by the waste contractor. Waste is transported in the sealed bin and disposed of at an off-site incineration (100% kill). The Avonmouth incinerator where the waste material is handled is licensed to process class 2 GMO waste and is registered under GMO reference centre number GM 779.

Decontamination of surfaces will be conducted using elevated levels of the disinfectant Distel spray.

Spill procedures are available for spills; in this event spill kits and PPE are used to treat all materials and surfaces with 1% Virkon for one hour. All contaminated materials would be collected securely and sent for off-site incineration as described above.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

No further comments.

Project Containment
Project Ref 3365/20.1

Date Ackn'd
08/01/2020

CU2 Project Title
Assessment of recombinant replication-incompetent and self-inactivating gamma retrovirus vector production isolated from human embryonic kidney (HEK293T) cells transiently transfected with CD20 CAR construct intended for ex vivo use for autologous modification of gamma delta T cells

Class
Class 2

CultureVol
1-50 Litres

Class CultureVol
Class 2 1-50 Litres

Consent Granted

Project notified under transitional arrangements

Withdrawn
N

Tick if notifying a connected programme of work
N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use
This work is a part of a process development program to help eXmoor's client to manufacture CD20 expressing γδ T-cell product for the treatment of haematological and solid tumours. The work in the lab involves cell culture using closed small scale multilayer cell stack system between 250ml and 10L working volume. The work will involve transient transfection of HEK293T cells using a three-plasmid transfection system (detailed below) in closed process conditions to generate the virus of interest which is subsequently clarified free of cells and cellular debris for further downstream purification and analysis of the material.

Recipient or parental organism

HEK293T, sourced from ATCC (CRL-3216) by eXmoor's client.

Host/vector system

Replication-incompetent and self-inactivating γ-RV encoding the CD20 CAR construct.

Origin & function

Replication-incompetent and self-inactivating γ-RV encoding CD20 CAR construct.
Genetic sequence of interest: of human origin and intended for autologous modification of γδ T cells.
γ-RV genes: gag-pol and env genes for packaging and encapsulation provided on separate plasmids to improve biosafety.
Sequences are available from eXmoor's client 100365.

Evaluation of foreseeable effects

γ-RV based on Moloney murine leukemia virus (MoMLV), pseudotyped with RD114 envelope (feline RD114 endogenous retrovirus envelope) will be packaged using transient transfection with the three-plasmid system (the transfer plasmid, a packaging plasmid and an envelope plasmid) into HEK293T cells to prevent recombination. The transfer vector contains modified long-terminal repeats (LTRs), the transgene and Ψ packaging signal. The other plasmids are a gag-pol packaging plasmid and a RD114 envelope plasmid. This multi-plasmid approach results in no single plasmid containing all the accessory genes necessary for viral replication. In addition, most of U3 is deleted from 3'LTR, making the viral promoter non-functional and self-inactivating after integration into the host genome which further negates the possibility of viral replication and improves biosafety.
Replication-incompetent and self-inactivating γ-RVs are non-infectious according to client 100365 information. Transiently transfected HEK293T cells pose no significant hazard for members of the laboratory or environment.
Closed processing and single use disposable equipment and components are used wherever possible to minimise personnel exposure in the lab and to contain process materials. A biological safety cabinet class II is used for all open processing steps.
The work will use standard laboratory methods and will not involve any processes that generate significant quantities of aerosols.
Post cell growth in small scale multilayer cell stack system, cells are transiently transfected in closed process conditions to generate the virus of interest which is subsequently clarified free of cells and cellular debris for further purification. Spent cell culture media and process fluids generated during downstream purification will be inactivated, along with single use materials (see section 12. below).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

A derogation request is sought for the secure storage, transport and incineration off-site of Class 2 solid waste. It is proposed that a licensed contractor will remove disinfected solid waste from the laboratory location where it is generated for off-site inactivation and disposal (also see section 12. below).

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
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Decontamination of surfaces will be conducted using elevated levels of the disinfectant Distel spray.
Spill procedures are available for spills; in this event spill kits and PPE are used to treat all materials and surfaces with 1% Virkon for one hour. All contaminated materials would be collected securely and sent for off-site incineration as described above.

Is an emergency plan required according to regulation 20? 

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

No additional comments.

Project Containment

<table>
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<tr>
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## GM Centre Number: 3366

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### Name

| INFEX THERAPEUTICS LTD |

### Name 2

### Department

### Campus Estate or Research Centre

#### Road Name

ALDERLEY PARK

#### District

MACCLESFIELD

#### Town

MACCLESFIELD

#### County

CHESHIRE

#### Postcode

SK10 4TG

#### Country

ENGLAND

### Tel Number

0161 274 9425

### Fax Number

0

### E-mail

blank

### HSE Division

### Comments

### Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The AMRC Safety Committee includes individuals with many years experience working in drug development including three medicinal chemists and two experienced microbiologists with expertise in molecular microbiology including, but not limited to recombinant DNA techniques and mutagenesis. These individuals have the necessary know-how to risk assess the use of GMOs at AMRC and find alternative approaches to negate the use of GMOs where possible.

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Other (please specify) Tick if confidential

Bacteriology Yes 
Parasitology 
Transgenic Birds 
Microbiology Research Yes
All agents will be handled in a cat 2 microbiology laboratory and handled in a Class 2 biological safety cabinet. All users are appropriately trained to handle these organisms, and utilise personal protective equipment including a howie lab coat, nitrile gloves and safety glasses at all times in the laboratory. The facility is equipped with appropriate waste streams for disposal of infectious materials and GMMs.

- Sharps - contaminated sharps are disposed of via sharps bins which are autoclaved using the Alderley park site facility (validated via annual thermocouple testing) and then disposed of as clinical waste and incinerated (via Veolia waste collection)
- Small plasticware - contaminated plastics such as pipette tips are inactivated using 1% ChemGene (http://medimark.co.uk/images/uploads/CHEMGENE_HLD4L_EN_TEST_SUMMARY.pdf)
- For recyclable glassware - a laboratory bench autoclave is used for inactivation of waste (validated via annual thermocouple testing). Inactivated material is then discarded as non-infectious ie. washed away and glassware washed and re-used.
- For all other solid waste - Solid waste is collected in autoclave bags and then inactivated using the Alderley Park site autoclave and then disposed of as clinical waste and incinerated (via Veolia waste collection).

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

<table>
<thead>
<tr>
<th>Virology</th>
<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
<th>Gene Therapy</th>
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</thead>
<tbody>
<tr>
<td>Mycology</td>
<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
</tr>
</tbody>
</table>

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

AMRC Safety Committee includes V S, D L, I C, N O, J K and S L. V S and N O have a collective experience of around 30 years in microbiology and molecular biology. Having worked in both academia and industry they have the necessary experience to assess the risk of utilising GMMs in the AMRC laboratory and minimise this risk. The committee meets every 3 months to discuss and review new biological agents for acquisition, GMMs if applicable, accidents/incidents/near misses where they have occurred, and observations from lab and office inspections.
### GM Centre Number: 3368

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**Name**

SPYBIOTECH LTD

**Name 2**

Department

**Campus Estate or Research Centre**

C2 7600 QUORUM

**Road Name**

OXFORD BUSINESS PARK NORTH

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX4 2JZ

**Country**

ENGLAND

**Tel Number**

01865 582088

**Fax Number**

01865 582089

**E-mail**

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Spybiotech GMO Biological Safety Committee has been established to review risk assessments and give guidance on all safety aspects of projects proposed within the company. It does so by identifying possible hazards associated with the proposed work that could compromise the safety to the general public, the risk of cross contamination or recombination, the risk of release into the community of GMOs and any ethical considerations that may be relevant. Its members are drawn from a variety of backgrounds and bring a diverse range of expertise both within and outside of the company itself. The Committee meets on an ad hoc basis whenever new GMO projects are proposed.

Level 1 (GMMs)  Yes
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial
Other (please specify) Tick if confidential
**Bacteriology** Yes

**Parasitology**

**Transgenic**

**Microbiology**

**Research**

**Virology**

Transgenic

**Animals**

**Gene Therapy**

**Mycology**

Transgenic

**Invertebrates**

**Other (please specify below)**

**Other(s)**

---

### For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste is disinfected with Distel at 1:10 dilution or Chemgene at 1:20 for a minimum of 12 hours before sink disposal.

Solid waste (including plasticwares) is double-bagged in yellow clinical waste bags, placed in a 1100L yellow eurobin with lock and removed weekly by waste management contractor for off-site treatment (autoclaving) and incineration.

Spillages of GMMs will be disinfected with Distel at 1:10 dilution or Chemgene at 1:20 for a minimum 10 minutes.

All disinfectant are used in strict accordance with the manufacturer's guidelines and efficacy data.

---

**Tick to confirm that you are attaching a summary of the risk assessment** Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

---

**Please enter comments of the GM safety committee on the risk assessment**

The GMO Biological Safety Committee reported that the proposed projects to be conducted at Spybiotech poses little to no risk to any of the company staff, the environment, or the general public. It is of a non-infectious, non-hazardous nature and the genes product have no inherent biological toxicity. The committee wanted to remind the company to keep all manufacturer's literature and product information for any vectors, organisms or cells that are being purchased, to add to the background safety information. The committee also noted that every risk assessment should be reviewed on a regular basis to ensure they are still accurate and applicable.
GM Centre Number: 3372

SOLENTIM LTD

Data Premises Notified (Originally) 25/07/2017

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

SOLENTIM LTD

Name 2

Department

Campus Estate or Research Centre

Building

SOLENT HOUSE

Road Name

JOHNSON ROAD

District

FERNSIDE BUSINESS PARK

Town

WINMBORNE

County

DORSET

Postcode

BH21 7SE

Country

ENGLAND

Tel Number 01202 798510

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>WIMBORNE</td>
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<td>BH21 7SP</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Senior Scientist/Laboratory Manager with 18 years laboratory experience including handling of category 1 cell lines, primary cells and tissue of human origin. Qualified to PhD level with Health and Safety training undertaken at DSTL, Porton Down, Nottingham University, Nottingham Trent University and Southampton University to include; Best Laboratory Practice, evaluating and writing Risk Assessments and Working in Containment Laboratories.

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Bacteriology       Parasitology       Transgenic Birds       Microbiology Research       Gene Therapy
Virology        Transgenic Animals   Transgenic Fish
For activities involving GMMs, describe the waste management measures which will apply to the activity

All contaminated solid waste will be inactivated by autoclaving (100% kill) prior to disposal by incineration, the autoclave will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

All contaminated liquids will be placed into a suitable waste container and disinfected by adding Virkon disinfectant to a final concentration of 1% Virkon. Leave for at least 12 hours before disposing down the sink with plenty of water.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

As the cell line(s) that will be used cannot survive outside of the culture conditions, are non-human, and are stable and non-infectious, it is considered by the GM safety committee that this work will be minimal/low risk
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| **Comments** | |

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<th>02/03/2022</th>
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<td>SK10 4TG</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The applicant has previously written risk assessments that were approved by the MRC's LMB GMAG Committee and by the Biological Safety Committee in the Faculty of Medicine and Health at the University of Leeds. He has also previously sought and obtained permission for contained use for his previous organisation, Aptuscan/Avacta Life Sciences Ltd. Additionally, advice has been sought from the Head of Health and Safety in the Department of Biological Sciences at the University of Leeds.

As the company grows, a GM Safety Committee will be established as a sub-committee of the Lab Safety Committee. The applicant is expected to Chair both Committees until sufficient experience has accumulated as the Company grows.

<table>
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Other (please specify) Tick if confidential

02/03/2022
Liquid waste that has been in contact with antibiotic-resistant micro-organisms must be decontaminated by autoclaving for at least 15 minutes at 121°C. Other biologically-contaminated liquid waste must be inactivated using an appropriate disinfectant (e.g. Virkon, Presept, Tegodyne) at a concentration and contact time appropriate to the level of contamination prior to disposal to the drains. Whilst chemical disinfection is not 100% effective, its use should result in at least a 4 to 5 log reduction in viable organisms, which will be monitored by plating on nutrient-rich agar. Where chemical disinfection is not possible (e.g. chemical incompatibility) then the liquids may be autoclaved. If neither of these methods is appropriate, consideration will be given to the use of a gelling agent to generate solidified waste that can be sent for incineration via our approved route for the disposal of clinical waste. Solid biological waste for disposal is double-bagged in biohazard autoclave bags (transparent). After autoclaving for at least 15 minutes at 121°C, the bags are closed and placed into heavy duty, opaque yellow bags for disposal and incineration via the clinical waste route using the BioCentre's approved contractor. Blades, needles, and glassware will not be used for biological work. Any contaminated pipettes (e.g. Stripettes) must be soaked in an appropriate disinfectant and then discarded into a UN approved Sharps-bin. Sharps bins must only be filled to the approved level (usually the three-quarters line). The lid must then be fastened and the bin securely stored to await collection and incineration via the clinical waste route. Incineration is well documented as killing 100% of viable organisms. Providing chemical disinfection is carried out in accordance with Appendix 1 then monitoring is not required.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

As the author of the risk assessment, I have taken every care to ensure that this meets both the needs of the legislation. Just as importantly, I am satisfied that the assessment correctly and conscientiously assigns risk, and clearly outlines measures that will minimise any hazard to MetaLinear's staff or visitors, and to the environment.
Antibiotic resistance of pathogenic micro-organisms is a major and growing problem in modern healthcare worldwide. metaLinear will be using genetic transformation of libraries of engineered proteins into pathogenic micro-organisms to screen for novel targets for antibiotics and for targets that break antibiotic resistance.

We are therefore seeking authorisation for the contained use of transiently transformed pathogenic micro-organisms, including Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeroginosa, and Enterobacter spp (the so-called KAPE organisms). We will also need to transform lab-disabled strains of E. coli, of Saccharomyces cerevisiae, and of Pichia pastoris with genes isolated from the pathogenic organisms that encode the candidate targets. These comprise Class 1 GMOs.

Class 2: Clinical strains of gram negative microbes, including E. coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeroginosa, Enterobacter spp. These strains are, by their very nature, able to infect and colonise human beings and animals. After their transformation with the engineered protein libraries, 3 classes of foreseeable effects must be considered: those resulting in loss of viability or pathogenicity (which are the effects we are seeking) and which will reduce the harmfulness of these micro-organisms; those that are without measurable effect, where the organisms remain Class II; and those that alter the expression of bacterial proteins to increase pathogenicity; as these genes pre-existed in the micro-organisms, there is no increased risk in working with the GMO compared to the parental organism. It is extremely unlikely that one of the engineered proteins, once expressed in the micro-organisms, would alter its biology in such a way as to make the organisms more pathogenic than they already are.

Class 1: Laboratory strains of Escherichia coli K12 and derivatives, and laboratory strains of Saccharomyces cerevisiae or Pichia pastoris. These will be used for the development and storage of the libraries of engineered proteins, and for the subsequent expression of bacterial proteins. The same three classes of foreseeable effects need to be considered, none of which will increase the risk of working with the micro-organisms following transformation. As with mutagenesis, the expression of random proteins in any organism is far more likely to be deleterious than advantageous to the organism. In addition, the library proteins will be controlled by an inducible or repressible transcription system, meaning that the engineered proteins are unlikely to be able to affect the GMO.

Bacteriophage are not properly a biological organism as they are incapable of independent replication. Nonetheless, they are included here as they will be recipients of engineered gene products and there is a risk of cross-contamination by mobilization.
The host strains for screens will be clinical strains of gram negative microbes, including E. coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp, each of which carries its own plasmids. The vector system will therefore need to be tailored for each screen, in each organism. The vector systems will need to be chosen so to minimise the likelihood of plasmid excision in each target strain, but will also be chosen so as to be non-mobiliseable, as far as predictable. All of these strains are Gram-negative bacteria, and will share mechanisms for the maintenance of plasmids, and for gene expression, which we anticipate to mean that we will be able to use common lab-safe vectors of the pBR and pET series (which are mobilisation defective) and pUC series, which are non-mobilisable. The hosts for protein expression will be common E coli K12-derived strains and yeast strains such as Pichia pastoris, together with their associated vector systems of the pBR322, pUC and pET series. These vectors are intrinsically non-mobilisable, although there is a minor risk of mobilisation should these vectors be transformed into the wild-type pathogenic KAPE strains. However, even if this happened, the vectors would only be carrying genes expressing proteins that are naturally present in these organisms (the protein targets for our therapeutic screens) or engineered proteins that were isolated by their ability to decrease the viability of the clinical strains. The risk of these escaping into the environment or infecting a human are therefore negligible.

Origin & function

Peptides constrained in three dimensions within the context of a constant, stable, folded scaffold protein interact with target molecules (usually but not exclusively proteins) with high affinity and specificity. Proteins comprising a scaffold and one or more constrained peptides are generically called peptide aptamers, and when engineered variants of the Stefin A scaffold is used the peptide aptamers are called Affimers. The genetic material to be used will be DNA encoding Affimers and Affimer libraries. To create collections of Affimers, oligonucleotides encoding a random collection of peptide sequences are inserted at defined positions within variants of the Stefin A open reading frame (ORF). Expression of the resulting collection of ORFs generates a library of proteins, the Affimers, randomized at specific regions on their surface. Libraries may be for work using phage display, yeast two hybrid screens, or manipulation of proteins within pathogenic micro-organisms. Following screening of any of these three libraries, larger amounts (µg - g) of individual Affimers may be produced, usually in E. coli or in Pichia pastoris or similar. The project will also include occasional cloning of open reading frames encoding target proteins for expression in E. coli or in S. cerevisiae or P. pastoris.

Evaluation of foreseeable effects

The foreseeable effects are that individual Affimer proteins expressed in a host cell will affect the biology of that cell. We are seeking Affimers that decrease the viability of the host cell (thereby identifying candidate antibiotic drug targets) or that break antibiotic resistance. By definition, these Affimers will not increase the risk of working with the transformed micro-organisms. Our experience is that the overwhelming majority of Affimers are without a positive effect of the growth of the host cell- out of 100s of millions of transformants we have worked with to date, we have never observed colonies with altered morphology, for example. Although it is conceivable that some Affimers may potentially lead to the expression of host genes that increase the viability and/or pathogenicity of the host cell, these genes are already present in the bacteria, and thus will not increase the hazard of working with them. Nonetheless, our risk assessment requires that all Petri dishes on which transformed E coli are grown be sealed with Parafilm, and never opened. Transformed bacteria will be inactivated by autoclaving within 3-5 days of transformation, and disposal will be by incineration by an approved contractor.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We are not requesting derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste. Cleaning and recycling of reusable laboratory equipment, such as glassware, will require a minimal 15 minute incubation with 2% Virkon prior to dishwashing (wash at 80 C, drying 65 C) and autoclaving. Glassware that has been contacted with bacteriophage will be treated with 2% Virkon. Note that glassware not be used when working with the pathogenic strains, to minimize risk of contamination by inoculation.

Liquid growth media contaminated with pathogenic strains will be inactivated by autoclaving. Other liquid waste will be inactivated overnight with 2% Virkon prior to disposal.
via the sink.

All bench surfaces, including the insides of the microbiological cabinets, will be cleaned with 1% Virkon or 70% ethanol (if no open flame is present). The resulting paper waste will be inactivated by autoclaving prior to disposal by incineration.

Incineration of inactivated waste will be performed off-site by the York Science Park’s approved Clinical Waste contractor.

Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min).

Project Containment

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<th>Growth Rooms</th>
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Project Ref 3373/18.1

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Antibiotic resistance of pathogenic micro-organisms is a major and growing problem in modern healthcare worldwide. metaLinear will be using genetic transformation of libraries of engineered proteins called RTeins into pathogenic micro-organisms to screen for novel targets for antibiotics and for targets that break antibiotic resistance.

We are therefore seeking authorisation for the contained use of transiently transformed pathogenic micro-organisms, including Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp (the so-called KAPE organisms).

We will also need to transform lab-disabled strains of E coli, of Saccharomyces cerevisiae, and of Pichia pastoris with genes isolated from the pathogenic organisms that encode the candidate targets. These comprise Class 1 GMOs.

Recipient or parental organism

Class 2: Clinical strains of gram negative microbes, including E. coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp. These strains are, by their very nature, able to infect and colonise human beings and animals. After their transformation with the engineered protein libraries, 3 classes of foreseeable effects must be considered: those resulting in loss of viability or pathogenicity (which are the effects we are seeking) and which will reduce the harmfulness of these micro-organisms; those that are without measurable effect, where the organisms remain Class II; and those that alter the expression of bacterial proteins to increase pathogenicity; as these genes pre-existed in the microorganisms, there is no increased risk in working with the GMO compared to the parental organism. It is extremely unlikely that one of the engineered proteins, once expressed in the micro-organisms, would alter its biology in such a way as to make the organisms more pathogenic than they already are.

Class 1: Laboratory strains of Escherichia coli K12 and derivatives, and
laboratory strains of *Saccharomyces cerevisiae* or *Pichia pastoris*. These will be used for the development and storage of the libraries of engineered proteins, and for the subsequent expression of bacterial proteins. The same three classes of foreseeable effects need to be considered, none of which will increase the risk of working with the micro-organisms following transformation. As with mutagenesis, the expression of random proteins in any organism is far more likely to be deleterious than advantageous to the organism. In addition, the library proteins will be controlled by an inducible or repressible transcription system, meaning that the engineered proteins are unlikely to be able to affect the GMO.

Bacteriophages are not properly a biological organism as they are incapable of independent replication. Nonetheless, they are included here as they will be recipients of engineered gene products and there is a risk of cross-contamination by mobilization.

**Host/vector system**

The host strains for screens will be clinical strains of gram negative microbes, including *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp*, each of which carries its own plasmids. The vector system will therefore need to be tailored for each screen, in each organism. The vector systems will need to be chosen so as to minimize the likelihood of plasmid excision in each target strain, but will also be chosen so as to be non-mobilizable, as far as predictable. All of these strains are Gram-negative bacteria, and will share mechanisms for the maintenance of plasmids, and for gene expression, which we anticipate to mean that we will be able to use common lab-safe vectors of the pBR and pET series (which are mobilization defective) and pUC series, which are non-mobilizable.

The hosts for protein expression will be common *E. coli* K12-derived strains and yeast strains such as *Pichia pastoris*, together with their associated vector systems of the pBR322, pUC and pET series. These vectors are intrinsically non-mobilizable, although there is a minor risk of mobilization should these vectors be transformed into the wild-type pathogenic KAPE strains. However, even if this happened, the vectors would only be carrying genes expressing proteins that are naturally present in these organisms (the protein targets for our therapeutic screens) or engineered proteins that were isolated by their ability to decrease the viability of the clinical strains. The risk of these escaping into the environment or infecting a human are therefore negligible.

**Origin & function**

Peptides constrained in three dimensions within the context of a constant, stable, folded scaffold protein interact with target molecules (usually but not exclusively proteins) with high affinity and specificity. Proteins comprising a scaffold and one or more constrained peptides are generically called peptide...
aptamers, and when engineered variants of the Stefin A scaffold is used the peptide aptamers are called RTeins. The genetic material to be used will be DNA encoding RTeins and RTein libraries.

To create collections of Affimers, oligonucleotides encoding a random collection of peptide sequences are inserted at defined positions within variants of the Stefin A open reading frame (ORF). Expression of the resulting collection of ORFs generates a library of proteins, the RTeins, randomized at specific regions on their surface. Libraries may be for work using phage display, yeast two hybrid screens, or manipulation of proteins within pathogenic micro-organisms.

Following screening of any of these three libraries, larger amounts ($\mu$g - g) of individual RTeins may be produced, usually in E. coli or in Pichia pastoris or individual RTeins may be produced, usually in E. coli or in Pichia pastoris or similar.

The project will also include occasional cloning of open reading frames encoding target proteins for expression in E. coli or in S. cerevisiae or P. pastoris.

**Evaluation of foreseeable effects**

The foreseeable effects are that individual RTein proteins expressed in a host cell will affect the biology of that cell. We are seeking RTein proteins that decrease the viability of the host cell (thereby identifying candidate antibiotic drug targets) or that break antibiotic resistance. By definition, these RTeins will not increase the risk of working with the transformed microorganisms.

Our experience is that the overwhelming majority of RTeins are without a positive effect of the growth of the host cell: out of 100s of millions of transformants we have worked with to date, we have never observed colonies with altered morphology, for example. Although it is conceivable that some RTeins may potentially lead to the expression of host genes that increase the viability and/or pathogenicity of the host cell, these genes are already present in the bacteria, and thus will not increase the hazard of working with them. Nonetheless, our risk assessment requires that all Petri dishes on which transformed E coli are grown be sealed with Parafilm, and never opened. Transformed bacteria will be inactivated by autoclaving within 3-5 days of transformation, and disposal will be by incineration by an approved contractor.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Alderley Park operates a full decontamination, sterilisation and incineration service that we propose to use for solid waste only. Double-bagged solid waste will be placed in sturdy yellow sterilisation bags within the lab, and then placed in yellow wheeble bins stored in locked cupboards immediately
outside the lab entrance. Trained Alderley Park personnel collect these bins and take them via a specified and approved route to the facility for sterilisation and incineration.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste. Cleaning and recycling of reusable laboratory equipment, such as glassware, will require a minimal 15 minute incubation with 2% Virkon prior to dishwashing (wash at 80 C, drying 65 C) and autoclaving. Glassware that has been contacted with bacteriophage will be treated with 2% Virkon. Note that glassware not be used when working with the pathogenic strains, to minimize risk of contamination by inoculation.

Liquid growth media contaminated with pathogenic strains will be inactivated by autoclaving. Other liquid growth media contaminated with pathogenic strains will be inactivated by autoclaving. Other liquid waste will be inactivated overnight with 2% Virkon prior to disposal via the sink.

All bench surfaces, including the insides of the microbiological cabinets, will be cleaned with 1% Virkon or 70% ethanol (if no open flame is present). The resulting paper waste will be inactivated by autoclaving prior to disposal by incineration.

Incineration of waste will be performed o-site by Alderley Park's approved Clinical Waste contractor, Veolia Ltd.

Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121ºC for 20 min).

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**Please enter comments on the GM safety committee on the risk assessment**

metroLinear Ltd is currently too small to form a committee. Every employee is working in the lab on these projects, and is required to read and sign off on the Risk Assessment attached. This risk assessment is based on that used by Avacta Life Sciences, from whom the RTein technology has been licensed, and that was written by Paul Ko Ferrigno and approved by Avacta's GM Safety Committee and Lab Safety Committee.

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**Project Containment**

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<th>Growth Rooms</th>
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Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- Composition of Biological/GM safety Committee
  - Person responsible for overall OHS (NNRCCO Head of Institute)
  - Biological Safety Officer
  - Management representatives (Department Managers for all teams using GMOs)
  - Representative from Operations
  - General staff representative(s) chosen by and from all members of the department who have access to genetic modification

The committee meets regularly (quarterly), and also as required, to review and make recommendations on proposed work to be carried out at the company's premises.

The GMSC will meet once yearly to review existing GM risk assessments.

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The work will be carried out in compliance with good laboratory practice and local safety rules for GM work in a containment level 2 facility.

Bacterial cultures:
All solutions and consumables are subjected to overnight Virkon treatment. Liquid waste will be discharged to drain waste stream, according to established practises in Centre.

Cell cultures:
All solutions and consumables are treated by Virkon only, before entering the drain waste stream, according to established practises in Centre.

Autoclave-specific instructions:
Consumables (mainly plastic ware e.g. pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via black bag waste stream.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via black bag waste stream.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturer’s guidelines)

Work conducted at the NNRCO will aim to identify novel type 2 diabetes drug targets.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The work will be carried out in compliance with good laboratory practice and local safety rules for GM work in a containment level 2 facility.

Bacterial cultures:
All solutions and consumables are subjected to overnight Virkon treatment. Liquid waste will be discharged to drain waste stream, according to established practises in Centre.

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All solutions and consumables are treated by Virkon only, before entering the drain waste stream, according to established practises in Centre.

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Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturer’s guidelines)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Work conducted at the NNRCO will aim to identify novel diabetes and metabolic disease drug targets via medium throughput cellular screens and smaller gene-specific studies. The genome of mammalian cells will be targeted by 'gene editing' tools such CRISPR, which cleave double-stranded DNA, and stimulate genomic repair via nonhomologous end joining (introducing indels), or homologous recombination (upon provision of an appropriate oligo template). This approach will allow study of endogenous gene knockout, as well as the effect of introduced/corrected mutations. Complimentary gene-targeting approaches using modified ('dead') Cas9 tethered to transcriptional regulators will also be used to enhance or repress gene expression. Similar approaches will also be used to generate reporter lines and genetically-encoded cell-signalling sensors. Functional characterisation of the resulting cells will involve standard laboratory techniques including (but not restricted to): secretion assays, reporter assays, kinetic assays, fluorescent microscopy, mass spec analysis, DNA sequencing, and gene expression studies.

Recipient or parental organism
Mammalian cell lines have been sourced from-, and well characterised by, commercial vendors. They are free of mycoplasma, bacteria and fungi, and human viruses (CMV, EBV, HBV, HCV, HIV1, HIV2, HPV, HSV1, and HSV2).
Risk = low. See list of example cell lines in Appendix 1.
Human embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) lines have been characterised extensively within Novo Nordisk, and are free of mycoplasma, bacteria and fungi, and human viruses (CMV, EBV, HBV, HCV, HIV1, HIV2, HPV, HSV1, and HSV2). Risk = low.
Human primary cells are considered especially disabled hosts under the Genetic Modification Regulations and are therefore minimal risk (considered to be ADCP hazard group 1). However, there is the potential for blood borne pathogens and as such samples will be handled at containment level 2 under CoSHH regulations. Where commercially-sourced, primary cells will also be tested by serology for the major human pathogens RPR (syphilis), Hepatitis B, Hepatitis C and HIV. All primary cell handlers will be vaccinated against hepatitis B purely for precautionary reasons (and in line with University of Oxford safety policy). All samples will be stored, used, and disposed of in line with the Human Tissue Act. Risk = low.
E.coli to be used in plasmid replication are ADCP category 1, as well as being inactivated and incapable of colonising the gut. Risk = low. See list of example bacteria in Appendix 2.
Virus or viral vectors have been well characterized. Third generation lentiviral vectors are safer as they are replication incompetent and are always SIN (self-inactivating). Adenovirus doesn't integrate into the genome and is not replicated during the cell cycle. Adenovirus spread from cells to cells. Risk = low.
Manipulation of endogenous genomic sequence will be achieved by the action of the site-specific nuclease delivered to the cell as a complex of nuclease protein and its cofactor nucleic acid, frequently an RNA molecule (RNP [ribonucleoprotein]).

CRISPR via viral transduction:
Manipulation of endogenous genomic sequence will be achieved by the action of the site-specific nuclease delivered to the cell via viral expression vector. This same vector will also express guide-RNA molecules which will be sequence-specific and target the nuclease to the desired genomic coordinates.

Additional exogenous DNA for insertion into the genome:
Examples include but are not limited to:
1) Linear single stranded oligonucleotides which are homologous to the region of interest and include the required sequence changes, e.g. point mutation changes or small sequence alterations.
2) Double stranded DNA as standard plasmids and BAC backbones harbouring:
   a) mammalian (e.g. human or mouse) genomic sequence
   b) mammalian (e.g. human or mouse) cDNA mini genes driven by tissue-specific promoters (e.g. human or mouse genomic promoter sequence), from inducible operons (e.g. the Lac or Tet bacterial operons), from ubiquitous promoters (e.g. viral CMV immediate early promoters) or a combination of promoter, exon, intron, splice acceptor elements (CAAGS promoter sequences)
   c) synthetic shRNAs and microRNAs driven by tissue specific or human ubiquitous Poilil promoter.

CONTAINMENT AND CONTROL MEASURES - OVERALL CONTAINMENT LEVEL 2
Under Genetic Modification regulations, cell lines are considered to be especially disabled hosts. However, as a precaution, all cell lines used will be handled at Containment Level 2 under COSHH Regulations. For aseptic purposes all cell lines will be cultured in a Class II microbiological safety cabinet located in a tissue culture laboratory (containment level 2). Risk=low
Primary cells are considered especially disabled hosts under the Genetic Modification Regulations and are therefore minimal risk (considered to be ADCP hazard group 1). However, there is the potential for blood borne pathogens and as such sample will be handled at Containment Level 2 under CoSHH Regulations. For aseptic purposes all cell lines will be cultured in a Class II microbiological safety cabinet located in a tissue culture laboratory (containment level 2). Risk=low

All work will take place in CL2 laboratories (leased from University of Oxford), and all local rules and University Biosafety policies will be followed whilst working with GMOs. Use of sharps will be avoided if possible and the generation of aerosols kept to a minimum.
Viral vectors/particles will only be used by trained individuals and contained within designated areas of the laboratory. Any procedure that can generate aerosols or splashes will be conducted inside a biological safety cabinet. All viral waste will be inactivated and disposed of in alignment with local (University of Oxford) safety rule (see below).

Expected biological action of inserted DNA/RNA or transcribed/translated gene product:
Normal mammalian cellular functions (as well as non-functional gene fragments and selective alterations of said gene/protein). Transgenes also include but are not limited to fluorescent, luminescent, or histochemical reporter genes, and previously-characterised recombinase and integrase enzymes. Inserts are not expected to affect pathogenicity of cloning.
CRISPR-Cas9:
The Streptococcus pyogenes Type II CRISPR-Cas system consists of the nuclease Cas9, and a guide (g)RNA. This gRNA determines DNA binding/cleavage specificity. Target DNA must immediately precede a 5'-NGG (PAM) sequence, with specificity directed by altering the 20-nt guide sequence within the sgRNA. The D10A Streptococcus pyogenes Cas9 nuclease mutant introduces single strand nicks at differing sites on the sense and antisense DNA strands. Other mutant 'dead' Cas9 proteins (with no nuclease activity) can be tethered to transcriptional regulators to modify gene expression levels at targeted/gRNA-specific loci. This nuclease, when directed against human genomic sequences, has the potential to be mutagenic, although the preparation would have to cross the cell membrane. Electroporation acts as an engineering control for this potential risk, as this makes it virtually impossible for the researcher to inoculate themselves by accident. When this nuclease is to be introduced into cells via an active transfection reagent (e.g. lipofection or viral infection), the risk is increased to containment level 2, and the appropriate handling and disposal rules will apply (see below).

As well as the intended, predictable on-target mutagenesis, the tolerance for some limited mismatches have been reported for site-specific nucleases, which suggests an additional risk of off-target mutagenesis. To minimize these unpredictable effects, all commercially-supplied gRNAs have been designed using proprietary algorithms that minimise predicted off-target events. As an additional safety measure, a list of all gRNAs and predicted off-targets will be kept, assessed, and reviewed on a regular basis.

Of note, there is hugely conflicting evidence in the field as to whether there is any, and if so what level risk there is, of off-target mutagenesis. Whilst studies involving many millions of cells report this phenomenon (doi:10.1038/nbt.2623, doi:10.1038/nmeth.4293), there are also many in which off-target mutagenesis is refuted (doi:10.1016/j.stem.2014.06.011, doi:10.1016/j.stem.2014.04.020). This refutation is was recently supported by opinion-leaders in the field (doi: https://doi.org/10.1101/153338).

Evaluation of foreseeable effects
All genetically modified E. coli, viruses, and some transfected lines will be antibiotic resistant for clonal selection; however this will not increase the risk from these GMOs, as such antibiotics would not be used in clinical treatment. All viruses and RNP-complexes (when transfected only) will be handled at containment level 2 to minimise risk of accidental inoculation. Generation of aerosols will be kept to an absolute minimum. Use of sharps will be avoided when using virus or RNP-complexes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
n/a

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

A disinfection policy is in place within all NNRCO laboratory space (Appendix 5).

WASTE MANAGEMENT MEASURES
Liquids (eg samples, culture supernatants, tissue culture media)
  Disinfect with 1 % Virkon for 1 hour, discharge any excess liquid to drains.
Consumables (mainly plasticware eg pipettes, flasks, tubes)
Health and Safety
Executive
  Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (the building discard autoclave sterilisation
cycle is 134nC for 5 minutes), discharge excess liquid to drains, dispose of solids via the industrial (black bag) waste stream for landfill.

Agar plates

Autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (see above), discharge excess liquid to drains, dispose of solids via the black bag waste stream for landfill.

Routine disinfection

Wash down benches and Class 2 Biosafety cabinets before and after each use and wipe clean equipment using 5% CHEMGENE and 70% EtOH. Deep clean Class II Biosafety cabinets with 5% CHEMGENE, followed by 70% EtOH, once Imonth.

Degree of Kill

Autoclaving I effectively 100% kill (annual validation)

Incineration I effectively 100% kill (licensed incinerator)

Chemical disinfection I effectively 100% kill (Rely+On Virkon)

Sharps (eg needles, syringes, scalpels) - NOTE: SHARPS WILL NOT BE USED WITH VIRUS/RNP COMPLEXES

Collect in suitable Sharps Bins for disposal via the usual building sharps waste stream; autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (see above) then dispose via clinical waste stream for incineration. Sharps must not be used for viral work.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This notification and the information included within has been written and reviewed by NB, QW, SE, JJ, and AK. It aligns with safety policy already in place at the University of Oxford.

Project Containment

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Biological Safety Officer (BSO) reviewed all the GMOs. BSO has a PhD in biochemistry with 17 years research experience. BSO has previously written GMO risk assessments for similar work. The BSO understands the science around the work carried out.

Health and safety consultant reviewed the GMOs. The consultant has over 20 years experience in risk assessments and has health and safety qualifications.

EH&S manager reviewed the GMOs. All GMO risk assessments were reviewed independently and externally by the EH&S manager to look for inconsistencies and to highlight potential risks.

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<td>Tick if confidential</td>
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02/03/2022
Bacterial cultures are treated with 1% freshly made Virkon or Presept and decontaminated for 1 hour before flushing down the sink with plenty of water. Virkon powder is an irritant, so only compressed tablets are to be used, or prepared 1% solutions, that were prepared in a fume hood. All glassware containing GMO are treated as above. All plasticware containing GMO’s are taken offsite by a specialist waste contractor for disposal by incineration.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Biological Safety Officer (BSO) reviewed all the GMOs. BSO has a PhD in biochemistry with 17 years research experience. BSO has previously written GMO risk assessments for similar work. The BSO understands the science around the work carried out. Health and safety consultant reviewed the GMOs. The consultant has over 20 years experience in risk assessments and has health and safety qualifications. EH&S manager reviewed the GMOs. All GMO risk assessments were reviewed independently and externally by the EH&S manager to look for inconsistencies and to highlight potential risks.

Project Ref 3376/17.1

Date Ackn'd CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4
31/08/2017 Selection, design and engineering of antibodies and TCRs and their analysis for Class 2 < 1 Litre
We are a biotechnology company focused on developing therapeutics for cancer by developing antibodies, TCRs and vaccines to halt tumour growth.

### Host/vector system
- pIONTAS1/TG1
- pSANG10/Various E. coli strains
- pPEP vectors/CHO cells
- pMX vector series, TOPO-TA, TOPO-Blunt, pTZ57R/T/Various E. coli strains
- B95-8 marmoset cell line or ATCC® VR-1492™/primary cell lines

### Recipient or parental organism
- BL21 E. coli (New England Biolabs: C2527H)
  - DH10B E. coli (ThermoFisher: 12331013)
  - TG1 E. coli (Lucigen: 60502-2)
- DH5α E. coli (ThermoFisher: various catalogue numbers based on competency)
- TOP-10 E. coli (ThermoFisher: C404006)
- Mammalian CHO (Chinese Hamster Ovary) cell line for expression of soluble and membrane expressed antigens (transient and/or stable) (ATCC® CCL-61)
- Expi293F (ThermoFisher: A14527)
- Primary human B cells
- AKD10R3 cells
- 1624-5 cells
- Jurkat cells
- K562 cell lines
Retroviral vectors/AKD10 and 1624-5 cells
pET21/Various E.coli cells
pET-DUET vectors/ Various E.coli cells
Lentiviral vector - pLS018/primary cells, K562, Jurkat cells
Lentiviral vector - pCDH/primary cells, K562, Jurkat cells
Lentiviral vectors - LENTIGUIDE puro and pLENTICRISPRv2/primary cells, K562, Jurkat cells

Human scFv
Human Antibodies and fragments
Soluble antigens from different species (human, mouse, non human primate)
EBV viral genome
Human, mouse and chimeric TCRs
Luciferase
eGFP and GFP
TCRs/CARTs (human)
MHC-peptide complex (human)

Evaluation of foreseeable effects
HEK293 cells
Human adenovirus is a common virus for humans, that can cause illness. The virus is transmitted into the tissue via soft mucous membranes. It causes respiratory disease, the symptoms of which are a sore throat, runny nose and eyes, sneezing, headaches, cough and fever. In some persons infection with adenovirus may also cause croup or bronchitis. Some infections may also lead to conjunctivitis (pink-eye), skin rash, diarrhoea and bladder infections. Laboratory scientists who are displaying signs of adenoviral infections regularly when working with HEK cells need to notify the GMO safety committee and be referred to occupational health.
In HEK cells, the adenovirus is incomplete, and it would be able to replicate if other genes from the adenovirus were present. So, if a laboratory worker was already harbouring the adenovirus, this could combine with E1A and result in further active viral particles. This has the potential to infect other health humans if the virus was not contained and will harm the environment. All HEK cell work must take place in a class 2 microbiological safety cabinet. This would mean any possible aerosols created from HEK cultures would be HEPA filtered before leaving the laboratory. A class 2 MSC hood would protect the user from possible exposure to any harmful effects of the cell line. All members of staff will be trained on how to use a class 2 MSC effectively to minimise exposure to themselves and the environment. Along with our biological safety procedures.
EBV
The use of primary human tissue/cells along with EBV is the most hazardous GMO. EBV is the causative agent for infectious mononucleosis and is
associated with several malignancies in man, such as African endemic Burkitt's lymphoma, nasopharynx carcinoma of the undifferentiated form, B cell lymphomas in immunosuppressed individuals, and Hodgkin's disease. Infection with EBV commonly occurs during childhood in an asymptomatic form, leading to a rapid increase in EBV antibodies. Seroepidemiological data from various regions of the world demonstrate a prevalence of anti-EBV antibodies of 85–95% at the age of 20 years. Thereafter, primary EBV infections are a rare event, but are still observed. This suggests that some individuals remain EBV− due to natural resistance. It is unclear what would happen if the immortalized B cell was accidentally injected or was able to enter the systemic circulation via the mucous membranes. It could potentially cause malignancies, but the number of cells that need to systemically administered to observe that is unclear. Use of sharps is prohibited with this cell line. All work must also take place in a class 2 MSC. The immortalized cell line can also synthesise new virus, so levels of exposure to the virus could be high. So volumes of cells and virus must be kept low to less than 1L.

AKD10 cells
The AKD10R3 cell lines with TCRs transduced into them. This cell has the intracellular signalling of a mouse T-cell, but the extracellular receptors for human. So human antigens could trigger a signalling response. The human triggers for such a signalling response can only be cells expressing MHC-peptide with beta-macroglobulin molecules. These are found at such low concentrations, that the signalling response will be weak. In addition, the TCR can only signal when there is a specific MHC-peptide complex, so the likelihood that there is a clear match is very low. This is not a dangerous cell line, to the environment or human health, Our good laboratory practise is to wear appropriate PPE when handling this cell line and to work within a Class 2 biological safety cabinet to reduce any possible exposure risks.

Lentiviruses
The biosafety of lentiviruses has been looked at in great detail and is well documented (Debyser, 2003. Curr. Gen. Ther). The highest risk of infection of lentivirus is through sharps and scratches. Mucous membrane exposure is also very high, and can be a source of infection for laboratory scientists. The use of adequate use of biosafety cabinets and PPE can prevent this risk. Use of sharps in class 2 laboratories is prohibited. When lentivirus is made, it is centrifuged and filtered before infecting cells. The virus particles will be spun in a high speed centrifuge in the laboratory and all buckets must be taken into the MSC and bucket seals opened in the MSC to minimise aerosol formation. All tubes must also be opened in the MSC. Filtration via a 0.4 uM syringe filter also has the potential to spray aerosols. All this must be done in an MSC. PPE must be adhered to at all times.
General
All staff must be made aware of the class 2 GMOs generated in this RA. Immunocompromised individuals must be made aware of the HEK risk to themselves, and be monitored for any adverse effects by our occupational health consultants. When used in a Class 2 MSC, the likelihood of exposure of the GMO’s in this Risk assessment will be low.
PPE will be adhered to. Spot safety checks (monthly) and continual laboratory surveillance will be carried out to ensure that staff are adhering to Agenus’ PPE laboratory requirements, and disposal of class 2 GMOs will be monitored by the inspections also. All appropriate PPE will meet current the current ISO/BS/EN standard.
Hepatitis B vaccination is only required for laboratory scientists working on primary human cells and human tissue. Cells bought from ATCC are not screened for Hepatitis B. It is recommended that all scientists working with any human cells should be vaccinated for Hepatitis B. This will be a requirement for Agenus-UK, and immunisation surveillance will be enforced.

EBV Specific
Laboratory scientists working on this project must be made aware of the health risks to human health when working with EBV. There are no vaccinations available to EBV. There are strictly no sharps allowed in the class 2 laboratories. Laboratory scientists working with EBV should report any symptoms of mononucleosis (fever, sore throat, body aches) to the BSO or health and safety committee, at which the scientist may be referred to the GP or hospital for a blood cell count. EBV antibody tests can also be performed in hospitals to monitor scientists who are immune compromised or at high risk of catching EBV. EBV can only be transmitted by saliva. Immune compromised scientists should wear protective facial masks that are HEPA filtered to prevent exposure.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste coming out of the CL2 laboratories will be inactivated before removal (GMO level 1 and 2). All liquid waste and pipette tips will be submerged in 1% Virkon solution for a minimum of 1 hour before strained and disposed of in double sealed autoclave bags. The 1% Virkon solutions will be kept in the Class 2 MSC to prevent removing any liquid waste from the MSCs. (Virkon has been validated to show it will inactivate any human cells and viruses). All waste will be decontaminated further by autoclaving, before taken offsite by a specialist biohazard waste company for incineration and final disposal. The disposal of plasticware that is not routinely subject to Virkon or autoclaving must will be double sealed in biohazard bags collected and stored in plastic biohazard waste bins before transfer to a specialist biohazard waste company.

Is an emergency plan required according to regulation 20?  
N

If yes, tick to confirm that it is attached to this form
N

Tick to confirm that you have attached a risk assessment to this form  
Y
The only class 2 GMO many RAs is the use of HEK cells. The GMOSC highlighted the importance of face to face training on the use of HEK cells, particularly the waste streams and handling of the cell line. All liquid waste is disposed of with virkon and all solid waste autoclaved onsite before removal. This may change if we find a supplier to decontaminate our class 2 material. There will be an additional web based training for all new hires, and all scientific staff will be responsible for adhering to Agenus biological safety procedures.

EBV Specific

GMOSC agreed that this GMO is the most hazardous. Any intent to work on EBV must be provided in writing to the GMOSC prior to any work started, stating when the work will start, how long EBV will be in culture and when the work is to finish. Progress on the timelines should be given regularly to the GMOSC. The GMOSC is responsible for notifying all staff via email that EBV is being cultured, and in which laboratories. The GMOSC will also be responsible for sticking up notifications on the doors of these laboratories, to minimise scientist exposure. Sharps is strictly prohibited. Scientists working with EBV will also be required to wear double gloves, where outer pairs will be removed when moving from centrifuges to the class 2 MSC. Centrifuge bucket seals will be purchased for these laboratories, and any breakage of tubes would require scientists to keep the centrifuges closed for at least 2 hours to allow aerosols to settle before opening. All tubes and bucket seals must be open in the class 2 MSC. Weekly cleaning procedures must be adhered to, as specified in the Agenus biologicals procedures documents, and this will be monitored by laboratory champions. All scientific staff will be required to adhere to Agenus biological procedures.

Lentivirus Specific

The GMOSC agreed that although viruses are used in this RA, they are well understood and deactivated and are safer to use. There are risks, particularly with incubating the virus in the cold room, ultra-centrifugation of the virus, incubation in shaking incubators and syringe filtering. These are hazards that can produce scientist exposure and aerosol formation. All filtering must occur under a class 2 MSC. Lentivirus that is live and CL2 in the incubators require a poster fitted to the front of the incubator to make other scientists aware of the hazards. The same with the centrifuges and cold room. Double gloves are also required to reduce the spread of contamination. Centrifuge bucket seals will be purchased for these laboratories, and any breakage of tubes would require scientists to keep the centrifuges closed for at least 2 hours to allow aerosols to settle before opening. All tubes and bucket seals must be open in the class 2 MSC. Weekly cleaning procedures must be adhered to, as specified in the Agenus biologicals procedures documents, and this will be monitored by laboratory champions. All scientific staff will be required to adhere to Agenus biological procedures.

Project Containment

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**Name**

THE JUDD SCHOOL

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

BROOK STREET

**District**

**Town**

TONBRIDGE

**County**

KENT

**Postcode**

TN9 2PN

**Country**

ENGLAND

**Tel Number** 01732770880

**Fax Number** N/A

**E-mail**

**HSE Division** EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

**[ ]**

Give brief details of the genetic modification safety committee:

Senior Lecturer - Faculty of Molecular Biophysics - School of Biociences - Kent University, Canterbury
Principal Investigator for Kent university’s iGEM team.

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| Other (please specify) |                       |             |             |             | Tick if confidential [ ]

Bacteriology [ ] Yes
Parasitology
Transgenic Animals
Transgenic Fish
Transgenic Birds
Microbiology Research [ ] Yes
Virology
Gene Therapy

02/03/2022
Both liquid and solid waste will be autoclaved with 100% expected degree of kill. Our autoclaves are examined and validated every three years. Virkon disinfectant will be used to deal with any spills in the laboratory.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The E coli strains for plasmid construction and maintenance are non-pathogenic laboratory strains (E.coli DH5alpha). The proposed genetic modifications will not change the infectivity of the microorganisms or allow them to persist in the environment. Although the plasmids used in the cloning procedures contain an antibiotic resistance marker that can be used for selection, the antibiotics used are not the same ones used in frontline patient treatment.

The proposed waste disposal by autoclaving should be sufficient to decontaminate the waste produced during the experiments.
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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

Axitan is a tenant company of the Imperial College Translation and Innovation Hub. The Axitan representative is the company director who is an experienced researcher holding both masters and doctoral qualifications. Axitan has sought advice from both the biological safety officer of the Department of Biochemical engineering at University College London and the Safety and Biorisk manager of Imperial College. Both individuals provided advice with respect to the risk assessment and HSE notification. In addition various literature sources and 3rd parties using the same materials were also consulted.

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Tick if confidential: **No**
Waste Disposal
- Virkon is used as it is a validated cleaning method for material and transfected cells if used in the following way under manufacturers recommendations
- Hard surfaces: A solution containing 1% Virkon for 1 hour contact time, 10mins for metal parts (longer can cause corrosion)
- Safety cabinets: 1% Virkon (10mins)
- Discard jars, plastic flasks, glassware: A solution containing 1% Virkon.
- Ensure all surfaces are in contact with the disinfectant (10mins). Added to sealed bags for autoclaving at a minimum of 121 degrees C, 1.15bar, 20 mins hold.
- Supernatants/liquid waste: 3% Virkon diluted 2:1 in culture medium
- All autoclaved solid waste is to be placed into hazardous waste bags. There is one autoclave available and the equipment is regularly serviced, and an annual multi point thermo-couple test carried out with a suitable representative load, to ensure that it maintains its ability to provide a validated method of waste inactivation.
- Full bags are to be transported to designated hazardous waste bins in the storage area
- Bins are collected for off site incineration by waste management company

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The Translation and Innovation Hub (I-HUB) provides space for companies to work alongside Imperial researchers. It's facilities support businesses large and small and, include start-up and fast-growth companies as well as established biotech and scientific research organisations. Through the Imperial College safety department, I-HUB provides health and safety assistance to ensure that tenants are assessing the risks associated with the work appropriately and to disseminate best practice among researchers. Imperial College Biological Safety Officer Ian Hackford has reviewed and advised Axitan on its GMO risk assessment and are satisfied they have considered the risks in terms of GM organisms and scale of work. They have appropriate control measures to contain the work and appropriate procedures for the class of GMO activity for waste disposal.

Below are specific comments from the Imperial Biosafety officer that have since been addressed:

1. Section 6 of the CU1. I would recommend changing the following sentence in section 6 of the CU1 referring to the autoclave from "There is 1 autoclave available and the equipment is regularly serviced to ensure it maintains its ability to be a validated cleaning method" to "There is one autoclave available and the equipment is regularly serviced, and an annual multi point thermo-couple test carried out with a suitable representative load, to ensure that it maintains its ability to provide a validated method of waste inactivation”

2. Section 6 of the CU1. In the first sentence Virkon is missing the “r”

3. Section 1.2 of the risk assessment. You state that "none of the genes would be expected to make C. reinhardtii pathogenic or increase persistence in the environment relative to parent strain". I notice that you are introducing endolysin and antimicrobial genes into the Algae. I appreciate that the host organism is not capable of establishing an infection in a human host but could you please provide a brief explanation as to why these modifications would not provide the GM algae with a selective advantage in the environment if it were to be accidentally released?

4. Section 1.1 of the risk assessment. I appreciate that you may be inserting multiple variations of Bacteriophage endolysin genes, antimicrobial peptide genes, and pathogen antigen protein genes and you do not wish to make this assessment to narrow. However in order to help set the limits on the scope of this work could you please give a few examples of each gene. I appreciate that the risk of harm is low but are there any genes in these groups that you would not introduce?
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**Name**

SAGENTIA LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

HARSTON MILL

**District**

HARSTON

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB22 7GG

**Country**

ENGLAND

**Tel Number**

01223 875220

**Fax Number**

0

**E-mail**

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**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

**Yes**

Give brief details of the genetic modification safety committee

- PhD Chemist with 6 years experience
- IOSH Biological Safety Training (attended in 2017)

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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
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Non-microbial: Yes

Other (please specify) Tick if confidential

- Bacteriology: Microbiology
- Parasitology: Research
- Transgenic Birds: Gene Therapy
- Transgenic Animals: Gene Therapy
- Transgenic Fish: Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

All contaminated substances will be either sterilised (by autoclave) or transferred as chemical waste to an approved waste disposal company for incineration

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Work undertaken will use GMO corn supplied by an external 3rd party. The resulting corn has no hazards over and above unmodified corn. Sufficient disposal methods have been put in place and will be adhered to.
### Data Premises Notified
- **Origin**: 06/10/2017

### Transferred from 1992 Regs?
- N

### Transitional Premises
- **Class**:  
- **Non-GMMs**: N
- **Withdrawn**: N

### Name
- **ARTIOS PHARMA LTD**

### Name 2
- **Department**

### Campus Estate or Research Centre
- **Building**: BABRAHAM RESEARCH CAMPUS

### Road Name
- **District**

### Town
- **Country**: ENGLAND

### Tel Number
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### Fax Number
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### E-mail
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### HSE Division
- **Comments**

### Date at Which Additional Info Submitted
- **02/03/2022**
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Committee will be made up of the CEO, CSO, Head of Discovery Biology, Biological Safety Officer and Senior Scientist.

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Tick if confidential

02/03/2022
### Waste Management Measures

Any solid microbiological and cell biological laboratory waste (e.g. bacterial agar plates, plastics, gloves) from our class 1 GM work is collected in laboratory waste bins (Eurobins) which are sealed and labelled by our staff. The sealed bins are then removed by the Babraham campus waste team to a central collection point where they will be placed into labelled 210 litre yellow Eurobins. The 210 litre bins are then collected by a nearby (off site) registered clinical/GM waste company and incinerated (see derogation application below).

Liquid class 1 GM culture waste (e.g. cultures and spent media after isolation of cells) is inactivated by treatment with Virkon. After full inactivation (1% Virkon treatment overnight), liquid waste is disposed of into the waste water supply. Associated plastics will then be disposed of as the solid waste.

Surfaces will be routinely cleaned and decontaminated with 2% Chemgene.

### Risk Assessment

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Any solid microbiological and cell biological laboratory waste (e.g. bacterial agar plates, plastics, gloves) from our class 1 GM work is collected in laboratory waste bins (Eurobins) which are sealed and labelled by our staff. The sealed bins are then removed by the Babraham campus waste team to a central collection point where they will be placed into labelled 210 litre yellow Eurobins. The 210 litre bins are then collected by a nearby (off site) registered clinical/GM waste company and incinerated (see derogation application below).

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Surfaces will be routinely cleaned and decontaminated with 2% Chemgene.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

### Project Ref

**Project Ref** 3380/18.1

**Date Ackn’d** 03/01/2018

**CU2 Project Title** Generation and use of retroviral and lentiviral vectors for gene expression, editing and

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Project Additional Information

Purposes of the contained use

To enable the generation of lentiviral and retroviral vectors and use them in cell lines for the expression of:

1) Open reading frame (ORFs) (including but not limited to oncogenes, tumor suppressor genes, Cas9 proteins), cDNA, peptides and other gene sequences
2) synthetic RNA sequences for gene expression regulation (including but limited to microRNA, short hairpin RNA, and short interfering RNA)
3) synthetic RNA for gene editing (including but not limited to synthetic guide RNAs for CRISPR/Cas9 technologies, template for gene editing)

Recipient or parental organism

The recipient organism will be of 2 types:
1) cell line used for virus production, either as a) helper cell lines (including but not limited to HEK293T and HEK293FT) into which the different plasm ids for virus production (encoding viral vector proteins and genome) will be transiently co-transfected, or b) packaging cell line stably expressing the proteins required to assemble virus particles that will be transiently transfected with plasm ids encoding the viral vector genome (including but not limited to GP2-293, Platinum, 293RTV, Phoenix cell lines). These cell lines will be used for the transient production of viral particles that will be released into the cell growth media. These viral particle suspensions could provide a risk for infection for the personnel handling them.
2) recipient cell line in culture that will be infected with the viral particles produced as described above. This includes (but are not limited to): immortalised cell lines, cancer cell lines, and other previously established cell lines and long term culture of human and animal origin. For the first few days after contact with virus, the infected cultures could provide a potential risk for infections for the personnel handling them.

Host/vector system

The retroviral vectors are derived from Xenotropic murine leukaemia virus-related virus, while the lentiviral vectors are derived from HIV-1 virus (Naldini et al., 1998). All the viral vectors that we will generate and use are devoid of the proteins required for virus replication and pathogenicity, and as so, they are replication-incompetent vectors that after
integration into the host cell genome cannot propagate nor replicate. Additional safety features of the retroviral and lentiviral vectors that we will use are:
- viral vectors are devoid of pathogenic accessory genes (vif, vpr, vpu and nef) required for viral replication and the genes from the parental virus strain contained in the viral vector is reduced to just the structural proteins: gag, pol, env (Naldini et al., 1998).
- genes encoding the structural and other components required for packaging the viral genome are separated onto 2 or more plasmids. All plasmids have been engineered to limit the regions of homology with each other so that undesirable recombination events that could lead to the generation of a replication-competent virus are minimised (Dull et al., 1998; Naldini et al., 1998).
- none of the packaging plasmids that allow expression in trans of proteins required to produce viral progeny, nor the constructs stably expressing the viral proteins in packaging cell lines, contain LTRs or the 4' packaging sequence. This means that none of the virus structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
- whenever possible, we will use viral vector constructs that are "self-inactivating": those vectors contain a deletion in the 3' LTR (I'U3) that does not affect generation of the viral genome in the producer cell line, but results in "selfinactivation" of the viral vector after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998; Naldini et al., 1998). Once integrated into the transduced target cell, the viral genome is unable to transcribe a packageable viral genome, further reducing the possibility of production of any infectious viral progeny.
Despite the above safety features, use of these viral vectors (which include WPRE sequence) falls within SACGM 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form selfreplicating virus, the possibility of insertional mutagenesis, and the expression of potentially oncogenic genes. Also, the virus will be packaged with envelope proteins (including but not limited to VSV-G) that allow the transduction of a wide variety of animal species and cell types. This means that these viruses could potentially infect a number of species, including human beings. Despite this, the viral vectors will not be able to propagate nor produce in any way an infective progeny, due to the safety measures applied as described above.

Origin & function

Selectable markers - examples (but not restricted to):
- Ampicillin resistance: E.coli derived
- Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
- Puromycin resistance (PAC): Puromycin acetyl transferase is derived from Streptomyces alboniger
- Blasticidin resistance: Streptomyces griseochromogenes-derived
- Hygromycin resistance: Streptomyces hygroscopicus-derived
- Zeocin resistance: Streptomyces verticillus derived

Reporter proteins such as (but not restricted to):
- Fluorescent proteins as reporters: GFP derived from the jellyfish Aequorea victoria and variants of this (BFP, YFP, CherryFP, CFP ...)
- Luciferase - class of oxidative enzymes used in bioluminescence; renilla luciferase derived from the Sea pansy (Renilla renifomris); firefly lu ciferase derived from the firefly Photinus pyralis.

Open reading frames, peptides, cDNAs and gene sequences from different species such as (but not restricted to):
- oncogene, tumor suppressor genes, CRISPR/Cas9 proteins for knockout, gene inactivation (CRISPRi), gene overexpression (CR ISPRa), TALEN proteins, endonuclease proteins, gene involved in DNA damage response, DNA repair, cell cycle, cell proliferation; and templates for gene editing.
- RNA sequences such as (but not restricted to): microRNA, short hairpin RNA, and short interfering RNA; synthetic guide RNAs for CRISPR/Cas9 techologies and templates for gene editing.
The viral vector that we will produce are, at worst, amphotropic or pseudo typed with VSV-G protein, either of which confers a broad host tropism including human cells. However, all the viral particles will be replication-incompetent and, when possible, self-inactivating, therefore there is no possibility of viral replication nor propagation further. Since some of the inserted DNA could code for potentially hazardous RNA or protein, the work is assessed as Class 2. This accords with HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.18-20). Even for the non oncogenic inserted DNA, there is a slight but non negligible risk due to the presence in some of the viral vectors of the Woodchuck Post-transcriptional Regulatory Element. The WPRE containing vector DNA will be treated as potentially oncogenic and is assigned to class 2 (see HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.13».

However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle:
- The genes encoding structural and other components of the viral genome have been separated. These genes and the plasmids encoding them have been engineered to minimise the risk of recombination that could lead to production of a replication-competent virus.
- The packaging cell lines allow expression of proteins, required to produce progeny virus, but the transfer vector (not encoding the structural viral proteins) is the only genetic material transferred to the target cells, consequently these recipient cells cannot produce the proteins which are essential for viral assembly and infectivity.
- The viral vector production in helper/packaging cell line will be transient, i.e. the component for the assembly of infective viral particles will be co-expressed exclusively for the few days of persistence of the transiently transfected plasmid encoding the viral genome.

Procedures and controls measures will therefore follow HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.30-36) i.e. using multiple plasmids with minimum sequence homology (e.g. our 2nd or 3rd generation lentiviral or retroviral vector systems), gloves will be worn, use of class II safety cabinets, sharps avoided and all wastes be rendered harmless before disposal etc.

It is not thought that the modified virus would pose a serious risk to animals or plants in the environment. Although the VSV coat protein permits transduction of other mammalian cells, as in the case of humans, infection would be restricted to primary cells and productive virus would not be produced. In addition the control measures to protect human health will minimise release of virus to the environment. Therefore the environmental risk is low.

The viral vectors will be used to generate a cell population stably expressing the gene or RNA sequence(s) of interest. For the first week after infection, or until at least both 3 replications and 3 media changes have occurred, the cell population infected with the viral particle will be treated as potentially infective. After this period, these cell cultures and the derivative products will be considered as Class I since the viral particles will be cleared from the culture.

Evaluation of foreseeable effects

N/A

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM1 05/4.1 (see also section 12 below). Reasons for adopting this method of waste disposal are:
1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.
All waste material will be inactivated by treatment with 5% (w/v) Chemgene solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization is GM898) according to disposal notification GM1 05/4.1. Waste from our GM work at Class2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM1 05/4.1. Reasons for adopting this method of waste disposal are:

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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

### Project Containment

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**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

Stevenage Bioscience Catalyst (SBC) has a 10-member GM committee which includes representative tenants from each company working in the SBC. The chair of the committee has 25 years’ experience working with GMOs and has previously served as GM Officer in the Dept of Life Sciences at Imperial College. The GM committee has a wide range of experiences in GMO R&D.

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</table>
### Bacteriology
- Yes

### Parasitology

### Transgenic
- Birds
- Animals
- Fish
- Invertebrates
- Plants

### Microbiology
- Research

### Virology
- Yes

### Transgenic
- Animals

### Gene Therapy
- Yes

### Mycology
- Transgenic
- Invertebrates

### Other (please specify below)
- Microbial culture to be used to generate plasmid starting materials

---

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

- Virkon is used as it is a validated cleaning method for viral vector material, transfected or transduced cells if used in the following way under manufacturers recommendations
  - Hard surfaces: A solution containing 1 % Virkon or Distel spray for 1 hour contact time, 10mins for metal parts (longer can cause corrosion)
  - Safety cabinets: 1 % Virkon (10mins) or Distel spray
  - Discard jars, plastic tissue culture flasks, glassware: A solution containing 1 % Virkon.
  - Ensure all surfaces are in contact with the disinfectant (1 Omins). Added to sealed bags for autoclaving at a minimum of 1210C, 1.15bar, 15 mins hold.
  - Supernatants/liquid waste: 3% Virkon diluted 2: 1 in culture medium
  - All autoclaved solid waste is to be placed into hazardous waste bags. There are 2x autoclaves available and the equipment is regularly serviced to ensure it maintains its ability to be a validated cleaning method. Chart recorder or display screen is attached to autoclave to monitor treatment.
  - Full bags are to be transported to Gyroscope's hazardous waste bins in the SBC storage area
  - Bins are collected for offsite incineration by Grundons waste management

---

**Please enter comments of the GM safety committee on the risk assessment**

The chair and the SBC GMO committee have advise Gyroscope Therapeutics limited on evaluating GMO handling and waste disposal risks and are satisfied that they are acting appropriately, responsibly and with the best practice of the SBC. The R&D does not contain anything exceptional that requires special consideration. The SBC GM Committee have approved the associated risk assessment and support this notification.

---

**Premises**

---

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

---
**Project Additional Information**

**Purposes of the contained use**

Research and Development of viral vectors as a cellular therapy relies on animal and in vitro cellular work to inform on the distribution and characterisation of viral vectors containing proprietary transgenes. The specialist nature of this administration means that Gyroscope utilises CROs and academics external to our premises at Stevenage, UK to generate AAV transduced cells or tissues from animals or primary human cells. To enable importation of tissues and biological fluids across International Borders our premises requires a CU2 designation to receive these tissues to enable research into the distribution and characterisation of the viral vectors containing our proprietary transgenes.

**Recipient or parental organism**

Humans and animals (rodent, dog, porcine, non human primate and lagomorpha). All animals used for Research Purposes at CRO's and academics will be obtained from reputable sources and designated as specific pathogen free.

HEK293, HEK293T and ARPE19 cells.

Human tissue samples will be generated as part of the human safety and efficacy studies conducted to support the development of this gene therapy.

**Host/vector system**

We utilise a proprietary replication incompetent virus: Adeno Associated Virus (AAV) serotype 2 for expression of proprietary transgenes to restore functionality of proteins deficient in ocular diseases.

**Origin & function**
All of the vectors used in this project are being developed for human therapies and thus have been significantly engineered to alter parts of the genetic code to create proprietary vectors that have a better safety profile than their wild type counterparts.

The key alteration is that all vectors have been altered to be replication incompetent. This means that they pose a minimal concern to the environment should they be released as they cannot replicate in any cell or organisms they are able to infect. The vectors used also pose minimal to no chance to infecting plant or livestock as they are developed/chosen to transfect human cells.

**Evaluation of foreseeable effects**

**AAV** is not currently known to cause disease. These vectors have the ability to infect humans via a number of methods e.g. direct contact with skin, mucous membrane of the eye, nose and mouth, ingestion, or injection. Infection could lead to the expression of the transgene and cause a very mild immune response.

Gene therapy vectors using AAV can infect both dividing and quiescent cells and persist in an extrachromosomal state without integrating into the genome of the host cell, although in the native virus some integration of virally carried genes into the host genome (albeit at a specific chromosomal locus) does occur in wtAAV2. The proprietary version used in this project has been altered to be replication incompetent and contain a benign reporter transgene or native human genes. In the SACGM Compendium of guidance Part 2: Risk assessment of genetically modified microorganisms (other than those associated with plants) wild-type (replication competent) AAVs are not categorised by ACDP and therefore, Containment Level 1 will be sufficient and should be adopted as a minimum requirement when handling wild-type virus.

If infection were to occur, by their nature and design the vectors are replication incompetent and so will be unable to propagate throughout an individual. There is the possibility that if an organism is also infected with a replication competent wild type vector that the transgene vector will also gain this ability. However control measures implemented as outlined below make this a highly unlikely event.

**HEK293 and HEK293T** were generated in 1973 by transformation of cultures of normal embryonic kidney cells with sheared adenovirus 5 DNA. This cell line is used to propagate the viral vectors which have been engineered to be replication incompetent. HEK cell lines pose minimal potential damage to the environment as they have been adapted to survive in an artificial cell culture environment under laboratory conditions. They are unlikely to thrive in the external environment if released.

**Control measures to be adopted:**

- Records and tracking of materials used are kept as part of Gyroscope’s good lab practice which involves both hard copy and electronic copies of lab books. All materials are clearly labelled and stored in specific safe storage areas within the facilities.
- PPE: lab coats, gloves and goggles to be worn at all times. This is to reduce the possibility of any physical contact with the vector.
- To avoid exposure to aerosol/droplets where feasible viral manipulations should be conducted in biosafety Level 2 (BSL2) cabinets with HEPA filters, conforming to a Containment Level 2 (CL2) to prevent release and possible inhalation.
- No sharps will be used – removes the possibility of introduction into the blood stream
- Centrifugation must be conducted in closed containers and using sealed rotors. Rotors must be opened in a Biosafety cabinet.
- Once the vector has been disrupted during preparation for nucleic acid or protein analysis it is safe to be used outside of the cabinet (PPE as outlined above should still be used).
- All wastes treated to destroy GMO material (see disposal section below)
- Volumes of dilute virus material to be kept under <20L of 1E13 virus particles to make waste handling and disposal easier and to reduce the severity of any adverse events
- Volumes of concentrated virus material to be kept under 20mL of 1E15 virus particles. This allows easy handling in the biosafety hood.
- HEK 293, HEK 293T, ARPE19 cell lines (volumes less than 20L). Once cells have been transduced or transfected they should only be handled in closed containers or in the CL2 cabinet
- All vector material when stored in fridge/freezer will be clearly labelled as to its contents.
- All experiments take place in the Stevenage Bioscience Catalyst which is on a secure site as part of the Glaxo SmithKline Campus. All areas where the virus is stored are only accessible by Gyroscope employees (via keycard or lock and key). Visitors are supervised at all times in the facility.
- Training for use of the vector material will be conducted by users who have prior experience of handling the vector material. The completion of training will involve the adding of the individuals name to the appropriate risk assessment. The signature will serve as a record of the training and be kept on file as part of the Gyroscope training
Where vector material is transported this will only be done in sealed containers which are labelled and placed inside sealed plastic packaging. These in turn will be put into larger boxes, sealed and the box labelled with Gyroscope name, sender’s name, address and contact details with the instruction not to open unless authorised.

The Gyroscope Health and Safety Policy covering lab practice and use of COSHH is to be followed at all times.

First Aid:
EYE EXPOSURE FROM SPLASH OR AEROSOLS: Rinse a minimum of 15 minutes in eye wash available at all sink hand wash areas or flush area with water. Seek medical attention.

CONTACT WITH SKIN: wash thoroughly with disinfectant. Hand sinks available in all labs for washing.

INHALATION EXPOSURE FROM AEROSOLS: Seek medical attention.

NEEDLESTICK AND/OR SHARPS EXPOSURE: No sharps are to be used

For personnel working with human Biological samples full health screening with Immunisation against Hepatitis B is made available by Gyroscope.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Rationale of Classification: After a review of the information sources and discussions with internal experts and SBC GMO committee.

CLASS 1
Vector: the rationale that vectors are replication incompetent and express what were considered to be biologically inert transgenes suggest a low risk for this biological material. Cell Lines: HEK293, HEK293T and ARPE cells are engineered to be suited to laboratory based cell culture conditions and are highly unlikely to survive in the external environment. E.Coli strain Stabl3 (Thermofisher) is a K-12 derivative used to propagate the plasmid and are non-pathogenic.

CLASS 2
The import and utilisation of animal tissues transduced with or without AAV vector determines that the laboratories where the characterisation of viral vectors and the analysis of transgene identity and activity/potency within animal and cellular tissues should be designated as Class 2 containment level.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Chemgene is used as it is a validated cleaning method for viral vector material and transfected cells if used in the following way under manufacturers recommendations

- Hard surfaces: A solution containing 1:20 and 1:40 Chemgene for 2 minutes contact time will precipitate the nucleic acids immediately.
- Safety cabinets: Wiping down of the safety cabinet with a solution containing 1:20 and 1:40 Chemgene for 2 minutes contact time will be used as this is known to precipitate nucleic acids immediately.
- Discard jars, plastic tissue culture flasks, glassware: A solution containing 1:20 and 1:40 Chemgene to a final concentration of 1:20 will be added to the glassware for 5 minutes
- Supernatants/liquid waste: Chemgene in culture medium to a final solution of 1:20.

All autoclavable solid waste is to be placed into hazardous waste bags. Sealed bags for autoclaving will be autoclaved at a minimum of 1210C, 1.15bar, 15 mins hold. A Chart recorder or display screen is attached to autoclave to monitor treatment. To verify the autoclave hold time/temp/pressure is achieved on every run an indicator will be used on each run.

There are 2x autoclaves available and the equipment is regularly serviced to ensure it maintains its ability to be a validated cleaning method.
Full bags are to be transported to Gyroscope's hazardous waste bins designated for CL2 waste in the SBC storage area. Bins are collected for off site incineration by Grundons waste management.

Accidental Release:
In a contained area allow aerosols to settle; wearing protective clothing, gently cover spill with paper towel and apply 10,000 ppm sodium hypochlorite or Chemgene solution, starting at perimeter and working towards the centre; allow sufficient contact time before clean-up (30 min). Alternatively, Chemgene can be applied all over the spill area and mopped up with absorbent non-hazardous material. This solid waste is then to be autoclaved as above.
In an uncontained area, all persons should evacuate the area to avoid contact with any potential aerosols formed. Only after 1 hour the room might be entered (to allow any aerosols to settle) and the procedures should be the same as in a contained area.
The floor is sealed and the lab is built to CL2 level.

Is an emergency plan required according to regulation 20? [N]
If yes, tick to confirm that it is attached to this form [N]
Tick to confirm that you have attached a risk assessment to this form [Y]
Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
The Stevenage Bioscience Catalyst (SBC) is an enterprise hub for small and emerging Biotechnology start-up and spin-out companies. It is a UK Government backed (Innovate UK) in collaboration with the Wellcome Trust and GSK. The SBC provides technical, business, organisational and commercial support to its tenant companies and also monitors Health and Safety Compliance. It has set up a GMO committee to ensure that tenants are assessing the risks associated with their work appropriately and to disseminate best practice amongst the researcher tenants. Dr Deonarain is the chairman of this committee and has 25 years experience working with GMO's in both academic and commercial settings and was chairman of Imperial College Life Sciences GMO committ for 4 years.
The SBC committee has reviewed and advised Gyroscope Therapeutics Limited on their GMO risk Assessment associated with Class 2 contained use activities involving animal tissues and are satisfied that we have in place appropriate control measures to contain the work and the appropriate procedures for GMO waste disposal and are acting appropriately, responsibly and with the best practice of the SBC.

Project Containment

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<tr>
<th>Laboratory Activities</th>
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<th>Growth Rooms</th>
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**Name**

AGLARIS LTD

**Campus Estate or Research Centre**

F30 INCUBATOR

**Building**

STEVENAGE BIOSCIENCE CATALYST

**Road Name**

GUNNELS WOOD ROAD

**District**

**Town**

STEVENAGE

**County**

HERTFORDSHIRE

**Postcode**

SG1 2FX

**Country**

ENGLAND

**Tel Number**

01438 906785

**Fax Number**

0

**E-mail**

blank

**HSE Division**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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<td>SG1 2FX</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Stevenage Bioscience Catalyst (SBC) is an incubator hub for small and emerging biotechnology start-up and spin-out companies. It is a UK Government backed (Innovate UK) enterprise in collaboration with the Wellcome Trust and GSK. The SBC provides technical, business, organisational and commercial support to its tenant companies and also monitors Health and Safety compliance. It has set up a GMO committee to ensure that tenants are assessing the risks associated with their work appropriately, implementing the correct procedures for containment, safety and waste disposal and to disseminate best practice among researchers. The SBC GMO committee's chairman has advised us on our GMO risk assessments and the entire committee has reviewed and approved the risk assessment.

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<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>
Cell therapy using genetically modified Mesenchymal stem cells.

For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A

Dr M D is the chairman of a 10-member SBC GM committee. Dr D has 25 years' experience working with GMOs and has previously served as GM Officer in the Dept of Life Sciences at Imperial College. The GM committee has a wide range of experiences in GMO R&D.

Dr D and the committee have advised Algaris Ltd on evaluating GMO handling and waste disposal risks and are satisfied that they are acting appropriately, responsibly and with the best practice of the SBC. The R&D does not contain anything non-standard or exceptional that requires special consideration. The SBC GM Committee have approved the associated risk assessment and support this notification.

Use of genetically modified Mesenchymal stem cells: hTMSC19 cells

Consent Granted

Project notified under transitional arrangements
The immortalized mesenchymal stem cells (hTMSC19 cells) which have been genetically modified are being used in the development of a bioreactor for adherent cell culture activities. Please note that these cells are only being used for cell culture purposes no further genetic modification or testing in humans or animals will be conducted.

Parental organism: Human
Cell type: Mesenchymal stem cells from a 18 year old male donor.

Host/vector system
Non-replicative, self-inactivating (third-generation) lentiviral vector.

Origin & function
Origins of cells: The donor cells were purchased from a stem cell bank in Spain and the genetic modification was conducted in a Spanish research institute.
Functions of the genetic material: The lentiviral vector contains HIV-1 non-coding cis-acting sequences which are non-functional outside of the vector producer cells. The integration of the vector in the genome results in the transcriptional inactivation of both LTRs (lentiviral promoters). It also contains an expression cassette under the control of the human cytomegalovirus (CMV) immediate-early enhancer/promoter region, a viral sequence commonly used to promote expression of cloned inserts. The codifying region is human telomerase reverse transcriptase (hTERT). This protein is responsible for catalyzing the addition of nucleotides in a TTAGGG sequence to the ends of a chromosome’s telomeres. The addition of these repetitive DNA sequences prevents degradation of the chromosomal ends following multiple rounds of replication. The constitutive expression of hTERT allows human cells, that would otherwise become postmitotic and undergo apoptosis over several passages in culture, to exceed the Hayflick limit and become potentially immortal without altering their phenotype or becoming tumorigenic in animal experimental models.

Evaluation of foreseeable effects
The GMO is immortal therefore allowing the cells to proliferate limetelessly. Although originating from class 2 pathogens, these viral sequences do not burden host cells with a phenotype that could cause illness to humans or could infier damage to the environment. The donor was tested negative for blood-borne human viruses including HIV-1, HIV-2, HBV and HCV and the cells have been tested and confirmed to be free from mycoplasma.
The cells will be primarily used to test different in vitro culture conditions, and will not be used in any in vivo studies, either in humans or animal models. No further genetic modification of the cells will be performed. Cell cultures will be handled inside a biosafety class II cabinet. Additionally, cells (in multi-well plates or tubes at volumes of ≤ 100mL) could be used to perform characterization studies, such as staining, cytometry (phenotyping) or PCR analysis. In these cases, cells (transported in double-contained containers) will be worked with inside a fume cabinet and immediately fixed with 4% formaldehyde or with protein-denaturizing agents which inactivate the relevant and known adventitious contaminating pathogens. These cells have been tested for the most common viruses thus the risk of viral contamination to the operator is very low. Any work to be done on the bench using open live cultures should be kept to the absolute minimum (≤ 50mL). Operators must wear all appropriate PPE (laboratory coat, lab gloves and eye protection), receive appropriate training, no work should be done using sharps or needles, any cuts or grazes must be covered with appropriate protection prior to work, cultures should be transported in sealed containers and cell suspensions should never be poured (aspirators should be used).
should be conducted in a manner which prevents the formation of aerosols, any spillages should be decontaminated immediately using virkon, and hands should be washed after any laboratory work is conducted.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

As stated in the risk assessment for the use of hTMSC19 the majority of work will be carried out in class II biosafety cabinets. However, it is requested that some work is conducted under biosafety containment level I. The work to be conducted at biosafety containment level I will be kept to the minimum and would include tasks such as live cell counting and staining for flow cytometry which require cells to be live so they cannot be deactivated prior to analysis. The cells have been tested negative for HIV-1, HIV-2, HBV, HCV and were modified using a non-replicative lentiviral vector which will not cause any harm to humans or the environment for this reason the risk of viral contamination of the operator is deemed to be low. However, measures will still be put in place to protect the operator when work is conducted outside of biosafety containment level II. These measures are detailed in full in the risk assessment under section 3E.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- **Liquid waste:** All biological liquid waste will be treated with a tablet of virkon for every 500mL (1% virkon solution) of liquid waste for a minimum of 10 minutes this has been confirmed by the manufacture of virkon to kill by oxidizing key structures and compounds, such as proteins in bacteria cell walls and viral structures leading to widespread irreversible damage and subsequent deactivation/destruction of the microorganism. Once liquid waste has been killed it will be discarded with an excess of water into the drain.
- **Solid dry waste (including contaminated cultureware):** All contaminated dry waste will be disposed in double bagged sealed biohazard bags and autoclaved (at 121°C, 1.15 bar with a 15 minute hold) and then disposed of in solid bins provided by the Stevenage Bioscience Catalyst and collected by Grundon management for off site incineration. Pipette tips and stripettes are disposed in biobins and when full sealed and placed in the same biohazard bags for autoclaving. The autoclave is regularly serviced which ensures that it consistently performs validated cleaning and there is a display screen confirming if a autoclave run has been successful with the additional use of autoclave tape which changes colour to confirm the correct temperature was reached during an autoclave cycle. Contaminated sharps/glassware: No work with needles will be done with these cells however all broken contaminated laboratory glassware is disposed of in 5L sharp containers. Once full the sharps bins are closed permanently and labelled and then placed in large yellow bins which are collected by Grundon waste management for incineration.
- **Contaminated surfaces and safety cabinets:** Surfaces are cleaned with a 1% virkon solution for a minimum of 10 minutes (no longer if cleaning metal sufraces as virkon is corrosive) and any materials (tissues/wipes) used to clean the area are autoclaved as described above.
- **Spillages:** In the event of a spillage a 1% solution of virkon is prepared and sprayed on the spillage to ensure full coverage and left for a contact time of 10 minutes. The material along with any materials used to clean the area are then disposed in biohazard bags and autoclaved as described above.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment
Dr Mahendra Deonarain is the chairman of a 10-member SBC GM committee. Dr Deonarain has 25 years’ experience working with GMOs and has previously served as GM Officer in the Dept of Life Sciences at Imperial College. The GM committee has a wide range of experiences in GMO R&D.

Dr D and the committee have advised Algaris Ltd on evaluating GMO handling and waste disposal risks and are satisfied that they are acting appropriately, responsibly and with the best practice of the SBC. The R&D does not contain anything non-standard or exceptional that requires special consideration. The SBC GM Committee have approved the associated risk assessment and support this notification.

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Animal Units

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**Name**

HARRISON BIO

**Name 2**

**Department**

**Campus Estate or Research Centre**

THE DIAGNOX LABORATORY

**Building**

CHERWELL INNOVATION CENTRE

**Road Name**

**District**

**Town**

UPPER HEYFORD

**County**

OXFORDSHIRE

**Postcode**

OX25 5HD

**Country**

ENGLAND

**Tel Number** 07920 422796

**Fax Number**

0

**E-mail**

**HSE Division** blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The main project holder (Principal Scientist) has 20 years experience of working with GMMs and has previously successfully applied to perform Cat 1 and Cat 2 GMO work, HSE has completed detailed risk assessment for project included GMOs for many years. A new risk assessment will be performed whenever a new project is started and current risk assessments will be reviewed at least once a year.

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
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All waste material containing GMMs or exposed to GMMs will be treated according to standard operating procedure for biohazardous waste. It will be decontaminated by treatment with a suitable disinfectant prior to disposal by a suitable route depending on the nature of the liquid. If these methods are deemed not to be suitable for particular items, they will be made safe by autoclaving in the on site autoclave prior to disposal by incineration. The estimated degree of kill by these combined methods is extremely close to 100%.

Virology

Transgenic Animals

Transgenic Fish

Mycology

Transgenic Invertebrates

Transgenic Plants

Gene Therapy

Other (please specify below)

Other(s)

Mammalian cell lines will be transfected with gene vectors for research scale production

For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste material containing GMMs or exposed to GMMs will be treated according to standard operating procedure for biohazardous waste. It will be decontaminated by treatment with a suitable disinfectant prior to disposal by a suitable route depending on the nature of the liquid. If these methods are deemed not to be suitable for particular items, they will be made safe by autoclaving in the on site autoclave prior to disposal by incineration. The estimated degree of kill by these combined methods is extremely close to 100%.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

none. The risk assessment was approved by all members of the committee.
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**Name**

BARBICAN LOGISTICS LTD

**Campus Estate or Research Centre**

PO BOX 425

**Road Name**

**District**

**Town**

**County**

**Postcode**

**Country**

0800 612 6701

**Fax Number**

N/A

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Bio Safety Officer, 1- Transport Manager, 1-DGSA, 1- ADR, 1- H&S Officer

<table>
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<tr>
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<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</table>
**Containment measures, addressing the relevant areas for Class 1 activity, in Schedule 8 Table 2 are as follows**;

Serial 1 - The GM waste will be held in containers. These containers will be sealed and security tagged, no work processes will take place involving this waste, it will remain sealed in the containers up to, and including, incineration.
Serial 4 - No sample collection will take place.
Serial 5 - No bulk culture fluids will be worked with; all other waste will be contained in sealed containers prior to collection from the waste producer, during transportation and during the incineration process.
Serial 7 - The controlled area has been designed to contain spillage of the entire contents through the use of sealed containers and absorption material.
Serial 11 - All waste will be contained in sealed containers prior to collection from the waste producer. No benches or surfaces will be exposed to the waste during transportation or storage.
Serial 12 - All waste will be contained in sealed containers prior to collection and will not be removed from these containers throughout these Contained Use processes.
Serial 17 - Work clothing will be worn at all times; this will include gloves when manual handling of the sealed containers takes place.
Serial 20 - The waste will be collected and transported direct to the incineration. A validation certificate for destruction will be signed by the disposal plant and copies provided to the waste producer and the transport company.

The Class 1 activity waste will be destroyed by High Temperature Incineration of +1000°C; 100% Kill.

CU 2 2015 (rev 11/15) Page 3 of 9

For GMMs only - application for any derogation from full containment (Schedule 8) (Measures and justification) (note 7)

We request a derogation from Schedule 8 for the following:
1. We will not carry out Class 1 activity GM work in Laboratories; Schedule 8, Part 2, page 68, Table 1 a.
2. Containment measures will not involve micro-organisms in plant growth facilities; Schedule 8, Part 2, page 71, Table 1b.
3. Containment measures will not involve micro-organisms in animal units; Schedule 8, Part 2, Page 72, Table 1 c.
Schedule 8, Part 2, Table 2 will be addressed above

**Tick to confirm that you are attaching a summary of the risk assessment**

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

Please enter comments of the GM safety committee on the risk assessment
The safety committee have concluded that the risk assessments attached were deemed sufficient for collecting Class 1 activity waste, from a GM registered waste producer, transporting and, if necessary, storing Class 1 activity waste, prior to incineration at the GM registered waste disposal site. At all times the Class 1 activity waste will be contained in the requisite UN Approved packaging for UN3291, following Packaging Regulations P621, strictly following the regulations for the Carriage of Dangerous Goods by Road. Class 1 activity waste will be held in sealed containers from collection, throughout transportation (and if necessary, storage), up to and including incineration. As stated on the attached risk assessment, in the event of an emergency, all surfaces exposed, or potentially exposed, to the Class 1 activity will be disinfected using a broad spectrum clinical disinfectant (e.g. Virkon 2%), using absorbent material. This will then be disposed of in a clinical bin and sealed.
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| Tel Number                        | 020 7554 4070       |
| Fax Number                         | 0                   |
| E-mail                             |                      |

| HSE Division                      | blank               |

Comments

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Yes

Give brief details of the genetic modification safety committee

- UCL Genetic Modification Safety Committee

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- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research
Solid contaminated GMM waste of tissue culture plastics, tips and pipettes are placed in yellow clinical waste bags and placed in yellow bins provided by UCL estates. The bags are then taken off site and disposed of by an approved GM waste management company. Liquid waste is disinfected using 2% Virkon and treated overnight before disposed of down the designated laboratory sink. Virkon is a multi-component peroxyge-based oxidising agent. It is effective against bacteria, fungi, and viruses. Virkon is widely used for sterilization in laboratory or hospital settings. The UCL medical school laboratory disinfection code of practice suggests Virkon (1%) as suitable for use in laboratories performing low risk microbiological or Class 1 genetic modification work.

Bacterial-vased plasmid amplification, generating viral vectors for transduction of mammalian cells

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

UCL GMSC approved risk assessment with no additional comments.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

We have been in correspondence with a GMO Notification Officer from the Microbiology & Biotechnology Unit of the HSE.

The University of Suffolk GMM Safety Committee will meet as a sub-committee of the thrice yearly Health and Safety Committee. The UoS HSC consists of one member of each department at UoS and GMM sub-committee will include N B (Trained Biological Safety Supervisor) and C M Laboratory Health and Safety Officer as well as T S and K W the head of H&S Department

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02/03/2022
All liquid waste from bacterial and vertebrate cell cultures are treated with 1% Virkon for 12hrs prior to discarding.
All solid waste is treated with 1% Virkon for 12hrs and then autoclaved (121°C for 30 minutes) within the department before incineration off campus.
To assess bacterial and vertebrate culture inactivation and subsample (1:100 v/v) of the Virkon treated culture will be incubated in fresh media and left to grow for 24hrs.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Dr B has been a Biological Safety Supervisor for the Franklin Wilkins Building and Nutritional Sciences Department from 2005 - 2017 and sat on both the Biomedical and Medicine Biological Safety committee at King's College London ~ prior to his move to University of Suffolk in January 2017. C M. 10 years of experience in biological laboratory work and risk assessments writing, as well laboratory management and H&S advisor.
Tick if notifying a connected programme of work  N

**Project Additional Information**

**Purposes of the contained use**
In relation to the project areas mentioned in section 5
To protect the operator from transfer of genetic material from the cells/tissues utilised and to prevent the transfer of such material to other organisms.

**Recipient or parental organism**
In relation to the project areas mentioned in section 5
1-The iPSCs are derived from the UK Stem cell bank and ECACC that hold all due licences for dealing with GMOs. The cells were obtained via reprogramming with episomal vectors and/or Sendai virus which are deemed as 'non integrative'. The cells are distributed as free from any residual reprogramming system, as part of the quality control operated by the distributors. As a further precaution, all liquid waste contacting these cell cultures is treated with 1% Virkon for 12 h prior to discarding. All solid waste is treated with 1% virkon for 12 h and then autoclaved (121°C for 30 minutes) within the department before incineration off campus. The ESCs are not classified as GMOs.
2-The c20A4/Tc82 cells are derived from ECACC that holds all due licences for dealing with GMOs. Such cells are produced at the origin by transfecting primary cultures of rib chondrocytes from a 5-year-old male with vectors.

**Host/vector system**
In relation to the project areas mentioned in section 5:
1-nothing to declare
2-noting to declare
3-nothing to declare
4- Sendai virus-based reprogramming kits commercially available will be used and other viral-based vectors. Individual risk assessments are attached detailing the particulars of each vector system. Foreseeable effect are discussed in the next sections.

**Origin & function**
Sendai infects cells by attaching itself to cell surface receptor sialic acid, present on the surface of many cell types of different species, the vectors are able to transduce a wide range of cells. However, they are no longer capable of producing infectious particles from infected cells, because the viral genome lacks the F-gene. In addition, the presence of functional mutations such as temperature sensitivity in the amino acid sequence of several SeV proteins (SeV/TSΔF, SeV/TS12ΔF, and SeV/TS15ΔF) renders the vectors easily removable from transduced cells. To minimise any risk, manipulation of Sendai and any material coming in touch with Sendai will be treated under category 2 conditions. The sterile nature of the category 2 laboratory areas, together with the use of dedicated abcoats, hirnets, clogs and srupulous routines of disinfection and cleansing protocols will ensure that any risk of
Transfer to the environment or personnel is absolutely minimised. The same applies to other viral vectors that may be used as reporter systems in the generated iPSCs, to include pBABEneo, pPUROneo or pCIGNAL Lenti-TRE reporters that will be used under a biosafety hood, with operator protected by labcoat, gloves and relevant PPE, together with the use of filtered tips and filtered serological pipettes. Materials and cell waste coming in contact with such vectors will be neutralised as discussed above.

Evaluation of foreseeable effects

Cells derived from human tissues may contain blood borne pathogens such as Hepatitis B & C, HIV, HTLV and Cytomegalovirus. Primary human cells have the greatest risk of contamination, compared to the established MG-63, SW-882, C20A4/TC82 human cell lines. hESCs and iPSCs are grown in media antibiotic-free, and have an even greater chance of getting contaminated with bacteria and fungi.

Primary bovine cells may contain pathogens (Prions) but pose a low risk.

Retroviral expression of pBABE-neo containing the SV40 Large T Antigen has the potential to be oncogenic.

SeV is transmitted by aerosol and contact with respiratory secretions. The virus is highly contagious, but the infection does not persist in immunocompetent animals, moreover this virus is not pathogenic for humans. So far known hosts are rats, mouse, guinea pigs, and hamsters.

Given the condition of work, the level of operator protection and training, and the precautions associated with category 2 work procedures, including rigorous disinfection and cleaning and GLP adherence we foresee a minimum risk for operators, animals and environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste and cell waste (from both eukaryotic and prokaryotic cell models discussed above) is treated in a 10% solution of Vikron. This is followed by autoclaving at 121 Degrees Celsius for 20 minutes. Finally, the waste is placed in biohazard bags and collected by an external company for incineration off-campus. According to the procedure explained, all GMOs will be destroyed safely on site.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The University GMM safety committee has revised the risk assessment and no comments were added.

Project Containment
<table>
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Animal Units

Large Scale Activities

Human Clinical Applications
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Name

MEIRAGTX UK II LTD

Name 2

Department

Campus Estate or Research Centre

Road Name

92 BRITTANIA WALK

District

OLD STREET

Town

LONDON

County

GREATER LONDON

Postcode

N1 7NQ

Country

ENGLAND

Tel Number

07561064561

Fax Number

0

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>GMP Facility &amp; supporting labs</td>
<td></td>
<td>92 Britannia Walk</td>
<td>OLD STREET</td>
<td>London</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

GMOs which may harbour various genes of interest and molecular cloning derivities used as transport vectors are already in use by our contract process development and manufacturing client. As such, a competent member of the client team (Senior Development Lead) has provided relevant safety information for use in our own risk assessment, and has reviewed the completed risk assessment and visited the lab itself. Oftentimes these transport vectors are used as raw materials in larger scale manufacture of recombinant viral vectors.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

One autoclaves are available for relevant waste. Solid/contaminated waste will be disposed into biohazard bins/boxes, sealed and placed into lockable clinical waste containers and disposed of by GMO registered waste management company (Example: Labwaste).

Decontamination of surfaces will be conducted using elevated levels of the disinfectant ChemGene HLD4L and 70%(v/v) Klercide spray. Inactivation of liquids will be performed using Virkon tablets as per manufacturer instructions.

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving, H2O2 gas (VHP) or chemical treatment using an anti-viral agent (Virkon) or Presept chlorine tablets to achieve a 100% kill, prior to disposal of waste or cleaning and recycling of reusable laboratory/manufacturing equipment, such as glassware. Inactivated liquid waste is disposed of down the sink with multiple volumes of water and pumped to waste handling room. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

MeiraGTx - the company are actively working in the gene therapy field, with the Assessment of AAV viral production isolated and harvested from transfected HEK293 cells with viral vector carrying gene replacement therapy related genes. This work is a part of a process development roadmap of work helping us to take AAV products through the clinic to commercial manufacture. It builds upon existing work contracted out by our CDMO partner at UCLB to a UK academic centre with a Cell and Gene Therapy GMP production capacity, within which Risk Assessments for the plasmid and host cell have already been completed and which are routinely upgraded.

Nativly AAV are a low risk organism, no reported pathologies have ever risen from AAV infection or mode of action. By performing an RA for AAV and HEK293 cells (the 2 biologicals at site), we postulate that we will be compliant within HSE expectations and guidelines.
**Project Ref** 3392/18.1

<table>
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**Date Project Ceased**

**Historical Significant Changes**

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

The purpose of the controlled use is to reduce risk of GMM/GMO misuse and allows Biotech companies comply with their legal duties in relation to working with GMOs in contained facilities. This application and risk assessment register shall serve to describe and set out the containment measures and other controls that need to be considered and explains the role of the competent authority.

All identified risk class 2 category organisms are;

- Host cell of choice (HEK293),
- BioBall™ from Biomerieux
- rAAV 2/5/8 Reference standard material (RSM)
- Adenovirus 5 (Ad5) material
- HeLa[RC32] cells (for infectious assay)

MeiraGTx may use reference material from previously made recombinant Adeno-Associated Virus (rAAV) serotype 2/5/8 batches in order to quantify and validate upcoming in-house batches. MeiraGTx also plan to utilise BioBall™ for on-going environmental monitoring purposes, the BioBall contains a predetermined number of microorganisms in a water-soluble ball delivering unprecedented accuracy for Quantitative Microbiological Quality Control in 4 usable format ranges. [20 cfu -10e8 cfu].

Adenovirus 5 standard material (consists of purified Adenovirus, Type 5 (wild type adenovirus, see ATCC VR-5) formulated as a sterile liquid in 20 mM TRIS, 25 mM NaCl, 2.5% glycerol, pH 8.0) which will/may be utilised in order to correctly titre viral vector batches and used as a positive control infectious spike during our infectious titre QC assays. The volume and concentration is relatively low at low working volumes [100-1000ul] – and is contained within a Laminar flow cabinet and segregated.
from the manufacturing core.

MeiraGTx will recombinant Adeno-Associated Virus (rAAV) at present using the host cell type Human Embryonic Kidney (HEK293) cells to propagate the rAAV, these hosts do not enter the Downstream processing (DSP) unit operations. These cells have been widely used without any reported safety or health issues globally for years.

MeiraGTx may intend to one day manufacture Retro/Lentivirus as our GMM viral vector of choice, due to its higher packaging capacity for larger genetic fragments (i.e: Genes that are too large to package into AAV – 4.7kb), this allows us to cater for more patients whom have genetic defects resultant from larger wild type genes.

Furthermore, MeiraGTx aim to utilise a secondary cell line HeLa[RC32] cells. These cells contain human papilloma virus 18 (HPV-18) and are a derivative of HeLa parental cells. These cells generate rAAV preparations with high titres of infectious particles which are essentially free of adenoviruses for biological, preclinical, clinical or pharmaceutical applications. Being devoid of adenovirus, they are susceptible to infection by MeiraGTx Ad5 material and serve as a suitable control cell line for the infectious QC assay.

These biological materials can be classified as risk group 2 or BSL2 (low risk), and they can be safely contained within the MeiraGTx facility in London and the supporting laboratories based on the Risk assessment and cross referencing the control measures stipulated by the GMO Regulations and SACGM guidance appendices.

Recipient or parental organism

There exists a clear demarcation of wild type AAV (wtAAV) and recombinant (rAAV). By utilising rAAV we immediately reduce/attenuate the native virus by transitioning its genome (essentially 2 genes REP and CAP) to a separate plasmid which can be delivered in trans and replacing this genome with a transgenic gene of interest (GOI). This greatly reduces the risk of selfreplication (REP) of the transgene plasmid.

rAAV has long been established as a non-pathogenic cat 1 organism which has no know reports of disease causing effects. It is used as a docile vehicle for administering a therapeutic construct to a target tissue (natural ability of AAV to target certain tissue types).

The resulting rAAV approach does further require the presence of ‘helper’ genes provided from an adenovirus. These genes are well characterised and established regions which confer full packaging and permit rAAV assembly/maturity in the host cells.

Furthermore, as we also use native Adenovirus (Ad5) – it was highlighted by the MeiraGTx Biological safety committee (BSC) as the most hazardous organism. It is ubiquitous, causes only a mild respiratory disease in humans when exposure is very high which is self-limiting and does not require any specific treatment. This is minimised due to having very small volumes (1mL at any given time within a LAF cabinet) for use in a QC assay as a surrogate infectious virus to measure infectious particles of AAV.

Host/vector system

MeiraGTx utilise HEK293 host cells in order to propagate viral vectors. They
are derived from Human embryonic Kidney cells and are a well characterised and defined manufacturing cell line. They have also been stably generated with key Ad5 genes in order to recapitulate rAAV and other viral vectors. This was historically done to reduce the genetic burden on mammalian host cells via transfection as the genes can be quite large in base pair size. We may also use a variant HEK293T cell line or CHO cells in future process which will also be governed by GMP, GMO, and controlled use 2 (cu2) use handling and rigor. All these cell lines cells typically die/degrade rapidly outside the artificial environment created within the laboratory and culture vessels.

Furthermore, MeiraGTx have currently created a master cell bank (MCB) of these HEK293 cells from an approved and reputable source. From this MeiraGTx are generating a working cell bank (WCB) which provides a consistent seeding platform and removes variability from clonality. These cell banks will undergo GMP QC scrutiny before being released for use in the beginning of each campaign (which begins with a single WCB vial thaw). This initial culture is then grown up to increase biomass for propagating the virus to a predetermined expectant yield.

The genetic inserts that MeiraGTx have in their pipeline are human in origin and poses no risk to animal health or the environment if accidentally released. This genetic material is designed to replace faulty genes in the recipient after the vector has been propagated to sufficient quantities in the host cell (HEK293). As a worst case, Viruses could, depending on your gene insert, be potentially hazardous. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes in vivo. For these reasons, due caution must be exercised in the production and handling of any recombinant viruses. Follow all applicable guidelines for research involving recombinant DNA. Take appropriate safety measures when producing or handling recombinant AAV, including working in a biological safety cabinet and wearing protective laboratory coats, face protection, and gloves.

Justifiably MeiraGTx inserted (GOI) genes have been carefully selected from preliminary experiments and have shown to biologically active in vitro but in very specific tissues. In principal this means that for example an ocular gene insert is used as the GOI then exposure of this gene into say the liver, will in theory have no harmful effect due to tissue specific nature of not only the virus but the gene itself. Additionally, the viral backbone never changes apart from the capsid protein (which gives it its tropism specificity), so the viral vector is simply a vehicle for the chosen gene insert (active component) to displace the faulty gene. Their properties as far as MeiraGTx are concerned thus far, relate to ocular
and neurological phenotypes only and have been extracted using best practice from cultured human cells. At administration level, Due to all our viral gene inserts being specific to the eye or CNS. Neither of these organs can be accessed (or accidentally exposed to rAAV) without specialised administration (Sub-retinal injection or Lumbar puncture) which shall be performed by a clinician in an operating theatre.

Evaluation of foreseeable effects

There is minimal risk for environmental and personnel effects due to handling and use of Cat 2 organisms. We have a dedicated H+S officer along with a dedicated GMO officer for which to continually assess and monitor GMO activities.

Level 2 containment measures will be in operation and the principles of Good Microbiological Practice will be applied. In conjunction with this access to the laboratory will be restricted when work with infectious agents is in progress. Persons at increased risk of acquiring infection or for whom infection may have serious consequences will not be allowed to enter the laboratory. A biohazard sign will be posted at the laboratory entrance bearing appropriate information including the agent(s) in use, containment level, the investigator’s name and telephone number, personal protective equipment requirements and exiting procedures if any. Biosafety procedures will be incorporated into Standard Operating Procedures (SOPs) or the biosafety manual and personnel will be advised of special hazards. All work will be done with the approval of the safety sub-committee.

Biological spills and GMO accidental release may happen from time to time even though measures exist to reduce this. If this happens, the Biological Safety Committee (BSC) will raise an incident and the remedial steps such as clean up, and reporting will be done in accordance with guidance set forth by the BSC.

We do not envisage any long-lasting effects detrimental to staff or technicians in our facility.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

We have safeguarded the facility with SOPs, spill kits, dedicated AHUs, personnel flows, transfer hatches which all maintain controlled use of GMMs to minimise risk and avoid cross contamination. In addition to having our viral manufacturing suites being segregated and having dedicated AHUs, while soliciting during every changeover, a bio-decontamination cycle of VHP to inactivate any residual virus that may arise from the manufacturing batch. As such, no transmittal of virus should occur from one suite or campaign to another.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving, Vapour Hydrogen Peroxide gas (VHP) or chemical treatment using an anti-viral agent (Virkon) or Presept chlorine tablets to achieve a 100% kill, prior to disposal of waste or cleaning and recycling of reusable laboratory/manufacturing equipment, such as glassware. Inactivated liquid waste is disposed of down the sink with multiple volumes of water and pumped to waste handling room. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.

OR

All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory/manufacturing equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121°C for 20 min). All waste will be carefully monitored and volumes accounted for.

Is an emergency plan required according to regulation 20?  Y

If yes, tick to confirm that it is attached to this form  Y

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The Risk Assessment herein has been detailed by the Biological Safety Committee (BSC) and the risk for staff and public exposure to harmful GMOs is minimal.

Project Containment

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02/03/2022
GM Centre Number: 3393

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Name

LOCATE BIO LTD

Name 2

Campus Estate or Research Centre

MEDICITY

Road Name

THANE ROAD

Town

NOTTINGHAM

Tel Number

0115 784 0041

Fax Number

0

E-mail

HSE Division

blank

Comments

Name change from Locate Therapeutics Ltd 13/11/2018

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

We have received advice from Webster's Biosafety, who specialize in High Biological Containment and have also established a genetic modification safety committee which meets every 2 months. This is comprised of:

- **Senior Manager-** Accountable to Board of Directors; experienced life sciences executive with over 15 years' working across the regenerative medicine, drug delivery and medical device sectors. Industrially trained pharmacist, with an Executive MBA and a PhD in tissue engineering.
- **Biological Safety Officer-** Previously managed pharmacology facilities with responsibility for health and safety/GMO policy implementation and staff training as well as sitting on many safety committees throughout their career.
- **Principal Investigator-** PhD educated, interdisciplinary scientist with over 10 years of research experience and 2 years of experience in managing a clean room environment. Has a sound understanding of complex research actions such as: stem cell isolation and culture; cell imaging and characterisation techniques; scaffold design and fabrication for tissue engineering; cell and tissue cryopreservation methods; the application of quality-by-design to cell product development and manufacture. Appreciates the significance of adhering to legislative demands and health & safety requirements; understands the detrimental impact non-compliance can have in relation to tasks and projects.
- **Company Safety Officer and Quality Systems Manager-** Has over 35 years of experience working in research and development in both academia and industry. Holds a PhD in the field of regenerative medicine and has held the role of Company Safety Officer for 3 years. Oversees the Company training programmes and has implemented a quality management system certified to ISO-13485:2016.
- **Administrator-** Office manager; minute taker.

<table>
<thead>
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Level 4 (GMMs)

Non-microbial

Other (please specify) 

Tick if confidential

Bacteriology

Parasitology

Transgenic

Microbiology

Research

Virology

Transgenic

Animals

Transgenic

Fish

Gene Therapy

Yes

Mycology

Transgenic

Invertebrates

Transgenic

Plants

Other (please

specify below)

Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment


Please enter comments of the GM safety committee on the risk assessment

All of the GM safety committee members have reviewed and discussed the Class II risk assessment and agree with the controls and classification of the activities detailed.

Project Ref 3393/18.1

Date Ackn’d 04/01/2018

CU2 Project Title Modulation of gene expression in mammalian (including human) cells and stem cells

Class 2

CultureVol

Class 2 1-50 Litres

ClassVolumeClass3-4
Project Additional Information

Purposes of the contained use

Our company is interested in a number of areas:
Using mammalian (including human) pluripotent stem cells, adult stem cells and differentiated cells to help understand tissue formation.
Improving differentiation of stem cells.
Developing future therapies using the above cell classes.

Recipient or parental organism

Non-mobilisable plasmid vectors will be constructed and amplified in E.coli for one of following purposes:
1. To constitutively express reporter genes to trace transfected mammalian cells.
2. To tissue-specifically express reporter genes from promoters or endogenous loci to trace mammalian cell behaviour.
3. To express modulators of gene expression and behaviour in cultured mammalian cells.
E.coli (lentiviral)
• Non-mobilisable plasmid vectors based on the pUC family of cloning vectors will be constructed and amplified in disabled E.coli.
• The three separate plasmids, previously approved (under assessment C07.01, Project Ref. 470/07.1) will be used to transfect Human Embryonic Kidney 293T cells to produce lentivirus. The lentivirus will then introduce the genes of interest into mammalian cells. For newly constructed systems 3rd and 4th generation lentivirus will be used.
• The genes will be reporters such as coloured fluorescent proteins, luciferases, differentiation markers or signal transduction markers, which will report on our cells in our tissue engineered constructs.
• Some primary cells will be immortalised.
Mammalian (lentiviral)
The host cells for lentiviral infection have no dormant viral auxiliary genes such as vpr, vif, vpu and nef which could reactivate lentiviral infectivity. The cell lines include human adult stem cells i.e. mesenchymal stem cells bought from a reputable supplier.
Cell selection will be via co-introduction of selectable markers neomycin, puromycin or thymidine kinase, or by FACS selection.
### Host/vector system

pUC based cloning vectors (eg pBluescript, pGEM and pCR-TOPO vector families) will be used to generate constructs in disabled E. coli strains. For introduction of genes into mammalian cells, a second generation, inducible lentiviral expression system may be used, which requires three separate plasmids. Newly generated constructs will use 3rd or 4th generation systems.

HOSTS to produce lentivirus
- Human Embryonic Kidney 293T cells

HOSTS to be genetically modified E.coli, and cultured human mesenchymal stem cells.

### Origin & function

| E. coli | Non-colonising, non-pathogenic laboratory strains of K-12 derived E.coli: DH5a, HB101, Stb13. BL21(DE3)pLysS: ACDP Hazard Group (HG) 1. Mammalian (assigned to ACDP HG 1): Mouse embryonic fibroblasts NIH3T3 Genetic modification: None- NON-GMO Source: ATCC CRL-1658 Mouse myoblast C2C12 Genetic modification: None- NON-GMO Source: ATCC CRL-1772 Mouse preosteoblast MC3T3-E1 sub-clone 4 Genetic modification: None- NON_GMO Source: ATCC CRL-2593 Mouse areolar and adipose fibroblasts L929 (NCTC) Genetic modification: None- NON-GMO Source: European Collection of Authenticated Cell Cultures Rat mesenchymal stem cells Genetic modification: None- NON-GMO Source: Invitrogen; S1601-100 Mammalian (assigned to ACDP HG 2): Human bone marrow mesenchymal stem cells from healthy donors and Type II diabetic patients Genetic modification: None — NON-GMO Source: Axol Biosciences, ax9002 and ax9017 Human bone marrow mesenchymal stem cells from healthy donor Genetic modification: None- NON-GMO Source: Rooster Biosciences, MSC-001 Human adipose mesenchymal stem cells from Type I and II diabetic patients Genetic modification: None — NON-GMO Source: Lonza, PT-5007 and PT-5008 Human immortalised mesenchymal stem cells (hiMSCs) Genetic modification: Lentiviral overexpression of hTERER and HPV E6/E7 as per Okamoto et al., 2002. Source 1: Generated in-house at University of Nottingham from hMSCs from Lonza PT-2501 Source 2: JCRB Cell Bank; JCRB1149 UE6E7T-11 Human immortalised mesenchymal stem cells expressing constitutive GFP. |
Genetic modification: Lentiviral overexpression of GFP in hiMSCs (source 1)
Source: Generated in-house at University of Nottingham from hMSCs from Lonza PT-2501
Human osteosarcoma cells MG-63
Genetic modification: None- NON-GMO.
Source ATCC CRL-1427

1) Constitutive or Tissue-specific Reporters:
GFP, mRFP and other colour/stability/localisation variants, luciferase, Iron binding reporters expressed from
constitutive (e.g. PGK, EF1a etc) and tissue-specific (e.g. Bry/T, Oct4, Nestin, Osteocalcin etc) promoters, or to insert
these reporters site specifically to control expression from endogenous loci (e.g. Bry/T, etc).
2) Selection markers:
Positive (e.g. neomycin or puromycin resistance genes etc) or negative (e.g. thymidine kinase [HSV-TK] gene etc)
selectable marker genes driven by the sequences described in point 1.
3) Gene Modulators and Modifiers (listed in Table 1):
Mammalian promoter driven vectors (e.g. PGK, EF1a etc) delivered by transfection with lipids, peptides or via
lentiviruses (described in 4.2 ‘Details of vectors’ section). These will express:
1. Transcription factors (e.g. RUNX2, GATA-4, NKX2.5, MEF-2C, TBX-5 etc).
The will program cells to change fate and identity, for example, RUNX2 will program cells to become
bone/osteoblasts.
2. Growth factors (e.g. BMP2, VEGF and PDGF). These will be used in cell culture models of angiogenesis and bone
formation in which growth factors are secreted locally by the cells and will act on recipient cells within the culture.
3. Reprogramming factors (e.g. NANOG, OCT4, SOX2 etc).
The will program cells to revert to earlier developmental stages and will allow them to be programmed to other fates
(i.e. skin cell to an iPSC which are similar to hESCs). These genes confer genome-wide epigenetic and gene
expression changes. These will also promote survival and increase cell proliferation.
4. Immortalisation/cell survival factors (e.g. TERT, CDK4, E6/E7, SV40T, KLF4 etc).
The will promote changes to cell growth or survival. These genes confer genome-wide epigenetic and gene
expression changes.
N.B. This will include the use of oncogene overexpression and knockdown of tumor suppressor genes.
Proteins produced are covered in COSHH assessment; they do not increase risk, hazard or consequence in class II
facilities.

Evaluation of foreseeable effects

The most dangerous combination of inserted material would be expression of a transcription factor or immortalisation
factor (such as TBX5 or TERT respectively) that promotes growth or cell survival. However, the episomal vectors we
will use are non-mobilisable and should not provide any fitness advantage to the bacterium and no increase in
pathogenicity is likely. Similarly, the viral system we will employ is second generation and replication defective.
Moreover, we will employ 3rd or 4th generation systems within new vectors to further minimise risk.
Consequently, it is unlikely that these modifications will materially alter the pathogenicity and virulence of the GMM
than prior to transfection. The use of non-mobilisable vectors and E. coli strains unable to survive out of the laboratory
mitigates the risk of these traits transferring to pathogenic strains.
The most hazardous cell types to be used are human cells with a theoretical, but not specific, risk of human
pathogens (e.g. primary fibroblasts, embryonic stem cells).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any biological waste generated will be either autoclaved at 125°C for 20mins (cell culture flasks and plates, centrifuge tubes, tissue paper used for cleaning up any spills inside the biological safety cabinet, gloves, sleeve covers and shoe covers), cycle validated using 12 point thermocouple test; or treated with Distel validated disinfectant, used at 1:50 (pipette tips, stripettes and general cleaning of safety cabinet, cell culture lab and equipment); 1:20 for rapid disinfection of high risk areas and high risk equipment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

All of the GM safety committee members have all reviewed and discussed the Class 2 assessment and agree with the controls and classification of the activity.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

Project Ref 3393/19.1

Date Ackn'd 07/03/2019

CU2 Project Title Modulation of gene expression in mammalian (including human) cells and stem cells

Class Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4

Non-GMM Consent Granted

Date Project 02/03/2022
**Purposes of the contained use**

Our company is interested in a number of areas:
- Using mammalian (including human) pluripotent stem cells, adult stem cells and differentiated cells to help understand tissue formation.
- Improving differentiation of stem cells.
- Developing future therapies using the above cell classes.

**Recipient or parental organism**

A) Non-mobilisable plasmid vectors will be constructed and amplified in disabled E.coli for one of the following purposes:
1. To constitutively express reporter genes to trace transfected mammalian cells.
2. To tissue-specifically express reporter genes from promoters or endogenous loci to trace mammalian cell behaviour.
3. To express modulators of gene expression and behaviour in cultured mammalian cells.
B) Propogation of disabled adenoviral and adeno-associated virus will be performed using HEK293 cells. These are well characterised and authenticated tissue culture lines used for this purpose.
C) We will use a variety of mammalian (including human) cell lines and primary cultures for transduction:
Mammalian cells (assigned to ACDP HG1):
Mouse embryonic fibroblasts (NIH3T3), mouse myoblast (C2C12), Mouse pre-osteoblast (MC3T3- E1 sub-clone 4),
Mouse areolar and adipose fibroblasts L929 (NCTC), rat mesenchymal stem cells.
Mammalian cells (assigned to ACDP HG2):
Human bone marrow mesenchymal stem cells (from healthy and diseased patients), human immortalised mesenchymal stem cells, human osteosarcoma cells (MG-63) and human tissue, not limited to but including, T cells, liver, lung and neural cells and haematopoetic stem cells. Cell lines are well characterised with a history of safe use and are purchased from a reputable supplier. Human cells/tissues are supplied from a reputable, HTAlicenced biobank or source.

**Host/vector system**

Adeno-associated virus (AAV) vector system:
AAV is member of the Paroviridae family that has no know link to any human disease. AAV is inherently
replication defective, requiring co-infection of a transduced cell with a helper virus (typically Adenovirus or Herpes simplex virus) in order to replicate. Wild-type AAVs are not categorised by ACDP and thus may be handled at Containment Level 1 (SACGM Compendium Of Guidance, 2007 Part 2:72; www.hse.gov.uk/ukbiosafety/gmo/acgm/acgmcomp/).

AAV based gene transfer vectors are generated by utilising the capsid protein shell of the wild-type virus, but replacing the genome of the natural virus with a recombinant DNA insert (typically a transgene expression cassette consisting of heterologous promoter, gene or cDNA of interest and a polyadenylation signal), retaining only the viral inverted terminal repeats (ITRs). Transduction with AAV vectors does not result in integration of the virus. Consequently, the main hazards associated with the use of AAV vectors relate to the properties of any inserted genetic material. AAV vectors are widely used to transduce mammalian cell culture systems, animal models and (in the context of clinical trials) humans; their use is associated with an excellent safety and efficacy profile (Wright, J.F. 2009 Human Gene Therapy 20:698).

The majority of genetic modification work involving AAVs has involved the use of the ITRs and capsid from AAV serotype 2. However, other serotypes are also available and the use of hybrid viral particles harbouring the ITRs of one serotype and the capsid of another are commonly used (Vandenberghe et al., 2009 Gene Therapy 16:311). AAV vectors to be used will typically utilise the capsid of AAV1-9.

AAV vector production will typically be performed by multiple plasmid transient transfection, where the helper viral functions required for packaging are provided by one or more plasmids. Alternatively stable producer cell lines harbouring the necessary AAV and helper-virus factors may also be developed. If only some of the required elements are included within a particular cell line, viral production may require transient transfection with the missing components. AAV vector production methods that rely on infecting producer cells with helper virus will not be utilised.

The main hazards associated with the use of AAV vectors relate to the properties of any inserted genetic material. Where non-harmful inserts are used, AAV vectors are considered a low-risk GM activity and can be handled at Containment level 1 (SACGM Compendium Of Guidance, 2007 Part 2:76;). However, as standard, viral work will be performed at Containment Level 2.

Adenoviral vector system:

The adenoviral vectors to be used are attenuated through deletion of the E1 region of the genome (containing E1A and E1B) and through deletions within the E3 gene that inactive it. This has several affects on the virus. Firstly deletion of the E1A gene eliminates the potential for viral transformation of cells, since the E1A gene product is absolutely required for this process. It is also required for activation of all other early genes (E1B, E2, E3 and E4). The E1B gene product (also deleted) co-operates with E1A in transformation. The E2 region (E2A and E2B) contains DNA binding (E2A) and DNA polymerase (E2B) activities that are absolutely essential for viral replication. The E3 gene product helps in viral avoidance of the immune system by binding to the major histocompatibility complex MHC polypeptides in the endoplasmic reticulum. However, this gene is also inactivated in this strain of adenovirus, resulting in the virus being highly susceptible to immune surveillance. The E4 gene product is required for formation of an active complex between the E1B gene product and the E4 gene product which can prevent apoptosis in the host cell.

The above characteristics means that the recombinant virus is unable to replicate in E1A-deficient cells of any organism. Under normal circumstances no human or animal cells contain the E1A gene product, resulting in the inability of this virus to replicate in any naturally occurring organism (human or non-human mammalian). Under conditions where an organism (human or non-human mammalian) was already suffering from an adenoviral infection, it is possible that the recombinant virus could be replicated and packaged. Since this is likely to be a
relatively rare event, and not one that could sustain a population of recombinant virus, the risks are extremely low. Additionally, any wild-type virus would be at a significant growth advantage with respect to the recombinant virus (for the reasons mentioned above concerning attenuation of the virus), meaning that the wild-type virus would outgrow the recombinant one very quickly.

The attenuated virus cannot recombine with wild-type virus in any way that produces viable virus (Becker T.C., et al Methods Cell. Biol. (1994) 43,161-89). Recombination with a wild-type virus would, by the nature of the recombination event, remove the E1 region from the wild-type virus thereby inactivating its ability to replicate. Taken together, the above means that the recombinant virus is essentially unable to propagate in the external environment. The wildtype adenovirus serotype 5 causes sub-clinical infections and is categorised by the ACDP as Hazard Group 2. Based on the information given above, the attenuated adenoviral vector can be classified as ACDP Hazard Group 1.

The vectors to be used will contain one of the following inserts:

1) Constitutive or Tissue-specific Reporters:
GFP, mRFP and other colour/stability/localisation variants, luciferase, Iron binding reporters expressed from constitutive (e.g. PGK, EF1a etc) and tissue-specific (e.g. Bry/T, Oct4, Nestin, Osteocalcin etc) promoters, or to insert these reporters site specifically to control expression from endogenous loci (e.g. Bry/T, etc).

2) Selection markers:
Positive (e.g. neomycin or puromycin resistance genes etc) or negative (e.g. thymidine kinase [HSV-TK] gene etc) selectable marker genes driven by the sequences described in point 1.

3) Gene Modulators and Modifiers (listed below):
Mammalian promoter driven vectors (e.g. PGK, EF1a etc) delivered by transfection with lipids, peptides or via lentiviruses (described in 4.2 'Details of vectors' section). These will express:
A. Transcription factors (e.g. RUNX2, GATA-4, NKX2.5, MEF-2C, TBX-5 etc).
These will program cells to change fate and identity, for example, RUNX2 will program cells to become bone/osteoblasts.

B. Growth factors (e.g. BMP2, VEGF and PDGF). These will be used in cell culture models of angiogenesis and bone formation in which growth factors are secreted locally by the cells and will act on recipient cells within the

C. Reprogramming factors (e.g. NANOG, OCT4, SOX2 etc).
These will program cells to revert to earlier developmental stages and will allow them to be programmed to other fates (i.e. skin cell to an iPSC which are similar to hESCs). These genes confer genome-wide epigenetic and gene expression changes. These will also promote survival and increase cell proliferation.

D. Immortalisation/cell survival factors (e.g. TERT, CDK4, E6/E7, SV40T, KLF4 etc).
These will promote changes to cell growth or survival. These genes confer genome-wide epigenetic and gene expression changes.

E. Gene editing: not limited to but including CRISPR/Cas9 and transposon systems such as Sleeping Beauty.
N.B. This will include the use of oncogene overexpression and knockdown of tumor suppressor genes. Proteins produced are covered in risk assessment; they do not increase risk, hazard or consequence when handled in class II facilities.

In some instances, the insert may be divided across two or more MV vectors. This approach relies on the natural propensity for MV genomes to concatamerise, which with appropriate placing of intronic sequences allows reconstitution of an expression cassette by cis- or trans-splicing (Hirsch et al., 2010 Molecular Therapy 18:6).
Evaluation of foreseeable effects

Insertion of the constructs will not alter the tissue tropism, or increase the infectivity or pathogenicity of the recipient vector. Scope for recombination with wild type virus is limited and, due to the packaging limits of adenovirus and AAV, if any such recombinants did arise they would be non-viable. Based upon there being no likelihood of any effect on the phenotypic characteristics of the recipient and the non-harmful nature of the inserts, the resulting replication defective viral/gene vector can be considered equivalent to the recipient vector in terms of hazard status, i.e., hazard group 1. Exception to this is perhaps GM viruses expressing a transcription factor or immortalisation factor (such as TBX5 or TERT respectively) that promotes growth or cell survival. However, the viral systems we will employ are replication defective and the Class II containment we will use as standard guarantees the minimised risk associated with the use of these vectors.

In the unlikely event of a release from the Class II Containment facility, adenovirus could remain viable in the environment for a similar period of time to the wild-type virus but AAV cannot survive if accidentally released. If the virus were to infect a susceptible host, then expression of the recombinant genes would occur in the infected cells. Since the virus cannot propagate (except in exceptional circumstances as detailed above), expression would be limited to the cells initially infected (i.e., once the cell divides, only ONE daughter cell will contain the adenovirus DNA. Eventually, this would result in loss of the DNA due to natural degradation of the episomal DNA. Additionally, the attenuated virus cannot recombine with wild-type virus in any way that produces viable virus (Becker T.C., et al Methods Cell. Biol. (1994) 43, 161-89). Recombination with a wild-type virus would, by the nature of the recombination event, remove the E1 region from the wild-type virus thereby inactivating its ability to replicate. Taken together, the above means that the recombinant virus is essentially unable to propagate in the external environment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Any biological waste generated will be either autoclaved at 125°C for 20m ins (e.g. cell culture flasks and plates, centrifuge tubes, tissue paper used for cleaning up any spills inside the biological safety cabinet, gloves, sleeve covers and shoe covers), cycle validated using 12 point thermocouple test; or treated with Distel validated disinfectant, used at 1 :50 (e.g. pipette tips, stripettes and general cleaning of safety cabinet, cell culture lab and equipment); 1 :20 for rapid disinfection of high risk areas and high risk equipment.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment
All of the GM safety committee members have reviewed and discussed the Class 2 assessment and agree with the controls and classification of the activity.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
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<td>L3 L4</td>
<td>L2 L3 L4 L2 L3 L4</td>
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02/03/2022
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**Name**

PROCTOR & GAMBLE TECHNICAL CENTRE

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

452 BASINGSTOKE ROAD

**District**

**Town**

READING

**County**

BERKSHIRE

**Postcode**

RG2 0QE

**Country**

ENGLAND

**Tel Number**

01784474900

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

This committee was put together as part of the project scope as GMM are not currently used at the site. The committee is made up of five team members, the site biological safety leader (qualified internally as the European Biosafety leader, and externally via the Biosafety Practitioner level 1 (foundation) SCQF level 11 BTI - ISTR registered), the site HS&E manager (NeBosh General Cert, NVQ level5 Occupation Health and Safety, P&G HSE Programme leader), microbiology management representative (PhD in Biotechnology, MSc in Microbial Genetics, Qualified Corporate QA Assessor), microbiology project leader (PhD in Biochemistry, MSc in Life Science, University of Edinburgh) and project technical leader (BSc Biological Sciences (Hons- Microbiology), PhD Chemical Engineering (Mammalian cell culture), Industrial co-supervisor (EPSRC, BBSRC, Marie Curie PhD's and Post docs) on biofilms related research. Panel D member BBSRC responsive mode.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>
It should be noted that the inserted material does not pose any additional hazard from the parent non-GMO strain. This results in no increased risk to the stability or survivability of the organisms and therefore the consequences of environmental release are negligible given the existing systems present in the lab for the disposal of BSL 2 organisms. Following the GMO Contained Use regulations, any GMO waste shall be inactivated prior to disposal. In this case, all GMO waste shall be autoclaved at 126°C for 30 minutes (validated cycle) prior to disposal in the biohazardous waste stream. Subsequent waste management is managed by contractors approved for the handling of "BSL 2 biohazard waste". Degree of kill = 100%.

For activities involving GMMs, describe the waste management measures which will apply to the activity.
Visualisation of hygiene benefits for R&D purposes and the use of generated images as potential commercial/publication material to develop and support superior products for consumers.

GMO GFP proteins will be used under standard EN European Antibacterial (AB) disinfection claims (e.g. BS EN 1276, BS EN 13697, BS EN 16615) methods and CDC reactor methods (e.g. ASTM E2562-17). GFP bacteria will be imaged using standard lab techniques such as microscopy and confocal imaging.

GMO BACTERIAL STRAIN
- Pseudomonas aeruginosa ATCC (Xen05) level 2
- Pseudomonas aeruginosa PAOI (Xen41) level 2
- Staphylococcus au reus ATCC 8325-4 (Xen8.1 ) level 2
- Staphylococcus au reus ATCC 12600 (Xen29) level 2
- Staphylococcus au reus ATCC 33591 (Xen31) level 2
- Staphylococcus aureus ATCC 49525 (Xen36) level 2
- Staphylococcus aureus UAMS-I (Xen40) level 2
- Escherichia coli WS2572 (Xen14) level 1
- Escherichia coli WS2583 (Xen16) level 1
- Salmonella typhimurium FDA 11 (Xen33) level 2

Recipient or parental organism
- Pseudomonas aeruginosa ATCC: Photorhabdus luminescens lux operon on the bacterial chromosome.
Pseudomonas aeruginosa PA01: Photorhabdus luminescens lux operon on the bacterial chromosome.
Staphylococcus aureus ATCC 8325-4: Photorhabdus luminescens lux operon on the bacterial chromosome.
Staphylococcus aureus ATCC 12600: Photorhabdus luminescens lux operon on the bacterial chromosome.
Staphylococcus aureus ATCC 33591: Photorhabdus luminescens lux operon on the bacterial chromosome.
Staphylococcus aureus ATCC 49525: Photorhabdus luminescens lux operon on the native plasmid.
Staphylococcus aureus UAMS-I: Photorhabdus luminescens lux operon on the bacterial chromosome.
Escherichia coli WS2572: Photorhabdus luminescens lux operon on the bacterial chromosome.
Escherichia coli WS2583: Photorhabdus luminescens lux operon on the bacterial chromosome.
Salmonella typhimurium FDA 11: Photorhabdus luminescens lux operon on the bacterial chromosome.

Origin & function

Supplier: Perkin Elmer.
940 Winter St, Waltham, MA 02451, United States.
+44 800-89-60-46
www.perkinelmer.com
Product references from Perkin Elmer:
Pseudomonas aeruginosa (Xen05)
Pseudomonas aeruginosa (Xen41)
Staphylococcus aureus (Xen8.1)
Staphylococcus aureus (Xen29)
Staphylococcus aureus (Xen31)
Staphylococcus aureus (Xen36)
Staphylococcus aureus (Xen40)
Escherichia coli (Xen14)
Escherichia coli (Xen16)
Salmonella typhimurium (Xen33)
119228
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119235

Evaluation of foreseeable effects

It should be noted that the inserted material does not pose any additional hazard than the parent non-GMO strain resulting in no increased risk to human health and safety. Other than the inclusion of the fluorescent tags the GMO strains have not been altered, retaining the same genetic and growth characteristics of the parental strain with the Biosafety categorisation remaining unchanged.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not Applicable
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

It should be noted that the inserted material does not pose any additional hazard from the parent non-GMO strain. This results in no increased risk to the stability or survivability of the organisms and therefore the consequences of environmental release are negligible given the existing systems present in the lab for the disposal of BSL 2 organisms. Following the GMO Contained Use regulations, any GMO waste shall be inactivated prior to disposal. In this case, all GMO waste shall be autoclaved at 126°C for 30 minutes (validated cycle) prior to disposal in the biohazardous waste stream. Subsequent waste management is managed by contractors approved for the handling of “BSL 2 biohazard waste”.

Degree of kill = 100%.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Committee members:
- Biological Safety Leader
- Health Safety and Environment Manager
- Management Representative - (Egham Microbiology Section head)
- General Staff Representative - (Project Microbiologist)
- Technical Expert - (Microbiology Principal Scientist)
- Project Risk Assessment reviewed with the Site Biosafety leader and content approved for the overall assessment, including containment facility use, and waste management procedures.

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Name

4D PHARMA RESEARCH LTD

Name 2

Department

Campus Estate or Research Centre

FORESTERHILL CAMPUS

Building

LIFE SCIENCE INNOVATION BUILDING

Road Name

CORNHILL ROAD

District

Town

ABERDEEN

County

ABERDEENSHIRE

Postcode

AB25 2ZS

Country

SCOTLAND

Tel Number

01224 900 460

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

We have received advice from the Foresterhill Genetic Modification Safety Committee, which is constituted of University of Aberdeen biological safety officers, safety advisers and research group members.

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02/03/2022
GMMs will be inactivated by autoclaving, using a cycle of 121 °C, 15 psi for 60 min. Autoclave is a routinely used and validated method for inactivation bacterial waste. We expect a 100% degree of kill, it was previously shown that autoclaving for 15 min or more at 121 °C kills 100% bacteria. Moreover, we have demonstrated that a heat treatment at 80°C for 30 min was sufficient to yield no viable cells for our recipient strains. Additionally, we will confirm inactivation by confirming the absence of viable cells after inactivation (swabs of from plates and liquid media will be plated on nutrient agar).

There is an autoclave dedicated to biological waste inactivation in the building. Pressure and temperature inside the autoclave are closely monitored during each cycle to ensure that the content reaches the appropriate temperature. The autoclave used for waste inactivation is tested and serviced yearly.

Inactivated liquid waste will be disposed down the drain. Inactivated solid waste will be placed in orange bags and collected regularly by NHS Grampian.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

We have submitted an application to the University of Aberdeen Foresterhill Genetic Modification Safety Committee for approval to carry out work with genetically modified micro-organisms. The Foresterhill Genetic Modification Safety Committee has reviewed our application and officially approved it on 13/10/2017.

Comments from the committee: "To be acceptable by the committee after minor modifications. It is a Commercial and requires a CU2 form."

The requested amendments were made and final approval was received on 13/10/2017.
The aim of this project is to inactivate bacterial genes involved in host interaction, adhesion and immunomodulation in anaerobic bacteria in order to gain an understanding of their biological function. The work will involve generating a number of targeted single gene knock-out mutants in a strain isolated from the gut microbiota of a healthy human. Gene inactivation will be performed using targeted gene disruption by plasmid insertion (through site-specific homologous recombination).

The recipient organism is a Gram-positive bacterial strain isolated from a healthy human. E. coli strains will be used as cloning hosts. A non-replicating vector will be used for gene disruption and generation of insertion mutants.

The genetic material will be extracted from the recipient organism. This project will focus on the genetic manipulation of genes involved in host interaction, adhesion and immunomodulation in order to gain an understanding of their biological function.

The genetically-modified strains to be generated are predicted to have reduced ability to colonise and interact with the host in comparison to the wild-type strain. We do not foresee any additional hazardous effects arising from the generation of those strains. Risks associated with the genetic manipulation of the wild-type strain and the resulting GMMs have been assessed and are predicted to be low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GMMs will be inactivated by autoclaving, using a cycle of 121 °C, 15 psi for 60 min. Autoclave is a routinely used and validated method for inactivation bacterial waste. We expect a 100% degree of kill, it was previously shown that autoclaving for 15 min or more at 121 °C kills 100% bacteria. Moreover, we have demonstrated that a heat treatment at 80°C for 30 min was sufficient to yield no viable cells of the recipient strain. We do not expect the proposed mutations to enhance the recipient strain's ability to survive exposure to the above conditions. Additionally, we will confirm inactivation by confirming the absence of viable cells after inactivation (swabs of from plates and liquid media will be plated on nutrient agar).

There is an autoclave dedicated to biological waste inactivation in the building. Pressure and temperature inside the autoclave are closely monitored during each cycle to ensure that the content reaches the appropriate temperature. The autoclave used for waste inactivation is tested and serviced yearly. Inactivated liquid waste will be disposed down the drain. Inactivated solid waste will be placed in orange bags and collected regularly by NHS Grampian.

Is an emergency plan required according to regulation 20? N

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Tick to confirm that you have attached a risk assessment to this form Y

Please enter comments on the GM safety committee on the risk assessment

We have submitted an application to the University of Aberdeen Foresterhill Genetic Modification Safety Committee for approval to carry out work with genetically modified micro-organisms. The Foresterhill Genetic Modification Safety Committee has reviewed our application and officially approved it on 13/10/2017. Comments from the committee: "To be acceptable by the committee after minor modifications. It is a Commercial and requires a CU2 form."

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Project Containment

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Animal Units

Large Scale Activities

Human Clinical Applications

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**Name**

EXCELerate BIOSCIENCE LTD

**Name 2**

Department

**Campus Estate or Research Centre**

BIOCITY

**Building**

INNOVATION BUILDING

**Road Name**

PENNYFOOT STREET

**Town**

NOTTINGHAM

**County**

NOTTINGHAMSHIRE

**Postcode**

NG1 1GF

**Country**

ENGLAND

**Tel Number**

0115 824 8243

**Fax Number**

0

**E-mail**

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**HSE Division**


**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

Safety committee consists of a group of ex-industry leaders (from Novartis), University Professor (Nottingham University) and external University Associate Professor (Nottingham University) who have worked in the relevant field for many years and have previous sat on such committees in the past. The committee will meet bi yearly, and additionally as necessary, to ensure that current measures and risk assessments are up to date and that new regulations are implemented.

Terms of reference:
1. Review all risk assessments for work involving genetic modification
2. Advise on laboratory GMM procedures, based on current legislation
3. Review laboratory inspection outcomes and monitoring arrangements, to ensure implementation of safe working practice related to GMM

Level 1 (GMMs)
Yes

Level 2 (GMMs)

Level 3 (GMMs)
Established chemical (disinfectant) or physical (autoclaving) inactivation methods will be used for all GMM associated materials prior to disposal via off-site incineration. Chemical inactivation routes will use Chemgene I Distel, with each batch checked against manufacturer’s test data to validate expected kill rates and recommended conditions. For example, Chemgene HLD4L at 1:100 dilution provides >510 gl 10 bacteriocidal activity for E Coli on surfaces or in suspension, under dirty (high BSA) conditions, following room temperature incubation for 5 min. Manufacturer data demonstrates chemical inactivation is suitable for all GMOs involved in this work. Our chemical disinfection procedures also exceed manufacturer standards significantly by routinely using more concentrated solutions (1:50, 2%) for an extended period of time (typically overnight). Physical inactivation by autoclaving will use validated cycle (121 - 126°C for 15 min) and an autoclave sited within our laboratory space. Agar plates I solid waste in contact with GM bacteria will also be spayed with 70% IMS prior to autoclaving.

Summary of inactivation methods for GM modified mammalian eukaryotic cells. Waste tissue culture media will be incubated with 2% Chemgene overnight, prior to drain disposal. Spent tissue culture flasks will be incubated with 2% Chemgene for >30 min, the disinfectant discarded to the drain, and flasks to bagged waste for incineration.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Established chemical (disinfectant) or physical (autoclaving) inactivation methods will be used for all GMM associated materials prior to disposal via off-site incineration. Chemical inactivation routes will use Chemgene I Distel, with each batch checked against manufacturer’s test data to validate expected kill rates and recommended conditions. For example, Chemgene HLD4L at 1:100 dilution provides >510 gl 10 bacteriocidal activity for E Coli on surfaces or in suspension, under dirty (high BSA) conditions, following room temperature incubation for 5 min. Manufacturer data demonstrates chemical inactivation is suitable for all GMOs involved in this work. Our chemical disinfection procedures also exceed manufacturer standards significantly by routinely using more concentrated solutions (1:50, 2%) for an extended period of time (typically overnight). Physical inactivation by autoclaving will use validated cycle (121 - 126°C for 15 min) and an autoclave sited within our laboratory space. Agar plates I solid waste in contact with GM bacteria will also be spayed with 70% IMS prior to autoclaving.

Summary of inactivation methods for GM modified mammalian eukaryotic cells. Waste tissue culture media will be incubated with 2% Chemgene overnight, prior to drain disposal. Spent tissue culture flasks will be incubated with 2% Chemgene for >30 min, the disinfectant discarded to the drain, and flasks to bagged waste for incineration.
Glass and plastic pipettes, pipette tips, pots and cryovials in contact with GM cells will be soaked in 2% Chemgene overnight (with disinfectant drawn into pipette barrels), then to incineration bag or sharps bin waste. Equally experimental microtitre plates containing GM whole cells will be soaked in 2% Chemgene overnight, then to transferred to incineration waste disposal.

Summary of inactivation methods for GM modified E Coli. Agar plates, and other solid waste in contact with GM E Coli (e.g. DNA preparation columns) will be surface sprayed with 70% IMS, then autoclaved prior to bag disposal for incineration. Liquid bacterial cultures, and waste media will be incubated with 2% Chemgene overnight, followed by drain disposal. Eppendorf tubes, pipettes and pipette tips will be soaked in 2% chemgene overnight, followed by incineration (sharps).

Any spill procedures will use more concentrated disinfectant (10% Chemgene, in contact with associated surfaces for >15 min), with associated towel and solid waste being autoclaved.

Waste disposal procedures and monitoring will form an essential element of staff training in handling GMOs, with the appropriate routes for different types of waste clearly displayed in all relevant laboratory areas. For chemical disinfection procedures, manufacturer testing records will be checked for each batch of disinfectant used, together with product expiry and weekly monitoring of replacement of the appropriate stock dilutions. There will be daily replacement of 2% soaking solutions for plasticware and microtitre plate decontamination. Staff will be trained in the use of the autoclave, verification of the thermocycle and the need to repeat the autoclave cycle if uncertain. Annual servicing of the autoclave will validate sterilisation cycle parameters. On site testing of a small sample of treated media (by autoclave or chemical disinfection) will occur biannually, inoculating on solid agar or cell culture liquid media and assessing for overnight growth - with results reported to safety committee.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Prepared in consultation and reviewed by all GM safety committee members; procedures for containment and risk assessments were considered appropriate for class I.
### GM Centre Number: 3398

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### Name

CONCEPT LIFE SCIENCES INTEGRATED DISCOVERY & DEVELOPMENT SERVICES LTD

### Name 2

### Department

### Campus Estate or Research Centre

### Building

4TH FLOOR

### Road Name

SPRING GARDENS

### District

### Town

MANCHESTER

### County

GREATER MANCHESTER

### Postcode

M2 1FB

### Country

ENGLAND

### Tel Number

0161 836 2760

### Fax Number

0

### HSE Division

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### Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

1) Principal Bioscientist with >30 years experience of generating and handling GMMs; previously responsible for biological safety within the Carcinogenesis (1992-2011) and Drug Discovery Groups (2011-2016) at the CRUK Manchester Institute.

2) Safety, Health and Environment Officer - IOSH qualified

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</table>

Other (please specify): **Tick if confidential**

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

| Disinfectant used is Biocleanse (Teknon) - Passes BS EN 1726 at 1%. Biohazard solid waste is stored in lined biohazard bins until removal by waste management staff, then transported by vehicle to the onsite permitted waste transfer station where it is autoclaved at 121°C; the process takes up to 2 hours to complete. Liquid waste: add equal volume of 10% biocleanse solution (Teknon) for minimum 30 minutes (final concentration =5%) - Wash down sink with at least 5x volume of water. Disposable plastics: disinfect with 1% biocleanse solution (30 mins) prior to incineration. Glass/plastic: disinfect with 1% biocleanse solution (30 mins), wash with plenty of water. Solid biohazard waste (e.g. gloves): send for autoclave disposal (100% kill) Surfaces: Spray with 1% biocleanse, wipe away with tissue, discard into autoclave waste. Spillage: Absorb onto paper towels, send for autoclave disposal. Swab area with 1% biocleanse, discard paper towels into autoclave waste. Spill in centrifuge: If breakage occurs during run, do not open lid. Leave for 30 mins to allow aerosol to disperse. Label centrifuge as to hazard. On opening, soak liquid onto paper towel and place in autoclave bag. Immense plastic parts in 1% biocleanse (30 mins). Swab bowl, rotor and buckets with 1% biocleanse. Remove rotor and/or bucket and rinse with water. Rinse bowl with water. Discard all towels into autoclave waste. |

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

This project has been approved as containment level 1, class 1 activity.
GM Centre Number: 3400

Data Premises Notified (Originally) 22/02/2018

Transferred from 1992 Regs? N

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name
LABGENIUS LTD

Name 2

Department

Campus Estate or Research Centre

Road Name
ST JAMES'S SQUARE

Building
SUITE 1, 3RD FLOOR

District

Town
LONDON

Country
ENGLAND

County
GREATER LONDON

Postcode
SW1Y 4LB

Tel Number 07946403074

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- CSO - LabGenius Health and Safety Officer
- Automation engineer - Experienced in risk assessments up to class II contained use.
- Research scientist - Experienced writing risk assessments up to class I contained use.
- Business development officer - expertise in risk assessments and lab health and safety at Johnson Matthey.

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- Microbiology Research |
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<th>Gene Therapy</th>
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<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
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All contaminated liquid waste (e.g. contaminated media, buffers), will be disinfected using Rely+On Virkon disinfectant according to the manufacturers instructions. This involves dissolving Rely+On Virkon into the liquid waste, such that the final solution of Rely+On Virkon is 1% (i.e. 10 grams per litre). Once fully dissolved, the waste is left at room temperature for 10 minutes before being sewer. Virkon reportedly kills 99.999% of microorganisms in less than 10 minutes. To monitor this efficacy, a sample will be taken from one in every 30 liquid waste loads. Before disinfection, the concentration of microbes will be measured via a OD600 reading. Following disinfection, the sample will be plated on nutrient agar and incubated at 37°C for 2 days. Any colonies that have grown on the plate will be counted. Comparing the concentration of cells pre-disinfection to the number of colonies on the agar plate will allow us to calculate a kill percentage.

All contaminated solid waste (e.g. pipette tips, plastic tubes, agar plates) will be sterilised in bags using an autoclave. The content of the autoclave will reach 121°C for 20 minutes in saturated steam. To ensure that the centre of the load reaches this temperature for the requisite time, the sterilisation cycle will run for 60 minutes. Using this method, 100% of microorganisms are killed. To monitor this, once a month, a Thermalog Steam Chemical Integrator will be placed in the centre of the waste load. Once the autoclave cycle is complete, the indicator will reveal whether the centre of the load has reached the requisite temperature to entirely sterilise it.

Tick to confirm that you are attaching a summary of the risk assessment

Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment


Please enter comments of the GM safety committee on the risk assessment

The genetic modification safety committee confirms that the summary risk assessment accurately explores the risk associated with the work at LabGenius. and has established appropriate precautions to mitigate these risks.

Project Ref 3400/19.1

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Project Additional Information

Purposes of the contained use

To transiently express genetically modified proteins, nanobodies and interleukins, from Homo sapiens, Mus musculus and Macaca fasciculari in characterised mammalian cell lines.

Recipient or parental organism

Recipient cells will be characterised mammalian cell lines - FreeStyle™ 293-F Cells (HEK293) and/or Expi293F™ Cells (HEK293) and/or FreeStyle™ CHO-S Cells and/or ExpiCHO-S™ Cells. The work involves generation of biomolecules selected for their therapeutic potential – inflammatory cytokines, their receptors and modified nanobodies designed to bind these proteins.

HEK293 cells were created using Adenovirus 5 DNA. Adenovirus type 5 is one of the most common viruses associated with lower respiratory tract infections. It is likely that lab personnel have already been exposed previously to a similar strain in childhood, so the likelihood of severe illness is very low. In the case of immuno-compromised individuals there is a risk of development of pneumonia. However, as this virus is attenuated is it highly unlikely to cause any infection.

HEK293 cells have been found to be tumorigenic in nude-mice, however there are no documented cases in humans. These cells are classified as non-hazardous under regulation (EC) No 1272/2008.

FreeStyle™ CHO-S Cells are not considered to be hazardous to human health (classified as non-hazardous under regulation (EC) No 1272/2008)

Host/vector system

List of vectors:
1. PSF-CMV-PURO-NH2-BM40-6HIS-EKT
2. PSF-CMV-PURO-NH2-6HIS-MBP-EKT
3. PSF-CMV-NH2-HIS-EKT-NCOI

Whilst the vectors used will have mammalian promoters, the GMM risk is considered to be low, working with nonhazardous
targets selected for their therapeutic potential. The expression vectors themselves are commercial DNA plasmids and are considered non-pathogenic. The cloned DNA products are unlikely to alter the pathogenicity of the host.

### Origin & function

The work involves generation of biomolecules selected for their therapeutic potential, as enzyme or binder to human proteins.

The genetic material involved are inflammatory cytokines, their receptors and modified nanobodies designed to bind these proteins. Proteins modified from Homo sapiens, Mus musculus and Macaca fasciculari.

### Evaluation of foreseeable effects

Interleukins and antibodies have been used in the clinic for a long time for immunotherapy with evidence of safety. Nanobodies clinical trials to assess safety have also been conducted with success. Gene transfer is a remote possibility. As inflammatory cytokines and their receptors are already present in the host, it is unlikely they would create an immune response, however they are linked in an increase in inflammation so could increase risk of inflammatory conditions. It is unknown the effect of nanobodies tailored against inflammatory cytokines and their receptors, however they are unlikely to pose much risk when compared to prior clinical trials on safety effects of nanobodies. Overall the risk of gene transfer and their effects are considered to be VERY LOW.

The recipient strains will not survive, and the vector used only contains mammalian promoters. It is highly unlikely this vector would survive and enter a viable host. Gene transfer into environmental bacteria is a remote possibility, however would be unlikely to provide a survival advantage to the host. The inserted sequences are not expected to represent a hazard to other organisms. None of the considered modifications are expected to alter the host range or infectivity of the vector.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste - Plasticware (i.e. tips, flasks, pipettes) - All potentially infected solid waste will be discarded in an approved autoclave bag. When the bag is ¾ full it will be closed with autoclave tape and placed within a second autoclave bag. Care will be taken to ensure that bags are not sealed, to ensure that steam will penetrate the waste inside the bag. Waste will be autoclaved at 121°C for 20 minutes. Autoclaved material will be put in the appropriate bins and disposed of with an authorized removal company.

Liquid waste - Mammalian cell cultures - Cell cultures will be treated with freshly prepared 1% Virkon disinfectant for at least 1h as per manufacturer’s recommendations.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022
Please enter comments on the GM safety committee on the risk assessment

## Project Containment

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<th>Glass Houses</th>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

External consultant - previous experience includes more than 10 years employment as a Biosafety Advisor/Manager for a multinational pharmaceutical company and 5 years as a self-employed consultant with extensive experience in risk assessment and biorisk management for work with biological agents in general and GMMs in particular.

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<th>Laboratory</th>
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<th>Large Scale</th>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All potentially contaminated GMM waste material (needles, syringes, wipes/swabs, PPE) and waste vaccine product will be placed into containers provided by GMO accredited/licenced clinical waste contractor, safely stored and then removed from our premises by said companies for destruction in the required manner, as described in the information from our waste contractor below.

Waste handling for GM Class 1 products

Under Schedule 8 of the Genetically Modified Organisms (Contained Use) Regulations 2014 Table 2 details the containment measures to be applied for non laboratory type facilities. Line 20 requires that inactivation of GMMs in contaminated material and waste is required by validated means where and to the extent the risk assessment shows it to be required. The waste is such that it cannot be disinfected and there is no available autoclave. The information detailed below indicates how the waste will be packaged to send directly to incineration.

Contaminated materials such as gauzes, bandages, gloves used during administration of product

All waste will be placed inside a yellow hazardous waste bag. This bag will be sealed with a plain cable tie. The bag is then placed inside a second yellow hazardous waste bag and sealed with another cable tie. The double-bagged
waste is then placed inside a 30L yellow hazardous waste burn bin containing at least 2 inches of vermiculite to act as spillage absorbent material. Clicking the lid closed seals this burn bin. The yellow burn bin is a UN approved biohazard waste bin used for transport of waste on public highways. The maximum weight of each burn bin must not exceed 20Kg.

Operationally, a yellow burn bin containing 2 inches of vermiculite in its base and 2 yellow bags (one inside each other) will be placed out at the start of each day. At the end of each day or when the container is three quarters full, the first bag will be sealed, the second bag sealed and then the lid of the burn bin closed. This will be performed in the room where the waste has been generated. Each burn bin is tagged with a uniquely numbered locking plastic tag. The tag reference is recorded.

The burn bin is labelled with a UN3373 label. Regular collections are arranged with the waste management contractor, Hazport. During collection the unique tag reference numbers on the will be noted onto the consignment note and confirmed to the hazardous waste safety advisor in order for a certificate of destruction to be requested. This waste must remain segregated from other burn bin waste generated from the building.

Sharps Bins

All sharps are placed inside a sharps bin. Needles are not resheathed.

A small sized sharps bin such as 1 L should be used. Once three quarters full or after 1 week, the sharps bin is locked closed and placed inside a second larger sharps bin eg 22L.

Multiple small sized sharps bins can be placed inside the larger sharps bin. This should be removed from the area at least monthly.

Each large sharps bin is tagged with a uniquely numbered locking plastic tag. The tag reference is recorded. A UN3373 label is applied to each large sharps bin.

Regular collections are arranged with the waste management contractor, Hazport. During collection the unique tag reference numbers on the will be noted onto the consignment note and confirmed to the hazardous waste safety advisor in order for a certificate of destruction to be requested. This waste must remain segregated from other sharps bin waste generated from the building.

Hazport Ltd

The waste is removed from the collection point and handed to Hazport waste management company as per the agreed schedule. The waste is transported by Hazport to Tradebe Ltd, Marlborough Road, Wrexham Industrial Estate, facility where it is processed direct through their High Temperature Clinical Waste Incinerator (CWI).

Hazport will leave the UN containers at Tradebe where they are placed into 770 L bins and wheeled onto the bin line where a mechanical handling device feeds them into the incinerator.

Waste in the primary combustion chamber burns for a minimum of six hours and then passes into the secondary combustion chamber which reaches temperatures of 1,100°C to burn off pharmaceutical and chemical agents. Off gases are cleaned through a gas scrubbing system to ensure that emissions meet European standards.

The Wrexham facility is licenced to process class 1 GMO, however Class 2 waste must be notified to the Home Office and prior agreement must be obtained.
The last sentence of Section 1.2 of the risk assessment states "Safety data (e.g. reactogenicity, AEs) of the HAV vaccine and TDV will be assessed by collecting data from all subjects during the course of the study". While there is strong data supporting the safety of either vaccine when administered on its own, this statement indicates that there is less certainty about this where there is co-exposure to both.

If the likelihood of co-exposure to both vaccines of persons administering the vaccines is negligible then this potential concern does not arise. I note that any such risk to workers can be completely eliminated by ensuring that if anyone administering either of the vaccines is accidentally exposed (e.g. needlestick) they will not be involved in administering the other and I recommend this is incorporated into relevant procedures.

Subject to the additional implementation of the foregoing requirement, the designation as a risk class 1 activity is appropriate. Implementation of that requirement together with the control measures specified in the risk assessment document should ensure that exposure of persons and the environment is minimized so far as is reasonably practicable consistent with the Principles of Good Microbiological Practice and Good Occupational Safety and Health.
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**Name**

NATIONAL PHYSICAL LABORATORY

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

HAMPTON ROAD

**District**

**Town**

TEDDINGTON

**County**

MIDDLESEX

**Postcode**

TW1 0LW

**Country**

ENGLAND

**Tel Number**

0208 9437102

**Fax Number**

0

**E-mail**

**HSE Division**

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**Comments**

Date at Which Additional Info Submitted:

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities [Y]

Give brief details of the genetic modification safety committee

Genetic modification safety committee, following the ethics committee guidelines. Committee gathers once per year and is led by the deputy CEO, representative director, a communications representative and other senior scientists. Further to this, any future meetings will include the biological safety advisor (BSA). All risk assessments and forms prepared for this license have been produced by the current and future biological safety advisor.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  
Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

02/03/2022
Consumables (mainly plasticware i.e.: pipettes, flasks, tubes) - disinfect with 2% Virkon for at least 30 minutes, autoclave using a make safe cycle (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (i.e.: samples, culture supernatants, tissue culture media) – disinfect with 2% Virkon for at least 30 minutes, autoclave using a make safe cycle (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (i.e.: needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection with Virkon, used according to manufacturer’s instructions under standard conditions, manufacturers validation log reduction ([99.998]% kill).

For activities involving GMMs, describe the waste management measures which will apply to the activity

Production of recombinant protein/peptides in bacteria, yeast, insect, HEK cells and cell free sys

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All forms and risk assessments have been prepared by the future biological safety advisor and discussed with the current biological safety advisor, genetic modification safety committee and health & safety department. The current team carrying this work and future biological safety advisor (who prepared the documents) are academically educated in molecular biology and hold expertise in the subject.
**Project Additional Information**

**Purposes of the contained use**
Small aliquots of wild type Salmonella and mutants will be received and grown so that their outer membrane lipids can be extracted for mass spectrometry identification.

**Recipient or parental organism**
Salmonella typhimurium LT2

**Host/vector system**
N/A

**Origin & function**
We would receive the Salmonella mutants - we won't be modifying the bacteria here. These have been reported to be resistant to antimicrobial peptides (Lofton et al 2013).

**Evaluation of foreseeable effects**
Mutants were reported to be more resistant than wild type, yet these advantage was also reported to come along with sacrifices to the fitness of the bacteria. Therefore, it is possible these mutants would not survive or replicate in the wild.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware i.e.: pipettes, flasks, tubes) - disinfect with 2% Virkon for at least 30 minutes, autoclave using a make safe cycle (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.
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Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection with Virkon, used according to manufacturer’s instructions under standard conditions, manufacturer’s validation log reduction ([99.998]%) kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All forms and risk assessments have been prepared by the future biological safety advisor and discussed with the current biological safety advisor, genetic modification safety committee and health & safety department. The current team carrying this work and future biological safety advisor (who prepared the documents) are academically educated in molecular biology and hold expertise in the subject.

Project Containment

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Animal Units | Large Scale Activities | Human Clinical Applications

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**Name**

GIFFORD BIOSCIENCE LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

THE BIOHUB BIRMINGHAM

**Building**

BIRMINGHAM RESEARCH PARK

**Road Name**

VINCENT DRIVE

**District**

**Town**

BIRMINGHAM

**County**

**Postcode**

B15 2SQ

**Country**

ENGLAND

**Tel Number**

0121 396 1288

**Fax Number**

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**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

University of Birmingham GMSC

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research |
Virology | Transgenic Animals | Transgenic Fish | Gene Therapy |
For activities involving GMMs, describe the waste management measures which will apply to the activity

Non-radioactive (Very Low Level radioactive Waste VLLW) disposed of in BioHub clinical waste stream.
Radioactive (>VLLW) transferred to our radioactive waste management company (SRCL). Both for incineration.
Liquid waste from tissue culture, mixed with household bleach, disposed of to sewer.
Inactivation not required per Schedule 8 of the Constrained Use Regulations; Part 2; Table 1a; row 18 (inactivation at CL-1 "required by validated means where and to extent the risk assessment shows it is required."). The cell lines cannot survive outside the specialist cell culture environment and pose minimal risk to both human health and the environment.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

There were a few minor comments from the GMSC such as "Effects on human health needs "none" entering in the people at increased risk box." and "There is only one worker listed on page 8. What would be the training procedure if other staff were to be recruited or will this never happen?".
These have been addressed within the body of the assessment, which is attached.
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

The Safety Committee at Blacktrace Holdings Ltd will conduct and document risk assessments for all contained use of GMOs within Blacktrace. It includes individuals with qualifications in Molecular and Cell Biology, Immunology and Genomics and who has been working with GMOs within the laboratory since 2001. In addition, it will include the Site Health & Safety Officer who will also function as Biological Safety Officer. He has over 30 years of lab experience working with a range a biological hazards.

| Level 1 (GMMs) | Yes |
| Level 2 (GMMs) |     |
| Level 3 (GMMs) |     |
| Level 4 (GMMs) |     |
| Non-microbial  |     |
| Other (please specify) | The Safety Committee at Blacktrace holdings Ltd will conduct and document | Tick if confidential |
The work at Blacktrace will not involve GMMs, but all GMO-related waste will be disposed as prescribed in the guidance from the Department of Health’s “Environment and sustainability Health Technical Memorandum 07-01: Safe management of healthcare waste”.

<table>
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<tr>
<th>Bacteriology</th>
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<th>Microbiology Research</th>
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<td>Mycology</td>
<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
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**Other(s)**

- Contained use within laboratory at Blacktrace will make use of Class 1 GMOs (mammalian cell lines).

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

The work at Blacktrace will not involve GMMs, but all GMO-related waste will be disposed as prescribed in the guidance from the Department of Health’s “Environment and sustainability Health Technical Memorandum 07-01: Safe management of healthcare waste”.

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

**Please enter comments of the GM safety committee on the risk assessment**

The Safety Committee at Blacktrace Holdings Ltd will conduct and document risk assessments for all contained use of GMOs within Blacktrace. It has personnel with qualifications in Molecular and Cell Biology, Immunology and Genomics and has been working with GMOs within the laboratory since 2001. In addition, it will include the Site Health & Safety Officer who will also function as Biological Safety Officer. He has over 30 years of lab experience working with a range a biological hazards.
GM Centre Number: 3406

Data Premises Notified (Originally) 16/04/2018

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

FREELINE THERAPEUTICS

Name 2

Department

Campus Estate or Research Centre

STEVENAGE BIOSCIENCE CATALYST

Building

Road Name

GUNNELS WOOD ROAD

District

Town

STEVENAGE

County

HERTFORDSHIRE

Postcode

SG1 2FX

Country

ENGLAND

Tel Number 01438 906870

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Dr M D is the chairman of a 10-member SBC GM committee. Dr D has 25 years' experience working with GMOs and has previously served as GM Officer in the Dept of Life Sciences at Imperial College. The GM committee has a wide range of experiences in GMO R&D.

<table>
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<td>Tick if confidential</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste Disposal
- Glassware- decontaminated with 5% Distel for 1 hour contact time
- TC plasticware and agar plates- places in yellow clinical waste bags and transported via high sided trolley to Freeline's waste collection bins in the SBC storage area. Bins are collected by Grundons waste management and taken off site for incineration as offensive waste for final treatment.
- Liquid waste- treated with 5% distel for 1 hour contact, then disposed of down the sink.
- Hard surfaces: Distel spray for 1 hour contact time, 10mins for metal parts (longer can cause corrosion)
- Safety cabinets: Distel spray for 10 minute contact time

Distel is a laboratory disinfectant: Broad spectrum efficacy within short contact times-30 seconds to 5 minutes; Sporicidal, mycobactericidal, virucidal, fungicidal, and bactericidal efficacy; Performance validated with a wide range of peer-reviewed scientific publications; acknowledged and recommended by professional societies, public and private healthcare instruction.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Dr D and the committee have advised Freeline Ltd on evaluating GMO handling and waste disposal risks and are satisfied that they are acting appropriately, responsibly and with the best practice of the SBC. The R&D does not contain anything non-standard or exceptional that requires special consideration. The SBC GM Committee have approved the associated risk assessments and support this notification.
Adeno-Associated Viral vectors will be used to deliver therapeutic genes

Recipient or parental organism
HEK-293 cells and HEK-293T cells

Host/vector system
Adeno Associated Virus: The proprietary versions of these vectors have been modified from the wild type to make them safe for use in human therapeutics. They have been altered to be replication incompetent and contain a benign reporter transgene (green florescent protein) GFP - minimising concerns for both operator and environment.

Origin & function
The transgene will be either a reporter gene for example GFP or a gene for a protein which will restore functional levels in human disease.

Evaluation of foreseeable effects
The vectors used in this project are being developed for human therapies and thus have been significantly engineered to alter parts of the genetic code to create proprietary vectors that have a better safety profile than wild type.

The key alteration is that all vectors have been altered to be replication incompetent. This means that they pose a minimal concern to the environment should they be released as they cannot replicate in any cell or organisms they are able to infect. The vectors used also pose minimal to no chance to infecting plant or livestock as they are...
developed/chosen to transfect human cells. These vectors have the ability to infect humans via a number of methods e.g. direct contact with skin, mucous membrane of the eye, nose and mouth, ingestion, or injection. Infection could lead to the expression of the transgene; insertional mutagenesis or an immune response.

However the transgenes of the vectors used do not encode for oncogenes, growth factor receptors or toxin molecules. The reporter transgenes used in this work will be green fluorescent protein and beta gal to minimise the chance of harm to either the environment or personnel. If an individual is infected the vectors are replication incompetent and so will be unable to propagate throughout an individual.

There is the possibility that if an organism is also infected with a replication competent wild type vector that that transgene vector will also gain this ability. However control measures implemented as outlined below make this a highly unlikely event.

HEK-293 cells and HEK-293T cells
HEK 293 cells were generated in 1973 by transformation of cultures of normal human embryonic kidney cells with sheared adenovirus 5 DNA. As this cell line includes adenovirus genes it allow the propagation of the viral vectors used which have been engineered to become replication incompetent.

The HEK cell lines pose minimal potential damage to the environment as they have been adapted to an artificial cell culture environment under laboratory conditions and so are unlikely to thrive in the external environment if released.

It is not the aim of this project to propagate virus for experimentation but rather use these cell lines to test the infectivity of the purified vectors via quantification of successfully transfected reporter transgene. Therefore the adherent cultures will be grown in multiwall plate formats of well columns less than 5mL (<500,000 cells per well). And not more than 1L of cell culture volume total. These wells will be infected with low viral concentrations (anticipated to be less than 10E6 per well). This makes handling and destruction of the viral infected cells easier to control, therefore minimising risk to operator or environment.

Rational of classification:
After a review of the information sources and discussions with our GMO committee:

Class 1
Vector: the rationale that vectors are replication incompetent and express what were considered to be biologically benign transgenes suggest a low risk for this biological material.
Cell lines: HEK 293 and HEK 293T cells are engineered to ideal lab based cell culture conditions and are highly unlikely to survive in the external enviroment. E.Coli strains DH5 alpha (New England Biolabs) and Stabl3 (Thermofisher), are both K-12 derivatives used to propagate the plasmid and are non-pathogenic.

Class 2
The import and investigational use of animal tissues with or without AAV vector use determines that the laboratory should maintain a Class 2 containment level.

Based on one essential category requiring Class 2, our laboratory will apply Class 2 containment.

Control measures adopted:
Records and tracking of materials used are kept as part of Freeline good lab practice which involves hard copy lab books. All materials are clearly labelled and stored in specific safe storage areas within the facilities.
- PPE: lab coats, gloves and goggles to be worn at all times. This is to reduce the possibility of any physical contact with the vector.
- To avoid exposure to aerosol/droplets viral manipulations should be conducted in biological safety cabinets with HEPA filters, conforming to a containment Level 2
(CL2) to prevent release and possible inhalation.
- Sharps used in the laboratory are restricted to 1; Dialysis bag filling 2; Electrophoresis Gel Cutting.
- Centrifugation must be conducted in closed containers and using sealed rotors. Rotors must be opened in a Biosafety cabinet.
- Once the vector has been disrupted during preparation for nucleic acid or protein analysis it is safe to be used outside of the cabinet (PPE as outlined above should still be used).
- All wastes treated to destroy GMO material (see disposal section below)
- Volumes of dilute virus material to be kept under <1L of 1E13 virus particles to make waste handling and disposal easier and to reduce the severity of any adverse events
- Volumes of concentrated virus material to be kept under 20mL of 1E15 virus particles. This allows easy handling in the biosafety hood.
- HEK 293, HEK 293T cell lines (volumes less than 1L). Once cells have been transfected they should only be handled in closed containers or in the CL2 cabinet
- All vector material when stored in fridge/freezer will be clearly labelled as to its contents.
- All experiments take place in the Stevenage Bioscience Catalyst which is on a secure site as part of the Glaxo Smith Kline Campus. All areas where the virus is stored are only accessible by employees (via keycard or lock and key). Visitors are supervised and wear badges to identify them.
- Training for the use of vector material will be conducted by users who have prior experience of handling the vector material. The completion of training will involve the adding of the individuals name to this risk assessment. The signature will serve as a record of the training and be kept on file as part of the Freeline training records.
- Where vector material is transported this will only be done in sealed containers which are labelled and placed inside sealed plastic packaging. These in turn will be put into larger boxes, sealed and the box labelled with Freeline’s name, address and contact details with the instruction not to open unless authorised.
- There will be no lone, of out of hours, work for this project.
- The Freeline Health and Safety Policy covering lab practice and use of COSHH is to be followed at all times.

Protein Purification in the AKTA Pure system
- The AKTA pure system does not have an enclosed fraction collector therefore the system is housed inside a CL2 cabinet, where the work is conducted.
- Viral material loaded to the loop inside the CL2 cabinet;
- Parafilm should be wrapped around the connections;
- Delta pressure alarm should be less than 2 MPa;
- To avoid the formation of aerosols, 10 minutes should be the minimum of time between the last fraction collected and before opening the drawer.

Together the implementation of these control measures make this project a “LOW” risk.

First Aid:
EYE EXPOSURE FROM SPLASH OR AEROSOLS: Rinse a minimum of 15 minutes in eye wash available at all sink hand wash areas or flush area with water. Seek medical attention.
CONTACT WITH SKIN: wash thoroughly with disinfectant. Hand sinks available in all labs for washing.
INHALATION EXPOSURE FROM AEROSOLS: Seek medical attention.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Disposal
- Vikon is used as it is a validated cleaning method for viral vector material and transfected cells if used in the following way under manufacturers recommendations.
- Distel is used as it is a validated cleaning method for viral vector material and transfected cells if used in the following way under manufacturers recommendations.
• Hard surfaces: A solution containing 5% Distel for 1 hour contact time, 10mins for metal parts (longer can cause corrosion)
• Safety cabinets: 5% Distel (10mins) followed by 70% IMS.
• Discard jars, plastic tissue culture flasks, glassware: A solution containing 1% Virkon.
• Ensure all surfaces are in contact with the disinfectant (10mins). Added to sealed bags for autoclaving at a minimum of 1210°C, 1.15bar, 15 mins hold.
• Supernatants/liquid waste: 1% Virkon with minimum of 1 hour contact time.
• All autoclaved solid waste is to be placed into hazardous waste bags. There are 2x autoclaves available and the equipment is regularly serviced to ensure it maintains its ability to be a validated cleaning method. Chart recorder or display screen is attached to autoclave to monitor treatment.
• Full bags are to be transported to Freeline’s hazardous waste bins in the SBC storage area
• Bins are collected for off site incineration by Grundons waste management.

Accidental Release:
In a contained area Distel should be applied all over the split area. The solid waste is then to be autoclaved as above.
In an uncontained area, all persons should evacuate the area to avoid contact with any potential aerosols formed. Only after 1 hour the room might be entered (to allow any aerosols to settle) and the procedures should be the same as in a contained area.
The floor is sealed and the lab is built to CL2 level.

Project Containment

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02/03/2022
### Project Ref 3406/19.2

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- **Withdrawn**: N
- **Tick if notifying a connected programme of work**: N

#### Historical Significant Changes

#### Historical Date of Additional Info

#### Significant Change ID

#### Date of Significant Change

### Project Additional Information

#### Purposes of the contained use

Our project aims to use recombinant AAV to treat rare disorders such as Haemophilia and Lysosomal storage disorders. rAAV constructs will be evaluated to create the vector with the best performance to take forward to clinical trial.

- **Containment level 2 for working with a Group 2 biological agent and class 2 activities.**
- **Development of novel AAV capsid variants by a directed evolution approach.**
  - This system requires the use of human adenovirus type 5 to facilitate virus library replication.
  - Basic procedure requires cells in culture to be infected with a shuffled AAV vector library that is capable of replicating. Co-infection with a helper virus (Adenovirus 5) facilitates library replication. After successive rounds of infection, AAV capsid DNA is isolated and sequenced to identify novel capsid variants. These capsid variants will then be vectorised into the recombinant AAV (rAAV) system for evaluation.
- **Target validation / In vitro disease models**
  - This requires modulation of protein expression in vitro cell culture systems. Work involve using self-inactivating replication incompetent lentivirus/retrovirus, either overexpress or knockdown genes encoding for lysosomal hydrolases (e.g. GLA, GBA) in order to modulate protein expression in various primary cells or cell lines.
  - Lenti (2nd and 3rd generation) will be used to maximize laboratory biosafety procedures.
**Recipient or parental organism**

- HEK-293 and HEK-293T cells
- Hepatic cell line Huh-7
- other mammalian primary cells or cell lines

**Host/vector system**

- **E.Coli**
  - Strains DH5 alpha (New England Biolabs) and Stabl3 (Thermofisher), are both K-12 derivatives. These strains are non-colonising, disabled, and are non-pathogenic to humans and animals. These E.Coli strains will be used to propagate plasmids.

- **Wild type AAV**
  - Adeno-associated virus is a small (approximately 25 nm in diameter), nonenveloped, icosahedral, non-pathogenic parvovirus. AAV infects cells through a receptor-mediated binding and uptake, endosomal release, trafficking of viral particles to the nucleus and unloading of the viral DNA in the nucleus.

- **Recombinant AAV**
  - Recombinant AAVs (rAAV) are generated by gutting the AAV viral genome of the rep and cap genes and replacing with a proprietary cassette which includes a promoter, transgene and poly adenylation signal. The transgene cassette is flanked by the inverted terminal repeats, which are the only remaining viral DNA sequences. In order to produce rAAV HEK 293 or 293T cells are transiently transfected with a double or triple plasmid combination to provide the transgene cassette, the rep, Cap and adenoviral helper functions. In Freeline’s proprietary vectors the transgene will be either a reporter gene for example GFP or luciferase; or a gene for a protein which will restore functional levels in human disease, for example Factor IX for hemophilia. All the transgenes used will encode modified versions of nononcogenic, non-toxic proteins. Promoters used will either drive constitutive expression, or a tissue specific expression. Freeline Therapeutics is currently using a novel capsid S3 which is derived from two naturally occurring AAVs and developing novel capsids which are better suited to transducing human tissues.

- **Wild type Adenovirus Ad5** will be purchased from ATCC.
  - Strain: Adenoid 75 Classification: Adenoviridae, Mastadenovirus.
  - Adenoviruses are nonenveloped viruses with a double stranded DNA virus. They have a broad range of hosts.

- **Lentivirus**
  - Self-inactivating replication incompetent lentivirus (viral particles or plasmids) based on viral vector such pLKO-1 backbone will be purchased from established vendors like Sigma-Aldrich, Applied Biological Materials, or Origene. The shRNA expression is controlled by the U6 promoter in the case of the shRNAs or the human cytomegalovirus (CMV) immediate-early enhancer and promoter or liver specific promoters (LSP) to knockdown or...
overexpress the genes of interest, respectively.
pCMV-dR8.2 dvpr (e.g. Addgene plasmid #8455) and pCMV-VSVG (e.g. Addgene plasmid #8454). HEK 293T cells are used to produce lentivirus.

Origin & function

The transgene will be either a reporter gene for example GFP or a gene for a protein which will restore functional levels in human disease

AAV
1. The plasmid for the transgene cassette will contain either a tissue specific promoter, typically a liver specific promoter or a constitutively active promoter. The transgene will encode a protein variant of a human factor IX protein, or an antibody fragment. Reporter genes used will typically be commercially available GFP or luciferase which are categorised as nononcogenic. Sequences will be validated by the vector development team.
2. Plasmids will contain the antibiotic resistance genes ampicillin or kanamycin for selection. These resistance genes will not be incorporated into the rAAV.

There are no inserted transgenes within the AAV replication competent library. The only gene products are Rep and Cap proteins associated with normal virus life cycle.

Adenovirus
3. There are no inserted transgenes within the Adenovirus. The only gene products will be proteins associated with normal virus function.

Lentiviral vectors (LV)
4. The viral vector carries a promoter such as U6 or a Liver specific promoter or strong/medium promoters such as CAG, PGK, CMV for stably expression cell line creation.
5. Vectors carrying an shRNA or a cDNA sequence to modulate the expression of a particular gene e.g. lysosomal enzyme genes such as GLA and GBA.

DNA sequence to be included in rAAV will be obtained by gene synthesis synthetic promoters may be used to drive expression of gene of interest. DNA sequence to be included in the AAV library will be derived by a combination of gene synthesis (ITRs and Rep) and PCR (shuffled capsid genes).

DNA sequence is to be included in lenti- or retrovirus will be obtained by gene synthesis. Synthetic promoters will be used to drive expression of genes of interest. Genes will be obtained by gene synthesis and will correspond to genes present in the human genome.

Evaluation of foreseeable effects

AAV
The final GMO (transduced cells) pose very low or no hazard. The elimination of 94% of the viral DNA reduces the probability of homologous recombination with related viruses that could lead to variants of the GMO.
Like the parent organism, AAV, the vector is non-pathogenic. As the Freeline vectors have had all viral genes removed it is assumed they are also nonpathogenic. The vector currently has fully wild-type capsid proteins and the immune response is not expected to be different from that of the parental virus.

Ad5/rAAV infected cells: Risk Ad5/AAV infected cells pose minimal risk. There will not be any genetic inserts (such as oncogenes etc) present that pose any risk to human health. The majority of people in Europe are seropositive for AAV and Adenovirus neutralising antibodies. If infections were to occur, it is likely an acute and self limiting immune response will take place to clear the virus. A risk of a more serious infection from Adenovirus can occur in immunocompromised individuals therefore an individual showing a compromised immune system will not work with this system. LV transduced cells should not acquire any pathogenicity or potential harm. The vectors used in this project are being developed for human therapies and thus have been significantly engineered to alter parts of the genetic code to create proprietary vectors that have a better safety profile than wild type. HEK-293 cells and HEK-293T cells
HEK 293 cells were generated in 1973 by transformation of cultures of normal human embryonic kidney cells with sheared adenovirus 5 DNA. The HEK cell lines pose minimal potential damage to the environment as they have been adapted to an artificial cell culture environment under laboratory conditions and so are unlikely to thrive in the external environment if released.

Rational of classification:
After a review of the information sources and discussions with our local GMO committee:
Class 1
rAAV Vector: the rationale that vectors are replication incompetent and express what were considered to be biologically benign transgenes suggest a low risk for this biological material.
Cell lines: HEK 293 and HEK 293T cells are engineered to ideal lab based cell culture conditions and are highly unlikely to survive in the external environment.
E.Coli strains DH5 alpha (New England Biolabs) and Stabl3 (Thermofisher), are both K-12 derivatives used to propagate the plasmid and are nonpathogenic.
Class 2
The import and investigational use of animal tissues with or without AAV vector
The use of Lentiviral vectors for target validation: this requires modulation of protein expression in vitro cell culture systems. Lenti (2nd and 3rd generation) will be used to maximize laboratory biosafety procedures.
Ad5/AAV co-infection for capsid development: basic procedure requires cells in culture to be infected with a shuffled AAV vector library that is capable of
replicating. Co-infection with a helper virus (Adenovirus 5) facilitates library replication. After successive rounds of infection, AAV capsid DNA is isolated and sequenced to identify novel capsid variants. These capsid variants will then be vectorised into the recombinant AAV (rAAV) system for evaluation.

Control measures adopted:
All materials are clearly labelled and stored in specific safe storage areas within the facilities.
• PPE: lab coats, gloves and goggles to be worn at all times. This is to reduce the possibility of any physical contact with the vector.
• To avoid exposure to aerosol/droplets viral manipulations should be conducted in biological safety cabinets with HEPA filters, conforming to a containment Level 2 (CL2) to prevent release and possible inhalation.
• All wastes treated to destroy GMO material (see disposal section below)
• HEK 293, HEK 293T cell lines (volumes less than 1L). Once cells have been transfected they should only be handled in closed containers or in the CL2 cabinet
• All vector material when stored in fridge/freezer will be clearly labelled as to its contents.
• Training for use of the vector material will be conducted by users who have prior experience of handling the vector material. The completion of training will involve the adding of the individuals name to this risk assessment. The signature will serve as a record of the training and be kept on file as part of the Freeline training records.
• The Freeline Health and Safety Policy covering lab practice and use of COSHH is to be followed at all times.
Together the implementation of these control measures make this project a "LOW" risk.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Disposal
• Vikon is used as it is a validated cleaning method for viral vector material and transfected cells if used in the following way under manufacturers recommendations.
• Distel is used as it is a validated cleaning method for viral vector material and transfected cells if used in the following way under manufacturers recommendations.
• Hard surfaces: A solution containing 5% Distel for 1 hour contact time, 10mins for metal parts (longer can cause corrosion)
• Safety cabinets: 5% Distel (10mins) followed by 70% IMS.
• Discard jars, plastic tissue culture flasks, glassware: A solution containing 1% Virkon.
• Ensure all surfaces are in contact with the disinfectant (10mins). Added to sealed bags for
autoclaving at a minimum of 1210°C, 1.15 bar, 15 mins hold.
• Supernatants/liquid waste: 1% Virkon with minimum of 1 hour contact time.
• All autoclaved solid waste is to be placed into hazardous waste bags. There are 2x autoclaves available and the equipment is regularly serviced to ensure it maintains its ability to be a validated cleaning method. Chart recorder or display screen is attached to autoclave to monitor treatment.
• Full bags are to be transported to Freeline’s hazardous waste bins in the SBC storage area
• Bins are collected for off site incineration by Grundons waste management.

Accidental Release:
In a contained area Distel should be applied all over the split area. The solid waste is then to be autoclaved as above.
In an uncontained area, all persons should evacuate the area to avoid contact with any potential aerosols formed. Only after 1 hour the room might be entered (to allow any aerosols to settle) and the procedures should be the same as in a contained area.
The floor is sealed and the lab is built to CL2 level.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The Stevenage Bioscience Catalyst (SBC) is an incubator hub for small and emerging biotechnology start-up and spin-out companies. It is a UK Government backed (Innovate UK) enterprise in collaboration with the Wellcome Trust and GSK. The SBC provides technical, business, organisational and commercial support to its tenant companies and also monitors Health and Safety compliance. It has set up a GMO committee to ensure that tenants are assessing the risks associated with their work appropriately and to disseminate best practice among researchers. Dr Deonarain is the chairman of this committee and has 25 years experience working with GMO in academic and commercial settings and was Chairman of Imperial College Life Sciences GMO committee for 4 years.
The SBC committee has reviewed and advised Freeline on its GMO risk assessment and are satisfied that they have considered the risks in terms of GM organisms and scale of work. They have appropriate control measures to contain the work and appropriate procedures for the class of GMO activity for waste disposal.

Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Name**

EVOX THERAPEUTICS LTD

**Campus Estate or Research Centre**

**Building**

MEDAWAR BUILDING 2, 2ND FLOOR

**Tel Number**

01865 819140

**Fax Number**

0

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

![Yes]

Give brief details of the genetic modification safety committee

Several meetings held and advice received for genetic modification work from a contracted Safety Consultant with many years experience of microbiology, genetic modification work and biosafety management.

A company Genetic Modification Safety Committee has been formed. This comprises of the Biological Safety Officer, Health and safety officer, Head of research at Evox Therapeutics, and a contracted Safety consultant and staff representatives. All members have at least 10 years of biological laboratory experience, and some of whom have over 10 years industrial level BSL2 experience.

The committee will meet at least quarterly.

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 3 (GMMs)</td>
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</table>
All liquid waste generated during bacterial culture or will be autoclaved at 121°C and 100kPa, for 15 minutes. The sterile liquid will be disposed to drain. All liquid waste generated during mammalian cell culture will be treated with 1% Virkon (or equivalent disinfectant used at the manufacturer's recommended concentration) for a minimum of 4 hours, then disposed to drain. No more than 10L of either culture will be generated per week.

All solid wastes generated will be disposed of via a special waste contractor (Grundon) and sent for incineration or alternative treatment. All sharps will be placed into sharps containers and removed via the special waste contractor for incineration.

Members present:
Justin Hean (Biosafety Officer) Over a decade of experience in academic and industrial and academic BSL1 and BSL2 labs.
David Virley (Head of Research) 15+ years experience in BSL2 research within an industrial setting.
Mark Thornton (H&S Officer) 15+ years experience in BSL2 research within an industrial setting.
Sharon Wood (H&S experienced consultant)

Members of the committee were satisfied with the BSL1 risk assessment. Sharon Wood noted to define the parameters of liquid waste treatment – Virkon 1% final concentration for 4 hours.
The use of retroviral and lentiviral vectors to facilitate transgene expression in mammalian cell and tissue culture systems.

Purposes of the contained use

Here, we aim to generate stably expressing cell lines using 3rd or higher generation lentiviral or retroviral transduction systems. The cell lines may be of common commercial, academic or primary origins. The stable cell lines will express fusion proteins which generate exosomes and extracellular vesicles enriched with therapeutics proteins of interest.

Recipient or parental organism

Both parental (viral progenitor cell line) and recipient (transduced cell line) are auxotrophic cell lines and is deemed extremely unlikely for the cells to survive within the environment. Cell lines are sourced from commercial or academic sources, and where possible, are deemed fit for use under containment level 1. Where appropriate, cell lines requiring containment level 2 will be cultured and treated according to the associated CBAl notification.

Host/vector system

Plasmid DNAs will be transfected into mammalian cells to facilitate the production of retroviral and lentiviral particles. The packaging cell lines are of murine or human origin and are well characterised and obtained from academic or commercial sources. The inserted viral sequences permit the production of retroviral and lentiviral particles. The viral sequences are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of the packaging cell
The viral sequences are from either HIV, SIV, FIV, EIAV or other commonly used retro- or lentiviruses. Wild type EIAV is a specified animal pathogen however in this system deletion of a number of genes (52, tat, and rev) renders the particles replication defective and recombination events could not replace the deleted genes. This makes the virus unable to cause a productive infection and therefore not pathogenic.

Third or later generation lentiviral systems will be used, incorporating a self-inactivating 3' L TR (SIN) U3 deletion. These systems typically utilise 4 or 5 separate plasmids; one encoding viral gag and pol, another (where necessary) encoding viral rev and one or two plasmids encoding the envelope protein(s). These together with the recombinant viral 6 genome, containing the insert of interest (for over expression or silencing) are transfected into the packaging cell line, typically 293T or similar cells, enabling the production of replication defective, infective particles. The recombinant viral genome may be constructed with or without the Polypurine Tract (cPPT) and/or the Woodchuck Posttranscriptional Regulatory Element (WPRE) or similar sequences to enhance expression levels of the insert and/or viral titre. Envelopes that facilitate transduction of a wide range of cell types will be used including the commonly used ecotropic and amphotropic envelopes (Morgan et al., 1993, Journal of Virology 67:4712), VSV-G envelope (Emi et al., 1991, Journal of Virology 65:1202), the SeV/HN/SeV-F envelope system that has similar tropism to V5V-G (Kobayashi et al., 2003, Journal of Virology 77:2607) and baculovirus GP64 envelope that has similar tropism to V5V-G (Kumar et al., 2003 Human Gene Therapy 14:67).

Origin & function

Genetic material is derived from commercial sources or is synthesised through appropriate vendors, (such as IOT). Where deemed necessary, genes, expression cassettes or open reading frames are subjected to codon optimisation technology through appropriate online vendors.

Genetic material encoding exosome enrichment factors - sequences are derived from either mammalian or viral sources. Expression products are demonstrated to enrich within the endosomal/exosomal pathway, thus associate with exosomes and extracellular vesicles. As part of the fusion technology, these components are responsible for driving the enrichment of therapeutic products into exosomes and extracellular vesicles. Sequences, their derivatives and products are deemed non-harmful to humans and the environment.

Genetic material encoding investigative tools - These sequences may originate from a variety of sources, e.g. eGFP from Aequorea victoria and Luciferase from Phytinus pyralis. These tools are frequently used in molecular biology assays to determine exosome loading efficiency, cellular uptake efficiency and biodistribution studies. Sequences, their derivatives and products are deemed non-harmful to humans and the environment.

Genetic material encoding therapeutic products - In most instances, therapeutic product sequences will originate from Homo sapiens, however in some instances other mammalian sequences may be used (where sequence homology appropriate). Furthermore, therapeutics sequences may be derived from viruses (albeit unlikely). Following the exosomal enrichment of the therapeutic product via its fusion with the endosomal enrichment factor, application of the purified, loaded exosomes induces a cargo dependent response in recipient cells. Again, as the loaded proteins would be deemed of therapeutic in nature, their sequences and/or derivatives are considered non-harmful to humans and the environment.

Evaluation of foreseeable effects

The retroviral and lentiviral particles produced will have a broad tropism and be capable of infecting and transducing human and other mammalian cells, inserting viral and mammalian and/or reporter gene sequences (see below) into the host genome. This is an anticipated risk associated with the use of retroviral and lentiviral gene transfer vectors. The viral particles have however been rendered non-replicative by a number of safety features:

1) The viral genomes are self-inactivating, carrying a deletion in the U3 region of 3’ L TR. During integration into the host cell genome, the viral 3’ L TR is copied to the 5’ of viral genome rendering it transcriptionally inactive and unable...
1) To function as a replicative retroviral genome.
2) The viral genome does not contain any of the viral packaging or structural genes necessary for viral replication.
3) The viral packaging and structural genes factors necessary for viral particle production are supplied in the producer cell line in trans, either by the producer cell line itself or by co-transfection with packaging plasmids. Regions of homology (e.g. LTRs or packaging sequences) in the viral particle genome have been minimised to eliminate undesirable recombination events that could lead to the generation of replication competent virus (RCV).
4) In the case of lentiviral vectors, the viral particle genomes contain exogenous promoter sequences to permit transgene/RNAi factor expression. This allows viral tat gene, essential to wildtype lentiviral replication, to be completely eliminated from the packaging system. Thus no RCV can be produced.

Consequently, only retroviral and lentiviral particles which are unable to replicate, but which can deliver the transgene/RNAi insert of choice will be produced. These viral particles cannot contain additional viral genes as their sequences lack the LTR or packaging sequences necessary for their sequences to be incorporated into the viral particles.

Accidental human exposure to viral packaging cells carries minimal risk as the inserted sequences are not expected to affect the pathogenicity of the cells. It is anticipated that any cells would be rapidly cleared by the complement/immune system of any exposed individual.

Accidental human exposure to viral particles could lead to viral infection and the insertion of viral and mammalian and/or reporter gene sequences into the host genome. Importantly, in the context of human gene therapy clinical studies, high doses of retroviral and lentiviral vector particles have been administered in vivo with no observed complications. However, in the worst case scenario, integration within a tumour suppressor gene or the biological activity of the integrated sequences could lead to the generation of a tumour. Indeed, leukaemia-like tumours have been observed in a small number (~10%) of individuals infused with bone marrow stem cells that had previously been treated ex vivo with retroviral vectors. Despite these observations it is considered highly unlikely that accidental human exposure to the viral particles described in this risk assessment would lead to the generation of a tumour. Major differences between the described worse case scenario and any accidental exposure exist including large differences in transgene/host interactions, likely exposed viral particle numbers, in vivo rather than ex vivo delivery route, consequent exposure of viral particles to complement/immune system, likely access to relevant precursor stem cells and immune status of individuals involved.

Consequently, it is not anticipated that accidental human exposure to viral particles is associated with significant risk. However, given the nature of some of the mammalian gene sequences (discussed above), gene transfer could be hazardous.

The viral particles are infectious but cannot replicate. There is no evidence for vector transmission. The consequence of the hazards being manifest is considered to be low and the likelihood of manifestation is low when containment assigned above is taken into account. In all cases, taking into account the control measures assigned, the overall risks to the environment from the genetically modified micro-organisms produced in this work is effectively zero. Therefore no additional containment or control measures are considered necessary to protect the environment other than those described to protect human health and safety.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or alternative treatment.

Liquids (e.g., bacterial and mammalian culture media, culture supernatants, viral samples) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or disinfect with 2% Virkon for at least 30 minutes, discharge to drains.

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or disinfect with 2% Virkon for at least 30 minutes, discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or alternative treatment.

Degree of kill
- Autoclaving, effectively 100% kill (annual validation)
- Incineration, effectively 100% kill (licensed incinerator)
- Chemical disinfection with Virkon, used according to manufacturers instructions under standard conditions, manufacturers validation [e94.79] 109 reduction (log 99.998)% kill.

Members present: Justin Hean (Biosafety officer), Mark Thornton (H&S officer), Sharon Wood (H&S consultant).

Members of the meeting were satisfied with the risk assessment and CU2 notification.

Sharon Wood further noted to confine agar plate waste disposal to autoclaving, incineration and alternative treatment.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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<thead>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

The Directors of Biomakespace Ltd have appointed a Safety Committee (SC) which is responsible for reviewing and providing advice on contained use risk assessments to the management. The SC consists of the Biological Safety Officer (BSO), two Directors of Biomakespace Ltd, and any additionally appointed Deputy Biological Safety Officers with relevant expertise as necessary. Principal investigators are required to submit risk assessments to the SC, who will review and provide advice on adequacy of the risk assessment. The BSO will integrate this advice into the risk assessment, approve or not and give final advice to the person responsible. The members of the SC represent a balance of experienced scientists, management, and lab users. The committee members include individuals with extensive postgraduate biological research experience. The BSO, particularly, has an extensive background working in class 1 and 2 contained use settings.

Through partnerships with local universities and other scientific institutions, the SC has access to a wide range of experts from who are willing to provide advice on risk assessments for contained use. In particular, we have sought specific advice from the Biological Safety Officers of the School of Clinical Medicine and the Department of Pathology at the University of Cambridge.

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All wet lab users with GM projects receive an induction detailing the procedures for the handling and containment of GMOs, and this includes a section on waste management and monitoring measures. These procedures are regularly reviewed and updated by our internal safety committee, and also by external review by competent assessors and are detailed in our Biological Safety Policy. Compliance is enforced by the BSO with the full support of the management.

According to established practice all waste (solid and liquid) that have come into contact with GMOs are decontaminated by one of two means, dependent on the risk assessment:

1) Disinfected by Virkon according to manufacturer’s instructions. According to manufacturer’s testing this provides 99.999% kill efficacy using a wide variety of contact times, temperatures and organic challenge levels that are consistent with the organisms and conditions proposed.

2) Autoclaved at 121 C at 15 psi for at least 15 minutes with effectively 100% kill. Autoclaving is performed within the laboratory suite and the autoclave is tested annually to ensure that it is functioning to the manufacturer’s specification, and this is done in two ways, firstly using a programmed pressure/vacuum test is run and secondly autoclave test strips are used to ensure that the necessary temperature has been obtained. The results of these tests are recorded in a log book. A back-up autoclave is also available on-site.

These requirements are codified in our policies and SOPs and all lab users are required to follow them strictly.

Surfaces, worktops and equipment that may have come into contact with GMOs are cleaned with 1% Virkon solution before and after a protocol is performed, as per manufacturer’s instructions.

In the case of unanticipated spillages and similar accidents in the lab, the spillage will be covered with Virkon powder, left for three minutes, scraped into a safe receptacle and the area washed with 1% Virkon as per manufacturers instructions and as detailed in the Biological Safety Policy.
The Safety Committee reviewed the risk assessment and asked for further detail in terms of the project proposed. Following submission of these details they approved the risk assessment and approved the level of contained use and procedures described.
**GM Centre Number: 3409**

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02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

Chief Technology Officer, Laboratory Manager, Senior Scientist

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Tick if confidential

No
**Bacteriology** | Yes | **Parasitology** |  | **Transgenic** | Birds | **Microbiology** | Yes | **Research** |
---|---|---|---|---|---|---|---|---|
**Virology** |  | Transgenic | Animals |  | Transgenic | Fish |  | Gene Therapy |
**Mycology** | Transgenic | Invertebrates |  | Transgenic | Plants | Yes |  | Other (please specify below) |
**Other(s)** |  |

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Clear clinical waste bags for solid biological waste only (including plates etc.) In autoclavable boxes with lids in each area where Class1 work is carried out. Collected and autoclaved daily. Liquid biological waste in glassware will be decontaminated with Bleach or by autoclaving via the Kill Boxes. Liquid biological waste in plastic containers will be decontaminated with bleach. Plant material will be autoclaved or steamed. Autoclave Thermologs will be used to test loads once per month and after servicing. Autoclave charts should always be used when operating the autoclaves.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

**Please enter comments of the GM safety committee on the risk assessment**

The committee that the risk of causing arm is minimal as long as GLP and all safety precautions are taken.
Good laboratory practice is to be adhere to and all stuff will be instructed in GLP.
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**Name**

| MINA THERAPEUTICS LTD |

**Campus Estate or Research Centre**

| TRANSLATION & INNOVATION HUB |

**Road Name**

| 80 WOOD LANE |

**Town**

| LONDON |

**County**

| GREATER LONDON |

**Postcode**

| W12 0BZ |

**Country**

| ENGLAND |

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**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022 |
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Y**

Give brief details of the genetic modification safety committee

- Laboratory Manager- holder of NEBOSH Certificate in General Health and Safety with previous experience of GMM containment and risk assessment, Chief Executive Officer, Chief Scientific Officer, Clinical Trial Manager, Senior Research Scientist, Laboratory Technician

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Waste disposal will be carried out according to an SOP. All solid waste is to be autoclaved before disposal carried out via Imperial College Innovation & Translation Hub central facility by incineration using a licenced outside contractor. All liquid waste is disinfected using a commercially available proprietary disinfectant solution and following the manufacturers instructions for maximum kill. Non-disposable labware is disinfected and washed prior to re-use. All spills to be cleaned using proprietary reagents e.g. spill kits. No further monitoring is deemed necessary due to the low/zero hazard presented by the GMM involved.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

For activities involving GMMs, describe the waste management measures which will apply to the activity

The disposal of waste was discussed, and the committee made aware of the use of Virkon as the disinfectant of choice. No other comments.
**GM Centre Number: 3411**

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

Advice on the contained use has been provided by the Biological Safety Officer of Talisman Therapeutics. The BSO has experience in assessing the risk of GMOs and carrying out GMO work. The BSO has a track record as postdoc of planning, supervising, managing and assessing the risk of GMO work. Additional advice has been provided by the Chief Scientific Officer who is also a research group leader at the Gurdon Institute of the University of Cambridge and has a long-standing track record in planning, supervising and assessing the risk of GMO work. Additionally, a GMO committee has been formed at Talisman Therapeutics consisting of the BSO, Talisman's Head of Research and the Health and Safety Officer. The GMO committee convenes quarterly or more frequently if required. It provides advise on risk assessment, organises training, reviews and updates local rules and ensures compliance with regulations.

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Tick if confidential

Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research |

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste is inactivated by autoclaving using an autoclave in the same building. Liquid waste is transported to the autoclave using secondary containment. For GMM inactivation a 'liquid cycle' at 121 degree Celsius for 15 minutes is used. Waste treatment by autoclaving ensures practically 100% inactivation. Inactivated liquid waste is then disposed of via the lab sink. The autoclave is serviced and maintained annually to ensure it is in proper working order. We propose to inactivate solid plastic waste by incineration (details are provided below).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMO committee has reviewed the company's GM procedures and the risk assessments performed. The GMO committee confirmed the work to be carried out is Class 1 and were satisfied with the assessment of risks to both human health and the environment. The control and containment measures in place are appropriate for class 1 activities.
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**Name**

NORWICH RESEARCH PARTNERS LLP

**Name 2**

**Department**

**Campus Estate or Research Centre**

NORWICH RESEARCH PARK MANAGEMENT OF

**Building**

CENTRUM

**Road Name**

NORWICH RESEARCH PARK

**District**

**Town**

NORWICH

**County**

NORFOLK

**Postcode**

NRW 7UG

**Country**

ENGLAND

**Tel Number**

01603 673683

**Fax Number**

0

**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Discussions have taken place with a Biological Safety Officer (Level 1 Biosafety Practitioner and M-ISTR) from the Norwich Research Park. Discussions included classification of GM work and appropriate containment measures that should be employed and risk assessment of work activities.

Further advice has been taken from other members of the Biological Safety Committee from the Norwich Research Park, all possess the appropriate knowledge, experience and training in the scientific area in question.

The risk assessment has been reviewed and it has been agreed to be appropriate and provides sufficient assessment of the risks relevant to the activities being undertaken.

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<td>Non-microbial</td>
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</table>
Containment Measures and disinfection routes. M15 Specified disinfection procedures in place. REQUIRED where and to the extent the risk assessment shows it is required. For small volume spills, 70% ethanol will be used to disinfect. Larger volume spills 50 ml-2 L, paper towels for absorbing the liquid and then wipe down with 70% ethanol. Paper towels can be autoclaved. Containing larger volume spills will depend on the surface type and surface area of the spill. 2-10 L, a specified sponge and mop & bucket will be in place to absorb liquid. The liquid will be treated with a Virkon (to 1-2% w/v; NB any liquid with Virkon will not be autoclaved as it can produce sulphur dioxide gas upon heating) or a hypochlorite compound to manufacturer's validated specifications (this varies depending upon the product; such as Virkon, hypochlorite solutions will not be autoclaved). The mop, sponge and bucket once rinsed can be autoclaved and re-used. The large spills from fermenter leakage, 10-50 L, will be treated similarly, but the potential spill area will be limited by rubber strips surrounding the fermenter. All treated liquid will be stored for 24 hours before disposal.

Fermenters and dyeing machinery will have disinfection routines as per manufacturer's specifications. Normally this is equivalent to internal autoclaving.

NB: Disinfection is defined as 10^5 reduction in live organisms (i.e. 99.999% kill).

Result: CL 1

Waste handling
M16 inactivation for handwashing effluent NOT REQUIRED
M17 inactivation of microorganisms in contaminated material (see above)

As the recombinant microbes will be Hazard Group I organisms, after inactivation via validated means (e.g. 1-2% Virkon, 70% ethanol or autoclaving at the previously validated regime(s); see above) the disinfected material can be disposed of as non-hazardous waste

Autoclave - Any solid waste (paper towels, mop, bucket, etc) will be autoclaved on a validated cycle to ensure complete inactivation of any microbiological material. Media containing bacterial cultures will be inactivated using the same equipment. All autoclave loads will be treated at 121 C for a minimum of 20 minutes, but the length of the cycle will be dependent on the validation that is performed. For denser loads the length of time is likely to be increased to 30 minutes. The cycle will be validated to ensure that the kill requirements have been achieved before operation.

Controls (procedural and system) exist to ensure loads are processed to the required settings (time, temperature and pressure).
Please enter comments of the GM safety committee on the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
| Data Premises Notified (Originally) | 10/05/2018 | Transferred from 1992 Regs? | N | Transitional Premises Class | N |
| Data Premises Closed | Transitional Premises | N | Non-GMMs | N | Withdrawn | N |

**Name**
NUCLERA NUCLEICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**
137 CAMBRIDGE SCIENCE PARK

**Road Name**
MILTON ROAD

**Town**
CAMBRIDGE

**Road Name**
MILTON ROAD

**District**

**Town**
CAMBRIDGE

**County**
CAMBRIDGESHIRE

**Postcode**
CB4 0GD

**Country**
ENGLAND

**Tel Number**
07724898040

**Fax Number**
0

**E-mail**

**HSE Division**
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**Comments**

**Date at Which Additional Info Submitted**
02/03/2022
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Y

Give brief details of the genetic modification safety committee

Nuclera's genetic modification safety committee comprises the company's CTO and CCO. The CTO has 6 years of experience working with level 1 GMMs and the CCO has 10 years of experience working with level 1 GMMs. The CTO and CCO have performed risk assessments of level 1 GMM work primarily in academic environments.

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Yes

All class 1 GMM waste will be disposed of in clinical waste containers, EWC Code 18.01.04 for yellow sack waste and 18.01.01 for sharps waste.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

All class 1 GMM waste will be disposed of in clinical waste containers, EWC Code 18.01.04 for yellow sack waste and 18.01.01 for sharps waste.

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment:

The genetic modification safety committee comprises the CTO and CCO, with 6 and 10 years experience respectively in GMM level 1 work. The committee meets on a quarterly basis to ensure safe working conditions for employees and appropriate disposal measures to ensure zero impact on the environment.
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**Name**

VACCITECH LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

MAGDALEN CENTRE

**Building**

OXFORD SCIENCE PARK

**Road Name**

1 ROBERT ROBINSON AVENUE

**District**

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX4 4GA

**Country**

ENGLAND

**Tel Number**

01865 819 012

**Fax Number**

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**E-mail**

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**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Biosafety officer has 30 years of experience with laboratory work, and has been a member of two other biosafety committees.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Degree of kill: 100%

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The BSO (T E) confirms that he has reviewed the risk assessments relevant for this application and agrees with the assigned classifications.

### Project Ref 3415/19.1

**Date Ackn'd** | **CU2 Project Title** | **Class** | **Culture Vol**
--- | --- | --- | ---
20/03/2019 | Generation and research use of viral vectored vaccines | Class 2 | 1-50 Litres

---

[^3]: Page 14385 of 15326
Historical Significant Changes

Project notified under transitional arrangements

Historical Date of Additional Info

Date of Significant Change

Project Additional Information

Purposes of the contained use

To produce viral vectored vaccines against infectious diseases and cancer by insertion of transgenes into vector backbones and testing immunogenicity of these vectors.

Recipient or parental organism

1) MVA (modified vaccinia virus Ankara). This highly attenuated orthopox virus is not capable of replication in mammalian cells, except for baby hamster kidney (BHK) cells. MVA will only replicate in chicken embryo fibroblasts (primary CEF, or DF1 cell line) or BHK cells. MVA is not capable of causing an infection in humans or animals, although it will transduce human and animal cells (i.e. the virus will enter the cytoplasm of a cell and express the recombinant protein, but no infectious viral particles can be produced).

2) replication-deficient adenoviruses of human or simian origin. These viruses have been made replication-deficient by removal of the E1 gene and can only replicate in cell lines that express E1. The E3 gene has also been deleted. The vector is therefore not capable of causing an infection in humans or animals, although it will transduce human and animal cells (i.e. the virus will enter a cell and express the recombinant protein, but no infectious viral particles can be produced).

Host/vector system

see above. MVA or adenovirus, produced in MVA-producer cells (CEF or DF1) or adenoviral producer cells (HEK293 derivatives)

Origin & function
commercially synthesised DNA encoding human cancer antigens, specifically, cancer-testis antigens (CTA), for example of the MAGE family. The Melanoma Antigen Gene (MAGE) protein family is a large, highly conserved group of proteins that share a common MAGE homology domain. A subset of these >40 human proteins are classified as cancer-testis antigens (CTA), and are aberrantly expressed in cancer where they can be immunogenic. MAGE-A3 expression may promote tumorigenesis and can cause transformation of fibroblasts ex-vivo. However, oncogenesis is a multistep process requiring mutations in more than one gene; the cell becoming more tumorigenic as the changes accumulate. A single event, such as the overexpression of one gene, is unlikely to result in oncogenic transformation.

Evaluation of foreseeable effects

All our viral vectors are classified as group 1 agents, so it is only the insertion of certain antigens that will elevate them to group 2 agents. We believe that the above-named cancer-testis antigens (CTA) qualify as group 2 agents. It is conceivable that they might contribute to a cancer phenotype (i.e. oncogenesis) when expressed in normal tissue.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid and liquid waste to be decontaminated by soaking for 2+ hours in a 1% Virkon solution, which has been shown to kill poxviruses and adenoviruses. Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration. Liquids (eg samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains. Degree of kill: 100%

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
Our Genetic Safety Committee has reviewed the risk assessment and agrees with the assigned classification.

**Project Containment**

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- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

**Project Ref** 3415/19.2

**Date Ackn'd**: 13/12/2019

**CU2 Project Title**: Preparation and use of recombinant replication deficient viral vectors

**Class**: Class 2

**Culture**: 1-50 Litres

**Culture Volume**: Class 3-4

**Non-GMM**: Consent Granted

**Project notified under transitional arrangements**: N

**Withdrawn**: N

**Tick if notifying a connected programme of work**: N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

To generate candidate viral vector vaccines.

**Recipient or parental organism**
To produce plasmids, the recipient micro-organisms will be derivatives of the K-12 strain of E.coli e.g. DH 1 DB and DH5alpha. To produce viral vectors, the recipient cell lines will be those suitable for the production of Adenoviral and Modified Vaccinia virus Ankara (MVA) viral vectors: For adenoviral production, these include HEK293 cells and TR293 cell lines and for the production of MVA, these include DF1 and CEF cells.

**Host/vector system**

Adenoviral Vectors e.g. ChAdOX1

Vaccinia viral vectors e.g. MVA

**Origin & function**

The DNA constructs are either a pUC-based shuttle plasmids (encoding a mammalian antigen expression cassette and/or reporter gene), or a (bacterial artificial chromosome) which encodes all or part of elements of the viral genome including a mammalian antigen expression cassette. The producer cell lines express genes that can facilitate the packaging of the viral vectors after transfection/infection with the necessary components. The wildtype MVA viral genome and the MVA shuttle vector containing the recombinant antigen plus a reporter gene, recombines with the viral genome inside the cytoplasm of the cell, by means of a single cross-over event between the plasmid and virus genome. Recombinant viruses can be identified by the expression of the marker gene, which is a fluorescent protein. Further passaging of the virus allows a second recombination event to occur, which deletes the marker gene from the recombinant virus genome and permits the isolation of recombinant viral vectors encoding the antigen cassette.

Antigens encoded in the EVA fusion protein constructs, are previously undiscovered polypeptides that are encoded by previously uncharacterized RNA molecules. These proteins are elements of proteins that are specifically upregulated in human cancer tissues and designed to illicitate an immune response towards tumour cells when delivered as a viral vector.

**Evaluation of foreseeable effects**

MVA (modified vaccinia virus Ankara) is a highly attenuated orthopox virus, and is not capable of replication in mammalian cells, except for permissive cell lines. MVA will only replicate in restricted cell types e.g. chicken embryo fibroblasts. MVA is not capable of causing a symptomatic infection in humans or animals, although it will transduce human and animal cells (i.e. the virus will enter the cytoplasm of a cell and express the recombinant protein (antigen), but no infectious viral particles can be produced). Importantly, MVA cannot replicate in humans. There is a long history of the safe use of MVA and vectors produced on this platform e.g. Unmodified MVA is currently licenced as a third generation smallpox vaccine (Imvanex). Replication-deficient adenoviruses e.g. ChAdOx1 are viral vectors engineered to be replication-deficient by removal of the E1 gene and can only replicate in cell lines that express E1 in trans. The E3 viral gene has also been deleted. These regions of the adenovirus are essential for viral transmission and the vector is therefore not capable of causing any clinical symptoms in humans or animals, although it will transduce human and animal cells (i.e. the virus will enter a cell and express the recombinant protein, but no infectious viral particles can be produced).

There is an unknown hazard from expression of the EVA antigen encoded in these vectors. It is therefore conceivable that toxicity or oncogenicity from either the RNA transcript or the translated polypeptide could result from the antigen expression, although the likelihood is very low. While there is no evidence that the EVAs have any intrinsic biological activity, and thus no evidence that the resulting fusion protein would be oncogenic, the lack of information regarding the function or effect of the antigens on cell growth indicates that a cautious approach should be taken and the
vectors encoding these antigens, should be considered to be potentially harmful until evidence proves otherwise.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Agents will be decontaminated on site using validated methods e.g. Autoclaving or suitable disinfectants (Virkon) prior to disposal through the usual clinical waste routes. Clinical waste is collected by an approved and recognised waste disposal provider e.g. Grundon.

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 deg.C for at least 15 minutes or 126-130 deg.C for at least 10 minutes or 134-138 deg.C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids.

Liquids (e.g. samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125 deg. C for at least 15 minutes or 126-130 deg. C for at least 10 minutes or 134-138 deg.C for at least 3 minutes) or treatment with a suitable validated disinfectant e.g. Virkon, according to manufacturers specifications. Discharge to drains post inactivation.

Solids - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/disposal.

Degree of kill: >99%

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

There is a theoretical risk of toxicity or oncogenicity arising from the expression of the transgenes, although the likelihood of this is low. While there is no evidence that the EVAs have any intrinsic biological activity, and thus no evidence that the resulting fusion protein would be oncogenic/toxic, the lack of information regarding the function or effect of the transgene on cell growth indicates that a cautious approach should be taken and that the transgene should be considered to be potentially harmful. Based on this, despite the vectors themselves being designated as Hazard Group 1 agents, we recommend that the Vectors encoding the EVA antigens should be designated as Hazard Group 2 agents and therefore handled under CU2 until potential hazards are proven otherwise.

We consider this to be a new program of work separate from the usual Vaccitech programmes. As such, we consider therefore consider this a significant change to current CU2 notification and a new notification is required.
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02/03/2022 Page 14391 of 15326
**GM Centre Number: 3416**

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**Name**

QKINE LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

80 TENNIS COURT ROAD

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 1GA

**Country**

ENGLAND

**Tel Number**

01223 491486

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Experienced scientific lead with >20 years experience in risk assessment for GMM contained use.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential
All solid waste is autoclaved in the in-house sterilisation facility and record of sterilisation temperatures are kept. All liquid waste is treated with at least 1% Virkon solution for a minimum of 20 minutes. This has been shown to be effective for E.coli in several tests as evident from the manufacturers data. Autoclaving: Effectively 100% kill at this programme as shown by microbiological testing. Certificated testing of all autoclaves is carried out annually and records are held by the host Cambridge University Department. Printed readouts from each run are retained to ensure temperatures within the autoclave were maintained during the cycle. Liquid waste: Virkon is a total spectrum disinfectant prepared and used as per the manufacturer's instructions. Effectively 100% kill. Routine Disinfection: working surfaces are washed with 1% Virkon solution. Spillage: 1% Virkon solution, or Virkon powder directly. 1% Virkon solution has been shown to effective against the recipient organisms by the manufacturer. All spillages are recorded in a log book.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

All solid waste is autoclaved in the in-house sterilisation facility and record of sterilisation temperatures are kept. All liquid waste is treated with at least 1% Virkon solution for a minimum of 20 minutes. This has been shown to be effective for E.coli in several tests as evident from the manufacturers data. Autoclaving: Effectively 100% kill at this programme as shown by microbiological testing. Certificated testing of all autoclaves is carried out annually and records are held by the host Cambridge University Department. Printed readouts from each run are retained to ensure temperatures within the autoclave were maintained during the cycle. Liquid waste: Virkon is a total spectrum disinfectant prepared and used as per the manufacturer's instructions. Effectively 100% kill. Routine Disinfection: working surfaces are washed with 1% Virkon solution. Spillage: 1% Virkon solution, or Virkon powder directly. 1% Virkon solution has been shown to effective against the recipient organisms by the manufacturer. All spillages are recorded in a log book.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessments have been reviewed and a class 1 contained use deemed appropriate.
## GM Centre Number: 3417

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### Name

OXSYBIO LTD

### Name 2


### Department


### Campus Estate or Research Centre


### Building


### Road Name


### District


### Town

Oxford

### County

OXFORDSHIRE

### Postcode

OX11 0QX

### Country

ENGLAND

### Tel Number

01865 817071

### Fax Number

NA

### E-mail


### HSE Division

EAST AND SOUTH EAST

### Comments


### Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The combined GMSC of the STFC, MRC Harwell, Diamond lightsource has assessed the risk relating to contained use. OxSyBio's biological safety officer (BSO), Sharon Webster from Websters Biosafety has also assessed the risk.

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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<tr>
<td>Non-microbial</td>
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Other (please specify) Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research |
|-------------|--------------|------------------|---------------------|
Virology | Transgenic Animals | Transgenic Fish | Gene Therapy |
Disinfection of liquid waste is carried out using 1-2% Virkon for 10-15 min. In case of spillage, absorb liquid using paper towels or other absorbent material (collect solid waste in biohazard bag). Treat the area for 10-15 min with 1-2% Virkon prior to cleaning and disposal. Alternatively use 70% ethanol when the spill is on a metal surface as Virkon degrades metal. Containment level 2 (CL-2) biological safety cabinets (BSC) are disinfected with 70% ethanol followed by UV treatment for 30 minutes (if UV lamp is installed). Solid waste is collected in biohazard bags and autoclaved. Virkon-treated liquid waste is disposed into sink. Where possible, oil waste will be collected for incineration. Small volumes of oil (<10 mL) containing artificial tissues are collected in a tube. This oil is mixed with Virkon at 1:3 ratio and treated with ultrasonic noise (40 kHz, 15 min) to create a fine emulsion, with biological material exposed to Virkon within the emulsion. The emulsion will be left to stand until phase separated and the aqueous solution is disposed of the down the sink and the oil is collected with standard oil waste which is incinerated.

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**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

**Please enter comments of the GM safety committee on the risk assessment**

1) This seems reasonable to me, I am happy with the form.
2) This work appears to have been considered carefully. As long as the previous comments were addressed I am happy with the assessment.
3) No additional comments from me, I’m happy with the assessment and its classification.
4) The only thing I have spotted here is the mention of adding oil samples to Virkon to disinfect the cells. If the cells are surrounded by oil how will the Virkon be able to act on the cells? Is it not better to dispose of these samples via autoclaving/ burn bins? Otherwise I think that this is OK.

(updated to include new oil disposal method- JG, 27-4-18)
GM Centre Number: 3418

Data Premises Notified (Originally) 07/06/2018

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

Phytoform Labs Ltd

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

12 GLENMORE ROAD

District

Town

SALISBURY

County

WILTSHIRE

Postcode

SP1 3HF

Country

ENGLAND

Tel Number 07704643551

Fax Number n/a

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022

Page 14398 of 15326
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

### Give brief details of the genetic modification safety committee

The committee consists of two PhD level scientists, employees of Phytoform Labs, with a combined experience of more than 10 years working with cloning and GM organisms. The committee will regularly inspect work areas and advise on improved safety procedures. All new employees will receive inductions and follow on safety information during their employment period. There will be an accident report procedure in place for employees. The committee will meet every quarter to discuss best safety practices and any reported accidents that have occurred.

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<tr>
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Tick if confidential
Both solid and liquid waste will be treated with a confirmed kill procedure. Autoclaving of the liquid and solid material containing GMMs will be performed by a trained person in a serviced, validated autoclave. The autoclave is regularly inspected by third party engineers to ensure serviced functional autoclave that meets necessary conditions for confirmed kill.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

Genetic Safety Committee has reviewed the risk assessment and approves of the genetic modification risk assessment form.

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Page 14401 of 15326
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

We have been advice form safety specialist from our collaborators in the University of Bradford, and Sheffild Hallam.

Additionally, I have been working with genetically modified microorganism for more than 15 years in UK and other european institutions

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Non-microbial</td>
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</tbody>
</table>

Other (please specify) Tick if confidential

Bacteriology Yes Parasitology Transgenic Birds Microbiology Research Yes

Virology Transgenic Animals Transgenic Fish Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

- Chemical disinfection (contaminated liquids)
- Autoclaving (contaminated solids)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessments in place and laboratory safeguards for containment are adequate for the purpose of intended use.

---

**Project Ref 3419/19.1**

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<td>Evaluation of resistance of S. epidermidis AZT-04 strain against challenge with S. aureus on Labskin1.1</td>
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<td>&lt; 1 Litre</td>
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Tick if notifying a connected programme of work

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
**Project Additional Information**

**Purposes of the contained use**

In vitro test. Contracted external research.

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Class II cabinets for handling the microbes.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Autoclave for solid disinfection.
Chemical disinfection of liquids.

**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

**Please enter comments on the GM safety committee on the risk assessment**

Risk assessments in place and laboratory safeguards for containment are adequate for the purpose of intended use.

**Project Containment**
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<th>Laboratory Activities</th>
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02/03/2022
GM Centre Number: 3421

Date Premises Notified (Originally) 16/08/2018
Transferred from 1992 Regs? N
Transitional Premises Class

Data Premises Closed
Transitional Premises
Emergency Plan Required? N
Non-GMMs N
Withdrawn N

Name
ENESI PHARMA LTD

Name 2

Department

Campus Estate or Research Centre

Road Name
45B WESTERN AVENUE

District
MILTON PARK

Town
ABINGDON

County
OXFORDSHIRE

Postcode
OX14 4RU

Country
ENGLAND

Tel Number 01235 577125
Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted 02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

The genetic modification safety committee comprises of the VP of Product Development, who also is responsible for ensuring the biosafety of the employees, alongside the Health & Safety Lead for the Laboratories and the Health & Safety representative of the employees. The team has extensive experience in a wide variety of pharmaceutical and biological safety within non-GMP laboratories for over a decade. As per the H&S Standard Operating Procedure (SOP) in-house the genetic modification safety committee meet at least bi-yearly to assess the general H&S implications of handling biological agents within the laboratories. The team assess the COSHH pertinent to the biological agent envisaged to be handled within the Containment Level 2 laboratory prior to commenting on the suitability of the designed procedure outlined in the COSHH as per the in-house H&S SOP document.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale

- **Level 1 (GMMs)**
- **Level 2 (GMMs)**: **Yes**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**
- Non-microbial
- **Other (please specify)**

Tick if confidential: **No**

02/03/2022
As advised in note 6, CU2 form provided. Sections 6 to 8 not applicable as activity planned is Level 2

Formulation of the provided GMO into new dosage form. Determination of the viable GMO virus titre

For activities involving GMMs, describe the waste management measures which will apply to the activity

As advised in note 6, CU2 form provided. Sections 6 to 8 not applicable as activity planned is Level 2

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

As advised in note 6, CU2 form provided. Sections 6 to 8 not applicable as activity planned is Level 2

Project Ref 3421/18.1

Date Ackn'd 16/08/2018  
Date Project Ceased

Handling of MVA vaccines  

Class Culture Vol

Class 2 ≤ 1 Litre

Consent Granted

Tick if notifying a connected programme of work

Project notified under transitional arrangements

Withdrawn

Historical Significant Changes
**Project Additional Information**

**Purposes of the contained use**

Enesi is performing this work in order to demonstrate that existing vaccines can be formulated into solid doses for use with Enesi's proprietary solid dose injection system. These solid doses are predicted to have improved stability and efficacy over the current product(s).

**Recipient or parental organism**

Examples include Replication-defective vaccinia virus (Copenhagen strain) and Modified Vaccinia Ankara (MVA) propagated in chicken cells, so chicken ("parental organism") - but they're going into mice ("recipient").

**Host/vector system**

Examples include Mammalian cells and specific pathogen-free chicken embryo fibroblasts. Vector is MVA.

**Origin & function**

Examples to such include genes from peanut (Arachis hypogea) associated with peanut allergy, chikungunya and zika virus structural proteins, Discosma sp. fluorescent reporter genes, mouse cytokine and human ubiquitin C as well as the following:

**Origins of MVA:**

MVA is a highly attenuated vaccinia virus developed specifically for use as a "safer" smallpox vaccine. MVA originated from the Dermovaccinia strain CVA, which was used in Germany in the 1950s as a smallpox vaccine. Dr. Mayr and colleagues passaged CVA more than 570 times to attenuate it further. In the process of serial passages in CEF, 9% of the DNA molecular weight of the viral DNA was lost from the original CVA strain and the virulence for mammalian cells was greatly reduced.

**Origins of inserts:**

The vaccine inserts constitute the genetic material that is added to MVA to create the recombinant MVA vaccines. The vaccine inserts encode antigens from the target pathogens. Each of the vaccine inserts was created in vitro using gene synthesis techniques and was then inserted into a plasmid and amplified in E. coli. The purified plasmid DNA from the E. coli cultures was used as the source material for the recombination processes that created the...
recombinant MVAs. The vaccine inserts were designed based on published sequences of the viral pathogens. For Lassa fever, we used the sequence of a well-characterized strain of Lassa virus. For Ebola, we used the sequence of the Makona strain of Ebola virus. For Zika, we used the sequence of a 2015 Suriname isolate.

**Intended functions of MVA:**
The MVA sequences serve to allow replication of the genetic material in cell culture, to allow an abortive infection of the cells of the recipient, to allow expression of the antigen sequences, and to elicit innate immune responses.

**Intended functions of inserts:**
The inserts encode the vaccine antigens, which elicit adaptive immune responses against the target pathogens.

### Evaluation of foreseeable effects

Some typical characteristics include:
Vaccinia virus is considered stable within the environment and has a broad host range. Vaccinia viruses have the potential to infect human cells, however, in the unlikely event of the GMO being unintentionally released into the environment, the likelihood of infection remains low since the virus would require direct contact with a mucosal membrane or an open wound to produce an infection. If this were to happen, infection of healthy individuals with vaccinia virus is generally mild and may include a rash or fever. In most cases, it would be expected that the person's immune response would rapidly clear infected cells and the virus. This work proposes use of a modified Vaccinia virus that is replication-incompetent and unable to produce progeny viruses. Therefore, consequences of infection with this modified Vaccinia are therefore likely to be less severe than with the parental virus and there is little risk of the virus spreading in the environment. Expression of foreign genes inserted into the replication-incompetent vaccinia virus are not foreseen to cause harm but intended to elicit protective immune responses. However, the effects are as yet untested, and persons with peanut allergy are potentially at risk of exposure to allergens.

Additionally, evaluation of foreseeable effects also include:
The foreseeable effects of the proposed research are: (1) the intended effect of generating data on the immunogenicity and efficacy of these vaccines in mice and (2) the unintended effect of generating small quantities of biohazardous waste, which will be disposed of properly.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All operations will be performed by suitably trained personnel within the containment level II laboratory. Appropriate signage warning of the biohazard risk will be placed on all doors to the laboratory. All formulation, manufacturing and analytical activities involving the vaccine will take place in a Cytotoxic Level 2 hood (unless the COSHH assessment indicates more stringent protection is required). All waste material potentially contaminated with vaccine (powder, wipes, gloves etc.) will be double-bagged in clinical waste bags, closed, and sealed in a clinical waste bin for disposal by an approved waste-handling agent. All potentially contaminated surfaces will be cleaned with Klercide Biocide B and either 70% denatured ethyl alcohol (ethanol) (DE) or 70% isopropyl alcohol (IPA) and left to dry. All equipment and parts potentially contaminated will be washed down with Klercide Biocide B and either 70% DE or IPA, or autoclaved at suitable conditions (typically 121°C for 30 minutes at 15 PSI but this will be adjusted for the weight and size of the item) to ensure sterility. All lab users will wash and dry their hands thoroughly before leaving the laboratory. All other lab procedures will be followed as per the Enesi’s Standard Operating Procedure for General Laboratory.
Control.

The risk assessment completed on the GMMs was approved

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick to confirm that it is attached to this form

Is an emergency plan required according to regulation 20?  

Yes

Project Containment

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**Name**

MEDICINES DISCOVERY CATAPULT

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

BLOCK 35

**Road Name**

MERESIDE

**District**

**Town**

ALDERLEY EDGE

**County**

CHESHIRE

**Postcode**

SK10 4TG

**Country**

ENGLAND

**Tel Number**

01625 238 734

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GM safety committee at the Medicines Discovery Catapult consists of 2 Lead Scientists (PhD, BSc) one of whom is the Biological Safety Officer (BSO) and a Head of Department (PhD). Together, they have >45 years of combined experience in the Academic, Biotech and Pharmaceutical sectors. All 3 have created GM risk assessments in their previous roles at AstraZeneca (AZ). Two of the 3 were Departmental representatives at AZ's internal GM Safety Committee charged with reviewing and aproving GM risk assessments. In addition to this internal expertise, MDC has retained the services of an exetrnal Safety Consultant Specialist who has lead the development of risk assessment process for GMO organisms and who has lead AZ's GM risk assessment committee.

Upon creation of a GM risk assessment by an MDC scientist, the GM committee will review then challenge or approve (as required) the proposed risk classification. Upon subsequent formal approval, the MDC scientist may commence the associated activities. GM RAs will then be indexed, stored and periodically reviewed. At each quarterly MDC H&S committee meeting, GM risk assessments are a standing agenda item. The BSO is a permanent member of the MDC H&S group and acts as the link to the GM risk assessment committee.

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All liquid cell culture waste will be disposed of by mixing with Virkon to maintain an active concentration of >1%. This has become an accepted standard disposal measure. Liquids are left at room temperature for at least 6 hrs prior to discard. The manufacturer's website gives details of the application range of Virkon.

For activities involving GMMs, describe the waste management measures which will apply to the activity

- Consequences of exposure are low given the confined replication status of the vector and the low cytotoxicity nature of the expressed RFP protein.
- The likelihood of exposure in the laboratory setting is low given the procedures used, PPE for the operator, low volumes used and the complete absence of sharps.
- Viral titres of less than or equal to 5 x 10^6 per ml will be used
- As the consequences of RFP expression in humans have not been specifically addressed, the effect of transgene expression is uncertain.
- While it is possible that unwanted integration event could change the oncogenic potential of the affected cell, the control[s] in place make this event a low probability occurrence.
The aim of this project is to produce lentiviral particles in order to genetically modify mammalian cell types including but not exclusive to induced pluripotent stem cells (iPSC), iPSC-derived microglia, astrocytes and neurons, primary cells including but not exclusive to neurons, astrocytes, microglia and endothelial cells and cells lines including but not exclusive to SH-SY-5Y, A549, U87, PANC1, U2OS and BT474. The lentiviral particles encode reporter proteins either in response to activation of a signalling pathway or constitutive expression for whole cell labelling. A selectable marker gene, puromycin N acetyltransferase (PAC), will be co-expressed. These reporter cells will allow the operator to monitor and analyse morphological/signalling changes in response to injury or compound treatment via microscopy/quantitative plate based assay (luciferase). The inclusion of the puromycin resistance gene allows for selection and expansion of cells that have been transduced and are stably expressing the transgene cassette. Lentivirus technology is the most appropriate method for generating stable cell cultures in difficult to transfect cells. Lentivirus offers long term expression, which is required to monitor cells over a period of weeks or months. Moreover, lentivirus often infects with high efficiency which is also important as it can be difficult to generate large numbers of primary/iPSC derived cell types.

Recipient or parental organism

Recipient- Mammalian cells: HEK293T ATCC ® CRL-3216 iPSC-derived microglia, neurons and astrocytes as well as primary neurons, astrocytes, microglial Mammalian cell lines (e.g. BT474, U2OS, PANC1, A549, SH-SY-5Y, U87) The commercially available 293T cell line is a highly transfectable derivative of human embryonic kidney 293 cells, containing the SV40 T-antigen and is recommended for use when generating lentivirus particles. The main function of the SV40 T antigen is to facilitate increased expression of transgene of viral plasmids with an SV40 origin of replication by retaining/replicating plasmids in the cell after a transfection (although this is transient). This will in
Plasmids (obtained from Aldevron): pALD-GagPol (cat 5043), pALD Rev (cat 5033), pALD VSV-g (cat 5037). Derivative of pALD-LentiEGFP (cat 5031) (modified to encode reporter cDNA/PAC) The vector system is lentivirus production via plasmid transfection and the particles will be produced in HEK293T cells, only cells that receive all four plasmid will produce viable infectious particles. We intend to use the third generation lentiviral vector as this is considered the most safe available system although lower viral titers are expected than second generation. To increase the safety of the lentivirus, the components for viral packaging and production are split across separate plasmids; VSVg, Gag-Pol, Rev and the transfer plasmid containing the transgene of interest (reporter and puromycin resistance gene (PAC)). The VSV-g plasmid encodes the envelope of the virus and is broad spectrum so can infect many cell types. Gag antigens form the viral core structure, Pol encodes reverse transcriptase and pol and Rev facilitates the export of transcripts to the cytoplasm. The transfer plasmid contains reporter cDNA and PAC, under the control of a constitutive native mammalian promoter, EF1α or a minimal promoter to monitor signalling pathway cascades. The transgenes are flanked by long terminal repeats (LTRs), which are identical sequences of DNA that repeat and are necessary for viral integration into the genome. The transfer plasmid also contains the packaging signal for packaging into the viral genome. Plasmid DNA encoding the essential genes to produce functional virus will be transfected into 293T using standard lipid-based method (Lipofectamine 3000). The packaging and envelope plasmids are purchased commercially (Aldevron - pALD GagPol, pALD VSV-G, pALD Rev). Successful transfection of all 4 plasmids into a cell will result in the transient production of lentiviral particles that are isolated from the spent tissue culture media.

Origin & function
Lentiviral packaging plasmids are derived from the HIV-1 lentiviral genome and are required for the production of replication deficient viral particles with a broad tropism. The transgenes contained within the infectious lentiviral particles produced are luminescent and fluorescent proteins including but not exclusive to green fluorescent protein (GFP) and mCherry, a red fluorescent protein isolated from Discosoma sp.(derived from 'DsRed') and luciferase. GFP, isolated from Aequorea victoria, is a widely used reporter protein, exciting at 395nm and emitting at 509nm. mCherry is a widely used reporter protein that is highly stable and rapid to mature and is not known to cause toxicity (Shaner et al, 2004, Shemiakina et al, 2012). mCherry absorbs light between 540-590nm and emits light in the range of 550-650nm. Luciferase, a widely used quantitative enzyme that is responsible for the bioluminescence of fireflies and click beetles, will be expressed downstream of a relevant minimal promoter.
promoter to track cell signalling changes. The enzyme catalyses the oxidation of luciferin in the presence of ATP and oxygen. The inserted enzyme is not known to cause photodynamic toxicity in cancer cells (Schipper, Patel and Gambhir, 2006).

The above reporters will be used to either track whole cells in co-cultures by fluorescence microscopy or quantify intracellular signalling events in response to a compound or other stimuli (luciferase).

The other transgene encoded within the reporter cassettes, PAC, confers resistance to puromycin, an antibiotic toxic to eukaryotic cells. PAC is a bacterial enzyme produced by Streptomyces A and has been purified and characterised (Vara J. et al, 1985). PAC inactivates puromycin by acetylating the amino position of its tyrosinyl moiety. Having this gene co-expressed with mCherry, allows rapid selection of microglia harbouring the transgene cassette as all microglia that have not been transduced will die off upon exposure to puromycin.


Evaluation of foreseeable effects

The recombinant viral vectors do not have any regulatory or accessory genes ensuring that the particles produced cannot replicate. Although the VSV-G confers a wide tropism meaning a wide range of cell types can be infected, Evaluation of foreseeable effects confers a wide tropism meaning a wide range of cell types can be infected, the replication deficient properties of the lentivirus renders the risk minimal to human health. Control measures in place (containment within class II biological safety cabinet, secure access laboratories, absence of any sharps in the procedures and disinfectant processes) are appropriate to mitigate any risk associated with generating lentivirus. The transgenes encoded within the virus do not interfere with the properties of the virus.

Genetic modification of mammalian cells with reporter lentivirus produced is not expected to alter the properties or characteristics of the cells. The reporter proteins or PAC should not interfere with any of the cells endogenous pathways or induce toxicity (Sheminkina et al, 2012, Schipper, Patel and Gambhir, 2006).

Effects to human health - It is unlikely that the inserted genetic material will
have any direct consequences on human health as transduction and
eexpression of the transgene is restricted to microglia. A drawback to the
lentiviral system is that it is not possible to control the site of integration of
the vectors as lentivirus does not integrate into a safe harbour site. There is a
small chance that the integrations may cause insertional mutagenesis, a
process by which genes close to the integration site are deregulated. It is
possible that an oncogene may be switched on the proliferation or function of
the cells may be altered. However, this poses little to no risk to human
health, only to the recipient cell line which will be decontaminated and
discarded if an obvious change in cell cycling occurs. GM cells will be
monitored for their proliferation rate and that they maintain their regular
phenotype and function.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All lentivirus production and transduction will be performed in a class II ducted biological safety cabinet
within access controlled laboratories. Liquid waste (cell media) be produced during the lentivirus production process, maximum of 100ml.
Contaminated liquid waste will be decontaminated with Virkon™ S tablets to maintain an active concentration of 2% (supplemented with water). Virkon™ S tablets are
known to be effective against HIV-1 and it is non-corrosive and non-bleaching (if used correctly). Liquid waste will be left at room temperpature for 2 hours before disposal
down the sink. HEK293T cells that are used to produce the virus
temperature for 2 hours before disposal down the sink. HEK293T cells that are used to produce the virus
will also be incubated with Virkon™ S tablets in the flask prior to discarding down the sink also
Solid waste (from flasks, tubes etc) will be produced during the procedures. Contaminated solid waste
will be decontaminated with Virkon™ S tablets to maintain an active concentration of 2%
(supplemented with water) and will be left at room temperature for 2 hours before final disposal in a
clinical waste bin destined for autoclave followed by incineration.
All cells transduced with virus will be tested one week post transduction to ensure they are not
producing any viral particles (p24 viral capsid ELISA assay) (in media).

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment
This risk assessment is sufficient to address the risks associated with retroviral vector production for the assessment of mammalian cells. The classification as a CLASS 2 activity is appropriate given the scale of vector production and use proposed (upto 100ml) and therefore the corresponding requirement to minimise aerosol formation in order to fully protect human health.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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<tr>
<th>Laboratory</th>
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Other (please specify) Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 3424/18.1

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<td>Generation of a genetically modified human Natural Killer (NK) cell line</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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Class Culture Vol: Class 2, Culture Volume: < 1 Litre

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn: N

Tick if notifying a connected programme of work: N

---

**Project Additional Information**

---
Purposes of the contained use

1) As primary cells reach senescence after a limited number of population doublings, cell immortalization is presented as a promising option to solve this problem. In this way, our purpose is to immortalise primary human NK cells by human Telomerase Reverse Transcriptase (hTERT) expression in order to establish a new NK cell line.

2) The second part of the project consists on the generation of a more efficient NK cell line. It is known that NK cells activation is determined by the balance of inhibitory and activating receptor stimulation. In this study, our purpose is to knock down two inhibitory NK receptors by CRISPR/Cas9.

Thereby, the final objective of this project is to generate a more active immortal NK cells, which would be tested as a powerful treatment against cancer.

Recipient or parental organism

Host/vector system

1) NK immortalization:
Vector: human Immunodeficiency Virus (HIV) - Lentivirus Expression System (Lenti-CMV-hTERT-GFP-2A-Puro Virus from abm (Cat# LV623)
Host: Peripherial blood CD56+ NK cells from Allcells (Cat# PB012F)

2) NK cells knock down:
Vector: Third generation lentiviral transfer vector from Oxford Genetics. CRISPR/Cas9 system. Single guide RNA (sgRNA) targeting the killer cell lectin like receptor (KLRC1) and LeukocyteImmonogobulin like receptor B1 (LILRB1) genes.
Host: NK cells

Origin & function

1) The most recently discovered approach to cell immortalization is through the expression of Telomerase Reverse Transcriptase protein (TERT), particularly for cells that are most affected by telomere length, such as human cells. This protein is inactive in most somatic cells, but when hTERT is exogenously expressed, the cells are able to maintain sufficient telomere lengths to avoid replicative senescence. Analysis of several telomerase immortalized cell lines has verified that the cells immortalized by hTERT over expression maintain a stable genotype and retain critical phenotypic markers. In this way, in this project human TERT protein will be overexpressed in primary peripheral blood NK cells using a lentiviral system, so NK cell senescence is expected to be blocked and its replicative capacity increased.

2) The modification of NK cells by knocking down two of its inhibitory receptors is planned to be developed by the CRISPR/Cas9 method using a third generation lentiviral transfer vector. This system is intended for the expression of a specific single guide RNA (sgRNA) along with the Cas9 protein in a target human cell. In our case, the sgRNAs will be targeted to the Killer cell lectin like receptor (KLRC1) and Leukocyte Immunogobulin like receptor B1 (LILRB1), which will allow the Cas9 protein to cut the DNA at the KLRC1 and LILRB1 locations. On the one hand, KLRC1 gene encodes the Natural killer cell receptor NKG2A protein, which pairs with CD94 to form a heterodimer that recognizes the nonclassical MHC class I molecule, HLA-E, in humans. On the other hand, LILRB1 encodes LIR-1 protein that normally binds to a wide range of MHC-I alleles, being the strongest MHC-I ligand, HLA-G. In this sense, the natural function of both is to induce tolerance against self blocking the killing activity of NK cells when they bind to their target antigens.

In brief, the intended functions of the genetic material involved are to increase the proliferative capacity and to reduce the stimulation of the inhibitory signals of the NK cells, thus generating an infinite source of active NK cells.

Evaluation of foreseeable effects

A) Lentiviral Expression Systems include the following safety features to ensure lack of Replication-Competent Lentivirus (RCL):
• An enhancer deletion in the U3 region of 3′LTR ensures self-inactivation of the lentiviral vector following transduction and integration into the target cell's genomic DNA
• The number of lentiviral genes necessary for packaging, replication and transduction is limited
to three (Gag/Pol/Rev), and their expression is derived from different plasmids, all lacking packaging signals. These plasmids share no significant homology to the expression vector, thus preventing the generation of replication-competent virus by recombination events.

- None of the Gag, Pol, or Rev genes will be incorporated into the packaged viral genome, thus making the mature virus replication-incompetent.

In this sense, the risks during lentivirus transduction of target cells are minimized, because a virus can then efficiently transduce target cells but, once the transduction has taken place, the virus cannot produce new virions.

B) In the theoretical case that the immortal and modified NK cell would reach the worker’s bloodstream the cells would be recognised as “non-self” by the immune system and eliminated. However, there is a low risk of these cells escaping the immune system and becoming a threat to the individual. The principal route of transmission of GM NK cells to laboratory workers bloodstream is by inoculation. Therefore, use of sharps will be prohibited. Other possible routes of transmission, including inhalation, ingestion and eye splashes, are less likely to happen because of the use of good laboratory practices and personal protection equipment.

Consequently, we would evaluate the risks of working with the GMO as medium/low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Celixir has planned a waste disposal program which consists on:

1) The solid waste will be placed in a double-bagged bin with a lid and once full, it will be sealed and placed in a sure locking system with a permanent hermetic seal bin.
2) The liquid waste, such as culture media or supernatants, will be aspirated by the vacuum system to the Vacsax (3L) until it is ¾ full. Then, Vacsax is closed, shaken, to achieve it becomes jellified, and placed in a sure locking system with a permanent hermetic seal bin.
3) Properly closed sure locking systems with permanent hermetic seal bins will be autoclaved by a safe-cycle of 126°C for 20 minutes.
4) The external waste management contractor, Initial, will finally collect the waste for their incineration.

Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by recording of the temperature/time profile.

Degree of kill
- Autoclaving, effectively 100% kill
- Incineration, effectively 100% kill

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
A Genetic Modification Safety Committee was created and the first meeting was focused on discussing the Use of Lentiviral system for human cell transduction Risk assessment. The main comments were:
1) To emphasize what people are at risk - Staff, cleaners, engineers.
2) To specify the use of personal protective equipment - To use lab coat and gloves that comply with regulatory rules to work with virus, and it was recommended to use different colour lab coat and gloves from the rest of activities.
3) Waste management - The external contractor to be validated
4) To create and Standard Operating Procedure (SOP).

Then, an SOP explaining the use of the Containment Level 2 room in Celixir Laboratories has been created and it is also attached to this notification.

Project Containment

<table>
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<tr>
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<td>Non-GMMs</td>
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**Name**

EVONETIX LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

UNIT 9A, COLDHAM'S BUSINESS PARK

**Road Name**

NORMAN WAY

**Building**

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB1 3LH

**Country**

ENGLAND

**Tel Number**

01223 930 300

**Fax Number**

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**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Evonetix genetic modification safety committee (GMSC) consists of a first person with over 15 years of experience working in academic laboratories conducting genetic modification of microorganisms. This person was responsible for the preparation of biological risk assessments at a Cambridge University lab. The second member of the committee has extensive experience in a UK industrial lab setting with regular GMM work. This member will not be directly involved in GMM work at Evonetix, giving the member independence and professional distance. A third member has 10 years of experience with GMM work (in academic labs) and will be directly involved with GMM work in the lab. A fourth member, who is also the chairperson of the Evonetix Chemistry Safety Committee, will ensure full integration of the GMSC within the Health and Safety Committees of Evonetix. A fifth member will represent higher management.

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To inactivate liquid waste, Virkon powder is to be added to a final 1% concentration. Virkon is a total spectrum disinfectant prepared and used as a 1% virkon solution as standard procedure. It has an effective 100% kill rate. Solid waste is to be disposed of in yellow clinical waste burn bins provided by contractor Grundon. The contractor will treat the waste by incineration, resulting in an effective 100% killing-off of micro-organisms.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Other(s)

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Other (please specify) Tick if confidential

Bacteriology Yes
Parasitology
Transgenic Birds
Microbiology Research
Virology
Transgenic Animals
Transgenic Fish
Gene Therapy
Mycology
Transgenic Invertebrates
Transgenic Plants
Other (please specify below)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

It was noted that there would be no need to use glass pasteur pipettes for biological work as this would represent an unnecessary sharps risk, given the availability of plastic pasteur pipettes. Therefore the Risk Assessment was amended to reflect that recommendation.
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**Name**

ADRESTIA THERAPEUTICS LTD

**Name 2**

Department

**Campus Estate or Research Centre**

BABRAHAM RESEARCH CAMPUS

**Road Name**

BABRAHAM

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB22 3AT

**Country**

ENGLAND

**Tel Number**

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**Fax Number**

N/A

**HSE Division**

EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee:

- **Genetic modification safety committee:**
  - CEO (chair) - PhD, Frederick James Quick Professor of Biology, FRS
  - Laboratory manager (biological safety officer) - MSc Molecular Biotechnology
  - Scientific Adviser (member) - PhD in human genetics
  - Research Scientist (member) - PhD in Cell and Molecular Biology

Any work involving GMO must first be approved by the genetic modification safety committee. In order to do so, all the necessary risk assessments must be completed or read and sign. Furthermore, all personal carrying on such work must have received all the necessary training to ensure safe work by the biological safety officer. The genetic modification safety committee meets as necessary on a regular basis with a frequency no less than 3 times a year.

| Level 1 (GMMs) | Yes |
| Level 2 (GMMs) | Yes |
| Level 3 (GMMs) |     |
| Level 4 (GMMs) |     |
Solid waste will be inactivated by autoclave (degree of kill effectively 100%) and liquid waste treated with Distel, or other approved desinfectant according to manufactures’s instructions. Autoclave is tested and certified annually, and during each run is shown to hold temperature correctly. Sharps will be collected in a sharps disposal bin and disposed of by University-approved contractors.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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<tr>
<td></td>
<td>Invertebrates</td>
<td>Plants</td>
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</tbody>
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Other(s) Genetically modified mammalian cell lines (standard laboratory use)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Risk assessments approved on 03.09.18

Project Ref 3428/18.1

<table>
<thead>
<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>CultureVolumeClass3-4</th>
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<tbody>
<tr>
<td>08/09/2018</td>
<td>Generation of Cas9 stably-expressing U20S and RPE-1</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>
# Project Additional Information

## Purposes of the contained use

CRISPR-Cas9 mediated knock-outs generation for genetic screens.

## Recipient or parental organism

Standart laboratory used mammalian cell lines, such as, but not limited to: U20S and RPE-1.

## Host/vector system

Second or third generation lentiviral vector system.

## Origin & function

AAV1-EF1ahCas9 (Cas9 expressing plasmid) and pCLlP-dual-SFFV-ZsGreen screening sgRNA library from Transomic Technologies (guide RNAs expressing library for CRISPRiCas9 mediated generation of knock-outs) were provided by Prof. Steve Jackson's laboratory at Gurdon Institute, Cambridge, UK. Both plasmidic vectors will be packaged into lentiviral vectors in HEK293T cells and delivered to host cells (U20S and RPE-1) by transduction aiming to generate Cas9-stably expressing RPE-1 cells and CRISPR/Cas9-mediated U20S and RPE-1 knock-out cells to be screened for sensitivity to DNA damaging agents.

## Evaluation of foreseeable effects

Lentiviruses are retroviral vectors derived from Human immunodeficiency virus type 1 and 2 (HIV-1/-2), classified as HG3. However, replication incompetent lentiviral vectors bear little resemblance to wild-type HIV. The infected cell lines cannot survive outside of sterile tissue culture environment. The lentiviral vectors do not encode for any oncogene or transgene.

## Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

---

**Date Project Ceased:**

<table>
<thead>
<tr>
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<th>Non-GMM</th>
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<tr>
<td></td>
<td>geneX/-/- human cell lines and subsequent lentiviral transduction of such cell line with focused sgRNA libraries for CRISPR-based editing and genetic screen.</td>
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**Withdrawn:**

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**Project notified under transitional arrangements:**

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<tr>
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</table>

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

---

02/03/2022
**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Solid waste will be inactivated by incubation in distel or bleach solution followed by autoclave treatment in autoclavable bags and then disposed according to the university of Cambridge procedure (degree of kill effectively 100%).

Liquid waste will be treated with Distellbleach, or other approved disinfectant according to manufacturer's instructions and disposed in the sink.

Autoclave is tested and certified annually, and during each run is shown to hold temperature correctly.

---

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L2 L3 L4 L2</td>
<td>L2 L3 L4 L2</td>
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<td>L2</td>
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**Project Ref** 3428/20.1

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<th>CultureVolumeClass3-4</th>
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<td>22/10/2020</td>
<td>Generation and use of lentiviral particles for mediating gene modulation and genetic editing in mammalian cells</td>
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**Risk assessments approved on (04.09.2018)**

---

**Is an emergency plan required according to regulation 20?** N

**If yes, tick to confirm that it is attached to this form** Y

**Tick to confirm that you have attached a risk assessment to this form**

**Tick if you are claiming exemption from disclosure for section of the risk assessment** N
**Project Additional Information**

**Purposes of the contained use**

To enable the generation and use of recombinant self-inactivating second and third generation lentiviral particles (by commercial research organisations or in house using commercially available 2nd and 3rd generation lentivirus systems) encoding:

1. Open Reading Frames (ORFs), cDNAs,
2. Specific gene sequences
3. Short hairpin RNAs (shRNAs) for the knockdown of Open Reading Frames (ORFs), cDNAs or specific gene sequences by RNA Interference (RNAi)
4. guide RNAs sequences for CRISPR/Cas9-based gene editing for knockout cell lines generation.

To enable the use of such cell lines for genetic high-throughput screens; specifically: a) production of lentiviral particles carrying DNA sequence coding for Cas9 for the generation of Cas9 expressing human cell lines.

**Recipient or parental organism**

To enable the generation and use of recombinant self-inactivating second and third generation lentiviral particles (by commercial research organisations or in house using commercially available 2nd and 3rd generation lentivirus systems) encoding:

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4. guide RNAs sequences for CRISPR/Cas9-based gene editing for knockout cell lines generation.

To enable the use of such cell lines for genetic high-throughput screens; specifically: a) production of lentiviral particles carrying DNA sequence coding for Cas9 for the generation of Cas9 expressing human cell lines.

**Host/vector system**

The lentiviral vectors which will be used are derived from HIV-1, which is an ACDP Hazard Group 3 biological agent.

However, second and third Generation lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase biosafety. These include the vit, vpr, vpu and nef accessory genes which are not required for in vitro replication. The tat gene is also deleted and the Tat-responsive promoter present in the S’L TR is replaced with heterologous promoters, for example with the Rous sarcoma virus U3 region. An additional biosafety feature is achieved by deletion of the rev gene from the viral transfer vector. Viral packaging is achieved by providing three helper constructs in trans containing gag, pol and rev sequences (figure 1).
An additional biosafety feature is that these vectors are self-inactivating (SIN), whereby the U3 region of the 3' L TR (which contains the major viral promoters and enhancers) is copied to the S' end of the provirus during reverse transcription. Deletion of enhancer and promoter elements from the 3' U3 region in the vector construct will result in a provirus that is entirely devoid of U3 enhancer sequences, therefore reducing the potential for transactivation of cellular genes as a result of insertion. Furthermore, such vectors are not easily mobilisable as a result of a superinfection with wild-type virus.

For example, third generation Lentiviral Expression Systems include the following key safety features:
- The Lenti expression vectors contain a deletion in the 3' L TR (.6.U3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
- The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., (998).
- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK-293FT or HEK-293-LentiX producer cell line, none of them contain LTRs or the '+' packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication competent virus can be produced.

Despite the above safety features, use of these lentiviral vectors (which include WPRE) falls within SACGM 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. Also, the virus will be packaged by transfecting transfer vector into specific amphoteric ‘helper’ cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types.

This means that the viruses produced for this experiment could potentially infect a number of species, including human.

Origin & function

- **Selectable markers - examples (but not restricted to):**
  - Ampicillin resistance: E.coli derived
  - Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
  - Puromycin resistance (PAC): Puromycin acetyl transferase is derived from Streptomyces alboniger
- **Reporter proteins such as (but not restricted to):**
  - GFP derived from the jellyfish Aequorea victoria and variants of this
  - LuCiferase - class of oxidative enzymes used in bioluminescence
- **Open reading frames, cDNAs and gene sequences and I or shRNAs, gRNAs and cyclic peptides - all human derived.**

Evaluation of foreseeable effects

The lentiviruses are, at worst, amphotropic or pseudo-typed with VSV-G protein, either of which confers a broad host tropism including human cells. However, infectivity is unstable and infection is only obtained by co-cultivation of the packaged viruses with the recipient cells. The retrovirus is self-inactivating and there if thus no possibility of
further transfer.
Since some of the inserted DNA codes for potentially hazardous RNA or protein, the work is assessed as Class 2. This accords with HSE SACGM Compendium of Guidance for retroviruses (Part 2, para 18-20).
Even for the non-oncogenic inserted DNA, there is a slight but non-negligible risk due to the presence in the lentiviral vector of the Woodchuck Post-transcriptional Regulatory Element. The WPRE-containing vector DNA will be treated as potentially oncogenic and is assigned to class 2 (see HSE SACGM Compendium of Guidance for retroviruses (Part 2, para 13)).
However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle:

- The genes encoding structural and other components of the viral genome have been separated. These genes have been engineered to minimise the risk of recombination that could lead to production of a replication-competent virus.
- The packaging cell lines allow expression of proteins, required to produce progeny virus: But the transfer vector a.

Third generation or Self INactivating vectors retrovirus vectors will be used in all experiments.
Procedures and controls measures will therefore follow HSE SACGM Compendium of Guidance for retroviruses (Part 2, para 30-36) i.e. using multiple plasmids with minimum sequence homology (e.g. our 3rd generation SIN lentivirus vector system), gloves should be worn, use of class II safety cabinets, sharps avoided and all wastes be rendered harmless before disposal etc.

It is not thought that the modified virus would pose a serious risk to animals or plants in the environment. Although the VSV coat protein permits invasion of other mammalian cells, as in the case of humans, infection would be restricted to primary cells and productive virus would not be produced. In addition the control measures to protect human health will minimise release of virus to the environment. Therefore the environmental risk is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company. Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor Vetspeed (GM authorisation number GM898).
This disposal method is expected to achieve 100% inactivation of the GMM.
The data sheets describing inactivation by Virkon are attached. No Sharps or glass will be used and plastics are collected in disposal bin and disposed of by approved contractors.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
Risk assessments approved on (07.10.2020)

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
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Animal Units

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<th>Human Clinical Applications</th>
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GM Centre Number: 3429

Data Premises Notified 07/09/2018 (Originally)

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

BioEscalator

Name 2

Department

Campus Estate or Research Centre

Building

INNOVATION BUILDING

Road Name

ROOSEVELT DRIVE

District

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX3 7LF

Country

ENGLAND

Tel Number 01865618801

Fax Number N/A

E-mail

HSE Division EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Genetic modification safety committee (GMSC) comprises of three individuals. The Biological safety officer (BSO) who holds the lab manager position for the BioEscalator and two Divisional safety officer from the University of Oxford expertise in biological safety. The specific role of the BSO will be the management and implementation of health and safety legislation (Management of Health & Safety at Work Regulations) by inspecting the laboratory, ensuring sufficient stocks of disinfectants etc and observing workers and will write regular reports to ensure a safe working environment. The BSO will implement genetic modification (GM) safety induction and compliance with GM safety procedures. The BSO will be immediately informed if there are GM incidences and containment measures will be taken and a report written and filed for HSE inspection. In the context of the GM safety and Contained Use Regulations, meetings between the BSO and Divisional safety officers will be held six monthly to review safety measures, disposal issues and implementation of further safety measures will be implemented and a written report will be filed.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
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<tr>
<td>Level 1 (GMMs)</td>
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Tick if confidential

- Bacteriology: Yes
- Parasitology: Yes
- Transgenic Birds: |
- Microbiology Research: Yes
- Virology: Yes
- Transgenic Animals: |
- Gene Therapy: Yes
- Mycology: Yes
- Transgenic Invertebrates: |
- Other (please specify below): |
- Transgenic Fish: |
- Other (please specify below): |
Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>The company will use Class 1 organisms that will include bacteria (E. coli). To safeguard human health measures for inactivation of microbes will be done with an autoclave on site, disinfectants and cleaning materials will be available, laboratory coats and gloves will be worn (Personnel Protective Equipment (PPE)). Waste management: Contaminated solid waste will be placed within a biohazard bag which will be removed by the BSO or lab technician for autoclaving at 134C for 15 minutes (for inactivation) and disposed for incineration by a licence waste contractor Barbican Logistics. Liquid waste will be treated primarily with 2% wlv virkon solution for inactivation. In case of spillage, a solution of disinfectant (Virkon 2% wlv in water-stable for 7 days) will be used. Spills of infected material will be attended to immediately by laboratory personnel (wearing gloves and lab coats) followed by treatment of the affected area with diluted Virkon disinfectant.</th>
</tr>
</thead>
</table>

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

GM safety committee seeks to maintain good microbiological practice for handling of E. coli, i.e. containment measures for level 1 organisms. K12 commercial E. coli used are not considered pathogenic to humans or animals. The E. coli strains used are very unlikely to survive or thrive in the environment as they have auxotrophic requirements or other debilitating mutations. The E. coli strains used would not be hazardous to the environment even if they did survive. The GMOs are unlikely to be any more harmful or better able to survive than the original host. There are no special measures necessary to protect the environment & details of waste disposal and emergency clean-up procedures have been described in section 6. Agar plates, batch cultures and all other contaminated materials will be autoclaved before disposal. E. coli K12 strains are recognised as non-colonising to humans and are unlikely to persist in the gut, lung, or survive outside of the special culture medium which provides their auxotrophic requirements.
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<td>Date at Which Additional Info Submitted</td>
<td>02/03/2022</td>
</tr>
</tbody>
</table>
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

More than 10 years experience of GMO class 1 and 2 organisms. Writing of multiple risk assessments for the use of GMO in different facilities. Submission of previous Notification of premises applications to HSE and been GMO safety officer.

<table>
<thead>
<tr>
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Non-microbial

Other (please specify)  

Tick if confidential

Bacteriology  

Parasitology  

Transgenic Animals  

Transgenic Fish  

Microbiology Research  

Virology  

Transgenic Birds  

Transgenic Fish  

Gene Therapy  

Mycology  

Transgenic Invertebrates  

Transgenic Plants  

Other (please specify below)  

Other(s)  

Microbiology testing  

For activities involving GMMs, describe the waste management measures which will apply to the activity.
Biological waste is disposed of in a yellow waste Eurobin, with an identifier sticker and then placed at the collection point for removal to the incinerator. Liquid waste is inactivated with 1% virkon overnight (>6 hours) before disposal in the sink with copious amounts of water. All the waste disposal procedures are overseen by the Babraham Campus where DefiniGEN is located.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

HEK 293 cell, the Human Embryonic Kidney 293 cells are class 1 GMO organisms. This line was originally derived from human embryonic kidney cells grown in tissue culture. HEK293 cells are available from Sigma Aldrich and are easy to grow and transfect cells and have been widely used for cell biology research and also used by the biotechnology industry. This line has been extensively used for several years and is also thought to be safe.
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**Name**

PROKARIUM LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

LONDON BIOSCIENCE INNOVATION CENTRE

**Road Name**

2 ROYAL COLLEGE STREET

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

NW1 0NH

**Country**

ENGLAND

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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<td>2 ROYAL COLLEGE ST</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Prokarium is relocating to new premises in London following spinning out from Cobra Biologics (Keele, Staffordshire) and a former Biological Safety Officer of Cobra Biologics (Prokarium's Chief Scientific Officer) will be the Biological Safety Officer of Prokarium, with over 15 years’ experience serving in a Genetic Modification safety committee. As a newly separate entity, Prokarium will set up a GM safety committee that will meet periodically when there are new GM risk assessments to be discussed, with a committee secretary to be appointed and minutes taken.

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<td></td>
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<td>Birds</td>
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</table>
Solid waste contaminated with GM Class 1 material will be sealed in autoclavable clinical waste bags, autoclaved by LBIC and collected by a licensed waste contractor. Liquid waste will be inactivated by the addition of sodium hypochlorite to a final concentration of 1% and minimum incubation time of 1 hour, resulting in a six order-of-magnitude reduction in viability, and disposed to drains.

For activities involving GMMs, describe the waste management measures which will apply to the activity
<table>
<thead>
<tr>
<th>Name</th>
<th>CELL CUIDANCE SYSTEMS LTD</th>
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GM Centre Number: 3432

Data Premises Notified (Originally) 02/02/2022

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed Transitional Premises Emergency Plan Required? N

Non-GMMs  N Withdrawn  N

Comments
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Yes

Give brief details of the genetic modification safety committee

The Safley committee consists of three PhD level scientists who each have experience in performing risk assessments

<table>
<thead>
<tr>
<th>Laboratory</th>
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Tick if confidential

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

- Disposal of waste
- Contaminated plastics in in biohazard bins,
- Liquids by addition of bleach 100%
- Liquids by addition of 70% ethanol 100%
- Spills by Virkon followed by 70% ethanol 100%

Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: 

Please enter comments of the GM safety committee on the risk assessment:

Genetic modification in relation to an organism is defined as "the altering of the genetic material in that organism by a way that does not occur naturally by mating or a natural recombination or both". Techniques involving direct introduction of heritable genetic material, including methods such as particle bombardment, direct injection of naked DNA into an animal and the use of other gene delivery system e.g. liposomes are considered to be genetiC modification where the introduced material is intended to be incorporated into the organisms genetic material in a reasonably stable way. All work that is covered by this definition must have a risk assessment carried out and approved by the genetic modification safety committee prior to the work beginning.
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Date at Which Additional Info Submitted

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Advice was sought from the REPROCELL Clinical Alliance Manager (PhD).

- Over 20 years of experience within academic research institutions working mainly within the field of pathogen research, laterally as a Research Fellow and Project Investigator.
- Extensive experience of preparing and assessing risk assessments associated with contained use and pathogen research.
- Prepared or contributed to past applications for consideration by University GM committees and the HSE.

<table>
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Other (please specify) Tick if confidential
All liquid cell culture waste is inactivated with 1% Virkon for at least 30 minutes prior to disposal (greater than 99.999% kill, manufacturer validated). Liquid waste is stored in a sealed vessel during inactivation, prior to disposal via liquid drain waste with copious volumes of running water. Any spillages are covered with Virkon powder for at least 3 minutes before being scraped into a safe receptacle and the area is washed with 1% Virkon (greater than 99.999% kill, manufacturer validated). Single use/disposable labware e.g. cell culture vessels, serological pipettes, pipette tips etc. are used where possible and reusable labware is decontaminated with 1% Virkon (greater than 99.999% kill, manufacturer validated) after each use.

Incineration

All solid laboratory waste is destined for incineration (100% kill).

Waste Disposal

All solid laboratory waste is treated as clinical waste and is uplifted by an approved waste disposal contractor (registered under the GMO contained use regulations 2001 to 2010) for disposal by incineration (100% kill).

For activities involving GMMs, describe the waste management measures which will apply to the activity

A copy of the risk assessment was provided for review. Based on the information provided I would concur that risks associated with this body of work are low and that all work should be performed under containment level 2 conditions. I agree with notification of this work to HSE as Class I.

Please enter comments of the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Other(s) Genome Editing and Reprogramming Technologies
### GM Centre Number: 3434

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**Name**

UNIVERSITY HOSPITAL SOUTHAMPTON NHS FOUNDATION TRUST

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

TREMONA ROAD

**Town**

SOUTHAMPTON

**District**

**County**

HANTS

**Postcode**

SO6 6YD

**Country**

ENGLAND

**Tel Number**

023 8077 7222

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

Date at Which Additional Info Submitted

02/03/2022
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

University of Southampton Genetic Modification and Biological Safety Committee have agreed to review and provide advice for risk assessments from University Hospital Southampton.

The University of Southampton GMBSC sits three times a year. The Committee is Chaired by a senior Professor and Committee members are from academic research departments within the Faculty of Medicine, Faculty of Environmental and Life Sciences and the Faculty of Engineering and Physical Sciences. The University Biological Safety Adviser is also a Committee member and can pre-approve any activity class 1 risk assessments prior to formal approval at a GMBSC meeting.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Any waste or remaining GMO will be destroyed according to the local operating procedures governing the destruction of genetically modified organisms under contained use regulations.

All GMM waste handled by the CRF is transferred to the PHE labs according to local SOP for autoclaving.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

GMBSC-0221 A Phase 3 Open-Label, Single-Arm Study To Evaluate The Efficacy and Safety of BMN 270, an AdenoAssociated Virus Vector-Mediated Gene Transfer of Human Factor VIII

It was noted that this project had been submitted by the trust under the agreement that the University of Southampton Genetic Modification & Bio-Safety Committee review their projects.

It was highlighted that a specific sentence in the risk assessment stating that the virus did ‘too little to do any harm’ made no sense.

Project Ref 3434/21.1

<table>
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<tr>
<th>Date Ackn’d</th>
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<td>23/09/2021</td>
<td>Gene therapy product RP1 (Common name rHSV-hGM-CSF/GALV-GP) is an oncolytic immunotherapy product that is developed for the treatment of solid tumours</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</table>

Date Project 02/03/2022
The purpose of the release is to perform an Open-Label, Multicenter, Phase 1/2 Study of RP1 as a Single Agent and in Combination with PD1 Blockade in Patients with Solid Tumors (IGNYTE). The Phase 1 portion of the study has completed enrollment and includes patients with advanced solid tumours. The Phase 2 portion of the study assesses the safety and efficacy of RP1 using the recommended Phase 2 dosing (RP2D) from Phase 1 in combination with nivolumab.

RP1 (rHSV-hGM-CSF/GALV-GP) is a selectively replication competent Herpes Simplex Virus-1 (HSV-1). The virus contains a codon-optimised sequence for human granulocyte macrophage colony stimulating factor (hGM-CSF) and a codon optimised sequence for the gibbon ape leukemia virus surface glycoprotein (GALV-GP) with the R- sequence deleted (R-) [GALV-GP-R-]. GALV-GP-R- expression leads to cell to cell fusion (syncytial) formation in infected tumour cells through binding to the constitutively expressed PiT-1 receptor for GALV. This results in the death of the cells by membrane fusion and is also intended to enhance the spread of the virus through the tumour. Since the RP1 selectively replicates in tumour cells, the expression of the GALV-GP-R- is minimised in normal tissues. The oncolytic destruction of tumour cells leads to the release of tumour associated antigens that are intended to engender an antitumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further enhanced through GALV-GP-R- mediated killing, fusion associated cell death which also results in the production of the highly immunogenic exosomes, which is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, uninjected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

RP1 (rHSV-hGM-CSF/GALV-GP) is derived from the RH018 strain of Herpes Simplex Virus-1. RP1 is produced in Vero cells and virus is released into the culture media by budding from the cells and during cell lysis, prior to purification.
RP1 expresses the immune stimulatory protein GM-CSF, which augments therapeutic activity. GALV-GP-R- binds to the PIT1 receptor, which is widely expressed on mammalian cells including human tumour cells. PIT1 is also critical for cell proliferation, and its expression is therefore unlikely to be lost or down-regulated in response to cancer treatment. The truncated R- version of the protein provides constitutive fusion activity without GALV (i.e. the virus) itself. Expressing GALV-GP-R- together with GM-CSF is expected to increase clinical activity as compared to only expressing GM-CSF. As well as causing direct tumour cell death by cell to cell fusion, cell to cell fusion followed by death is highly immunogenic and includes the release of Immunogenic tumour antigen-containing exosomes. Expression of GALV-GP-R- from an oncolytic virus is therefore expected to improve systemic, immune mediated, effects, as well as effects in the directly treated tumour thereby increasing synergy with other immune-mediated approaches to cancer therapy such as immune co-inhibitory pathway blockade.

Evaluation of foreseeable effects

As described above (under Recipient or Parental Organism), the oncolytic destruction of tumour cells (upon transduction with RP1) leads to the release of tumour associated antigens that are intended to engender an antitumour immune response, enhanced by the local expression of GM-CSF. This is intended to be further amplified through GALV-GP-R- mediated killing, fusion associated cell death which results in the production of the highly immunogenic exosomes and is expected to contribute to this immune effect. The immune response generated may then lead to immune destruction of distant, un.injected tumours, and/or delay the progression of distant disease, and/or vaccinate against relapse. RP1 is intended for direct injection into solid tumours.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Ill used and unused vials of RP1 will be disposed of per institutional policies and procedures and in line with the RP1 IS.

As per the wild-type HSV-1 virus, the recombinant HSV-1 vector particles that represent RP1 are highly susceptible to dehydration, rapidly inactivated outside the host and easily inactivated (for example with 1% Virkon solution). All materials contaminated with RP1 will be disposed of in compliance with local institutional policies and SOPs. Incineration is appropriate.

How must infected waste be treated before disposal and how has this been validated as being effective?

Liquid waste - All potentially contaminated material will be inactivated by chemical disinfection (1% Virkon for 30 minutes) and/or autoclaving at 121°C for a minimum of 15 minutes.

Solid waste - All potentially contaminated material will be inactivated by chemical disinfection (1% Virkon for 30 minutes) and/or autoclaving at 121°C for a minimum of 15 minutes.

How is waste to be disposed of after it has been made safe by validated means?

Treated Liquid waste - Liquid waste would have been placed in a bucket containing freshly made 2% Virkon solution for a minimum of 30 Min. At the end of this time the liquid contents will be carefully Upped through a fine sieve into the lab sink; the contents of the sieve will be placed into a blue waste box for disposal via incineration.

Treated Solid waste - Disposal of GMO waste in dedicated wheeled bins for Incineration.

All staff identified as at risk of exposure will be trained appropriately during the trial setup process during trial
Uncontrolled if printed 29 April 2020 Page 9 of 18

setup either at the site initiation visit or at separate dedicated meetings; records of this training will be kept as per local SOPs and GCP guidance in the training log kept in the site file.

In the event of exposure to broken skin or needle stick, the site will be cleaned thoroughly with soap and water or a skin disinfectant. A physician for monitoring signs of infection and occupational health will be contact for support.

Acyclovir or other anti-herpes drugs may be administered prophylactically.

Patients will be provided full instruction sand support from the Principal and co-investigators which will include care of the lesions and advice on shedding. Patients will be provided with spare dressings, instructions for use and safe disposal equipment which will be returned to the clinical site. In addition, the patients are given out of hours contact numbers and advice will be available from the clinical site team, if needed. All advice given will be in line with the sponsor's instructions and the study protocol.

The GM herpes virus is replication deficient in normal healthy individuals.
Risks considered and appropriate measures taken to prevent accidental transmission of virus.

Looks like all sensible precautions are in place for AC2 contained use.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [Y]

Please enter comments on the GM safety committee on the risk assessment

The GM herpes virus is replication deficient in normal healthy individuals.
Risks considered and appropriate measures taken to prevent accidental transmission of virus.

Looks like all sensible precautions are in place for AC2 contained use.

Project Containment

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<th>Laboratory Activities</th>
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Name

EMPYREAN THERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre

BABRAHAM RESEARCH CAMPUS

Building

BUILDING 250

Road Name

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB22 3AT

Country

ENGLAND

Tel Number

0223 804380

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Person with extensive experience (17 years) working with GMMs/GMOs in the cell therapy research and development and AAV/CRI/SPR area who has also set up new procedures and risk assessment previously. Extensive experience working with primary human cells.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Gene Therapy</td>
</tr>
</tbody>
</table>

02/03/2022
Incubation in 1% w/v virkon and 70% ethanol is used to disinfect areas and for disposal of cells once experiments have been completed. Disposable plastics, such as pipette tips, are incubated in virkon before being incinerated by Babraham Research Campus waste disposal service.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

This work is considered to be low hazard (class 1) to the environment and human health due to the following
1. No virus is constructed on site (obtained through a commercial supplier)
2. The virus is replication incompetent so the infection cannot spread beyond the cells initially exposed
3. The genes involved are non hazardous to human health (eg reporter genes such as GFP)
4. The infected human cells are not able to survive outside of the specific incubator environment (5% CO2, 37°C)
5. The risk to human health is minimised as no sharps are used, in addition all samples will be handled in a class 2 tissue culture hood to maintain experiment purity, thus further minimising risk to human health via aerosols.
6. All waste will be handled correctly minimising risk to the environment.
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**Name**

ORBIT DISCOVERY LTD

**Name 2**

Department

**Campus Estate or Research Centre**

BROOKES INNOVATION HUB

**Road Name**

GIPSY LANE

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX3 0BP

**Country**

ENGLAND

**Tel Number**

01865 484 057

**Fax Number**

0

**E-mail**

HSE Division

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Advice has been sought from Orbit Discovery Ltd employees (graduate and post doctoral research scientists with both academic and industrial backgrounds) with relevant experience working with GMMs at biosafety levels 1, 2 and 3 and additionally we have sought advice from an external biosafety consultant.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
All waste materials (liquid cell cultures, frozen cell stocks, plasticware such as pipette tips, culture flasks etc) will be soaked in a suitable disinfectant (eg Virkon 2%). Liquid waste will then be disposed of via an approved laboratory sink (thoroughly flushed with water) all other materials will be double bagged and disassembled of by incineration (carried out by external laboratory waste management specialist).

Projects:
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
- Mycology
- Transgenic Invertebrates
- Transgenic Plants
- Other (please specify below)
- Other(s)

Peptide Discovery - discovery of peptide candidates for therapeutic use

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

All work currently carried out by Orbit Discovery Ltd and any future work planned falls into biosafety level 1. This work includes the use of well characterised mammalian (human and non human eg CHO, HEK293) continuous cell lines that may have been genetically modified to express proteins of human origin (eg G protein coupled receptors - GPCRs), such cell lines are purchased from commercial sources (ECACC, ATCC).
None of the cell lines used will be modified by or exposed to pathogenic agents. Wont integrating lentivirus into the genome count as modification? We also use chemicals that are pathogenic?
Orbit Discovery currently have and will have at the new site (The Schrodinger Building, Heatley Road, Oxford, OX4 4GE) laboratory facilities suitable for biosafety level 2 and the appropriate processes and systems in place to handle biosafety level 1, and level 2 in the future if required. All cell cultures will be routinely monitored for the presence of bacterial or mycoplasma contamination.
Lentiviral transduction of mammalian cell lines, recombinant protein expression and generation of stable cell lines

The overall aim is the generation of mammalian cell lines for use as tools to screen for active peptides. Cell lines will be generated that overexpress a cell membrane receptor [predominantly G-protein coupled receptors (GPCR)] along with a reporter system to allow for detection of receptor activation. More specifically, mammalian cell lines (such as CHO-K1, HEK293T, HEK293, A549, TO2J) will be transduced with lentivirus carrying sequences encoding for a cell membrane receptor (e.g. GPCR) and/or an inducible reporter construct (e.g. cAMP response element linked GFP). Different combinations of receptor and reporter constructs will be used and most of the sequences will include an antibiotic resistance cassette (such as Puromycin or Hygromycin) in order to select for cells that were successfully transduced. The lentiviral particles (3rd or 4th generation) will be sourced from commercial suppliers and will arrive pre-packaged with the RNA cargo. Commercially available, well characterized mammalian cell lines such as (but not limited to) CHO-K1 and HEK293 will be used. Mammalian cells have very stringent requirements for growth and are very susceptible to dehydration and to exposure to ultraviolet radiation. Outside of the animals from which they are derived, growth and survival requirements can only be met by using specialised media, the correct temperature range, optimum pH and an adequate oxygen concentration. These constraints mean that cell lines will pose minimal risk to both human health and the environment. In addition, due to immune rejection of non-self-tissue, it is highly improbable that accidental exposure would result in survival and replication in normal healthy individuals. However, there is some possibility that any cell culture could contain yet unidentified adventitious agents, thus cells will be handled in a class II biological safety cabinet meaning that the risk to human health is low.
Lentiviral constructs are well characterised vectors that enable insertion of genetic material into host cell genomes. A 3rd or 4th generation lentiviral vector will be used for transduction of mammalian cells, this generation of vectors are not considered harmful due to being replication incompetent (self-inactivating). Lentiviral particles will be sourced from commercial suppliers pre-packaged with the RNA cargo and pseudotyped with VSV-G (or similar). The VSV-G pseudotyped lentivirus has a broad tropism and thus could infect a variety of cell types (most mammalian cells). A high concentration of virus is present in culture for a short time and will be washed away over progressive media changes and passages. Once the insert has been incorporated into the cell, the virus is no longer infectious. The main risk to laboratory staff comes from accidental inoculation or entry through broken skin while handling the virus. No sharps will be used in this procedure, gloves and a class II cabinet will be used to prevent exposure. If exposure did occur the lack of ability for the virus to replicate would result in Infection of cells at the site of injection only (in vivo experiments have shown diffusion of infectious particles no more than millimetres from the injection site), the nature of the insert is such that it will pose minor risk. Overall, the risk of an infection with a lentivirus particle is very low as:
• the virus and the transduced cells will be handled in a class II biological safety cabinet,
• small quantities of virus are used (< 1 ml of stock virus in used in smaller aliquots, ~3-4*10^8/ml TU, diluted/remaining virus in up to 30 ml medium).
• the packaged viral vector will be sourced commercially,
• the virus is replication defective and present in the cultures for a limited time
• Sharps are not used when virus particles are present and are otherwise avoided.

Origin & function

The proteins expressed in the transduced mammalian cells will pose no risk, as these will be mostly Human GPCRs, GPCR signal pathway molecules or reporter proteins. These proteins do not possess oncogenic properties and do not classify as toxins.

Evaluation of foreseeable effects

The resulting GMO will be a cell line that over-expresses a cell surface receptor and reporter construct, no virus particles will remain, so the risk from the GMO will be equivalent to that for mammalian cell lines. Mammalian cells have very stringent requirements for growth and are very susceptible to dehydration and to exposure to ultraviolet radiation. Outside of the animals from which they are derived, growth and survival requirements can only be met by using specialised media, the correct temperature range, optimum pH and an adequate oxygen concentration. These constraints mean that cell lines will pose minimal risk to both human health and the environment. PPE must be worn, and personnel must be trained in handling mammalian cells and virus.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For waste where virus particles are present in culture:
The presence or absence of virus particles in culture waste will be calculated using using the Viral particle reduction rate calculation (Dautzenberg et al. 2020):
Reduction ratio = (Wash^W x (Wash x Tryp)^I x 2^(HL x T)) / (10 x Ci)
where “Wash” represents the reduction factor upon a wash step of the transduced cells (50 for 1x6w, 20 for 10 cm dish) and “W” signifies the number of times the cell culture was washed. The parameter “Tryp” indicates the vector-dependent reduction factor for treatment of the cells with trypsin (for VSV-G: 1), where “I” is the amount of trypsin treatments after a wash step. “HL” is the reduction factor based on the half-life of the envelope-pseudotyped vector particles at 37 °C in culture medium (for VSV-G: 0.7) and “T” the total culture time in days since the transduction of the cells. The parameter “Ci” represents the measured amount of infectious vector particles in the inoculum. The formula includes a safety margin of 10-fold to correct for the underestimation of infectious titre of the inoculum by multiplying the amount of initial vector particles in the culture medium (Ci) by 10. All waste generated where Viral particles could be present will be treated as follows.

Solid waste and liquid waste will be disinfected inside a biological safety cabinet using a final concentration of at least 1 % Virkon, with a contact time of 1h. All disinfected solid waste material will be double bagged in autoclavable bags or put in Bio-bins and then autoclaved. This includes any materials used for handling leakages, spillages or breakages. This waste will be later incinerated by a GMO licenced waste company. Disinfected liquid waste will be drained down an approved sink with excess of water. The autoclave will be validated by yearly service and the success of each run will be monitored by recording run temperature and time.

For waste where virus particles are no present:
All solid material or waste that comes into contact with cells will be soaked in a final concentration of at least 1 % Virkon for at least 15 min before draining off the Virkon and disposing of in the lab waste that is later incinerated by an external laboratory waste management specialist.
All liquid waste/media in contact with cells is inactivated in a final concentration of 1 % Virkon for at least 15 min before draining down an approved sink with excess of water.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment has been read and discussed at a meeting held by the GMO biological safety committee, they are satisfied that the proposed measures will provide adequate containment.

Project Containment

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<tr>
<th>Laboratory Activities</th>
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Animal Units | Large Scale Activities | Human Clinical Applications

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| Comments                          |            |

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The University of Manchester Faculty of Science and Engineering genetic modification and biological safety committee.

<table>
<thead>
<tr>
<th>Laboratory</th>
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Tick if confidential

- Bacteriology: Yes
- Parasitology
- Transgenic Birds
- Microbiology Research
- Transgenic Animals
- Gene Therapy
- Transgenic Fish
For activities involving GMMs, describe the waste management measures which will apply to the activity

Sterilisation of small amounts of solid contaminated waste (eg tips, gloves), and small cultures (<10L) will be performed by on site autoclaving (Prestige Medical Classic, 12L capacity electrical autoclave, steam disinfection type). Waste is then disposed of through domestic routes.

Large volume liquid culture will be pumped into external waste containers (large IBC drum) and treated with virkon (1% for a minimum of 1 hour) prior to being removed by specialist chemical disposal company. Equipment that has been in contact with GMMs will be sanitised by treatment with sodium hypochlorite (0.5% for minimum 30 minutes to 1 hour). For the fermenter vessel 0.5% sodium hypochlorite (~50L) will be recirculated throughout the entire vessel for one hour. Vessel with then be extensively flushed with water to remove bleach. Liquid waste from this procedure will be pumped in to external waste containers (large IBC drum) for removal by specialist chemical disposal company. Site is not connected to domestic drains so any liquid waste will go into IBC waste drums. Confirmation of effective sterilisation/sanitisation will be performed by periodic test swabbing the laboratory equipment and culturing on GMM-specific agar plates, which will be performed in the MIB.

The fermenter will have 100% liquid external containment capability (bunding) in the case of a spillage or leak. For small spills, these will cleaned up with absorbant paper or mats, from spills kits, which are then autoclaved. The area is then sanitized with 1% virkon. Gaseous emissions will be filtered (0.2 um) to eliminate the risk of accidental GMM aerosol exposure to the air.

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Please enter comments of the GM safety committee on the risk assessment

All genetic manipulations will be performed at the Manchester Institute of Biotechnology. A similar small-scale project (University of Manchester GM Project no. NSC1803) has already been approved by the faculty of science and engineering GM and biological safety committee, and the work undertaken at the MIB. Small starter cultures will be produced in the MIB, then transported by courier from the MIB with the correct packing and labelling.

No genetic manipulations will be performed on site, only small and large-scale fermentations of the recombinant microorganisms (20-300 L).

This project will be assessed by the University of Manchester faculty of science and engineering GM and biological safety committee, in order to ensure a level of oversight on the biological safety aspects of the project, although none of the large-scale fermentation work will be completed on university premises.
GM Centre Number: 3438

Data Premises Notified: 22/10/2018 (Originally)
Transferred from 1992 Regs?: N
Transitional Premises Class: N
Data Premises Closed: N
Transitional Premises Emergency Plan Required?: N
Non-GMMs: N
Withdrawn: N

Name
CHAIN BIOTECHNOLOGY LTD

Name 2

Department

Campus Estate or Research Centre
CENTRE FOR BIOMOLECULAR SCIENCES

Building
UNIVERSITY OF NOTTINGHAM

Road Name
UNIVERSITY BOULEVARD

District

Town
NOTTINGHAM

County
NOTTINGHAMSHIRE

Postcode
NG7 2RD

Country
ENGLAND

Tel Number
07966 172134

Fax Number
0

E-mail

HSE Division
blank

Comments

Date at Which Additional Info Submitted:
02/03/2022
**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

GMSC comprises of two individuals:
- Project Manager/Health and Safety Officer
- Lab/Qality Manager has several years experience conducting risk assessment in pharmaceutical microbiology laboratories/pharmaceutical manufacturing.
- Containment, monitoring and accidental release procedure are documented in the following SOP's CB2.6 Accident Reporting CB2.14 Laboratory Entry/Exit Procedure CB4.1 Culture Handling CB6.1 Housekeeping CB6.2 Environmental Monitoring CB6.3 Waste Diposal CB6.4 Spill Procedure

Meetings/inspections will be held at least every six months.

4.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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</table>
All biological laboratory waste will be autoclaved prior to disposal (with the exception of discared gels which will be sealed ahead of specialist disposal by Labwaste). The autoclave is located within the suite of laboratories where the work is being conducted. The waste cycle will be run at 121°C for 45 minutes which will achieve a kill of at least 10^5 or higher. After autoclaving, the waste will be stored in a locked Eurobin before collection by a specialist waste removal firm (we will be using Labwaste part of TradeBe). The autoclave will be under a service contract to ensure it is functioning as required. The autoclave run will be monitored by the use of the onboard software. Preuse and every three months after, the kill level will be verified by the use of Geobacillus stearothermophilus biological indicator spore strips containing a known number of spores. Each run will be verified by the use of autoclave indicators such as autoclave tape and Browne tubes or similar.

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<tr>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

GMSC comprises of two indivduals. Project Manager/Health and Safety Officer holds a PhD in Microbiology, is an expert on gut pathogens and has extensive industrial experience in Human Medical Diagnostics. Lab/Quality Manager has 18 years experience in GMP/Pharmaceutical Microbiology and CL 1/2 microorganisms.
## Project Additional Information

### Purposes of the contained use

Development of live biotherapeutic products and analysis of in vitro and in vivo experimental samples.

### Recipient or parental organism

C. butyricum, C. sporogenes, C. novyi. All non-toxigenic variants

### Host/vector system

N/A

### Origin & function

Genetic material is derived from genom sequences of other bacteria or humans. All material is synthesised and introduced into bacterial strains to confer a metabolic, anti-inflammatory, anti-microbial function.

### Evaluation of foreseeable effects

Expression of therapeutic metabolites and peptides from recipient organisms. Does not confer any growth or niche benefit to strains.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Directly contaminated waste (petri dishes, liquid media) will be autoclaved where possible to achieve a kill of 10^5. After autoclaving, the waste is stored in either a drum or locked clinical waste bin until full. Once full, it is taken off site by a specialist waste carrier. Where it is not possible to autoclave the waste e.g. where the vessel size exceeds the capacity of the autoclave, the waste is chemically neutralised using a validated procedure, the waste is then taken off site by specialist waste carrier.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form N

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment and relevant information is freely available to all members of CHAIN staff. CHAIN have seven members of active laboratory staff who are all satisfied that the control measures in place are sufficient to ensure their safety and the safety of others.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
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<tr>
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<td>L4</td>
</tr>
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Animal Units

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<th>Human Clinical Applications</th>
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GM Centre Number: 3440

Data Premises Notified (Originally) 02/11/2018

Transferred from 1992 Regs? N

Transitional Premises

Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

SIXFOLD BIOSCIENCE LTD

Name 2

Department

Campus Estate or Research Centre

TRANSLATION & INNOVATION HUB

Building

Road Name

80 WOOD LANE

District

Town

LONDON

County

GREATER LONDON

Postcode

W12 0BZ

Country

ENGLAND

Tel Number

07513463372

Fax Number

0

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
# Premises Addresses

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</tbody>
</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

Sixfold Biosciences is advised by the head of health and safety at the Francis Crick institute, who has vast experience in leading and implementing risk assessments for the use of genetically modified organisms.

### Laboratory

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
<th>Level 4 (GMMs)</th>
<th>Non-microbial</th>
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### Animal Unit

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<th>Bacteriology</th>
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<th>Microbiology Research</th>
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<tbody>
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<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
</tr>
</tbody>
</table>

02/03/2022  Page 14478 of 15326
The cell lines that we are using require specific conditions in order to survive (e.g. specific media, temperature and CO2 concentration). They should not survive after disposal outside those conditions, but to ensure cell killing we will use a biocide such as Virkon (1%) to decontaminate tissue culture fluid in a vacuum reservoir prior to disposal. This will ensure 100% of cells are killed.

<table>
<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
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<th>Other (please specify below)</th>
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<tr>
<td></td>
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</table>

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

None

---

**Project Ref 3440/20.1**

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
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<tr>
<td>20/03/2020</td>
<td>The use of genetically modified organisms and other containment level 2 (CL2) work carried out in Sixfold cell culture labs primarily in laboratory G10L</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
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</table>

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID
## Project Additional Information

### Purposes of the contained use

The purpose of the notified activity relates to the usage of lentiviral vectors for stably transfecting mammalian cells for performing preclinical studies.

### Recipient or parental organism

Mammalian cells derived from homo sapiens, mus musculus or other rodent species.

### Host/vector system

Second or third generation lentiviral plasmids (such as psPAX2 (Addgene plasmid #12260) and pMD2.G (Addgene plasmid #12259)) will be used to transflect HEK293T cells and generate viral particles.

### Origin & function

Multiple methods will be used to modify the cellular expression of certain proteins by incorporating DNA sequences onto the genome. The vectors used may contain an shRNA sequence, a guide RNA, Cas9 protein or a sequence coding for full or part of proteins present in homo sapiens, mus musculus or other rodent and non-human primate species. The function of the genetic material will be to modify (up and downregulate) the expression of certain proteins involved in the development of a variety of diseases, with an initial focus on oncology indications.

### Evaluation of foreseeable effects

2. Healthy volunteers screened blood purchased from a commercial source: it may be wrongly screened or contain diseases it has not been screened for.
3. Growth media and supplements: Cells are cultured in growth media supplemented with Penicillin, streptomycin, fetal calf serum and L-glutamine. Additional biological agent supplements such as insulin, hydrocortisone and recombinant cholera toxin may be used as required.
4. Biocides such as Virkon (1%) that is used to decontaminate tissue culture fluid in a vacuum reservoir prior to disposal can cause irritation to skin and respiratory tract.
5. Puncture injuries from disposal of tips that may be contaminated with cells or cell culture fluids.
6. Use of vacuum reservoir for aspiration of biological fluids
7. Chemical agents in use. DMSO, used for freezing down cells lines in classed as non toxic.
8. 2-propanol. Mr. Frosty freezing container are filled with 2-Propanol which is highly flammable, can cause serious eye irritation and may cause dizziness or drowsiness. Appropriate PPE required and use in Fume hood.

10. Cells may be plated on glass coverslips in tissue culture dishes for use in immunofluorescence. The coverslips are delicate and break easily which can result in sharp debris left in the hood.

11. Scissors used to open buffy coat packs.

12. Reagents IL-2, IL-4, GM-CSF and M-CSF, are biological agents and potential irritants to the skin and eyes, however, they are not hazardous in the concentrations used.

All the work will be performed under a strict CL2 risk assessment that will aim to mitigate the aforementioned effects on the people and the environment (attached).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No larger GMOs will be used in these premises.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste:
Pipette tips and stripettes will be disposed in a plastic waste bin within the BSL-2 cabinet and sealed prior to removal from the biosafety cabinet. Waste will be transported in sealed containers prior to autoclaving. All contaminated materials will be decontaminated by autoclaving (100% kill) prior to disposal of waste. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature/time profile. Following autoclaving the non-hazardous waste will be sent for incineration following local regulations. Glass coverslips will be handled on tissue. In case of breakage, all the pieces can be gathered up in the tissue and disposed of in a sharps bin.

Liquid waste:
Liquid waste with low contamination risk (e.g. routine cell culture media) will be aspirated into a bottle containing Virkon, a peroxygen based disinfectant, which will be used according to the manufacturer’s instructions. Following a 24-hour chemical inactivation, the resulting non-hazardous waste will be disposed of in an excess of water. Liquid waste with higher contamination risk (e.g. human blood products and excess viral supernatants) will be incubated with Presept disinfection according to the manufacturer’s instructions for at least 24 hours. The resultant non-hazardous waste (100% kill) will then be disposed of with an excess of water.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
The risks associated with the use of CL2 GMMS have been appropriately identified and mitigated on the risk assessment attached. Sixfold Bioscience Ltd. has systems in place to review and update those on a yearly basis or before if there are any improvements suggested or significant changes on the R&D processes.

Please enter comments on the GM safety committee on the risk assessment

The risks associated with the use of CL2 GMMS have been appropriately identified and mitigated on the risk assessment attached. Sixfold Bioscience Ltd. has systems in place to review and update those on a yearly basis or before if there are any improvements suggested or significant changes on the R&D processes.

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<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
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GM Centre Number: 3441

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<table>
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Date at Which Additional Info Submitted: 

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

University of Oxford Bioescalator Safety Office

Tick if confidential


<table>
<thead>
<tr>
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<th>Gene Therapy</th>
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<tr>
<td>Virology</td>
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<td></td>
<td>Yes</td>
<td>Yes</td>
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</tbody>
</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Disinfection measures: A 2% Virkon solution will be prepared prior to working with viruses and will be available should a spillage occur. After use the consumable and liquids containing virus will be exposed to 2% Virkon for a minimum of 30 minutes, as will all materials (e.g. tips, tubes) used during the experiment to ensure complete decontamination. Excess liquid is disposed of down the drain. Plasticware and consumables will then be entered into tissue culture waste and autoclaved (see below).

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection, used according to manufacturers instructions under standard conditions, manufacturers validation (99.998% kill)

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The safety committee agreed with the assessment of risk, containment facilities and disposal routes.

---

Project Ref 3441/18.1

Date Ackn’d 14/11/2018

Date Project 02/03/2022

CU2 Project Title Expression of reporter genes in adenovirus for laboratory research

Class Class 2

CultureVol Class 2 < 1 Litre

Class Volume Class 3-4

Non-GMM

Consent Granted
Adenoviruses have been studied in laboratories for many decades in order to gain insights into the biology of both viruses and cells. To help understand how they infect or replicate in cells under different conditions, it is common practice to add in reporter genes. These genes are non-hazardous and facilitate observation by techniques such as fluorescent microscopy.

In this project, we intend to modify adenoviruses to express fluorescent proteins, enzymes such as luciferase and control proteins to help with understanding adenovirus infection and replication in cancer cells.

Recipient or parental organism

Adenoviruses modified with reporter genes will be evaluated in laboratory tissue culture experiments. The recipient cells will be standard laboratory cancer cell lines and immortalised cells available from national biobanks. The reporter gene will be used to monitor the progress of adenovirus in short-term replication experiments. For comparison, some studies will be conducted in culture normal cells.

Host/vector system

Adenoviruses from group 8-G, for example adenovirus type 5, group C will be modified to express innocuous reporter genes, such as beta-galactosidase, tags (such as His tags or HA) green fluorescent protein (GFP; or similar fluorescent protein) or luciferase. Reporter genes will be driven by conventional promoters (e.g. CMV immediate early gene promoter) or by inserting IRES or P2A sites to link expression of the transgene to that of specific virus proteins. Alternatively, the transgene is under control of the virus endogenous promoters or splice acceptor sites.

Origin & function

1. Beta-galactosidase is a bacterial exoglycosidase that hydrolysos end terminal beta-galactopyranosyl residues. Natural substrates include lactose; the enzyme is also active e.g. ONPG, or X-gal which is used to produce an intense blue colour which can be used to visualise the distribution of the enzyme e.g. in different cells on a tissue culture plate or in tissue sections; it is widely used as a reporter gene and is considered innocuous.
2. Green fluorescent protein is a naturally fluorescent protein from the jellyfish, several variants of the natural gene have been produced which encode a protein with modified adsorption/emission characteristics and which have been optimised for higher level expression, e.g. the "EGFP". GFP's or similar fluorescent proteins are widely used...
and no hazardous properties have been reported.

3. Luciferase from firefly and sea pansy are widely used as reporter genes and are not considered to have harmful properties.

**Evaluation of foreseeable effects**

The addition of reporter genes will not have any incremental safety risk to the virus already in routine use in research laboratories and handled under category 2 containment conditions. When used in appropriately equipped laboratories by trained staff adenovirus (wild type or expressing reporter genes) do not pose a significant health risk to workers or the environment.

The virus does not integrate its DNA into the host genome as part of its life cycle.

Adenovirus are species specific and are not fully permissive in animals and the reporter genes are non-hazardous.

There should be no risk to the environment.

All work will be carried out by trained staff in category 2 conditions.

A full risk assessment has been written and has been signed off by the institutional health and safety committee.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

No derogations applied for

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Disinfection measures: A 2% Virkon solution will be prepared prior to working with viruses and will be available should a spillage occur. After use the consumable and liquids containing virus will be exposed to 2% Virkon for a minimum of 30 minutes, as will all materials (e.g. tips, tubes) used during the experiment to ensure complete decontamination. Excess liquid is disposed of down the drain. Plasticware and consumables will then be entered into tissue culture waste and autoclaved (see below).

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration/dispose of solids via the industrial (black bag) waste stream for landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

Degree of kill

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical disinfection, used according to manufacturers instructions under standard conditions, manufacturers validation (99.998% kill).

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y
The safety committee agreed with the assessment of risk, containment facilities and disposal routes.

**Project Containment**

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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**Project Ref** 3441/19.1

**CU2 Project Title**

Use of human adenoviruses as highly selective anti-cancer agents

**Class** CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre

Non-GMM Consent Granted

**Date Ackn'd**

18/07/2019

**Date Project Ceased**

**Withdrawn**

N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

Adenoviruses have been studied in laboratories for many decades to gain insight into the biology of both viruses and
cells.
The purpose of this project is to identify adenovirus genome sequences that facilitate selective lysis of cancer cells and attenuated activity in normal cells.

Recipient or parental organism

Parental: Human adenoviruses
Recipient: Standard laboratory cancer cell lines or immortalised cell lines available from national biobanks.

Host/vector system

Human adenovirus group 8-G (e.g. group C Ad5)

Origin & function

In this project we will use established genetic engineering techniques (restriction enzyme cloning, Gibson assembly, CRISPR/Cas9) to develop cancer specific adenovirus variants by identifying genome segments across adenovirus serotypes with utility against cancer. Adenovirus chimeras in specific gene regions important for oncolytic potential, notably E1-4 and L 1-5 will be created. Oncolytic candidates with modified genomes that show the highest therapeutic index (activity in cancer cells vs normal cells) will be taken forward for future studies.

For clarity, this project does not include the insertion of transgenes into adenovirus that may have additional associated risks. The use of transgene to individual oncolytic viruses identified in this project will be the subject to specific risk assessment in the future.

Evaluation of foreseeable effects

Viruses will be handled under category 2 containment conditions. When used in appropriately equipped laboratories by trained staff adenoviruses do not pose a significant health risk to workers or the environment. The virus does not integrate its DNA into the host genome as part of its life cycle. Adenoviruses are species specific and are not fully permissive in animal models. There should be no risk to the environment.

All work will be carried out by trained staff in category 2 conditions. A full risk assessment has been written and has been signed off by the institutional health and safety committee.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogations applied for

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Disinfection measures: A 2% Virkon solution will be prepared prior to working with viruses and will be available should a spillage occur. After use the consumables and liquids containing virus will be exposed to 2% virkon for a minimum of 30 minutes. As will all material (e.g. tips, tubes) used during these experiments to ensure complete decontamination. Excess liquid is disposed of down the drain. Plasticware and consumables will be entered into tissue culture waste and autoclaved.

Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS
2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration. The black bags are collected by select environmental services and are incinerated for energy and not landfill.

Liquids (eg samples, culture supernatants, tissue culture media) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), or inactivate by chemical means (following manufacturers guidelines) then discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration. The black bags are collected by select environmental services and are incinerated for energy and not landfill.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration.

The safety committee agreed with the assessment of risk, containment facilities and disposal routes.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The safety committee agreed with the assessment of risk, containment facilities and disposal routes.

Project Containment

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<tr>
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<th>Growth Rooms</th>
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Animal Units

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Project Ref  3441/21.1

Date Ackn’d  22/04/2021

Date Project  02/03/2022

CU2 Project Title  Use of replication-defective retroviral and lentiviral vectors expressing reporter genes
Project Additional Information

Purposes of the contained use

We plan to generate and use recombinant replication-defective retroviral and lentiviral particles expressing genes of interest (e.g. reporter genes) using a 3rd generation lentivirus plasmid vector system. This work will be performed in a research laboratory, to assist the development of new safe and effective cancer therapies. This work will use a 3rd generation lentivirus plasmid vector system which further improves on the safety of the 1st and 2nd generations. The vectors produced will not be used in any human or animal experiments, and will not be transferred outside the laboratory area or into the wider environment.

Recipient or parental organism

Recipient cells will be standard laboratory cancer cell lines and immortalised cells available from national biobanks.

Host/vector system

Lenti-SFFV is a typical lentivirus plasmid, which may be engineered to introduce reporter transgenes and promoters and then co-transfected into packaging cells with other plasmids providing the proteins. Providing the packaging genes on three separate plasmids minimises the opportunity for recombination leading to replication competent virus (RCV). Only one plasmid contains packaging signals and hence only this plasmid, including any transgene, is packaged into the vectors produced.

Origin & function

Transgenes will include only reporter genes and/or selectable markers. No other proteins will be encoded (for example there will be no toxins, super-antigens or oncogenes). Transgenes may include:

- Reporter genes: Photinus (firefly) and renilla luciferase, Beta-galactosidase, Secreted Alkaline Phosphatase, Chloramphenicol acetyltransferase, fluorescent proteins from marine organisms (CFP, EGFP and YFP). These naturally fluorescent proteins emit light when excited by high-intensity light of specific wavelengths. Several variants of natural genes have been produced that encode a protein with modified adsorption/emission characteristics and which have been optimised for higher level expression, e.g. the “EGFP”. GFPs are widely used and no hazardous properties have been reported. Other proteins have been isolated from other organisms, however, each of these proteins has no cellular toxicity in normal cells, otherwise, they would not function as a successful reporter gene. Luciferase from
Firefly and Sea Pansy are widely used as reporter genes and not considered to have harmful properties. No hazardous effects are anticipated because all the parent proteins are without harmful properties.

- CRISPR genes targeted to virus genes for knockdown of virus function: The gene for the Cas9 endonuclease from the Streptococcus pyogenes Type II CRISPR/Cas system along with a guide RNA specific to Viral genomes and guide RNA scaffold for the Cas9 system.
- Mammalian selectable markers: Hygromycin resistance, Puromycin resistance, Zeomycin resistance, Neomycin resistance, Blasticidin resistance, and Geneticin resistance. Never shall any vector contain more than one of these markers. They do not function in bacteria. The promoter of the mammalian antibiotic resistance or selectable marker is recognised by the mammalian RNA polymerase and allows transcription of the gene of the mammalian antibiotic resistance or selectable marker in mammalian cells.
- Promoters: Different promoters will be used to regulate reporter gene expression in order to achieve different levels of expression. Typically these will include CMV, SFFV, SV40, U6 and EF1α promoters. There are no risks associated with the promoters, as they do not themselves encode any proteins, they merely influence the level of transgene expression achieved.

Evaluation of foreseeable effects

Only replication incompetent lentiviruses will be used under this contained use application. This reduces the potential for generation of replication-competent viral particles (RCPs). Extensive safety profiling has been carried out on lentiviral vector systems and they are commonly used worldwide for stable expression of transgenes.

With non-replicating vector systems the potential remains for possible insertional mutagenesis resulting from integration of the virus into the cell genome. This risk is mitigated to some extent when using self-inactivating vectors, handled at containment level 2 and with adequate waste disposal measures.

Risks to humans will be further mitigated through appropriate safety measures such as PPE to protect from splashes and, most notably, the strict prohibition of working with sharps at any stage of the process.

The addition of reporter genes will not have any incremental safety risk. All work will be carried out by trained staff in category 2 conditions. A full risk assessment has been written and has been signed off by the local genetic modification safety committee.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogations applied for.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumables (mainly plasticware eg pipettes, flasks, tubes) – treat overnight with Virkon following manufacturer’s guidelines, discharge any excess liquids to drains, dispose of solids into a solid sided yellow bin for licensed incineration.

Liquids (eg samples, culture supernatants, tissue culture media) – treat overnight with Virkon following manufacturer’s guidelines, discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (134C for 15 mins), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for incineration.

Degree of kill

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection with Virkon, used according to manufacturer’s instructions under standard conditions, manufacturers validation [e.g. 4.79] log reduction ([e.g. 99.998]% kill).

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Comment from the GMSC:
"Under waste management can you be specific to what decontamination method you will be using for solids, liquids and agar plates waste?"
This change has been made (the updated risk assessment is attached with this notification form) and the final version accepted.

Please enter comments on the GM safety committee on the risk assessment

Project Containment

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**Name**

ANTIVERSE LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

MEDICENTRE (UNIT 16)

**Road Name**

HEATH PARK

**Building**

**District**

**Town**

CARDIFF

**County**

**Postcode**

CF83 3GG

**Country**

WALES

**Tel Number**

00441223494388

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

**Biological Safety Officer**

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Other (please specify)  

Tick if confidential

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- Cultures, glassware, disposable plasticware and consumables exposed to non-transformed/phagemid carrying micro-organisms in the absence of helper phages will be autoclaved at 134°C for 30 min within 24 hours of the work being completed.
- Cultures and glassware that have been in contact with high titre cultures of phagemid/helper phage will be treated with solutions of Virkon S and allowed to stand overnight prior to autoclaving as above.
- Autoclaved solid waste will be disposed of properly in waste containers, while liquid waste (deactivated cultures) collected in sealed vessels will be disposed of via the main drainage system.
- Large volume spills of liquids containing high titre cultures of phagemid/helper phage will be contained using absorbent materials (e.g. paper towels), treated with undiluted Virkon S powder and left for 5 min. The waste will be disposed of by autoclaving and the area cleaned with Virkon S solution immediately following the spill.

These measures are based upon validated, well-established regimes for handling E. coli based GMMs and bacteriophage based work. Their implementation should result in complete elimination of GMMs and helper phage particles.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The proposed work will employ disabled, non-pathogenic E. coli host strains for cloning, sequencing, and protein expression of recombinant DNA sequences. They are generally recognised to be noncolonising and are unlikely to survive outside of a laboratory environment. The strains are not considered to pose any hazard to humans, animals or plants. The stated host will be used in conjunction with non-mobilisable vector systems (plasmids and phagemids) that have been widely used over many years and have a long history of safe application. Furthermore, the insert gene sequences are safe and non-pathogenic. Therefore, the overall materials used in this work could be classified as GM1.

Any potential risk of gene transfer arising from the combination of phagemid and helper phage has been addressed adequately in the risk assessment (section 3.5) and appropriate measures will be taken to avoid these.
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Name

MOA TECHNOLOGY LTD

Name 2

Department

Campus Estate or Research Centre

Road Name

9400 GARSINGTON ROAD

Town

OXFORD

District

OXFORD BUSINESS PARK

Town

OXFORD

District

OXFORD BUSINESS PARK

Postcode

OX4 2HN

Country

ENGLAND

Tel Number

01865 567302

Fax Number

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E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The BioEscalator GM safety committee is composed of a number of Life Science professionals, many of whom hold PhDs and NEBOSH General Certificates in Occupational Health and Safety. Members include a BioEscalator Biological Safety Officer (PhD qualified laboratory manager), senior Oxford University Safety Officers, and senior representatives from the companies within the Bioescalator unit. The committee meets in its official capacity on an annual basis. Agenda items for the GMSC include committee member updates, and risk assessment reviews and updates. Meetings are minuted with actions and emailed to the committee for comments and final approval. GM risk assessments are sent (via email) to the BioEscalator BSO for circulation to the GMSC members for comment. Once the forms have been approved by the GMSC, risk assessments are signed off by the BioEscalator BSO as record of final approval.

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<tr>
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Other(s)

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 3443/18.1

<table>
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<td>Production and characterisation of transgenic plant propagules derived from endogenous British plants</td>
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</table>
**Project Additional Information**

**Purposes of the contained use**

The aim of this project is to produce transgenic plant propagules of an endogenous British plant species. The transgenic propagules are to be used for:

1. Characterising the localization of the transgene's product, and/or
2. Characterising the function of the genomic locus where the transgene is located

Both wild type and transgenic T-DNA mutant lines (already available) will be transformed, using a disabled Agrobacterium tumefaciens strain GV3101. Plasmids used for transformation of plants with marker transgenes contain the coding sequences for the genes conferring resistance to kanamycin or spectinomycin in Agrobacterium and chlorsulfuron or hygromycin or geneticin in plants.

**Recipient or parental organism**

Endogenous British plant species and T-DNA mutant lines derivatives of the same.

**Host/vector system**

Agrobacterium tumefaciens and Gateway vector plasmids.

**Origin & function**

The intended function of the transgenes is to produce proteins that localize to cellular areas of interest or to perturb the function of neighbouring DNA sequence.

**Evaluation of foreseeable effects**

Characteristics of the GMOs and evaluation of the foreseeable effects are detailed in the attached risk assessment. Overall environmental effects are estimated to be low:

The unmodified plant species is native to Britain, and the inserted genetic material does not offer a selective advantage outside the laboratory unless antibiotics are in use. In addition, the fluorescent proteins used have no toxic effects. The vector (Agrobacterium tumefaciens) can infect plants, however, only disabled strains are to be used which are at a competitive disadvantage to wild type strains. The strain used here have not been associated with harm to the environment and will be handled in the laboratory under controlled conditions. Material is Virkon treated or autoclaved prior to disposal.

The genetically modified plant is not expected to be toxic to other plants nor to display increased invasiveness of natural habitats. The resulting plants are not expected to be pathogenic and are not expected to alter host plants.
resistance to other pathogens. It is unlikely that these genes could enhance weediness.
In the event that sensitivity to chemical compounds proves to be true, then the transgenic plants may have a selective
advantage over natural accessions in the context of agricultural environments where the relevant compound is used
for crop protection. The targeted editing is not expected to lead to plant changes outside the assessment of
associated risks.
Hazards to human health are estimated to be effectively zero:
The unmodified plant is non hazardous, the inserted genetic material is not hazardous, and the marker genes are not
hazardous. Antibiotic resistance genes do not offer a selective advantage outside the laboratory unless antibiotics are
in use. Fluorescent proteins have no toxic properties. The vectors are not hazardous to humans and none of the
transgenic plants are expected to be hazardous to humans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
All plants are grown in environmentally controlled cabinets and contained in sealed pots after fertilization to stop any
unlikely escape of plant material. Details of which are in the attached risk assessment.
These will be in laboratories with restricted access. All contents treated as GM.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
None

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Solid GM waste materials (agar, culture dishes and pots, plant material and soil etc) will to be collected and
autoclaved in a validated autoclave prior to disposal. Any non GM plant material grown in the same growth cabinets
as GM material will also be treated as GM waste and autoclaved prior to disposal. Solid waste will be disposed of via
the clinical waste stream for incineration. Pots or other containers in which GM plants have grown will be
decontaminated (soaking overnight in 2% Virkon solution) before being re-used.
Disinfection measures for Agrobacterium: A 2% Virkon solution will be prepared prior to working with Agrobacterium
and will be available should a spillage occur. After use the consumable and liquids containing vector will be exposed
to 2% Virkon for a minimum of 30 minutes, as will all materials (e.g. tips, tubes) used during the experiment to ensure
complete decontamination. Excess liquid is disposed of down the drain. Plasticware and consumables will then be
autoclaved (see below) and then disposed of via clinical waste stream for incineration.
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS
2646, Part 3, 1993 (134°C for 15 minutes) and then dispose of solids via clinical waste stream for incineration.
Sharps (eg syringes, slides, razor blades scalpels) will be autoclaved and then disposed of via clinical waste stream
for incineration
Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical disinfection, used according to manufacturers instructions under standard conditions, manufacturers
validation [eg4.79] log reduction ([eg99.998]% kill).
Approved by Bioescalator GM safety committee 26th October 2018.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Large Scale Activities

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Human Clinical Applications

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The premises has multiple companies as tenants. A genetic modification safety committee has been constituted from a representative from each company carrying out GMO work (all are or were until recently tenured academic staff at HE institutions and have experience in preparing risk assessments for GMO use) plus one staff representative elected by staff members, and the health and safety lead of Unit OX. The committee is chaired by an external HE academic who is a member of another genetic modification safety committee and has experience in reviewing and approving contained use activities. Two committee members have experience as HE biological safety officers and have had responsibility for reviewing contained use risk assessments.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Bacteriology  Yes  Parasitology  Yes  Transgenic Birds   |

Microbiology Research  Yes

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

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Highly contaminated material - tips and loops, and similar plasticware will be collected in the laboratory within small benchtop containers (waxed card waste bins, not biohazard marked). This waste will be combined with agar plates and larger volume plasticware and sterilised by autoclaving. Transport to autoclave will be in a leakproof container on a trolley. Following confirmation of autoclave cycle using TST strips waste will be disposed of to landfill by a licenced contractor. Failure of cycle as indicated by either autoclave or TST strips will mean waste is autoclaved again. Autoclaves are inspected and tested for temperature and time conformance annually. TST indicators are used as a failsafe.

Sharps will be contained within an autoclavable sharps bin, autoclaved as above, then collected by a licenced contractor for incineration.

Liquid waste will be sterilised by the addition of Virkon from stocks made up weekly. This will be added to a concentration of 2%, the mixture left to stand for 60 minutes before discharge to drain. Glassware will be autoclaved as above.

Low risk material - hand towels and gloves will be collected in yellow bags disposed of by incineration off site after collection by a licenced contractor.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Lab users should use PPE - lab coat with elasticated cuffs and high neck, and gloves at all times. Coats must not be worn outside of the laboratory. When transporting waste to autoclave this must be on a trolley.

TST indicators should be used in autoclave loads and these ensured to be specific to 121 C for 15 min at 15 PSI.

These have been incorporated into the risk assessment.
The overall aim of this project is to inform the development of gene-targeted therapeutic agents for the control of bacterial diseases. In the initial stages of this work, this will entail identification of gene targets by knockout and complementation within an individual bacterium and also expression of the target genes in a second bacterial species, usually the common expression vector E. coli. In order to develop diagnostic tests for the particular target bacteria and genes expression of partial and full length proteins is also necessary. This will enable the calibration of protein sequencing tests and ELISAs for the target pathogens, and identify cross-reactivity, sensitivity and specificity of such tests.

The significance of this work is that it is developing agents which can replace antibiotics in food animal production, as a response to the antibiotic resistance now commonly found in these bacteria. It is necessary to use GMMs as targets for the various therapeutics under test target individual genes, and without producing GMMs the target cannot be verified.

Specifically for this individual project, the aim is to establish the validity of such an approach across multiple bacterial species which are known to be animal and zoonotic pathogens. This is a key enabling step in informing the future design of gene-targeted agents.

### Recipient or parental organism
- Salmonella (non-typhoidal serotypes)
- E. coli serotypes
- Streptococcus spp.
- Staphylococcus spp.
- Campylobacter spp.

### Host/vector system
Standard antibiotic resistance cassettes will be used for gene disruption: ApR (ampicillin), KnR (kanamycin), Sm/SpcR (spectinomycin), TcR (tetracycline), CmR (chloramphenicol). Also, a range of standard plasmids (all nonconjugative) conferring antibiotic-resistance will be employed for cloning purposes. All routine cloning will be in E. coli K-12 using standard plasmids (pUC series and derivatives) and will include strains such as E. coli K-12 (BW25113, TOP10, DH5a, MC4100, W3110, MG1655, S17::pRP4), E. coli B (BL21/DE3). Gene expression will use standard commercially available backbones such as pOPIN-E/F, pBAD, pCDF-duet and may be under the control of inducible promoters such as arabinose and tetracycline. Green fluorescent and other FPs will be cloned in to assess expression in model systems. The commercial source is Clontech pGFPuv Vector.

### Origin & function

Partial or whole gene sequences from Salmonella (non-typhoidal serotypes), E. coli serotypes, Streptococcus spp., Staphylococcus spp. Campylobacter spp. Genes encoding toxins, or that enhance virulence, will be specifically excluded from the work. For expression outside E. coli, vectors may be altered by modifying the promoter and ribosome binding site and/or origin of replication by inserting artificially synthesised gene fragments.

### Evaluation of foreseeable effects

Expression of target genes in bacteria would not increase the virulence of any organisms containing them. Created strains would be either as virulent or less virulent as the parent organism, and would likely be less fit in terms of survival and competition in the environment. Forseeable effects would be as for the parental organisms. Plasmid carriage or knockout will render them less fit than the wild type, limiting their ability to compete and survive.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**NA**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

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Please enter comments on the GM safety committee on the risk assessment

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**Project Containment**

**Laboratory Activities**
- L2: Yes
- L3
- L4

**Glass Houses**
- L2
- L3
- L4

**Growth Rooms**
- L2
- L3
- L4

**Animal Units**
- L2
- L3
- L4

**Large Scale Activities**
- L2
- L3
- L4

**Human Clinical Applications**
- L2
- L3
- L4
### AVIAGEN UK LTD

#### Name 2

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#### Road Name

- **11 LOCHEND ROAD**
- **District**: RATHO STATION
- **Town**: NEWBRIDGE
- **County**: SCOTLAND
- **Postcode**: EH28 8SZ

#### Tel Number

- **0797 141 7877**

#### Fax Number

- **0131 333 3296**

#### E-mail

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#### HSE Division

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#### Date at Which Additional Info Submitted

- **02/03/2022**
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Company Health & Safety Manager

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Clinical Waste picked up by Specialist Waste Contractor, suitably packaged and sent for incineration

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Health & Safety Manager

**Project Ref 3445/18.1**

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Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**
**Purposes of the contained use**

1. For use as a positive control for PCR.
2. For use in vitro experiments to allow identification.
3. For use in diagnostic assays to allow colonisation.

**Recipient or parental organism**

1. non-pathogenic lab strain of E. coli DH5alpha - no foreseeable effects
2. E. coli strain - unlikely to result in infection in humans - minimal risk - proper procedures will prevent exposure
3. non-infectious chicken fibroblast cells - no foreseeable effects.

**Host/vector system**

1. and 2. Plasmid, plasmid may be transferrable to other E. coli. This has not been assessed. Procedures in place will prevent escape from the lab environment.

**Origin & function**

1. Origin: DTU, for use as a control strain for PCR only
2. Origin: ATCC, expression of GFP to identify E. coli from in vitro assays
3. Origin: not purchased yet. Under development at another lab. For use as a diagnostic tool. The addition of a cell surface receptor will make the cell vulnerable to infection by virus. These cells will be used to detect the presence of virus in diagnostic samples

**Evaluation of foreseeable effects**

no foreseeable effects.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

n/a

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

After the work has been completed these waste traps will be emptied into clinical waste theatres. Waste theatres are collected weekly by external contractors and incinerated. All growth media used will be disposed of in clinical waste theatres that are collected weekly by external contractors and incinerated. Any contaminated glass ware that will be re-used will be autoclaved.

**Is an emergency plan required according to regulation 20?**

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y
On the 5th December the Company Health and Safety Steering Group was updated by R O and J E on the use of GMO at our Lab in Broxburn. The steering group gave the matter consideration and decided to endorse the use of GMO under the control measures outlined in the risk assessment.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

N

### Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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**GM Centre Number: 3447**

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**Name**

LUMIRADX UK LTD

**Department**

**Campus Estate or Research Centre**

UNIT 1, BLOCK 4

**Road Name**

MANOR FARM BUSINESS PARK

**District**

MANOR LOAN

**Town**

**County**

STIRLING

**Postcode**

FK9 5QD

**Country**

SCOTLAND

**Tel Number**

01259 301309

**Fax Number**

0

**E-mail**

**HSE Division**

Blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

- Genetic modification safety committee members are drawn from a cross-section of the business in both senior and junior roles. Members are:
  - Scientist 2 - Virologist
  - Senior Scientist - Molecular Biologist
  - Technical Lead - Molecular Biology
  - H&S Manager
  - H&S Advisor
  - QA Engineer
  - Facilities Manager

<table>
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1.1. General

1.1.1 All material generated in working practices must be inactivated or autoclaved prior to removal from the CL3 laboratory. Procedures for inactivation and disposal of waste are detailed in this SOP.

1.1.2. The only exceptions being waste that cannot physically fit into the autoclave, and waste that would damage the autoclave (e.g. batteries, lightbulbs). For these exceptions, individual risk assessments should be made, before disinfection and disposal for incineration.

1.1.3. Liquid waste must be chemically inactivated overnight with Virkon (final concentration of no less than 1% w/v) prior to being discharged to drains and flushed with water. Discharge of inactivated liquid waste to drains must be logged on Liquid waste form, S_QMS-SOP-30143.F1.

1.1.4. Solid waste that has been in contact with infectious material (e.g. cell culture plastics) must be inactivated with Virkon (no less than 1% w/v) prior to being autoclaved. Gloves that have been used within MSCs must be sprayed with 70% IPA prior to being discarded and autoclaved. Other solid waste (e.g. paper, packaging, dirty disposable lab coats) can be autoclaved without prior chemical inactivation.
1.1.5. All solid waste must be autoclaved at 121°C for 15 minutes at the earliest opportunity. After autoclaving, solid waste will be bagged before being removed from the laboratory and sent for incineration. It is the responsibility of all personnel to ensure their waste is autoclaved at the earliest opportunity.

1.1.6. For trained personnel, it is their responsibility to ensure that their waste is decontaminated and disposed of appropriately, as per the guidelines laid out in this SOP, risk assessments and Code of Practice: Containment Level 3 (CL3) laboratory (S-OHS-SOP-30016).

1.1.7. If this policy is breached, the Lab manager must be notified immediately and a non-conformance must be raised in accordance with S-QMS-SOP-30005, Reporting of Quality Events.

2. Waste from within MSC
2.1. Liquid waste
2.1.1. Small volumes (<25ml) of liquid can be disposed of by pipetting or submerging their container directly into 2% w/v Virkon. Ensure the final concentration is no less than 1% w/v. Leave overnight prior to discharge to drains. Log decontaminated waste discharged to drain on Liquid waste disposal form, S-QMS-SOP-30143.F1.
2.1.2. Larger volumes (such as T75 and T150 flasks) can be poured gently directly into a suitable container containing 2% w/v Virkon. Ensure the final concentration is no less than 1% w/v. Leave overnight prior to discharge to drains. Log decontaminated waste discharged to drain on Liquid waste disposal form, S-QMS-SOP-30143.F1.

2.2. Solid waste

2.2.1. All solid waste from within a MSC has to be treated as potentially infectious.

2.2.2. Spray gloves used within MSCs with 70% IPA. Discard into an autoclavable waste box outside of the MSC for autoclaving.

2.2.3. Discard tissue and papers (e.g. wrapping) into an autoclavable waste box outside of the MSC for autoclaving.

2.2.4. Discard cell culture plastics which have been in contact with potentially infectious material into a tub/jar containing 2% w/v Virkon.

2.2.5. For larger items (such as flasks), add sufficient amount of 2% w/v Virkon to cover the surface area that has been in contact with biological material. Alternatively, add Virkon powder/tablets to reach final concentration of no less than 1% w/v. Rotate the item to ensure all surfaces have come in contact with disinfectant.
2.2.6. Discharge disinfectant to suitable vessel within the MSC. Disinfect exterior surface with 70% IPA and discard into an autoclavable waste box for autoclaving.

2.2.7. For Gilson style pipette tips, aspirate 2% w/v Virkon solution from the tub/jar into pipette tips. Ensure tips are full (to the level used in the experimental action) prior to expelling into a tub/jar containing 2% w/v Virkon.

2.2.8. For serological pipettes, discharge contents into 2% w/v Virkon solution and aspirate with 2% w/v Virkon using a volume greater than that used in the experiment prior to submerging in a jar inside the MSC containing 2% w/v Virkon.

2.2.9. Spray or wipe down racks, tip boxes, etc. used in the working procedure with 70% IPA prior to removal from MSC. Re-use or discard into an autoclavable waste box for autoclaving.

2.2.10. Loosely seal tubs/troughs. Label with date waste has been generated, user's initials and pathogen used. Store in the designated area until relevant disinfection time has passed, prior to discharging to drain as below.

2.3. Discharge of disinfected waste

2.3.1. Waste from MSCs (Gilson-style pipette tips, serological pipettes submerged in Virkon 2% w/v). Check date to ensure overnight disinfection (or longer) prior to discharging to drains through a colander/suitable sieve.

2.3.2. Log decontaminated waste discharged to drain on liquid waste form, S-QMS-SOP-30143.F1. After draining, place plasticware in autoclavable box for autoclaving.

2.4. Disposal of MSC HEPA filter

2.4.1. Contaminated HEPA filters are sterilised in situ during MSC fumigation.

2.4.2. When required, and after purging of fumigant, the filters are replaced by the external contractor.

2.4.3. Double wrap the contaminated filter in specifically designed large plastic bags and label as containing a biological hazard. Filters are not autoclaved but disposed of directly into autoclavable waste box.

3. Waste from Outwith MSC

3.1. Procedure

3.1.1. Dispose of solid waste, including dirty disposable laboratory coats and gowns, in an autoclavable waste box for autoclaving.

3.1.2. Discard liquid waste (from incubators, defrosting of fridge/freezer, etc.) in a container. Add Virkon powder/tablets to reach a final minimum concentration of 1% w/v. Leave overnight prior to discharging to drains.


4. Autoclaving

4.1. General

4.1.1. All solid waste is to be autoclaved at 121°C for 15 minutes prior to disposal, see S-QMS-WI-30232 for Operation of the Autoclave.


5. Waste Disposal

5.1. General

5.1.1. Post autoclaving, solid waste is incinerated.


5.1.3. Solid material is incinerated post autoclaving. This requires disposal via a Registered Waste Contractor.

5.1.4. Collect all autoclave waste in clinical waste bags and put into clinical waste bins at the rear of the facility.

5.1.5. Place all sterilised solid waste in plastic clinical waste bags and close with tie wraps. Put into clinical waste bins.

5.2. Clinical Waste

5.2.1. All clinical waste from a CL3 laboratory prior to leaving the site must be in clinical waste bags/containers as described above labelled with UN3291 and marked “for incineration”.

6. DOCUMENT

6.1. General

6.1.1. All Autoclave records and records of Decontaminated waste discharged to drain will be maintained in the laboratory for 6 months prior to being archived by the Lab manager according to S-QMS-SOP-30003, Submission and Archiving of Quality System Records.
The internal LumiraDx GMM safety committee is composed of a cross-functional group with expertise in Virology, Quality Assurance, Health and Safety, Environment, and Biological Sciences. Several members have experience in working in CL3 laboratories, and one member has 10 years particular experience working with GMMs. The risk assessment has been presented and agreed by the committee, and will be revised on an ongoing basis at least annually.
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Name
MENARINI BIOTECH UK BRANCH

Name 2

Department

Campus Estate or Research Centre
THAMES VALLEY SCIENCE PARK

Building
THE GATEWAY BUILDING

Road Name
1 COLLEGIATE SQUARE

District

Town
READING

County
BERKSHIRE

Postcode
RG2 9LH

Country
ENGLAND

Tel Number
01183041051

Fax Number
N/A

E-mail

HSE Division
EAST AND SOUTH EAST

Comments

Date at Which Additional Info Submitted
02/03/2022
Premises Addresses

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<td>Thames Valley Science Park</td>
<td>The Gateway Building</td>
<td>1 Collegiate Square</td>
<td>Shinfield</td>
<td>Reading</td>
<td>BERKSHIRE</td>
<td>RG2 9LH</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Menarini Biotech UK Branch is part of Menarini Biotech S.r.l, Pomezia (Rome), Italy which has an established Biological Safety Committee and reviews all GMOs in use at the Facility in line with regional legislation.

The UK branch of Menarini Biotech will only use GMOs that have been created for MBH Pomezia and are accompanied by an approved Biological Risk Assessment.

3 Document are attached to this Contained Use license application.


This is the Risk Assessment on cell line OBT_357NF_19C4, translated from Italian into English, approved for use on 25Sep2013. This document originally risk assessed OBT_357 as Cat 2.

Doc2. "report valutazione rCHO 8.5.2018_signed.pdf"

This native Italian language Risk Assessment updated the Risk Classification of all GMOs in use at MBH's Pomezia (Italy) facility as Cat 1.


The Menarini Biotech UK Branch facility will only work on GMO's that originate through the parent Italian organisation and have therefore been risk assessed by the parent organisation's Biological Safety Committee and by the responsible Biological Safety Officer.

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02/03/2022
An Astell autoclave (63L model AMA240) has been purchased/installed within the Menarini Biotech UK branch labs for the inactivation of relevant waste. Solid/contaminated waste will be disposed into biohazard bins/boxes, sealed and placed into a lockable clinical waste container and disposed of by a registered waste management company (Select Environmental Services).

Decontamination of surfaces will be conducted using elevated levels of the disinfectant ChemGene HLD4L, Distel and 70%(v/v) Kleercide spray. Inactivation of liquids will be performed using Virkon tablets as per manufacturer instructions.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Other(s) Cell culture of recombinant CHO cell lines and process development in shake flasks

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

In conclusion as stated in "Evaluation report rCHO_8.5.18_FINAL.pdf"*

“All of the above (...) refers to reasonable certainty about the attribution of the rCHO (modified) cell lines, in use by MBH, to the class 1 contained use.”
GM Centre Number: 3449

Data Premises Notified (Originally) 28/12/2019

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

MACROPHOX LTD

Name 2

Department

Campus Estate or Research Centre

ROOM F11

Building

MAGDALEN CENTRE

Road Name

1 ROBERT ROBINSON AVENUE

District

THE OXFORD SCIENCE PARK

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX4 4GA

Country

ENGLAND

Tel Number 01865 618825

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted
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<td>OX4 4GA</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

**Y**  

Give brief details of the genetic modification safety committee

We have received approval for our risk assessments from the University of Oxford, Innovation Building Biologica safety committee.

<table>
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Tick if confidential  

02/03/2022
GM bacteria and mammalian cells will be deactivated in virkon, such that all GMOs will be destroyed, before disposal in wastewater. It is expected that this will kill 99.98% (as per the manufacturer's validation) of bacteria and/or mammalian cells. Testing will consist of spread plate bacteria after virkon deactivation, and measuring remaining viable cells by trypan blue exclusion assay for virkon treated mammalian cells.

All plastic ware will be autoclaved in a BS 2646, Part 3, 1993 (either 121-125°C for a minimum of 15 minutes, or 126-130°C for a minimum of 10 minutes, or 130-136°C for a minimum of 3 minutes). Excess liquids will be discharged to the drains, solid wastes will be disposed of via the clinical waste stream for incineration/disposal. Any sharps used will be disposed of via clinical waste stream for incineration. Autoclave and incineration will deliver a degree of killing of 100%.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The University of Oxford, Innovation Buildings Biological safety committee was pleased to approve our risk assessments with no modifications.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The University of Oxford, Innovation Buildings Biological safety committee was pleased to approve our risk assessments with no modifications.

Project Ref 3449/19.1

Date Ackn'd 14/03/2019

CU2 Project Title Use of replication-defective retroviral and lentiviral vectors expressing reporter genes

Class CultureVolClass2 CultureVolumeClass3-4

Class 2 < 1 Litre
**Project Additional Information**

**Purposes of the contained use**

The purpose of our work is to develop improved cellular therapeutics for treatment of disseminated cancer. The risk assessments to human health and the environment show that the genetic material to be generated during this project has a relatively low risk, warranting using containment level 2. The final GM activity class is containment level 2.

**Recipient or parental organism**

**Working in vitro**

1. Typically cells will include human and murine cancer cell lines (e.g., human breast cancer cell lines such as MB-MDA-268 or human colorectal cancer cell lines such as HT29, HCT116 or murine colorectal CT26 and MC38 cell lines), primary human and murine blood cells and other cell lines such as 293 and THP cells.
2. All cells will be incubated in vitro with retro/lentiviral vectors at a multiplicity of infection (MOI) between 1 and 100, with virus left in place for at least 24h.
3. Cells are then washed three times to remove free virus and either evaluated for reporter gene expression (using flow cytometry or luminometry) or expanded to allow for selection of transduced cells to create enriched preparations of transduced cells.
4. Sometimes cells may be grown as single cell clones to allow for characterisation of expression and/or copy number and integration site analysis.

**Working in vivo**

Typically cells (including THP cells, monocytes, other leukocytes) will be incubated with virus in vitro using the procedures and PPE (see attached risk assessment for details). The transduced cells will be selected by treatment with puromycin, sorted by flow cytometry or subject to at least three media changes and used as a mixed preparation of transduced and untransduced cells. See below, or the attached risk assessment, for details of the protocol.

**Host/vector system**

Replication defective retro/lentiviruses are generated by transfection of producer cells (commonly HEK293 cells or derivatives of) with multiple plasmids which together provide the required genes for packaging the gene of interest into infectious replication defective viral particles.

The viral vectors are derived from numerous wild type viruses, such as MoMLV, HIV, SIV and EIAV. However non-essential genes have been removed from the vectors and the remaining genes split onto separate plasmids with minimal homology to reduce the risk of homologous recombination. For example lentiviral vectors derived from HIV are devoid of tat, vif, vpu, vpr nef and the envelope gene has been replaced by VSV-G envelope. Gag, pol and rev are generally on one plasmid, env on a second and the gene of interest is on a third plasmid along with cis-acting signals required for reverse transcription and integration. As the gag, pol, rev and env plasmids do not contain
the packaging sequence none of the genes are present in the package viral genome. The likelihood of these viral particles obtaining the missing genes is very low and therefore the risk of replication competent viruses being created is minimal.

In addition, some of the later generation viral vectors also include a self inactivating feature, a deletion within the 3’ LTR, which is transferred to the 5’ LTR after reverse transcription and integration in infected cells. This results in the transcriptional inactivation of the LTR in the provirus and reduces the risk of transactivating genes around the site of insertion.

Some concern has been raised with regards to the potential oncogenic activity of the post-transcriptional regulatory element from the woodchuck hepatitis virus (WPRE) which is frequently included in lentiviral systems. Following the guidance from the Health and Safety Executive these systems must be handled at Containment Level 2

Retro/lentiviral particles will be pseudotyped with common glycoproteins such as RD114 or Vesicular Stomatitis Virus glycoprotein G (VSV-G), which allow efficient gene transfer to a broad array of cell types and species.

Producer cells are especially disabled hosts having strict nutritional and environmental requirements for survival. The modifications are not expected to increase their survival in the environment, nor increase their ability to evade the immune system. Therefore the only risk is from the viral particles themselves.

The inserts are all thought to not increase the risk of the virus. Known oncogenes, proto-oncogenes or immunomodulatory genes are not covered by this risk assessment. No genetic alterations will be made to the retroviral/lentiviral packaging plasmids or retro/lentiviral backbone transfer vector plasmid

Assignment of provisional containment level: Containment level 2 with Good Microbiological Practice and Good occupational Hygiene.

Origin & function

Transgenes to be expressed include:

• Reporter genes: Photinus (firefly) and renilla luciferase, Beta-galactosidase, Secreted Alkaline Phosphatase, Chloramphenicol acetyltransferase, fluorescent proteins from marine organisms (CFP, EGFP and YFP). These naturally fluorescent proteins emit light when excited by high-intensity light of specific wavelengths. Several variants of natural genes have been produced that encode a protein with modified adsorption/emission characteristics and which have been optimised for higher level expression, e.g. the “EGFP”. GFPs are widely used and no hazardous properties have been reported. Other proteins have been isolated from other organisms, however, each of these proteins has no cellular toxicity in normal cells, otherwise, they would not function as a successful reporter gene. Luciferase from Firefly and Sea Pansy are widely used as reporter genes and not considered to have harmful properties. No hazardous effects are anticipated because all the parent proteins are without harmful properties.

• Mammalian selectable markers: Hygromycin resistance, Puromycin resistance, Zeomycin resistance, Neomycin resistance, Blasticidin resistance, and Geneticin resistance. Never shall any vector contain more than one of these markers. They do not function in bacteria. The promoter of the mammalian antibiotic resistance or selectable marker is recognised by the mammalian RNA polymerase and allows transcription of the gene of the mammalian antibiotic resistance or selectable marker in mammalian cells.

• Promoters: Different promoters will be used to regulate reporter gene expression in order to achieve different levels of expression. Typically these will include CMV, SFFV, SV40 and EF1α promoters. We will also include mammalian promoters associated with differentiated macrophages, such as the promoters for CD80 and CD86, CD206 and others. We may also include synthetic promoters. There are no risks associated with the promoters, as they do not themselves encode any proteins, they merely influence the level of transgene expression achieved.

Evaluation of foreseeable effects

Working in vivo

As above, typically cells (including THP cells, monocytes, other leukocytes) will be incubated with virus in vitro using the procedures and (see attached risk assessment).
The transduced cells will be selected by treatment with puromycin, sorted by flow cytometry or subject to at least three media changes and used as a mixed preparation of transduced and untransduced cells.

All animal work will be performed under the Project licence led by Prof L Seymour, currently PPL: 30/3391

Procedure: [19(b)]:2 Studies in tumour-bearing animals

METHODS:
1. Immune-compromised mice such as NSG will be used, suitable for engrafting human tumour xenografts and also administration of human immune cells.
2. Administration of tumour cells or mixtures of tumour and normal cells with/without matrigel (on one or two occasions; as suspension) by inoculation subcutaneously. Animals may receive two subcutaneous injections of tumour cells in different sites.
3. Following tumour growth, administration of retrovirus/lentivirus-treated therapeutic cells (+/- loaded with a fluorescent dye for in vivo tracking) by intravenous injection (i.v.) or inter-tumoral (i.t.)
4. Irradiation of tumour either given in a single fraction (maximum 30 Gy) or utilising a fractionated regime, up to a maximum of 50 Gy total (maximum five times per week, maximum 5 Gy per fraction). Mice will be anaesthetised throughout. Lead-lining or SAARP may be utilised to minimise dose to normal tissue.
5. Light imaging up to 12 times, 24hrs minimum interval between imaging sessions. Animals will be placed in an imaging camera for 5-30 minutes.
6. At the end of the experiment animals will be killed by Schedule 1 method or exsanguination under terminal anaesthesia with or without perfusion
7. Animals will be checked and scored daily for signs of discomfort, and weighed at least 3 times a week. If general condition becomes poor and recovery is deemed unlikely, animals will be killed by a schedule 1 procedure.

Transgenes and their potential risks
Transgenes will include only reporter genes and/or selectable markers. No other proteins will be encoded (for example there will be no toxins, super-antigens or oncogenes).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
n/a - We will not be generating GM animals or plants

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We will use full containment class 2, with no derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Please see specific control measures in Table 1 of the attached risk assessment
i) For E.coli work
The containment and control measure that will be applied for work with these genetically modified micro-organisms are shown on Table 1. Containment level 1 will be applied with Good Microbiological Practice and Good Occupational Hygiene. Class 1.

ii) For viral production work
The containment and control measure that will be applied for work with the genetically modified cell lines are shown on Tables 1. Containment level 2 will be applied with Good Microbiological Practice and Good Occupational Hygiene. A microbiological safety cabinet, eye protection and gloves will be used for protection and to maintain sterility. Class 2.

iii) For in vitro and in vivo work
The containment and control measure that will be applied for work with the genetically modified cell lines and the infectious vector preparations are shown on Tables 1.
Containment level 2 will be applied with Good Microbiological Practice and Good Occupational Hygiene. A microbiological safety cabinet, safety glasses and gloves will be used for protection and to maintain sterility. Class 2.

WASTE MANAGEMENT MEASURES

Consumables (mainly plasticware eg pipettes, flasks, tubes) – treat overnight with Virkon following manufacturer’s guidelines or autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for incineration.

Liquids (eg samples, culture supernatants, tissue culture media) – treat overnight with Virkon following manufacturer’s guidelines or autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for incineration.

Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration

Animal bedding – depending on the location. Will be autoclaved using make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes) and disposed as industrial (black bag) waste, or sent directly for incineration.

Animal carcases – Sent directly for incineration

Degree of kill

Autoclaving, effectively 100% kill (annual validation)

Incineration, effectively 100% kill (licensed incinerator)

Chemical disinfection with Virkon, used according to manufacturer’s instructions under standard conditions, manufacturers validation [eg 4.79] log reduction [eg 99.998]% kill.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The safety committee had no collective comments on our risk assessment, given the limited nature of our work, they were happy to approve the work without modifications.

Individual comments included:
"I agree with the classification and the assessment is comprehensive and well written." Dr Julie Hamilton, Divisional Safety Officer (Clinical) Medical Sciences Division, University of Oxford
"No comment from me. The RA looks really comprehensive."
"I have no comments as all have been answered now."
"No comment"
"This looks reasonable to me"

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**Name**

GW PHARMAACEUTICALS

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

BUILDING 760

**Road Name**

KENT SCIENCE PARK

**District**

**Town**

SITTINGBOURNE

**County**

KENT

**Postcode**

ME9 8AG

**Country**

ENGLAND

**Tel Number** 07824 150685

**Fax Number** 0

**E-mail**

**HSE Division** blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification committee consists of three members. The first member has a BSc and PhD in microbiology, and has 14 years’ post-qualification experience working with genetically modified micro-organisms. The second member also holds a PhD and has over four years’ experience working with genetically modified organisms. The third member Julian is the Environmental and Health Safety (EHS) representative for Building 760. The committee will meet every four months, or on an ad hoc basis where the need arises. The role of the committee is to confirm that the work being undertaken falls within Class 1 in that none of the host organisms or transgenes are likely to pose a risk to human health, and the correct procedures are followed for the handling and disposal of waste.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs) | Yes | | | |
Level 2 (GMMs) | | | | |
Level 3 (GMMs) | | | | |
Level 4 (GMMs) | | | | |
Non-microbial | | | | |
Other (please specify) | Tick if confidential | | | |
Liquid waste will be sterilized by either autoclaving or chemical disinfection. Where practically possible, autoclaving is the preferred method for the inactivation of liquid waste. For autoclaving, the liquid waste will be held at temperature equal to or greater than 121°C for at least one hour per gallon. For chemical disinfection, Presept tablets will be added to a final concentration of at least one 2.5 gram tablet per litre of liquid waste.

Solid waste will be disposed of by autoclaving for at least one hour at 121°C. Once sterilized, the autoclaved waste will be enclosed within clinical waste bins (which are incinerated).

To prevent risk of incomplete inactivation, colour-change indicators will be used for each autoclave run. The autoclave will also be validated annually during its service.

The work that will be undertaken will include the transfer of enzyme-encoding plant genes into micro...
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Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

Initial guidance on risk assessments was provided by an external biosafety consultant. Ongoing advice is provided by the Genetic Modification Safety Committee (GMSC).

The GMSC is composed of:

Committee Chair - Environmental, Health and Safety Team Leader
Committee secretary - Biological Safety Officer - Consultant Scientist Technical Services/Manufacturing Science (TSMS). PhD qualified scientist with accredited Biological Safety Officer Training (Biosafety Practitioner Level 1 validated by the University of Edinburgh)
Core Team Member - Environmental Advisor
Core Team Member - Development Team Leader, TSMS. PhD qualified scientist with management responsibility for process development

Attendance at the GMSC by other management and technical personnel is on an ad hoc basis according to the needs of the areas represented by those personnel at the time of the committee meeting.

The GMSC meet at least 6 times per annum on a scheduled basis. Additional ad hoc meetings are held if matters requiring GMSC advice and/or review arise outside of the planned meeting schedule.

A self inspection programme, led by the Biosafety Officer, is in place and a minimum of 6 inspections are carried out per annum.

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Level 4 (GMMs)</td>
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</table>
All wastes are inactivated before leaving the premises. Where at least a 5 log reduction in viable cells can be assured inactivated wastes are disposed of via normal drain or laboratory waste routes. Where at least a 5 log reduction in viable cells cannot be assured wastes are incinerated via an approved waste contractor.

At laboratory and pilot plant scale: Liquids and liquid contacting consumables are primarily chemically inactivated. Heat inactivation is used in some laboratories.

At plant scale bulk liquids are heat inactivated. Residues on plant processing equipment may be chemically and/or heat inactivated. Small liquid volumes, such as samples, and consumables which come in to contact with samples are chemically inactivated.

Spills are chemically inactivated. Where chemical inactivation is employed proprietary disinfectants are used which conform to standard CEN1276 and therefore assure at least a 5 log reduction in viable cells. As such no additional monitoring is proposed.

Where heat inactivation is used initial data are generated to demonstrate the level of inactivation achieved. Heat inactivation generally results in full inactivation. Ongoing monitoring is achieved through normal process and equipment monitoring and maintenance programmes.
All risk assessments are reviewed by appropriate technical and management personnel including the Biological Safety Officer and the GMSC Chair. Please refer to section 10 for the qualifications of the Biological safety Officer.

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Date Project Ceased: 17/12/2019

Non-GMM Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work: N

**Project Additional Information**

**Purposes of the contained use**
To produce a protein for development purposes

**Recipient or parental organism**
Aspergillus niger randomly mutated from ATCC 13496

**Host/vector system**
Several copies of the target gene have been integrated into the genome of the parent A. niger strain. No other foreign DNA or vectors are present in the final GMM.

**Origin & function**
The target gene has been inserted to allow the GMM to produce the protein of interest and secrete it extracellularly.
### Evaluation of foreseeable effects

The A. niger parent strain presents a low hazard to humans and to the environment. The changes made to the parent strain, to create the GMM, do not present any additional hazards to humans or the environment.

The product of the GMM is extracellularly expressed. It is generally recognised as safe for use in human food. It is not a skin allergen or sensitiser but may cause sensitisation and allergic response, by inhalation, in hypersensitive individuals. Given that the material is to be produced in liquid culture there is a low risk of inhalation.

The product of the GMM is not ecotoxic. It is water miscible and biodegradable and is unlikely to bioaccumulate or persist in the environment. It is produced by environmental microbes, consequently an inadvertent release from a laboratory is unlikely to increase the already low potential for impact to the environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation is requested.

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Flask seed cultures which are not transferred to the seed fermenter will be chemically inactivated with a proprietary disinfectant, to guarantee at least a 5 log reduction in viable cells, with 100 % inactivation being expected. The actual level of inactivation will be experimentally determined. The waste will not be disposed of until this determination has been made. Routinely, this chemically inactivated liquid waste will be sent to drain. Completed seed fermenter cultures, which will not be transferred to main fermenters, and completed main fermenter cultures, which will not be transferred to the downstream development laboratory, will be heat inactivated to guarantee 100 % inactivation. The heat inactivation temperature/time conditions will be experimentally determined. Until the heat inactivation conditions have been proven all fermenter cultures will be additionally sterilised at 121°C, or greater, for at least 15 minutes, to guarantee 100 % inactivation, before being cooled and sent to drain. Routinely, heat inactivated fermenter cultures will be sent to drain. The first step in the downstream process will be to remove biomass from the culture sample. This biomass will be autoclaved at 121°C for 15 minutes, on a small fluids load, to guarantee 100 % inactivation. This autoclave cycle has already been validated for 100 % inactivation of a worst case test organism, Bacillus stearothermophilus. Routinely, autoclave inactivated biomass waste will be sent to drain. Once the biomass has been removed, the downstream process stream will be considered non-GMM. Laboratory consumables, such as sample bottles, pipettes, cuvettes, nitrile gloves, which have been in contact with live cultures will be placed in an autoclavable waste bag and autoclaved on a 134 °C porous load cycle to guarantee 100 % inactivation. This autoclave cycle has already been validated for 100 % inactivation of a worst case test organism, Bacillus stearothermophilus. |

### Is an emergency plan required according to regulation 20?  

| N |

If yes, tick to confirm that it is attached to this form  

| N |

Tick to confirm that you have attached a risk assessment to this form  

| Y |
The GMSC has reviewed the risk assessment and approved it.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y

Project Containment

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Animal Units

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### Comments

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### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Dr A PhD, formerly chief scientist, managing director and CEO of TwistDx Ltd. 30 years experience in molecular biology techniques, biochemistry and development of in vitro DNA amplification technology.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tr>
<td>Level 1 (GMMs)</td>
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<td>Level 3 (GMMs)</td>
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<td>Other (please specify)</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

See risk assessment. All bacterial cultures will be inactivated by lysis prior to processing - either sodium hydroxide or via lysozyme freeze/thaw. Unused culture or culture residues to be disposed of into clinical waste for offsite treatment by commercial vendor, and/or treated 12 hours with 1-2% Virkon.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The sole user of this facility will be the applicant Dr A. The attached risk assessment describes the measures taken to isolate the facility, control access, etc, pertinent to category 1 work. "Risk Assessment Cat 1 GMO cloning and expression work in attenuated Ecoli"
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| Tel Number                       | 01625 460235 |
|                                  |              |
| Fax Number                       | 0            |
|                                  |              |

| E-mail                            | blank       |
|                                   |              |

| HSE Division                      | blank       |

| Comments                          | name change from Cryosphere Services Ltd notified 01/06/2020 |

| Date at Which Additional Info Submitted | 02/03/2022 |
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee
Risk assessments have been conducted by an experienced research scientist, with over 12 years experience of working in bioscience pharmaceutical industry, with extensive experience generating, expressing, analysing and disposal of genetically modified organisms, up to and including GMO level 2 organisms. Scientist has spent the past 2 years working as a member of a biosafety committee for a large pharmaceutical company.

Risk assessments and HSE applications have been reviewed by a biosafety committee comprising of:
- The author of the risk assessment - as outlined above
- a highly experienced molecular biologist and mammalian cell culture specialist with >25 years experience in the drug discovery of generating large scale mammalian and insect cell genetically modified organisms.
- a highly experienced protein expression scientist with >25 years experience in the pharmaceutical industry of generating and expressing genetically modified organisms in a range of host organisms, including bacteria, mammalian cell lines, yeast etc.
- A consult Biosafety Officer, with a background of >20 years working in oncology drug discovery in the pharmaceutical industry, supporting researchers to provide biosafety awareness training, legislative awareness, supporting bioscientists to risk assess both biohazards and genetically modified organisms for their respective experiments prior to initiating work.
- A Senior Lab Technician, currently supporting scientists in a large pharmaceutical company, but with no formal scientific qualifications past GCSE.

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

access. The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. To deactivate, 2% virkon strength for a minimum contact time of 2 hours will be implemented. As a result, there will be no viable genetically modified microorganisms (GMM) remaining in solid waste.

All samples within Cryosphere Services are treated as GMM level 2 waste, due to the samples we store being generated by third parties, and therefore we cannot guarantee their validity, for example the risk of mis-labelled tubes. As such, all solid GMM contaminated waste post deactivation is double bagged into biohazard autoclave waste bags and secured with autoclave tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste technician will transfer the bags using medibins and specifically designed wheeled clinical waste carrier, to an on-site and enclosed waste facility, where the waste is transferred into a nearby building, via an internal corridor, and into the autoclave for 100% sterilisation. The waste does not go outside until it has undergone complete deactivation.

All liquid waste will be disinfected with 2% virkon solution. Solutions will be left soaking for a minimum of 2 hours before disposal via the drain with copious amounts of water.

Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with waste protocols correctly as specified above.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
1. Given the remit of this risk assessment solely covering storage of genetically modified microorganisms, in well established facilities, with an existing support infrastructure in place, this is very low risk to both staff, downstream operatives and the environment.

2. Although the GMO level risk is captured upon receipt of each sample from a third party, and tracking on an inventory management system, is it possible to highlight the locations of these samples in the laboratory, or segregate them?

   Whilst the majority of our samples will not be genetically modified, or GMO risk class 1, our day-to-day storage processes are not impacted by their containment level, as we are only storing samples, not using them. However, should a container break and sample contents be released in the laboratory, our standard procedure is to treat all material at containment level 2, and disinfect and dispose of the sample and associated cleaning materials, to ensure 100% sample is killed. This is for two reasons;
   - We cannot wholly guarantee that our customer hasn't accidentally mis-labelled a vial, or contaminated a sample with a class 2 microorganism, and
   - The safety of our employees, downstream contractors and the environment is paramount, and due to the inability to guarantee the first point, we do not wish our staff to be complacent around samples, and not react with due diligence and haste should a sample be broken.

3. Is it possible to put GMO 2 level samples in an additional layer of storage?

   Samples are currently secured in bespoke made sample containers specific for the sample type and conditions under which they are being stored. Samples are visually inspected prior to placing in secure storage boxes, with sample container level tracking in place. Storage boxes are placed in purpose built storage boxes, with sample container level tracking in place. Storage boxes are placed in purpose built locked storage units, e.g. ultra-low temperature freezers, in a locked premises accessible only accessible to Cryosphere Services trained staff and contractors. Additional level of storage has been considered, however due to the purpose built, tight fit, nature of the storage containers and racking, it was deemed that additional containment, would make it cumbersome to handle and increase the risk of dropping samples.

4. How are you able to guarantee that no customer deposits class 3 or 4 material with you?

   Whilst we are not able to validate the authenticity of every sample we receive, we do have robust contracts in place, coupled with deposition forms, which we expect customers to declare the containment level associated for the samples. We reserve the right at any time to decline receipt of samples, return samples to the customers or ask for a copy of their detailed risk assessment, should we have any doubts about the information they have provided. We have worked in the industry for 13 years, and as such have a wealth of knowledge to understand the types, and risks, of a variety of samples. The industry we work in, equally is well connected and as such customers credibility is frequently known.

**Project Ref** 3454/19.1

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<th>Class</th>
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**Project Additional Information**

**Purposes of the contained use**

This proposal covers 'storage only' activities for all microorganisms, which would be assigned to risk classes 1 or 2 for normal laboratory operations. It covers only storage of microorganisms where no other activity is being conducted. Any work using relevant organisms, other than for storage will need to be the subject of a separate risk assessment conducted by the responsible scientist, for example in the third party company.

Customers may require Cryosphere Services to either:
- arrange transport of genetically engineered biological materials from Cryosphere premises to other sites or,
- arrange disposal of genetically engineered biological samples,

In both of these instances, Cryosphere Services employees will coordinate the transport, and/or disposal of samples via third-party contracting parties, specialising in handling and disposal of genetically modified organisms.

Cryosphere Services have investigated a number of contracting companies and have identified preferred suppliers, whom are experts in their fields and can guarantee the containment of the samples, ensuring safety of people and the environment.

**Recipient or parental organism**

Cryosphere Services will be providing storage service for third-parties and as such there will be multiple parental and recipient organisms.

**Host/vector system**

The cloning will be carried out by third parties, and not Cryosphere Services, and therefore various hosts and vector systems may be used.

Customers will likely have used cloning systems such as the following outlined below:

Non mobilisable and mobilisation-defective plasmids containing established mammalian expression and maintenance features. Some may also contain viral sequences for plasmid maintenance. Lentiviral and retroviral particles (or vectors) based on a range of human virus (HIV) or animal virus (eg. MMLV, MMTV and MSCV). Adenoviral and adeno associated viral vectors containing minimal adenoviral sequences. Also includes Bacmam vectors which are a modified form of the Baculovirus vectors containing mammalian promoters and other viral sequences. Viral vector
systems, such as second generation retroviruses contain minimal viral sequences and the transgene plasmid is separated from the packaging signal to minimise possibility of replication. Second generation lentiviruses are further de-risked by the transgene and packaging signals being split across three vectors. These modifications mean that the viral elements are covered under class 2 containment. Baculovirus vectors for insect cell expression - although replication competent are incapable of transducing human cells. Other vectors may be derived from viruses, e.g. Semiliki Forest virus and Alpha virus. Viral packaging where appropriate is achieved using plasmids containing minimal viral sequences. All viral vectors are extremely unlikely to be able to mobilise or replicate in human cells and are unable to survive in the wider environment. As such they are unable to present the pathogenesis associated with the originating pathogen.

Vectors may include some which are potentially mobilisable in an appropriate host background (i.e. mobilisationdefective) and which include antibiotic resistance genes for use as selection markers. Some vectors include transposons or encode transposase functions. They include non-expression and expression vectors. Expression vectors include those designed for expression in mammalian, insect, bacteria and yeast systems and include vectors used in the generation of recombinant viral particles. The resulting viral particles are all designed to be attenuated as described in the original proposals for their use. Where cells are capable of producing recombinant viral particles, these may be able to deliver to human cells, sequences able to express biologically active molecules. There may be harmful effects associated with these when expressed in an unregulated manner in cells in which expression is normally subject to tight control, or where they are expressed in cells in which they do not normally occur at significant concentrations. There is also a risk of activation and recombination events with native retroviruses, e.g. ecotropic, xenotropic, amphotropic and polytropic viruses. Considering the storage-only scope of this risk assessment, as long as material is treated under containment level 2 material and material handled accordingly, this is low risk. Where cells are designed to produce recombinant viral particles these are all designed to be attenuated and with the exception of recombinant baculoviruses, the viral particles are designed to minimise production of replication competent viruses.

Historically, replication competent viruses may have been employed to engineer certain cell lines and some laboratories may still use this system. As standard, all material stored within Cryosphere Services is treated as risk class 2 material.

Origin & function

Inserts are from a wide variety of sources including rodent and human tissues, and include both genomic and cDNA sequences. Both hazardous and non-hazardous modifications may be introduced either via insertion, deletion of sequences or the presence of mutations generated by genetic modification techniques, including but not exclusively CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). The properties of the proteins covered by this proposal as they exist in the native microorganisms cover a wide range of known protein functions including enzymes, receptors, and other proteins involved in regulation of normal cellular activities including cell growth and division. Details are set out in separate risk assessments, which are generated by the third-party depositing these micro-organisms for storage at Cryosphere Services. These task specific risk assessments are completed by the third-party scientist intending to use the cell lines.

There will be occasions where genes would be expressed in an active form, however this risk is not covered under this assessment, as only storage, of samples is considered under this proposal.

Some recombinant microorganisms are able to express products which may be oncogenic, toxic or allergenic, and human primary material, in particular, can also present adventitious agents which may affect a normal immune status. It is for this reason that all material is treated as risk class 2 and all Cryosphere Services employees are given the opportunity to be immunised for Hepatitis B. This is considered a low risk for this particular risk assessment given samples are to only be stored.
It is possible that under culture conditions, recombination events between viral vectors and host viruses may lead to resistant strains, however this is not in scope for this risk assessment, and therefore this is considered a low risk for this particular risk assessment given samples are only being stored.

**Evaluation of foreseeable effects**

None of the microorganisms would be classified as greater than risk class 2 in normal operations and most are risk class 1. Having regard to the restriction of the activities covered by this proposal to storage only, and the conditions under which they are stored as described elsewhere in this proposal, the risk of exposure of persons or the environment is negligible to low. Due to the high number of vials handled by employees on a daily basis, all material is treated at containment level 2, and personal protective equipment used, which include lab coats, safety glasses and gloves. Hand wash facilities are available in all laboratories. Cryosphere Services staff are also provided the opportunity to be immunised for Hepatitis B.

Employees undergo a rigorous training programme prior to working independently with samples. This includes reading and understanding of standard operating procedures, 1:1 hands-on training, including assessments and a Biosafety Awareness course. Upon completion of the training procedure, the employee signs off the training task to accept they have undergone the complete training programme and deem themselves competent to undertake the task on their own. The trainer then countersigns to confirm the employee has shown clear understanding, skill and competence to undertake the task independently, at which point they are able to work with the samples. All procedures and training are reviewed annually, and day-to-day working practices are reviewed and standards maintained.

The potential for dissemination of genetically modified microorganisms to the environment is negligible for storage only activities. Disposal of risk class 2 GMO is a consideration, upon unintentional breakage of vials, however samples are stored in purpose built storage containers, further secured in plastic boxes and secure metal racking, within the storage unit. Any disposal of samples is carried out using validated methods in deSignated laboratories or by specialist on-site waste management contractors, with whom Cryosphere Services have audited. Please refer to section 12 for details.

Breakage of vials in liquid nitrogen Dewars. This risk has been mitigated by the optimisation of the tower and box materials used. In the event of vials dropping to the bottom of the tank and being crushed by the weight of the existing towers, sample retrieval and decontamination procedure must be carried out by the Facility Manager. In this situation, all existing well-stored stock is transferred to a spare back-up freezer, samples from the bottom of the tank are retrieved using a long-handled scoop. All retrieved samples are immediately transferred to a double contained bag, kept at -1960C prior to transfer to the quarantine facility for decontamination with 2% virkon for a minimum of 2hrs, ensuring 100% kill.

Any moisture/spillage-is mopped up with 2% virkon infused blue towel, area wiped with water prior to a final wipe with 70% ethanol infused blue towel.

All inactivated liquid waste after virkon decontamination is washed down the sink with copious quantities of water.

Class 2 solid waste is chemically deactivated before leaving the laboratory. The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. To deactivate, 2% virkon strength for a minimum contact time of 2 hours will be implemented. As a result, there will be no viable genetically modified microorganisms remaining in the solid waste.

All solid genetically modified microorganism contaminated waste will then be double bagged into biohazard autoclave waste bags, and secured with autoclave tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste contractor will transfer the bags using medibins and a specifically designed wheeled clinical waste carrier, to the on-site, enclosed waste facility into the autoclave for 100% sterilisation. The waste will not leave the building until post autoclaving.

Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with
waste protocols correctly as specified above.
In the event of a sample breakage, Cryosphere Services are able to request a copy of the company's risk assessment for the sample to understand and investigate the biosafety impact of the incident.
All genetically modified organisms will be 100% killed prior to leaving the building.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable.
Cryosphere Services will only be offering storage services for genetically modified microorganisms.
Contained

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Not applicable.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
Class 2 solid waste will be chemically deactivated before leaving the laboratory. Access to the laboratory is restricted.
The solid waste will be comprised mainly of plasticware and contaminated tissues used in the decontamination process. To deactivate, 2% virkon strength for a minimum contact time of 2 hours minimum will be implemented. As a result, there will be no viable genetically modified microorganisms (GMM) remaining in the solid waste. All solid GMM contaminated waste will then be double bagged into biohazard autoclave waste bags, and secured with autoclave tape. Should there be any chance of liquid drip escape, absorbent pads are used within the bag. The bags are labelled and a trained waste technician will transfer the bags using medibins and a specifically designed wheeled clinical waste carrier, to an on-site and enclosed waste facility where the waste is transferred into a nearby building, via an internal corridor, and into the autoclave for 100% sterilisation. The waste does go outside until it has undergone complete deactivation.
All liquid waste will be disinfected with 2% virkon. Solutions will be left soaking for a minimum of 2 hours before disposal via the drain with copious amounts of water.
Procedures, relevant training, supervision and safety inspections are in place to ensure that all staff comply with waste protocols correctly as specified above.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
1. Given the remit of this risk assessment solely covers the storage of genetically modified microorganisms in well established facilities, with an existing support infrastructure, this is low risk.

2. Although the GMO level risk assessment for each sample is captured on the deposition form and tracking system, is it worth noting down in the laboratory clearly where the GMO samples are, or segregating their storage?

   - 1=

   Whilst the majority of our samples will not be genetically modified, or GMO risk class 1, our day-to-day storage processes are not impacted by their containment level. However, should a container break and sample contents be released in the laboratory, our standard procedure is to treat all material at containment level 2 and disinfect and dispose of the sample and associated cleaning materials accordingly, to ensure 100% sample is killed. This is for two reasons;

   1. We cannot wholly guarantee that our customer hasn’t accidentally mislabelled a vial, or contaminated a sample with a class 2 microorganism, and

   2. The safety of our employees and downstream contractors and the environment is paramount, and due to the inability to guarantee point 1, we do not wish our staff to be complacent around samples, and not react with due diligence and haste should a ‘non-GMO class 2’ sample be broken.

3. Is it possible to put GMO 2 level samples in an additional layer of storage?

   Samples are currently secured in bespoke made sample containers specific for the sample type and conditions under which they are being stored. Samples are visually inspected prior to placing in a secure storage box, with sample container level tracking in place. Storage boxes are placed in purpose built locked storage units, e.g. ultra-low temperature freezers, in a locked premises accessible only to Cryosphere Services trained staff and contractors. Additional level of storage has been considered, however due to the purpose built, tight fit, nature of the storage containers and racking, it was deemed that additional containment, would make it cumbersome and increase the risk of dropping of samples, due to the lack of dexterity when using thermal protective gloves.

4. How are you able to guarantee that no customer deposits class 3 or 4 material with you?

   Whilst we are not able to validate the authenticity of every sample we receive, we do have robust contracts in place, coupled with deposition forms, which we expect customers to declare the containment level associated for the samples. We reserve the right at any time to decline receipt of samples, return samples to the customer or ask for a copy of their detailed risk assessment, should we have any doubts about the information they have provided. We have worked in the industry for 13 years, and as such have a wealth of knowledge to understand the types, and risks associated with a variety of samples. The industry we work in, equally is well connected and as such customers credibility is frequently known.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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02/03/2022
# GM Centre Number: 3455

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**Name**

EXSCIENTIA LTD

**Campus Estate or Research Centre**

**Road Name**

36 ST GILES' ROAD

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX1 3LD

**Country**

ENGLAND

**Tel Number**

01865 818941

**Fax Number**

0

**E-mail**

blank

**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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<tr>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

This form, together with the CU2 form has been assembled under the guidance of:
- two experimental scientists with a combined experience handling prokaryote and eukaryote micro-organism GMOs of over 30 years, and;
- HSE consulting company with highly successful track record in HSE procedures in work-places, including laboratories and in HSE teaching in HE.

The GMSC comprises the Head of Protein Sciences and the Collaborations Pharmacology Director, both Exscientia employees.

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<td>Level 4 (GMMs)</td>
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</tbody>
</table>

02/03/2022
Deactivation of biological waste for level 1 activity is not necessary but is carried out in line with good practice procedures to deactivate all biological waste before disposal, except for waste securely stored for collection and incineration by the approved Exscientia clinical waste contractor.

Disinfection measures: A 2% Virkon solution will be prepared prior to working with viruses and will be available should a spillage occur. After use the consumable and liquids containing virus and GMOs will be exposed to 2% Virkon for a minimum of 60 minutes. Liquid waste will then be disposed of via an approved laboratory sink (thoroughly flushed with water).

Biologically contaminated glassware will decontaminated with a 2% Virkon solution for 20 minutes prior to disposal via an approved laboratory sink.

Biologically contaminated plasticware and consumables will be autoclaved (121°C for at least 15 minutes) and disposed of in the waste collection by the local authority.

Sharps (eg needles, syringes, scalpels) will be disposed via incineration (carried out by external laboratory waste management specialist).

Degree of kill
- Autoclaving, effectively 100% kill
- Incineration, effectively 100% kill
- Chemical disinfection, used according to manufacturer’s instructions under standard conditions, manufacturers validation (99.998% kill).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The person responsible for the biosafety has checked these documents and discussed our risk assessment. In addition, these documents have been reviewed by an HSE consultant. The GMSC has reviewed this document and associated Risk Assessment and found that they are adequate, suitable and sufficient.

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<td>Date Ackn'd</td>
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<td>CU2 Project Title</td>
<td>Functional studies of proteins for drug discovery projects</td>
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<td>Tick if notifying a connected programme of work</td>
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<td>Project notified under transitional arrangements</td>
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**Historical Significant Changes**

**Historical Date of Additional Info**

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**Project Additional Information**

**Purposes of the contained use**

To support drug discovery programs.

1. Expression of recombinant proteins, including potential therapeutic targets and anti-targets, in bacterial, insect and mammalian cells for purification and use in structural, biophysical and biochemical activity studies for drug discovery projects.

2. Expression and genetic knock-down of potential drug targets and anti-targets in mammalian cell lines for target characterisation and the development of screening assays. Functional characterisation of the resulting cells will involve standard laboratory techniques including (but not restricted to): secretion assays, reporter assays, kinetic assays, fluorescent microscopy, DNA sequencing, and gene expression studies.

**Recipient or parental organism**

Mammalian cells:

Standard laboratory mammalian cell-lines used are class 1 GMOs. These cell-lines have been widely used for
molecular and cell biology research and are also used by the biotechnology industry. The cell lines are sourced from and characterised by commercial vendors, e.g. ATCC or ECACC, and are provided pre-screened for the presence of blood-borne mycoplasma, bacterial, fungal and viral contaminants.

Insect cells:
The commonly used Hi5 and SF9 insect cell lines used are class 1 GMOs. These strains are harmless to humans and will not survive in the human body or the environment. Baculoviruses have only been isolated from invertebrates, primarily insect species. Although baculoviruses can be taken up by certain mammalian cells (notably hepatocytes cells, Boyce FM & Bucher NL, 1996, Proc.Natl. Acad. Sci USA 93, 2348- 2352; Hofmann et al. 1995, Proc.Natl. Acad.Sci.USA 92, 10099-10103) it is recognised they do not productively infect mammalian cells. Therefore, these viruses do not pose an inherent risk to humans and may be assigned ACDP hazard group 1.

Bacterial cells:
K12 commercial E. coli strains used are not considered pathogenic to humans or animals are recognised as noncolonising to humans and are disabled and thus unlikely to persist in the gut, lung, or survive outside of the special culture medium which provides their auxotrophic requirements. Thus the E. coli strains used would not be hazardous to the environment even if they did survive. Thus, they may be assigned to ACDP hazard group 1. E.coli BL21 is unlikely to be pathogenic and can be considered as broadly equivalent to K12 strains (Chart et al. J.Appl.Microbiol. 2000, 89 1048-1058). Therefore, for the benefit of this assessment it can be categorised as ACDP group 1.

Commercially sourced and a proprietary series of vectors (pEXS) using standard vector backbones will be employed, e.g., those containing and not limited to CoIE1/pMB1/pBR322/pUC, R6K, pACYC, p15A, pSC101 and pRSF1030 replicons. The backbones will include a range of fusion partners eg polyhistidine tags and selection (resistance) markers including but not limited to ampicillin, Zeocin, chloramphenicol and G418 as well as additional sequences such as promoters necessary for expression in specific host cells. The vector sequences are stored on a central drive. All plasmids are non-mobilizable.

Host cells including but not limited to:
Mammalian host cells: HEK293T, Expi293F, CHO, ExpiCHO, Jurkat, A549, MCF-7
Bacterial host cells: DE3 E. coli cells such as BL21, Lemo21, Rosetta 2 and additionally cloning strains such as DH5alpha.
Insect host cells: Sf9, SF21, ExpiSf, Hi5 and drosophila S2.
Transfer of DNA to heterologous hosts will be via transformation of E. coli, transfection and baculovirus mediated infection of insect cells, and transfection or baculovirus mediated infection (BAC-MAM) of mammalian cells. Under Genetic Modification regulations, the bacterial strains and cell lines to be used are considered to be especially disabled hosts. For aseptic purposes, all eukaryotic cell lines will be cultured in a Class II microbiological safety cabinet located in a tissue culture laboratory (containment level 2). The bacterial cells will be cultured in a safety cabinet in a preparative laboratory (containment level 2).

Origin & function
The genetic material will be derived from a range of species and have a variety of functions. This will include cytokines and growth factors, putative and known oncogenes and tumour-suppressor genes, cell signalling and cell cycle pathway genes and short hairpin RNA (siRNA) molecules intended to inhibit the expression of a range of genes including those known or suspected to be involved in pathways of oncogenesis. Genetic material is derived from mammalian or bacterial genomic DNA or cDNA, supplied from commercial sources.
or synthesised through appropriate vendors, (such as IDT). Where deemed necessary, genes, expression cassettes
or open reading frames are subjected to codon optimisation technology through appropriate online vendors.
Gene sequences will require approval internally by the company GMSC before work can start.
Gene functions will include but are not limited to:
1. cDNAs encoding wild-type or mutant known and candidate oncogenes, tumour suppressor genes, cytokines,
growth factors and genes involved in cell signalling.
2. Expression cassettes encoding siRNA molecules under the control of human or viral promoter. siRNAs will be
targeted primarily but not exclusively to genes that may be, or are known to be, involved in cancer development and
progression.

Evaluation of foreseeable effects

Specific hazards associated with inserted genes include:-
Expression/over-expression of known or potential oncogenes: while oncogenesis is known to be a multi-factorial
process, the risk of insertional activation of other oncogenes by the vector, or of insertional inactivation of tumour
suppressor genes is unpredictable, and, could act in concert to contribute to oncogenic pathways.
Expression of cell signalling and cell-cycle genes: constitutive expression of these genes at high levels could be
regarded as oncogenic: similar risk to oncogene expression, above.
Expression of siRNAs designed to knock down known or potential tumour suppressor genes: similar risk to oncogene
expression, above.
Expression of cytokines and growth factors: these proteins have the potential to cause inappropriate growth,
differentiation or apoptosis of cells, which are associated with oncogenesis. Growth factors and cytokines may also
be teratogenic, and have other effects on the immune response, so consideration will have to be given during the
individual risk assessment as to whether expectant mothers and workers with other health issues should be excluded
from such work.
Oncogenic, cytokine and growth factor sequences are capable of encoding biologically active proteins which are
potentially pathogenic to humans. Some of the prokaryotic and eukaryotic vector systems are optimised for high level
expression of protein. The sequences are highly unlikely to alter the pathogenicity or properties of bacterial host
cells. Enforced expression of such sequences in mammalian cells will, in some instances, affect the malignant
properties of the cells. Sequences encoding biologically active oncogenic proteins are classified as carcinogens for
which containment level 2 is appropriate.
Neither the DNA, encoded protein, bacterial or mammalian transfectants are likely to have any deleterious effect on
the environment and the work is at a small-scale (< 15 L). The GM microorganisms are incapable of surviving
outside of the laboratory environment. All laboratory procedures will follow good microbiological practice. GM
microorganisms will be manipulated in a Microbiological safety cabinet. Written records of staff training will be
obtained and checked before commencement of work by individuals. Handling and manipulation of naked DNA will
require the wearing of disposable gloves. Mammalian cell cultures for DNA transfection procedures will be handled in
a Class 2 cabinet. Where possible, sharps will be avoided in all operations; in the cases where it is necessary, sharp
handling training will be provided and all sharps will be disposed in yellow shar disposable bins that will be sealed
and collected for incineration by a trusted contractor. Disposable plasticware (pipettes especially) will be used for cell
culture.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Disinfection measures: A 2% Virkon solution will be prepared prior to working with viruses and will be available should a spillage occur. After use, consumables and liquids containing virus or GMOs will be exposed to 2% Virkon for a minimum of 60 minutes. Liquid waste will then be disposed of via an approved laboratory sink (thoroughly flushed with water).

Biologically contaminated glassware will be decontaminated with a 2% Virkon solution for 20 minutes prior to disposal via an approved laboratory sink.

Biologically contaminated plasticware and consumables will be autoclaved (121°C for at least 15 minutes) and disposed of in the waste collection by the local authority.

Sharps (e.g., needles, syringes, scalpels) will be disposed via incineration (carried out by external laboratory waste management specialist).

Degree of kill
- Autoclaving, effectively 100% kill
- Incineration, effectively 100% kill
- Chemical disinfection, used according to manufacturer’s instructions under standard conditions, manufacturers validation (99.998% kill).

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment

The Exscientia GMSC has reviewed this document and associated Risk Assessment and found that they are adequate, suitable and sufficient.

Project Containment

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| HSE Division | blank |

| Comments |                           |

| Date at Which Additional Info Submitted | 02/03/2022 |
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

The GMO safety committee consists of four members: The laboratory Head, a Senior Scientist and 2 Directors of Safety and Environment, one with responsibility for EMEA with specialist Sio-safety expertise. The team combines experience in biological safety and molecular biology/cell biology. This team will meet quarterly once the laboratory is operational.

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Yes

All biological material waste is autoclaved on site prior to disposal. Liquid waste may be treated by an appropriate disinfectant with approved concentration and contact time.

The principle of the work is “Research on the cellular pathways of ageing using a number of cell bio

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 3456/19.1

Date Ackn'd 31/07/2019

CU2 Project Title Transduction of a variety of cell types with replication incompetent and self-inactivating lentiviral vectors to generate reporter cells expressing GFP and luciferase

Class 2

CultureVolClass2 < 1 Litre

CultureVolumeClass3-4 Non-GMM Consent Granted
### Project Additional Information

**Purposes of the contained use**

The purpose of this project is to generate reporter cell lines that will fluoresce and express a secreted form of luciferase upon activation of a particular signalling pathway. 2nd generation replication incompetent, self-inactivating, VSV-G pseudotyped lentiviral vectors will be produced using HEK293T cells and then will be used to transduce different type of cell lines to generate reporter cells expressing GFP and luciferase.

**Recipient or parental organism**

The lentivirus will be used to transduce various different host cell lines.

**Host/vector system**

2nd generation replication incompetent, self-inactivating, VSV-G pseudotyped lentiviral vectors purchased from a commercial supplier.

**Origin & function**

Marker genes (e.g. GFP, Cypridina luciferase and Nanoluc luciferase).

**Evaluation of foreseeable effects**

Evaluation of foreseeable effects: Resulting GM reporter cell lines are not expected to possess harmful properties for humans, animals and the environment as the inserts do not encode any products with potentially harmful biological activity, for example toxins, cytokines, growth factors, allergens, hormones or oncogenes. Additionally the inserts do not encode viral specific proteins, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus as a vector.

In the event of an environmental release, neither the lentiviral particles nor transduced cultured cells are expected to survive outside culture medium. This rules out any possibility of survival, establishment or dissemination in the external environment. In the event of accidental release, viral particles are not capable of causing any infection in the host cells due to absence of viral genes and their replication incompetent nature. Transduction can only happen one round before virus inactivates itself and even if they manage to transduce cells, the resulting gene expression of GFP and luciferase is not expected to cause any harm.

CL2 control measures will be applied throughout the project in addition to local hygiene rules, good microbiology practices, and waste management. The control measures overall are accepted to be suitable and sufficient to reduce any residual risk to negligible/acceptable levels.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Plasticware vessels, tips, and stripettes will be immersed into 2% Virkon solution for at least 1 h and then they will be placed inside Bio-bins which later will be autoclaved and incinerated.

Manufacturer's validation states 100% kill when left in contact with 1% Virkon for a minimum of 10 minutes.

https://www.fishersci.co.uk/webfiles/uk/web-docs/SLSDG05.PDF

Contaminated PPE such as gloves and flasks will all be disposed of in Bio-bins and autoclaved and later incinerated. Waste is autoclave sterilised using Boxer 400/300V at 121°C for a minimum of 20 minutes. The sterilisation process is logged and checked for successful completion using a Thermo-log. The contents of the bin are then treated as clinical waste, re-bagged, sealed and labelled before disposal into the outdoor Silo bin.

Liquid cell media waste will be aspirated into sealed containers and then autoclaved as above.

Autoclaves undergo annual service, calibration and statutory inspections arranged by London Bioscience and Innovation Centre (LBIC).

No sharp waste will be generated in this project.

Solid laboratory waste that doesn't come into contact with any viable cells and lentivirus will be treated as clinical waste and incinerated via licenced waste contractors.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]

Tick to confirm that you have attached a risk assessment to this form [Y]

Tick if you are claiming exemption from disclosure for section of the risk assessment [N]

Please enter comments on the GM safety committee on the risk assessment

The project bioburden assessment was appraised by the Environment, Health and Safety (EHS) committee and minor revision was recommended. All recommended points were implemented and the Committee has concluded the existing control measures are suitable and sufficient to reduce remaining risk to negligible levels. Committee consent form is issued on 21 June 2019.

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| Comments                       |                   |

Date at Which Additional Info Submitted

02/03/2022
# Premises Addresses

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# Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

All activities will be peer risk assessed as a team of scientists with the advice of the Alderley Park Site Risk Manager.

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<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste will be streamed according to Alderley Park Site Waste guidelines
Contaminated solid waste will be denatured and deactivated in a centralised autoclave that operates under an environmental permit and is remote from these activities. All waste transported to the autoclave facility by trained waste operators in drop-sided wheelie bins. The autoclave sterilising cycle is 125°C for 30 minutes. Autoclave waste will be sent for incineration
Cell culture liquid waste will be inactivated in 2% Virkon for at least 1 hour as solution before disposal down drains and flushing with copious amounts of water
All activities are covered by appropriate COSHH Risk Assessments

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The proposal has been reviewed as a team of scientists including advice from the Site Safety Manager.
All are satisfied that the risk class identified is appropriate for the work to be carried out and the facilities meet the required standard
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Page 14571 of 15326

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

As a tenant company of Babraham Bioscience Technologies Ltd we have all of our risk assessments approved by the Babraham Biosafety Committee prior to any laboratory work and this covers correct procedures for genetic modification as well as contained use.

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Tick if confidential

Tick if confidential

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02/03/2022
Yes

For activities involving GMMs, describe the waste management measures which will apply to the activity

All biological waste must be inactivated by treatment with a 1% Virkon solution prior to disposal and yellow bins will be processed by incineration off-site by Vetspeed- GM authorisation number GM898. Incineration eliminates 100% infectious material. Treatment of culture fluids with 2% Virkon and soaking of recyclable lab ware in 1% Virkon for 16h eliminates 100% infectious material. Bench/cabinet surfaces will be wiped down with Chemgene or similar detergent. Small spills will be covered with paper towels, sprayed with 2% Virkon or Chemgene solution, left for 10min before transferring the paper towels to the designated containment level 2 bins. Excess liquid will be dried with additional paper towels using the same disposal method. Large spills will be contained by paper towels at the perimeter and Virkon powder will be spread over the spill. After 10 minutes the liquid waste will be absorbed by paper towels disposed as described previously.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

As tenants of Babraham Bioscience Technologies all laboratory work, including genetic modifications can only take place once it is approved by the Babraham Biological and Chemical Safety Committee which meets weekly.

Project Ref 3458/19.1

Date Ackn'd 31/01/2019

Date Project Ceased

CU2 Project Title Investigating transcriptional regulatory networks by modulating non-coding DNA and RNA

Class 2

Class Culture Volume

Volume Class 3-4

Consent Granted

Project notified under transitional arrangements

Tick if notifying a connected programme of work

Withdrawn
Our aim is to gain an understanding of transcriptional regulatory networks that determine cell fate choice based on a network model that we are developing from deep machine learning. This is a broadly applicable approach across multiple cellular systems. Our prototype experiments involve using mouse organoids to study the mechanisms of T-cell specification, which is also relevant to the development of leukaemia. We are initially targeting the non-coding region encoding ThymoD RNA, which is required for T-cell specification and causes leukaemia in mice when knocked down. We plan to use CRISPR to knock this down in our organoid system and investigate the phenotype in the broader context of our network model.

Our other prototype experiments involve using a human lung cancer cell line to dissect inducible epithelial to mesenchyme transitions in which several non-coding RNA have been implicated. The rationale behind targeting non-coding RNAs is their prevalence, high cell-type specificity and wide associations with disease phenotypes. In understanding their behaviour within a network we are working towards comprehensive therapeutics. After establishing our prototypes, we plan to move on to a variety of other cellular systems, developing 3D organoid cultures. This will involve using patient derived cells such as matched samples available from EBISC.

Established commercially available mouse (e.g. EML) and human (e.g. A-549 VIM RFP) cell lines. Primary human stem cells e.g. CD34+ blood stem cells from Stem Cell Technologies. Patient derived iPSC lines from resources such as the Cambridge Blood and Stem Cell Biobank, EBISC and HipSci. The likelihood of transfer to humans is minimal given the level of containment and aim to use transient, specific targeting.

Our current approach is to avoid vector based systems and deliver CRISPR reagents as a ribonuclease protein (RNP) complex by electroporation or (preferably) lipofection/vesicle transfer. However, we have consider other delivery systems for more challenging cells types which may require viral
delivery. In this case we will select harmless viral delivery systems such as recombinant Adeno-Associated Virus (rAAV) and second or (preferably) third generation lentivirus systems.

**Origin & function**

The non-coding RNAs that will be modified, either at the DNA or RNA level will be part of a suite of nucleic acids that will have been predicted to be critical for determining cell fate. For example, in our mouse prototype project to study T-cell specification, the non-coding RNA ThymoD is required for T-cell specification and knockdown in mouse results in leukaemia. Our other prototype project, studying the epithelial to mesenchyme transition of a human lung cancer cell line, is likely to predict oncogenic and tumour suppressor type properties of non-coding RNA which we will experimentally validate. Furthermore, future projects aim to functionally test novel noncoding transcripts that may prove to be oncogenic, tumour suppressors and/or convey alterations in growth kinetics. Therefore, until fully characterised, the genetic modifications that we are generating should be handled as possessing these potential properties. All modified cells will be contained at containment level 2 by trained personnel.

Beyond genomic insertions/deletions of targeted non-coding RNA sequences, there may be incorporation of the Cas9 protein sequence which has no known harmful effect. It may also be desirable to insert fluorescent reporter genes, which are biologically inert.

**Evaluation of foreseeable effects**

Our first choice CRISPR delivery system is a vector free RNP complex. This is highly specific, carries a nuclear localisation signal and designed with a single strand oligo donor DNA (ssODN) along with reagents to facilitate homology directed repair rather than inaccurate non-homologous end joining. This reduces off target effects in recipient cells, not least due to the transient presence of exogenous proteins and nucleic acids. Editing potential is therefore limited to a narrow time frame.

If this approach does not work for some difficult to transfect cells, safe harbour viral systems will be used. The non-pathogenic recombinant adeno-associated virus (rAAV) will be the first choice, but if larger constructs are necessary, second or third generation lentiviral systems will be used. The second generation viruses lack all accessory protein associated with virulence and cytotoxicity, while retaining Rev, Tat, Gag and Gag-pol in the packaging vector. A second (transfer) vector contains the viral LTRs (including a Tat-transactivated promoter), the targeted insert and a psi packaging signal, while a third (envelop) vector contains the envelop protein. The third generation lentivirus advances the safety features of the second generation packaging vector by splitting it into two. One vector contains Rev, and the other contains Gag and Gag-pol. Tat is eliminated and an alternative chimeric promoter is present in the transfer vector, independent of Tat
Transactivation. While safer, the third generation system can be cumbersome and so the second generation may have to be considered in difficult to transfect cells. In both systems, the multi-vector approach considerably reduces the probability of recombinant events producing a replication competent virus.

While this is highly unlikely, the potential hazard of a recombinant virus as well as a packaged virus prior to transfection of cells, or residual virus in media should be considered. A virus has the potential to infect multiple cell types via entry routes such as abraded skin and mucous membranes. These entry routes can be protected by standard PPE, gloves, lab coat, safety glasses. Infection is only likely at high titre and high titre viruses are only likely to be achieved by ultracentrifuge concentration, not a remit of our project plans.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All biological waste must be inactivated by treatment with a 1% Virkon solution prior to disposal and yellow bins will be processed by incineration off-site by Vetspeed - GM authorisation number GM898. Incineration eliminates 100% infectious material. Treatment of culture fluids with 2% Virkon and soaking of recyclable lab ware in 1% Virkon for 16h eliminates 100% infectious material. Bench/cabinet surfaces will be wiped down with Chemgene or similar detergent. Small spills will be covered with paper towels, sprayed with 2% Virkon or Chemgene solution, left for 10min before transferring the paper towels to the designated containment level 2 bins. Excess liquid will be dried with additional paper towels using the same disposal method. Large spills will be contained by paper towels at the perimeter and Virkon powder will be spread over the spill. After 10 minutes the liquid waste will be absorbed by paper towels disposed as described previously.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The Babraham Genetic Safety Committee have reviewed the risk assessment, and once the means of delivering genetic editing agents to the cells was established, approved the document. There is currently only one person in place to perform GM work and future personnel will be made fully aware of this risk assessment.

### Project Containment

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Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The Agentus Bioscience Ltd genetic modification committee will consist of:
1. The head of the Cambridge site who will serve as the chair of the committee and has over 30 years of relevant research experience.
2. Five Agentus staff who have PhDs in various relevant fields of research and more than 10 years research experience including genetic modification and GMOs.
3. The external biosafety consultant (see section 8).
4. The Agentus USA EH&S manager.

The Agentus GM committee agreed that the risk assessment included with this notification was suitable and sufficient. The committee will meet at least every 6 months to review risk assessments and GM procedures.

<table>
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Tick if confidential

02/03/2022
All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment
The Agentus GMO committee reviewed the GMO risk assessment (see attached). The Agentus GMO committee consists of 6 people who have PhDs in different fields with at least 10 years research experience including genetic modification and GMOs. The GMO committee have previously written GMO risk assessments for similar work prior to joining Agentus and they understand the science around the work carried out.

The Health and safety consultant reviewed the GMOs. The consultant has a B.Sc in Animal Sciences - Wye College (part of Imperial), London University, M.Sc. Animal Parasitology, Ph.D. The immunology and pathology of Sarcocystis ovicanis infections in sheep (both at U.C.N.W., University of Wales). Technical member of the Institution of occupational safety and health (Tech IOSH), NEBOSH National Diploma in Occupational Health and Safety (in progress), NEBOSH National General Certificate in Occupational Health and Safety, Member of the Institute of Safety in Technology and Research, MRC Biological Safety Officer Training Certificate. 1986 to 2010 at the Babraham Institute (Cambridge) where experience of handling pathogens and GMO obtained during research. 2006 made Babraham Research Campus Biological Safety Officer (part time plus research). 2009 onwards teach the genetic modification safety section of the One Nucleus 'Biological Safety: Management and Practice' course (IOSH approved). 2010 onwards Babraham Institute Health, Safety and Quality Assurance Manager and Babraham Bioscience Technologies (BBT) Biosafety Officer (Full time). 8 years experience in risk assessments and has health and safety qualifications.

All GMO risk assessments were reviewed independently and externally by the Agentus (USA) EH&S manager to look for inconsistencies and to highlight potential risks.

Project Ref 3459/19.1

Date Ackn'd 06/02/2019

CU2 Project Title The use of lentivirus vectors for mediating gene modulation in mammalian cells involved in immune responses and their subsequent use in vitro for screening, target identification and validation of targets for cancer therapies.

Class 2 CultureVol Class 2 CultureVolume

Consent Granted

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
Project Additional Information

Purposes of the contained use
The aim of this project is to use lentiviral delivery systems to introduce various mammalian DNA sequences into mammalian immune cells in culture. These sequences direct expression of either (i) RNA species (siRNAs or antisense RNAs) that interfere with the expression of various mammalian proteins or (ii) the corresponding mammalian proteins themselves; for in vitro use and for the generation of stable mammalian cell lines (and subsequent use of such stable lines in vitro).

Recipient or parental organism
Cloning, propagation and amplification of component plasmids for lenti-virus vector systems will take place in K12 and B strains of E. coli (with a history of safe use).
Mammalian cell lines (e.g. immortalized lines derived from human cancer tissues) fall into two types:

a) Packaging/helper cell lines into which plasmids containing lenti- or retroviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre (e.g. HEK296 cells). The media from these cell lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines, i.e. a negligible risk.

Host/vector system
The LENTIVIRAL vectors that will be used are derived from HIV-1, which is an ACDP Hazard Group 3 biological agent. However, second and third generation lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase biosafety and viral packaging is achieved by providing three helper constructs in trans containing gag, pol and rev sequences.
For example, second and third generation Lentiviral Expression Systems include the following key safety features:
In the second and successive generation lentiviral vectors several lentiviral accessory genes (vif, vpr, vpu and nef) are deleted from the transfer plasmid since they are not required for in vitro replication and the products they encode have cytotoxic activities.
In addition to this in the third generation lentiviral vectors:
- The Lenti expression vectors contain a deletion in the 3' L TR (LlU3) that does not affect generation of the viral genome in the producer cell line but instead results in "self-inactivation" of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a packageable viral genome.
- The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).
- Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain L TRs or the 4J packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication competent virus can be produced.
Despite the above safety features, use of these lentiviral vectors (which include WPRE) falls within SACGM 2
criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating
virus, or the possibility of insertional mutagenesis. Also, the virus will be packaged by transfecting
transfer vector into specific amphoteric 'helper' cell line (gag, pol and env stably incorporated into host
chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a
wide range of mammalian species (including humans) and cell types.
This means that the viruses produced for this experiment could potentially infect a number of species, including
man.

Origin & function

Selectable markers - examples (but not restricted to);
- Ampicillin resistance: E.coli derived
- Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
Puromycin resistance (PAC) : Puromycin acetyl transferase is derived from Streptomyces alboniger
Reporter proteins such as (but not restricted to);
- Fluorescent proteins as reporters;
- GFP derived from the jellyfish Aequorea victoria and variants of this
- Luciferase - class of oxidative enzymes used in bioluminescence
- renilla luciferase derived from the Sea pansy (Renilla reniformis)
- firefly luciferase derived from the firefly Photinus pyralis.
Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and / or
shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding
potential drug targets or therapeutic proteins) - all human derived.
This could include the expression of potentially harmful genes e.g. encoding known proto-oncogenes or genes with
known oncogenic mutations that can contribute to cellular transformation.

Evaluation of foreseeable effects

All viral vectors employed in this protocol exhibit broad tropism and potential to infect human. Risks conferred
following infection are identified as:

i) genetic insertion of viral sequences with potentially deleterious effects on endogenous genes: we will employ
transgene promoters and other viral sequences that may affect host gene function in a wide range of cell types
(e.g. CMV promoter). We assess retro-, lenti- or adenovirus infection might induce permanent changes in infected
cells including a risk for tumorigenesis. Risks conferred are previously described and categorised under Class 2
risks [SACGM compendium of guidance part 2, page 121]: "The effects of integration upon the infected cell
should be considered. For instance, promoter sequences present in the provirus might activate genes adjacent to
the integration site or, alternatively, insertion may disrupt genes and prevent their expression."

ii) expression of human-derived or homologous transgenes with potentially deleterious effects: various transgenes
may be employed, wherein intrinsic function of the transgene confers potentially deleterious effects. For example,
expression human oncoproteins could induce transformation of infected cells. Beyond endogenous homeostatic
mechanisms that may lessen this risk (e.g. apoptosis and other host tumor suppression responses), we assess that
standard precautions under Class 2 risk mitigation procedures are adequate to address such risks.

iii) expression of exogenous transgenes directed to host genes with potentially deleterious effects: multiple
transgene technologies (antisense, RNAi, CRISPR or related gene conversion) may be employed to modify the
function of endogenous genes with potentially deleterious effects. For example, RNAi or antisense RNA could
reduce the function of genes necessary to control cell apoptosis, potentially resulting in tumorigenesis. As above,
we assess that Class 2 risk management procedures adequately address these risks.

iv) expression of heterologous genetic sequences with potentially deleterious effects: we will employ diverse
collections of non-human derived, exogenous transgene sequences that carry potential risks following infection.
Risks from expression of these collected transgene sequences are mitigated and attenuated through several
means. First, known pathogenic transgenes systemically removed, and other exogenous gene products are
fragmented or rearranged, such that potentially pathogenic or other biologically deleterious genetic functions are
not recapitulated in their complete endogenous configuration. Second, potentially deleterious transgenes in any
infectious viral preparation are highly titrated, such that any single deleterious sequence comprises less than 1 part
in 100,000 parts of an inoculum with infectious potential. In total, we assess these risks are adequately managed
via the same Class 2 risk precautions employed above.
We assess environmental hazards are adequately addressed through the proposed personnel risk management
measures. We employ multiple attenuation strategies and protocols to severely limit independent virus propagation
beyond the intended use, and therefore anticipate minimal risks of GMM release.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute
described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting
this method of waste disposal are:
1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since
All liquid waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Solid waste
material will be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM
authorization is GM898) according to disposal notification GM1 05/4.1.
Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable (red lid to distinguish
them from Class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed
into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local
registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site
has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of
any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of
loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any
spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the
environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of
release would be as a result of a major RTA, however, the containers are designed to withstand this.
This disposal method is expected to achieve 100% inactivation of the GMM

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Solid waste
material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation
application above). Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable
bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210
litre yellow labelled "Eurobins".
Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.
This disposal method is expected to achieve 100% inactivation of the GMM

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment

The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the IPPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

Project Containment

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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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Name

BIVICTRIX THERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre

Road Name

MERESIDE

District

ALDERLEY PARK

Town

MACCLESFIELD

County

CHESHIRE

Postcode

SK10 4TG

Country

ENGLAND

Tel Number

0784 1533089

Fax Number

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E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

All activities will be risk assessed as a team of scientists working with the advice of a professional Site Risk Manager.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
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Tick if confidential

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<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<tbody>
<tr>
<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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</tbody>
</table>
Contaminated solid waste will be deactivated in a centralised autoclave that operates under an environmental permit. The autoclave has a sterilising cycle of 125°C for 30 minutes. Once denatured the waste will be sent for incineration. Liquid waste will be denatured using 1% Virkon for a period of at least 1 hour, before discharging to drain flushed with copious amounts of water.
### GM Centre Number: 3462

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### Name 2

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The person responsible has worked in biosafety level 2 laboratories for over 5 years, and has experience in risk assessment related to laboratory work and writing COSHH forms. They hold a PhD in Genetics and have been directly involved in the work of genetically modified organisms.

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Non-microbial

Other (please specify) Tick if confidential

Bacteriology | Yes         | Parasitology | Transgenic Birds | Microbiology Research | Yes
Virology     | Yes         | Transgenic Animals | Transgenic Fish | Gene Therapy |
The GMMs will be harvested by centrifugation and autoclaved at 121°C for 20 minutes to ensure 100% kill. The supernatant media will be neutralised with 1% virkon overnight before being discharged. In the event of spillage, the area can be effectively disinfected. Solid waste will be autoclaved to ensure 100% kill and then double bagged before discharged.

The person responsible has worked in biosafety level 2 laboratories for over 5 years, and has experience in risk assessment related to laboratory work and writing COSHH forms. They hold a PhD in Genetics and have been directly involved in the work of genetically modified organisms.
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Name

ALDERELY PARK LTD

Name 2

Department

Campus Estate or Research Centre

MERESIDE

Road Name

ALDERLEY PARK

Building

Town

ALDERLEY EDGE

County

CHESHIRE

Postcode

SK10 4TG

Country

ENGLAND

Tel Number

07899 076239

Fax Number

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E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities:  
- **Yes**

Give brief details of the genetic modification safety committee:

Work with genetically modified organisms will be managed as a team approach. All activities will be risk assessed as a team of scientists working with the advice of a professional OSH manager.

### Laboratory

<table>
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<tr>
<th>Level 1 (GMMs)</th>
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### Animal Unit

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### Growth Room

- **Tick if confidential**

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### Glass House

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### Microbiology Research

- Bacteriology: **Yes**
- Parasitology
- Transgenic Birds
- Microbiology Gene Therapy

### Virology Research

- Transgenic Animals
- Transgenic Fish
- Gene Therapy
Contaminated solid waste will be deactivated in a centralised autoclave that operates under an environmental permit. The autoclave has a sterilising cycle of 125°C for 30 minutes. Once denatured the waste will be sent for incineration. Liquid waste will be denatured using 1% Virkon for a period of at least 1 hour, before discharging to drain flushed with copious amounts of water.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Contaminated solid waste will be deactivated in a centralised autoclave that operates under an environmental permit. The autoclave has a sterilising cycle of 125°C for 30 minutes. Once denatured the waste will be sent for incineration. Liquid waste will be denatured using 1% Virkon for a period of at least 1 hour, before discharging to drain flushed with copious amounts of water.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Site Risk Manager has reviewed the proposal and is satisfied that the risk class identified is appropriate for the work to be carried out and the facilities meet the required standard.
**GM Centre Number: 3465**

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**Name**

CAMBRIDGE DISPLAY TECHNOLOGY LTD

**Campus Estate or Research Centre**

UNIT 12

**Name 2**

**Department**

**Building**

CARDINAL BUSINESS PARK

**Road Name**

CARDINAL WAY

**District**

**Town**

GODMANCHESTER

**County**

CAMBRIDGESHIRE

**Postcode**

PE29 2XG

**Country**

ENGLAND

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

<table>
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<th>Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities</th>
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Give brief details of the genetic modification safety committee

All risk assessments for new work involving the use of genetically modified materials are submitted for review and approval by the Biosafety Committee.

The approval/rejection of a risk assessment by the Biosafety Committee is recorded by the Biological Safety Officer together with any comments applicable.

The members of the CDT Biosafety Committee are:

- Senior Executive responsible for work using genetically modified materials
- Biological Safety Officer
- Senior Advisor, Health, Safety and Environment

The Senior Executive responsible for work using genetically modified materials has over 15 years research experience, including the assessment and review of risk assessments.

A Scientist has been appointed to the role of Biological Safety Officer (BSO). The Scientist has a BSc and MSc in Medical Engineering, a PhD in the field of Biosensors and 5 years' work experience in the field of biosensors, including the assessment of risk and writing of risk assessments. The appointed BSO has attended a Biosafety Management and Practice training course, focusing on work at ACDP Containment Levels 1 and 2 and work with GMOs. The training course is approved by the Institution of Occupational Safety and Health.

The Senior Advisor, Health, Safety and Environment has also completed a Biosafety training course which was provided by the Medical Research Council and has over 15 years of work experience providing health and safety advice in healthcare and scientific research environments.

<table>
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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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The requirements for inactivation of GMMs are considered and recorded in the risk assessments relevant to the work being carried out with those GMMs. The arrangements for waste disposal of GMMs are described in the local guidelines for work in the designated Biosafety Laboratory at CDT. All wastes containing GMMs are placed in sealed plastic containers and consigned as clinical waste to a licensed waste contractor for incineration. The following wastes are produced as a result of work with GMMs:
- contaminated solid wastes - pipette tips, wipes, petri dishes, tubes, t spreader and inoculation loop
- flammable solid wastes - alcohol wipes used to disinfect the work area
- aqueous waste - solutions containing microorganisms

Wherever it is possible only work with GMMs which are certified as inactive, and which therefore have a low risk of human infection, is carried out. The risk to human health and the environment from GMMs which are certified as inactive is negligible, and as such, wastes which are generated from the work with those materials are not subjected to a further inactivation process prior to consignment from site. Where work with active GMMs is carried out, then wastes from that work are subjected to a chemical inactivation process using a proprietary peroxygen compound (Virkon S) prior to consignment from site. Information from the manufacturer of the product that used to carry out the inactivation, or from a reliable source in the public domain, is used to substantiate a minimum 5-log reduction in viability and validate the inactivation of specific GMMs.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

- contaminated solid wastes - pipette tips, wipes, petri dishes, tubes, t spreader and inoculation loop
- flammable solid wastes - alcohol wipes used to disinfect the work area
- aqueous waste - solutions containing microorganisms

Wherever it is possible only work with GMMs which are certified as inactive, and which therefore have a low risk of human infection, is carried out. The risk to human health and the environment from GMMs which are certified as inactive is negligible, and as such, wastes which are generated from the work with those materials are not subjected to a further inactivation process prior to consignment from site. Where work with active GMMs is carried out, then wastes from that work are subjected to a chemical inactivation process using a proprietary peroxygen compound (Virkon S) prior to consignment from site. Information from the manufacturer of the product that used to carry out the inactivation, or from a reliable source in the public domain, is used to substantiate a minimum 5-log reduction in viability and validate the inactivation of specific GMMs.
The risk assessments completed for class 1 activities at CDT have been reviewed by the Biosafety Committee.
The arrangements in place for the storage, use and disposal of GMMs at CDT comply with the requirements of the legislation and the best practice described in the associated guidance for class 1 activities.
No additional arrangements were identified as necessary to control the risks to human health and the environment presented by the work specified in the assessments.
Approval of the risk assessments has been recorded.
The risk assessments are reviewed at least once a year or whenever it is required by changes in work conditions or procedures.
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**Name**

OMASS THERAPEUTICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

THE SCHRODINGER BUILDING

**Road Name**

HEATLEY ROAD

**District**

OXFORD SCIENCE PARK

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX4 4GE

**Country**

ENGLAND

**Tel Number**

01865 548358

**Fax Number**

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**E-mail**

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**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Safety consultant (Director of Safety, Health and Sustainability - The Francis Crick Institute)

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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
The risk assessment shows that the modified organisms will not survive outside of the laboratory environment and therefore no special waste treatment is required. If however at some point a risk assessment indicates that the waste requires treatment prior to final disposal then all solid waste will be disposed of in biohazard labelled bags. Liquid waste will be inactivated with freshly prepared 1% Virkon solution prior to disposal.

For activities involving GMMs, describe the waste management measures which will apply to the activity:
The risk assessment shows that the modified organisms will not survive outside of the laboratory environment and therefore no special waste treatment is required. If however at some point a risk assessment indicates that the waste requires treatment prior to final disposal then all solid waste will be disposed of in biohazard labelled bags. Liquid waste will be inactivated with freshly prepared 1% Virkon solution prior to disposal.

Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: 

Please enter comments of the GM safety committee on the risk assessment:
The risk assessments are suitable for the type of work which will be performed at the OMass laboratories, the work will continually be assessed and additional risk assessments will be written if required (along with the application for additional GMO licences if the nature of the work extends beyond Cat 1, although this is currently not anticipated).
GM Centre Number: 3468

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Campus Estate or Research Centre

BIOCITY SCOTLAND

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Tel Number

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Fax Number

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E-mail

HSE Division

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Comments

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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

*Y*

**Give brief details of the genetic modification safety committee**

BioAscent has formed a genetic modification safety committee comprising of a biological safety officer with over 20 years' GM and non-GM cell culture experience, two senior managers with experience of working in and managing GM cell culture facilities and one senior manager from a chemistry background with extensive knowledge of COSHH. The committee will meet regularly to review existing risk assessments and procedures and all new proposals to work with GMOs will be referred to the committee for review.

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Liquid waste will be inactivated by addition of 1% Virkon in accordance with manufacturers instructions before being discharged to the drains. Contaminated solid waste will be placed in robust leak-proof and biohazard labelled plastic bins while sharps will be disposed of in desingated sharps bins and both will be destroyed by high temperature incineration by a licenced waste disposal contractor.

Outline Protocol for Disinfection Validation
The composition of the culture fluid, cell density, contact time and final concentration must be documented. 5-log reduction (99.999% kill) must be achieved.

• Grow up micro-organism under the typical experimental conditions.
• Add disinfectant to a known culture volume to give the desired final concentration.
• Incubate at room temperature for specified time period.
• Take a sample from the incubation mixture
• Eliminate traces of disinfectant by washing cells into fresh culture medium.
• Attempt to grow any surviving micro-organisms on plates or in liquid culture. Note – for viruses attempt to grow in host cell line.
• Count survivors and calculate % kill.

All waste disposal and spill procedures are captured in written standard operating procedures and compliance will be monitored via regular lab inspections and staff appraisal.

Please enter comments of the GM safety committee on the risk assessment

The risk assessment comprehensively details the characteristics of any new GM cell line that BioAscent aims to work on and provides a framework for capturing the associated risks and details the control measures required to mitigate those risks. The committee requires the risk assessments to be reviewed on a biennial basis.
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Name

BIOMEDHA LTD

Name 2

Department

Campus Estate or Research Centre

BIOCITY

Building

INNOVATION BUILDING

Road Name

PENNYFOOT STREET

District

Town

NOTTINGHAM

County

NOTTINGHAMSHIRE

Postcode

NG1 1GF

Country

ENGLAND

Tel Number

0115 822 6464

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

We have received advice from our director (of invitro biology at BioMedha), who has previously managed cell biology bioscience department with responsibility of Health and Safety/ GMO/ HTA/ Waste management internal policy design, risk assessments, implimenatation and training staff. He also received Health & safety training with respect to laboratory research activities. He has been instrumental in assessing, writing and implementation of Risk Assessments and SOPs for handling of biological and chemical materials, Class 1 and 2 containment safe practices, GMOs safe and clean environment and waste management. He is very experienced cellular and molecular biology scientist with over 15 years working across drug discovery, invitro cell biology, molecular biology, cell culture, transfection, cell line generation, class 1 and 2 containment ,GMOs and HTA safe and effective working practices. Has extensive working knowledge of complex research activities such as cell culture, cell isolation, transfection, stable cell line generation, PCR, Cryopreservation and thawing, passaging, centrifugation, cell counting, secondary containment sample handling, cell imaging and characterisation. He appreciates the significance of adhering to legislative demands and health & safety requirements. He understands the detrimental impact of non-compliance can have in relation to people, environment, tasks and project.

Laboratory

- Level 1 (GMMs) Yes
- Level 2 (GMMs) Yes
- Level 3 (GMMs)
- Level 4 (GMMs)
Liquid waste from all cell culture/GMO activities will be inactivated overnight by using 2% (v:v) Chemgene/bleach/virkon. Once inactivated, liquids should be decanted into the 10L plastic bottles (labelled “aqueous inactivated waste”. When full, liquid waste is collected by an external contractor for proper waste treatment.

Solid waste (tissue culture plastic waste) from cell culture/GMO Class 1 & 2 containment facilities, will be sprayed thoroughly with 2% (v/v) Chemgene/bleach and 70% ethanol before being placed yellow biohazard bins. Once filled, solid waste biohazard waste bins will be collected by external contractor for proper waste treatment.

Sharps waste will be disposed of via the sharps bins and will be collected by external contractor for treatment.

All contaminated glassware will be treated with fresh 2% (v/v) Chemgene/bleach/virkon solution, sprayed with 70% ethanol prior to washing with detergent.

All working surfaces are sprayed with 2% chemgene/ vircnon/ bleach and followed by 70% ethanol and wiped with tissue and will be disposed of into yellow biohazard solid waste bins.
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**Name**

SOLENT UNIVERSITY

**Campus Estate or Research Centre**

**Road Name**

EAST PARK TERRACE

**District**

**Town**

SOUTHAMPTON

**County**

**Postcode**

SO14 0YN

**Country**

ENGLAND

**Tel Number**

02382013000

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The Solent University GMSC has been established to provide guidance and advise on the use of GMMs. The inaugural meeting was held on 8th March 2019 and will subsequently meet at least annually or as required to discuss newly received GM risk assessments and to review existing assessments. The committee comprises four members, including academic and technical staff and the University Biological Safety Officer.

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Virology

Transgenic Animals

02/03/2022  
Page 14609 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste (spent media) will be inactivated either by autoclaving at 121°C for 15 minutes, or alternatively treated with chemical disinfectant (e.g., 1% Virkon) at contact times designated by the manufacturer to achieve complete inactivation. Liquid waste is discarded to the drain with copious water. Solid waste (including gloves, petri dishes, and pipettes) will be autoclaved for 15 minutes at 121°C, and then discarded via the clinical waste route. These methods achieve an effective 100% kill.

For autoclaving, the temperature and time of the process is monitored and logged digitally, and confirmed by indicator tape and commercial indicator vials, and the autoclave is subject to six monthly servicing.

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

The genetic modification safety committee reviewed the risk assessment and requested minor changes, which have now been incorporated, and the risk assessment has subsequently been approved by the committee.
Data Premises Notified: 04/04/2019

Transferred from 1992 Regs: N

Transitional Premises Class: N

Data Premises Closed: N

Transitional Premises Emergency Plan Required: N

Non-GMMs: N

Withdrawn: N

Name

THE MILNER THERAPEUTICS INSTITUTE

Name 2

Department

Campus Estate or Research Centre

JEFFREY CHEAH BIOMEDICAL CENTRE

Building

UNIVERSITY OF CAMBRIDGE

Road Name

PUDDICOMBE WAY

District

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB2 0AW

Country

ENGLAND

Tel Number: 01223767111

Fax Number: 0

E-mail

HSE Division: blank

Comments

Date at Which Additional Info Submitted: 02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

A senior scientific advisor has reviewed the attached risk assessment.
The senior scientific advisor has experience working with GMM's in academic laboratories at a post-doctoral level.
They also have experience working with GMM's in the biomedical/pharmaceutical industry in the UK. Roles in industry include, Team Leader, Group Leader and Project Manager.

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Tick if confidential

Bacteriology Yes Parasitology Transgenic Birds Microbiology Research Yes
The Jeffrey Cheah Biomedical Centre building management team will manage the building's waste streams. CL1 solid waste management: CL1 solid waste will be treated as GMO waste and includes CL1 contaminated plastic consumables, stripettes, pipette tips etc. CL1 solid waste will be placed in orange bags in orange bins. The CL1 waste bins will be placed throughout MTI (Milner Therapeutics Institute) laboratory areas. The CL1 orange waste bags will be closed with cable ties when 3/4 full and deposited in a central laboratory area for collection twice a day by building services. This waste will be removed from site by Novus Environmental as 'clinical waste'. CL1 liquid waste management: CL1 liquid waste will be treated with Chemgene before disposal down the drain. CL1 liquid waste will be treated with a final concentration of 10% v/v Chemgene with a minimum contact time of 30 minutes, before being disposed of down the sink/drain.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Project Ref 3472/19.1

Date Ackn’d 10/04/2019
CU2 Project Title Expression of oncogenes and tumour suppressor genes using lentiviral vectors and < 1 Litre
Class 2
Culture Volume Class 2 10/04/2019

Comments of the senior scientific advisor (see section 3):
The attached risk assessment is fit for purpose and accurately describes a common GM process routinely conducted at CL1. The control measures and waste management streams stipulated are adequate to effectively manage the risks to humans and risks to the environment. This risk assessment, along with all other risk assessments issued in the institute, will be reviewed annually and updated/amended as required.
**Purposes of the contained use**

The purpose of this work is to modify mammalian cell lines (human and/or murine) with oncogenes, tumour suppressor genes and reporters in order to evaluate mouse models for paediatric high-grade gliomas.

**Recipient or parental organism**

Cells and cell lines:
- Hela, W12, S12, SiHa, CaSki, C33A derived from human cervix.
- U2OS, Saos-2 derived from human bone.
- FSK, NEK, HaCaT, NIKS, EF-1F derived from human skin.
- COS, CV-1, Vero derived from monkey epithelium.
- 293, 293T, 293TT, phoenix cells derived from human epithelium.
- RPTEC, ARPE19 commercially available primary human renal and retinal epithelial cells.
- U87-MG derived from human glioblastoma.
- J2 3T3, NIH 3T3, TK143 of mouse fibroblast origin.
- Neuro-2a of mouse neuroblastoma origin.
- Mouse cortical neurons.
- Mouse neural progenitors.
Cells will be routinely tested for microbiological and mycoplasma contamination.

**Host/vector system**

Vectors:
- TRIP-PGK-ATGm-MCS-WHV, PGIPZ or any second/third generation lentiviral expression vector.
- pMD.G or any second/third generation packaging system. Viral envelope (VSV-G) expression vector.
- pCMVdeltaR8.91 or any second/third generation packaging system. Gag and Pol expression vector.
- pcDNA3, pCMS-GFP, TOPO or any other commercially available backbone vectors commonly used for mammalian protein expression.

Bacterial strains:
Origin & function

Inserted sequences will be composed of either reporter genes such as GFP or Luciferase, or components of recombination systems that allow expression:
- PBase Piggybac transposase; A mammalian codon optimised version of the Piggybac transposase (Trichoplusia ni)
- Cre recombinase (P1-phage); A Type I topoisomerase that catalyzes the site-specific recombination of DNA between loxP sites.
- LoxP sequence (P1-phage); Target sequence for Cre recombinase which mediates the site-specific recombination of DNA.
- FRT sequence (Saccharomyces cerevisiae); Target sequence for Flp (flippase) catalyzes the site-specific recombination of DNA between FRT sites.
- CreERT2 ; Cre recombinase - estrogen receptor T2 fusion gene. Tamoxifen- inducible Cre recombinase.
- GFP and its derivatives (Aequorea victoria); Green fluorescent proteins.
- Firefly Luciferase (Lampyridae); A secreted reporter luciferase from firefly.
- Blasticidin resistance (Bacillus cereus); Blasticidin-S deaminase.
- Kanamycin resistance (Streptomyces kanamyceticus).
- Puromycin resistance (Streptomyces alboniger); puromycin N-acetyl-transferase.
- LacZ (Escherichia coli); β-galactosidase.

Reporter gene expression will be directed by:
- Human cytomegalovirus promoter (human cytomegalovirus)
- Mouse U6 Promoter (Mus musculus)
- TRE2 Promoter
- Lac promoter (Escherichia coli)

shRNA (small hairpin RNA) are small RNA molecules which form a hairpin structure. The hairpin structure will induce a normal cellular in vivo downstream sequence of processing events in response to foreign cellular single-stranded RNA intruder molecules that will result in the post-transcriptional downregulation of the gene to which the hairpin sequence is targeted.

PiggyBac vectors are non-viral/non-integrating expression vectors. PiggyBac vectors include two components expressed together in a cell; a vector containing a cassette of various genes that will be inserted into the genome at transposon sites, and a vector expressing transposase that will mediate the integration of the expression cassette at transposon sites.

H3.3 is a histone H3 variant that is expressed throughout the cell cycle.
DAXX and ATRX are both histone chaperones specific for H3.3.

Evaluation of foreseeable effects

Although recombinant Retro and Lentiviruses can infect humans, both are defective and unable to be propagated after infection because of the presence of self-inactivating deletions. Cell lines used for lenti/retrovirus packaging require the expression of replication genes from a separate cassette, which eliminates the possibility of autonomous virus production. In the unlikely event of infection, the viral particles will not be able to replicate as they lack the gene encoding the envelope protein, which is required for cell entry. The cell lines used as recipients of foreign genes are not hazardous, as they are unable to infect people or animals. Foreign genes will be expressed in mammalian cells from either the the CMV, TRE-tight, EF1F, CAG or TGK promoters, or from the Retro/Lenti virus promoter contained
within the viral LTR. Retro/Lentivirus production and infection will be carried out under containment level 2 conditions, and we will follow the standard code of practice for virus-handling.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All animal work will be carried out off premises at the Cancer Research UK Cambridge Institute - Biological Resources Unit.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| The Jeffrey Cheah Biomedical Centre building management team will manage two laboratory waste streams. One waste stream for CL1 waste and a second for all CL2 waste. CL2 solid waste management: CL2 solid waste includes items such as general tissue culture consumables used in CL2 (consumables having been in contact with or containing residual GM material) tissue culture plates, tissue culture flasks, tubes etc. Serological pipettes and pipette tips will be collected in Bio-bins initially. Once Bio-bins are full they will be sealed and placed into autoclave bags in clearly demarcated CL2 waste bins. When CL2 autoclave bags are 3/4 full, they will be closed using cable ties to reduce the risk of waste spillage. The 3/4 full cable tied bags will be removed from the laboratory and placed into red CL2 waste bins in a clearly demarcated collection area. When the red CL2 waste bins are full they will be closed with cable ties. The red CL2 waste bins will be collected at least once a day by the building services team who will transport them to the basement of the building to be autoclaved. The building's waste autoclaves will have maintenance schemes written by Bureau Veritas and will be inspected annually by Bureau Veritas and PSSR. Additionally, the buildings waste autoclaves will be serviced regularly and annually validated under a service contract. The autoclave will run at the pre-set guidelines for deactivation of CL2 waste, this will be validated on every run using indicator tape. Autoclaved waste will be transported off-site by Novus Environmental as 'offensive waste'. CL2 liquid waste, that is safe to dispose of down the drain after inactivation, including; used tissue culture media, PBS used to wash cells etc will be treated with Chemgene before disposal down the drain. Inactivation of liquid waste will be achieved by using a final concentration of 10% volume/volume Chemgene with a minimum contact time of 30 minutes, before being disposed of down the sink/drain. Chemical waste for disposal from CL2 tissue culture (that is not suitable for disposal down the drain) will be collected in bottles, treated with a final concentration of 10% volume/volume Chemgene for a minimum of 30 minutes. Bottles will be labelled with their contents and placed in the chemical fume hood for disposal by building services. All work surfaces shall be routinely disinfected by wiping with 5% Chemgene, left for 30 seconds before subsequently wiping with 70% ethanol. |

Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**

02/03/2022
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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**GM Centre Number: 3473**

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**Name**

MEDPHARM LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

UNIT 1 CHANCELLOR COURT

**Road Name**

50 OCCAM ROAD

**Town**

GUILDFORD

**County**

SURREY

**Postcode**

GU2 7AB

**Country**

ENGLAND

**Tel Number**

01483 457580

**Fax Number**

0

**E-mail**

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**HSE Division**

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Yes

Give brief details of the genetic modification safety committee

University of Surrey Biological Safety Officer

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For activities involving GMMs, describe the waste management measures which will apply to the activity

All GMO's will be inactivated by autoclaving at 123 degrees centigrade for at least 15 minutes on a validated cycle, including all solid and liquid contaminated waste.

Post autoclaving; solid waste is disposed of into an offensive waste bag and sent off-site for incineration via the approved waste management company. Liquid waste is disposed of down the sink with copius amounts of water. This flows to the main foul drain and onto a waste water treatment facility owned by Thames Water.

Spills/surfaces in contact with GMO will be disinfected using 1:10 Distel spray (tested and approved as bactericidal, fungicidal and destroy DNA and RNA).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Based on the information provided and discussion around the work, the work described is GM Class 1 because it is working with a non-pathogenic bacteria already modified to express wild type human proteins. It poses no foreseeable hazard to human health and to the environment. The laboratory where the work will be conducted meets Containment Level 1 criteria and is suitable for the activities proposed in the GMRA.
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**Name**

ENARA BIO LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

MAGDALEN CENTRE

**Building**

THE OXFORD SCIENCE PARK

**Road Name**

1 ROBERT ROBINSON AVENUE

**District**

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX4 4GA

**Country**

ENGLAND

**Tel Number**

020 7421 70070

**Fax Number**

0

**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

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<td>OXFORD</td>
<td>OX4</td>
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</tr>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

Bioescalator GMO committee meets once per year, and on an ad hoc basis for new applications and is composed of several PhD-level Bioscientists with experience in Biological safety and genetic modification and risk assessments thereof. Specifically the committee comprises 2 Divisional Safety officers for Oxford University clinical departments. A Professor and company director who is a founding director of the British Society for Gene and Cell Therapies, The Bioescalator Lab manager, and three laboratory directors and project managers with several years of experience in GMO work. Three are staff representatives from companies at the Bioescalator.

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For E.coli work
The containment and control measure that will be applied for work with these genetically modified micro-organisms are shown on Table 1 of the attached GMO risk assessment. Containment level 1 will be applied with Good Microbiological Practice and Good Occupational Health and Safety. Class 1.

For Virus work
The containment and control measure that will be applied for work with the genetically modified lentivirus or retrovirus and cell lines infected with lentivirus or retrovirus are shown on Tables 1 and 2 of the GMO risk assessment attached. Full containment level 2 will be applied with Good Microbiological Practice and Good Occupational Health and Safety. A microbiological safety cabinet and gloves will be used where appropriate. Class 2.

WASTE MANAGEMENT MEASURES
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3,1993 (either 121-1250C for at least 15 minutes or 126-1300C for at least 10 minutes or 134-1380C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the Bioescalator clinical waste route.
Liquids (eg samples, culture supernatants, tissue culture media) - add Virkon to 1 % w/v for at least 1 hour and then discharge to drains or autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes) and discharge to drains. Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the bioescalator clinical waste route.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation).
Incineration, effectively 100% kill (licensed incinerator).
Virkon disinfection, 99.99% kill. Virkon has been tested by independent laboratories and been proven to be effective against a total of 20 virus families (including HIV/AIDS)

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Project Additional Information

Purposes of the contained use

The aim of this project is to use self-inactivating, replication incompetent lentivirus-based expression vectors to generate stable cell lines expressing TCR derived from tumour-specific cytotoxic T cells. For practical purposes (e.g., rapid isolation by FACS sorting of transduced cells) we will mostly use lentivirus-based vectors that express the green fluorescent protein or blue fluorescent protein (GFP, BFP) derived from jellyfish, as part of a bi-cistronic RNA. A development of these studies will concern the use of similar self-inactivating, replication incompetent lentivirus-based vectors for stable inactivation of genes of interest by small interfering (si) RNA in tumour cell lines and cultured primary tumour cells. Lentiviral particles expressing the transfer vector will be used with widely used packaging vectors, such as those described herein. A second approach to inhibition of target transcripts in tumour cells will be to use deactivated CAS9 lentiviral particles in association with lentiviral particles expressing target gene-specific "guideRNAs". The combination of the two vectors allows extremely specific transcriptional inhibition in the targeted cells.

Recipient or parental organism

K12 or B derivatives of E. coli will be used as bacterial cloning hosts to generate plasmid expression clones in readiness for expression within specified packaging cell lines. These are disabled hosts that cannot colonise the human gut and have a history of safe use. These hosts may be considered equivalent to ADCP hazard group 1. The packaging cell lines 293T, Jurkat-76 and Hut-78 are of human origin, which are well characterised and authenticated and is obtained from commercial sources. 293T, Jurkat-76 and Hut-78 can be regarded as low hazard for GM activities and as hosts are suitable for containment level 1 precaution. However, in our laboratory they are discarded as in containment level 2 via the Bioescalator clinical waste route. Human primary lymphoid cells (e.g CD4+ blasts and/or CD8+) can be considered as especially disabled hosts and as such may be considered to be equivalent to ADCP hazard group 1 however the cells have the potential to contain adventitious agents and as such will be handled at containment level 2 under COSHH.

Host/vector system

a) Lentiviral transfer vectors

a-1) cDNAs encoding proteins encoding CD8 TCRs will be cloned into pHRSIN-BX-IRES-Em, a slightly modified version (Appendix 1) of the lentiviral plasmid pHRSIN-CSGW (Demaison, Parsley et al. 2002; Ikeda, Takeuchi et al. 2003; Palmowski, Lopes et al. 2004) (provided by Dr. Mary Collins, University College London). The latter is a tailored version (Demaison, Parsley et al. 2002) of the original pHR'SIN-CE, described in 1997 by Trono and co-workers (Zufferey, Nagy et al. 1997). pHRSIN-CSGW and modified versions are last generation of widely used lentiviral vectors (Demaison, Parsley et al. 2002; Ikeda, Takeuchi et al. 2003; Palmowski, Lopes et al. 2004; Gruh, Schwanke et al. 2005; Clements, Godfrey et al. 2006). pHRSIN-BX-IRES-Em contains the 5' HIV LTR and an inactivated deletion mutant of the 3' HIV LTR which lacks a 400-nucleotide and L TR activity(Zufferey, Dull et al. 1998). This inactivation improves significantly biosafety because it reduces the possibility that replication-competent retroviruses will originate in the vector producer and target cell. Within the 5' and the 3'O/OU3 elements, pHRSIN-BX-IRES-Em contains a packaging sequence (0), a Rev responsive element (RRE), followed by a central purine tract cis-active sequence (cPPT)(Zennou, Petit et al. 2000) that improves transduction, and a central termination sequence (CTS) (Demaison, Parsley et al. 2002). The U3 part of the spleen focus forming virus (SFFV) strain P long terminal repeat (SFFV-U3L TR) follows that drives sustained and strong mRNA transcription in cells of haematopoietic origin of the gene of interest (in pHRSIN-BX-IRES-Em, a bicistronic mRNA containing the gene of interest cloned into a BAH-1 or Xho-1, restriction sites followed by an internal
ribosomal entry site (IRES) and the gene coding for eGFP (called Emerald GFP, a point mutant version of GFP that makes it 5 times brighter). A Woodchuck hepatitis virus Post-translational Regulatory Element (WPRE) (Zufferey, Donello et al. 1999) is inserted downstream of eGFP stop codon and allow enhanced gene expression. The HIV derived part of the vector is terminated by the defective HIV 3' LTR (3'/D U3).

pHR'SIN-BX-IRES-Em derives from pHR'SIN-cPPT-SE (Demaison, Parsley et al. 2002) modified into pHR'SINCSGW by Collins and co-workers (Ikeda, Takeuchi et al. 2003) by inserting an IRES eGFP element under the control of the SFFV promoter and the 3' WPRE sequence. pHR'SIN-BX-IRES-Em contains in addition a BAH-1 or Xho-1 restriction sites. This modification should not have introduced any substantial changes to cause safety concerns.

We intend to stably express by Lentivirus-based vectors short-hairpin (sh) DNA segments encoding a sequence generating an siRNA capable of inactivating mRNA of tumour transcripts of interest. The long-term silencing in a majority of cell lines can be achieved through the use of shRNAs in a lentiviral transduction fashion. For this purpose, we will use the pLKO.puro system.

The transfer vector pLKO.1-puro (see appendix 1 B) is a lentiviral (HIV)-based plasmid. pLKO.1-puro (used in combination with two other vectors (so-called *third generation lentivirus vector with a conditional packaging system (Zufferey, Dull et al. 1998) (see appendix 1 B), contains contains HIV-1 cis-acting sequences and an expression cassette with U6 Pol-III-driven promoter for expression of shDNA. It is the only portion transferred to the target cells and does not contain wild-type copies of the HIV L TR (e.g., elimination of the majority of lentiviral genes, Dvpr, vif, vpu and nef). The 5' L TR is chimeric, with the enhancer/promoter of RSV replacing the U3 region (RSV/5'L TR) to rescue the transcriptional dependence on Tat. The 3' L TR has an almost complete deletion of the U3 region (SIN/3'L TR), which includes the TATA box (from nucleotides -418 to -18 relative to the U3/R border). As the latter is the template used to generate both copies of the L TR in the integrated provirus, transduction of this vector results in transcriptional inactivation of both L TRs; thus, it is a self-inactivating vector (SIN generation). Moreover, pLKO.1-puro contains an RNA packaging sequence (0 ), a Rev responsive element (RRE), followed, after the expression cassette, by a central purine tract cis-active sequence (cPPT)(Zennou, Petit et al. 2000) that improves transduction, and a central termination sequence (CTS) (Demaison, Parsley et al. 2002). The human phosphoglycerate kinase (PGK) promoter drives the puromycin-resistance gene (to be used for stable transfection) and it is followed by SIN/3'L TR. The rest of the sequence is common to other bacterial plasmids (Amp-resistance gene for bacterial selection, f1 origin of replication and a pUC origin of replication). The vector is regarded as safe due to its modified features (deletion of a number of accessory genes implicated in the virulence of HIV, minimal genome of the viral particles, nonreplicating and self-inactivation features), making it incapable of producing virus once infected into the host cell.

a3). pHR-SFFV-dCAS9-BFP-KRAB and plentiguide Puro

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system offers a general approach for RNA-guided regulation of transcription. Fusion of CRISPR-associated catalytically inactive dCas9 protein to distinct effector domains (e.g. p65AD or KRAB enables robust and efficient repression or activation of transcription in human cells, with the site of delivery determined solely by a co-expressed short guide RNA (sgRNA) which is encoded by a second lentiviral vector (Gilbert et al. 2013). Coupling of dCas9 to a transcriptional repressor domain can effectively silence expression of multiple endogenous genes, with no detectable off-targets as verified by RNA-seq analysis.

pHR-SFFV-dCAS9-BFP-KRAB like pHR'SIN-BX-IRES-Em is a third-generation lentiviral vector with self-inactivating 3' HIV-derived L TR (appendix 1 C). It encodes a human codon-optimized dCas9 from S. pyogenes fused to two copies of a nuclear localization sequence (NLS), an HA tag, and blue fluorescent protein (BFP). These are fused to the KRAB (Krlippel-associated box) domain of Kox1, which is a transcriptional repressor. These are cloned into the pHR vector backbone (Addgene).

pLentiguide-puro expresses S. pyogenes CRISPR chimeric RNA element with customizable synthetic guide RNA
(sgRNA) from U6 promoter and puromycin resistance from EF-1a promoter. Like the lentiviral vectors described above it has the safety features of the 3rd generation lentiviral backbone. The sgRNA expression plasmids are cloned by inserting annealed oligos into the lentiviral U6-based expression vector that was digested by BstXI and Xho1 (appendix 1 C). Cells to be targeted are transformed with two separate viruses; the dCAS9 vector and the lentiguide sgRNA vector.

Origin & function

In order to carry out these studies COB T cells which we identify as strong responders to the tumour antigens will be purified to the single cell level by flow cytometry and their T cell receptor (TCR) alpha and beta chain mRNA sequenced. The TCR sequences will then be constructed into cDNA and expressed in human T cell lines using the viral vectors described above. These T cell lines will then be used to characterise the efficiency and affinity of the purified TCRs.

Evaluation of foreseeable effects

i) For E.coli work
No significant hazards have been identified above. Insertion of the foreign sequences into these is not expected to result in harmful physiological or pharmacological properties or to affect the pathogenicity of the cloning host or normal human defence mechanisms. Gene transfer as described above is possible, but unlikely to be hazardous. The resulting GMO's are not expected to carry any additional risks compared to that of the un-modified recipients. Assignment to provisional containment: Containment level 1 with Good Microbiological Practice and Good Occupational Health and Safety.

ii) For viral work
For the transfected 293T cells there could be a significantly greater risk associated with the production of recombinant infectious Lentiviral particles. These therefore pose a risk to human health, but as they are self-inactivating replication deficient vectors, the risk is only from the insertion of the genetic material, described above. Although the two packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, tat, env) in the 293T producer cell line, none of them contain L TRs or the psi packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target T cell lines. No new replication-competent virus can be produced. Finally, none of the genetic inserts are likely to cause significant effect on an individual. Therefore, as they are replication defective and in accordance with ACGM Guidelines, no additional containment and control measures are required. However, it is theoretically possible for these Lentiviruses to integrate into the genome of an individual on exposure. As they are self-inactivating, they are not capable of activating gene transcription from the inactivated viral L TR. However, they could in theory disrupt the function of a gene at the site of insertion. The worst-case scenario could be the chance integration of the virus into a tumour suppressor, thus disrupting that gene's function. Even so this single event is unlikely to cause any tumour formation and so the overall risk is still low. Therefore, taking into account all of the above, it would be appropriate to lower the overall level of containment for these types of vectors, but increase the containment level for the transfected cells and the virus containing supernatants from these cells. Assignment to provisional containment: Containment level 2.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Consumables (mainly plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the Bioescalator clinical waste route.

Liquids (eg samples, culture supernatants, tissue culture media) - add Virkon to 1% w/v for at least 1 hour and then discharge to drains.

Agar plates - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-1250°C for at least 15 minutes or 126-1300°C for at least 10 minutes or 134-1380°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the Bioescalator clinical waste route.

Degree of kill:
Autoclaving, effectively 100% kill (annual validation).
Incineration, effectively 100% kill (licensed incinerator).
Virkon disinfection, 99.99% kill. Virkon has been tested by independent laboratories and been proven to be effective against a total of 20 virus families (including HIV/AIDS).

Please enter comments on the GM safety committee on the risk assessment

1. Change the containment of vectors containing Woodchuck hepatitis virus post translational regulatory element (WPRE) from class1 to class-II in line with the 2014 regulations.
2. Review the Risk Assessment to ensure the waste management provisions are in line with the Bioescalator standard operating procedures.
3. Highlight table one to make clear where the justification for class II containment arises from.

Project Containment

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<th>Growth Rooms</th>
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Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N
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Name

APTAMER GROUP LTD

Name 2

Department

Campus Estate or Research Centre

SUITE 2.78-2.89

Road Name

INNOVATION WAY

District

HELSINGTON

Town

YORK

County

YORKSHIRE

Postcode

YO10 5NY

Country

ENGLAND

Tel Number

01904 567790

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Risk assessment was compiled using information and advice provided by Sporegen Ltd, the supplier of the GMOs.

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<th>Laboratory</th>
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Bacteriology  | Parasitology  | Transgenic Birds | Transgenic Animals | Transgenic Fish | Microbiology Research | Gene Therapy | Yes |

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

All work is performed in a Class II microbiological safety cabinet, with all waste being treated with 1% Virkon (for 24 hours) and UV treated for 1 hour before being disposed of via clinical waste (and incineration).

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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessment was compiled using information and advice provided by Sporegen ltd, the supplier of the GMOs. - Risk assessment will be provided separately due to issue with attachments.

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**Project Ref 3477/19.1**

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Non-GMM Consent Granted

Tick if notifying a connected programme of work

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
### Project Additional Information

#### Purposes of the contained use

The aim of the project is to develop affinity ligands (known as aptamers) that are capable of specifically binding to surface epitopes on *Clostridium Difficile* spore. Spores which have had the surface proteins knocked out (GMO spore) will be used as controls to show the aptamer interaction is specific.

#### Recipient or parental organism

*Clostridium Difficile*

#### Host/vector system

The mutant spores were created using allelic exchange to delete most of the gene via homologous recombination. It is a clean deletion involving no antibiotic markers.

#### Origin & function

**Evaluation of foreseeable effects**

Native *Clostridium Difficile* have heightened risks to the following:

- Individuals on broad-spectrum antibiotics or several different antibiotics (which can include pregnant women).
- 65 years old
- Have underlying conditions such as IBD, cancer or kidney diseases
- Immunocompromised people (from diabetes, chemotherapy or steroid medication)
- Individuals on proton pump inhibitors (or similar)
- Individuals with digestive system related illness.

The vector system poses no risks, the *Clostridium Difficile* spores pose risks to anyone on proton pump inhibitors (or similar) or any stomach related illnesses, therefore by extension the knockout versions posing the same risk. The biological activity of the knockout spores is assumed to be the same as the native *Clostridium Difficile*.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

All work using the *Clostridium Difficile* spores (native or knockouts) is performed in a Class II microbiological safety cabinet, with all waste being treated with 1% Virkon (for 24 hours) and UV treated for 1 hour before being disposed of via clinical waste (and incineration). Cabinet is routinely cleaned with 1% Virkon and 70% IMS after each use.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All work using the *Clostridium Difficile* spores (native or knockouts) is performed in a Class II microbiological safety cabinet, with all waste being treated with 1% Virkon (for 24 hours) and UV treated for 1 hour before being disposed of via clinical waste (and incineration). Cabinet is routinely cleaned with 1% Virkon and 70% IMS after each use.
Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

No comments have been received by the genetic modification safety committee regarding the risk assessment. The Risk assessment was written using guidance from the supplier Sporegen Limited.

Project Containment

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### Name

**EQ LTD**

### Campus Estate or Research Centre

### Road Name

15 LAWRENCE HILL

### Town

NEWPORT

### County

GWENT

### Postcode

NP19 8AY

### Country

WALES

### Tel Number

07773 709045

### Fax Number

0

### Comments

### Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

An Intellectual Property and technology development professional at the University of Bradford; who has an undergraduate degree in Applied Biology with Biotechnology, and higher research degree in Biochemistry and Molecular Biology. The person has previously worked in drug development with Covance Laboratories, where they were the Biotechnology Gene Modification Risk Assessment Officer and member of the wider Gene Modification Safety Council within that organisation. Following this and prior to joining Bradford University, they have worked extensively developing early-stage biotechnologies and setting up the systems and procedures required for the delivery of these in a variety of positions, including acting as Chief Operating Officer of a biotechnology PLC.

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
All reagents, consumable supplies, or materials that contain, or have come in contact with, the micro-organism C003 will be sterilized via steam autoclaving where possible or bleach addition prior to disposal in the regular waste stream. Steam autoclaving of waste is performed at 121°C for 60 min under 0.24 MPa, and results in no growth of cultures on agar plates after 48 h at 37°C. Moreover, sterilization efficacy of the autoclave is confirmed weekly using sterility indicator spore paper strips (Sigma-Aldrich, Cat No. 74041-25TESTS). Alternatively, bleach is added to the waste [to 10% (v/v)] and left to stand for 1 hour to ensure complete sterilization. No effluents are expected to be produced that would contain the viable C003 bacteria.

Use of a GM variant (C003) of the well known and highly attenuated E.coli strain DH5-alpha

For activities involving GMMs, describe the waste management measures which will apply to the activity

It was suggest that the CU risk assessment include a secondary protocol of and appropriate % bleach solution for disinfecting a contaminated area in the event that the surface cannot be excavated and extracted, and suggested a 10% solution should be adequate for the specific organism. These suggestions were addressed and the CU risk assessment was amended appropriately.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

None

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For activities involving GMMs, describe the waste management measures which will apply to the activity:

Waste management:
Solid waste will be autoclaved at 121°C for minimum of 30 min.
Liquid waste will go through an acid (pH 1-3) excursion before a caustic (pH 10-12) excursion for a min of 30 min each time before being neutralised and autoclaved. This is to inactivate the antibiotic present but also aids in killing the bacteria.
Autoclave will be tested to ensure a kill of a minimum of 10^116 organisms ml at scale (2 ltr volume) as part of process validation.

Please enter comments of the GM safety committee on the risk assessment:

Personson involved in risk assessment are all protak employees. Karyn Godden (HSE and HR Person) Kate Marshal (Research ASSOCiate) and Paul Shortland (BSO and Technical Production Manager) and Phill Godden (CEO)
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**Name**

LIGHTCAST DISCOVERY LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

BROERS BUILDING

**Road Name**

21 J J THOMSON AVENUE

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB3 0FA

**Country**

ENGLAND

**Tel Number**

01223 358652

**Fax Number**

0

**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The genetic modification safety committee was formed as part of the process of establishing work with GMMs within Base4 Innovation Ltd.
The remit of the committee is to carry out risk assessments of all new processes/projects which utilise GMMs. A minimum of two committee meetings per year are to be held, or more frequently if required.
The genetic modification safety committee consists of:
- Base4 CEO
- Base4 H&S officer
- Base4 Biological Safety Officer
- Project lead(s) of the project(s) which use GMMs
- Employee(s) attached to the project(s) which use GMMs

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

N/A - notification is for class 2

---

**Project Ref 3483/19.1**

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<td>The generation and use of lentiviral particles for mediating gene modulation in mammalian cells and the subsequent use of these cells in microdroplets</td>
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Withdrawn N  

Tick if notifying a connected programme of work N  

Historical Significant Changes

02/03/2022  Page 14643 of 15326
Project Additional Information

Purposes of the contained use

To enable the generation and use of recombinant self-inactivating third generation lentiviral particles (by commercial research organisations or in house using commercially available 3rd generation lentivirus systems) encoding:
1. Open Reading Frames (ORFs), cDNAs, peptides
2. Specific gene sequences
3. Short hairpin RNAs (shRNAs) for the knockdown of Open Reading Frames (ORFs), cDNAs or specific gene sequences by RNA Interference (RNAi)

for in vitro use and for the generation of stable mammalian cell lines (and subsequent use of such stable lines in vitro) with the aim of:
1. Determining response to phenotyping reporters
2. Determining in-vitro immunomodulation responses
3. Efficiently generating cell models lines
4. Validating data from other experiments
5. Identifying and / or validating ORFs / cDNAs / or specific gene sequences as potential drug targets.

Recipient or parental organism

Recipient experimental systems are cells derived from mammalian organisms, predominantly in the form of immortalized cell lines derived from human cancer tissues.
The mammalian cell lines fall into two types:
a) A packaging/helper cell line into which plasmids containing lentiviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.
b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines i.e. a negligible risk.

Host/vector system

The lentiviral vectors which will be used are derived from HIV-1, which is an ACDP Hazard Group 3 biological agent.
However, “Third Generation” lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase
biosafety. These include the vif, vpr, vpu and nef accessory genes which are not required for in vitro replication. The tat gene is also deleted and the Tat-responsive promoter present in the 5’ LTR is replaced with heterologous promoters, for example with the Rous sarcoma virus U3 region. An additional biosafety feature is achieved by deletion of the rev gene from the viral transfer vector. Viral packaging is achieved by providing three helper constructs in trans containing gag, pol and rev sequences. An additional biosafety feature is that these vectors are self-inactivating (SIN), whereby the U3 region of the 3’ LTR (which contains the major viral promoters and enhancers) is copied to the 5’ end of the provirus during reverse transcription. Deletion of enhancer and promoter elements from the 3’ U3 region in the vector construct will result in a provirus that is entirely devoid of U3 enhancer sequences, therefore reducing the potential for transactivation of cellular genes as a result of insertion. Furthermore, such vectors are not easily mobilisable as a result of a superinfection with wildtype virus.

Third generation Lentiviral Expression Systems include the following key safety features:
- The Lenti expression vectors contain a deletion in the 3’ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in “self-inactivation” of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
- The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998). Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication competent virus can be produced.

Despite the above safety features, use of these lentiviral vectors (which include WPRE) falls within SACGM 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. Also, the virus will be packaged by transfecting transfer vector into specific amphoteric ‘helper’ cell line (gag, pol and env stably incorporated into host chromosome.
cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range of mammalian species (including humans) and cell types. This means that the viruses produced for this experiment could potentially infect a number of species, including man.

Origin & function

Selectable markers – examples (but not restricted to);
- Ampicillin resistance: E. coli derived
- Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
- Puromycin resistance (PAC): Puromycin acetyl transferase is derived from Streptomyces alboniger
- Reporter proteins such as (but not restricted to);
  - Fluorescent proteins as reporters;
  - GFP derived from the jellyfish Aequorea victoria and variants of this
  - Luciferase – class of oxidative enzymes used in bioluminescence
    renilla luciferase derived from the Sea pansy (Renilla reniformis)
    firefly luciferase derived from the firefly Photinus pyralis.

Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and/or shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins) – all human derived.

This could include the expression of potentially harmful genes e.g. encoding known proto-oncogenes or genes with known oncogenic mutations which can contribute to cellular transformation. Full length cDNA encoding wild type and disease relevant mutants of these types of genes will be expressed in third generation SIN lentiviral vectors. Any use of lentiviral particles encoding oncogenic inserts will require appropriate controls and operator training.

Evaluation of foreseeable effects

The lentiviruses are, at worst, amphotropic or pseudo typed with VSV G protein, either of which confers a broad host tropism including human cells. However, infectivity is unstable and infection is only obtained by co-cultivation of the packaged viruses with the recipient cells. The retrovirus is self-inactivating and there is thus no possibility of further transfer. Since some of the inserted DNA codes for potentially hazardous RNA or protein, the work is assessed as Class 2. This accords with HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.18-20).

Even for the non oncogenic inserted DNA, there is a slight but non negligible risk due to the presence in the lentiviral vector of the Woodchuck Posttranscriptional Regulatory Element. The WPRE containing vector DNA will be treated as potentially oncogenic and is assigned to class 2 (see HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.13)). However, bearing this in mind we should consider that the viruses that will be
used have been modified in a number of ways which will make them safer to handle:
- The genes encoding structural and other components of the viral genome have been separated. These genes have been engineered to minimise the risk of recombination that could lead to production of a replication-competent virus.
- The packaging cell lines allow expression of proteins, required to produce progeny virus: But the transfer vector is the only genetic material transferred to the target cells, consequently these cells cannot produce the proteins which are essential for viral assembly and infectivity.
Third generation or Self INactivating vectors retrovirus vectors will be used in all experiments
Procedures and controls measures will therefore follow HSE SACGM Compendium of Guidance for retroviruses (Part 2, para.30-36) i.e. using multiple plasmids with minimum sequence homology (e.g. 3rd generation SIN lentivirus vector system), gloves should be worn, use of class II safety cabinets, sharps avoided and all wastes be rendered harmless before disposal etc.
It is not thought that the modified virus would pose a serious risk to animals or plants in the environment. Although the VSV coat protein permits invasion of other mammalian cells, as in the case of humans, infection would be restricted to primary cells and productive virus would not be produced. In addition the control measures to protect human health will minimise release of virus to the environment. Therefore the environmental risk is low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure described below. The reason for adopting this method of waste disposal is that we do not have access to an autoclave of sufficient size within the premises.
All waste material will be inactivated by treatment with 2% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Grundun – their GM authorization is GM782/01.1).
If at any time inactivation of waste from GM work at class 2 using Virkon is not possible, the off-site incineration company is permitted to dispose of noninactivated GM waste.
Waste from our GM work at Class2 will be placed into suitably labelled hermetically sealable 30/60L UN 3291 approved bins. Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made once every four weeks or more frequently if required to prevent build-up. The site has 24/7 security.
The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Second containment is provided by 710L UN 3291 approved bins in which the 30/60L bins are placed during transport. In case of loss of the contractor's facilities, 4 weeks worth of waste can be stored on site. This is sufficient time to organise disposal through alternative contractor (Novus). The type of container means that the risk of any spillage of GM waste on site is negligible. However, as waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this.

This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material will be inactivated by treatment with 2% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable bins. Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.

This disposal method is expected to achieve 100% inactivation of the GMM.

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form  

Y

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment

The scope and particular aspects of safety risks described in the risk assessment were agreed upon by the GM safety committee, and consensus was achieved regarding the adequacy of the SOPs and risk management protocols. Pending notification and acknowledgement by relevant authorities, the risk assessment and related work activities were deemed suitable for these premises.

Project Containment

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**Name**

MOGRIFY LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

25 CAMBRIDGE SCIENCE PARK

**Road Name**

MILTON ROAD

**Building**

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 0FW

**Country**

ENGLAND

**Tel Number**

01223 734154

**Fax Number**

0

**E-mail**

**HSE Division**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The Safety Committee meets at least yearly and is formed by a Safety Committee Chairman and three Biological Safety Officers (BSOs). The Safety Committee Chairman has extensive experience working with GMMs/GMOs during undergraduate, PhD, Post doc and industrial experience, as well as setting up new GMM/GMO procedures, including risk assessments. The BSOs have ample experience (more than 20 years altogether) working with CAT1/CAT2 GMMs/GMOs and as BSO at an academic and industrial institutions developing and reviewing CAT1/CAT2 biological and GMM/GMO risk assessments.

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<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential: 

Bacteriology: Yes, Parasitology: Yes, Transgenic Birds: Yes, Microbiology Research: Yes

Page 14651 of 15326
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<td>Discovery Stage research &amp; development experiments in mammalian cells</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

GMO/GMM contaminated liquids must be placed into a container with sufficient Rely+On Virkon added (final concentration 1%, EPA Registration No. 39967-137; independent tests have shown Rely+On Virkon to have a broad spectrum of activity against viruses, some fungi and bacteria, including HIV and E. coli at dilution 1/100) to disinfect the waste. Leave for at least 12 hours before disposing using the drains with plenty of water.

GMO/GMM contaminated solid material or potentially contaminated material should either be bagged into autoclave bags (autoclave bags must be sealed at the top leaving sufficient air space for steam to penetrate, and autoclaved for 20 minutes at 121°C. Cycle records and autoclave tape will be checked to confirm sterilization) or disinfected with 1% Rely+On Virkon for at least 12 hours. Alternatively, containment level 1 contaminated material could be bagged into yellow sacks. The inactivated waste (or yellow sacks) will be removed from site by a professional specialised waste management company (e.g. Grundon) who performs high temperature incineration and disposal of the waste.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment
This work consists of growing bacteria for cloning purposes and the growth of mammalian cell lines for maintenance and transfection. Therefore this work is considered to be low hazard (class 1) to the environment and human health due to the following:

1. E. coli K12 derivatives are disabled, non-mobilisable and are not pathogenic to humans. They are unlikely to survive outside laboratory culture. They are assigned as ACDP hazard group 1. Mammalian sequences cloned into the bacteria are under the control of eukaryote promoters and thus will not be expressed through the bacteria culture and no potentially harmful proteins are produced. Inserts coding for RNAs and proteins that are physiologically expressed in mammals are to be found naturally in the environment from deceased bodies. Thus inserts are not expected to overcome disablement of the host organism, nor affect host specificity, tissue tropism or susceptibility to host defence mechanisms.

2. The inserts are not expected to alter the properties of the recipient. The environmental fitness will remain severely limited, thus it will be unable to survive. Resulting organisms are not harmful to the environment and the inserted sequences are non-mobilisable and offer no selective advantage to other organisms.

3. Mammalian cells will be transfected with plasmids (e.g. pUC-based plasmids, transposons, etc). Resulting transfected (non-viral) cell lines are not expected to gain selective advantage over other organisms. Additionally the inserts don't overcome disablement of the host organism, nor affect host specificity, pathogenicity, carcinogenicity, toxicity, tissue tropism, host range or susceptibility to host defence mechanisms.

4. Antibiotic resistance genes, for example Kan/AmpR, are not medically critical. Reporter genes including GFP, dsRED and Luciferase are thought to have no deleterious biological effect.

5. Cells containing oncogenes may be oncogenic but are incapable of colonising and causing disease. These cells could represent a genuine risk in the occurrence of accidental injection to an immuno deficient individual or to the corresponding primary cell donor but there will be no such opportunity for involved staff.

6. The transfected cells are not able to survive outside of the specific incubator environment (5% CO2, 37°C).

7. The risk to human health is minimised as no sharps are used, in addition all samples will be handled in a class 2 tissue culture hood to maintain experiment purity, thus further minimising risk to human health via aerosols.

8. All waste will be handled according to Mogrify Waste Management measures to minimise risk to the environment.

All the work mentioned above is to be carried out in containment level 1 laboratories. Future work at Mogrify Ltd. will require CAT2 premises and the CU2 application is being prepared. Therefore, the committee advises on applying for both level 1 and level 2 (GMMs) premises in the CU1 form.
**Project Additional Information**

**Purposes of the contained use**

Transcription factor mediated reprogramming of somatic cells to a pluripotent state showing key features of embryonic stem cells (e.g. induced pluripotent stem cells, iPSCs) is now well stablished. Based on this premise/technology, the overall goal of the research is to transdifferentiate cells from their origin cell type (e.g. hepatocyte, fibroblast, iPSC) to a desired cell type that can be then used in cell therapy or other applications. This will be achieved by a screening process using lentivirus, adenovirus (adenovirus and recombinant adeno-associated virus) and Sendai virus systems to discover which genes need to be overexpressed, downregulated or knocked out to allow such transdifferentiation. Transdifferentiated cells will be fully characterized to prove efficient transdifferentiation to the desired cell type (e.g. western blot, qPCR and flow cytometry of lineage specific markers; cell morphology and differentiation potential).

**Recipient or parental organism**

- GM E. coli K-12 derived: no insert expression due to eukaryotic promoters and transformed plasmid is non-mobilisable. Risk: effectively zero
- GM Mammalian cells in culture:
  - Packaging/helper cell line such as HEK/293, into which lentiviral/adenoviral transfer, packaging and envelope vectors will be introduced and from which infectious viruses will be secreted into the medium at high titre. This media possesses a risk because of the high viral titre. Risk: medium
  - Recipient cells such as cell lines, iPSC or primary cells will be used, depending on the project since transdifferentiation efficiency may be altered. Cells will be initially exposed to a high titre of virus to permit infection. Insert will be expressed, but vector is non replicative and mobilization is very unlikely and restricted. GM cells may be transformed/oncogenic but are still relatively fastidious and would be incapable of colonising and causing disease. These cells could represent a genuine risk to corresponding donor but such exposure would be unlikely. Risk: medium/low

**Host/vector system**

- Viral vectors (lentiviruses, adenoviruses and Sendai viruses): replication deficient vectors, amphotropes, viral infectious genes deleted, carry insert expression cassette, possible long term expression/insertional mutagenesis, transforming/oncogenic potential. Mobilization very unlikely (needs concomitant infection by similar wild type virus). Risk: medium

**Origin & function**

Several types of genetic material may be involved. Examples are listed below. Other inserts maybe used as required, which are similar to those discussed below.

1. Mammalian cDNA (+/- TAG sequences) coding for transcription factors, signal transducers, growth factors and surface receptors.
2. shRNA and miRNA sequences against cell endogenous coding sequences.
3. Reporter genes (e.g. GFP, LacZ, Luciferase, possibly fused to cDNA (type1 inserts)).
4. Selection genes (e.g. Ampicillin, NPT, PAC).
5. Functional non coding sequences (e.g. promoter, enhancer, insulators, IRES, WPRE, of mammalian or viral origin).
6. Neutral non coding sequences (such as introns, isolated exons, guides for CRISPR/Cas9 and similar genomic engineering techniques and tagging sequences).
7. Coding sequences of bacterial CAS9 and similar enzymes or alternatives (i.e. TALENs) with short sgRNA sequences will be used to express CRISPR systems. These systems are used for CRISPR gene editing techniques to knockout or knockdown gene sequences, to introduce specific sequences into the genome or to introduce point mutations. These modifications are expected to alter the development, differentiation, pluripotency, cell fate decision of the recipient host.

**Evaluation of foreseeable effects**

Work will be carried out in Class 2 containment laboratories in class II biological safety cabinets with routine disinfection of work surfaces. All staff working at Class 2 premises will be suitably trained to work in Class 2 containment laboratories, both in the experimental procedures undertaken for the study and in the case of accidental spillage or contamination. In addition, no glass or other sharps will be used, workers will be protected by PPE (a lab coat, gloves and eye protection) and skin lesions will be covered with a bandage in addition to the protective wear described above. Additionally, workers, contractors and visitors will be made aware of the risk assessments and
Control measures being applied in Mogrify Ltd. Consequently, risk of human infection with virus particles is very unlikely. In addition, no single plasmid contains all the genes necessary to produce packaged virus (lentiviruses, adenoviruses and Sendai viruses). Therefore, resultant particles are replication-incompetent and should the unlikely scenario of end-user infection occur, further virus production could not take place in infected cells.

Vectors/inserts are not expected to overcome disablement of the host organism, nor affect host specificity, toxicity, tissue tropism, host range or susceptibility to host defence mechanisms. Cells containing oncogenes may be oncogenic but are incapable of colonising and causing disease. These cells could represent a genuine risk in the occurrence of accidental injection to an immuno deficient individual or to the corresponding primary cell donor but this is most unlikely and additionally no glass or other sharps will be used, workers will be protected by PPE (a lab coat, gloves and eye protection) and skin lesions will be covered with a bandage in addition to the protective wear described above.

Control measures employed will minimise risks to the environment. These control measures include rendering all solid and liquid waste inactive within the laboratory by disinfection with Rely+On Virkon (final concentration 1%, EPA Registration No. 39967-137, independently proven to be effective against a wide range of viruses, bacteria and fungi including HIV and Escherichia coli at dilution 1/100). This waste will be disposed by a professional specialized waste management company who performs high temperature incineration. More detailed information on waste management procedures can be found on section 12.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All GM waste will be inactivated at the same site as the activity is taking place.

GMO/GMM contaminated liquids must be placed into a container and sufficient Rely+On Virkon added (final concentration 1%, EPA Registration No. 39967-137; independent tests have shown Rely+On Virkon to have a broad spectrum of activity against viruses, some fungi and bacteria, including HIV and E. coli at dilution 1/100) to disinfect the waste. Leave for at least 12 hours before disposing using the drains with plenty of water.

Pipette tips are to be disinfected with 1% Rely+On Virkon for at least 12 hours (EPA Registration No. 39967-137; independent tests have shown Virkon to be active at 1:100 for HIV and 1:1400 with Maedi and visna virus). After disinfection, material will be collected in burn bins (material used for viral work will be kept in closed containers). This inactivated solid waste will be then removed from site by a professional specialised waste management company (e.g. Grundon) who performs high temperature incineration and disposal of the waste.

Other solid waste such serological pipettes and culture plates will be disinfected with 1% Virkon and bagged into autoclave bags (autoclave bags must be sealed at the top leaving sufficient air space for steam to penetrate, and autoclaved for 20 minutes at 121°C. Cycle records and autoclave tape will be checked to confirm sterilization). This inactivated solid waste will be then bagged into offensive sacks to be removed from site by a professional specialised waste management company (e.g. Grundon) who performs high temperature incineration and disposal of the waste.

**Is an emergency plan required according to regulation 20?**  

N  

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

02/03/2022
The Genetic Modification Safety Committee (GMSC), formed by chair of the Committee, BSOs, confirmed the Risk Assessment as Containment Level 2. The risk to the human health and the environment was considered to be medium/low when applying the principles of Good Microbiological Practice and Good Occupational Safety and Hygiene. The project was classified as Class 2.

### Project Containment

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GM Centre Number: 3485

Data Premises Notified (Originally) 01/07/2019

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

CYTERA CELLWORKS

Name 2

Department

Campus Estate or Research Centre

TRANSLATION & INNOVATION HUB

Building

Road Name

80 WOOD LANE

District

Town

LONDON

County

GREATER LONDON

Postcode

W12 0BZ

Country

ENGLAND

Tel Number 07902924224

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

| Liquid waste to be bleached for at least 24h before disposal according to local regulations. |
| Solid waste to be autoclaved prior disposal according to local regulations for biohazardous waste. |
| Both types of waste will be cultured to verify complete killing and assess bioburden. |

### Premises Conditions

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Other (please specify)  
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Liquid waste to be bleached for at least 24h before disposal according to local regulations.
Solid waste to be autoclaved prior disposal according to local regulations for biohazardous waste.
Both types of waste will be cultured to verify complete killing and assess bioburden.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

No active genetic modification will be carried out on the premises. Cells will be purchased from Public Health England or equivalent vendor with the genetic modifications.
**GM Centre Number: 3487**

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**Name**

THE ANTIBODY COMPANY

**Name 2**

**Department**

**Campus Estate or Research Centre**

BIOCITY SCOTLAND

**Road Name**

BO’NESS ROAD

**Town**

MOTHERWELL

**County**

**Postcode**

ML1 5UH

**Country**

SCOTLAND

**Tel Number**

01698 534805

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

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**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

See minutes to convene a BSO attached

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**Level 1 (GMMs)**

**Level 2 (GMMs)**

Yes

**Level 3 (GMMs)**

**Level 4 (GMMs)**

**Non-microbial**

**Other (please specify)**

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
Yes

See relevant Risk assessments attached

Recombinant protein /antibody expression

For activities involving GMMs, describe the waste management measures which will apply to the activity

See relevant Risk assessments attached

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

See minutes from meeting to convene a BSO attached

Project Ref 3487/19.1

Date Ackn'd 25/07/2019

Date Project Ceased 20/12/2019

CU2 Project Title CHO Cell transfection for recombinant antibody/protein production

Class Class 2

Culture Vol Class 2 1-50 Litres

Non-GMM Consent Granted

Project notified under transitional arrangements

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information
**Purposes of the contained use**

Recombinant antibody/protein production

**Recipient or parental organism**

CHO cells to produce purified monoclonal antibody. Purified client antibodies used for research purposes (non-therapeutic)

**Host/vector system**

CHO K1 host/ pcDNA3.4-TOPO

**Origin & function**

Murine Hybridoma/ Plasmid to be transfected in to CHO cells to produce antibody

**Evaluation of foreseeable effects**

N/A

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

See associated Risk Assessments

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

See Minutes to convene a BSO attached

**Project Containment**

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<th>Building</th>
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<th>Post-code</th>
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<tr>
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<td>CELL &amp; GENE THERAPY CATAPULT</td>
<td>MANUFACTURING CENTRE</td>
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The TRUCS Therapeutics Ltd GMSC will consist of the following four committee members:
Company Chief Scientific Officer responsible for Research and Development at TRuCs Therapeutics who has a degree and PhD in biological sciences followed by over 20 years of experience leading R&D activities.
Company Vice President of Process Development at TRuCs Therapeutics who has a degree in chemistry and PhD in biological sciences followed by over 20 years of experience leading GMO R&D activities.
Company Operations Manager UK responsible for operations at TRuCs Therapeutics UK who has a degree in Physiology, a Masters and Ph.D in Biomedical Science followed by over 15 years of experience working in operational roles supporting R&D activities within biotechnology companies and over 6 years with GMO production. The Operations Manager UK is also the nominated BSO and has received training in biosafety and Health and Safety.
Company Vice President of Manufacturing responsible for the implementation and running of manufacturing at TRuCs Therapeutics UK has over 25 years of manufacturing and quality experience working in regulated biotech, pharmaceutical and healthcare sectors.

The Safety Committee meets at least yearly and is formed by the Biological Safety Officer (BSO) who acts as Committee Chair, VP of Process development, head of viral vector process development, director of QA and virology expert. Others may be invited as necessary. The Safety Committee Chairman has experience working with GMMs/GMOs during undergraduate, PhD, and industrial experience, as well as setting up new GMM/GMO procedures, including risk assessments. The members of the committee collectively have over 20 years of experience working with Class 1/Class 2 GMMs/GMOs within academic and industrial institutions developing and reviewing biological risk assessments for handling Hazard Group 1/Hazard Group 2 microorganisms and Class 1/Class 2 GMM/GMOs.

<table>
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<tr>
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</table>
The facility is designed to manufacture Advanced Therapy Medicinal Products under licence from the Medicines and Healthcare products Regulatory Authority (MHRA). The control measures listed below reflect the requirements of both Health and Safety legislation and the MHRA. CGTMC have designed and developed a range of control measures for the safe handling and inactivation of GM waste. TRUCS Therapeutics Ltd staff will be instructed and trained in these procedures and CGTMC staff will monitor and audit the collaborators to ensure compliance. Any near-miss or incidents will be reported to and assessed by the TRUCS Therapeutics BSO who will assign corrective and preventative actions.

Low Volume Liquid Waste (≤10L) and Solid Waste are inactivated by incineration using the following procedure:

Within the manufacturing module the operatives will contain low volume liquid waste (≤10L) within a sealed container in a leak free manner (via a screw cap or tubing seal or tie). The sealed liquid waste container and any solid waste will be placed in a secondary container (double biohazard bag or a biohazard bin) and then into a tertiary container (hard clinical waste bin) which will be sealed and labelled. They will then move the clinical waste bin through to the waste out section of the manufacturing module where the surface of the bin is cleaned using a spray and wipe technique with a broad spectrum biocide such as Virkon, Distel, IMS 70% and Klericide B. CGTMC technical support operatives will record the label details and transfer the clinical waste bins to the secure waste storage area located on the ground floor of the building where they will be stored until collected by CGTMCs approved waste disposal contractor (approved to handle GM waste). On the day of collection the CGTMC technical support team will transfer the clinical waste bins to the transport vehicle for transfer to the incineration facility and update the waste log.

Thames water trade effluent consent T.E. Case No: TRMD0BK2
The committee had no major concerns regarding the risk assessment. It was decided that the risk assessment should contain greater detail of our intended GM and so a greater depth of information on the modification has been added. The committee agreed there was minimal risk to human health or the environment and recommended that the material is classified as GM class 1, and our application will be made at GMO class 1.
<table>
<thead>
<tr>
<th>Name</th>
<th>SCANCELL LTD</th>
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<td>Date at Which Additional Info Submitted</td>
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| Data Premises Notified (Originally) | 15/08/2019 |
| Data Premises Closed              |            |
| Emergency Plan Required?          |            |
| Emergency Premises                |            |
| Transferred from 1992 Regs?       | N          |
| Transitional Premises Class       |            |
| Non-GMMs                         | N          |
| Withdrawn                         | N          |

Comments
### Premises Addresses

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<td>OX4 4GD</td>
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The GM committee comprises of the company's Head of Research, two senior scientists, senior project manager, an external consultant and a Biological safety officer.  
The committee meets once a year to discuss safety measures in place in the laboratory and approve Genetic Modification Risk Assessments.  
When scientists plan to use a new genetically modified organism, the committee meets ad hoc when required to review new projects, otherwise they meet once a year.

<table>
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<tr>
<td>Non-microbial</td>
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</table>
All waste from E.coli class 1 activity (e.g., used agar plates) will be sealed and placed into sealed double bagged biohazard bags and placed into yellow bags sealed for incineration, then disposed of with clinical waste for incineration through an appointed specialist waste management company: Stericycle. All solutions containing E.coli or GM E.coli will be disinfected by treatment with including but not limited to Virkon, ChemGene or Trigene according to manufacturer's specifications. All glassware will be disinfected prior to washing and sterilisation in designated autoclave.

Please enter comments of the GM safety committee on the risk assessment

The risk assessment was reviewed by the GMO committee on 04/07/2019. It considered that the risk assessment contained sufficient risk management to justify classification of the project as class 1. It was therefore approved.

Project Ref 3491/19.1

Date Ackn'd 16/08/2019  
CU2 Project Title Use of lentivirus and lentiviral vectors for expression of novel molecules in both E.coli  
Class CultureVolClass2 CultureVolumeClass3-4

Class 2
To use Lentivirus transduction technology to generate cells or cell lines that can be used to develop tools to screen novel TCRs.

Disabled/non colonising E.coli K-12 derivatives including Stbl3 strain.
Fully characterised, widely used, commercially available cell lines.
Primary cells isolated from whole blood.

Commercially acquired 3rd generation Lenti-ArtTM virus packaging system in conjunction with a Lentiviral transfer plasmid (Creative Biolabs) containing the receptors insert to generate infectious replication incompetent lentivirus particles. The vectors are listed below:
- Packaging plasmid pHelp1, which encodes for gag and pol (HIV-1 structural viral proteins and enzymes) under control of a CMV promoter.
- pHelp2, which encodes for VSV-G (Vesicular Stomatitis Virus envelope protein VSV-G) in replacement of the HIV-1 envelope. This further reduces the chances of recombination with viral genome and is also driven also by the CMV promoter.
- pHelp3, which encodes for Rev (HIV-1 regulatory gene), under control of the RSV promoter. The expression of the gag-pol sequence (present in plasmid pHelp1) is Rev dependent, thus preventing the expression of gag-pol in the absence of Rev.

- Human Leukocyte antigens (HLAs), class I and class II. HLA is expressed by the majority of human cells and confers no harmful properties.
- Native TCR molecules.
- Variant TCR molecules including higher affinity versions of Native TCRs containing point mutations to increase the
affinity of binding to HLA peptide complexes on surface of target cells.

- Marker and Reporter genes such as GFP, RFP and luciferase (these have been used in various transgenic animal systems and have been shown not to cause any harmful effects), antibiotic resistance genes (such as Puromycin, Zeocin) and cell surface markers (such as Rat CD2) for monitoring and selection of transduced cells.

- Genome editing molecules such as CRISPR Cas 9 proteins and gRNA to target and knockout specific genes. In this Lenti transfer vector the transgene would comprise of a human U6 promoter to drive expression of a gRNA alongside sequence encoding a Cas9 protein or derivatives of (spCas9) driven by a mammalian promoter such as EFS or EF1-a.

**Evaluation of foreseeable effects**

### Lentivirus:
All the lentivirus plasmids used are non-mobilizable. The lentiviral vectors are well characterised, with a history of safe use, and inherently safe. These vectors will all be self-inactivating and replication-incompetent, that is, each lentiviral particle infects and gives genomic modification of one cell, and that cell will not (and indeed cannot) subsequently produce and release further viral particles. This is due to a deletion in the 3’ L TR (TAT A deletion) within the transfer vector, which prevents excision after genomic integration. Lentiviruses are highly susceptible to dehydration. However, they can survive for long periods in high protein media. Lentiviruses are rapidly inactivated outside the host, as illustrated by the fact that close contact is required for transmission. The lentivirus used for this study cannot replicate and so the consequences of escape are considered negligible.

### TCR inserts
The TCR gene inserts encode for proteins known to be functionally expressed by T cells only, as they require the presence of cellular CD3 proteins only found in T cells. Expression of TCRs by cells other than T cells renders the TCR non-functional. In the case of cancer specific TCR genes, their expression in T cells should only induce a response to cells expressing tumour antigens. TCR genes specific for self-antigens or auto antigens do present a hazard to the worker performing the work but only if the workers own T cells were transduced to express the genes, for this reason the use of self-cells is strictly prohibited.

### Mammalian cells:
Primary T cells are isolated from blood following standard gradient centrifugation protocol. These cells can only survive under specialised laboratory culture conditions. Any T cell transduced using the Lentivectors vectors could present a potential hazard to the original T cell donor for this reason no laboratory staff are allowed to work on their own cells. These donor cells will be HLA mismatched and therefore present no hazard to the individual user. Inclusion of any TCR to these donor cells will not change this and therefore the cells can be considered specially disabled.

Human Leukocyte Antigens (HLA)

HLA is expressed by the majority of human cells and confers no harmful properties. The HLA transduced human cells produced will have a HLA tissue type different from the laboratory staff and hence will be detected and efficiently eliminated by the immune systems of any inadvertently contaminated worker.

### CRISPR machinery:
There is a slight risk if all the components of are injected into the operator, especially in conjunction with a lentiviral vector delivery system. To minimise risk, avoid route of direct infection, e.g. needle sticks and/or spills onto open wounds. Take caution when dealing with CRISPR-Cas9 machinery, especially if lentiviral vector delivery system is used. Resulting cell lines should not pose an increased risk to the operator over their parental cell lines.
We do not have any work planned with larger GMOs.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

GM CL2 liquid waste is inactivated in freshly prepared 1 % Virkon (final concentration) or Chemgene for at least one hour contact time before being poured down the foul drain.
GM CL2 solid waste (eg. plastic tissue culture flasks or plates) will be treated with 1 % Virkon or Chemgene, then double-bagged in strong autoclave bags, placed in a leak-proof container with a lid on, then disposed with clinical waste for incineration through an appointed specialist waste management company: Stericycle.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

GM CL2 liquid waste is inactivated in freshly prepared 1 % Virkon (final concentration) or Chemgene for at least one hour contact time before being poured down the foul drain.
GM CL2 solid waste (eg. plastic tissue culture flasks or plates) will be treated with 1% Virkon or Chemgene, then double-bagged in strong autoclave bags, placed in a leak-proof container with a lid on, then disposed with clinical waste for incineration through an appointed specialist waste management company: Stericycle.

13. • Is an emergency plan required according to regulation 211
DYes IZI

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  N

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The risk assessment and project was reviewed by the GMO committee on 04/07/2019. It considered that the risk assessment contained sufficient risk management to justify classification of the project as class 2. It was therefore approved.

Project Containment

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<td>L3 L4</td>
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<td>Human Clinical Applications</td>
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| Fax Number                         | 0                        |

| E-mail                             | blank                    |

| HSE Division                       | blank                    |

| Comments                           |                          |

Date at Which Additional Info Submitted: 02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

PolyProx has enrolled an expert to act as Biological Safety officer. (years of experience, qualification, etc)
The advisor has reviewed and advised us on our GMO risk assessments……….  

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Tick if confidential

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<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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</table>
Waste Disposal

- Presept is used as it is a validated disinfectant for transfected cells if used in the following way under manufacturers recommendations for 100% kill rate.
- Hard surfaces: A solution containing 2g/L for 1 hour contact time, 10mins for metal parts (longer can cause corrosion)
- Safety cabinets: 2g/L Presept (10mins)
- Supernatants/liquid waste: 2g/L Presept, for 1 hour, then flush down the drain with a lot of water.
- All solid waste is going to be incinerated.
- Full yellow euro bins (properly labelled and closed) are to be transported to the hazardous waste bins to the Babraham storage area by the state team. Howie type Lab coats, safety glasses and nitrile gloves will be used during the waste handling.
- The Babraham Research Campus has a derogation with the HSE so that clinical waste can be removed from site without being autoclaved. Waste is processed in accordance with the Babraham Institute disposal method according to notification GM 105.4.1.
- Bins are collected for off-site for incineration by Vetspeed Ltd._GM authorization number GM898.

Accidental Release:
In our laboratories the floor is sealed and the labs are built to CL2 level, all the MSC are Class 2.
In an uncontained area, all persons should evacuate the area to avoid contact with any potential aerosols formed.
Only after 1 hour the room might be entered (to allow any aerosols to settle) and the procedures should be the same as in a contained area.
In a contained area cover the spill with absorbent material, such as paper towels, and let the spill soak in. This helps prevent aerosolization of the contaminant.
Virkon powder should be applied all over the spill area. Allow 20 min contact time. The spillage is to be mopped up with damp towels, it must not be brushed. This solid waste is then to be incinerated as described before.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The BSO C MIOSH CBiol MRSB is the Director of Making Heath Safety Work Ltd.
The BSO has advised PolyProx Therapeutics on evaluating GMO handling and waste disposal risks and he is satisfied that we are acting appropriately, responsibly and with the best practice. The R&D does not contain anything exceptional that requires special consideration. The BSO approved the associated risk assessment and support this notification.
### Project Ref 3492/19.1

#### Date Ackn'd
21/08/2019

#### CU2 Project Title
Use of cell-lines for bioassays to demonstrate the activity of PolyProx's therapeutics (PROTACS comprising engineered natural proteins)

#### Date Project Ceased
31/03/2021

#### Class
Class 2

#### CultureVol
< 1 Litre

#### Consent
Non-GMM

#### Project notified under transitional arrangements
N

#### Significant Change ID

#### Date of Significant Change

#### Purposes of the contained use
To develop engineered natural proteins for therapeutic use.

#### Mammalian cell lines
- HEK293 LgBiT Stable Cell Line generated by PROMEGA
- Human embryonic kidney cells (HEK293)
- Human epithelial adenocarcinoma cell line (HeLa)
- Human lymphoma cell lines (KARPAS 299, SU-DHL-1 and SR-786)
- Human breast cancer cell line (MDA-MB-231)
- Human melanoma cell line (SK-MEL)
- Human colorectal carcinoma cell lines (DLD-1, HT29, HCT116, SW480, LS 180, LoVo)
- Human Pancreatic cancer cell line (MIA PACA 2, HPAF-II)
- Human embryonal carcinoma (NTERA-2)

#### Host/vector system

---

02/03/2022  Page 14679 of 15326
Set of plasmids for the Flp-In™ T-REx™ System, described on the attached RA, to induce expression the protein of interest with Tetracycline on the stable transfected cell lines. They are procured from ThermoFisher.
pCDNA3 plasmid to transiently express the proteins of interest. It is procured from Genscript.
The CRISPR/Cas9- dependent genome editing of cells does not involve viral vectors or plasmids. We will use it to tag the gene of interest with a reporter gene system (HiBiT) (described on the RA).

Origin & function

Evaluation of foreseeable effects

HEK293 LgBiT Stable Cell Line (commercially available at PROMEGA).
Further engineered HEK293 LgBiT Stable Cell Line to tag proteins of interest with HiBiT (generated by a third party using CRISPR).
Various commercially available cancer cell lines (listed in the RA) engineered to have HiBiT tagged proteins of interest using CRISPR.
Various commercially available cancer cell lines (listed in RA) engineered to express protein PROTACS under the tet-inducible system.
HEK-293 is commercially available, were generated in 1973 by transformation of cultures of normal human embryonic kidney cells with sheared adenovirus 5 DNA.
The transgenes (Protein PROTACS) do not encode for oncogenes, growth factor receptors or toxin molecules. The generation of all transgenes and cloning into expression vectors will be outsourced from commercial providers (e.g. Genscript). This includes all bacterial work.
The CRISPR/Cas9- dependent genome editing of cells does not involve any viral vector, nor plasmids.
The transfected HEK 293 and other cell lines used in this project pose minimal potential damage to the environment as they have been adapted to an artificial cell culture environment under laboratory conditions and so are unable to thrive in the external environment if released.

Control measures to be adopted:
• Records and tracking of materials used are kept as part of PolyProx Therapeutics’ good lab practice which involves up to date hard copies of lab books. All materials are clearly labelled and stored in specific safe storage areas within the facilities.
• PPE: lab coats, gloves and goggles to be worn always. This is to reduce the possibility of any physical contact with the transfected cells. Their handling will be conducted in biosafety Level 2 (BSL2) cabinets.
• No sharps to be used – removes the possibility of introduction into the blood stream
• All wastes treated to destroy GMO material (see disposal section below)
• HEK 293 and other cell lines (volumes less than 500ml) they should only be handled in closed containers or in the BSL2 cabinet.
• All experiments to take place in Building 580, which is on a secure site as part of the Babraham Research Campus. All areas where the cells are stored are only accessible by employees (via keycard or keypad). Visitors are supervised and wear badges to identify them.
• Training for use of the transfected cells will be conducted by users who have prior experience of handling them. The completion of training will involve the adding of the individuals name to this risk assessment. The signature will serve as a record of the training and be kept on file as part of the PolyProx Therapeutics training records.
• Where transfected cells are transported this will only be done while frozen, in sealed containers which are labelled and placed inside sealed plastic packaging. These in turn will be put into larger boxes, sealed and the box labelled with PolyProx’s name, address and contact details with the instruction not to open unless authorised.
There will be no lone, out of hours, work for this project.
The PolyProx Therapeutics Health and Safety Policy covering lab practice and use of COSHH is to be followed at all times.
Together the implementation of these control measures makes this project “LOW” risk
First Aid:
EYE EXPOSURE FROM SPLASH OR AEROSOLS: Rinse a minimum of 15 minutes in eye wash available at all sink hand wash areas or flush area with water. Seek medical attention.
CONTACT WITH SKIN: wash thoroughly with soap. Hand sinks available in all labs for washing.
INHALATION EXPOSURE FROM AEROSOLS: Seek medical attention.
NEEDLESTICK AND/OR SHARPS EXPOSURE: No sharps are to be used.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

<table>
<thead>
<tr>
<th>Waste Disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Presept is used as it is a validated desinfectant for transfected cells if used in the following way under manufacturers recommendations for 100% kill rate.</td>
</tr>
<tr>
<td>• Hard surfaces: A solution containing 1g/L for 1 hour contact time, 10mins for metal parts (longer can cause corrosion)</td>
</tr>
<tr>
<td>• Safety cabinets: 2g/L Presept (10mins)</td>
</tr>
<tr>
<td>• Supernatants/liquid waste: 2g/L Presept, for 1 hour soaking, then flush down the drain with a lot of water.</td>
</tr>
<tr>
<td>• All solid waste is going to be incinerated.</td>
</tr>
<tr>
<td>• Full yellow euro bins (properly labelled and closed) are to be transported to the hazardous waste bins to the Babraham storage area by the state team. Howie type Lab coats, safety glasses and nitrile gloves will be used during the waste handling.</td>
</tr>
<tr>
<td>• The Babraham Research Campus has a derogation with the HSE so that clinical waste can be removed from site without being autoclaved. Waste is processed in accordance with the Babraham Institute disposal method according to notification GM 105.4.1.</td>
</tr>
<tr>
<td>• Bins are collected for off-site for incineration by Vetspeed Ltd _GM authorization number GM898.</td>
</tr>
</tbody>
</table>

Accidental Release:
In our laboratories, the floor is sealed and the labs are built to CL2 level, all the MSC are Class 2.
In an uncontained area, all persons should evacuate the area to avoid contact with any potential aerosols formed.
Only after 1 hour may the room be entered (to allow any aerosols to settle) and the procedures should be the same as in a contained area.
In a contained area cover the spill with absorbent material, such as paper towels, and let the spill soak in. This helps prevent aerosolization of the contaminant. Virkon powder should be applied all over the spill area. Allow 20 min contact time. The spillage is to be mopped up with damp towels, it must not be brushed. This solid waste is then to be incinerated as described before.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment
We are happy that the above and attached RA, address the risk involved within this project.

Project Containment

<p>| Laboratory Activities | Glass Houses | Growth Rooms |</p>
<table>
<thead>
<tr>
<th>Data Premises Notified (Originally)</th>
<th>28/08/2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferred from 1992 Regs?</td>
<td>N</td>
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<tr>
<td>Transitional Premises Class</td>
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<tr>
<td>Data Premises Closed</td>
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<tr>
<td>Transitional Premises</td>
<td>N</td>
</tr>
<tr>
<td>Emergency Plan Required?</td>
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<tr>
<td>Non-GMMs</td>
<td>N</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>N</td>
</tr>
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</table>

Name

MIROBIO LTD

Name 2

Department

Campus Estate or Research Centre

Building

OXFORD SCIENCE PARK

Road Name

ROBERT ROBINSON AVENUE

District

LITTLEMORE

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX4 4GA

Country

ENGLAND

Tel Number

01865 618807

Fax Number

0

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

<table>
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<tr>
<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
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<th>Building</th>
<th>Road Name</th>
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<tr>
<td>15/06/2020 MIROBIO LTD</td>
<td>BIOESCALATOR</td>
<td>INNOVATION BUILDING</td>
<td>ROOSEVELT DRIVE</td>
<td>OXFORD</td>
<td>OX3 7FZ</td>
<td>N</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MIROBIO LTD</td>
<td>OXFORD SCIENCE PARK</td>
<td>ROBERT ROBINSON AVENUE</td>
<td>LITTLEMORE</td>
<td>OXFORD</td>
<td>OX4 4GA</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

BioEscalator GM Safety Committee:
The BioEscalator Innovation Building on Oxford University's Old Road Campus is home to a range of early stage life science companies. The GM Safety Committee is composed of representatives from these companies (Founders, research associates, and Designated Biological Safety Officers), the BioEscalator Laboratory Manager, and two Divisional Safety Officers from the Medical Sciences Division of the University of Oxford. Several of the committee members have extensive experience in the field of virology, and all have experience of working with or assessing the risk associated with working with GMM/GMO. The affiliation of the BioEscalator with the University of Oxford means that they adopt many of the practices of the University Safety Policy.
The committee meet annually. In the interim, when a new GM Risk Assessment is submitted to them for review, it is sent to each member of the committee for comments/approval. Their comments must be sent in writing to the assessor. Only once all committee members are satisfied that the Risk Assessment is appropriate for the containment class specified, can the HSE application be submitted.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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<td></td>
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<tr>
<td>Level 2 (GMMs)</td>
<td>Yes</td>
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<td></td>
</tr>
<tr>
<td>Level 3 (GMMs)</td>
<td></td>
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<tr>
<td>Level 4 (GMMs)</td>
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<tr>
<td>Non-microbial</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Other (please specify)</td>
<td>Tick if confidential</td>
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<tr>
<td>-----------------------</td>
<td>----------------------</td>
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<td></td>
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</tr>
<tr>
<td>Bacteriology</td>
<td>Yes</td>
<td>Parasitology</td>
<td>Yes</td>
<td>Transgenic Birds</td>
</tr>
<tr>
<td>Virology</td>
<td>Yes</td>
<td>Transgenic Animals</td>
<td>Yes</td>
<td>Transgenic Fish</td>
</tr>
<tr>
<td>Mycology</td>
<td>Yes</td>
<td>Transgenic Invertebrates</td>
<td>Yes</td>
<td>Transgenic Plants</td>
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<tr>
<td>Other(s)</td>
<td>Immunology</td>
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</tbody>
</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

Not applicable-First contained use will be Class 2 (see form CU2)

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>Culture Vol Class 2</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
</tr>
</thead>
<tbody>
<tr>
<td>29/08/2019</td>
<td>Assays for the assessment of immune receptor modulating antibodies</td>
<td>Class 2</td>
<td>1-50 Litres</td>
<td>Non-GMM</td>
<td>Consent Granted</td>
</tr>
</tbody>
</table>

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Not applicable

Project notified under transitional arrangements

Tick if notifying a connected programme of work

02/03/2022
### Project Additional Information

**Purposes of the contained use**

We will be using a lentiviral system to stably express immune cell surface receptors in recipient cell lines. The transduced cell lines will then be used in cell based assays to study immune response to modulating antibodies. The same lentivirus-based vector system will be used to express high titres of soluble forms of immune receptor proteins in a 293T cell line. These proteins will subsequently be purified from supernatant by affinity chromatography to be used in various assays required to characterise candidate antibodies (e.g. SPR and ELISA-based assays of antibody-receptor binding).

**Recipient or parental organism**

E.coli derivatives will be used to retransform lentiviral transfer plasmids. These cells are disabled such that they cannot colonise human gut. They are considered equivalent to ADCP hazard group 1.

HEK 293T cells will be transfected with a mixture of lentivirus transfer, packaging and envelope plasmids to produce the lentiviral particles.

The recipients are all cell lines of human or mouse origin. They include HEK 293T, Jurkat and BW5147. In some cases, the host Jurkat cell line may have already been genetically modified to contain a fluorescent reporter construct to report transcriptional activation via NFkB. The cell lines are all widely used and have a safe history of use. They are well characterised, authenticated, and are obtained from commercial sources. They are considered low hazard for GM activities and as such are considered Containment level 1 with good microbiological practice and good occupation hygiene.

**Host/vector system**

The vector system is a third generation HIV-1 based lentiviral system, using transfer plasmids pSF-Lenti-SFFV-EMCV-Blast-SV40ori and/or pSF-Lenti-SFFV-EMCV-Puro-SV40ori, along with ExceLenti LTX Lentivrus Packaging mix. The system is split over 4 plasmids, enhancing safety by reducing the likelihood that recombination events could produce replication competent virus. Several other features make this system incapable of producing replication competent virus; the system lacks the full complement of necessary genes to make replication competent virus, since the accessory proteins vpr, vif, vpu and nef are all absent, as is Tat; the transfer plasmid does not contain wild-type HIV-1 LTR, but has a mutated version which is self inactivating and uses a 5’ CMV rather than native promoter; HIV structural genes are provided in trans; LTR and psi packaging sequences are absent from the packaging plasmids containing these elements.

The transfer plasmid contains a WPRE sequence. This has a partial open reading frame for X Protein. Evidence suggest that this protein may play a role in promoting hepatocarcinogenesis in mice. Under the Genetically Modified Organisms Regulation 2014, work using vectors containing this sequence should be classified as Class 2.

**Origin & function**

The cDNA inserts will be ordered from commercial sources as gene strings and then cloned into the transfer plasmids using standard restriction cloning methods. These genes are all of mammalian immune cell origin and, along with the standard antibiotic resistance genes, have no oncogenic potential, and can be classified under Containment Class 1.
The transformed E.Coli confer no significant hazards. Expression of inserted genes does not present a hazard. The resulting GMO is no more hazardous to human health than the un-modified cells, since the changes do not alter the pathogenicity of the host. The resulting GMM would not survive outside laboratory conditions. Since it is non-colonising, and none of the inserted sequences would affect the level of risk, it would not be harmful to animals, plants or humans. Risk to the environment is effectively zero.

The inserts are manufactured synthetically as gene strings, so there is no risk associated with insert donor source.

The main risk to human health is from exposure to the lentiviral particles, which have wide tropism and are capable of infecting human cells. The transgenes are not toxic or oncogenic and therefore low GM risk, however there is a small oncogenic risk associated with the possibility that upon exposure to lentivirus, the transgene could potentially insert into a tumour suppressor gene, resulting in loss of function. The likelihood of this happening is extremely low. Good laboratory practice also minimises the risk of laboratory workers being exposed to virus.

Since the lentiviral system used produces replication incompetent vector, the resulting GMOs are incapable of producing replication competent virus and so genetic modifications cannot be transmitted from one individual to another or from one organism to another. As such, a combination of containment level 2 working and appropriate waste disposal methods (see below) means there is effectively zero risk to the environment from the resulting GMO. The lentiviral transfer plasmid contains a WPRE sequence that has not been mutated to scramble the open reading frame of the X protein, which may be oncogenic in nature. Under the Genetically Modified Organisms Regulation 2014, work using vectors containing this sequence should be classified as Class 2

Assignment to provisional containment: Containment Level 2

Evaluation of foreseeable effects

The transformed E.Coli confer no significant hazards. Expression of inserted genes does not present a hazard. The resulting GMO is no more hazardous to human health than the un-modified cells, since the changes do not alter the pathogenicity of the host. The resulting GMM would not survive outside laboratory conditions. Since it is non-colonising, and none of the inserted sequences would affect the level of risk, it would not be harmful to animals, plants or humans. Risk to the environment is effectively zero.

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Assignment to provisional containment: Containment Level 2

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Not applicable

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Consumable waste (mainly plasticware eg. Pipettes, flasks, tubes, tips)
Autoclave using make safe cycle as specified in BS 2646 Prt 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the black bags (for incineration) waste route following autoclaving
Liquid waste (samples, tissue culture medium, supernatants, washings etc)
Add Virkon to 1% W/V for at least 1 hour and then discharge to drains.
Agar plate waste
Autoclave using make safe cycle as specified in BS 2646 Prt 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the black bags (for incineration) waste route following autoclaving

Degree

Is an emergency plan required according to regulation 20?  

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

Please enter comments on the GM safety committee on the risk assessment
No concerns were raised by any member of the committee.
"I have no issues with this assessment and have no further comment."
"Waste management seems fine and I have no comments."
"I have no objections or concerns."
"This risk assessment looks appropriate for the activities covered."
"I thought the risk assessment looked good and very comprehensive."

## Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Animal Units</td>
<td>Large Scale Activities</td>
<td>Human Clinical Applications</td>
</tr>
<tr>
<td>L2</td>
<td>L3</td>
<td>L4</td>
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<tr>
<td><strong>Name</strong></td>
<td>ROYAL LIVERPOOL &amp; BROADGREEN UNIVERSITY HOSPITALS</td>
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<tr>
<td>----------</td>
<td>--------------------------------------------------</td>
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<td><strong>Campus Estate or Research Centre</strong></td>
<td>ROYAL LIVERPOOL UNIVERSITY HOSPITAL</td>
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<tr>
<td><strong>Road Name</strong></td>
<td>PRESCOT STREET</td>
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<tr>
<td><strong>Town</strong></td>
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<tr>
<td><strong>County</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>Tel Number</strong></td>
<td>0151 706 4861</td>
<td></td>
</tr>
<tr>
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<td>NORTH WEST</td>
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**Date at Which Additional Info Submitted**
02/03/2022
Premises Addresses

<table>
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<tr>
<th>Date Premises Closed</th>
<th>Name</th>
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<th>Name 2</th>
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<th>Town</th>
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<th>Post-code</th>
<th>Country</th>
<th>Withdrawn</th>
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<tbody>
<tr>
<td>02/03/2022</td>
<td>ROYAL LIVERPOOL &amp; BROADGREEN UNIVERSITY HOSPITAL</td>
<td>CLINICAL RESEARCH UNIT</td>
<td>ROYAL LIVERPOOL UNIVERSITY HOSPITAL</td>
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<td>Liverpool</td>
<td>LIVERPOOL</td>
<td>MERSEYSIDE</td>
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<td>ENGLAND</td>
<td>N</td>
</tr>
</tbody>
</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The BSO has provided advice research proposal has been reviewed by the ATIMP committee which at the Royal Liverpool performs the function of the GMSC (please see attached ToR). The following personnel form the committee:

- Clinical Director of RD&I
- H&S rep
- Human resources Rep
- Director of Phase 1 research unit
- Designated individual for Human tissue transplant
- Lab Rep - 1 x Trust, 1 x University of Liverpool
- BSO (honorary contract to us from LSTM)
- Infection control consultant
- RD&I rep
- Local CCG rep
- Pharmacy Rep

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

- Level 1 (GMMs) [Yes]
- Level 2 (GMMs)
- Level 3 (GMMs)
- Level 4 (GMMs)

02/03/2022
Non-microbial

Other (please specify)  
Tick if confidential [ ]

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<thead>
<tr>
<th>Bacteriology</th>
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<th>Transgenic</th>
<th>Gene Therapy</th>
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<th>Transgenic</th>
<th>Transgenic</th>
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<tr>
<td>Invertebrates</td>
<td>Plants</td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

Other(s)  
Vaccine without license for use in the EU

For activities involving GMMs, describe the waste management measures which will apply to the activity

Disposal will be by incineration off site using standard Royal Liverpool Hospital protocols

Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

Reviewed by BSO only as contained use as per contained use Genetically Modified Organisms (Contained Use) Regulations 2014. The following comments were raised:
1) Confirmation of contained use of GM should be sought regarding likelihood of shedding of live, infectious GMO from patients.
2) Evidence should be provided for the GMO confirming limited viraemia and virulence of infection.
3) Confirmation that GMO is no more likely to persist in the environment compared to the attenuated vaccine strains of Yellow Fever and Japanese Encephalitis vaccine strains
Manufacturer risk assessment provided in response to above queries and attached to this application
Review by ATIMP committee requested further details regarding inactivation of vaccine and whether manufacturer had sought license in EU - responses provided by Sanofi.
<table>
<thead>
<tr>
<th>Date Premises Notified</th>
<th>Transferred from 1992 Regs?</th>
<th>Transitional Premises Class</th>
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<table>
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<th>Transitional Premises Emergency Plan Required?</th>
<th>Non-GMMs</th>
<th>Withdrawn</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>N</td>
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</tbody>
</table>

**Name**

ALLOY THERAPEUTICS LTD.

**Department**

UNIT 3, GROUND FLOOR.

**Campus Estate or Research Centre**

GRANTA PARK

**Building**

MCCLINTOCK BUILDING

**Road Name**

GREAT ABINGTON

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB21 6GP

**Country**

ENGLAND

**Tel Number**

01223 734112

**Fax Number**

N/A

**E-mail**


**HSE Division**

EAST AND SOUTH EAST

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
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<td>Cambridge</td>
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<td>CB21 6GP</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The Alloy Therapeutics Ltd genetic modification committee will consist of:
1. The head of the Cambridge site who will serve as the chair of the committee and has over 30 years of relevant research experience.
2. Five staff who have PhDs in various relevant fields of research and more than 10 years research experience including genetic modification and GMOs.
3. The external biosafety consultant (see section 8).

The Alloy Therapeutics Ltd GM committee agreed that the risk assessment included with this notification was suitable and sufficient. The committee will meet at least every 6 months to review risk assessments and GM procedures.

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Invertebrates</td>
<td>Plants</td>
<td>specify below)</td>
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Other(s)  
Molecular biology including: Protein expression in mammalian and bacterial systems.

For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled “Eurobins”.

Collections for incineration will be made by the local registered clinical GM waste incinerator contractor.

This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The Alloy Therapeutics Ltd GMO committee reviewed the GMO risk assessment (see attached). The Alloy Therapeutics Ltd GMO committee consists of 6 people who have PhDs in different fields with at least 10 years research experience including genetic modification and GMOs. The GMO committee have previously written GMO risk assessments for similar work prior to joining AlloyTherapeutics and they understand the science around the work carried out.

The Health and safety consultant also reviewed the GMO risk assessment. The consultant has a B.Sc in Animal Sciences - Wye College (part of Imperial), London University, M Sc, Animal ParaSitology, Ph.D. The immunology and pathology of Sarcocystis ovicanis infections in sheep (both at U.C.N.W" University of Wales). Technical member of the Institution of occupational safety and health (Tech IOSH), NEBOSH National Diploma in Occupational Health and Safety (in progress), NEBOSH National General Certificate in Occupational Health and Safety, Member of the Institute of Safety in Technology and Research, MRC Biological Safety Officer Training Certificate. 1986 to 2010 at the Babraham Institute (Cambridge) where experience of handling pathogens and GMO obtained during research. 2006 made Babraham Research Campus Biological Safety Officer (part time plus research). 2009 onwards teach the genetic modification safety section of the One Nucleus 'Biological Safety: Management and Practice' course (IOSH approved).

2010 onwards Babraham Institute Health, Safety and Quality Assurance Manager and Babraham Bioscience Technologies (BBT) Biosafety Officer (Full time). 8 years experience in risk assessments and has health and safety qualifications.
### Purposes of the contained use

The aim of this project is to use MV, y-retrovirat and lentiviral delivery systems to introduce various mammalian DNA sequences into mammalian cells in culture. These sequences direct expression of either (i) RNA species (siRNAs or antisense RNAs) that interfere with the expression of various mammalian proteins or (ii) the corresponding mammalian proteins themselves; for in vitro use and for the generation of stable mammalian cell lines (and subsequent use of such stable lines in vitro) with the aim of generating stable cell lines expressing fluorescent proteins, potential drug targets or therapeutic proteins, etc.

### Recipient or parental organism

Cloning, propagation and amplification of component plasmids for MV, y-retro- and lenti-virus vector systems will take place in K12 strains of E. coli (with a history of safe use). Mammalian cell lines (e.g. immortalized lines derived from human cancer tissues) fall into two types:

a) Packaging/helper cell lines into which plasmids containing lenti- or retroviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

b) Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines, i.e. a negligible risk.

### Host/vector system

The AAV vector technology that we will use is based on a non-enveloped single-stranded DNA virus (Adeno Associated Virus - which are ACDP Hazard Group 1 biological agents) as vehicles to efficiently deliver and express genes in mammalian cells. MV viruses are taken up by endocytosis, released for transcription and expression following migration to the nucleus. Given the low pathogenicity of the recipient virus, the major hazards that will be posed by recombinant MV vectors will depend upon the properties of the inserted genetic material and any products that it may encode ..

The V-RETROVIRAL vectors which will be used are derived from either Moloney Murine sarcoma Virus (MoMLV), Mouse Mammary Tumour Virus (MMTV) or Feline Leukaemia Virus (FeLV), all of which are ACDP Hazard Group 1 biological agents. On the other hand, the virus will be packaged by transfecting transfer vector into specific amphoteric 'helper' cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types. This means that the viruses produced for this experiment could potentially infect a number of species, including man.

### Origin & function

Selectable markers - examples (but not restricted to);
- Ampicillin resistance: E.coli derived
- Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene

Puromycin resistance (PAC) : Puromycin acetyl transferase is derived from Streptomyces alboniger

Reporter proteins such as (but not restricted to);
- Fluorescent proteins as reporters;
- GFP derived from the jellyfish Aequorea victoria and variants of this
Luciferase - class of oxidative enzymes used in bioluminescence
  o renilla luciferase derived from the Sea pansy (Renilla reniformis)
  o firefly luciferase derived from the firefly Photinus pyralis.
Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and I or
shRNAs (designed to knockdown the expression of Open reading frames, cDNAs and gene sequences encoding
potential drug targets or therapeutic proteins) - all human derived.
This could include the expression of potentially harmful genes e.g., encoding known proto-oncogenes or genes with
known oncogenic mutations that can contribute to cellular transformation.

Evaluation of foreseeable effects

All viral vectors employed in this protocol exhibit broad tropism and potential to infect human. Risks conferred
following infection are identified as:

i) genetic insertion of viral sequences with potentially deleterious effects on endogenous genes: we will
employ transgene promoters and other viral sequences that may affect host gene function in a wide
range of cell types (e.g., CMV promoter). We assess retro-, lenti- or adenovirus infection might induce
permanent changes in infected cells including a risk for tumorigenesis. Risks conferred are previously
described and categorised under Class 2 risks [SACGM compendium of guidance part 2, page 121]:
  • ... The effects of integration upon the infected cell should be considered. For instance, promoter
sequences present in the provirus might activate genes adjacent to the integration site or,
alternatively, insertion may disrupt genes and prevent their expression."
ii) expression of human-derived or homologous transgenes with potentially deleterious effects: various
transgenes may be employed, wherein intrinsic function of the transgene confers potentially
deleterious effects. For example, expression human oncoproteins could induce transformation of
infected cells. Beyond endogenous homeostatic mechanisms that may lessen this risk (e.g., apoptosis
and other host tumor suppression responses), we assess that standard precautions under Class 2
risk mitigation procedures are adequate to address such risks.
iii) expression of exogenous transgenes directed to host genes with potentially deleterious effects: multiple
transgene technologies (antisense, RNAi, CRISPR or related gene conversion) may be employed to
modify the function of endogenous genes with potentially deleterious effects. For example, RNAi or
antisense RNA could reduce the function of genes necessary to control cell apoptosis, potentially
resulting in tumorigenesis. As above, we assess that Class 2 risk management procedures
adequately address these risks.
iv) expression of heterologous genetic sequences with potentially deleterious effects: we will employ diverse
collections of non-human derived, exogenous transgene sequences that carry potential risks
following infection. Risks from expression of these collected transgene sequences are mitigated and
attenuated through several means. First, known pathogenic transgenes systemically removed, and
other exogenous gene products are fragmented or rearranged, such that potentially pathogenic or
other biologically deleterious genetic functions are not recapitulated in their complete endogenous
configuration. Second, potentially deleterious transgenes in any infectious viral preparation are highly
titrated, such that any single deleterious sequence comprises less than 1 part in 100,000 parts of an inoculum with infectious potential. In total, we assess these risks are
adequately managed via the
same Class 2 risk precautions employed above.
We assess environmental hazards are adequately addressed through the proposed personnel risk management
measures. We employ multiple attenuation strategies and protocols to severely limit independent virus
propagation beyond the intended use, and therefore anticipate minimal risks of GMM release.
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:
1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization is GM898) according to disposal notification GM105/4.1.

Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from Class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/SI2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site has 24h Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM.

The data sheets describing inactivation by Virkon are attached.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

02/03/2022
The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

## Project Containment

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**Name**

CC BIOTECH LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

TRANSLATIONAL & INNOVATION HUB

**Building**

I-HUB, IMPERIAL WHITE CITY CAMPUS

**Road Name**

84 WOOD LANE

**District**

**Town**

LONDON

**County**

**Postcode**

W12 7RH

**Country**

ENGLAND

**Tel Number**

0750 0838793

**Fax Number**

0

**E-mail**

**HSE Division**

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**Comments**
### Premises Addresses

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<th>Building</th>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Responsible person for genetic modification safety is a qualified Microbiologist (B. Sc.) with a PhD in synthetic biology. This person (Chief Scientific Officer of the company) has extensive experience devising risk assessments for the genetic modification of bacteria, which will be the focus of this activity. This employee has created the risk assessments for the operations at the premises and is responsible for their implementation. All employees will meet with this person once a month to ensure continued compliance with existing SOPs and methods, and to assess operations for any further potential safety risks.

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<thead>
<tr>
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<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
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Our waste management procedures involve disposal of all GMM material in standard sealable yellow 60L eurobins. These bins will have ‘UN3245’ stickers (includes the biohazard triangle and Biohazardous class 1 waste) placed onto the box with the premises building and room numbers detailed, in accordance with the Babraham Institute (wider campus in which the laboratory is located) SOP 3 - disposal of GMM waste. When securely locked, the date will be applied to the bin and the bin transported for incineration using Babraham Institute approved waste disposal service contractors. Incineration will fully inactivate/kill the GMM waste.

The person responsible for biological safety (company CSO) will audit this procedure to ensure that all employees are disposing with GMM waste in accordance with this protocol, underpinned by the procedures described in the SOP.
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**Town**

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**Comments**

**Date at Which Additional Info Submitted**

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Babraham Institute Biosafety Consultant

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Tick if confidential

Bacteriology          | Parasitology | Transgenic Birds | Microbiology Research |
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02/03/2022
Approved disinfectants (Chemgene and Virkon) will be used for disinfection (SOP1: Use of Disinfectants in the containment 1 lab). A standard spill procedure will be used (SOP 27: Dealing with Biological Spills at containment 1) (see attachments for the risk assessment). When needed, an autoclave will be used, at 121 degree C for 15 minutes. Reflection Therapeutics will use the standard sealable yellow 60l eurobin for all Biological lab waste, including GM class 1 micro-organism, GM animal material and other low risk Biological waste or contaminated material.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Approved disinfectants (Chemgene and Virkon) will be used for disinfection (SOP1: Use of Disinfectants in the containment 1 lab). A standard spill procedure will be used (SOP 27: Dealing with Biological Spills at containment 1) (see attachments for the risk assessment). When needed, an autoclave will be used, at 121 degree C for 15 minutes. Reflection Therapeutics will use the standard sealable yellow 60l eurobin for all Biological lab waste, including GM class 1 micro-organism, GM animal material and other low risk Biological waste or contaminated material.

Please enter comments of the GM safety committee on the risk assessment

"(…) needs to notify the HSE that she will be working with GM material (follow links) this is CLASS 1"

"HSE requires that human pathogens are handled in accordance with the classification as per http://www.hse.gov.uk/pubns/misc208.pdf . HEK293 cells are not listed on this list, therefore they themselves are not classified and do not require notification under COSHH (via a CBA1). Whilst they contains genes from viruses (e.g. adenovirus, SV40), if they do not produce that virus during passage, they do not need notifying via a COSHH route (i.e. 1st use of hazard group 2 agents). You should apply this logic to your assessment for other cell lines which you intend to use."

Project Ref 3498/20.1

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<th>Date Ackn’ed</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVolClass2</th>
<th>Class CultureVolumeClass3-4</th>
<th>Non-GMM</th>
<th>Consent Granted</th>
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<td>The generation and use of Adeno Associated Virus (AAV), y-retrovirus and lentivirus vectors for TMN Therapeutics Ltd.</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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Tick if notifying a connected programme of work Y
### Project Additional Information

#### Purposes of the contained use

The aim of this project is to use AAV, γ-retroviral and lentiviral delivery systems to introduce various mammalian DNA sequences into mammalian cells in culture. These sequences direct expression of either (i) RNA species (siRNAs or antisense RNAs) that interfere with the expression of various mammalian proteins or (ii) the corresponding mammalian proteins themselves; for in vitro use and for the generation of stable mammalian cell lines (and subsequent use of such stable lines in vitro) with the aim of generating stable cell lines expressing fluorescent proteins, potential drug targets or therapeutic proteins, etc.

#### Recipient or parental organism

Cloning, propagation and amplification of component plasmids for AAV, γ-retro- and lenti- virus vector systems will take place in K12 strains of E. coli (with a history of safe use). Mammalian cell lines (e.g. immortalized lines derived from human cancer tissues) fall into two types:

- **a)** Packaging/helper cell lines into which plasmids containing lenti- or retroviral DNA will be introduced, and from which infectious virus will be secreted into the medium to high titre. The media from these cell-lines poses a risk because of the high viral titre. As such this could provide a risk of infection to personnel working with the media.

- **b)** Recipient cell lines in culture. These cells will initially be exposed to a high titre of virus to permit infection (virus derived from (a) above). However the infected cell lines will be incapable of themselves producing active virus and as such are no more dangerous than the parental cell lines, i.e. a negligible risk.

#### Host/vector system

The AAV vector technology that we will use is based on a non-enveloped single-stranded DNA virus (Adeno Associated Virus - which are ACDP Hazard Group 1 biological agents) as vehicles to efficiently deliver and express genes in mammalian cells. AAV viruses are taken up by endocytosis, released for transcription and expression following migration to the nucleus. Given the low pathogenicity of the recipient virus, the major hazards that will be posed by recombinant AAV vectors will depend upon the properties of the inserted genetic material and any products that it may encode.

γ-RETO/ROVIRAL vectors which will be used are derived from either Moloney Murine sarcoma Virus (MoMLV), Mouse Mammary Tumour Virus (MMTV) or Feline Leukaemia Virus (FeLV), all of which are ACDP Hazard Group 1 biological agents. On the other hand, the virus will be packaged by transfecting transfer vector into specific...
amphoteric ‘helper’ cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types. This means that the viruses produced for this experiment could potentially infect a number of species, including man.

However, bearing this in mind we should consider that the viruses that will be used have been modified in a number of ways which will make them safer to handle:

• The genes encoding structural and other components of the viral genome have been separated. These genes have been engineered to minimise the risk of recombination that could lead to production of a replication-competent virus.

• The packaging cell lines allow expression of proteins, required to produce progeny virus: But the transfer vector is the only genetic material transferred to the target cells, consequently these cells cannot produce the proteins which are essential for viral assembly and infectivity.

• Second, third generation or Self INactivating vectors retrovirus vectors will be used in all experiments (see SACGM compendium of guidance part 2, section 2.11 (Retroviruses) pp117

The LENTIVIRAL vectors that will be used are derived from HIV-1, which is an ACDP Hazard Group 3 biological agent. However, second and third generation lentiviral vectors have several of the lentiviral accessory genes (required for pathogenesis only) deleted to increase biosafety and viral packaging is achieved by providing three helper constructs in trans containing gag, pol and rev sequences.

For example, second and third generation Lentiviral Expression Systems include the following key safety features:
In the second and successive generation lentiviral vectors several lentiviral accessory genes (vif, vpr, vpu and nef) are deleted from the transfer plasmid since they are not required for in vitro replication and the products they encode have cytotoxic activities.

In addition to this in the third generation lentiviral vectors:

• The Lenti expression vectors contain a deletion in the 3’ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line but instead results in “self-inactivation” of the lentivirus after transduction of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing a packageable viral genome.

• The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).

• Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull et al., 1998).

• Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication competent virus can be produced.

Despite the above safety features, use of these lentiviral vectors (which include WPRE) falls within SACGM 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. In addition, the virus will be packaged by transfecting transfer vector into specific amphoteric ‘helper’ cell line (gag, pol and env stably incorporated into host chromosome cells). NOTE: amphotropic packaging will widen the host range of the virus and allow it to infect a wide range if mammalian species (including humans) and cell types. This means that the viruses produced for this experiment could potentially infect a number of species, including man

Selectable markers – examples (but not restricted to):
• Ampicillin resistance: E.coli derived
Neomycin resistance: bacteria derived aminoglycoside phosphotransferase gene
Puromycin resistance (PAC): Puromycin acetyl transferase is derived from Streptomyces alboniger
Reporter proteins such as (but not restricted to):
- Fluorescent proteins as reporters:
  - GFP derived from the jellyfish Aequorea victoria and variants of this
  - Luciferase – class of oxidative enzymes used in bioluminescence
    - renilla luciferase derived from the Sea pansy (Renilla reniformris)
    - firefly luciferase derived from the firefly Photinus pyralis.
Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and/or shRNAs (designed to knockdown the expression of
Open reading frames, cDNAs and gene sequences encoding potential drug targets or therapeutic proteins) – all human derived.
This could include the expression of potentially harmful genes e.g. encoding known proto-oncogenes or genes with known oncogenic mutations that can contribute to cellular transformation.

Evaluation of foreseeable effects

All viral vectors employed in this protocol exhibit broad tropism and potential to infect human. Risks conferred following infection are identified as:

i) genetic insertion of viral sequences with potentially deleterious effects on endogenous genes: we will employ transgene promoters and other viral sequences that may affect host gene function in a wide range of cell types (e.g. CMV promoter). We assess retro-, lenti- or adenovirus infection might induce permanent changes in infected cells including a risk for tumorigenesis. Risks conferred are previously described and categorised under Class 2 risks [SACGM compendium of guidance part 2, page 121]: “…The effects of integration upon the infected cell should be considered. For instance, promoter sequences present in the provirus might activate genes adjacent to the integration site or, alternatively, insertion may disrupt genes and prevent their expression.”

ii) expression of human-derived or homologous transgenes with potentially deleterious effects: various transgenes may be employed, wherein intrinsic function of the transgene confers potentially deleterious effects. For example, expression human oncogenes could induce transformation of infected cells. Beyond endogenous homeostatic mechanisms that may lessen this risk (e.g. apoptosis and other host tumour suppression responses), we assess that standard precautions under Class 2 risk mitigation procedures are adequate to address such risks.

iii) expression of exogenous transgenes directed to host genes with potentially deleterious effects: multiple transgene technologies (antisense, RNAi, CRISPR or related gene conversion) may be employed to modify the function of endogenous genes with potentially deleterious effects. For example, RNAi or antisense RNA could reduce the function of genes necessary to control cell apoptosis, potentially resulting in tumorigenesis. As above, we assess that Class 2 risk management procedures adequately address these risks.

iv) expression of heterologous genetic sequences with potentially deleterious effects: we will employ diverse collections of non-human derived, exogenous transgene sequences that carry potential risks following infection. Risks from expression of these collected transgene sequences are mitigated and attenuated through several means. First, known pathogenic transgenes systemically removed, and other exogenous gene products are fragmented or rearranged, such that potentially pathogenic or other biologically deleterious genetic functions are not recapitulated in their complete endogenous configuration. Second, potentially deleterious transgenes in any infectious viral preparation are highly titrated, such that any single deleterious sequence comprises less than 1 part in 100,000 parts of an inoculum with infectious potential. In total, we assess these risks are adequately managed via the same Class 2 risk precautions employed above.

We assess environmental hazards are adequately addressed through the proposed personnel risk management measures. We employ multiple attenuation strategies and protocols to severely limit independent virus propagation beyond the intended use, and therefore anticipate minimal risks of GMM release.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:
1. We do not have access to an autoclave within our area of the building.
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed – Their GM authorization is GM898) according to disposal notification GM105/4.1.

Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from Class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this.

This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class 1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins".

Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.

This disposal method is expected to achieve 100% inactivation of the GMM.

The data sheets describing inactivation by Virkon are attached.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

Project Containment

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02/03/2022
### GM Centre Number: 3499

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#### Name
RX BIOLOGICS LTD

#### Name 2

#### Department

### Campus Estate or Research Centre
BABRAHAM RESEARCH CAMPUS

#### Building
BUILDING 950

#### Road Name

#### District

### Town
CAMBRIDGE

#### County
CAMBRIDGESHIRE

#### Postcode
CB22 3AT

#### Country
ENGLAND

#### Tel Number
01223 839557

#### Fax Number
0

#### E-mail

#### HSE Division
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### Comments

#### Date at Which Additional Info Submitted
02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GMSC will be comprised of the Chief Scientific Officer/Safety Officer, two Scientific Officers. Note that we are a small company, and the Chief Executive Officer is also the Safety Officer. A quorum for the meeting is a minimum of two individuals.

The Committee will meet a minimum of once per year to discuss routine matters, review all existing risk assessments and audit the existing procedures. The GMSC will meet more frequently to discuss risk assessments etc. that require more urgent attention.

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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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All liquid waste from the GMMs will be aspirated into an appropriate disinfectant such as Virkon solution, such that the final concentration of Virkon remains above 1%, and is disinfected for at least 1 hour. The waste should then be disposed of down the sink with a copious amount of water. Contaminated solid waste such as plastic disposables, tissue paper and culture plates, etc will be disposed of in category 1 clinical waste disposal bins. The clinical waste disposal bins will be sealed, and disposed of together with other such material generated on the Babraham Research Campus by incineration off site. Sharps materials will be disposed of in yellow sharps containers, which are also disposed of by incineration off site.

The GMSC found that the risk assessment adequately stated the risks of carrying out the activities as described. The person responsible for supervision (in section 9 below) has confirmed the activity class of the work to be 1. FreeStyle 293-F will be used to express recombinant proteins. These are commercially available and are derived from HEK293 parental cells that have been adapted for suspension culture. The HEK293 lines are now well characterised with a history of safe use over several decades. They are an established host of choice for protein expression work. Work with the Freestyle 293-F cells are not believed to pose an increased hazardous risk compared to the parental line and containment within a cat 1 laboratory and good laboratory practice should be sufficient to minimise risk to health and the environment.

Use of genetically modified filamentous phage derived from well characterised parental phage strains

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GMSC found that the risk assessment adequately stated the risks of carrying out the activities as described. The person responsible for supervision (in section 9 below) has confirmed the activity class of the work to be 1. FreeStyle 293-F will be used to express recombinant proteins. These are commercially available and are derived from HEK293 parental cells that have been adapted for suspension culture. The HEK293 lines are now well characterised with a history of safe use over several decades. They are an established host of choice for protein expression work. Work with the Freestyle 293-F cells are not believed to pose an increased hazardous risk compared to the parental line and containment within a cat 1 laboratory and good laboratory practice should be sufficient to minimise risk to health and the environment.
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**Name**

BARKING, HAVERING & REDBRIDGE UNIVERSITY HOSPITALS NHS TRUST

**Name 2**

Department

**Campus Estate or Research Centre**

QUEEN'S HOSPITAL

**Road Name**

ROM VALLEY WAY

**Town**

ROMFORD

**County**

ESSEX

**Postcode**

RM7 0AG

**Country**

ENGLAND

**Tel Number**

01708 435 000

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

Date at Which Additional Info Submitted

02/03/2022
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**Name**

SHIFT BIOSCIENCE LTD

**Campus Estate or Research Centre**

CB1 BUSINESS CENTRE

**Road Name**

20 STATION ROAD

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB1 2JD

**Country**

ENGLAND

**Tel Number**

07898 636588

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Alloy Therapeutics Ltd genetic modification committee will consist of:

1. The COO/researcher at Shift Biosciences will serve as the chair of the committee and has over 10 years of relevant research experience.
2. As the company (Shift Biosciences) currently has only one member of staff, four other committee members have been co-opted from Babraham Bioscience Technologies (BBT) and one a local biosafety consultant. The co-opted members from BBT have experience in various relevant fields of research and more than 10 years research experience including genetic modification and GMOs.
3. The local biosafety consultant (see section 8).

The Shift Biosciences Ltd GM committee agreed that the risk assessment included with this notification was suitable and sufficient. The committee will meet at least every 6 months to review risk assessments and GM procedures.

<table>
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<tr>
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Human cell culture with treatment with a variety of drugs, followed by isolation of nucleic acids.

Waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below). Waste from our GM work at Class 1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.

This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are available.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The Shift Bioscience Ltd GMO committee reviewed the GMO risk assessment (see attached). The Shift Bioscience Ltd GMO committee consists of 6 people who have at least 10 years research experience including genetic modification and GMOs. The GMO committee have previously written GMO risk assessments for similar work and they understand the science around the work carried out.

The biosafety consultant reviewed the GMOs. The consultant has a B.Sc in Animal Sciences - Wye College (part of Imperial), London University, M.Sc. Animal Parasitology, Ph.D. The immunology and pathology of Sarcocystis oviscanis infections in sheep (both at U.C.N.W., University of Wales). Technical member of the Institution of occupational safety and health (Tech IOSH), NEBOSH National Diploma in Occupational Health and Safety (in progress), NEBOSH National General Certificate in Occupational Health and Safety, Member of the Institute of Safety in Technology and Research, MRC Biological Safety Officer Training Certificate. 1986 to 2010 at the Babraham Institute (Cambridge) where experience of handling pathogens and GMO obtained during research. 2006 made Babraham Research Campus Biological Safety Officer (part time plus research). 2009 onwards teach the genetic modification safety section of the One Nucleus ‘Biological Safety: Management and Practice’ course (IOSH approved). 2010 onwards Babraham Institute Health, Safety and Quality Assurance Manager and Babraham Bioscience Technologies (BBT) Biosafety Officer (Full time). 8 years experience in risk assessments and has health and safety qualifications.

<table>
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<td>03/10/2019</td>
<td>Analysis of gene expression for anti-ageing studies using the Ker-CT CRL-4048TM cell lines bought from ATCC®</td>
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<td>&lt; 1 Litre</td>
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Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Analysis of anti-ageing genes and drugs.
### Host/vector system

- One retroviral parent vector pSRαMSU expressing mouse Cdk4 that was obtained from C S (Memphis, TN, USA).
- One pBABE-puro plasmid containing the hTERT sequence. The construct was created at the JS’s laboratory at the University of Texas Southwestern Medical Center, USA.

### Origin & function

**Origins:** Ker-CT CRL-4048TM cell lines are from ATCC®. The cell lines are immortalised keratinocytes, and originally obtained in J S laboratory at the University of Texas Southwestern Medical Center. This cell line was created by using the following steps:

1. Retroviral parent vector pSRαMSU expressing mouse Cdk4 obtained from C S (Memphis, TN, USA)
2. Plasmid DNA transfection into the ecotropic packaging cell line PE501, followed by infection into the amphotrophic packaging cell line PA137 (Miller And Rosman, 1989)
3. Selected clones generated from the amphotrophic packaging cell line were used to produce viral supernatant to infect normal epidermal keratinocytes
4. For double infections, keratinocytes were infected with a second construct Cdk4+hTERT
5. Keratinocytes were infected with pBABE containing hTERT in the presence of polybrene
6. Selection with 350 ng/ml of puromycin

**Intended Functions:** DNA sequence and cell culture. Overexpression of CDK4 and hTERT in neonatal foreskin keratinocyte induced a dramatic upregulation of p16INK4a and milder upregulation of p53 and p21WAF1, which became unresponsive to UV irradiation. Despite the high levels of these checkpoint factors, Ker-CT cells divide in an apparently normal regulated fashion, are able to respond to changes in calcium levels, retain the stem cell phenotype, and fully differentiate and stratify in organotypic culture.

### Evaluation of foreseeable effects

This cell line is not known to cause disease in healthy adult humans. The cells have NOT been screened for Hepatitis B, HIV, or other adventitious agents unless otherwise stated in Certificate of Analysis. The cell line contains one promoter of the full sequence of the SV40 virus, therefore the insert should not cause a problem. In addition, the plasmid contains the MMVL sequence (derived from Moloney murine leukemia virus) but lacks the genes required for viral packaging and
transduction (these genes are carried by helper plasmids or integrated into packaging cells instead). As a result, the vectors have the important safety feature of being replication incompetent (meaning that they can transduce target cells but cannot replicate in them).

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:

1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed – Their GM authorization is GM898) according to disposal notification GM105/4.1.

Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from Class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor’s facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this.

This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid waste material such as cell culture supernatant and medium will be rendered non-viable by overnight decontamination with Virkon (1%) and autoclaving prior to skin disposal. 2% or 3% solutions are used for buffered solutions. A minimal contact time of 1 hour is allowed prior disposal to sewers as per manufacturer’s’ recommendation.

Contaminated solid waste, e.g. Class 1 biological waste & GM waste, DNA and protein gels, contaminated paper, cardboard and towels used to mop spills, contaminated pipette tips and plastics and nitrile gloves) will be bagged in appropriate waste bins (yellow euro bins). Bins should be placed in the waste compound ready for collection and disposal as clinical waste.

Contaminated solid ACDP 2 waste (contaminated plastic ware, cell pellets, contaminated tubes and falcons, tips, gloves, etc.) should be placed in a red-topped bin. When bins are full, the bin is sealed tight and appropriately labels for collection and rendered inactive by autoclaving (100% kill) in a validate machine located within the same building. The autoclave is performance validated on an annual basis.

Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior incineration. Any contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning.

Solid surfaces are disinfected with 1% Virkon solution.

Chemical waste does not go down the drain with the exception of deactivated tissue culture supernatants and quantities of acids and alkalis of less than 100 mL. Chemical waste should be
decanted into a suitable container and labelled with the content and company name. Chemical waste must not be mixed. Chemical waste must be taken to the chemical store at the rear of the general store. Bottles should be transported using a bottle holder. Containers should be no spill and securely sealed.

Please enter comments on the GM safety committee on the risk assessment

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.

This disposal method is expected to achieve 100% inactivation of the GMM.
The data sheets describing inactivation by Virkon are attached.

Project Containment

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<thead>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<td>L2 L3 L4 L2 L3 L4</td>
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#### Name

PHENOTYPECA LTD

#### Name 2

Department

#### Campus Estate or Research Centre

BIOCITY NOTTINGHAM

#### Road Name

PENNYFOOT STREET

#### Town

NOTTINGHAM

#### County

NOTTINGHAMSHIRE

#### Postcode

NG1 1GF

#### Country

ENGLAND

#### Tel Number

0115 7842331

#### Fax Number

0

#### E-mail

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#### Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

Phenotypeca's Biological Safety Officer and the University of Nottingham's Biological Safety Officer(s) for the Centre for Biomolecular Sciences and/or the School of Life Sciences have provided advice on GM risk assessments. All are scientists who have expertise in carrying out research using GMOs and are a good source of advice on risk assessment. Heads of Schools at the University of Nottingham where work with biological agents & GMOs is undertaken are required to appoint a person to act as Biological Safety Officer (BSO) to act as a local source of advice and support to researchers.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Other (please specify)</td>
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<td></td>
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<td>Tick if confidential</td>
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</table>
All solid waste and plastics exposed to GM material will be autoclaved prior to disposal. All liquid waste and glassware exposed to GM material will be autoclaved prior to disposal/wash up. Validated autoclaves are run at 126°C for 10 minutes (Centre for Biomolecular Sciences) and 128°C for 15 minutes (Life Sciences Building). Autoclaves are validated and serviced periodically with run cycle data recorded and retained, e.g. annual validation, 6-monthly service, annual insurance inspections and for each run a printout is obtained to validate the cycle, which is retained. Surfaces and spills will be disinfected (e.g. 2% Distel or 1% Virkon according to the manufacturers’ instructions) followed by disposal of cleaning materials by autoclaving.

For activities involving GMMs, describe the waste management measures which will apply to the activity

As reviewer of this application I am confident that the correct containment level has been determined for the work proposed. The yeast Saccharomyces cerevisiae is considered GRAS and QPS and the strains used in this proposal have been used safely for 10 years in numerous labs. They are disabled in that they have auxotrophies requiring nutrients making them unlikely to survive outside of the lab. They are benign except in cases of severe immune deficiencies or severe allergies which would be relevant to all yeast and not just these. The transient use of nonmobilisable vectors in disabled E. coli is also considered low risk. Any products made will be at small lab scale but should be assessed for potential toxicity or other detrimental effects. The scale of production in Phenotypewca is small and the risk of exposure is low. It is agreed that procedures within the company will ensure that containment at the required level is provided. All GM risk assessments, staff training, and GMO research will be compliant and aligned with the University of Nottingham policy.
**GM Centre Number: 3503**

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**Name**

ORBSEN THERAPEUTICS UK LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

**Road Name**

138 EDMUND STREET

**District**

**Town**

BIRMINGHAM

**County**

MIDLANDS

**Postcode**

B3 2ES

**Country**

ENGLAND

**Tel Number**

+353 915 28778

**Fax Number**

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**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

We have received advice from the Medical and Dental Sciences Genetic Modification Safety Committee at the University of Birmingham.

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02/03/2022
10% bleach solution or commercial disinfectant (i.e. Virkon) will be used to disinfect any working surface or equipment used to handle the cells. In the event of a spill on a work surface, equipment (i.e. spill from a tube, etc) or floor, spilled material must be contained by covering with paper towels and decontaminated immediately. Manufacturer recommends a minimum of 10 minutes contact time for sufficient disinfection. This will be used followed by 70% ethanol/IPA wipe. Both methods have been validated against viruses, bacteria and some fungi activities. Further information on the safety and effectiveness of bleach is published by CDC (https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html).

Virkon has been proven effective against 300 + strains/clinical isolates from 71 bacteria; 50 + strains/clinical isolates from 15 fungi; 70 + strains/clinical isolates from 33 viruses. Further detail on Virkon efficacy is provided in the following document: https://www.fishersci.co.uk/webfiles/uk/web-docs/SLSGD05.PDF

Waste solution from cell culture will be decontaminated using 10% bleach solution or commercial disinfectant (i.e. Virkon) with a minimum of 30 minutes contact time and is to be sluiced down the sink with warm water. Both bleach solution and Virkon are commonly used in laboratory environments for eliminating viable cells in cell culture and have been validated, as described above, to disrupt proteins and cell lipids, therefore can be used to effectively kill GMO cells (>99.9%) and denature proteins produced by them. The cell lines cannot survive outside the specialist cell culture environment. Solid waste, including plastic and gloves etc, will be disposed in a biohazard container. Once the container is full a lid will be placed to irreversibly seal the container, and a designated service (Stericycle) will collect them for disposal by incineration.

Spills and splashes on a work surface or equipment (i.e. spill from a tube, etc) must be contained by covering with paper towels and decontaminated immediately using the methods described above. In the event of a worker’s exposure to the GMO materials or waste via injuries, ingestion, or inhalation, treat immediately by making the site bleed (for cuts) and wash the exposed area for at least 15 minutes with water. Cover any small wounds with a waterproof dressing. Seek medical attention and contact Occupational Health if required. Uncontaminated personnel should be removed from the area while spills are dealt with by trained personnel wearing appropriate PPE with appropriate warning signs displayed throughout. Accidents must be reported following the company’s reporting procedure immediately and the appropriate Health and Safety body in the institution should also be informed.

For the use of a centrifuge, workers must follow the SOP to ensure a proper loading and use of appropriate lids. If a primary container has broken in a centrifuge without a closed rotor or bucket, immediately suspend use, notify lab staff and company head and request assistance from the company Biosafety Officer. For suspected or confirmed spills/breakage in any centrifuge, post a sign to indicate it cannot be used, wait at least 30 minutes after the centrifuge has stopped operating to allow aerosols to settle before initiating clean-up. If the spill is contained within a closed cup, bucket or rotor, spray the exterior with disinfectant and allow at least 10 minutes of contact time. Remove the carrier to the nearest biosafety cabinet (BSC). Carefully remove any unbroken tubes and place into a bin filled with disinfectant for 20 minutes. Wipe carrier/bucket with disinfectant. Spray the interior of the centrifuge chamber with a disinfectant, let sit for 20 minutes and then wipe down. In the event of a more significant failure (for example, a rotor failure) follow the above disinfection process and ensure equipment is clearly marked out of order until responsive maintenance by a qualified engineer.

Mammalian cell lines have stringent growth requirements and therefore are unlikely to survive outside the specialist cell culture environment posing minimal risk to both human health and the environment.
Genetic modification safety committee at The University of Birmingham had some minor comments which have been addressed within the body of the assessment. Examples of the comments are: 1) The assessment is generally well written but the significance of exposure to human health could be made clearer, ie. Effects on human health. 2) These types of modified cells are usually classed as Class 1 but handled at CL2. They are over classified. 3) Autoclaving is not used for waste inactivation and 30 minutes contact time with Virkon is deemed sufficient – there is a link to validation data but the relevant data about virkon could be pulled out.
### GM Centre Number: 3506

**Data Premises Notified (Originally)**: 21/10/2019

**Transferred from 1992 Regs?**: N

**Transitional Premises Class**

**Data Premises Closed**

**Transitional Premises**

**Emergency Plan Required?**

**Non-GMMs**: N

**Withdrawn**: N

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**Name**

ROYLANCE STABILITY STORAGE LTD

---

**Name 2**

**Department**

---

**Campus Estate or Research Centre**

BIOCITY

**Road Name**

BO NESS ROAD

**District**

NEWHOUSE

**Town**

MOTHERWELL

**County**

LANARKSHIRE

**Postcode**

ML1 5UH

**Country**

SCOTLAND

**Tel Number**

01698 539611

**Fax Number**

0

**E-mail**

**HSE Division**

blank

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**Comments**

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**Date at Which Additional Info Submitted**

02/03/2022
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

GMO committee contains members with pharmaceutical backgrounds, stability storage backgrounds and specific GMO experience. The final sign off on the risk assessment has worked in several GMO labs, therefore adhering to GMO guidelines. They also planned, developed and ran practical classes for students using GMO’s which included risk assessment production and review.

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</table>
As a storage facility we do not offer a disposal service in the instance of spillages or breakages of primary packaging out internal standard operating procedures will be followed for containment, cleaning, reporting and safety. This is performed with guidance from the MSDs or client instruction.

As Roylance Storage is a storage only site we cannot predetermine the nature of work we will receive.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

As a storage facility we do not offer a disposal service in the instance of spillages or breakages of primary packaging out internal standard operating procedures will be followed for containment, cleaning, reporting and safety. This is performed with guidance from the MSDs or client instruction.

Tick to confirm that you are attaching a summary of the risk assessment:

Tick if you are claiming exemption from disclosure for sections of the risk assessment:

Please enter comments of the GM safety committee on the risk assessment:

---

**Project Ref** 3506/19.1

<table>
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Date Project Ceased

Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

02/03/2022 Page 14732 of 15326
Historical Significant Changes

Historical Date of Additional Info

Significant Change ID 3506/19.1a

Date of Significant Change 26/08/2020

Project Additional Information

Purposes of the contained use
Roylance storage is a storage only facility. GMO samples will be received on site via a courier, stored in the appropriate conditions and pulled and despatched at client request

Recipient or parental organism
Unknown - as we are storage oly this could vary between client requirements before we receive samples we ask for MSDS and as much information on the product as possible. Ensuring clients have the appropriate GMO clearance

Host/vector system
As above

Origin & function
As above

Evaluation of foreseeable effects
N/A

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
As a storage only facility the GMO products will be contained, controlled and treated as per MSDS and client instructions. If GMO products are deemed too hazardous or high risk, the samples will not be accepted for storage - only level 1 or 2 will be accepted for storage. Additional handling and spillage covered in risk assessment

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
As per risk assessment - likely only GMO's to be stored

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
As a storage only facility we do not offer disposal services. In the instance of spillages or primary packaging breakage our internal standard operating procedures will be followed for containment, cleaning, reporting and safety. This is performed with guidance from the MSDs and client instruction.
Products deemed too hazardous or high risk will be assessed before the study is accepted and storage rejected if applicable.

Please enter comments on the GM safety committee on the risk assessment

GMO committee contains members with pharmaceutical backgrounds, Stability Storage backgrounds and specific GMO experience, The final sign off on the risk assessment has worked in several GMO labs, therefore adhering to GMO guidelines. They also planned developed and ran practical classes for students using GMO's which included risk assessment products and review

Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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<td>L2 L3 L4 L2 L3 L4</td>
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<td>Non-GMMs</td>
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Name

IOTA SCIENCES LTD

Name 2

Department

Campus Estate or Research Centre

CIE BUILDING

Road Name

BEGBROKE HILL, WOODSTOCK ROAD

District

BEGBROKE

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX5 1PF

Country

ENGLAND

Tel Number

01865 309 630

Fax Number

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E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

We have received advice from the previous Professor at the University of Oxford. Now retired, the Professor is an expert in cell biology, and has a 40 years experience in risk assessment of working with various cells, in particular genetically modified cells, and microorganisms. The Professor is still actively engaged in research as an 'Honorary Researcher' at Oxford University.

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</table>
Iota Sciences follows site regulations on the disposal of GM material. All records are kept on site. All waste listed below will be disposed of as Clinical Waste (collected and incinerated by licensed waste contractors, Grundon):-
- Liquid waste (eg. culture, media etc. is first inactivated by treating with Virkon (final concentration of 2% for 12 hours, according to manufacturers instructions) and discarded into laboratory sink via excess water.
- Plastic ware (eg. petri dishes, pipette tips, Eppendorf tubes etc.) are collected into yellow plastic bags inside 60ltr burn bins with lids.
- Gloves, paper towels etc. which have potentially been in contact with GM material are disposed of in yellow bags inside 60ltr burn bins with lids.
- Surfaces are wiped with 70% Ethanol (100% kill).
Waste bins are not accessible to public and located only in the laboratory and carried down directly to Waste Contractors upon collection.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The procedures outlined here are in accord with good laboratory practice (Good Microbial Practice, Good Occupational Health and Safety), and which are in use in the University of Oxford.

Project Ref 3508/19.1

Date Ackn’d  CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4
31/10/2019  Single-cell cloning with iotaSciences’ isoCel1  Class 2  < 1 Litre
**Project Additional Information**

**Purposes of the contained use**

- The purpose of the contained use is to study the efficiency, cell survival and growth kinetics of expanding single induced-pluripotent stem cells (iPSCs) into populations of -20-500 cells.
- iPSCs are genetically reprogrammed somatic cells which exhibit a pluripotent stem cell-like state similar to embryonic stem cells. iPSCs can be derived by inducing selected gene expression via various methods including sendai virus-mediated gene transduction.
- The respective iPSCs will be obtained from commercial entities, for example, Public Health England (PHE) and EBiSC (European Bank for Induced Pluripotent Stem Cells), which are shown to be free of sendai-virus as per PCR-based detection (https://cells.ebisc.org/).

**Recipient or parental organism**

- Of note, iPSCs will be obtained from commercial entities, for example, Public Health England (PHE) and EBiSC (European Bank for Induced Pluripotent Stem Cells). The iPSC cell line(s) were originally generated by academic institutions, for example, the Institute for Neurophysiology, Medical Faculty, University of Cologne (UKK) Germany. The iPSC-cells were derived by reprogramming somatic cells, for example, fibroblasts of dermis (for example, cell line UKKI020-D) or Peripheral Blood Mononuclear Cell (PBMC) (for example, cell line UKKI026B) using Sendai Virus-mediated delivery of reprogramming factors.

**Host/vector system**

- Sendai-virus has been used to reprogram somatic cells into induced pluripotent stem cells (iPSCs). The Sendai virus is a non-integrating virus, which in principle means it does not integrate into the cells genomic DNA. It replicates in the cytoplasm and delivers key genetic elements that produce proteins to reprogram mammalian cells (for example, fibroblasts) into iPSCs.
- Sendai-virus can infect mammalian cells, including human cells in petri dishes and respective laboratory conditions. Upon infection, cells are not capable of producing infectious particles.
Sendai-virus has been used to reprogram somatic cells into induced pluripotent stem cells (iPSCs). The Sendai virus is a non-integrating virus, which in principle means it does not integrate into the cells genomic DNA. It replicates in the cytoplasm and delivers key genetic elements that produce proteins to reprogram mammalian cells (for example, fibroblasts) into iPSCs.

- Sendai-virus can infect mammalian cells, including human cells in petri dishes and respective laboratory conditions. Upon infection, cells are not capable of producing infectious particles.

**Evaluation of foreseeable effects**

SeV (Sendai Virus) vectors used consist of viral proteins NP, P, M, F (activated), HN, and L, and the SeV genome RNA, from which the F gene is deleted.

- Because SeV infects cells by attaching itself to cell surface receptor sialic acid, present on the surface of many cell types of different species, the vectors are able to transduce a wide range of cells. However, they are no longer capable of producing infectious particles from infected cells, because the viral genome lacks the F-gene.

- Therefore, cells infected with SeV are not capable of producing infectious particles.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

- Liquid waste will be collected in a sealed container, and discarded into yellow Biohazard plastic bags inside 60L burn bins and sent for incineration.
- Solid and plastic ware (eg. petri dishes, pipette tips, tubes etc.) that have been in contact with cell culture biohazard waste will be collected into yellow biohazard plastic bags inside approved 60L burn bins with lids and sent for incineration.
- Other waste material that have been in contact with Biohazard materials (eg: tissue, gloves and other non-sharp items that will not puncture the bag) will also be collected into yellow biohazard plastic bags in 60L burn bins. The bag will be tied and carried inside the bin, and the bag handed directly to the Waste contractor for incineration.
- All waste will be packaged according to ADR regulations to minimise risk to human and/or animal health and the environment. All bins will be kept in the lab and handed directly to the Waste Management contractor upon collection.
- All waste will be incinerated through the Clinical Waste! GMO System provided by our Waste Management Contractor, Grundon (GM Authorization Number GM78201.1) that is permitted to accept Class 2 GMM that is noninactivated. Incineration Degree of kill: 100%.
- For routine sterility control of work surfaces: Wipe clean before and after use with 70% ethanol and/or ChemGene High level disinfectant. Degree of Kill: 100%.

**Is an emergency plan required according to regulation 20?**

No

**If yes, tick to confirm that it is attached to this form**

No

**Tick to confirm that you have attached a risk assessment to this form**

Yes
Iota Sciences Ltd. is a spin-out company from the Sir William Dunn School of Pathology, University of Oxford. We have applied procedures outlined here in accordance with good laboratory practice (Good Microbial Practice, Good Occupational Health and Safety), and which are also in use in the University of Oxford.

We have received advice from a Professor at the University of Oxford. Now retired, the Professor is an expert in cell biology, and has a 40 years experience in risk assessment of working with various cells, in particular genetically modified cells, and microorganisms. The Professor is still actively engaged in research as an 'Honorary Researcher' at Oxford University.

### Project Containment

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GM Centre Number: 3510

Data Premises Notified (Originally) 12/11/2019

Transferred from 1992 Regs? N

Transitional Premises

Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

SOUTH TYNESIDE & SUNDERLAND NHS FOUNDATION TRUST

Name 2

Department

Campus Estate or Research Centre

SUNDERLAND ROYAL HOSPITAL

Building

Road Name

KAYLL ROAD

District

Town

SUNDERLAND

County

TYNE AND WEAR

Postcode

SR4 7TP

Country

ENGLAND

Tel Number 0191 565 6256

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The South Tyneside and Sunderland NHS Foundation Trust has appointed an internal Biological Safety Officer (BSO) to advise management on contained use activities and provide approval for the risk assessments which are of GM Class 1 risk level as permitted under the contained use regulations. The BSO has been trained by external providers. The Queen Mary University of London (QMUL) GM Safety Committee and the QMUL BSO under contractual agreement provide expert peer review of risk assessments where risk level is deemed greater than Class 1 or contentious issues arise. The Sunderland Trust BSO is a co-opted member of the QMUL GM safety committee and participates in relevant meetings either in person or via video conferencing. The QMUL GMSC meets at least three times a year and conducts its activities under the terms of reference drawn up according to the contained use regulations and can be viewed at a specific webpage at http://www.hsd.qmul.ac.uk/

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Tick if confidential
Solid GM Class 1 non-sharp waste is either inactivated by an effective disinfection method (noted below) or directly placed into eco-lock bins for uplift and offsite high temperature incineration (900 deg C) by an authorised upper-tier clinical waste contractor (currently Sharp Smart; incinerator location CliniPower, Avonmouth, Bristol). Sharps waste is deposited directly into sharpsafe bins, sealed and uplifted for offsite high temperature incineration by the same clinical waste contractor / incinerator. High temperature incineration achieves 100% kill of the GMM.

Liquid waste (or as necessary, for solid waste) is disinfected by an effective disinfectant shown to give > 5 log kill of the GMM. Virkon (final concentration 1% w/v) is the current effective disinfectant shown by the supplier under defined ‘suspension’ standards for liquid samples to give 5 log kill of the viral GMMs proposed for use. Liquid waste will then be disposed with copious tap water down designated sluice / lab sinks, in line with the local Water Authority effluent permit conditions. Any new disinfectant to be used will be shown to provide 5 log kill or greater before authorised use.

Surface decontamination (e.g. lab / ward benches) is to be conducted with virkon (1% w/v). Emergency spillages will also be treated with virkon to a final concentration of 1% w/v.

Disinfection protocols are being set up and applicable personal protective equipment donned / worn during handling. Training of GM users / workers in the use of disinfection procedures is recorded and refreshed according to local policy.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The attached risk assessment for Class 1 work has been peer reviewed by the QMUL BSO and approved for submission. Future risk assessments and any amendment required to this notification will be peer reviewed by the QMUL BSO and where relevant, the QMUL GM safety committee (as per contractual arrangements noted in section 3).
## GM Centre Number: 3511

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### Name

**PA CONSULTING**

### Name 2

**Department**

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### Date at Which Additional Info Submitted

2022/03/02
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

As this is the first (and currently only) GMAC2 project at this premise, the GMSC has been constituted specifically for this project. The committee consists of:
- Health and Safety Manager (MSci, CMIOSH), who leads the compliance efforts in this area
- Member of PA's management team, with responsibility for quality and compliance in the group
- 3 members of the project team specialising in microbiology, biomaterials and physics
- Project team lead ('assignment manager') with direct responsibility for safety
- Microbiologist with PhD and BSO training independent from the project
- Independent member (group Head of Operations)

If further GM work is to be undertaken, the GMSC membership will be reviewed and it will be reconstituted as appropriate.

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</table>

02/03/2022
Liquid wastes will be chemically deactivated using a disinfectant with validated efficacy against the microorganism, typically Virkon. The deactivated waste will then be disposed of via clinical waste. Solid wastes will be sealed in a medibin in the laboratory then incinerated as clinical waste.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The comments of the GMSC have been incorporated into the risk assessment.

Project Ref 3511/19.1

Date Ackn’d 15/11/2019

Date Project Ceased

Withdrawn N

Tick if notifying a connected programme of work N

Project notified under transitional arrangements N
Project Additional Information

Purposes of the contained use

Functional testing of prototype designs and the final production system. GMMs only to be used when components/prototypes have been fully tested with surrogate materials.

Recipient or parental organism

Jurkat Clone E6-1 T-cells, or similar non-hazardous T-cell lines [commercially purchased]

Host/vector system

rLV.EF1.ZsGreen1-9, or similar lentiviral vectors encoding non-hazardous proteins [commercially purchased]

Origin & function

GFP marker protein

Evaluation of foreseeable effects

No foreseeable adverse effects in-vivo, however notification reflects the unlikely potential for re-acquisition of capsid genes, in line with the classification of the vector supplier.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid wastes will be chemically deactivated prior to incineration as clinical waste
Solids will be incinerated as clinical waste

The comments of the GMSC have been incorporated into the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Please enter comments on the GM safety committee on the risk assessment

The comments of the GMSC have been incorporated into the risk assessment

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

Large Scale Activities

Human Clinical Applications

02/03/2022
GM Centre Number: 3514

Data Premises Notified (Originally) 02/12/2019

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

BIT BIO LTD

Name 2

Department

Campus Estate or Research Centre

Building

THE DOROTHY HODGKIN BUILDING

Road Name

BABRAHAM RESEARCH CAMPUS

District

BABRAHAN

Town

CAMBRIDGE

County

CAMBRIDGEShire

Postcode

CB22 3FH

Country

ENGLAND

Tel Number 01223 787297

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>CB22 3FH</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Yes**

Give brief details of the genetic modification safety committee

GM committee comprises of members of staff at various levels in the company. Head of Research and Development, Head of Laboratory Operations who is the Biological Safety Officer, Senior Scientists.

Other members of the committee have worked with GM material and also have carried out the work in the past with viral vectors. We have a wide range of Knowledge working with biological materials from an academic research environment, industry and the NHS.

The biological safety committee will meet on a 6 monthly basis and will produce a report and send minutes to the main safety committee. This then in turn will be submitted to the board of directors.

Any risk assessments for review prior to committee meeting, a meeting will be set up to discuss.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 4 (GMMs)</td>
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</table>
### For activities involving GMMs, describe the waste management measures which will apply to the activity

All the waste generated will be handled by the Babraham Institute and with accordance to the regulations set out by Babraham Institute, and will seek derogation, however this is an outline:

- **Usual chloride containing disinfectant (e.g. 1% solution of Virkon) or other chemical disinfectant (e.g. Chemgene 2%) overnight, Autoclaving/incineration of contaminated labware or laboratory coats. Effectively 100% inactivation of virus.**
  - Autoclaves regularly serviced, temperature validated and calibrated
- **All disposable culture/labware from the CL2 laboratory decontaminated with 2% Chemgene or 1% Virkon prior to being placed in yellow Eurobin (red lid) before leaving the building for off-site incineration.**
- **All highly contaminated material i.e. all items that have been in contact with viruses (pipettes, tips, culture plates) will be separately decontaminated (2%**
Chemgene. 1% Virkon) before placing in red lid yellow Eurobin.

- Liquid waste capture bottles have 1% Virkon added overnight before autoclaving. This then to be discarded to drain flush with copious amounts of water.
- Small spills will be removed after surface soaking with 2% Chemgene, left to deactivate for at least 10 minutes, followed by further decontamination with 70% ethanol.
- Large accidental spills will be sprinkled with neat Virkon powder before cleaning after 10 minutes.
- Bench and cabinet surfaces will be wiped down with 2% Chemgene and 70% Ethanol.
- Large spill outside of MSCII will be covered up with Virkon powder and the room then evacuated and entry be restricted for 1 hour to allow for 6 air changes to remove any aerosols.
- All GM modified cell cultures will be destroyed (soaking with 1% Virkon overnight and the placed in Red Lid Yellow Eurobin for incineration) or chemically fixed (e.g. 1-4% Formaldehyde, guanidine isothyocianate, RIPA) before leaving containment.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
information is on the CU2 notification application form.

---

**Project Ref**  3514/19.1

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<td>13/12/2019</td>
<td>Differentiation of mammalian cells by cellular programming approaches: genetic intervention by recombinant viral vectors, episomal or other DNA plasmids to generate relevant cell models. For basic and medical research</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
<td>Non-GMM</td>
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</table>

Tick if notifying a connected programme of work Y

Withdrawn N

Historical Significant Changes
### Purposes of the contained use

Our main objective is to understand the molecular mechanisms controlling lineage differentiation from pluripotent stem cells in order to develop cells relevant to basic and medical research and applications. The research projects will include transduction of a variety of genes with expected biological effects into mammalian cell lines and primary cells by the means of replication deficient recombinant viral vectors episomal or other DNA plasmids.

Use of recombinant viral vectors, episomal and DNA plasmid vectors to introduce genetic material into mammalian cell lines, including primary and iPSCs. The cells are derived from human tissue expanded outside of the body and also various mammalian species including bovine, porcine and rodent.

Our main objective is to understand the molecular mechanisms controlling lineage differentiation from pluripotent stem cells. The research projects will include transduction of a variety of genes with expected biological effects into mammalian cell lines and primary cells by the means of replication deficient recombinant viral vectors (including lentiviral, Sendai viral vectors) and also episomal and DNA plasmid vectors.

By identifying and understanding fundamental development processes, our projects should allow us to better control in ex vivo differentiation protocols and improve the production of therapeutically relevant cell types, for example neurons, muscle cells and blood platelets.

After the R&D processes the final iPSC lines will be made commercially available for use in research, drug discovery and eventually therapeutically. These cells will be free from viral vectors however they will be genetically modified.

### Recipient or parental organism

- Escherichia coli, K12 derived strains
- Mammalian cells: iPSC (human and other mammals)
- Mammalian cells: Primary Human origin (Skin fibroblasts, blood cells, immortalised cell lines)
- Mammalian cells: Bovine/porcine Primary Inhouse

### Host/vector system

- Vector (1): replication deficient recombinant lentiviral vectors
- Vector (2): Sendai: F temperature sensitive derived vectors. (Cytotune, SeV-hNanog)
Origin & function

- cDNA (Type1 inserts) will code for transcription factors, signal transducers, growth factors and receptors which are expected to play a role in pluripotency, cell fate decision and differentiation from pluripotent stem cells (PSCs).
- shRNA (Type2 inserts) will produce non-coding RNA sequences targeting endogenous mRNA from above mentioned families (Type1 list). Expression of both insert types may enhance/block commitment and differentiation of PSCs towards one of the three primitive germ layers and further differentiated progeny.
- Reporter genes (Type3 inserts) will code for proteins with fluorescent or enzymatic properties allowing easy identification of genetically modified (GM) cells. They are thought to have no deleterious biological effect on expressing cells (for example EGFP, ECFP, EBFZ, EYFP, dTomato, LSSorange, LacZ, Luciferase, possibly fused to cDNA (type1 inserts)).
- Selection genes (Type4 inserts) will produce enzymatic proteins able to inactivate specific antibiotic families allowing selection of GM cells. They are thought to have no deleterious biological effect on expressing cells (for example NPT, PAC, Puro, Neo, Hygro, Blast).
- Functional non-coding sequences (Type5 inserts) will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES). They will be used (independently or in combination) into viral expression cassettes to achieve best control of transgene expression depending on targeted cells (for example promoter, enhancer, insulators, IRES, WPRE, of mammalian or viral origin).
- Neutral non-coding sequences (Type6 inserts) will be used in particular for gene targeting projects to promote homologous recombination at defined genomic loci (intrinsic/exonic genomic sequences). They should not have biological effect by themselves.
- Expression cassettes for guide RNA and CAS9 DNA nucleases (Type 7 inserts) variants of the CAS9 system, eg, dCAS9, dCAS9-KRAB

Evaluation of foreseeable effects

TYPE1, cDNA Type1 inserts will code for transcription factors, signal transducers, growth factors and receptors which are expected to play a role in pluripotency, cell fate decision and differentiation from pluripotent stem cells (PSCs). It is difficult to predict in vivo consequences of ectopic expression of all these genes on human health. However, since they are key regulator of cell identity, their overexpression could lead to pathological modifications of cell phenotype/function. Moreover, several of these genes have been involved in oncogenic processes and uncontrolled expression could subsequently initiate tumoral transformation. However, malignancy is a complex multistep process which involves multiple genomic alterations and a single "hit" is unlikely to trigger alone the oncogenic process. Experiments involving co-transduction or even co-expression by the same vector of several inserts should consequently be considered at higher risk for the worker. Notably, reprogramming experiments of somatic cells toward pluripotency (co-transduction with the 4 factors OCT4, SOX2, MYC, KLF4) could imply a specific risk since accidental genetic modification of worker cell could theoretically lead to teratoma formation.

TYPE2, shRNA/miRNA Type2 inserts will produce non-coding RNA sequences able to inhibit protein expression from the group of genes mentioned as Type1 insert. As for Type1 insert, it is difficult to predict in vivo consequences of ectopic expression of these sequences on human health. However, since they target key regulators of cell identity, their expression could lead to pathological modifications of cell phenotype/function. Notably, several miRNA have been implied in oncogenic processes.

TYPE3, reporter gene Type3 inserts will code for proteins with fluorescent or enzymatic properties allowing easy identification of genetically modified (GM) cells. They are thought to have no deleterious biological effect on expressing cells excepted acute toxicity at very high concentration.

TYPE4, selection gene Type4 inserts will code for enzymatic proteins able to inactivate specific antibiotic families allowing selection of GM cells. They are thought to have no pathological effect on expressing cells excepted acute toxicity at very high concentration.

TYPE5, functional non-coding sequence Type5 inserts will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES). They will be used (independently or in combination) into the expression cassette to achieve best control of insert expression depending on the targeted cell. No direct pathological effect is expected for these sequences. However, indirect deleterious effects should be considered for promoter and enhancer sequences through retrovirus mediated genomic integration in the host cell and potential insertional mutagenesis. Indeed, strong
promoter/enhancer could lead to ectopic activation of neighbour endogene expression. Moreover, the WPRE sequence has been shown to have oncogenic properties by itself.

TYPE6, neutral non coding sequence Type6 inserts do not have any foreseeable deleterious biological effect. They will be used in particular for gene targeting projects to promote homologous recombination at defined genomic loci.

Type 7 inserts expression cassettes for guideRNA and CAS9 DNA nickase (and variants of the CAS9 system, eg, dCAS9, dCAS9-KRAB). The coded proteins have nuclease or epigenetic remodelling activities which might create DNA double strand breaks and mutagenesis, or impact on cell gene expression profile respectively, possibly leading to biological perturbations.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

The hazardous waste is managed by the Babraham Institute which has a derogation see below. If bit bio operates at a separate site in the future the derogation below will only apply to bit bio facilities located on the Babraham Institute site.

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM 105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:

1. We do not have access to an autoclave within our area of the building

2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004. All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed -Their GM authorization is GM898) according to disposal notification GM105/4.1. Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site has 24/7 Security.

The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Please see information in section 11 as all the waste generated will be handled by the Babraham Institute and with accordance to the regulations set out by Babraham Institute.

- Usual chloride containing disinfectant (e.g. 1% solution of Virkon) or other chemical disinfectant (e.g. Chemgene 2%) overnight, Autoclaving/incineration of contaminated labware or laboratory coats. Effectively 100% inactivation of virus.

Autoclaves regularly serviced, temperature validated and calibrated
- All disposable culture/labware from the CL2 laboratory decontaminated with 2% Chemgene or 1% Virkon prior to being placed in yellow Eurobin (red lid) before leaving the building for off-site incineration.

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- Bench and cabinet surfaces will be wiped down with 2% Chemgene and 70% Ethanol.

- Large spill outside of MSCII will be covered up with Virkon powder and the room then evacuated and entry be restricted for 1 hour to allow for 6 air changes to remove any aerosols.

- All GM modified cell cultures will be destroyed (soaking with 1% Virkon overnight and the placed in Red Lid Yellow Eurobin for incineration) or chemically fixed (e.g. 1-4% Formaldehyde, guanidine isothiocyanate, RIPA) before leaving containment.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment  

Please enter comments on the GM safety committee on the risk assessment
Q1. How should the tubes be handled before moving to storage? Chemgene and ethanol?

All tubes should be wiped with 2% chemgene before moving in to storage as per RA justification to move out of containment.

Q2. How are the cells generated from vector batches tested for RCR? and how do you know they are free from RCR?

This is carried out by quality testing by transducing Supp-T1 cell lines with 5% of the vector batch, leave to grow for 14 days and monitor P24 levels in supernatant at day 7+14. The P24 levels are detected by ELISA.

Q3. Final handling of GM at containment level 1 or 2 needs to be explained further.

This has now been addressed in the RA by the following statement:

"The final handling of the resultant GM cell line must then revert back to the containment level that the cell line assigned due to and other biological factors, set out in the subsequent biological risk assessment that is summarised in the table on page 19. Therefor if the resultant GM handling can subsequently be moved from CL2 to CL1 and the biological risk assessment has assigned the cell line to containment level 2 then the cell line must be used at the containment level originally designated."

Q4. Justification of removal of material from containment for imaging?

The committee would like this to be reduced to a minimum and that imaging system in the CL2 lab may reduce the risk. This has been addressed in the risk assessment as follows:

Removal form CL2 is to be reduced to minimum, possibly by having imaging systems in CL2 labs. Ideally, this should not happen with culture recently exposed to viral vectors where viral load in medium is still significant. If necessary, additional measures like not opening the secondary container for >5min to protect from any aerosols generated, and ensuring no spills happened during transport should be considered.

An emergency procedure for spillage at CL1 should also be in place.

**Project Containment**

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<thead>
<tr>
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**Name**

WREN THERAPEUTICS LTD

**Campus Estate or Research Centre**

**Road Name**

CLARENDON ROAD

**Town**

CAMBRIDGE

**Country**

ENGLAND

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 8FH

**Tel Number**

01223 763282

**Fax Number**

0

**E-mail**

blank

**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Yes

Give brief details of the genetic modification safety committee

- Departmental and Biological Safety Officer
- Department of Chemistry, University of Cambridge
- Departmental and Health and Safety Officer
- Department of Chemistry, University of Cambridge
- Wren Biological Safety Officer, Wren therapeutics Limited, Cambridge

#### Laboratory

- Level 1 (GMMs)
  - Yes
- Level 2 (GMMs)
  - Yes

#### Animal Unit

#### Growth Room

#### Glass House

#### Large Scale

Other (please specify) Tick if confidential
<table>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

All biological waste is treated and properly disposed of.
For liquid waste, inactivation is done with Virkon (1% solution stored away from direct sunlight and changed weekly or once colour is lost; if it is not pink, it does not work).
Surfaces and spills can be cleaned with 70% Ethanol.
Appropriately labelled red bins are present in the labs for Autoclave waste and incineration with an external contractor Novus Environmental.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All biological work is in compliance with the Department of Chemistry and Wren Therapeutics safety instructions.
**GM Centre Number: 3516**

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**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

![Y]  

Give brief details of the genetic modification safety committee  

| Genetic Modified Safety Committee at the Bioescalator innovation building, part of Oxford University.  
| Chair person; Bioescalator Laboratory Manager  
| Committee consists of various representatives from start-up companies in the Innovation building. And representative safety officers from the University of Oxford |

<table>
<thead>
<tr>
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<th>Animal Unit</th>
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<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
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Other (please specify)  
Tick if confidential  

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
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<th>Microbiology Research</th>
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### For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
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<tr>
<th>Mycology</th>
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<th>Transgenic Plants</th>
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<td>Yes</td>
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<tr>
<td>Other(s)</td>
<td>Transgenic and CRISPR edited mammalian cell lines, and human primary cell lines</td>
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</table>

All contaminated solid waste (plasticware e.g. pipettes, flasks, tubes) inactivate by autoclave using a make safe cycle at 134°C for at least 3 minutes and then collected by Waste contractor for incineration.

All contaminated Liquid waste (e.g. samples, culture supernatants, tissue culture media) inactivated with Virkon to 2% w/v for at least 60 minutes and then discharge to drains.

For the GMO risk assessment all materials are classified as class I, however the laboratories meet the standard for Containment Level 2 due to other laboratory work. All work performed with good laboratory practices and observation of established health and safety local rules for containment and disposal of GMOs.

Tick to confirm that you are attaching a summary of the risk assessment: Yes

Tick if you are claiming exemption from disclosure for sections of the risk assessment: No

### Please enter comments of the GM safety committee on the risk assessment

The Bioescalator GMSC concluded the proposed work present low risk work using commercially available materials and that the use of Containment Level 1 measures would be adequate to reduce the risks to negligible. Therefore the GMSC thought that the proposed work with genetically modified micro-organisms should be classified as Class 1. Further, it was noted the laboratories in the Bioescalator Innovation building exceed the requirements and meet containment level 2.
<table>
<thead>
<tr>
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**Name**

| PORVAIR SCIENCES LTD |

**Name 2 Department**

| |

**Campus Estate or Research Centre**

| UNIT 73 |

**Road Name**

| CLYWEDOG ROAD SOUTH |

**District**

| WREXHAM INDUSTRIAL ESTATE |

**Town**

| WREXHAM |

**County**

| |

**Postcode**

| LL13 9XS |

**Country**

| WALES |

**Tel Number**

| 01978 661144 |

**Fax Number**

| 0 |

**E-mail**

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**HSE Division**

| |

**Comments**

| |

**Date at Which Additional Info Submitted**

| 02/03/2022 | Page 14764 of 15326 |
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

<table>
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<tr>
<th>Level 1 (GMMs)</th>
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<td>Yes</td>
<td>Yes</td>
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</table>

Webster’s Biosafety Ltd have reviewed and commented on the risk assessment. Webster’s Biosafety has over 20 years experience in reviewing GM and Wild type risk assessments.

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

All contaminated materials, including waste destined for incineration, will be inactivated by onsite autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be calibrated and serviced annually by an approved supplier and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Rodwell, TST class 6 emulating indicator 121°C for 20 min). Autoclaving will be carried out using the autoclave located in the room adjacent to the Microbiology laboratory and all waste will be placed in autoclave bags and sealed before transfer between the two rooms.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Assessment was reviewed and comments made. The reviewer agreed with the classification of the activity as a Class 1 GMM.
### GM Centre Number: 3519

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#### Comments

**Date at Which Additional Info Submitted**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Sense GMO committee with three members of staff including representatives of research and development, management and health and safety. All committee members are highly experienced in research and development and laboratory management gained at multiple different organisations. Representatives hold extensive first-hand experience in microbiology and health and safety procedures for cloning and protein expression using Class 1 microorganisms.

<table>
<thead>
<tr>
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<th>Animal Unit</th>
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<th>Large Scale</th>
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Tick if confidential

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<th>Microbiology Research</th>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

GMM contaminated material and all liquid waste will be deactivated by 5000ppm Presept solution. All solid waste will be autoclaved in the same laboratory as GMM handling. Autoclave designed to operate at 121 °C for 28 minutes, for the sterilization of glassware and consumables used with GMM's. Non-autoclavable materials, and laboratory consumables contaminated with cell culture material, shall be disposed of in a solution of 5000ppm Presept solution and soaked overnight. Presept disinfectant will be made up fresh just prior to use. Waste decontaminated materials should be double bagged in yellow hazardous waste bags and sent for incineration. Appropriate process testing carried out on deactivated material to validate safe waste management.

Tick to confirm that you are attaching a summary of the risk assessment ☐

Tick if you are claiming exemption from disclosure for sections of the risk assessment ☐

Please enter comments of the GM safety committee on the risk assessment

Risk assessment reviewed in detail by Sense GMO committee and approved.
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**Data Premises Closed**

**Emergency Plan Required?**

**Transitional Premises**

**Non-GMMs**

**Withdrawn**

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**Name**

MOTE RESEARCH LTD

**Name 2**

MILNER THERAPEUTICS INSTITUTE

**Campus Estate or Research Centre**

JEFFREY CHEAH BIOMEDICAL CENTRE

**Building**

UNIVERSITY OF CAMBRIDGE

**Road Name**

PUDDICOMBE WAY

**District**

CAMBRIDGESHIRE

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 0AW

**Country**

ENGLAND

**Tel Number**

01223 804060

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

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**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
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</table>

Give brief details of the genetic modification safety committee

The Mote Research Ltd genetic modification committee will consist of:

1. The biosafety officer who has extensive experience of genetic modification issues, being currently in charge of biosafety for a large research institute. The biosafety officer will chair all genetic modification committee meetings.
2. The Chief Executive Officer has 30+ years experience of genetic modification and previously ran research groups in academia. His function on the committee is to advise and provide the necessary resources for the actions of the committee to be implemented. He will write the minutes of the meetings.
3. Postdoctoral scientist who has >10 years experience of genetic modification in E. coli and mammalian cells. He will provide advice and ensure the actions arising from the committee meetings are implemented.

The committee will meet every 6 months to review biosafety and whenever a new risk assessment is required. The first meeting will review the existing risk assessments.

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<td>Level 2 (GMMs)</td>
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Level 4 (GMMs)

Non-microbial

Other (please specify)  

Tick if confidential  

Bacteriology  Yes  Parasitology  Transgenic Birds  Microbiology  Research Virology  Transgenic Animals  Transgenic Fish  Gene Therapy Mycology  Transgenic Invertebrates  Transgenic Plants  Other (please specify below)  Yes

Other(s)  

In addition animal cell culture will be performed using the immortal cell line….

For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

Project Ref  3520/20.1

Date Ackn'd  02/03/2022  

CU2 Project Title  Class  CultureVolClass2  CultureVolumeClass3-4
The culture and use of mammalian cells believed to harbour Epstein Barr Virus (EBV)

Purposes of the contained use
Part of our work is aimed at selectively killing EBV-containing cells. For this reason, we need to be able to grow such cells so that we can study them.

Recipient or parental organism
The mammalian cell lines include human-derived lines such as Daudi (ATCC® CCL-213), a well-known and widely used B lymphoblast cell line. This cell line is known to harbour EBV.

Host/vector system
In the first instance, we will not be using any viral (replicable) vectors with these cells. Instead, they will be transfected with plasmids not capable of replicating in mammalian cells.

Origin & function
Selectable markers – examples (but not restricted to);
Ampicillin resistance: E. coli derived, and not expected to be expressed in mammalian cells (this is a selectable marker used for preparing plasmids in E. coli).
Green Fluorescent Protein: this is a fluorescent protein, expected to be expressed in mammalian cells, and serving (by fluorescence) to confirm that the plasmid has entered the mammalian cell.

Evaluation of foreseeable effects
The only foreseeable effects arise from the Epstein Barr Virus (EBV) present in this cell line, rather than from any modification or work done on the cell line. EBV is an extremely common virus (approximately 90% of adults worldwide have had EBV) which usually causes no or mild symptoms (fever, fatigue) in adults contracting it for the first time; symptoms normally resolve without treatment. Individuals who have already had EBV are not susceptible.
Given the abundance of the virus in the general population, working with EBV-infected cells does not create a risk to
the wider population.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:

1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed – Their GM authorization is GM898) according to disposal notification GM105/4.1.

Waste from our GM work at Class2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this.

This disposal method is expected to achieve 100% inactivation of the GMM.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.

This disposal method is expected to achieve 100% inactivation of the GMM.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The safety committee was convened on 15 December 2019. The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities, the risk assessment and related work activities were deemed suitable.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
<tr>
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02/03/2022
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Name

CORNATURAL LTD

Name 2

Department

Campus Estate or Research Centre

TRANSLATIONAL & INNOVATION HUB

Building

IMPERIAL COLLEGE WHITE CITY CAMPUS

Road Name

80 WOOD LANE

District

Town

LONDON

County

GREATER LONDON

Postcode

W12 0BZ

Country

ENGLAND

Tel Number

07548 868254

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Lecturer and Principle Investigator and at the Centre for Synthetic Biology, Imperial College London with more than 10 years of lab experience working with genetically modified organisms. He heads a lab group composed of 15 scientists where he is responsible for the genetically modified organisms and conducts a thorough risk assessment for their use every year. Collectively his experience allows him to understand the risks to both human health and the environment arising from the proposed contained use activity and should any uncertainty arise from the activity, he has sufficient experience to be able to judge the adequacy of the risk assessment and the controls that are being put in place and have recourse to external expertise if required. As CorNatural is based in the Imperial I-Hub, access to advice from the Imperial College GM committee (HSE reference GM31) is permitted if required.

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<tr>
<td>Non-microbial</td>
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Other (please specify) Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity

For Liquid waste - Virkon is added to 1% final solution. Incubated for 20 minutes for E coli cultures and 30 minutes for yeast.
For Solid waste - Dispose of solid waste in the autoclave bins provided. The bins will be changed when 3/4 full. The bag will be gathered at the top, sealed loosely to allow steam penetration, and placed in a plastic box before transportation to the autoclave facility. The autoclave is calibrated and then validated annually by a UKAS accredited service engineer with a worst case representative waste load to check that it achieves 121 degrees for 15 minutes throughout the chamber during a waste cycle.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

During the development of the risk assessment, the following recommendations were made, which were subsequently implemented:

2.14. - Mention the boundaries of the work - That we will not introduce any genes that would make the host organisms pathogenic, alter their host / environmental range, increase their ability to survive in the environment or in any other way make them more harmful to human health or the environment than the original host organisms.

2.18 - Add that no frontline therapeutic antibiotics will be used.

7.1 - Solid waste. Also state that the autoclave is calibrated and then validated annually by a UKAS accredited service engineer with a worst case representative waste load to check that it achieves 121 degrees for 15 minutes throughout the chamber during a waste cycle.

7.6 - Elaborate on proper disposal and how waste is dealt with.

Besides this no further comments agreed GM Class 1.
GM Centre Number: 3522

Data Premises Notified (Originally) 31/12/2019

Transferred from 1992 Regs? N

Transitional Premises

Emergency Plan Required? N

Data Premises Closed

Transitional Premises Withdrawn N

Non-GMMs N

Name

ECHA MICROBIOLOGY LTD

Name 2

Department

Campus Estate or Research Centre

UNIT 22 & 23 WILLOWBROOK TECHNOLOGY

Road Name

LLANDAGO ROAD

District

Town

ST MELLONS

County

CARDIFF

Postcode

CF3 0EF

Country

WALES

Tel Number 02920 365 930

Fax Number 0

E-mail

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Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Biological Safety Officer,
Prokarium,
London Bioscience Innovation Centre,
2 Royal College St,
London,
NW1 0NH,
UK.

<table>
<thead>
<tr>
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<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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</table>

Tick if confidential

02/03/2022
Solid waste contaminated with GM Class 1 material will be segregated from other non-GMO waste generated in the laboratory.

Class 1 GMO waste will be sealed in autoclavable waste bags and inactivated by autoclaving at 134degC and 15 psi for 15 minutes; after which the solid waste will be placed in clinical waste bins.

Liquid waste will be inactivated by the addition of sodium hypochlorite to a final concentration of 1% and minimum contact time of 1 hour (resulting in a six order-of-magnitude reduction in cell viability), after which the deactivated waste will be placed in clinical waste bins.

Sealed clinical waste bins will be collected by a licenced waste carrier.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Bacterial strains have been administered to human volunteers and were well tolerated (Hindle et al. 2002 Inf Imm. Vol. 70-7 pg 3457–3467; Tennant & Levine 2015 Vaccine C36-C41; Hien et al. 2010 Plos One 5: e11778; Darton et al. 2016 Plos NTD 10: e0004926) and severely immunocompromised adults chronically infected with hepatitis B virus (He 2007, Trends Biopharma Ind 3: 40-45). These direct evidences in humans enable these mutants to be classified as GM Class 1.
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</table>

**Name**

AMPHISTA THERAPEUTICS LTD

**Campus Estate or Research Centre**

**Road Name**

158-160 NORTH GOWER STREET

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

NW1 2ND

**Country**

ENGLAND

**Tel Number**

07888 728644

**Fax Number**

0

**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

There is a genetic modification safety committee in place with 4 members comprised of the COO, Laboratory Manager, the department head and a member of the laboratory staff. They meet quarterly to discuss the use and containment of GMs within the lab. The committee members collectively have significant experience in microbiology, cell biology and virology, including but not limited to the proper handling, storage and disposal of GMMs and associated materials. All risk assessments are reviewed to ensure they are up to date along with ensuring personnel training is up to date for the safe handling of GMs within the lab.

<table>
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</table>
Liquid decontamination - once the GMs have been used and are no longer required, they are decontaminated in 1 %
virkon for 60 minutes as per manufacturers instructions and flushed down the sink along with excess cold water.
Waste Procedure - the contaminated labware is placed in an orange ridged sided box so it cannot pierce through and
is sent to our waste management firm for disposal. Contaminated personal protective equipment is placed into
orange clinical bags for pick up by the same waste management firm.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The Genetic Modification Safety Committee have> 25 years combined experience handling various GMOs, up to
biosafety category 3, and have written risk assessments on each of these organisms.

Date Ackn’d
CU2 Project Title
07/08/2020
Genetically modified mammalian cells for drug discovery research
Class CultureVol
Class2 CultureVolume
Consent Granted
Non-GMM < 1 Litre

Project notified under transitional arrangements
### Purposes of the contained use

The purpose is to generate, maintain and characterise stably transfected or CRISPR-engineered mammalian cells that express proteins of interest to our drug discovery work. Additional work may involve the knockdown of proteins of interest using shRNA. These all include proteins that are directly relevant to therapeutic development as well as those that would be considered tool proteins required for specific experiments.

### Recipient or parental organism

Genetic modifications will be carried out on frequently used immortalised mammalian cell-lines relevant to drug discovery and specific therapeutic indications. This will include cell-lines derived from myeloid cells, colorectal, lung, pancreas, prostate, liver, and breast cancers, and the HEK293T cell-line. In some cases, these cell-lines will have already undergone genetic modification by a commercial supplier using the CRISPR/Cas9, and then procured prior to subsequent in-house modification of the form discussed in this document. Infected cells will be selected for using culture medium supplemented with appropriate antibiotics (such as neomycin, puromycin, hygromycin or blasticidin).

### Host/vector system

Lentiviral particles carrying cDNA or shRNA constructs will be procured from commercial suppliers (e.g. SMARTvector Lentiviral Non-targeting control shRNA, Horizon VSC7078) and the obtained lentiviral vectors are self-inactivating, making them replication deficient. When procurement is not possible, a Trans-Lentiviral ORF Packaging System from Dharmacon will be utilised, according to manufacturer guidelines. This is a multicomponent, 4th generation system that minimises the possibility of producing replication competent viruses. In this system, constructs of interest will be cloned into commonly used lentiviral vectors (i.e. pLenti7), which will then be transfected alongside plasmids encoding lentiviral packaging genes, representing 4th generation technology. As a safety precaution, viral packaging components are separated onto five plasmids and expression of gag-pro and tat-rev are under the control of the conditional tetracycline-responsive promoter element (TRE), limiting expression of these viral components strictly to cells expressing the tetracycline transactivator. The HEK293T cell-line will be used for viral packaging.

### Origin & function

The genetic material will be cDNA constructs (designed either for gene expression or shRNA-mediated gene silencing) encoding a number of human genes. These genes are well studied and characterised, with information on them available in the public domain. These genes will typically be either relevant to clinical disease (i.e. oncogenes) or significant to our specific drug discovery work. In some cases, commercially available, proprietary cDNA constructs from Promega's NanoLuc system will be used.
These constructs are originally from the shrimp Oplophorus gracilirostris but have since undergone significant modification by Promega. Such genetic material will, once introduced into immortalised cell-lines, act as ‘tool proteins’ and aid in our drug discovery work.

### Evaluation of foreseeable effects

The introduction of gene-expressing cDNA or gene-silencing shRNA constructs is not expected to cause any hazards to human health. These modifications could change the phenotype of the modified cell, potentially turning a normal cell into a cancerous one. However, these modifications are being made in already immortalised mammalian cells predominantly derived from cancers. These cell-lines are, by default, not capable of colonising healthy individuals nor are the genetic modifications likely to change this.

Cells modified using Lentiviral transfection are not expected to release infectious viral particles. Commercially sourced Lentiviral vectors will be acquired in a replication deficient state. Lentivirus vectors produced in-house will have been prepared using a commercially used 4th generation packaging system which encodes the required helper, structural and scaffolding genes into 5 separate pTLA1 plasmids lacking homology with the pLenti7 vector. This system also places the necessary gag-pro and tat-rev genes under tetracycline control (preventing expression in cells lacking the tetracycline transactivator gene). This packaging system has been tested for virus production with negative results.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable for this project

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

M8 - There is an autoclave available in the same building, but does not possess sufficient capabilities to meet our waste output. Contaminated labware and PPE is placed in yellow ridged sided boxes or orange clinical bags so it cannot pierce through for pick up by and off-site incineration by our waste management firm.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Liquid decontamination - once the GMs have been used and are no longer required, they are decontaminated in 2% virkon for 60 minutes as per manufacturers instructions and discarded into liquid waste.

Waste Procedure - the contaminated labware is placed in a yellow ridged sided box so it cannot pierce through and is sent to our waste supplier for disposal. Contaminated personal protective equipment is placed into orange clinical bags for pick up by the same waste management firm.

### Is an emergency plan required according to regulation 20?

N

If yes, tick to confirm that it is attached to this form

N

Tick to confirm that you have attached a risk assessment to this form

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment

Y

Please enter comments on the GM safety committee on the risk assessment
The Genetic Modification Safety Committee have > 25 years combined experience handling various GMs, up to biosafety category 3, and have written risk assessments on a large variety of organisms.

The risk assessments are prepared by proposers according to HSE and SACGM guidelines. Together with the appropriate GM Notification/assessment form, the proposals are then circulated to all members of the GMSC who return their comments to the proposers for their response and possible amendment of the proposals or risk assessments. The final submissions are discussed by the GMSC members and the required containment level is agreed.

For the proposal herein, agree to Class 2, Containment Level 2. Notifiable to the HSE.

**Project Containment**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

**Y**

Give brief details of the genetic modification safety committee

Director of Development and Scientific Operations. Qualifications - BSc, MSc, PhD. 16 years of experience specific to use and risk management of category I GMOs. Two years experience as a member of the University of Nottingham genetic modification safety committee. Biological Safety Officer Practitioner qualification - Public Health England.

Senior Scientist - BSc, MSc. 8 years of experience working in a GM laboratory.

Laboratory Manager - BSc, TechIOSH. 10 years of general laboratory experience, NEBOSH general certificate.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

- Media & broths - 1/50 or 2 % concentration of approved disinfectant (Distel/Chemgene) to media/broth with a contact time of 60 minutes, then disposal down laboratory sink.

- Plastics, agar plates and cell pellets - deposit in clinical waste bins which are double bagged prior to sterilisation by autoclave using discard program (130 °C for 10 minutes).

- Tips & sharps - Deposit in yellow sharps bins, then sterilise in autoclave using discard program (130 °C for 10 minutes).

- Serological pipettes - Deposit in 'Biobins', then sterilise in autoclave using discard program (130 °C for 10 minutes).

- Flasks and glassware - 1/50 or 2 % concentration of approved disinfectant (Distel/Chemgene) to empty flasks, contact time of 60 minutes, then sterilise in autoclave.
using equipment programme (121 °C for 15 minutes).

Autoclave: Automated built in jacketed vacuum autoclave (Astell ASB300) for use by Oncimmune staff (following training & SOP) in Oncimmune service laboratory. Two cycles (discard & equipment) will be validated prior to use (externally by installation engineers using thermocouple) to determine degree of kill >5 log. Programmes will also be re-validated annually. Autoclave will also be serviced annually. Each run will be verified (print out record stored) to ensure each cycle has met the required criteria for successful sterilisation of each load. All loads will then be transported off site for incineration (service provider to be confirmed).

Disinfectant: Distel or chemgene will be used to inactivate GMMs and DNA/RNA from media/liquid, glassware and spillages. The disinfectant concentrations quoted are at or higher than the recommended concentration by the manufacturer/supplier of both disinfectants to result in 5 log reduction/ degree of kill in the timescales quoted. The websites below detail the studies carried out to determine contact times but none have not been verified in house, this is deemed to be acceptable considering the low containment level (s) of the GMMs used.

distel-high-level-disinfectant_PF-SL-154479.html?gclid=Cj0KCQiA89vzBRDoARtASOlPgEy19_GbHUE8e9_6LAL92pzejwzNvmMckjDa2KB462z8r2cHkfcaAjyyEALw_wcB#tab=downloads

chemgene-high-level-disinfectant_PF-SL-154637.html#tab=downloads

Please enter comments of the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Y

Director of Development and Scientific Operations - Novel and dangerous pathogens training course, Biological Safety Officer Practitioner level 1 course passed - Public Health England, accredited by The Institute of Safety and Technology 3rd - 7th June 2019. Over 16 years experience working with E.Coli GMMs.

Laboratory Manager - BSc, TechIOSH: Specific experience relating to risk assessments - NEBOSH general certificate, and five years’ experience producing and conducting risk assessments.
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Name

ADAPTATE BIOTHERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre

THE WESTWORKS

Road Name

195 WOOD LANE

District

WHITE CITY

Town

LONDON

County

GREATER LONDON

Postcode

W12 7FQ

Country

ENGLAND

Tel Number

07837 651890

Fax Number

0

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The GMSC for Adaptate Biotherapeutics Ltd was established in January 2020 and consists of the following committee members:
- Company Chief Scientific Officer responsible for Research and Development at Adaptate Biotherapeutics who has a degree and PhD in Biological Sciences followed by over 15 years of experience leading R&D activities in companies.
- Company Scientist & lab manager who has a degree in biological sciences, has received training in biosafety and Health and Safety and has 5 years experience in initiating and maintaining start-up company health and safety programmes.
- An external Safety Advisor who is laboratory based and a chartered member of the Institution of Occupational Safety and Health (over 12 years). They are a member of a University GMSC.

Level 1 (GMMs)  
Level 2 (GMMs)  
Level 3 (GMMs)  
Level 4 (GMMs)  
Non-microbial
For activities involving GMMs, describe the waste management measures which will apply to the activity

- Any surface/equipment which is known or thought to be contaminated with GMOs will be disinfected using 2% Chemgene spray solution. A contact time of at least 5 minutes is required. After contact time, any residue is wiped away using absorbent tissue, which is then disposed of via clinical waste bags. Liquid wastes are to be inactivated by adding Chemgene to a final concentration of 5% (contact time in solution of at least 2 hours). After contact time, liquid wastes are disposed of via a designated laboratory sink, flushing with plenty of water.
- At a concentration of 5%, Chemgene solution has a greater than 4 log degree of kill on a range of bacterial, fungal and viral strains.
- Consumables or re-usable equipment (e.g. forceps, spatulas) contaminated with GMO waste will be autoclaved with a holding time of 15 minutes at 121 degrees celsius. The autoclave is 12-point thermocouple and load tested annually. Inactivated bagged waste will be transported inside a sealed wheelie bin designated for use of clinical waste only. The autoclave is present in the GammaDelta Therapeutics (HSE Centre GM Number 3363) facility which directly adjoins the Adaptate Biotherapeutics laboratory on the same corridor.
- Untreated waste is stored securely until autoclaved by GammaDelta staff under a contract with Adaptate Biotherapeutics.
- After waste is autoclaved by Adaptate staff it is sent for incineration at a site certified to deal with clinical waste.

Tick to confirm that you are attaching a summary of the risk assessment  
Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment
With regards to the associated risk assessment:

The GMSC requested clarification on:

2.1- Additional information on downstream processes
2.2- Remove use of other cell lines
2.8 – Risk to environment
3.1c – Antibiotics and cell lines
4.1 – When aerosols are generated
4.3 & 4.4 – Waste and disinfection procedures

Also – in section 4.6 to add the UN number that GMOs will be transported under.

Further clarification was provided and the risk assessment was amended appropriately. The GMSC had no further comments.
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**Name**

| ENDOCRYNE LTD |

**Name 2**

**Department**

**Campus Estate or Research Centre**

| BIOCITY |

**Road Name**

| BO'NESS ROAD |

**Building**

**District**

**Town**

| MOTHERWELL |

**County**

| LANARKSHIRE |

**Postcode**

| ML1 5UH |

**Country**

| SCOTLAND |

**Tel Number**

| 01506 841 632 |

**Fax Number**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022 |

| Page 14796 of 15326 |
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Endocrin has received advice from a chartered member of the Institute of Occupational Safety and Health and the Institute of Safety in Technology and Research, with significant in-depth knowledge of Health and Safety systems which are relevant to working at high biological containment. This member currently has experience serving with the Biosafety Steering Group of the ISTR.

The chartered member's experience has been gained and developed over 20 years and includes periods of time spent as Head of Health and Safety at the Institute for Animal Health, Head of Risk & Assurance and Biological Safety Officer at The Pirbright Institute and as a Specialist Inspector within the Biological Agents Unit of the Health and Safety Executive. The member has also worked with BBSRC supporting all of their Institutes.

Prior to this the member worked at the Health Protection Agency (HPA), Porton Down, initially as a scientist working predominately at Containment Level 3 (CL3) then as a Safety Advisor where the member took the role of Biological Safety Officer (BSO) and was responsible for the Research Division which included the high containment animal facility and the TSE, CL3 and CL4 facilities.

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste material will be inactivated in a decontamination autoclave, which has previously been validated (outsourced, independent validation) for each material load. We will maintain the waste loading to the maximum validation set point (e.g., validation of 5 L, will see no more than 5 L of material autoclaved at one time). Solid waste will be routinely separated from liquid waste and both will be autoclaved separately before being disposed of. We will treat all waste whether solid or liquid, as a potential biohazard. Once autoclaved it will be considered non-hazardous and disposed of as offensive waste, however, to ensure IP protection and uphold company reputation, an external contractor will be consulted to dispose of it through incineration. Chemical waste (e.g., acids, metals) will be neutralised, if possible, according to the COSHH assessment, and then disposed of via an external chemical waste contractor or as specified in the chemical’s COSHH. We will write into the lab policy/code of conduct that no liquid waste will disposed of into any sink, compliance will be mandatory for all workers. For routine cleaning and inactivation of microorganisms we will use a sporicidal (e.g., Klercide or similar) chemical to disinfect work areas before and after experimentation and an appraisal in the way of a report, on the efficacy of this sporicide (complete killing of a highly dense culture representative of any routine fermentations, 0 0 600 >200) will be conducted on all Endocryne strains. To test sterilisation, killing and other sterility checks, we will routinely assess the growth of cultures (plating, turbidity checks) prior and post-inactivation to confirm decontamination has been successfully performed.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Endocryne’s Managing Director and the appointed external Biological Safety Officer convened to discuss the risk assessment, which had been prepared by Endocryne’s scientific team. The Biological Safety Officer concurred with the risk assessment conducted and no further comments were made.
| Data Premises Notified (Originally) | 25/02/2020 | Transferred from 1992 Regs? | N | Transitional Premises Class | N | Non-GMMs | N | Withdrawn | N |

| Data Premises Closed | N | Transitional Premises Emergency Plan Required? | N | Non-GMMs | N | Withdrawn | N |

**Name**

QUELL THERAPEUTICS LTD

**Campus Estate or Research Centre**

TRANSLATIONAL & INNOVATION HUB

**Building**

LEVEL 3

**Road Name**

80 WOOD LANE

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

W12 0BZ

**Country**

ENGLAND

**Tel Number**

0207 096 9012

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

Guidance has been taken from a Safety Consultant working for a major London academic institution specifically experienced in the use of GMOs. In addition, a Genetic Modification Safety Committee has been formed for Quell Therapeutics Limited consisting of the Biological Safety Officer, VP Product Delivery, Office Representative and Scientific Lab Representative (note that the GMSC also acts as the internal Health and Safety Committee). The Committee will meet four times per year (as a minimum) to review the work of Quell Therapeutics. This will include periodic review of the GM risk assessment. Ad hoc meetings will be scheduled should more frequent risk assessment updates be required.

All members of the Committee have significant experience in the field of GM research with the exception of the Office Representative who acts as the lay person.

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Other (please specify)  

Tick if confidential  

N
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For activities involving GMMs, describe the waste management measures which will apply to the activity

No waste treatment is required but live cells will be inactivated with 1% Virkon and all solid waste will be disposed of as clinical waste (yellow bags) in line with standard lab practice. Clinical waste bags will be stored/transported in designated leak proof containers and be collected by the approved clinical waste contractor appointed by the building landlord, Imperial College London ThinkSpace.

---

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

---

Please enter comments of the GM safety committee on the risk assessment

The GMSC has reviewed the enclosed risk assessment and considers that it accurately reflects the activities and risks relevant to Quell's operations.
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**Name**

OXFORD NANOIMAGING LTD

**Name 2**

Department

**Campus Estate or Research Centre**

LINACRE HOUSE

**Building**

JORDAN HILL BUSINESS PARK

**Road Name**

BANBURY ROAD

**District**

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX2 8TA

**Country**

ENGLAND

**Tel Number**

07930342122

**Fax Number**

0

**E-mail**

**Comments**

**HSE Division**

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**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The ONI GMSC has provided guidance on risk assessments relating to contained use, and has also received guidance from appropriately qualified biosafety officers based at the University of Oxford.

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Waste Management Plan

WASTE DISPOSAL Waste disposal procedures were specified for all work carried out in the ONI laboratory in the lab safety policy (which can be provided on request)

Biological waste
Any waste containing or exposed to biological materials, such as microorganisms and tissue culture, will be treated with procedures that are appropriate to their containment level prior to disposal.

Procedures planned to follow
Autoclaving (121°C, 13psi and 30 minutes duration), or a one-hour soaking in Decon 90. Using Vikron solutions to clean up the work benches which are used for blood products and body fluid samples Using Chemgene to disinfect tissue culture area 70% ethanol for general disinfection

Waste containers
Yellow bins Chemically contaminated, hazardous solid wastes will be disposed in clinical waste bins provided by our approved waste management service providers

Blue bins
Empty solvent bottles are discarded after evaporating the residual substances in fume hood overnight. Large broken glass bottles can also be discarded in the blue bins

Sharp bins
Glass slides, Needles, Coverslips, etc. will be discarded in these bins

General waste bins
Non-hazardous, household waste

Recycling bins
Recycling bins will be kept away from the lab work area and will be categorised as plastic waste, electrical waste and cardboard recycling waste

Refill racks recycling
Designated for recycling pipette tips refill racks

Jerry cans
Three different Jerry cans will be kept for disposing:
Non halogenated solvents
Halogenated solvents
Aqueous waste

Jerry cans will be stored in the fume hood

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Please enter comments of the GM safety committee on the risk assessment

Head of BioR&D
Operations Manager

### Project Ref 3529/20.1

<table>
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<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
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<tbody>
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<td>26/03/2020</td>
<td>Contained use of GMOs, blood/blood products, body fluids and tissue samples at Oxford Nanoimaging Ltd shortly called as ONI Ltd</td>
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<table>
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Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

### Historical Significant Changes
- Historical Date of Additional Info
- Significant Change ID
- Date of Significant Change

### Project Additional Information

#### Purposes of the contained use
All the GMOs, tissue samples, cell lines and body fluids are used exclusively for research purpose only. There are no clinical trials, animal studies or sales from the R&D department.

#### Recipient or parental organism
The GMO which will be used at ONI Ltd, is only a low risk source of any infection. This strain of E. Coli is not a pathogen, and was developed for laboratory cloning use. This strain was developed by D. Hanahan as a cloning strain with multiple mutations that enable high-efficiency transformations. The mutations that the DH5-Alpha strain has are: dlacZ Delta M15 Delta(lacZYA-argF) U169 recA1 endA1 hsdR17(rK-mK+) supE44 thi-1 gyrA96 relA 1. These mutations correspond to the distinct characteristics that make the DH5-Alpha strain excel in laboratory cloning procedures.

lacZ Delta M15 mutation: Allows for blue-white screening for recombinant cells.
endA 1 mutation: Allows for lower endonuclease degradation which ensures higher plasmid transfer rates.
recA 1 mutation: reduces homologous recombination for a more stable insert.

**Host/vector system**

- **GMO**: Escherichia coli - DH5 alpha competent cells
- **Body fluids**:
  - Cerebrospinal fluid (CSF) - These are samples of neurological origin which may contain disease forming prion proteins
  - Saliva
  - Blood and blood products - Risk of viral infections
  - Human tissue samples - Samples have high chance of containing blood and therefore is considered infectious for the risk of HIV, HBV, HCV and other smaller group of viruses such as HTLV1 and paravirus B19
- **Commercially purchased cell lines from ATCC**:
  - Jurkat: Source - Human Cell type - T lymphocytes
  - HaCAT: Source - Human Cell Type - Keratinocytes
  - HEK Cells: Source - Human Cell Type - Kidney cells
  - Risk - Contains adenoviral DNA sequences
  - COS-7 Cells: Source - Monkey Cell Type - Kidney cells
  - Risk - Contains SV40 viral sequences
  - 3T3 Cells: Source - Mouse Cell Type - Fibroblast
  - N2ACells Source - Mouse Cell Type - Neuroblastoma
  - Exosomes - will be purchased from Fujifilm COL021
  - Mesenchymal placental exosomes in saline - will be purchased from Kimera
  - From academic collaborators CT26 - colorectal tumour samples from mouse and human

**Origin & function**

Research purposes only.

**Evaluation of foreseeable effects**

- Risk of HIV, HBV, HCB, and other viruses from bodily fluids and tissue samples.
- Exposure to blood-borne pathogens.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No large GMOs are used.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- High-level disinfectants such as Chemgene and Virkon will be used to clean the work benches and other surfaces/equipment that has come into contact with possibly infectious agents.
- Appropriate sodium hypochlorite bleach (Clorox) and hydrogen peroxide (Virox) solutions will be used to kill bloodborne pathogens.
- All bodily fluid will be dispose in a biohazard bag and sterilised prior to disposal in a chemical wast bin, collected by our hazardous materials collection service provided, Grundon.
Please enter comments on the GM safety committee on the risk assessment

General Company Health and Safety
Laboratory Health and Safety

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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<tbody>
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**Name**

VIROLOGY RESEARCH SERVICES LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

BUILDING 500, LAB S13/ S14

**Building**

GROUND FLOOR, SOUTH BLOCK

**Road Name**

DISCOVERY PARK

**District**

RAMSGATE

**Town**

SANDWICH

**County**

KENT

**Postcode**

CT13 9FE

**Country**

ENGLAND

**Tel Number**

01304 799790

**Fax Number**

0

**E-mail**

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**HSE Division**

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Tick if confidential

Give brief details of the genetic modification safety committee

The Biological Safety Committee of Virology Research Services Ltd (VRS) is comprised of the company Directors, Health & Safety and Facilities Manager and other staff members. Most committee members have post-graduate degrees in a biological sciences discipline (virology, molecular cell biology and imaging sciences), and each has over a decade of experience of working with human pathogens and genetically modified microorganisms in containment level 1- 3 facilities. Furthermore, in their previous employment at University College London and King's College London, committee members were active participants in matters of health and safety, particularly those pertaining to biological and GM safety, training, compiling risk assessments, and writing codes of practice and standard operating procedures.

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<th>Large Scale</th>
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Non-microbial

Other (please specify) | Tick if confidential

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02/03/2022
For inactivation of Class 1 GMMs we will use an aqueous solution containing 10% Distel high level laboratory disinfectant for equipment, surfaces, solid and liquid waste.

All solid waste items that have been in contact with GMMs will be soaked overnight in 10% Distel. Solid waste will be placed into a rigid clinical waste container and sent for incineration. For this purpose we use Stericycle Waste Management.

For liquid waste Distel will be added to containers before use, such that the final volume will result in a 10% solution of disinfectant. When containers are 2/3 full they will be sealed and left overnight to ensure inactivation of GMMs. Waste will then be poured down a general waste sink and flushed with copious amounts of water.

Using the parental MDCK cell line, we have previously shown that 1 hour incubation in a solution of 10% Distel renders all cells non-viable by Trypan Blue exclusion. We will confirm this with MDCK-SIAT1 cells after we receive authorisation for GM work from the HSE.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

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<tr>
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</table>

Please enter comments of the GM safety committee on the risk assessment

Representatives of Virology Research Services Ltd, Biological Safety Committee are satisfied that the attached risk assessment "Use of MDCK SIAT1 Cells for Producing Human Influenza Viruses" and the work proposed therein comply with the provisions of The Genetically Modified Organisms (Contained Use) Regulations 2014, and that notification should be given to the Health and Safety Executive. Work must not commence until receipt of notification is acknowledged.

Furthermore, we are satisfied with the information provided in the risk assessment and agree that it is suitable and sufficient for the proposed work.
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Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The University of Nottingham Health Service (UNHS) Genetic Modification Safety Committee has been formed as part of the process for initiating a study to determine the efficacy, safety and immunogenicity of a candidate COVID-19 vaccine in healthy adult volunteers. The inaugural meeting was held on May 4th 2020. The initial remit of the committee was to review the risk assessment of the GMM-based trial vaccine, and going forward, is to review risk assessments relating to any additional GMMs which may be used at the premises. Membership of the committee, which is chaired by the organisation's Research Lead, includes, amongst others, a Consultant Microbiologist, the Senior Nurse Manager, a general practitioner representative and a trial nurse representative. A PhD-level qualified, laboratory-based, external advisor with 20+ years of practical GMO and University Safety Committee membership experience also provided external expert review and attended the UNHS GMSC. Further committee meetings are to be held on an annual basis (or more frequently, if required). Reporting arrangements are to the Chief Operating Officer with copy to UNHS Safety Committee for information.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Level 1 (GMMs)</th>
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<tr>
<td>Level 4 (GMMs)</td>
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</table>
The GMM is incapable of surviving outside a host cell and is susceptible to common disinfectants. All surfaces to be disinfected with Clinell single-use spill wipes (which generate peracetic acid once wet) to disinfect non-soiled surfaces/small spills, and large spillages using Haz-tab granules/solution (granules to soak up the spill and tablets which when dissolved provide chlorine solution to clean the area; manufacturers validation >5 log10 reduction). Spillages will be reported as per local SOPs. All solid GMO waste will be collected and stored in designated leak-proof containers and autoclaved as specified in BS2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes; 100% kill), prior to incineration by validated means through a licensed waste contractor (Sharpsmart Ltd). Backup autoclave facilities are available onsite at the University of Nottingham, if required.

For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic</th>
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<tr>
<td>Mycology</td>
<td>Transgenic</td>
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<td>Other (please specify)</td>
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</table>

Other(s) Human trials to determine efficacy and immunogenicity of Class 1 GMM-based vaccines

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The UNHS GMSC met to review the submitted GM risk assessment (and related SOPs) at their meeting on 04 May 2020. It was noted that the GMM had been previously assessed and classified as Class I by a GMSC at the GMM manufacturers.

After recommending that the GMM be transported from the storage freezer to clinical areas in a sealed rigid container to ensure that, in the unlikely event of being dropped, the GMM would be contained, category 1 work involving the GMM on the premises was approved.
GM Centre Number: 3536

Data Premises Notified (Originally) 12/05/2020
Transferred from 1992 Regs? N
Transitional Premises Class

Data Premises Closed
Transitional Premises Emergency Plan Required? N
Non-GMMs N
Withdrawn N

Name
LONDON VISION CLINIC

Name 2

Department

Campus Estate or Research Centre

Road Name
138 HARLEY STREET

District

Town
LONDON

County
GREATER LONDON

Postcode
W1G 7LA

Country
ENGLAND

Tel Number 020 7224 1005
Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

- The London Vision Clinic has appointed an internal designated GMO individual to advise management on contained use activities and provide approval for the risk assessments which are of GM Class 1 risk level as permitted under the contained use regulations. The GMO individual holds a BSc in Biomedical Sciences and previous experience in cell and molecular biology. The genetic modification safety committee (GMSC) comprises of this designated GMO individual, a staff nurse and a member of clinic management, and meets on an ad hoc basis to review all risk assessments and conducts its activities under the terms of reference drawn up according to the contained use regulations. The GMO individual and GMSC have received additional training and advice from external providers.

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</table>

Tick if confidential: 

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid GM Class 1 non-sharp waste directly placed into eco-lock bins for uplift and offsite high temperature incineration (900 deg C) by an authorised upper-tier clinical waste contractor (currently Medisort; incinerator location CliniPower, Avonmouth, Bristol).

Sharps waste is deposited directly into sharpsafe bins, sealed and uplifted for offsite high temperature incineration by the same clinical waste contractor/incinerator. High temperature incineration achieves 100% kill of the GMM.

Liquid waste (or as necessary, solid waste) on site is disinfected by Klericide CR Sterile Biocide E which is an effective disinfectant shown to have broad spectrum virucidal activity. Liquid waste will then be disposed with copious tap water down designated sluice/lab sinks, in line with the local Water Authority effluent permit conditions.

Any new disinfectant to be used will be shown to provide effectiveness against GMM before authorised use.

Surface decontamination (e.g. lab/ward benches on site) is to be conducted with Klericide CR Sterile Biocide E.

Emergency spillages will also be treated with Klericide CR Sterile Biocide E.

Disinfection protocols are being set up and applicable personal protective equipment donned/worn during handling.

Training of GM users/workers in the use of disinfection procedures is recorded and refreshed according to local policy.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

GMSC felt there was minimal risk to human health or the environment and recommended that the material is classified as GM class 1
GM Centre Number: 3537

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**Name**

MAGNITUDE BIOSCIENCES LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

NETPARK PLEXUS

**Road Name**

THOMAS WRIGHT WAY

**District**

SEDGEFIELD

**Town**

STOCKTON - ON - TEES

**County**

CLEVELAND

**Postcode**

TS21 3FD

**Country**

ENGLAND

**Tel Number**

01740 625250

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<th>Building</th>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

This risk assessment is essentially identical to a risk assessment that has been approved by Durham University GM Safety Committee. It was drawn up by a person with over 20 years experience working in laboratories with relevant work and risk assessments in place.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
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<tr>
<td>Non-microbial</td>
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Other (please specify) Tick if confidential: **Yes**

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

02/03/2022
There will be three routes of disposal. All organisms will either be:
1. Autoclaved using a standard laboratory autoclave with recording to confirm sterilisation conditions, and disposed of via normal waste
2. Liquid cultures will be exposed to bleach overnight and washed down the sink
3. Disposed of via a clinical waste route.

This risk assessment is essentially identical to that approved by the Durham University Health and GMO committee.

For activities involving GMMs, describe the waste management measures which will apply to the activity

---

**Project Ref**  3537/22.1

**Date Ackn'd** 02/03/2022  
**CU2 Project Title** Co-culture of bacterial strains (class I and class 2), with C. elegans nematodes, to assess effects on growth, development, mobility, survival and behaviour

**Class** Class 2  
**CultureVolClass2** < 1 Litre  
**CultureVolumeClass3-4** Non-GMM  

**Consent Granted**

---

**Withdrawn** N  

**Historical Significant Changes**

---

**Date 02/03/2022**  
**Page 14820 of 15326**
**Project Additional Information**

**Purposes of the contained use**

Examples include, but are not limited to use of GM lab strains of Pseudomonas aeruginosa, E.coli and other bacteria to investigate biofilm formation and infection in C. elegans. GM bacteria carrying fluorescent markers will be used to monitor the formation of biofilm during infection of C. elegans as an alternative to cell or mammal based assays.

**Recipient or parental organism**

Pseudomonas aeruginosa is an aerobic, class 2 bacterium. Escherichia coli will include class 1 or class 2 strains.

**Host/vector system**

The bacteria is modified by transforming with plasmids for the expression of GFP or RFP (fluorescent proteins) under bacterial promoters. These plasmids are derived from commonly used plasmids and sequences and also confer resistance to an antibiotic such as kanamycin or ampicillin.

**Origin & function**

**Evaluation of foreseeable effects**

Genetic modification of the bacteria will not increase risk. The bacteria will express fluorescent proteins so they can be imaged under a fluorescent microscope.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

C. elegans are kept in agar plates or liquid media. If they manage to crawl out of their containers they are quickly killed by desiccation. All animals not killed in experiments will be killed by autoclaving or by soaking overnight in bleach or through a clinical waste route.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

There will be three routes of disposal. All organisms will either be:
1. Autoclaved using a standard laboratory autoclave with recording to confirm sterilisation conditions, and disposed of via normal waste
2. Liquid cultures will be exposed to bleach overnight and washed down the sink
3. Disposed of via a clinical waste route.

Is an emergency plan required according to regulation 20? [N]

If yes, tick to confirm that it is attached to this form [N]
This risk assessment is essentially identical to a risk assessment that has been approved by Durham University GM Safety Committee. It was drawn up by a person with over 20 years experience working in laboratories with relevant work and risk assessments in place.

Please enter comments on the GM safety committee on the risk assessment

This risk assessment is essentially identical to a risk assessment that has been approved by Durham University GM Safety Committee. It was drawn up by a person with over 20 years experience working in laboratories with relevant work and risk assessments in place.

### Project Containment

<table>
<thead>
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<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**Name**

CAITHNESS BIOTECHNOLOGIES LTD

**Name 2**

Department

**Campus Estate or Research Centre**

UNIT W9

**Road Name**

72 BOSTON ROAD

**Town**

LEICESTER

**County**

LEICESTERSHIRE

**Postcode**

LE4 1HB

**Country**

ENGLAND

**Tel Number**

0116 326 3802

**Fax Number**

0

**E-mail**

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**HSE Division**

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

**Y**

**Give brief details of the genetic modification safety committee**

Advice was obtained from an external biological safety officer (BSO) with >10 years experience of managing the health and safety of several academic groups actively involved in the genetic modification of micro-organisms, and the preparation of risk assessments for such processes in a university setting. The RA was prepared, with help from the external BSO, by the company BSO, who has >15 years post-doctoral practical experience of the genetic modification of micro-organisms up to CL2, including writing GM risk assessments for such work.

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<tr>
<td>Microbiology Research</td>
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</table>

Tick if confidential |
Solid biological waste is sequestered from waste of non-biological origin, by placing into biohazard labelled yellow autoclave bags. Bags are folded in such a way as to allow steam to enter during autoclaving. Solid waste is then sterilised by autoclaving (20 minutes at 121°C), using an autoclave sited within the laboratory. This cycle is expected to result in essentially complete kill of the types of microbe cultured in the laboratory (E. coli DH5α and BL21(DE3)). The sterilised waste is then collected for incineration by an approved contractor. Autoclave function will be tested monthly by including a glass vial containing E. coli DH5α cells at a concentration of 10^8 CFU/ml in a representative sterilisation run, and streaking the suspension after the autoclaving cycle is complete on an LB agar plate. If any colonies grow on the test plate, the autoclave should be repaired and the run repeated before waste is sent for disposal.

Liquid waste is inactivated within the vessel in which the organisms were cultured or their waste products collected, by treatment with Distel disinfectant at a final concentration of 1% for at least 12 hours before disposal to drain with copious water flushing. This treatment is expected to result in at least a 5-log reduction in viable E. coli within 5 minutes, according to the manufacturer’s information.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The external advisor's comments were as follows:
The risk assessment is adequate for the planned activities, which are appropriately classified as class 1 contained use. The outlined containment measures are also appropriate to the level of risk. However, the risk assessment could benefit from further clarification with respect to the following items:
• Include further information with respect to the rationale for the assumption that E. coli expressing the proteins of interest will not be more likely to cause infection than the unmodified cloning strain
• Give an estimate of the expected efficiency of sterilisation of the E. coli cloning strains by the disinfectant to be used for liquid waste
• Indicate the maximum culture volumes
• Provide a list of target genes to be cloned
<table>
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<th><strong>GM Centre Number:</strong> 3539</th>
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| **Data Premises Notified (Originally)** | 15/06/2020 |
| **Transferred from 1992 Regs?** | N |
| **Transitional Premises Class** | |
| **Data Premises Closed** | |
| **Transitional Premises Emergency Plan Required?** | N |
| **Non-GMMs** | N |
| **Withdrawn** | N |

Name

MEDIMAB BIOTHERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre
BIOESCALATOR

Building
INNOVATION BUILDING, UNIVERSITY OF OXFORD

Road Name
ROOSEVELT DRIVE

District

Town
OXFORD

County
OXFORDSHIRE

Postcode
OX3 7FZ

Country
ENGLAND

Tel Number
0792 086 4588

Fax Number
0

E-mail

HSE Division
blank

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes [Y]

Give brief details of the genetic modification safety committee

CU 2 2015 (rev 11/15) Page 2 of 9
Premises Notification
3. * Check to confirm that you have received advice from a person or a genetic modification safety committee with expertise in risk assessments relating to contained use
* Give brief details of the person or genetic modification safety committee - no actual names should be given (note 3)

<table>
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</tbody>
</table>

02/03/2022
All cell lines and primary cells will be deactivated in 2% virkon for 20 minutes, such that all GMOs will be destroyed, before disposal in wastewater. It is expected that this will kill 99.98% (as per the manufacturer's validation) of mammalian cells. Testing can be done by measuring remaining viable cells by trypan blue exclusion assay after virkon treatment.

All plastic ware will be autoclaved in a BS 2646, Part 3, 1993 (either 121-125°C for a minimum of 15 minutes, or 126-130°C for a minimum of 10 minutes, or 130-136°C for a minimum of 3 minutes). Excess liquids will be discharged to the drains, solid wastes will be disposed of via the clinical waste stream for incineration/disposal. Any sharps used will be disposed of via clinical waste stream for incineration. Autoclave and incineration will deliver a degree of killing of 100%.

The University of Oxford, Innovation Buildings Biological safety committee was pleased to approve our risk assessments with no modifications.

The University of Oxford, Innovation Buildings Biological safety committee was pleased to approve our risk assessments with no modifications.
Purposes of the contained use

The risk assessments to human health and the environment show that the genetic material to be generated during this project has a relatively low risk, warranting using containment level 2.
The final GM activity class is containment level 2.

Recipient or parental organism

GMO will be standard cancer model cell lines (available from the ATCC) that have been genetically modified to overexpress specific natural receptors of interest. Working in vitro, typically cells will include human and murine cancer cell lines (e.g., human breast cancer cell lines such as MB-MDA-268 or human colorectal cancer cell lines such as HT29, HCT116 or murine colorectal CT26 and MC38 cell lines).

Host/vector system

We will use transfection reagents, such as lipofectamine, or electroporation to generate GM target cell lines

Origin & function

Transgenes to be expressed include:

• Reporter genes: Photinus (firefly) and renilla luciferase, Beta-galactosidase, Secreted Alkaline Phosphatase, Chloramphenicol acetyltransferase, fluorescent proteins from marine organisms (CFP, EGFP and YFP). These naturally fluorescent proteins emit light when excited by high-intensity light of specific wavelengths. Several variants of natural genes have been produced that encode a protein with modified adsorption/emission characteristics and which have been optimised for higher level expression, e.g., the “EGFP”. GFPs are widely used and no hazardous properties have been reported. Other proteins have been isolated from other organisms, however, each of these proteins has no cellular toxicity in normal cells, otherwise, they would not function as a successful reporter gene. Luciferase from Firefly and Sea Pansy are widely used as reporter genes and not considered to have harmful properties. No hazardous effects are anticipated because all the parent proteins are without harmful properties.

• Mammalian selectable markers: Hygromycin resistance, Puromycin resistance, Zeomycin resistance, Neomycin resistance, Blasticidin resistance, and Geneticin resistance. Never shall any vector contain more than one of these markers. They do not function in bacteria. The promoter of the mammalian antibiotic resistance or selectable marker is recognised by the mammalian RNA polymerase and allows transcription of the gene of the mammalian antibiotic
resistance or selectable marker in mammalian cells.

Evaluation of foreseeable effects

As above, typically cells (as described above) will be transduced using the procedures described in the attached risk assessment. The transduced cells will be selected by treatment with puromycin, sorted by flow cytometry or subject to at least three media changes and used as a mixed preparation of transduced and untransduced cells.

Transgenes and their potential risks

Transgenes will include only reporter genes, naturally occurring human receptors (such as PD-1, CTLA4, TIGIT, LAG-3, or TIM3) receptor, and/or selectable markers. No other proteins will be encoded, for example there will be no toxins, super-antigens or oncogenes.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

n/a

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

We will use full containment class 2, with no derogation.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>The containment and control measure that will be applied for work with the genetically modified cell lines and the infectious vector preparations are shown on Tables 1. Containment level 2 will be applied with Good Microbiological Practice and Good Occupational Hygiene. A microbiological safety cabinet, safety glasses and gloves will be used for protection and to maintain sterility. Class 2.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WASTE MANAGEMENT MEASURES</td>
</tr>
<tr>
<td>Consumables (mainly plasticware eg pipettes, flasks, tubes) – treat overnight with Virkon following manufacturer’s guidelines or autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the industrial (black bag) waste stream for incineration.</td>
</tr>
<tr>
<td>Liquids (eg samples, culture supernatants, tissue culture media) – treat overnight with Virkon following manufacturer’s guidelines or autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.</td>
</tr>
<tr>
<td>Sharps (eg needles, syringes, scalpels) - dispose via clinical waste stream for incineration</td>
</tr>
<tr>
<td>Degree of kill</td>
</tr>
<tr>
<td>Autoclaving, effectively 100% kill (annual validation)</td>
</tr>
<tr>
<td>Incineration, effectively 100% kill (licensed incinerator)</td>
</tr>
<tr>
<td>Chemical disinfection with Virkon, used according to manufacturer’s instructions under standard conditions, manufacturers validation [eg 4.79] log reduction ([eg 99.998]% kill).</td>
</tr>
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Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The safety committee had no comments on our risk assessment, given the limited nature of our work, they were happy to approve the work without modifications.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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**GM Centre Number: 3540**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
**Premises Addresses**

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<th>Department</th>
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<th>Building</th>
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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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<tr>
<th>Level 1 (GMMs)</th>
<th>Level 2 (GMMs)</th>
<th>Level 3 (GMMs)</th>
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<th>Non-microbial</th>
<th>Other (please specify)</th>
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Give brief details of the genetic modification safety committee

Exonate has established a local genetic modification safety committee who have reviewed and approved this notification and GM risk assessment. The committee is comprised of a Senior Scientist with expertise in GM work in Universities and Exonate, a Principal Scientist with experience in reviewing risk assessments in Universities and Exonate and a Professor with extensive expertise in risk assessments relating to genetically modified organisms who is Head of Department and Director of a Research Centre at the University of Nottingham.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale |
|------------|-------------|-------------|-------------|-------------|

- Bacteriology: Yes
- Parasitology: Yes
- Transgenic Birds: Yes
- Microbiology Research: Yes
- Transgenic Animals: Yes
- Transgenic Fish: Yes
- Gene Therapy: Yes

Tick if confidential

02/03/2022
All cultures and infected liquids will be sterilised by incubation in 1% Distel for at least 2 hours and disposed of through designated sinks. Validated data supplied by the manufacturer indicates that this will result in inactivation of all organisms Exonate uses in class 1 activities.

Contaminated glassware and solid waste, including plastics, tubes, pipettes, agar plates will be decontaminated by the addition of 1% Distel (final concentration) and incubated at room temperature for 2 hour. This will give 100% kill. Objects which are likely to puncture the bags will be placed in a BioBin or similar container. The inactivated waste will be stored in a sealed and labelled wheele bin with biohazard symbols displayed until removal by a registered waste contractor (Stericycle) for incineration.

Work surfaces will be wiped down with 70% ethanol after use.

Sharps material is not required, and it will be avoided.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment Y

Exonate were working in University GM approved laboratories and have recently moved to new laboratories at MediCity. The new labs meet all criteria for containment level 2 but will initially be used for class 1 activities upon acknowledgement of this premises notification followed by class 2 activities upon notification and approval from HSE. The risk assessment for the initial class 1 activities meets our approval for containment of low risk activities.
Project Additional Information

Purposes of the contained use

The GMM originated in this project will be used in R&D and small molecules screening for kinase inhibitors discovery and development.

Recipient or parental organism

Primary RPE cells, iPS-derived RPE cells, RPE cell line (ARPE19), embryonic kidney cell line (HEK-293T) and other immortalized or cancer cell lines detailed in section 17. The recipient for plasmids transformation and amplification will be E. coli DH5alpha.

Host/vector system

Vector system: mammalian expressing plasmids (chemical transfection and nucleofection), transduction of replication-defective lentivirus, Nucleofection of ribonucleoprotein complex. More detailed information in following section.

Origin & function

1. For knock-in modifications
For integration of the tags sequences in the genome, cells will be nucleofected with Cas9 protein and gRNA ribonucleoproteins (RNP) purchased from Sigma or ThermoScientific and the HDR (homology directed repair) donor plasmid with homology arms and tag coding sequences (in vitro synthesised by GeneScript). HDR-plasmids are pUC-based vectors and are non-mobilisable. They contain bacterial origin of replication and selection gene for ampicillin resistance. They do not contain markers of mammalian selection. As donor template for small knock-ins or point-mutations, single-stranded oligo DNA nucleotides will be used instead of plasmid.

2. For knock-out modifications
Knock-out cell lines will be generated by chemical transfection of dual-vector plasmids based on commercially available construct pSpCas9(BB)-2A-Puro (PX459). These are non-mobilisable plasmids that express both Cas9 endonuclease and guide RNA (gRNA) sequences targeting SRSF1, "Target X (detailed in section 17)" or SRPK1 genes and the antibiotic selection markers ampicillin and puromycin.pSpCas9(BB)-2A-Puro (PX459) plasmid was purchased from GeneScript and gRNA were cloned in house.

Mammalian expressing plasmids (detailed in Section 17) are develop by the Custom Assay Services team within Promega. The vectors are non-mobilisable. They contain bacterial origin of replication and bacterial selection genes such as Kanamycin and Chloramphenicol resistance. They also contain markers of mammalian selection such as Neomycin and Blasticidin resistance. Transfection of mammalian cells will be accomplished using chemical reagents or by nucleofection. None of the mammalian vector systems can be used to generate viruses.

02/03/2022
4. For shRNA mediated knock-down
Mammalian cells will be infected with shRNA Lentiviral Transduction Particles targeting SRPK1, Target X or SRSF1. Commercially available self-inactivating recombinant lentiviral vectors with enhanced biosafety will be used.

**Evaluation of foreseeable effects**

1. Overview of the different types of GMM that will be constructed (detailed list in section 17).
   - Knock-in Stable Cell Lines (by CRISPR)
     Stable cell line expressing tagged-SRPK1 + tagged-SRSF1 OR tagged-TargetX + tagged-SRSF1.
     Cells: HEK-293T, ARPE19 and others (section 17).
   - Knock-out Stable Cell Lines (by CRISPR)
     Modification: SRPK1 or “Target X” or SRSF1 knock-out.
     Cells: HEK-293T, ARPE19, and others (Section 17).
   - Kinase-dead mutant cell lines (by CRISPR)
     Modification: kinase-dead point mutations in catalytic domain of SRPK1 or “Target X”.
     Cells: HEK-293T, ARPE19, and others (Section 17).
   - Knock-down cell lines (by shRNA-lentiviral infection)
     Modification: SRPK1, “Target X” or SRSF1 shRNA.
     Cells: HEK-293T, ARPE19, primary RPE cells, iPS-derived RPE cells and others (section 17).
   - Human cells transiently transfected with tagged SRPK1, SRSF1, “Target X”
     Cells: ARPE19, HEK-293T, primary RPE cells and iPS-RPE cells.
   - Human cells stably expressing transfected tagged SRPK1, SRSF1, “Target X”
     Cells: (section 17).
   - E. coli DH5α cells transformed with plasmids for amplification and cryopreservation

2. Function of inserted sequences and knock-out genes (complete list in Section 17)
   - SRPK1
     Serine–arginine protein kinase 1 phosphorylates proteins containing serine–arginine rich (SR) domains which are involved in the regulation of several mRNA processing pathways, including alternative splicing.
   - SRSF1
     Serine/Arginine Splicing Factor 1 regulates key aspects of mRNA metabolism, such as mRNA splicing, stability, and translation, as well as other mRNA-independent processes, such as miRNA processing, protein sumoylation, and the nucleolar-stress response. SRSF1 is a proto-oncogene that appears to exert its tumorigenic effects via a number of mechanisms, particularly alternative pre-mRNA splicing (8).
   - Cas9 endonuclease
     Guided by a guide RNA, the Cas9 endonuclease breaks DNA at a target sequence. Imprecise repair of the double strand break can result in insertion or deletion mutations, while repair pathways can be engineered to introduce specific point mutations or insertions.

3. Nature of the risks
The work involves the genetic modification of human cell lines, primary cell cultures and bacteria, manipulation of naked DNA encoding CRISPR machinery components, the handling of Cas9 purified protein complexed with gRNA and overexpression of proteins with oncogenic properties. Therefore, risk assessment for human health and
environment is necessary.

4. Risk assessment for human health

4.1 Mechanisms by which the GMM might pose a hazard to health

4.1.1 What are the hazards associated with the vectors?

- shRNA Lentiviral Transduction Particles targeting SRPK1, Target X (Section 17) or SRSF1. Commercial viral particles developed by 3rd-generation of lentiviral vector system (Sigma MISSION®) will be purchased. Particles are replication-incompetent and deletion in the U3 portion of the 3' LTR eliminates the promoter-enhancer region, further negating the possibility of viral replication and they lack of virulence genes which are not necessary for shRNA packaging. These features combined have improved biosafety and handling. Nevertheless and, though there are no known incidents of third generation systems producing replication competent virus, it is advisable to handle replication-incompetent lentiviral particles as Risk Group-Level 2.

Lentiviruses are generally transmitted via exposure to contaminated material or percutaneous inoculation and generally not transmitted via the airbone route. Therefore, accidental piercing of the skin or other surface tissues with virus containing objects represents the main potential route by which accidental infection could occur. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

DNA Plasmids

Dual Cas9-gRNA plasmids. The vectors are non-mobilisable and are unable to generate viral particles. Cas9 and gRNA will not be expressed in bacteria as they are under regulation of a eukaryotic promoter.

Mammalian expression vectors. The vectors are non-mobilisable and are unable to generate viral particles. "Target X", SRSF1 and SRPK1 will not be expressed in bacteria as they are under regulation of a eukaryotic promoter.

Antibiotic resistance markers expressed in E. coli: These cassettes have been widely utilised for research purposes and these antibiotics are not used for treatment of natural infections by the recipient organisms. Besides, they will be expressed only transitory during the amplification of the plasmids, rather than in the final GMO product and it does not constitute any risk to human or animal health.

4.1.2 What are the hazards associated with the recipient cells?

- Primary human RPE cells developed in house. Human ocular (choroid/iris/retina) endothelial cells will be isolated from posterior segments. Despite donor tissues being submitted to stringent health screens for transplant suitability and negative for Hepatitis B and VIH, this organism would be considered class II due to the potential risk of infection.

- iPS-RPE. Retinal Pigment Epithelial cells developed from human induced pluripotent stem cells purchased to Phenocell. This product should be treated as potentially infectious and only used in biological safety level 2 premises and conditions.

- HEK-293T cell line is classified as ACDP Hazard Group 2 because it was originally initiated by the transformation and culturing of normal HEK-293 cells with sheared adenovirus 5 DNA. A portion of human adenovirus 5, nucleotides 1-4344, is integrated into chromosome 19 but they do not contain the complete viral genome and therefore, the risk of generation of these viruses by these cells is extremely low. However, the possibility that wild-type virus might recombine and mobilize these viral genes into infective particles can't be excluded. HEK-293T cells also contain Simian Virus 40 large T antigen (SV40LT).

- Immortalized cell line detailed in section 17. The cells were immortalized by transduction with human papilloma virus 16 (HPV-16) E6/E7 genes and contain papilloma viral DNA sequences, therefore it will be considered as biosafety level 2.

- All the other cells mention in section 17 except one, are well characterized human cells and do not represent any hazard to human health or the environment and are categorised as Biosafety level 1:

  - E. coli DH5 alpha cells are avirulent and considered to be ACDP biological agents hazard group 1 because they have an established record of safety in the laboratory with no adverse effects on human, animal or plant health or the environment. Furthermore E. coli DH5alpha is incapable of survival outside the laboratory.

4.1.3 What hazards are associated with the inserted genetic material or genetic modifications?

Knock-ins: The genetic insertions generated by HDR are stable in the genome and plasmid maintenance is not required. Knock-ins tags are not pathogenic and have no direct effect on cellular processes. The resistance markers encoded in the backbone of the HDR-donor will not be permanently integrated in the genome, as no selection will be used. While the risk of random integration of fragments of the plasmids in the genome of the human cell lines is very low, this cannot be completely excluded. SRPK1, "Target X" and SRSF1 Knock-out/knock-down: Depletion or nonsense mutations in SRSF1, "Target X" and SRPK1 are unlikely to induce oncogenic transformation or pathogenicity of the cell lines, or to suppose a risk for the worker. Nevertheless, SRSF1 is classified as proto-oncogene(8) and Wang et al. (9) report that SRPK1 can function as both an oncogene and a tumour suppressor in mouse embryonic fibroblasts. "Target X" can act as a tumour suppressor or its amplification can contribute to carcinogenesis depending on cellular context(10). The greatest risk would be associated with accidental infection of someone during handling of the shRNA viral particles.
and therefore, viral particles will be handled at containment level 2. Also, Cas9-mediated editing can generate coding mutations with undescribed phenotype but cells carrying those mutations won’t be amplified or stored.

4.4 What hazards are associated with the mutations in SRPK1, "Target X" and SRSF1 genes?

Whilst the risks of the knock-ins generating a transformed phenotype seem very low, this cannot be completely excluded as the effect of tagging these proteins to SRPK1, "Target X" and SRSF1 has never been investigated. SRSF1 is a proto-oncogene and both, oncogenic and tumour-suppressor characteristics have been attributed to SRPK1 and "Target X". Therefore, even though tumorigenesis is a multistep process, genetic modification of these genes could induce transformation of the cell lines. However, with the precautions in place and the use of Class II Microbiological safety cabinet, the GM cell lines will not present a hazard for humans.

4.5 What hazards are associated with the stable overexpression of SRPK1, "Target X" and SRSF1 in human cell lines?

SRPK1 is a proto-oncogene and both, oncogenic and tumour-suppressor characteristics have been attributed to SRPK1 and "Target X". While development of a cancer is acknowledged to be a multistep process requiring a number of genetic lesions to generate a malignant tumorigenic cell, expression of some genes can allow proliferation or confer an extended life span upon primary cells. This may predispose a cell to accumulating oncogenic lesions and might be one step nearer to forming a cancer. For this reason, all the primary cells used in this study will be handled as class 2 organisms after being stably transfected with SRPK1, "Target X" or SRSF1 expressing vectors. The stable overexpression of oncoproteins in already cancerous cell lines is not expected to significantly increase its safety characteristics, however class 2 safety guides are advisable to handle these cells.

4.6 What hazards are associated with the potential off-targets effects?

There is the potential of off-target DNA double strand breaks following transduction with Cas9 and gRNA, which could potentially alter the cell phenotype, however the reported incidence of this is extremely low. This will be minimised by careful gRNA selection with as little sequence homology as possible to off-target genes. This risk is lower when transfecting Cas9-gRNA RNP complex instead of plasmids because Cas9-gRNA RNP complex is rapidly cleared from the cell, minimizing the chance of off-target cleavage events.

Similarly, shRNA potential off targets will be minimised by careful design with as little sequence homology as possible to off-target genes, including BLAST searches against genome databases.

4.7 Likelihood of genetic transfer to other GMM or organisms

Gene transfer is possible as the commercial lentiviral vector will be able to infect a wide range of animal cells, including human cells. However, after transduction and integration into genomic DNA of the target cells, gene transfer possibility is insignificant due to the viruses being replication defective.

4.2 Containment level needed to sufficiently protect human health

HEK-293T, iPS-RPE and the cell line immortalized with HPV (Section 17) require biosafety level 2. CRISPR-Cas9 resulting modifications could have the effect of deletion, repression or upregulation on the target gene. This could potentially alter the cell phenotype, for instance increasing the ability to survive or proliferate. There is also the potential of off-target DNA double strand breaks, however the reported incidence of this is extremely low. Biosafety level 2 containment is also advisable when working with or handling any lentiviral vector particles and when stably expressing proto-oncogenes in human cells. Consequently, appropriate containment level 2 control measures will be used to minimize exposure.

Hazard evaluation of the activities and measures to follow are summarized in Table 1 in the attached risk assessment.

4.3 Likelihood that the GMM will be a risk to human health and safety

With all the precautions in place and following good laboratory practices the likelihood that the GMM will be a risk to human health and safety is close to negligible for the following reasons:

The CRISPR-Cas9 plasmids, E. coli cells and all the human cells used in this project except HEK-293T, are not able to infect humans or survive in the environment. HEK-293T cells do not contain the complete adenoviral genome and therefore, the risk of generation of these viruses by these cells is extremely low. HEK-293T cells have been widely used in research labs during years and to the best of our knowledge, no infections have been described in humans. Following proper microbiological techniques will avoid wild-type virus to infect HEK-293T cells, recombine and mobilize their viral genes into infective particles.

Third generation lentiviral particles are self-inactivating recombinant lentiviral vectors with enhanced biosafety. The greatest risk would be associated with accidental infection of staff during handling of the shRNA viral particles and therefore, viral particles will be handled at containment level 2.

5. Risk assessment for the environment

5.1 Likelihood that the GMM will be a risk to the environment

Risk associated with these activities to the environment is deemed low. First, the cells will not be cultured on a large scale, there will be no risk of any large spillages.
Consequently, it is highly unlikely that either cells or viruses would be released into the environment. In the unlikely event of escape, neither the GMM nor the lentivirus are capable of surviving outside of the laboratory environment since they require supplementation with growth factors and specific temperature and atmospheric conditions. The virus does not replicate so will not amplify in the cells. Secondly, neither the DNA, encoded proteins, bacterial or mammalian transfectants are likely to have any deleterious effect on the environment.

5.2 Containment level needed to protect the environment
Containment level 1 is sufficient to prevent release and protect the environment. A higher level of containment has been assigned to protect human health, but the environmental risk is essentially effectively zero.

6. Review procedures and control measures
Human infection/colonisation by cultured cells is mitigated by the use of microbiological safety cabinets class II, secondary containment during transport, and appropriate centrifuge spill/leak protocols. Proper microbiological techniques will be used so as not to contaminate HEK-293T and HK-2 cells with wild-type virus that might recombine and mobilize their viral genes into infective particles. Handling of lentiviral particles must be done in Class 2 cabinets and sharps/glass will not be used in the procedure. Environmental exposure of all GMMs will be limited. Any waste generated must be inactivated by validated disinfection before being sent for incineration.
A more detail review of the procedures and measures implemented can be found in Table 1 in the attached risk assessment.

6.1 Record staff training
End user will be required to read, understand and sign the SOPs involved in this activity, the risk assessments related with the reagents, assays and genetic modifications involved and Exonate's Code of Practice for work with Biological Agents and Genetically Modified Micro-Organisms.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All cultures and infected liquids will be sterilised by incubation in 1% Distel for at least 2 hours before disposed of through designated sinks. Validated data supplied by the manufacturer indites that this will result in inactivation of all organisms used or generated in this project. Contaminated glassware and solid waste, including plastics, tubes, pipettes, agar plates will be submerge in 1% Distel (final concentration) and incubated at room temperature for 2 hours. This will give 100% kill. Objects which are likely to puncture the bags will be placed in a BioBin or similar container. The inactivated waste will be stored in a sealed and labeled wheeble bin with biohazard symbols displayed until removal by a registered waste contractor (Stericycle) for incineration. When Distel is not suitable for inactivation, for instance for the decontamination of agar plates, contaminated items will be autoclaved. Exonate has access to an autoclave on site, where items will be transported to in labeled and sealed bags. Spillages will be immediately absorbed into paper towels soaked in undiluted Distel, surface wiped down with Distel 1%. The use of 70% ethanol is not recommended for spill clean-up due to evaporation. As the cells will not be cultured on a large scale, there will be no risk of any large spills. Work surfaces will be wiped down pre and post carrying out GMO work use using 70% ethanol or 1% Distel, except when working with lentiviral particles, in which case ethanol should not be used to avoid evaporation. Sharps material is not required and must be avoided during GMO work.

Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  Y
Exonate were working in University GM approved laboratories and have recently moved to new laboratories at MediCity. The new labs meet all criteria for containment level 2 and we have already notified the premises to carry class 1 activities and are waiting for the confirmation letter and HSE reference number. We are submitting now an application to additionally execute class 2 activities. The risk assessment for this class 2 activity meets our approval for containment of medium risk activities.

**Project Containment**

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Please enter comments on the GM safety committee on the risk assessment
GM Centre Number: 3541

Data Premises Notified (Originally) 29/06/2020

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

PATHWAY INTERMEDIATES LTD

Name 2

Department

Campus Estate or Research Centre

UNIT 1, HARLESCOTT BUSINESS PARK

Road Name

HARLESCOTT LANE

Town

SHREWSBURY

County

SHROPSHIRE

Postcode

SY1 3FG

Country

ENGLAND

Tel Number 01743761067

Fax Number 0

E-mail

HSE Division blank

Comments

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The person performing the risk assessments is a senior scientist within the organisation, who has used GMMs during their PhD and two post-doctoral positions. This individual has written previous risk assessments for Class 1 GMM work for internal approval at a previous employer, and was the post-doctoral representative on a GMO safety committee at a previous employer. This individual has worked as a post-doctoral scientist in derogated Category 3 conditions with genetically modified organisms covered by a SAPO license at a previous employer.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

Yes
All liquid waste will be treated with an appropriate disinfectant (CHEMGENE HLD4L; final dilution of 1:100, Virkon; final concentration of 1%, or equivalent). This will be left at room temperature for a minimum of 2 hours to ensure full deactivation of the GMMs. Deactivated liquid waste can then be disposed of down the sink with plenty of water. Solid waste will be autoclaved (121°C, 20 minutes) in an autoclavable biohazard bag. This is then subsequently bagged in black bin liners and sent to landfill. The autoclave is serviced annually, but the success of each run is monitored by integrated sensors, and TST (time, steam and temperature) test strips. Spillages can be safely contained within the laboratory and treated with either concentrated CHEMGENE HLD4L or 70% ethanol. These will be mopped with blue roll, placed in autoclave bags and then deactivated as contaminated waste, as detailed above.
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**Name**

DEEP BRANCH TECHNOLOGY LTD

**Campus Estate or Research Centre**

BIODISCOVERY INSTITUTE

**Building**

UNIVERSITY OF NOTTINGHAM

**Road Name**

UNIVERSITY PARK

**Town**

NOTTINGHAM

**County**

NOTTINGHAMSHIRE

**Postcode**

NG7 2RD

**Country**

ENGLAND

**Tel Number**

07732211998

**Fax Number**

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**E-mail**

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**HSE Division**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes  

Give brief details of the genetic modification safety committee

Our genetic modification safety committee consists of two people, the head of strain development and the chief operating officer of Deep Branch Biotechnology.

Head of strain development: PhD in molecular microbiology, 6 years working with GMOs in the laboratory.

Chief Operating Officer: PhD in molecular microbiology, 5 years working with GMOs in the laboratory.

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Tick if confidential

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<th>Microbiology Research</th>
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</table>

02/03/2022  Page 14845 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste potentially contaminated with GM material will be autoclaved at 126°C for 10 minutes using the biodiscovery institute (formerly centre for biomolecular sciences) building system. Autoclaves are validated and serviced and each run cycle data is recorded and analysed. Autoclave servicing is performed 6-monthly, inspections occur annually. Laboratory surfaces and equipment will be cleaned and disinfected using for instance 2% distel of 1% virkon.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All of the bacteria intended to be modified are considered ACDP HG1.
GM Centre Number: 3543

Data Premises Notified
(Originally) 03/07/2020

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

C3 BIOTECHNOLOGIES LTD

Name 2

Department

Campus Estate or Research Centre

THE RAILWAY GOODS YARD

Road Name

MIDDLETON IN LONSDALE

District

Town

KIRBY LONSDALE

County

CUMBRIA

Postcode

LA6 2NF

Country

ENGLAND

Tel Number 015242 76575

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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- Bacteriology: Yes
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy

Manchester Institute of Biotechnology Safety and Risk Manager and assistant biological safety advisor.

02/03/2022
All contaminated biological waste will be fully inactivated (sterilised) prior to disposal through standard drainage systems. Sterilisation will be performed by either on site autoclaving or chemical treatment (1% Virkon).

The fermenter will have 100% liquid external containment capability in the case of a spillage or leak. Gaseous emissions will be filtered (0.2 um) to eliminate the risk of accidental GMM aerosol exposure to the air. Equipment that has been in contact with GMMs will be sanitised by treatment with 1% Virkon and/or dairy hypochlorite. Confirmation of effective sterilisation/sanitisation will be performed by periodic test swabbing the laboratory equipment and culturing on GMM-specific agar plates.

For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
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</table>

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessment has been approved and is attached. Work will involve intermediate to large scale (20-1000L) growth of Level 1 GMMs. Large scale containment level 1 measures will be in place.
**GM Centre Number: 3544**

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**Name**

BIOLOGIC TECHNOLOGIES LTD

**Name 2 Department**

Campus Estate or Research Centre

**Road Name**

9B THE SHADE

**District**

SOHAM

**Town**

ELY

**County**

CAMBRIDGESHIRE

**Postcode**

CB7 5DE

**Country**

ENGLAND

**Tel Number**

07951746679

**Fax Number**

0

**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

BiologIC is currently a small startup so has a BSO on the executive committee rather than a full GM safety committee. The BSO has 15+ years hands on experience in the handling of GMOs and infectious diseases, and has headed up commercial laboratories up to BSL3 including with safety and biosecurity remit.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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</table>
All waste is treated to ensure 100% kill prior to disposal. For class 1 activities, the primary inactivation is by disinfectant as per the suppliers instructions e.g. Virkon. All contaminated materials that cannot be suitably disinfected will be inactivated by autoclaving (100% kill) at 121°C or 134°C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Each run will be monitored using chemical indicators e.g. steam sterilisation indicator tape.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The BSO has 15+ years hands on experience in the handling of GMOs and infectious diseases, and has headed up commercial laboratories up to BSL3 including with safety and biosecurity remit.
## GM Centre Number: 3545

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02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

The Cardiff And Vale University Health Board GMSC evaluated and approved the risk assessment. The GMSC meets every first Wednesday of the month and is composed of a Chair and a Secretary, plus a collection of members with various skill and expertises, in no particular order a Biological Safety Officer, a Health and Safety Advisor, a Consultant Microbiologist, a Consultant Haematologist, a Consultant Pharmacologist, a Nurse representative, a Occupational Health representative, a Rn'D representative and a Technical Expert.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs) | Yes | | | |
Level 2 (GMMs) | | | | |
Level 3 (GMMs) | | | | |
Level 4 (GMMs) | | | | |
Non-microbial | | | | |
Other (please specify) | | | | |

Tick if confidential  

Bacteriology | Yes | Parasitology | Transgenic Birds | Microbiology Research |
---|---|---|---|---
Yes | | | | Yes
Class 1 GM waste will be separated from non-GM waste and inactivated on site by means of autoclave sterilisation (i.e., 134°C for 30 minutes), then sent off site for incineration. A record of autoclave run reports will be kept as proof of effective sterilisation. Autoclaves are regularly maintained by the Estate Department of Cardiff and Vale University Health Board and serviced by a third party company.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

A specific SOP and training documents related to storage, handling and disposal of GMMs were requested by the GMSC in order to ensure that laboratory staff operate following the relevant GM regulations.
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

1) Laboratory Manager with >15 years experience of handling GMMs. NEBOSH qualified.
2) Head of Operations with >15 years experience of handling GMMs.
3) Principal Bioscientist with >30 years experience of generating and handling GMMs.
4) Safety, Health and Environment Officer - IOSH qualified

<table>
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<th>Growth Room</th>
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<th>Large Scale</th>
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Bacteriology Parasitology Transgenic Birds Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<tr>
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<td>Storage, use and disposal of genetically modified mammalian cell lines.</td>
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All liquid waste from sample processing will be disinfected with 2% Virkon for at least 15 minutes before disposal to the drain with copious volumes of water.
All potentially contaminated solid materials will be inactivated by autoclaving prior to disposal of waste.
This will include any material used for handling leakages, spills or breakages. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile.
Surfaces will disinfected with 1% Virkon spray after use, and tissue discarded into autoclave waste.
Spillage will be absorbed onto paper towels and sent for autoclave disposal. The area will then be disinfected with 1% Virkon as above.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The measures outlined in the attached risk assessment should adequately control the hazards in this project.

**Project Ref** 3547/20.1

<table>
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<td>Non-GMM</td>
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Purposes of the contained use

Chimeric Antigen Receptor (CAR) -T cells are engineered T cells that specifically express a T cell receptor to a defined antigen, allowing for the activation of the cells by this target antigen. Engineering of Treg in this manner has implications in the treatment of autoimmune and inflammatory indications. The lentiviral and retroviral vectors provided by the client for this project will allow the generation of test CAR-T regulatory cells (Treg) that target MOG antigen. The activity of these T cells will then be verified in vitro (human cells) or in vivo (murine cells).

Recipient or parental organism

Each virus stock solution contains a pure recombinant, replication-defective lentivirus/retrovirus population. Virus constructs contain a chimeric antigen receptor to enable T cells to recognise a specified antigen (constructs outlined in attached RA).

Host/vector system

Host cells will be purified murine or human T cells. These cells have a regulatory phenotype, therefore any inadvertent exposure or entry of these cells into a human or other vertebrate should not instigate any aberrant immune responses. Lentivirus and retrovirus constructs are well characterised vectors that enable insertion of genetic material into host cell genomes. Third generation construct vectors are not considered harmful as they are replication incompetent.

Origin & function

The inserted gene product is not in itself harmful and encodes T cell receptors that can recognise a peptide sequence within MOG protein. T cell receptors are ordinarily expressed on T cells and therefore, no adverse biological activity should be present. Activation of the receptor in the model T cells would require exposure to the specific antigen.
Evaluation of foreseeable effects

Sequence will be stably transduced into target cells and so there should be no risk of sequence being transferred from transduced CAR-T cells. Supernatants from transduced T cells, and any excess viral vector solutions, will be inactivated (by addition to Virkon) prior to disposal and so there should be no further risk of accidental transfer of sequence. Each virus stock solution is a replication-defective, well characterised vector and so is unlikely to cause any harm to human health. The chimeric antigen receptor that will be inserted into the host T cells is specific for protein expressed within the central nervous system which is not normally visible to the mammalian immune system (due to the blood brain barrier). Therefore, even if lab workers were accidentally exposed to these transduced T cells, these would provide no harm to human health in immunocompetent people (with an intact blood brain barrier). Murine CAR-T cells generated in this project will provide no additional risk to human health. In terms of oncogenic potential, the vectors used in this study pose no additional harm over standard vectors. Pregnant women, new mothers and/or immunocompromised employees must not handle class 2 lentiviral and retroviral vectors.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste from sample processing will be disinfected with 2% Virkon for at least 15 minutes before disposal to the drain with copious volumes of water. All potentially contaminated solid materials will be inactivated by autoclaving prior to disposal of waste. This will include any material used for handling leakages, spills or breakages. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile. Surfaces will disinfected with 1% Virkon spray after use, and tissue discarded into autoclave waste. Spillages will be absorbed onto paper towels and disposed of after autoclaving. The area will then be disinfected with 1% Virkon as above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

02/03/2022
The measures outlined in the attached risk assessment should adequately control the hazards in this project.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Animal Units

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<th>Human Clinical Applications</th>
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Project Ref 3547/20.2

Date Ackn'd: 23/07/2020

CU2 Project Title: Handling live genetically modified Mycobacterium bovis

Class: Class 2

CultureVolClass2: < 1 Litre

Consent Granted

Withdrawn: N

Tick if notifying a connected programme of work: N

Historical Significant Changes

Project notified under transitional arrangements: N

Project Additional Information

Purposes of the contained use:

Genetically modified mycobacterium will be supplied by the client: VPM1002BC is a live genetically modified Mycobacterium bovis (M. bovis) BCG strain, generated in order to direct mycobacterial
antigens to the major histocompatibility complex (MHC) class I pathway. The project will utilise cancer
cell lines and PBMC to understand whether genetically modified material can alter the immune
responses and directed killing of cancer cells by BCG-activated PBMC.

Recipient or parental organism

VPM1002BC is a genetically modified version of BCG-medac, that has been
categorised by the suppliers as biological substance risk group 1. Nonmodified
BCG-medac belongs to biological substance risk group 2, which
can lead to disease in humans and pose a danger to employees. Both
substances will be handled with the same precautions, making the overall
activity class 2.

Host/vector system

Client will provide stably modified VPM1002BC (and control BCG-medac).
No genetic modification will take place within Concept Life Sciences, just
stimulation of primary (human) cells in vitro with these mycobacterium
preparations.

Origin & function

VPM1002BC is a live, attenuated BCG strain that has been modified in order
to direct mycobacterial antigens to the major histocompatibility complex
(MHC) class I pathway. This should provide no additional risk to human
health.

Evaluation of foreseeable effects

The genetic modification of BCG-medac to produce VPM1002BC reduces its
pathogenicity by improving any resultant immune response upon accidental
exposure.
Exposure to VPM1002BC and BCG-medac can lead to disease in humans
and pose a danger to employees. If the following precautions are followed by
trained personnel, then risk to human health is low:
• Persons handling BCG-medac and VPM1002BC must use BSCs and
should additionally wear gloves and lab coats. This will protect users from
any hazard posed by the mycobacterium and ensure sterility of cellular
samples is maintained.
• After work, all waste and surfaces must be disinfected with Virkon solution
(or a similar suitable disinfectant).
• After completion of work, PPE must be removed and hands thoroughly
cleaned before users leave the laboratory area.
Pregnant women, new mothers and/or immunocompromised employees
must not handle VPM1002BC or BCG-Medac product.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste from sample processing will be disinfected with 2% Virkon for at least 15 minutes before disposal to the drain with copious volumes of water. All potentially contaminated solid materials will be inactivated by autoclaving prior to disposal of waste. This will include any material used for handling leakages, spills or breakages. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile. Surfaces will be disinfected with 1% Virkon spray after use, and tissue discarded into autoclave waste. Spillages will be absorbed onto paper towels and disposed of after autoclaving. The area will then be disinfected with 1% Virkon as above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The measures outlined in the attached risk assessment should adequately control the hazards in this project.

Project Containment

<table>
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<tr>
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Project Ref 3547/20.3

Date Ackn’d CU2 Project Title Class CultureVolClass2 CultureVolumeClass3-4

02/03/2022
Transduction of cell lines using genetically modified adenovirus

Replication deficient adenoviruses will be used to transduce human urinary bladder transitional cell papilloma RT4 cells. The project will use these transduced cells and HLA-1 matched PBMCs to understand IFN alpha expression can increase tumour antigen-specific T cell responses using a series of in vitro cell based assays.

VQAd EMPTY-eGFP is a recombinant adenovirus type 5 encoding eGFP.
VQAd hIFNa2.GFP is a recombinant adenovirus type 5 encoding human IFNa2 and eGFP.

Host cells will be RT4 cells which are Biosafety level 1.

Adenoviridae are non-enveloped, icosahedral virions, 75-80 nm diameter, doubled stranded, linear DNA genome. The recombinant viruses are based on human adenoviral backbone (dl309) which is deleted in the essential E1 gene as well as the E3 gene. The viruses produced are replication deficient. Vectors were produced by ViraQuest, Inc. Both vectors have tested negative for E1 detection and RCA titre.

Both vectors contain GFP expressing gene while one of the vectors also contains an IFNa expressing gene. IFNa is involved in the innate immune
response to viral infections. As IFNα is immunomodulatory, exposure to the recombinant adenovirus expressing IFNα could result in an immune response. Danger can be caused by penetration through injured or unprotected softened skin, spraying over the eye and mucous membranes and/or swallowing.

Each virus stock solution is a replication-defective, well characterised vector and so is unlikely to cause a large infection risk. However, the risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Infection can vary in clinical manifestation and severity; symptoms can include fever, rhinitis, pharyngitis, cough and conjunctivitis. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.

<table>
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<tr>
<th>Containment and control measures for GMOs that are not microorganisms (e.g. GM animals &amp; plants)</th>
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<tbody>
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**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

<table>
<thead>
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<th>Described the waste management measures which you will apply to the activity (including type &amp; form, treatment, ultimate form &amp; fate)</th>
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| All liquid waste from sample processing will be disinfected with 2% Virkon for at least 15 minutes before disposal to the drain with copious volumes of water.  
All potentially contaminated solid materials will be inactivated by autoclaving prior to disposal of waste. This will include any material used for handling leakages, spills or breakages. Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile.  
Surfaces will disinfected with 1% Virkon spray after use, and tissue discarded into autoclave waste. Spillages will be absorbed onto paper towels and disposed of after autoclaving. The area will then be disinfected with 1% Virkon as above. |

| Is an emergency plan required according to regulation 20? | N |

**Tick to confirm that it is attached to this form**

| Tick to confirm that you have attached a risk assessment to this form | Y |

**Tick if you are claiming exemption from disclosure for section of the risk assessment**

<table>
<thead>
<tr>
<th>Please enter comments on the GM safety committee on the risk assessment</th>
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</thead>
<tbody>
<tr>
<td>The measures outlined in the attached risk assessment should adequately control the hazards in this project.</td>
</tr>
</tbody>
</table>
Purposes of the contained use

Genetically modified lentivirus, retrovirus or adenovirus will be supplied by our clients and will be used to transduce human or rodent cells. These will either be primary cells (from healthy human donors or WT animals) or stable cell lines. Engineering of cells in this manner has implications in the treatment of autoimmune and inflammatory indications and will allow us to use a range of in vitro assays to further elucidate the function of the molecules being targeted by our clients in health, and in disease.

Recipient or parental organism
Each virus stock solution will be provided by our clients and will contain a pure recombinant, replication-defective lentivirus, retrovirus or adenovirus population.

### Host/vector system

Host cells will be purified primary human (isolated from PBMCs from healthy blood donors), or rodent (isolated ex vivo from wild-type mice or rats) immune cells; or established cell lines. These cells are not considered harmful and therefore any inadvertent exposure or entry of these cells into a human, or other vertebrate, should not instigate any aberrant immune responses.

### Origin & function

Each virus stock solution will be provided by our clients and will contain a pure recombinant, replication-defective lentivirus, retrovirus or adenovirus population. The inserted gene product will not in itself be harmful and will not include allergens, oncogenes or cytokines.

### Evaluation of foreseeable effects

Sequence will be stably transduced into target cells and so there should be no risk of sequence being transferred from transduced cells. Supernatants from transduced cells, and any excess viral vector solutions, will be inactivated (by addition to Virkon) prior to disposal and so there should be no further risk of accidental transfer of sequence. Each virus stock solution is a replication-defective, well characterised vector and so is unlikely to cause any harm to human health. In terms of oncogenic potential, the vectors used in these studies pose no additional harm over standard vectors.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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disinfected with 1% Virkon as above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

All liquid waste from sample processing will be disinfected with 2% Virkon for at least 15 minutes before disposal to the drain with copious volumes of water.

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Project Ref 3547/21.2

Handling genetically modified Herpes Simplex Virus-1 (HSV-1)

Date Ackn'd 04/03/2021

Date Project Ceased 04/03/2021

Class 2

< 1 Litre

Non-GMM Consent Granted
Purposes of the contained use

Genetically modified Herpes Simplex Virus-1 (HSV-1 - KGα2 -4 E6-B hKt) will be supplied by the client. The virus is an IL13Rα2-targeted oncolytic virus (OV) with eGFP. This virus will be used to infect A375 and A549 cell lines, investigating the effect of this construct upon immunogenic cell death in vitro.

Recipient or parental organism

An IL13Rα2-targeted oncolytic virus expressing eGFP will be provided by our client.

Host/vector system

Host cells will be established A375 and A549 human cell lines (class 1). These cells are not considered harmful and therefore any inadvertent exposure or entry of these cells into a human, or other vertebrate, should not instigate any aberrant immune responses.

Herpes simplex virus-1 (HSV-1) belongs to the sub family Alphaherpesviridae in the family Herpesviridae, genus Simplexvirus. It is 120-300 nm in diameter and consist of a linear, double stranded DNA genome (152 Kb) enclosed within an icosahedral capsid, surrounded by a phospholipid rich envelope.

Origin & function

The GM HSV-1 used in this project (HSV-1 - KGα2 -4 E6-B hKt) has genetically modified glycoproteins gD, gB, and gK. The mode of infection, and transmission capabilities, are considered to be more limited than that of wild-type HSV-1.

gD is essential for HSV-1 entry into cells. Amino acid 2 to 24 of gD are deleted and a point mutation Y38C is introduced to eliminate its ability to bind to its natural receptors HVEM and nectin-1. A single-chain variable fragment (scFv) targeting a human cancer-testis antigen IL13Rα2 is inserted into the deletion region of gD. These gD modifications enable the GM HSV-1 to enter only cells actively expressing human IL13Rα2.
Two point mutations (D285N, A549T; refers to as NT mutations) are introduced in gB to enhance the entry of the GM HSV-1 into target cells. Syncytial mutations (point mutations R858H in gB and A40T in gK; refers to as BhKt mutations) are introduced to confer hyperfusogenicity to the GM HSV-1, resulting in enhancement of the cytopathic effect in IL13Ra2-positive cells.

The coding sequence for enhanced green fluorescent protein (eGFP) under the CMV promoter is inserted between UL3 and UL4 genes. eGFP has no known hazards and is not a dangerous substance according to GHS classification.

Evaluation of foreseeable effects

Infection with WT HSV-1 can lead to Herpes labialis/ cold sores; Herpetic whitlow; infections of the eye and encephalitis in children and adolescents. The GM HSV-1 used in this project (HSV-1 - KGNα2 -4 E6-B hKt) has genetically modified glycoproteins gD, gB, and gK. The mode of infection, and transmission capabilities, are considered to be more limited than that of wild-type HSV-1.

NT and BhKt mutations may enhance the virulence of the GM HSV-1; however, this effect is limited to infection of IL13Ra2-positive cells. Considering that virus entry and replication of the GM HSV-1 are strictly limited to cells expressing IL13Ra2, which is known as a cancer-testis antigen and is not expressed in normal tissues except for the testis, the potential for harm to human health is considered low.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste from sample processing will be disinfected with 2% Virkon for at least 15 minutes before disposal to the drain with copious volumes of water.

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Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile.

Surfaces will disinfected with 1% Virkon spray after use, and tissue discarded into autoclave waste. Spillages will be absorbed onto paper towels and disposed of after autoclaving. The area will then be disinfected with 1% Virkon as above.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N
The measures outlined in the attached risk assessment should adequately control the hazards in this project.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

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Comments

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| 02/03/2022 |
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<td>UK</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Site has a safety committee structure. Departmental reviews take place. This activity will form part of the existing governance overview. Regular vaccine task force meetings have been taking place between Wokhardt, AstraZeneca and Oxford's Jenner Institute, where collaboration has taken place, including discussion of health and safety matters, e.g the risk assessment was developed using data from the Jenner Institute at Oxford University.

Level 1 (GMMs)
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
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<tr>
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**PREMISES IS NOT USED FOR GENETIC MODIFICATION - Storage of substance only.**
The potential to generate waste would result only as a consequence of a spill. This outcome has been assessed as being highly unlikely due to the method of packaging of the substance (an article), The substance itself is defined in the SDS as not being hazardous in terms of exposure. The safety data sheet advises that the adenovirus vector has no risk of pathogenicity and virulence in the case of occupational exposure. It is not classified as being harmful to the environment. Wockhardt has procedures to deal with spills for hazardous substances and materials (HSOP-029-2874) and waste disposal (ENV-007-04-1857). These procedures would allow for any risk of contamination that could potentially render the substance to become hazardous to be safely managed- the risk assessment for the defined activities also identifies that such a scenario is also highly unlikely. Hazardous, non-hazardous and clinical waste is disposed of via approved licensed contractors.

Tick to confirm that you are attaching a summary of the risk assessment 

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

EHS Manager; Programme Manager; Warehouse and Operations Lead;
EHS Consultant - Members who participated in the risk assessment
<table>
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<tr>
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<th>27/07/2020</th>
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**Date at Which Additional Info Submitted**

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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- Yes

**Give brief details of the genetic modification safety committee**

University of Birmingham Medical and Dental Sciences Genetic Modification Safety Committee.

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**Tick if confidential**

- No
For activities involving GMMs, describe the waste management measures which will apply to the activity

Inactivation of GMMs in waste and subsequent disposal:
Plasticware, gloves and tissues etc. will be disposed into autoclave bags (double-bagged), autoclaved (121°C for 15 minutes; programmed and validated on the Biohub autoclave which reports that the cycle has passed or failed [if a cycle fails, the fault would be rectified and the contents treated as hazardous and the load run again successfully]; this autoclave is serviced every 6 months with annual UKAS calibration and supported by a rolling service contract) and disposed of as clinical waste by incineration. Expected degree of kill 100%.

Liquid waste will be incubated in 1% Chemgene or 1% Virkon or PreSept tablets before solidification with vernagel and disposed of in clinical waste for incineration. Expected degree of kill 100%.

The cell lines are not expected to survive outside the specialist cell culture environment.

Monitoring of waste inactivation methods:
As cell lines are so fragile and cannot survive outside specialist cell culture environment growth is not expected following inactivation. The autoclave waste cycle used for inactivation has been validated and reports a ‘pass’ or ‘fail’. The autoclave is serviced every 6 months with annual UKAS calibration and supported by a rolling service contract along with regular monitoring by Biohub staff.

 Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM safety committee commented on the disinfection process for plasticware. We will decontaminate plasticware by disposal into autoclave bags (double-bagged), autoclaving (121°C for 15 minutes; programmed and validated on the Biohub autoclave which reports that the cycle has passed or failed [if a cycle fails, the fault would be rectified and the contents treated as hazardous and the load run again successfully]; this autoclave is serviced every 6 months with annual UKAS calibration and supported by a rolling service contract) and disposed of as clinical waste by incineration. Expected degree of kill 100%. This information has been updated within the risk assessment.

In addition, they asked:
"Mention autoclave and how its runs are monitored."

We have added details of the autoclave waste cycle, how it is serviced, and how runs are monitored into the risk assessment (121°C for 15 minutes; programmed and validated on the Biohub autoclave which reports that the cycle has passed or failed [if a cycle fails, the fault would be rectified and the contents treated as hazardous and the load run again successfully]; this autoclave is serviced every 6 months with annual UKAS calibration and supported by a rolling service contract).
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#### Date at Which Additional Info Submitted

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

An earlier version of the Risk Assessment associated with this application was reviewed by a previous member of the University Genetic Modification and Biological Agents Committee, based in the School of Biosciences at Cardiff University. Cellesce is a small, independent, start-up company (SME) originating from Cardiff and Bath Universities. Cellesce has offices and labs in a Cardiff University-run facility that supports new biotechnology businesses. The “Lead Scientist” has more than 15 years experience in the assessment of risk related to academic and commercial use of GMOs. The senior staff members of the company also have many years of experience working with GMOs. As required by the Safety Representatives and Safety Committees Regulations 1977, Cellesce employees who are not covered by trade union safety representatives have been consulted, according to the Health and Safety (Consultation with Employees) Regulations 1996. This application has been reviewed and approved by Cellesce’s “Genetic Modification Safety Committee” GMSC. This comprises Cellesce’s Lead Scientist, the Lab Operations Manager and a Professor at Cardiff University who is Deputy Head of Molecular Biosciences, Director of organoid biology at Cellesce, and has been working with GMOs for more than 20 years.

Level 1 (GMMs)  
Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)
Procedures for waste management are based on the principles of Good Microbiological Practice, COSHH regulations, and the British Standard (BS EN 12740: 1999 Biotechnology. Laboratories for research, development and analysis. Guidance for handling, inactivating and testing of waste). The SACGM guidance on risk assessment (part 2) and waste inactivation before disposal (part 3, section 3.5) was also considered. The waste management system is appropriate to the overall management of the waste produced as part of the laboratory's activities.

Commercially available cell-lines are negative for mycoplasma. Cells are transported into the lab, contained within suitable packaging and stored at the appropriate temperature (depending on whether they are cryopreserved or live), humidification and carbon dioxide as necessary. Personal Protective Equipment; lab coat, gloves and safety glasses, are worn at all times in the laboratory area.

All staff working within the laboratory are competent, properly trained and informed. Training records are kept and updated regularly. Hands will be washed in the event of a suspected contamination and on leaving the laboratory. Lone working is not permitted. All accidents and incidents are recorded and reviewed regularly.

The handling of the cell cultures is performed in Class II Biological Safety Cabinets. Cabinets are serviced annually.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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02/03/2022
Surfaces are decontaminated with 70% ethanol before use and the insides of the cabinet are disinfected weekly using "Virkon" (Du Pont), a high-level surface disinfectant proven to effectively tackle all known pathogens; fungi, viruses and bacteria. Equipment within the hoods such as pipettes, are decontaminated regularly with 70% ethanol. Equipment is decontaminated thoroughly with 70% ethanol before repair or servicing. Manual culture materials are single-use. All contaminated solids, such as used plastics, single-use pipettes and paper (used for decontamination of work surfaces and equipment with 70% ethanol) are treated as hazardous and placed in designated, clearly labelled, biohazard waste bins, double-lined with brightly coloured, clearly labelled, biohazard waste bags. The partially filled bags are removed regularly and double sealed with tape. They are removed to a large, locked container at the back and exterior of the building, where all of the waste is stored. Media from tissue culture is aspirated into sealed, impermeable, rigid cannisters, containing a gelling agent to solidify the liquid. This container is sealed when full, to maintain the required impermeability and integrity. It is then placed in a biohazard bag and sealed at the top with tape. The bag is placed in the bin at the back of the building with the rest of the bio-hazard waste. Keys for the container are held by Cellesce and by the commercial waste disposal company, Greener Options Ltd, who collect the rubbish on a monthly basis for incineration. This is the recommended method for solids and is 100% effective. The use of sharps is not normally necessary for this procedure. If absolutely necessary, these are placed in commercially available "sharps bins" that are sealed when full and stored with the biohazardous waste, for collection and incineration. Non-contaminated material, for example cardboard or packaging from sterile tissue culture plastics, is classified as non-hazardous waste and placed in clear bags for recycling where possible or in in black bags in designated black bins, for removal to the household waste facility at the rear of the building. The recycling and nonhazardous waste is collected twice weekly by the local council.

This project does not require an emergency action plan as the risk to people and to the environment is negligible.

Tick to confirm that you are attaching a summary of the risk assessment Y
Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The work proposed is very low risk and is appropriate for the category level proposed.
GM Centre Number: 3551

Data Premises Notified (Originally) 28/07/2020

Transferred from 1992 Regs? N

Transitional Premises

Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

BRITISH AMERICAN TOBACCO (INVESTMENTS) LTD

Name 2

Department

Campus Estate or Research Centre

REGENTS PARK ROAD

Road Name

MILLBROOK ROAD

District

Town

SOUTHAMPTON

County

Postcode SO15 8TL

Country ENGLAND

Tel Number 0336 741 1079

Fax Number 0

E-mail

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Comments

Date at Which Additional Info Submitted

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The team is currently located in Cambridge, where a local Sub-Committee holds Biological Safety Team Meetings four times a year. There are currently three members on the committee, which cover EH&S requirements, Nagoya compliance, Biological safety, Material received/sent inspections.

Our procedures are captured in the internal Biological Safety Handbook, which collects all the information required to work with:

- Working with and keeping live organisms (plants, bacteria, yeast, cell cultures etc.)
- Biological compounds such as certain DNA plasmids or viral RNAs that can act as biological agents.
- Importing biological material into R&D Cambridge
- Exporting biological material from R&D Cambridge
- Disposal of biological material

We also have a Risk assessment for "Working with Biological Materials including Class 1 GMOs", and SOPs for the different procedures.

The person responsible for Biological Safety is a Senior Scientist in R&D New Sciences. They have expertise in microbiology, molecular biology, biotechnology and agronomy. They have worked for over 10 years with plants, microbes, and GM material in laboratory controlled environment condition and greenhouses (up to BSL2)."
For activities involving GMMs, describe the waste management measures which will apply to the activity

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Waste containing plant material, including greenhouse waste and waste from growth chambers, and plant growth medium are disposed of in the sealable, rigid clinical waste bins. These are sealed when full and stored in the waste chemical store for licensed special waste disposal.

Waste material and laboratory glassware, plastic-ware or other materials contaminated with GMMs are to be inactivated by validated means: Domestos bleach to be used at a final concentration of 10% solution (needs to be made up fresh daily) or freshly made 1% Hypochlorite (final concentration) for disinfecting liquid cultures, cleaning benches, surfaces and floors. Laboratory sharps (scalpel blades, syringe needles, glass pipettes etc.) are all to be collected in labelled clinical sharps containers and disposed of as clinical waste by incineration, whether likely to be contaminated or not. Alternatively, they can be autoclaved with a discard cycle (121C at 15psi for 30min), after which they can be safely disposed of.

Simple spills are to IMMEDIATELY be covered with a paper towel. Wear disposable gloves and use paper towels to absorb all free liquid. Finally, decontaminate surfaces with undiluted disinfectant. All infected towels and gloves must be put into an autoclave bag for autoclaving or in a yellow labwaste bin for incineration.

When infected materials in bottles, flasks or petri-dishes are dropped and broken, the debris is be IMMEDIATELY covered with a paper towel soaked in undiluted disinfectant. Avoid bringing the face into contact with the aerosol cloud. Absorb any free liquid with paper towels (DO NOT use sweeping brushes because these can cause an aerosol). Use the dustpan and plastic scoop or forceps to collect the debris and place in a container to be autoclaved or yellow labwaste bin for incineration. Broken glass must NOT be placed in autoclave bags. It has to be placed in a yellow labwaste bin for incineration. The area is to thoroughly with undiluted disinfectant. All debris, used towels and gloves are then to be autoclaved before discarding.

Working surfaces are disinfected after each use, staff is to inspect each laboratory for accidental spills before start of work and clean those as stated above. Incidents are to be tracked and reported immediately.

Additionally, the general rules for working in laboratory environment apply.

Tick to confirm that you are attaching a summary of the risk assessment  
Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment
The person responsible for Biological Safety is a Senior Scientist in R&D New Sciences. They have expertise in microbiology, molecular biology, biotechnology and agronomy. They have worked for over 10 years with plants, microbes, and GM material in laboratory controlled environment condition and greenhouses (up to BSL2).
## GM Centre Number: 3552

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The London Clinic has appointed an internal Biological Safety Officer (BSO) to advise management on contained use activities and provide approval for the risk assessments which are of GM Class 1 risk level as permitted under the contained use regulations. The BSO has been trained by external providers. The Queen Mary University of London (QMUL) GM Safety Committee and the QMUL BSO under contractual agreement provide expert peer review of risk assessments where risk level is deemed greater than Class 1 or contentious issues arise. The QMUL GMSC meets at least three times a year and conducts its activities under the terms of reference drawn up according to the contained use regulations and can be viewed at a specific webpage at http://www.hsd.qmul.ac.uk/

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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Tick if confidential
Solid GM Class 1 non-sharp waste is either inactivated by an effective disinfection method (noted below) or directly placed into yellow, sealable UN approved containers for uplift and offsite high temperature incineration (900 deg C) by an authorised upper-tier clinical waste contractor Harrison Benn. High temperature incineration achieves 100% kill of the GMO. The clinical waste contractor's quarterly returns for certification of solid waste destruction by incineration will be held for 3 years at the site, in accordance with UK environmental legislation. Sharps waste is not expected since no needles will be used during the product administration procedure. Liquid waste (or as necessary, for solid waste) is disinfected by an effective disinfectant. Chlor-Clean for inactivation of the GMO is proposed for use. Chlor-Clean contains triclosene sodium, which is a strong oxidant and chlorating agent. Liquid waste will then be disposed with copious tap water down designated sluice / lab sinks, in line with the local Water Authority effluent permit conditions. Surface decontamination (e.g. lab / ward benches) is to be conducted using Clinell Universal Wipes (in didecyl dimethyl ammonium chloride which is an antiseptic/disinfectant and polyhexamethylene biguanide which has antiviral and antibacterial properties). Emergency spillages will be treated using Chlor-Clean. Disinfection protocols are being set up and applicable personal protective equipment donned / worn during handling. Training of GM users / workers in the use of disinfection procedures is to be recorded and refreshed according to local policy. Any residual GMO cell stocks will be disposed into clinical waste sharp bins (yellow or white lid) for high temperature incineration by the authorised upper-tier clinical waste contractor Harrison Benn. Autoclaving of waste is not required at this risk level, although in case of emergencies, an autoclave at The London Clinic for waste inactivation by autoclaving at 121 deg C for 15 min holding time is available. All waste bags and containers are to be transported from clinical area to waste collection bin location in robust closed leak proof containers (on trolley if bulky or heavy).

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

---

**Tick to confirm that you are attaching a summary of the risk assessment**  

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

---

Please enter comments of the GM safety committee on the risk assessment
The attached project risk assessment for Class 1 work and this notification has been peer reviewed by the QMUL BSO and approved for submission. Future risk assessments and any amendment required to this notification will be peer reviewed by the QMUL BSO and where relevant, the QMUL GM safety committee (as per contractual arrangements noted in section 3).
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Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Current Faculty Biological Safety Officer at UK Higher Education Institution and non-employee of the notifying organisation. Good understanding of the legislation, guidance, procedures and best practice relating to the management of the risks arising from biological agents, including GMOs. Formal Biological Safety Officer training: Institute of Safety in Technology and Research registered Biosafety Practitioner level 1 and level 2 course, Biological Safety Officer network, conferences and workshops, continuing professional development.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
--- | --- | --- | --- | ---
Level 1 (GMMs) | Yes | | | |
Level 2 (GMMs) | | | | |
Level 3 (GMMs) | | | | |
Level 4 (GMMs) | | | | |
For activities involving GMMs, describe the waste management measures which will apply to the activity

Primary containment of solid and liquid waste containing GMMs in clear plastic sack marked "Autoclave Waste Only" containing absorbent material held in appropriate bag stand. Deactivation of contents is achieved by closing the bag loosely with Autoclave Indicator Tape prior to sterilisation and autoclaving (121°C for 60 minutes) before disposal as non-infectious clinical waste through laboratory waste management contractor (e.g. Grundon).

Spillages treated with standard cleaning materials (e.g. absorbent towels) and surface cleaned with appropriate disinfectant such as Distel Trigene (or similar commercial reagent). Any contaminated cleaning materials (e.g. paper towels) will be disposed of according to procedure for disposal of solid and liquid waste containing GMMs.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
<table>
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**Name**

ALCHEMAB THERAPEUTICS LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

EAST SIDE, OFFICE 1.02

**District**

KINGS CROSS

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

N1C 4AX

**Country**

ENGLAND

**Tel Number**

020 7961 0300

**Fax Number**

0

**E-mail**

blank

**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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#### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

**Y**

Give brief details of the genetic modification safety committee

The Alchemab Ltd genetic modification committee will consist of:

1. **GMO Supervisor** - The Head of Research has extensive experience of genetic modification, having 20 years+ experience of working in a biotechnology/pharmaceutical industry setting, working in antibody and protein engineering research, involving phage display and protein expression/modification in mammalian cells and E.Coli, with management oversight for a research group.

2. **Biological Safety Officer** - The Head of Translational and Clinical Science who has who has 20+ years experience of working in a biotechnology/pharmaceutical industry setting working in pre-clinical bioscience, translational biology and early clinical settings, including use of mammalian cell culture, primary cells and human samples, and management of a research group. The biosafety officer will chair all genetic modification committee meetings.

3. **The Chief Scientific Officer** has 20+ years experience of genetic modification and was previously site lead for a large multinational pharmaceutical company. Her function on the committee is to advise and provide the necessary resources for the actions of the committee to be implemented.

4. **2x Senior Research Scientists** who are either specialists in mammalian cell culture or molecular biology, both with 5+ years experience in an academic/pharmaceutical industry/biotechnology setting. These members will provide advice and will rotate writing the minutes of the meeting between them.

The committee will meet every 6 months to review biosafety and whenever a new risk assessment is required. The first meeting will review the existing risk assessments.

<table>
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</tbody>
</table>
At Illumina Accelerator:
All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal, and autoclaved onsite before being placed in sealed bins and removed by registered waste specialists. Liquid waste (Virkon inactivated) will be decanted into suitably labelled liquid waste vessels before being removed from the site by registered waste specialists. (Veolia)

At Babraham Meditrina premises:
All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below). Waste from our GM work at Class 1 will be placed into suitably labeled sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical GM waste incinerator contractor.
Both of these disposal methods are expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

 Tick to confirm that you are attaching a summary of the risk assessment

 Tick if you are claiming exemption from disclosure for sections of the risk assessment
Given the lack of mechanism for transmission to, or expression in, human hosts there is effectively zero likelihood of harm to human health even in the event of exposure.
- Hosts are standard, non-pathogenic E.coli lab strains, such as TG-1 and BL-21, which are hazard group 1
- the genes are human-derived and therefore normally expressed and tolerated in human hosts
- no vector capable of transmitting DNA into humans is involved in the work described
- the human genes are under the control of a bacterial promoter (not a mammalian promoter) so even if plasmids were present in a human host the genes would not be expressed

There is minimal risk to environment.
If the GM microorganism (GMMO) were to leave the lab environment, it would be expected to lose the plasmid encoding the foreign gene within a few cell divisions unless maintained on antibiotic selection. Only in the scenario where the foreign gene confers a growth advantage would the GM microorganism be able to survive and potentially displace other organisms. The only organisms likely to receive the DNA vectors and express the gene inserts are closely related bacterial species so no harm to animals or plants is anticipated. Bacterial expression vectors are designed for optimal function within lab strains of E.coli but expression of the human gene products in related gram negative bacteria eg other strains of E.coli or Salmonella species is possible. If the human gene products are toxic to the recipient bacteria then these bacteria will rapidly self-eliminate without passing the vector any further. Even if the gene products are not toxic but change bacterial biology in some other way, they will not be maintained over multiple cell divisions in the absence of the antibiotic used to maintain the plasmid so the vector's persistence would be expected to be short-lived. Only if the gene product confers a selective advantage to the bacterial recipient will it be maintained and potentially passed to other bacterial hosts.
**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

The contained use purpose covers the generation and use of third generation lentivirus particles that encode individual open reading frames (ORFs), gene sequences, cDNAs and/or shRNAs, and their subsequent use to generate cell lines for in vitro studies. These studies will assist with understanding disease biology, and the role of specific target proteins by generating cell lines for in vitro screens. Such screens can include: (i) Binding assays and flow cytometry (ii) Pull down and Immunoprecipitation studies (iii) Imaging assays (iv) Proliferation assays (v) Phenotypic screens (vi) Generation of cell lines with constitutive reporter proteins or inducible signalling reporter cell lines. This work will assist in the validation and discovery of novel antigenic targets relevant to human health. The overall purpose is to develop antibody therapeutics.

**Recipient or parental organism**

Mammalian cell lines from Hazard group 1 & 2 and Activity Class 1 & 2 genetically modified cells. All mammalian cells will be covered by appropriate risk assessments prior to their use for this project.

**Host/vector system**

For successful viral production, the third-generation lentiviral system requires 4 separate plasmids to be co-transfected into a host mammalian cell line (e.g. HEK293T). HEK293T, is a subclone of the transformed human embryonic kidney cell line, HEK 293, which is highly transfectable and supports high levels of viral protein expression. The cell line also constitutively expresses the simian virus 40 (SV40) large T antigen.

The plasmids required for lentiviral production are:
- The transfer plasmid (encoding the genetic insert)
- Two packaging plasmids (one encoding Gag and Pol and another encoding Rev)
- One envelope plasmid encoding VSV-G

Commercial packaging and envelope plasmids will be used, for example pPACKH1 HIV Lentivector Packaging Kit 200ul (Systems Biosciences #LV500A-1).

The lentiviral vector together with the packaging plasmids comprise the third generation lentiviral expression system. The HIV-based lentivectors are based on the vectors developed for gene therapy applications by Dr. J. G. Sodroski (US patent #5,665,577 and # 5,981,276).

The HIV-based lentivector systems are designed to maximize their biosafety features, which include:
- A deletion in the enhancer of the U3 region of 3’LTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
- The RSV promoter (in HIV-based vectors) upstream of 5’LTR in the lentivector allow efficient Tat-independent production of viral RNA, reducing the number of genes from HIV-1 that are used in this system.
- Number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev), and the corresponding proteins are expressed from different plasmids (for HIV-based packaging plasmids) lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector, to prevent generation of recombinant replication-competent virus.
- None of the HIV-1 genes (gag, pol, rev) will be present in the packaged viral genome, as they are expressed from packaging plasmids lacking packaging signal—therefore, the lentiviral particles generated are replication-incompetent.
- Third generation lentiviral vectors are also not readily mobilizable as a result of a superinfection with a wild-type virus.

**Origin & function**

Open reading frames (ORFs), cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and / or shRNAs (designed to knockdown the expression selected gene products) will be expressed in third generation, self-inactivating, lentiviral expression vectors. ORFs could include DNA encoding potentially ‘harmful inserts’, which can be defined as a sequence that may have harmful biological activity such as oncogenes, toxins,
cytokines, transcription factors, receptor tyrosine kinases, growth factors and immunomodulatory proteins. For example, the expression of an oncogene, proto-oncogene, or sequence with oncogenic mutations may result in cellular transformation. In addition, the use of shRNAs to knockdown tumour suppressor gene expression could also result in cellular transformation.

Lentiviral particles will also include selectable markers which may include (but are not restricted to):
- Ampicillin resistance: derived from E.coli
- Neomycin resistance: aminoglycoside phosphotransferase gene derived from bacteria
- Puromycin resistance (PAC): Puromycin acetyl transferase is derived from Streptomyces alboniger

### Evaluation of foreseeable effects

In lentivirus transfer plasmids, the encoded genetic insert sequence is flanked by long terminal repeat (LTR) sequences, which facilitate integration of the transfer plasmid sequences into the host genome. Typically, it is the sequences between and including the LTRs that is integrated into the host genome upon viral transduction.

3rd-generation transfer plasmids have chimeric 5'LTR that includes an RSV promoter and are self-inactivating (SIN) vectors. SIN vectors have a deletion in the 3'LTR of the viral genome that is transferred into the 5'LTR after one round of reverse transcription. This deletion abolishes transcription of the full-length virus after it has incorporated into a host cell.

Examples of elements found in the vector system include, but are not limited to, the following:

<table>
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<tr>
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<tbody>
<tr>
<td>3'SIN LTR 3' self-inactivating long terminal repeat (increase safety)</td>
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<tr>
<td>5'LTR (truncated) RSV Chimeric 5'LTR that includes a RSV promoter</td>
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<tr>
<td>WPRE</td>
<td>Enhances the stability and translation of transcripts</td>
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<tr>
<td>RRE (Rev Response Element)</td>
<td>Increases efficient packaging of full-length viral genomes</td>
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<table>
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<th>Plasmid backbone elements</th>
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<tbody>
<tr>
<td>pUC ori High copy replication and maintenance in E.coli</td>
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<tr>
<td>Selection marker e.g. AmpR Bacterial selectable marker</td>
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Two potential safety concerns for use of Lentivirus are:
1. Potential for generation of replication-competent lentivirus (RCL)
2. Potential for oncogenesis.

Replication-defective lentiviral vectors, such as the 3rd generation vector, are not known to cause any diseases in humans or animals. Non-essential viral accessory genes have been removed and the remaining ones split across 4 plasmids so that 4 separate recombination events would have to occur simultaneously to generate replication competent lentiviruses.

In addition, lentivirus particles pose some risks because they can transduce primary human cells and can integrate into the host cell genome, thus still posing some small risk of insertional mutagenesis if able to transduce cells of the user. Genome-wide studies of viral integration have shown that lentiviruses most often integrate into actively transcribed genes, and that this preference is conserved across target species. For this reason, the product should be handled at containment level 2.

Open reading frames (ORFs), cDNAs and gene sequences encoding potential drug targets or therapeutic proteins and / or shRNAs (designed to knockdown the expression selected gene products) will be expressed in third generation, self-inactivating, lentiviral expression vectors.

ORFs could include DNA encoding potentially 'harmful inserts', which can be defined as a sequence that may have harmful biological activity such as oncogenes, toxins, cytokines, transcription factors, receptor tyrosine kinases, growth factors and immunomodulatory proteins. For example, the expression of an oncogene, proto-oncogene, or sequence with oncogenic mutations may result in cellular transformation. In addition, the use of shRNAs to knockdown tumour suppressor gene expression could also result in cellular transformation.

The expression of lentiviral encoded transgenes is controlled by a promoter. Some promoters, such as human cytomegalovirus (CMV) promoter, are considered strong mammalian expression promoters. Hence, the expression levels of 'harmful inserts' could be reasonably high in the transduced cells. An example of this is high expression of growth factors or transcription factors (e.g. myc) resulting in induced cell proliferation. Uncontrolled proliferation of cells in a human host can result in cancer.

Overexpression of cytokines in human hosts could result in autoimmunity or cytokine storm.

Random integration of the lentivirus encoded genetic insert (or transgene) into the human genome can be hazardous irrespective of the transgene as it may disrupt essential genes or inactivate tumour suppressor genes.

There is also the risk of transfer of the lentivirus to other cell lines used for in vitro work, if they are exposed to the lentiviral particles. This could impact experimental work using these cells are they may not behave as parental lines.
Lentivirus transfer plasmids that encode genetic inserts/transgenes are designed for optimal function within lab strains of E.coli and in mammalian cells in combination with co-transfection with packaging and envelope plasmids to produce lentivirus. If the plasmids were transferred to related microorganisms, for example, related gram negative bacteria eg other strains of E.coli or Salmonella species, they will not be maintained over multiple cell divisions in the absence of Ampicillin antibiotic. Antibiotic selection is used to maintain the plasmid so the vector’s persistence would be expected to be short-lived. Only if the gene product confers a selective advantage to the bacterial recipient will it be maintained and potentially passed to other bacterial hosts.

Within mammalian cells, once a 3rd generation lentivirus has transduced the cell and integrated within the host genome, it is incapable of generating more lentivirus particles due to the safety mechanisms used. The lentiviral particles produced by third generation lentivirus systems are replication incompetent. A deletion in the 3’ LTR (U3) results in “self-inactivation” (SIN) of the lentivirus after transduction and genomic integration of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). This alteration renders the lentiviral genome incapable of producing packageable virus following host integration.

Transduced cell line pools or stable cell lines generated will be treated as hazard group 2 (especially when the nature of the inserted transgene or location of insertion is unknown).

If a ‘hazardous insert’ was transferred to an immunocompromised individual or pregnant female host, and the insert was correctly incorporated into the host genome and subsequently expressed at high levels, or resulted in insertional mutagenesis, these individuals may be more susceptible to its downstream effects; however, the likelihood of this is very low using containment level 2 practices. The initial bacterial strain encoding the lentiviral transfer plasmid (with a genetic insert/transgene) is unlikely to transfer the genetic material to other organisms. Once a mammalian cell has been transduced with a third-generation lentiviral system it is unable to generate lentivirus particles. Therefore, it will not be able to transfer genetic material to other organisms.

Lentivirus particle encoded inserts can integrate into the genome of a host cell. This poses a risk of insertional mutagenesis if the cells of a user were to be transduced with lentivirus. There is a risk that the integration of the encoded insert may disrupt, or mutate, essential genes or tumour suppressor genes. Genome-wide studies of viral integration have shown that lentiviruses most often integrate into actively transcribed genes, and that this preference is conserved across target species. Following containment level 2 practices, the likelihood of transduction of cells of a user is low and therefore the risk of genetic instability is also low.

The NIH guidance ‘Biosafety Considerations for Research with Lentiviral Vectors’ states that ‘laboratory scale’ use of lentivirus (as opposed to mass production scale used in cell therapies or commercial production) pose a low risk. Replication-defective lentiviral vectors, such as the 3rd generation vectors, are not known to have ever caused any diseases in humans or animals.


If the GM microorganism (GMMO) E.coli host were to leave the lab environment, it would be expected to lose the plasmid encoding the foreign gene within a few cell divisions unless maintained on antibiotic selection. Only in the scenario where the foreign gene confers a growth advantage would the GM microorganism be able to survive and potentially displace other organisms.

Human hosts may be infected by lentivirus through injection, or direct contact with mucous membranes, such as inhaling airborne droplets/aerosols. The initial bacterial strain encoding the lentiviral transfer plasmid (with a genetic insert/transgene) is unlikely to transfer the genetic material to other organisms.

The potential for the transfer of genetic material between organisms is greatest during the production of lentiviral particles and transduction of mammalian cell lines where airborne droplets may be produced. To minimise this risk, segregation of the lentiviral work from standard cell culture work should be in place (e.g. strip cleaning class II MBSC hoods with distel and 70% ethanol after working with lentivirus). HEK293T cells transfected with lentiviral plasmids and host cells initially transduced with lentiviral particles should also be separated from standard cell culture cell lines (e.g. in a separate incubator). Once transduced cells have been washed with PBS, subjected to a media change and passed into new plastic flasks or plates, they can be moved back to an incubator with standard cell culture stocks, as the risk of transfer of lentiviral particles are low. In addition, all serological pipettes used with lentivirus particles should be deactivated with 1% Virkon (final concentration) in the class II MSC to reduce the spread of particles outside the hood and reduce aerosol/droplet transmission. Pipette tips which cannot be easily disinfected with 1% Virkon will be collected inside the MBSC hood inside a BioBin container. The BioBin will then be sealed inside the MSC hood and placed in a sealed plastic yellow CL2 waste bin, with a category 2 sticker (includes UN 3373 diamond sign). The bin will then be collected for incineration. Laboratory costs will be autoclaved prior to laundry.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Lentiviruses have been identified to infect humans and other primates, cats, cattle, sheep, goats and horses. Genetic inserts may include human ORFs and therefore the expression of human genes in the animals may result in harmful immune reactions. Moreover, similar to humans, overexpression of ‘harmful inserts’ and/or insertional
mutagenesis could also cause harm. The experimental work outlined utilises third generation vectors with no known history of causing harm in animals, will adhere to containment level 2 procedures and no animals will be present in the environment where this work will be carried out. The risk of generation of unanticipated genetic modification of animals and subsequent harms are therefore extremely unlikely. As lentivirus is unable to infect plant cells, there is no mechanism for transmission of the lentivirus plasmid to plant hosts.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM 105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:
1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.
All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization is GM898) according to disposal notification GM105/4.1.
Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins".
Collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution inside a Class 2 microbial safety cabinet prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below).
Waste from our GM work at Class 2 will be placed into suitably labeled sealable bins. They will be sealed by our staff, notified on a central electronic logging systen and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins".
Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.
Laboratory coats will be autoclaved prior to laundry.
All of these disposal methods are expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon were attached at notification of premises (CU1).

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

Please enter comments on the GM safety committee on the risk assessment
The Risk Assessment was approved after the following comments had been addressed:
- Remove reference to use of generated cell lines for in vivo use as this is not currently planned
- Remove reference to 'Generic' in Risk Assessment as this RA applies to specific packages of work
- Insert additional description of the in vitro assays that will use the generated cell lines (in particular, the use of generation cell lines with constitutive reporters and inducible signaling reporters)
- Use 'serological pipettes' to remove confusion between disposable stripettes and pipetting equipment
- Lab coats should be autoclaved prior to laundry
- Include risks of random integration to Section 3.2.6 in Risk Assessment
- Add descriptors of harm to Risk Matrix

Project Containment

<table>
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Name

SOLUS SCIENTIFIC SOLUTIONS LTD

Name 2

Department

Campus Estate or Research Centre

SCOTTISH ENTERPRISE TECHNOLOGY PARK

Road Name

RANKINE AVENUE

District

EAST KILBRIDE

Town

GLASGOW

County

LANARKSHIRE

Postcode

G75 0QF

Country

SCOTLAND

Tel Number

01355 228016

Fax Number

N/A

E-mail

HSE Division

SCOTLAND

Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Experienced UK University professor who has years of experience working with bacteriophages and has an extensive publication record.

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Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Transgenic Animals | Transgenic Fish | Microbiology Research | Gene Therapy
| Yes         |             |                |                  |              |                    |            |
For activities involving GMMs, describe the waste management measures which will apply to the activity

All plates or tubes containing the genetically modified bacteriophage will be soaked in virkon. All waste will then be transferred into theatre bins which are collected by a commercial waste management company. No genetic modification work is being performed at the Solus lab. The genetically modified bacteriophage used in this diagnostic technique are supplied by the manufacturer of the diagnostic assay.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Experienced UK University professor who has years of experience working with bacteriophages and their risk assessment. He was clear that this work is low risk work as these phages pose no risk to humans, animals or plants and correlates with Levell (GMM's).

---

**Project Ref** 3555/21.1

**Date Ackn’d** 16/04/2021

**CU2 Project Title** Culture Biomerieux Bioball Luminate Salmonella Typhimurium Green Fluorescent Protein, BTF 131, Catalogue # 422190 and test in the Solus Scientific Salmonella ELISA kits

**Class** Class 2

**CultureVol** 1-50 Litres

**Class 2 Volume** Class 3-4

**Non-GMM** Consent Granted

**Project notified under transitional arrangements** N

**Withdrawn** N

Tick if notifying a connected programme of work N

**Historical Significant Changes**

**Historical Date of Additional Info**
Recipient or parental organism
Salmonella Typhimurium Bioball Luminate is a commercial product manufactured and sold by Biomerieux.

Host/vector system
Bioball Luminate is a commercial product manufactured and sold by Biomerieux.

Origin & function
Commercially available Salmonella Typhimurium which is tagged with a Green Fluorescent Protein (GFP) marker used as a QC control strain in food testing labs as it is easy to distinguish from external contaminants.

Evaluation of foreseeable effects
The genetic modification is not expected to effect the growth of Salmonella Typhimurium and poses no greater risk than working with Salmonella Typhimurium (Class 2 organism with class 2 containment measures)

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
No derogation from Class 2 containment

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)
All waste will be placed in theatre bins and be collected by a waste management company. Treatment involves sterilising and shredding the waste, heat penetrating treatment and more shredding so the end product is not distinguishable as waste, it's more like fluff which is then sent for deep burial.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The genetic modification is not expected to effect the growth of Salmonella typhimurium and poses no greater risk than working with Salmonella typhimurium (Class 2 organism with class 2 containment measures)

## Project Containment

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**Name**

RENASCI LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

BIOCITY

**Road Name**

PENNYFOOT STREET

**Building**

**District**

**Town**

NOTTINGHAM

**County**

NOTTINGHAMSHIRE

**Postcode**

NG1 1GF

**Country**

ENGLAND

**Tel Number**

01559 124260

**Fax Number**

N/A

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Biological Safety Officer advice available through parent company - Sygnature Discovery with access to their safety committee as necessary.

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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

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- Spillages are likely to be small volumes only – the area will be disinfected with 1% Virkon (contact time 10 minutes); absorbed onto absorbent pad and disposed in clinical waste bin.
- Stock AAV will be disinfected with 1% Virkon for at least 10 minutes before being absorbed onto absorbent material and disposed in the clinical waste stream.
- Sharps and other consumables in direct contact with the AAV will be bagged/ sealed and autoclaved at 121°C for 30-45 minutes in line with manufacturer’s instructions.
- Bedding/ enrichment materials will be autoclaved at 121°C for 30-45 minutes before undergoing normal washing procedures.
- Carcasses will be disposed by incineration via the licensed establishment's GMO clinical waste stream.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Agree with assessment and subsequent risk containment measures.
<table>
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**Name**

COLORIFIX LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

NUFFIELD ROAD

**Town**

CAMBRIDGE

**District**

**County**

CAMBRIDGESHIRE

**Postcode**

CB4 1TF

**Country**

ENGLAND

**Tel Number**

07735 368814

**Fax Number**

n/a

**E-mail**

**HSE Division**

EAST AND SOUTH EAST

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>cb4 1TF</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

GM specialist - Biological Safety Practitioner level 1, gained in 2018. Full Member of ISTR. 7 years experience as a Biological Safety Officer up to CL3 in both academia and industry settings.

<table>
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<tr>
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<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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Other (please specify) Tick if confidential

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<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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02/03/2022
Small scale culture (<10 litres): All wild type and GMM organisms in culture will be autoclaved or chemically inactivated prior to disposal. Standard autoclaving for cycle should be 121°C for at least 30 minutes by using saturated steam under at least 15 psi of pressure. Chemical inactivation will take place using validated disinfectants according to the manufacturer’s instructions. Large scale culture: Validated inactivation methods that have been shown to prevent release of the GMO to protect human health and the environment will be used. As part of the industrial process the culture mixture is heated to 80 °C for at least 30 seconds. Internal validation has demonstrated this to be an effective inactivation step. Annual validation of the process is required and monitoring of the waste takes place after production of 1000 L of material. Subsequently the material is treated with a chlorination step. Chlorination is by far the most common method of wastewater disinfection and is used worldwide for the disinfection of pathogens before discharge into receiving streams, rivers or oceans. Chlorine is known to be effective in destroying a variety of bacteria, viruses and protozoa, as well as effectively destroying DNA contamination.

Monitoring
Monitoring of process organisms in the waste water is required to ensure compliance with procedures to protect human health and the environment and to ensure that containment is maintained. The monitoring protocol for process organisms is performed according to ISO standard 9308-1 (membrane filtration method). This is a standard monitoring method used across the pharmaceutical, food and beverage industry to measure bioburden. Briefly; a sample of the spent dye liquor is filtered through a membrane designed to retain microorganisms. The membrane collects cellular debris as well as any intact microorganisms. This is then placed onto a selective media which promotes the growth of our process organisms and incubated at optimal temperatures to allow their growth. If any process organisms remain intact and are collected by the membrane they will form growth colonies on the media. Monitoring frequency is dependent on volumes of dye liquor being produced and so will take place after every 1000 L volume of fermentation dye liquor produced rather than on a weekly or monthly basis.

The biological safety committee accepted the risk assessment of the activity to be complete. The risk assessment has carefully considered the risks that the project may pose to both human health and the environment and the controls indicated by the risk assessment as sufficient to protect human health and the environment.
GM Centre Number: 3559

Data Premises Notified (Originally) 09/10/2020  
Transferred from 1992 Regs? N  
Transitional Premises Class

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Transitional Premises  
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Non-GMMs N  
Withdrawn N

Name  
TRANSINE THERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre

Road Name  
30 BROAD STREET

District CAMBOURNE

Town CAMBRIDGE  
County CAMBRIDGESHIRE  
Postcode CB23 6HJ  
Country ENGLAND

Tel Number 01223 804 067  
Fax Number 0

E-mail

HSE Division blank

Comments

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Level 1 (GMMs)

Level 2 (GMMs)

Laboratory

Animal Unit

Growth Room

Glass House

Large Scale

The TranSINE Therapeutics Ltd genetic modification committee will consist of:

- Vice President of Research and Operations: Has 22 years experience working with mammalian cell culture projects, including the use of GMMs. She has lead and managed groups of scientists and advised on biosafety matters. Her function on the committee is to advise and provide the necessary resources for the actions of the committee to be implemented.

- Research Director: Will chair the TranSINE H&S committee. Has 20 years experience working with mammalian cell cultures, including genetically modified cell lines and bacteriological work, in both academia and industry. He has managed teams of lab scientists and advised on safety aspects of work. His role on the biosafety committee is to provide advice and ensure that actions arising from the committee are implemented.

- Senior Scientist: Is an expert in mammalian cell culture and molecular biology, with 8 years of experience. She will provide advice and implement the outcomes of the Biosafety Committee in the laboratory.

The committee will meet every 6 months to review biosafety and ad hoc whenever a new risk assessment is required.
All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below).

Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins".

Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below).

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Use of adeno-associated virus or lentivirus in hazard group 1 and 2 cell lines to investigate the therapeutic potential of natural or synthetic long non-coding RNA molecules

SINEUPs are natural or synthetic long non-coding (Inc) RNA molecules that can upregulate the protein synthesis of target genes in mammalian cells without altering the endogenous mRNA levels of the target gene. As such these could have therapeutic applications in diseases associated with decreased expression of key proteins.

A standard SINEUP project takes the following format:

SINEUPs versus a specific cellular target gene are designed in silico and synthesised.

In certain instances adeno-associated virus (AAV) or lentivirus containing vectors to express SINEUPs will be obtained from 3rd party vendors in small aliquots and used to transduce hazard group 1 or 2 mammalian cells.

The effects of SINEUP transcription upon protein expression will be monitored for 1-28 days.

SINEUP-transduced cells will be used for a range of biochemical, cell biology and cell-based assay experiments including, but not limited to, lysis for western blot and quantitative PCR, fluorescence microscopy and immunocytochemistry on live and fixed cells, cell death quantification, luciferase assays, GFP assays, ATP assays, mitochondrial membrane potential assays and other functional readouts.

To facilitate a full understanding of the biology around the activity of SINEUPs, there will also be the need to use AAV or lentivirus to generate in vitro cellular models which may have:

- Stable overexpression of exogenous genetic sequence
- Reduced expression of target genes by expressing shRNA
- Knockout or modification of endogenous genes using CRISPR/Cas9 or related methodologies to:
  - introduce specific amino acid changes
  - add a tag such as but not limited to GFP, luciferase, FLAG & HA
- Introduction of reporter gene systems to allow the measurement of functional effects on key cellular signalling
Recipient or parental organism

Well characterised immortalised cell lines.
A number of immortalised cell lines are available that can be used as model systems for validating and initial assessment of target activity. Due to the nature of the work cell lines known to support the propagation and production of replication competent lentivirus particles (RCLs) will not be used. The Biosafety level 1 or 2 cell lines will be obtained from reliable sources (commercial and academic) with a clear history of safe use and a well understood mechanism of immortalisation to remove the risk of recombination events leading to production of RCLs.

Primary human cells
The main risk is the presence of adventitious pathogens.
Ideally, cells will have been screened for major human pathogens with the potential to harm health and shown to be negative.
In some instances however, consent to screen for major human pathogens is not sought at the time of cell donation. Screening the cells for major human pathogens would have potentially significant clinical health implications for patients (e.g. HIV), for which explicit consent has not been given at the time of donation. As such, for cells where consent has not been given, these cannot be screened for human pathogens and must therefore be handled at Biological Safety Level 2. All donors however, must have no previous known history of infectious disease and will be from epidemiologically low-risk populations.

These mutations are naturally occurring in the genome of these individuals, they are not mobile genetic elements and thus present a very low biosafety risk.

hiPSC derived from primary human cells, most commonly fibroblasts.
The hiPSC that will be used may have been either generated using 3rd or 4th generation self-inactivating (SIN) non-mobilisable integrating retroviral systems based on , but not limited to, mouse Maloney murine leukaemia virus (MMLV), Maloney murine sarcoma virus (MMSV), myeloproliferative sarcoma virus (MPSV), or human HIV. Some of the original donor material will not have been screened for human pathogens but donors will have a known medical history and will be classified as being low risk for retroviral pathogens. As such the hiPSC are highly unlikely to be producing RCLs.

Reprogramming of the hiPSC is achieved using a variety of factors, that are integrated into the cell genome by the retroviral vectors, that induce pluripotency in the donor cells. A combination of the following factors may be present in the supplied hiPSCs:

- **SOX2**: is a transcription factor that is essential for maintaining the pluripotency of undifferentiated embryonic stem cells.
- **KLF4**: is a transcription factor that regulates proliferation, differentiation, apoptosis and somatic cell reprogramming. KLF4 may also act as a tumour suppressor gene.
- **OCT4**: is a homeodomain transcription factor of the POU family. This protein is critically involved in the selfrenewal of undifferentiated embryonic stem cells and is used as a marker for undifferentiated stem cells. It is indispensable for generating iPSC.
- **CMYC**: is a transcription factor that plays a role in cell cycle progression, apoptosis and cellular transformation. Mutated c-Myc is found in many cancers, where it is constitutively expressed, leading to the unregulated expression of many genes, some of which are involved in cell proliferation leading to oncogenesis. This factor poses a risk if mobilised into RCL.
- **NANOG**: is a transcription factor critically involved with self-renewal of undifferentiated embryonic stem cells.
- **LIN28**: encodes a microRNA-binding protein; overexpression of which in mice, can cause gigantism and a
delay in the onset of puberty. Human GWAS studies indicate the LIN28B gene to be associated with human height and the timing of puberty. The biosafety risk is low for adults.

- GLIS1: is a highly promiscuous transcription factor, positively or negatively regulating the expression of a number of genes.
- shp53: short hairpin RNA that suppresses expression of p53. P53 is a known tumour suppressor gene and binds to DNA where it regulates the expression of anti-mutagenesis genes. Mutations in the p53 gene leading to a loss of activity are the most commonly found single mutation in human cancer.
- Mir302/367 cluster: microRNAs are a subclass of small non-coding RNAs that fine-tune the regulation of gene expression at the post-transcriptional level, with a particular role in establishing cellular pluripotency.

HiPSCs can also be generated using episomal approaches and if suitable will be used preferentially. Where cells are reprogrammed using non-viral episomes, there is no integration of genetic material into the resulting hiPSC genome and no viral sequences are introduced into the cells. Episomes are lost as the reprogrammed hiPSC proliferate, at an approximate rate of 5% per generation. Therefore by approximately 12-13 proliferation cycles, the episomal transgenes have been entirely lost. This eventually results, at the time of use, in “footprint-free” cells that do not contain the original reprogramming sequences. In this respect the hiPSC are not genetically modified but do have a changed gene expression profile and a pluripotent phenotype.

**Host/vector system**

In the case of hiPSC generated using integrated retrovirus the factors and vectors used will be fully described prior to receipt and be reviewed by the Biological Safety Committee (BSC) at TranSINE Therapeutics Ltd (TTX) which also has the responsibility for any work involving genetic modification. In this case subsequent lentiviral vectors will be non-homologous with the integrated sequences to mitigate the small risk of a recombination event leading to mobilisation of genetic material, such as the myc oncogene or immunomodulatory genes, that might pose a risk to human health. The integrated murine-based lentivirus pose no additional risk to the environment. It is also planned to transduce immortalised cell lines, primary cells and episomally generated hiPSCs with lentiviral vectors. The recipient cells will not be known to harbour viral material that could be mobilised by introduction of lentiviral vectors and where viral material is present it will not support the production of RCLs.

Secondary transduction would either transiently modulate the protein expression of a target protein at the translational level, modify the cells to explore the underlying disease process or to generate tools for drug discovery. To this end the following could be used based upon the needs of drug discovery programs:

- Integration of shRNA constructs to ‘knock down’ expression of a gene of interest
- The over-expression of particular genes
- Reporter genes under the control of selected transcriptional regulatory sequences to study effects on cellular pathways.
- The lentivirus used will be obtained from commercial sources and generated using third of fourth generation vector systems. The vector backbone and promoters used to drive vector generation in these systems will be consistent. Due to the nature of the work, genes may be selected that will include growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors and proteins known to be involved in neurological disorders. The vectors will be generated using a non replication-competent HIV based lentiviral system that upon integration into the host genome loses part of its long terminal repeat (LTR) preventing excision at a later time point. Such self-inactivating (SIN) vectors are designed to produce stable gene expression in target mammalian cells. The viral particles can only be generated upon co-transfection of a packaging cell line (usually HEK-293T) with at least four
separate plasmids, three of which provide the minimal set of genes required for viral production (gag-pol, rev and env). The env gene used is from the vesicular stomatitis virus (VSV-G) to increase tropism. The final plasmid provides the genetic material to be supplied to the target cell and as such contains packaging sequences directing it to be incorporated into nascent viral particles. Only the minimum amount of lentiviral genome is used in the system and all of the plasmids lack homologous sequences to minimise any chance of recombination. The resultant lentiviral particles are VSV-G pseudotyped, SIN and replication incompetent in mammalian cells. Small aliquots of no more than 1x10^8 viral particles in 5 to 10 microlitres will be used at anytime, further reducing the risk.

Third Generation Systems use 4 standard bacterial plasmid vectors. Three plasmids encode for proteins required for production and packaging of full length viral RNA (for example: pCgpV, pRSV-Rev and pCMV-VSVG). The gene of interest is contained in a fourth plasmid (e.g., pSMPUW). This is the only plasmid that contains the packaging sequence for incorporation into the virus particle.

Fourth Generation Systems use 6 standard bacterial plasmid vectors. The system uses tetracycline to control when viral particles are produced adding yet another level of control to production. For example, pTRE-gag-pro, LTR HIV2-vpr-pol, penv(VSV-G), pTet-Off and ptat-IRES-rev contain the sequences that code for proteins that are responsible for the tetracycline control and packaging of the viral genetic material. As above the last plasmid (e.g., pSMPUW) only contains the genetic material to be packaged into the vector and no other lentiviral gene sequence. An integrase deficient version of the fourth generation system is available which contains a mutation in the sequence encoding the viral integrase.

In some cases the broad tropism of MV vectors will be utilised to transiently introduce genetic material of interest into hiPSC cell lines, primary cells or immortalised cell lines. MV vectors are replication incompetent. The vectors used will be incapable of integration into the host genome as they will not contain the rep or cap genetic sequence. The genetic material will remain epichromosomal and will be progressively lost as cell continue to divide until essentially no introduced genetic material remains. These vectors pose negligible risk to human health. These vectors will be obtained from third party sources.

### Origin & function

As mentioned above there may be a requirement to look at genes encoding growth factors, their receptors, tropic factors, putative oncogenes, confirmed oncogenes, tumour suppressors and proteins associated with neurological disorders. Any target gene will need the approval of the TranSINE Therapeutics BSC and the vectors used will have their sequence checked to ensure lack of homology.

- **a-Vector containing SINEUP** - each vector will encode a sequence expressing a SINEUP that is designed to enhance the translation of a specific protein. This will be under the control of a polymerase II or III promoter.

- **b-Vector containing shRNA** - each vector will code for one shRNA transcript that will be designed to target one mRNA/protein of interest (or a scrambled shRNA in the case of negative control). This will be under the control of a polymerase II or III promoter.

- **c-Vector containing CRISPR nucleases** - Programmable nucleases function by binding to, and cleaving, user-defined target DNA sequences. This instigates a robust DNA repair response which can either be used to disrupt the target sequence by deletion at the cleavage site or to introduce user-supplied genetic material via recombination with a vector containing complementary sequences to the target gene.

For Cas9/CRISPR; nuclease target specificity is determined by a guide RNA, which consists of a short (18-20 nucleotide) sequence homologous to the target gene and an additional short sequence that forms a complex with the Cas9 enzyme. The vector will encode Cas9/CRISPR plus a guide RNA under the control of a Polymerase III promoter.

Zinc finger nuclease target specificity is dependent upon the zinc finger protein sequence which will be under the
control of a polymerase II promoter. A fluorescent protein or enzyme under the control of a polymerase II promoter or the promoter of a gene of interest may be included. Control vectors only expressing fluorescent protein/enzyme will also be used.

d-Reporter or overexpression gene vector - The vector will contain either sequence coding for a reporter protein, a protein to be overexpressed under the control of regulatory elements of a promoter of a gene of interest or a polymerase II promoter. An appropriate selection marker i.e.; neomycin could also be included to allow the selection of cells stably containing the introduced genetic material. The efficacy of lentiviral vectors will be assessed first using cell lines that routinely require biosafety level 1 containment (BSL 1). Application of lentiviral particles will be done at Biosafety Level 2 containment. At least 24 hours post transduction the cells will be washed three times to remove any residual viral particles and then treated as BSL 1 as the potential risk will have been minimised. The efficacy of the genome manipulation will be assessed using in vitro assays. AAV vectors will be tested in a similar manner but only require BSL 1 containment and will look at the effect of the expression of the introduced genetic material upon the cell phenotype within several cell generations before the proportion of the cell population still containing the episomal vector becomes too small. hiPSC will be transduced with validated lentivirus. All procedures will be conducted at BSL2. Stable cell lines will be documented. Resultant cells will be studied to further understand disease processes or used for drug discovery efforts. This will be done by phenotypic analysis using a range of common in vitro assays, including but not limited to RT-PCR, Western blotting, immunocytochemistry and imaging, cellular bioenergetics or electrophysiology. All culturing of viable BSL2 cell lines will be conducted at Biosafety Level 2. Cells that are rendered non-viable (i.e., fixed, lysed etc.) will not pose a risk. This material does not need Biosafety Level 2 containment.

Evaluation of foreseeable effects

hiPSC are pluripotent and are capable of uncontrolled proliferation. Lentiviruses belong to the Retroviridae virus family and are capable of infecting both human and animal species. The lentiviral vector system that will be used is based on HIV-1. Retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airborne route. Piercing of the skin represents the main potential route by which material could be accidentally introduced into an individual. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring. Accidental introduction of iPSC into healthy individuals should not lead to the formation of teratomas because the immune system will rapidly destroy these cells. As mentioned above the pathogen status of some cells is not known. However the material will not be derived from high-risk clinical patients and procedures are in place to deal with needle stick incidents (although no sharps will be used). Individuals with compromised immune systems are not permitted to work with this material. The genetic material to be incorporated into the viral particles is flanked by non-coding retroviral LTRs. No retroviral genes are encoded on this plasmid, minimising the chance any will be packaged into viral particles. As the system requires the co-transfection of three to five additional separate plasmids into a permissive cell line the chance of recombination occurring that leads to the incorporation of any or all of the retroviral genes necessary for the production of a RCL is very low and the risk is therefore low. The lentiviral Expression System includes the following key safety features:

- Only the vector containing the genetic sequence of interest includes LTRs and the packaging signal sequence required for incorporation into viral particles and integration into the genome of a transduced cell. A hybrid 3’LTR is used that does not affect generation of the viral genome in the producer cell line, but upon integration into the genome of a target cell, the 3’LTR SIN which prevents production of viral genomic material that can be packaged and reduces the chance of mobilisation due to secondary infection with wild-type lentivirus. No transcriptionally active LTRs are present in the system and an SV40 polyA sequence is included after the hybrid 3’LTR to reduce the potential for transactivation of cellular genes due to an insertion event that might promote inappropriate gene expression.
expression leading to oncogenic effects.

* The LTR has been modified to enable lentiviral production independent of Tat expression. HIV-1 devoid of Tat is known to be replication incompetent. This decreases the risk of RCL occurring.

* The essential genes encoding the proteins required for packaging of the viral genome are separated onto three to five different plasmids. These express the proteins (gag, pol, rev, env) required to generate viral particles transiently in a permissive packaging cell line (e.g. HEK-293T). Other retroviral structural genes are not present in the system. None of the plasmids have regions of homology which will prevent undesirable recombination. As multiple recombination events would be required to generate a RCL the risk of this happening is very low and has so far not been observed in large-scale production and testing of lentiviral vectors.

* In most systems expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Expression requires the presence of rev, which is supplied by a separate plasmid. The Rev/RRE system is highly conserved among lentiviruses and loss of the RRE sequence and associated splice donor/acceptor sequences results in a loss of transduction efficiency.

* Lentiviral vectors have a very low potential to cause immunogenicity.

* The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction of target cells. Whilst pseudotyped VSV-G is used as the envelop protein to increase the cell types that can be infected this increase in risk is mitigated by the SIN non-replication nature of the viral vectors generated. Due to the random nature of integration into the genome insertional events that disrupt gene function could occur and, in some instances, might lead to undesirable effects which could be oncogenic.

The resultant lentiviral vectors contain about 20% of the original HIV-1 genome. Recombination with wild-type HIV-1 is still potentially possible and could result in mobilisation of the transgene. This is highly unlikely because:

1. Wild-type HIV-1 is not used with in the laboratory environment.
2. Primary cells or hiPSC are not derived from donors either known to be HIV positive or from a high-risk population; so HIV contamination is highly unlikely.
3. Wild type HIV-1 cannot rescue a SIN HIV-1 based lentivirus once integrated into the host genome.
4. It is likely that any recombination would actually decrease the ability of HIV-1 to infect other cells or to replicate.
5. Even if a worker was already or became HIV-1 positive and was accidentally infected with lentiviral vector any recombination would require infection of the same cells. Generation of RCL would require homologous recombination to be in the right regions to enable mobilisation and incorporation to the HIV-1 genome without loss of replication ability. If such a rare event were to occur it is likely to be self limiting and the HIV-1 infection itself is of greater concern to the worker.

The use of lentiviral vectors is an efficient manner with which to deliver genetic material to, and integrate into, the genome of numerous cell types. This stable integration is of great benefit in scientific research. The design of the vectors minimised the chance for subsequent mobilisation of transgenes and inappropriate activation of endogenous oncogenes. Insertional events can lead to harmful side effects but are still very unlikely. As such the greatest risk from these vectors comes with their production where much larger volumes and numbers of vectors are being generated. The risk here is mitigated by the correct use of containment measures and the absence of sharps. Lentiviral vectors are susceptible to dehydration and loss of viability if they are not stored in high protein conditions which reduces the risk to workers and the wider environment.

MV expression system has the following safety features:

1. The vector lacks the rep and cap sequences preventing integration at the specific MVS1 integration site in the human genome.
2. The vector is replication incompetent and only forms epichromosomal double stranded DNA (dsDNA)
concatemers in the nucleus of a transduced cell.

3- In dividing cells this epichromosomal dsDNA is not replicated and gets progressively lost/diluted out in the daughter cells until eventually it is effectively not present in the cell population. In this way the expression system is transient and self limiting.

4- Recombination is highly unlikely as the dsDNA is not replicated and even if it was to occur all of the structural genes have been removed (contained in the cap sequence) and replication is also reliant upon co-infection with another virus that could support this process (e.g., adenovirus). This is a highly unlikely event in the laboratory setting.

The use of MV vectors is an efficient manner with which to transiently deliver genetic material to a wide range of dividing and quiescent cells and in a self limiting manner. There is negligible risk from insertional events. Even if the vector was introduced into a worker the effect would be localised and self limiting. There is negligible risk even if the co-worker had a co-infection with a permissive virus as this would need to be in the same cell and the AAV vector lacks the necessary gene sequence to support capsid generation and hence formation of new virus particles. Additionally wild-type MV is not known to be pathogenic.

Transgenes to be delivered to target cells:

Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cell lines, primary cells or hiPSCs.

The introduced SINEUP sequence is aimed to increase the translation of a target gene and can be viewed as potentially the same as introducing the equivalent genetic code (though whether the effect is equivalent is not yet known). As such SINEUPs to growth factors, their receptors oncogenes and proteins associated with disease all carry an increase in risk and must be approved by the SSC before work can commence.

Gene products to be overexpressed may include growth factors, oncogenes and proteins associated with disease. The risk that these represent is minimised by the use of single use aliquots of MV and lentiviral vectors in a controlled biosafety level 2 environment. Any protein to be expressed will require justification to and gain SSC approval before work can commence.

The 'knock-down' of specific gene expression using shRNA can lead to off-target and immunomodulatory responses in vivo. shRNA sequences will be screened against databases of known mammalian/mRNA sequences during the design stage to avoid such complications. Control vectors will also be used that contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to and therefore does not target any known mammalian sequences. Such sequences are unlikely to pose any safety risks for the environment or human health.

Programmable nucleases: Cas9/CRISPR will be directed against genes of therapeutic interest. In isolation, these sequences are unlikely to pose any safety risks for the environment or human health. Programmable nucleases may be used to generate isogenic controls lines by correcting disease causing mutations back to wildtype sequence. This is low risk because the sequence is aimed to revert back to non-disease forms of the gene. In the case of adding a tag to monitor/follow gene expression there is no anticipated change to the function of the gene and the risk is low.

Reporter genes: Commonly used fluorescent proteins or enzymes under the regulatory control of promoters of interest may be used to monitor effects on cellular pathways. Such proteins are not known to cause any human or animal disease and pose no-risk.

In all cases justification for any target must be submitted to the TranSINE Therapeutics SSC for approval prior to any work commencing.

Summary

Primary human cells and hiPSC will be derived from low risk patient populations but may not be screened for
human pathogens. Only well characterised immortalised cell lines will be used. The lentiviral systems are replication incompetent and SIN, minimising the potential for transgene mobilisation and propagation. The AAV system is transient, non-integrating and replication incompetent, having a negligible potential for transgene mobilisation. The transgenes that will be selected will on their own have a low risk to human health. Infection of mucosal cells may occur via aerosols but is highly unlikely due to the use of microbiological safety cabinets and secondary containment. Such infection is self limiting due to the natural shedding of epithelial cells coupled with the replication incompetent nature of the vectors. The most likely route of accidental infection with an AA V, lentivirus or with an hiPSC line will be via inadvertent percutaneous inoculation via stick injury or open wound. The likelihood of this occurring will be minimised by following standard BSL2 containment practices. Infection of the community and environment with AA V or lentivirus particles is highly unlikely due to the small quantities used, their intrinsic instability and rapid loss of viability with time. hiPSC can not survive outside of the laboratory environment so pose no wider risk. Therefore the risks towards workers, co-workers, the public and the environment associated with the use of these AA V and lentiviral vectors with hiPSC will be low and BSL2 containment is sufficient.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM1 05/4.1. Reasons for adopting this method of waste disposal are:
1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vets peed - Their GM authorization is GM898) according to disposal notification GM105/4.1. Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Waste Handling
Solid waste will be chemically inactivated by treatment with a 1 % Virkon solution, after which the material will be placed in suitable hermatically sealed bins. These bins are collected from a central point and sent off-site for incineration via a registered waste disposal company. All liquid waste will be inactivated for at least 6 h with a 1 % (final concentration) Virkon solution prior to disposal to drains via a designated sink. These disposal methods
achieve 100% inactivation of the GMM. Virkon is a synergised oxidising system that works by physical destruction of pathogens by acting on proteins. It is resistant to inactivation by organic material. Shown to be effective against bacteria, viruses and fungi including all major human pathogens. Recommended to be used at 1% concentration for all activities. Chemgene HLD4L is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporidical. It is recommended to be used at a 1:100 dilution (1%) for general purposes, 1:20 dilution (5%) for high risk areas, and for disinfection of blood and biohazard spillages.

General cleaning procedures
Surfaces will be thoroughly cleaned with a 5% Chemgene solution (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these antimicrobial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with 5% Chemgene before being incinerated.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
It is noted that the primary human cell lines used for the generation of hiPSCs will be taken from patient populations with a known medical history meaning that there is a low risk of material containing harmful human pathogens. This does not rule out the presence of latent viral infection but the use of third or fourth generation integrating lentiviral systems that are self-inactivating and essentially non-mobilisable makes it highly unlikely that infective replication competent virus will be generated. This is important due to the presence of the potentially oncogenic MYC sequence. Additional rounds of lentiviral transduction should not be able to mobilise any genetic elements because self-inactivating systems will be used that lack the necessary genes for viral replication and packaging. As an additional measure the GMSC will have a clear understanding of how each cell line was generated and steps will be taken to ensure overlapping, therefore potential sites for recombination are not present in lentiviral vectors. These vectors are for the introduction of further modifications. Those aimed at correcting disease mutations do not pose a risk to health. The introduction of non-disease causing genetic material for exogenous expression is not seen as a risk either. In the instance where immune-modulators may be targeted the risk, although increased, is well contained by the avoidance of sharps during transduction procedures. Once cells are transduced and virus is removed the risk posed here is very low. The use of iPSC derived from primary human cells and integrating lentiviral vectors dictates propagation, differentiation and further transduction of cell lines at BSL2. Subsequent testing of the cells will also continue at BSL2 although the genetic modification of the cells is of very low risk.
## Project Containment

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### Additional Categories

- **Animal Units**
- **Large Scale Activities**
- **Human Clinical Applications**

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**Name**

LINEAR DIAGNOSTICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

THE BIRMINGHAM BIOHUB

**Building**

BIRMINGHAM RESEARCH PARK

**Road Name**

97 VINCENT DRIVE

**District**

**Town**

BIRMINGHAM

**County**

MIDLANDS

**Postcode**

B15 2SQ

**Country**

ENGLAND

**Tel Number**

01218090646

**Fax Number**

0

**E-mail**

**HSE Division**

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**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: ☑️

Give brief details of the genetic modification safety committee:

University of Birmingham Medical and Dental Sciences Genetic Modification Safety Committee

<table>
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<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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</table>
All waste GMM material e.g. cellular material pellets in plastic tubes from centrifugation, will be autoclaved. The procedure to be used will be autoclaving at at least 121 degrees centigrade for 15 minutes. This method has been validated by the manufacturer and the machine itself is serviced by the manufacturer on a regular basis as organised by the BioHub management. When carried out correctly the degree of kill is expected to be 100%. Glassware: Soaked in 1% VIRKON solution overnight and then washed with washing up liquid the next day. Plasticware e.g. tips, will be soaked in 1% VIRKON solution overnight then disposed of in the laboratory waste which is sent for incineration. Effective use of VIRKON is monitored using the scientifically determined standards and protocols (see the VIRKON background information Report, AMTEC 1994). The autoclave is maintained by the manufacturer via 6-monthly maintenance visits where cycles are tested and calibrated. The machine is self-reporting if any errors occur during the cycle and will alert the user if the sterilization was unsuccessful. In the event of an error, the waste will be treated as contaminated until the error is fixed and then run again.

**Tick to confirm that you are attaching a summary of the risk assessment** [ ]

**Tick if you are claiming exemption from disclosure for sections of the risk assessment** [ ]

---

### Please enter comments of the GM safety committee on the risk assessment

**Reviewer 1**

"Emergency procedures
I don’t think the detail is really necessary – what are the chances of the culture being ingested?"

Actions taken by LD: In the section ‘Emergency procedures - Is an emergency plan required?’ The text has been modified to reflect a more practical approach to any spillage which more accurately reflects the risks involved.

"Final containment section
I think there is some confusion about the conclusions as its come out as GM Class 1, but the guidance on the form is clear"

Actions taken by LD: In the section 'Assign your final containment level/GMM Class (1/2)' the risk was correctly assessed as GM class 1 but due to the requirement from the Biohub facility, Containment Level 2 procedures will be implemented at all times. This has been clarified in the text.

**Reviewer 2**

"Soaking pipette tips in Virkon prior to disposal is usually only performed when working with class 2 viral vectors because it can be difficult to safely separate the tips from Virkon. Is the method used in this procedure considered straightforward and safe?"

Actions taken by LD: 'In the section Inactivation of GMMs in waste, and subsequent disposal ' we have clarified the reasons and process for decontaminating tips.
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**Name**

MICROGRAPHIA BIO LTD

**Name 2**

**Department**

Campus Estate or Research Centre

**Road Name**

20 DAWES ROAD

**District**

London

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

SW6 7EN

**Country**

ENGLAND

**Tel Number**

07595544409

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

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Give brief details of the genetic modification safety committee

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Other (please specify) Tick if confidential

Bacteriology    Parasitology    Transgenic Birds    Microbiology Research

Virology        Transgenic Animals Transgenic Fish Gene Therapy

Mycology        Transgenic Invertebrates Transgenic Plants Other (please specify below)
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
## GM Centre Number: 3562

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### Name

**NEUROGENEUS LTD**

### Name 2

### Department

### Campus Estate or Research Centre

### Building

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### Town

**LONDON**

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### Tel Number

**07494959704**

### Fax Number

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### E-mail

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### HSE Division

### Comments

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

ADVICE HAS BEEN PROVIDED BY A HEALTH & SAFETY ADVISOR WITH EXTENSIVE EXPERIENCE WITH THE LIFE SCIENCES AND GM SAFETY COMMITTEES. THIS INDIVIDUAL HAS SERVED AS A HEALTH & SAFETY ADVISOR AT A UK RESEARCH UNIVERSITY FOR OVER 20 YEARS AND WILL SERVE ON THE COMPANY'S GM SAFETY COMMITTEE GOING FORWARD

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**THE COMPANY DEVELOPS GENE THERAPIES FOR NEUROLOGICAL DISORDERS.**

For activities involving GMMs, describe the waste management measures which will apply to the activity

All solid waste is placed into a double yellow bag and taken offsite for incineration (by Rentokil Initial). Rentokil Initial disposes GM class 1 waste under through Tradebe Healthcare National Limited (at the Rochester Clinical Waste Treatment Facility) under the permit number EPR/WP3036ZR.

Liquid waste will be treated with 1% Chemgene. Supernatants must be siphoned via a pump into a flask containing 1% Chemgene. After finishing work, Chemgene will be added to the flask to 1% and left for at least 60 min prior to disposal. All treated liquid waste is emptied down laboratory sink with excess water.

All experiments using viral particles will be carried out in a Class 2 microbiological safety cabinet, where applicable aerosol-resistant tips will be used.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

GMSC Comments:

GMSC requested:

1. Clarification on use of sharps
2. Clarification on most hazardous GMO
3. Clarification on which disinfectant used & efficacy
4. Updates to waste handling

Final Assigned GM Class: Approved as Class 1 GM project

02/03/2022
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**Name**

THE ROSALIND FRANKLIN INSTITUTE

**Name 2**

Department

**Campus Estate or Research Centre**

HARWELL CAMPUS

**Road Name**

**Building**

**District**

**Town**

DIDCOT

**County**

OXFORDSHIRE

**Postcode**

OX11 0FA

**Country**

ENGLAND

**Tel Number**

01235 445000

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

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<td>THE ROSALIND FRANKLIN INSTITUTE (R113)</td>
<td>HARWELL CAMPUS</td>
<td>DIDCOT</td>
<td>OXFORDSHIRE</td>
<td>OX11 0FA</td>
<td>N</td>
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</table>

## Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

Yes

**Give brief details of the genetic modification safety committee**

For the purposes of this initial Class 1 application, the guidance of our appointed BSO has been sought to review and approve the Risk Assessment. She is a Chartered member of the Institute of Occupational Safety and Health and a Member of the Institute of Safety in Technology and Research, and previously Chair of the Biosafety Steering Group of ISTR for 4 years.

Recommendations and actions from the Risk Assessment are conveyed direct to staff.

For potential future Class 2 activities, the organisation shall join the on site genetic modification safety committee covering Diamond Light Source (DLS), Research Complex at Harwell (RCaH) and Rutherford Appleton Laboratory (RAL).

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

All personnel will be trained in the activity. All personnel working in the laboratories are required to wear protective clothing (gloves, lab coat and safety glasses) when working and observe good laboratory practice. No contaminated material will leave the lab other than as waste, including protective clothing, and all surfaces and equipment will be decontaminated after use.

For contaminated equipment that can be reused such as bacterial culture flask, spatula and centrifuge tube will be decontaminated by Virkon solution and then washed with detergent and water.

For disposal of cells & contaminated material/equipment such as bacterial culture dish, pipette tips and one-off culture tubes will be decontaminated with Virkon solution to ensure no risk of live cells remains, then dispose to bio-hazard waste bag/bin for removal to secure local storage, and sent for incineration via a licenced waste carrier. Alternatively, waste is collected into autoclaveable bags, autoclaved and disposed of as non hazardous waste.

Sharps will be placed into a labelled sharps bin, sealed after use and sent for disposal via a licensed waste carrier.

For used culture media, it will be decontaminated by Virkon first to ensure no risk of live cells remains, then pour into the sink.

For maintenance of a clean working environment, free from bacterial contamination, all working surfaces (bench, table, centrifuge, balance) will be regularly decontaminated by Virkon disinfectant. Wash hands before leaving the lab.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
Webster’s Biosafety Ltd have reviewed and commented on the risk assessment. Webster’s Biosafety has over 20 years experience in reviewing GM and Wild type risk assessments.

### Project Ref 3564/21.1

<table>
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<tr>
<th>Date Ackn’d</th>
<th>CU2 Project Title</th>
<th>Class</th>
<th>CultureVol</th>
<th>CultureVolume</th>
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<tbody>
<tr>
<td>19/08/2021</td>
<td>Generation, usage and storage of material, including genetically modified material, from Hazard Group 2 agents for scientific research only</td>
<td>Class 2</td>
<td>&lt; 1 Litre</td>
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</tr>
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</table>

- **Non-GMM Consent Granted**
- **Project notified under transitional arrangements**

#### Project Additional Information

**Purposes of the contained use**

The Rosalind Franklin Institute is a scientific research organisation for the public good. It is primarily funded by the UK research council (UKRI) and Wellcome Trust. The organisation is a charity (regulated by the charity commission) and a company limited by guarantee. The Trustees are drawn from the Industry and Academia. Its researchers are predominantly post PhD with some PhD students. The Franklin has a strict code of safety covering all activity.

The purpose of the contained use is for the Franklin’s work with Hazard Group 2 (HG2) organisms which include those that may be genetically modified. The work would involve the generation, growth, imaging and storage of material. Expression of the material (i.e. protein) will be in either prokayotic or eukaryotic organisms which will be no greater than HG2 and maybe GM material. The expressed material is to be prepared at RFI for usage, primarily imaging. Samples will be generated, stored, imaged and disposed of at the RFI. Assessment will be completed at a local level for every sample. This assessment will detail the experiment to be completed, including details of hosts, vectors, inserts and mutations to be used.

This completed assessment will be submitted to the Genetic Modification Safety (GMS) management committee. This committee is already in operation for all organisations on the "STFC" campus, ISIS, Diamond, Research Complex at Harwell and Central Laser Facility. The attached risk assessment identifies these organisations.
By functioning as part of the established campus system, we will ensure that our procedures meet or exceed these already in operation and approved.

The GMS management committee will review and make a recommendation to the Director of the Rosalind Franklin for proposed organism/sample and work. The Director will then make a final determination as to whether to allow the work by Franklin staff (employees, secondees or students) subject to the correct safety assessments being in place.

No organism or sample that is NOT approved by the GMS management committee will be considered by the Director and no such organism or sample allowed in the Franklin or be permitted to be handled by Franklin staff.

The samples that we be submitted for approval could be any of the following:

- Non-hazardous proteins genetically modified with a tag for structural and bio-chemical characterisation
- Non-hazardous proteins originating in higher than Hazard Group 2 agents classification
- Prokaryotic material from no greater than HG2 classification
- Eukaryotic material from no greater than HG2 classification
- Virus no greater than HG2 classification

The assessment by GMS will include all the proposed work in addition to organism. Only work approved by GMS will be considered by the Director and no other work will be allowed.

Any and all work involving the following will be rejected:

- Any biological agent to be used is classified as HG3 or HG4
- Any biological agent to be used is a SAPO agent
- Any biological agent is listed on ATCSA Schedule 5
- In the case of experiment using multiple live bio-material, there should not have more than one biological agent from the same domain carrying a genetic modification (i.e. the experiment will be rejected where a GM virus is used in co-infection with non-GM/GM virus but will be accepted where an experiment using a GM virus infected a GM mammalian cell line).
- If the genetic modification confers potentially harmful biological activity (e.g. virulence factor, toxin, determinant of immune evasion)
- If the genetic modification increases the pathogenicity and/or fitness of the sample.

Material which is no longer the subject of active study will be destroyed by steam autoclave and disposed of according current regulation.

**Recipient or parental organism**

Any prokaryotic or eukaryotic organisms

**Host/vector system**

Any prokaryotic or eukaryotic organism.

Vector systems used will include vectors from prokaryotic or viral sources. All vectors used will be commercially available and include a replication origin and a drug-resistance gene. All vectors will be avirulent and have a good safety profile (e.g. has a long documented history of safe use) both of which will be evidenced in the GM assessment required to be completed prior to starting any work. For example, bacterial vectors will be non-mobilisable. Viral vectors will be disabled and incapable of establishing a transmissible infection in humans.

A list of prohibitions for the insert is detailed in the CU2 notification.

Work will be prohibited if the parental strain of the GMO is a human HG3 or HG4, even where the modification attenuates the strain.

**Origin & function**
The genetic material will originate from either a prokaryotic or eukaryotic organism. The inserted genetic material will be expressed in either a prokaryotic and eukaryotic system and will not be permitted if it can

- Confer potentially harmful biological activity (e.g. a virulence factor, a toxin, an allergen or determinant of immune evasion)
- Increase its pathogenicity and/or fitness.
- Alter the host range
- Alter the susceptibility to prophylaxis
- Confer potentially harmful activity to the environment
- Increase its survival fitness.

Any work to be completed must not increase the virulence, pathogenicity or robustness of the recipient host (classification up to HG2 prokaryotic or eukaryotic organism)

Manipulation using sharps will be minimised wherever possible, however the use of sharps is unavoidable in some cases (e.g. some imaging tools) Sharps must be handled only by members of staff with experience in and trained for using these tools

Any manipulation of an organism that can cause harm by the airborne route must be able to be handled in a microbiological safety cabinet or the proposed work will be rejected. The Franklin has 12 Class II safety cabinets and these are inspected as per the manufacturers requirements.

If an antibiotic is an option for the treatment of any infection with that organism, it must not be used in the work. (i.e. antibiotic resistance selection cannot use the same antibiotic that could be used for any treatment of an infection with that organism).

**Evaluation of foreseeable effects**

Access to the laboratory is tightly controlled to authorised personnel (managed by photo card swipe). All those entering the labs to work must have completed mandatory training, must have personal protective equipment and completed appropriate risk assessments.

Visitors to the lab areas are only permitted when escorted and must also wear personal protective equipment. All visits are risk assessed and in the event of grant of CU2 permission is given, notices will be displayed prominently at all lab entry points.

The labs are well maintained and regularly cleaned. All biological waste is autoclaved in the laboratory. We do not see any prospect of accidental release outside the lab area. Lab cleaning protocols ensure that any spill inside the lab area is dealt with properly, including disinfection where required.

Exposure to aerosols will be avoided by using sealed flasks, handling in class II safety cabinets, using lysis buffer rather than mechanical lysis methods wherever possible (where this not possible any such mechanical lysis will be inside the safety cabinet).

No specific additional hazards are foreseen to arise from our work since we are not creating new pathogenic traits as any mutation must not confer any pathogenic traits or alter the host range.

No specific hazards are envisaged as the inserted material will have been scrutinised to ensure it does not encode any hazardous products.

Finally to avoid transfer of genetic material, in the case of experiment using multiple live bio-material, there will not be more than one biological agent from the same domain of life carrying a genetic modification as part of the experiment. All material no longer subject to study will be destroyed. Any material that is stored will be kept to the absolute minimum required for further experiments.
No use of larger GMOs will take place

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

No derogation measures required

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

<table>
<thead>
<tr>
<th>The work will produce both solid waste and liquid waste. Solid waste: Will be treated by disposal of into autoclave bags or bins and autoclaved in a cycle validated by a 12 point thermocouple test prior to either off site incineration or disposal to landfill. In the case of lab coats, they will be treated by autoclaving in a validated cycle followed by washing in the general laundry route. Autoclaves are serviced and validated annually. Liquid waste: Will be treated in one of the following ways depending on the concentration and organism in use.</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Immersion in 1% virkon for 15 mins (final concentration)</td>
</tr>
<tr>
<td>• Immersion in Sodium hypochlorite for at least 30 mins. Concentration dependent on organism used and work being completed</td>
</tr>
<tr>
<td>• Immersion in Chemgene for 15 mins. Concentration dependent on organism used and work being completed.</td>
</tr>
<tr>
<td>• Autoclaving in a cycle validated for by a 12 point thermocouple test.</td>
</tr>
<tr>
<td>Surfaces and tools will be decontaminated with one of the following depending on the concentration and organism in use:</td>
</tr>
<tr>
<td>• 70% ethanol</td>
</tr>
<tr>
<td>• 1% virkon</td>
</tr>
<tr>
<td>• Sodium hypochlorite. Concentration dependent on organism used and work being completed.</td>
</tr>
<tr>
<td>• Chemgene. Concentration dependent on organism used and work being completed.</td>
</tr>
</tbody>
</table>

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
The enclosed risk assessment is broad overview that can be used to guide future individual assessments. It is based upon a previous CU2 application accepted by HSE on the 4/09/2019 with the reference GM972/19.1. The GM972/19.1 application was accepted on the basis of future support and review to be provided by the site GMS management committee of which the RFI has become a member. As such, all future work will first be reviewed through the site GMS management committee a well established GM and BioSafety committee supporting work on the Rutherford Appleton Laboratory Site. The committee is composed of representatives from Diamond Light Source (DLS), ISIS, Research Complex at Harwell (RCaH), Central Laser Facility (CLF), STFC, partner companies, universities and RFI with support from our consultant BioSafety Officer, Sharon Webster. Some of these representatives are also members of the onsite safety, health and the environment (SHE) group (https://staff.she.stfc.ac.uk/pages/staff/home.aspx). All work will have an individual supporting GM/bioCOSH assessment that requires approval from the aforementioned GMS management committee before it can take place. This will enable a consistent and coordinated approach to all notifications and risk assessments completed by staff in the member institutes.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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### GM Centre Number: 3566

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#### Name

**LOQUUS23 THERAPEUTICS LTD**

#### Name 2

#### Department

#### Campus Estate or Research Centre

**RIVERSIDE**

#### Building

**BABRAHAM RESEARCH CAMPUS**

#### Road Name

#### District

#### Town

**CAMBRIDGE**

#### County

**CAMBRIDGESHIRE**

#### Postcode

**CB22 3AT**

#### Country

**ENGLAND**

#### Tel Number

**07467825333**

#### Fax Number

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#### E-mail

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#### HSE Division

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#### Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

BSO and CSO, LoQus23 Therapeutics

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<td>Other (please specify)</td>
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Tick if confidential

Bacteriology, Parasitology, Transgenic Birds, Microbiology Research, Virology, Transgenic Animals, Transgenic Fish, Gene Therapy
### Other(s)

- Genetically modified immortalised cell lines

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

- Disinfection (precept, alcohols, virkon), sterilisation and incineration as appropriate

**Tick to confirm that you are attaching a summary of the risk assessment**

- Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

- 

**Please enter comments of the GM safety committee on the risk assessment**

- (BSO): Risk assessments support the use of CL1 facilities
**GM Centre Number: 3567**

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02/03/2022
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</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Expertise in risk assessment pertaining to contained use is obtained from a Biosafety Consultant with a specialism in GM. The individual is a Former HSE Principal Specialist Inspector of Biological Agents, University Biological Safety Officer and research scientist in government, academic, healthcare and industrial containment laboratories.

4.

<table>
<thead>
<tr>
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<td>Yes</td>
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</tbody>
</table>
Solid waste will be inactivated with overnight soaking in 10% Distel solution, before being placed in sharps bins and biohazard-marked bags, which will be sealed and collected by Initial Medical Services for incineration. Liquid waste will be sterilised by the addition of Virkon tablets or 10% Distel solution in leak-proof containers. This will be left to stand overnight before discharge to drain. Expected degree of kill is 100%.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Our biosafety consultant has reviewed the attached risk assessment and concluded that the work is of no or negligible risk and suitable for assignment to class 1. They have concluded that the control measures and waste management processes are appropriate for the activity being undertaken.

Project Ref 3567/21.1

Date Ackn’d 18/06/2021

Date Project Ceased

CU2 Project Title Use of retroviral and lentiviral vectors to transduce murine and human cells

Class 2

CultureVolClass2 < 1 Litre

Class VolumeClass3-4 Non-GMM Consent Granted
**Project Additional Information**

**Purposes of the contained use**

The overall aim of this project is to generate genetically modified murine and human cells in vitro, for example but not limited to Chimeric Antigen Receptor (CAR)-T and CAR-NK cells, which will be used in combination with CytoSeek’s technology for in vitro and in vivo pre-clinical studies. All genetic modifications will involve the addition of a targeting receptor for a tumour antigen, and will not involve inserting any known oncogenes. CytoSeek’s technology, which consists in adding artificial membrane-binding proteins on the GM cells, will not alter the potential risks of the generated GMOs. In adoptive cell therapy, immune cells are genetically engineered to express a receptor targeting a defined antigen, allowing the activation of the cells by this target antigen. Engineering of T cells and other immune cells in this manner has implications in the treatment of liquid and solid tumours. The use of viral vectors will allow the generation of engineered immune cells that target tumour associated antigens. Genetically modified immune cells will be combined with CytoSeek's technology and their safety and efficacy will be tested in vitro and in vivo using mouse models of cancer. It would not be possible to effectively assess the functionality of the technology in vitro or in vivo without using GM cells in this way. This study will generate data relevant to clinical application. The work is significant as the technology being developed by CytoSeek is the first of its kind and may be useful in medicinal therapies that use genetically altered immune cells.

**Recipient or parental organism**

We will genetically modify three types of cells:

1. **Bacteria**: standard laboratory strain of E. coli will be transformed with recombinant plasmids, containing genes that encode for tumour antigen-specific receptors (CARs), and/or retroviral/lentiviral genes, as well as genes providing antibiotic resistance genes to the bacteria to aid selection. This will facilitate molecular cloning and allow us to grow up sufficient quantities of plasmids for transfection of packaging cells (e.g. 293T cells). The competent bacteria used are commercially available.

2. **Retroviral and Lentiviral packaging cell lines** such as the human HEK293T cell line and its derivatives (e.g. Phoenix Amphi cell line, Phoenix-Eco cell line containing chromosomally integrated helper functions and encoding amphotropic or ecotropic envelope proteins respectively, and 293FT cells for co-transfection with helper plasmids in lentivirus rescue).

3. **Human and mouse immune cells** (eg T and NK cells) and mammalian cell lines (eg Jurkat) will be transduced with the above recombinant retroviral/lentiviral vectors.

**Host/vector system**

Replication-defective, genetically modified retroviruses (MoMuLV- or derived), with amphotropic or ecotropic envelopes, and 3rd generation self-inactivating (SIN) lentivirus vectors.

The retrovirus system uses two plasmids, the pMP71 retroviral expression plasmid that carries the CAR transgene, and a pCL Amphi or Eco packaging vector to increase expression of the appropriate envelope protein in the packaging cell line. These plasmids are transfected into the Phoenix packaging cell line. The Phoenix Amphi cell line is a high titre amphotropic retrovirus-producing cell line that was generated by stable transfection of 293T cells with a Moloney GagPol-IREs-Lyt2 construct with a Rous sarcoma virus (RSV) promoter and a pPGK hygro selectable marker. 293T cells are human embryonic kidney cells transformed with the E1 region of adenovirus type 5;
and subsequently also stably transfected with SV40 T-antigen. These cells were then stably transfected with the Moloney amphotropic envelope gene (Phoenix-Ampho) or ecotropic envelope gene (Phoenix-Eco) driven by a CMV promoter and coselected with a diphtheria toxin resistance gene (pHED-7). The separation of the gag-pol and the env plasmids within the Phoenix cells is designed to ensure a very low likelihood of generating replication-competent retrovirus.

The lentiviral system uses four plasmids, where Rev and Gag/pol genes have been separated on two separate plasmids, with the third plasmid encoding envelope proteins. The Tat gene has been deleted. The transfer plasmid will contain the transgene of interest (eg CAR gene) driven by a promoter (eg human elongation factor 1 alpha promoter (EF1alpha)). The plasmids are as described in the attached risk assessment.

These split packaging systems are designed to minimise the chance of recombination events that could incorporate the helper functions to generate a replication-competent virus. For lentivirus, only “self-inactivating” (SIN) vectors will be used; these have a deletion within the U3 region of the 3’LTR, which removes enhancer and promoter sequences. Upon completion of one life-cycle from the RNA genome of the virus particle, via reverse transcription to integration into chromosomal DNA, the deleted U3 region is copied to the 5’LTR. As a result, the 5’LTR becomes incapable of transcribing full length viral genomes. This further reduces the possibility of onward viral transmission. Lentiviruses will generally be pseudo-typed using the VSV-G envelope glycoprotein; this is widely used as it permits efficient transduction of a wide variety of cell types and makes the virus particles relatively resistant to shear stress, facilitating their concentration by centrifugation if required to increase the titre.

Origin & function

The sequences that will be inserted into recipient eukaryotic cells will include chimeric antigen receptors (CAR) as described in the attached risk assessment. In general, the CAR constructs will contain:

- a sequence encoding a receptor able to specifically recognise a tumour-associated antigen (eg a single chain variable fragment (scFv) constructed from a tumour antigen-specific mouse antibody). Other examples are in the attached risk assessment;
- a hinge region (eg from CD8);
- a transmembrane region (eg from CD8 or CD28);
- a series of intracellular signalling moieties, including costimulatory domains (eg from CD28 and/or 41BB), followed by CD3ζ.

The plasmids containing the tumour-targeting construct and the retro/lentiviral genes will be expanded in competent bacteria and purified to transfect packaging cells that will allow the formation of the viral particles.

Viral particles will be harvested from the supernatant fraction of the packaging cell cultures and may be concentrated to transduce human and murine immune cells.

Human and murine immune cells, for example but not limited to primary T and NK cells from peripheral blood of healthy human donors or murine models, and mammalian cell lines, will be transduced with the viral stock solution containing a pure recombinant, replication-defective lentivirus/retrovirus population. The integration of the tumour-targeting construct in the genome of human and murine cells will enable these cells to stably express this receptor and thereby respond to a specific tumour-associated antigen.

Evaluation of foreseeable effects

None of the proposed genetic alterations are expected to cause a significant increase in the risk to human health or to the environment. As with any retroviral vector, there is the potential hazard of generating a replication competent virus by recombination with helper sequences in the packaging cells. However this risk has been minimised by the design of the “split” packaging cells used, and by the use of equivalent split helper functions, and SIN vectors, for lentivirus vectors.

Retrovirus and lentivirus infection also carries an associated risk of insertional mutagenesis. Such insertional mutagenesis has resulted in oncogene activation and leukaemia in some patients in a gene therapy clinical trial for X-SCID, however this was in the context of deliberate administration of very high doses of virus expressing the cytokine receptor gamma chain to haematopoietic stem cells. The lack of such adverse effects in the vast majority of other gene therapy trials including a large number of CAR T cell therapy trials using retroviral or lentiviral systems and involving hundreds of patients, indicates that the chromosomal insertion of replication-defective forms of these viruses is generally unlikely to cause overt harm.
The retroviruses or lentiviruses will be used in vitro to transduce human or mouse immune cells or cell lines (e.g. Jurkat) which are not known to carry any other virus and which are not expected to complement the replication-deficiency of the vectors. The Phoenix cells are widely used and any adventitious retrovirus contamination of these cells would have been widely publicised if the problem had arisen. There is a substantial history of safe use of such vectors in these and other human cells, with no apparent interaction with the endogenous, ancestral, degenerate retrovirus-like sequences in the human genome. If the vectors or cells transduced with the vectors are used in mice, there is also a theoretical risk of complementation/recombination with endogenous viruses, but again there is a substantial history of safe use of such vectors.

The introduction of CAR genes into T lymphocytes should result in expression of the CAR at the cell surface and could potentially result in activation of the T cell if it encounters the target antigen. The CARs used are specific for tumour-associated antigens, so activation is more likely to be beneficial to the host rather than harmful, although if the target antigen is expressed on healthy cells this could result in immune-mediated pathology in the host.

In all cases, cells derived from the operator will not be used in these studies and therefore should the operator be accidentally inoculated with any of these genetically modified cells they will be identified as “foreign” cells and eliminated by the host’s immune system. For the same reason any murine genetically modified cells generated in this project will provide no additional risk to human health. Pregnant women, new mothers and/or immunocompromised employees will not handle lentiviral and retroviral vectors.

If the viral vectors were introduced directly into the host by accidental exposure and transduce T cells in situ this could result in autologous T cells with auto-reactive potential. Although transduction in this manner is likely to be very inefficient, it is important that sharps are avoided when handling the virus vectors to control the risk of injecting yourself. The main hazard with these viral vectors is the possibility of oncogene activation at the site of integration. Consideration of the experiences from human gene therapy clinical trials suggests this risk is particularly low when using T cells as the host, and any accidental exposure of humans to recombinant virus is likely to involve many logs fewer virus particles. Nevertheless precautions to avoid exposure are appropriate.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N.A.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None. CytoSeek has access to an autoclave on site at Unit Dx and the University of Bristol if needed.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated pipette tips, serological pipettes, cell culture plates, flasks, tubes and similar plasticware will be collected in a class II cabinet within a category 2 laboratory, where it will be inactivated with overnight soaking in 1% Virkon solution. After overnight soaking, disinfected plasticware will be rinsed and disposed of within biohazard-marked 60L sharp bins. Sharps will be contained within the same sharp bins. Sharp bins will be sealed and collected by a specialised clinical waste licensed contractor for incineration. Soft waste, such as hand towels and gloves, will be collected in hazard-marked bags which will be disposed of by incineration off-site after collection by a licenced contractor. If any soft waste is known to be contaminated with virus (e.g after a spill), this will be soaked overnight in 1% Virkon before disposal in hazard-marked bags. Liquid waste will be collected in 1L sealed buckets in a class II cabinet within a category 2 laboratory and inactivated overnight by the addition of Virkon to make up 1% solutions. Deactivated liquid waste will be then discharged to drain.

The manufacturer’s information indicates efficacy of Virkon against HIV (at dilutions from 1:100 – 1:1400), and against the oncovirus Avian Leukosis Virus (at 1:200). CytoSeek has access to an autoclave on site at Unit Dx. Autoclaving achieves effectively 100% kill of all GMMs.

**Is an emergency plan required according to regulation 20?** N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

02/03/2022  Page 14954 of 15326
Our biosafety committee has reviewed the attached risk assessment and concluded that the measures outlined should adequately control the hazards in this project, which is suitable for assignment to class 2.

**Project Containment**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Genetic Modified Safety Committee (GMSC):
1) Qualified Person (QP) who has an education and background working with Genetically Modified Organisms (GMO's).
2) Head of Operations, Institute of Occupational Safety and Health qualified (IOSH).
3) Packaging Room Supervisor.
4) Distribution Support Coordinator, Dangerous Goods trained (IATA)

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Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research

02/03/2022
The GMO is a Recombinant Adeno-Associated Virus.

Inactivation Methods:
1) Trained staff will use a specific GMO spill kit and wear appropriate PPE. This includes: Safety Glasses, FPP2 Face Mask, Labcoat, and Shoe Covers.
2) In the event of a breakage, any broken glass will be collected using a disposable scoop and placed into clinical waste bags.
3) Absorbent paper will be used to soak up any liquid. This will be repeated until there is no liquid in the contaminated area. Contaminated absorbant paper will be placed into clinical waste bags.
4) Hypochlorite/chlorine bleach will be poured over the contaminated area and left for 20 minutes.
5) The area will then be thoroughly wiped with water to remove any traces of disinfectant.
6) Any wipes or other cleaning materials which came into contact with the spill will be placed into clinical waste bags ready for destruction.*
7) Items from the GMO spill kit which are used during clean-up will be replaced to ensure the contents of the kit are appropriate for next use.
   * Xerimis Ltd. have a service agreement with a destruction company who are able to dispose/destroy infectious clinical waste.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Receipt, Storage and Distribution of a GMO to UK clinical sites on behalf of a sponsor company.

Tick to confirm that you are attaching a summary of the risk assessment 

Tick if you are claiming exemption from disclosure for sections of the risk assessment 

Please enter comments of the GM safety committee on the risk assessment
1) Qualified Person (QP) who has an education and background working with Genetically Modified Organisms (GMO's).
2) Head of Operations, 28 years of working in pharmaceutical sector. Institute of Occupational Safety and Health qualified (IOSH).
3) Packaging Room Supervisor.
4) Distribution Support Coordinator, Dangerous Goods trained (IATA)

The GMSC group worked through a Failure Mode and Effects Analysis (FMEA) risk assessment process, identified risks were scored and mitigating actions identified, in review of the residual risks post implementation of the mitigating actions, no high risks remained. The remaining risks were for the majority to be determined low, with a few medium risks, with the controls in place these remaining risks were deemed by the committee to be acceptable.

The GMO is a Recombinant Adeno-Associated Virus which is pre-packed before entering the facility. The sponsor company anticipates less than 50 active patient kits will be supplied to Xerimis UK.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

More than 10 years experience of GMO class 1 organisms. Writing of multiple risk assessments for the use of GMO and non-GMO in different facilities. Submission of previous notification of premises applications to HSE and been part of risk assessment committees.

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Tick if confidential

☐
1. Waste such pipettes, stripettes, falcon tubes, eppendorfs etc. are treated as "biologically hazardous" and collected and incinerated off-site by a waste disposal company accredited to manage hazardous material.

2. Sharps used for the dissection of tissues are disposed of in the assigned sharps disposal container. Plus their use is reduced by the usage of scissors.

3. Live cells and culture media waste is decontaminated with 3% Virkon for more than 2 hours and washed down the sink under running water.

For activities involving GMMs, describe the waste management measures which will apply to the activity

- Sendai virus reprogramming kit CytoTune is class 1 GMO organism. This kit has been developed to reprogram somatic cells into induced pluripotent stem cells ad it is available from Thermo Fisher Scientific and has been widely use for cell biology research. The kit and lines produced have been used for several years and are also though to be safe.
GM Centre Number: 3571

Data Premises Notified (Originally) 18/11/2020

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

MULTUS BIOTECHNOLOGY LTD

Name 2

Department

Campus Estate or Research Centre

Building

TRANSLATIONAL & INNOVATION HUB

Road Name

84 WOOD LANE

District

Town

LONDON

County

GREATER LONDON

Postcode

W12 0BZ

Country

ENGLAND

Tel Number 07902906830

Fax Number 0

E-mail

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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

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| Level 4 (GMMs) | |
| Non-microbial | |
| Other (please specify) | Tick if confidential |
| Bacteriology | Yes |
| Parasitology | |
| Transgenic Birds | |
| Microbiology Research | Yes |
For activities involving GMMs, describe the waste management measures which will apply to the activity

Effective inactivation and disposal of waste containing genetically modified organisms will be done as follows:

-Solid waste: All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature/time profile. Waste will be transported in sealed containers and following autoclaving, the resulting non-hazardous waste will be packaged into "Biohazard"-labelled bags and be sent for incineration following local regulations.

-Liquid waste: All liquid waste generated will be aspirated into a bottle containing Virkon, a peroxygen based disinfectant, which will be used according to the manufacturer's instructions. Following a 24-hour chemical inactivation, the resulting non-hazardous waste will be disposed of in an excess of water.

Tick to confirm that you are attaching a summary of the risk assessment  Y  
Tick if you are claiming exemption from disclosure for sections of the risk assessment  Y 

Please enter comments of the GM safety committee on the risk assessment

1.) Background in Biotechnology and Biomedical Engineering. Extensive experience from working with the same organisms as described in this activity.
2.) Background in Microbiology with focus in Synthetic Biology. Relevant experience in the microbiological and genetic procedures involved.
3.) Background in Molecular Bioengineering specifically Bioprocesses. Relevant experience with completing risk assessment and similar filings.
### GM Centre Number: 3572

| Data Premises Notified     | 18/11/2020 | Transferred from 1992 Regs? | N |
| Data Premises Closed       |            | Transitional Premises Class |   |
| Data Premises Notified (Originally) |           | Transitional Premises Emergency Plan Required? | N |
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The GMSC consists of the CEO (Chair), CSO, Laboratory Manager, a Senior Scientist and Lab Technician (acting as a lay person). Collectively, the GMSC represents decades of experience in safe laboratory practices including working safely with Class I GMMs. The Laboratory Manager has specific IOSH qualifications related to drafting and implementing laboratory risk assessments.

<table>
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<th>Glass House</th>
<th>Large Scale</th>
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Solid wastes and small volumes of liquid are disposed of in dedicated lined yellow plastic snap-top 60L bins that are treated by autoclaving at 121 degrees C for 15 minutes in the contained use laboratory. An indicator strip is included with each autoclave run to monitor peak temperature of each run. Bins are numbered and collected by a third party waste disposal company for incineration off-site. Paperwork is signed off upon collection of designated, autoclaved laboratory waste. Liquid wastes including microbiological cultures are inactivated by contact with 1% bleach (working final concentration) for 1 hour before disposal down the designated laboratory sink. Laboratory surfaces are wiped down at least daily with chempgene first followed by 70% ethanol. Swabs will be taken from designated locations/surfaces of the laboratory twice a year with growth on agar and agar supplemented with the antibiotics ampicillin and kanamycin (swabs on two separate plates). Results from these swabs to be documented with any mitigation requirements noted for areas that are identified as having a detectable level of contamination with presumed GMM organisms.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

All experiments to be conducted on site involving GMMs are considered to fall under Class I. As such, standard good laboratory practice applies with a defined waste disposal methodology and safe methods of work documented within the companies risk assessment and SOPs.
GM Centre Number: 3574

Data Premises Notified (Originally) 01/12/2020

Transferred from 1992 Regs? N

Transitional Premises

Class

Data Premises Closed

Transitional Premises

Emergency Plan Required?

Non-GMMs N

Withdrawn N

Name

OCHRE BIO LTD

Name 2

Department

Campus Estate or Research Centre

BIOESCALATOR

Building

INNOVATION BUILDING

Road Name

ROOSEVELT DRIVE

District

Town

HEADINGTON

County

OXFORDSHIRE

Postcode

OX3 7FZ

Country

ENGLAND

Tel Number 07595702453

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

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Give brief details of the genetic modification safety committee

The BioEscalator has a Biological safety officer who has formed a genetic modification safety committee (GMSC), comprising of two University divisional safety officers who have expertise in biological safety as well as a representative from each company that is currently doing GMO work.

- **Laboratory**
  - Bacteriology: Yes
  - Virology: Yes

- **Animal Unit**
  - Parasitology
  - Transgenic Animals: Yes

- **Growth Room**
  - Transgenic Birds
  - Transgenic Fish

- **Glass House**

- **Large Scale**
  - Microbiology Research
  - Gene Therapy: Yes
The studies being undertaken are for improvement of cellular metabolism and stress response.

For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment [ ]
Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment.

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**Project Ref:** 3574/20.1

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Withdrawn [N] 
Tick if notifying a connected programme of work [N]

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Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

---

**Project Additional Information**
The proposed work will identify gene modifiers of dysregulated processes that result in poor outcome of steatotic liver transplants. This will involve interrogation of gene candidates utilising standard overexpression and knockdown viral vectors, e.g. lentiviral vectors encoding the gene of interest or vectors expressing CRISPR systems that target genes for inactivation. The impact of these genetic edits will then be assessed using in vitro and ex vivo systems.

Recipient or parental organism

The recipients include

(a) bacterial cultures to enable growth of the DNA required to produce the viral vectors. E.coli (K12 or B derivatives) are disabled and unable to colonise the human gut and have a history of safe use and can be assigned to ADC hazard group 1. The vectors are non-mobilisable. The inserted genetic material does not increase the level of risk for the GMMs.

(b) mammalian cell cultures (e.g. human hepatoma cell lines) These are are well characterised, continuous cell lines that have an established history of safe use (regarded as low hazard for GM activities and are suitable for use under containment level 1, though are handled at containment level 2 for CoSHH regulations), and additional modification are not considered capable to increasing the risk of these cells lines.

(c) primary human cells (e.g. hepatocytes) These are less well characterised but are considered especially disabled hosts under the Genetic Modification Regulations and are therefore of minimal risk (considered to be ADCP hazard group 1). However, due to the potential presence of adventious agents they are handled at containment level 2 under CoSHH regulations and additional control measures are included (e.g. the requirement of HepB vaccination and records of training) - this risk is reduced through the use of prescreened donors negative for HIV, HBV and HCV.

Host/vector system

For this work self-inactivating, third generation lentiviral system will be used, generated by transient transfection of the human 293T cell line. Although this vector system is based on the pathogenic human immunodeficiency virus, it has an excellent safety rating due to the removal of accessory genes, the lack of expression of any viral genes in transduced cells, the split production plasmids with no homology to prevent the recreation of replication competent retroviruses and reduced insertional mutagenesis though mutation of the viral U3 promoter. However, the vector does incorporate the WPRE, elevating its potential for tumourogenesis due to the presence of a truncated HBV X protein, is pseudotyped with VSV-g to increase its tropism, and the transgenes used in this proposal (human genes and components of the CRISPR system) increase the risks of altered cellular behavior (both cell death and tumourogenesis) if exposed and transduced by these vectors.

Origin & function

Genes for overexpression will be of human origin generated from cDNA, and as such will not be toxins but do have the potential to act as oncogenes or other modifiers of cellular behaviour in cells that are transduced by the vector.

CRISPR components (e.g. Cas9 and sgRNA) are of bacterial origin and are designed to introduce a mutagenic double stranded break in the human genome. Gene targets could include tumor suppressor genes, thereby increasing the risk of tumourogenesis associated with the vector. However, the two components will be separated into two vectors in order to reduce the likelihood of this event (i.e. it would require dual exposure to result in this event).

Evaluation of foreseeable effects

The bacterial used in this proposal are disabled and introduction of additional plasmids for their upgrowth does not alter their risk to the environment or human health.

The use of third generation lentivirus systems has many advantages, notably all accessory genes are removed and the essential viral proteins, gag, pol, and rev are provided in trans by additional plasmids (optimised to harbour no homology) and the envelope gene is replaced with VSV-g, all of which reduce the likelihood of producing replication competent or infectious virus effectively to zero (and there is no documented evidence of this occurring in third-generation systems). However, upon transduction of a cell the genetic vector is integrated at random into the host genome, which has the potential to disrupt the expression of a host gene or to enhance the expression of neighbouring genes through insertional mutagenesis, and the use of the VSV-g envelope expands the cell types that the vector can access. Additionally some vectors harbour the WPRE to enhance transgene expression. The WPRE may result in the expression of the HBV X protein, which as been associated with tumourogenesis in
certain models. The impact of these insertional mutagenic events is reduced through the use of self-inactivating vectors in which the viral promoter is mutated, and standard laboratory safety measures (class II safety cabinets, PPE etc), not producing large quantities (>1l) of virus or concentrating viral stocks to very high titre (not >100 fold), and not using sharps in any procedure involved with vectors makes the use of these vectors of negligible risk on their own. Finally, they are self-inactivating so do not increase the risk of cells transduced by them, so such cells/cell lines can be considered of the same category of risk as the parental cells (generally class 1 for cell lines and class 2 for primary cells).

That said the transgenic inserts that are carried to the recipient cells using lentiviral vectors do increase the potential risk to human health. The overexpression of human genes can have numerous impacts but importantly could act as oncogenes, immunomodulators, apoptotic regulators etc, all of which can enhance the potential for tumourogenesis. Additionally the use of the CRISPR system to target gene inactivation not only has the potential to eliminate important tumour suppressor control elements of the cell but also act as a general mutagen through off-target double strand break induction. However these risks would only be realised through self-inoculation through percutaneous or mucosal routes that are minimised through the elimination of sharps usage during vector procedures, use of good laboratory practice and PPE, and ensuring adequate documented training.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste i.e. consumables such as plasticware eg pipettes, flasks, tubes are rinsed in 5% chemgene or 10% distel prior to being autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (134-138oC for at least 15 minutes). Any excess sterilised liquids will be discharged to drains and the resultant sterile waste is disposed via the industrial (black bag) waste stream for incineration to produce electricity (Select environment Ltd) or clinical waste stream for incineration.

Liquids (eg samples, culture supernatants, tissue culture media) are exposed to a final concentration of 2% Virkon/ 10% distel/ or 2% chemgene for 30 minutes at room temperature prior to discharge to drains.

Agar plates are autoclaved using a make safe cycle as specified in BS 2646, Part 3, 1993 (see above), excess liquid discharged to drains, and solids discarded via the black bag waste stream for landfill.

All GM containing material must be deactivated or decontaminated before leaving the premises. Sharps bins containing GM material, that has not been decontaminated prior to being placed in a sharps bin, must be autoclaved before being placed in clinical waste.

Degree of kill
Autoclaving, effectively 100% kill (annual validation)
Incineration, effectively 100% kill (licensed incinerator)
Chemical, effectively 100% kill (following manufacturer’s guidelines)

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

02/03/2022  
Page 14973 of 15326
We don't use 10% distel or 5% chemgene or 2% trigene as disinfectant in TC lab, its % Virkon and 70% ethanol. The autoclave waste discard cycle here is 134C for 15 minutes not 5 minutes mentioned in the RA. The black bags are collected by Select environmental Ltd and are not landfill but incinerated to produce electricity.

All GM containing material must be deactivated or decontaminated before leaving the premises. Sharps bins containing GM material, that has not been decontaminated prior to being placed in a sharps bin, must be autoclaved before being placed in clinical waste. This should be written in the waste procedures.

Project Containment

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Genetic Modification safety committee has not yet been established. Risk assessments were produced by Axovia Therapeutics.

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Tick if confidential

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

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2. Solid waste - Treatments used Autoclave, Chemical and Incineration
   Disposables used in aliquotting the vector (ependorphs, pipette tips) and injecting the vector (glass micropipettes, needles). Animal carcasses will be disposed of via incineration.
   Any materials that contain or have come into contact with viral particles will be decontaminated by soaking in the DEFRA-approved virucide TriGene Advance 10% for 5 minutes and then subjected to autoclaving. Autoclaving (132 degrees celsius, 15 minutes holding time) will give 100% kill at this temperature and time. Animal carcasses will be disposed of via incineration.

3. Liquid Waste - Treatments Used Autoclave, Chemical.
   The vector will be aliquoted into 10 ul volumes and stored at -80 degrees celcius. Between 5-10 ul will be injected in vivo. Any spare vector will be disposed.
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Tick to confirm that you are attaching a summary of the risk assessment [ ]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]
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Axovia shall be responsible for:
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RVC shall be responsible for:
• Providing risk assessments associated with husbandry of standard rodents
• Providing risk assessments associated with BSU shared areas

Project Ref 3576/20.1

Date Ackn’d 24/12/2020

CU2 Project Title Gene and Cell Therapy to treat GM mice

Class 2

Culture Vol Class 2 Culture Volume Class 3-4

Class

Culture Vol Class 2 Culture Volume Class 3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

With withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Bardet-Biedl Syndrome (BBS) is a rare genetic condition that presents complex clinical manifestations, where multiple systems are affected; polydactyly, retinal degeneration, cystic kidneys, obesity, auditory deficiencies and. Perhaps the most clinically relevant feature is the retinal degeneration that appears progressively starting as night blindness and progressing to complete loss of sight and registered blind usually before puberty. So far 18 genes have been linked with BBS and most of them form complex known as BBS that is involved in the function of the cilium.
The particular and molecular mechanism of how and why the retina degenerates progressively still remains unclear. So far all knock-down mouse models lacking complete BBS gene function, reproduce the same pattern of the human progressive degeneration. In this project we have aim to a new gene therapy approach to for the study and treat the loss of sight. We have to our disposition a new knock-in BBS1 M390R knock-out mouse lines both replicating the human retinal phenotype. To pin point how the retinal function improves with gene therapy we propose to use to treat them with intravenous injections of adeno-associated virus (AAV), as vectors containing a human wild-type copies.

Viral vectors also often incorporate reporter proteins that allow us to determine the success and extent of expression, as well as offering the possibility to trace the anatomy of retinal cells affected by experimental perturbations. Two main questions will be answered by this project; first, which are the molecular functions involve in the death of retinal cells and how good is our gene therapy restoring the photoreceptors and the different layers of the retina.

The assessment should review for the first time the future use of AAV as a genetic vector in the Procedure rooms at the Biological services, located at LBIC and RVC. The new activity risk assessment will include the perinatal injections on GM mice and the cleaning and disposal of bench, material, tools and animal carcasses.

Recipient or parental organism

Adeno-associated virus (pAAV). Note that vectors will not be handled, as the already-packaged virus particles (replication-deficient).

Genetic modified mice with gene modificaton to mimic ciliopathies and BBS.

Host/vector system

The host are the genetic modified mice and the vector system will be the adeno-associated virus (AAV).

Origin & function

H.sapiens cDNAs will be cloned or purchased with the aim to replace mutated gene expression in the GM mouse models.

Evaluation of foreseeable effects

None of these are hazardous as they are replication deficient virus particles. The donor organism is not pathological or pathogenic. While AAVs can survive in the environment for protracted periods of time (they are resistant to dehydration). However, they would not be able to replicate in the absence of helper viruses (which are not used in the process of virus packaging - see above) or other helper functions.

Insertion of the gene sequences listed above will not affect the pathogenicity or toxicity of the AAVs. Transmission of AAV can occur through ingestion, inhalation of aerosol droplets, mucous membrane contact and accidental injection. However, for all of the above reasons, the AAV will be unable to replicate.

The infected cells (which might be in the epithelia or lung) will only express the recombinant protein transiently.

When transduced secondary cells divide, the expressed protein is diluted and quickly lost. We are also highly trained in the use of 'sharps' under aseptic conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Mouse Handling-

All animal handling is performed according to Home Office guidelines and local area safety codes. Suitable training in the form of a Home Office approved animal handling
course is provided for all individuals working with animals. Protective clothing, masks and gloves are worn. Additional respiratory protection is available for allergic individuals to reduce exposure. Western labs/UCL Central facility users will have the standard six monthly health check. Regular lung function tests are monitored by Occupational Health. Occupational Health advice and protocols will be followed in the event of sensitisation. If an individual is significantly sensitised, all contact with live animals/ animal dander will be avoided and a substitute person will undertake parts of the research requiring direct animal contact.

Use of Mouse Tissues-

Mouse tissues will be collected by a personal licence holder or staff once they have attended the Home Office approved animal handling course and passed to staff, in order that exposure to allergen is minimized. Use of needles and other sharps will be minimized. Gloves, lab coat and protective eye wear will be worn at all times whilst handling specimens. Any cuts or abrasions will be covered with Elastoplast or similar material. Additional respiratory protection is available for allergic individuals to reduce exposure. Facility users will have the standard six monthly health check. Regular lung function tests are monitored by Occupational Health. Occupational Health advice and protocols will be followed in the event of sensitisation. If an individual is significantly sensitised, all contact with live animals/ animal dander will be avoided and a substitute person will undertake parts of the research requiring direct animal contact. Solid waste material will be disposed of into designated waste bags and then autoclaved before being incinerated. Any liquid waste will be treated with sodium hypochlorite solution before disposal.

Other personnel: Hazardous waste will be properly bagged, labelled and removed from the labs to allow safe disposal by cleaning staff. Maintenance staff and visitors may enter the lab only by appointment with the lab manager and will be accompanied by trained personnel of the facility.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

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- Providing risk assessments associated with BSU shared areas.

Please enter comments on the GM safety committee on the risk assessment

In accordance with regulation 3 of the Management Regulations 1999, risk assessments are required for all Axovia Activities.

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**Project Containment**

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<th>Growth Rooms</th>
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#### Name

MELLIZYME BIOTECHNOLOGY LTD

#### Name 2

#### Department

#### Campus Estate or Research Centre

ICI

#### Building

LIVERPOOL SCIENCE PARK

#### Road Name

MOUNT PLEASANT

#### District

#### Town

LIVERPOOL

#### County

MERSEYSIDE

#### Postcode

L3 5TF

#### Country

ENGLAND

#### Tel Number

0151 482 9611

#### Fax Number

0

#### E-mail

#### HSE Division

blank

#### Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Mellizyme have sought advice from GMBSC Ltd Biosafety consultancy who will act as our GMSC. Our Advisor was a former Principal Specialist Inspector of Biological Agents for HSE and a University BSO and so is well equipped to advise our biological health and safety needs. GMBSC Ltd will work with two of our Lead Scientists to develop comprehensive GM Risk assessments which will be reviewed on a quarterly basis (or more frequently if the need arises).

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
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<th>Large Scale</th>
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Other (please specify) Tick if confidential

Bacteriology Yes Parasitology Transgenic Birds Microbiology Research

02/03/2022

Page 14983 of 1532
Autoclaving is the preferred route for inactivation of GMMs and where possible, cultures will be inactivated in the autoclave located in LSP wash up room at 121°C for 20 minutes. The autoclave is serviced, calibrated and validated once a year by Select Engineering Services, 13 Hurst Close, Over Hulton, Bolton BL5 1DT. Inactivated cultures will be poured down the laboratory sink and flushing copiously with water.

Where autoclaving is not possible, Virkon disinfectant will be added to final concentration of 1% w/v and left for at least 8 hours before being poured down the laboratory sink and flushing copiously with water.

Contaminated single use pipette tips are placed into yellow "Bio-Bins" for incineration. These are labelled and sealed before they leave the laboratory.

Contaminated plates and single use tubes are disposed of via yellow incineration bags, labelled and sealed with a cable tie. These are transported to the appropriate clinical waste bins in IC1, Liverpool Science Park in a clearly marked UN-compliant CL2 transport container.

Glassware will be disinfected with 1% w/v Virkon disinfectant and left for at least 8 hours before being poured down the laboratory sink and flushing copiously with water. The empty glassware will then be sterilised by autoclave.

Virkon is a safe and effective decontaminant shown to be active against 300 + strains/clinical isolates from 71 bacteria and resulting in >5-log reduction in viable organisms when used as a 1% dilution (Hernandez et al., 2000 [J. Hosp. Inf. 46(3)203-209], ‘Virkon Background Information’ https://www.fishersci.co.uk/webfiles/uk/webdocs/SLSGD05.PDF).

Chemgene will be used as an alternative to Virkon. It is a high-level laboratory surface disinfectant combines enhanced active ingredient molecules with micelle cleaning technology to provide swift penetration of the cell walls to ensure rapid cell death. The synergistic blend of technology and active ingredients enables Chemgene to work quicker than most disinfectants and ensure that apoptosis (cell death) is achieved rather than merely suspending activity. Chemgene is a safe and effective decontaminant shown to be active against E. coli K-12 cloning strains when using a 2% solution (Technical details can be obtained here: http://medimark.co.uk/industry/product/chemgene-hld4l-laboratory-disinfectant)

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Tick to confirm that you are attaching a summary of the risk assessment **Y**

Tick if you are claiming exemption from disclosure for sections of the risk assessment **☐**

02/03/2022
I have reviewed the proposed work and conclude that class 1 is appropriate. The initial proposed work involves using disabled E.coli strains transformed with fluorescent protein markers, which is of no or negligible risk to human health or the environment. I have requested that the users include information on the mobilisation status of the plasmid vectors to be used.

As this is the first genetic modification project to be undertaken by the company, this risk assessment should be submitted to HSE along with a CU1 notification form to register the premises for GM work.
<table>
<thead>
<tr>
<th>Name</th>
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Date at Which Additional Info Submitted

02/03/2022
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Give brief details of the genetic modification safety committee

Head for Pharmacy, Cancer Department, HCA Healthcare UK.

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02/03/2022
Once KTE-X19 is administered to the patient, empty bags and the used delivery system components (e.g., guide tube, cannula, injection needles and syringes), gauzes, personal protective equipment (e.g. gloves etc.) and any other components that have been in contact with the product before and during administration will be disposed of as medical waste. Spillage of product will be treated with 10000ppm free chlorine solution as per local biological product spillage guidance. Unused product will be returned to the manufacturer in the shipper.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Staff will wear appropriate PPE, including glasses, plastic gown and gloves. Patients are in single rooms and current waste management and spillage guidance is satisfactory. The product presents a negligible risk to staff, patients and the environment based on the Gilead risk assessment and the current practices and control measures are sufficient.
### GM Centre Number: 3580

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**Name**

ORCHID CELLMARK LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

16 BLACKLANDS WAY

**District**

ABINGDON BUSINESS PARK

**Town**

ABINGDON

**County**

OXFORDSHIRE

**Postcode**

OX14 1DY

**Country**

ENGLAND

**Tel Number**

01235528609

**Fax Number**

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**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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</table>

### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Advice has been sought from an individual with more than 20 years of academic and industrial experience. They are fully trained and registered with ISTR as a Biosafety Practitioner Level 1, and will act as the advisory BSO for Orchid Cellmark Ltd.

<table>
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<td>Other(s)</td>
<td>Genotyping of genetically modified immortalised cell lines and mouse tissue samples.</td>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity:

- Disinfectant solutions (Presept, Virkon and alcohols) will be used to clean work areas and deal with any spillages.
- An autoclave is available within the same building.
- Solid waste will be bagged as offensive waste and incinerated using a licensed waste disposal firm.
- Sharps or pipettes will be disposed of via sharps bins and incinerated using a licensed waste disposal firm.

Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: 

Please enter comments of the GM safety committee on the risk assessment:

The BSO has assisted with, and agreed the final draft of the attached risk assessment.
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**Name**

VIVIMED SPECIALITY INGREDIENTS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

PO BOX B3

**Road Name**

LEEDS ROAD

**Building**

**District**

**Town**

HUDDERSFIELD

**County**

YORKSHIRE

**Postcode**

HD1 6BU

**Country**

ENGLAND

**Tel Number**

01484320307

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Employee of Vivimed Labs
Possesses PhD in Biological Sciences
8 years experience working with GMOs

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Other (please specify)

Tick if confidential

Bacteriology
Parasitology
Transgenic Birds
Microbiology Research

Yes

02/03/2022
An autoclave is present in Biotechnology Laboratory. GMMs and associated waste will be deactivated completely by physical means via autoclave (1210°C, 2 bar pressure for 30 minutes). Autoclave is on a service contract and is maintained by a qualified engineer. Autoclave efficiency to be verified on a monthly basis via a validated spore test according to manufacturer's instructions.

For activities involving GMMs, describe the waste management measures which will apply to the activity

An autoclave is present in Biotechnology Laboratory. GMMs and associated waste will be deactivated completely by physical means via autoclave (1210°C, 2 bar pressure for 30 minutes). Autoclave is on a service contract and is maintained by a qualified engineer. Autoclave efficiency to be verified on a monthly basis via a validated spore test according to manufacturer's instructions.

Tick to confirm that you are attaching a summary of the risk assessment □

Tick if you are claiming exemption from disclosure for sections of the risk assessment □

Please enter comments of the GM safety committee on the risk assessment

The risk assessment is adequate for the safe containment of class 1 GMMs
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Name

MACOMICS LTD

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

137A GEORGE STREET

District

Town

EDINBURGH

County

Postcode

EH2 4JY

Country

ENGLAND

Tel Number

07795394442

Fax Number

0

E-mail

HSE Division

blank

Comments

 Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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<td>CB4 0GJ</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

This Notification and the accompanying Risk Assessment have been checked and revised by the person who has been a biological safety officer at the mid-size biotech company in Cambridge for several years and completed the Biological Safety: Management and Practice (IOSH Approved) course. If needed, the assessor has agreed to be identified by name.

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Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
Bacterial cells in suspension will either be lysed using detergents (SDS) and strong base solutions (e.g. during the process of plasmid DNA extraction) or inactivated by treatment with Virkon with a final concentration of at least 1% Virkon solution for at least 1h. Bacterial cultures growing on solid media will be autoclaved. Mammalian cells and tissue cultures will be treated Virkon solution with a final concentration of at least 1% for at least 1h. These methods of treatment provide virtually 100% inactivation of GMMs. Following inactivation, solid waste will be disposed using a professional waste management firm (registered contractor.) Liquid waste will be disposed of via a sink following inactivation with Virkon with a final concentration of at least 1% Virkon. Drains, sinks etc. do not pose a mode of transmission to the environment, as liquid waste has been inactivated.

All personnel who intend to undertake activities involving the use of GMMs, will be required to demonstrate the knowledge of the rules for work under containment and if necessary the relevant training will be provided.

The risk assessment has been reviewed by an independent Biological Safety Officer who agrees that the work outlined within falls within the brackets of level 1 GMM work.
GM Centre Number: 3583

Data Premises Notified: 15/01/2021

Transferred from 1992 Regs?: N

Transitional Premises Class: N

Data Premises Closed: N

Transitional Premises Emergency Plan Required?: N

Non-GMMs: N

Withdrawn: N

Name

STRATOSVIR LTD

Name 2

Department

Campus Estate or Research Centre

LAB G16, INNOVATION BUILDING

Building

STEVENAGE BIOSCIENCE CATALYST

Road Name

GUNNELS WOOD ROAD

District

Town

STEVENAGE

County

HERTFORDSHIRE

Postcode

SG1 2FX

Country

ENGLAND

Tel Number: 01438906906

Fax Number: 0

E-mail

HSE Division: blank

Comments

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The safety committee comprises of three experienced scientists that each have held/hold positions of responsibility leading laboratory activities at academic and industrial sites. Each have expertise in virology and GMM. The chair has previously held responsibility as CSO for biological safety for a company with over 20 bench scientists. Stratosvir's safety committee for genetic modification reviews codes of practice, COSHH and risk assessments and meets regularly (every quarter or as necessary) to review policies, procedures and conduct audits to ensure this is integral to the company's health and safety systems.

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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

**Solid waste:**
Contaminated general Class I waste will be disposed of in yellow autoclave bags. This includes plasticware (except stripettes) and soiled tissues/packaging which have been contaminated with biological/GM agents. The bags will be sealed with a cable tie when no more than 3/4 full and sent for incineration through a recognised clinical waste disposal provider.
Glass and sharps (including stripettes) which will be disposed of in rigid plastic containers which will be sealed and incinerated when full.

**Liquid waste:**
Liquid waste will be disposed of in the following manner: Biological liquid waste (not exceeding Class I) should be properly treated before disposal down the sink. Bacterial cultures/supernatants will be treated by adding Virkon to a final concentration of 1% and left for 1 hour followed by discarded down sink and rinsed with cold water. Cell cultures (Class I) will be treated with be treated with 1% Virkon for at least 1 hour and discard down sink with cold water.
Animal fluids (Class I) will be treated with 1% Virkon for at least 1 hour. Discard down sink with cold water.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The risks have been assessed and can be sufficiently managed using the procedures outlined in this document.

Project Ref    3583/21.1
**Project Additional Information**

**Purposes of the contained use**

Please see section 17. We do not wish to publicly disclose this information because of intellectual property rights.

**Recipient or parental organism**

The parental organism is the vaccine strains of vaccinia virus, Western Reserve, IHD-J, Copenhagen, Lister and Modified Vaccinia Ankara. These strains have been widely used to eradicate smallpox and are considered safe in terms of their pathogenicity, infectivity, toxicity, virulence, allergenicity. Attenuated strains such as MVA, Copenhagen, Wyeth and Lister strains are considered ACDP hazard group 1 (SACGM Compendium Table 2.10.2) whereas wild type vaccinia virus strains are considered ACDP hazard group 2. Western Reserve and IHD-J are more virulent and will be considered ACDP hazard group 2 unless further attenuated through TK deletion. MVA MVA is widely used as recombinant vector for vaccination or gene therapy. MVA corresponds to an attenuated laboratory virus developed by Professor Anton Mayr. It is derived from the Chorioallantois Vaccine Ankara (CVA) strain of the vaccinia virus. The attenuated strain was renamed MVA after the 516th passage of CVA strain on primary chicken embryo fibroblasts (CEF). Genomic studies have revealed that, as a consequence of these longterm passages, the resulting virus lost approximately 15% of its genome compared to the parental CVA strain. As a result of these deletions and disruptions, MVA no longer encodes many of the known poxviral immune...
evasion and virulence factors, making the virus defective for replication in human cells and avirulent in test animals. MVA growth is restricted to a few cell lines in vitro and doesn’t replicate in vitro due to the deletions. (Verheust 2012 Vaccine 30, 2623-2632)

Lister Lister or Lister/Elstree strain was developed at the Lister Institute in the United Kingdom. From 1968 to 1971, the Lister strain became the most widely used vaccine throughout the world. Lister was deposited to ATCC ® by Dr. James H. Nakano at the Centers for Disease Control in 1978, after isolation from the skin of a sheep. The Lister (Elstree) strain was widely used during the World Health Organization (WHO) program on the eradication of smallpox, but this can induce rare but severe adverse effects.

Western Reserve This strain is derived from serial passage of the original smallpox vaccine strain New York City Board of Health (NYCBH) through 50 rounds of intracerebral infection in mice. Although WR was never used as a human vaccine, its parental strain (NYCBH) had the lowest rate of complications among vaccine strains. WR is pathogenic in mice replicating in nose, lungs, brain, ovaries, and skin. It can provide immunization in mice by scarification. WR has been adopted as the laboratory strain of VACV, it is the standard used in most poxvirus laboratories and the vast majority of mutants in laboratories and the field at large have been generated in this background.

IHD-J IHD-J: This strain is also derived from passage of the VACV NYCBH strain through 51 rounds of intracerebral infection in mice followed by four passages on egg chorioallantoic membranes. Like WR IHD-J was never used in humans as a vaccine but is pathogenic in mice replicating in nose, lungs, brain, ovaries, and skin and can provide immunization by scarification. This strain is commonly used in laboratories for comparative studies with WR.

Copenhagen This is the original smallpox vaccine used in Denmark. It led to intermediate adverse affects. Cop replicates poorly in mice, but provides immunization by scarification. This strain is commonly used in laboratories for comparative studies with WR.


Further modifications are detailed in section 17 as we do not wish to disclose these publicly for reasons of intellectual property rights.

**Host/vector system**

DNA plasmids derived from pUC (e.g. pUC18, pUC119, pBluescriptIIKS) with ampicillin resistance and neomycin resistance/kanamycin resistance will be used to shuttle gene sequences into the viral genome. Flanking regions will allow recombination of the gene with sites in the virus which have been deleted or replaced with marker genes.

Plasmids will be amplified in E. coli K-12 derivatives such as Novablue,
which are recognised as non-colonising and disabled, and may be considered to be equivalent ACDP hazard group 1. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture.

Mammalian cell lines permissive to viral infection will be used. As a precaution, cell culture will be performed in a BSL2 laboratory. These cell lines include the following: HeLa (Human: ECACC 93021013; ATCC CCL 2) - HeLa cell lines were derived from cervical cancer cells taken in 1951 from Henrietta Lacks. The cells are characterized to contain human papillomavirus type 18 (HPV-18) and BSL2 requirements are recommended.

Vero cells (Monkey: ECACC 88020401; ATCC CCL 81) - Established from the kidney of a normal adult African Green monkey. Susceptible to a wide range of viruses including polio, rubella, arboviruses and reoviruses. BSL1.

MRC-5 (Human: ATCC CCL-171) – normal lung fibroblast. BSL1

RK13 (Rabbit: ECACC 00021715, ATCC CCL-37) – Rabbit kidney cells. RK13 has been derived from kidney cells of a 5 week old rabbit. Cells contain Bovine Viral Diarrhea Virus (BVDV) therefore BSL2 requirements are recommended.

HEK293T - (Human ECACC 12022001) - Human embryonic kidney SV40 transformed, genetically modified. BSL1

Origin & function

Please see section 17 for further details. We do not wish to publicly disclose this information because of intellectual property rights.

Evaluation of foreseeable effects

These are detailed in section 17 as we do not wish to disclose these publicly for reasons of intellectual property rights.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All vaccinia stocks are stored in a locked -80C freezer within a biosafety level 2 facility for designated personnel only.

Routine infection work will occur in biological safety class II safety cabinets. Disposable polystyrene aspirating pipets will be utilized for aspirating virus containing media into proper disposal containers containing 1% Distel (final volume). All aspiration will be followed by disinfection of Disposable polystyrene aspirating pipettes through aspiration of 5% Virkon which results in a degree of killing of 100% (Butcher and Uleato 2005, Contact inactivation of orthopoxviruses by house hold disinfectants). All disposable polystyrene aspirating pipettes will then be autoclaved.
All hard plasticware waste will be placed in an autoclave bag inside a rigid plastic bin which will be collected and placed directly in the autoclave (121°C, 15 PSI, 35 minutes). Following autoclaving they are disposed of via the clinical waste route of incineration. Contaminated tips are placed in standard plastic screw-top containers, which are autoclaved when full and then incinerated. Pipettes and pastettes will be flushed with 5% Virkon which has 100% kill on contact.

Liquid cultures are sterilised by addition of 1% v/v Distel followed by autoclaving. Vaccinia virus can be 100% inactivated upon autoclaving occurs at 15 PSI for 15 min therefore standard conditions (121C, 15 PSI, 35 minutes) can be used (Espy M.J. et al. 2002: Detection of vaccinia virus by LightCycler by PCR after autoclaving: implications for biosafety of bioterrorism agents).

Autoclaves in the Centre are serviced twice yearly, inspected and validated annually. Should a load fail it is reautoclaved in a different machine and the cause of the failure investigated and rectified. In case of spillage, the area of the laboratory in which the spill has occurred will be blocked off. Virkon powder can be used to soak up the spilled liquid and absorbent disposable material (paper roll) containing in 5% Virkon, which has 100% killing activity upon contact, will be applied to the spill to soak up any remaining visible spillage. The area of the spill and its reasonable surrounding area will then be thoroughly wiped a second time with absorbent material containing in 5% Virkon. The area will be wiped dry and all absorbent material used for clean-up will then be disposed of in autoclave bags for inactivation.

In case of personal injury, the wound will be treated by decontamination of wounded area with 70% EtOH, followed by bandaging of area and transport of the injured person to the Queen Elizabeth Emergency Department. Cidofovir and ST246 are USDA approved prophylactic agents available for post-exposure treatment of VACV infection or vaccination.

The risks have been assessed and can be sufficiently managed using the procedures outlined in this document. The main risk from the activity would be through piercing of the skin and subsequent viral infection. This is minimised by the lack of any procedure using sharps. Biological safety level 2 activity.

**Project Containment**

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**Name**

FAETH THERAPEUTICS

**Name 2**

**Department**

**Campus Estate or Research Centre**

CRUK CAMBRIDGE INSTITUTE

**Road Name**

RONINSON WAY

**Building**

**District**

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 0RE

**Country**

ENGLAND

**Tel Number**

01223769500

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Advice has been received from the Health and Safety Operations Manager in the Institute. A genetic modification risk assessment has been written (Ref: FAETH_GM_01v1), along with a procedural risk assessment (Ref: FAETH_01v1_CL2) for this work. These risk assessments have been reviewed and approved by the CRUK Cambridge Institute's Biological Safety Committee. The Committee is composed of:
1. Chairperson - Senior Scientist
2. Scientific Advisors
3. Biological Safety Officer
4. Occupational Health Physician
5. Members of: Management Team, Scientific Technical Staff and Staff Representatives.

The committee will meet as required but at least annually to review current projects.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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All liquid waste shall be mixed with no less than an equal volume of a 2% solution of the peroxygen chemical inactivation agent DuPont RelyOn Virkon, for at least 60 minutes, prior to disposal to drain via a sink designated for this purpose (as per the manufacturer’s recommendation) within a GM lab. This procedure will inactivate all cells and other biological agents present (100% kill). Any spills shall be treated with an equal volume of 2% Virkon for at least 10 minutes. Work surfaces shall be routinely disinfected with 1% Virkon for at least 10 minutes.

Bags will be placed in a GM-labelled leak-proof rigid container, then autoclaved on site to inactivate all biological agents (100% kill). This is then disposed of by incineration as hazardous ‘clinical’ waste by a licensed contractor (SRCL) at a licensed site.

The efficacy of the autoclaving procedure will be monitored as follows:
1. Monitoring of the autoclave parameters (temperature, time etc.) displayed by the autoclave, to ensure successful completion of the autoclave cycle on each occasion.
2. Autoclave print outs are produced on each occasion, documenting the details of the autoclave run, and maintained on site by the Laboratory Management Facility.
3. Autoclave indicator strips are added to the run in 2 places (for example top/bottom or front/back) to provide a visual confirmation the run had sterilised, on each occasion.

The autoclaves are serviced quarterly and thermometric tested every 6 months.
The genetic modification risk assessment (Reference: FAETH_GM_01v1) and procedural risk assessment (Reference: FAETH_01_v1_CL2) were assessed by the CRUK Cambridge Institute Biological Safety Committee. The Committee suggested to include the reference number of the genetic modification risk assessment (FAETH_GM_01v1) in the procedural risk assessment. The Committee subsequently approved the risk assessments.

Project Ref 3584/21.1

Date Ackn'd 20/01/2021  CU2 Project Title Modulation of energy metabolism in cancer cells to uncover therapeutic vulnerabilities

Class 2  CultureVolClass2 < 1 Litre  CultureVolumeClass3-4

Non-GMM Consent Granted

Withdrawn N  Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

This project aims to uncover factors that govern the energy metabolism of cancer cells. The long term aims of the project are to define novel therapeutic targets which will inform the development new anticancer medicines and strategies. The project will make use of a number of standard cancer cell lines together with gene modulation by CRISPR/Cas9 approaches. These modulations will be achieved by using lentiviral vectors and subsequently characterised in vitro.

Recipient or parental organism

Commonly used immortalised cancer cell lines (for example HeLa, Jurkat, etc).

Host/vector system

Commercially available recombinant 2nd or 3rd generation lentiviral vectors (e.g. pC-Pack2), pseudotyped with VSV-G envelopes, will be used. All vectors are non-mobilisable and the transfer vector contains a self-inactivating (SIN) unit. Furthermore, the lentiviral vectors used in this project are non-functional outside of the producer cells (e.g. 293T cells). This ‘split genome’ conditional packaging acts as an inherent safeguard against generation of productive recombinants. An example of a second generation vector system is described in Naldini L et al (1996) Science 272, 263-267.

Origin & function

The genetic material will be sets of CRISPR guide RNAs constructs computationally designed to disrupt target genes. cDNA encoding Cas9 will need to be introduced into the cell lines, to enable target gene disruption. Cas9 originates from bacterial species such as S. pyogenes, and has no known virulence and is not toxic. cDNAs encoding selection markers such as GFP or puromycin resistance may need to be used. Furthermore, vectors encoding different selection markers such as RFP, BFP, mCherry or blasticidin may have to be used. None of these components are known to induce oncogenic effects in cells.

Evaluation of foreseeable effects

The CRISPR constructs are designed to disrupt target genes. The foreseeable effect of this will be the removal of the corresponding protein from the cells transduced with the virus. In most cases, it is anticipated that the disruption of a component of a pathway will impair the ability of that pathway to function. In other cases, it is likely that disruption of certain genes may have no overall effect on a pathway. In rare cases, it is possible that disruption of certain genes may lead to activation of a pathway, for example, if a negative regulator of a pathway is disrupted. However, these effects are likely to be deleterious to the cell's ability to grow and it is not anticipated that cells transduced with the viruses will achieve a selective advantage relative to their parent cells. Indeed, the genes of interest in this work, are largely comprised of metabolic genes and enzymes. It is therefore very much predicted that disruption of such genes will impair pathways and lead to a selective disadvantage to cell growth and survival. An example of this phenomenon is outlined by Arroyo ey al (2016), Cell Metabolism, 24, 875-885. Consequently, the potential risks to human health and the environment are not increased as a result of these modifications, and are likely to be decreased. Furthermore, the viral vectors described in this assessment are self-inactivating, replication incompetent and non-mobilisable. They require two or three different vectors for packaging (that cannot themselves be packaged), and in the case of third generation vectors require upstream elements and in trans complementation. They cannot survive outside of closed controlled cell culture conditions, are rapidly inactivated by dehydration or other environmental insults. Spread to the wider human
and/or animal populations is thus very unlikely, and the risks to the environment and human health are extremely low. The GM cells themselves are similarly rapidly inactivated by any environmental insult, and are unable to survive outside of a closely controlled cell culture setting. A fuller description can be found in the attached Genetic Modification risk assessment.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste shall be mixed with no less than an equal volume of a 2% solution of the peroxygen chemical inactivation agent DuPont RelyOn Virkon, for at least 60 minutes, prior to disposal to drain via a sink designated for this purpose (as per the manufacturer's recommendation) within a GM lab. This procedure will inactivate all cells and other biological agents present (100% kill). Any spills shall be treated with an equal volume of 2% Virkon for at least 10 minutes. Work surfaces shall be routinely disinfected with 1% Virkon for at least 10 minutes.

All solid waste shall be collected in double autoclave bags, which are sealed by autoclave tape. These bags will be placed in a GM-labelled leak-proof rigid container, then autoclaved on site to inactivate all biological agents (100% kill). This is then disposed of by incineration as hazardous 'clinical' waste by a licensed contractor (SRCL) at a licensed site.

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The autoclaves are serviced quarterly and thermometric tested every 6 months.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
All liquid waste shall be mixed with no less than an equal volume of a 2% solution of the peroxygen chemical inactivation agent DuPont RelyOn Virkon, for at least 60 minutes, prior to disposal to drain via a sink designated for this purpose (as per the manufacturer's recommendation) within a GM lab. This procedure will inactivate all cells and other biological agents present (100% kill). Any spills shall be treated with an equal volume of 2% Virkon for at least 10 minutes. Work surfaces shall be routinely disinfected with 1% Virkon for at least 10 minutes.

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**Project Containment**

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| Comments |               |

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

Pneumagen Ltd operates on a site within the University of St Andrews Biomedical Sciences Research Centre (BSRC) and complies with the health and safety procedures of the University. All GM work in the BSRC is assessed by the building Health and Safety Committee. This committee is comprised of the building Safety Coordinator and the Director of the BSRC facility. After initial approval, GM projects are submitted to the Chemical and Biological Hazards Sub-committee (which acts as the Genetic Modification Safety Committee for the University) for ratification, before work commences.

<table>
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- Bacteriology: Yes
- Parasitology: |
- Transgenic Birds: |
- Microbiology Research: Yes
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<tr>
<th>Virology</th>
<th>Transgenic Animals</th>
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<th>Gene Therapy</th>
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E. coli waste medium will be autoclaved at 121°C for 30 min. If this is not possible, the waste will be treated with 1% (w/v) Virkon for a minimum of 1 h prior, to disposal via the drain with excess water. Glassware/plasticware will be soaked in 1% (w/v) Virkon and washed out with to the drain with excess water.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment is very straightforward. The project involves expressing carbohydrate binding domains from a number of bacterial proteins in E. coli using standard cloning and expression systems that are well documented and have a long history of use. The protein subdomains being expressed are isolated modules with no enzymatic activity. As such, and taking into account the decontamination and handling procedures that will be used, I am content that this is a level 1 activity as indicated.
## GM Centre Number: 3587

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Chief Scientific Officer - 32 years experience of managing commercial laboratories conducting GM work including the use of bacterial, mammalian, retroviral, lentiviral and baculoviral vectors for protein expression.
- Chief Operating Officer - 11 years experience of managing business operations
- Director, Biology - 9 years experience of academic and 3 commercial laboratory work/management including the use of mammalian primary and cell lines
- Director, Drug Discovery - 20 years experience of generating GMO’s in commercial laboratories including the use of bacterial, mammalian, retroviral, lentiviral and baculoviral vectors for protein expression.
- Director, Oncology - 21 years experience of academic laboratory work/management including the use of baculoviral, adenoviral and lentiviral vectors for protein expression and functional genomics.
- Laboratory Manager - 6 years experience of health and safety and risk assessments in academic laboratories
- Director of Safety Services - >39 years’ experience in healthcare, >29 years’ experience in health and safety / biosafety, 18 years working in microbiology laboratories (diagnostic microbiology and microbiological research at CL2 and CL3)

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PPE (lab coat, gloves and goggles) will be used at all times as according to Engitix Laboratory Standard Operating Procedures. The standard procedures for mammalian cell culture will be appropriate for handling the GMM (all cell culture is carried out inside a Biological Safety Cabinet). Sharps and glassware will not be used within this project. Solid waste materials (e.g. gloves, plastic ware, cell pellets etc) that have come in contact with GMOs are segregated from other tissue culture waste and are autoclaved (100% kill) using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes) in a validated machine located in room 4.04 (Utility room). Excess liquids (culture and medium) are discharged to drains following disinfection by the addition of Virkon to give a 3% final working concentration. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation). Alternatively, a 10% bleach solution in place of Virkon may be used. Solid waste is disposed via the clinical waste route in yellow bags and goes for incineration. The autoclave is performance validated by 12-point thermocouple tests on an annual basis. Records are kept on site.

Virkon is routinely used as per the manufacturer’s recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to placing in yellow bags for incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Biosafety Committee discussed the project and agreed that the control measures were appropriate, and that it could be approved as Class 1, Containment Level 1. Points highlighted were:

a) waste management and the clinical waste route - this was confirmed with the waste contractor.
b) the numbering system for GM Risk Assessments – change to make a clear separation between GM and non-GM work.

Other(s)
To permit the generation of DNA vectors and use them in mammalian cell lines
**Project Additional Information**

**Purposes of the contained use**
To permit the generation of lentiviral vectors and use them in mammalian cell lines for the expression of:
1) Open reading frame (ORFs), including but not limited to oncogenes, tumor suppressor genes, Cas9 proteins, cDNA, peptides and other gene sequences.
2) synthetic RNA sequences for gene expression regulation, including but limited to microRNA, short hairpin RNA, and short interfering RNA.
3) synthetic RNA for gene editing, including but not limited to synthetic guide RNAs for CRISPR/Cas9 technologies, template for gene editing.

**Recipient or parental organism**
The recipient organism will be of 2 types which may be classified as ACDP hazard group 2 or below:
1) cell line used for virus production, such as HEK293T and into which the different plasmids for virus production (encoding viral vector proteins and genome) will be transiently co-transfected for the transient production of viral particles that will be released into the cell growth media. These viral particle suspensions could provide a risk for infection for the personnel handling them.
2) recipient cell line in culture that will be infected with the viral particles produced as described above. This includes (but is not limited to): immortalised cell lines, cancer cell lines, and other previously established cell lines and long term culture of human and animal origin, classed as ACDP hazard group 1. For the first few days after contact with virus, the infected cultures could provide a potential risk for infections for the personnel handling them.
Lentivirus parental organism – Human Immunodeficiency virus (HIV-1) is classified as ACDP hazard group 3.

**Host/vector system**
The lentiviral vectors used are multi-attenuated meaning they are devoid of all potentially pathogenic HIV-1 encoded functions. In addition, they are replication-defective which means that the vector cannot multiply on its own unless supplied by certain proteins in trans. These are provided on at least two separate plasmids to limit the risk of unintended viral replication. They are self-inactivating which means that the viral promoter in the U3 region of the 5’ LTR has been disabled by genetic manipulation. Only a very unlikely event of multiple recombinations during the process of generation of the viral vector can expose the person to a wild type HIV-1. The probability of such an event is extremely low.

Origin & function

The mammalian cell lines used herein are from commercial suppliers (ATCC, DSMZ, RIKEN, CLS). The genetic material is obtained from commercial suppliers eg. tetracycline-inducible lentiviral expression vector pTMONB-TRE-MCS-12-RFP-EFS-rtTA-2A-Blast, lentiviral packaging plasmids pS Pax2 and pMD2.G, sgRNA expression vectors such as pSF-Lenti-U6-guide-Puro. Overexpression studies may utilise similar lentiviral expression systems from other suppliers. cDNAs/ORFs for genes of interest will be purchased from commercial suppliers.

The lentiviral vectors are derived from HIV-1 virus. All the viral vectors that we will generate and use are devoid of the proteins required for virus replication and pathogenicity, and as so, they are replication-incompetent vectors that after integration into the host cell genome cannot propagate nor replicate.

Additional safety features of the lentiviral vectors that we will use are:
- viral vectors are devoid of pathogenic accessory genes (vif, vpr, vpu and nef) required for viral replication and the genes from the parental virus strain contained in the viral vector is reduced to just the structural proteins: gag, pol, env.
- genes encoding the structural and other components required for packaging the viral genome are separated onto 2 or more plasmids. All plasmids have been engineered to limit the regions of homology with each other so that undesirable recombination events that could lead to the generation of a replication-competent virus are minimised.
- none of the packaging plasmids that allow expression in trans of proteins required to produce viral progeny, nor the constructs stably expressing the viral proteins in packaging cell lines, contain LTRs or the 5’ packaging sequence. This means that none of the virus structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
- whenever possible, we will use viral vector constructs that are "self-inactivating": those vectors contain a deletion in the 3’ LTR that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation"of the viral vector after transduction of the target cell. Once integrated into the transduced target cell, the viral genome is unable to transcribe a packageable viral genome, further reducing the possibility of production of any infectious viral progeny. Despite the above safety features, use of these viral vectors (which include WPRE sequence) falls within Class 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, the possibility of insertional mutagenesis, and the expression of potentially oncogenic genes. Also, the virus will be packaged with envelope proteins (including but not limited to VSV-G) that allow the transduction of a wide variety of animal species and cell types. This means that these viruses could potentially infect a number of species, including human beings. Despite this, the viral vectors will not be able to propagate nor produce in any way an infective progeny, due to the safety measures applied as described above.

Evaluation of foreseeable effects

The viral vectors that we will produce will be pseudo typed with VSV-G protein, which confers a broad host tropism including human cells. However, all the viral particles will be replication-incompetent and, when possible, self-inactivating, therefore there is no possibility of viral replication nor propagation further. Since some of the inserted DNA could code for potentially hazardous RNA or protein, the work is assessed as Class 2. Even for the non-oncogenic inserted DNA, there is a slight but non negligible risk due to the presence in some of the viral vectors of the Woodchuck Post-transcriptional Regulatory Element. This sequence has been linked to liver tumours in mice in a pre-clinical study using lentiviral vectors. Under the Genetically Modified Organisms Regulations 2014, work using vectors containing this sequence should be classified as Class 2.

However, the viruses that will be used have been modified in a number of ways which will make them safer to handle:
- The genes encoding structural and other components of the viral genome have been separated. These genes and the plasmids encoding them have been engineered to minimise the risk of recombination that could lead to production of a replication-competent virus.
- The packaging cell lines allow expression of proteins, required to produce progeny virus, but the transfer vector (not encoding the structural viral proteins) is the only
genetic material transferred to the target cells, consequently these recipient cells cannot produce the proteins which are essential for viral assembly and infectivity.

- The viral vector production in helper/packaging cell line will be transient, i.e. the component for the assembly of infective viral particles will be co-expressed exclusively for the few days of persistance of the transiently transfected plasmid encoding the viral genome.

Procedures and controls measures will therefore follow HSE SACGM Compendium of Guidance for retroviruses (Part 2, para. 30-36) i.e. using multiple plasmids with minimum sequence homology (e.g. 2nd or 3rd generation lentiviral vector systems), lab coats, eye protection and gloves will be worn, use of class II safety cabinets, sharps avoided and all wastes will be rendered harmless before disposal etc.

It is not thought that the modified virus would pose a serious risk to animals or plants in the environment. Although the VSV coat protein permits transduction of other mammalian cells, as in the case of humans, infection would be restricted to primary cells and productive virus would not be produced. In addition the control measures to protect human health will minimise release of virus to the environment. Therefore the environmental risk is low.

The viral vectors will be used to generate a cell population stably expressing the gene or RNA sequence(s) of interest.

For the first week after infection, or until at least both 3 replications and 3 media changes have occurred, the cell population infected with the viral particle will be treated as potentially infective. After this period, these cell cultures and the derivative products will be considered as Class I since the viral particles will be cleared from the culture.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Solid waste materials that have come in contact with GMOs (e.g. gloves, plastic ware, cell pellets etc) are segregated from other tissue culture waste and autoclaved (100% kill) in a validated machine located within the same building using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125oC for at least 15 minutes or 126-130oC for at least 10 minutes or 134-138oC for at least 3 minutes). Subsequently the solid waste enters the yellow bag clinical waste route and goes for incineration. The autoclave performance is validated by 12-point thermocouple tests on an annual basis. Records are kept on site.

Virkon is routinely used as per the manufacturer’s recommendations: Solid surfaces are disinfected with 1% Virkon solution. Plastic ware that can be effectively disinfected is treated with 1% Virkon for a minimum of 1 hour prior to incineration. Contaminated glassware is treated with 1% Virkon solution for a minimum of 1 hour prior to cleaning. Liquid waste (culture and medium) is disinfected by the addition of Virkon to give a 3% final working concentration. 2% or 3% solutions of Virkon are used for buffered solutions. A minimal contact time of 1 hour is allowed prior to disposal to sewers (as per manufacturer’s recommendation). Alternatively 10% bleach solution in place of Virkon may be used.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
The GM Committee agreed that the control measures were appropriate and that the work can be approved as Class 2, Containment Level 2. Points highlighted by the committee:

a) Plasmid maps to aid understanding

## Project Containment

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**Name**

EXACT SCIENCES INNOVATION

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

THE SHERARD BUILDING

**District**

LITTLEMOORE

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX4 4DQ

**Country**

ENGLAND

**Tel Number**

01865618817

**Fax Number**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

We are represented on and receive guidance from the BioEscalator GMO Safety Committee, this committee sits within the BioEscalator facility of the University of Oxford and is attended by the Biosafety Officer. We have also engaged an external safety consultant with expertise in GM safety.

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

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<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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Waste media and contaminated lab ware (plastics & consumables) will be decontaminated / sterilised using either the addition of Virkon (as manufacturer's instructions) or via autoclaving. The kill will be 100%. Viability testing to ensure efficacy of the killing / sterilisation process will be carried out. Autoclave validation, testing the ability of the autoclave to decontaminate a typical load, will be performed at least annually by a competent engineer using calibrated equipment in accordance with the relevant British Standard. Autoclave operation will be monitored while in use via chart recordings to ensure correct performance (pressure / temperature / cycle time) as measured via integral thermocouple and they will be operated such that they reach and maintain a temperature of 121°C for at least 30 minutes by using saturated steam under at least 15 psi of pressure. Following autoclaving, solid materials will be packaged in yellow laboratory waste bags and enter the waste stream for incineration. Sterilised liquids will be disposed via the drain. In the event of both main and auxiliary autoclaves failing simultaneously any waste which is normally autoclaved will be packaged in accordance with the approved requirements and incinerated.

All sharps will be discarded into appropriate sharps boxes meeting the requirements of BS 7320 and the boxes will be incinerated.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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| **Comments** | |
| **Date at Which Additional Info Submitted** | 02/03/2022 |

Page 15026 of 15326
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

**Y**

Give brief details of the genetic modification safety committee

We have received approval for our risk assessments from the Sitryx GMO safety committee. Committee comprises of head of biology, Biology group lead, 2 senior scientist, operations director, and operations manager. Combined the is over 50 years’ experience working in research in CU1 and CU2 laboratories.

- **Laboratory**
- **Animal Unit**
- **Growth Room**
- **Glass House**
- **Large Scale**

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- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
- **Virology**
- **Transgenic Animals**
- **Transgenic Fish**
- **Gene Therapy**

02/03/2022
For routine culturing of genetically modified cells either purchased commercially or produced in-house using non-viral vectors, all contaminated solid waste will be double-bagged and then sealed in leak-proof solid, rigid 60L bins; hard plastic waste that may puncture bags such as pipette tips and stripettes are first sealed in a cardboard Bio-Bin® or dispo-jar before being placed in bags/plastic bins. Plastic bins will be collected from site on a regular basis by a Certified Waste Management company, Grundon Waste Management Ltd (GM Centre 782), and disposed of under EWC classification 18 01 03*.

All solid waste will be collected live (without prior inactivation) and transported directly for high temperature incineration, providing 100% kill as monitored by Grundon (Certificates of Destruction are available on request). Alternatively, solid waste may first be autoclaved using make safe cycles (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes) before being double bagged and stored in a lockable container within the lab until collection by a Certified Waste Management company, such as Grundon Waste Management Ltd. (GM Centre 782), and disposed of under EWC classification 18 01 03 (Human) or 18 02 02 (murine). All solid waste will be collected live (without prior inactivation) and transported directly for high temperature incineration, providing 100% kill as monitored by Grundon (Certificates of Destruction are available on request).

Mammalian cells and liquid waste will first be deactivated by treatment with 2% Virkon for 60 minutes prior to either disposal to wastewater via lab sinks with copious amounts of water in accordance with trade effluent license limits, or solidified using LabSorb or similar absorbent material before disposal as solid waste by a certified Waste Management company (Grundons see details above). Alternatively, liquid waste may first be autoclaved using make safe cycles (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes) before being discharged to waste and monitored.

Contaminated laboratory glass or plasticware for re-use will be soaked in 2% Virkon for 1 hour then rinsed in water before passing through dishwasher. Virkon, when used according to manufacturers’ instructions under standard conditions, has been validated to deliver 99.998% kill. Disposal of Virkon via laboratory sinks is recorded and full training, supervision and regular safety checks ensure compliance with the Sitryx Waste Management SOP.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment Y

Please enter comments of the GM safety committee on the risk assessment

The Sitryx Therapeutics Biological Safety Committee was consulted, and risk assessments were reviewed. The Committee had no additional comments on any of the final risk assessments submitted.
Developing therapeutics targeting immunometabolism, autoimmunity, cancer and metabolic disorders using mammalian cell systems expressing reporter genes or overexpression/silencing of normal mammalian genes

Sitryx is a pharmaceutical company focused on developing novel therapeutics targeting immunometabolism across therapeutic areas such as autoimmunity and cancer. GMMs will be used to ascertain the effects of known and novel compounds on the immunometabolism and phenotypic profile of the cells of interest.

Recipient or parental organism

A list of cells to be used can be found in Appendix I of the attached "Contained use of GMMs (stably transfected cell lines, and transiently transfected primary tissues arising from transgenic mice and humans)" risk assessment. Commercially available and pre-characterized cells from collaborators will be used to ascertain the effects of known and novel compounds on the immunometabolism and profile of the cells for use in therapeutic areas such as cancer and autoimmunity. Typical examples of cells to be used include human and mouse cancer cell lines such as THP1 (myeloid leukaemia), Jurkats (lymphoid leukaemia), A549 (adenocarcinoma) and HEK 293 (embryonic kidney). Some cells from collaborators may also have been altered to overexpress specific mammalian genes of interest or reporter genes such as LacZ, GFP or luciferase; these may be maintained under the control of antibiotic genes. Primary murine cells arising from blood or tissues such as bone marrow and spleens, from wildtype or genetically modified mice to overexpress or knock out mammalian genes typically related to immunometabolism, may be cultured ex-vivo. Some cells may also express reporter genes such as LacZ, fluorescent constructs or luciferase. These cells will be obtained from external sources and no in-house work involving animals will take place within the Sitryx laboratories. Primary human blood cells and tumour samples and tissues may be cultured ex-vivo and may have been altered to overexpress certain mammalian genes or reporter genes such as LacZ, GFP or luciferase. These cells will be obtained from external sources. All cells to be used have strict in vitro growth requirements and are therefore incapable of growth outside normal laboratory conditions.

Host/vector system

GMMs purchased as commercially available stably transfected cell line/tissues or provided by collaborators may contain standard replication deficient/incompetent viral vectors, which are unlikely to be hazardous. Non-viral vectors Non-vector systems (e.g. CRISPR-Cas9 mediated targeted deletion of endogenous genes) or non-hazardous standard plasmid or phage vector systems will be used for in-house modification in Sitryx laboratories.
Viral vectors
To properly interrogate relevant biology and the effects of compound treatment, cells may have to be modified to differentially express certain mammalian genes; this may be done using retro/lentiviral vectors. Should this work occur, cells would be incubated with the replication incompetent virus overnight, at an MOI of 1-100 infectious virus particles per cell. Cells would then be washed to remove free uninternalized and unbound virus (media to be treated with Virkon), and cells would then be allowed to recover for a few days before being evaluated for transgene expression or markers determined by FACS or plate reader assays. Cells may be grown as single cell clones to allow for characterization of expression in subpopulations, by copy number and integration assays.
Replication incompetent viruses are generated by transfecting producer cells (commonly HEK293 cells or derivatives) with multiple plasmids which together provide the required genes for packaging the gene of interest (be it reporter or transgene) into infectious replication defective viral particles. The viral vectors are derived from numerous wild type viruses, such as MoMLV, HIV, SIV and EIAV. However, all non-essential genes are removed from the vectors with the remaining genes split onto separate plasmids with minimal homology thus reducing the risk of homologous recombination. For example, lentiviral vectors derived from HIV are devoid of tat, vif, vpu, vpr nef and the envelope gene has been replaced by VSV-G envelope. Gag, pol and rev are generally on one plasmid, env on a second and the gene of interest is on a third and fourth plasmids along with cis-acting signals required for reverse transcription and integration. As the gag, pol, rev and env plasmids do not contain the packaging sequence none of the genes are present in the package viral genome. The probability of these viral particles obtaining the missing genes is very low and therefore the risk of replication competent viruses being created is minimal.
In addition, some of the later generation viral vectors also include a self-inactivating feature, a deletion within the 3’ LTR, which is transferred to the 5’ LTR after reverse transcription and integration in infected cells. This results in the transcriptional inactivation of the LTR in the provirus and reduces the risk of transactivating genes around the site of insertion. Some concern has been raised with regards to the potential oncogenic activity of the post-transcriptional regulatory element from the woodchuck hepatitis virus (WPRE) which is frequently included in lentiviral systems. Following the guidance from the Health and Safety Executive these systems must be handled at Containment Level 2. Retro/lentiviral particles will be pseudotyped with common glycoproteins such as RD114 or Vesicular Stomatitis Virus glycoprotein G (VSV-G), which allow efficient gene transfer to a broad array of cell types and species. Producer cells are especially disabled hosts having strict nutritional and environmental requirements for survival. The modifications are not expected to increase their survival in the environment, nor increase their ability to evade the immune system. Therefore, the only risk is from the viral particles themselves. No genetic alterations will be made to the retroviral/lentiviral packaging plasmids or retro/lentiviral backbone transfer vector plasmids.

Origin & function
Our primary focus is on genes reported in the literature or implicated by collaborative research to be involved in human immunometabolism and related processes. Therefore, inserts typically code for normal mammalian genes, selective alterations of those genes (including targeted editing of endogenous genes e.g. CRISPR/Cas9 mediated gene targeting), or targeted inhibition of endogenous gene expression (e.g. miRNA and shRNA) or viral vectors. In addition to these, typical genetic inserts to facilitate include:
• Standard reporter genes such as lac Z/beta-galactosidase, secreted alkaline phosphatase, and chloramphenicol acetyltransferase, fluorescent proteins from marine organisms (e.g. CFP, EGFP, RFP and YFP), and Photinus (firefly) and renilla luciferase. These naturally occurring fluorescent or luminescent proteins either emit light when excited by high-intensity light of specific wavelengths, or generate light from catalysis. Each of these reporters is widely used and no cellular toxicity in normal cells or harmful properties have been reported. Therefore, no hazardous effects are anticipated.
• Mammalian selectable antibiotic markers such as Hygromycin resistance, Puromycin resistance, Zeomycin resistance, Neomycin resistance, Blasticidin resistance, and Geneticin resistance may be present in altered cells to maintain gene expression. The promoter of the mammalian antibiotic resistance or selectable marker is recognised by the mammalian RNA polymerase and allows transcription of the gene of the mammalian antibiotic resistance or selectable marker in mammalian cells. These resistance markers are not functional in bacteria.
• Various promoters may be used to regulate gene expression in order to achieve different levels of expression. Typical examples of these promoters include CMV, SFFV, SV40 and EF1α promoters, as well as synthetic promoters. We will also include mammalian promoters associated with cell types of interest in specific states, such as the promoters for CD60, CD206, iNOS, IL-10 in differentiated macrophages. There are no risks associated with these promoters, as they do not themselves encode any proteins; they merely influence the level of transgene expression achieved.
• Overall, these genetic inserts are not expected to have harmful physiological or pharmacological properties or to affect the pathogenicity of cloning host or normal human defence mechanisms. Gene transfer is possible, but unlikely to be hazardous.

Evaluation of foreseeable effects
Risk assessments (attached) have determined that the resulting GMMs used and generated during this project carry no additional hazards compared with those already.
present in the environment and are of a relatively low risk to human health and the environment, warranting the use of containment level 2. All cells will be cultured and propagated in fully serviced Class II Microbiological Safety Cabinets and incubators accordance with good microbiological practice and good occupational safety and hygiene, and in accordance to SACGM Compendium of guidance part 3 p18-21 and any additional advice referred to in the relevant Material Safety Data Sheets (e.g. US Dept Labor Universal Precautions 29 CFR 1910.1030). Resulting GMMs have strict in vitro growth requirements and are therefore incapable of growth outside normal laboratory conditions. Any transfer of genetic material to other organisms would be of minimal hazard.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

We request a derogation for the Class 2 Containment requirement for use of an autoclave. Mammalian cells and liquid waste will first be deactivated by treatment with Virkon for 60 minutes prior to either disposal to waste water via lab sinks with copious amounts of water in accordance with trade effluent license limits. Alternatively it will be solidified using LabSorb or similar absorbent material before disposal as solid waste by a certified Waste Management company (Grundons see details below). All contaminated solid waste will be double-bagged and then sealed in leak-proof solid, rigid 60L bins; hard plastic waste that may puncture bags such as pipette tips and stripettes are first sealed in a cardboard Bio-Bin® or dispo-jar before being placed in bags/plastic bins. Plastic bins will be collected from site on a regular basis by a Certified Waste Management company, Grundon Waste Management Ltd (GM Centre 782), and disposed of under EWC classification 18 01 03 (Human) or 18 02 02 (murine). All solid waste will be collected live (without prior inactivation) and transported directly for high temperature incineration, providing 100% kill as monitored by Grundon (Certificates of Destruction are available on request).

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

For routine culturing of genetically modified cells either purchased commercially or produced in-house using non-viral vectors, all contaminated solid waste will be double-bagged and then sealed in leak-proof solid, rigid 60L bins; hard plastic waste that may puncture bags such as pipette tips and stripettes are first sealed in a cardboard Bio-Bin® or dispo-jar before being placed in bags/plastic bins. Plastic bins will be collected from site on a regular basis by a Certified Waste Management company, Grundon Waste Management Ltd (GM Centre 782), and disposed of under EWC classification 18 01 03* and 18 02 02. All solid waste will be collected live (without prior inactivation) and transported directly for high temperature incineration, providing 100% kill as monitored by Grundon (Certificates of Destruction are available on request).

Solid waste that has been used for viral work will be first be inactivated by treatment with Virkon (2% final concentration for 1 hour) prior to being disposed of as mentioned above. Alternatively, solid waste may first be autoclaved using make safe cycles (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes) before being double bagged and stored in a lockable container within the lab until collection by a Certified Waste Management company, such as Grundon Waste Management Ltd. Liquid waste will first be deactivated by treatment with Virkon for 60 minutes prior to either disposal to waste water via lab sinks with copious amounts of water in accordance with trade effluent license limits. Alternatively it will be solidified using LabSorb or similar absorbent material before disposal as solid waste by a certified Waste Management company (Grundons as previously described). Contaminated laboratory glass or plasticware for re-use will be soaked in 2% Virkon for 1 hour then rinsed in water before passing through dishwasher. Virkon, when used according to manufacturers’ instructions under standard conditions, has been validated to deliver 99.998% kill. Disposal of Virkon via laboratory sinks is recorded and full training, supervision and regular safety checks ensure compliance with the Sitryx Waste Management SOP.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
The Sitryx Therapeutics Biological Safety Committee was consulted, and risk assessments were reviewed. The Committee had no additional comments on the final risk assessments for "Contained use of GMMs (stably transfected cell lines, and transiently transfected primary tissues arising from transgenic mice and humans)" and "Contained use and generation of lentiviral vectors and virally transduced GMMs".

### Project Containment

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<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment Y
**GM Centre Number: 3590**

|------------------------------------|------------|-----------------------------|---|-----------------------|------|----------------------|-----------------------|--------------------------|---|---------|---|-----------|---|

**Name**

RESOLUTION THERAPEUTICS LTD

**Campus Estate or Research Centre**
8 BLOOMSBURY STREET

**Road Name**

**District**

LONDON

**Town**

**County**

GREATER LONDON

**Postcode**

WC1B 3SR

**Country**

ENGLAND

**Tel Number**

0131 6519545

**Fax Number**

0

**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- [ ] Yes

Give brief details of the genetic modification safety committee

The person that provided us with expert advice has been working in the GM field for 10+ years. He advised several academic research groups and small companies on matters related to risk assess (RA), use and contain genetically modified organisms.

He will keep working with us as a consultant to ensure we have appropriate RA, training and recall/revision system in place.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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<td>Non-microbial</td>
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Other (please specify)

 Tick if confidential   [ ]

- Bacteriology: Yes
- Parasitology
- Transgenic Birds
- Microbiology Research
Disinfection:
The liquid waste will be chemically disinfected by soaking in 1% w/v Virkon or 1,000ppm Presept (four 0.5g tablets into 1L of liquid) for 24h before discharging via drains.

Autoclaving:
All contaminated materials will be inactivated by autoclaving (100% kill) at 121C or 134C prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autocalves will be validated by annual (at least) thermocouple mapping and each run will be monitored using thermal indicators (e.g. Browne TST indicator test strip).

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
The risk assessment (RA) has been conducted in a thorough fashion, considering:
- effect of the genetic modification on the environment
- effect of the genetic modification on human health

Here below is a brief sum-up of the main considerations behind the risk assessment.

The risk for the environment is low because:
- the genetically modified mice will be bought in from a commercial partner and not produced in-house
- the bacteria used for plasmid propagation are derivatives of E.coli K-12 and B-strains, which have been demonstrated to be non-pathogenic, incapable of colonising mammalian hosts, and auxotrophic, so dependent on nutrients in the media for survival
- the genes inserted in the plasmid are under a human promoter, therefore unable to be expressed by bacteria
- sequences will be checked carefully to avoid the unintentional introduction of a cryptic bacterial promoter

The risks for human health are minor because:
- the genetically modified mice cannot pass any disease or genetic modification as a consequence of being genetically modified themselves and they will be humanely culled at the end of each experiment
- the transfected genes will be expressed in a transient and non-integrating manner, so it is highly unlikely that any transfected gene product will cause long term harm upon occasional contact
- no oncogene or tumour-suppressor gene will be targeted during the project
- the material will be appropriately inactivated and disposed of after the end of the experiments as described above, and it will therefore not be hazardous

More details on the transfected targets are provided in section 12.
### GM Centre Number: 3591

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**Name**

GRAYSHILL LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

MOLLINS ROAD

**District**

**Town**

CUMBERNAULD

**County**

LANARKSHIRE

**Postcode**

G68 9BA

**Country**

SCOTLAND

**Tel Number**

01236-823138

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- Yes [ ]

Give brief details of the genetic modification safety committee

- There is no current genetic modification safety committee.
- Individual providing advice with expertise in risk assessments relating to contained use has MSc distinction Safety and Risk Management 2007.
- Corporate member of professional body IOSH 2005 then CMIOSH 2007 to date i.e. current member.
- Experienced and qualified in HACCP with REHIS Intermediate Certificate in Food Hygiene.
- Extensive training European Community Food Hygiene Regulations H123 i.e. circa 2006 at Govt Agency.
- Previous expertise on GMO Contained Use Regs 2000 i.e. circa 2006 at Govt Agency.

#### Laboratory

- Level 1 (GMMs) [ ]
- Level 2 (GMMs) [ ]
- Level 3 (GMMs) [ ]
- Level 4 (GMMs) [ ]
- Non-microbial [ ]
- Other (please specify) [ ]

#### Animal Unit

- Tick if confidential [ ]

#### Growth Room

- Bacteriology
- Parasitology
- Transgenic
- Birds

#### Glass House

- Microbiology
- Research

#### Large Scale

- Yes [ ]

02/03/2022
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<th>Gene Therapy</th>
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<td>Transgenic Invertebrates</td>
<td>Transgenic Plants</td>
<td>Other (please specify below)</td>
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</table>

Other(s)  
To confirm: there shall be absolutely no work undertaken at the premises....

For activities involving GMMs, describe the waste management measures which will apply to the activity

Taking cognisance of note 6:
The proposed work shall, I understand from GMO (CU) Regs, involve non-notifiable work involving larger GMOs.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Comments on the person with expertise on the risk assessment process is that the responsible person at the organisation receives advice from an individual whom has knowledge in, among other things, risk assessments relating to confined use to the level, as required in these matters, regarding CU Level 1. This knowledge was acquired while employed with a Govt Agency working with animals. Among other abilities, training, knowledge and experiences possessed by this individual there are various supporting qualifications e.g. an MSc distinction in Safety & Risk Management together with being a current member of a professional safety body with designation CMIOSH.
| Data Premises Notified (Originally) | 04/02/2021 | Transferred from 1992 Regs? | N | Transitional Premises Class |  |
| Data Premises Closed |  |
| Transitional Premises | N | Non-GMMs | N | Withdrawn | N |

**Name**

| VETQUEST RESEARCH LTD |

**Name 2**

| Department |

**Campus Estate or Research Centre**

| Building |
| INTERNATIONAL HOUSE |

**Road Name**

| District |
| SILVERTOWN |

| Town |
| LONDON |

| County |
| GREATER LONDON |

| Postcode |
| E16 2DQ |

| Country |
| ENGLAND |

| Tel Number |
| 0845 468 0027 |

| Fax Number |
| 0 |

**E-mail**

| HSE Division |
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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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<td>E16 2DQ</td>
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

UK Research Institute and Local University GMSCs have been consulted regarding risk assessments. These GMSCs are long standing committees that regularly review risk assessments and have members that are experienced in GMMs and GMOs.

<table>
<thead>
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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

---

**Project Ref** 3592/21.1

**Date Ackn’d** 11/02/2021

**CU2 Project Title**
The evaluation of recombinant avian herpesvirus (HVT) expressing foreign genes

**Class** Class 2

**Culture Vol** < 1 Litre

**Consent** Non-GMM Consent Granted

**Date Project Ceased**

**Withdrawn** N

**Tick if notifying a connected programme of work** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**
### Project Additional Information

#### Purposes of the contained use

**Recipient or parental organism**

The parental organisms are viral vectors.

**Host/vector system**

The host vector system is a Marek's disease virus, such as Rispens or Herpes Virus, which has been modified to express one or more transgenes.

**Origin & function**

The parental viral vectors have been supplied by a UK research institute. The transgenes to be evaluated were synthesised by a commercial gene synthesis company using Gibson assembly and were then integrated into the viral vector by the UK research institute.

**Evaluation of foreseeable effects**

Avian herpesviruses have a tropism to avian species. It is highly unlikely that avian herpesviruses can be transferred into humans. Thus, it is not expected that the GMM described in this document could be hazardous to the human. Avian herpesviruses are strictly restricted to the avian species and expected to be either attenuated relative to wildtype, or at worst no more hazardous than wildtype, and thus pose no risk to the environment. Furthermore, cellassociated nature of HVT restricts its chances for transmission. The modified viruses, as is the case with wild type avian herpesviruses, are strictly avian pathogens, with infection and replication restricted to avian cells. Moreover, HVT is cell associated, making any exposure unlikely. These characteristics including their strict host restriction, coupled with the likely disabling mutations in gene knock out constructs make them extremely low risk to human health.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

HVT-contaminated materials (e.g. glassware, plastics, tips, flasks, media and cultures) will be inactivated (e.g. exposure to at least 1% virkon for a minimum of 30 minutes or autoclaving). Testing and monitoring methods will include quantitative PCR.

---

**Is an emergency plan required according to regulation 20?** 

**If yes, tick to confirm that it is attached to this form**
Risk assessment was accepted and no comments were received.

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Risk assessment was accepted and no comments were received.

### Project Containment

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| Fax Number                           | 0                                  |

| E-mail                               | blank                             |
| HSE Division                         | blank                             |

| Comments                             |                                    |

| Date at Which Additional Info Submitted | 02/03/2022 |
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

We are a 7-person team and the Chief Scientific Officer is responsible for compliance with GMO processes as outlined by the HSE. They have complete oversight of all laboratory work performed by the laboratory team.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

02/03/2022 Page 15046 of 15326
We only work with non-pathogenic microbial GMOs (E. coli and mammalian cells) to express and purify recombinant monoclonal antibodies. All microbial GMOs are disposed of using high-level disinfectants such as Virkon.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

We only work with non-pathogenic microbial GMOs (E. coli and mammalian cells) to express and purify recombinant monoclonal antibodies. All microbial GMOs are disposed of using high-level disinfectants such as Virkon.

**Tick to confirm that you are attaching a summary of the risk assessment**

Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**


Please enter comments of the GM safety committee on the risk assessment

In our laboratory we handle non-pathogenic E. coli (strain K-12), which we use to make preparations of plasmids. We use these E. coli to produce plasmids which are subsequently used for the expression of non-pathogenic monoclonal antibodies. None of the components of these plasmids are derived from any infectious pathogen, and none of them contain any known oncogenes. The genetic modifications do not contribute to pathogenicity of the host E. coli, or to any other environmental micro-organisms.

Appropriate disinfection and disposal procedures have been put in to place to minimise the risk of any accidental release of GMO organism in to the environment.

Appropriate PPE and training is available to protect laboratory staff from the host species itself, which while non-pathogenic (BSL 1) should be handled with care.

**Project Ref**

3593/22.1

**Date Ackn'd**

02/02/2022

**CU2 Project Title**

Introduction and knock-down of cell surface proteins into mammalian cell lines or

**Class**

Class 1

**CultureVol**

1-50 Litres

02/03/2022

Page 15047 of 15326
The purpose of the project is to use lentiviral vector expression systems for the expression of cell surface proteins in order to determine the functional characteristics of purified monoclonal antibodies. The use of this vector system would also allow the use of a more extensive array of cells for screening, allowing the modification of cell lines without established transfection tools. It would also further allow the use of shRNA to allow for the modulation of existing cell surface proteins.

Recipient or parental organism

Lentiviruses are complex retroviruses that cause the transmissible immunodeficiency syndromes.

Host/vector system

Mammalian cells/Lentivirus vectors

Origin & function

Lentivirus: The origin of the lentiviral vector requested in this application is HIV-1. Modern Lentivirus systems which would be used are attenuated so as to reduce the risk of infection or oncogenesis should an employee be exposed. These mechanisms of attenuation include: Deletion of the enhancer region of the 3' U3 of LTR. This results in a transcriptionally inactive vector that cannot be converted into a full length RNA. The deletion also reduces the risk of tumorigenesis via promoter insertion. In addition to loss of the regulatory/accessory genes (tat, vpr, vpu, vif and nef), pLKO.1 is also deficient for the gag, pol, rev, and env genes of HIV-1, which must be provided on three non-homologous plasmids expressed in a packaging cell-line. These highly attenuated lentiviral vectors have shown sufficient biosafety for their insertion in ongoing human clinical trials as therapeutic vectors.

Evaluation of foreseeable effects

The generation of replication competent virus has to be viewed as a potential major safety issue. However, the probability of the lentiviral vectors reverting to wild-type is extremely low. Therefore, although these viral vectors can potentially infect a wide range of cell types there is a minimal risk of viral replication in infected cells. The only potential safety concern for retroviral-based vectors is insertional mutagenesis of essential genes. Retroviral integration can lead to oncogenesis but appears restricted to stem cells additionally inherent characteristics of LVs and safety modifications considerably reduce the risk of genotoxicity with no reports of genotoxicity-related adverse effects to date in any of the LV-based gene and cell therapies (Munis A., 2020). Although the cell surface proteins themselves do not cause a major risk due to their natural presence on mammalian cells, their modification may in the worst case scenario induce autoimmune disease.
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

- Virkon is a synergised oxidising system that works by physical destruction of pathogens by acting on proteins. It is resistant to inactivation by organic material. Shown to be effective against bacteria, viruses and fungi including all major human pathogens. Recommended to be used at 1% concentration for all activities.
- Chemgene HLD4L is a commercial validated disinfectant. It is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1%) for general purposes, 1:20 dilution (5%) for high-risk areas.
- Following all work solid waste (including tips, pipettes, and flasks) will be bagged into an autoclavable bag as the waste is generated. After work has been completed it will then be double bagged before being sealed and immediately then autoclaved prior to being bagged and sent into the clinical waste stream for incineration with a registered waste contractor.
- It will be routine practise that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Liquid waste will be inactivated with Virkon for 24 hours prior to disposal. In some instances, the liquid waste will be disposed to sewers via a designated laboratory sink or solidified with Labsorb (or equivalent product) and disposed of as solid waste as above.
- Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L before being incinerated.

The Biological Safety Committee (BSC) agrees with the assessment that this work can be conducted at BSL2. The BSC notes that any work with primary cell lines should be taken from population with a known medical history to minimise risk of the presence of harmful human pathogens. The BSC agrees that the use of the latest generation of lentiviral systems, which are self-inactivating and in effect non-mobilisable reduces the risk of potential infection to near zero, however they have stressed the need to follow the procedures set forth in the risk assessment to prevent an exposure as there is still a potential risk of oncogenesis which cannot be completely ablated. Lentiviral transduction into immortalised cell lines should not be able to mobilise any genetic elements because self-inactivating systems will be used that lack the necessary genes for viral replication and packaging. The BSC agrees the requirement for each new piece of work to have an individual risk assessment completed prior to commencement to ensure the safety of the work. Where new vectors or hosts are to be used this risk assessment must assess the potential sites for recombination and ensuring they are not present in the lentiviral vector. Once cells are transduced and virus removed the risk posed here is very low. Due to some cell lines already requiring biosafety level 2 containment and the use of integrating lentiviral vectors this dictates continued use at BSL2. BSL1 cell lines once transduced, and lentivirus removed at BSL2 could be used at BSL1, however it is the opinion of the BSC that they should be maintained at BSL2.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N
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**Name**

MESTAG THERAPEUTICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**SUITES 15/16, SCIENCE VILLAGE**

**Road Name**

**CHESTERFORD RESEARCH PARK**

**Town**

**SAFFRON WALDEN**

**County**

**CAMBRIDGESHIRE**

**Postcode**

**CB10 1XL**

**Country**

**ENGLAND**

**Tel Number**

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**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

GMSC includes the SVP Research and Early Development, Cellular and Molecular Biologists and Cell Culture Scientists, all with experience of working at activity class 1 and 2. Collectively many decades worth of experience in similar organisations undertaking preclinical research and development. Where additional support is required the staff needed will be added/adapted to allow for the most relevant expertise and input. External advice from H&S Consultant/Biosafety Practitioner Level1 (ISTR) is contracted and used where necessary. The GMSC meet to review risk assessments and discuss GM matters, these are minuted and also GM is a standing item on the H&S committee agenda.

<table>
<thead>
<tr>
<th>Level 1 (GMMs)</th>
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<th>Level 2 (GMMs)</th>
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At the conclusion of an experiment, cells and culture media will be inactivated by 1% Virkon
(final concentration) disinfectant for 30 minutes as per the manufacturer’s instructions. This
protocol has been shown to reduce organisms by 5 log. Solid waste will be decontaminated by
the submersion in 1% virkon final concentration for 30 mins, drained and collected in waste
bags for incineration with a licensed waste contractor as infectious waste.

Please enter comments of the GM safety committee on the risk assessment

The GMSC met to review this assessment 05/Feb/2021.
The GMSC agreed to the application of activity class 1. This was concluded after discussion of
the project, hazards and risk. The need for CL2 controls were discussed and it was concluded
they would be unnecessary to protect human health or the environment. This was based on
the low risk due to use of hosts that are pre-screened and inactivated, inactive 2nd generation
lentivirus, low oncogenic risk and use of electroporation techniques for modification at the
premises.
It was agreed that this risk assessment would form the supporting evidence so the HSE GM
Premises notification at Activity Class 1
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Molecular & cellular biologist with more than 20 years industry experience. Trained as a Biological Safety Officer for more than 5 years. Previously set up new laboratories for use with genetically modified organisms

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Liquid waste will be chemically inactivated for 24 h prior to disposal via designated sinks. Solid waste will be collected in 60 L clinical burn bins which will be hermetically sealed before being sent for incineration with a registered waste company.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The proposed work posses negligible risk to human health or the environment. The laboratories are built to a high specification and processes are in place to contain and inactive any material generated.

Project Ref 3595/21.1

Date Ackn’d
14/05/2021

Date Project Ceased

CU2 Project Title
Genetic Modification of Cell Lines Requiring Biosafety Level 2 Containment Including the use of Lentiviral Vectors

Class
Class 2

CultureVol
< 1 Litre

Class Culture Vol

Consent Granted

Non-GMM

Project notified under transitional arrangements

Withdrawn

Tick if notifying a connected programme of work

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022

Page 15056 of 15326
**Project Additional Information**

**Purposes of the contained use**

O2 Healthcare is seeking to develop novel therapeutics focused on human disease. To facilitate a full understanding of the biology of disease it is anticipated that, there will be the need to generate in vitro cellular models which may have:

- Stable overexpression of exogenous genetic sequence.
- Reduced expression of target genes by expressing short hairpin RNA (shRNA).
- Knockout or modification of endogenous genes using CRISPR/Cas9 or related methodologies to:
  - Introduce specific amino acid changes.
  - Add a tag such as but not limited to GFP, luciferase, FLAG & HA.
- Introduction of reporter gene systems to allow the measurement of functional effects on key cellular signalling pathways.
- The target mammalian cell lines may be categorised as hazard group 1 & 2.
- These modifications maybe introduced into cells using expression plasmids and standard non-viral transfection methodologies.
- In some circumstances the nature of the enquiry will be better addressed by transducing the most appropriate cell line using lentivirus. Where possible these will be obtained from 3rd party vendors in ready to use aliquots but there will be instances where the lentiviral material will need to be generated on site.

**Recipient or parental organism**

**Cell lines for packaging lentivirus**

Where lentivirus cannot be reasonable obtained from commercial sources it will need to be produced internally using a packaging cell line, such as HEK-293T capable of supporting the production of lentiviral particles. Packaging cell lines are only used for the production of lentiviral particles and are not used to develop cellular models.

Well characterised immortalised cell lines for in vitro cellular models

A number of immortalised cell lines are available that can be used as model systems for validating and assessment of target activity and used subsequently in developing molecules to modify that activity. Due to the nature of the work cell lines known to support the propagation and production of replication competent lentivirus particles (RCLs) will not be used in this area of the work. The Biosafety level 1 or 2 cell lines will be obtained from reliable sources (commercial and academic) with a clear history of safe use and a well understood mechanism of immortalisation to remove the risk of recombination events leading to production of RCLs.

**Primary human cells**

The main risk is the presence of adventitious pathogens.

Ideally, cells will have been screened for major human pathogens with the potential to harm health and shown to be negative.

In some instances, however, consent to screen for major human pathogens is not sought at the time of cell donation. Screening the cells for major human pathogens would have potentially significant clinical health implications for patients (e.g., HIV), for which explicit consent has not been given at the time of donation. As such, for cells where consent has not been given, they cannot be screened for human pathogens and must therefore be handled at Biological Safety Level 2. All donors, however, must have no previous known history of infectious disease and will be from epidemiologically low-risk populations.

Recipient cells will be from healthy individuals, or from patients with a disease caused by genomic mutations. As these mutations are naturally occurring in the genome of these individuals, they are not mobile genetic elements and
thus present a very low biosafety risk.

HiPSC derived from primary human cells, most commonly fibroblasts. The hiPSC that will be used may have been either generated using 3rd or 4th generation self-inactivating (SIN) nonmobilisable integrating retroviral systems based on, but not limited to, mouse Maloney murine leukaemia virus (MMLV), Maloney murine sarcoma virus (MMSV), myeloproliferative sarcoma virus (MPSV), or human HIV. Some of the original donor material will not have been screened for human pathogens but donors will have a known medical history and will be classified as being low risk for retroviral pathogens. As such the hiPSC are highly unlikely to be producing RCLs.

Reprogramming of the hiPSC is achieved using a variety of factors, that are integrated into the cell genome by the retroviral vectors, that induce pluripotency in the donor cells. A combination of the following factors may be present in the supplied hiPSCs:

- **SOX2**: is a transcription factor that is essential for maintaining the pluripotency of undifferentiated embryonic stem cells.
- **KLF4**: is a transcription factor that regulates proliferation, differentiation, apoptosis, and somatic cell reprogramming. KLF4 may also act as a tumour suppressor gene.
- **OCT4**: is a homeodomain transcription factor of the POU family. This protein is critically involved in the self-renewal of undifferentiated embryonic stem cells and is used as a marker for undifferentiated stem cells. It is indispensable for generating iPSC.
- **CMYC**: is a transcription factor that plays a role in cell cycle progression, apoptosis, and cellular transformation. Mutated c-Myc is found in many cancers, where it is constitutively expressed, leading to the unregulated expression of many genes, some of which are involved in cell proliferation leading to oncogenesis. This factor poses a risk if mobilised into RCL.
- **NANOG**: is a transcription factor critically involved with self-renewal of undifferentiated embryonic stem cells.
- **LIN28**: encodes a microRNA-binding protein; overexpression of which in mice, can cause gigantism and a delay in the onset of puberty. Human GWAS studies indicate the LIN28B gene to be associated with human height and the timing of puberty. The biosafety risk is low for adults.
- **GLIS1**: is a highly promiscuous transcription factor, positively or negatively regulating the expression of a number of genes.
- **shp53**: short hairpin RNA that suppresses expression of p53. P53 is a known tumour suppressor gene and binds to DNA where it regulates the expression of anti-mutagenesis genes. Mutations in the p53 gene leading to a loss of activity are the most commonly found single mutation in human cancer.
- **Mir302/367 cluster**: microRNAs are a subclass of small non-coding RNAs that fine-tune the regulation of gene expression at the post-transcriptional level, with a particular role in establishing cellular pluripotency. HiPSCs can also be generated using episomal approaches and if suitable will be used preferentially. The cells are reprogrammed using non-viral episomes, there is no integration of genetic material into the resulting hiPSC genome, and no viral sequences are introduced into the cells. Episomes are lost as the reprogrammed hiPSC proliferate, at an approximate rate of 5% per generation. Therefore, by approximately 12-13 proliferation cycles, the episomal transgenes have been entirely lost. This eventually results, at the time of use, in “footprint-free” cells that do not contain the original reprogramming sequences. In this respect the hiPSC are not genetically modified but do have a changed gene expression profile and a pluripotent phenotype.

**Host/vector system**

In the case of hiPSC generated using integrated retrovirus the factors and vectors used will be fully described prior to receipt and be reviewed by the Biological Safety Committee (BSC) at O2 Healthcare (O2H) which also has the responsibility for any work involving genetic modification. In this case subsequent lentiviral vectors will be nonhomologous.
with the integrated sequences to mitigate the small risk of a recombination event leading to mobilisation of genetic material, such as the myc oncogene or immunomodulatory genes, that might pose a risk to human health. The integrated murine based lentivirus poses no additional risk to the environment. It is also planned to transduce immortalised cell lines, primary cells and episomally generated hiPSCs with lentiviral vectors. The recipient cells will not be known to harbour viral material that could be mobilised by introduction of lentiviral vectors and where viral material is present will not support the production of RCLs. Secondary transduction would either transiently modulate the protein expression of a target protein at the translational level, modify the cells to explore the underlying disease process or to generate tools for drug discovery. To this end the following could be used based upon the needs of drug discovery programs:

- Integration of shRNA constructs to ‘knock down’ expression of a gene of interest
- The over-expression of particular genes
- Specific modification of the host genome using programmable nucleases:
  - Cas9/CRISPR
  - Reporter genes under the control of selected transcriptional regulatory sequences to study effects on cellular pathways.

Introduction of genetic material on expression plasmids using chemical or physical transfection methods has a negligible risk of introducing such material into humans or the wider environment. The main risk is associated with the host cell line and its altered form. Cell lines will be used in accordance with their biosafety level status. As lentivirus have a much greater ability to transduce cells this risk assessment will focus on their use. The lentivirus obtained from commercial sources will have been generated using third of fourth generation vector systems. The vector backbone and promoters used to drive vector generation in these systems will be consistent. Due to the nature of the work, genes may be selected that will include growth factors, tropic factors, quiescence factors, confirmed oncogenes, tumour suppressors and proteins known to be involved in neurological disorders. The vectors will be generated using a non-replication-competent HIV based lentiviral system that upon integration into the host genome loses part of its long terminal repeat (LTR) preventing excision at a later time point. Such self-inactivating (SIN) vectors are designed to produce stable gene expression in target mammalian cells. The viral particles can only be generated upon co-transfection of a packaging cell line (usually HEK-293T) with at least four separate plasmids. Three of which provide the minimal set of genes required for viral production (gag-pol, rev and env). The env gene used is from the vesicular stomatitis virus (VSV-G) to increase tropism. The final plasmid provides the genetic material to be supplied to the target cell and as such contains packaging sequences directing it to be incorporated into nascent viral particles. Only the minimum amount of lentiviral genome is used in the system and all of the plasmids lack homologous sequences to minimise any chance of recombination. The resultant lentiviral particles are VSV-G pseudotyped, SIN and replication incompetent in mammalian cells. Small aliquots of no more than 1x10e8 viral particles in 5 to 10 microlitres will, further reducing the risk.

Third Generation Systems use 4 standard bacterial plasmid vectors. Three plasmids encode for proteins required for production and packaging of full-length viral RNA (for example: pCgpV, pRSV-Rev and pCMV-VSVG). The gene of interest is contained in a fourth plasmid (e.g., pSMPUW). This is the only plasmid that contains the packaging sequence for incorporation into the virus particle.

Fourth Generation Systems use 6 standard bacterial plasmid vectors. The system uses tetracycline to control when viral particles are produced adding yet another level of control to production. For example, pTRE-gag-pro, LTRHIV2-vpr-pol, penv(VSV-G), pTet-Off and ptat-IRES-rev contain the sequences that code for proteins that are responsible for the tetracycline control and packaging of the viral genetic material. As above the last plasmid (e.g., pSMPUW) only contains the genetic material to be packaged into the vector and no other lentiviral gene sequence. An integrase
deficient version of the fourth-generation system is available which contains a mutation in the sequence encoding the viral integrase.

Lentiviral Vector Expression System

If lentiviral particles do need to be generated internally either a 3rd or 4th generation system will be used as above and purified using either commercially available kits, or well-established methods (detailed below). Lentiviruses belong to the Retroviridae virus family which are capable of infecting both human and animal species. As the lentiviral vector system that will be used in these studies is based on HIV-1, a theoretical risk to human health exists. However, retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airborne route. Therefore, accidental piercing of the skin or other surface tissues with virus containing objects represents the main potential route by which accidental infection could occur. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

In all of the modified pSMPUW plasmids (containing protein-coding sequences, miRNA, shRNA, or programmable nucleases or reporter gene sequences), the sequences inserted are flanked by non-coding retroviral LTRs, and no retroviral genes are encoded on the modified pSMPUW plasmids. Therefore, no retroviral genes will be transferred into generated viral particles. This construct is packaged into particles using a HEK293T cell based packaging system, which requires the co-transfection of these cells with three to five additional separate plasmids (as detailed above). The additional plasmids express the envelope protein from VSVg and the non-structural proteins of the virion, and, importantly, none of these genes will be transferred into the assembled viral vectors, since they lack the packaging signal (Psi), which is only present on the modified pSMPUW plasmids.

The lentiviral Expression System what will be used include the following key safety features:

- All of the pSMPUW contain a hybrid 3’LTR that does not affect generation of the viral genome in the producer cell line, but results in “self-inactivation” (SIN) of the vector after transduction of target cells. Once integrated into a transfected target cell, the lentiviral genome is no longer capable of producing viral genomic material that can be packaged. Moreover, presence of an SV40 polyA after the hybrid 3’LTR in the vector construct will result in a provirus which should reduce the potential for transactivation of cellular genes due to an insertion event. Furthermore, the development of self-inactivating vectors improves the biosafety of vectors, as they are less likely to be mobilised following a superinfection with wild-type virus (HIV).
- The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 or other retroviral envelope genes. Heterologous envelopes, like VSV-G, typically broaden the tropism and allow gene transfer into a broad variety of cells. The risk will mitigated by the use of self-inactivating virus and limiting the number of viral particles that will be handled at any one time.
- Sequences encoding the proteins required for packaging of the viral genome are separated onto three to five plasmids, and all of the 4-6 plasmids used in the system have been engineered not to contain any regions of homology with each other so as to prevent undesirable recombination events that could lead to the generation of a replication-competent lentivirus (RCL), which could potentially be harmful to humans. It is important to note that no such RCL has ever been observed despite large-scale production and testing of lentiviral vectors.
- All of the pSMPUW containing plasmids/vectors will be used are devoid of all viral sequences apart from essential cis-acting sequences, including the LTRs and the packaging signal Psi. Although the packaging plasmids used in these systems allows for the expression in trans of protein required to produce viral progeny (e.g. gal, pol, rev, env) in the HEK293T producer cell lines, none of them contain LTRs or the Psi packaging sequence. Several of the lentiviral accessory genes (vif, vpr, vpu and nef) that are dispensable for lentiviral vector production/transduction have been deleted from the packaged construct. Therefore, none of the retrovirus structural genes will actually be present in the packaged viral genome, and they will never be expressed in the transduced target cells, which means that no new
RCL can be produced.

- The lentiviral particles produced in this system are replication-incompetent, only carry the sequences of interest, and no other viral species are produced.
- Expression of the gag and pol genes from pgag-pol-RRE has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Addition of the RRE in these plasmids prevents gag and pol expression in the absence of rev, which is contained in the pRSV-REV plasmid only. The Rev/RRE system is highly conserved among lentiviruses, and removal of the RRE sequence and associated splice donor/acceptor sequences result in a loss of transduction efficiency.
- LTR has been modified so as to increase lentiviral vector production, and also to allow lentiviral vector production to be independent of tat expression. It is known that Tat-deleted mutants of wild-type HIV-1 are not replication competent. Therefore, the deletion of Tat should decrease the risk of generating a putative RCL.
- Lentiviral vectors have a very low potential to cause immunogenicity.
- The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction.

While the lentiviral vectors that are produced using this system contain only about 20% of the original genome of HIV-1, there is a very small risk that subsequent infection of cells already infected with the lentiviral genome of HIV-1 could lead to a rare recombination event in which the transgene is transferred to a replication-competent virus. Thus, the sequences in the vector that will be expressed could potentially be transferred to surrounding cells. This event is, however, extremely unlikely to occur for a number of reasons:

1- The lentiviral vector is replication-incompetent and self-inactivating. In the case of a subsequent HIV-contamination, wild-type HIV-1 cannot rescue a self-inactivating HIV-1 based lentivirus once integrated into the host genome, although non-self-inactivating vectors can be.
2- In order for the spread of the gene of interest to occur following accidental infection (assuming that this has lead to viral integration), a series of unlikely events have to occur:
   a- The worker would have to become infected with HIV-1 or to be already infected with the virus.
   b- The viral and lentiviral genomes would have to integrate into the host worker’s genome in the same cells and in a position where they could interact to effect homologous recombination (point 1 above)
   c- Recombination would have to occur in just the right regions to allow for transfer of the gene of interest from the lentivirus to the HIV-1 genome, which could also involve the transfer of the HIV-1 genes to the lentiviral genome. In that case, it is conceivable that a non-self-inactivating HIV could be generated that contained the gene of interest but not the rest of the genome it requires. The other gene products could be provided in trans from the lentiviral genome that may now contain the HIV-1 genes or from other HIV-1 integrants.
   d- The situation in c could conceivably lead to the production of an infectious particle containing the gene of interest, but again no genes necessary for subsequent replication. In order for another round of infection to occur, the process would have to begin again.

In these circumstances, the effects of lentiviral infection are likely to be minor in comparison to the effects of the HIV-1 infection, which would be required to affect the spread of the gene of interest. In addition, the scenario described is essentially equivalent to the rescue of the lentiviral genome from the host, which has already been shown not to occur.

3- It is extremely unlikely that any worker would infect themselves with a significant dose of lentivirus as the volumes that are used in transduction experiments are small (aliquots contain a maximum of 10 μl of vector solution).
4- Moreover, insertional mutagenesis into the host genome may be considered as an oncogenic risk. We cannot rule out the possibility that, when the lentiviral genome integrates into the host genome, it will not lead to the activation of
an endogeneous oncogene. However, all transcriptionally active long-terminal repeats (LTRs) have been removed as well as all promoter-like elements that required to drive expression of the transgene. This should prevent unforeseen activation of such genes. It is noted that deletion of retroviral enhancer in self-inactivating systems reduces the risk of activation but not of disruption, therefore, retroviral infection might still have permanent effects upon a cell (including oncogenic effects).

Importantly, we do not consider that the use of these lentiviral vectors will result in a significant increased risk of oncogenic activation compared to the risk possessed by any other viral delivery system. Moreover, the risk of transduction leading to tumourigenesis or other untoward harm following exposure is related in part to the titre of the viral vectors; exposure of workers to quantities of virus high enough to cause such effects would be unlikely during standard laboratory-based manipulations of lentiviral vectors.

For both commercial and internally generated lentivirus the fourth plasmid (e.g., pSMPUW) will contain the sequences that code for protein, miRNA, shRNA, or programmable nuclease sequence, or reporter gene sequence under the promoter of the gene of interest. These may include growth factors, tropic factors, quiescence factors, confirmed oncogenes or tumour suppressors.

Origin & function

• Gene overexpression
Some genes/proteins may be overexpressed in order to investigate their cellular function and role in disease mechanisms. The genes/proteins that will be overexpressed may include any growth factors, confirmed oncogenes, tumor suppressors, immunomodulators or proteins known to be involved in disease. The BSC will be informed of the full nature of, and justification for, any proposed gene/protein overexpression, and have final say on whether it is acceptable to proceed with the proposed modification.

• shRNA
With the use of shRNA-expressing viral vector systems, the expression of specific mammalian genes/proteins can be knocked-down, in order to study their functions. Potential deleterious effects such as off-target and immunomodulatory responses can be minimised through screening designed shRNA sequences against databases of known mammalian/mRNA sequences during the design stage. The genes/proteins of interest that will be targeted by shRNA may include growth factors, tropic factors, quiescence factors, confirmed oncogenes, or tumor suppressors. Some of the viral vectors that will be used contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to any known mammalian sequences. This sequence will adopt a hairpin structure as with any shRNA, but it should not target any mRNA of human, mouse or rat origin. Consequently, these sequences are unlikely to pose any safety risks for the environment or human health. The BSC will be informed of the full nature of and justification for, any proposed shRNA in cell line in order to seek approval and will have final say on whether it is acceptable to proceed with the proposed modification.

• Programmable nucleases: zinc fingers or Cas9/CRISPR
CRISPR/Cas9 gene editing will be directed against genes of therapeutic interest. Since the purpose of programmable nucleases is to cause mutations in defined DNA sequences the primary hazard is that such a mutation would lead to deleterious cellular effects. The genes/proteins that will be targeted may include growth, trophic factors, confirmed oncogenes, tumor suppressors or immunomodulators. In isolation, these sequences are unlikely to pose any safety risks for the environment or human health. Programmable nucleases may also be used to generate isogenic controls lines, whereby mutations are reverted to the common wild-type sequence. Generation of isogenic lines poses a low risk to human health as the sequences being targeted are the rare mutated sequences and they are being reverted back to the normal, functional version of the gene. The BSC will be informed of the full nature of and justification for, any proposed programmable nuclease-based modifications in cell lines in order to seek approval and will have final say on whether it is acceptable to proceed with the proposed modification.
Reporter genes: Fluorescent protein or enzymes

The reporter gene could be a fluorescent protein or an enzyme. Fluorescent proteins, for example GFP or dsRed, are not known to cause any diseases in human or animals and have no direct effect on cellular processes. Enzymes used frequently in reporter gene assay ie luciferase of beta-galactosidase are not known to cause any diseases in humans or animals and as no direct effect on cellular processes. The expression of fluorescent protein or enzymes under control of a promoter of a gene of interest is unlikely to pose any safety risks for the environment or human health. The BSC will be informed of the full nature of, and justification any proposed reporter gene expression in cell lines in order to seek approval and will have final say on whether it is acceptable to proceed with the proposed modification.

Evaluation of foreseeable effects

HiPSC are pluripotent and are capable of uncontrolled proliferation. Lentiviruses belong to the Retroviridae virus family are capable of infecting both human and animal species. The lentiviral vector system that will be used is based on HIV-1. Retroviruses are generally transmitted via exposure to contaminated body fluids or percutaneous inoculation and generally not transmitted via the airborne route. Piercing of the skin represents the main potential route by which material could be accidentally introduced into an individual. Appropriate risk reduction measures will be implemented to reduce the likelihood of this occurring.

Accidental introduction of hiPSC into healthy individuals should not lead to the formation of teratomas because the immune system will rapidly destroy these cells. As mentioned above the pathogen status of some cells is not known. However the material will not be derived from high-risk clinical patients and procedures are in place to deal with needle stick incidents (although no sharps will be used). Individuals with compromised immune systems are not permitted to work with this material.

The genetic material to be incorporate into the viral particles is flanked by non-coding retroviral LTRs. No retroviral genes are encoded on this plasmid minimising the chance any will be packaged into viral particles. As the system requires the co-transfection of three to five additional separate plasmids into a permissive cell line the chance of recombination occurring that leads to the incorporation of any or all of the retroviral genes necessary for the production of a RCL is very low and the risk is therefore low.

The lentiviral Expression System includes the following key safety features:

* Only the vector containing the genetic sequence of interest includes LTRs and the packaging signal sequence required for incorporation into viral particles and integration into the genome of a transduced cell. A hybrid 3’LTR is used that does not affect generation of the viral genome in the producer cell line, but upon integration into the genome of a target cell, the 3’LTR SIN which prevents production of viral genomic material that can be packaged and reduces the chance of mobilisation due to secondary infection with wild-type lentivirus. No transcriptionally active LTRs are present in the system and an SV40 polyA sequence is included after the hybrid 3’LTR to reduce the potential for transactivation of cellular genes due to an insertion event that might promote inappropriate gene expression leading to oncogenic effects.

* The LTR has been modified to enable lentiviral production independent of Tat expression. HIV-1 devoid of Tat is known to be replication incompetent. This decreases the risk of RCL occurring.

* The essential genes encoding the proteins required for packaging of the viral genome are separated onto three to five different plasmids. These express the proteins (gag, pol, rev, env) required to generate viral particles transiently in a permissive packaging cell line (e.g. HEK-292T). Other retroviral structural genes are not present in the system. None of the plasmids have regions of homology which will prevent undesirable recombination. As multiple recombination events would be required to generate a RCL the risk of this happening is very low and has so far not been observed in large-scale production and testing of lentiviral vectors.

* In most systems expression of the gag and pol genes has been rendered Rev-dependent by virtue of the HIV-1 RRE (Rev Responsive Element) in the gag/pol mRNA transcript. Expression requires the presence of rev, which is supplied...
by a separate plasmid. The Rev/RRE system is highly conserved among lentiviruses and loss of the RRE sequence and associated splice donor/acceptor sequences results in a loss of transduction efficiency.

- Lentiviral vectors have a very low potential to cause immunogenicity.
- The central poly purine tract (cPPT), from the pol ORF, is included in all of the viral vectors that will be used to improve the nuclear import of the proviral DNA and hence accelerate transduction of target cells.

Whilst pseudotyped VSV-G is used as the envelop protein to increase the cell types that can be infected this increase in risk is mitigated by the SIN non-replication nature of the viral vectors generated. Due to the random nature of integration into the genome insertional events that disrupt gene function could occur and, in some instances might lead to undesirable effects which could be oncogenic.

The resultant lentiviral vectors contain about 20% of the original HIV-1 genome. Recombination with wild-type HIV-1 is still potentially possible and could result in mobilisation of the transgene. This is highly unlikely because:

1. Wild-type HIV-1 is not used with in the laboratory environment.
2. Primary cells or hiPSC are not derived from donors either known to be HIV positive or from a high-risk population; so HIV contamination is highly unlikely.
3. Wild type HIV-1 cannot rescue a SIN HIV-1 based lentivirus once integrated into the host genome.
4. It is likely that any recombination would actually decrease the ability of HIV-1 to infect other cells or to replicate.
5. Even if a worker was already or became HIV-1 positive and was accidentally infected with lentiviral vector any recombination would require infection of the same cells. Generation of RCL would require homologous recombination to be in the right regions to enable mobilisation and incorporation to the HIV-1 genome without loss of replication ability. If such a rare event were to occur it is likely to be self limiting and the HIV-1 infection itself is of greater concern to the worker.

The use of lentiviral vectors is an efficient manner with which to deliver genetic material to and integrate into the genome of numerous cell types. This stable integration is of great benefit in scientific research. The design of the vectors minimises the chance for subsequent mobilisation of transgenes and inappropriate activation of endogenous oncogenes. Insertional events can lead to harmful side effects but are still very unlikely. As such the greatest risk from these vectors comes with their production where much larger volumes and numbers of vectors are being generated.

The risk here is mitigated by the correct use of containment measures and the absence of sharps. Lentiviral vectors are susceptible to dehydration and loss of viability if they are not stored in high protein conditions which reduces the risk to workers and the wider environment.

Transgenes to be delivered to target cells:

Additional hazards could arise from the properties of the genetic material that will be inserted into the viral vectors and expressed in transfected cell lines, primary cells or hiPSCs.

- Gene products to be overexpressed may include growth factors, oncogenes and proteins associated with disease. The risk that these represent is minimised by the use of single use aliquots of AAV and lentiviral vectors in a controlled biosafety level 2 environment. Any protein to be expressed will require justification to and gain BSC approval before work can commence.

- The 'knock-down' of specific gene expression using shRNA can lead to off-target and immunomodulatory responses in vivo. shRNA sequences will be screened against databases of known mammalian/mRNA sequences during the design stage to avoid such complications. Control vectors will also be used that contain a sequence of interest that has been scrambled (scrambled shRNA), and which is not complementary to and therefore does not target any known mammalian sequences. Such sequences are unlikely to pose any safety risks for the environment or human health.

- Programmable nucleases: Cas9/CRISPR will be directed against genes of therapeutic interest. In isolation, these sequences are unlikely to pose any safety risks for the environment or human health. Programmable nucleases may...
be used to generate isogenic controls lines by correcting disease causing mutations back to wild-type sequence. This is low risk because the sequence is aimed to revert back to non-disease forms of the gene. In the case of adding a tag to monitor/follow gene expression there is no anticipated change to the function of the gene and the risk is low.

- Reporter genes: Commonly used fluorescent proteins or enzymes under the regulatory control of promoters of interest may be used to monitor effects on cellular pathways. Such proteins are not known to cause any human or animal disease and pose no-risk.

In all cases justification for any target must be submitted to the BSC for approval prior to any work commencing.

Summary

Primary human cells and hiPSC will be derived from low risk patient populations but may not be screened for human pathogens. Only well characterised immortalised cell lines will be used. The lentiviral systems are replication incompetent and SIN minimising the potential for transgene mobilisation and propagation. The AAV system is transient, non-integrating and replication incompetent having a negligible potential for transgene mobilisation. The transgenes that will be selected will on their own have a low risk to human health. Infection of mucosal cells may occur via aerosols but is highly unlikely due to the use of microbiological safety cabinets and secondary containment. Such infection is self limiting due to the natural shedding of epithelial cells coupled with the replication incompetent nature of the vectors. The most likely route of accidental infection with lentivirus or with an hiPSC line will be via inadvertent percutaneous inoculation via stick injury or open wound. The likelihood of this occurring will be minimised by following standard BSL2 containment practices. Infection of the community and environment with lentivirus particles is highly unlikely due to the small quantities used, their intrinsic instability and rapid loss of viability with time. hiPSC can not survive outside of the laboratory environment so pose no wider risk. Therefore the risks towards workers, co-workers, the public and the environment associated with the use of these lentiviral vectors with hiPSC will be low and BSL2 containment is sufficient.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMOs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Instead of autoclaving waste in the building as per the guidance we wish to chemically inactivate all of our waste and then send it for disposal off site via a registered waste contractor.

All waste material will be inactivated by treatment with a validated disinfectant prior to disposal. For example 5% (v/v) Chemgene solution or 2% final Virkon concentration in the case of material with a high protein concentration such as waste liquid media. Liquid waste will be inactivated for 24 hours prior to either disposal to sewers via a designated laboratory sink or being solidified and treated as solid waste. Solid waste material will then be placed in sealed bins and incinerated by an off-site incineration company.

Waste from our GM work at Class 2 will be double bagged and placed into suitably labelled hermetically sealable (yellow lid to distinguish them from class 1 waste) 60 litre UN approved (to Class 3) bins. Sealed bins are placed into secondary 210 litre yellow labelled “Eurobins”. Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be routinely be made to prevent a buildup. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major road traffic accident, however, the containers are designed to withstand this.

This disposal method is expected to achieve 100% inactivation of the GMM.
### Waste Handling

- Following all work requiring biosafety level 2 containment solid waste will be neutralised by soaking in a validated disinfectant solution for at least 20 minutes, after which the material will be “double bagged”, and, ultimately placed in hermetically sealed burn bins for incineration offsite with a registered waste contractor. It will be routine practise that all liquid waste material be neutralised before disposal, and for the neutralised waste to be transported in leak-proof containers to prevent spillages. Liquid waste will be inactivated with Chemgene HLD4L* (or equivalent product, i.e. Virkon) for 24 hours prior to disposal. In some instances, the liquid waste will be disposed to sewers via a designated laboratory sink or can be solidified with Labsorb (or equivalent product) and disposed of as solid waste as above.

- Virkon is a synergised oxidising system that works by physical destruction of pathogens by acting on proteins. It is resistant to inactivation by organic material. Shown to be effective against bacteria, viruses and fungi including all major human pathogens. Recommended to be used at 1% concentration for all activities.

- Chemgene HLD4L is a commercial validated disinfectant. It is formulated as a micelle cleaning technology that enables the active molecules in the product to be carried rapidly through the cell walls or micro-organisms. Swift penetration of the cell walls ensures cell death is equally rapid. Chemgene HLD4L has been reported to be bactericidal, fungicidal as well as virucidal (HIV, hepatitis B, Herpex Simplex, Norovirus and Coronavirus) and sporicidal. It is recommended to be used at a 1:100 dilution (1%) for general purposes, 1:20 dilution (5%) for high-risk areas, and for disinfection of blood and bio-hazard spillages.

### General cleaning procedures

- Surfaces will be thoroughly cleaned with Chemgene HLD4L 5%* (or an equivalent product) at the end of each experiment or after the occurrence of an accidental spillage of infectious material. Application of these anti-microbial agents will totally inactivate any viral vectors that might be present. According to the biosafety literature, all the viruses to be used are susceptible to these disinfectants. Anything that might have potentially come into contact with the virus will be treated with Chemgene HLD4L* before being incinerated.

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**Is an emergency plan required according to regulation 20?**  
N

**If yes, tick to confirm that it is attached to this form**  
N

**Tick to confirm that you have attached a risk assessment to this form**  
Y

**Tick if you are claiming exemption from disclosure for section of the risk assessment**  
N

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*Page 15066 of 15326*
The Biological Safety Committee (BSC) agrees with the assessment that this work can be conducted at BSL2. It is noted that the primary human cell lines used will be taken from patient populations with a known medical history meaning that there is a low risk of material containing harmful human pathogens. This does not rule out the presence of latent viral infection but the use of third or fourth generation integrating lentiviral systems that are self-inactivating and essentially non-mobilisable makes it highly unlikely that infective replication competent virus will be generated. Important due to the potential use of oncogenic sequences. Lentiviral transduction into hiPSC or immortalised cell line should not be able to mobilise any genetic elements because self-inactivating systems will be used that lack the necessary genes for viral replication and packaging. As an additional measure the BSC will have a clear understanding of how each cell line was generated and steps will be taken to ensure overlapping and therefore potential sites for recombination are not present in lentiviral vectors. These vectors are for the introduction of further modifications. Those aimed at correcting disease mutations do not pose a risk to health. The introduction of nondisease-causing genetic material for exogenous expression is not seen as a risk either. In the instance where oncogenic or immune modulators may be targeted the risk although increased is well contained by the avoidance of sharps during and subsequent to the transduction procedure. Once cells are transduced and virus removed the risk posed here is very low. Due to the use of iPSC derived from primary human cells, cell lines already requiring biosafety level 2 containment and the use of integrating lentiviral vectors this dictates propagation, differentiation of cell lines at BSL2. BSL1 cell lines once transduced, and lentivirus removed at BSL2 could be used at BSL1.

### Project Containment

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### Date of Last Update

| Page Reference | 15068 of 15326 |
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Advice and consultation received from 5 PhD educated senior scientists with backgrounds in molecular and microbiology from Oxford and Imperial universities. Across our committee we have over a cumulative 80 years of safe scientific laboratory practise and knowledge. The committee is well versed in writing and assessing risk assessments for leading academic and industrial laboratories.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
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</table>
The work will be carried out in compliance with good laboratory practice and local safety rules for GM work in a containment class 2 facility.

Solid media will be autoclaved at 121 degrees C and 15 psi for 30S mins to achieve 100% inactivation. Autoclave is present in the laboratory.

Print outs from the autoclave and autoclave indicator tape will be monitored and recorded for successful runs. All autoclave cycles have been validated for the actual load types used. Successful completion of every load will be checked prior to disposal.

Decontaminated solid media will be placed into lab waste bags, cable tied and stored in lidded carts and stored in a separate dedicated waste storage room prior to collection from site by professional waste removal subcontractor.

Liquid media will be treated with 1% solution of Virkon for minimum of 30 mins as per Virkon manufacturer instructions to achieve 100% inactivation. Decontamination will occur on a dedicated lab bench distinct from the rest of the lab to ensure safe working procedures. Virkon provides a colourmetric identification to ensure successful and correct usage. Decontaminated liquid media will be safely disposed through the lab sink drain. Any accidental spillage of liquid waste will be cleaned with 2% virkon and paper towels, followed by 70% ethanol.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

The risk assessment provided is comprehensive and adequate for use of class 1 contained GMM usage. Appropriate risks have been identified and counter measures have been placed to ensure safe working and avoid any contamination risk to human health of users, staff and also risk to the building and local environment.

Project Ref 3596/21.1
Bacterial and fungal strains isolated from soil will be cultured alongside bioluminescent reporter strains for novel metabolite production.

**Project Additional Information**

**Purposes of the contained use**

Novel non GM biological agents isolated from soil are screened against class 1 reporter stains and cultured in laboratory on a small scale (0-1 OOmL volume) to extract primary and secondary metabolites. Bioluminescent reporter strains (currently all class 1) have had the lux operon integrated into genome to produce luminescence. The reporter strain is transferred to a 96-well plate containing the novel bacterial species in a BSC level II safety cabinet. The plate is then sealed and luminescence is measured over a period of 24 - 72 hrs in a sealed TECAN cryoSPARK plate reader.

**Recipient or parental organism**

No class 2 GMOs currently present.

**Host/vector system**

No class 2 GMOs currently present.

**Origin & function**

No class 2 GMOs currently present.

**Evaluation of foreseeable effects**

No class 2 GMOs currently present.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

No larger GMO work will be conducted at the premises.

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
No derogation from full containment is requested

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The work will be carried out in compliance with good laboratory practise and local safety rules for GM work in a containment class 2 facility.
Solid media will be autoclaved at 121 degrees C and 30 psi for 15 mins to achieve 100% inactivation
Autoclave is present in the laboratory.
Print outs from the autoclave and autoclave indicator tape will be monitored and recorded for successful runs.
All autoclave cycles have been validated for the actual load types used.
Successful completion of every load will be checked prior to disposal.
Decontaminated solid media will be placed into lab waste bags, cable tied and stored in lidded carts and stored in a separate dedicated waste storage room prior to collection from site by professional waste removal subcontractor.
Liquid media will be treated with 1 % solution of Virkon for minimum of 30 mins as per Virkon manufacturer instructions to achieve 100% inactivation.
Decontamination will occur on a dedicated lab bench distinct from the rest of the lab to ensure safe working procedures.
Virkon provides a colourmetric identification to ensure successful and correct usage.
Decontaminated liquid media will be safely disposed through the lab sink drain.
Any accidental spillage of liquid waste will be cleaned with 2% virkon and paper towels, followed by 70% ethanol.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Whilst no class 2 material is currently present, the risk assessment and procedures are appropriate against any foreseeable future class 2 GM work

Project Containment

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02/03/2022
**GM Centre Number: 3597**

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**Campus Estate or Research Centre**

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**Comments**

**Date at Which Additional Info Submitted**

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Person with expertise in risk assessments is a qualified biological safety consultant with experience advising academic institutions and biopharma organisations on implementing robust safety, governance and control procedures.

<table>
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<tr>
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Tick if confidential

02/03/2022
Standard organism decontamination procedure used at our laboratory site is to use Virkon disinfectant as a 1-2% solution to be subsequently disposed of in the laboratory sink. Virkon is validated against a number of typical lab and hospital pathogens with extremely high killing capability (greater than 99.999% kill) and is one of the most reliable and commonly used disinfectants on the market. Random periodic sampling and plating of disinfected surfaces, equipment and culture flasks will be performed to monitor and demonstrate total cell death. After disinfecting with Virkon, culture flasks will be autoclaved.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

"I think this [risk assessment] looks fine."
"You have provided well reasoned justification so can assign it Class 1."
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**Name**

MAXION THERAPEUTICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

ROOM 2019, MONETA BUILDING

**Road Name**

BABRAHAM RESEARCH CAMPUS

**Town**

CAMBRIDGE

**County**

CAMBRIDGEShire

**Postcode**

CB22 3AT

**Country**

ENGLAND

**Tel Number**

01223 497429

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

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<thead>
<tr>
<th>Level 1 (GMMs)</th>
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<tbody>
<tr>
<td>Yes</td>
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The Maxion Therapeutics Ltd genetic modification committee will consist of:

1. The biosafety officer who has extensive experience of genetic modification issues, being currently in charge of biosafety for a large research institute. The biosafety officer will chair all genetic modification committee meetings.
2. The Chief Executive Officer has 30 years experience of genetic modification and previously ran research groups both in industry and academia. His function on the committee is to advise and provide the necessary resources for the actions of the committee to be implemented.
3. The Chief Scientific Officer who has 11 years experience of genetic modification in E. coli and mammalian cells. He will provide advice and ensure the actions arising from the committee meetings are implemented.
4. Senior Research Scientist who is a specialist in mammalian cell culture has 7 years experience. He will provide advice and write the minutes of the meetings.

The committee will meet every 6 months to review biosafety and whenever a new risk assessment is required. The first meeting will review the existing risk assessments.
All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below).

Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins".

Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.
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Date at Which Additional Info Submitted

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The ArkVax Ltd genetic modification committee will consist of:
1. The CEO and biosafety officer who has extensive experience of genetic modification issues, having worked at a University and being the academic in charge of a category 3 laboratory. He has over 20 years of experience of genetic modification of E. coli and other bacterial cells. The biosafety officer will chair all genetic modification committee meetings.
2. The Co-founder has 20 years experience of genetic modification and previously ran research groups in academia. His function on the committee is to advise and provide the necessary resources for the actions of the committee to be implemented.

The committee will meet every 6 months to review biosafety and whenever a new risk assessment is required. The first meeting will review the existing risk assessments.

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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02/03/2022
All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled “Eurobins”. Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Project Ref 3599/21.1

Date Ackn’d 25/02/2021

CU2 Project Title Engineering of veterinary vaccine candidates targeting bacterial diseases

Class CultureVol

Class 2 < 1 Litre

Consent Granted

Non-GMM
Purposes of the contained use

To genetically modify strains of E. coli K-12 derivatives or ACDP category 2 pathogens in order to create attenuated vaccine candidates. The mutation target site will generally render the organism unable to establish an infection (example, removal of O antigen or capsular polysaccharide) in a host in addition to disabling growth outside of rich culture medium (such as the removal of Aro genes, responsible for creating aromatic amino acids). These live weakened bacteria will also be modified to carry vaccine immunodominant antigens such as capsular polysaccharide or protein antigen that have been demonstrated to be efficacious immune system stimulants. Vaccine candidates will be cloned into pathogens such as (i) immunodominant antigens such as surface proteins (ii) the O (LPS) antigen from several bacteria, (iii) the capsule from several bacteria. These will be introduced into the host bacteria on their chromosomes or on plasmids for initial proof of concept studies. The cloned antigens will be tested by whole cell western blotting but also purified for further analysis. None of the cloned antigens are classified as active toxins.

Recipient or parental organism

Subproject 1: Vaccine candidates will be cloned into E. coli K12 such as (a) immunodominant antigens (eg AcrA and OmphH1 from Campylobacter jejuni, sortase and sorted proteins from Clostridium spp. and detoxified proteins (b) adherence proteins such RsCC from Yersinia pseudotuberculosis and PEB3 from Campylobacter jejuni (c) the O (LPS) antigen from several bacteria, (d) the K (capsule) antigen from several bacteria. The nucleic acid sequences of the genes to be cloned from disease causing bacteria such as Campylobacter spp., Salmonella spp., Streptococcus spp., Staphylococcus spp., Actinobacillus spp., Haemophilus spp., Brucella spp., Yersinia spp. Edwardsiella spp. Pseudomonas spp. E. coli.

Subproject 2: Construction of attenuated mutants in (Yersinia pseudotuberculosis, Yersinia enterocolitica, Yersinia ruckeri, Salmonella spp., Shigella spp. Actinobacillus pleuropneumoniae, Streptococcus suis, Haemophilus parasuis, Pasteurella spp. Mycoplasma hyopneumoniae, Edwardsiella spp. and wild type E. coli). These are all category 2 organisms. Of note, no category 3 members of the genus will be worked on such as Y. pestis.

Vectors with the following specified origins of replication will be employed in this work: pMB1/ColE1, p15A, pSC101/R100/FII, incW, pBBR1, RK2/ori/IncP and carrying the following antibiotic resistance markers: Tetracycline/kanamycin/ampicillin/chloramphenicol/Zeocin/trimethoprim. The majority of the vaccine assembly work such as cloning and protein expression will first be carried out in E. coli K12 category 1 laboratory strains. However when working on direct attenuation of a live organism we will carry out as follows:

**Origin & function**

For the construction of defined mutants, in Gram positive organisms, the pMTL and pSET low copy shuttle vectors will be used to introduce DNA into test pathogens such as Streptococcus suis. No high copy number vectors are required to introduce DNA into the test pathogens. Low copy number plasmid pBBR1MCS3, pACYC and pWKS will be used to introduce DNA into competent E. coli K12 derivative cells. Selection will be through tetracycline, trimethoprim, ampicillin, chloramphenicol, zeocin, kanamycin resistance. Low copy number plasmids such as pUOA18 will be used to introduce DNA in the attenuated S. typhimurium and other Salmonella strains (Wyszynska A, et al., Vaccine. 2004 Mar 29;22(11-12):1379-89). The Salmonella typhimurium crp-, cya-, asdA1 mutant, is a lethal-balance vector-host system, in which the asd deletion introduced to the chromosome of the host is complemented by the asd gene present on the plasmid, that enables cloning of foreign genes without antibiotic selection.

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Instead of autoclaving waste in the building we propose to follow the procedure used by the Babraham Institute described in their disposal notification GM 105/4.1 (see also section 6 above for description). Reasons for adopting this method of waste disposal are:

1. We do not have access to an autoclave within our area of the building
2. The waste disposal system used by the Babraham Research Campus is safe and has been running since October 2004.

All waste material will be inactivated by treatment with 1 % (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (Vetspeed - Their GM authorization Is GM898) according to disposal notification GM105/4.1. Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The
only real risk of release would be as a result of a major RT A, however, the containers are designed to withstand this.  
This disposal method is expected to achieve 100% inactivation of the GMM.  
ArkVax is applying for its own derogation.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled “Eurobins”.  
Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. 
This disposal method is expected to achieve 100% inactivation of the GMM. 
The data sheets describing inactivation by Virkon are attached under SOP2 'use of disinfectants in containment level 2 laboratories'.

| Is an emergency plan required according to regulation 20? | N |
| If yes, tick to confirm that it is attached to this form | N |
| Tick to confirm that you have attached a risk assessment to this form | Y |
| Tick if you are claiming exemption from disclosure for section of the risk assessment | N |

Please enter comments on the GM safety committee on the risk assessment
In the very improbable event that the GMOs should be outside the ACDP category 2 designated area, they are unlikely persist, disseminate and cause disease for the following reasons.

1. Invariably modifications will result in an attenuated less virulent strain.
2. The transmission of these organisms is usually dependent upon an animal host. For example, Y. enterocolitica, Y. pseudotuberculosis and S. typhmurium are transmitted through the consumption of meat products. Attenuated strains are less likely to have the same level of transmissibility as the wild type strain.
3. Y. enterocolitica and Y. pseudotuberculosis require several nutrients to grow outside the host, have a slow growth phase compared with most enterobactericiae (eg E. coli) and generally do not persist. In addition, Y. enterocolitica, Y. pseudotuberculosis, S. typhmurium, Edwardsiella ictaluri, Mycoplasma hyopneumoniae, Actinobacillus pleuropneumonia, Streptococcus suis and Haemophilus parasuis are not spore formers and all are unlikely to survive well outside a nutrient rich environment. Again attenuated strains are less likely to survive than the wildtype strain.
4. The wildtype/parent strains used for allele replacement are passaged through several in vitro rounds of growth. Invariably the wild type strain will have lost properties to allow it to persist, transmit and cause disease compared with fresh clinical isolates. Thus even the wild type strain is unlikely to be as pathogenic as strains causing disease.
5. Actinobacillus pleuropneumonia, avian pathogenic E. coli, Mycoplasma hyopneumoniae, Streptococcus suis, Pasteurella multocida and Haemophilus parasuis are invariably pathogens associated with pigs, and not a direct threat to humans.

Undertaking good laboratory practice and waste disposal in ACDP 2 lab conditions will minimize the risk to humans and the environment. Taking all of these factors into consideration, we consider that there is a negligible consequence of environmental contamination and the estimation of risk is effectively zero.

Y. ruckeri, Yersinia pseudotuberculosis, Yersinia enterocolitica, Salmonella typhimurium cause gastroenteritis that is either self-limiting or can be treated with a range of antibiotics. None of the pathogens are transmitted by aerosol delivery.

### Project Containment

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**Name**

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**Campus Estate or Research Centre**

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**Tel Number**

| 0 |

**Fax Number**

| 0 |

**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022 |
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Forms were approved by the the Biological Agents and Genetic Manipulation Safety Committee (BAGMSC) at the Pirbright Institute

Laboratory  Animal Unit  Growth Room  Glass House  Large Scale

Level 1 (GMMs)  Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)  Tick if confidential

Bacteriology  Parasitology  Transgenic Birds  Microbiology Research

Virology  Transgenic Animals  Transgenic Fish  Gene Therapy

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

A validated disinfectant, for example; 1% Virkon for 10 minutes or 10% Distel for 10 minutes, 2% Oettol30 minutes.
1 :100 FAM30 30 minutes) will be used to disinfect any exposed surfaces including equipment.
Absorbent material (Blue roll or similar) will be used to stem the spread of spills.
Accidental spillages of liquid waste will be deactivated with a double strength concentration of a validated disinfectant.
Contaminated dothing and shoes should be removed and advice sort on its decontamination from the Biological safety officer
All liquid waste will be inactivated by a validated disinfectant and contact time and then disposed to drain or autoclaved on a validated cycle. Users are trained in waste disposal prior to starting work.
All solid waste will be placed into double bagged autoclave bags and placed directly into a metal autoclave tin before being transported directly for autoclaving. Following autoclave the waste is incinerated.

Please enter comments of the GM safety committee on the risk assessment

BAGMSC comments: Reviewed by Holly Shelton and Luke Alphey
This RA has been reviewed by the Pirbright Institute's Biological Agents and Genetic modification Safety Committee (BAGMSC) for 272810 under it's capacity as a GM safety committee as defined in the GM(CU)R 2014. this Committee do not endorse or own any of the work being undertaken.2728io will notify all and any GM work to the HSE under their own volition and under their own GM center number and Pirbright accepts no liability for any issues, or incidents ariSing from this work. Graeme Harkess. Head of Blorisk. and Site BSO. 1210212021
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Name
PURESPRING THERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre
ROLLING STOCK YARD

Road Name
188 YORK WAY

District

Town
LONDON

Country
ENGLAND

County
GREATER LONDON

Postcode
N7 9AS

Tel Number
0207 691 1122

Fax Number
0

E-mail

HSE Division
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Comments

Date at Which Additional Info Submitted
02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Purespring Therapeutics is a new gene therapy company which has resulted from work currently being carried out at the University of Bristol by the investigators cited in the risk assessment. The Scientific Officer of Purespring Therapeutics is also currently a member of their University of Bristol GMSC and has been the Deputy Biological Safety Officer for the Bristol Medical School for over 10 years. The Scientific Officer has reviewed and advised on this class 1 risk assessment as the competent person to do so for the company. When required, the duties of a GMSC will be undertaken by a Safety Committee being established by Purespring Therapeutics, chaired by the Scientific Officer, with relevant experience represented, including the lab manager and representatives from both the research and technical staff.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs) | Yes |
Level 2 (GMMs) |
Level 3 (GMMs) |
Level 4 (GMMs) |
All solid waste will initially be inactivated by exposure to 1% Virkon for at least 30 minutes. All solid waste (pipette tips, animal tissues, including slides with sections) will be placed in autoclavable bags or an appropriate CinBin, sealed, collected and disposed of appropriately by Novus-Environmental (registration number is GMGM898). Liquids (e.g. samples, culture supernatants, tissue culture media) should made safe by 1% Virkon (for at least 30 minutes) and discharged to drains. Autoclaving effectively 100% kill (annual validation). Incineration effectively 100% kill (licensed incinerator). Chemical disinfection with Virkon, used according to manufacturer's instructions under standard conditions, manufacturers validation (e.g. 99.998 % kill). Animal waste – carcasses will be disposed of by incineration following the RVC Animal Services Unit rules.

Purespring Therapeutics is a new gene therapy company which has resulted from work currently being carried out at the University of Bristol by the investigators cited in the risk assessment. The original risk assessment has been reviewed by the University of Bristol Genetic Modification Safety Committee for work carried out on University of Bristol premises. The attached risk assessment has now been reviewed and updated by the Purespring Therapeutics Scientific Officer so that it is relevant to the processes and procedures being implemented at Purespring Therapeutics premises. The scope, risks and control measures have not changed from those reviewed by the University GMSC because of this review and remain appropriate for work undertaken by Purespring Therapeutics.
Purespring are developing novel gene therapy tools for the kidney. This includes using Lentiviral expression in kidney cell lines to validate our tools.

Recipient or parental organism

AAV, Ad5 & Lentivirus
Species Organ Tissue or cell Source
Human Kidney HEK 293T Cells Commercial
Human Cervix HeLa cells Commercial
Human and Mouse Kidney Podocytes Isolated from kidney tissue
Human and Mouse Kidney Glomerular Endothelial Cells Isolated from kidney tissue
Human and Mouse Kidney Mesangial cells Isolated from kidney tissue
Human and Mouse Kidney Proximal Tubular Epithelial Cells Isolated from kidney tissue
Human and Mouse Kidney Glomeruli Isolated from kidney tissue
AAV and Ad5 will be administered to mice by systemic injection or targeted kidney injection

Host/vector system

Other vector components Description of safety features, control sequences (e.g. promoters), antibiotic resistances
Human NPHS1 promoter Podocyte-specific promoter
Mouse NPHS1 promoter Podocyte-specific promoter
CMV promoter Constitutive promoter
WPRE (Woodchuck Hepatitis Virus Posttranscriptional regulatory element) Shown to improve gene expression.
MSCV promoter (Murine Stem Cell Virus) Constitutive promoter, effective in most mammalian cell lines, optimal for pluripotent cell lines

Origin & function
The lentiviral constructs are all 3rd/4th generation and have been purchased from Addgene. AAV and Ad5 are also from commercial sources. Promotors have been designed in house and proprietary to Purespring.

Evaluation of foreseeable effects

Lentivirus
The 3rd generation Lentivirus is recombinant deficient but can integrate into the human host. The lack of such adverse effects in most other gene therapy trials using retroviruses indicates that the chromosomal insertion of replication-defective retroviruses is generally unlikely to cause overt harm. There will be no change to other properties of the GMMs after modification, and they will be unable to engraft in a normal immunocompetent human host. Some of the vectors will encode standard reporter genes (GFP, LacZ, Luciferase), which are routinely used without safety issues. Other vectors will express proteins important in kidney diseases under a podocyte-specific promoter. It is unlikely to have any toxic effects. Potential to express within a human but should have no detrimental effect.

AAV
The recombinant AAV vectors are replication defective and have none of the wild type AAV genes. They are replication defective and incapable of propagating in a human host. There will be no change to other properties of the GMMs after modification, and they will be unable to engraft in a normal immunocompetent human host. Some of the vectors will encode standard reporter genes (GFP, LacZ, Luciferase), which are routinely used without safety issues. Other vectors will express proteins important in kidney diseases under a podocyte-specific promoter. It is unlikely to have any toxic effects. Potential to express within a human, but should have no detrimental effect.

Adenovirus Type 5
The recombinant Ad5 vectors are 3rd generation and are recombinant deficient. There will be no change to other properties of the GMMs after modification, and they will be unable to engraft in a normal immunocompetent human host. Some of the vectors will encode standard reporter genes (GFP, LacZ, Luciferase), which are routinely used without safety issues. Other vectors will express proteins important in kidney diseases under a podocyte-specific promoter. It is unlikely to have any toxic effects. Potential to express within a human but should have no detrimental effect.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

• Handling mice that have been infected with recombinant viral vectors poses no extra risk to staff at the BSU.
• Where appropriate, an animal restrainer will be used.
• Only experienced and licensed staff will undertake any regulated procedures required for this part of the project.
• All solid waste will initially be inactivated by exposure to 1% Virkon or a suitable, validated alternative for at least
30 minutes. All solid waste (pipette tips, animal tissues, including slides with sections) will be placed in autoclavable bags or an appropriate CinBin, sealed, collected and disposed of appropriately by Novus-Environmental (registration number is GMGM898).

- Gloves and PPE will be disposed via standard clinical waste routes. No autoclaving or disinfection is required prior to disposal.
- Animal bedding is considered minimal risk and will be disposed as normal landfill. No autoclaving or disinfection is required prior to disposal.
- Animals carcasses will be disposed by incineration.
- Animals will not pose additional health risks if released into the environment as the recombinant viral vectors are replication deficient and cannot be passed down through reproduction as it does not integrate.
- The animal house is a high security facility with many measures in place to prevent escape of mice into the environment.
- Mice are housed in secure cages in rooms designated to one individual research group per room.
- Rodent barriers exist to prevent mice from escaping and travelling between rooms and exits.
- Animal bait traps are placed throughout the facility and regular checks are made to ensure that if any cracks/holes in walls, doors etc appear they are repaired immediately.
- Mice will only be injected with viral vectors by appropriately trained staff.
- The doses of recombinant viral vectors being used for mouse transduction are several orders of magnitude lower than that required for human transduction even in the event of inadvertent intravenous puncture.
- Only appropriately trained personnel will use sharps.
- Sharps will never be re-sheathed.
- Sharps will be appropriately disposed of in a sharps bin that will never exceed being ¾ full, which is then disposed via normal waste routes.
- Health surveillance is conducted on all staff that conduct work within the BSU (e.g. annual lung function test).
- The BSU will ensure adequate ventilation.
- Where appropriate local exhaust ventilation is used.
- If an operator experiences any negative reactions, e.g. an allergic reaction, they report this immediately.

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All solid waste will initially be inactivated by exposure to 1% Virkon for at least 30 minutes. All other solid waste (pipette tips, animal tissues, including slides with sections) will be placed in autoclavable bags or an appropriate CinBin, sealed, collected and disposed of appropriately by Novus-Environmental (registration number is GMGM898).

Liquids (e.g. samples, culture supernatants, tissue culture media) should made safe by 1% Virkon (for at least 30 minutes) and discharged to drains. Autoclaving effectively 100% kill (annual validation). Incineration effectively 100% kill (licensed incinerator) Chemical disinfection with Virkon, used according to manufacturer's instructions under standard conditions, manufacturers validation (e.g. 99.998 % kill). Animal waste –carcasses will be disposed of by incineration following the RVC Animal Services Unit rules.
Please enter comments on the GM safety committee on the risk assessment

**Project Containment**

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Comments

Date at Which Additional Info Submitted

02/03/2022

Page 15097 of 15326
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

The advisor has over ten years experience as a Biological Safety Officer, having previous served as the BSO to two biotechnology companies: the contract manufacturing organisation Cobra Biologics Ltd, and most recently the vaccine and cancer therapy development company Prokarium Ltd (BSO for six years). The advisor has reviewed numerous risk assessments at GM Class 1 and 2 for various species of bacteria (including modified pathogens), viruses and cell lines.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tbody>
<tr>
<td>Level 1 (GMMs)</td>
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<td>Other (please specify)</td>
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Tick if confidential: 

- Bacteriology: Yes
- Parasitology: 
- Transgenic Birds: 
- Microbiology Research: Yes
- Virology: 
- Transgenic Animals: 
- Transgenic Fish: 
- Gene Therapy: 

02/03/2022
Accidental spillages will be dealt with by swabbing with 70% IPA spray which is bactericidal.

Contaminated, solid, bacteria waste such as plastic disposables, tissue paper, culture plates, etc., are bagged in autoclave bin bag and closed with autoclave bin bag. The bag is autoclaved at 121°C for 20 mins, 1 bar pressure. The autoclave tape has to turn black before being placed into a yellow bin bag and tied up. The bags are finally placed in large yellow waste bin.

Approximately 2L of liquid culture medium is treated by adding to Virkon or bleach to achieve a final concentration of 10% and left to stand overnight before discharge to the sewer.
<table>
<thead>
<tr>
<th>Name</th>
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<tbody>
<tr>
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<tr>
<td>Road Name</td>
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**Date at Which Additional Info Submitted**

02/03/2022
### Premises Addresses

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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- Yes

**Give brief details of the genetic modification safety committee**

We have received advise from consultants at Health and Safety works Ltd

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Non-microbial</td>
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**Other (please specify)**

- Bacteriology
  - Yes
- Parasitology
  - Transgenic Birds
- Transgenic Animals
- Microbiology Research
  - Yes
- Virology
  - Yes
- Transgenic Fish
- Gene Therapy
  - Yes

02/03/2022
Contaminated solid waste is autoclaved at 128 celcius and 15 PSI for 20 mins, under these conditions sterilisation will be achieved, thus ensuring inactivation of the the micro organisms. The autoclave is calibrated annually and serviced every 6 months, during the service the cycle is tested to ensure sterilisation is achieved.

Liquid waste is inactivated by treatment with 5% solution of chlorine. For example, the waste is treated with 5% virkon for 48 hours before disposal. The manufacturer has verified that a 10 min contact time with a 1% solution is sufficient contact time to inactivate 99.999% of the micro-organisms.

Saccharomyces cerevisiae solid cell pellet waste from 10 litre fermentation is inactivated by treatment with 5% Janitol solution for 48 hours before disposal. This has been tested in house and no growth of S. cerevisiae was observed after treatment (R&D/R-0345).

<table>
<thead>
<tr>
<th>Mycology</th>
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<td>Other(s)</td>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity:

- Contaminated solid waste is autoclaved at 128 celcius and 15 PSI for 20 mins, under these conditions sterilisation will be achieved, thus ensuring inactivation of the the micro organisms. The autoclave is calibrated annually and serviced every 6 months, during the service the cycle is tested to ensure sterilisation is achieved.
- Liquid waste is inactivated by treatment with 5% solution of chlorine. For example, the waste is treated with 5% virkon for 48 hours before disposal. The manufacturer has verified that a 10 min contact time with a 1% solution is sufficient contact time to inactivate 99.999% of the micro-organisms.
- Saccharomyces cerevisiae solid cell pellet waste from 10 litre fermentation is inactivated by treatment with 5% Janitol solution for 48 hours before disposal. This has been tested in house and no growth of S. cerevisiae was observed after treatment (R&D/R-0345).

Tick to confirm that you are attaching a summary of the risk assessment: Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment: Y

Please enter comments of the GM safety committee on the risk assessment:

This project has been reviewed and approved by the health and safety committee.

Project Ref 3606/21.1

<table>
<thead>
<tr>
<th>Date Ackn'd</th>
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<td>Non-GMM</td>
<td>Consent Granted</td>
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Date Project Ceased

Withdrawn: N

Tick if notifying a connected programme of work: N

Tick if notifying a connected programme of work: N

02/03/2022
**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Contaminated solid waste is autoclaved at 128 celcius and 15 PSI for 20 mins, under these conditions sterilisation will be achieved, thus ensuring inactivation of the the micro organisms. The autoclave is calibrated annually and serviced every 6 months, during the service the cycle is tested to ensure sterilisation is achieved.

Liquid waste is inactivated by treatment with 5% solution of chlorine. For example, the waste is treated with 5% virkon for 48 hours before disposal. The manufacturer has verified that a 10 min contact time with a 1 % solution is sufficient contact time to inactivate micro-organisms.
This project has been reviewed and approved by the health and safety committee

Please enter comments on the GM safety committee on the risk assessment

Tick if you are claiming exemption from disclosure for section of the risk assessment

Tick to confirm that you have attached a risk assessment to this form

Tick if you are claiming exemption from disclosure for section of the risk assessment

---

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
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Animal Units | Large Scale Activities | Human Clinical Applications

| L2 | L3 | L4 | L2 | L3 | L4 | L2 | L3 | L4 |

**Project Ref** 3606/21.2

**Date Ackn’d** 19/03/2021

**CU2 Project Title** Transduction of mammalian cells with rAAV

**Class** Class 2

**CultureVolClass2** < 1 Litre

**Consent Granted** Non-GMM

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

Withdrawn

Tick if notifying a connected programme of work

---

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form

Tick to confirm that it has been reviewed and approved by the health and safety committee

---

**Project notified under transitional arrangements** N
Project Additional Information

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated solid waste is autoclaved at 128 celcius and 15 PSI for 20 mins, under these conditions sterilisation will be achieved, thus ensuring inactivation of the the micro organisms. The autoclave is calibrated annually and serviced every 6 months, during the service the cycle is tested to ensure sterilisation is achieved.

Liquid waste is inactivated by treatment with 5% solution of chlorine. For example, the waste is treated with 5% virkon for 48 hours before disposal. The manufacturer has verified that a 10 min contact time with a 1% solution is sufficient contact time to inactivate the micro-organisms.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
This project has been reviewed and approved by the health and safety committee.

### Project Containment

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### Project Ref 3606/21.3

- **Date Ackn'd**: 19/03/2021
- **CU2 Project Title**: Transduction of mammalian cells with Lentivirus
- **Class**: Class 2
- **Culture Vol Class 2**: ≤ 1 Litre
- **Non-GMM Consent Granted**: Yes
- **Project notified under transitional arrangements**: No

### Additional Information

- **Purposes of the contained use**: 
- **Recipient or parental organism**: 

---

02/03/2022
Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Contaminated solid waste is autoclaved at 128 celcius and 15 PSI for 20 mins, under these conditions sterilisation will be achieved, thus ensuring inactivation of the the micro organisms. The autoclave is calibrated annually and serviced every 6 months, during the service the cycle is tested to ensure steri lisation is achieved

Liquid waste is inactivated by treatment with 5% solution of chlorine. For example, the waste is treated with 5% virkon for 48 hours before disposal. The manufacturer has verified that a 10 min contact time with a 1 % solution is sufficient contact time to inactivate micro-organisms

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment

This project has been reviewed and approved by the health and safety committee

Project Containment

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<th>Laboratory Activities</th>
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Animal Units Large Scale Activities Human Clinical Applications

02/03/2022

Page 15107 of 15326
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**Name**

FUTURE HEALTH TECHNOLOGIES LTD

**Name**

T/A BIO-DOCK

**Campus Estate or Research Centre**

NOTTINGHAM SCIENCE & TECHNOLOGY PAR

**Building**

10 FARADAY BUILDING

**Road Name**

UNIVERSITY BOULEVARD

**District**

**Town**

NOTTINGHAM

**County**

NOTTINGHAMSHIRE

**Postcode**

NG7 2QP

**Country**

ENGLAND

**Tel Number**

+44 115 907 8613

**Fax Number**

0

**E-mail**

**HSE Division**

Blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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<td>10 FARADAY BUILDING</td>
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

N/A - GMM to be stored inside a contained tank as part of a third party storage agreement with a BioDock client. No processing of any type to be taking place on site. A risk assessment for the storage of GMM materials is attached.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
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<tbody>
<tr>
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<td>Other (please specify)</td>
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Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research | Gene Therapy
Virology | Transgenic Animals | Transgenic Fish | | |
<table>
<thead>
<tr>
<th>Mycology</th>
<th>Transgenic Invertebrates</th>
<th>Transgenic Plants</th>
<th>Other (please specify below)</th>
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<td>Storage under a third party agreement with a BioDock client.</td>
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</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

N/A - Client is responsible for the disposal of any material.

Tick to confirm that you are attaching a summary of the risk assessment

Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment


Please enter comments of the GM safety committee on the risk assessment

N/A - GMM to be stored inside a contained tank as part of a third party storage agreement with a BioDock client. No processing of any type to be taking place on site.
A risk assessment for the storage of GMM materials is attached.
### GM Centre Number: 3611

<table>
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<td>Emergency Plan Required?</td>
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<tr>
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**Name**

NOVALGEN LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Building**

ARGYLE HOUSE

**District**

NORTHWOOD HILLS

**Town**

LONDON

**Road Name**

JOEL STREET

**County**

GREATER LONDON

**Postcode**

HA6 1NW

**Country**

ENGLAND

**Tel Number**

0207 431 7976

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022

Page 15112 of 15326
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Head of CMC at NovalGen has over 15 years of contained use experience in the industry. They served on Health and Safety committees in manufacturing and development sites in biopharma as well as sitting on a GMO safety committee. Their experience covers the use of viral vectors, microbial and mammalian cells, some of which required the use of negative pressure isolators to protect the operators and environment from the expresssed compounds of interest.

<table>
<thead>
<tr>
<th>Laboratory</th>
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<th>Glass House</th>
<th>Large Scale</th>
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Bacteriology | Parasitology | Transgenic | Microbiology |
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02/03/2022
Yes

All waste containing viable GMM is to be inactivated by validated means prior to leaving site for incineration. Solid waste, including contaminated materials like plasticware, pipette tips, gloves and tissue paper, is to be rendered safe by autoclaving. Contaminated sharps will be placed in sealed sharps bins and disposed of by incineration. The autoclave will be available within the same building as the laboratory. The autoclave is to be validated and checked annually. Given the thermal sensitivity of the organisms which will be used on site a 6-log kill cycle will be used, its performance monitored on each use (e.g. with indicator tape or probes) and the inactivation documented. Autoclaved inactivated GMM waste still be treated as hazardous waste (180103) and will be collected by an approved contractor for incineration. Liquid waste containing viable GMM (including spent culture media) is to be inactivated chemically with Distel 1:20, Virkon or Precept before discarding to achieve 6 log kill. As with the solid waste this will be sent off site for incineration. Inactivation of GMM waste and subsequent disposal will be detailed in the Waste Management SOP.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The current NovalGen laboratories location gives us access to the risk assessments and GMO safety committee of our landlord. Consequently, NovalGen staff are familiar with the relevant procedures for working with GMMs and have access to risk assessments performed and reviewed by the GMO safety committee. The GMMs we propose to use are a subset of those detailed in these risk assessments.
Project Additional Information

Purposes of the contained use

Research, development and analysis of antibodies or antibody-like molecules with biopharmaceutical potential (e.g. bispecific antibody T-cell engager for targeting hematological cancers such as Chronic Lymphocytic Leukemia and Mantle Cell Lymphoma, and for targeting solid tumors). Research and development of Chimeric Antigen Receptor (CAR) T cell therapeutic solutions by lentiviral or retroviral vector transduction of human T cells in culture. Research and development of AAV vectors for gene transfer. The contained use includes molecular biology techniques using recombinant bacteria, phage display, yeast display, and cell culture techniques for expression of recombinant proteins and for biological assays.

Recipient or parental organism

The microorganisms used are standard, well characterised, strains commonly used the area of molecular biology (e.g. DH5alpha, K1 and L21 disabled E.coli strains, bacteriophage M13, yeast strain EBY100). Standard, well characterised and widely used mammalian cell lines will also be used (e.g. human HEK293 or HEK293T cells, animal cells such as hamster CHO and other immortalised cell lines). The intended function of the lentiv- and retroviral vectors is CAR T cell engineering for development of adoptive immunotherapy for blood cancer and/or solid tumour cancers, or engineering of standard mammalian cell lines as research tools. AAV viral vectors would be used for gene transfer. Hybridoma cells (fusions of mouse B cells and the well known SP2/0 cells) are cultured for expression of monoclonal antibodies.

Host/vector system

Standard DNA cloning vectors (e.g. pUC derivatives) and mammalian expression vectors for production of antibodylike molecules in cell culture. All cloning will be performed using commercial bacterial strains which are disabled. The inserted sequences may originate from human, mouse, or other mammalian genomic DNA or cDNA and encode engineered antibody binding arms. For example, the bispecific antibody NVG-111 has two "arms", one arm binds ROR1 molecule on cancer cells, and the other arm binds body's immune cells directing them to kill the cancer cells. The NVG-111 drug has been approved for clinical trial. Antibody gene libraries for phage and/or yeast display of antibody fragments allowing for discovery of molecules with therapeutic potential. Hybridoma cells cultured for the
production of monoclonal antibodies are a well known and established technology. Standard lentiviral vectors will be used for engineering of CAR T cells, including packaging plasmid pCMVR8.74 and VSV-G envelope plasmid pMD-G. The lentivirus is a 3rd generation (or safer, 4th generation) HIV vector and 2nd generation FIV vector with virulence genes deleted, on split plasmids. Recombinant AAV (rAAV) vectors are the gold standard for studying in vivo gene function. It is forceable that AAV vector engineering and virus generation may be performed on site. However, the “use” of the virus would be restricted to an authorised external animal facility only. The vector capsid and backbone are derived from wild type AAV. Such plasmids have type AAV genes replaced with a transgenic cassette with an inserted sequence encoding the molecule of interest or a reporter transgene like GFP or luciferase. For virus production helper plasmids are required such as pHelper (HGTI), rep/cap, Ad5Rep2G (adenoviral functions), AAV cap/rep expressing plasmids - 2/8, 2/5 LKO3, AA3L, transgene plasmids with transgene flanked by AAV inverted terminal repeat sequences, such as pAV plasmids.

**Origin & function**

| N/A |

**Evaluation of foreseeable effects**

As the bacterial strains are disabled, they are not harmful to health and environment. The strains are widely used in academic and commercial laboratories. Following transformation with plasmids the bacteria gain resistance to antibiotics. The release of such a microorganism may mean that it spreads to the environment. Such bacteria should have no environmental advantage over wild type bacteria. However, due to the possibility of bacterial recombination and DNA transfer to recipient strains, all cultures are to be inactivated by validated means prior to discarding. Peripheral blood and isolated peripheral blood mononuclear cells from normal human donors and patients with haematological malignancies can harbour potentially unknown pathogens. Samples and cultures of animal or human cells are handled in biosafety class II A1/A2 microbiological safety cabinets with class H14 HEPA/ULPA filters to protect the operators and are inactivated by validated means prior to discarding. Operators must not work with their own blood cells as autologous cells could potentially pose a major risk in the unlikely event of infected or otherwise modified cells reaching their blood streams through injuries. The viral vectors are not pathogenic as they have all virulence genes deleted. These vectors can potentially integrate into human DNA which poses a low risk of impact on health of a laboratory operator directly handling the virus. However, there are no such cases known. The lentiviral vector particles include a vesicular stomatitis virus G (VSV-G) viral fusion protein, which results in broad host specificity and may increase susceptibility of different cells. However, lentiviral vectors insert preferentially into the coding regions of DNA rather than into more risky promoter or control regions. The risk of insertional mutagenesis is reduced by the use of self-inactivating (SIN) vectors. Overall, the lentiviral vectors are considered low risk. The AAV viruses are not known to cause disease and are a known platform for gene therapy. Chimeric antigen receptors (CARs) are artificial receptors consisting of an antibody specific to a tumour target, fused to T cell receptor signalling domains. Autologous T cells can be isolated from patients and modified to express a CAR directing the T cell response against tumour cells with a specific marker. Such cells have therapeutic potential when readministered to the patient and need to be studied in vitro and potentially in vivo to determine the safety and efficacy.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

| N/A |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All waste containing viable GMM is to be inactivated by validated means prior to leaving site for incineration. Solid waste, including contaminated materials like plasticware, pipette tips, gloves and tissue paper, is to be rendered safe by autoclaving. Contaminated sharps will be placed in sealed sharps bins and disposed of by incineration. The autoclave will be available within the same building as the laboratory. The autoclave is to be validated and checked annually, and its performance monitored during each use (e.g. with indicator tape) and documented. Given the thermal sensitivity of the organisms which will be used on site a 6-log kill cycle will be used, its performance monitored on each use (e.g. with indicator tape or probes) and the inactivation documented. Autoclaved inactivated GMM waste still be treated as hazardous waste (180103) and will be collected by an approved contractor for incineration. Liquid waste containing viable GMM (including spent culture media) is to be inactivated chemically with Distel 1:20, Virkon or Precept before discarding to achieve 6 log kill. As with the solid waste this will be sent off site for incineration. Inactivation of GMM waste and subsequent disposal will be detailed in the Waste Management SOP. Risk Assessment and training is to be made available to all staff dealing with GMM waste.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

The current NovalGen laboratories location gives us access to the risk assessments and GMO safety committee of our landlord. Consequently, NovalGen staff are familiar with the relevant procedures for working with GMMs and have access to risk assessments performed and reviewed by the GMO safety committee. The GMMs we propose to use are a subset of those detailed in these risk assessments.

Project Containment

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**Name**

ZYGOSITY LTD

**Campus Estate or Research Centre**

LI KA SHING CENTRE

**Road Name**

ROBINSON WAY

**Town**

CAMBRIDGE

**County**

CAMBRIDGESHIRE

**Postcode**

CB2 0RE

**Country**

ENGLAND

**Tel Number**

+44 7570 610119

**Fax Number**

0

**E-mail**

blank

**HSE Division**

blank

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Cancer Research UK Cambridge Institute Biological Safety Committee & Cancer Research UK Cambridge Institute Heath and Safety Operations Manager

<table>
<thead>
<tr>
<th>Laboratory</th>
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Non-microbial

Other (please specify)  
Tick if confidential

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</table>
All biological waste produced will be inactivated in a 2% Virkon soln (where necessary) for at least 10 min. Solid biological waste (for example the bacterial pellet) will then be disposed of in the biohazard waste. Liquid waste will be disposed of down the sink with copious amounts of running water.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Attached are procedural and GM risk assessments to cover work to be carried out on bacterial transformation and plasmid DNA prep & generic retrovirus work.

Project Ref 3612/21.1

Date Ackn’d 23/04/2021

CU2 Project Title Preparation of editing vectors

Class Class 2

Culture Vol Class 2 < 1 Litre

Culture Volume Class 3-4

Non-GMM Consent Granted

Project notified under transitional arrangements N

Withdrawn N

Tick if notifying a connected programme of work N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

02/03/2022
## Project Additional Information

### Purposes of the contained use

- Bacterial transformation and plasmid DNA prep & generic retrovirus work

### Recipient or parental organism

- Continuous human cell lines (i.e. Hela, HEK293, tumour-derived cell lines)
- Continuous rodent cell lines (i.e. 3T3, CHO)
- Primary mouse tissue (from animals hosted in the Biological Resource Unit and devoid of pathogens)
- Patient-derived xenografts (passed in rodent after initial transplantation)

### Host/vector system

- Retrovirus, standard (a plasmid containing the viral genome, in a self-inhibiting cassette, is transfected into a packaging cell line (i.e. PlatA, Phoenix) containing the viral capsid, polymerase and accessory proteins. Optionally, the VSV-G envelope protein can also be added through a separate plasmid. The insert can contain the WPRE transcriptional element.

### Origin & function

The vectors used will all be self-inactivating and replication-incompetent, that is, each retroviral vector particle infects and gives genomic modification of one cell, and that cell will not, and indeed cannot, subsequently produce and release further viral particles.

### Evaluation of foreseeable effects

Retroviruses give infection of, and stable genomic integration into, dividing cells of a wide variety of tissue types with relatively high efficiency, including human and mouse. These vectors derived from retroviruses provide an excellent vehicle of achieving the required genomic modifications and will be useful tools with which to carry out the genetic manipulation of cells.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

All cell lines, human and mouse, are considered GM Class 1 before the packaging/infection. While the GM classification of the infected cells is creased to GM Class 2 during the described process, these cells can return to containment level 1 after they have been washed or passaged 4 times after the infection. The rationale behind this 'demotion' is that the retroviral GM construct is self-inactivating and non-replicative, and therefore infected cells lack the ability to produce new virus. The virus originally used for infection is diluted at every subsequent wash, and rigorous testing has shown that no virus remains after 4 passages.

GM Class 2 agents and GMOs shall only be handled and stored in Containment Level 2 laboratory, set up and operated according to the appropriate laws and requirements.

Routine CL2 control measures include but are not limited to: restricted access, occupational health clearance of workers, training and supervision requirements, no sharps, no vortex mixing, use of Class II microbiological safety cabinets conforming to the appropriate standards, closed rotor/bucket centrifuges opened in the safety cabinet, disposable nitrile gloves conform to appropriate standards, CL2 dedicated lab equipment, separate lab coats for CL2, hands washed before leaving CL2 lab, and emergency and contingency arrangements.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste shall be mixed with not less than an equal volume of a 2% solution of the peroxygen disinfectant DuPont Rely On Virkon for at least 10 minutes prior to disposal to drain via a sink designated for this purpose, and this shall all be done within the CL2 lab. This will inactivate all viral particles, cells and other biological agents.
liable to be present. All solid waste shall be collected in double autoclave bags in an appropriately labelled leak-proof rigid container, then autoclaved on site (within the premises of the Cambridge Institute but not within the laboratories) to deactivate all biological agents, and this then disposed by incineration as hazardous 'clinical' waste by a licensed contractor at the licensed site. The only exceptions are any sharps and pipettes, which shall be collected in appropriately labelled rigid sharps bins designed to collect such articles, and thence autoclaved and incinerated as for the other solid waste. Any spills shall be treated with an equal volume of 2% Virkon for at least 10 minutes. Work surfaces shall be routinely disinfected with 1% Virkon for at least 10 minutes.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Attached are procedural and GM risk assessments to cover work to be carried out on bacterial transformation and plasmid DNA prep & generic retrovirus work.

Project Containment

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GM Centre Number: 3613

Data Premises Notified (Originally) 04/05/2021

Transferred from 1992 Regs? N

Transitional Premises

Class

Data Premises Closed

Transitional Premises

Emergency Plan Required?

Non-GMMs N

Withdrawn N

Name

NUNTIUS THERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre

UNIT 013

Building

WESTBOURNE STUDIOS

Road Name

242 ACKLAM ROAD

District

PORTOBELLO

Town

LONDON

County

GREATER LONDON

Postcode

W10 5JJ

Country

ENGLAND

Tel Number 07515362573

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The person in charge has 17 years' experience in carrying out biological research related to contained use. He has work in both academia and industries and has been conducting risk assessment and training others to perform procedures relating to contained use materials. He will perform annual review on the risk assessment.

<p>| Level 1 (GMMs) | Yes |
| Level 2 (GMMs) |
| Level 3 (GMMs) |
| Level 4 (GMMs) |
| Non-microbial  |
| Other (please specify) | Tick if confidential |</p>
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<td>Transgenic Plants</td>
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For activities involving GMMs, describe the waste management measures which will apply to the activity

The GMM in our case will be mammalian cells transfected with plasmid DNA. To kill cells, Virkon solution (1 tablet of Virkon=1% solution when diluted in 500ml of water) will be added to the cells (1:1 v/v to medium). The liquid waste will be kept in contact with Virkon for at least 30 minutes before disposing it down the sink.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The procedures suggested should be enough to minimise the risk of conducting the GMM work.
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| Comments                           |             |

| Date at Which Additional Info Submitted | 02/03/2022 |
Premises Addresses

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The person used is a Professor of Molecular Virology at the University of Liverpool. They have 34 years experience of genetic manipulation of viruses, including pox viruses, Chair a genetic modification safety committee at the University of Liverpool and Chair the University of Liverpool Biohazards Committee.

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
-------------|-------------|-------------|-------------|--------------
Level 1 (GMMs) | Yes | |
Level 2 (GMMs) | |
Level 3 (GMMs) | |
Level 4 (GMMs) | |
Non-microbial | |
Other (please specify) | | Tick if confidential |
Bacteriology | Parasitology | Transgenic Birds | Microbiology Research |
Virology | Yes | Transgenic Animals | Gene Therapy |
Transgenic Fish | |
For activities involving GMMs, describe the waste management measures which will apply to the activity

Bison, contained on the farm on the Rhug Estate will be inoculated by a veterinary surgeon experienced with handling bison with MVA recombinant(s) expressing OvHV-2 glycoproteins. The bison will be handled by the farm staff using purpose built handling pens feeding onto a curved chute terminating in a Warwick cattle crush (squeeze type). This crush can safely restrain bison of all sizes being easily adjusted by the operator.

Inoculation will take place to deliver up to 1 x 10^9 PFU of recombinant MVA into the neck of bison via the intramuscular route. The bison will be accessed through a door in the side of the crush safely permitting access to the neck whilst restraining the bison. We will use a sterimatic gun for inoculation e.g. http://www.molevalleyfarmers.comlmvf/storeJproducts/molecare-injector-gun-2ml This consists of a high quality Simcro syringe with metalluer-lock and selectable dose in 0.2ml increments. The Sterimatic system automatically protects and cleans the needle with 2.5% glutaraldehyde, this helps provide user safety and reduces potential injection site abscesses and disease transmission between animals. Injection site will be swabbed after vaccination with alcohol. After individual vaccination each bison will be released into the handling pens before being housed in a shed adjacent to the handling system. Housing will take place for one week after vaccination with forage supplied to the bison from outside the pen via a feed passageway.

Solid waste: Steeping overnight in a solution of 2% w/v virkon. Virkon has been validated as an effective decontaminant for MVA. Disposable PPE will be incinerated. Decontaminated glass vials and other sharps will be disposed of in sharps waste. Non-disposable PPE e.g. overalls, wellingtons will be washed after decontamination.

Liquid waste: Steeping overnight in a solution of 2% w/v Virkon. Virkon has been validated as an effective decontaminant for MVA. Accidental spills of vaccine will be either soaked up with absorbent material and the area decontaminated with 2% Virkon or by covering in virkon powder prior to wiping up with absorbent material.

All equipment and protective clothing will remain overnight in the crush area being soaked in Virkon for 24 hours. The vaccine vial will be immersed in Virkon together with the injection gun and the needles. The crush area will be sealed off from the rest of the farm for 24 hours. Following removal of the equipment the crush area will be cleaned and disinfected with Virkon and sealed off from the rest of the farm for a further 24 hours.

We will also perform environmental sampling to detect MVA DNA in bison secretions and in the containment environment. To do this, samples will be collected using boot socks and then analysed for the presence of MVA using a peR assay for virus DNA.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
This work was risk assessed by the Dept of Infection Biology, University of Liverpool GMSC on 21/1/20 and approved for use as a class I activity. The virus is based on modified vaccinia Ankara (MVA; ACDP 1) with the insertion of coding sequences for the green fluorescent protein and glycoprotein B from ovine herpesvirus 2 neither of which were deemed likely to increase the pathogenicity or tropism of the virus. Given the extensive knowledge and use of MVA and recombinant derivatives there are therefore negligible risks to either the vaccinated animals, humans or the environment. The premises and procedures for vaccination and waste disposal were inspected by members of the GMSC and deemed appropriate for contained use for the purposes of vaccination with the MVA recombinant.
<table>
<thead>
<tr>
<th>Name</th>
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<tr>
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<tr>
<td>BABRAHAM RESEARCH CAMPUS</td>
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**Emergency Plan Required?**
- N

**Transitional Premises**
- N
- Non-GMMs: N
- Withdrawn: N

**Data Premises Notified (Originally)**
- 14/05/2021

**Date at Which Additional Info Submitted**
- 02/03/2022
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The laboratory safety committee comprises the CEO, CTO and chair of the scientific advisory committee, all have PhD plus > 20 years laboratory management and safety experience. Specific advice to the committee on contained use of GMM provided by consultant with PhD + > 20 years laboratory experience in the use of GMMs as well as in consultation with the Babraham Research Campus Biological Safety Officer. Health and safety is reviewed approximately monthly and as required by work ongoing.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 1 (GMMs)</td>
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<td>Parasitology</td>
<td>Transgenic Birds</td>
<td>Microbiology Research</td>
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</table>

Tick if confidential

02/03/2022
Solid biological laboratory waste (e.g. bacterial agar plates, plastics, gloves) from our class 1 GM work is collected in laboratory waste bins (Eurobins) which are sealed and labelled by our staff. The sealed bins are then removed by the Babraham campus waste team to a central collection point where they will be placed into labelled 210 litre yellow Eurobins. The 210 litre bins are then collected by a registered clinical/GM waste company and incinerated (see derogation application below).

Liquid class 1 GM culture waste (e.g. cultures and spent media after isolation of cells) is inactivated by treatment with Virkon. After full inactivation (1% Virkon treatment overnight), liquid waste is disposed of into the waste water supply. Associated plastics will then be disposed of as the solid waste. Surfaces will be routinely cleaned and decontaminated with 2% Chemgene.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Comments of the Safety Officer: proposed work is low risk and risks and control measures adequately identified in the risk assessment. As a tenant of BBT Ltd we have all of our risk assessments approved by the Babraham Biosafety Committee prior to any laboratory work and this covers correct procedures for genetic modification as well as contained use.
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Name

ELEVEN THERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre
THE CRUK INSTITUTE

Building
LI KA SHING CENTRE

Road Name
ROBINSON WAY

District

Town
CAMBRIDGE

County
CAMBRIDGESHIRE

Postcode
CB2 ORE

Country
ENGLAND

Tel Number
01223 769825

Fax Number
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E-mail

HSE Division
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Comments

Date at Which Additional Info Submitted

02/03/2022

02/03/2022

Page 15134 of 15326
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Copies of the GM project and risk assessments were circulated to members of the CRUK biological safety committee for consideration prior a meeting on the 16th of March 2021. The project was subsequently approved by the committee and signed by the chair.

<table>
<thead>
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Other (please specify) Tick if confidential

Bacteriology

Parasitology

Transgenic Birds

Transgenic Animals

Transgenic Fish

Microbiology Research

Gene Therapy

Virology

Transgenic Birds

Transgenic Animals

Transgenic Fish

02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

All liquid waste shall be mixed with not less than an equal volume of a 2% solution of Virkon for at least 10 minutes prior to disposal to drain via a sink designated for this purpose. This will inactivate all cells and other biological agents liable to be present.

All solid waste shall be collected in biohazard bags in an appropriately labelled rigid container, then autoclaved on site (within the premises of CI but not within the laboratories) to deactivate all biological agents, and this then disposed by incineration as hazardous ‘clinical’ waste by a licensed contractor at a licensed site. The only exceptions are any sharps and pipettes, which shall be collected in appropriately labelled rigid sharps bins designed to collect such articles, and thence autoclaved and incinerated as for the other solid waste.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Copies of the GM project and risk assessments were circulated to members of the CRUK biological safety committee for consideration prior a meeting on the 16th of March 2021.

The project was subsequently approved by the committee and signed by the chair.

Project Ref 3618/21.1

Date Ackn'd 26/05/2021

Date Project Ceased

Genetic Modification of cell lines using lentiviral vectors

Class CultureVol

Consent Granted

Project notified under transitional arrangements
The objective of this project is the modification of mammalian cell lines (human and/or murine) or avian cell lines with transgenes expressing a short hairpin RNA (shRNA), or specific proteins involved in the cellular RNA silencing mechanism to allow high-throughput RNAi screening. A broad range of our projects are aimed at the investigation of the cellular processes regulating the biogenesis and function of small and long non-coding RNAs in mammals. Such projects often require the production of genetically modified cells in which a) key components of the physiological pathways (i.e., small RNA biogenesis, long non-coding RNAs, piRNA clusters) are knocked down by means of RNA interference, or removed by disruptive mutation) components of the pathway are overexpressed or modified, or c) components of the pathway are modified by fusing them with a protein reporter or tag, allowing their detection by imaging and/or their purification for further biochemical studies.

Categories of gene inserts / gene modifications:
- Selection cassettes (e.g. neomycin, hygromycin, puromycin, thymidine kinase)
- Reporter genes and tags (i.e. EFGP, Discosoma fluorescent proteins, and their variants. Hemagglutinin tag, FLAG tag, myc-tag, SNAP-tag, HALO-tag, His-tag)
- Genes coding for small RNAs (i.e. shRNAs, miRNAs, piRNAs).

Recipient or parental organism
Mammalian cell lines including commercially available primary cells and well characterised cell lines (immortalised) of human origin. All without a significant risk of transmitting pathogens to human.

Host/vector system
LENTIVIRUS, THIRD GENERATION - Four plasmids are co-transfected on 293T / 293FT cells or similar. The plasmids contain: 1) The gag and pol genes, plus other accessory viral genes 2) The rev gene, 3) an envelope protein gene (i.e. VSV-G), 4) the viral genome and insert, in a self-inactivating (SIN) unit. The insert can contain the WPRE transcriptional element.
LENTIVIRUS, SECOND GENERATION - Three plasmids are co-transfected on 293T/293FT cells or similar. The plasmids contain: 1) The gag, pol and rev genes, together with other viral accessory genes, 2) an envelope protein gene (i.e. VSV-G), 4) the viral genome and insert, in a self inactivating (SIN) unit. The insert can contain the WPRE transcriptional element.
RETOVIRUS - A plasmid containing the viral genome, in a self-inhibiting cassette, is transfected into a packaging cell line (i.e. PlatA, Phoenix) containing the viral capsid, polymerase and accessory proteins. Optionally, the VSV-G envelope protein can also be added through a separate plasmid. The insert can contain the WPRE.
Origin & function

We will use PCR amplification to amplify approximately 10 regions across the viral genome in separate reactions. These regions will include the 5'UTR, the entire Orf1_ab segment, Spike, Orf7, Orf8, the Nucleocapsid region and the 3'UTR. In addition, we will create a GFP/Luciferase reporter construct under the control of the 'E' 5'UTR of the SARS-CoV-2 genome. The left primer for the 5'UTR will include a T7 promoter sequence. The final product will have the SARS-CoV-2 genome with two important differences: (i) the E (Envelope) and the M (Membrane) genes will be totally absent from the replicon and the Spike protein will have multiple loss of function mutations to prevent its expression and a full deletion of the Furion Cleavage site that is required for pathogenicity. These two genes are essential for particle assembly and therefore the virus will be deleterious (ii) Instead of the 'E' gene, the replicon will have a reporter construct.

T7 RNA polymerase will be used for transcription of the replicon in vitro.

RNA from the T7 polymerase reaction will be transfected into recipient cells using RNA transfection reagents (e.g lipofectamine)

Evaluation of foreseeable effects

CELL CULTURE

This project will make use of commercially available primary cell or continuous mammalian cell cell lines, all with a history of safe use and where possible screened as free from HepB, HepC and HIV. Some examples of such lines are A549, 293, HeLA, etc. In such cells, adventitious pathogens are unlikely to be present, and even if present originally, are likely to have been deactivated or diluted away. The chances of such cells, on entering the body, evading immune surveillance and giving colonisation etc are remote.

LENTIVIRAL / RETROVIRAL VECTORS

The lentiviral and retroviral vectors used will all be well characterised, and with a history of safe use.

These vectors will all be self-inactivating and replication-incompetent, that is, each lentiviral/retroviral vector particle infects and gives genomic modification of one cell, and that cell will not (and indeed cannot) subsequently produce and release further viral particles. This is due to a deletion in the 3' LTR (TATA deletion) which prevents excision after genomic integration.

The genetic material inserted in the destination cell line through the GM modification here proposed include selection cassettes (producing genes that confer selective immunity to cytotoxic agents), reporter genes (i.e. fluorescent proteins), regulatory sequences (promoters, enhancers, etc) and other accessory sequences.

These inserts do not present any significant hazard to human health in the form and amounts in which they are used, either when expressed alone or fused to endogenous proteins.

The hazards arising from an inserted transgene can be divided into two categories: hazards due to the insertion of the transgene(s) directly into the human genome and hazards due to cultured cells that have been genetically modified by the insertion of the transgene(s).

Direct insertion of the transgene in the human genome would require a contact between the original lentivirus/retrovirus stock and a human host. The presence of the VSV-G envelope protein on the viral particles described in this assessment results in an increased tropism, and therefore enhances this hazard.

While the transgenes described in this document would NOT result, if integrated, in nocive effect, the insertion itself could result in the inactivation of an endogenous genes (insertional mutagenesis), with potential harmful consequences.

While extremely unlikely, this could, in a worst-case scenario, result in the inactivation of a tumour-suppressor gene and in the production of tumorigenic cells. It has to be noted, however, that in most cases a single mutation is not sufficient to result in tumorigenesis, and that such aberrant cells would most likely be cleared by the immune system.

Since the viral genome used in this project is “inactivated”, and not able to replicate past a first round of infection, any infected cell would not be able to propagate the infection. Indeed, the insertion of any transgene in a human host would require a direct contact with the original viral stock, during its manipulation. The risk of such an event can be minimised by adequate control measures.

A second category of hazard is the hazard due to cultured cells that have received the transgene via the lentivirus/retrovirus. As mentioned above, the transgenes used in this study do not result in any significant hazard.

As for the case of a direct infection of a human host, the insertion of the transgene in cultured cells could increase their proliferative potential due to insertional mutagenesis. However, these GM cells, even if made tumorigenic in nature, would not be able to colonize the human body due to the activity of the immune system.

02/03/2022
All the inserts used in this project could include the WPRE element, which can result in an increase of the expression level of other transgenes present in it. This could potentially exacerbate transgene-related hazards.

Cell lines expressing biologically active proteins could lead to accumulation of molecules in the media. However, experiments are small scale and short term and not designed to produce large amounts of active proteins. Again, the risk is low and can be minimised by adequate control measures.

This project makes use of genetic inserts that might be hazardous in nature, including short hairpins targeting onco-suppressor genes. Examples of onco-suppressor genes include BRCA1, TP53, RB1 etc.

The insertion of these transgenes in human cells via a primary infection could result in potentially serious health hazards: a worst-case scenario is that the virus might insert a dominant acting oncogene under the control of an active promoter, activate an endogenous oncogene, or knock-down a tumour suppressor, thus contributing to the genesis of human cancers. However, this would require penetration of the human body, cellular uptake and integration into the genome of the relevant tissue. The risk is fairly low, since the transgenes themselves are unable to mobilize from the GM cells once inserted. The risk can be further minimised by adequate control measures (Good Microbiological Practice, avoid work if having an open wound, avoiding aerosols and sharps and wear personal protective clothing.)

The insertion of these transgenes in cultured cells would increase their tumorigenic potential if these cells invade a human host. This even, however, is extremely remote due to the activity of the immune system, that would recognise and destroy the invading cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Biological waste – inactivate with an equal volume of 2% Virkon for a minimum contact time of 10 minutes. Discard the Virkon solution down the sink. Solid waste like used agar plates, TC flasks, falcon tubes and other plastic ware (excluding pipettes) will be placed in yellow waste disposal bags and incinerated by a licensed contractor.

Chemical waste: discard allowed substances (pbs, salts, non-hazardous substances) down the sink with plenty of water. Discard regulated or hazardous chemicals in an appropriate chemical waste bottle for proper disposal. If the chemical is contained in cell culture medium, inactivate with an equal volume of 2% virkon first, and then dispose as chemical waste.

Solid waste – cell pellets, extracted tissues, including gels, eppendorfs, placed in yellow waste bins for incineration.

Tips – dispose of in plastic jars or in white cardboard waste containers. When full, dispose of the containers in yellow waste bags for incineration.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

Copies of the GM project and risk assessments were circulated to members of the CRUK biological safety committee for consideration prior a meeting on the 16th of March 2021.

The project was subsequently approved by the committee and signed by the chair.
**Project Additional Information**

**Purposes of the contained use**

A SARS-COV-2 replicon system provides a safe alternative to study the virus and examine anti-viral therapies. We aim to use the replicon system to test the effect of our novel therapy strategies on viral replication.

**Recipient or parental organism**

The starting material is extracted RNA of a SARS-CoV-2 virus. E coli host will be E coli K12 derivatives (e.g. HB101, XL1B, DH5a, Y1088, 1089, 1090, C600) Mammalian cell lines including commercially available primary cells and well characterised cell lines (immortalised), all without a significant risk of transmitting pathogens to
**Host/vector system**

We will use PCR to amplify 10 regions across the viral genome in separate reactions. The final product will be cloned into pUC based plasmid backbones which are non-mobilised. This includes pUC 12, 18, 19, pSP65, pBSBluescript and pTOPO-PCR. Transfection of mammalian cells will be accomplished by RNA mediated transfection.

**Origin & function**

We will use PCR amplification to amplify approximately 10 regions across the viral genome in separate reactions. These regions will include the 5'UTR, the entire Orf1_ab segment, Spike, Orf7, Orf8, the Nucleocapsid region and the 3'UTR. In addition, we will create a GFP/Luciferase reporter construct under the control of the ‘E’ 5'UTR of the SARS-CoV-2 genome. The left primer for the 5'UTR will include a T7 promoter sequence.

The final product will have the SARS-CoV-2 genome with two important differences: (i) the E (Envelope) and the M (Membrane) genes will be totally absent from the replicon and the Spike protein will have multiple loss of function mutations to prevent its expression and a full deletion of the Furion Cleavage site that is required for pathogenicity. These two genes are essential for particle assembly and therefore the virus will be deleterious (ii) Instead of the “E” gene, the replicon will have a reporter construct.

T7 RNA polymerase will be used for transcription of the replicon in-vitro.

RNA from the T7 polymerase reaction will be transfected into recipient cells using RNA transfection reagents (e.g lipofectamine)

**Evaluation of foreseeable effects**

This work will make use of an extracted/inactivated RNA of a positive sample for SARS-CoV-2. This material will arrive at the CI from external collaborators already inactive, and won’t be inactivated on the premises. Purified SARS-CoV-2 RNA is non hazardous and is routinely processed at CL1 in the institute (i.e. to perform qPCR).

As part of our replicon construction, we will delete the E (Envelope) and M (Membrane) genes from the SARS-CoV-2 genome and will introduce loss of function mutations to the Spike gene.

The M gene is the most abundant protein structural protein in coronaviruses and exists in all branches of Coronavirinae (Masters and Perlman, Fields Virology, 2013). A segment of its transmembrane domain is highly conserved and even found in torovirus, the closet outer group to Coronavirinae (Masters and Perlman, Fields Virology, 2013). The protein is required for virion production in coronavirus (Schoeman and Fielding, Virology, 2019).

The E gene is less conserved but required to maintain pathogenesis of the virus (Masters and Perlman, Fields Virology, 2013). Studies with SARS-CoV have shown that a deletion of the E gene resulted with highly attenuated virus (Schoeman and Fielding, Virology, 2019). Moreover, studies with viral-like particles in SARS-CoV have demonstrated that both E and M are necessary for the formation of virus like replicons and a single expression of each does not produce any viral particles (Mortola and Roy, FASEB, 2004). Taken together, these lines of evidence show that these genes are highly essential for the viability of SARS-CoV-2.

The Spike protein is essential for receptor binding cellular entry (Masters and Perlman, Fields Virology, 2013). We will create a premature stop codon within the first 10 aminoacids of the ~1000aa of this protein and will also introduce a premature stop codon. Moreover, we will remove the Furion Cleavage Site. A recent Nature paper showed that without this site the SARS-CoV-2 virus is substantially attenuated and does not create any pathology in an hamster model (Johnson et al., Nature, 2021).

To further demonstrate the deleterious effects of these two genes, we analyzed the mutation profile of over 26,000 SARS-CoV-2 genomes in GISAID. We calculated the probability to observe a nonsense mutation in each gene adjusted to its length. Our analysis shows that E and M and among the top three structural proteins with the smallest number of nonsense mutations. Other proteins such as ORF8, ORF6, and N exhibited 4x to 5x fold increase in these loss of function mutations. Again, this shows that deleterious effects and the importance of these genes for viral survival.

As stated above, a replicon system have no ability to produce viral particles and therefore become infectious. One extremely unlikely and theoretical event is that the replicon system will undergo a recombination with a coronavirus that somehow infected the cells to acquire the missing proteins. This scenario is partially impossible. The specific SARS-CoV-2 Spike protein is absolutely essential for its increased pathogenicity compared other coronaviruses (Xia et al., Signal Transaction and Targeted Therapy, 2020). The replicon system has no Furion Cleavage Site and its Spike has two loss of function mutations. Thus, even if somehow a low pathogenicity coronavirus (“common cold”) infects the cells, it lacks the SARS-CoV-2 Spike protein and any recombinant product

As stated above, a replicon system have no ability to produce viral particles and therefore become infectious. One extremely unlikely and theoretical event is that the replicon system will undergo a recombination with a coronavirus that somehow infected the cells to acquire the missing proteins. This scenario is partially impossible. The specific SARS-CoV-2 Spike protein is absolutely essential for its increased pathogenicity compared other coronaviruses (Xia et al., Signal Transaction and Targeted Therapy, 2020). The replicon system has no Furion Cleavage Site and its Spike has two loss of function mutations. Thus, even if somehow a low pathogenicity coronavirus (“common cold”) infects the cells, it lacks the SARS-CoV-2 Spike protein and any recombinant product
will not be able to produce back the SARS-CoV-2 spike. The only way for the replicon to acquire this protein is a recombination with SARS-CoV-2. However, that means that the cells were exposed to SARS-CoV-2, which creates a hazardous condition regardless of the replicon system. Moreover, as we are working in a CL2 conditions, the personal protective equipment and the hoods should prevent human-to-cell transmission of any coronavirus.

Finally, before T7 transcription, we are going to evaluate to deeply sequence the DNA of the replicon system in order to ensure that all the changes above were made. Only after verification, we will transcribe and transfect the cells.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| N/A |

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

| Biological waste – inactivate with an equal volume of 2% Virkon for a minimum contact time of 10 minutes. Discard the Virkon solution down the sink. Solid waste like used agar plates, TC flasks, falcon tubes and other plastic ware (excluding pipettes) will be placed in yellow waste disposal bags and incinerated by a licensed contractor. Pipettes and stripettes will be placed in a dedicated pipette waste bin prior to incineration. Chemical waste: discard allowed substances (pbs, salts, non-hazardous substances) down the sink with plenty of water. Discard regulated or hazardous chemicals in an appropriate chemical waste bottle for proper disposal. If the chemical is contained in cell culture medium, inactivate with an equal volume of 2% virkon first, and then dispose as chemical waste. Solid waste – cell pellets, extracted tissues, including gels, eppendorfs, placed in yellow waste bins for incineration. Tips – dispose of in plastic jars or in white cardboard waste containers. When full, dispose of the containers in yellow waste bags for incineration. |

Is an emergency plan required according to regulation 20?  

| N |

If yes, tick to confirm that it is attached to this form  

| N |

Tick to confirm that you have attached a risk assessment to this form  

| Y |

Tick if you are claiming exemption from disclosure for section of the risk assessment  

| N |

Please enter comments on the GM safety committee on the risk assessment  

Copies of the GM project and risk assessments were circulated to members of the CRUK biological safety committee for consideration prior a meeting on the 16th of March 2021. The project was subsequently approved by the committee and signed by the chair.

Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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</thead>
<tbody>
<tr>
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GM Centre Number: 3619

Data Premises Notified (Originally) 03/06/2021

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises

Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

UNIVERSITY OF THE HIGHLANDS & ISLANDS

Name 2

Department

Campus Estate or Research Centre

Building

Road Name
12B NESS WALK

District

Town INVERNESS

County

Postcode IV3 5SQ

Country SCOTLAND

Tel Number 01463279000

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022

Page 15144 of 15326
### Premises Addresses

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee  

Y
The Division of Biomedical Science GMO safety committee (GMOSC) oversees all GMO projects within the Division. The GMOSC will provide a review and advice on the following issues:

- All genetically modified organism risk assessments.
- Biological agent risk assessments for COSHH notifiable projects.
- Any other relevant hazardous work that the GMOSC determines is needed to be reviewed and approved by the committee.

The GMOSC will check that all documents and procedures required at a local level within the Division have been completed prior to commencement of the project and reported. The GMOSC will also investigate and report accidents and incidents, when required.

It is anticipated that the number of GMO projects within the Division will be small (less than 3 per annum) therefore the GMOSC will meet on an ad hoc basis when required. There will be at least one physical meeting of the GMOSC per annum to review relevant documents and procedures. All meeting agendas and minutes will be issued at least 2 days before every meeting and minutes reviewed for accuracy prior to approval, electronic copies will be kept. The committee will report regularly to the School of Health research committee and the University Executive Office Health and Safety committee.

The GMOSC consists of:

**Chair (academic representative):**
This position is filled by a Reader in the Genetics and Immunology Department. This individual has a PhD and 20 years' experience in academic bioscience research laboratories. This individual has substantial practical experience of working at containment level 2, cell and tissue culture and GMO projects in human cell lines and micro-organisms. This individual has considerable experience of health and safety and risk assessment in a research laboratory.

**Head of Division (management representative):**
This position is filled by a Professor in the Free Radical Research Facility. The individual has a PhD and over 30 years experience in academic research laboratories in several HEIs in the UK. A proportion of his research has involved human and mammalian cell culture and treatment and he has overall responsibility for Health and Safety in the Division. This individual has considerable experience of health and safety and risk assessment in a research laboratory.

**Staff (technical) representative:**
The staff representative position is filled by the Genetics and Immunology research technician. This individual has a BSc (Hons) in Life Sciences with more than 20 years experience in academic research labs and 5 years' experience in industry in quality control. This individual has substantial experience in molecular biology techniques and in working at containment level 2 cell culture with several different cell types including human and trypanosomatid cell lines. GMO experience includes working with microorganisms and trypanosomatid cell lines. This individual has considerable experience of health and safety and risk assessment in a research laboratory.

**GMO safety officer (Divisional health and safety officer):**
This position is filled by the laboratory manager. This individual has a PhD and over 25 years experience in academic bioscience research labs and is currently responsible for health and safety in the Division. This individual has considerable previous hands-on experience of working at containment level 2, cell culture and GMO projects in mammalian cell lines and microorganisms with responsibility for many aspects of the projects including health and safety, waste disposal and record keeping. This individual has considerable experience of health and safety and risk assessment in a research laboratory.
Committee member:
This individual is a senior scientist with a PhD and more than 30 years experience of working in biomedical sciences research. This person has extensive experience of tissue culture working with cell lines, primary cells and tissues at containment level 2, as well as some experience of GMO work using mammalian cells and microorganisms. This individual also has additional responsibility for research governance and compliance. This individual has considerable experience of health and safety and risk assessment in a research laboratory.

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<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<td>Non-microbial</td>
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Other (please specify)  
Tick if confidential  

Bacteriology  
Parasitology  
Transgenic
Birds

Virology  
Transgenic
Animals
Transgenic
Fish

Mycology  
Transgenic
Invertebrates
Transgenic
Plants
Other (please specify below)  

Tick if confidential

For activities involving GMMs, describe the waste management measures which will apply to the activity

All plasticware and solid lab waste used in GMO projects will be autoclaved (100% kill) at 121C and 15 psi for 30 minutes, prior to disposal. The autoclave servicing and temperature validation will be completed annually by suitably qualified external contractors. A printed record with time and date will be kept for each autoclave run for GMO waste.

Liquid waste will be inactivated with 2% Rely-On+ Virkon solution for 24 hours and then be discarded to drain with water.

Solid waste will be disposed of via a licensed waste contractor to be incinerated as clinical waste. Accidental spillages of GMO waste will be inactivated with 2% Rely-On+ Virkon solution. Benches and the immediate will be cleaned using 2% virkon solution after each use.

Tick to confirm that you are attaching a summary of the risk assessment  

02/03/2022
The GMO safety committee had some comments regarding transport, the procedure for spillages and clarity around stating that the project uses commercially purchased GMO cell lines. These concerns have been addressed in the risk assessment to satisfaction of the GMO safety committee.
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Name

ROCKEND LTD

Campus Estate or Research Centre

Building

Road Name

3RD FLOOR, 1 ASHLEY ROAD

Town

ALTRINCHAM

County

CHESHIRE

Postcode

WA14 2DT

Country

ENGLAND

Tel Number

07446932081

Fax Number

0

E-mail

HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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<td>PUDDICOMBE WAY</td>
<td>UNIVERSITY OF CAMBRIDGE</td>
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Y

Give brief details of the genetic modification safety committee:

Our expert was previously head of GM and biosafety at a known Cambridge institute.

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<tr>
<td>Bacteriology</td>
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<td>Transgenic</td>
<td>Microbiology Research</td>
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<tr>
<td>Birds</td>
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We will make use of Chemgene and Virkon to sterilize and kill any live material. Chemgene 2% spray will be used for the sterilization of surfaces by spraying, and replaced after 1 month. It will be applied to surfaces for >5 minutes to ensure biological material killing. Virkon 1% will be used to treat cultures, pipette tips, and any cultureware that comes into contact with cells. Appropriately treated waste (i.e. Virkon 1%) will be disposed of in standard sealable yellow 60L eurobins. A UN3245 sticker will be placed on the bin, including biohazardous symbol. The bin will not be overfilled and will be replaced after 1 month. The bin will be marked with date sealed. The sealed bin will be placed in the building's clinical waste bin area, where it will be removed and taken to the incinerator by central Babraham Research Campus services.

For activities involving GMMs, describe the waste management measures which will apply to the activity

We will make use of Chemgene and Virkon to sterilize and kill any live material. Chemgene 2% spray will be used for the sterilization of surfaces by spraying, and replaced after 1 month. It will be applied to surfaces for >5 minutes to ensure biological material killing. Virkon 1% will be used to treat cultures, pipette tips, and any cultureware that comes into contact with cells. Appropriately treated waste (i.e. Virkon 1%) will be disposed of in standard sealable yellow 60L eurobins. A UN3245 sticker will be placed on the bin, including biohazardous symbol. The bin will not be overfilled and will be replaced after 1 month. The bin will be marked with date sealed. The sealed bin will be placed in the building's clinical waste bin area, where it will be removed and taken to the incinerator by central Babraham Research Campus services.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

This CU1 notification along with the attached risk assessment have been reviewed by the Rockend biological safety officer (BSO), Rockend's head of discovery, Rockend's Chief Scientific Officer (together - Rockend's genetic modification safety committee) as well as the BBT Biosafety committee.
Our objective is to understand the molecular mechanisms involved in blood cell formation (haematopoiesis) from different stem cell sources (haematopoietic stem cells, pluripotent stem cells). The research projects will include transduction of genes with expected biological effects into mammalian cell lines and primary cells by the means of CRISPR based editing.

By identifying and understanding fundamental development processes, our projects should allow us to better control in ex vivo differentiation protocols and improve the production of therapeutically relevant cell types, for example haematopoietic stem cells.

The recipient microorganism is especially disabled, non-colonising and unlikely to survive outside laboratory culture. The hosts can be assigned to ACDP hazard group 1. iPSCs are considered especially disabled, unable to survive outside the laboratory environment. However, they have been derived from human primary tissue with associated low risk of pathogen contamination. Their culture has been assigned CL2.

- Mammalian primary cells are considered especially disabled, unable to survive outside the laboratory environment. However, they have been derived from primary tissue with associated low risk of pathogen contamination. Their culture has been assigned CL2.
- Mammalian cell lines are considered especially disabled.

The vector system will be purified CRISPR components and guide RNAs. These vector systems are completely inert and incapable of further replication. They will be transduced into cells by nucleofection in a dedicated apparatus. In the absence of these apparatus, the CRISPR components are unable to edit or self propagate (and are just purified protein and RNA components). Where transgenes are integrated into the host, the code for these genes will be present on naked plasmids only, and also delivered to the hosts by nucleofection.

Type 1 inserts

None of the inserted sequence will generate product presenting acute toxicity (e.g. toxin).
cDNA will code for genes, for example transcription factors, signal transducers, growth factors and receptors which are expected to have a biological role in transduced recipient cells (cell growth, death, differentiation).

Type 2 inserts
shRNA will produce non-coding RNA sequences targeting cell endogenous mRNA in the recipient host. Downregulation of these genes are expected to have a biological role in transduced recipient cells (cell growth, death, differentiation).

Type 3 inserts
Reporter genes will code for proteins with fluorescent or enzymatic properties allowing for easy identification of genetically modified (GM) cells. These are thought to have no deleterious biological effect on expressing cells.

Type 4 inserts
Selection genes will produce enzymatic proteins able to inactivate specific antibiotic families allowing for selection of GM cells. These are thought to have no deleterious biological effect on expressing cells.

Type 5 inserts
Functional non-coding sequences will act as promoters of transcription (promoters and enhancers), local chromatin structure modifiers (enhancers and insulators), RNA transcript stabilizers (WPRE) or translation regulators (IRES, 2A). They will be used (independently or in combination) with viral expression cassettes to provide control of transgene expression, type used depending on targeted cells. Type 5 inserts are not expected to have a biological effect separately from their associated coding sequence (type 1-3). There remains the unlikely exception of genomic integration events which could lead to endogene dysregulation.

Type 6 inserts
Neutral non-coding sequences will be used in particular for gene targeting projects to promote homologous recombination at defined genomic loci (intron/exon genomic sequences). Use of type 6 inserts should not have biological effect on the recipient host by themselves.

**Evaluation of foreseeable effects**

The GMOs themselves are not capable of surviving outside of the tissue culture environment and thus there is little to no risk of environmental contamination.

As we do not plan on using viral GM methods there is no risk of our approach producing any propagative particles. GM cells may be transformed/oncogenic but are incapable of colonising and causing disease. These cells could represent a genuine risk in the occurrence of accidental injection to an immune-deficient individual or to the corresponding primary cell donor but there will be no such opportunity for involved staff.

We do not expect any harmful products resulting from the increased transcribed/translated genes.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The Jeffrey Cheah Biomedical Centre building management team manages two waste streams. One waste stream for CL1 waste and a second for all CL2 waste.

CL2 solid waste management:
CL2 solid waste includes items such as general tissue culture consumables used in CL2 (consumables having been in contact with or containing residual GM material) tissue culture plates, tissue culture flasks, tubes etc. Serological
pipettes and pipette tips will be collected in Bio-bins initially. Once Bio-bins are full they will be sealed and placed into autoclave bags in clearly demarcated CL2 waste bins. When CL2 autoclave bags are 3/4 full, they will be loosely closed with autoclave tape to reduce the risk of waste spillage. The 3/4 full cable tied bags will be placed into red CL2 waste bins and lids secured with cable ties. The red CL2 waste bins will be collected at least once a day by the building services team who will transport them to the basement of the building to be autoclaved. The building's waste autoclaves will have maintenance schemes written by Bureau Veritas and will be inspected annually by Bureau Veritas and PSSR. Additionally, the buildings waste autoclaves will be serviced regularly and annually validated under a service contract. The autoclave will run at the pre-set guidelines for deactivation of CL2 waste, this will be validated on every run using indicator tape. Autoclaved waste will be transported off-site by Novus Environmental as ‘offensive waste’. CL2 liquid waste, that is safe to dispose of down the drain after inactivation, including; used tissue culture media, PBS used to wash cells etc will be treated with Chemgene before disposal down the drain. Inactivation of liquid waste will be achieved by using a final concentration of 10% volume/volume Chemgene with a minimum contact time of 30 minutes, before being disposed of down the sink/drain. Chemical waste for disposal from CL2 tissue culture (that is not suitable for disposal down the drain) will be collected in bottles, treated with a final concentration of 10% volume/volume Chemgene for a minimum of 30 minutes. Bottles will be labelled with their contents and placed in the chemical fume hood for disposal by building services. All work surfaces shall be left for 30 seconds before subsequently wiping with 70% ethanol.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

This CU2 notification along with the attached risk assessment have been reviewed by the MTI biological safety officer (BSO), and MTI Drug Discovery and Onco-Innovation Programme Manager. This CU2 application will be noted at the next MTI Safety Committee Meeting, which includes our biological/genetic modification safety committee function.

Project Containment

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Yes

Give brief details of the genetic modification safety committee

CSO, Biological Safety Officer and GMSC chair of Phenotypeca Ltd  
Professor of Genetics with the following relevant experience:  
Served on the Genetic Modification Sub Committee (2001-2005) for the University of Leicester and chaired the subcommittee from 2003-2005. Currently serves on the EFSA microorganism working group and is familiar with the current EU regulations. Extensive experience modifying yeast (S. cerevisiae) and E. coli for 30 years under GMM approval at the Universities of Oxford, Leicester and Nottingham

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Bacteriology | Parasitology | Transgenic Birds | Microbiology Research | Yes

02/03/2022
We sub-let laboratory space within the Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS). As such, our rental agreement gives us access to the building’s waste disposal and sterilisation facilities. Therefore, all waste generated through project activities will be disposed of in accordance with practice enforced by SIPBS. All biological and GMM waste is treated as clinical waste; therefore any waste derived from our work processes that involve GMM will be processed as clinical waste. Solid waste such as agar plates, non-recyclable plastic, paper towel, absorbent powder, nitrile gloves placed into autoclave bags within yellow clinical waste bins. Small volumes of contaminated liquid waste (<50 mL) can also be placed into autoclave bags. Each bag of clinical waste contained in autoclave bags will be sterilised by use of a validated autoclave. Each bag of autoclavable clinical waste must have a yellow label with the name and floor number of the person generating the waste. This is to ensure that the waste can be traced back to the user in case of any problems.

Large volumes of contaminated or spent media will decanted into dedicated rigid containers marked waste and autoclaved (using validated autoclave) prior to disposal as clinical waste.

Control measures such as the use of autoclave tape and temperature charts (to track autoclave temperature and hold times) will be used to ensure waste is correctly sterilised.

Autoclave operating conditions for sterilisation of waste: 126 degrees centigrade, 15 psi, hold time 30 minutes

Disinfection of reusable items will be achieved by soaking the items in high level disinfectant such as Virkon or Chemgene HLD4 (prepared and used as directed by the manufacturer) for a sufficient time. Monitoring of this (to ensure thorough disinfection) will involve taking samples from the process and plating out onto non-selective agar plates in order to detect re-growth and therefore failed disinfection.

For activities involving GMMs, describe the waste management measures which will apply to the activity

As reviewer of this application, I am confident that the correct containment level has been determined for the work proposed. The yeasts Saccharomyces cerevisiae and Pichia pastoris and the specific E. coli strains are considered GRAS and QPS and the strains used in this proposal have been used safely for decades in numerous labs. They are disabled in that they have auxotrophies requiring nutrients making them unlikely to survive outside of the lab. The yeast are benign except in cases of severe immune deficiencies or severe allergies which would be relevant to all yeast and not just these. The use of non-mobilisable vectors in disabled E. coli is also considered low risk. Any products made will be at small lab scale but should be assessed for potential toxicity or other detrimental effects. The scale of production in Xias Bio Limited is small and the risk of exposure is low. It is agreed that procedures within the company will ensure that containment at the required level is provided. All GM risk assessments, staff training, and GMO research will be compliant and aligned with the University of Strathclyde policy.
### GM Centre Number: 3623

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**Name**

GLYKOGEN LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

DOUBLEDYKES ROAD

**Town**

ST ANDREWS

**District**

**County**

FIFESHER

**Postcode**

KY16 9DR

**Country**

SCOTLAND

**Tel Number**

01224 395048

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**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The Biological Safety Officer, The James Hutton Institute, Invergowrie, 002 5DA. The BSO has more than thirty years of experience working with GMMs, principally GM plant pathogens. The BSO has been in position for more than five years, was a deputy BSO for ten years and served on the institute's GMSC prior to that. The BSO has qualified as a Biosafety Practitioner Level 1 on an ISTR approved course.

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Tick if confidential  

02/03/2022  
Page 15160 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

Waste is inactivated in the building by autoclaving at 123°C for sixty minutes to give 100% kill of laboratory E. coli strains. A load probe is used as appropriate. Waste runs are monitored using 3M™ Comply™ Thermalog™ Steam Chemical Integrator Strips and/or an independent temperature logger and/or a biological indicator. Autoclaved solid waste is passed to a contractor for incineration.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

The adviser considered the proposed activities as commonplace low risk work. The adviser thought that the cloning of low-risk mammalian antibody fragment sequences and their expression using phage display systems, with a long history of use, and vectors based on commercial expression vector systems as chimeric antibody sequences in disabled E. coli K12 derived strains presented a low risk to human health and the environment. The adviser thought that using containment level 1 measures would reduce the risks to human health and the environment to negligible levels. Therefore, the adviser thought that the work should be classified as Class 1.
# GM Centre Number: 3624

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Main person has approximately 26 years of people management, including managing facilities teams.
Experience of setting up new laboratories and SOPs in areas such as life sciences and forensics.
Lots of IS09000 and GLP experience.
And will be auxiliated by the lab management that has 8 years' experience working with opportunistic pathogens and
GM. Experience in developing and implementing SOPs for testing antimicrobial properties in medical devices. 2 years' experience working under IS013485 and IS09001

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|-------------|-------------|-------------|-------------|-------------|-------------|
Level 1 (GMMs) | Yes | |
Level 2 (GMMs) | |
Level 3 (GMMs) | |
Level 4 (GMMs) | |
Non-microbial | |
Other (please specify) | Tick if confidential | |

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research
---|-------------|------------------|---------------|-------------|-------------|-------------|
02/03/2022 | Page 15163 of 15326
For activities involving GMMs, describe the waste management measures which will apply to the activity

Biological waste. The volumes of biological samples used are very small (below 200μl). The typical experiment will require volumes of 1-10μl of the biological material (depending on titer). Most of the contaminated material will be small volumes of diluted sample contained in Eppendorf tubes or equivalent tubes, glass coverslips and pipette tips.

- Liquid waste: due to the small amounts of liquid used and the nature of the biological material. Contaminated material will be disposed into appropriate bins for biological waste, identified for the purpose.
- Spills will be cleaned using inactivating chemical agents, such as bleach or other appropriate liquid for decontamination.
- Sharps, such as glass coverslips, will be discarded into puncture-resistant containers designed for the purpose of sharps disposal.

Biological waste bins and sharps are disposed using a 3rd party specialised in biological waste.

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment Y

Please enter comments of the GM safety committee on the risk assessment

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**Name**

NORTH OF ENGLAND ZOOLOGICAL SOCIETY

**Campus Estate or Research Centre**

CHESTER ZOO

**Road Name**

CAUGHALL ROAD

**District**

UPTON-BY-CHESTER

**Town**

CHESTER

**County**

CH2 1LH

**Postcode**

ENGLAND

**Tel Number**

01244 380280

**Fax Number**

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**E-mail**

HSE Division

NORTH WEST

**Date at Which Additional Info Submitted**

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

### Give brief details of the genetic modification safety committee

Advice received from Faculty of Health and Medical Sciences, University of Surrey

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- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
Modified vaccinia virus Ankara (MVA) is a replication deficient virus. It has lost its ability to replicate in most mammalian cells and cannot revert to virulence, even in irradiated animals that are otherwise immunocompromised. Antigens expressed by MVA are not expected to alter the ability of the attenuated vectors to infect or replicate in mammalian cells or their ability to survive in the environment. The trial will not amplify or modify the GMM, it will apply and ultimately destroy it. Any items in contact with the substance (vials, syringes, needles) will be disposed of as clinical waste for incineration.

For activities involving GMMs, describe the waste management measures which will apply to the activity

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Other(s) Vaccine trial

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Royal College Veterinary Surgeons (RVCS) registered
## VACCINE MANUFACTURING & INNOVATION CENTRE ((VMIC))

### Name

**VACCINE MANUFACTURING & INNOVATION CENTRE ((VMIC))**

### Campus Estate or Research Centre

**THOMPSON AVENUE**  
**HARWELL CAMPUS**

### Town

**OXFORD**

### County

**OXFORDSHIRE**

### Postcode

**OX11 0GD**

### Country

**ENGLAND**

### Tel Number

**07946716227**

### Fax Number

**0**

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### Date at Which Additional Info Submitted

**02/03/2022**
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

VMIC has a biological safety committee, that reports through the VMIC safety officer to the VMIC executive management. The committee meets throughout the year to deal with matters arising from biological safety including genetically modified organisms.

<table>
<thead>
<tr>
<th>Laboratory Level 1 (GMMs)</th>
<th>Animal Unit Level 1 (GMMs)</th>
<th>Growth Room Level 1 (GMMs)</th>
<th>Glass House Level 1 (GMMs)</th>
<th>Large Scale Level 1 (GMMs)</th>
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Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
All waste liquids are collected in two 20,000 litre treatment tanks and heat inactivated prior to release to public sewer or to contracted registered road haulier waste disposal company.

Laboratory solid waste is collected in airtight sealable containers for disposal via a registered waste management company. Laboratory liquid aqueous waste is chemically inactivated prior to entering the building liquid waste streams. Liquid organic waste is collected and disposed using a registered waste management company.

Manufacturing solid waste is either autoclaved onsite prior to being bagged and disposed in landfill or double bagged and removed for incineration by a registered waste management company.

The VMIC production facility consists of both laboratory research and development and large scale vaccine manufacturing activities using GMMs. The classification of GMO's handled within the facility is limited to biological safety level 2 and contained accordingly. The traceability of all GMMs within the facility is achieved from receipt to disposal using electronic systems (manufacturing activites using a Laboratory Information Management System and research and development using an Electronic Laboratory Notebook system). GMM storage is in secured cold storage with controlled access.

Mechanical segregation of the GMMs from staff is maintained during use using biological safety cabinets and closed systems with onsite air-quality monitoring for the manufacturing areas. Liquid waste stream treatment is built into the building design via two 20,000 litre treatment tanks prior to release to public sewer or collection by contracted registered specialist waste company. A rotational disinfectant program is used throughout the facility to control microorganism contamination, with manufacturing areas being maintained as clean rooms with air quality between class B and class D. Solid contaminated waste is autoclaved prior to disposal or collected by a registered commercial waste operator for incineration.

It is the conclusion of the VMIC Biological Safety Committee that the VMIC facility is designed to control all the potential risks from working with genetically modified organisms to a classification of category 1.
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Name  
RSK ADAS LTD

Name 2

Department

Campus Estate or Research Centre

Road Name
172 CHESTER ROAD

Town
HELSBY

County
CHESHIRE

Postcode
WA6 0AR

Country
ENGLAND

Tel Number
01159 229249

Fax Number
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E-mail

HSE Division
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Comments

Date at Which Additional Info Submitted

02/03/2022

Page 15171 of 15326
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The work to be carried out is a continuation of many years (20+) R&D by the organisation albeit when we were based within an academic collaboration and worked within University departments (this work has previously been agreed as class 1 activity at the level of University GMSC). The ADAS research team are now in premises of their own and are looking to continue similar research using the same techniques. The person with overall responsibility for the research team is the director of the business, trained to the level of PhD and has been working in scientific research for the last 25 years. Previously this individual was involved in the writing and maintenance of the GMO RAs within academic departments and has also been involved in a consultancy role for a CRO that has periodically had to notify HSE of studies where clients have wanted them to test products containing GMOs. For this contained used application the GM risk assessment has been based on a similar form that we had used within a department of the University of Nottingham. In this application the GMO RA has been updated and discussed within the ADAS research team. At present this has been discussed with the person acting as GMSC meeting chair and the site BSO (who is also the proposer of the GMRA). In this instance-and because the class 1 use has been assessed before, it has was agreed that further staff involvement for the activity was not necessary on this occasion. Between the BSO and the chair there is 40+ years experience of working with GMOs and specifically phage display technology- the subject of the RA that supports this notification of premises. It is anticipated that the GMO RA will be reviewed on a 6 monthly basis with the same two individuals plus a ADAS senior research scientist, to ensure that the RA is still valid, up to date and within scope for the R&D activities that are being carried out. To ensure that these are not missed they will be an agenda item to be added to the 6 monthly quality meetings that we currently have planned.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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02/03/2022
The decontamination and disposal routes set out below have been used by our laboratory personnel (when working within an academic department) for over 20 years. We have discussed this again within our internal GMSC and see no reason to change this approach.

All contaminated disposable plasticware will be collected in appropriately labelled containers with a disposable liner. Once the container is full, the liner will be removed and placed in an autoclave bag closed with autoclave tape for autoclave sterilisation. These items will be inactivated on an autoclave cycle at 121°C for 15 mins, demonstration that the cycle had been successful would be by the autoclave cycle report and the visual indicator check of the autoclave tape. Inactivated waste will be disposed of via the laboratory waste stream (bagged waste to landfill).

Liquid cultures (phage and E.coli) and including spent media supernatants will be inactivated by the addition of the halogenated tertiary amine solution Trigene to the manufacturers recommended concentration. Inactivated solutions will be disposed of via liquid waste using copious flushing with fresh water.

Glassware and any reusable plasticware will initially be treated with Trigene to the manufacturers recommended dilution before extensive washing and then sterilisation by autoclaving.

Benches, cabinets and incubator surfaces will be periodically surface sterilised by wiping down with 70% alcohol solution, any spills will be treated by addition of Trigene to the spill, before mopping up with a spill kit and its disposal after autoclave.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment  

No comments.
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

The University of Warwick HSE and Genetic Modification Biosafety Committee

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<th>Level 1 (GMMs)</th>
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Non-microbial

Other (please specify): Tick if confidential

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</table>
All GMM work undertaken by NanoSyrinx Ltd at the University of Warwick premises will be done in compliance with existing long-standing biosafety compliance. Measures for the management of waste include:
1. Non-biological waste (e.g. used gloves, tissue, labware not used for culture etc) is disposed of in to hazardous waste bins which are clearly demarcated (red bins with yellow liners). This waste is collected in to a secure compound and removed by a University approved 3rd party contractor.
2. Contaminated biological waste is autoclaved on-site. Waste is added to metal autoclave tins which are double lined with autoclave bags. University central technical support actions the disposal of waste after autoclaving in line with established protocols.
3. All other routine waste (such as paper towels from handwashing, office waste etc) is disposed off in the institutes regular waste.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Risk assessments are due to be carried over from previous academic lab work based at the University, of which this research is a continuation. The existing risk assessments have been approved by the University HSE and biosafety committee for academic work. We have not yet formally adopted the risk assessments via approval from the University of Warwick HSE, but as the research we are conducting is a direct continuation of academic research, within the same containment facility, we expect the existing risk assessments to be appropriate for our research activity and acceptable by the committee.
We are awaiting final approvals/revisions for the risk assessments at the present time, and so will provide these in a follow-up email.
GM Centre Number: 3631

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Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: Yes

Give brief details of the genetic modification safety committee:

Biological Safety Officer at a UK academic institution with experience in GMM/O risk assessments.

<table>
<thead>
<tr>
<th>Level</th>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
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All liquid waste arising from/contaminated with cell cultures (of E.coli and cyanobacteria) will be made unviable/inactive by 1-2% Virkon disinfectant for 30 minutes. All solid waste arising from/contaminated with cell cultures will be made unviable/inactive by use of autoclave at 121-125°C for 15-30 minutes (as per manufacturer's instructions). All waste to be collected in biohazard bins to be disposed of by a specialized, licenced contractor.

For further details on the following, please see attached risk assessment:

[All E. coli strains and all cyanobacteria strains to be used are classified as ACDP Hazard Group 1 (low hazard). The transformed (genetically engineered) cyanobacteria strains have no additional harmful properties in comparison the wild-type strains. As such, according to guidelines of “The Genetically Modified Organisms (Contained Use) Regulations 2014”, transformed cyanobacteria strains have Risk Classification Class 1 with only Containment Level 1 required.]

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Comments from Biological Safety Officer at a UK academic institution:

"The nature of the experiments justifies the classification of containment level and biohazardous group."

"For good practice, the risk assessment should be assessed case by case, meaning that if the inserted genetic material to be expressed results in higher pathogenicity or toxicity (however unlikely), the biohazardous group and containment level would proportionally increase. In such a scenario, further notification to HSE would be required"
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- **Yes**

**Give brief details of the genetic modification safety committee**

Hoxton Farms has a genetic modification safety committee consisting of two experts: a company director who holds a DPhil (PhD) degree in molecular biology and has ten years' experience conducting genetic modification experiments and associated risk assessments in industrial and academic laboratory settings; and (2) an external advisor who is the facilities manager at a 450-person university molecular biology department in England. The committee meets on a quarterly basis (or more frequently if the need arises) to develop and review comprehensive GM risk assessments.

**Laboratory**

- **Level 1 (GMMs)**
  - Yes

**Animal Unit**

- **Level 2 (GMMs)**
- **Level 3 (GMMs)**
- **Level 4 (GMMs)**

**Growth Room**

- **Non-microbial**

**Glass House**

- **Other (please specify)**

**Large Scale**

- **Tick if confidential**

**Bacteriology**

- **Parasitology**

**Transgenic Birds**

**Microbiology Research**

- **Yes**
Autoclaving is the preferred route for inactivation of GMMs. Cultures will be sterilized in the autoclave located in the Hoxton Farms laboratory at 121°C for 20 minutes. The autoclave is serviced, calibrated and validated once a year by Astell Scientific Ltd., 19 - 21 Powerscroft Road, Sidcup, Kent DA14 5DT. Inactivated cultures will be poured down the laboratory sink while flushing copiously with water.

Where autoclaving is not possible, Virkon disinfectant will be added to a final concentration of 1% w/v and left for at least 8 hours before being poured down the laboratory sink and flushing copiously with water.

Contaminated single use pipette tips, serological pipettes, flasks and other single-use plasticware are placed into yellow “Bio-Bins”. These are labelled and sealed before they leave the laboratory. Waste is collected weekly by Initial Medical Services for incineration as offensive clinical waste.

Glassware will be disinfected with 1% w/v Virkon disinfectant and left for at least 8 hours before being poured down the laboratory sink and flushing copiously with water. The empty glassware will then be sterilised by autoclave.

Virkon is a safe and effective decontaminant shown to be active against 300 + strains/clinical isolates from 71 bacteria and resulting in >5-log reduction in viable organisms when used as a 1% dilution (Hernandez et al., 2000 [J. Hosp. Inf. 46(3)203-209], ‘Virkon Background Information’ https://www.fishersci.co.uk/webfiles/uk/webdocs/SLSGD05.PDF).

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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**Name**

BETTER DAIRY LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Building**

OPEN CELL

**Road Name**

15 PENNARD ROAD

**District**

**Town**

LONDON

**County**

GREATER LONDON

**Postcode**

W12 8DW

**Country**

ENGLAND

**Tel Number**

07903127870

**Fax Number**

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**E-mail**

**HSE Division**

blank

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

We have submitted risk assessments on our GMO work to both the Imperial College Innovation Hub and the OpenCell Biotech Campus, which were approved. The Innovation Hub laboratory manager has over seven years experience as a safety officer in a synthetic biology division, and twenty years experience running laboratories.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
<td>Level 1 (GMMs)</td>
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Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology
- Transgenic Animals
- Transgenic Fish
- Gene Therapy
For activities involving GMMs, describe the waste management measures which will apply to the activity

Spillages will be dealt with by swabbing with 70% alcohol spray which is fungicidal.

Contaminated, solid waste such as plastic disposables, tissue paper, culture plates, etc., are bagged in autoclave bin bag. The bag is autoclaved at 121°C for 20 mins, 1 bar pressure. The autoclave tape has to turn black before being placed into a yellow bin bag and tied up. The bags are finally placed in large yellow waste bin.

Liquid culture medium is treated by adding to Virkon or bleach to achieve a final concentration of 10% and left to stand overnight before discharge to the sewer.

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

Please enter comments of the GM safety committee on the risk assessment

The advisor has reviewed the accompanying risk assessment and regards the proposed work as GM Class 1 requiring Containment Level 1.
GM Centre Number: 3634

Data Premises Notified: 02/08/2021  (Originally)

Transferred from 1992 Regs?: N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required?: N

Non-GMMs: N

Withdrawn: N

Name

FABRICNANO LTD

Name 2

Department

Campus Estate or Research Centre

Building

Road Name

4TH FLOOR, 184-192 DRUMMOND STREET

District

Town

LONDON

County

GREATER LONDON

Postcode

NW1 3HP

Country

ENGLAND

Tel Number

07565809262

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The committee comprises of people with years of experience working with GMOs (Post-doc and PhD level) and a person that has undertaken the Biosafety Practitioner Level 1 (Foundation) online course provided by the Biosafety Training Institute (BTI) at The University of Edinburgh.

<table>
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The waste management measures include use of chemical disinfectant and autoclave (steam based decontamination) in the lab, where the work is conducted. Solid bio-wastes will be autoclaved and given to a licensed company for disposal. Liquid wastes will be collected and autoclaved using the decontamination cycle described below. Alternatively, appropriate amount of disinfectant will be added and allow sufficient time to be effective before disposing.

Examples of chemical disinfectants:
- Virkon (peroxygen): Effective against a range of bacteria, fungi and viruses. To be used at 1% w/v, i.e. 10 g Virkon in 1L liquid waste for at least 30 mins.
- Methylated spirits (alcohols): Effective against bacteria, and fungi (but not bacterial spores). To be used at 70% v/v for at least 10 mins.
- Bleach (not household) (chlorine): Effective against vegetative bacteria, virus, fungi. To be used at 2500-5000 ppm (or 0.25-0.5% v/v) for at least 30 mins.

Autoclave decontamination cycle:
The decontamination (waste treatment) cycle will include 121-124°C for 15 mins, followed by 134°C for 3 mins. Autoclaves is fitted with chart recorders or alternative devices for recording run-time parameters (temperature, time, pressure etc). These will be checked and kept for each sterilisation run to ensure that the autoclave continues to perform satisfactorily. Use of autoclave tape is an indicator that the desired temperature has been reached.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:
The strains that will be used have been demonstrated to be avirulent, have a long history of safe use and the genetic lesions are well understood. The genetic material involved includes genes from bacteria that translate specific proteins. These proteins do not have any harmful biological activity and thus it is unlikely that they will give rise to any harm.

As the range of proteins expressed in our lab will increase, the nature and biological activity of every new protein will be reviewed and appropriate safety measures will be followed.

Project Ref: 3634/21.1

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<td>Production of recombinant proteins from bacterial or yeast hosts for use in biocatalysis</td>
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<td>1-50 Litres</td>
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## Project Additional Information

### Purposes of the contained use

Overexpression of recombinant proteins in bacterial or yeasts hosts. Cells will be homogenised and the lysate will be used directly or purified to afford the protein of interest, which then will be used to catalyse chemical reactions.

### Recipient or parental organism

**Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae, Kluyveromyces lactis, Pichia pastoris, Streptomyces coelicolor**

### Host/vector system

**Host:** Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae, Kluyveromyces lactis, Pichia pastoris, Streptomyces coelicolor  
**Vector:** Plasmids for expression in bacteria (such as pET) and in yeasts (such as Ylp, YEp, YCp)

### Origin & function

The genetic material involved includes genes from bacteria that translate specific proteins. These proteins do not have any harmful biological activity (e.g. toxins, cytokins, growth factors, allergens, hormones or oncogenes) and thus it is unlikely that they will give rise to any harm. The intended function includes utilizing these proteins for biocatalytic reactions in vitro.

### Evaluation of foreseeable effects

Variants of E. coli strains K-12 and B will be used. The strains that will be used have been demonstrated to be avirulent, have a long history of safe use and the genetic lesions are well understood. The gene inserts that will be used do not have harmful biological activity (e.g. toxins, cytokins, growth factors, allergens, hormones or oncogenes). Thus, it is very unlikely that the gene products will give rise to any harm.  
As the range of proteins expressed in our lab will increase, the nature and biological activity of every new protein will be reviewed and appropriate safety measures will be followed.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

No larger GMOs will be used.

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)
Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The waste management measures include use of chemical disinfectant and autoclave (steam based decontamination) in the lab, where the work is conducted. Solid bio-wastes will be autoclaved and given to a licensed company for disposal. Liquid wastes will be collected and autoclaved using the decontamination cycle described below. Alternatively, appropriate amount of disinfectant will be added and allow sufficient time to be effective before disposing.

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Virkon (peroxygen): Effective against a range of bacteria, fungi and viruses. To be used at 1% w/v, i.e. 10 g Virkon in 1L liquid waste for at least 30 mins

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Is an emergency plan required according to regulation 20?  N

If yes, tick to confirm that it is attached to this form  Y

Tick to confirm that you have attached a risk assessment to this form  Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  N

Please enter comments on the GM safety committee on the risk assessment

The strains that will be used have been demonstrated to be avirulent, have a long history of safe use and the genetic lesions are well understood. The gene inserts that will be used do not have harmful biological activity (e.g. toxins, cytokins, growth factors, allergens, hormones or oncogenes). Thus, it is very unlikely that the gene products will give rise to any harm.

As the range of proteins expressed in our lab will increase, the nature and biological activity of every new protein will be reviewed and appropriate safety measures will be followed.

Project Containment

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02/03/2022
GM Centre Number: 3635

Data Premises Notified: 02/08/2021
Transferred from 1992 Regs?: N
Transitional Premises Class: N
Data Premises Closed: N
Transitional Premises Emergency Plan Required?: N
Non-GMMs: N
Withdrawn: N

Name
ACHILLES THERAPEUTICS UK LTD

Name 2

Department

Campus Estate or Research Centre

Building

Road Name
245 HAMMERSMITH ROAD

District

Town
LONDON

County
GREATER LONDON

Postcode
W6 8PW

Country
ENGLAND

Tel Number: 020 8154 4600
Fax Number: 0

E-mail

HSE Division: blank

Comments

Date at Which Additional Info Submitted: 02/03/2022
**Premises Addresses**

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**Premises Conditions**

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: Yes

- **Give brief details of the genetic modification safety committee**
  
  Consultant BSO has given advice.

- **Laboratory**
  - Level 1 (GMMs): Yes
  - Level 2 (GMMs)
  - Level 3 (GMMs)
  - Level 4 (GMMs)
  - Non-microbial

- **Animal Unit**
  - Other (please specify)

- **Growth Room**

- **Glass House**

- **Large Scale**

- **Tick if confidential**: No

- **Bacteriology**
  - Parasitology
  - Transgenic Birds

- **Parasitology**

- **Transgenic**
  - Animal
  - Fish

- **Transgenic**

- **Microbiology**
  - Research

- **Microbiology**

- **Gene Therapy**: Yes

- **Transgenic**

- **Birds**

- **Virology**
  - Transgenic Animals

02/03/2022
Solid waste will be placed in bags for autoclaving. Pipettes will be placed in yellow bio bins before placing in the bags. Liquid waste will be inactivated with 2% virkon for a minimum of 30 minutes before disposing either down the laboratory sink and flushing thoroughly or collected into a waste container and taken off-site for disposal by a licensed waste contractor. Waste bags will be sealed and labelled as GM waste. Bags will be autoclaved using the autoclave in the building before they are collected for disposal by the authorised hazardous waste disposal company, Albus Environmental.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
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</table>

**Name**

ETCEMBLY LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

OXFORD SCIENCE PARK

**Building**

MAGDALEN BUILDING

**Road Name**

ROBERT ROBINSON AVENUE

**District**

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX4 4GA

**Country**

ENGLAND

**Tel Number**

07515 574182

**Fax Number**

0

**E-mail**

**HSE Division**

blank

**Comments**

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

**Premises Address**

**ETCEMBLY LTD**

**Address:**
- OXFORD SCIENCE PARK
- MAGDALEN BUILDING
- ROBERT ROBINSON AVENUE
- OXFORD OX4 4GA

**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

[ ] Yes

Give brief details of the genetic modification safety committee

Person: Ph.D. Qualified in Infection and Immunity followed by over 5 years in academic medical research then 20 years in the Biotech sector including a 10 year spell as Biological Safety Officer.

Professional qualifications/courses

Health and Safety Qualification: National General Certificate: NEBOSH Level 3 (Pass with Credit).

<table>
<thead>
<tr>
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**Other (please specify)**

Tick if confidential

[ ]

- Bacteriology: Yes
- Parasitology
- Transgenic Birds
- Microbiology Research: Yes

02/03/2022
Yes
Yes

Well characterised laboratory strains of E. coli and commercially obtained mammalian expression cells (related to CHO cells) will be used in this study.

Biohazard group 1:

Non-pathogenic E. coli (such as BL21, Rosetta, TOP10, DH5a, K12)

ExpiCHO-S™ Cells

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will be placed in sealed bins and incinerated by an off-site incineration company. Waste from our GM work at Class1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the genetically modified entities. The biocidal and viricidal activity of Virkon can be reviewed here: https://virkon.us/#downloads

Virkon solution will be prepared fresh to maximise biocidal activity.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Well characterised laboratory strains of E. coli and commercially obtained mammalian expression cells (related to CHO cells) will be used in this study.

Biohazard group 1:

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Virkon solution will be prepared fresh to maximise biocidal activity.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The chosen E. coli strains and ExpiCHO cell lines are considered non-harmful to human health or the environment. They both have exacting growth requirements and would rapidly perish outside of controlled laboratory conditions. The gene inserts confer no pathogenic traits to the recipient cells.
Name

CELOMATICS BIOSCIENCES LTD

Name 2

Campus Estate or Research Centre

BIOCITY

Road Name

PENNYFOOT STREET

Town

NOTTINGHAM

Building

119-122 STEWART ADAMS BUILDING

District

County

NOTTINGHAMSHIRE

Postcode

NG1 1GF

Country

ENGLAND

Tel Number

0115 7870081

Fax Number

0

E-mail

HSE Division

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Date at Which Additional Info Submitted

02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

GMO safety committee is formed of the following members:
1) BSO - individual with extensive experience of genetic modification issues (>20 years), fully certified and trained by completing the full Biosafety Practitioner course "Biological Safety Officer's Workshop", accredited by the ISTR Biosafety Accreditation Scheme. The BSO is external to the applicant organisation and will provide advice to the committee.
2) Committee chair - non-executive director of the applicant organisation, currently holds a full Professorship in a reputed academic institution. Has extensive experience of genetic modification procedures (>30 years) and currently runs an academic research group.
3) Research scientists - one Scientist and one Senior scientist with >12 years of combined experience in genetic modification procedures in microorganisms (e.g. E. coli and S. cerevisiae) and human cells. These members will draft and implement Risk Assessments to carry out Level I and Level II GMO research activities.

The committee will meet (in person or via videocall) every 6 months or whenever a new Risk Assessment is required, to review existing procedures. In case face-to-face meetings are not possible or not-advised due to COVID restrictions, an email-based review and approval system will be established.

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02/03/2022
For activities involving GMMs, describe the waste management measures which will apply to the activity

To minimise the risk of escape of GMO’s to the environment, all contaminated waste streams generated during the course of this work will be decontaminated within the laboratory block. Cultures will be grown within leak proof vessels. Teknon™ Biocleanse disinfectant will be added to liquid waste and allowed to stand for 24 hours prior to disposal. Solid waste will be autoclaved prior to incineration. This will achieve 100% inactivation of the lentiviral particles as well as all the materials, reagents and biological samples in contact with them.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Risk assessment by the BSO and genetically modified organisms safety committee supports the use of CL1 containment.

Project Ref 3637/21.1

Date Ackn’d 19/08/2021
Date Project Ceased
CU2 Project Title Transduction of human genetically-modified U20S cells with lentiviral particles containing shRNA library

Class 2
Culture Volume Class 2 < 1 Litre
Class 3-4 Non-GMM Consent Granted
Tick if notifying a connected programme of work  

Project notified under transitional arrangements  

Historical Significant Changes
Historical Date of Additional Info
Significant Change ID
Date of Significant Change

**Project Additional Information**

**Purposes of the contained use**

**Recipient or parental organism**

**Host/vector system**

**Origin & function**

**Evaluation of foreseeable effects**

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

Not applicable

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

Not required - the disinfectant (Teknon Biocleanse) and equipment (autoclave) are available on site and as such all the suitable waste management processes described in section 12 can be effectively implemented on site.

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

To minimise the risk of escape of GMO’s to the environment, all contaminated waste streams generated during the course of this work will be decontaminated within the laboratory block. Cultures will be grown within leak proof vessels. Teknon™ Biocleanse disinfectant will be added to liquid waste and allowed to stand for 24 hours prior to disposal. Solid waste will be autoclaved prior to incineration. This will achieve 100% inactivation of the lentiviral particles as well as all the materials, reagents and biological samples in contact with them.
Risk assessment by the BSO and genetically modified organisms safety committee supports the use of CL2 containment.

Please enter comments on the GM safety committee on the risk assessment

Risk assessment by the BSO and genetically modified organisms safety committee supports the use of CL2 containment.

### Project Containment

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Is an emergency plan required according to regulation 20? **N**

If yes, tick to confirm that it is attached to this form **N**

Tick to confirm that you have attached a risk assessment to this form **Y**

Tick if you are claiming exemption from disclosure for section of the risk assessment **N**
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Name

EXOGENE LTD

Name 2

Department

Campus Estate or Research Centre

CORE LAB 1, BIOESCALATOR

Building

INNOVATION BUILDING

Road Name

696 ROOSEVELT DRIVE

District

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX37FZ

Country

ENGLAND

Tel Number

07861889657

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

- A) Divisional Safety Officer, (Clinical) Medical Sciences Division, University of Oxford, Boundary Brook House, Churchill Drive, Headington, Oxford, OX3 7GB
- B) Laboratory Manager, BioEscalator, Innovation Building, University of Oxford, Roosevelt Drive, Oxford, OX3 7FZ

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- Bacteriology Yes
- Parasitology
- Transgenic Birds
- Microbiology Research
- Virology Yes
- Transgenic Animals
- Transgenic Fish
- Gene Therapy Yes

02/03/2022
The waste proposal will strictly follow the COSHH procedures and genetically modified organism regulations. We have submitted a waste disposal form to the BioEscalator, which has been approved already.

For contamination level 1 waste, the waste will be decontaminated using chemical disinfectant, such as Virkon. The validation of decontamination will rely on the manufacturer's data and standard protocols for chemical disinfection. Infected liquid cultures will be decontaminated with Virkon (2-5%) for at least 2 hours and then dispose of in sink.

The safety committee has approved our GMO work and there are no further comments/concerns.
**Project Additional Information**

**Purposes of the contained use**

The aim of the project is to build up a novel AI model to accelerate T cell receptor (TCR) discovery with better specificity and accuracy. As training the AI model requires a large amount of data, we will adopt high-throughput methods mainly for TCR discovery. This involves the use of well-established methods for TCR discovery, including soluble expression of proteins and surface expression of proteins on mammalian cells (mammalian display). DNA plasmids will be transfected into mammalian expression systems for soluble expression of proteins of interest. Lentivectors will be transfected into mammalian cells to produce non-replicating lentiviral particles which will in turn be transduced into mammalian cells for stable surface expression.

**Recipient or parental organism**

1. Expi293F cells (derived from 293, immortalised human embryo kidney cell line)
2. HEK293T cells (derived from 293, immortalised human embryo kidney cell line)
3. Jurkat cells (immortalised T cell line)

**Host/vector system**

DNA plasmids and non-replicating lentivirus particles. An example of DNA plasmids used include:

1. pD649 - a expression vector plasmid used in expression vectors such as HEK and CHO, contains CMV promoter and puromycin resistance for selection in mammalian cells
2. pHR-SFFV - a lentivector used for cloning in gene of interest, contains SFFV promoter
3. pRSV-Rev or similar - a packaging lentivector plasmid that encodes for Rev
4. pMDLg/pRRE or similar - a packaging plasmid that encodes for Gag and Pol
5. pMD2.G or similar - a packaging plasmid that encodes Env

Vectors 2-5 combine to produce a non-replicating lentivirus.

**Origin & function**

DNA plasmid vectors and gene fragments have either been purchased commercially or obtained via Material Transfers Agreement from scientific collaborators. For transient protein expression, DNA plasmids containing genes of interest will be transfected into Expi293F cells to produce soluble proteins. The proteins will be used as reagent tools for functional assays. For stable protein expression, DNA plasmids containing genes of interest will be transfected into HEK293T cells, along with lentiviral packaging vectors to produce lentivirus particles. Lentiviral particles will be transduced into Jurkat cells for stable protein expression on cell surface. These proteins will be used for high-throughput screening of binders against a specific target (mammalian display).

All proteins produced are of human origin and non-toxic to humans.

**Evaluation of foreseeable effects**

The human cell lines described above are classified as risk group 2. 293-derived cell lines are known to contain DNA derived from adenoviruses which are pathogenic to humans. However, due to the viral genome being incomplete, the risk of viral generation is extremely low. The Jurkat cell line is not known to contain any DNA that could cause
pathogenesis to humans. Regardless, all cell cultures have the potential to carry as yet unidentified adventitious agents and thus, any cell culture work will only be performed in a class II biosafety cabinet by an experienced, fully-trained scientist.

Most of the lentiviruses used are derived from HIV. They have the ability to integrate into host chromosomes, infect dividing and non-dividing cells and have high mutation rates. The major risks associated with lentiviruses are:

- Potential for generation of replication-competent lentivirus (RCL)
- Potential for oncogenesis

To circumvent this, we will be using 3rd generation production protocols for lentiviruses that are developed with enhanced safety by eliminating virulence genes and splitting key genes among different vectors and will also be taking extra precautions when handling the lentiviruses (namely extra PPE and double-containment).

More information can be found in our risk assessment which has been written and has been signed off by the institutional health and safety committee.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

| N/A |

### For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

| No derogations applied for. |

### Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

For lentiviral work and mammalian cell culture, liquid waste will be chemically inactivated by incubating with 2% Virkon for 2 hours and disposed of in the sink.

Contaminated solid waste will be double-bagged and autoclaved at 134°C using GMO option and then disposed of in black bag (general waste route) which will be collected by Select Environmental Ltd for incineration.

**Degree of kill:**
- **Autoclaving:** effectively 100% kill (annual validation)
- **Chemical disinfection,** used according to manufacturer’s protocols under standard condition: manufacturer's validation of 99.99% kill

### Is an emergency plan required according to regulation 20?  

| N |

If yes, tick to confirm that it is attached to this form

| N |

Tick to confirm that you have attached a risk assessment to this form

| Y |

Tick if you are claiming exemption from disclosure for section of the risk assessment

| N |

### Please enter comments on the GM safety committee on the risk assessment

The BioEscalator GMO safety committee has approved our risk assessment of class II work, including risk assessment, containment facilities and disposal route.

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**Name**

BITROBIUS GENETICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

LINNARDS LANE

**District**

WINCHAM

**Town**

NORTHWICH

**County**

CHESHIRE

**Postcode**

CW9 6ED

**Country**

ENGLAND

**Tel Number**

07780678648

**Fax Number**

N/A

**HSE Division**

NORTH WEST

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Give brief details of the genetic modification safety committee

The advisor has over ten years experience as a Biological Safety Officer, having previous served as the BSO to two biotechnology companies: the contract manufacturing organisation Cobra Biologics Ltd, and most recently the vaccine and cancer therapy development company Prokarium Ltd (BSO for six years). The advisor has reviewed numerous risk assessments at GM Class 1 and 2 for various species of bacteria (including modified pathogens), viruses and cell lines.

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For activities involving GMMs, describe the waste management measures which will apply to the activity

Solid waste contaminated with GM Class 1 material will be sealed in autoclavable clinical waste bags, autoclaved and collected by a licensed waste contractor. Liquid waste will be inactivated by the addition of sodium hypochlorite to a final concentration of 1% and minimum incubation time of 1 hour, resulting in a six order-of-magnitude reduction in viability, and disposed to drains. Spills will be treated with sodium hypochlorite or 70% ethanol, adsorbed with paper towels and disposed via the solid waste route.

Tick to confirm that you are attaching a summary of the risk assessment  

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The advisor has reviewed the accompanying risk assessment and regards the proposed work as GM Class 1 requiring Containment Level 1.
**GM Centre Number: 3640**

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**Tel Number**

| 07887622153 |

**Fax Number**

| N/A |

**E-mail**

| NORTH WEST |

**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022 |
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### Premises Conditions

- **Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**: Yes

- **Give brief details of the genetic modification safety committee**
  
  All aspects of Health and Safety are managed by the campus Director at Daresbury including Genetic Manipulation work. A link to the support team details is given below.
  
  [https://www.sci-techdaresbury.com/sectors/biomedical/](https://www.sci-techdaresbury.com/sectors/biomedical/)

- **Laboratory**
  - Level 1 (GMMs): Yes
  - Level 2 (GMMs): 
  - Level 3 (GMMs): 
  - Level 4 (GMMs):
  - Non-microbial: 

- **Animal Unit**
  - Other (please specify): 

- **Growing Room**
  - Other (please specify): 

- **Glass House**
  - Other (please specify): 

- **Large Scale**
  - Tick if confidential: 

- **Other (please specify)**
  - Bacteriology: 
  - Parasitology: 
  - Transgenic Birds: 
  - Microbiology Research: 

02/03/2022
The work involves use of standard molecular biology E.coli strains and extracts for micro-scale expression of recombinant enzymes.

All spent cultures will be autoclaved and disposed of via the waste sluice. All smaller volumes (<1ml) will be diluted with bleach and disposed of following site regulations. The largest volume of cultures used will not exceed 1 litre and will typically be 1-5ml.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The applicant has recently retired from running a research laboratory in the area of molecular genetics at the University of Sheffield. He previously held the position of Head of the Department of Molecular Biology and Biotechnology and was a member of the University over-arching Health and Safety Committee. He has considerable experience of managing work in this area in both academic and commercial sectors.

The Daresbury campus has an exemplary track record of supporting Health and Safety and Recombinant DNA technology in this general area.
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Name

BLOOM BIOTECHNOLOGIES LTD

Name 2

Department

Campus Estate or Research Centre

THE JAMES HUTTON INSTITUTE

Road Name

ERROL ROAD

District

MYLNEFIELD

Town

INVERGOWRIE

County

DUNDEE

Postcode

DD2 5DA

Country

SCOTLAND

Tel Number

01382 568853

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

- Genetic Modification Safety Committee appointed consisting of 5 Plant Scientists.
- One Scientist served on GMSC at James Hutton Institute for 15 years.
- Three Scientists have between 10 and 30 years experience conducting GM experiments and preparing risk assessments for GM plants.
- One Scientist has 15 years experience working with GM mouse, viruses and other microorganisms.

| Level 1 (GMMs) | Yes | |
| Level 2 (GMMs) | Yes | Yes |
| Level 3 (GMMs) | Yes | Yes |
| Level 4 (GMMs) | Yes | Yes |
| Non-microbial | Yes | Yes |

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research

Yes

02/03/2022
Solid waste will be collected in autoclave bags and liquid waste in clearly marked glass bottles for autoclaving. Lab waste is inactivated by autoclaving at 121 degrees C for 30 minutes to kill 100% of E.coli and Agrobacterium strains. A load probe is used as appropriate. Waste runs are monitored using 3M Comply Thermalog Steam Chemical Integrator Strips. All waste is contained and autoclaved within the GM containment building (AN). Autoclaves are thermometrically tested. Following autoclaving, all incinerable material is collected for incineration offside.

For activities involving GMMs, describe the waste management measures which will apply to the activity

The GMSC thought the proposed cloning of plant DNA fragments, that do not encode known toxins or allergens, into plasmid vectors for sequencing and subsequent manipulation for expression and silencing (RNAi) in transgenic plants as routine, low risk work. The GMSC thought that as disabled Escherichia coli laboratory strains and disarmed strains of Agrobacterium tumefasciens, which are not pathogenic to humans, would be used and the introduced sequences would be under the control of eukaryotic, not prokaryotic, regulatory sequences with the plant derived sequences containing introns to prevent expression in bacteria the risk to human health was minimal and that the use of Containment Level 1 measures would be adequate to reduce the risks to negligible. Therefore the GMSC thought that the proposed work with genetically modified micro-organisms should be classified as Class 1. Further, as the cloned genes do not encode known toxic or allergenic proteins and the introduced genes are not predicted to increase the levels of toxic or allergenic compounds in the transformed plants, the GMSC thought that the transgenic plants produced would present no greater risk to human health than the untransformed, progenitor plants.
**GM Centre Number: 3644**

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**Name**

| VENOMTECH LTD               |

**Campus Estate or Research Centre**

| Building                   | GROUND FLOOR, BUILDING 500 |

**Road Name**

| DISCOVERY PARK             |

**Town**

| SANDWICH                   |

**County**

| KENT                       |

**Postcode**

| CT13 9FF                   |

**Country**

| ENGLAND                    |

**Tel Number**

| 01304 892694               |

**Fax Number**

| 0                          |

**E-mail**

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**HSE Division**

| blank                      |

**Comments**

**Date at Which Additional Info Submitted**

| 02/03/2022                  |
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Prior to this notification we have not used GMOs and therefore have not had a GMSC. Our new GMSC will consist of:
Venomtech CEO, CSO, operations manager, post doctoral researcher and senior technician
The CSO has relevant expertise in health and safety and will chair the committee
The GMSC convened to complete and approve this CU1 (including risk assessment) and will meet formally once every three months and also ad hoc.

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<th>Glass House</th>
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Other (please specify) Tick if confidential

Bacteriology | Parasitology | Transgenic Birds | Microbiology Research

02/03/2022
All waste cell culture media is denatured in 2% DEFRA approved quaternary amine detergent and then disposed of to waste water treatment. Live cells will be Autoclaved by registered waste handler prior to incineration.

Early stage drug discovery, using transiently and stably transfected mammalian cell lines

Tick to confirm that you are attaching a summary of the risk assessment  Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment  

We have reviewed the risks associated with working with established cell lines including genetic modification and we feel they are adequately controlled. We routinely carry out non GM cell work and thus have proven containment and safe systems of work, so we feel the addition of genetic modification to these cells will pose no measurable extra risk to our staff or the environment in which they work.
GM Centre Number: 3645

Data Premises Notified: 01/10/2021

Transferred from 1992 Regs?: N

Transitional Premises Class: N

Non-GMMs: N

Withdrawn: N

Name: SAMSARA THERAPEUTICS LTD

Name 2

Department

Campus Estate or Research Centre: WOOD CENTRE FOR INNOVATION (WCFI)

Building: QUARRY PARK

Road Name: STANSFELD ROAD

District: HEADINGTON

Town: OXFORD

County: OXFORDSHIRE

Postcode: OX3 8SB

Country: ENGLAND

Tel Number: +44 1865 546406

Fax Number: 0

E-mail

HSE Division: blank

Comments

Date at Which Additional Info Submitted: 02/03/2022
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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Biological Safety Committee includes its chair (Head of Biology) and Senior Scientists within Samsara Therapeutics. It meets quarterly to approve new scheme of works and to review associated risk assessments, which involve genetically modified organisms. The committee is also tasked with auditing compliance to all risk mitigation strategies. Samsara Therapeutics consult with an individual with Health and Safety and Risk Management expertise, particularly within biosafety and biosecurity. The consultant is a director in safety training and an non-executive director in governance, strategic planning and risk management and works with a variety of small to medium biotechnology companies within the UK.

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<td>Other (please specify below)</td>
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Other(s) Basic DNA cloning using bacterial and tissue culture procedures with mammalian cells.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Plastic Consumables (e.g. pipettes, flasks, tubes, agar plates) - incineration (licensed incinerator). Plastic wear is sealed within BioBins before disposal and incineration.

Liquids (e.g. samples, culture supernatants, tissue culture media) - inactivate by chemical means (e.g. Virkon following manufacturers guidelines 1-2 % final concentration for 30 minutes.) Virkon will be added to liquid in class II hood to minimise risk of aerosol exposure.

Sharps (e.g. needles, syringes, scalpels) - dispose via clinical waste stream for incineration in sharps bin.

All waste will be appropriately labelled. Solid waste will be placed in double bio-hazard plastic bags for incineration.

Degree of kill:

Incineration, effectively 100% kill (licensed Incinerator)

Chemical, effectively 100% kill (following manufacturers guidelines)

Tick to confirm that you are attaching a summary of the risk assessment Y

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

This risk assessment is approved by Head of Biology with input from external Health and Safety consultant.
GM Centre Number: 3646

Data Premises Notified (Originally) 04/10/2021

Transferred from 1992 Regs? N

Transitional Premises Class

Data Premises Closed

Transitional Premises Emergency Plan Required? N

Non-GMMs N

Withdrawn N

Name

PHYCOBLOOM LTD

Name 2

Department

Campus Estate or Research Centre

Building

OPEN CELL

Road Name

OLD LAUNDRY YARD

District

Town

LONDON

Country

GREATER LONDON

Postcode

W12 8EZ

Country

ENGLAND

Tel Number 07826377563

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

We have submitted risk assessments on our GMO work to both the Imperial College Innovation Hub and the OpenCell Biotech Campus, which were approved. The Innovation Hub laboratory manager has over seven years experience as a safety officer in a synthetic biology division, and twenty years experience running laboratories.

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Bacteriology | Parasitology | Transgenic Birds | Microbiology Research

Yes
Spillages will be dealt with by swabbing with 70% alcohol spray and diluted bleach spray which are microbiocidal. Contaminated, solid waste such as plastic disposables, tissue paper, culture plates, etc., are bagged in autoclave bin bag. The bag is autoclaved at 121°C for 20 mins, 1.5 bar pressure. The autoclave tape has to turn black before being placed into a yellow bin bag and tied up. The bags are finally placed in large yellow waste bin. Liquid culture medium is treated by adding to Virkon or bleach to achieve a final concentration of 10% and left to stand overnight before discharge to the sewer.

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</tbody>
</table>

For activities involving GMMs, describe the waste management measures which will apply to the activity

The advisor has reviewed the accompanying risk assessment and regards the proposed work as GM Class 1 requiring Containment Level 1.

Tick to confirm that you are attaching a summary of the risk assessment [Y]

Tick if you are claiming exemption from disclosure for sections of the risk assessment [ ]

Please enter comments of the GM safety committee on the risk assessment

The advisor has reviewed the accompanying risk assessment and regards the proposed work as GM Class 1 requiring Containment Level 1.
<p>| | | | |</p>
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**Name**

COSYNE THERAPEUTICS LTD

**Name 2**

**Department**

**Campus Estate or Research Centre**

**Road Name**

30 UPPER HIGH STREET

**District**

THAME

**Town**

OXFORD

**County**

OXFORDSHIRE

**Postcode**

OX9 3EZ

**Country**

ENGLAND

**Tel Number**

07497784533

**Fax Number**

0

**E-mail**

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**HSE Division**

**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
Premises Addresses

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</table>

Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Health and Safety Committee is responsible for H&S Strategy at the Milner Therapeutics Institute (MTI) and consists of Chair of Safety Committee, School Biology Sciences Representative, Department Safety Officer, Biological Safety Officer, Building Services Manager, Functional Genomics Centre Representative and MTI Senior Scientific Advisor. Risk assessments are reviewed and approved by the biological safety committee (BSO, DSO and Senior Scientific Advisor) and reported to the H&S Committee. Members of the biological safety committee have experience working with GMM's in academic laboratories at a postdoctoral level. They also have experience working with GMM's in the biomedical/pharmaceutical industry in the UK and abroad. Roles in academia/industry include, Team Leader, Group Leader and Project Manager.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
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Tick if confidential
The Jeffrey Cheah Biomedical Centre building management team will manage the building's waste streams. Surfaces used for GMM work will be disinfected using 70% EtOH and 5% Chemgene. Care is taken to assure correct concentration of the chemicals used as disinfectants. Agar plates with bacterial colonies will be autoclaved for disposal. Degree of kill is sterilisation. Waste management personnel is fully trained in waste handling. Autoclaves are periodically serviced.

CL1 solid waste will be treated as GMO waste and includes CL1 contaminated plastic consumables, stripettes, pipette tips etc. CL1 solid waste will be placed in orange bags in orange bins. The CL1 waste bins will be placed throughout MTI (Milner Therapeutics Institute) laboratory areas. The CL1 orange waste bags will be closed with cable ties when 3/4 full and deposited in a central laboratory area for collection twice a day by building services. This waste will be removed from site by Novus Environmental as 'clinical waste'.

CL1 liquid waste will be treated with Virkon 2% or Chemgene 10% for at least 30 min before disposal down the sink/drain.

### For activities involving GMMs, describe the waste management measures which will apply to the activity

For activities involving genetically modified of non-pathogenic E. coli and derivatives

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**Tick to confirm that you are attaching a summary of the risk assessment**

Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**

N

**Please enter comments of the GM safety committee on the risk assessment**

The attached risk assessment is fit for purpose and accurately describes a common GM process routinely conducted at CL1. The control measures and waste management streams stipulated are adequate to effectively manage the risks to humans and risks to the environment. This risk assessment, along with all other risk assessments issued in the institute, will be reviewed annually and updated/amended as required.
Protein coding gene inactivation in human brain tumour cells via lentiviral transduction of inducible gRNA/Cas9 (CRISPR) and shRNA. Gene down-regulation will be confirmed by quantitative PCR.

**Project Additional Information**

**Purposes of the contained use**
The purpose is to test the effect of gene down-regulation on viability and proliferation of human brain tumour cells.

**Recipient or parental organism**
Cultured mammalian cells, usually human brain tumour cells.

**Host/vector system**
The oligos (shRNA or gRNA) complementary to protein coding gene of interest are cloned into existing retroviral vectors (i.e., pLV[gRNA]-EGFP/Puro-U6 or Tet-pLKO-puro) that are based on HIV lentivirus (3rd generation). The pLV vector is a self-inactivated vector carrying a deletion in the U3 region of the 3' LTR, which eliminates the promoter activity of LTR. The vectors lack the viral gag, pol and env genes. The viruses will be packaged into replication incompetent particles using HEK 292T cells. The infected cells will be selected using puromycin.

**Origin & function**
The genetic material will be oligonucleotide sequences (guide-RNA or short hairpin RNA) complementary to known protein coding genes.

**Evaluation of foreseeable effects**
We expect expression of the doxycycline inducible sgRNA/Cas9 constructs or shRNA in human brain tumour cells to down-regulate a protein coding gene of interest, which in turn should result in reduced proliferation or cancer cell death. The cells are not expected to release infectious viral particles. The retroviral particles are incapable of replication due to removal of the viral pol, gag and env genes. The virus particles are capable of infecting human cells, but will not replicate. The expression of Cas9 and shRNA is doxycycline dependent. The primary concerns are formation of aerosols and use of sharps. We will carry out all manipulations in Class II microbiological safety cabinet.
and will not use sharps or sonication of solutions containing virus. The cancer cell-lines are not viable outside tissue culture conditions.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The Jeffrey Cheah Biomedical Centre building management team has an established CL2 waste stream: CL2 solid waste includes items such as general tissue culture consumables used in CL2 (consumables having been in contact with or containing residual GM material) tissue culture plates, tissue culture flasks, tubes etc. Serological pipettes and pipette tips will be collected in Bio-bins initially. Once Bio-bins are full they will be sealed and placed into autoclave bags in clearly demarcated CL2 waste bins. When CL2 autoclave bags are 3/4 full, they will be closed using cable ties to reduce the risk of waste spillage. The 3/4 full cable tied bags will be removed from the laboratory and placed into red CL2 waste bins in a clearly demarcated collection area. When the red CL2 waste bins are full they will be closed with cable ties. The red CL2 waste bins will be collected at least once a day by the building services team who will transport them to the basement of the building to be autoclaved. The building’s waste autoclaves will have maintenance schemes written by Bureau Veritas and will be inspected annually by Bureau Veritas and PSSR. Additionally, the buildings waste autoclaves will be serviced regularly and annually validated under a service contract. The autoclave will run at the pre-set guidelines for deactivation of CL2 waste, this will be validated on every run using indicator tape. Autoclaved waste will be transported off-site by Novus Environmental as ‘offensive waste’. CL2 liquid waste, that is safe to dispose of down the drain after inactivation, including; used tissue culture media, PBS used to wash cells etc will be treated with Chemgene before disposal down the drain. Inactivation of liquid waste will be achieved by using a final concentration of 10% volume/volume Chemgene with a minimum contact time of 30 minutes, before being disposed of down the sink/drain. Chemical waste for disposal from CL2 tissue culture (that is not suitable for disposal down the drain) will be collected in bottles, treated with a final concentration of 10% volume/volume Chemgene for a minimum of 30 minutes. Bottles will be labelled with their contents and placed in the chemical fume hood for disposal by building services. All work surfaces shall be routinely disinfected by wiping with 5% Chemgene, left for 30 seconds before subsequently wiping with 70% ethanol.

Is an emergency plan required according to regulation 20?  

Y

If yes, tick to confirm that it is attached to this form  

N

Tick to confirm that you have attached a risk assessment to this form  

Y

Tick if you are claiming exemption from disclosure for section of the risk assessment  

N

Please enter comments on the GM safety committee on the risk assessment

The committee confirmed the risk assessment as Containment Level 2 and HSE will need to be notified.
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**Name**

FOLIUM FOOD SCIENCE LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Road Name**

1010 CAMBOURNE ROAD

**Town**

CAMBRIDGE

**Road Name**

1010 CAMBOURNE ROAD

**District**

CAMBOURNE

**Town**

CAMBRIDGE

**District**

CAMBOURNE

**County**

CAMBRIDGESHIRE

**Postcode**

CB23 6DW

**Country**

ENGLAND

**Tel Number**

07561046620

**Fax Number**

0

**E-mail**

**HSE Division**

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**Comments**

Date at Which Additional Info Submitted

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

The risk assessments have been prepared or reviewed by an external HE academic who is a member of another genetic modification safety committee and has experience in reviewing and approving contained use activities with GMOs and at with ADCP group 2 and 3 pathogens.

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Tick if confidential
For activities involving GMMs, describe the waste management measures which will apply to the activity.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

The risk assessments have been prepared or reviewed by an external HE academic who is a member of another genetic modification safety committee and has experience in reviewing and approving contained use activities with GMOs and at with ADCP group 2 and 3 pathogens.

Project Ref 3648/21.1

Date Ackn’d: 03/11/2021
CU2 Project Title: Development of novel methods for the diagnosis and control of intestinal and skin pathogens

Class: Class 2
Culture Vol: 1-50 Litres

Consent Granted
Project notified under transitional arrangements

Withdrawn: No
Tick if notifying a connected programme of work: No
## Purposes of the contained use

The overall aim of this project is to inform the development of gene-targeted therapeutic agents for the control of bacterial diseases. In the initial stages of this work, this will entail identification of gene targets by knockout and complementation within an individual bacterium and also expression of the target genes in a second bacterial species, usually the common expression vector E. coli. In order to develop diagnostic tests for the particular target bacteria and genes expression of partial and full length proteins is also necessary. This will enable the calibration of protein sequencing tests and ELISAs for the target pathogens, and identify cross-reactivity, sensitivity and specificity of such tests.

The significance of this work is that it is developing agents which can replace antibiotics in food animal production, as a response to the antibiotic resistance now commonly found in these bacteria. It is necessary to use GMMs as targets for the various therapeutics under test target individual genes, and without producing GMMs the target cannot be verified.

Specifically for this individual project, the aim is to establish the validity of such an approach across multiple bacterial species which are known to be animal and zoonotic pathogens. This is a key enabling step in informing the future design of gene-targeted agents.

## Recipient or parental organism

- Salmonella (non-typhoidal serotypes)
- E. coli serotypes (non-toxigenic)
- Streptococcus spp.
- Staphylococcus spp.
- Campylobacter spp.
- Clostridium spp.
- Edwardsiella spp.
- Klebsiella spp.
- Enterobacter spp.
- Enterococcus spp.
- Listeria spp.
- Pseudomonas spp.
- Providencia spp.
- Yersinia spp. (intestinal strains, not pestis)
- Vibrio spp. (not cholerae)

## Host/vector system

Standard antibiotic resistance cassettes will be used for gene disruption, eg ApR (ampicillin), KnR (kanamycin), Sm/SpcR (spectinomycin), TcR (tetracycline), CmR (chloramphenicol). Also, a range of standard plasmids (all nonconjugative) conferring antibiotic-resistance will be employed for cloning purposes.

Crispr-Cas systems derived from ACDP group 1 bacteria, or components of these, systems will be introduced on plasmids.

All routine cloning will be in E. coli K-12 using standard plasmids (pUC series and derivatives) and will include strains such as E. coli K-12 (BW25113, TOP10, DH5α, MC4100, W3110, MG1655, S17::pRP4), E. coli B (BL21/DE3). Gene expression will use standard commercially available backbones such as pOPIN-E/F, pBAD, pCDF-duet and may be
under the control of inducible promoters such as arabinose and tetracycline. Green fluorescent and other FPs will be cloned in to assess expression in model systems. The commercial source is Clontech pGFPuv Vector.

### Origin & function

Crispr-Cas sequences from ACDP group 1 bacteria targetted to bacteria. Bacetrially-sourced or synthetic resistance genes. Genes encoding toxins, or that enhance virulence, will be specifically excluded from the work. For expression outside E. coli, vectors may be altered by modifying the promoter and ribosome binding site and/or origin of replication by inserting artificially synthesised gene fragments.

### Evaluation of foreseeable effects

Expression of target genes in bacteria would not increase the virulence of any organisms containing them, Crispr-Cas genes will be on plasmids and large size of construct will render the created strains less fit than wild type. Created strains would be either as virulent or less virulent as the parent organism, and would likely be less fit in terms of survival and competition in the environment. Forseeable effects would be as for the parental organisms. Plasmid carriage or knockout will render them less fit than the wild type, limiting their ability to compete and survive.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

**None**

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

None

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

Highly contaminated material - tips and loops, and similar plasticware will be collected in the laboratory within small benchtop containers (waxed card waste bins, not biohazard marked). This waste will be combined with agar plates and larger volume plasticware and sterilised by autoclaving. Transport to autoclave will be in a leakproof container on a trolley. Following confirmation of autoclave cycle using TST strips waste will be disposed of to landfill by a licenced contractor. Failure of cycle as indicated by either autoclave or TST strips will mean waste is autoclaved again.

Autoclaves are inspected and tested for temperature and time conformance annually. TST indicators are used as a failsafe.

Sharps will be contained within an autoclavable sharps bin, autoclaved as above, then collected by a licenced contractor for incineration.

Liquid waste will be sterilised by the addition of Virkon from stocks made up weekly. This will be added to a concentration of 2%, the mixture left to stand for 60 minutes before discharge to drain. Glassware will be autoclaved as above.

Low risk material - hand towels and gloves will be collected in yellow bags disposed of by incineration off site after collection by a licenced contractor.

**Is an emergency plan required according to regulation 20?**  

If yes, tick to confirm that it is attached to this form

Tick to confirm that you have attached a risk assessment to this form
Lab users should use PPE — lab coat with elasticated cuffs and high neck, and gloves at all times. Coats must not be worn outside of the laboratory. When transporting waste to autoclave this must be on a trolley. TST indicators should be used in autoclave loads and these ensured to be specific to 121°C for 15 min at 15 PSI. These have been incorporated into the risk assessment.

### Project Containment

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<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
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Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

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Tick if confidential

Bacteriology
Parasitology
Transgenic
Birds
Microbiology
Research
Virology
Transgenic
Animals
Transgenic
Fish
Gene Therapy
Mycology
Yes
Transgenic
Invertebrates
Transgenic
Plants
Other (please specify below)

Other(s)
Gene Edited Invertebrates (as well as Transgenic Invertebrates)

For activities involving GMMs, describe the waste management measures which will apply to the activity

02/03/2022
Note: the first contained use is to be for a “non-notifiable activity involving larger GMOs” not GMMs. However Note 6 says that section 6-8 should be completed. Inactivation of the GMOs will be carried out by freezing them at -20 °C in a dedicated freezer for 72 hours. This will attain a near 100% kill percentage. After inactivation by freezing the GMOs will be placed for 48 hours into a sodium hypochlorite solution with a minimum of 13 % sodium hypochlorite by weight (i.e. 13 g of sodium hypochlorite for every 100 g of solution) to ensure destruction of the GMOs. The waste produced after inactivation and destruction will be category 3 waste under Regulation (EC) 1069/2009 and will be stored at betabugs limited until disposal in an authorised landfill in line with regulations.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

“The risks presented are negligible to both humans and the environment. In discussing the project proposal there were no concerns raised regarding the risk of carrying out any of these [GE/GM] procedures”
**GM Centre Number: 3650**

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<td>EAST AND SOUTH EAST</td>
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<td>Comments</td>
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Date at Which Additional Info Submitted: 02/03/2022
Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Health and Safety/Biosafety Consultant with 18 years’ experience working in microbiological laboratories at CL2 and CL3, and more than 20 years’ experience advising on health & safety and biosafety including genetic modification.

- Laboratory
- Animal Unit
- Growth Room
- Glass House
- Large Scale

Level 1 (GMMs) Yes

Level 2 (GMMs)

Level 3 (GMMs)

Level 4 (GMMs)

Non-microbial

Other (please specify)

Tick if confidential

- Bacteriology
- Parasitology
- Transgenic
- Birds
- Microbiology
- Research

- Virology
- Transgenic
- Animals
- Transgenic
- Fish
- Gene Therapy

- Mycology
- Transgenic
- Invertebrates
- Transgenic
- Plants
- Other (please specify below) Yes

Other(s) Cell biology- To access cell function and phenotype through the analysis of various cellular markers

For activities involving GMMs, describe the waste management measures which will apply to the activity
**Disinfection**

The disinfectant of choice (Virkon) is used in strict accordance with the manufacturer's (Antec's) guidelines to inactivate liquid and solid waste.
- Liquid waste is treated with 1% Virkon solution overnight and disposed of to drain with copious amounts of water.
- Surfaces and equipment will be decontaminated with Virkon (1% w/v).
- Emergency spillages will also be treated with Virkon to a final concentration of 1% w/v.

Virkon has been validated as being effective within 10 minutes at this concentration and Virkon stocks are routinely tested by the manufacturer ([http://www.antecint.co.uk/go.htm](http://www.antecint.co.uk/go.htm)).

**Waste disposal**

**Solid waste:** All solid waste will be collected live on a weekly basis and transported directly for high temperature incineration (HTI), providing 100% kill as monitored by Grundon (Certificates of Destruction are available on request).
- Solid waste will be double-bagged and then sealed in leak-proof solid, rigid 30L burn bins.
- Hard plastic waste that may puncture bags such as pipette tips and stripettes are first sealed in a Bio-Bin® before being placed in sharp bins (Volumes available 5L, 11.5L, and 22L).
- Biological samples (containing blood samples without prior inactivation) will be disposed into burn bins (30L) containing vermiculite granulates and sealed before reaching 20kg – Such bins will be separated from the rest of the lab waste and labelled as GMO Class 1 – A certificate of destruction can be provided for waste sent via Grundon.

*Alternatively, solid and liquid contaminated waste can be treated by autoclaving for 30 minutes at 121°C on a validated cycle resulting in 100% kill. This is not the preferred route as the autoclave is small/medium size and the waste could be accumulated during long periods resulting more hazardous to the people and it is unnecessary as the samples are classified as GMO 1. The autoclave is serviced, calibrated, and validated once a year by Astell Scientific Ltd.*

**Liquid waste:** liquid waste will first be deactivated by treatment with 1% Virkon overnight at room temperature prior to disposal to wastewater via lab sinks with copious amounts of water in accordance with trade effluent license limits.

---

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

---

Please enter comments of the GM safety committee on the risk assessment

The risks from the GMMs potentially present in the clinical samples (from trials using Class 1 GMMs) is low to negligible, so the work can be approved as a Class 1 activity and carried out at Containment Level 1. It is noted that all clinical samples are routinely handled at Containment Level 2.
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities  

Y

Give brief details of the genetic modification safety committee

The MTI Health and Safety Committee has delegated the GMO Risk Assessment responsibilities to the MTI-BSO since it has the expertise to advise the committee in the review and approval of not only biological and chemical but also GMO risk assessments. The committee is also supported by the Safety Officer in the School of Biological Sciences at the University of Cambridge. The committee will receive the advise and will in turn make the decision on approving or make suggestion for modifications in the risk assessment.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Other (please specify)  

Tick if confidential  

Bacteriology | Yes | Parasitology | Transgenic | Transgenic | Microbiology | Other (please specify below)  

Virology | Yes | Transgenic | Transgenic | Gene Therapy |

Mycology | Transgenic | Transgenic | Other (please specify below)  

Other(s)  

Production and use of genetically modified of non-pathogenic E. coli and derivatives

For activities involving GMMs, describe the waste management measures which will apply to the activity
All GM waste is inactivated at the same site as the activity is taking place. Liquid waste will be inactivated with 2% Virkon or similar for at least 1 h (or overnight) before disposal to sink. All contaminated labware (pipettes, tips and culture plates) are separately decontaminated using Virkon 2% or ChemGen 5% before being disposed in clinical waste bins for incineration. All GM modified cell cultures (flasks with cells and no media) will be chemically inactivated by soaking with Virkon 2% for at least 1h (or overnight). Once inactivated this waste will be placed in clinical waste bins for disposal by incineration. Small spills will be removed immediately by surface soaking with Virkon 2% and further decontamination using 70% Ethanol. Large accidental spills will be sprinkled with Virkon powder before cleaning and immediately reported to the Biological Safety Officer. Bench and cabinet surfaces will be wiped down with Distel/Chemgene/Virkon 2% and further decontamination using 70% Ethanol.

The attached risk assessment is fit for purpose and accurately describes a common GM process routinely conducted at CL1. The control measures and waste management streams stipulated are adequate to effectively manage the risks to humans and risks to the environment. This risk assessment, along with all other risk assessments issued in the institute, will be reviewed annually and updated/amended as required.

Project Ref 3651/21.1

Date Ackn’d 22/11/2021

CU2 Project Title Generation and use of lentiviruses and retroviruses to introduce genetic material into mammalian cell lines, iPSCs and primary cells

Date Project Ceased

Class 2 CultureVol

Class2 CultureVolumeClass3-4

Non-GMM Consent Granted Not Applicable

Project notified under transitional arrangements N

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change
### Purposes of the contained use

Our research aims at understanding the molecular signalling pathways underlying the communication between the lymphoma microenvironment and primary CLL cells and how this affects the selection of drug resistant clones. HEK293T cells are being used to produce recombinant amphotropic/VSV-G pseudotyped recombinant lentivirus and retrovirus particles containing specific transgenes. These particles may then be used for transduction of primary cells, cultures, cell lines.

### Recipient or parental organism

**Mammalian cells: primary cells and cell lines (including haematopoietic progenitor cells, leukemic cell lines, skin fibroblasts)**

In vitro cultured mammalian cells are considered especially disabled and unable to survive outside the lab environment.

### Host/vector system

<table>
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<tr>
<td>Vector containing insert:</td>
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</table>

Vectors contain coding sequences for antibiotic resistance genes and reporter genes that are used for selection. Antibiotic resistance genes used, for example Kan/AmpR, are not medically critical. Reporter genes, for example GFP, dsRED, Luciferase, are thought to have no deleterious biological effect.

**Inserts**

Several types of inserts may be used:

- **Type 1** - Human, murine, xенopus cDNA (+/- tag sequences) coding for genes including, but not restricted to, transcription factors, signal transducers, growth factors, oncogenes and surface receptors
- **Type 2** - shRNA and miRNA sequences against endogenous coding sequences of the recipient host
- **Type 3** - Reporter genes (i.e. EGFP, ECFP, EBFB, EYFP, LacZ, Luciferase, possibly fused to cDNA (type1 inserts))
- **Type 4** - Selection genes (i.e. NPT, PAC, Puromycin, Neomycin, Hygromycin, Blasticidin)
- **Type 5** - Functional non-coding sequences (promoters, enhancers, insulators, IRES, WPRE, of mammalian or viral origin)
- **Type 6** - Neutral non-coding sequences (introns, isolated exons)
- **Type 7** - expression cassettes for guide-RNA and CAS9 DNA nickase (and variants of the CAS9 system, e.g. dCAS9, dCAS9-KRAB)

### Origin & function

**Transient transfection of HEK293T** with these plasmids will result in the production of recombinant lentiviral or retroviral particles containing transgenes of interest that will be harvested from the cell culture media for subsequent over-expression, knock-down or knock-out experiments.

### Evaluation of foreseeable effects

The modification is not expected to overcome disablement of the host organism, not affect host specificity, tissue tropism or susceptibility to host defence mechanisms. Co-transfected HEK293T cells will produce amphotrope/ VSV-G pseudotyped recombinant lentiviral or retroviral particles containing the transgene of interest, which will be secreted into the culture medium.

Insert expression, non -replicative vector, vector mobilization very unlikely and restricted

Generation of replication competent lentiviral particles (RCL) in a host cell is a theoretical possibility. Oncogenes (or potential oncogenes) altered through insertional mutagenesis could potentially lead to pathological modifications of mammalian cell phenotype/function which may be oncogenic.

The use of 3rd generation lentivirus systems has decreased this risk to a negligible level. However, if oncogenes or genes of unknown function are being inserted into hosts using 2nd generation lentivirus systems this risk is increased.
To decrease the likelihood of infection of an individual, standard measures to decrease percutaneous as well as aerosolised transmission of the virus, such as no use of sharps and work to be performed within a Class 2 microbiological safety cabinet, will be used. Lentiviral and retroviral virulence genes (e.g. vpr, vpu, vif and nef) have been deleted. The derived vectors are therefore unable to replicate once they have transduced a target cell. In addition, third generation lentivirus production systems are deleted for the tat gene and carry a SIN deletion of the 3’ LTR (ΔU3). This results in “self-inactivation” of the lentivirus following the transduction of the target cell.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

The Jeffrey Cheah Biomedical Centre building management team will manage two laboratory waste streams. One waste stream for CL1 waste and a second for all CL2 waste.

CL2 solid waste includes items such as general tissue culture consumables used in CL2 (consumables having been in contact with or containing residual GM material) tissue culture plates, tissue culture flasks, tubes etc. Serological pipettes and pipette tips will be collected in Bio-bins initially. Once Bio-bins are full they will be sealed and placed into autoclave bags in clearly demarcated CL2 waste bins. When CL2 autoclave bags are 3/4 full, they will be closed using cable ties to reduce the risk of waste spillage. The cable tied bags will be removed from the laboratory and placed into red CL2 waste bins in a clearly demarcated collection area. CL2 waste bins will be collected everyday by the building services team who will transport them to the basement of the building to be autoclaved. The building’s waste autoclaves will have maintenance schemes written by Bureau Veritas and will be inspected annually by Bureau Veritas and PSSR. Additionally, the building’s waste autoclaves will be serviced regularly and annually validated under a service contract. The autoclave will run at the pre-set guidelines for deactivation of CL2 waste, this will be validated on every run using indicator tape. Autoclaved waste will be transported off-site by Novus Environmental as ‘offensive waste’.

CL2 liquid waste, that is safe to dispose of down the drain after inactivation (i.e. used tissue culture media, PBS used to wash cells) will be treated with 2% Virkon for at least 30 min for inactivation before disposal down the drain.

Chemical waste for disposal from CL2 tissue culture (that is not suitable for disposal down the drain) will be collected in bottles labelled with their contents/concentrations, and will be placed in the chemical fume hood for disposal by building services. All work surfaces shall be routinely disinfected by wiping with 5% Chemgene, left for 30 seconds before subsequently thoroughly wiping with 70% ethanol.

Is an emergency plan required according to regulation 20?  

If yes, tick to confirm that it is attached to this form  

Tick to confirm that you have attached a risk assessment to this form  

Tick if you are claiming exemption from disclosure for section of the risk assessment

This CU2 application along with the attached risk assessments (Biological and GMM) have been reviewed and approved by the MTI biological safety officer (BSO) and presented it to the safety committee. The risk assessment supporting this CU2 application covers the specific containment measures needed at this particular level (CL2) and all work with GMMs apply the principles of good microbiological practice (GMP) and good occupational safety and hygiene (GOSH).
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GM Centre Number: 3653

Data Premises Notified
(Originally) 22/11/2021

Transferred from 1992 Regs? N

Transitional Premises
Class

Data Premises Closed

Transitional Premises
Emergency Plan Required?

Non-GMMs N

Withdrawn N

Name

T-CYPHER BIO LTD

Name 2

Department

Campus Estate or Research Centre

OXFORD SCIENCE PARK

Building

3RD FLOOR, THE SHERARD BUILDING

Road Name

EDMUND HALLEY ROAD

District

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX4 4DQ

Country

ENGLAND

Tel Number 07528310901

Fax Number 0

E-mail

HSE Division blank

Comments

Date at Which Additional Info Submitted

02/03/2022
**Premises Addresses**

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**Premises Conditions**

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

External Health and Safety/Biosafety Consultant with 18 years’ experience working in microbiological laboratories at CL2 and CL3, and more than 20 years’ experience advising on health & safety and biosafety including genetic modification. Member of ISTR and EBSA.

<table>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity:

Bacteria cultures will be in the order of 2 to 4L on a weekly basis or less often.
Bacterial waste and utensils that come into contact with it will be inactivated by soaking in 1% Virkon for at least 30min. This will include:
- media and flasks used for growth (2 to 4L approximately) - dissolve Virkon tablets to 1%
- utensils used to collect and purify cell pellets and the supernant waste (5-50ml) from cell lysis
- small culture aliquotes used for analysis (1-2ml), or small scale cultures (50ml) will be poured into a container with 200ml of final concentration of 1% Virkon.
Small disposable containers (1 to 50ml tubes, plates) that have come into contact with bacteria or bacteria lysis waste will be autoclaved for 20mins at 121 degree Celsius (15psi) and disposed via waste contractor holding a GM waste carriers licence.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The risk assessment has been reviewed and as the risks for human health and the environment are minimal the work can be classified as a Class 1 activity.
### GM Centre Number: 3656

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#### Building

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### Comments

#### Date at Which Additional Info Submitted

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# Premises Addresses

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# Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

- **Yes**

Give brief details of the genetic modification safety committee

Accession Therapeutics Ltd is a biotechnology company focussed on developing treatments for cancer. The technology and basis for the work originates from work registered previously (GM130/15.2) and will continue at Accession Therapeutics Ltd.

Accession Therapeutics Ltd has established a Biological and Genetic Modification Safety Committee with membership comprised of corporate and scientific leadership, scientific operations and bench science, documentation and operations personnel, and is chaired by the Head of Preclinical Development who is a member of the company leadership team and reports directly into the CEO. Members of the committee represent all functional areas of the company and facilities.

The Committee meets on a quarter-year basis or as required more frequently, with agenda and minutes and documentation managed through a Quality Management System.

The scientific leadership team have extensive expertise in genetic modification and virology, with the lead projects coming from 15 years of GMO committee-approved research at a leading University from which the Chief Investigator is also a member of this scientific leadership team and on this GMO committee. Other leadership team members also have previous experience with attending and leading GMO and general health and safety committees.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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The first notification will be for class 2 GMM work, detailed in the accompanying cu2 notification. Class 1 and 2 work will be carried out in fit-for-purpose laboratories adhering to appropriate risk assessments and following our SOP for waste management which outlines waste management routes including Accession's contracted waste removal firms.

Commercially available human and animal cell lines (class 1 or 2), primary human blood and tissues, and viruses (class 2) will be maintained in an environment designed to prevent aerosol spread (biosafety level 2 tissue culture hoods, under negative pressure). All used plastics shall be kept sealed/closed except when decanting and pipetting which will be conducted in tested class II safety cabinets, and virus containing waste media will be treated immediately using sodium dichloroisocyanurate (HAZ-TABS).

The use of sharps in the process of purifying viral vectors from Caesium gradients is unavoidable, and is essential to the procedure, but will be kept to minimum use possible. Initial disinfection will be with HAZ-TABS then disposed of through commercial sharps bins.

Liquid waste will be disinfected with HAZ-TABS. Expected degree of kill 100%. Haz-Tab Tablets have been proven through independent EN Standard testing to have broad spectrum biocidal activity, including against viruses.

Solid waste will be autoclaved unless involving human tissue, which would then be disposed of through clinical waste routes by Accession's commercial clinical waste partner.

Details of any other control measures to be used:

Recombinant viruses will at all times be maintained in an environment designed to prevent aerosol spread. All flasks containing the virus shall be kept closed except when decanting and pipetting which will be conducted in tested class II safety cabinets. Centrifugation steps shall be performed in sealed buckets that will be opened only in safety cabinets.

Contaminated pipettes will be immersed in Sodium Dichloroisocyanurate for a minimum of 4hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with chlorine-based disinfectant or Virkon (1%) according to an approved protocol. Access to laboratories employed for virus work will be restricted.
The first notification is for a class 2 activity, therefore no risk assessment for a class 1 activity is attached. Please see accompanying cu2 form for class 2 activity and risk assessment.

### Project Ref 3656/21.1

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**Tick if notifying a connected programme of work** Y

**Project notified under transitional arrangements** N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**

**Date of Significant Change**

## Project Additional Information

### Purposes of the contained use

The purpose of the proposed project is to use the existing Ad5-Null-A20 platform (described in GM130/15.2) to express therapeutic genes that may be beneficial for cancer treatment.

We have previously modified the tropism of both oncolytic and replication-deficient adenoviruses to more selectively target cancer cells (See GM130/15.2). We now propose to use these viruses to express therapeutic genes.

### Recipient or parental organism

We have previously generated oncolytic Ad5-Null virus (this form of the virus does not infect, or replicate in, healthy...
cells) by standard modifications of the Ad5 genome. These include the delta 24 mutation in the E1A protein (1) and the potency enhancing T1 mutation (2). We have also modified the tropism of both oncolytic and replication deficient virus to more selectively target cancer cells using the A20 peptide, from foot and mouth disease, so that the Ad5-Null-A20 form of the virus is only able to infect cells that express αvβ6 integrin (a cancer selective marker). We now propose to use these viruses to express therapeutic genes (please see box 17 for non-disclosure).

Host/vector system

Preliminary vector manipulations will be performed in disabled E.coli strains (DH5a, JM109, XL-1 blue, BJ5813, SW102).

Viruses will be grown in mammalian helper cell lines where required (e.g. 293 cells), or non-helper cell lines where viral replication can occur without complementation (using e.g. A549 cells, HeLa cells).

The viruses will be used to infect various αvβ6 integrin positive and negative cells, including cancer cell lines, cell lines representing healthy tissues and primary blood cells from healthy volunteers.

Origin & function

Evaluation of foreseeable effects

The envisaged worst-case scenario would be that a worker would inadvertently self-inoculate with a large dose of conditionally replicating virus that expresses a biologically active gene that may have benefit in cancer treatment. “Leaky” expression of this gene (which should be minimal due to the inability to infect non-αvβ6 expressing cells) may result in expression of immune modulators in off target tissues, and potentially transient autoimmune responses. However, due to the altered tropism of Ad5-Null-A20 (8), and the very limited expression of its ligand, αvβ6 integrin, in healthy tissues (9), it is very unlikely that the Ad5-Null-A20 virus with the intended encoded transgene would infect any cells in a healthy person. Additionally, the Ad5-Null-A20 virus has been modified to be conditionally replicative by incorporation of a DL24 mutation in E1A (1). Thus, even if a low level of the virus was able to infect healthy cells, it would not be able to replicate in the presence of pRB (a tumour suppressor gene). Finally, even if the virus was to infect αvβ6 integrin positive tumour cells, the expression of the transgene would be highly localised, and would be very unlikely to reach the serum levels associated with dose limiting toxicity as observed when these therapeutic proteins have been administered clinically.

Mitigation:

To minimise the potential for infection, all work will be conducted under containment level 2 conditions, including the use of class 2 level biosafety cabinets.

References:
3-7 can be found in box 17 for non-disclosure
Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

N/A

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Will a safety cabinet be used? Yes
Will any procedures use sharps? Yes
The use of sharps in the process of purifying viral vectors from Caesium gradients is unavoidable, and is essential to the procedure. Initial disinfection will be with sodium dichloroisocyanurate then sent for autoclaving in a sealed container.
Will liquid waste be autoclaved or disinfected? Liquid waste will be disinfected:
Product Name: HAZ-TABS
Generic Chemical Name: Sodium Dichloroisocyanurate
Expected degree of kill 100%. Haz-Tab Tablets have been proven through independent EN Standard testing to have broad spectrum biocidal activity, including against viruses.
Will solid waste be autoclaved or disposed of as clinical waste? Solid waste will be autoclaved unless involving human tissue, which would then be disposed of through clinical waste routes by Accession's commercial clinical waste partner.
Details of any other control measures to be used:
Recombinant viruses will at all times be maintained in an environment designed to prevent aerosol spread. All flasks containing the virus shall be kept closed except when decanting and pipetting which will be conducted in tested class II safety cabinets. Centrifugation steps shall be performed in sealed buckets that will be opened only in safety cabinets.
The use of sharps when handling virus preparations will be minimised.
Contaminated pipettes will be immersed in Sodium Dichloroisocyanurate for a minimum of 4hr prior to transferring to a container for autoclaving. The tissue culture cabinet and any potentially contaminated area will be disinfected with chlorine-based disinfectant or Virkon according to an approved protocol. Access to laboratories employed for virus work will be restricted.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

Please enter comments on the GM safety committee on the risk assessment
The risk assessment has been reviewed and deemed appropriate by the Accession Therapeutics Biological and Genetic Modification Safety Committee. The work here at Accession Therapeutics will follow biosafety level 2 processes, waste routes and controls the same as the previous lab as the linked program GM130/15.2. The work will be carried out by, under the supervision of, or trained by the same investigator and scientist as GM130/15.2.

**Project Containment**

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Name

**HULL UNIVERSITY TEACHING HOSPITALS NHS TRUST**

Name 2

Department

Campus Estate or Research Centre

Road Name

**ANLABY ROAD**

District

Town

**HULL**

County

Postcode

**HU3 2JZ**

Country

**ENGLAND**

Tel Number

01482875875

Fax Number

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E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Hull University Teaching Hospitals NHS (Castle Hill Hospital) recently established a dedicated GMSC for the initial purpose of clinical trials with GMO-based vaccines and the approval of corresponding risk assessments. Upon risk assessment, the proposed vaccine clinical trials are considered to be ‘contained Use’ which falls within the remit of the GMO (Contained Use) Regulations 2014.

The HUTHGMSC includes representatives from different departments within the local hospital Trust with various levels of expertise of working with GMOs. In addition, an honorary Biosafety Advisor has been appointed, who is part of the GMSC. The Biosafety Advisor has extensive work experience (competency) with GMOs and is currently the University Biological Safety Officer and Chair of Genetic Modification Committee of the University of Hull.

The composition of the GMSC is as follows: Chair and vice-Chair, Biosafety Advisor, Director of Infection Prevention and Control, Clinical Trials Pharmacist, R&D Quality Manager, Trust Safety Manager, different consultant clinicians for Infectious Diseases including a Virologist, and Employee Union representatives from Unison, Unite, RCN and BMA. The GMSC is chaired by infectious diseases consultant clinician who has recent experience with GMO-based clinical trials and laboratory research.

The HUTH Trust GMSC will meet a minimum of annually with more frequent meetings when required. The GMSC reports to the Trust Patient Safety and Effectiveness Committee, which sits under the Quality Committee, which reports to the Trust Board.

Risk assessments will be reviewed and approved by the GMSC on an annual basis or earlier when proposed change(s) affect the risk level and classification of the GM work.

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<tr>
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<th>Large Scale</th>
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Following risk assessment, current work with GMOs at the HUTH Trust has been classified as Class I genetic modification work. Following the requirements of the GMO (Contained Use) Regulations 2014 and the SACGM Compendium of Guidance on the use of genetically modified organisms within clinical settings (part 6), all GMO-containing waste must be inactivated before entering the clinical waste stream and leaving the hospital premises as (accumulated) infectious clinical waste (incineration route, appointed contractor).

Main method of biological inactivation of the current GMOs is the use of chemical disinfectants, in this case the chlorine-dioxide based disinfectants Tristel FUSE and Tristel DUO. These are the recommended disinfectants to be used within the clinical settings of the HUTH NHS Trust. According to the manufacturer’s specifications, both formulations of Tristel have a confirmed high efficacy against Adenovirus and Vaccinia virus of which disabled/non-replicative versions are used as vectors for the GMO-based vaccines.

Non-sharps solid waste will be inactivated by submerging in the by the manufacturer prescribed working concentration of Tristel FUSE (50 ml sodium chlorite activator combined with 50ml citric acid base and diluted in 5 litres of water for a final concentration of chlorine dioxide of up to 125ppm) for at least the recommended contact time of 5 minutes. Following inactivation, the disinfectant phase is disposed of via a sluice and the remaining solid waste will be disposed of

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For activities involving GMMs, describe the waste management measures which will apply to the activity
via the clinical waste stream (accumulated infectious clinical waste, incineration route).

Sharps containing waste will be submerged in Tristel FUSE for an extended time (longer than recommended by the manufacturer) as for example needles are/can be less accessible to disinfectants. For practical reasons, this incubation will be overnight (minimum of 8 hours) before the disinfectant phase is disposed of via a sluice and the sharps bin containing the disinfected sharps is disposed of via the cytotoxic waste route.

Laboratory surfaces are disinfected by using Tristel DUO (sodium chlorite activator and citric acid base combined at the time of use in a foam by a dispenser for a final concentration of chlorine dioxide of 200-600ppm) for at least the manufacturer recommended contact time of 1 minute.

Accidental spills will be inactivated with an excess of Tristel FUSE at the minimum recommended contact time of 5 minutes before disposal of the materials/used paper towels via the clinical waste route. It is further standard practice to disinfect (wipe down) the external surface of waste bags with Tristel FUSE before disposal via the clinical waste stream.

Dedicated SOPs for all waste and spill management processes are in place. All staff handling GMOs will confirm they have read and understood the risk assessment and waste management measures (SOPs) before they can commence GMO work.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

A risk assessment was conducted as set out under the GMO (Contained Use) Regulations 2014 and the SACGM Compendium of Guidance (part 6, working with GMMs in clinical settings). The GMSC and Biosafety Advisor agreed that the risk assessment (attached) is satisfactory, that all regulatory requirements have been fulfilled and that the proposed work is to be considered as a Class 1 genetic modification work activity with no risk to humans, animals or the environment. A copy of the respective risk assessment is attached.
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**Data Premises Notified (Originally):** 20/12/2021

**Transferred from 1992 Regs:** No

**Emergency Plan Required:** No

**Transitional Premises:**
- **Class:**
- **Non-GMMs:** No
- **Withdrawn:** No

**Data Premises Closed:**

**Transitional Premises:**

**Comments:**

**Date at Which Additional Info Submitted:** 02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

- Director of Safety Services
- Medical Director
- Head of Sites
- Director of Operational Development and Compliance
- Pharmacist Team member
- Operational Facilities Senior Management
- Project Management

<table>
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<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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02/03/2022
Decomtamination of all used vials on site using known/recommended method of disinfectant (0.9% Virkon S (> 5 min contact time)). All GMO disposable waste that can't be inactivated on site as the site doesn't have a waste autoclave will be placed into an appropriate, dedicated, and lockable GMO waste container. The location of this waste container will be within a secure/limited access consulting room within the Panthera site.

Non-disposable materials are disinfected with appropriate disinfectants as mentioned above.

All disposable GMO waste will be sent for destruction using a registered GMO waste supplier and a Note of destruction will be maintained.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Decontamination of all used vials on site using known/recommended method of disinfectant (0.9% Virkon S (> 5 min contact time)). All GMO disposable waste that can't be inactivated on site as the site doesn't have a waste autoclave will be placed into an appropriate, dedicated, and lockable GMO waste container. The location of this waste container will be within a secure/limited access consulting room within the Panthera site.

Non-disposable materials are disinfected with appropriate disinfectants as mentioned above.

All disposable GMO waste will be sent for destruction using a registered GMO waste supplier and a Note of destruction will be maintained.

Please enter comments of the GM safety committee on the risk assessment
The Panthera BSO & GMSC can confirm, after review of the EU approved Application form for the use of the vector in humans, the likelihood that the Ad26.RSV.preF clinical vector is shed to the environment in relevant quantities upon administration, beyond its initial potential presence at the injection site (injection site leakage) is negligible, taking into account:

• vector DNA is only infrequently detected, and if so, is only found at very low levels and does not contain infectious virus particles.

• the potential for shedding is considered to be independent of the transgene insert, as the transgene insert does not have an impact on the vector particle, and thus does not change cell tropism.

• the same routes (i.e. intramuscular injection) and dose (1 × 1011 VP or below) of inoculation will be used for vaccination as have been used for previous shedding studies.
**GM Centre Number: 3659**

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**Name 2**

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**Comments**

**Date at Which Additional Info Submitted**

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Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Enplas Europe Ltd., Lifescience Lab genetic modification committee will consist of:
1. One member of staff who has a PhD in a relevant field of research and more than 25 years research experience including genetic modification and GMOs, and will act as chair. This person has IOSH Biosafety Officer training.
2. Two other employees with approximately 2 years each experience of generating and handling GMOs.
3. The external biosafety consultant (see section 8).

The Enplas Europe Ltd., Lifescience Lab GM committee agreed that the risk assessment included with this notification was suitable and sufficient. The committee will meet at least every 6 months to review risk assessments and GM procedures.

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<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Tick if confidential

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For activities involving GMMs, describe the waste management measures which will apply to the activity

All waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application below). Waste from our GM work at Class 1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor. This disposal method is expected to achieve 100% inactivation of the GMM. The data sheets describing inactivation by Virkon are attached.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The Enplas Europe Ltd., Lifescience Lab GMO committee reviewed the GMO risk assessment (see attached). The Enplas Europe Ltd GMO committee consists of one person who has a PhD in a relevant field with more than 25 years research experience including genetic modification and GMOs together with IOSH Biological Safety Officer training, plus two others with 2 years experience with GMOs each. The GMO committee have previously written GMO risk assessments for similar work prior to joining Enplas Europe Ltd., and they understand the science around the work carried out.

The Health and safety consultant reviewed the GMOs. The consultant has a B.Sc in Animal Sciences - Wye College (part of Imperial), London University, M.Sc. Animal Parasitology, Ph.D. The immunology and pathology of Sarcocystis ovicanis infections in sheep (both at U.C.N.W., University of Wales). Technical member of the Institution of occupational safety and health (Tech IOSH), NEBOSH National Diploma in Occupational Health and Safety (in progress), NEBOSH National General Certificate in Occupational Health and Safety, Member of the Institute of Safety in Technology and Research, MRC Biological Safety Officer Training Certificate. 1986 to 2010 at the Babraham Institute (Cambridge) where experience of handling pathogens and GMO obtained during research. 2006 made Babraham Research Campus Biological Safety Officer (part time plus research). 2009 onwards teach the genetic modification safety section of the One Nucleus ‘Biological Safety: Management and Practice’ course (IOSH approved).

2010 onwards Babraham Institute Health, Safety and Quality Assurance Manager and Babraham Bioscience Technologies (BBT) Biosafety Officer (Full time). 10 years experience in risk assessments and has health and safety qualifications.
The generation and use of AAV, Adenoviral, Baculoviral, HSV-1 viral, Retroviral and Lentiviral particles for knock-in/knock-out of oncogenes and other potential cancer therapeutic targets to study and confirm mechanisms of action, and efficacy of anti-cancer agents.

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Non-GMM Consent Granted

Project notified under transitional arrangements

Withdrawn

Historical Significant Changes

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

**Project Additional Information**

Purposes of the contained use

Recipient or parental organism

Host/vector system

Origin & function

Evaluation of foreseeable effects

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

Not applicable

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution then autoclaved prior to disposal. Solid waste material will be placed in sealed bins.
and incinerated by an off-site incineration company (Stericycle – Their GM authorization is GM779) according to disposal notification GM.779/01.1. Waste from our GM work at Class 2 will be placed into suitably labelled hermetically sealable (red lid to distinguish them from Class 1 waste) 60 litre UN approved (to Class 3) Type 3H21Y30/S/2003 bins. Sealed bins are placed into secondary 210 litre yellow labelled "Eurobins". Contents of the bin are recorded and collections by the local registered clinical/GM waste incinerator contractor will be made three times a week to prevent a build-up. The site has 24/7 Security. The contractor uses solid bodied transport and carries portable disinfection equipment in case of any spillage. Autoclave facilities will be available in an adjacent building, as required by the Regulations, in case of loss of the contractor's facilities. The type of container and the secondary Euro container means that the risk of any spillage of active GM waste on site is negligible. However, as active waste will leave the site, the risk to the environment and health is possibly marginally higher than autoclaving prior to incineration. The only real risk of release would be as a result of a major RTA, however, the containers are designed to withstand this. This disposal method is expected to achieve 100% inactivation of the GMM.

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

All liquid waste material will be inactivated by treatment with 1% (w/v) Virkon solution prior to disposal. Solid waste material will then be placed in sealed bins and incinerated by an off-site incineration company (see derogation application above). Waste from our GM work at Class 1 will be placed into suitably labelled hermetically sealable bins. They will be sealed by our staff and removed to a central collection point where they will be placed into 210 litre yellow labelled "Eurobins". Collections for incineration will be made by the local registered clinical/GM waste incinerator contractor.

This disposal method is expected to achieve 100% inactivation of the GMM.

The data sheets describing inactivation by Virkon are attached.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment Y

The scope and particular aspects of safety risks described in the risk assessment were agreed upon, and satisfactory consensus was achieved regarding the adequacy of the SOPs, COPs and risk management planning protocols. Pending notification and acknowledgement by relevant authorities.

**Project Containment**

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
</thead>
<tbody>
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<td>L2 Yes</td>
<td>L3</td>
<td>L4</td>
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<th>Large Scale Activities</th>
<th>Human Clinical Applications</th>
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Name

ADVENT BIOSERVICES LTD

Name 2

Department

Campus Estate or Research Centre

Road Name

SAWSTON BUSINESS PARK

District

SAWSTON

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB22 3JG

Country

ENGLAND

Tel Number

+44 1223 903100

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
Premises Addresses

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Advent Bioservices has a GMSC consisting of members of the Senior Management Team, including the Quality Department, a contracted Safety Consultant and the H&S lead for the company. This committee meets quarterly, with additional meetings as required if a new client is interested in the service.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
Virology Transgenic Animals Transgenic Fish Gene Therapy

02/03/2022
Yes

For disposal, all materials are sealed into yellow hazard bins (which are hermetically sealed) and sent for incineration (FCT-SOP-4). Any spills of materials would be managed as per standard procedure (FCT-SOP-7) which requires that any spills involving biological materials are treated with a suitable disinfectant to ensure inactivation of the biological material. Incineration is outsourced to Stericycle (GM Centre Number 779). We are applying for derogation as Advent Bioservices does not have a waste autoclave.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

For disposal, all materials are sealed into yellow hazard bins (which are hermetically sealed) and sent for incineration (FCT-SOP-4). Any spills of materials would be managed as per standard procedure (FCT-SOP-7) which requires that any spills involving biological materials are treated with a suitable disinfectant to ensure inactivation of the biological material. Incineration is outsourced to Stericycle (GM Centre Number 779). We are applying for derogation as Advent Bioservices does not have a waste autoclave.

**Tick to confirm that you are attaching a summary of the risk assessment**

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**Non-GMM Consent Granted**

**Project notified under transitional arrangements**

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**Significant Change ID**

**Date of Significant Change**

**Project Additional Information**

**Purposes of the contained use**

Advent Bioservices offers off site storage to external clients at a range of cold temperatures. Advent Bioservices do not in any way manipulate GMO cells. The service offered includes Receipt, Storage and Distribution. This includes waste management if applicable.

**Recipient or parental organism**

Not applicable. Advent Bioservices offer Receipt, Storage and Distribution only. Manipulation performed by Client as per their own Risk Assessment and Notification to HSE.

**Host/vector system**

Not applicable. Advent Bioservices offer Receipt, Storage and Distribution only. Manipulation performed by Client as per their own Risk Assessment and Notification to HSE.

**Origin & function**

Not applicable. Advent Bioservices offer Receipt, Storage and Distribution only. Manipulation performed by Client as per their own Risk Assessment and Notification to HSE.

**Evaluation of foreseeable effects**

Not applicable. Advent Bioservices offer Receipt, Storage and Distribution only. Manipulation performed by Client as per their own Risk Assessment and Notification to HSE.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

N/A

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

N/A

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

For disposal, all materials are sealed into yellow hazard bins (which are hermetically sealed) and sent for incineration (FCT-SOP-4). Any spills of materials would be managed as per standard procedure (FCT-SOP-7) which requires that any spills involving biological materials are treated with a suitable disinfectant to ensure inactivation of the biological material. Incineration is outsourced to Stericycle (GM Centre Number 779). We are applying for derogation as Advent Bioservices does not have a waste autoclave.

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N
The Genetic Modification Safety Committee (GMSC) have affirmed that Advent Bioservices is equipped with the appropriate facilities and systems to store and distribute external clients GMO material(s). These include appropriate waste and spill procedures. No manipulations will be performed. To undertake this work we are required to notify the HSE using the CU2 form. All clients must have their own risk assessment and Notification to the HSE prior to commencement of contracted work. This work is deemed low risk, please see attached Risk Assessment.

### Project Containment

<table>
<thead>
<tr>
<th>Laboratory Activities</th>
<th>Glass Houses</th>
<th>Growth Rooms</th>
</tr>
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- **Animal Units**
  - L2
  - L3
  - L4

- **Large Scale Activities**
  - L2
  - L3
  - L4

- **Human Clinical Applications**
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  - L3
  - L4
**GM Centre Number: 3661**

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**Name**

UNIVERSITY OF CAMBRIDGE, DEPARTMENT OF RADIOLOGY

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**Campus Estate or Research Centre**

SCHOOL OF CLINICAL MEDICINE

**Road Name**

BOX 218, CAMBRIDGE BIOMEDICAL CAMPUS

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**E-mail**

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**Comments**

Date at Which Additional Info Submitted

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: **Y**

Give brief details of the genetic modification safety committee:

- **Biosafety Officer** for the department of Radiology, University of Cambridge
- **Clinical School Safety Officer**, University of Cambridge

<table>
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<th>Animal Unit</th>
<th>Growth Room</th>
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Other (please specify): Tick if confidential: **N**

- **Bacteriology**
- **Parasitology**
- **Transgenic Birds**
- **Microbiology Research**
We will use commercially available prostate cancer cell lines (LNCaP) to knock-out two genes.

For activities involving GMMs, describe the waste management measures which will apply to the activity

Leftover cells not used in experiments will be detached from their respective flasks/containers and aspirated to a sealed reservoir containing pure cold sterilant solution (actril), according to the manufacturer's specifications, which achieves a kill percentage of 100%. Once inactive has been achieved, this solution can be safely disposed of via the main drain pipes in the lab.

Any flask/container where cells were present will be sealed and placed in appropriate biohazard bags, which will be autoclaved prior to disposal.

Any potential spills will be cleaned with appropriate wipes plus sprayed with 70% ethanol solution. These wipes will be also sprayed with ethanol and left under the microbial safety cabinet, exposed to UV light overnight, to further inactivate any potential leftover cells. These wipes will then be placed in appropriate biohazard bags and autoclaved prior to their disposal.

Cells used in terminal experiments will be actively lysed and destroyed according to the experimental protocol.

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Tick to confirm that you are attaching a summary of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

It is expected by both the Biosafety officer of the department of Radiology as well as an external Safety officer that this genetic modification will not pose any danger. In fact, the expectation is that knockdown of these genes would probably lead to a decrease in cell viability and proliferation, making these cells even less dangerous than normal.
**GM Centre Number: 3663**

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| Tel Number                         | 07779804913                |
| Fax Number                         | 0                          |
| E-mail                             |                            |
| HSE Division                       | blank                      |

| Comments                           |                            |

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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

The Skylark Therapeutics Ltd Genetic Modification Safety Committee will consist of

1. The CSO who has 15 years+ experience working in basic and translational research, including use of mammalian cell culture/primary samples and management of research teams.
2. The COO who has 15 years+ experience working in the biotechnology industry, managing teams focused on genetic engineering/synthetic biology across microbial and mammalian systems, and working with viral vector engineering and manufacture
3. External Consultant - Currently a Safety Advisor (CMIOSH) at a UK Research University with > 20 years experience in Biological and GM Safety.

The Committee will meet quarterly to review GM biosafety and whenever a new GM risk assessment is submitted

Laboratory | Animal Unit | Growth Room | Glass House | Large Scale
---|---|---|---|---
Level 1 (GMMs) | Yes |
Level 2 (GMMs) |
Level 3 (GMMs) |
Level 4 (GMMs) |
Non-microbial |
Other (please specify) | Tick if confidential |
Solid Waste disinfection and disposal
- All solid waste (i.e., tissue culture plates, stripettes, tips etc) will be double bagged in clear plastic autoclave bags at the end of each session.
- At designated daily time slots, autoclave bags will be taken to the autoclave room in the communal lab (F35) by the individual TC user (trained by competent person) where they will be processed immediately. The TC user is responsible for the loading and unloading of the autoclave.
- A backup autoclave is located in the adjacent Accelerator building in cases when the primary autoclave is not in use.
- Autoclave settings: 121°C, (holding time 20mins). Autoclaves will be validated by annual (at least) thermocouple mapping and each run will be monitored by continuous chart or digital recording of the temperature / time profile.
- Waste will be removed from site and incinerated by Grundon CWI, Clinical waste incinerator, Lakeside road, Colnbrook, Slough, SL3 0EG (who are licensed to handle clinical waste).

Liquid Waste disinfection and disposal
- Liquid will be collected in an autoclavable screw top plastic container.
- The container will be labelled with the date and company name. The liquid waste will be chemically disinfected by adding to 1:20 diluted Chemgene HLD4L for 1 hour before discharging via the drains with excess water in the lab sink within the TC suite. (see https://www.starlabgroup.com/Documents/eng/339784.pdf). The containers are reusable and should be rinsed with excess water and left to dry on the sink within the TC suite. Labelling will be removed at point of waste disposal.

Waste disposal routes
- All solid and liquid waste will be decontaminated/disposed of by the user at the end of every TC session. Liquid waste will be disinfected as described above.
- Solid waste will be autoclaved before final disposal by incineration.
- Waste will be removed from site and incinerated by Grundon CWI, Clinical waste incinerator, Lakeside road, Colnbrook, Slough, SL3 0EG (who are licensed to handle clinical waste).

Inactivation Controls
1. Autoclaving is a validated method and provides 100% cell kill.
2. All solid waste will be double bagged at the end of each session, prior to autoclaving.
3. All liquid waste will be decontaminated/disposed of by the user at the end of every TC session.
4. Surfaces will be sprayed 1% Trigene/1:20 Chemgene HLD4L followed by 70% Ethanol after use.

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment
This project has now been approved by Skylark GMSC as a Class 1 project subject to the following amendments:
- Clarification on downstream analysis;
- Source of iPSCs and continuous cell lines;
- Statement on health hazard of transient gene expression;
- Clarification on loading and removal of waste in the autoclave;
GM Centre Number: 3664

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Name

ARCTORIS LTD

Name 2

Department

Campus Estate or Research Centre

OXFORD BUSINESS PARK

Road Name

9400 GARSINGTON ROAD

Town

OXFORD

County

OXFORDSHIRE

Postcode

OX4 2HN

Country

ENGLAND

Tel Number

07713828576

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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| ARCTORIS LTD | OLYMPIC AVENUE | MILTON PARK | ABINGDONE | OXFORDSHIRE | OX14 4SA | N |

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

Experienced cell / molecular biologist with PhD and several (5+) years working in different laboratory settings with GM material including generating stable cell lines and performing cloning.

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| Level 2 (GMMs) | | | | |
| Level 3 (GMMs) | | | | |
| Level 4 (GMMs) | | | | |

Non-microbial

Other (please specify)

Tick if confidential

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<td>Virology</td>
<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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02/03/2022
Liquid GM class 1 waste is treated with 1% virkon for a minimum of 30 minutes before disposal to the drains as per manufacturer's instructions. Virkon is known to be an effective against viruses, bacteria and mycoplasma under these conditions (manufacturer's data) in the case of any infection that has contaminated the cell culture. Cultures are mycoplasma tested regularly. All solid waste (contaminated plastics, gloves etc) that has been in contact with GM class 1 material is either submerged in 1% Virkon for at least 30 minutes, or autoclaved at 121 degrees for a minimum holding time of 15 minutes before final disposal into yellow sacs as biohazard waste which is taken for incineration off-site by a licenced waste carrier. All waste is double contained. This should give a 100% kill, as established by Collins, CH (1993), Laboratory Acquired Infections, 3rd edition. Autoclaves undergo annual service and calibration and inspections. Small volume spillages will be cleaned up using 1% virkon. Due to the nature of the work there is no likelihood of large volume spillages. Benches are cleaned after use with 70% denatured ethanol or isopropyl alcohol.
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### Name

TRANSITION BIO LTD

### Name 2

### Department

### Campus Estate or Research Centre

### Road Name

JJ THOMPSON AVENUE

### Building

MAXWELL CENTRE

### District

### Town

CAMBRIDGE

### County

CAMBRIDGESHIRE

### Postcode

CB3 0HE

### Country

ENGLAND

### Tel Number

07944162996

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### HSE Division

### Comments

### Date at Which Additional Info Submitted

02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

We have received guidance from a health and safety consultant in the preparation of relevant risk assessment documentation and procedures. The risk assessment has been approved by an internal biological safety committee, which comprises the biological safety officer, scientific director and chief operating officer of the company and which meets every three months. The biological safety officer is an experienced biological scientist with >20 years experience working in and being responsible for risk assessment within CL1 and CL2 laboratories.

Laboratory Animal Unit Growth Room Glass House Large Scale

Level 1 (GMMs) Yes
Level 2 (GMMs)
Level 3 (GMMs)
Level 4 (GMMs)
Non-microbial

Other (please specify) Tick if confidential

Bacteriology Parasitology Transgenic Birds Microbiology Research
For activities involving GMMs, describe the waste management measures which will apply to the activity

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<td>Other(s)</td>
<td>Production of recombinant proteins.</td>
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Solid GM waste will be inactivated via autoclave (136°C for 4 minutes, 100% kill) prior to disposal via waste disposal contractor. The autoclaves are tested and calibrated annually and shown to hold temperature during the runs.

Liquid waste will be chemically inactivated using 1-5% Virkon for 60 minutes (or other approved disinfectand according to manufacturer's instructions).

The contingency procedure (for example in the event of autoclave malfunction) is the removal of biohazard waste for external incineration.

This work will be carried out within the Physics of Medicine building, Department of Physics, University of Cambridge. As such, the Department is responsible for waste management, the steps outlined above are the same as departmental requirements.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

We have received guidance from a health and safety consultant in the preparation of relevant risk assessment documentation and procedures. The risk assessment has been approved by an internal biological safety committee. One item of feedback from the committee was that the autoclave run time should be clarified to be four minutes, rather than forty as was originally stated. The safety committee comprises the biological safety officer, scientific director and chief operating officer of the company and which meets on a bimonthly basis.

The biological safety officer is an experienced biological scientist with >20 years experience working in and being responsible for risk assessment within CL1 and CL2 laboratories. The BSO will be attending an IOSH-approved programme in biological safety, management and practice.
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**Name**

FUTUREMEDS LTD

**Name 2**

Department

**Campus Estate or Research Centre**

**Building**

SOHOS ROAD HEALTH CENTRE

**Road Name**

247-251 SOHO ROAD

**District**

**Town**

BIRMINGHAM

**County**

MIDLANDS

**Postcode**

B21 9RY

**Country**

ENGLAND

**Tel Number**

07531298249

**Fax Number**

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**E-mail**

**HSE Division**

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**Comments**

**Date at Which Additional Info Submitted**

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

External consultant - previous experience includes more than 10 years employment as a Biosafety Advisor/Manager for a multinational pharmaceutical company and 8 years as a self-employed consultant with extensive experience in risk assessment and biorisk management for work with biological agents in general and GMMs in particular.

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Bacteriology  Parasitology  Transgenic  Microbiology
Birds  Research
All potentially contaminated GMM waste material (needles, syringes, wipes/swabs, PPE) and waste vaccine product will be placed into suitable clinical waste containers for collection by our GMO accredited/licenced clinical waste disposal contractor. In the unlikely event of a small spill, this will be absorbed onto an absorbent pad and disposed of as above. The area of the spill will then be cleaned using either sodium hypochlorite or Virkon™ disinfecant at the appropriate concentration as set out in the relevant SOP and risk assessment. Surfaces will then be cleaned using standard cleaning procedures.

For activities involving GMMs, describe the waste management measures which will apply to the activity

<table>
<thead>
<tr>
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<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
<th>Gene Therapy</th>
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Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

I am satisfied that risk class 1 is appropriate for this work and that the control measures set out in the risk assessment should ensure that exposure of persons and the environment is minimized so far as is reasonably practicable consistent with the Principles of Good Microbiological Practice and Good Occupational Safety and Health.
GM Centre Number: 3667

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Name

SOMASERVE LTD

Name 2

Department

Campus Estate or Research Centre

Building

BUILDING 580

Road Name

BABRAHAM RESEARCH CAMPUS

District

BABRAHAM

Town

CAMBRIDGE

County

CAMBRIDGESHIRE

Postcode

CB22 3AT

Country

ENGLAND

Tel Number

01223496790

Fax Number

0

E-mail

HSE Division

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Comments

Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

**Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities**

- Yes

**Give brief details of the genetic modification safety committee**

Advice was sought from the Health and Safety and QA manager, Babraham Institute, Babraham Research Campus. Somaserve GMO committee is composed of non-technical staff, and experienced scientific staff including members of the senior management team. The Health and Safety manager, provides both administrative and management support to the committee.

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- Tick if confidential

- Bacteriology
- Parasitology
- Transgenic Birds
- Microbiology Research
For class 1 contained use involving the propagation and transfection (with plasmid DNA or RNA) of mammalian cells; liquid waste from mammalian cell culture will be treated with Chemgene high level biodegradable, non-corrosive surface detergent, to a final concentration of 10% for a minimum of 10 minutes, as per manufacturers recommendations. Liquid waste will then be disposed of into the waste water system. Tissue culture flasks will be decontaminated with 10% solution of Chemgene detergent, as recommended by the manufacturer. Chemgene high level laboratory surface disinfectant combines enhanced active ingredient molecules with micelle cleaning technology to provide swift penetration of the cell walls to ensure rapid cell death. 'Treated, decontaminated' plastic waste will be rinsed with tap water and placed in waste bins reserved for 'treated decontaminated' plastic waste, and will be processed as recycled plastic. This procedure is applicable to large tissue culture flasks and is in line with current policies on plastic recycling and clinical waste reduction measures. Contaminated sharps will be placed in 'sharps bins', which are clearly labeled and disposed of according to Somaserve (POL003) and site waste handling policies (SOP BIO3). Benches, Microbiological Safety Cabinets (MSC) and other surfaces will be cleaned with 1% Chemgene solution and if required, 70% ethanol/water mix. Contaminated plastic consumables, for example serological pipettes and disposable tips, not suitable for recycling will be placed in autoclave bags and transferred to yellow 60L, leak proof eurobins, designated for 'clinical waste'. Bins are clearly labeled with biohazard symbols (UN3245 label), location and company name. Sealed yellow bins are surface decontaminated with 1% Chemgene detergent prior to removal from the containment facility for off-site incineration, according to Somaserve (POL003) and site waste handling policies (SOP B1O3). Autoclaves are not available for disposal of clinical waste on the Babraham Research Campus. A derogation from full containment has been granted to Babraham Research Campus. All clinical waste is incinerated in an off site facility. Off site incinerated site: Novus Environmental - Specialist Waste Management Experts (novus-environmental.co.uk)

GMGM898

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment
Project Ref 3667/22.1

Date Ackn'd 09/02/2022  CU2 Project Title
Date Project Ceased

Evaluation of Polynaut (https://somaserve.com/polynaut-technology), vesicles, prepared from biodegradable or stable polymers for the delivery of plasmid DNA to various cell types

Class 2  CultureVol 2  CultureVolume 3-4

Non-GMM  Consent Granted

Project notified under transitional arrangements N

Withdrawn  N

Tick if notifying a connected programme of work  N

Historical Significant Changes

Tick if notifying a connected programme of work

Historical Date of Additional Info

Significant Change ID

Date of Significant Change

Project Additional Information

Purposes of the contained use

Routine culture and cell banking of continuous mammalian cell lines (human and mouse), which have been transformed with known oncogenic sequences. Preparation and culture of primary murine glial cells from tissue and short term culture. Primary cells have an increased risk of carrying endogenous pathogens. Mice are obtained from 'pathogen free' colonies.

Cells will be transfected with plasmid DNA, here described as the cargo, in order to evaluate the expression of selected genes in vitro. Cells will be transfected using a novel technology, Polynaut (https://somaserve.com/polynaut-technology), vesicles, prepared from biodegradable or stable polymers. Polynaut will be evaluated alongside more traditional transfection protocols eg Lipofectamine and Electroporation.

Recipient or parental organism

GMO Cell Lines: Human endothelial kidney (HEK293T) cells, although a frequently used laboratory cell line are derived from HEK293 cells and result from sequential transformation events with viral genetic material: human adenovirus (Ad5) DNA and SV40 virus large T antigen. This modification allows continuous growth in culture and plasmids carrying the SV40 virus origin of replication to replicate to high copy in the transfected cell. BV-2 is a murine microglial cell line obtained from the C57/BL6 mouse strain. The BV2 cells are transformed by vraf/v-myc. BV2 express nuclear v-myc and the cytoplasmic v-raf oncogene products as well as the env gp70 antigen.
(J2 retrovirus) at the surface level. BV2 microglia cell line retains microglia morphological and functional characteristics. Cell lines transformed with known oncogenic sequences carry increased hazard of causing harm. Primary murine glial cells. Primary cell lines carry additional hazards, primary cell lines are more likely to carry adventitious agents, mycoplasma, and virus pathogens. Primary cells will be sourced from 'pathogen free' animals from established colonies.

### Host/vector system

Cell lines and primary cells will be transfected with 'reporter plasmids' pcDNA3.1 NL which carries the nano luciferase gene, amp and neo resistance markers and CMV promoter. The plasmid is non-mobilisable.

R-pre-GFP which carries the green fluorescent protein, kan and neo resistance markers and CMV promoter. The plasmid is non-mobilisable.

### Origin & function

The reporter plasmids have been prepared in an Ecoli host system, but it is not intended that this document includes the preparation of the reporter plasmids. Reporter plasmids are sourced from commercial/contractual suppliers.

- GFP reporter protein is from the species Aequorea victoria
- Nano luciferase (nanoLuc) reporter protein is from the species Oplophorus gracilirostris.

The GFP protein has been modified to locate to the cell surface. No additional hazard is anticipated with this modification.

The reporter plasmid will allow the efficiency of 'transfection' to be measured. Luciferase expression in transfected cells will be measured using the "luminol" assay. GFP expression will be measured by microscopy and western blot.

### Evaluation of foreseeable effects

Expression of the inserted reporter genes will be transient and therefore limited. It is not anticipated that expression of the GFP or nanoLuc protein will modify the tropism of the cell or change/increase the underlying hazard of working with a transformed cell line.

The GMO cell line requires specialised growth media and can not survive out side the laboratory environment.

### Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

- **For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**
  - Class 2 waste is disposed of as described in policy documents Somaserve POL003 and BRC SOP BIO3. Briefly, class 2 waste (solid) is placed in EURO bins which are sealed with a red lid and surface cleaned and incinerated off site. Off site incineration is managed by on site contractors, Babraham Research Campus. Autoclaves are not available for disposal of clinical waste on the Babraham Research Campus. A derogation from full containment has been granted to Babraham Research Campus. All clinical waste is incinerated in an off site facility. Off site incinerated site: Novus Environmental - Specialist Waste Management Experts (novus-environmental.co.uk) GMGM898

- **Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**
  - For class 2 contained use involving the propagation and transfection (with plasmid DNA or RNA) of BSL2 mammalian cells; liquid waste from mammalian cell culture will be treated with Chemgene high level biodegradable, non-corrosive surface detergent, to a final concentration of 10% for a minimum of 10 minutes, as per manufacturers
recommendations. Liquid waste will then be disposed of into the waste water system. Chemgene high level laboratory surface disinfectant combines enhanced active ingredient molecules with micelle cleaning technology to provide swift penetration of the cell walls to ensure rapid cell death. Chemgene detergent is biodegradable. Tissue culture plastics (solid waste) and other contaminated plastic waste will be placed directly in yellow EURO bins and sealed with a red lid and designated as class 2 'clinical waste'. Contaminated plastic consumables, (solid waste) for example serological pipettes and disposable tips, not suitable for recycling will be placed in autoclave bags and transferred to yellow 60L, leak proof EURO bins, and sealed with a red lid, and designated as 'class 2 clinical waste'. Bins are clearly labelled with biohazard symbols (UN3245 label), location and company name. Sealed yellow EURO bins are surface decontaminated with 1% Chemgene detergent prior to removal from the containment facility for offsite incineration, according to Somaserve (POL003) and site waste handling policies (SOP B1O3). Large tissue culture vessels, may be treated with 10% chemgene solution. 'Treated', decontaminated plastic waste will be rinsed with tap water and placed in waste bins reserved for 'treated decontaminated' plastic waste, and will be processed as recycled plastic. This procedure is applicable to large tissue culture flasks and is in line with current policies on plastic recycling and clinical waste reduction measures. Contaminated sharps will be placed in leak proof 'sharps bins', which are clearly labelled and disposed of according to Somaserve (POL003) and site waste handling policies (SOP BIO3). Sharps bins are incinerated at the offsite facility, Novus Environmental. Benches, Microbiological Safety Cabinets (MSC) and other surfaces will be cleaned with 1% Chemgene solution and if required, 70% ethanol/water mix.

Is an emergency plan required according to regulation 20? N
If yes, tick to confirm that it is attached to this form N
Tick to confirm that you have attached a risk assessment to this form Y
Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

no comments

Project Containment

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02/03/2022
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Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Give brief details of the genetic modification safety committee

Safety Committee comprises the following personnel:

- Site and Health & Safety Manager
- Director of R&D Delivery
- Biological Safety Officer
- Subject Matter Expert - Cell Culture and Viral Vectors
- Viral Vector - Research Scientist

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### Virology
- Transgenic Animals
- Transgenic Fish

### Mycology
- Transgenic Invertebrates
- Transgenic Plants

### Gene Therapy
Yes

### Other(s)
- Other (please specify below)

**For activities involving GMMs, describe the waste management measures which will apply to the activity**

n/a

**Tick to confirm that you are attaching a summary of the risk assessment**

Y

**Tick if you are claiming exemption from disclosure for sections of the risk assessment**


**Project Ref 3668/22.1**

**Date Ackn'd**
11/02/2022

**CU2 Project Title**
Production of plasmid DNA from E. Coli as well as production of viral vectors; Adeno associated virus (AAV) and Lentivirus to 10L scale. Purification of same and infectivity assays will be run on the vectors post purification

**Date Project Ceased**

**Class**
- Class 2

**CultureVol**
- 1-50 Litres

**Non-GMM Consent Granted**

**Historical Date of Additional Info**

**Project notified under transitional arrangements**

N

**Withdrawn**

N

**Tick if notifying a connected programme of work**

N

**Historical Significant Changes**

**Historical Date of Additional Info**

**Significant Change ID**
### Purposes of the contained use

Vectors and plasmid DNA generated will be used to characterise novel chromatography adsorbents for vector purification. To assess the purification of vectors for use in gene therapy.

### Recipient or parental organism

Lentivirus is a retrovirus and may be infective:

[https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5152689](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5152689)

AAV used will be non competent and therefore will not be infective.

The E. coli host will be E. coli K12 derivatives - DH5 alpha, ER2738 (F+) for phage display and BL21-Gold (DE3) for subcloning and protein expression (F-).

### Host/vector system

Mammalian cell lines will include a number of well characterised (immortalised) cell lines of human or murine origin. Transgenes/ reporter genes will be expressed in HT 1080, for example, and limited to GFP/ Luciferase for infectivity testing purposes to limit possibility of harm.

Mammalian cell lines will include a number of well characterised (immortalised) cell lines of human or murine origin. Transgenes/ reporter genes will be expressed in HT 1080, for example, and limited to GFP/ Luciferase for infectivity testing purposes to limit possibility of harm.

### Origin & function

Cell lines will be purchased from commercial vendors.

### Evaluation of foreseeable effects

The recombinant viral vectors are disabled vectors. They have had regulatory and accessory genes deleted, ensuring that viral particles produced in packaging cell lines
are replication incompetent. As a result, whilst they pose an infection risk they are unable to initiate further rounds of replication/infection cycles. The probability of seroconversion is minimal.

Whilst the VSV-G envelope extends the cellular tropism and confers greater stability and environmental survivability, it is thought there is NO significant increase in the likelihood of transfection via the airborne route of exposure. Control measures utilised are appropriate to guard against the associated residual risks.

The target genes do not encode viral specific protein, nor do they interfere with known activities of the virus and so are unlikely to have any effect upon the basic nature of the virus.

Whilst the VSV-G envelope confers greater stability and environmental survivability, control measures employed will minimise risks to the environment. These control measures include - (1) rendering all solid or liquid waste inactive within the building (either by autoclaving or use of Virkon), (2) work will be carried out in Containment level 2 laboratories, (3) work will be conducted within Class 2 microbiological safety cabinets, (4) routine disinfection of work surfaces and (5) all staff are suitably trained in GM work and employing the necessary risk control measures.

**Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)**

NA

**For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)**

n/a

**Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)**

**Solid waste (i.e. gloves, plastic consumables, tissue) -**

Will be collected in labelled autoclavable biohazard bags in leak proof containers.
Waste will be autoclaved at 121°C for an appropriate time then placed into heavy duty biohazard bags and sealed.
Waste will then be placed into a locked incineration waste bin to be collected by an approved waste management company (i.e. Grundons) and incinerated accordingly.

**Solid waste (i.e. sharps) -**

Will be collected in dedicated autoclavable approved sharps containers.
Waste will be autoclaved at 121°C for an appropriate time then sealed and placed into heavy duty biohazard bags and sealed.
Waste will then be placed into a locked incineration waste bin to be collected by an approved waste management company (i.e. Grundons) and incinerated accordingly.

**Liquid waste -**

Will be treated via a suitable chemiclal process (i.e. 1% Virkon for a minimum of 15 minutes) prior to disposal down the sink; flushed with plenty of water.
Spillages will be contained and treated via a suitable chemical process (i.e. ChemGene).

**Is an emergency plan required according to regulation 20?**

N

**If yes, tick to confirm that it is attached to this form**

N

**Tick to confirm that you have attached a risk assessment to this form**

Y
Safety Committee comprises the following personnel:

- Site and Health & Safety Manager
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- Animal Units
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GM Centre Number: 3669

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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Yes

Give brief details of the genetic modification safety committee

The risk assessment was examined and approved by the BioEscalator Genetic Modification Safety committee comprising at least 4 Biological Safety Officers from other biotech companies within the building.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
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<tr>
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Other (please specify)  
Tick if confidential

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<th>Microbiology Research</th>
<th>Transgenic Animals</th>
<th>Transgenic Fish</th>
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Yes

Generation of replication-deficient genetically modified viral vectors

For activities involving GMMs, describe the waste management measures which will apply to the activity

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

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Purposes of the contained use

As a result of intellectual property implications, the identity of the vector and the inserts will need to remain confidential. The wild-type agent from which the replication-deficient viral vectors are derived is classed as ADCP Group 2, with low propensity for mutation, recombination or integration. The natural route of infection for the wild-type replicating agent is via the oral, mucosal and inhalation route. The wild-type agent is attenuated by removal of key viral replication genes and can only replicate in cell lines that express the complementary viral genes. It is therefore not capable of causing an infection in humans or animals, although it will transduce human and animal cells (i.e. the virus will enter a cell and express the insert, but no infectious viral particles can be produced). There are no reports of shedding of the constructs into the environment upon in vivo administration and it has been reported to be deactivated by chlorinated drinking water.

Plasmids containing the truncated viral genome (destination vectors) will be recombined in vitro with entry vectors containing the genetic sequence of interest to generate plasmids containing the viral genome and the genetic sequence of interest. These will be transfected into cell lines expressing the complementary viral genes to produce viral particles that also express the recombinant protein of interest. These recombinant, replication-deficient viruses will be tested in vitro and in vivo in appropriate animal facilities.

Recipient or parental organism

Standard packaging cell lines (eg PerC6 or 293) from commercial sources. These are recombinant human cells with part of the virus genome integrated, so that the missing viral proteins are expressed.

Host/vector system

Destination vectors:
- p-Dest
- plasmid with recombinant replication deficient viral genome (See Appendix 1a for p-Dest vector map)

Entry vectors:
- p-Entry
- A plasmid with appropriate promoter, multiple cloning site to insert immunogen sequence of interest and polyA signal sequence (See Appendix 1b for pEntry vector map)

Origin & function

Transcription in the recipient cell line expressing the complementary viral proteins allow formation of infectious recombinant viral particles. These can be purified for use in other experiments. The recombinant virus particle lacks key viral genes, so that replication is only possible in the recipient cell line. None of the products of the introduced genetic sequence will increase the required containment level above BSL2.

The generated viral particles will transduce animal cells and express the recombinant genetic material. If injected into animals, an immune response will be generated against the recombinant genetic sequence. However no further infectious particles can be formed.

Evaluation of foreseeable effects

Evaluation of the foreseeable effects of

i) the recipient micro-organism: No hazard identified from recipient human cells

ii) the inserted (donated) genetic material: The inserted genetic material will be typically manufactured as a synthetic sequence; these will consist of DNA sequences derived from human genes or genes from other mammalian or micro-organisms. There is no risk associated with the inserted donor source. Likewise, the products of these sequences would not be toxic or oncogenic to animals or humans.
iii) the vector: The recombinant viral genome can only form infectious particles in the recipient cells, which contain the missing viral genes, not in other human or animal cells, and cannot therefore cause a disseminated infection or disease.

iv) the resulting genetically modified micro-organism: The recombinant replication deficient virus would contain the inserted gene. Expression of the genetic sequence is driven by the CMV promoter. This does not generate additional hazards.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)

N/A

For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

1. Used consumables (plasticware eg. Pipettes, flasks, tubes, tips)
   Autoclave using make safe cycle as specified in BS 2646 Prt 3, 1993 (134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the black bags (for incineration) waste route following autoclaving.

2. Culture fluids (tissue culture medium, bacterial growth medium, washing etc) will be inactivated with virkon solution (Virkon concentration 2% W/V) for at least 60 mins and then discharged to drains.

3. Agar plates are autoclaved using make safe cycle as specified in BS 2646 Prt 3, 1993 (134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via the black bags (for incineration) waste route following autoclaving.

4. Spillage: In the event of a spill of culture fluid, virkon granules should be added to the spill. Mop up using tissue and autoclave all contaminated waste.

Degree of kill:
   Autoclaving, effectively 100% (annual validation)
   Incineration, effectively 100% (licensed incinerator)
   Virkon disinfection, 99.99% kill. Virkon has been tested by independent laboratories and been proven to be effective against 58 viruses including the virus family of the vector.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment
Please note that as a result of the comments of the committee below we have modified the containment level to BSL2.

Happy with the revisions

While it is stated that the agent is class 2, the form is still listed as class 1 and this line "None of the products of the introduced genetic sequence will increase the required containment level above BSL1" reads as though the work will be performed at BSL1. My understanding is that if an agent is class 2 then it needs to be handled under BSL2 conditions. Additionally, human cells lines like 293 cells are also required to be handled/manipulated under BSL2 conditions. Hope this makes sense.

Thanks for this. It seems a little bit strange they are reluctant to name their type of virus (particularly when they say they are using 293 and PerC6 cell to package, which implies it's an adenovirus; and the genome size in the plasmid supports that). Nevertheless the risk assessment appears reasonable to me - except the university used to have a guideline that 293 and PerC6 cells (because they harbour a known oncogene) have to be handled at category 2.

Looks good to me except the virus name and the usual route of infection for the virus used (inhalation, oral, skin contact) is not described. This is useful to know to show safety methods in place are sufficient to prevent exposure or clean up after an incident. Though the risk assessment does clearly show even if someone was exposed its highly likely to be extremely harmless.

The rest of the members had no comments.

### Project Containment

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#### Name

**SMITHS DETECTION WATFORD LTD**

#### Name 2

**Department**

#### Campus Estate or Research Centre

#### Road Name

**MAYLANDS AVENUE**

#### Town

**HEMEL HEMPTEDD**

#### County

**HERTFORDSHIRE**

#### Postcode

**HP2 7DE**

#### Country

**ENGLAND**

#### Tel Number

01923 65 8000

#### Fax Number

0

#### E-mail

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#### HSE Division

blank

#### Comments

**Date at Which Additional Info Submitted**

02/03/2022
## Premises Addresses

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## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

- UK SDW BioSafety Officer / UK SDW Deputy BioSafety Officer / UK SDW HSE Manager / UK SDW Director of Technology / US SDI Chief Scientist, BioTechnology / US SDI Senior Microbiologist
- The committee comprises the following skills:
  - 4 members of the committee hold degrees in biological sciences
  - 1 is a professional safety manager
  - 1 is a senior manager with experience in managing safety critical systems and materials.
- All have multiple years experience in their respective fields of work.
- Initial review held, quarterly meetings scheduled

<table>
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<td>Tick if confidential</td>
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</table>
Liquid wastes will be treated with bleach (1% sodium hypochlorite) and left for 24 hours prior to disposal via laboratory sinks into waste water management systems. Used pipette tips and vessels such as bottles and beakers will be soaked for 24 hours in 1% hypochlorite prior to rinsing in water: i) The single use items, such as disposable pipette tips, will be placed in sharpsafe canisters and sealed prior to disposal as hazardous waste for incineration. ii) The re-usable items, such as glass bottles and beakers, will, after treatment with 1% sodium hypochlorite for 24 hours, be placed in a laboratory dish washer for cleaning prior to re-use. Spills will be sprayed with 1% hypochlorite and wiped up with tissues which will then be placed in an autoclave bag for subsequent autoclaving prior to disposal as hazardous waste for incineration. Other solid wastes, such as tissues used to wipe up spills, will be autoclaved prior to disposal as hazardous waste for incineration.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

The GM Committee reviewed the CU1 form and risk assessment and the cells used, E. coli K12 and immortalised lymphocytes, are both confirmed low risk Containment Level 1 materials and the inserted sequences for aequorin, coelenterazine and antibodies are Class 1 GM modifications with the final modified material presenting negligible risk to humans and the environment. Further the control measures and decontamination methods used are suitable for the selected materials.
### GM Centre Number: 3671

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#### Name

PENCIL BIOSCIENCES LIMITED

#### Name 2

#### Department

#### Campus Estate or Research Centre

OFFICE 3F38

#### Building

OFFICE 3F38

#### Road Name

ALDERLEY PARK

#### Town

MACCLESFIELD

#### County

CHESHIRE

#### Postcode

SK10 4TG

#### Country

ENGLAND

#### Tel Number

01625 704040

#### Fax Number

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#### HSE Division

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#### Comments

#### Date at Which Additional Info Submitted

02/03/2022
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### Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities: 

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Tick if confidential:

- Bacteriology: Yes
- Parasitology: Yes
- Transgenic Birds: Yes
- Microbiology Research: Yes
Inactivation methods include autoclaving (up to 121°C). Solid waste disposed in clear biohazard waste bags which are submitted to Alderely Park Waste management for autoclave. Liquid waste is disposed of by using 1% virkon.

For activities involving GMMs, describe the waste management measures which will apply to the activity:

Inactivation methods include autoclaving (up to 121°C). Solid waste disposed in clear biohazard waste bags which are submitted to Alderely Park Waste management for autoclave. Liquid waste is disposed of by using 1% virkon.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment:

All bioagents are Biosafety Level 1 and the GMMs produced lead to modifications that do not lead to any functional advantages of biological significance.

Transfection of protein/DNA or RNA encoding Cas9, Cas9 nickases or our in-house genomic editing tool with BSL 1 agents, that target endogenous genes producing either knock in or knock out GMMs. Genes targeted do not lead to any functional advantages of biological significance.

Inserts are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms.

The GMMs pose no threat to humans or the environment and are unable to survive outside of a laboratory environment.

All GMMs will be inactivated by autoclave before disposal and hand over to waste management at Alderely Park.

Risk Assessments are attached for more details.
Our studies are carried out by transfecting RNP (Ribonucleoprotein)/RNA or plasmid DNA insertions that target and edit a selected endogenous gene producing either a tag or knock in/out GMM. No known loss or gain of function is expected to cause an effect of any biological significance. Recombinant DNA methods are used to analyse gene characteristics, for example by subcloning and sequencing DNA obtained from screening libraries or by PCR. Recombinant methods are also used to make constructs to express normal or mutant proteins in tissue culture.

Recipient or parental organism
Gibco® Episomal Human iPSC line
& Human iPS Cell Line
Although the cell lines are not known to contain any agents capable of harm to humans the possibility of a contaminant, adventitious virus can rarely be excluded. Therefore, these are recommended as a BioSafety Level 2 agent

Host/vector system
For DNA inserts delivery is via standard commerical-based plasmid expression vectors. DNA inserts from vectors are integrated through transient expression.

Origin & function
The insertion is either the protein/plasmid DNA/mRNA inserts that encode for proteins that are known (Cas9 and Cas9 nickases) or supposed (our in-house tool) to function as a genomic editing tool. These include enzymes that target and modify a selected endogenous gene. Either short nucleic acid/amino acid or dsDNA templates are introduced to aid the restoration or disruption of the targeted gene. Inserts are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms.

Evaluation of foreseeable effects
The delivery of the DNA inserts produce either knock in or knock out of a pre-selected endogenous gene. This will not lead to any advantageous gain in function compared to the orginal host cells. The resulting GMMs are unable to survive beyond the laboratory environment and after appropriate heat inactivation or disinfection pose no risk to the environment. Therefore they remain a BioSafety Level 2 agent and will be contained as such.

Containment and control measures for GMOs that are not microorganisms (e.g. GM animals & plants)
GMO includes Biosafety level 2 agents (iPSC) cell lines only. All cell lines are fixed, all work to be carried out using a Class II Biosafety cabinet. This level of containment is sufficient for these cells. Cells once transfected are grown for 1-2 days and then sacrificed and the gDNA extracted using general molecular biology techniques.
For only GMMs - application for any derogation from full containment for the Class of activity. (Measures & Justification)

Described the waste management measures which you will apply to the activity (including type & form, treatment, ultimate form & fate)

Specified disinfection procedures in place, inactivation of liquid waste by autoclave or 1% vikron, solid waste disposal in clear biological waste bags for autoclave by waste management at Alderley Park.

Standard molecular biology techniques. Sonicator or chemical lysis used for breaking cells and centrifuge for pelleting cells. Maximum culture volumes - cell culture/plasmid preps - less than 0.1 L

Standard molecular biology analysis and processing such as PCR or DNA sequencing.

Is an emergency plan required according to regulation 20? N

If yes, tick to confirm that it is attached to this form N

Tick to confirm that you have attached a risk assessment to this form Y

Tick if you are claiming exemption from disclosure for section of the risk assessment N

Please enter comments on the GM safety committee on the risk assessment

iPSC cell lines are designated Biosafety Level 2. Mostly to protect both user and cells. Therefore Level 2 biosafety hood required to handle the cell line. Genetic modifications to be carried out on the cell lines produce knock in/knock out GMMs with no advantageous functionality of biological significance.

Endogenous gene target have been specifically selected (gene PD1) to be deleted or restored resulting in a GMM that are not harmful to the environment or humans. These GMMs are unable to survive outside of the laboratory environment.

Project Containment

<table>
<thead>
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<th>Glass Houses</th>
<th>Growth Rooms</th>
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Name

BRADFORD TEACHING HOSPITALS FOUNDATION TRUST

Department

Campus Estate or Research Centre

Road Name

DUCTWORTH LANE

Town

BRADFORD

District

Buildings

BRADFORD ROYAL INFIRMARY

County

Postcode

BD9 6RJ

Country

ENGLAND

Tel Number

01274 383383

Fax Number

0

Email


HSE Division

blank

Comments

Date at Which Additional Info Submitted

02/03/2022

Page 15324 of 15326
## Premises Addresses

<table>
<thead>
<tr>
<th>Date Premises Closed</th>
<th>Name</th>
<th>Department</th>
<th>Name 2</th>
<th>Campus</th>
<th>Estate or Research Centre</th>
<th>Building</th>
<th>Road Name</th>
<th>District</th>
<th>Town</th>
<th>County</th>
<th>Post-code</th>
<th>Country</th>
<th>Withdrawn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRADFORD</td>
<td>TEACHING</td>
<td>HOSPITALS</td>
<td>FOUNDATION</td>
<td>TRUST</td>
<td>PATIENT RECRUITMENT CENTRE</td>
<td>BRADFORD ROYAL INFIRMARY</td>
<td>GATE 4, SMITH LANE</td>
<td>BRADFORD</td>
<td>BD9 6RJ</td>
<td>N</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Premises Conditions

Tick to confirm that you have established a GM safety committee to advise on risk assessments of contained use activities

Y

Give brief details of the genetic modification safety committee

Director of pharmacy

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Animal Unit</th>
<th>Growth Room</th>
<th>Glass House</th>
<th>Large Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 (GMMs)</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 2 (GMMs)</td>
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<tr>
<td>Level 3 (GMMs)</td>
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<tr>
<td>Level 4 (GMMs)</td>
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</table>

Non-microbial

Other (please specify) Tick if confidential

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Transgenic Birds</th>
<th>Microbiology Research</th>
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<td>Transgenic Animals</td>
<td>Transgenic Fish</td>
<td>Gene Therapy</td>
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<tbody>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
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</table>
For activities involving GMMs, describe the waste management measures which will apply to the activity

Standard GMO clinical waste disposal will apply. As per the R&I COP, any material that has been in contact with the product will be disposed/packaged by the team undertaking the trial, in yellow Econix bins (for soft waste) and/or yellow sharps bins (for sharps waste) as appropriate.

The disposal of waste following administration will follow the waste stream for non-infectious waste. A UN 3245 label and a GMO label should be placed on these bins. Two copies of the GMO Waste Disposal Record Sheet will be maintained by the research team with a copy given to waste disposal team so tracking to external disposal/inactivation is possible.

Level 1 waste stream will be adopted at pharmacy during handling of the IMP.

GM waste will not be inactivated on site. They will be incinerated offsite by an external contractor.

Tick to confirm that you are attaching a summary of the risk assessment

Tick if you are claiming exemption from disclosure for sections of the risk assessment

Please enter comments of the GM safety committee on the risk assessment

Total GMO Centres included in this report = 1,195